SUMMER WORKSHOP ON BIOAFM MICROSCOPY 2023, BRNO CZECH REPUBLIC

Integration of AFM with other techniques

Dr Alexander Dulebo
Application Scientist

Outline

1 AFM and optical microscopy

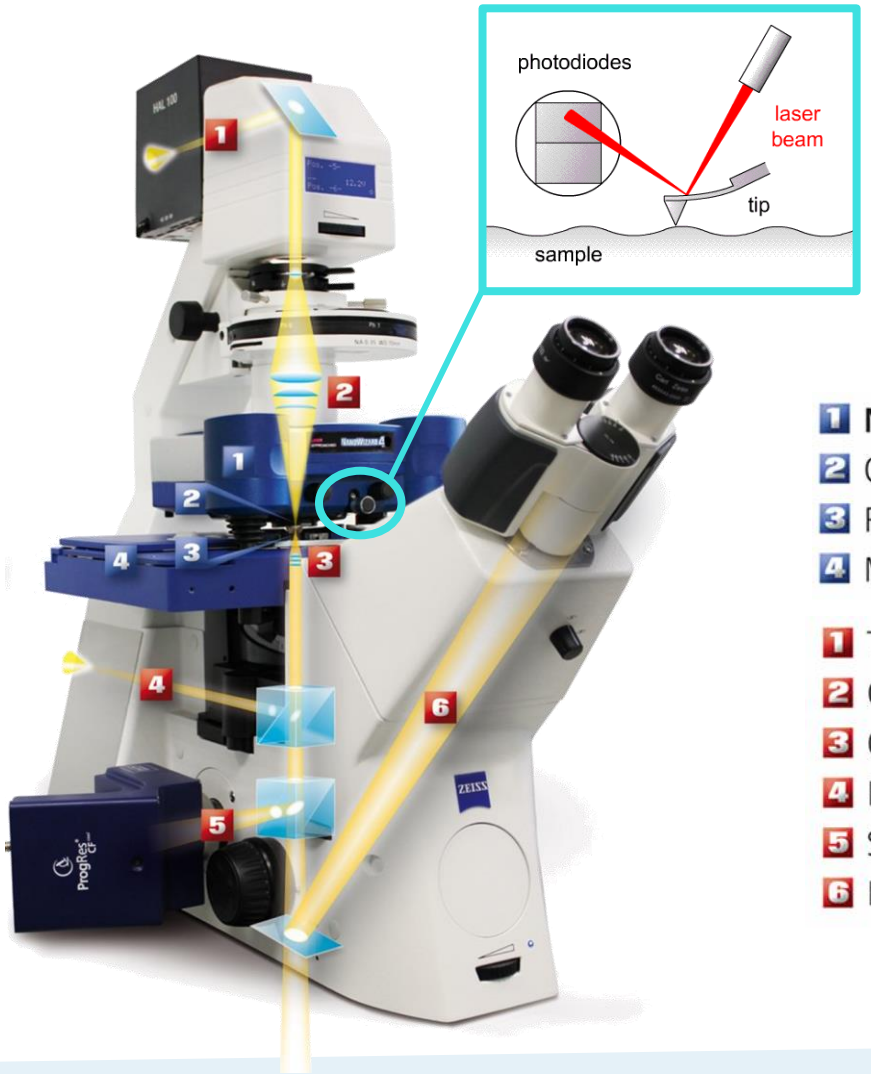
2 AFM and Raman

3 AFM and nanoIR

4 AFM and FluidFM

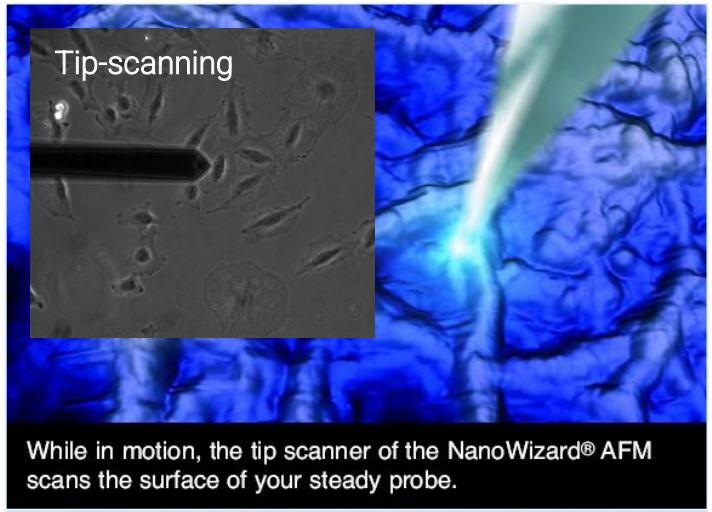
01 AFM and optical microscopy

BioAFM and optical microscopy integration

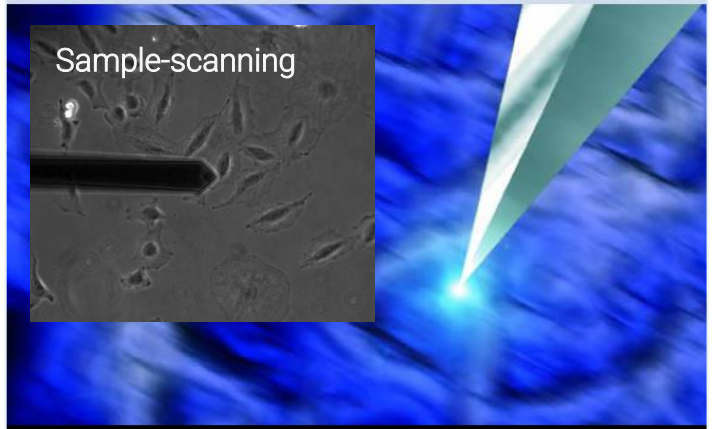


1. **NanoWizard®** head
2. Cantilever holder
3. Petri dish
4. Motorized stage

1. Transmission light beam path
2. Condenser lens
3. Objective
4. Fluorescence excitation path (backport)
5. Side port with fluorescence camera
6. Eye piece beam path

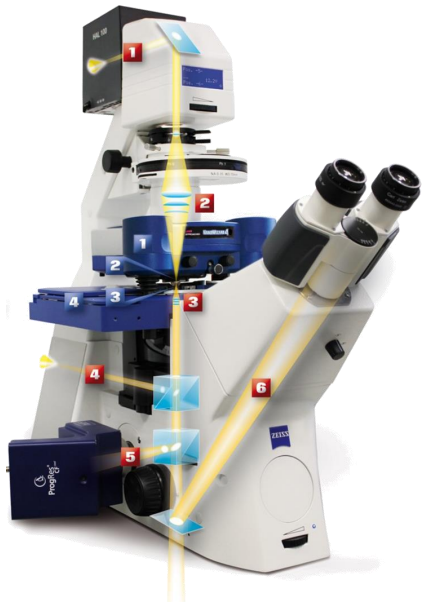


While in motion, the tip scanner of the NanoWizard® AFM scans the surface of your steady probe.



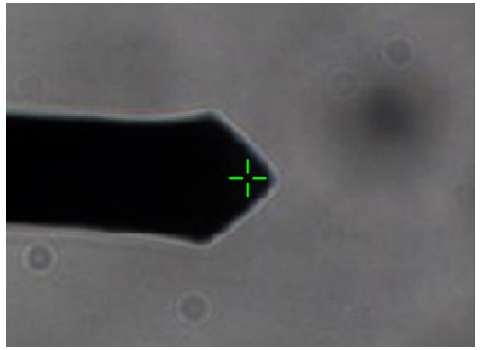
While scanning the surface, a sample scanning AFM moves the sample holder.

