CEITEC
Central European Institute of Technology
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MUNI

AFM Data processing

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CIISB
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MŠMT

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měřicí technika morava

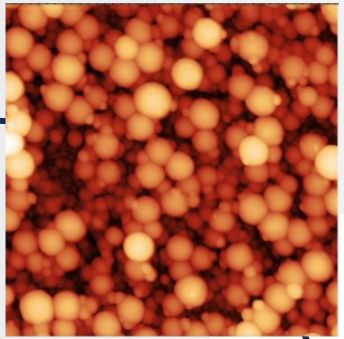
Summer workshop on BioAFM microscopy 2023

13.09.2023

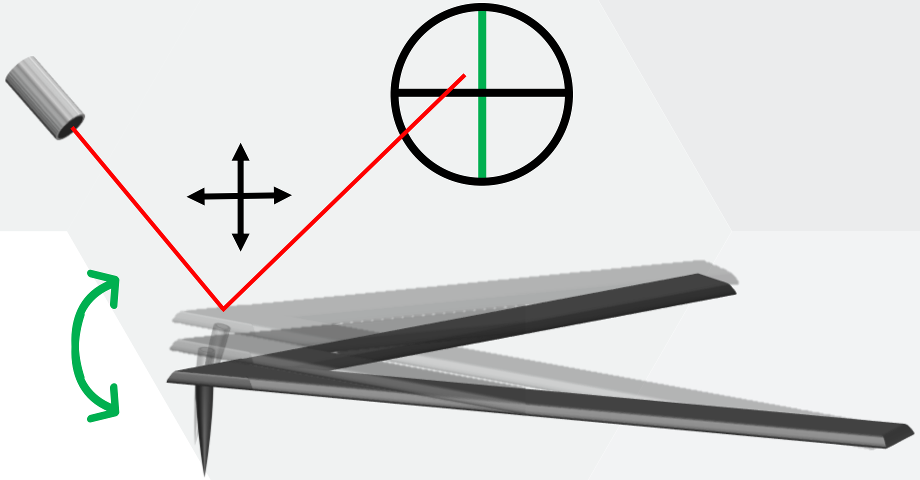
Layout of the presentation

- Types of AFM data
- Imaging
  - AFM images – leveling data, artefacts, surface reconstruction, masks, analysis, LAFM
  - Real time scans of vertical deflection – contraction properties of CMs, peaks detection, HRV analysis
- Force spectroscopy
  - What is a force distance curve (FDC)?
  - Young`s modulus – models, analysis
  - Alternative analysis of FDCs – SMFS, thickness of lipid bilayers, rupture events
- Indentation
  - Rheology analysis, viscoelasticity

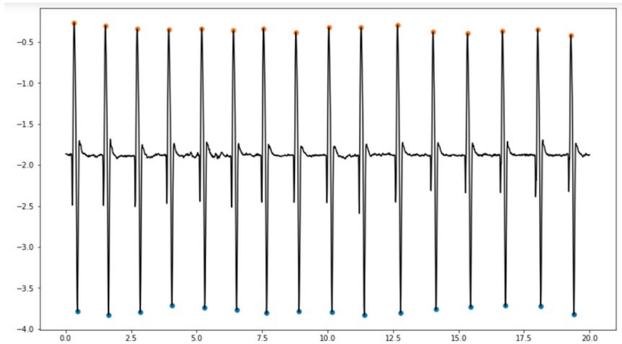
Types of AFM data



AFM Images

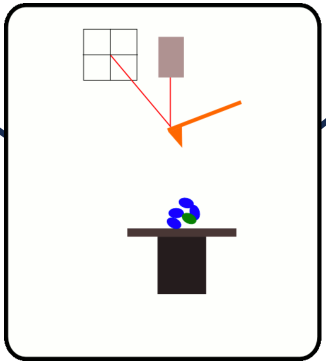


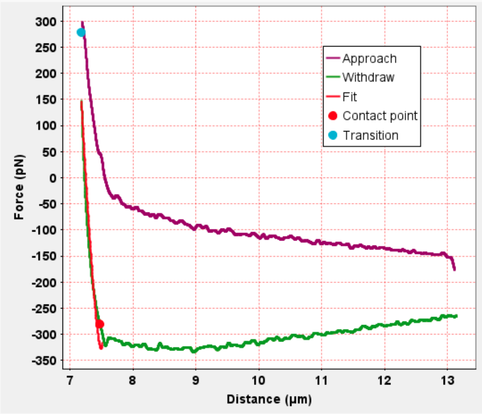
Monitoring
of vertical
deflection



Force mapping,
Peakforce QNM,
QI etc.

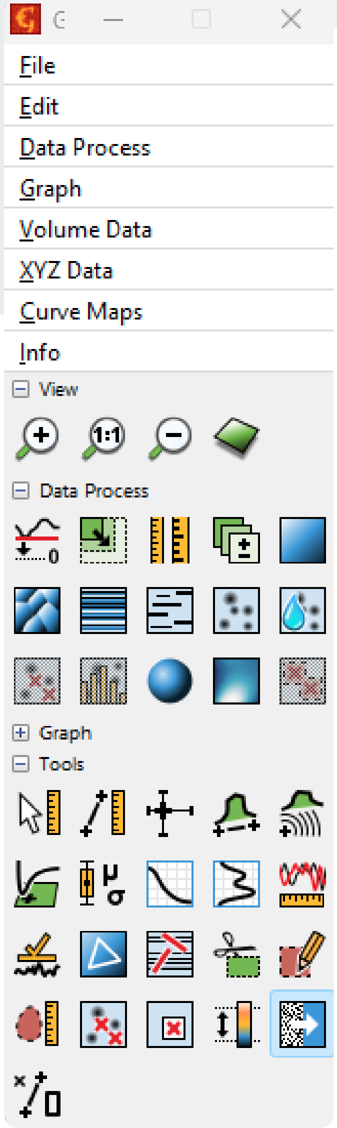
AFM
Spectroscopy

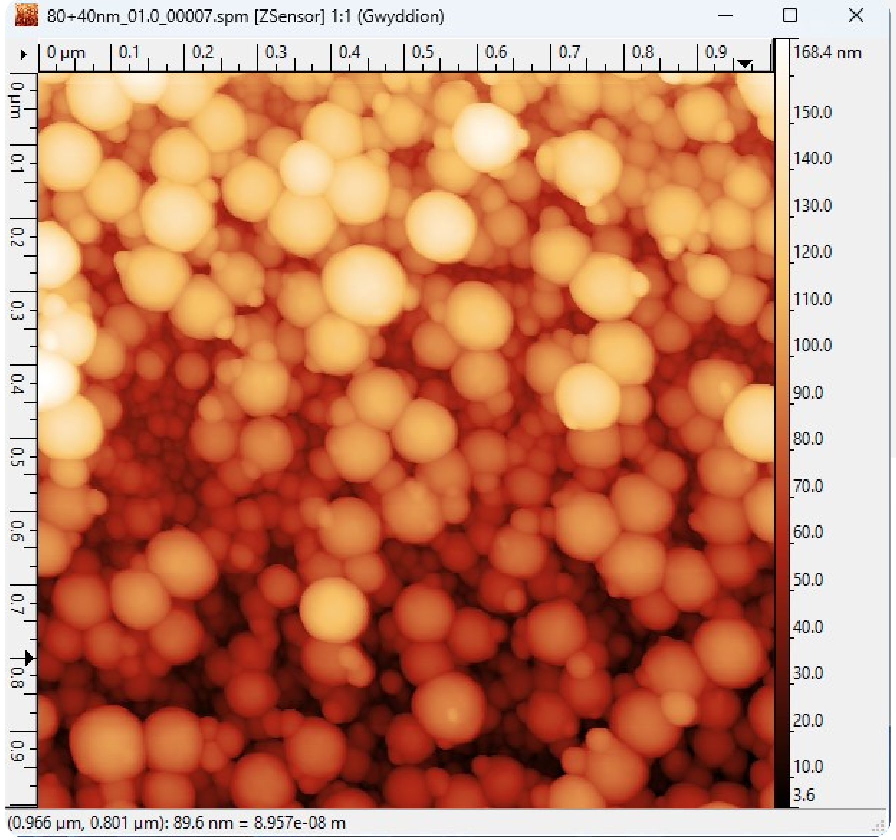




Leveling data

- Gwyddion is a program for AFM data visualization and analysis.

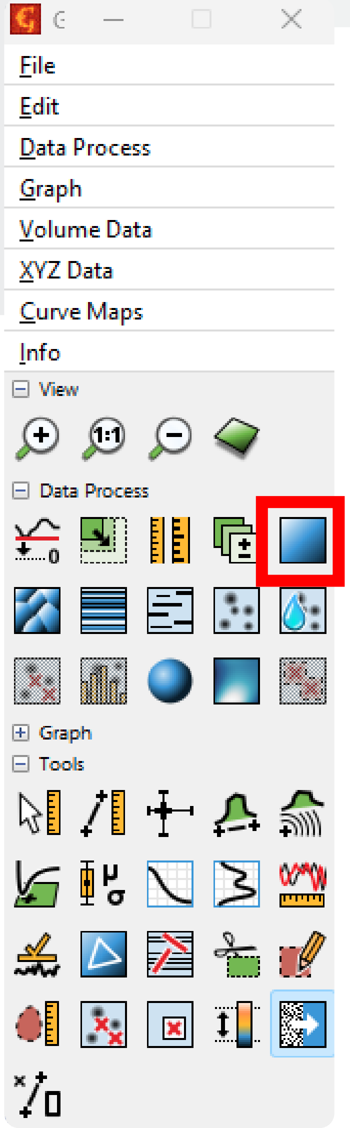


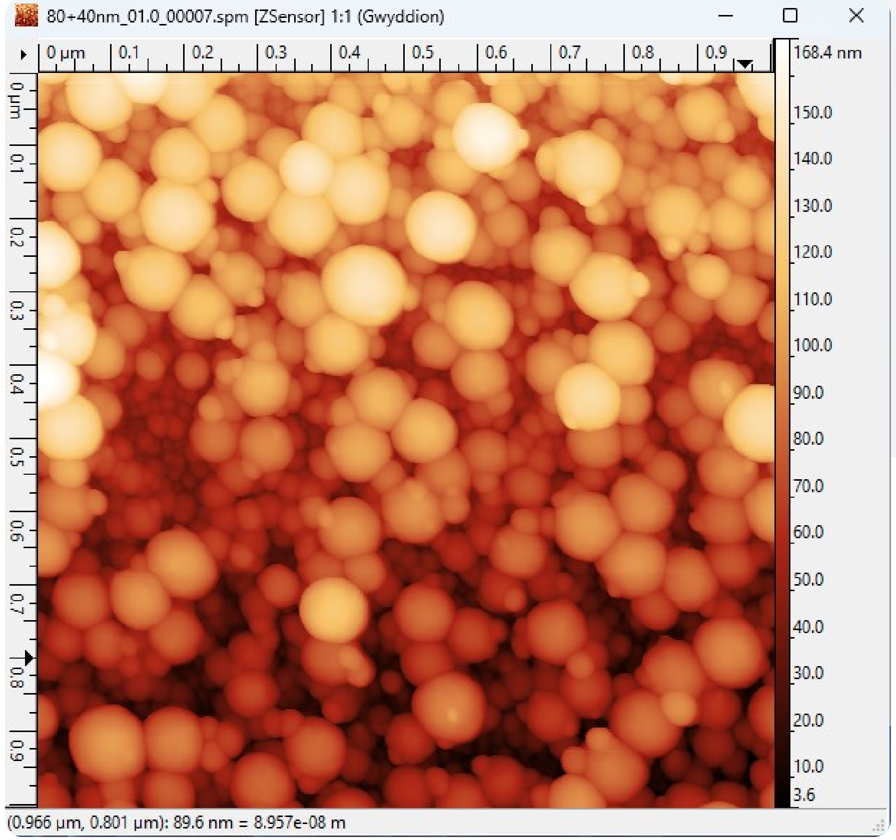


(0.966 µm, 0.801 µm): 89.6 nm = 8.957e-08 m

Leveling data

- Gwyddion is a program for AFM data visualization and analysis.
- Plane level - based on mean plane substraction

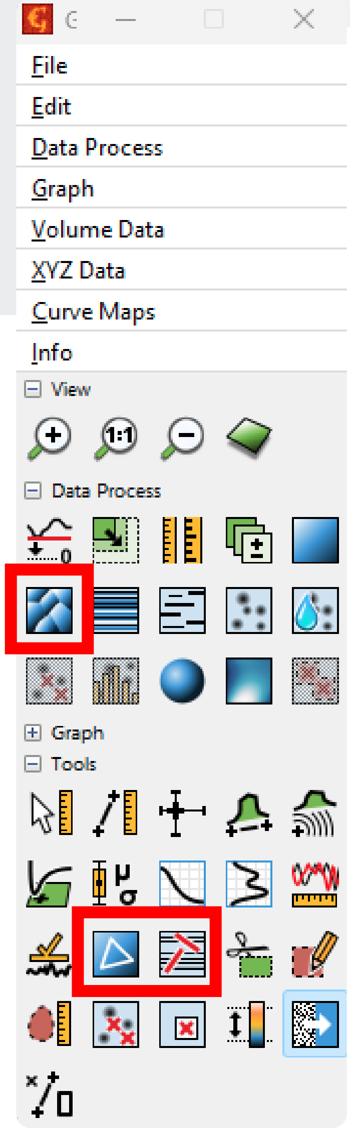


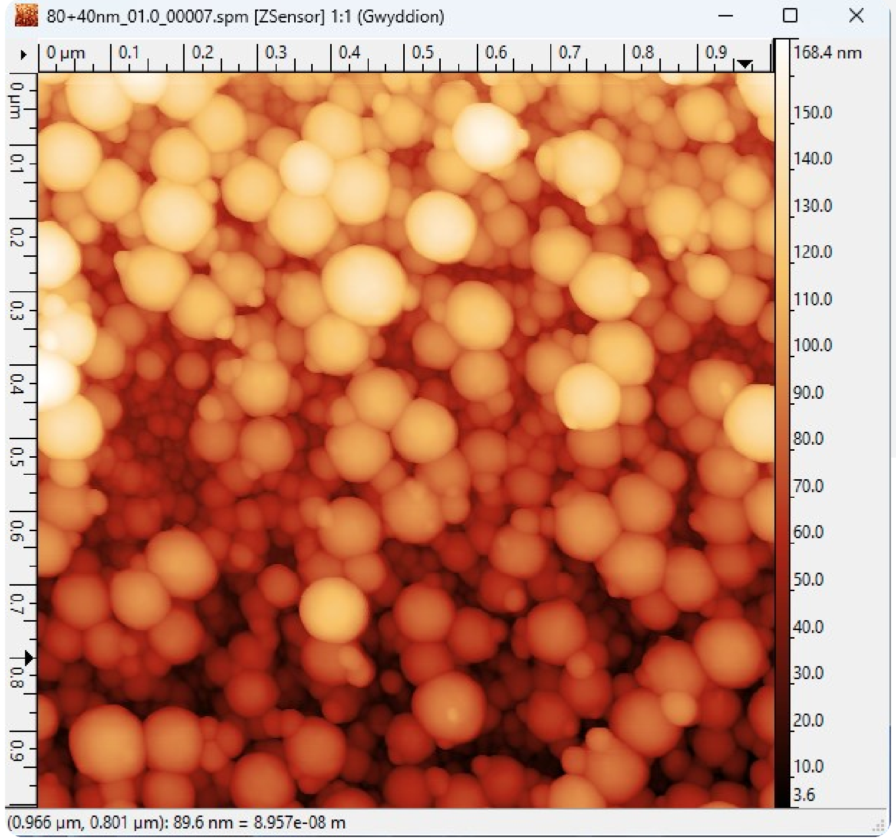

80+40nm_01.0_00007.spm [ZSensor] 1:1 (Gwyddion)

(0.966 µm, 0.801 µm): 89.6 nm = 8.957e-08 m

Leveling data

- Gwyddion is a program for AFM data visualization and analysis.
- Plane level - based on mean plane substraction
- *Facet levelling*
- *Three-point levelling*
- *Lines intersection levelling*





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Leveling data

- Gwyddion is a program for AFM data visualization and analysis.
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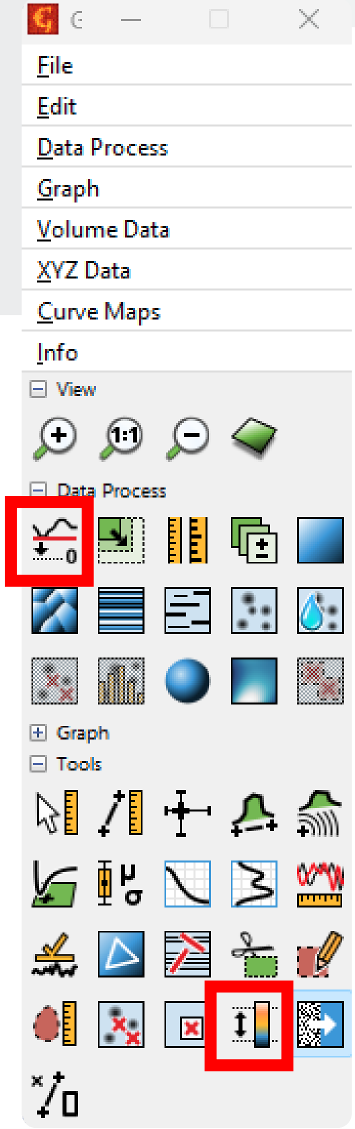
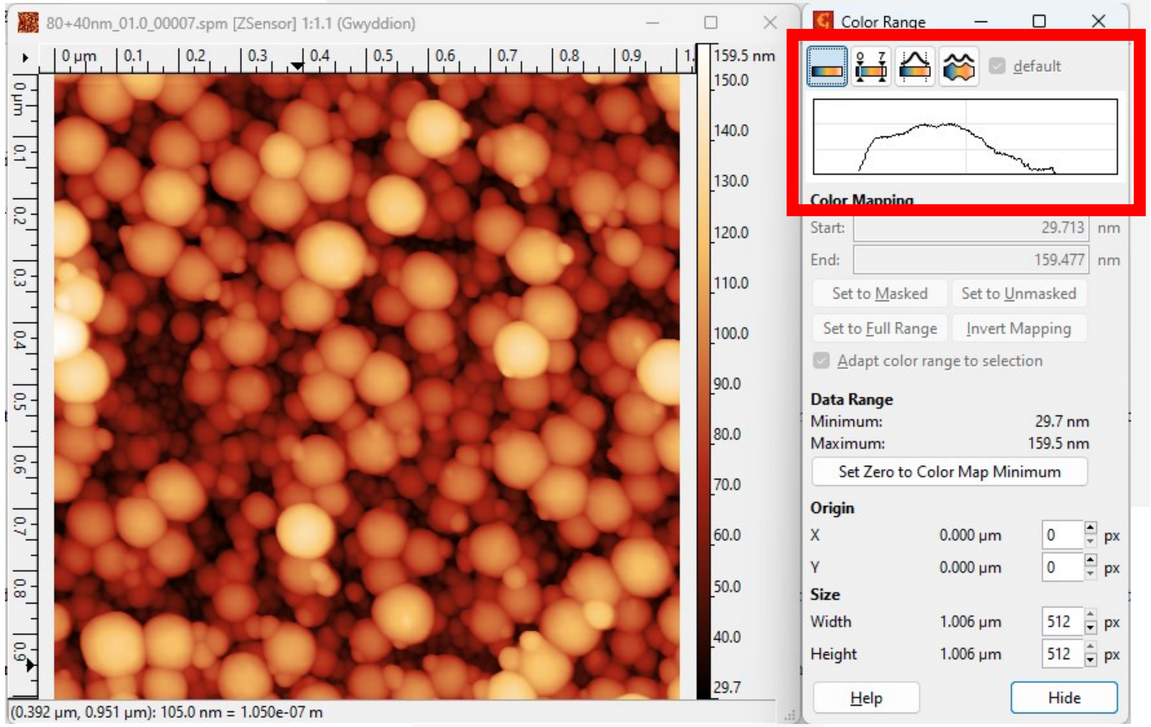
- Facet levelling
- Three-point levelling
- Lines intersection levelling
- Colour range and Fix zero




80+40nm_01.0_00007.spm [ZSensor] 1:1.1 (Gwyddion)

