Flex-Bio

Versatile Research AFM System for Life Science

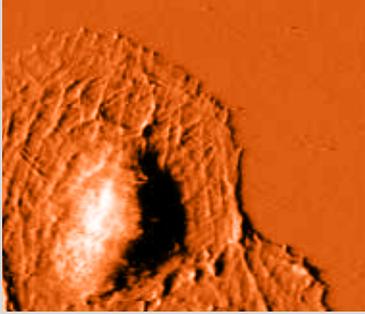| Measurement capabilities in air and in liquid | Versatile in applications and modes | Compatible with inverted microscopes |

![nanosurf]

AFM image of living Rat-2 fibroblast cells showing details of the cell's cytoskeleton discernible through the cell membrane.

Origami DNA on silicon.

Single molecule force spectroscopy of bacteriorhodopsin (BR).
The force–distance curve reports the controlled C-terminal unfolding of a single membrane protein from its native environment, the purpTTle membrane from Halobacterium salinarium. Solid and dashed orange lines represent the WLC curves corresponding to the major and minor unfolding peaks observed upon unfolding BR, respectively. The contour length of the stretched poly-peptides of the major unfolding peaks is given in amino acids (aa).

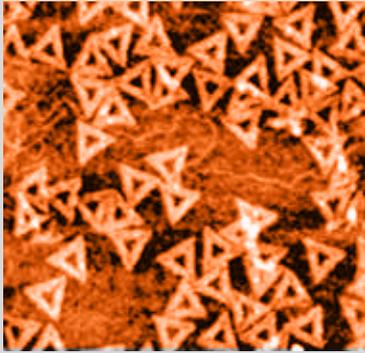## Versatile research AFM system for Life Science

For success in Life Science research, scientists depend on professional tools that can readily provide the information needed, regardless of the tasks at hand. By combining key technologies and components, Nanosurf has made the Flex-Bio system one of the most versatile and flexible atomic force microscope systems ever, allowing a large variety of biological and life science applications to be handled with ease. With the included C3000 controller, new levels of accuracy can be achieved with the FlexAFM scan head.
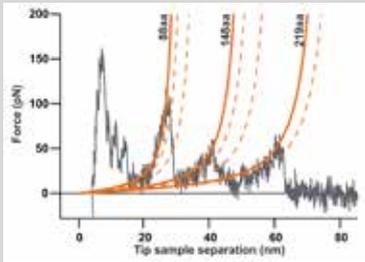
Key features & benefits

- Flat and linear scanning thanks to flexure-based scanner technology
- True flexibility with exchangeable cantilever holders for specialized tasks:
  - Cantilever Holder Air Only: AFM measurements in air
  - Cantilever Holder Liquid/Air: AFM measurements in air and liquid
  - Cantilever Holder Scanning Thermal: Scanning thermal microscopy
  - Cantilever Holder FluidFM: Fluid Force Microscopy applications
- More measurement versatility with the FlexAFM's scanning capabilities in liquid and its additional measurement modes:
  - Lateral Force Microscopy
  - Kelvin Probe Force Microscopy
  - Scanning Thermal Microscopy
  - Fluid Force Microscopy
- Compatible with inverted microscopes:
  - FlexAFM Inverted Microscope Option provides a seamless integration with many types of inverted microscopes
  - Readily combine AFM and optical data (fluorescence/phase contrast/bright field)