Optical integration perfected



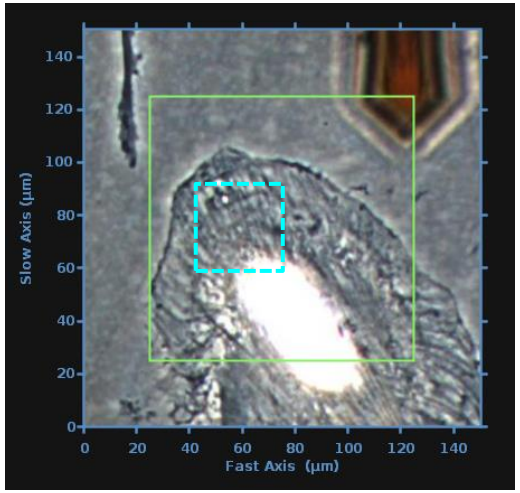
- Tip-scanner AFM design means sample does not move while AFM is scanning
- Standard condenser strongly recommended, particularly for living cells
- Perfect integration with inverted optical microscopes
- Compatible with optical super-resolution techniques (STED, STORM/PALM, SIM)
- Fully simultaneous operation with fluorescence, even for TIRF, FRET, FLIM, FRAP, FCS, Raman, SNOM...

DirectOverlay™ 2 - optical image calibration

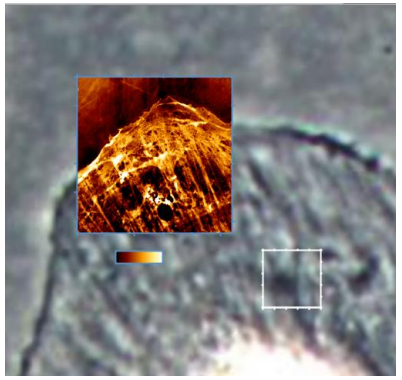


Automatic detection of the tip position in the optical image

→ Correlation of optical and AFM space

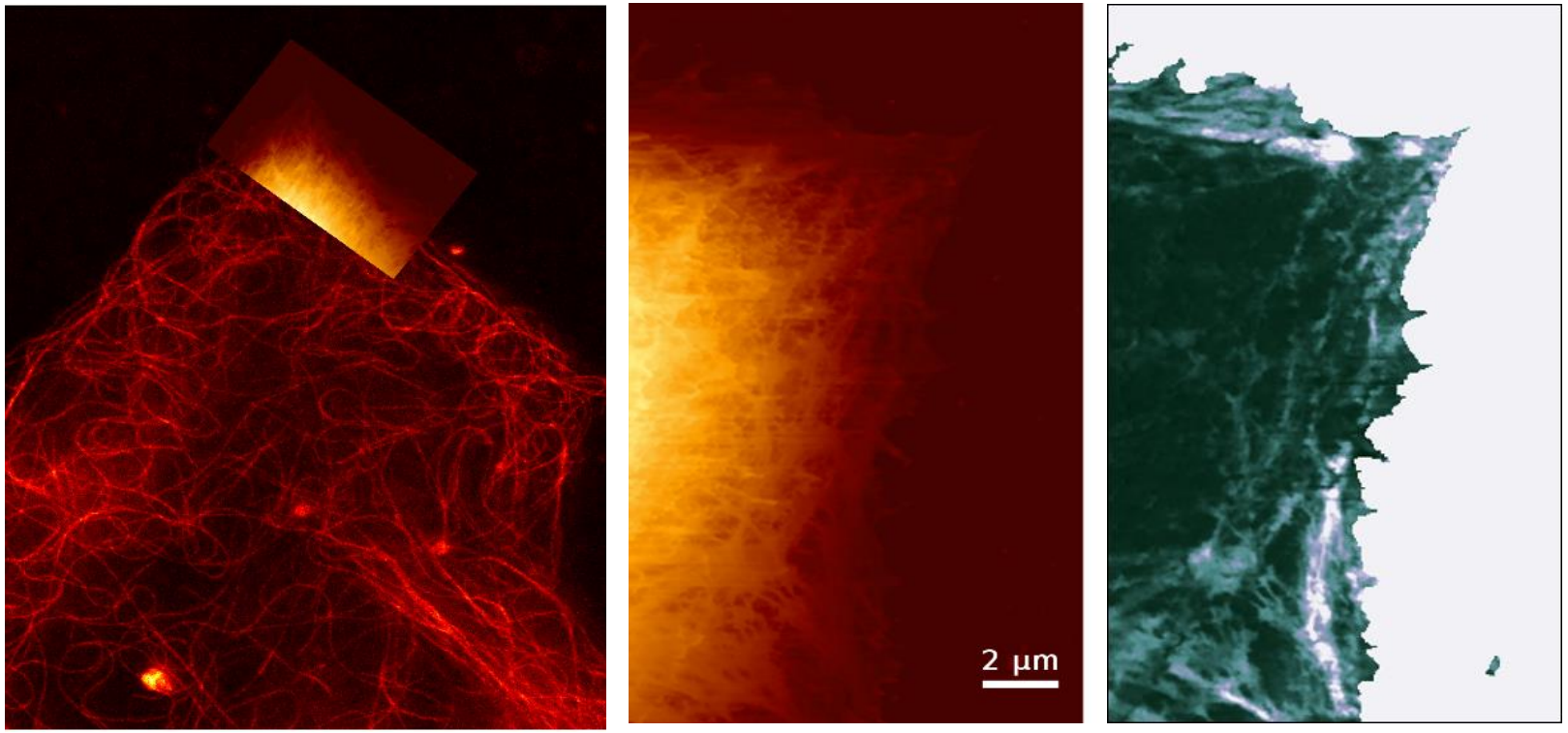


- Import optical image into the AFM software
- Select region of interest and start scanning



All AFM images can be selected in the optical image

AFM & STED on living human lung cancer cells (A549)



- Living A549 cells imaged at 37°C in medium.
- Left: STED image of microtubules labelled with silicon rhodamine overlayed with AFM topography
- Mid: AFM QI topography image at 240 pN imaging force (height range 3.5 µm)
- Right: Corresponding Young's modulus image (z range 100 kPa)

Collaboration with Abberior Instruments – STEDYCON on Zeiss Axio Observer

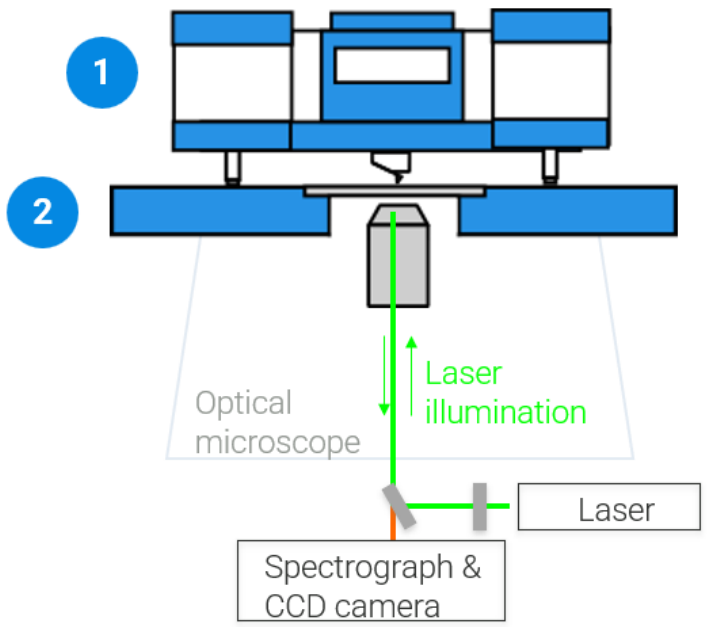
Sample courtesy of A. Hermann group, HU Berlin, DE

