Measurement of ex vivo porcine lens shape during simulated accommodation before and after fs-laser treatment

J Hahn, Laser Zentrum Hannover e.V., Hanover, Germany, j.hahn@lzh.de
M Fromm, ROWIAK GmbH, Hanover, Germany, m.fromm@lzh.de
S Besdo, Institut für Kontinuumsmechanik, Leibniz Universität Hannover, Hannover, Germany, s.besdo@ikm.uni-hannover.de
H Lubatschowski, ROWIAK GmbH, Hanover, Germany, h.lubatschowski@rowiak.de
A Krüger, Laser Zentrum Hannover e.V., Hanover, Germany, a.krueger@lzh.de
T Ripken, Laser Zentrum Hannover e.V., Hanover, Germany, t.ripken@lzh.de

Introduction

Accommodation is the ability of the human eye to adjust from far to near vision by deforming the eye lens. With age, the lens material hardens, which leads to a decrease in flexibility and therefore a decrease of accommodation ability, called presbyopia. To study the possibility to treat presbyopia with the application of fs-laser pulses in the crystalline lens (fs-lentotomy), the deformability due to the laser treatment was evaluated in this experimental study.

Methods

Ex vivo porcine lenses were used to evaluate the deformability during simulated accommodation with a lens stretching device before and after the fs-lentotomy. To study a possible increase of deformability the parameters lens thickness, diameter, anterior and posterior radius were measured by analysing cross-sectional and volumetric imaging data acquired with optical coherence tomography (OCT). Furthermore the optical power was calculated and the lens shape was parameterised by using the measured lens parameters and photographs of the isolated lens. The results of a control group without laser treatment were used for comparison.

Results

The findings show a significant improvement of deformability of the treated lenses due to the parameters lens thickness, diameter, and anterior radius after the laser treatment. The change of posterior radius amplitude shows ambiguous results with no statistical significance. There also was no significant increase in the calculated optical power due to the laser treatment. All parameters of the control group showed no statistically significant changes.

Conclusion

Although there was no significant effect on the calculated optical power standing for the accommodation ability, our results show a positive effect on the overall lens deformability of porcine lenses due to the fs-laser treatment. These findings support the basic understanding of fs-lentotomy as a treatment method for presbyopia.
Chromatically encoded high-speed photography for the analysis of cavitation bubble oscillation inside inhomogeneous ophthalmic tissue

N. Tinne, S. Veith, R. Lorbeer, A. Krüger and T. Ripken; Laser Zentrum Hannover e.V. (Biomedical Optics Department); Hannover, Germany, e-mail: n.tinne@lzh.de

Introduction

The presented study describes a method of chromatically encoded photography for the high-speed analysis of cavitation bubble oscillation generated by femtosecond laser-induced optical breakdown in inhomogeneous aqueous media like ophthalmic biological tissue.

Interaction effects between single laser pulses and biological tissue have been studied extensively in water as an accepted sample medium. Here, the effect itself is highly reproducible so that the method of time-resolved photography is well applicable. Analyzing cavitation mechanisms inside more solid and inhomogeneous media like ophthalmic tissue the reproducibility significantly decreases. Hence, to analyze single events a high-speed photographic approach is necessary.

Methods

To fulfill restrictions in the effect’s time scale a high-speed imaging system was developed. For illumination of the region of interest within the sample medium the light paths of various LEDs of different emission wavelengths are combined. The diodes are externally triggered with an individual delay for each LED. Furthermore, for detection, the various illumination wavelengths are chromatically separated on the chip of the camera. Hence, a time-sequence of images of a single interaction event is generated.

Results and Discussion

A sufficiently short LED flash with a duration below 100 ns was achieved with MOSFET-electronics. Furthermore, a compact laboratory setup was realized. Flash LEDs were combined chromatically as well as spatially for illumination. Chromatically separation was applied for detection, correspondingly. First results in different sample media like water and HEMA show the considerable advantages of this novel illumination technique in comparison to the one of conventional time-resolved photography.

Conclusion

In Conclusion, the results of this study are of great interest for the fundamental analysis of the laser-tissue-interaction inside inhomogeneous biological tissue as well as for the prospective optimization of the surgical process with high-repetition rate fs-lasers. Additionally, this application is also suitable for the investigation of other ultra-fast events in transparent inhomogeneous materials.
Echo State Networks for Granulopoietic Cell Recognition in Histopathological Images of Human Bone Marrow

P. Kainz¹, M. Mayrhofer-Reinhartshuber¹, H. Burgsteiner², M. Asslaber³, H. Ahammer¹
¹Institute of Biophysics, Medical University of Graz, Austria
²Institute for eHealth, Graz University of Applied Sciences, Austria
³Institute of Pathology, Medical University of Graz, Austria
Corresponding author: philipp.kainz@stud.medunigraz.at

Abstract

The assessment of the cellularity in bone marrow is an essential step in diagnostic processes in pathology. The quantification of cell maturity at microscopic level is a tedious and error-prone task and heavily relies on the experience of the pathologists. The inter-observer variability in cellularity estimation may be reduced by employing supervised machine learning methods on digital histopathological images. The main goal of this paper is to examine echo state networks (ESN) for quantitative cell recognition. We show that a properly designed and trained ESN is able to discriminate early and late granulopoietic cells in histopathological images of the human bone marrow with overall mean accuracy (±SD) of 0.846 (±0.013). This work gives strong indication that ESNs are able to work even with raw image patches directly and do not necessarily require image pre-processing, or feature extraction prior to classification.

1 Introduction

Within the last years, digital histopathological slides have become a popular alternative to conventional glass slides [1] and are now used more frequently in diagnostics and research [2]. Histomorphometry of bone marrow tissue is a fundamental step in a wide range of diseases, including leukemia, or lymphoma. If undetected, both blood cancers can be lethal, but an early detection of atypical biological behaviour may increase patient survival tremendously. Currently, pathologists are using glass slides and conventional light microscopy, or digital slides for the assessment of histological specimen. A multi-potent stem cell is the origin of all types of bone marrow cells: erythrocytes, granulocytes, monocytes, megakaryocytes and their corresponding progenitor cells, as well as macrophages and mast cells [3]. Granulopoiesis refers to the maturation of granulocytes in bone marrow and comprises six major stages. A single slide may contain thousands of cells belonging to different classes, where the pathologist has to determine an overall statistic of the cellularity in terms of hypo-, normo-, or hypercellular marrow in order to support a final diagnosis. This qualitative assessment within a single specimen is a tedious and error-prone activity and heavily depends on the experience of the observer [4,5]. Additionally, the determination of different maturity stages is inherently difficult, since evolution is continuous. Subjective emphasis of morphological criteria may lead to an under- or overestimation of the cellularity. Consequently, in certain cases both intra- and inter-observer variability may be quite high. Gurcan et al. [6] pointed out the need for robust quantitative methods in disease grading and that computer-aided methods are substantial to modern diagnostics in pathology departments. While the human visual system is highly sophisticated in localizing semantically coherent regions and objects in images, teaching computers to see from examples has always been a hard task. However, machine learning provides tools for automated tissue analysis in digital pathology [7]. Procedures for automated blood cell classification mostly focused on peripheral blood smear samples [8,9] and did not attribute for cell evolution, but for quantification of mature cells.

1.1 Research Problem and Goals

Early and late granulopoietic cells exhibit a high intra-class variability and a low inter-class distance. They differ in color and shape, but unfortunately, static image processing methods cannot generalize to unseen instances. Fig. 1 shows examples of both classes to be distinguished.

![Figure 1](image_url)

**Figure 1** Single cell examples of early and late granulopoiesis, where promyelocytes (a) and myelocytes (b) are attributed to early, and metamyelocytes (c) and band cells (d) are attributed to late granulopoiesis.

Classical quantitative image processing methods [10] mostly rely on texture, and geometrical or statistical features for classification [6], but the discrimination of objects based on rigid texture models is often not sufficient for biological material. Thus, we propose a machine learning approach for the discrimination of two maturity stages. This work presents a proof-of-concept study on the application of echo state networks for the discrimination between early and late granulopoiesis in histopathological images.
1.2 Supervised Machine Learning and Echo State Networks

The purpose of supervised machine learning is to design algorithms to learn a model or function from some examples without explicitly defining inference rules [11]. Besides training examples, supervised learning requires the desired output to be learned by an algorithm, i.e. a class label (classification) or a continuous function (regression). Reservoir computing (RC) refers to a recent paradigm in computational intelligence and deals with separate training of recurrent neural networks and its readouts [12]. Echo state networks (ESN) [13] are one possible realization of RC and consist of an input layer, a pool of randomly interconnected artificial neurons (“reservoir”) and a readout layer. The input layer is connected to the reservoir via a sparse random synaptic weight matrix $W^{\text{in}}$. ESNs are characterized by a set of hyperparameters, which have to be chosen carefully and individually for each problem. The most important parameters of the reservoir are the number of units $N$, the leaking rate $\alpha$, and the spectral radius of the weight matrix $\rho(W)$. In order to be an universal function approximator, ESN reservoirs require to exhibit the echo state property [13], where $\rho(W) < 1$. ESN have been successfully applied to handwritten digit recognition from trajectories [14], speech recognition [15], image classification [16], and in generative approaches, use biologically realistic spiking neuron models in the reservoir and operate on spike trains, but are more complex to evaluate.

2 Methods

2.1 Material and Image Acquisition

Bone marrow material was harvested from the iliac crest and the tissue was embedded in acrylate at Graz University Hospital. The section was stained with Hematoxilin-Eosin (HE) and digitized by an Aperio ScanScope T3 (Leica Biosystems, Vienna, Austria) scanner. Fig. 2 shows a cropped region of a scanned slide at 40x magnification. Average dimension of the JPEG compressed 24-bit RGB images was 25,000×50,000 px.

Figure 2 HE-stained non-pathological human bone marrow tissue at 40x magnification.

Images of non-pathological bone marrow were used for the proof-of-concept experiments. In the current approach, we omitted an initial automated localization and extraction step of single cells from histopathological whole slide images. However, small image patches containing single cells had to be extracted in order to learn the corresponding class. An experienced pathologist assigned a single label, either ‘Class 1’ to late, or ‘Class 2’ to early granulopoietic cells using ImageJ (v1.46g [19]). Labeled cells $C_i$ were extracted in IQM (v3.0.2, [20]) as RGB patches using a manually set, minimum square bounding box. Due to the varying sizes of the cells and the fixed size of the ESN input layer (see section 2.2), RGB patches were scaled to a fixed dimension of 20×20 px by bicubic interpolation. RGB color channels were averaged to 8-bit and converted from integer [0,255] to double range [0,1] for subsequent classification.

2.2 ESN Architecture and Training

Usually, ESN work with time-dependent signals, but in our case we were facing static 2D images. Our approach to convert static images into a set of concurrent 1D signals was based on two considerations: (i) Dynamic input was required to exploit the reservoir’s high dimensional spatio-temporal feature space, and (ii) rotation invariance. Thus, we rotated each scaled and labeled cell patch centrically for an angle $\varphi = 0, ..., 359^\circ$. This formed the temporal input stream $\theta_i$ of a single cell $C_i$. We concatenated all $\theta_i$ and introduced zero signals of random length as first and last signal, and between all $\theta_i$. This procedure was used for generation of training and test input streams $U$.

The input layer consisted of 332 linear input units, since cropping the incircle from the 20×20 px patch resulted in $l = 1, ..., 332$ visible pixels $P_l$. The 2D patch was vectorized. The readout layer consisted of two linear units, no feedback connections were employed, see Fig. 3.

Figure 3 Architecture of the echo state network with 2 linear readout units without feedback connections.

The 332 dimensional input stream $U$ was fed into the reservoir at discrete time steps $t$. The hyperbolic tangent activation function was applied to compute the output of the non-spiking leaky integrator units in the reservoir. All time-discrete reservoir responses of an input sample

$$x(t + 1) = (1 - \alpha) x(t) + \alpha \tanh(W^{\text{in}} u(t) + W x(t))$$

were recorded along the actual input $u(t)$ (dashed grey line in Fig. 3) in a state matrix $X$ over time. While input and reservoir weights remained fixed, training of reservoir-
to-readout weights $W_{c}^{\text{out}}$ was performed in batch mode for each class $c$ using ridge regression:

$$W_{c}^{\text{out}} = Y_{d,c}X^{+}(X^{T}X + \beta I)^{-1}$$

where $Y_{d,c}$ denotes the desired output (target function) of class $c$, $X^{T}$ the transpose of $X$, $\beta = 10^{-8}$ the regularization coefficient, and $I$ the identity matrix. For each output unit, a binary 1D target function $Y_{d,c}$ was 1 for correct patches, and 0 for breaks and patches of the other class.

We used simulated annealing [21] to determine a suitable set of hyperparameters. All models were evaluated in a k-fold cross validation (CV) [22] to assess their generalization capabilities. The final class of a patch was determined in a one-vs-all manner. The winner unit in the readout layer is determined as the one, where the mean output during image presentation was a maximum. We used receiver operating characteristics (ROC) analysis [22] and accuracy measures based on the confusion matrix to assess the classification performance. Overall performance was quantified by averaging single class performances. ESN experiments were conducted using MATLAB® (v8.1, [23]).

### 3 Results

A total of $n = 176$, $n_{c1} = 63$ (late) and $n_{c2} = 113$ (early), expert-labeled cell patches were extracted from two scanned histopathological slides. Both data sets were merged and shuffled randomly, training and validation input streams were prepared for 10-fold CV. Important ESN hyperparameters resulting from simulated annealing are listed in Tab. 1. Connectivity of $W_{\text{in}}$ and $W$ refers to the percentage of non-zero values in the input and reservoir weight matrix, respectively.

#### Table 1 ESN hyperparameters used in the experiment.

<table>
<thead>
<tr>
<th>Connectivity</th>
<th>$W_{\text{in}}$</th>
<th>Input Shift</th>
<th>Connectivity</th>
<th>$N$</th>
<th>$\rho (W)$</th>
<th>$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$W_{c}^{\text{out}}$</td>
<td>0.3</td>
<td>-0.25</td>
<td>0.1</td>
<td>200</td>
<td>0.8</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Figure 4 depicts activities of 10 reservoir units. These activities represent response dynamics of the reservoir according to the visual input. Each cell patch is presented for 360 discrete time steps (i.e. $q$, see section 2.3), followed by constant zero input of random length between two consecutive cell patches. We observed that for fixed reservoir architectures, a shift of the input signal towards a more non-linear region of the sigmoid activation function, the network’s generalization capability increased. Learned reservoir-to-readout weights in a single CV are illustrated in Fig. 5 as an example. Other CV runs showed similar distributions in weights without outliers. 532 degrees of freedom were determined by the ridge regression for each readout unit.

**Figure 5** Reservoir-to-readout weights $W_{c}^{\text{out}}$ of one CV. Direct input-to-readout weights are not shown.

The classification performance of this ESN architecture on early and late granulopoiesis has been examined in 50 independent experiments. The classes of interest could be discriminated with an overall mean accuracy (±SD) of 0.846 (±0.013).

**Figure 6** ROC curve and AUC of a single experiment.

Fig. 6 contains ROC curves (plots of true positive rates (TPR) against false positive rates (FPR)) of each class in one of the 50 experiments. Despite the unbalanced data set, the classification performance is similar for both classes. The overall average (±SD) area under the ROC curve (AUC) is 0.915 (±0.008).
4 Conclusion

In this paper, we have shown the successful application of a recurrent neural network to the maturity quantification of cells in granulocytopenia. ESNs are able to extract relevant discriminative information directly from raw 8-bit grey value images. Hence, an explicit feature extraction step like in classical cytometry approaches [24] can be omitted, which reduces the effort in engineering applications for cell recognition. Since the present two-class problem could be solved with acceptable overall accuracy and precision even on an unbalanced data set, our future work will focus on multi-cell recognition. An increase of the accuracy is expected, as the ESN is trained on a more comprehensive data set. Furthermore, the results of this proof-of-concept study will contribute to the progress in digital pathology by demonstrating the potential of computer-assisted histomorphometry.

5 References

Eyelid Detection in Eye-Tracking Experiments

J.A. Nagel¹, P. Hevesi², C. Beck³, U. Gengenbach¹, G. Bretthauer³
¹Institute for Applied Computer Science, Karlsruhe Institute of Technology (KIT), Germany
²Embedded Intelligence, German Research Center for Artificial Intelligence (DFKI), Kaiserslautern, Germany
³Institute of Applied Computer Science/Automation, Karlsruhe Institute of Technology (KIT), Germany

Abstract

The power consumption of novel eye lens implants such as the Artificial Accommodation System highly depends on the pattern of eye movements. In order to minimize the overall power consumption, studies about the frequency and amplitude of accommodative gaze shifts as well as blinks are required. Within this paper an algorithm is described to extract the eyelid closure from eye-tracker data. The proposed algorithm is based on a beam bending approach. Within this approach the points of the eyelids are constrained in a way that they are influenced by their neighbours. The eyelids are then fitted by means of a cubic polynomial. The validation of the algorithm shows that 92.3% of the points of the upper and 83.6% of the points of the lower eyelid were tracked with very good accuracy.

1 Introduction

Presbyopia and cataract are increasing concerns in the aging society. Both age-related ailments go along with a loss of accommodation as neither the stiffened human lens nor a conventional intraocular lens is able to change its refractive power. A new approach to restoring the patients’ ability to accommodate is the Artificial Accommodation System [1]. The Artificial Accommodation System will be implanted into the capsular bag of the eye to replace the human crystalline lens. It measures the actual need for accommodation, and autonomously adapts the refractive power of its integrated optical element correspondingly. Power consumption of the system is highly influenced by the user’s behaviour. One challenge in estimating the power profile is to simulate the power consumption prior to implantation tests to optimize power consumption of the Artificial Accommodation System. Therefore knowledge of the accommodation amplitude and accommodation frequency during everyday life activities is required. Furthermore, knowledge about eyelid closure plays a significant role in this context.

To our knowledge, investigations of gaze or gaze-shifts under various viewing conditions mainly focus on corresponding features of the targets attracting the subject’s attention. Only few studies investigate the corresponding viewing distance [2-3]. To investigate on the accommodation amplitude and accommodation frequency as well as the eyelid closure behaviour an optical eye-tracker is being developed [4]. This work focuses on the implementation of Algorithms to robustly detect eyelids.

2 Methods

2.1 Used hard- and software

The eye-tracker is mainly comprised of monochrome cameras from ImagingSource (DMM 22BUC03-ML) delivering up to 87 frames per second with a resolution of 640x480 Pixels with 8 bit depth. The cameras are connected to a host computer via USB 2.0 and are synchronized by a programmable trigger device. Within the eye-tracker the cameras are mounted above the eyes of the subject. The lenses of the cameras are standard IR-corrected mount lenses with a focal distance of 8 mm. In front of the lenses an IR low pass filter with a cut-off wavelength of 830 nm (Edmund optics #54-663) is mounted. The optical path is deflected by use of a dichroitic mirror reflecting infrared light and transmitting the visible spectrum. The working distance of the camera is approx. 70 mm. The eyes are illuminated by infrared LEDs emitting light with a wavelength of 850 nm.

For the analysis, image data of both eye cameras were processed by a personal computer embodying an Intel i3-2120 processor and running Microsoft Windows7®. The image processing software has been developed in Microsoft VisualStudio® 2012 by use of the OpenCV library 2.4.3 from 2nd of November 2012.

2.2 Eyelid extraction algorithm

The eyelid extraction algorithm is subdivided into three sections. It comprises

- Detection of the inner and outer eye corners
- Detection of characteristic points on the eyelid
- Polynomial fit of the eyelid geometry

2.2.1 Detection of the corners of the eye

Pre implementation experiments showed that the detection of the nasal corner of the eye can be performed in a much more robust way than the temporal corner of the eye. To robustly fit both eyelids we assume that

- the width of the eye remains constant during experiments,
- the horizontal position of the nasal corner of the eye stays unaltered.
Therewith the vector \( \mathbf{r}_{NT} \) between both corners of the eye remains constant and the position of the temporal corner of the eye can be calculated by:

\[
\mathbf{r}_{\text{temporal}} = \mathbf{r}_{\text{nasal}} + \mathbf{r}_{NT}
\]

During experiments the nasal corner of the eye is tracked by means of template matching. The template is determined by the operator by manually marking the nasal corner of the eye in the first frame of the experiment. Examples for the used templates are shown in image 1.

(a) Temporal corner of the eye
(b) Nasal corner of the eye

**Image 1**  Examples for templates of corresponding corners of the eye

The templates are matched within a dynamic search region as depicted in image 2.

**Image 2**  Visualization of the region of interest to search for the nasal template

2.2.2 Tracking of the characteristic points on the eyelid

To obtain an initial condition of both eyelids, the operator has to mark several points \( P_i \) of each eyelid. Within the initialization, a feature set \( M(P_i) \) is computed for each eyelid point \( P_i \). The features are selected in a way to be easily and fast computable. The feature vector can be written as:

\[
M(P_i) = \begin{bmatrix}
\tilde{f}(P_i) \\
I(P_i) \\
G_{s_n}(P_i) \\
G_{s_n}(P_i)
\end{bmatrix}
\]

- A geometric constraint \( \tilde{f}(P_i) \)
- Intensity of the selected pixel \( I(P_i) \)
- Intensity gradient along \( n \) axis by use of Sobel operator \( G_{s_n}(P_i) \)
- Intensity gradient along \( u \) axis by use of Sobel operator \( G_{s_n}(P_i) \)

The used geometric constraint \( \tilde{f}(P_i) \) is quite similar to the active contour models described in [5]. It can be interpreted as an elastic bending beam which connects two points on the eyelid. A point lying in between these points is only able to move proportional to its surrounding points. During initialization, the exact contour of the eyelid is known due to the operator’s input. In the first step the corners of the eye are connected by a straight line and the perpendicular distance of the neighbouring points to this line is calculated by the following formula where \( P_1 \) and \( P_2 \) are start and end points of the line:

\[
\tilde{f}(P_i) = \frac{P_1 P_2}{\|P_1 P_2\|} \left( \frac{P_1 P_2}{\|P_2 P_i\|} - \frac{P_1 P_i}{\|P_1 P_i\|} \right)
\]

The geometric relation is depicted in image 3. After calculating the perpendicular distance of the points of the eyelid next to the corners of the eye, these points are connected with a new straight line and the next inner points’ distance is calculated. This is iteratively done for all points.

**Image 3**  Visualization of the calculation of the perpendicular distance of a point to a straight line

To process the next frame, first of all the positions of the corners of the eye are updated with the method described in 2.2.1. Based on the bending beam analogy, if the ends of the beam are moved, without changing the force applied to it, the whole beam moves without changing its shape. Based on this, the estimated positions of the eyelid points are corrected. After that, the new positions of the eyelid points are searched for by means of their known reference features. To reduce calculation power and to omit search algorithms, the coordinates of the possible candidates points \( P_{i,k} \) are taken from the column the former point \( P_i \) was from \( (k \) is in range of 0 to the number of image rows). For each candidate \( k \) a feature vector \( M(P_{i,k}) \) is calculated. Then the candidates’ feature vectors are compared to the reference vector \( M(P_i) \) by means of Euclidean norm.

\[
e(k) = \| M(P_i) - M(P_{i,k}) \|
\]

Here a weighting of the individual features can be introduced. As a valid successor \( P_{i,new} \) the point with the smallest \( e(k) \) is selected. The feature vector for the lid point \( i \) is updated with the feature vector of the successor point to represent the changes between observed frames.

\[
M(P_{i,new}) = M(P_{i,k})
\]

This new feature vector will be used at the next detection cycle. Additionally the adaptation of the feature vectors could be performed by specific rules, which limit the
changes for specific features, to prevent the model from drifting into an invalid state. The search for new candidates is performed for all inner eyelid points from outer to inner as already described earlier while calculating the initial feature vectors.

2.2.2 Polynomial fit of the eyelid geometry

After updating all eyelid points with new candidates, the eyelid contour is fitted by a polynomial fit. The eyelids are modelled by a cubic polynomial

\[ v = p_3 u^3 + p_2 u^2 + p_1 u + p_0 \]

where \( v \) is the vertical coordinate and \( u \) is the horizontal coordinate. The polynomial is fitted to the tracked points of the eyelid by means of least squares method.

3 Results

For evaluation of the eyelid detection algorithm more than five hundred frames of a recorded scene were manually labelled to obtain a ground truth. Image 4 shows a screenshot of the labelling process while the operator marks points of the eyelids. For every frame five points of an eyelid must be selected as equally distributed along the horizontal axis as possible.

Image 4  Operator marks points corresponding to eyelid.

The vertical uncertainty of manual marking was investigated and could be determined to lie within ±20 pixel. That means if two operators mark the same eyelid on the same frame their selected points vary within this interval. This results from some eyelashes and small shadows covering the edge of the eyelid and making the decision difficult. For accuracy evaluation, the vertical distances of the manually marked lid points relative to the polynomial fit were computed. Based on the resulting distance a single observed point is categorized into one of the following accuracy categories:

1. good: marked point is within ±20 pixels of the polynomial
2. fair: marked point lies in range of 20 to 60 pixels away from the polynomial
3. insufficient: the point distance from the polynomial is more than 60 pixels.

The accuracy results for both eyelids are presented in Table 1 and Table 2.

<table>
<thead>
<tr>
<th></th>
<th>Good</th>
<th>Fair</th>
<th>Insufficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right eye</td>
<td>2543</td>
<td>91</td>
<td>21</td>
</tr>
<tr>
<td>Left eye</td>
<td>2222</td>
<td>283</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>4765</td>
<td>374</td>
<td>26</td>
</tr>
<tr>
<td>Percentage</td>
<td>92.3%</td>
<td>7.2%</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

Table 1  Distribution of the detection results for upper eyelid.

<table>
<thead>
<tr>
<th></th>
<th>Good</th>
<th>Fair</th>
<th>Insufficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right eye</td>
<td>2268</td>
<td>261</td>
<td>11</td>
</tr>
<tr>
<td>Left eye</td>
<td>1996</td>
<td>561</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>4264</td>
<td>822</td>
<td>14</td>
</tr>
<tr>
<td>Percentage</td>
<td>83.6%</td>
<td>16.1%</td>
<td>0.3%</td>
</tr>
</tbody>
</table>

Table 2  Distribution of the detection results for lower eyelid.

As can be seen in image 5, the algorithm is able to fit a third grade polynomial to the upper and lower eyelid for different eyelid positions.

Image 5  Example results of the fit of the upper and lower eyelid.

4 Conclusion

In this work an approach for detecting the upper and lower eyelid was introduced. The algorithm was developed for an eye tracker to investigate on the accommodation amplitude and accommodation frequency as well as the eyelid closure behaviour. The algorithm performs in most cases with a neglectable error. As the evaluation shows, the accuracy was very good in 92.3% of all upper eyelid points and in 83.6% of all lower eyelid points. Analysis of the errors showed that the erroneous detections are mainly in the region of the temporal eye corner. This suggests that the assumption, of both eye corners staying aligned all the time does not hold in all cases. It can be assumed that a better computation of the eye corners without the limitations presented above could improve the results of the presented method for eyelid detection.
4 References


Continuously monitoring regional ventilation distribution in lavage ARDS pigs under high frequency oscillatory ventilation

Songqiao Liu1,*, Zhanqi Zhao2,*, Li Tan1, Yingzi Huang1, Ling Liu1, Lihui Wang1, Jianfeng Xie1, Jingyuan Xu1, Chun Pan1, Yi Yang1, Knut Möller3 and Haibo Qiu4

1 Department of Critical Care Medicine, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, China,  
2 Department of Critical Care Medicine, Furtwangen University, Jakob-Kienzle Straße 17, D-78054 VS-Schwenningen, Germany

*Songqiao Liu and Zhanqi Zhao contributed equally to this study. Correspondence to Haibo Qiu.
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Abstract

High frequency oscillatory ventilation (HFOV) is considered to be more lung protective for patients with acute respiratory distress syndrome (ARDS). In the present study, we examined the potential of electrical impedance tomography (EIT) in continuously monitoring regional ventilation distribution during HFOV. ARDS was introduced to five healthy pigs by lung lavages. The pigs were under conventional mechanical ventilation (CMV) and HFOV in a random sequence. Decremental pressure titration were performed after a recruitment maneuver. Cardio output and oxygenation were monitored. EIT measurements were performed at transthoracic level 5cm above the xyphoid. Mean tidal variation (MTV) of relative impedance at different regions of interest and MTV differences between different pressure levels were obtained. EIT measurements were performed at transthoracic level 5cm above the xyphoid. Mean tidal variation (MTV) of relative impedance at different regions of interest and MTV differences between different pressure levels were obtained in real time from the EIT device. No significant differences were found in cardio activities between the two ventilation modes. High pressure level during HFOV introduced less overdistention and more recruitment compared to that during CMV. Online information available in the EIT device provided useful insight of regional ventilation distribution. This study confirms that EIT is able to capture the respiratory signal despite of high variation frequency and low amplitude.

1 Introduction

Patients with acute respiratory distress syndrome (ARDS) in critical care require mechanical ventilation. Despite of all advance treatments, mortality rate of severe ARDS is still very high [1]. Improper settings of the ventilator may lead to hypoxemia, cyclic recruitment/derecruitment and biaxial [2]. Lung protective ventilation demands low tidal volume and appropriate airway pressure to open the atelectatic lung regions without overinflating other lung regions. Recently, high frequency oscillatory ventilation (HFOV) gains more research interest. Under this mechanical ventilation mode, relative high constant distending pressure (CDP) but small tidal volume are applied. Therefore, it is considered to be more lung protective compared to conventional mechanical ventilation (CMV) for ARDS [3].

Up to now, optimization of CDP is based on oxygenation criteria [4]. In order to develop lung protective ventilation strategies, regional distribution of ventilation in mechanically ventilated patients is also very important. However, hardly any well-accepted clinical methods can fulfill this task. Computed tomography (CT) has high spatial resolution and is considered as a gold standard for assessment of aeration in injured lungs [5]. With radiation and relative low temporal resolution, CT is not suitable for bedside capturing dynamic information of respiration. Electrical impedance tomography (EIT) is the first bedside imaging technique that enables monitoring air distribution of the lungs [6]. The basic idea of EIT is that changes in regional air content modify electrical impedance of lung tissue [7]. Small alternating electrical currents are applied at the chest wall surface during measurement and the resultant potential differences are recorded. The distribution of electrical impedance within the thorax then can be calculated. Since EIT has a relative high sampling rate (20Hz – 50Hz), it is capable of monitoring ventilation distribution in the lungs under HFOV, despite of high respiratory rate (3Hz-15Hz) [8-9].

Although EIT was originally designed for bedside use, unfortunately, the analysis of the EIT data in most of the studies was offline. To our best knowledge, there are no HFOV studies that utilize the real time information (without offline analysis) from the EIT devices. In the present study, we compared the differences of regional ventilation distribution between CMV and HFOV using the real time information obtained from the EIT device and examined the potential of EIT as a bedside monitoring device for HFOV.

2 Materials and methods

The study was randomized crossover self-control study, approved by the Science and Technological Committee and the Animal Use and Care Committee of the Southeast University School of Medicine. All experiments were performed according to the Guidance for the Care and Use of Laboratory Animals [10].
2.1 Animal preparation

A total of five healthy male pigs (weight 51.2 ± 1.9 kg, mean ± SD) were included in the present study (Southeast University Medical College Laboratory Animal Center, Nanjing, China). Pigs were anesthetized with an intramuscular injection of ketamine hydrochloride (3 mg/kg), atropine (2 mg/kg) and Fentanyl citrate (2 mg/kg), followed by a continuous intravenous infusion of Propofol (1-2 mg/kg/h), Fentanyl citrate (0.5-1 µg/kg·h), and midazolam (0.1 mg/kg·h), and atracurium (0.4 mg/kg·h). They were placed, in supine position, on a thermo-controlled operation table to maintain body temperature at about 37.5°C. 20 ml/kg 0.9% saline solution was administered during the anesthesia. After tracheostomy, the animals received CMV by a computer controlled ventilator (Servo-i ventilator, Solna, Sweden.). Respiratory frequency was set at 30 breaths per minute (I:E=1:2) and a positive end-expiratory pressure (PEEP) of 5 cm H2O. FiO2 and tidal volume (VT) were 0.4 and 6 ml/kg, respectively. A central venous catheter (Arrow International, Reading, PA) was inserted through the internal jugular vein and used to measure the central venous (CVP). A thermistor-tipped PiCCO catheter (Pulsion Medical System, Munich, Germany) was advanced through the right femoral artery to monitor the mean arterial pressure (MAP). Additionally, arterial blood samples were collected from a PiCCO catheter.

A continuous infusion of a 5 ml/kg·h balanced electrolyte solution was administered during the experiment, and the MAP was maintained above 60 mmHg with rapid infusions of 0.9% saline solution at up to 20 ml/kg, if required.

2.2 Experiment protocol

After the initial preparation, the pigs were stabilized for 30 min and baseline measurements (Tbaseline) were taken. then ARDS was induced by performing bilateral lung lavages with 30 ml/kg of isotonic saline (38 °C). The saline was infused through a funnel while the chest was gently massaged. After the maneuver, the excessive fluid was allowed to drain by gravity and was removed by negative pressure suction through the proximal portion of the endotracheal tube. The alveolar lavages were repeated once every 10 min until the PaO2/FiO2 ratio decreased to less than 100 mmHg and remained stable for 30 min (TARDS) with unchanged ventilatory parameters.

After the saline lavage-induced ARDS, the animals were randomly assigned to HFOV groups or CMV group according to a random digital table. When the first round finished, the animals were stabilized for 30 min, and switched to opposite group.

The lung recruitment maneuver was performed repeatedly until the PaO2/FiO2 was greater than 400 mmHg or the increase in PaO2/FiO2 was less than 10%. This condition was regarded as full lung inflation. After full recruitment, the PEEP (30 cmH2O) or mean airway pressure (mPaw) (36 cmH2O) levels were reduced at a decrement of 3 cmH2O every 5 min until ZEEP or minimal mPaw.

Hemodynamic and gas exchange indices were determined and recorded at the following time points: Tbaseline, TARDS (after the lung injury period), and TRM (post-recruitment maneuver), as well as at every pressure level during the experimental period.

![Screenshot of the device display indicates different regions of interest (ROIs) and the parameter settings.](image)

2.3 EIT measurements and analysis

EIT measurements were performed during the whole procedure described above (PulmoVista 500, Dräger Medical, Lübeck, Germany). An EIT electrode belt with 16 electrodes was placed around the thorax 5cm above the xyphoid level and one reference electrode was placed at the abdomen. The frequency of injected alternating current was selected automatically according to the noise spectrum. The images were continuously recorded and reconstructed at 40 Hz, and displayed in real time on the EIT monitor. Status image was set to “Minute Image” displaying mean tidal variation over the last minute (MTV). Regions of interest (ROIs) divided the status image into four anteroposterior layers with equal height (Fig. 1). Due to low ventilation-related signal component in the ventral and dorsal regions [11], we examined only the changes in mid-ventral and mid-dorsal regions. Given MTV of all pixels in the image to be 100%, percentages of impedance changes in mid-ventral and mid-dorsal regions were indicated on the display. Relative impedance at each mPaw levels or PEEP levels were compared in “Trends Mode” of the EIT device.

The data are presented as the means ± SD (standard deviation). All analyses were performed using the SPSS 16.0 statistical package (SPSS Inc., Chicago, IL).

3 Results

Mean arterial pressure (MAP) in the both groups were significant lower, while central venous pressure (CVP) were significantly higher after recruitment maneuver and at the beginning of pressure decremental (high airway pressure phase) (p < 0.05). The heart rate, MAP, cardiac output, CVP revealed no significant difference in the same pressure step of two groups.
Compare to T_{ARDS}, the \( \text{PaO}_2/\text{FiO}_2 \) ratios were significantly improved in both groups after recruitment and decreased during the pressure decremental period (\( p < 0.05 \)).

Regional distribution of ventilation could be observed in status image. MTV of one pig at highest and lowest pressure levels during CMV and HFOV are plotted in Fig. 2. The pressure levels refer to PEEP (for CMV) and mPaw (for HFOV). The differential images indicate region volume gain and loss, when pressure level decreased from the highest one to the lowest one. At low PEEP level during CMV (Fig. 2, top middle), ventral regions were well ventilated (indicated by large white areas) while most of the dorsal regions were not ventilated (indicated by large black areas). At high PEEP level (Fig. 2, top right), part of the dorsal regions were recruited. In the mean time, part of the ventral regions were overinflated. Similar scenario was found during HFOV (Fig. 2, bottom). But by comparing the differential images, more recruitment in dorsal regions and less overdistention in ventral regions can be observed (Fig. 2, left).

Tidal variation during HFOV was smaller than that during CMV (e.g. refer to colorbar in Fig. 2). In order to compare the trends of tidal variation change between two different ventilation modes during decremental pressure titration, MTV values were normalized to the corresponding maxima in each mode (Fig. 3, left). Volume distributed in mid-ventral region and mid-dorsal region at different pressure levels are also plotted in Fig. 3, right. MTV during CMV increased when PEEP level decreased. During HFOV, MTV in four pigs slightly decreased or stayed unchanged when mPaw level decreased, while MTV in the other pig increased, which leads to relative high standard deviation. For both CMV and HFOV, the percentage of MTV in mid-ventral and mid-dorsal regions differed as pressure levels decreased.

4 Discussion and conclusions

In the present study, we confirmed that EIT was able to capture the respiratory signal despite of high variation frequency and low amplitude. Online information available in the EIT device provided insight of regional ventilation distribution. High pressure level during HFOV introduced less overdistention and more recruitment compared to that during CMV.

Visualization of aeration in the lungs was difficult due to the dynamic of respiration. EIT attracted more attention in the field of critical care because of its high temporal resolution compared to traditional imaging techniques. Although many studies showed that regional tidal variation can be monitored by EIT [11, 12], no studies have proved that EIT can also be applied to subjects under HFOV without offline signal processing. With the presented EIT device settings, physicians should be able to examine the regional ventilation distribution during HFOV with the help of status images and Trends Mode.

We found it interesting that MTV during CMV increased as PEEP level decreased, while MTV during HFOV kept at a relative high level (>0.7, Fig. 3, left) regardless the change of mPaw. At high PEEP levels, percentages of
MTV in both ROIs were close (Fig. 3, right). At the highest PEEP level, percentage in mid-dorsal region was even larger than that in mid-ventral region. With respect to the information from Fig. 3, left, we concluded that at high PEEP levels, ventilation distribution seemed more homogeneous since the recruitment in dorsal regions and overdistention in ventral regions occurred at the same time. Overinflated regions due to higher PEEP and conventional $V_T$ overwhelmed recruited regions (decrease of $V_T$), although relative small tidal volume as recommended by previous studies (6 ml/kg) was applied [13]. Since tidal volume in HFOV was much smaller than that in CMV, decrease of $V_T$ due to overdistention was similar to increase of $V_T$ due to recruitment. Percentage changes in the ROIs were not as extreme as during CMV (Fig. 3, dashed lines). Titration of optimal mPaw should be examined in further studies.

One limitation of the study was that both CMV and HFOV pressure titrations were done in the same pigs. Although we managed to get PaO$_2$/FiO$_2$ to the same levels before the start of maneuvers, we could not evaluate the influence of the order of the maneuvers within this small number of animals.

In conclusion, EIT is an interesting novel method for determining regional volume changes in HFOV. HFOV introduced less overdistention and more recruitment compared to that during CMV.

5 References

Elastosonography and elastic modulus in healthy young heel pads

S. Matteoli¹, L. Forzoni², F. Vannetti³, A. Virga¹, A. Corvi¹, R. Molino-Lova³
¹Department of Industrial Engineering, University of Florence, Florence, Italy, sara.matteoli@unifi.it
²Esaote S.p.A., Florence, Italy ³Don Gnocchi Foundation, Rehabilitation Center IRCCS, Florence, Italy

Abstract

The aim of this study is to investigate whether is possible to find a correlation between heel pad elastic properties by means of sonoelastography and a compression device specifically designed. To date sonoelastography was not used for clinical correlation of heel pad elasticity. Sixteen healthy subjects (9F/7M, 31-35 years old) underwent B-mode ultrasound (for heel pad thickness measurement), sonoelastography (for soft tissue investigation) and compression tests (for elastic modulus assessment) on both heel pads. Findings showed that females had lower elastic modulus than males, that corresponded to a higher percentage of soft tissue.

1 Introduction

The interest in quantifying the mechanical properties of human soft tissues is an important aspect of diagnosing diseased tissues. Knowledge of the mechanical properties of heel pad tissue could be used in tools for screening patients for the purpose of preventing further complications in the foot (e.g. ulcerations in diabetics [1]) as well as of obtaining validated examination methods for medico-legal purposes (e.g. falanga torture [1]).

Sonoelastography is a non-invasive method to support the physician in assessing tissue elasticity. This technology provides additional information to standard B-Mode, a better definition of the lesion area, and it is suitable for diagnosis and follow-up. Moreover, it gives information on the tissue elasticity by associating different chromatic patterns. Real-time Esaote (Esaote S.p.A., IT) sonoelastography (ElaXto) is based on the concept of elastic strain: an object, subject to stress, distorts proportionally to the intensity of the applied stress and depending on the material. It is known that tissue elasticity, in different districts, is correlated to pathologies [2]. Palpation, which is routinely used in clinical exams, is based on this assumption. In order to perform the sonoelastographic exam, the user has to apply a perpendicular pressure through rhythmic movements on the tissue under exam. Thanks to the pressure given by that action, it is possible to evaluate the modification of the echo signal and thus to compute how the different tissues distort (if they are soft) or move (if they are hard) compared to the probe position. The result of this calculation, computed in real-time, is shown by a color image overlapped to the B-Mode image. The deformability degree is given by a chromatic scale [2]. ElaXto is a qualitative analysis where the estimation of strain information is computed in relation to the surrounding tissue. The computed strain information is dependent on the tissue of the Region of Interest (ROI) [2]. To date, sonoelastography was not applied on in vivo heel pad for tissue elasticity investigations.

The human heel pad is a portion of the plantar foot tissue located between the heel skin and the tuberosity of the calcaneus bone. It acts as an efficient shock absorber, smoothing the effects of impact forces during gait. The heel pad exhibits non-linear visco-elastic behavior as characteristic of soft biological tissues. Due to the visco-elastic nature, when a loading/unloading cycle is applied a load-deformation curve is obtained showing a hysteretic behavior.

The aim of this study is to investigate, on a group of healthy young subjects, whether is possible to find a correlation between heel pad elastic properties by means of sonoelastography and a compression device specifically designed.

2 Methods

2.1 Subjects

Sixteen healthy subjects (9F/7M, 31-35 years old) were enrolled in this study (Table 1). Both feet were considered so that 32 heels were investigated. All subjects declared to be in healthy conditions and to have never had injuries/trauma to any of the feet. The enrolled subjects had different lifestyles, including some being sporty and some following a more sedentary routine. Subjects engaged in professional sport were not included in this study.

Before starting the experimental procedure (which included B-mode ultrasound (US), sonoelastography and compression tests), the volunteer was asked to give information about age, weight, height, nature of physical activity and hours per week, as well as shoe size. All participants were volunteers and were informed about the conditions of the test that involved no harmful procedures or physical pain.

2.2 Ultrasonography investigations

The same expert operator performed ultrasonography examinations using a portable US system (MyLabAlpha, Esaote S.p.A., IT) by following always the same protocol. Specifically, the right foot was always the first to be scanned and the ultrasound images were performed with the probe in both longitudinal and transversal positions (Image 1). B-mode US acquisitions (performed to meas-
ure the heel pad thickness) were followed by sonoelastography scans.

Table 1 Subjects characteristics

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>16</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>28±6</td>
<td>27±5</td>
<td>29±7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66±13.9</td>
<td>57.3±7.8</td>
<td>77.1±11.9</td>
</tr>
<tr>
<td>Height (m)</td>
<td>171.0±9.4</td>
<td>165.3±5.1</td>
<td>178.3±8.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.4±3.2</td>
<td>20.9±2.2</td>
<td>24.3±3.4</td>
</tr>
<tr>
<td>Shoe size</td>
<td>40±3</td>
<td>38±1</td>
<td>44±1</td>
</tr>
<tr>
<td>Sport (h/week)</td>
<td>3.3±2.4</td>
<td>2.4±2.3</td>
<td>3.9±2.3</td>
</tr>
</tbody>
</table>

Image 1 Position of the probe on the heel during ultrasound examinations

A large amount of ultrasound gel (Parker Aquasonic 100, Parker Laboratories, USA) was used in order to avoid compression of the probe coupling with the heel skin. Indeed, it is known that even a small compression changes the shape and dimension of the heel tissues. The technique adopted consisted in starting to couple the probe (SL1543, 3-13 MHz, Esaote S.p.A., IT) with the heel tissue up to complete coupling of the probe on the center part of the echographic image sector. The probe movement towards the tuberosity of the heel stopped at the moment the first tissue compression was noticed. The heel pad thickness (UHPT), defined as the shortest distance between the calcaneus tuberosity and the heel skin, was calculated for each heel as the mean value between the longitudinal and transversal measurements.

During the activation of sonoelastography modality, the ROI was positioned over the heel tissues between the longitudinal and transversal measurements. The probe movement towards the tuberosity of the heel stopped at the moment the first tissue compression was noticed. The height of the soft layer was measured from each sonoelastography acquisition.

2.3 Compression device & test procedure

A compression device [3,4] (Image 2) was used to apply a compression (up to 30 N) on the subjects’ heel pads by means of a piston with a diameter of 40 mm (Image 3a). The main components of the device are: a load cell (model 31, RDP Electronics Ltd., UK), a linear transducer (LVDT, RDP Electronics Ltd., UK), two amplifiers (E725, RDP Electronics Ltd., UK), a laser optical displacement sensor (optoNCDT1300, Micro-Epsilon, DE).

The same procedure was applied to each subject, who removed shoes and socks, and then laid down on an adjustable hospital bed with both legs completely straight and relaxed. The compression device was fixed on an table in front of the hospital bed.

First the rigt foot, then the left one, was positioned in front of the device, so that the anterior part touched the vertical aluminium plate, with the heel pad in front of the cylinder. Specifically, the heel pad was placed with the center almost coincident with the center of the piston (Image 3a). Once the foot was well positioned, it was blocked with five Velcro fasteners (three to strap down the anterior part of the foot, one to keep the heel in front of the cylinder, and one to stabilize the ankle), as shown in Image 3b. When necessary, a cushion was positioned under the calf in order to better position the heel pad in front of the cylinder. The subject was asked to remain as relaxed as possible and to maintain the foot in the same position for the duration of the test.

A program made with LabView (version 2009, National Instruments, USA) was used to control the entire measurement (on/off of system, start/stop of stepper motor, direction of rotation of the stepper motor). The velocity of the piston was set to about 2.4 mm/s. Before starting the compression test on the heel pad, an idling test was done in order to verify the system functionality. As soon as the subject was completely relaxed and ready to be tested, the examiner ran the LabView program controlling each step of the measurement procedure. For each subject, the compression test was repeated M=3 times with one-minute break between each trial, to allow the heel pad tissue to return to its initial shape.
The value of the displacement determining the point of inversion of rotation of the stepper motor was fixed at 9 mm while the superior limit of the force was fixed at 30 N. The compression device as well as the procedure applied was not intended to reproduce any conditions of walking and/or running, but rather to minimize any discomfort and sensation of being strapped in order to be usable on patients with heel pain. Due to this main requirement, the foot could not be completely fixed. Therefore, involuntary muscular and neuronal movements and/or tensions may occur during the entire procedure, even though the volunteer was asked to remain relaxed. In fact, as the compressions/decompression are made on in vivo heel pads, it is almost impossible to consider the foot completely still. The most critical part is the ankle joint because it is only stabilized by using two straps on the lower part of the leg. In order to verify and quantify the involuntary movements of the ankle during the entire duration of the test, a laser optical displacement sensor was used. Specifically, an aluminium plate was strapped around the ankle (Image 3c), so that the laser could hit its surface during the compression/decompression of the heel pad and maximum movement of the ankle could be calculated (Image 4). Therefore, it was possible to measure the real displacement of the heel pad (subtracting the movement of the ankle from the piston displacement) and its deformation (by knowing the UHPT). Load-displacement curve were obtained and the Energy Dissipation Ratio (EDR) as [5]:

\[
EDR = \frac{A_{l} - A_{u}}{A_{l}} \times 100
\]

where \(A_l\) is the area under the loading curve, and \(A_{un}\) is the area under the unloading curve.

Stress-strain curves were obtained by knowing that:

\[
\sigma = \frac{\text{Load}}{\text{Piston area}}, \quad \varepsilon = \frac{\text{UHPT}}{\text{heel pad displacement}}
\]

From these curves, the elastic modulus (E) was calculated as the slope of the first and last 30% of the loading curves, where they are almost linear (Image 5).

### Results

#### 3.1 B-mode ultrasound

An example of heel pad scanned by US is shown in Image 6, where the yellow dashed line indicates the UHPT. Table 2 shows UHPT values of both feet grouped by gender and expressed as mean ± one standard deviation.

<table>
<thead>
<tr>
<th>Table 2: Heel pad thickness</th>
<th>All</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>UHPT (mm) right heel</td>
<td>15.7±2.7</td>
<td>14.9±2.5</td>
<td>16.8±2.6</td>
</tr>
<tr>
<td>UHPT (mm) left heel</td>
<td>15.6±2.0</td>
<td>15.2±1.9</td>
<td>16.2±2.1</td>
</tr>
</tbody>
</table>

Paired t-test showed no significant difference between right and left heel pads in UHPT (P-value=0.67); unpaired t-test showed no significant difference between females and males (P-value=0.17).

#### 3.2 Sonoelastography

Sonoelastography images showed two characteristic layers: a soft one (red color) close to the bone, and a hard/medium one (blu/green colors) close to the heel skin. This finding confirm the anatomy of the heel pad which consists of macrochambers (near the bone) and microchambers layers (near the heel skin) [6].

Due to the difference between images (Image 7), it was necessary to divide them into three classes depending on the homogenity of the soft tissue, considering the soft (red) and medium hardness (blu/green) area. The homogeneity was evaluated depending on the visual control of the density of the central area of the heel tissue: 1) high homogeneity - red area more present than the blu/green one (Image 7a); 2) medium homogeneity (Image 7b) - red area comparable to the blu/green one; 3) scarce or absent homogeneity (Image 7c) - more blu/green area than the red one. Paired t-test showed no significant difference between right and left heel pads (P-value=0.93); unpaired t-test showed no significant difference between females and
males (P-value=0.13). However, females tended to have a higher percentage of soft tissue in the heel pads than males.

![Image 7](https://via.placeholder.com/150)

**Table 3** Percentage of soft tissue measured from elaxto images

<table>
<thead>
<tr>
<th>Soft tissue right heel (%)</th>
<th>All</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>38.3±6.42</td>
<td>40.4±6.64</td>
<td>35.6±5.99</td>
</tr>
<tr>
<td>Soft tissue left heel (%)</td>
<td>38.5±3.18</td>
<td>38.2±2.89</td>
<td>38.8±3.72</td>
</tr>
</tbody>
</table>

### 3.2 Elastic modulus and EDR

Table 4 shows numerical results of EDR and E, expressed as mean ± standard deviation. Paired t-test showed no significant difference between right and left EDR (P-value=0.22), nor between right and left E (P-value=0.33). When comparing males with females, unpaired t-test showed no significant difference in EDR (P-value=0.69), and a significant difference in E (P-value=0.40). Specifically, females had lower elastic modulus than males especially in the right foot.

![Table 4](https://via.placeholder.com/150)

**Table 4** Energy dissipation ratio (EDR) and elastic modulus (E)

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDR right heel (%)</td>
<td>0.32±0.05</td>
<td>0.30±0.03</td>
<td>0.29±0.07</td>
</tr>
<tr>
<td>EDR left heel (%)</td>
<td>0.32±0.04</td>
<td>0.31±0.05</td>
<td>0.33±0.03</td>
</tr>
<tr>
<td>E right heel (kPa)</td>
<td>0.07±0.03</td>
<td>0.06±0.02</td>
<td>0.09±0.02</td>
</tr>
<tr>
<td>E left heel (kPa)</td>
<td>0.07±0.02</td>
<td>0.07±0.03</td>
<td>0.07±0.01</td>
</tr>
</tbody>
</table>

### 4 Conclusions

The aim of the present study was to investigate whether there was a correlation between sonoelastography and the heel pad elastic modulus calculated by means of a compression device. To date, sonoelastography was not used for clinical correlation of heel pad elasticity. Our findings suggest that this correlation does exist. Indeed, females showed a lower elastic modulus than males, that corresponded to a higher percentage of soft tissue.

A portable US system with the capability of sonoelastography modality and high level of image quality was used due to its transportability. Indeed, many of the subjects with heel pain or heel injuries have often limited mobility. Moreover, sonoelastography is a non-invasive method to support the physician in assessing tissue elasticity. US diagnostic technology is in general cost effective, real-time and not-ionizing therefore enabling also repetitive follow ups, widely available within different clinical settings and suitable, in case of portable systems, also for home diagnosis. Although our findings confirm our expectations, it is necessary to point out some peculiar aspects. Possible “discrepancies” between the results obtained from sonoelastography with respect to the measurement with the compression device could be explained by the fact that the two technologies “measure” different aspects of the elastic properties of the heel. Sonoelastography enables the clear differentiation of soft and hard structures with the desired ROI, while the compression device measures the elastic module of the whole heel pad (plus skin), considering both soft, medium and hard structures together (those structures/tissues can have different “weight” which are “separated” graphically within sonoelastographic images while they are considered as a whole with the piston-based system. Moreover, sonoelastography is a relatively-quantified technology: tissues are shown harder or softer in a relative not in an absolute manner, therefore, tissues soft are softer than the average value of the tissues within the ROI while they can be hard if compared to other tissues. Furthermore, there are no positioning tools for the “centering” of the piston above the major cusp of the heel. The US can visually check whether the probe is positioned over the higher heel cusp, while the piston-based system cannot. This pilot study paves the way for a more sophisticated analysis of the heel pad to be applied on survivors of flanga torture.

