Research article

Shiqi Jiang, Ronny Förster, Malte Plidschun, Jens Kobelke, Ron Fatobene Ando and Markus A. Schmidt*

Three-dimensional spatiotemporal tracking of nano-objects diffusing in water-filled optofluidic microstructured fiber

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Abstract: Three-dimensional (3D) tracking of nano-objects represents a novel pathway for understanding dynamic nanoscale processes within bioanalytics and life science. Here we demonstrate 3D tracking of diffusing 100 nm gold nanosphere within a water-filled optofluidic fiber via elastic light scattering–based position retrieval. Specifically, the correlation between intensity and position inside a region of a fiber-integrated microchannel has been used to decode the axial position from the scattered intensity, while image processing–based tracking was used in the image plane. The 3D trajectory of a diffusing gold nanosphere has been experimentally determined, while the determined diameter analysis matches expectations. Beside key advantages such as homogenous light-line illumination, low-background scattering, long observation time, large number of frames, high temporal and spatial resolution and compatibility with standard microscope, the particular properties of operating with water defines a new bioanalytical platform that is highly relevant for medical and life science applications.

Keywords: biosensing; confined diffusion; elastic light scattering; nanofluidics; resistance coefficient; 3D tracking of single nanoparticle.

1 Introduction

Measuring the trajectory of diffusing nano-objects that have dimensions substantially below the diffraction limit represents a powerful approach particular within bioanalytics and life science. This allows us to understand processes at the nanoscale level, examples of which include protein folding [1] or for measuring virus concentrations [2]. Various types of microscopy-based image processing techniques for object localization and tracking with both high temporal resolution, as well as localization precision have been developed [3, 4]. However, most currently employed schemes only track the object of interest in the image plane, while accessing the axial dimension, i.e., conducting full three-dimensional (3D) tracking, is considerably more challenging. Promising experiments using multifocal plane microscopy [5] or point spread function engineering [6, 7] have been conducted, while standard microscopes cannot be directly used due to the need for additional components (e.g., additional camera [8], phase masks [9]).

Recently microstructured optical fibers have been introduced as a novel platform for the tracking of diffusing nano-objects over very long time scales using elastic light scattering [9–13]. Here tracking by means of elastic light scattering includes key advantages such as unlimited photon budget, fast data acquisition [14, 15] and avoidance of labeling [16], all of which are difficult to achieve with fluorescence-based schemes. As recently demonstrated, one configuration of the fiber-based approach allows full 3D tracking of a diffusing nanosphere over thousands of frames at kHz frames rates via elastic light scattering–based position retrieval [13]. This approach uses a microstructured optical fiber including a liquid-filled microchannel (diameter $D^m \approx 1 \mu\text{m}$) running parallel to the light guiding glass core (with a center-to-center distance of about $2 \mu\text{m}$) allowing to retrieve the position of the nano-object along the direction of the microscopic detection via the intensity of the light scattered at the evanescent field of the core mode. Specifically, the successful tracking of a diffusing 50-nm

***Corresponding author: Markus A. Schmidt**, Leibniz Institute of Photonic Technology, Albert-Einstein-Str. 9, 07745 Jena, Germany; Otto Schott Institute of Materials Research (OSIM), Friedrich Schiller University Jena, Fraunhofer Str. 6, 07743 Jena, Germany; and Abbe Center of Photonics and Faculty of Physics, Friedrich-Schiller-University Jena, Max-Wien-Platz 1, 07743 Jena, Germany, E-mail: markus.schmidt@leibniz-ipht.de. <https://orcid.org/0000-0002-5324-6405>

Shiqi Jiang, Ronny Förster, Malte Plidschun, Jens Kobelke and Ron Fatobene Ando, Leibniz Institute of Photonic Technology, Albert-Einstein-Str. 9, 07745 Jena, Germany. <https://orcid.org/0000-0003-2017-6006> (S. Jiang)

gold nanosphere along all three spatial directions over seconds was demonstrated. Particular remarkable is the very large number of continuously recorded frames ($N_f = 8000$) greatly exceeding the track lengths of other reported evanescent-based 3D tracking schemes, examples of which include the tracking of 200 nm polystyrene sphere nanoparticles inside water ($N_f = 67$) [17] or nil red molecules inside poly(acrylamide) (PAA) gel ($N_f = 8$) [18]. Here is it important to note that as the standard deviation of the determined diffusion coefficient scales with $1/\sqrt{N_f}$ [19–21], the fiber-based approach shows an unprecedented statistical significance regarding the determination of hydrodynamic diameters. Compared to commonly used nanoparticle tracking analysis (NTA), which due to low number of frames gives a precision (i.e., standard deviation) per ensemble, the fiber approach yields a very high precision per nano-object. This allows for principally studying the dynamics of single nano-objects, an issue that is challenging to achieve with the commonly used implementations of NTA. Other advantages of the fiber approach include high and homogeneous illumination intensity along the entire field of view (i.e., diffractionless light-line illumination), confinement of the nano-objects to the area of illumination and the focal plane of the microscope, reduced readout time and increased frame rates due to the smaller imaging window (35 pixel height) on the camera chip, principal compatibility with fiber circuitry, very small sample volumes (<200 pL) and, in contrast to fluorescence, no photodegradation.

In spite of these benefits mentioned above, this fiber-based 3D tracking approach demands a specific value of refractive index (RI) inside the microchannel to achieve an exponential evanescent field and an unambiguous correlation between scattered intensity and position. Such RI adjustment has been achieved using a composite liquid

containing water and dimethyl sulfoxide (DMSO), although the use of a mixture is unfavorable for bioanalytical applications typically requiring a water environment.