02 AFM and Raman

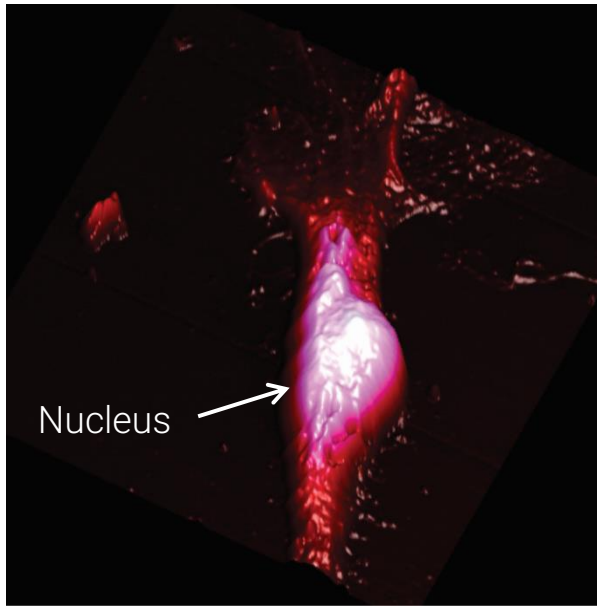
AFM and Raman

What is Raman spectroscopy?

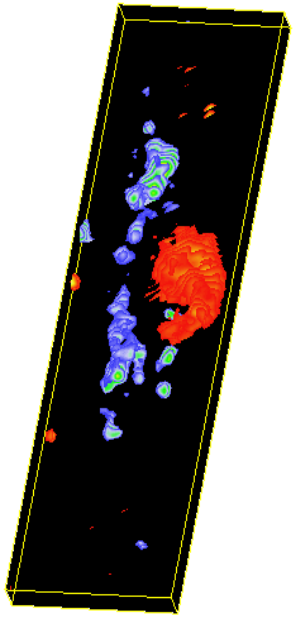
- Inelastic scattering of photons -> shift in wavelength -> vibrational modes of molecules
- Non-destructive



AFM-Topography

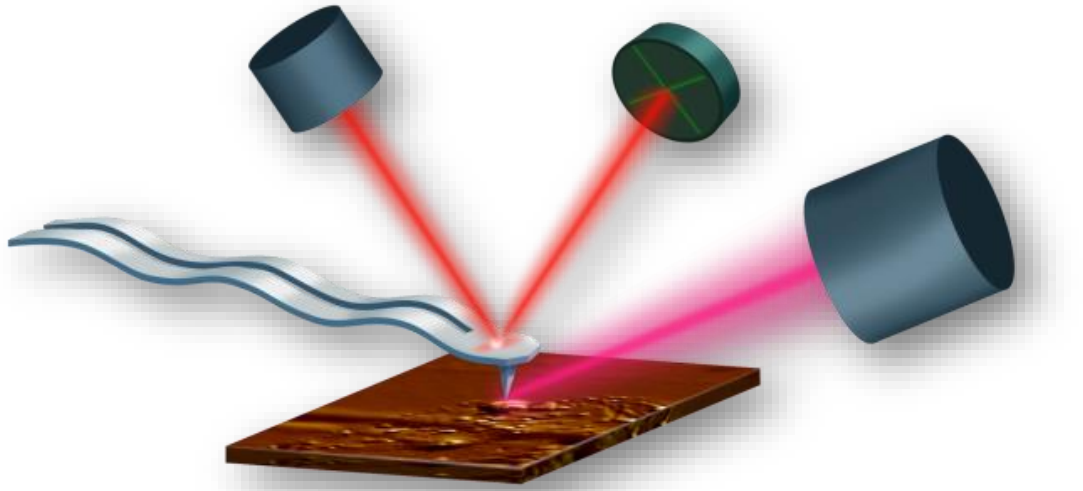


Raman-Map



03 AFM and nanoIR

Nanoscale IR spectroscopy in the life sciences



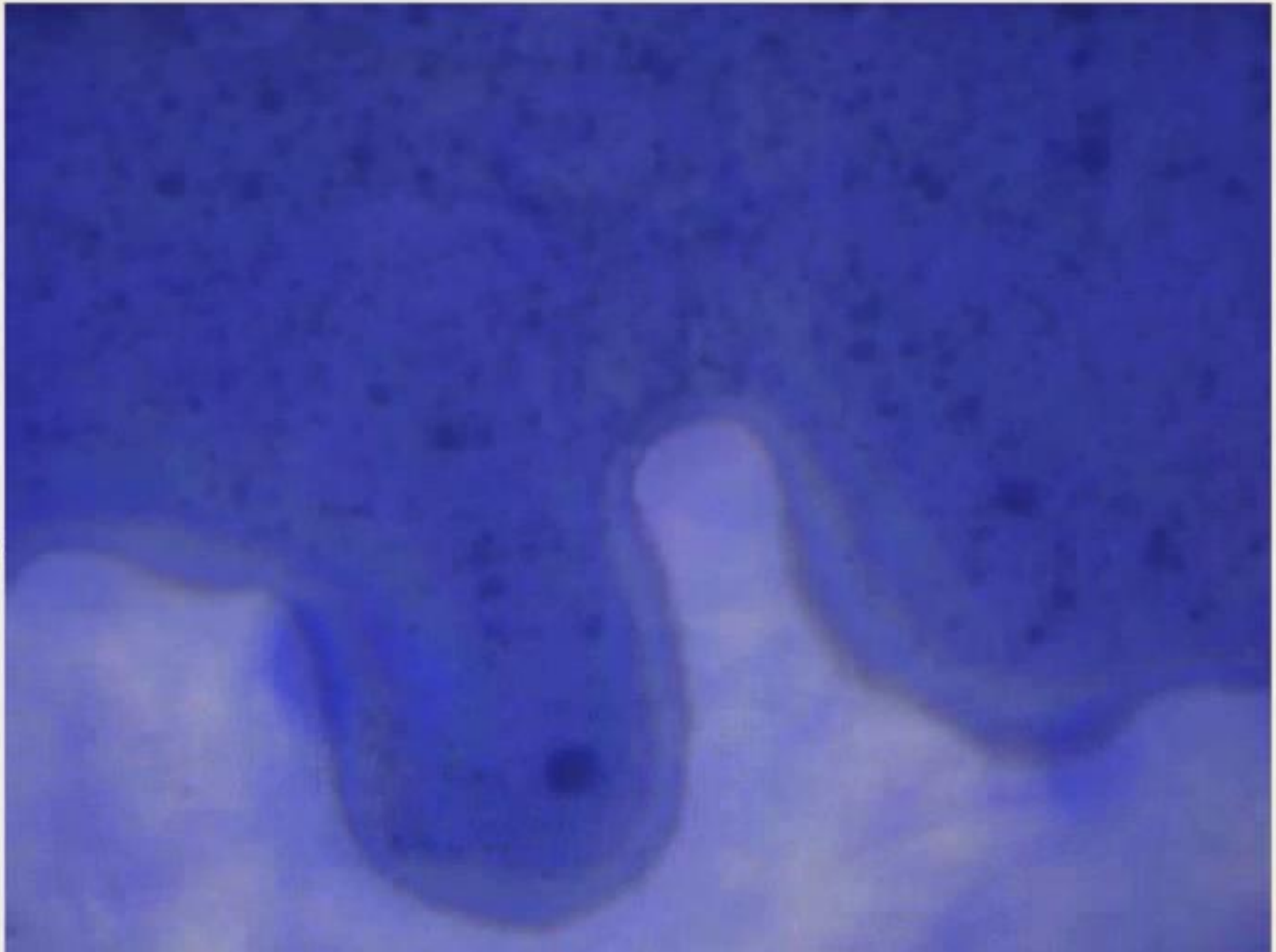
ANASYS INSTRUMENTS
The nanoscale analysis company

