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Dark field particle tracking with enhanced sizing precision by confining particles

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Abstract

The size of micro- and nanoparticles in liquid can be measured by tracking of their Brownian motion on video recordings. By matching the height of the liquid sample with the focal depth of the optical system, particles stay in focus and very long particle tracks can be acquired which in turn enhances the sizing precision. A microfluidic chip with suitable chamber height was used in a custom dark field video microscopy setup to detect and size suspended particles. Unlike conventional microscopy setups, the optical components were arranged horizontally to achieve a more compact and stable setup and allow simultaneous measurements of particle diffusion and sedimentation. We describe the design, fabrication, and characterization of the microfluidic chips as well as the sensor setup and apply the method for measuring the size distribution of metallic wear particles in base oil.

Key words: micro-/nanoparticle tracking, Brownian motion, microfluidic chip, dark field microscopy setup, sizing precision

Introduction

The detection of micro- and nanoparticles (NP) is a relevant task in a variety of industrial and scientific areas [1,2]. Besides invasive and complex laboratory methods (e.g., electron microscopy), there are several electrical, mechanical, and magnetic sensing principles to measure the size distribution of small particles in liquid [3-5]. Some also have the potential for continuous online operation, but they are often limited due to the properties of the particles or the liquid (e.g., non-conducting liquids for resistive pulse sensing [2]).

Optical detection methods, however, can be used to detect and characterize particles in liquids over a wide range of sizes and materials. Dynamic light scattering (DLS) is a widely used method that measures the fluctuation of the scattering signal of a particle ensemble, which is caused by Brownian motion, and calculates an average ensemble size by autocorrelation. Unfortunately, the peak resolution can be insufficient when multiple size modes (multiple particle sizes) are present in a sample [6].

Nanoparticle tracking analysis (NTA) is based on dark field video microscopy to visualize and record suspended particles moving due to Brownian motion [7]. In contrast to DLS, individual particles are tracked by software and the diffusive movement is related to the particle size. However, when large measurement chambers are used, the particles can easily leave the microscope focus and only short particle trajectories can be recorded [7,8]. To address this issue, the dark field visualization and tracking of nanoparticles has been improved, as described in our previous publications [8,9] and in the present work.

Visualization, tracking, and sizing principle

The basic dark field arrangement is depicted in Fig. 1a. The sample is illuminated from above in an angle so that no reflected light can enter the objective, which has a long working distance and low numerical aperture (NA=0.3) to achieve a large focal depth. The sample is confined between two glass substrates to create a 10 µm thick liquid film and the diffusing particles are in the focus for the whole observation time (Fig. 1b). Particle images (i.e., diffraction limited spots) spread over several camera pixels are tracked in every frame of a recorded sequence. A MATLAB script based on the code of Crocker and Grier [10] is used for 2D-tracking with sub-pixel resolution. The individual diameters are calculated according to Eq. 1 and 2, assuming spherical particles:

$$\rho(n) = 4 \cdot D \cdot n\Delta t \qquad (1)$$

$$d_p = \frac{k_B T}{3\pi\eta D} \qquad (2)$$

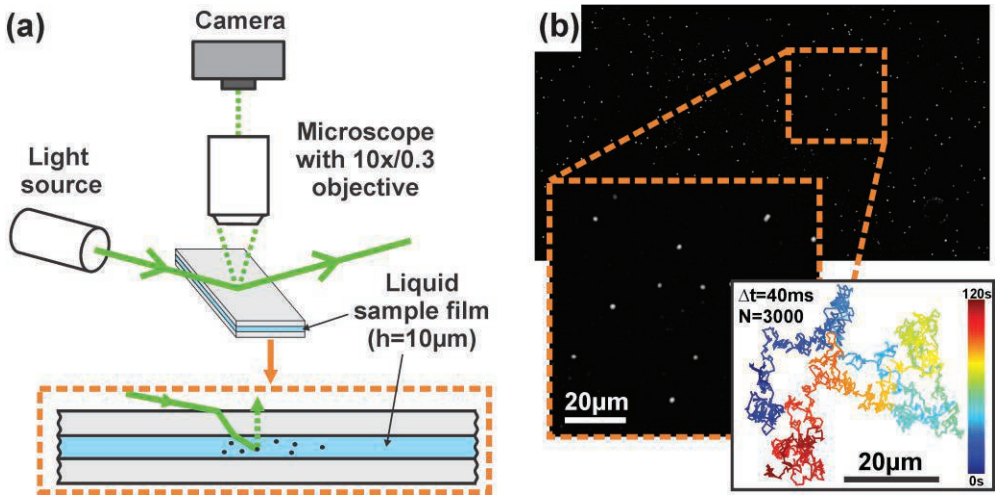

Fig. 1: (a) Schematic of the dark field arrangement and diffusing particles confined between two glass substrates. (b) All particles are constantly in focus and very long trajectories can be acquired, as depicted for a single 100 nm particle over a period of 120 s (i.e., N=3000 positions for a frame rate of 25 fps) [8].