Beyond images

A true research AFM is only as powerful as its ability to acquire and combine sample measurement data from multiple techniques. This is where the FlexAFM can do plenty! In the experiment below, this is nicely demonstrated. On an inverted microscope, and in a single setup, the FlexAFM combines bright field analysis, immunofluorescent detection, AFM topography, and force mapping of the internal limiting membrane (ILM) of the human retina into state-of-the-art research towards methods for early tissue diagnostics:
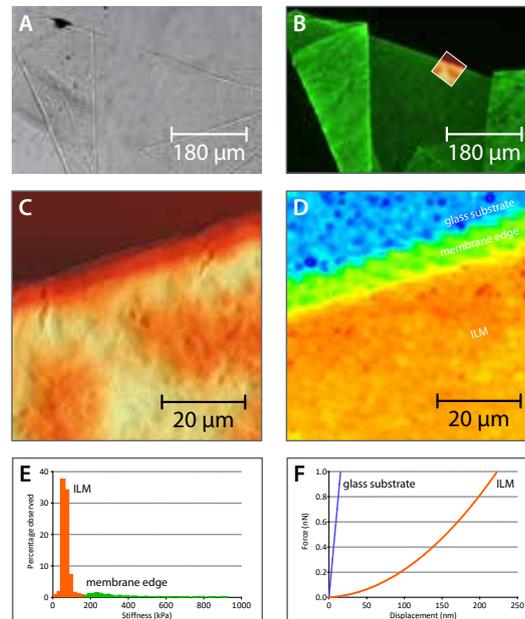
(A) Bright field image of isolated ILM in a physiological buffer. (B) Fluorescence image of the same section showing anti-laminin staining. (C) AFM topograph of a subsection of the ILM; also shown as overlay in B. (D) AFM stiffness map of the same subsection. The color for each point represents the local stiffness value as calculated from force curves recorded at the respective positions. (E) Histogram of the stiffness data shown in D. (F) Typical force–displacement curves obtained on the ILM and on the glass substrate. These curves are converted to force–indenta-tion data, which then allows calculation of the stiffness. Stiffness distribution of biological tissues has been shown to be a marker for diseases such as age-related macular degeneration, arthritis and can-cer. Data: Marko Loparic, Marija Plodinec, Philip Oertle & Paul B. Henrich, Biozen-trum/SNI/UHBS, University of Basel.

Typical Flex-Bio setup

A typical Flex-Bio setup consists of the FlexAFM near-infrared scan head (either with 10- or 100-μm scan range), the C3000 controller, the motorized sample stage for inverted microscopes, an inverted microscope, a vibration isolation table, and a PC with control software. Adapters for many types of inverted microscopes are available. The motorized sample stage provides an easy way to combine inverted microscopy (bright field, phase contrast, and fluorescence) with AFM. It allows exact and reproducible position of microscope samples. In addition to the motorized sample stage shown above, a manual version is also available.

Alternative stages:

The ATS 204 is an automated translation stage that allows movement of the sample in X, Y, and Z via the Nanosurf stage control unit and accompanying software. Additionally, it can optionally contain a high-resolution 100-μm Z-actuator with position sensor that is ideally suited for force spectroscopy measurements.

The ECS 204 allow to perform simultaneous AFM imaging and electrochemical measurements on electrodes and samples immersed in electrolyte solutions. It has in integrated micrometer stage and together with the electro-chemical cell it allows to work in oxygen free atmosphere and can accommodate a true reference electrode, flat or rod type samples and allows for liquid exchange.

With the additional standard sample platform it functions as a normal sample stage.

The ECS 204 was developed to facilitate electrochemical corrosion and deposition AFM studies using the FlexAFM. It features an inert liquid cell embedded in a solid steel frame, a small protected compartment for oxygen-free atmosphere above the solution, and an Integrated micrometer stage for lateral positioning (2 mm range).

The Cantilever Holder

As central part of the AFM detection system, the cantilever holder contains cantilever alignment structures for exact cantilever positioning and all optics related to Nanosurf's top and side view technology. It is magnetically attached to the scanner unit to allow quick removal from the scan head for easy cleaning and fast cantilever exchange. Four cantilever holder models are currently available, each optimized for its own specific task.

Top   Side

Cantilever holder air only
The cantilever holder air only is the default cantilever holder for any FlexAFM system. Its CantiClip spring provides a very convenient way to hold and exchange a cantilever.

Air   Liquid

Cantilever holder liquid/air
With its SureAlign™ optics, the cantilever holder liquid/air adds liquid measurement capabilities to your FlexAFM. In addition to measuring in air and in a liquid droplet (as illustrated in the images to the right) it can dive directly into a layer of liquid with up to 6 mm in height, e.g. in a standard cell culture dish.

Holder   Thermal Probe

Cantilever holder scanning thermal
Optimized for use with Anasys Instruments thermal probes, the cantilever holder scanning thermal allows scanning thermal microscopy as well as nano-TA measurements to be performed.
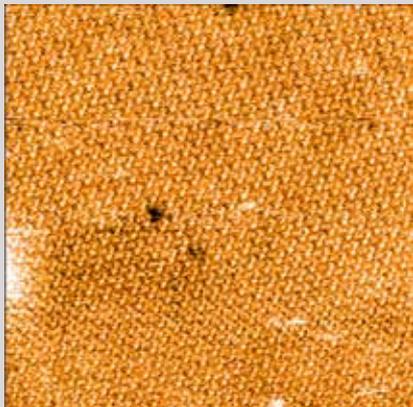

