Force measurements on oil and liposomes with the NanoTracker™ 2 optical tweezers system

The versatile NanoTracker™ 2 system is designed for the optical trapping of dielectric particles and optically refractive objects. Sizes range from sub-micrometer polystyrene and silica particles (beads) to living cells with diameters of >10µm. After automated calibration of the trap-particle system, the precise and reliable measurement of piconewton forces is possible. Applications include single-molecule mechanics and the micro-rheology of fluid and soft matter materials.

In different fields of research, interest in the investigation of lipid and oil-water emulsion systems has grown. Micrometer sized, lipid enclosed cargo droplets play decisive roles in intracellular transport and metabolism processes, and the properties of oil/water or liposome emulsions are of great interest for oil production as well as cosmetic and medical drug delivery applications.

In this application note, the handling of and force measurements with oil and lipid droplets in aqueous solution using the NanoTracker™ 2 are introduced, further extending the range of possible applications.

Multilamellar lipid vesicles (MLVs)

In biological cells, lipid vesicles (liposomes) are involved in many transport processes including inter-neuronal signaling and the uptake of extracellular material. These vesicles typically consist of a spherical single- or double-layer of lipids which enclose a solution of proteins or other cargo. Lipids themselves are stored and transported in cells in so called lipid droplets or lipid bodies.These organelles contain different types of lipids, cholesterol and proteins and have previously been used to measure individual motor protein forces with optical traps [1]. Also a number of pharmaceutical processes like the delivery of drugs to individual cells [2] or the controlled cellular uptake of liposomes [3] have been investigated using optical tweezers systems. Multilamellar lipid vesicles (MLVs) serve as a model system for these liposomes in the measurements described in this note. MLVs consist of several

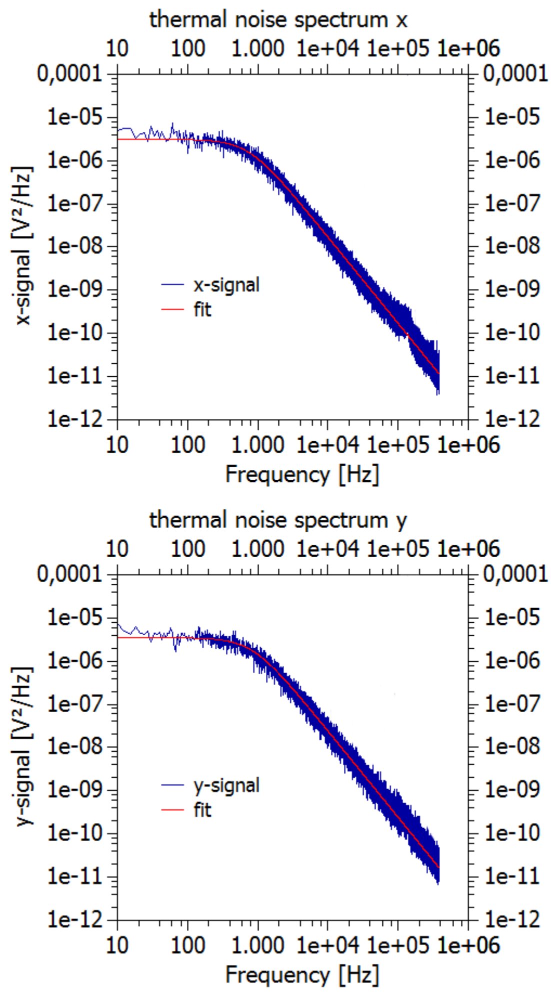

Figure 1 *Thermal noise spectra (x- and y-direction) of a trapped MLV*

concentric spherical lipid bilayers. They assemble spontaneously in an aqueous solution as a result of hydrophobic effects which organize the amphipathic lipid molecules in this particular order. We used DPPC (16:0 phosphatidylcholine, Avanti Polar Lipids) in purified H_2O, prepared by short sonication which produced MLVs with a large range of sizes, varying from tens of nanometers to micrometers. Individual micrometer sized MLVs were identified in the optical camera image, which also allows a coarse approximation of their diameter.

