Micro-rheology of cells and soft matter with the NanoTracker™

Introduction

In micro-rheological measurements, the complex deformation or flow of viscoelastic systems under small external forces is investigated. From the dynamic response to well defined external stress, the mechanical characteristics, i.e. the contributions of viscous and elastic components to the material properties can be derived.

Typically, deformations are in the microscopic range of 0.1…10 µm displacements and sub nN forces on a timescale of milliseconds to seconds.

With the NanoTracker™ optical tweezers setup, spherical particles (beads) with well-defined size and optical properties are used as force sensors and actuators. This ensures the reliability and reproducibility required for mechanical measurements on the smallest scales. For a detailed description of the physical mechanisms underlying optical trapping, please refer to our application note "Quantitative force measurements with optical tweezers: the JPK NanoTracker™" which can be downloaded from www.jpk.com. Briefly, the interaction between focused laser light and the small refractive beads results in optical forces that stably hold the particle in the focal region. Displacements of the particles from their stable position in the harmonic trap potential can be detected by analyzing the transmitted laser light. Once the force constant ("trap stiffness") has been determined in a convenient one-click trap calibration, the displacement data can be converted into units of force. In our fully calibrated NanoTracker™ system, force and displacement of the particle as well as trap position information are recorded at the same time making the relation between force and deformation readily accessible in the acquired data.

This application note describes different types of micro-rheological measurements that can be performed with the NanoTracker™ optical tweezers system, and the basic principles of their analysis using the integrated data processing software.

For more detailed theoretical background information, please have a look at our technical note "Micro-Rheology Measurements with the NanoTracker™, which you can find on www.jpk.com.

Viscosity measurements

To determine the viscosity of a fluid, a suspended, micron-sized spherical particle is optically trapped. Unlike most standard calibration routines, the advanced method implemented in the NanoTracker™ control software allows determination of the trap stiffness without knowing the mechanical properties of the sample – a prerequisite for micro-rheological measurements. The trapped particle is moved relative to the fluid with a known velocity v (flow velocity), which results in a viscous drag force F_d that depends on the fluids viscosity η following the Stokes' equation:

$$F_d = 6\pi\eta r v \qquad (1)$$

where r is the radius of the sphere. According to equation (1), the force is directly proportional to the relative (flow) velocity between fluid and particle. Figure 1 A shows the measured drag force on a $2\ \mu m$ polystyrene (PS) bead as a function of flow speed and, as expected, the linear relationship is well reproduced. The slope can be written as

$$\frac{F_d}{v} = 6\pi\eta r \qquad (2)$$

and thus

$$\eta = F_d / 6\pi r v \qquad (3)$$

Figure 1 B shows the measured viscosities of different glycerol-water mixtures at 40°C. The data was acquired using two different calibration methods. First, measurements were performed after the trap had been calibrated in pure water using the standard thermal noise calibration method. In many tweezers systems, this is the only available calibration method, the disadvantage being that the viscosity of the medium must be known to calculate the trap stiffness. Thus, this type of calibration is not suitable for solutions with unknown viscosities. The trap stiffness (among other parameters) depends on the

refractive index of the fluid that is used. For lower glycerol concentrations, the refractive index of the mixture is sufficiently close to that of water. So the trap stiffness measured in water is close to that in the mixture and the measured viscosities agree very well with literature values.