In this work, we resolve the mentioned issue by an extended data processing procedure of the measured trajectories, allowing for 3D tracking inside a half semicircle region of the microchannel of the optofluidic fiber in case it is filled with water (Figure 1a). The hydrodynamic diameter of the nanosphere within the confined channel is estimated by applying the resistance coefficient for the three directions separately. The presented 3D tracking approach includes all mentioned advantages of fiber-based 3D tracking and additionally is able to handle a water environment, making this system in particular relevant for bioanalytics applications.

2 Concept and working principle

The working principle of the waveguide-based 3D tracking scheme discussed here relies on a combination of localization microscopy within the image plane (xz -plane, Figure 1a inset) and scattering light-based position retrieval along axial direction (y -direction, Figures 1b and 1a inset).

The waveguide used is a microstructured single-mode graded index fiber (MGIF) (Figure 1a) consisting of a central waveguide core with a longitudinal water-filled microchannel (diameter $D^m \approx 1 \mu\text{m}$) running parallel to the glass core. The nano-objects diffusing inside the water-filled microchannel are homogeneously illuminated by the evanescent field inside the channel across the entire field of view. They are localized within the image plane via the elastically scattered light that is sidewise detected by a microscope. The position of the particle along y -direction can be retrieved from the scattered intensity due to the

Figure 1: The concept of three-dimensional (3D) tracking of nano-objects inside a water-filled modified graded index fiber. (a) Illustration of the concept (light blue: water-filled microchannel, red: optical core). The yellow elements represent the nano-objects. The inset is a sketch of the fiber cross-section within the yz -plane showing the modal intensity distribution of the core mode along the y -direction. The intensity based y -position retrieval can be achieved in the highlighted channel region (nondashed area). (b) Sketch of fiber cross-section (xy -plane) and experimental configuration (i.e., relative orientation of core, micro-

channel and microscope objective) used for 3D tracking. (c) Scanning electron micrographic (SEM) image of the cross-section of the implemented microstructured fiber.

relation between the y -coordinates and local field intensity $I = I(y)$. Therefore, this special fiber geometry provides a platform for 3D tracking by retrieving the depth location (y -axis) from the scattered intensity which commonly cannot be measured. The position within the xz -plane is determined by image processing, i.e., via center-of-mass analysis in the recorded images.

In order to decode the axial position from the scattered intensity, an bijective function between the local intensity distribution of the evanescent field and the y -coordinate ($I = f(y)$, $y = f^{-1}(I)$, (f^{-1} is the reverse of f) is required, i.e., the y -position can be uniquely determined from one certain intensity. In contrast to the water-DMSO mixture used in our previous work (RI $n_r = 1.44$ at a wavelength of $\lambda = 640$ nm) [13], in which the field intensity can be described by a uniform exponential function within the whole channel, the intensity distribution (simulated by finite-element modeling using COMSOL) of the evanescent field inside the microchannel in case of pure water filling is substantially more complex regarding the following three aspects: (i) First, using water leads to a distinct variation of the intensity inside the microchannel not only along the y -axis but also along the x -direction in contrast to our previous work using DMSO/water. Consequently, the mathematical function that relates y -position and intensity must be determined separately for each x -position. (ii) Secondly, the function between intensity and y -position is surjective (i.e., double-valued) at certain x -positions within a certain part of the low-intensity region (red-shaded area in Figure 2a), yielding an irreversibility from intensity to y -position. Therefore, the intensity position recovery can only be achieved exclusively in the single-valued region of the channel (nonred-shaded area in Figure 2a). (iii) Another feature is the nonexponential decay of the evanescent field for each x -position particular in the low-intensity semicircle of the microchannel (meshed area in Figure 2a). Here the deviation from a single exponential decay is significant in contrast to the upper semicircle (Figure 2c).

In order to circumvent the mentioned issues, the 3D tracking addressed here is restricted to the upper semicircle of the microchannel ($I = I(x, y \geq 0)$, nonmeshed area in Figure 2a and b), i.e., high-intensity region which is partitioned into small bins along x -direction (Figure 2b). This choice yields a bijective (i.e., single-valued) function between intensity and position in each bin. A detailed investigation of the mode field in y -direction within defined spatial bins has revealed that a single exponential function including two parameters can be used to approximate the field behavior in that region (Figure 2c):

$$I_i(y) = e^{(p_i y + b_i)} \quad (1)$$

Figure 2: (a) Simulated spatial intensity distribution (logarithmic color scale including iso-intensity contour lines) in a circular water-filled microchannel (diameter $D^m = 1 \mu\text{m}$, $\lambda = 642$ nm). The correlation between the local field intensity and the y -coordinates is ambiguous in the red-shaded area and position retrieval is performed in the upper semicircle. (b) Close-up of the intensity distribution in the upper semicircle of the microchannel. The white vertical lines indicate the boundaries between the adjacent bins along the x -direction. In each bin, the highest scattered intensity occurs when the particle is at the upper channel wall indicated by y_{max} (center of the particle). The median scattered intensity is expected to be at $y_{med} = 0$ (see text for details). (c) Intensity distribution along the y -direction of selected bins in case of 100 bins partitioning (bin width 10 nm). Exponential fitting is applied to the points in the upper semicircle (nonhashed area) for each bin, representing by the dashed curves in corresponding darker colors.

with the slope and local intensity parameters $p_i = p_i(x)$ and $b_i = b_i(x)$, respectively, that both depend on the bin index i . Examples of intensity distributions for selected bins including the respective fit using Eq. (1) are shown in Figure 2c, confirming the applicableness of a single exponential function with x -dependent parameters. As a consequence, the locations of a nano-object along the y -direction within the upper semicircle of the microchannel cross section can be retrieved from its scattered intensity bin by bin.

