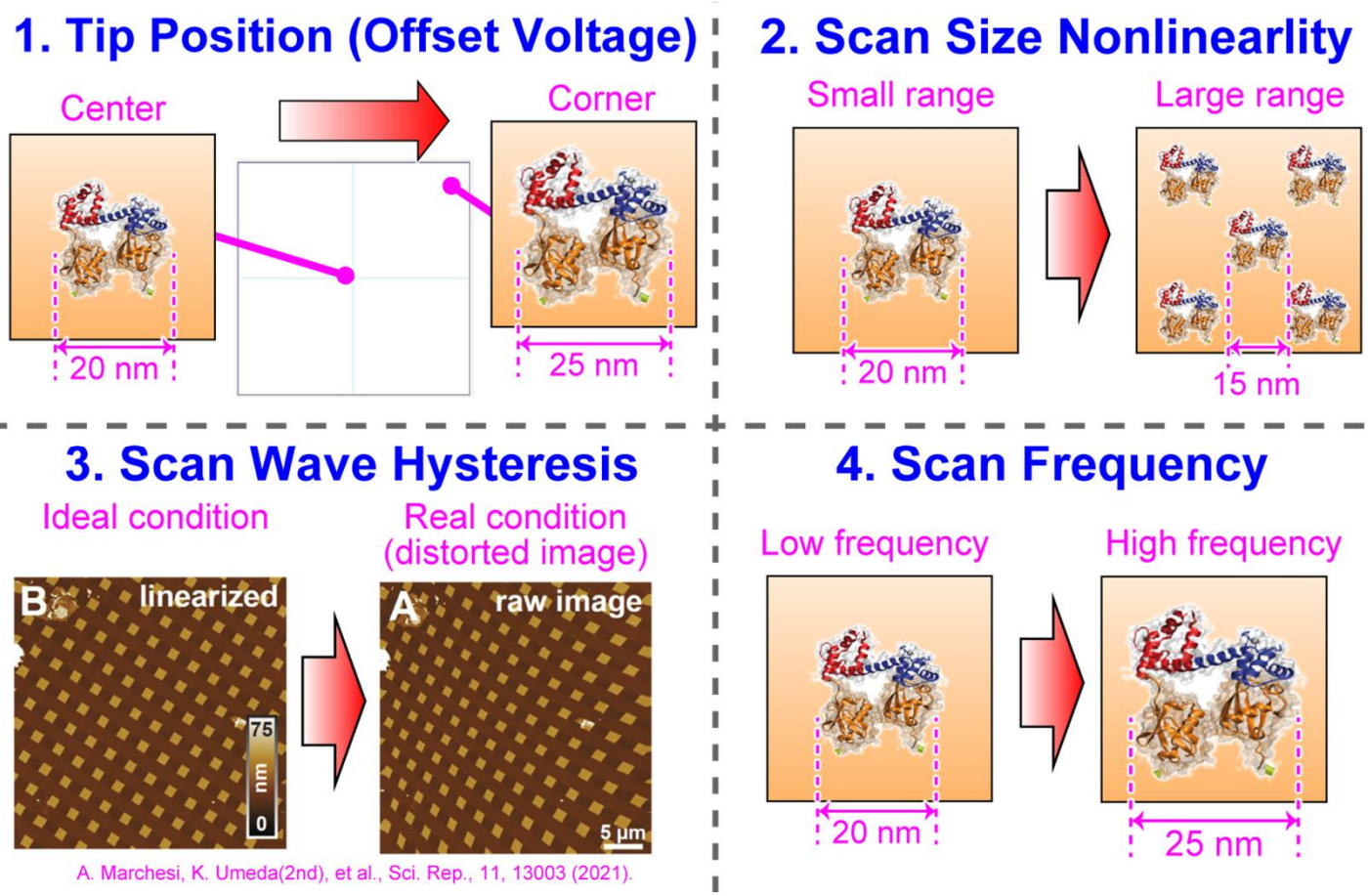
Principle of Piezo Nonlinear Correction

There are four major factors for the piezo nonlinearities as shown in the following figure.
Software corrects all these factors but the parameter must be calibrated only for

1. Tip Position
2. Scan Size Nonlinearity

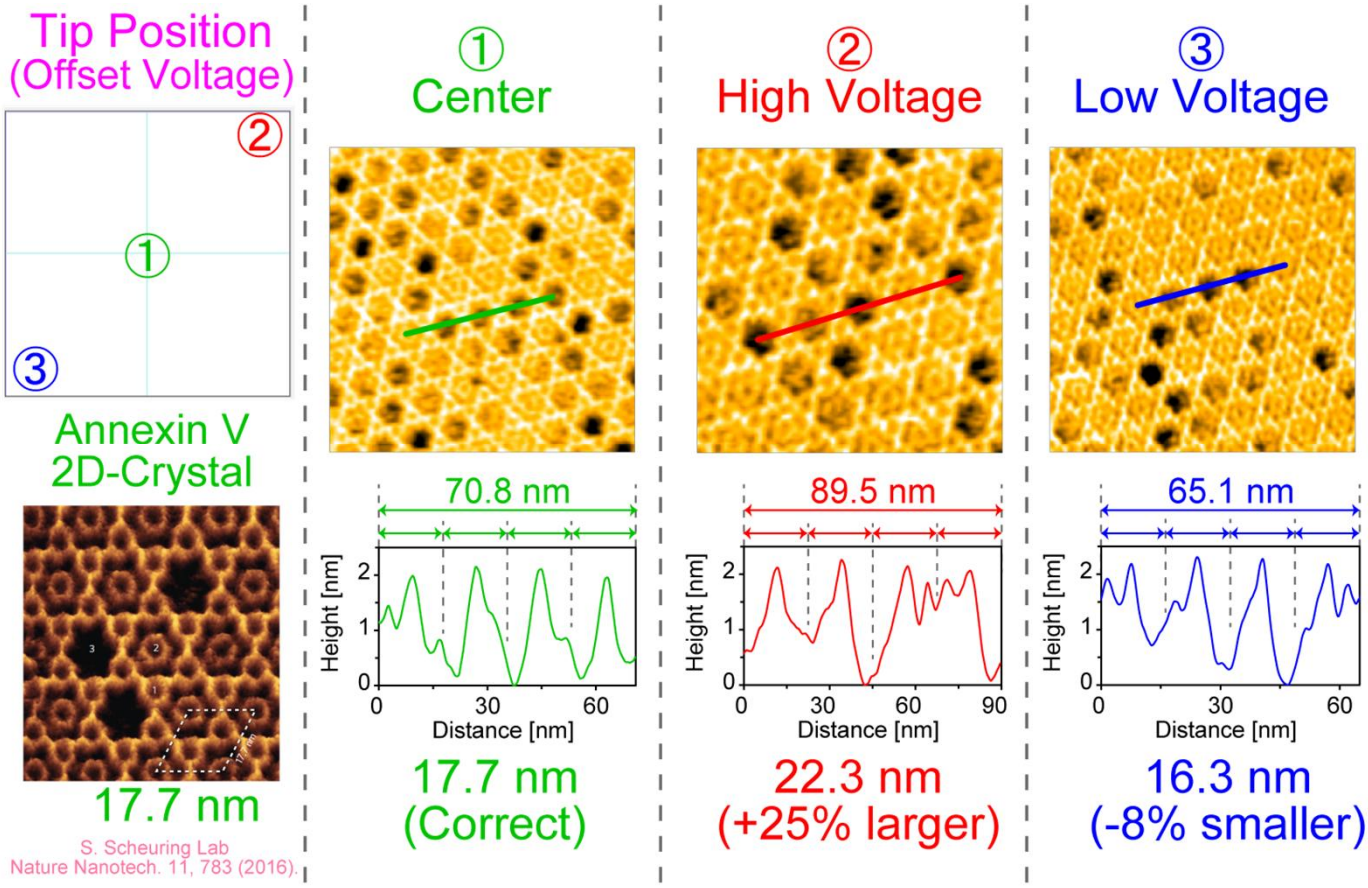
while regarding 3 and 4, typical values measured using a laser displacement meter in advance are used without calibration because they are hard to be accurately estimated solely from the AFM image.



A. Marchesi, K. Umeda(2nd), et al., Sci. Rep., 11, 13003 (2021).

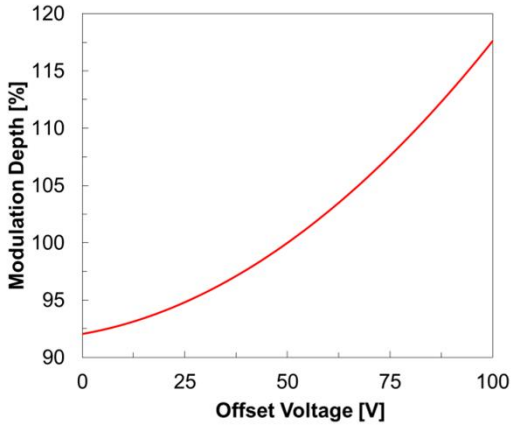
1. Tip Position (Offset Voltage)

The piezo constant should be normally calibrated at the center of stage (V=50 V). However, to seek desired target molecules, the offset voltage must be changed to move the tip position. Unfortunately, this also alters the actual piezo constant and apparent molecular size. As described in the following figure, when the tip is located at the center of the stage (V=50 V), the observed lattice constant of Annexin-V is an ideal 17.7 nm. However, when the offset voltage is high (V=100 V), it is increased by 25 %, and when offset voltage is low (V=0 V), it is reduced by 8%.



1. Tip Position (Offset Voltage)

As described in the following figure, the optimum modulation depth for the scan range can be approximated by a quadratic function of the offset voltage. In the actual calibration procedure, the normal piezo constant is first calibrated at the center of the stage and nonlinear factors (γ+ and γ-) are then calibrated at the upper and lower ends or the stage, respectively. From these three parameters, the parameters α and β can be obtained and the modulation depth in all the positions can be predicted.