Here, $\rho(n)$ is the two-dimensional mean squared displacement (MSD) of a particle. It is determined from the tracked particle positions over a sequence of frames using different time intervals $n\Delta t$. The temporal resolution Δt is defined by the video frame rate. The diffusion coefficient D is determined by linear fitting of $\rho(n)$. Eventually, the particle diameter is calculated with Eq. 2, where k_B is the Boltzmann constant, T is the absolute temperature, and η is the dynamic solvent viscosity.

Thanks to the extended particle visibility, very long trajectories can be acquired (Fig. 1b), which effectively increases the sizing precision. This is particularly important when more than one peak occurs in the size distribution. Although crucial for many applications, a resolution of close particle sizes is not possible with methods like DLS, which can typically distinguish particles with a diameter ratio of 1:4 (e.g., 100 nm and 400 nm). Fig. 2 shows the measured size distribution of a mixture of 100 nm and 150 nm gold particles in water having the same concentration [8]. Either N=100 or N=1000 frames of the recorded sequence had been evaluated in order to demonstrate the effect of long particle trajectories. It is evident that for N=100 a broad peak with a modal diameter corresponding to 100 nm particles and a tail towards larger diameters dominates the size distribution. For N=1000, and hence increasing sizing precision, the peak corresponding to 150 nm particles can also be observed. Both modal diameters corresponded to the diameters measured for monodisperse samples of the respective particles. It was further demonstrated that for a film thickness of 10 µm the proximity of solid substrates does not significantly influence the diffusion of particles with a diameter in the order of 100nm, because particles are sufficiently well separated from the walls thanks to electrostatic repulsion [8].

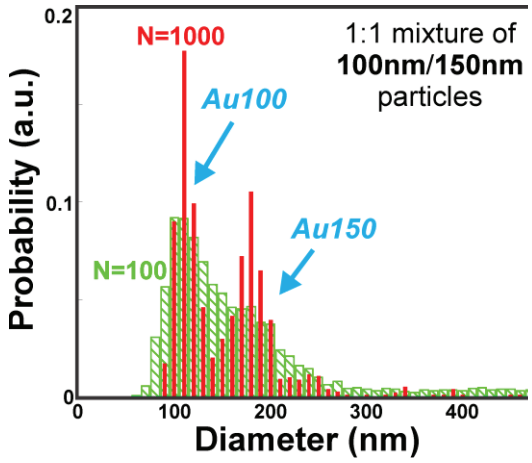

Fig. 2: A bimodal particle mixture (AuNP with 100 nm and 150 nm diameter) can be resolved when long trajectories are evaluated (N=1000). For shorter trajectories (N=100) a resolution of both peaks is not possible [8].

Based on these findings, the previously described arrangement using a standard brightfield microscope with external laser illumination was adapted in order to construct a custom dark field microscopy setup (see next section). Furthermore, we elucidate the design and fabrication process of a microfluidic Si-glass chip that can be used instead of a disposable glass sample cell and describe exemplary particle size measurements.

Novel dark field microscopy setup

In contrast to conventional microscopy, here the optical components are aligned horizontally and the sample chamber is placed on a vertical

