C-Trap™ | Product Brochure

Truly correlative high-resolution optical tweezers and fluorescence microscopy

LUMICKS
Capture Molecular Interactions

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History

The field of single-molecule biophysics has radically changed over the past decade, owing to major technological breakthroughs enabling landmark experiments. Now that the single-molecule methods have reached maturity, they are ready to make a long-lasting impact on the field of biological research.

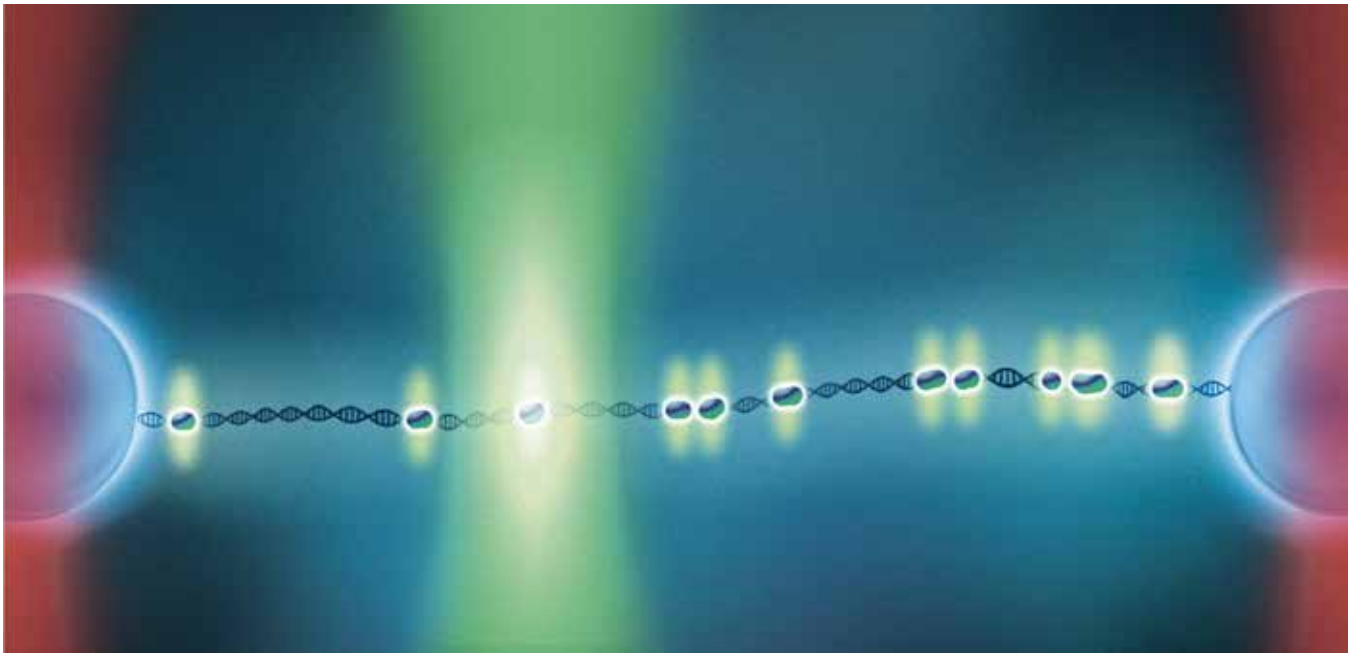
Focusing on these new methods, LUMICKS brings to market the revolutionary C-Trap™ Correlative Tweezers-Fluorescence Microscope (CTFM) and the Acoustic Force Spectroscope (AFS™). LUMICKS is rooted in the research groups of Prof. Gijs Wuite and Prof. Erwin Peterman at the VU University Amsterdam, and its instruments for single-molecule studies allow the disruptive method of live measurement and imaging of interactions at the molecular level.

Mission

LUMICKS provides ready-to-use single-molecule instrumentation, allowing you to focus on the science. Understanding complex biological processes at the single-molecule level is key for prevention and cure of cancer and other diseases. To this purpose, live observation and measurement have proven game changing, and LUMICKS aims to make these enabling technologies available to biologists to create a better world.


LUMICKS

C-Trap™ Technology



Confocal fluorescence scanning along a single DNA molecule between optically trapped microspheres

