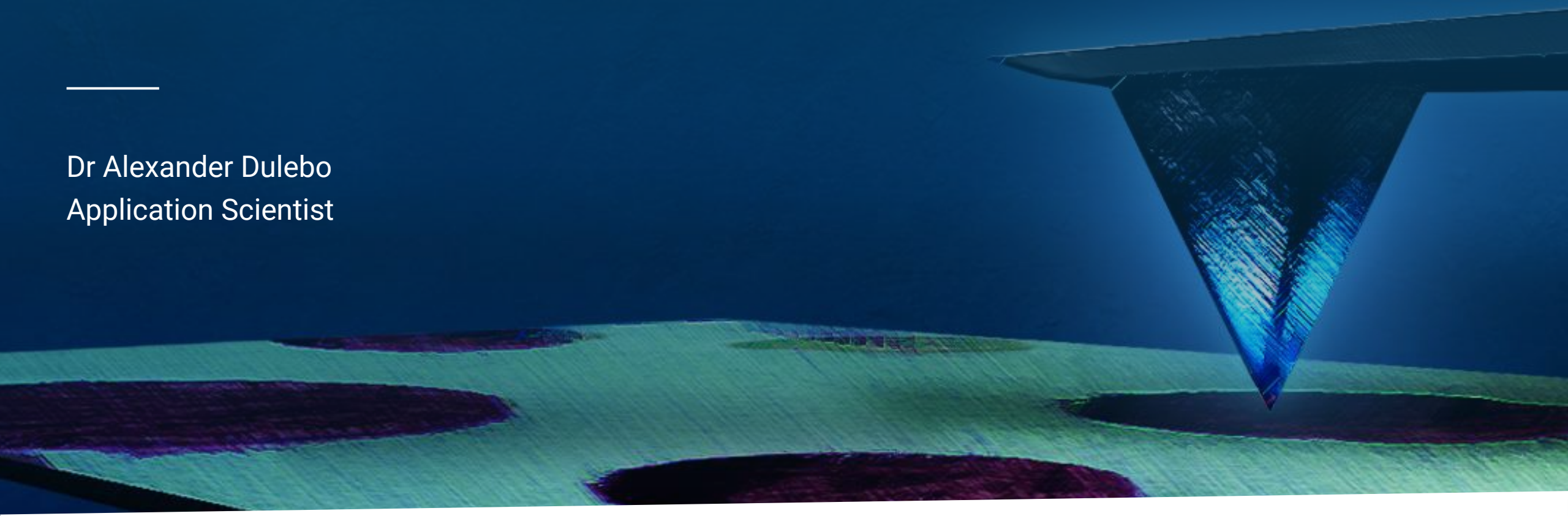
SUMMER WORKSHOP ON BIOAFM MICROSCOPY 2023, BRNO CZECH REPUBLIC

The Fundamentals of AFM Probe Selection

Dr Alexander Dulebo
Application Scientist



Outline

1 Manufacturing a probe

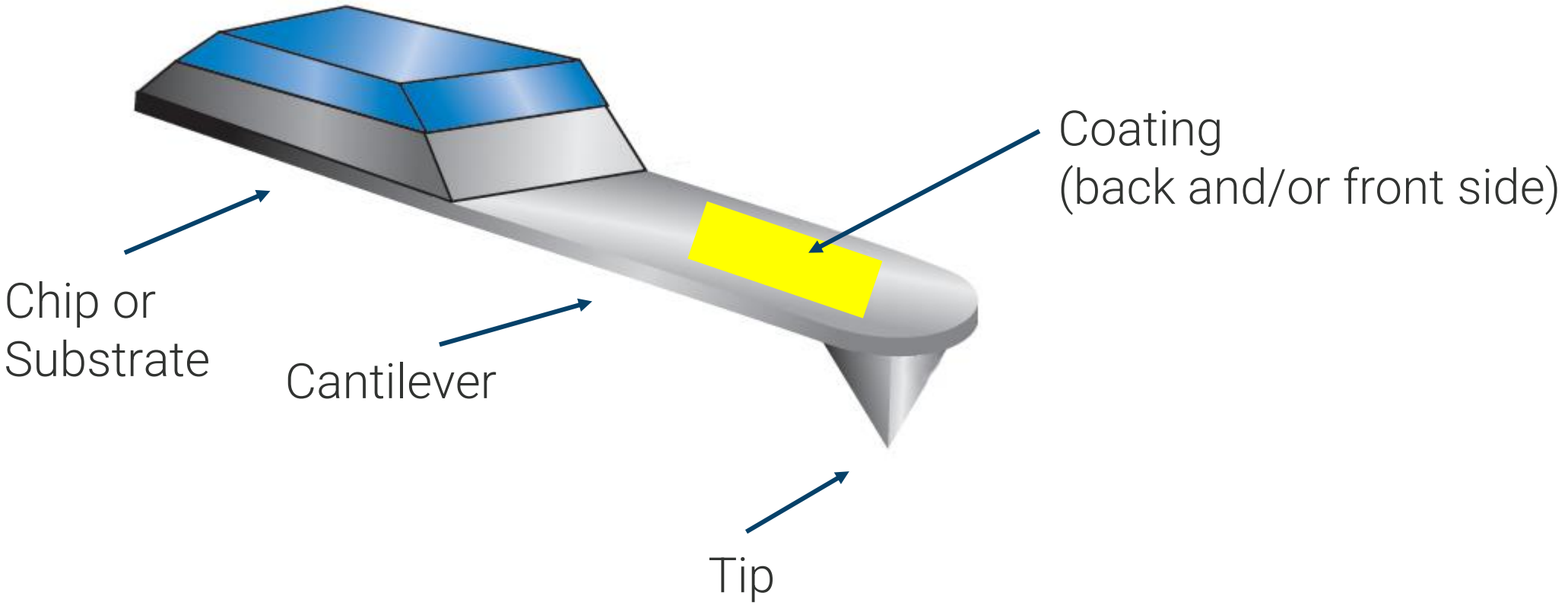
2 How to select a probe

3 Recommended probes for bio applications

4 Probe artefacts, probe cleaning

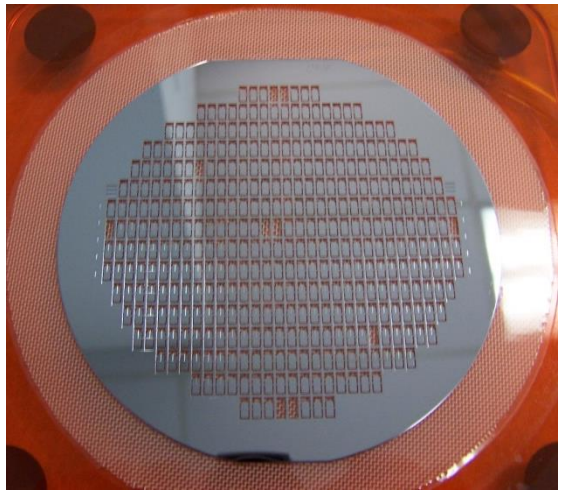
01 Manufacturing a probe

Anatomy of the probe



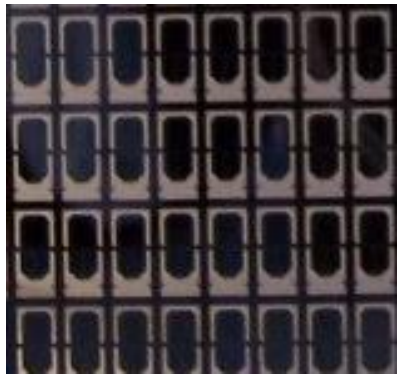
Bruker Nanofabrication Center in Camarillo, CA, USA

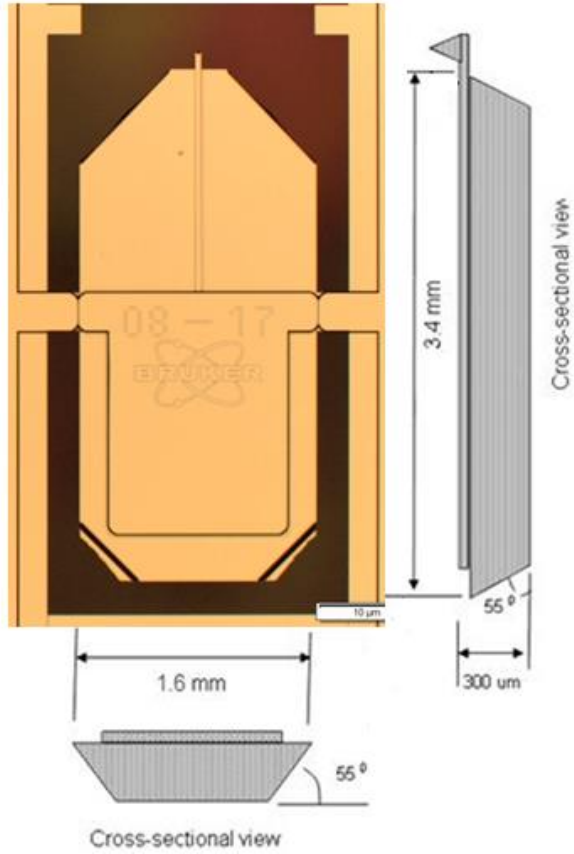
Probes Fabrication – batch fabrication



4-inch wafer

Zoom in





- Probes are made by batch fabrication
- Each 4-inch wafer holds about 400 probes

Step 0

- Wafer 4“ <100>

Step 1

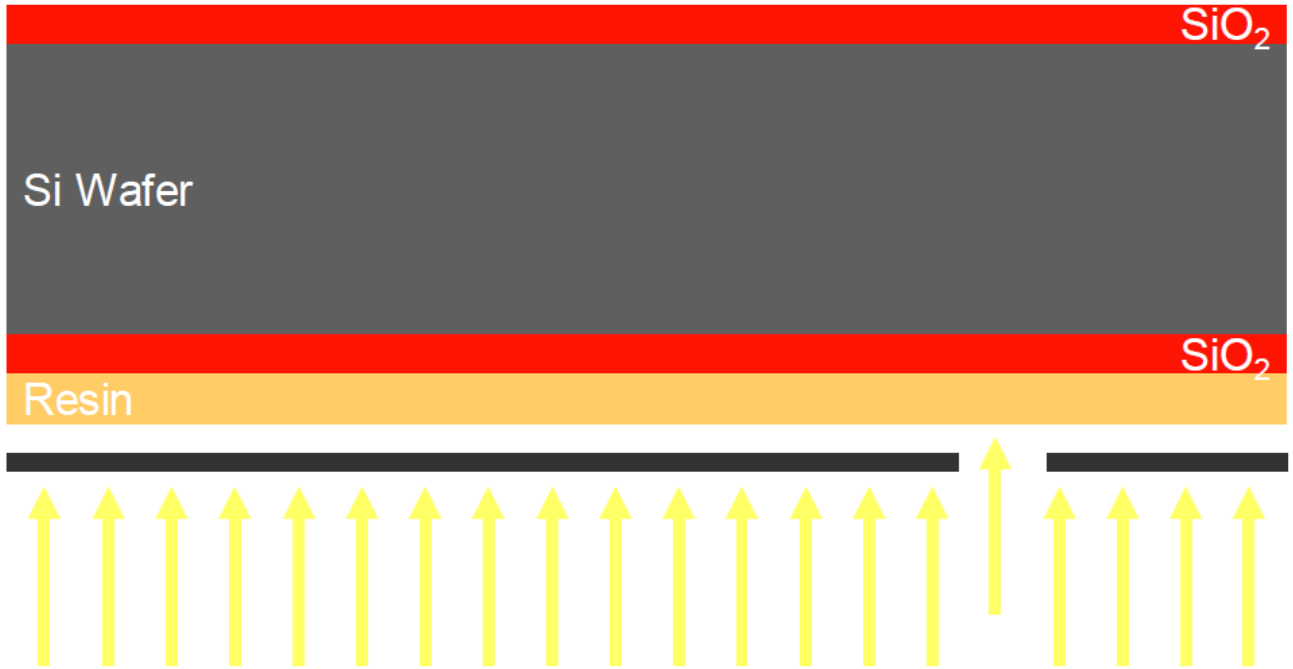
- Oxidation

Step 2

- Backside Photosensitive resin coating

Step 3

- Photolithography (Photosensitive resin is exposed through a Chrome/Quartz mask)



Step 4

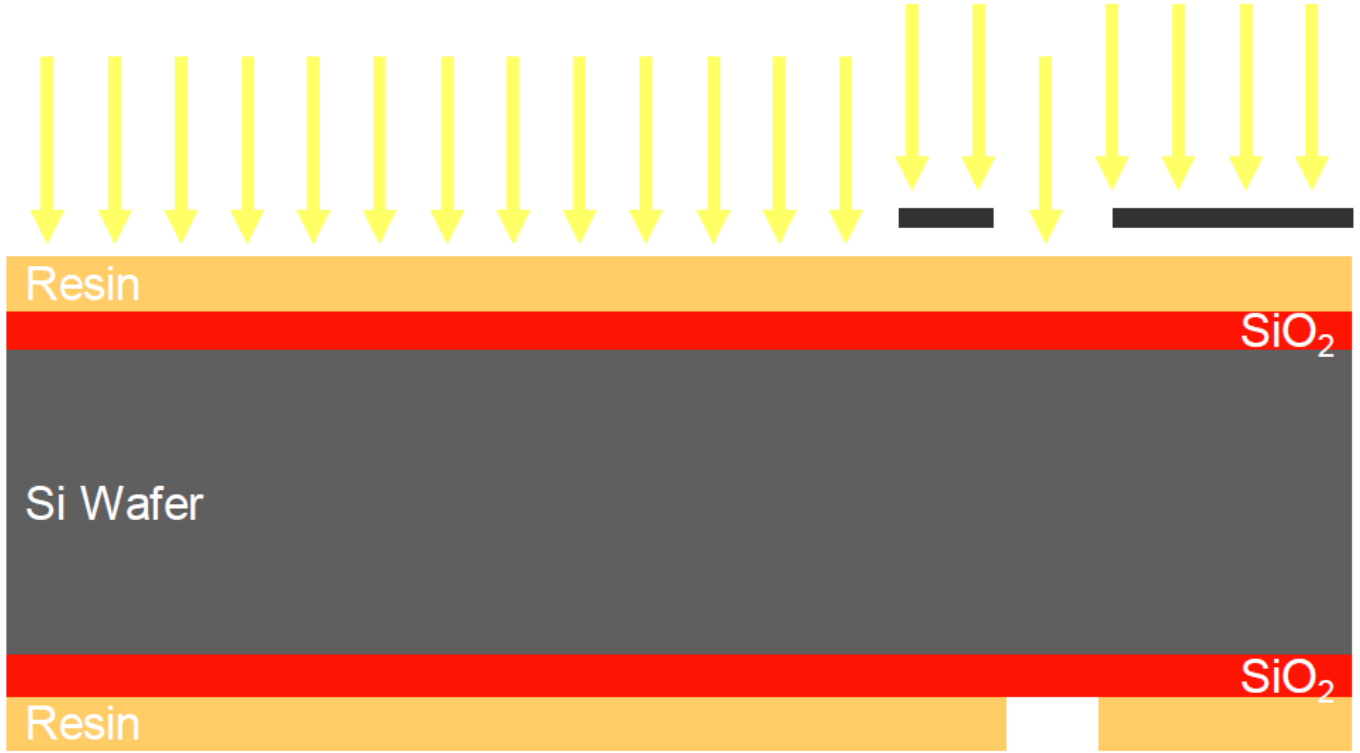
- Lift off

Step 5

- Front side photosensitive resin coating

Step 6

- Photolithography (photosensitive resin is exposed through a chrome/quartz mask)



