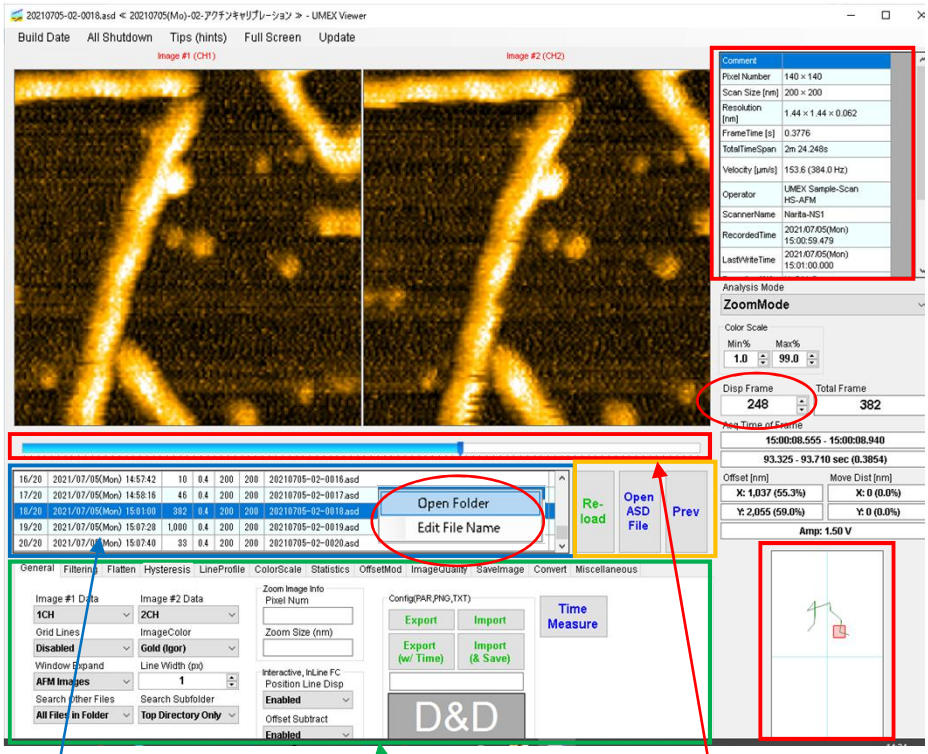
Quickstart Guide

UMEX Viewer Quick start Guide

1. Open ASD file



File name display field

・Drag & Drop the File, select the File name and left-click to display the Image.

Parameter edit tab field

Position slider

Click & Drag, ▲▼ of Disp Frame to move.

- It is also possible to input in Disp Frame.
- You can also use the arrow keys on your keyboard to move.

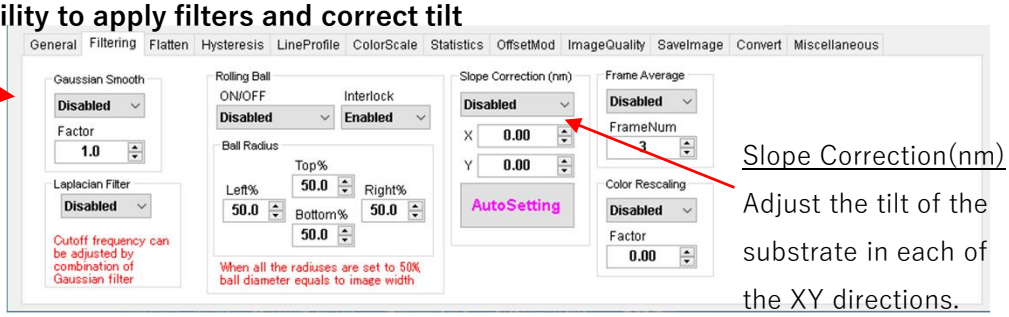
[Shift + →] = fast forward

[Shift + Ctrl + →] = Further fast forward

2. Smoothing and Slope Correction

Filtering

Ability to apply filters and correct tilt



Gaussian Smooth

Adjust the degree of blurring with the Factor value ▲▼.

Slope Correction(nm)

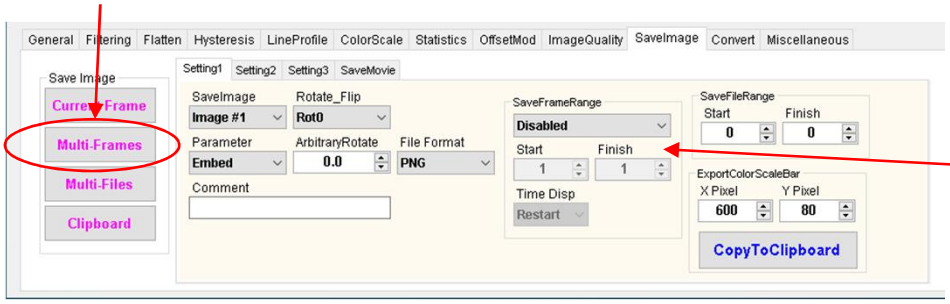
Adjust the tilt of the substrate in each of the XY directions.

3. Save image

Save Image Image save settings.

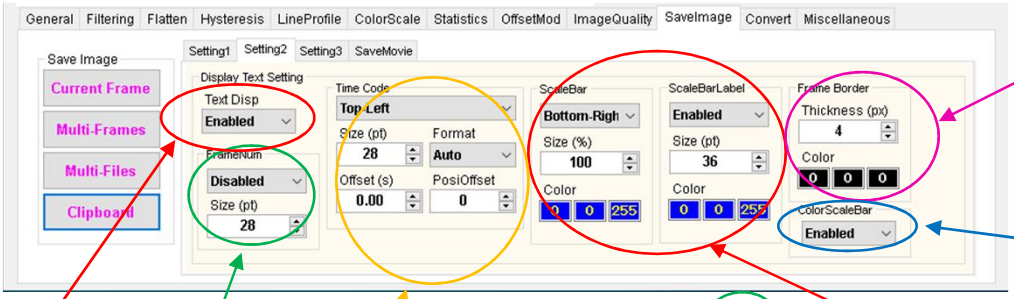
Save Image

Multi-Frames…Save all frames specified by SaveFrameRange of the open file.



Save Frame Range
Specify the Frame range to save.

Setting 2　表示するテキストの設定



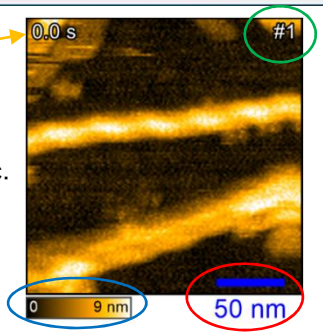
Specify the border width and color.

Specifies the display of the color scale bar.

Change to Enabled first.

Specifies the display of the frame number.

Specify the position to display time, display style, etc.



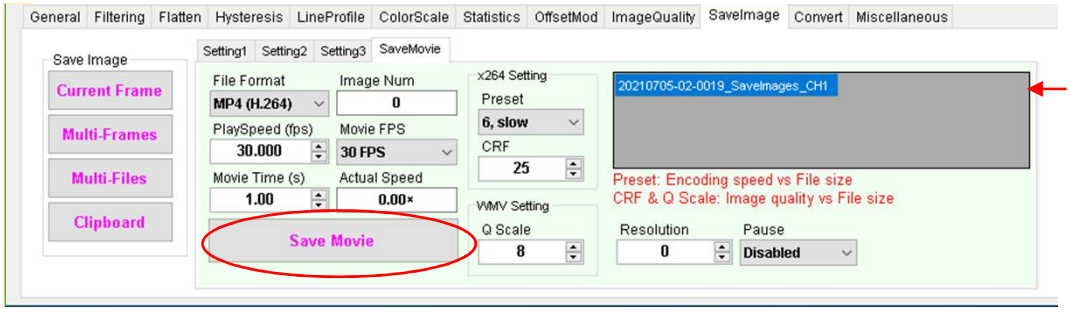
Specify the position, size, and color to display the scalebar and scalebar labels.

4. Save video

Save Image

Video save settings (Settings for creating videos based on the images previously saved)

*When using the Save Movie function, first install [UMEX Viewer Installer .exe]
[ffmpeg.exe] must be unzipped in the config folder.



Drag&Drop
Automatically set when saving an image with Multi-Frames.
Alternatively, you can D&D the saved folder.

File Format
MP4 has a higher compression rate, but it may not play well when embedded in PowerPoint, so in that case, WMV is recommended.

PlaySpeed（fps）＆Movie Time（s）
Works with Image Num. Set either Speed or Time.

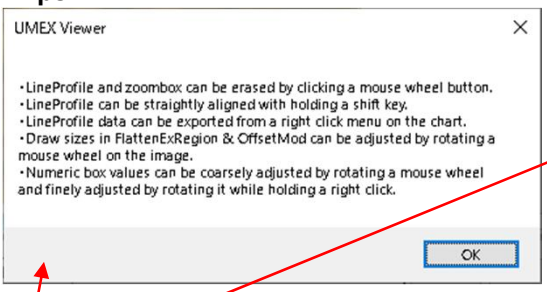
Full Instruction Manual

UMEX Viewer Guide

- LineProfile and zoombox are cleared with the mouse wheel button.
- LineProfile can be a straightly alighned with holding a Shift key.
- LineProfile data can be exported from the right-click menu in the chart.
- The drawing size of FlattenExcludeRegion and OffsetMod can be adjusted by rotating the mouse wheel on the image.
- The value of the numeric box can be coarsely adjusted by rotating the mouse wheel, and finely adjusted by rotating the wheel while holding the right-click.

＜1＞ Main screen

Tips

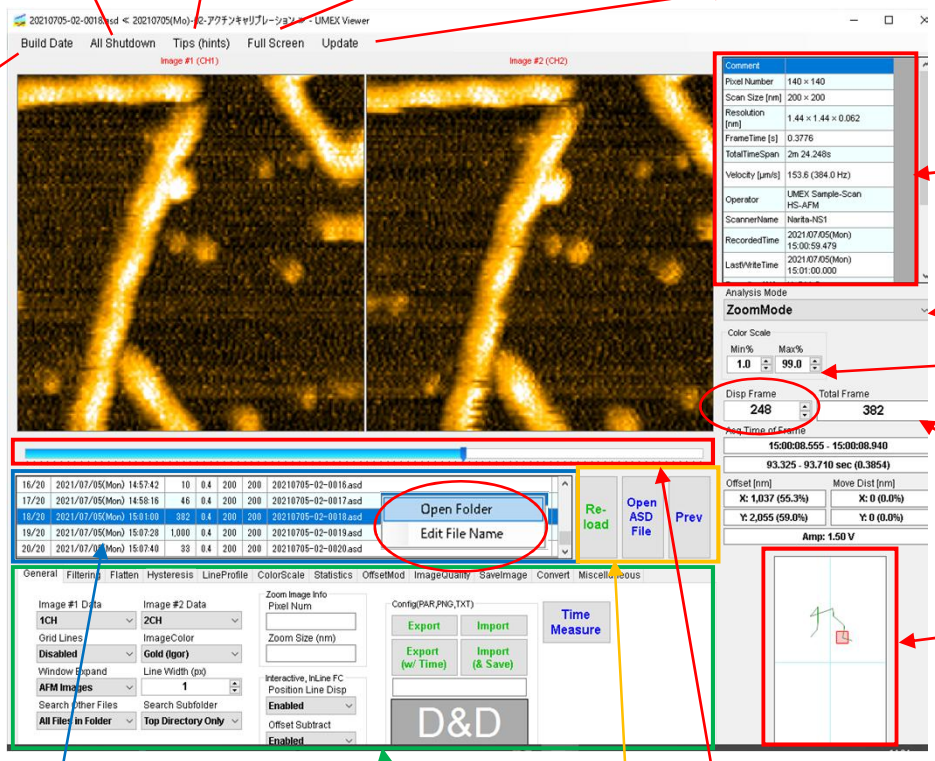


All Shutdown
All windows can be closed at once.

Full Screen
Image part is enlarged and displayed. Useful for display on low-resolution laptops and projectors.

Update
You can download the latest version.

Build Date
You can open the folder with the program by clicking the path name.



ASD file information

Analysis mode

Color Scale

Display frame

Tip position

Parameter edit tab field

File name display field
・When you drag and drop a file, the file name, saved date/time, number of frames, frame time, and scan size are displayed.
・If you do D&D for each Folder, all ASD files in the Folder will be read.
・Select the file name and left-click to display the image. If you right-click, you can open the folder containing that file, or change the file name or folder name.

