Non-Contact Tapping SOP

Dimension FastScan Programmed Move Survey Scans of DNA Nanostructures

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Introduction to Non-Contact Mode AFM

Amplitude modulation (AM) non-contact mode can be thought of as an extremely "soft" or "gentle" version of normal tapping (intermittent contact or AC) mode AFM. By carefully choosing a drive frequency that is slightly higher than the free space natural resonance frequency of the probe and employing an extremely small (~1-3 nm) oscillation amplitude, it is possible to keep the probe entirely within the attractive regime of the tip-sample potential throughout the engage process and imaging. As a result, the probe never comes into "contact" with the surface being imaged. This enables accurate Z (height) measurements, minimizes damage to the sample, and keeps the AFM probe sharp, maintaining lateral resolution over large scan areas and throughout long periods (hours) of continuous scanning. By leveraging the superior capabilities of the Bruker FastScan AFM head, NanoScope V controller, and FastScan-A probes, it is possible to rapidly obtain large area (100-400 μm^2) images with extremely high lateral resolution (2-4 nm). When combined with the Dimension system's Programmed Move capabilities, even larger area high resolution images can be obtained by stitching together multiple overlapping high resolution images. This SOP provides step-by-step instructions, best practices, and tips regarding how to set up and carry out non-contact mode imaging to acquire large area high resolution survey scan images. In addition, it describes the necessary procedures and precautions for conducting automated Programmed Move imaging in non-contact mode.

Setting up for Success: Mounting the Probe

While mounting the AFM probe may seem extremely simple and elementary, this step is in fact essential to ensuring a good tuning curve with a sufficiently high Q/low drive amplitude for successful non-contact mode imaging. For rapid acquisition of high resolution non-contact mode images, it is standard to use Bruker's FastScan-A probes due to their high natural resonance frequency (> 1 MHz). Note that these probes will only work with the Dimension FastScan head because of the need for an equally fast dither piezo and feedback electronics.

Probe Mounting Procedure

1. Obtain a new FastScan-A probe and remove the FastScan probe holder (also known as the FastScan Z scanner, **Figure 1**) from its protective Pelican case.

Figure 1. The FastScan probe holder (also known as the FastScan Z scanner) and probe holder mounting block for the Dimension FastScan AFM.

2. Using tweezers, gently place the probe in the slot on the probe holder. Take care not to squeeze the probe too tightly with the tweezers, as that may generate small shards of SiN that can lodge between the probe and the probe holder, leading to problems when tuning. Gently swing the spring clip into the locked position to secure the probe (**Figure 2**).

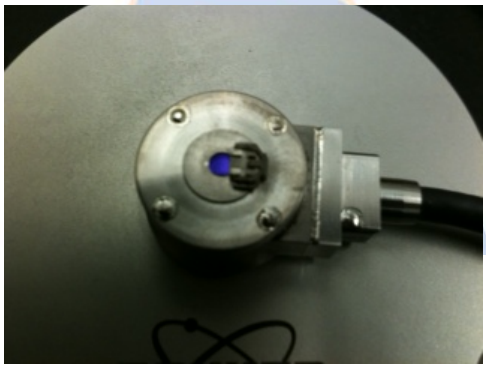

Figure 2. FastScan probe holder with spring clip secured in the locked position.

3. Use the optical microscope to view the probe's alignment within the slot on the probe holder. Carefully align the probe so that the substrate does not directly contact any of the three edges of the channel and is roughly equidistant from all three edges. Take care to only move the probe substrate a small distance off the back edge of the probe holder's groove. If the probe is either too far from or too close to the back edge of the groove, the tuning curve will be poor.
4. Make sure the probe holder is clean and free of debris. As mentioned above, the probe can generate SiN debris if it's not handled with absolute care. Debris lodged under the substrate will negatively impact the tune. If debris is visible when viewing under the microscope, remove the probe and gently clean the probe holder with a bulb blower, a gentle stream of ultrapure dry nitrogen, or a lens tissue wetted with spectroscopic grade ethanol. Be careful with the optical window and probe holder clip while cleaning; they can easily be damaged.