Second-order Taylor polynomial at V = 50 V

$$\text{ModulationDepth}: \gamma = 1 + \alpha x + \beta x^2$$

$$x = \frac{V - 50}{100}$$

$$\alpha = \frac{\gamma_+ - \gamma_-}{2}$$

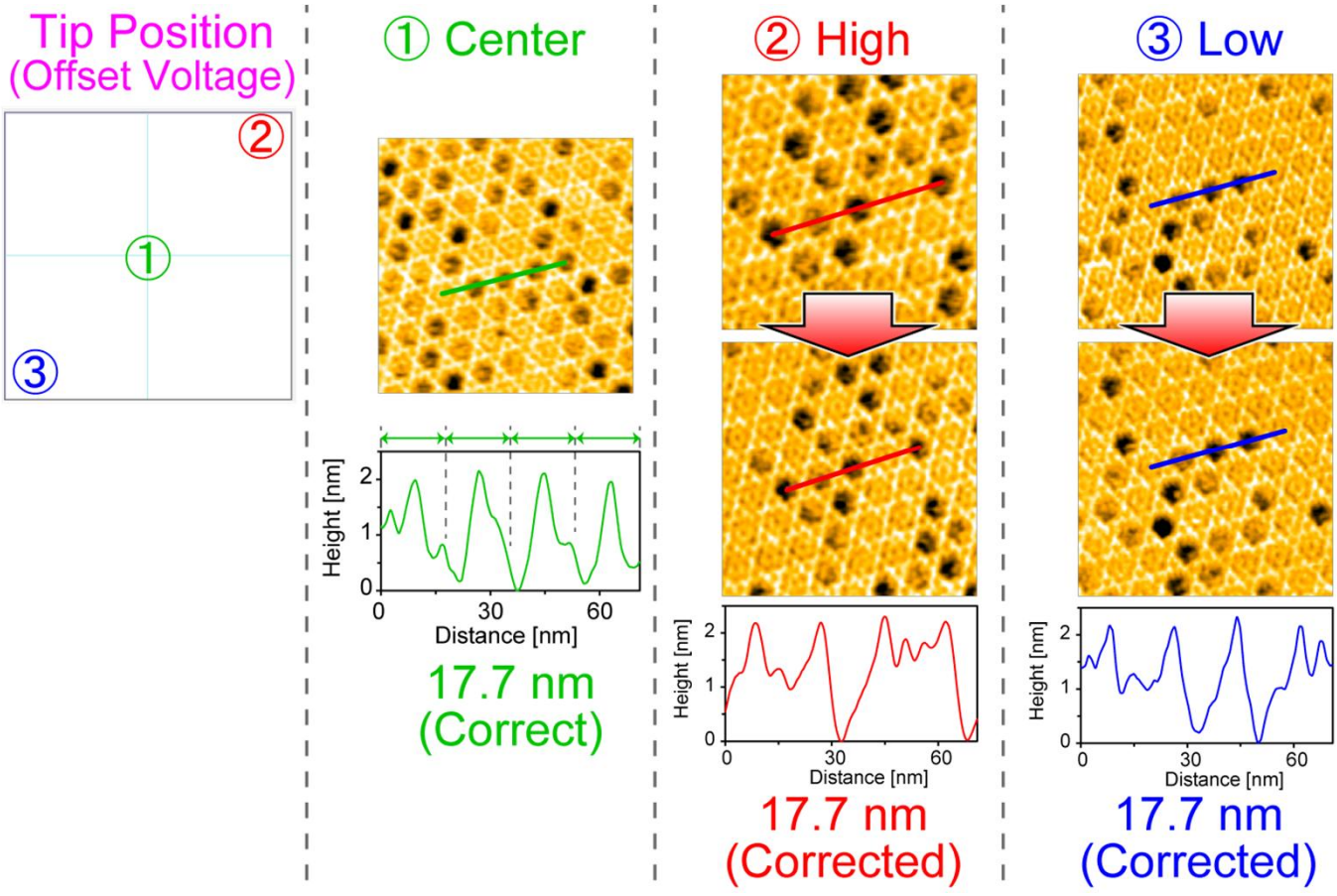
$$\beta = \frac{\gamma_+ + \gamma_-}{2} - 1$$

$$\gamma = \gamma_- \left(\text{when } V = 0 \text{ V}\right)$$

$$\gamma = 1 \left(\text{when } V = 50 \text{ V}\right)$$

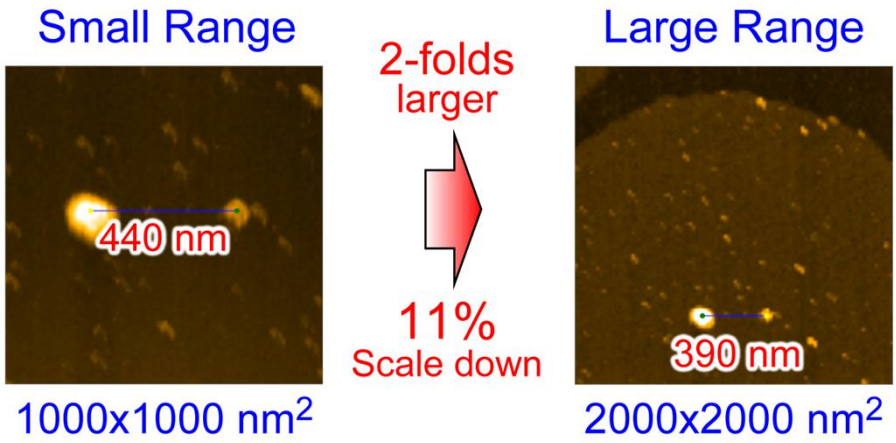
$$\gamma = \gamma_+ \left(\text{when } V = 100 \text{ V}\right)$$

As described in the following figure, by doing the above procedure, the correct lattice constant was successfully obtained even at the corners of the stage.

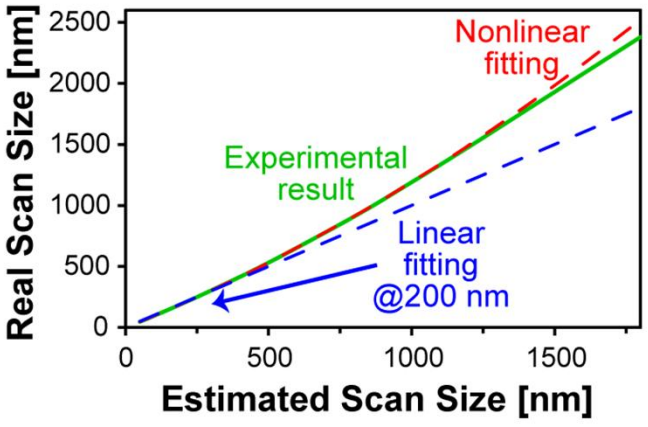


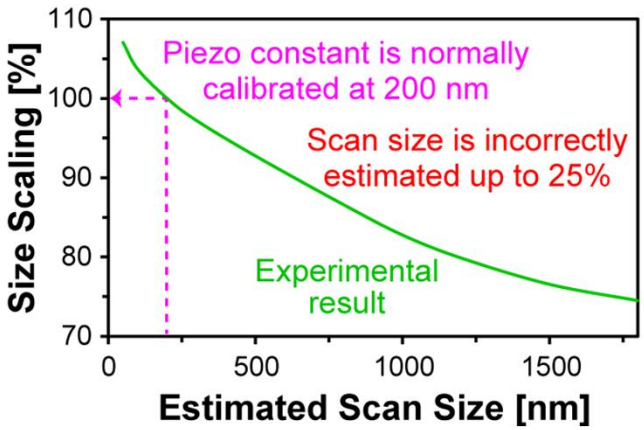
2. Scan Size Nonlinearity

The piezo displacement is not linear function of the voltage but a nonlinear function of the voltage. So the scan range also nonlinearly increases with increasing the scanning voltage. As demonstrated in the following figure, the apparent intermolecular distance becomes smaller with increasing the scanning range.



By examining the real piezo response using a laser displacement meter, the real scan size was confirmed to be nonlinearly increased with increasing the scan size programmed. The piezo calibration is normally performed at 200x200 nm^2 scan size, and hence, as indicated by the blue broken line, the real scan size significantly deviates from the estimated scan size in the high scan range. It reaches down to 75% as shown in the right figure. By fitting the experimental result onto a second-order polynomial, a better agreement was obtained (See the red broken line).





Linear equation

$$x = \alpha_{\text{Linear}} V$$

Second-order Taylor polynomial

$$x = \alpha_{1\text{st}} V \left(1 + \beta_{2\text{nd}} V\right)$$

Definition of Variants

Scan Range : x

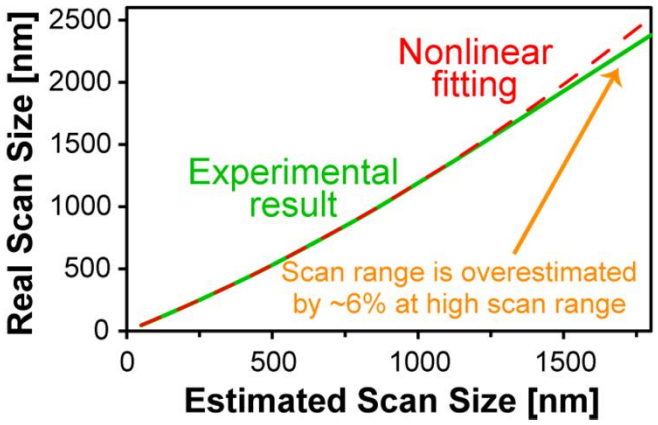
Linearized Piezo Const : α_{Linear}

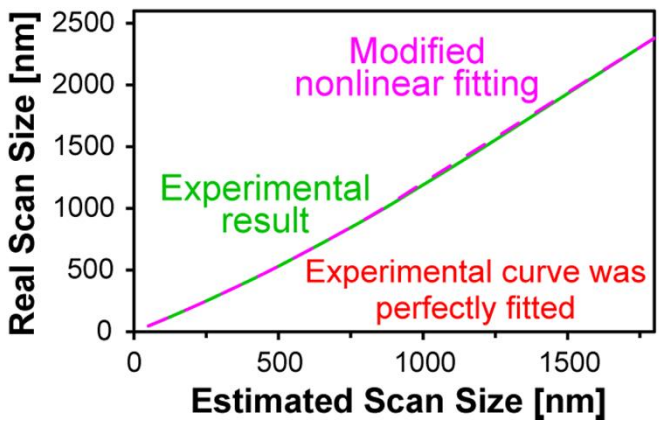
1st-order ScalingFactor : $\alpha_{1\text{st}} = \dfrac{\alpha_{\text{Linear}}}{1 + \beta_{2\text{nd}} \dfrac{200}{\alpha_{\text{Linear}}}}$

2nd-order ScalingFactor : $\beta_{2\text{nd}} \approx 0.5$

2. Scan Size Nonlinearity

However, by taking closer look at the chart in the high scanning range, we can see that the red curve slightly overestimates the nonlinearity of the scan range (~6%). A possible solution is the use of higher-order polynomial (e.g., more than 2nd-order), but it cannot be used because an explicit form of the inverse function of the higher-order polynomial cannot be derived. This becomes problematic when it is implemented in the software. Therefore, the software adopts a linear/nonlinear hybrid calculation method. When the scan range is less than 50% of the maximum voltage, a 2nd-order polynomial is used while when it is higher than 50%, a linear equation extrapolated from the nonlinear equation to the higher scan range is used. Using this hybrid method, as shown in the right figure, a perfect agreement between the experiment and fitting has been successfully obtained.





