Micro-Rheology Measurements with the NanoTracker™

JPK's NanoTracker™ optical tweezers system is a versatile high resolution force measurement tool. It is based on the principle of optical trapping and uses the interaction between light and refractive particles to generate and measure forces on these particles in the range of a single piconewton to hundreds of piconewton (pN).

Among many applications including single molecule force spectroscopy and biomechanical measurements of individual cells and their components, the NanoTracker™ provides excellent options to probe the mechanical properties of all kinds of materials on a local, microscopic scale and to relate their viscous, elastic, and especially viscoelastic contributions to their macroscopic behavior. This field of research is termed *micro-rheology* (from Greek: 'rheo' – to flow) and finds wide applications in material science and soft matter physics. It complements the results gained from macro-rheological measurements using larger volumes of material and forces as well as comparatively slow deformation rates.

Optical tweezers as a force measurement tool

In an optical tweezers system, a tightly focused laser beam generates a local intensity gradient of light. A refractive particle entering this gradient experiences a force that moves it to the focal region of the beam where the intensity is the highest and the distribution has rotational symmetry (*gradient force*). In this focal spot, the net force is zero and the particle is stabilized in three dimensions. As soon as external forces act on the particle (e.g. the Brownian interaction between the particle and the molecules of the surrounding fluid), it is displaced from the trap center. The optical trap acts like a mechanical spring and pulls the particle back to the trap center with the gradient force. For small displacements, this force is directly proportional to the particle displacement (harmonic potential). The advanced detection system of the NanoTracker™ can detect these displacements and forces with a sensitivity of single nanometers (nm) and piconewtons, respectively, at microsecond (µs) time resolution.

Micro-rheological measurements

As the name suggests, rheological measurements aim to analyze and quantify the "flow" of fluids and soft materials under the influence of external forces. In general, these forces are applied at different loading rates or oscillation frequencies. Three classes of matter can be distinguished: viscous, elastic, and viscoelastic materials. While **viscous fluids** (e.g. water) permanently change their shape under external force, **elastic materials** (e.g. cross-linked polymers, rubber) temporarily deform and resume their original shape when the external force vanishes (high forces will cause long-term plastic deformation or rupture of these materials). **Viscoelastic materials** (e.g. polymer hydrogels) show characteristics of both, depending on the loading rate, i.e. the speed of the mechanical probe. Slow deformation ($\omega \rightarrow 0$) results in a different material response than a fast application of force. The characteristics of this frequency-dependent behavior depend on the microscopic organization of the material (e.g. polymer size, cross-linking, etc.), and thus rheological analysis can give insight into its internal structure.

There are four major types of micro-rheological measurements that can be performed with optical tweezers:

(i) Viscosity measurements quantify the viscous drag force on a spherical particle that is moving relative to a fluid. The analysis is based on the Stokes equation and allows determining the viscosity of a fluid.

(ii) Elasticity measurements quantify the Young's modulus of an elastic material. The sample is indented with a micrometer-sized particle and the force-indentation curve is analyzed to calculate mechanical properties.

(iii) *Passive* viscoelastic measurements analyze the Brownian motion of a particle in a viscoelastic fluid. The Brownian motion inherently covers a wide frequency spectrum and thus contains information about the mechanical response of the surrounding fluid for different loading rates. The power spectral density (PSD) of the Brownian motion is analyzed to derive information about the frequency-dependent viscous and elastic modulus.

(iv) ***Active*** **viscoelastic measurements** use a well-defined periodic force to deform the material with different frequencies. In particular, the trap holding a particle embedded in the material of interest is oscillated resulting in a sinusoidal force. The response (motion) of the particle to the force is recorded. From the phase difference between the trap movement, the force and the particle motion measured at different driving frequencies, the frequency-dependent ratio between storage (elastic) and loss (viscous) modulus of the material can be calculated.

Micro-rheology data acquisition with the NanoTracker™

The NanoTracker™ control software supports the previously described micro-rheology measurements as well as their analysis and facilitates the workflow with one-click trap calibration and automated data acquisition.

