

protein positions protein diffusion We provide you with the experimental capabilities.

otein conformational changes

rheology and surface tension protein effect

protein position and effect DNA breathing

equilibrium dynamics

You lead the next wave of discoveries.

Discover what you can do next by exploring:





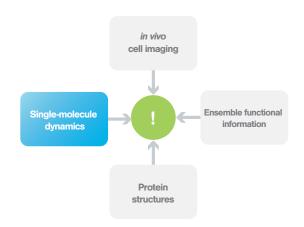


DNA/RNA structural

Faster and more impactful science by adding the missing link to your molecular toolbox

Many life science methods and tools are used today to approximate and extract molecular function. However, these tools give either detailed structural or functional information — but rarely both. Since the current tools do not allow for observation of the molecular processes in real time, they are often unable to reveal the **crucial mechanistic details of molecular factors** at play. This lack of direct observation of the underlying dynamic processes often results in **ambiguity in the obtained results**.

What if you could have access to technology that enables you to **observe molecular processes in action and obtain direct, indisputable evidence** of the underlying molecular mechanisms? An easy-to-use instrument that revolutionizes the way we look at biology. A state-of-the-art system that offers you data from the rarest, fastest, and most transient processes and eliminates ambiguity from your studies, resulting in more **impactful studies** with **shorter time to results.** A solution that can help you gain insights into of the root of disease development at the molecular level and accelerate therapeutic breakthroughs.



The C-Trap® is the only dynamic single-molecule microscopy product line that allows you to obtain direct evidence of how the mechanisms and dynamic processes of biomolecular processes work. Its unique Nobel Prize-wining technology enables –within the same experiment– to:

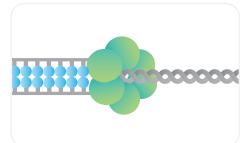
1. Directly visualize the dynamics of individual proteins in **real-time**.

2. Control and observe **stepwise assembly** of the biological complex.

3. Modulate the molecular system to test the model under different conditions.







Ultra-high performance



The highest spatiotemporal force resolution and sensitive single-molecule visualization ensure that you can measure the **smallest**,

most transient and fastest changes happening in your biological system.

One experiment to get a 360° view



Seamless correlation between force measurements and molecular visualization providing you multiple data streams in parallel, while at the same time, you can manipulate and sequentially assemble your molecular complex.

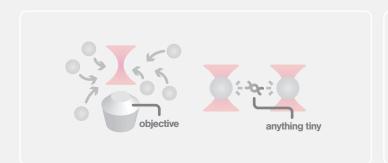
Ease of use

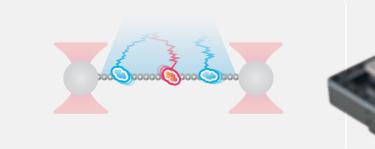


You don't need to be a trained biophysicist to use the C-Trap and interpret your data. Its intuitive controls, automation, and data analysis features ensure the **highest ease** of use and reliable data for all users.

Revolutionizing biology with optical tweezers, microfluidics, and imaging

Revealing the full picture of the working principle behind the C-Trap, a Nobel Prize-winning technology, and what results it can deliver for your experiments through **3 simple steps**.





Sten 1

Optical tweezers

Catch and manipulate your molecule of interest

The focused light generated by the optical tweezers is strong enough to trap any tiny object. You can trap your molecule of interest directly (e.g., protein droplets) or microscopic beads that can tether your molecule, e.g., a DNA between two beads. You can study the properties of your captured single molecules by manipulating them in different conditions. For example:

- Stretch and release your molecule to study conformational properties.
- Expose your molecules to interacting proteins (e.g., endonucleases or motor proteins) to measure their effects on your captured construct.
- Fuse your droplets to study their density and viscosity.

Step 2

Fluorescence or label-free imaging

Record your captured molecules in real time

The correlated microscopy systems offer you real-time visualization to follow the relevant interactions while you measure their associated processes.

Distinguish between multiple fluorescently labeled molecules at once, track and quantify foci formations based on intensity, or measure the dynamics of label-free microtubules. Whether you choose fluorescence or label-free microscopy, you'll extract high-resolution images with detailed information about your molecule.

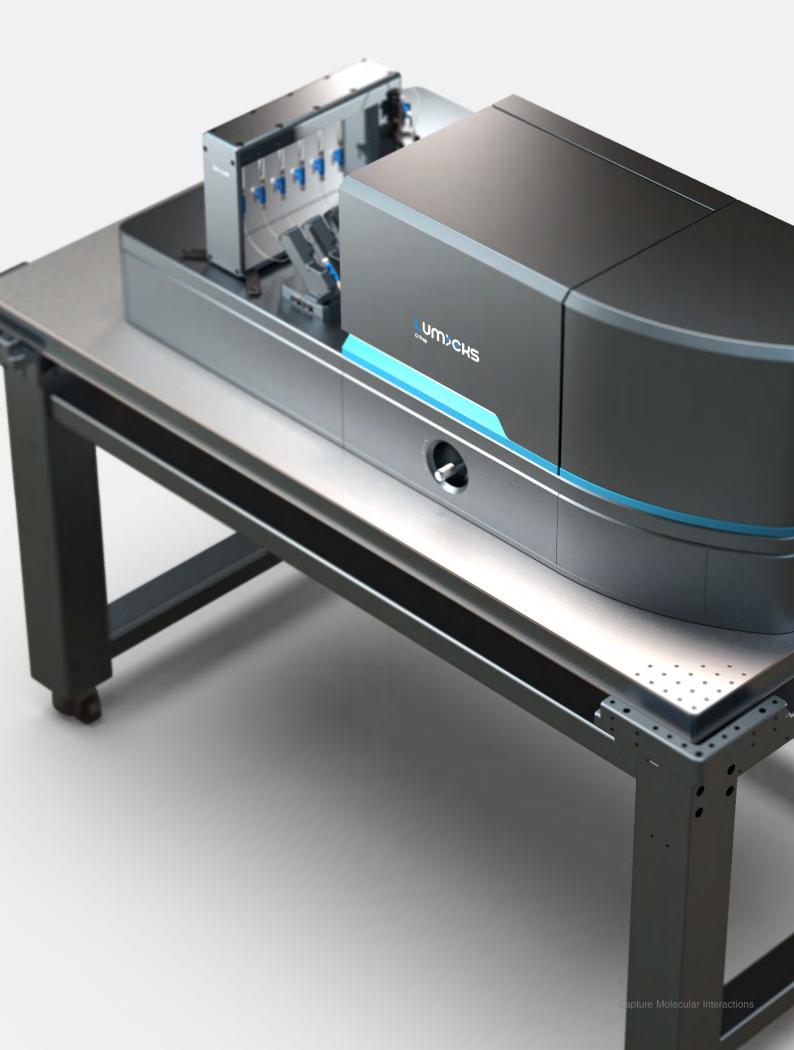


Step 3

Laminar flow microfluidics

Introduce, assemble, and position your reagents

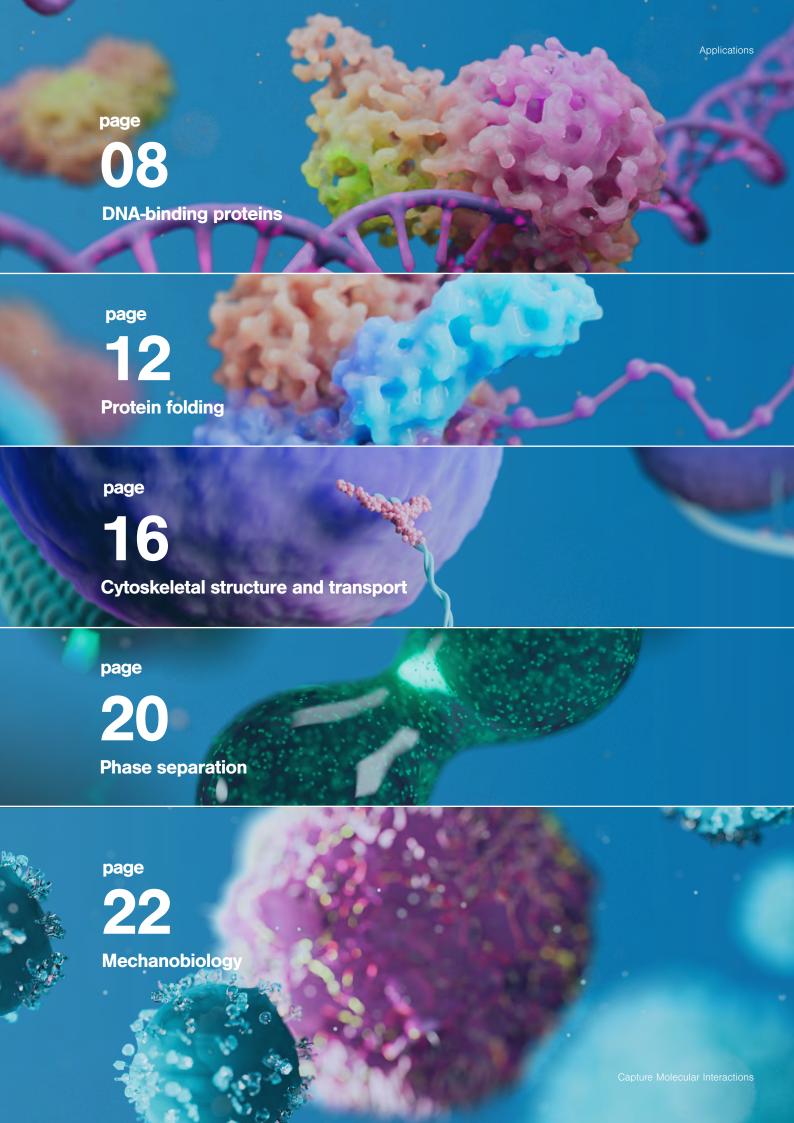
The integrated microfluidics system separates your reagents into neighbouring laminar flow channels, which are separated without physical barriers. Since the optical traps can move between channels, they are perfect for introducing and assembling your reagents in separate channels and easily evaluating your construct under different conditions.