Problem of 3rd-order polynomial

$$x = \alpha_{1st} V \left(1 + \beta_{2nd} V + \gamma_{3rd} V^2\right)$$

Inverse function of cubic equation cannot be analytically derived

Low Scan Range (V < W)

$$x = \alpha_{1st} V \left(1 + \beta_{2nd} V\right)$$

$$V = \frac{\sqrt{1 + \frac{4\beta_{2nd} x}{\alpha_{1st}}} - 1}{2\beta_{2nd}}$$

High Scan Range (V ≧ W)

$$x = \alpha_{1st} \left[\left(1 + 2\beta_{2nd} W\right) V - \beta_{2nd} W^2\right]$$

$$V = \frac{x + \alpha_{1st} \beta_{2nd} W^2}{\alpha_{1st} \left(1 + 2\beta_{2nd} W\right)}$$

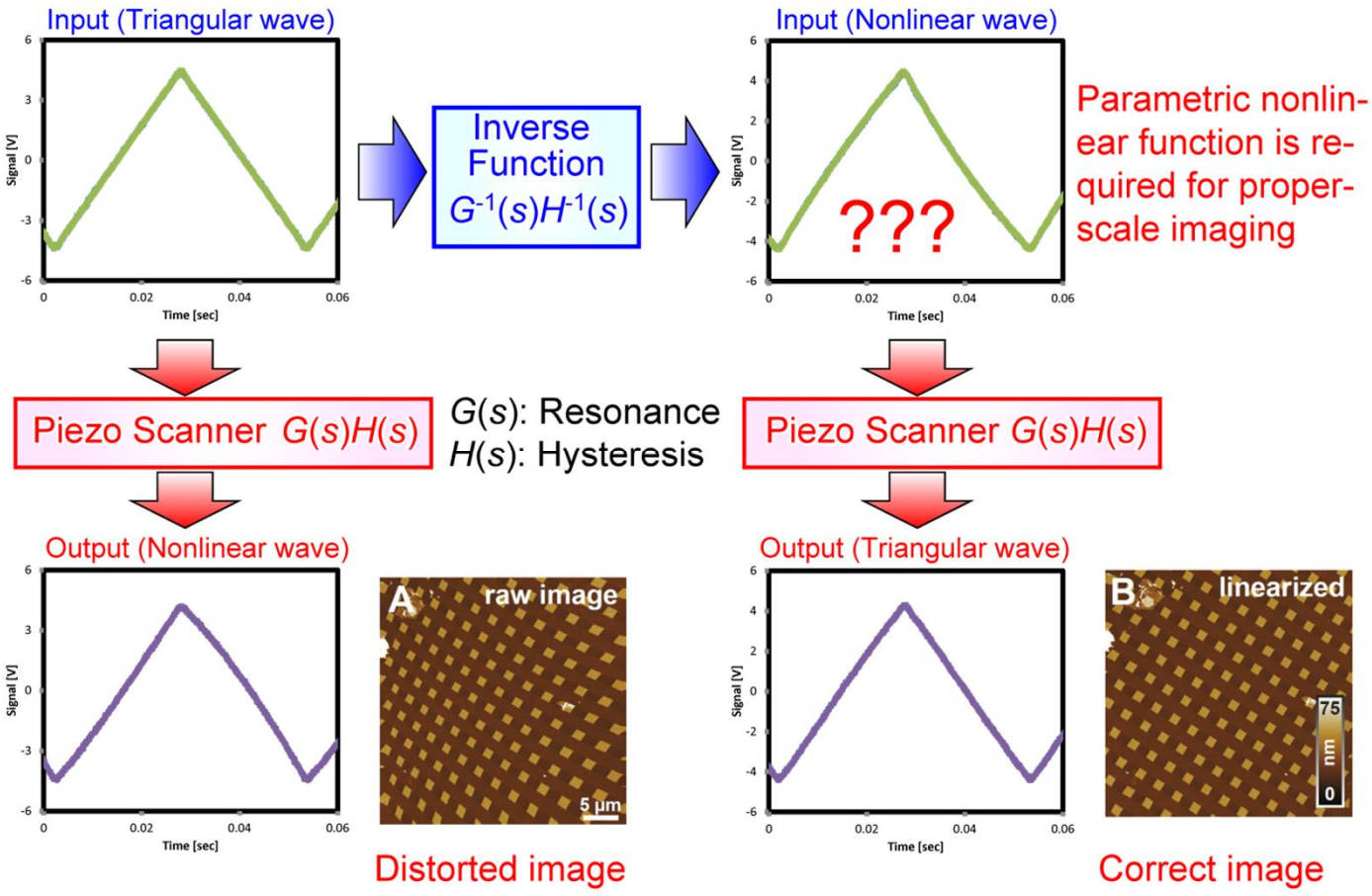
Linear equation was obtained by extrapolating 2nd-order polynomial to high scan range

3. Scan Wave Hysteresis

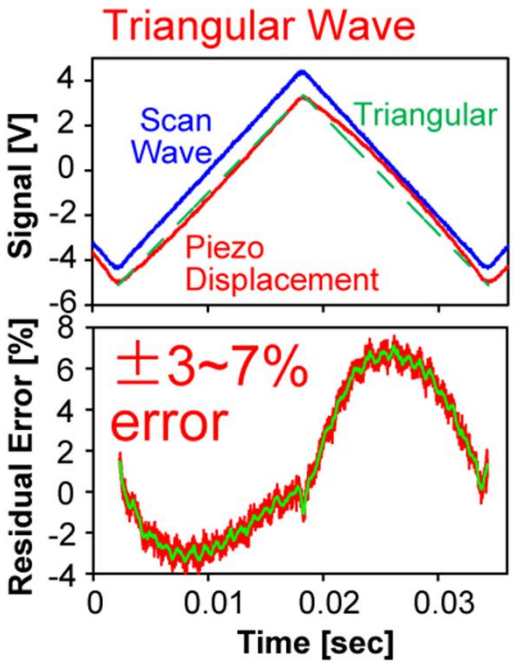
To drive the X piezo scanner, a triangular wave form of voltage is normally used. However, the actual piezo movement is affected by the transfer functions of resonance and hysteresis characteristics of the piezo, and hence the resultant displacement becomes nonlinearly distorted. To correct this unwanted effect, by applying the inverse function of these two transfer functions to the driving voltage, a straight triangular waveform of the piezo displacement can be obtained.

The transfer function of the resonance characteristics can easily be analyzed by a laser displacement meter and compensated by the X-feedforward function. This mainly affects the measurements only when the scanning frequency becomes large.

However, to compensate the hysteresis characteristics, some mathematical models, e.g., Preisach, have been proposed, but an easier method is desirable. The HS-AFM instruments adopt a hard piezo, which indicates much less hysteresis than a soft piezo, and a more simple analytical equation would be alternatively used.



3. Scan Wave Hysteresis



As described in the left figure, there are 3~7% residual errors when a triangular waveform is used. As clearly seen in the figure, the hysteresis is asymmetric and the backward scan exhibits a larger nonlinearity compared to the forward scan.

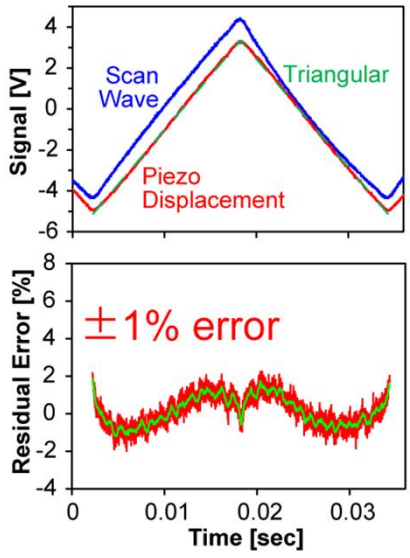
To construct an analytical nonlinear equation, a prerequisite condition is that the derived equation can be expanded to a Fourier series equation to reduce the resonance characteristics.

As shown in the following figure, a nonlinear waveform based on an exponential function has been successfully derived. It can reduce the residual error down to 1%.

To reduce the error further, another equation which considers a asymmetry using a sinusoidal waveform was also derived.

Scan Waveform1

$$f(x)=\begin{cases}\dfrac{1-\exp\left(-\dfrac{\beta_1}{\alpha}x\right)}{1-\exp(-\beta_1)} & \cdots\ 0<x\le\alpha \\ 1-\dfrac{1-\exp\left[-\beta_2\left(\dfrac{x-\alpha}{1-\alpha}\right)\right]}{1-\exp(-\beta_2)} & \cdots\ \alpha<x\le 1\end{cases}$$



Fourier Series Expansion

$$f(x)=a_0+2\sum_{n=1}^{\infty}\left[a_n\cos(n\omega t)+b_n\sin(n\omega t)\right]$$

$$a_0=\alpha\left(\frac{1}{\exp\beta_1-1}-\frac{1}{\beta_1}+1\right)+(\alpha-1)\left(\frac{1}{\exp\beta_2-1}-\frac{1}{\beta_2}\right)$$

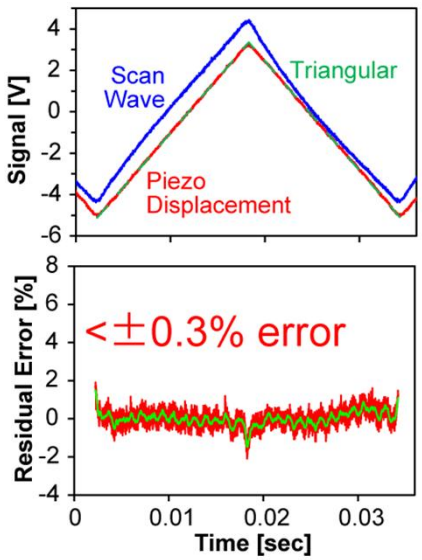
$$a_n=\frac{1}{2(\exp\beta_1-1)}\left\{\frac{\exp\beta_1\sin(2\pi\alpha n)}{\pi n}-\frac{2\alpha\left[-\beta_1\cos(2\pi\alpha n)+2\pi\alpha n\sin(2\pi\alpha n)+\beta_1\exp\beta_1\right]}{(2\pi\alpha n)^2+\beta_1^2}\right\}$$
$$+\frac{\exp(-\beta_2)}{1-\exp(-\beta_2)}\left\{-\frac{\sin(2\pi n)-\sin(2\pi\alpha n)}{2\pi n}+\frac{(\alpha-1)}{\left[2\pi(\alpha-1)n\right]^2+\beta_2^2}\left\{\begin{matrix}\beta_2\left[\cos(2\pi n)-\exp\beta_2\cos(2\pi\alpha n)\right]\\+2\pi(\alpha-1)n\left[\sin(2\pi n)-\exp\beta_2\sin(2\pi\alpha n)\right]\end{matrix}\right\}\right\}$$

$$b_n=\frac{1}{\exp\beta_1-1}\left\{\frac{\exp\beta_1\sin^2(\pi\alpha n)}{\pi n}+\frac{\alpha\left[2\pi\alpha n\left[\cos(2\pi\alpha n)-\exp\beta_1\right]+\beta_1\sin(2\pi\alpha n)\right]}{(2\pi\alpha n)^2+\beta_1^2}\right\}$$
$$+\frac{\exp(-\beta_2)}{1-\exp(-\beta_2)}\left\{-\frac{\cos(2\pi\alpha n)-\cos(2\pi n)}{2\pi n}+\frac{(\alpha-1)}{\left[2\pi(\alpha-1)n\right]^2+\beta_2^2}\left\{\begin{matrix}\beta_2\left[\sin(2\pi n)-\exp\beta_2\sin(2\pi\alpha n)\right]\\-2\pi(\alpha-1)n\left[\cos(2\pi n)-\exp\beta_2\cos(2\pi\alpha n)\right]\end{matrix}\right\}\right\}$$

