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JPK BIOAFM Application Note

Atomic Force Microscopy
Automated Force Spectroscopy
Optical Tweezers/Optical Trapping
Cell/Tissue Mechanics & Cell Adhesion

Manipulation of Jurkat T-cell membrane tethers with NanoTracker™ 2 Optical Tweezers

Introduction

Light photons have both linear and angular momentum. This fundamental property plays a key role in the physical phenomenon of optically trapping objects with a highly focused laser beam. The Optical Tweezers (OT) technique utilizes this property of light and allows the direct trapping and manipulation of objects on the scale of several tens of nanometers up to tens of micrometers. Furthermore, OT enable non-invasive, nanometer-precise manipulation of biological samples both *in vitro* and *in vivo*, while simultaneously applying and recording the piconewton forces acting on the measured systems.

The last two decades have seen OT develop from a proof-of-principle experiment to an essential quantitative force spectroscopy method for the analysis of biological samples ranging from single molecules to complex processes, and interactions in living cells [1].

JPK NanoTracker™ 2 Optical Tweezers

For the past decade, the NanoTracker™ has provided researchers worldwide with a versatile, force-sensing OT platform [2]. The latest generation NanoTracker™ 2 system features a highly sensitive and easy-to-use platform, suitable for a wide range of OT applications. Furthermore, its capabilities can be extended to enable novel areas of application (Fig. 1). The hardware specifications and software features make it a particularly powerful research tool for studying living cells, their organelles, and outer membranes, as well as viruses and bacteria [3]. The wavelength of the NanoTracker's trapping laser (1064 nm), which is close to the minimum light absorption spectrum of water, is particularly suitable for the non-invasive, long-term measurement of living cells [4]. The choice of several different laser power levels and fine-tuning intensity control enables researchers to optimise the laser exposure of sensitive cell samples during the experiment.

Fig. 1: *Dual-trap optical tweezers NanoTracker™ 2 combined with Nikon's laser scanning confocal microscope.*

A central feature of the NanoTracker™ 2 is the implementation of an optical layout that ensures compatibility with standard inverted microscopes (all major manufacturers are supported). In this way, it can be reliably combined with the majority of standard optical microscopy techniques including, e.g., epifluorescence imaging [5], differential interference contrast microscopy (DIC) [6], total internal reflection fluorescence imaging (TIRF) [7], and laser scanning confocal microscopy [8] for simultaneous applications.

The force and spatial detection scheme of the NanoTracker™ 2 is based on the well-established back focal plane interferometry method, where fast and sensitive quadrant-photodiodes (QPDs) are utilized to detect bead displacement in the optical trap, reaching 1 nm resolution

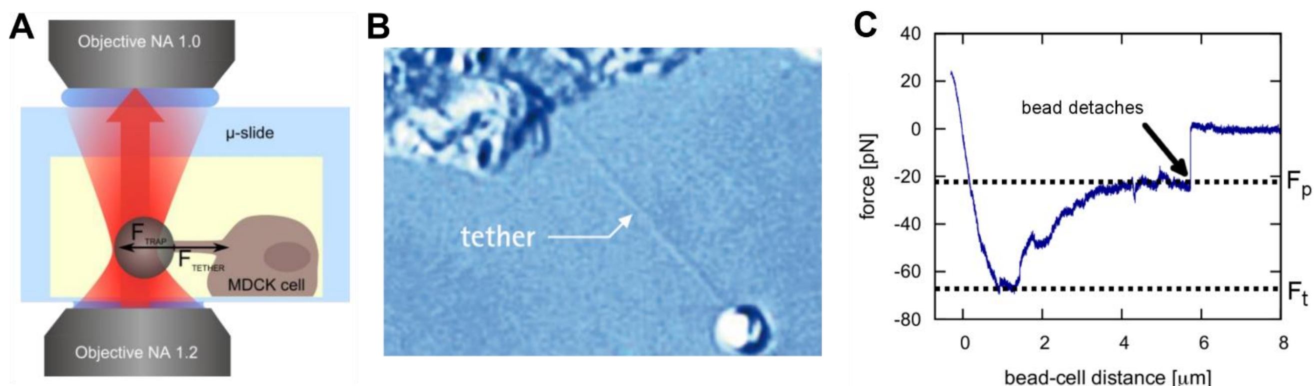

***Fig. 2:** Pulling membrane tethers with a NanoTracker™ 2. A bead is attached to the membrane of a cell (A). Pulling it away from the cell surface results in the formation of so-called membrane tethers (B). The characteristic forces F_t (tether formation force) and F_p (plateau force) allow the calculation of mechanical membrane characteristics (C).*

and sub-piconewton forces at megahertz sampling rates [9]. Accessories, specifically developed for handling cell samples, such as the PetriDishHeater™ or LaminarFlowCell Heater™, make the NanoTracker™ 2 a truly universal tool for live cell applications.

