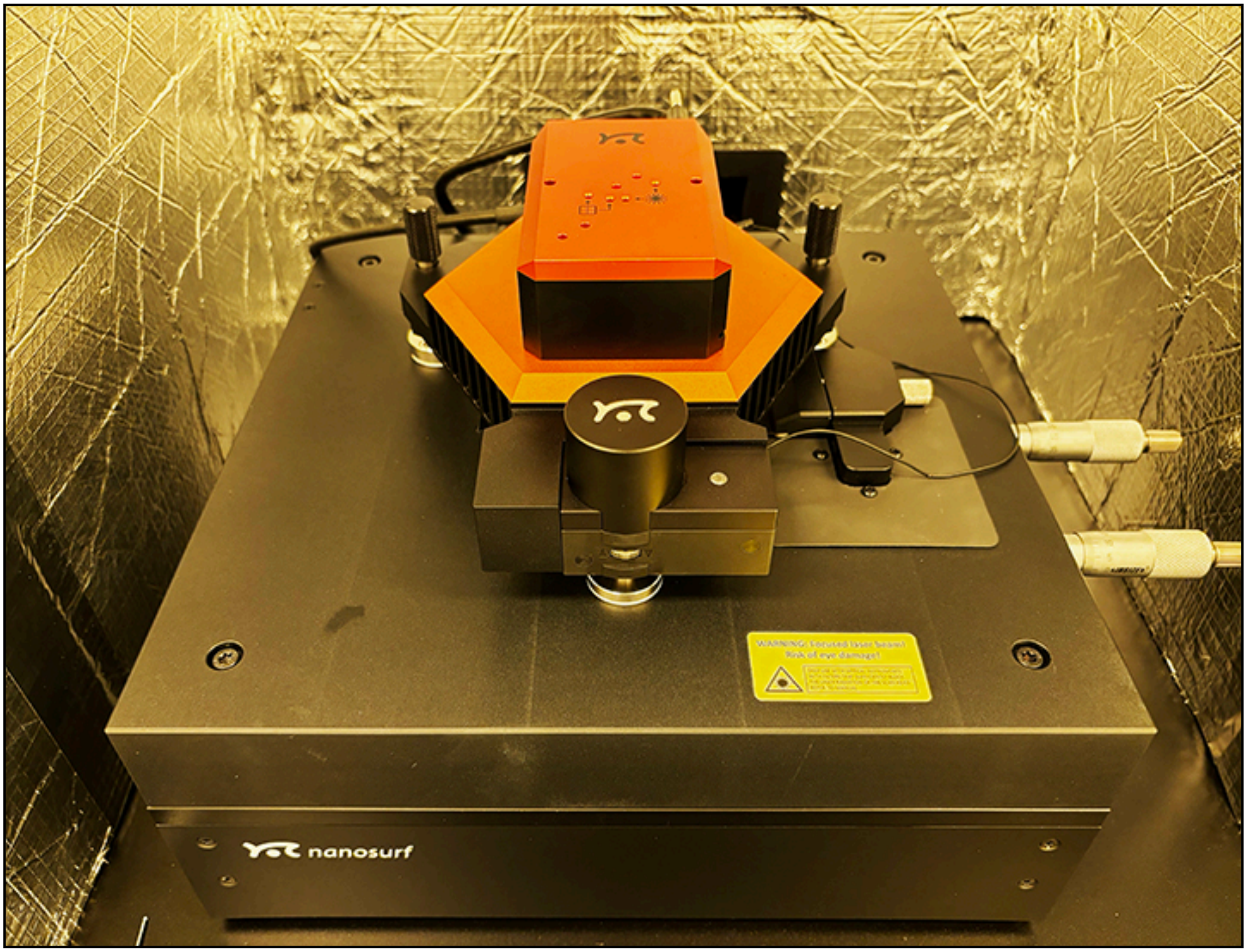
Standard Operating Procedure for the Nanosurf FlexAFM

Version: 03 SEP 2025



Read and understand this SOP completely before attempting to operate this tool. Training by KUNF personnel is required before use.

For detailed descriptions of the hardware and software elements, refer to the FlexAFM Operating Instructions available in the Help panel of the View tab.