Position slider
Click & Drag, ▲▼ of Disp Frame to move.
- It is also possible to input in Disp Frame.
- You can also use the arrow keys on your keyboard to move.

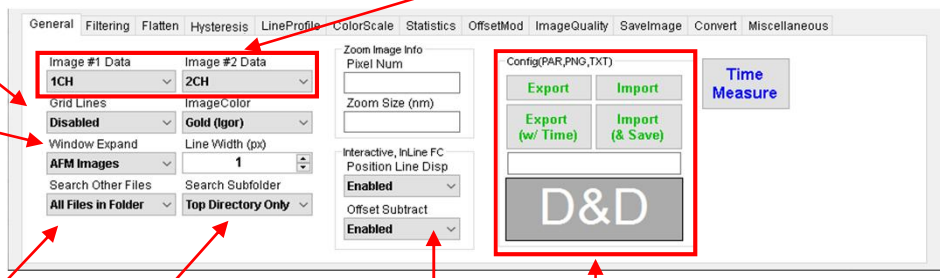
[Shift + →] = fast forward
[Shift + Ctrl + →] = Further fast forward

Reload…Reload the new files created in the same folder.
Open ASD File: Open the specified File.
Prev... Open the File read previously.

＜2＞ Parameter edit tab details

General

General settings for File display



Grid Lines
Select the number of grid lines.

Image # 1 Data　Image # 2 Data
Select the channel to display.

Window Expand
Select the display frame you want to enlarge when you change the window size by dragging the mouse.

Search Other Files
Select whether to select all files in the folder or only selected files when dragging and dropping files.

Search Subfolder
Setting for import of subfolder when dragging and dropping a file.

Interactive, InLine FC
Functions used when measuring in Interactive mode. Changes the strength with which the sample is struck. You can see where it was when it was suppressed.

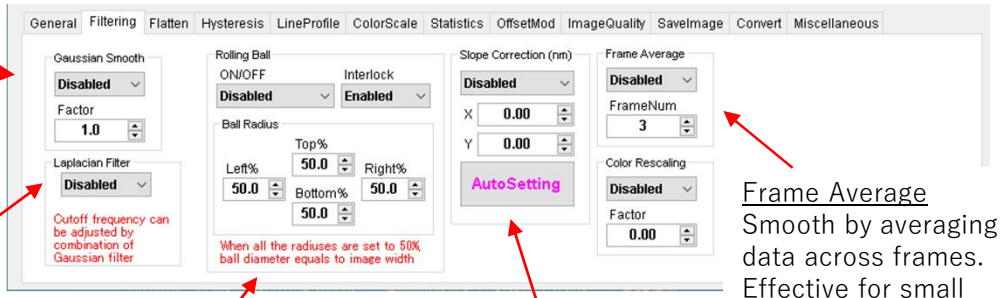
Config(PAR,PNG,TXT)
Export and import parameter settings.

Export…Overwrite save
Export (w/Time): Save with an alias with time.
Import… Read the D&D parameter file.
Import (& Save) … Read settings and save Image.

TextBols…Text can be added to the File name when exporting.
D&D: Drag & Drop the PAR file you want to import.

Filtering

Functions to apply filters and correct tilt



Gaussian Smooth
Adjust the degree of blurring with the Factor value ▲▼.

Laplacian Filter
Used to even out the contrast of molecules on surfaces at different heights. Or use it to emphasize contours. Used in combination with Gaussian Smooth.

Rolling Ball
A filter that suppresses the undulations of the background by subtracting the trajectory from the original data after rolling the ball while contacting it from under the surface topography image. This is effective for uneven surfaces, such as on lipid membranes.

Slope Correction(nm)
Adjust the tilt of the substrate in each of the XY directions.

Frame Average
Smooth by averaging data across frames. Effective for small motion data. (Triangular can reduce afterimages more than rectangular)

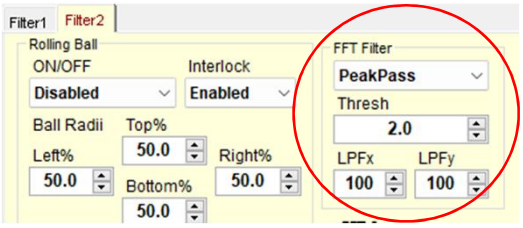
FFT Filter

Usage Instructions

After converting the data to the frequency domain using FFT, the data points above or below the threshold specified by *Thresh* are replaced with zero data. The image is then transformed back to real space using an inverse FFT.

Select either *PeakPass* or *PeakStop*, and adjust the *Thresh* value manually so that the resulting image reaches the desired condition. You can also apply a low-pass filter to the image by enabling the *LPF* option.

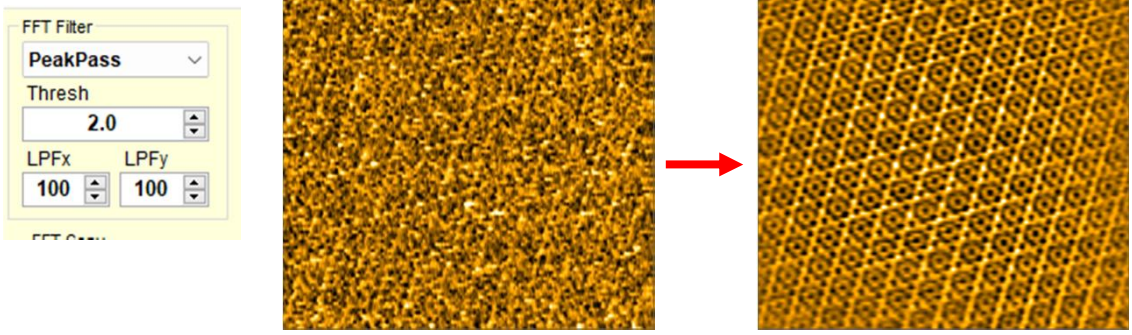
Important: Be sure to apply *Flatten* beforehand; otherwise, the process may not function.



PeakPass

In the frequency domain, all data except for peaks whose magnitudes exceed the specified threshold are set to zero.

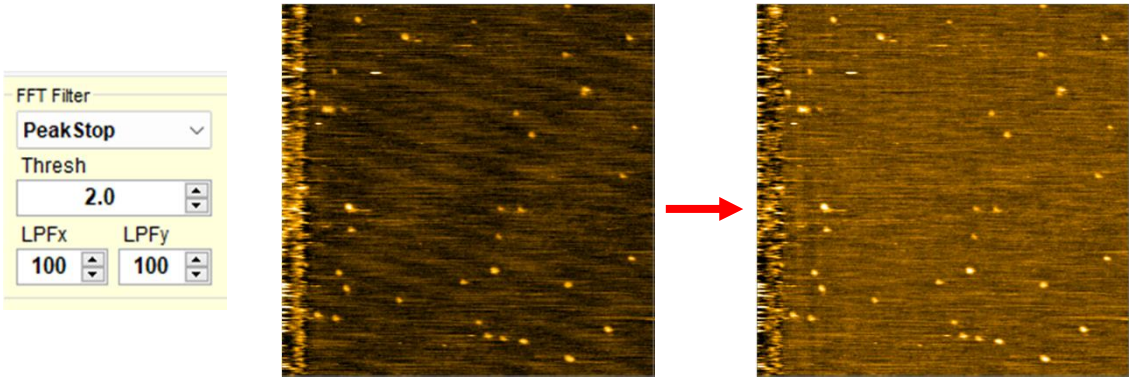
This function is useful for clearly visualizing periodic structures such as two-dimensional crystal lattices when poor probe conditions make the lattice difficult to observe. It is especially helpful for scanner calibration using Annexin-V samples.



PeakStop

In the frequency domain, peaks with magnitudes exceeding the specified threshold are set to zero.

This function is used to remove periodic noise from images caused by instrument malfunctions or mechanical vibrations. If possible, it is recommended to identify and resolve the source of the noise rather than relying solely on this function.



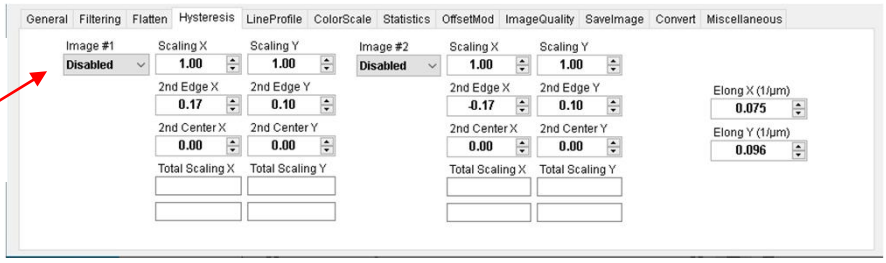
Flatten

This function is used when ringing is severe during high-speed scanning or when Tip-Scan AFM is used. →Explanation will be created in the future.

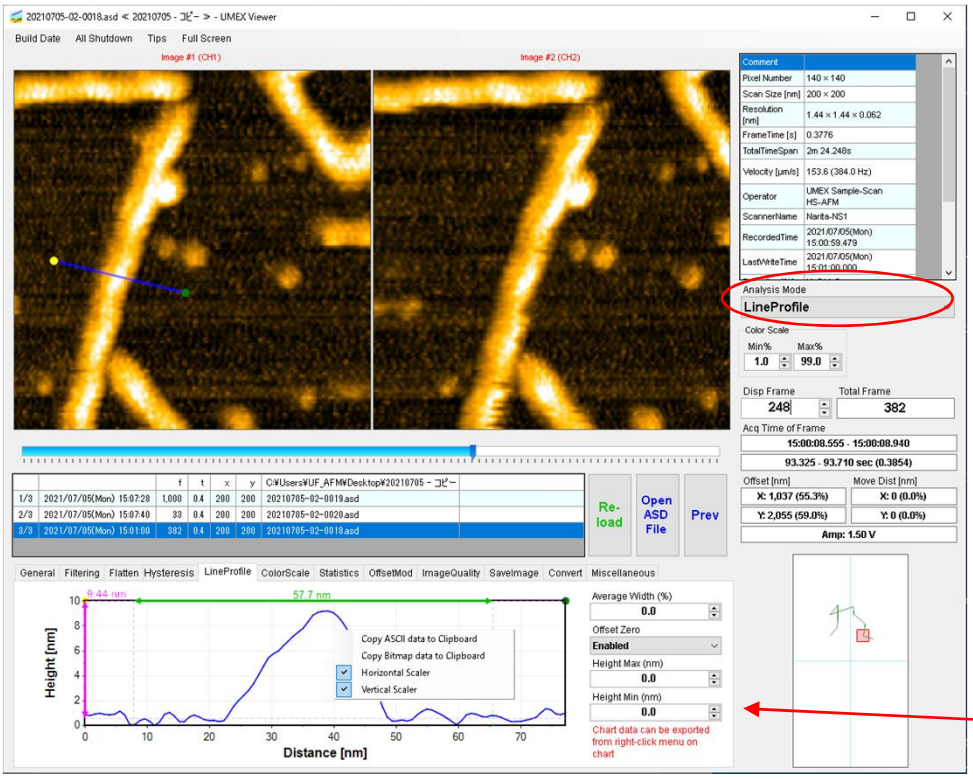
Hysteresis

Corrects image distortion and rescaling due to piezo hysteresis.

It is used to compensate for piezo non-linearity that appears prominently in large scan ranges. It is corrected when it is set to Enabled.



LineProfile Get the height variation along the specified line.



You can change the display range by specifying Height Max and Height Min.

→Set Analysis Mode to LineProfile.
→ Specify where you want to measure.
Set start point: Left click
Set middle point: Left click
Set end point: Right click
Clear all points: Wheel click

Insert point: Enter key while holding a point using mouse left click
Delete point: Delete key while holding a point using mouse left click

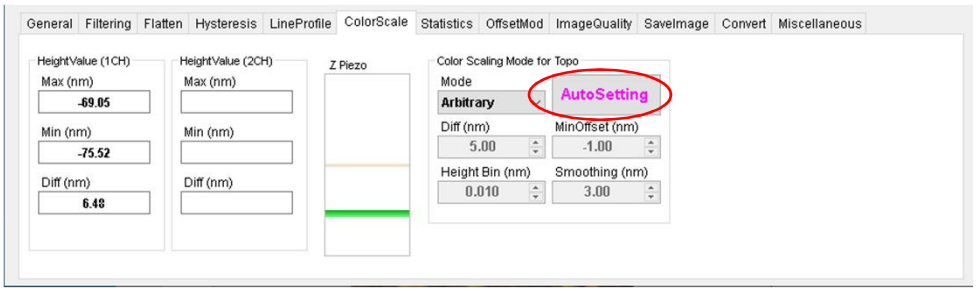
・Right-clicking in the graph displays a context menu.