Trap calibration

A proper calibration of the trap characteristics is crucial for the reliable evaluation of mechanical material properties. The standard calibration method implemented in the NanoTracker™ control software is based on the analysis of the thermal noise signal resulting from the thermally driven Brownian motion of the particle. This in turn depends on temperature, particle size and the medium viscosity which need to be known in order to receive proper calibration results. For micro-rheological measurements of fluids with unknown mechanical properties, this method is only of limited use since viscosity is one of the quantities the experiment is supposed to determine. This is why we implemented an advanced calibration method that combines the analysis of the particle's response to periodic external forces with that of the thermal motion. As a result, the system's detection sensitivity as well as the force constant α (trap stiffness) can be determined independent of medium viscosity or size of the particle.

Once trap stiffness and detection sensitivity have been determined, the different rheological measurements can be performed.

(i) For **viscous drag measurements**, the trapped particle is moved with constant velocity through the fluid of interest using the "absolute" mode of the force spectroscopy software tool. This is achieved either by moving the trap holding the particle or by moving the sample stage using the integrated x/y/z piezo scanner (Figure 1). In both cases, velocity and displacement can be set independently. After successful trap calibration, the viscous drag force acting on the particle can be measured directly (Figure 2).

The data analysis is straightforward: The Stokes' equation allows direct calculation of the viscosity η from the measured drag force F_d when the velocity v and the radius r of the used particle are known.

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

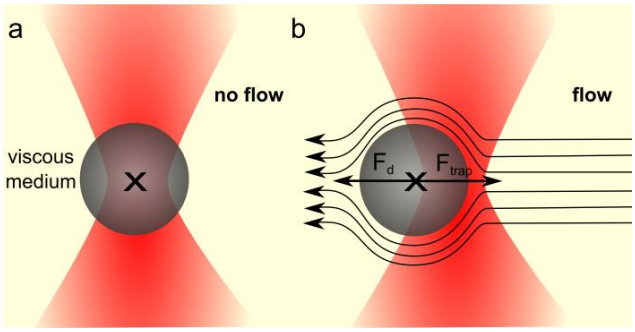


Figure 1 a) *Principle of a viscosity measurement.* ***b)*** *Motion of the fluid surrounding a trapped particle generates viscous drag resulting in the displacement of the particle. The (Stokes') drag force can be measured with high accuracy and with known parameters like bead diameter and relative velocity, the viscosity of the fluid is calculated.*

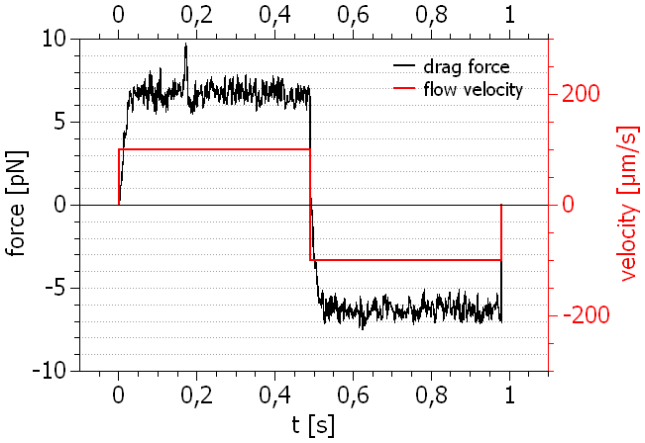


Figure 2 Viscous drag measurement *in 1:1 glycerol-water mix. The flow of* $\mathbf{100\ \mu m/s}$ *is generated by moving the sample with a piezo scanner. With a bead of diameter* $\boldsymbol{d = 2\ \mu m}$ *the viscosity calculated with equation (1) is* $\boldsymbol{\eta \approx 3.44\ mPa\ s}$

(ii) The **elastic deformation** of a material is achieved by indenting the object with a trapped micro-bead. Again, the NanoTracker™ offers multiple options to generate the relative motion. Either the sample or the trap can be moved with high accuracy to create reproducible conditions and to acquire reliable data. Before the bead and sample come in contact, the particle only performs thermal fluctuations around the stable trapping position (

Figure 3, top). At the low velocities typically used ($v \approx 0.5 \ldots 2\ \mu m/s$), the fluid drag force can be neglected. As soon as it starts indenting the sample, it is displaced from the trap center due to the elastic restoring force of the material. Then the trap position is no longer identical with the average particle position.

