
AFS™ | Product Brochure

A compact solution for highly parallel single-molecule measurements





History

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

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

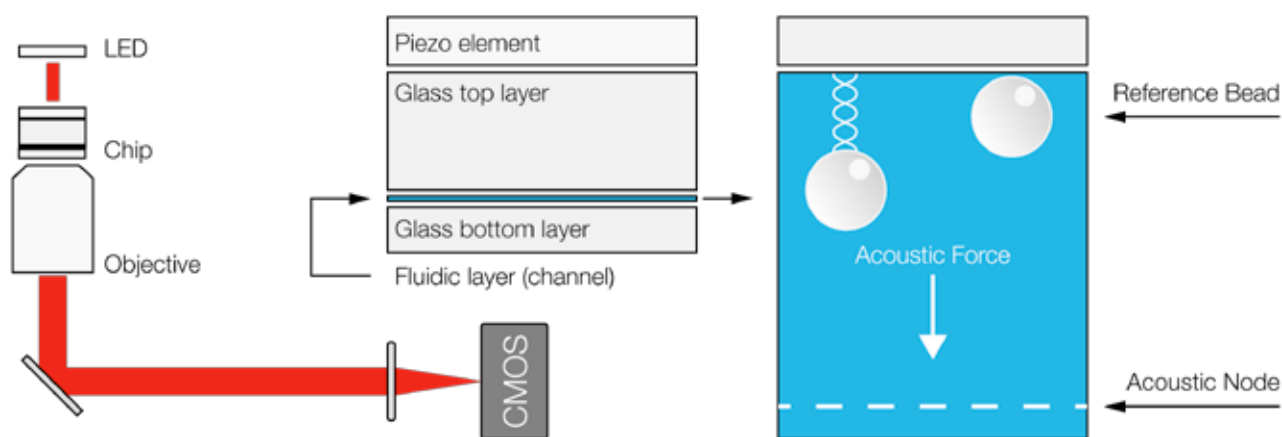
Mission

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



AFS™ Technology

Acoustic Force Spectroscopy, or AFS™, is the first commercially available highly parallel single-molecule manipulation method. AFS™ is a stand-alone instrument that includes an easy-to-use dedicated microscope for turnkey operations and measurements. The AFS™ technology is based on a powerful yet cost effective lab-on-chip device capable of applying forces on thousands of biomolecules in parallel with high precision.



AFS™ chip build-up

AFS™ Explained

AFS™ technology consists of a glass microfluidic chip and transparent piezoelectric transducers to generate resonant acoustic waves. In the AFS™ chip the acoustic waves (ultrasound) are employed to exert forces from sub-pN to hundreds of pNs on thousands of biomolecules (such as DNA, RNA or proteins) in parallel, with sub-millisecond response time and inherent stability.

AFS™ represents an ideal tool to perform a wide variety of experiments and decipher the details of molecular interactions at the level of nucleic acid-protein, drug-protein and antigen-antibody. AFS™ enables scientists to investigate structure-function relationships of proteins, new biological mechanisms, and cell mechanics. Finally, free energy diagrams, kinetic rates and intermediate states in reaction pathways can also be studied and identified.

LUMICKS offers the AFS™ technology in a stand-alone package, including a inverted optical microscope with software, workstation and electronics. AFS™ enables you to perform single-molecule experiments from day-one in an efficient and user-friendly environment.