is a part of Bruker

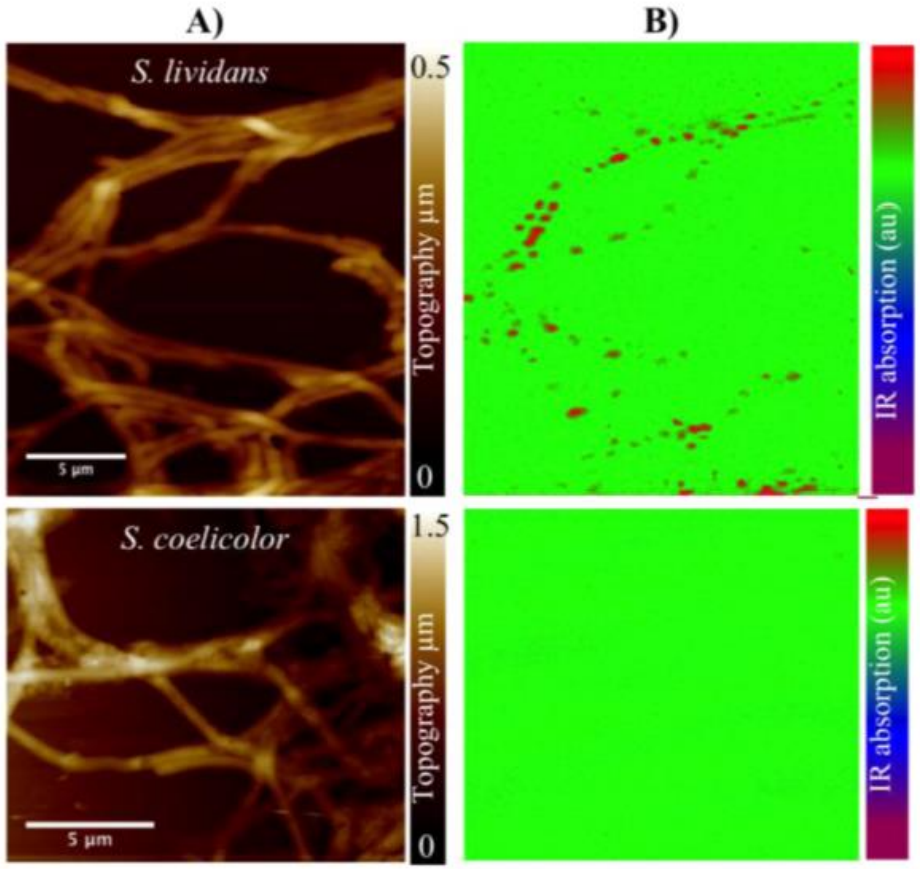
Dimension IconIR

nanoIR3™
nanoIR platform with Tapping AFM-IR

Optical microscope view



Accumulation of TriAcylGlycerols in *Streptomyces* Species



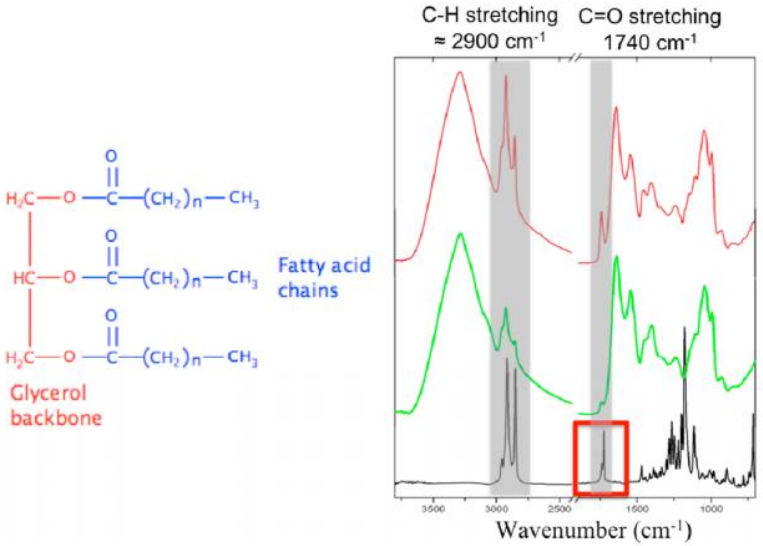


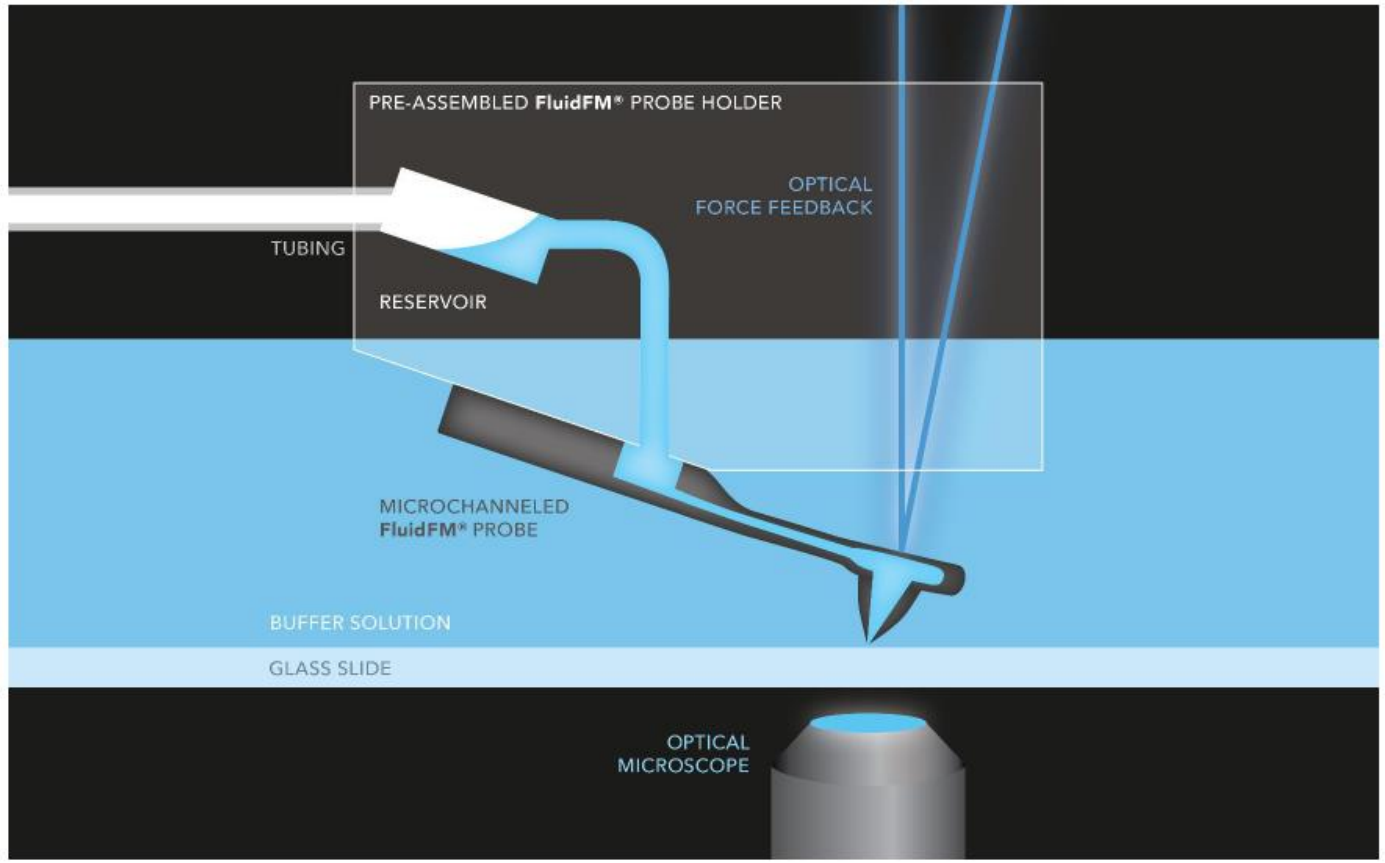
Figure 3. (A) AFM topography and (B) chemical mapping at 1740 cm^{-1} for the two strains.