Copy ASCII data to Clipboad... Copy the distance and height data from the starting point to the clipboard. (Use by pasting into Excel).

Copy Bitmap data to Clipboad... Copy the Bitmap data to the clipboard.
Horizontal Scalar...distance measurement bar display (distance can be measured by dragging the ⇔ length.)
Vertical Scalar... height measurement bar display (height can be measured by dragging the ⇔ length.)

ColorScale

Adjust color scale.

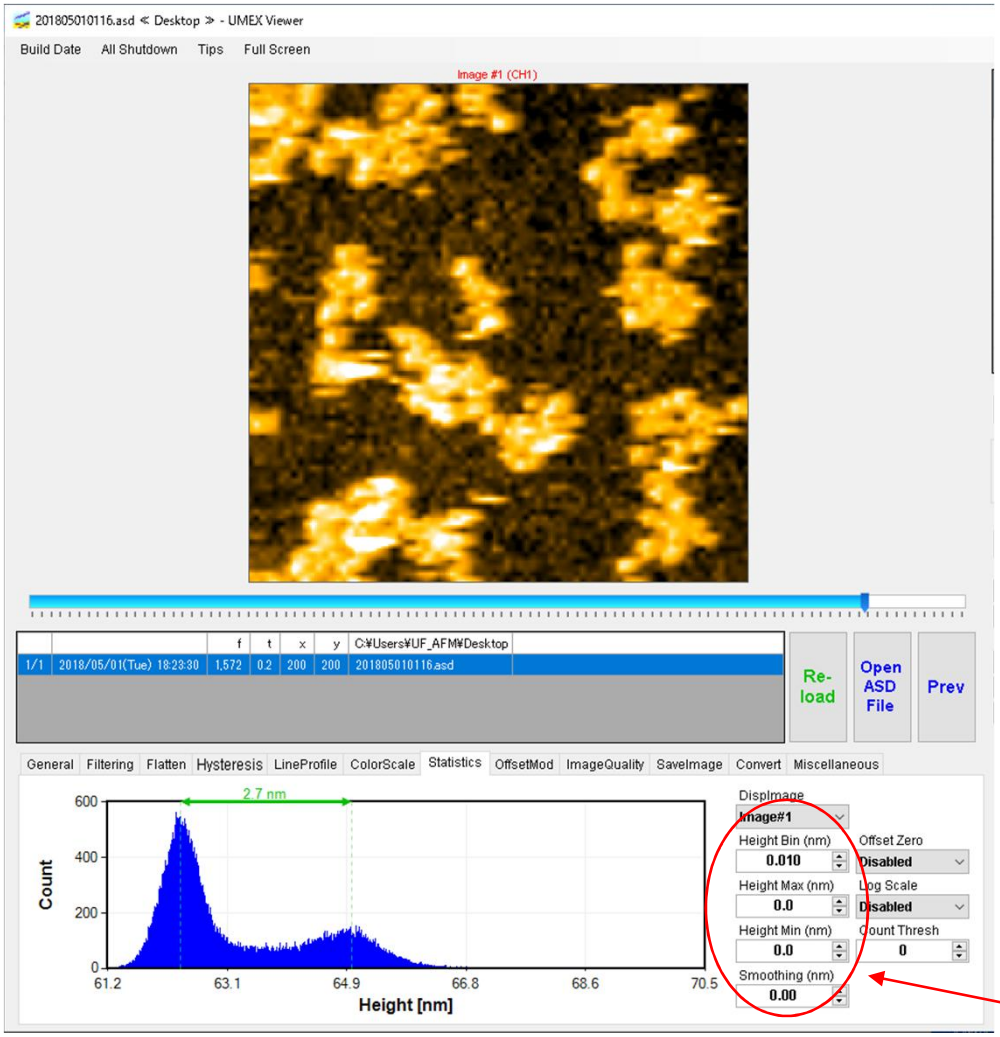


Color Scaling Mode for Topo

Auto Setting: Adjust the brightness automatically in Peak Ref Mode. (Effective when there is a flat substrate appears) If you cannot adjust well with Auto, try changing the Mode.

Statistics

Obtain the height histogram of the image.



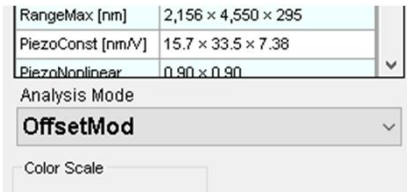
You can change the display range. You can smooth the graph with Smoothing.

In the histogram, the left peak reflects the substrate height, and the right peak reflects the molecular height. Therefore, difference in the two peak positions reflects the height difference between the substrate and the. (The major premise is that the substrate is not tilted.)

* Used for cases where two or more flat surfaces are exposed at the same time, such as lipid membranes. It can also be used to estimate surface roughness.

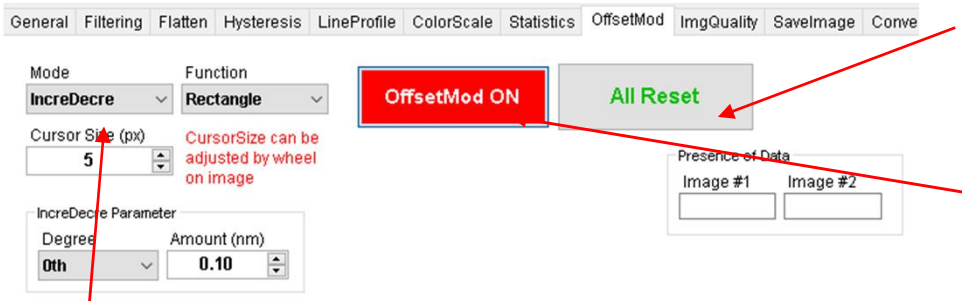
OffsetMod

Image height correction function. It is useful when you want to eliminate line noise or when Flatten does not work well due to excessive ringing.



First, change Analysis Mode to OffsetMod, then cursor on image is changed to a blue square, allowing you to manually adjust the height of the clicked area.

- Left click…modify the height.
- Right click…erase the modification locally
- Middle click…clear all the modification in the current frame
- Wheel…change the cursor size



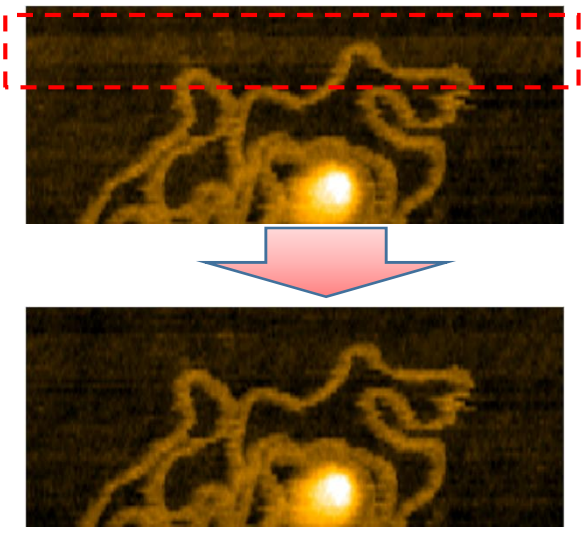
All Reset
Clear the OffsetMod settings for all frames.

OffsetMod ON
Turn it OFF if you want to temporarily hide the OffsetMod changes.

Mode
Select one of the following three modes.

- IncreDecre…Increase or decrease the height of the entire clicked line. Set the amount of increase or decrease with the Amount setting.
- Flatten … Apply local flatten only to the clicked line. Set the polynomial degree with the Degree setting. HeightThresh is a setting for excluding Flatten from areas higher than the set threshold.
- Interpolate … Substitute data interpolated from upper and lower pixel data for the clicked line. Equivalent to WSxM's "Remove Lines".

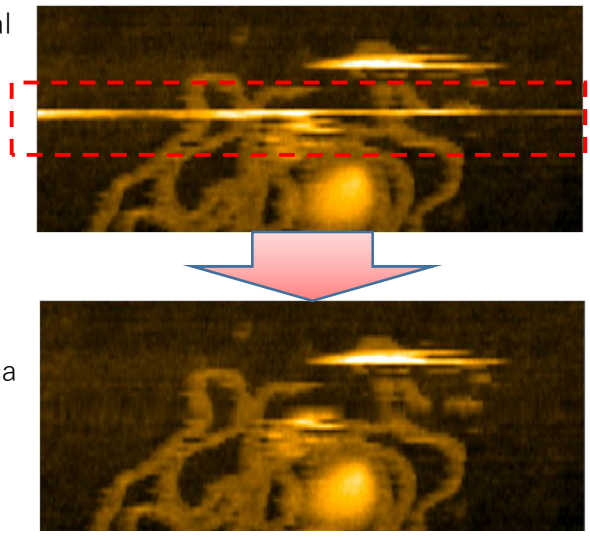
Case suitable for IncreDecre or Flatten(Surface shape can be seen on line noise)



Original data

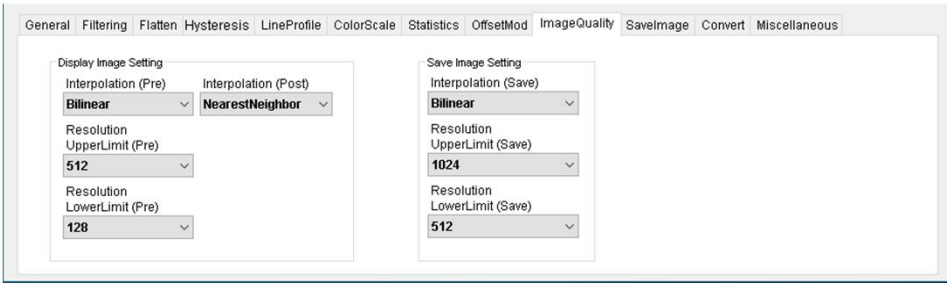
After modification

Case suitable for Interpolate(Surface shape is not seen on line noise)



The OffsetMod information can be saved with the Export button of Config and restored with the Import button after restarting the software.

ImageQuality　Set image quality.



When the number of pixels is too small, interpolate to make it larger.

Set the number of Pixels below which LowerLimit should be interpolated.
Set the maximum number of pixels to interpolate with UpperLimit.

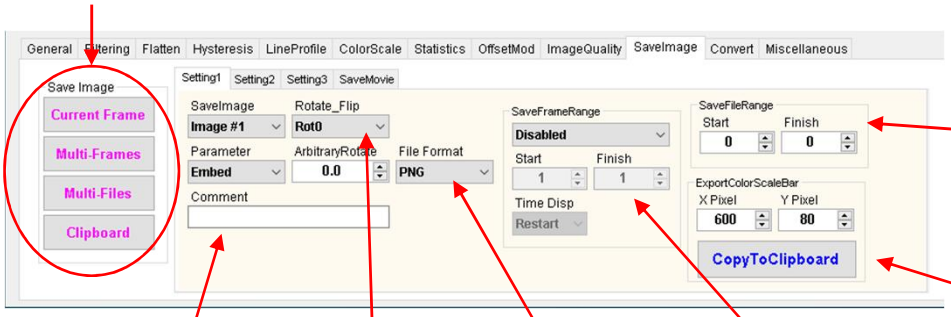
Display Image Setting
Select the interpolation method with Interpolation▼▲.
(The lower you go, the smoother it gets.)
However, in the case of data with a lot of noise, the image becomes unnatural, so Bilinear is better.

Interpolation　▼▲
Auto... automatic adjustment
NearestNeighbor... the nearest point
Bilinear... 2 points each for vertical and horizontal lines
Lanczos2...4 points
Lanczos3...6 points

Save Image　画像の保存設定。

Setting1　Save style settings

Save Image
Current Frame: Save the currently displayed Frame.
Multi-Frames…Save all frames specified by SaveFrameRange of the open file.
Multi-Files……Save all files specified in SaveFileRange.
Clipboard…… Copies the current Frame to the clipboard.



Save File Range
Specify the File number to save

Export Color Scale Bar
Specify the size of the color scale bar and copy it to the clipboard. Paste it into Illustrator and use it.