### 5 Reference


New Approach for 3D Reconstruction of Femur using 2D Conventional X-Ray Images

E. Jamshidi¹, U. Nackenhorst²

¹Institute of Mechanics and Computational Mechanics, Leibniz Universität Hannover, Germany, ehsan.jamshidi@ibnm.uni-hannover.de

²Institute of Mechanics and Computational Mechanics, Leibniz Universität Hannover

Abstract

Acquisition of 3D shapes from 2D x-ray images is of great interest for researchers. In this study, a new method to reconstruct 3D femur geometrical model from 2D x-ray images will be presented. The presented method does not need any marker in the x-ray image to acquire the 3D image. In comparison with former methods, it considers more geometric properties of the target bone which can be valuable in pathological. The method consists of four sequential steps, which have been implemented in MATLAB software.

1 Introduction

Acquisition of 3D shapes from 2D x-ray images has a number of important features including low radiation exposure on patients. 2D image acquisition instruments are also more accessible than CT scanners and not as expensive as them. Furthermore, in cases that the patient has severe femur damage and needs individual made implants, a 3D shape of the femur is essential for the manufacturer. In this study, a new method to reconstruct the 3D shape of a femur from 2D x-ray images will be presented. The difference between the present work and former approaches are 1) we don't use markers in x-ray images which makes it possible to use former x-ray images which don’t have markers. 2) Besides considering relation between points and geometrical parameters of the bone, this method also considers relation between lines of borders. It means more geometrical characteristics of the bone will be used. A similar approach suggested in [1] has considered only the geometrical parameters of the target image and uses its established relationships.

2 Method

To generate the 3D model of femur, four sequential steps are needed: (1) Segmentation of target x-ray image (2), reconstruction of a 3D geometrical shape model from CT images, (3) finding the most similar 3D bone to the target bone and (4) 3D reconstruction using produced curves. All four steps have been implemented in MATLAB (version R2013b, MathWorks, Natick, MA).
3 Result

In figure (1) the first, middle and final steps of the segmentation procedure for a sample CT image are shown. Although the developed program is semi-automatic for most CT images, in the case of presence implants which lead to artifacts, the user decision about the accuracy of segmentation is also needed. For an accurate reconstruction about 140 CT slices for each person are needed.

![Figure 1: Three sample steps during segmentation](image1)

To find the most similar bone in the dataset to the target bone, their borders are required. This could be achieved using DRR methods. In figure (2) the DRR of former CT images is depicted.

![Figure 2: Representation of a CT dataset after conducting DRR on it.](image2)

In figure (3) a reconstructed femur from the former CT images has been shown. According to [3], the femoral head has been considered a half-sphere.

![Figure 3: Two different views of reconstructed femur bone.](image3)

4 Conclusion

In this study a femur geometry model reconstruction method based on conventional x-ray images and a database of CT images is presented. It is remarkable that no markers are needed for the x-ray images. Moreover, as it combines methods which consider only the outer contour of the bone and methods which are based on internal geometrical parameters of the bone, it works well also for bones with pathologies.

5 References


Cross-correlation based detection of nanoparticles in SEM images from sedimentation cell experiments

M Simon1, 2, E M Wülfers1, Antoine Tavernier1, S Fritsch-Decker3, E Müller2, J Seiter2, C Weiss3, O Dössel1, D Gerthsen2, G Seemann1

1Karlsruhe Institute of Technology, Institute of Biomedical Engineering, Karlsruhe, Germany, publications@ibt.kit.edu
2Karlsruhe Institute of Technology, Laboratory for Electron Microscopy, Karlsruhe, Germany
3Karlsruhe Institute of Technology, Institute of Toxicology and Genetics, Karlsruhe, Germany

Abstract

To meet the increasing demand for reliable and fast experimental methods for nanoparticle investigation, we developed a combination of a scanning electron microscopy (SEM) imaging procedure and a computer program for counting nanoparticles (SiO\textsubscript{2}) on cells (A549) in sedimentation experiments. Our method is based on cross-correlation and is called “template-based cross-correlation”. We have tested this method of nanoparticle detection regarding its ability to quantify nanoparticles on cells in SEM images of sedimentation experiments. Our iterative template-based algorithm starts with a given disk template and generates a template of the nanoparticles via cross-correlation. After multiple additional cross-correlations for improving the template, the algorithm performs a final cross-correlation with the most improved template. We found that this special kind of algorithm is useful to detect single particles on plain surfaces but fails on agglomerated particles or complicated background scenarios, e.g. cell-surfaces. Due to this, advanced programs for analyzing and quantifying nanoparticles in SEM images have to use multiple methods to bridge the gaps between the particular shortcomings of the single methods. Furthermore, a semi-automatic program design with the ability of interactive correction is probably the most reliable way to deal with the challenging demands of nanoparticle analysis in SEM images.

1 Introduction

Over the past few years, nanoparticles (NP) have become part of our daily-life products. Especially so-called engineered NPs have gained a significant role in modern manufacturing of industrial and daily-life products. Due to this increasing exposure to NPs during everyday life and fabrication processes, the demand of profound hazard assessment of NPs has increased. As a result of the pharmacological principle that the impact of an agent depends on its concentration in the target tissue, today’s research should not only aim for the absolute dose of NP uptake, but especially for the so-called target tissue dose of NPs to determine the real biological impact on living tissue [1].

One step towards understanding the behavior of NP uptake is the experimental investigation of sedimentation and diffusion of NP settling on cells. The common techniques for the investigation of NPs on cells have often been (scanning) transmission electron imaging of thin cell sections. The main disadvantages of these methods are the time-consuming preparation of samples and the complicated capability of reliable quantification of NPs on cell surfaces. Here, new scanning electron microscopy techniques can provide a fast and easy-to-use method of surface investigation of NPs on cells. Using scanning electron microscopy (SEM) at low primary electron energies, we developed image processing algorithms that helped us compensate the emerging experimental and investigative problems of SEM imaging of NPs on cells. The quantification of NPs without automated computer-based frameworks is a time-consuming and exhausting task that interferes with the aim of fast and reliable NP examination, e.g. see the large amount of NPs in Figure 1. Thus, our approach of SEM combined with computer-based analyzing algorithms aims to simplify and accelerate further experimental NP research. In this paper, we want to focus on our novel “template-based cross-correlation” algorithm for quantification of NPs.

Figure 1 SEM, secondary electron image of SiO\textsubscript{2} NPs (100 nm diameter) on Indium-Tin-Oxide coated glass substrates and an A549 cell.
2 Methods

2.1 Sedimentation experiments and SEM imaging

For the sedimentation experiments, we used A549 lung cells. These cells were seeded onto Indium-Tin-Oxide (ITO) coated glass substrates, in particular “Brand ITO Coated Substrates” from SPI Supplies, West Chester, USA and “ITO-Coatings on High Quality Glass Substrates CEC010S” from PGO Germany, Iserlohn. The thickness of the used ITO coating varied from approximately 40 nm to 180 nm, complying with the experimental demands. Further, we used monodisperse “Silica Nanoparticles” (fluorescence-labeled) from Postnova, Landsberg am Lech. The monodisperse NPs had different diameters, in particular 70 nm, 100 nm, 200 nm and 500 nm. The used concentration varied between approximately 0.3 – 50 µg/ml, respective to the diameter of the NPs and the specific experiment. A sketch of the experimental conditions is shown in Figure 2.

A well of approximately 2 – 3 mm height was filled with cell culture medium (DMEM) supplemented with serum (Fetal Calf Serum, FCS). For the cell experiment, SiO$_2$ particle stock suspensions were diluted in deionized water at 1 mg/ml and dispersed by vortexing. Working suspensions were prepared by further dilution in cell culture medium at the indicated concentrations. NP suspensions were added and started to settle on cells via sedimentation and diffusion. The settling process is mainly influenced by agglomeration, NP-protein-interaction and electrostatic forces of NPs. After a specific time, the sedimentation process was stopped by removing the NP-FCS-mixture.

The adherent cells were washed twice with PBS (phosphate buffered saline) and fixed with freshly prepared 4% paraformaldehyde (w/v) for 10 min at room temperature. After washing with PBS, we used a combination of cell dehydration with graded ethanol series (50%, 70%, 95% and 100%) and critical point drying to preserve the samples for SEM [2].

For our investigations, we used an FEI Quanta FEG 650 Environmental SEM (ESEM) from FEI, Hillsboro, USA. For consideration of sample charging and contrast interpretation we had to operate the SEM at low primary electron energies at approximately 3keV. One experimental problem at these low energies is the complicated behaviour of emission coefficients for secondary and back-scattered electrons for small particles on an inhomogeneous substrate. Besides this, the main problems of computer-based imaging were the difficult image contrast of NPs settled on cells due to background signals, agglomerates of NPs and contaminations of the same diameter and shape as the settled NPs.

2.2 Template-based cross-correlation algorithm for SEM image processing

In order to quantify and count the NPs, we have developed a template-based cross-correlation algorithm in Matlab (The MathWorks, Inc., Natick, USA). The algorithm creates an averaged template of the searched NPs and cross-correlates the image with this template. Cross-correlation is a mathematical operation that compares two functions – in this case two images – and balances the outcome of this comparison according to the similarity of both functions. Where the functions are similar by their shape, the cross-correlation yields a high value. Where the functions differ massively from each other, the cross-correlation yields nearly zero [3].

As first step, our template-based algorithm computes the intensity histogram of the image. The background intensity is determined by the mode of the histogram and all values less or equal to the mode are set to zero (black). Second, the algorithm performs a cross-correlation with a pre-defined initial disk-shaped template. The diameter of this disk template is fitted to the diameter of the NPs in the image via extracting the required pixel-meter conversion data from the header of the SEM image. In the next step, it estimates the positions of the NPs at the maxima of the cross-correlation result. To avoid multiple detections of the same NP due to proximal (local) maxima in the cross-correlation results, the algorithm uses a Gaussian filter ($\sigma = $ NP diameter) to smooth proximal maxima. Furthermore, all cross-correlation maxima with an intensity less or equal to 50% of the maximal value of the cross-correlation are ignored to avoid that small contaminations are detected as NP.

With the estimated positions of the NPs, the algorithm determines the centroid of the particles via area analysis in the original image: Symmetrically around each detected maximum, a quadratic cut-out of approximately 130% the size of a NP is extracted from the original image. The gradient of the extract is binarized with a threshold of 40% of its maximal intensity. From the binary image, all connected regions with an area of 10 pixels or less are re-
moved. Holes (i.e. connected areas of background pixels that can not directly be reached from the image edges) in remaining structures are filled. The center of mass of the remaining structure is determined. A cut-out of approximately 110% the size of an NP is extracted around this center from the original image. The new template is created as the average of these cut-outs.

The next step improves the template by performing a new cross-correlation on the original image with this template. The results of this new correlation are averaged to a better template to improve the further detection. By repeating the last correlation step — normally 5 or 10 times — we generate an improved template of the NPs in the image. The number of maxima in the final cross-correlation result is our estimate for the number of NPs in the image.

3 Results

To evaluate the template-based algorithm for SEM imaging, we conducted several analyses. First we only used a cross-correlation with the pre-defined disk template to count NPs on cells in several images. All template adapting functions were deactivated. Then we executed the same correlations with our iterative algorithm and compared the results. We have discovered that especially single particles on plain backgrounds can reliably be found by cross-correlation methods (Figure 3). Our template-based approach barely improved the quantification results of single particles on plain background. Furthermore, agglomerated particles got hardly quantified by neither the cross-correlation with the disk-template, nor the template-based approach.

Many errors occurred during NP counting in agglomerates due to false positives and not-detected NPs. Especially, if insufficient single NPs were present in the image to create a good template of NPs, the number of false positive or not-detected NPs increased. Single NPs in front of more difficult and varying cellular background were often not detected by the cross-correlation algorithms (see Figure 4). In addition, especially round cell organelles caused many false positive counts. Not to mention that agglomerates in front of difficult cellular background caused fails in both cross-correlations (disk and template).

Figure 3 SEM, secondary electron image of SiO2 NPs (500 nm diameter) on plain ITO substrate without A549 cells. The small crumbs all over the substrate are unwelcome contaminations. The template-based algorithm reliably marked only the single NPs and ignored the smaller contaminations. Agglomerates however were quantified unsatisfactory.

Figure 4 SEM, secondary electron image of SiO2 NPs (100 nm diameter) on cell. The difficult cell background causes false positive (black arrow) detections and detection fails (white arrow).

4 Conclusion

We could show that this procedure is successful for single isolated NPs but performs rather unsatisfactory if the particles are agglomerated or in front of difficult cellular backgrounds. Taking into consideration that agglomerates are even hard to quantify by human perception, the fail of the template-based correlation could have been expected. Furthermore, the histogram-based background reduction turned out to be ineffective when dealing with complicated cellular backgrounds. Here, we can presumably achieve better outcomes by using low- and high-pass filters that remove certain spatial frequencies of the background.

To conclude, the template-based correlation for SEM imaging is a reliable and fast way to identify single NPs on plain backgrounds. Complicated backgrounds or agglomerates cause many mistakes in the quantification due to false positives or not-detected NPs. Advanced programs for NP analysis in SEM images have to cover a wide range of demands. By using only one method it is rather difficult to meet all the challenges provided by the difficult SEM contrasts. With our experiences with template-based cross-correlation we are in the process of developing an analysis tool for NP quantification that uses multiple quantification methods combined with a comparison
of secondary electron (SE) and back-scattered-electron (BSE) images to determine contaminations: Using an image of SE and an additional image of BSE of the same sample area, we try to use the advantages of both imaging methods. Whereas SE images deliver mainly topographic contrasts, BSE images represent mostly element-specific contrasts.

The images of both SEM modes are formally treated the same way by using methods of template-based cross-correlation, wavelet transformation, contrast analysis and high- and low-pass-filtering. After some automated evaluation procedure of the individual findings of the different methods, the user will be able to manually correct the results. After this, the individual results of SE and BSE images are brought together and the outcomes are balanced to distinguish contaminations from NPs. Although the use of different kinds of quantification methods and an automated scoring to evaluate the particular outcomes of each method can help to bridge the shortcoming of using only one single method, manual user-interaction and semi-automatic program designs will probably turn out as most efficient and reliable to deal with the difficult demands of SEM investigation of NPs on cells.

Our integrative use of SEM imaging techniques and corresponding image analysis shows that advanced imaging methods can be improved by using digital image processing. The interdisciplinary work of experimental and information-processing researchers opens a wide range of future possibilities in NP research.

5 References


Correction of image artifacts caused by refractive cylindrical surfaces in Scanning Laser Optical Tomography.

G. C. Antonopoulos1,*, D. Pscheniza2, R.-A. Lorbeer1, M. Heidrich1, K. Schwanke2, R. Zweigerdt2, T. Ripken1, H. Meyer1,3

1 Biomedical Optics Department, Laser Zentrum Hannover e.V., Hannover, Germany; 2 Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover Medical School, Hannover, Germany; 3 Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover Medical School, Hannover, Germany
* gantonopoulos@lzh.de

Abstract

Advancements in cell biology have made it possible to generate large quantities of human pluripotent stem cells (hPSC) in three-dimensional cultures in bioreactors. Volumetric imaging of hPSC cell aggregates with Scanning Laser Optical Tomography can provide insight into their physiology and growth behavior. Containing cell clusters in a glass capillary for in vitro imaging introduces a non-vanishing refractive index gradient into the field of view that will produce artifact in reconstructed tomographic images. The effects of refraction are studied by means of numerical simulations. Furthermore we present a method of correcting for the effects of refraction utilizing a reordering step for the projections before tomographic reconstruction. Removal of artifacts is successfully demonstrated on experimental data of human pluripotent stem cell (hPSC) clusters. This will enable long term in vitro studies of hPSC clusters with Scanning Laser Optical Tomography.

1 Introduction

Human pluripotent stem cells (hPSC) present an attractive cell source to generate lineage-specific progenies for cell therapies, tissue engineering and in vitro disease modeling. Therapeutic and industrial applications of hPSCs, however, will require large cell quantities generated in defined, well controlled and scalable culture conditions. It was recently shown that hPSCs can be expanded as cell-only, spherical aggregates (cell clusters) in serum-free suspension culture regardless of matrix supplementation [1]. Volumetric imaging of these cell clusters provides direct insight into the physiological behavior of three-dimensional cell cultures and their response to chemical or mechanical stimulation [2,3]. Imaging of three-dimensional cell cultures sets new challenges and imposes specific requirements concerning the choice of a suitable microscopy technique. Scanning Laser Optical Tomography (SLOT) is a highly efficient 3D microscopy technique for biological samples ranging from sub millimeter size up to a few centimeters [4]. It is an offspring of Optical Projection Tomography (OPT) [5]. In that sense it can be considered computed tomography (CT) with laser light in the visible spectrum. Volumetric datasets of a sample are generated by digital reconstruction from a series of parallel ray projections. A way of mounting cell clusters for 3D in vitro imaging is to contain them in capillaries and supply them with culture medium [2]. However, this introduces a non-vanishing refractive index gradient into the field of view. This in turn will create artifacts in the reconstructed volumetric images, effectively preventing the generation of meaningful data. Here we overcome this problem by adapting a sinogram reordering method that was originally proposed to reconstruct images from fan beam projections in X-ray computed tomography.

1.1 Radon transform and volumetric reconstruction

The problem of reconstructing a 3D object from a set of parallel ray projections can be separated to a set of 2D problems in the x-y-plane. The Radon Transform is the mathematical foundation for tomographic reconstruction [6].

The Radon Transform describes the projection of a function $g(x, y)$ along a set of straight lines $L(\rho, \theta)$ where $\rho$ is the perpendicular distance of a line to the origin and $\theta$ is the angle included by its normal vector and the x-axis. The Radon Transform $R(\rho, \theta)$ of a function $g(x, y)$ is expressed as

$$R(\rho, \theta) = \int_{L(\rho, \theta)} ds \ g(x, y),$$

(1.1)

where the integration is carried out along each line. $R(\rho, \theta)$ for a fixed $\rho$ can thus be considered the parallel ray projection of the function $g$ from an angle $\theta$. The graph of $R(\rho, \theta)$ is called a sinogram. This is due to the fact that a value of the function $g$ at the coordinates $(x_0, y_0)$ contributes to $R(\rho, \theta)$ exactly along the curve

$$\rho(\theta) = \rho_0 \cdot \sin(\theta + \theta_0),$$

(1.2)
where \( \rho_0 = \sqrt{x_0^2 + y_0^2} \) and \( \theta_0 = \tan^{-1}(y/x) \). Any violation of this property due to refraction will cause artifacts in reconstructed data. This will be studied in section (3.1).

To obtain a function \( g(x, y) \) from a set of projections \( R(\rho, \theta) \) the inverse Radon Transform is applied to the sinogram. There exist a number of computationally efficient implementations for the inverse Radon Transform [6]. Applying the inverse Radon Transform to projection data is called reconstruction.

\section{Methods}

\subsection{Scanning Laser Optical Tomography (SLOT) experimental setup}

![Image 1](https://example.com/image1.png) Schematic of the Scanning Laser Optical Tomography (SLOT) experimental setup, modified from [7].

The setup and working principle of SLOT will be explained briefly, see image 1. For a detailed discussion refer to Lorbeer et al. [4]. A cuvette filled with a bath liquid acts as the sample chamber. A capillary containing immobilized cell clusters is attached to a rotation stage (M60, Physik Instrumente (PI) GmbH & Co. KG, 76228 Karlsruhe/Palmbach, Germany) and is suspended in the cuvette. The cuvette and the bath liquid provide a plane window of entrance for the illuminating laser beam, so that refraction will only occur at the inner and outer surfaces of the capillary. Refraction will change the direction of beam propagation.

For illumination a multi-wavelength diode based laser source is coupled into the system with a single mode optical fiber. Light exiting the collimator with a 10X motorized zoom lens (1/2" 12-120mm f1.8, computar, CBC (AMERICA) Corp., Commack, NY 11725, USA) was then filled with this matching liquid and tomographic imaging of the cell spheroids was performed. The numerical aperture (NA) of the system was adjusted so that the whole outer diameter of the capillary was within the depth of field. This resulted in a theoretical optical resolution of \( \Delta x = 11 \mu m \). The outer and inner diameters of the capillary were measured to be 2.25 mm and 1.60 mm, respectively. The illuminating laser wavelength was 532 nm and the scattering signal of the sample was collected with the PMT.

For the first experiment a matching liquid for the capillary was produced by mixing two silicone oils (AP 150 Wacker and AR 20, Sigma-Aldrich, Missouri, USA) so that the capillary appeared invisible in the liquid. The refractive index of the matching liquid was \( n_{\text{cap}} = 1.486 \). The cuvette was then filled with this matching liquid and tomographic imaging of the cell spheroids was performed. For the second experiment the same culture medium that was used for the cell clusters inside the capillary was chosen as the bath liquid. The refractive index of the medium was \( n_{\text{med}} = 1.339 \). The NA of the system was again adjusted to image the whole capillary within the depth of field keeping the optical resolution practically unchanged compared to the first experiment (\( \Delta x = 12 \mu m \)).

\subsection{Numerical simulations}

The refractive index of the capillary was assumed to be constant along the z-direction, so that the reconstruction could be carried out for each x-y-plane individually as in section 1.2. This assumption is valid as long as the capillary is perpendicular to the optical axis. This allowed us to limit simulations to the two dimensional case in the x-y-plane.

Simulations were based on a simplified model: the capillary in the x-y-plane was modelled as two concentric circles with outer radius \( R_{\text{out}} \) and inner radius \( R_{\text{in}} \). The rota-
tional axis was placed in the center of the capillary. A piecewise constant refractive index distribution was assumed given by \( n_{\text{bath}}, n_{\text{cap}} \) and \( n_{\text{med}} \) being the refractive indices of the bath liquid, the capillary glass and the medium inside the capillary, respectively. In this model these five parameters are sufficient to describe all refractive effects. Geometric ray tracing was then used to calculate the path of each incident light ray within the capillary. This allowed for the formation of digital projection images. Furthermore the effect of refraction by the capillary on the measured sinogram was studied. This was achieved by simulating the trajectory of a single point near the inner capillary surface in the projection images. The radial coordinate of this point is set to \( r_0 = 0.999 \cdot R_{\text{in}} \) to simulate close proximity to the inner capillary wall.

Two special cases were considered corresponding to the investigated experimental conditions: In the first case the refractive index of the bath liquid is matched to the refractive index of the capillary, i.e. \( n_{\text{bath}} = n_{\text{cap}} \). For the second case \( n_{\text{bath}} = n_{\text{med}} \) was assumed. Simulations were performed using \( n_{\text{cap}} = 1.486, \; n_{\text{med}} = 1.339, \; R_{\text{in}} = 0.8 \text{mm} \) and \( R_{\text{out}} = 1.125 \text{mm} \) as measured in section 2.1.

### 2.3 Image reconstruction with sinogram reordering before reconstruction

Any straight ray entering the capillary from the outside propagates as a straight ray within the capillary due to the constant refractive index distribution inside the capillary. Due to refraction at the capillary surfaces an incident ray \( L(\rho, \theta) \) will propagate as a refracted ray \( L(\rho', \theta') \) in the capillary. Therefore the measured Radon Transform \( R_{\text{meas}}(\rho, \theta) \) is related to a parallel ray Radon Transform as defined in (1.1) according to

\[
R_{\text{meas}}(\rho, \theta) = R(\rho', \theta'),
\]

with \((\rho, \theta)\) and \((\rho', \theta')\) being the coordinates of the incident and refracted ray, respectively. Using digital ray tracing as detailed above for each incident ray \( L(\rho, \theta) \), the coordinates of the refracted ray \( L(\rho', \theta') \) were calculated. We could show that there exists a simple expression relating the radial coordinates of the incident and refracted ray:

\[
\rho' = n_{\text{bath}} / n_{\text{med}} \cdot \rho.
\]

There is no simple closed form expression for \( \theta' \). However, due to the rotational symmetry of the model the difference \( \Delta \theta := \theta - \theta' \) is independent of \( \theta \), that means for fixed parameters of refraction:

\[
\Delta \theta := \theta - \theta' = \Delta \theta(\rho).
\]

To correct for the effects of refraction in the measured sinogram a reordering strategy was employed \[8\]. A measured sinogram is reordered according to equation (2.1) to give an equivalent parallel ray sinogram previous to the reconstruction step. The reconstruction of the reordered sinogram was performed using a standard Filtered Backprojection algorithm. Similar techniques were applied in X-ray CT for fan beam projections \[6\] and in Optical Projection Tomography by Birk et al. \[9\].

### 3 Results

#### 3.1 Numerical Simulations

Refraction leads to a distortion of the trajectory of a single point in the projection, see image 3. Trajectories of a single point near the capillary wall were calculated for \( n_{\text{bath}} = n_{\text{med}} \) (blue) and \( n_{\text{bath}} = n_{\text{cap}} \) (red). Also shown is a projection without refraction (dashed black line) which is the sine curve given by equation (1.2).

![Image 3 Influence of refraction on the trajectory of a single point near the inner capillary surface.](image)

The trajectory in the refracted projections differs from the sine curve for two reasons: A radial scaling and a distortion in the angular coordinate. The radial scaling is explained by equation (2.2). This scaling will only occur if the bath liquid and the medium inside the capillary have different indices of refraction. A radial scaling of this form alone does not cause discernable artifacts in a reconstructed image. It will only effect an isotropic scaling of the reconstructed image. The reason for this is that the trajectory of a point in the projection is still a sine curve when only a radial scaling is present. However the trajectory of a single point in the refracted projections does not follow a sine shape. This is due to the addition of a \( \rho \)-dependent phase term \( \Delta \theta \) to the phase of the sine in equation (1.2). It can be shown that for a fixed \( \rho \) the phase term \( \Delta \theta \) will be larger for the case \( n_{\text{bath}} = n_{\text{cap}} \) than for \( n_{\text{bath}} = n_{\text{med}} \). This distortion in turn will lead to image artifacts as, see image 4 for experimental data. This explains why artifacts due to refraction are more pronounced in the case where the capillary refractive index is matched by the bath liquid.

#### 3.2 Experimental data

Projection images of cell clusters were acquired as described in section 2.1. Reconstructions of the tomographic measurements were first performed without and with prior sinogram reordering, see image 4: A and C show reconstructions without prior sinogram reordering for the cases
respectively, while images B and D show the effects of sinogram reordering before reconstruction. When using sinogram reordering before reconstruction the image artifacts caused by the refraction at the capillary surfaces are removed.

![Image 4](image_url) Application of sinogram reordering before reconstruction in a single plane of an imaged hPSC cell cluster.

It was then possible to reconstruct the cell clusters inside a capillary plane by plane. Stacking the planes produced a volumetric image of the cell clusters, see image 5.

![Image 5](image_url) Rendering of hPSC clusters in a capillary using sinogram reordering before reconstruction.

## 4 Conclusion

Sinogram reordering before reconstruction was shown to be a suitable technique for removal of image artifacts caused by mounting samples in a capillary. This is a first step in enabling long term in vitro studies of stem cell cluster development with Scanning Laser Optical Tomography (SLOT). We expect SLOT to be a viable tool for monitoring the growth and physiology of hPSC aggregates in 3D bioreactors in vitro. Due to high collection efficiency of excited fluorescence and scattering signal SLOT could be an excellent tool for monitoring cell clusters with minimal photo toxicity and thus enable long term studies.

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## 6 References


Multimodal Image Segmentation of Cellular Fragmentation Using Edge Detector and Morphological Operators
A. Khan1, C. Weiss2, B. Schweitzer2, I. Hansjosten2, R. Mikut1 and M. Reischl1
1Institute for Applied Computer Science, Karlsruhe Institute of Technology, Germany
2Institute of Toxicology and Genetics, Karlsruhe Institute of Technology, Germany

Abstract
Image processing and analysis pipelines can be adapted to solve challenging biological cell segmentation problems. Occasionally, the image segmentation outcomes of images containing cells are unsatisfactory and insufficient if done alone in a single channel e.g. when staining the nucleus. An efficient method using morphological operators and edge detectors using information from an additional bright field (BF) channel is able to assign conflicting cases when segments of nuclei belonging to a single cell are detected artificially as independent objects by nuclear staining. This method is shown to improve the image segmentation accuracy of cell segmentation using the information from multiple channels in comparison to only using nuclear staining.

1 Introduction
Currently, the focus on using image processing techniques and methods in order to develop adequate comprehension of biological processes has become downright attractive, both to cell biologists and toxicologists. In multicellular organisms, different forms of cell death (such as apoptosis) are of lasting significance for various diseases such as cancer. Image data sets of cell populations often consist of multiple modalities, e.g. a BF channel and a channel showing the nuclear staining and are named according to the dye used e.g. Hoechst (see Fig. 1). The latter channel (i.e. Hoechst) or a channel that measures labeled green fluorescent protein (GFP) with a nuclear marker are predominantly used in research for studying cell morphology and consequent feature quantification (e.g. the total number of segments detected) [1, 2]. However, if cell nuclei are fragmented due to apoptosis, each cell consists of multiple segments (fragmented nuclei), which cannot be assigned to each other by only using the information given in the Hoechst channel. An example is given in Fig. 1. The image on the top left shows the BF channel, the bottom left image shows the nuclear staining (Hoechst channel) and the images on the right show cases where stained nuclei cannot easily be recognized as fragmented nuclei belonging to one and the same or multiple cells.

The work done related to segmentation of cells with fragmented nuclei and segmentation in the BF channel can be seen in [3, 4, 5, 6]. However, combining multichannel information for the correct assignment of segments obtained from a single channel needs to be explored in more detail. Here, we propose a method to improve the segmentation process for fragmented segments. In cases where multiple neighboring nuclear fragments occur, cell shape features from the BF channel can be effectively used. This method includes information from both Hoechst and BF channels especially in cases where segments cannot be infallibly assigned. The BF channel exhibits distinctive features due to shadows arising from membrane blebbing or cellular protrusions. The main idea is to use the segments in the BF channel as a measure for cellular integrity and combine this information with the segments representing fragmented nuclei in the Hoechst channel.

2 Methods
The image processing pipeline implemented for this task is shown in Fig. 2. BF channel segment information is extracted by applying a Sobel edge detector to the BF channel image. The extracted segments are further processed sequentially using morphological operators i.e. image closing, hole-filling and image opening respectively (Fig. 3).

In the next step, binary large objects (BLOBs) detected by
Figure 2: Complete image processing pipeline for assignment of Hoechst segments to their BF counterparts

Figure 3: BF segmentation (steps 1-2 from Fig. 2): BF (section of original BF image), 1 (edge detector), 2a (image closing), 2b (hole filling) and 2c (image opening). Red box highlights an example of a potentially large BLOB according to plausibility criteria and therefore would be segmented further using step 4 in Fig. 2 i.e. watershed segmentation (see Fig. 4).

the operations 1-2 in Fig. 2 are further checked for plausibility (step 3 in Fig. 2). Criteria based on irregular size i.e. too small/big segments (approx. ranging from 200 to 1500 pixels) are implemented, thereby removing small objects and further segmenting big objects. A watershed segmentation seeking for gradients in brightness is known to perform well in cell separation [7, 8]. However, this segmentation cannot achieve the desired results due to inconsistencies caused by cellular fragmentation in both the channels. Therefore, we use the distance map of each BLOB. As a result all found BLOBs are within a given area range and show convex properties (see Fig. 4).

For Hoechst segmentation, we apply a segmentation routine described in [9, 10]. As a result we obtain $i = 1, \ldots, n$ segments in the BF channel and $j = 1, \ldots, m$ segments in the Hoechst channel. The pixel positions $(x/y)$ belonging to the segments are gathered in the sets $B_i$ for BF segments and $D_j$ for Hoechst segments. The sets $D = \{D_1, \ldots, D_m\}$ and $B = \{B_1, \ldots, B_n\}$ define all found Hoechst and BF segments respectively.

If Hoechst segments belong to the same BF segment, they are annotated to the same class. Therefore, the portion $p_j$ of positive BF segment pixels in a Hoechst segment $j$ is calculated and the most affecting BF segment is denoted as $n_j$ (see Eq. 1, 2 below).

$$p_j = \max_i \frac{\text{card}(D_j \cap B_i)}{\text{card}D_j}, \quad (1)$$

$$n_j = \arg \max_i \frac{\text{card}(D_j \cap B_i)}{\text{card}D_j}. \quad (2)$$

If $p_j \geq 0.1$, segment $D_j$ is removed from the Hoechst segments and transferred to a set $\text{HOECHSTposBF segments} DB$. The set $\text{HOECHSTposBF}$ contains segments in Hoechst which are also segmented in the BF channel. All segment sets being affected by the same BF segment are merged:

$$D = \{D_j | p_j < 0.1\}, \quad (3)$$

$$DB = \{DB_1, \ldots, DB_n\} \quad (4)$$

with

$$DB_i = \bigcup_{n_j=i, D_j>0.1} D_j. \quad (5)$$

Empty sets in Eq. 4 are removed. All subsets in Eq. 3 denote BF-negative segments, all subsets in $DB$ denote BF-positive segments. In this way segment reassignment is done (see step 5 in Fig. 2).

1If more than one BF segment affects the Hoechst segment, the most affecting BF segment will be chosen.
3 Results

The devised pipeline is applied to a biological dataset consisting of cis-platin treated human lung cancer cells (A549) acquired as described earlier [11]. This dataset contains some images of fragmented nuclei from cells that also show some distinctive features in the BF channel. Some images in this dataset contain 5 to 10% of such cells. Therefore, room to improve the segmentation accuracy is evident. The results are shown in Fig. 5 and 6. The goal of this processing pipeline is to annotate all segments correctly and assign only one label to all those nuclear segments that belong to one cell while giving each nucleus a unique label. All such segments belonging to each other are assigned the same number and exact same color in the final annotated image (a section of such an image is shown in Fig. 5). Here, the label 77 and specific red color tone is assigned to two segments that belong to each other. Therefore, it is easier for users to instantly see which segments belong to each other and vice versa.

Some critical cases using different channels separately and combined are shown in Fig. 6. Critical cases are those that require immediate assignment but were not obtained by using only Hoechst segments. The critical cases are a combination of:

1. fragmented nuclei (as defined by manual inspection) which however were labeled differently from each other using Hoechst segmentation only

2. oversegmentation of the nuclei thus generating a number of Hoechst segments not corresponding to the true number of nuclei

In Fig. 6, each column belongs to one of the several critical cases. The first row shows critical nuclear segments in the Hoechst channel and their corresponding appearance in the BF channel is given in the second row. The overlaid images are displayed in the third row and show the segments from Hoechst in red over the grayscale BF image. The resulting annotation shows segments that belong to each other encircled together in the final row. From Fig. 6, it is clear that with such a method critical cases can be resolved (also see Tab. 1).

However, the improvement in segmentation results is highly dependent upon the number of critical cases with respect to the total number of segments present in an image. If a higher number of fragmented nuclei belonging to the same cell is present, such an algorithm can highly improve the segmentation result (see Tab. 1).

4 Conclusion and Outlook

This new method enables us to improve the nuclear segmentation outcome using morphological information from another channel thereby avoiding errors in counting fragmented nuclei. The number of fragmented nuclei would be underestimated in absence of such a method. In our next steps, the algorithm needs to be applied to a bigger dataset (including shading, noise etc.) to show robustness and performance improvement. Furthermore, implementation of automatic parameter tuning (as in big image processing pipelines) for optimization of such a method is required.

5 Acknowledgement

We express our gratitude to DAAD (German Academic Exchange Service) for funding this research work of A. Khan.
Table 1: Results of segment reassignment and segmentation improvement. The first column shows names of images containing critical cases. Some of these cases could be seen in Fig. 6. The second column shows the number of nuclear segments found using the Hoechst channel only. The third column shows the number of critical cases solved by using segmentation in the BF channel in addition to the already available Hoechst segmentation (e.g. the three critical cases shown in Fig. 6). Here, number of cases represents the total number of critical cases in a given image and number of segments represents the total number of segments that are involved in critical cases. The fourth column shows the total number of segments (belonging to critical cases) that are now assigned correctly using this methodology e.g., in column 1 and row 4 of Fig. 6 the two lower segments are now correctly assigned to the same cell shown within green encirclements. However, not in all critical cases the improved algorithm could correctly assign the segments as manual inspection revealed (listed in Tab. 1 as incorrect reassignment). The last column shows the improvement in segmentation results using this method. Here, the first sub-column is assigned to the % improvement in segmentation results using the total number of correct segments with respect to the total number of segments detected originally in a given image. This percentage is simply calculated using: 100 × (1 - (total Hoechst segments without using BF channel - difference of correct and incorrect number of segments after reassignment)) / total Hoechst segments without using BF channel. The second sub-column is dedicated to segmentation success with respect to the number of critical cases solved. This percentage is simply calculated as: 100 × (segments reassigned correctly - segments reassigned incorrectly) / total number of reassigned segments per image. NOTE: no. and w.r.t are the abbreviations for number and with respect to respectively.

<table>
<thead>
<tr>
<th>Image name</th>
<th>total segments from Hoechst</th>
<th>critical cases</th>
<th>segments reassigned</th>
<th>segmentation correction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no. of cases</td>
<td>no. of segments</td>
<td>correct</td>
<td>incorrect</td>
</tr>
<tr>
<td>E1P2</td>
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<td>11</td>
<td>24</td>
<td>9</td>
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</tr>
<tr>
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<td>4</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
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<td>7</td>
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<td>5</td>
<td>11</td>
<td>5</td>
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</table>

6 References


Sparse recovery for 3D electrical impedance tomography involving large number of finite elements: A simulation study

Ashkan Javaherian, Benjamin Schullcke, Knut Möller

Abstract

This study deals with the problem of computational complexity in recovering conductivity field over a large number of finite elements in three-dimensional electrical impedance tomography (EIT). Typically, 2D EIT covers solely the plane encompassed by the electrodes ring, thus ignoring the fact that electrical current is inherently spread in three dimensions. The 2D reconstruction is thus subject to artefacts produced by off-plane contrasts. 3D EIT has been paid much attention recently, but its application to medical diagnosis is limited because of the need for large number of finite elements to cover the whole volume. Even if the ill-conditioning imposed by the large number of unknown parameters can be addressed suitably, the problems arising from storage space and computational cost will still remain. The present study shows great potential of a sparse regularized approach, the so-called Gradient Projection for Sparse Reconstruction (GPSR), for 3D EIT. The results show that the GPSR improves the spatial resolution and shape preservation compared to the commonly-used quadratic regularized conjugate gradient (CG) codes, at the same time significantly reducing the computational cost. To better evaluate the performance of the competing solvers, they are applied to a data set collected from a simulated 3D volume matching a human chest.

1 Introduction

Electrical Impedance Tomography (EIT) is an imaging modality in which an image of conductivity field inside an object is inferred from surface electrical measurements [1]. This technique has many applications for diagnostic purposes in medicine, e.g., monitoring of lung function, detection of tumours in breast or imaging of brain activity [2-4].

A number of electrodes are attached to the skin of the subject, a small alternating current is successively applied to some or all the electrodes and the resulting electrical potentials are measured from the remaining electrodes [1]. EIT is an efficient tool for monitoring lung function since there is a large contrast between the conductivity of the air and that of the encompassing tissues [5]. There are some sources of error during the measurement. Breathing action or posture changes may cause some unappealing electrode movements during the measurement, which can deleteriously affect the recovered conductivity field [6]. To deal with such problems, time difference imaging is often given precedence over the absolute reconstruction. Employing the time difference reconstruction, the objective is to infer conductivity changes arising from difference between two boundary data sets taken in different time points [7].

EIT is inherently three-dimensional since electrical current cannot be confined to flow solely in the plane encompassed by the electrodes. As a result, 2D EIT is subject to artefacts produced by off-plane inhomogeneities. Therefore, 3D EIT with multiple-plane electrode arrangements have been studied in the recent decades [8-12]. 3D EIT not only significantly increases the ill-posedness of the problem as a result of the large size of unknown parameters, but also leads to an increase in computational cost [13]. Therefore, the advantage of real time monitoring with EIT in comparison to other imaging modalities for functional imaging will be lost.

Typically, image reconstruction in EIT involves a non-linear forward problem for calculating the surface voltages from the conductivity field and a severely ill-posed inverse problem for updating the conductivity field. To linearize the problem, the Jacobian matrix is computed around a homogenous conductivity [1]. To deal with the high ill-posedness of the problem, a priori assumption around the conductivity field is considered [14]. Classical quadratic regularized methods typically stabilize the problem at the cost of imposing some smoothness on the solution, so precisely detecting sharp discontinuities over the conductivity profile will be impossible in this way [15]. Among these techniques, Conjugate Gradient (CG) solvers best suit the 3D EIT, as classical Newton’s methods need calculation of inverse Hessian [16-17].

Finding sparse solutions to large-size ill-conditioned linear systems of equations has recently attracted much interest in mathematics and image/signal processing. The presence of $\ell_1$ norm as the regularization term of the objective function encourages small components of the unknown parameters to become exactly zero, thus promoting sparse solutions [18,19]. Employing sparse feature of the unknown parameters typically provides two benefits to inverse problems. Firstly, it enables the inverse solver to reduce, in some cases significantly, the number of degrees of freedom of the problem. Secondly, a better distinction of the true signal information from the recovery artefacts will be possible during the reconstruction.

2 Method

Many different approaches have been proposed to seek sparse solutions to large linear systems of the form...
\[ y = Ax + n, \text{ where } y \text{ is the observation and } n \text{ is noise.} \]

There has been much interest in solving the unconstrained form of the problem, i.e.,
\[
\min_x \|y - Ax\|^2_2 + \|x\|_1, \tag{1}
\]
where \( \lambda \) is the regularization parameter [18,19].

This study has been focused on a class of sparse optimization algorithms that do not need \( A \) to be stored explicitly, but only needs matrix-vector products including \( A \) or its transpose. Here the application of so-called Gradient Projection for Sparse Reconstruction (GPSR) algorithm is tailored to large-size 3D EIT [19]. The generalized form of the algorithm is modified solely for application to 3D EIT.

The benchmark is the solution to the quadratic regularized problem, i.e.,
\[
\min_{\lambda, \sigma} \frac{1}{2} \|y - F(\sigma + \eta)\|^2_2 + \lambda R_\gamma(\sigma), \tag{2}
\]
where \( R_\gamma(\sigma) = \frac{1}{\gamma} \|\sigma\|^2_2 \).

By choice of \( \gamma = 2 \), the problem will be in the classical quadratic form, which is solved by the PCG solver here. For further information, the reader is referred to [13,16-17].

2.1 Forward and inverse model

The nonlinear form of the forward problem is linearized around an arbitrary conductivity \( \sigma_0 \) via forming the Jacobian \( J \).

Applying the time difference reconstruction, the objective is to calculate conductivity changes \( \Delta \sigma \) derived from difference between two data frames collected at times \( t_0 \) and \( t_1 \). The linearized form of the forward problem will thus be \( \Delta \sigma = J \Delta \sigma \). The inverse model is then to calculate the optimization problem
\[
\min_{\Delta \sigma} \| \Delta \sigma - \Delta \sigma \|^2_2 \text{ st. a priori on } \Delta \sigma \tag{3}
\]
The unconstrained Tikhonov form of the problem can be written as
\[
\min_{\Delta \sigma} \| \Delta \sigma - \Delta \sigma \|^2_2 + \lambda R_\gamma(\Delta \sigma), \tag{4}
\]
where \( R_\gamma(\sigma) = \frac{1}{\gamma} \|\sigma\|^2_2 \).

By choice of \( \gamma = 2 \), the problem will be in the classical quadratic form, which is solved by the PCG solver here. For further information, the reader is referred to [13,16-17].

2.2 Gradient Projection for Sparse Reconstruction (GPSR)

The choice of \( \gamma = 1 \) conducts the problem to (1), i.e., the unconstrained \( \ell_1 \) regularized optimization problem. This paper applies the GPSR approach to infer sparse solution of the conductivity field [19,20]. The base of this method is to split the unknown vector \( \Delta \sigma \) into its positive and negative parts, i.e.,
\[
x = u - v, \quad u \geq 0, \quad v \geq 0 \tag{5}
\]

Considering a mesh made up of \( n \) finite elements, for all \( i = 1, \ldots, n \),
\[
u_i = (\Delta \sigma)_+, \quad v_i = (- \Delta \sigma)_+, \tag{6}
\]

where \( (x)_+ = \max(0, x) \). By considering the penalty function in the form of
\[
\|x\|_1 = u^T + v^T, \tag{7}
\]

the optimization problem will be written as
\[
\min_{u,v} \|\Delta \sigma - (u - v)\|^2_2 + \lambda \|u\|^2 + \lambda \|v\|^2 \text{ st. } u, v \geq 0 \tag{8}
\]

Supposing
\[
z = \begin{bmatrix} u \\ v \end{bmatrix}, \quad c = \lambda \begin{bmatrix} 2n \end{bmatrix} + \begin{bmatrix} -J^T \Delta \sigma \\ J^T \Delta \sigma \end{bmatrix}
\]

\[
B = \begin{bmatrix} J^T J & J^T J \\ J^T J & J^T J \end{bmatrix},
\]

the problem (7) will be casted in the form of
\[
\min_{z} \begin{bmatrix} c^T z + \frac{1}{2} z^T B z \end{bmatrix} \text{ st. } z \geq 0 \tag{9}
\]

Now, the well-known Gradient Projection (GP) method is applied to problem (9). Given \( z^{(k)} \), we search along the negative gradient \( -\nabla F(z^{(k)}) \) by choosing \( \alpha^{(k)} > 0 \) as follows [19].
\[
w^{(k)} = \begin{bmatrix} z^{(k)} + \alpha^{(k)} \nabla F(z^{(k)}) \\ z^{(k)} - \alpha^{(k)} \nabla F(z^{(k)}) \end{bmatrix}, \tag{10}
\]

\[
\sigma^{(k)} = \begin{bmatrix} z^{(k)} + \tau^{(k)}(w^{(k)} - z^{(k)}) \\ z^{(k)} - \tau^{(k)}(w^{(k)} - z^{(k)}) \end{bmatrix} \tag{11}
\]

\( \tau^{(k)} \) is chosen so that \( F(z^{(k)}) \) is minimized over the interval \( \tau^{(k)} \in [0,1] \). Employing the so-called Barzilai and Borwein approach (BB), at each iteration, \( \tau^{(k)} = -H^{(k)} \nabla F(z^{(k)}) \) is calculated, where \( H^{(k)} \) is an approximate estimation of the Hessian of \( F \) at \( z^{(k)} \), i.e., \( H^{(k)} = \eta^{(k)} \mathbf{I} \), and \( \eta^{(k)} \) must be chosen so that the following relationship is satisfied.
\[
\nabla F(z^{(k)}) = \nabla F(z^{(k)}) - \nabla F(z^{(k)}) = \eta^{(k)} \lVert \sigma^{(k)} - z^{(k-1)} \rVert^2_2 \tag{12}
\]

The criterion applied for terminating the algorithm has been motivated by Linear Complementarity Problems (LCP) [18,19]. The algorithm is terminated when \( \min(z, \nabla F(z)) \leq \text{tol} \). Here the parameter \( \text{tol} \) is heuristically set to be 0.01.

Algorithm 1 (GPSR-BB)

Set \( k = 0 \)

Choose \( \alpha^{(0)} = [\alpha_{\min}, \alpha_{\max}] \)

While \( \min(z, \nabla F(z)) \geq \text{tol} \) do

Calculate \( w^{(k)} \) according to (10)

Calculate \( \sigma^{(k)} = w^{(k)} - z^{(k)} \)

Line search: Find \( \tau^{(k)} \) that minimizes \( F \) at \( z^{(k+1)} \)

according to (11)

Set \( z^{(k+1)} \)

Update \( \alpha \) based on the BB-method:

Calculate \( \eta^{(k)} = \nabla F(z^{(k)}) - \nabla F(z^{(k-1)}) \)

Calculate \( \gamma^{(k)} = \sigma^{(k)} - z^{(k-1)} \)

If \( \gamma^{(k)} = 0 \), Set \( \alpha^{(k+1)} = \alpha_{\max} \)

Otherwise:

\[
\alpha^{(k+1)} = \min \left\{ \alpha_{\min}, \frac{\|\gamma^{(k)}\|^2}{\|\gamma^{(k+1)}\|^2}, \alpha_{\max} \right\}
\]

Set \( k = k + 1 \)

End while
3 Results

Here the performance of the GPSR technique was compared to that of the classical PCG algorithm. A 3D mesh was made up of 161021 tetrahedral elements to simulate the geometry of a human chest. Thirty two circular electrodes were installed around the chest in two rings aligned by axial planes 0.33 and 0.66, as the height of the 3D volume is normalized to 1. The radius of electrodes was set to 0.05. An electrical current with peak-peak amplitude of 1mA was successively injected into the simulated chest and the induced electrical potentials on the remaining electrodes were measured according to the planar configuration, the well-known protocol in 3D EIT. The collected data was contaminated with a 20db white Gaussian noise. To avoid the so-called inverse crime, the inverse solver was applied to a coarser mesh made up of 20955 elements, with the same geometry. Image 1(a) shows the 3D view of the homogenous mesh with a conductivity of $1 \text{S}\cdot\text{m}^{-1}$, and image 1(b) shows the top view of the mesh after simulating lungs having a conductivity of $0.3 \text{S}\cdot\text{m}^{-1}$. To simplify comparison between reconstructed images in different height levels, the conductivity field is fixed in different levels. The PCG scheme was applied by regularization parameter $\lambda = 10^{-3}$ and was terminated when the residual norm becomes smaller than threshold $\text{tol} = 10^{-2}$. These parameters were heuristically selected to produce the optimal image. The GPSR-BB was also optimized with $\lambda = 10^{-3}$ and $\text{tol} = 10^{-2}$. Image 2 shows reconstructed images in different height levels. The left column pertains to images reconstructed by the PCG, while the right column represents images reconstructed by the GPSR technique. To make a fair comparison between the competing algorithms, the images in both columns are represented by the same colourbar. According to the left column, the PCG produced great unappealing artefacts over the different slices. The results show that the PCG failed to accurately determine sharp variations over the conductivity field, leading to a misleading estimation of lungs’ boundaries. From the right column, employing the GPSR, the reconstructed images better represent the true conductivity field. The reconstructed images include much less artefact, and better match the true simulated conductivity, in comparison to those reconstructed by the PCG, in all different levels.

Although the PCG solver is significantly more efficient than other classical quadratic reconstructions with regard to computational cost and storage space, but the large size of the unknown parameters has still led to a time consuming reconstruction. By employing Intel R Core TM i3-3220 processor ($3.30 \text{GHz}$) with a RAM of 4 GB and a 64-bit system type, and also ignoring the time spent on calculation of the Jacobian, the CPU time consumed by the PCG was $6.47 \text{Sec}$, while the CPU time spent on calculation of the GPSR was only $0.61 \text{Sec}$.

4 Conclusion

The results indicate superiority of the sparse reconstruction over the classical quadratic regularized solvers in dealing with a large numer of finite elements in 3D EIT. Such cases arise in medical applications such as monitoring lung function, where the need for an acceptable spatial resolution prevents us from considerably reducing the number of finite elements over the inverse mesh. The large number of required finite elements then increases the ill-condition of the Jacobian as a result of an increase in the ratio of number of the unknown parameters to data size. The performance of inverse solvers such as the PCG is thus affected deleteriously, leading to an image including severe artefacts in different height levels. Another issue is that employing the CG solvers for calculating such large number of unknown parameters considerably increases the CPU time. It is worth pointing out that the application of classical Newton’s methods to such large-size problems is very limited due to the need for calculation of the inverse Hessian, which is bulky and time-consuming. In contrast to the quadratic regularized solvers like the PCG, presence of $\ell_1$ norm as the regularization term in the objective function encourages small components of the conductivity field to become exactly zero. The algorithm then deals with...
solely the nonzero components of the conductivity change, rather than the whole domain, thus significantly reducing the computational cost. Furthermore, the GPSR solver, which was applied in this study, requires only matrix-vector products involving \( J \) and its transpose, which makes another benefit to the CPU time. Another issue is that the capability of the GPSR algorithm in iteratively nulling the small components of the conductivity field has led to a more accurate shape in comparison to the solution calculated by the PCG, better determining the sharp variations over the conductivity profile.

5 Acknowledgement

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[20] Instituto de Telecomunicacoes, www.it.pt
Signal Chain Optimization in Magnetic Particle Imaging

A. Behrends, M. Graeser, J. Stelzner, T. M. Buzug
Institute of Medical Engineering, Universität zu Lübeck, Lübeck, Germany
{behrends, buzug}@imt.uni-luebeck.de

Abstract
In Magnetic Particle Imaging (MPI) resolution strongly depends on the achievable gradient strength of the magnetic field that establishes the field-free line. To generate sufficiently strong magnetic fields it is necessary to apply huge electrical currents to the generating coils. Due to the inductive properties of the coils the apparent power needed for this task is a lot higher than the effective power dissipated in the coils. Furthermore, to efficiently transfer power from the amplifiers to the coil setup, the coils complex impedances have to be matched to the amplifiers desired load resistance. This paper presents a signal chain that has been optimized for low power consumption by compensating these drawbacks through power factor correction and impedance matching.

1 Introduction
Magnetic Particle Imaging (MPI) is an imaging technique proposed in 2005 by Weizenecker and Gleich [1] based on the nonlinear response of Superparamagnetic Iron Oxide (SPIO) nanoparticles. By exciting the SPIOs with a sinusoidal magnetic field the spatial distribution of the nanoparticles can be reconstructed from distortions caused by their nonlinear magnetization.

