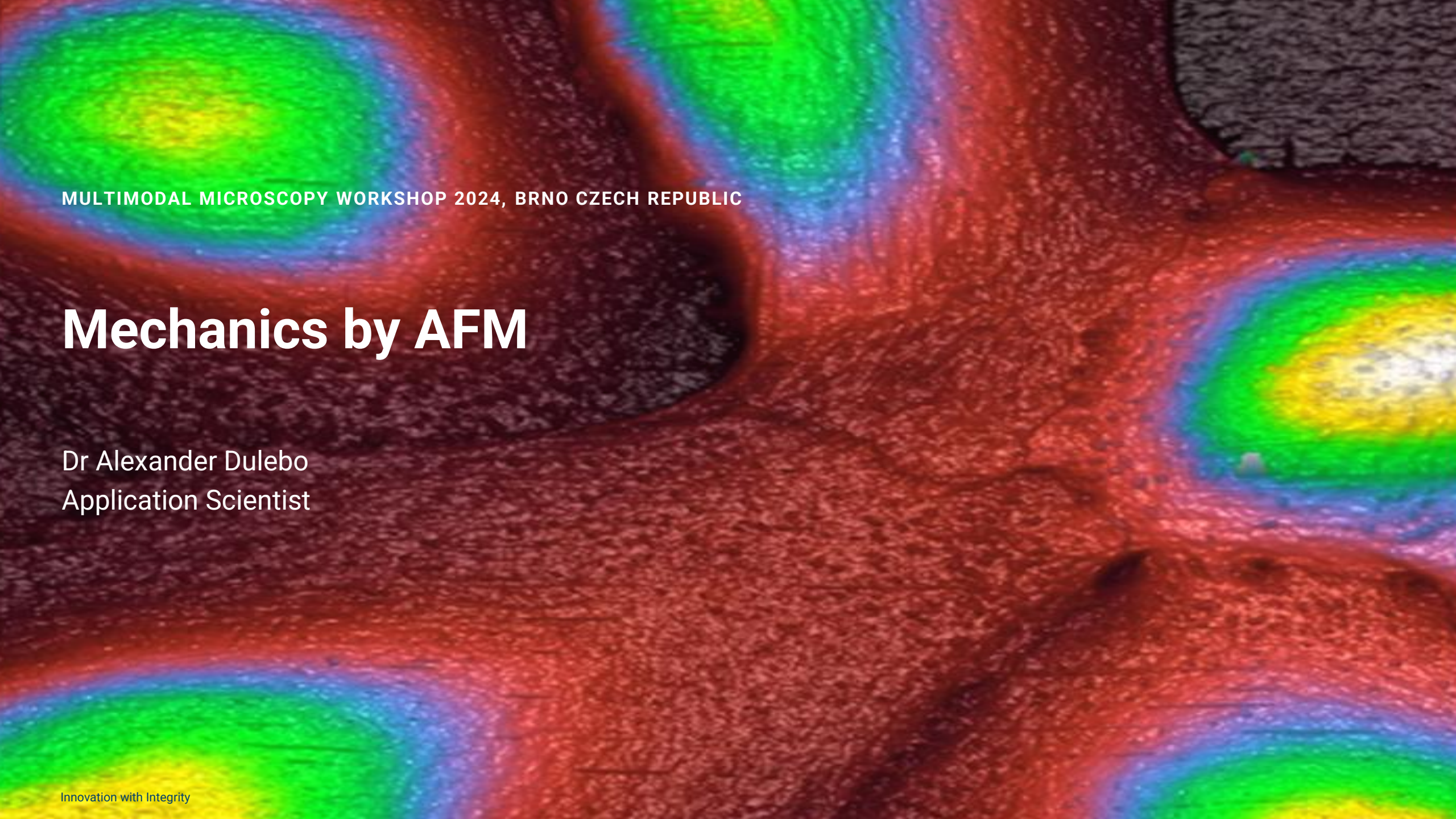
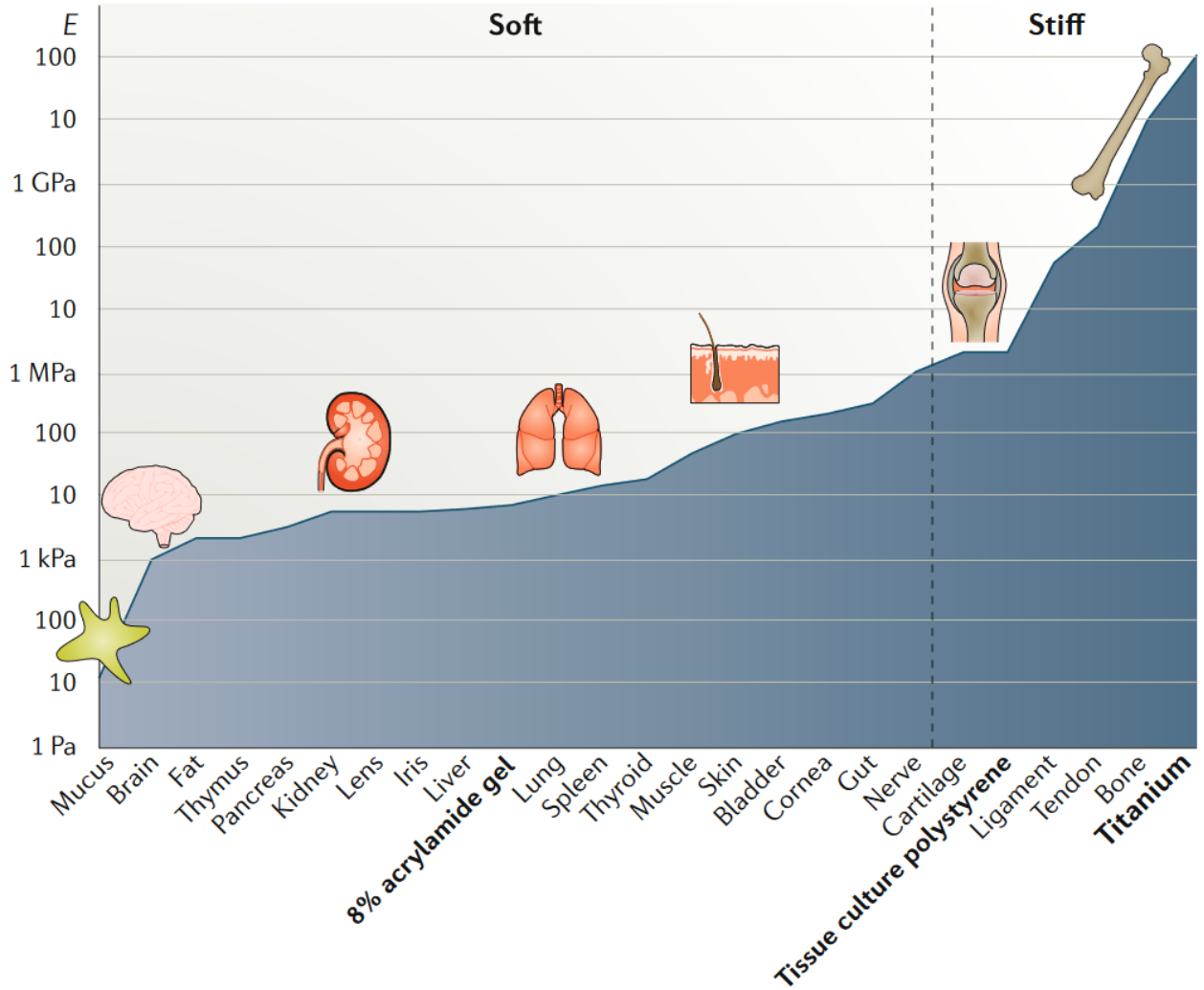


MULTIMODAL MICROSCOPY WORKSHOP 2024, BRNO CZECH REPUBLIC

Mechanics by AFM

Dr Alexander Dulebo
Application Scientist

The stiffness of living tissues spans a wide range

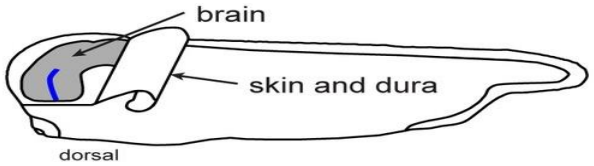


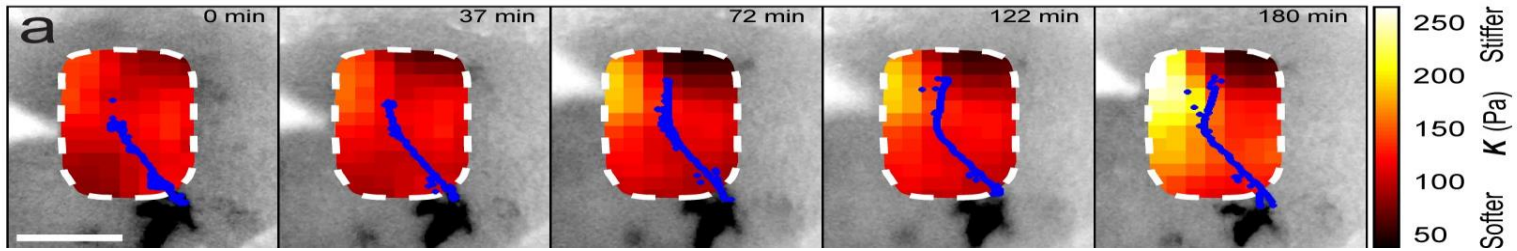
Adapted from Guimaraes et al. 2020, Nat Rev Mat. 5, 351–370

- Cancer, pathological alterations or developmental differentiation can change tissue:
  - Elasticity
  - Topography
  - Adhesion behaviour.
- Mechanics becomes a Biomarker.

Mechanics Becomes a Biomarker

- Development



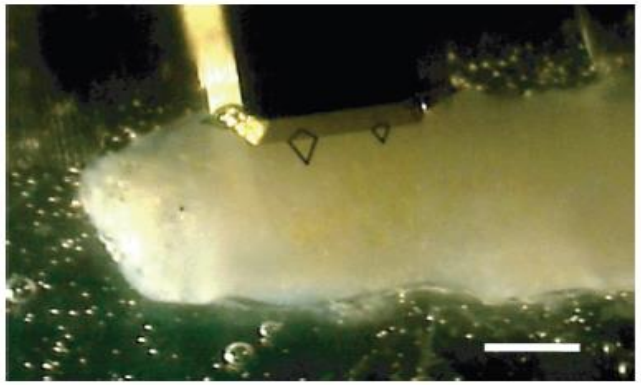


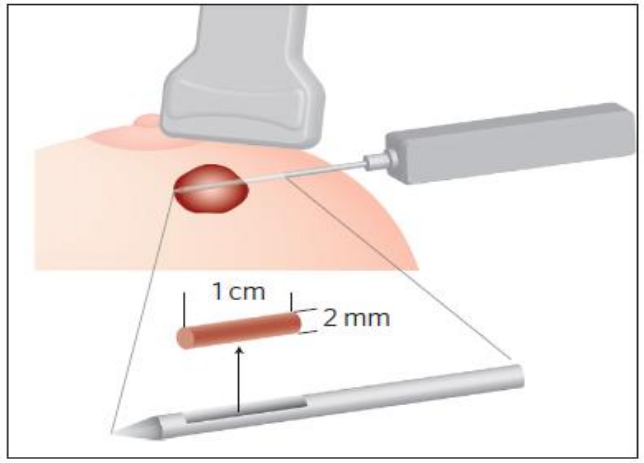
Adapted from Thompson et al. 2019, eLife. 8:e39356

Rapid changes in tissue mechanics regulate axon behavior in the developing embryonic brain.

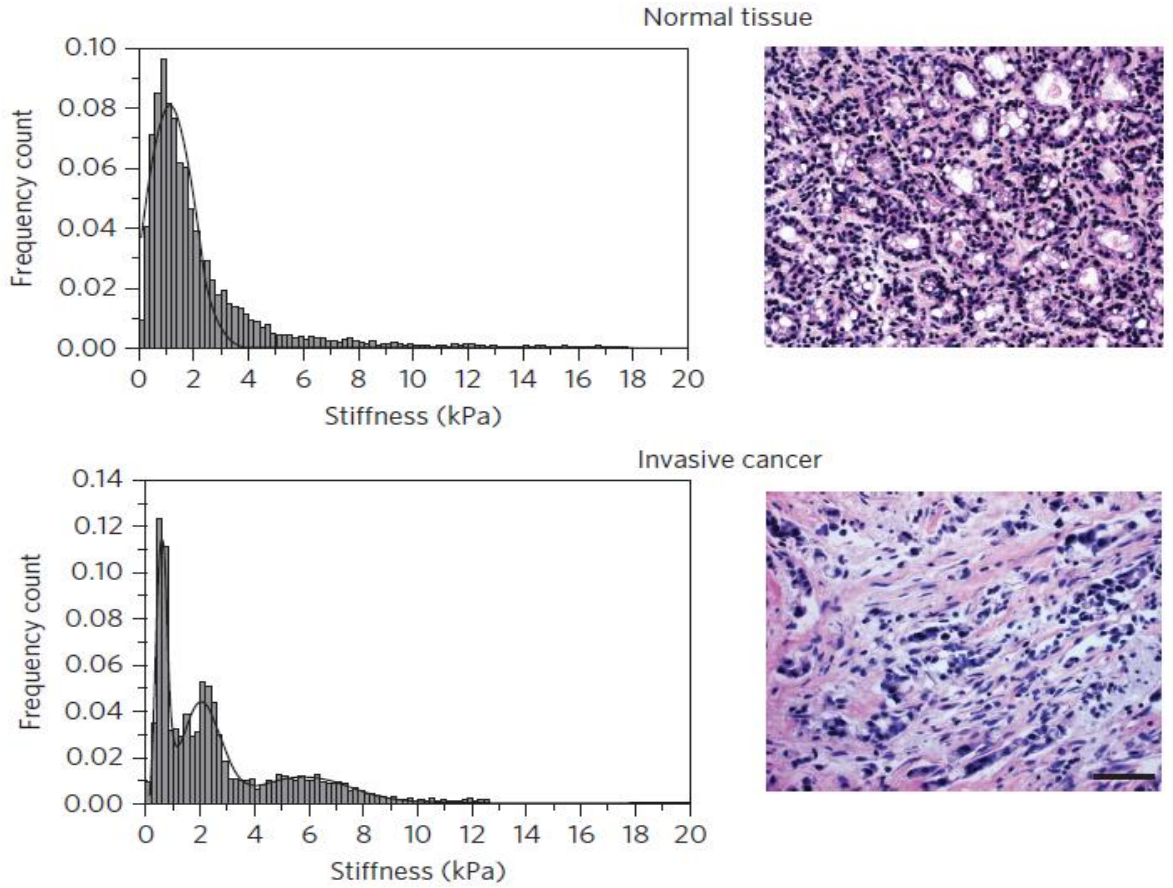
Mechanics Becomes a Biomarker

- Disease



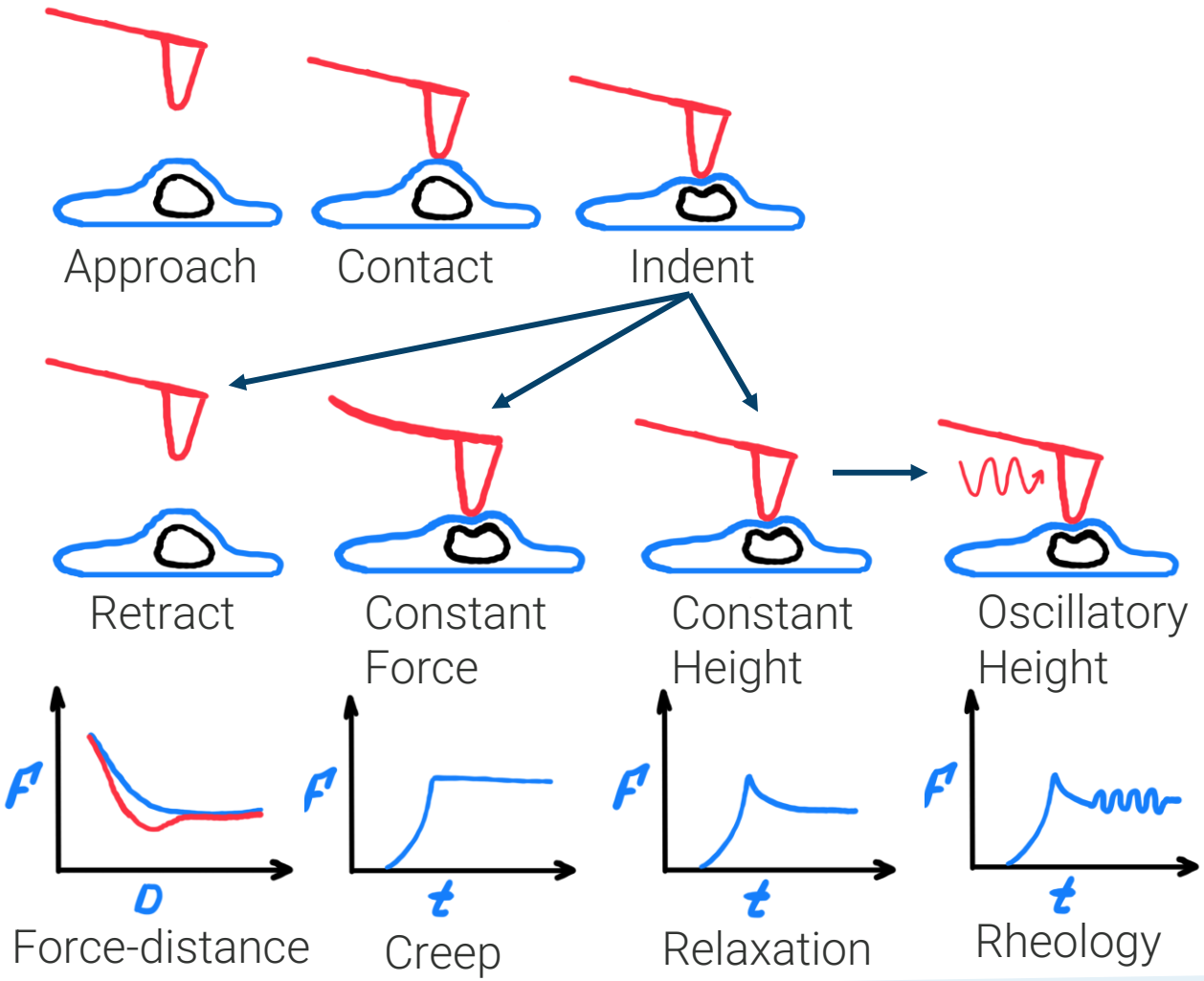


Adapted from Plodinec et al. 2012, Nat Nanotechnol. 7(11):757-65

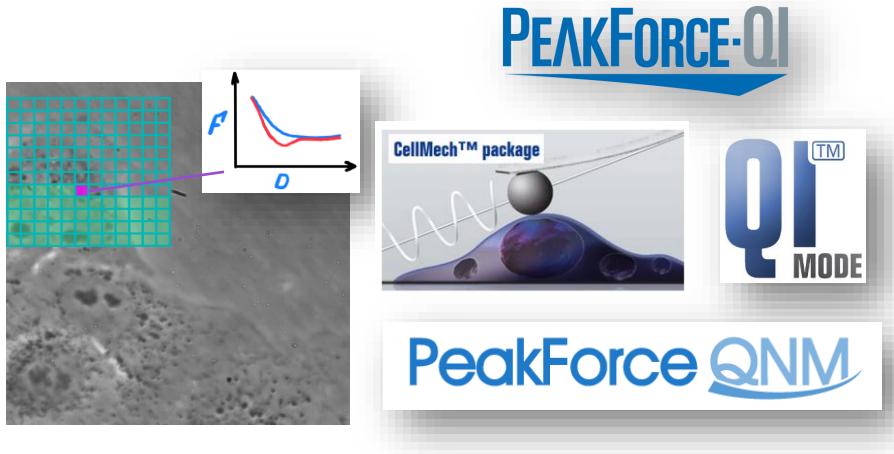


Breast cancer malignant tissues display a broader stiffness distribution than their healthy counterparts.

