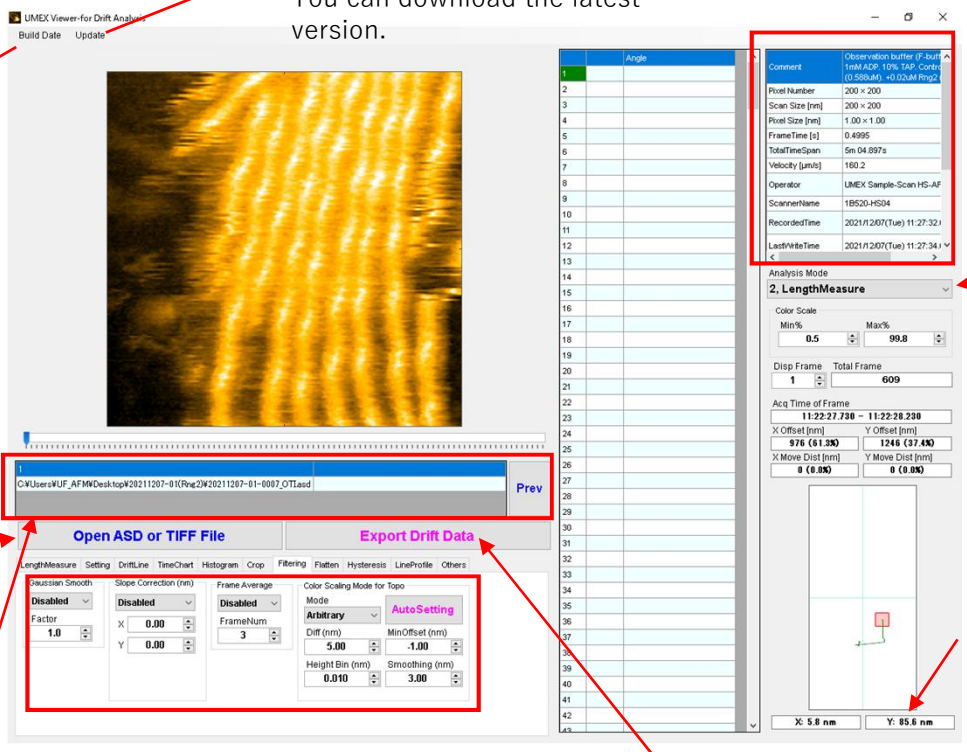
Quick Guide for UMEX Viewer for Line Analysis

< Main screen/common tab >



Update
You can download the latest version.

Build Date
Click on the pathname to open the folder containing the program.

Basic image information

Analysis mode

※You can also open the file by dragging and dropping it to the icon before opening the software.

Represents the XY coordinates of the cursor position with the origin at the bottom left of the Image.

File name display field
- When you drag and drop a file, the file name is displayed.
-Right-click on a file name to open the folder containing that file.

Export Drift Data
Export the results as csv.File.

Filtering

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

Slope Correction(nm)
Adjust the tilt of the surface in each of the X and Y directions.

Frame Average
Smooth the data by averaging it between frames. Effective for data with small movements. (triangular can suppress afterimages better than rectangular)

Color Scaling Mode for Topo

Auto Setting…Automatically adjust brightness in Peak Ref Mode. (Effective when there is a flat surface)
If you cannot adjust the settings properly with Auto, try changing the Mode.

Time Chart

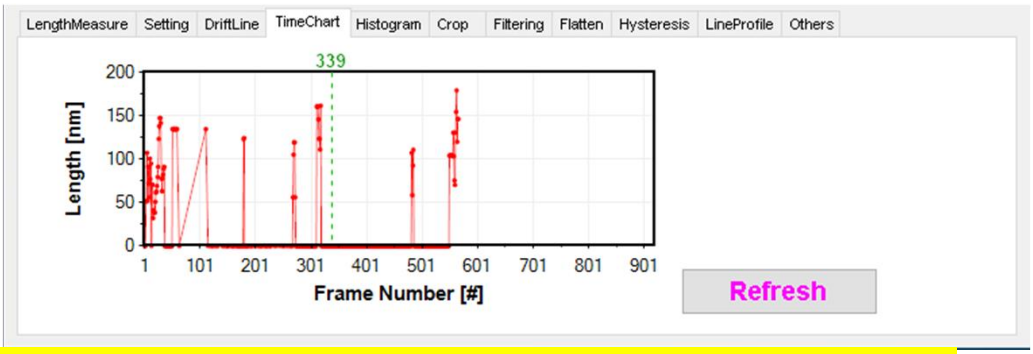


Diagram visualizing frames with data and measured values.
・You can jump to the target frame by dragging the green dotted line.

Others

Display Image Setting
Set the interpolation method.
(Bilinear is better if the data is noisy, as the image will look unnatural.)
Interpolation(Pre) ▼▲
Auto··· automatic adjustment
NearestNeighbor··· nearest point
Bilinear···2 points each in the vertical and horizontal directions
Lanczos2···4points
Lanczos3···6points

Config(DRI)
Export and import settings such as parameters.
Export (w/Time)...Save under a different name with time included.
Import...Load the D&D parameter file.
⬜ ···Drag & Drop the file you want to import.

Save Image
Save the displayed Frame.