1.1 Basic principle of Magnetic Particle Imaging
The nonlinear magnetization curve of the SPIOs is well described by Langevin’s Theory of paramagnetism [1]. Image 1 shows the magnetization curve and that excitation of the particles with a sinusoidal magnetic field $H$ leads to a magnetization $M$ of the particles. Due to nonlinear saturation effects, a measurement $s(t)$ of this magnetization response will not only result in the sinusoidal signal that was applied, but a combination of this excitation signal and its higher harmonics. These are shown in the signal spectrum in Image 1. By measuring these harmonics it is possible to calculate the particle concentration of an examined sample. The sinusoidal field that causes the particle response is called drive field and works at a frequency of $f_0 = 25 \text{ kHz}$. Frequencies in the spectrum are given as multiples of the base frequency as $n \omega_0 = n \cdot 2 \pi f_0$ with $n = f / f_0$.

1.2 Spatial encoding and generation of the selection field
To achieve spatial encoding a so-called selection field is used that contains either a field-free point (FFP) or a field-free line (FFL). This field is produced by one or more pairs of Maxwell coils. In the center of these Maxwell coils the magnetic field strength vanishes. Depending on the setup of the Maxwell coils this area is either a point (FFP) [1] or a line (FFL) [4],[5]. If a second sinusoidal magnetic field, named drive field, is applied, the particles inside the FFL or FFP will respond in the above mentioned way. Otherwise, if the particles are not inside the FFL or FFP, they are already in magnetic saturation and the drive field will only lead to a small magnetization change thus producing only a vanishingly small excitation response. Image 2 shows the behaviour of the particles outside the field-free line. The selection field has non-zero field strength. Thus, the particles are already in magnetization and superimposing the drive field only leads to small magnetization changes subsequently inducing only a small voltage $U(t)$. To improve the resolution in MPI a high gradient of the selection field is preferred. A steep gradient field whatsoever can only be achieved by either putting the Maxwell coils closer together or by increasing the magnetic field strength generated by the coils. The distance depends of the fixed size of the sample. Therefore, the remaining solution to get the desired gradient is to increase the magnetic field strength produced by the coils. The relation of the magnetic flux density $\mathbf{B}$ to the current density $J$ and the electric field $\mathbf{E}$ is given by Ampere’s law

$$\nabla \times \mathbf{B} = \mu_0 \left( J + \varepsilon_0 \frac{\partial \mathbf{E}}{\partial t} \right),$$

with $\mu_0$ being the permeability of free space and $\varepsilon_0$ the permittivity of free space. As the current density is the electrical current per unit area and the magnetic flux density is proportional to the magnetic field strength, it follows that for a constant geometry and thus a constant wire...
cross-sectional area a higher current leads to a stronger magnetic field. In this paper, a method to apply a high current to the selection field coil is proposed.

2 Material and Methods

The optimization of the signal chain will be discussed in general and specifically for a certain example setup that is used in our scanner setup. Thus the used equipment will be introduced in the following section. Table 1 shows the summary of these specifications.

2.1 Hardware and Signal Specifications

1) Signal: The signal will be a sine wave with frequency $f = 100 \text{ Hz}$, which is produced by a PC and further amplified within the signal chain.

2) Amplifiers: Three AETechron 7796 Amplifiers are used to amplify the signal. The desired load for one of these amplifiers is $2 \Omega$. As we use our amplifiers in series the desired load for the amplifier setup is $R_{\text{OptLoad}} = 3 \cdot 2 \Omega = 6 \Omega$. The amplifiers are set in voltage-controlled mode and can provide a maximum voltage of $U_{\text{amp}} = 100 \text{ V}$ per amplifier. This sums up to a maximum voltage of $U_{\text{max}} = 300 \text{ V}$. The maximum current the amplifier can supply at this voltage is

$$I_{\text{max}} = \frac{U_{\text{max}}}{R_{\text{OptLoad}}} = 50 \text{ A},$$

leading to a maximum power of

$$P_{\text{max}} = \frac{U_{\text{max}}^2}{R_{\text{OptLoad}}} = 15 \text{ kW}.$$  

3) Selection Field Coil: The imaging setup needs two channels to produce a field-free line. The resistance of each coil is $R_L = 44 \text{ m}\Omega$ the inductance $L = 507 \mu\text{H}$ and the electrical current that is needed to produce the desired field strength is $I_L = 454 \text{ A}$.

### Table I

<table>
<thead>
<tr>
<th>Element</th>
<th>Property</th>
<th>Value</th>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Current (RMS)</td>
<td>$I_L = 454 \text{ A}$</td>
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</table>

2.2 Power Factor Correction

To calculate the power needed to produce the current $I_L$ one has to evaluate the magnitude of the selection field coils impedance at the given frequency. The impedance is a complex quantity consisting of a real resistance and an in imaginary reactance, which can be represented by a vector in $\mathbb{R}^2$. For a coil as an inductive component the reactance is given by

$$X_L = \omega L = 2\pi f L.$$  

(3)

The magnitude of the impedance can be calculated by the Euclidean norm of its representing vector

$$||Z|| = \sqrt{R_L^2 + X_L^2} = \sqrt{R_L^2 + (2\pi f \cdot L)^2} = 321 \text{ m}\Omega.$$  

(4)

and the apparent power needed to produce the desired field strength is

$$P = I_L^2 \cdot ||Z|| = 66 \text{ kVA}.$$  

(5)

Most of the impedance is caused by the inductance of the coil. To minimize the magnitude of the selection field coils impedance one can use a capacitor in series for compensation. For capacitive components the reactance is given by

$$X_C = -\frac{1}{\omega C} = -\frac{1}{2\pi f C}.$$  

(6)

To compensate the inductive reactance the following equation should hold

$$X_L + X_C = 0,$$  

(7)

by substituting (3) and (6) into (7) the needed capacitance can be calculated by

$$C = \frac{1}{\omega^2 L} = \frac{1}{4\pi^2 f^2 L} = 5 \text{ m}\Omega.$$  

(8)

After applying the power factor correction the combined impedance of the selection field coil and the power factor correction capacitor is purely resistive, as the resistance of the capacitor is negligible. The total load of the impedance at a frequency of 100 Hz results in $R_{\text{Load}} = 44 \text{ m}\Omega$. Connecting this low ohmic load to the amplifiers directly would set off the shortcut protection of the amplifiers. To avoid this, one may use impedance matching to properly adjust the load to the desired load of the amplifier.
2.3 Impedance Matching

The concept of impedance matching is to match the input impedance of a certain load to the desired load of a source. The impedance matching will make use of a so-called matching transformer. Generally, a transformer is used to transform voltage of a primary circuit to a voltage used in a secondary circuit preserving the signal waveform. This behavior is characterized by the transformer voltage ratio

\[
\alpha = \frac{U_p}{U_s} = \frac{N_p}{N_s}
\]  

(9)

with \(U_p\) and \(U_s\) being the primary voltage and secondary voltage as well as \(N_p\) and \(N_s\) being the number of primary windings and the number of secondary windings. Image 3 shows the currents and voltages in a transformer.

\[\text{Image 3} \text{ Voltages and currents in a transformer.}\]

The power losses in a transformer are usually small enough that with a good approximation one can assume

\[
P_p = U_p \cdot I_p = U_s \cdot I_s = P_s
\]

(10)

with \(I_p\) and \(I_s\) being the primary current and secondary current. And \(P_p\) as well as \(P_s\) the primary power and secondary power. By solving (9) for \(U_p\) and substituting in (10) one gets

\[
\alpha \cdot U_s \cdot I_p = U_s \cdot I_s \Rightarrow \alpha = \frac{I_s}{I_p}
\]

(11)

Multiplication of (9) with (11) gives

\[
\alpha^2 = \frac{U_p I_s}{U_s I_p} = \frac{U_p I_s}{I_p U_s} = \frac{Z_p}{Z_s}
\]

(12)

a relation of an impedance in the primary circuit \(Z_p\) to an impedance in the secondary circuit \(Z_s\). As these impedances are known where \(Z_p = R_{\text{OptLoad}}\) and \(Z_s = R_{\text{Load}}\) one can determine the transformer voltage ratio as

\[
\alpha = \frac{\sqrt{\frac{R_{\text{OptLoad}}}{R_{\text{Load}}}}}{1.168}
\]

(13)

As the impedance matching is complete the voltage and the current in the secondary circuit follow according to (9) and (11) as \(U_s = 25.7\, \text{V}\) and \(I_s = 584\, \text{A}\) making use of the maximum power \(P_s = 15\, \text{kW}\) of the amplifier setup. One can further improve the system using only a fraction of the maximum power. Therefore, the lowest voltage the amplifiers have to work at to produce the desired current in the selection field coil has to be determined.

2.4 Minimum Voltage-Output Estimation

Using the maximum power of the amplifiers may give the biggest field strength but leads to harmonic distortion effects in our signal. These effects appear due to nonlinear components in the amplifiers. Higher harmonics will be added to the signal and consequently distort the magnetic fields. Possible reasons for nonlinear behavior are temperature dependency of the used components or the use of semiconductors, which are main parts in modern amplifiers. Nevertheless a minimum current is needed and one has to know the minimum voltage-output of the amplifiers to supply the selection field coil with the desired current. Ongoing from the discussion in section 2.3 this value can be derived by taking into account Ohms law for the primary circuit and (11)

\[
U_{\text{min}} = R_{\text{OptLoad}} \cdot \frac{I_s}{\alpha} = 233\, \text{V}
\]

(14)

it follows that the minimum power equals

\[
P_{\text{min}} = \frac{U_{\text{min}}^2}{R_{\text{opt}}} = 9.05\, \text{kW} = 0.6 \cdot P_{\text{max}}
\]

(15)

This means the used amplifiers could work at about 60% of its maximum power to generate the desired currents in the selection field coil. The power needed is slightly below the power limit of 10 kW which could be delivered by 2 amplifiers. Although one could assume the use of 2 amplifiers is sufficient it has to be considered that in a real-life setup components are not ideal and one has to deal with losses in the components as well as production tolerances.

2.5 Feed-Through Filtering

To further improve the signal chain it is necessary to take disturbances occurring due to electromagnetic induction into account. These can originate from radio, wireless transmission or mobile communication as well as other parts of the imaging setup. However, most of these interferences are part of the higher frequency spectrum going from several kilohertz to gigahertz. To avoid these interferences most of the imaging setup is placed in an electromagnetically shielded chamber. Inside this chamber external electromagnetic induction is negligible. Nonetheless some parts of the signal chain have to be placed outside this chamber including the PC, the amplifiers and the wiring of these components, thus exposing them to electromagnetic induction. To secure minimum distortion of the signal direct feed-through filtering [6] is used to eliminate eventually induced electromagnetic distortions. To preserve the signal the filter has to be a low-pass filter with an attenuation of less than 1 dB at the used frequency of 100 Hz and should have an attenuation of at least 40 dB at 25 kHz and above. This requirement originates from the fact that this frequency is used in the drive field generation and any disturbance would contribute to our drive field in a negative way. Frequencies above 25 kHz especially higher harmonics of this frequency are crucial in the measurement process. Therefore, any disturbance produced by electromagnetic induction at those frequencies will cause problems in the measurement of the SPIO concentration in the sample.
2.6 Thermal Considerations

The relatively high currents cause thermal dissipation in the capacitors. Heating in capacitors originates from 2 different aspects. The first effect arises due to losses in the resistance of the capacitor, the second effect originates from dielectric losses. The thermal dissipation losses are then given by the sum of both losses. The thermal losses can be calculated as

\[ P_T = I_{\text{rms}}^2 \cdot ESR + Q \cdot \tan \delta_0 \]  

(16)

where ESR is the equivalent series resistance and \( I_{\text{rms}} \) the current. The dielectric dissipation factor \( \tan \delta_0 \) for polypropylene is \( \tan \delta_0 = 2 \cdot 10^{-4} \) and \( Q \) is the reactive power of the capacitor which can be calculated by

\[ Q = \frac{I_{\text{rms}}^2}{2\pi \cdot f \cdot C} \]  

(17)

with \( I_{\text{rms}} \) again being the current, \( f \) being the frequency and \( C \) being the capacitance of the capacitor. Most capacitor manufacturers provide a thermal resistance considering the dissipation losses as a heat current. The temperature difference that is important for a non-cooled setup lies between the capacitor hotspot and the ambient room temperature therefore the following thermal resistance \( R_{th} \) always relates to these spots. The temperature difference follows now as

\[ \Delta T = P_T \cdot R_{th} \]  

(18)

To minimize the current in the capacitor not one but a few capacitors in parallel connection are chosen, thus splitting up the current \( I_c \) in smaller currents. This is necessary as capacitors providing the desired capacitance whilst withstand the used currents are virtually not available.

3 Results and Discussion

Image 4 Proposed Signal Chain.

From the considerations discussed in this publication the signal chain shown in Image 4 was derived. It consists of a PC as signal source and three AETechron 7796 amplifiers to provide the power needed to generate the desired selection field. The amplified signal will then be filtered by a low-pass feed-through filter to decrease electromagnetically induced high frequency signals. For an optimized power distribution a transformer with a voltage-ratio of 11.68 will work as an impedance match between the amplifiers and the selection field coil. Finally a capacitor with a capacity of 5 mF will act as a power factor correction to minimize the apparent power in the selection field coil due to its inductive properties. Using these methods the power needed to generate the desired selection field decreases from 66 kW to \( P_{\text{min}} = 9 \) kW, which is an improvement of 86 % compared to the apparent power used by directly connecting the selection field coil to the amplifiers. Nevertheless one has to consider that this result was obtained assuming some idealizations such as the use of an ideal transformer in the impedance matching and an ideal capacitor in the power factor correction. Therefore the result in an actual implementation will be slightly different.

4 Conclusions

Simulation of the used techniques shows a good performance to minimize losses in our signal chain. However this has to be evaluated in a real-life implementation which will be done in the near future. If the implementation confirms the theoretical results it is possible to efficiently generate strong magnetic fields and thus steep gradient fields which lead to an increasing resolution in Magnetic Particle Imaging and more detailed images of examined samples.

5 Acknowledgements

The specifications for the selection field coil are provided by Gael Bringout (Institute of Medical Engineering, Universität zu Lübeck).

6 References

A distributed active NMR sensor array for artifact correction in ultra high field MRI applications

J. Handwerker1, A. Hoffmann1, M. Eschelbach2, K. Scheffler2, M. Ortmanns1, J. Anders1

1Institute of Microelectronics, University of Ulm, Germany, jonas.handwerker@uni-ulm.de
2High-Field MR Center, Max Planck Institute for Biological Cybernetics, Tübingen, Germany

Introduction

We present a distributed sensor array for the real-time monitoring of magnetic field imperfections in magnetic resonance imaging (MRI) scanners. These imperfections occur due to hardware limitations originating from non-ideal gradient coils as well as patient motion and lead to artifacts which limit the achievable imaging quality especially for ultra high field scanners. By monitoring these imperfections, the artifacts can be corrected by either a predistortion of the gradient waveforms or during image reconstruction.

Methods

The presented sensor array consists of four active transmit/receive (TX/RX) field probes and signal conditioning electronics on a printed circuit board (PCB). The field probes consist of a glass capillary ($d_o = 800 \mu m$) filled with a liquid NMR sample surrounded by a solenoid TX/RX coil which is connected via a tuning/matching network to a homodyne quadrature transceiver. The proposed system is an extension of the work presented in [1] to an array of sensors which allows for an artifact correction based on first order spherical harmonic base functions. Furthermore, we use a $^{19}F$ instead of a $^1H$ NMR sample to reduce coupling between sensor and imaging object and a significantly enhanced the transceiver architecture and layout. The field probes are connected using differential, impedance-matched cables to the signal conditioning board which provides line drivers and anti-aliasing filters and interfaces to a commercial data acquisition system (USB-6366, National Instruments) with 2 MS/s and 16 bit resolution.

Results

The sensor array has an input amplitude ranging from $<2.2 \mu V_{RMS} - 78.4 mV_{RMS}$ and accepts input frequencies between 175 MHz - 660 MHz, corresponding to field strengths of 4.4 T - 16.4 T for $^{19}F$ samples. The detector gain can be adjusted between 21 dB and 81 dB with a noise figure of 2.74 dB for quadrature detection. The on-board transmitter generates a peak power of 18.7 dBm, resulting in a 90° pulse time $<10 \mu s$. The sensor array was successfully tested in a 9.4 T whole-body scanner and a 11.7 T small animal scanner and achieved a frequency resolution $<5$ ppb.

Conclusion

In contrast to previously published RX-only [2] and TX/RX [3] field probes, the active field probe array presented here eliminates the need for long RF cables inside the scanner due to a local generation of the RF signal required for excitation and downconversion of the NMR signal, reducing the crosstalk with the imaging experiment and therefore improving the accuracy of the recorded data. Currently, we are working on an implementation of the field probe electronics as a custom designed integrated circuit to further reduce crosstalk and power consumption and improve system performance.

References


A metric to ensure high image quality in parallel breast MR imaging

M Ariens1, C Liess2, B Kremers1, C Kuhl1, S Schrading1
1Radiology, University Hospital RWTH Aachen, Germany, mariens@ukaachen.de
2Philips Healthcare, Hamburg, Germany

Abstract
The purpose of this preliminary MRI study was to design a metric for the determination of the best practicable parallel imaging acceleration (SENSE) factors in routine breast MRI, that allows complete anatomic coverage whilst ensuring diagnostically adequate SNR. 18 healthy women with different breast sizes were examined at 1.5 T MRI. SNR values were measured as a function of breast size and SENSE factor. Small breasts gave SNRs of 38-21, Medium breasts gave SNRs of 55-29 and Large breasts gave SNRs of 73-40 for SENSE factors of 1.0–2.0. Large breasts displayed an SNR necessary for clinical diagnosis for all SENSE factors, whereas Small breasts suffered from a lower SNR. In fact too high SENSE factor yielded images of SNR too low for routine clinical diagnosis. We provide guidance for the adjustment of SENSE factors to ensure diagnostic imaging quality for all breast sizes.

1 Introduction
MRI is an important tool for breast cancer diagnosis, and to significantly more sensitive than mammography especially in dense breast. Pulse sequences such as a dynamic T1w gradient echo sequences (before and after contrast agent) are essential in routine MRI breast protocol. For dynamic scans fast sequences are compulsory, and these may be sped up further by selecting high parallel imaging acceleration (SENSE) factors. On the other hand using parallel imaging techniques results in a loss in SNR. Especially in breast imaging the SENSE factor is chosen by the technical staff depending on the size of the anatomy (breast size) and the number of slices to cover the whole breast. Hence technical staff is faced with the question of selecting the correct SENSE factor to yield sufficiently high SNR and hence good image quality whilst ensuring the whole breast is covered.

The SENSE method is an MRI technique to reduce the image acquisition time, and it helps to reduce artefacts, e.g. motion artefacts. The SENSE method can be applied to many sequences such as dynamic T1, T2 and DWI of the clinical breast protocol. Using SENSE in these sequences will speed up the acquisition and reduce motion artefacts.

2 Methods
20 healthy women (age: 28 ± 9) with different breast sizes were scanned on a Philips Achieva 1.5 T (Philips Healthcare, Best, The Netherlands) with a 4-channel breast coil with SENSE factor ranging from 1.0 (no sense) to 2.0 (acceleration of 2) in steps 0.1. SENSE factor 2.0 is the maximum SENSE factor, available with our hardware. The following data applies to the dynamic T1 sequence of the routine breast MRI protocol; however the results may be extrapolated to any other sequence using SENSE. One characteristic of the T1w sequence is that fat is hyperintense and glandular breast tissue is hypointense. Small breasts tend to have less fat and more glandular tissue. Large breasts tend to have more fat tissue, hence are comparatively more signal-intense.

A T1-FFE sequence with parameter of TR= 313ms, TE= 4.6ms, flip angle= 90° was used. The number of slices (35-25) and size of the FOV (380 mm x 380 mm -300 mm x 300 mm) were adjusted to the breast size, as in the normal clinical routine. Two women were excluded from the study cohort due to either claustrophobia or previous treatment intervention, such that n=18 women were analysed.

Women were divided into three groups depending on the size of their breasts, small (cupsize A-B), medium (C-D) and large (E-F).

Breast volumes by drawing a region of interest (ROI) in each breast (left and right) using ImageJ (NIH, http://imagej.nih.gov.ij), the number of pixels counted in the ROI was referenced to the field-of-view, matrix size and slice thickness to yield the volume of the whole breast. The same method of drawing ROIs was used to measure the signal; the latter was measured in these ROIs using an average of all intensities of all pixels in all slices. All slices were analysed because of the spatially varying breast densities from caudal to cranial which result in different signal intensities, see figure 1.
The SNR was calculated with this formula:

\[
SNR_{std} = \frac{signal}{std.\, deviation\, noise}
\]

The SENSE factor is relevant for the calculation of SNR:

\[
SNR_{sense} = \frac{SNR_{std}}{g \times \sqrt{R}}
\]

R is the SENSE factor and varies between 1.0 and 2.0 in our study. G reflects the local coil geometry factor. In all scans the same coil with the same geometry was used and g was assumed to be constant.

Figure 2 shows the ROI placement for the central slice. An average SNR was calculated based on the left breast for each patient and SENSE factor. The noise and std. deviation of the noise was taken from an ROI positioned just outside the breast.

We defined threshold values for clinically acceptable SNR that ensure sufficient image quality for diagnosis. Various radiologists in-house deemed a SNR from 40-30 as an adequate value for diagnosis. To account for individual reader preferences, the following analysis is carried out for three different SNR threshold values; these were chosen to be 40, 35 and 30.

3 Results

In table 1 the mean breast volumes and SNR of the categories for the three groups are shown. The SNR of the Small group is less than the SNR of the Large group.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>volume</th>
<th>cupsize</th>
<th>SNR of SENSE 1.0</th>
<th>SNR of SENSE 2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large (n=5)</td>
<td>5</td>
<td>1270 cm³</td>
<td>E-F</td>
<td>73 ± 7</td>
<td>40 ± 4</td>
</tr>
<tr>
<td>Medium (n=7)</td>
<td>7</td>
<td>753 cm³</td>
<td>C-D</td>
<td>55 ± 8</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>Small (n=6)</td>
<td>6</td>
<td>380 cm³</td>
<td>A-B</td>
<td>38 ± 3</td>
<td>21 ± 2</td>
</tr>
</tbody>
</table>

Table 1 The averages of volume, SNR and signal of the different categories are shown.

In table 2 the SNR values dependent on breast size and the SENSE factor are shown. The SNR decreases in group Large by 45%, in group Medium by 47% and in group Small by 45% from SENSE factor 1.0 - 2.0. The geometry circle indicates the best SENSE factor to reach a SNR of 40. In the Large group is that 2.0 and in the Medium group 1.4. The Small group doesn’t reach a SNR of 40, even with sense factor 1.0. The geometry square indicates the best SENSE factor to produce a SNR of 35. In the Medium group is this 1.6 and in Small 1.1. The triangle shows the best SENSE factor to get a SNR of 30. This is in group Medium 1.9 and in group Small 1.3.

<table>
<thead>
<tr>
<th>SENSE factor</th>
<th>1.0</th>
<th>1.1</th>
<th>1.2</th>
<th>1.3</th>
<th>1.4</th>
<th>1.5</th>
<th>1.6</th>
<th>1.7</th>
<th>1.8</th>
<th>1.9</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large (n=5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium (n=7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>Small (n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 The SNR results of the SENSE factor 1.0 to 2.0 are shown in the different groups: large (E-F), medium (C-D) and small (A-B). The geometry circle indicates the best SENSE factor to produce a SNR of 40. The square is the best factor to get a SNR of 35 and the triangle for a SNR of 30. Std. deviations were left out for better readability, but were in the order of 10%.

In table 3 the duration of the sequences is shown for the defined SNR thresholds and breast groups. The best SENSE factors for the groups were transferred from table 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>SNR 40</th>
<th>SNR 35</th>
<th>SNR 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large (n=5)</td>
<td>2 (64 s ± 7)</td>
<td>2 (64 s ± 7)</td>
<td>2 (64 s ± 7)</td>
</tr>
<tr>
<td>Medium (n=7)</td>
<td>1.4 (91 s ± 8)</td>
<td>1.6 (79 s ± 7)</td>
<td>1.9 (67 s ± 6)</td>
</tr>
<tr>
<td>Small (n=6)</td>
<td>-</td>
<td>1.1 (104 s ± 10)</td>
<td>1.3 (88 s ± 8)</td>
</tr>
</tbody>
</table>

Table 3 shows the duration of the sequences and the best SENSE factor to achieve the desired SNR values from 40, 35 and 30 of the different groups.

The Large group always exceeds an SNR of 40, independent of the SENSE factor. The SENSE factor required to reach a SNR of 40 in the Medium group however is only 1.4. The Small group never reaches a SNR of 40, even with a SENSE factor of just 1.0. In the Medium group a
SNR of 35 is possible with a SENSE factor of 1.6 and in the Small group by using a SENSE factor of 1.1. A SNR of 30 can be produced in the Medium group with a SENSE factor of 1.9 and in the Small group with a factor of 1.3.

Figure 3 shows, that if the required SNR is 40, the sequence time of the Large group will be 64 sec. and in the Medium group 91 sec. The small group didn’t reach a SNR of 40. The best SNR without SENSE was 38. The duration of this sequence for a SNR of 35 is for the Large group 64 sec., Medium group 79 sec. and Small group 104 sec. A SNR of 30 takes time up to 64 sec for the large group, 67 sec. for the Medium group and 88 sec. for the Small group. The time of the Large group is always the same, because the maximum SENSE factor can be used already by a desired SNR of 40.

Figure 4 The duration of the sequence in the large group is always the same, because the SNR of 40 is achieved with the highest SENSE factor of 2.0. In group Medium the time decrease from SNR 40 to SNR 35 by 12 seconds (13%) and from SNR 40 to SNR 30 by 24 seconds (26%). In the Small group the SNR of 40 can’t be achieved. The duration of the sequence decrease from SNR 35 to SNR 30 by 26 seconds (25%).

4 Conclusion

In this study the SNR of different sizes of breast (group large (cupsize F-E), medium (C-D), small (A-B)) and different SENSE factors from 1.0 to 2.0 were compared with each other, in order to obtain sufficient image quality, coverage of the breast anatomy depending on breast size, and speed of the acquisition. We have shown that SENSE factors should be adapted to the size of the breast, in order to guarantee images of sufficient SNR for clinical diagnosis. Whilst large breasts intrinsically yield higher SNR values and can thus be acquired with the highest SENSE factor 2.0. Smaller breasts suffer from lower SNR, often precluding the use of higher SENSE factors.

The SENSE factor reduces the sequence duration, this is especially important for breast imaging where a dynamic sequence with a high temporal resolution essential in order to detect breast cancer. But also the other sequences like T2 and diffusion based sequences benefit from parallel imaging, e.g. artefacts can be reduced.

Table 1 and table 2 demonstrate a decreasing the SNR with decreasing breast size. This is, due to the coil geometry small breasts give less signal as larger sizes. The coil used in our study has 4 Elements, two on each breast. Because the women lie in prone position on the coil, one element of the coil is on the upper part of the body and the other element in the direction of the nipple from the breast. If the breast has a small volume, the breast is far away of the second coil element, resulting in a SNR reduction. Furthermore, smaller breast consist of more dense glandular tissue and less fat. Fat is hyperintens in a T1 sequence and can thus be acquired with the highest SENSE factor 2.0. Smaller breasts suffer from lower SNR, often precluding the use of higher SENSE factors.

Table 3 shows the time savings due to the sense factor. For the sequences of the larger breast more slices of the image were used to cover the whole breast. But due to a high sense factor this time loses of more slices can be compensate and these sequences are the fastest one with the best SNR. Small breasts need only a few slices and so these sequences are faster. But due to their intrinsically low signal (see above) a low SENSE factor is preferred. So the image acquisition time is slower than that of the Medium or the Large group.

The 4-channel breast coil is not able to achieve a SENSE factor higher than 2.0. This is because the SENSE factor is limited by the number of coil elements per breast. With containing more elements coil, a higher SENSE is possible. Based on our study, especially women with larger breasts may benefit from the higher number of coil elements, as SNR tends to be higher and may be treeded for faster image acquisition. There are breast coils available with more elements which addressed this issue.
This study was performed using our in-house MRI protocol. Breast MRI protocols in other institutions may also benefit from our optimisation; note however the resulting SNR values will most likely be different.

Limitation of our study is the small sample size. In conclusion we have shown that the SENSE factor should be chosen based on the size of the breast is maybe a baseline to develop a metric guiding for radiologist and technical staff in the selection of the best SENSE factor to ensure breast images of diagnostic quality are acquired every time.

5 References

[1] Sinha S, Sinha U; Recent advances in breast MRI and MRS; NMR Biomed. 2009; 22:3-16
Abstract—We investigated a stripe artifact in diffusion weighted MRI on a clinical system. It manifests itself as a periodical variation in the global intensity level of the imaged slices, where every n-th slice position is associated with higher intensity. Several measurements with varying scan parameters were conducted to analyze the artifact. The origin of the artifact has been found to be a correction for the field drift effect which inserts a delay between each measurement of a diffusion direction. Deactivating this correction may lead to a reappearing fat signal or mislocation of voxels during longer measurements. Conducting a measurement of 30 minutes with deactivated field drift correction showed no such problems. It is therefore advisable to avoid activating the field drift correction at least for scans shorter than 30 minutes to prevent this kind of artifact from appearing.

I. INTRODUCTION

The contrast of Diffusion-Weighted Imaging (DWI) [1] and all its more advanced applications depend on the extent of water diffusion induced by Brownian motion in the direction of the diffusion sensitization gradients. This is the basis for more advanced techniques like Diffusion Tensor Imaging (DTI) [2] and Diffusion Kurtosis Imaging (DKI) [3] providing details on tissue microstructure. This information is used to to map out the orientation in space of the white matter tracts in the brain (tractography) [4] and to extend the diagnostic capabilities for the detection of a number of diseases including but not limited to ischaemic stroke [5], multiple sclerosis [6] and schizophrenia [7].

Enabling this enhanced diagnostic capability requires quantitative analysis of the diffusion data e.g. to calculate the diffusion tensor in DTI for fiber tracking or calculate apparent diffusion maps in DWI. An artifact can lead to wrong derived measures which can affect the outcome of any post-processing steps substantially e.g. fibers may be wrongly tracked [8].

The artifact investigated in this work manifests as systematic variation of mean slice signal intensity in an entire stack. It appears as a stripe pattern when combining a stack of axial slices to a sagittal view of the imaged volume. An example for this is shown in Fig. 1. It is not to be confused with artifacts caused by Gibbs-Ringing where high-contrast boundaries lead to dark-bright variations in a single slice due to “truncation” of high frequency parts of the sampled signal.

In this article, the artifact is investigated in in-vivo images. The influence of different parameters on the artifact are tested and described, as well as a possible mechanism presented that might create the artifact. Finally, suggestions on how to avoid it are provided.

II. MATERIAL AND METHODS

All image data was acquired on a Philips Achieva 3.0 T MR system (Philips Medical Systems Nederland BV, Best, The Netherlands). An 8-channel head receive coil with sensitivity encoding (SENSE) [9] was used for all acquisitions. In order to test the influence of scanning parameters on the form and magnitude of the artifact data from 1 volunteer without known history of neurological disorder was acquired. Informed written consent from the volunteer was acquired prior to...
the measurement. All measurements were performed with a diffusion-weighted spin echo echo-planar-imaging sequence. TABLE I shows the parameters. Any deviation from the reference protocol will be explicitly stated in the descriptions of the performed scans. With partial Fourier acquisition enabled not the entire k-space is acquired but only a little over half (in this case 65.7 %) in the phase encoding direction. This is possible because of the Hermitian symmetry between negative and positive k-space data. Using partial Fourier acquisition allows for shorter TE and therefore increases SNR.

SPIR (Spectral Presaturation with Inversion Recovery) is a fat suppression technique employing spectrally selective RF pulses [10].

The slice scan order (SSO) defines the temporal order in which slices are excited. The following ordering methods were investigated (The numbers represent the spatial order in which the slices are located in the volume):

1) 2 step interleaved (default): A mechanism is applied which tries to maximize the time between the measurement of every pair of adjacent slices. For 8 slices the resulting scan order would be 1, 3, 5, 7, 2, 4, 6, 8.

2) Variable interleaved: The slices are measured in an interleaved order, i.e. the time delay between any two neighboring slices is always large to prevent crosstalk between slices. A step size is calculated by round(√number of slices). For 70 slices the resulting slice step size is 8 and the scan order is 1,9,17,⋯,65; 2,10,18,⋯,66; 3,11,19,⋯,67; 4,12,20,⋯,68; 5,13,21,⋯,69; 6,14,22,⋯,70; 7,15,23,⋯,63; 8,16,24,⋯,64.

3) Ascending: Slices are measured in a linearly ascending order e.g. 1, 2, 3, 4, 5.

4) Descending: Slices are measured in a linearly descending order e.g. 5, 4, 3, 2, 1.

The parameter dynamic stabilization employs repeated measurements of the resonance frequency of water (called f₀ for brevity) during longer scans. In the preparation step before each diffusion gradient direction change, f₀ is measured and used to adjust the receive and transmit frequencies during scanning. Changes of f₀, due to temperature changes of the hardware, are then automatically corrected.

In order to understand the influence of the slice scan order a series of measurements was performed with slice scan order set to 2 step interleaved, variable interleaved and ascending. Descending was not tested since it is the same as ascending but in the opposite direction.

Another series was acquired with 4 b-values (625 s/mm², 1250 s/mm², 1875 s/mm², 2500 s/mm²) to investigate whether the artifact depends on the diffusion gradient strength.

Partial Fourier acquisition is set to off in another series to test, if not enough k-space data is acquired or that the interpolation of missing data points is causing regular formed artifacts. Daniel Gallichan et al. [11] have previously shown that low frequency vibrations induced from the switching of gradient coils with partial Fourier acquisition may cause severe artifacts.

A fixed TR of 20000 ms was used to test if slices experience a loss of signal due to pre-excitation from a radio frequency pulse targeting an adjacent slice. Using such a long TR makes sure that the slice has completely returned to thermal equilibrium by the time the next excitation happens.

Finally, a measurement with dynamic stabilization off was conducted to asses, what the influence of the reoccurring field drift correction is. The purpose of dynamic stabilization is to prevent changes of the resonant frequency f₀, due to temperature changes of the hardware.

Therefore, a 30 minute measurement with disabled dynamic stabilization was conducted to investigate whether any ghosting or mislocated voxels arises.

III. RESULTS AND DISCUSSION

A. Influence of Parameters

TABLE II shows an overview over all tested parameters and their influence on the stripe artifact.

<table>
<thead>
<tr>
<th>Parameter Influence on artifact</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 step interleaved slice scan order Manisfests as bright dark stripe pattern.</td>
</tr>
<tr>
<td>Variable interleaved slice scan order Bright stripes separated by constant step size.</td>
</tr>
<tr>
<td>Ascending slice scan order Does not appear.</td>
</tr>
<tr>
<td>Full Fourier acquisition Much less pronounced but regular pattern still visible.</td>
</tr>
<tr>
<td>Increased TR to 20000 ms Reduced magnitude but regular pattern still visible.</td>
</tr>
<tr>
<td>Diffusion gradient direction and strength No influence.</td>
</tr>
<tr>
<td>Dynamic stabilization off Does not appear.</td>
</tr>
</tbody>
</table>

TABLE II shows an overview over all tested parameters and their influence on the artifact.

Variations of the SSO do affect the form of the artifact. While SSO “2 step interleaved” results in a bright-dark zebra pattern (see Fig. 1), changing it to interleaved shows a completely different pattern, as shown in Fig. 2. The plot of slice mean intensities versus slice number shows that the bright stripes are separated by a constant step size. This step size is in fact the same that was calculated to maximize the time spacing. This implies that the artifact is only visible in the first batch of slices acquired i.e. 1,9,17,⋯,65. Any subsequent
slice batches do not show the artifact. Ascending SSO leads to artifact free images with a slight decrease in SNR because of crosstalk between neighboring slices.

The resulting images of the measurement with full Fourier acquisition (see Fig. 3) show the artifact much less pronounced, but the plot of the mean slice intensity reveals the regular pattern of the artifact. While the partial Fourier acquisition parameter has an influence on the artifacts appearance it does not cause it.

Increasing $TR$ significantly reduces the magnitude of the artifact but the regularly spaced spikes are still visible in the slice mean intensity plot, similar to the previous measurement. This implies that the artifact is caused by a different mechanism and the increased $TR$ happens to mitigate the effect.

It appears that the artifact is not dependent on the diffusion direction and diffusion gradient strength, because no difference in form or magnitude of the artifact could be observed for these measurements. This makes low frequency vibrations from the switching of gradient coils an unlikely source for the artifact.

Disabling dynamic stabilization instead results in completely artifact free images. This makes it the most likely candidate for the appearance of the stripe artifact.

**B. Mechanism behind dynamic stabilization**

As mentioned before, dynamic stabilization introduces measurements of $f_0$ in between each diffusion direction measurement to prevent the field drift effect. Through the strong gradient fields applied while scanning, temperature of passive shim elements in the scanner rise. These passive shim elements are used to enhance the homogeneity of the magnetic field and are chosen once, when the scanner is installed. When the temperature of these elements rises, their magnetic properties change. Therefore the homogeneity of the magnetic field is reduced which has several implications: A change in homogeneity leads to changes of the resonance frequency of the molecules affected, which in turn can lead to a false location of voxels containing these molecules. On the other hand the fat suppression may be less effective for longer measurements. For the fat suppression to work reliably in general and for longer measurements especially good $B_0$ homogeneity is required. When the resonance frequency of fat molecules changes due to a changing $B_0$ field, the frequency-selective fat suppression pulse becomes ineffective, because it misses the changed fat frequency. This may cause the fat signal to reappear, which leads to ghosting artifacts overlapping brain signal. The problem itself is the time needed to perform the measurement of $f_0$ in each preparation step before the slices of the next direction get scanned. After analyzing the sequence with and without dynamic stabilization it is apparent that in addition to the time needed to perform the measurement of $f_0$ itself, there is a two second long waiting period before that. A possible reason for the extended waiting period may be that the time is needed to make sure that there are no eddy currents present which would otherwise cause magnetic field inhomogeneities and alter the resonance frequency.

Due to an imperfect pulse profile not only the actually selected slice is excited but also adjacent slices. When the partially excited slice is again excited after $TR$ for the next diffusion gradient direction, the magnetization has not fully reached thermal equilibrium and has therefore less magnetization to flip into the transverse plane. The delay introduced by the field drift measurement leads to higher slice mean intensities, because more time is available to reach thermal
equilibrium. Slice mean intensities would be reduced in slices where both or at least one adjacent slice was excited after the pause so that not enough time passed to let the magnetization fully recover. A way to verify this is to categorize every slice into one of three categories:

1) Both adjacent slices were excited before the delay. The magnetization has more time to fully reach thermal equilibrium.
2) One adjacent slice was excited before and the other after the delay.
3) Both adjacent slices were excited after the delay. Magnetization has not reached thermal equilibrium.

The slices in the first category should have a significant higher intensity than slices in both other categories.

Doing this for the measurement with variable interleaved \( SSO \) shows that only both adjacent slices to 1, 9, 17, \( \cdots \), 65 were excited before the pause. Fig. 4 illustrates that by plotting the slice mean intensity of all slices separated into their respective categories. It is apparent that all slices of the first category have indeed a significantly higher mean intensity. This makes the delay added by the field drift measurement a very likely cause for the origin of the stripe artifact.

After a visual assessment of the 30 minutes measurement without dynamic stabilization no dislocated fat signal or chemical shift artifacts could be observed. This leads to the assumptions that it is safe to disable the parameter at least for measurements of 30 minutes and less to prevent the artifact.

IV. CONCLUSIONS

It is advisable to refrain from the use of dynamic stabilization even for scans taking half an hour. This prevents the stripe artifact reliably. When measuring for 30 minutes no decrease in SNR or the appearance of other artifacts could be observed, which suggests that it is safe to disable this parameter for scans of 30 minutes or less. As an alternative, changing the slice scan order to a linear mode like ascending or descending prevents the artifact as well but a decrease in SNR has to be taken into account.

REFERENCES


Visualizing Microscopic Hemorrhages with Susceptibility-Weighted Imaging (SWI) for Forensic Applications

A. Biber, M. Meyer, M. A. Koch

Abstract—There are several pathological conditions which are combined with hemorrhages caused by physical illnesses like shaken baby syndrome. Until recently main clinical examination for these conditions is Computed Tomography, which is not successful in all cases and emits ionizing radiation. A new and reliable technique is SWI, which is a postprocessing step performed on a T2*-weighted image. SWI enhance the image contrast and makes tissue visible that contains deoxyhemoglobin – an iron-containing transport protein. Blood-Oxygen-Level-Dependent imaging is already integrated in clinical application but SWI is still at the research stage and shows much potential. Our goal in this project was to investigate brain samples, which contain hemorphages, by using SWI. The results show that this technique works very sensitive to hemorrhages and makes them clearly visible, which opens a new area for the forensic pathology. However, it needs further studies to support the results and to investigate the scope of SWI.

I. INTRODUCTION

Magnetic resonance imaging (MRI) is a commonly used non-invasive method to visualize anatomy and physiology of the body. For this purpose MRI uses the nuclear magnetic resonance (NMR) and a strong magnetic field to form detailed images of tissue. Until recently, most diagnostic MRI applications relied on reading the magnitude information, in which the intensity of a pixel corresponds to the magnitude of the MR signal at the respective location. The phase image, which can also be reconstructed from the raw data, contains the respective phase of the spins between \(-\pi\) and \(+\pi\) in each pixel. However, the phase image is basically used for velocity encoding and flow imaging or in inversion recovery sequences [1], [2].

In 1990 Ogawa et al. [3] investigated the MRI contrast dependent on blood oxygenation. They found that deoxyhemoglobin in venous blood has a significant susceptibility difference to surrounding tissue. This effect gets stronger by using a gradient echo sequence. It is based on the fact that diamagnetic oxyhemoglobin becomes paramagnetic when it releases its oxygen. The paramagnetic property of blood produces bulk susceptibility differences between blood vessels and surrounding tissue. This causes a change in the resonance frequency of water and reduction of the signal intensity so that the voxel appears darker. This technique is called blood oxygen level-dependent (BOLD) imaging [4].

In 1997 J.R. Reichenbach et al. developed first approaches to use the phase information [2]. The phase image can be used to enhance contrast of the magnitude image different from that of proton density. One problem of this technique is the presence of background local field inhomogeneities that influence the effect of local phase changes and cause unwanted phase shifting of low spatial frequencies within the image [5], [6]. Therefore, the first step of SWI is to remove the low-frequency components of the background by using a high-pass filter. Second is to create a phase mask of the processed phase image and multiply it by the original magnitude image. The phase mask is a filter that is designed to suppress certain phases of pixels. Figure 1 shows different schemes of phase masks.

The negative phase mask (eq: 1) is designed to be

\[
f(x) = \begin{cases} \frac{\phi(x) + \pi}{\pi}, & \text{phase} < 0 \\ 1, & \text{otherwise} \end{cases}
\]

for a minimum phase of interest of \(-\pi\). If the maximum phase of interest is \(+\pi\) the positive phase mask (eq: 2) is
designed to be
\[ f(x) = \begin{cases} \frac{\pi - \phi(x)}{\pi}, & \text{phase > 0} \\ 1, & \text{otherwise} \end{cases} \tag{2} \]
where \( \phi(x) \) is the phase at location \( x \). The triangular phase mask is a combination of the negative and positive phase mask [5], [7].

The benefit of SWI is that it is very sensitive to susceptibility differences caused by variable tissue composition. These requirements for SWI are constituted by venous structures and iron in the brain and make it possible to study or visualize some pathologic conditions like Traumatic Brain Injury (TBI) [8], Multiple Sclerosis (MS) [9], brain tumor [10], etc.

The motivation of this pilot project is to investigate the possibility of visualization and characterization of SBS (Shaken Baby Syndrome). SBS, also called abusive head trauma, is a form of child abuse. It often occurs when caregiver of an infant gets angry and shakes it by the arms, legs, chest or shoulder. This leads to an uncontrolled acceleration of the brain tissue and the cranial bone. The result is a movement of the brain and the cranial dura matter in opposite directions, which causes a laceration of the perpendicular blood vessels. Retinal bleedings and diffuse brain injuries are additional consequences. Our goal in this study is to visualize microscopic hemorrhages, which can be caused by SBS, diffuse axonal injury (DAI) or other forms of brain damage, whose consequences can range from development disorders and hearing, speech or visual defects to death [11], [12]. It would be an advantage to be able to diagnose these injuries by use of MRI for forensic medicine and in clinical applications for early therapy or interventions.

As described above, deoxygenated venous blood has a different susceptibility than surrounding tissue like white or gray matter. Therefore the phase information of blood at the respective location differs from the rest of the brain. In an image postprocessing step we can use this phase information and enhance the contrast of the magnitude image in order to visualize these veins or microscopic hemorrhages in case of venous injuries. It is important to choose an appropriate MR sequence. First we need a fast MR method to avoid deterioration of the image caused by patient movement, which is important in case of clinical applications. Typical methods are gradient echo or spin echo sequences. Both sequences are useful to visualize microscopic hemorrhages, but the gradient echo sequence is faster and more robust with T1/T2-contrast [13], [14]. It is important to get a T2* based image contrast and for that reason to have a long echo time (TE), a long repetition time (TR) and a short flip angle [15]. With these acquisition parameters and the postprocessing steps mentioned above it should be possible to visualize microscopic hemorrhages with very small diameter.

II. MATERIAL AND METHODS

In this project two brain samples from a deceased 51 year old man were used, who committed suicide by shooting himself in the head. The samples are taken from the Forensic Department of the Universitätsklinikum Schleswig-Holstein Lübeck. The time duration between shooting and death is not exactly known. Therefore, the pathophysiological processes that have occurred cannot accurately be described. The pressure from the gun barrel and the non-linear triangle of the ammunition led to damages in the brain and caused diffuse injuries of the blood vessels.

The brain samples are cut from different areas and contain hemorrhages with various sizes. Both are separately stored in little plastic containers filled with phosphate-buffered formalin for fixation and preservation. In Fig. 2 is shown the image of the respective brain sample. The hemorrhages can already be seen as dark spots at the edge of the laterally positioned brain sample. In the brain sample in Fig. 2(b) there can be seen the white matter in the middle and the gray matter in the outer area. In the bottom right corner there is also a large dark spot.

All images were acquired on a Philips Achieva 3.0 T MR system (Philips Medical Systems Nederland BV, DA Best, The Netherlands). For the measurement we used a SENSE Head coil 8. SENSE is an abbreviation for sensitive encoding and represents a form of an image based parallel acquisition technique (PAT). It is a method that enhances the performance of MRI by means of arrays of multiple receiver coils and saves much scan time without reducing the image resolution [16].

To visualize the microscopic hemorrhages we used an MRI protocol which is based on gradient echo sequence to increase the sensitivity to local susceptibility differences. It is a 3D multishot Fast Field Echo sequence to create a T2*-weighted image contrast. The measurement were acquired with different parameter settings. In order to keep a high resolution and a short acquisition time the images were acquired with the parameter settings shown in TABLE I.

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARAMETER SETTINGS OF SUSCEPTIBILITY-WEIGHTED IMAGING.</td>
</tr>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>voxel size AP</td>
</tr>
<tr>
<td>voxel size RL</td>
</tr>
<tr>
<td>voxel size FH</td>
</tr>
<tr>
<td>FOV AP</td>
</tr>
<tr>
<td>FOV RL</td>
</tr>
<tr>
<td>FOV FH</td>
</tr>
<tr>
<td>TR</td>
</tr>
<tr>
<td>TE</td>
</tr>
<tr>
<td>Flip angle</td>
</tr>
</tbody>
</table>
For a strong T2*-weighting the used gradient echo pulse sequence requires a long TE. The main advantage of this sequence, however, is its short TR to provide acquisition speed. Because in conventional gradient echo sequences TE is shorter than TR the application of this technique is limited. Therefore, the method used in this sequence is PRESTO that allows TE to exceed TR by using an echo shifting [17] and makes a rapid T2*-weighted scanning available.

We also acquired T1-weighted images for comparison. We used a T1-weighted fast field echo protocol with short TE and TR. The acquisition parameters are shown in TABLE III.

### TABLE II

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voxel size AP</td>
<td>1 mm</td>
</tr>
<tr>
<td>Voxel size RL</td>
<td>0.72 mm</td>
</tr>
<tr>
<td>Voxel size FH</td>
<td>2.5 mm</td>
</tr>
<tr>
<td>FOV FH</td>
<td>120 mm</td>
</tr>
<tr>
<td>FOV RL</td>
<td>95 mm</td>
</tr>
<tr>
<td>FOV AP</td>
<td>45 mm</td>
</tr>
<tr>
<td>TR</td>
<td>145 ms</td>
</tr>
<tr>
<td>TE</td>
<td>4.6 ms</td>
</tr>
<tr>
<td>Flip angle</td>
<td>10°</td>
</tr>
</tbody>
</table>

The idea was to compare the susceptibility-weighted images with the anatomical images to distinguish between hemorrhages, blood vessels and air bubbles.

### III. RESULTS AND DISCUSSION

As expected, the images of both brain samples show dark spots as in Fig. 2. In Fig. 3 is exemplarily shown a slice of the frontal lobe samples from different views.

![Fig. 3. Susceptibility-weighted images of the hippocampus sample from different views. It contains several dark spots with different sizes. The sample is in a plastic container filled with formalin.](image)

They are distributed irregularly but most of them occur in white matter. They also have different sizes and unsharp contours. None of them have a cylindrical form like a vessel. Therefore all dark spots are probably hemorrhages caused by the gunshot injury. However, there are a number of problems in the interpretation of the images. First, the air-container boundary, which appears dark in the air side of MR image, influences the signal intensity in the samples. This effect can especially be observed in the coronal (left) and sagittal (middle) views of Fig. 3. Second, the edge of the brain sample is hardly distinguishable from the formalin background, if there is a hemorrhage.

This can clearly be seen in Fig. 3, too. This makes the interpretation of the dark spots difficult, whether they are hemorrhages or air bubbles in liquor.

In Fig. 4(a) is shown a susceptibility-weighted image in which many dark spots can be seen. On every view is one dark spot marked with a white arrow. As before these are probably hemorrhages. In order to support this statement we compare Fig. 4(a) with the anatomical images in Fig. 4(b).

![Fig. 4. Comparison between the contrast enhanced susceptibility-weighted images (a) and the T1-weighted images (b) of the hippocampus sample. All white arrows point to the same location in the brain sample.](image)

In the T1-weighted images we expect dark spots at the same locations if they are caused by air bubbles. The resolution of the anatomical images of sagittal and transversal views were not very good, because of the low Food-Head resolution (see TABLE I and TABLE III). However, at the locations marked with white arrows, which point all to the same location in the brain sample, the anatomical images are very bright and show no air bubbles. All other slices in that image and the frontal lobe sample (Fig. 5) show the same circumstances.

![Fig. 5. Comparison between the contrast enhanced susceptibility-weighted images (a) and the T1-weighted images (b) of the frontal lobe sample. All white arrows point to the same location in the brain sample.](image)

A counting of the dark spots has shown that they have variable sizes. Table 2 shows the order of magnitude and the
count of these dark spots.

TABLE III
ORDER OF MAGNITUDE OF THE DARK SPOTS.

<table>
<thead>
<tr>
<th>Volume</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 15 mm³</td>
<td>10 - 15</td>
</tr>
<tr>
<td>15 - 30 mm³</td>
<td>1</td>
</tr>
<tr>
<td>30 - 60 mm³</td>
<td>2</td>
</tr>
<tr>
<td>&gt;60 mm³</td>
<td>1</td>
</tr>
</tbody>
</table>

Due to the spatial proximity of the dark spots to each other and their uneven structure, some of the small dark spots are hardly distinguishable. Whether the volumes of the dark spots represent the exact sizes of the hemorrhages is not conclusively known.

IV. CONCLUSIONS

Contrast enhancement via SWI with T2*-weighted sequences seems to be a successful technique to visualize hemorrhages. From the introduction we know that deoxygenated blood causes significant susceptibility differences. The question is whether visualization of oxygenated blood can be performed with this technique. However, high-resolution SWI is potentially usable for clinical application. In the forensic medicine SWI can guide histology to probable locations of hemorrhages. This can make the histology simpler and can help to optimize the investigation of the samples.

V. ACKNOWLEDGMENT

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REFERENCES

Development and Validation of a Tool for Pulse Wave Velocity Measurements in MRI Phase Contrast Data

A.Timmermeyer1, M. A. Koch1, A.Frydrychowicz2
1Institute of Medical Engineering, Universität zu Lübeck, Lübeck, Germany (anja.timmermeyer@miw.uni-luebeck.de)
2Klinik für Radiologie und Nuklearmedizin, Universitätsklinikum Schleswig-Holstein, Lübeck, Germany

Abstract

The pulse wave velocity (PWV) is the speed at which the pulse wave travels through the arterial vasculature, driven by the periodic motion of the beating heart. PWV serves as an indirect marker of vascular health. The PWV can be determined non-invasively if blood flow in the aorta is measured using flow sensitive MRI. Here, a tool for the extraction of the time-dependent blood flow and vessel length and for the computation of the PWV was developed. The tool's robustness was validated by intra- and interobserver comparison using exemplary patient data. Overall mean PWV was 6.44 m s\(^{-1}\) ± 3.33 m s\(^{-1}\), which included outliers. Results agreed well with expected physiological ranges and with previous studies. Inter- and intraobserver comparison resulted in mean differences of (−0.24 ± 0.83) m s\(^{-1}\) and (−0.17 ± 0.84) m s\(^{-1}\). This small range implies applicability for further testing in a clinical setting and comparison with a reference standard to test for accuracy.

