Basic operation

1. Press the Browse button to set the save folder. You can use the Open button to open the configured folder in Explorer.
2. Set the scan size and number of pixels. By default, Constant Velocity is enabled, and when Frame Time (sec) is changed, Scan Velocity is set. After that, when you change the scan size or number of pixels, the Frame Time is automatically set so that the Velocity does not change. Scan Velocity is usually set in the range of 50 to 150 μm/s, if you want to observe molecules without destroying them, set it to about 50 μm/s, and if you want to observe high-speed phenomena, set it to about 150 μm/s. .
3. The set parameters can be saved in Config. After restarting the program, you can restore it using the Import button. Entering your name in the text box is convenient for multiple users to use.
4. To start scanning, press the "Scan" button. To stop scanning, press the Stop or Forced Stop button. The Stop button ends immediately after acquiring one image. The Forced Stop button ends immediately after you press it (so the last frame is saved halfway). If the Frame Time is small, it is sufficient to use only the Stop button, but if the Frame Time is large, it may become necessary to use the Stop button separately.

Important note

1. The image aspect ratio can be set using the Y Pixel Rate (%). In this case, *Y Pixels (R = 100%)* specifies the pixel count when the Y Pixel Rate is 100%, and the actual Y Pixels value is automatically calculated as *Y Pixels (R = 100%) × Y Pixel Rate*. Therefore, you do not need to adjust Y Pixels manually.
2. For example, if you set the Y Pixel Rate to 50%, X Pixels to 100, and Y Pixels (R = 100%) to 50, the actual resolution will be 100 × 25 pixels, which may not be what you intend.
3. Even if you want non-square pixels, you do not need to adjust the pixel count manually, as the system automatically sets it when *Nonsquare Pixel* is set to values such as 2× or 3×.

Basic operation
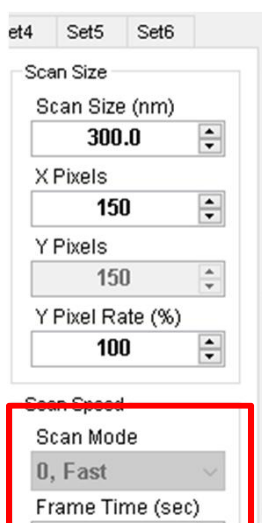

Normally, rather than directly changing scan settings in the imaging window, it is more convenient to set them in the parameters of the preset window and use the "Set" button. The parameters set here can also be saved in Config and restored after restarting the software.
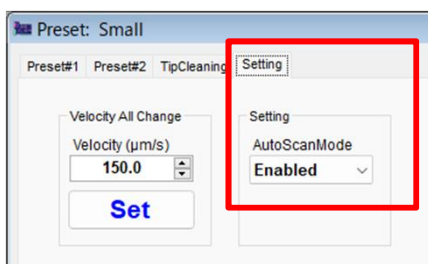
Scan mode

There are two scan modes: Fast and HighReso.

Fast is a method of acquiring data for each image. The maximum number of DA converter memories is 524,288, so the number of pixels can only be increased to a maximum of 500x500 pixels. Also, since the lower limit of the sampling frequency is 3.5 kHz, low-speed scanning is not possible when using an Ultra-Large scanner.

In HighReso mode, data is acquired for each X scan line. Therefore, the problem of the upper limit of the number of data items mentioned above is eliminated, and instead, the upper limit is 12 bits (=4096), which is the resolution of the DA converter. Therefore, it is possible to increase the number of pixels to a maximum of about 20000x4096 pixels. Also, when using an Ultra-Large scanner, it is possible to scan at low speed. However, there is a slight latency when acquiring data, so the frame rate will be slightly slower than the set value. Additionally, since the scan stops momentarily when acquiring data, there is a problem that the left end of the piezo backward image becomes discontinuous. Also, since slope correction cannot be applied, image processing such as flattening is required.

The problem with the Igor program was that it could only use Fast mode, which meant it couldn't use large pixel counts.

With the default settings, switching between Fast and HighReso is done automatically according to the number of pixels and frame rate, so users do not need to be concerned it.
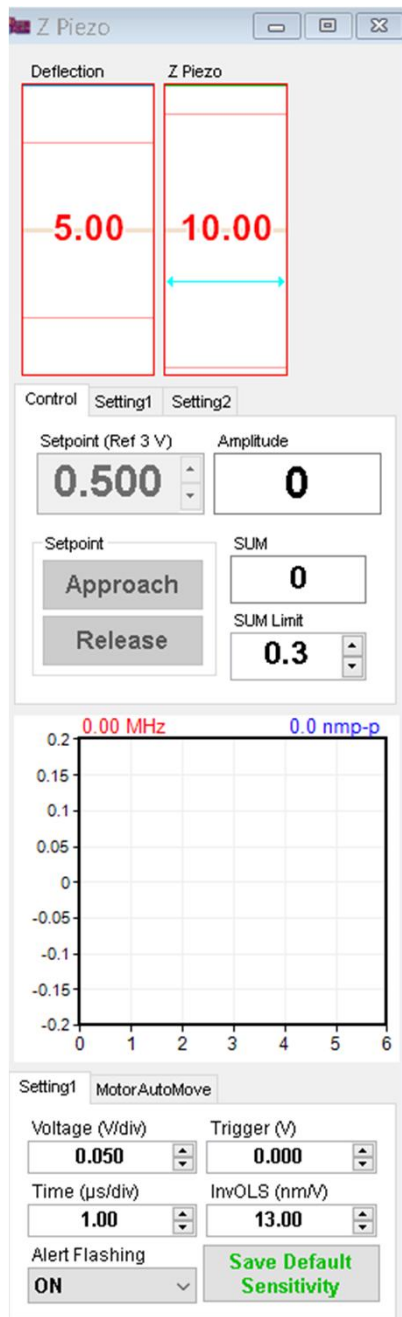If you want to switch manually, change AutoScanMode to Disabled in the Setting tab of the Preset window.

Enlarge window

When observing a large scan area with a high pixel count, it becomes difficult to see using a normal imaging window, but using an enlarge window is convenient because you can enlarge the window.

Measuring instrument meter

Signals that need to be constantly monitored during AFM observation are displayed in the Z Piezo window right next to the AFM image window, so you can check them without having to look to the side or back during measurement.

Deflection: Graviton Subtract or Quotient is displayed
Z Piezo: The needle on the front panel of the feedback circuit of Tsuji Electronics is displayed.
Amplitude: Displays the amplitude value of the amplitude measuring instrument.
Sum: Graviton's Sum is displayed

Furthermore, when the data reaches the upper or lower limit, the window flashes red to notify you. The upper and lower limit values can be set in Setting1 and Setting2. If you want to disable this red flashing feature, just turn off Alert Flashing at the bottom of the window.