Step 7

- Lift off

Step 8

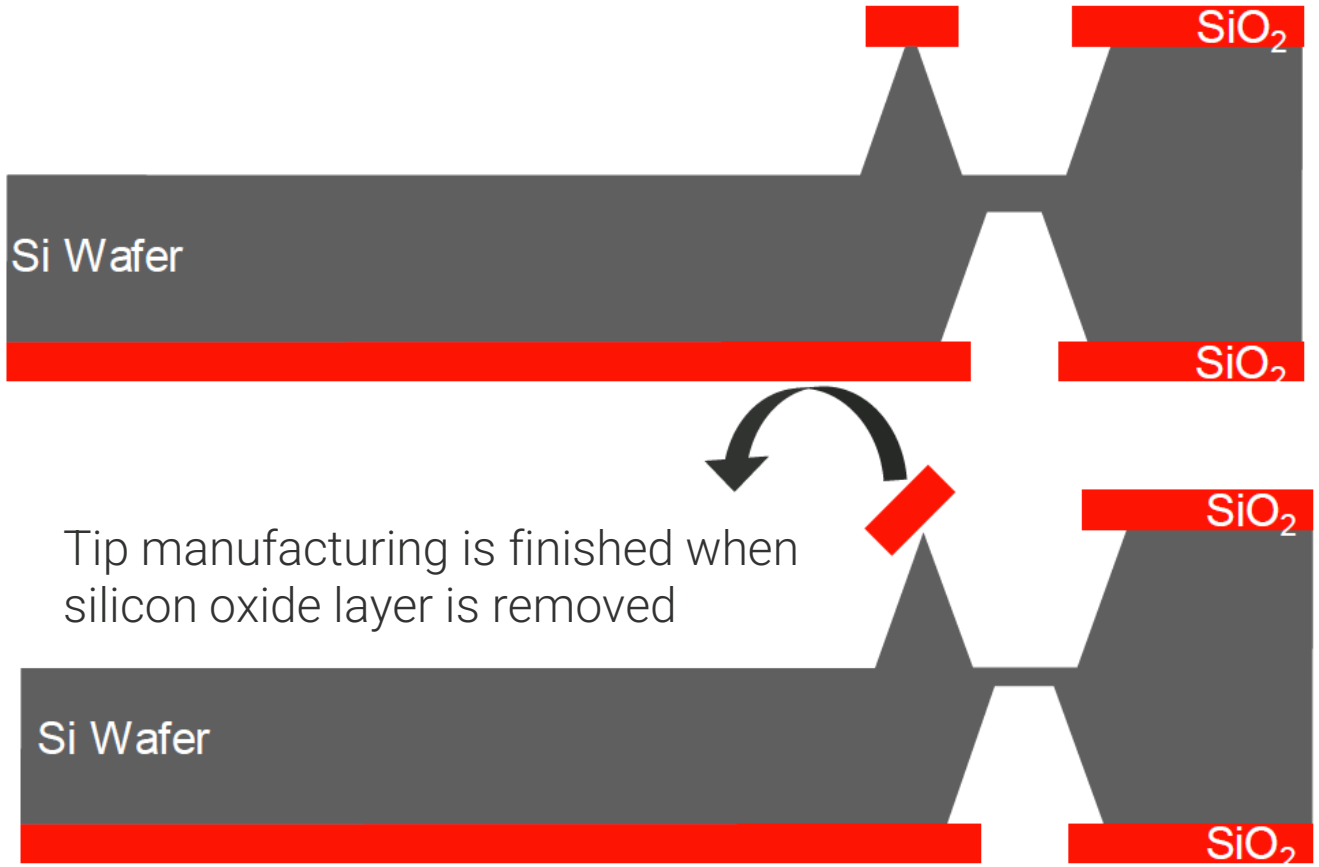
- Silicon Oxide isotropic wet etching with BHF

Step 9

- Photosensitive resin dissolved in acetone

Step 10

- Silicon anisotropic wet etching with KOH (this is critical and made in several step)



Step 11

- Silicon oxide isotropic wet etching with BHF

Step 12

- Silicon nitride coating to protect the tip

Step 13

- Silicon anisotropic wet etching with KOH (cantilever thickness is controlled there)

Step 14

- Silicon nitride isotropic wet etching with H_3PO_4

Step 15

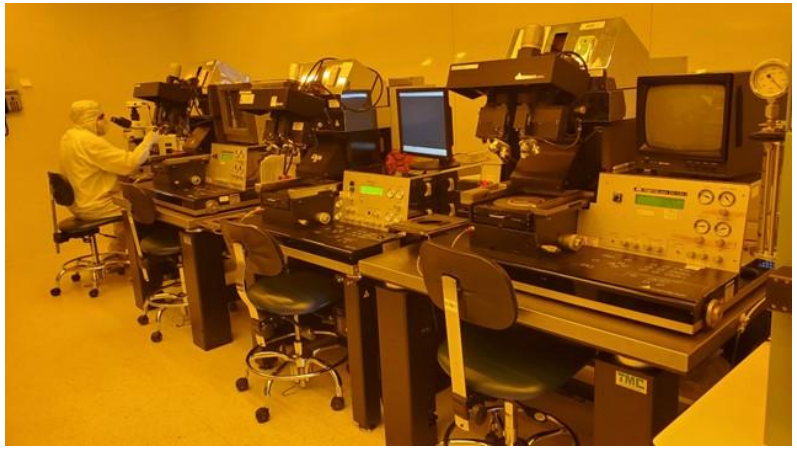
- Silicon nitride isotropic wet etching with H_3PO_4

Wafer Fab

- Process Capabilities:
  - Lithography: From < 1 mm to 35 mm Photoresist Thicknesses and 1 mm Linewidths
  - Stochiometric and Super Low Stress Silicon Nitride
  - Thermal Oxidation
  - Wet Etch: Silicon, Oxide, Silicon Nitride, Metals
  - Dry Etch: Silicon, Oxide, Silicon Nitride
  - Metallization: Magnetic Films, Titanium, Chrome, Aluminum, Gold, Platinum, & Metal Alloys
  - Focused Ion Beam
  - Electron Beam Deposition

FIB and EBD System

Wet Process Stations



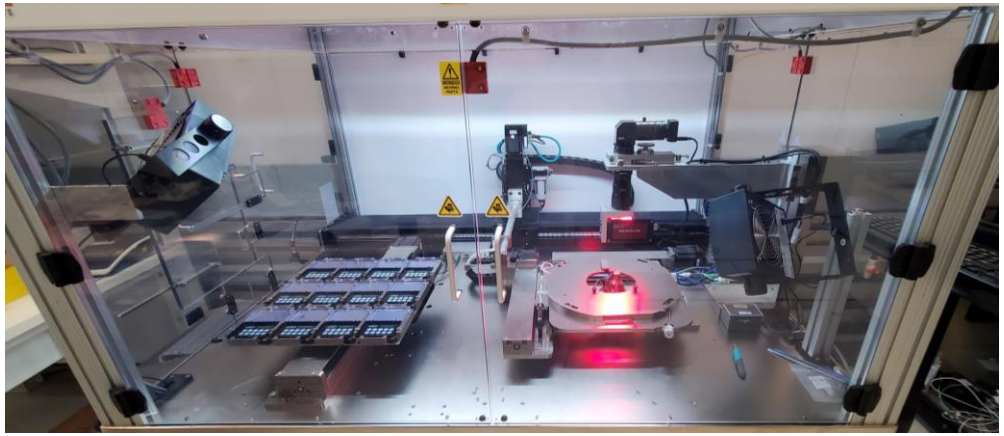
Contact Aligners

Photoresist Spin Coat & Developer Track

Final test and assembly room

AFM & Vibrometer

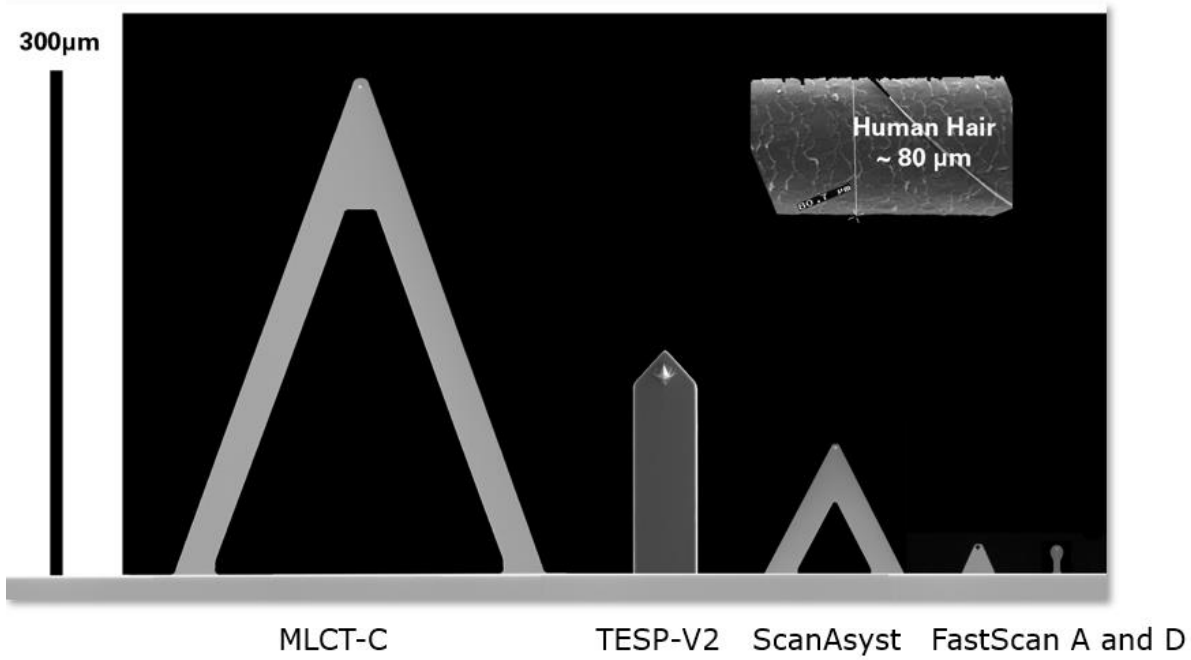
SEM & ContourGT

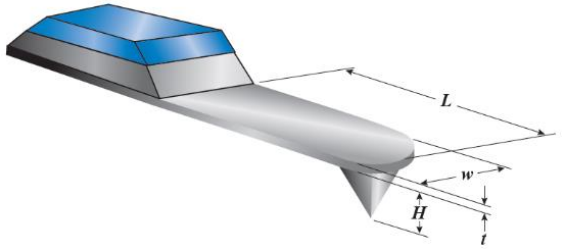


Pick & Place System

02 How to select a probe

How to select a probe: Cantilever shape affects sensitivity and hydrodynamic



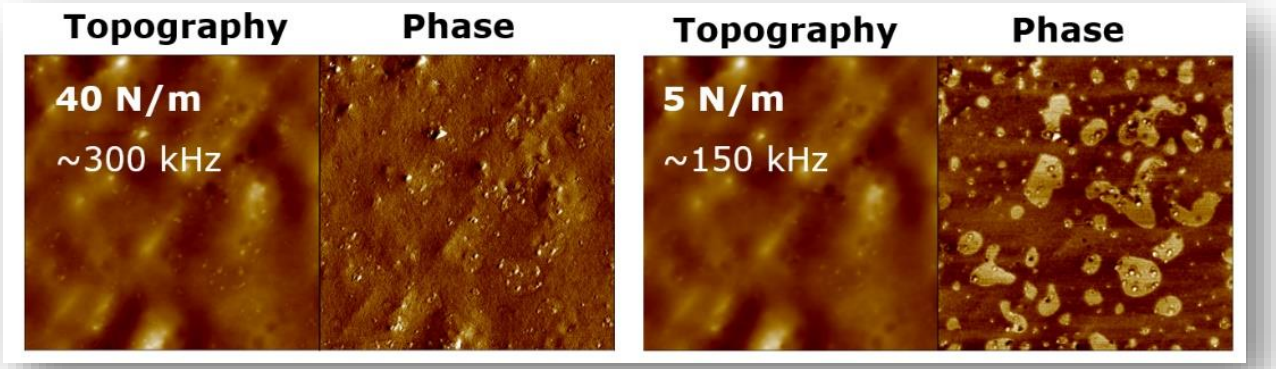


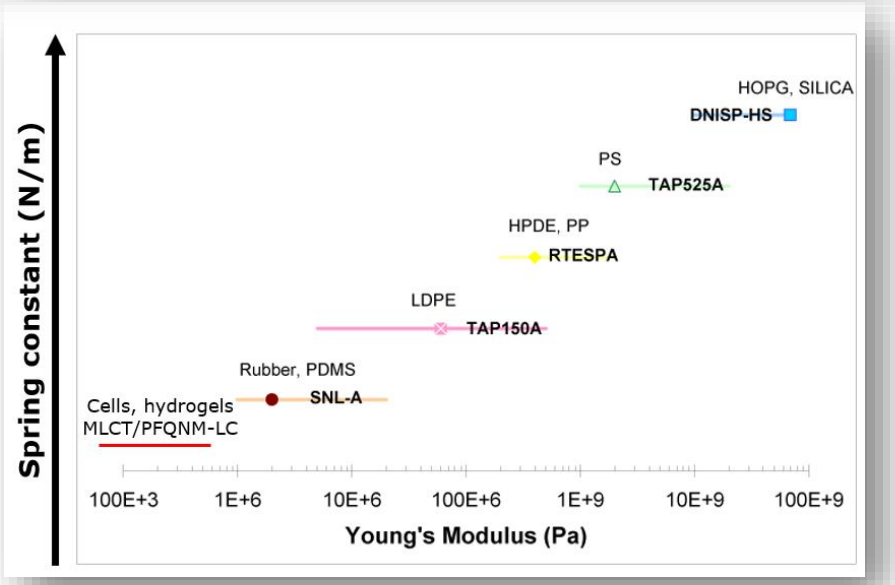
- The probe's cantilever is usually rectangular, triangular, or special
- The width (W), length (L), and thickness (t) of the cantilever will influence spring constant k (N/m), resonance frequency f (kHz), and deflection sensitivity d (nm/V)
- Short cantilevers are usually better
  - Have lower deflection sensitivity
  - Have lower hydrodynamic drag in liquid
  - Be aware of laser spot size!

Model	Length	Defl. Sens.
FastScan-C	40 µm	12 nm/V
ScanAsyst-Fluid+	70 µm	22 nm/V
SNL-C	120 µm	30 nm/V

How to select a probe: Spring constant (k, N/m) affect imaging force

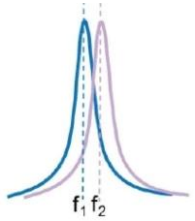
- **Contact mode:** low spring constant (<1N/m)
- **Tapping mode air:** high spring constant (>20N/m)
- **Tapping mode air (phase imaging):** medium spring constant (4-40N/m)
- **Tapping mode liquid:** low-high spring constant (depends on other factors)
- **PeakForce Tapping/ScanAsyst/QI:** low spring constant (<1N/m)
- **PeakForce QNM/QI/Force Mapping:** spring constant to match material modulus



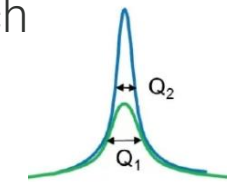


How to select a probe: Resonance frequency (kHz-MHz) and Q affect imaging speed

- Resonance frequency (f_{res}): speed to reach equilibrium oscillation



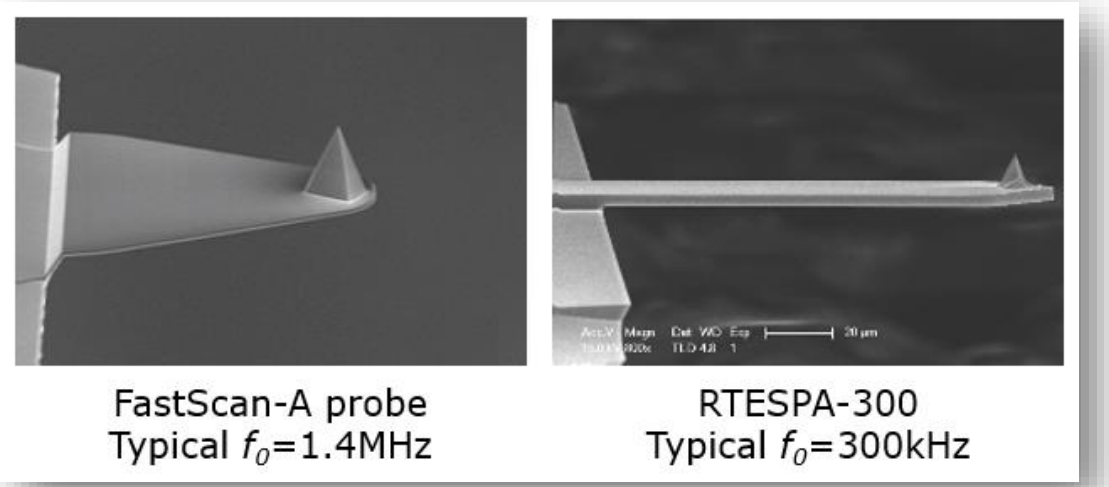
- Quality factor (Q): number of cycles to reach cantilever equilibrium



- Cantilever bandwidth (BW): determine the imaging speed

$$BW = \frac{\pi f_{res}}{Q}$$

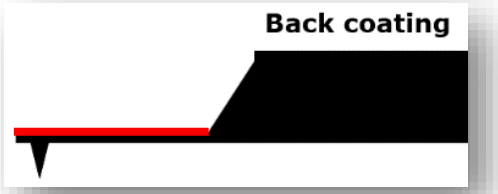
- How to achieve that? Smaller cantilever!



