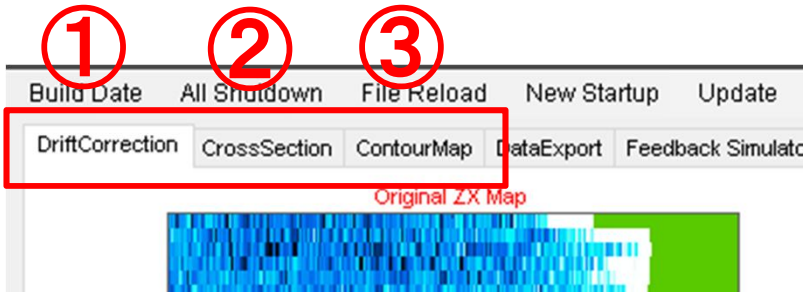
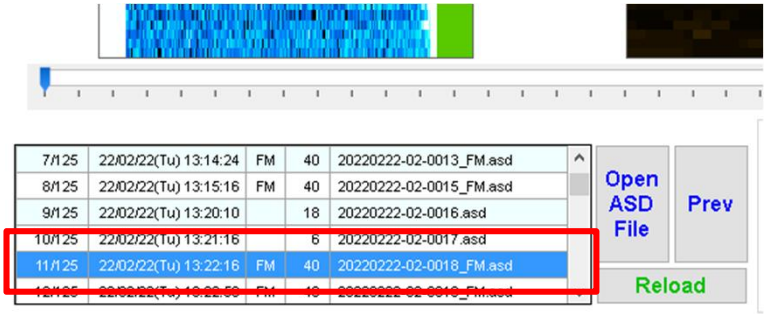
Quick Guide for UMEX 3D Force Map Viewer

1. Overview of Analysis Process

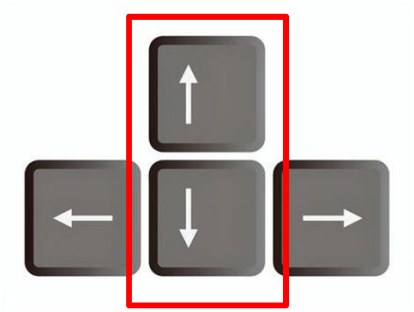


Data is analyzed in order of
①DriftCorrection
②CrossSection
③ContourMap

2. Open of ASD File



Drag-and-drop an ASD file onto anywhere on software to open it



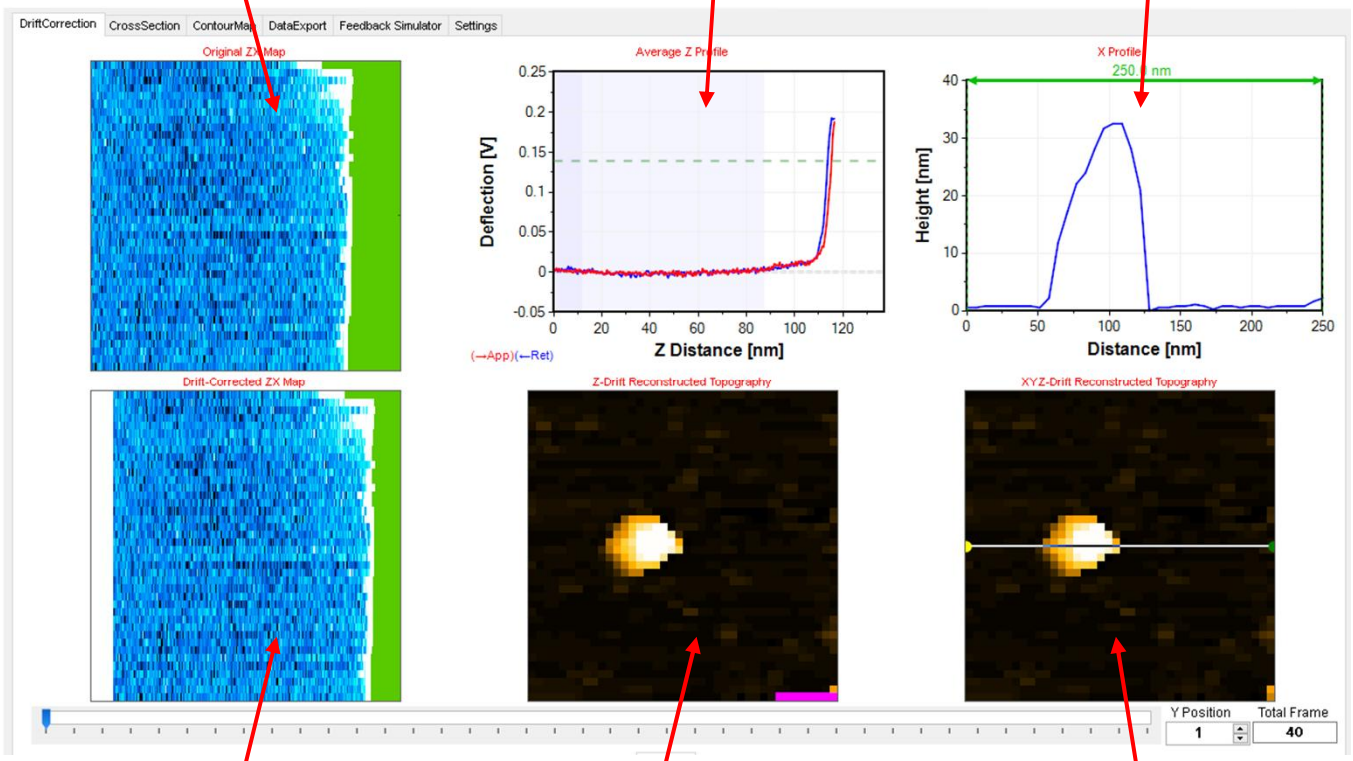
Opened file can be changed by up and down keys in any tabpage.

3. DriftAnalysis

ZX cross section image before Z drift correction

Force curve averaged over displayed ZX map

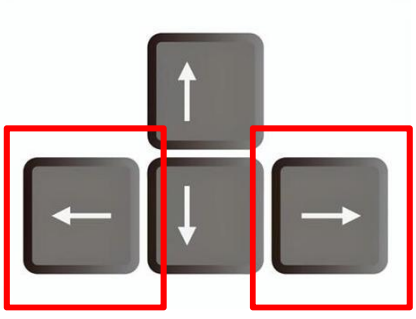
Line profile extracted at the line on the image below



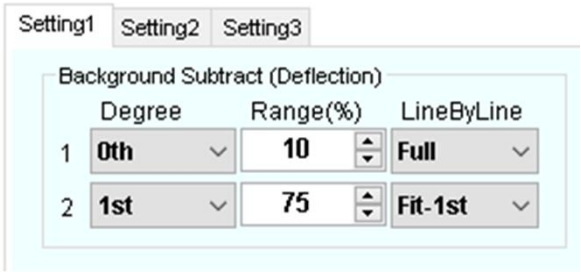
ZX cross section image after Z drift correction

Topographic image reconstructed at the height where the signal was triggered

Reconstructed image after shear correction



Slide bar can be controlled by left and right keys.