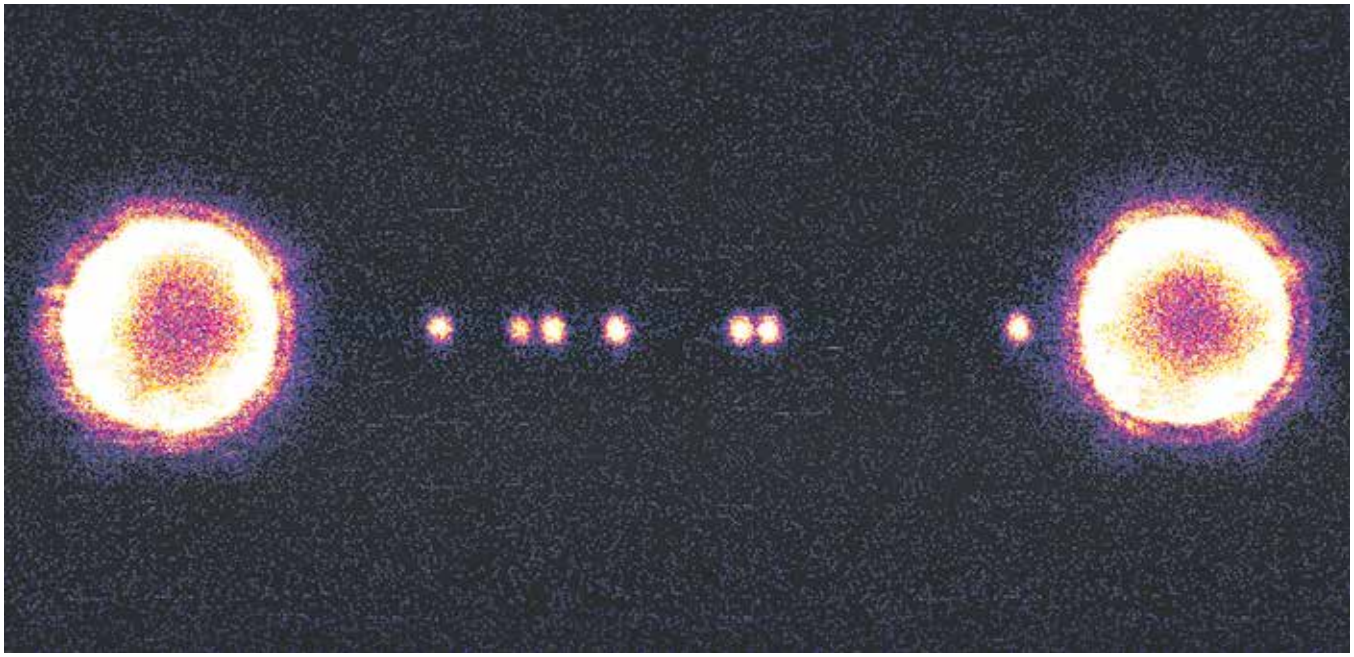
C-Trap™ Explained

The C-Trap™ is the world's first instrument that combines optical tweezers, confocal microscopy and STED (for SuperC-Trap™) nanoscopy and an advanced microfluidics system in a truly integrated and correlated way. The C-Trap™ is capable of live, simultaneous and correlative manipulation and visualization of biomolecules with sub-picoNewton force resolution and sub-nm localization.

C-Trap™ is based on more than 15 years of research and has been designed conforming to the highest quality standards. Developed by top-level scientists, the patented technology provides researchers the ability to improve and validate biological models with unprecedented clarity.

Using the C-Trap™ and SuperC-Trap™, fluorescent molecules interacting with optically trapped biomolecules (such as DNA, RNA, protein filaments or other) can be visualized with single-molecule and single-photon sensitivity, at localization accuracy down to 10 nm, resolution below 50 nm (for SuperC-Trap™) and an imaging rate of 200 Hz. Simultaneously, force-spectroscopy measurements are performed with sub-picoNewton force resolution, limited only by the fundamental Brownian noise limit.

Both in the C-Trap™ and SuperC-Trap™ a single laser source is used to generate multiple continuous, independently movable optical tweezers. 3D position and stiffness of each trap is controlled by separate motorized precision elements for maximum single-molecule experimental flexibility.

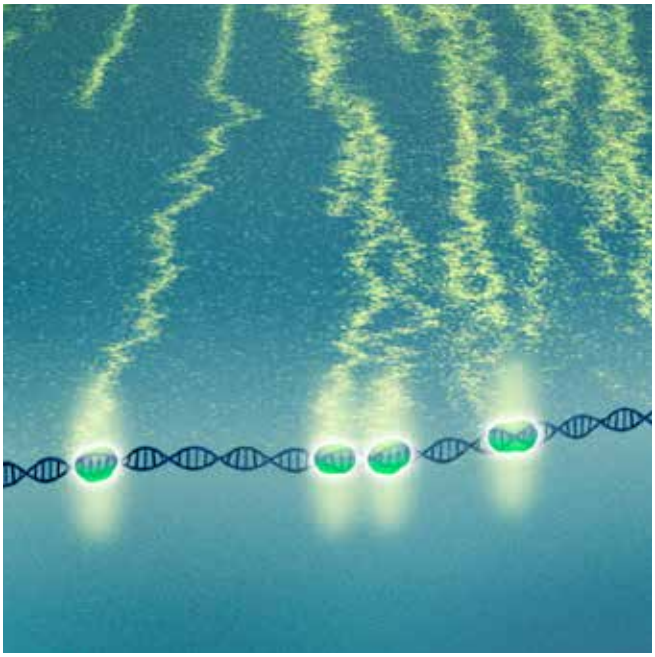


Detection of sIngle fluorescent proteins bound to a single DNA molecule using the C-Trap™