Q=300
BW=14,6 kHz

Q=300
BW=3,1 kHz

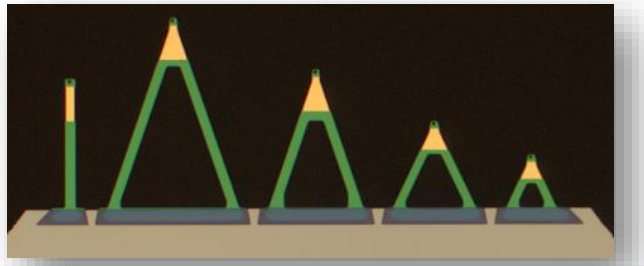
How to select a probe: Coatings enable specific property measurements



- Back side coating (Al, Au), improve laser reflection
- DO NOT use aluminum backside coated probe in fluid

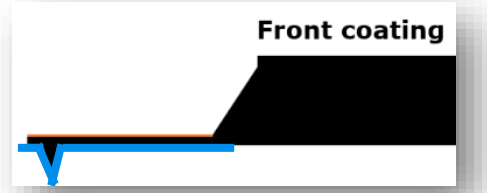
Cantilever Thickness (Nom):	0.65 µm
Cantilever Thickness (RNG):	0.6 - 0.7 µm
Back Side Coating:	Reflective Aluminum

- Back side coating could cause cantilever to bend when temperature changes

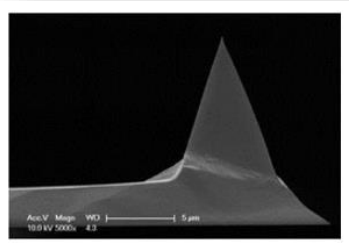


MLCT-BIO-DC probe –

partial coating minimize thermal drift

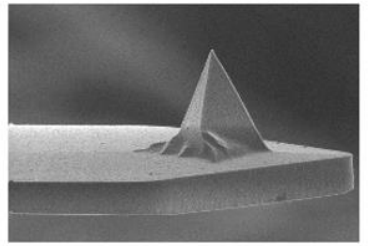


- Front side coating (conductive, chemical, hardened) enable specific applications (electrical, magnetic measurements)



SCM-PtSi

Tip SetBack (TSB)(Nom):	15 µm
Tip Set Back (TSB)(RNG):	5 - 25 µm
Tip Coating:	Conductive PtSi

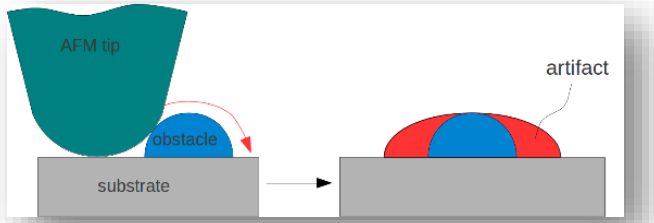


DDESP

Tip SetBack (TSB)(Nom):	14 µm
Tip Set Back (TSB)(RNG):	11 - 16 µm
Tip Coating:	Conductive Diamond

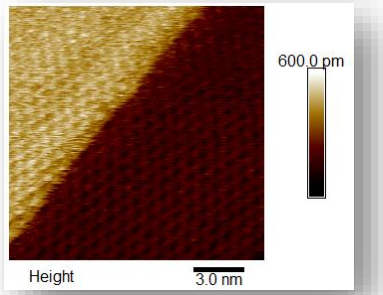
How to select a probe: Tip shape and radius determine resolution

- AFM topography is always a convolution of tip shape and sample surface structure

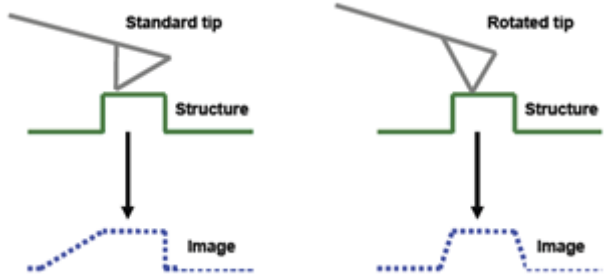


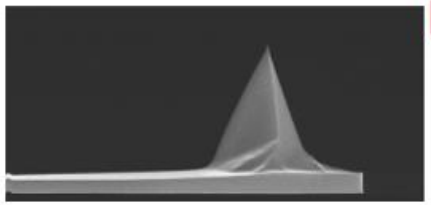
Wikipedia©

- High resolution on rough samples require a sharp tip (e.g. DNA double helix)
- High resolution on flat samples is possible with a regular tip (e.g. atomic resolution on calcite)



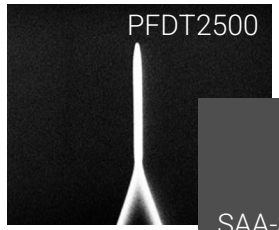
- Rotated tip for accurate topography tracking



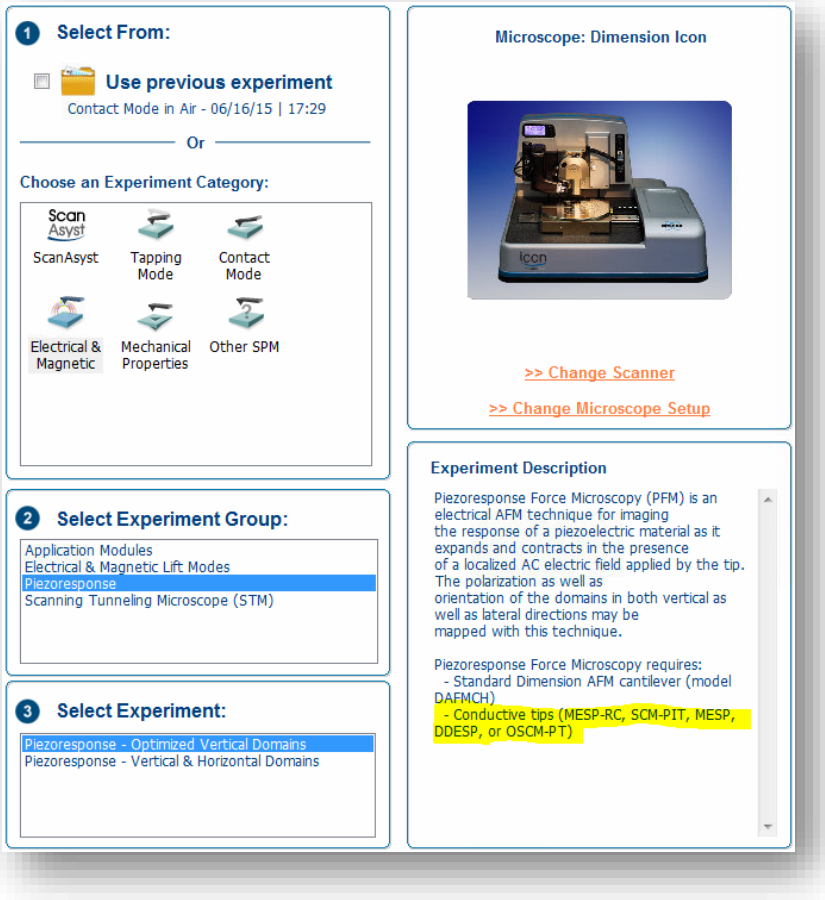


Geometry:	Rotated (Symmetric)
Tip Height (h):	10 - 15µm
Front Angle (FA):	15 ± 2°
Back Angle (BA):	25 ± 2 °
Side Angle (SA):	17.5 ± 2 °
Tip Radius (Nom):	8 nm
Tip Radius (Max):	12 nm

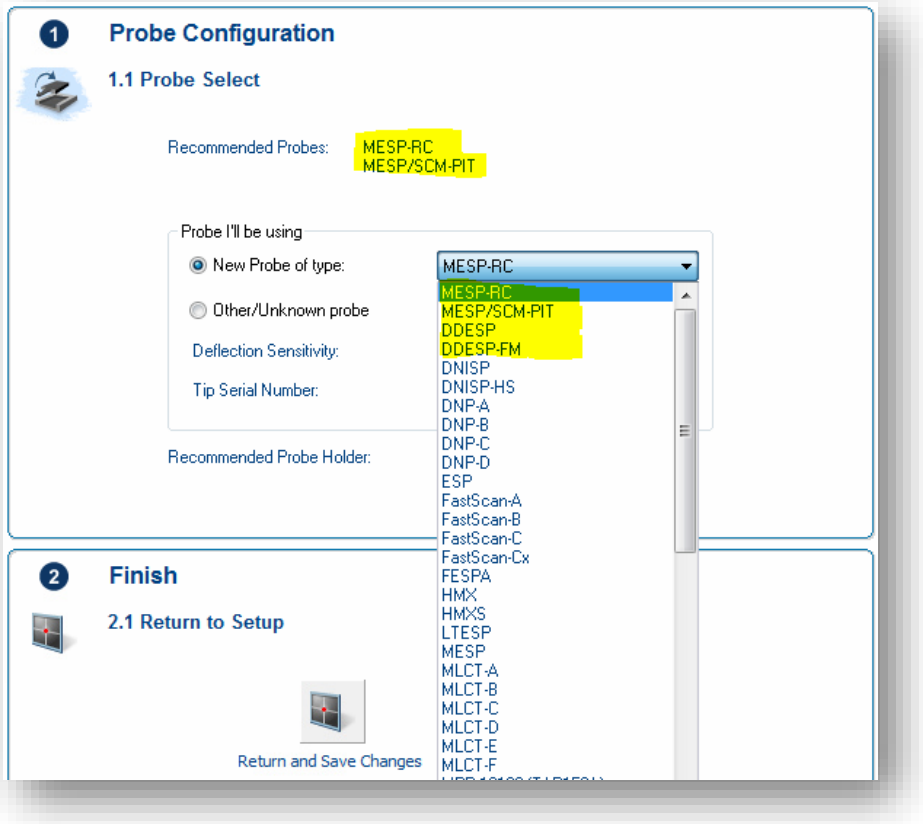
- High aspect ratio
- Tipless
- Spherical



How to select a probe: Guidance from the AFM software

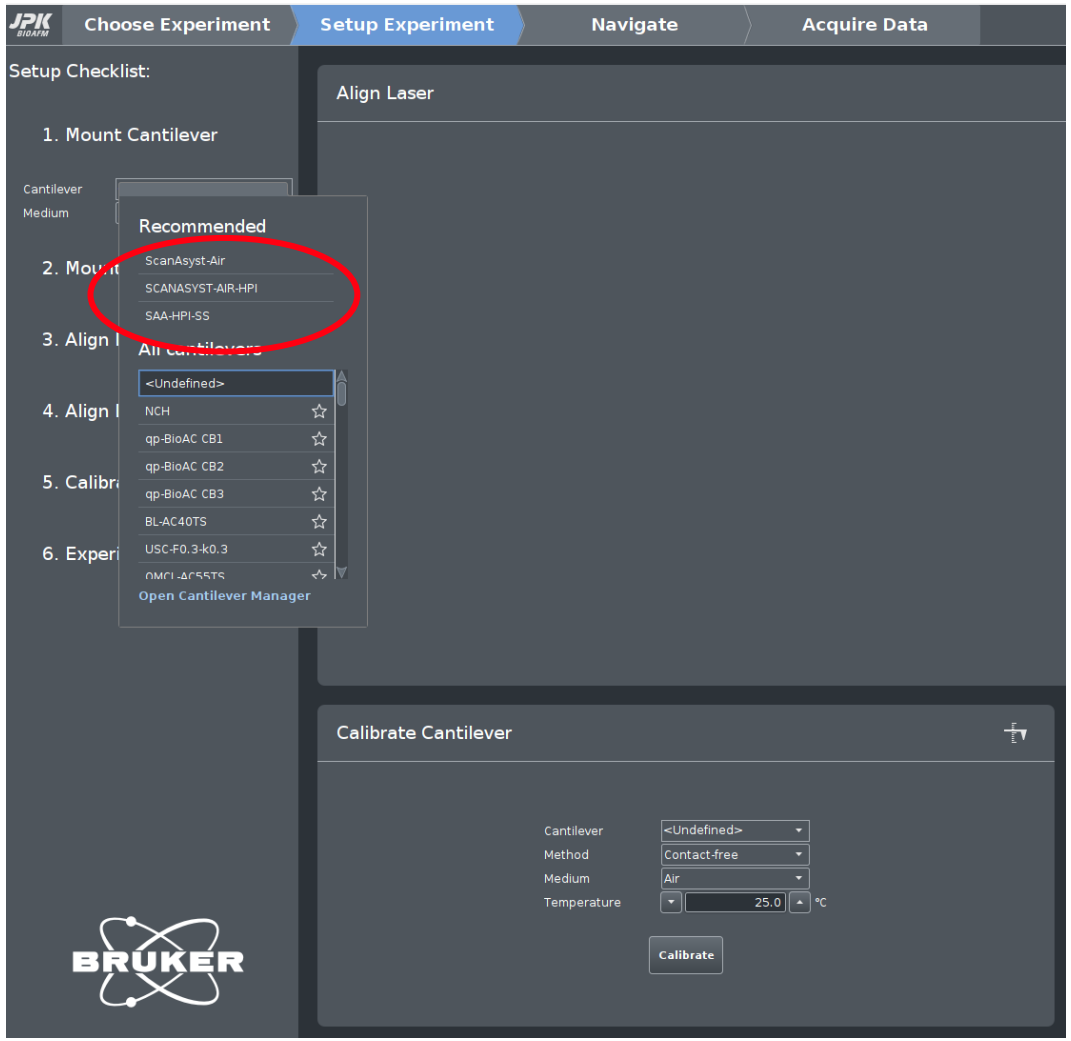


- Probe Suggestions in Loading Experiment



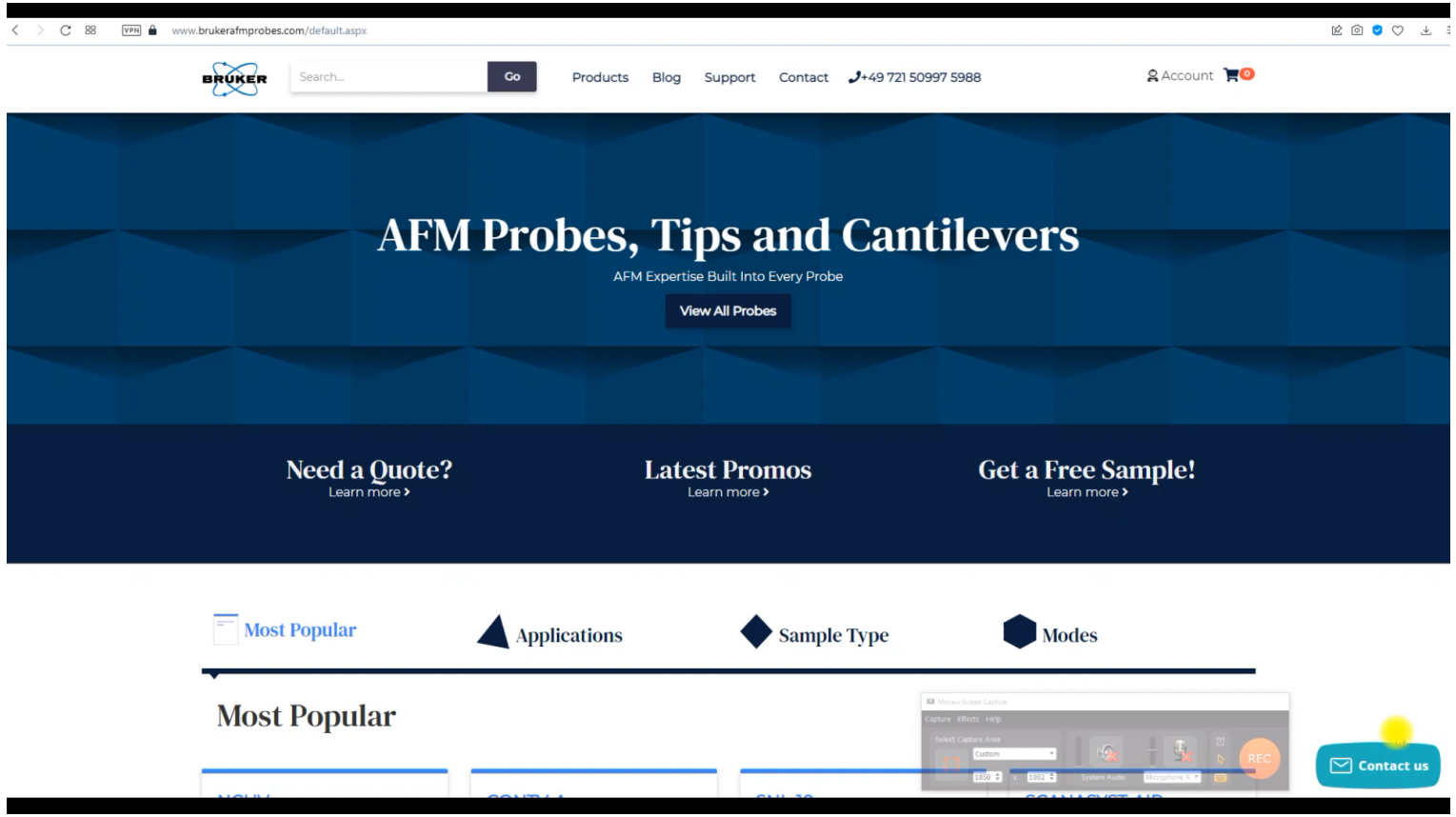
- Probe Suggestions in Probe Configuration

How to select a probe: Guidance from the AFM software



- Probe Suggestions in BioAFM probe database (version 8.0 to be released)

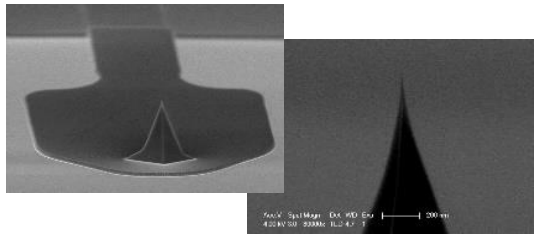
How to select a probe?
Probe selection on www.BrukerAFMProbes.com