1 Introduction

As a result of the contraction of the heart, the blood pressure rises within the proximal aorta. This pressure propagates through the elastic arterial system and is immediately followed by a decrease of the blood pressure due to the relaxing of the heart. This periodic behaviour creates a pulse wave on every heart beat [1]. The elasticity of the arterial vessel wall influences the speed of the pulse wave (pulse wave velocity, PWV) [2]. Due to degenerative changes like arteriosclerosis, the vessel wall may become stiffer [3], which leads to a higher PWV [4]. Therefore, the PWV is used for the assessment of vascular health and was shown to be predictive, i.e., in assessing stroke risk in hypertensive patients [5]. As the pulse wave moves through the vessel, the blood flow varies due to the changing pressure which leads to a time dependency of the blood flow. The PWV can be calculated if the local blood flow at two different vessel locations is measured with sufficient temporal resolution. By comparing the time-dependent flow of both locations, the time delay, \(\Delta t\), of the pulse wave between the two locations can be determined. With given distance, \(\Delta s\), between those two locations along the vessel, the PWV is given by [6]

\[
\text{PWV} = \frac{\Delta s}{\Delta t}.
\]  

Information about both pulse wave time delay and vessel length can be obtained by catheter based intra-arterial pressure measurements [7], which are invasive measurements and are potentially associated with unwanted complications. A non-invasive method like magnetic resonance imaging (MRI) is therefore preferable for clinical testing [6]. Using cardiac-gated flow sensitive MR imaging with high temporal resolution, the velocity, \(v_{\text{blood}}\), of the blood inside of a vessel at time \(t\) can be obtained. If the cross section, \(A\), of the vessel is known (see image 1), the time-dependent blood flow can be considered as the volumetric flow rate, \(Q\), which is given by the surface integral

\[
Q(t) = \int_A v_{\text{blood}}(x,y,t)dxdy. 
\]

Considering that high blood flow is related to high blood pressure, the time delay of the pulse wave can be found by comparing the blood flow information of both vessel locations (image 2) [8]. The distance between those two points can be obtained using a centerline in the vessel specified on an image covering the full course of the vessel (image 3). However, explicit specification of the vessel border and its centerline is required to estimate the PWV.

In this study, the examiner interactively sets these specifications. To evaluate the user dependence and reliability of this technique in this pilot study, we compared PWV between users (interobserver comparison) as well as repeated PWV measurements of the same user (intraobserver comparison) for various algorithms. The calculations were performed with a Matlab (The MathWorks, Natick, MA, USA, R2011a) based tool developed in-house.

2 Methods

2.1 Human Subjects

Ethical approval was given by the local ethics committee and written informed consent was obtained from all patients. This study includes 7 patients (55 ± 25 years, range: 21-83 years, 3 female, 4 male). Two of these patients were diagnosed with arteriosclerosis and coronary artery disease. For the other five patients myocarditis or pericarditis was suspected while arteriosclerosis and coronary artery disease were ruled out.
### 2.2 MRI
All scans were performed on a clinical 1.5 Tesla MR system (Achieva, Philips Healthcare, Best, The Netherlands) using a 16 elements torso coil array.

To obtain blood flow information, a T1-weighted RF-spoiled FLASH 2D phase contrast (PC) [9] sequence with throughplane velocity encoding and retrospective cardiac gating was acquired at two different parts of the thoracic aorta: ascending aorta (AAO) at the level of the right pulmonary artery and descending aorta (DAO) at the level of the diaphragm. Imaging parameters vary with heart rate, breathhold capabilities, and patient size adapted to 60 acquired time frames distributed within the cardiac cycle. Typical values were: TR: 3.93 ms, TE: 2.43 ms, temporal resolution: 16 ms, flip angle: 15°, FOV: 27x27 cm², Matrix: 224x224, reconstructed to an in-plane voxel-size of 1.21x1.21 mm², slice thickness: 5 mm. Velocity encoding sensitivity \( v_{enc} \) was chosen in the range of 150-180 cm s\(^{-1}\).

A sagittal oblique imaging plane through AAO, DAO and aortic arch was used to create the "candy cane" view using a T2-weighted turbo spin echo sequence (PHASTE) [10].

### 2.3 PWV Calculation
To calculate the PWV, a Matlab based software tool was developed. For volumetric flow rate calculation, a region of interest (ROI) covering the whole vessel lumen has to be chosen interactively by the user in each PC-MRI frame (image 1). Due to motion of the aorta between frames, the size and position of the ROI has to be adapted in each frame. Since PC-MRI provides blood velocities in voxels, equation (2) leads to

\[
Q(t) = \sum_j v_j(t) A_{\text{Voxel}}
\]

where voxels comprise an area of \( A_{\text{Voxel}} \) and \( v_j \) equals the blood velocity in voxel j if the voxel lies within the ROI and zero otherwise.

To calculate the time shift of the pulse wave between two vessel locations, different algorithms have been proposed and evaluated. Here, different characteristics of the time dependent flow curves are compared (image 2). These characteristics are: time to peak (TTP, time to maximal flow), time to foot (TTF, time to the intersection of a linear fit to the rising part of the 20-80 % section of the curve with the zero line), and time of upstroke (TTU, time of maximum of the first time derivative in the rising part of the curve) as described in [8]. Alternatively, the maximum of the cross correlation (xcorr) of both curves provides the time shift [11].

The vessel length between the two vessel locations is determined in the sagittal oblique image containing the entire vessel course (image 3). Since the aorta can elongate with age and can show a tortuous course, the vessel shape has to be taken into account when measuring its length. Therefore, the user selects an individually chosen number of points in the center of the vessel. The centerline is then estimated using spline interpolation between the chosen points as previously described [12]. The distance between the two flow sensitive acquisitions is given by the length of the interpolated centerline.
2.4 Statistical Evaluation

In light of a missing reference standard, the reliability of the tool was to be tested using inter- and intraobserver comparison. The evaluation was performed separately for all four algorithms to determine their respective inter- and intraobserver variability. Results were compared with literature values.

2.4.1 Interobserver Variability

For investigating the interobserver variability, three different users determined the PWV. One of those users was familiar with the PWV determination using the described tool. The other two users performed a test run that is not part of the results. Every user performed a PWV determination on each patient’s data set using all four algorithms.

The results were compared using a Bland-Altman plot [13]: Differences between the results of the user familiar with the software and the grouped results of the other two users were plotted against the mean of the compared results. Mean differences ± 2SD (standard deviation) of the difference were plotted. The mean difference can be used to distinguish between systematic and random errors. If the mean difference equals zero, positive and negative deviations between users compensate each other which indicates random errors. Otherwise, a one-directional shift between results caused by systematic errors (i.e. observer bias) can be assumed.

2.4.2 Intraobserver Variability

One user familiar with the software evaluated each patient’s data set on two different days to investigate the intraobserver variability. The results depending on the evaluation day are statistically compared and visualized in a Bland-Altman plot [13] as described above.

3 Results

A tool was created to allow PWV estimations from 2D PC-MRI data using four different algorithms. Derived PWV values (mean ± SD = 6.44 m s⁻¹ ± 3.33 m s⁻¹, table 1) were in agreement with values established in literature [8] and indicated physiological results for all patients with the exception of two participants which were excluded due to acquisition problems (see below). Inter- and intraobserver comparison indicated that TTU and xcorr were most reliable as they showed the smallest differences between observers.

Table 1 Results of PWV determination and observer bias in m s⁻¹.

<table>
<thead>
<tr>
<th>Algorithm</th>
<th>PWV Mean ± SD</th>
<th>Intraobs. Bias Mean ± SD</th>
<th>Interobs. Bias Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTF</td>
<td>10.58 ± 9.01</td>
<td>-2.18 ± 3.99</td>
<td>-0.33 ± 1.80</td>
</tr>
<tr>
<td>TTP</td>
<td>8.16 ± 6.54</td>
<td>-2.63 ± 5.78</td>
<td>-3.00 ± 5.13</td>
</tr>
<tr>
<td>TTU</td>
<td>6.25 ± 3.21</td>
<td>-0.30 ± 0.39</td>
<td>0.00 ± 0.88</td>
</tr>
<tr>
<td>xcorr</td>
<td>6.02 ± 1.77</td>
<td>-0.12 ± 0.64</td>
<td>0.07 ± 0.34</td>
</tr>
<tr>
<td>all</td>
<td>6.44 ± 3.33</td>
<td>-1.31 ± 3.44</td>
<td>-0.82 ± 2.95</td>
</tr>
</tbody>
</table>

Detailed investigation of data of the excluded participants led to the conclusion that inaccuracies during retrospective ECG-gating of data may have caused incorrect results. This problem could be overcome by using only one acquisition near the aortic arch containing both ascending and descending aorta. In that case, the user can place the two ROIs in the same slice image, which eliminates the risk of a different gating offset of two different acquisitions. However, this might be associated with inaccuracies due to the small distance between the locations used for PWV estimation.

The interobserver comparison using data of 5 different patients and all four algorithms resulted in a mean PWV difference ± 2 SD of –0.82 m s⁻¹ ± 5.9 m s⁻¹ as visualized in image 4. This indicates that the actual difference between two users is likely to be in a range between –6.72 m s⁻¹ and 5.08 m s⁻¹ (limits of agreement).

The Bland-Altman plot in image 5 shows the intraobserver variability. Here, 5 patients were evaluated by one user on two different days using all four algorithms. This resulted in mean difference ± 2 SD of –1.31 m s⁻¹ ± 6.88 m s⁻¹ and limits of agreement of –8.19 m s⁻¹ and 5.57 m s⁻¹. These limits of agreement might be too broad to ensure...
accurate diagnosis. However, a few outliers could be identified in both tests. Since each patient is marked with an individual marker, images 4 and 5 indicate that only PWV values for two patients showed a high difference in both diagrams. The outliers marked with a triangle in both diagrams revealed a higher mean value as well as a higher difference than other points. Re-evaluating the PC-MRI acquisitions of these values, we identified aliasing due to wrongly set $v_{en}$ as a source of error.

If aliasing occurs within the selected ROI, the calculated volumetric flow rate is underestimated. Therefore, the flow curves are affected and the resulting PWV is inaccurate. In this study, the users were advised not to place the ROI in aliasing-affected areas and to use a smaller ROI that does not cover the whole vessel lumen instead. Consequently, the volumetric flow rate is still underestimated because it is calculated considering a smaller area. A possible solution could be an aliasing correcting algorithm applied after a segmentation of the affected area to restore the true velocity.

Further investigation of the outlying data points showed that the data collected from the patient marked with a circle is prone to misinterpretation when examined with the TTP algorithm. This problem is caused by a deformation of the pulse wave in the distal vessel location resulting in a flow curve with two peaks. A second peak could be the consequence of a superposition of the flow curve with its peripheral reflection, a phenomenon caused by increased peripheral vascular resistance. Since both peaks have similar amplitudes in this case, little changes of the ROI’s position may influence which peak is considered the highest. The TTP algorithm is based on a single peak detection and therefore specifically dependent on the temporal resolution and absence of additional peaks. The three other algorithms are less influenced by the double peak condition. This may also be considered the reason why the TTP algorithm shows the highest spread in data in table 1. The TTF algorithm also seems to be affected by the outliers, especially when aliasing occurs. TTU and xcorr appear to be more stable, which is congruent with previously published work [8], [12].

For better evaluation of the data apart from the previously described errors, the limits of agreement were recalculated under exclusion of the outliers. Here, the mean differences ± 2SD were $-0.24 \text{ m s}^{-1} \pm 1.70 \text{ m s}^{-1}$ (inter-observer variability) and $-0.17 \text{ m s}^{-1} \pm 1.68 \text{ m s}^{-1}$ (intra-observer variability). In this evaluation, inter- and intraobserver variability seem to be on the same order of magnitude underlining the importance to choose the optimal algorithm. These results appear to be influenced mostly by random errors which could be reduced by automatic segmentation algorithms unifying the ROI and centerline placements. Manual placements can cause uncertainties and therefore decrease the result’s repeatability, which requires further investigation (e.g. evaluating the influence of ROI size and shape). Future validation of the tool will include comparison of results with invasive PWV measurements (true reference standard) and larger patient/volunteer collectives to test for assumable physiological age and gender dependencies.

4 Conclusion
An effective tool that allows for a simple estimation of the PWV was created and tested for observer bias. The detected bias was most likely caused by acquisition-based shortcomings and random errors that may be overcome by extending the tool to automatic segmentation. Deviations from physiological values were traced back to imaging errors like aliasing. A validation of the tool using intraarterial pressure measurement as a reference standard is planned.

5 References
Endoscopic optical coherence tomography for a handheld laryngoscope

A. Krüger, S. Donner, S. Bleeker, T. Ripken, Laser Zentrum Hannover e.V., Biomedical Optics Department, Hannover, Germany, s.donner@lzh.de

Introduction

Optical coherence tomography (OCT) enables non-contact optical imaging of tissue with a resolution in the ten µm range and a penetration depth of several hundred microns. Therefore it is an effective tool for the investigation of superficial tissue layers.

Methods

To apply this imaging technique in head and neck diagnosis OCT was adapted to a rigid endoscope. For indirect laryngoscopy rapid working distance adjustment was implemented. Motions throughout the examination are caused by movement of the operator. To keep the sample in the imaging depth of the device the motion is tracked and compensated for in the reference arm of the OCT laryngoscope.

Results

Tests were performed on porcine samples to validate the algorithms. The autofocus and motion compensation are important tools to gain OCT images with a handheld laryngoscope in the awake patient.

Conclusion

This work therefore is an important step towards clinical use of OCT to provide optical tissue sections prior to or instead of histology with the risk of scar formation.
Imaging of lung tissue dynamics during continuous artificial ventilation with Optical Coherence Tomography and Intravital Microscopy

C. Schnabel, M. Gaertner, L. Kirsten and E. Koch
Technische Universität Dresden, Faculty of Medicine Carl Gustav Carus, Germany
christian.schnabel@tu-dresden.de


Abstract
Visualization of lung tissue structure and morphology with 3D optical coherence tomography (OCT) and intravital microscopy (IVM) allows new insights into lung function at an alveolar level during artificial ventilation in animal experiments. The detailed knowledge about tissue behaviour during different ventilation conditions and parameters can support the development of new ventilation manoeuvres and treatment strategies for patients depending on artificial ventilation support. For gathering comprehensive information about lung tissue, a more challenging topic is the imaging of the dynamic tissue movement during uninterrupted ventilation. Therefore we developed a continuous measurement mode for Fourier-domain OCT imaging in combination with a tuneable focus lens for IVM. Furthermore, to increase the image quality for OCT we used the concept of total liquid ventilation (TLV). The combination of new dynamic measurement modes for OCT and IVM in combination with the enhanced OCT image quality during TLV allows new insights into lung tissue behaviour within three dimensions.

1 Introduction
Artificial ventilation and ventilation support is the most important procedure for patients with severe lung diseases in case of emergency and for long-term treatments. To prevent the sensitive lung tissue from ventilator induced injury and to perform artificial ventilation as gentle as possible the choice of appropriated ventilation strategy and parameters is essential. However, a proper decision can only be taken based on detailed knowledge of lung tissue behaviour during artificial ventilation, but this knowledge often depends on the expertise of the attending physician. Optical coherence tomography (OCT) is a suitable tool to investigate lung tissue morphology during artificial ventilation in animal experiments on an alveolar level [1]. The visualization of dynamic processes is a more challenging task which is currently solved by using high-speed OCT systems based on Fourier-domain mode locked laser systems for example with A-scan rates higher than 60 kHz [2]. In this work we present a continuous OCT scanning mode with a conventional system (A-scan rate 12 kHz) triggered by our costume made ventilator for artificial ventilation. Simultaneously acquired intravital microscopy (IVM) images show the tissue movement with the aid of an adjustable focus lens to track the lung surface. Furthermore, to increase OCT image quality the concept of total liquid ventilation (TLV) was used [3].

2 Methods

2.1 Imaging system
The developed imaging system combines Fourier-domain OCT and IVM simultaneously by using the same beam path [1]. It allows the acquisition of 2D IVM images and 3D OCT data. The superluminescence diode for OCT is centered at 845 nm with a full width at half maximum of 50 nm. The system provides a resolution of 10 µm and 7 µm axial and lateral in air, respectively. The A-scan rate is 12 kHz, which allows the acquisition of a cross section (320 x 512 Pixel, 1.28 x 2.56 mm) within 29 ms.
A 2 megapixel CMOS camera (SMX-M72, Sumix, USA) in combination with an adjustable focus lens (EL-10-30, Optotune AG, Switzerland) is used for IVM. The electrically tunable lens is current driven via a 20 kHz pulse width modulation signal and has a response time of 20 ms, which, in addition with the exposure time for image acquisition, limits the IVM frame rate to 20 frames per second. In combination with the four-fold magnification optic a focal shift of 5 mm can be readjusted.

2.2 Image acquisition and postprocessing
To record a conventional 3D OCT stack, two galvanometer scanners (x and y direction) are necessary. For the measurement of a single 3D stack, the x scanner deflects the near-infrared light beam in one direction to acquire cross sectional B-scans. Adjacent B-scans can be measured by tilting the second scanner resulting in 3D OCT data.
images. For continuous image acquisition to visualize dynamic processes, both scanners were controlled separately. During one ventilation cycle B-scans were grabbed at the same position showing different states of the movement with a time gap of 29 ms (compare to 2.1). After the ventilation cycle, a trigger signal from the ventilator is used to set the second scanner one step further and B-scans were acquired again continuously at the same position. Each B-scan is consecutively numbered and the acquisition time as well as the point in time at which a trigger occurs is saved to a data log file. After all B-scans are acquired, they are resorted by using their indices and the trigger time to form the different 3D OCT stacks each showing one phase of the sample movement. That means that the resulting 4D OCT stack shows the average tissue movement of one breath measured over several adjacent ventilation cycles.

As an example: A rat lung is pressure-controlled ventilated with 60 breaths per minute (bpm) from 2 to 11 mbar. Each OCT stack consists of 320 cross sections. Within one second of each ventilation cycle 34 B-scans were acquired (cycle time/acquisition time). The total measurement is done after 320 ventilation cycles (equal to 5.3 minutes measurement duration). During the rearrangement, the first B-scan of the first ventilation cycle and the first B-scan of the second ventilation cycle and so on up to cycle 320 were put together to form the first 3D OCT stack (compare to image 1). The resulting 4D OCT data set consists of 34 different 3D stacks showing the tissue movement in steps with a temporal resolution of 29 ms.

To reduce measurement duration for lower ventilation rates (12 bpm shown in image 1) during total liquid ventilation, the scanning mode acquires small volumes, consisting of several cross sections (typically 4 to 8), instead of single B-scans at the same position. It depends on the pressure difference from end-inspiration to end-expiration and the breathing frequency how many cross sections can be taken for the small volume scans. In our case, during total liquid ventilation with 12 bpm and a pressure difference of 9 mbar the maximum number was set to 4 B-scans, so the pressure difference within this small volume is kept marginal to prevent disturbing motion artifacts.

The adjustment of the focal plane for IVM is done by tracking the lung surface movement. Therefore, the travel range is extracted from the OCT cross sections by measuring the difference of the maximum intensity index, which corresponds to the lung surface in two successive B-scans. By using a calibration curve obtained from preliminary test, the current for the new focus position is set and an image is grabbed. All images were saved as a video stream with the timestamp of their acquisition.

2.3 Total liquid ventilation

All experiments were performed on rats in accordance with the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 7th edition, 1996). The setup of the used ventilator prototype is shown in image 2 and described in detail by Schnabel et al. [3]. The supply unit maintains temperature and oxygen saturation of the used breathing medium. Ventilation itself is performed by two independent custom-made syringe pumps and four pinch valves. The control unit visualizes ventilation settings and measured parameters such as pressure and volume and allows online user interactions. The device provides a 5 V TTL trigger output at the beginning of each ventilation cycle, which is used for the continuous OCT scanning mode. Conventional ventilation is performed by using air whereas perfluorodecaline (C_{10}F_{18}; F2 Chemicals Ltd) is used for total liquid ventilation because it has nearly the same refractive index as water and allows refractive index matching to increase OCT image quality by reducing scattering loss of the near-infrared light [3].
### Experimental setup and ventilator device.

The ventilator prototype (yellow background) is used in a pressure controlled manner for continuous artificial ventilation on rats. ECG and arterial blood pressure is measured to obtain vital parameters of the animal. To trigger continuous OCT data acquisition, a 5 V TTL signal from the ventilator device is generated at the beginning of each ventilation cycle. Other components: 1..syringe pump; 2..manual valve; 3..stepper motor; 4..pressure transducer; 5..operation table; 6..peristaltic pump; 7..membrane oxygenator; 8..CO2 absorbents; 9..gas pump.

### Results

The continuous OCT scanning mode and the surface tracking algorithm with the adjustable focus lens for IVM were tested at a loudspeaker with sinusoidal signal from a function generator up to 1.5 Hz (data not shown). For the experimental measurements during total liquid ventilated rats, the breathing frequency was set to 12 breaths per minute to prevent lung tissue from induced damage due to the incompressibility of the liquid in a pressure controlled manner from 2 to 11 mbar. A selection of the acquired OCT and IVM images is shown in image 3. The first row illustrates an IVM image sequence without adaptive focus lens. Only image (B) is in the focal plane at end-inspiratory pressure of 11 mbar. Tissue structures become unsharp and show less contrast during ventilation cycle. The second row shows an image sequence with adaptive focus lens. Due to the tracking algorithm, all images offer same sharpness and contrast and can be used for 2D analysis of lung dynamics and structural changes during ventilation. The third row is an 4D OCT image sequence showing tissue changes during one ventilation cycle from 2 mbar to 11 mbar. All volume stacks were aligned to the same level of height and the pleura was hidden. With the continuous scanning mode 42 OCT volume stacks can be acquired showing an 1.3 x 1.3 mm² area during a ventilation cycle of 5 sec with a pressure step resolution of 0.26 mbar. The measurement duration was 6.7 minutes. Both imaging techniques show the same region of interest indicated by distinctive structures (blue and yellow arrows).

### Discussion

To investigate dynamic processes like lung tissue movement during uninterrupted artificial ventilation with OCT and IVM one needs a suitable image acquisition technique. We showed that a continuous OCT scanning mode for a conventional OCT system with an A-scan rate of 12 kHz in combination with an electrically tunable lens for IVM enables the multimodal visualization of the lung tissue movement during ventilation without any disturbing motion artifacts. The presented experimental setup provides a high temporal resolution for both imaging modalities. Certainly, the OCT scanning mode needs an external trigger signal, in our case generated by the ventilator device, to allow the correct realignment of the continuously acquired B-scans. Furthermore, the measurement duration of several minutes prones this technique to motion artifacts due to different movements of the tissue from one cycle to the next. As long as no effects occur disturbing the regular movement of a sample, as it is the case during artificial ventilation, this algorithm is a suitable tool to investigate high dynamic processes without the need of further or expensive equipment.
5 References


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High resolution frequency domain optical coherence tomography in the Vis-NIR spectral range

Felix Köttig1*, Peter Cimalla1, Maria Gärtner1, Madalena Carido2, Kai Postel2, Marius Ader2, Mike Karl2,3, Edmund Koch1
1Technische Universität Dresden, Faculty of Medicine Carl Gustav Carus, Clinical Sensing and Monitoring, Germany, *f.koettig@gmail.com
2Center for Regenerative Therapies Dresden, Germany
3German Center for Neurodegenerative Diseases, Dresden, Germany


Abstract

In spatially encoded frequency domain optical coherence tomography (spectrometer based FD OCT), the axial resolution depends on the effective spectral range of the detected interference spectrum. We present high resolution FD OCT in the Vis-NIR spectral range of nearly 400 nm spectral bandwidth centered around 734 nm. An axial resolution in air of 1.3 µm and a sensitivity of -103.2 dB at 12.8 kHz A-scan rate were measured. A fiber-coupled scanner yields a telecentric scan pattern and a measured lateral resolution of 7 µm. This scanner was modified for in vivo imaging of the mouse eye and typical results are presented.

1 Introduction

In spatially encoded frequency domain optical coherence tomography (FD OCT), a low-coherent light source creates distinct spectral interference patterns in a two-beam interferometer, which result from optical path differences between the reference arm and sample arm. The minimal observable optical path difference and thus the axial resolution of the OCT system are defined by the coherence length of the light source, which is inverse proportional to its spectral bandwidth, whereas the measurement range depends on the resolution of the spectrometer. Hence, high resolution FD OCT requires a large spectral bandwidth of the light source and demands for a broadband high resolution spectrometer to sample the interference patterns.

We present high resolution FD OCT in the Vis-NIR spectral range featuring a SuperK Versa Super Continuum Source (Koheras A/S, Denmark) and a broadband high resolution astigmatism corrected spectrometer in Czerny-Turner configuration adapted from Lee et al. [1]. The spectral range on the detector (Basler spl4096-140km, Basler, Germany; two lines with 4096 pixels each, 10 µm x 10 µm pixel size) was measured to be 541 nm – 927 nm, resulting in an axial resolution of 1.3 µm in air and a maximal measurement depth in air of 1.3 mm. The spectrometer induced sensitivity roll off over 90 % of the measurement range (65 µm distance to both zero delay and Nyquist limit) was measured to be -10.8 dB. At an A-scan rate of 12.8 kHz, the sensitivity was measured to be -103.2 dB.

2 Spectrometer Design

For the desired Vis-NIR spectral range of 540 nm – 940 nm, a spectrometer design with spherical mirrors in Czerny-Turner configuration is favorable since lens based spectrometers suffer from severe chromatic aberrations within this broadband spectral range. A spectrometer in Czerny-Turner configuration is built with two spherical mirrors under off-axis illumination and a plane diffraction grating. A spherical mirror under off-axis illumination introduces spherical aberrations, coma, astigmatism, field curvature and distortion. However, only the first three aberrations are dominant and there exists a geometric condition under which coma can be corrected, the Shafer equation [2]. Austin et al. presented that, under divergent illumination and certain conditions for the distance from the diffraction grating to the focusing mirror and the detector angle, astigmatism is corrected to first order in wavelength [3]. Still, this is not sufficient for a broadband high resolution OCT spectrometer. Therefore, Lee et al. presented a broadband astigmatism corrected spectrometer in Czerny-Turner configuration for the 600 nm – 1000 nm spectral range with an additional tilted cylinder lens [1]. This design has been adapted to meet our requirements for the 540 nm – 940 nm Vis-NIR spectral range.
In Czerny-Turner configuration, the difference in focal positions of the tangential and sagittal imaging planes is calculated as follow [4]:

\[ \Delta z = \frac{r_1}{2} \sin \alpha_1 \tan \alpha_1 + \frac{r_2}{2} \sin \alpha_2 \tan \alpha_2, \]

with the radius of curvature of the collimating and focusing spherical mirror, \( r_1 \) and \( r_2 \), respectively, and the incidence angles to the collimating and focusing spherical mirror, \( \alpha_1 \) and \( \alpha_2 \), respectively. The incidence angle to the focusing spherical mirror \( \alpha_2 \) varies with wavelength according to the grating equation:

\[ \sin \theta_i + \sin \theta_o = g \lambda, \]

with the incidence angle to the grating and the diffraction angle, \( \theta_i \) and \( \theta_o \), respectively, and the grating constant and wavelength, \( g \) and \( \lambda \), respectively. Note that only the first diffraction order is considered. A cylinder lens with focal length \( f \) is placed before the detector and introduces a sagittal focus shift \( z_s - z'_s \) as follows:

\[ \frac{1}{f} = \frac{1}{z_s} + \frac{1}{z_s}, \quad \text{(thin lens formula),} \]

\[ z_s - z'_s = z_s - \frac{f z_s}{f + z_s}. \]

The cylinder lens also introduces a tangential focus shift \( z'_t - z_t \) as follows:

\[ z'_t - z_t = \frac{n-1}{n} t \quad \text{(plane parallel plate),} \]

with the refractive index \( n \) and center thickness of the cylinder lens \( t \). The astigmatism is corrected when the astigmatic focal difference inherent in the Czerny-Turner configuration equals the focal difference introduced by the cylinder lens:

\[ \Delta z = z_s - z'_s + z'_t - z_t. \]

As presented by Lee et al. [1], this condition can be fulfilled to first order in wavelength and thereby effectively corrects astigmatism over a broad spectral range.

3 OCT System Design

The spectrometer based FD OCT system has been adapted from Cimalla et al. [5], and the short wavelength band spectrometer has been replaced with the Vis-NIR spectrometer.

Figure 2 OCT system with light source spectral shaping (not shown, for details see Cimalla et al. [5]), applicator with Michelson interferometer and Vis-NIR spectrometer. The dichroic mirror (DM) separates a second band in the NIR spectral range with 300 nm bandwidth centered around 1250 nm, which is currently not used for imaging. FC1, FC2 … fiber collimator, BS1, BS2 … beam splitter, RL … reference arm lens, RM … reference arm mirror, GSX, GSY … galvanometer scanner in x- and y-direction, DFM … deflection mirror, SL … scan lens, TL … telescope lens, PH … pinhole, DM … dichroic mirror, CM … collimating mirror, DG … diffraction grating, FM … focusing mirror, CYL … cylinder lens, DET … detector.
In FD OCT, the inverse Fourier transform requires interference patterns sampled equidistant in wavenumber to yield a Fourier transform limited peak width. However, the presented spectrometer samples interference patterns (approximately) linear in wavelength. Resampling to equidistant samples in wavenumber (k-resampling) is performed with a spectrometer calibration that was measured using the method presented by Witte et al. [6]. A residual dispersion mismatch between the reference arm and sample arm of the interferometer causes peak broadening after the inverse Fourier transform. Within the same procedure as was used to measure the spectrometer calibration, a residual dispersion mismatch of 30 rad was measured, which is numerically corrected before the inverse Fourier transform of each A-scan as presented by Köttig et al. [7].

4 Performance Measures

In OCT, axial and lateral resolution are independent. While the axial resolution depends on the light source characteristics and the sampling of the spectral interference patterns, the lateral resolution depends on the spectral range and the scanner optics. A fiber-coupled scanner emits an approximately Gaussian beam. In our setup, this beam is deflected by a pair of galvanometer scanners in x- and y-direction, which are approximately placed in the pivot point of the scan lens and yield a telecentric scan pattern. The lateral resolution was measured to be 7 µm (FWHM) using a nanoparticle target with Fe₂O₃ spheres (mean diameter of 400 nm) in polyurethane, that was generously provided by Peter Tomlins. A lateral Gaussian fit was used to measure the (depth, respectively focal, dependent) lateral resolution [8]. The axial resolution, the spectrometer induced sensitivity roll off and the sensitivity were measured within one setup: a glass plate was placed at the sample arm of the interferometer and the length of the reference arm was altered to cover the whole measurement depth in steps of approximately 65 µm. The acquired A-scans are shown in Fig. 3. From the A-scans shown in Fig. 3, the axial resolution is derived:

$$\Delta z_{\min} = \frac{\pi}{n \Delta k_{\text{eff}}},$$

(7)

with the sample refractive index $n$ and the effective wavenumber range on the detector:

$$\Delta k_{\text{eff}} = \frac{\Delta k}{w_{-6 \text{ dB}}}$$

(8)

where the -6 dB width $w_{-6 \text{ dB}}$ corresponds to the FWHM definition. To reduce spectral leakage, a Hann window is applied before the inverse Fourier transform of an A-scan, which ideally leads to a -6 dB width of two Fourier bins, i.e. $w_{-6 \text{ dB}} = 2$.

**Figure 3** A-scans acquired from a defocused glass plate (-14 dB reflectivity plus -23.7 dB attenuation by defocusing) and resulting spectrometer induced sensitivity roll off versus measurement depth. Over 90 % of the measurement depth range (65 µm distance to both zero delay and Nyquist limit), the spectrometer induced sensitivity roll off was measured to be -10.8 dB. The grating based spectrometer samples interference patterns (approximately) linear in wavelength, which results in partial aliasing of a portion of the spectrum before the actual Nyquist limit. This effect starts at measurement depths exceeding approximately 50 % of the maximum measurement depth.

**Figure 4** Measured axial resolution in air, derived from the A-scans shown in Fig. 3. Due to partial aliasing of a portion of the spectrum, the axial resolution degrades ($w_{-6 \text{ dB}} > 2$) with increasing measurement depth from 1.3 µm at 65 µm distance to zero delay, to 1.8 µm at 65 µm distance to the end of the depth measurement range (Nyquist limit).
5 In Vivo Mouse Eye Retinal Imaging

In vivo mouse eye retinal imaging was performed with a fiber-coupled ophthalmic scanning unit for small animal models adapted from Cimalla et al. [9]. In the current set-up, the commercially available fiber collimator with an aspheric lens was replaced by a custom-made fiber collimator with a plano-convex lens and a Hastings triplet. The OCT sample beam power at the distal end of the scanning unit, i.e. on the mouse’s cornea, was measured to be approximately 1 mW, which is sufficiently low to avoid light damage or phototoxicity effects on the retina. Typical results are shown in Fig. 5 and Fig. 6.

![Figure 5](image1.png) Mouse eye in vivo retinal imaging. Average of five B-scans with 1000 A-scans each, acquired at 12.8 kHz A-scan rate. The image is cropped in depth-direction to 43 % of the maximum measurement depth.

![Figure 6](image2.png) Mouse eye in vivo fundus imaging by depth-projection (z-projection) of 480 B-scan with 1000 A-scans each, acquired at 12.8 kHz A-scan rate.

6 Conclusion

We presented high resolution FD OCT in the Vis-NIR spectral range from 541 nm – 927 nm using a high resolution broadband astigmatism correction spectrometer in Czerny-Turner configuration. With an axial resolution in air of 1.3 µm, sub-micron axial resolution in tissue is in reach. A spectrometer induced sensitivity roll off of -10.8 dB over 90 % of the measurement range and a sensitivity of -103.2 dB at 12.8 kHz A-scan rate prove that the presented spectrometer design is highly suitable for high resolution FD OCT with a large spectral bandwidth.

7 References

Doppler optical coherence tomography for the investigation of the human tympanic membrane ex vivo

J. Walther*1,2, A. Burkhardt*2, L. Kirsten2, J. Golde2, T. Stoppe3, M. Kemper3, M. Bornitz3, T. Zahnert3, E. Koch2
1Technische Universität Dresden, Medizinische Fakultät Carl Gustav Carus, Medical Physics and Biomedical Engineering, Dresden, Germany, julia.walther@mailbox.tu-dresden.de
2Technische Universität Dresden, Medizinische Fakultät, Clinical Sensing and Monitoring, Dresden, Germany
3Technische Universität Dresden, Medizinische Fakultät, Clinic of Otorhinolaryngology, Dresden, Germany
* both authors contributed equally to this paper

Abstract

Optical coherence tomography (OCT) in combination with Doppler analysis (DOCT) is used to image the vibration response of the human tympanic membrane (TM). Free-field sound excitation was applied repeatedly in an acoustic frequency range of 0.4 to 5.1 kHz. In this study, two ex vivo TM samples were used – one non-pathological and one thickened prior around the manubrium of malleus. In spite of the minor oscillation amplitude of the pathological TM, comparable vibration modes can be identified even though occurring at lower acoustic excitation frequencies.

1 Introduction

Conductive hearing loss is typically caused by functional disorders of the tympanic membrane and ossicular chain. Well established diagnostic methods rely solely on ear microscopy of the tympanic membrane and audiometric measurements specially of the sound conduction apparatus. However, so far there is no clinical routine instrument to separately determine the acousto-mechanical properties of the tympanic membrane. Optical coherence tomography (OCT), a high resolution non-invasive imaging technique for the three-dimensional visualization of structure and function of biological tissue is able to comply with these demands. Combining OCT imaging and Doppler analysis, the three-dimensional structural information can furthermore be extended with information on the vibrational properties of the tympanic membrane. As there is a lack of appropriate diagnostic instruments, OCT might become significant for the diagnosis of otogenic diseases such as tympanosclerosis, otosclerosis as well as acute and chronic otitis media, and the quality of coupled prostheses.

1.1 Tympanic membrane diagnostics

Otoscopes and ear microscopes are well established tools in otorhinolaryngology for diagnostics of diseases of the tympanic membrane. They allow for two-dimensional top-view visualization of the tympanic membrane. However, typically only well trained and experienced eyes can conclude in a diagnosis based on these methods. Otoscopy is faster and cheaper compared to an ear microscope but does not provide the high resolution of a microscopic examination. In general, both methods are neither invasive nor harmful but they do not allow for three-dimensional imaging of the tympanic membrane. For functional diagnosis of the middle ear, there are well established and new methods of audiometry, such as tympanometry, laser-doppler-vibrometry (LDV) and holography, applied in clinical and research examinations. Comparing the audiometric signals of air and bone conduction (air-bone gap), disorders of the middle ear (conductive hearing loss) can in principle be detected. However, there is no clear differentiation between different pathologies. The method of tympanometry can only reliably diagnose a functional disorder of the Eustachian tube and a middle ear effusion (cf. glue ear). Vibrational examination of the tympanic membrane (e.g. at the coupling of the umbo) using LDV or holography can diagnose different pathological changes of the ossicle [1,2]. However, LDV only gathers information of a spot on the tympanic membrane, which limits the sensitivity of this method due to a typical location-dependency of the signal. This disadvantage can only be improved by scanning LDV measuring multiple spots or by holographic measurements, which are technically challenging, very time consuming and thus costly for clinical routine.

1.2 OCT at the tympanic membrane

Optical coherence tomography (OCT) is a non-invasive, contactless and high resolution (< 10 µm) imaging technique for the three-dimensional investigation of biological tissue. In the last years, different studies of the structure of the tympanic membrane have been conducted using OCT. The clinical benefit of OCT for the in vivo examination of the mucous membrane of the tympanic cavity for the detection of cholesteatoma [3] and for the distance measurement of middle ear prostheses [4] has been pointed out. Using laser interferometry and two-dimensional OCT otoscopy, in vivo studies for the detection of bacterial layers behind the tympanic membrane due to inflammation of the middle ear have been presented [5]. Regarding functional examinations of the tympanic membrane, the depth resolved visualization of the vibration of the ossicle ex vivo using OCT at a frequency of 500 Hz has been reported [6].
2 Methods

2.1 Optical coherence tomography

This study was performed with an optical frequency domain imaging (OFDI) setup developed in our work group [7]. There, a broadband tunable lightsource with a center wavelength of 1300 nm, a scanner head based on a modified Michelson interferometer and a detection unit is used in a fiber-coupled configuration. By the use of a wavelength tunable lightsource, where the spectrum is temporally encoded, a spectrometer in the detection arm of the OCT system is thus not necessary. The light source is a Fourier domain mode locked (FDML) laser [7], which is a ring laser with a resonator of a few kilometer long optical fiber. The wavelength in the fiber ring is swept sinusoidally by an integrated Fabry Perot filter. The developed OFDI system provides a center wavelength of 1300 nm and a wavelength range of 120 nm. As usual for OFDI systems, the interferometric signal as a function of the wavelength is detected in time. After spectral shaping and conversion to equidistant in wave number, a Fast Fourier transformation (FFT) is used to determine the amplitude of the backscattered light of the sample as a function of depth. The resulting depth scan is the so-called A-scan. By scanning the sample beam laterally over the sample surface, two- and three-dimensional imaging is achieved. The FDML OCT system of our work group provides A-scan rates of 60 kHz. The whole setup of the laser and the OCT detection unit is fiber-coupled. Fast OFDI systems and Doppler imaging without signal damping and nonlinear Doppler signal [8] are favorable for imaging moving samples. Furthermore, due to the high depth scan rate, the system is less sensitive for image artifacts caused by sample movements making it well suited for in vivo applications.

Besides structural imaging, OCT offers the ability to resolve even marginal movements of the sample. This method relies on phase information of the backscattered light and the resulting Doppler shifts caused by moving structures, which is known as Doppler OCT (DOCT). OCT is therefore suitable to simultaneous morphological and functional imaging of the tympanic membrane. A feasibility study of our group proved the applicability of a FDML-based OCT system with DOCT extension for visualizing the movement of the tympanic membrane ex vivo [9].

2.2 Measuring principle

The measuring principle developed uses the stimulation of the tympanic membrane (TM) by a loudspeaker (BETA-8, Eminence Speaker LLC) in the free field configuration using a customized soundproofed chamber. There, the TM specimen was positioned on a three-axis translation stage in combination with a two-axis rotation stage for the parallel alignment relative to the fiber-coupled scanner head performing telecentric scanning. Because the positioning unit of the TM preparation and the scanner head is arranged within the soundproofed chamber, the essential sound-reflecting surfaces were covered with acoustic foam plastic to damp unwanted reflections of the incident sound field. The sound excitation is geometrically realized by a freely suspended loudspeaker with an angle of 30° relative to the TM and along the manubrium of malleus towards the umbo. The loudspeaker was driven with a frequency chirp in the range of 0.4 to 5.1 kHz. The sound pressure level amounts to 100 dB measured at the TM. The oscillating movements of the TM is measured by Doppler OCT. Simultaneously, the applied sound pressure was detected as reference signal by a probe tubemicrophone (ER-7C, Etymotic Research, Elk Grove Village, USA), placed above the border of the TM to secure the telecentric 3D OCT imaging by the scanner head. The algorithm to determine the oscillatory movement of the TM encoded in the measured Doppler signal is described in detail by a previous publication of our group [9]. Transfer functions are measured on a grid of 25x25 scan points to reconstruct the oscillation pattern of the TM for different acoustic frequencies in the range of 0.4 to 5.1 kHz. A complete determination of the vibrations at 625 points took only 5.3 s, which demonstrates the in vivo capability of this method.

3 Results and Discussion

In contrast to the presented OCT methodology by using a normal TM specimen [9], we exemplarily present results of the comparison of a healthy left TM to a pathologically altered atrophic right TM with a mucotympanum in this study. Image 1 and 2 show the structure of both specimen by an average intensity projection over depth (z-projection) and cross-sectional imaging along the manubrium of malleus (MM). Because in 1(a) the TM of a right and in (b) of a left ear is used, anterior and posterior parts are inverted. Additionally, one can see a strong tissue buildup already in the en face view in 1(b) in comparison to (a). In the pathologically normal left TM in 2(a), the cone shape of the membrane and typical regions like the manubrium of malleus (MM) and the umbo are clearly visible. The examined normal TM was approximately 100 µm in thickness and had a nearly oval shape.

**Image 1** En face OCT image (z-projection) of the two exemplary samples used (a) without pathology and (b) showing a thickening of the tympanic membrane (TM). Description of the regions on the TM: as - anterior superior, ai - anterior inferior, ps - posterior superior, pi - posterior inferior, asterisk - manubrium of malleus (MM)
In contrast, the typical structural behavior is hardly identifiable in the pathologically modified right TM in 2(b) showing a strong thickening of about 400 µm. There, the surface appeared uneven with sedimentation upon it and the exact position of the MM was difficult to determine.

Image 2 Cross-sectional image along the manubrium of malleus (asterisk) showing the umbo as deepest point of the TM of the normal preparation (a) and the pathologically altered atrophic one (b). Scale bars correspond to 1 mm.

The oscillation pattern of both TM preparations at different acoustic frequencies were determined by the imaging protocol resulting in the spatially resolved frequency response functions at the lattice points covering the entire TM. Image 3 shows three significant oscillation patterns of the physiologically normal TM (cf. Image 1(a),2(a)) at different acoustic frequencies. There, the instantaneous displacement with the phase of highest positive amplitude is presented. In the upper right part of each image, the average intensity projection over depth is presented with added mask, shown overlayed in red, to exclude Doppler information of negligible regions as the bony parts. At an acoustic frequency of 663 Hz, the entire TM vibrated in phase, where the largest deflection can be identified at the anterior superior part. Going to higher excitation frequencies, we additionally observed travelling waves besides stationary vibration patterns. A wave propagating towards the pars flaccida (PF) becomes most apparent at 1725 Hz (not shown in Image 2). In literature, PT is described as a balancing point, which prevents the pars tensa (PT) from excessive displacements under large pressure in the middle ear [10]. Considering the coexistent stationary wave at 1725 Hz, one can identify a phase delay between the anterior and the posterior part. At an acoustic frequency of 2123 Hz, the TM moves in phase again with exception of the umbo showing only a small, inversely phased displacement. At higher excitation frequencies, several waves superimpose and different areas move out of phase. A significant pattern becomes apparent at 5175 Hz showing a situation prior the circular oscillation pattern expected at a slightly higher frequency above the upper frequency limit of the speaker, where many propagating motions moved from outside towards the MM. The behaviour of the normal TM preparation is comparable to the results of previous studies at healthy TMs [6].

In comparison to the results of the functional measurement at the normal TM, three significant vibration patterns were also identified for the pathologically altered specimen.

Image 3 Displacements for three characteristic frequencies showing significant oscillating patterns of the normal left TM. The scale of the patterns vary between each plot, because of a variation of the acoustic pressure in the order of one magnitude over the entire frequency range.

Since the thickened TM originates from a right ear, the generally highly vibrating anterior part is pointing forward in the plot in Image 4 compared to Image 3. Generally for the entire acoustic frequency range, the Doppler amplitude is smaller by a factor of 2.5 compared to the normal TM. At 663 Hz, the stationary fundamental oscillation of the whole TM becomes apparent, where the anterior and pos-
terior part vibrate in phase. Contrary to the normal TM, a strong phase shift of about 45° between anterior and posterior region occurs already in the stationary oscillation at a low excitation frequency of 929 Hz (not shown). Additionally, a small propagating wave towards the PF appears and becomes more distinctive up to 1725 Hz. At higher frequencies, this wave becomes a circular oscillation. A beginning circular vibration pattern with a small travelling wave towards the PF overlayed arises at 2123 Hz and is completely developed at 3583 Hz.

Generally for all excitation frequencies applied to the pathological atrophic TM, only regions with small thickening at the border area of the TM show distinct oscillations and can be used to identify vibrational modes, whereas parts with strong tissue buildup prior around the MM exhibit almost no movement. In a direct comparison of the two exemplary preparations, first one for a TM with normal behavior and second one with pathological thickening, it becomes obvious that the vibration modes of the TM thickened around the MM as presented in Image 2(b) are occurring already at lower acoustic excitation frequencies, which is probably caused by the additional mass.

4 Summary and Conclusion

In this study, imaging of structure and oscillation behaviour of two specimen of human TM ex vivo by means of OCT and Doppler OCT (DOCT) were presented. Here, the vibration characteristics of a normal TM to a pathological thickened one as a response to a free field excitation by a mid-range loudspeaker in an acoustic frequency range of 0.4 to 5.1 kHz were compared. As a result, both examined samples show comparable vibration pattern whereas the atrophic TM with tissue thickening and mucotympanum has a lower oscillation amplitude. Furthermore, the vibration modes of the thickened TM occur already at lower acoustic frequencies compared to the healthy one. For future research, further pathologies of ex vivo preparations should be examined with DOCT, primarily to gain and improve knowledge on the specific vibration responses of the entire TM and further differentiate emerging diseases and pathologies at an early stage. With this, adapting the system to an endoscopic configuration for the clinical in vivo application will be a promising diagnostic tool in future.

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Dispersion Encoded Full Range Fourier Domain Optical Coherence Tomography for Image-Guidance of Fs-Laser Lens Surgery

B. Matthias¹, T. Ripken¹, A. Krüger¹
¹Laser Zentrum Hannover e.V. (Biomedical Optics Department), Hannover, Germany, e-Mail: b.matthias@lzh.de

Abstract

Fs-laser surgery is an emerging technique for cutting of the crystalline lens, e.g. in assisting cataract surgery and for presbyopia treatment through lentotomy. An intraoperative depth resolved imaging is required for precise targeting the nucleus and cortex while keeping of safety distance to the capsule. Spectrometer based Fourier domain optical coherence tomography (OCT) at 840 nm is customized for a 1040 nm fs-laser surgical system in order to guide focus positioning in the anterior eye. The OCT sample beam and the fs-laser beam share the same scanner and focusing optics. Conventionally, FD-OCT images suffer from disturbing mirror artifacts due to an complex ambiguity processing the real-valued measured spectral signal. We make use of the dispersion encoded full range (DEFR) algorithm to suppress these complex conjugate artifacts exploiting a dispersion mismatch between sample and reference arm and thereby increase the usable imaging range. The C++ implemented processing and display is performed in real time on the graphics processing unit. The developed spectrometer provides a single-sided measurement range in air of 11.96 mm at average axial resolution of 14.6 µm. The -3dB-limit of the spectrometer is 2 mm and sensitivity decreases further with about -3.3dB/mm. By means of DEFR mirror artifacts in the OCT images are suppressed and imaging the whole anterior segment of porcine eye is demonstrated. Based on the obtained image information subsequent surgical procedures can be defined. Utilizing DEFR technique reduces complexity of FD-OCT-guided fs-laser surgery systems and increases system stability. Moving and error-prone parts for artifact suppression and image depth extension are unnecessary. Costly hardware, such as phase shifting elements, can be spared. Real-time visualization is a critical characteristic in image-guided laser surgery and provides competitive advantages in the market.

1 Introduction

Ophthalmic fs-laser surgery is clinically established in the field of corneal flap cutting during femtoLASIK (laser in situ keratomileusis) [1,2]. More recent developments have made the crystalline lens accessible as a target for fs-laser surgery. It is increasingly used for cutting of the crystalline lens assisting cataract surgery [3] and has potential for presbyopia treatment through lentotomy [4]. Different from corneal flap cutting, a reference plate can not be brought into direct contact to the crystalline lens. Moreover, interindividual anatomical differences lead to variations of the lens position and its geometry. Accordingly, fs-laser surgery on the lens requires the integration of depth resolved imaging for targeting desired internal structures while keeping safety zones to the capsule. Optical coherence tomography (OCT) [5] has been originally introduced as such a measurement technique for intraocular distances and has evolved into a versatile diagnostic ophthalmic imaging tool.

Fourier domain OCT (FD-OCT) provides crucial advantages with respect to imaging speed and signal-to-noise ratio (SNR) compared to conventional time domain OCT (TD-OCT) [6-8] and is nowadays means of choice. However, FD-OCT has two major drawbacks that restrict the measurement depth. First, both kinds of FD-OCT systems (spectrometer based: SD-OCT, and swept source: SS-OCT) show a sensitivity roll-off to the end of the measuring range [9-11]. Therefore, the most sensitive measuring range is close to the reference plane or zero delay position, where the OCT interferometer arms are matched in length. In addition, conventional FD-OCT images suffer from disturbing mirror artifacts due to an complex ambiguity in the Fourier analysis of the real-valued measured spectral signals and the effective measuring range is halved. Different full range OCT methods, that eliminate the mirror artifacts and effectively double the usable imaging range, are reported in the literature. Most of them use phase shift techniques which can roughly be classified into alternating phase shifts between stationary A-scans (Inter-A-Phaseshift) [12-15] and continuous phase shifts during lateral scanning the sample (BM-mode-scan) [16-19]. These techniques try to generate or reconstruct a complex-valued analytical signal which is unambiguous under Fourier transform. Phase shifting methods increase the complexity of the imaging system. Especially in the case of moving samples, dense and fast sampling is required to accomplish high efficiency of mirror artifact removal. High phase stability is essential for the algorithms. Another approach to generate artifact-free OCT images is the dispersion encoded full range (DEFR) technique [20-23]. By means of a dispersion mismatch between the two interferometer arms and numerical dispersion compensation, the true structure and mirror terms get distinguishable and mirror artifacts can be suppressed iteratively. Moving and error-prone elements for artifact suppression are unnecessary and system complexity is reduced. One drawback of DEFR is its high computational effort. However, using graphics processing unit for signal analysis enables real-time visualization of acquired tomograms [24].
2 Methods

2.1 System overview
A schematic diagram of the OCT-guided fs-laser surgical instrument is depicted in image 1.

Image 1 Setup of the OCT-guided laser surgery system for cutting of the crystalline lens. SLD: superluminescence diode.

The beam of the 1040 nm fs-laser system is directed via an attenuation unit, a scanning unit (x-y and z), and a focusing unit with patient interface onto the patient’s eye. The imaging module for laser beam guidance is a custom made spectral domain optical coherence tomography system. The light of a fiber coupled superluminescent diode ($\lambda_c = 841$ nm, $\Delta \lambda = 48$ nm, SLD-371-HP1-DIL-SM-PD, Superlum, Ireland) enters a fiber based Michelson type interferometer with splitting ratio of 90:10. An optical circulator is used to prevent damage of the SLD through backreflected light. The reference arm consists of a moveable deflection unit to adjust path length difference and a reflecting end mirror. Optical elements for dispersion compensation are deliberately omitted. The intrinsic dispersion imbalance between reference and sample arm is used to remove mirror artifacts from the OCT image by means of the dispersion encoded full range algorithm. The coupling interface of the OCT and the surgical system is a dichroic mirror. The OCT sample beam runs in parallel with the fs-laser beam sharing the scanning unit and the focusing optics. The effective NA is reduced compared to the cutting fs-laser beam. The common path configuration for imaging beam and surgical laser beam reduces the system complexity and effort for coalignment and geometrical calibration.

The detection arm consists of a fiber coupled spectrometer containing a collimator, a volume phase holographic grating ($g = 1200$ lines/mm), and an achromatic doublet lens (EFL = 486.3 mm). The spectral interference fringes are captured by a 12-bit CMOS line-scan camera with 2048 pixel (spl2048-140km, Basler, Germany). A simple line scan of the OCT beam produces a B-mode image. The C++ implemented image processing and display is performed in parallel on the graphics processing unit for a whole B-mode frame in real time.

2.2 Signal processing
As a first step the background signal was estimated from the measured raw frame averaging all single A-lines. Autocorrelation terms and fixed pattern noise are removed via background subtraction. Afterwards the resulting spectra are recalibrated to equally k-spaced data and filtered for spectral shaping. The mirror artifacts are removed iteratively from the OCT image by means of DEFR. We implemented the algorithm similar to [22] and [24]. Because of the dispersion mismatch of the OCT interferometer, numerical dispersion compensation has an asymmetric effect on structure and mirror terms; structure terms get sharp and mirror terms broaden further. Employing peak detection structure terms can be extracted and corresponding mirror terms can be removed from the OCT image. At the end the the amplitude of the full range tomogram is logarithmized and converted into gray scale values to be displayed as the columns in the B-mode images.