1. **General Information and Precautions**
   a. The FlexAFM is configured for basic imaging and spectroscopy, as well as small-area lithography.
      i. The following **Imaging Modes** are available:
         - Dynamic Force (“Tapping”) — Dynamic mode is a technique that images the sample topography by scanning the surface with an oscillating cantilever. Changes in the dynamic behavior of the cantilever are detected by measuring changes in its vibration amplitude when it is excited with a sinusoidal signal with a frequency close to the free resonance frequency of the cantilever.
         - Phase Contrast — The phase contrast mode is an extension of the dynamic force mode. Therefore, the same cantilevers can be used as for dynamic force mode. In addition to the vibration amplitude, the phase shift between the cantilever vibration and a reference signal is measured. This phase shift changes when the resonance characteristic of the cantilever changes due to changes in the tip–sample interaction.
         - Static Force (“Contact”) — Static mode is a technique that can obtain topographic information on a wide range of sample types. As the scanner traces the tip across the sample, the repulsive contact forces between tip and sample cause the cantilever to bend to accommodate for changes in topography. Deflection of the cantilever is used as the error signal for the Z-controller for feedback.
         - Force Modulation — The force modulation mode is an extension of the static force mode. The static force acting on the cantilever is still used to produce a topography image of the sample. Simultaneously, the cantilever is excited and the resulting vibration amplitude measured. Thus, material contrast is highlighted when there is a significant difference in the stiffness of the tip–sample contact of these materials. Consult *Appendix A: Parameters for Static Mode Extensions* for instructions.
         - Spreading Resistance — The spreading resistance mode is an extension of the static force mode. The static force acting on the cantilever is used to produce a topography image of the sample. Simultaneously, changes in the spreading resistance from the cantilever tip to a ground contact on a sample can be imaged by measuring changes in tip current while a fixed voltage is applied to the tip. Consult *Appendix A: Parameters for Static Mode Extensions* for instructions.
         - Lateral Force — The lateral force mode is an extension of the static force mode. Lateral force mode measures lateral deflections (twisting) of the cantilever that arise from forces working on the cantilever parallel to the plane of the sample surface. Lateral force mode is useful for imaging variations in surface friction that can arise from inhomogeneity in surface material, and also for obtaining edge-enhanced images of any surface. Consult *Appendix A: Parameters for Static Mode Extensions* for instructions.
      ii. For a description of the available **Spectroscopy Modes** and instructions, see *Appendix B: FlexAFM Spectroscopy Modes.*
      iii. For a description of the **Advanced Lithography Mode** and instructions, see *Appendix C: Advanced Lithography Mode*.

b. Probe Tip Holder, Scan Head and Motorized Stage:

i. The probe tip holder is mounted to a piezoelectric motor in the scan head, which has a maximum X-Y scan range of 100 μm and a maximum Z range of 10 μm.

ii. The scan head height from the sample holder can be adjusted to accommodate samples of different thickness. Three 100 TPI adjusters (one motorized) provide a maximum scan head Z travel of 5 mm.

iii. The sample holder sits on a motorized stage with maximum X-Y travel of 20 mm.

c. Probe Tips:

i. The FlexAFM is designed to hold standard AFM probe tip assemblies with alignment grooves. *Probe tips without alignment grooves will not work with the supplied tip holder*. See Figure 1 for details.

ii. Generally:

- Shorter and stiffer cantilevers are used for Dynamic Force and Phase Contrast modes.
- Longer and more flexible cantilevers are used for the Static force modes.
- Conductive tips are required for Spreading Resistance Mode.

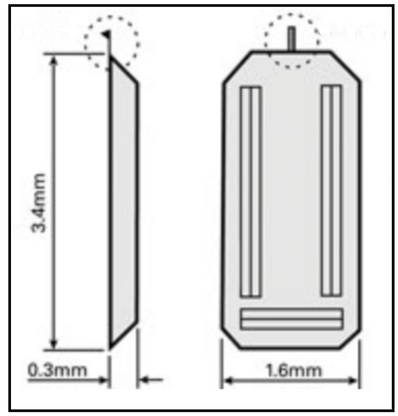


Fig. 1

iii. Users are encouraged to purchase their own probe tips in bulk.

iv. The maximum allowable probe tip resonance frequency is 750 kHz.

v. Probe tips for tapping mode imaging are available for individual purchase.

vi. A grounding strap is provided for those working with ESD-sensitive tips.

d. Laser and Sensor Alignment:

i. Both the laser and the sensor in the scan head must be readjusted when the probe tip is changed.

ii. The adjusting screws for both the laser and sensor are extremely sensitive. Use great care when inserting and turning the driver.

iii. Do not use any tool other than the provided driver to adjust the laser and sensor.

USE CAUTION WHEN ADJUSTING THE LASER AND SENSOR.
Damage to the laser or sensor adjustors will require that the scan head be returned to the manufacturer for service.

e. Sample Holding:

i. The FlexAFM sample stage is designed to accommodate a variety of samples, with clamps for slides or wafers, and a magnet for metal specimen discs.

ii. 15 mm specimen discs are available for free upon request. Alternate specimen or sample supports must be provided by the user.

iii. All samples must be clean, dry and dust free prior to analysis.

iv. Consult *Appendix D: Measuring Liquid Samples* if measuring in a liquid environment.

f. Vibration and Acoustic Isolation:

i. The FlexAFM stage is set on a “Spike Guard” active isolation base; its controller is connected to the AFM C3000i controller. If a vibration spike is detected mid-measurement, the AFM will re-scan the affected line.

   ii. The FlexAFM and active isolation base are set within an acoustic isolation chamber. The chamber protects the AFM from both acoustic noise and air currents from the filter fan units.