Cell membrane properties

Due to the complex nature of the cellular environment, performing quantitative force spectroscopy on living cells is still a challenging task. However, studies focusing on the mechanical properties of cells, their sub-cellular organelles, and the cell's molecular components are becoming more frequent [10].

One important aspect of live cell research is the characterization of lipid membrane properties, which play an important role in regulating cellular mechanobiology, e.g., shape, motility, division, and differentiation.

The outer cell membrane is a highly complex structure consisting of a lipid bilayer and sterol molecules with embedded isolated or clustered proteins. It fulfills a variety of functions and directly interacts with other cellular components and organelles via the cytoskeleton.

The mechanical properties of the cell membrane (tension, viscosity) are essential for many processes. An important method for investigating cell membrane elasticity is pulling out long nanotubes known as membrane tethers [11]. Tethers can be either spontaneously formed by cells or artificially extracted (pulled) from the cell surface by applying a local force.

Formation of a membrane tether is achieved by attaching an optically trapped polystyrene or silica microparticle to the membrane and moving either the sample stage or the trap itself (Fig. 2). The force spectroscopy curves obtained can be analyzed using models to determine the mechanical properties of the cell membrane [12,13].

Typically, the so-called tether formation force F_t must be exceeded for a tether to form. This threshold can be ascribed to the membrane-cytoskeleton interactions that need to be overcome. A lower force plateau is then reached where the tether is elongated at a constant force as membrane reservoirs on the cell surface provide material for the growing tube. The typical force spectroscopy curve of a tether pulling process is displayed in Fig. 2C. The plateau force F_p depends on the tether elongation speed and increases for higher velocities. Knowing F_p and the pulling velocity v allows the calculation of the effective membrane viscosity η_{eff} [12], where F_0 is the force required to hold the tether stable at zero velocity ($v = 0$) and R is the tether's cross-sectional radius:

$$\eta_{eff} = \frac{F_p - F_0}{2\pi v} \quad (1)$$

and the effective membrane tension γ_{eff} [10]:

$$\gamma_{eff} = \frac{F_p - F_0}{4\pi R} \quad (2)$$

Results from tether formation experiments have provided detailed insight into mechanisms as diverse as cell motility, vesicle trafficking and intercellular signaling, and are currently the focus of many research labs [14].

Pulling membrane tethers from Jurkat T-cells

NanoTracker™ 2 optical tweezers integrated into a Zeiss Axio Vert.A1 inverted microscope were used to pull membrane tethers from living Jurkat T-cells. The optical tweezers setup was as follows: 1064 nm continuous wave trapping laser (3 W maximum power); two independently steerable optical traps, where one trap is controlled by a piezo-mirror and the other by acousto-optic deflectors (AODs); a sample nano-positioning module with a scan range of 100×100×100 µm (piezo table); and two state-of-the-art QPDs for simultaneous and independent detection in both traps. To maintain the Jurkat cells under appropriate physiological conditions, they were kept in a designated incubator at 37 ˚C and in a suitable cell growth medium at all times. Directly before the force spectroscopy experiments with the NanoTracker™ 2, 100-200 µl of the cell suspension were diluted with a pre-warmed phosphate-buffered saline (PBS) buffer to a final volume of 1-2 ml in a glass bottom Petri dish (35 mm diameter). The sample was then placed in a PetriDishHeater™, which kept the cells at the required physiological temperature at all times during tweezing experiments.

An example of a typical membrane tether puling experiment with the corresponding force spectroscopy curve is shown in Fig. 3. To begin, it is necessary to locate a cell attached to the bottom of the Petri dish by surveying the sample in XY using the NanoTracker's motorized stage (15×15 mm travel range) while imaging under the bright-field conditions of the microscope. This force spectroscopy experiment was performed using the NanoTracker's standard "Absolute Force Spectroscopy" mode which allows the selection of the moving scanner (1st trap, 2nd trap or the sample scanner in our case) and consists of two motion segments – approach and withdraw.