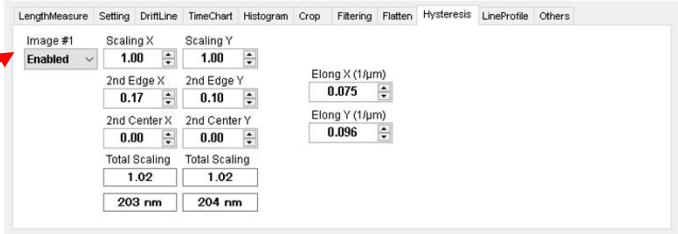
Clipboad
Copy the displayed Frame to the clipboard.

Save Point Data
Save the data of the displayed frame.

Hysteresis

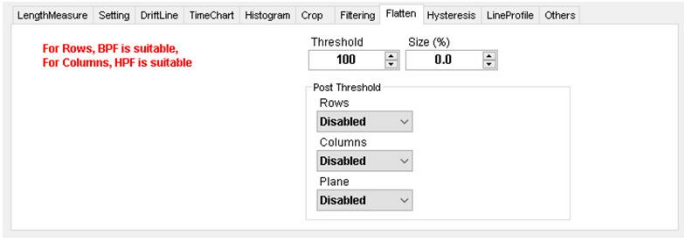
Corrects image distortion and scaling caused by piezo hysteresis.

It is better to use this because it becomes noticeable when scanning a large scanning range of 1 μm or more.
It is corrected when it is enabled.



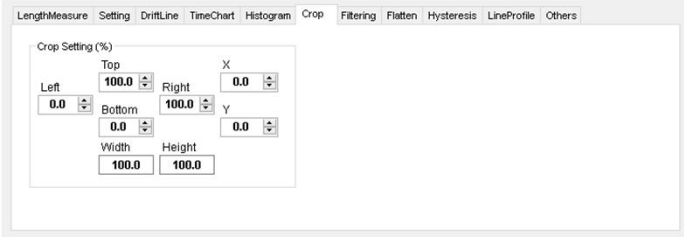
Flatten

Used to eliminate ringing.



Crop

Used to enlarge only a portion of an image.



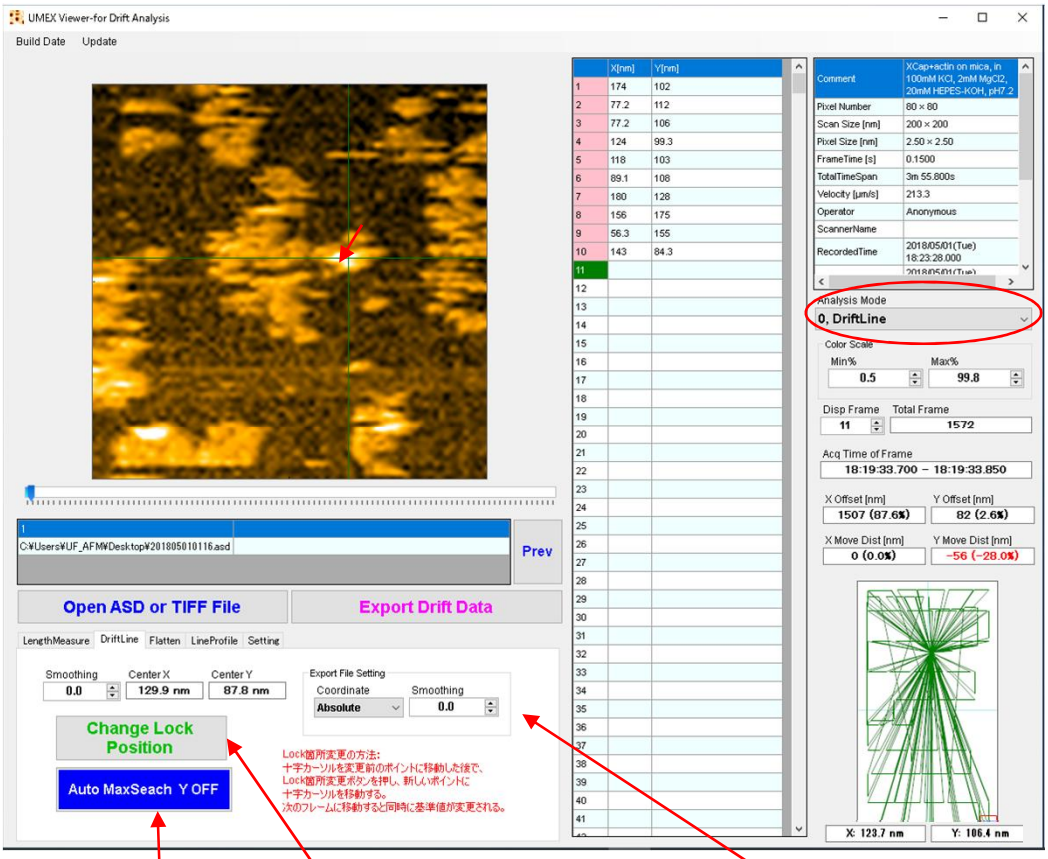
< Analysis Mode 0 _Drift Line >

Drift Line

Measure the XY position (coordinates) with the origin 0 at the top left of the image.

・Obtained by moving the intersection point of the green line crosshairs for each frame.

・Be careful as the coordinates at that time will be overwritten when you move the frame.