(0.392 µm, 0.951 µm): 105.0 nm = 1.050e-07 m

Color Range

☑ default

Color Mapping

Start: 29.713 nm

End: 159.477 nm

☑ Adapt color range to selection

Data Range

Minimum: 29.7 nm

Maximum: 159.5 nm

Origin

X 0.000 µm 0 px

Y 0.000 µm 0 px

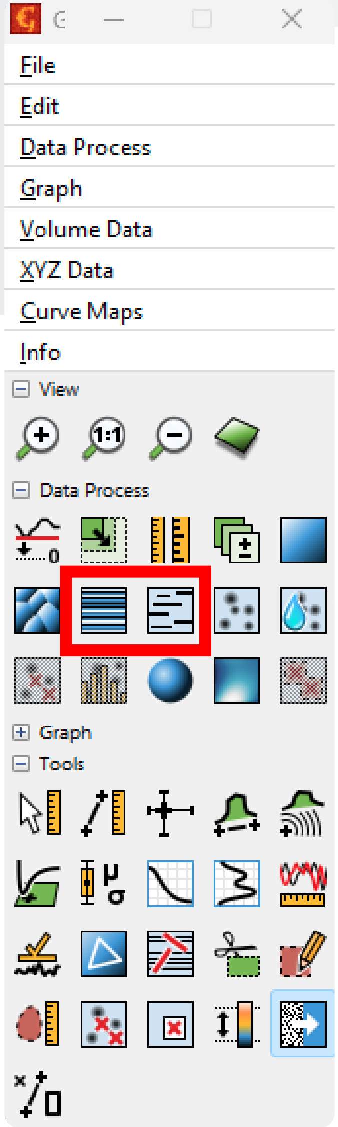
Size

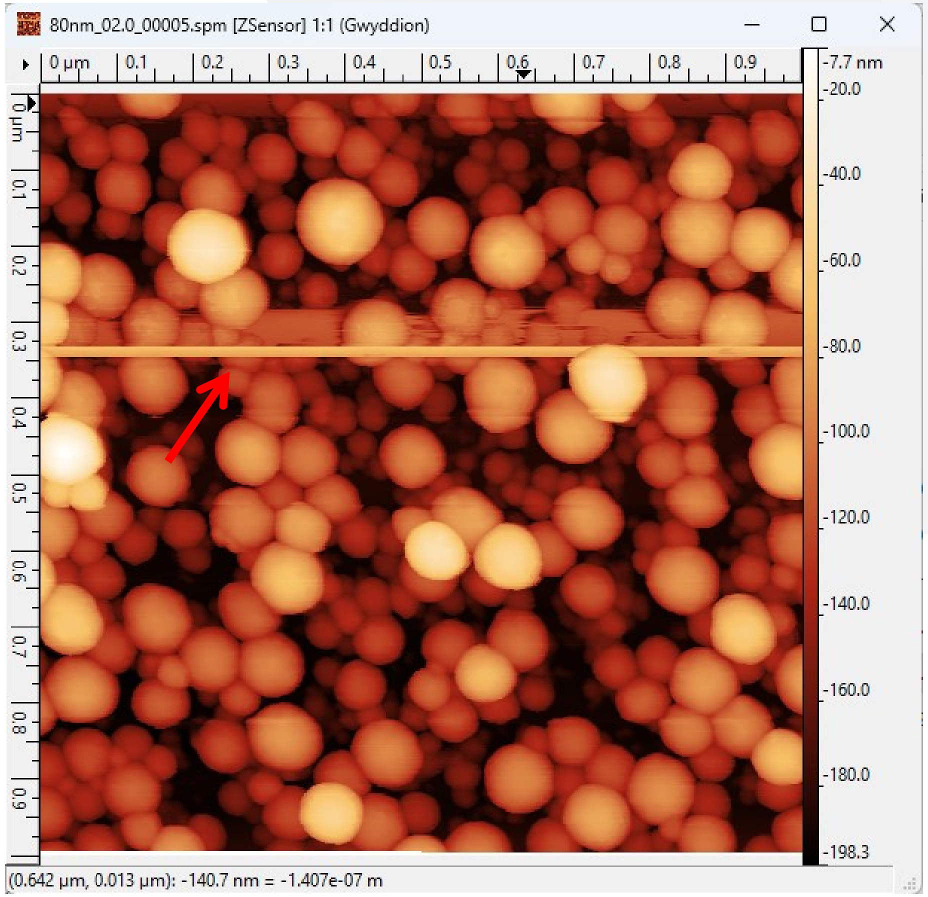
Width 1.006 µm 512 px

Height 1.006 µm 512 px

Line artefacts

- Removing AFM artefacts in from the image
  - Align rows using different methods
  - Correct horizontal scars

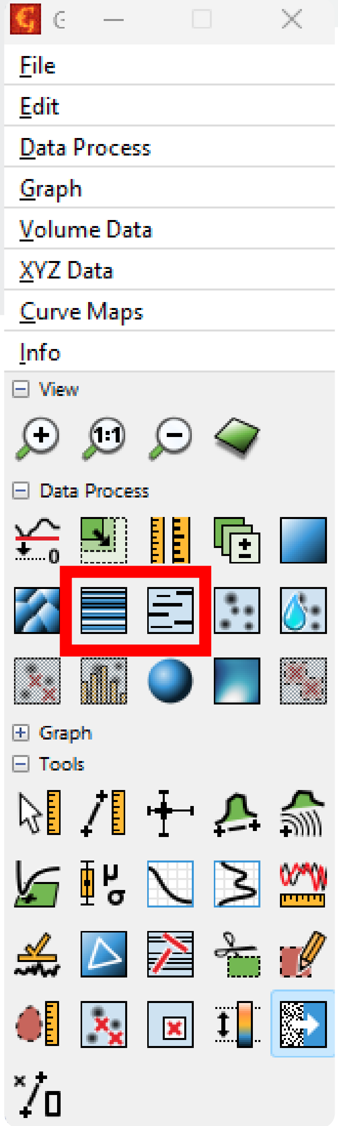


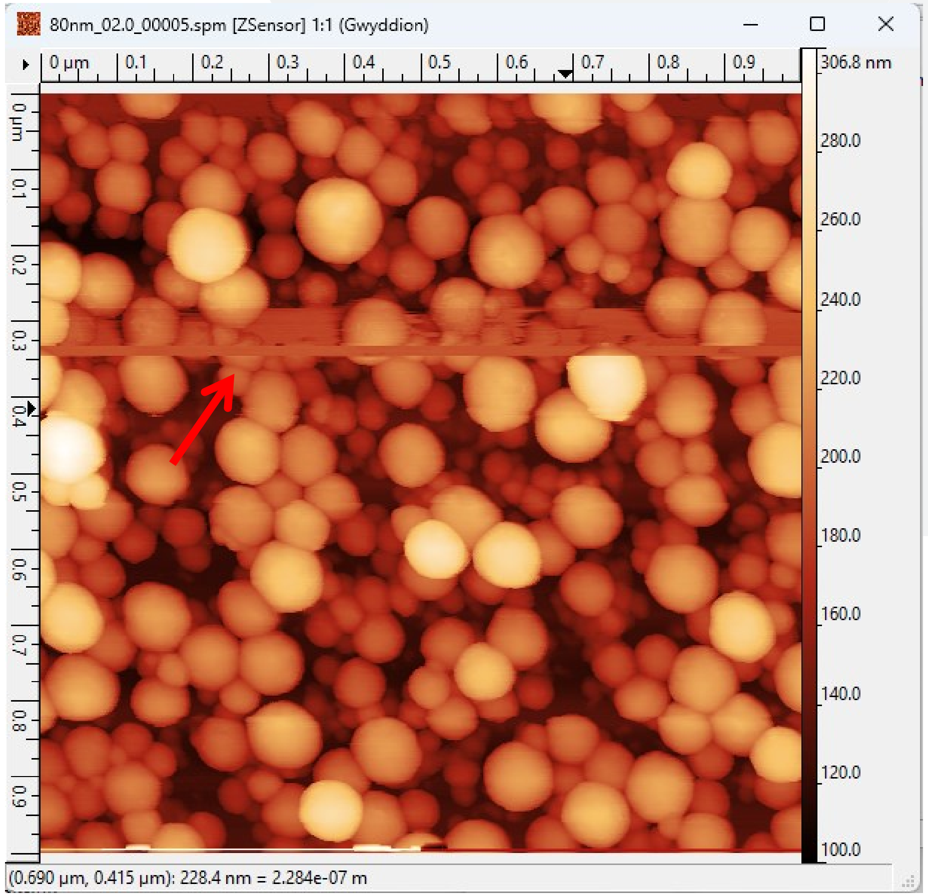


(0.642 µm, 0.013 µm): -140.7 nm = -1.407e-07 m

Line artefacts

- Removing AFM artefacts in from the image
  - Align rows using different methods
  - Correct horizontal scars

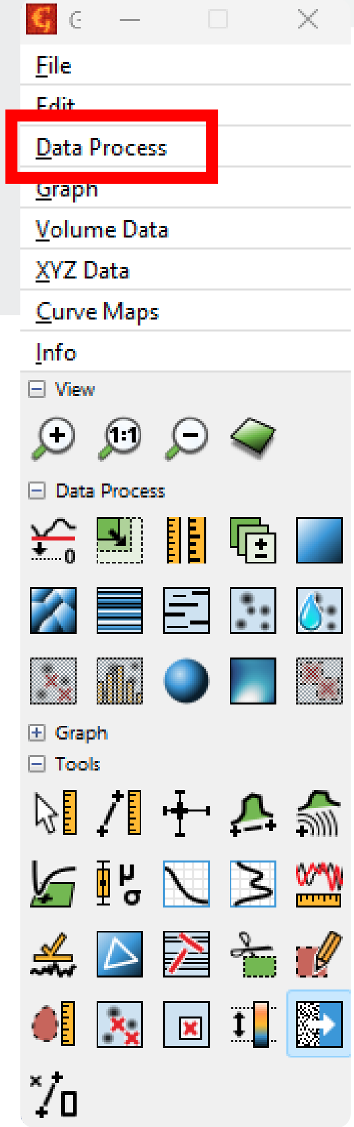


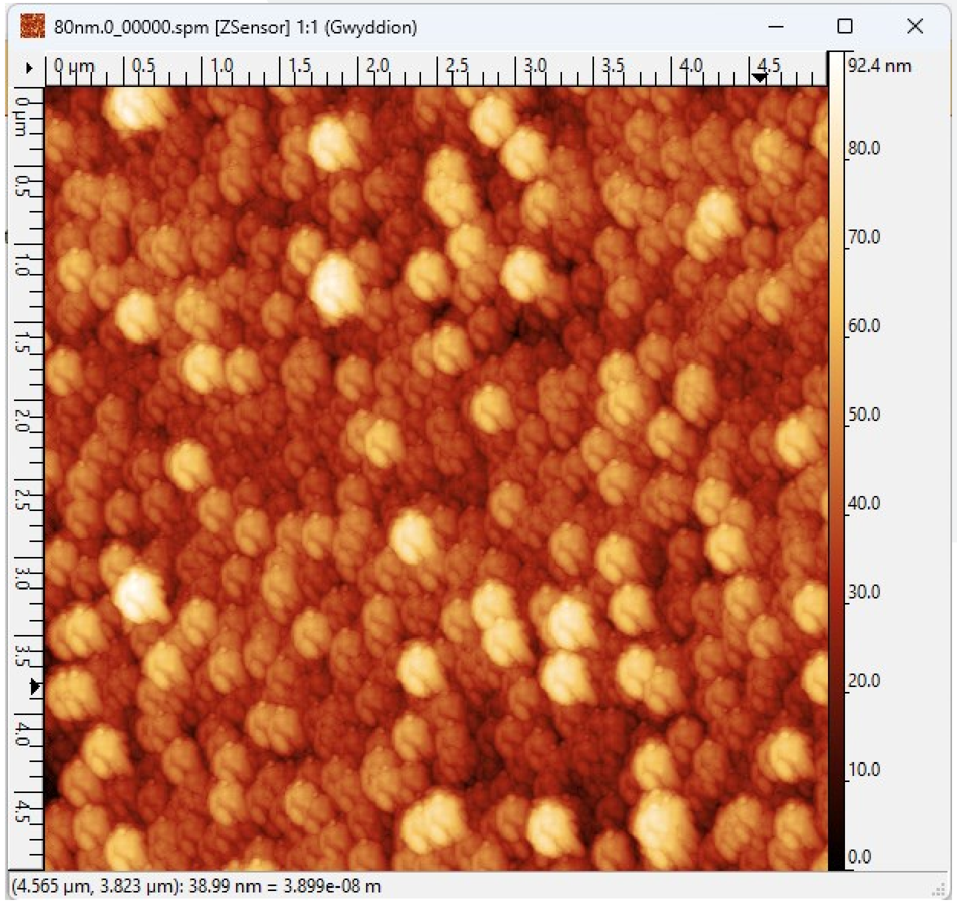


(0.690 µm, 0.415 µm): 228.4 nm = 2.284e-07 m

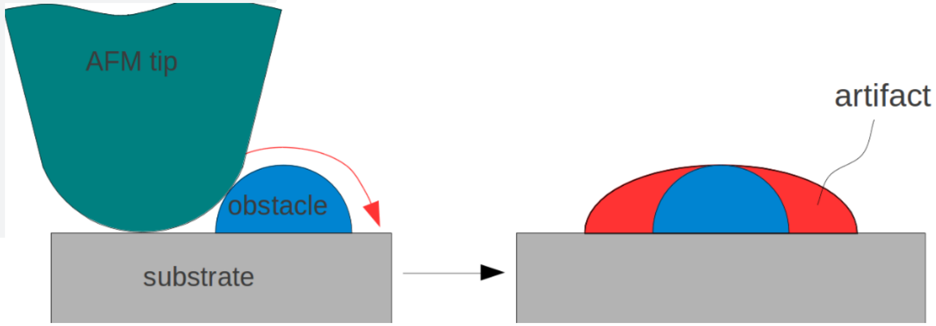
Tip convolution artefacts

- Object can be seen bigger due to broken or dirty AFM tip
- In most cases its better to change tip but it can be corrected after to an extend



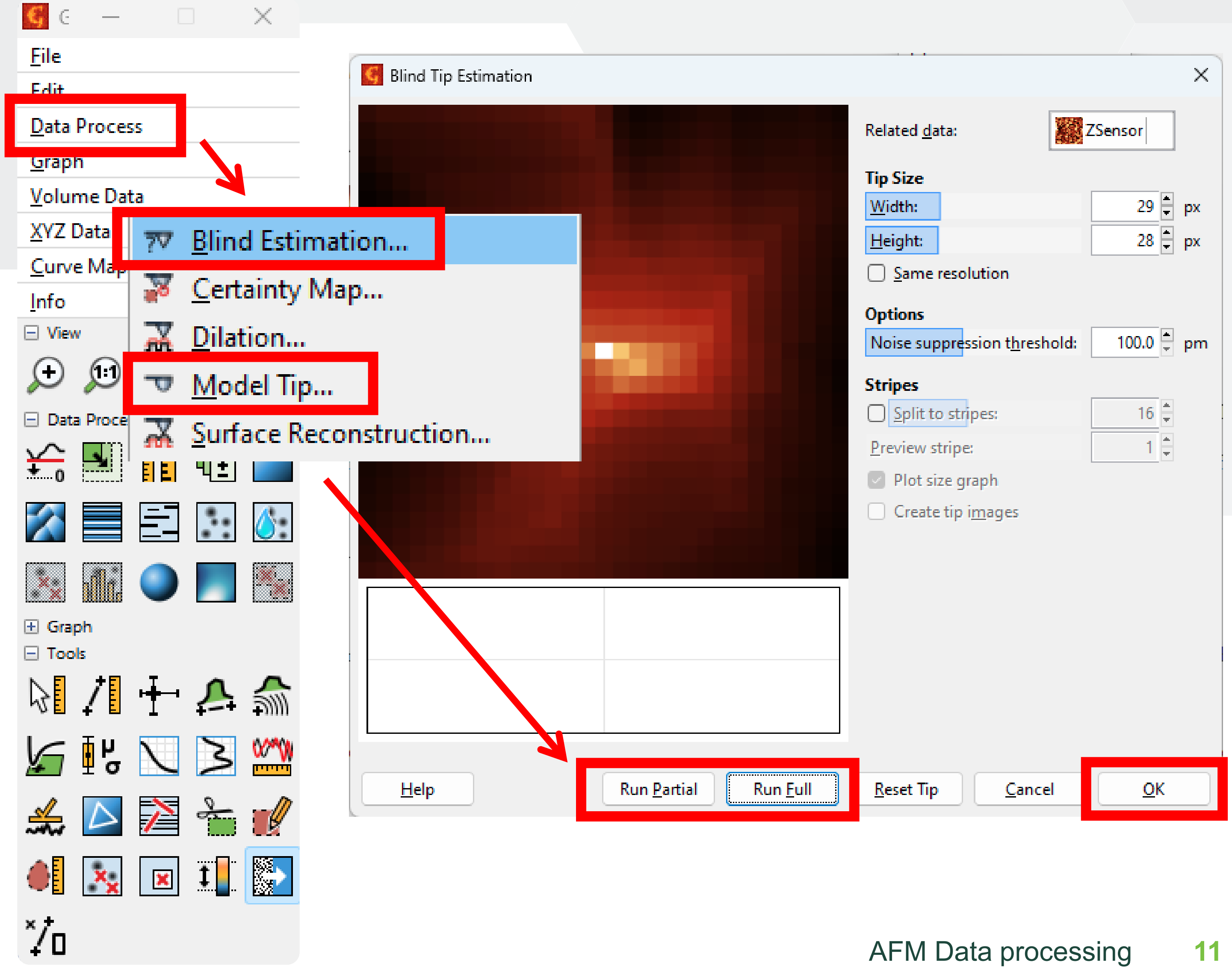


(4.565 µm, 3.823 µm): 38.99 nm = 3.899e-08 m



Tip convolution artefacts

- *Data process > Tip and Indentation > Blind estimation*
- Or *Model Tip*, where we put dimensional information about the tip



Related data: ZSensor

Tip Size

Width: 29 px

Height: 28 px

☐ Same resolution

Options

Noise suppression threshold: 100.0 pm

Stripes

☐ Split to stripes: 16

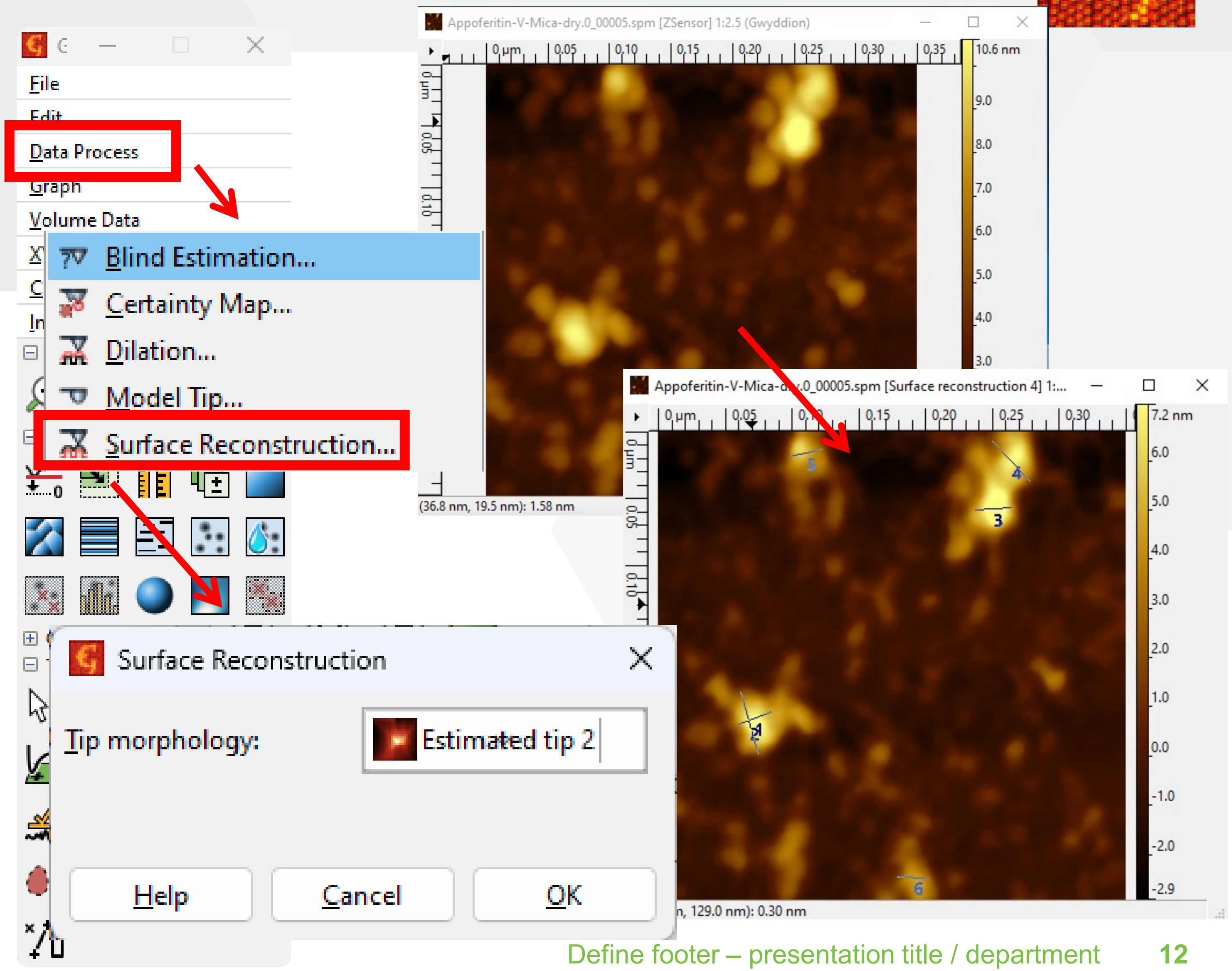
Preview stripe: 1

☑ Plot size graph

☐ Create tip images

Tip convolution artefacts

- *Data process > Tip and Indentation > Blind estimation*
- Or *Model Tip*, where we put dimensional information about the tip
- *Data process > Tip and Indentation > Surface reconstruction*