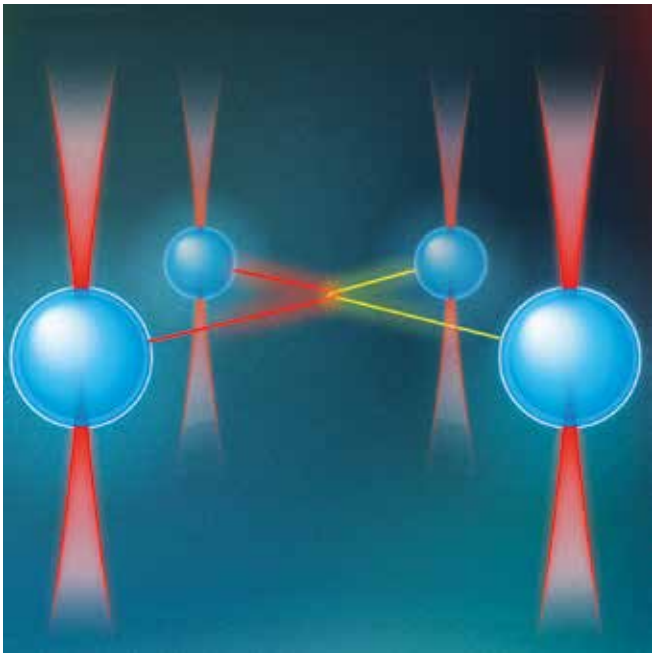
Technology Features

- Correlative workflow: Move an optical trap to a target in the fluorescence image and vice-versa: The user can select a region of interest in the bright field image of the beads and record fluorescence images from that area.
- Designed as integrated solution with correlated signals of force, distance and fluorescence.
- Live observation of biological interactions (proteins-DNA, proteins-RNA, etc…) in physiologically relevant conditions.
- Background rejection for single-molecule sensitivity up to 100 nM protein concentrations.
- Fast (200Hz) imaging with Super-resolution performances: 50 nm resolution.
- Ultimate protein localization accuracy: <10nm (SuperC-Trap™ <5nm).
- Ultra-high force resolution: <0.1 pN @ 50 Hz on 8kb DNA.
- Manipulation of biomolecules with sub-picoNewton force resolution and sub-nanometer-positioning.
- Monolithic glass microfluidics for laminar flow allows easy buffer exchange and controlled triggering of biochemical reactions.
- Automated trap calibration, force-stretching and stage movement through integrated software.
- Simultaneous force measurements using multiple traps with a bandwidth of up to 200 kHz, allowing force feedback.
- State-of-the-art closed-loop piezo system for trap positioning.
- Automated trap-motion sequencing and constant force mode.

Key Features



Confocal Line Scanning



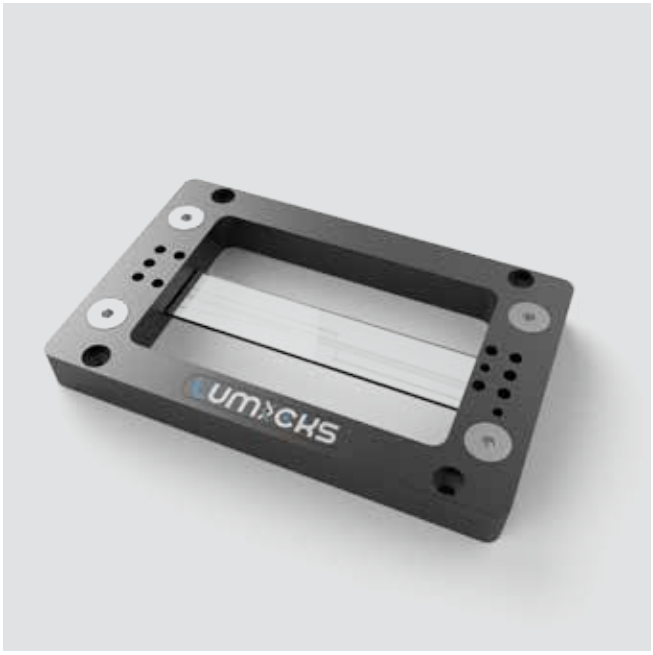
Quadruple Optical Tweezers

Confocal Microscopy

- Multi-colour confocal scanning fluorescence microscopy. Up to 10 different wavelengths available in the range 488nm - 647nm.
- Ready for alternating excitation scheme for FRET applications.
- Confocal scanning up to 200 lines per second, microsecond to seconds dwell-times with nm-precise spot positioning, jitter-free and sub-nm repeatability.
- Ultra-low light detection and single photon counting for the most demanding single molecule applications. Capable of single eGFP detection.
- Full integration with optical tweezers for correlative measurement and user interaction.
- Upgradable to super-resolution performance using pulsed-STED (SuperC-Trap™). High resolution enables studies of e.g. densely covered DNA and improves localization accuracy; background rejection enables single-molecule sensitivity in high fluorescence background conditions.

Optical Tweezers

- Two to four optical traps with XY force detection on two traps (independent or differential).
- Continuous optical traps (no time sharing) for higher optical trapping stability and precision.
- Optical traps with a very high escape force and low force noise. Suitable for biological systems with a large dynamic range
- Absolute 3D trap positioning with sub-nm repeatability.
- Single hardware steering of multiple optical traps ensuring identical movement of two optical traps.
- Full integration with confocal and optional STED microscopy for correlative measurements and user interaction.



U-Flux™ Microfluidics

C-Trap™ Software

Microfluidics

- Monolithic glass fluidics, reusable and leak-free allows high-throughput: short time to results.
- Laminar flow cell with multiple channels without physical barriers; for performing complex multi-step assay assembly and biomolecular reactions *in situ*.
- Ultra-stable flow using passive pressure system allows detection of sub-nm trap displacement in laminar-flow environment.
- Automated valves with zero-volume displacement design for remote operations and zero-disturbance of single-molecule measurements.
- Integrated with Pt100 electrodes for local temperature sensing during measurements.

Innovative Software & Workflow

- Truly correlative tweezers-fluorescence data acquisition and interaction.
- Developed from the user perspective.
- Programmable navigation through multi-channel laminar flow cell enabling fast and reproducible assembly of single-molecule assays.
- Automation of procedures and personalized user profiles.
- Workflow optimized for high throughput single molecule experimentation: e.g. trapping microspheres, tethering and subsequent manipulation and imaging are done within minutes.