5. After ensuring the probe is aligned properly in the desired location and the probe holder is clean/free of debris, (re)close the spring clip to lock the probe firmly in place.

Tuning and Engaging the Probe

The fundamental difference between non-contact and normal tapping mode AFM is how the probe is tuned (i.e., drive frequency and target amplitude). As mentioned in the introduction, in non-contact mode an extremely low drive amplitude is chosen to achieve a target oscillation amplitude of ~1-3 nm. In addition, a drive frequency slightly to the right of the peak of the free space tuning curve (i.e., a frequency higher than the probe's natural resonance frequency, $f > f_0$) is chosen to ensure the probe engages in the attractive regime of van der Waals forces (<10 nm from the surface). This is in contrast to normal tapping mode, wherein a larger oscillation amplitude (~50-200 nm) and an offset of ~5% to the left of the natural resonance frequency (i.e. to lower frequency, $f < f_0$) is typically employed so that the probe engages and operates in the repulsive regime. Following are instructions regarding how to tune the probe, as well as typical values of various parameters for non-contact tuning.

Non-Contact Tuning Procedure

1. After loading the probe and ensuring the probe holder is free of debris (see Probe Mounting Procedure), install the Z scanner on the FastScan AFM. Ensure the probe type selected in software matches the one you are using.

WARNING: Prior to installing the Z scanner, ensure that the High Voltage indicator light on the FastScan head is not illuminated. Failure to do so could result in electrical shock and/or damage to the AFM head. If the NanoScope software is already open, you should always use the "Change Scanner" or "Change Probe" option in software, as these automatically disable the high voltage and alert the software that you are installing the Z scanner and/or a new probe.

a) Select "Setup" in the Workflow Toolbar column. Click "Change Probe" (**Figure 3**).
b) The "Change Probe" dialog box will appear. Click the "Move to probe loading position" icon (**Figure 4**). This will move the stage away from the head so that the probe holder can be safely loaded.
c) Select the type of probe loaded (e.g., FastScan-A) from the "New Probe of type" dropdown menu (**Figure 4**).
d) Ensure the laser spot size selected via the switch on the right side of the FastScan heads matches the size "recommended" in software for your chosen probe type (**Figure 3** and **Figure 4**).