3 Methods

The fiber used in the experiments consists of a graded index GeO_2 -doped silica core (1.4 μm diameter) with a maximum RI of $n_r = 1.49$ (maximum doping concentration is 24 mol %) [22] in the center and a parallel running microchannel with pitch (center-to-center distance)

of $\Lambda = 2 \mu\text{m}$ (Figure 1b). The fiber is single-mode at the operation wavelength $\lambda = 642 \text{ nm}$. SEM imaging (Figure 1c) reveals that the microchannel is slightly elliptical with the principal extensions along the x - and y -directions of $D_x^m = 1.2 \mu\text{m}$ and $D_y^m = 1.1 \mu\text{m}$, respectively.

The solution used consists of water of buffer medium and spherical gold nanoparticles (particle concentration of 5.6×10^9 particles/mL). Prior to the tracking experiment, the diameter distribution of the nanosphere ensemble has been characterized using a commercially available device (Malvern Zetasizer Nano ZS), showing an average hydrodynamic diameter and a standard deviation of $d_{NP}^m = 104.3 \text{ nm}$ and $\sigma_{NP} = 0.66 \text{ nm}$, respectively. Here we use the z -average as mean nanoparticle diameter, which is typically considered in the literature within the context of Zetasizer measurements, i.e., dynamic light scattering (DLS) experiments. The standard deviation stated has been obtained here from statistically analyzing a series of Zetasizer measurements (here nine) on the same sample. The choice of nanosphere diameter represents a compromise between the intensity decay across the water-filled microchannel, the in-fiber power, the scattering cross section and the frame rate, allowing the nanosphere to be tracked along the transverse direction at any time. The particle diameter considered here is larger compared to our previous work [13], imposed by a smaller scattering coefficient and a faster field decay, both of which result from the smaller RI of water compared to the liquid used in our previous work.

For the tracking experiments, red laser light (Thorlabs HL6366DG, $\lambda = 642 \text{ nm}$) is coupled into the fiber core via an objective ($20\times$, $NA = 0.4$) from one end of the fiber. This solution is introduced into the microchannel from the output side of the fiber by capillary action within several minutes until the respective observation region of the microscope objective is filled with the nano-object solution (a sketch of the experimental setup and a plot of the calculated filling time can be found in the Supplementary material). Note that the required sample volumes are extremely small ($<200 \text{ pL}$). In order to avoid the impact of drift the tracking experiment was not started until the liquid flow was vanished. To confirm the absence of flow, the trajectories of the nano-object along z -direction is plotted in Figure 3a, showing no direction-biased movement. The corresponding histogram of the probability of the displacement along the z -direction at different time intervals, as well as the related normal distribution fittings (red curves in Figure 3c) that are centered on the origin further confirming the absence of flow. The sidewise scattered light from the gold nanoparticles (measured at a distance of 7 cm from the output side) is

collected by an oil-immersed $\times 60$ objective and imaged onto a sCMOS camera (ANDOR Zyla 4.2 Plus). The raw video of the tracking experiment is presented in the Supplementary material and three selected frames are shown in Figure 3b. Note that for the 3D tracking, the fiber is oriented such that the connection line between the centers of core and microchannel coincides with the axis of the objective (Figure 1b). Within the presented experiments, the guided power in the fiber was about 2.4 mW and the camera frame rate was $\nu = 2741 \text{ Hz}$ with the exposure time of 0.36 ms . The input power was chosen on the basis of considering the trade-off between the saturation of signal in the upper semicircle and the detecting the nanoparticle at any location in the lower semicircle.

The tracking procedure within the image plane (i.e., xz -plane) involves frame-by-frame center-of-mass localization using the Trackpy (v. 0.3.3) package of Python. As examples, the determined positions of the particle in 2000th, 8000th and 11,000th frames are marked by the yellow dashed circles in Figure 3b. The final result is a data set containing over 10 thousands of entries, each of which includes the x -, z -locations and the corresponding scattered intensity (entry $_j = \{x, z, I\}$ with the frame index j). Optimization of the various parameters leads to a transverse localization accuracy of $\sigma_{tr} = 3.06 \text{ nm}$.

The determination of the parameters required for Eq. (1) mainly requires the two following data processing procedures: (i) partitioning the trajectory along the x -axis in bins and (ii) selection of data entries that are within the upper (high-intensity) semicircle (non-meshed area in Figure 2a). Note that the data processing does not rely on the simulated mode field patterns but rather uses the measured trajectory, thus circumventing the impact of improper knowledge on RI distribution and/or geometric parameters.

Step 1. Binning: The first step – the binning – involves dividing the data entries into bins of constant width along the x -direction with the aim to obtain the parameters p_i and b_i within each bin (central bin position $\bar{x}_i$, index of bin i). The correct choice of bin width Δx_{bin} is essential for a comparison of experimental results and finite-element simulations, since a smaller bin width leads to smaller deviation between the single exponential function approach (Eq. (1)) and the distribution of values of the fitting parameter across the bin, while the accuracy of the

Figure 3: Longitudinal trajectory of the single gold nanosphere considered here.

