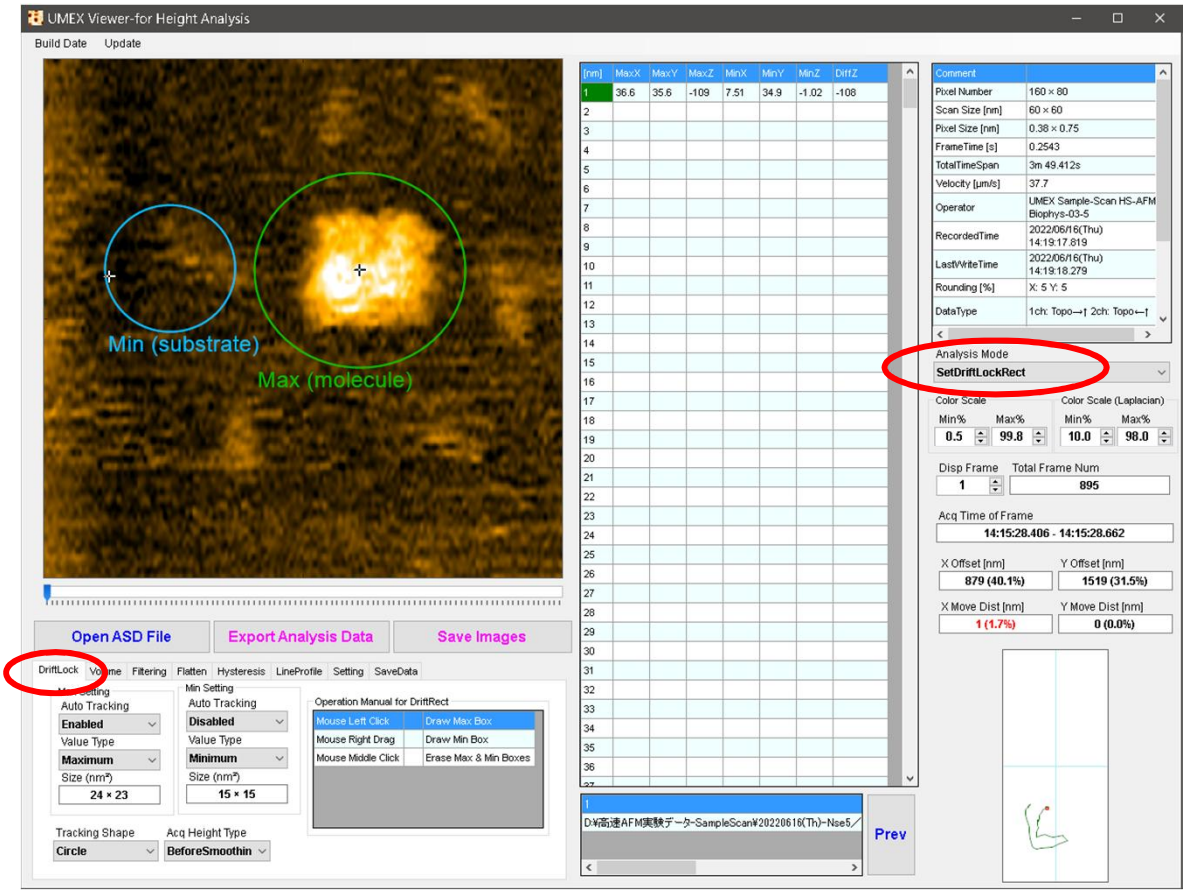
Quick Guide for UMEX Viewer for Height Analysis

< Analysis Mode _Set Drift Lock Rect >

This mode is used for tracking the time course of the maximum height (z) and position (xy) of a molecule. The moving molecule can also be automatically tracked



By the mouse left drag, set the positions of Max and Min boxes in the AFM image. The height and positions of each cross point and difference between two of heights are saved to the table.

Max Box Setting : It detects the position at the maximum height in the box (for molecule).
Min Box Setting : It detects the position at the minimum height in the box (for substrate).

