m-Trap™

Dedicated High Resolution Optical Tweezers

m-Trap™ I Product Brochure

The m-Trap™ is the first dedicated optical tweezers instrument specifically developed for high-resolution single-molecule force spectroscopy research. Ultra-high force resolution and stability, with incredible throughput & ease of use, all at an unprecedented price level. This enables the characterization of molecular structural transitions and interactions at the Ångström scale, in a quick and reliable manner.

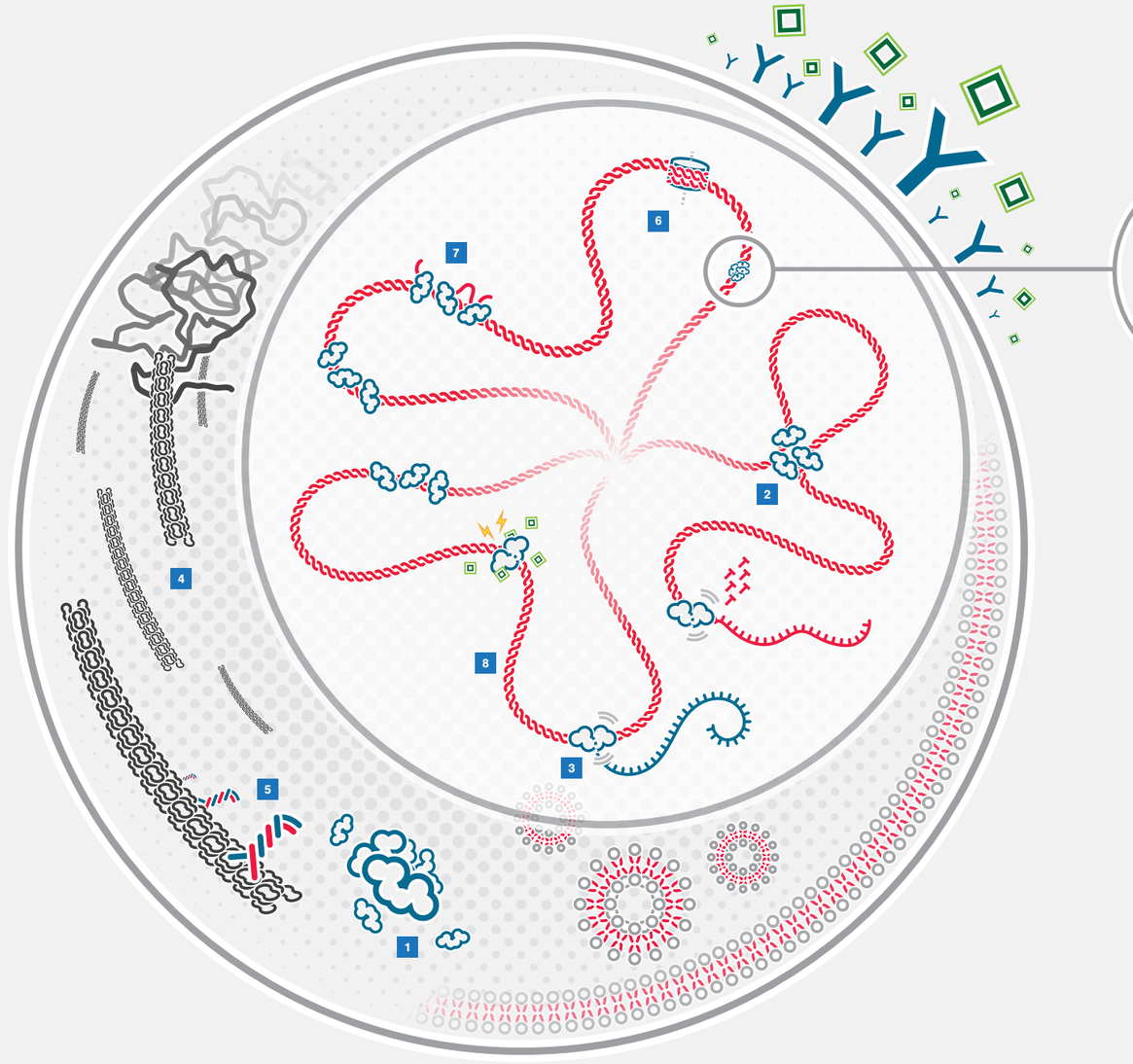
A new milestone for single-molecule research.

The characterization of biology at the single-molecule level has led to novel insights into the crucial molecular and cellular processes underlying life, disease, and successful therapy. As science is evolving towards the smaller scale, single-molecule research is rapidly emerging to play an even bigger role in the fields of life science and pharmaceutics.

Our dream is to unlock single-molecule analysis.

Understanding the root causes of diseases at the molecular level is one of the greatest scientific challenges of today. Expanding the knowledge of biological processes that are at the basis of diseases is key for their prevention and the development of future cures. We aim to create the best possible tools for researchers to perform high-quality, high-throughput single-molecule experiments in the most accessible manner.

With this in mind, we developed the m-Trap™: a dedicated high-resolution optical tweezers instrument which lowers the price barrier for access to state-of-the-art single-molecule force spectroscopy without any compromise on key performance specifications.