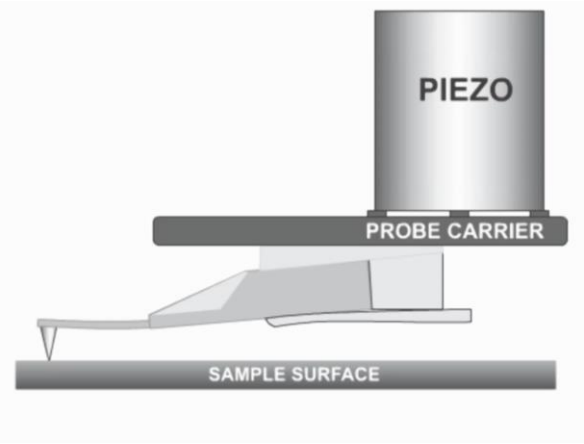
AFM-based Mechanical Measurements

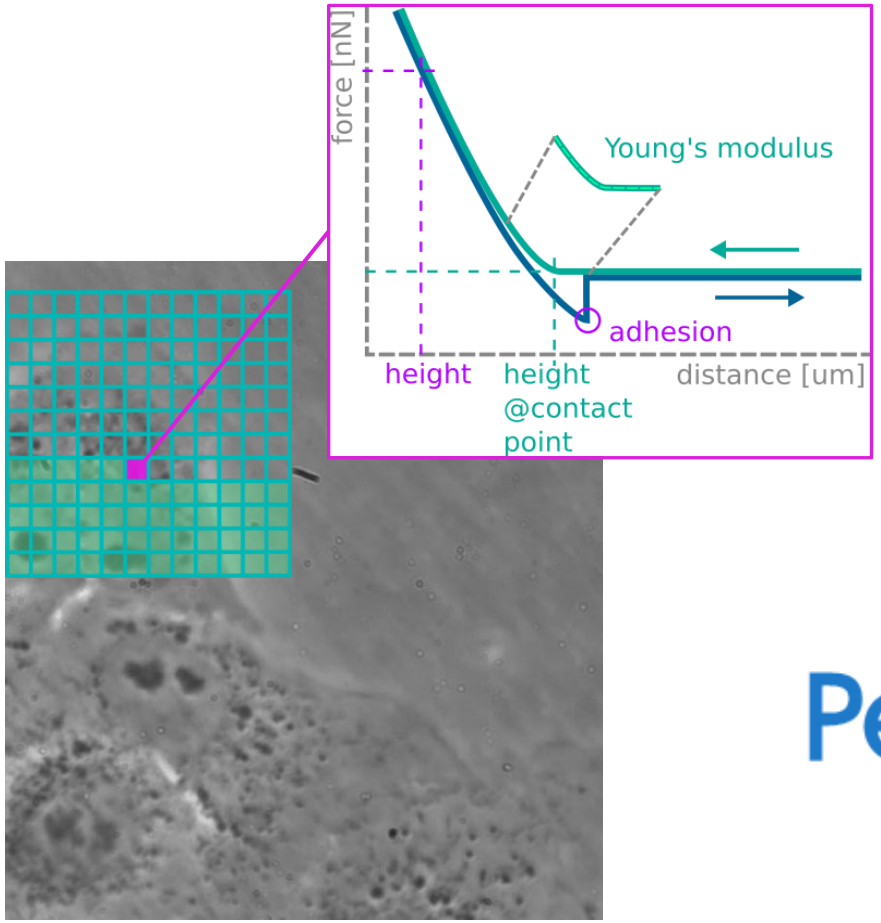


- **What do we measure?**
  - **Force distance curves** elastic (Young's) modulus, deformation, adhesion, work of adhesion, energy loss (dissipation)
  - **With delay** relaxation time, creep, storage and loss moduli



A Full Set of Mechanical Modes

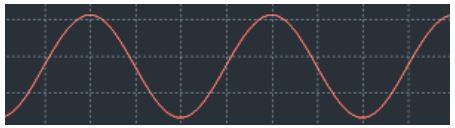




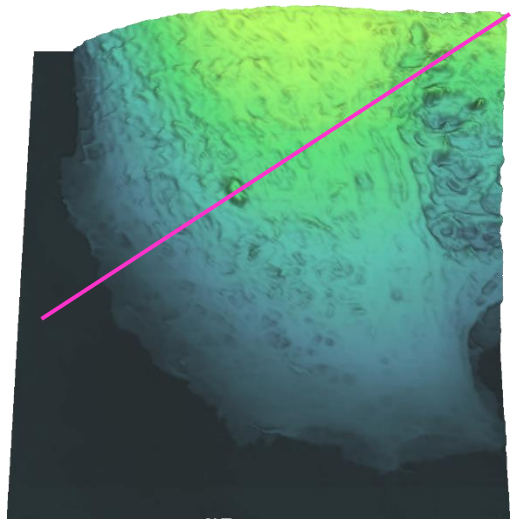
QI™ MODE

PEAKFORCE-QI

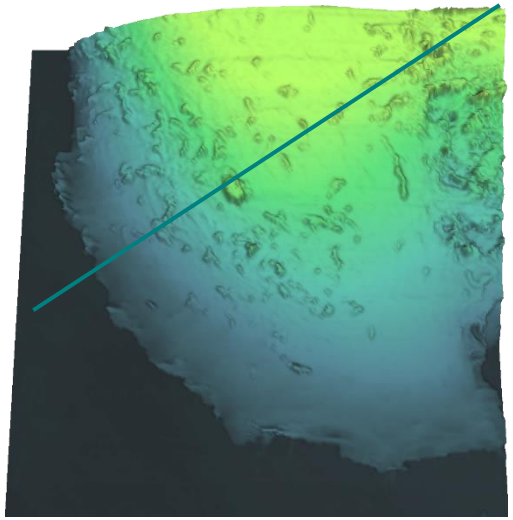
PeakForce QNM



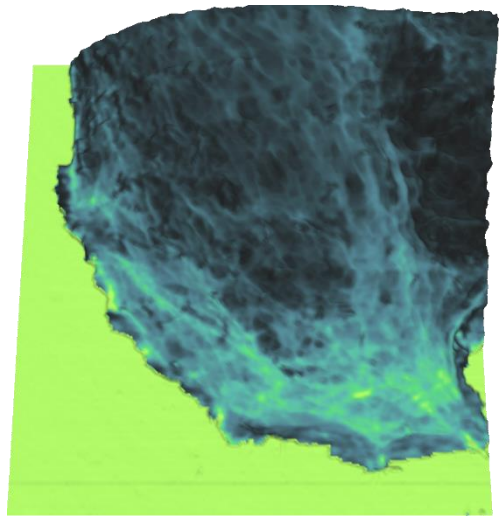
Multiparametric imaging of living Vero cells



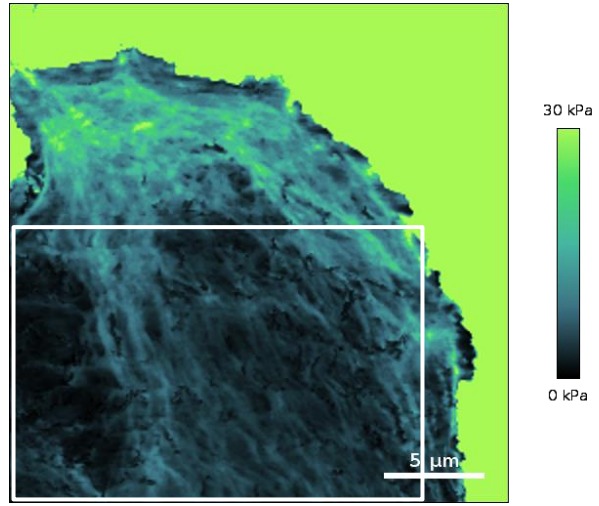
3D-height at 240 pN



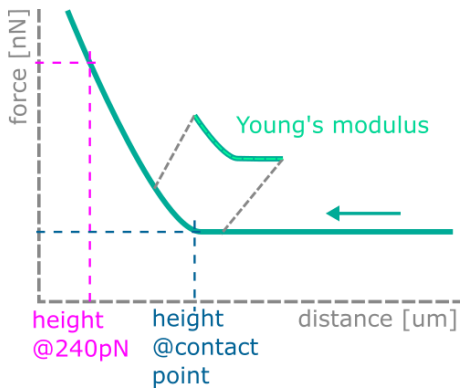
3D-contact point image

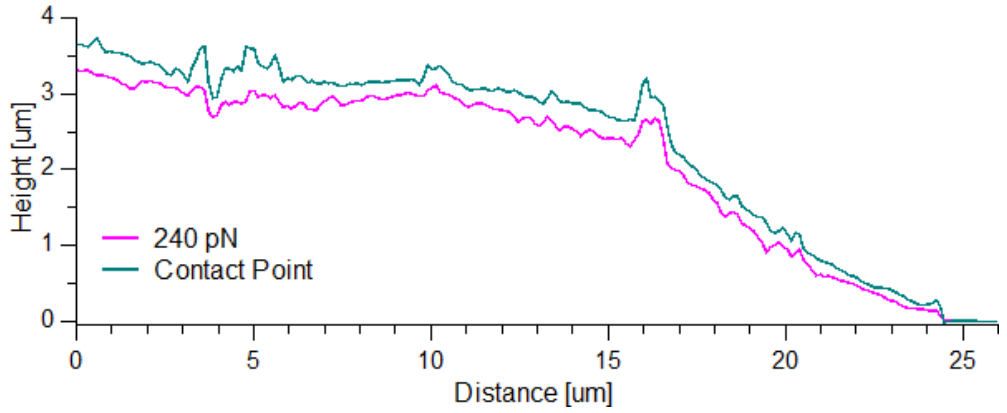


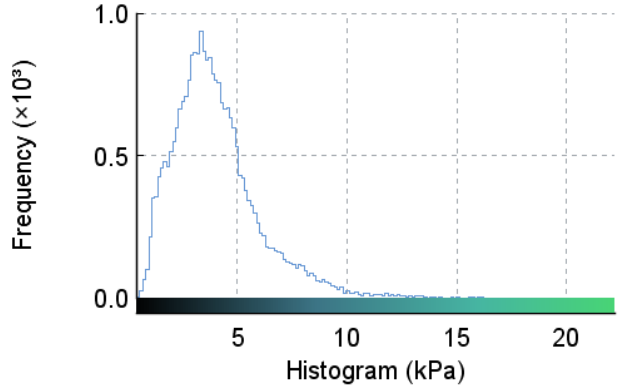
Apparent Young's modulus image
Over 3D-height



Apparent Young's modulus

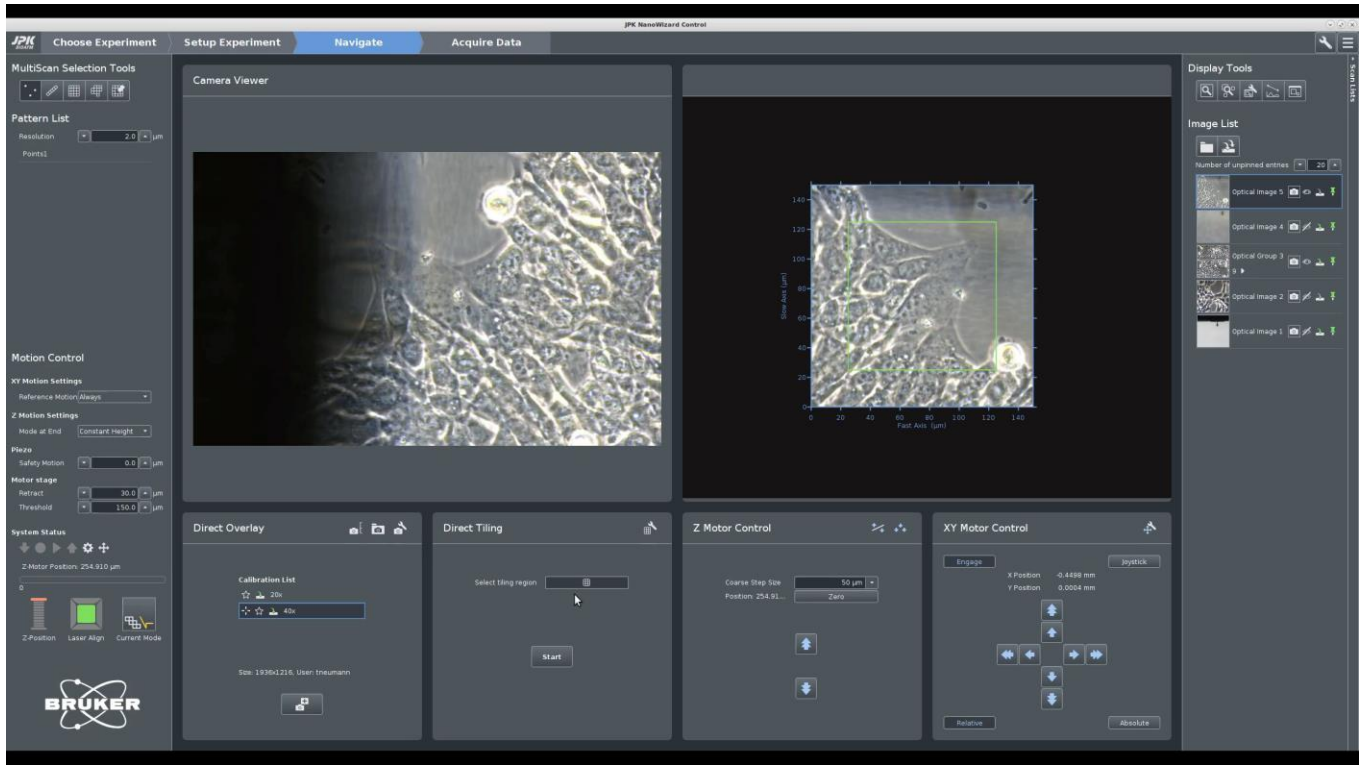


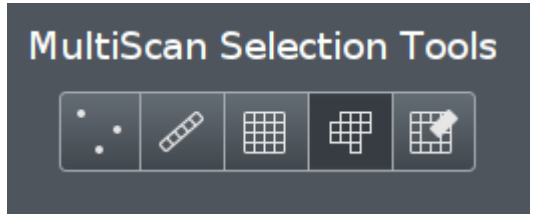


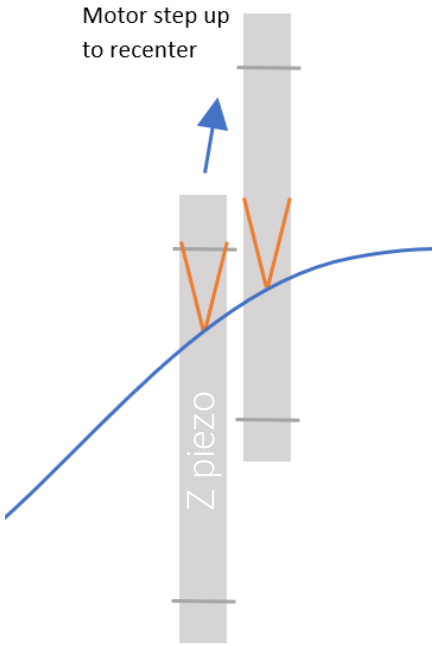


Sample courtesy of A. Hermann group, HU Berlin, DE

SmartMapping – Flexible, Large Area Nanomechanical Testing



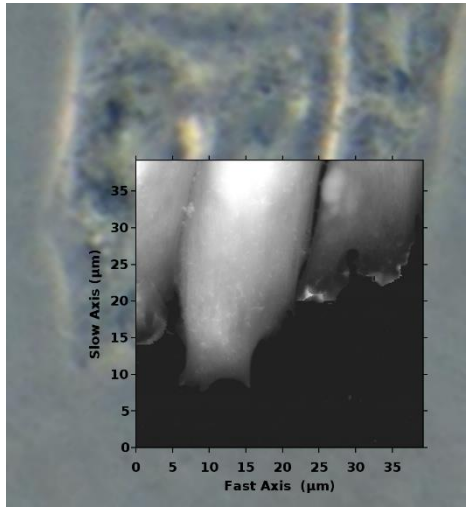


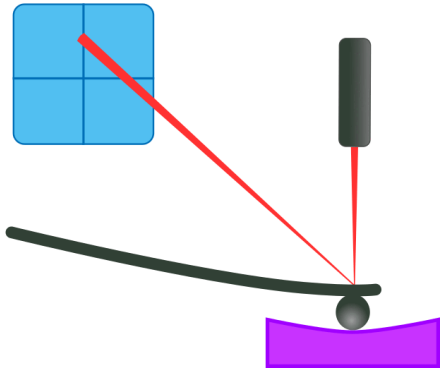


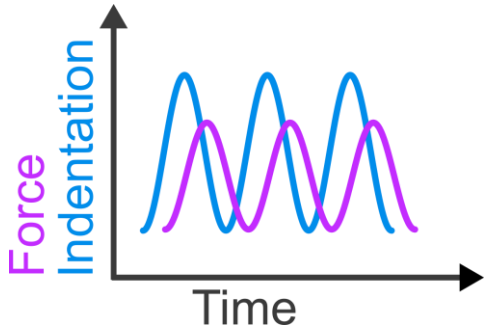
Available for:

NanoWizard 4XP and above
(SPM ver. 7.0 and above)

Viscoelastic mapping: living fibroblast cells



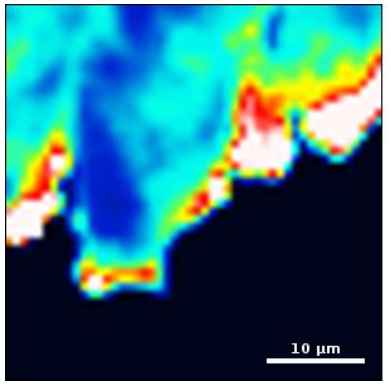


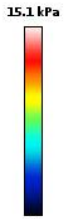


Phase shift → Viscosity

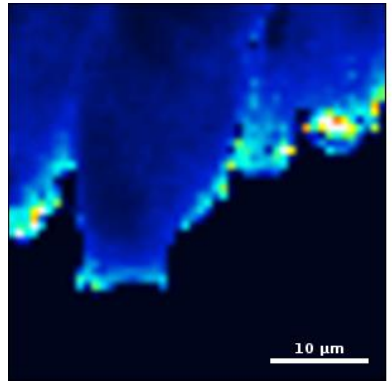
Amplitude → Elasticity

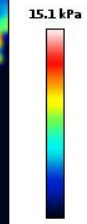
Storage modulus E‘:

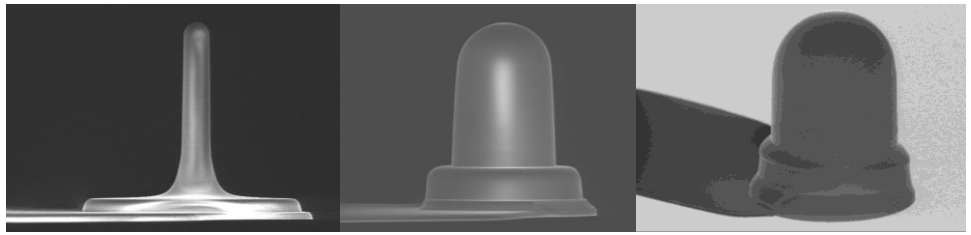




Loss modulus E“:

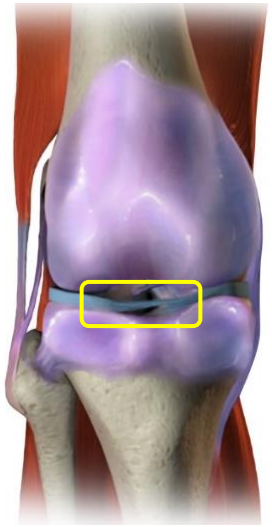


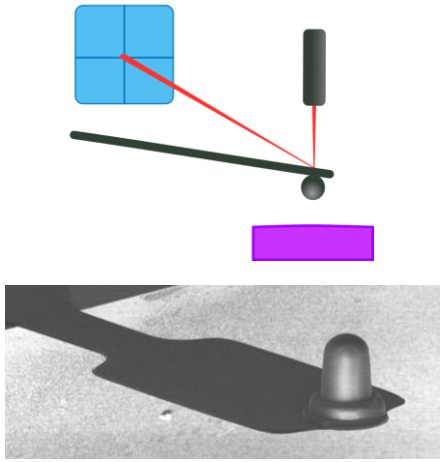




Hemispherical Probes – SAA-SPH-1/5/10-UM

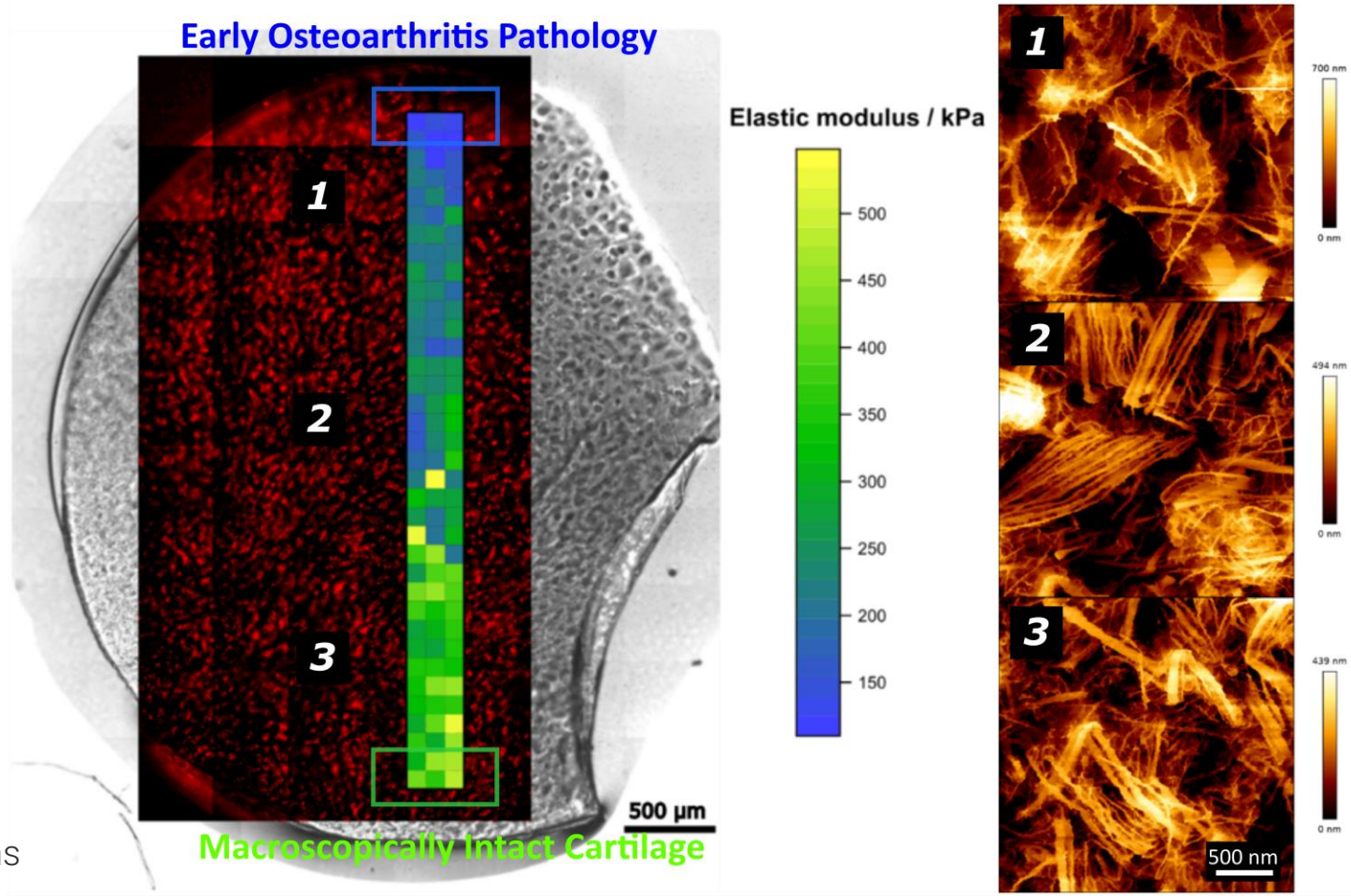
Probing the elastic modulus of human osteoarthritic articular cartilage





https://en.wikipedia.org/wiki/Osteoarthritis
M. Engelhardt, DZ Sportmedizin 54/6 (2003)

- 6 × 10 fluorescence images with optical tiling
- 3 × 36 maps (100 × 100 µm^2)
- Large Scale Mapping using colloidal probe (r=5 µm)
- Loss of Nanoscale Surface Stiffness in early OA regions
- Clear loss of fibre alignment in arthritic areas
- Associated with cartilage remineralisation

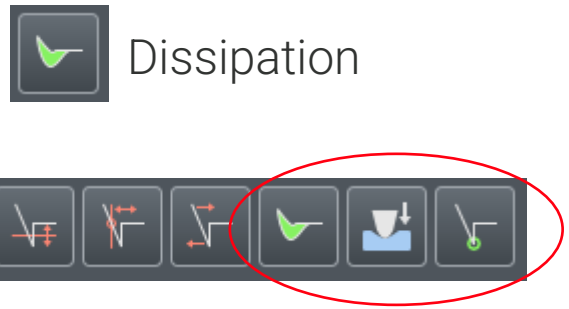


[1] Wieland HA et al., Nat Rev Drug Discov 4 (2005) 331-44

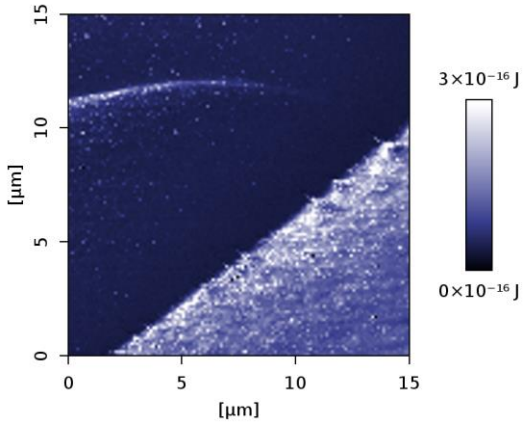
Tschaikowsky M et al., Acta Biomater 126 (2021) 315-325

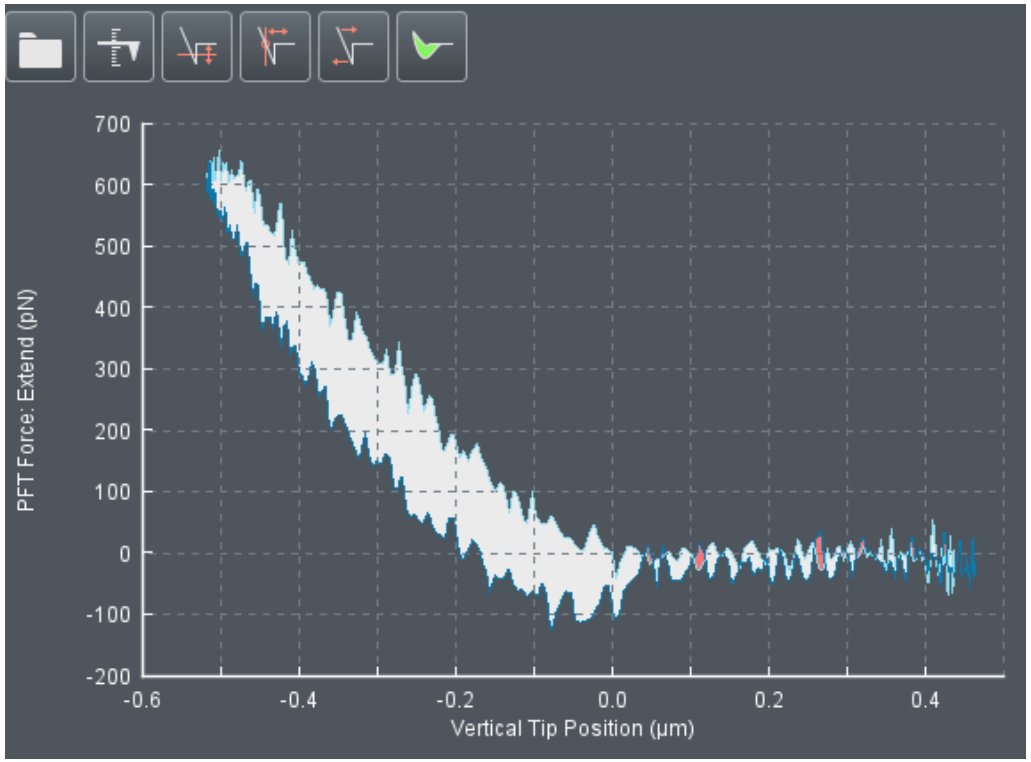
[2] Sample courtesy of T. Hugel group (Freiburg, DE)

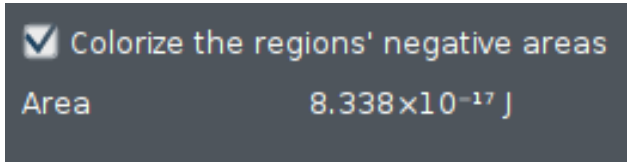
Data Processing



Dissipation

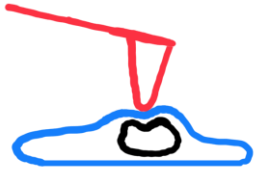




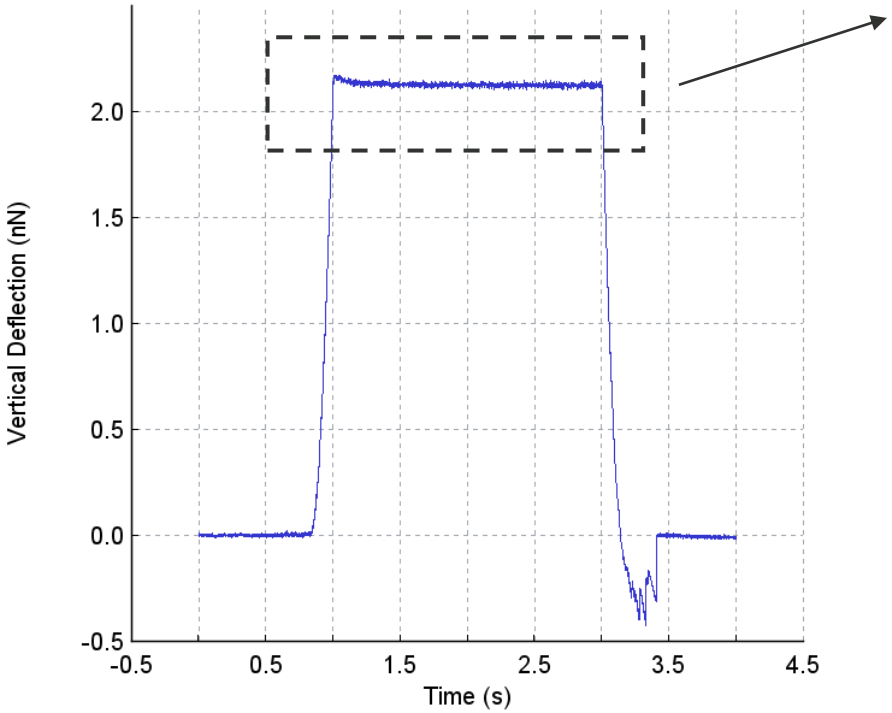


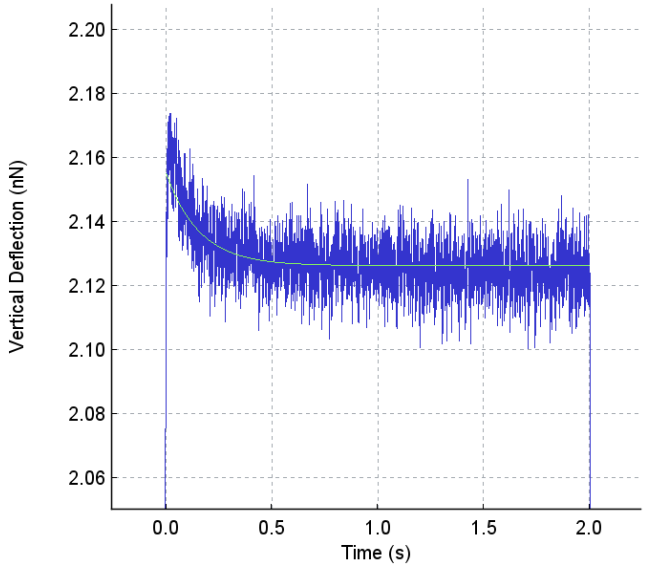
1 kPa hydrogel in PBS SAA-SPH-5UM probe

Data Processing



Exponential fit





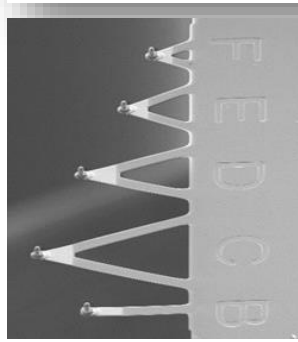
Exponential Fit

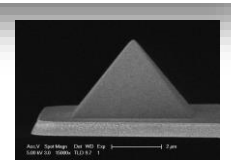
Fit exponential decay to data.
The model is
y = y_b + A exp[-(t-t_start)/t_decay]

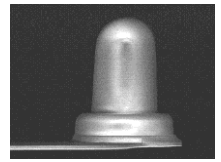
Fit Results

Baseline	2.125 nN
Amplitude	36.30 pN
Start Time	250.0 µs
Decay Time	156.3 ms
Residual RMS	7.615 pN