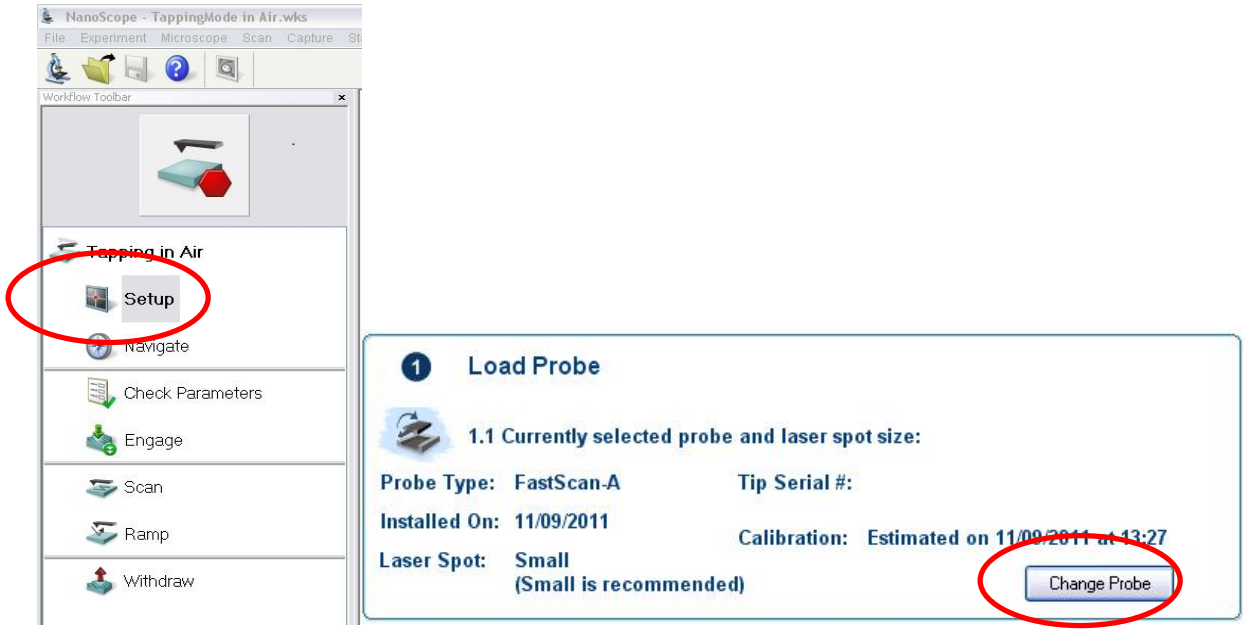

Figure 3. Workflow Toolbar column with Setup circled (left). Within Setup, Load Probe option with the Change Probe button circled (right).

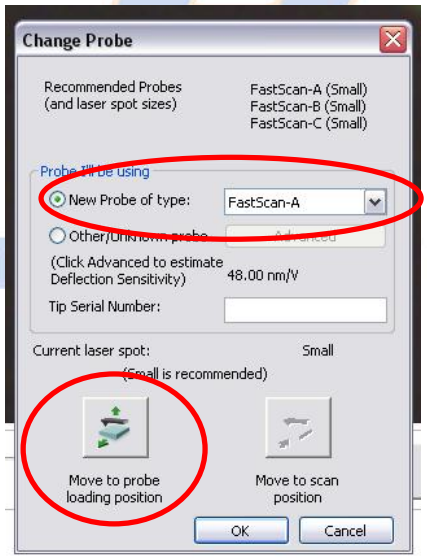

Figure 4. The Change Probe dialog box.

2. After the Z scanner has been mounted and the probe type selected, align the laser in the center of the forward end of the cantilever. The closer the laser is to the apex of the cantilever, the longer the lever arm and the more sensitive the AFM will be to deflection of the probe.

   NOTE: **You should periodically recheck the laser alignment as the laser spot can drift over time, drastically reducing the sum.**

3. While in Setup, click on Manual Tune.
   a) Set the Auto Tune start and end frequencies to the range listed on the probe box (e.g., 800-2000 kHz for a FastScan-A).
   b) Set the Target Amplitude to 200 mV. This is a good default starting value to enable comparisons between different probes and evaluate the quality of the probe/tune.
   c) Decrease the Peak offset to 2%.
4. Click Auto Tune to sweep the frequency range and obtain a tuning curve for your probe. The tuning curve (blue trace, **Figure 5**) should roughly overlap the fast thermal tune data (pink crosses and corresponding fit, **Figure 5**).
5. Look at the resultant Drive Amplitude (**Figure 5**). For non-contact mode to work well it should be ≤***75 mV*** (the lower, the better). If it is **>75 mV**, your probe is likely misaligned in the holder or touching some debris. Alternatively, it may be a bad probe (less likely). Try realigning the probe, cleaning the probe holder, and/or replacing the probe. Repeat until a sufficiently low Drive Amplitude is achieved.