1 **Protein Unfolding**

See Page 6

2 **DNA Replication Activity**

See Page 7

3 **Transcription Activity**

4 **Polymers & Protein Filaments**

5 **Cytoskeletal Motors Activity**

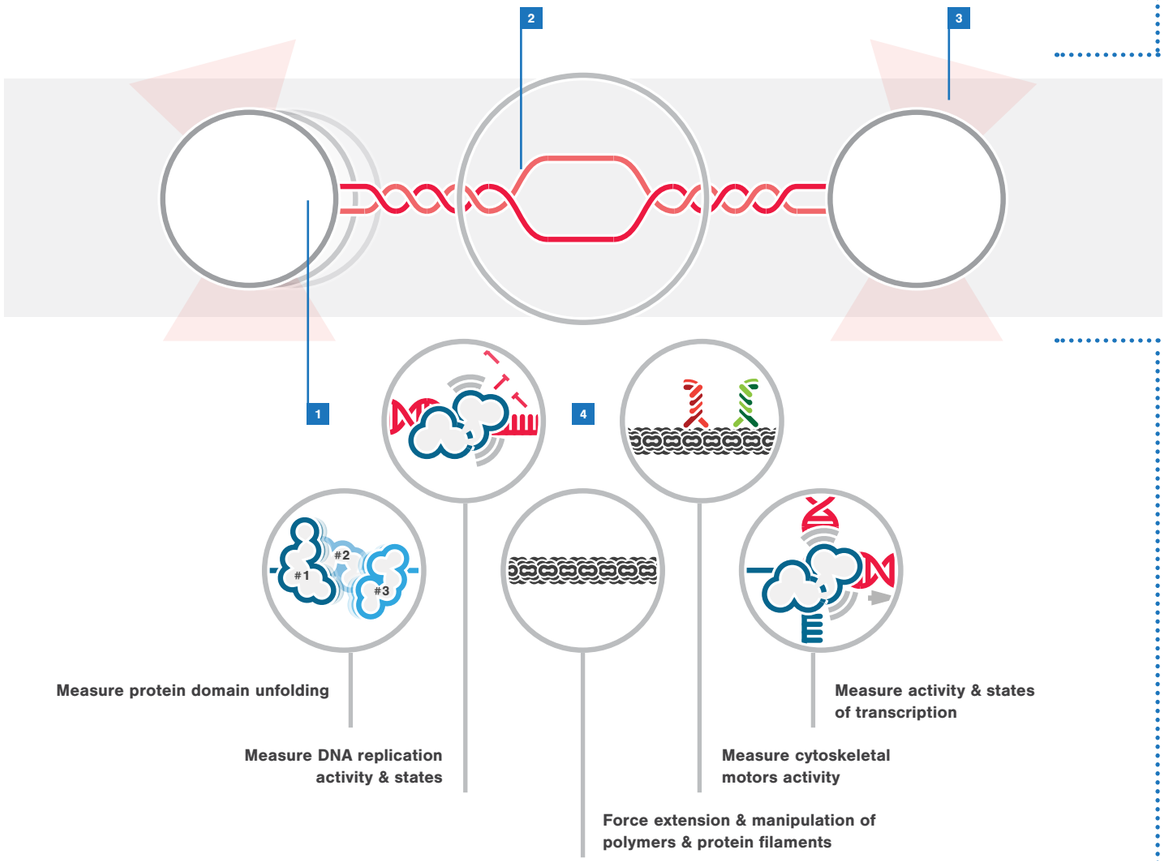
6 **Conformational Changes of DNA Organization**

7 **DNA Repair Force Measurements**

8 **DNA/RNA Structural Dynamics**

POSSIBILITIES

DIscover the power of optical tweezers.

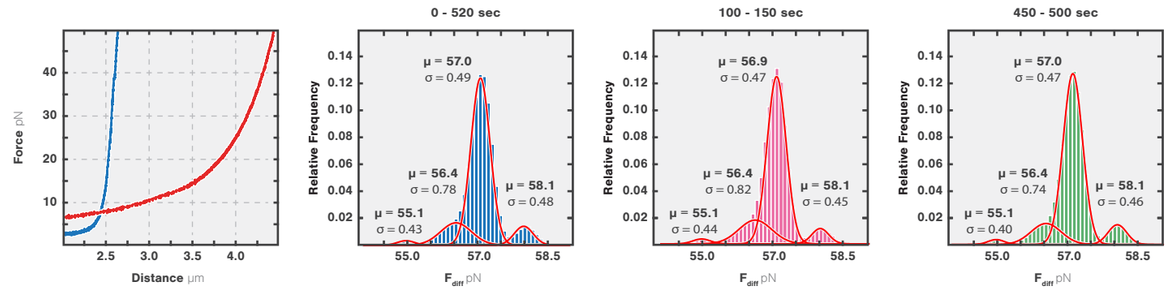


1 Microsphere or “bead” trapped in the focal point of a laser beam, called the optical trap.

2 Biomolecule tethered between two optically-trapped beads. In the example presented here, a double-stranded DNA is captured.