www.brukerafmprobes.com
recommended probes







1 kPa hydrogel in PBS SAA-SPH-5UM probe

MLCT-BIO-DC

MLCT-SPH-1UM-DC

MULTIMODAL MICROSCOPY WORKSHOP 2024, BRNO CZECH REPUBLIC

Integration of AFM with other techniques

Dr Alexander Dulebo
Application Scientist

Outline

1 AFM and optical microscopy

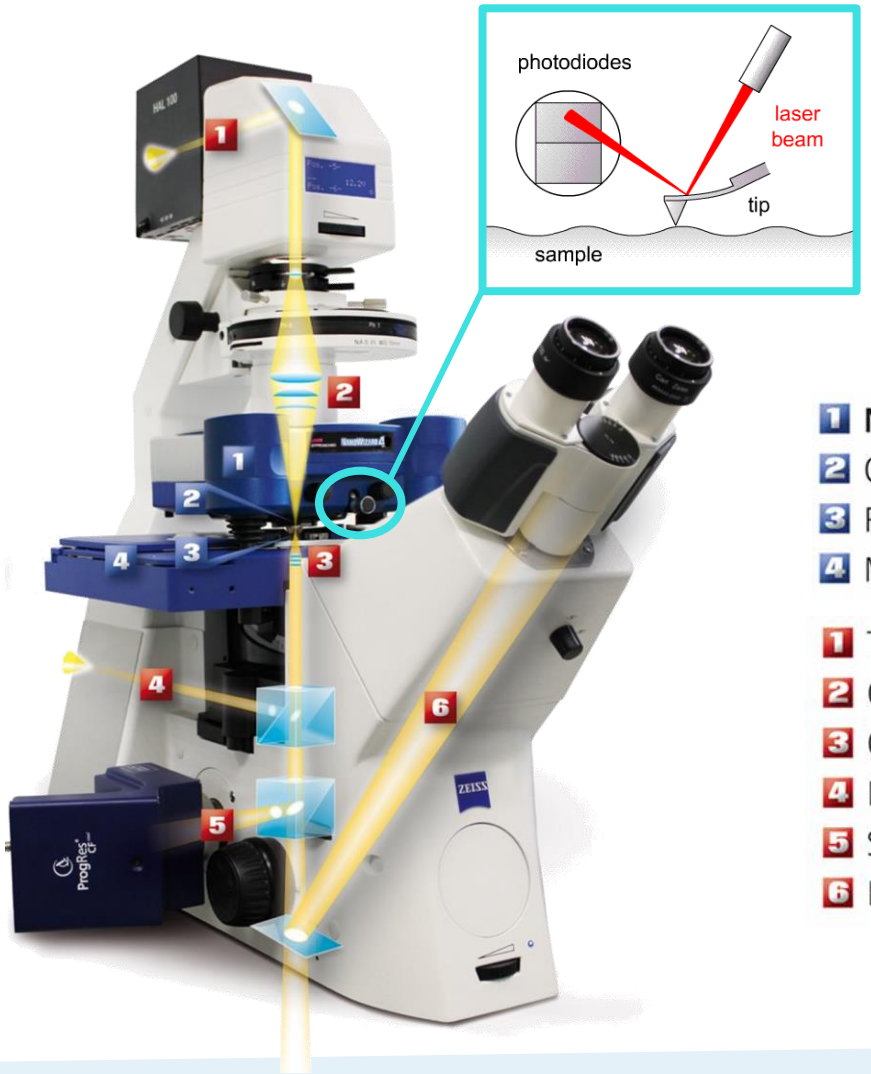
2 AFM and Raman

3 AFM and nanoIR

4 AFM and FluidFM

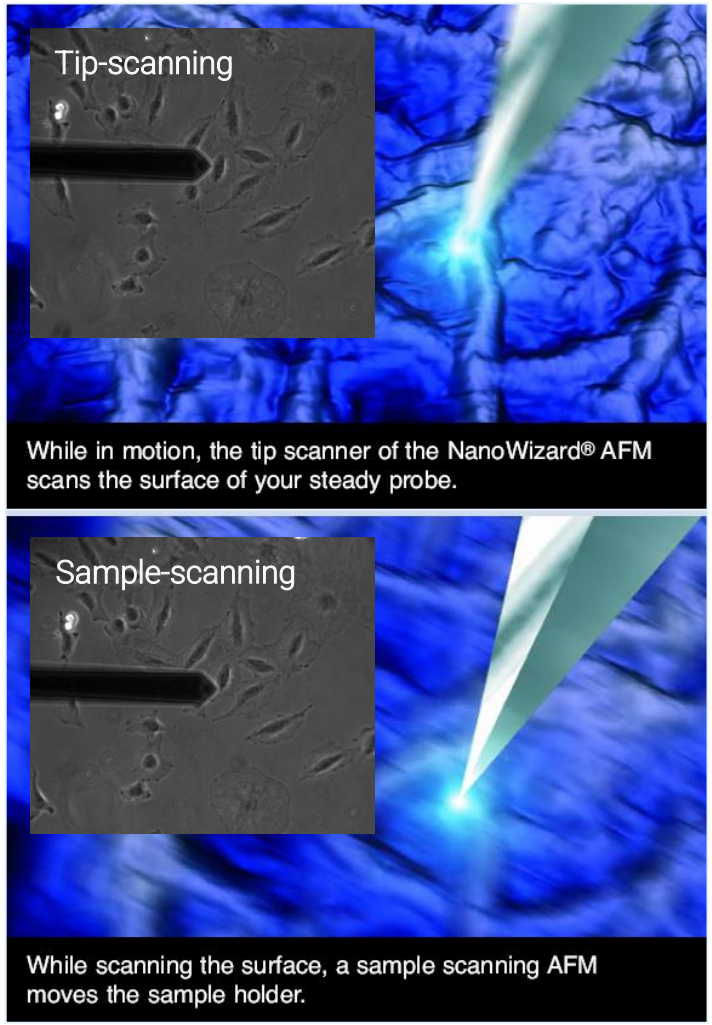
01 AFM and optical microscopy

BioAFM and optical microscopy integration

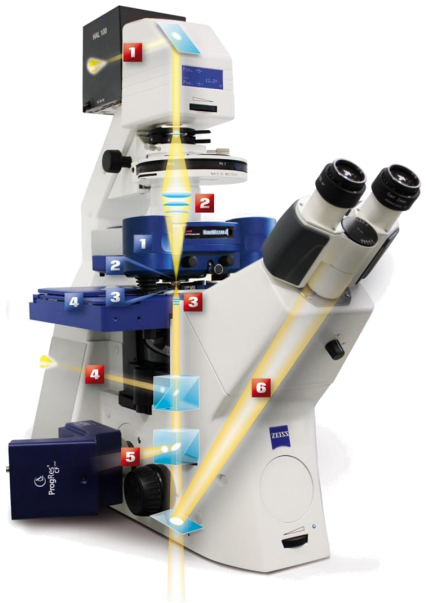


1 **NanoWizard®** head
2 Cantilever holder
3 Petri dish
4 Motorized stage

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

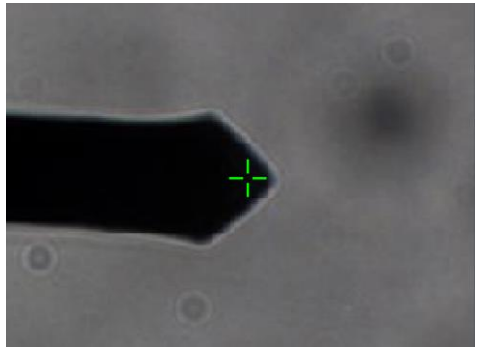


Optical integration perfected



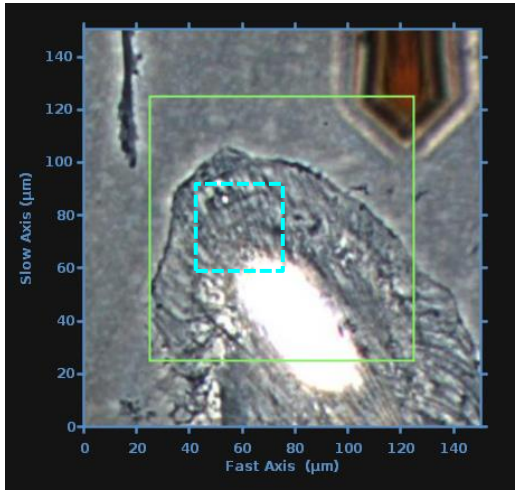
- Tip-scanner AFM design means sample does not move while AFM is scanning
- Standard condenser strongly recommended, particularly for living cells
- Perfect integration with inverted optical microscopes
- Compatible with optical super-resolution techniques (STED, STORM/PALM, SIM)
- Fully simultaneous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

DirectOverlay™ 2 - optical image calibration

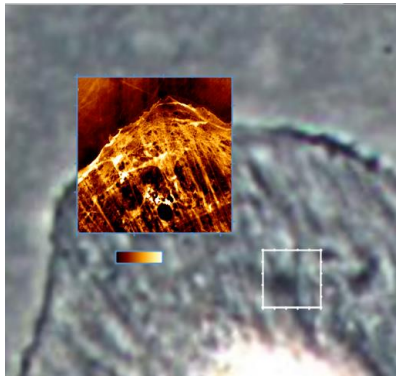


Automatic detection of the tip position in the optical image

→ Correlation of optical and AFM space

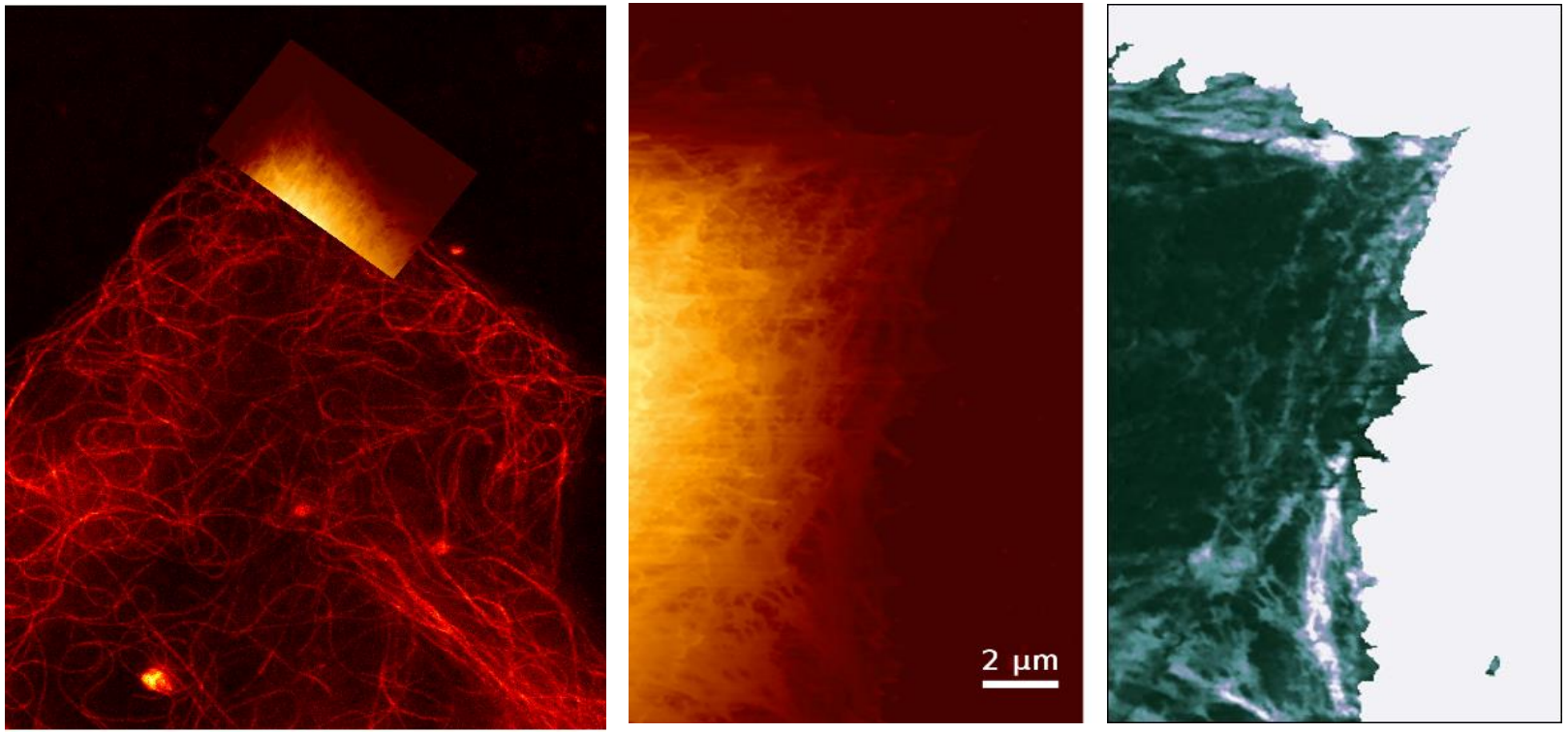


- Import optical image into the AFM software
- Select region of interest and start scanning



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

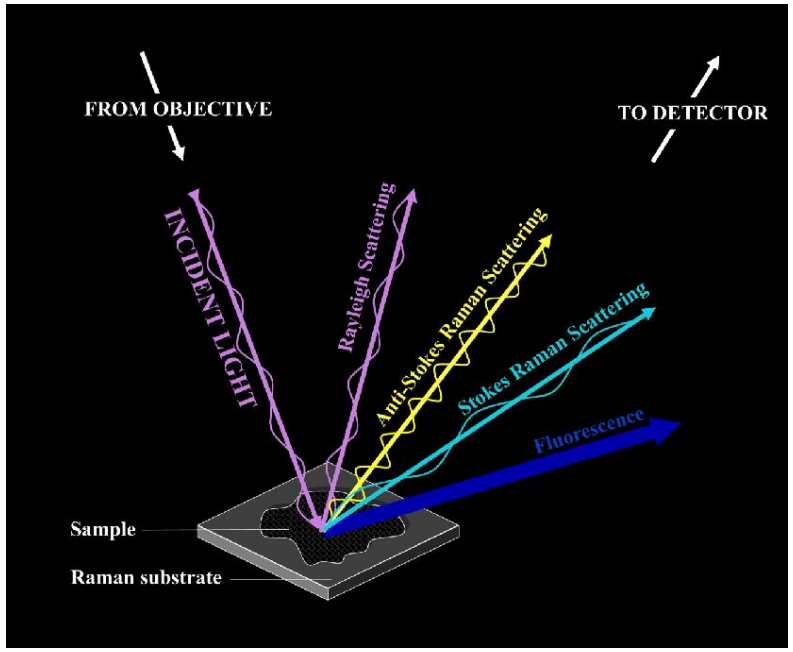
Sample courtesy of A. Hermann group, HU Berlin, DE

02 AFM and Raman

Raman spectroscopy

What is Raman spectroscopy?

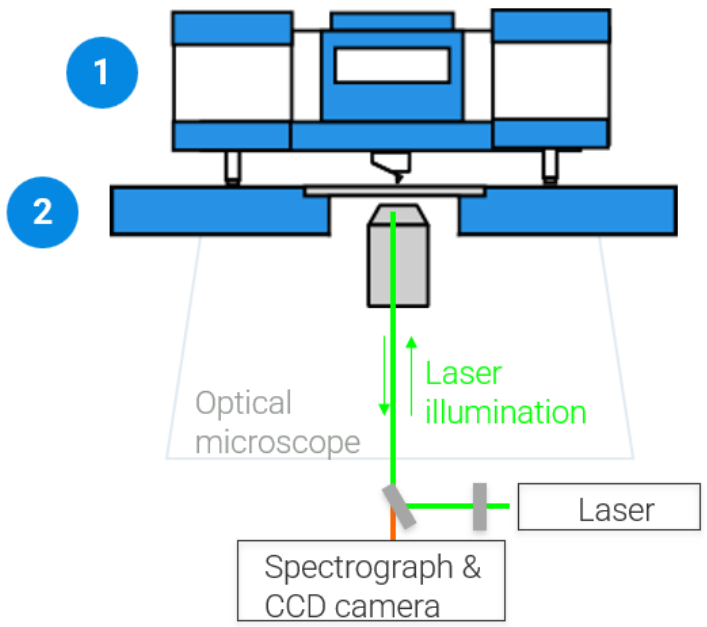
- Chandrasekhara Venkata Raman in 1928
- Inelastic scattering of photons → shift in wavelength → vibrational modes of molecules
- Non-destructive (30 mW)



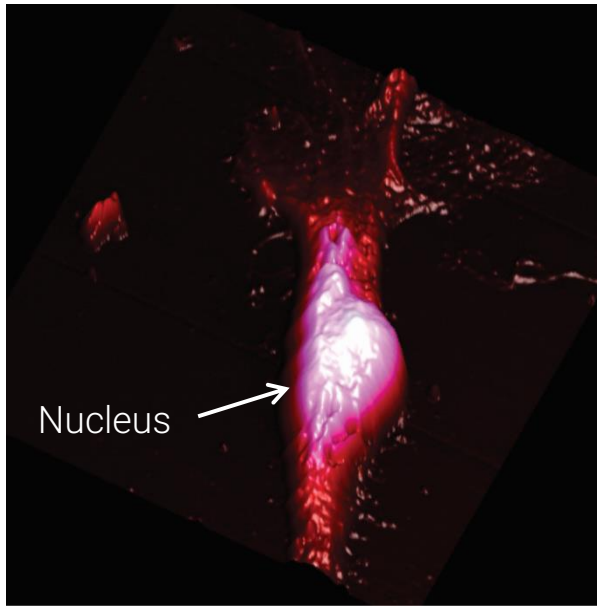
Why is it interesting for integration?

