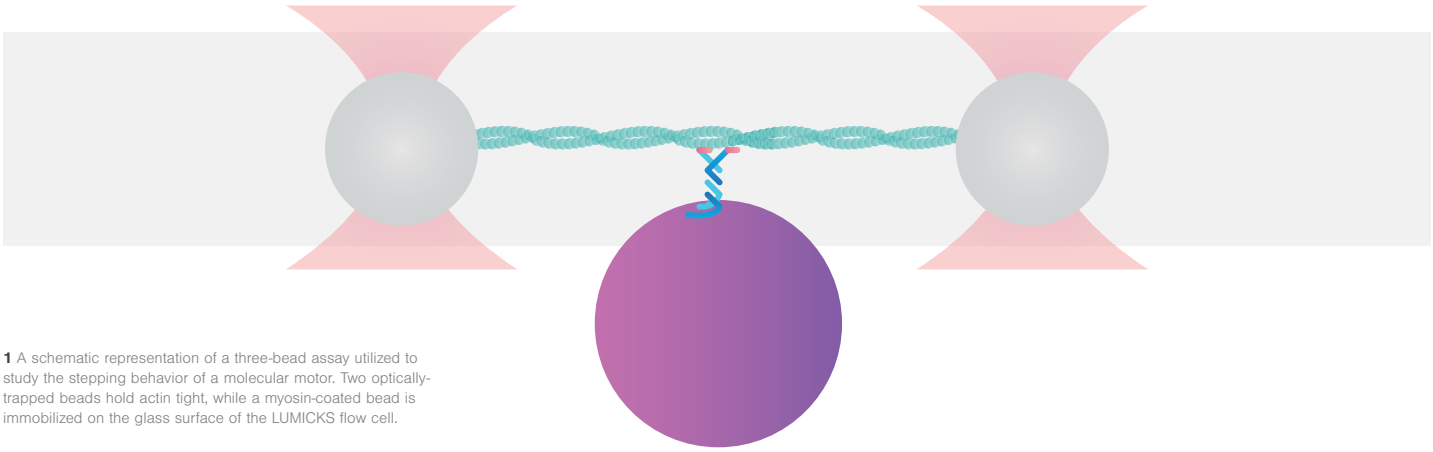
High-throughput observation of molecular motor activity and dynamics

Motor protein
Application note - C-Trap® Edge

2018

LUMICKS

MOTOR PROTEIN
Application Note



1 A schematic representation of a three-bead assay utilized to study the stepping behavior of a molecular motor. Two optically-trapped beads hold actin tight, while a myosin-coated bead is immobilized on the glass surface of the LUMICKS flow cell.

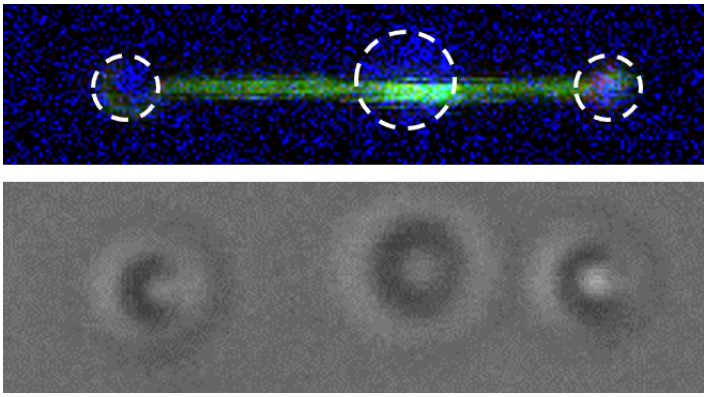
Investigation of myosin VI activity at the single-molecule level

The cytoskeleton and its many molecular motors are major players in a variety of cellular functions. The interconnected networks of cytoskeletal filaments act as tracks directing molecular motor transport and provide structural support and organization to cells.

