Max B. Schäfer*, Sophie Weiland, Kent W. Stewart and Peter P. Pott

Compact Microscope Module for High-Throughput Microscopy

Abstract: Microscopy is an essential tool in research and science. However, it is relatively resource consuming regarding cost, time of usage, and consumable supplies. Current low-cost approaches provide good imaging quality but struggle in terms of versatility or applicability to varying setups. In this paper, a Compact Microscope Module for versatile application in custom-made setups or research projects is presented. As a first application and proof of concept, the use of the module in a High-Throughput Microscope for screening of samples in microtiter plates is shown. The Compact Microscope Module allows for simple and resource-efficient microscopy in various applications while still enabling relatively good imaging qualities.

Keywords: Microscopy, Compact Microscope Module, High-Throughput Microscopy, Scanning, Microtiter Plate

https://doi.org/10.1515/cdbme-2020-3136

1 Introduction

1.1 Motivation

In science, education, and medicine, light microscopy is one of the most prevalent used tools. However, microscopy is still relatively resource consuming considering cost, time of usage, and consumable supplies. Applying low-cost approaches in the field of microscopy might limit imaging quality, but also has the potential to elevate this crucial tool to a new level of dissemination or even to new applications. An example for an approach, focusing on a more balanced trade-off between costs and imaging capabilities, are smartphone-based microscopes. Utilizing the potent camera systems of modern smartphones for analytic purposes in combination with only few and low-cost additional components, already has led to promising results [1–3], not only in microscopy but also for other methods such as immunoassays [4]. Even though the mentioned low-cost microscopy approaches result in relatively good imaging qualities, they are mostly focused on a dedicated application, such as transmitted light microscopy of samples on a conventional specimen slide. A more universal setup would provide benefits in terms of applicability to various fields not only regarding the way of illumination but also the implementation in different research projects or setups.

A small and low-cost microscope module could be an adequate solution to provide easy-to-use microscopy. With a small microscope module, providing sufficient imaging capabilities, numerous initial investigations and/or experiments could be performed on a low level regarding costs and complexity. However, this might have even more effect if the field of screening microscopy is considered. Modern day biomedical research for example, requires frequent observation of cell populations which are cultivated in small cavities on microtiter plates to determine for example growth and confluency of the cells [5]. Screening of either a high number of samples and/or of samples over a certain time interval is a limited resource since relatively expensive microscopes are occupied for the complete imaging procedure resulting in a tightly scheduled utilization. Aiming on a small and low-cost microscope module could therefore lower the barrier to entry and make screening microscopy more accessible.

In this paper, a Compact Microscope Module (CMM) for versatile application is presented. As a first application and proof of concept, the use of the module in a High-Throughput Microscope (HTM) is shown.

1.2 Form Factor Requirements

To ensure a high applicability of the CMM to various setups, an appropriate form factor is crucial besides imaging requirements. To meet high standards, the limited dimensions of a standard microtiter plate with up to 96 wells [6] are considered (see Fig. 1). Assuming the potential requirement of arranging 96 CMMs in the present area of such a microtiter

***Corresponding author: Max B. Schäfer:** Institute of Medical Device Technology, University of Stuttgart, Pfaffenwaldring 9, Stuttgart, Germany, max.schaefer@imt.uni-stuttgart.de
Sophie Weiland, Kent W. Stewart, Peter P. Pott: Institute of Medical Device Technology, University of Stuttgart, Germany

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plate, leads to maximum size of 9 mm in width and length of the optical system with no specified limit in height. Commonly, there is only limited space close to the sample, but further off, slightly more space is available. This can lead to an approach where the optical system is designed as small as possible, whereas the electronic part is placed slightly away.

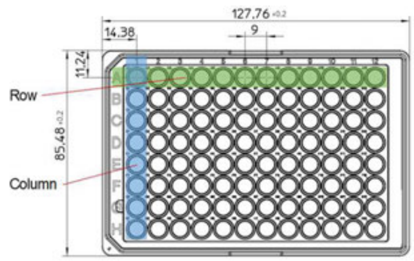

Figure 1: Standard microtiter plate with 96 wells [7].