Deniset-Besseau, et al, Chem. Lett., 5 (4) 654–658 (2014)

04 AFM and FluidFM

FluidFM technology

pressure control



FluidFM

- 300 nm - 8 µm aperture
- ~5 pL volume
- Femtoliters per second flow

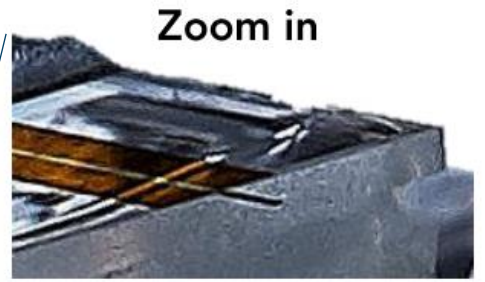
ETH zürich

CYTOSURGE®

FluidFM Probes

Probe = consumable

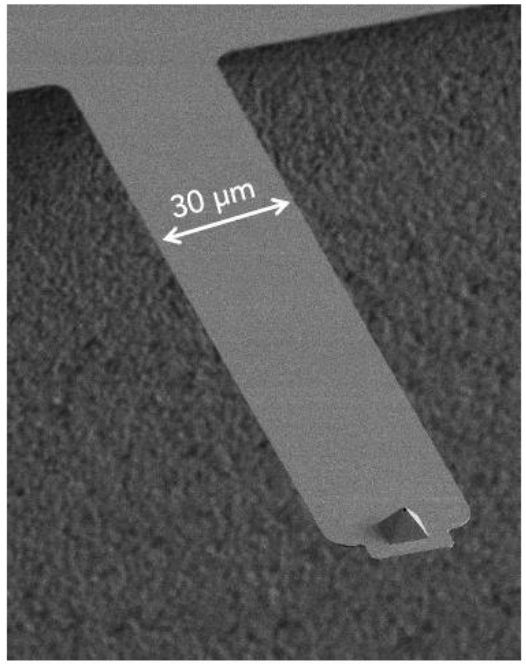
- Pre-mounted
- Easy to use
- Reliable

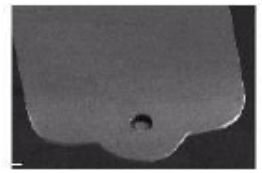


FluidFM probe blister

- Clean and robust
- Convenient
- QR-code

FluidFM probes

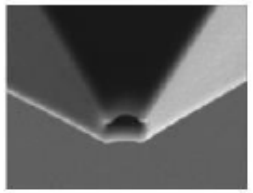




FluidFM micropipette

0.3 – 4N/m, aperture sizes: 2, 4, 8 µm

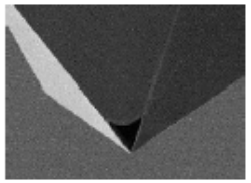
Single cell manipulation, colloids, local dispensing & single cell isolation and adhesion



FluidFM nanopipette

0.6 – 2N/m, aperture sizes: 300 nm

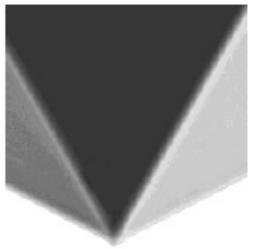
Nano-printing, manipulation of sub µm particles, bacteria adhesion



FluidFM nanosyringe

2 N/m, aperture sizes: 800 nm

Injection into & extraction from Single cells



FluidFM prototyping probe

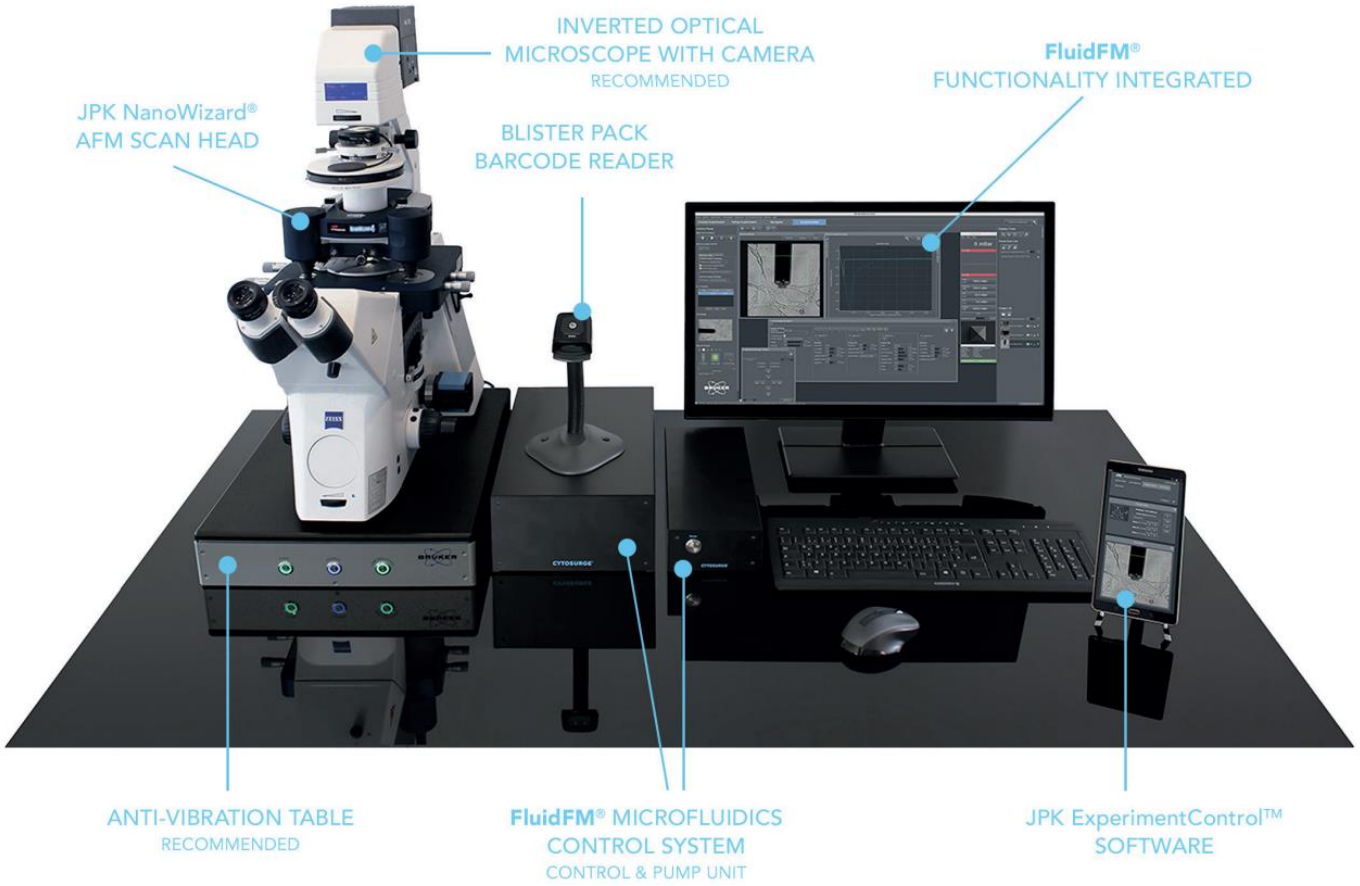
Aperture can be customized with Focused Ion Beam (FIB)

