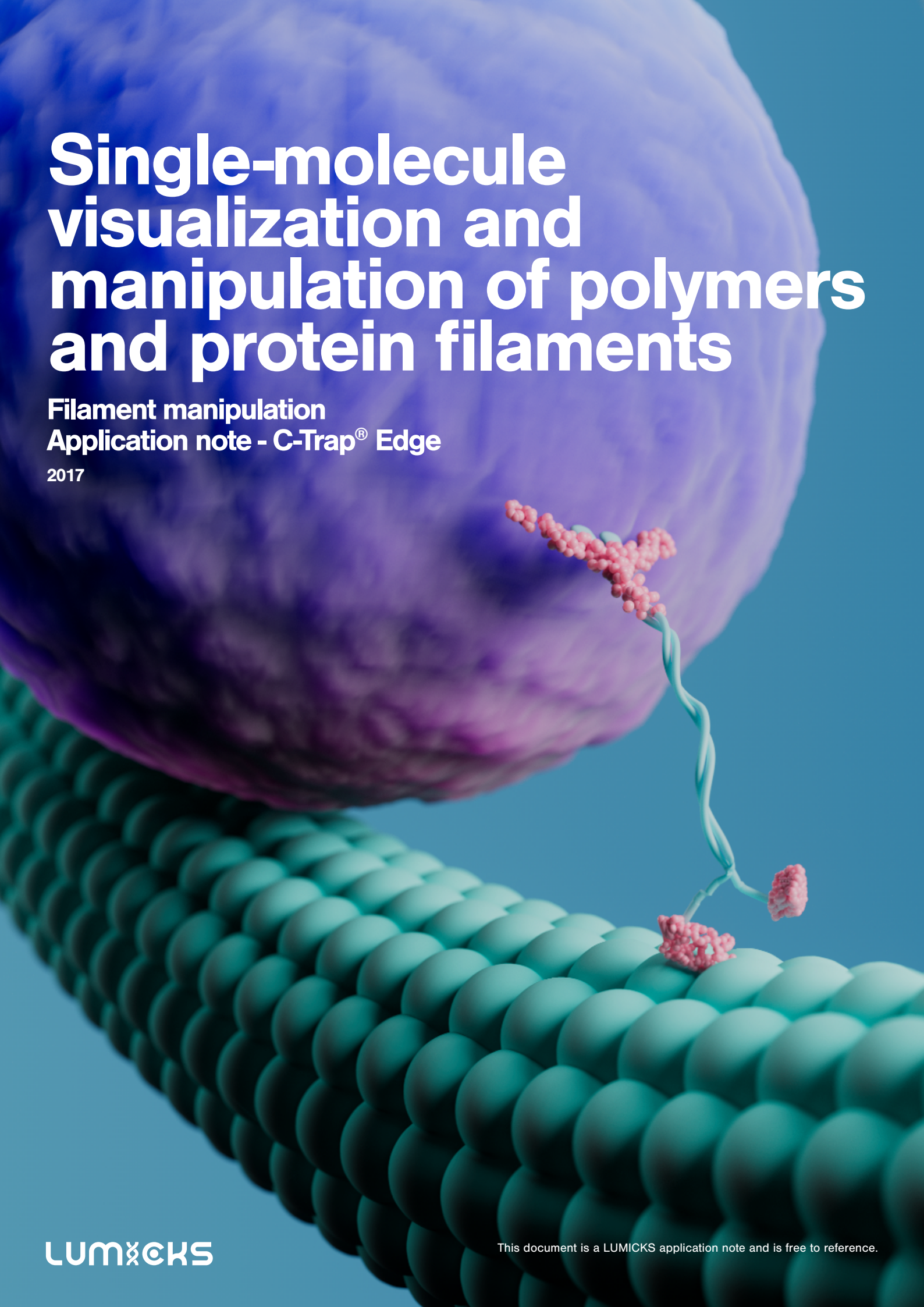
Single-molecule visualization and manipulation of polymers and protein filaments