Applications

in dynamic single-molecule studies

Today's scientific trends are racing toward smaller scales and experiments that provide both structural and mechanistic insights. Here, we showcase the applications that can help you get there.





Follow individual DNA-binding proteins in real time

Overview of applications:

- Track individual DNA-binding proteins in molecularly crowded environments and assess their dynamic properties under biological conditions
- Introduce multiple fluorescently labeled proteins and follow their interactions with a DNA or RNA molecule to study their activity.
- Follow protein events over time and reveal the number, position, diffusion, and duration of each interaction.





Data courtesies

[1] Prof. Dr. Gijs Wuite and Prof. Dr. Erwin Peterman at the VU University Amsterdam



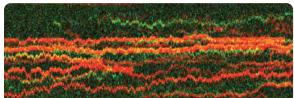
Check out the papers

[2] Brouwer et al. (2016) Nature [3] Heller et al. (2013) Nature Methods

Real-time tracking of DNA-protein interactions to evaluate how highly dynamic NHEJ repair proteins assemble, bind to DNA, and ligate double-strand breaks

Expose a DNA molecule to multiple interacting proteins to measure and visualize their interactions and activity in real time. The kymograph in **Figure 1** shows the protein-specific binding and dynamics of fluorescently-tagged XRCC4 and XLF repair proteins on a DNA molecule over time [1, 2]. With this setup, you can also study how different conditions affect DNA-protein interactions by applying force to manipulate the DNA conformation or regulating the protein composition.





1 Kymograph showing the binding position of XRCC4 (green, 9% of the total events), XLF (red, 62% of the total events), and XRCC4–XLF complexes (yellow, 29% of the total events) along a DNA molecule.

Distinguish individual proteins in molecularly crowded contexts to decipher physiological conditions of DNA-processing proteins

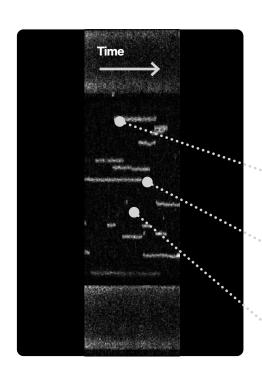
Correlate optical-tweezers experiments with integrated stimulated emission depletion (STED) microscopy to follow fluorescently labeled transcription factors on a DNA molecule in highly crowded environments [1, 3]. The high image resolution enables you to track individual enzymes on a protein-dense DNA molecule and study protein diffusion. A comparison between the binding events using confocal or STED microscopy elegantly shows the improved resolution (**Figure 2**).



STED microscopy (right).







How to read a kymograph

The kymograph offers you a detailed graphical representation of individual binding events occurring on a single DNA molecule over time and provides direct information into the $k_{\mbox{\tiny on}}$ and $k_{\mbox{\tiny off}}$ values of your DNA-binding protein.

Single fluorescent protein binds to the DNA

Single fluorescent protein unbinds from the DNA

Short-lived binding event (< 10ms)

Measure DNA-protein interactions while inducing DNA structural changes

Overview of applications:

- Manipulate DNA structures and conformations by stretching the molecule beyond its contour length.
- Study DNA-protein interactions under conformational conditions that reflect DNA structures established during transcription and repair.
- Reveal the properties of inter- and intramolecular DNA interactions induced by DNA-binding proteins, such as stability, formation, and rupture of cohesin or nucleosome bridges.





Check out the papers

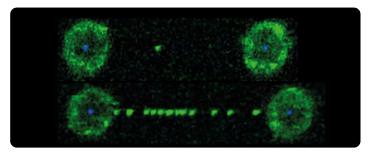
[4] Newton et al. (2019) Nat Struct Mol Biol. [5] Gutierrez-Escribano et al. (2019) Science Advances [6] Leicher et al. (2020) PNAS

Study how structural changes on DNA regulate the binding and catalytic activity of gene-editing enzymes

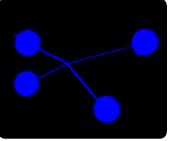
The C-Trap enables you to visualize sequence-specific gene-editing proteins while you manipulate the structure of your target DNA. You can study how conformational changes on the DNA, such as DNA breathing, affect the target specificity of Cas9 complexes [4]. In this example, you find the binding events of fluorescently labeled catalytically dead Cas9 (dCas9) coupled to a DNA-specific guide RNA (gRNA) as the optical tweezers stretch the tethered DNA molecule with increasing forces (**Figure 3**). Pulling the tethered DNA induces conformational changes and results in an increased number of off-target binding events. These results indicate that naturally occurring structural transitions, such as DNA breathing, make the DNA permissive to off-target binding by dCas9–gRNA complexes.

Reveal intermolecular or intramolecular DNA interactions induced by DNA-binding proteins

A quadruple-trap configuration on the C-Trap allows you to test intermolecular DNA bridging properties by manipulating two DNA molecules simultaneously [5]. Here, researchers maintained two DNA strands in close proximity, while being exposed to cohesin complexes that induce intermolecular bridging. Subsequently, moving the DNA molecules in different orientations revealed the strength and sliding properties of the established bridges, which they followed in real time (**Figure 4**). Similarly, they quantified the stability, formation, and rupture of intramolecular cohesin bridges by exposing a single DNA, tethered in a dual-trap configuration, to cohesin complexes.



3 A DNA molecule trapped between two beads with a fluorescently labeled dCas9-gRNA complex (green) bound to the target site (top). The dCas9-gRNA complex binds to increasing numbers of off-target sites upon stretching with 20 pN forces or higher (bottom). Image was adapted from Prof. David Rueda's data published on https://www.youtube.com/watch?v=rm1TfNgMOXI



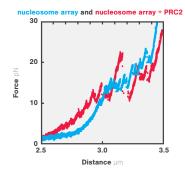
Gutierrez-Escribano et al. (2019) Science Advances (CC-BY).



Manipulating nucleosome arrays to identify the effects of epigenetic proteins on DNA condensation profiles and stability

You can study DNA condensation and organization, such as nucleosome bridging, by tethering nucleosome arrays between optically trapped beads and exposing them to epigenetic modifiers. In this example, researchers stretched an array of nucleosome repeats in the absence and presence of epigenetic modifiers to uncover polycomb-induced bridging. The relative contour-length changes and DNA transitions upon unwrapping can reveal the respective bridging profiles of specific modifiers, such as polycomb repressive complex 2 (PRC2).

The force–extension curves in **Figure 5** show the resulting transitions during the stretching procedure of the respective array, each transition representing the unwrapping of single nucleosomes [6]. The prominent peak observed in the PRC2-exposed array reveals a distal bridging interaction between two nucleosomes, causing an abrupt lengthening of the DNA template.





5 A representative figure of two force–extension curves upon stretching nucleosome arrays in unexposed conditions (blue) or exposed to PRC2 (red). Adapted from Leicher et al. (2020) PNAS.

Extract high-resolution data about the functions and mechanisms of DNA-processing enzymes

Overview of applications:

- Manipulate, measure, and visualize transient and intricate stepping activities of motor proteins processing a DNA molecule.
- Assess enzymatic activities with base-pair resolution to extract detailed information about stepping and DNA-packaging activities and reveal their functional properties and mechanisms.





Check out the papers

[7] Marchetti et al. (2019) NanoLetters



Sample courtesy

[8] Prof. Dr. Carlos Bustamante at IJC Berkeley

Reveal the hidden properties of DNA-processing enzymes with base-pair resolution

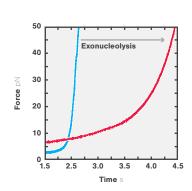
The C-Trap offers you base-pair resolution readouts to assess the activity of enzymes as they process a DNA molecule. As unwinding double-stranded DNA to single-stranded DNA increases its length, you to track the activity of enzymes on the DNA. **Figure 6** shows the exonuclease activity of T7 DNA polymerase as it converts double-stranded DNA to single-stranded DNA.

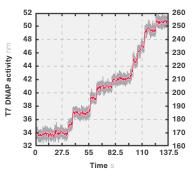
In this experiment, optical tweezers maintained a DNA molecule tethered between two beads at a force that induced exonucleolytic activity of T7 DNA polymerase. The data shows that the enzyme exerted short activity bursts of 3 to 10 nucleotides interspersed by frequent pauses of varying duration.

Define the DNA packaging characteristics of motor proteins with base-pair resolution

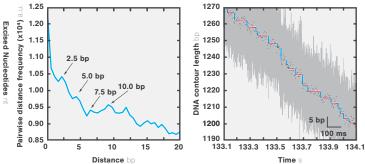
Identify DNA packaging features to understand how motor proteins process DNA during, for instance, viral infections. To this aim, researchers tether a DNA packaging motor protein, such as $\varphi29$, and its corresponding double-stranded DNA substrate between two optically trapped beads. The decreasing distance between the two beads highlighted the DNA packaging with base-pair resolution (**Figure 7**).

Specifically, these high-resolution readouts can reveal the dynamic functions of a single motor protein, such as motor stepping, packaging cycle durations, and amount of packaged DNA [7, 8].









7 Left: Pairwise distribution function of the packaging trajectory, revealing the size of the φ29 motor power strokes, corresponding to ~2.5 bp. **Right:** Trace of DNA packaging trajectory shows the different phases of the mechanochemical cycle in red (dwells) and blue (translocation). Raw data was collected at 78.125 Hz (grey) and decimated to 250 Hz (black dots).

Discover the mechanisms of DNA processes C-Trap® Dymo

Obtain direct evidence of protein function

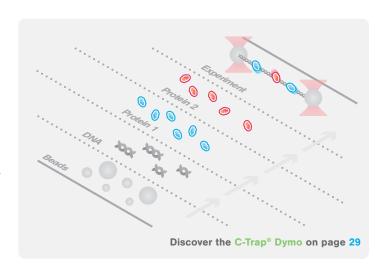
Direct visualization of individual protein dynamics in real-time provides the most direct evidence of protein function.