2 Materials and Methods

2.1 Optical System

The optical principle described in [1] is used. Therefore, an inverted smartphone camera lens is combined with an off-the-shelf camera module designed for use with a Raspberry Pi single-board computer (Raspberry Pi Foundation, UK). This optical setup, shown in Fig. 2, enables microscopy with resolutions of a few micrometers while providing a relatively large field of view (FOV).

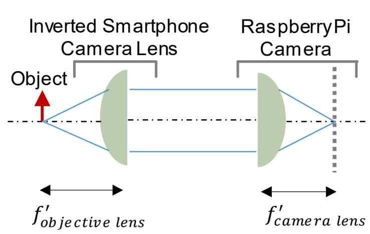

Figure 2: Optical principle of the Compact Microscope Module.

While both lenses have focal lengths in the same order of magnitude, only limited optical magnifications can be achieved (see Eq. 1). However, a digital magnification is enabled due to the high number of camera pixels in the Raspberry Pi camera.

$$\boldsymbol{M} = -\frac{f'_{objective\ lens}}{f'_{camera\ lens}} \tag{1}$$

A camera module designed for the Raspberry Pi was used. It features an 8-megapixel IMX219 CMOS sensor (3280 × 2464 pixels) and a focal length of 2.96 mm. A Raspberry Pi v2.1 camera board and a Raspberry Pi 3B+ single-board computer were used to control the camera. For the inverted smartphone camera lens, a Samsung Galaxy S4 (Samsung Electronics Co. Ltd., Suwon, KR) front camera lens with a focal length of 1.90 mm or an Apple iPhone 5S (Apple Inc., Cupertino, CA, US) rear camera lens with a focal length of 4.12 mm was used, aiming on varying working distances depending on the application.

2.2 Evaluation of the Optical System

A MIL-STD-150A US Air Force optical resolution test target was used to determine the resolution of the optical system. Expecting a Michelson contrast of at least 10 % in horizontal and vertical orientation between the white and black bar elements on the target [8, 9], analysis of the contrast gradient was performed to determine the achievable resolution. To assess the FOV, images of graph paper were taken and measured with knowledge of the respective pixel size of the camera sensor. In order to determine the distance between the front lens surface and the focal plane, referred to as working distance, the optical system was positioned relative to graph paper on a specimen slide using a micrometer screw. Initially, the optical system was moved until the front lens had contact with the graph paper. By moving back until the graph paper was in focus, the travelling range was obtained from the scale of the micrometer screw.

3 System Design

3.1 Compact Microscope Module

A CMM was developed consisting of a small optical unit and a slightly larger handle mount to provide housing for the electronics but more importantly to enable ease of handling. The optical unit holds the camera and the additional lens in place and provides a dovetail connection to allow fast attachment to other setups (see Fig. 3a). Camera and lens can be mounted without any tools due to press-fit tolerances. A rectangular and flat shaped handle mount allows for better handling of the small optical unit (see Fig. 3b). The optical unit is attached by the dovetail joint and locked by a bolt. Additionally, the handle provides space for the camera board and ventilation slots for heat dissipation of the electronics. Due to a sufficient cable length, the optical unit can be taken off the handle if space around the sample of interest is limited.

Manufacturing of housing components was done with a Prusa i3 MK3 3D printer (Prusa Research s.r.o., Prague, CZ) using PLA filament.

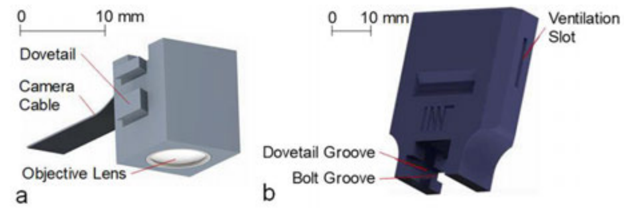

Figure 3: The Compact Microscope Module (a) can be attached to the handle mount (b), which is housing the camera electronics.