Change Lock Position

The function to estimate drift changes for the entire image rather than individual molecule trajectories. When the molecule you're tracking fades out of the image, you can move the reference point there by pressing the button to relock to another molecule and move to the next frame.

Export File Setting

＞Coordinate
Specify whether to save the coordinates in the table on the right as they are, or relative values based on the coordinates in frame #1, as the coordinates of the exported CSV data.
＞Smoothing
Smooth the graph of exported CSV data.

Auto MaxSeach Y ON

When turned on, when moving the frame, it will automatically move to the highest point on the same Y axis.

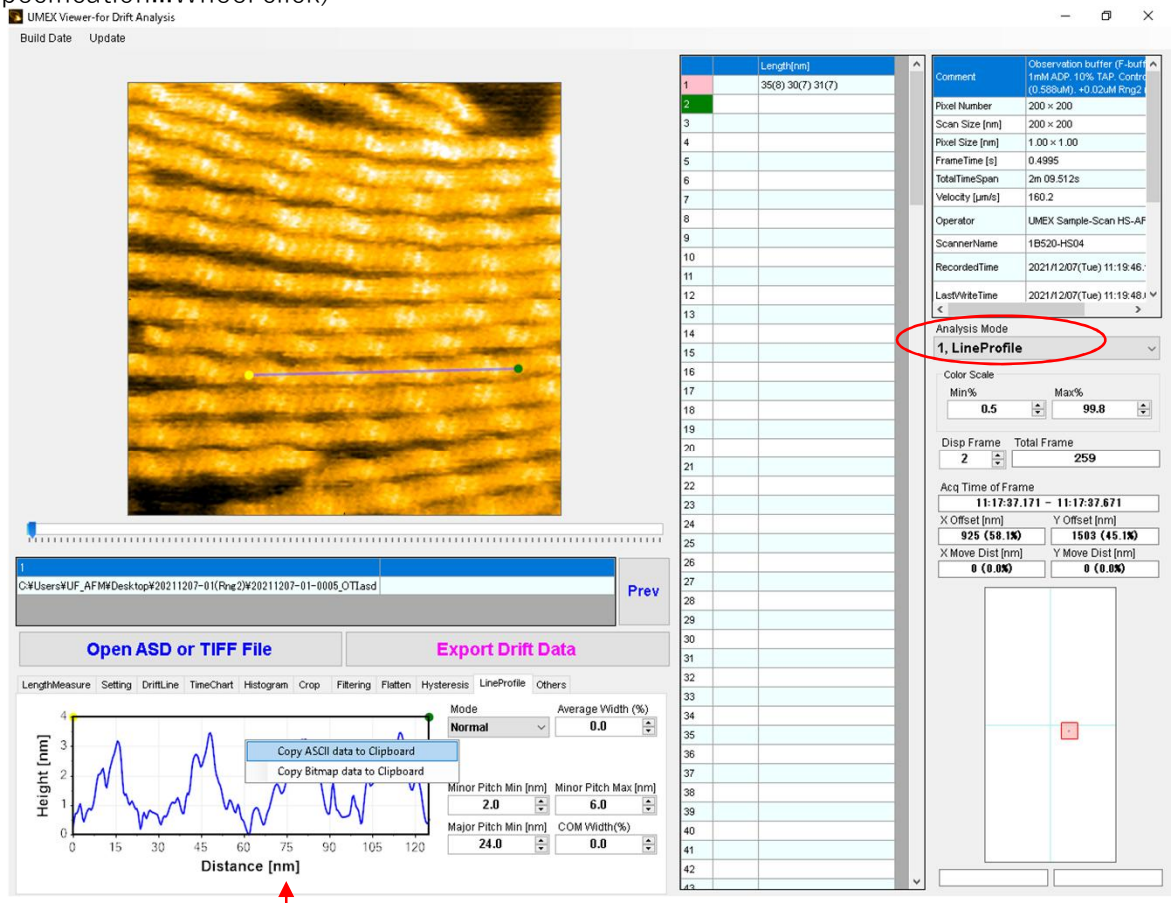
< Analysis Mode 1 _Line Profile >

Line Profile

Get the height and distance of the specified line on Image.

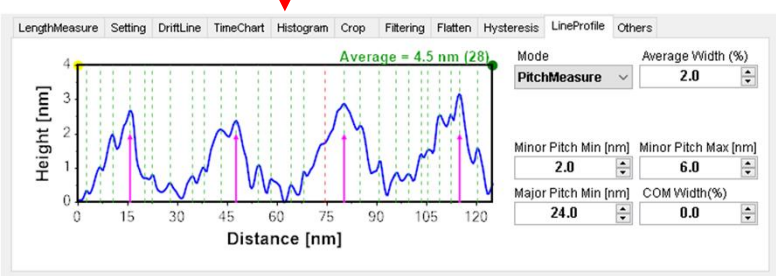
→Set Analysis Mode to LineProfile.

→Specify the area you want to measure. (Start point…Left click, End point…Right click, Cancel specification…Wheel click)



Mode

【Normal】…Measure height and distance.
【Pitch Measure】…Detects the main pitch (purple line) and the minor pitch (green line) between them, and measures the distance between the main pitches and the number of pitches between them.



※Right-click inside the graph to display the context menu.
Copy ASCII data to Clipboad
… Copy the distance and height data from the starting point to the clipboard.

Copy Bitmap data to Clipboad
…Copy Bitmap data to the clipboard.