In the next step, the trap with the MLV was calibrated to determine the system's detection sensitivity and the stiffness of the trap. The advanced calibration routine available in the NanoTracker™ 2 control software is able to determine these parameters without knowing the trapped particle's size or the viscosity of the solvent. This is particularly suitable for the present applications since the medium may contain residual lipid molecules that can influence its hydrodynamic properties. Furthermore, the exact MLV diameter is also not known. A periodic external force is briefly applied to the trapped particle by moving the sample chamber with a known frequency and amplitude. From the signal response to this force and the spectral analysis of the particle's thermal motion in the trap potential, it is possible to calculate the detection sensitivity σ and trap stiffness κ [4]. Thus we refer to this as the *oscillating thermal noise* calibration method. The two thermal noise spectra for a trapped MLV with the respective Lorentzian fit curves that are used for the calculation of σ and κ are shown in Figure 1 (blue and red curves, respectively).

With known trap parameters, it is possible to directly measure forces acting on the trapped MLV. A standard experiment often used as an alternative calibration method (with the drawback that it requires information about the particle size and medium viscosity) is the measurement of viscous drag, where the medium is moved relative to the particle. For the measurements presented here, this was done by moving the sample chamber with a three-axis piezo scanner while holding the trap position constant. According to Hooke's law, the drag force F_d on a spherical particle depends on its radius r, the fluid viscosity η and the relative speed v:

$$F_d = 6\pi\eta r v$$

The force data collected during such a measurement are shown in Figure 2. The red and green curves were recorded using the same sample chamber displacement scheme (as indicated by the blue curve showing the sample scanner's x position) but with MLVs of different sizes.

If we now assume that the viscosity η of the medium is approximately that of water ($\eta \approx 0.89 mPas$), we can estimate the radius of the two trapped MLVs from the force measurements. The calculation with $v = 100\frac{\mu m}{s}$, $F_1 \approx 4\,pN$ and $F_2 \approx 1\,pN$ yields $r_1 \approx 2.5\,\mu m$ for the larger MLV (green curve) and $r_2 \approx 0.6\mu m$ for the smaller one (red curve).

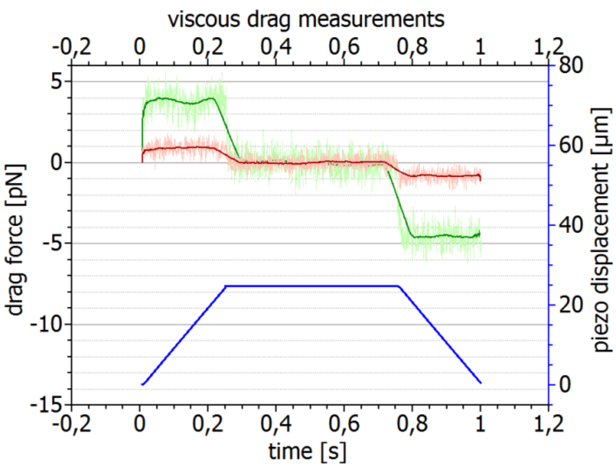

Figure 2 *Measurements of the viscous drag acting on trapped MLVs of different size. The blue curve represents the sample position. It moves with a constant velocity of 100 µm/s, pauses, and moves back. The corresponding drag forces on two trapped MLVs are in the range of 1 and 4pN.*

Oil/water emulsions

Oil/water emulsions are used in many cosmetic and medical applications as well as in oil production, where so called drilling or completion fluids are used to extract the oil from the surrounding ground material. In both cases the emulsion stability and fusing behavior of individual oil droplets is of great interest. A growing number of studies use optical tweezers to investigate these properties and, for example, the influence of surfactants on droplet fusion and stability [5].

A prerequisite for optical trapping of emulsion oil droplets is that their refractive index is higher than that of the aqueous phase. For representative measurements we used immersion oil (Zeiss Immersol™ 518F) typically employed with high numerical aperture microscopy objectives. It has a refractive index of $n \approx 1.52$ which allows the trapping of individual droplets in water. The emulsion was prepared simply by mixing immersion oil in water followed by two minutes of sonication. This procedure produces micrometer-sized oil droplets which can be easily trapped and used for further investigations. Two of these droplets held by the NanoTracker™ 2 optical traps are shown in Figure 3. As expected (due to their high refractive index), the trap stiffness is rather high. The oscillating thermal noise calibration described above delivers approx. $0.147\,pN/nm$ at a laser power of $50\,mW$ to the sample. After successful calibration, viscous drag force measurements (Figure 4A) were conducted at different drag velocities v. The results are shown in Figure 4B where the measured drag force is plotted against the velocity. The expected linear relationship $F_d \propto v$ is nicely reproduced in the measurement data. The slope of the linear fit curve in turn is proportional to the droplet radius which is calculated to be approx. $0.53\mu m$.