Degree of polynomials:

By increasing the degree, curved lines can be corrected straightly, but the curve near the surface is often wrongly corrected.

1st step is always set to 0th, and 2nd step is set to 1st or 2nd depending on the curvature of profile.

Overview:

The force curves are flattened by fitting polynomials and subtracting it in the far region of force curve.

Although two-step subtractions are conducted, only setting of the second step should be adjusted.

Range:

Set the range of polynomial fitting. Only the region where the tip-sample force is not occurred.

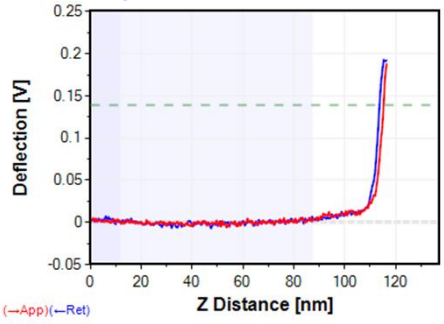
LineByLine:

Set whether to flatten the profile each X position or not

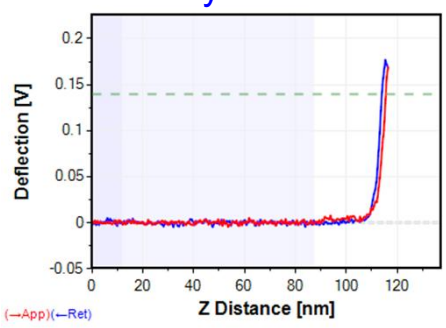
Fit-1st –3rd: After polynomial flattening, the obtained polynomial factors of each force profile are fitted by polynomial and the site dependency is subtracted.

Full: Polynomial flattening is conducted in each Z profile. Disadvantage of Full mode is that when the degree is 1st or 2nd, the flattening often oversubtract the fluctuation.

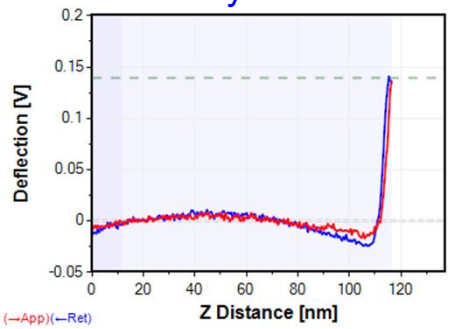
Original Force curve



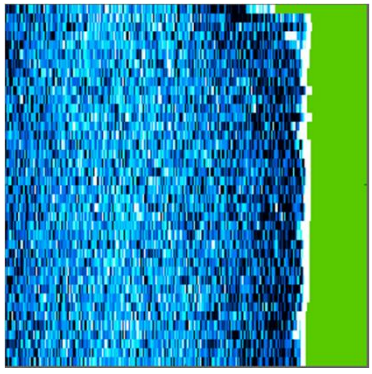
Correctly Fixed Curve



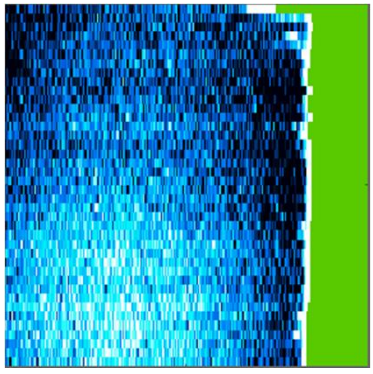
Incorrectly Fixed Curve



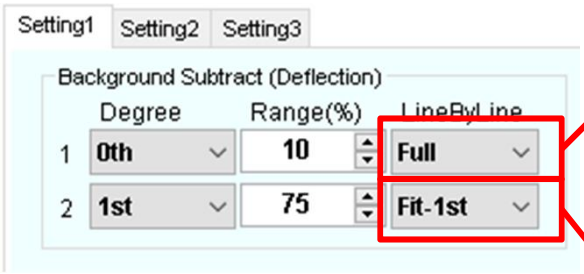
Correctly Fixed Map



Incorrectly Fixed Map



Cautions

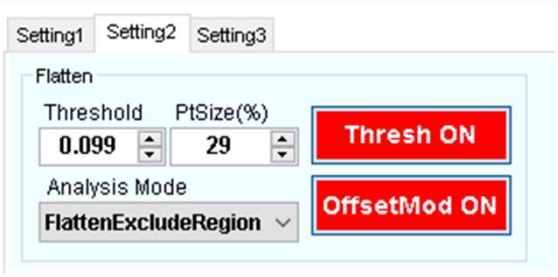


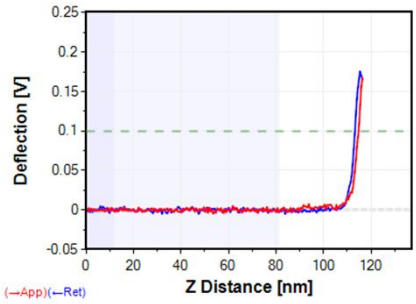
Keep this fixed to Full at all times.

Set the option to anything other than Full whenever possible.
Use Full only as an exception when the noise level is too high and other settings do not work.

For Background Subtract, “LineByLine” in section #1 should always be set to “Full.”
In contrast, setting “LineByLine” in section #2 to “Full” can introduce artifacts in data such as energy dissipation when analyzing surfaces with large height variations, even if the setting appears to work correctly at first glance.
The Full option in section #2 should be used only when the variation in force curves is so large that non-Full settings cannot produce a flat background.
In such cases, you must check the force curves to ensure that the analysis is valid.

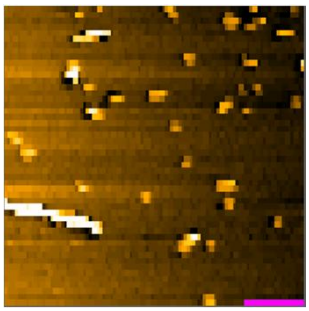
Flatten





Position of threshold value (green broken line in the chart) is aligned to the same height so that offset height of each X line is flattened. Threshold should normally be set to 50% of maximum deflection.