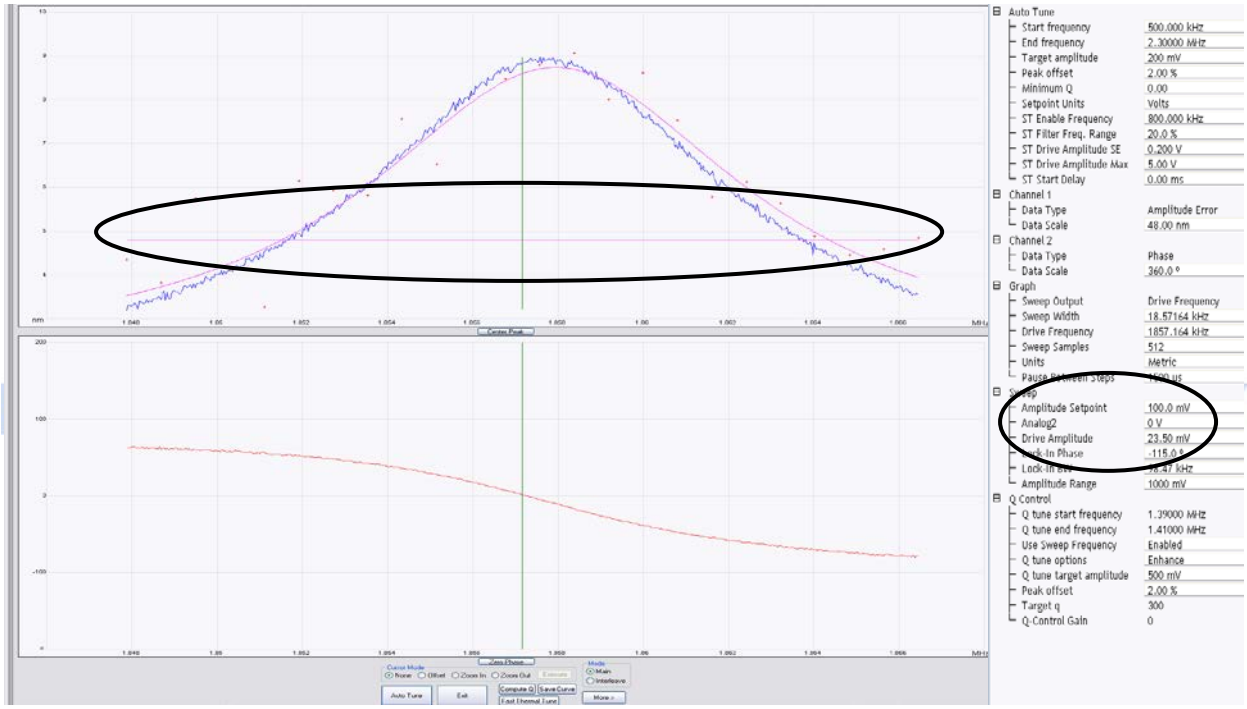

Figure 5. Example of an ideal FastScan-A manual tune. The Drive Amplitude and Amplitude Setpoint parameters are circled in black, as is the pink line indicating the current Amplitude Setpoint value.

6. Set the Target Amplitude (peak of the tuning curve) to ~1-3 nm. Note the corresponding Drive Amplitude; it should be ***<30 mV***.
7. Select the Offset button in the Manual Tune window. Grab the green vertical cursor line and move it to select a Drive Frequency to the right of the peak (i.e., a higher frequency, $f > f_0$). Choose a frequency approximately midway between the peak of the tuning curve and its right hand

inflection point. The amplitude at this frequency will be ~80% of the peak amplitude. Click Execute to offset to this frequency.

8. Once the Drive Amplitude and Frequency are set, manually adjust the Amplitude Setpoint until the horizontal pink cursor line demarcating it intersects the tuning curve (blue trace) at the Drive Frequency (green vertical line) chosen in Step 7 above (**Figure 6**).