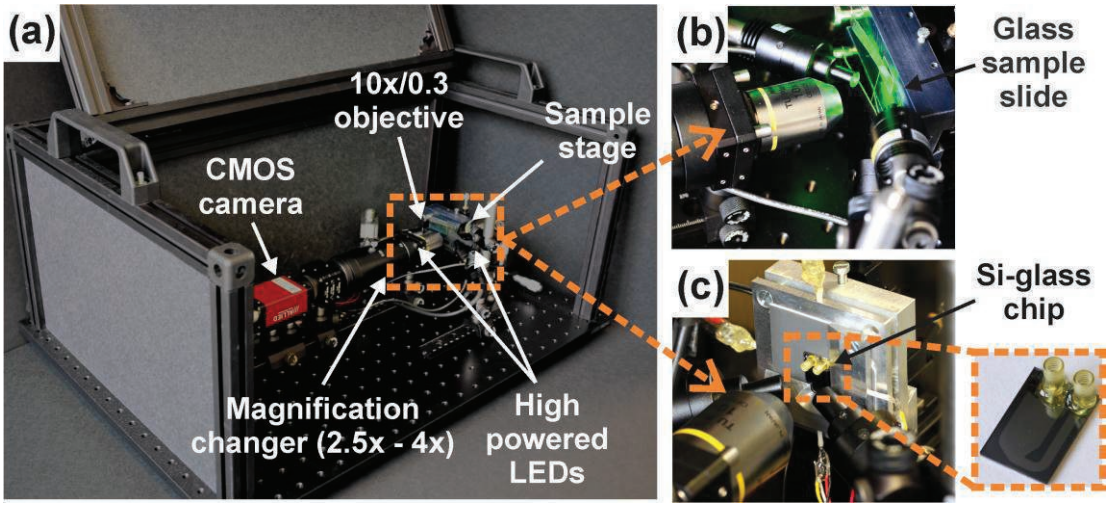

Fig. 3: (a) Portable measurement setup containing all optical and electronic components. The close-ups (b,c) depict how the objective, LEDs, and sample stage are arranged for the two possible sample cell configurations: (b) Sample liquid can be sandwiched between glass slides and mounted on the stage to perform room temperature measurements. (c) A microfluidic chip (Si-glass) with fluidic connectors on top is placed on the aluminum chip holder containing a Peltier element and Pt100 sensors for temperature control. Chip dimensions are 10×15×0.85 mm³. Tubings can be attached to the fluidic connectors to inject sample liquid with a syringe.

stage (Fig. 3). Therefore, both the particle diffusion in x- and y-direction as well as potential sedimentation movement in negative y-direction can be observed, while the liquid film thickness is constricted in the direction of the optical axis (z-axis). Additionally, the setup is more compact and vibration-stable compared to a portable upright arrangement.

The sample is illuminated with high powered spot LEDs from the same side as the 10×/0.3 observation objective and therefore allows dark field microscopy of non-transparent samples. The spot LEDs are arranged in an angle of approximately 20° to the plane of the sample and can be placed very close because of their small face size (diameter of 8 mm) and the objective's working distance, thus achieving a high light intensity and making collimator lenses for the LEDs obsolete.

Glass slides can be placed on the custom platform made of matte black polycarbonate with a recess in the middle including stage clips (Fig. 3b). This platform can be replaced with an aluminum holder to mount a reusable microfluidic chip (Fig. 3c) and control its temperature. A Peltier element and Pt100 temperature sensors are incorporated into the holder. The Si-glass chip is placed on top of a compliant heat conducting pad and clamped with an L-shaped aluminum piece. The 45×60×30 cm³ large enclosure of the microscopy setup is made of optical blackout material and includes a lid to ensure eye safety during operation. Electronic components (LED driver, power supply units, temperature logger, Peltier controller) are situated within a separate compartment at the back of the housing. The camera and other components are connected to an external measurement PC via Ethernet and USB, respectively. Temperature logging and, if required, temperature control is performed with proprietary software provided by the device manufacturers. Recorded frames are analyzed with custom MATLAB routines.

Microfluidic chip design and fabrication

The sample liquid can be contained in a disposable sample chamber made of two microscopy cover slips without the need of a spacer element by placing an adequate amount of liquid on one glass, sandwiching with a second cover slip, and sealing with glue or lacquer [8]. It is easy to prepare and very suitable for diffusion measurements in low viscosity samples (e.g., aqueous media), but allows only measurements at ambient temperature and no exchange of the sample liquid in the chamber. However, the majority of liquids show an exponential decrease of viscosity with increasing temperature, which in turn enhances the extent of Brownian motion of suspended particles and facilitates their sizing. Positioning a sample cell with a non-transparent bottom on a flat heating element prevents the visualization of out-of-focus particles from the surface below. While a variety of technologies exist for the fabrication of microfluidic chips (e.g., rapid prototyping with Ordyl dry film resist and adhesive bonding at low temperatures [9,11]) it was chosen to fabricate shallow measurement chambers in Si and seal them with glass by anodic bonding (Fig. 4). As a consequence, a very high strength permanent