- label-free identification of molecules

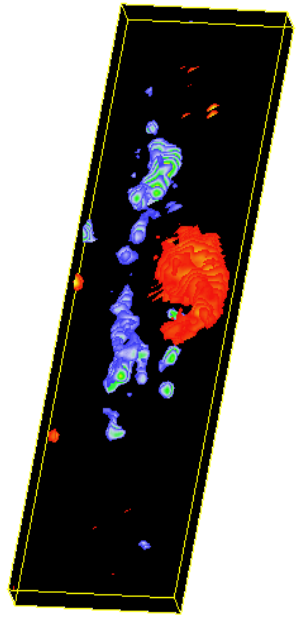
AFM and Raman



AFM-Topography

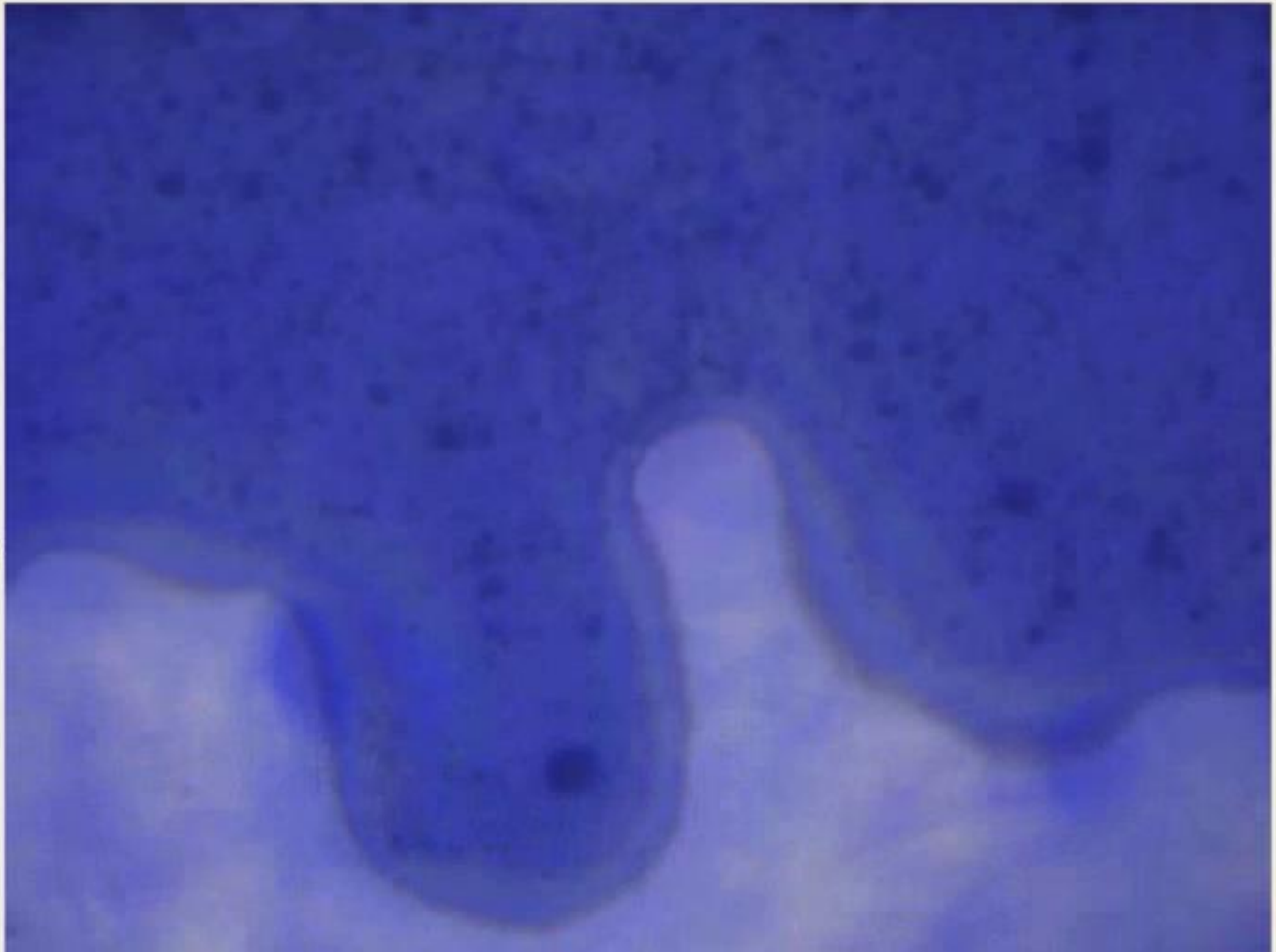


Raman-Map

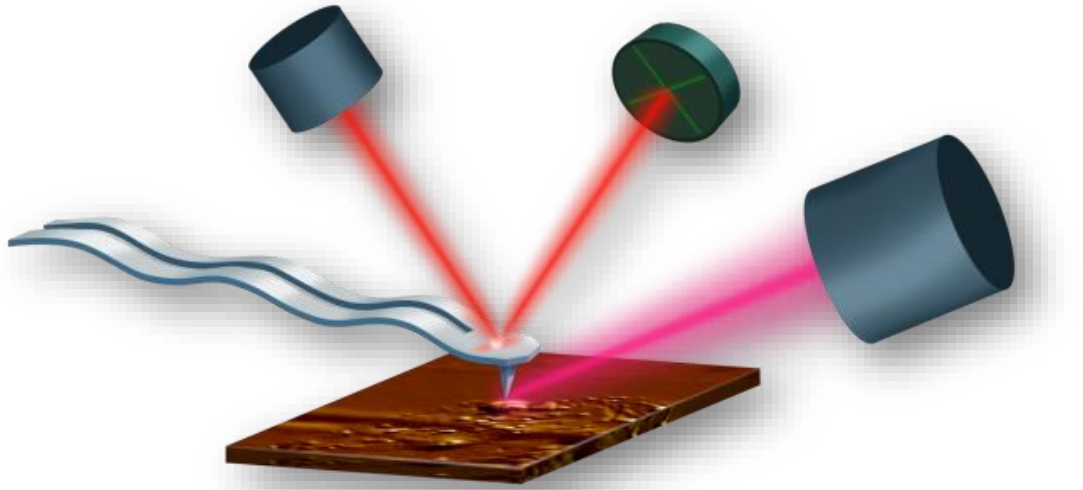


03 AFM and nanoIR

Optical microscope view



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

Accumulation of TriAcylGlycerols in *Streptomyces* Species

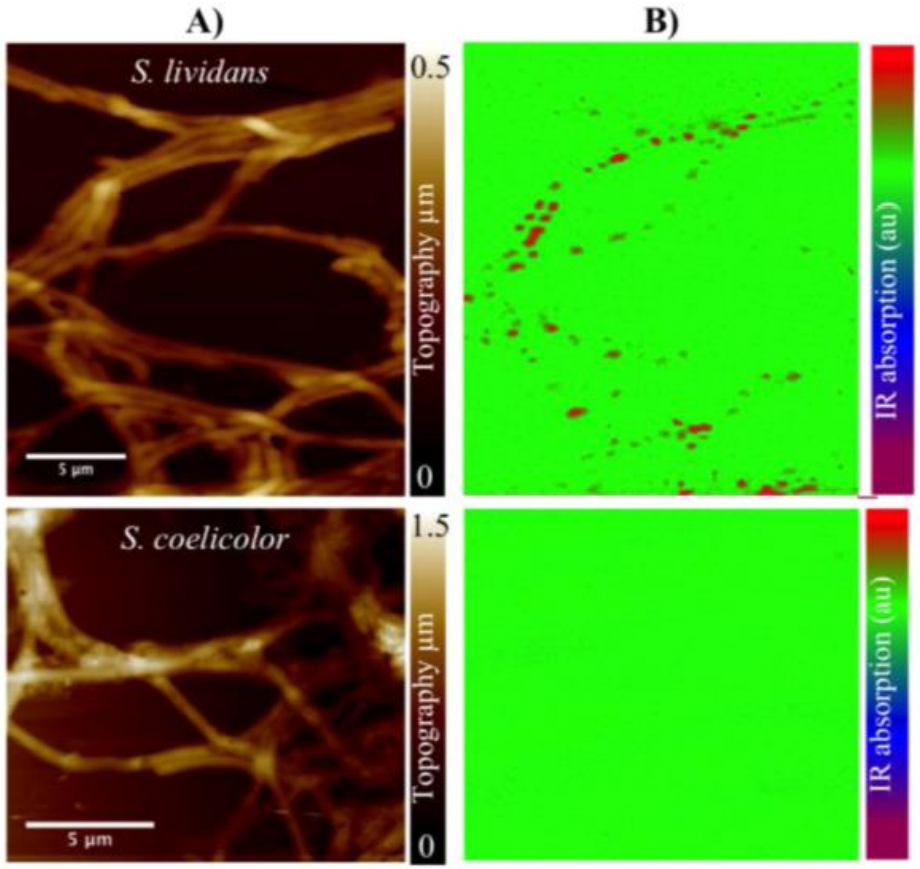
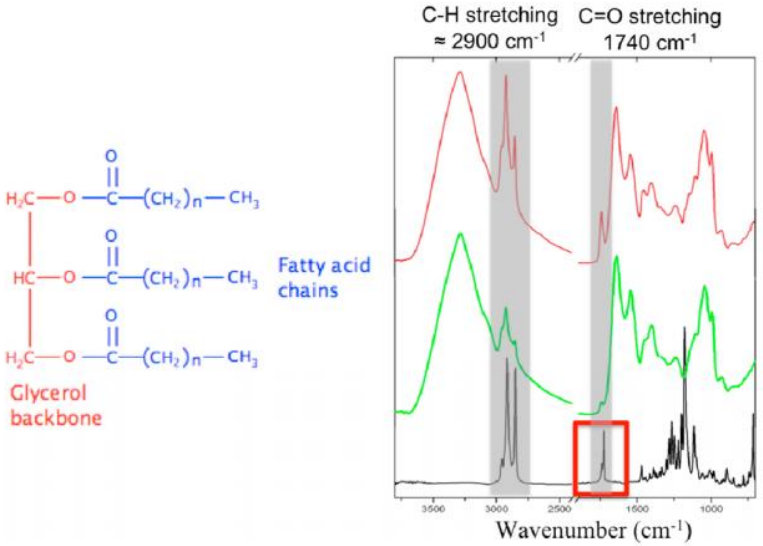


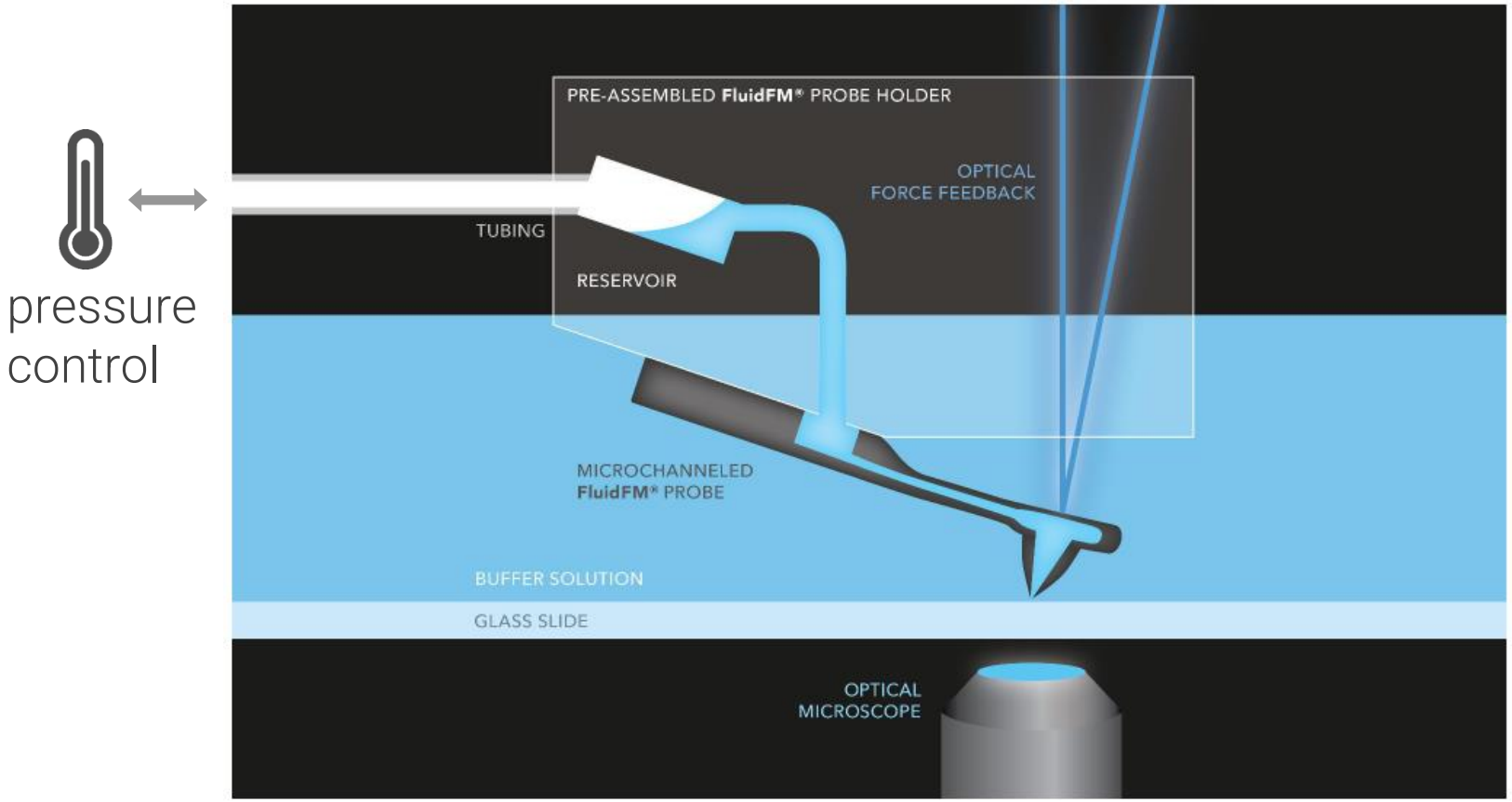
Figure 3. (A) AFM topography and (B) chemical mapping at 1740 cm^{-1} for the two strains.



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

04 AFM and FluidFM

FluidFM technology



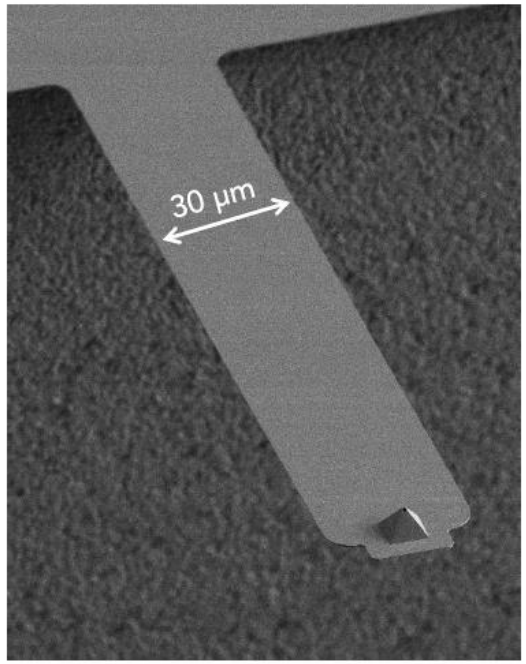
FluidFM

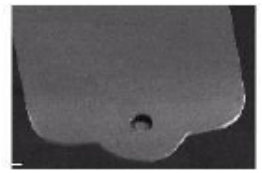
- 300 nm - 8 µm aperture
- ~5 pL volume
- Femtoliters per second flow

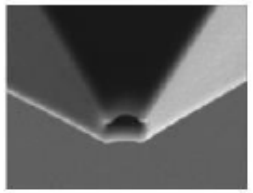
ETH zürich

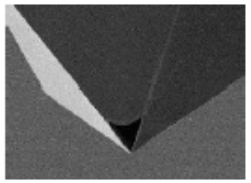
CYTOSURGE®

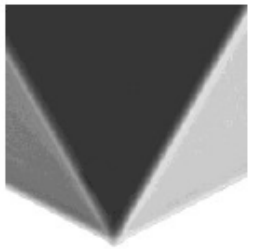
FluidFM probes











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

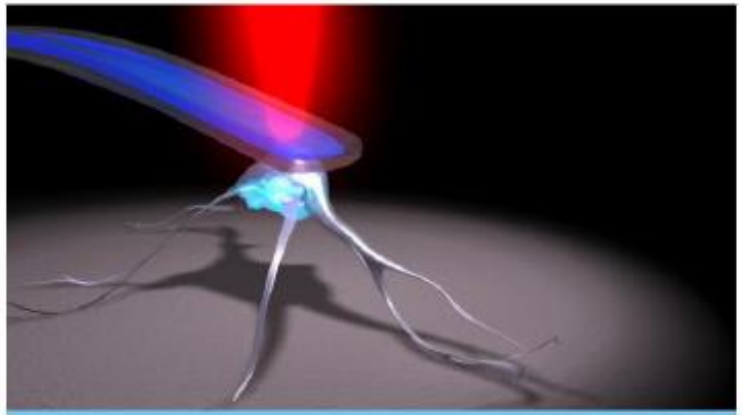
FluidFM prototyping probe

Aperture can be customized with Focused Ion Beam (FIB)

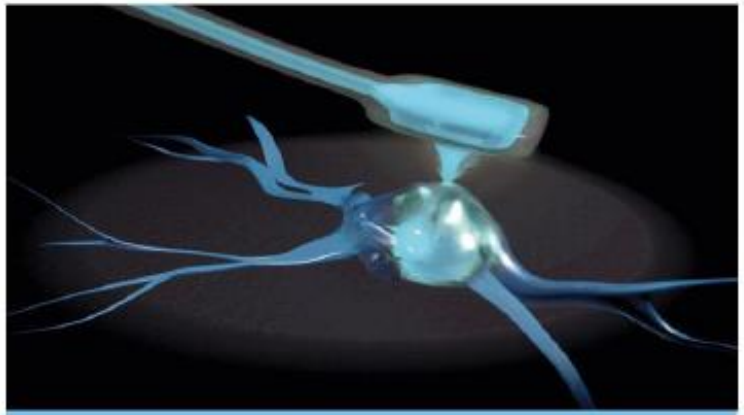
0.6 – 2 N/m, 30+ nm

Application depending on the customization

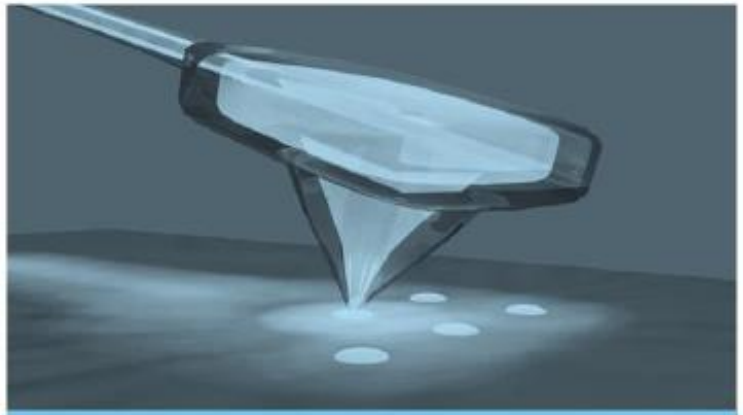
Major FluidFM applications



FluidFM micropipette
PICK & PLACE MICRO OBJECTS

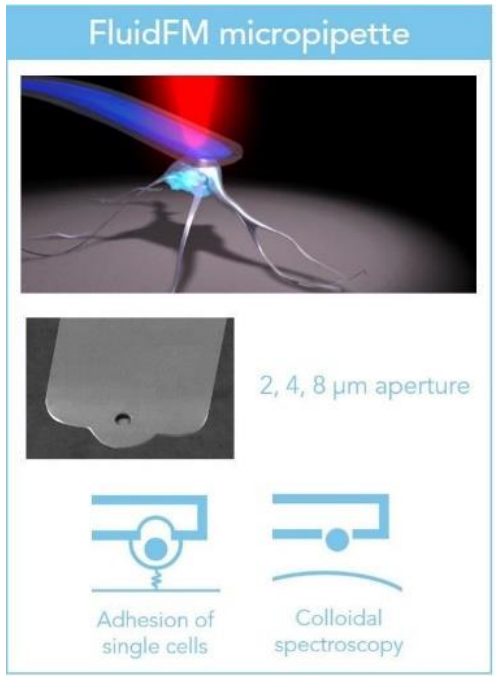


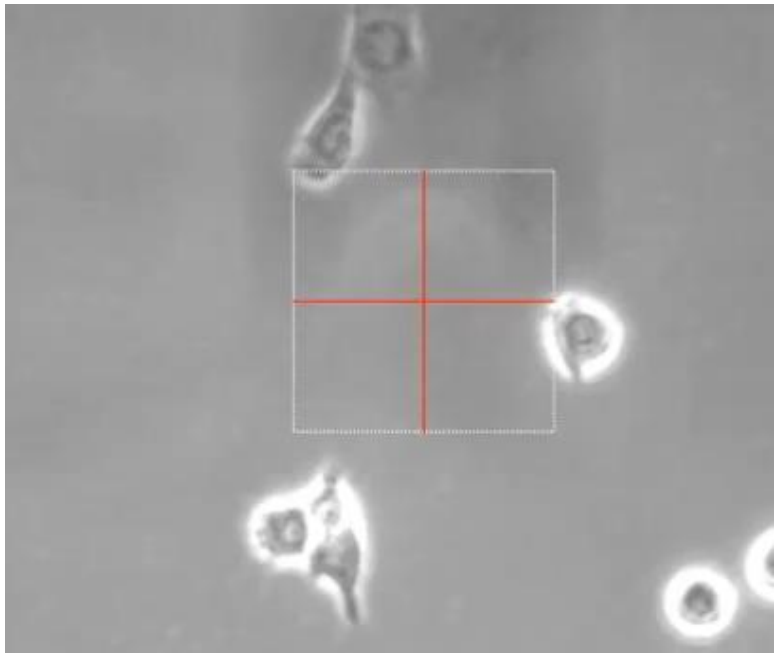
FluidFM nanosyringe
NANO-INJECT & NANO-EXTRACT



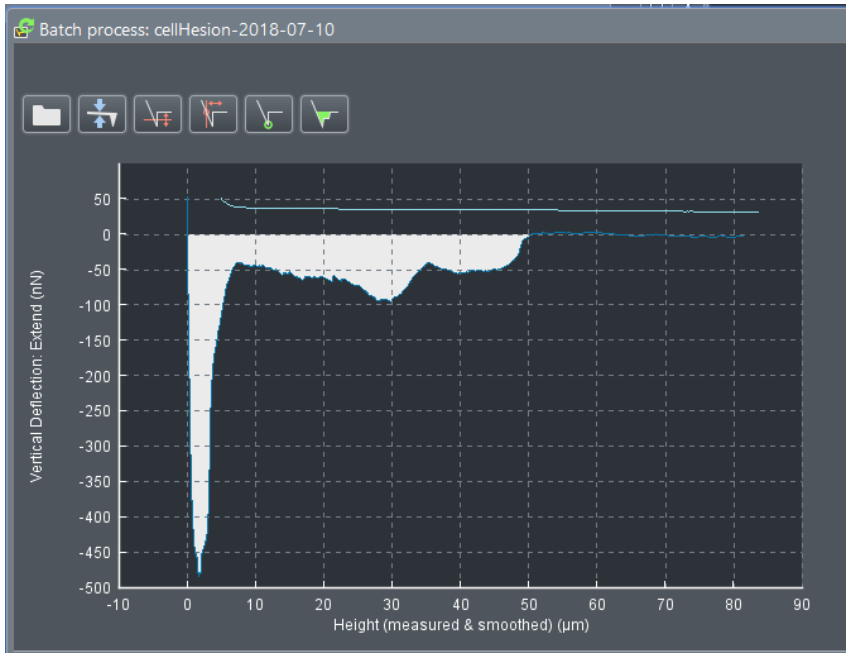
FluidFM nanopipette
BIOPRINT & METAL 3D PRINT

Cell adhesion/separation of adherently growing cells





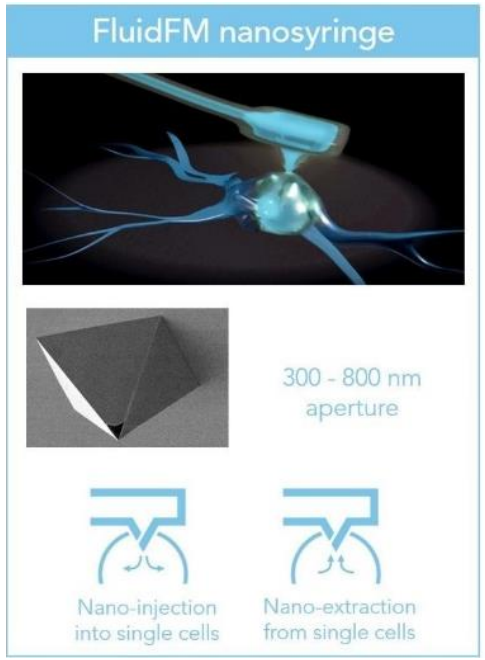
Phase contrast of living cells. A 4µm micropipette is used to separate the cell from the substrate.

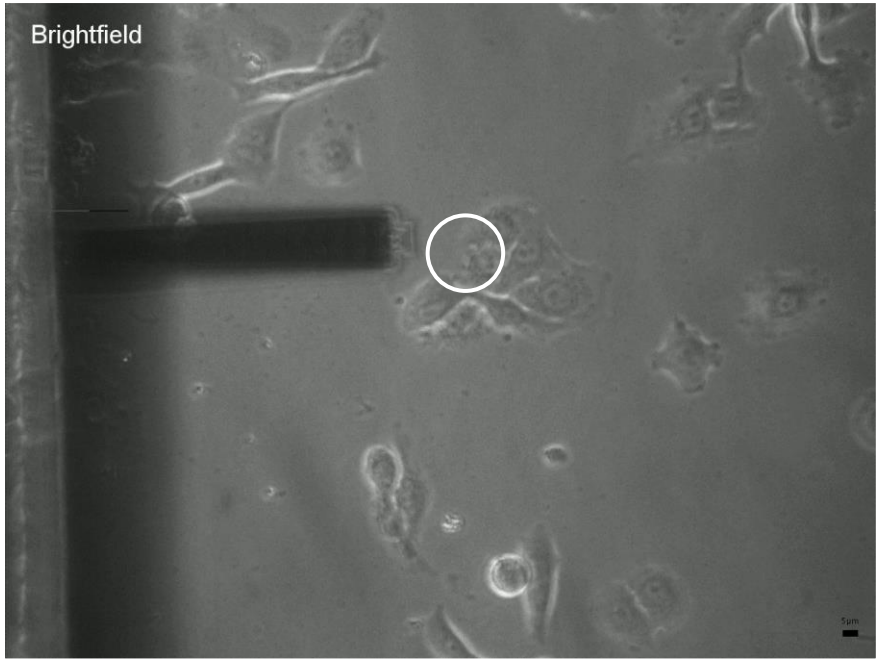


Resulting force distance curve.