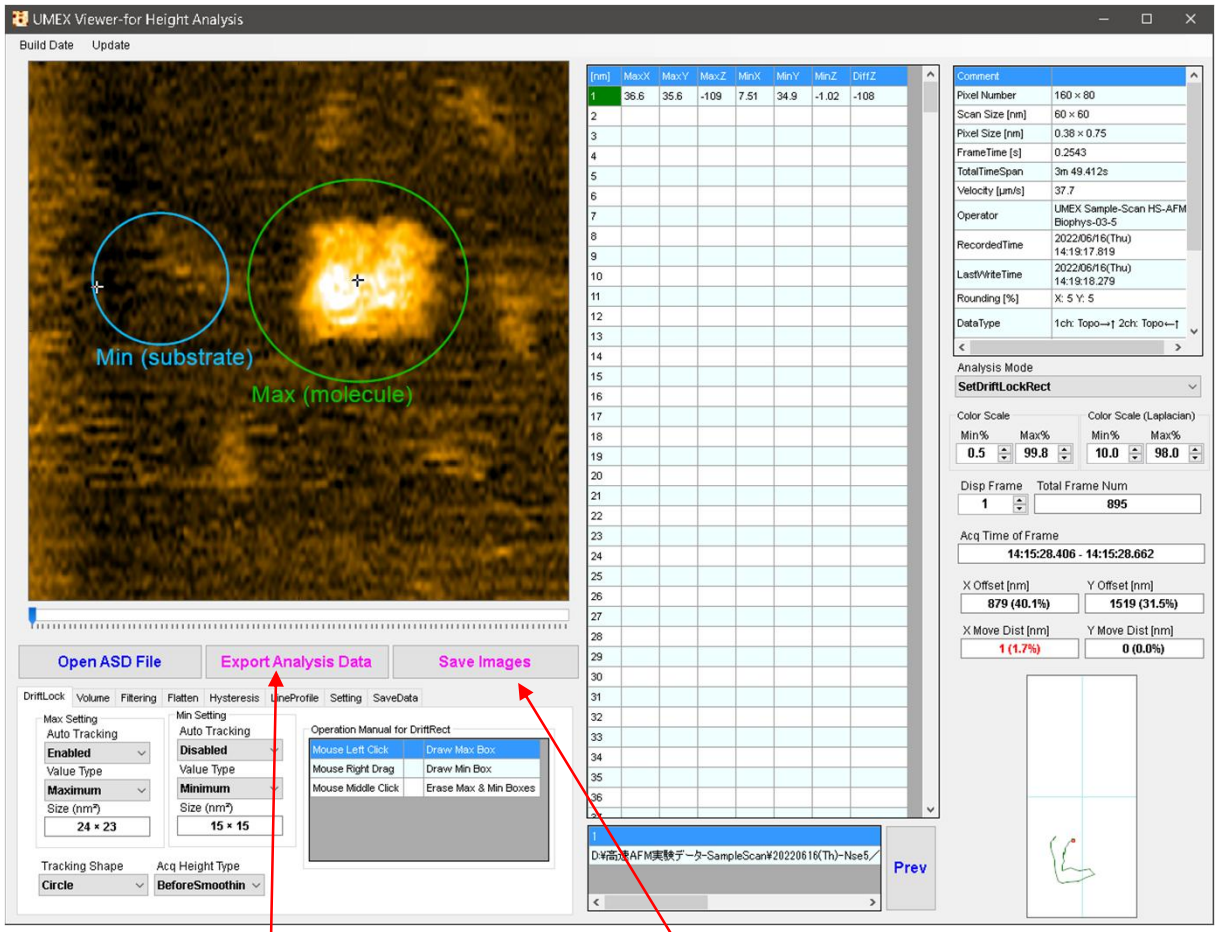
AutoTracking: It determines whether the center position of the individual box is automatically adjusted to the max or min position during the frame change.
Value Type: When it is Maximum or Minimum, maximum or minimum height is acquired (the position is indicated by the cross hairs in the AFM image)
When it is Average, the overall average height in the box is acquired (this is used for substrate.).

Operation Manual for DriftRect :
Left drag…draw Max Box（green, molecule)
Right drag…draw Min Box （blue, substrate)
Middle click…Erase boxes.

Tracking Shape : It determines the shapes of the boxes.

Acq Height Type :

It determines whether the height is acquired before or after the Smoothing. Before : maximum and minimum height is prone to be affected by image noise. After : the effect of the noise can be suppressed, but the apparent height becomes smaller. Therefore, if Smoothing factor is high, “before smoothing” is recommended, and if it is low, “after smoothing” is recommended.