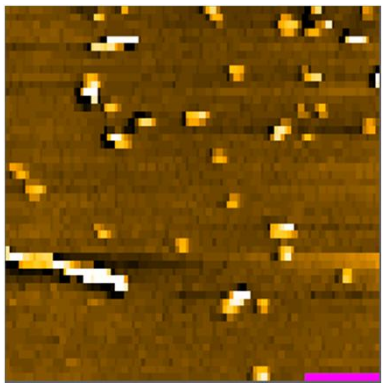
Tilt Correction



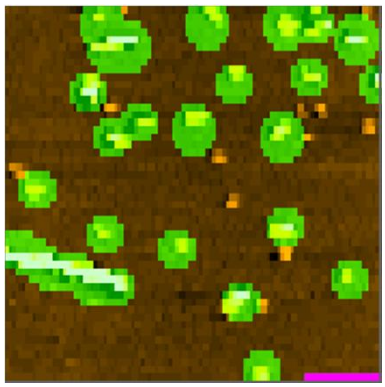
Tile Correction is used for correcting a tilt of surface as shown in the left image.
Use Global setting and adjust Offset value when the tilt is not varied in all the Y positions.
Use LineByLine when the tilt is varied in each X line due to a severe drifting.

LineByLine TiltCorrection often causes an artificial line noise at the line where molecules exist. In such a case, the molecules should be excluded from the TiltCorrection by dragging a left mouse button to set the exclusion region. The size of drawing can be adjusted by a mouse wheel. By pressing a right or middle click button, the excluded region can be deleted.
This operation is annoying, and the force map experiment is better to be performed with no drifting condition.

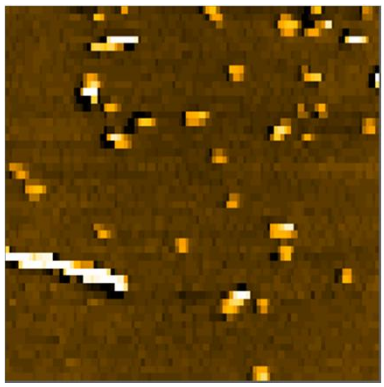
Original Image



Exclude Region



Hide Exclude Region

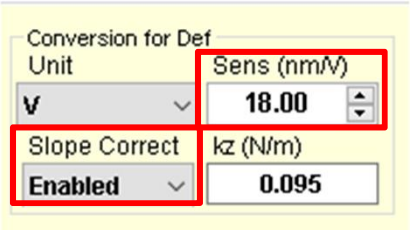


4. CrossSection

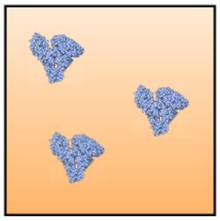
The deflection sensitivity and spring constant kz calibrated using FFT spectrum are saved in ASD files and displayed in Brownian Info. Normally, these parameters do not need to be changed.

The deflection sensitivity should be calibrated by a force curve measurement on a hard surface such as mica, and they should be saved as a default value.

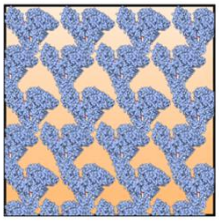
For HS-AFM, normally, a quotient signal, which is deflection signal normalized by the sum value, is used, and the sensitivity does not significantly changed in each experiment. When the quotient gain is set to 3.0, the sensitivity is in the range of 10~20 nm/V.

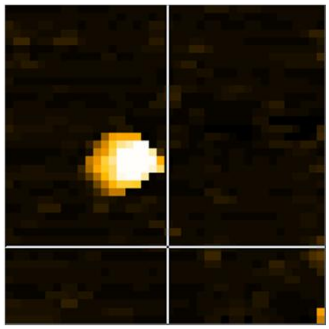


When a hard mica surface is exposed to the surface, the sensitivity can be measured from 3D force map data.



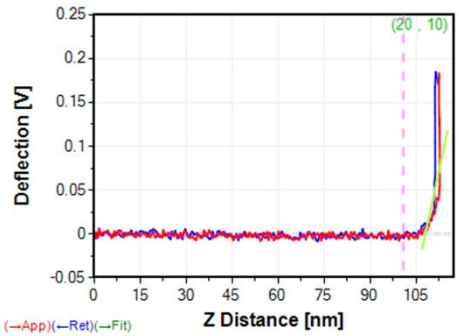
However, when soft molecules cover a whole surface, the sensitivity should be calibrated on mica that is cleaved again after 3D force map experiments.



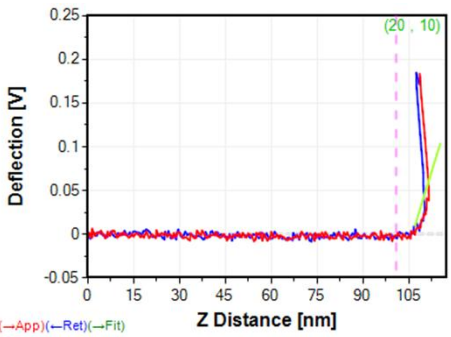


When the mica surface appears, the sensitivity can be calibrated using the 3D force map data.
By positioning the crossing lines on AFM image to mica surface, the force curve on mica can extracted.
After enabling the Slope Correct, Sen(nm/V) should be adjusted so that the slope of force curve becomes straight.

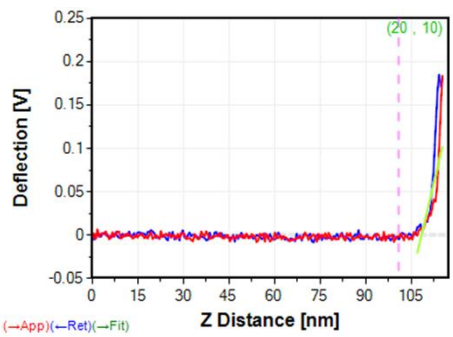
Good Setting



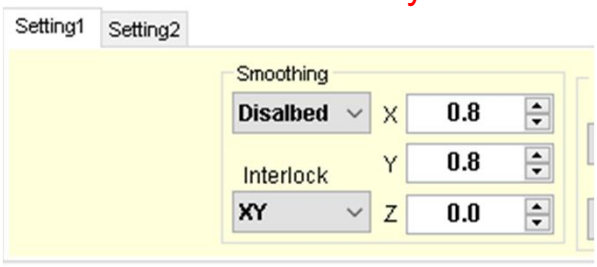
Bad Setting



Bad Setting



This must be disabled during quantitative analysis.