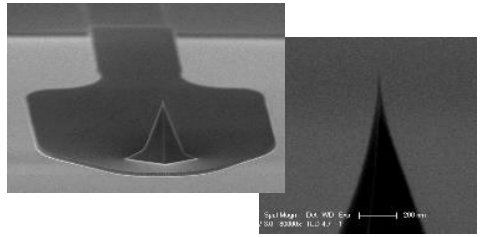


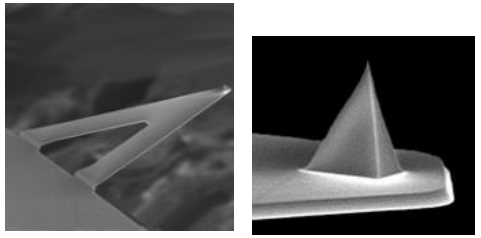
03 Recommended probes for bio applications

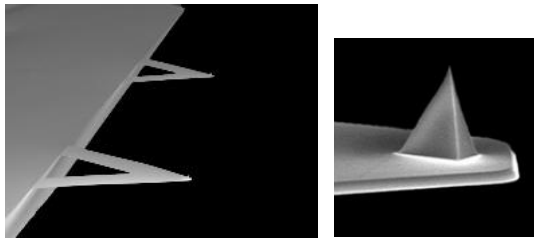
Bio Applications – recommended probes
High resolution imaging

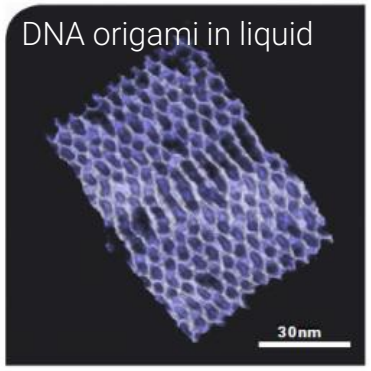
	PEAKFORCE-HIRS-F-A	PEAKFORCE-HIRS-F-B	ScanAsyst-Fluid+	SNL
Purpose	Extreme resolution, 2D crystals, DNA origami	Extreme resolution, single molecules	High resolution, general	High resolution, general
End radius	1-2 nm	1-2 nm	2-12 nm	2-12 nm
Spring constant	0.35 N/m	0.12 N/m	0.7 N/m	0.06-0.35 N/m
Mode	PFT/ScanAsyst/Tapping/QI	PFT/ScanAsyst/Tapping/QI/CM	PFT/ScanAsyst/Tapping/QI	PFT/ScanAsyst/Tapping/QI/CM

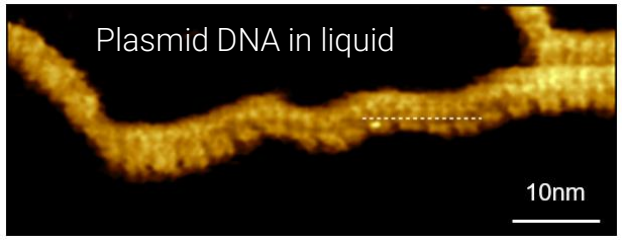


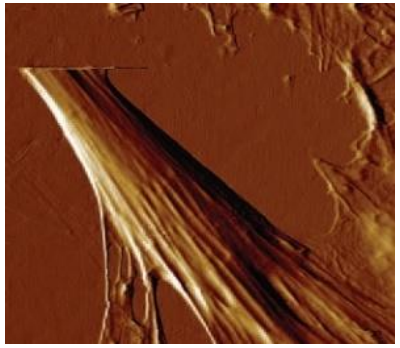






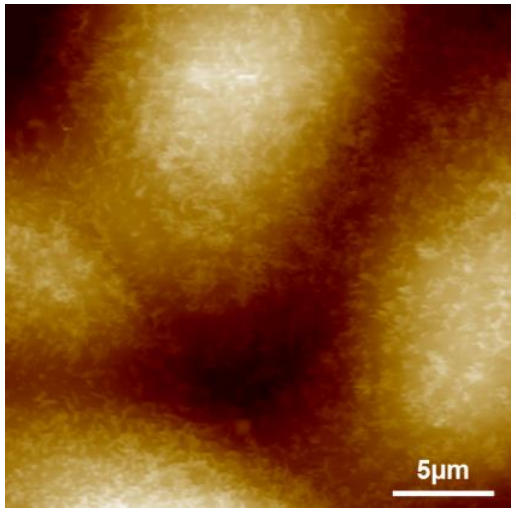




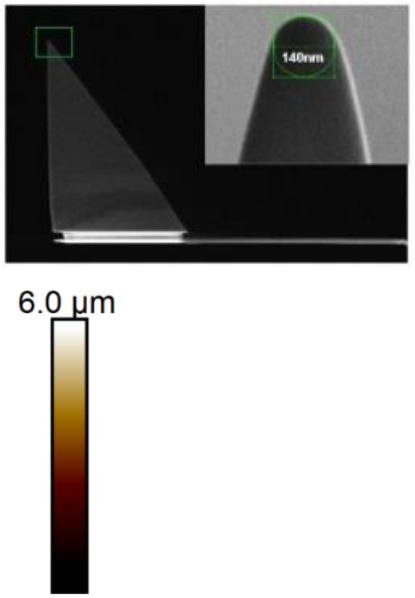


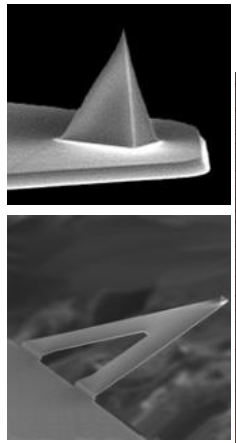
Bio Applications – recommended probes
Imaging cells

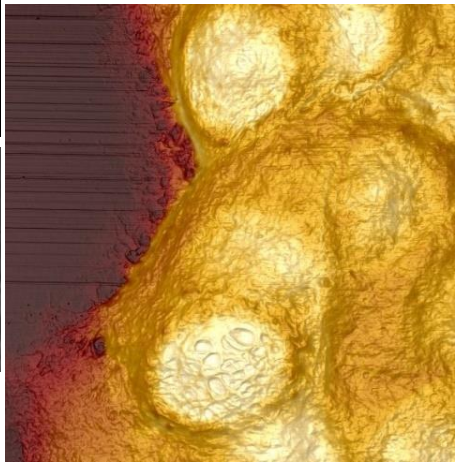
	PFQNM-LC-A-CAL	ScanAsyst-Fluid	MLCT-(BIO-DC)
Purpose	Living cells, bacteria	Fixed cells, yeasts, bacteria	Various cells
End radius	70 nm	20-60 nm	20-60 nm
Spring constant	0.1 N/m (pre-calibrated)	0.7 N/m	0.01-0.6 N/m
Mode	PFT/ScanAsyst/Tapping/QI	PFT/ScanAsyst/Tapping/QI	PFT/ScanAsyst/Tapping/Contact mode



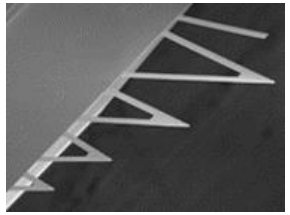
Living MDCK cells

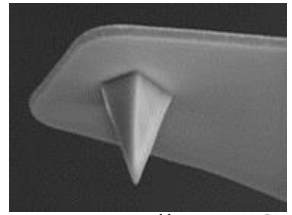




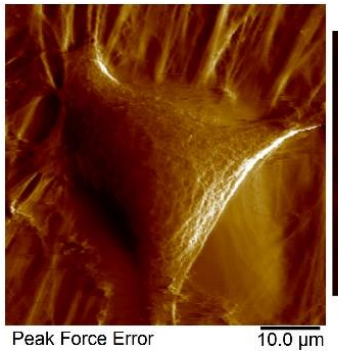


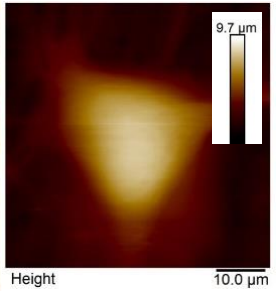
Fixed MCF7 cells





Live cell on ECM

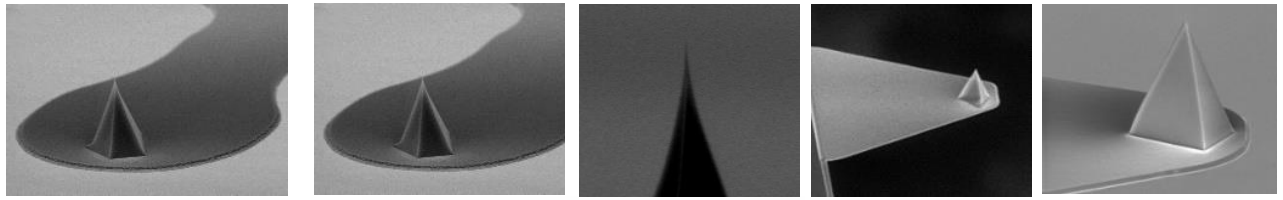


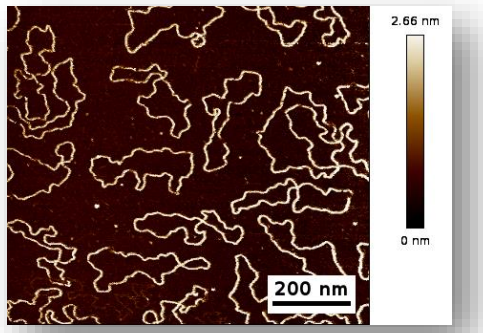


Bio Applications – recommended probes
High speed imaging

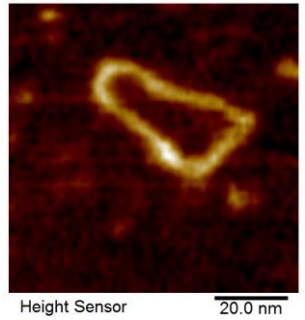
	FASTSCAN-D	FASTSCAN-D-SS	FASTSCAN-C	HIGHSPEED-FLUID
Purpose	Extreme resolution, 2D crystals, DNA origami	Extreme resolution, single molecules	High resolution, general	High resolution, 2D crystals, DNA origami
End radius	5-8 nm	1-2 nm	5-12 nm	4-12 nm
Spring constant, f	0.25 N/m, 110kHz liquid	0.25 N/m, 110kHz liquid	0.7 N/m, 70kHz liquid	0.35 N/m, 120kHz liquid
Mode	PFT/ScanAsyst/Tapping	PFT/ScanAsyst/Tapping	PFT/ScanAsyst/Tapping	PFT/ScanAsyst/Tapping

E. Coli bacteria





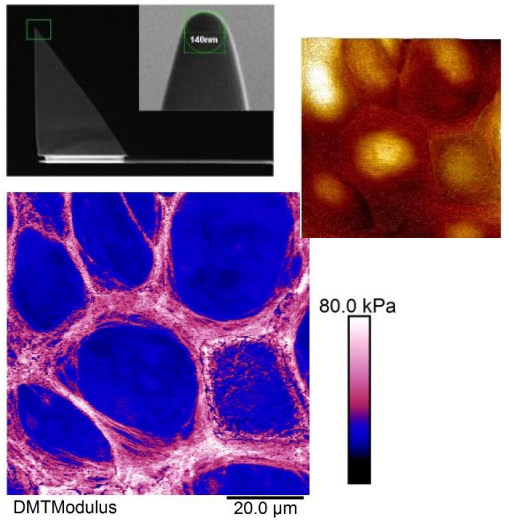
Plasmid DNA



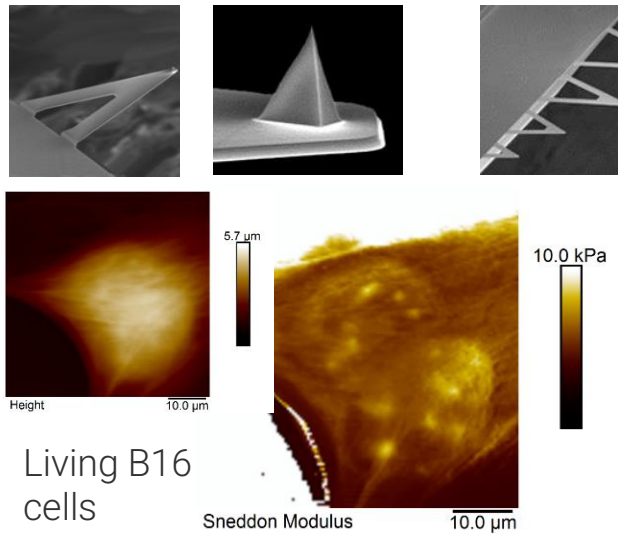
DNA mini circles. Data courtesy of Dr. Alice Pyne, University of Sheffield, UK

Bio Applications – recommended probes Mechanical mapping

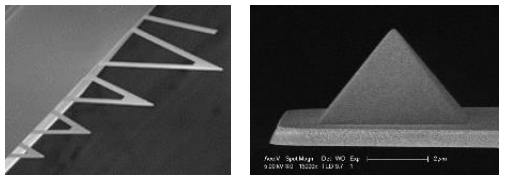
	PFQNM-LC-A-CAL	ScanAsyst-Fluid	MLCT-BIO-(DC)	SAA-SPH
Purpose	Living cells, hydrogels	Bacteria, fibrils	Living cells, hydrogels	Cells, tissues, hydrogels
End radius	70 nm	20-60 nm	20-60 nm	1, 5, 10 µm
Spring constant	0.1 N/m (pre-calibrated)	0.7 N/m	0.01-0.6 N/m	0.17 N/m (pre-calibrated)
Mode	QNM/QI/Force Volume/Force mapping/Force Curves	QNM/QI/Force Volume/Force mapping/Force Curves	QNM/QI/Force Volume/Force mapping/Force Curves	Force Volume/Force mapping/Force Curves

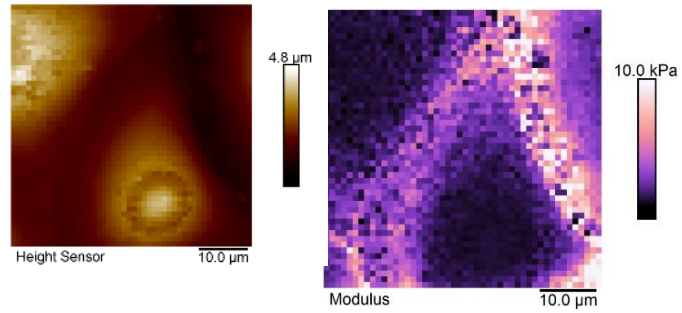


Living H431 cells



Living B16 cells

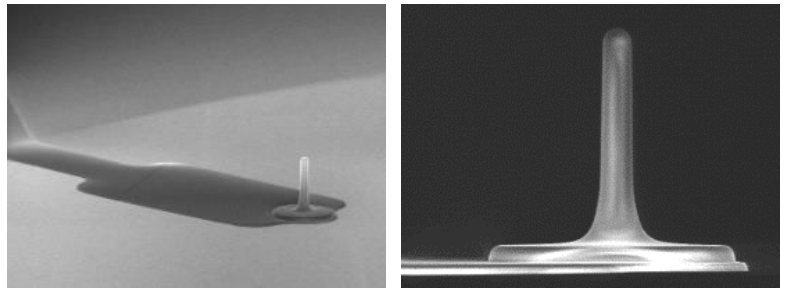




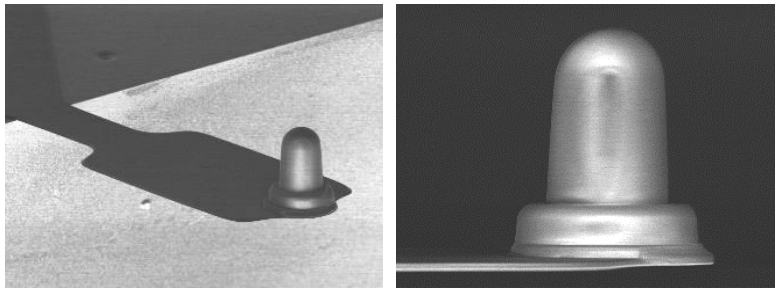
Living H431 cells

Hemispherical probes: Accurate mechanical and rheological measurements

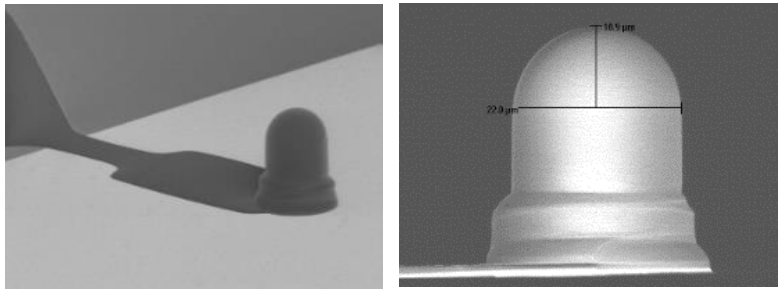
- SiN probe on SiN lever design
- No glue used
- Tall tip (19-28 µm) - minimal interference between cantilever and high roughness cellular or tissue samples
- Soft lever (k~0.25N/m) always comes LDV pre-calibrated
- Dedicated to very soft samples (sub-kPa to 100kPa)
- Different radii (R=1, 5, 10µm)
- Patented
- Available at www.brukerafmprobes.com