Tip morphology: Estimated tip 2

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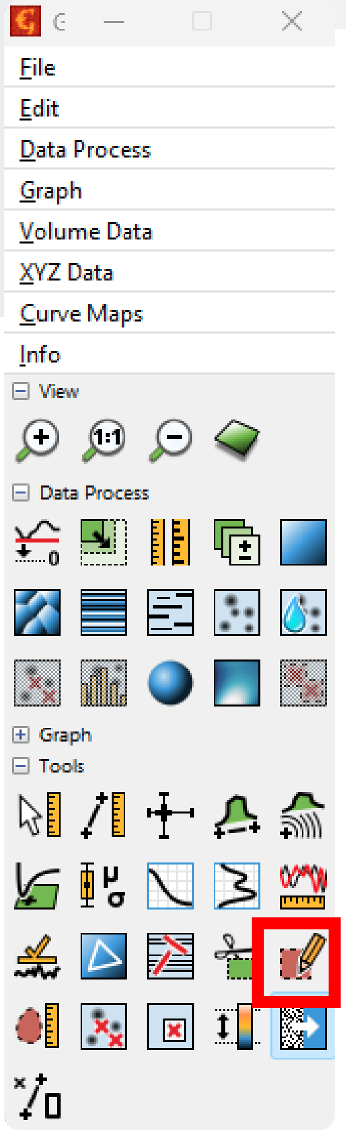
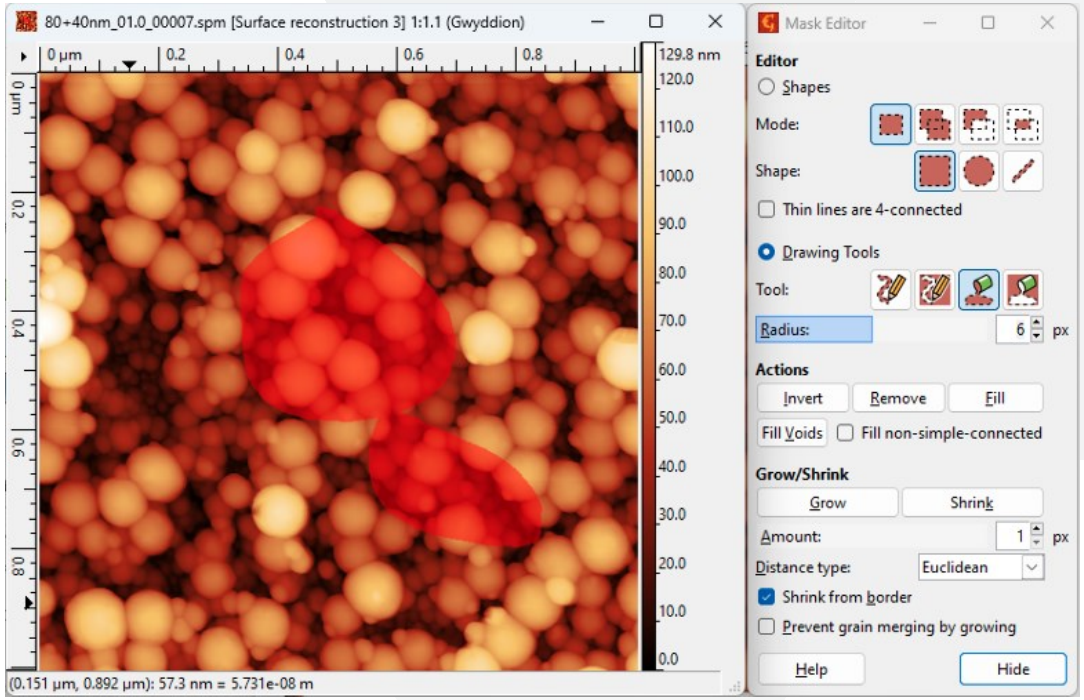
Masks

- Mask editor can help you define masks with drawing tools or shapes.





(0.151 µm, 0.892 µm): 57.3 nm = 5.731e-08 m

Editor

○ Shapes

Mode:

Shape:

☐ Thin lines are 4-connected

● Drawing Tools

Tool:

Radius: 6 px

Actions

☐ Fill non-simple-connected

Grow/Shrink

Amount: 1 px

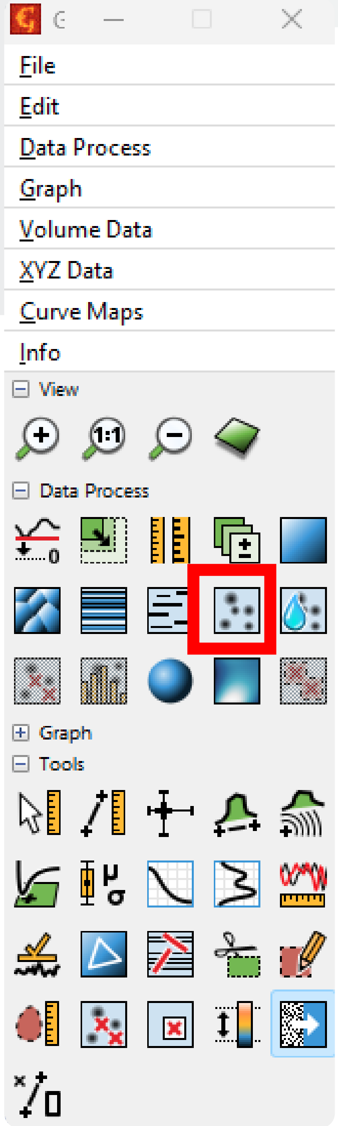
Distance type: Euclidean

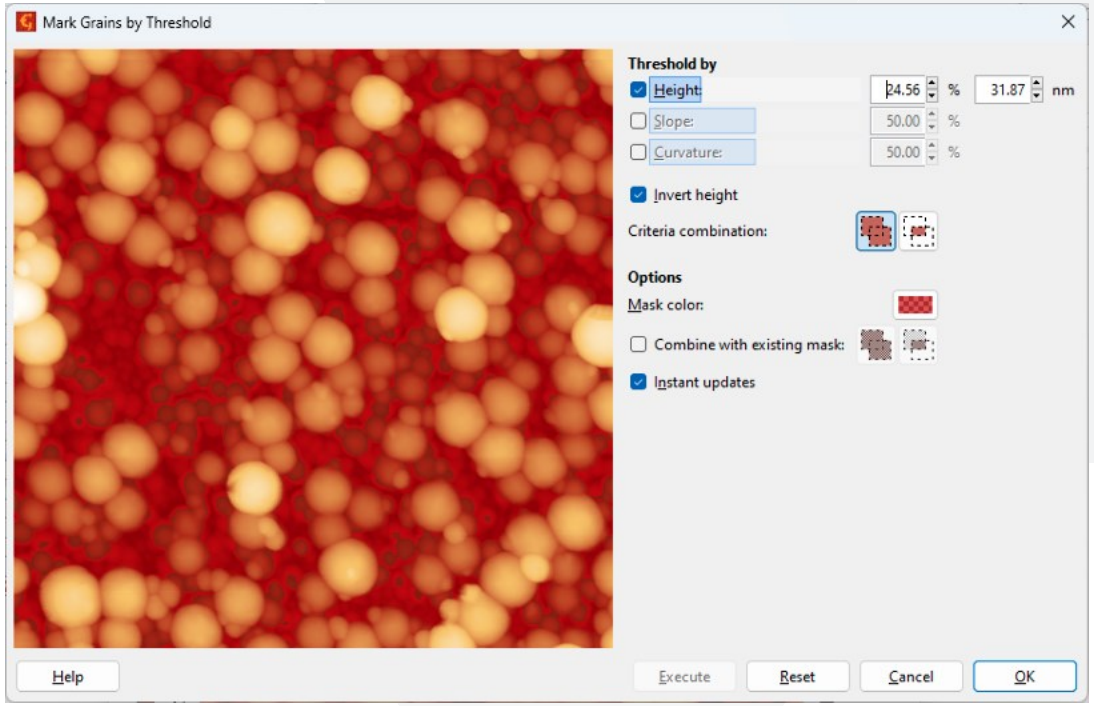
☑ Shrink from border

☐ Prevent grain merging by growing

Masks

- Mask editor can help you define masks with drawing tools or shapes.
- *Mark Grains by Threshold* tool can define mask based on physical dimension (Height, *E*)





Threshold by

☑ Height: 24.56 % 31.87 nm

☐ Slope: 50.00 %

☐ Curvature: 50.00 %

☑ Invert height

Criteria combination:

Options

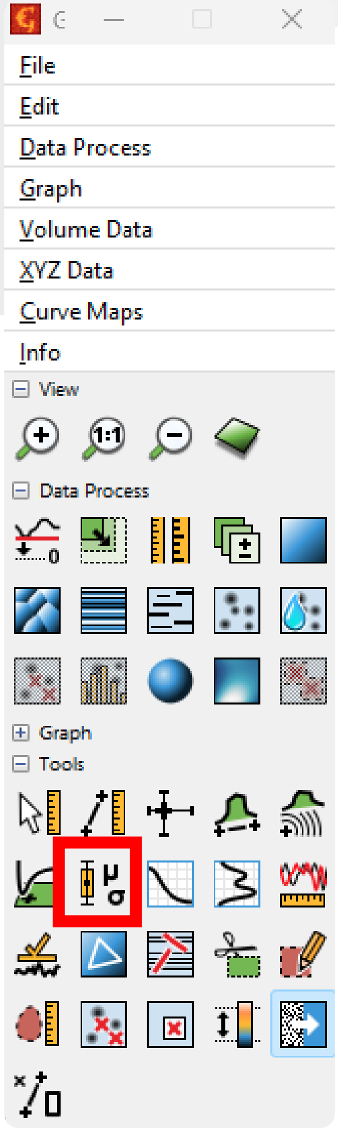
Mask color:

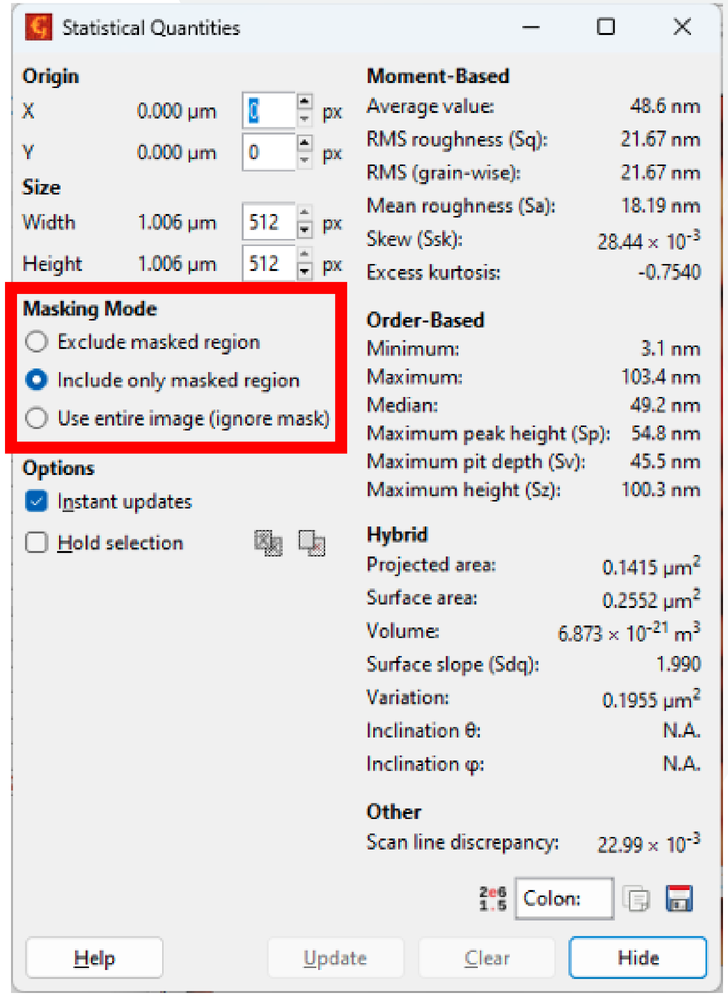
☐ Combine with existing mask:

☑ Instant updates

Masks

- Mask editor can help you define masks with drawing tools or shapes.
- *Mark Grains by Threshold* tool can define mask based on physical dimension (Height, *E*)
- You can extract statistical information about masked/ non-masked regions.




Statistical Quantities

Origin
X 0.000 µm 0 px
Y 0.000 µm 0 px

Size
Width 1.006 µm 512 px
Height 1.006 µm 512 px

Masking Mode
○ Exclude masked region
● Include only masked region
○ Use entire image (ignore mask)

Options
☑ Instant updates
☐ Hold selection

Moment-Based
Average value: 48.6 nm
RMS roughness (Sq): 21.67 nm
RMS (grain-wise): 21.67 nm
Mean roughness (Sa): 18.19 nm
Skew (Ssk): 28.44×10^{-3}
Excess kurtosis: -0.7540

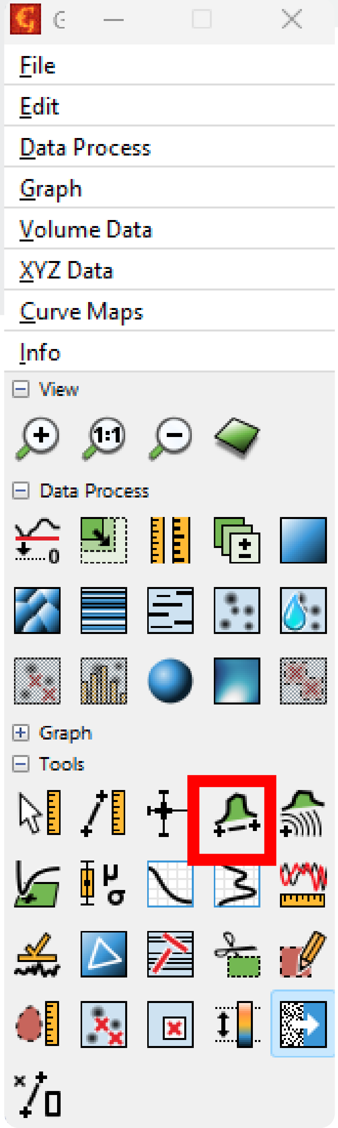
Order-Based
Minimum: 3.1 nm
Maximum: 103.4 nm
Median: 49.2 nm
Maximum peak height (Sp): 54.8 nm
Maximum pit depth (Sv): 45.5 nm
Maximum height (Sz): 100.3 nm

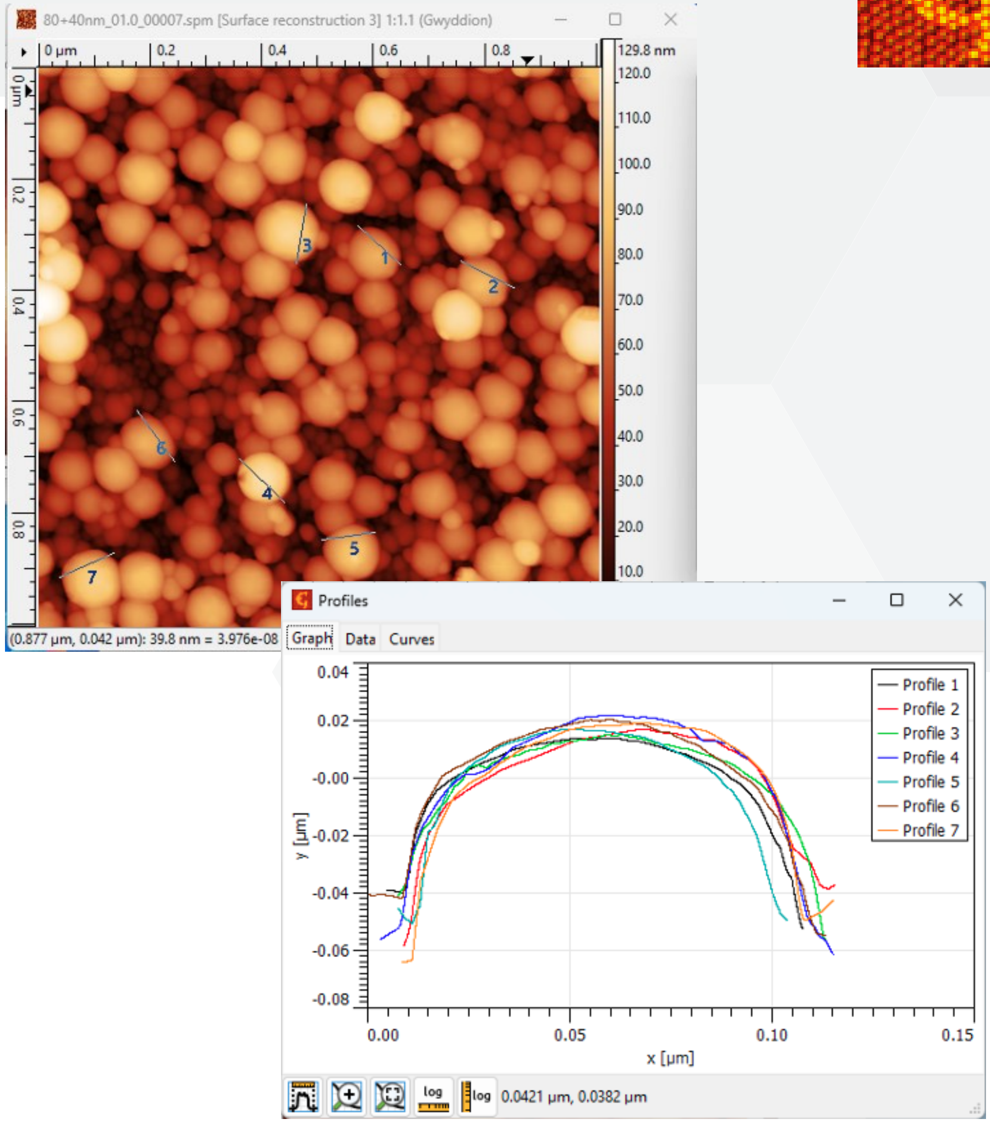
Hybrid
Projected area: $0.1415\ \mu m^2$
Surface area: $0.2552\ \mu m^2$
Volume: $6.873 \times 10^{-21}\ m^3$
Surface slope (Sdq): 1.990
Variation: $0.1955\ \mu m^2$
Inclination θ: N.A.
Inclination φ: N.A.

