# Live cell measurements with the JPK NanoTracker™ optical tweezers platform and ibidi®* µ-Slides

The precise measurement of the small scale forces acting within living cellular systems have gained increasing interest in a growing community of biologists, bio-physicists and medical researchers. These measurements include the quantification of intracellular transport processes, as well as the micro-mechanical characterization of living cells and tissues. In combination with ibidi's well-established microfluidic µ-Slide technology for small volume cell culture and high quality imaging, JPK's NanoTracker™ optical tweezers platform provides a wide range of live cell applications.

## The NanoTracker™ system

The optical trapping technique relies on the transfer of momentum from light entering a refractive particle to the particle itself. Focused laser light generates a symmetric potential for stable three-dimensional trapping of nano- to microscale objects. Highly sensitive detection systems enable the measurement of the forces acting on the trapped objects down to single piconewtons. JPK's NanoTracker™ optical tweezers platform offers a multitude of configurations, ranging from basic microparticle and cell manipulation to the precise detection of forces and displacements in the piconewton and nanometer range, at highest resolution and sampling rates. Dual-beam and trap multiplexing options allow complex experimental designs and JPK's sophisticated, user-friendly control software facilitates workflow automation.

## ibidi µ-Slides

With their product range of µ-Slide flow chambers, ibidi has developed high quality equipment for live cell experiments and high-resolution imaging, including all kinds of standard and super-resolution fluorescence techniques. Easy handling and small channel volumes strongly reduce the consumption of costly material and establish highly reproducible experimental conditions. The superior optical properties and biocompatibility of the µ-Slide product family makes them suitable for state-of-

***Figure 1 Top left:*** *ibidi Heating System with Temperature Controller, Heated Plate and Heated Glass Lid.* ***Top right:*** *ibidi Gas Incubation System with Gas Mixer and Humidifying Column.* ***Left:*** *Ibidi µ-Slide Luer*

the-art live cell experimental techniques, including optical tweezers for manipulation and force measurements.

## Experimental setup

The NanoTracker™ uses a tightly focused laser beam to generate the strong intensity gradients required for a stable trapping potential. Therefore, objectives with a high numerical aperture (NA) of 1.2 to 1.4 are typically used. Strong focusing inherently limits the working distances of these objectives to less than 300 µm. This in turn demands thin, planar and optically homogenous sample substrates like the µ-Slide product family.

During force and displacement measurements, the light that passes through the trapped object is collected with a second objective and projected onto a detection system comprising of one quadrant photo diode (QPD, for x/y detection) and one high sensitivity photo diode (for z detection) for each trap. In order to focus through the



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thicker top cover of the chamber, a water dipping objective with a working distance of 2.2 mm has been used with ibidi's µ-Slide microfluidic channels (see Figure 2).

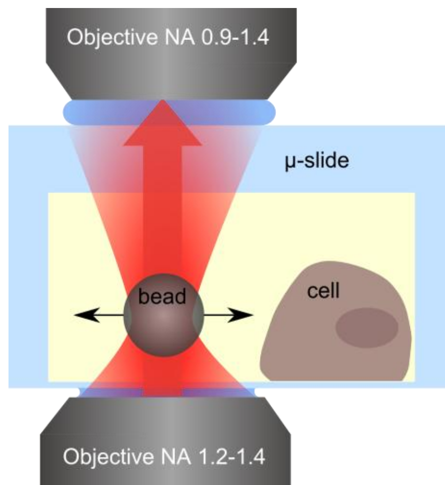


***Figure 2*** *Setup for optical trapping and signal detection with µ-Slide microfluidic channel systems. The laser light (coming from below) is tightly focused, generating a stable optical trap inside the sample channel. After it passes through the particle, the light is collected by a second objective and projected onto a photodiode detection system (not shown).*

## Trap quality and calibration

The stability and strength of an optical trap strongly depends on the quality of the optical components involved. High-end optics employed in the NanoTracker™ laser steering and detection units ensure the superior signal quality and stability required for high-resolution force measurements.

A prerequisite for the detection of forces is a correct calibration of the trap signals based on the thermal fluctuations of the particle in the trap potential. For this purpose, a frequency spectrum of the thermal noise is recorded, which is subsequently fitted by a Lorentz function to determine the detection sensitivity and trap stiffness. Figure 3 shows two spectra of polystyrene beads (d = 1.5 µm). One is recorded in a sample chamber assembled from thickness #1 coverslips, the other is recorded inside an ibidi µ-SlideVI-flat. The direct comparison shows that there is no significant difference in the quality of the spectra and the trap stiffness is even slightly higher in the µ-Slide.

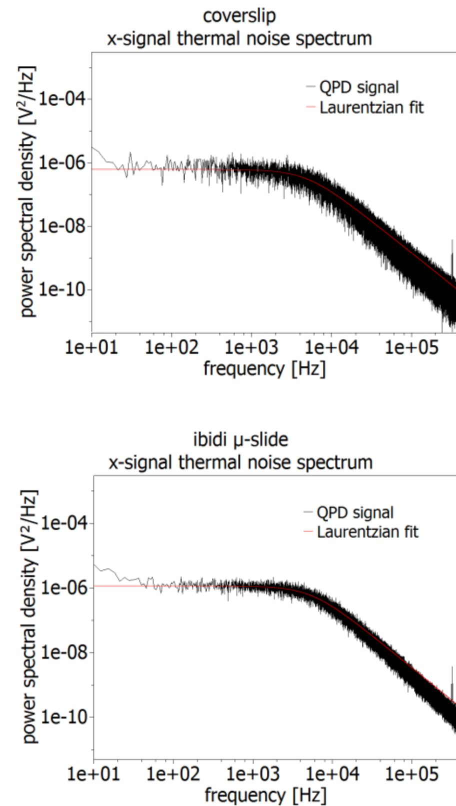


***Figure 3*** *Comparison of thermal noise spectra used for trap calibration with 1.53 µm polystyrene beads. Top: spectrum recorded in a sample chamber made from glass coverslips. Bottom: Same spectrum recorded in a µ-Slide. The characteristics of the spectra are identical in both cases; the calculated trap stiffness is slightly higher in the µ-Slide (0.44 pN/nm) than in the glass chamber (0.40p N/nm). The red curve displays the fit function used for calculating the trap stiffness.*