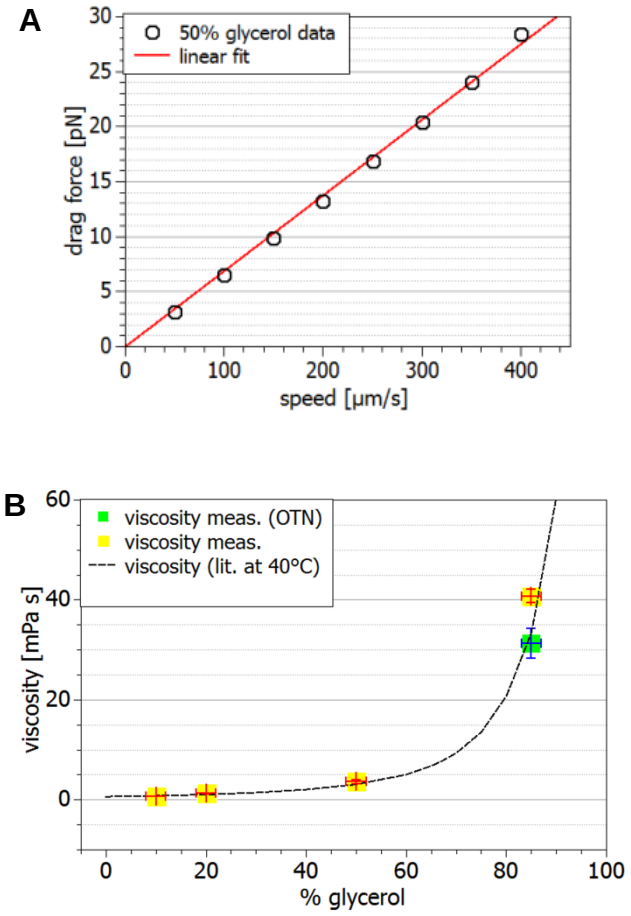

Figure 1 Viscous drag measurements in glycerol water mixture. A *The viscous drag force F_d is measured at different flow speeds v. The red line represents a fit of the linear relation of the Stokes equation (1). With this, the viscosity η can be derived.* ***B*** *Measurements of η for water mixtures with increasing glycerol content. The yellow squares represent measurements based on a trap calibration in water. For low glycerol content, these agree well with literature values. At high glycerol content, the oscillating thermal noise method (green square) provides better results as it can be performed in the fluid under investigation and is independent of the fluid viscosity.*

For 85% glycerol, however, we find a deviation due to the change in refractive index. For this concentration, an additional measurement with the so called *oscillating thermal noise* (OTN) calibration method was performed directly in the 85% glycerol mixture. This medium-independent method is integrated in the NanoTracker™ control software and is the only suitable method to perform reliable viscosity measurements. The result of the second measurement much better reproduces the expected value (deviation from literature value reduced by a factor of 3).

Elasticity measurements

Elastic materials deform reversibly under the influence of external stress. Thus, the well–defined application of forces to these materials and simultaneous monitoring of their deformation is the method of choice for determining the mechanical properties. For complex materials like biological and other very soft matter, the precise application and measurement of the smallest forces is of great importance for reliable results. Microscopic strains in the range of less than one micrometer require high resolution in force and deformation sensing in order to obtain the desired force-deformation relation.

The NanoTracker™ offers multiple options for the nanometer-precise positioning of trapped particles and surface-bound samples. In combination with high speed force detection in the 0.1…500pN range, the optical tweezers platform is well-suited for this type of analysis. During the measurement, a trapped particle was moved towards the sample surface. After they came in contact, the sample was indented and the force-indentation curve recorded. The relative position of the sample and bead was calculated from the known position of both (trap: calibrated beam deflection system / sample: closed-loop piezo scanner) and the measured position of the bead inside the trap. Figure 2 shows a schematic of the setup.

