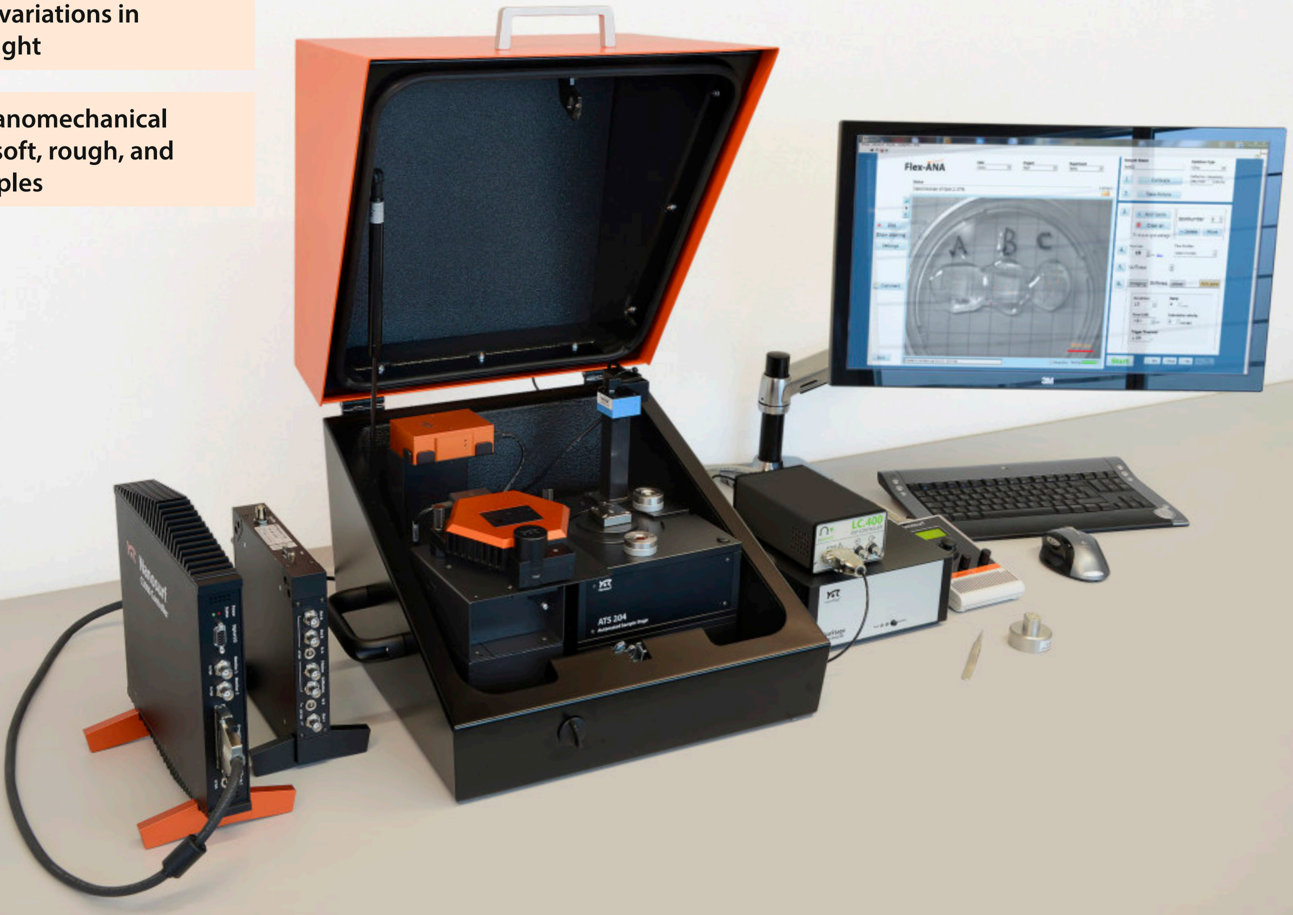
Flex-ANA

AFM for automated nanomechanical analysis

- Real-time, automated data collection and analysis
- Proprietary algorithm to cope with large variations in sample height
- Ideal for nanomechanical testing of soft, rough, and sticky samples



nanosurf

Flex-ANA

Automated nanomechanical data acquisition and analysis

The Nanosurf Flex-ANA system is an automated solution for AFM-based nanomechanical analysis. It is designed to investigate the nanomechanical properties of materials such as cells, tissues, scaffolds, hydrogels, and polymers on multiple or large samples in an easy-to-use fashion.