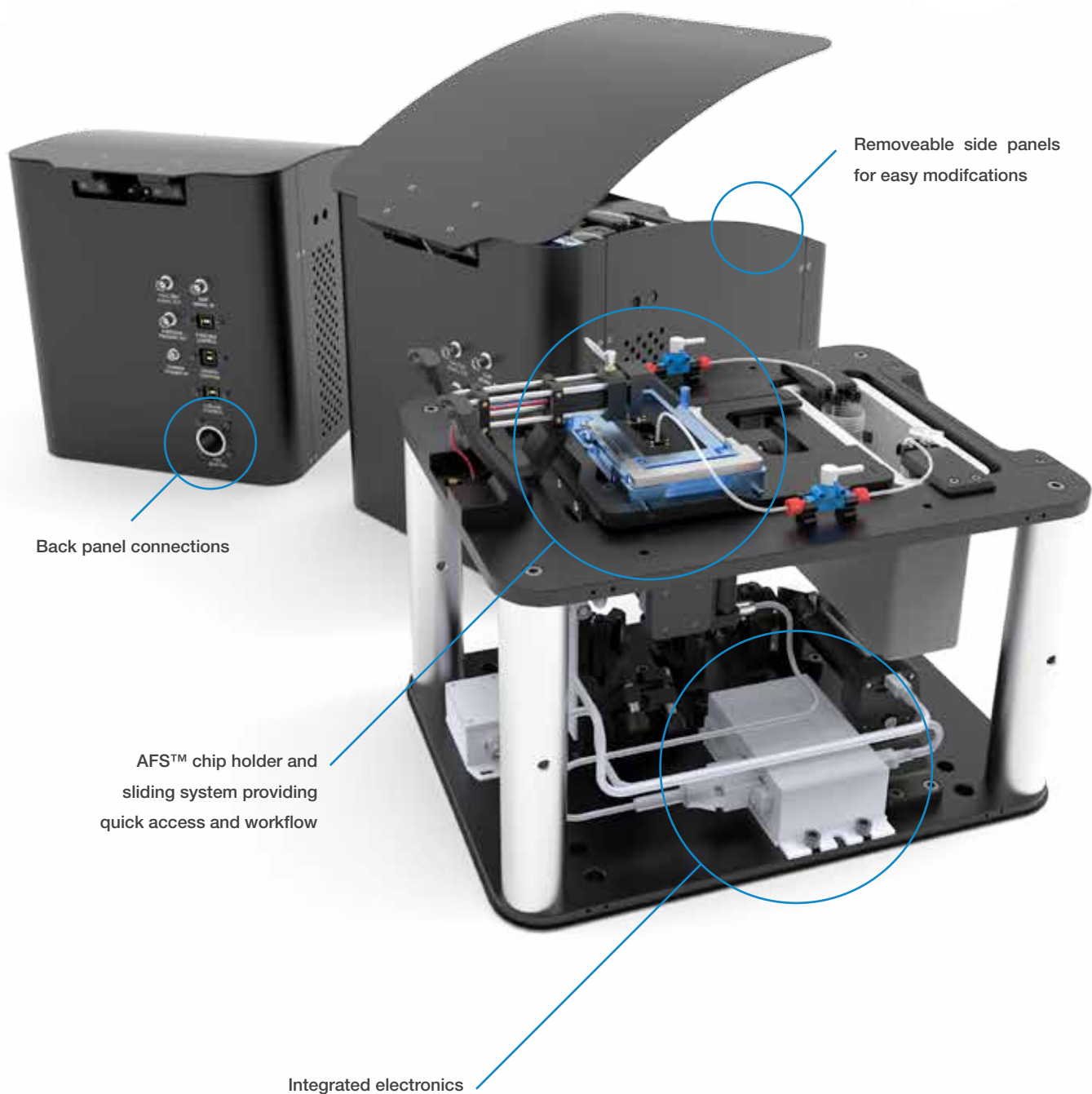
AFS™ chip, the core of the AFS™ technology

Key Technology Features

- Lab-on-a-chip
- Single-molecule manipulation
- Highly parallel
- Sub-millisecond response time
- Inherently stable
- Safe and user friendly
- Cost-effective

Further Reading

For more information about the AFS™ technology, please consult G. Sitters et al. "Acoustic Force Spectroscopy" in Nature Methods 2015.



Developed For Everyone

AFS™ is designed as a highly flexible single-molecule instrument. The LUMICKS designed microscope platform gives the users ample options of customization and personalization.