Holder   CytoClip

Cantilever holder FluidFM
The cantilever holder FluidFM and matching CytoClips with premounted hollow cantilevers can be coupled to a microfluidics pressure control system to allow Fluid Force Microscopy in single cell applications and beyond.
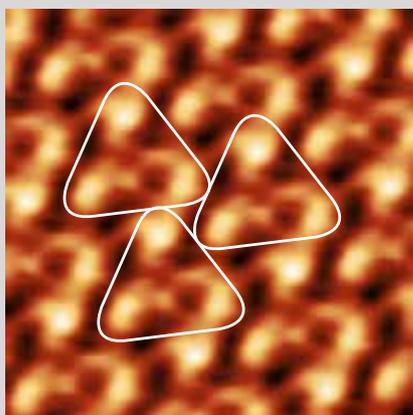
High-resolution imaging of the cytoplasmic side of bacteriorhodopsin:

Unfiltered overview image with linear background correction. Scan size: 140 nm.
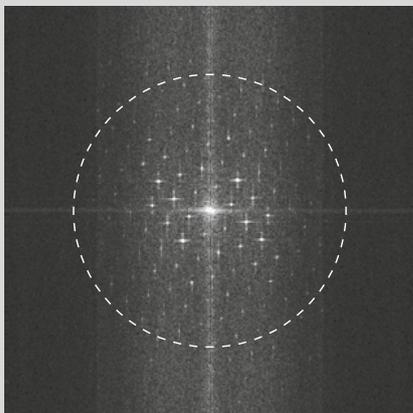
Power spectrum of the crystal latice, showing a lateral resolution well beyond 1 nm (dashed white circle).

Correlation average, with 3 trimers highlighted in white. Fourier analysis and cross-correlation averaging were performed using the IPLT software (available at www.iplt.org).

High-end AFM controller for more performance and precision

The versatility and performance of the FlexAFM scan head is brought to its full potential by the C3000 controller. With this AFM controller's fully digital internal data processing, 24-bit ADC/DAC conversion depth, and programmable FPGA CPU, it is a huge step up from the standard Easyscan 2 controller. It allows high-speed data acquisition, dynamic filtering and analysis, and real-time signal monitoring directly from within the C3000 control software.

Through soft- and firmware changes, the C3000 controller can be updated and upgraded to support new options and features at any time!

Main features

- All digital data processing in FPGA

- 24-bit DACs for accurate scanning with widely varying scan ranges

- 24-bit ADCs and adaptive filters for high-resolution and low-noise data

- Fast and sensitive digital Z-feedback and spectroscopy

- Fully equiped with integrated thermal tuning, data monitoring, user I/O and signal access, advanced operating modes

Additional options

Available C3000 controller options/packages include: advanced spectroscopy, signal modulation, advanced lithography, scripting interface, external synchronization.

Accessories that extend the capabilities of your Flex-Bio research system

Halcyonics_i4
Vibration-free measurements

- State-of-the-art active vibration isolation system

- Ideal for isolating your Flex-Bio system from building vibrations and other disturbances

- Low-profile carbon-design, straightforward handling, easy operation

- Two versions for a variety of applications

- Isolation effect starts at 0.6 Hz and achieves max. performance of –40 dB at 10 Hz, where 99.0% of the vibration is isolated

ARTIDIS Upgrade
Nanomechanical tissue diagnostics and soft material analysis

- Fully automated measurements on rough and non-even surfaces

- Quantitative analysis of tissues and soft materials alike

- Fast, objective, and routine sample categorization

- Touchscreen interface for intuitive operation

Coverslip Holder and Bio Heater
Maintain control over your cells

- Conveniently grow cells on coverslips prior to your experiments and then place them in the coverslip holder for analysis

- Possibility to perfuse the cell reservoir with cell culture medium or buffer solutions

- Possibility to add ligands or other reagents through separate channels

- Bio sample heating with accurate temperature control of coverslip holder and medium (via dual temperature sensors)

- Liquid pre-heater available as a further option

Halcyonics_acoustic enclosures
Protection from airborne noise

- Useful accessories to the halcyonics_i4

- Designed to match halcyonics vibration isolation systems, but also fit with other setups.

- Protect your equipment from airborne noise emitted by air conditioning, venting, door slamming etc.

- Enable you to perform undisturbed experiments with your Flex-Bio setup.

- Available for all products, sizes, and applications.