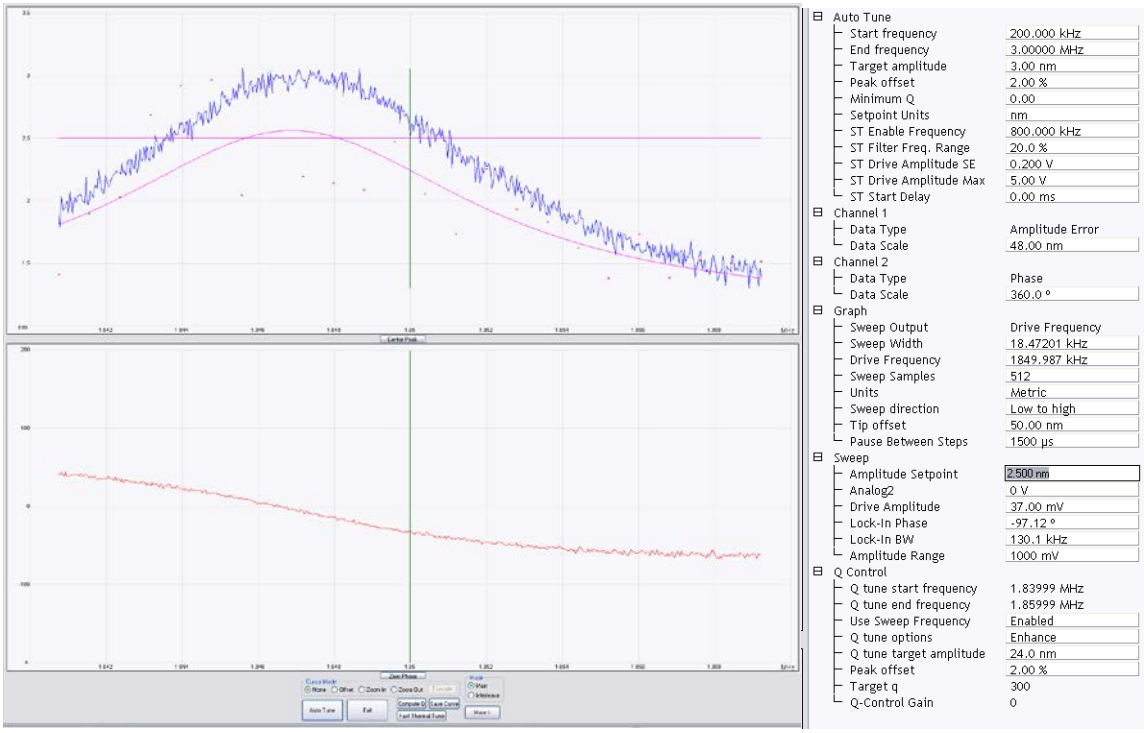

Figure 6. Tuning curve after decreasing the Target Amplitude, selecting the frequency Offset, and setting the Amplitude Setpoint.

9. Now that the probe is properly tuned, switch to the Navigate window in the Workflow Toolbar and move to the desired scan location above your sample.
10. If this is *not* an automated Programmed Move (i.e., you will be present during the engage process), go to Engage Settings (under the Microscope dropdown menu heading) and set the ***Engage Setpoint*** to ***1.00-1.10*** and the ***Engage Type*** to ***Standard***. This will ensure that the probe doesn't dull immediately by engaging the surface too hard (larger Engage Setpoint values correspond to a "gentler" engage).

    NOTE: False engages are more likely for Engage Setpoint >1.0. Thus an Engage Setpoint of 0.9 is recommended if you will be conducting an automated Programmed Moves as noted in the appropriate section at the end of this SOP.

11. After checking to ensure that the Sample Clearance and SPM Safety Margin are set to 1,000 µm and 100 µm, respectively, exit the Engage Settings dialog box. Prepare to engage as in normal

tapping mode, using either Focus on the Sample or Focus on the Tip Reflection (as appropriate to your sample) to align your probe at the correct height above the surface.