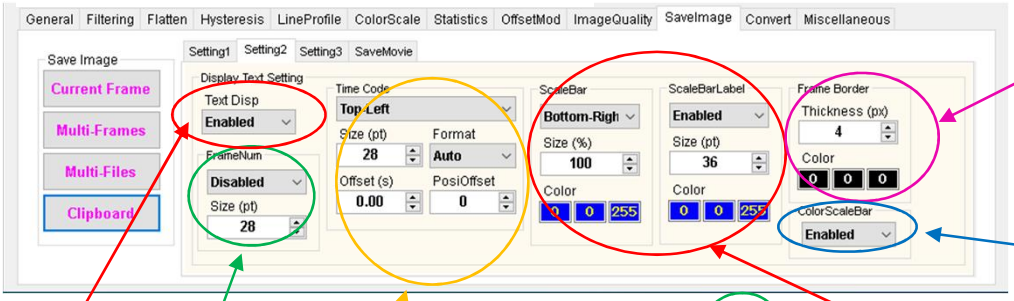
File Format
Specify image format.

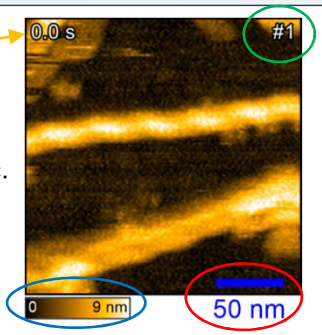
Rotate_Flip
Change the orientation of the image.

Comment
Add a comment to the File name.

Save Frame Range
Specify the Frame range to save.

Setting 2　Setting the text to display





Specify the border width and color.

Specifies the display of the color scale bar.

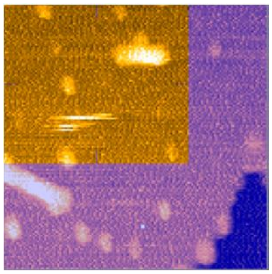
Change to Enabled first.

Specify the position to display time, display style, etc.

Specifies the display of the frame number.

Specify the position, size, and color to display the scalebar and scalebar labels.

Setting 3　Crop Setting



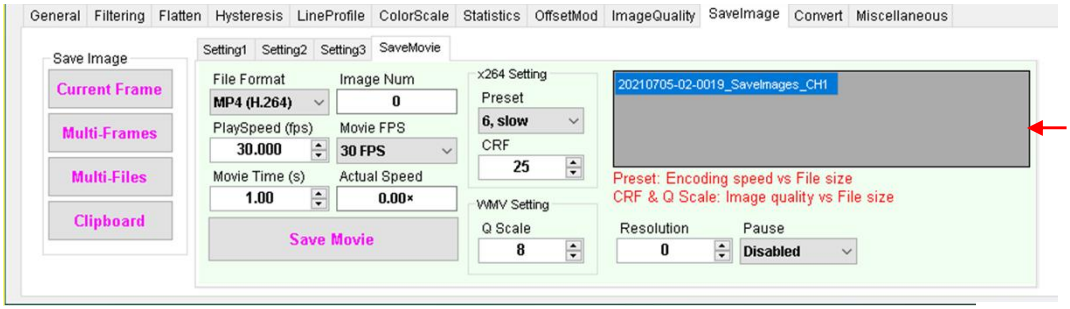
Crop Setting
Specify the display range of the image and save it.

Bottom and Top of the image represent 0-100%, Left and Right each represent 0-100%.

*Convenient when you want to always display the drifting object in the center (correction and save one by one).

Video save settings (Settings for creating videos based on the images previously saved)

*When using the Save Movie function, you must first extract [ffmpeg.exe] from [UMEX Viewer Installer .exe] to the config folder.



Drag&Drop
Automatically set when saving an image with Multi-Frames. Alternatively, you can D&D the saved folder.

File Format
MP4 or MOV (H.264) has a high compression level, but it may not play well in PowerPoint, so in that case, WMV is recommended.

Set either PlaySpeed (fps) or Movie Time(s).

Image　Num
Number of image files.

Movie FPS
Generally 30-60.

Save Movie
Save a movie with specified conditions.

x 264Setting Preset
The slower the preset is, the longer the encoding takes and the smaller the file size.

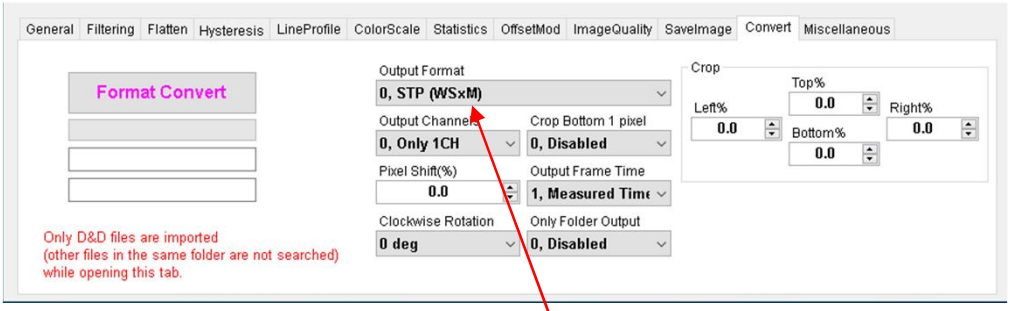
CRF
The lower the value, the better the image quality but the higher the file size.

WMV Setting
Q-Scale
The higher the number, the lower the image quality but the higher the file size.

Resolution
Used when you want to reduce the File size.

Convert

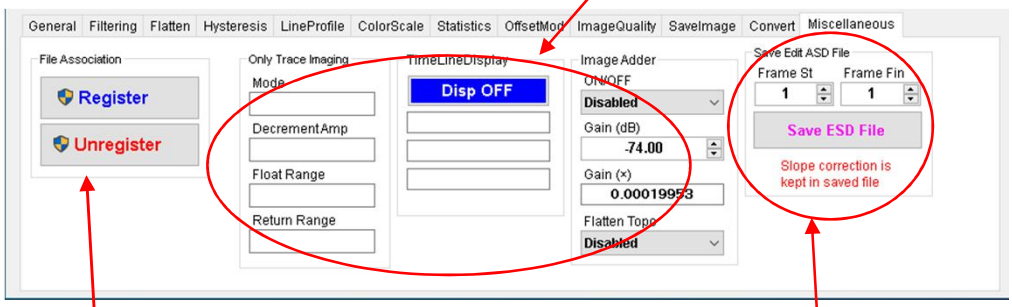
Change ASD File to another file format.



Output Format
1.STP (WS×M)
2.ASC (SPIP,Gwyddion)
3.GSF (Gwyddion)
4.CSV (Mathematica)
5.XLSX(Time Line)
After specifying the format, press Format Convert button.

Miscellaneous

Other settings.



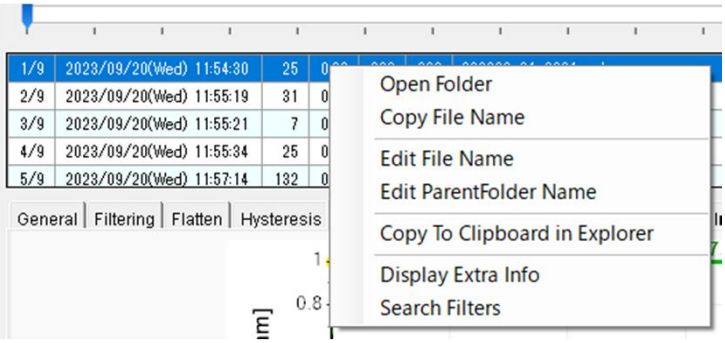
Settings not normally used

File Association
Register UMEX Viewer as software to open when ASD File is double-clicked on Explorer.

Save Edit ASD (ESD) File
Extract and save only the necessary frames from ASD File. Slope Correction can be kept in the file.

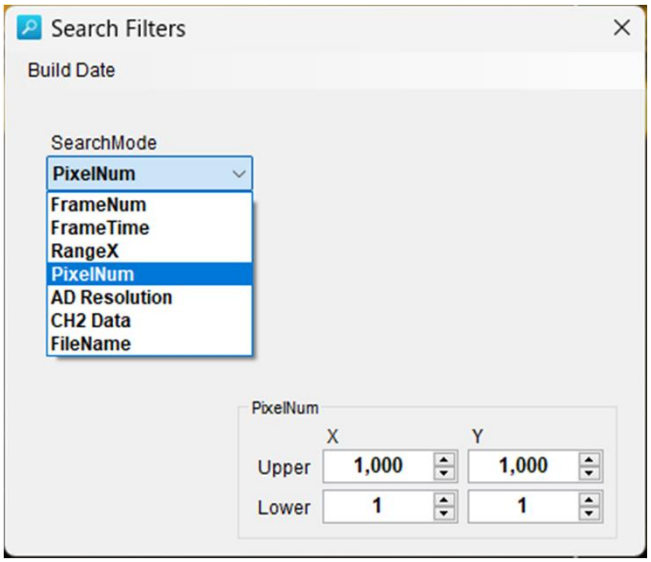
Open with ImageJ

File List Context Menu



Right-clicking on the file list will display a context menu.

- Open Folder……Opens the folder containing the selected ASD file in Explorer.
- Copy File Name……Copies the file name of the selected ASD file to the clipboard. If multiple files are selected, copies the file names of all selected files.
- Edit File Name……Displays a dialog box for editing the file name (used to write notes on data while analyzing data).
- Edit ParentFolder Name……Displays a dialog box for editing the name of the folder containing the ASD file.
- Copy To Clipboard in Explorer ……Copies the selected ASD file. You can copy the selected file to another folder by selecting "Paste" from the right-click menu in Explorer. (Use this if you want to pick up files and collect them in another folder while analyzing.)
- Display Extra Info ……Displays information that is not normally displayed, such as the scanner name and scan speed, in the file list.
- Search Filters ……When a folder is loaded by drag and drop, only files that match the set parameters are displayed in the file list.

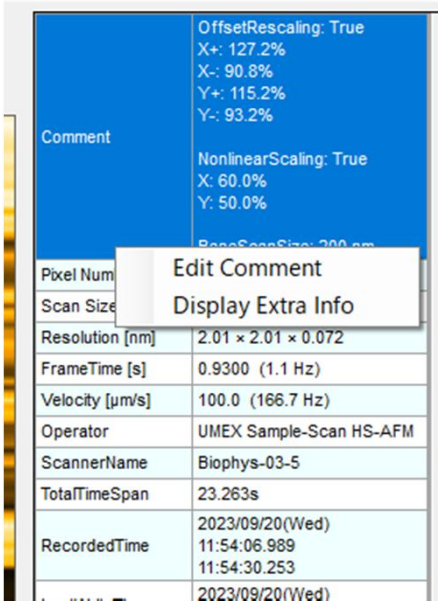


Search Filters Dialog

Select options such as
- FrameNum
- FrameTime
- RangeX
- PixelNum
- AD Resolution
- CH2 Data
- FileName

to set upper and lower limits.

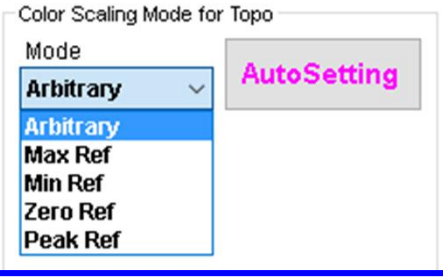
File Property Context Menu



Right-clicking on the file properties will display a context menu.

- Edit Comment …… Edit the comment and rewrite the original ASD file.
- Display Extra Info …… Displays information such as AD Range and AD Resolution that is not normally displayed.