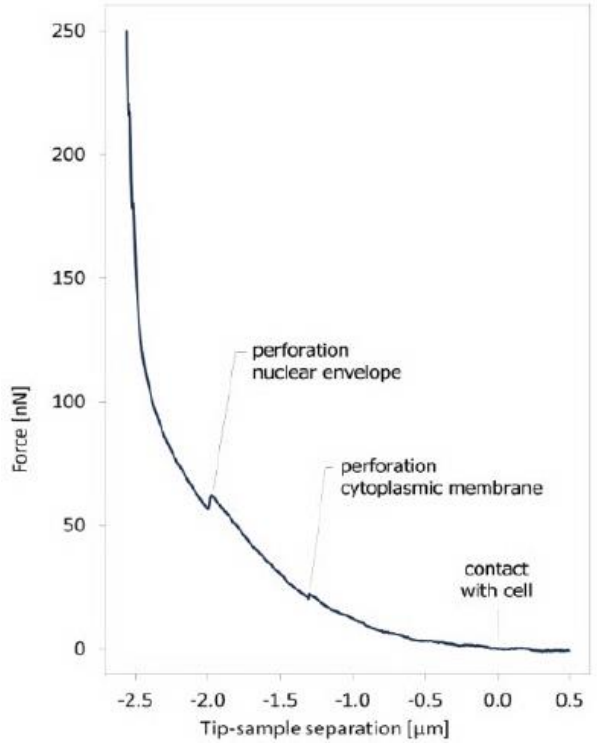
30 to 200	nN to µN	pN
CELLS PER DAY	PICK UP ANY CELL	RESOLUTION

Cell injection and extraction



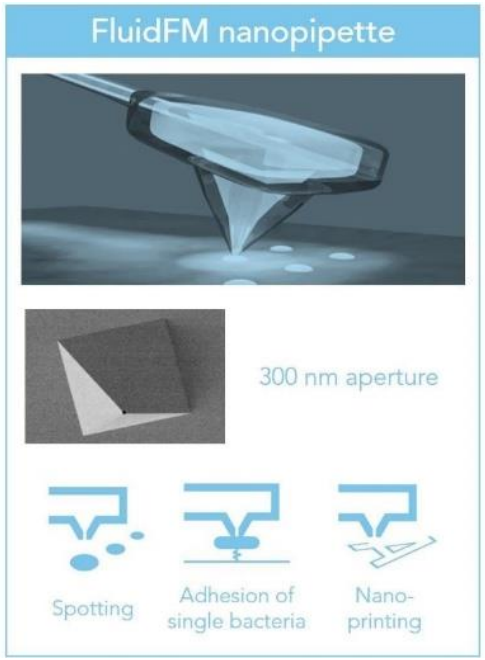


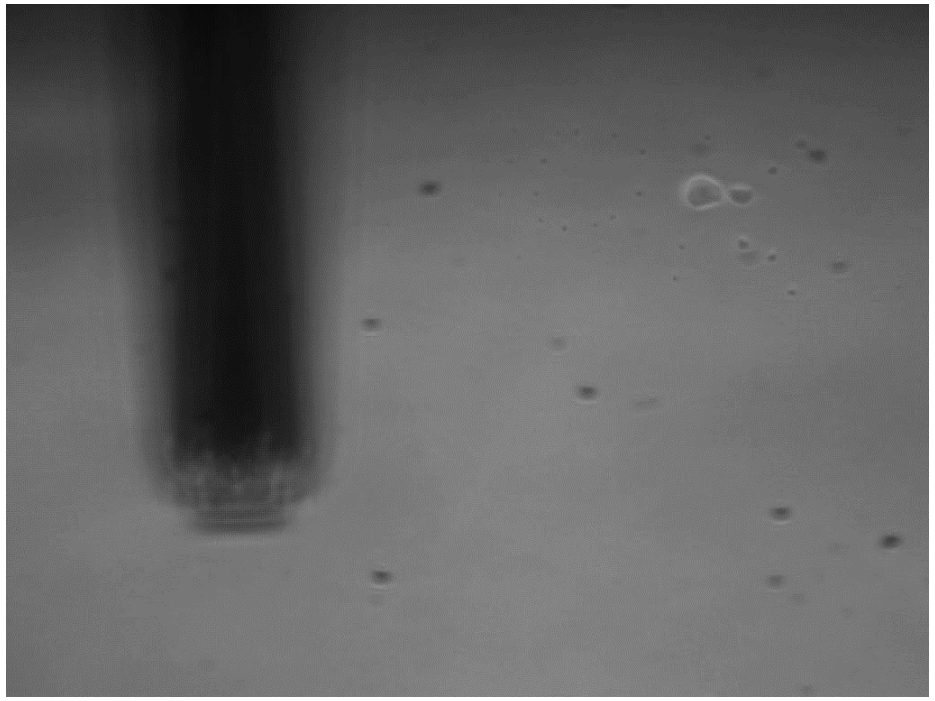
Phase contrast and epifluorescence imaging of living CHO cells. A nanosyringe is used to inject Propidium iodide into the indicated cell



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

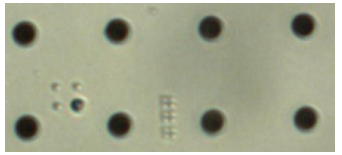
Nano spotting



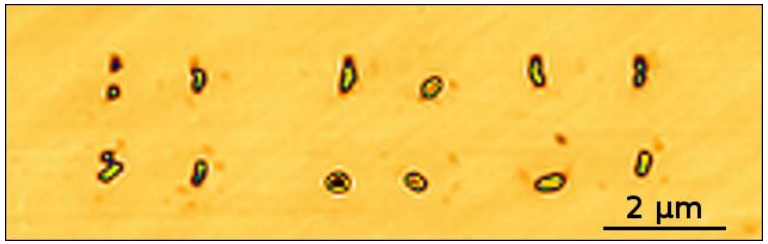


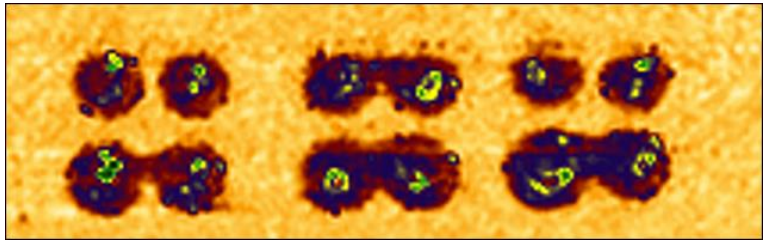
Phase contrast while spotting a glycerol/water mixture on glass.

High reproducibility using the NanoWizard®



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

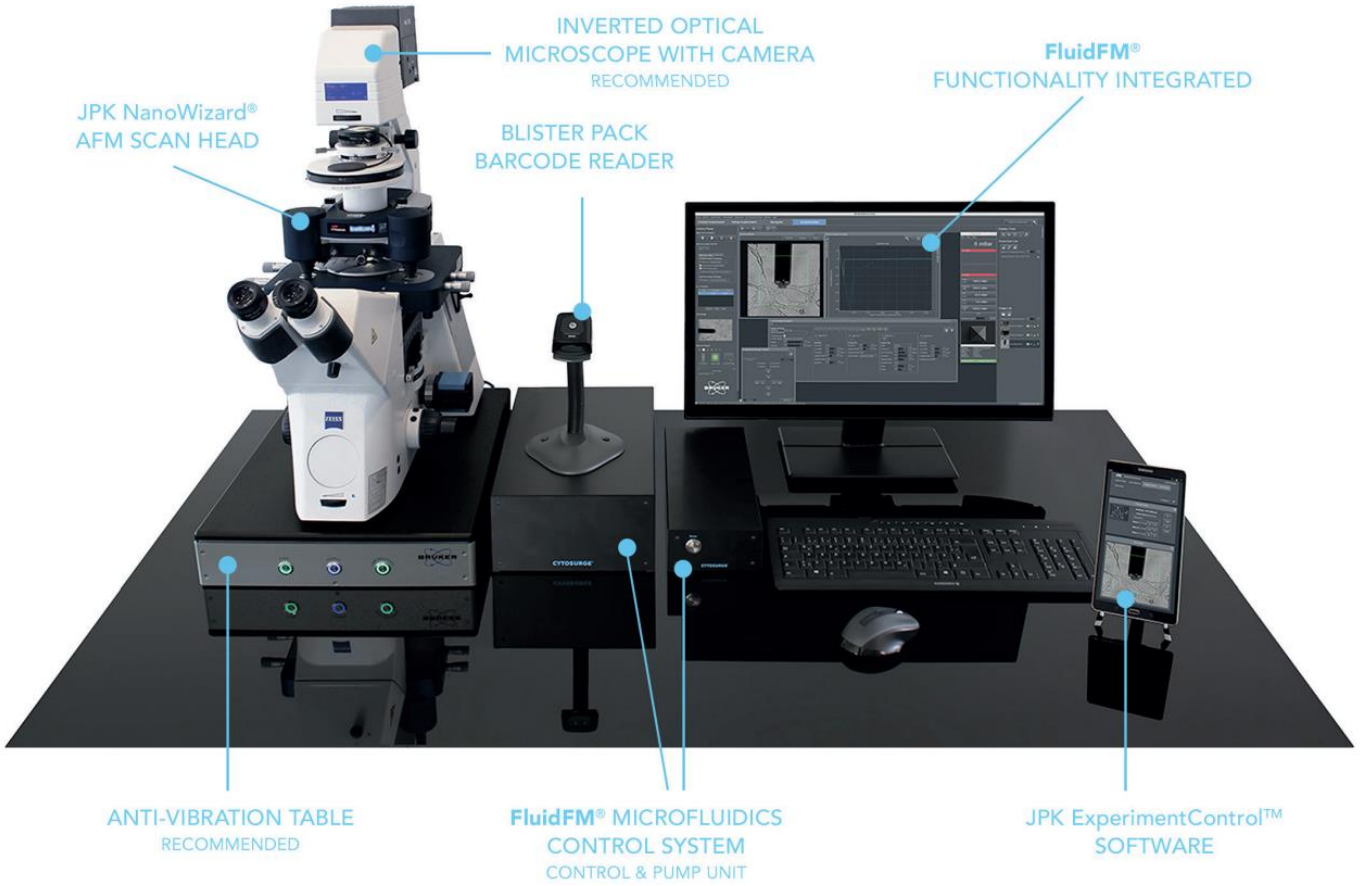




Slope channel useful to calculate the spotting area and volume.

Technical implementation on NanoWizard AFM

Cantilever holder with Cyto clip mounted on AFM head

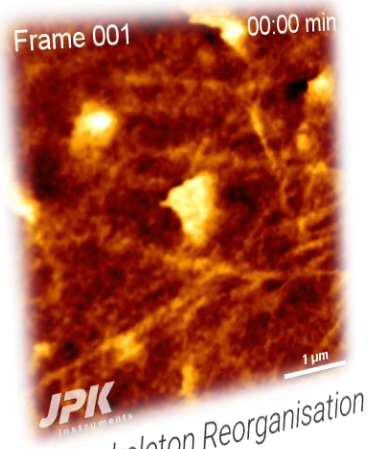


MULTIMODAL MICROSCOPY WORKSHOP 2024, BRNO CZECH REPUBLIC

High-Speed AFM

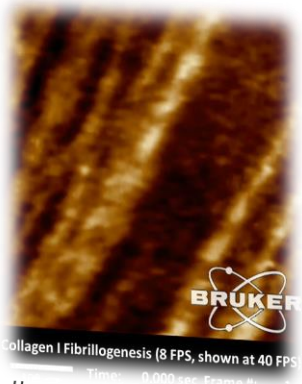
Dr Alexander Dulebo
Application Scientist

Cellular and Molecular Dynamics – Across Multiple Timescales



Cytoskeleton Reorganisation

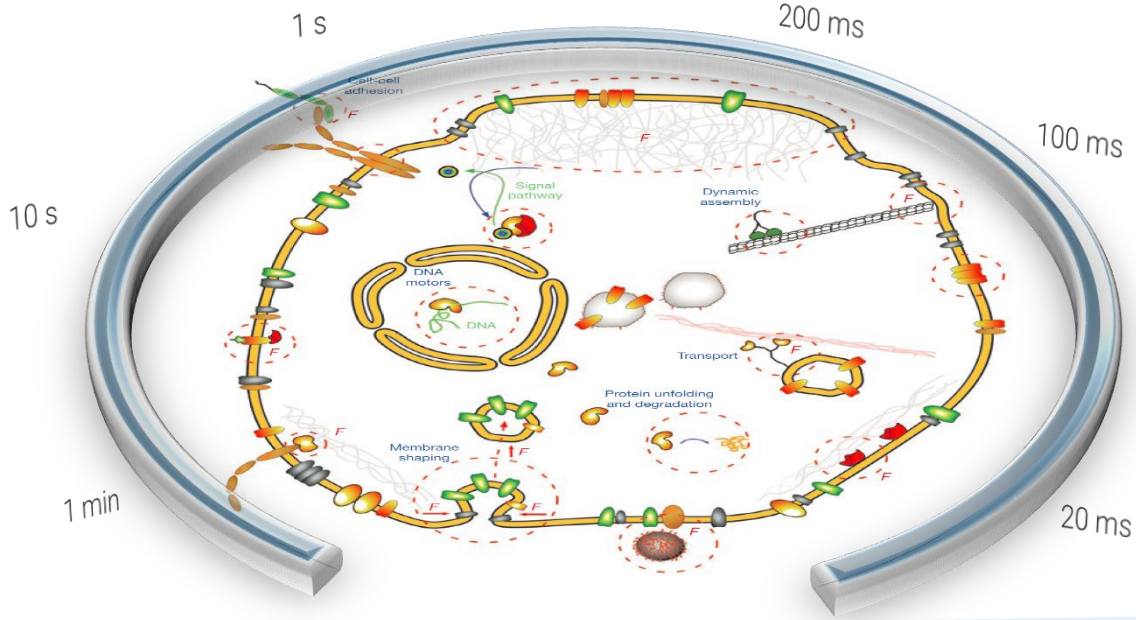
Lipid Phase Transitions



Collagen Fibrillogenesis

Protein Binding Kinetics

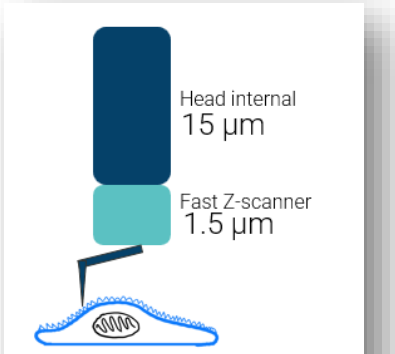
Membrane Vesiculation

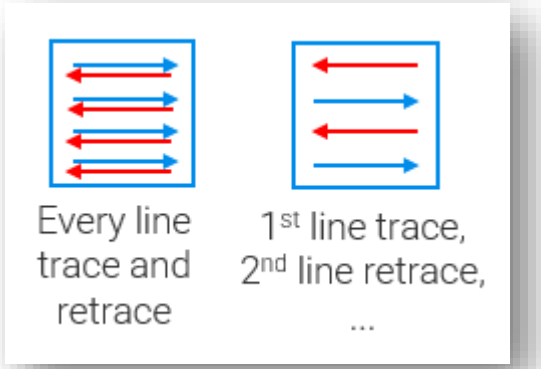


DNA Rehybridization

Speed of scanning

- **Nested scanner** technology (NW5-4XP fast, NWUS2-3)
- **Bi-derectional scanning** (NW5-4XP fast, NWUS2-3, NanoRacer, FastScanBio, Resolve, MultiMode, Icon)
- **Active balancing** (NWUS3, NW5 fast) faster scanning over large scan ranges
- **3D acceleration sensor** near the probe and **feed-forward** technology (NWUS3)





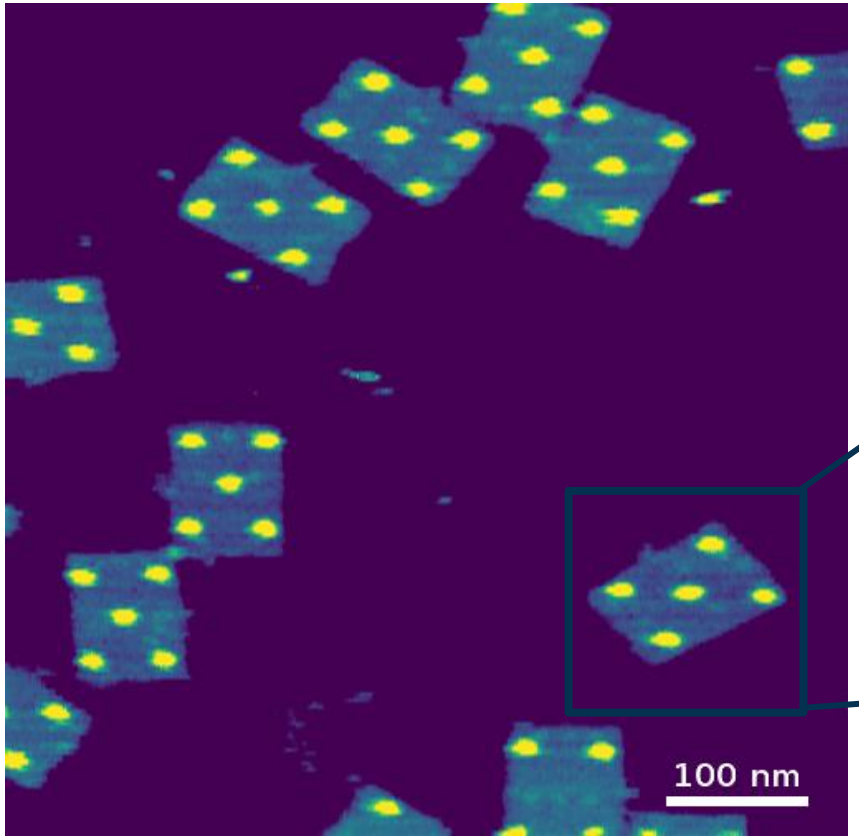
FastScan Bio

NanoWizard UltraSpeed 3

NanoRacer

Specific Biotin-Streptividin Binding Dynamics in DNA Nanostructures for Targeted Cell Stimulation