SAA-SPH-1UM



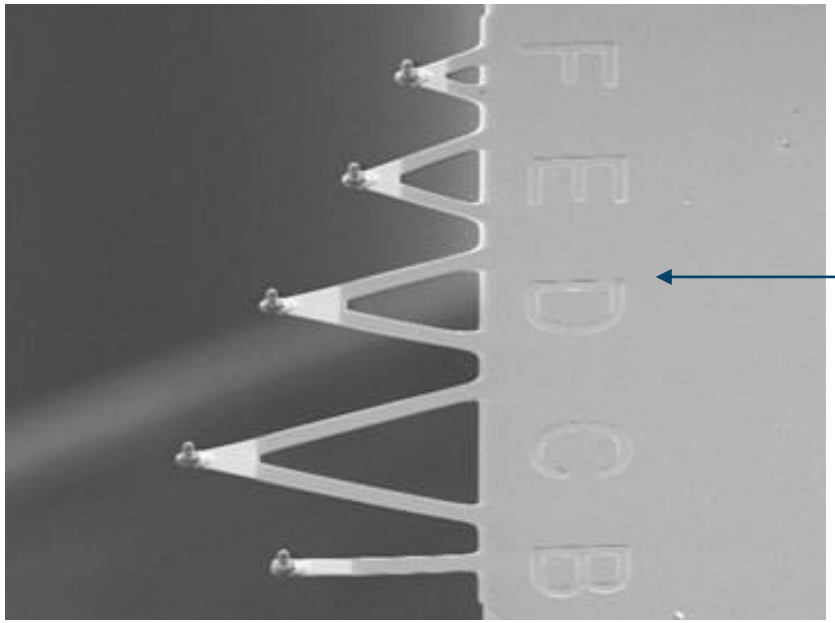
SAA-SPH-5UM

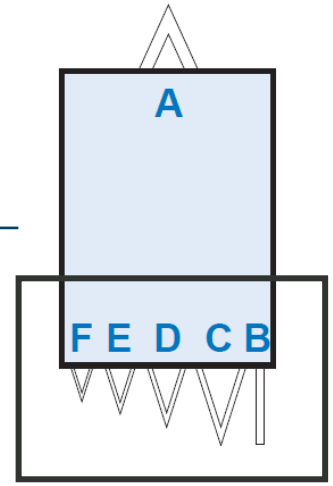


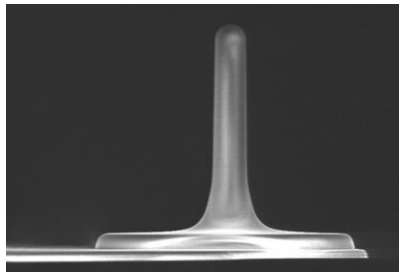
SAA-SPH-10UM

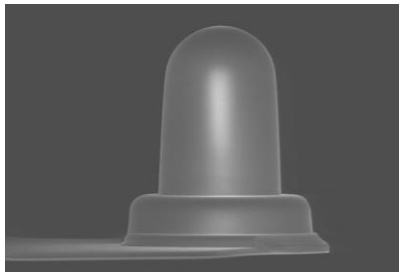
New MLCT-DC-SPH

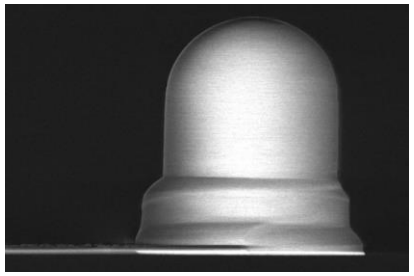
- For extremely soft samples
- 6 hemispherical probes on one chip!
- 6 different spring constants
- All levers LDV pre-calibrated
- Drift Compensated (DC) levers
- Different radii (R=1, 5, 10µm)
- Available at www.brukerafmprobes.com



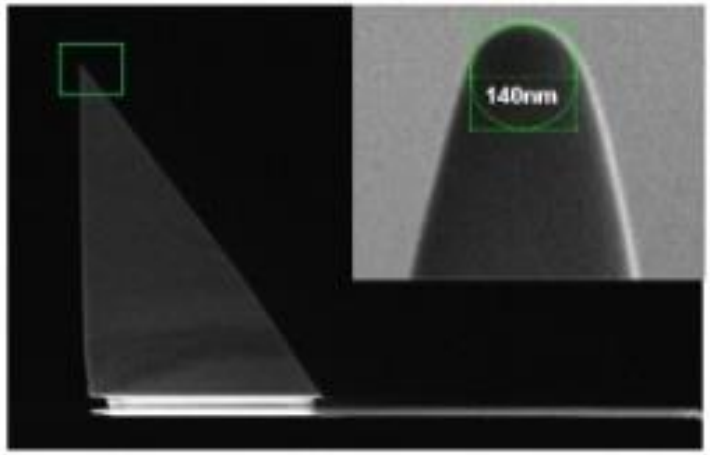




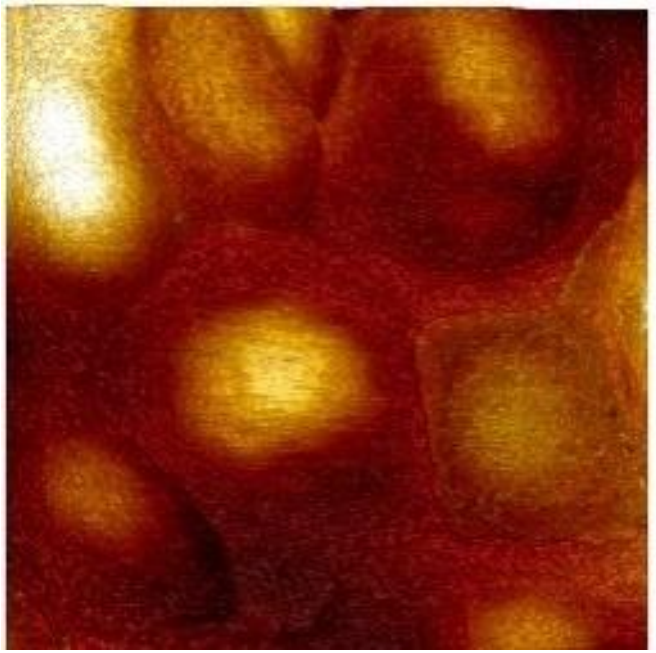




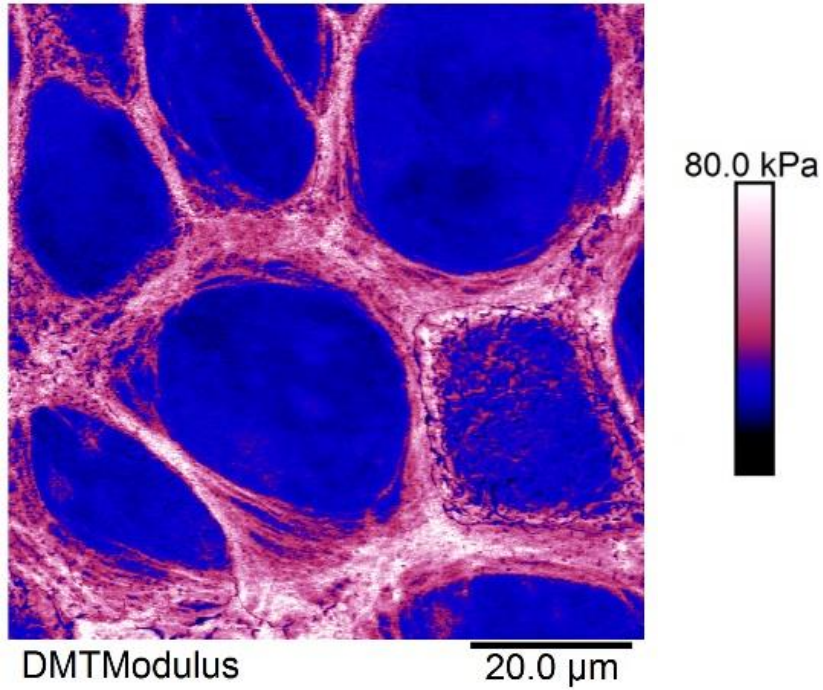
PFQNM-LC-A-CAL probe for cell imaging and mechanical mapping



PFQNM-LC-A-CAL probe



Height Sensor

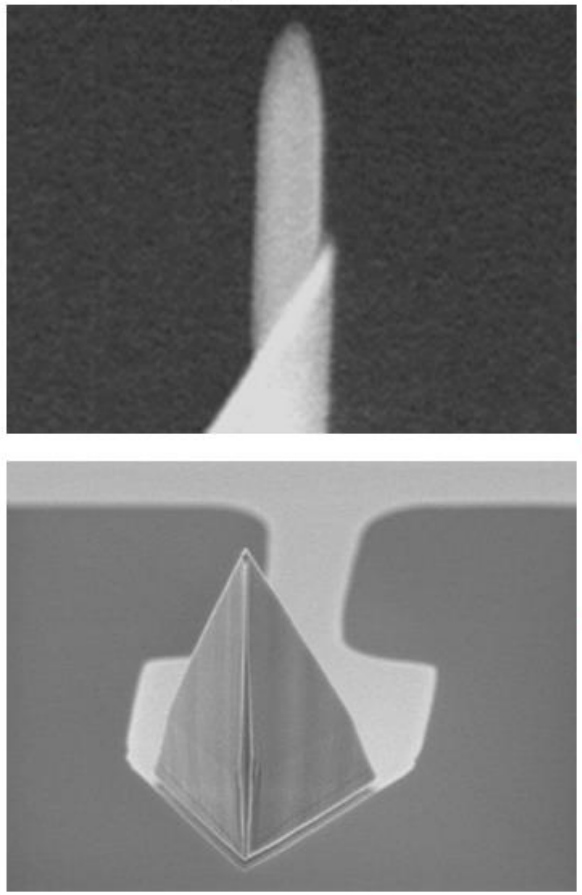


Living H431 cells

NOT at www.brukerafmprobes.com but available for order!

PFQNM-LC-version 2

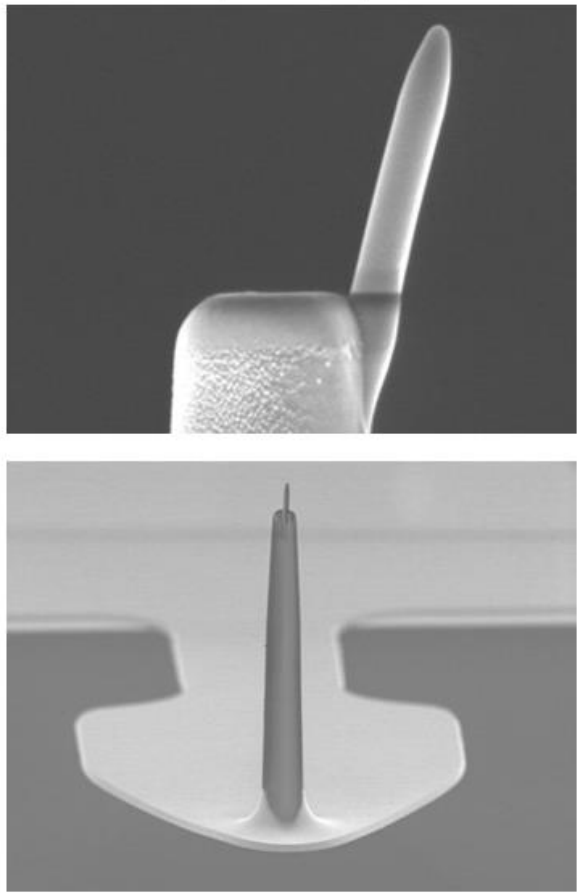
(Old)



PFQNM-LC-A-CAL

- Technically a Visible Tip Probe, but users still need to perform some alignment of tip-to-sample.
- Tip has large mass, resulting in lower Frequencies.
- Tip is not tilted, so it doesn't compensate for the tilt in holder.
- The fabrication process is very complicated, resulting in added cost and lower yields.

(New)

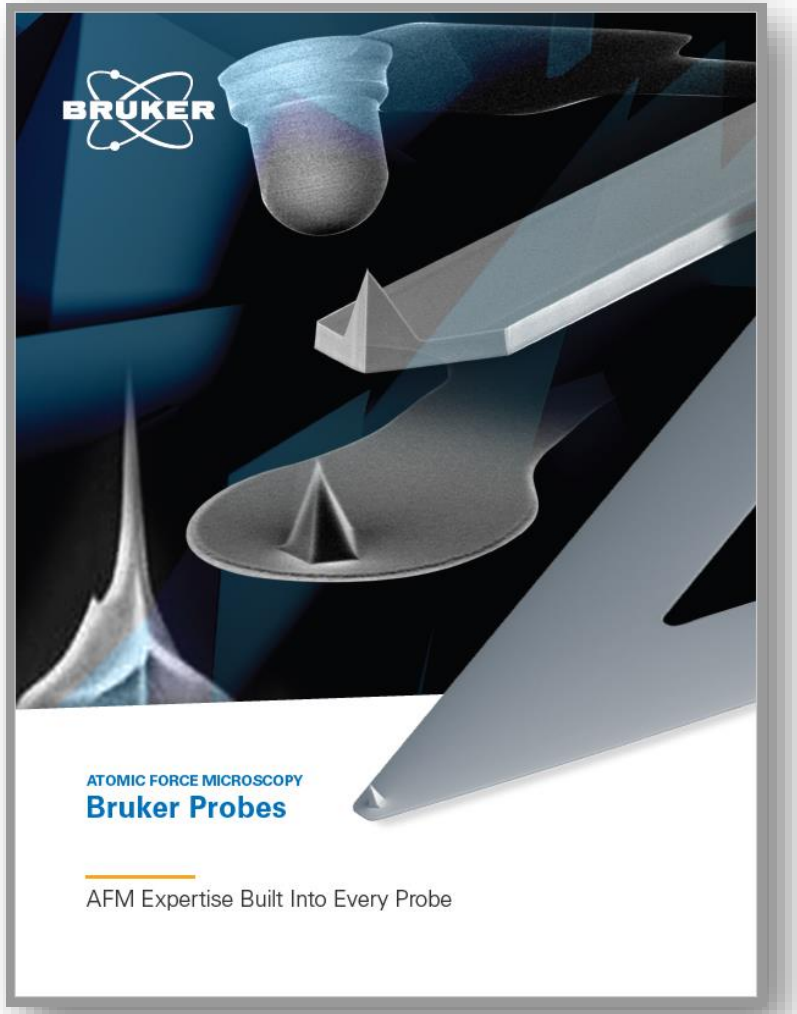


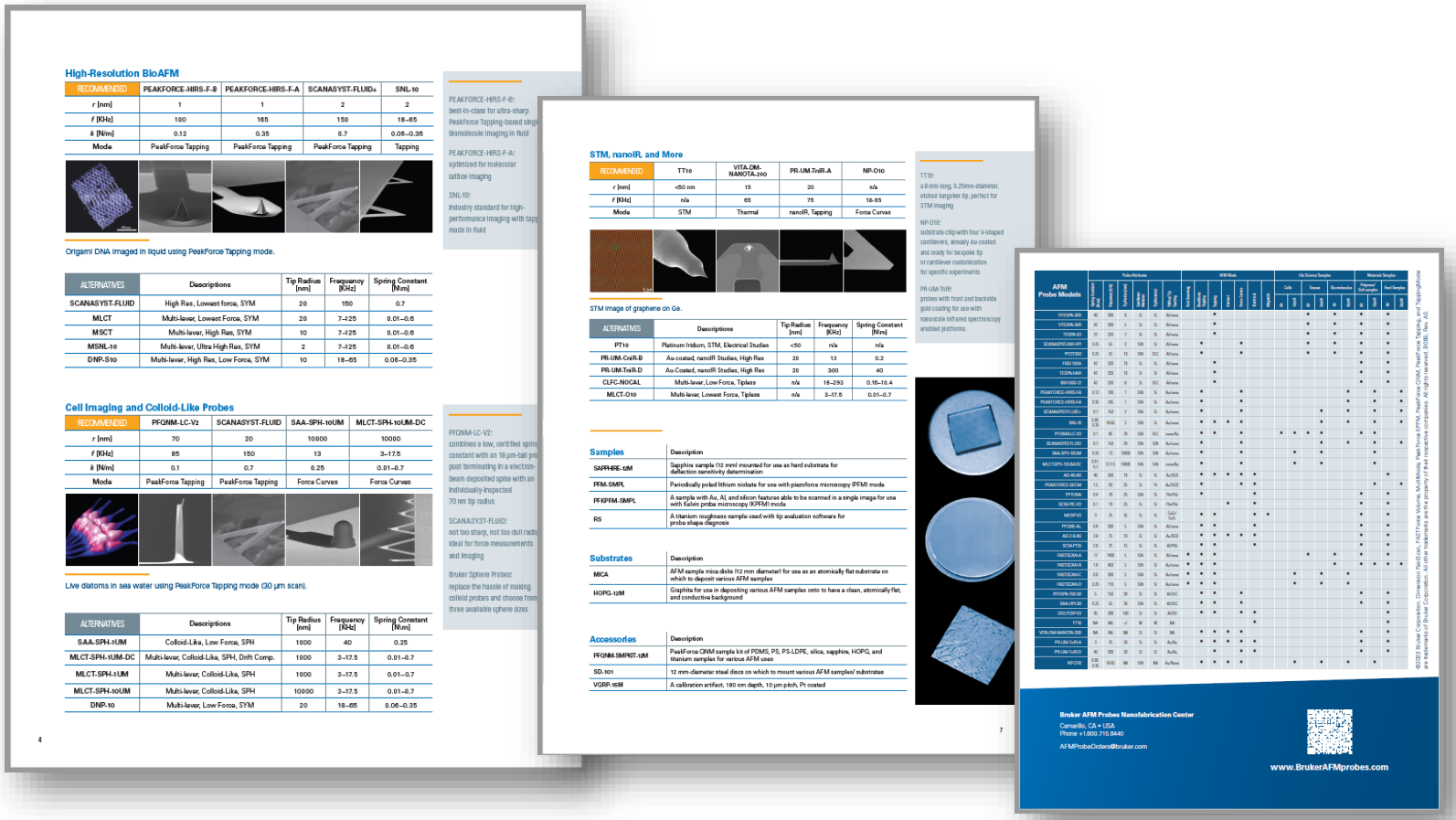
PFQNM-LC-V2

- Tip has very small mass, resulting in nearly 2X higher Frequency.
- Tip is tilted 11° to compensate for the 10° – 12° holder tilt.
- The fabrication process is very simple, and cuts fabrication time by 50%.
- Not a Visible Tip design, but Tip Set Back is minimal, averaging about 3μm.