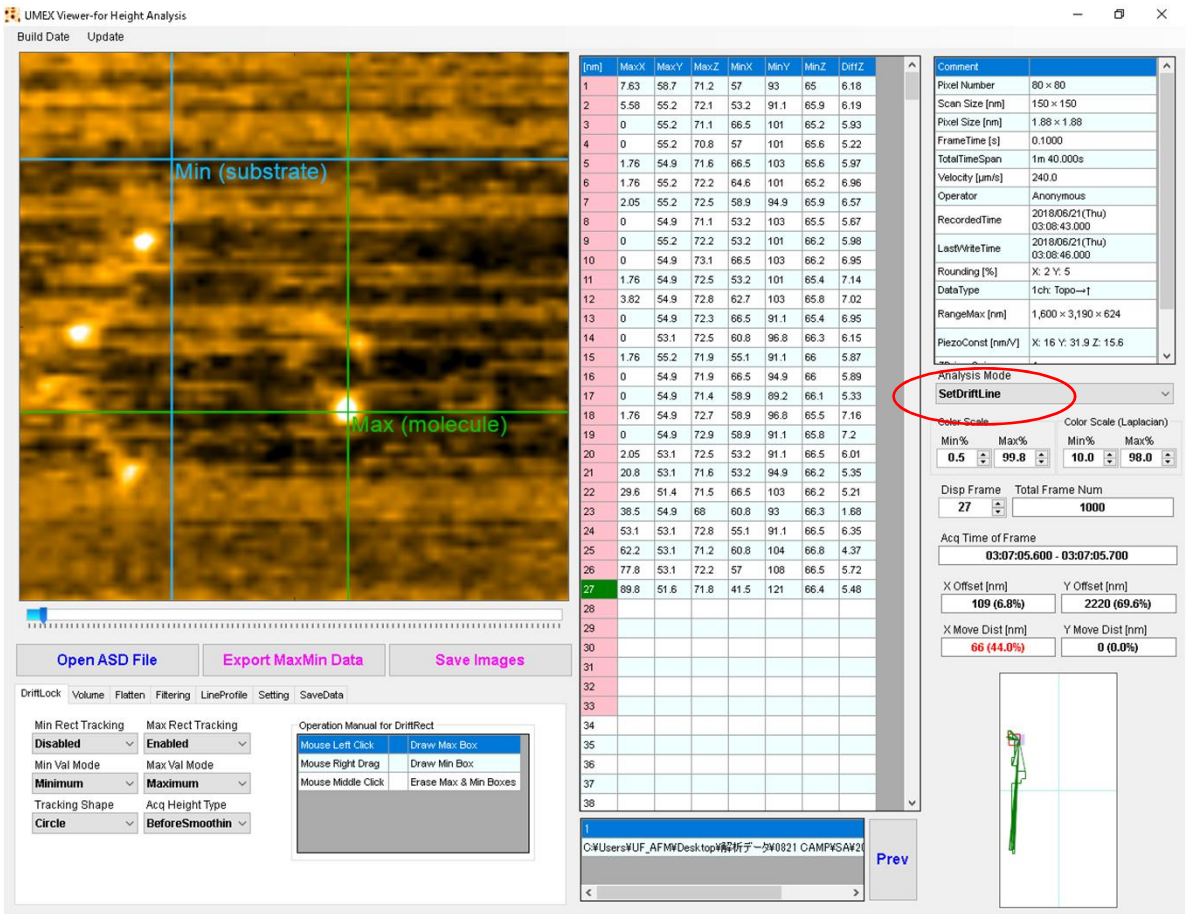
Export Analysis Data
It is used for exporting the analysis data as CSV File.

Save Images
All frame images are saved.
→One frame saving can be done in the SaveData tab.

< Analysis Mode_Set Drift Line>

This mode is used for manually measuring the time course of the maximum height (z) and position (xy) of a molecule. Use this mode when Drift Lock Rect does not work properly.

By the mouse left drag, set the positions of Max and Min Lines in the AFM image. The height and positions of each cross point and difference between two of heights are saved to the table.