Overview



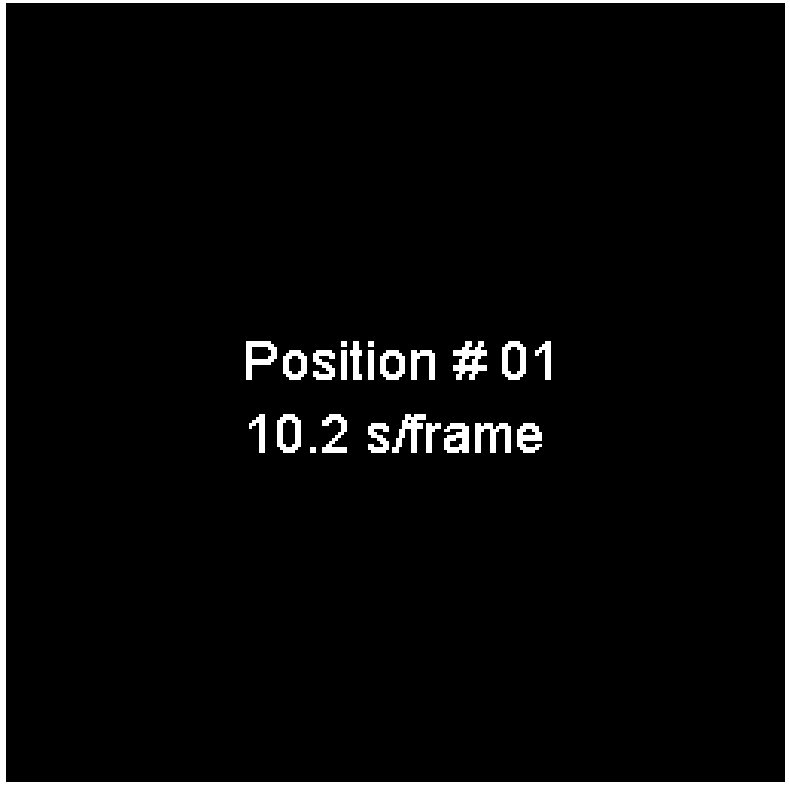
Topography: z-range 3.1 nm, scan speed: 4 sec/frame

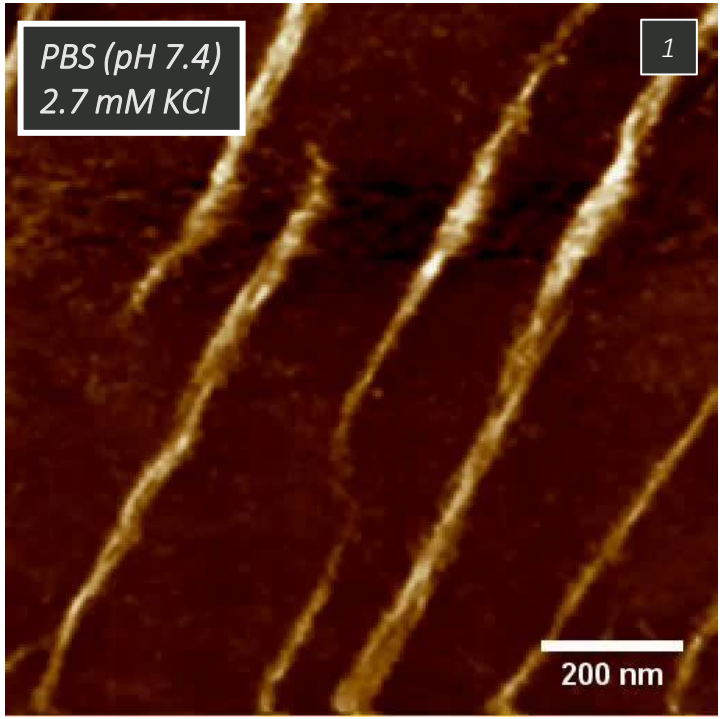
Video – binding dynamics

Topography: x,y-range 150 nm, scan speed: 10 frames/sec

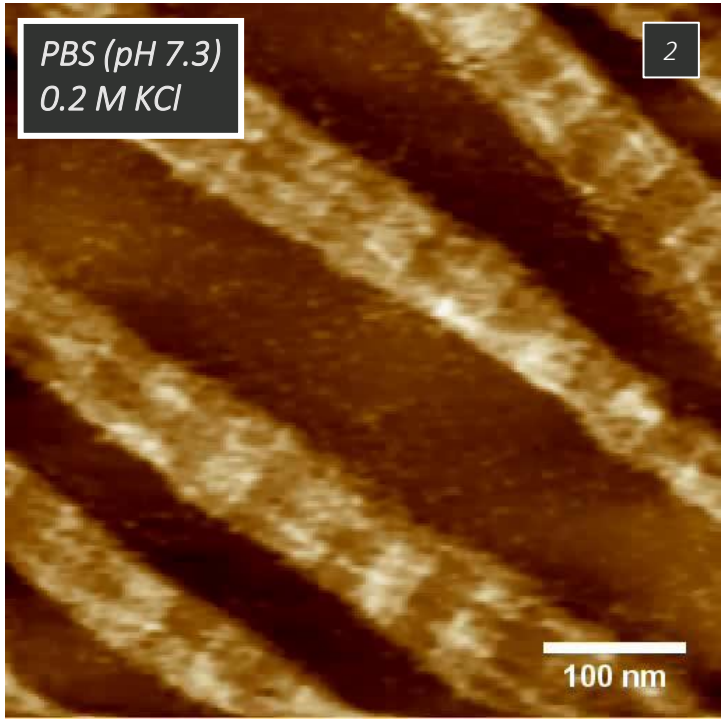
in collaboration with C.M. Domínguez, C.M. Niemeyer, Institute for Biological Interfaces (IBG-1), KIT (Germany).

Dynamics of Collagen I Fibrillogenesis





Cant: USC-f0.3-k0.3, **Z:** 3 nm, **Linerate:** 15 Hz, **Pixels:** 256x256
Env: 1x PBS (2.7 mM KCl) at pH 7.4, **Ch:** Height, **Videorate:** 10x Sped Up



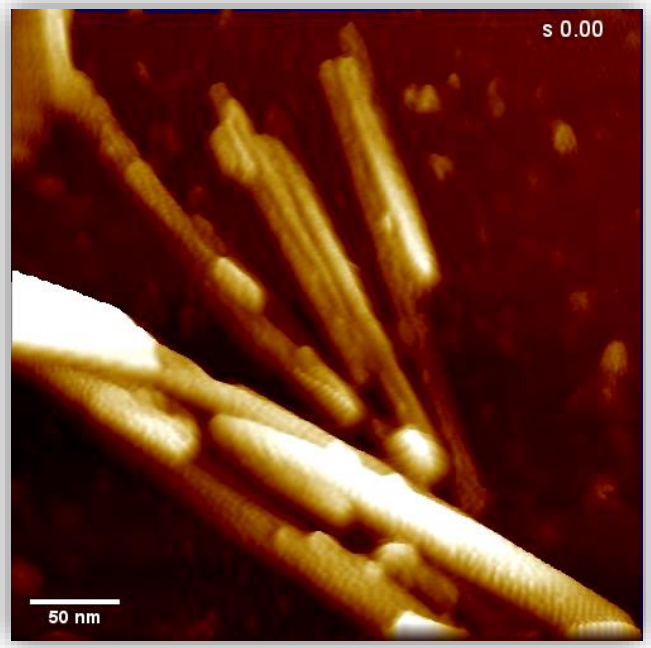
Cant: USC-f0.3-k0.3, **Z:** 3 nm, **Linerate:** 32 Hz, **Pixels:** 256x256
Env: 1x PBS (0.2 M KCl) at pH 7.3, **Ch:** Height, **Videorate:** 10x Sped Up

- Reduced fibrillogenesis kinetics at pH 9.2 (high ionic strength of KCL lowers IEP of collagen I fibrils)
- Faster Assembly Kinetics at pH 7.4 (no additional Gly)
- Higher [K+] are critical for the proper D-banding packing

Stamov DR et al., Ultramicroscopy (2015) 86-94

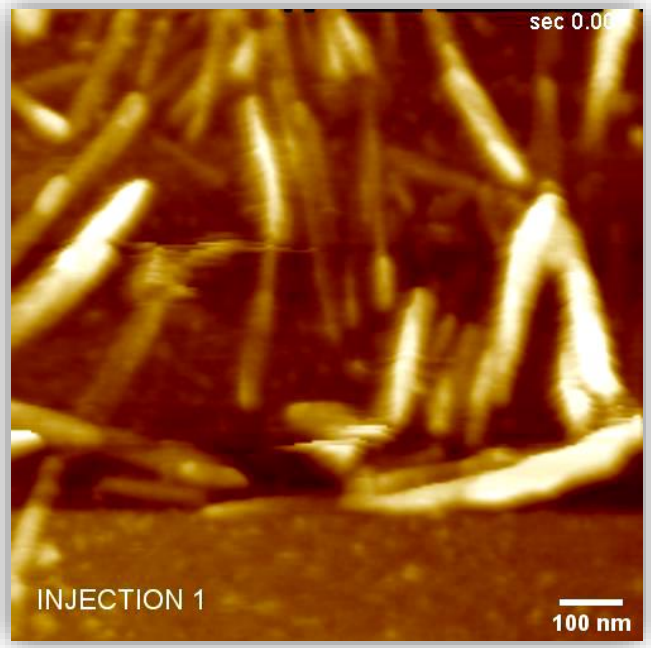
Observing amyloid fibrils disassambly *in situ*

- Before injection



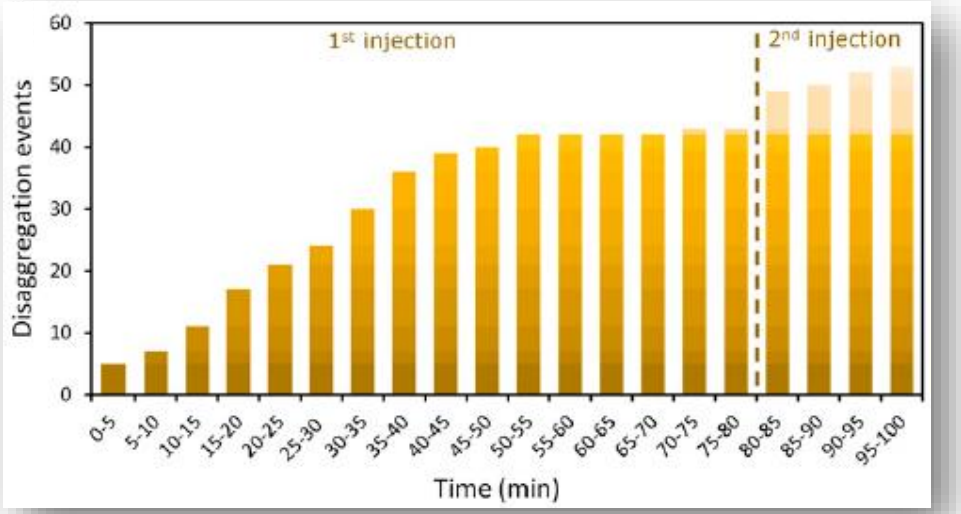
- α-syn fibrils observed with HS-AFM before chaperone and ATP injection (Full image size: 350 × 350 nm; imaging rate: 300Hz, total time: 16 min 9 s).

- After injection



- Chaperone-induced fibril depolymerisation after two consecutive injections of ATP and ATP-regeneration system (Full image size: 1000 × 1000 nm; imaging rate: 100Hz, total time: 90 min).

- *From: PNAS September 7, 2021 118 (36) e2105548118*



- Cumulative disaggregation events were plotted as a function of time

- Fast and stable imaging if big and loosely bound (poly-l-lysine) fibrils for minutes.

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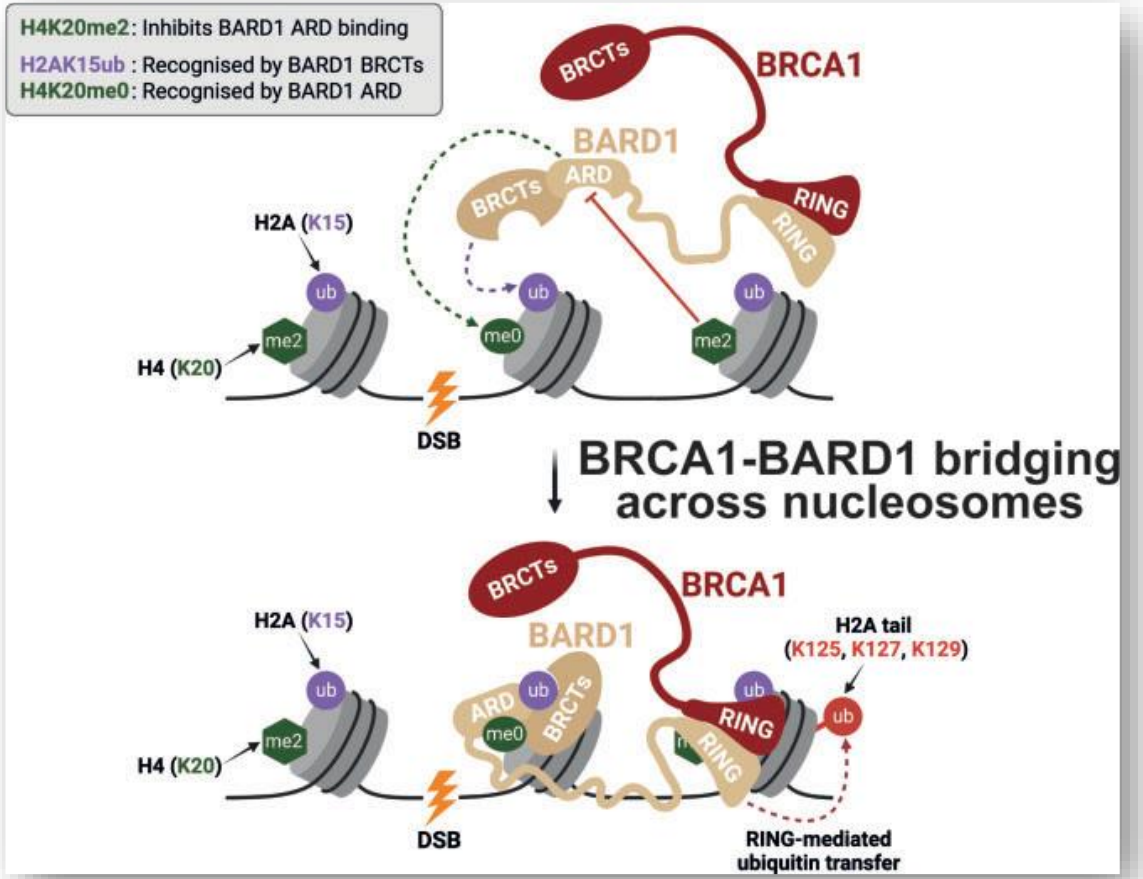
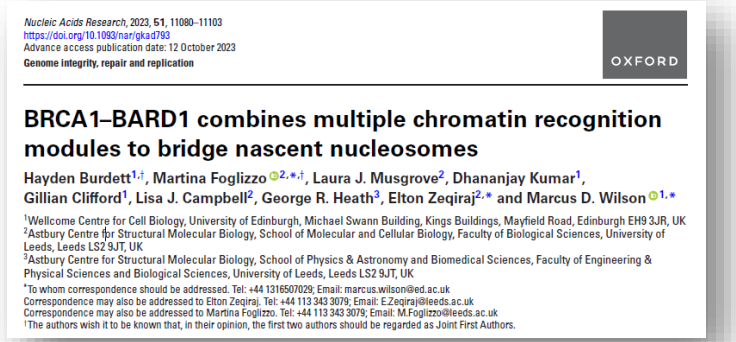
High-Speed AFM molecule analysis example





Nucleic Acids Research, 2023, **51**, 11080–11103
https://doi.org/10.1093/nar/gkad793
Advance access publication date: 12 October 2023
Genome integrity, repair and replication

OXFORD

BRCA1–BARD1 combines multiple chromatin recognition modules to bridge nascent nucleosomes

Hayden Burdett[1,†], Martina Foglizzo[2,*,†], Laura J. Musgrove[2], Dhananjay Kumar[1], Gillian Clifford[1], Lisa J. Campbell[2], George R. Heath[3], Elton Zeqiraj[2,*] and Marcus D. Wilson[1,*]

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[2]Astbury Centre for Structural Molecular Biology, School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK
[3]Astbury Centre for Structural Molecular Biology, School of Physics & Astronomy and Biomedical Sciences, Faculty of Engineering & Physical Sciences and Biological Sciences, University of Leeds, Leeds LS2 9JT, UK
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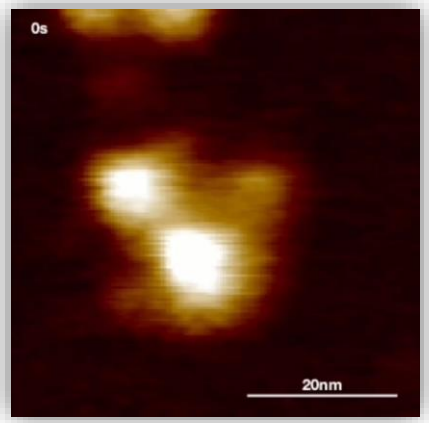
†The authors wish it to be known that, in their opinion, the first two authors should be regarded as Joint First Authors.