3. Scan Wave Hysteresis

Scan Waveform2

$$f(x)=\begin{cases}(1+2\gamma_1)\left[\dfrac{1-\exp\left(-\beta_1\dfrac{x}{\alpha}\right)}{1-\exp(-\beta_1)}\right]+\gamma_1\left[\cos\left(\pi\dfrac{x}{\alpha}\right)-1\right] & \cdots\ 0<x\le\alpha\\ (1+2\gamma_2)\left[1-\dfrac{1-\exp\left[-\beta_2\left(\dfrac{x-\alpha}{1-\alpha}\right)\right]}{1-\exp(-\beta_2)}\right]-\gamma_2\left[\cos\left(\pi\dfrac{x-\alpha}{1-\alpha}\right)+1\right] & \cdots\ \alpha<x\le 1\end{cases}$$



Fourier Series Expansion

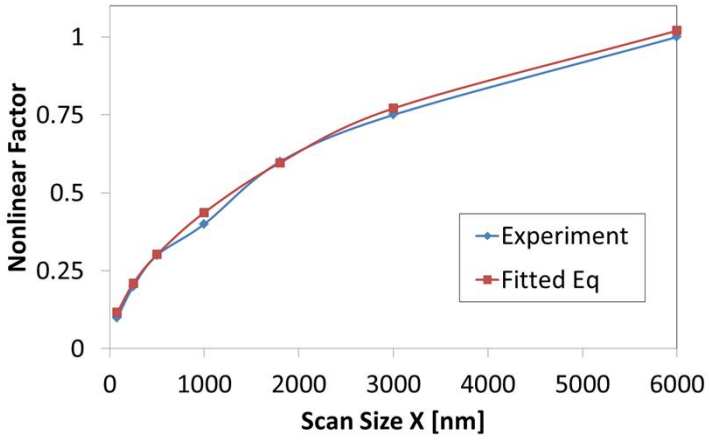
$$f(x)=a_0+2\sum_{n=1}^{\infty}\left[a_n\cos(n\omega t)+b_n\sin(n\omega t)\right]$$

$$a_0=(1+2\gamma_1)\left[\alpha\left(\frac{1}{\exp\beta_1-1}-\frac{1}{\beta_1}+1\right)+(\alpha-1)\left(\frac{1}{\exp\beta_2-1}-\frac{1}{\beta_2}\right)\right]-\alpha\gamma_1+(\alpha-1)\gamma_2$$

$$a_n=(1+2\gamma_1)\left\{\begin{aligned}&\frac{1}{2(\exp\beta_1-1)}\left\{\frac{\exp\beta_1\sin(2\pi\alpha n)}{\pi n}-\frac{2\alpha\left[-\beta_1\cos(2\pi\alpha n)+2\pi\alpha n\sin(2\pi\alpha n)+\beta_1\exp\beta_1\right]}{(2\pi\alpha n)^2+\beta_1^2}\right\}\\&+\frac{\exp(-\beta_2)}{1-\exp(-\beta_2)}\left\{-\frac{\sin(2\pi n)-\sin(2\pi\alpha n)}{2\pi n}\right.\\&\left.+\frac{(\alpha-1)}{\left[2\pi(\alpha-1)n\right]^2+\beta_2^2}\left\{\begin{aligned}&\beta_2\left[\cos(2\pi n)-\exp\beta_2\cos(2\pi\alpha n)\right]\\&+2\pi(\alpha-1)n\left[\sin(2\pi n)-\exp\beta_2\sin(2\pi\alpha n)\right]\end{aligned}\right\}\right\}\end{aligned}$$
$$-\frac{\gamma_1\left[2(2\alpha n)^2-1\right]\sin(2\pi\alpha n)}{2\pi n\left[(2\alpha n)^2-1\right]}+\gamma_2\frac{\left[8(\alpha-1)^2n^2-1\right]\sin(2\pi\alpha n)}{2\pi n\left[4(\alpha-1)^2n^2-1\right]}$$

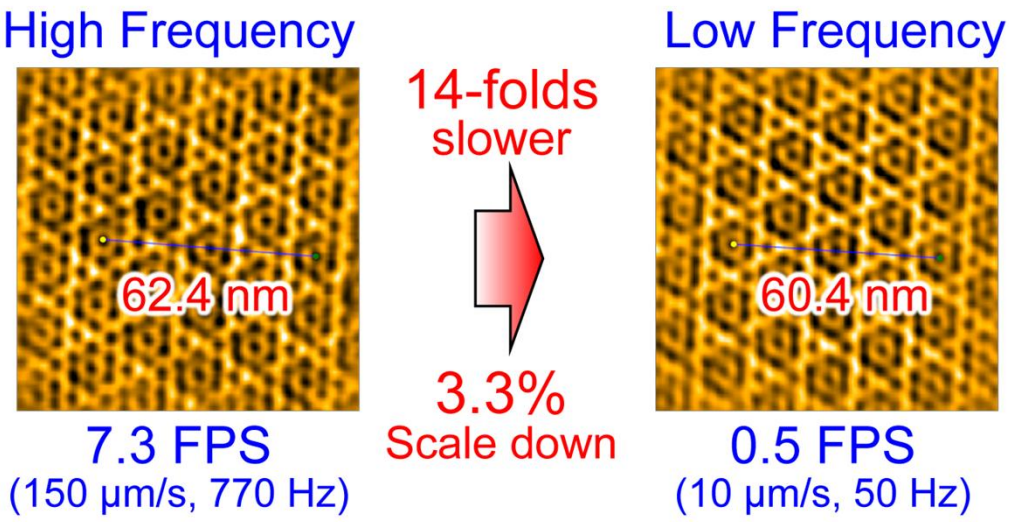
$$b_n=(1+2\gamma_1)\left\{\begin{aligned}&\frac{1}{\exp\beta_1-1}\left\{\frac{\exp\beta_1\sin^2(\pi\alpha n)}{\pi n}+\frac{\alpha\left[2\pi\alpha n\left[\cos(2\pi\alpha n)-\exp\beta_1\right]+\beta_1\sin(2\pi\alpha n)\right]}{(2\pi\alpha n)^2+\beta_1^2}\right\}\\&+\frac{\exp(-\beta_2)}{1-\exp(-\beta_2)}\left\{-\frac{\cos(2\pi\alpha n)-\cos(2\pi n)}{2\pi n}\right.\\&\left.+\frac{(\alpha-1)}{\left[2\pi(\alpha-1)n\right]^2+\beta_2^2}\left\{\begin{aligned}&\beta_2\left[\sin(2\pi n)-\exp\beta_2\sin(2\pi\alpha n)\right]\\&-2\pi(\alpha-1)n\left[\cos(2\pi n)-\exp\beta_2\cos(2\pi\alpha n)\right]\end{aligned}\right\}\right\}\end{aligned}$$
$$+\gamma_1\frac{\left[2(2\alpha n)^2-1\right]\cos(2\pi\alpha n)+1}{2\pi n\left[(2\alpha n)^2-1\right]}-\gamma_2\frac{\left[8(\alpha-1)^2n^2-1\right]\cos(2\pi\alpha n)+1}{2\pi n\left[4(\alpha-1)^2n^2-1\right]}$$

The nonlinearly of the hysteresis is obviously a function of the scan size. By parametrizing the nonlinear factor, the following equation has been derived. However, the calibration of the hysteresis factor of the scan size dependence is difficult, and hence they are used as a fixed value in the software.



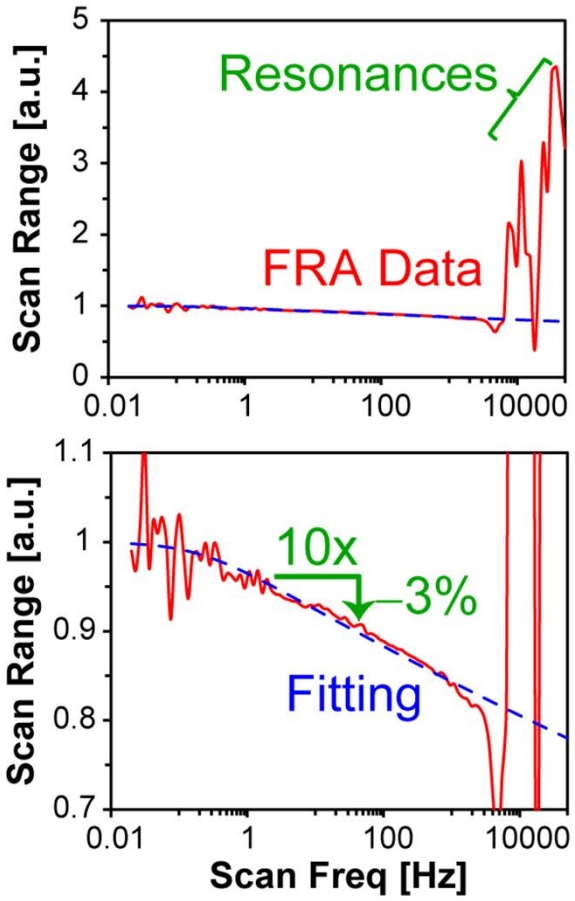
$$\beta_x=\underbrace{\gamma\left(\frac{\text{ScanSize}}{\text{MaxSize}}\right)^{0.5-0.25\frac{\text{ScanSize}}{\text{MaxSize}}}}_{\text{Scan Size Dependency}}\times\underbrace{\left\{1+\varepsilon\left[\left(0.5-\frac{\text{Offset}}{\text{MaxSize}}\right)^{\kappa}-0.5^{\kappa}\right]\right\}}_{\text{Offset Dependency}}$$

4. Scan Frequency



The displacement of the piezo originates from the polarization of ferroelectrics, of which the response becomes smaller with increasing the driving frequency. Therefore, the actual scan range at the high frequency becomes smaller than that at the low frequency and apparent molecular size becomes larger.

To examine this effect, the frequency characteristics measured by a laser displacement meter is shown in the following figure. In the high frequency range, multiple peaks of the resonance appeared while in the low frequency range, the scan range gradually decreased with increasing the scan frequency. By fitting this response by a simple equation below, the cutoff frequency and decay factor were determined to 0.2 Hz and 0.03. this parameters do not significantly differ in each scanner and they are used as fixed parameters in the software.