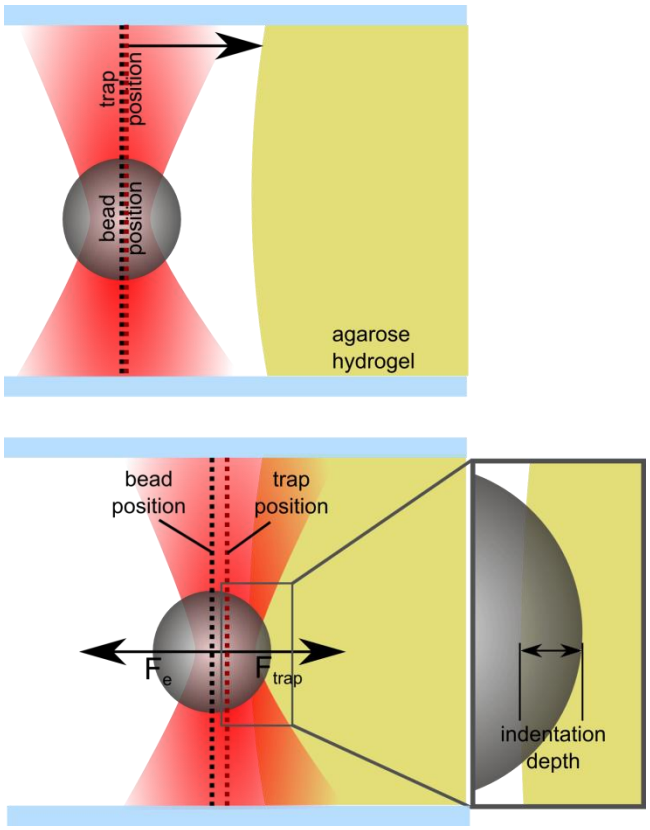

Figure 2 Hydrogel indentation measurement. *A trapped bead is used to indent the hydrogel. After it contacts the surface, a force is exerted and the trap and bead position no longer coincide. For data analysis purposes, this shift has to be considered in order to calculate the correct indentation depth.*

The indentation axis in Figure 3 represents the distance between the bead and the agar surface. For indentation values >0, the data can be fitted using the Hertz model which considers the geometry of the indenter and the resulting change in contact area [1]. So the typical process of indentation data analysis is as follows:

Data acquisition
↓
Calculation of the bead-sample distance
(JPK Data Processing software)
↓
Fit with Hertz model
(JPK Data Processing software)

The Hertz model is applied to describe the indentation of an elastic half-space and a sphere. These assumptions can be made for sample surfaces and volumes much bigger than the bead used for the indentation. It relates force F and indentation depth δ as follows:

$$F(\delta) = -\frac{4\sqrt{R}}{3}E^*\delta^{\frac{3}{2}} \qquad (4)$$

where E^* is the effective modulus of the bead-sample system and depends on the Young's moduli E of the bead and sample and their Poisson ratios v. For beads much harder than the sample, it reduces to

$$E^* \approx \frac{E_{sample}}{1 - v_{sample}^2} \qquad (5)$$

For elastic materials, the Poisson ratio v is typically set to 0.5.

The green curve in Figure 3 represents a Hertz fit used to calculate the Young's modulus E of a water-swollen agarose hydrogel.

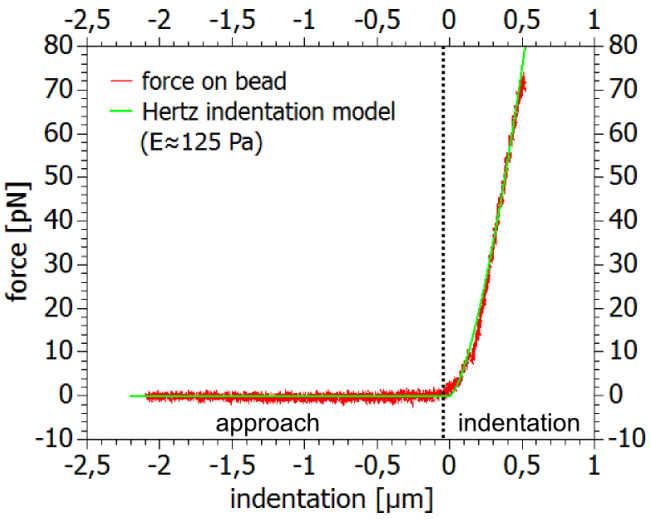

Figure 3 Elasticity measurements using the Hertz model. The measured force-indentation data (red curve) was fitted with the Hertz model (green) taking into account the shape and size of the indenting particle. The calculated Young's modulus E for the sample is 125 Pa.

Viscoelastic systems

Soft matter, biological materials in particular, usually require more advanced analysis methods than those

previously described, to fully capture their complex mechanical properties. Composite materials like polymer solutions, whole cells or their components (e.g. the cytoskeleton, cytosol or cell membrane) typically combine viscous and elastic contributions to a complex, rate- (or frequency-) dependent mechanical response to external mechanical perturbations. In these cases, the material response to linear deformation at one given rate does not yield enough information to characterize the mechanical properties adequately. The NanoTracker™ optical tweezers system utilizes two different approaches for investigating load rate or frequency dependent mechanical properties, so called *passive* and *active* microrheology. Both use optically trapped particles embedded in the material of interest as force sensors.