Average Width(%)
Set the average line width.
Minor Pitch Min(nm)
【Recommended value ：2.0nm】
?
Minor Pitch Max(nm)
【Recommended value ：6.0nm】
? It will be automatically displayed as a red line, but you can ignore it.
Major Pitch Min(nm)
【Recommended value ：24nm】
?
※By setting the maximum value and minimum value of the pitch detection width detection failures can be compensated for.

How to manually add or delete a minor pitch line (green line)…With the line selected, press Enter and Delete keys.

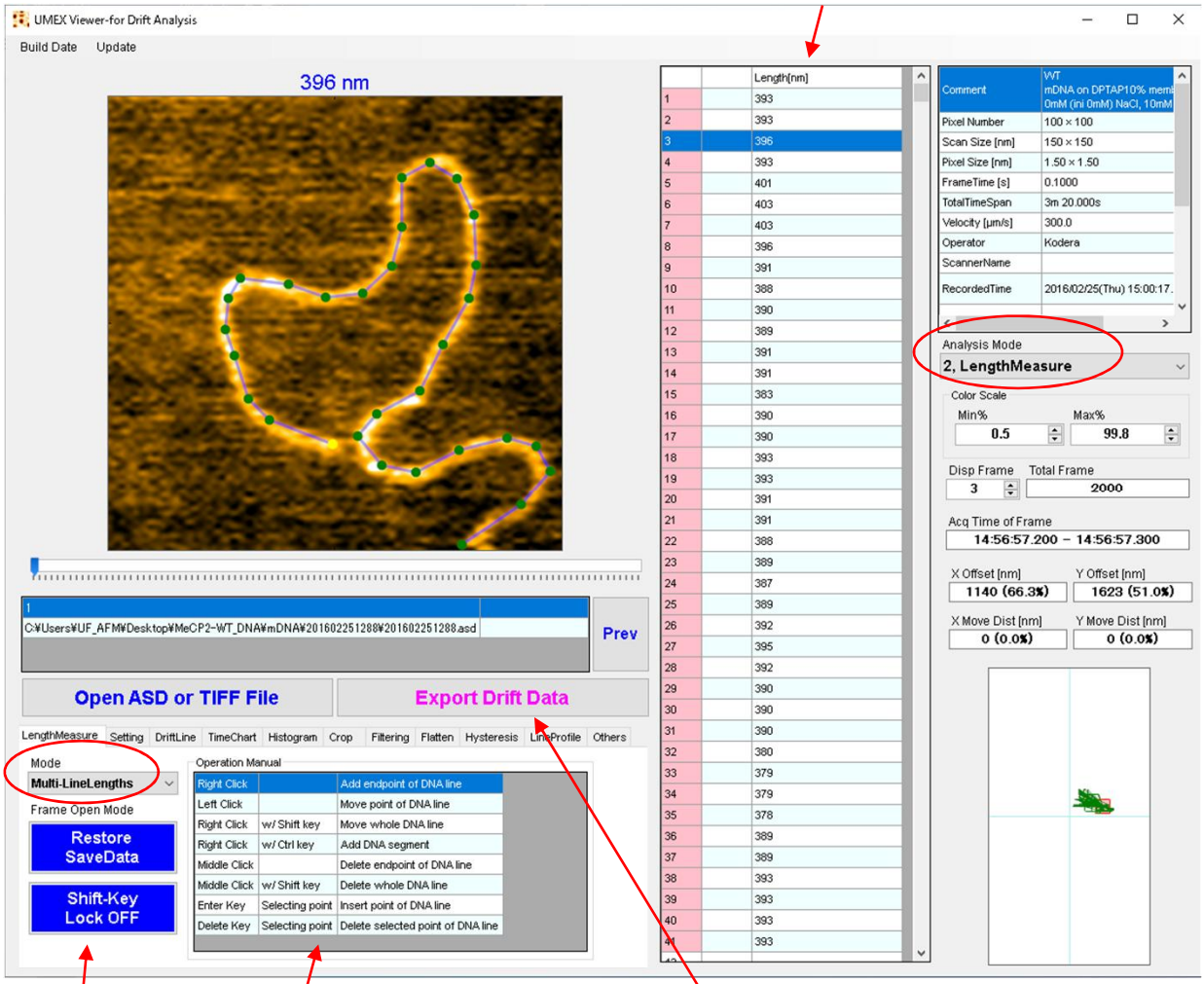
< Analysis Mode 2 _Length Measure >

LengthMeasue

A function that analyzes the time course of the length and curvature of linear molecules such as DNA, and the position of molecules bound to DNA.

Mode… There are 4 modes.Multi-Line Lengths,Adsorbate Position,CircularLine AdPosi,Multi-Angle.

Data is displayed and saved when moving to the next Frame. （Move with keyboard ← →, Disp Frame ▲▼, slider ）



Operation Manual
The drawing can be changed freely according to the operation manual.
*Operations differ depending on the mode.

Export Drift Data
Export as csv.File.

Frame Open Mode

Restore Save Data — Return to original data. (Recommendation)

Overwrite Save Data — The previously drawn drawing is restored.