(a) Trajectory along z -direction. The three red dots mark the examples of frames which are shown in (b). (b) Selected frames from the tracking video, with the determined position of the particle marked by the yellow dashed circles. The number indicates the frame. (c) Histogram of the probability of the nanosphere displacement along the z -direction at different time intervals. The lines refer to the corresponding normal distribution fitting curves (red curves).

determined parameters reduces simultaneously due to the lower number of points per bin. Considering that trade-off, we decided the total bin number to be $N_{\text{bin}}^{\text{tot}} = 100$, providing a small bin width ($\Delta x_{\text{bin}} \approx 10$ nm) as well as sufficient sampling points per bin ($N_{\text{bin},i} \geq 20$). Note that the trajectories in every symmetric pair of bins along the x -direction are combined within one bin which is a valid approach considering the geometric symmetry of the microchannel (Figure 2c). Note that due to the small number of points in the first bin ($N_{\text{bin},1} = 4$), we consider the first and second bins as one bin; therefore the number of practically analyzed bins is 49 considering the symmetric combination.

Step 2. Selection: The second step – the entry selection – relies on dividing the entire data set into a higher and lower intensity subset corresponding to the upper and lower semicircles of the microchannel (Figure 2b). Here the unique properties of the MGIF environment play an essential role: due to the transverse confinement and the light-line illumination, the diffusing gold nanoparticles can be tracked for very long time (here, $\tau = 5.8$ s) at kHz frame rate (here, $\nu = 2741$ Hz), leading very large number of total frames ($N_f = 16,000$ in the present case). Taking into account the statistical nature of Brownian motion, an even and homogenous distribution of the spatial locations of the nano-object within the microchannel cross section can be anticipated. Based on this assumption, the probability of the nano-object to appear in either the upper (Figure 2b) or the lower semicircle should be 1/2, allowing us to divide the data in each bin into two classes by sorting the individual entry with respect to the median intensity value $I_{\text{med},i}$ that has been obtained bin by bin. As a result, the high-intensity subset ($I_{j,i} > I_{\text{med},i}$) corresponds to nano-object positions inside the upper semicircle ($y > 0$), while the complementary ($y < 0$) holds for the low-intensity subset ($I_{j,i} < I_{\text{med},i}$).

With the known $y_{\text{med},i}$ (set to be at $y = 0$ μm as the reference position) and $I_{\text{med},i}$, the local intensity parameter b_i can be obtained by inserting these values into Eq. (1). The slope parameter p_i can be calculated accordingly by using the highest intensity in each bin with the corresponding relative y -position $y_{\text{max},i}$ (calculated from the elliptic equation $y_{\text{max},i}^2/d_y^m + \tilde{x}_i^2/d_x^m = 1$ describing the shape of the microchannel) and b_i . Here the geometry parameters d_x^m and d_y^m take into account the physical size of particle (about 100 nm diameter) and are therefore smaller than the channel axes length D_x^m and D_y^m . The length $d_x^m = 1.1$ μm is calculated from the experimentally measured maximum transverse distance the nanoparticle has traveled along

x -axis while $d_y^m = 1$ μm , estimated from $D_y^m = 1.1$ μm obtained from SEM imaging.

4 Results

The spatial distribution of the parameters p and b obtained from the bin-by-bin procedure of the tracked data (red dots) are compared in Figure 4 to corresponding values from simulations using the same data treatment (dashed blue lines). The slope parameter obtained from experiment p_{exp} shows a very good agreement with its simulated counterpart p_{sim} ($D_{x,\text{sim}}^m = 1.2$ μm , $D_{y,\text{sim}}^m = 1.1$ μm) (Figure 4a) which is remarkable considering that this parameter has been determined from two pairs of values only and that the simulations include several assumptions such as a perfect ellipse shape of the channel or a simple concentric RI distribution in the core. More pronounced deviations are visible at lower bin indices, i.e., positions closer to the boundaries of the microchannel. We attribute this to the lower number of frames available (inset of Figure 4a). Statistically speaking, more frames available within one bin imposes a higher probability that the nano-object is located at $y = 0$ and $y = y_{\text{max}}$, thus leading to higher probability to obtain more accurate intensity values at these two positions ($I_{\text{med},i}$ and $I_{\text{max},i}$). Note that another potential source of error is the limited spatial resolution of the SEM imaging impacting the determination of the extension of the channel along the y -direction. Within the context of the analysis presented, the uncertainty in the determination of d_y^m is $\Delta d_y^m = \pm 80$ nm, imposing an error in the estimation of y_{max} with this impact being strongest in the center of the semicircle ($x_i = 0$). Besides, the non-ideal ellipse shape of the channel also contributes to the error which is not considered in both simulations and experimental data analysis.

As the parameter b scales with the local field intensity, b_{sim} can be adapted to the range of b_{exp} as shown in Figure 4b showing a good agreement in terms of evolution and dynamic range between simulation and experiment.

4.1 3D trajectories recovery

To obtain continuous correlations between the parameters and the x -position ($p = p(x)$ and $b = b(x)$) independent of simulations, we applied 2nd order polynomial fitting to the experimentally obtained parameters p_i and b_i in each bin with the corresponding central bin position $\tilde{x}_i$ (black lines in Figure 4a and b). Note that the feasibility of using second order polynomial fitting to the parameters is confirmed in simulations as shown in the Supplementary material.

Figure 4: Dependence of (a) slope and (b) local intensity parameters (p and b) on bin index along the x -direction. The cyan dots and the black line refer to the data obtained from the tracked data and the corresponding 2nd order polynomial fit. The red lines result from the finite element simulation. The corresponding number of available frames used in the respective bin $N_{\text{bin},i}$ is plotted in the inset of (a).