Passive micro-rheology

In the passive case, the power spectral density (PSD) of the trapped particle's Brownian motion is analyzed. The thermal noise of the position signal is the superposition of fluctuations at different frequencies and thus inherently yields information about the frequency-dependent material properties. For this measurement, the laser power is adjusted to the lowest possible level in order to minimize the influence of the trapping laser on the particle's fluctuations. Nevertheless, for the calculation of the elastic component, the trap stiffness has to be taken into account as it introduces an additional term into the effective elasticity of the system. The high speed data acquisition of the NanoTracker™ system allows recording of the PSD for frequencies of up to several MHz. Details of the mathematical analysis can be found in publications by MacKintosh, Schmidt et al. [2,3] Brau et al. [4] and others.

Active micro-rheology

The active method uses a periodic external force on the sample that is applied at different frequencies to capture the load dependent material mechanics. The trap holding the particle is oscillated in a sinusoidal movement with an amplitude of typically several hundred nanometer. This is repeated at different frequencies while the position of the trap and bead, as well as the force acting on the bead, is recorded. The behavior at a given frequency is characterized by the phase shift between the trap and bead movement, and the trap movement and force, respectively. Figure 4 shows the data for a slow oscillation (10 Hz) of a 2 µm polystyrene bead in 1% (w/v) methylcellulose solution.

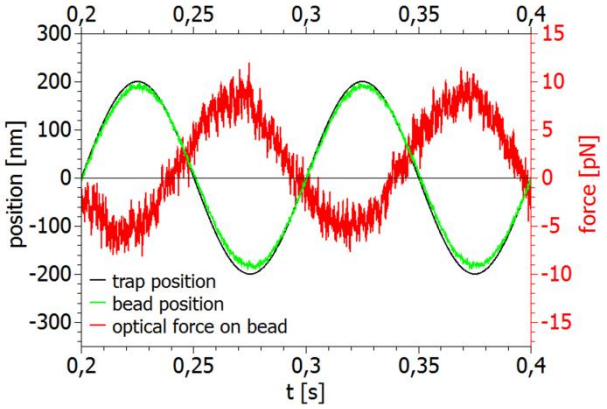

Figure 4 Active micro-rheology. *A trap holding a PS bead performs a sinusoidal movement with $f = 10\,Hz$ and $200\,nm$ amplitude. The oscillations of the trap (black) and bead (green) position, as well as the force (red), are phase shifted. The magnitude of the shift changes with increasing frequencies and is characteristic for the viscoelastic properties of the fluid.*

The measurement was repeated for a number of frequencies and the phase shift data was extracted using the built-in sine fit function of the JPK data processing software. The phase shift difference $\Delta\theta = \theta_b - \theta_f$ relates the phase shifts to the elastic (storage) modulus G' and the viscous (loss) modulus $G"$:

$$\Delta\theta(\omega) = \theta_b(\omega) - \theta_f(\omega) = \tan^{-1} G"(\omega)/G'(\omega) \quad (6)$$

The angular frequency dependence of $\Delta\theta$ of the same solution is displayed in Figure 5. For lower frequencies of up to approx. 50 Hz (~300 rad), the polymer solution behaves like a classical Newtonian fluid with predominantly viscous properties. At higher frequencies and thus loading rates, the polymer chains in the solution disentangle and the effective viscosity drops (shear thinning). The active rheology method has been successfully applied to purified polymer solutions [2,4] as well as to the inside of living cells [5].

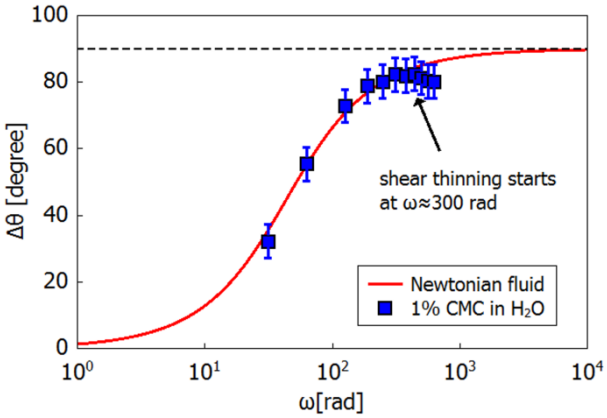

Figure 5 Frequency dependence of the phase shift in 1% carboxy-methylcellulose (CMC) solution. *The phase difference Δθ increases with increasing (angular) frequencies. At approx. 300 rad, the behavior deviates from that of a purely viscous Newtonian fluid, due to the shear thinning effect. The filaments in the polymer solution disentangle at these rates, which influences the mechanical properties of the material.*