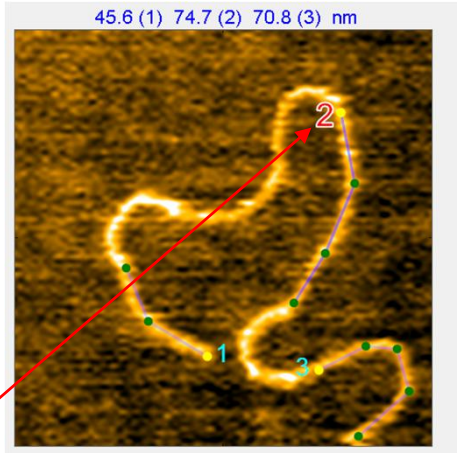
Shift-Key Lock OFF — Overwrite with current drawing. （Convenient when making corrections all at once ）

Shift-Key Lock ON — Control whether to lock the Shift-key.

1. Multi-Line Lengths

A mode for analyzing temporal changes in DNA length and curvature.

Point each frame on the image and measure the entire length of the object.

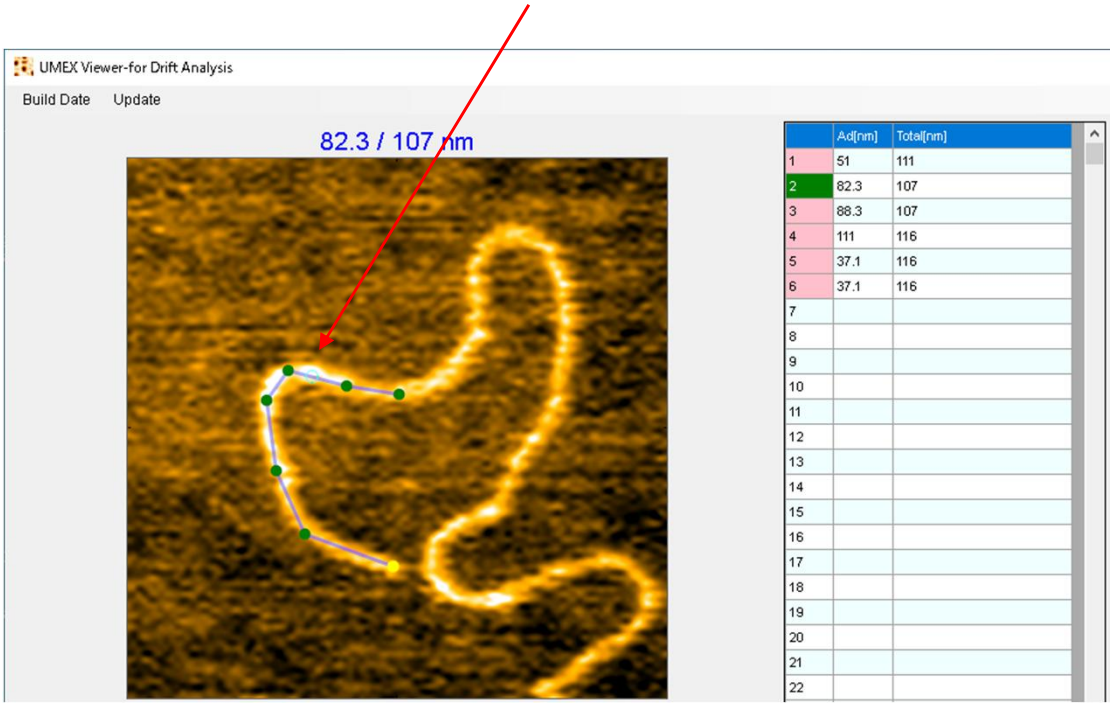


If there are multiple drawings, you can select them by clicking on the starting point (yellow point).
In this case, drawing 2 is selected.

2. Adsorbate Position

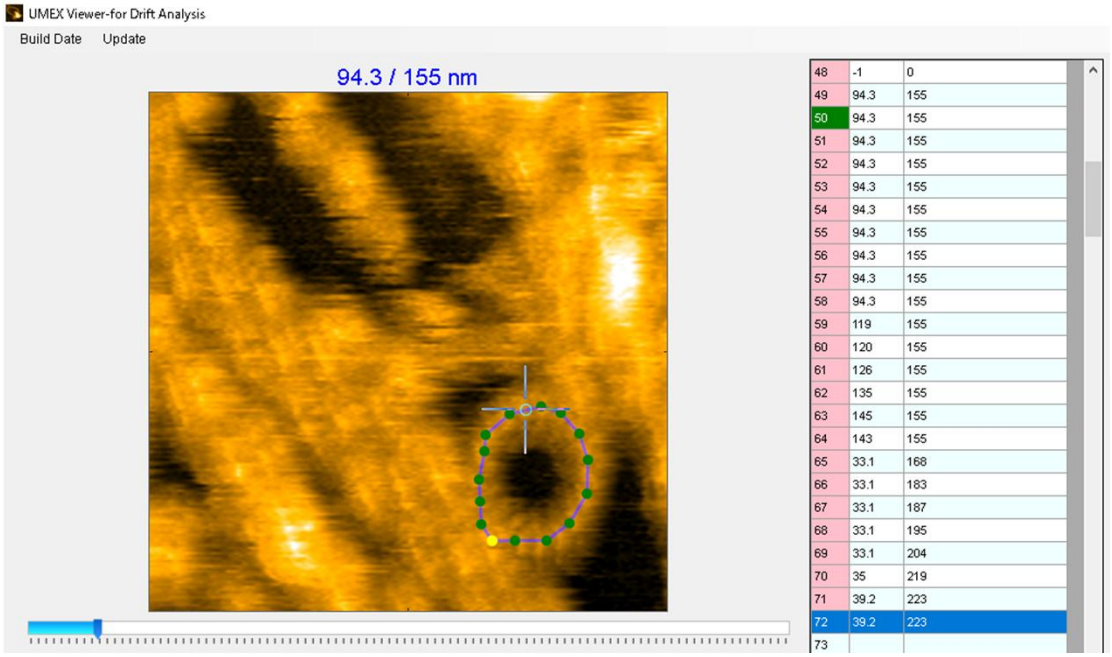
A mode for analyzing temporal changes in the position of molecules bound to DNA.