NOT at www.brukerafmprobes.com but available for order!

2023 AFM Probes Selector Guide





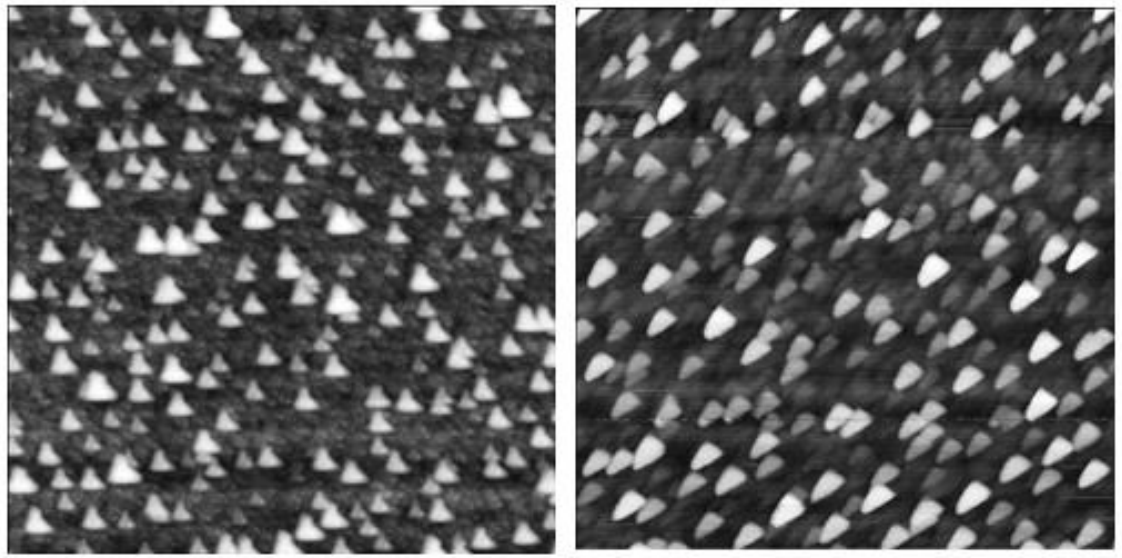
Available at www.brukerafmprobes.com

04 Probe artefacts, probe cleaning

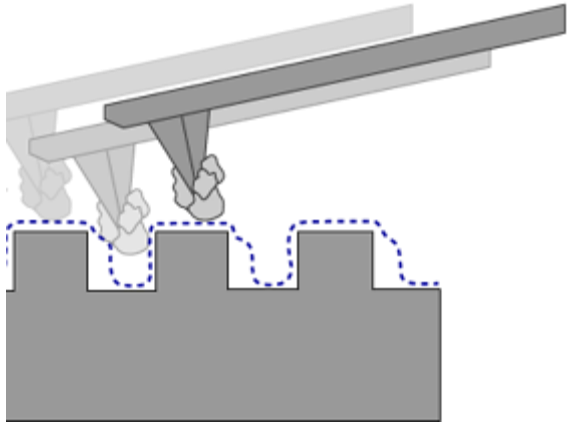
Tip artefacts

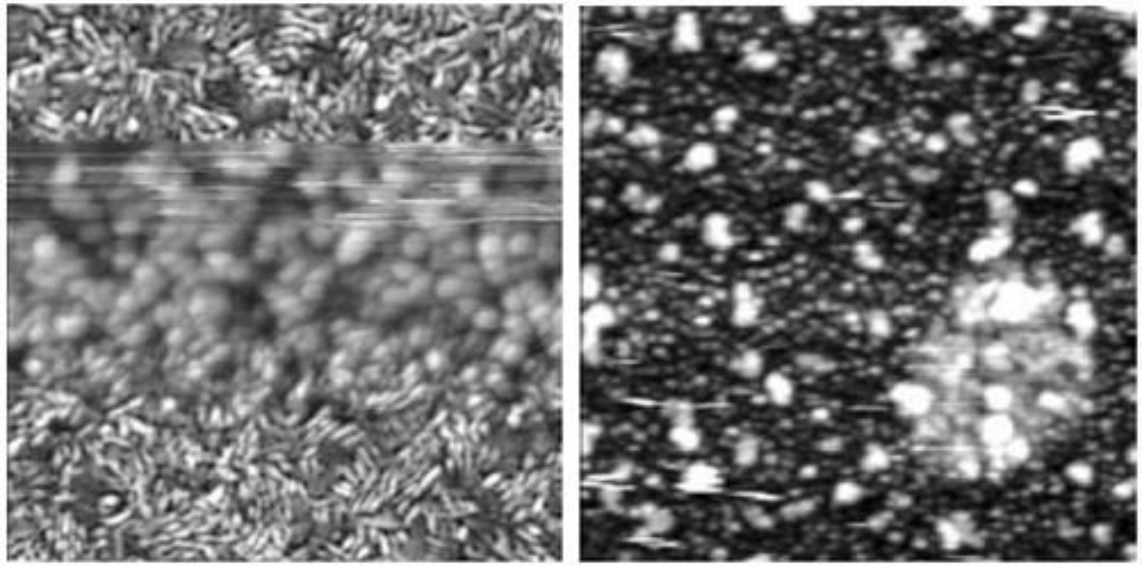
Double tip image of DNA

Tip artefacts



Dull or dirty tip

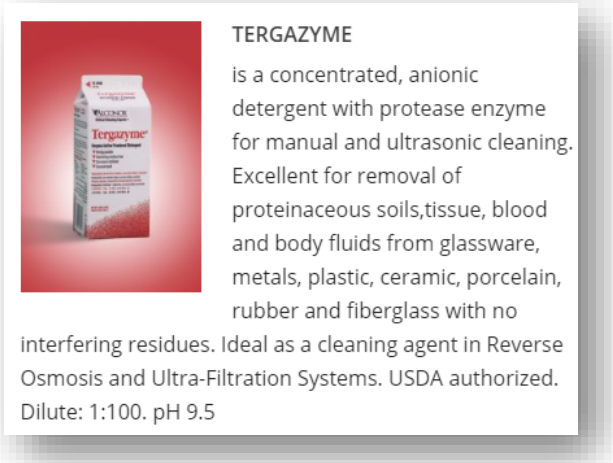




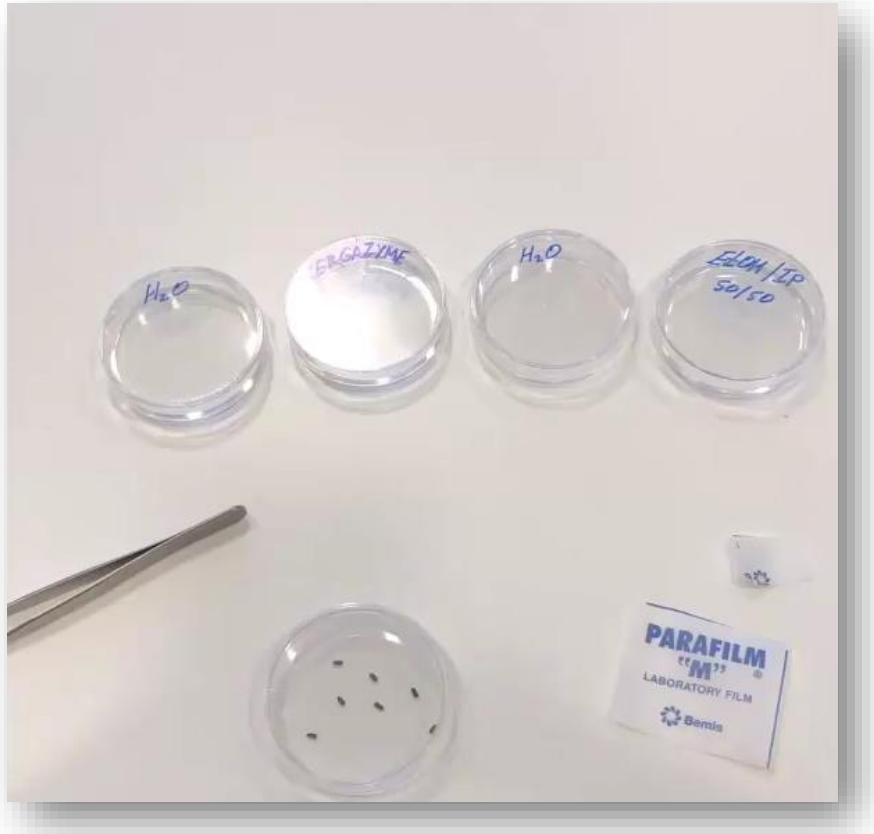
Tip contamination

Probes cleaning

- Yes, you can clean probes
- Yes, you can re-use probes
- Cleaning solutions: water, Tergazyme®, organic solvents (EtOH/IP), “piranha”, peroxide, etc.
- Physical treatments: UV/ozone, plasma

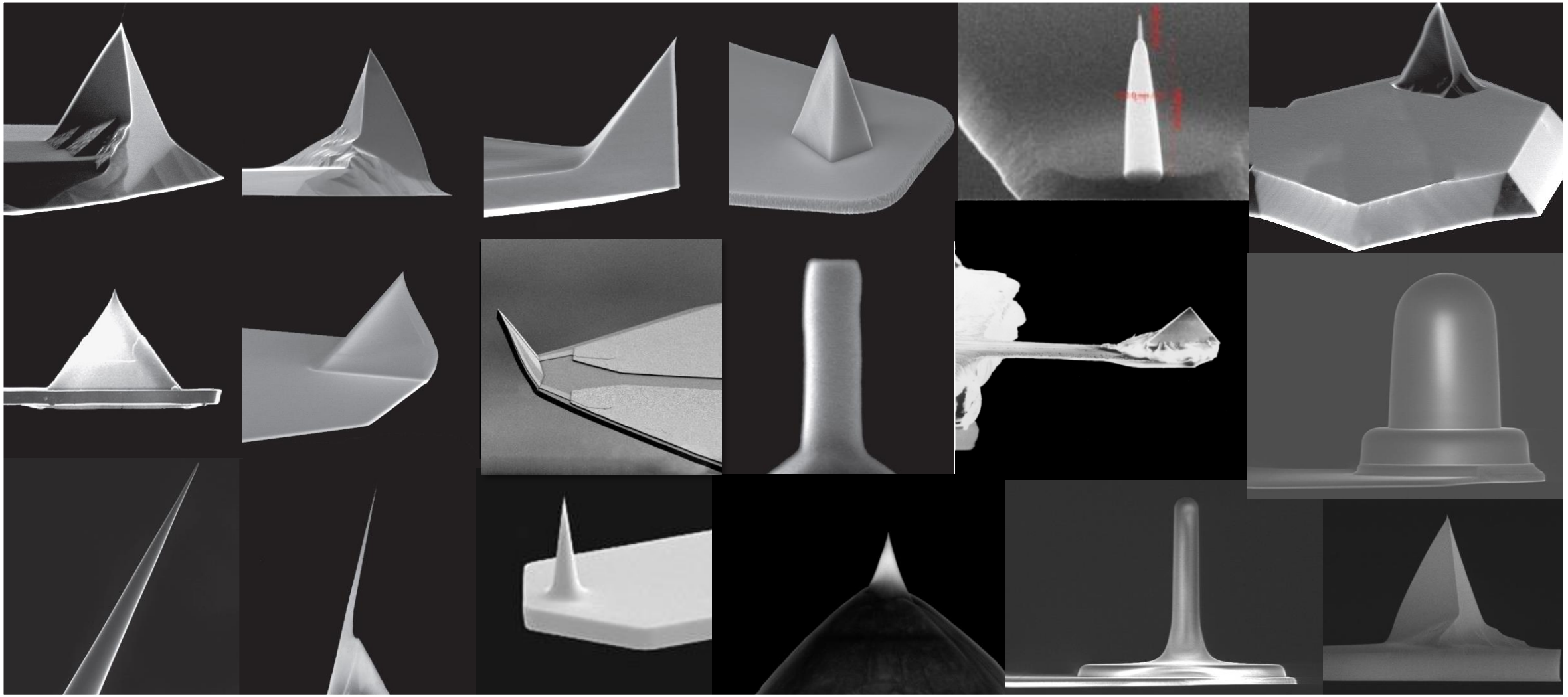


https://alconox.com/tergazyme

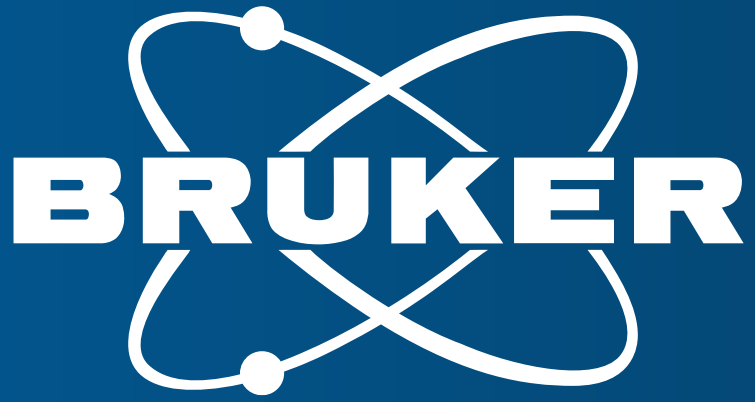


Example of cleaning procedure

AFM image is a combination between tip shape and sample topography!



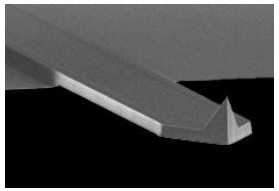
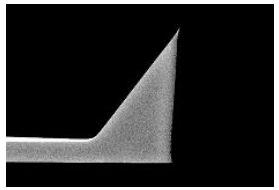
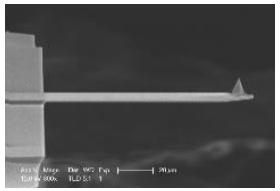
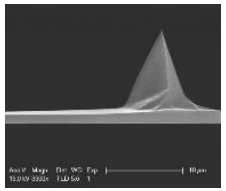
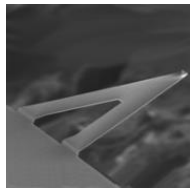
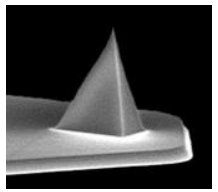
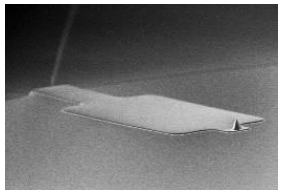
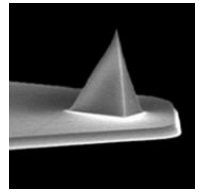
Thank you!