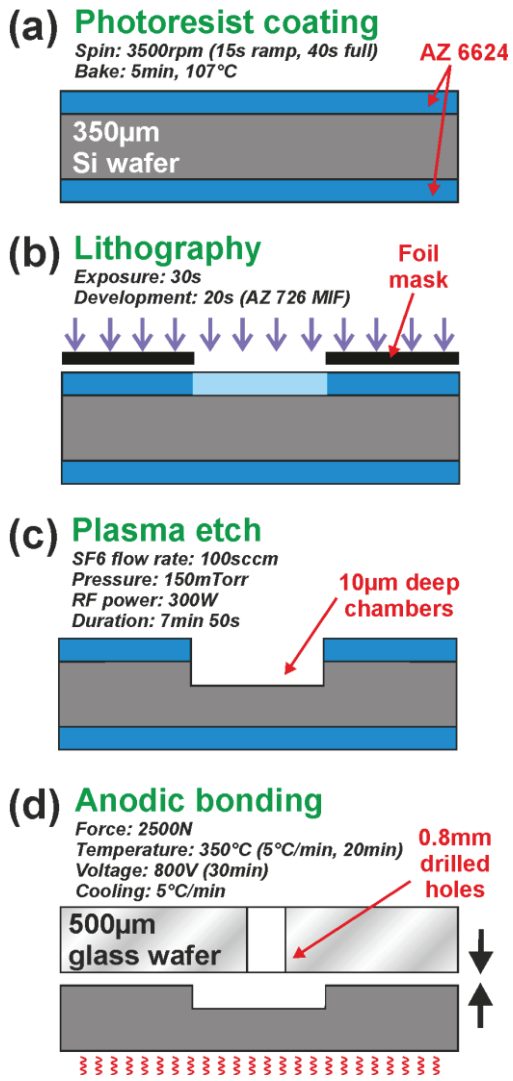

Fig. 4: Chip fabrication steps: (a,b) Photoresist is spun on the Si wafer and structured using a low resolution foil mask, which is sufficient for the large sample chambers. (c) 10 µm deep chambers are dry etched in the silicon. (d) Anodic bonding of the structured Si wafer and a glass wafer with drilled inlet holes.

bond is generated and the chamber surfaces are resistant to typical aqueous and organic solvents. Also, the high thermal conductivity of Si ensures fast heating of the sample. Importantly, the surface roughness in the chambers after the short etching process is only marginally higher than the one of polished Si and much lower compared to PMMA, which was used previously [9,11].

The chambers comprise most of the chip area (10×15mm²) because only a small contact area is required to achieve a strong anodic bond. Consequently, there are many spots within the chamber where it is possible to perform measurements, while close to the walls strong

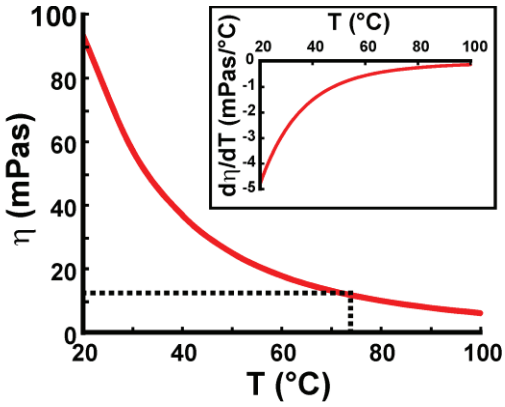

Fig.5: Dynamic viscosity η of PAO8 base oil. The sample was heated to 73 °C to reduce the viscosity. Inset: first derivative of η.

scattering of the Si structure would occur. Since the chip is placed flat on the Al-holder, the holes to access the chamber and hence also fluidic connectors are positioned on top of the chip (see details in Fig. 3c). Flexible tubings are tightly attached with screw fittings. Because of the large working distance of the objective, connectors and fittings with a total height of 10 mm extending from the top of the chip can be used.

Experimental

We have previously worked with Au reference particles in aqueous solutions to confirm the sizing method [8,9] and here we subsequently extend the range of samples to metal wear particles in mineral oil which has a much higher viscosity. Those particles originated from a tribological experiment of AlSi cylinder liner against steel piston ring lubricated with base oil (PAO8). A tribometer with linear reciprocating movement was used with the following test parameters: normal force 10 N, sliding distance 1.5 mm, frequency 50 Hz, oil temperature 120 °C, test duration 2 h. The undiluted oil sample was shaken thoroughly to prevent unevenly distributed particles in the liquid. Measurements were done at elevated temperatures to reduce the oil viscosity and observe more pronounced Brownian motion. The camera frame rate was 25 fps and the size of a pixel on the recordings was 328 nm. A DLS reference measurement was performed and the dynamic viscosity of PAO8 over temperature was determined with a Stabinger viscometer (Fig. 5).