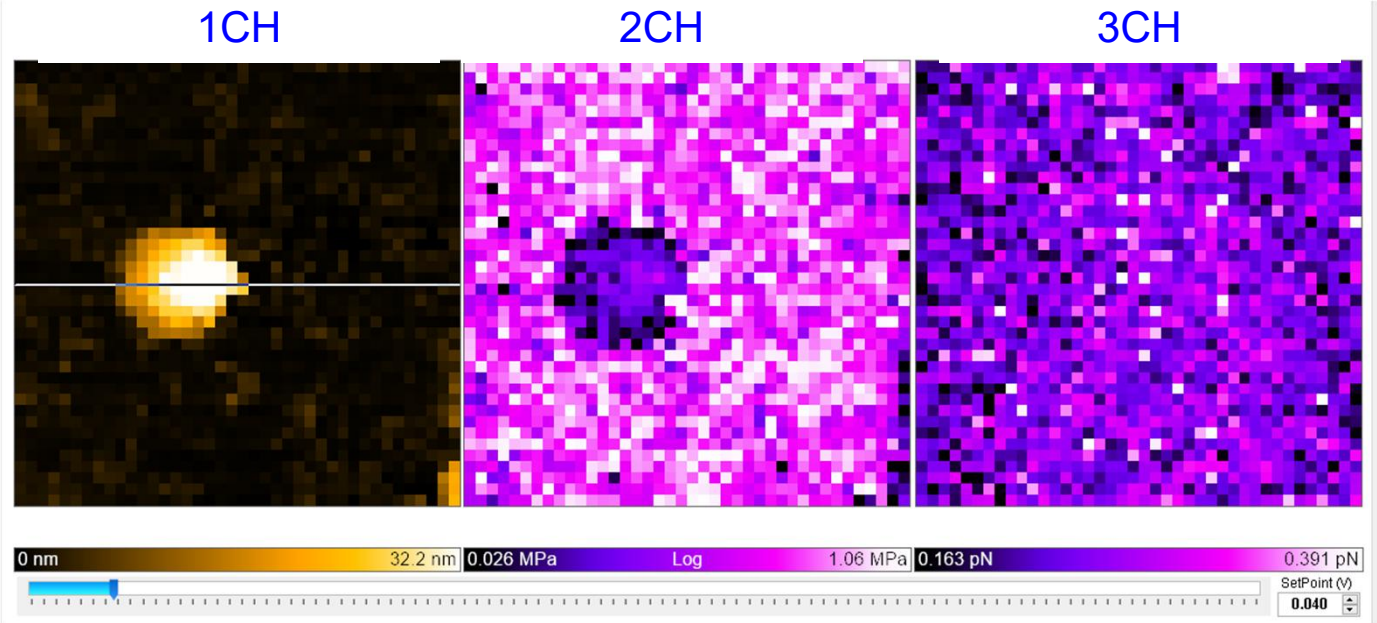


A Gaussian smoothing is effective for reducing the image noise. But this deteriorates the accuracy of the Hertz fitting of the Young's modulus, and hence it should be disabled when quantitative measurement is needed.

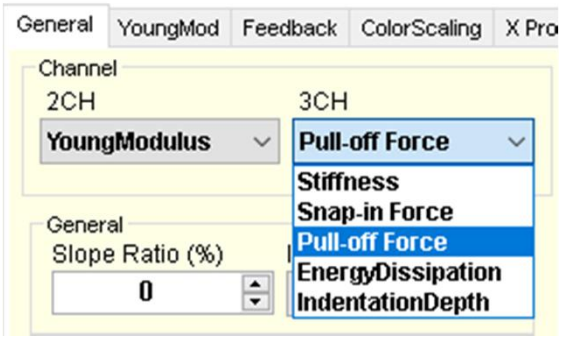
Cautions

When XY smoothing is enabled, averaging is applied across neighboring force curves in the lateral direction. As a result, if there is a large difference in the surface position between adjacent force curves—for example, near steps or molecular edges—the repulsive region from a neighboring line may appear at a position separated from the actual sample surface. This can interfere with quantitative evaluation. Therefore, this function should be set to Disabled during standard quantitative analysis, and should be enabled only when visualizing an XYZ map.
Smoothing in the Z direction does not inherently cause problems; however, setting a large value may affect the analysis. Thus, the Z setting should also be set to Disabled whenever possible.

5. ContourMap

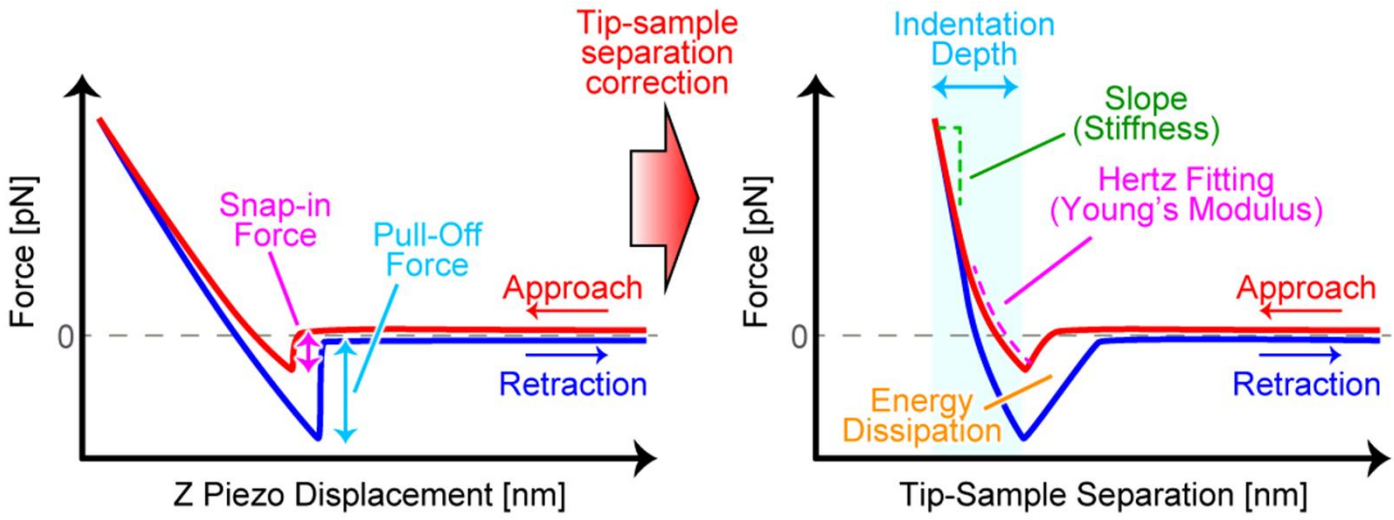


1CH: ContourMap of deflection is displayed.
Setpoint used can be set by a slider below the images.