Quick & Easy Workflow

C-Trap™ offers a quick and simple workflow that starts at the very beginning of your experiment. From the loading of the sample to the start of the single-molecule measurement, each step has been repeatedly tested and optimized thoroughly. The software provides a navigation interface in which you are able to easily guide the traps between different channels; picking up beads, biomolecules and different proteins with a simple click.

Fast Track to Results

Lumicks provides ready-to-use single-molecule instrumentation; for the fastest track to results, allowing you to focus on science. The C-Trap™ offers the ability to be up to speed with Tweezers – Fluorescence for real single-molecule experimentation in a matter of days.

Why buy if you can build?

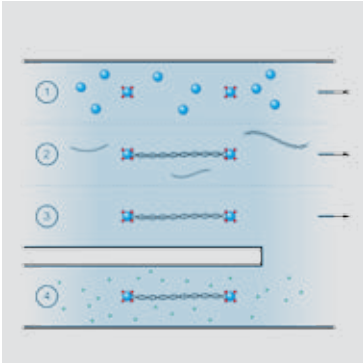
- C-Trap™ is alignment free. No PhD in physics is needed to operate the system. Scientists can focus on the applications and produce discoveries.
- Designing and building high-resolution single-molecule instruments is time consuming, drastically limiting scientific output. With C-Trap™, the focus on science starts at day one.
- Safety and ease of use can be an issue with home built systems. LUMICKS' C-Trap™ enables broader application in a multi-user environment or as a shared facility.
- Home built academic set-ups are flexible and can be easily altered or upgraded, but it is hard to maintain reproducibility, performance and alignment. The C-trap™ has been designed after extensive optical modelling and the entire opto-mechanics has been fabricated into a single block of aluminium in a single step (MonoBloc design); ensuring high performance and extreme stability and reproducibility.
- LUMICKS provides guaranteed performance, uptime and support, whereas with home built instruments the actual knowledge of the instrument and its maintenance can be a problem on the long run – the PhD who built it is likely to leave.

1. Sample loading

Insert microspheres, buffers, biomolecules and proteins of interest in dedicated syringes placed in the pressure box. These syringes are connected via fluidic valves and tubing to the microfluidic laminar flow cell.

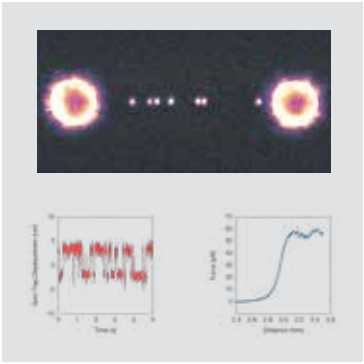
2. Setting up your experiment

Multi step single molecule assay assembly and measurements can easily be set up in the software. Microsphere and biomolecule pick-up as well as protein incubation and imaging settings are set in an intuitive manner. A simple command scripting feature enables the user to easily automate these steps, for increased throughput, or repeated experiments.



3. Semi-automatic assay assembly

The microfluidic flow cell creates several laminar flow channels next to each other while they do not mix (no physical barriers are involved). To create the experimental assay in situ, the following semi-automatic steps are typically executed from the software: 1) catching of two microspheres; 2) attaching a biomolecule, such as DNA, between the microspheres; 3) checking the integrity of the DNA; 4) loading proteins on the DNA and performing a force-fluorescence measurement. These steps can be executed automatically through scripting, or manually through simple clicks on the joystick.



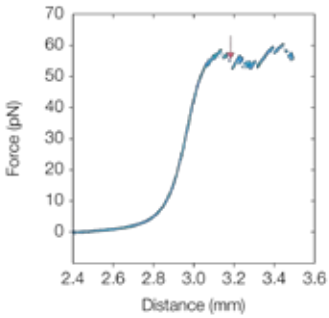
4. Results

The force, extension and fluorescence data are simultaneously visualized on the screen in real time and saved as a correlative dataset that include all instrument settings.

Applications

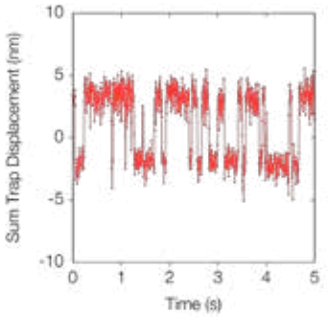
Probing the structural dynamics of proteins and nucleic acids with the C-Trap™

The high force resolution of the C-Trap™ makes it an excellent instrument for precision single-molecule studies of protein folding, RNA folding, DNA processing enzymes and molecular motors. Here we show how it enables the study of DNA structural dynamics under high tension in a DNA overstretching assay.



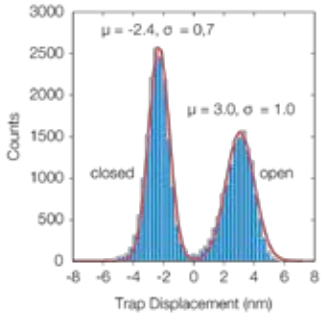
Force-distance curve of DNA

Force extension curve showing the overstretching of a DNA construct with a single free end. In the overstretching region the free end starts to unpeel. Unpeeling proceeds in bursts as first reported by Gross P. et. al. Nature Physics 7, 731-736 (2011).