A Versatile Instrument

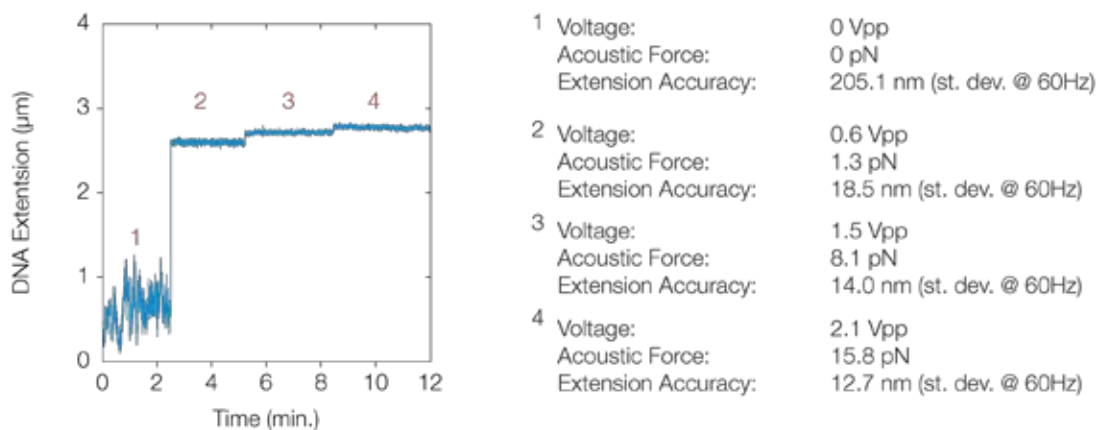
- Different tube lens mounting capabilities at distances of 200 mm, 150 mm, 75 mm and 50 mm to change optical magnification
- Removable side panel(s) allowing access to the optical path for easy modifications
- Standardized metric 25mm grid pattern for component mounting
- External fluidics capability e.g. syringe pump
- Designed for external amplifier possibilities, providing a quick and easy connection
- Special chip holder providing a secure fluidic & electronic connection to the AFS™ chip, allowing fast and simple (re)placement
- Sliding mechanism for risk-free positioning and locking the sample above the objective
- Clamps to secure standardized fluidic components on your work area, providing multiple flow capabilities such as gravity or syringe-driven flow

AFS™ Applications

AFS™ technology can be employed in numerous different applications. Here we demonstrate three single-molecule measurements that demonstrate the precision and multiplexing capabilities of AFS™.

Applications include:

- Dynamic force spectroscopy
- Constant-force measurements
- Force-distance curves
- Biopolymer mechanics
- Bond Rupture
- Micro-rheology
- Cell mechanics
- Mechanical properties of hydrogels



High Precision Force and Length Measurement

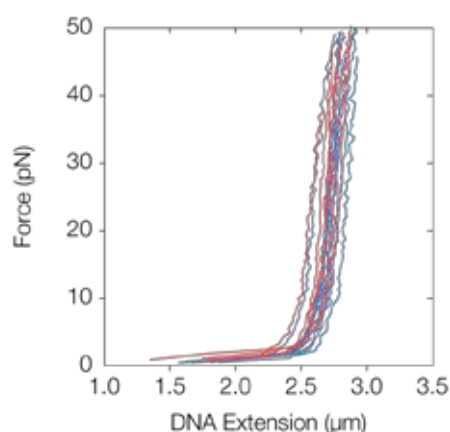
AFS™ is an ideal tool to perform high-precision force measurements on single biomolecules. Here we demonstrate the accuracy AFS™ by applying various level of tension on a single DNA molecule and measure its length using the AFS™ tracking software.

The experiment is performed on a single DNA molecule (8.4 kbps in length) tethered to a streptavidin coated polystyrene microsphere (4.5 μm in diameter).

Prior to the measurement a look-up-table is generated and the microsphere's height can be tracked with nm-accuracy.

During the measurement we increase the amplitude of the driving voltage from 0 to 2.1 Volt Peak-to-Peak (Vpp), corresponding to an increase in the acoustic force from 0 to 15.8 pN. The AFS™ software determines the DNA length at various forces in real-time to detect biological events, e.g. protein-DNA interactions.

The 14bit vertical resolution of the function generator allows generating constant acoustic forces with sub-pN precision, stability over hours and sub-ms response time.

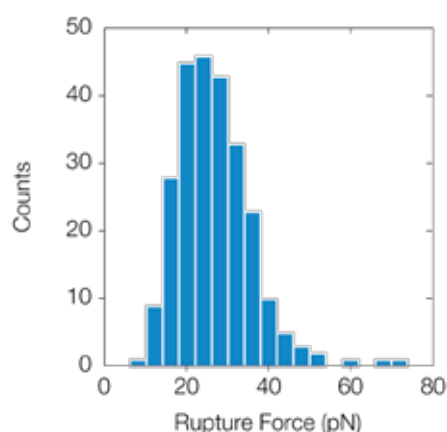


Measuring Mechanical Properties in Parallel

AFS™ has the powerful and unique capability of performing single-molecule measurements in a highly parallel fashion. This is of pivotal importance since often many independent measurements are needed to distinguish heterogeneous behavior and rare events from intrinsic stochasticity caused by thermal fluctuations. In this example we perform a single-molecule experiment in which we simultaneously apply tension and measure the mechanical properties of 20 DNA molecules in parallel.