g. **Image Data Storage and Data Analysis:**
   i. All image data is temporarily saved to each individual user's folder at: C://Users/Public/Documents/AFM Data/*User Group*/*User Name*
   ii. All data is backed up to the KUNF ResFS in folders matching the user's KUID.
   iii. Post-scan image analysis and editing is accomplished using 3rd-party software.
      - Nanosurf recommends **Gwyddian** for image analysis, which is free to download from: https://sourceforge.net/projects/gwyddion/
      - **AtomicJ** is an alternate analysis software that is specifically designed for Force analysis. It is free to download from: https://sourceforge.net/projects/jrobust/

2. **Powering on the System**
   a. Power on the C3000i controller, the Isostage 300 active isolation table controller, and the motorized stage joystick controller.
   b. If necessary, adjust the spike guard knob so that it is just below the spike threshold. To do this, slowly turn the knob clockwise until the red LED illuminates, then turn the knob back slightly until it goes out. The red LED should flash if the rigid table is lightly tapped.
   c. Once the C3000i and isolator controllers are on and completely initialized, start the C3000 software by logging in using the KUNF AFM logging software.
   d. Once the C3000 software has initialized, you'll see a message popup with a green background; click OK. If you see a yellow or red message field, contact KUNF personnel for assistance.
   e. In the C3000 software, click the **View** tab, then **Stage**. The **Stage** window will open at the right of the screen. Click **Search Reference** to initialize the motorized stage.

3. **Installing and Removing a Cantilever**
   NOTE: The cantilever holder is held to the scanning head with three small magnets. A white dot on the side of the holder is used to determine correct orientation when fixing it to the scanning head or setting it in the storage case. See Figure 2.
   a. If it is not already done, unlock and open the acoustic isolation chamber.
   b. Locate the storage case for the probe tip holder, and open the lid. Installing the cantilever can be done either in the box or on the table top. A zoom microscope is available nearby to aid in viewing the installation process.
   c. Make sure that the holder is completely free of debris by blowing it off with nitrogen. The cantilever must be perfectly flat when installed.
   d. Using the forceps, carefully place the probe tip assembly into the recess under the spring clamp. See Figure 3 for a correctly-installed cantilever.

Fig. 2

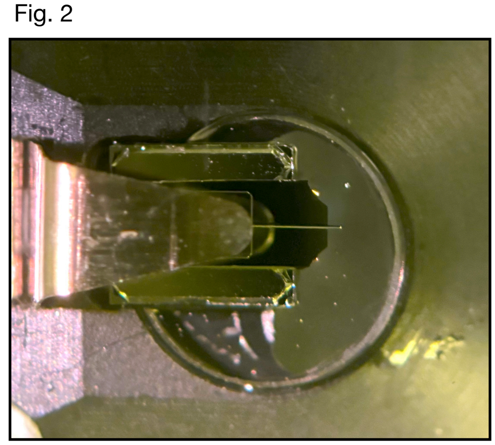


Fig. 3

e. Using the forceps, carefully push down on the spring clamp until it clicks into its home position. See Figure 4.
f. Tilt up the scanning head to install the probe tip holder, making sure that the white dot is oriented with the corresponding symbol. Carefully set the scanning head back down, making sure that the ball-feet of the two rear legs are correctly seated.
g. Removing the probe tip is done in the reverse order, using the forceps to lift the spring clamp using the small tab on the back (see Figure 4).

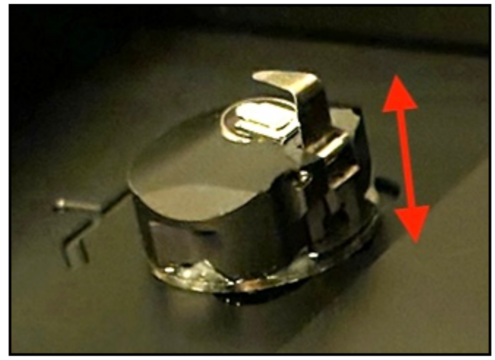


Fig. 4

h. If you are done working with the AFM, leave the empty probe tip holder in its case with the clamp up. Close the case and place it in the acoustic isolation chamber in front of the stage.

4. Sample Placement and Rough Cantilever Positioning

a. In the C3000 software, click the **View** tab, then **Video**. The **Video** window will open at the right of the screen. **Top View** and **Side View** camera images are available. To view both camera views simultaneously, click **Show in Separate Window** in the tool bar.
b. Click the **Acquisition** tab. In the Approach section of the tool ribbon, click **Home** to fully retract the cantilever holder.
c. Carefully tilt back the scan head and place your sample on the stage, positioning it so that the area that you want to scan is generally below where the probe will fall.
    i. Absolute precision is not necessary, as final positioning can be done later with the micrometers.
    ii. Larger samples can be held with the clamps or Magic “Scotch” Tape. If using tape, all fragments of tape and/or tape residue must be completely removed later.
    iii. Smaller samples will need to be mounted to a metallic specimen disc.
    iv. All samples must be clean, dry and dust free.
    v. Measurements in liquid are possible; consult *Appendix D: Measuring Liquid Samples* for instructions.