For the optimal collection of force data, it is recommended that the sample scanner is selected as a moving scanner to move the cell towards the trapped bead and withdraw afterwards, and to keep the trap static during the experiment. This ensures the best force detection and minor force signal offsets caused by a moving trap are avoided. Scanner displacement during the approach should be adjusted so that after the initial cell-bead contact a pushing force of up to 100 pN is applied (Fig. 3B, dark blue curve). This ensures that the contact of the cell membrane with the bead is tight enough to allow successful tether pulling in the subsequent withdrawal segment.

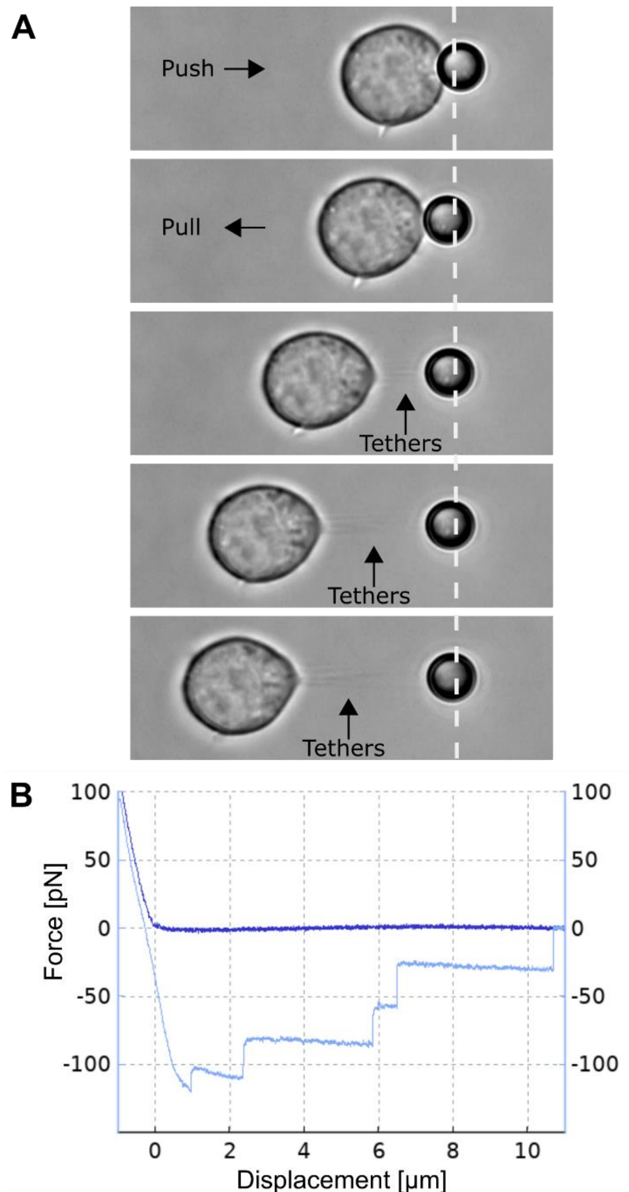

Fig. 3: Multiple membrane tether pulling experiment.
A) *The Jurkat T-cell attached to the glass bottom Petri dish is moved by the piezo table towards the trapped 4.5 µm polystyrene bead and pushed (upper panel) with a force of approx. 100 pN, thereby facilitating a non-specific attachment. Following this, the cell and bead are separated at a constant speed of 0.5 µm/s, thus forming and pulling multiple membrane tethers.*
B) *The plot shows a typical force spectroscopy curve collected during approach of the cell to the bead (dark-blue curve) and its withdrawal (light-blue curve), showing a tether formation force of approx. 120 pN and multiple rupture events with 35-40 pN steps in the plateau force.*

Another important experiment parameter that can be controlled with the NanoTracker software is the scanner motion speed. For the collection of the force spectroscopy curves presented here, the speed was set at 0.5-1.0 µm/s for the stable pulling of tethers to lengths of up to several tens of micrometers. Collecting force spectroscopy curves at different velocities and determining the F_t and F_p provides a more detailed characterization of the membrane's viscoelastic properties.