Other
Scan line discrepancy: 22.99×10^{-3}

Analysis

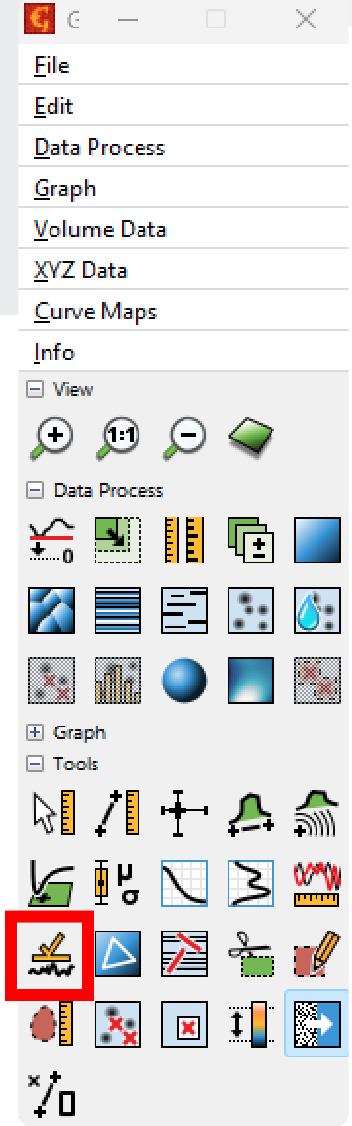
- Profile sections can be obtained and then exported using *Extract profiles*

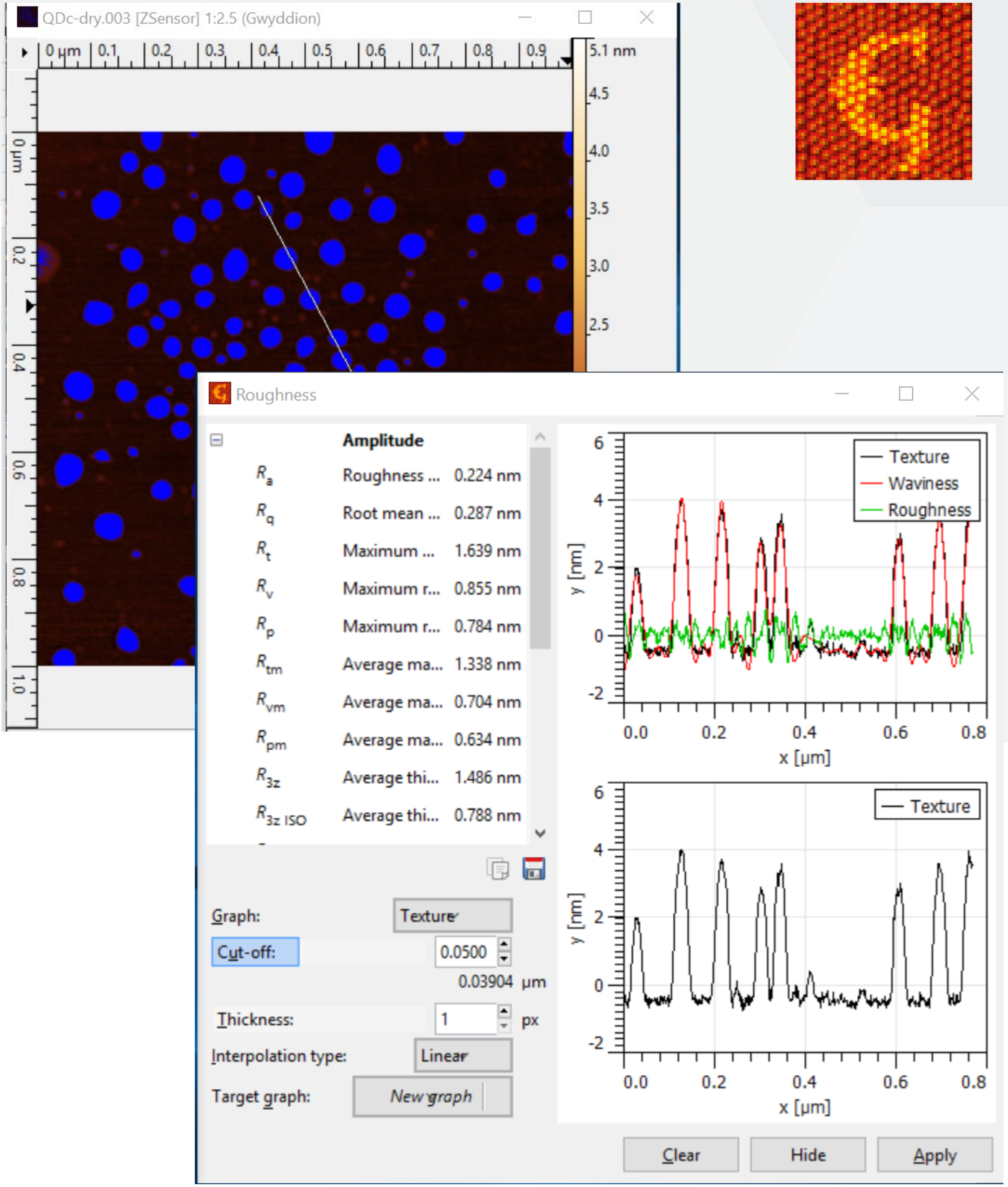




Analysis

- Profile sections can be obtained and then exported using *Extract profiles*
- Standardized one-dimensional roughness parameters.





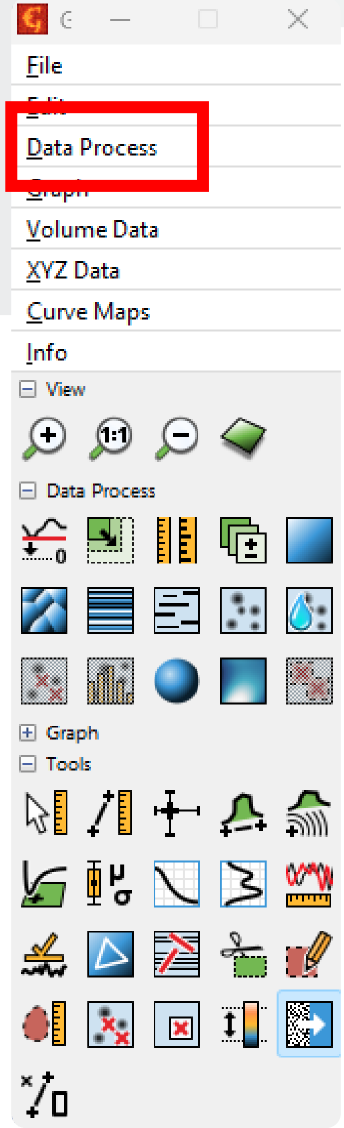
Amplitude

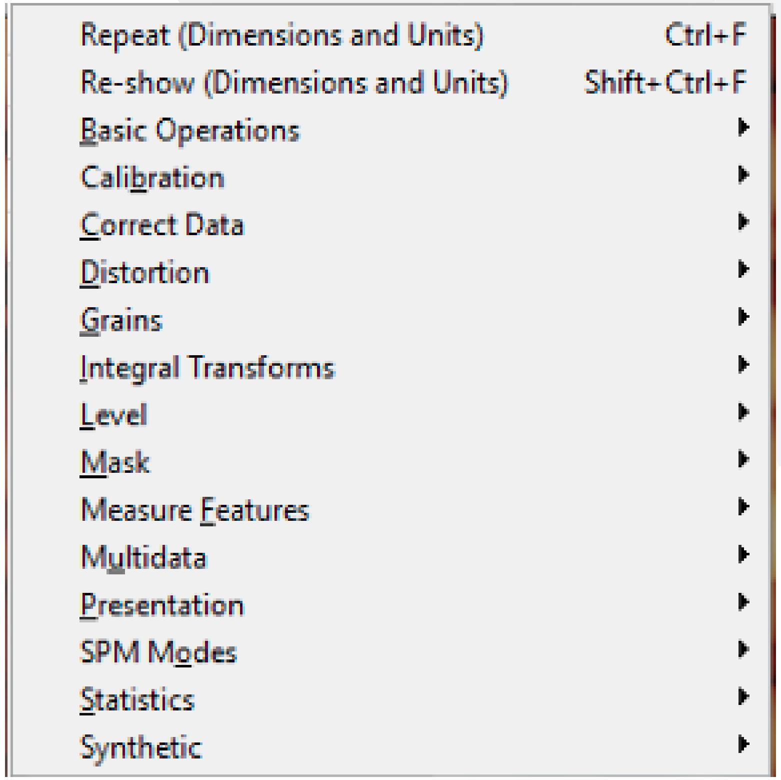
R_a	Roughness ...	0.224 nm
R_q	Root mean ...	0.287 nm
R_t	Maximum ...	1.639 nm
R_v	Maximum r...	0.855 nm
R_p	Maximum r...	0.784 nm
R_{tm}	Average ma...	1.338 nm
R_{vm}	Average ma...	0.704 nm
R_{pm}	Average ma...	0.634 nm
R_{3z}	Average thi...	1.486 nm
R_{3z} ISO	Average thi...	0.788 nm

Graph: Texture
Cut-off: 0.0500
0.03904 µm
Thickness: 1 px
Interpolation type: Linear
Target graph: New graph

Analysis

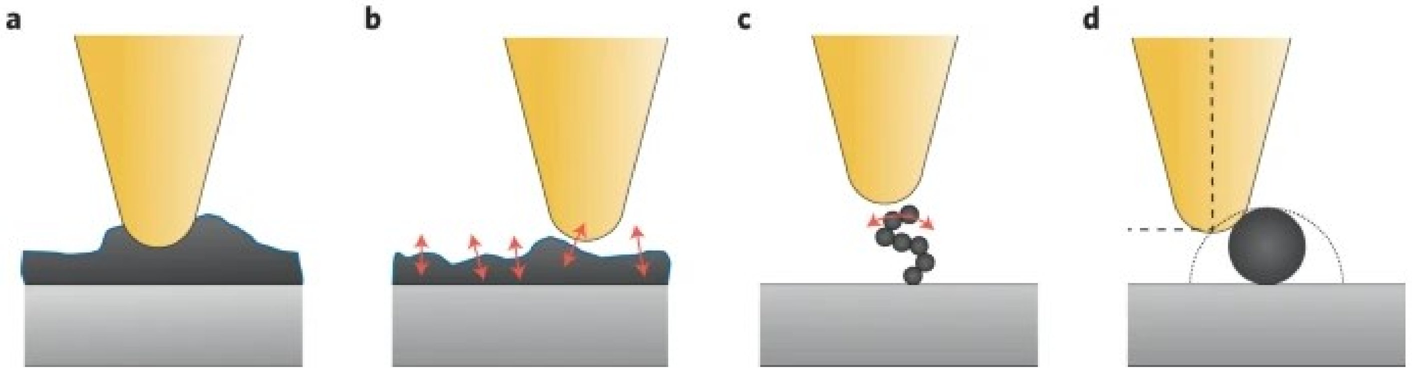
- Profile sections can be obtained and then exported using *Extract profiles*
- Standardized one-dimensional roughness parameters.
- And many more…
- *Info > User guide*





Localized AFM

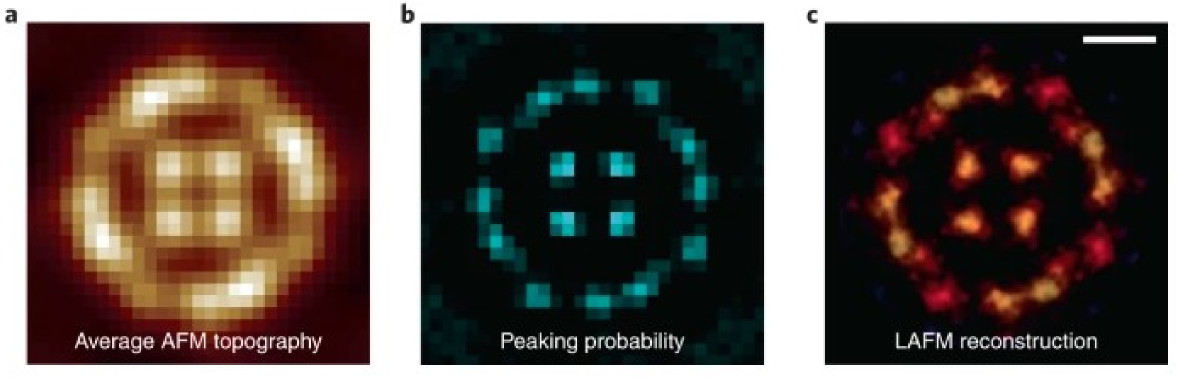
- Factors that can limit spatial resolution of AFM: adhesion, mobility of scanned molecules, tip artefacts

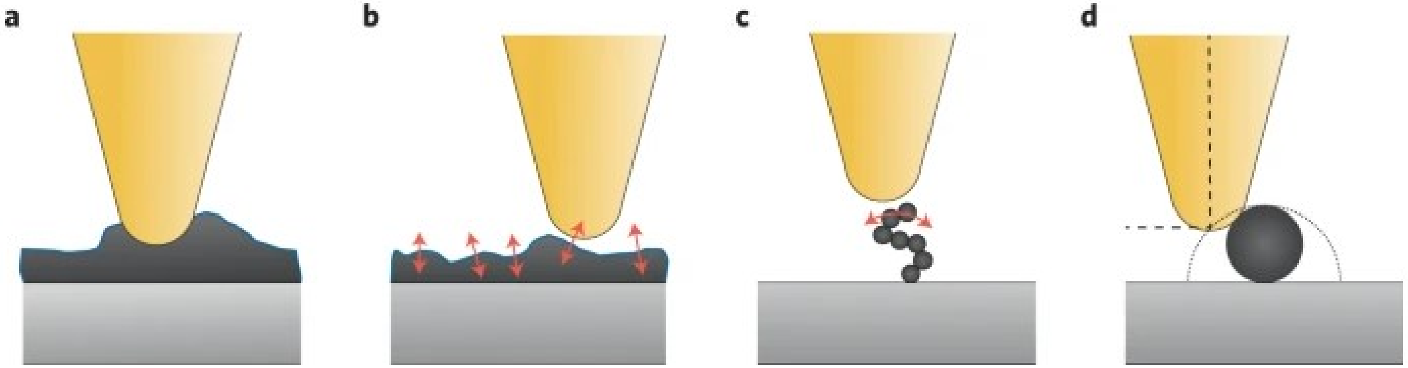


Hoogenboom, B.W. Stretching the resolution limit of atomic force microscopy. *Nat Struct Mol Biol* **28**, 629–630 (2021). https://doi.org/10.1038/s41594-021-00638-x

Localized AFM

- Factors that can limit spatial resolution of AFM: adhesion, mobility of scanned molecules, tip artefacts
- Instead mapping the sample height, we can generate local height maxima in the repeated AFM images and plot **peaking-probability map > Extrapolate**
- More in presentation by D. Kabanov, Today at 15:00





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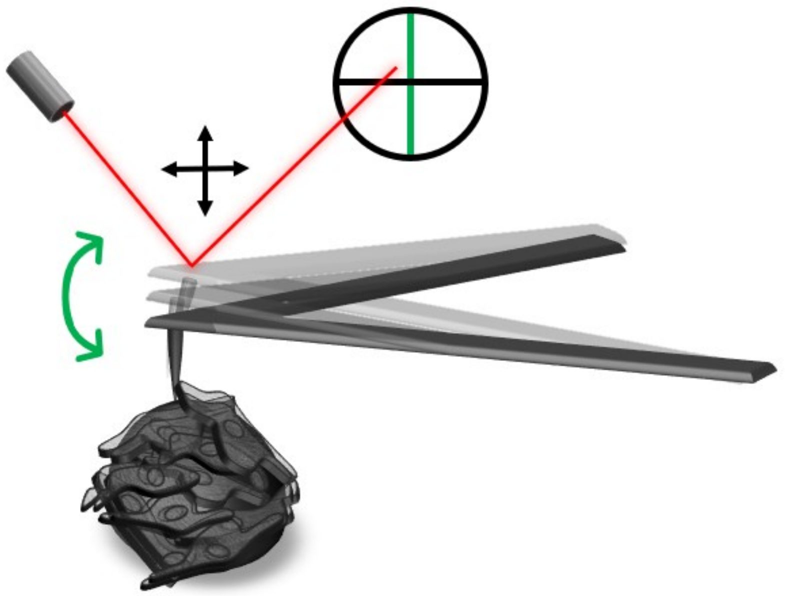
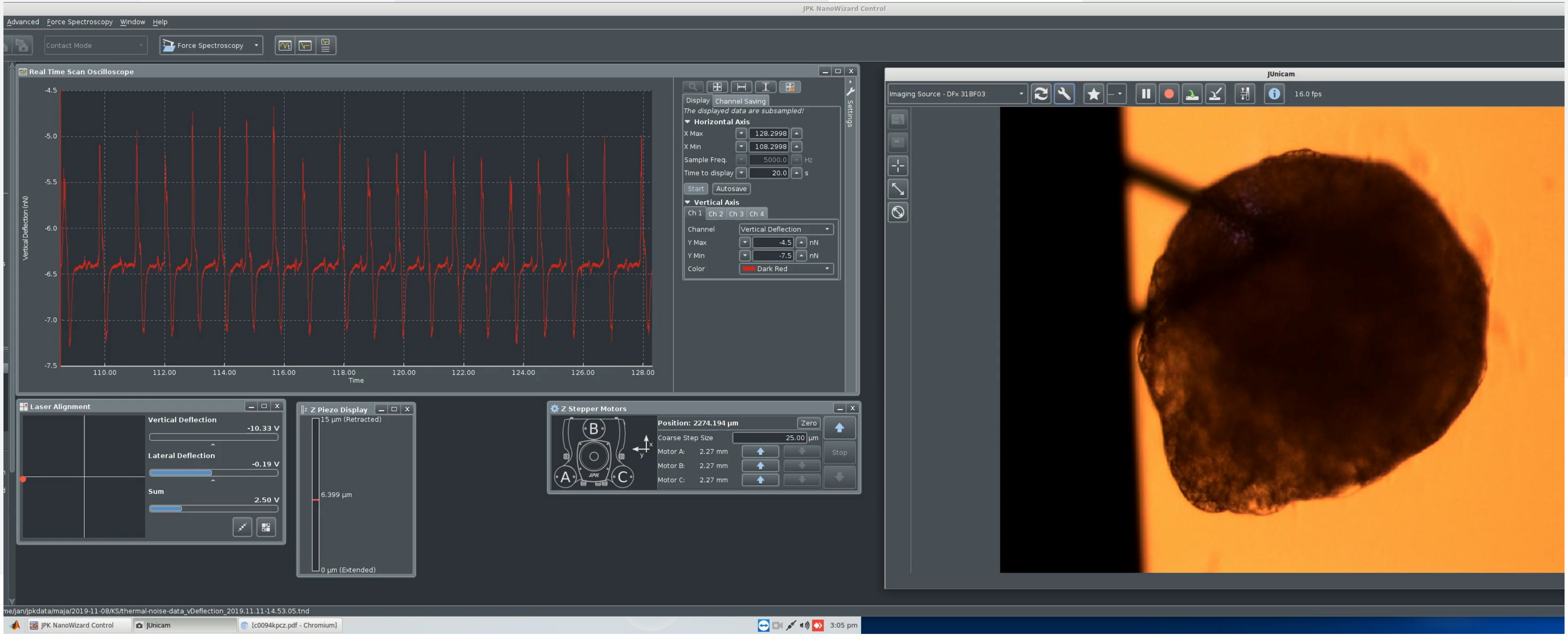
Contraction properties of CMs

- Time recording of vertical deflection
- (hPSC-CMs) differentiated in form of cellular clusters – Embryoids bodies
- AFM-based biosensor setup



Contraction properties of CMs



The displayed data are subsampled!