⚠ **If the tip of the cantilever makes contact with your sample while lowering the scan head, the tip will likely be damaged or destroyed.** ⚠

d. Slowly and carefully lower the scan head over your sample. As you are doing so:
    i. Make sure that the ball-feet of the two rear legs are correctly seated.
    ii. In the **Top View** and/or **Side View** panes in the **Video** window, use the camera to monitor the position of the cantilever over your sample as you lower the scan head.
    iii. Use the switch (see Figure 5) on the front of the scan head to extend or retract the front leg, *making sure that the cantilever tip does not touch your sample*.

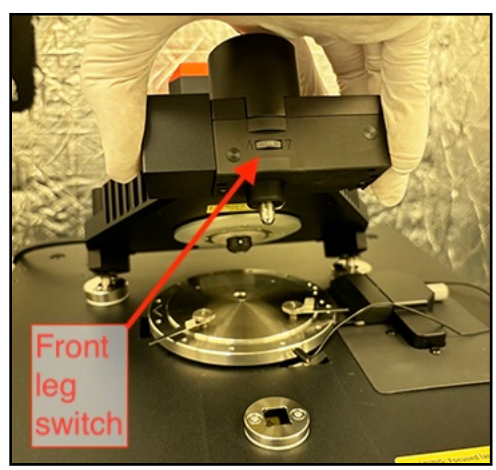


Fig. 5

iv. If necessary, adjust the height of the rear legs with the knobs. When adjusting, make note of the detent markings on the tops of the knobs and turn them both equally.
v. If necessary, check that the scan head is parallel to the stage using the bubble level. Repeat steps **4.d.iii** and **4.d.iv** as needed.
vi. When correctly set, the distance between the probe tip and your sample should be less than 300 µm. In the **Top View** tab in the **Video** window, the shadow of the cantilever should be slightly less than the width of the cantilever.

USE CAUTION WHEN ADJUSTING THE LASER AND SENSOR.
Damage to the laser or sensor adjustors will require that the scan head be returned to the manufacturer for service.

5. Aligning the Laser and Detector

a. If the Video window is not already open, click the **View** tab, then **Video**. The **Video** window will open at the right of the screen.
b. Select the **Acquisition** tab, then click the **Laser Align** button. If the laser spot (diameter is slightly less than the cantilever width) is at the tip of the cantilever, proceed to step **5.f**. If not, it will need to be repositioned.
c. Carefully drop the 1.5 mm driver into one of the two ☀ adjuster holes. See Figure 6. *Do not push or in any way firmly place the driver into the hole.*

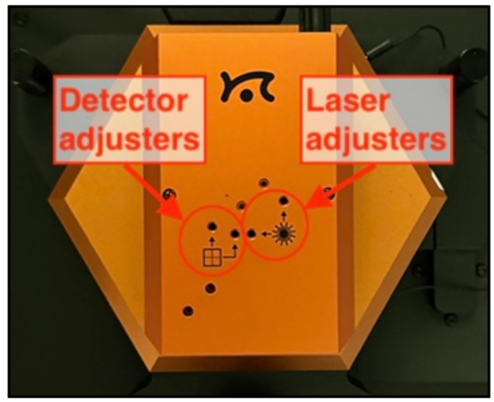
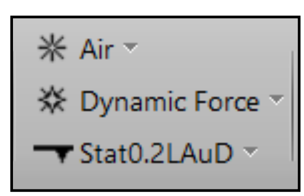


Fig. 6

d. Carefully nudge the driver one way or another until you find the spot. *Do not turn the driver more than a small fraction of a turn, otherwise the spot will be lost.* Do not be confused by reflection halos; the spot is distinct and about the width of the cantilever.
e. Continue back and forth between the two X and Y adjusters until the laser spot is positioned at the end of the cantilever.
f. In the **Laser Alignment** window, make note of the green spot. If it is in the center of the window, at the crosshairs, proceed to step **5.j**. If not, it will need to be repositioned.
g. Carefully drop the 1.5 mm driver into one of the two ⊞ adjuster holes. *Do not push or in any way firmly place the driver into the hole.*
h. Carefully nudge the driver one way or another until it is centered on the X or Y axis. *Do not turn the driver more than a small fraction of a turn, otherwise the spot will be lost.*
i. ontinue back and forth between the two X and Y adjusters until the green spot is positioned at the center of the crosshairs.
j. Once the detector is aligned, close the **Laser Alignment** Window.
k. **Close and lock the lid of the acoustic isolation chamber.**