0.6 – 2 N/m, 30+ nm

Application depending on the customization

Technical implementation on NanoWizard AFM

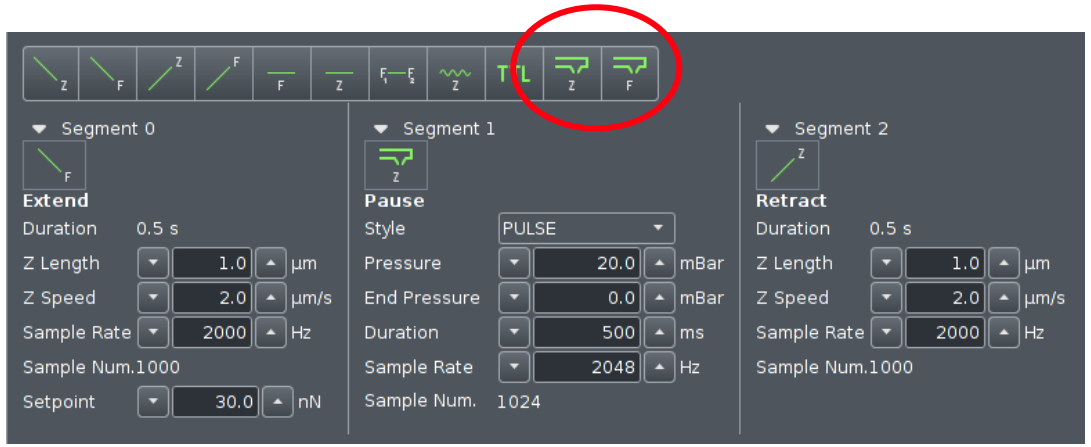
Cantilever holder with Cyto clip mounted on AFM head

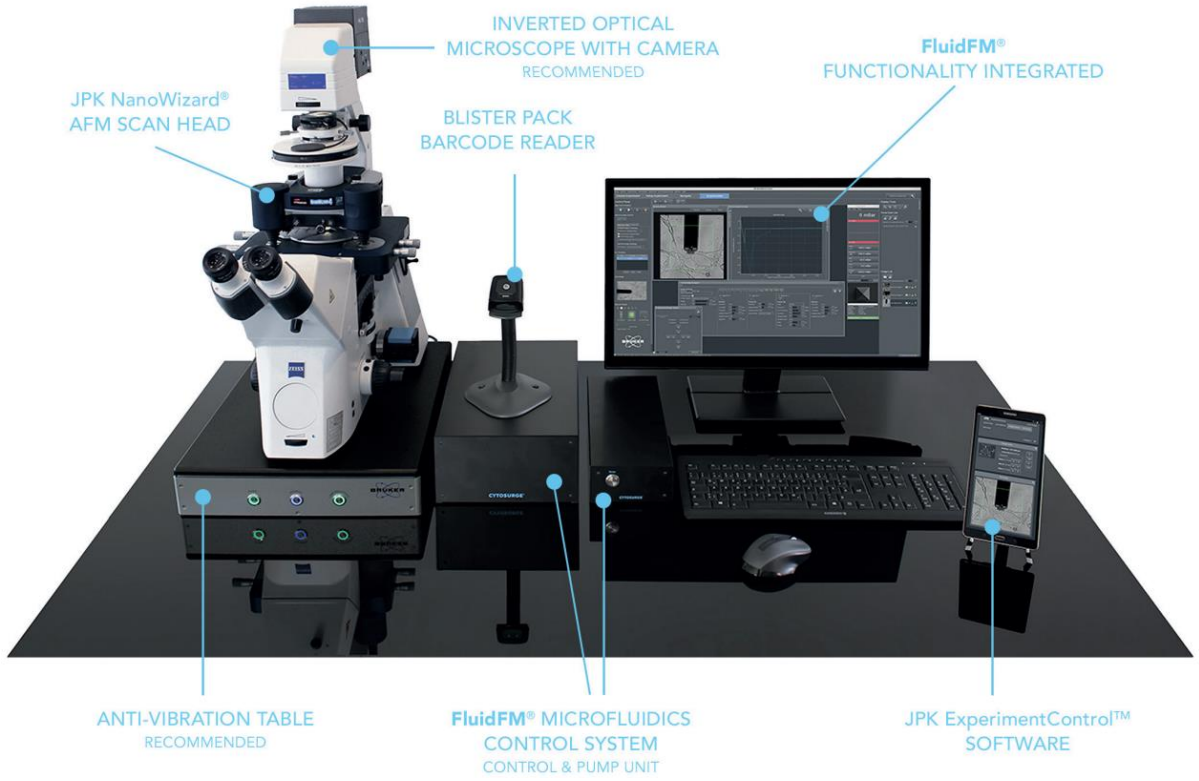


Technical implementation on NanoWizard

Software integration:

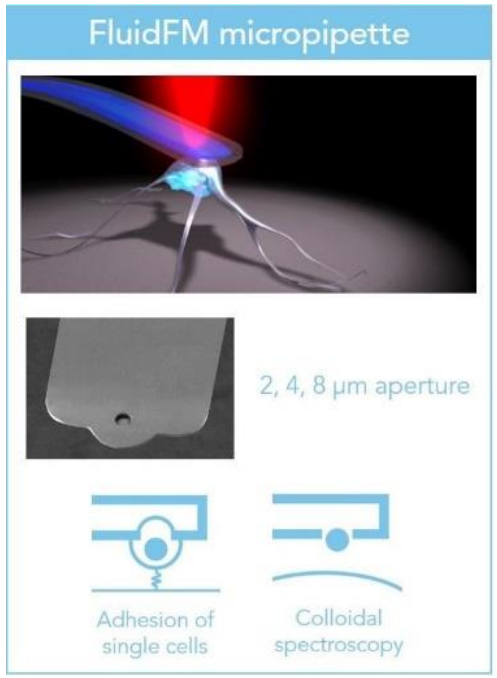
- Manual application of negative/positive pressure
- Pulses or continuous pressure
- Integration of pressure application in force ramps/automated force experiments.