Measure the position of the adsorbed object for each frame.
After pointing on the image and drawing the entire length, specify the position of the attracting object.
The position of the adsorbate (light blue circle) is expressed by the distance from the starting point (yellow point).



3. Circular Line 、 Circular Line AdPosi

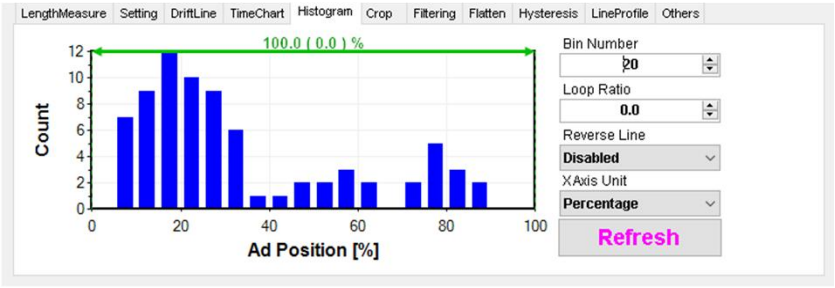
A mode that analyzes temporal changes such as changes in the curvature of plasmid DNA and the position of molecules bound to DNA.



48	-1	0
49	94.3	155
50	94.3	155
51	94.3	155
52	94.3	155
53	94.3	155
54	94.3	155
55	94.3	155
56	94.3	155
57	94.3	155
58	94.3	155
59	119	155
60	120	155
61	126	155
62	135	155
63	145	155
64	143	155
65	33.1	168
66	33.1	183
67	33.1	187
68	33.1	195
69	33.1	204
70	35	219
71	39.2	223
72	39.2	223
73		

Histogram

Display the measurement results of the Adsorbate position in a Histogram.

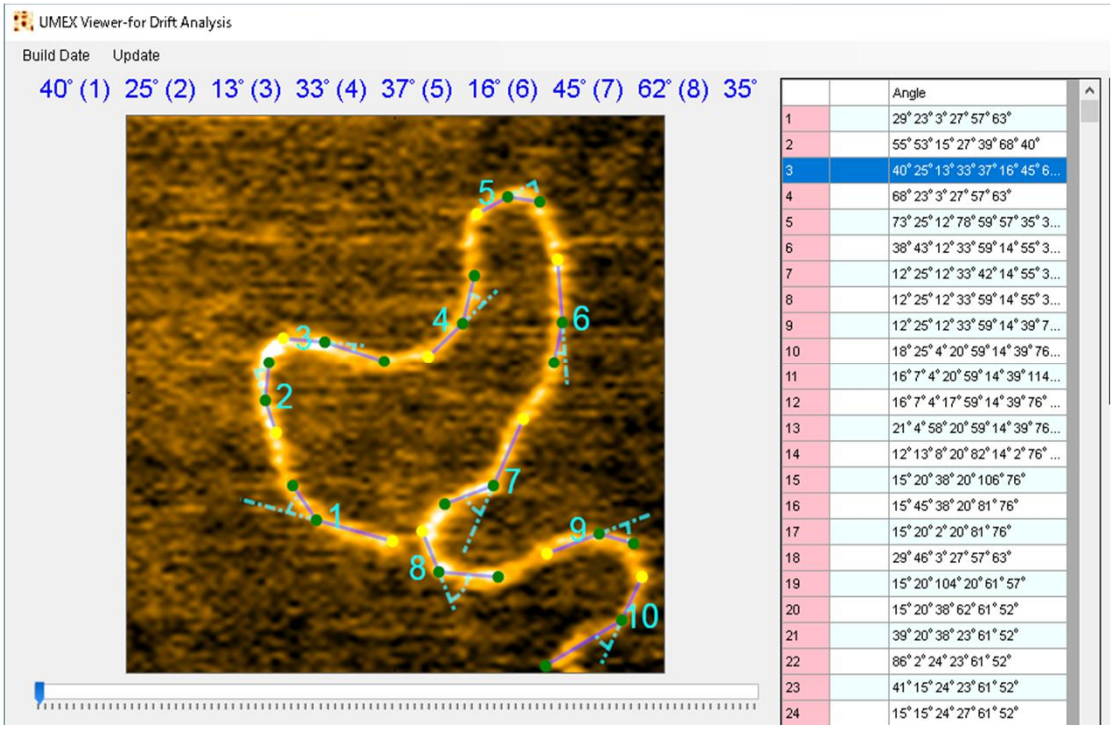


Bin Number
Adjust Bin size.
Loop Radio
?
Reverse Line
?
X Axis Unit
Specify the unit of the X axis.
Refresh
?