2CH:
Only Young's modulus is used

3CH:
Select signals from the list below
Stiffness
Snap-in Force
Pull-off Force
Energy Dissipation
Indentation Depth



Young's Modulus (Pa):
A mechanical property that reflects the material elasticity. They can be compared among different morphologies and materials. To quantitatively estimate the value, the exact tip radius and surface morphology are required. The force profile near a surface is fitted to the Hertz model using the least-square method. There are three theoretical models, Hertz, JKR, and DMT, but currently, only the Hertz model is implemented because of its wide applications.

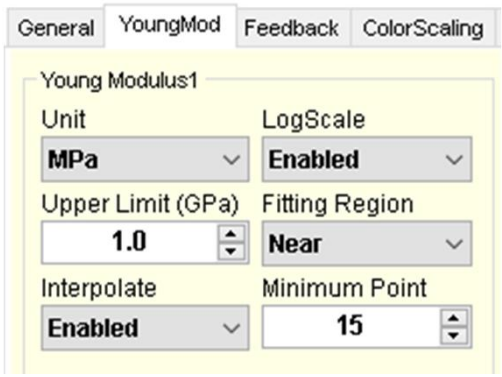
Stiffness (N/m):
A mechanical property that is similar to Young's modulus.
This can be calculated from the slope of force profile after the tip indents into the surface more deeply than Hertz fitting region. Young's modulus is inherent to materials while the stiffness reflect the total elasticity including the tip radius and surface morphology. This is frequently utilized for virus, cell, and exosome experiments because they do not have a uniform Young's modulus due to a hollow structure. Although using Thin-Shell model, Young's modulus of such hollow structures can be obtained, notice that it does not provide a whole property but the membrane's property.

Snap-in Force (N):
Attractive force of the jump-to-contact in the approach force curve. This reflects the van der Waals and electric double layer forces. It is also used to estimate the breakthrough force that occurs when the probe penetrates and destroys a molecule. In liquid measurements, Van der Waals and electrostatic forces are screened by the electrolyte, so it is often used to estimate the breakthrough force.

Pull-Off Force (N):
Attractive force of the jump-off in the retraction force curve. It is frequently utilized for studying the adhesive property of a sample surface and obtaining rupture force and energy landscape of antigen-antibody interactions.

Energy Dissipation (J):
Difference between the integrals of the approach and retraction force curve. This can be used for examining the hysteresis and energy loss during a force curve cycle. It is used for estimating the adhesion and bonding energy similar to the pull-off experiment.

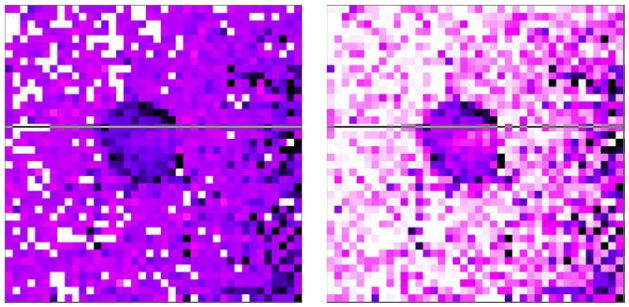
Indentation Depth (m):
Difference between the positions of onset on the force increase and end of force profile. On soft surfaces, the tip can penetrate into the surface more deeply compared to hard surfaces, and it reflects the elasticity of the surface.



Unit:
Set the unit of Young's modulus.

UpperLimit(GPa):
On a hard surface with a GPa order, e.g., mica, the estimated Young's modulus is susceptible to the noise and fluctuation of force profile. So Young's modulus artificially varies even on a uniform surface (left figure). By setting an upper limit for Young's modulus, such artificial distribution can be suppressed.

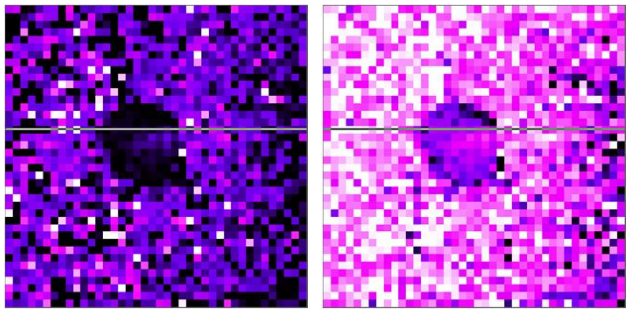
UpperLimit 4 GPa | UpperLimit 1 GPa



MinimumPoint:
Set minimum distance point of Hertz fitting. This parameter needs to be adjusted only when the fitting does not work properly.

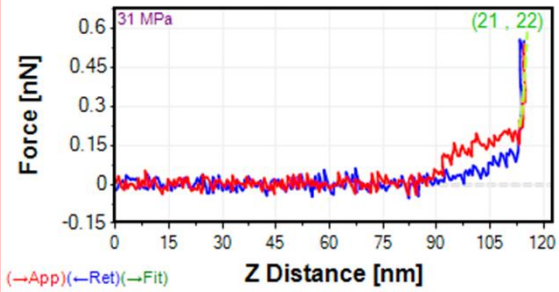
LogScale:
Young's modulus varies in logarithmic scale, and the contrast is often lost when a linear scale is used. So normally LogScale should be enabled.

LogScale Disabled | LogScale Enabled

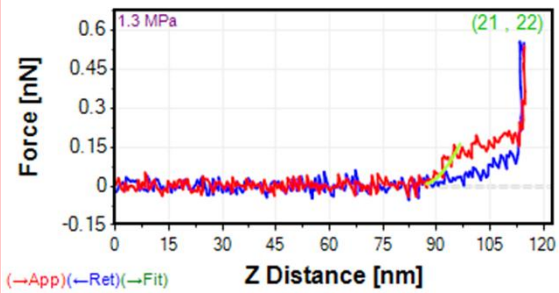


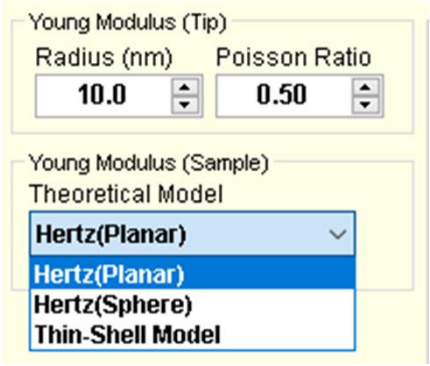
FittingRegion:
In most of the cases, use Near.
When an experiment of sample having a shell structure, e.g., exosome, the force profile does show a monotonic increase. In such cases, if you want to measure the shell, use Far. If you want to measure the inner structure, use Near. When Thin-Shell Model is used, this is automatically set to Far.