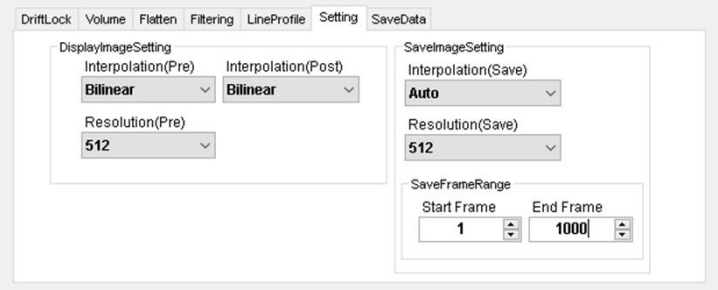
＜ Analysis Mode_Line Profile ＞

Line Profile

Analysis Mode をLineProfileにすると、Image上で指定したLineの高さと距離を見ることができる。

※ここでは、記録および保存はできない。

Setting



Display Image Setting
Interpolation…Image表示の補間方法を指定する。
Resolution…Imageの解像度を指定する。

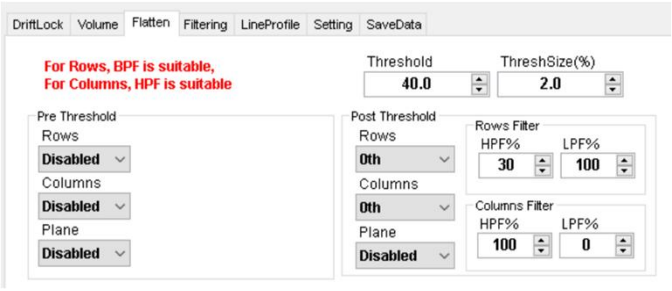
Save Image Setting
Imageを保存するときの表示方法やFrame範囲を指定する。

Save Data

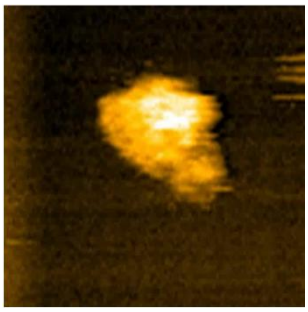
Save Current Image…現在表示されているImageをpng.Fileで保存する。
Save Averaged Height data…Frame全体の高さの平均値をcsv.Fileで保存する。

Flatten

When the line noise is significantly large or substrate slope varies over time, Flatten must be used, in which the line noise can be removed LinebyLine.

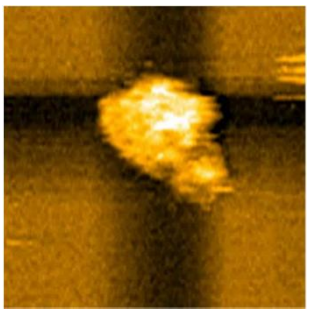


1. Original Data



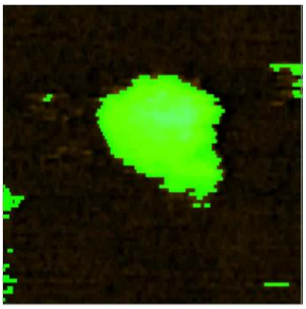
Brightness fluctuates line by line due to unstable scanning condition.

2. Normal flatten



Normally, adjust the parameters in “Post Threshold” box to process the flatten filter. However, when an isolated molecule appears in the AFM image, a artificial dark line appears on the sides of the molecule because the filter justifies the average height of each scan line.

3. Detecting molecular regions

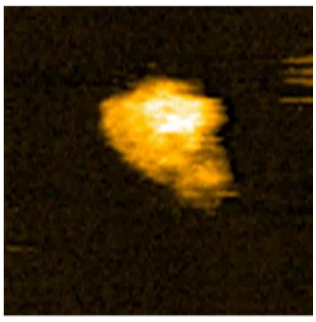


To avoid this problem, the molecular region should be excluded from the Flatten filter. To do this, the Flatten should be processed in two steps. After the first step, molecular region is detected by the height threshold, for which parameters is in “Thresh Image1” and “Thresh Image2” groups. The parameters for the first Flatten is in “Pre Threshold” group.

Typical setting is that Threshold↑ is 40~60% and Size is 0~3%.

Thresh Disp button: By turning it on, the excluded region can be displayed on the AFM image just for checking. Note that this does not cause any effect to the image processing.
Mode: Byte%, the threshold values indicate brightness value normalized to 0~100% range. nm, the threshold value indicate the absolute height of AFM image in the unit of nm. Normally, only Byte% is used, but when a large object appears only in certain frames, the nm mode provides a better result.
Threshold↑: Upper exclusion threshold mainly used for molecules and higher terraces.
Threshold↓: Lower exclusion threshold mainly used for hall and cracks.
Size(%): This enlarges the detected exclusion region, which is used for excluding the parachute effect or scratch noise.
Smoothing: This blurs the exclusion region, which is used when the substrate region is inevitably included in the exclusion region due to the noise.