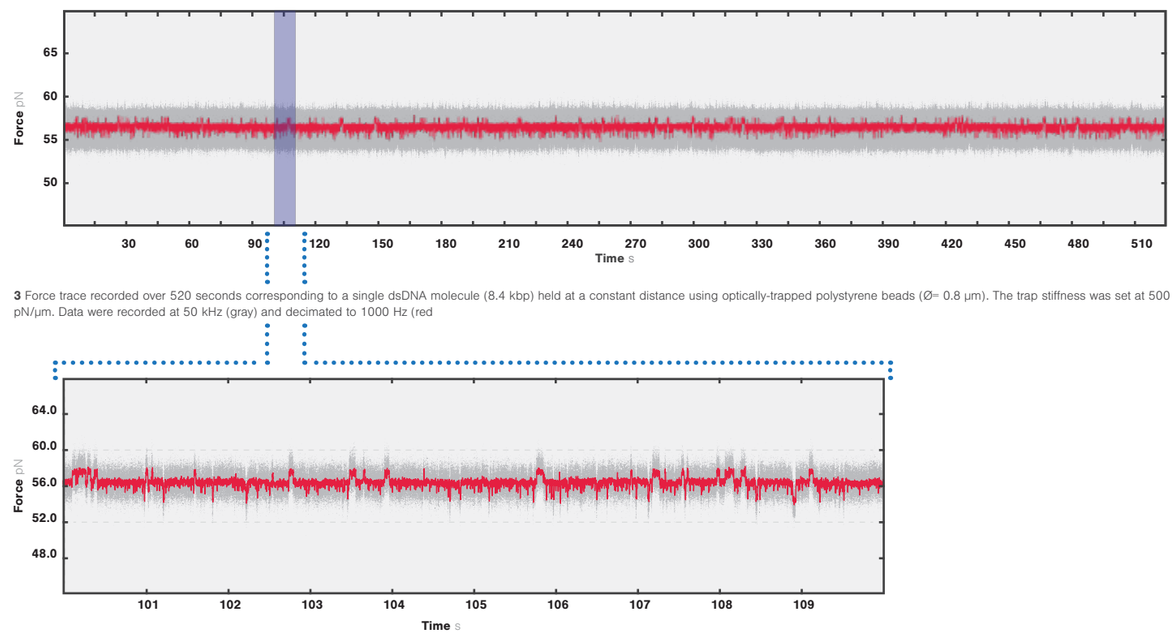
3 The biomolecule can be manipulated by moving one of the beads, while the force and extension are measured at the same time.

4 A great variety of biomolecular samples can be manipulated and measured using optical tweezers



1 Force-distance curves of double-stranded DNA (blue) and single-stranded DNA (red)

2 Histograms of the force values collected for the full trace and for values collected during two different fragments of 50 seconds. The mean and sigma values are reported for each peak obtained from a Gaussian fit.



3 Force trace recorded over 520 seconds corresponding to a single dsDNA molecule (8.4 kbp) held at a constant distance using optically-trapped polystyrene beads (Ø= 0.8 µm). The trap stiffness was set at 500 pN/µm. Data were recorded at 50 kHz (gray) and decimated to 1000 Hz (red

4 Fragment with a duration of 10s of the trace shown within the blue inset in figure 2

Manipulation and force spectroscopy measurements at the single-molecule level.

Using LUMICKS' pioneering m-Trap optical tweezers, scientists of all biological disciplines and backgrounds are able to study and discern the high complexities of molecular processes with base-pair resolution.

The structural dynamics of biomolecules can be measured in a matter of minutes, while long- and short-lived conformational states within a biomolecular structure can be recorded over long periods of time. With the m-Trap, scientists are now able to reveal novel detailed information on a wide range of biomolecular mechanisms, which ultimately lead to groundbreaking discoveries.

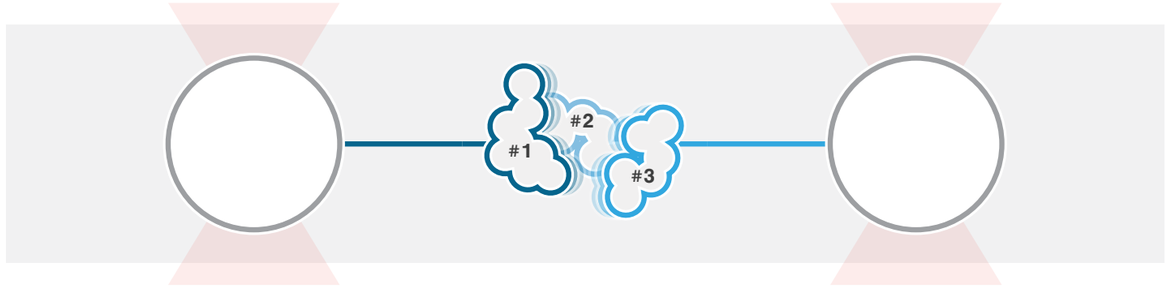
In the **example presented here**, optical tweezers are used to trap beads and a double-stranded DNA (dsDNA) molecule is tethered in-between. By stretching the dsDNA it becomes possible to obtain the characteristic **force-distance curve (Figure 1)** and determine the mechanical properties of the tethered biomolecule.