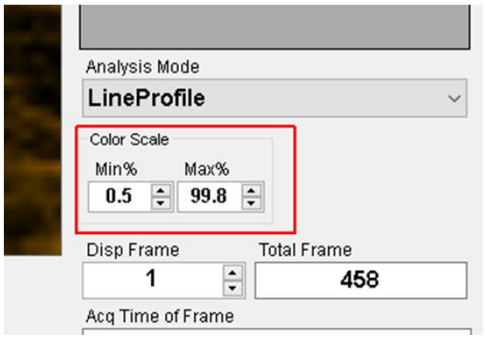
Image Color Adjustment 1

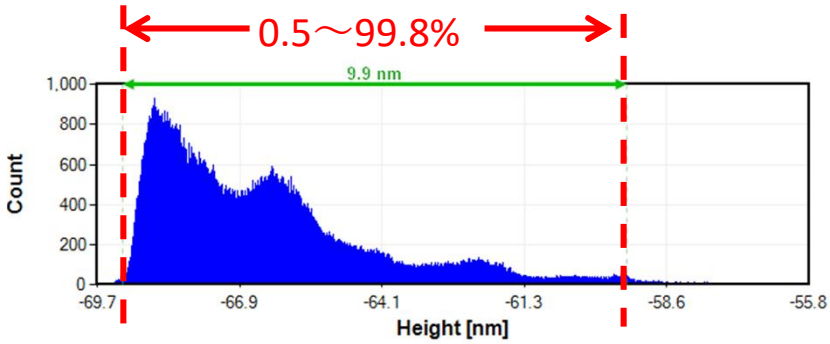


- Arbitrary
- Max Ref
- Min Ref
- Zero Ref
- Peak Ref

A user can select five modes as in the left list. For routine analyses, Arbitrary mode should be used, and only when you need to fix the color scale in every frames, the other modes should be used.

• Arbitrary

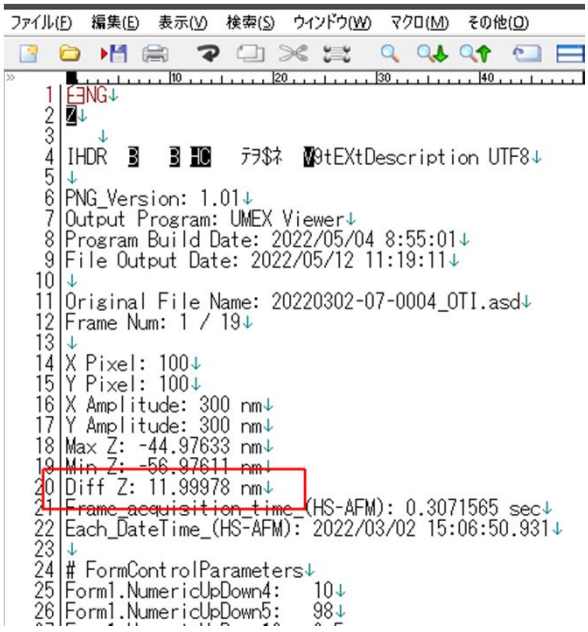




The color contrast can be adjusted by the ColorScale setting on the right end of the window. In this setting, the Min% and Max% values set here are obtained by taking a brightness value histogram as shown in the right figure, and when the total number of pixels is taken as 100%, the pixel with the lowest brightness value is 0.5% of the pixels with the minimum luminance value. (=0), which means that 99.8% of the pixels counted from the highest point have the maximum brightness value (=255). Therefore, setting Min% and Max% to 0% and 100% respectively means that the minimum brightness in the image is set to 0 and the maximum brightness is set to 255.

When image files (PNG or BMP) are saved with Arbitrary mode, the height scale differs in each frame. If you want to check the height scale, drag-and-drop the image file onto a text editor, then you will find the height information in the row of Diff Z.

In the case of a PNG file, the height information is described in the file header.



In the case of a BMP file, the height information is described in the file footer.

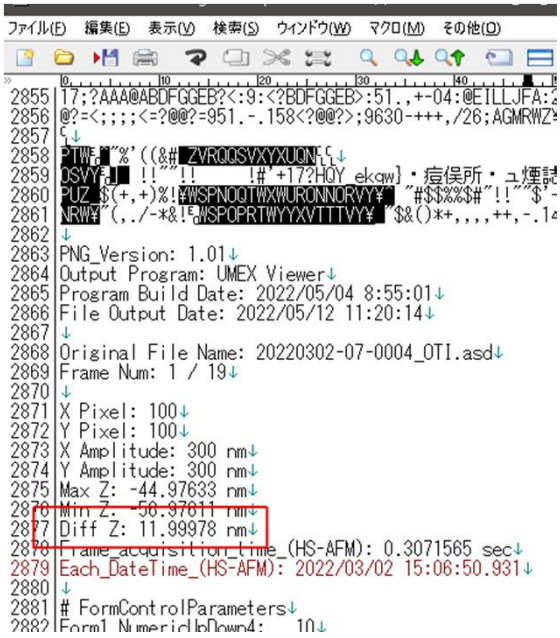
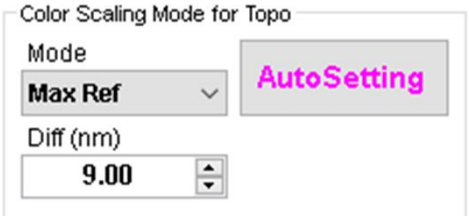
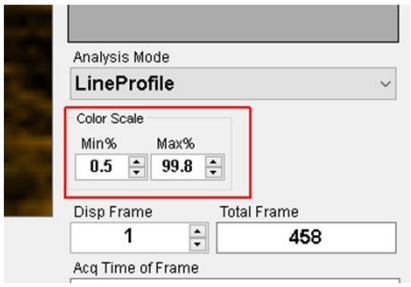
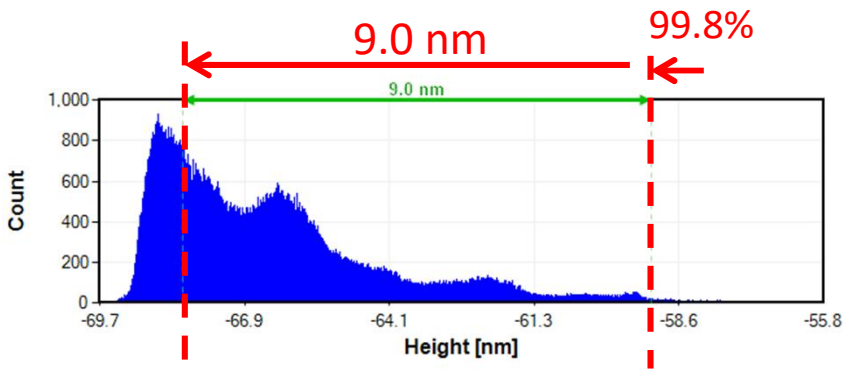


Image Color Adjustment 2

• Max Ref

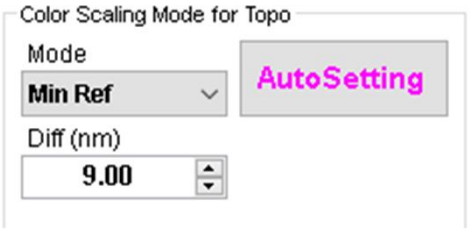


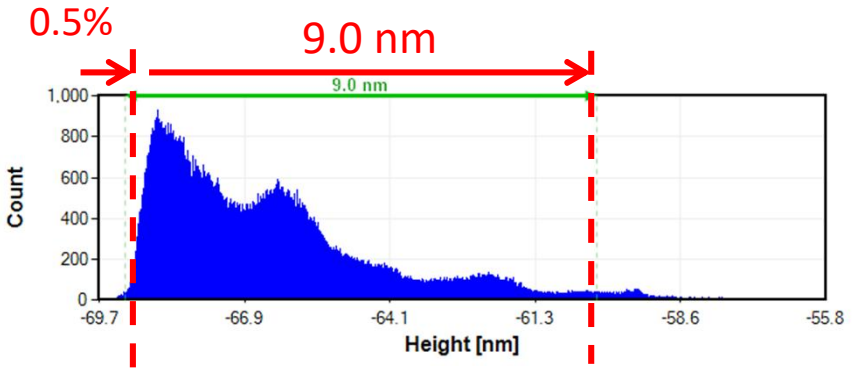




This mode takes the brightness value set in Max% of "Color Scale" as the maximum brightness value (=255), and takes the minimum brightness value (=0) so that the height scale is the value set in "Diff(nm)". You can apply an offset to the entire brightness value with Offset(nm).

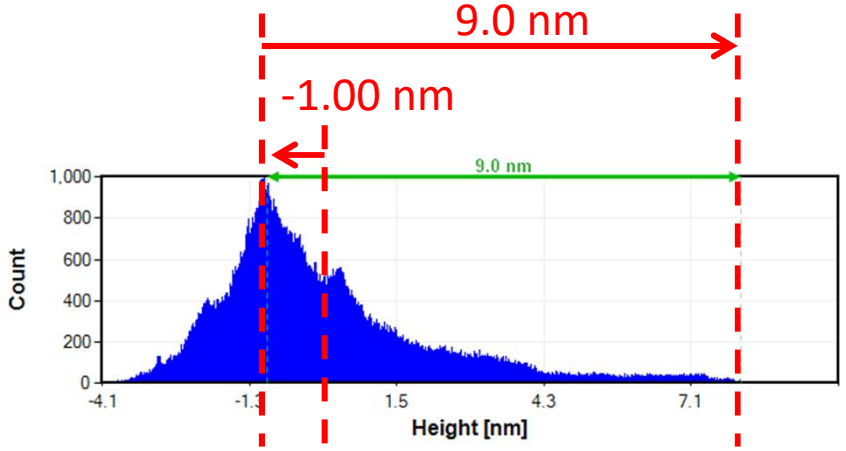
• Min Ref





This mode takes the brightness value in Min% of "Color Scale“ as the minimum brightness value (=0), and takes the maximum brightness value (=255) so that the height scale becomes the value set in "Diff(nm)". You can apply an offset to the entire luminance value with Offset(nm).

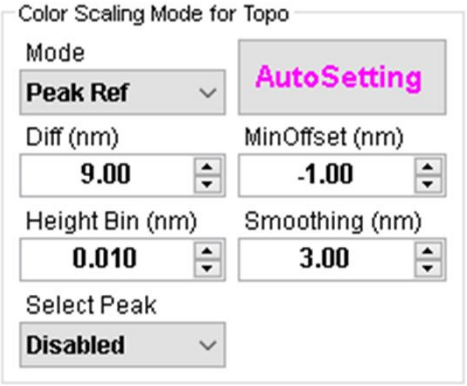
• Zero Ref

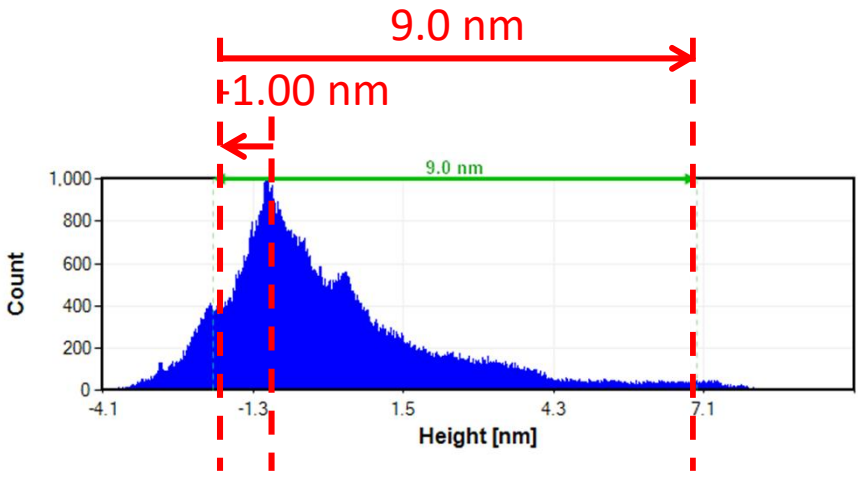


From the zero height point set by Flatten, the height obtained by subtracting "MinOffset (nm)" is the minimum brightness value (= 0), and the height obtained by adding "Diff (nm)“ is the maximum brightness value (=255). Flatten must be enabled in this mode.