Regarding the Z Piezo signal, if you use the default BNC connection with 0.5x of the Q controller turned on, only ±5 V of the ±10 V output voltage will be clipped and displayed. Therefore, it is not possible to monitor the full voltage range. Therefore, as shown in the diagram below, by default the PID Monitor signal on the back of the HS-AFM Controller Box is connected to PCI-AD4, but the MOCK-OUT output of Z Q-Con is connected to the T-shaped plug. You need to branch out and connect to PCI-AD4 instead.
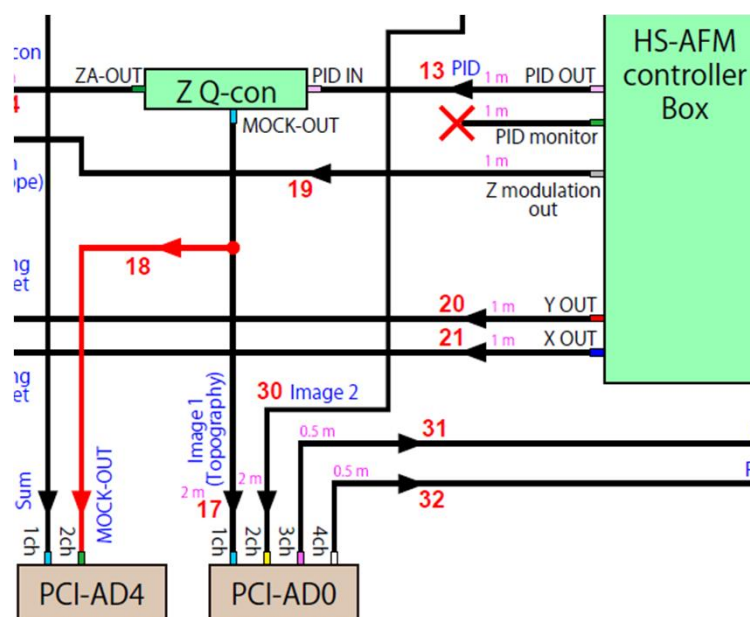
Keyboard operation
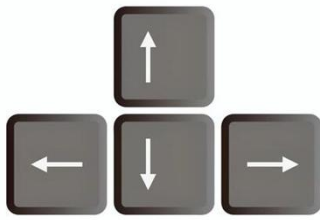
1. The tip position can be moved using the cursor keys on the keyboard. Coarse movement is possible by holding down the shift key and operating the cursor keys.
2. The probe driving speed can be set in the stage window.
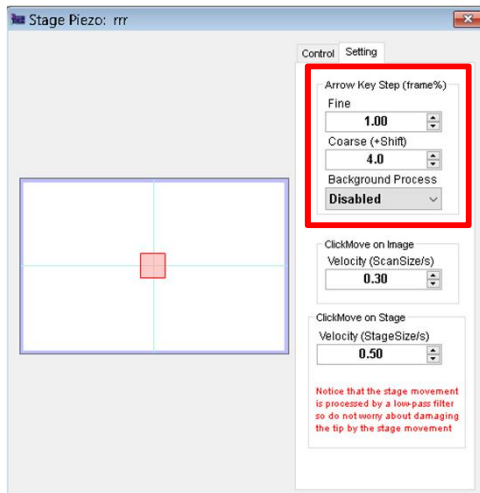
Mouse operation

1. Since left clicks are used to select numerical boxes and combo boxes, miscellaneous functions are assigned to right click and middle (wheel) click.

Right Click

1. By right-clicking on the AFM image, you can move the probe position so that the clicked position is in the center.
2. By right-clicking on the stage screen, you can move the probe position to the clicked position.
3. You can use the arrow keys on your keyboard to control the tip position, but if you want to make a large movement, you may use right-click the mouse, and if you want to make fine adjustments, such as when chasing a drifting molecule, you may use the keyboard. .

<table>
<tr><td>AFM image</td><td>stage screen</td></tr>
</table>
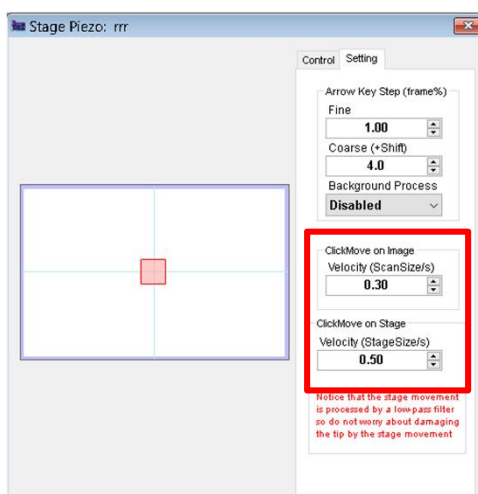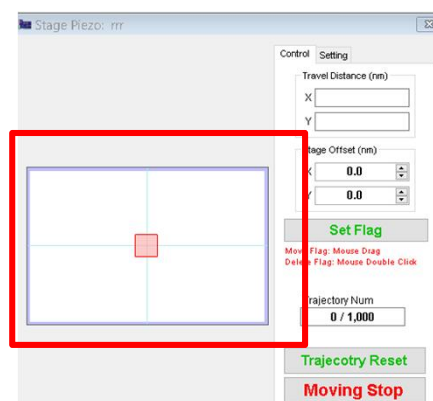
5. The probe driving speed can be set in the stage window.

Mouse operation (continued)

Middle click
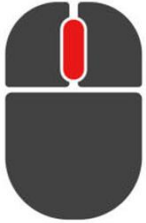
1. If you press and hold the middle click anywhere in the program, you can automatically run the stepping motor so that the current position is the light blue arrow on the Z piezo meter. Depending on whether the sample is approaching or moving away, Slow Approach or Slow Release is automatically selected. The position of the arrow can be set in Setting 2 of the Z Piezo window.
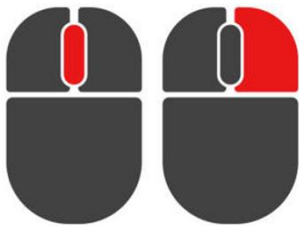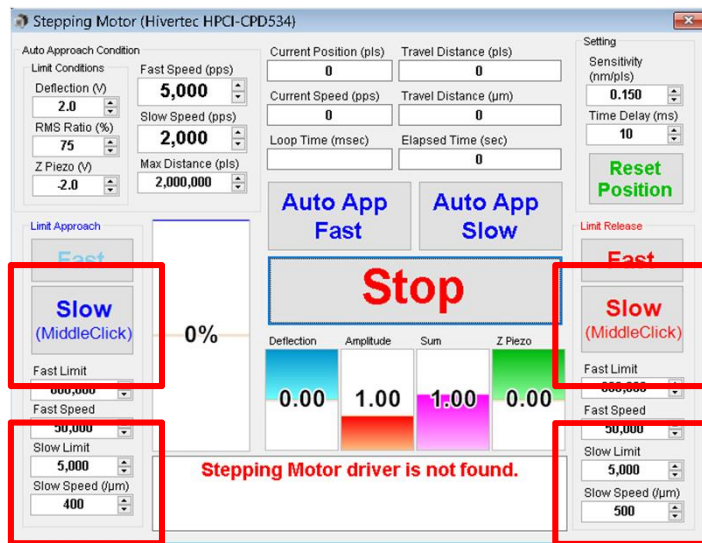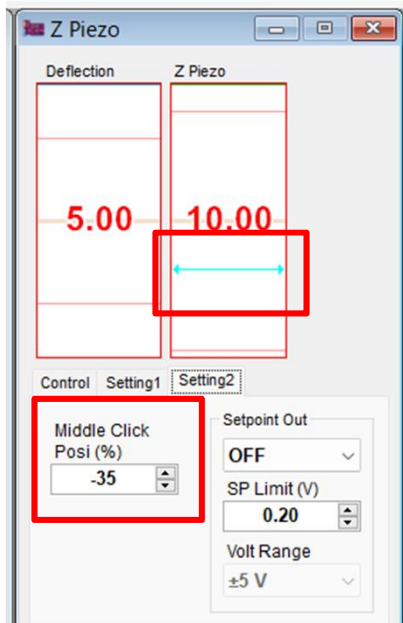
Right-click wheel operation

1. When setting a numerical value box such as scan size, you can input it directly using the keyboard, but you can also use the mouse wheel. If you turn the wheel normally, you can make coarse adjustments. If you hold down the right click and turn the wheel, you can make fine adjustments.

Motor operation

When approaching,
1. With the Fast Speed set to 5000 pps (pulse per second), start the approach with the "Auto App Fast" button.
2. As the sample approaches, the amplitude value will gradually increase, so always check the time change of the amplitude value in the Time Course window. As the sample approaches further, the amplitude value decreases rapidly, so press the "Auto App Fast" button several times while increasing the gain of the amplitude measuring instrument.
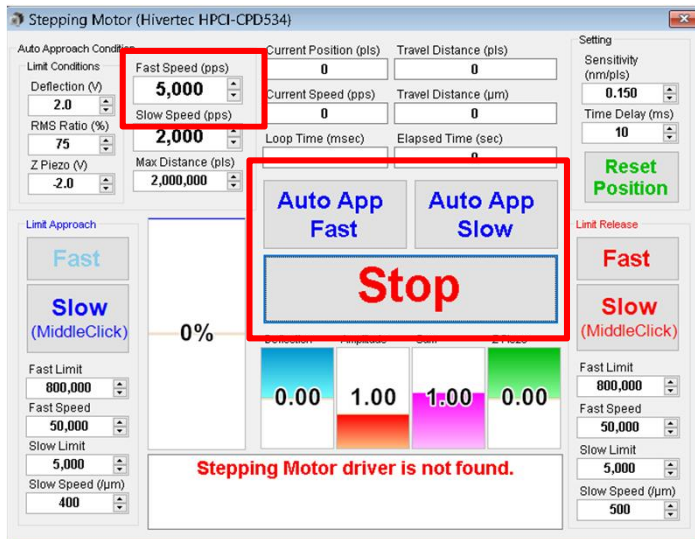
After the experiment, at the time of release,
1. Run Fast Limit Release with the default settings, wait until the meter reaches 50-100%, and press the Stop button.