Near



Far





Young's Modulus Setting

Set the radius and Poisson ratio of the tip.
Since it is difficult to quantitatively estimate the tip radius, only AFM data obtained with the similar tip condition should be analyzed.

Theoretical model
Hertz(Planar) : flat surfaces (e.g., lipid membrane)
Hertz(Sphere) : spherical biomolecules
Thin-Shell Model: hollow structures (e.g., exosome and cell). Parameters of membrane thickness, height and width are required.

References:
https://en.wikipedia.org/wiki/Contact_mechanics

$$F_{\text{Hertz}} = \frac{4E^*}{3} R^{1/2} \delta^{3/2}, \qquad R = R_{\text{tip}} \quad \ldots \text{PlanarSample}$$

$$\frac{1}{E^*} = \frac{1-\nu_s^2}{E_s} + \frac{1-\nu_{\text{tip}}^2}{E_{\text{tip}}} \qquad \frac{1}{R} = \frac{1}{R_s} + \frac{1}{R_{\text{tip}}} \quad \ldots \text{SphericalSample}$$

Thin-Shell Model

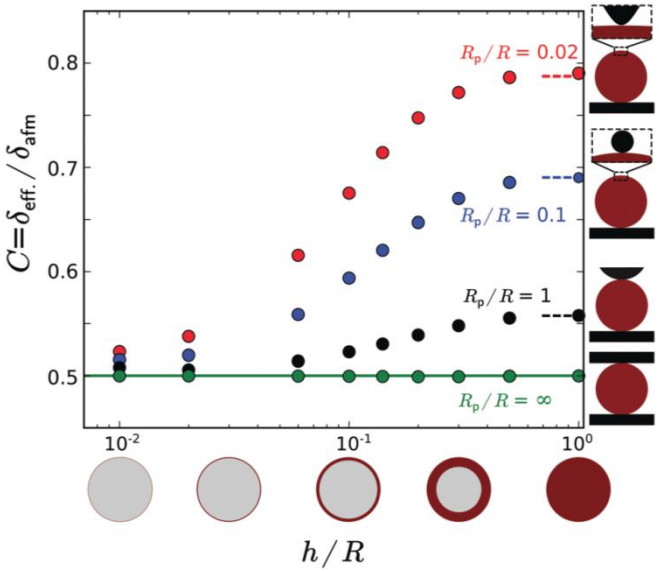
$$F_{\mathrm{TSM}} = \frac{4CEt^2}{R_{\mathrm{C}}\sqrt{3\left(1-\nu^2\right)}}\delta,$$

$$R_{\mathrm{C}} = \frac{h^2 + w^2/4}{2h}$$

Eq.(3) in Langmuir(2017)

Here, the parameters of shell capsule,
t: shell thickness;
w: capsule width
h: capsule height,
Rc: the radius of curvature of capsule
v: capsule Poisson's ratio
C: bottom effect correction factor

Unlike the Hertz model, parameters such as the Young's modulus, radius of curvature, and Poisson's ratio of the tip are not required. Also, the function is linear with respect to the distance, not 3/2.



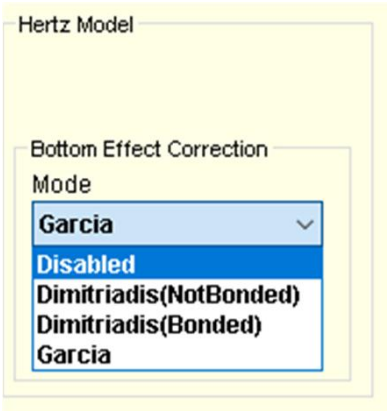
Excerpt from Soft Matter, 13, 1943-1947 (2017)

C represents the Correction factor of Bottom-effect. Since there is no analytical solution for this, it can only be obtained by numerical calculation. As shown below, in the case of exosomes, the value is about 0.55, so a fixed value of 0.55 is used following Langmuir (2017).

References :

Soft Matter 5, 4944 (2009).
Physical Review Letters 109, 144301 (2012)
Nanoscale 6, 2275-2285 (2014).
Langmuir 33, 5117−5126 (2017)
Soft Matter, 13, 1943-1947 (2017)

Bottom Effect Correction (Reaction force)



When the indentation depth is sufficiently small compared to the molecular size, the deformation is localized near the contact point, allowing Hertz theory to be simply applied. On the other hand, when the indentation depth exceeds a few percent of the molecular size, the deformation extends to the substrate, and the reaction force from the substrate causes the apparent Young's modulus to be overestimated. As a result, molecules adsorbed on a hard substrate are influenced by the substrate, leading to an overestimation of the Young's modulus by approximately 1.5 to 2 times the actual value. This setting determines whether to perform a calculation to correct for this substrate effect. Since the García model is an improved version of the Dimitriadis model, using the García model is generally sufficient.

The Dimitriadis model has two variations: bonded and not-bonded. The bonded condition refers to a state where the observed membrane is fixed to the substrate and cannot slip. When the probe indents the membrane, forces act not only in the vertical direction but also in the lateral direction. Since the bonded condition does not allow lateral force relaxation, the Young's modulus appears higher.

It should be noted that these theories were originally developed for flat surfaces and cannot be directly applied to spherical shapes. However, when the object's radius is sufficiently larger than the probe's curvature radius, the surface can be approximated as nearly flat, making these models a reasonably good approximation. Conversely, when the object's radius becomes comparable to or smaller than the probe's curvature radius, this correction becomes less meaningful.

$$F_{\mathrm{Hertz}}^{\mathrm{brfc}} = \frac{4E^{*}\alpha_{\mathrm{brfc}}}{3} R^{1/2}\delta^{3/2} \qquad \chi = \frac{\sqrt{R\delta}}{h}$$

Dimitriadis model（bonded）

$$\alpha_{\mathrm{brfc}} = 1 + 1.133\chi + 1.283\chi^{2} + 0.769\chi^{3} + 0.0975\chi^{4}$$

Dimitriadis model（not-bonded）

$$\alpha_{\mathrm{brfc}} = 1 + 0.884\chi + 0.781\chi^{2} + 0.386\chi^{3} + 0.0048\chi^{4}$$

Garcia model

$$\alpha_{\mathrm{brfc}} = 1 + 1.133\chi + 1.497\chi^{2} + 1.469\chi^{3} + 0.755\chi^{4}$$

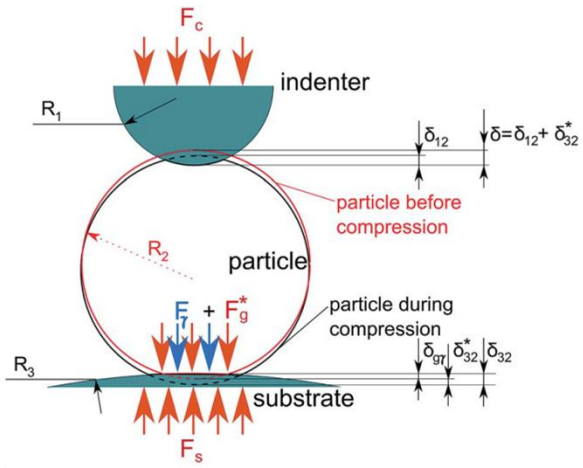
References：
Biophysical Journal 82, 2798 (2002).
Nature Nanotechnology 7, 733 (2012).
Biophysical Journal 114, 2923 (2018).
RSC Advances 10, 19258 (2020)
ACS Nano 15, 20574 (2021).