Image Color Adjustment 3

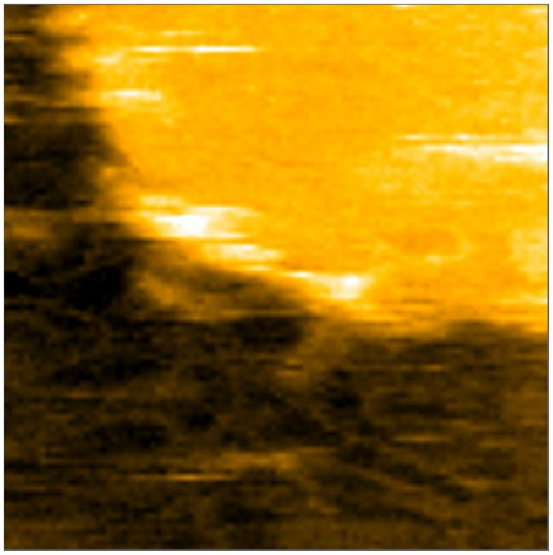
• Peak Ref

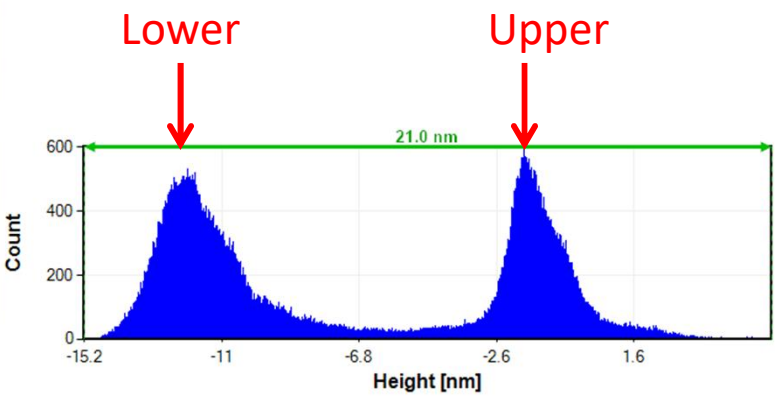




This mode first takes a height histogram and detects the largest peak. The height obtained by subtracting "MinOffset (nm)" from the maximum peak position on the histogram is the minimum brightness value (= 0), and the maximum brightness value (=0) is calculated by adding "Diff (nm)“ to minimum height. The default values for "Height Bin (nm)" and "Smoothing (nm)" are basically fine, but if color scaling does not work well, try changing the values appropriately.

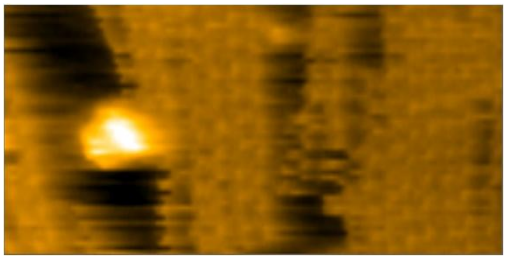
"Select Peak" can be left as Disabled when there is only one peak (that is, when only one substrate surface is visible). If two surfaces appear at the same time, as in the image below, two peaks may appear and the maximum peak may transition from image to image. Only in such cases, use "Select Peak" to set whether to use the higher peak (Upper) or the lower peak (Lower) as the reference.



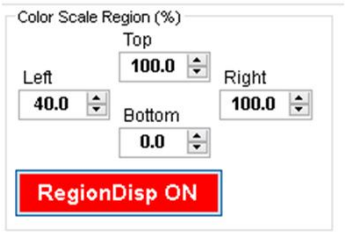


• ColorScaleRegion

When an large dirt or surface crack is in the image, the color scaling is strongly affected by such unwanted objects. Using Color Scale Region, you can exclude the region where the brightness is extracted for the color scaling and get appropriate color scaling.



Same as the Crop setting in Save mode, Bottom, Top, left, and right can be set from 0% (left or bottom end of the image) to 100% (right or top end of the image).



By turning on the RegionDisp, the excluded region can be checked. This function makes appropriate color scaling by excluding the large object.

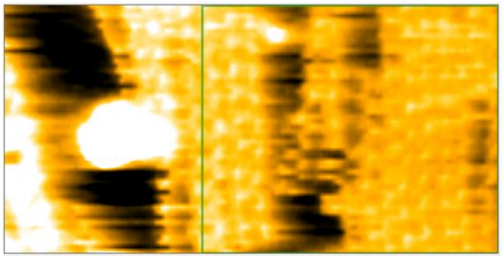
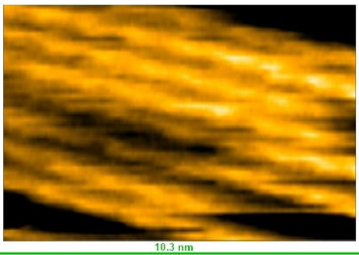


Image Color Adjustment 4

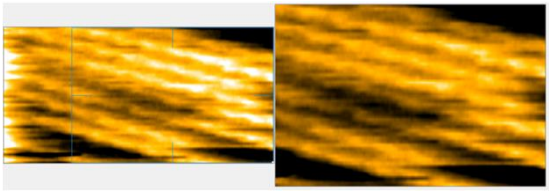
• Setting when molecules cover the surface

PeakRef is not effective when the entire surface is covered with molecules, e.g., actin filaments, as shown in the right figure, the histogram peak becomes broad. Therefore, in such cases, the peak cannot be detected well and the contrast changes from frame to frame.

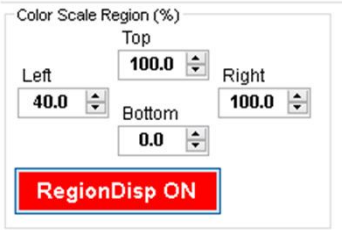


In such cases, use Max/MinRef. Below, MaxRef is described as an example.

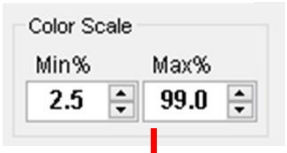
1. When using OTI mode, a bright area appears on the left edge of the image, so use Zoom mode to crop the left part.



2. If bright areas appear on the left or right edge of the image due to OTI mode or ringing only in some frames, cropping using Zoom mode will reduce the angle of view to normal frames as well. In that case, the exclusion area should be set in the Crop Scale Region. A setting of 10% for Left and 90% for Right usually works well.



3. Display the frame you want to optimize, and go to Arbitrary mode, adjust Min% and Max% of Color Scale to just optimize the image contrast.

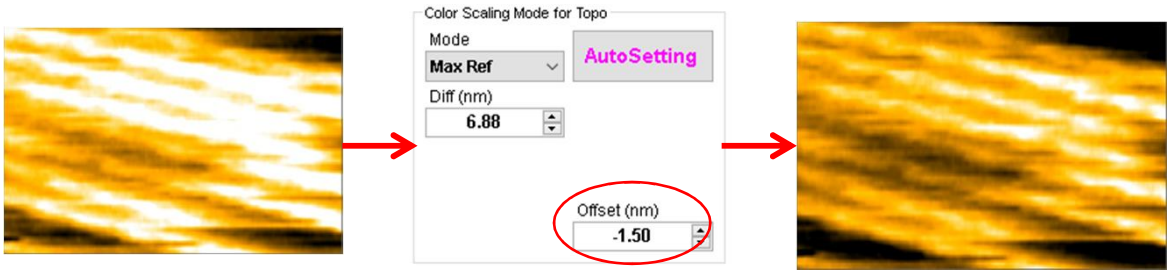


4. Change the ColorScale mode to MaxRef and press the AutoSetting button. Then, the difference between the maximum height and minimum height is automatically entered in Diff in the optimized color contrast.

5. Then, move the frame to check if the contrast does not change. If the adsorbate appears and disappears on actin, it is necessary to remove the brightness value of the adsorbate. Therefore, reduce Max% in ColorScale to about 80 to 90%. Since the Max% differs depending on the range of the adsorbate covering the surface, it is necessary to adjust it later.

• Setting when molecules cover the surface (continue from prev page)

6. Decreasing Max% will make the image brighter and overexposed. Therefore, reduce the Offset(nm) and adjust the image to optimize the brightness. Finally, move the frames to see if the contrast does not change from frame to frame.

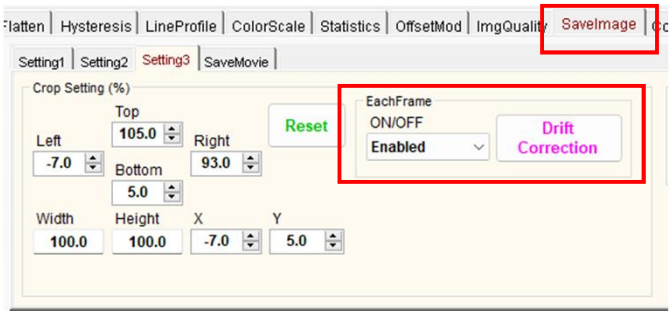


7. If the contrast still changes, adjust Max%.

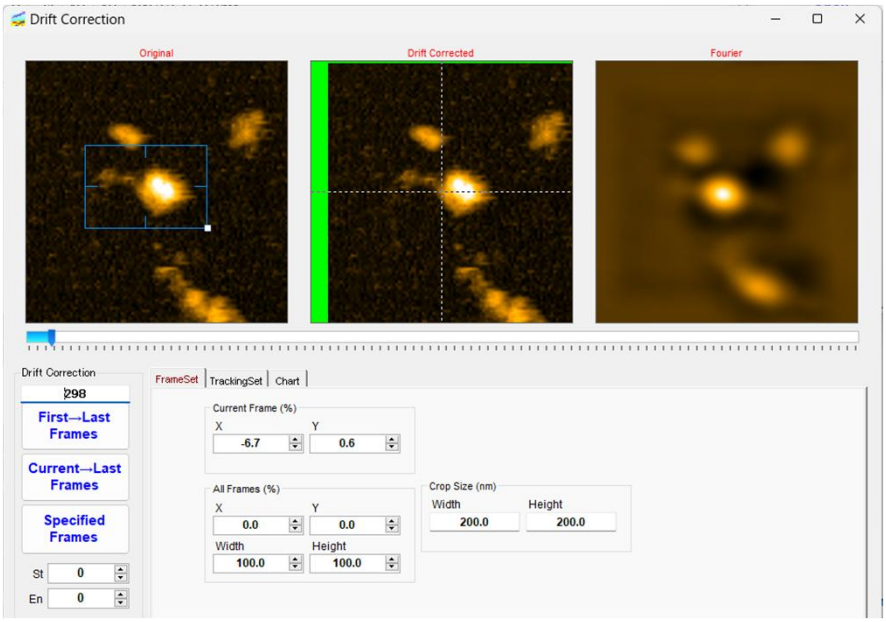
To do this, check the frame where the contrast changes. In the case of the image where the adsorbed molecules disappears, Max% is too small, so try increasing it to 85-95%. Conversely, in the case of an image in which adsorbed molecules appear, Max% is too large, so try lowering it to 60-70%. After that, move the frame and adjust Max% while checking that the contrast does not change. If you can confirm that there is no change, adjust the Offset again. If that doesn't work, try MinRef.

Drift Correction

When creating a movie file, if the position of the molecule changes every frame, it looks bad, so it is preferable to perform drift correction so that the molecule is always located at the center of the image. It is advisable to compensate for drift manually as much as possible during the experiment and then perform drift correction in the software.



1. Open the Setting3 tab of the SaveImage tab.
2. You can set the crop area of the image with CropSetting.
3. By default, EachFrame is Disabled, and the crop settings are reflected for all frames at once. By setting this to Enabled, you can save the crop settings for each frame.
4. If it is difficult to adjust manually, perform automatic tracking using template matching. First, set Flatten, Smoothing, etc., then press the Drift Correction button.

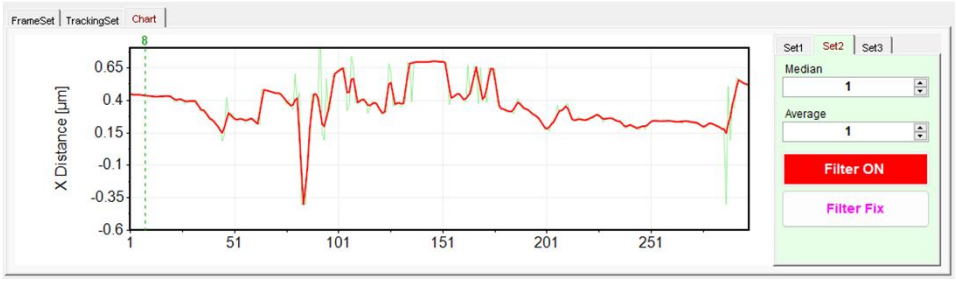


1. The images are displayed in the upper half of the window. From the left, they are the original AFM image, the drift-corrected AFM image, and the 2D Fourier image.
2. Set the Width and Height of All Frames in the FrameSet tab to the image size you want to output (you can also change this after tracking).
3. First, in the leftmost window, manipulate the bounding box and adjust the size and position so that it covers the molecule you want to track.
4. Tracking can be started by pressing any of the three buttons at the bottom left of the window.
    1. First→Last Frames button: Tracks from the start frame to the last frame
    2. Current→Last Frames button: Tracks from the currently open frame to the last frame
    3. Specified Frames button: Tracks only the frames in the range set by St or En below the button
5. The drift-corrected image is displayed in the central image, so use the cursor keys to check that the correction has been successful.