3 Results

3.1 Spectrometer characteristics
The properties of the spectrometer were measured with a free-space Michelson interferometer with 50:50 beamsplitter and two end mirrors. The path length difference between the interferometer arms was varied by means of a translation stage with micrometer resolution. The roll-off of the signal-to-noise ratio with depth is shown in image 2.

Image 2 Depth dependent roll-off of the signal-to-noise ratio of the spectrometer. At approximately 2 mm the signal amplitude has decreased by -3dB.

The axial resolution is determined as the full width at -3dB amplitude of the peaks. The axial resolution versus measuring depth of the spectrometer is depicted in image 3. The average axial resolution is (14.55±0.58) $\mu$m.

3.2 Full range imaging
Imaging of the anterior eye segment is performed by composing two B-scans at two different z-focus; one B-scan is acquired in the front and one in the rear part of the anterior segment. The reference plain remains unchanged, roughly in the middle of the crystalline lens.
Depth dependent axial resolution of a series of single reflection peaks. The average axial resolution is 
\(14.55\pm0.58\) \(\mu\)m.

Image 4 depicts the OCT B-scan of a porcine eye ex vivo without mirror artifact suppression. In order to increase contrast for display the depicted image is an average of five consecutive scans. Indeed the mirror artifacts are blurred due to numerical dispersion correction but still disturb the OCT image and impede the correct interpretation of the image.

Applying the implemented DEFR algorithm disturbing mirror artifacts can be suppressed and full range OCT imaging is possible (image 5). The true structure is clearly visible. Again the depicted image is an average of five consecutive scans to improve image contrast for display.

4 Conclusion

Utilizing DEFR technique makes full range FD-OCT imaging possible without additional error-prone and moving parts for mirror artifact suppression and reduces the system complexity. The true structure of the sample (patient’s anterior eye segment) can be clearly obtained. By means of the full range tomogram a surgeon is capable to define the following surgical procedure on the lens reliably, e.g. for cataract or presbyopia treatments. Setting up the fs-laser and the OCT system to share the same scanner and focusing optics reduces the system complexity and facilitates the extraction of the correct transfer parameters for laser incisions. Real-time visualization, here implemented by signal processing on the GPU, is an important feature in image-guided laser surgery concerning the patient safety and the convenience of the laser surgical treatment.

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6 References


Optical Coherence Tomography (OCT) Guided Inner Ear Decalcification, Fast and Safe Method

Saleh Mohebbi1,2, Jesus Diaz Diaz3, Mark Philipp Kühnel4, Martin Durisin1, Thomas Rau1, Marjan Mirsalehi1,2, Tammo Ripken5, Heiko Meyer5,6, Thomas Lenarz1, Omid Majdani1

1. Otolaryngology Department of Hannover Medical School, Hannover, Germany (Mohebbi.saleh@mh-hannover.de)
2. Brain and Spine Injury Research Center (BASIR), Tehran University of Medical Science, Tehran, Iran
3. Institute of Mechatronic Systems (IMES), Leibniz University Hannover, Hannover, Germany
4. Institute of Functional and Applied Anatomy, Hannover Medical School, Hannover, Germany
5. Laser Zentrum Hannover, Biomedical Optics, Hannover, Germany
6. Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover Medical School, Germany

Abstract

Introduction: The labyrinth organ shelters the sensory systems for hearing and balance. It consists of membranous, soft tissue organelles, covered by a bone shell. The intactness of the intracochlear structure in different diseases and during cochlear implantation is of high interest in the development of new treatment methods and cochlear implant electrodes for residual hearing preservation by cochlear implant surgery. Different histological and imaging systems have been described for the evaluation of the labyrinth organ. For best visualization of the intracochlear membranous fine structures, a decalcification of the labyrinth organ is required. Chemical decalcification is a long lasting process. However, combined chemical and mechanical treatment can accelerate this process, but endangers the membranous structure integrity as e.g. by reshaping the bone. Using Optical Coherence Tomography (OCT) - a high resolution, noninvasive, non-contact imaging technology based on laser-application – the safety of the fast combined chemico-mechanical decalcification can be enhanced.

Aim: We utilized OCT as a supportive tool for qualitative analysis during the decalcification process of the labyrinth organ.

Material and Method: Four explanted cadaveric human temporal bones were decalcified by softening the bone in decalcifying medium using OCT as guidance.

Result: The labyrinth bones were fully decalcified within 7 hours. In summary the study has shown that OCT is a valuable technology for navigation to decalcify the temporal bone safely without inner ear destruction.

Keyword: OCT, inner ear, decalcification

Introduction

The Labyrinth is composed of the cochlea and the labyrinth organ, the sensory systems for hearing and balance. This sensory organ consists of membranous, soft tissue organelles, placed in a boney tunnel system. Detection of the intactness of the microstructure of the cochlea is of high interest in residual hearing preservation by cochlear implant surgery. Cochlear implant surgery is a therapeutic method to enhance the speech understanding of hearing impaired patients. Different histological and imaging systems have been described for evaluation of the labyrinth organ, as e.g. Computer Tomography (CT), Cone Bean CT (CBCT), µCT, MRI and µ-Grinding. Optical Coherence Tomography (OCT) is an all optical high resolution, noninvasive, non-contact imaging technology based on laser-application. Decalcification of the labyrinth enables the examination of the membranous microstructures of the cochlea, but this is time consuming and may take several days to perform.

First medical use of optical coherence tomography (OCT) has been described by Huang et al in 1991. They reported the use of this technology in retina and coronary artery imaging.

OCT is a non-invasive and non-contact imaging system using low coherence interferometry. This technique is similar to ultrasound but instead of sound it is detecting light. Reflected light from the subsurface is detected by OCT scanner and based on this information high resolution images are created. The lateral resolution of OCT can reach the level of 10 µm. OCT uses near infra-red light which is nondestructive to the body. The specimen or subject also does not need any preparation. The limitation of OCT is the depth of penetration and lateral resolution because of scattering.

In the Otolaryngology, OCT has been used primarily in diagnosis of larynx diseases, in the ear for ossiculoplasty and stapedectomy, tympanic membrane imaging and cochlear implant surgery. In a recent study Tona et al presented the use of OCT for the in vivo visualization of cochlea in mice. Development of new imaging technologies such as Scanning Laser Optical Tomography (SLOT) and µCT could help to increase the knowledge about inner ear anatomy. SLOT is only able to scan small and translucent objects. For anatomical inner ear studies the whole decalcified inner structure was needed, but this method is a time consuming and difficult process, taking several days or
weeks when the specimen is placed in the Ethylenediaminetetraacetic (EDTA) solution for decalcification only. When milling the superficial part of the bone which has been softened in EDTA solution, the process can be done faster, but injuring the membranous structure is a risk as the sub-milimetric amount of the thickness of the bone cannot be estimated under microscopic view only. We aimed to use OCT to estimate the residual thickness of the bone during the decalcification of the bone. This should facilitate the milling process and reduce the risk of penetrating the membranous structures.

Material and method

Five fresh frozen explanted temporal bone specimens were cut in smaller cube by cutter to simplify the procedure. The specimen were cut lateral to tympanic membrane, medial to internal auditory canal, and below the middle ear.

CBCT and μCT scans were acquired to show bone density and anatomy. Specimen were trimmed to ensure their weight were similar. Then labyrinth bones were preserved overnight in 4% formol at -4°.

On the next day the specimen was placed in 50 ml 20% solution of Ethylenediaminetetraacetic acid (EDTA) on a rotator plate. Each 90 minutes the specimen was taken out and the softened part of bone was removed by milling. During the drilling process the very fragile specimen was simply hold manually. Before each milling step OCT scans were acquired from different sides of the specimen using the Thorlabs OCT Spectral Radar System (image 1). The thickness of the bony shell of the membranous structures was measured using the OCT. Afterwards the samples were washed with phosphate buffered saline (PBS) three times and replaced into fresh EDTA medium. The specimen was checked by OCT intermittently to show the thickness of the remnant bone and the depth of the membranous cochlea structures. This procedure was repeated every 90 minutes. Bone removal was stopped when a residual thickness of the bone was less than 500µm. The samples decalcification was verified by X-ray. Some important hints on the whole procedure are listed in table 1.

As a control we decalcified one sample without OCT in the same medium and same time only by mechanical bone removing of bone (drilling).

Result

Decalcification process of the sample without OCT took approximately nine hours. In spite of special care to preserve the labyrinth, the specimen was still damaged in three sites in the semicircular canals and inferior side of cochlea.

Samples which were decalcified under OCT guidance showed fewer damages (image 3). First bone was decalcified within 14 hours’ time. But the following three samples were decalcified in less than 8 hours. 4 samples remained completely intact. Only in one sample a small area of violation was noted in inferior side of posterior semicircular canal. Wherever the bone thickness was less than half millimeter inner ear structure, such as in the region of scala vestibuli and tympani, basal membrane was visible by OCT (image 4). In most sites of decalcified cochlea remain bone thickness was about 300-400 µm.

Discussion

OCT is a B-mode modality based on near infra-red light. It can penetrate bone structures by approximately 500µm with a lateral resolution of approximately 10µm and axial resolution of approximately 3µm. It also has the capability to be used as a navigation system in real time surgery11. Like in ultrasound, reflections at the borders of different tissue materials are detected and visualized best with OCT. In OCT guided decalcification the differentiation of air and fluid filled structures can be visualized on the screen, e.g. air cells, cochlea and semicircular canal. Air -bone reflectivity by 1,300 nm light is 0.04646 and water- bone reflectivity 0.0064211, therefore the differentiation is possible.

When the inner ear was opened accidentally during drilling or during manipulation on the stapes some air bubbles were introduced into the fluid filled cochlea. The samples were set in a vacuum chamber to remove small air bubbles. Violation to the membranous structures of the cochlea can destroy the complete architecture of the inner ear and further studies cannot be performed on those samples. Decalcified samples of the cochlea can ideally be visualized with novel imaging techniques as e.g. SLOT.

OCT has been used previously for detection of the temporal bone structures and for high precision image guided surgery previously11,13. We used OCT to measure the remaining thickness of the bone at different sites of the labyrinth organ during the decalcification process. By this, critical zones with very shallow boney coverage of the membranous structures could be defined early and could be protected during drilling of the remaining bone structures at neighbor sites.

We could reduce the time consuming procedure and decalcified complete human cochleae on a reasonable time scale. We reached the fully decalcification of the inner ear within seven hours. This time is comparable with another report that take in average of 13 hours 3. However, the most important advantage of using OCT in this process was to identify already thinned bone parts and avoid further removement of bone structures on those as risky defined areas. Thus, the rate of intact final probes without any violation to the membranous structure of the cochlea was raised. By reducing the sample size, e.g. if only the cochlea would be targeted for decalcification, the preparation time could be reduced even to approximately less than 4 hours.

In this study, drilling of temporal bone was done by an otolaryngologist; anatomical knowledge is the first requirement for drilling the structures and cannot be replaced by OCT imaging.
Table 1: Some important point for successful decalcification of temporal bone

- First the tympanic membrane, malleus and incus have to be removed. The over structure of the stapes needs to be removed, but the footplate needs to be left intact.
- Mastoid air cells are removed carefully from the inferior surface and facial using a cutting burr until only the otocapsula remains.
- After each step of chemical decalcification in EDTA solution milling is performed, each milling step takes approximately 15-20 minutes.
- A suitable diamond burr is appropriate for milling off the softened bone.
- Drilling in the area of the round window and posterior semicircular canal is critical and needs more attention.
- Only firm bone should be removed.
- Be careful not to break the cochlea at the weak connection between cochlea and vestibule.

Conclusion:

Inner ear decalcification is time consuming procedure and difficult. Decalcified inner ear probes can be examined by novel imaging techniques for investigation of the membranous structures of the cochlea. While chemical decalcification of the cochlea takes several months, a combined procedure of chemical decalcification and milling of the softened parts of the otocapsula can reduce the preparation time considerably. However the milling process of the otocapsula without depth control of the remaining boney shell leads often to the accidentally violation of the membranous structures. We decided to use OCT system as guidance for depth control during the decalcification procedure. Hereby we could reduce the preparation time considerably and the membranous structures of the labyrinth organ remained intact.

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References:


Extracellular Space Contribution to the Double Wave Vector Diffusion-Weighted Signal

P. Ulloa, Institute of Medical Engineering, University of Luebeck, Lübeck, Germany, ulloa@imt.uni-luebeck.de
V. Wottschel, Queen Square MS Centre, UCL Institute of Neurology, University College London, London, United Kingdom, viktor.wottschel.12@ucl.ac.uk
M. A. Koch, Institute of Medical Engineering, University of Luebeck, Lübeck, Germany, koch@imt.uni-luebeck.de

Introduction

Using two independent diffusion periods between excitation and acquisition, sometimes known as double wave vector (DWV) diffusion-weighted MRI, it is possible to obtain tissue structure information for which no other non-invasive technique exists. Comparing the signal between different relative diffusion gradient orientations, the antiparallel-parallel ((SĹĻ-SĹĹ)/S₀) and parallel-perpendicular ((SĹĹ-Sŏ)/S₀) differences bear information on pore size and shape, respectively. However, evaluating the former in the corticospinal tract (CST) yield values above the axon diameter expected here. This may indicate contributions of extracellular space. The intracellular compartments have the shape of circular cylinders, while the extracellular space is rather represented by cylinders with irregular base. Hence, assessing the pore shape yields information on the extracellular contribution.

Methods

DWV diffusion-weighted echo-planar-imaging was performed on one healthy volunteer with a 3 T whole-body MR system, using 4 diffusion gradient directions (each with parallel and antiparallel orientations) in the x-y plane. Regions of interest in left and right CST were delineated manually. Diffusion tensor images (DTI) were acquired to estimate the fibre axis direction. A smaller DWV signal with perpendicular gradients indicates a noncircular cross section. However, this may arise from an inclination of cylinders with circular base. When geometrically averaging the signal of measurements with diffusion gradients rotated by 90°, the difference between parallel and perpendicular directions should vanish for elliptic and tilted circular cylinders. If the difference remains, more than one orientation of eccentric cylinders, or circular cylinders with more than one inclination is present.

Results

The DTI-derived fibre direction was approximately aligned with the z-axis. The observed positive parallel-perpendicular difference was hardly affected when applying the geometric mean between perpendicular gradients.

Conclusion

Given that different inclinations are unlikely, it is concluded that the signal-dominating compartment is shaped irregularly. This is consistent with the extra-axonal compartment being the origin of the measured signal.
A Novel System Calibration Method in Photoacoustic Tomography

M. Oeri¹, W. Bost¹ and M. Fournelle¹, Fraunhofer Institute for Biomedical Engineering, Biomedical Ultrasound Research, St. Ingbert, Germany, milan.oeri@ibmt.fraunhofer.de

Introduction

(Phot-)acoustic tomography (PAT) allows to overcome the limitation regarding the anisotropic image resolution that is characteristic for conventional reflection mode imaging. However, achieving a high resolution requires precise information on the transducer position to accurately calculate delays and reconstruct smallest structures in tissue. Due to inevitable alignment imperfection such as transducer tilt and spatial shift, this modality suffers — uncalibrated — a poor system response. We present a mathematical calibration method in order to improve the image resolution in any tomographic setup based on linear transducers.

Methods

We first reconstruct photoacoustic data of a linear transducer of an uncentred, rotated point source under arbitrary but defined angles in one spatial plane. Due to transducer tilt and shift the following tomographic reconstruction yields to a characteristic circular artefact. We derive an overdetermined system of linear equations (SLE) by the expression of the backtransformation of the measured coordinates into a global system as a function of the degree of tilt and shift. By computing the coefficients from the SLE we receive the distance of the detector to the rotation axis and the tilt degree. Once obtained, the misalignment of the investigated detector and arising time-of-flight shifts during data acquisition are compensated within the reconstruction algorithm, i.e. the detector alignment is adapted to the actual setup. The mathematical calibration method is evaluated on simulated and experimental data.

Results

Our findings verify the dependence of the performance of a PAT-system on the knowledge of the de facto transducer tilt and position and proved to enhance the image quality. Once calibrated, the spatial resolution can be improved significantly.

Conclusion

In this work we demonstrate a mathematical calibration method to compensate for acoustic transducer tilt and spatial shift when performing tomographic imaging by using linear transducer arrays. Our findings are generally applicable for acoustic transducers arranged on an arbitrary shape.
Open Healthcare: Transferring Research to Everyday Cardiology with Free Software

B. Schwarz¹, M. Kaiser², L. Mursina²,³, A. Rolf⁴, H. Schneider²,³
¹Technische Hochschule Mittelhessen – University of Applied Sciences, Giessen, Germany, bjoern.schwarz@mni.thm.de
²Technische Hochschule Mittelhessen – University of Applied Sciences, Giessen, Germany
³Competence Centre for Information Technology, Giessen, Germany
⁴Kerkhoff Klinik gGmbH, Bad Nauheim, Germany

Introduction

Atrial arrhythmias are typically treated with RF ablation procedures. While RF energy treatments potentially cure the arrhythmias, a large number of patients need to return for second or even third treatments to be successfully cured. None of the currently available solutions to visualize and improve this treatment is suitable for adoption in a clinical routine workflow [1]. The development of a simplified clinical workflow procedure and the implementation of an OsiriX DICOM viewer [2] plugin is elaborated here.

Methods

In order to develop an adapted workflow, MR angiography and delayed enhancement as well as electroanatomic mapping (EAM) data are evaluated. The workflow features a multi-stage process to join the different data sets and allow RF treatment visualization. The necessary steps include the segmentation of MR angiography image data to obtain a reliable cardiac surface model, the registration of the MR and EAM based datasets, and the use of maximum intensity projections for visualizing fibrosis.

Results

An open source project has been set up to allow further development of the current prototype. Currently, the segmentation of MR images, NavX™ data import and the visualization of all available data has been completed, and a foundation for the registration was successfully created. It has been proven that the transfer of research into clinical workflows can be easily achieved by relying on open source software.

Conclusion

The presented plugin and associated open source project do not yet implement the full visualization process, but have shown enough promise to be considered a proof of concept for the approach of bringing up-to-date research into a tool for clinical routine.

A model for improving patient care and transferring current research to daily clinical routine has been shown, that employs open source software to create a highly customizable yet reliable foundation for the implementation.

References

Three-dimensional TV Minimization Algorithm using Total Curvature

Eric Wisotzky, Fraunhofer IPK, Berlin, Germany, eric.wisotzky@ipk.fraunhofer.de
Marc Käseberg, Fraunhofer IPK, Berlin, Germany
Erwin Keeve, Fraunhofer IPK and Charité–Universitätsmedizin Berlin, Berlin, Germany

Introduction
Computed tomography (CT) is essential in modern surgery. For intraoperative CT a free access to the patient is achieved at the cost of a limited image acquisition angle. Additionally, it is attempted to receive good reconstruction results with even a limited number of projections and a reduced patient dose. These limitations cause noisy data and reconstruction artifacts. In this work we present an iterative algorithm which is able to reduce noise and artifacts to provide good image quality.

Methods
We propose a TV minimization algorithm that operates right after the volume reconstruction. It is based on the Rudin-Osher-Fatemi (ROF) problem and minimizes the total variation of the reconstructed volume $f$. This minimization is solved via its Euler-Lagrange equation. In the ROF-problem the diffusion coefficient $D = |\nabla f|^{-1}$ only depends on the strength of the level lines. In our algorithm, $D$ is enhanced by total curvature. The advantage of curvature is that it has the potential to reconnection separated parts of broken objects far apart. To preserve three dimensional structures in the correction process the algorithm was extended with a specialized 3D filter kernel.

Results
The described algorithm is able to reduce various types of artefacts like noise and metal artifacts. First simulations with 50 projections show a volume quality improvement from 0.908 to 0.946 in terms of normalized-cross-correlation. Nevertheless further evaluations are needed for limited data sets. The runtime takes about 3s on CPU for a single iteration. To achieve a good artefact reduction about 30 to 50 iterations are needed.

Conclusion
The presented algorithms shows good results in volume restoration and artefact reduction, especially for limited data problems as in image-guided surgery. Nevertheless the runtime on the CPU takes to long for an effective usage, therefore we want to speed up the algorithm by implementing it on the GPU.
Large scale in vivo imaging of the corneal sub-basal nerve plexus by guided eye movements

S. Allgeier1,*, B. Köhler2,*, S. Maier1, S. Peschel3, K.-M. Reichert2, O. Stachs3, J. van Wezel4, R. Mikut2, G. Bretthauer1,2

1 Institute for Applied Computer Science/Automation, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany
2 Institute for Applied Computer Science, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany
3 Department of Ophthalmology, University of Rostock, Rostock, Germany
4 Steinbuch Centre for Computing, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

* S. Allgeier and B. Köhler contributed equally to this work
stephan.allgeier@kit.edu

Introduction

The densely innervated cornea is the only tissue of the human body in which nerve fibres are accessible in vivo by confocal microscopy. Numerous studies were aimed at establishing morphometric features of the corneal sub-basal nerve plexus (SNP) as a sensitive marker for various ocular and systemic conditions and diseases. However, due to considerable variability in local nerve fibre density across the corneal area, evaluation of a single image with a typical field of view of about 0.15 mm² is insufficient for reliable morphometric characterization. Mosaicking approaches have been proposed to examine the SNP on a larger scale. We present a technique that significantly facilitates the generation of mosaic images of the SNP by a high degree of automation.

Methods

A computer-controlled moving fixation target on a display located in front of one eye guides the patient’s gaze in an outward-spiralling pattern, while the contralateral eye is being examined by corneal confocal microscopy. Due to the synchronicity of human eye movements, the examined eye performs identical movement patterns below the microscope, which thus captures an image sequence of a continuously increasing area of the SNP. Specifically developed image processing software registers the sequence, removes motion artefacts, and fuses the acquired images by weighted averaging. The prototype system was evaluated using 12 volunteers.

Results

All volunteers involved in this study tolerated the examinations well with no relevant adverse effects. A single examination took an average of 65.3 s (range 14.6-139.2 s) and the resulting mosaic images cover an average of 9.94 mm² (range 1.68-18.35 mm²).

Conclusion

The presented easy-to-use and fast technique for imaging extended areas of the SNP could pave the way for a more robust and reliable morphometric analysis of corneal nerve fibres with potential use in diabetic neuropathy diagnosis and provide new insight into the living human nervous system.

Acknowledgement

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New robotic phantom: Validation of performance in respiration triggered medical imaging

H. Arenbeck, Institute of Automatic Control, RWTH-Aachen University, Aachen, Germany
H.Arenbeck@irt.rwth-aachen.de

A. Duffe, Institute of Automatic Control, RWTH-Aachen University, Aachen, Germany
A.Duffe@irt.rwth-aachen.de

O. H. Winz, Clinic for Nuclear Medicine, University Hospital Aachen, Aachen, Germany
owinz@ukaachen.de

C. Schubert, Clinic for Radiooncology and Radiotherapy, University Hospital Aachen, Aachen, Germany
caschubert@ukaachen.de

N. Escobar Corral, Clinic for Radiooncology and Radiotherapy, University Hospital Aachen, Aachen, Germany
nescobarcorral@ukaachen.de

M. J. Eble, Clinic for Radiooncology and Radiotherapy, University Hospital Aachen, Aachen, Germany
MEble@ukaachen.de

F. M. Mottaghy, Clinic for Nuclear Medicine, University Hospital Aachen, Aachen, Germany
fmottaghy@ukaachen.de

D. Abel, Institute of Automatic Control, RWTH-Aachen University, Aachen, Germany
D.Abel@irt.rwth-aachen.de

Introduction

Four-dimensional techniques in medical imaging allow measurement and compensation of tissue motion and have gained increasing importance in both research and clinical practice. Validation of these techniques can be realistically performed with robotic (4D-) phantoms, which resemble structure, physical properties and motion of a human body environment. A 4D-phantom that features a unique combination of high performances in human equivalence of structures and motion, measurement capabilities, universality of setup and efficiency of practical use was recently presented and successfully applied to validation of PET imaging and dose application in radiotherapy. To enable validation of a complete process chain of 4D-radiotherapy, including surrogate based respiratory gating and tracking techniques, motion-synchronous and customizable replication of respiration surrogates by this 4D-phantom is necessary.

Methods

An add-on motion unit that allows excitation of most common surrogate sensors (breathing belt, breathing pad, optical markers, etc.) was developed and integrated into motion customization and control of the 4D-phantom. Using this unit, prospective 4D-CT scans of end-inspiration and end-expiration positions of the phantom target featuring motion according to a Lujan cos^4 trajectory in superior/inferior direction was performed.

Results

The 4D-phantom scan could be efficiently performed in accordance with the workflow of clinical practice. No artefacts, distortions or unexpected phenomena were noticeable in both the CT-images and the respiratory signal. The end-expiration image featured high accuracy and sharpness, the end-inspiration image featured slight blurring as a result of the higher motion range during end-inspiration phase.

Conclusion

The 4D-phantom with the new add-on motion unit appended has proven technologically and practically feasible for respiration triggered imaging and thus realistic and comprehensive validation of a 4D-radiotherapy process chain. Next, the unique features of the 4D-phantom will be employed for the investigation of the effects of motion in radiotherapy and the design of new quality assurance concepts that capture the demands of next generation radiotherapy techniques.
Comparison of OpenCL and OpenGLSL for real-time reconstruction of ultrasound images

S Maas, T Dithmer, H M Overhoff, Department Electrical Engineering and Applied Natural Sciences, Westphalian University of Applied Sciences, Gelsenkirchen, Germany, stefan.maas@w-hs.de

Introduction

Image reconstruction with individual algorithms is possible for ultrasound devices with receive raw data access, e.g. Esaote MyLab 70 XVG with ART.LAB©. At a frame rate of 60 images/sec, up to 48 MByte/sec raw data are acquired. A real-time processing at this data rate can only be performed on graphics processing units (GPUs) using languages like e.g. “C” for CUDA”, “OpenCL C” or (Open)GLSL. While GLSL is designed for visualization purposes, CUDA and OpenCL is conceptualized for “General Purpose Computing on GPU” (GPGPU). Obviously for image reconstruction CUDA or OpenCL is recommended. For a SAFT-based reconstruction, that directly exploits the receive data stream, we tested which implementation is performed faster. Since CUDA is NVIDIA-specific we only compared OpenCL and OpenGLSL.

OpenGL and OpenCL use different contexts. Context switches are necessary when OpenCL starts and finishes calculation. This is a bottleneck in the program execution (Kilgard 2012). Bypassing OpenCL should slow down the reconstruction but speeds up the data flow from reconstruction to visualization resulting in higher frame rates.

Methods

The calculation was performed using an Intel Core2Duo, 2.6 MHz CPU PC and different graphics cards (table 1). A primarily build OpenCL implementation was ported to GLSL 4.3. Identical shaders were used for visualization of the resulting ultrasound images of both implementations. To keep the calculations comparable the OpenCL algorithm were ported to compute shader unchanged. Only language specific differences were regarded.

Results

<table>
<thead>
<tr>
<th>Graphics card</th>
<th>Frame rate [fps]</th>
<th>Speed advantage GLSL [in %]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OpenCL</td>
<td>GLSL</td>
</tr>
<tr>
<td>Nvidia GeForce GT 525M</td>
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<td>624</td>
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</tr>
<tr>
<td>Nvidia GeForce GTX 760</td>
<td>1968</td>
<td>2457</td>
</tr>
</tbody>
</table>

Table 1: Implementations performance on different graphics cards

Conclusion and future work

The faster the graphics card the higher the speed advantage for GLSL (table 1). The implemented SAFT-based reconstruction is as the say, “close to the pixel”. Therefore, GLSL benefits from its graphical specialization. Additionally the bottleneck was avoided. Both conduces to the speed advantage. Further work is addressed to the determination of the exakt parameter when GLSL is preferable to OpenCL.

References

Evaluation of reproducibility and variability of a perfusion phantom

S. Gugel, P. Mier, A. Boese, G. Rose, Department of Medical Engineering, Otto-von-Guericke-University, Magdeburg, Germany, sebastian.gugel@ovgu.de

Supported by Forschungscampus STIMULATE (BMBF 03FO16103A), Magdeburg, Germany,

Introduction

To test the performance of algorithms for calculating the tissue perfusion of 4D CT datasets, a dynamic phantom was developed based on fluid dynamics simulations [1]. First results of the manufactured phantom showed a high similarity to the computational data and a good reproducibility and homogeneity of the results [2]. In this work we tried to investigate the performance of the phantom in a larger extent.

Methods

The phantom was evaluated using a clinical CT scanner (SOMATOM Definition AS+) with a standard perfusion protocol (80 kV, 30 s measurement time, 68 slices). The phantom was mounted to a fixed position after proper alignment in the gantry. A variation of the flow rate (0.4 and 0.6 l/min) and the amount of injected contrast agent (15 and 10 ml) was varied. The measurement was repeated three times. The data was evaluated by plotting time-attenuation-curves (TAC) of the resulting data. The TACs of different regions of interest within the phantom were compared to evaluate the homogeneity of the phantom. The reproducibility was evaluated by the comparison of successive measurement with the identical setup.

Results

Consecutive measurements showed almost identical characteristics in a temporal and spatial context. In Figure 1 the TACs of a repeated measurement are illustrated exemplarily. A variation of the amount of contrast agent lead to a proportional shift of the TAC, as demonstrated in Figure 2. The variation of the flow rate lead to a broadening/narrowing of the TAC.

Conclusion

The developed perfusion phantom could be a valuable tool for the assessment of new perfusion algorithms. The measurements showed a high reproducibility with a reasonable variation possibility to adjust the TACs. Further measurements and calibrations must be performed to utilise the phantom for the quantitative assessment of tissue perfusion.


Figure 1: Regions of Interest in the Perfusion slice; TAC of two successive measurements

Figure 2: TACs for 0.6 l/min and 0.4 l/min overall flow; TACs for 15ml and 10ml contrast agent
Lensless Live Cell Imaging with thermoelectric cooled Cell-Microscope

M. Hubl, M. Blechert, M. Engelhardt, E. Jung, Fraunhofer IZM, Berlin, Germany
K.D. Lang, TU Berlin, Germany
Corresponding Author: Moritz.Hubl@izm.fraunhofer.de

Lensless Chip-Microscopy

We presented on the BMT 2012 a Lensless Chip-Microscope for characterisation of cells and microparticles, which we improved for live cell imaging inside the incubator. Therefore an automated imaging software, a thermoelectric cooler and an advanced analysis tools were implemented (see Figure 1).

Live Cell Imaging

The lensless microscopy is based on holographic imaging. A red laser diode emits coherent light, which scatters at the cells and overlays with the unscattered laserlight (see Figure 2). A CMOS sensor digitalizes the resulting individual holograms. The lensless Cell-Microscope fits easily in the incubator due to its minimal size of 12x6x10cm. A picture every 10 minutes enables time-laps recording of cell cultures over several days. In order to avoid thermal effects on the cells, the camera temperature is kept to 37°C by peltier cooling. Because the system is not limited by lenses, the resulting field-of-view equates to the sensor dimensions of 32 mm². The collected data can be used for further image processing like growth curves, cell counting and confluence analysis.

Cell Culture Analysis

The captured images show beside the cell location and dimension also the adhesion status. While adherent cells have a bright internal spot, nonadherent cells have a globular shape with different refraction resulting in a dark circle (see Figure 3). This is an useful fact for detecting dividing or dead cells and check the division rate of the observed cell culture. Continuous cell counts of a fibroblast culture reveal no influence of the Cell-Microscope on the cell growth. Further more this method shows high significance due to monitoring up to 15000 cells in just one image. With the large field-of-view it is also more likely to observe rare events like tripolar cell division of mutated cells.

Outlook

The lensless cell microscopy system thus offers great potential of analysing the cell behaviour for automated cell monitoring, cell assays, cytotoxicity test and drug testing.
Figure 1. The initial lensless Chip-Microscope (left) and new design with integrated thermoelectric cooling (right).

Figure 2. Two dividing cells under 10x microscope (top), as hologram and as digitally reconstructed image (down). Entire field of view from 4x and 10x microscopes compared with the lensless Cell-Microscope (right).

Figure 3. Cell division of incubating fibroblasts cells (left, scale = 100 µm) and counted fibroblast cell growth in a microfluidic channel, observed with the lensless Cell-Microscope (right).
**Parameterization of the complex MPI signal using a set of coefficients of polynomial base functions**

Daniel Schmidt¹, Florian Palmethofer¹, Uwe Steinhoff¹  
¹Physikalisch-Technische Bundesanstalt, 10587 Berlin, Germany

**Abstract**

We present a new approach to describe the Magnetic Particle Imaging (MPI) signal via a set of coefficients of polynomial base functions. Contrary to the description via a Taylor series we are not limited to a small radius of convergence or the phase shift. Using this method, we describe a simulated MPI signal and use the resulting series to investigate the creation of harmonics and the impact of the availability of a limited number of harmonics on the signal quality.

**1 Introduction**

Magnetic Particle Imaging (MPI) is an imaging modality developed to visualize magnetic nanoparticles in the body. It is based on the detection of higher harmonics induced by the nonlinear magnetization curve and time lag of an ensemble of magnetic nanoparticles (MNP) in an oscillating magnetic field. An additional gradient field creates different harmonics at every location. Decomposing the signal into the characteristic part of each voxel allows a spatial reconstruction of the MNP amount.

To analyze the MPI signal one approach is to use polynomials developed with a Taylor series. This approach has the main advantage that the creation of harmonics can easily be observed via the single polynomial terms and basically would be a powerful tool to investigate the connection between magnetization curve and MPI-spectrum. Yet, there are disadvantages: The approach is not capable of projecting the time lag of the response of MNP and therefore could only describe (quasi)static signals, it is restricted to the use of the Langevin equation and it has a radius of convergence of (-π, +π) for the expansion of the Langevin equation, caused by the polynomial description of $f(x)=\coth(x)$.

We present a new approach to describe the measured or simulated MPI signal using a set of polynomial coefficients enabling us to express the phase shift and are not limited to the small radius of convergence.

In this paper, we will show how we use a simple mathematical model to derive the set of polynomial coefficients for a given number of polynomials and compare the results to the Fourier transform of the used signal.

**2 Method**

As a very simple approximation the MPI signal produced by the alternating magnetic moment of an ensemble of MNP can be described by the Langevin function $L(x)$

$$L(x) = \coth(x) - 1/x$$  

with

$$x := \xi = \frac{mH\mu_0}{k_B T}$$

Here, $m$ is the magnetic moment, $H$ is the magnetic field strength, $\mu_0$ is the magnetic vacuum permeability, $k_B$ is the Boltzmann constant and $T$ is the temperature. The magnitude of $x$ is directly proportional to the magnetic moment $m$ and the magnetic field strength $H$ and therefore determines the steepness of the flipping of the magnetic moment of an MNP ensemble. It can be seen that this model does not consider the anisotropy and therefore the time lag of the magnetic response is not taken into account. Still for some applications this model is sufficient and for that case, it is possible to derive the Taylor expansion of $L(x)$ around the point $x=0$:

$$L_T(x) = \frac{x}{3} - \frac{x^3}{45} + \frac{2x^5}{945} - \frac{x^7}{4725} + \frac{2x^9}{93555} + O(x^{11})$$

Using $L_T(x)$ it is possible to investigate the creation of harmonics up to $x_{max}=\pi$ using a sine excitation for $x$ and the relation

$$\sin(x)^n = \sum_{n=0}^{N} K_n \sin(nx)$$

For more complex applications this simple approach does not suffice. On real MNP samples the field dependency of the magnetic moment $m(H)=m(H_0=m(H_0...H_0)$ may differ from the Langevin function either in measured data or in calculations based on more complex models. We describe the function $m(x)=m(x(H))$ via:

$$\begin{bmatrix}
m(x_1) \\
m(x_2) \\
m(x_3) \\
\end{bmatrix} = \begin{bmatrix}
a_1 \\
a_2 \\
a_3 \\
\end{bmatrix} \begin{bmatrix}
\sin(x_1)^1 & \cdots & \sin(x_1)^n \\
\vdots & \ddots & \vdots \\
\sin(x_n)^1 & \cdots & \sin(x_n)^n \\
\end{bmatrix}$$

$$\begin{bmatrix}
m(x_1) \\
m(x_2) \\
m(x_3) \\
\end{bmatrix} = \begin{bmatrix}
a_1 \\
\vdots \\
a_n \\
\end{bmatrix} \begin{bmatrix}
\cos(x_1)^1 & \cdots & \cos(x_1)^n \\
\vdots & \ddots & \vdots \\
\cos(x_n)^1 & \cdots & \cos(x_n)^n \\
\end{bmatrix}$$
Based on this equation, the terms $a_1 \ldots a_N$ and $b_1 \ldots b_N$ can be calculated by a linear estimation technique. This calculation results in the description of the magnetic moment as a polynomial series of sine and cosine terms without limitations regarding $x_{\text{max}}$ or the time lag in relation to the excitation field.

If we combine the results of (5) into

$$m(x) = \sum_{n=1}^{N} a_n \sin(x)^n + b_n \cos(x)^n$$

it is possible to use (4) and its cosine counterpart to calculate the harmonic fraction of every polynomial.

$$m_N^* (x) = \sum_{n=1}^{N} K_{n,1} \sin(nx) + K_{n,2} \cos(nx)$$

Summing up the fractions of $K_{n,1}$ and $K_{n,2}$ for a given $n$ of all polynomials we get the overall components for the $n$-th harmonic $C_{n,1}$ and $C_{n,2}$.

$$C_{n,1} \sin(nx) + C_{n,2} \cos(nx)$$

$$= \sum_{n=1}^{N} K_{n,1} \sin(nx) + K_{n,2} \cos(nx)$$

Those can be written in the classic harmonic notation:

$$C_{n,1} \sin(nx) + C_{n,2} \cos(nx)$$

$$= C_{n,1} \sin(nx) + C_{n,2} \sin\left(nx + \frac{\pi}{2}\right)$$

(9)

$$= \sqrt{C_{n,1}^2 + C_{n,2}^2} \sin\left(nx + \arctan\left(\frac{C_{n,2}}{C_{n,1}}\right)\right)$$

$$= C_n \sin(nx + \varphi)$$

This way, we found a polynomial expression of the MPI signal and can either relate the fraction of every polynomial of the series expansion to the creation of harmonics or calculate the overall harmonic composition. In the next chapter, we will apply this method on a simulated MPI signal and compare the results to the Fourier transform of the signal.

### 3 Results

In image 1 we see a simulated MPI signal in comparison to its expression in polynomials. Since the simulation is not based on the Langevin function but on a more complex approach, the time lag in relation to the excitation field has to be taken into account, which would not have been possible with a standard Taylor expansion.

**Image 1:** A simulated MPI signal in comparison to its polynomials expansion

This mapping was done with a polynomial expression up to the 5th term which corresponds to to $\sin^9(x)$ or $\sin(9x)$, respectively (4). It can be seen that it was possible to map the simulated signal nearly perfectly with our method and would have been even better using more terms.

**Image 2:** Comparison of harmonics derived from a Fast Fourier Transform and our polynomial expansion

Image 2 depicts the absolute values of the harmonics, as calculated via a Fast Fourier Transform of the simulated signal compared to the accumulated $\sin(nx)$ values, calculated from the polynomials using (9), while Image 3 shows the respective phase angles.

**Image 3:** Comparison of phase angles derived from a Fast Fourier Transform and our polynomial expansion
As expected from Image 1, the harmonics coincide nearly completely as well as the respective phase angles. Calculations showed that the correlation decreased when we used too few polynomials to describe the signal. This gives us a tool to investigate the necessary amount of harmonics above noise level.

Using this method, it is possible to investigate the influence of single polynomials on the creation of harmonics. As [1] already mentioned, this was not possible using Taylor Polynomials due to the limitation of the convergence radius and the restriction of the Langevin function.

The n-th polynomial corresponds to a contribution of n harmonics. It can be seen that the expansion of harmonics is an oscillating process. Regarding the first harmonic we observe only a small decay of contributions during the first five polynomials up to \( \sin(9x) \) which indicates that the introduction of higher harmonics than \( \sin(9x) \) still affects the overall magnitude of the first harmonic. This means that the number of available harmonics directly influences its respective magnitudes. It remains to be seen whether the accumulated magnitude of lower harmonics reaches a steady state with the construction of more polynomial terms.

### 4 Conclusion

We introduced a new method to express a phase shifted MPI signal via a polynomial expansion by solving an algebraic matrix equation. This expression is similar to the Taylor polynomial approach but does not have some of its limitations. Using this method we described a synthetic MPI signal that was similar to a real measured signal by expressing it in polynomial coefficients. In the evaluation of these polynomials we showed the influence of the number of available harmonics on the magnitude of each harmonic. Similarly, it is also possible to investigate the necessary amount of harmonics to express a signal with a certain correlation.

Overall, this method seems to be a valuable tool to explore the connection between the number of available harmonics and their creation during the measurement process due to a limited amount of harmonics which might influence resolution and imaging quality.

### 5 References


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**Image 4**: Contribution of single polynomials to the overall harmonic magnitude

Image 4 depicts the contributions of the every polynomial to the overall harmonic spectrum from \( \sin^1(x) \) to \( \sin^9(x) \).
Image fusion of histological images to generate high resolution datasets of the human middle and inner ear structures

S. Hügl1, G.J. Lexow1, Th.S. Rau1, Th. Lenarz1, O. Majdani1
1Department of Otolaryngology, Hannover Medical School, Hannover, Germany, huegl.silke@mh-hannover.de

Abstract

Preoperative planning of a surgical pathway for minimally invasive and computer-assisted cochlear implantation strongly depends on high resolution imaging of bony and soft tissue middle and inner ear structures. Since conventional medical imaging lacks information on delicate soft tissue structures inside the cochlea, sets of histological data were generated using a serial cross section imaging technique in order to prepare the development of a model-based segmentation algorithm. To increase the image resolution and to provide more detailed morphological information a currently developed 3D histology imaging approach was enhanced by a data processing with integrated automatic image fusion. Finally the generated datasets were converted into the DICOM standard. The presented study (n=15) evaluates the results of the image fusion based on the fiducial registration error (FRE).

1 Introduction

Patients with severe to profound hearing loss can be implanted with a cochlear implant (CI) to regain an auditory impression as long as their acoustic nerve is still fully functional. During the surgical treatment the electrode carrier of a CI system is inserted into the scala tympani, which is a compartment inside the cochlea surrounded by delicate soft tissue structures like the basilar membrane. Considering the tiny dimensions it poses a major challenge not to damage these structures in order to preserve the patient’s residual hearing. This indicates the need for a precise preoperative planning for a computer-assisted surgical intervention with regard to soft tissue structures. One option is the generation of an anatomical atlas which presents bony and soft tissue structures. To support preoperative planning this atlas may be combined with patient-individual conventional imaging like computed tomography to add the missing information on the location of soft tissue structures inside the inner ear through a model-based approach.

Since conventional medical imaging techniques lack either the necessary spatial resolution or the possibility to visually differentiate the thin soft tissue structures of the inner ear a serial cross section imaging procedure has been developed [3][4]. This procedure uses embedded human temporal bone specimens mounted into a custom-made specimen holder to ensure a defined amount of abrasion per slice, in order to finally generate a dataset of cross-sectional images with exactly known distances in between. After image processing these datasets provide high resolution 3-dimensional (3D) information on the bony and soft tissue structures of the middle and inner ear, thus building a digital anatomical atlas.

The highly accurate registration of the images requires artificial markers, which are placed outside the structures of interest. All of these markers need to visible in the image, which requires a larger field of view, i.e. a lower magnification. This conflicts with the demand for high resolution images of the structures of interest. Therefore, a procedure has been developed to combine these conflicting needs, which is presented in the following.

2 Methods

Freshly cut human temporal bone specimens were used to generate the above mentioned datasets. They were dehydrated, embedded in epoxy resin, grinded and dyed with acid fuchsin, following the procedure described in [4]. As the accuracy of such an atlas depends on the fact that only structures within the present surface are visible but not below the grinding surface, a white coloured epoxy resin has been used. The abrasion distance was 100µm and the diameter of the marker 3.2 mm. Deviating from the original procedure, two images have been taken of each slice: one with a large field of view containing all markers and one at a high magnification showing only the structures of interest.

After the registration of the image stack using the artificially added registration markers, the resulting dataset was cut to the relevant structures of the inner ear. The next step was to rescale this dataset to reach the magnification level of the corresponding images that provide the high resolution information on the soft tissue structures.

The two images (low and high magnification) of each slice were registered using a point-based algorithm. The characteristic points were generated by the program “autopano-sift-c” (APSCcpp, enhanced Autopano-sift-c, version 2.5.1). Generally this software is used to join several photographs to form one panoramic picture. It is based on the SIFT algorithm (Scale-Invariant Feature Transform) [2], which identifies local features in every image and is invariant to rotation, scaling and changes in illumination. The program was called from a MATLAB script.
(MATLAB, Version 7.12.0, Mathworks, Natick, Massachusetts) and up to 100 control points per image pair were calculated. Addressing the possibility of duplicates and singular defective control points, the detected control points were reduced to ten, which were used in the registration of the corresponding images.

The registration of the images was performed through affine transformations, in this case translation, rotation and scaling. The fiducial registration error (FRE) has been used to evaluate the registration results [1]. Finally the generated datasets were converted into the DICOM standard.

3 Results

The study includes 15 embedded, dissected and digitalized specimens which could all be fused successfully. The postprocessing of a total of 2476 images was conducted according to the method explained above. The FRE for each image fusion was evaluated and averaged for each sample (see Image 2). Calculated over all images the FRE in pixels is 0.47 px respectively 2.08 µm.

The pixel spacing within the images before image fusion is 16.3 µm/px on average. After the image fusion the pixel spacing of the samples 1 to 14 is 4.6 µm/px on average whereas sample 15 reaches 2.9 µm/px due to a higher magnification used with the images of the structures of interest.

4 Conclusion

Datasets of images acquired through serial microgrinding – one with a large and the other one with a small field of view – were fused using the SIFT algorithm, which has been embedded in a custom-made MATLAB script. Due to the image fusion the achieved resolution is more than three times better than without it, which results directly in a more detailed representation of the structures of interest (see Image 3).

The invariance of the SIFT algorithm to rotation is especially relevant to the presented microgrinding process, since, due to the manual execution, the orientation of the specimens on the photographs may vary between the slices. During an additional experiment the images were fused twice – both times based on the same registered dataset – comparing translation, rotation, scaling and FRE for both fusion results. This showed a high reproducibility of the presented fusion process, which is a clear advantage over other methods for image fusion which are based on manual inputs and thereby can vary between different users.

The DICOM converted datasets are now available for use with commercial medical imaging and segmentation software to be applied for preoperative planning of the surgical approach for the cochlear implantation.

5 Acknowledgements

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6 References


Using Principal Component Analysis and IIR-Filtering for Detecting the Position of the Heart in Electrical Impedance Tomography at different Stimulation Frequencies

B Schullcke, A Javaherian, K Möller
Institute of Technical Medicine, Furtwangen University, VS-Schwenningen, Germany, sben@hs-furtwangen.de

Abstract

Data of four normal breathing volunteers were recorded using an EIT system. The data were collected at different belt positions and the stimulation frequency of the EIT system was systematically varied during recording. After filtering the collected data with an IIR filter and performing Principal Component Analysis (PCA) the position of the heart could be detected in all datasets. Calculating the centre of gravity demonstrates that the reconstructed position of the heart does not vary considerably at different stimulation frequencies.

1 Introduction

EIT is used to image the conductivity change within a body. Applied to the thorax EIT is used e.g. to monitor the regional ventilation of the lung. Beside noise there is also the heart activity that influences the reconstructed data of the voltage measurements at the surface skin. As the heart moves upwards and sideways when PEEP decreases the information about the position of the heart using EIT is valuable, since the weight of the heart can cause atelectasis and surfactant depletion in dorsal and caudal regions [1]. Due to the different frequency behaviour of cardiac and respiratory signals [2], the stimulation frequency of the EIT system was varied during data acquisition.

2 Methods

2.1 Data collection

Thousand five hundred EIT frames of four healthy volunteers were recorded with a Pulmo Vista 500 EIT system (Dräger Medical, Lübeck, Germany). The volunteers performed normal breathing in sitting position. Data were collected in three stimulation frequencies i.e. 80kHz, 105kHz and 130kHz. The frame rate of the EIT-System was set to 50frames/sec.

2.2 Filtering of data

To filter frequency dependent components out of a set of digital data an IIR-Filter can be used. In order to realize a sharp edge at the cutoff frequency a Chebyshev filter is approximated. Filter coefficients were calculated using Scilab (Scilab Enterprises, France).

To find the frequency components of the collected data a FFT-analysis was performed on every pixel. As expected the frequency components depend on the position of the analyzed pixel. At pixels which are located around the assumed position of the heart the FFT shows two peaks, one at the breathing frequency and another at the heart rate (Image 1).

Image 1: FFT of a pixel around the assumed heart position

The determined frequency of the heart can be used to design an IIR-Filter with a cutoff frequency between the breathing frequency and the heart rate. In order to investigate the possibility to detect the position of the heart, the filter is realized as a high-pass filter with a ripple of +/-3dB in the passband and <40dB in the stopband. Let $X'$ denote the filtered signal.

$$X' = IIR(X_{M\times N})$$

Where $M = 1, ..., 1024$ (number of pixel) and $N = 1, ..., 1000$ (number of frames).
2.3 Performing PCA

PCA is a multivariate statistical method which is used to extract the most relevant components out of a multidimensional dataset. By identifying similarities, redundancy and difference in data, PCA can be used to reduce the dimension of a dataset by focusing on linearly uncorrelated variables. It has already been presented, that the detection of cardiac related signals using PCA is possible [3].

Principal Component Analysis is applied using the covariance matrix of the filtered data $X'_{N\times N}$. This leads to the eigenvector $P_{N\times 1}$ which corresponds to the highest eigenvalue $\lambda$ of the covariance matrix.

$$cov(X') \cdot P_{N\times 1} = \lambda \cdot cov(X')$$

Applying the eigenvector $P$ to the non-filtered data by calculating

$$X_{heart} = X \cdot P$$

leads to an image that shows the position of the heart (Image 2). Clearly the left and the right ventricles can be identified.

For standardization reasons pixels corresponding to the heart are in the following characterized by a negative value.

Image 2: Position of the heart using PCA

3 Results

The position of the heart can be detected using IIR filtering and PCA. As it can be seen in Image 3 the heart shape and the position of the ventricles do not significantly depend on the applied stimulation frequency, as long as the frequency is in the range between 80kHz – 130kHz.

Calculating the centre of gravity of the heart related pixels shows the difference in heart position detection at above mentioned frequencies. As the heart position is characterized by negative values, pixels are assigned as related to the heart when:

$$x_{m,n} < \beta \cdot \min(x_{heart}), \quad \text{for } 0 < \beta < 1$$

The threshold factor $\beta$ was heuristically set to 0.2.

For volunteers 1-3 the values for the centre of gravity show a mismatch of maximum 1.4 pixels in horizontal direction and 0.3 pixels in vertical direction at frequencies between 80kHz and 130kHz. At this limited set of data no correlation regarding the mismatch and the stimulation frequency is observable.

The maximum difference at centre of gravity for volunteer 4 is 4.2 pixel in horizontal direction and 1.1 pixel in vertical direction, which is related to higher noise in the recorded EIT data.

Image 3: Position of the heart at different stimulation frequencies

Image 4: Centre of gravity of heart position for all volunteers

The above described procedure was performed at different thoracic levels, leading to results displayed in Image 5.
Heart detection at different thoracic levels

At 7th intercostal space the two ventricles can not be distinguished any more. Measurements at 3rd and particularly at 5th intercostal space of some volunteers clearly show both ventricles.

Applying only IIR filtering or only PCA seperately does not lead to comparable results. Also applying PCA on filtered data does not ensure that the position of the heart is visible.

Image 6 shows the effect of perfoming PCA on different data. Applying PCA on original data (upper) shows the lungs, as this area has the highest variance. When applying the eigenvector $P$ to the filtered data $X'$ as it is usually done in PCA (middle) the heart is only visible at the stimulation frequency of 130kHz. At stimulation frequencies of 80kHz and 105kHz the shape of the lung can be quite good extracted. This result varies with different volunteers. On some volunteers the heart shape is also visible at all stimulation frequencies. The best results regarding the position of the heart is achieved when applying the eigenvector $P$ to the non-filtered data $X$ as described above (lower). Then the heart shape is visible at all stimulation frequencies.

Data displayed in Image 6 was taken at 5th intercostal space.

4 Conclusion

Using PCA for detecting the position of the lung and the heart is possible. This may be used for optimizing image reconstruction techniques and making images easier to interpret.

Future tests will consider the relative change of conductivity of the heart to the lung and also distinguish between abdominal breathing and breathing via the chest. Also the mismatch of the reconstructed heart position using the described method and the real position using e.g. ultrasonography will be evaluated.

Further investigations regarding the dominance of the lung shape or the heart shape when applying the eigenvector $P$ to the filtered data $X'$ as shown in Image 6 is necessary, in order to identify the effect of perfusion of the lungs.

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6 References


Development and evaluation of passive autofocus algorithms in the field of automated microscopy

D. Erpenbeck¹, Th. Wittenberg¹, Ch. Münzenmayer¹, M. Benz²
¹Image Processing and Medical Engineering Department, Fraunhofer Institute for Integrated Circuits IIS, Erlangen, Germany, erpenbdl@iis.fraunhofer.de, michaela.benz@iis.fraunhofer.de

Abstract
This paper presents a comparison of different focus functions applied to microscopic images of blood smears. At the beginning of the article, standard methods for calculating the sharpness of an image are presented. In addition, these methods which are typically based on calculations for grayscale images are extended to the analysis of color images. Furthermore, the influence of calculating these metrics on small “Region of Interests” (ROI) instead of considering the whole image is investigated. For evaluating the results, the focus curves of several focus stacks have been analyzed. Therefore, some criteria for describing the quality of a focus curve are introduced as well. The evaluation shows, that especially in the far focus area the performance of sharpness calculations can be improved by considering color aspects. Furthermore, the choice of selected ROIs is confirmed by the acceleration of these calculations without loss of information.