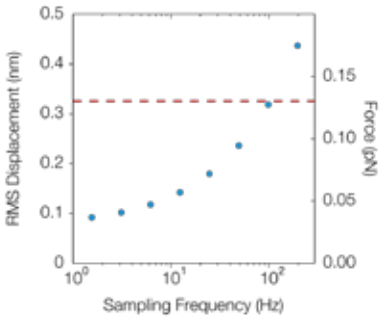
Dynamic structural transitions in DNA overstretching

A single DNA molecule is held at 60 pN and equilibrium fluctuations in extension reflecting folding/unfolding transitions can be observed in real-time. The distance between the traps alternates between two stable conformations as the same patch of nucleotides melts and re-anneals repeatedly. This graph represents a 5 s period from a total observation time of 150 s. The sampling frequency was 50 kHz and is shown here filtered at 200 Hz.



Detection of open and closed states

Histogram of trap displacements for the whole 150 s dataset. The red solid line shows a double Gaussian fit of the data. The mean displacement for the closed (annealed) state is -2.4 nm with a standard deviation of 0.7 nm (at 200 Hz). The mean displacement for the open (melted) state is 3.0 nm with a standard deviation of 1.0 nm (at 200 Hz). The 5.4 nm distance between the states corresponds to the melting/annealing of a patch of 22 base pairs.



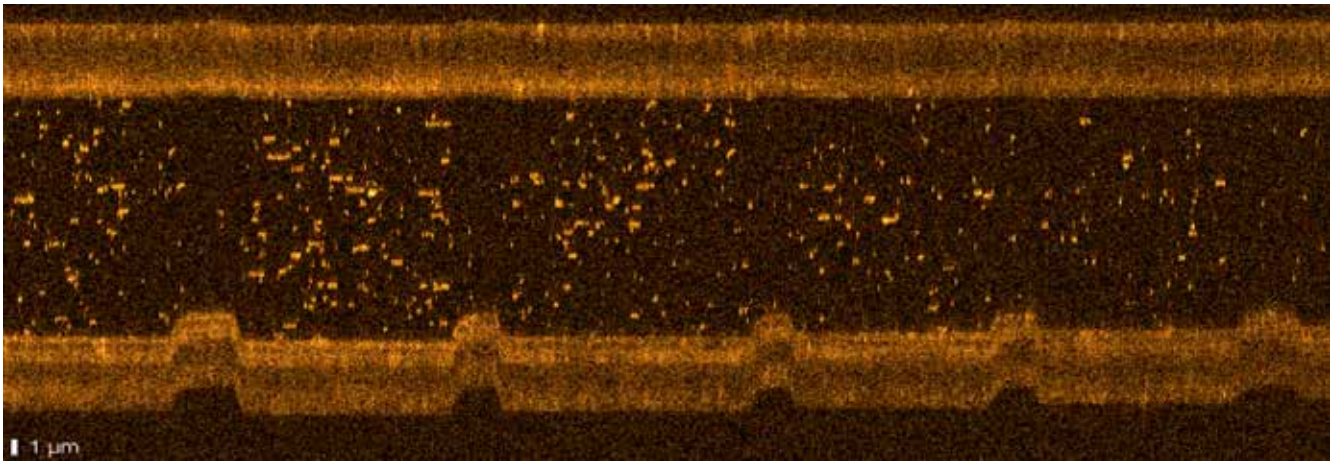
Limit of Distance Resolution

Root-Mean-Squared (RMS) displacement of a single trapped bead observed for 15 minutes at 200 Hz. Boxcar filtering was applied to demonstrate improved sensitivity at lower bandwidth. The RMS displacement of 0.43 nm at 200 Hz is reduced to less than 0.15 nm at 10 Hz. The dotted blue line indicates the average distance of a single base pair. The trap stiffness was 0.39 pN/nm.

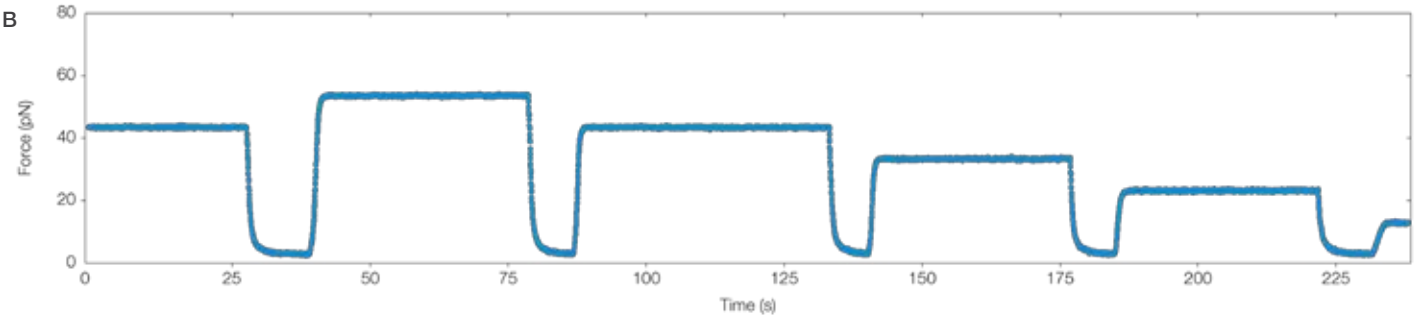
Force Fluorescence Correlation Measurement: Fluorescent Intercalators on DNA