12. Switch to the Check Parameters window in the Workflow Toolbar. Select the "Expanded" view (red puzzle pieces on the toolbar menu). Set ***Scan Size*** and ***X*** and ***Y Offsets*** to ***0*** in the parameter list.
13. Click Engage in the window in the Workflow Toolbar. As soon as the computer beeps to indicate the tip has engaged, click on the tuning fork icon in the toolbar to retune near the surface. Use an ***Offset*** of ***50 nm*** and check to make sure the tuning curve hasn't changed significantly. Make adjustments as necessary.
14. After retuning, the Z piezo meter bar graph may be red. This means that the AFM has false engaged and the probe is not tracking the surface. If this is the case, slowly lower the Amplitude Setpoint, ~1 mV at a time, until the Z piezo meter just turns green. Otherwise continue to the next step.
15. Halve the Lock-In Bandwidth to improve the signal to noise ratio.
16. Gradually lower the Amplitude Setpoint (~0.5-2 mV or ~0.1 nm steps) until the probe just begins tracking the surface. The voltage indicated on the Z piezo meter should stop changing significantly at this point.
17. Once the probe is just barely tracking the surface, lower the Amplitude Setpoint another ~1-2 mV to ensure the probe will track well. Lower it further to improve tracking, but be warned that this may cause the tip to dull more quickly.

Setting up Survey Scans

For the most part, setting up survey scans is relatively straightforward once you have properly tuned the probe and engaged the surface. As noted in the introduction, non-contact mode is particularly useful for conducting large area (100-400 μm^2) scans at lateral resolutions close to the limit imposed by the probe's tip geometry (~2-4 nm) and the NanoScope V controller's maximum file size (5120 x 5120 data points). The following instructions will provide some guidance as to typical parameter values for high-resolution non-contact survey scans of DNA nanostructures on mica or high optical quality glass slides.

Typical Parameter Values

Scan Size

- Scan size is dictated by the needs of the image recipient and the number density of DNA nanostructures on the surface.
- For quick surveys designed to simply check for successful formation/verify the structure of DNA nanostructures, a **5 µm** scan size is recommended, assuming a relatively high sample density.
- For statistical surveys, **10-15 µm** scan sizes are recommended. However, the total number of images needed will depend on the DNA nanostructure density and the number of structures necessary for statistical considerations.

- The largest recommended scan size is **~20-22 µm**. Above this size, the image resolution (due to max of **5120 Samples/Line**) will be worse than the tip radius of curvature (nominally 5 nm for FastScan-A probes, but in practice typically closer to 2 nm) and the DNA nanostructures will begin to appear pixilated. Furthermore, more empty mica will be imaged, which can result in faster tip wear.

Samples/Line and Lines/Scan

- These are determined by the combination of the Scan Size and the lateral resolution needed. If fine details of structure are needed, then more Samples/Line and Lines/Scan will obviously be necessary. The max value for Samples/Line and Lines/Scan is 5120 (assuming a square image with an Aspect Ratio of 1.0).
- Recall that ultimately the resolution is limited by the tip geometry or the pixel size, whichever is greater. For FastScan-A probes, the nominal tip radius of curvature is approximately 5 nm, making 5 nm the lower limit of resolution. However, this value is based upon the idea that the end of a tip will be dulled to ~5 nm upon engaging the surface in normal tapping mode. When engaging and operating in non-contact mode, the actual tip radius is typically closer to 2 nm.
- For large scans (10-20 µm square), standard settings for ***Samples/Line are between 3000-5120.*** Note that the Lines/Scan will adjust automatically based on the set Aspect Ratio (typically 1.0).
- The higher your resolution, the more noise is introduced into your image at a given scan speed (less signal averaging due to fewer surface interactions per point/pixel). Choose your resolution based on the purpose of image. If you just need to count nanostructures, use a lower resolution. If the image is for publication or presentation, go with a higher resolution.