Derived frequency characteristics

$$\text{ScanRange} = \frac{1}{\left(1+\frac{\text{ScanFreq}}{\text{CutoffFreq}}\right)^{\frac{\alpha}{2}}}$$

$$\approx 1-\alpha \log_{10}\left(2\frac{\text{ScanFreq}}{\text{CutoffFreq}}\right)$$

$$\text{CutoffFreq} = 0.2 \text{ Hz}$$

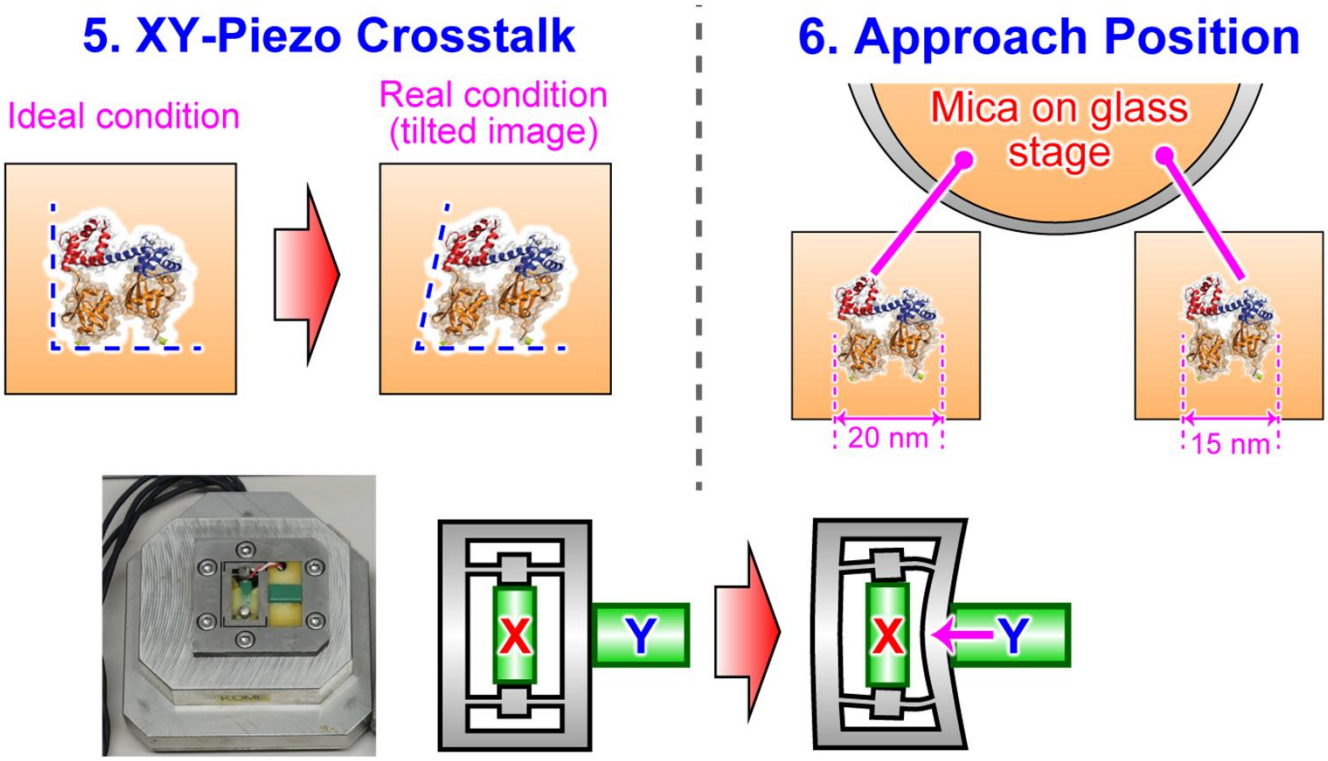
$$\alpha = 0.03$$

With increasing scan frequency 10-folds, Scan range decreases by ~3%

5. Other Factors

Besides the effect described above, the cross-talk between X and Y piezo brcomes sometimes problematic for the quantitative distance measurement. This effect is caused by the distortion of the scanner flexure of the X piezo by the force exerted by the Y piezo extension. This also cause the variation of the piezo constant depending on the approach position of the mica stage.

However, these effects become remarkable only when a scanner made of duralumin is used (~3%) and it can be significantly reduced using a scanner made of SUS (~1%) due to its greater rigidity. So please do not use a scanner made of duralumin when you want to do a quantitative distance measurement.



Manual for Scanner Nonlinear Calibration Using Annexin-V Crystal

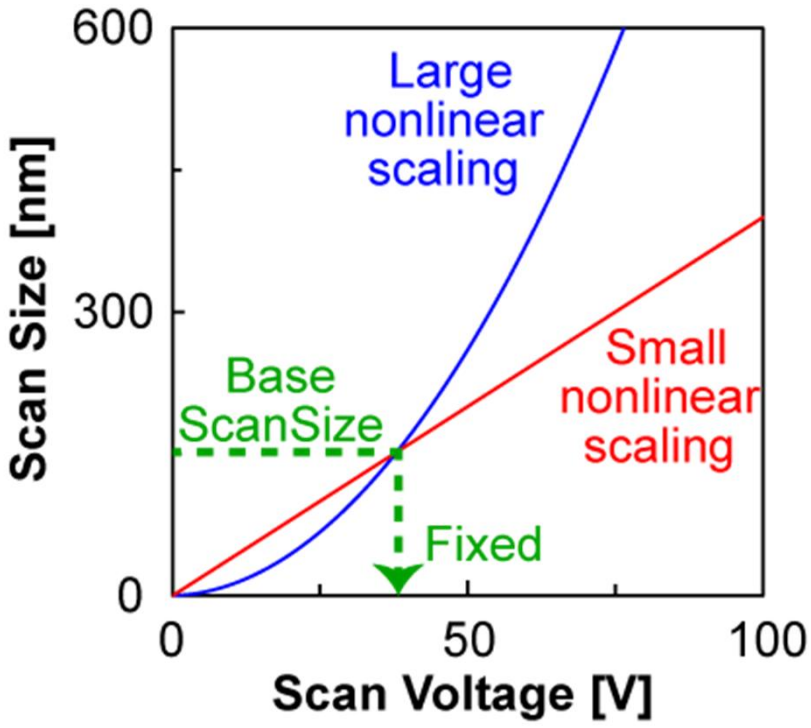
Selecting Calibration Scan Size

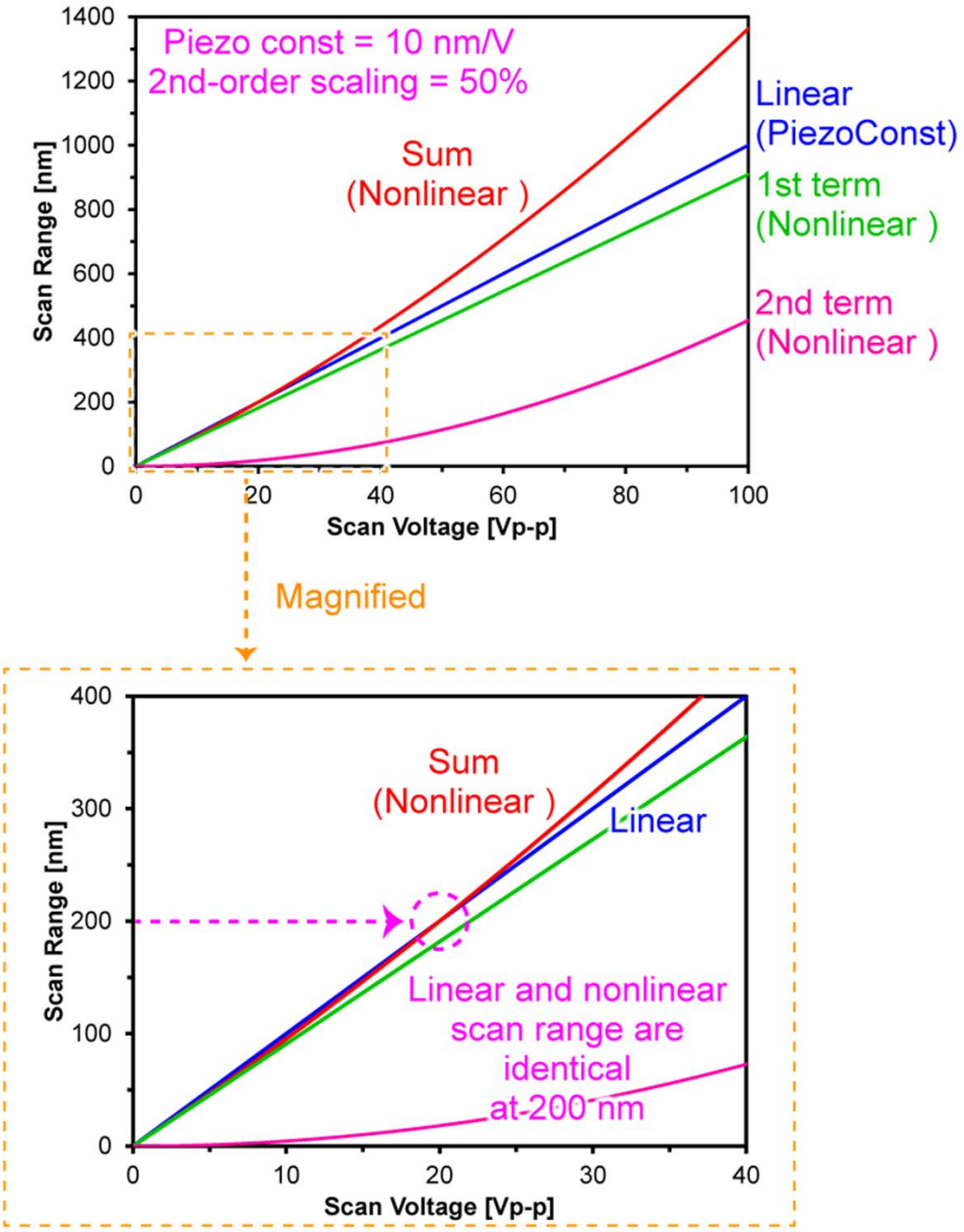
The calibration should be performed with the ScanSize that will be mainly used for the practical AFM experiments because the distance accuracy is highly optimized for the ScanRange used in the calibration. A user can select two different ScanSizes.

Normally, the ScanSize below 500 nm is used for most of the biomolecular imaging, and hence this manual describes the procedure using 200 and 500 nm ScanSize.
First, enter 200 nm to BaseScanSize and calibrate it with 200 nm ScanSize. Then, with 500 nm ScanSize, calibrate Parameter3_Nonlinear Scaling for Scan Size(%).

When using Annexin-V, the molecular lattice is hardly measured in the ScanSize other than 200~500 nm. Therefore, if you want to calibrate it with another ScanSize, you have to use another suitable sample.