4. Only-substrate flatten



If only the molecular region is excluded from the Flatten filter, substrate region can be perfectly flattened.

< Analysis Mode _Volume Measure >

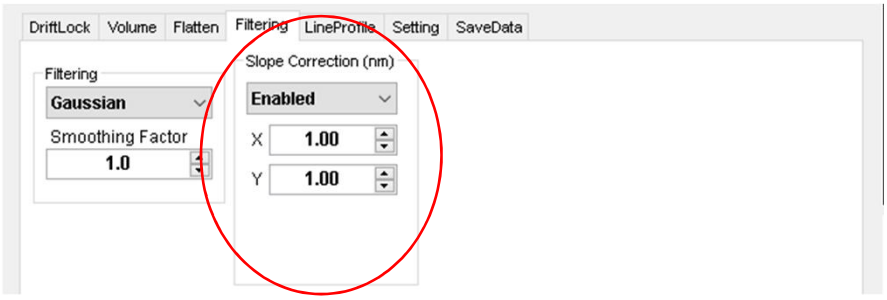
This mode is used for tracking the time course of the volume change of a molecule or morphology.

- First, slope correction or flattening filter should be processed because it is impossible to accurately measure the molecular volume unless the substrate surface is not perfectly flat with slope.

Step 1

Slope Correction or Flatten

Method 1: Slope Correction

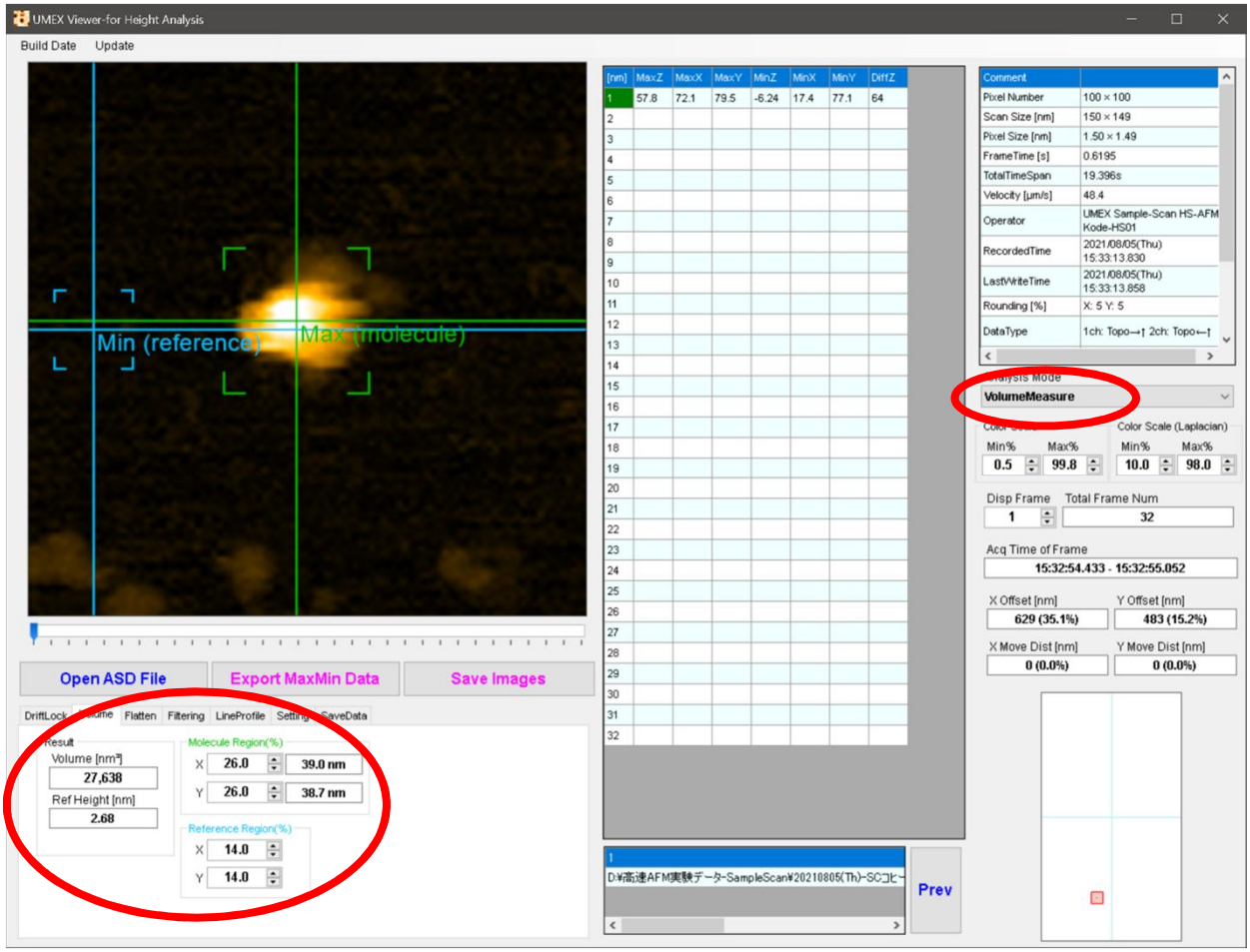


Make the substrate surface flat by adjusting the slope correction values.

Method 2: Flatten

Described in the previous page.

Step 2



Volume calculation method

- First, the average height of the Reference region is calculated by dividing the integrated height over the Reference region by the area.

$$\overline{z}_{\text{reference}} = \frac{\iint z_{\text{reference}}(x,y)\,\mathrm{d}x\mathrm{d}y}{\iint \mathrm{d}x\mathrm{d}y}$$

- Second, the volume of the Molecule region is calculated by integrating the height on the basis of the averaged height of the Reference region.

$$V_{\text{molecule}} = \iint z_{\text{molecule}}(x,y) - \overline{z}_{\text{reference}}\,\mathrm{d}x\mathrm{d}y$$

How to calculate the Radius of Gyration (RoG)

- A mathematical concept for quantifying the compactness of an object.
- As shown in the following formula, it is equivalent to the root mean square (RMS) of the distance of each pixel from the center of the object.
- When the object is a perfect circle, it takes a value of $1/\sqrt{2}$ times the radius of the object.

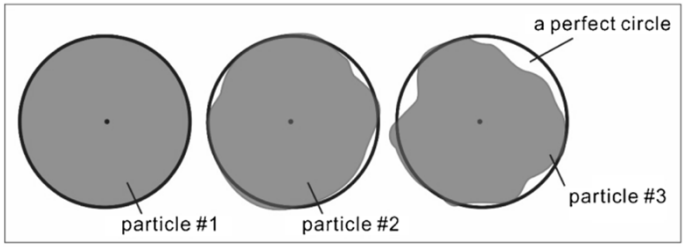