Horizontal Axis

X Max 128.2998
X Min 108.2998
Sample Freq. 5000.0 Hz
Time to display 20.0 s

Vertical Axis

Ch 1 | Ch 2 | Ch 3 | Ch 4

Channel Vertical Deflection
Y Max -4.5 nN
Y Min -7.5 nN
Color Dark Red

Vertical Deflection -10.33 V
Lateral Deflection -0.19 V
Sum 2.50 V

15 µm (Retracted)
6.399 µm
0 µm (Extended)

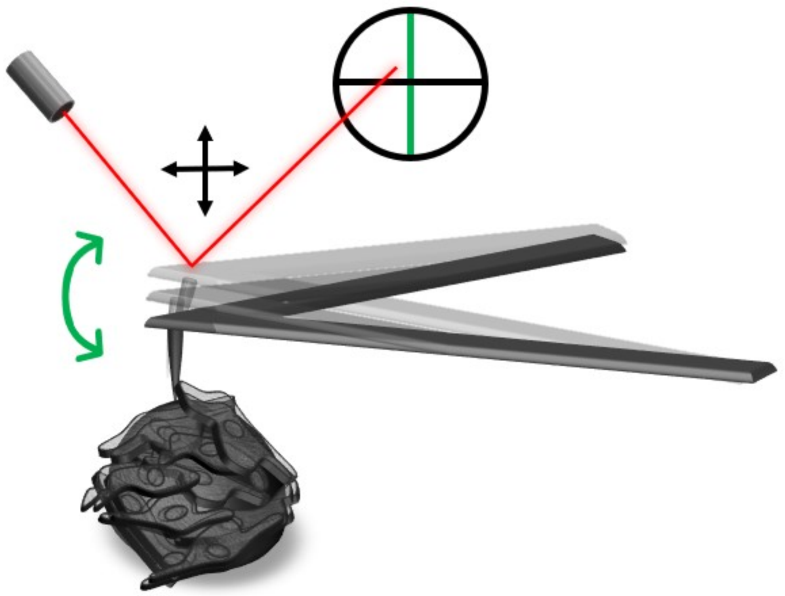
Position: 2274.194 µm
Coarse Step Size 25.00 µm
Motor A: 2.27 mm
Motor B: 2.27 mm
Motor C: 2.27 mm

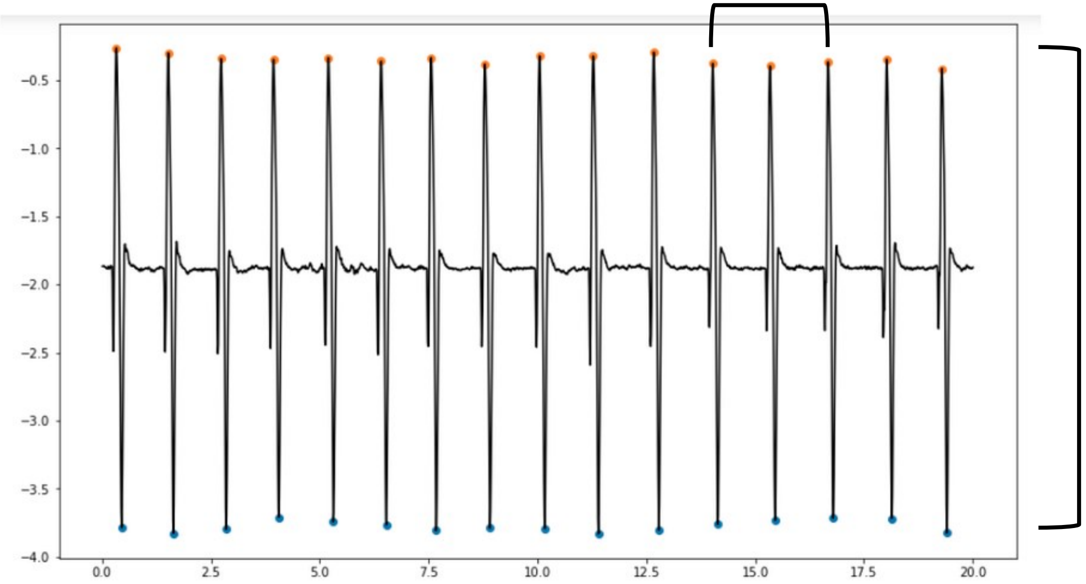
Imaging Source - DFx 31BF03
16.0 fps

me/jan/jpkdata/maja/2019-11-08/KS/thermal-noise-data_vDeflection_2019.11.11-14.53.05.tnd

Contraction properties of CMs

- Time recording of vertical deflection
- Python-based script for detection peaks
- Basic parameters – **Beat rate** and **contraction force**
- **Heart rate variability** - variation in the beat-to-beat interval.





Beat rate (bpm)

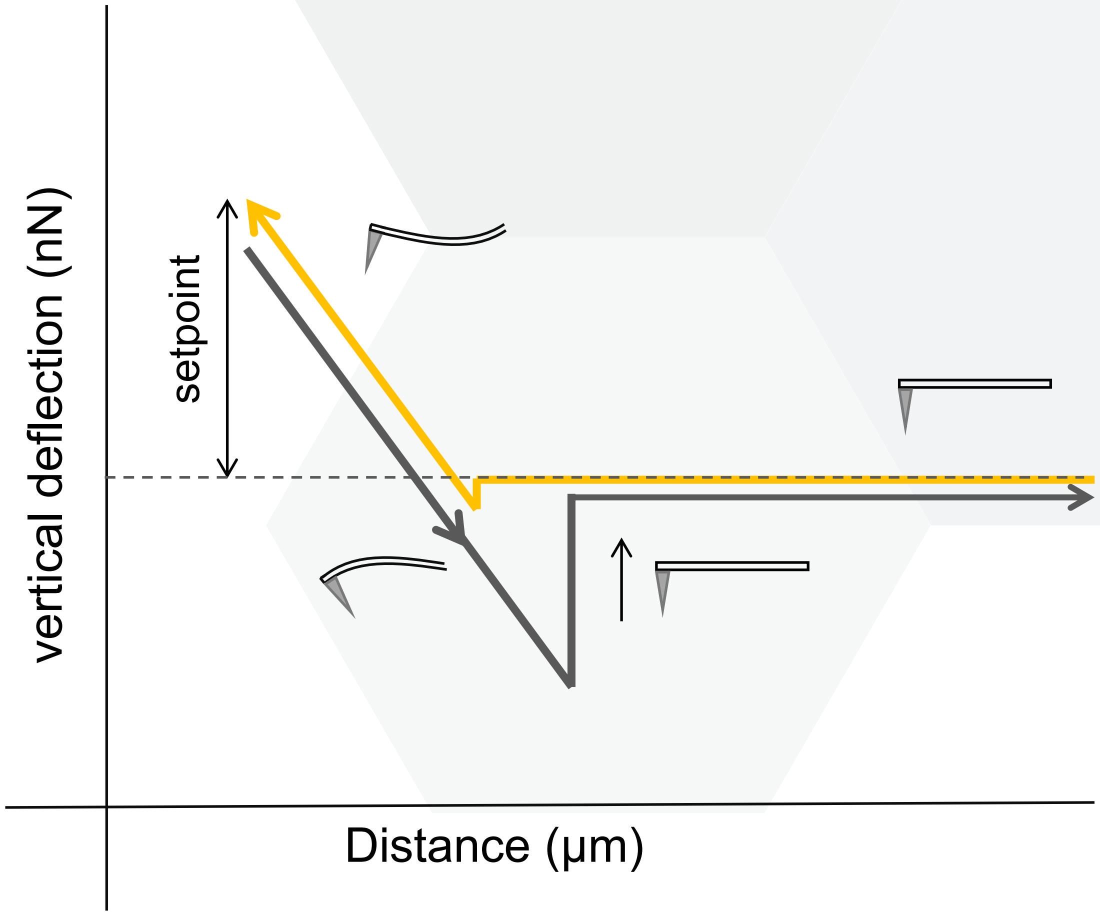
Contraction force (nN)

Layout of the presentation

- Types of AFM data
- Imaging
  - AFM images – leveling data, artefacts, surface reconstruction, masks, analysis, LAFM
  - Real time scans of vertical deflection – contraction properties of CMs, peaks detection, HRV analysis
- Force spectroscopy
  - What is a force distance curve (FDC)?
  - Young`s modulus – models, analysis
  - Alternative analysis of FDCs – SMFS, thickness of lipid bilayers, rupture events
- Indentation
  - Rheology analysis, viscoelasticity

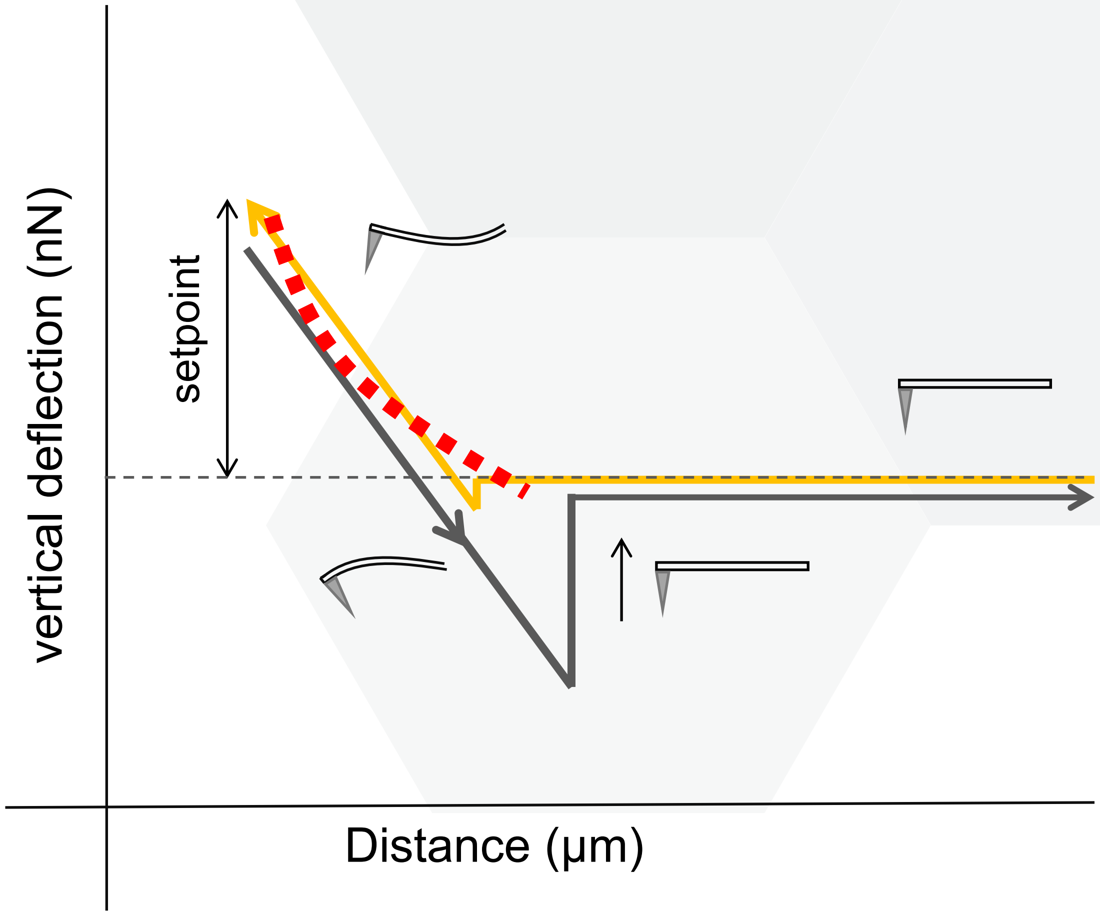
Force distance curve (FDS)

- Cantilever approach the surface, press with defined force (setpoint) and the withdraw
- Resulting vertical deflection vs. distance (height) curve is FDS



Force distance curve (FDS)

- Cantilever approach the surface, press with defined force (setpoint) and the withdraw
- Resulting vertical deflection vs. distance (height) curve is FDS
- We can obtain Young‘s modulus (*E*) by fiting the interaction part with non-linear function



Force distance curve (FDS)

- Cantilever approach the surface, press with defined force (setpoint) and the withdraw
- Resulting vertical deflection vs. distance (height) curve is FDS
- We can obtain Young‘s modulus (*E*) by fiting the interaction part with non-linear function
- Single point or multiple point (Force imaging, Peakforce, QI, etc.)

Models for processing FDCs

- Basic models for elastic deformation are **Hertz** and **Sneddon**, but they are neglecting surfaces forces and adhesion – very prevalent with biological samples
- For more adhesive samples advanced models such as **JKR, DMT** are used.

$$P = \frac{4E}{3(1-\nu^2)}\sqrt{R}\,\delta^{\frac{3}{2}}$$

Hertz

$$P = \frac{E}{(1-\nu^2)}\left(\frac{R^2+a^2}{2}\,\mathrm{Log}\left(\frac{R+a}{R-a}\right) - a\,R\right)$$

$$\delta = \frac{a}{2}\,\mathrm{Log}\left(\frac{R+a}{R-a}\right)$$

Sneddon

$$P = \frac{4E\sqrt{R}}{3(1-\nu^2)}\,\delta^{3/2} - 2\pi\gamma R$$

DMT

$$P = \frac{4Ea^3}{3R(1-\nu^2)} - 2\sqrt{2\pi\frac{E\gamma a^3}{(1-\nu^2)}}$$

$$\delta = \frac{a^2}{R} - \sqrt{\frac{2\pi a\gamma}{\frac{E}{(1-\nu^2)}}}$$

JKR

Sharp pyramidical tips ⟷ **Large spherical tips**

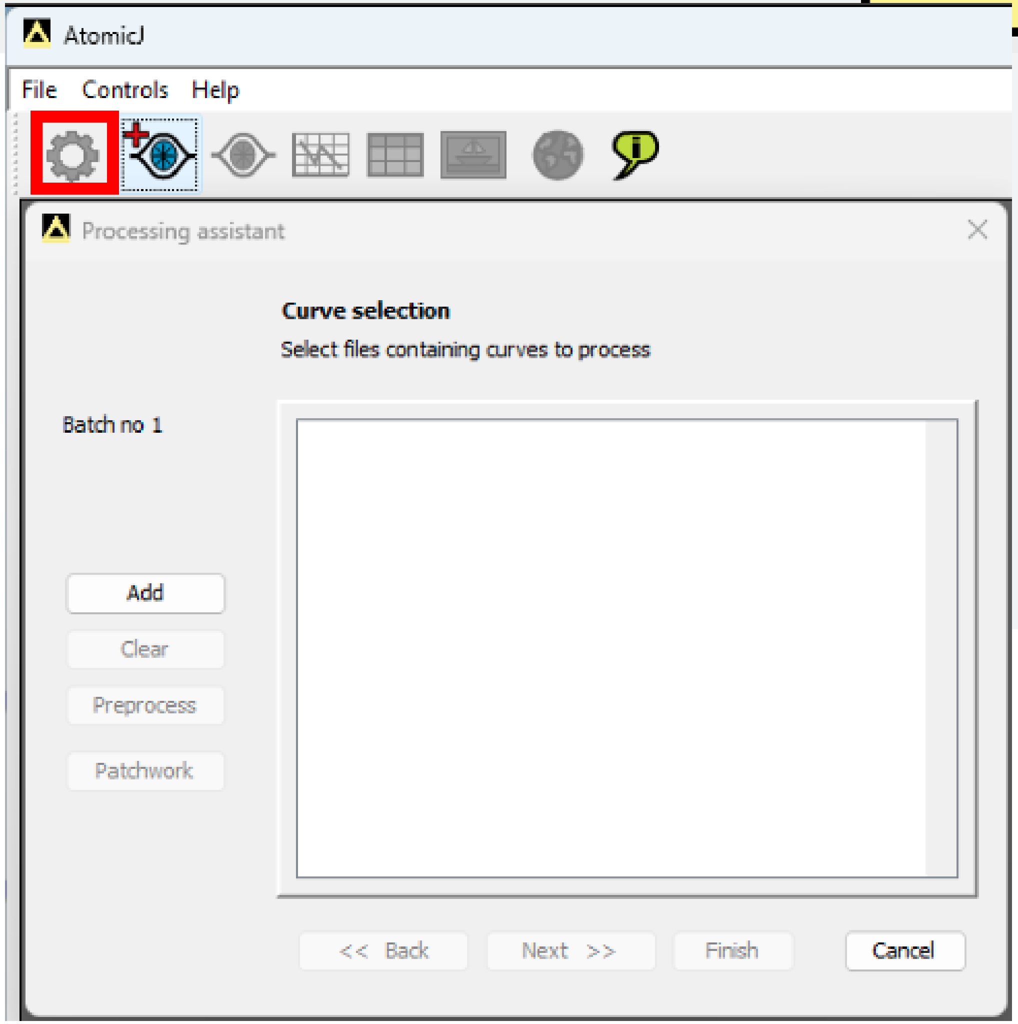
Adhesion

Force distance curve (FDS)

- Cantilever approach the surface, press with defined force (setpoint) and the withdraw
- Resulting vertical deflection vs. distance (height) curve is FDS
- We can obtain Young's modulus (*E*) by fiting the interaction part with non-linear function
- Single point or multiple point (Force imaging, Peakforce, QI, etc.)
- Software: **AtomicJ**, JPK Processing, Nanoscope, Mountain SPIP

Processing FDCs

- High quality third-party software for processing AFM mechanical data



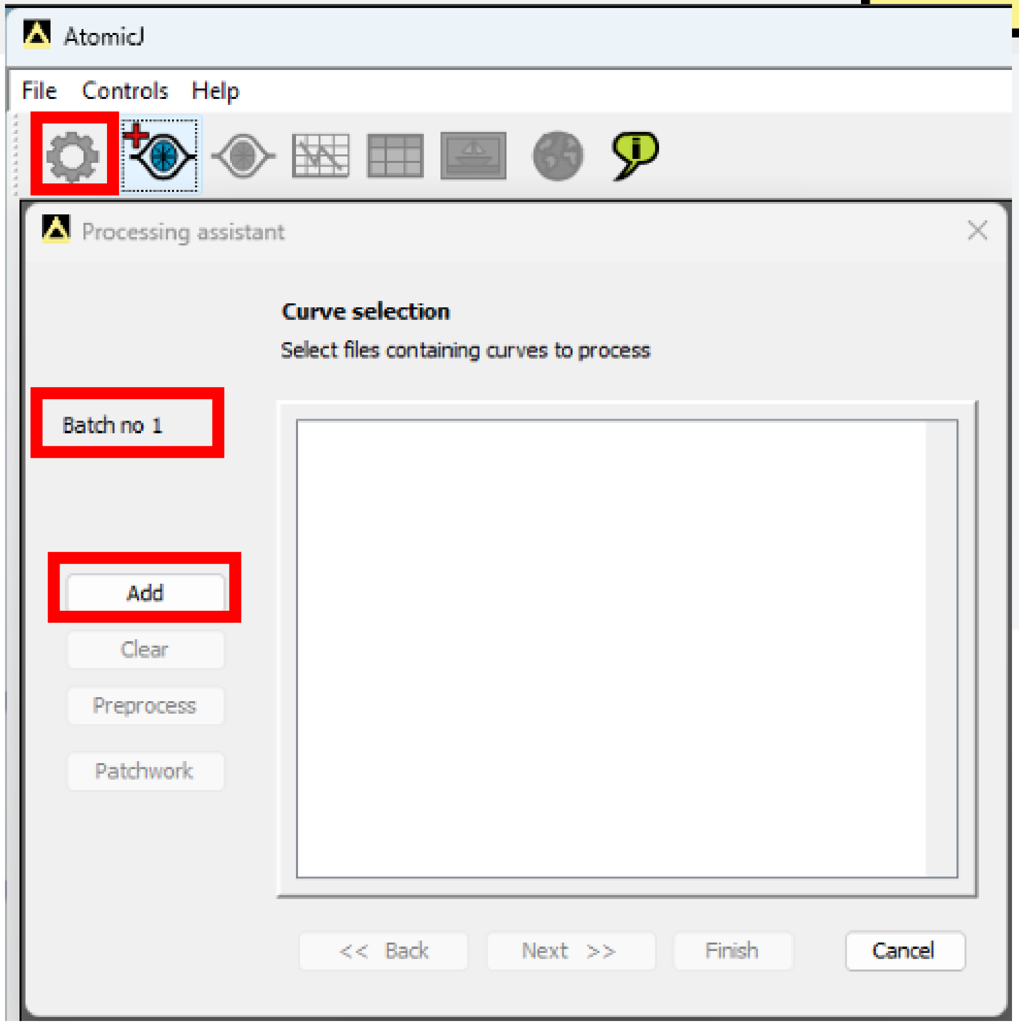
Curve selection

Select files containing curves to process

Batch no 1

Processing FDCs

- High quality third-party software for processing AFM mechanical data
- Suported data files: JPK, Nanoscope, Innova, Asylum, NT-MDT and Park
- Batch processing