Filament manipulation
Application note - C-Trap® Edge

2017



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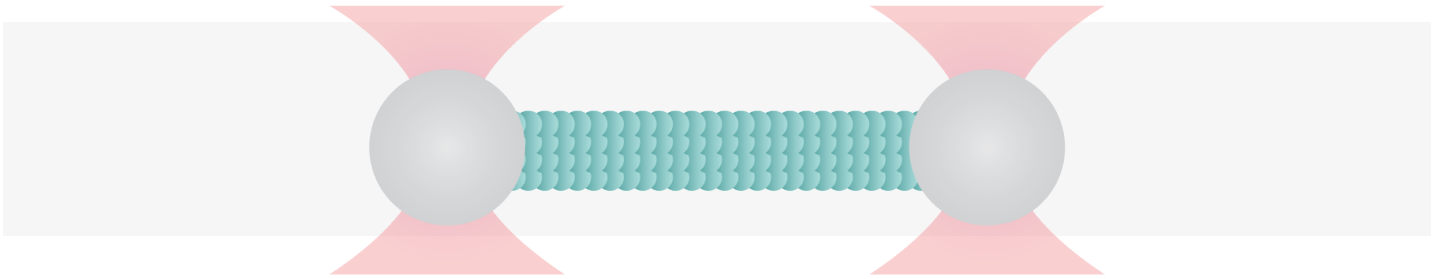
FILAMENT MANIPULATION
Application Note



1 A schematic representation of a microtubule tethered between two optically trapped beads.

Polymer and Protein Filament Measurements and Visualization

The cell is in constant and rapid flux between attaining stasis and remaining dynamic to perform its functions like polarization, migration and cytokinesis. These changes arise from various mechanical and molecular stimuli, but ultimately converge to the molecules of the force-generating cytoskeletal machinery.

The polymeric cytoskeletal filaments are extremely versatile, forming bundles and networks, which define cellular morphology, govern transport, and provide stability and resilience to cells and tissues. The physical characteristics of these filaments, like viscoelasticity, tension, etc. are governed by their unique encoded structure, dimensions and their interaction with other filaments and molecules.

Changes in the structure of microtubules, for example, generates cytoplasmic forces and is the first step for re-orientation of cytoplasm, cell movement, shape change and subsequently tissue organization. Forces exerted by microtubular spindles are important for cell division. Actin generated forces are important for endocytosis and subsequent trafficking. Cells depend on their force transducers to be able to interrelate with their surrounding and respond to stimuli.

These filaments have been studied indirectly using various approaches of fluorescence microscopy in cells and tissues and more directly, using force spectroscopy on single filaments or filament bundles. LUMICKS' C-Trap® provides for a unique approach which combines the strength of both techniques, to give a truly correlative method of studying and manipulating cytoskeletal filaments.

Filament Characterization

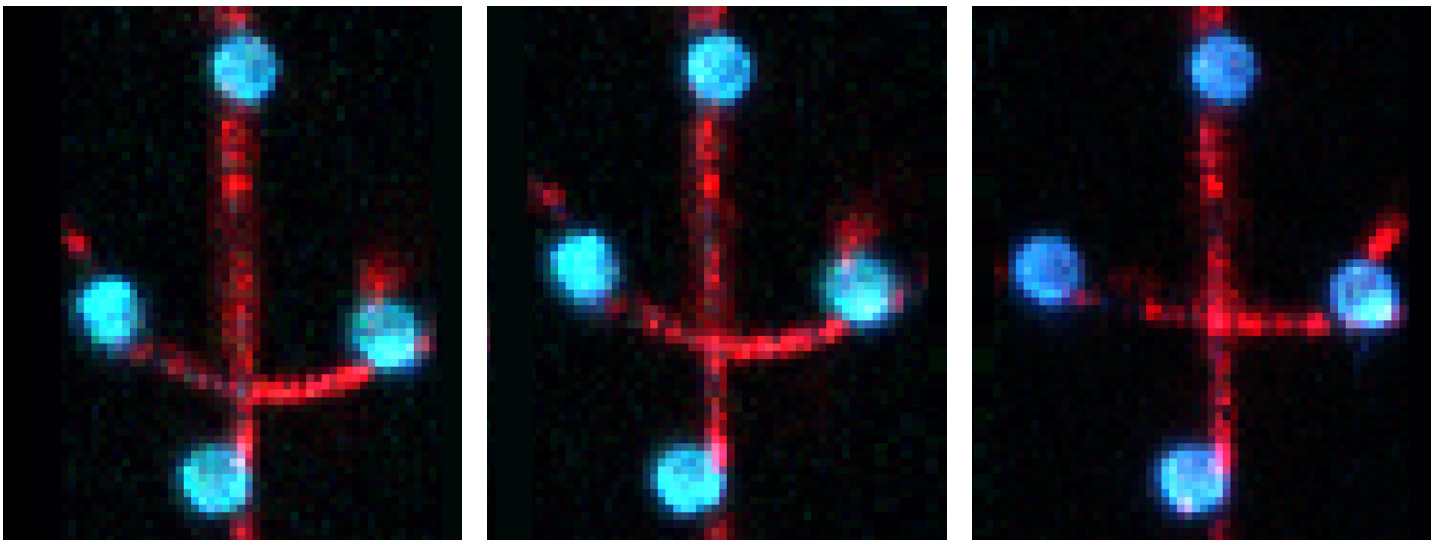
Biophysical tools like optical tweezers are capable of directly measuring the mechanical characteristics of these filaments and manipulating them to understand their thresholds.