6. Cantilever Settings and Final Positioning



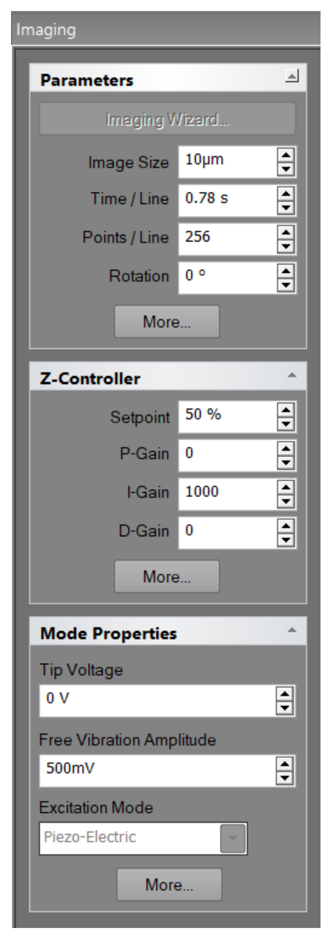
a. In the C3000 software, select the **Acquisition** tab.
b. In the Preparation section of the tool ribbon, select the measuring environment from the drop-down: **Air** or **Liquid**.
c. Select the **operating mode** (Dynamic, etc.) from the drop-down.
d. Select your **cantilever** type from the next drop-down. If you do not see your cantilever:

   i. Select **Cantilever Browser**. A window will pop up with a data table containing the currently saved cantilevers.
   ii. Click **New.** The **Cantilever Editor** window will pop up, allowing you to create an entry for your cantilever.
   iii. Enter the Properties specific to the cantilever. This data is provided by the cantilever manufacturer. Some of this data will be overwritten during fine tuning (Section 6.e).
   iv. Click **OK** to save the data, then **Close** the Cantilever Browser.

e. Fine tune your cantilever.
   i. For Dynamic Modes:
   - Click the **Freq. Sweep** button. The **Vibrational Frequency Sweep Tuning** window will open.
   - Make sure that the **Free Vibration (Amplitude)** is set to 500 mV
   - Click the **Find Vibration Frequency** button at the top-right of the window. Once complete, a graph showing the resonant frequency will be displayed.
   - Click **OK** to save the data and close the window.

   ii. For Static Modes:
   - Click the **Thermal Tuning** button. The **Thermal Tuning** window will open.
   - Click the **Start** button at the top-right of the window. Once complete, a graph of the noise spectrum will be displayed, which is used to determine the precise spring constant (k) of the cantilever.
   - Click the **Use Calculated Result** button to save the data.
   - Click **Close** to close the window.

f. In the Approach section of the tool ribbon, click the **Approach** button. The cantilever holder will be lowered to its final, optimal position. An “Approach Done” message will appear; click **OK**. If the message reads “Approach at max limit”, then repeat the steps in Section 4.d to lower the scan head more. Then, press **Approach** again.



7. Scanning a Sample for Imaging

NOTE: At first, it is recommended to make a large area image of your sample at higher scan speeds and/or lower resolutions. This image can be saved as a **Master Image** and used as a reference map to find areas of interest for high resolution image capture or spectroscopy. Capturing an image of any size or resolution is typically a prelude to getting spectroscopic data (Appendix B).

a. At the bottom left of the screen, select the **Imaging** tab.
b. If you have a scan settings that you would like to load, select the **File** tab → **Parameters** → **Load Parameter Settings**. Then proceed to **step f.**
c. Select the **Acquisition** tab. In the Parameters pane, set the **Image Size**, **Time / Line** (scan speed), **Points / Line** (resolution) and **Rotation** (0° to 90°). For advanced Parameters settings, such as Line Scanning modes (see *Appendix E: Line Scanning Modes for Imaging*), click the **More** button.
d. In the Z-Controller pane, **Setpoint** and **PID Gain** settings can be adjusted to improve image quality.
   i. In Dynamic and Phase Contrast modes, the Setpoint is the

    percentage of the Free Vibration Amplitude that the tip is *not in contact* with the sample surface. Start with 70%, and adjust as needed. Limit the range to 50-to-90%.
    ii. In Static Mode (and its extensions), the Setpoint is the downward force deflection in Newtons. A higher value correlates with a higher downward force.
    iii. Generally, only the I (Integral) -Gain PID setting will have a noticeable effect. For Dynamic Mode, start with an I-Gain setting of 3000. For Static Mode, start with an I-Gain setting of 1000.

e. In the Mode Properties pane:
    i. Set the **Tip Voltage** to 0.
    ii. For Dynamic and Phase Contrast modes, the **Free Vibration Amplitude** should be set to 500 mV**.**

f. Once the initial scan settings have been entered, click the **Start** button in the Imaging section of the tool ribbon.

g. After the scan has been started, the settings in the Z-Controller pane can be readjusted to improve image quality. Note the Z-Axis graph below the corresponding image; adjust the settings so that the white and black lines closely match.

h. When the Z-Controller settings have been optimized, click the **Stop** button in the Imaging section of the tool ribbon. Then, click the **Start** and **Finish** buttons to make a complete scan using the optimized settings. **Note:** if you do not click the Finish button, the AFM will scan up and down infinitely.