3.2 High-Throughput Microscope

To demonstrate a first possible application of the principle of the CMM, an HTM for automated imaging of samples in a 96-well microtiter plate was designed. Since the HTM is only an example for application of the designed CMM, only technical details, which are necessary for the description of the working principle, are given. Instead of positioning the samples relative to a single microscope, eight optical units were used to reduce scanning time. Since some cell cultures are sensitive to movement, the optical unit instead of the vessel is positioned in all three dimensions. Positioning in x- and y-axes is accomplished by conventional linear spindle drives. To align the focal plane of the optical units with the structure of interest, z-axis positioning is achieved through a solid-state joint lever mechanism [1]. This focusing mechanism is actuated by a geared DC motor with a spindle.

In order to ensure a congruent and robust alignment of all optical units, the CMMs are manufactured as a connected line of eight optical modules (see Fig. 4). Since the 96-well microtiter plate comprises eight rows with twelve cavities each, a line of eight combined CMMs allows for simultaneous recording of a complete column of cavities.

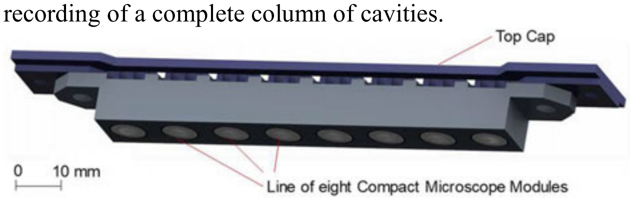

Figure 4: Microscope assembly comprising eight Compact Microscope Modules.

4 Results

4.1 Compact Microscope Module

For the evaluation, the CMM was equipped with a Samsung Galaxy S4 lens, resulting in a magnification of -1,56 which enables the identification of structures with a size of 2.46 µm. Further, a FOV of 3,5 mm^2 and a working distance of 0.3 mm is achieved. The optical unit solely has a size of $9 \times 9 \times 11$ mm^3 (length $\times$ width $\times$ height), whereas the handle with the attached optical unit has a size of $38 \times 27.5 \times 13.2$ mm^3. The cable which connects the optical unit with the camera board in the handle has a length of 70 mm, allowing for versatile placement of the CMM. The setup is shown in Fig. 5.

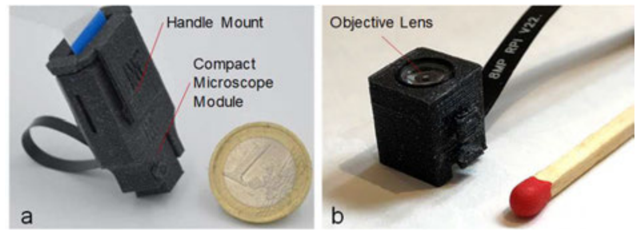

Figure 5: Handle mount containing electronics with Compact Microscope Module (CMM) attached (a) and CMM removed for use when space is limited (b).

Manual positioning of the CMM relative to the sample by hand is possible if the heel of the hand is placed on a stable surface while guiding the CMM between index finger and thumb. Further, natural ambient light is sufficient to achieve a satisfying image quality.

4.2 High-Throughput Microscope

Based on the CMM, the HTM enables scanning of standard microtiter plates with up to eight optical units working simultaneously. With overall dimensions of $294 \times 178 \times 85$ mm^3, the HTM is relatively compact. A sufficient working distance was achieved - overcoming the thickness of the glass bottom of the microtiter plates and enabling in-focus images of larger samples in the cavities – by equipping each CMM with an iPhone 5S rear lens, resulting in a magnification of -0,72 which enables the identification of structures with a size of 3.48 µm in a FOV of 21.2 mm^2 and a working distance of 1.0 mm. The HTM is shown in Fig. 6, whereas Fig. 7 shows exemplary images recorded with the CMMs used.