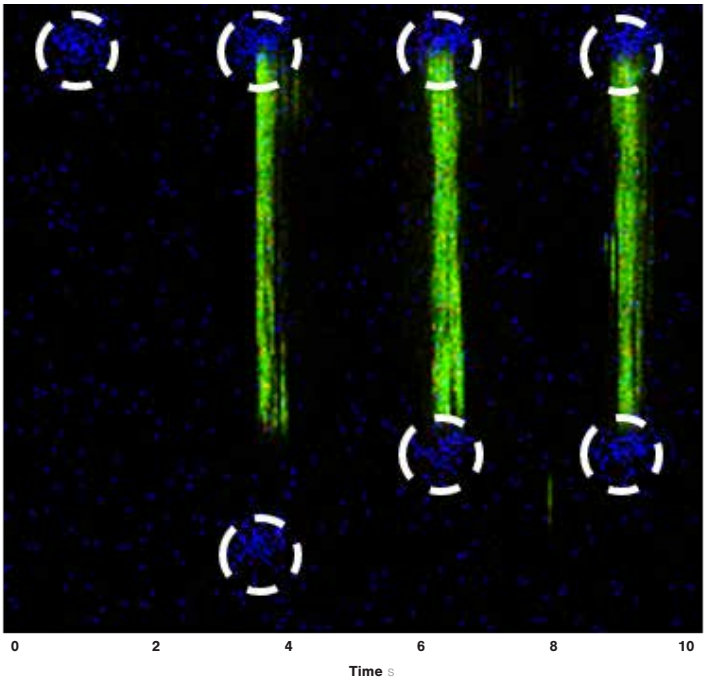
One example of a class of motor proteins is the myosin superfamily, a large family of proteins whose members are involved in diverse cellular processes, including cellular transport, migration and structure maintenance. A dimeric myosin motor is capable of processive ATP-dependent hand-over-hand motion employing actin filaments as tracks.

The stepping mechanics of molecular motors are force-dependent (pN), nanoscopic (1-100 nm) and often very fast (~1-100 Hz). Thus, the investigation of discrete steps and the subsequent mechanics of myosin motion on actin demands the use of high-resolution optical tweezers.

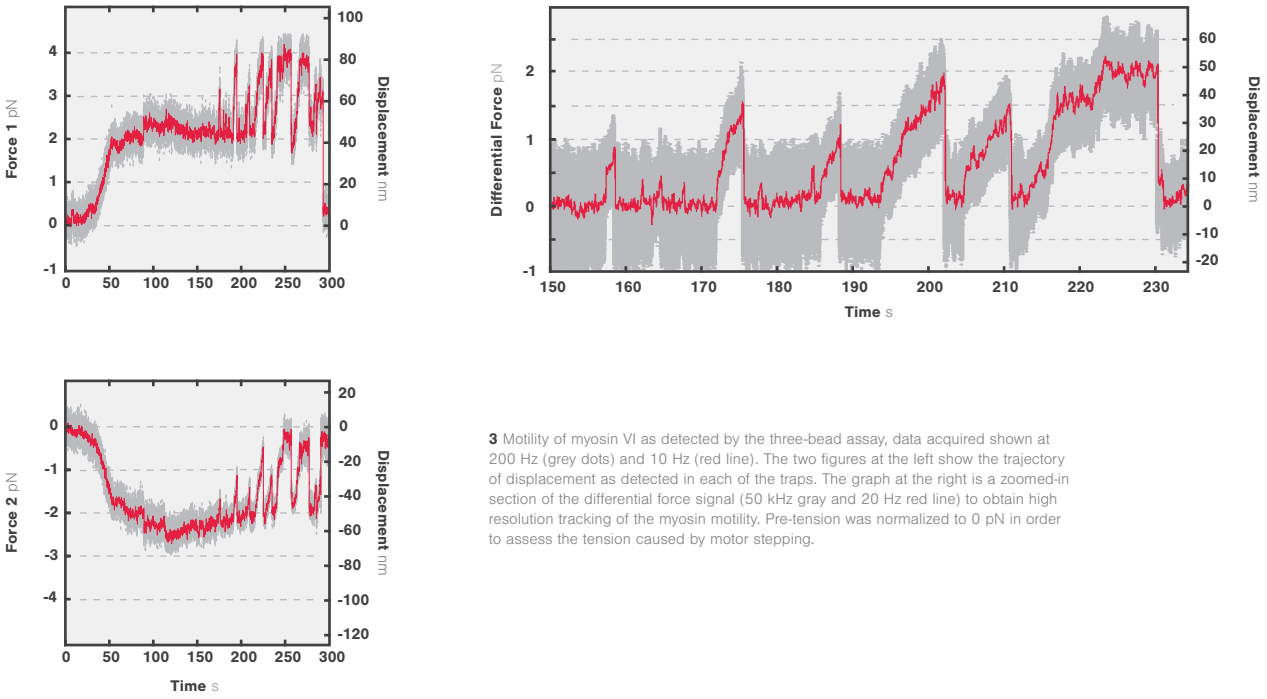
With optical tweezers, we are capable of resolving sub-nanometer displacements and applying pico-Newton forces on single proteins. This force spectroscopy approach has proven extremely useful in elucidating the mechanics and kinetics of molecular motors involved in intracellular transport. With LUMICKS' C-Trap™, it is possible to observe myosin activity, the processive motion, the binding kinetics along actin, and to determine its thermodynamic properties at the single-molecule level.