Equilibrium dynamics showing the transition between short-lived states can be studied because of the intrinsic distance clamp which keeps the traps at a fixed distance, while force fluctuations are measured. Furthermore, owing to the m-Trap’s ultra-high stability it is possible to characterize the properties of conformational transitions during extremely long periods of time.

Figure 3 shows spontaneous DNA conformational transitions occurring for almost 10 minutes. In this example, the observed fluctuations reveal spontaneously formed transient bubbles within the structure of the DNA molecule, a phenomenon otherwise known as DNA breathing. When looking at a 10 s set of the complete trace, **fast transitions between multiple states** are clearly visible (**Figure 4**). The histogram analysis for both the complete 520 s trace and two 50 s sections distributed along the main trace (**Figure 2**) show identical features, indicating that the experiment was performed without altering the transition kinetics due to unwanted force drift.

PROTEIN UNFOLDING

Study protein unfolding steps and equilibrium dynamics.

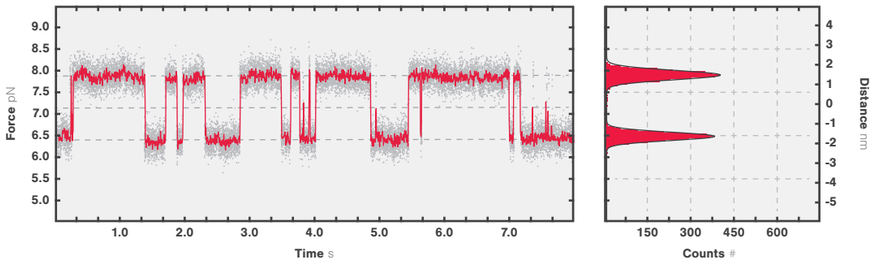


Multi-domain Protein Unfolding

The m-Trap enables measuring the unfolding dynamics of individual protein domains at the single-molecule level. Unknown structural domains are revealed and **protein (un)folding pathways** can be studied under different biological circumstances.

Optical tweezers are used to trap beads while a protein is tethered using DNA-handles. The (un)folding of the protein is controlled by moving the beads while the force and distance are measured simultaneously.

Equilibrium dynamics showing the transition between short-lived intermediate states can be studied because of the intrinsic distance clamp which keeps the traps at a fixed distance, while force fluctuations are measured. When this is applied to calmodulin, the equilibrium fluctuations and relative probabilities between the states can be observed with a force resolution of <0.1 pN. **Figure 1** shows that calmodulin switches between two major states, without a clear preference, and that intermediate steps can be resolved as calmodulin occasionally jumps to a third state for short periods of time, as shown by the dashed grey lines.



1 Force trace of equilibrium measurements over 10 seconds displaying the structural fluctuations of a single calmodulin molecule. Grey data is shown at a 2.5 kHz sampling rate while the red line shows raw data decimated to 200 Hz. The histogram on the right shows the two major states, while the third intermediate state becomes negligible due to its transitory existence.

Sample obtained with courtesy of UC Berkeley, Bustamante Lab.

ACTIVITY AND CONFORMATIONAL CHANGES OF DNA-PROTEIN INTERACTIONS

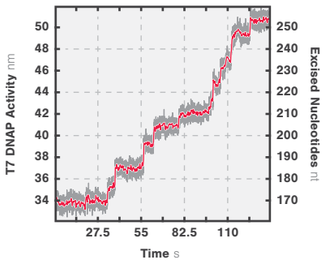
Study the activity and conformational changes of DNA-protein interactions.

Activity & Conformational Changes

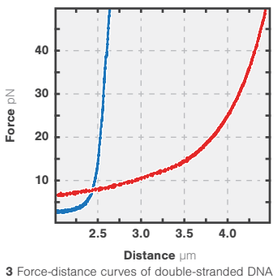
Optical tweezers can be used to measure and visualize the activity and states of motor proteins, such as **DNA** and **RNA polymerase**. Single-molecule measurements of stepping behavior of biomolecular motors will supply important new information about their enzymatic mechanisms.

A DNA molecule can be caught and stretched at a constant force using the optical tweezers, while a single DNA polymerase protein replicates the DNA. As the DNA polymerase incorporates nucleotides, single-stranded DNA becomes double-stranded; thus, the end-to-end length of the DNA molecule and the distance between the two traps becomes smaller, which can be measured in high resolution. This way the activity events of the polymerase can be measured.

Figure 2 shows measured data of the activity of T7 DNA polymerase, which participates in DNA replication. Optical tweezers hold a DNA construct (8.3 kbp), tethered between two beads (Ø =1.86 µm) at a force of 45 pN to observe force-induced exonucleolysis at the single-molecule level. The stiffness of the DNA changes as more double-stranded DNA becomes single-stranded (**Figure 3**), allowing the activity of the polymerase to be measured. Short activity bursts of 3 to 10 nucleotides are observed interspersed by frequent pauses of varying duration.



2 Activity bursts of DNA polymerase.



3 Force-distance curves of double-stranded DNA (blue) and single-stranded DNA (red).

FEATURES & OPTIONS
A look into the m-Trap system.