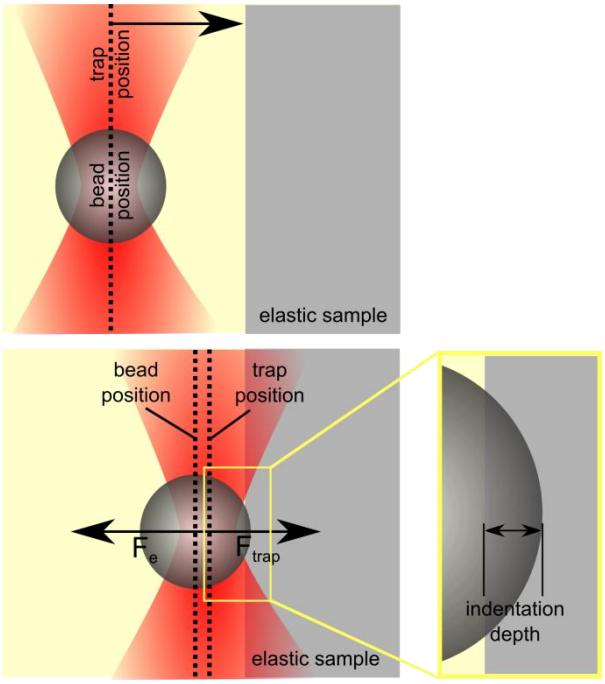


Figure 3 Indentation Measurement

Upper panel: The trapped particle is brought to the surface of an elastic sample. Lower panel: As soon as the particle deforms the sample, it is displaced from the trap center and the force can be detected. The maximum indentation depth is typically <10% of the particle diameter. From the relation between indentation and force, the Young's modulus of the material can be calculated.

When analyzing the force-indentation curves of such measurements, it is important to use the correct bead-sample distance which is a combination of the trap position and the displacement of the bead inside the trap. Since indentation depths (see Figure 3 bottom) are in the same order of magnitude as the displacement of the bead from the trap center (or even smaller), it is of great importance to apply this correction. JPK's data processing software can take this automatically into account eliminating a potential source of error.

The corrected force-indentation curve can then be fitted easily with the Hertz model to calculate the Young's modulus E of the sample material [1]:

$$F = \frac{4}{3}\frac{E}{1-\nu^2}\sqrt{R}\,(\delta - \delta_0)^{3/2} \qquad (2)$$

where δ is the indentation depth relative to the contact point δ_0, R is the particle radius and ν is the material's Poisson's ratio ($\nu = 0.5$ for incompressible materials).

Also advanced measurement modes like the rate-jump method are conveniently integrated in the NanoTracker™ control software. Here, the load rate (i.e. the indentation speed) is varied and the analysis delivers a consistent, load-rate normalized elastic modulus that better reflects the intrinsic mechanical properties of the sample [2].

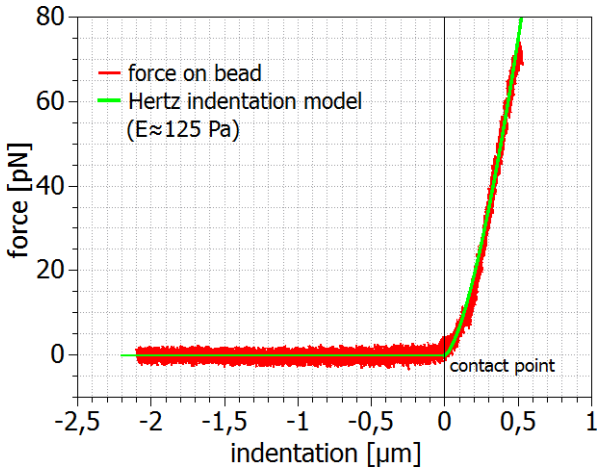
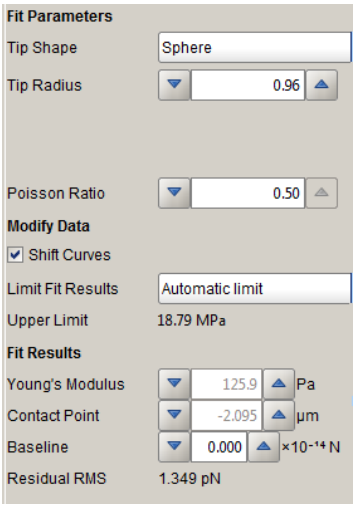