Reveal how molecular complexes are built

Sequential assembly control and visualization uniquely reveals how molecular complexes are built and activated.

Control and measure local DNA structures

Manipulation and sensing of DNA tension is the easiest way to control and measure local DNA structure



Reveal conformational changes of proteins, DNA, and RNA and their energy landscapes

Overview of applications:

- Measure short-lived, long-lived, and rare conformational changes of single proteins, DNA, or RNA molecules in real time and under different biological conditions.
- Combine optical tweezer measurements with FRET to correlate the mechanical properties of the protein to local structural changes.
- Stretch and relax the captured biomolecule to evaluate its unfolding and folding properties and map the associated energy landscapes.



P

Sample¹ and data² courtesies

[1] Prof. Dr. CarlosBustamante at UC Berkeley[2] Prof. Dr. Hang Shi atTsinghua University



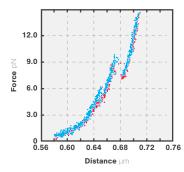
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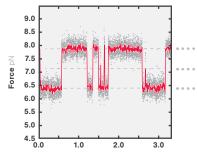
[3] Pelz et al. (2016) Nature Communications [4] Wruck et al. (2021) Communications Biology

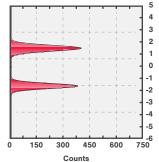
Tracking major and "hidden" conformational states of proteins

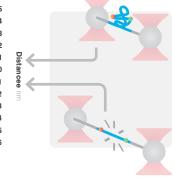
Characterize highly dynamic conformational states of enzymes to improve the selection of protein-specific drug candidates. In this study [1], we tethered the calcium-binding messenger protein calmodulin between two optically trapped beads to study the unfolding and folding properties. The resulting force—distance curve derived upon pulling and subsequent relaxation highlights a reversible two-step unfolding process, corresponding to two helix-loop-helix domains on the enzyme (**Figure 1**).

The protein transition states, observed when holding the optical traps at a constant distance, revealed stochastic fluctuations between two major states (open and closed). The assay also distinguished a third, brief intermediate state (**Figure 2**).







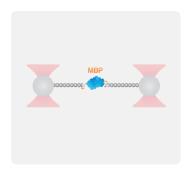


1 Force-distance curves of stretching (blue) and relaxing (red) cycle of a calmodulin protein reveal that the protein unfolds and refolds in two steps.

2 Left: A force trace of equilibrium measurements over 3 s displaying the structural fluctuations of a calmodulin molecule. The gray data show a 50 kHz sampling rate, while the red line shows data decimated to 200 Hz. Center: Histogram representing the major states upon holding the beads at a constant distance. The 3rd intermediate state is negligible due to its transitory profile. Right: Schematic representation of the two states observed in the histogram.

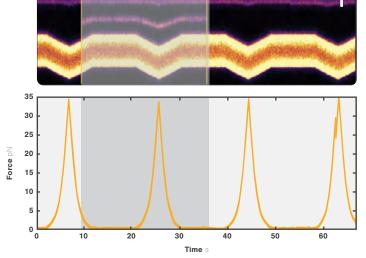
Real-time sensing and imaging of protein-protein interactions upon conformational changes

Visualize the binding of a chaperone to a protein and study the molecular interactions during protein complex formation. In this study, researchers tethered a maltose-binding protein (MBP) between two beads and monitored the binding events of fluorescently labeled trigger factor, a chaperone protein that interacts with MBP, while stretching and relaxing the tethered protein [4]. They could follow how the chaperone bound MBP upon stretching (unfolding) and how it remained bound during cycles of stretch and release (**Figure 3**).



3 Top right: Trace of optically trapped beads with a tethered MBP, showing its unfolding and refolding upon stretching with up to 35 pN and subsequent releasing. Atto647N-labeled trigger factor (excited with 638 nm laser) binds to MBP after unfolding and remains bound (thin line).

Bottom right: A representation of the stretching forces over time. Adapted from Avellaneda et al. (2020) Communications Chemistry (CC BY).



Investigate the unfolding and folding properties of RNA hairpins and uncover their different conformational states

Characterize the stability of DNA and RNA hairpins to understand their physiological properties and improve gene-silencing tools. You can tether an RNA hairpin between two beads using DNA/RNA hybrid handles [2]. In this example, we stretched and relaxed the RNA molecule at a constant pulling speed while determining the resulting mechanical and structural properties (**Figure 3, left**). An approximately 15 nm long unfolding rip appeared at about 8 pN. Relaxing the molecule initiated a protein-folding process that followed the unfolding trace – including the 15-nm rip at 8 pN – which demonstrates the reversibility of RNA hairpin unfolding and its structural stability.

Further evaluation of the RNA structural transitions over time, indicating the structural equilibrium, revealed two major states at tensions around 6.7 pN and 7.7 pN. The assay also exposed a third conformational state, occasionally appearing between the two main states (**Figure 3, right**).

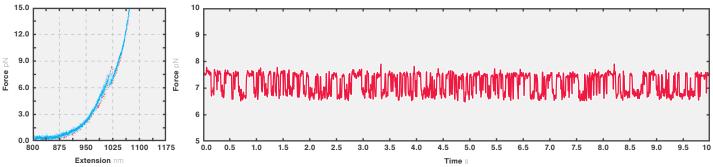
Prof. Dr. Tony Hyman and Prof. Dr. Stephan Grill

Max Planck Institute of Molecular Cell Biology

"The correlation of force traces with fluorescence observation provides very powerful insights for our research. The team has been fighting over system time!"

Photo copyright of MPI-CBG



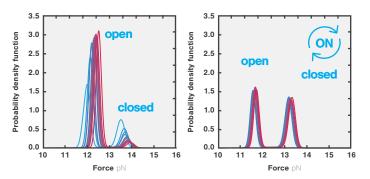


3 Left: Extension (red) and retraction (blue) of a tethered RNA molecule upon increasing pulling forces. Right: Ten-second time trace of a single RNA molecule held at an initial tension of 7.5 pN. Data were decimated to 200 Hz from the high resolution dataset collected at 50 kHz.

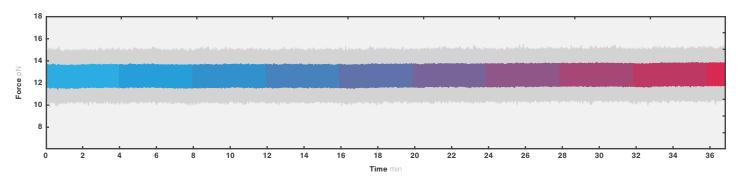
Active trap stabilization improves long-term measurements of conformational changes by increasing stability

Measuring small conformational changes and rare states of DNA, RNA, and proteins requires extremely stable optical traps, as even the smallest drift in the trap positions can confound the outcome of your measurements. To overcome this challenge, the active trap stabilization feature of the C-Trap implements a closed-loop feedback mechanism that actively compensates trap—trap distance drift, independent of the sample and force read-out. These features result in unprecedented stability in equilibrium measurements.

As a proof of principle, we measured the equilibrium dynamics of a single DNA hairpin with the feedback mechanism activated for >35 mins. With active trap stabilization active, we found that the population distribution remained stable throughout the complete experiment (**Figure 4** and **5**), while measurements without active drift compensation significantly shifted the intial population distribution. The data emphasize the power of trap distance lock in enabling you to keep your molecule in the exact same energetic state and perform true, long-term equilibrium measurements.



4 Probability density functions showing the open and closed state distributions of the different time intervals as shown in Figure 5. DNA hairpin pulled at 13 pN pre-tension with a constant trap-trap distance and the active trap stabilization off (left graph) or on (right graph). The initial timepoints (0–4 min) are highlighted in light blue and the last timepoints (36–37 min) in red.

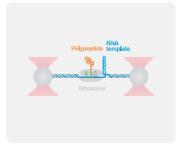


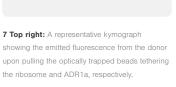
5 Equilibrium measurements of the force changes over time at a constant trap-trap distance and activated trap-distance lock. The tethered DNA hairpin was pulled with 13 pN pre-tension and the measurements were performed for 37 minutes. Raw data (78kHz) shown in light gray, downsampled (100 Hz) data is binned in 4 minutes intervals.

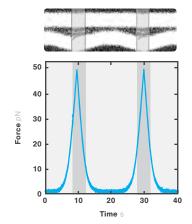
Validate elusive protein folding properties inside the ribosome with amino-acid resolution by combining optical tweezers and single-molecule FRET

Combining single-molecule force spectroscopy with fluorescence measurements through single-molecule Förster resonance energy transfer (smFRET) creates a powerful tool to evaluate protein folding inside a ribosome. Such assessments can be exemplified by tethering a 29-amino-acid protein domain (ADR1a) with incorporated fluorophores to an optically trapped bead and the protein-holding ribosome to a second bead [4].

Pulling the ADR1a-ribosome complex reveals protein folding within the ribosome both through the resulting contour length and fluorescence emission from the donor fluorophore upon unfolding (**Figure 7**). Stretching the complex resulted in an approximate distance of 29 amino acids between fluorophores and fluorescent emission, consistent with in-ribosome unfolding. Combined data from force spectroscopy and smFRET can help you deciphering processes in "hard-to-reach" environments.



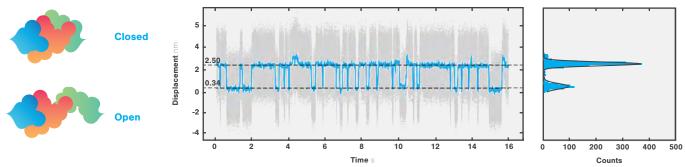




Bottom right: A representation of the stretching forces over time. The gray highlights indicate areas of high tension between the beads. Adapted from Wruck et al. (2021) Communications Biology (CC0).