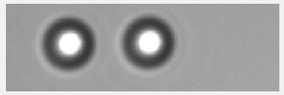
The m-Trap was developed to lower the price barrier of advanced single-molecule force spectroscopy instrumentation without compromising on key performance characteristics. For this, every single component was considered and measured to be truly useful and deliver the most complex force application. By extension, we designed an instrument that is easy to use, requires no alignment and can produce state-of-the-art experiments, while offering accessibility to scientists of all disciplines and backgrounds.

The m-Trap has been created after extensive optical modeling and manufactured to ensure extremely stable high performance and experimental reproducibility. LUMICKS is committed to standing by you to ensure your instrument performs to specification throughout its lifetime, and to provide you with access to our experts for application support and service.

1 MANIPULATION

Dual optical traps

The system is equipped with two ultra-stable optical traps for manipulating a single biomolecule. The m-Trap optical tweezers configuration consists of one trap that can be operated individually in x and y-direction and one trap with a fixed position. The extreme stability of less than 0.3 pN over minutes enables long acquisitions at a constant distance revealing even the rarest states, otherwise hidden by Brownian motion or instrumental drift.



Dual trap
Imaged with brightfield microscopy

2 FORCE DETECTION

The force on the sample is measured with sub-picoNewton resolution on one of the traps through an ultra-sensitive position sensing detector. Tension values ranging from a few tenths of pNs to the nN level and beyond can be applied and measured. This allows for monitoring extremely small steps on a broad regime, relevant for example in protein unfolding experiments.

3 LAMINAR FLOW MICROFLUIDICS

The m-Trap can be integrated with u-Flux – a solution that allows to reliably perform single-molecule experiments in a laminar flow environment. The u-Flux microfluidics system consists of a highly-stable passive pressure system, automated fluidic regulation, and a laminar flow cell.

The pressure system feeds multiple channels into the laminar flow cell where up to five adjacent flows are created. No physical barriers separate the highly stable flows. Free navigation between DNA, protein and other solutions, with accommodation for precisely triggering enzymatic reactions by specific buffer components, is allowed.

4 SAMPLE MOVEMENT

A high-resolution piezo-controlled nanostage enables the repeatable and absolute positioning of the sample in X, Y and Z and can be used for surface measurements with sub-nanometer accuracy.

5 M-TRAP FLEX

The m-Trap Flex option provides access to the optical path, allowing for the integration of custom hardware. Imaging and spectroscopy techniques can be added to the m-Trap offering full flexibility to combine locally developed optical hardware with the m-Trap's ultra-high force resolution and stability.

6 STABILIZATION

The m-Trap can be delivered with a pressure-stabilized optical table. Depending on your requirements, LUMICKS can find the optimal solution for ultra-stable experiments with minimal noise.

U-FLUX MICROFLUIDICS

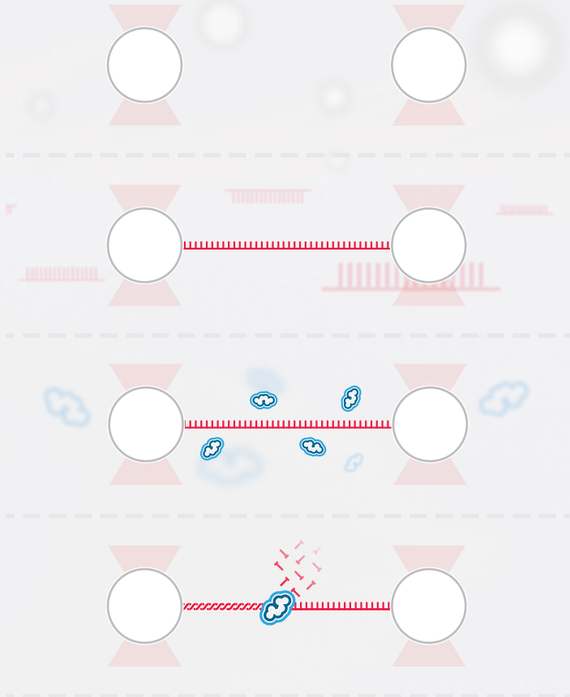
A highly stable microfluidic system for single-molecule experiments.

1 u-Flux pressure system including all electronics for regulating the pressure and laminar flow with high precision.

2 Twist-lock syringes which can individually be filled with your samples.

3 Automated fluidic valves for remote and automated control over the fluidics.

4 Custom-designed flow cell containing five channels as its standard, but can be optimized to fit your specific experimental needs.



1 Bead Trapping
The first channel is filled with beads, which are trapped in the focal point of the optical tweezers, allowing you to manipulate and exert high forces on your sample.

2 Tether Formation
The traps are moved to the second channel, filled with a biomolecule of interest – in this example DNA. In this channel, the biomolecule is tethered between the beads.

3 Protein Incubation
DNA is incubated in the third channel with a protein or multiple proteins of interest, in this case a DNA polymerase.

4 Force Measurement
The fourth channel contains a buffer solution and is used to perform measurements. The activity of the proteins on the DNA events are quantified by the force measurement.

Single-molecule experiments within minutes

u-Flux has been developed as an easy-to-use high throughput solution dedicated for single-molecule applications. The microfluidic flow cell provides multiple adjacent laminar flow channels that do not mix (no physical barriers are involved). Flow channels can be independently switched on and off through automated fluidic valves.