Here we measure the DNA intercalation kinetics of cyanine dye Sytox-Orange using simultaneous fluorescence, force and distance measurements. In the experiment, a single bacteriophage lambda DNA molecule is moved to different tension using a force-clamping algorithm while fluorescence (A), force (B) and DNA elongation (C) are simultaneously measured. The single-dye sensitivity of the C-Trap™ allows resolving that the duration of individual DNA-binding events of Sytox-Orange increases strongly with tension. This in turn implies that Sytox-Orange unbinding rate is strongly tension dependent while the binding rate is not. This is also visible by comparing the graphs in figure B and C: when the tension is suddenly lowered, the DNA elongation reaches an equilibrium value slowly, indicating that Sytox-Orange unbinding is the rate-limiting step of the process.[1]

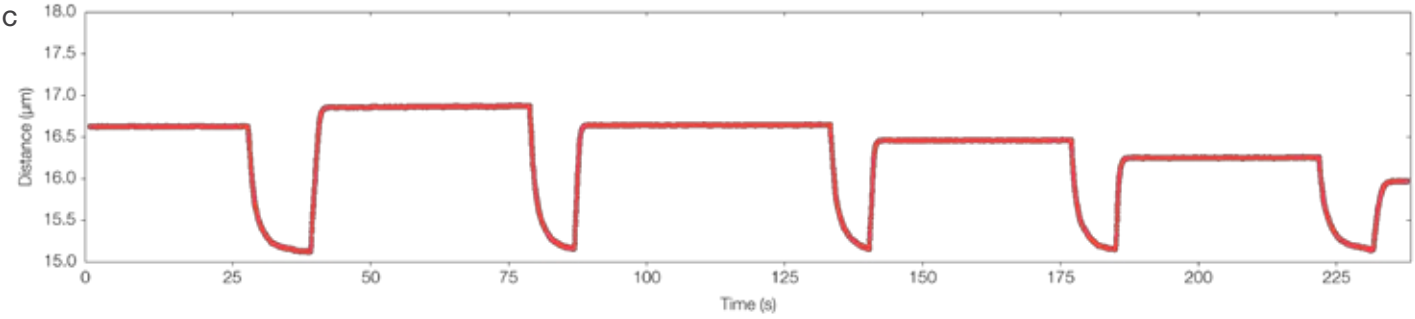
A



B



C

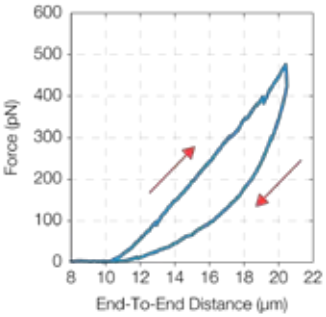


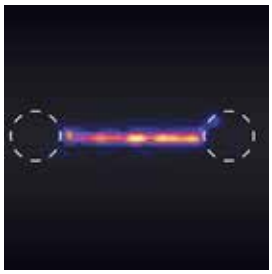
[1] For more information, refer to: Biebricher A.S. et. al. Nature Communications. 6, 7304 (2015).

Applications

Cytoskeletal Proteins & Intermediate Filaments

Cytoskeletal proteins and Intermediate filaments play a fundamental role in cell morphology through their ability to generate and sustain forces. Here we show exploratory measurements on the nano-mechanical properties of intermediate filaments (vimentin) using the C-Trap™ technology. These measurements were performed by the group of Sarah Köster at University of Göttingen, Germany.





Extension Curve & Trapped Vimentin Filament

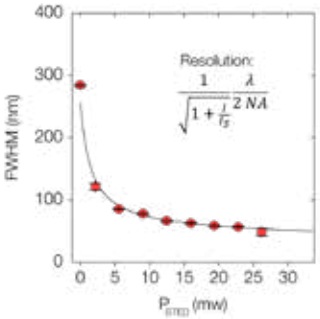
On the left a force-extension curve of a single vimentin filament is shown. Due to remodelling of the vimentin filament under high tension the backward curve shows clear hysteresis. The extremely high escape force of the C-Trap™ makes it possible to probe biomolecules at forces up to the nanoNewton regime.

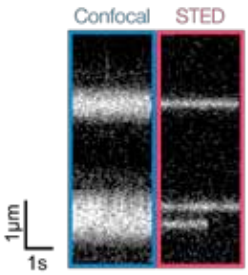
An individual vimentin filament is held between two optically trapped microspheres in the right image. Confocal fluorescence imaging of the filaments enables the user to distinguish the amount of filaments tethered between the microspheres and resolve intra-molecular motion due to filament remodelling.