2a Fluorescent image (top) and brigh tfield image (bottom) of a single actin filament tethered between two trapped beads that has been brought into contact with a third bead, functionalized with myosin molecules (top).



2b Fluorescent images of two polystyrene beads and an actin filament at different time-points (right). The total amount of time needed to catch a single filament and tether it between two beads is typically 10 seconds.



3 Motility of myosin VI as detected by the three-bead assay, data acquired shown at 200 Hz (grey dots) and 10 Hz (red line). The two figures at the left show the trajectory of displacement as detected in each of the traps. The graph at the right is a zoomed-in section of the differential force signal (50 kHz gray and 20 Hz red line) to obtain high resolution tracking of the myosin motility. Pre-tension was normalized to 0 pN in order to assess the tension caused by motor stepping.

Figure 1 illustrates a "three-bead assay" comprising two optically-trapped beads holding a single actin tether and a third bead, functionalized with individual myosin molecules and fixed on the glass surface of the flow cell.

Fluorescently tagged actin filaments can be visualized using the C-Trap's correlated confocal microscopy capability which significantly simplifies and accelerates the process of forming a suitable tether (**Figure 2a**).

The formation of actin tethers in a classic three-bead assay is often a tedious process. In the C-Trap, force measurements can be performed simultaneously with fluorescence imaging, which together with the integrated microfluidics streamlines the assay significantly, consequently increasing the reliability, speed and throughput of measuring molecular motor mechanics. We were able to successfully form actin tethers within 10 seconds (**Figure 2b**), while readily rejecting undesired molecules, such as multiple or short tethers, which were easily discernable through the fluorescent images.

The actin tether is subsequently lowered towards the third bead to provide a close contact between the actin filament and the myosins. The myosin heads then dynamically interact with the actin substrate in the presence of ATP and generate translocating forces on the filament. The myosin-driven translation of actin results in a displacement of the attached and optically trapped beads. Owing to the C-Trap's high spatial and temporal resolution, the processive motion of myosin can be studied in detail.

In this experiment, we used the C-Trap to investigate the displacement of actin with respect to wild type myosin VI by measuring the correlated displacements of the two beads trapped in the dual optical trap. **Figure 3** shows that myosin pulls the actin filament in a unidirectional manner with a measurable force. When looking at the independent force channels (**Figure 3**, left), we observed an anti-correlated force signal when pulling the actin filament only to a pre-tension of 2 pN (t = 0-50 seconds). Once contact was established between the actin filament and the myosin VI-coated platform bead (at 175 seconds), the signals of both force channels appeared correlated, indicating directional displacement caused by myosin activity. As shown in **Figure 3** (right), the resolution of force-displacement measurements can be significantly increased when calculating the differential force between the two traps.