Force - Extension Curve

Stretching curves of 20 individual DNA molecules in parallel. We apply increasing tensions from 0.1 to 48 pN to record the force-extension profiles. The curves are obtained in parallel, simultaneously and in a single field of view. The ability of multiplexing mechanical measurements at the single-molecule level can be readily applied to different assays such as protein and RNA unfolding and nucleic acids processing enzymes.



Highly Multiplexed Bond Rupture Measurement

To characterize the strength of antibody-antigen interactions statistical analysis and high data throughput is of crucial importance. Due to the massive multiplexing capabilities of AFS™, a large number of bonds can be probed in parallel, greatly reducing measurement times.

Here we show how AFS™ is able to obtain a complete rupture-force distribution of the Dig::anti-Dig bond in a single measurement of 251 single-molecule measurement in parallel.

Rupture Force Histogram

251 individual Dig::anti-Dig bonds are stretched with a loading rate of 2.3 pN/s. The rupture force of each Dig::anti-Dig bond is recorded and plotted into a rupture force histogram. This histogram was obtained in a single experiment under identical conditions, insuring experiment reliability and reproducibility. AFS™ is able to apply loading rates that span several orders of magnitude (10^{-4} pN/s to 10^3 pN/s), making it an ideal tool for highly parallel dynamic force spectroscopy measurements.

Specifications

The AFS™ is a true turnkey single-molecule tool that includes a dedicated inverted optical microscope, fluidics and necessary on-board electronics in a small (300mm x 375mm x 200mm) and cost-effective footprint.

AFS™ Dedicated Microscope

Monochromatic LED Illumination

- 660 nm wavelength

CFI Achromat Air Objective (upgradable)

- Nikon 40x, NA 0.65, WD 0.65mm
- Corrected for chromatic aberrations, spherical aberrations, coma and image flatness

Motorized Z Objective Stage (upgradable)

- High-performance 2-phase stepper micro-translation stage 5 mm travel range, 50 nm step size, sub-nm stability

USB 3.0 CMOS Camera (upgradable)

- 1280 x 1024 pixel (pixel size 5.3 μm), 1.31 MP
- Real-time parallel measurements up to 60 Hz in full field of view

Manual XY Sample Stage

- Micrometer precision 20 mm travel range
- Double-dovetail guide enabling the user to measure at different locations within the sample
- Equipped with fine-thread spindles with graduated buttons

On-board Function Generator (upgradable)

- Standing resonant acoustic waves up to 10Vpp (Volt peak-to-peak) at 50 Ω impedance
- Allows for dynamic force spectroscopy and force sweeps on different timescales from 10⁻⁵ Hz to the kHz range and beyond
- Exceptional stability and reproducibility
- Both static and dynamic force measurements with 14bit vertical resolution

AFS™ Chips

Three individual AFS™ chips are included with the AFS™ system. Each AFS™ chip is pre-calibrated by LUMICKS and the resonance frequencies are made known to the buyer.

- Range of loading rates 10⁻⁴ pN/s to 10³ pN/s
- AFS™ chip size (configurable) 45 mm x 15 mm x 1.275 mm
- Maximum force applicable >200 pN (4.5 μm polystyrene microspheres, using voltage amplifier)

New AFS™ Chip Designs

We continue to improve our AFS™ chips every day. We work closely together with AFS™ users to improve the chip performance, features and usability.



AFS™ Workstation & Software (upgradable)

AFS™ includes a LabView2013 software package and a powerful workstation. The AFS™ software is capable of real-time, nanometer resolution tracking of thousands of microspheres in parallel.

Key Features

- Real-time parallel 3D tracking, 60-300 microspheres at 25 Hz
- Real-time parallel 2D tracking, 300-1500 microspheres at 25 Hz
- Real-time position tracking accuracy (X,Y) of 2 nm at 25 Hz
- Real-time height tracking accuracy (Z) of 5 nm at 25 Hz
- Z-drift correction through reference bead differential tracking
- Automated look-up-table calibration

AFS™ Amplifier (optional)

AFS™ capabilities can be expanded by using a voltage amplifier. Amplifying the voltage increases the maximum force that can be applied on the biomolecules.

The amplifier can be easily connected to the AFS™ via the back panel. All relevant software and drivers have been pre-installed on the AFS™ computer to accommodate the voltage amplifier.



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