Whole cell rheology

The active micror-heology method has also found application in the evaluation of whole cell mechanical properties. For most cell types, these are closely related to cellular function and deviations can lead to severe diseases. In particular the mechanics of red blood cells (RBCs) that have a relatively simple structure - compared to tissue cells - and their relation to malfunctions have been studied with optical tweezers systems [6–9]. The method we present here applies two beads attached to opposite points on the cell edge (see Figure 6 A). One trap fixed at a constant position was used to measure the force and displacement only, while the second trap (and bead) oscillated with constant amplitude and increasing frequencies. As shown in Figure 6 B, the amplitudes of trap, bead 2 (oscillating trap) and bead 1 (stationary trap) differ depending on the deformation of the attached cell. The phase shift between the movement of bead 1 and 2 can also be ascribed to this dynamic behavior.

In analogy to the rheological measurements in viscoelastic fluids, the oscillation frequency was increased in order to determine load rate dependent mechanical properties of the RBC. Figure 7 A displays the force-elongation data recoded for 100 deformation

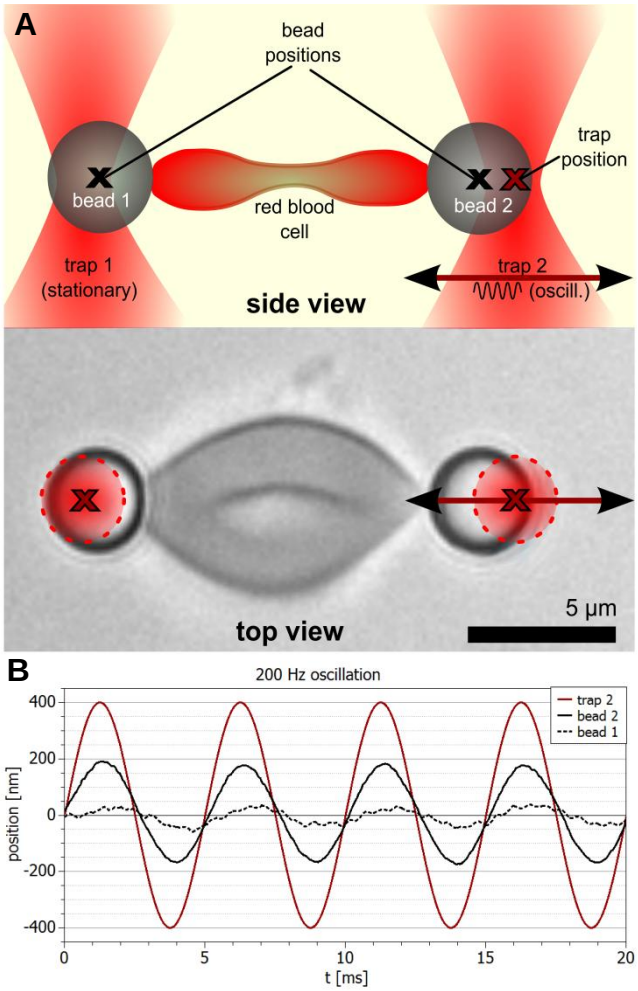

Figure 6 Active cell rheology. A *Two trapped particles in a dual beam setup are attached to the opposite sides of a red blood cell (RBC). One trap is held at a constant position while the other oscillates at increasing frequencies. Measurements were performed in a glass bottom Petri dish at 37°C using the JPK PetriDishHeater™.* ***B*** *Recorded data of the oscillating trap and bead positions at f =200 Hz. The amplitude of the bead opposite to the driving force is much smaller and time shifted due to the dynamic mechanical deformation of the cell.*

cycles at three discrete frequencies, 50, 200, and 450 Hz. While the amplitude of the trap oscillation was constant for all three measurements, the maximum diameter of the RBC decreased with higher frequencies, as the bead in the oscillating trap was not able to follow

the fast movement of the trap through the full cycle. In addition to the cells slow deformation, the viscous friction between the bead and the buffer solution influences the bead motion.