FluidFM Upgrade
An enabling technology for micro-manipulation of single cells and other small objects, surfaces and tissues

- Nanofluidics through a hollow cantilever combined with the positional accuracy and force control of the Nanosurf FlexAFM

- Specialized application modules for different applications as injection, pick-and-place, adhesion force spectroscopy, elasticity measurements, and spotting

Environmental control chambers and glove boxes
Control temperature, humidity, and atmosphere

- Various control possibilities and chamber sizes available from Nanosurf or one of its partners

- We will be happy to assist you in finding the right system for you, or to design a custom solution that fits your exact application needs

Standard functionality and specifications of the Flex-Bio system

Scan head version 3

FlexAFM NIR scan head features

General design	Tripod stand-alone scan head, flexure-based electromagnetically actuated XY-scanner, decoupled piezo-based Z-scanner
Cantilever alignment	Automatic self-alignment for cantilevers with alignment grooves. Manual laser adjustment possible for special cantilevers.
Laser adjustment	No adjustment required upon immersion of cantilever into liquid because of SureAlign™ laser optics (patent pending). Near-infra red laser.
Electrical connection to tip	Available
Sample observation	Top and side view in air and liquid
Sample illumination	White LEDs (brightness 0–100%); axial illumination for top view
Operating modes	Static Force, Lateral Force, Dynamic Force, Phase Contrast, Magnetic Force, Electrostatic Force, Kelvin Probe Force, Scanning Thermal, Spreading Resistance, Force Modulation, Multiple Spectroscopy modes, Lithography and Manipulation modes. Some modes may require additional controller options.

FlexAFM NIR scan head specifications

Scan head type:	NIR 100-µm	NIR 10-µm
Laser class (wavelength)	Class 1M laser product (850 nm)	
Maximum Petri dish height (fluid level)	9 mm (6 mm)	
Manual approach range	30 mm	
Automatic approach range	1.1 mm	
Maximum scan range	100 µm [1]	10 µm [1]
Maximum Z-range	10 µm [2]	3 µm [1]
Drive resolution in XY	0.006 nm [3]	0.0006 nm [3]
Drive resolution in Z	0.0006 nm [3]	0.0002 nm [3]
XY-linearity mean error	< 0.1%	
XY-flatness at maximum scan range	typ. 5 nm	typ. 1 nm
Z-measurement noise level (RMS, dynamic mode in air)	typ. 0.03 nm	
Scan head dimensions	143 × 158 × 53 mm	
Scan head weight	1.25 kg	

(1) Manufacturing tolerances ± 5%
(2) Manufacturing tolerances ± 10%
(3) Maximum theoretical resolution; calculated by dividing the maximum range by 24 bits

C3000 controller standard features

Standard imaging operating modes	Static force, dynamic force, phase contrast, MFM, friction force, force modulation, spreading resistance
Standard imaging functions	• Up to 8000×8000 data points with 24-bit zoom in • 8 acquisition channels with dynamic digital filters • 2× user input measurements, constant height mode • Sample XY-slope correction
Standard spectroscopy operating modes	• Force–distance, amplitude–distance, phase–distance • Tip current–tip voltage, 2× user output modulation • 2× user input measurements

Standard spectroscopy functions	• Setup wizard for each spectroscopy mode
	• XY-position table: point, line, grid, and free mode
	• Maximum number of curves: limited to 64
	• 3 Spectroscopy phases:
	   1. Move to start offset (absolute or relative to surface)
	   2. Forward modulation and acquisition
	   3. Backward modulation and acquisition

Standard lithography operating modes	• Free vector object drawing or real-time drawing by mouse • List of vector objects on layers with individual litho. parameters • Tip lift or force control during movement from point to point
Sample approach	• Fast home, retract, and advance movement • Automatic approach with definable final end position • Continuous or step-by-step approach mode

Advanced spectroscopy and lithography functions available as separate C3000 controller options

Compatible cantilevers

For use with the Cantilever Holder Air Only and Liquid/Air, cantilevers should have:

• Grooves compatible with the alignment chip used by Applied Nanostructures, BudgetSensors, NanoSensors, NanoWorld, Nascatec, and VISTAprobes
• A width of 40 µm or more and a nominal length of 225 µm or more (shorter cantilevers must either be of the XY-alignment series type, or require laser adjustment)
• A coating on the backside of the cantilever that reflects red light

Compatible options and equipment

C3000 controller, C3000 advanced spectroscopy, C3000 signal modulation, C3000 advanced lithography, C3000 scripting interface option, C3000 external synchronization, FlexAFM Video Camera, FlexAFM Inverted Microscope Option, FluidFM Option, Artidis Options, Thermal Measurement Option, Acoustic Enclosure 100, 300, and 500, Environmental Control Chamber, Isostage, Automated translation stage 204, Electrochemistry stage 204, FlexAFM Micrometer Translation Stage, AFM Extended Sample Kit, Small Sample Heater.