## Live cell measurements

Most cell types require well-defined and stable environmental conditions, in particular a constant temperature is crucial for most experimental applications. The live cell NanoTracker™ measurements presented in this paragraph were all performed with Madin-Darby Canine Kidney (MDCK) cells in a µ-Slide I Luer mounted on the feedback-controlled ibidi Heated Plate (multi-well



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format) with the suitable insert. The temperature was set to 37°C and held stable throughout the experiments.

Typical force measurements with live cells include so called tether pulling and surface binding experiments, where a functionalized particle is attached to the cell surface and subsequently moved away with the optical trap. During this movement, the breaking of bonds between the bead and surface or the formation of so called membrane tethers or tubes can be observed (schematically shown in Figure 4).

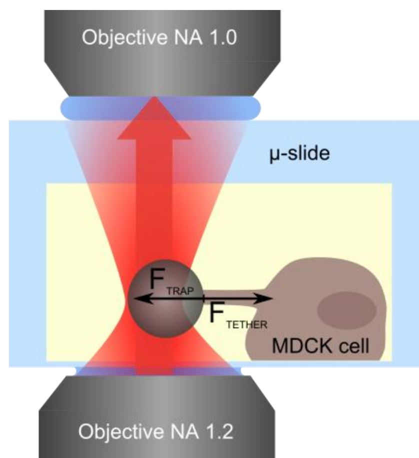


***Figure 4*** *Schematic representation of the tether pulling experiment. A carboxylated bead is optically trapped and attached to the cell surface. By moving the cell away from the trap, a tether can be formed and elongation forces can be measured.*

Figure 5 shows the force curve recorded during a tether pulling experiment. Since the binding of the bead to the cell surface is unspecific in this case (carboxylated bead surface), the exact binding conditions remain elusive. The relatively high force of 240pN indicates the formation of multiple tethers that rupture individually, which is reflected in the steps in the force curve.

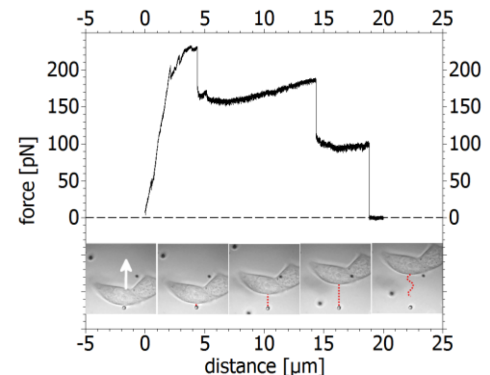


***Figure 5*** *Tether pulling experiment with MDCK cells in a µ-Slide. A carboxylated polystyrene bead (d = 2 µm) is optically trapped and attached to the cell surface. Pulling of presumably multiple membrane tethers from the surface requires a force of approx. 240 pN. The three steps in the force curve correspond to the detachment of tethers or bonds from the bead surface. The micrographs show a series of snapshots (*$\Delta t = 4$ *s) of the experiment: The bead is attached to the cell which is moved away from the trap with a piezo-controlled sample scanner. The red dashed line indicates the assumed position of the membrane tether(s).*

The local mechanical properties of cells can also be investigated by means of optical tweezers. Besides micro-rheological measurements inside cells, indentation experiments are a powerful tool for learning more about the visco-elastic properties of cells and cellular components.

Figure 6 shows a force-distance curve that was recorded while a polystyrene bead was approaching the surface of an MDCK cell. Interestingly, the force acting on the trapped particle increases by more than 1µm just before the bead comes in contact with the cell body, as can be seen in the respective microscopy images. The surface of MDCK cells is typically covered with protrusions termed microvilli that most likely interact with the trapped particle.



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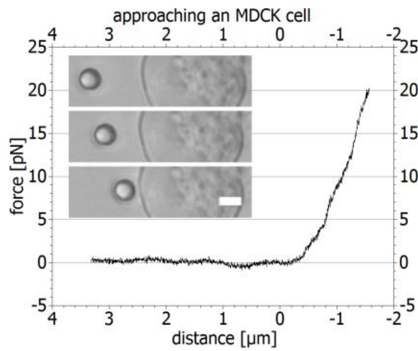


***Figure 6*** *Force-distance curve recorded approaching an MDCK cell with a 2µm polystyrene bead. The increase in force occurs before the bead comes in contact with the main cell body. This indicates interactions of the bead with the extracellular matrix or microvilli that are typically found on the surface of MDCK cells. Indenting this extracellular material by approx. 1.5 µm requires a force of 20pN. Scale bar in the micrograph: 2 µm*

## Conclusion

JPK's versatile NanoTracker™ optical tweezers platform enables the high resolution measurement of extra- and intracellular forces, mechanical properties of different cellular components and the investigation of binding processes in live cell experiments. By using the ibidi Heating System and µ-Slides for microfluidic cell culture that meet the highest optical standards, these measurements can be performed with high reproducibility under well-controlled environmental conditions. By combining expertise from different fields, JPK and ibidi offer a broad range of solutions for the precise measurement of the smallest forces in living cells with an easy-to-use setup for the most complex experimental designs.

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