Figure 4 a) Force-indentation curve of a soft agarose gel. *After the particle has contacted the surface (indentation = 0), the deformation starts. The positive part of the curve was fitted with a Hertz model (green line) to calculate the material's Young's modulus, E.*



b) *Fit control in the NanoTracker™ data processing software. With only few intuitive parameters, the force-deformation curve can be approximated with a Hertz fit delivering* $E \approx 125\ Pa$ *for the investigated agarose gel (see equation (2)).*

(iii) In **passive viscoelasticity measurements**, a low-power trap at a constant position is used to monitor the particles Brownian motion (

Figure 5). The fluctuations of the trapped bead in this configuration depend on multiple factors: the temperature and the resulting thermal force,F_{th}, the trap stiffness, α, and the viscoelastic properties of the surrounding medium.

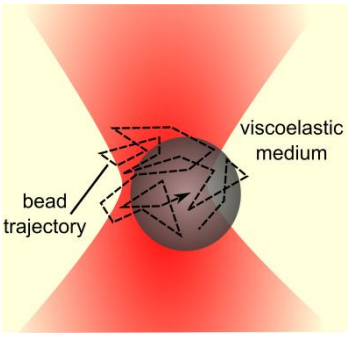


Figure 5: Passive rheology measurement. *The trajectory of a particle under the influence of collisions with thermally accelerated fluid molecules (Brownian motion) is recorded.*

During the measurement, the bead position signal $x(t)$ is recorded for a certain time, T. The power spectral density (PSD, see Figure 6) $S(\omega)$ of the particle motion is calculated via

$$S(\omega) = \lim_{T\to\infty} \frac{2}{T}\left|\int_0^T x(t)e^{i\omega t}dt\right|^2 \qquad (3)$$

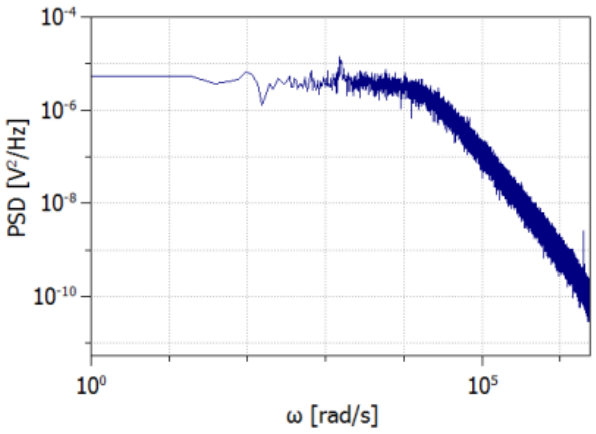


Figure 6 Power spectral density *(PSD) of a trapped particle's Brownian motion along one direction. The fast electronics in the NanoTracker™ controller allow the detection of particle oscillations up to an angular frequency of* $\boldsymbol{\omega \approx 2.4\ x\ 10^6\ rad}$ $\boldsymbol{(f \approx 380 kHz)}$. *For single spectra, frequencies of up to* $\boldsymbol{3.5\ MHz}$ *can be achieved.*

The Fourier transform of the particle motion $x(t)$ and the thermal force $F_{th}(\omega)$ are related via the complex transfer function $h(\omega) = h'(\omega) + ih''(\omega)$ [3]:

$$x(\omega) = \frac{F_{th}(\omega)}{6\pi r G^*(\omega)} = h(\omega)F_{th}(\omega) \qquad (4)$$

where $G^*(\omega)$ is the complex shear modulus $G^*(\omega) = G'(\omega) + iG''(\omega)$ and ω is the angular frequency. G' and G'' are the storage (elastic) and loss (viscous) moduli of the material.

The real and imaginary parts of the complex transfer function h can be determined using the dissipation-fluctuation theorem [4,5] and the Kramers-Kronig dispersion relation [5,6], respectively.