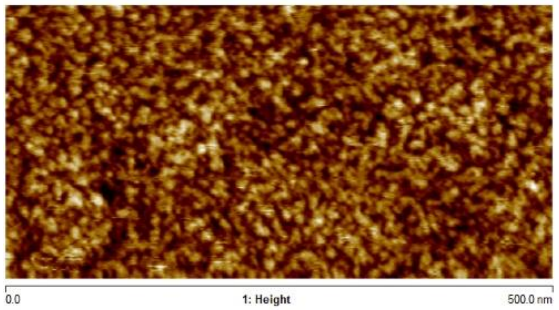


Innovation with Integrity

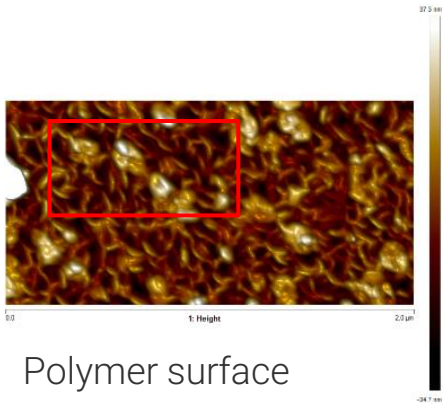
Material Science – recommended probes Topography

	VTESP-300	RTESP-150	ScanAsyst-Air	ScanAsyst-HPI
Purpose	General use	Mechanical phase contrast	General use	General use/laser interference reduction
End radius	5-12 nm	8-12 nm	2-12 nm	2-12 nm
Spring constant, f	40 N/m, 300kHz	5 N/m, 150kHz	0.4 N/m, 70kHz	0.25 N/m, 55kHz
Mode	Tapping	Tapping/QI	PFT/ScanAsyst/Contact/QI	PFT/ScanAsyst/Contact/QI

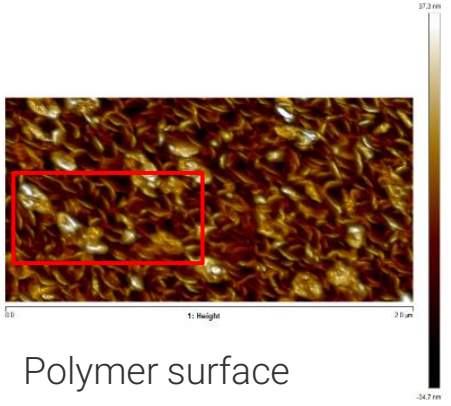











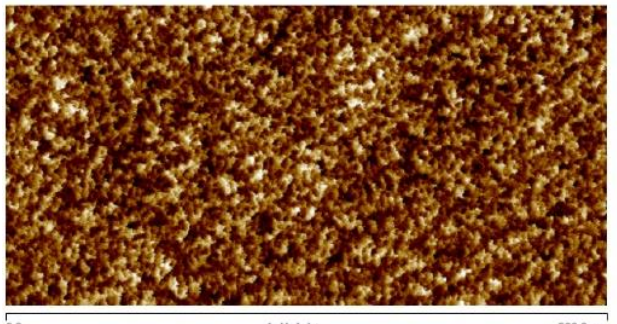
Silicon oxyde



Polymer surface



Polymer surface

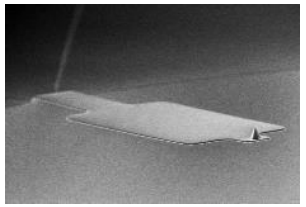
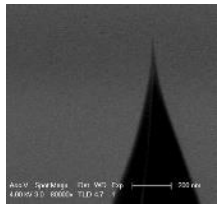
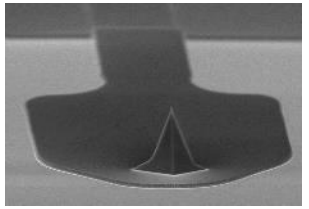
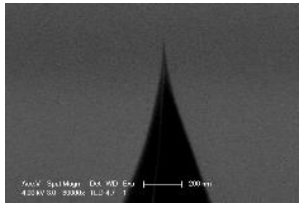


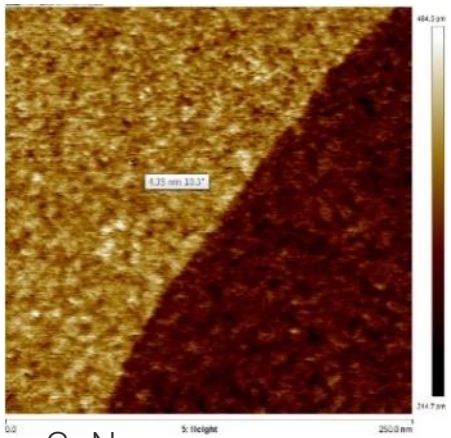
Silicon oxyde

Material Science – recommended probes
High resolution imaging

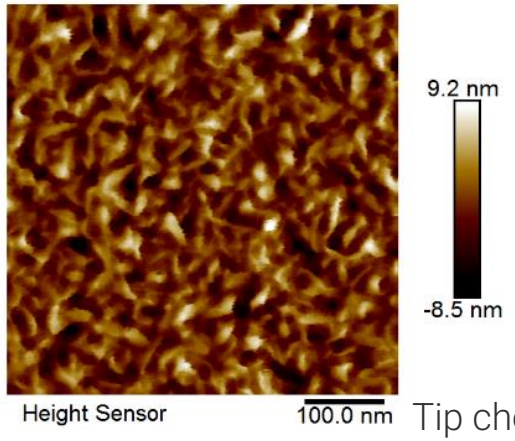
	TESP-SS	SAA-HPI-SS	PEAKFORCE-HIRS-SSB
Purpose	Extreme resolution	Extreme resolution-widest samples range	Extreme resolution-delicate samples
End radius	2-5 nm	1-2 nm	1-2 nm
Spring constant, f	40 N/m, 300kHz	0.25 N/m, 55kHz	0.12 N/m, 100kHz
Mode	Tapping	PFT/ScanAsyst/QI	PFT/ScanAsyst/QI



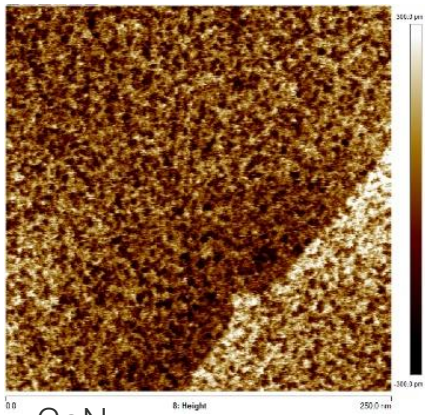







GaN



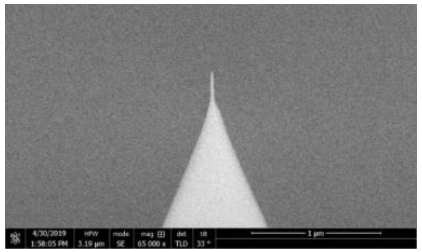
Tip check

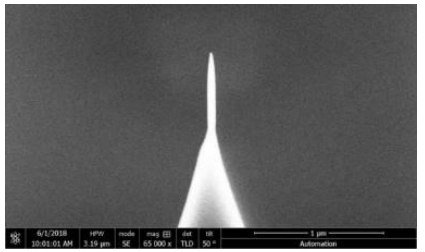


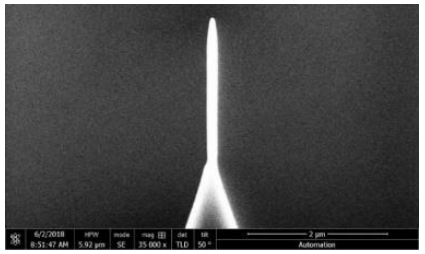
GaN

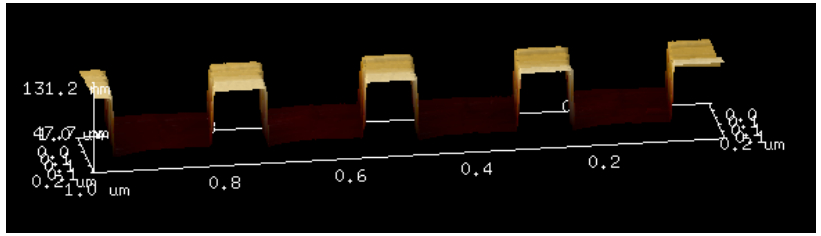
Material Science – recommended probes
Trenches and lines

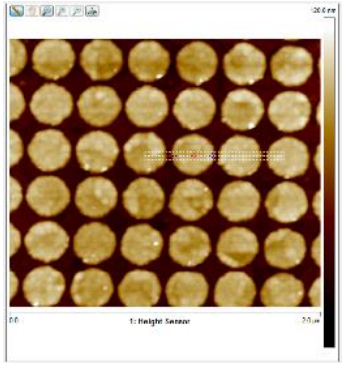
	FIB series	PFDT350	PFDT750	PFDT2500
Spike length	1-7µm	350 nm	750 nm	2500 nm
Spike base width	100-400nm	30 nm	65 nm	150 nm
End radius	10 nm	10 nm	10 nm	30 nm
Mode	Tapping	PFT/ScanAsyst/QI	PFT/ScanAsyst/QI	PFT/ScanAsyst/QI

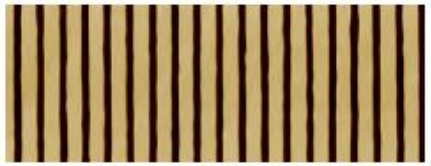










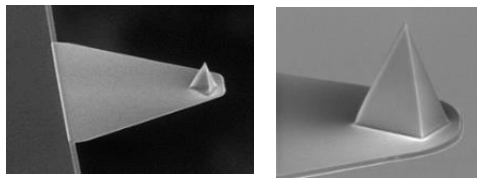


Period 190-200nm
Depth 38-100nm
Top surface 120nm
Bottom 40-80nm

Period ~600nm
Depth 700nm
Top ~120nm
Bottom ~330nm

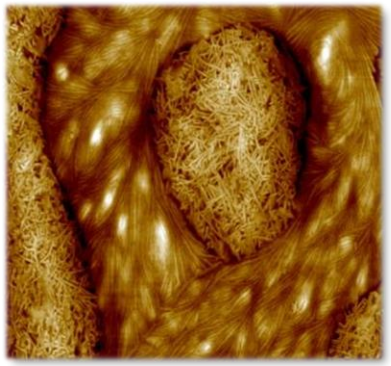
Material Science – recommended probes
High speed imaging

	FastScan-A	FastScan-B	FastScan-C	ScanAsyst-AIR-HR
Purpose	High speed, general use	High Speed, phase contrast	High speed, general use	High speed, general use
End radius	5-12 nm	5-12 nm	5-12 nm	2-12 nm
Spring constant, f	18 N/m, 1400kHz	1.8 N/m, 450kHz	0.8 N/m, 300kHz	0.4 N/m, 130kHz
Mode	Tapping	Tapping	PFT/ScanAsyst/QI	PFT/ScanAsyst/QI

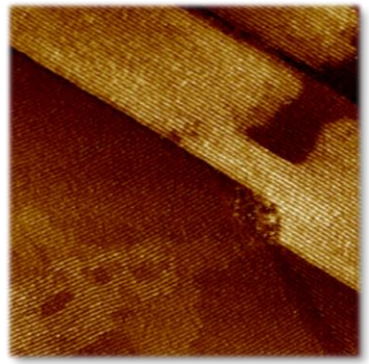


PHBV crystallization (sample courtesy of Dr. Jamie Hobbs)

Polymer melting



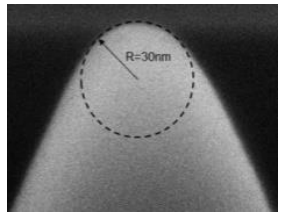
Syndiotatic Polymer

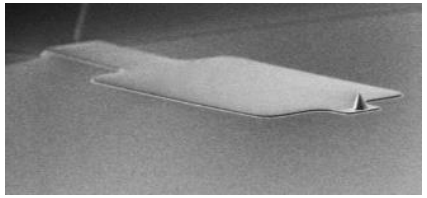


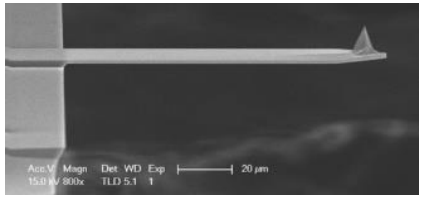
Alkane

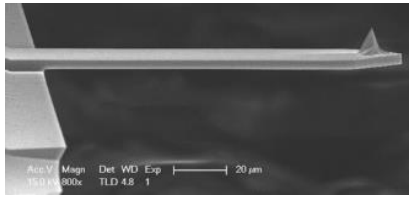
Material Science – recommended probes
Force curves based mechanical measurements

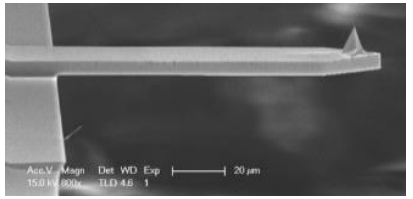
	SAA-HPI-30	RTESPA-150-30	RTESPA-300-30	RTESPA-525-30
Modulus range	100kPa-15MPa	10-500MPa	300MPa-10GPa	8GPa-100GPa
End radius	30 nm	30 nm	30 nm	30 nm
Spring constant, f	0.25 N/m, 55kHz	5 N/m, 150kHz	40 N/m, 300kHz	200 N/m, 525kHz
Mode	QNM/QI/Force Volume/Force Curves	QNM/QI/Force Volume/Force Curves	QNM/QI/Force Volume/Force Curves	QNM/QI/Force Volume/Force Curves

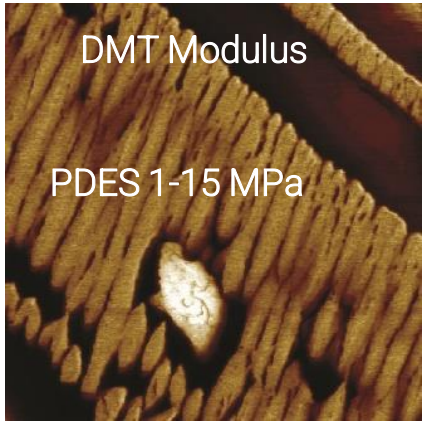


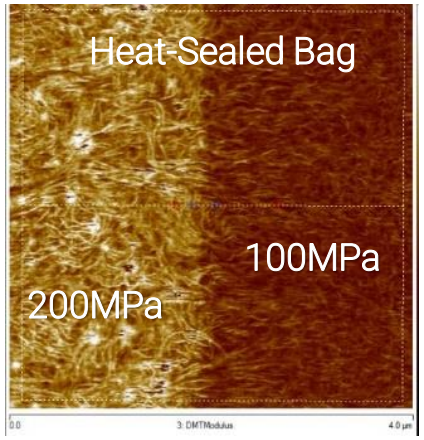


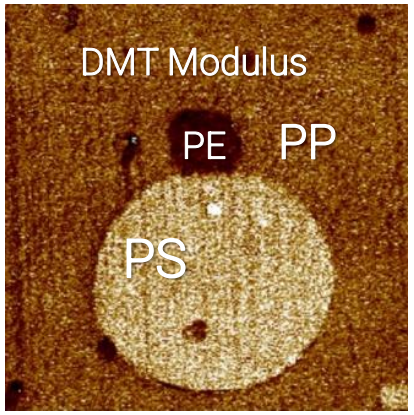


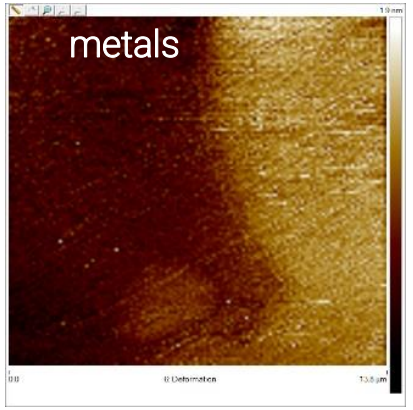










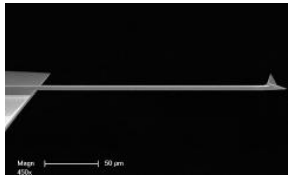


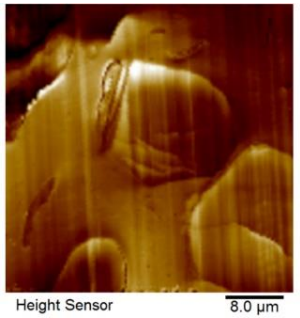
Material Science – recommended probes
Resonance based mechanical measurements

	DDLTESP-V2	DDESP-FM-V2	DDRFESPA40
Modulus range	3-300 GPa	0.5-50 GPa	0.1-10 GPa 10-200 GPa Mode2
End radius	100 nm	100 nm	100 nm
Spring constant	95 N/m, 280 kHz	6 N/m, 105 kHz	2 N/m, 60 kHz
Mode	Contact resonance	Contact resonance	Contact resonance

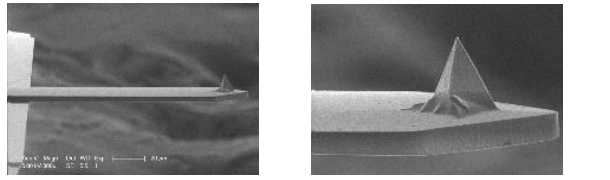
Contact Resonance module for Icon AFM

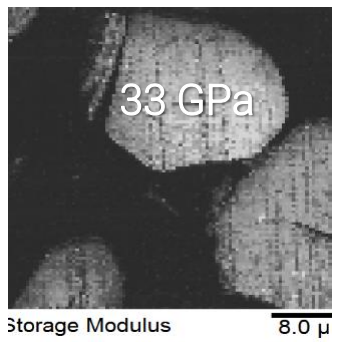
Contact Resonance module for NanoWizard AFM