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Processing FDCs

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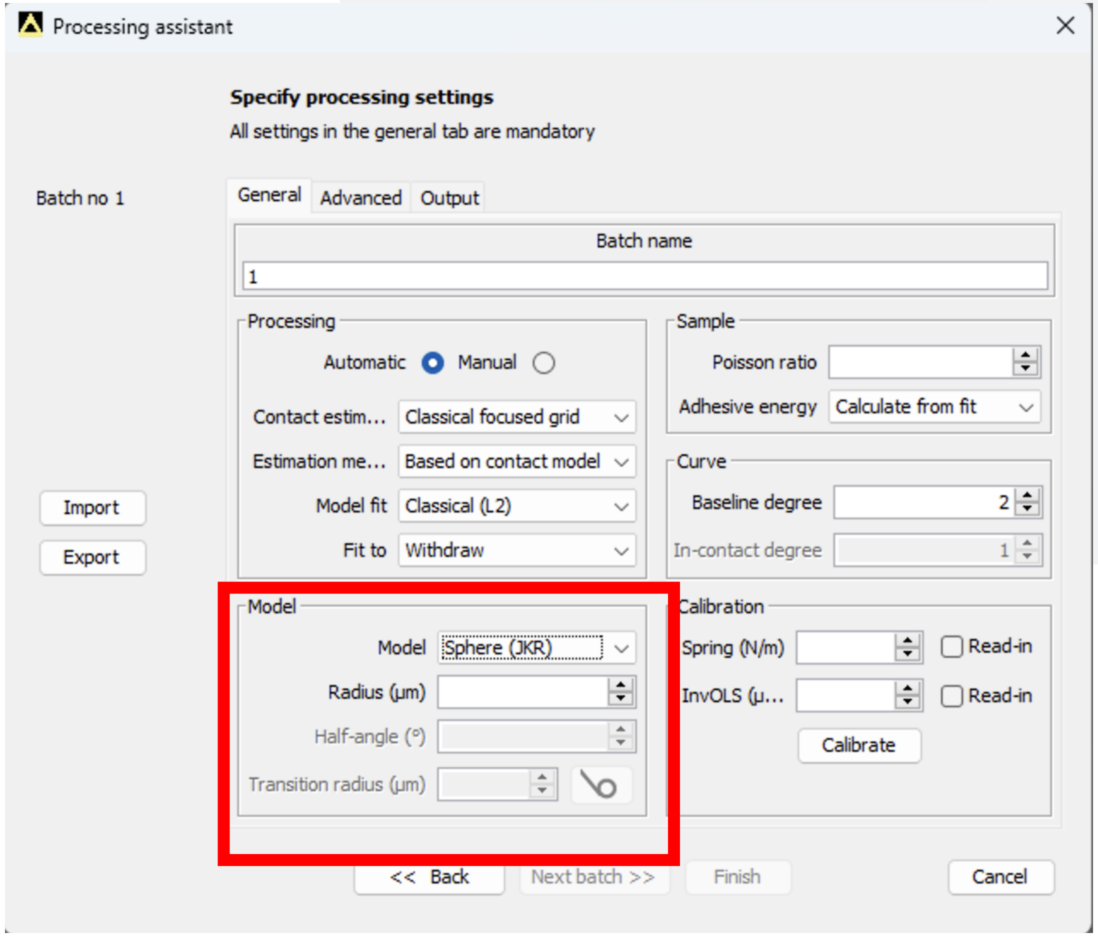
- Various models for sphere, pyramidical, conical, hyperboloid tips etc.



Processing assistant

Specify processing settings

All settings in the general tab are mandatory

Batch no 1

General | Advanced | Output

Batch name: 1

Processing

Automatic ● Manual ○

Contact estim… Classical focused grid

Estimation me… Based on contact model

Model fit Classical (L2)

Fit to Withdraw

Sample

Poisson ratio

Adhesive energy Calculate from fit

Curve

Baseline degree 2

In-contact degree 1

Model

Model Sphere (JKR)

Radius (µm)

Half-angle (°)

Transition radius (µm)

Calibration

Spring (N/m) ☐ Read-in

InvOLS (µ… ☐ Read-in

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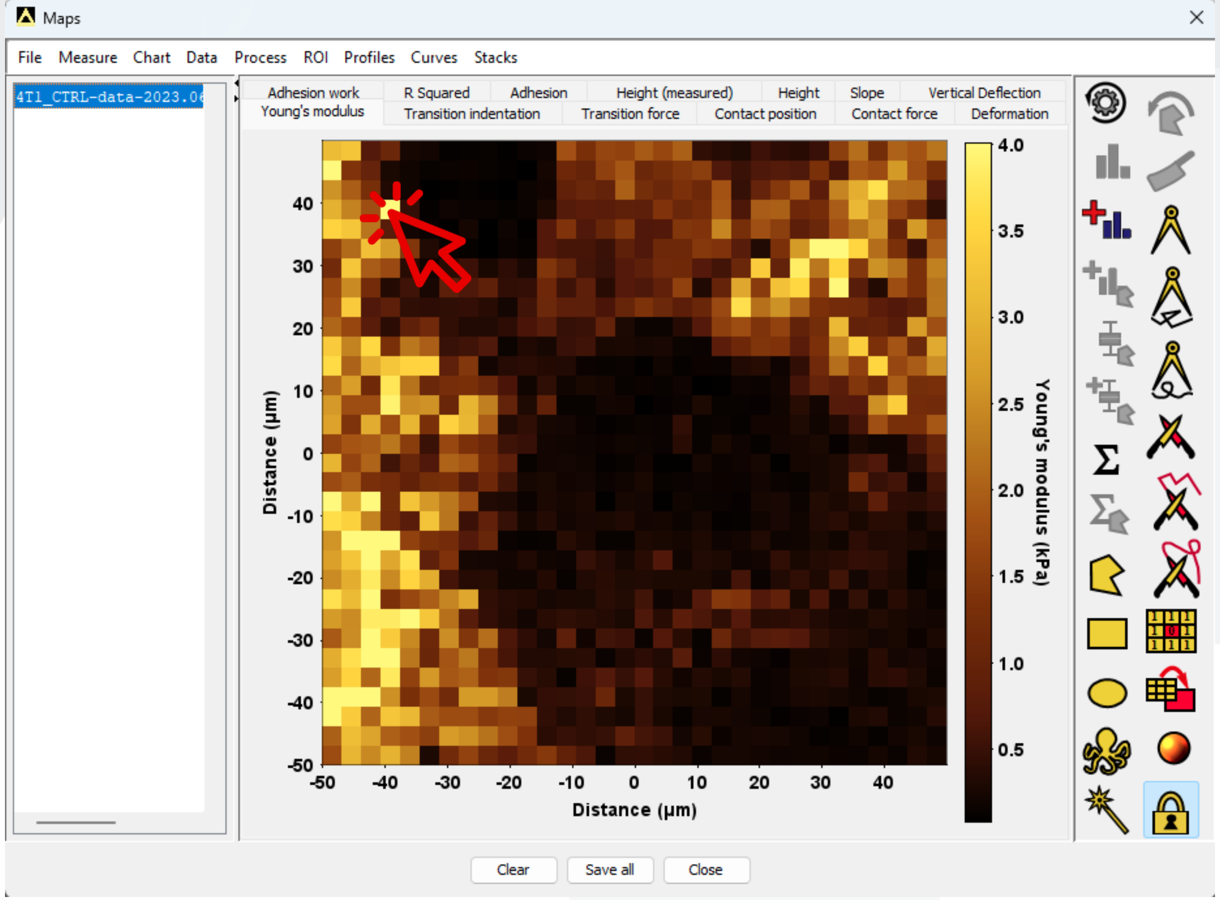

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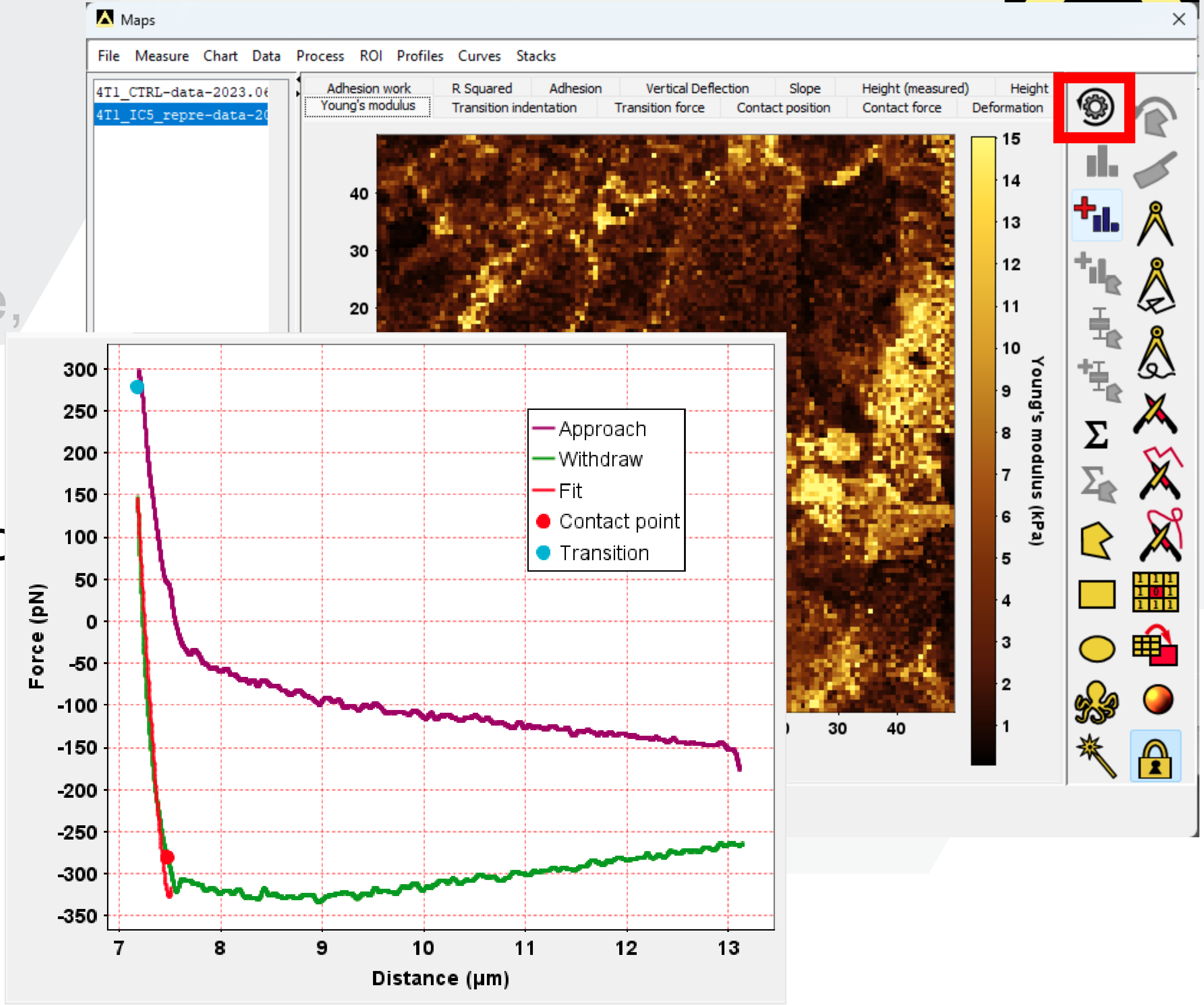

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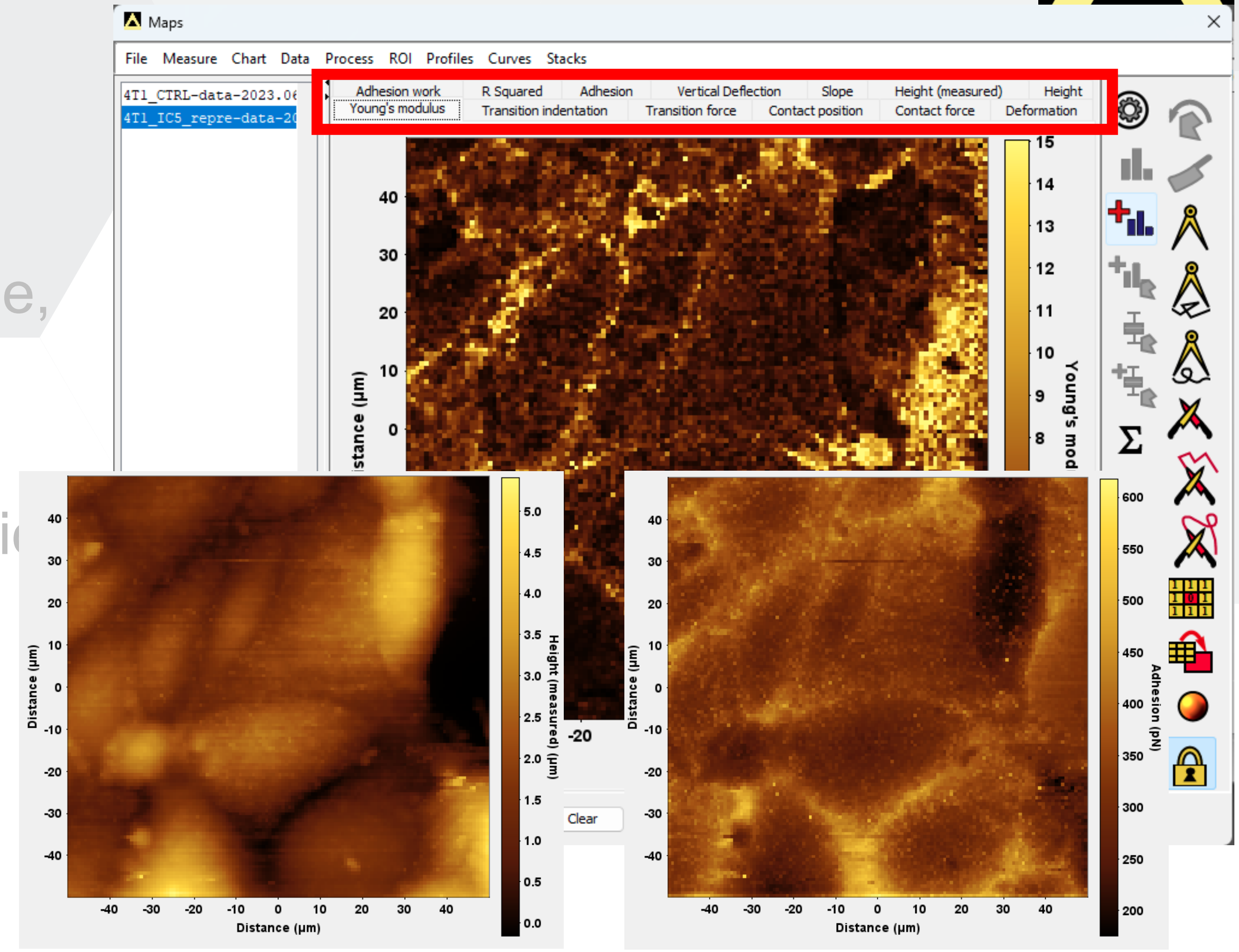
- High quality third-party software for processing AFM mechanical data
- Suported data files: JPK, Nanoscope, Innova, Asylum, NT-MDT and Park
- Batch processing
- Various models for sphere, pyramidi conical, hyperboloid tips etc.
- **Program calculate lots of different channels**