Distinguishing Single Proteins on DNA

SuperC-TRAP™ Exclusive

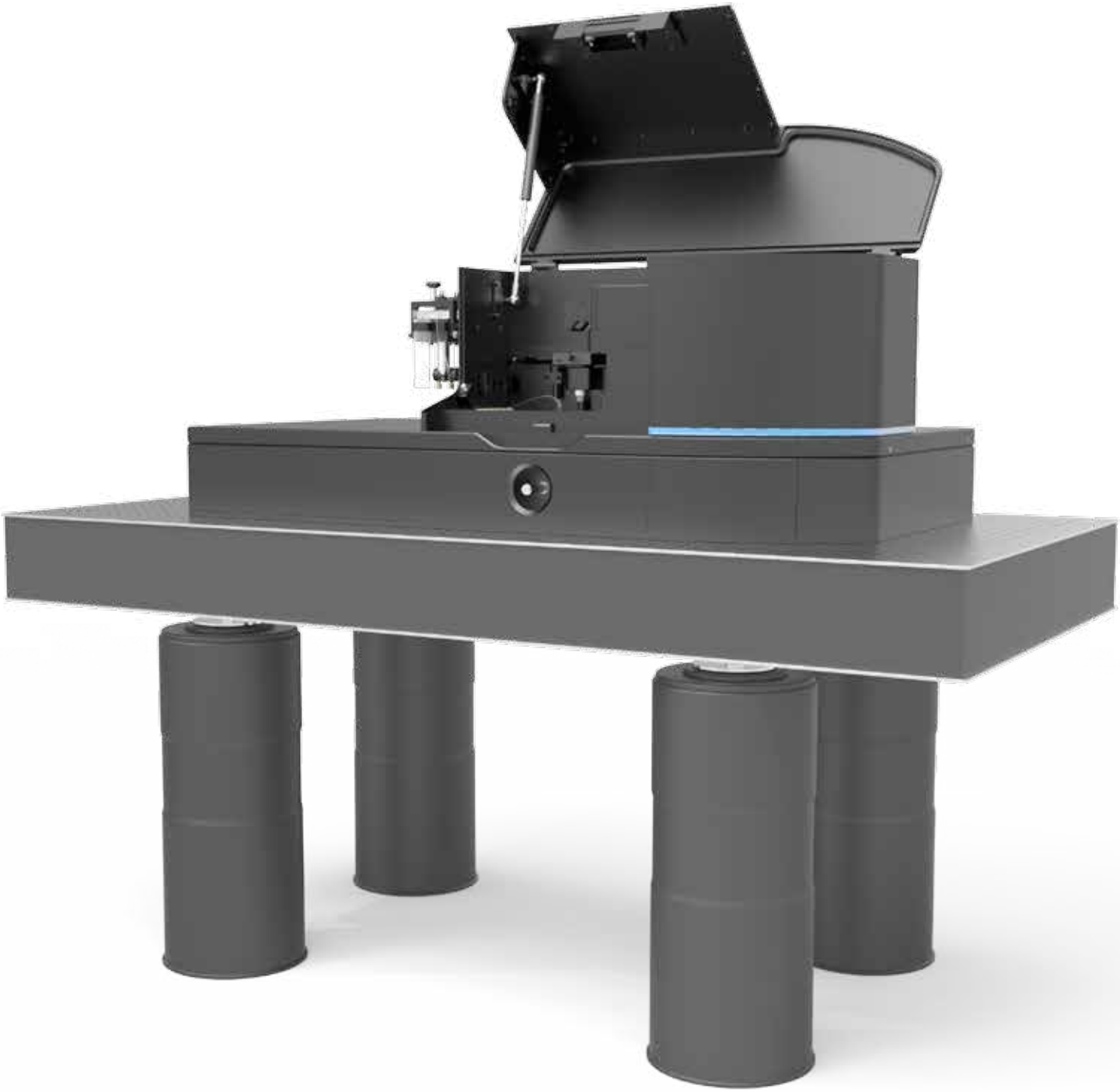
In the crowded cellular environment proteins densely cover DNA. Being able to distinguish single proteins under these biologically relevant conditions is essential for linking in vitro experiments with the in vivo situation. Combining optical tweezers with multicolour confocal and stimulated emission depletion (STED) enables the visualization of individual DNA-binding proteins on densely covered DNA with unprecedented resolution and clarity.





Extension Curve & Kymograph

Scaling of the effective resolution with the STED power used (SuperC-Trap™ only) is shown on the left. On the right we see a Kymograph showing three Atto-647N labelled restriction proteins (BsoBI) on optically stretched DNA, imaged partially with confocal microscopy (red border) and partially with STED nanoscopy (PSTED = 6 mW, blue border)). The kymograph demonstrates that STED combined with optical tweezers enables scientists to distinguish single proteins in crowded environments with superresolution performance.



Specifications

Key Component Specifications

Both C-Trap™ and SuperC-Trap™ include a high quality pressure-stabilized optical table and a powerful computer workstation. In addition, a tower containing all necessary electronic modules to power and control the system are included.

Service

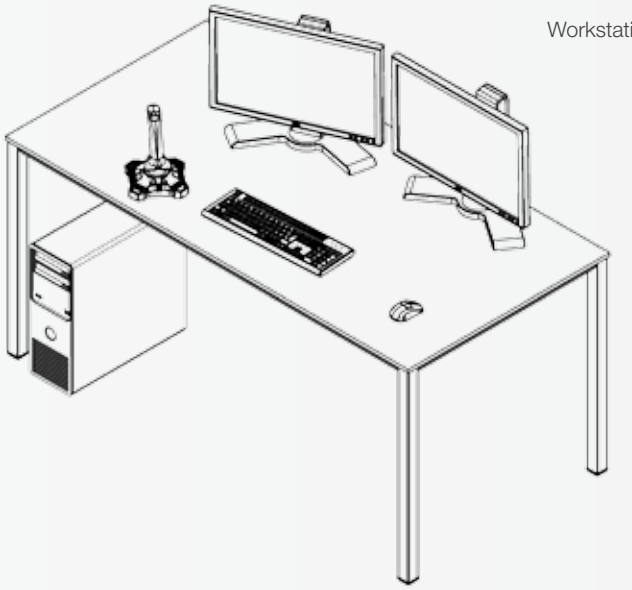
C-Trap™ and SuperC-Trap™ systems include a one-year warranty, which includes full service. LUMICKS instruments include access to premium expertise with our application specialists and service support. We stand with you to deliver unparalleled performance through the lifetime of your LUMICKS Correlative Tweezers-Fluorescence instrument.