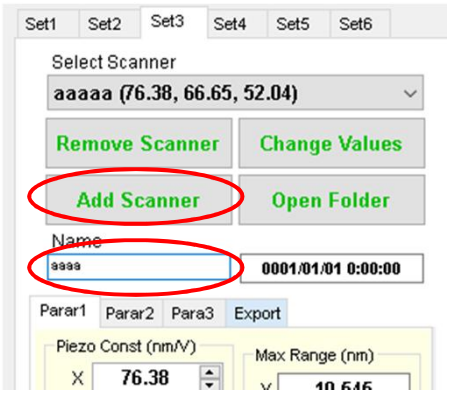
When using Ultra-Large Scanner, a microscale scan size, i.e, 35 µm, is often mainly used. So to calibrate it, micron-size calibration gratings must be used. First, enter 35,000 nm to BaseScanSize and calibrate the PiezoConst at 35,000 nm ScanSize. If you do not need to acquire AFM images with the ScanSize besides this, you do not have to calibrate the nonlinear parameters. But if you want to measure the AFM images with another ScanSize, enter the ScanSize that will used for experiments and calibrate Parameter3_Nonlinear Scaling for Scan Size(%).



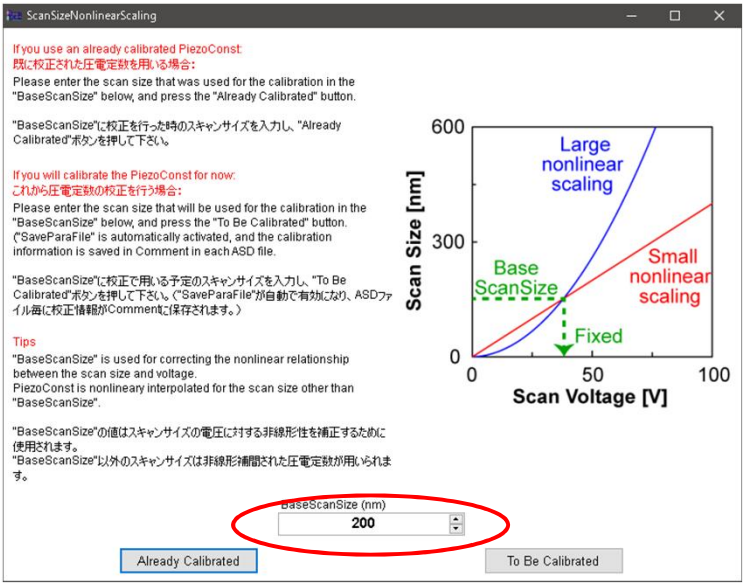

Piezo const = 10 nm/V
2nd-order scaling = 50%
Sum
(Nonlinear)
Linear
(PiezoConst)
1st term
(Nonlinear)
2nd term
(Nonlinear)
Scan Range [nm]
Scan Voltage [Vp-p]
Magnified
Sum
(Nonlinear)
Linear
Linear and nonlinear
scan range are
identical
at 200 nm
Scan Range [nm]
Scan Voltage [Vp-p]

Setting before Calibration

1. When adding a new Scanner

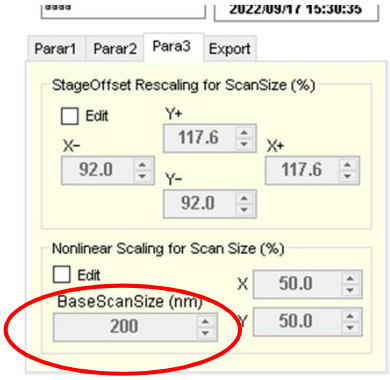


After entering a new scanner name to the Name textbox, by pressing “Add Scanner” button, the following dialogue box will appear.



Please input a scan size used for the calibration imaging to BaseScanSize (nm) shown at the bottom of the dialogue. When you use an Annexin-V sample, typically 200 nm scan size is recommended. By changing the Nonlinear Scaling for Scan Size(%), the nonlinearity of the scan size as a function of scan voltage is changed. As shown in the left figure, the scan voltage corresponding to the BaseScanSize (nm) is fixed, and hence at first please calibrate the piezo constant at the BaseScanSize (nm), and then calibrate the Nonlinear Scaling for Scan Size(%) at the different scan size (i.e., 500 nm). Then quantitative imaging will be realized in all the scan range.

2. When recalibrating an existing scanner



Please check that the scan size used for the calibration is set to BaseScanSize (nm). If a different scan size is set, please enter a check mark to the Edit box and enter the correct scan size to BaseScanSize (nm).

Setting before Calibration

A thing to check before observation

Please confirm that the offset voltage knobs of the XY piezo driver are set to 50 V. Please do not change these settings in all the experiments because the calibration becomes incorrect if these offset voltages are changed.

When Nonlinear Calibration is not necessary

If the quantitative distance measurement is not essential, the nonlinear calibration is not required. For this case, enter the ScanSize used for calibration to the BaseScanSize and leave the checkbox of Prameter3 StageOffset Rescaling for ScanSize と Parameter3_Nonlinear Scaling for Scan Size(%) blank. The,n typical nonlinear parameters are automatically used in experiments. The dependence of the nonlinearity on each scanner is only 20%, and the distance accuracy would be enough for most of the cases.

Preparation of Calibration Sample

Calibrate the scanner using the 17.7 nm lattice constant of Annexin-V 2D crystal.

Flow Overview
①Calibrate at the stage center (determine Prameter1_Piezo Const(nm/V))
②Calibrate at the right, left, top, and bottom ends of the stage (determine Prameter3_StageOffset Rescaling for Scan Size(%))
③Calibrate at a different Scan Size (determine Prameter3_Nonlinear Scaling for Scan Size(%))

Preparation of sample

・Buffer 150mM NaCl, 2mM $CaCl_2$, 10mM HEPES-NaOH.pH7.4
・ Lipid DOPC:DOPS:biotin-capDOPE=70:20:10
1mg/ml in 150mM NaCl, 2mM $CaCl_2$, 10mM HEPES-NaOH.pH7.0
※please put a lid on a mica stage to avoid the liquid evaporation for all the waiting times
・Lipid can be reserved for one month in a freezer at -20℃ .
・Please reserve Annexin-V sample at 4 ℃ (do not freeze)after taking it from the original tube.

❶
▼Sonicate a lipid solution for 5 min.
▼Put a 2µl lipid solution onto a mica stage and wait for 3 min (after cleaving a mica, put a lipid as soon as possible)
▼Add MQW2µl and wait for 3 min (lipid membrane likely to be attached to mica)
▼Add MQW2µl, stir it using a pipette and suck out 2µl solution. Wait for a few min
▼Rinse with MQW20µl × 5 times
▼Rinse with Buffer20µl × 1 times
▼Add 1µM Annexin-V 1µl and stir using a pipette. Wait for 5 min
▼ Rinse with Buffer20µl × 1 times （10µl is also fine. By increasing this step, the number of vacancy of the crystal increases.）

❷
▼Sonicate a lipid solution for 3 min.
▼Put 1µl lipid solution onto a mica stage and wait for 3 min (after cleaving a mica, put a lipid as soon as possible)
▼Add MQW3µl and stir using a pipette and wait for 3 min (lipid membrane likely to be attached to mica)
▼Absorb a solution slightly using a filter paper and put a few µl from MQW20µl. Wait for 30 sec
▼Rinse with remaining MQW
▼Rinse with Buffer20µl × 2 times (to avoid drying, the number of the rinse is reduced)
▼Add 1µM Annexin-V 1µl and stir using a pipette. Wait for 5 min
▼ Rinse with Buffer20µl × 1 times （10µl is also fine. By increasing this step, the number of vacancy of the crystal can be increased.）

❸
▼Sonicate a lipid solution for 3 min.
▼Put 1µl lipid solution onto a mica stage and wait for 3 min (after cleaving a mica, put a lipid as soon as possible)
▼Add MQW3µl and stir using a pipette and wait for 3 min (lipid membrane likely to be attached to mica)
▼Put a few µl from MQW20µl. Wait for 30 sec
▼Rinse with the remaining MQW
▼Rinse with Buffer20µl × 2 times (to avoid drying, the number of the rinse is reduced)
▼Add 1µM Annexin-V 1µl and stir using a pipette. Wait for 5 min
▼Not rinse with Buffer

HS-AFM Observation Condition

Observation setting

UMEX Sample-Scan

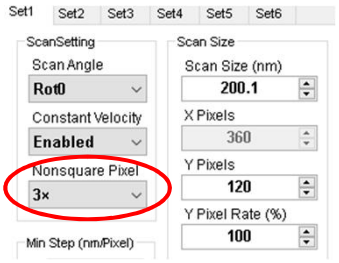
・imaging＞Set1 Nonsquare Pixel → 3×

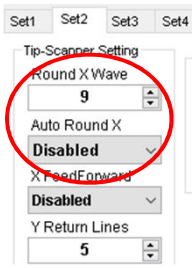
・Scan Size 200x200 nm^2
Pixel 360x120
FrameTime 0.33sec

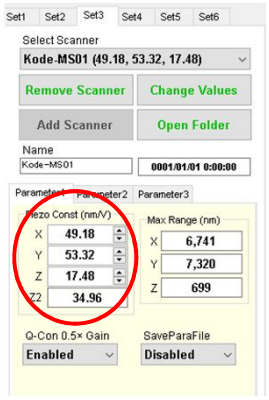
・Imaging＞Set2 Round X Wave → 9
Auto Round X → Disabled

・ imaging＞Set3 Select Scanner

If you have predetermined calibration values, please enter them.







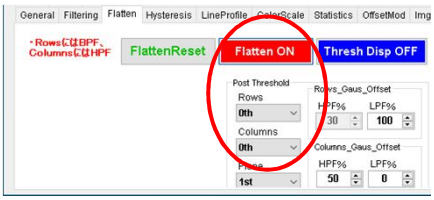
Note
To avoid the tip damage, slightly release the tip using stepping motor when the analysis using viewer and Excel sheet is performed.

UMEX Viewer

・Flatten ON

・Post Threshold Rows 0th Columns 0th

・FFT Filter Thresh 1～2



手順詳細

① Calibration of Parameter1 Piezo Const

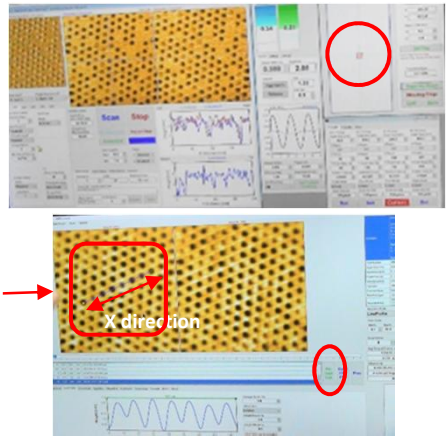
1　Move to the center of the stage. Acquire the AFM images until the thermal drift settles down

2　Load the ASD file using UMEX Viewer. Measure a horizontal distance between a couple of vacancies (6~7 lattices) drawing a line profile. Press Reload button when you load a newly created file in the same folder.