2. In the case of the same scanner and glass stage, it is sufficient to stop at about 50%. When changing the scanner or glass stage, the position of the sample surface is likely to change compared to the previous time, so raise it to 100%.

Color contrast
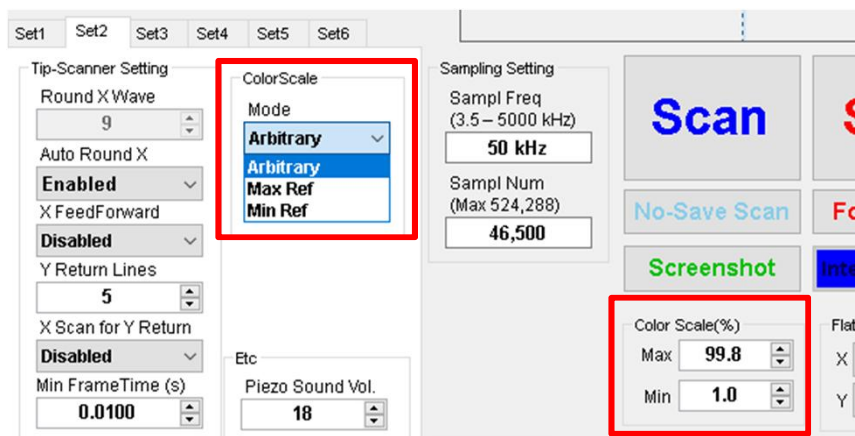
You can switch the color scale mode with "ColorScale" in Set 2 of the imaging window. The default is Arbitrary, and you can set the upper and lower color limits with "ColorScale(%)" below the Scan button. After acquiring the brightness values for the entire image and sorting them in order of brightness value, scaling is performed so that pixels with brightness values within the range of Max to Min have the maximum to minimum brightness values. That is, if Max is set to 100% and Min is set to 0%, the maximum and minimum brightness values in the image are displayed as the maximum and minimum brightness values of the actual displayed image. Since there is noise in the image, it is better to shift it slightly from 100-0%, such as 99.8-1.0%.

Basically, there is no problem if you use Arbitrary, but in cases where adsorbed molecules appear and disappear, or when there is a structure larger than the molecule you want to observe, it may be easier to see if you fix the color scale. Occasionally. In that case, use MinRef or MaxRef.

MinRef calculates the lower limit of the brightness value using the same method as Arbitrary, and then uses the value obtained by adding the lower limit value by the height set in Diff as the upper limit of the brightness value.
MaxRef calculates the upper limit of the brightness value using the same method as Arbitrary, then subtracts the height set in Diff from the upper limit value and uses the value as the lower limit of the brightness value.

When observing molecules on a flat molecule, it is necessary to use the brightness value of the substrate as a reference, so MaxRef is rarely used.

Non-square pixels

Enabled ∨
Nonsquare Pixel
Disabled ∨

Min Step (nm/Pixel)
X 2.01 / 0.34
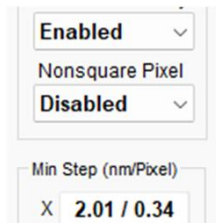
For example, if you observe 100×100 nm2 with a setting of 100×100 pixel2, the pixel size will be 1x1 nm/pixel2, which is a square pixel. On the other hand, if you observe 100×100 nm2 with a setting of 200×100 pixel2, the pixel size will be 0.5×1 nm/pixel2, which is a rectangular pixel. In AFM measurements, the frame time increases depending on the number of pixels in the Y direction, but has the characteristic that it does not depend on the number of pixels in the X direction. Therefore, by increasing only the number of pixels in the X direction, it is possible to improve only the spatial resolution without lowering the frame rate.

By setting Arbitrary, you can set any pixel independently for X and Y.
However, Arbitrary is only used for special measurements.
In normal measurements, a fixed constant such as 2 or 3 times the Y pixel is often set for the X pixel. Because of this, it is tedious to enter values into two number boxes every time you change the number of pixels, so by using 2x, 3x, or 4x, when you enter the Y pixel, the value multiplied by the fixed constant is automatically entered for the X pixel, so it is usually recommended to use this.

However, if non-square pixels are used, the image will be noisy unless Gaussian smoothing is applied in the viewer, and there is also the problem that the saved file size will be large. Normally, it is recommended to disable non-square pixels and enable them only when you want to take a beautiful image.

Flatten
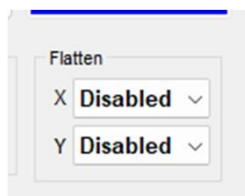
This is an image processing method that performs polynomial fitting on the AFM image for each X and Y line, and reduces the height difference of the background to zero. It is not necessary for normal measurements, but it is effective when there is severe scanner ringing, or when you want to easily see small molecules on a surface with height differences.

The settings are reflected in the image processing only on the image, and are not reflected in the saved ASD file. Therefore, if you want to use Flatten during data analysis, you need to enable Flatten on the viewer.

X-Rounding

A triangular wave is usually used to scan the X-piezo. However, when the triangular wave is Fourier transformed, it contains components up to high frequencies as follows:

$$f(x) = \frac{4}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \sin\left(\frac{n\pi}{2}\right) \sin(nx)$$

$$= \frac{4}{\pi^2}\left[\sin(x) - \frac{1}{9}\sin(3x) + \frac{1}{25}\sin(5x) - \frac{1}{49}\sin(7x) + \cdots\right]$$
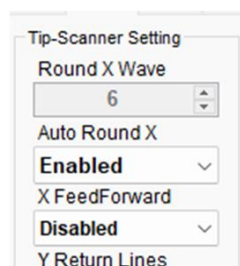
Therefore, even with a slow scan frequency, the piezoelectric resonance may be hit, causing ringing to appear on the image. For this reason, the effects of ringing can be suppressed by using a waveform in which the high-frequency components of a triangular wave have been removed.
In X-Rounding, this sets the maximum order of the high-frequency components. The default is set to 5. If you set it to 0, the triangular wave will be set as is.
When Auto Round X is Enabled, the order is automatically set between 5 and 9 depending on the scanner's resonant frequency and the scan frequency.

Brownian Fitting
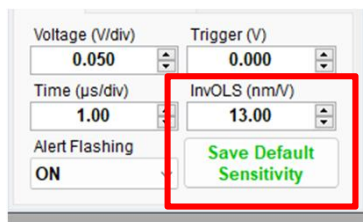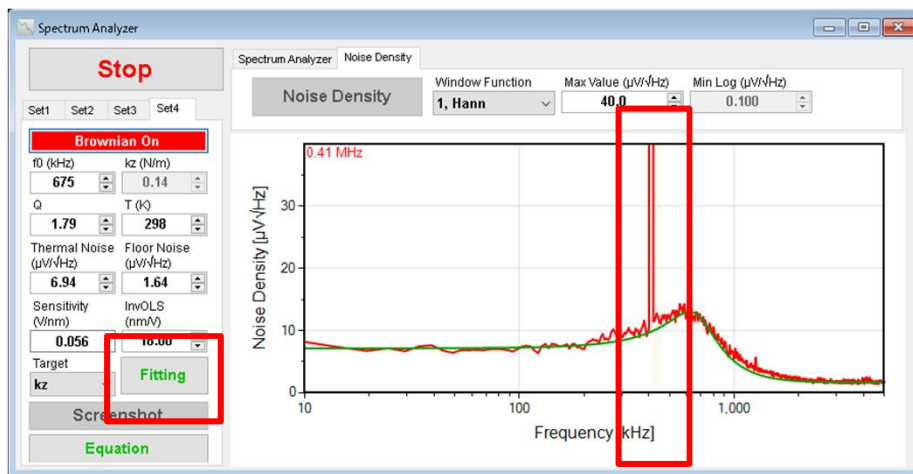
Calibration of the Cantilever Spring Constant

By pressing the "Fitting" button, the theoretical equation can be fitted to the Brownian noise of the cantilever. If a calibrated InvOLS has been entered, the spring constant (kz) can be measured. InvOLS performs measurements using a force curve on mica, and by entering a value in the numeric box at the bottom of the "Z Piezo" window and pressing the "Save Default Sensitivity" button, the software can be automatically restored the next time it is started.