We found that, in this case, using plain or carboxylated polystyrene beads didn't lead to the formation of stable tethers in the Jurkat cells. Hence, to induce a good nonspecific binding required for successful tether pulling, the beads and cells were treated with Concanavalin A. The recommended protocol is to incubate a batch suspension of the beads with Concanavalin A (5-10 µl Con A to 1 ml of the bead solution). After a 100-fold dilution, the beads can be introduced to the Petri dish with the cells. Similarly, the beads can be coated with immunoglobulin G (IgG) antibodies.

Fig. 3A represents an example of multiple tether formation. For many types of living cells, very long membrane tethers measuring tens of micrometers can easily be pulled. In turn, the thickness of such tethers is typically in the range of several hundred nanometers in diameter. This is at the limit of the optical resolution of conventional bright-field microscopy and additional efforts are required to improve observation of the tethers in the optical image. The tethers in Fig. 3A can be seen as a result of a digital background subtraction algorithm implemented in the NanoTracker's camera software, which can dramatically improve the signal-to-noise ratio of the image collected with a digital camera.

The corresponding force spectroscopy curve (Fig. 3B) demonstrates a characteristic multi-step change in force with increased cell-bead separation. These are the rupture events of individual tethers in the bunch. Caution is advised in the interpretation of data in such experiments, as it is difficult to judge whether an event is due to the breakage of a single tether or several tethers. However, in this experiment, we observed a very similar rupture force of about 35-40 pN across all force jumps, which allows us to determine with a high degree of certainty that these represent a single tether rupture event.

An example of pulling a single membrane tether from a Jurkat T-cell is shown in Fig. 4. In contrast to the experiment

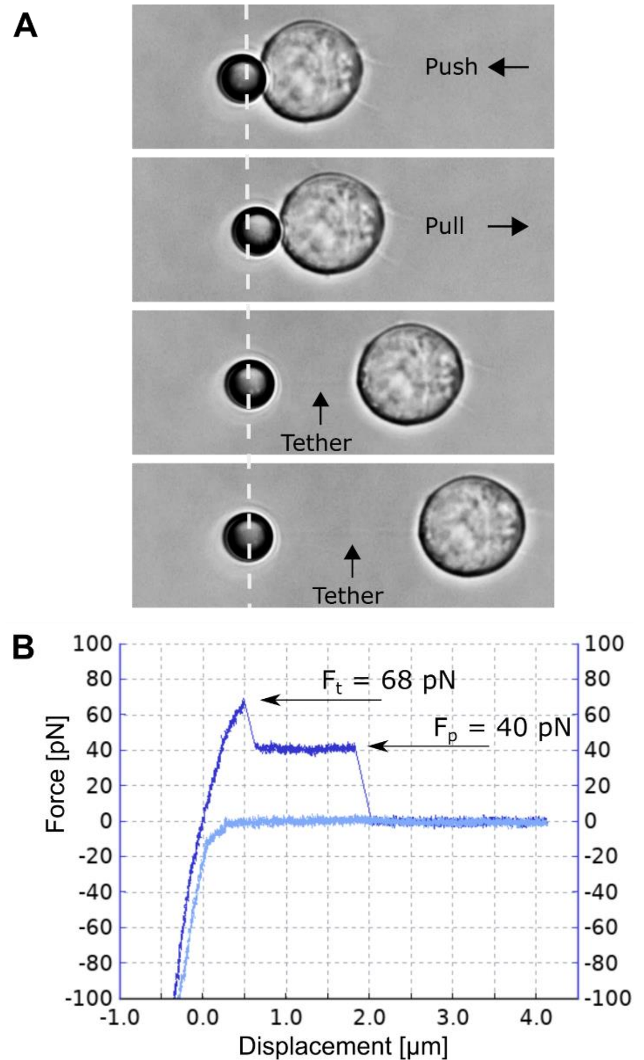

Fig. 4: A single membrane tether pulling experiment.
A) *A Jurkat T-cell attached to the glass bottom Petri dish is moved and pushed against the 4.5 µm polystyrene bead, facilitating nonspecific attachment. Separating the cell and the bead at a constant speed of 0.5 µm/s induces a single membrane tether formation and its pulling is recorded.*
B) *The corresponding force spectroscopy curve for the single tether formation and pulling (light blue curve for approach; and dark blue for separation).*

shown in Fig. 3, the cell and bead are separated in the reverse direction, which enables the tether (and the deflecting bead) to be pulled to the right-hand side of trap. In the NanoTracker software, this direction is selected as a positive axis for the force detection. Therefore, in figures 3 and 4, the pulling forces are read as negative and positive respectively. From the force spectroscopy curve in Fig. 4B, F_t can be clearly observed and measured at 68 pN. When the tether is formed and initially extruded from the cell

membrane, the force is reduced by 28 pN and remains at a constant F_p of 40 pN, while the length of the tether increases. Increasing the cell-to-bead separation to about 1.8 µm ultimately results in tether rupture.