Therefore, the y -position for frame j in the upper half channel region can be retrieved by solving Eq. (1) for y , leading to $y_j = (\log(I_j(x)) - b(x))/p(x)$.

Together with the tracked positions in the xz -plane, the frame number–sorted 3D trajectory of the nanoparticle ($\{x, y, z\}_k$ with entry index of $k = 1 \dots N_{\text{up}}$ [N_{up} : number of frames in the upper region]) within the high-intensity semicircle is obtained. The projection of nanoparticle positions onto the xy -plane (fiber cross-section plane) is shown in Figure 5b with 97.23% of retrieved y -positions being located within the upper semicircle. Note that the remaining 2.77% trajectories are out of the upper semicircle due to the polynomial fit used for the parameters of Eq. (1) (Figure 4). The plot reveals an homogenous nanoparticle distribution across the upper semicircle and thus post *a priori* confirming the assumption that was required to determine $I_{\text{med},i}$ and $I_{\text{max},i}$. The 3D trajectory in the upper semicircle is visualized in Figure 5a, confirming the feasibility of the scattered-intensity–associated 3D tracking approach. Note that once the nanoparticles enters the lower intensity region ($y < 0$), tracking along the y -direction is not feasible via the use of Eq. (1), i.e., the 3D tracking procedure is only valid in the upper semicircle. To highlight this procedure,

Figure 5a shows the continuous parts of the trajectory of the nanosphere that are solely located in the upper semicircle. Here each subtrajectory is shown in one single color. The number of continuous trajectories of the nanoparticle in the upper semicircle is $N_{\text{con}} = 492$, while the averaged and maximum number of frames per continuous trajectory are $N_{\text{ave}} = 16$ and $N_{\text{max}} = 262$.

4.2 Mean squared displacement analysis and Stokes–Einstein relation

One important application of NTA is diameter determination of deep subwavelength nano-objects via mean squared displacement (MSD) analysis [23–25]. This analysis is associated with the statistical nature of Brownian motion, i.e., diffusion and yields a linear dependence between the second momentum of the position change $\langle x_q^2 \rangle$ (with the spatial coordinate $q = x, y, z$) and the lag time Δt [26]. Here we apply MSD analysis along the three spatial directions independently yielding:

$$\langle x_q^2 \rangle = 2D_q \Delta t + \sigma_q, \quad (2)$$

Figure 5: Three-dimensional (3D) tracking of a 100 nm gold nanosphere inside the water-filled mode graded index fiber (MGIF). (a) Parts of the 3D trajectory of a single diffusing nanosphere in three spatial dimensions within the upper part of the microchannel (indicated by the semi-transparent red background). Note that the position retrieval along the y -direction is only valid within the upper semicircle and therefore only this part is presented in (a).

Each color represents a continuous subtrajectory and the color scale refers to relative position of the respective subtrajectory inside the entire track ensemble (e.g., 100% refers to $N_{\text{con}} = 492$). (b) Transverse locations of the nanoparticle within the upper semicircle of microchannel, i.e., projection of the entire data set onto the xy -plane. The boundaries of channel and center of particle are separately indicated by the red solid and red dash lines. The border between upper and lower half of the channel ($y = 0 \mu\text{m}$) is illustrated by the blue dashed line. The color of each point refers to the location of the nanoparticles at a specific time.

with the direction-dependent diffusion coefficient D_q and corresponding potential errors summed up in σ_q . The error of the diffusion coefficient σ_{D_q} is related to the number of frames f and number of lag times n_{lt} involved in the MSD analysis by: $\frac{\sigma_{D_q}}{D_q} = \sqrt{\frac{2n_{lt}}{3 \cdot (f - n_{lt})}}$ [19–21], which are very small in this work due to the large frame number (16,000 for x -, z -direction and 6879 for y -direction in calculation). Here it is important to note that even though the position retrieval procedure along the y -direction is only valid in the upper semicircle, the nano-object can always be tracked within the image plane and identified as the same particle. This allows us to use the trajectories within the upper semicircle ($y > 0$) for the MSD analysis in the y -direction and all the trajectories within the whole channel for MSD analysis in the x - and z -directions (details in the Supplementary material). Here we have used the first three lag times for fitting Eq. (3) to the MSD data (Figure 6a), which includes 7473, 7148 and 6879 trajectories along y -direction and 15,999, 15,998 and 15,997 trajectories along x - and z -directions. Note that generally the first lag times include the statistically smallest errors (details in [27]) and are therefore most relevant for a precise determination of the diffusion coefficient of the nano-object, while using more lag times does not improve statistics. The hydrodynamic diameters of the nano-object along the three spatial directions d_q are obtained via the Stokes–Einstein relation [23]:

$$d_q = \frac{2k_B T}{6\pi\eta D_q}, \quad (3)$$

with the Boltzmann constant k_B , the temperature T and the viscosity η . Note that d_q is an auxiliary quantity and refers to the diameter in the case of confinement, which is not the diameter of the free diffusion situation. As shown in

Figure 6: Measured (dots) dependence of mean squared displacement (MSD) on lag time for the gold nanosphere investigated along the three spatial directions (x : yellow, y : blue and z : green). The solid lines show the corresponding linear fits using Eq. (2).

Ref. [29] the confinement provided by the microchannel substantially alters diffusion compared to the unconfined case, i.e., leads to different values of d_q along the transverse and longitudinal directions which in the context of this work is accounted for by using the hindrance factor as shown in the subsequent section (Sec. 4.3). Here we assume a temperature of $T = 293$ K and a corresponding viscosity of $\eta = 1.001$ mPa · s [28].