Easy-to-use twist-lock syringes

Sample loading is easily performed by directly pipetting your sample(s) into the syringes. The twist-lock syringe adaptor with bayonet fitting allows for quick and easy refilling of individual syringes.

Remote pressure & valve control

Automated flow control and valve switching allow for an optimal remote operation to perform robust measurements with high throughput. Using the software, you are able to regulate the pressure and control each of the channels with simple clicks or with a scripting plugin for automation.

Reliable and precise experiments

The pressure driven flow in combination with the monolithic glass flow cell provides an extremely stable and repeatable experimental environment. The laminar flow permits the sequential assembly of single-molecule assays and the controlled triggering of biochemical reactions by exposing the molecule of interest to different buffer environments at specific time-points.

Designed for repeated use

The monolithic glass design allows for re-use. Even highly-concentrated chemical solutions can be cleaned quickly and effectively.

SOFTWARE

An intuitive software suite for high-throughput experiments.

1 Live video tracking of the trapped beads, allowing independent distance tracking.

2 Plugins and scripting functionality enable a broad range of automation options.

3 Force-extension data shows mechanical properties of the sample in real-time.

4 Fully correlated data in our the timeline window, providng access to all data streams.

An intuitive software suite for high-throughput experiments.

Designed from the ground up, our newly released software suite provides intuitive features that bring you closer to your experiment. Manipulate your sample directly with simple mouse and joystick movements, and automate your measurement with our powerful Python scripting engine. The new timeline feature ensures you can focus on the data that matters, and never lose anything of value.

An open source initiative

We believe in the power of raw unedited data. This is why our software provides access to all system parameters and data streams. Perform data analysis, automate your workflow and improve the quality of your research with custom plug-ins. Any code that touches your data is part of our open source suite and can be fully inspected, modified, and re-used under an open-source license.

Automation and scripting

Repeatable experiments are key for gathering statistically relevant amounts of data and publishable results. The m-Trap software allows for automation through the implementation of:

- Plugins that control and automated basic procedures such as trap calibration, predefined force-extension measurements, force-clamp experiments and predefined sample-stage trajectories, making measurements faster and less error-prone.
- Scripting with full access to all relevant system parameters and data streams to allow the user to automate any kind of repeated experimental procedure.

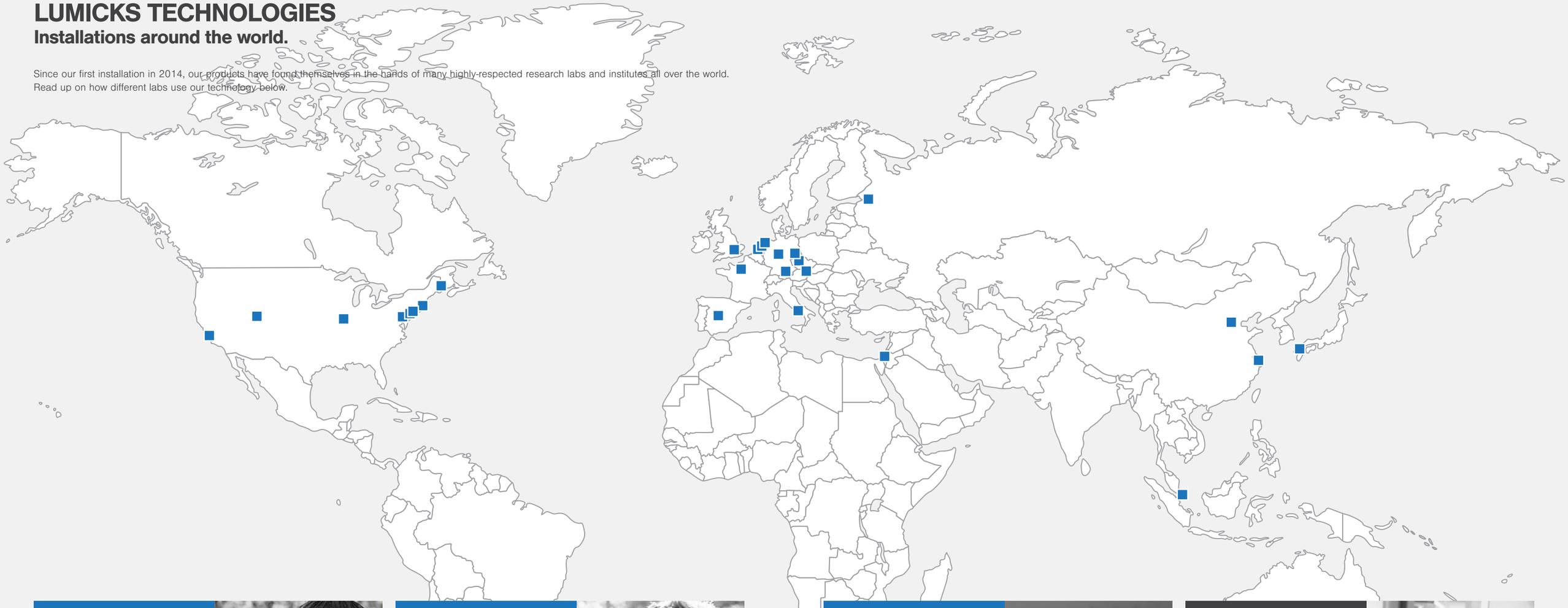
Data interface and raw data

Our novel data-centric user interface automatically generates a structured overview of the state of your experiment and allows for easy & intuitive navigation through your timeline of acquired data with click and scroll-to-zoom. Furthermore, by storing extensive metadata about your experiments and saving the data in an open file format, LUMICKS ensures better reproducibility of your experiments and raw data that are easily accessible.