1 Introduction
To diagnose and differentiate blood diseases, examination of blood preparations under the use of microscopes is essential and a standard procedure. Modern microscopes integrate computers, which opens the possibilities to apply image processing and pattern recognition tools in order to provide a computer-aided diagnosis (CAD) to the user. Thereby, it is essential that the automated microscopy system acquires well focused and sharp images as they are the basis for all further image analysis computations. The core step of an autofocus algorithm is to evaluate the sharpness of a given image. A variety of metrics can be found in literature and the crucial point is to choose the optimal metric for a particular field of application. In this contribution a comparison of different sharpness metrics for microscopic images of blood smears, as well as color variations of sharpness metrics will be presented.

2 Material and Methods
In this section first the image material used for evaluation and the means of image acquisition will be described. In addition, the investigated focus functions and their color extension will be explained. Finally, the evaluation method applied in this work will be described.

2.1 Material
Two applications in the field of hematology are considered here. The first one (sample 1) considers blood smears which are commonly used for blood differentials, displaying leukocytes and erythrocytes. For these blood smears May Grünwald Giemsa (MGG) staining is used. The second application (sample 2) are blood smears which are prepared with Giemsa staining for the diagnosis of Malaria and therefore can contain malaria parasites (plasmodia). For both applications the images have been obtained by a brightfield microscope (SCube, Fraunhofer IIS). In this type of microscopy, white light is transmitted through the sample [1]. Here, the sample is irradiated from below by a LED illuminator and the transmitted light is captured by a magnifying lens and a camera. Objects, such as cells, therefore, appear dark compared to the background (see Figure 1).

Figure 1 Comparison of dye agents (left: sample 1, MGG staining; right: sample 2, Giemsa staining).

Figure 2 Five images out of a z-stack acquired at different z position.

Using this equipment, 177 focus stacks (z-stacks) have been recorded. Each stack shows multiple images of one hematological scene at different z-Positions of the microscope. Hence a focus stack contains images that show the same scene but differ in sharpness (see Figure 2). In addition, this work distinguishes between focus stacks with a
large and a small step size of the z-coordinate (far focus and near focus image stacks). With a small step size, the variation in the sharpness is small between two images. A focus stack with a large step size however can contain strongly defocused images, where it’s hard to recognize any content.

2.2 Focus functions

In this section, state-of-the-art methods for the calculation of the image sharpness are presented. They can be divided in gradient based methods, histogram based methods and statistical techniques. Furthermore these grayscale methods are extended for calculations on color images.

2.2.1 State-of-the-art methods

Gradient based methods operate on the basis that in well focused images the image energy computed from edges is higher than in insufficiently focused scenes. The best method known from literature is the so-called Brenner Gradient [2]. It determines the gradient value between a pixel and a neighbor pixel, as e.g. given as:

\[ f_{\text{Brenner}} = \sum_H \sum_W (I(x + 2, y) - I(x, y))^2 \]  

(Eq. 1)

The variables H and W give the image height and width, while \( I(x,y) \) denotes the intensity of a pixel at position \((x,y)\). Only when the gradient value exceeds a certain threshold, the value contributes to the sum:

\[ (I(x + 2, y) - I(x, y))^2 > \vartheta \]  

(Eq. 2)

The standard method based on a statistical measure uses the global image variance to determine the sharpness of an image and is calculated as follows [2]:

\[ f_{\text{Variance}} = \frac{1}{HW} \sum_H \sum_W (I(x,y) - \bar{I})^2 \]  

(Eq. 3)

Here, \( \bar{I} \) denotes the mean intensity of the image.

The entropy of an image is a histogram based technique and its value describes the amount of information in this image. The better an image is focused, the more information is recognizable in the image [3]. The following equation shows the calculation [2]:

\[ f_{\text{Entropy}} = -\sum_{i=256}^{255} p_i \log_2(p_i) \]  

(Eq. 4)

The variable \( i \) corresponds to the number of histogram bins (default 256) and \( p_i \) stands for the relative frequency of a gray value \( i \). \( p_i \) itself is calculated by the following equation:

\[ p_i = \frac{h(i)}{HW} \]  

(Eq. 5)

The number of pixels with gray value \( i \) is characterized by \( h(i) \).

2.2.2 Extensions for analyzing color images

Traditionally, the standard focusing methods work with gray values. That means, if color images are used, a conversion to a grayscale image is needed before applying the sharpness metrics. One way to make better use of the color informations is to analyze every RGB color channel separately instead of mixing up the information within a grayscale conversion. Another possible extension is to operate on the channels of the HSV color space which showed to perform better than the RGB values. The HSV color space has been designed to represent the perception of colors in a way more descriptive to human perception [6] by hue, color saturation and intensity (value). In contrast to the RGB space, the individual color channels correlate less strongly with each other. The exact conversion between RGB and HSV color space can be found in [6].

A further possibility is to use customized grayscale conversions. One technique applied in this work is derived from a method for the detection of leukocytes and erythrocytes. It was shown that the ratio of green and blue channel is a convenient feature for the segmentation of cell nuclei [4]. When plotting the green and blue values of leukocyte cell nuclei and cytoplasm as well as erythrocytes against each other, the separation of the cluster nucleus and the cluster cytoplasm/erythrocytes can be reached by a line through the origin which is described by the angle between this line and the x-axis [4]. This angle can be determined from the arctangent of the ratio between the x and y axis. Hence, the following transformation is performed before applying the sharpness metrics:

\[ I_p(x,y) = \frac{\arctan(I_{\text{green}}(x,y))}{\arctan(I_{\text{blue}}(x,y))} \times 255 \]  

(Eq. 6)

\( I_p \) denotes the new pixel value which is afterwards used for the sharpness calculations. \( I_{\text{green}} \) and \( I_{\text{blue}} \) are the green and blue values of the pixel \((x,y)\).

2.3 Determination of selected ROIs

Besides the investigation of the color aspect, the influence of calculating the image sharpness on selected ROIs only instead of considering the whole image has been analyzed as well. Therefore, two types of ROIs were used. For focus stacks with a large step size, four rectangular ROIs were placed around the image center as shown in Figure 3. For the images of focus stacks with a small step size, it is possible to perform a leukocyte (Figure 4a) or plasmodia (the malaria parasite) detection (Figure 4b) beforehand. The resulting ROIs (see Figure 4) form the basis for generating the selected ROIs, which are represented by a rectangular box around its center of gravity. Thus, the number of selected ROIs depends on the amount of detected leukocytes or plasmodia in the image.
Once the ROIs have been defined, the sharpness measure is calculated for each ROI. The total image sharpness can then be determined by the average value of all detected ROIs.

2.4 Evaluation methodology

In order to evaluate the quality and suitability of a sharpness measure, the focus curves of the sharpness metrics are analyzed. A focus curve is obtained by calculating the sharpness for each image of a focus stack (see Figure 5). The "width" of the focus curve is one of the applied evaluation criterions. A sharp peak of the focus curve is preferred for an autofocus algorithm in the near focus area [3]. Therefore, sharpness metrics resulting in focus curves with a small width should be chosen.

The second criterion that was used in this work is called "accuracy" and is an indicator how well the maximum of the focus curve corresponds to the sharpest image of the stack [3]. For this purpose, a number of five test persons determined the position of the sharpest image for every focus stack with small step size. From all the judgments, the average value was calculated, which formed the ground truth. The accuracy is determined by calculating the distance between the ground truth and the focus curves maximum position. The smaller this distance, the more accurate is the localization of the focus position using this metric. For all considerations, emphasis was put on the accuracy criterion, which, however, can be error prone because it is based on subjective judgements of the test persons.

In addition to these criteria, the computation time was also considered. A powerful autofocus algorithm must come to the best focus position very quickly and in almost real-time. Therefore, a fast calculation of the image sharpness is absolutely necessary.

3 Results

All methods described have been applied to 61 focus stacks with small step size (0.3 µm) and 37 focus stacks with large step size (5 µm) of sample 1. Furthermore 50 focus stacks with small step size and 29 focus stacks with large step size of sample 2 have been evaluated. Every focus stack contains in total 15 images at different z-positions. The detailed results are described in [7].

The analysis of the focus stacks of sample 1 (MGG staining) showed that for a small step size, on average, the Brenner Gradient works best. Furthermore, due to the higher contrast between blood cells and background (see Figure 6), green channel images are also suited much better than using the red or blue channel images.

It has nevertheless been shown that in this case it is still best to compute the sharpness with gray values. This can be explained by the standard grayscale conversion. Here, the green channel gets the most emphasis[5] as it is shown in Equation 7. In addition, the analysis shows that the blue channel again works worse than the red channel.

\[
I = 0.229I_{\text{Red}} + 0.587I_{\text{Green}} + 0.114I_{\text{Blue}} \quad \text{(Eq. 7)}
\]
The results also revealed that the HSV color space should be used when working with a large step size within a focus stack. The S-channel results in a fast loss of information across the focus stack, which can be recognized clearly by applying the entropy or variance metric. The results for the stacks of sample 2 differ due to their different staining. Although the two just mentioned methods again perform well (whether small or large step size), the conversion from Equation 6 proved to be a good alternative to derive a grayscale image and to use it as input image for the calculation of the Brenner Gradient or entropy and variance. The results for both small and large step sizes were rather precise. The studies on the determination of the sharpness based on selected ROIs showed that working with ROIs is much faster than calculating the sharpness on the whole image. Additionally, it was important to see that working with pre-selected ROIs has no influence on the quality of the sharpness metrics, although smaller image sections were considered (as long as the selected ROI depict content, and not only white background). For observations with small step sizes, the calculations on ROIs were even more accurate. For this, however, a leukocyte and plasmodia detection is necessary, that adds to the computation time. Nevertheless, in the far focus case the calculation is performed on predefined ROIs resulting in a faster autofocus algorithm. If the microscope lens at the beginning of the focus search is far away from the focus position, i.e. the images are still very blurred (this was simulated in the work by focus stacks with a large step size), predefined ROIs could be used. Applying this methodology will speed up the process to find the near focus area.

4 Conclusion

Recapitulating the results of this work shows that the introduced color extensions yield better results than the common sharpness metrics especially for the large step size focus stacks. For the field of blood smear examination (especially when focus stacks with a small step size respectively sharp images of sample 1 are present) the evaluation of green channel images or images with a corresponding thereto weighted greyscale conversion show very precise results. Accordingly, for the images of sample 2 the transformation of Equation 6 is a good variant for grayscale conversion to obtain accurate results. For stacks with a large step size, even the change to the HSV color space may improve the results. Furthermore, the quality of the results depends strongly on the application and the chosen calculation method (e.g. Brenner Gradient performs best in case of small step size sample 1 images whereas Entropy/Variance based on HSV values yields the best results for large step size focus stacks of both samples). Therefore, it can be noted that for an automated microscope system that should handle different fields of application, an individual sharpness metric for each addressed application should be used for the autofocus algorithm. For this purpose, the scope should be extended by further studies (e.g. for the field of histological tissue sections or bone marrow samples).

A further improvement of autofocus algorithms can be achieved by working on ROIs. As shown in the results section, calculating the image sharpness on small sub images for focus stacks with a large step size accelerates the determination of the sharpest image without a loss of quality. Depending on the application, the size and number of ROIs can be optimized. Selecting ROIs containing leukocytes and plasmodia for images near the focus position (simulated in this work by focus stacks with a small step size) yields better result but has the disadvantage that a potentially time-consuming detection step is necessary.

5 Acknowledgement

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6 References

Assessment of the plant health status based on hyperspectral and color image analysis towards the cGMP-compliant large-scale production of biopharmaceuticals in plants

C. Dach¹, D. Erpenbeck¹, Th. Rademacher², A. Reimann², Th. Wittenberg¹, Ch. Münzenmayer¹, M. Benz¹
¹Image Processing and Medical Engineering Department, Fraunhofer Institute for Integrated Circuits IIS, Erlangen, Germany, michaela.benz@iis.fraunhofer.de
²Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Aachen, Germany

Abstract

Plant-based systems offer a promising alternative for the production of biopharmaceuticals and, just like classical production systems, have to comply with cGMP regulations if the biopharmaceuticals are intended for human use. To allow a more robust and reproducible process for the large-scale production of pharma plants in a novel automated plant production facility at the Fraunhofer IME a monitoring system for the assessment of the plant health status was evaluated. Here we describe preliminary results of a plant monitoring system for the automatic detection of chlorotic and necrotic areas on tobacco leaves. Different analysis methods based on hyperspectral and RGB color images have been investigated to decide whether a hyperspectral monitoring system is required for the given application or not. The evaluation on 204 leaf samples shows that all tested methods are suitable for the detection of necrotic and chlorotic areas and therefore the hyperspectral monitoring system can be reduced to a more cost-saving setup consisting of conventional RGB cameras.

1 Introduction

Since the late 1980s [1] plant-based production systems emerged as promising alternative for the production of biopharmaceuticals offering advantages such as natural resistance to human pathogens, low production costs and high scale-up potential [2]. Furthermore, the recent approval of Elelyso [3], a glucocerebrosidase produced in transgenic carrot cells for the treatment of Gaucher disease, proved the suitability of plant-based systems for the commercial production of biopharmaceuticals. Within the Fraunhofer Malaria-Vaccines Project, a multidisciplinary project of the Fraunhofer Future Foundation focusing on the development of novel vaccine candidates and their cGMP-compliant large-scale production in an automated, plant-based production facility, a monitoring system for the assessment of the plant health status was evaluated. Here we describe preliminary results of a plant monitoring system that is able to automatically detect chlorotic and necrotic areas on tobacco leaves. Thereby various image analysis approaches based on hyperspectral and RGB color images have been investigated.

2 Material and Methods

The following sections describe the data acquisition, preprocessing and analysis methods applied to determine the health status of the tobacco leaves.

2.1 Material

To mimic potential plant diseases as basis for the computer-assisted assessment of the plant health status different necrotic and chlorotic areas were artificially induced on Nicotiana tabacum and Nicotiana benthamiana leaves by applying different stress stimuli to the leaves, such as sodium hydroxid solution, herbicide (phosphinotricyn), antibiotic (kanamycin) and hot needles.

![Figure 1: Hyperspectral measurement setup with a Nicotiana tabacum leaf placed on the positioning stage for measurement.](image_url)
a transmission spectrograph (IST-VIS 0.38100, inno-spec GmbH) and an active cooled CCD camera (Exi Aqua, Q-Imaging). For the measurement the leaves were cut off the plants and placed on a positioning stage where they were fixed by clamps (see Figure 1). The hyperspectral data cube is obtained by a relative movement between the leaf and the hyperspectral camera acquiring at each position one measurement of 1392 wavelength bands for each pixel of the 1040 pixels in one spatial raw. The total number of measurements depends on the size of the leaf. A typical amount of measurements are 700 per leaf resulting in a hyperspectral data cube of 1392 x 1040 x 700 values. For this study a total amount of 204 tobacco leaves have been recorded. As an example in figure 2 the spectra of different leaf areas (healthy, chlorotic and stem) are depicted.

![Figure 2: Comparison of mean spectra from selected regions in one hyperspectral measurement of one Nicotiana tabacum leaf. The selected regions are marked in a derived RGB color image.](image)

### 2.2 Data pre-processing

In order to obtain appropriate classification results, image pre-processing steps such as radiometric correction, masking and smoothing (Savitzky-Golay [4]) were conducted. For visualization and color image analysis, RGB images were derived from the pre-processed hyperspectral data (see Figure 2, left column). The three-channel-computation was based on CCD-sensitivity curves ranging from 400 to 700 nm integrating all measured intensity values in this range weighted by the corresponding normalized sensitivity of a conventional RGB CCD camera.

### 2.3 Data analysis and classification

In the following sections four different image analysis approaches will be described. Two methods are based on the hyperspectral data and two on the RGB color images, extracted from the hyperspectral image data. All methods have in common that they compare a certain query vector to a set of pre-classified reference vectors representing healthy and stressed states of the leaves. Hence a graduation for each pixel is derived to belong to one or the other of this two classes. Hereby the class “stressed state” sums up chlorotic as well as necrotic pixels. Afterwards the graduations of the two classes “healthy” and “stressed” are visualized in one representative picture by applying the cross table method referred to in Table 1.

#### Table 1: Cross table used for visualization of the classification results.

<table>
<thead>
<tr>
<th>Region</th>
<th>stressed grade 2</th>
<th>stressed grade 1</th>
<th>stressed grade 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>healthy grade 0</td>
<td>very stressed</td>
<td>stressed</td>
<td>transient region</td>
</tr>
<tr>
<td>healthy grade 1</td>
<td>stressed</td>
<td>transient region</td>
<td>healthy with deviation</td>
</tr>
<tr>
<td>healthy grade 2</td>
<td>transient region</td>
<td>healthy with deviation</td>
<td>healthy</td>
</tr>
</tbody>
</table>

### 2.3.1 Hyperspectral analysis

Distinguishing the health status of tobacco leaves based on hyperspectral image data was approached by two different methods: (1a) spectral angle mapper (SAM), and (1b) the computation of vegetation indices.

SAM (method 1a) is a physic-based spectral classification that interprets a spectral signature consisting of n bands as an n dimensional vector and calculates the angle between a query vector and a reference spectra to decide how good they match (a value of zero indicates perfect match). In this study three sets of different mean reference spectra (representing ‘healthy’, ‘chlorosis’, ‘necrosis’) were obtained by averaging various representative leaf samples. The algorithm determines the spectral similarity between two spectra by calculating the angle between the spectra and treating them as vectors in a space with dimensionality equal to the number of bands (here 1000 bands were selected out of 1392 from the original measurement) [5]. Based on the similarity to the reference vectors, different grades were defined for the two classes “healthy” and “stressed”. Thereby it was not differentiated between “chlorotic” or “necrotic” – all pixel with similar spectra to the “chlorotic” or “necrotic” reference spectra were classified as “stressed”. The classification results were then visualized by applying the cross table method (see Table 1 and Figure 3, center column).

The second method (1b) that was applied is based on vegetation indices (VIs). VIs are combinations of reflectance values at two or more wavelengths and can be indicators for different parameters of leaf health state such as greeness, water content, leaf pigments or “Light Use Efficien-
More than hundred VIs can be found in scientific literature but only some have a substantial biophysical basis. Two well-established greenness indices are for example the “Normalized Differentiation Vegetation Index” (NDVI) [6] (see Equation 1) and Red Edge Normalized Difference Index (mND705) [7] (see Equation 2):

\[
NDVI = \frac{R_{860} - R_{680}}{R_{860} + R_{680}} \quad \text{(Equation 1)}
\]

\[
mND_{705} = \frac{R_{750} - R_{705}}{R_{750}} \quad \text{(Equation 2)}
\]

where \( R_\lambda \) stands for spectral reflectance values measured at the wavelength \( \lambda \) (measured in nanometers). Both take advantage of the high reflectance difference in the visual spectrum (low because of chlorophyll absorption) and near-infrared spectrum (high because of mesenchyme scattering) and were chosen in this study for further classification.

Similar to method 1a, the ratings of the two indices are depicted in one picture using the cross table method. The classification into the both classes “healthy” and “stressed” was conducted by using thresholds that were derived from a reference dataset.

### 2.3.2 Color image analysis

Furthermore, the health assessment of tobacco leaves was performed by analysing the three-channel RGB color images that were derived from the hyperspectral data. Again, two different methods were applied: (2a) color angles relative to reference colors, (2b) Principal Component Analysis (PCA).

Method 2a calculates for each pixel the angle between its RGB color vector and the RGB reference vector of each of the three clusters (‘healthy’, ‘chlorosis’ and ‘necrosis’) based on the scalar product of the according normalized vectors [8]. This approach is similar to method (1a, SAM), whereas the dimensionality of the color space is now reduced to three. The RGB reference values were obtained from several representative leaf samples that were annotated manually. Similar to the SAM (method 1a), for each pixel on a leaf different grades were defined for the two classes “healthy” and “stressed” based on the calculated angles with respect to the three reference clusters. Again the cross table method is applied and the visualization of these results in one image can be seen in Figure 4.

Method 2b uses the Principal Component Analysis (PCA) [9] for analysing and determining the distance of the RGB-color vector of each pixel to the three reference clusters (‘healthy’, ‘chlorosis’ and ‘necrosis’). In this case, for distinguishing the health statuses of each pixel of a leaf, all calculations were performed in the HSV color space. Therefore, all image data was converted from the RGB to the HSV color space [10]. Afterwards all calculations are performed using the cartesian representation of the cylindric HSV coordinates. The color vector of each pixel is transformed into the Eigensystem of each reference cluster and scaled with the according Eigenvalues. Afterwards, the distance to the cluster center is calculated. This distance is taken as a measure of similarity. Subsequently for each pixel different grades of “healthy” and “stressed” are defined and the cross table method can be applied. The results are shown in Figure 4.

### 3 Results

All four methods have been applied to 204 tobacco leaf samples and the results have been compared qualitatively. Two leaf samples and the classification results obtained by each of the four methods are depicted in Figures 3 and 4. The results differ in the detected degree of stress, but all four examined methods recognize the deviation from the healthy status reliably for all 204 leaf samples. Considering all 204 samples the methods 1a and 2a based on spectral respectively color angels (SAM) obtained slightly better results than the both other methods. Method 1b based on vegetation indices tended to judge stressed regions too positive whereas method 2b (PCA) judged slightly too negative. Nevertheless, the graduation can be influenced for each of the methods by the choice of thresholds and can be adapted.

One difficulty arises for all of the methods from the similarity of the spectra of chlorotic areas and the spectra of pixels belonging to the leaf stem. Therefore these pixels are often misclassified as “stressed” (see Figures 3 and 4).
Fig. 4: Health assessment of two leaf samples (left: RGB visualization; middle: classification result based on the calculation of color angles; right: classification result using PCA).

4 Conclusion

The comparison of the hyperspectral data analysis versus the RGB image data analysis revealed that the RGB data is sufficient for the robust detection of necrosis and chlorosis within the presented application. Therefore, a hyperspectral measurement setup is not required and thus could be replaced by a significantly more cost-efficient setup consisting of a conventional RGB camera instead of a hyperspectral camera. The integration of such a monitoring system into a large-scale plant production facility will provide a more robust and reproducible basis for the assessment of the plant health status and ultimately facilitate to establish a cGMP-compliant production process.

Regarding the misclassification of pixels belonging to the leaf stem further investigations are required. Neither color nor spectral information alone have been able to differentiate robustly these areas from chlorotic areas. Therefore, knowledge-based methods that include spatial information and identify the position of the leaf stem in advance e.g. based on symmetry considerations have to be investigated.

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6 References

Gaussian mixture models for unsupervised classification of perfused blood vessels in intraoperative thermography

N. Hoffmann¹, Y. Radev², J. Hollmach¹, C. Schnabel¹, M. Kirsch², G. Schackert², U. Petersohn¹, E. Koch¹, G. Steiner¹

¹Technische Universität Dresden, Klinisches Sensoring und Monitoring, Dresden, Germany, nico.hoffmann@tu-dresden.de
²Technische Universität Dresden, Klinik und Poliklinik für Neurochirurgie, Dresden, Germany
³Technische Universität Dresden, Angewandte Wissenverarbeitung, Dresden, Germany

Structure: 1. Introduction / 2. Vessel classification / 3. Results / 4. Conclusion

Abstract

Thermography allows real-time and high-frequency capturing of small temperature variations of the exposed cortex during neurosurgical operations. One cause of temperature gradients depicts the cerebral blood flow, which leads to cyclic temperature variations. We now propose a unsupervised method to identify perfused blood vessels from thermographic image sequences by their characteristic pattern. For this purpose we employ the discrete wavelet transform on thermographic sequences and analyze its wavelet coefficients by a Gaussian mixture model. This allows the classification of cortical vessels for the analysis of cortical blood flow and correlation with white light imaging. The proposed approach is further independent of haemodynamic parameters, resulting in a fast and robust scheme for intra-operative use.

1 Introduction

Thermal imaging is a contactless, marker-free, white light independent and non-invasive method for online measurement of temperature variations up to $30\,\text{mK}$. Current generation devices use infrared microbolometer focal plane arrays (FPA) sensing temperature variations at spatial resolutions of $250\mu\text{m}$ at 50 frames per second. These FPAs detect the electromagnetic radiation in the long-wavelength infrared range ($7.5 – 14\mu\text{m}$ wavelength). A black body with a temperature above $0\text{K}$ emits electromagnetic radiation in the long infrared spectral range. The power of this radiation depends on the temperature (Stefan-Boltzmann law) and in case of non-black body objects on its emissivity. The infrared radiation is then computed into temperature values and stored into a three-dimensional data cube. This data cube contains the spatial and time-resolved temperature distribution of the recorded scene.

The registered temperature variations are caused not only physiologically but also by extrinsic and environmental effects. In medical application, physiological causes are assumed to support the diagnosis of pathologies, like fever, breast cancer and vascular disorders [1]. In bransurgery the causes of temperature variations can be divided into perfusion- and neuronal activity related heat transfers. Thus, thermography has been employed for the detection of functional areas and brain tumors [2],[3]. Because of its time resolution, thermography allows inference of diagnostic information like triggers of focal epilepsies or to distinguish functional from pathological tissue. In a previous work, we have shown that there’s a direct link between temperature gradients and an injected cold bolus (ice-cold saline solution), which was used to quantify the cerebral blood flow [4]. This method enables the analysis of the cortical perfusion under cerebral ischemia. However, thermal images are usually characterized by low gradients and exhibit weak constraints of morphological features (see Image 1). Also boundaries do not necessarily correspond to morphological edges as seen in visible light and show dynamic behaviour due to heat transfers in several frequency bands. During OP the surgeon usually has an augmented microscopic view to the cerebral cortex enabling the matching of white light images with for example fluorescence microscopy. In this work, we demonstrate a method to extract vessels from thermographic recordings, by exploiting blood flow specific artifacts. This approach depicts the first step towards the unsupervised multimodal image fusion of white light or microscopic to thermal images. It is further possible to monitor the perfusion state of vessels without any contrast agent.

2 Vessel classification

This section at first focuses on the temperature variations of a single pixel and how to exploit its characteristics. Afterwards we develop a mechanism to automatically identify vessels in thermographic image sequences. Each pixel’s timeseries of temperature values typically inhibit a non-stationarity nature while it is influenced by time varying frequencies with differing amplitude and period. Therefore we employ the wavelet transform making the multiscale analysis of signals with non-constant frequency components tractable. Hereby we are able to detect dynamic frequency components at specific points in time.
2.1 Discrete wavelet transform

The one-dimensional wavelet transform decomposes a given signal \( x[t] \) into a space spanned by the signal convolved with some chosen base vectors (wavelet) \( \psi \). The wavelet’s shape and scale mainly determine its time-frequency resolution. Small scales result in coarse frequency localization capabilities but good time localization.

The discrete wavelet transform (DWT) of a discrete-time signal descibes a computationally efficient method for expanding \( x[t] \) as wavelet series composed of weighted time localized wavelet functions. Describes this transform as decomposition of \( x[n] \) onto \( J + 1 \) scales \( j = 0, \ldots, J \) and \( k \in \mathbb{Z} \) sampling points, as of

\[
x[n] = \sum_{j=0}^{\infty} \left( \sum_{k} c_{j,k} h[n - 2^j k] + \sum_{k} b_{j,k} g[n - 2^j k] \right)
\]

(1)

\[
c_{j,k} = \sum_{n} x[n] h^*[n - 2^j k]
\]

(2)

with \( c_{j,k} \) being detail wavelet coefficients and \( b_{j,k} \) being approximation wavelet coefficients with corresponding “synthesis wavelet” \( h_j[.] \) and “scaling sequences” \( g_j[.] \).

The fast discrete wavelet transform algorithm is a fast implementation of the DWT that applies the transformation by successive decimation and convolution operations. The frequency filter characteristics are mainly determined by the chosen discrete wavelet. The transform results in a multi-scale analysis of \( x[n] \), wherein each scale \( j \) spans a subspace \( V_j \) with

\[
L^2(\mathbb{R}) \supset V_J \supset V_{J-1} \supset \ldots \supset V_0 \supset 0
\]

(3)

Each subspace contains information about frequency components at half nyquist frequency of the superordinate subspace. This allows the time localized characterization of dynamic events.

2.2 Modeling wavelet coefficients

The wavelet transform depicts a multi-resolution analysis of a signal. At scale (frequency band) \( j \) there are \( K = 2^j < n \) time localized wavelet coefficients to be evaluated. In comparison the fourier transform characterizes each frequency by one single coefficient, hereby hampering inference about dynamic signals.

Since wavelet coefficients at scale \( 1 \leq j \leq J \) originate from decimating \( x[n] \), we have to adjust the heart rate with respect to scale \( j \)

\[
f_{HR} = f_{HR} : f_s/(J - j + 2)
\]

(4)

with sampling rate \( f_s \) and heart rate \( f_{HR} \). Using equation (4) we can describe the squared wavelet coefficient of scale \( j \) at time \( k \) as

\[
w_{j,k}^2 = s \sin^2 \left( 2\pi k / f_{HR}^j \right)
\]

(5)

Image 2 Sum of squared wavelet coefficients at \( j_{HR} \).

Pixels with high values exhibit high activity in the heart rate frequency band. The strongest activity reveals a structure that resembles the vessels of Image 1.

2.3 Cluster analysis of wavelet coefficients

Using the described model we now propose a Gaussian mixture model for classification of pixels with cyclic patterns. Gaussian mixture models (GMM) depict a well understood approach and provide a good compromise between fast computations and model capabilities. The general definition of a GMM is given as follows:

\[
p(w_j|\theta) = \sum_{i} \pi_i p_i(w_j|\theta_i)
\]

(6)

with \( w_j \in \mathbb{R}^K \) describing all wavelet coefficients at scale \( j \), \( \theta = \{ \theta_i | i \geq 1 \} \) with \( \theta_i = \{ \mu_i, \Sigma_i, \pi_i \} \) consisting of
mean vectors $\mu_i \in \mathbb{R}^K$, covariances $\Sigma_i \in \mathbb{R}^{K \times K}$ and component weights $\pi_i$. As we expect only heart rate artifacts and background activity at scale $j$ we model two components. Component 1 depicts the heart rate pattern with mean $\mu_1 = \sin^2(2\pi f/\tilde{f}_{HR})$ of equation (5) and covariance matrix $\Sigma_1 = \sigma_1 I_K$. The background component has mean $\mu_2 = 0$ and covariance matrix $\Sigma_2 = \sigma_2 I_K$.

By omitting the squares, equation (5) also allows the introduction of specific phase parameters, allowing further differentiation between cortical arteries and veins.

The model is fitted to the data using the expectation maximization (EM) algorithm [7]. The EM algorithm is a well developed method for parameter estimation of models with unobservable variables $Z$. It estimates the parameter vector $s \theta$ and hereby the segmentation $Z$ by iteratively maximizing the data likelihood with respect to $\theta$ and $Z$. Derivations in the case of gaussian mixture models are described elsewhere [8].

3 Results

The method has been tested on a set of intra-operative recordings, which originated from patients with brain tumors. The thermal camera was mounted on the operation table, which only allows the application of uncooled thermal cameras that unlikely induce a temperature drift into the data. The temporal drift is compensated by modeling this low-frequent time behavior by a smooth penalized spline with polynomial basis functions.

Since equation (5) depends on the heart rate $\tilde{f}_{HR}$, we have to estimate it. Let $\mathcal{F}(f)_+$ denote the maximum fourier coefficient of frequency $f$ computed at $m$ pixels as of

$$\mathcal{F}(f)_+ = \max \{ \mathcal{F}(f)_1, \mathcal{F}(f)_2, \ldots, \mathcal{F}(f)_m \} \quad (7)$$

the heart rate can be recovered by solving

$$\tilde{f}_{HR} = \arg \max_{f \in [0.5, 2]} \{ \mathcal{F}(f)_+ \} \quad (8)$$

The origin and progression of cerebral heat transfers are not well understood as there is no deterministic model about future temperature gradients. The heat transfers typically induce spatially and temporally varying components below 0.1 Hz. Another component of thermographic timeseries depict heart rate and respiratory activity related artifacts. During OP, the heart rate can vary by up to 10 beats per minute depending on the pathology whereas the respiration rate is quasi-constant.

Cortical vessels located in the subarachnoid space move on a pixel to subpixel level at pulse rate inducing specific temperature variations in the respective time series which are measured and allow the segmentation of the affected pixels. In image 3, the segmentation result of detected heart rate pattern is shown in red. The structured elements turn out to match vessels as seen in the white light image. The detected vessels are a subset of the visible vessels. This is caused by the fact, that smaller vessels are located on top of the cortex and thus motion below the spatial resolution or temperature gradients below camera sensitivity might occur. We further expect the periodic motion pattern to occur dominantly at pixels of cortical arteries, whereas veins are less affected.

Yet the method reveals vessel structures making following multimodal image fusion possible. Comparing to white-light imaging, further quantification and evaluation of the cerebral blood flow gets possible without injecting any fluid just by passively recording the cortical temperature variations. In case of aneurysm or cerebral ischemias this knowledge could be used to infer information about the pre- and post treatment perfusion state of vessels.

4 Conclusion

The temperature of blood vessels exhibits a characteristic pattern of cyclic temperature variations caused by cerebral blood flow. For analysis, the wavelet transform is applied to account for non-stationary frequencies. The subsequent classification of the cyclic pattern is done by a computationally efficient 2-Gaussian mixture model. This model is fitted to the data by the expectation maximization algorithm. The classification result depicts a map of blood vessels located in the subarachnoid space. The approach provides a fast and scalable solution to the identification of vessels in temperature time series without the need to pre-specify any haemodynamic parameters.

In further studies, this map will be employed to infer the arrival time and characteristic pattern of thermal tracers (cold bolus approach as demonstrated in [4]) in order to quantify its spatio-temporal distribution. Another application depicts the image fusion of thermal images with white light and microscopic views using the determined vessel structure. In white light imaging thresholding wrt. to the vessel’s color characterics yields a binary representation consisting of vessels and background. By non-linear registration methods the extracted vessels of the thermographic- and white light image can then be joined providing a transformation matrix allowing the unsupervised fusion of both modalities. This enables monitoring the perfusion state of blood vessels by a combination of blood flow driven ther-
mographic sequences with morphological information from white light images.

References


Single-mode fiber based polarization sensitive swept source optical coherence tomography using alternating sweep polarizations

L. Kirsten¹, P. Rottmann¹, A. Burkhardt¹, M. Gaertner¹, J. Waltherr¹,², E. Koch¹
¹Technische Universität Dresden, Faculty of Medicine Carl Gustav Carus, Clinical Sensing and Monitoring, Dresden, Germany, lars.kirsten@tu-dresden.de
²Technische Universität Dresden, Faculty of Medicine Carl Gustav Carus, Medical Physics and Biomedical Engineering, Dresden, Germany


Abstract

In comparison to standard optical coherence tomography (OCT), polarization sensitive OCT provides an additional image contrast revealing optical properties of tissue, which supports the differentiation of tissue and extends the diagnostic potential of OCT. In this study, a polarization contrast is obtained using a standard single-mode fiber based swept-source OCT-system with a buffered Fourier domain mode locked laser, where the wavelength sweeps are adjusted, in order to exhibit an alternating polarization. The sweep polarization is measured using a self-made high-speed polarimeter. This approach of utilizing a buffered laser source provides the advantage that a polarization contrast can be realized in standard OCT-systems without any complex extensions of the OCT-system. An imaging example illustrates the polarization contrast obtained from the alternating sweep polarization.

1 Introduction

Optical coherence tomography (OCT) is a non-invasive imaging technique, which provides two- and three-dimensional images with a spatial resolution in the range of 10 µm. Broadband light in the near-infrared spectral range is used for the measurement of interference spectra utilizing an interferometer, where reference light is superimposed with light, which is reflected or backscattered from the sample. In optical frequency domain imaging, also called swept source OCT, interference spectra are acquired temporally resolved using a tunable laser, whose wavelength is swept repetitively. Since fast tunable lasers, like Fourier domain mode locked lasers [1], are available, swept source OCT is one successful approach for high-speed imaging with a high temporal resolution.

Standard OCT provides information about the intensity of the light, which is backscattered from the sample and interferes with the reference light. A functional extension is polarization sensitive OCT (PSOCT), where information about the polarization of the light is evaluated for visualizing polarization changing properties of tissue, for instance birefringence. This information reveals an additional image contrast, which can facilitate the differentiation of biological tissue. However, conventional PSOCT requires an extended OCT-setup, which allows the detection of polarization properties of the light. This is mostly realized either by using polarization diverse detection [2-4], which requires a more complex optical setup, or by using polarization modulating/frequency multiplexing techniques [5, 6]. Also polarization maintaining fiber based buffered Fourier domain mode locked lasers have been suggested as potential light sources for PSOCT [7]. All these techniques require modification of the standard OCT-setup, for instance by employing polarization maintaining fibers, active optical elements or multiple detection channels.

Swept source OCT-systems provide the advantage, that in buffering setups [8] an alternating polarization of the laser can easily be adjusted, which can be used to obtain a polarization contrast using standard swept source OCT-systems without modifying the optical setup and without using polarization maintaining fiber based components. In this preliminary study, a buffered Fourier domain mode locked laser was used to realize PSOCT imaging in a standard, single-mode fiber based OCT-system. The measurement of the manually adjustable alternating polarization states was carried out using a self-constructed high-speed polarimeter.

2 Methods

2.1 OCT-system

The OCT-system utilizes a Fourier domain mode locked (FDML) laser as a swept laser source, which is depicted in image 1. Due to the single-mode fiber spools (km) inserted into the ring resonator, the optical round trip time is 16.7 µs corresponding to a round trip frequency of 60 kHz. The wavelength in the ring is swept sinusoidally over a total sweep range of 120 nm around a center wavelength of 1300 nm using a tuning frequency, which is nearly matching the optical round trip frequency. A semiconductor optical amplifier initializes the laser activity and amplifies the light, which is transmitted by the Fabry-Perot filter. During one period, a forward sweep with increasing wavelength and a backward sweep with decreasing wavelength are generated. The buffered FDML principle [8] is used to
replace the backward sweep with a copy of the forward sweep, which exhibits a lower intensity noise and better signal-to-noise ratio for OCT-imaging. First, the backward sweep is suppressed by modulating the amplifier current synchronously with a rectangular waveform. The remaining forward sweep is coupled out at fiber coupler FC1. Out coupling additionally at fiber coupler FC2 yield a copy of the forward sweep, which is delayed by one half of the round trip time due to the single-mode fiber between FC1 and FC2. Thus, the two outputs FC1 and FC2 can be combined with fiber coupler FC3 providing a total sweep rate of 120 kHz with two alternating sweeps. Since the polarization of these alternating sweeps can be adjusted differently using manual fiber polarization controllers (PC), the buffering principle can be utilized for achieving a polarization contrast during OCT-imaging.

The single-mode fiber based high-speed OCT-system is sketched in image 2 and was described previously [9]. The laser light is propagating via a circulator to the scanner head which incorporates a modified Michelson interferometer. The reflected or back scattered light interferes with the light from the reference arm and propagates to a balanced detector. A Mach-Zehnder interferometer is used as a reference interferometer allowing rescaling the interference spectra in wave number domain and correcting the jitter between subsequent sweeps numerically. Both interference spectra are acquired using a two-channel digitizer (250 MS/s).

### 2.2 Sweep polarization measurement

To achieve a polarization contrast, it is necessary to set the polarization of the two alternating sweeps to be different, for instance opposite or orthogonal on Poincaré sphere. Therefore, it is necessary to measure the polarization of the sweep temporally and accordingly spectrally resolved. In order to measure not only the average polarization but also the polarization change and uniformity within the sweeps, a polarimeter, which allows a sufficient sample rate of more than 5 fps, is required. A self-made motorized polarimeter, based on the rotating quarter-wave plate method [10] was used. The light is propagating through an achromatic quarter-wave plate and a linear polarizer (image 2). The intensity of the transmitted horizontally polarized light is measured with a detector. During the rotation of the quarter-wave plate, a time period of about 20 µs is recorded at each of 2000 equidistant positions per rotation cycle. Each record consists of 1024 samples detected with 50 MHz sample rate, which corresponds to the acquisition of the spectrum of the laser (sweep and copy) with a resolution of about 840 data points. The trigger from the laser and an encoder wheel is used for controlling the acquisition temporally. The Fourier analysis described in [10, 11] is carried out for each of the 1024 data points per record, yielding the Stokes parameters $S_0$ (intensity), $S_1$ (horizontal/vertical polarization), $S_2$ (+45°/-45° polarization) and $S_3$ (positive/negative circularly polarized light), for each wavelength of the spectrum. The motor-driven polarimeter allows the calculation of the Stokes vectors with a rate of 7 fps which is sufficient for fast manual adjustment of the polarization using manual polarization controllers. The main measurement uncertainty is caused by the imperfect retardance of the quarter-wave plate, which depends not only on the wavelength but mainly on the temperature. This was considered by an extended calculation of the Fourier components/Stokes parameters taking the actual retardance into account [10]. After a calibration measurement of the retardance, which is carried out by placing a linear polarizer between the collimator and the wave plate, the measurement uncertainty of the polarimeter was 2% for the degree of polarization (DOP) and 2° for the angle on the Poincaré sphere.

The polarimeter was connected to the single-mode fiber, which is otherwise connected to the scanner head during OCT-imaging. Due to the relocation and bending of the fiber during the connection and positioning of the scanner head, it is not possible to predict the exact incoming polarization in the Michelson interferometer. But the angle between the polarization of the forward sweep and its copy...
remains constant on the Poincaré sphere, which is important to ensure a polarization contrast between the corresponding OCT depth-scans.

3 Results and discussion

The polarization, which was used for OCT-imaging is shown in image 3. The forward sweep (from 4 µs to 10 µs) has a circular/elliptical polarization and the copy of the forward sweep (from 12 µs to 18 µs) has a dominant vertical polarization. These polarizations are approximately orthogonal on Poincaré sphere. The different intensities (S₀ component) are corrected numerically via normalization.

OCT-imaging was carried out using the above described high-speed OCT-system. During beam deflection over the sample, the lateral distance between adjacent depth-scans (between forward sweep and its subsequent copy and vice versa) was 2 µm, which is small compared to the beam width in the focus, which is 13 µm. After Fourier-transformation of the interference spectra, the amplitude and the phase information for both sweeps, $A_1 \cdot \exp(i\phi_1)$ and $A_2 \cdot \exp(i\phi_2)$ can be evaluated. During processing of the interference spectra, the jitter between subsequent spectra was numerically corrected using the interference spectra delivered from the Mach-Zehnder interferometer, resulting in a stabilized phase without visible artifacts in the phase evaluation. From the alternating depth-scans, two OCT-images can be extracted, one composed of the forward sweeps ($A_1$) and one composed of the copied sweeps ($A_2$). These two images (image 4A and 4B) represent standard OCT images of a human finger nail. From these two amplitudes, a compound intensity image ($\sqrt{(A_1^2 + A_2^2)}$) was calculated (image 4C). The difference between these two images can be visualized by calculating the amplitude ratio ($\tan^{-1}(A_1/A_2)$) and the phase difference ($\phi_2 - \phi_1$), which are presented color-coded in image 4D and 4E. The OCT-images in image 4 were scaled using a refractive index of 1.45 [12, 13].

In the region of the nail, OCT images 4A and 4B reveal different information. Due to the birefringence, the polarization of the light is changed with increasing penetration depth resulting in alternating bright and dark horizontal stripes in image 4A, which are occurring only in the nail, but not in the neighboring epidermal or dermal tissue. These stripes are not visible in image 4B and only marginal in the compound intensity image 4C. The amplitude ratio, shown in image 4D shows this difference color-coded.
from 0 (blue, dominating amplitude of second sweep) over \( \frac{\pi}{4} \) (white, no amplitude difference) to \( \frac{\pi}{2} \) (red, dominating amplitude of first sweep). The nail exhibits a considerably alternating intensity ratio with increasing depth, which is visible in the rapid color change. In contrast to this, the amplitude ratio in the epidermal or dermal tissue exhibits only minor changes with increasing penetration depth. The phase difference in image 4E shows an equivalent behavior.

Although it has been shown before that birefringence is the reason for the stripes in the OCT-images of finger nails [14], the imaging example illustrates the enhanced information content in PSOCT images in contrast to the standard OCT image. Obviously, in standard OCT images, as shown in image 4A and 4B, the optical properties of the sample is not directly accessible and different incident polarizations can result in different intensity images, depending on the incident polarization and on the orientation of the sample, which can complicate the interpretation of standard OCT images. The presented measurement of the polarization using a high-speed polarimeter allows the adjustment of alternating polarizations. The presented method is applicable to swept-source based OCT-systems, which are using buffered swept laser sources.

4 Conclusion

This study demonstrates that polarization sensitive OCT can be implemented in standard single-mode fiber based swept source OCT-systems, if buffered swept lasers are used, which provide alternating sweeps, whose polarization can be adjusted differently. A self-made motorized polarimeter was employed for measuring the polarization of the wavelength sweeps temporally resolved and for monitoring the adjustment of the polarization. The amplitude ratio as well as the phase difference contain information about the optical properties of the sample and provide an additional tissue contrast. Additionally, the polarization contrast can support the interpretation of assumed changes in the backscattering amplitude in standard OCT-images, whose origin remains unclear without polarization sensitivity.

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References

Recording the Movement Behaviour of a Bolus on Variation of the Bolus Density in the Rumen of Cattle with a Magnetic Monitoring System

T. Reuter¹, M. Beck¹, S. Lindner², H. Richert³, S. Wangemann³, R. Holzhey³, M. Hoffmann¹
¹fzmb GmbH, 99947 Bad Langensalza, Germany, treuter@fzmb.de
²Matesy GmbH, 07745 Jena, Germany
³Innovent e.V. Technologieentwicklung Jena, 07745 Jena, Germany

Abstract

Current research at the fzmb deals with the development of a monitoring system for the continuous pH-measurement in vivo in the rumen of cattle in order to optimize the feed application and improve animal health in cattle farming. Here, an important aspect is the position of the pH-measuring bolus in the rumen. Within the scope of this work the movement pattern of the bolus depending on the bolus density (1.5 - 3 g/cm³) was evaluated and recorded with a 3D magnetic monitoring system for 50 minutes during feeding.

1 Introduction

The ruminal acidosis is one of the most important feeding-conditioned diseases of the dairy cow [1, 2]. The disease manifests itself in a huge number of clinical symptoms and leads to considerable economic losses in the dairy cattle industry. The monitoring of the state of health of the cattle by an objective and diagnostically conclusive measuring methodology is urgently necessary particularly in the case of livestock farming in big herd. Current research projects of the fzmb deal with the development of a monitoring system for the continuous pH-measurement in vivo in the rumen of cattle in order to optimize the feed application and improve animal health in cattle farming. The position of the pH-measuring bolus plays an essential role for the continuous pH-measurement in vivo in the rumen, because its position changes constantly by the reticulorumininal motility and the independent movement of the animal. On this account the movement pattern of the bolus was evaluated and recorded with a magnetic monitoring system for 50 minutes during feeding on variation of the bolus density.

1.1 Rumen movement

In the course of evolution ruminants developed a fore-stomach system consisting of fermentation chambers in which protozoa, bacteria and fungi macerate the forage under anaerobic conditions. Functionally, reticulum and rumen form a unit called reticulorumen. An important prerequisite for effective microbial digestive processes is the constant mixing of the ingesta in the reticulorumen. The contraction of the ruminal pillars, which project into the lumen of the rumen as strong muscle bulges, allows a more intense circulation of the ingesta in the reticulorumen. After swallowing, the coarsely chewed feed is pushed dorsally by reticulorumininal motility into a thick mat of roughly structured digesta in the dorsal sac. The rumi-
solution of the inverse field problem. The minimization of
the difference between the field distribution resulting from
a simulation of the capsule position and the actually mea-
ured magnetic field distribution due to the change of the
capsule position yield the three dimensional position and
spatial orientation of the capsule.

2.2 Experimental approach
At first the magnetic monitoring system was calibrated. It
was fastened with screw connection to an aluminium rack
and placed saddle-like on the back of the cattle. Thus, the
magnetic monitoring system was at a level with the ventral
sac as well as with the opening between rumen and reticu-
"lum (Ostium ruminoreticulare). Through a surgically made
rumen access (fistula) a permanent magnet (m = 12 Am²;
NdFeB magnet; geometry 5.5 x 2 x 1 cm; magnetisation
through the width 2) was introduced into the ventral sac of
the cow. The shell of the magnet (ruminal bolus) resem-
bles the rumen cage magnets used in cattle practice. The
dimensions of the ruminal bolus were 35 mm in diameter
and 140 mm in length (see Image 1). The density of the
ruminal bolus was 2.94 g/cm³ (experiment I), 2.17 g/cm³
(II), and 1.45 g/cm³ (III), respectively.

2.3 FFT Analysis
In the motion analysis the long time FFT is used to inquire
into the dominant frequency in a time interval and win-
dowed or color FFT to depict the time-resolved dominant
frequency. The long time FFT carries out a Fourier analy-
sis on an area selected in the time diagram and returns the
result in a 2-D diagram. In this diagram the strongest fre-
quency with its amplitude is marked. The windowed or
color FFT also carries out a Fourier analysis on a selected
area. However, these views return a representation of the
analysis, in which several Fourier analyses in successive
"time windows" of the selected area are carried out and
applied on a 3rd time axis. Therefore the changes of the
prevailing frequency within a certain time become visible.

3 Results
In Image 3 the motion of the ruminal bolus during experi-
ment III is shown. The insertion of the ruminal bolus (ver-
tical red arrow) and the motion of the ruminal bolus in the
ventral sac are recognised very well.

Image 1 Ruminal bolus

During the 50 minutes experiment the cow was halter-
ted, tethered and fed hay. The fistulated cow with the magnetic
monitoring system is depicted in Image 2.

Image 2 Cattle with fistula and magnetic monitoring sys-
tem

The Images 4 and 5 display the distance covered and the
velocity of the ruminal bolus in x-, y- and z-direction dur-
ing feeding, respectively. The color FFT and the long time
FFT analyses are depicted in Images 6 and 7. Comparing
the three experiments, the measured distances and velocities
differ considerably. This may be attributed firstly to
the variation of the density of the rumen bolus and sec-
ondly to the individual feeding and standing behaviour of
the cattle during the experiments. The distances covered
(absolute value over all directions in space) and the mean
velocities are given in Table 1.

Image 3 Movement pattern of the ruminal bolus III in the
rumen during feeding

<table>
<thead>
<tr>
<th>Image 4 and 5</th>
<th>Distance covered and velocity of the ruminal bolus in x-, y- and z-direction during feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Image 6 and 7</td>
<td>Color FFT and long time FFT analyses are depicted</td>
</tr>
</tbody>
</table>

Table 1

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This indicates that the distance and the mean velocity are the lowest at a density of 2.94 g/cm³. With decreasing the density to 2.17 g/cm³ the distance and the mean velocity are doubled. However, with another decrease in density to 1.45 g/cm³ the distance and the mean velocity do not increase further. Distance and velocity in experiment III are only slightly increased in comparison to experiment I (density of 2.94 g/cm³). With the help of the long time FFT the dominant frequency in z-direction may be calculated. In Table 2 the dominant frequencies for the experiments I – III are shown. The dominant frequencies in x- and y-direction are calculated to be 0.59 min⁻¹ in all experiments. The color FFT shows no changes in the prevailing frequency in z-direction over the time.

### Table 2 Overview long time FFT over the movement in z-direction

<table>
<thead>
<tr>
<th>experiment (density ρ [g/cm³])</th>
<th>long time FFT z-direction [1/min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (2.94)</td>
<td>2.52</td>
</tr>
<tr>
<td>II (2.17)</td>
<td>1.70</td>
</tr>
<tr>
<td>III (1.45)</td>
<td>2.34</td>
</tr>
</tbody>
</table>

From Table 2 is evident that the dominant frequency decreases with decreasing density. However, the dominant frequency at a bolus density of 2.17 g/cm³ is about 37% lower than at 1.45 g/cm³.

### 4 Conclusion

The localisation as well as the movement pattern of a ruminal bolus in the rumen of the cattle was recorded with the magnetic monitoring system during a 50 minutes examination. The present results confirm another experimental study [8]. Hence, the exact measuring place of a pH-measuring bolus could be determined with the help of this system. The movement of the ruminal bolus occurred exclusively in the ventral sac. The calculated frequencies were lower than denoted in literature. In future works the frequency results will be validated by auscultatory findings. The results of the density variation of the ruminal bo-

---

**Image 4** Distance covered by the ruminal bolus III in the rumen during feeding (raw data)

**Image 5** Velocity of the ruminal bolus III in the rumen during feeding (raw data)

**Image 6** Color FFT analysis over the movement in z-direction of the ruminal bolus III in the rumen during feeding

**Image 7** Long time FFT analysis over the movement in z-direction of the ruminal bolus III in the rumen during feeding
lus are affected particularly by the individual feeding and standing behaviour of the animal during the experiment. Hence, no trend could be determined in dependence of the density with regard to distance, mean velocity, or frequency analysis. Further works will deal with the repeatability and standardization of the experiments as well as the analysis of the pH-value distribution within the reticulorumen.