The linear dependence of the measured MSD data (Figure 6a) justifies the applicability of the linear fitting procedure on the basis of Eq. (2) and reveals that within the first three lag times, transverse confinement imposed by the microchannel does not change the functional shape of the dependency (fitting results shown in Table 1). This choice is justified by estimating the time t_d a nanoparticle requires to freely diffuse from the center of channel to the wall with a length of $L = 0.5$ μm, which roughly represents the average radius of the microchannel.¹ Assuming a 100 nm nanoparticle and neglecting σ_q in Eq. (2), Eq. (3) leads to $t_d = \frac{3L^2 d^{NP} \pi \eta}{k_B T} \approx 58$ ms, which is must longer than three lag times (1.09 ms). The value of σ_y^{NP} is larger compared to those of the tracked directions which is expected due to the stronger susceptibility of the intensity-mediated position retrieval procedure on experimental inaccuracies (for details see the study by Jiang et al. [13]).

4.3 Application to available data set: resistance coefficient

As investigated in a study by Higdon et al. [29], the microchannel imposes a transverse confinement which alters the viscosity field of the fluid in all dimensions by a resistance coefficient R_q ($q = x, y, z$) in comparison to free diffusion (details can be found in the Supplementary material). Therefore the diffusion property of the nanoparticle is consequently modified by R_q , leading to:

$$D_q = \frac{2k_B T}{6\pi \cdot (\eta \cdot R_q(a_q, r_0)) \cdot a_q} = \frac{C}{R_q^{\text{avg}}(a_q) \cdot a_q} = \frac{C}{d_q},$$

where a_q is the hydrodynamic diameter of the particle in free diffusion which we would like to determine. Note that R_q is a function of particle size a_q and radial position of particle r_0 . In the particle size estimation process using Eq. (3), this dependence of R_q needs to be taken into account as otherwise an incorrect estimation of the particle size via $d_q = a_q \cdot R_q^{\text{avg}}$ would be obtained. Here R_q^{avg} is the averaged

¹ This time relates to a probability of 68% that the nano-object is located in the interval $0 \leq r_q \leq L$.

Table 1: Results of the MSD analysis ($q = x, y$ and z).

Direction	D_q [$\mu\text{m}^2/\text{s}$]	d_q [nm]	R_q^{avg}	a_q [nm]
x (transverse, tracked)	2.69 ± 0.03	159.01 ± 1.78	1.50	106.06 ± 0.90
y (recovered)	2.59 ± 0.04	165.12 ± 2.82	1.51	109.13 ± 1.40
z (longitudinal, tracked)	3.32 ± 0.04	128.90 ± 1.44	1.33	97.27 ± 0.88

MSD, mean squared displacement.

resistance coefficient within the whole channel cross-section which is a function of particle size a_q as well (details in the Supplementary material). To circumvent inaccuracies, the true particle diameter a_q can be obtained by iteratively solving the function:

$$d_q - a_q \cdot R_q^{\text{avg}}(a_q) = 0.$$

Detailed explanations and calculations can be found in Supplementary material. The true hydrodynamic diameters of the nanosphere a_q determined along the three dimensions are shown in Table 1. Note that the determined diameter for the y -direction including the resistance coefficient matches those of the image processing based directions. All obtained values are located within the range of the ensemble measurement ($\tilde{d}_{NP} = 104.3$ nm), again confirming the validity of our approach. Note that the Zetasizer measurements and the fiber approach discussed here rely on different types of evaluations (Zetasizer: DLS [ensemble analysis]; this work: fiber-based nano-object tracking [single object analysis]) and therefore standard deviations cannot be compared directly.

5 Discussion

The presented 3D tracking scheme relies on a unique platform – the optofluidic microstructured fiber – for analyzing the diffusion of nanoscale objects combining a confinement of the nano-object to a micrometer region with a well-defined light-line illumination that is identical at any point along waveguide within the FoV. This combination allows for the acquisition of trajectories with thousands of frames, yielding statistical evidence with very high significance within the context of NTA analysis. Here it is important to note that the precision of the determined diameter (i.e., standard deviation σ^{NP}) scales inversely with the number of frames $\sigma^{NP} \propto 1/\sqrt{N_f}$ [19–21]. Moreover the opportunity to track nano-objects for thousands of frames is essential for our 3D tracking scheme, as it allows

for intensity binning along the x -direction with each bin containing a significant number of frames.

The presented method to determine slope and local intensity parameter relies on the measured intensity values at the top and bottom edges of upper semicircle within each bin. Even though the good match between experiments and simulation (Figure 4a and b) future evaluation strategy will target to include more points into the analysis potentially via using more mean intensity values within each bin.

Establishing mode fields inside the microchannel with bijective function between position and local intensity that ideally can be fitted by a single exponential function across the entire fiber cross-section represents an obvious target for improving the device performance. Via simulations the geometric fiber parameters within realistic intervals have been swept, showing that improved device performance can be reached. For instance, the domain of ambiguous correlation can be reduced or even vanishes in some scenarios that are currently investigated. Specifically, when the core diameter is increased to about $6\ \mu\text{m}$ with a similar type of RI distribution, the unambiguous region does not exist anymore for the fundamental mode while; however, the fiber simultaneously gets multimode. This emphasizes the flexibility of the fiber approach, while future research targets to identify the physical origin of the multivalued intensity. Here we believe that this presumably results from a formation of a Mie-type resonance in the water-filled channel hybridizing with guided mode in the glass core.