LUMICKS TECHNOLOGIES

Installations around the world.

Since our first installation in 2014, our products have found themselves in the hands of many highly-respected research labs and institutes all over the world. Read up on how different labs use our technology below.



Rockefeller University
New York City, United States
C-Trap

In the heart of Manhattan, the group led by Dr. **Shixin Liu** at Rockefeller University uses the C-Trap to study the coordination and competition between macromolecular machines involved in gene regulation processes.

MPI-CBG
Dresden, Germany
C-Trap

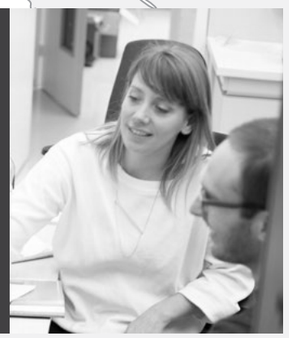
The C-Trap at MPI-CBG is installed in a multi-user environment where different research groups—including those of Prof. Anthony Hyman, Prof. Stephan Grill and Dr. **Marcus Jahnel**—are using it to answer research questions related to protein droplets, membrane protein unfolding and the enzymatic activity of RNA polymerase.

ShanghaiTech
Shanghai, People's Republic of China
C-Trap

The group of Dr. **Bo Sun** at ShanghaiTech University uses the C-Trap for their research focused on understanding the mechanisms of molecular motors and their influence of DNA replication, repair and transcription.

LUMICKS HQ
Amsterdam, The Netherlands
m-Trap, C-Trap, AFS, u-Flux

At our headquarters in Amsterdam, we use our instruments to perform demo's, hands-on workshops, and even contracted research jobs. Interested in how the m-Trap works and what it can do for your research? Reach out to us and come by to experience the instrument yourself!



VU University
Amsterdam, The Netherlands
C-Trap

BIOCEV
Prague, Czech Republic
C-Trap

University of Groningen
Groningen, The Netherlands
C-Trap

FOM Institute AMOLF
Amsterdam, The Netherlands
C-Trap

Max F. Perutz Laboratories
Vienna, Austria
AFS

Leiden University
Leiden, The Netherlands
AFS

National University of Singapore
Republic of Singapore
AFS

Kyushu University
Kyushu, Japan
AFS

Johns Hopkins University
Baltimore, United States
C-Trap

Göttingen University
Göttingen, Germany
C-Trap

St. Petersburg University
St. Petersburg, Russia
AFS, u-Flux

Imperial College London
London, United Kingdom
AFS

Ludwig Maximilian University
Munich, Germany
AFS

Chinese Academy of Sciences
Beijing, China
AFS

UC Berkeley
Berkeley, United States
u-Flux

Harvard University
Cambridge, United States
u-Flux

SPECIFICATIONS OVERVIEW
Unique & enabling features.

Optical tweezers

Dual optical tweezers; for manipulating single biomolecules with high stability and precision.

Force detection; measured on one trap with an extremely high escape force (>1000 pN), force stability (<0.3 pN over 2 minutes) and force resolution (<0.1 pN at 100 Hz); for probing different biological systems with a large dynamic range, while measuring very small force steps.

Trap steering; providing >20nm positioning accuracy accessible over a range of 50 µm; for providing trap movement over a large field-of-view.

Video based bead-tracking; with an accuracy <3nm at 100 Hz for independent distance tracking.

Integrated software suite; with optimized workflow; for high throughput single-molecule experimentation by trapping microspheres, tethering and subsequently manipulating biomolecules within minutes.

Standard sample holder; to clamp microscope slides and/or cover slips containing the sample.

Microfluidics

Multi-channel laminar flow microfluidics system; without physical barriers between the channels; for introducing precious reagents in a controlled manner and in-situ assembly of a wide range of complex, multi-step single-molecule assays.

Remote fluidics valve control; for programming the software user interface for high data throughput applications.

General

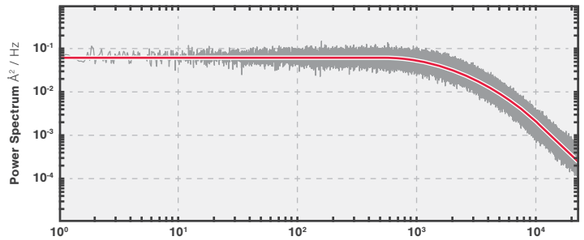
High-resolution piezo-controlled nanostage; for surface assays and repeatable and absolute positioning of the sample.

On-site application and scripting training; for expert support from our specialists dedicated to the customer's scientific application.