Equation (4) can then be solved to find the real and imaginary part of the complex shear modulus, G^*. Here one has to take into account that the trap itself adds a storage modulus to the system that depends on the particle radius r and the trap stiffness, α. Thus the material modulus G' has to be calculated as follows:

$$G' = G'_{obs} - \frac{\alpha}{6\pi r} \qquad (5)$$

Finally, the empirical Cox-Merz [7] rule can be used to relate a viscoelastic medium's G' and G'' to its complex viscosity:

$$\boldsymbol{\eta^* = \left[\left(\frac{G'(\omega)}{\omega}\right)^2 + \left(\frac{G''(\omega)}{\omega}\right)^2\right]^{1/2}} \quad (6)$$

The above analysis of the thermal particle fluctuations depends in many details on the conditions in the sample and the formula shown here can only depict the general principle of the method. JPK's control software delivers the raw data at the highest possible sample rate in a convenient text file format. For a thorough analysis, more aspects of the investigated material need to be considered and we thus recommend further reading in respective publications describing analysis routines for different samples to implement custom-tailored solutions for individual experiments. These includecytoskeletal polymers [8], filamentous virus solutions [9], and different types of polymeric hydrogels [3].

(iv) In **active rheology measurements**, the periodic force on the particle generated by the oscillating trap causes an oscillation of the bead. The trap movement is described by

$$x_t(t) = A_t \sin(\omega t) \quad (7)$$

where A_t is the amplitude of the oscillation.

The phase of the bead movement is shifted against the trap movement (illustrated in

Figure 7, data shown in Figure 8 a) and can be approximated by

$$x_b(t) = A_b \sin[\omega t - \theta_b(\omega)] \quad (8)$$

with A_b the amplitude and $\theta_b(\omega)$ the phase of the bead response relative to the trap motion x_t.

Using the trap stiffness α that was determined by previous calibration of the trap, the periodic force on the particle can be calculated:

$$f(t) = \alpha[x_t(t) - x(t)] = F sin[\omega t - \theta_f(\omega)] \quad (9)$$

where F is the magnitude of the force, $x(t)$ is the displacement of the particle inside the trap and θ_f is the resulting phase of the force relative to x_t [3].

The frequency dependent phase angle difference

$$\Delta\theta(\omega) = \theta_b(\omega) - \theta_f(\omega) \quad (10)$$

can now be used to determine the relative contributions of the loss and storage moduli via

$$\Delta\theta(\omega) = \arctan\left[\frac{G''(\omega)}{G'(\omega)}\right] \quad (11)$$

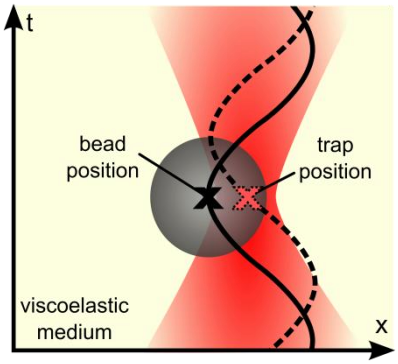


Figure 7 Trap oscillation method. *The trap position is modulated with a sinusoidal signal which results in a periodic force on the trapped particle. The response (movement) of the particle depends on the frequency of the modulation and the viscoelastic properties of the surrounding material.*

The NanoTracker™ data processing (DP) software supports the phase analysis of oscillation measurements with a user-friendly sine fit feature. Any data recorded (e.g. trap position, bead displacement and force) can be fitted easily with a sine function to get information about amplitude and phase of the measured curve at a glance (Figure 8 b). This facilitates the analysis of rheological sample properties using the active micro-rheology approach. The example curves in Figure 8 were recorded with a trapped polystyrene bead ($d = 2\ \mu m$) in 1% methyl cellulose solution. The trap position was oscillated with a frequency of $f = 10\ Hz$ and amplitude, $a = 200\ nm$. The phase shift θ_b between the bead and trap positions is approx. $16°\ (0.28\ rad)$.

Together with the phase shift between force and trap position θ_f(data not shown), the ratio G''/G' can be determined using equation (11). We find $\Delta\theta \approx 0.96\ rad$ and $^{G''}/_{G'} = \tan\Delta\theta \approx 1.45$ which indicates that for this particular methylcellulose concentration and driving frequency the loss modulus G'' (related to the viscosity) slightly dominates the response of the system.