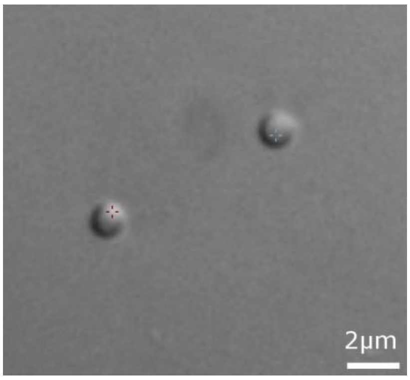

***Figure 3** Brightfield microscopy image of oil droplets optically trapped with the NanoTracker™2 setup.*

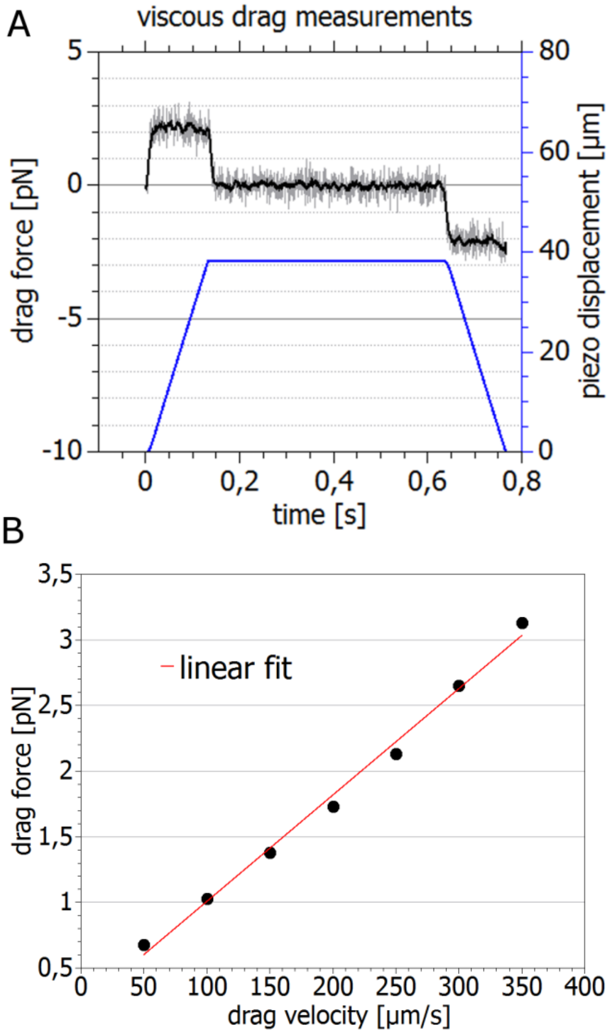

***Figure 4** (A) Single viscous drag measurement of a trapped oil droplet in water. The sample is moved repeatedly with $350\ \mu m/s$ which generates a drag force of $\sim 5\,pN$. (B) Drag forces measured at different sample velocities between 50 and $350\ \mu m/s$.*

Surface interaction forces

The viscous drag force measurements described above serve to cross-check calibration results and deliver information about interactions between the oil and water phase (generally, the dispersed and continuous phase) of the emulsion. For many applications, however, interactions between individual droplets are the focus of investigation, e.g. to characterize the influence of surfactants or other additives on emulsion stability on a single-droplet level. In their proof-of-concept study, Nilsen-Nygaard et al. used the NanoTracker™ system to compare the behavior of rigid polystyrene spheres and deformable oil droplets as they are brought in contact with the optical traps [6].

They were also able to quantify adhesion between individual droplets in a sodium dodecyl sulphate (SDS) containing solution which they ascribe to the so called depletion effect [1]. In further measurements they found a positive correlation between droplet size and the work required to separate aggregated droplets.

[1] The underlying mechanism of the *depletion interaction* is the exclusion/depletion of SDS micelles in the continuous phase from a volume surrounding the droplets, called the depletion layer. Coagulation or fusion of droplets brings the system to an energetically favorable state [7].

Conclusion

The NanoTracker™ 2 optical tweezers system is well suited for manipulating and investigating individual oil or lipid droplets in emulsions and colloids. Applications include droplet fusion experiments, the determination of viscous properties, investigations on intracellular transport and trafficking as well as the influence of surfactants or polymer additives on emulsion properties.

Literature

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