Software support; At LUMICKS, we have a software team working relentlessly to continuously optimize and adapt the m-Trap software for novel applications. We work with our users to implement features that help them improve their research capabilities, enhancing the possibilities of the system with every release.

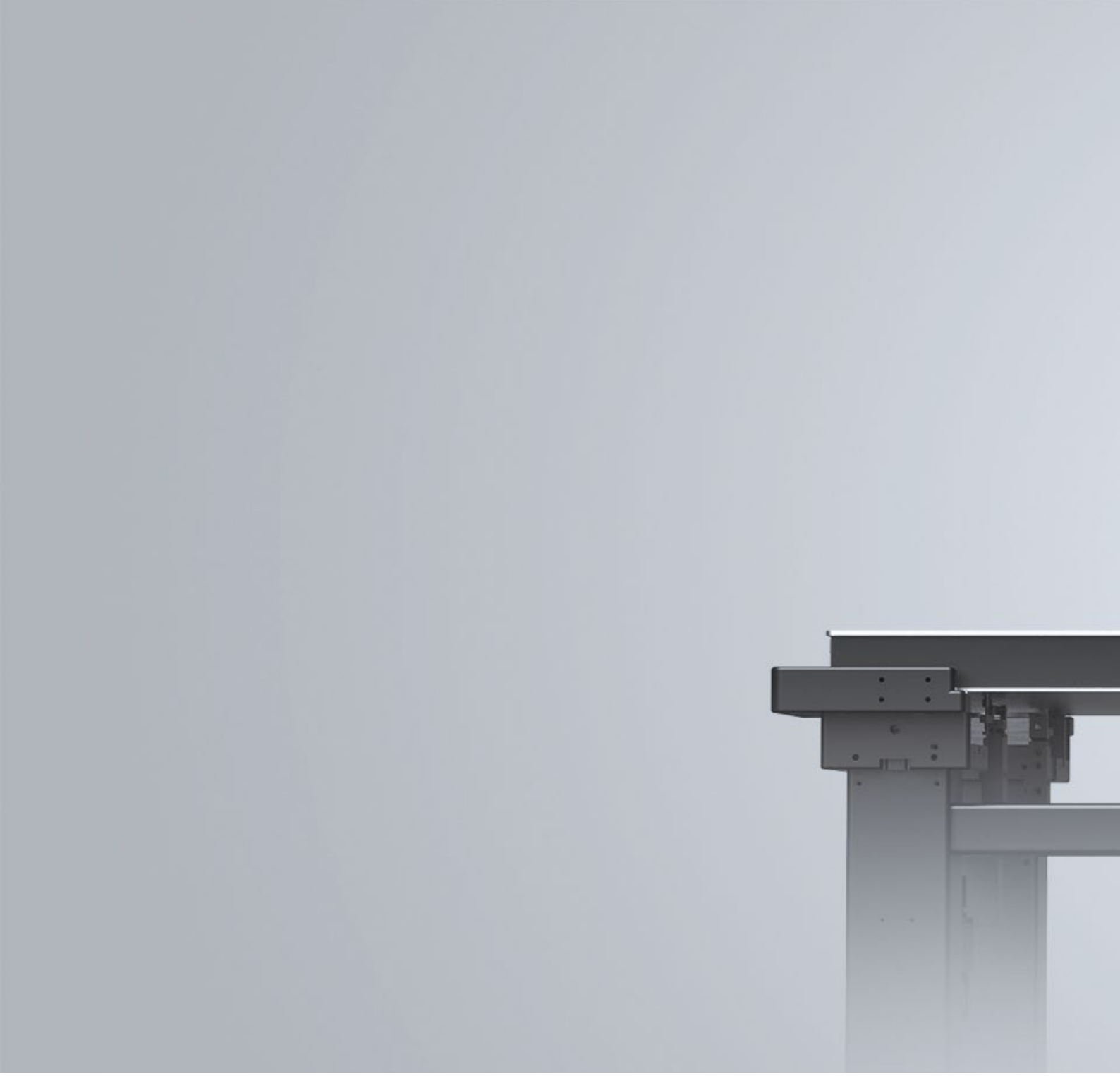
Optical tweezers

Force resolution	<0.1 pN at 100 Hz (1 µm beads at 0.3 pN/nm trap stiffness)
Maximum escape force	> 1000 pN using 4.5 µm polystyrene beads
Force stability	< 0.3 pN over 2 minutes
Minimal incremental step size	20 nm (software tuneable to <10nm)
Trap distance resolution	< 0.3 nm at 100 Hz
Live bead tracking accuracy	< 3 nm at 100 Hz using video analysis
Field of movement (FoM)	50 µm x 50 µm (X, Y)
Trap type	Continuous wave for unparalleled stiffness, stability and precision
Number of independent traps	2 (one fixed, one moveabl)
Force acquisition rate	50 kHz



Quick & Reliable Calibration

The m-Trap's software automatically calibrates the trap stiffness using power spectral analysis of the Brownian bead motion, following the state-of-the-art standards. The figure shows a power spectrum (acquisition time of 5 minutes) of the Brownian motion in the X-direction of an optically-trapped 1.00 µm polystyrene bead. Raw data (dark grey) was binned in 20 points bins (light grey), which were then used for performing a 2-step fit (red).



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