3　Enter the measured distance to an Excel calibration sheet

4　Enter a corrected value to Parameter1 Piezo Const

5　Repeat the 1~4 steps until the Residual Error turns to OK（±0.8%）（copy&paste the previous rows to the new rows in an Excel sheet）



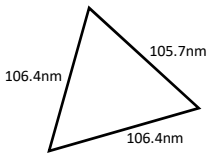
	Current PiezoConst(nm/V)	LineProfile Distance(nm)	Lattice Number	Theoretical Distance(nm)	Corrected PiezoConst(nm/V)	Residual Error
Experimental	16.51	126.3	7	123.9	16.19627078	NG 1.937%
Value	16.3	124.9	7	123.9	16.1694956	NG 0.807%
	16.24	106.4	6	106.2	16.20947368	OK 0.188%

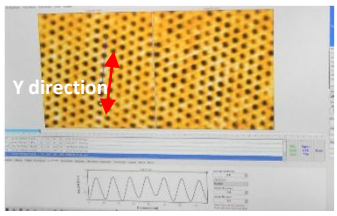
Enter this corrected value to software

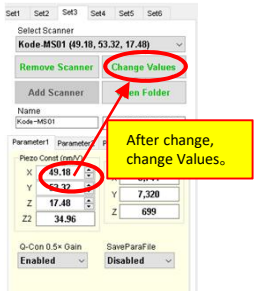
6　Calibrate the Y Piezo Const by doing the same procedure

7　Check and make a note another side of triangular lattice (image is slightly tilted due to the cross-talk between X and Y piezo especially when using a scanner made of duralumin

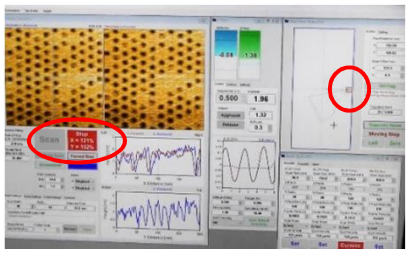
8　Save the parameters by pressing “Change Values” button







② Calibration of Prameter3 StageOffset Rescaling for ScanSize (at the right, left, top, and bottom ends of the stage)



1 Move to the right center of the stage. Acquire the AFM images until the thermal drift settles down. When change the tip position while imaging, correction values appear on the Stop button. Press the Stop and Start button again to correct the scan range.

2 Measure a horizontal distance between a couple of vacancies (6~7 lattices) by drawing a line profile in UMEX Viewer.

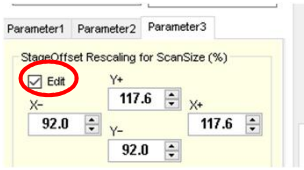
3 Enter the measured distance to an Excel calibration sheet

4 Enter a check to ☐Edit and change the scaling factor.

5 Repeat the 1~4 steps until the Residual Error turns to OK （±0.8％）
（copy&paste the previous rows to the new rows in the Excel sheet）

	Current ScalingFactor(%)	LineProfile Distance(nm)	Lattice Number	Theoretical Distance(nm)	Corrected ScalingFactor(%)	Residual Error
46						
47	実験前の値 → 117.6	121	7	123.9	114.8474576	NG 2.341%
48	115	123.8	7	123.9	114.9071832	OK 0.081%
49	115	107.1	6	106.2	115.9745763	NG 0.847%
50	115.4	124.2	7	123.9	115.6794189	OK 0.242%
51	92	110.7	6	106.2	95.89830508	NG 4.237%
52	94.5	107.5	6	106.2	95.65677966	NG 1.224%
53	95.5	106.4	6	106.2	95.67984934	OK 0.188%
54						

Enter this corrected value to software



※ When the tip position is left or right center of the stage, Y piezo constant does not change from that in the center position. So check Y+ and Y- are correct just roughly.

6 Check that three sides of triangular lattice are the same.

7 Move to the left center of the stage and calibrate X- factor.

8 Move to the top center of the stage and calibrate Y+ factor.

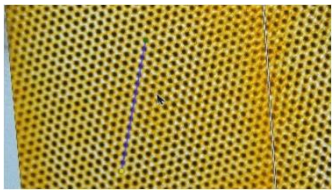
9 Move to the bottom center of the stage and calibrate Y- factor.

10 Press “Change Values” button to save the parameters

③ Calibration of Parameter3_Nonlinear Scaling for Scan Size(%)

0　Move to the center of the stage. Confirm the Piezo const is correct at Scan Size of 200nm.

Scan size 500nm



1　Change the Scan Size to 500nm (Pixel is 120~140). Acquire the AFM images until the thermal drift settles down.

2　Measure a horizontal distance between a couple of vacancies (12~14 lattices)

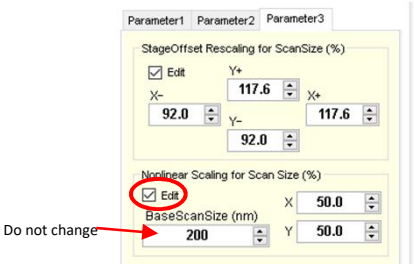
3　Enter the measured distance to an Excel calibration sheet

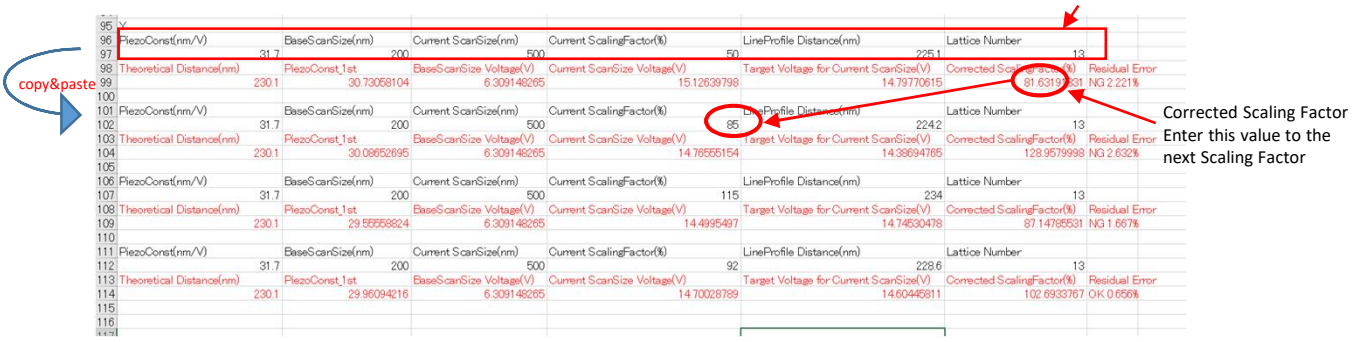
4　Enter a check to ☐Edit and enter the corrected X scaling factor.

5　Repeat the 1~4 steps until the Residual Error turns to OK （±0.8％） （copy&paste the previous rows to the new rows in Excel File）

6　Do not change the Y scaling factor.

7　Press “Change Values” button to save the parameters



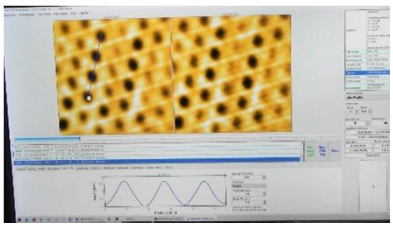


PiezoConst(nm/V)	BaseScanSize(nm)	Current ScanSize(nm)	Current ScalingFactor(%)	LineProfile Distance(nm)	Lattice Number	
31.7	200	500	50	225.1	13	
Theoretical Distance(nm)	PiezoConst_1st	BaseScanSize Voltage(V)	Current ScanSize Voltage(V)	Target Voltage for Current ScanSize(V)	Corrected Scaling Factor(%)	Residual Error
230.1	30.73058104	6.309148265	15.12639798	14.79770615	81.6319231	NG 2.221%
PiezoConst(nm/V)	BaseScanSize(nm)	Current ScanSize(nm)	Current ScalingFactor(%)	LineProfile Distance(nm)	Lattice Number	
31.7	200	500	85	224.2	13	
Theoretical Distance(nm)	PiezoConst_1st	BaseScanSize Voltage(V)	Current ScanSize Voltage(V)	Target Voltage for Current ScanSize(V)	Corrected ScalingFactor(%)	Residual Error
230.1	30.08652695	6.309148265	14.76555154	14.38694765	128.9579998	NG 2.632%
PiezoConst(nm/V)	BaseScanSize(nm)	Current ScanSize(nm)	Current ScalingFactor(%)	LineProfile Distance(nm)	Lattice Number	
31.7	200	500	115	234	13	
Theoretical Distance(nm)	PiezoConst_1st	BaseScanSize Voltage(V)	Current ScanSize Voltage(V)	Target Voltage for Current ScanSize(V)	Corrected ScalingFactor(%)	Residual Error
230.1	29.55558824	6.309148265	14.4995497	14.74530478	87.14785531	NG 1.667%
PiezoConst(nm/V)	BaseScanSize(nm)	Current ScanSize(nm)	Current ScalingFactor(%)	LineProfile Distance(nm)	Lattice Number	
31.7	200	500	92	228.6	13	
Theoretical Distance(nm)	PiezoConst_1st	BaseScanSize Voltage(V)	Current ScanSize Voltage(V)	Target Voltage for Current ScanSize(V)	Corrected ScalingFactor(%)	Residual Error
230.1	29.96094216	6.309148265	14.70028789	14.60445811	102.6933767	OK 0.656%

◎Fill in the form of the calibration file

※To calibrate the Z piezo, another sample, e.g., an actin filament sample must be used

Scan size 100nm



Calibration of ultra large scanners

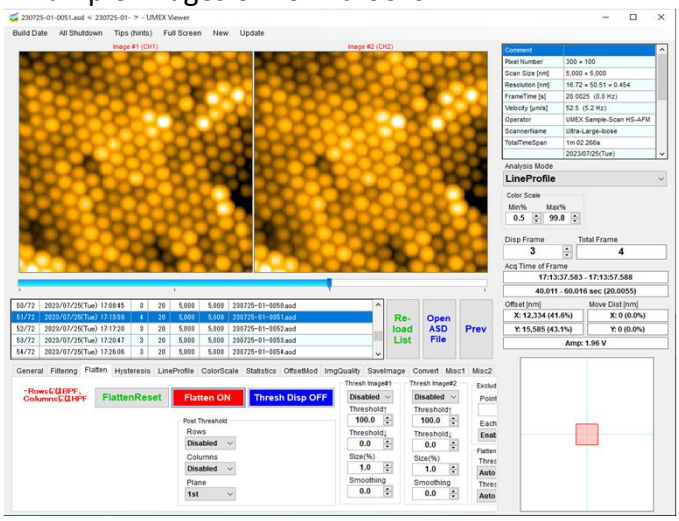
[When using 350nm beads (recommended)]

① Dilute the 350nm polystyrene bead stock solution to 1/10 with MQ.
*Shake the stock solution well and then transfer it to a tube.
②Apply ultrasonic for 1 min.
③ Cleave mica, put 0.5 µl of solution on it, and let it dry naturally. (several minutes)
④ Vacuum for 30 minutes.
⑤ Observe and calibrate with SA-Buffer (150mM NaCl, 2mM $CaCl_2$, 10mM HEPES-NaOH.pH7.4).
*The calibration procedure follows "Nonlinear calibration using annexin."
*Set Base Scan Size to 5,000 nm.
*Make the Q value control circuit ultra wide.
*It is best to approach the area where the bead is densely packed.