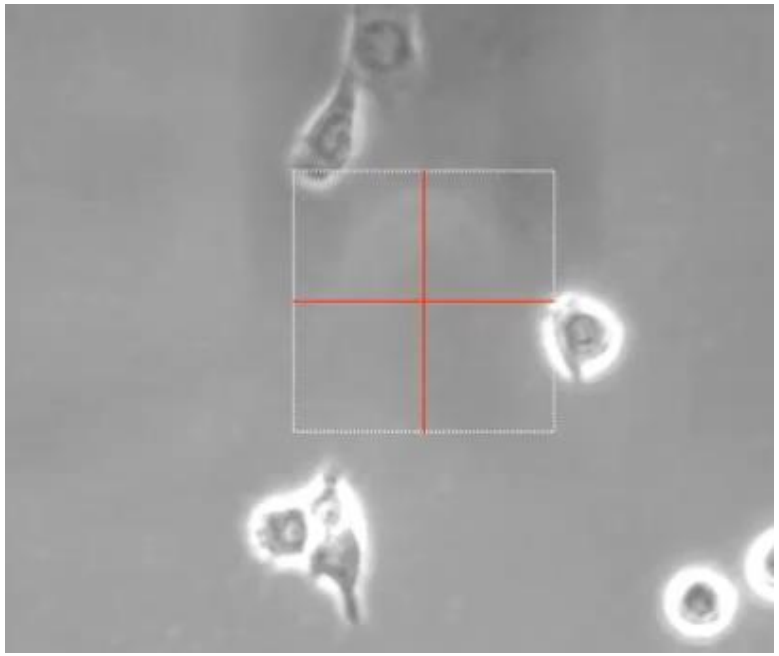




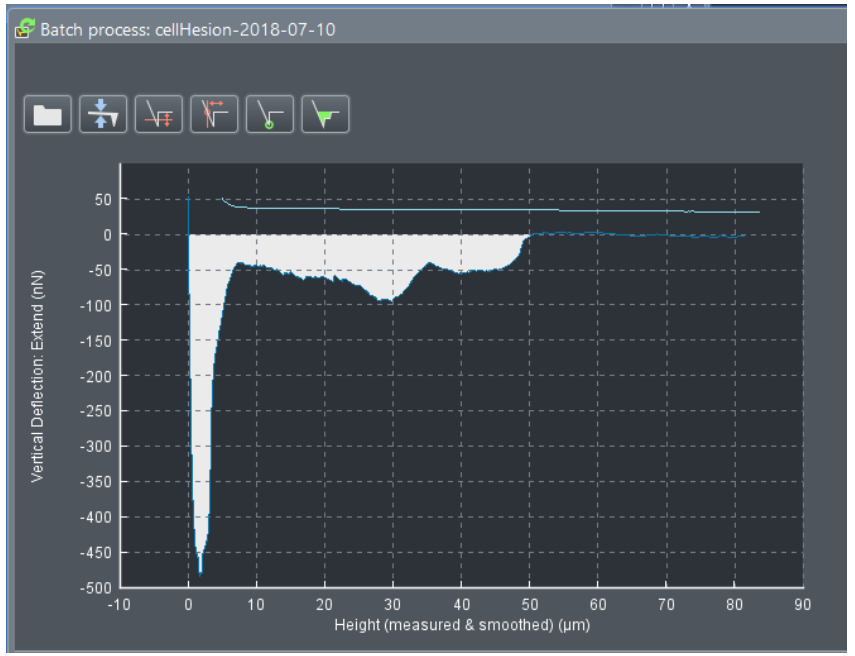
Major FluidFM applications

Cell adhesion/separation of adherently growing cells





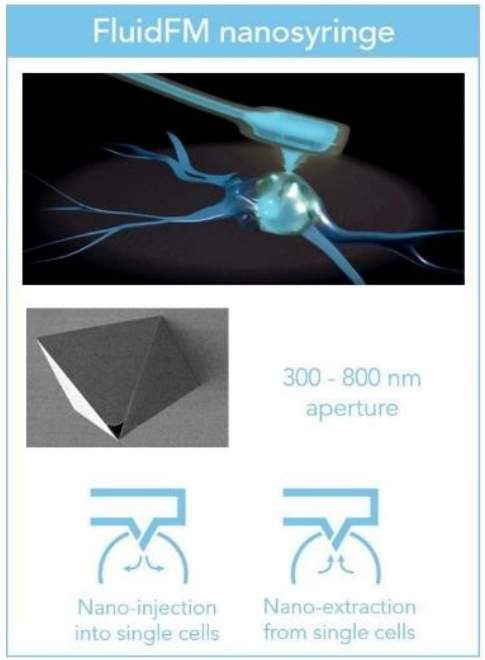
Phase contrast of living cells. A 4µm micropipette is used to separate the cell from the substrate.

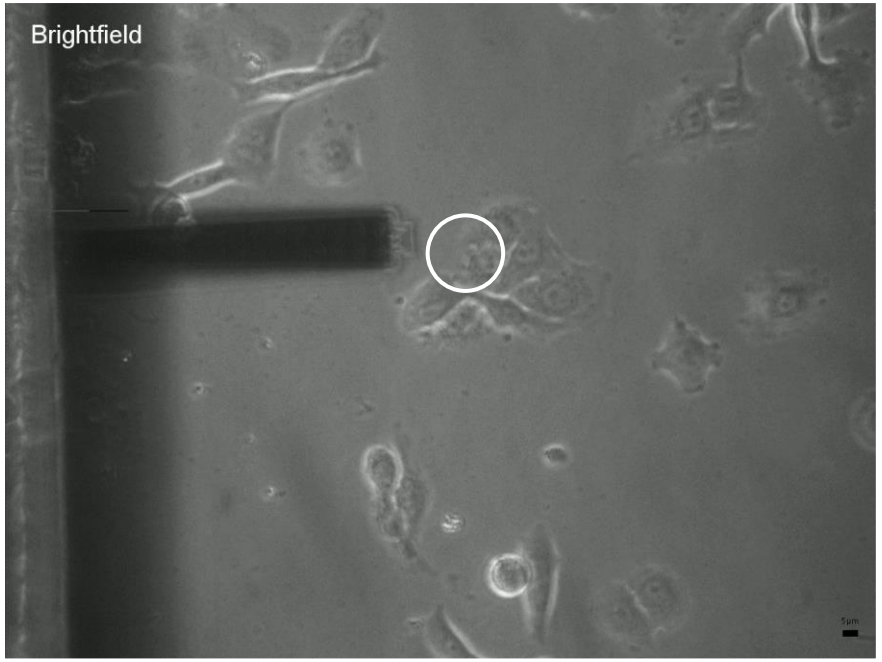


Resulting force distance curve.

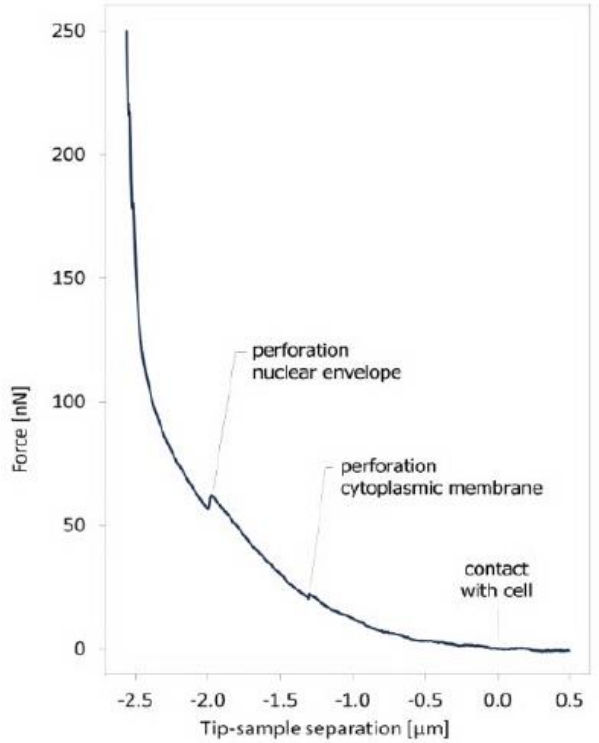
30 to 200	nN to µN	pN
CELLS PER DAY	PICK UP ANY CELL	RESOLUTION