Optimization of the Excitation Frequency

It is also possible to optimize the excitation frequency. In Amplitude-Modulation (AM)-AFM, driving the cantilever at a frequency slightly lower than the resonance peak provides optimal force detection sensitivity, allowing fragile molecules to be imaged without damage.
When performing fitting, the optimal excitation frequency is indicated by the orange square on the right spectrum. First, adjust the excitation frequency of the amplitude detector to fall within this square. Then, fine-tune the frequency within this range to achieve the highest excitation efficiency, thereby obtaining the optimal force detection sensitivity.

Reference
Physical Review Applied 23, 034065 (2025)

Interactive Mode1
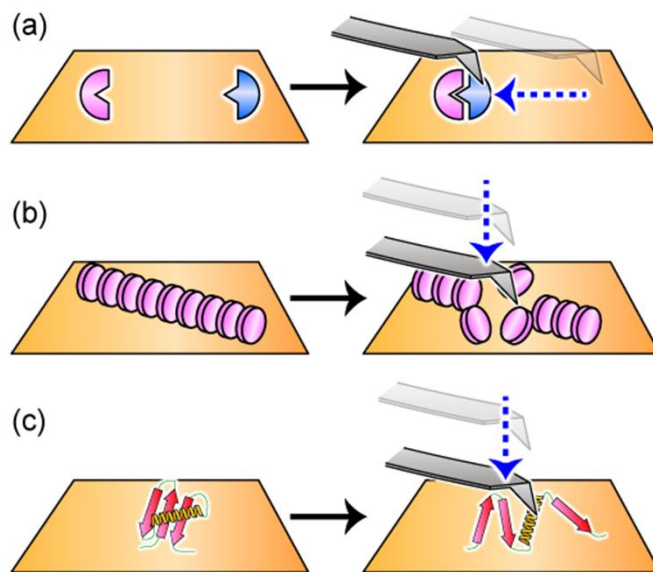
The interactive mode allows the tip to touch the sample surface at a targeted position during imaging, which can be used to manipulate molecules on the surface, for example to bind other molecules, break molecular assemblies, or denature proteins.
The in-line force curve mode also allows you to obtain force curves at targeted positions during imaging.

Setting for Controller

Back panel of Tsuji feedback circuit.

Please confirm that the "Sig. Modulation in"switch is flipped on before the measurement.

Interactive Mode2

Control Software

5. Scan Direction

1. Interactive Mode ON/OFF

2. Increase in voltage of amplitude (reduced setpoint)

3. After one shot is done, interactive mode is automatically disabled

6. Position of Interactive mode (Position can be changed by mouse drag)

4. Range of applying force region

7. Interactive mode ON/OFF (Space key can be also used during imaging)

Analysis Software

Region where the force is applied is displayed

Display of applying force region ON/OFF

Increased voltage is displayed
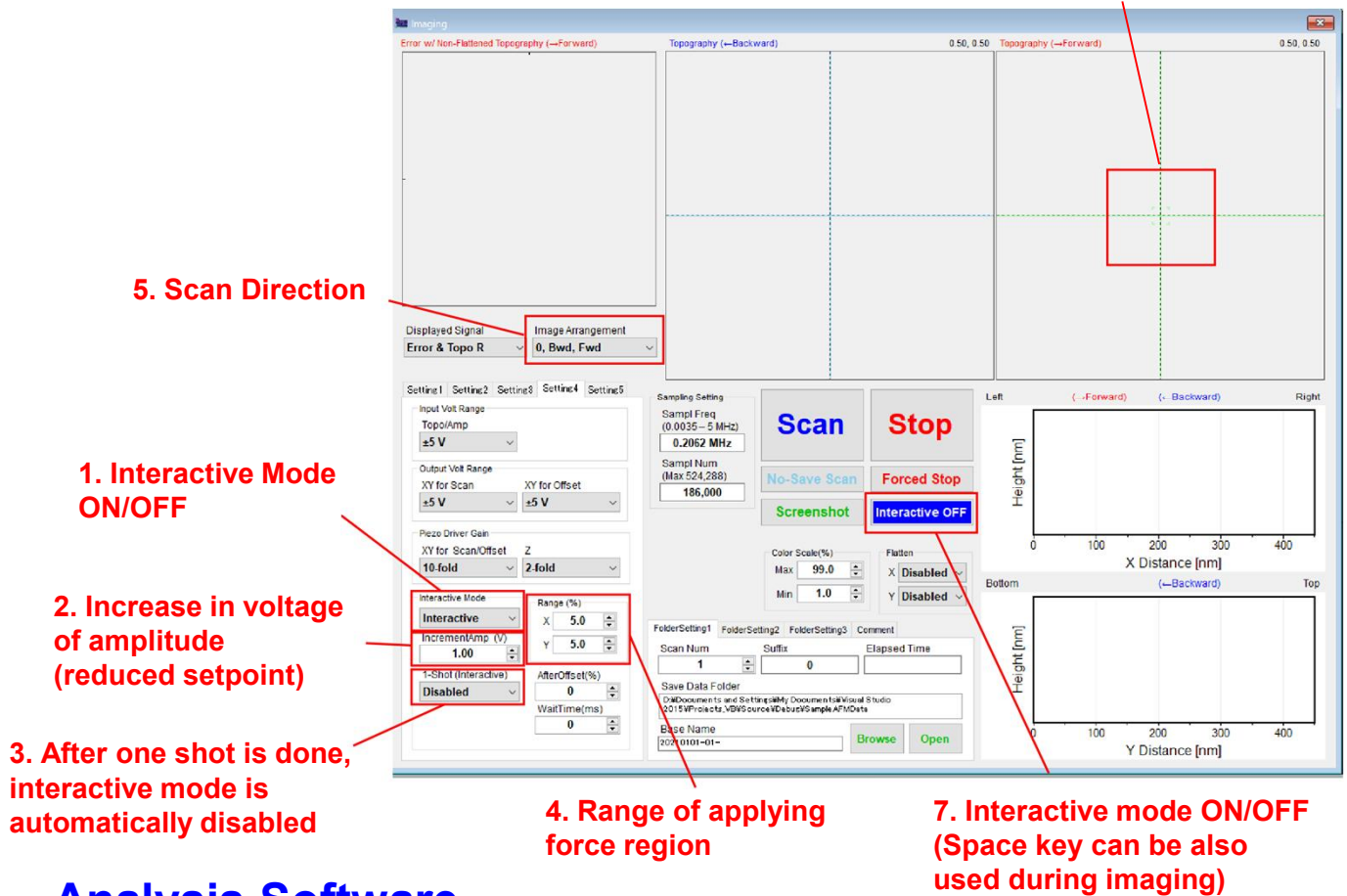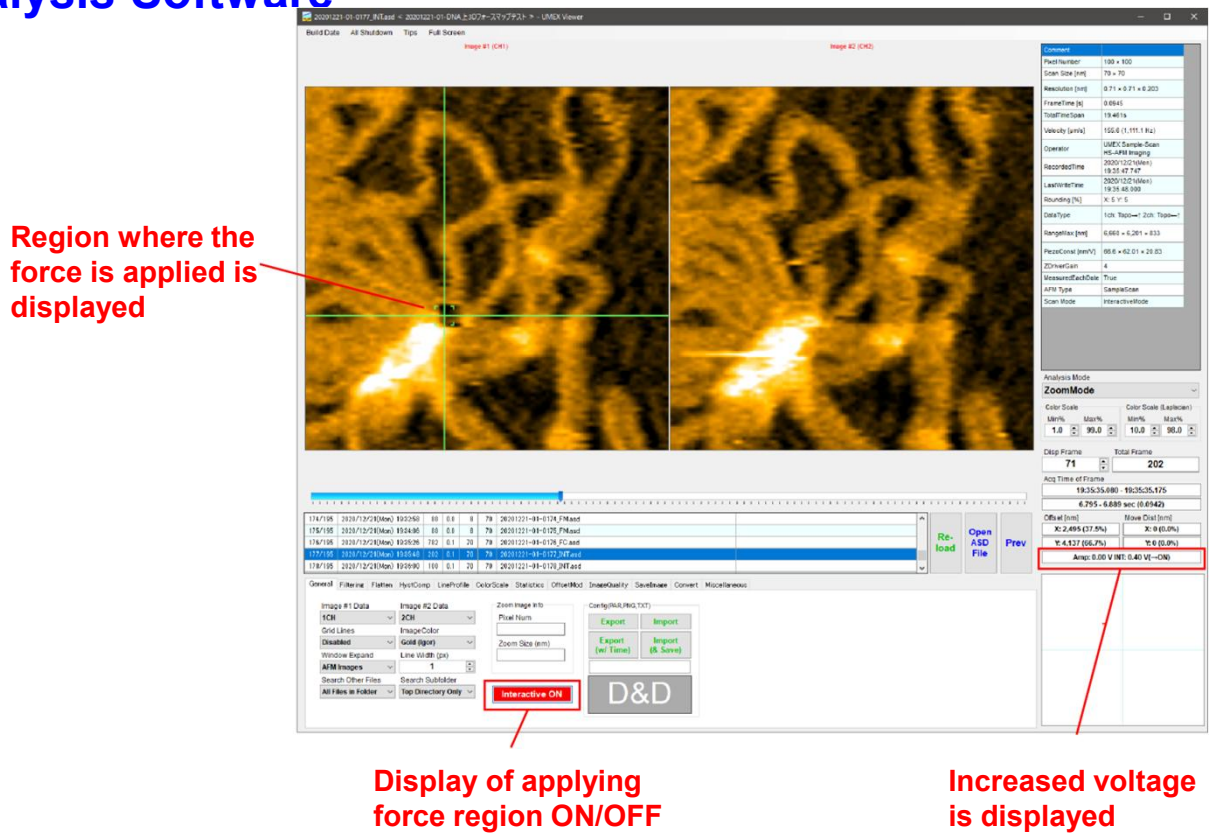
Principle