The major advantage of confining particles to a thin liquid film is the acquisition of very long trajectories. Even if the particles are permanently present in the focus, they can show intensity variations like blinking (e.g., due to asymmetric shape [1,8]) and hence vanish

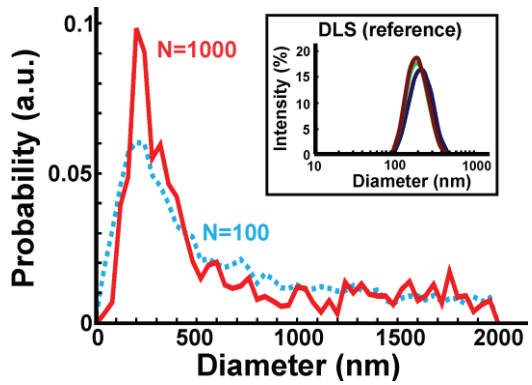

Fig. 6: Size distribution of wear particles in PAO8. Trajectories with a maximum number of N=1000 and N=100 positions have been evaluated. Values are weighted with the trajectory length and normalized by the total number of collected tracks. Inset: DLS measurements of the sample.

and reappear so that no long trajectory can be formed. Especially smaller particles are subject to this effect. To take into account also these particles, short tracks are evaluated as well.

Fig. 6 shows the size distribution measured by our tracking method at 73 °C for maximum trajectory lengths of N=1000 and N=100. Minimum length was 500 and 50, respectively, as elucidated in the Supplementary Information of ref. 8. The histogram peaks show the predominant particle size while individual larger particles are visible as a tail towards larger sizes. The peak diameters determined from a Kernel density fit in the range 1 nm – 1000 nm are 242 nm and 222 nm, respectively, while the FWHM peak widths are 290 nm and 390 nm. The narrower peak for N=1000 shows that the precision is higher for larger N, while the lower peak diameter for N=100 can be attributed to small particles that are not sufficiently acquired when only long trajectories are used. As a comparison, the DLS reference measurements show modal diameters in the range of 200 nm to 230 nm. The peak resolution of DLS is typically very limited, but by tracking we can ascertain that the wear particles in the sample comprise a unimodal size distribution. Moreover, the average of the mean displacements of all particles over all frames was zero, which indicates that there was no drift motion in any direction and that the random Brownian motion exceeded any sedimentation movement of particles.

To estimate the sizing uncertainty, the temperature dependent viscosity of the medium has to be considered. Eq. 2 and Fig. 5 suggest that the viscosity can strongly influence the diameter calculation. The temperature of the holder was measured at four different locations below and above the chip (i.e., inside of the Al base plate and clamp, respectively). Average temperature ± range was 73±2 °C; the average viscosity was therefore 12mPas. The first derivative of the viscosity (Fig. 5 inset) corresponds to the uncertainty related to temperature variations. Values range from -0.31 to -0.36 mPas/°C in this temperature region. As a consequence, for ±2 °C a deviation of the peak diameter of ~15 nm can be determined. Measurements at low temperatures, however, would introduce a larger uncertainty due to the higher viscosity variation over temperature (e.g., -2.7 mPas/°C at 30 °C). Thus, it can be of advantage to perform particle size measurements in media like base oil or fully formulated engine oil at elevated temperatures.

Conclusions

We have described particle size measurements based on dark field visualization and tracking of Brownian motion of particles confined to a thin liquid sample film that was matched with the focal depth of the microscope objective. A novel measurement setup and microfluidic chips were used to determine the size distribution of metallic wear particles in base oil, which were produced during a tribometer test of engine components. The arrangement of the sample in the setup also allows concurrent measurements of diffusion and sedimentation, which can be used for particle density measurements in the future.

The newly-developed particle measurement method can be applied in many scientific and technical fields where the measurement of nanoscopic particles is a necessity; examples include fields such as pharmacy and medicine or mechanical engineering and tribology, where this method can be used to study fundamental wear mechanisms. There is also the potential for condition monitoring and early detection of incipient material damage in tribological contacts of heavy machinery.

Acknowledgements

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