6 Conclusion

Tracking of nano-objects along all three spatial dimensions with high spatial localization accuracy and temporal resolutions represents a novel pathway for the precise understanding of nanoscale processes within bioanalytics and life science. Here we demonstrate 3D tracking of diffusing nano-objects within a water-filled optofluidic microstructured fiber via elastic light scattering based position retrieval. Specifically, the bijective function between

intensity and position inside the upper semicircle of the microchannel was used for the tracking along the direction of the microscopic detection, while image processing based tracking was used in the image plane. The mentioned 3D tracking approach includes advantages such as a large numbers of frames per trajectory or a high spatial localization accuracy and extends the current capabilities of fiber-based 3D tracking toward a water environment, making this system in particular relevant for bioanalytical applications. The approach allows the employment of commonly used microscopes, is straightforward to use and yields continuous trajectories with hundreds of frames. The capability of the fiber approach was demonstrated by the determination of the hydrodynamic diameter of a diffusing gold nanosphere using MSD analysis, leading statistically significant values along all three dimensions with very high significance due to the very large number of frame. Additionally, we show that the strong confinement provided by the microchannel significantly impacts diffusion, while this impact is different along the transverse and longitudinal directions.

The presented platform represents an extension of NTA, combining this well-established technology with the benefits of a flexible integrated optofluidic waveguide (e.g., light-line illumination and nano-object confinement) and extending its capabilities along all three spatial dimensions while the number of frames per trajectory exceeds those of typically used systems. Moreover, the water environment adaption of this work make this novel sensor platform, which includes further highly relevant advantages such as the measurement of the dynamics of nano-scale processes on single object level or the compatibility with fiber circuits and microscopy, for the first time highly attractive for bioanalytical and medical applications such as virus detection or understanding dynamic biological processes.

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Appendix of symbols

Parameter	Description	Unit
a	Hydrodynamic diameter of nanosphere in free diffusion	nm
b	Local intensity parameter	Counts
d	Hydrodynamic diameter of nanosphere calculated from MSD	nm
$\bar{d}_{NP}$	z-average hydrodynamic diameter of nanosphere ensemble measured from zetasizer	nm
d^m	Movable range of the nanosphere center	μm
D^m	Dimension (length of principle axes/diameter) of microchannel	μm
D_0	Bulk diffusion coefficient	$\mu\text{m}^2/\text{s}$
D	Diffusion coefficient within confinement	$\mu\text{m}^2/\text{s}$
j	Frame index	/
i	Bin index	/
I	Light intensity	Counts
k	The index of the retrieved y-position sequence	/
k_B	Boltzmann constant	$\text{m}^2 \cdot \text{kg} \cdot \text{s}^{-2} \cdot \text{K}^{-1}$
n	The index of lag time/MSD	/
n_r	Refractive index	/
N_{ave}	Averaged number of frames per continuous trajectory	/
$N_{\text{bin},i}$	Number of available frames used in the i th bin;	/
$N_{\text{bin}}^{\text{tot}}$	Total number of bin	/
N_{con}	Number of continuous trajectories	/
N_f	Number of recorded frames	/
N_{max}	Maximum number of frames per continuous trajectory	/
N_n	The number of sub-trajectories that contribute to the n th MSD	/
N_{up}	Number of frames in the upper half of channel	/
p	Slope parameter	Counts/ μm
q	$q = x, y, z$ indicating different directions	/
r_0	Radial position of nanoparticle in the microchannel	μm
R	Local resistance coefficient	/
R^{avg}	Averaged resistance coefficient within the whole microchannel cross section	/
Δt	Lag time	s
T	Temperature	K
ν	Camera frame rate	Hz
$\bar{x}_i$	Central bin position	μm
Δx_{bin}	Bin width	nm
x_q^2	Mean squared displacement along q ($q = x, y, z$) direction	μm^2
η	Viscosity	mPa·s
λ	Operation wavelength	nm

(continued)

Parameter	Description	Unit
λ_h	Relative particle size	/
Λ	Pitch (center-to-center distance) between core and microchannel	μm
σ_{N_p}	Standard deviation of z-average particle diameter	nm
σ_q	Sum of potential errors in q direction MSD estimation	μm^2
σ_{tr}	Transverse localization accuracy	nm
τ	Tracking time	s

MSD, mean squared displacement.