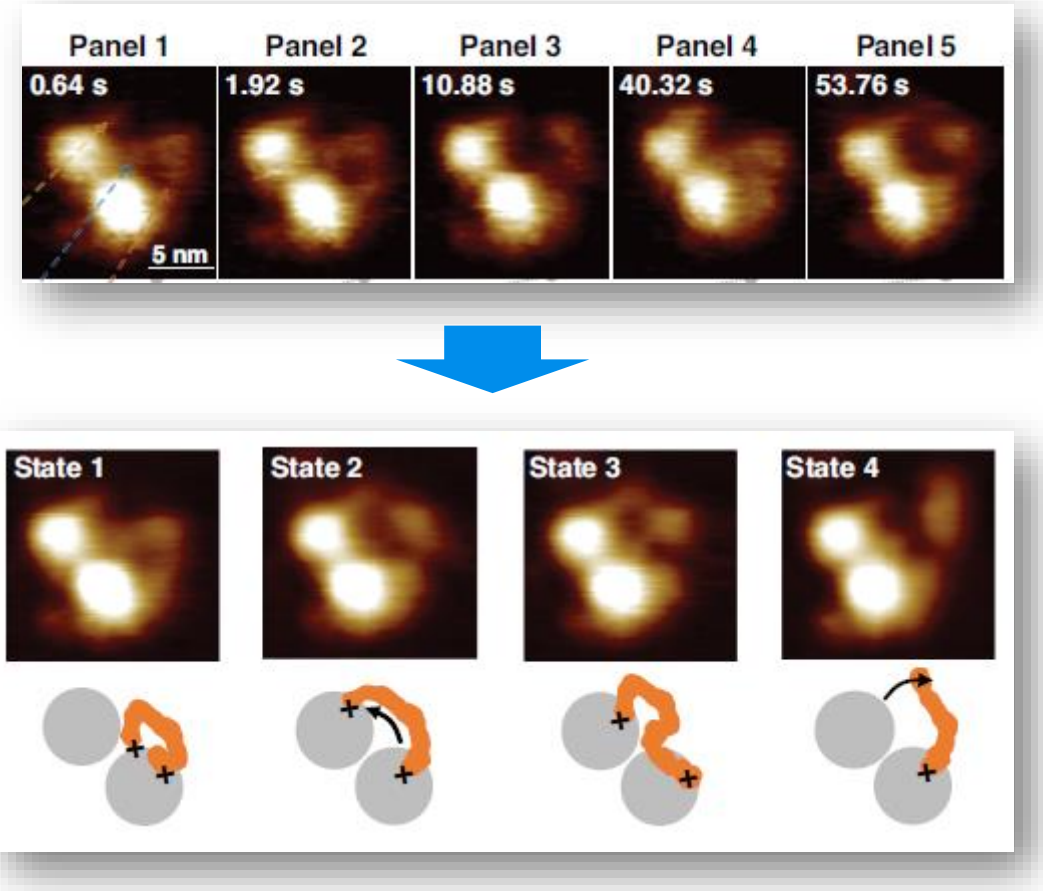
H. Burdett, M. Foglizzo, et al., *Nucleic Acids Research*, 2023, Vol. **51**, No. 20



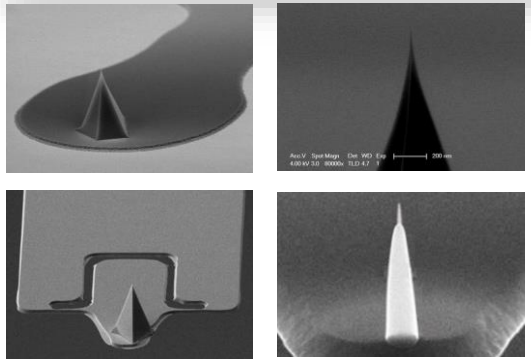
1.56 fps

Adapted from: H. Burdett, M. Foglizzo, et al., *Nucleic Acids Research*, 2023, Vol. **51**, No. 20

High-Speed AFM molecule analysis example
Image averaging



www.brukerafmprobes.com
recommended probes



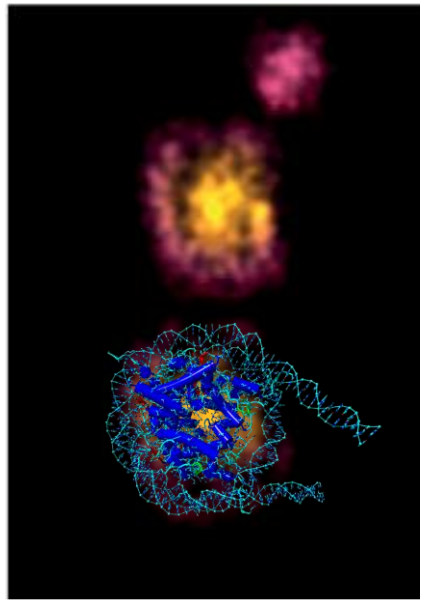
FASTSCAN-D
FASTSCAN-D-SS
HISPEED FLUID

Adapted from: H. Burdett, M. Foglizzo, et al., *Nucleic Acids Research*, 2023, Vol. **51**, No. 20

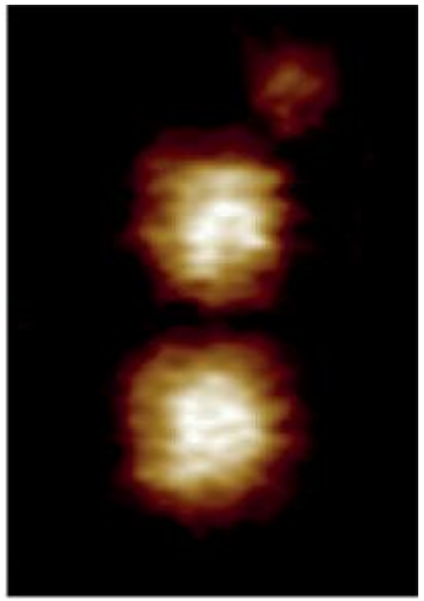
High-Speed AFM molecule analysis example
Localisation AFM (LAFM)

BRUKER

Localisation AFM

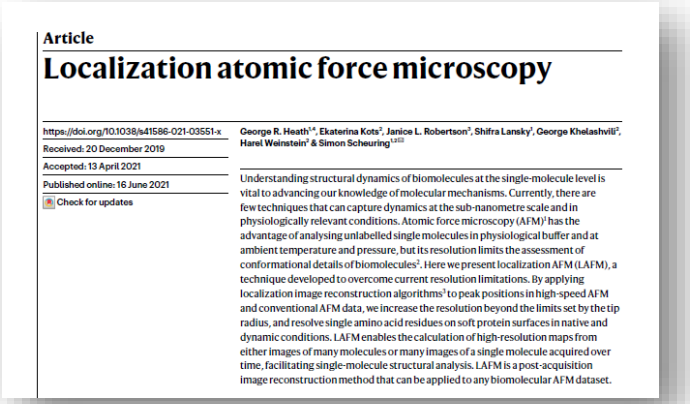


AFM



"Localisation AFM (LAFM) images of di-nucleosomes were generated using 273 HS-AFM images of a single di-nucleosome captured at 3 pixel/nm and processed with bicubic subpixel localisation."

Adapted from: H. Burdett, M. Foglizzo, et al., *Nucleic Acids Research*, 2023, Vol. 51, No. 20



Article

Localization atomic force microscopy

https://doi.org/10.1038/s41586-021-03551-x
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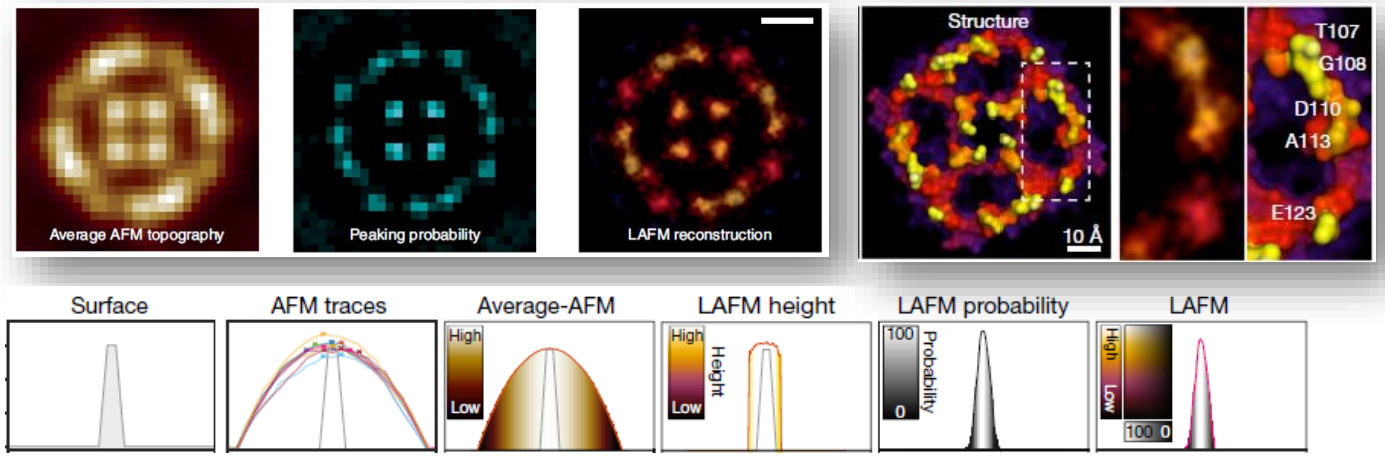
Check for updates

George R. Heath[1,4], Ekaterina Kots[2], Janice L. Robertson[2], Shifra Lansky[3], George Khelashvili[2], Harel Weinstein[2] & Simon Scheuring[1,2]

Understanding structural dynamics of biomolecules at the single-molecule level is vital to advancing our knowledge of molecular mechanisms. Currently, there are few techniques that can capture dynamics at the sub-nanometre scale and in physiologically relevant conditions. Atomic force microscopy (AFM)[1] has the advantage of analysing unlabelled single molecules in physiological buffer and at ambient temperature and pressure, but its resolution limits the assessment of conformational details of biomolecules[2]. Here we present localization AFM (LAFM), a technique developed to overcome current resolution limitations. By applying localization image reconstruction algorithms[3] to peak positions in high-speed AFM and conventional AFM data, we increase the resolution beyond the limits set by the tip radius, and resolve single amino acid residues on soft protein surfaces in native and dynamic conditions. LAFM enables the calculation of high-resolution maps from either images of many molecules or many images of a single molecule acquired over time, facilitating single-molecule structural analysis. LAFM is a post-acquisition image reconstruction method that can be applied to any biomolecular AFM dataset.

Heath, G.R., Kots, E., Robertson, J.L. et al. Localization atomic force microscopy. *Nature* 594, 385–390 (2021)

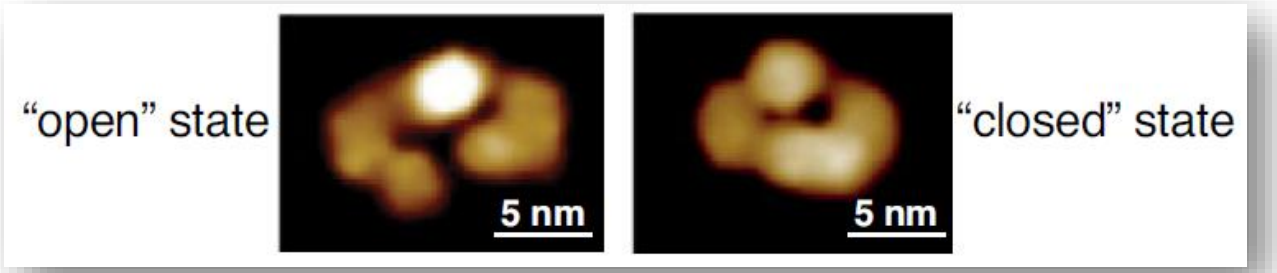
aquaporin AqpZ



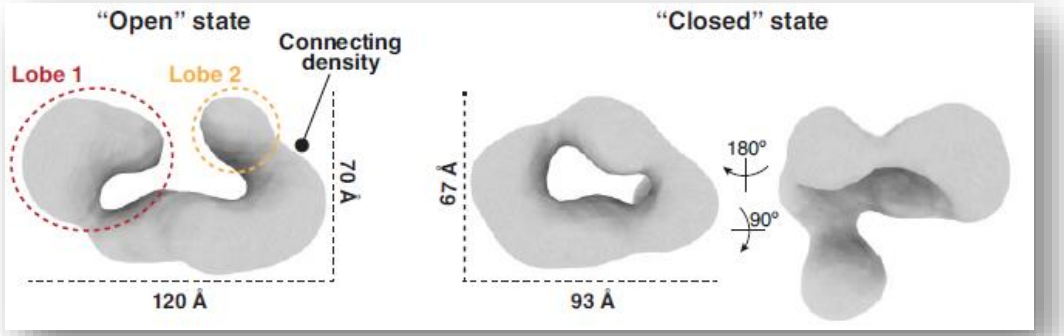
High-Speed AFM molecule analysis example

Simulated AFM images

Simulated AFM (BRCA1:BARD1)



Cryo-EM maps



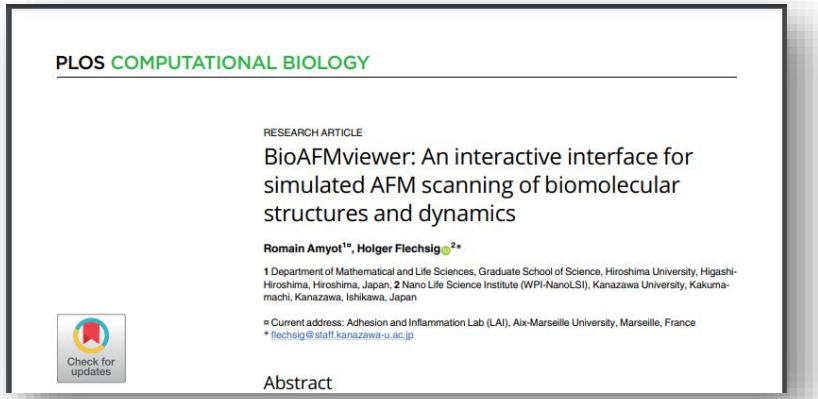
“Simulated topographies were generated using Mat-SimAFM software available at: github.com/George-R-Heath/Mat-SimAFM”

Adapted from: H. Burdett, M. Foglizzo, et al., *Nucleic Acids Research*, 2023, Vol. 51, No. 20

High-Speed AFM molecule analysis example

Simulated AFM images using Biomolecular AFM Viewer

BRUKER



PLOS COMPUTATIONAL BIOLOGY

RESEARCH ARTICLE

BioAFMviewer: An interactive interface for simulated AFM scanning of biomolecular structures and dynamics

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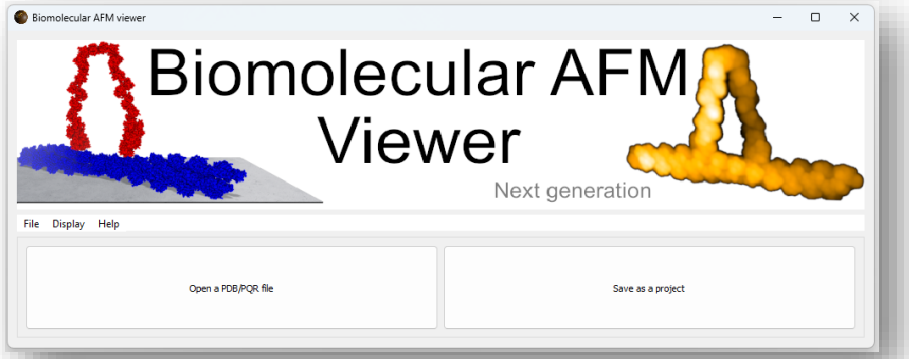
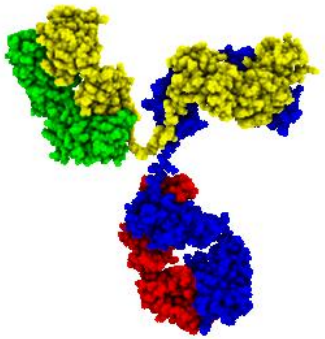
Check for updates

Abstract

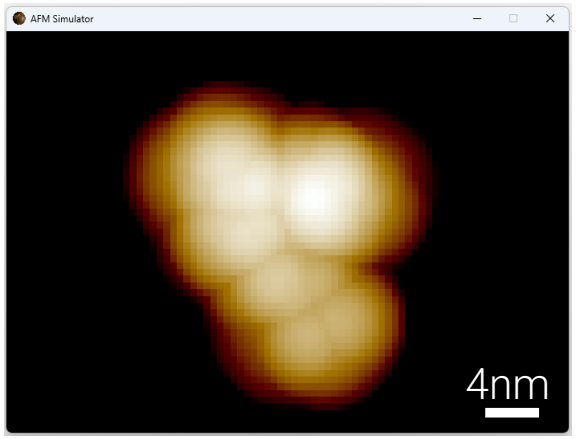
Amyot R, Flechsig H (2020) BioAFMviewer: An interactive interface for simulated AFM scanning of biomolecular structures and dynamics. PLOS Computational Biology 16(11): e1008444



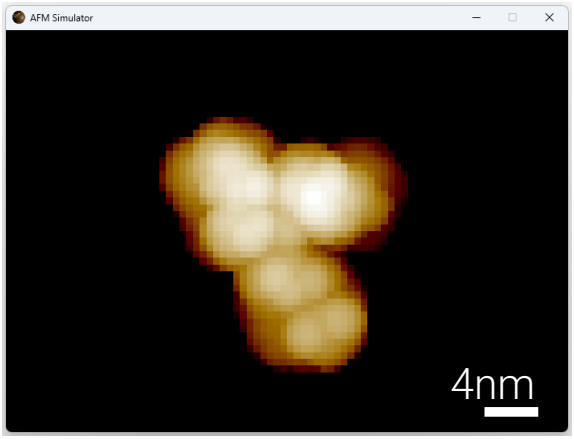
www.bioafmviewer.com



Mouse immunoglobulin IgG2a (PDB ID:1IGT)

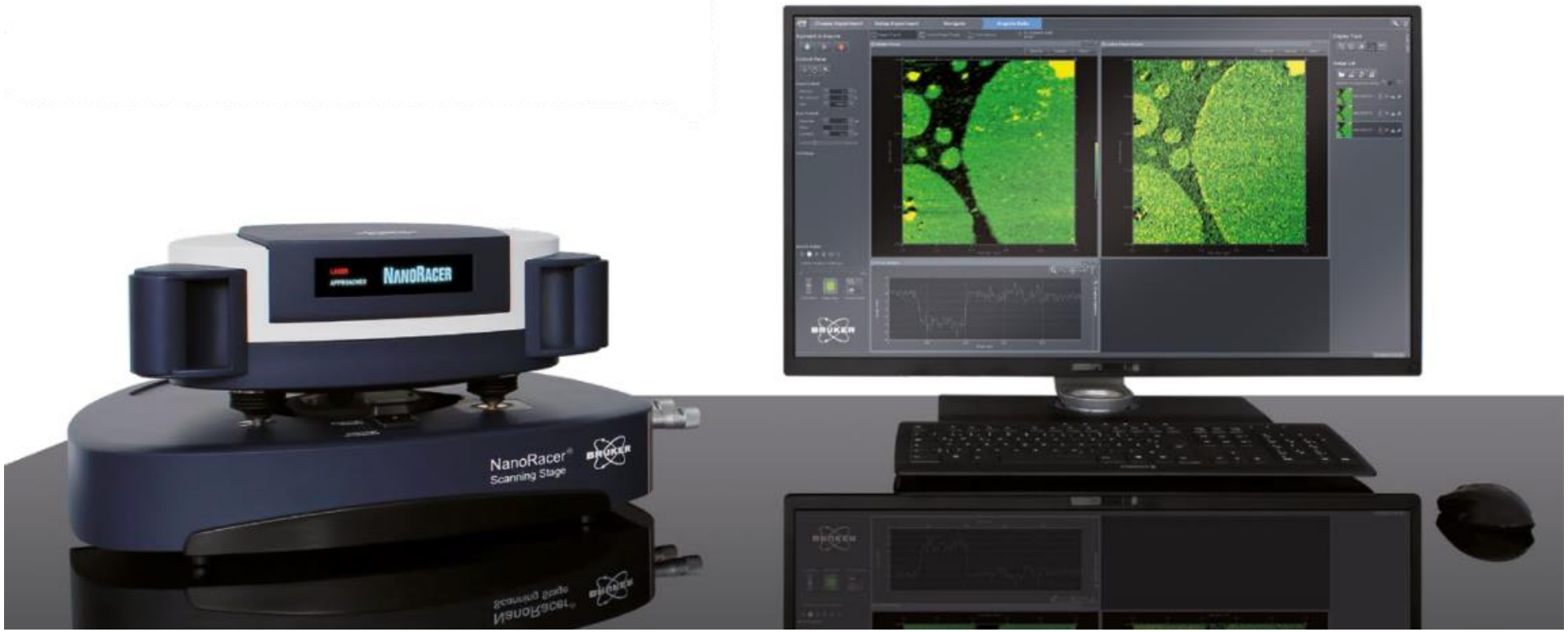


Tip radius: 5 nm



Tip radius: 2 nm

NanoRacer High-Speed AFM



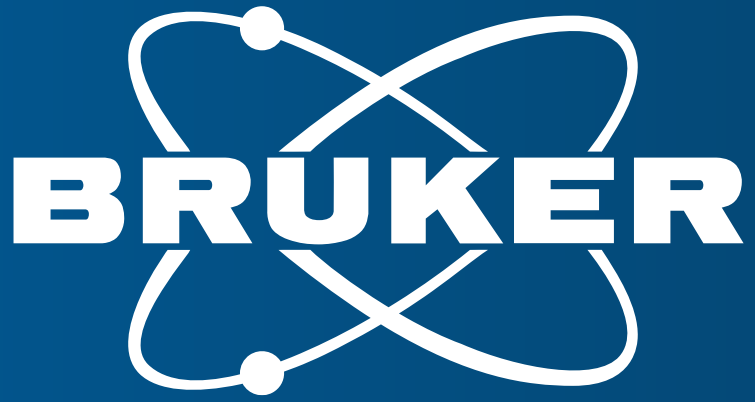
NanoRacer High-Speed AFM

NanoRacer head + stage + scanner

NanoRacer head flipped up
+ stage + scanner

NanoRacer Head + Stage + portable
scanner unit detached

Thank you!



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