Recording an enzyme with nanometer precision to reveal inhibitor-induced conformational properties

Characterize highly dynamic conformational states of enzymes to understand their fundamental dynamics and improve the selection of protein-specific drug candidates. By capturing an enzyme, such as phosphotransferase enzyme AdK between two beads, you can test its conformational properties based on the distance-changes between the trapped beads. To evaluate the equilibrium dynamics associated with conformational states, we pulled an AdK protein at low forces (between 6 and 10 pN) in the presence of a substrate analog (AP5A). Exposing AdK with this small-molecule inhibitor induced conformational fluctuations with a bead-distance range of approximately 2 nm, corresponding to the open and closed states (**Figure 8**). Hence, with the C-Trap, you can extract data from highly dynamic processes with nanometer precision to reveal protein conformational properties in varying conditions.



8 Graph depicting the two-state displacement of the protein-tethering beads in the presence of 50 nM of APSA upon pulling. The dashed lines represent the average bead displacement in the closed and opens states, respectively. The biochemistry protocols used here for tethering the protein to the DNA handles were developed in collaboration with the lab of Prof. Matthias Rief (TUM) and were based on Pelz et al. (2016) Nature Communications [3]

Discover the mechanisms of protein folding with the C-Trap® Dymo

Measure the smallest conformational changes

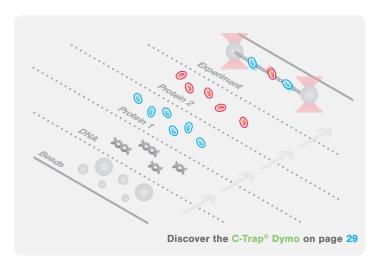
With the superior stability and resolution of the C-Trap, you can observe protein equilibrium fluctuations and folding steps.

Measure protein conformational changes and map the energy landscape

Perform measurements over long periods in true equilibrium of time to identify protein folding and unfolding pathways and map the associated energy landscape.

Correlate global mechanical properties of the protein with local structural information

Combine high-resolution optical tweezer measurements with FRET to follow protein-protein interactions or to spot folding events within a proteins' specific domains.

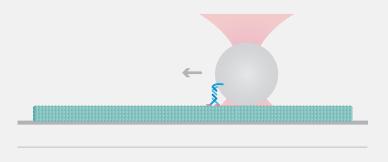




Follow highly dynamic filament and motor protein processes in real time

Overview of applications:

- Assess highly dynamic processes of cytoskeletal remodeling and motorprotein transportation in all 3 dimensions that are essential for molecular and cellular functions.
- Image filaments, motors and other proteins at the single molecule level with high sensitivity and at video rate
- Observe and measure hard-to-capture filament processes, such as microtubule growth and determine the effects of motor proteins.



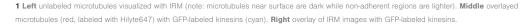


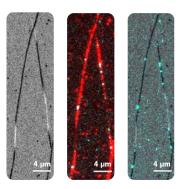
Sample courtesies

[1] Dr. Arne Gennerich at Albert Einstein College of Medicine

Reveal dynamic cytoskeletal interactions using simultaneous label-free IRM and TIRF imaging of motors on microtubules

The unique combination of TIRF microscopy and interference reflection microscopy (IRM) integrated into the C-Trap Edge enables you to study the individual activities of motor proteins on cytoskeletal filaments. **Figure 1** shows the imaging results from GFP-labeled kinesins on label-free microtubules [1]. These images show how correlative imaging techniques enable the study of dynamic cytoskeletal interactions at the single-molecule level with high contrast and spatial resolution. Thanks to the label-free capacity of IRM microscopy there is no need to label the microtubules, reducing sample preparation costs, abolishing photobleaching of the filaments, and preventing potential undesired effects of labels affecting on the behavior of the motors on the filaments.

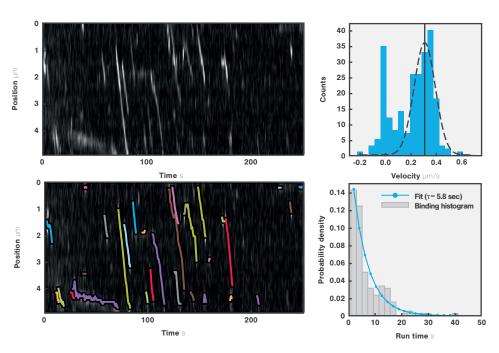




Obtain speed and residence times of motors under different conditions and identify velocity of kinesins walking along a microtubule

In this experiment, we characterized the velocity of kinesins by analyzing the kymographs of GFP-labeled kinesin (**Figure 2**, **left top and bottom**) on individual microtubule filaments [1]. From these data, we then calculated the population of velocities and modelled them as a combination of two Gaussian distributions, one for moving and one for static motors (**Figure 2**, **top right**). The moving motors had an average velocity of 299±82 nm/sec. Note that some motors appeared to display a negative velocity, which is likely due to a crossing microtubule having motors moving in the opposite direction.

Next, we calculated a histogram of the residence time of the motor and fitted a single exponential, which led to a mean lifetime of 5.8 seconds, with 95% confidence intervals (**Figure 2, bottom right**). This example demostrates that the C-Trap can serve as an ideal tool to easily and quickly obtain speed and residence times of different motors under different conditions.



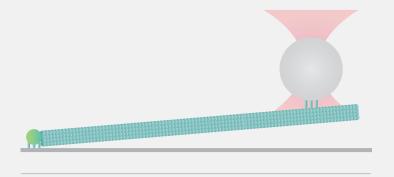
2 Left top and bottom: Single-molecule traces of kinesin motors walking along the microtubules, imaged using TIRF and IRM, and automated identification and characterization of individual kinesins identified.

Bottom left: Quantification of kinesin velocity from a number of traces (N=234). Bottom right: Dwelltime distribution of moving motors (gray) reveal a time constant of 5.8 seconds (95% bootstrap confidence interval between 5.0 and 6.7 seconds). Tracked kymograph lines with a speed <50 nm/sec were excluded from the analysis.

Measure and visualize cytoskeletal properties as a response to manipulation

Overview of applications:

- Capture filaments and manipulate their structure to measure their stabilities.
- Correlate mechanical properties of the microtubule with local structural changes by combining optical tweezers with fluorescence microscopy.





Data^{1,3} and sample² courtesies

- [1] Prof. Dr. Sara Köster at the University of Göttingen
- Prof. Dr. Erwin Peterman at the VU University Amsterdam

Bend cytoskeletal filaments to identify functions and

mechanisms that influence cell motility and division

cytoskeletal filaments and their associated motor proteins.

Manipulating filaments to assess the point of bending and rupture can reveal

mechanical properties that show the regulation of cell motility or division.

Here, we used a kinesin-coated bead to capture a microtubule filament [2]. Figure 4 shows the force exerted on the microtubule upon bending (4-6

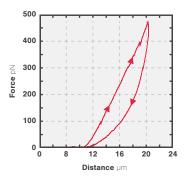
seconds) with subsequent disruption of the complex (6-8 seconds). You can

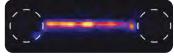
use similar assays to investigate the functions and mechanisms of different

[3] Dr. Zdeněk Lánský at BIOCEV Prague

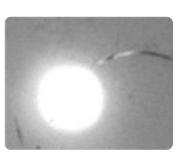
Evaluate mechanical properties of cytoskeletal filaments to learn how they affect cell motility

Measuring the mechanical properties of filaments can reveal how they affect the motility of healthy and malignant cells. To measure these characteristics in a filament, you can stretch and relax the captured molecule to determine its resulting hysteresis [1]. In this experiment, the mechanical properties of vimentin were established by pulling the filament while measuring the force and extension (Figure 3). We used simultaneous confocal fluorescence imaging to resolve the intramolecular remodeling of the vimentin.









3 Left: Force-distance curve of a vimentin filament while stretching and relaxing at a slow speed, allowing for structural equilibrium, revealing hysteresis due to remodeling of the vimentin under high tension. Right: Simultaneous confocal fluorescence imaging of the vimentin labeled with ATTO 647N.

4 Left: The graph shows a force-time curve of microtubule bending. We can observe the disruption event between the kinesin-coated bead and the filament at a force of ~27 pN. Data are shown at 30 kHz (gray) and decimated to 50 Hz (red). Right: The figure shows a snapshot of microtubule bending.

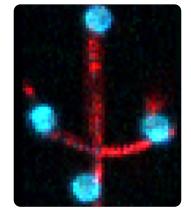
Dr. Neil Kad University of Kent "The combination of force and single-molecule fluorescence, in an easy-to-use system, will accelerate experiments that take days to just take minutes. Making complex tools easier to use, will make ours and the scientific community's science move faster."

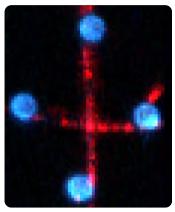
Time

Evaluate how cross configurations of cytoskeletal filaments affect interactions with associating proteins and properties of the cell cortex

In this experiment, researchers used a quadruple trap configuration to measure filament-filament interactions between two fluorescently labeled microtubules in solution [3]. The trapped microtubules were arranged in a cross pattern, and one filament was dragged across the other with a specific force. The configuration served to measure the resulting friction between the two filaments through the surface interaction, as well as electrostatic and ionic forces (Figure 5).

5 Filament interactions studied by dragging one microtubule across the other with a known force. Snapshots show how the friction force between the filaments causes one microtubule to adopt a curved conformation. Once the dragging movement halts, the microtubule slowly relaxes and straightens.

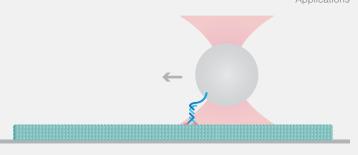




Investigate stepping mechanisms and activities of individual motor proteins

Overview of applications:

- Study molecular motions at the single-molecule level and in real time by tracking the motor proteins with unprecedented resolution and sensitivity.
- Study discrete steps and dynamics of motors in 3 dimensions and perform the experiments under relevant conditions.
- Measure the smallest mechanical changes on a variety of complex molecular assays for long periods of time.