Key Software Features

- Straight-forward experimental workflow

  Only a few steps from sample mounting to results
- One-click cantilever calibration

  Automatic cantilever deflection sensitivity and spring constant (Sader method) measurement
- Real-time data analysis

  Young's modulus, adhesion, and indentation are calculated and displayed during the measurement
- Proprietary algorithms control the sample height and facilitate nanomechanical measurements on demanding samples

  Sample position is adjusted to always keep the sample within optimal reach of the Z-piezo

Key Hardware Features

- Overview camera with large field of view

  Define measurement locations within a 32 mm x 32 mm area
- Motorized XY movement facilitates automated measurements on multiple samples or different areas of a large sample

  Precisely address pre-defined measurement areas automatically
- Up to three independent Z-actuators allow addressing smooth, rough, sticky and stiff samples

  Proprietary algorithms monitor and synchronize the motion of all Z-actuators to cope with large sample height variations (100 µm within a measurement location and 4 mm across the sample)

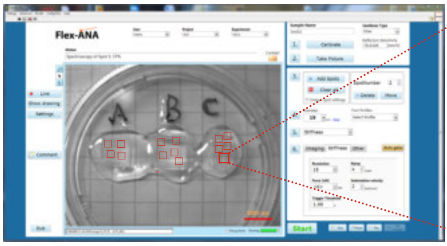
  Address soft and sticky samples employing a long-range Z-scanner (100 µm)
- Reduced viscoelastic effects

  Nanoindentation measurements are performed at appropriate indentation velocities to allow relaxation of the sample

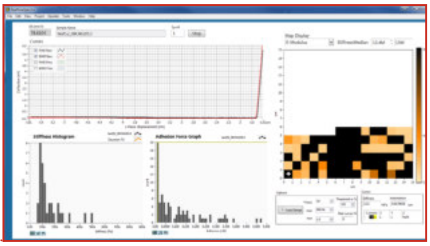
  Automation allows collecting a large number of data without operator presence
- Small indentation depths and forces

  Nanoindentation can be performed with indentation depths and forces significantly lower than possible with conventional nanoindenters

  Force resolution below 7 pN (in liquid)

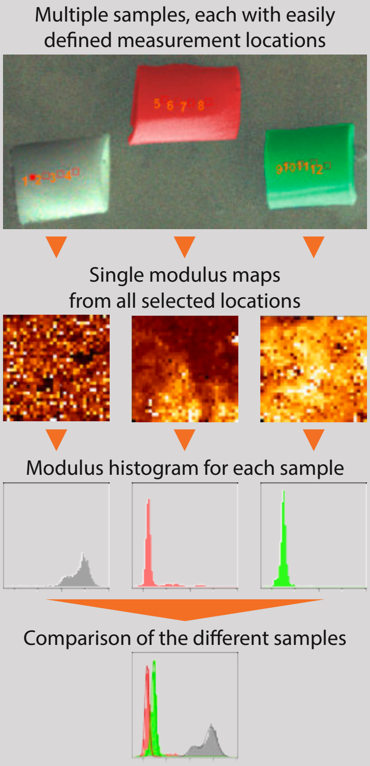


User interface. Touchscreen spot selection.



Real-time data acquisition and analysis.

Flex-ANA workflow:



Multiple samples, each with easily defined measurement locations

Single modulus maps from all selected locations

Modulus histogram for each sample

Comparison of the different samples

- Flex-ANA retains full FlexAFM functionality and can thus be used as a research AFM without limitations
- Existing FlexAFM systems can be easily upgraded to Flex-ANA via system add-ons

Flex-ANA — System composition
Nanosurf FlexAFM scan head
Nanosurf C3000 controller
Nanosurf ATS 204 motorized translation stage
Nanosurf Isostage
Nanosurf FlexAFM video camera
Sample overview camera
Dedicated PC for data acquisition and analysis
Flex-ANA control software
Flex-ANA analysis software
Optional 15-µm Z-range for the FlexAFM scan head
Optional 100-µm Z-actuator for the motorized stage