A simple characterization of such a cellular filament like microtubules is achieved by holding this microtubule between two trapped microspheres in a dual beam optical trap (C-Trap®), the fluorescently labeled tubulin is visible as a red filament using confocal microscopy, allowing for correlative force and fluorescence spectroscopy (**Figures 1, 2**).

The two microspheres can now be moved independent of each other in a three-dimensional space with a known velocity, hence a controlled force can be applied on the microtubule. Depending on the direction and the magnitude of applied force, a microtubule can be stretched or bent to desired configurations.

The C-Trap® has a high temporal (>MHz) and spatial resolution (<1 nm), essential for measuring single molecule effects on the filament structure. The dual detection system combined with 3D manipulation provides an unparalleled range of trap stiffness allowing experiments with single molecules, filaments or bundles of filaments.

The C-Trap® seamlessly combines optical tweezers with fluorescence microscopy (3 color, confocal, or STED) to observe and correlate the effect of binding and motion of single molecules on filament mechanics. The built-in 4-(5-) channel microfluidics system allows one to acquire reliable data with a high throughput, while simplifying experiments with variable molecules and buffer conditions.

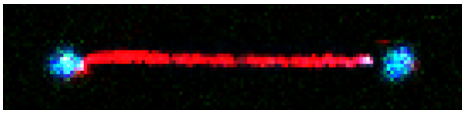


3 Two fluorescently labelled microtubules held in a crossed pattern using avidin-biotin chemistry on polystyrene microshpheres. A microtubule is dragged across another microtubule with a known force. A characteristic curve is observed due to the friction force between the two filaments (Left and Middle). The microspheres are now held stationary; the microtubule slowly relaxes (Right).

Filament Interaction

The characterization of cytoskeletal filaments has the potential to unlock several key problems in cellular physiology.

As discussed before, the cytoskeletal filaments interact with other similar and dissimilar filaments to define cellular morphology, networks and signaling. The surface properties of these filaments, the electrostatic and ionic forces and specific interactions, can generate friction when two filaments interact. This friction can be measured using a device such as the C-trap™ configured for quadruple optical trapping.