OnlyTraceImaging Mode2

Control Software
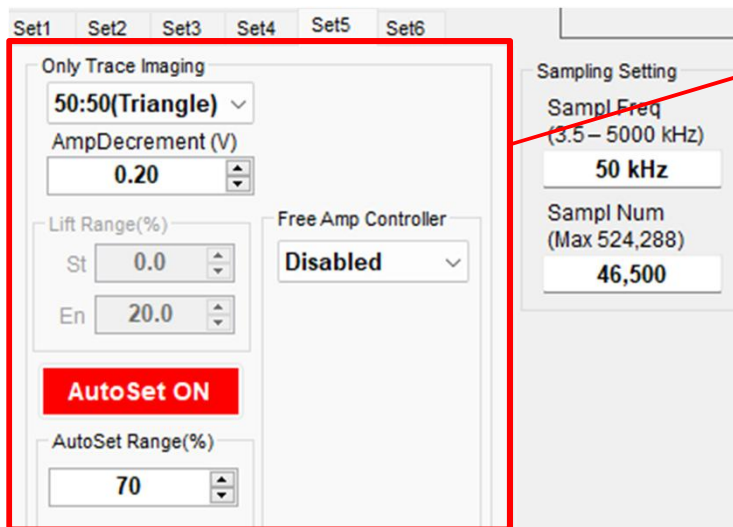
OTI control setting can be found here

By enabling Extended Header setting, the parameters for OTI can saved in the ASD files. They can be checked using UMEX viewer after experiments. They can be opened by Kodec & UMEX Viewer.
However, some compatibility problems occur in the following software.
Falcon Viewer (Igor)
 - The ASD file can be opened and viewed, but edited files cannot be properly exported.
ImageJ with Load_ASD_32bit Plug-in
 - To open the ASD file, a modified version of Plug-in must be installed.
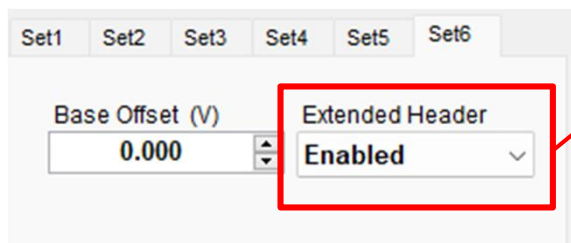
Analysis Software
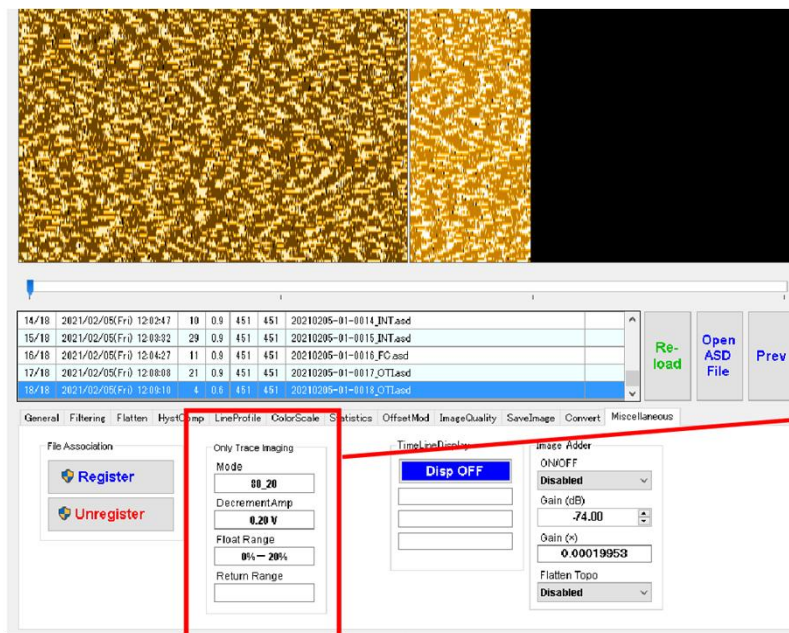
Parameters used for OTI mode in the individual frame are saved in ASD file, so that they can be checked using UMEX viewer

Free Amp Controller
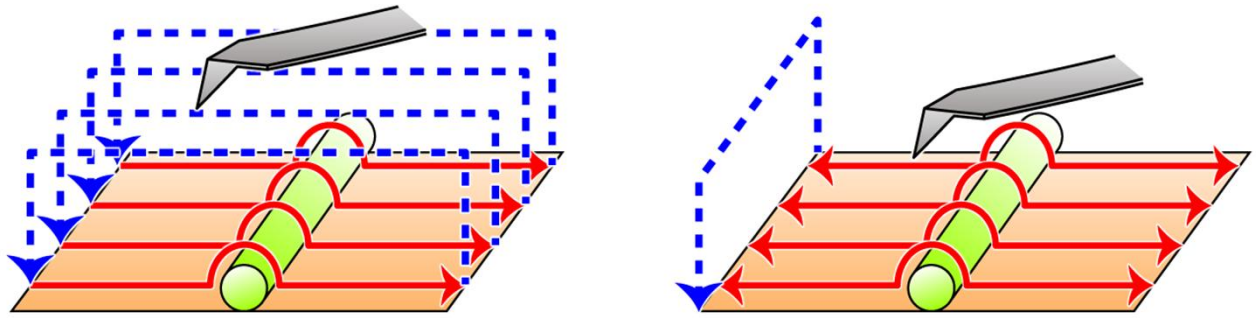
Rev. Sci. Instrum. 87, 053705 (2016)

AM-AFM has a problem that the force applied to the sample changes even with the same set point because the free amplitude value fluctuates over time during measurement. Therefore, by temporarily lifting up the probe during measurement, measuring the free amplitude value, and automatically adjusting the excitation voltage, it becomes possible to perform continuous imaging over a long period of time.

Method 1 (left)
In the original literature, the probe is lifted up during backward scanning, but this method has disadvantages such as insufficient lift-up when scanning quickly, and the need to adjust the lift-up settings along with the feedback parameter settings.