$$R_G = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(\mathbf{r}_i - \mathbf{r}_{CM}\right)^2} \qquad \mathbf{r}_{CM} = \frac{1}{N}\sum_{i=1}^{N}\mathbf{r}_i$$

How to calculate Circularity

- A mathematical concept for quantifying the unevenness of an object's contour.
- It is a parameter expressed by the following formula, which takes a value of 1 when the object is a perfect circle and a value less than 1 otherwise.
- However, because the pixels in an actual AFM image are quantized, errors occur in the calculation of Perimeter. As a result, even for a perfect circle, the value ends up being around C=0.9.

1. Circularity [-] c
2. Area [nm^2] A
3. Perimeter [nm] P

$$c = \frac{4\pi A}{P^2}$$



C=1 C=0.955 C=0.883

Area, radius of gyration, and circularity are mathematical concepts that share some common aspects, but they are used differently as follows:

	Characteristic	Suitable Experimental System	Application Example
Area	Value determined solely by size, regardless of shape	Cases where object size changes need to be quantified when the object has a consistent shape or when shape is irrelevant	Lipid membrane domains Droplets or aggregates
Radius of gyration	Value reflecting both shape and size (influenced by both area and circularity)	Cases where object compactness needs to be quantified.	DNA supercoils DNA compaction caused by DNA-binding proteins
Circularity	Value determined solely by shape, regardless of size (same shape takes the same value, even with different sizes)	Cases where object shape roughness needs to be quantified when the total size remains unchanged, and only the shape changes.	RNA secondary structures

1. Area Shape

- Select Area shape from Rectangle, Circle, and MousePaint.