C3000 controller specifications

X/Y/Z-axis scan and position controller	3× 24-bit DAC (200 kHz sampling rate)
X/Y/Z-axis position measurement	3× 24-bit ADC (200 kHz sampling rate)
Excitation & modulation outputs	4× 16-bit DAC (20 MHz sampling rate)
Analog signal input bandwidth	0–5 MHz (20 MHz sampling rate) 0–20 kHz (200 kHz sampling rate)
Main input signal capturing	2× 16-bit ADC (20 MHz sampling rate) 2× 24-bit ADC (200 kHz sampling rate)
Additional user signal outputs	3× 24-bit DAC (200 kHz sampling rate)
Additional user signal inputs	3× 24-bit ADC (200 kHz sampling rate)
Additional monitor signal outputs	2× 24-bit ADC (200 kHz sampling rate)
Digital synchronization	2× digital out, 2× digital in, 2× I2C Bus
FPGA module and embedded processor	ALTERA FPGA, 32-bit NIOS CPU, 80 MHz, 256 MB RAM, multitasking OS
Communication	USB 2.0 Hi-Speed to PC and scan head interface
System clock	Internal quarts (10 MHz) or external clock
Power	90–240 V AC, 70 W, 50/60Hz

Specifications of C3000 controller options

Options can be activated via software activation codes

C3000 advanced spectroscopy option

Additional spectroscopy functions	• Additional "Stop by input value reached" modulation mode
	• Automatic cantilever drift recalibration
	• 5 Spectroscopy phases:
	  1. Move to start offset (absolute or relative to surface)
	  2. Forward modulation and acquisition
	  3. Forward pause (feedback on/off, sampling on/off)
	  4. Backward modulation and acquisition
	  5. Backward pause (feedback on/off, sampling on/off)

Spring constant calibration	• Free resonance detection via thermal tuning • Q-Factor calculation • Spring constant calculation by Sader method • FFT spectrum analyzer, various windowing modes, averaging • Frequency range: 0–5 MHz (resolution 1–100 Hz)
Deflection sensitivity calibration	• Wizard for deflection sensitivity calculation from force–distance measurements • Automatic mode or user-defined parameters

C3000 signal modulation option

Additionatl imaging operating modes	• Kelvin probe force microscopy (KPFM) • Dynamic friction force microscopy • User defined modulation of many outputs (incl. user outputs) and measurement of amplitude and phase of such modulations at any input
Additional spectroscopy operating modes	• Amplitude–frequency measurements
Second lock-in	• Frequency range: 1 kHz–5 MHz • Demodulation bandwidth: 125 Hz–68 kHz • Amplitude resolution: 24 bit • Phase range: ±90° (resolution: 0.00005°) • Reference phase shift: 0–360° (digital) • X/Y (I/Q) and Amplitude/Phase output modes
Second function generator	• Frequency range: 0–5 MHz (resolution: 71 nHz) • Amplitude resolution: 18/16 bit • Output signals: tip voltage, sample voltage, excitation, 3× user output
Second amplitude controller	• PID-feedback controller (200 kHz) • Input signals: second lock-in X, Y, amplitude, or phase, 2× user input • Output signals: tip voltage bias, 2× user output

C3000 advanced lithography option

Additional lithography modes	• Vector-based lithography with objects • Bitmap-based drawing mode
Additional lithography features	• CAD vector graphics import (GDS files) • Multiple vector object layers, each with their own lithography parameter set • Bitmap graphics import

C3000 scripting interface option

Internal scripting	• Visual Basic script editor • Ribbon drop-down menu to access user scripts
External COM-API	Full control of the measurement process and data analysis via objects: • Approach, Imaging, Spectroscopy, Lithography • Video, Signal I/O, Operating Modes • NID-Documents, Measured Data, Charts, DataInfo
Compatibility	• All applications that support the Microsoft COM Automation standard • LabVIEW, MATLAB, Scilab, C++, All .Net languages (C#, Visual Basic, etc.) • Excel, Word, any many other applications

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