Method 2 (right)

Therefore, a method of lifting up during Y Return was also implemented. This method allows you to obtain and set the free amplitude value stably. However, this method also has a problem, and the bottom end of the image may be slightly lower. This is hardly a concern for uneven surfaces, but for flat surfaces, it may be necessary to apply Flatten in post-processing.

Line Scan Mode
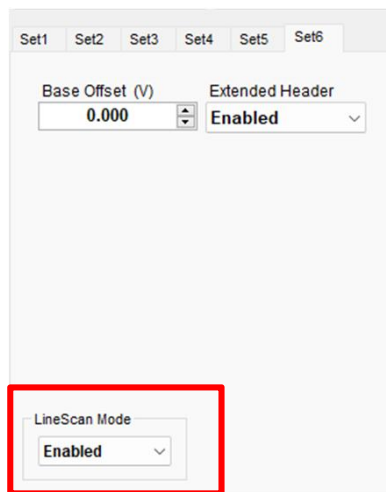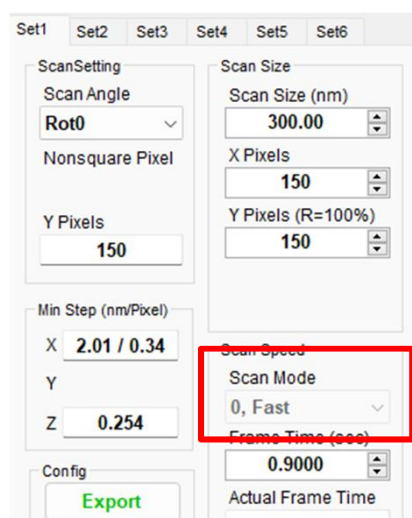
Overview

Unlike conventional topographic imaging, this method scans only in the X-direction without performing a Y-direction scan. Although it has the drawback that only spatial information along the X-direction can be obtained, it enables measurements with approximately 100 times higher temporal resolution, allowing phenomena on the millisecond timescale to be visualized. Prof. Simon Scheuring's group refers to this mode as HS-AFM line scanning (HS-AFM-LS), and it is also known as a kymograph.

Applications
➢ This technique is typically used to evaluate kinetics in systems where two-dimensional information is unnecessary or where the entire surface is fully covered by a two-dimensional crystal or fibers, so that drift is not an issue.
➢ It is also suitable for observing processes such as molecular adsorption/desorption reactions, where molecules randomly adsorb or desorb on the surface.
➢ If high-speed scanning is required but line scanning is not suitable due to issues such as drift or insufficient probe positioning accuracy, it is often better to perform conven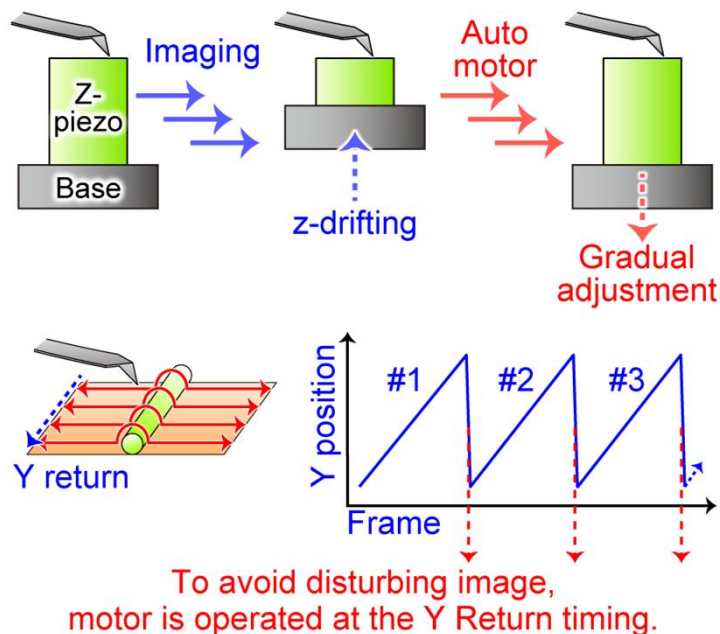tional imaging with the Y Pixel Rate set to around 10% and the Y Pixels set to 5–10 pixels, rather than using line scanning.

Scan Settings

➢ Y Position (%) sets the position in the Y-direction where the line scan will be performed. A setting of 0% corresponds to the bottom edge of the image, 50% to the center, and 100% to the top edge. Note that even if the Y Rate is set to a value other than 100%, it is treated as 100%. Therefore, the bottom edge corresponds to -X Scan Size / 2, and the top edge to +X Scan Size / 2.
➢ When a value other than 0% is specified for Y Position (%), the probe moves to the specified position at the start of the scan. As a result, due to effects such as piezo creep, the Y-piezo may slightly drift immediately after the scan begins. For this reason, it is recommended to discard the first second or so of data.
➢ As with standard imaging, you can set the scan size and the number of pixels. When *Scan Mode* is set to Fast, a time lag of approximately 100 microseconds occurs for each acquired frame. Therefore, if you need continuous data acquisition, increase the number of Y pixels accordingly. The maximum total number of pixels (X Pixels × Y Pixels) is limited to 520,000. For example, if *X Pixels* is set to 100, the maximum *Y Pixels* is 2,600.
➢ If you try to set *Y Pixels* beyond this limit, the *Scan Mode* will automatically switch from Fast to HighReso. In HighReso mode, data is acquired line by line, allowing up to 300,000 Y pixels. However, please note that a small time lag occurs for each line.

References
1. Nature Communications 9, 4983 (2018)
2. Nature Communications 11, 5016 (2020)
3. Nature Communications 12, 7225 (2021)
4. Nature Communications 16, 5055 (2025)

MotorAutoMove

To avoid disturbing image, motor is operated at the Y Return timing.

During imaging, the motor drifts in the Z direction, and imaging becomes impossible when it reaches the end of the Z range, so it is necessary to use a stepping motor to correct the Z position, which can be very troublesome. Z drift becomes particularly severe when the glass stage is not properly bonded. Furthermore, there is a problem with the image being distorted while the motor is moving.

MotorAutoMove automatically controls the motor during imaging, eliminating the need for manual operation. If the motor operates during imaging, line noise will occur only during that time, so it is synchronously controlled with the scan so that the motor only drives during Y Return.

The motor will automatically drive if it deviates too far from the blue line of the Z Piezo bar. When set to Auto, it will move in both up and down directions, and when set to AppOnly or RetOnly, it will only drive in one direction.

The motor has backlash, so it needs to move an extra amount to retract after approaching. Furthermore, drift is in one direction in most cases. Therefore, it is better to use AppOnly or RetOnly.

In Step, you set the amount of drive for one time. There is no need to change Velocity or WaitTime from the default settings.

MotorAutoMove

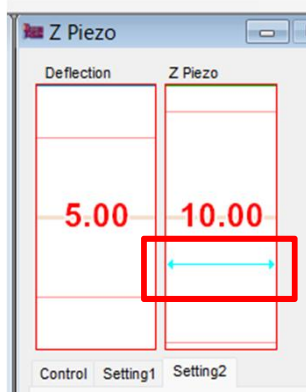To avoid disturbing image, motor is operated at the Y Return timing.

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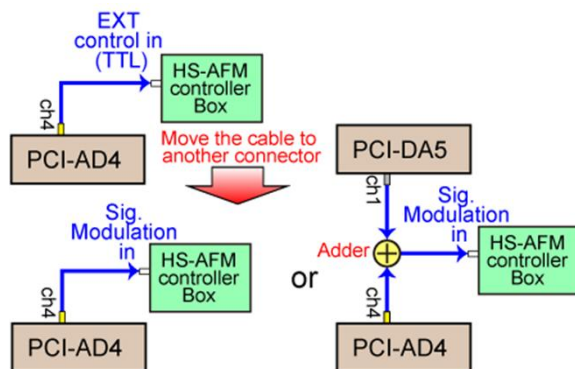
In Step, you set the amount of drive for one time. There is no need to change Velocity or WaitTime from the default settings.

Setpoint Out #1

Normally, the setpoint is controlled by the feedback circuit, but it can also be set in the software.
In the Z Piezo window, switch the Set3 tab to ON to enable setpoint adjustment in the Set1 tab.