Scan Rate

- Scan Rate is paramount in determining how well your probe tracks the sample surface: slower scan rates will result in better tracking. However, scan rate also determines how long your scan will take to complete.
- Changing the Scan Rate changes the Tip Velocity. Tip Velocity is really the key parameter in obtaining a high quality image. Tip Velocity is determined by the product of the Scan Rate and the Scan Size. The lower the Tip Velocity, the better the tracking. However, lower tip velocities mean it will take longer for the scan to finish.
- The FastScan head has the ability to scan at very high rates, allowing for relatively quick imaging/short scan times. However, at very high scan rates (above ~4Hz) small details (such as DNA nanotubes and Au nanoparticles) in large scans become distorted (smeared or streaked) because the tip velocity is too high, negatively impacting tracking.
- For smaller scans (< 2 µm on a side), "good" images can be obtained using ***Scan Rates*** as high as ***2-5 Hz (Tip Velocity = 8-20 µm/s)***.
- For larger scans, Scan Rate and acquisition time must be balanced, based on what is needed from the image.
    - For quick surveys where slight image distortion is not critical, a ***Scan Rate*** of ***1.5-3 Hz***, corresponding to a **Tip Velocity** of **~60 µm/s**, can be used.

  - For high quality, low distortion large area scans, a ***Scan Rate of 0.2-1 Hz (Tip Velocity = 8-20 µm/s)*** must be used.
- Overall, there are two general rules of thumb for Scan Rates:
  - *The smaller the scan, the faster the scan rate can be, and vice versa. (In the end, what matters is the Tip Velocity, which is the product of the Scan Rate and twice the Scan Size.)*
  - *The slower the Scan Rate, the better the tracking (provided the I and P Gains are set correctly, as described below).*

Integral and Proportional Gains

- The I and P (Integral and Proportional) Gains are used to control and adjust how much/how quickly the probe responds to changes in sample height/topography. The higher the gains, the greater the feedback response to a given change in topography. Thus the gains can be used to improve sample tracking and reduce distortion of features in your image.
- Integral Gain has a larger effect than Proportional Gain.
- As the gains are increased, measurement noise is amplified; eventually the added noise outweighs the improved tracking. The noise can be viewed on the Amplitude Error channel.
- As a general rule, the lower the gains that can be used while still obtaining good tracking, the better the measurement/image obtained (due to lower noise).
- You should adjust the gains based on the quality of sample tracking you need.
- To fully resolve fine details such as Au NPs (nanoparticles), QDs (quantum dots), and DNA nanostructures, you should increase both gains until any streaking or distortion of features are gone (or at least minimized).
- How much gain is needed depends on both the Scan Rate and Scan Size. Faster and/or larger scans will usually require higher gains, while slower/smaller scans will require less gain to properly track the sample surface.
- Optimal gains will vary between samples (and potentially even between scans of different areas of the same sample).
- For large area, high resolution non-contact scans:
  - ***Integral gain is usually in the 1.5-3.0 range (but is Scan Rate dependent).***
  - ***Proportional gain is usually in the 7.00-10.00 range and ~2-10x the Integral Gain.***

Z limit

- This setting is used to control height resolution and inherent noise in the measurement.
- The lower the Z limit, the lower the noise and the greater the height resolution.
- However, reducing the Z limit imposes a restriction on how much the Z piezo can move.

Caution: If a feature in your scan exceeds the Z limit, it can stress the Z piezo to the point of damaging it (or the probe). Make sure the Z limit is set so that all features present in the scan can be imaged without damage, taking into account sample tip/tilt and thermal drift of the Z piezo with time as the electronics and sample chamber heat up.

- As noted above, the Z piezo is subject to upward thermal drift. This is particularly pronounced if the acoustic hood is shut, preventing convective cooling. Therefore if the Z limit is very low and the scan time is long, the tip will eventually lose track of the surface.
- Also as noted above, the necessary Z piezo range can also be affected by how flat the substrate is glued on the puck (i.e., how perpendicular the sample surface is to the probe). As a general rule, the larger the Scan Size, the larger your Z limit will need to be due to sample tip and tilt.
- The relative flatness of the sample can be seen on the height channel. Change *RT Plane Fit* to *None* and observe the change in height across the sample at its top and bottom using the Scan Down and Scan Up features.
- For large area, high resolution non-contact scans on relatively flat samples with limited (i.e., low height) topographical variation (e.g., DNA nanostructure surveys) the ***Z limit should be ~1.5-2.75 µm.***
- Only use low Z limits (≤1 µm) when the scan is relatively small/quick and it is certain that the sample is relatively flat (limited tip/tilt) and has no large topographical features.