i. To scan a zoomed-in area of a **Master Image**.
    i. Click the **View** tab at the top of the screen.
    ii. Select **Online** in the ribbon; the Online frame with two windows — **Scan Position** and **Master Image** — will appear at the right side of the workspace.
    iii. Select **Tool** in the ribbon; the Tool frame with two windows — **Cursor Position** and **Tool Status** — will appear at the right side of the workspace.
    iv. In the **Master Image** frame, select **Load**. Your most recent scan will appear.
    v. In the **Master Image** frame, select **Zoom**, then draw a square within the image.
    vi. Click the **View** tab at the top of the screen.
    vii. Select **Tools** in the ribbon.
    viii. In the Tool Status window (at right), click the **Zoom** button. **Image Size** in the Parameters pane (left column) should match your selection size.
    ix. If necessary, adjust the other settings in the Parameters and Z-Controller panes under the **Acquisition** tab.
    x. Click **Start** in the tool ribbon of the **Acquisition** tab to initiate the zoomed scan.

j. To monitor raw signal data during a scan:
    i. Click the **View** tab at the top of the screen.
    ii. Select **Monitoring**; the Monitoring frame will appear at the right side of the workspace.
    iii. Click the  symbol to open the Monitoring Configuration popup window.
    iv. Tick the boxes for the channels that you wish to monitor.
        - **Main Lock-In Amplitude** is recommended for Dynamic mode(s).
        - **Normal Deflection** is recommended for Static mode(s).

k. To save your scan, click the **Capture** button in the Imaging toolbar (Auto Chart, etc.). An image and all of your scan data will appear at the right. Your scan data will be saved to the following location: C://Users/Public/Documents/*User Group*/*User Name*

l. To scan a zoomed-in area of the current scan:
   Note: this is a different method than the one outlined in section **7.i**. The scan does not need to be “Finished”. Once a zoomed-in scan has been started using this method, a new low-resolution scan will be required to subsequently zoom into different areas of the sample.
   i. If you haven’t already, click the **Stop** button in the Imaging section of the tool ribbon.
   ii. In the Imaging toolbar (Auto Chart, etc.), select **Zoom**.
   iii. Draw a box around the area that you want to (re)scan.
   iv. If necessary, adjust the scan **Parameters** (the Image Size will update once you complete **step 6.n.vi**).
   v. Click the **View** tab at the top of the screen, then select **Tool.** The **Zoom Results** window will appear at the right.
   vi. Click the **Zoom** button to initiate a new scan of the zoomed-in area.

m. To scan an area slightly adjacent to the current scan area:
   i. If you haven’t already, click the **Stop** button in the Imaging section of the tool ribbon.
   ii. In the Imaging toolbar (Auto Chart, etc.), select **Move**.
   iii. Click a point in the scan area, and then draw a line in the direction that you want to move the image. The scan area will move in the direction of the line and at a distance of the length of the line.
   iv. If necessary, adjust the scan **Parameters.**
   v. Click the **View** tab at the top of the screen, then select **Tool.** The **Move Results** window will appear at the right.
   vi. Click the **Move** button to initiate a new scan of the selected area.

n. To zoom out to the maximum scan area (centered on the current scan area):
   i. If you haven’t already, click the **Stop** button in the Imaging section of the tool ribbon.
   ii. If necessary, adjust the scan **Parameters.**
   iii. In the Imaging toolbar (Auto Chart, etc.), select **Full**.
   iv. Click the **Start** button to initiate a new scan.

o. To scan an area of your sample that is beyond the maximum scan area of 100 μm:
   i. Click the **Withdraw** button in the Approach section of the tool ribbon.
   ii. Reposition the sample using the motorized stage joystick controller. See the *Joystick Controller Quick-Reference Guide* for details.
   iii. Click the **Approach** button in the Approach section of the tool ribbon.
   iv. Repeat **steps f through h** in this section to make a new scan.

p. To save your scan settings for future use, select the **File** tab → **Parameters** → **Save Parameter Settings**. Save the settings to your user folder.

q. To view your saved scans, click the **View** tab, then **Gallery** from the Panels section of the tool ribbon.

r. If you require spectroscopic data, consult *Appendix B: Collecting Spectroscopic Data using the FlexAFM*. Otherwise, continue to Section 8.

8. Basic Image Analysis

Note: Advanced analysis and image adjustment can be done using the **Gwyddian** or **AtomicJ** software. Instructions for use of this software is beyond the scope of this SOP. **Note:** For descriptions of the operations available in the **Analysis** tab, see *Appendix E: Analysis Tools*. **Note:** Analysis of spectroscopic data is described in *Appendix B: FlexAFM Spectroscopy Modes.*