2. Position Setting

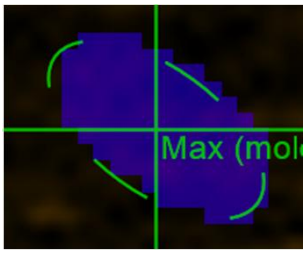
- Set the Molecule and Reference position by moving the green and blue lines using mouse drag operation. **(Important point) in most of the cases, there is significant variation along the Y axis, and hence the Molecule and Reference regions are better to be aligned along the Y direction as shown in the figure in the manual.**

2-1. Reference Setting

- Set the range of Reference area in the “Reference Area” groupbox. The calculated average height is displayed in the “Ref Height”. Confirm that “Ref Height” does not significantly changed when moving the position of Reference region. If not, the surface slope is not properly corrected.

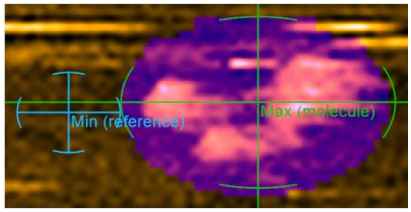
2-2. Molecule Setting （Rectangle, Circle）

- By adjusting the Molecule Range, fit the blue range to the whole molecular region in the AFM image. The calculated volume is displayed in the “Volume”.



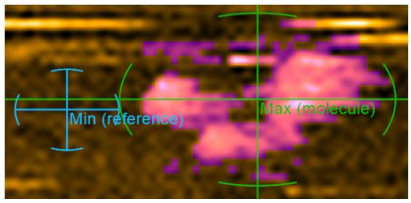
The inclination angle of Rectangle and Circle can be adjusted

Disabled Threshold



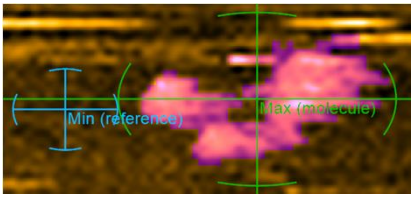
Analyzes all selected regions, and it is necessary to fit the selected region exactly to the molecular region.

All Objects



Analyzes only the pixels where the height exceeding the Threshold Height, and the molecular region can be detected automatically. This is good for analyzing multiple molecules, but it also detects noise, so it is better to use “Largest Object” when analyzing a single molecule.

Largest Object



Detects and analyzes only the largest object among the pixels exceeding the Threshold Height of the selected area. Ideal for analyzing single molecules.

2-3. Molecule Setting （MousePaint）

- Molecular region is set by mouse control in the AFM image.
- Left click：Draw Molecule region
- Right click：Erase Molecule region
- Wheel：Change drawing region
- Middle click：Reset drew region

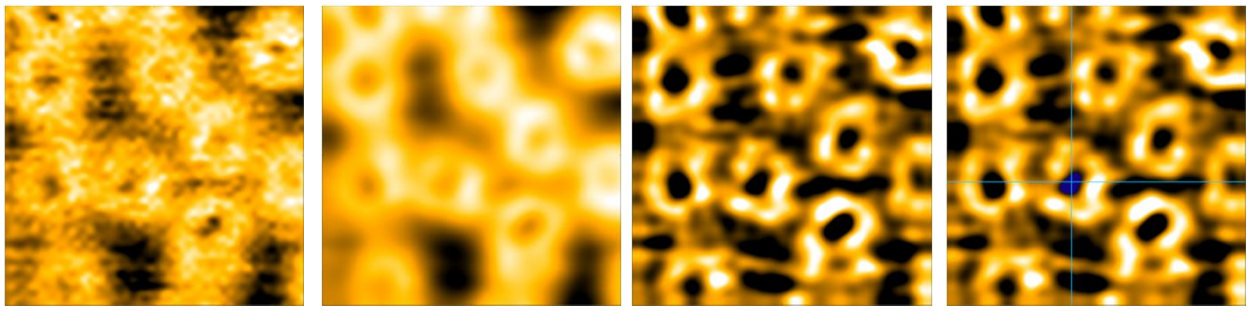
< Analysis Mode _Blob>

Mode for measuring shape of holes on surface.

- The shape of the hole is measured by performing the following procedure in the software.