Wiring setup

Since the Tsuji feedback circuit has no external setpoint control terminal, the amplitude value is controlled via Sig. Modulation in, which substitutes for setpoint control.
When setting the setpoint from the software, change the wiring as shown in the left diagram: connect the PCI-AD4 ch4 output, normally used for feedback ON/OFF control, to Sig. Modulation in.
With this wiring, OTI and Interactive Mode cannot be used. To use them simultaneously, combine the signal from PCI-DA5 ch1 with the above output using an adder, and input the summed signal

Software setting 1
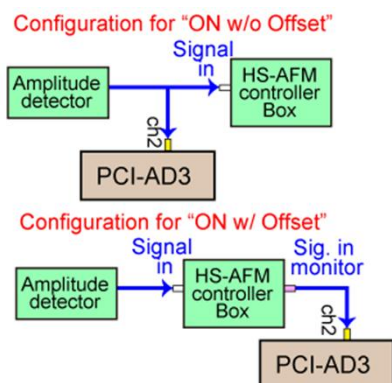
There are two options for setpoint configuration:
1. ON w/o Offset
2. ON w/ Offset
If the amplitude signal is directly acquired through AD3 CH2, select *1. ON w/o Offset*.
On Kanazawa University instruments, the amplitude signal is acquired by AD3 CH2 with the "Sig. Modulation in" signal superimposed and displayed in the software. To control the setpoint and display the raw amplitude signal, the setpoint signal must be subtracted. In this case, select ON w/ Offset.
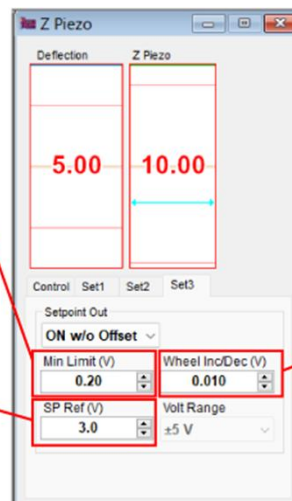
Setpoint Out #2

Software setting 2

Min Limit (V)
Sets the lower limit of the setpoint when adjusting it using the mouse wheel.

SP Ref (V)
Since the setpoint control is substituted by amplitude control, the hardware setpoint must be fixed. To prevent the probe from crashing in case the software stops unexpectedly, the default setpoint is set to 3.0 V.
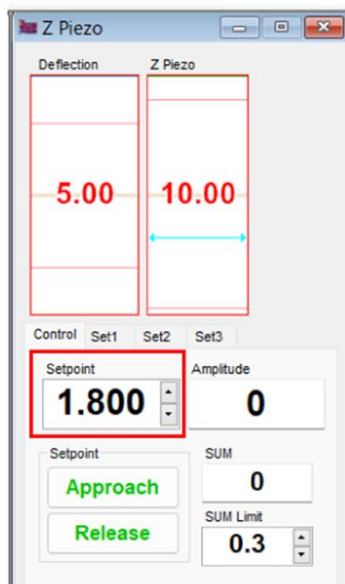
Wheel Inc/Dec (V)
Sets the increment/decrement step when adjusting the setpoint using the mouse wheel.

Requirement before experiment
Set the voltage specified in "SP Ref" as the reference value for the feedback circuit.

Setpoint adjustment during experiment

Turn the mouse wheel over the Setpoint box to adjust the value.
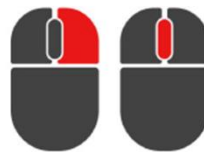
Hold the right mouse button while turning the wheel to make fine adjustments.

Approach:
Set the setpoint to the current amplitude minus ("Wheel Inc/Dec" × 2).

Release:
Apply the value set in "SP Ref" as the setpoint to retract the probe to a distant position.

X Scan Feedforward1

Data headers in the 1st row are skipped in the import. But please avoid including numbers in the header characters.

	A	B	C	D	E	F	G
1	Frequency	GAIN [−]	Phase [de	GAIN [dB]	Phase [deg	Frequency [kHz]	
2	10	1	2.162048	−7.66008	177.838	0.01	
3	10.2329	1.054278	0.870193	−7.20098	179.1298	0.010233	
4	10.4713	1.067164	1.077667	−7.09546	178.9223	0.010471	
5	10.7152	1.038132	1.450851	−7.33503	178.5491	0.010715	
6	10.9648	1.04619	0.334167	−7.26787	179.6658	0.010965	
7	11.2202	1.047085	0.557877	−7.26044	179.4421	0.01122	
8	11.4815	1.025129	0.228317	−7.44451	179.7717	0.011482	
9	11.749	1.048017	2.102875	−7.25272	177.8971	0.011749	
10	12.0226	1.02381	1.235214	−7.45569	178.7648	0.012023	
11	12.3027	1.061627	2.638184	−7.14064	177.3618	0.012303	
12	12.5893	1.063989	0.548981	−7.12134	179.451	0.012589	
13	12.8825	1.053069	0.820068	−7.21094	179.1799	0.012883	
14	13.1826	1.030342	1.559601	−7.40045	178.4404	0.013183	
15	13.4896	1.005755	−0.50813	−7.61024	180.5081	0.01349	
16	13.8038	1.027651	−0.17462	−7.42317	180.1746	0.013804	
17	14.1254	1.002221	1.57811	−7.64081	178.4219	0.014125	
18	14.4544	0.977218	1.509628	−7.86025	178.4904	0.014454	
19	14.7911	0.981273	0.222946	−7.82428	179.7771	0.014791	
20	15.1356	1.004225	0.292511	−7.62346	179.7075	0.015136	
21	15.4882	0.994451	2.353302	−7.70841	177.6467	0.015488	
22	15.8489	0.972137	0.973724	−7.90553	179.0263	0.015849	
23	16.2181	0.997187	0.15892	−7.68446	179.8411	0.016218	
24	16.5959	1.020932	1.510025	−7.48014	178.49	0.016596	
25	16.9824	1.001347	1.557709	−7.64839	178.4423	0.016982	
26	17.378	1.021065	1.977631	−7.47901	178.0224	0.017378	
27	17.7828	0.932079	3.936951	−8.27103	176.063	0.017783	
28	18.197	0.963701	−2.5899	−7.98124	182.5899	0.018197	
29	18.6209	1.025855	1.195663	−7.43836	178.8043	0.018621	
30	19.0546	1.008468	1.068787	−7.58684	178.9312	0.019055	
31	19.4984	1.007291	1.366577	−7.59698	178.6334	0.019498	
32	19.9526	0.978542	−0.07738	−7.84849	180.0774	0.019953	
33	20.4174	1.00411	0.697952	−7.62446	179.302	0.020417	
34	20.893	0.981433	1.14888	−7.82286	178.8511	0.020893	
35	21.3796	0.984818	1.486572	−7.79296	178.5134	0.02138	
36	21.8776	0.981836	0.26062	−7.8193	179.7394	0.021878	
37	22.3872	1.008676	1.221039	−7.58504	178.779	0.022387	
38	22.9087	1.028453	0.995407	−7.41639	179.0046	0.022909	

Frequency response data should be stored in the 1st ~ 3rd columns.

1st column: Frequency (Hz, normally 10 Hz ~ 100,000 Hz)

2nd column: Reciprocal Gain (Reciprocal of linearized dimensionless gain, it approaches asymptotically to 1 at the lowest frequency and it shows minima at the resonance peaks)

3rd column: Phase (deg, it approaches asymptotically to 0 at the lowest frequency and it positively increases with increasing the frequency.)

Since the data from the 4th column onward are not imported, the original data can be backed up here.