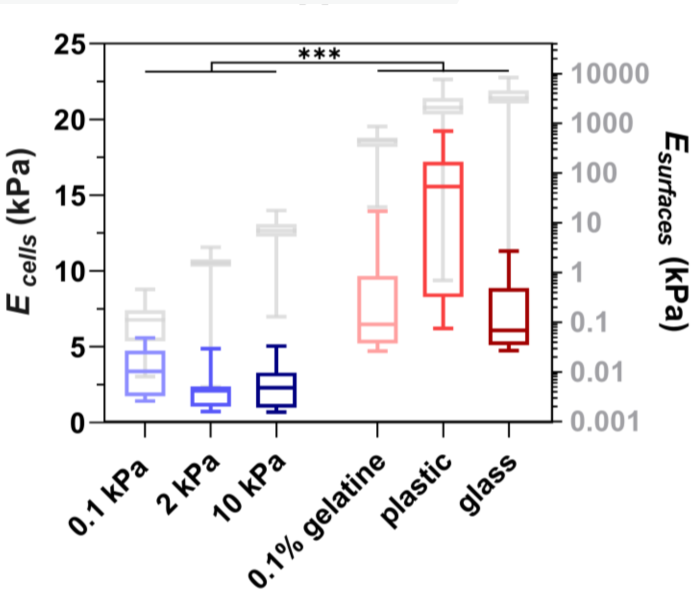


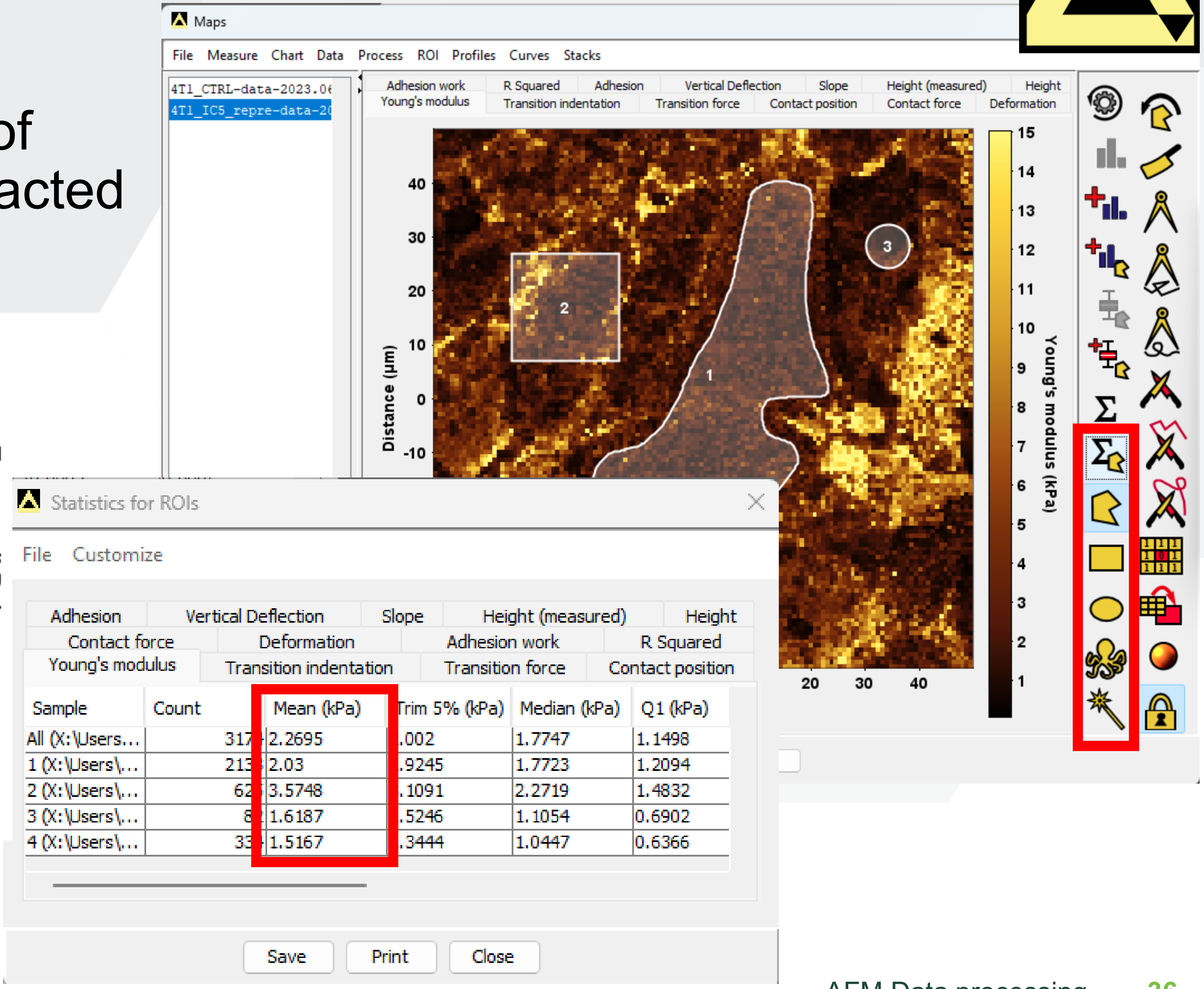
Height

Adhesion

Processing FDCs

- Many tools for selecting regions of interest from which data can extracted

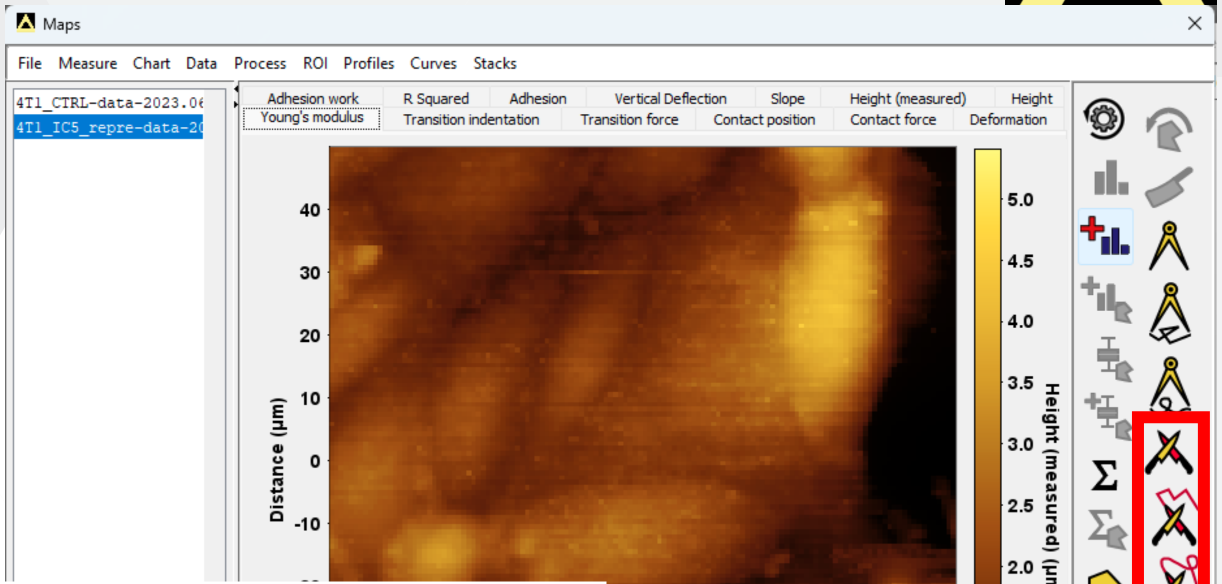


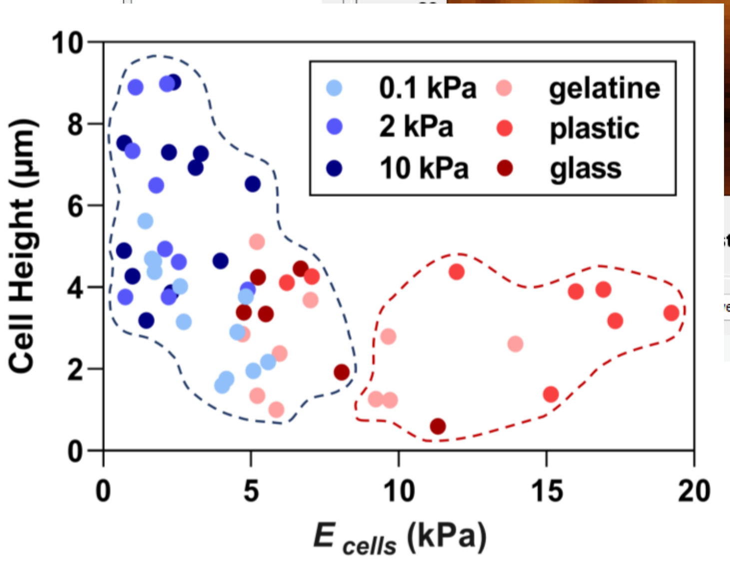


Sample	Count	Mean (kPa)	Trim 5% (kPa)	Median (kPa)	Q1 (kPa)
All (X:\Users…	317	2.2695	.002	1.7747	1.1498
1 (X:\Users\…	213	2.03	.9245	1.7723	1.2094
2 (X:\Users\…	62	3.5748	.1091	2.2719	1.4832
3 (X:\Users\…	8	1.6187	.5246	1.1054	0.6902
4 (X:\Users\…	33	1.5167	.3444	1.0447	0.6366

Processing FDCs

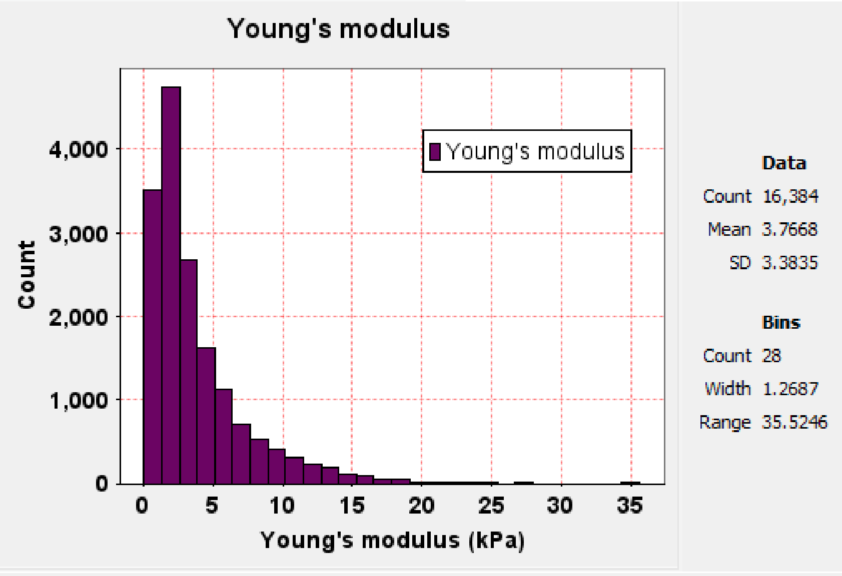
- Many tools for selecting regions of interest from which data can extracted
- Line profiles measurements





Proccesing FDCs

- Many tools for selecting regions of interest from which data can extracted
- Line profiles measurements
- Histograms and box plots

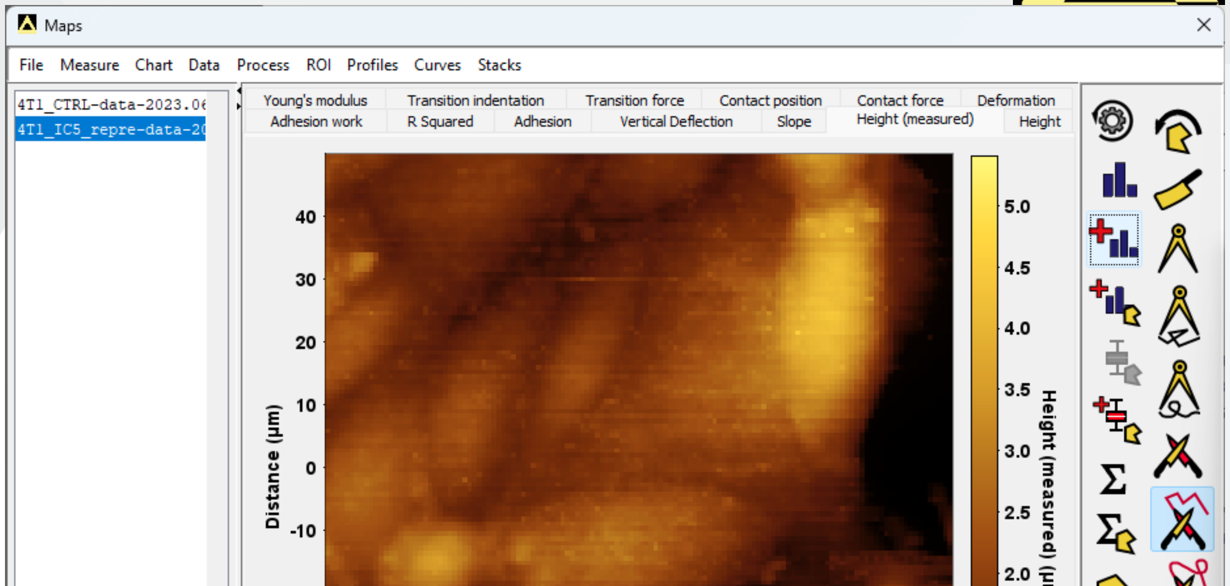


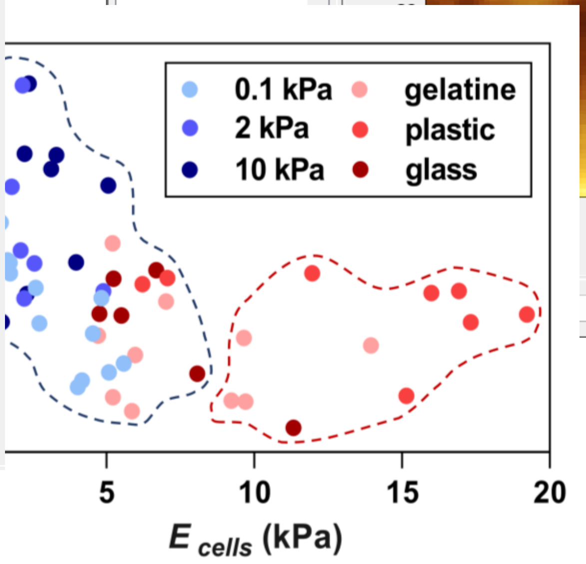
Data

Count	16,384
Mean	3.7668
SD	3.3835

Bins

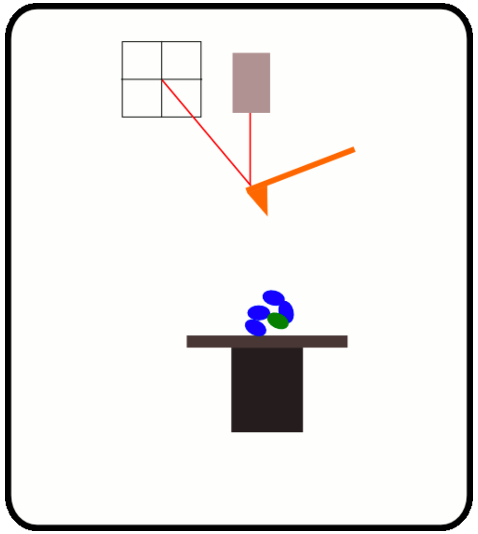
Count	28
Width	1.2687
Range	35.5246

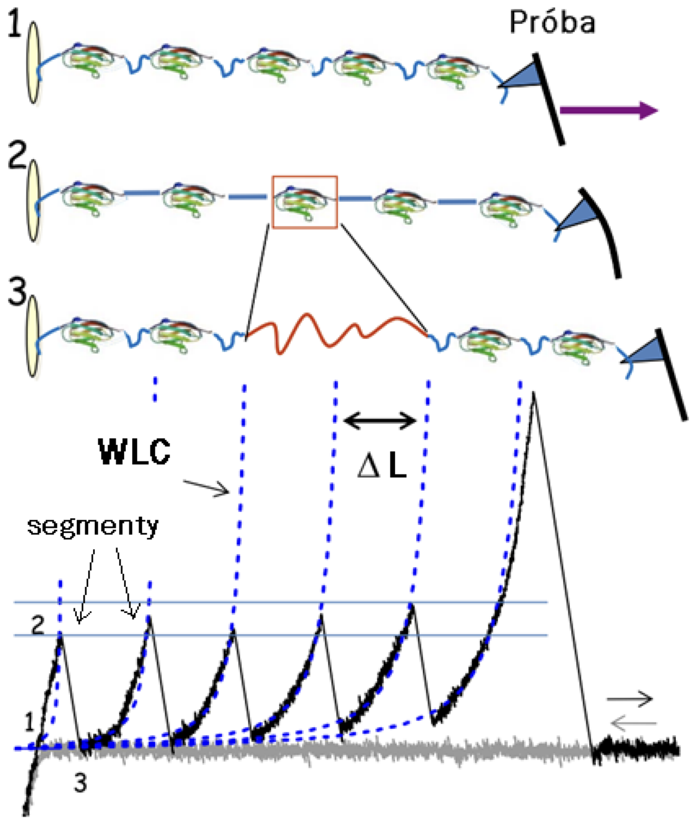




Single molecule force spectroscopy (SMFS)

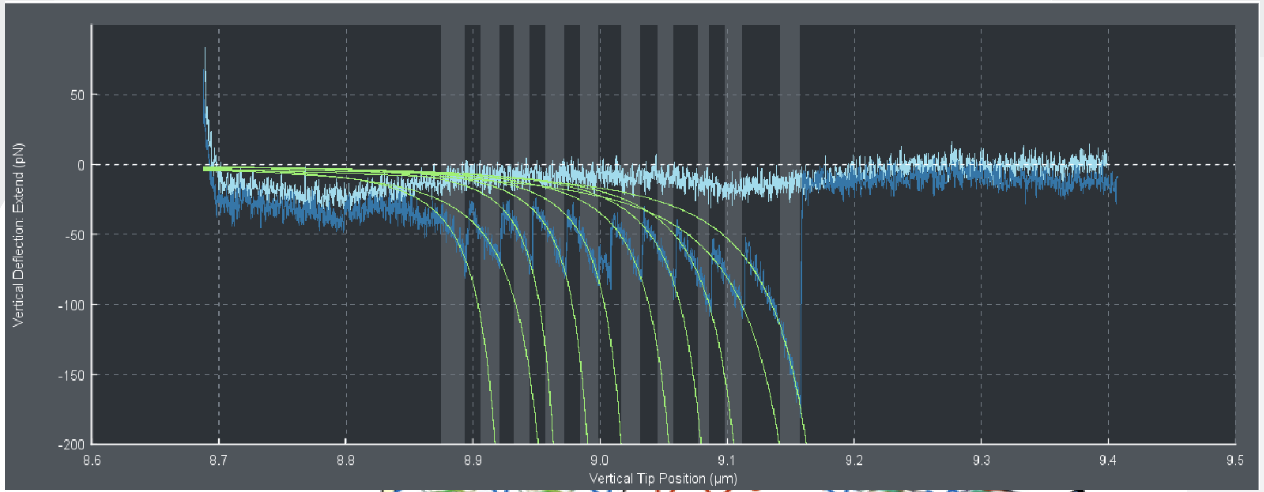
- Protein domains unfolding by means of AFM.
- Usually, protein is immobilized on the surface with golden tip cantilever approaching.

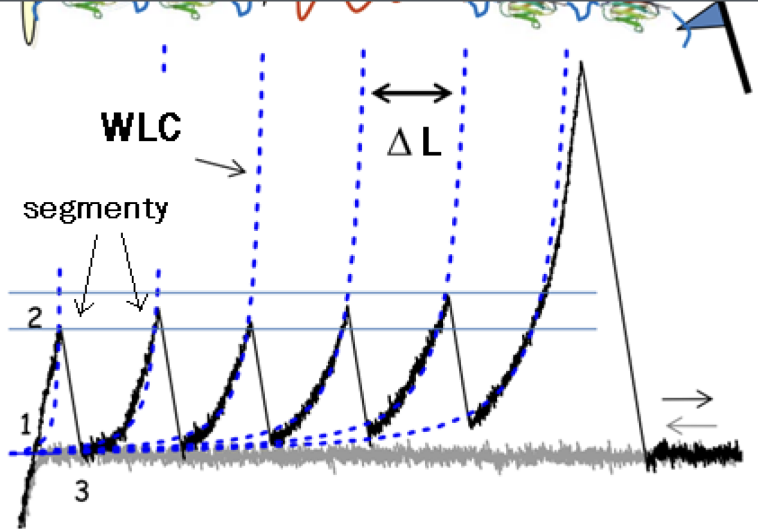




Single molecule force spectroscopy (SMFS)

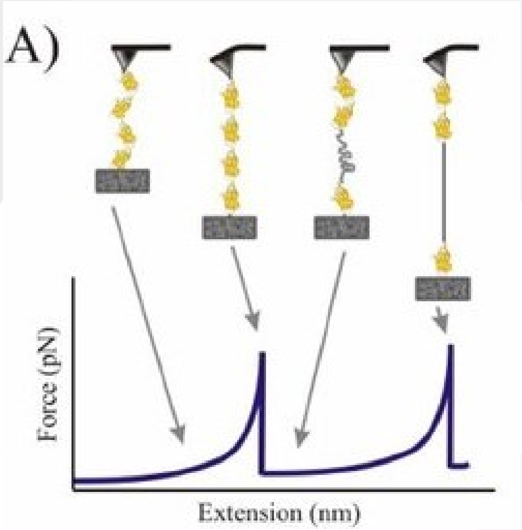
- Protein domains unfolding by means of AFM.
- Usually, protein is immobilized on the surface with golden tip cantilever approaching.
- FDCs have typical ”sawtooth pattern” representing each domain folding event
- Curve can be fitted with Worm-like chain model in JPK Processing software.
- Unfolding force, Length

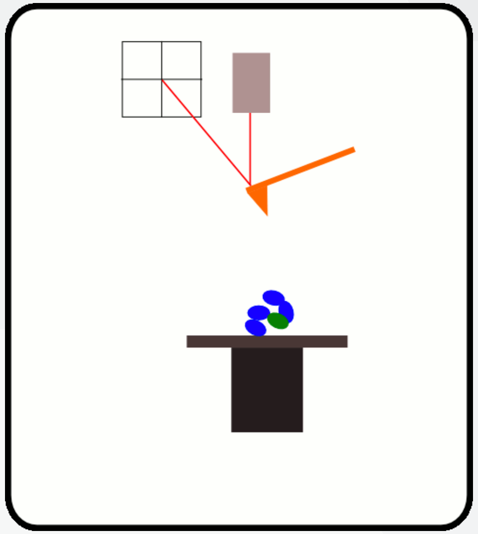


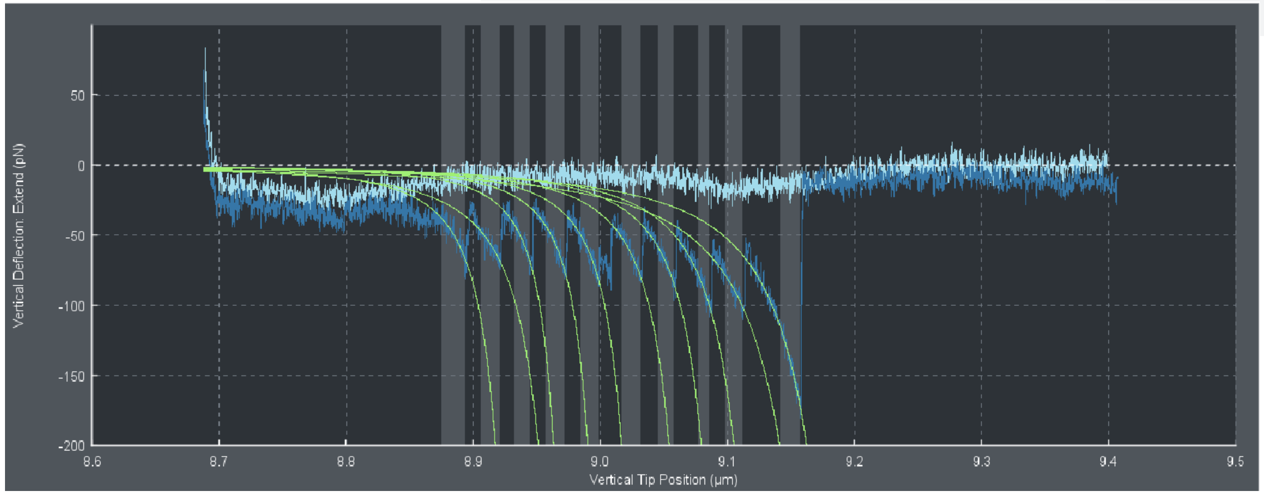


Single molecule force spectroscopy (SMFS)