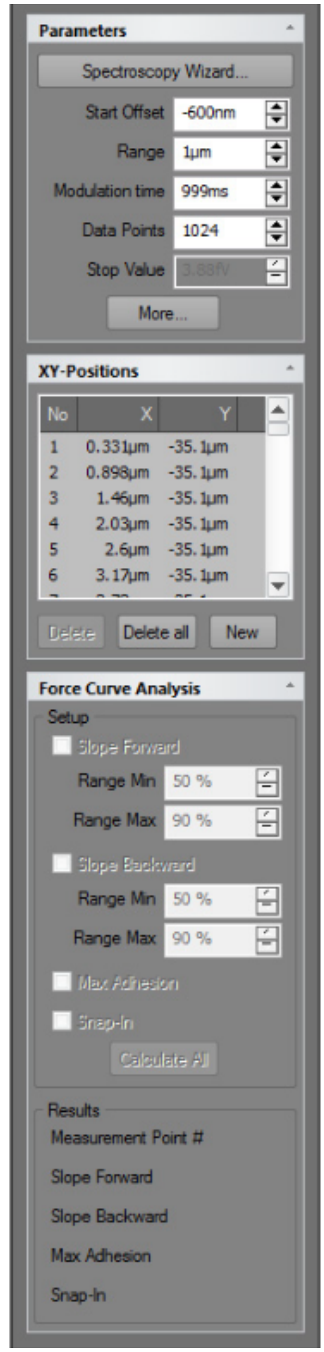
a. Select your image (.nid filetype) for analysis.
   i. Click the **View** tab, and then select **Gallery**.
   ii. In the Gallery pane, select your image from either the **History** or **File Browser** tab.
   iii. To find the file location of an image in the **History** tab, right click the image and select **Show in Explorer**.
b. Once selected, your image data will appear in a window on the screen.
   i. In the window, different charts —color maps, line graphs and 3D views— of your scanned sample will be displayed.
   ii. Different chart data can be displayed by selecting the title dropdown (Z-Axis…, etc.).
   iii. Data filtering for each chart can be adjusted by selecting the associated dropdown (Raw data, Mean fit, etc.).
   iv. Color scales and data ranges can be adjusted using the vertical sliders at the right of each data chart.
   v. Chart properties can be viewed and edited by clicking the  icon.
      - Click the [+] or [–] buttons at top to add or removed charts.
      - Click the [<] or [>] buttons to view properties for different charts.
   vi. To view metadata of your image or change display settings, click either the yellow **Data Info** or the **Tools** tab, respectively, at the right of the window.
c. Select the chart in the image window that you want to analyze (it will be outlined in blue).
d. Click the **Analysis** tab at the top of the screen.
e. Select the desired tool from the ribbon. Refer to the *Appendix E: Analysis Tools* for detailed descriptions of each tool and how to use them.
f. Define the required data points in the chart (the mouse pointer will appear as a quill). Data for the analysis tool will be displayed in the Tools pane (**View** tab → **Tools**).
g. To save the data, click the **Report** button.

9. Sample Removal and System Shutdown

a. Once your scan is complete —after pressing either the **Finish** or **Stop** buttons— click the **Withdraw** button in the Approach section of the tool ribbon to retract the cantilever holder.
b. Click the **Home** button in the Approach section of the tool ribbon to extend the scan head front leg completely
c. Unlock and open the acoustic isolation chamber.
d. Carefully tilt back the scan head and remove the cantilever according to **Section 3**.
   i. Leave the cantilever holder with the clamp up in its storage box for the next user. Store the box in the acoustic enclosure.
   ii. Store your cantilever in your personal storage in the cleanroom.
   iii. Remove your sample from the stage. If you used tape to secure the sample to the stage, *be sure to remove all pieces of tape and clean up any residue*.
e. Set the scan head back on the stage, making sure that the rear legs are properly seated in the detents.
f. Close and lock the acoustic isolation chamber.
g. Close the C 3000 software.
h. Power off the active isolation table controller.
i. Power off the C3000i controller.
j. Log out of the KUNF AFM logging software.

Appendix B: FlexAFM Spectroscopy Modes

1. **General Information and Precautions**
    a. A spectroscopic measurement is a measurement of an input signal as a function of a modulated output.
    b. The following **Spectroscopy Modes** are available:
        i. Force-Distance Spectroscopy — single point measurements in which the cantilever approaches and “pokes into” the sample, and then withdraws. During this measurement the cantilever deflection vs. piezo movement is measured, and this can ultimately be converted to a force vs. tip sample separation measurement that provides mechanical information about the sample. Available in Static, Spreading Resistance and Lateral Force scanning modes.
        ii. Amplitude-Distance Spectroscopy — Also known as Phase-Distance spectroscopy. Similar to Force-Distance spectroscopy, except that the cantilever is oscillated. Available in Dynamic and Phase Contrast scanning modes.
        iii. Tip Current-Distance Spectroscopy — In this mode, the tip current is measured while the tip is moved over the sample. Only available in Spreading Resistance scanning mode. **Note:** *the sample must be grounded to the scan head*.
        iv. Voltage Spectroscopy — Also known as Current-Voltage Spectroscopy. In this mode, the tip is not moved (remains in contact with sample), but the tip voltage is changed over time while the current signal is measured. Only available in Spreading Resistance scanning mode. **Note:** *the sample must be grounded to the scan head*.
        v. KPFM-Voltage Spectroscopy — Similar to Voltage Spectroscopy, except the AC-EFM/Lock-In X Signal is acquired as a function of Applied Voltage. Only available in Spreading Resistance scanning mode. **Note:** *the sample must be grounded to the scan head*.
    c. The following **Modulation Methods** are available:
        i. Fixed Length: This type of modulation has a fixed start and end value. A Fixed Length spectroscopy measurement is typically divided into four distinct phases: 1. approach (contact with sample); 2. forward modulation (distance above the sample, defined by Range); 3. backward modulation (makes contact with sample, measuring deflection); and 4. remain approached (returns to the original Z position from step 1). If multiple points are selected (Line or Grid), these points will be subsequently measured.
        ii. Stop by (value): This type of modulation has a fixed speed from a start value until a certain measurement value is reached. A Stop By… spectroscopy measurement is typically divided into six distinct phases: 1. start from a retracted Z position; 2. forward modulation (makes contact with sample, measuring deflection); 3. forward stop/pause (stop at a defined deflection value, defined by Distance Range); 4. backward modulation (move to distance above the sample while measuring deflection); 5. move to the next point(s) (Line or Grid) and repeat steps 2 through 4; 6. retract to the Z position defined in step 1.
    d. **Force Curve Analysis** allows setting up analysis functions that produce results as each force curve is measured. This allows immediate inspection of numerical analysis results, graphical representation of the analyzed parameters in a Force Curve graph, and producing a color map of analyzed parameters during the measurement.