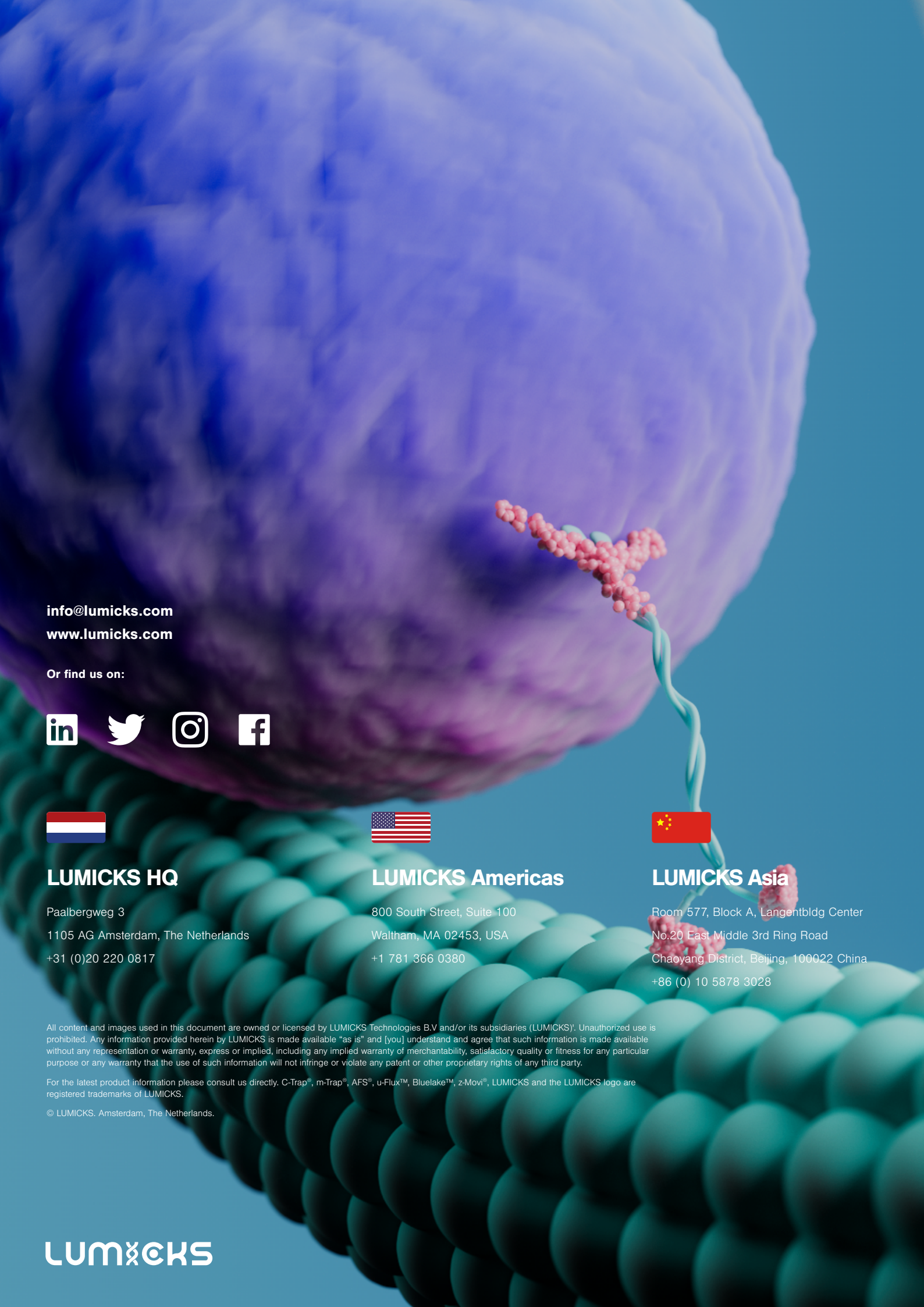
2 A biotinylated fluorescent microtubule held by two avidin coated, optically trapped microspheres, was observed using a three-colored confocal microscope. The mictotubule is fully stretched to be linear. The microsphere on the right can be moved towards the microsphere on the left with a known force causing the microtubule to buckle.

Data courtesy of BIOCEV Prague, Dr. Zdeněk Lánský's group.

The quadruple C-Trap® can modulate four microspheres relative to each other in a 3D space, while retaining the high measuring parameters and ease of use of a C-Trap® (**Figure 3**).

It is known that protein complexes use shapes, shear and stress on individual filaments as molecular switches. The correlated quadruple optical tweezers with fluorescence microscopy allow for real-time visualization of filament-filament, filament-protein and filament-protein-filament interaction between cytoskeletal filaments, under biologically relevant conditions with high spatial and temporal resolution. Especially in combination with stimulated emission depletion or STED (Super C-Trap®), direct observation of individual protein dynamics is made possible.

Tools like the C-Trap® in its dual or quadruple-trap configuration can enable modern researchers to ask novel questions aimed at investigating how the spatial organization and dynamics of cytoskeletal components are controlled by linkages to the cytoskeleton; and in turn, how these structures affect single protein functions.



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