- *Lorem i*

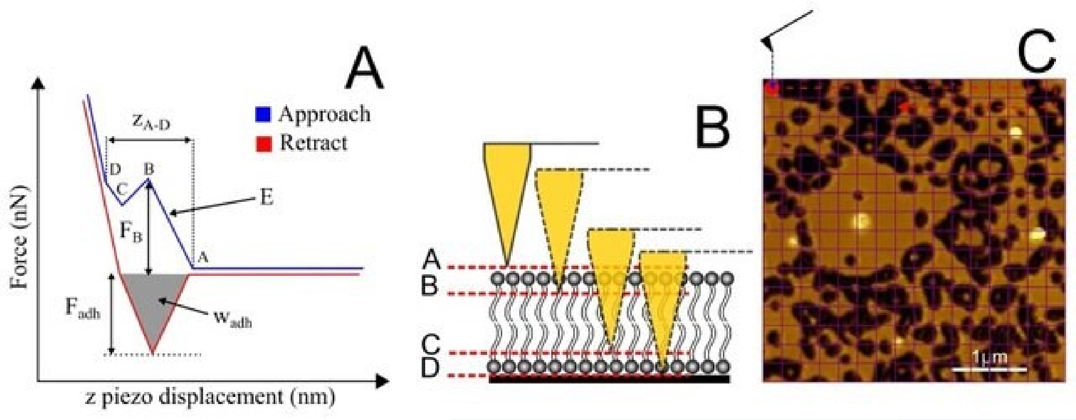


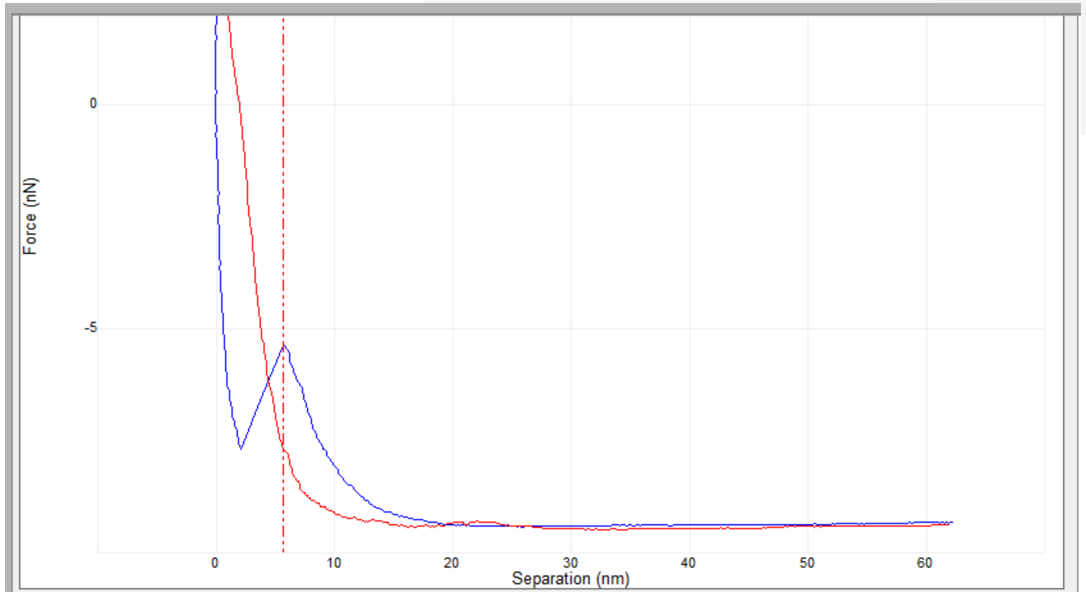




Scanning of lipid bilayers by AFM

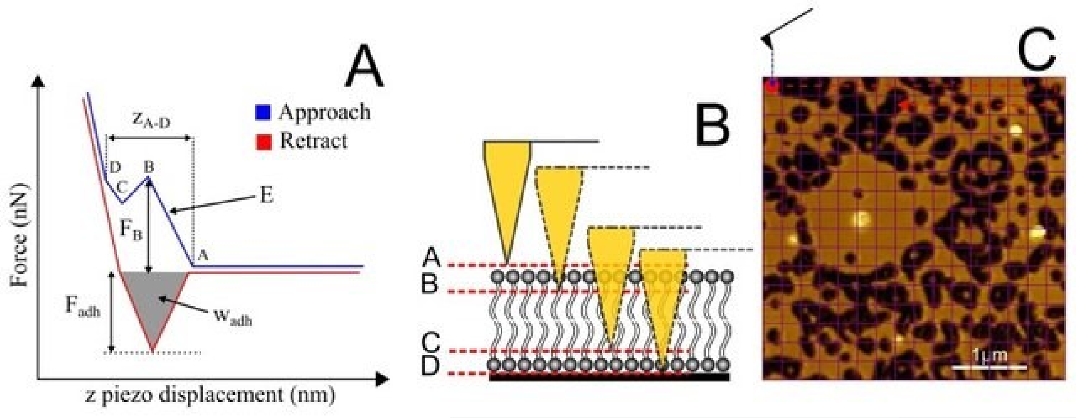
- Scanning of phospholipidic bilayer (PLB) by AFM.
- When applying force during FDC rupture of PLB occur (= rupture event)

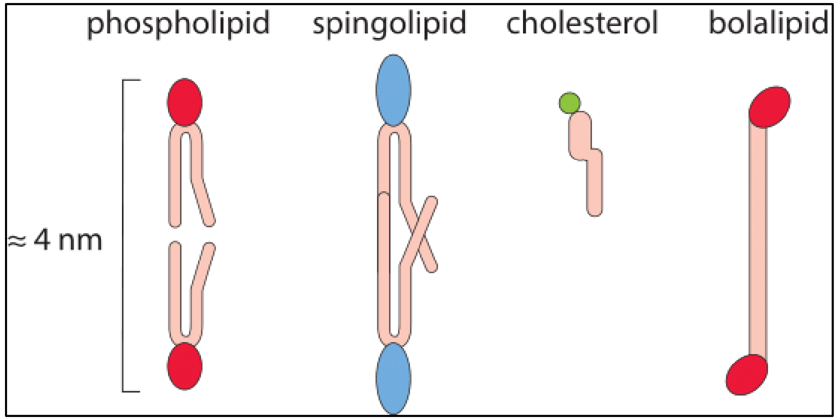


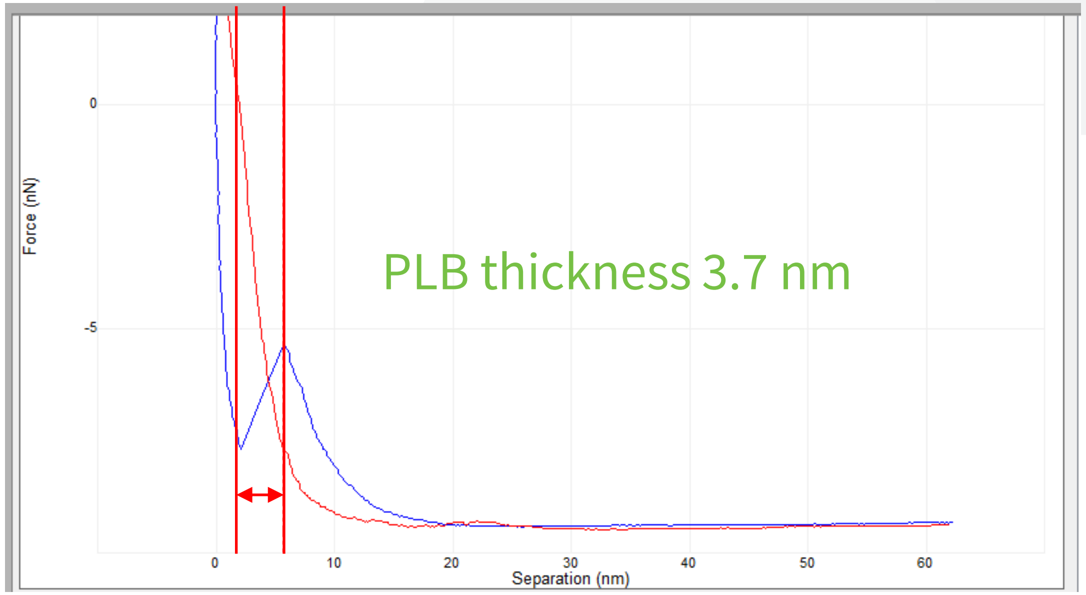


Scanning of lipid bilayers by AFM

- Scanning of phospholipidic bilayer (PLB) by AFM.
- When applying force during FDC rupture of PLB occur (=rupture event)
- Difficult analyzing but we are now working on automatization of the process.







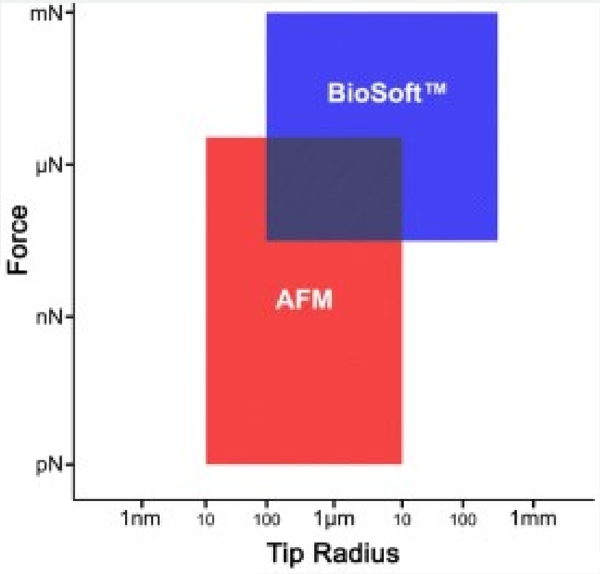
PLB thickness 3.7 nm

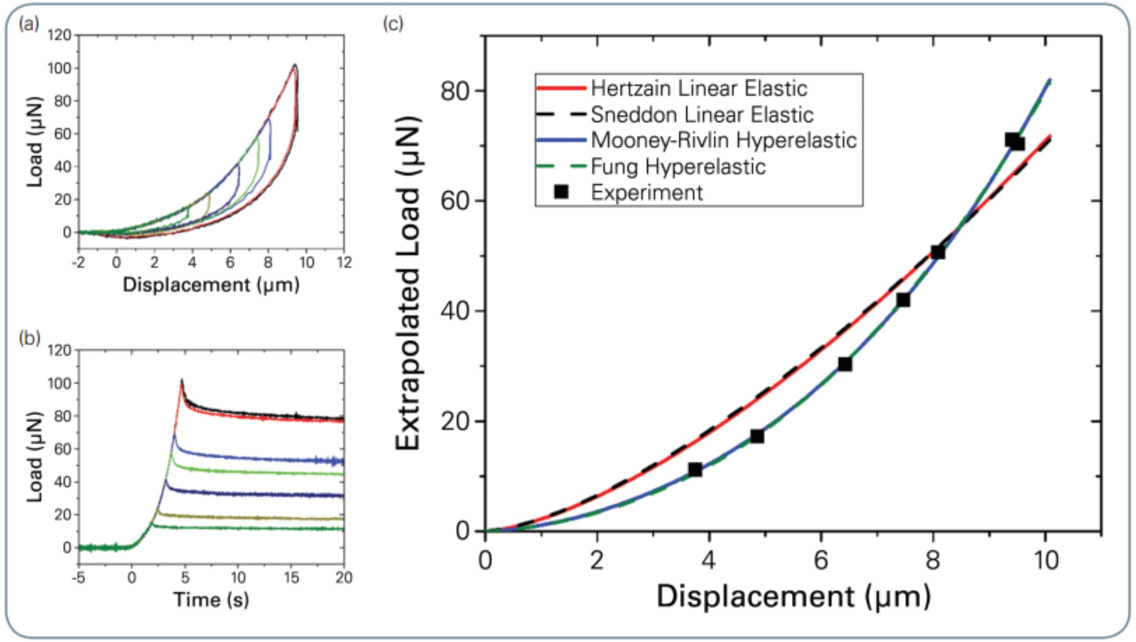
Layout of the presentation

- Types of AFM data
- Imaging
  - AFM images – leveling data, artefacts, surface reconstruction, masks, analysis, LAFM
  - Real time scans of vertical deflection – contraction properties of CMs, peaks detection, HRV analysis
- Force spectroscopy
  - What is a force distance curve (FDC)?
  - Young`s modulus – models, analysis
  - Alternative analysis of FDCs – SMFS, thickness of lipid bilayers, rupture events
- Indentation
  - Rheology analysis, viscoelasticity

Indentation analysis

- Mechanical characterization of soft materials
- Indentation are usually in μm, where hyperelastic effects are more pronounced and normal elastic models are not correct.





Indentation analysis

- Mechanical characterization of soft materials
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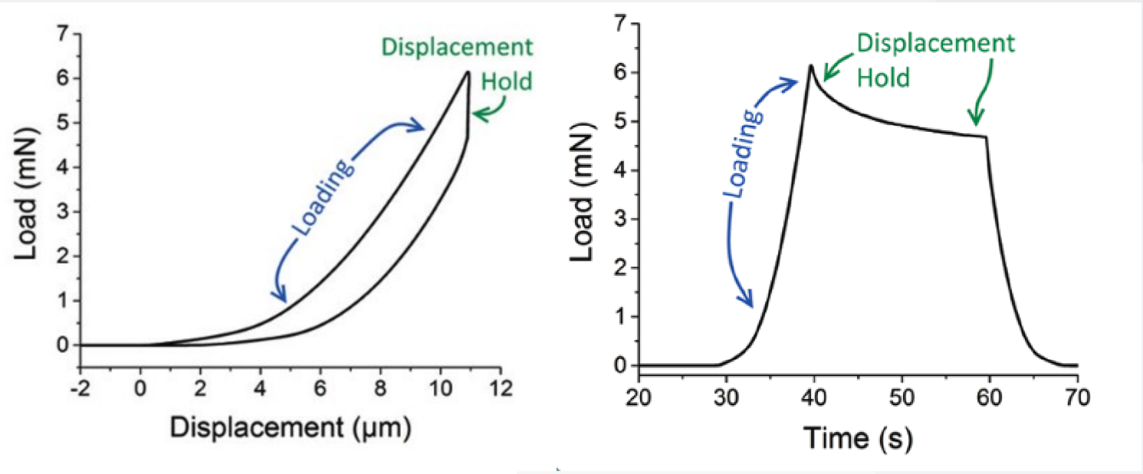
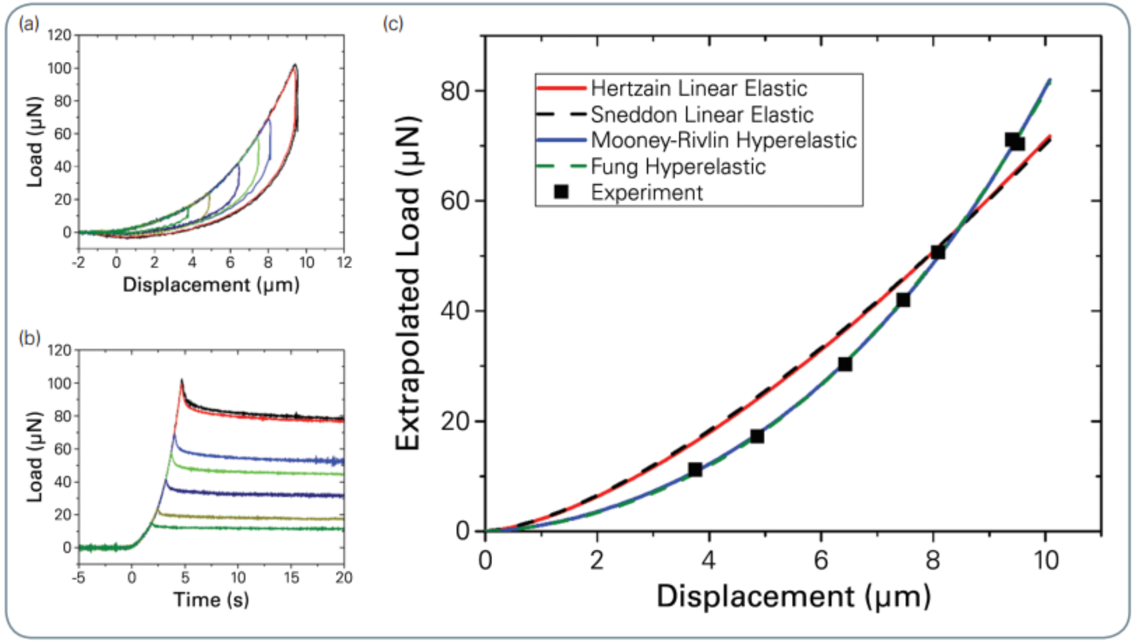
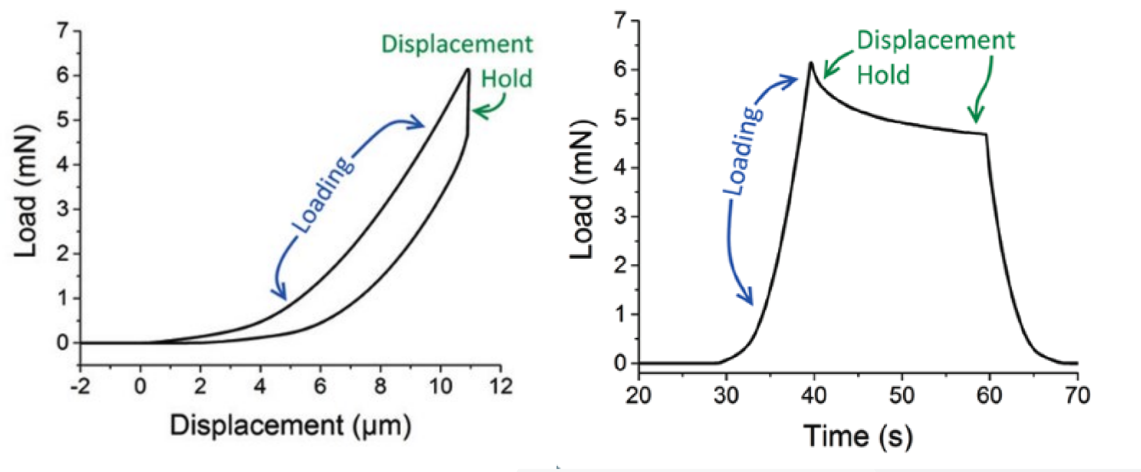
- Additional hold segment is measured and fitted with hyperelastic models

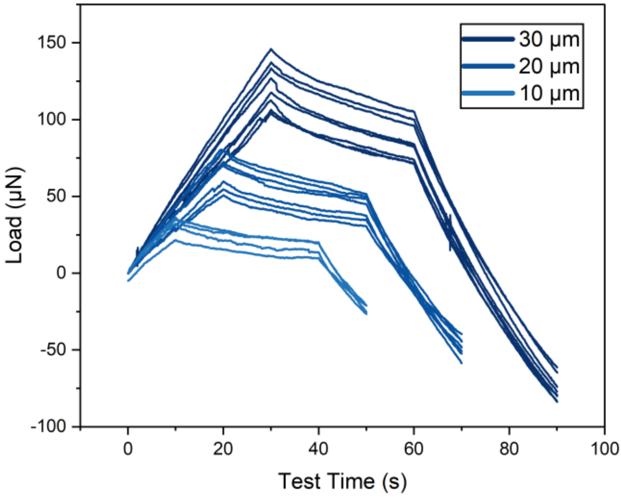


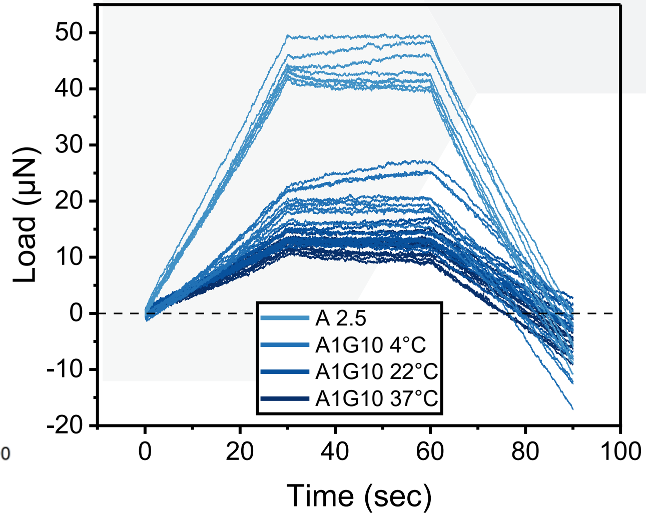


Indentation analysis

- Indentation are usually in µm, where hyperelastic effects are more pronounced and normal elastic models are not correct.
- Additional hold segment is measured and fitted with hyperelastic models
- Typical samples are **hydrogels**, cartilage samples, tissue sample
- More information in practical session Thursday at 13:00 – L. Pařízek, MTM







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Thank you for your attention!

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