• Cases where drift correction does not work

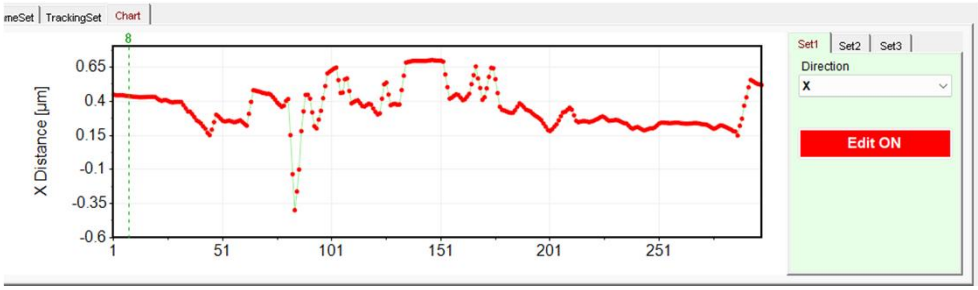
1. If tracking works well up to some frames, but tracking is lost in subsequent frames → Press the Current → Last Frames button from the frame where tracking was lost to perform tracking again.
2. If the amount of drift is larger than the bounding box, or the amount of drift is extremely small → Open the TrackingSet tab, adjust the Search Region, and perform tracking again.
3. If some frames move in a pulsating manner → Open the Set2 tab on the Chart tab and adjust parameters such as Median and Average.
4. If tracking is lost in some frames even after adjusting the filter → Open the Set1 tab on the Chart tab, switch the Edit button to ON, and adjust the frames that have shifted on the chart.
5. If this does not work for the target molecule, try tracking it due to dust or other debris, then adjust the position of the target molecule in the All Frames tab of the FrameSet tab so that it is in the center of the image.

• Chart Filter



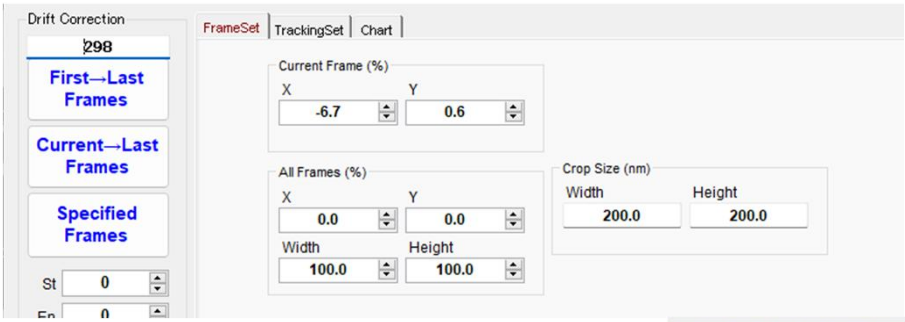
1. The trajectories before and after the filter are displayed as green and red lines respectively. You can move to the desired frame by dragging the dashed line on the chart.
2. Adjust the Median and Average filter values so that the line is smooth.
3. If there is a lot of pulsating noise, increase the Median.If there is a lot of random fluctuation, increase the Average. If the values are too high, it will not track large drifts, so be careful not to make them too high.

• Chart Edit

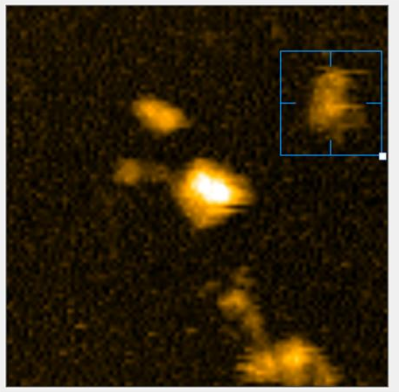


1. You can directly edit the chart using the mouse.
2. Turning the Edit button ON makes the chart editable. If a filter is enabled, a dialog will be displayed. Pressing the OK button will reflect the filter settings in the original data, overwriting the original data, so edit the chart after you've finished adjusting the filters.
3. The chart will change to red circles and green lines. You can adjust the position of each frame by dragging the red circles. You can also select and operate multiple points simultaneously by holding down the Shift key and dragging the mouse.

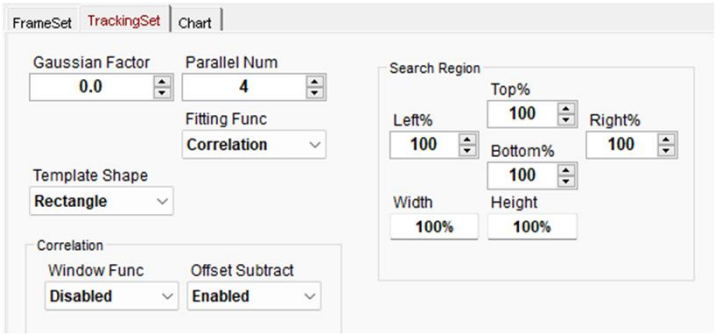
• Frame Set



1. Current Frame allows you to adjust the position of the currently open frame. You can also edit it in Chart Edit, but here you can edit it numerically.
2. All Frames allows you to adjust the position and size of all frames.
3. If you have trouble tracking the target molecule properly, it is a good idea to track some debris or something as shown in the image on the right, and then adjust All Frames so that the target molecule is in the center.



• Tracking Set



1. The settings on the left half are for development purposes and are not generally used.
2. The Search Region on the right allows you to set the molecular search range. With the default setting of 100%, when searching for a molecule in a certain frame, the search is narrowed down to an area the same size as the bounding box, centered on the molecular position in the previous frame. You can change the search range by setting this value to a value other than 100%.
3. If the drift is large compared to the size of the molecule, set a value larger than 100%.
4. If there is a molecule close to the target molecule and tracking shifts to that molecule, set a value smaller than 100%.

How to Remove Frame Footer

In the ASD file format, the frame header is allocated 32 bytes by default. Since 32 bytes is not enough to save the information for each frame in interactive mode or OTI mode, it was necessary to expand the frame header size. The ASD file header has a section for specifying the frame header size, so it is expandable. However, Kodec was not able to read this frame header size properly. Therefore, to maintain compatibility with Kodec, I decided to keep the frame header size fixed at 32 bytes and introduce a frame footer instead.

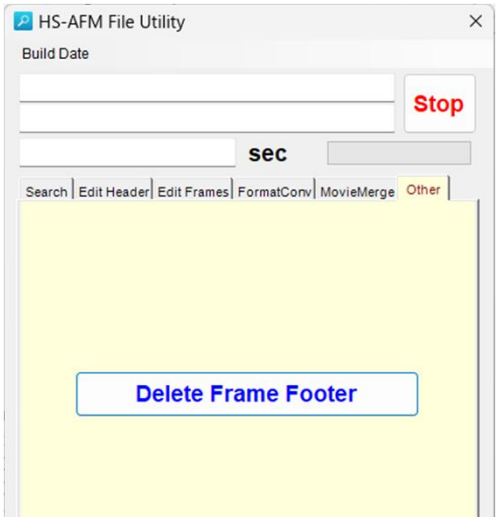
The frame header size in the header stores the frame header (= 32 bytes) + frame footer size.

While a ImageJ plugin and other programs support this, some software, such as NanoLocz, do not.

Therefore, deleting the frame footer makes it possible to use non-compatible software.

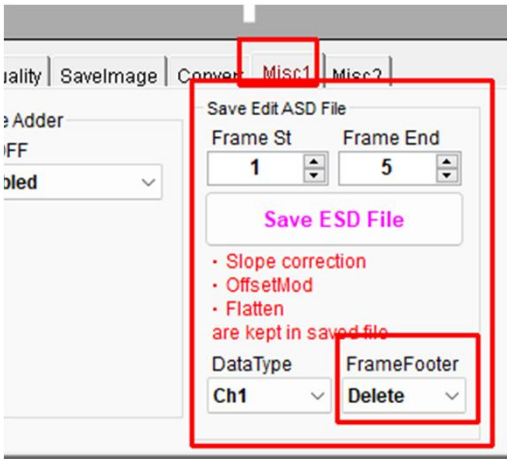
• When converting multiple files

Using the "HS-AFM File Utility", you can convert multiple files or folders at once by pressing the Delete Frame Footer button.



• Converting a single file

Using "UMEX Viewer" to save an ESD file using "Save Edit ASD File," you can set the “FrameFooter” option to “Delete” to save the file with the frame footer deleted.

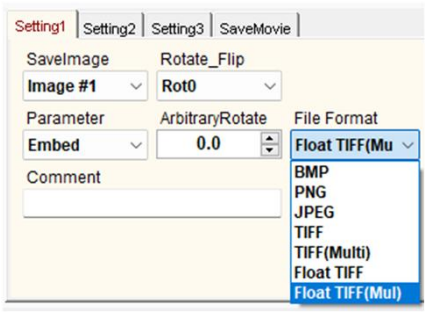


How to Open Data in ImageJ

ImageJ offers a wide range of analysis functions, making it very convenient to use.
By default, ImageJ cannot open ASD files directly, but they can be opened using the methods described below.
In all cases, the files can be saved with applied processing such as filtering and drift correction.

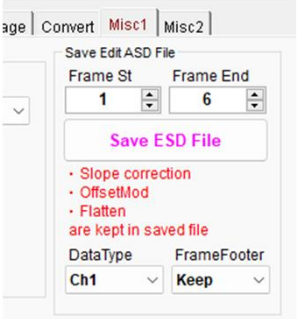
How to Save as Float TIFF

When saving an image using *SaveImage*, you can choose formats such as BMP or PNG, which can be opened directly with standard image viewers. While these formats are widely compatible, they limit the brightness gradation to 255 levels, which reduces the resolution in the Z direction and may result in loss of data in very bright or dark regions. Therefore, they are not suitable for quantitative analysis in ImageJ.
To preserve the full resolution of the data, it is recommended to save the image in *Float TIFF* or *Float TIFF (Mul)* format. These formats store the data in floating-point format, maintaining the original data precision, which is ideal for analysis in ImageJ.
Please note that Float TIFF files are saved uncompressed. If saved at high resolution, the file size can become very large, and saving may fail if your PC does not have sufficient memory. In such cases, follow the instructions shown in the message box during saving and save at the original resolution instead.