References

- [1] M. J. Saxton and K. Jacobson, "Single-Particle Tracking: applications to membrane dynamics," *Annu. Rev. Biophys. Biomol. Struct.*, vol. 26, pp. 373–399, 1997.
- [2] P. Kramberger, M. Ciringer, A. Štrancar, and M. Peterka, "Evaluation of nanoparticle tracking analysis for total virus particle determination," *Viro. J.*, vol. 9, p. 265, 2012.
- [3] J. Ando, A. Nakamura, A. Visootsat, et al., "Single-nanoparticle tracking with Angstrom localization precision and microsecond time resolution," *Biophys. J.*, vol. 115, pp. 2413–2427, 2018.
- [4] R. E. Thompson, D. R. Larson, and W. W. Webb, "Precise nanometer localization analysis for individual fluorescent probes," *Biophys. J.*, vol. 82, pp. 2775–2783, 2002.
- [5] S. Ram, P. Prabhat, J. Chao, et al., "High accuracy 3D quantum dot tracking with multifocal plane microscopy for the study of fast intracellular dynamics in live cells," *Biophys. J.*, vol. 95, pp. 6025–6043, 2008.
- [6] H. P. Kao and A. S. Verkman, "Tracking of single fluorescent particles in three dimensions: use of cylindrical optics to encode particle position," *Biophys. J.*, vol. 67, pp. 1291–1300, 1994.
- [7] M. A. Thompson, M. D. Lew, M. Badieirostami, and W. E. Moerner, "Localizing and tracking single nanoscale emitters in three dimensions with high spatiotemporal resolution using a double-helix point spread function," *Nano Lett.*, vol. 10, pp. 211–218, 2010.
- [8] G. Corkidi, B. Taboada, C. D. Wood, A. Guerrero, and A. Darszon, "Tracking sperm in three-dimensions," *Biochem. Biophys. Res. Commun.*, vol. 373, pp. 125–129, 2008.
- [9] S. R. Pavani, M. A. Thompson, J. S. Biteen, et al., "Three-dimensional, single-molecule fluorescence imaging beyond the diffraction limit by using a double-helix point spread function," *Proc. Natl. Acad. Sci. USA*, vol. 106, pp. 2995–2999, 2009.
- [10] S. Faez, Y. Lahini, S. Weidlich, et al., "Fast, label-free tracking of single viruses and weakly scattering nanoparticles in a nanofluidic optical fiber," *ACS Nano*, vol. 9, pp. 12349–12357, 2015.
- [11] S. Faez, S. Samin, D. Baasanjav, et al., "Nanocapillary electrokinetic tracking for monitoring charge fluctuations on a single nanoparticle," *Faraday Discuss.*, vol. 193, pp. 447–458, 2016.
- [12] R. Förster, S. Weidlich, M. Nissen, et al., "Tracking and analyzing the brownian motion of nano-objects inside hollow core fibers," *ACS Sens.*, vol. 5, pp. 879–886, 2020.
- [13] S. Jiang, J. Zhao, R. Förster, et al., "Three dimensional spatiotemporal nano-scale position retrieval of confined diffusion of nano-objects inside optofluidic microstructured fibers," *Nanoscale*, vol. 12, pp. 3146–3156, 2020.
- [14] X. Nan, P. A. Sims, and X. S. Xie, "Organelle tracking in a living cell with microsecond time resolution and nanometer spatial precision," *ChemPhysChem*, vol. 9, pp. 707–712, 2008.
- [15] A. R. Dunn and J. A. Spudich, "Dynamics of the unbound head during myosin V processive translocation," *Nat. Struct. Mol. Biol.*, vol. 14, pp. 246–248, 2007.
- [16] J. A. Gallego-Urrea, J. Tuoriniemi, and M. Hasselöv, "Applications of particle-tracking analysis to the determination of size distributions and concentrations of nanoparticles in environmental, biological and food samples," *TrAC Trends Anal. Chem. (Reference Ed.)*, vol. 30, pp. 473–483, 2011.
- [17] K. D. Kihm, A. Banerjee, C. K. Choi, and T. Takagi, "Near-wall hindered Brownian diffusion of nanoparticles examined by three-dimensional ratiometric total internal reflection fluorescence microscopy (3-D R-TIRFM)," *Exp. Fluids*, vol. 37, pp. 811–824, 2004.
- [18] R. M. Dickson, D. J. Norris, Y. L. Tzeng, and W. E. Moerner, "Three-dimensional imaging of single molecules solvated in pores of poly (acrylamide) gels," *Science*, vol. 274, pp. 966–968, 1996.
- [19] M. J. Saxton, "Single-particle tracking: the distribution of diffusion coefficients," *Biophys. J.*, vol. 72, p. 1744, 1997.
- [20] K. Fogelmark, M. A. Lomholt, A. Irback, and T. Ambjörnsson, "Fitting a function to time-dependent ensemble averaged data," *Sci. Rep.*, vol. 8, p. 698, 2018.
- [21] H. Flyvbjerg and H. G. Petersen, "Error estimates on averages of correlated data," *J. Chem. Phys.*, vol. 91, pp. 461–466, 1989.
- [22] J. W. Fleming, "Dispersion in $\text{GeO}_2\text{-SiO}_2$ glasses," *Appl. Opt.*, vol. 23, pp. 4486–4493, 1984.
- [23] A. Einstein, "Über die von der molekularkinetischen Theorie der Wärme geforderte Bewegung von in ruhenden Flüssigkeiten suspendierten Teilchen," *Ann. Phys.*, vol. 322, pp. 549–560, 1905.
- [24] H. Qian, M. P. Sheetz, and E. L. Elson, "Single particle tracking. Analysis of diffusion and flow in two-dimensional systems," *Biophys. J.*, vol. 60, pp. 910–921, 1991.
- [25] R. A. Dragovic, C. Gardiner, A. S. Brooks, et al., "Sizing and phenotyping of cellular vesicles using nanoparticle tracking analysis," *Nanomed. Nanotechnol. Biol. Med.*, vol. 7, pp. 780–788, 2011.
- [26] X. Bian, C. Kim, and G. E. Karniadakis, "111 years of Brownian motion," *Soft Matter*, vol. 12, pp. 6331–6346, 2016.
- [27] X. Michalet and A. J. Berglund, "Optimal diffusion coefficient estimation in single-particle tracking," *Phys. Rev. E*, vol. 85, p. 061916, 2012.
- [28] The International Association for the Properties of Water and Steam, *Release on the IAPWS formulation 2008 for the viscosity of ordinary water substance*, 2008. Available at: <http://www.iapws.org>.
- [29] J. J. Higdon and G. P. Muldowney, "Resistance functions for spherical particles, droplets and bubbles in cylindrical tubes," *J. Fluid Mech.*, vol. 298, pp. 193–210, 1995.

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