Examples

Polymeric coatings on medical tubing

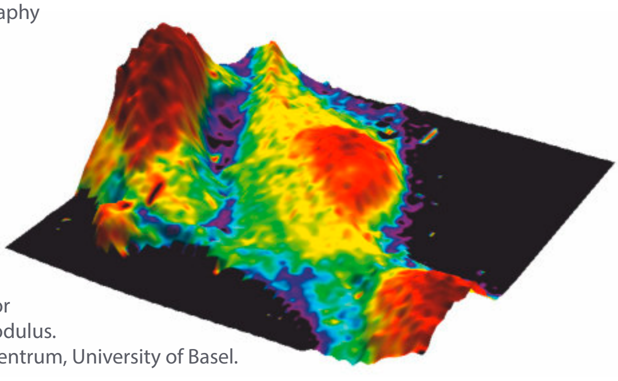
- Tuning surface coatings for improved biocompatibility
  - Biocompatibility is crucial for many devices and consumables used in modern medicine, i.e. surfaces must be compatible with the tissue they interact with
  - Surface coatings made from organic polymers are widely used to confer biocompatibility
  - Polymer properties can be tailored to meet the requirements of the application, e.g. drug release or minimized blood coagulation and foreign body response
  - Polymers provide surfaces with mechanical properties that mimic the generally soft tissue environment
- Coated medical tubing analyzed with Flex-ANA
  - The nanomechanical properties of uncoated and two differently coated tubings were investigated using Flex-ANA in a single run on multiple locations for each tubing.
  - Tubings were fully immersed in buffer solution to allow dwelling of the coatings
  - The thin coatings required low indentation forces and depths
  - Surface coatings significantly lower the elastic modulus of the tubing surface to levels comparable those found in human tissue

Hydrogels for tunable cell culture substrates

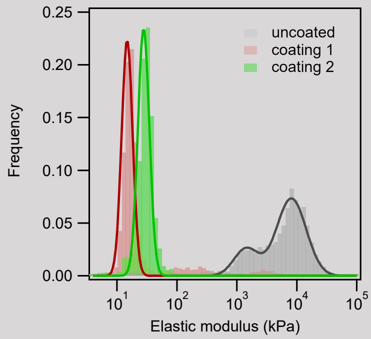
- Cellular microenvironment
  - Cells respond to the mechanics of their microenvironment
  - *In vivo*, cells experience elastic modulus of their surrounding ranging from less than 1 kPa to more than100 kPa, depending on the tissue
  - Cell lineage differentiation can be driven by the mechanics of the environment
- Hydrogels for cell culture
  - A large number of different hydrogels is available
  - Hydrogels can be tuned in their mechanical surface properties to mimic the biological tissue environment of cultured cells
- Analysis using Flex-ANA
  - Automated measurement on four different hydrogel samples at pre-defined locations
  - Hydrogel moduli vary depending on the gelatin concentration
  - The softest hydrogel showed an elastic modulus below 1 kPa

Cell and tissue mechanics

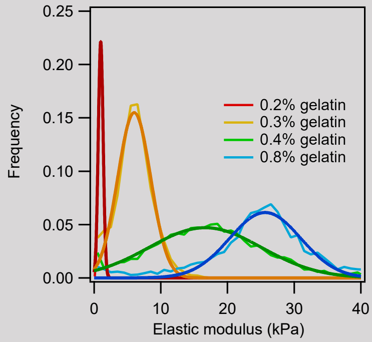
- Diseases often show mechanical phenotypes
  - Changes in cell and tissue stiffness are often related with disease states, e.g. atherosclerosis, fibrosis, osteoporosis, and cancer
  - Metastatic potential of cancer cells correlates with cell stiffness
- Flex-ANA for cell and tissue elasticity measurements
  - Especially tissue samples can be very rough, with several hundreds of micrometers height variation along a tissue section
  - The Flex-ANA system provides sample height adjustment algorithms to overcome the limits of the scan head Z-axis
  - The nanomechanical profile of cells and tissues can be recorded alongside and correlated with the unperturbed sample topography



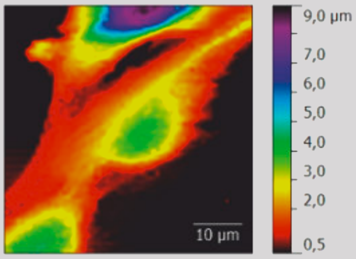
3D representation of the cell topography overlaid with the color scale representing the Young's modulus.
Data courtesy Philipp Oertle, Biozentrum, University of Basel.