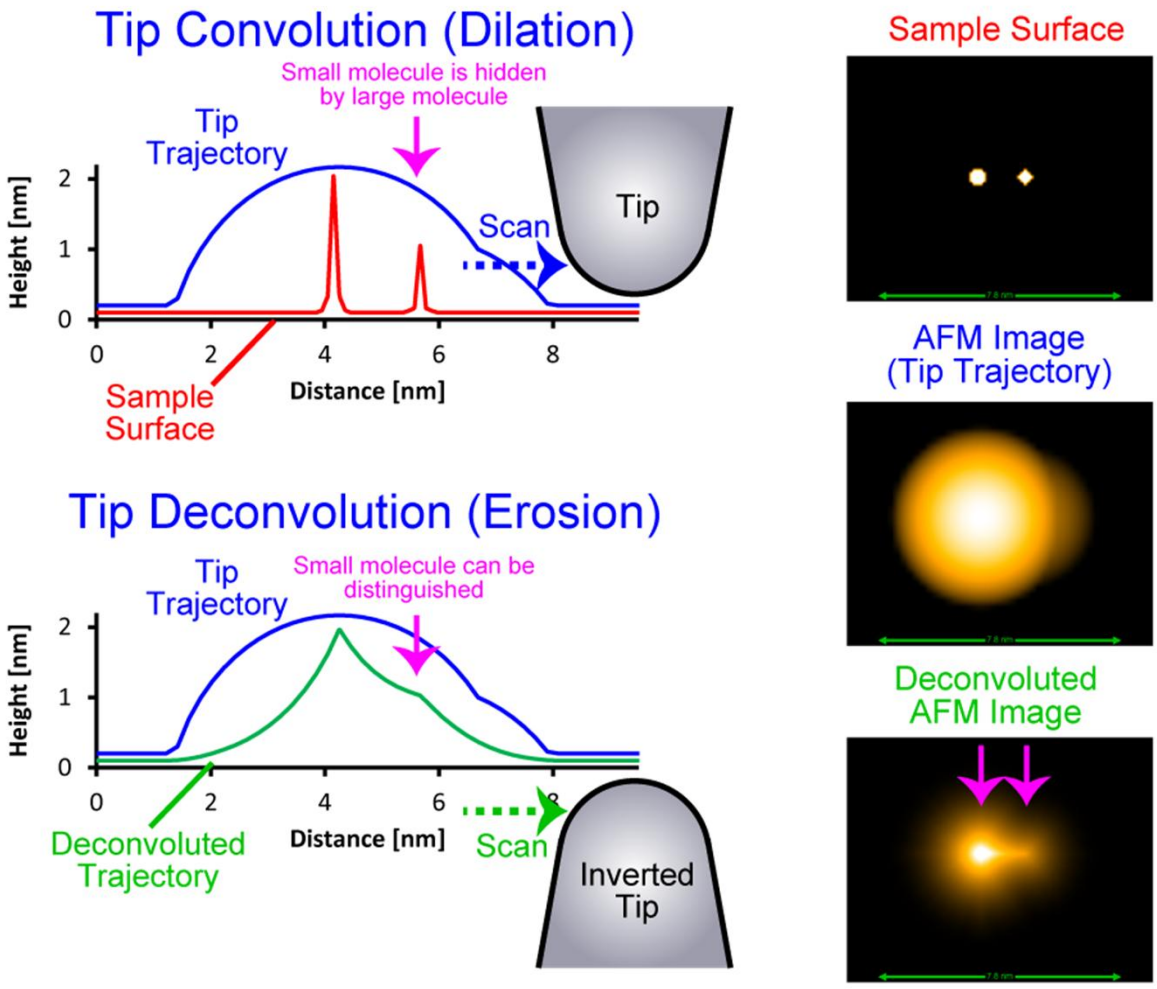
How to Open ASD Files Directly

By installing the Load_ASD_32bit_FooterEnabled_RetraceEnabled.java plugin in ImageJ, you can open ASD files directly in ImageJ. Additionally, by utilizing ESD format, only the specified frames can be saved, which makes subsequent analysis more convenient.



Tip Deconvolution1

Case for Small biosample



In AFM, a probe with a radius of curvature of several nanometers is used to trace the surface of the sample for measurement. Due to the tip effect, molecules are often hidden and the location of small molecules is hardly distinguished from the image. The process in which the apparent surface shape of the sample looks different due to the tip effect is called "Tip Convolution (Dilation)".

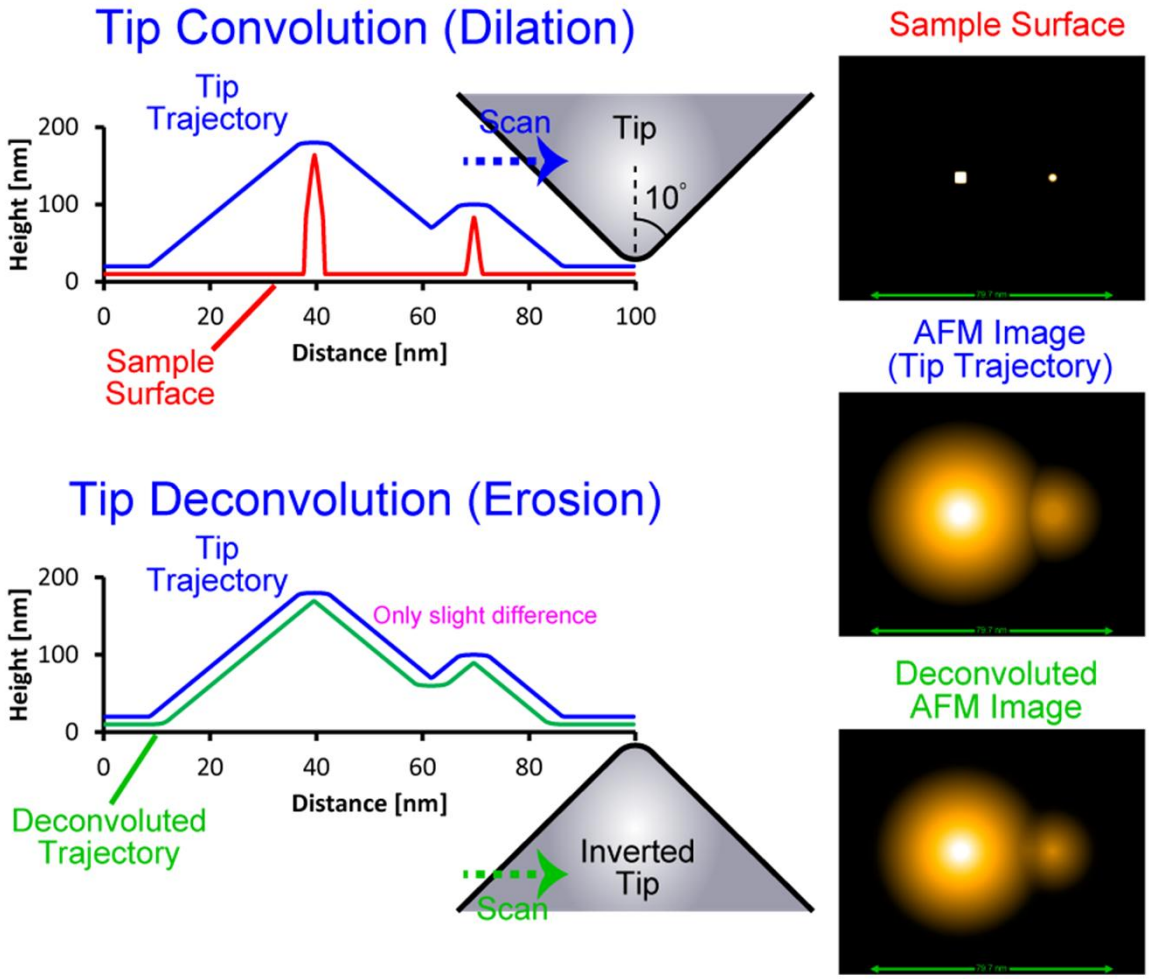
Conversely, the process of removing this tip effect is called "Tip Deconvolution (Erosion)". In the Tip Deconvolution filter, an inverted probe is used to trace the obtained surface topography image from below. The probe trajectory obtained by this can obtain an image from which the probe effect is removed. Since the probe cannot get closer to the surface just beside the molecule, the original shape of the molecule cannot be completely restored, but it is effective for the detection of the position of the hidden small molecule.

UMEX software assumes a structure in which a hemispherical probe is attached to the tip of a cone-shaped needle, but in actual measurements, probe anisotropy and double tips are often problematic. Therefore, an algorithm called "Blind Tip Reconstruction " has been developed to estimate the tip shape from the AFM image. However, unlike the simulated image, the experimental AFM image often has non-ideal effects such as noise and feedback errors, resulting in unstable resulted tip geometry. Also, even if the tip shape can be perfectly estimated, as mentioned above, the original sample surface cannot be completely restored, so a filter that assumes a simple tip shape is sufficient for the time being.

Application examples: Measurement of coiled-coil length bound to molecular complexes, analysis of time-course of volumes of molecular complexes, etc.

Tip Deconvolution2

Case for large biosample



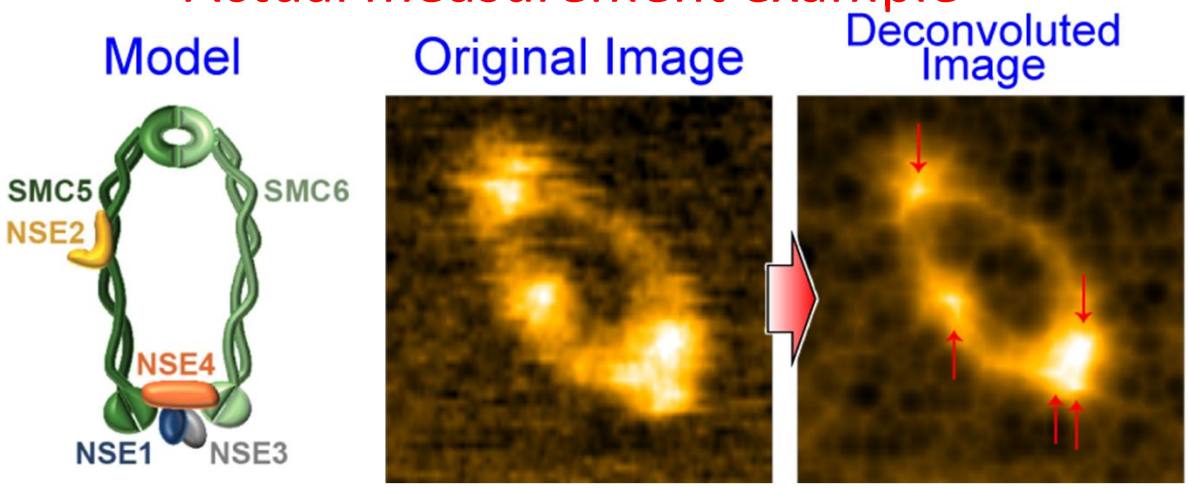
On the other hand, when the observed molecule is 100 nm or more, sufficiently larger than the radius of curvature of the probe, the image obtained mainly reflects the shape of the conical part of the tip. When the tip is spherical, tip deconvolution works effectively, but when it is conical, the difference between the images before and after tip deconvolution becomes quite small, and a large effect cannot be obtained. Therefore, this filter is an effective only for small molecular systems.

Reference

- J. Res. Natl. Inst. Stand. Technol. 102 (1997) 425.
- Ultramicroscopy 94 (2003) 19–29.

Tip Deconvolution3

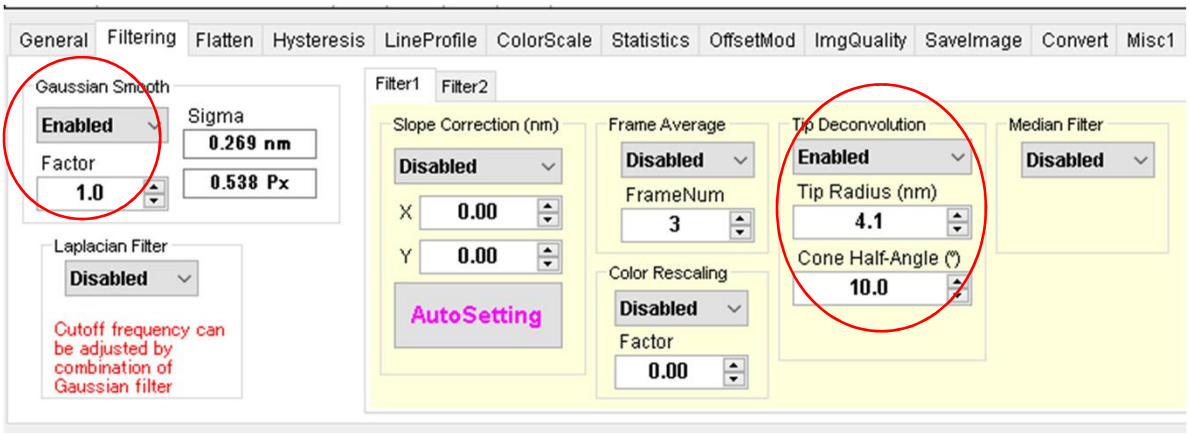
Actual measurement example



The results of SMC5/6 will be explained as an example. This molecule consists of hexamers and forms a complex of Nse4, Nse1 and Nse3. In order to analyze the molecular length such as the length of the coiled-coil domain, it is necessary to specify the central position of the domains. However, since the bright spots of these domains are spread by the radius of the probe curvature and these bright spots are overlapped, it is difficult to specify the exact position of the center of the domain from the AFM image.

By applying the Tip Deconvolution filter to this experimental result, the size of the bright spot becomes smaller, and the central position of the bright spot can be easily identified. This result indicates that the true central position of the molecule is slightly shifted from the apparent position of the bright spot.

How to use



First, set Gaussian Smooth to Enabled and Factor to 1.0.

After that, set the Tip Deconvolution filter to Enabled. Basically, adjust only the Tip Radius so that the image becomes natural. Typically, a value of about 2-6 nm should be used.

Cone Half-angle can also be adjusted, but as long as the EBD probe is used, there is no need to change it to anything other than 10° .

If a flat surface looks like a spider web due to experimental noise, you can reduce this effect by enabling the Median Filter.