Conclusion

We have successfully used the latest generation NanoTracker™ 2 optical tweezers to study the mechanical properties of Jurkat T-cells. It was demonstrated that the instrument can be used in membrane tether pulling experiments on living cells while, non-invasively, measuring the tether formation force and plateau force with a high resolution and reproducibility. The cellular mechanical properties obtained this way vary for different type of cells. Membrane tension can fluctuate in different regions of the same cell, in moving cells, and as a result of differences in the composition of the plasma membrane and the surface of the substrate [10].

New applications, especially in combination with high-resolution fluorescence microscopy, are continuously being developed with the aim of shedding more light on the fascinating interplay between cellular mechanics, dynamics and signaling. For further reading on these topics, we recommend our technical notes on fluorescence and confocal integration with the NanoTracker™ optical trapping system which can be found at
https://www.bruker.com/en/products-and-solutions/microscopes/optical-tweezers.html

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References

1. Wozniak, A., Van Mameren, J. and Ragona, S., 2009. Single-Molecule Force Spectroscopy Using the NanoTracker™ Optical Tweezers Platform: from Design to Application. Current pharmaceutical biotechnology, 10(5), pp.467-473.

2. van Mameren, J., Eggert, H., Behme, G. and Jaehnke, T., 2011, April. NanoTracker: force-sensing optical tweezers for quantitative single-molecule nanomanipulation. In Novel Techniques in Microscopy (p. JTuA28). Optical Society of America

3. Rauch, P., & Jähnke, T., 2014. Optical Tweezers For Quantitative Force Measurements And Live Cell Experiments. Microscopy Today, 22(4).

4. Ashkin, A., Dziedzic, J.M. and Yamane, T., 1987. Optical trapping and manipulation of single cells using infrared laser beams. Nature, 330(6150), pp.769-771.

5. JPK Instruments, Optical Tweezers Combined with Multi-Fluorescence Microscopy, Application Note. https://www.jpk.com/app-technotes-img/Optical-Tweezers/pdf/jpk-tech-fluorescence-integration.1.pdf

6. JPK Instruments, Quantifying cell-virus interactions using NanoTracker™ optical tweezers. Application Note. https://www.jpk.com/app-technotes-img/Optical-Tweezers/pdf/jpk-app-nt-tethers.1.pdf

7. Lansky, Z., Braun, M., Lüdecke, A., Schlierf, M., ten Wolde, P.R., Janson, M.E. and Diez, S., 2015. Diffusible crosslinkers generate directed forces in microtubule networks. Cell, 160(6), pp.1159-1168.

8. Johansen, P. L., Fenaroli, F., Evensen, L., Griffiths, G., & Koster, G. (2016). Optical micromanipulation of nanoparticles and cells inside living zebrafish. Nature communications, 7(1), 1-8.

9. JPK Instruments, Characterizing quantitative measurements of force and displacement with optical tweezers on the NanoTracker™. Application Note. https://www.jpk.com/app-technotes-img/Optical-Tweezers/pdf/jpk-nt-tech-calibration.1.pdf

10. Arbore, C., Perego, L., Sergides, M. and Capitanio, M., 2019. Probing force in living cells with optical tweezers: from single-molecule mechanics to cell mechanotransduction. Biophysical reviews, pp.1-18.

11. Nussenzveig, H.M., 2018. Cell membrane biophysics with optical tweezers. European Biophysics Journal, 47(5), pp.499-514.

12. Hochmuth, F.M., Shao, J.Y., Dai, J. and Sheetz, M.P., 1996. Deformation and flow of membrane into tethers extracted from neuronal growth cones. Biophysical journal, 70(1), pp.358-369.

13. Nowak, S.A. and Chou, T., 2010. Models of dynamic extraction of lipid tethers from cell membranes. Physical biology, 7(2), p.026002.

14. Diz-Muñoz, A., Fletcher, D.A. and Weiner, O.D., 2013. Use the force: membrane tension as an organizer of cell shape and motility. Trends in cell biology, 23(2), pp.47-53

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