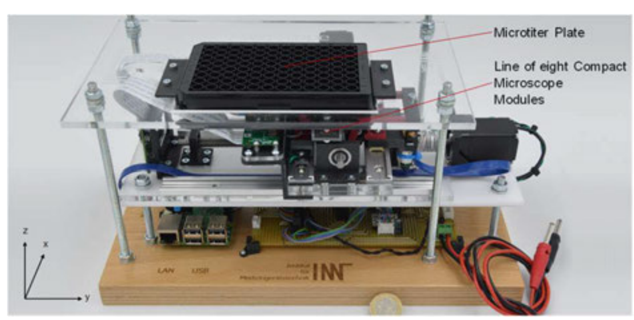

Figure 6: High-Throughput Microscope based on eight Compact Microscope Modules arranged in a line to record samples in microtiter plates.

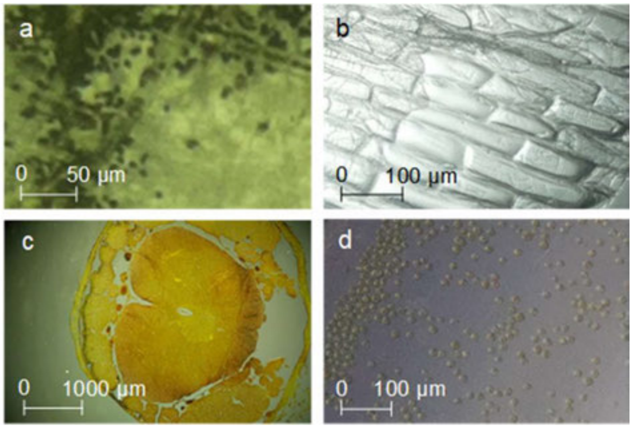

Figure 7: Example images recorded with the Compact Microscope Module equipped with the iPhone 5S lens showing laser printed lines on graph paper (a), onion cells (b), spinal cord (c), and freshwater algae (d). Samples were illuminated by a LED array placed above the microtiter plate.

5 Discussion

A CMM was developed, enabling widespread use of microscopy in various research projects under challenging space requirements. A relatively high resolution of up to 2.46 µm (Samsung Galaxy S4 lens) and a large FOV of up to 21.2 mm^2 (iPhone 5S lens) could be achieved while still being relatively low in cost due to the use of off-the-shelf and simple 3D printed components. Both lenses used have their specific advantages of either providing a high resolution but a small FOV and working distance, or a limited resolution but therefore the possibility of capturing a large FOV with a larger working distance. The CMM can easily be equipped with the desired lens due to tool-free press-fit connections only. As a result, a fast and simple adaption of the CMM to different applications is possible.

Wang et al. present a microscope based on a similar camera module and therefore with comparable dimensions [10]. However, this system allows for identification of a line pair of 10 µm distance on a limited FOV of 1 mm^2. A similar resolution compared to the CMM showed in the present paper is achieved by Tristan-Landin et al. [11]. Aiming for fluorescence microscopy, a high number of components is necessary resulting in larger overall dimensions.

For a proof of the versatility of the CMM, the usage in a custom designed HTM was performed. The challenges in space and alignment could be accomplished and a dataset of sample images was successfully recorded. However, due to the required larger working distance, a remarkable trade-off in image resolution had to be accepted. Regarding the resulting limits in identification of small structures, further investigations are necessary to improve the optical setup. However, the accomplished implementation of the CMM in a complex research setup around scanning microscopy reveals its potential as a versatile module for various setups.

Author Statement

Research funding: The author state no funding involved. Conflict of interest: Authors state no conflict of interest. Informed consent: Informed consent is not applicable. Ethical approval: The conducted research is not related to either human or animals use.

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