5 References


Acknowledgement

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Intraoperative perfusion imaging of the cerebral cortex by time-resolved thermography

J. Hollmach¹, Y. Radev², N. Hoffmann¹, C. Schnabel¹, S. Sobottka², E. Koch¹, G. Schackert³, G. Steiner¹ and M. Kirsch²

¹Technische Universität Dresden, Klinisches Sensing und Monitoring, 01307 Dresden, Germany, julia.hollmach@tu-dresden.de
²Technische Universität Dresden, Klinik und Poliklinik für Neurochirurgie, 01307 Dresden, Germany

Abstract

Time-resolved thermography is a novel method to image temperature gradients of tissue by detecting infrared radiation emitted by the object. It is a sensitive, noninvasive and fast method to investigate blood perfusion. Therefore, usability of thermography for perfusion imaging during neurosurgeries is studied. The motivation of this study is the characterization of the cortical perfusion by intraoperative thermographic imaging and detection of an intravenously applied cold bolus of ice-cold normal saline solution. In this study, thermographic cold bolus detection and the standard indocyanine green (ICG) technique are compared. ICG videoangiography and time-resolved thermography were performed simultaneously in order to investigate the time of cold bolus appearance in cortical vessels of a patient who suffered from an arteriovenous malformation. We defined as occurrence of the cold bolus the minimum temperature of the cold bolus profile in vessels and the half maximum intensity of the ICG fluorescence. Both imaging methods resulted in a time of appearance between 20 – 25 s after injection. For cold bolus detection, the mean arrival time amounts to 21.6 s (±2.7). ICG fluorescence showed a mean of 23.4 s (±0.5). The results show that the temporal properties of thermographic cold bolus detection and ICG videoangiography are in accordance. Therefore, thermography is a suitable tool for label-free and repeated imaging of the cerebral blood flow.

1 Introduction

In neurosurgery, 2D and 3D imaging modalities are essential for the surgical management of a range of brain disorders. Preoperative computer tomography (CT) and magnetic resonance imaging (MRI) provide 3D image data of the brain and enable the localization and demarcation of pathologies, like degenerated tissue and abnormal vessel growth. Other achievements of medical engineering are the various methods used for perfusion scanning, like perfusion MRI, CT and single-photon emission computed tomography (SPECT). All these methods mentioned above are mainly performed preoperatively and are not suitable to provide fast and repeated perfusion imaging during surgery. For intraoperative purposes, Doppler ultrasound and indocyanine green (ICG) angiography are the current methods of choice. While Doppler ultrasound is used to display larger vessels within the parenchyma, ICG angiography can visualize the cortical vessels. However, a major drawback is inherent due to the single contrast application delaying a second investigation for at least 20-30 min. Thermographic imaging overcomes these challenges. It is a fast, contactless, label-free and noninvasive technique which acquires a series of thermal images by detecting the infrared radiation emitted by the cortex. Therefore, it is capable of imaging weak temperature variations both in a high temporal and spatial resolution.

Thermography was already used in the field of medical imaging. For example, Gorbach et al. analyzed cortical temperature gradients associated with changes in cerebral blood flow caused by tumors¹ and neural activation.² Nakagawa et al. and Okada et al. performed infrared blood flow monitoring of the brain to examine the success of bypass surgeries.³ Steiner et al. have demonstrated the potential of thermography for cortical perfusion imaging.⁴ During recording a series of thermal images of the cortex, a bolus of ice-cold normal saline solution was applied through a central line (cold bolus approach), providing a thermographic contrast. Furthermore, the quantifiability of the cold bolus signal was shown by calculating the occurring temperature differences in cortical arteries (Hollmach et al.).⁵

In this study, we analyzed thermographic cold bolus detection and simultaneously performed ICG videoangiography in particular with regard to the time of appearance in cortical vessels. By comparing this novel method to an extensively tested and established method like fluorescence angiography, the reliability of the thermographic cold bolus approach could be confirmed.

2 Methods

Intraoperative time-resolved thermography and fluorescence angiography were performed simultaneously in order to assess the comparability of both imaging modalities. Indocyanine green was added to a bolus of 50 ml ice-cold normal saline. The solution was injected intraoperatively. A sequence of thermal images and a fluorescence video of the exposed cortex were recorded during injection. The method was evaluated based on eight patients and will be...
demonstrated with an example. Figure 1 shows cerebral cortex images of a patient who suffered from a cerebral arteriovenous malformation (AVM). An AVM is characterized by an abnormal connection of arteries and veins without the involvement of capillaries. Thus, the blood flow reaches these vessels early, and both the cold bolus signal and the fluorescence signal are of significant strength. In figure 1(a) a microscopic image of the brain surface is displayed. The large blood vessels are part of the AVM. The darker vessels on the top left contain coagulated blood and are not perfused. Figure 1(b) depicts an ICG angiographic image of the same cortex. Black regions represent the thrombosed vessels. A thermographic image of the cortex is shown in figure 1(c). The warmer cortex (colour coded from yellow to red) contrasts with the colder non-cortical region (blue). Larger vessels can be identified by dark red structures. The colder green area represents the thrombosis.

Figure 1. (a) Microscopic image, (b) ICG fluorescence image and (c) thermographic image of the cerebral cortex.

2.1 ICG angiography
ICG videoangiography was performed using an OPMI® Pentero® surgical microscope from Carl Zeiss Surgical GmbH with an integrated INFRARED 800 fluorescence module. The fluorescence module is designed for excitation in the wavelength range of 700 to 780 nm and detects emitted fluorescence light in the wavelength range from 820 to 900 nm. The fluorescence dye (ICG Pulsion®) was diluted in 50 ml physiological sodium chloride solution resulting in a concentration of 1 mg/ml.

2.1.1 Preprocessing of fluorescence data
The intensity profiles for each pixel of the ICG fluorescence video were extracted. In order to eliminate high frequency noise and background information the data was decomposed into its principal components using principal component analysis. Components with a high variance and a resulting cumulative sum of above 0.95 were selected to recompose the time series. After an offset subtraction, sharp intensity decreases caused by the auto gain function of the recording system had to be corrected. The intensity profiles showed a characteristic saw tooth pattern, which was removed by an intensity correction at corresponding time points. Afterwards, the time series, now forming smooth curves, were normalized to the overall maximum.

2.2 Time-resolved thermography
Thermographic recordings were performed with an infrared camera system from InfraTec GmbH (Dresden, Germany). It is capable of imaging small temperature variations by detecting infrared radiation in the spectral range of 7.5 – 14 µm. It attains a thermal resolution of 0.03 K (at 30°C) and a spatial resolution of 250 x 250 µm per pixel. Images of 640 x 480 pixels are recorded with a frame rate of 50 Hz.

2.2.1 Preprocessing of thermographic data
Thermographic image files were converted into ASCII format and data preprocessing and analysis were carried out with MATLAB Version 8.2. Discrete wavelet transform was performed using WaveLab, a MATLAB wavelet library available from the Department of Statistics, Stanford University, USA.

In a single thermographic image cortical pixels were selected manually. The time series were subjected to a discrete wavelet filter in order to eliminate frequencies higher than 0.2 Hz and standardize them to the mean. Afterwards they were subsampled to 10 Hz and a spatial binning (2x2) was applied in order to reduce the amount of data.

2.2.2 Cold bolus detection
For assessment and quantification of the cold bolus signal, an inverse skewed Gaussian function was approximated to the subsequence of each time series where the probability of the cold bolus signal was highest. A skewed Gaussian function is defined by

\[ f(x) = h \cdot e^{-\frac{(x-t_0)^2}{2\sigma^2}} + y_0 \quad \text{if} \quad t < t_c \]
where $t_c$ depicts the position of the peak, $\sigma_1$ is the standard deviation of the half Gaussian function left to the peak and $\sigma_2$ is the standard deviation of the half Gaussian function right to the peak. The height is given by $h$. The skewness factor was set to be 0.3 in order to force the falling edge to be steeper than the rising one. The base $y_0$ equals zero. An example of a skewed Gaussian function is shown in figure 2.

Figure 2. Skewed Gaussian function.

3 Results

We defined the time point of minimum temperature in the cold bolus profile (demonstrated in figure 3) as time of appearance of the cold bolus in vessels, for ICG half maximum intensity of the fluorescence was selected. Exclusively pixels containing a cold bolus signal within the first 25 s after injection were considered. These pixels correspond to arteries or vessels which are part of the arteriovenous malformation. A similar restriction was made for the fluorescence data set. Here, only pixels reaching a real fluorescence maximum within 31 s after injection were examined, since the recording was stopped after 32 s. Further, the fluorescence maximum of individual pixels have to amount half of the overall maximum. These constraints also result in a pixel subset, which covers vessels related to the AVM.

The time of appearance for both imaging modalities was calculated for each pixel subset. The results are shown in figure 4.

Figure 3. Thermal profiles (black) and approximated inverse skewed Gaussian function (red). The minimum temperature is given by the fit and is reached at $t = 20$ s.

Figure 4. Image overlay of microscopic image of the exposed cortex and calculated time of appearance for (a) ICG fluorescence and (b) thermographically detected cold bolus signal.

Figure 4 displays overlays of the microscopic image of the cortex and the calculated appearance time for both imaging modalities. The half maximum of the fluorescence intensity was reached within 20 to 25 s seconds after injection, as shown in figure 4(a). The same applies for the maximum temperature difference of the cold bolus signal, as can be seen in figure 4(b). Two exceptions are recognizable (displayed in blue). One affects a vessel, the other involves regions of coagulated blood.

The images in figure 4 demonstrate the temporal correspondence of the calculated features for the evaluated imaging methods. Nevertheless, the results show some discrepancies. The appearance time of the cold bolus signal scatters more strongly around a lower mean of 21.6 (±2.7) than the fluorescence signal 23.4 (±0.5). The cold bolus seems to appear earlier in some upstream vessels, but shows the same arrival time in vessels downstream. The ice-cold solution is strongly affected by thermodilution and warming, whereas the fluorescence dye is basically influenced by dilution. Therefore, the temperature profile of the cold bolus could be more biased by the blood flow and the temperature of surrounding tissue, which may lead to a shift of the minimum temperature in the cold bolus signal.

A closer inspection of figure 4(b) reveals that pixels attributed to vessels are slightly different to pattern in figure 4(a). This could be attributed to the constraints set for pixel selection. These constraints depend on the occurrence of the wanted signal within a given time and
strength, and are therefore strongly dependent on precise
detection of the signal. In spite of the difficulties men-
tioned above, the calculated temporal features of both im-
ageing methods show good agreement.

4 Conclusion

We compared the signal appearance of a thermographi-
cally detected cold bolus with a simultaneously performed
ICG videoangiography of the cerebral cortex. We were
able to show that the temporal signals properties of both
imaging methods are in good agreement. Therefore, fluo-
rescence angiography can be used as a reference method to
establish the reliability of the thermographic cold bolus
approach for perfusion imaging. Existing discrepancies
should not be declared as failures a priori, but need further
investigation, since they may contain additional infor-
mation about the blood flow.

5 Acknowledgement

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Influence of thin non-conducting layers in electromagnetic body-phantoms for imaging radar

J Schmid1, C Marzi1, V Lupici-Baltzer1, M Jalilvand2, O Dössel1

1Karlsruhe Institute of Technology, Institute of Biomedical Engineering, Karlsruhe, Germany, publications@ibt.kit.edu
2Karlsruhe Institute of Technology, Institute of High Frequency Technology and Electronics, Karlsruhe, Germany, publications@ibt.kit.edu

Abstract

In the process of developing new diagnostic procedures for human health purposes a lot of tests on e.g. phantoms are necessary before the procedures can be approved to be used in human health care systems. In the context of the higher-level project a phantom will be developed to testify imaging procedures based on electromagnetic waves. Mixtures of gelatin, sugar, water and salt can be used to approximately model most of the electromagnetic properties of different tissues within the human body. Problematic with those mixtures is the fact that they are prone to diffuse to each other especially on edges with strong concentration gradients. To prevent them from doing this a waterproof layer can be used. Unfortunately, these layers usually show a significant difference in permittivity. But if the thickness of the layer is only a very small fraction of the wavelength of the frequencies finally used, the influence should be negligible. To prove this an experiment and a computer simulation were made.

1 Introduction

In the process of developing new diagnostic devices for human health many preliminary tests are necessary to comply with the legislation and more importantly to reduce the risk of adverse health effects. One alternative to taking measurements on real patients are computer simulations. But in many cases these simulations need a very high degree of complexity to be realistic. In this project a system for radar imaging of the human head will be developed in order to detect and distinguish stroke. Especially in the case of imaging radar the models are often very complex and as we need one simulation for each antenna very many simulations have to be done, too. All this leads to very long computation times [1], in some cases longer than one month. As an alternative to simulations phantoms can be used. These phantoms need a good similarity of electromagnetic properties compared to their physiological analogues. Phantoms are also needed to verify the computational results. As it is shown in [2] and [3] a mixture of e.g. water, gelatin, sugar and other substances can be used to approximate most of the soft tissues.

Since these mixtures are based on water and soluble substances it cannot be excluded that these substances leak out into neighboring mixtures or into a matching liquid during measurement.

This results in an unwanted change of permittivity and conductivity near the boundary layer. But especially the contrast between the tissues is necessary to get realistic information from the scattered fields.

One option to prevent the phantom from mixing up is to integrate a thin layer of a waterproof material such as a leaf of polyethylene (PE) between the different gel-mixtures. The first thought that comes up when doing this is that it could increase the reflection in an unphysiological manner as the permittivity of most synthetic materials is in the range of 2.0 to 4.0. PE has a permittivity of 2.2. However, the tissues and therefore their representing gel-mixtures have a high conductivity in a way that the construction of the layer and the gel-mixtures can be seen as a capacitor. As generally known on a high frequency level the influence of capacitors is very low. Due to the fact that the devices, which are developed within this project are operating on high frequencies (above 500 MHz) and due to the fact that the thickness of the layer is only a small fraction of the wavelength, the assumption that it does not have any influence is justified. To measure the influence of such a PE-layer an experiment with two simply shaped models was made.

2 Methods

2.1 Model and measurement unit

To measure the reflectivity of a thin non-conducting layer within a conducting phantom of human tissue two cylindrically shaped models with a diameter of 13 cm were made. Each of it was filled up with 6 cm of the same gelatin mixture which represented brain grey matter tissue. After the first mixture had become solidified a second cylinder of 6 cm with a gelatin mixture which represented brain white matter tissue was filled in. In one of the models a thin layer of about 16 µm thickness made of PE was used as a barrier between the different materials. On each side of the cylindrical models a Bowtie antenna was used to measure the transmitted and reflected signals [4]. The setup of the experiment is visible in figure 1. For the measurements a network analyzer from Agilent type E8363 was used. In general, Network analyzers return the scattering parameters. In this experiment the scattering parameters are measured in frequency domain within the frequency range between 0.5 and 5 GHz.
In a third experiment a thin non-conducting layer was placed near to one antenna and in between a matching liquid and a tissue representing gel-mixture. In order to remove remaining air a few drops of a matching liquid were put between the layer and the model. Finally a 9 mm thick layer of the same matching liquid was applied to the PE-layer. One measurement was made with the described setup and a second one after the removal of the PE-layer but still with the matching liquid. The antenna was placed in contact to the matching liquid.

### 2.2 Calculations and comparison

The scattering parameters for a two port system in general are calculated by

$$S_{sv} = \left. \frac{b_v}{a_w} \right|_{a_w=0}.$$

The variable $b_v$ can be interpreted as the arriving wave on port $v$ and the variable $a_w$ is the in-sent wave on port $w$ under the constraint that there is no wave sent from port $v$. The variables $a$ and $b$ have the unit of an energy. That means the parameter $S_{11}$ can be interpreted as the factor of reflected energy and the parameter $S_{21}$ as the factor of the transmitted energy.

Under the assumption that the thin layer does not have any influence on the results the scattering parameters of both measurements/simulations should be equal.

To have a more robust value to compare the results the average factor of transmitted power $a_e$ in dB was calculated by

$$a_e = 10 \cdot \log \left( \frac{1}{N} \sum_{n=1}^{N} |\text{Real} \{s_{21}(n)\}| \right),$$

in which the values $s_{21}(n)$ are the discrete frequency domain values of the scattering parameter and $N$ is the total number of frequency samples.

As in practical experiments a lot of things could cause differences and hence can influence the results an additional computer simulation of the experiment with the tissues barrier was made. As simulation framework the commercial software SEMCAD X was used. The solver integrated in SEMCAD is based on the well-known FDTD algorithm. To achieve correct results the spatial resolution was set to a level so that the thin layer consisted of at least two voxel in width. The simulation setup is visible in figure 2.

### 3 Results

The simulated scattering parameters $S_{11}$ and $S_{21}$ are shown in figure 3 and figure 4 and the results of the experiment are shown in figure 5 and 6. The average factor of transmitted power in the experiment with the model with the non-conducting layer was $a_e = -89.116606 \, dB$ and the model without the non-conducting layer was $a_e = -89.737694 \, dB$. The results of the third experiment can be seen in figure 7 and 8. The results of the experiment with the barrier-free model and the matching liquid (one with a separating PE-layer and the other without it) are depicted in the figures 7 and 8. The average factor of transmitted power in this experiment was $a_e = -77.104738 \, dB$ with the PE-layer and $a_e = -77.417272 \, dB$ without the PE-layer.

### 4 Conclusions

The results of simulations show - within numerical accuracy - that the influence of such a PE-layer is nearly zero. The results of the experiment with the matching liquid and the barrier free model lead to the same conclusion.

Unexpectedly, the results of the experiment with the different models (one of it with a PE-layer in between) are not as clear as the other ones. As it can be seen in figure 5 and 6 the models have a differing damping at different frequencies but the amount of power passing through is within the same range. Another important fact can be extracted from this experiment: The frequencies that can be used for brain imaging can barely be higher than $1.5 \, GHz$ (visible at figure 6). Due to the fact, that in this model no shielding effects
Figure 3 $S_{11}$ from the simulations with and without a barrier

Figure 4 $S_{21}$ from the simulations with and without a barrier

Figure 5 $S_{11}$ from the experiment with the model with and without a barrier

Figure 6 $S_{21}$ from the experiment with a model with and without a barrier

Figure 7 $S_{11}$ from the experiment with the model without the barrier, matching liquid and with and without a thin PE-layer near the antenna

Figure 8 $S_{21}$ from the experiment with the model without the barrier, matching liquid and with and without a thin PE-layer near the antenna
caused by skull and cerebrospinal fluid were regarded and in addition to that the length of the model (12 cm) is smaller than the diameter of a real human brain, the upper limit of the frequency is even lower than 1.5 GHz for systems measuring in transmission. The peak at approximately 3.8 GHz visible in the results of the simulation (figure 4) is not visible in real experiment. The reason for this is that in reality the conductivity is growing with increasing of the frequency and for the simulation used here the material was set to a constant permittivity and conductivity. Summing up, we can assume that the influence of a thin PE layer in a phantom for UWB radar imaging is indeed negligible.

5 References


Semi-Automated Detection and Fractal Characterization of Myocardial Fibrosis in Histological Images

M. Mayrhofer-Reinhartshuber, P. Kainz, D. Sánchez-Quintana, Y. Macías, E. Hofer, H. Ahammer
1Institute of Biophysics, Medical University of Graz, Graz, Austria,
2Department of Anatomy and Cell Biology, University of Extremadura, Badajoz, Spain
Corresponding author: michael.mayrhofer-reinhartshuber@stud.medunigraz.at

Abstract
Histological images of 14 human hearts were analyzed by semi-automated image analysis. An image set of 40 images was used to quantify the amount of myocardial fibrosis caused by structural ischemic heart disease. Two different segmentation algorithms were tested and compared, both showing comparable results with statistically significant (p<0.001) differences between the investigated groups. Fractal image analysis using a new and efficient pyramid method was applied to the segmented images and revealed a fractal dimension of (1.65 ± 0.10) for the investigated myocardial fibrosis, which is in the same range as results obtained by fractal characterization of liver fibrosis.

1 Introduction
In heart disease, structural as well as electrical remodeling occurs regularly. In most of the cases, an increased collagen deposition in the heart is observed. The amount of the resulting fibrosis is a significant parameter for arrhythmia. Furthermore, the texture of fibrosis (interstitial, compact, patchy, diffuse, ...) influences the incidence of arrhythmias [1,2]. Besides these well known facts, open questions regarding fibrosis and its influence on impulse propagation, especially on a microscopic level, still remain [1]. Although fibrosis is often classified, a uniform and exact categorization of different types is debatable. A deeper understanding of the connection between electrical excitation spread and the amount, distribution and type of fibrosis is highly desirable. Assessing the vulnerability for arrhythmias could be possible with a detection and characterization of collagen in the heart. Hence, our work is focused on the development of image analysis methods for these tasks. In addition to the detection and quantification of fibrosis, also the characterization of different types and grades should be possible with fully automated algorithms. The herein presented results represent an important milestone on the way towards this goal.

1.1 Myocardial Fibrosis
Cardiac tissue mainly consists of cardiomyocytes, which are surrounded by extracellular matrix (ECM). The major component of the extracellular matrix is collagen. Different types of collagen exist, and the fibrillar collagen types I and III are most abundant in the myocardium (~80% and ~11%). These collagen fibers form a collagen network that provides cardiac strength during contractile forces, and play a role in myocyte-myocyte connections [3,4]. A change of the interstitial status may be induced by disease, (mechanical) stress, inflammation or the hormonal environment. Fibroblasts are metaplased into myofibroblasts, which results in a relatively higher amount of type I collagen and more fiber cross-linking. Early changes of the composition and structure of the interstitium may be physiological due to a response to current demands [1,4,5,6]. However, persisting and combined changes alter myocardial properties and may lead to arrhythmia and functional impairment and further to morbidity and mortality. Especially excessive synthesis and deposition of (type I) collagen seems to play a major role in the enhancement of myocardial fibrosis that accompanies the appearance of heart failures. The type and hence also the pattern of the occurring fibrosis is partly disease specific [1,4,7,8].

1.2 Imaging of Myocardial Fibrosis
Since the risks for the patients are nearly negligible, non-invasive techniques for the imaging of cardiac fibrosis would be preferable. The most promising approaches seem to be late gadolinium enhanced cardiovascular magnetic resonance (LGE CMR) imaging [9,10] or 2D strain imaging by speckle-tracking echocardiography (STE) [11,12]. Although they are able to reveal a rough shape in addition to the amount of fibrotic tissue, both are known for their suboptimal visualization of some types of fibrosis (e.g. diffuse fibrosis) [13,14]. Hence, histological analysis of biopsies of cardiac tissue is still the gold standard in the detection, quantification and characterization of fibrosis [14,15]. For the improvement of non-invasive techniques, high quality, observer independent and very well defined data sets from histology are necessary, which may be gained from algorithms based on the presented methods.

1.3 Fractals in Medicine
As noticed in a study of subcellular membrane systems by Paumgartner et al. [16] in 1981, the outcome of image analysis methods depends strongly on the resolution of the investigated images. By using the concept of fractals [17], they successfully explained the observed behavior. One main advantage of fractal analysis is, that the complexity and (dis)order of tissue can often be described with a single value: the fractal dimension. Hence, fractal analysis has become an important part in medical image analysis.
For instance, our group was able to classify different grades of anal intraepithelial neoplasia [18]. Very recently, fractal measurements were used to determine whether chemotherapy applied on patients with breast tumors is effective [19]. In contrast to cardiac fibrosis, liver fibrosis has been investigated by fractal methods very well [20-22].

2 Methods

2.1 Image Acquisition
The images investigated in this study show different regions of 14 human hearts, namely parts of the interventricular septum and of the left papillary muscles (anterior and posterior). All patients were diagnosed with structural ischemic heart disease. Tissues were impregnated with paraffin wax before 7 μm sections were cut on a microtome. To achieve a good contrast between the different types of tissue (especially myocytes and connective tissue), the sections were stained with Masson’s trichrome. From each heart, one or two slices showing nonfibrotic regions were assigned to the control group. Slices showing fibrotic regions were assigned to the fibrosis group. For digitalization a stereomicroscope (Nikon model SM 1500, 5× magnification) was used. In total the image set contains 40 images (20 control, 20 fibrosis) with a dimension of 2560×1920 pixels and a pixel size of 0.68 μm × 0.68 μm in the TIFF file format. All images were saved uncompressed in 24-bit true color RGB space.

2.2 Segmentation
The aim of the segmentation process was to differentiate between three main contents of the images: Connective tissue (fibrosis), myocytes and lumen. Hence, for each original image, three binary images \((I_{fib}, I_{myo}, I_{lum})\) were created. For each created image the sum of white pixels was calculated, which yielded single numbers for fibrosis \(N_{fib}\), for myocytes \(N_{myo}\), and for lumen \(N_{lum}\). Two different segmentation algorithms (Color Thresholding and RGB Relative, implemented as ImageJ [23] and IQM [24] plugins) were used and compared.

2.2.1 Color Thresholding
Color Thresholding [25] has been successfully tested for cardiac tissue segmentation elsewhere [26]. As a first step the images were transformed from RGB color space to the CIE L*a*b* color space. To separate the different parts, empirically determined thresholds were applied. All pixels with a value between the minimum and maximum threshold were set to 0, all remaining were set to 1. An illustration of this process is depicted in fig. 1.

2.2.2 RGB Relative
This algorithm segments images based on differences of the three different channels of an RGB image. The parameters were set empirically by analyzing the whole set of images and selecting the values, which identified the tissue compartments best.

2.3 Pixel Ratios
After segmentation, three different pixel ratios \(r_{1,2,3}\) were calculated to find the best parameter to discriminate between both groups.

\[
r_1 = \frac{N_{fib}}{A_{total}}
\]

\[
r_2 = \frac{N_{fib}}{(N_{fib} + N_{myo})}
\]

\[
r_3 = \frac{N_{fib}}{(A_{total} - N_{lum})}
\]

\(A_{total}\) denotes the total area of the investigated image. All subsequent statistics were performed with SPSS [27].

2.4 Fractal Methods
Although a broad variety of fractal methods exist, for binary images usually the Box-Counting-Method (BCM) is used [17]. Lately, a new method for the determination of the fractal dimension, namely the Pyramid Method (PM), has been developed by our group [28,29]. We showed that the obtained values for the fractal dimensions of artificially generated images had the same quality as when determined with the traditional BCM. In addition, they were calculated within significantly shorter computational times. [28]. Hence, the PM was also the method of choice in the present study and applied to the binary images resulting from segmentation algorithms.

2.4.1 Pyramid Method
The PM uses sequences of identical images at different sizes (image pyramids) to determine the so-called Pyramid Dimension \(D_p\). The bottom of the pyramid is given by the original, binary image, which has \(N_0\) object (white) pixels. Successively smaller images are generated, ending with an image, which is no more than one pixel, i.e. the top of the pyramid. For each size the number of object pixels \(N\) is counted. The slope of the double-logarithmic plot \((N/N_0\) vs. scaling variable) yields the fractal dimension \(D_p\). A more detailed description as well as possible influences due to interpolation methods can be found in [28] or [29].
3 Results

3.1 Fibrosis Detection
After segmentation, the pixel ratios introduced in section 2.3 were calculated to find the best parameter to discriminate between both groups. Furthermore, they were tested on normal distribution by using the Shapiro-Wilk (S-W) test with a level of significance α=0.05. Subsequently, mean values and standard errors of the means were calculated. All approaches showed remarkable differences of means between both groups. The highest difference was obtained for $r_2$. In fig. 2, the results from all approaches are depicted for Color Thresholding (threshold values are shown in tab. 1). Fig. 3 shows the results of the two methods used for segmentation (Color Thresholding, RGB Relative). The outcomes for the best discriminative ratio $r_2$ are compared. Both methods yielded comparable results and a statistically significant differentiation between control and fibrosis was possible (Mann-Whitney U test, two-tailed, p<0.001). The difference was higher for Color Thresholding (control: $r_2$=4-6%, fibrosis: $r_2$=41-47%) than for RGB Relative (control: $r_2$=6-8%, fibrosis: $r_2$=38-44%).

Table 1 Values for Color Thresholding. The myocyte-image was inverted after thresholding to obtain $I_{myo}$.

<table>
<thead>
<tr>
<th></th>
<th>fibrosis, $I_{fib}$</th>
<th>myocytes, $I_{myo}$</th>
<th>lumen, $I_{lum}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$L^<em>$ $a^</em>$ $b^*$</td>
<td>$L^<em>$ $a^</em>$ $b^*$</td>
<td>$L^<em>$ $a^</em>$ $b^*$</td>
</tr>
<tr>
<td>min</td>
<td>201 0 0</td>
<td>0 124 133</td>
<td>0 138 0</td>
</tr>
<tr>
<td>max</td>
<td>255 255 255</td>
<td>255 255 255</td>
<td>208 255 255</td>
</tr>
</tbody>
</table>

Figure 2 Different approaches ($r_1$, $r_2$, $r_3$) to differentiate between the investigated groups (control and fibrosis).

Figure 3 Discriminatory power of $r_2$ on different segmentation algorithms (Color Thresholding vs. RGB Relative).

3.2 Fractal Characterization
Fractal characterization, i.e. the Pyramid Method was applied to those binary images, which only contained the segmented connective tissue. An S-W test was performed to prove the normal distribution of the obtained values for the fractal dimension (level of significance α=0.05).

The mean value for the fractal dimension of the control group was found to be $D_p$=(0.95 ± 0.20), for the group of images assigned to fibrosis $D_p$=(1.65 ± 0.10) was obtained. By applying Student’s t-test it was shown, that also $D_p$ is a statistically significant parameter to discriminate between both groups (two-tailed, p<0.001). The results are shown in fig. 4 together with the standard deviations of the means. Compared to the results for liver fibrosis (1.58 ± 0.07) [22], our results are slightly higher, but still in the same range. The quite high variance of the control group may arise from the fact, that the control images were unavoidably created from the same hearts as the images of the fibrosis group. Hence, there may be connective tissue with a different shape than in completely healthy hearts. However, the detected difference in fractal dimension is significant and may be also a good parameter to differentiate between different types of fibrosis in an extended image set.

Figure 4 Fractal dimensions (Pyramid Method) of the investigated groups (control vs. fibrosis).

4 Conclusion
Image processing and fractal analysis were applied to histological images of human hearts, which had suffered from ischemic heart disease. Two segmentation algorithms (Color Deconvolution and RGB Relative) were used to detect myocardial fibrosis. A comparison of the control with the fibrosis group showed statistically significant (p<0.001) differences of the amount of connective tissue for both methods (control: $r_2$=4-8%, fibrosis: $r_2$=38-47%). Furthermore, fractal analysis (binary Pyramid Method) was used to characterize the segmented connective tissue. While the control group has a fractal dimension of about 1, the analysis of the images showing fibrosis resulted in a significantly different (p<0.001) fractal dimension of (1.65 +/- 0.10), which is in the same range as results obtained for liver fibrosis [22]. Based on these fundamental findings, a more precise characterization and a differentiation of different types of myocardial fibrosis seem achievable with an extended image set.
5 Acknowledgements

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6 References

Improving the Consistency of Manual Deep Brain Structure Segmentations by Combining Variational Interpolation, Simultaneous Multi-Modality Visualisation and Histogram Equilisation

F. Bernard1,2,3, bernard.florian@chl.lu
P. Gemmar1, p.gemmar@hochschule-trier.de
A. Husch1,2,3, husch.andreas@chl.lu
C. Saleh1, saleh.christian@chl.lu
H. Neb1, h.neb@hochschule-trier.de
G. Dooms1, dooms.georges@chl.lu
F. Hertel1, hertel.frank@chl.lu

1Centre Hospitalier de Luxembourg, Luxembourg City, LUXEMBOURG
2Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Esch-sur-Alzette, LUXEMBOURG
3Trier University of Applied Sciences, Trier, GERMANY

Abstract

The manual segmentation of structures in 3D medical imagery is essential for the evaluation of automatic segmentation methods and for the creation of statistical shape models. However, amongst others, shortcomings are that the standard approach of slice-wise manual annotation in 3D imagery takes a large amount of time and in most cases the resulting 3D objects are affected by inconsistencies. In order to reduce the time required for manual segmentations and to improve the consistency of the resulting segmentations a tool for better manual segmentation that makes use of variational interpolation, simultaneous multi-modality visualisation and histogram equilisation has been developed. We show an increased consistency of manual segmentations with this approach compared to slice-wise 2D segmentations. Furthermore, the total time required for manual segmentation is decreased.

1 Introduction

The process of labelling voxels in 3D medical images, also known as segmentation, is an important task for many clinical treatments (e.g. radiosurgery) as well as for research related to medical imaging. This process is frequently performed manually by clinicians or researchers. The standard approach to manual segmentation is the creation of a label-image by selecting for each label the voxels that belong to it. In 3D images this is either performed on a per-slice basis by using a 2D brush (or 2D contours that are then filled automatically) or by using a 3D brush that is able to annotate several consecutive slices simultaneously. In both cases this requires a large amount of time. Furthermore, by using a 2D brush the objects are in most cases only labelled in a single image plane by the user, which results on the one hand in a stair-case like appearance of the resulting 3D objects and on the other hand in objects that are consistent only in the image planes where they have been segmented. Whilst using a 3D brush reduces these effects, on the contrary the accuracy of the resulting segmentation decreases due to the fact that not all voxels that are labelled at a time can be visualised simultaneously. Additionally, labelling each voxel individually (or a group of voxels) allows for very high degrees-of-freedom, resulting in many pseudodetails which are rather segmentation artefacts than actual anatomical details. Also, a high variability between different raters is likely to occur with voxel-wise manual annotation (e.g. documented in [1] for the segmentation of deep brain structures).

For reducing the mentioned deficiencies numerous automatic and semi-automatic segmentation methods have been proposed. Among the most powerful automatic segmentation methods are statistical shape models (SSMs) [2], where low contrasts, partial occlusions, missing object parts or noisy images can be effectively dealt with by restricting the resulting segmentations only to plausible shapes according to a statistical shape distribution. This statistical shape distribution is generally learnt from annotated training data.

Furthermore, for the evaluation of automatic segmentation methods usually a comparison to a manually created ground truth is performed. Therefore, for automatic segmentation approaches the availability of manual segmentations is essential. Semi-automatic segmentation approaches are powerful tools that can unburden the user. One can further distinguish between semi-automatic segmentation and interactive segmentation [3]. In semi-automatic segmentation the user merely gives some input to a segmentation algorithm at the beginning without any further interaction (such as setting seed points for region-growing). In contrast, inter-
active segmentation allows for repeated user input in an iterative manner. The latter is in particular useful for clinical applications, where the physician always needs to have the last choice.

A very promising interactive method is 3D interpolation, where the user draws a sparse set of planar contours and the interpolation method then generates the resulting 3D segmentation [3]-[5]. In this paper we describe a tool that makes use of such an interpolation method. In conjunction with a simultaneous visualisation of multiple image modalities and histogram equilisation we show that the consistency between manual segmentations can be improved.

2 Methods

In order to enable efficient, consistent and more objective manual segmentations a tool for the manual annotation of 3D images has been developed using MeVisLab.

To decrease the time required for manual annotations the volumetric representation of each structure is interpolated from a sparse set of 2D contours using variational interpolation [3] (see Fig. 1). Variational interpolation is the extension of 2D thin-spline interpolation to arbitrary dimensions. In variational interpolation a smooth surface is interpolated from a set of control points by minimising an energy functional based on radial basis functions.

So instead of annotating all voxels in all image slices where the object is present the user merely has to place a few contours in some image slices. In our case all raters where asked to place at least one contour in the axial plane and the coronal plane and then refinements can be performed by adding additional contours, if necessary.

In general, the planar contours can be placed in each image plane. Thus the segmentation is de-facto performed in 3D, avoiding the stair-case like appearance of the resulting objects and leading to more natural object shapes.

In order to increase the consistency of segmentations up to four image modalities are visualised simultaneously, enabling the user to draw contours in each of them and checking the plausibility of a segmentation in all image modalities at the same time (see Fig. 2).

Furthermore, histogram equilisation is performed within a region-of-interest containing the structure to be segmented, resulting in an enhanced and objective contrast within this region.

3 Results

We were able to observe a reduced inter-rater variability in manual deep brain structure segmentations compared to our first approach based on conventional manual segmentation using ITK-SNAP [6]. In the following we refer to this as slice-wise method because the segmentation was performed slice-wise using a 2D brush in axial flow, only a single modality (SWAN) was visualised at a time and each user was allowed to adapt the contrast freely.

Using the proposed manual segmentation method, segmentations of two deep brain structures (subthalamic nucleus and substantia nigra as single object, SNr+STN; nucleus ruber, NR; each for both hemispheres) have been performed by four raters (one neurosurgeon, one resident in neurology and two computer scientists trained in medical imaging). The images of seven patients have been segmented using three MR modalities (T1, T2, SWAN) simultaneously. For the evaluation of the slice-wise method four patients have been segmented. For the evaluation of the proposed method three additional patients have been considered.

For each individual object the volumetric overlap for all patient’s segmentations and all pairs of raters (number of all possible pairs of 4 raters is 6) have been computed. So, in our previous study there was a total number of 4*6=24 pair-wise comparisons and in our new study there is a total number of 7*6=42 pair-wise comparisons.

The Dice Similarity Coefficient (DSC) has been used for computing the volumetric overlap:

\[
DSC(X,Y) = \frac{|X \cap Y|}{0.5(|X| + |Y|)}
\]

\text{X and Y denote binary images of a particular object segmentation. Essentially, the DSC computes the ratio between all the voxels that have been segmented in both images and the average number of voxels that have been segmented.}

Results of the evaluation are shown in Table 1. It can be seen that our proposed method is in all but one cases better than the standard slice-wise approach. We have observed a average DSC improvement of 0.06 (0.03), respectively a relative improvement of 8.2% (4.6%) compared to the slice-wise method using our proposed method with 7 patients (with 4 patients).

<table>
<thead>
<tr>
<th>Method</th>
<th>SNr+STN_L</th>
<th>SNr+STN_R</th>
<th>NR_L</th>
<th>NR_R</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>slice-wise</td>
<td>0.64±0.10</td>
<td>0.68±0.13</td>
<td>0.76±0.13</td>
<td>0.72±0.13</td>
<td>24</td>
</tr>
<tr>
<td>proposed</td>
<td>0.70±0.10</td>
<td>0.74±0.09</td>
<td>0.74±0.12</td>
<td>0.75±0.07</td>
<td>24</td>
</tr>
<tr>
<td>proposed</td>
<td>0.72±0.10</td>
<td>0.76±0.10</td>
<td>0.77±0.10</td>
<td>0.78±0.07</td>
<td>42</td>
</tr>
</tbody>
</table>

Table 1 Mean±standard deviation DSC of N pair-wise manual segmentations. For N=24 the same four patients have been considered.
4 Conclusion

We have proposed a segmentation tool that allows faster and more consistent segmentations compared to standard slice-wise segmentations using a 2D brush.

The 3D interpolation of a sparse set of 2D contours accounts on the one hand for a reduced user input, directly resulting in a lower amount of time required for manual segmentations. We have observed that the time saving depends on each user. Generally, we have found that, after approximately 1-1.5 hours of training and familiarisation, the manual segmentations have been conducted 2-3 times faster with the proposed method compared to the slice-wise method. It is expected that this number increases further if larger objects are to be segmented in high resolution images.

Furthermore, provided that the user has drawn sensible 2D contours, the 3D interpolation ensures that the resulting 3D object is a smooth object. On the contrary, when performing slice-wise segmentations with a 2D brush it is nearly impossible to ensure that the resulting segmented object is smooth in all directions. In Fig. 3 a comparison of the resulting segmentations using slice-wise and the proposed manual segmentation method are shown.

For further increasing consistency and correctness we enable to visualise multiple (co-registered) image modalities at the same time. With that, contours can be drawn in the image modality that best visualises object boundaries and a drawn contour can be checked in each image modality for consistency and plausibility.

By performing histogram equilisation in a user-defined region-of-interest an optimal and more objective contrast compared to manual contrast adaptation is given.

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6 References


Entropy based image blending for endoscopic panorama imaging in cystoscopy

Tobias Bergen, Jonas Trost, Thomas Wittenberg
Fraunhofer Institute for Integrated Circuits IIS, Erlangen, Germany, tobias.bergen@iis.fraunhofer.de

Abstract

The usual clinical procedure for early detection of cancer in the urinary bladder is the visual inspection with an endoscope (cystoscope). The very limited field of view provided by the cystoscope impedes orientation for the surgeon, thus making it challenging to ensure, that the interior bladder wall has been examined completely. Panorama imaging techniques can be used to assist the surgeon and provide a larger view field. Creating endoscopic panorama images by means of stitching techniques require image blending to provide smooth transition between fused input frames. Several blending algorithms have been proposed in the past with the goal to suppress hard transitions between images and at the same time keep a maximum amount of image structure. In this contribution, we discuss several state of the art blending techniques applied to cystoscopy images and present a new approach based on a local entropy measure. We show that local entropy is a suitable measure to achieve a good balance between transition smoothness and structure preservation. A quantitative comparison with two well-established methods shows the efficacy and efficiency of the proposed method.

1 Introduction

Cancer of the urinary bladder is considered one of the most frequent types of cancer worldwide. The accepted guidelines for early detection are based on cystoscopy, being the visual examination of the inner bladder wall with an endoscope (cystoscope) inserted through the urethra. Both, rigid and flexible endoscopes are currently used for this examination. The very limited field of view provided by these endoscopes, as well as their restricted maneuverability pose challenges for the surgeon. The biggest challenge is to ensure, that the bladder wall has been examined completely and no lesion has been overseen.

Using image mosaicking techniques, a broader field of view can be generated by stitching together sequential frames from the endoscopic video stream in order to calculate a panorama image. Several methods for cystoscopic image stitching have been proposed in the past. Behrens et al. developed a system for real-time mosaicking to provide an extended field of view of the bladder wall for fluorescence cystoscopy [1]. Miranda-Luna et al. use image stitching to provide a panorama image of the bladder for later visual assessment [2]. Daul et al. describe a mosaicking algorithm for visually coherent maps, providing an extended field of view. They also present preliminary results towards 3D surface reconstruction of a bladder phantom [3]. Soper et al. presented a reconstruction approach to build a texturized three-dimensional model of the bladder [4]. In [5], we have presented a real-time stitching approach to incrementally generate panorama images during endoscopic inspections.

Most approaches follow a standard processing pipeline (cf. Fig. 1): First, all input video frames undergo a series of pre-processing steps (valid image region masking, compensation for distortion caused by wide-angle-lenses and inhomogeneous illumination, detection of specular reflections, etc.). Afterwards, successive and non-successive video frames are registered in a pair-wise manner using either pixel-based or feature-based techniques. The output consists of a set of inter-frame transformations, which geometrically align the images. Usually, affine or perspective transformations are used. From these, a global transformation can be estimated for every video frame, aligning it in a common coordinate system, thus projecting every image onto a common surface model (most commonly a plane). Due to inhomogeneous illumination and inaccuracies during frame alignment, seams between the images are visible in the final mosaic. Thus, blending techniques are used to combine pixel values in overlapping image regions to minimize visible seams and at the same time preserve as much image structure as possible.

For cystoscopic images, typically the image center is much more illuminated than the outer regions (cf. Fig. 2). As a consequence, contrast is usually higher and a blending scheme for application in cystoscopy should be able to account for this a priori information.

2 Image blending

Several blending algorithms have been proposed in literature. For any part of the final mosaic where two or more input images overlap, the final pixel value can be calculated by either choosing one of the available input pixel values or by calculating a weighted average of all input pixel values. An early contribution by Milgram [6] proposes to determine a path through the overlap region from top to bottom and minimize the edge between the left and right part in a line-wise manner. All pixels left of this path are copied to the output image from the left input image and all pixels to the right of the path from the right image, respectively. Davis [7] extends the idea of determining an
optimal path through the overlap region by applying Dijkstra's algorithm to find a path along which the input images are similar. A gain, pixel values for the resulting mosaic are chosen from the input images and no averaging is performed. These approaches can be regarded as optimal in the sense of preserving image structure and clinical details, as no smoothing takes place. Nevertheless, visible seams will most likely not be removed completely, giving rise to a different group of approaches, which calculate weighted mean values of the input images to produce the output pixel values.

The most prominent of these approaches is linear alpha blending [8]. Assuming that the transformed images \( I_A \) and \( I_B \) overlap within the region \( I_{AB} \), pixel values of the combined mosaic image \( I_M \) in this region are calculated as

\[
I_M = \alpha I_A + (1 - \alpha) I_B,
\]

where \( \alpha \) denotes a weighting factor or transparency map. For \( \alpha = 0.5 \) for all pixel positions a simple averaging is performed. In order to remove seams along the image edges, a common choice for alpha is based on a distance transform, assigning \( \alpha = 0 \) to pixel positions along the edges and increasing \( \alpha \) to \( \alpha = 1 \) towards the image center. This is also referred to as feather blending.

Burt [9] observes that it is hard to construct a proper transparency map \( \alpha \), which is appropriate to preserve image structure at different frequencies. He therefore suggests multi-scale blending (or pyramid blending), which performs alpha blending on the different levels of the Laplacian pyramids of the input images. Let \( L_1 \) and \( L_2 \) be the Laplacian pyramids of \( I_A \) and \( I_B \) and \( G_a \) the Gaussian pyramid of the transparency map \( \alpha \). Then the output image is calculated from the combined Laplacian pyramid \( I_M \):

\[
L_M = G_a L_A + (1 - G_a) L_B.
\]

Both, linear alpha blending and multi-scale blending are suitable for endoscopic applications, since they can easily be modeled to emphasize the central image region over the outer ones. Wald et al. [10] have successfully implemented linear alpha blending for endoscopic images, generating a circular alpha mask, which suppresses the outer regions of the image. A modification of pyramid blending is presented by Behrens et al. [11] for mosaicking of fluorescence cystoscopy images. They perform multi-scale blending and adapt the alpha mask according to the average image intensity of the contributing images, thus favoring brighter image regions over darker ones. This is based on the observation, that the intensity distribution resembles a monotonically decreasing function from the image center towards the edges.

When applying the discussed blending approaches to our cystoscopic video material, several observations can be made, which motivate our proposition of a blending scheme that incorporates an entropy measure into the weighting function. Linear alpha blending is based on a transparency map, which is kept constant over time and does not account for the scale structure visible in the images. It can therefore be expected to decently smooth out seams along image edges (if the rim suppressed by the alpha mask is chosen sufficiently large), but at the same time suppress relevant image structure and thus reduce their information content. We follow the argument of Burt, that a multi-scale approach is preferable, when image structure is present at different scales in the image. This is the case for cystoscopy images, because the vessel structure by its nature varies from wide to very fine vessels (cf. Fig. 2). Moreover, due to the motion of the cystoscope, zooming effects frequently occur during an inspection, leading to changes in the scale of the vessel structure between images. For fluorescence images, Behrens et al. exploit the fact, that relevant image content correlates with its brightness, since relevant structures are highlighted by the fluorescence technology. For white-light cystoscopy this observation does not hold. In contrary, often quite bright regions with low contrast appear in the images due to over exposure effects. These regions are of little relevance and should not dominate the final mosaic image. Instead of image intensity we seek for a more general measure of relevance to guide the blending process. We have found local image entropy to be an evident and suitable measure for information content. We therefore extend Burt's multi-scale blending by a transparency map which encodes the local entropy of the input images and favors regions with high information content over those with low information content.

### 3 Entropy-based image blending

The information contained within an image region can be encoded by a local image entropy measure, defined as...
Figure 3 Measurement of local entropy with and without over exposure. Left: Images acquired with an endoscope. Right: Local entropy measure as heat map (blue = low values, red = high values).

$$H_{\Omega} = - \sum_{g \in \Omega} p_g^2 \log_2 p_g^2,$$

where $\Omega$ is a local neighborhood in the image and $p_g^2$ the relative frequency of graylevel $g$ from the valid set of graylevels $Z$ within the neighborhood $\Omega$.

We conducted a series of experiments to assess local entropy in the context of cystoscopic imaging. A target was created showing a random noise pattern, so that the information content is approximately equal across the target (cf. Fig. 3). A series of images were taken with an endoscope from varying distances, angles and illumination. The local entropy was calculated for each image within a local neighborhood of circular shape with radius $r = 5$ pixels. Fig. 3 depicts two result images. As expected, the general assumption holds that the entropy decreases towards the edges of the image, due to the inhomogeneous illumination, caused by the endoscopic light source. On the other hand, the entropy also decreases towards bright regions which tend to be over-exposed. Since this scenario is frequently found within cystoscopic images, local entropy can be used as a steering mechanism for the blending algorithm during image stitching.

Due to the setup of most rigid cystoscopy systems, the images usually depict useful information within the aperture of the ocular. This strong edge between foreground and background leads to high values of local entropy in its neighborhood. To compensate for this artefact, we multiply the entropy map with a function $\omega(x, y)$, which is defined as 1 in the central part of the image and decreases linearly to zero towards the circular border. Consequently, we define the blending mask $\alpha$ at pixel position $(x, y)$ as

$$\alpha(x, y) = \omega(x, y) \tilde{H}_{\Omega}(x, y). \quad (2)$$

$\tilde{H}_{\Omega}$ is the local image entropy normalized to the range of $[0,1]$ and $\omega$ defines the weighting function to suppress the values near the border. Fig. 4 depicts a cystoscopy image, the blending mask calculated from local entropy and the final mask composed from local entropy and border suppression.

For the blending algorithm, we use the previously defined mask $\alpha$ within a multi-scale blending approach:

1. Calculate the blending masks for the input images within the overlap region as described above.
2. Calculate the Gaussian pyramids of the input images and the blending masks by iteratively using binomial filtering and subsampling for each pyramid level.
3. Calculate the Laplacian pyramid of the input images as differences of the respective Gaussian pyramid levels.
4. Calculate the Laplacian pyramid of the blended image according to equation (1).
5. Reconstruct the blended image from the Laplacian pyramid by iteratively expanding and summing the pyramid levels.

Since the blending mask depends on the image content it is calculated for every image. Therefore, an efficient implementation is essential to make the approach suitable for a real-time stitching application. Porikli [12] presented a fast method to calculate local image entropy based on integral histograms. Integral histograms are closely related to integral images. While integral images allow the calculation of pixels sums over squared image regions in constant time (i.e. independent of the region size), integral histograms provide the histogram of a local neighborhood by four addition/subtraction operations on the integral histogram.

4 Experiments and results

We compare the proposed approach with the above described linear alpha blending and multi-scale blending. For all three methods, the weighting function is chosen equally, i.e. for linear alpha blending $\alpha = \omega$ (cf. equ. (3)), for multi-scale blending, the same $\alpha$ is used and incorporated into the multi-scale blending scheme and for entropy-based blending $\alpha$ is used as defined by equation (2). From a cystoscopy recording, we selected six images, which under-
went the stitching pipeline (cf. Fig. 1), including feature-based registration with SIFT features, robust estimation of inter-frame homographies with RANSAC and projection onto a planar surface to generate the mosaic image, blended by the respective method. To quantitatively evaluate the result, two measures were defined. The first one relates to edge intensity along frame to frame transitions. It is defined as the mean gradient amplitude (MGA) along all border pixels between overlapping frames:

$$MGA = \frac{1}{|B|} \sum_{(x,y) \in B} \| \nabla I_M(x, y) \|_2,$$

where $B$ denotes the set of border pixels. A smooth transition between images will lead to a low MGA value. The second measure specifies the preservation of image structure in the sense of similarity (Sim) between the input images and the blending result within the overlap region:

$$Sim(A) = \frac{1}{|O|} \sum_{(x,y) \in O} |I_1(x,y) - I_M(x,y)|$$

with $O$ being the set of all pixels within the overlap region (excluding the set of border pixels). This measure is calculated for both contributing input images $I_1$ and $I_B$. The final measure Sim is defined as the mean value of $Sim(A)$ and $Sim(B)$. Finally, a blending method is evaluated by calculating the mean $MGA$ and $Sim$ over all input images of the sequence. The results are depicted in Table 1. Fig. 5 compares the blending results for two of the six input images. A as a reference, we added the results obtained without any blending algorithm.

The results show, that all methods perform comparably well in terms of smooth inter-frame transitions, as can be concluded from the $MGA$ values. Without any blending a clear seam is noticeable both visually and in terms of $MGA$. All blending methods were able to reduce this effect significantly. At the same time, it can be observed that any blending scheme will reduce the mean similarity between input and output images due to their smoothing effect. In terms of $Sim$, not using any blending leads to optimal results. The closest to this is achieved by the entropy-based blending, thus making it among the evaluated methods the preferred one for the task of cystoscopic image stitching.

Table 1 Results of different blending methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>$MGA$</th>
<th>$Sim$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No blending</td>
<td>50.692</td>
<td>5.589</td>
</tr>
<tr>
<td>Linear alpha blending</td>
<td>17.746</td>
<td>6.271</td>
</tr>
<tr>
<td>Multi-scale blending</td>
<td>17.564</td>
<td>6.085</td>
</tr>
<tr>
<td>Entropy-based blending</td>
<td>17.495</td>
<td>5.869</td>
</tr>
</tbody>
</table>

Table 1 Results of different blending methods.

## 5 Conclusion

We presented a modified blending method for creating panorama images from cystoscopic image sequences. The approach is based on an established multi-scale blending method with the extension to favor high information content in the images. The information content is calculated through a local entropy measure, which can be implemented very efficiently using integral histograms. A quantitative comparison between two well-established blending methods shows the advantage of the new method. It outperforms the other methods in terms of reducing seams along inter-frame borders and at the same time preserving image content within overlap regions.