Sample courtesies

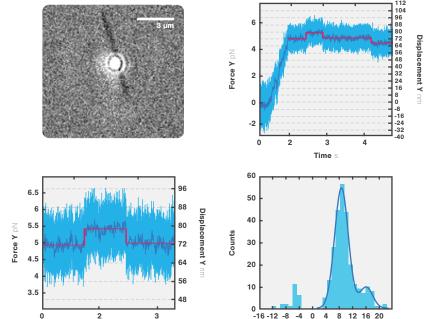
[1] Dr. Arne Gennerich at Albert Einstein College of Medicine

High-resolution measurements to distinguish singlestepping events of motor proteins and stall force

In addition to passively observing kinesin motion, the C-Trap Edge can be used to calculate step size and force generation of kinesin motors in real time. In this example, a kinesin motor was conjugated to a bead and brought into close proximity of a microtubule [1]. The microtubule was visualized by IRM, which does not require fluorescent labeling of the microtubule (**Figure 6. top left**).

We then proceeded to calculate the stall force, which is relevant when trying to understand the kind of cargo a motor can translocate within a cell, or how different cofactors might affect the physical behavior of the motors. In this example, the trap was held static as a kinesin attempted to translocate (**Figure 6, top right**). The results showed approximately 6-5 pN stall forces. Additionally, at the stalling force, the data also revealed the forward and backward stepping of the motor with a step size of 8 nm (**Figure 6, bottom left**).

Next, to study the force that individual motors can exert we followed the motion of the motor. In this example, the motor was walking against a constant, backward force of ~2pN applied by the trap and its motion followed (**Figure 6, bottom right**). This approach revealed how the motor walks with 8-nm steps and provided information on the number and duration of pauses or the instantaneous speed at which the motor walks.



6 Top left: A sample screenshot from IRM timelapse imaging of a kinesin-coated microsphere walking along a microtubule. Top right: Force vs. time trace of a single kinesin against a static trap, showing a stalling force at ~5pN. Bottom left: Forward and backward 8-nm steps are observed from single kinesin stepping against a static trap. Bottom right: Histogram of 2D force clamp experiment following the motion of the motor, revealing 8 nm and 16 nm steps, N=285. Force and displacement data was recorded at 78 kHz (light blue) and noise was reduced by down-sampling to 200 Hz (dark blue.

Time

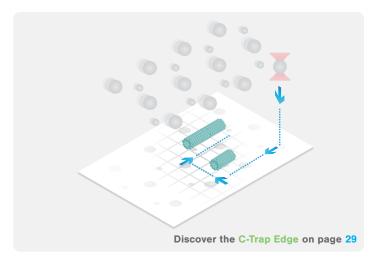
Discover the mechanisms of cytoskeletal processes with the C-Trap® Edge

Perform one-of-a-kind experiments to investigate the cytoskeletal motors and filaments

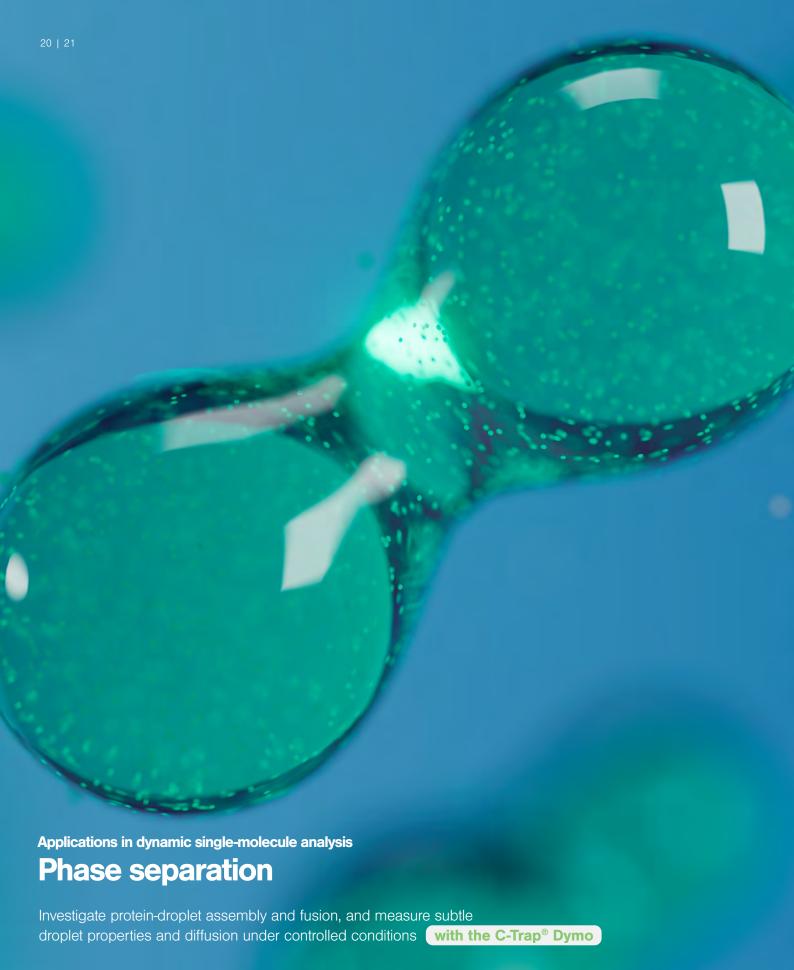
Optical tweezers have the highest force resolution in all dimensions and optimized stability near the surface. This enables not only measuring the smallest steps and load on cytoskeletal motors, but also measure filament bending.

Easier and faster time to impactful publication with our unique user-friendly workflow providing sequential-molecule throughput

Dedicated workflow, label-free visualization of filaments, active surface stabilization, and correlated data ensures the fastest time to the most impactful results.



Step size nm



Measure protein-droplet assembly, fusion, and viscosity in real time

Overview of applications:

- Manipulate, measure, and visualize dynamic droplet properties in different conditions.
- Investigate changes to the phase of matter of protein droplets to understand condensate-associated cell functions and the development of pathological structures.



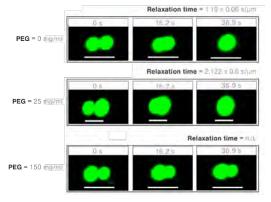


Check out the papers

[1] Kaur et al. (2019) Biomolecules [2] Jawerth et al. (2018) Phys Rev Lett, [3] Alshareedah et al. (2021) Nature Communications

Directed droplet fusion can reveal the structural properties of protein droplets in different molecular conditions

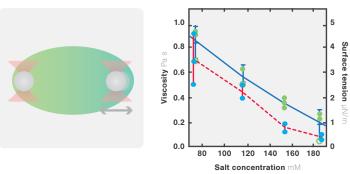
Fusion experiments are excellent indicators of protein-droplet rigidity, a characteristic that can affect cellular processes. By trapping protein droplets and direct them into adhesive contact, we can induce and time the fusion of individual droplets [1]. The assay can test how external or internal queues influence the structural properties of individual droplets, including the effects of molecular crowders or different protein variants. **Figure 1** shows how different concentrations of molecular crowder polyethylene glycol (PEG) affect the fusion times between droplets.



1 Images showing time-lapses of FUS droplet fusion as a function of PEG8000 molecular crowder concentrations. Droplet fusion was induced by bringing the trapped droplets into proximity at constant velocities in the presence of PEG8000. Adapted from Kaur et al. Biomolecules, 2019 (CC BY).

Microrheology reveals the viscosity of a single protein droplet in different conditions

Microrheology offers reliable insight into the viscosity of protein droplets, a material property that affects cellular functions. To measure the microrheological properties of droplets, you trap two beads and bring them into adhesive contact with a single protein droplet [2]. While maintaining one of the traps static, the second bead deforms the droplet by bidirectional movements. Through this assessment, you can directly measure droplet stiffness and viscosity in different environmental conditions, for example, varying salt concentrations (**Figure 2**).

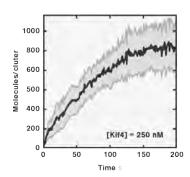


2 Left: Schematic representation of a microrheological experiment performed with optical tweezers.
Right: Graph representing changes in viscosity (red) and surface tension (blue) as a function of increasing salt concentrations. Recreated from Jawerth et al. (2018) Phys Rev Lett.

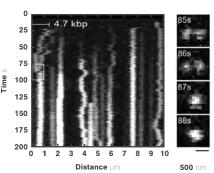
Record the development of sequence-dependent condensates of transcription factors on DNA using optical tweezers with real-time fluorescence imaging

Combine optical tweezers and fluorescence microscopy to study the condensation of transcription factors on DNA and understand how it affects DNA organization inside the cell nucleus. Exposing a captured DNA molecule to fluorescently tagged transcription factors, such as Klf4 [3], gives you real-time data on the concentration-dependent foci formation.