Setting up Programmed Moves

The Programmed Move feature allows users to automate imaging of multiple locations on an individual sample or multiple samples (with the optional multi-sample chuck), potentially even in the user's absence. The following is provided to help users set up Programmed Moves, but **this should not be done without first consulting the lab manager** about the imaging needed to ensure a Programmed Move is the best option. This is because unattended Programmed Moves are risky; piezoelectrics can easily be overextended/retracted and damaged during prolonged imaging, thereby necessitating expensive repairs, if the following steps and precautions are not rigorously followed.

Before setting up a Programmed Move, first determine the desired scan parameters for imaging (see the Typical Parameter Values section above). Change the Engage Setpoint to 0.9 (rather than the more typical 1.0-1.1) to ensure the probe will engage the surface every time. Engage the surface and check that the probe engages when the stepper motor is at ~100 µm (if using the Standard Engage with an SPM Safety Margin of 100 µm). Optimize the I and P gains for your desired Scan Size and Scan Rate (i.e., Tip Velocity). Withdraw from the surface. You are now ready to set up the Programmed Move.

Programmed Move Procedure

1. Once you have completed the steps outlined in the paragraph above, select Navigate from the Workflow Toolbar column. If desired, use Set References (under the Stage dropdown menu) to Mark Point as Origin.
2. Select Programmed Move from the Stage dropdown menu. A new box will appear beneath the optical image of the sample.
3. Select New Program. Name your Programmed Move and save it in the same directory and folder where your captured images will be saved.

4. Select Add Step to add the current (X, Y, Z) location to the Programmed Move file. Make sure the sample surface is in focus!
5. Navigate to the next location to image. Be sure to take into account the mechanical backlash/hysteresis of the stage motor (up to ~2 µm), especially if attempting to obtain overlapping images. Refocus on the surface! Engage this location, and ensure that the same parameters as used in the previous location are sufficient to image the new location.

   NOTE: **Users will not be able to change any parameters once the Programmed Move is underway; all images must use the same scan parameters (Scan Size, Scan Rate, Gains, etc.).**

6. Withdraw the probe. Return to the Navigate window and select Add Step to add the second location.
7. Continue this process for each location you wish to image with the Programmed Move.

   NOTE: **Make sure to refocus on the surface at each location; the Z value for each (X, Y) location should be different.**

8. Prior to running the Programmed Move, make sure all scan parameters are set at the desired values (including scan size – should not be 0). As noted above, the same values will be used for ALL images in the program. Also be sure to set up the file nomenclature and capture folder for saving images prior to running the program.
9. Click the Run button in the Programmed Move window. You MUST run the program through the Programmed Move window in the Navigate screen; **DO NOT SELECT ENGAGE** – the program will automatically move to the first location and engage on its own when you click run.

   NOTE: **After starting the Programmed Move, stay and observe AT LEAST the engage and imaging of the first location, along with the engage of the second location before leaving the AFM unattended. The AFM will automatically capture and save all images, then withdraw at the conclusion of the Programmed Move.**

Conclusion/Disclaimer

By following the above general guidelines, experienced/qualified AFM users should be able to obtain excellent quality high resolution non-contact mode AFM images. Keep in mind that the precise optimal values of the various scan parameters will depend on the individual sample/scan and desired image quality. It therefore requires practice to become proficient and obtain the best images possible.

This document is intended as a guide to help experienced AFM users new to non-contact mode AFM. Users should always take caution to preserve and protect the AFM from unnecessary damage or stress. Most importantly, if a user is unsure of how to perform a specific task in non-contact mode they should **ALWAYS ASK FOR HELP OR SEEK ASSISTANCE FROM THE SSL STAFF AND/OR A MORE EXPERIENCED USER BEFORE ATTEMPTING ANYTHING!**