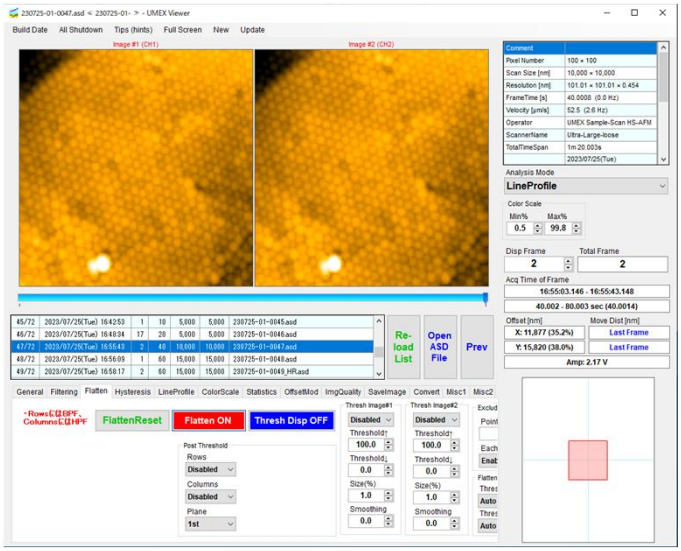
Vacuuming
(within 225 drafts)

The beads used ：
Polybead®
Polystyrene 0.35
Microspheres
Cat # 07306
Polysciences,Inc.

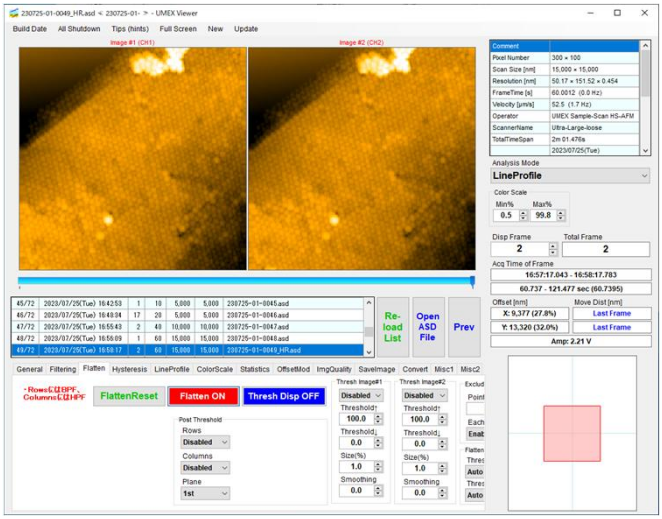
・Example images of how it looks



ScanSize_5,000nm



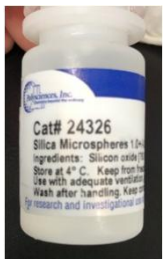
ScanSize_10,000nm



ScanSize_15,000nm

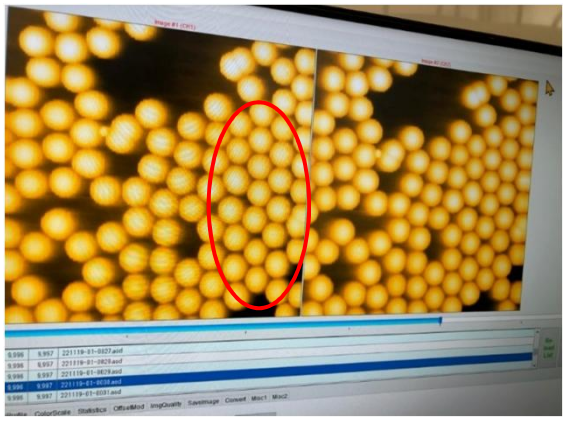
[When using 1 µm beads (optional)]

① Dilute 1 µm-Silica beads stock solution to 1/100 with MQ.
*Shake the stock solution well and then transfer it to a tube.
② Cleave mica, place 2 µl of 1/10000 APTES, and wait 3 min.
③Rinse with 60µl MQ.
④ Ultrasonicate the dilute bead solution for 1 min.
⑤Put 10µl of bead solution on mica. (while rinsing)
⑥ Dry naturally. (Leave it until it dries out. It will take more than an hour.)
⑦ Vacuum for 1 hour.
⑧ Observe and calibrate with MQ.
*Set Base Scan Size to 10000.
*Other methods are the same as when using 350nm beads.

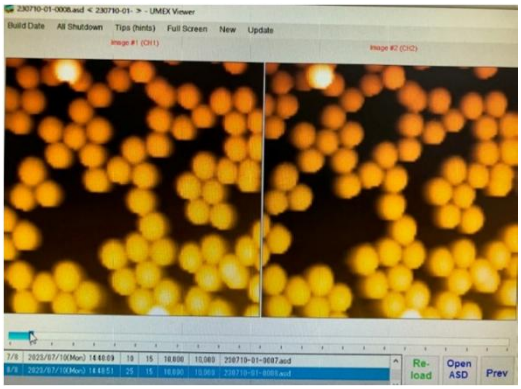


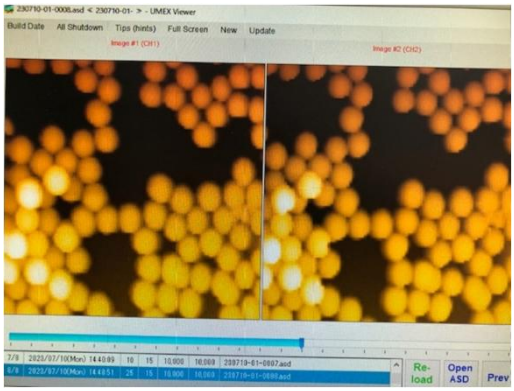
The beads used ：
Polybead®
SillicaMicrospheres
1.0+/-0.05micron
Microspheres
Cat # 24326
Polysciences,Inc.

・Reference image of how it looks



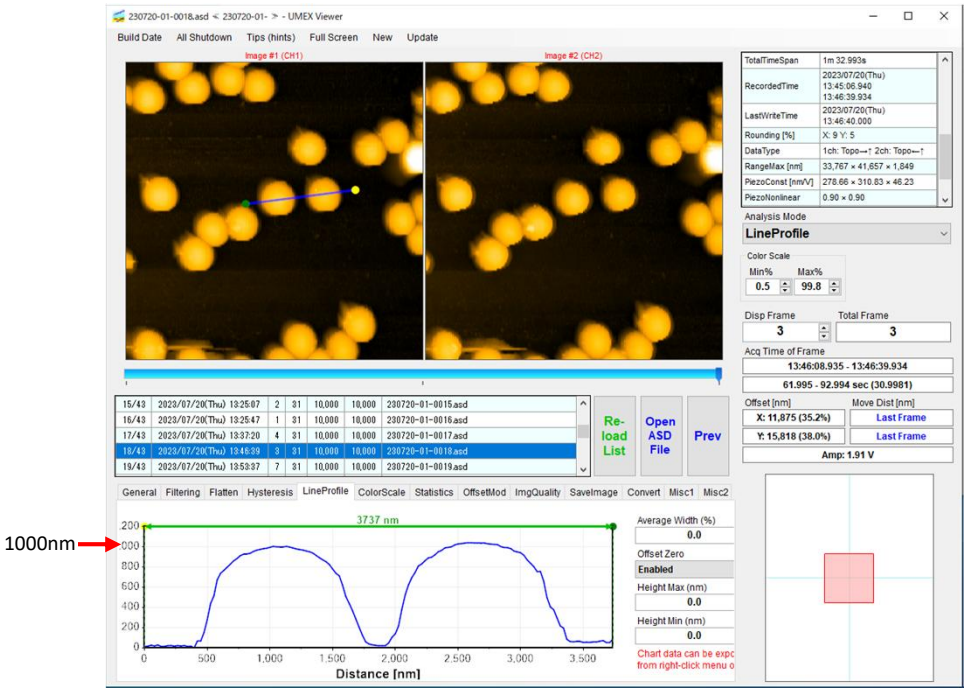
Calibrate XY by looking for the area circled with a circle that is densely packed.



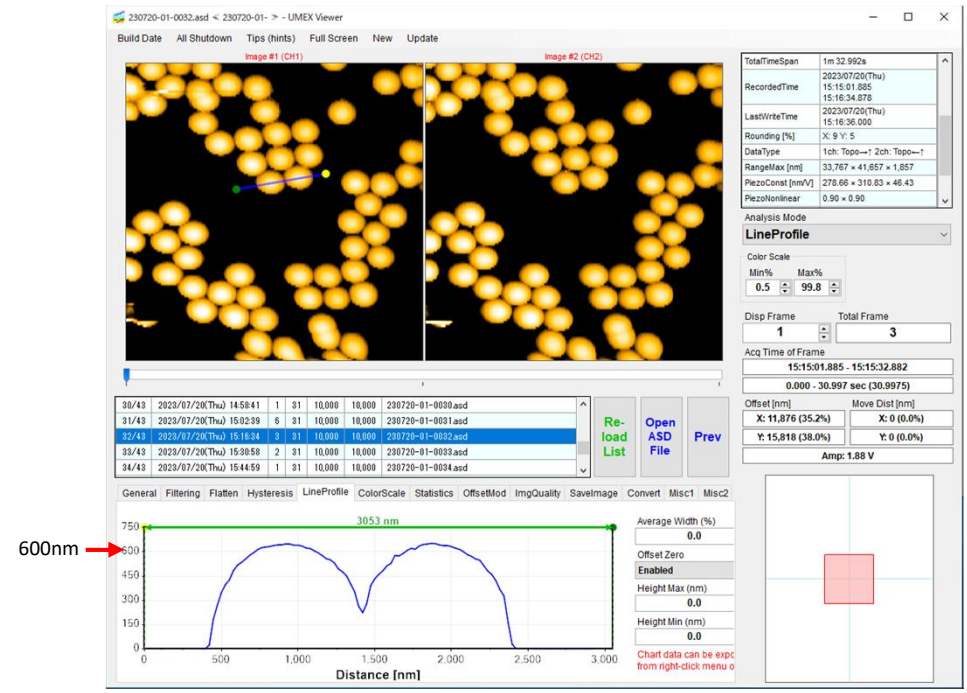


Not crowded.
Sparse.

[Reference: Z calibration]
Z calibration was performed using sparse 1 µm beads.



The tip has reached the substrate. Height 1000nm.



The tip does not reach the substrate.
Please note that even with the same sample and similar Piezo Const, the height is 600 nm, which is a completely different result.