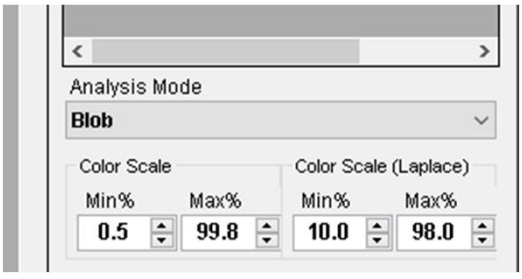
1. Remove high frequency noise using Gaussian smoothing
2. Laplacian filter is processed to obtain the second-order derivative of the height distribution. Since the second-order derivative takes a large value on the surface such as molecules and holes, the molecules and holes can be recognized by setting a threshold value.
3. By clicking on the image, a crosshair will appear where you clicked.
4. The area around the clicked pixel that is smaller than the second-derivative threshold is displayed in blue.

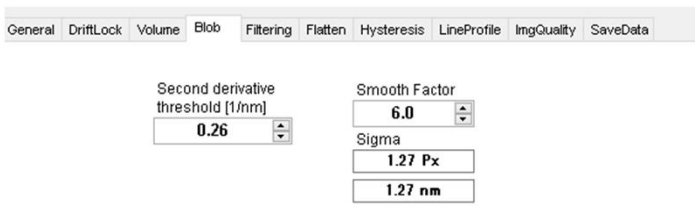
1. Original image | 2. Gaussian smoothing | 3. Laplacian filter | 4. Threshold detection



Step 1

1. Change Analysis Mode to Blob.
2. Open the Blob tab.





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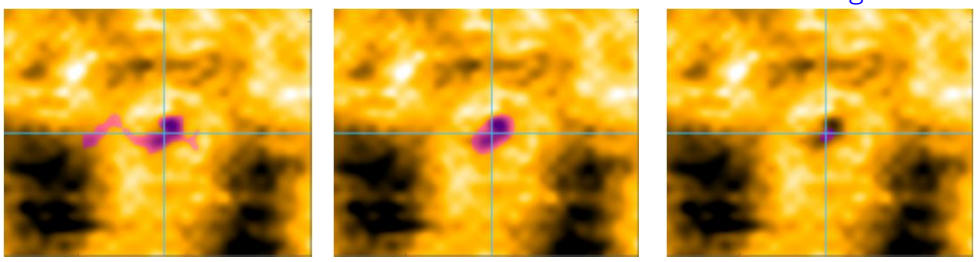
Step 2

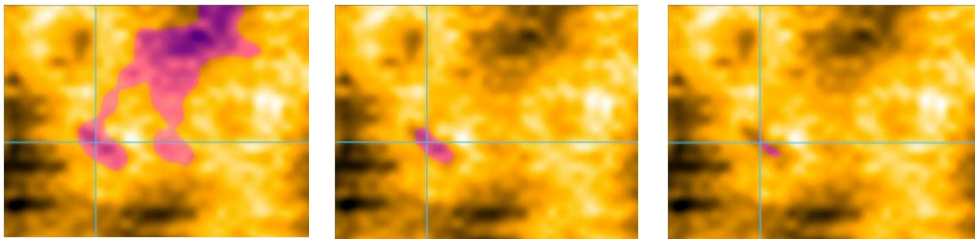
1. Smooth Factor of Gaussian Filter

Too small | Moderate | Too large



2. Second derivative threshold [1/nm] of Laplacian filter

Too small | Moderate | Too large



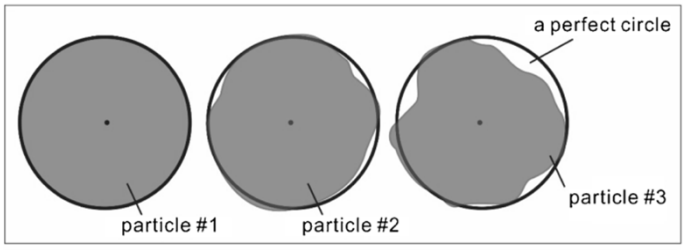
Step 3

- By pressing the [Export Analysis Data] button, the following parameters can be saved to a CSV file.

Circularity is a parameter expressed by the following formula, and takes a value of 1 when the circle is perfect, and a value of less than 1 otherwise.

1. Circularity [-] c
2. Area [nm^2] A
3. Perimeter [nm] P

$$c = \frac{4\pi A}{P^2}$$



C=1 C=0.955 C=0.883