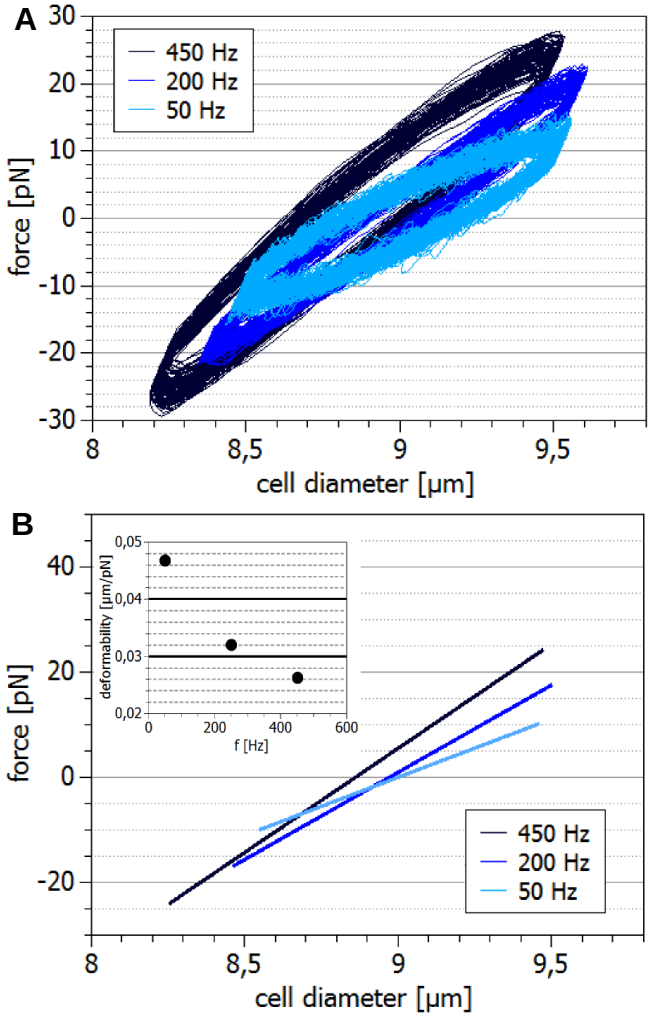

Figure 7 Frequency dependent deformability of RBCs.

A *Deformation cycles at different oscillation frequencies show that the maximum cell diameter as well as the force-elongation relation depends on the applied frequency. The cells were deformed 100 times per frequency.* ***B*** *The slopes of the deformation curves directly represent the cell's deformability. As shown in the insert, it decreases with increasing oscillation frequencies.*

The different slopes of the force-deformation curves shown in Figure 7 B indicate that at higher loading rates, RBC effectively become increasingly stiffer, i.e. the deformability decreases (see inset in Figure 7 B).

A thorough analysis of the data requires a correction term that considers the aforementioned interaction between the trapped particles and the surrounding viscous fluid in order not to over-estimate the mechanical response of the RBC.

Conclusion

With micro-rheological measurements we gain insight into the fundamental processes that contribute to a material's mechanical behavior on microscopic scales. While the data analysis for simple systems dominated by either purely viscous or elastic behavior is rather straightforward, viscoelastic matter requires more in-depth analysis taking the special features of the individual sample into account. In a biological context, local mechanical properties are often related to specific functions including signaling, growth and tissue development.

Being a highly developed optical tweezers system, the NanoTracker™ is particularly suitable for small scale samples like biological cells or other very soft matter since it provides the required sensitivity and resolution in time, force and position measurements. JPK has implemented a toolbox of micro-rheological methods for convenient use in terms of hard- and software, as well as environmental control options for delicate samples, including living cells.

Literature

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