LUMICKS guarantees instrument performance within specifications and supports scientists to solve application problems by maintaining high reliability and uptime.

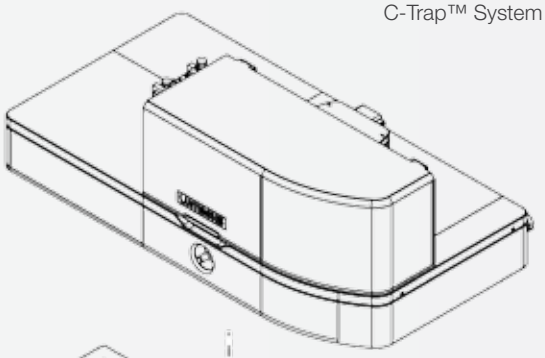
Specifications

Field of movement (FOM)	50 µm x 50 µm x 10 µm (x,y,z)
Minimum incremental step	<0.1 nm
Force resolution	<0.1 pN @ 50 Hz
Distance resolution	<0.3 nm @ 50 Hz
Force stability	<1 pN over 15 minutes
Maximum escape force	>1000 pN using 4.5 µm polystyrene beads
Trap corner frequency	0.5 kHz to 10 kHz (depending on configuration)
Number of independent traps	1 to 4 continuous traps steerable in 3 dimensions
Live bead tracking accuracy	<3 nm @ 100 Hz using video analysis
Fluorescence excitation laser	Up to 3 (default 488nm, 532nm, 635nm)
STED depletion laser	745 nm pulsed laser (20 MHz, 100 ps width)
Resolution (on 48 kB DNA protein with 4pN force)	<50 nm using 25 mW STED power
Scanning speed	200 Hz line scanning, jitter free
Background rejection limit	100 nM @ 1ms integration time
Spot position accuracy and repeatability	<5 nm

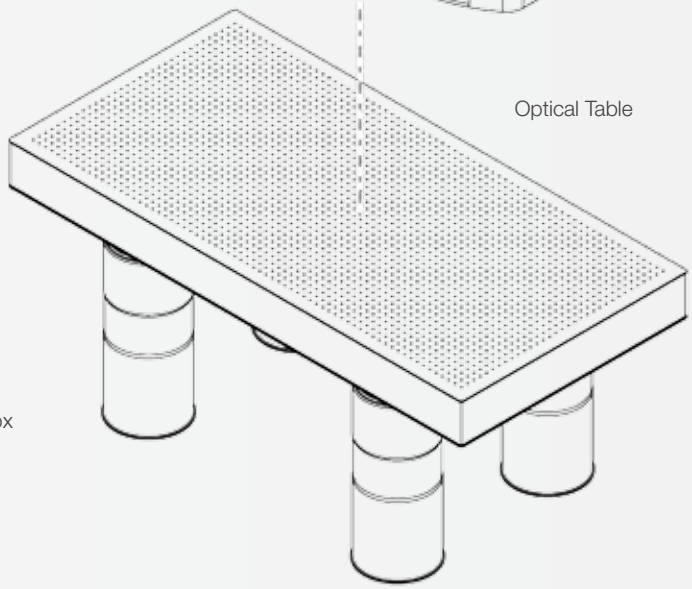
Workstation



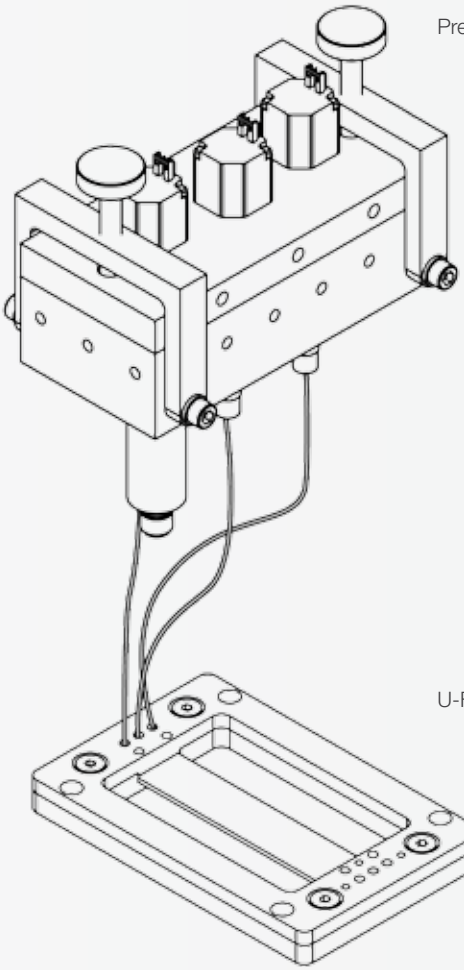
C-Trap™ System



Optical Table

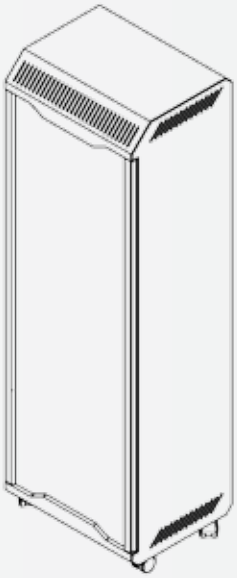


Pressure Box



U-Flux™ Flowcell

Module Rack



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