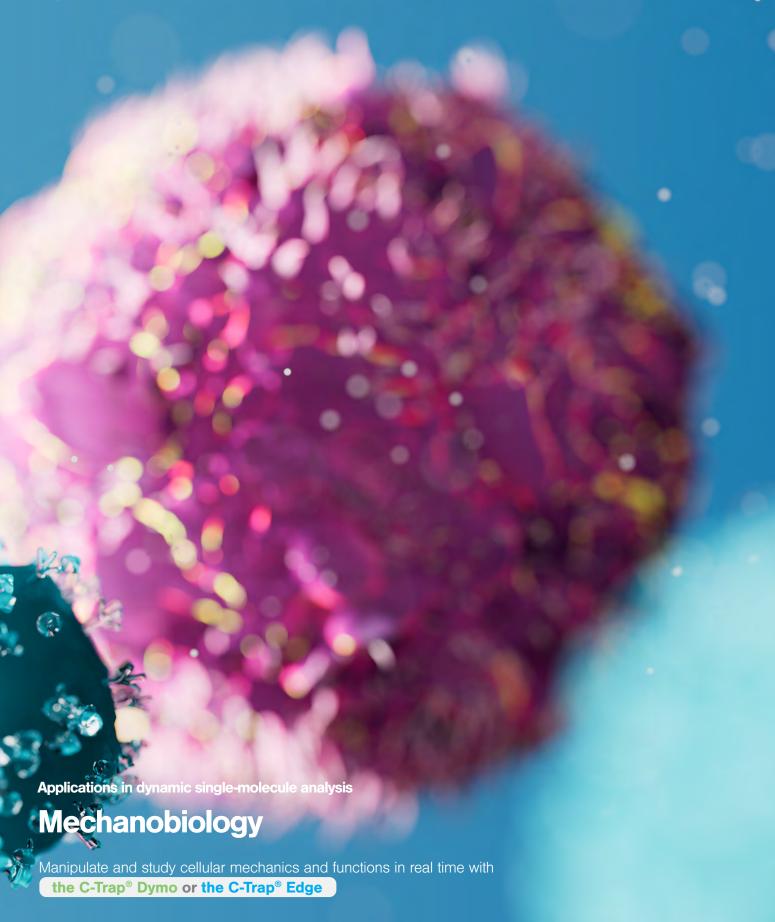
The kymograph in **Figure 3** shows how Klf4 forms growing foci along the tethered DNA molecule at physiological concentrations. Throughout their growth, these condensates fuse and change position until they reach saturation. Following the clustering in real time through fluorescence imaging and comparing excitation intensities allowed the researchers to determine the relative number of condensing Klf4 molecules per cluster and their sequence-dependent positions over time.







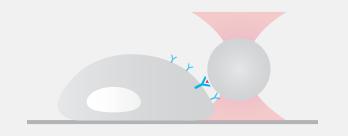
3 Left: Plot presenting the mean increase (black line) in number of GFP-labeled Klf4 molecules per focus over time. Gray area represents the standard error of the mean. **Right:** A kymograph showing the foci formation of Klf4 condensates. The vertical panels present the fusion observed in the kymograph (white box). Adapted from Morin et al. (2021) bioRxiv (CC-BY).



Investigate cellular responses in living cells and organisms

Overview of applications:

- Study how mechanical stimuli or stresses affect cell receptor responses by applying forces in the 100s of pN range to manipulate the cell surface and by following multiple cellular components with multi-color microscopy
- Follow filopodia formation and actions in real time using fluorescence and track forces associated with cellular interactions and environment.





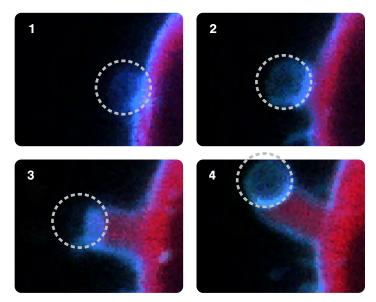
Sample courtesy

[1] Prof. Dr. Jonathan Javitch at Columbia University [2] Prof. Dr. Margaret Titus at the University of Minnesota [3] Alshareedah et al. (2021) bioRxiv (preprint)

Study how mechanical stimuli or stresses affect cell receptor responses using correlated visualization with manipulation and force readouts

Make use of the C-Trap to understand the properties involved in themechanical activation of transmembrane receptors [1]. Applying a controlled stimulus using optical tweezers, allows you to monitor the activation of intracellular responses and signals in real time.

To demonstrate, we started by placing a bead into contact with the surface of a human HEK293 cell and a cytosolic marker (red signal). We then moved the bead away from the cell and followed the changes on the membrane using confocal microscopy. At the same time, we tracked the forces exerted on the membrane and identified four distinct regimes. **Figure 1** shows how moving the bead away from the cell with high caused deformation of the membrane and accumulation of receptors at the site of manipulation. As we proceeded, minimal applied forces on the cell membrane were enough to pull out a thick membrane tube. The results suggest a correlation between membrane deformation and local accumulation of receptor protein (blue signal).



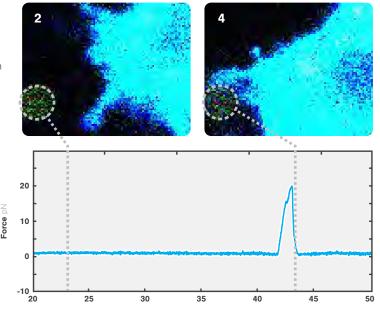
1 Top Confocal microscopy images of a cell expressing fluorescently labeled biotinylated receptors (blue) and a cytosolic marker (red) showing changes in cell membrane, receptor and cytosol-associated activity, upon manipulation of the polystyrene bead with a laser (grey dashed circle), show cell membrane deformation and receptor accumulation at the site of manipulation.

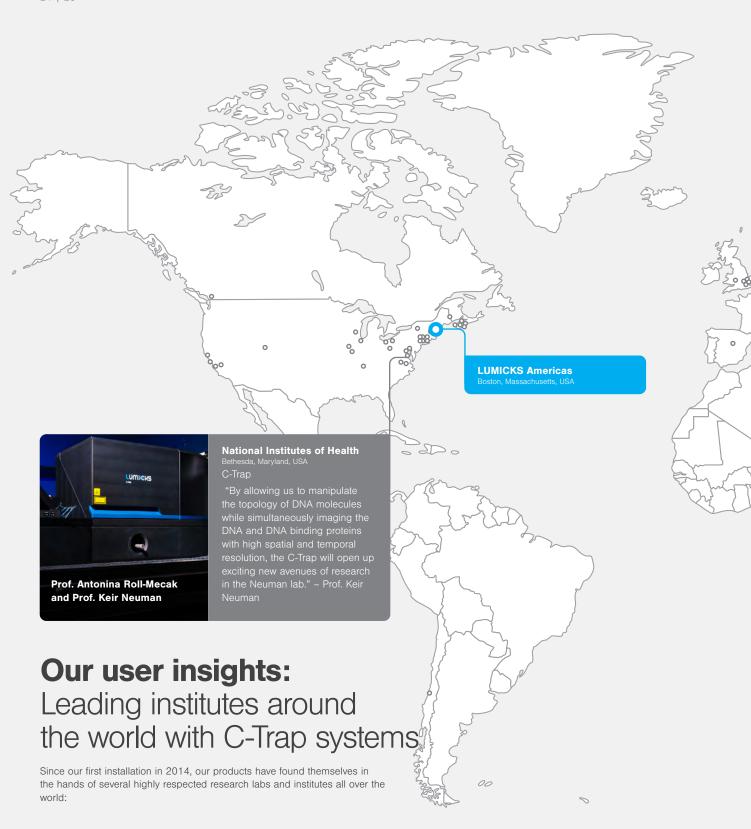
Track forces linked to filopodia formation and functions

To highlight the forces and kinetics associated with filopodia formation, we performed an experiment together with the lab of Prof. Margaret Titus [2]. To assess the process, we studied Dictyostelium discoideum cells, a eukaryotic amoeba cell and model system that is commonly used to understand human cell processes.

In this experiment, we placed a bead in proximity to the D. discoideum cell, ectopically expressing GFP-myosin 7 (blue signal) and actin filament marker RFP-LifeAct (**Figure 2**). The cell moved in the direction of the trapped bead, extending multiple protrusions towards it upon sensing the proximity of the object. Each time the cell engulfed the bead we observed a characteristic signal and found an occasional force spike right before bead engulfment (complete force graph shown in **Cellular mechanics and functions** application note). These results indicate that the peak force value shown on the graph (around 20 pN) corresponds to the force that a single protrusion exerted over the foreign object.

2 Confocal microscopy images of a cell expressing fluorescently labeled biotinylated receptors (blue) and a cytosolic marker (red) showing changes in cell membrane, receptor and cytosol-associated activity, upon manipulation of the polystyrene bead with a laser (grey dashed circle), show cell membrane deformation and receptor accumulation at the site of manipulation





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Cambridge University

Hong Kong University of Science and technology (HKUST)

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Emory University

Max Planck Institute Dresden (MPI-Dresden)

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Westlake University

Francis Crick Institute

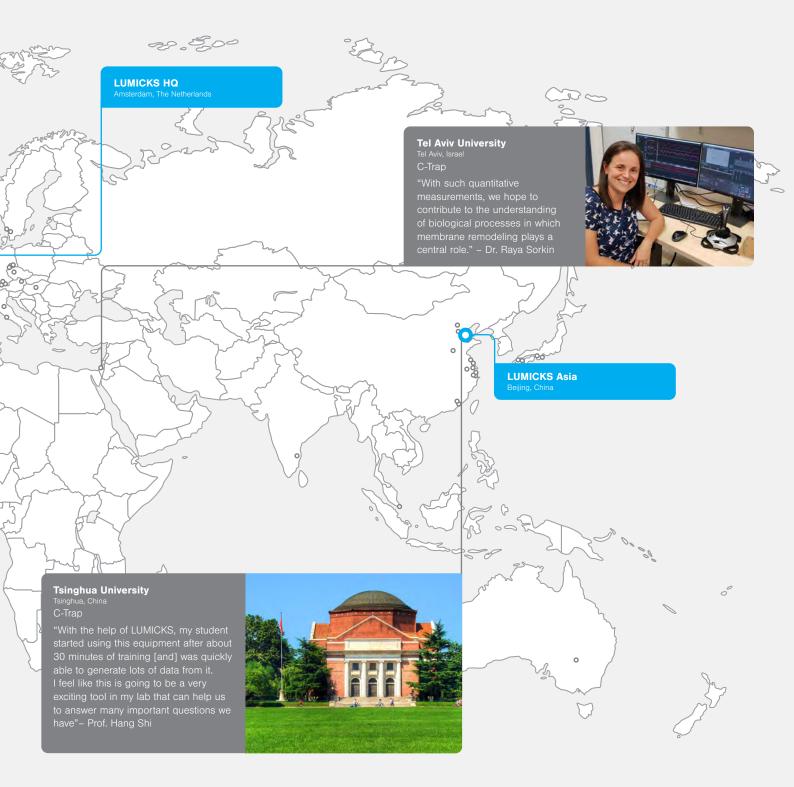
The University of Chicago

University of Zurich

University of Groningen

Bethesda, Maryland, US

CSIC Madrid



Features and options

With the desire to constantly produce breakthrough discoveries, you need an easy-to-use instrument, fully-optimized for your applications. It should bring unprecedented insights with high precision, accuracy, and reliability, in short time.