Spectrum data in the CSV file format, which has the scanner name as the file name, should be placed in the config folder.
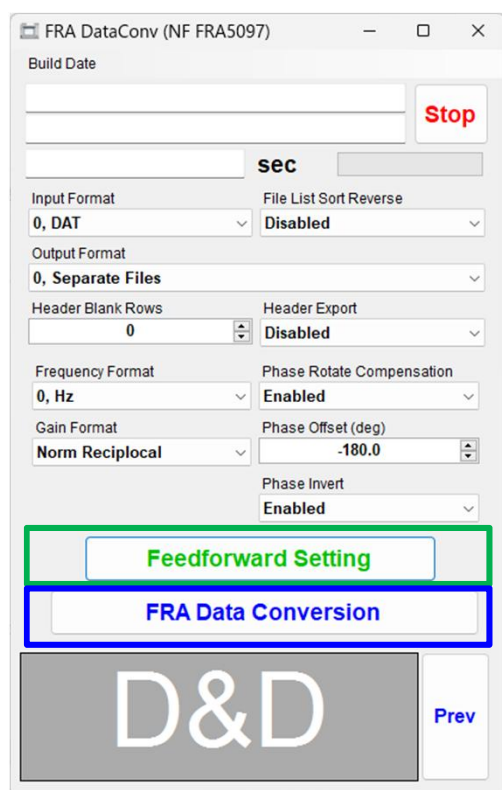for example,
C:¥xxx¥desktop¥config¥Kode-MS01.csv
It is automatically imported when the scan is started.
Although data format is the same as the one used in Igor program, the data header in the 1st row should be deleted for using it in the Igor program.
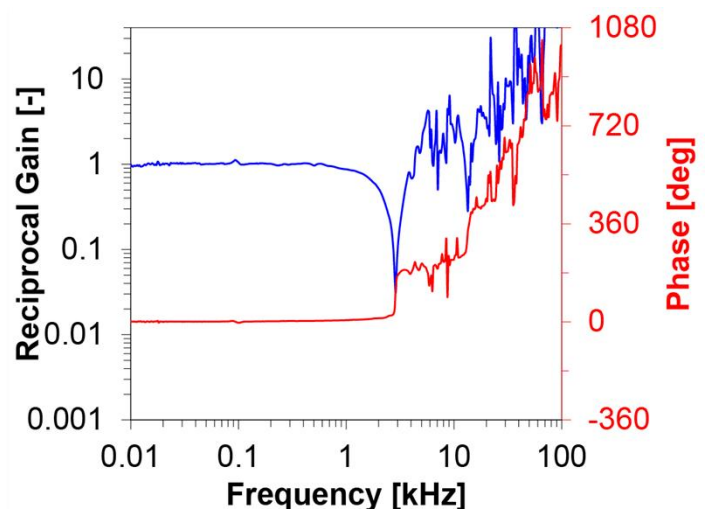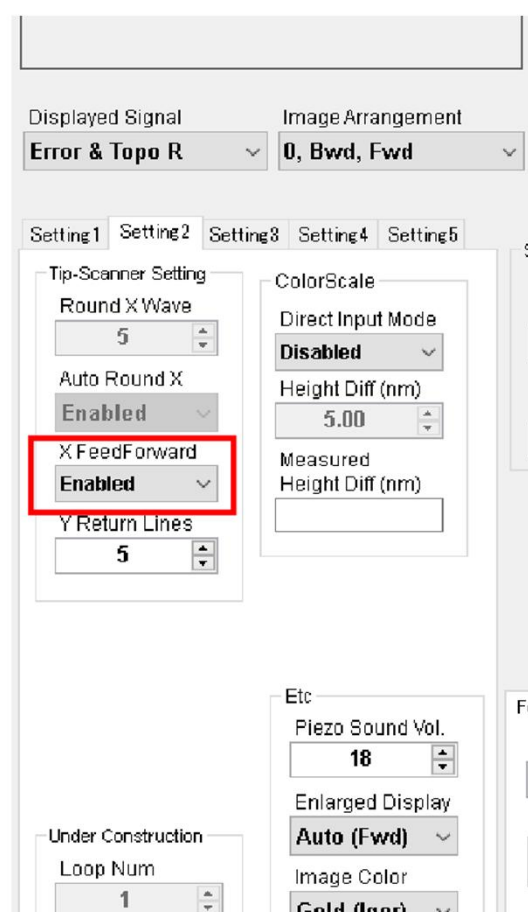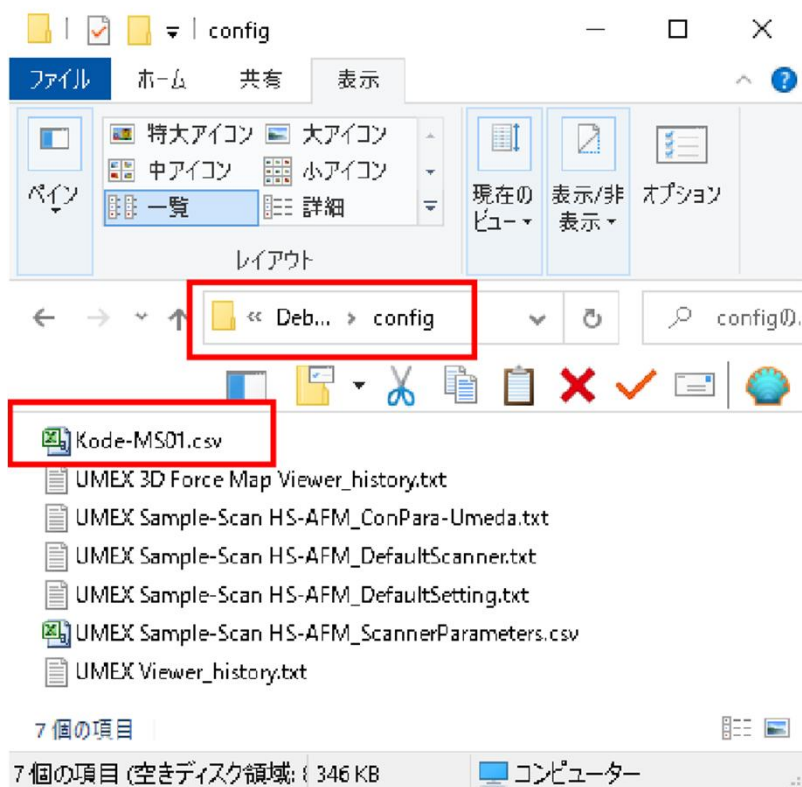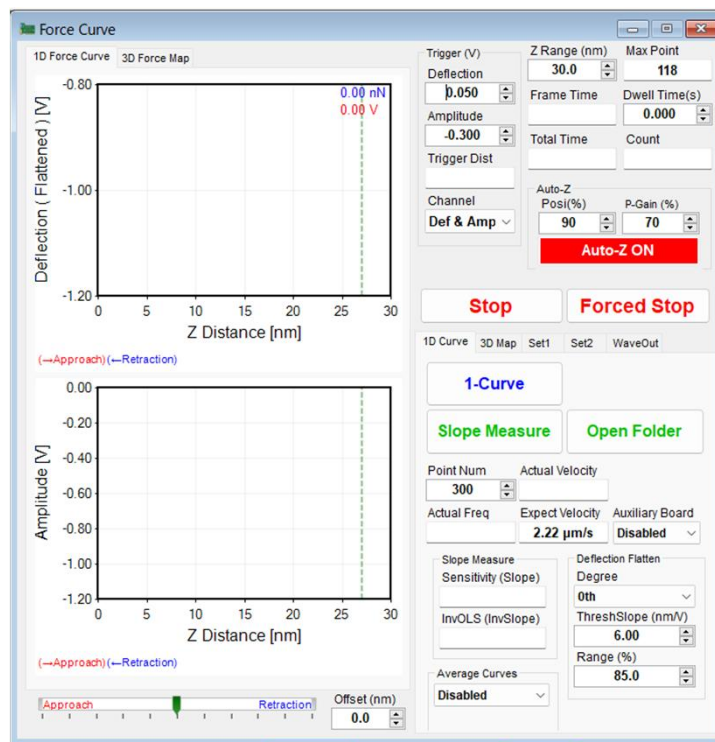
X Scan Feedforward2

When using spectral data acquired using the NF FRA5097, it is necessary to convert the binary data to an ASCII CSV file using the FRA DataConv program.

① **Press the "Feedforward Setting" button to restore the Feedforward settings.**

② **Then, press the "FRA Data Conversion" button to output a CSV file.**

Force Curve & 3D Force Map

It is used when you want to quantitatively measure the viscoelasticity and mechanical properties of the sample surface. For details, see the separate manual files "UMEX 3D Force Mapping _Guide" and "UMEX 3D Force Map Viewer _Guide".