Iterative Hard Thresholding for the Reconstruction of an Undersampled System Matrix in Magnetic Particle Imaging

Matthias Kleine 1,2, Anselm von Gladiß1, Thorsten M. Buzug1,
1 Institute of Medical Engineering, Universität zu Lübeck, Germany
2 Graduate School for Computing in Medicine and Life Sciences, Universität zu Lübeck, Germany
[kleine, buzug]@imt.uni-luebeck.de

Abstract

The system matrix in magnetic particle imaging relates the measured signal with the particle concentration and is required for image reconstruction. Its exact determination is a time-consuming process, which is accomplished by measuring a small delta sample at each spatial position. Recently, a method for its reconstruction from only few sampling points has been published that takes advantage of the sparsity of its cosine transform. In this paper an alternative algorithm is suggested, which is based on the iterative hard thresholding scheme. The results of the algorithm are compared to the results of the lately proposed fast iterative shrinkage-thresholding algorithm and to the known ground truth of an magnetic particle imaging measurement. Qualitative and quantitative evaluation reveal that the suggested algorithm achieves a better interpolation of the undersampled system matrix. At the lowest examined sampling rate of 5%, image quality of the reconstructed images is clearly superior compared to the fast iterative shrinkage-thresholding algorithm.

1 Introduction

Magnetic particle imaging (MPI) is a new medical imaging modality, which was first introduced by Gleich and Weizenecker in 2005 [1]. It visualizes the distribution of superparamagnetic nanoparticles inside a three-dimensional (3D) field of view and combines high spatial and temporal resolution with high sensitivity. Furthermore, no ionizing radiation is applied. An introduction into the functional principle of MPI is given in [2].

The distribution of the magnetic nanoparticles $c$ and the Fourier-transformed measured voltage signal $\hat{u}$ are related by a linear equation, $\hat{u} = \hat{S}c$, where $\hat{S}$ denotes the MPI system matrix in frequency space. Given that the system matrix is known, the distribution of the nanoparticles is obtained as the solution of the linear system.

Even though there are approaches to determine a mathematical model to describe the MPI system matrix [3], a sufficient model has not been found yet. Therefore, the MPI system matrix has to be obtained by a time-consuming calibration scan, which is accomplished by successively measuring a small delta sample at each spatial position. The required acquisition time depends on the size of the image volume and is typically in the range of several hours to several days. Recently, a reconstruction scheme for the system matrix has been proposed, which requires only a small number of calibration scans [4]. The method exploits the sparse structure of the transformed system matrix and uses the fast iterative shrinkage-thresholding algorithm (FISTA) from [5].

In this work, the restoration of the system matrix is formulated as an inpainting problem. The term inpainting denotes the process of restoring missing or corrupted parts of a 2D or 3D signal. From the large class of inpainting algorithms, an algorithm is used, which exploits equally the sparsity of the transformed system matrix.

2 Methods

It has been shown that the discrete cosine transform (DCT) allows for a sparse approximation of the individual system matrix rows (system functions) [6]. In order to consider frequencies in both spatial directions, the 2D DCT is used in this work. As the number of elements of each system function $s_k$ is equal to the number of sampling points, each $s_k$ can be reshaped to an image of the size of the sampling grid. Inpainting is accomplished individually on each system function considered as a 2D image.

For the ease of notation, let images be represented by an 1D vector by stacking the matrix columns on top of each other. Taking this into account, the synthesis of each $s = s_k$ can be written as a matrix-vector product $s = \Phi \alpha$, where $\Phi^T$ denotes the matrix performing a 2D DCT on the system function $s$ stored as a column, and $\alpha$ represents the cosine coefficients. The analysis of the signal is given by $\alpha = \Phi^T s$.

The sparse approximation can be obtained by solving the following minimization problem for $0 \leq p \leq 1$: 
\[
\min_{\alpha} \|\alpha\|_p \quad \text{s.t.} \quad \|s - \Phi \alpha\|_2 \leq \sigma,
\]
where \(\|\alpha\|_p\) is the sparsity promoting prior. For an orthogonal basis \(\Phi\) and for \(p \in \{0, 1\}\), the closed form solution of Eq. 1 can be obtained by a simple thresholding operation. In order to adapt Eq. 1 for inpainting, a binary diagonal mask \(M\) indicating existing pixels with 1 and missing pixels with 0 can be introduced. The resulting modified minimization problem can be stated as

\[
\min_{\alpha} \|\alpha\|_p \quad \text{s.t.} \quad \|s - M\Phi \alpha\|_2 \leq \sigma. \quad (2)
\]

A similar approach for inpainting using sparse representations is proposed in [7] where the image is divided into different layers and each layer is approximated by an individual transform. As the 2D DCT achieves a sparse approximation of the MPI system functions, only this transform is used in this work. With this simplification, the algorithm from [7] becomes the iterative hard threshold (IHT) algorithm with an inserted mask in the residual step. The crucial step is the thresholding operation \(\text{TH} \delta\) with an exponentially decreasing threshold \(\delta\). Algorithm 1 summarizes the different steps of the used algorithm.

**Algorithm 1: IHT**

**Data:** Undersampled system function \(s\), mask \(M\), number of iterations \(N\), stopping threshold \(\sigma\).

**Result:** Interpolated system function \(\hat{s}\).

Initialization: \(s^{(0)} = 0\), \(\alpha^{(0)} = \Phi^T s\), \(\delta^{(0)} = 0.98 \cdot \max(|\alpha^{(0)}|)\).

for \(i = 0\) to \(N - 1\) do

\[
\begin{align*}
\alpha^{(i)} &= \Phi^T (\hat{s}^{(i)} + r^{(i)}) \\
\alpha^{(i)} &= \text{TH} \delta \alpha^{(i)} \\
\hat{s}^{(i+1)} &= \Phi \alpha^{(i)} \\
\delta^{(i+1)} &= \delta^{(i)} \cdot \left(\frac{\sigma}{\max(|\alpha^{(i)}|)}\right)^{1+\frac{1}{\alpha}} \\
\end{align*}
\]

end

\[
\hat{s} = s + (1 - M) \hat{s}^{(N-1)}
\]

The algorithm is capable of filling in missing values as the large starting threshold \(\delta_{\text{max}}\) leads to a coarse approximation of the system function at the first iterations. The missing samples are interpolated due to the overlapping support of the cosine basis functions. At further iterations, the threshold is decreased exponentially and the approximations are refined step-by-step.

In accordance to [8], it was experienced that hard thresholding leads to better results compared to soft thresholding. Therefore, the hard thresholding operator defined by

\[
\text{TH} \delta (\alpha_k) := \begin{cases} 
\alpha_k & \text{for } |\alpha_k| \geq \delta \\
0 & \text{otherwise}
\end{cases} \quad (3)
\]

for a vector \(\alpha = (\alpha_1, \ldots, \alpha_n)^T \in \mathbb{C}^n\), a threshold \(\delta\) and \(k = 1, \ldots, n\) is used in this work. The stopping threshold \(\sigma\) is chosen data-dependent and set to a fraction of the maximum of the absolute value of the known data points. Various stopping threshold denominators are tested and evaluated in terms of the difference to the known original system functions. As an error measure the normalized root mean square error (NRMSE) is used. For the determination of the optimal stopping threshold, all system functions with a signal to noise ratio (SNR) > 100 are considered. The iteration number for each system function is set to \(N = 200\). The same dataset as in [4] with an image size of \(68 \times 40\) pixels is used in this work. The algorithm is evaluated on 3 different random grids with a sampling rate of 20%, 10% and 5%, resulting in 544, 272 and 136 known data points. The used sampling grids are displayed in Fig. 1.

**Figure 1:** The used sampling patterns with a sampling rate between 5% and 20%.

The inpainting results and the corresponding reconstructions are compared to the known ground truth as well as to the results of the FISTA algorithm, which is the current state of the art. The FISTA algorithm is executed with a regularization parameter \(\lambda = 10^{-3}\) and 20 iterations. Both algorithms utilize the same sampling grids. For reconstruction, all system functions with an SNR > 10 and the iterative Kaczmarz method were used.

**3 Results**

The mean NRMSE of the interpolated system functions with respect to the stopping threshold denominator for the 3 different sampling grids is plotted in Fig. 2. The lowest NRMSE is achieved with the maximum of the known data points multiplied by 1/128 in case of the 20% sampling grid. In case of the 10% and 5% sampling grids, the optimal stopping threshold denominators are determined to be 64 and 32 respectively.

**Figure 2:** The NRMSE for 3 different sampling grids and various stopping threshold denominators \(\lambda\).
The inpainting results from the IHT with optimal stopping thresholds and the FISTA algorithm are shown in Fig. 3 to 5. For each sampling rate and both algorithms, 4 different inpainted system functions are displayed and compared to the ground truth. For the 20% and 10% sampling grid, both algorithms lead to comparable inpainting results. At 5% sampling rate, the results of the IHT algorithm appear smoother and achieve a far better recovery of the displayed system functions compared to the FISTA algorithm. The visual impression is confirmed by the numerical evaluation regarding the NRMSE between the interpolated and the fully sampled system functions with an SNR > 100. The NRMSE between the interpolated results of the IHT algorithm and the original system functions is lower than the NRMSE of the results of the FISTA algorithm for each evaluated sampling grid. The comparison of the NRMSE of the two evaluated algorithms is plotted in Table 1.

Table 1: The NRMSE between the inpainting results and the original system functions with SNR > 100.

<table>
<thead>
<tr>
<th>Sampling Rate</th>
<th>IHT</th>
<th>FISTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>20%</td>
<td>0.0148</td>
<td>0.0318</td>
</tr>
<tr>
<td>10%</td>
<td>0.0497</td>
<td>0.0603</td>
</tr>
<tr>
<td>5%</td>
<td>0.0975</td>
<td>0.1339</td>
</tr>
</tbody>
</table>

The reconstructions of the interpolated system functions are shown in Fig. 6 to 8. All images were reconstructed with the same regularization parameter and are displayed in the same gray value window. Comparable to the inpainting results, the reconstructions of the P-Phantom appear similar for the 20% and 10% sampling case for both algorithms. For the 5% sampling case, the reconstruction of the IHT result is clearly superior to the reconstruction of the FISTA result. Whereas the different points of the P-Phantom are still distinguishable after inpainting with the IHT algorithm, the reconstruction of the FISTA result is stronger affected by noise and suffers from a lower resolution.

Fully implemented in MATLAB, one system function is processed in about 0.3 seconds (200 iterations), resulting in a runtime of about 2.5 minutes for 1332 system functions with SNR > 10 when running parallel on 3 cores. Parallelization is feasible as each system matrix row can be processed independently. Consequently, further acceleration of the inpainting process can be achieved by executing the algorithm on a larger number of cores and by using a native programming language.

4 Conclusion

In this work it is shown that the modified IHT algorithm achieves a smooth interpolation and is suitable to restore an undersampled MPI system matrix. The algorithm is very fast as each iteration contains mainly the application of the 2D DCT and the inverse 2D DCT, which provide fast implementations. The algorithm outperforms the FISTA algorithm when the sampling rate is reduced down to 5%. Given that the purpose of this work is the reduction of the calibration scan time, the performance at lower sampling rates is mostly relevant. Hard thresholding appeared to be advantageous compared to soft thresholding in this paper. A quantitative evaluation of this observation is pending. The stopping threshold was chosen data-dependent in this work. Even though this approach seems to be reasonable, further validation is planned.
Figure 5: Inpainting results for the sampling rate 5% compared to the original system functions.

Figure 6: Reconstruction results for the sampling rate 20%.

Figure 7: Reconstruction results for the sampling rate 10%.

Figure 8: Reconstruction results for the sampling rate 5%.

References


Characterization of Superparamagnetic Nanoparticles using a Micro-CT Phantom: Estimation of Iron Concentration in Ferrofluids

C. Debbeler, J. Müller, and K. Lüdtke-Buzug
Institute of Medical Engineering, Universität zu Lübeck, Lübeck, Germany, debbeler@imt.uni-luebeck.de

Abstract

This paper introduces micro computed tomography (micro-CT) as a method for the determination of iron concentration of ferrofluids and thus an alternative to photometry or atomic absorption spectroscopy. A phantom has been designed and an evaluation software has been implemented, which enable the user to determine the concentration without chemical processing of the ferrofluid. First results raise expectations that an accurate determination of the iron concentration is possible with micro-CT. This approach is of particular interest in the field of medical imaging, where it could be used for technical validation of new iron-tracer based modalities as magnetic particle imaging, promising quantitative images. One application with great potential is the use of superparamagnetic iron oxide tracers in lymph node biopsies instead of radioactive tracers. However, before the method can be applied in human imaging, CT could be used to determine the iron concentration in lymph nodes of a mouse model, without depending on invasive techniques as histologic staining.

1 Introduction

Today, several methods are known to allow for high resolution medical imaging. X-ray, computed tomography (CT), or magnetic resonance imaging (MRI) are the most common used in daily routine. If it is necessary to improve the image contrast and thus make diagnostics easier, contrast agents are administered to patients before the imaging process. In case of X-ray and CT, these diagnostic agents consist of iodine-based material. For MRI gadolinium solutions or superparamagnetic iron oxide nanoparticles (SPION) are used [1].

An emerging technology in the field of medical imaging is magnetic particle imaging (MPI), which detects the spatial distribution of superparamagnetic nanoparticles in a volume of interest [2]. For this imaging method, ferrofluids based on superparamagnetic iron oxide are used as contrast agents, which are administered into the patient [3].

Even though iron is one of the mineral nutrients, it can get highly toxic if injected in high concentrations, which may result for example in cirrhosis of the liver or coronary artery diseases. Commonly used methods for the determination of the iron concentration in a tissue matrix are photometry or atomic absorption spectroscopy (AAS). Both require time consuming laborious preparations of the tissue using staining reagents [4].

The approach presented here is to simplify this process. The feasibility of a concentration measurement using micro computed tomography (micro-CT) based on the X-ray attenuation coefficient has been analysed, using the Hounsfield scale as correlation between the X-ray attenuation coefficients and the grey values. A phantom has been constructed which keeps artefacts as small as possible, avoids evaporation of the SPION suspension, and meets the requirements of the small measuring chamber. In a second step, the phantom measurements have been calibrated by correlation of the X-ray attenuation values with known iron concentrations of the SPION suspension.

2 Material and Methods

2.1 Micro-CT phantom

In the initial design of a SPION CT phantom, three major considerations have been taken into account. It has to meet the requirements of the micro-CT measurement, should avoid evaporation, and allow for multiple measurements in a single CT sequence. A first generation phantom has been produced with a combination of a screwable lid and a toric joint to avoid evaporation. Six SPION chambers with a volume of approximately 15 µl each enabled multiple measurements in a single step.

First measurements with ferrofluids indicated that discrimination of different iron concentrations is possible. However, a problem of the first phantom generation was the multiple components it consists of. Another disadvantage of the first generation phantom was the metal device used for fixation of the phantom in the measurement chamber of the micro-CT.

The required measurement time was determined using this first generation phantom. The measurement time was set to 15 minutes, taking the needed number of averages into account to reduce noise influence.

Based on the results obtained with the first generation phantom, an improved phantom has been designed. CAD draw-
nings of the phantom are shown in Image 1. To be able to increase the image resolution, the size of the phantom was decreased to a height of 20 mm and diameters of 16 mm at the top and the bottom (a) and 5 mm in the middle (b), which results into a volume of approximately 300 \( \mu \text{l} \). The number of chambers has been reduced to one, especially since first measurements had shown that data acquisition would take only about 15 minutes. A third alteration resulted into the removal of the lid. The chamber is now sealed with wax, although evaporation is unlikely to occur because of the short acquisition time. And, as a last improvement, a special fixation device has been designed, which replaces the metal device.

![Image 1: CAD drawing of the second generation phantom.](image1)

The second generation phantom as well as the fixation device have been produced out of polyoxymethylene. This phantom has been used for all measurements presented in this contribution.

### 2.1.1 Micro-CT measurements

For the measurements, a Skyscan 1172 high-resolution micro-CT was used (Bruker microCT, Belgium). It has a fully shielded measurement chamber and uses the cone-beam X-ray geometry. As opposed to clinical CTs, in micro-CTs the mounting stage rotates between the source and the two-dimensional flat-array detector, which are both fixed.

In a first step, appropriate measurement parameters were determined. Several measurements have been carried out, alternating either the voltage, the measurement time, or the X-ray source filters. The chamber was either filled with air, distilled water, or a highly concentrated SPION-based ferrofluid.

For 3D reconstruction of the measured data the software NRecon was used, which is also provided by Skyscan. This software uses the Feldkamp, Davis and Kress approach ([5, 6]) for the volume reconstruction. An image of one reconstructed slice of the phantom is shown on the left in Image 2. In the middle, the chamber with the ferrofluid is located (a), the ring around the chamber shows the plastic of the phantom (b), which is surrounded by air (c).

![Image 2: Reconstructed image of one slice of the second phantom generation (Image 1, (c)), filled with highly concentrated ferrofluid.](image2)

In order to compare measurements with different iron concentrations, the mean grey value was used as a measure for the X-ray attenuation coefficient. The mean grey value was determined over the areas of both masks and then displayed. The final algorithm automatically assigns a concentration to the calculated mean grey value. One crucial aspect for the approach chosen in this project is the choice of imaging parameters, which has to remain constant in order to guarantee for an accurate determination of the iron concentration in the ferrofluid.

### 2.2 Photometry measurements

Photometry measurements of the SPION suspensions have been performed in order to verify the CT measurements. As preparation, the iron oxide particles were chemically freed of their dextran shell, deoxidized to bivalent iron, and a prussian blue complex formation was triggered. This made it possible to measure the light absorption of the solution at a wavelength of 690 nm. With the help of a calibration curve, the detected absorption could be matched to a specific concentration [7].

### 3 Results and Discussion

#### 3.1 Measurement parameters

To find the optimal CT measurement parameters for the concentration determination, several measurements with
varying measurement time, X-ray source filters, and voltages were performed. The source-object distance was set to be as small as possible while still imaging over the whole phantom diameter (Image 1, (c)).

To improve the concentration determination, different filters and voltages were tested. For these, the phantom was either filled with a ferrofluid of an iron concentration of 0.5 mol/l or with distilled water (0 mol/l). There were three options for the X-ray source filtering: either no filter, a 0.5 mm aluminium filter, or an aluminium-copper filter. The voltage was increased from 40 kV to 100 kV in steps of 20 kV.

The results for the mean grey value of the chamber (mask a) have been plotted in Image 3. The mean grey value decreases with increasing filter efficiency and voltages. Additionally, there is a difference detectable between high and low iron concentrations, which indicates that the determination of the iron concentration of ferrofluids using micro-CT is possible.

Image 3: Mean grey value at different voltages for varying filters and iron concentrations.

The further significance of these measurements becomes obvious, when the proportion of the mean grey values at different concentrations is plotted, which is shown in Image 4. In order to determine a concentration as accurate as possible, a large difference between the highest and lowest detectable concentration would be desirable. This is the case for measurements with a voltage of 40 kV and a 0.5 mm aluminium filter, for which reason those parameters were chosen for all further measurements.

The measurement time is mostly influenced by the chosen filter and the number of averagings. With the chosen 0.5 mm aluminium filter and an averaging of 9 images, which reduces the noise in the image, it is about 15 minutes.

3.2 Measurements with different iron concentrations

Using the previous set of parameters, first measurements with varying iron concentrations have been performed. A serial dilution with concentrations between 0 mol/l and 0.5 mol/l has been prepared, which was then measured three times. Image 5 displays the results of these measurements.

Image 5: Mean grey value of the chamber and the plastic, measured at increasing iron concentrations over three independent measurements with 40 kV and a 0.5 mm aluminium filter. The abscissa shows the calculated concentration of the measured ferrofluid.

The mean grey values measured in the chamber increase proportionally with the iron concentration, which again promises an accurate determination of the ferrofluid concentration based on micro-CT. The mean grey values of the plastic material (mask b) do not change significantly. Small displacements probably result from the use of two different phantoms, because earlier comparisons of both phantoms already showed small differences in the mean grey values of the plastic.
3.3 Verification of the prepared iron concentrations

In a second measurement, the concentrations of the serial dilution used for the measurements presented in section 3.2 were determined using photometry, which are shown in Table 1.

Table 1: Iron concentrations of the second serial dilution, determined by photometry

<table>
<thead>
<tr>
<th>Volume ratio of ferrofluid / distilled water</th>
<th>Concentration in mol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 / 1.0</td>
<td>0.000</td>
</tr>
<tr>
<td>0.2 / 0.8</td>
<td>0.090</td>
</tr>
<tr>
<td>0.4 / 0.6</td>
<td>0.165</td>
</tr>
<tr>
<td>0.6 / 0.4</td>
<td>0.240</td>
</tr>
<tr>
<td>0.8 / 0.2</td>
<td>0.325</td>
</tr>
<tr>
<td>1.0 / 0.0</td>
<td>0.445</td>
</tr>
</tbody>
</table>

The results of the corresponding micro-CT measurement are displayed in Image 6, using the concentrations determined by the photometry measurements for the scaling of the abscissa. The increase of the concentration again causes an almost linear increase of the mean grey value. This should make the calculation of calibration curves possible, which can be used to determine the iron concentration using micro-CT measurements.

Image 6: Mean grey value of the chamber and the plastic versus measured concentrations of the ferrofluid (Table 1), measured at increasing iron concentrations over two independent measurements with 40 kV and a 0.5 mm aluminium filter.

4 Conclusion

In this paper, micro-CT is introduced as a new method for the determination of the iron concentration of ferrofluids. This offers an alternative to photometry or atomic absorption spectroscopy, because micro-CT measurements could avoid the time consuming chemical altering of the ferrofluid.

A phantom has been constructed, which meets the requirements of micro-CT measurements, avoiding evaporation and artefacts and heeds the dimensions of the X-ray measurement chamber. An evaluation software has been implemented, which enables the user to determine the concentration using the X-ray attenuation coefficient. First results indicate that a determination of the iron concentration is possible with micro-CT.

One aspect which requires further investigation, is the increase of temperature during very long or a large number of measurements and its effect on the viscosity and the attenuation coefficient and thus the detected mean grey value. First experimental measurements on this topic show no influence of the temperature though.

The approach is of particular interest in the field of medical imaging, where it could be used to combine different imaging modalities, for example magnetic particle imaging and computed tomography.

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5 References

Impact of electrode positioning on EIT data interpretation

S. Krueger-Ziolek, Z. Zhao and K. Moeller
Institute of Technical Medicine, Furtwangen University, Villingen-Schwenningen, Germany, krue@hs-furtwangen.de

Abstract

The aim of this study was to demonstrate the impact of the measurement electrodes positioning on electrical impedance tomography (EIT) data. EIT measurements were conducted at three different thoracic levels (denoted as cranial, middle and caudal) during normal breathing and vital capacity manoeuvres in five healthy volunteers. Changes in relative impedance to volume changes were calculated for expiratory reserve volume (ERV), tidal volume (VT) and inspiratory reserve volume (IRV). Furthermore, we investigated the ratio between relative tidal impedance changes and tidal volume (ΔI/VT/VT) in mechanically ventilated female patients with healthy lungs during an incremental positive end-expiratory pressure (PEEP) trial, because we suspected to find a tendency towards more caudal belt positions. Results showed that ΔI/ΔV differed in cranial-caudal direction for healthy non-ventilated volunteers. Since atelectasis and overdistension were not present in this group of volunteers, we suggested that changes in ΔI/ΔV might be caused by the shift of lung tissue in cranial-caudal direction during respiration. For mechanically ventilated female patients ΔI/VT/VT increased with rising PEEP, showing a similar trend like ΔI/ΔV in the caudal chest plane of the healthy volunteers. Since changes in ΔI/ΔV are often related to recruitment in patients with lung diseases, which was not the case in our study, EIT data might be misinterpreted without considering electrode plane positioning.

1 Introduction

Electrical Impedance Tomography (EIT) is a noninvasive imaging technique which enables a radiation-free monitoring of regional lung aeration at the bedside and provides information of the regional lung function. Utilizing varying electrical properties of the lung tissue induced by changes in air content and blood flow according to the breathing cycle [1], cross-sectional images of impedance distribution within the human thorax could be generated. Electrical potentials caused by excitation of a small alternating current are measured between surface electrodes placed in a transverse plane around the studied thorax section. Resulting potential differences are used to reconstruct 2-dimensional EIT images [2]. Usually one array of surface electrodes are added around the thorax between the 4th and 6th intercostal space based on the fact that artificial ventilated patients frequently show anesthesia-associated atelectasis in dependent juxta-diaphragmatic lung regions [3]. Although gaining helpful indices of atelectasis, overdistension and tidal recruitment during mechanical ventilation, the information content is restricted to the chosen thorax section. Since the position of the electrodes is fixed to the thorax surface and the lung tissue is constantly moved due to inspiration and expiration imaging averages over a certain, non-definable part of the lung.

There are some EIT studies, including EIT measurements at different thoracic levels, showing that during mechanical ventilation regional ventilation distribution differs within a studied thorax plane depending on parameters like positive end-expiratory pressure (PEEP) and tidal volume (VT) [4, 5]. Another EIT study including healthy non-ventilated adults reveals that posture has a significant influence on regional ventilation distribution within the thorax varying by ventilation mode and measuring plane [6]. During mechanical ventilation differences in ratios of relative impedance changes to changes in volume (ΔI/ΔV) between various chest planes are often attributed to different recruitment rates of the studied thorax sections. Furthermore, it never can be ruled out that other organs like plethysmographic measurements depend on the volunteers’ coop-
eration, at least three FRC values that agree within 5% have to be measured according to guideline [7]. At the same time EIT measurements (PulmoVista 500, Dräger Medical, Germany) were conducted successively at three different thoracic levels (labelled as cranial, middle and caudal) using an electrode belt consisting of 16 surface electrodes (Fig. 1). The first thoracic level was defined right under the armpits (around the 3rd to 4th intercostal space). The second level was set around the 5th and the third level around the 7th intercostal space. A purpose-built system composed of a body plethysmograph and an electrical impedance tomograph enabled the simultaneous performance of lung function tests and EIT measurements.

Figure 1 EIT data of one healthy volunteer during normal breathing (including four plethysmographic occlusion pressure manoeuvres) and vital capacity manoeuvre. EIT measurements were performed at the 5th intercostal space (denoted as middle thorax section). ERV: expiratory reserve volume, VT: tidal volume, IRV: inspiratory reserve volume, AU: arbitrary units.

Plethysmographic FRC measurements were carried out to verify that a healthy volunteer has breathed at similar ventilation levels during EIT measurements at different thoracic planes. Measurements were repeated three times for each thoracic level (in total nine plethysmographic/EIT measurements per test person). On each thoracic level the measurement with the highest cooperation of the volunteers i.e. showing highest vital capacity, was chosen for further calculations. Ratios between relative impedance changes and corresponding volumes were calculated for ERV, VT and IRV (ΔERV/ERV, ΔVT/VT and ΔIRV/IRV).

2.2 EIT measurements on mechanically ventilated patients

EIT data of three mechanically ventilated female patients with healthy lungs (30.7 ± 8.4 y, mean age ± SD, 67.7 ± 4.5 kg, mean body weight ± SD, 170.0 ± 6.0 cm, mean height ± SD) during an incremental PEEP trial were retrospectively investigated. Female patients were selected from this study, because we expected to find a tendency towards more caudal EIT belt positions. Please refer previous studies for detailed information regarding patients and study protocol [8].

According to the protocol the electrode belt which is composed of 16 electrodes (EIT Evaluation KIT 2, Dräger Medical, Germany) was placed around the thorax at the 5th intercostal space. The ratio between relative tidal impedance changes (ΔIVT) and tidal volume (VT) was calculated for every PEEP step (PEEP from 0 to 28 mbard, 2 mbard steps).

3 Results

3.1 EIT data analysis (spontaneously breathing volunteers)

During the body plethysmographic measurements all five healthy volunteers showed a high level of cooperation. At least three satisfying occlusion pressure manoeuvres were performed to determine mean FRC. In total nine body plethysmographic measurements (3 measurements per studied thoracic level) were performed by each volunteer. FRC and VC values varied less than 5% within the nine measurements. ΔERV/ERV, ΔIVT/VT and ΔIRV/IRV were calculated for every test person in each studied thoracic level (Fig. 2). All three patients showed an increasing relative tidal impedance to tidal volume ratio with rising PEEP. At a PEEP level of approx. 22 mbard a slight decrease in ratio was discernible.

3.2 EIT data analysis (mechanically ventilated patients)

Ratios of relative tidal impedance changes and tidal volume (ΔIVT/VT) were calculated for three healthy mechanical ventilated female patients during an incremental PEEP trial (Fig. 2). All three patients showed an increasing relative tidal impedance to tidal volume ratio with rising PEEP. At a PEEP level of approx. 22 mbard a slight decrease in ratio was discernible.

Figure 2 Ratios of relative tidal impedance changes (ΔIVT) and tidal volume (VT) of three mechanically ventilated female patients (P1-P3) during an incremental positive end-expiratory pressure (PEEP) trial.
4 Discussion and Conclusion

In the present study we demonstrated that during vital capacity manoeuvres, including a deep expiration to residual volume (RV) and a full inspiration to total lung capacity (TLC), ratios between relative impedance changes and corresponding volumes (ΔI/ΔV) differ in various thoracic levels in healthy volunteers.

Since healthy non-ventilated volunteers were investigated, atelectasis and overinflation were excluded as possible explanations for varying ratios. Therefore, we suggested that changes in ratios might be caused by different amounts of lung tissue captured during the measurements. There were distinct differences between ΔERV/ERV and ΔIERV/ERV in the cranial and caudal thoracic plane during deep expiration and full inspiration, assuming that the movement of the respiratory muscles (mainly diaphragm) shifted the lung tissue within the chest which in turn caused differences in regional impedance.

As the variation within the measured FRC values was less than 5% for each volunteer, we concluded that every test person tried to perform the VC manoeuvres with nearly the same effort for each studied chest plane. We could not exclude that other intrathoracic organs might be captured during the EIT measurements in the studied chest planes as well.

Additionally, we confirmed that under mechanical ventilation the ratio of relative tidal impedance change and tidal volume (ΔIVT/VT) increased during an incremental PEEP trial. An increase of PEEP leads to an inflation of the lung which will manifest in EIT imaging like an inspiration. ΔIVT/VT showed a similar trend during rising PEEP (Fig. 2) like ΔERV/ERV, ΔIVT/VT and ΔIERV/ERV in the caudal thorax section of the healthy volunteers (Fig. 3). The slight decrease of the ratios at higher PEEP levels (starting from approx. 22 mbar) might be caused by an overdistension of the lung. Since the positioning of the electrode belt at the 5th intercostal space on a female thorax is complicated by the female anatomy, electrode belts are often placed slightly lower on the female thorax, which could be suspected by our results. Since the increase of ΔI/ΔV might correlate to recruitment in patients with lung diseases, which is not the case in the presented study, the EIT data might be misinterpreted due to the positioning of the EIT electrodes. Therefore, it is important to consider the positioning of the electrode plane for EIT data interpretation.

One limitation of the study was that EIT data analysis was restricted to five healthy volunteers and three mechanically ventilated patients. Additional measurements are required for statistical evaluations. EIT measurements on mechanically ventilated female patients were limited to one thoracic plane. There was no information on ΔI to ΔV ratios of other thoracic planes (e.g. 3rd to 4th intercostal space).

5 Acknowledgement

This work was partially supported by the Federal Ministry of Education and Research under grant no. 03FH3813 (MOSES) and grant no. 01PL11008 (TREFFER).

6 References


Design and Construction of an Ultrasound Transducer Positioning and Fixation Device

Dennis Sandkühler, Linda Kopp, Ewald Bonberg, Anke Poelstra and Heinrich Martin Overhoff
Medical Engineering Laboratory, Westphalian University of Applied Sciences, Gelsenkirchen, Germany
dennis.sandkuehler@w-hs.de

Abstract

Ultrasound-based precision measurements require a precise alignment of the ultrasound transducer. At the example of analyzing the backscatter signal from a point reflector in a homogeneous medium, problems of misalignment were discussed and on this basis a transducer positioning and fixation device was designed and constructed. The device allows a fixation of the transducer to a phantom. After the fixation, the transducer could be adjusted to compensate misalignments. The construction design for adjusting the most common used transducers types is explained in this article.

1 Introduction

Ultrasound imaging has been proven over the past decades and is now the most used imaging modality in medical diagnostics. Several kinds of ultrasound-based precision measurements demand for precise alignment of a clinical used transducer vs. a measurement object. Regarding the case of analyzing for example the backscatter signal from a point reflector in a homogeneous medium. Figure 1 demonstrates the transducer alingment relative to a phantom coordinate system.

An ultrasound backscatter signals can be caused by main or side lobes of the transmitted ultrasound wave [5, 1, 4]. The main and side lobes differs in their amplitude and frequency domains [6, 2]. Due to small pitch and roll misalignment of the transducer the backscatter signal from the point reflector might be more influenced by the side lobe then by the main lobe. An example of the effect due to misalignment is presented in figure 2. To avoid erroneous measurements the exactly placement of an ultrasound transducer over a scattering structure is necessary.

Figure 1: Transducer alingment relative to a phantom coordinate system.

Figure 2: Ultrasound image excerpt of the point reflector (color coded). Misalignment of the transducer and side lobes effects leads to the asymmetrical imaging.

2 Methods

The main problem in positioning the ultrasound transducers is the misalignment of the pitch and the roll angle. As the alignment of the piezoelectric elements can only approximately derived from the transducers shell. Rotation around the transducer’s z-axes and also 3D translation can be realized by accurately fitting of the phantom in a rack.

The range of adjustment of the pitch $\delta_{\text{pitch}}$ and the roll angle $\delta_{\text{roll}}$ can be obtained from the resolution of the ultrasound transducer. The resolution of an ultrasound trans-
ducer consists of three single resolutions for each spatial directions. These resolutions are called axial (in transmit direction of the ultrasound wave), lateral (in the image plane and perpendicularly to the transmit direction) and elevational (perpendicularly to the image plane and to the transmit direction) resolution. For the adjustment of the pitch and the roll angle the lateral and elevational resolution are important. As the elevational resolution is estimated to be up to five times wider than the lateral resolution, the range of adjustment is oriented in the minimum of roll angle that is necessary in order to cover the width of elevational resolution in a given depth. Also the resolution changed within the transducers frequency $f$. The widest elevational resolution can be found for a conventional $f = 2.5$MHz transducer with $d_{\text{elevational}} \approx 9.5 \text{mm}$ [3] and $s = 100 \text{mm}$ depth of penetration of the ultrasound wave. To detect a change in the received signal or the reconstructed ultrasound image while adjusting the roll angle, a range of roll adjustment $\delta_{\text{roll}}$ should be greater then:

$$\delta_{\text{roll}} > \arctan\left(\frac{d_{\text{elevational}}}{2s}\right).$$

With the given parameter values a range of roll adjustment of at least

$$\delta_{\text{roll}} > \arctan\left(\frac{9.5 \text{mm}}{2 \cdot 100 \text{mm}}\right) = 3.02^\circ$$

has to be implemented in the construction design. As the actual elevational resolution of transducers is estimative, the range of roll and pitch adjustment is chosen to $\delta_{\text{roll}} = 3^\circ$ and $\delta_{\text{pitch}} = 6^\circ$.

In the last decades, 2D ultrasound imaging is extended to 3D (spatial ultrasound volume) and 4D (3D varying in time), therefor the positioning and fixation device should also be customizable to connect the most transducers types. For this purpose, an ultrasound transducer positioning and fixation device with the following properties and assemblies is desired:

- Connection of transducers (2D - 4D) to the device.
- Adjustment of pitch and roll angle within $\pm 6^\circ$.
- Rack for positioning the transducer to a phantom.

At present, the positioning and fixation device is designed and constructed for the following transducers from Esaote S.p.A. (Genoa, Italy) and Vermon (Tours, France)

<table>
<thead>
<tr>
<th>Transducer</th>
<th>Type ID</th>
<th>Distributor</th>
</tr>
</thead>
<tbody>
<tr>
<td>linear 2D</td>
<td>LA523</td>
<td>Esaote Biomedica</td>
</tr>
<tr>
<td>convex 2D</td>
<td>CA431</td>
<td>Esaote Biomedica</td>
</tr>
<tr>
<td>linear mech. 3D</td>
<td>BL433</td>
<td>Esaote Biomedica</td>
</tr>
<tr>
<td>convex mech. 3D</td>
<td>BC431</td>
<td>Esaote Biomedica</td>
</tr>
<tr>
<td>linear mech. 3D</td>
<td>custom</td>
<td>Vermon</td>
</tr>
</tbody>
</table>

Table 1: List of applied transducers.

3 Results

3.1 Connection of the transducers

The positioning and fixation device should be able to use with several kinds of transducers. That means, a massive electronically 4D transducer should also be attachable as a mechanical, vibrating 3D transducer and a small 2D transducer. To transmit the desired rotational movements (pitch and roll) to the transducers, the transducer must be clamped in a holder. A form-fitting casing for a transducer designed from the transducers shell seems to be recommended. Therefor the shells of all transducers from tabular 1 was digitize using an non-contact measurement system (ScanArm, FARO, FL, USA ) of accuracy $\pm 35 \mu\text{m}$ to obtain points from the transducer’s shell. From this points the surface models of each transducer were reconstructed. Figure 3 exemplify the obtain points and the reconstructed surface model.

![Figure 3: Point cloud and reconstructed surface of the transducer shell.](image)

Due to the mostly ellipsoidal shape of the transducers two half shells which are the negative print of the transducers surface on the inside (elements 1a and 1b in figure 4) was constructed. This construction leads to a form-fitting fixation. The outside of one of the shells is mounted to the mechanical pitch adjustment component.

3.2 Adjustment of pitch and roll angle

The pitch adjustment was realized mechanically by two L-brackets (elements 2a and 2b in figure 4) based on the principle of a mirror mount device. The upper L-bracket (2a) is used to connect to the half shell (1a). With a metric fine pitch screw (2c), the degree of inclination up to $\pm 6^\circ$ can be set. Here, the ultrasound transducer is at $0^\circ$ position exactly in the horizontal. In the lower L-bracket (2b) a hardened cylinder is pressed in, serves as a counter bearing for the metric fine pitch screw. The mechanical link is realized with four tension springs (2d) connecting the L-brackets and two rolling elements (2e). The metric fine pitch screw works against the spring force. Through the rolling elements only the upper L-Brackets can roll when turning the metric fine pitch screw. The roll adjustment assembly realized a rotation of the transducer perpendicular to the image plane. The assem-
bly enables a first rough rotational alignment of the trans-
ducer and the related ultrasound image to allow several
image orientations and also a fine rotary movement for
precise adjustment. The roll adjustment basically con-
sists of five elements shown in figure 5 and 6:

- Socket (3a) connected to the lower L-bracket (2b),
- element for rough roll alignment (3b),
- housing (3c) connected to the rack,
- a tension spring (3i),
- and a metric fine pitch screw (3j) for precise adjust-
ment.

The rough rotational alignment was realized by a loose fit
between the socket (3a) and the element (3b). Both can
be fixed to each other by a knurled head screw (3f), that
ensures a nonslip traction between the element (3b) and
the socket (3a). At the socket (3a) the upper L-bracket
(2b) will be fixed. The socket is bedded in the housing
(3c). Thus a mechanical adjustment of the roll angle can
be done, a tension spring (3i) is mounted between the ele-
ment (3b) and the housing (3c) to put them under tension.
By adjusting the metric fine pitch screw (3j), the socket
(fixed to the element (3b)) could be rotated against the
housing (3c) in the range of $\pm 6^\circ$. The housing (3c) will
be fixed to the rack later on.

In figure 7 the three dimensional engineering drawing of
the entire adjustment assembly is presented.

3.3 Mounting of assemblies

From the construction design, all assemblies were pro-
duced. For all transducers from tabular 1 the half shells
has been made in a rapid prototyping process using a
polyoxymethylene plastic. Figures 8 and 9 shows exem-
plary the manufactured half shell for the linear mecha-

- nical transducer BL433 from Esaote Biomedica.
The adjustment assembly was mounted in a rack to place
the transducer over a phantom. An basin, as shown in fig-
ure 10, was made were phantom objects can be placed in
homogeneous media later on. The basin could be placed
in a defined position to the transducer.

4 Conclusion

A positioning and fixation device for ultrasound trans-
ducers was constructed to achieve the desired adjustment
properties. In a first application it was asserted that the
adjustment of the yaw angle could be also of interests,
if the design tolerances of a phantom has to be comp-
pensated or if the piezoelectric elements were also mis-
aligned about the $z$-axis of the transducer. Accuracy
evaluation of the whole positioning device is currently in
progress.
Acknowledgement

We thank the Ministry of Work, Social Integration and Welfare in collaboration with the Ministry of Economic Affairs, Energy and Industry and the Ministry of Innovation, Science and Research of Northrhine-Westfalia, Germany for the funding of this work in the Med in.NRW program (grant no. 005-GW01-78A).

References


Utilization of Shape and Texture features with Statistical Feature Selection Mechanism for Classification of Malignant Tumors in MR Images

Ankit Vidyarthi¹, Namita Mittal²
¹²Department of Computer Engineering, Malaviya National Institute of Technology Jaipur, India
¹2012rcp9514@mnit.ac.in, ²nmittal@mnit.ac.in

Malignant Brain Tumor is one of the life threaten diseases in the field of medical science. On the basis of World Health Organization (WHO) reports among all types of tumor malignant brain tumors are most dangerous tumor. Every year lot of individuals lost their life because of either misjudgement in analysis of tumor type in its early stage or unpredictable tumor type at first sight. Proper identification for these tumors in their early stage is an extremely difficult clinical task that must depend upon information gathered through non-invasive techniques like biopsy and spectroscopy. In this paper a new hybrid feature extraction mechanism is proposed which help in classification of malignant tumors like Central Neuro Cytoma (CNC), Glioblastoma Multiforme (GBM), Gliomas, Intra Ventricular Malignant Mass, Metastasis in its early stage of development. The proposed feature extraction mechanism uses the shape and textural features for classification of malignant tumors. Among the extracted features, relevant features are identified using cumulative frequency based features selection mechanism. K-Nearest Neighbour (KNN), a supervised classification, algorithm is used for analysis of the results. For experimental purpose 150 brain tumors MR images are used having 30 images each for Central Neuro Cytoma, Glioblastoma Multiforme, Gliomas, Intra Ventricular Malignant Mass, Metastasis. The classification accuracy achieved for each type of malignant tumors are 90%, 93%, 93%, 90%, 93%. The overall accuracy achieved is nearly 92% for whole dataset of malignant brain MR images.

Keywords—Brain Tumors; Feature Extraction; Feature Selection; Supervised Classification; KNN;

I. INTRODUCTION

Brain tumors in human body comprise a diverse group of neoplasm that vary in their behaviour depending on variety of factors as cell of origin, site of occurrence, morphology and pattern of spread. An abnormal growth of the cells formed a solid mass inside the body which is termed as tumor by radiologist. As per the World Health Organization (WHO) there are almost 120 types of brain tumors which are recognized till date [1]. These identified tumors can be further classified into benign and malignant tumors [2].

Since the locality of tumor is not specific to any pre defined location so it can affect any of the brain lobes where it initiates. Clinical methodologies regarding the treatment of brain neoplasm shows trust on variety of imaging modalities like CT scan, MRI, fMRI, PET etc. Each of the imaging modality has a specific feature role of disease diagnosis. Among the various imaging modalities Magnetic Resonance Imaging (MRI) is the most widely used and radiological relied imaging modality in clinical diagnosis at various stages of the treatment. MRI helps in visualization of the soft tissues more apparently than other imaging techniques.

For the classification of brain tumors in [3] author uses genetic algorithm for feature extraction which was used by fuzzy c-means for segmentation purpose. While intensity based shape features was identified using Fourier [4] analysis for classification of the breast tumors. A more robust feature extraction model is defined in [5] based on the concept of GARCH series. The wavelet based parameters was used to calculate the feature vector followed by PCA and LDA for selection of relevant features. Another approach for generating intensity based features using Discrete Wavelet Transformation (DWT) was proposed in [6] for classification of brain tumors using Bayesian Neural Network (BNN).

In tumor classification basically three domain features are extracted like spatial domain, frequency domain and intensity based features [7]. These features vector represent the overall imaging statistics which was used by the classifier for its categorization. Wavelet based feature extraction [5, 8] was mainly used with non linear classifiers and neural network variants. In [9], author describes the comparative study for the classification of brain tumors which was done in past for the duration of 2009 to 2013. In [12], author gives the idea about use of intensity, shape and texture spatial domain features for the segmentation of posterior fossa tumor. These imaging features effectiveness is shown with feature selection mechanism for the segmentation of tumor region from MR image. A decision forest based tumor segmentation using symmetric Texture and symmetric intensity based features is shown in [16]. While a frequency based features classification using Discrete Wavelet Transformation (DWT) is described in [13].

A probabilistic neural network model PNN with textural features also used for brain tumor characterization [14]. While a modification based on iteration of ANN is done in [15], which improves the performance rate of abnormal to normal brain MR image classification.

The rest of this paper is organized as follows: Section II discusses about proposed methodology for tumor classification. Section III, gives the experimental results. Section IV, gives the conclusion of the paper and future work.
II. PROPOSED APPROACH

To identify the degree of malignancy and type of malignant tumor in brain MR image a new hybrid multi-parametric features set based on shape and texture is used. This hybrid feature set is incorporated via a pattern classification mechanism for brain neoplasm classification. Proposed approach uses four phases for the identification of tumor type i.e., Pre-processing, Feature extraction, Feature selection and Classification. The input image is initially pre-processed, whose output image is used for feature extraction. The extracted hybrid feature set uses feature selection method for selecting relevant subset of image features, which is used by the classifier for training and testing of brain images. Figure 1 shows the block diagram of proposed approach.

A. Input Dataset

Proposed approach is examined over 150 Malignant brain MR images which includes 80 images of male and 70 images of female. The collected dataset consists of 5 types of malignant tumors which were named as Central Neuro Cytoma, Glioblastoma Multiforme, Gliomas, Intra Ventricular Malignant Mass and Metastasis, each of them having 30 images.

The experimental images are generated using 3.0 Tesla GE MRI Scanner at SMS Medical college Jaipur, Rajasthan, India. All the patients whose images are used for dataset generation were imaged using same imaging system and environment variables. The obtained images have following specifications: axial 3D T1-Weighted, 3D T2-weighted image and Fluid Attenuated Inversion Recovery (FLAIR) each of having size 256*256*3. In proposed work T2 weighted axial imaging modality is used for examination purpose having dimensionality of 256*256*3.

B. Pre-processing

In this step, an input MR image is initially being pre-processed for removing any time of noise generated during image generation. Noise removal helps us to discard unwanted signals which can lead to certain errors during processing. For noise removal median filter is used as it preserves edges while removing noise. The main idea of median filter is to scan pixel by pixel of the image and replacing each entry with the median value of the neighborhood pixels. In our work, a 3x3 box window is used as a median filter. Next, the filtered image is converted into 8 bit gray level for reducing the hands of image. An original 3 band color image is converted to corresponding intensity image ranging between 0 – 255. After pre-processing the input image will become noise free intense image of size 256*256 which acts as an input to next step.

C. Feature Extraction

In this phase, the gray image of previous step is used to extract the features of an MR image. Proposed approach uses a large number of features vector having (69) features for neoplasm classification which includes tumor texture based features and tumor shape based features.

Tumor shape based features -
Six simple shape descriptors [24] based features (s1, s2, s3, s4, s5, s6) of the tumor image are taken into consideration which includes feature like Convexity, Principal axis ratio, Compactness, Circular variance, elliptic variance and angle. Also seven invariant moments shape features (Φ1, Φ2, Φ3, Φ4, Φ5, Φ6, Φ7) are calculated [7]. Moments can be used to analyze the shape by reducing the dimension of its depiction. These features are invariant about rotation, scaling, and translation.

Tumor texture based features -
A set of 14 textural features calculated from the tumor MR image. All such features are extracted from each of the gray-tone spatial dependence matrices [25] and includes features like second order angular moments, contrast, correlation, sum of square: variance, inverse difference moment, sum average, sum variance, sum entropy, entropy, difference
variance, difference entropy, information measures of correlation and maximum correlation coefficients. Above features are calculated on various angular scale value of $\theta$ i.e. $\theta = \{0, 45, 90, 135\}$ degrees. Thus all texture related features sum up to 56 features in total.

Finally the extracted feature vector consists of 69 features which are used for brain neoplasm classification.

### D. Feature Selection

Here a mathematical model based on Cumulative variance frequency is proposed which is used for filtering out the subset of relevant features from the large feature vector generated through feature extraction mechanism. The detailed algorithm for feature selection is given below:

---

**Algorithm: Proposed statistical feature selection algorithm**

**Input:** Feature vector (V)

**Output:** Relevant feature subset (RFS)

**Procedure:**

1. for each vector $v_i \in V$
   
   - $\mu_i \leftarrow \text{Col_mean}(v_i)$
   
   - $D_i \leftarrow (v_i - \mu_i)$

2. end for

3. $C \leftarrow \text{Find_cov}(D)$

4. $Ev \leftarrow \text{Find_eigenVec}(C)$

5. $Var \leftarrow \text{Find_var}(Ev)$

6. $SEv \leftarrow \text{Sort}(Var)$

7. $Cv \leftarrow \text{cumulative}(SEv)$

8. $RFS \leftarrow \text{T_test}(Cv)$

return (RFS)

---

**Detailed Description**

In feature selection the input is the vector ‘V’ i.e., $V = \{v_1, v_2, \ldots, v_n\}$ returned by the feature extraction step. The vector is of size $1 \times n$ where each $v_i$ is of dimension $p$ and $n = \text{no. of data elements and } p = \text{no. of features.}$ Initially we find out the mean of each column vector which is represented by the $\mu_i$. Then the data normalization is done by subtracting the mean from each column vector. For this normalized column vector, a covariance matrix is generated.

The generated covariance matrix is of size $p \times p$. An Eigen vector is calculated from the covariance matrix using equation 1 as given below:

$$Ev1*\text{Cov}*Ev = D \quad \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots 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\cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cd \n
Here, in this work a whole malignant brain neoplasm dataset ‘D’ is divided into two equal subsets i.e. D0 and D1. Firstly the training of the classification model is being done using D0 and testing of the model using D1. Then the training and testing dataset are swapped i.e. training of the model using D1 and testing using D0. Similarly the process iterates for 5 times with two classification accuracy results on each run. The dataset D0 and D1 is selected randomly on every run which helps the model to learn about almost all the dataset images. This also results in removing the biasing among dataset images. Final accuracy of the dataset is claimed as the mean of all the accuracies obtained in each run.

### III. EXPERIMENTAL RESULTS

The dataset consists of 5 types of malignant tumors named Central Neuro Cytoma, Glioblastoma Multiforme, Gliomas, Intra Ventricular Malignant Mass, and Metastasis having 30 images of each type. The detailed number of features selected for each malignant tumor type is shown in Table 1.

<table>
<thead>
<tr>
<th>Input Dataset</th>
<th>No. of Features Extracted</th>
<th>No. of Features Selected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Neuro Cytoma</td>
<td>69</td>
<td>22</td>
</tr>
<tr>
<td>Glioblastoma Multiforme</td>
<td>69</td>
<td>19</td>
</tr>
<tr>
<td>Gliomas</td>
<td>69</td>
<td>19</td>
</tr>
<tr>
<td>Intra Ventricular Malignant Mass</td>
<td>69</td>
<td>22</td>
</tr>
<tr>
<td>Metastasis</td>
<td>69</td>
<td>17</td>
</tr>
</tbody>
</table>

Table 1. Feature Selected through Cumulative frequency

The above selected features are the combination of both texture based and shape based of an image. These selected features are now used by the KNN classifier for training and testing for brain neoplasm dataset. The performance of the KNN classifier of each type of brain neoplasm abnormality is represented with the help of confusion matrix as shown in table 2. In confusion matrix each type of malignant tumor is denoted as T1- Central Neuro Cytoma, T2- Glioblastoma Multiforme, T3- Gliomas, T4- Intra Ventricular Malignant Mass and T5- Metastasis.

Table 2. Confusion Matrix

<table>
<thead>
<tr>
<th>Input Dataset</th>
<th>No. of input images</th>
<th>No. of images Correctly identified</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Neuro Cytoma</td>
<td>30</td>
<td>27</td>
<td>90%</td>
</tr>
<tr>
<td>Glioblastoma Multiforme</td>
<td>30</td>
<td>28</td>
<td>93%</td>
</tr>
<tr>
<td>Gliomas</td>
<td>30</td>
<td>28</td>
<td>93%</td>
</tr>
<tr>
<td>Intra Ventricular Malignant Mass</td>
<td>30</td>
<td>27</td>
<td>90%</td>
</tr>
<tr>
<td>Metastasis</td>
<td>30</td>
<td>28</td>
<td>93%</td>
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Table 3. Malignant Tumor Type Classification Results

The overall accuracy achieved for malignant tumors is 92%. Some other performance analysis measures are also calculated with the help of confusion matrix which is given in table 4.

### IV. CONCLUSION

This paper presents a classification idea for differentiating malignant brain neoplasm MR images using a hybrid feature extraction mechanism based on Texture and Shape based features. The hybrid feature vector generated is of size 69 features which includes 13 shape based features and 56 texture based features. The feature vector is further reduced by using cumulative frequency and t-test at 99% significance level based feature selection mechanism. The reduced feature set is used by KNN classifier for class tagging. 5 x 2 Cross validation mechanism is used for partition the dataset into training and testing subsets. The proposed approach was tested on 5 categories of malignant brain tumors. The classification accuracy achieved for each malignant tumor MR image is shown in table 3. The overall accuracy achieved for complete dataset is 92%. The proposed methodology was tested on different values of k, but the best result is obtained at k=7. Proposed approach is also tested on various performance measures using confusion matrix whose results are shown in table 4.

Acknowledgment

We are thankful to the Sawai Man Singh (SMS) Medical College for providing us the original brain tumor images in DICOM format. We would also like to thanks Dr. Sunil, Department of Radiologist for helping us in verifying the results.
REFERENCES

[2]. Brain Tumor basics, Brain Tumor Primer. chapter 2 page 9.

<table>
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<tr>
<th></th>
<th>Precision</th>
<th>Neg. predict value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>False +ve rate</th>
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<td>T1</td>
<td>0.90</td>
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Table 4. Performance parameter analysis Table