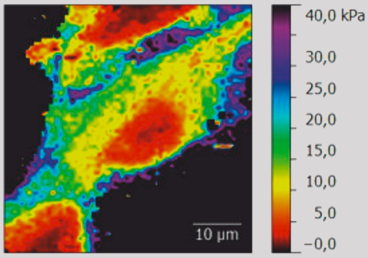
Elastic modulus distributions obtained from three different medical tubings (see inset). While one tubing was uncoated, two tubings exhibited a soft polymeric coating. Histograms represent data obtained during a single Flex-ANA experiment on 3-4 different locations on each tubing. Samples were fully hydrated and immersed in buffer solution during the experiments.



Flex-ANA analysis of fully hydrated gelatin hydrogel cell culture substrates. Histograms represent data recorded as single measurements on 3 different locations for each hydrogel.



The unperturbed sample topography of single cells grown on a plastic Petri dish can be obtained by calculating the contact point of the cantilever with the sample for each point of the force map.



Young's modulus map of single cells.

Flex-ANA

Flex-ANA system workflow

Mounting and calibrating the cantilever	Automated measurement and calculation of deflection sensitivity and spring constant Spring constant calibration based on the Sader Method Resonance frequency and Q-factor extraction from thermal noise power spectrum Frequency range 0–5 MHz Calibration can be performed on the scan head parking station that also facilitates cantilever alignment in liquid and for NIR scan heads using an integrated camera
Sample loading and overview image	35 mm × 35 mm sample platform Overview image covering up to 80 mm × 60 mm
Definition of measurement locations and conditions	Measurement locations can be defined on the optical overview image Measurement conditions, e.g. contact force, indentation velocity or resolution, can be defined in the Flex-ANA software
Start of the automated measurement	Autonomous measurements at defined locations Automatic sample approach at each location with an approach range >5 mm Real-time data analysis (elastic modulus in the range of 0.1 kPa – 20 GPa (1), adhesion, indentation , height, slope of contact region) Map and histogram are available for each analysis parameter Proprietary algorithms adjust the sample height to cope with large height variations on rough and uneven samples
Analysis optimization	Integrated data browser with data preview Various contact mechanics models (Hertz, Sneddon, Oliver-Pharr, DMT, JKR, and user-defined power-law) Batch data processing of multiple data sets Force curve quality check
Data grouping and comparison of different samples	Definition of regions of interest (ROIs) Grouping of multiple data sets or ROIs Comparison of multiple data sets or groups
Results	Export of final histograms and maps as data or image files

(1) Accessible modulus range depends on choice of cantilever

ATS 204 specifications

Range (X / Y / Z)	32 / 32 / 5 mm
Optional δZ range	100 µm
Positioning accuracy (X / Y / Z / δZ)	<1 / <1 / <1 / <0.001 µm
Repositioning accuracy (X / Y / Z)	<2 µm

FlexAFM scan head features

General design	Tripod stand-alone scan head, flexure-based electromagnetically actuated XY-scanner, decoupled piezo-based Z-scanner
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, Non-Contact, Magnetic Force, Electrostatic Force, Kelvin Probe Force, Scanning Thermal, Spreading Resistance, Force Modulation, Multiple Spectroscopy modes, Lithography and Manipulation modes, Liquid, EC-AFM, Conductive AFM, Lift mode, Contour mode, Nanoindentation. Some modes may require additional controller options.

FlexAFM scan head specifications

Laser class (wavelength)	NIR: Class 1M (840 nm) Red: Class 2 (650 nm)
Maximum Petri dish height (fluid level)	9 mm (6 mm)
Automatic approach range	2 mm
Maximum XY-scan range	100 µm (1)
Maximum Z-range	10 µm (2)
Drive resolution in XY	0.006 nm (3)
Drive resolution in Z	0.0006 nm (3)
Z-sensor resolution noise (@3kHz)	typ. 180 pm, max. 200 pm
Force noise	<7 pN (4)
XY-linearity mean error	<0.1%
XY-flatness at maximum scan range	typ. 5 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
(4) Measured with a qp-CONT cantilever in PBS at 10 kHz sampling rate

C3000 controller specifications

X/Y/Z-axis scan and position controller	3× 24-bit DAC (200 kHz)
X/Y/Z-axis position measurement	3× 24-bit ADC (200 kHz)
Additional user signal outputs	3× 24-bit DAC (200 kHz)
Additional user signal inputs	3× 24-bit ADC (200 kHz)
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
Power	90–240 V AC, 70 W, 50/60Hz

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