Cell injection and extraction



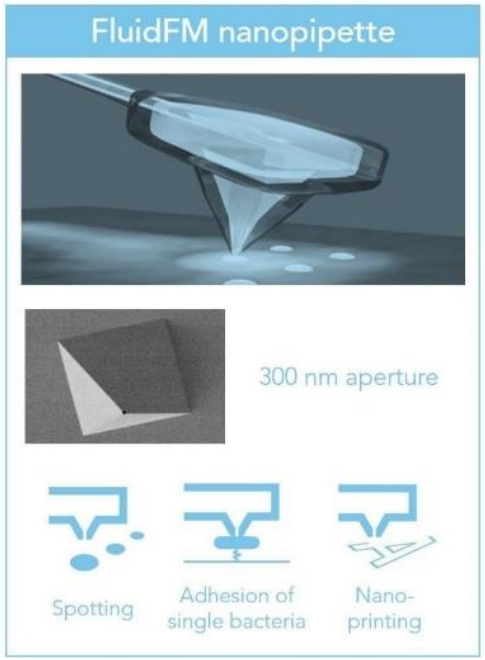


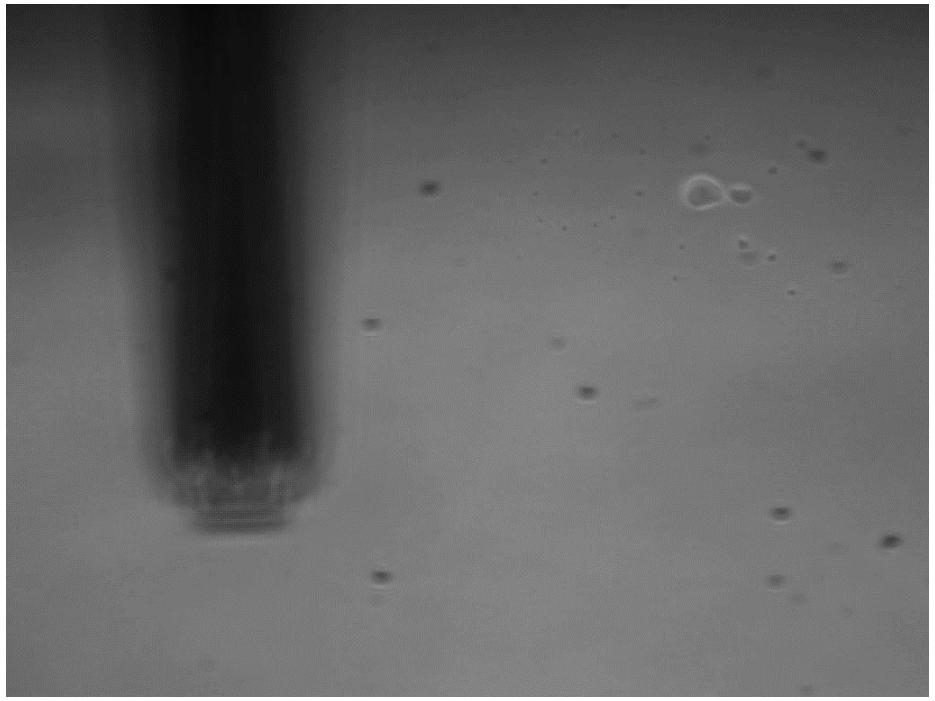
Phase contrast and epifluorescence imaging of living CHO cells. A nanosyringe is used to inject Propidium iodide into the indicated cell



10+	90%+	95%+
CELLS/HOUR with AFM	SUCCESS RATE	VIABILITY

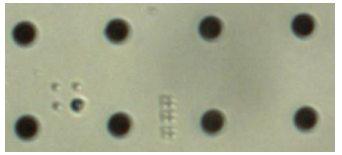
Nano spotting



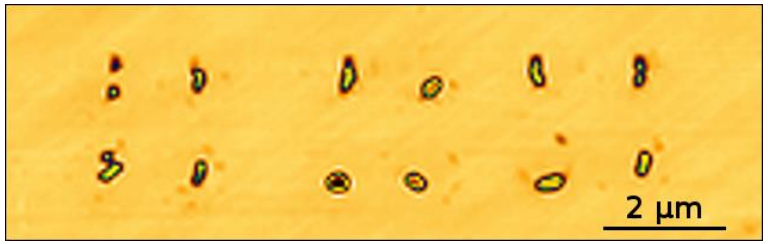


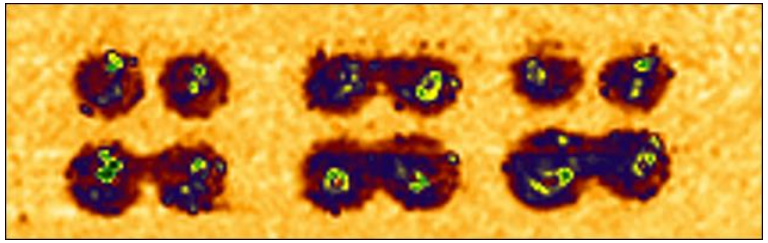
Phase contrast while spotting a glycerol/water mixture on glass.

High reproducibility using the NanoWizard®



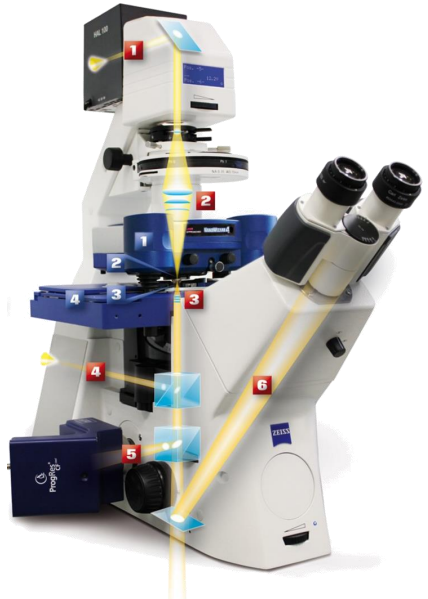
Optical image of the deposited spots: 2x2 maps with 3x3µm², gap 1.5 µm.



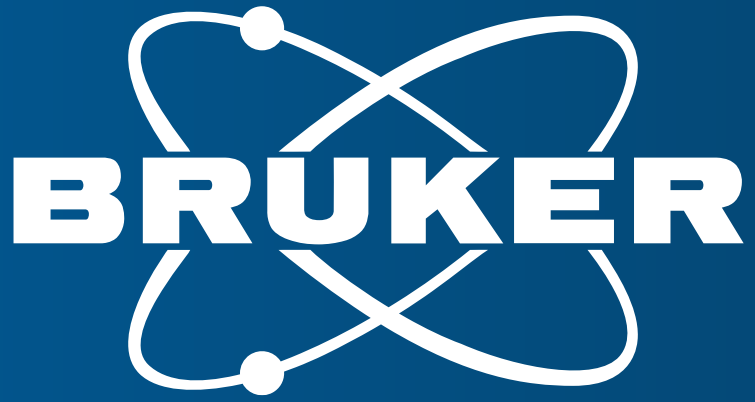


Slope channel useful to calculate the spotting area and volume.

BioAFM is a versatile add-on to any type of microscopy



Thank you!



Innovation with Integrity