4. Multi-Angles

A mode for analyzing changes in angle over time at multiple points.

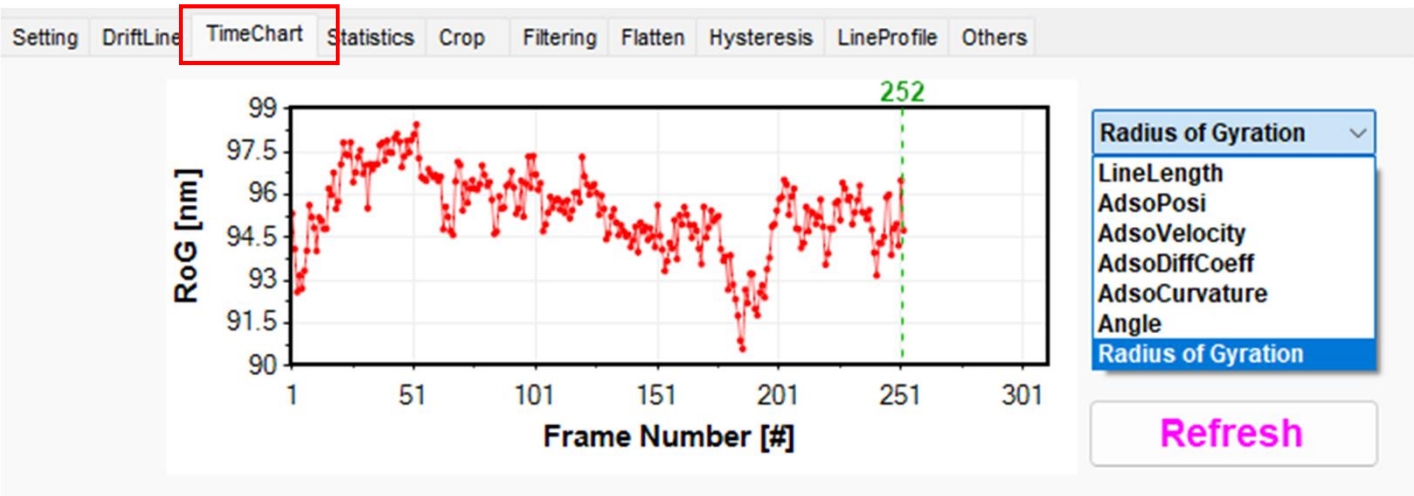
Specify three points and measure the angle between the straight line connecting the first and second points and the straight line connecting the second and third points, assuming that the line connecting the first and second points is 0 degrees.
*If there are multiple drawings, click on the starting point of the drawing to select it.
The selected drawing can be changed freely.



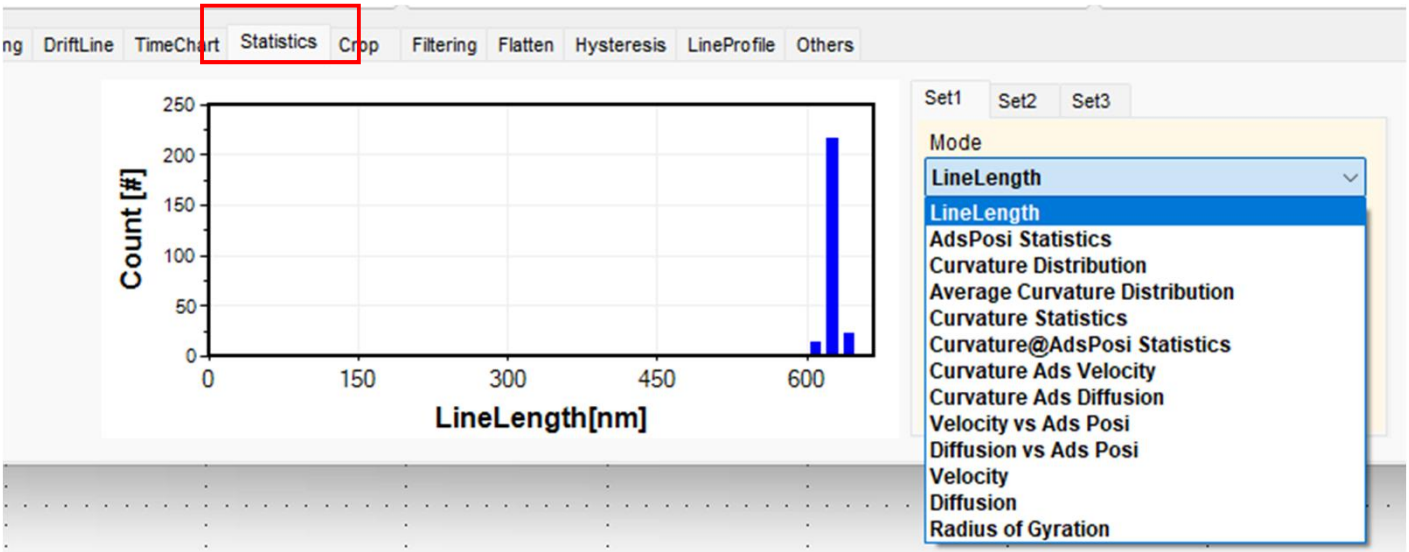
Setting

Angle can be measured by fixing the length.
→Enable Fixed Length and specify the length.