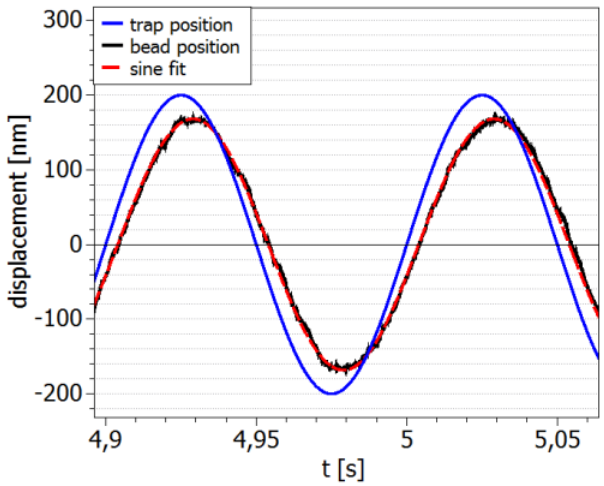
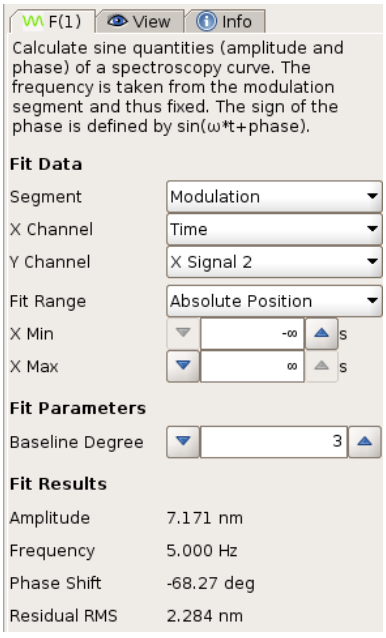


***Figure 8 a) Sinusoidal movement of the trap and the delayed bead response in 1% methylcellulose solution**. In active rheology measurements the trap position (blue curve) is oscillated at different frequencies (here: $\boldsymbol{f = 10Hz}$). The bead movement is phase delayed due to the mechanical interactions between the particle and the viscoelastic fluid. The red dashed line is a cosine fit to the bead movement that shows a phase delay of approx. $\boldsymbol{0.28\ rad}$ against the trap position.*



b) Sine fitting in the NanoTracker™ data processsing software.
With the advanced fitting routines signal offsets and baseline drifts can be captured and corrected easily. The baseline can be fitted with a polynomial of up to 9th degree. The Fit Results section shows all fitting parameters and a quantification of the fit quality residual root mean square, RMS.

Conclusion

Optical tweezers technology offers numerous approaches to investigate the microscopic rheological properties of small soft samples. These are of great interest in the field of material science and in biologic and biophysical research since the function of biological systems is often closely related to the local mechanical conditions. In JPK's NanoTracker™ system, the workflow of both, the measurement of relevant data as well as the extraction of micro-rheological information, is conveniently implemented in the control and data processing software. With the available sample handling options like the PetriDishHeater™ and the temperature controlled microfluidic integration (LaminarFlowCell™), temperature dependent rheology measurements of biological and other materials become easy and a statistically meaningful amount of data is generated in short time.

References

[1] K. Johnson, *Contact Mechanics* (Cambridge University Press, 1987).
[2] B. Tang and A. H. W. Ngan, Soft Matter **8**, 5974 (2012).
[3] R. R. Brau, J. M. Ferrer, H. Lee, C. E. Castro, B. K. Tam, P. B. Tarsa, P. Matsudaira, M. C. Boyce, R. D. Kamm, and M. J. Lang, J. Opt. Pure Appl. Opt. **9**, S103 (2007).
[4] L. D. Landau, E. M. Lifshitz, and L. P. Pitaevskii, *Course of Theoretical Physics* (Butterworth-Heinemann, Oxford, 1980).
[5] F. Gittes, Phys. Rev. Lett. **79**, 3286 (1997).
[6] K. Ohta and H. Ishida, Appl. Spectrosc. **42**, 952 (1988).
[7] O. Manero, F. Bautista, J. F. A. Soltero, and J. E. Puig, J. Non-Newton. Fluid Mech. **106**, 1 (2002).
[8] B. Schnurr, F. Gittes, F. C. MacKintosh, and C. F. Schmidt, Macromolecules **30**, 7781 (1997).
[9] K. M. Addas, C. F. Schmidt, and J. X. Tang, Phys. Rev. E **70**, 021503 (2004).