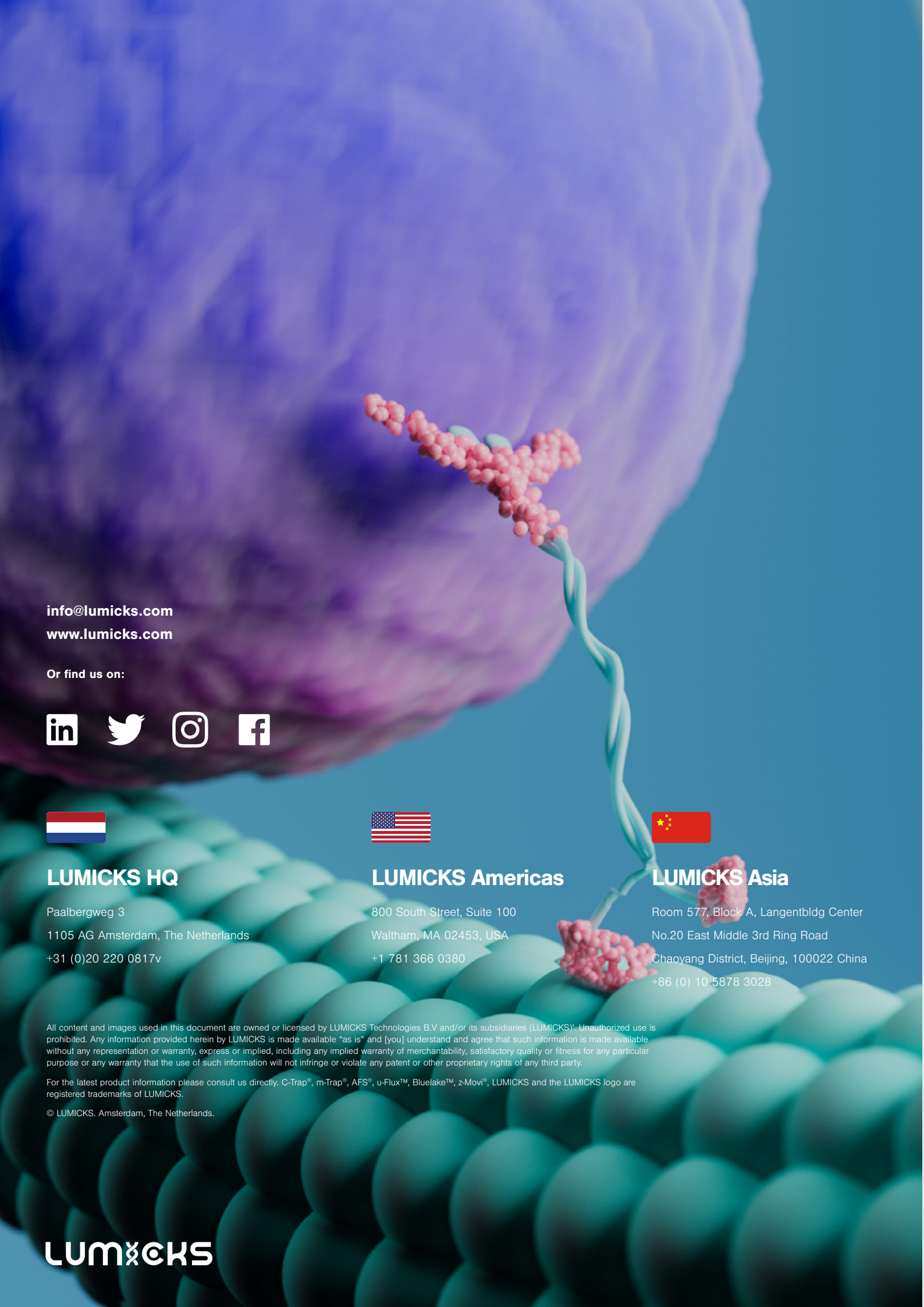
The integral microfluidic system of the C-Trap allows for not only the quick optical trapping of beads and actin tether formation but also permits studying motor motion at different ATP and salt concentrations while retaining the same molecule over the course of these assays, thus increasing the consistency of the experiment.

With the C-Trap it is possible to visualize and track the kinetics of molecular motors along cytoskeletal proteins, and determine their thermodynamic properties at the single-molecule level.

The exact kinetics of the motor can be observed by measuring the relative translocation of the filament with respect to the motor and the related forces. From the resulting force-time traces, the speed and processivity of the motor can be derived.

This, in combination with the possibility to visualize fluorescently-labeled motor proteins, provides highly precise real-time measurement of myosin motor activity. This is a prerequisite for accurate analysis and the study of additional experimental parameters such as the force-dependence of structural transitions and how these processes are coupled to the chemical hydrolysis of ATP.

Data collected using samples courtesy of P. Ruijgrok and Z. Bryant, Stanford University.



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