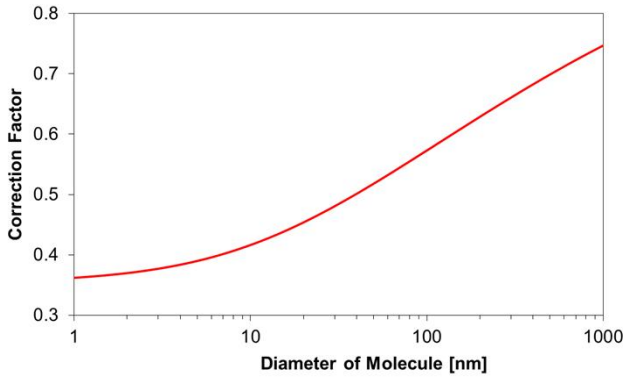
Bottom Effect Correction for Displacement of Spherical Molecules

Hertz's theory is strictly valid only when two freely placed objects come into contact due to their own gravity or external pressure. In AFM measurements, the molecule being observed is adsorbed onto the substrate, so the bottom effect caused by the substrate needs to be corrected. In the case of flat membrane structures, only the bottom effect described on the previous page needs to be considered. However, for spherical molecules, an additional bottom effect must be taken into account. As shown in the figure below, when a particle adsorbed on a flat surface is indented by a probe, both the top and bottom surfaces of the molecule deform simultaneously. Therefore, even if the probe is indented by 1 nm, the actual indentation into the molecule will be approximately 0.5 nm, which is half of the probe's indentation. As a result, in contrast to the bottom effect described on the previous page, the apparent Young's modulus will be underestimated. The coefficient for this bottom effect depends only on the sizes of the probe and molecule, and does not vary with indentation distance. After fitting, Young's modulus can be corrected in Excel.

$$F_{\mathrm{Hertz}}^{\mathrm{bdc}} = \frac{4E^{*}\alpha_{\mathrm{bdc}}}{3} R^{1/2}\delta^{3/2}$$

$$\alpha_{\mathrm{bdc}} = \left[\frac{\left(R_{\mathrm{tip}} / R_{\mathrm{smpl}} + 1\right)^{1/3}}{\left(R_{\mathrm{tip}} / R_{\mathrm{smpl}} + 1\right)^{1/3} + \left(R_{\mathrm{tip}} / R_{\mathrm{smpl}}\right)^{1/3}} \right]^{3/2} = \frac{1}{\left[\left(\frac{R_{\mathrm{tip}}}{R_{\mathrm{tip}} + R_{\mathrm{smpl}}} \right)^{1/3} + 1 \right]^{3/2}}$$





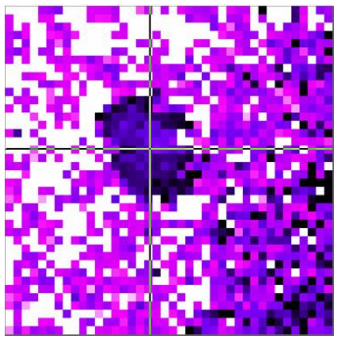
The correction coefficient calculated by using the probe curvature radius of 5 nm and the molecular height (diameter) as a parameter is found to range from approximately 0.4 to 0.7.

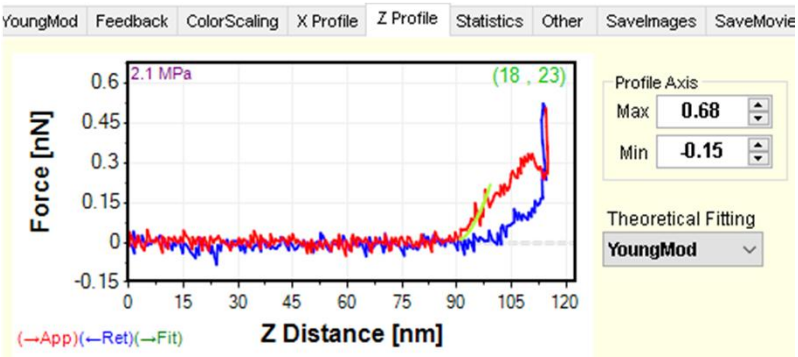
On the other hand, this theory assumes that the particle under observation is a perfect sphere. However, in reality, due to adsorption on the substrate, the surface on the substrate side has already been deformed by the substrate interaction, even if there is no interaction from the probe, and is expected to resemble a structure closer to flatness. Therefore, the impact of this correction coefficient is expected to be small and close to 1. It can be concluded that the effect will be limited to about 20-30%.

参考文献:
Soft Matter, 2014, 10, 6732–6741
Langmuir 2014, 30, 7206−7212
Soft Matter, 2017,13, 1943
Nanomaterials 2023, 13, 1916

Z Profile

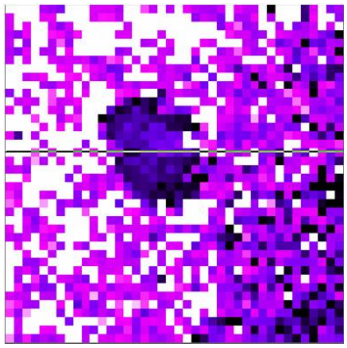
Z profile can be extracted at the position where the cross lines are crossover. The green curve is Hertz curve which is fitted to the red approach curve. This is useful for checking whether the Hertz fitting works correctly.