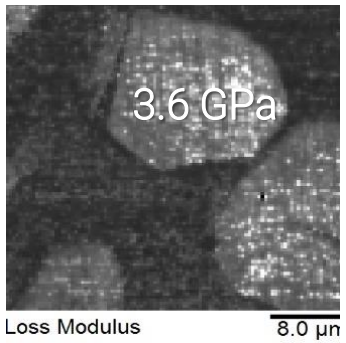


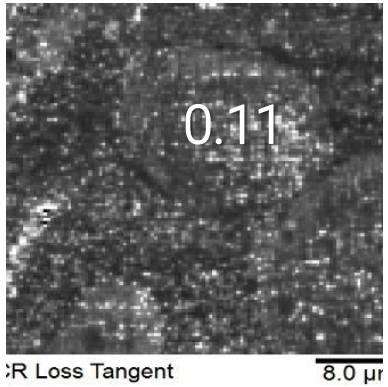


Fiber epoxy blend



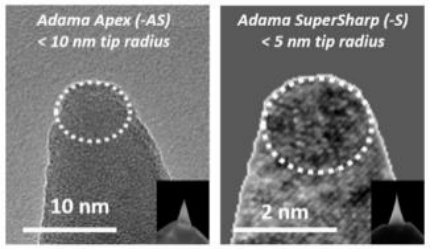


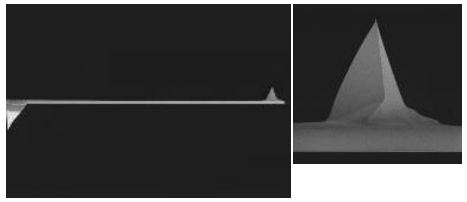


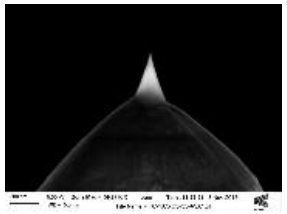


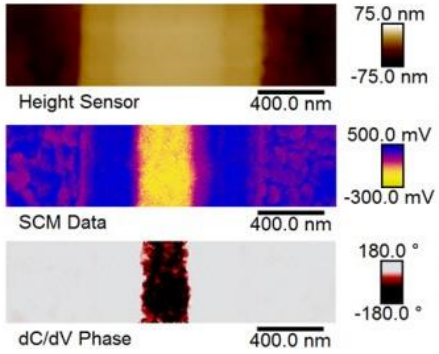
Material Science – recommended probes
Nanoelectrical contact mode based modes

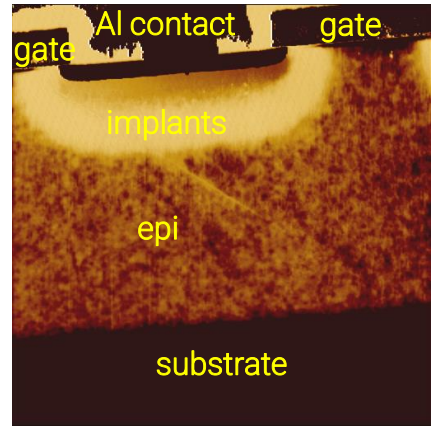
	SCM-PIT-V2	AD-0.5	AD-2.8	AD-40
Purpose	General nanoelectrical	High resolution, tip lifetime, soft sample	High resolution, tip lifetime	High resolution, tip lifetime
End radius	25 nm	10 nm	10 nm	10 nm
Spring constant, f	3 N/m, 75 kHz	0.5 N/m, 30 kHz	2.8 N/m, 65 kHz	40 N/m, 300 kHz
Mode	C-AFM, TUNA, PFM, SCM, SSRM	C-AFM, TUNA, PFM	C-AFM, TUNA, PFM, SCM	TUNA, SSRM

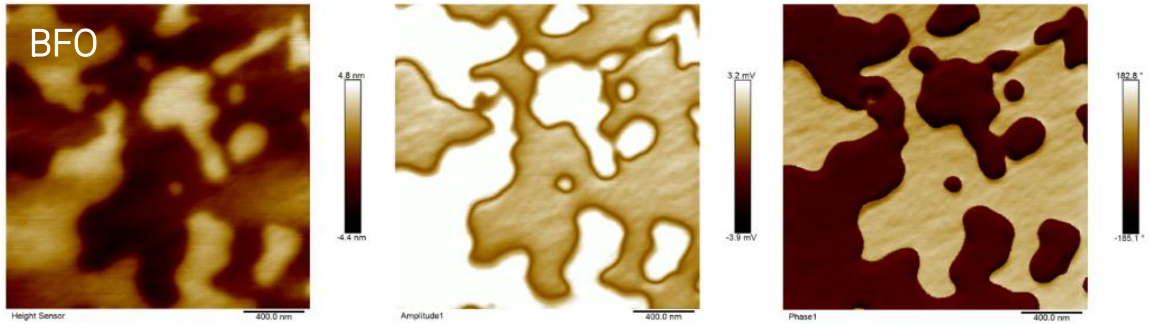






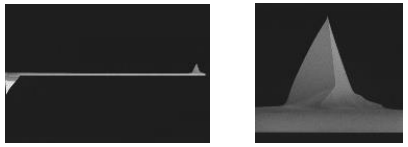
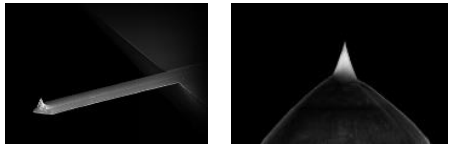
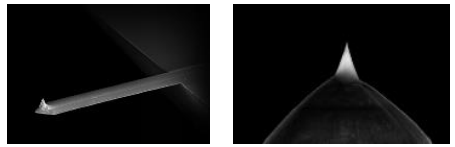
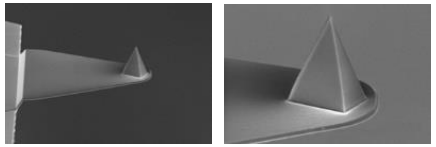


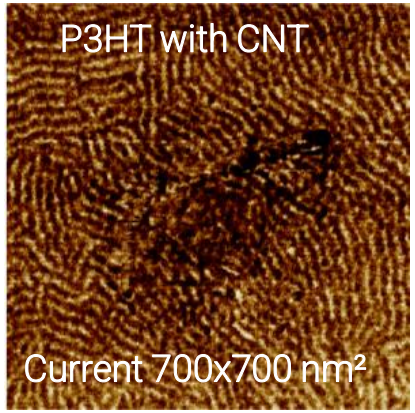


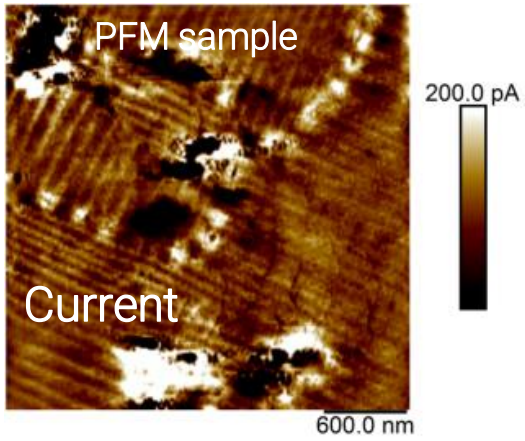


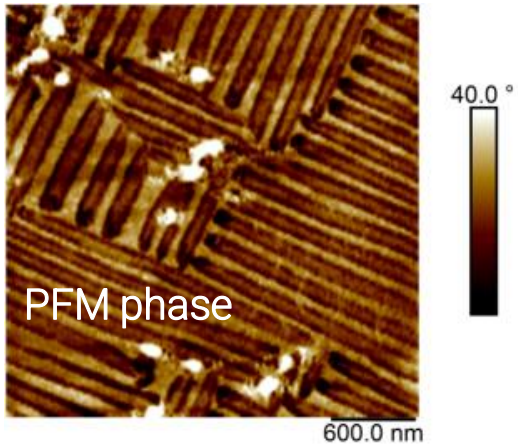
Material Science – recommended probes
Nanoelectrical intermittent-contact based modes

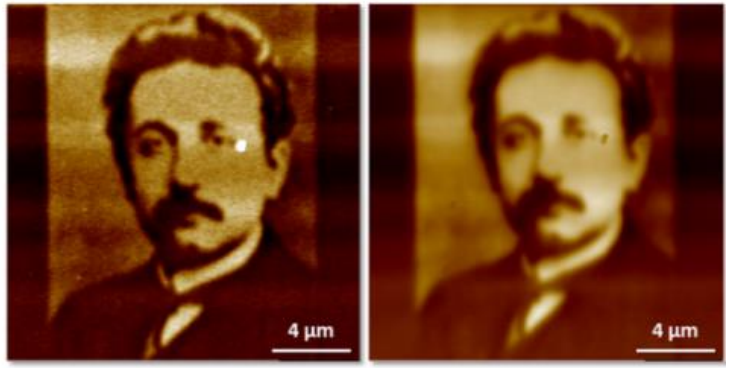
	PF-TUNA	AD-0,5	AD-2,8	PFQNE-AL
Purpose	General nanoelectrical	High resolution, tip lifetime, soft sample	High resolution, tip lifetime	High resolution, tip accuracy
End radius	25 nm	10 nm	10 nm	30 nm
Spring constant, f	3 N/m, 75 kHz	0.5 N/m, 30 kHz	2.8 N/m, 65 kHz	0.4 N/m, 300 kHz
Mode	C-AFM, TUNA, PFM, SCM, SSRM	C-AFM, TUNA, PFM	C-AFM, TUNA, PFM, SCM, SSRM	Surface potential (KPFM)



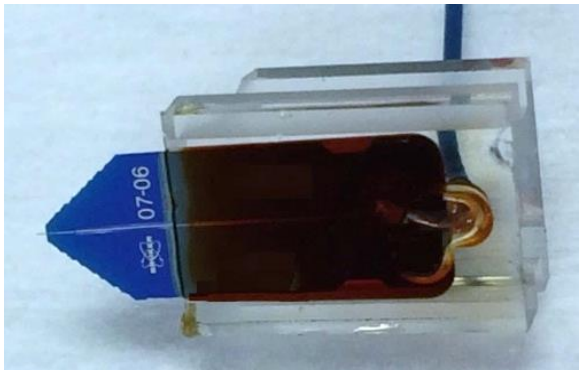


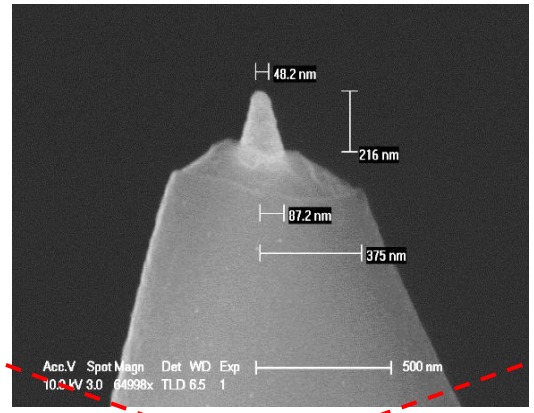


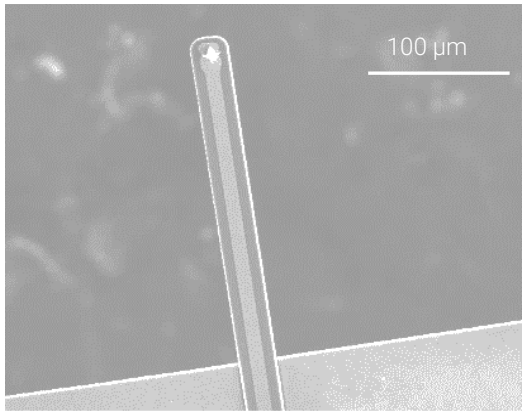


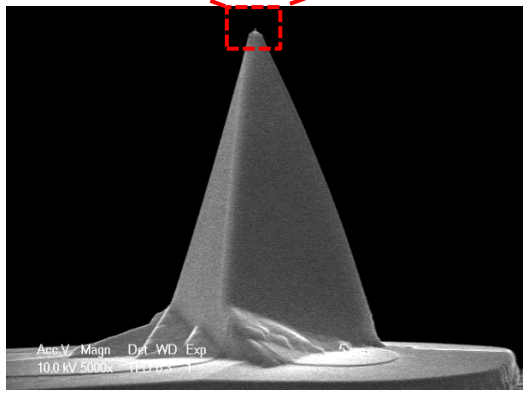
Material Science – recommended probes Electrochemical characterization

- Probe
  - Exposed tip height: ~ 200 nm
  - End tip diameter: ~ 50nm
  - Exposed tip material: Platinum
  - Passivation: silicon dioxide
  - Conducting path technique
- Package
  - Fully isolated
  - Encapsulated in two parts glass
  - Easy to handle package
  - Chemical resistant epoxy



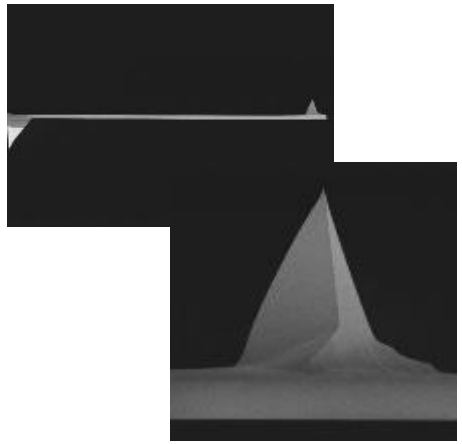


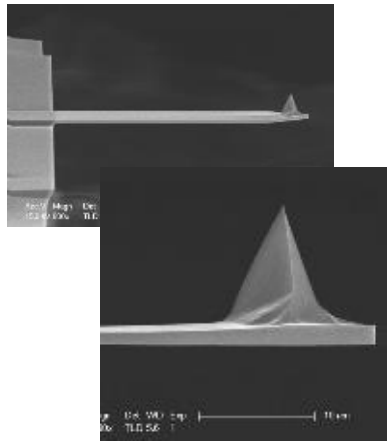


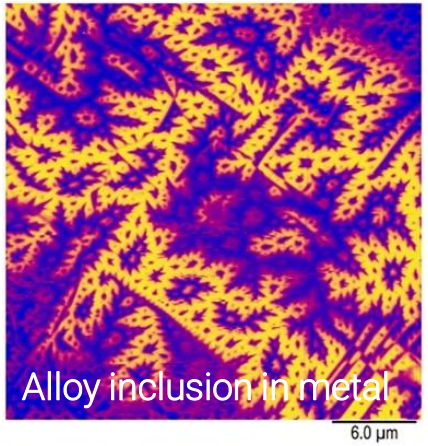


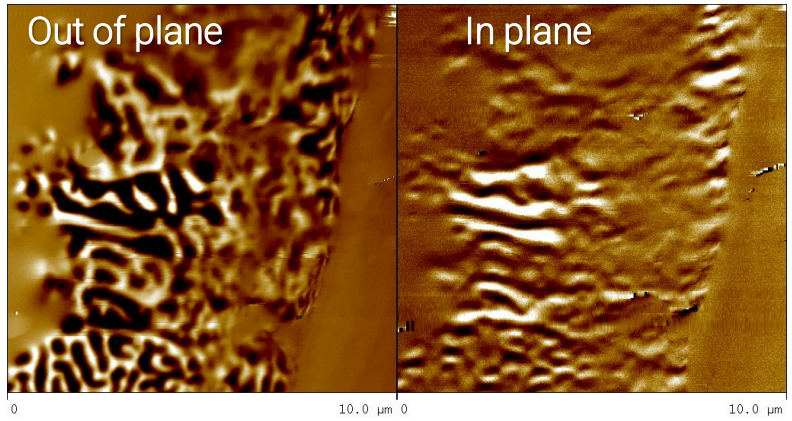
Material Science – recommended probes
Magnetic characterization

	MESP-V2	MESP-RC-V2	MESP-LM-V2	MESP-HM-V2	MESP-LC-V2
Purpose	General MFM	Higher sensitivity	avoid modification/motion of magnetic walls	High Moment	image low magnetized materials
End radius	35 nm	35 nm	35 nm	35 nm	35 nm
Moment/coercivity	400 Oe/1e-13 EMU	400 Oe/1e-13 EMU	<400 Oe/0,3e-13 EMU	400 Oe/3e-13 EMU	<10 Oe/<1e-13 EMU
Spring constant, f	3 N/m, 75 kHz	5 N/m, 150 kHz	3N/m, 75 kHz	3 N/m, 75 kHz	3 N/m, 75 kHz









Material Science – recommended probes
Thermal characterization

	VITA-Nano-TA-200	VITA-Nano-TA-300	VITA-GLA-1
Purpose	Nano Thermal Analysis	Nano Thermal Analysis	Scanning Thermal Microscopy (SThM)
End radius	<30 nm	<30 nm	<100 nm
Spring constant, f	2 N/m, 65 kHz	0.3 N/m, 20 kHz	0.5 N/m, 50 kHz