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Explore C-Trap® Dymo and Edge

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High-throughput experiment workflow



Introducing the new C-Trap products

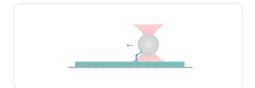
Dymo & Edge

Get unprecedented insights in a short time with the C-Trap

The C-Trap gives you access to three key features: stable and precise sample manipulation and measurements, a wide variety of visualization capabilities, and a high-throughput experiment workflow for both solution and surface assays. Explore below the different optical-tweezers configurations of the C-Trap and learn all about its manipulation and visualization features that allow you to get the most out of your sample.

Single trap

For highly stable assays at the surface



Dual trap

For your assays in solution



Quadruple trap

Excellent for studying complex interactions



Confocal fluorescence, upgradable to STED Super Resolution

Confocal: With its fast 1D scanning capabilities, confocal is optimized for 1D constructs, such as DNA or filaments. Up to 3-color confocal fluorescence for visualizing multiple proteins at the same time.

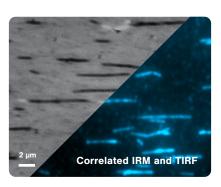
STED: Upgrade to C-Trap **SR** for stimulated emission depletion (STED) nanoscopy to distinguish between individual proteins in close proximity and perform experiments with unprecedented resolution (< 35 nm).

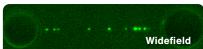
TIRF, Widefield fluorescence and IRM

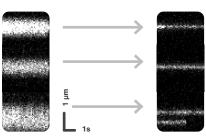
TIRF: Suitable for visualization on surfaces as it eliminates background fluorescence outside the focal plane.

IRM (Interference reflection microsopy) allows you to visualize microtubules without the need for fluorescence labeling.

Widefield: Image biomolecular processes in solution at high acquisition rates.







STED Super Resolution



C-Trap® Dymo:

For resolving DNA and protein dynamics and interactions

The C-Trap Dymo is purposefully built and optimized to provide the ultimate tool for solution assays. Available with confocal or widefield fluorescence and optional STED, with two or four traps, it is the perfect tool for the specialist deep-diving into one specific application or the facility providing access to users working on a variety of different solution assays.



DNA-binding proteins

Study molecular mechanisms involved in DNA repair, repair, replication, editing, transcription, and chromatin organization.



Protein folding

Study how proteins fold correctly and undergo conformational changes to accomplish their biological function.





Phase separation

Measure protein droplet assembly, fusion, and properties in real-time.

For multi-user facilities

Use widefield imaging to unlock solution

C-Trap® Edge:

For unprecedented cellular and cytoskeletal insights

The C-Trap Edge is a next generation tool developed specifically for ultra-stable surface assays at the single-molecule level. Designed from the bottom-up, this solution enables scientists to investigate the cytoskeleton from a new dynamic perspective. With imaging on the surface (smTIRF, IRM) and workflow tools that enable surface assays, it brings the best of surface imaging and optical tweezers tweezers into a fully integrative platform.



Cytoskeletal structure and transport

Study the activity and mechanical properties of



Mechanbiology

Manipulate and study cellular mechanics and



High-throughput experiment workflow

Load your sample and analyze your data set in less than 30 minutes.

1

Load your sample into the flow

with fast and simple pipetting

For solution assays, load your samples and conditions into the syringes of the 5-channel automated microfluidics system. Pipetting each reagent takes seconds thanks to the twist-and-lock syringe adaptor, with which you can quickly and easily refill individual syringes.

For surface assays, simply coat a coverslip with your sample, apply your protein and bead solution, seal, and load the reagents into the flow cell holder.

Regulate the pressure and ontrol each of the channel with a mouse click

2

Assemble your assay without physical barriers

with our optimized workflows for solution and surface assays

For solution assays, you can seamlessly move your optical traps to gather beads, move them between the 5 microfluidics lanes, and assemble your constructs.

For surface assays, double-click on a bead to trap it. You can easily locate your samples using, for example, IRM or fluorescence microscopy. The high-resolution piezo-controlled nanostage, allows you to accurately move between positions (x,y, and z) to place the trapped bead at the end of the microtubule and start your experiment.

3

Perform and automate your experiments

with our Bluelake software suite

BluelakeTM is the C-Trap's intuitive and user-friendly software suite. It contains functions that simplify, automate, and enhance single-molecule experiments and real-time data gathering.

Repeatable experiments are key for gathering statistically relevant quantities of data and publishable results. Bluelake allows for automation through the implementation of scripting with full access to all relevant system parameters and data streams. This way, any user can fully automate all kinds of repeated experimental procedures, enabling experiments to be performed autonomously.

It takes roughly 80 seconds for Bluelake to assemble an assay and run an experiment. In just half an hour, it's possible to get 18 useable sets of data, as we demonstrate here on the right.

Automate your experiments

4

Organize and analyze your data

with a dedicated software suite

Generate a structured data overview with a fast and smooth navigation through multiple days of

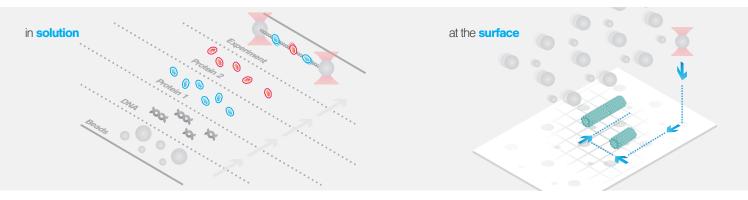
View, compare, and export your fully correlated data stream during or after the experiment. The dedicated software automatically stores all your metadata so you never lose valuable information and always have the option to reproduce your experiments.

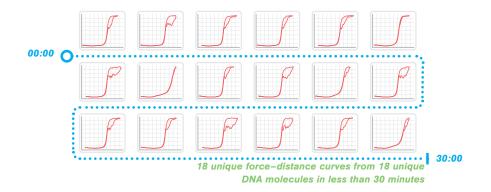
Analyzing and repeating experiments has never been easier, as our analysis software comes with tutorials and sample notebooks that can serve as a scaffold for your own analyses.

Use the open-source **Harbor** platform to upload, download, and review user scripts for free:

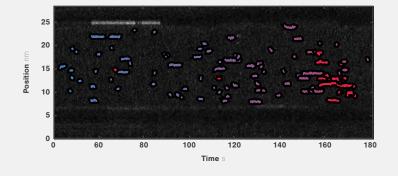
https://harbor.lumicks.com







Kymotracker is a data analysis package that automatically tracks individual fluorescent traces on a recorded kymograph, allowing you to easily quantify protein-binding events and evaluate them over time.







Beyond the product

Faster, easier, and more reproducible with our new biochemistry services and script-sharing platform—Dynamic single-molecule research has never been so accessible to so many backgrounds.

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Harbor: The meeting place for DSM scripts

page

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Reagents, kits, and services store

page

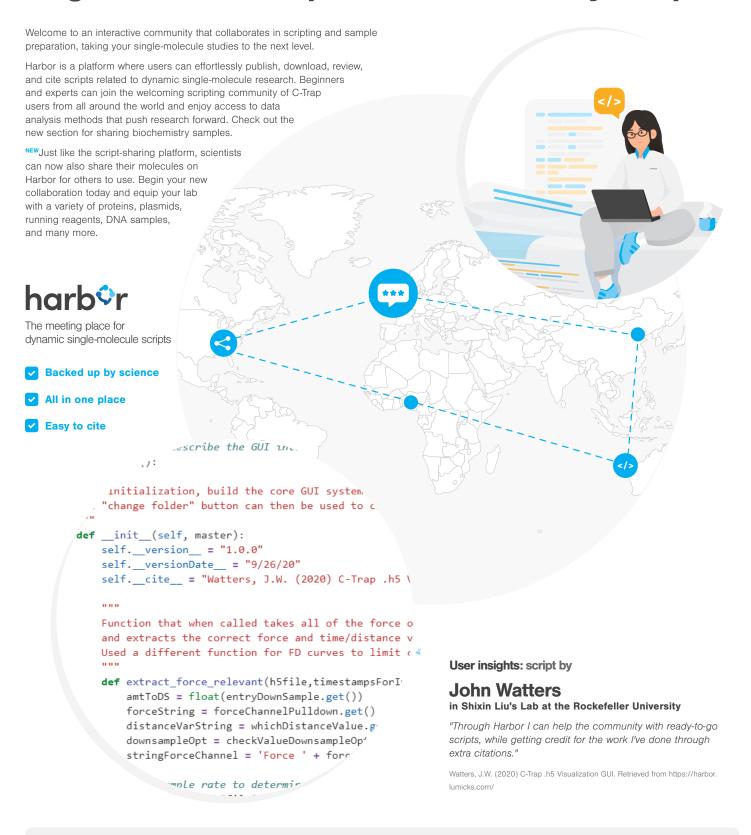
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Publications and resources

E:He.



Harbor: the meeting place for dynamic single-molecule scripts and biochemistry samples



Join for free today www.harbor.lumicks.com

Reagents, kits, and services store

The reagents, kits, and services in the LUMICKS Store helps C-Trap users to generate data faster by optimizing the time spent on the system.

All items in our store have been selected to simplify the user experience and enhance the efficacy of our instruments through:

- Ready-to-use products that allow you to start your experiments quicker by eliminating the need to invest time, money, and personnel to set up experiment workflows.
- Established, tested, and optimized protocols that ensure your instrument is optimally used – no loss of time or materials for troubleshooting. The samples work right away with a high tethering efficiency.
- Standard experiment kits that facilitate the practical training of new users and necessary application testing.