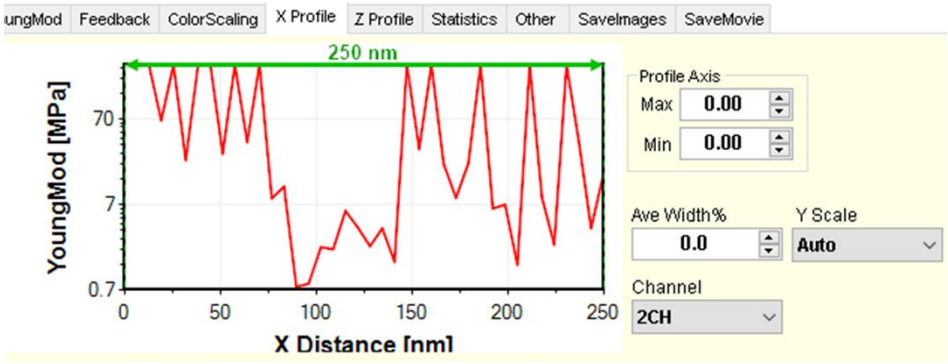




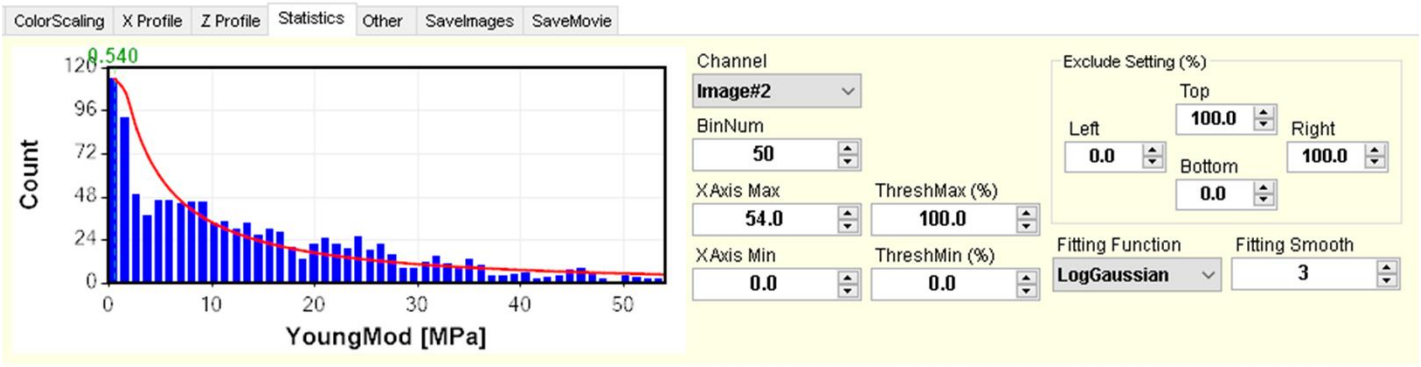
X Profile

X profile can be extracted at the position where the vertical line indicates. This is useful for checking the distribution of Young's modulus quantitatively.





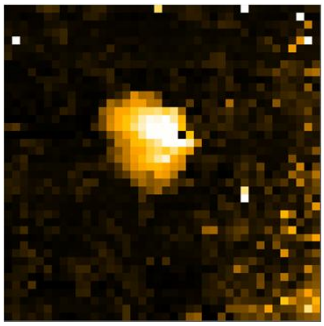
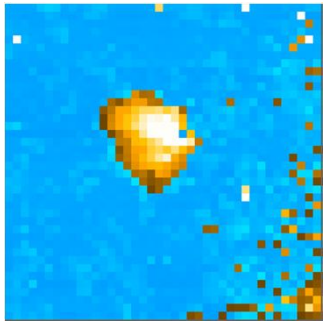
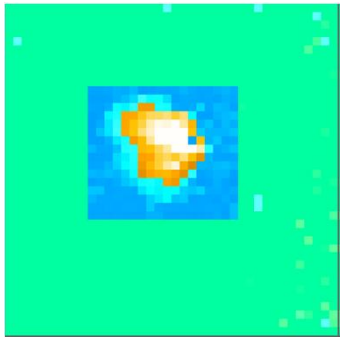
Statistics



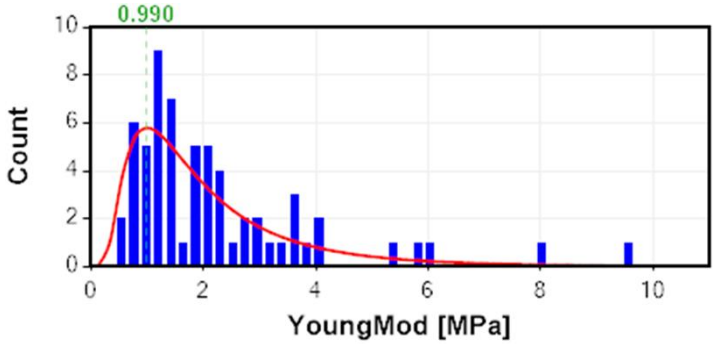
This function is for obtaining statistics of young's modulus.
To obtain statistics data only from adsorbed molecules, the substrate region must be excluded from the analysis.
To exclude a low region, ThreshMin(%) is adjusted.
To exclude a high region, ThreshMin(%) is adjusted.
The blue region in the AFM image indicates the excluded region.

When the ThreshMin&Max cannot cover all the specified region, manually adjust the Exclude Setting (the green region apprears).

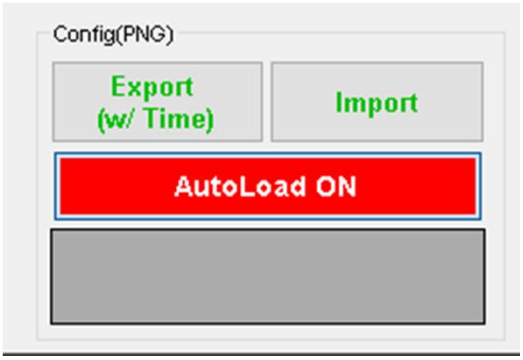
LogGaussian

Original Image	ThreshMin Image	ExcludeSetting Image
		

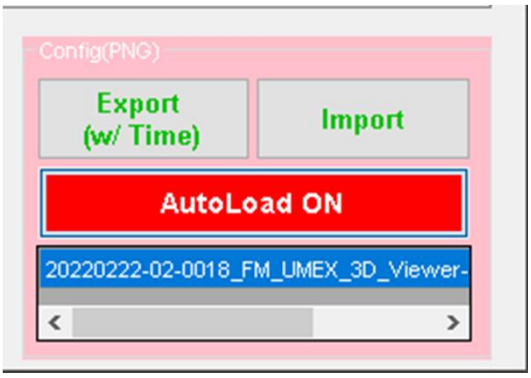
After these settings are done, a single peak with a LogGaussian function can be obtained. The Peak value is displayed above the chart (in this case 0.990 MPa).



Config Export



The parameters used can be saved by Export button. The exported PNG parameter file can be restored by drag-and-drop and Import button.



By enabling AutoLoad ON, an associated parameter file is automatically imported in case that it exists. If the parameter file is automatically imported, Config box changes the color to pink as shown in the left figure.