TimeChart

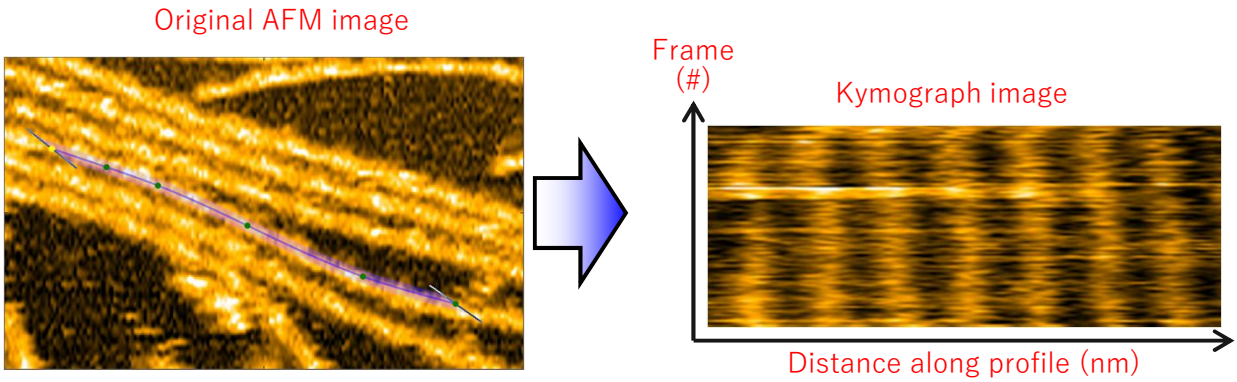


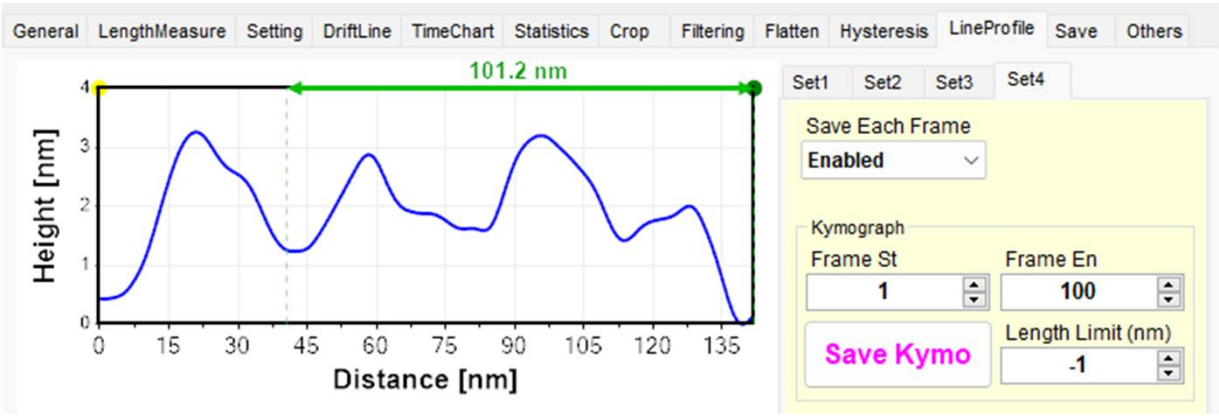
Statistics



Kymograph Analysis1

Line profiles for each frame are stacked vertically to generate kymograph data with distance on the horizontal axis and frame number (time) on the vertical axis. Data is saved in PNG and ESD file formats.





Basic Operation

1. Use Flatten or Slope Correction to correct the slope in the analyzed frame. (setting of Color scaling is not necessary.) Set Gaussian Smoothing if necessary.
2. Change Analysis Mode to LineProfile.
3. Go to the Set4 tab in the LineProfile tab and change “Save Each Frame” to Enabled. Then, a line profile for each frame will be recorded.
4. Set the line profile for each frame that you want to perform kymograph analysis.
5. The yellow circle indicates the starting point, which is the reference point for each profile, and so must be set accurately for each frame, but it is difficult to fine-tune on the image. Therefore, you can set the starting point more accurately by dragging the left-end Horizontal Scaler displayed in the line profile for each frame.
6. Enter the numbers of the start and end frames being to perform kymograph analysis in “Frame St” and “Frame En”, respectively.
7. Press the “Save Kymo” button to output the analyzed data.
8. It is recommend to save the position information of the line profile that you set by pressing the Export button in Config (DRI) in the Others tab. The saved data can be restored by pressing the Import button even after restarting the software.

Kymograph Analysis2

Additional Operations

1. To erase all the recorded data, first set "Save Each Frame" to Disabled, then change it back to Enabled.
2. Kymograph data often become noisy, so setting Average Width and Gaussian Smoothing to reduce the noise.
3. By default, -1 is set in Length Limit (nm), which means that the shortest distance among the line profiles of the analyzed frame is output as the upper limit. Line profiles with long distances are output with the end point side cropped (the yellow circle represents the starting point side).
4. By setting a positive value into Length Limit (nm), data is output with the specified distance as the upper limit. It is recommend to set an upper limit when comparing kymographs with different conditions.
5. To change the aspect ratio of the output image, use Illustrator to modify it losslessly. Alternatively, open the ESD file in UMEX Viewer and adjust the Scaling Y of Hysteresis to modify it.