User insights Prof. **Kasia Tych**University of Groningen

"The kit is very easy to use and worked right away. I saved a lot of time compared with setting up the whole biochemistry production workflow myself."

What's in store for you?

DNA/RNA-protein interaction kits

- Ready-made ssDNA and dsDNA samples, ideal for localizing fluorescent DNA-binding proteins or measuring local conformational changes of the DNA molecule.
- Custom constructs such as DNA and RNA hairpins, DNA with specific binding sites, or sitespecific fluorescent markers for FRET measurements.



Protein folding and conformational changes

- Protein-tethering master kit, including DNA handles and oligonucleotides, labeling protocol, quality control test protein, beads, and all reagents needed for your single-molecule experiments.
- Custom protein service taking care of the full package: design, purification, and labeling of your
 protein of interest. Give us your protein wish list, we'll take care of the rest.



Running reagents and consumables

- Ultrapure filtered buffers of the highest quality.
- · Ready-made solutions optimized for single-molecule tethering.
- Functionalized beads in multiple sizes, to cover all of your needs.





Start shopping today www.store.lumicks.com

Publications and resources to inspire your future optical tweezer experiments

Some recent publications with the C-Trap

- Belan et al. <u>Single-molecule analysis reveals cooperative stimulation of Rad51 filament nucleation and growth by mediator proteins.</u>

 Molecular Cell 2021
- Budaitis et al. Pathogenic Mutations in the Kinesin-3 Motor KIF1A Diminish Force Generation and Movement Through Allosteric Mechanisms. JCB 2021
- Kaur et al. <u>Sequence-encoded</u> <u>and Composition-dependent</u> <u>Protein-RNA Interactions</u> <u>Control Multiphasic Condensate</u> <u>Morphologies</u>. Nat. Commun. 2021
- Mei, L. et al. <u>Molecular mechanism</u> for direct actin force-sensing by α-catenin. eLife 2020

- Kraxner et al. Post-Translational
 Modifications Soften Vimentin
 Intermediate Filaments. Nanoscale
 2020
- Avellaneda et al. Processive
 extrusion of polypeptide loops by a
 Hsp100 disaggregase. Nature 2020
- Khawaja et al. <u>Distinct pre-initiation</u> steps in human mitochondrial translation. Nat. Commun. 2020
- Sorkin et al. <u>Synaptotagmin-1 and</u>
 <u>Doc2b Exhibit Distinct Membrane-Remodeling Mechanisms</u>.

 Biophysical Journal 2020
- Zhang et al. <u>Dynamics of</u>
 <u>Staphylococcus aureus Cas9</u>
 <u>in DNA target Association and Dissociation</u>. EMBO Rep 2020

C-Trap publications: an average impact factor of

12





"After one week of experiments in the LUMICKS headquarters during our visit, we had generated enough preliminary results to support our grant application but also to complement the ensemble data in our paper which was published in Nature Communications"



Check out the paper

Kosinski et al. Sites of high local frustration in DNA origami. Nature Communications 2019



User insights Prof. Luis Aragón

Principal Scientist at London Institute of Medical Sciences (MRC)

"C-Trap is a unique and powerful tool that helped us unravel precise molecular mechanisms on how cohesin tethers chromatids. By using the shared instrument located at the Imperial College London together with our collaborators, we were able to perform valuable measurements and build the data presented in our publication in a few weeks of measurements."



Check out the paper

Gutierrez-Escribano et al. A conserved ATP- and Scc2/4-dependent activity for cohesin in tethering DNA molecules. Science Advances 2019

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Visit www.lumicks.com to find exclusive interviews, webinars, and video testimonials from our customers and experts on dynamic single-molecule around the world, sharing their views and their research DSM analysis.



On-demand webinars

Educational webinars with key guest speakers, experimental webinars and more.



DSM symposium series

Key speakers discussing their work, the C-Trap and the benefits of the instrument in their work.



Video testimonials

Training sessions testimonials, production workflow introductions and more.

Our application notes

Designed to inspire your next optical-tweezers experiments

Check out our application notes to learn more about the possibilities of dynamic single-moecule research: https://lumicks.com/knowledge/dsmapplications/, but here's a sneak peek.



Real-time detection of kinase conformational changes in the presence of a small-molecule inhibitor

Perfect for protein research, as it showcases how you can use the C-Trap to measure protein conformational changes in response to different conditions. Find out:

- How we tether a single protein to optically trapped beads.
- How we measure conformational changes of AdK upon exposure to smallmolecule inhibitor AP5A.
- The workflow and features that make these types of experiments fast and straightforward, regardless of your previous experience level.

Manipulate and study protein droplet dynamics and properties in realtime to understand phase separation

Four published experiments describe the fundamental processes linked with phase separation through C-Trap data. Learn how the C-Trap correlated optical tweezers and fluorescence microscopy can measure and visualize assembly, fusion, and solidification processes of protein droplets in real time:

- Which factors regulate liquid protein droplets and their transitions to solid aggregates.
- The relationships between protein droplets and irreversible solid amyloid fibril structures, linked with certain diseases.
- How the material properties of protein droplets change during solidification.

Analyze Cas9 binding and cleavage properties in real-time while manipulating DNA structures

We show you how the lab of Prof. Dr. David Rueda obtained high-resolution, real-time data with the C-Trap revealing Cas9-targeting properties upon structural changes of a target DNA molecule. The assessed features include:

- · Genomic binding specificity.
- Off-target dwell times.
- Sequence-dependent binding properties.
- DNA structure dependent binding properties.
- Cleavage kinetics.

Specifications of the C-Trap Dymo

	Iweezers
:	Optical

Force resolution (x,y)	< 0.1 pN @100 Hz*
Force stability (x,y)	< 0.3 pN over 2 minutes*
Force acquisition rate (x,y)	78 kHz on two traps
Maximum escape force in dual trap mode (x,y)	> 1000 pN for 4.5 µm polystyrene beads
Bead displacement resolution using force signal	< 0.3 @100 Hz
Bead displacement resolution using live brightfield bead tracking	< 3 nm @100 Hz
Minimum incremental step size	0.5 nm
Field of movement (x,y,z)	50 μm x 50 μm x 9 μm
Numer of independent traps	1-2 or 1-4°

Confocal fluorescence**

Contocal resolution	Dilitaction little
1D STED resolutiond	< 35 nn
Spot position accuracy	< 5 nn
Localization precision	< 15 nn
Scanning speed	Up to 100 H
Sensitivity	Optimized for single fluorophore detection
Dark counts	< 0.025 photons per pixel dwell time (typically 0.1 ms
Background rejection limit	100 nM at 1 ms integration time
Photon time tagging accuracy	10 n:
Field of view (x,y)	50 μm x 35 μn
Number of confocal colors (default: 488 nm. 561 nm. 638 nm)	1ª or up to 3

Sample handling

Microfluidics	Advanced microfluidics with
Temperature control absolute accuracy ^b	0.2°C
Temperature control stability ^b	0.05°C
Temperature control range ^b	Room temperature to 45°C

Specifications of the C-Trap Dymo options

Force resolution (z)	< 0.1 pN @100 Hz***
Force stability (z)	< 0.3 pN over 2 minutes***
Surface assay toolkit	Nanostage, near surface force calibration, height determination
*** for 1 µm beads at ≥ 0.05 pN/nm trap stiffness	

Short tether package

Trap distance stability (x,y) using active trap stabilization	< 0.1 nm drift over 10 minutes
Short tether assay toolkit	Nanostage with active calibration, short tether piezo tracking, baseline correction

- Dymo 200
- Only available for Dymo 300, 400w, 400, 700
- Only available for Dymo 400w, 400, 700
- d. Only available for Dymo 700

Specifications of the C-Trap Edge

Optical Tweezers

Force resolution (x,y,z ^b)	< 0.1 pN @100 Hz*
Force stability (x,y,z ^b)	< 0.3 pN over 2 minutes*
Force acquisition rate (x,y,z ^b)	78 kHz on two traps
Maximum escape force in dual trap mode (x,y)	> 1000 pN for 4.5 µm polystyrene beads
Bead displacement resolution using force signal	< 0.3 @100 Hz
Bead displacement resolution using live brightfield bead tracking	< 3 nm @100 Hz
Minimum incremental step size	0.5 nm
Field of movement (x,y,z)	50 μm x 50 μm x 9 μm
Numer of independent traps	1-2 or 1-4°

^{*} for 1 μ m beads at \geq 0.35 pN/nm (x,y) and at \geq 0.05 pN/nm (z b) trap stiffness

Widefield/TIRF fluorescence

Widefield/TIRF resolution	Diffraction limited
Pixel size	~ 72nm
Camera frame rate at full FOV	> 125 Hz
Field of view (x,y)	45 μm x 45 μm
Number of TIRF colors (488 nm, 561 nm, 638 nm)	1ª or up to 3 ^b
Number of widefield colors (488 nm, 561 nm, 638 nm)	1 ^a or up to 3 ^b

IRMº

IRM resolution	Diffraction limited
Pixel size	~ 77nm
Camera frame rate at full FOV	> 25 Hz
Field of view (x,y)	> 25 µm x 25 µm

Sample nandling

Microfluidics	Advanced microfluidics with automated valves
Temperature control absolute accuracy ^b	0.2°C
Temperature control stability ^b	0.05°C
Temperature control range ^b	Room temperature to 45°C
Surface assay toolkit	Nanostage, near surface force calibration, height determination ^b
Surface stability (z) ^b	< 3 nm over 2 minutes

Specifications of the C-Trap Edge options

ether	age
hort t	pack

Trap distance stability (x,y) using active trap stabilization	< 0.1 nm drift over 10 minutes
Short tether assay toolkit	Active calibration, short tether piezo tracking, baseline correction

- a. Edge 250
- b. Only available for Edge 350, 450
- c. Only available for Edge 450

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