e. Prior to making spectroscopy measurements, it is recommended to get an image or topography data of your sample. See **Section 7** in the FlexAFM SOP.

2. Scanning a Sample for Spectroscopy

a. At the bottom left of the screen (Acquisition Pane), make sure that the **Spectroscopy** tab is selected.

b. In the tool bar click Load to load an image.

c. If you have a scan settings that you would like to load, select the **File** tab → **Parameters** → **Load Parameter Settings**. Then skip to step **2.j.**

d. Select the **Acquisition** tab.

e. If you are unsure of how to set the **Parameters**, click the **Spectroscopy Wizard** button and follow the directions outlined below. Otherwise, skip to step **2.i.**

f. The **Spectroscopy Wizard** window will open. Select the **Spectroscopy Measurement** mode (some options will be grayed out, depending on selected **operating mode** (Dynamic, Static, etc.)).

g. Select the **Measurement Subtype (Modulation Method):**

   i. **Fixed length**… or **Stop By**… (see section 1.c)

   ii. **Point** or **Grid** (a single data point, or multiple)

   …, then click **Next.**

h. In the next window, set the:

   i. Start Offset — The Z-axis offset applied at the start of each modulation cycle.

   ii. (**Distance) Range** — this value defines the change of the output value during the modulation phase. The range can be positive or negative. **Note:** in Voltage Spectroscopy mode (Spreading Resistance), this field is **Voltage Range**, where the voltage is modulated from -(value)/2 to +(value)/2.

   iii. **(Modulation) Time** — The time used to change the modulated output from its the start to end value

   iv. **Data Points** — the resolution of the scan.

   v. **Stop at Maximal Force/Minimal Amplitude (Optional) —** the cantilever stops its forward approach movement as soon as the defined value is reached.

   vi. **Sampling Rate** — Shows the number of data points measured per second during the respective modulation phase. This value is calculated form the Data Points and Time parameters (see above).

   vii. **(Move) Speed** — the speed at which the modulation output is changed. The move speed can be positive or negative (also see Important (page 209)). This value is calculated from the modulation Range and Time parameters (see above).

   …then click **Next.**

i. In the next window, click **Finish**. Then, skip to step **2.j**.

j. In the Parameters pane (left column at top), set the **Start Offset**, **Range**, **Modulation Time**, **Data Points** and **Stop Value**. For advanced Parameters settings, click the **More** button.
k. The **XY Positions** pane lists the points where in the Z-Axis image pane your measurements are to be performed:
    i. Use the **Point**, **Line** or **Grid** tools in the Imaging toolbar (Auto Chart, etc.) to help you to define these positions graphically using the mouse. **Note:** When using the Line or Grid tools, define the number of measurement positions in the Tool Status field in the Tool panel (click View tab, then Tool if not shown).
    Or…
    ii. Select **New**. The XY-Positions window will open; enter the X and Y-Position coordinates manually, then select OK.
    iii. Repeat Steps j.i or j.ii as needed.
l. In the Force Curve Analysis pane (optional; see section 1.d):
    i. Tick either or both of the **Slope Forward** and **Slope Backward** boxes to adjust the **Range Minimum %** and **Range Maximum %**. This limits the slope of the part of the forward/backward force curve in the part where the tip touches the sample (force is positive). Min defines the smallest force that is evaluated, Max defines the largest force. The 0% value corresponds to 0 N deflection signal; 100% corresponds to the maximum repulsive Deflection signal in the curve. Usually, Min is larger than 0% for the curve moving in contact with the surface, to ignore effects that occur when the contact is made. Max can be chosen smaller than 100% for the curve moving out of contact of the sample, to ignore effects caused by turning around the motion direction while in contact with the sample.
    ii. If desired, tick the **Max Adhesion** box. When selected, the graph limits the signal to the smallest deflection value of the force curve while moving away from the sample.
    iii. If desired, tick the **Snap-In** box. When selected, the graph limits the signal to the smallest deflection value of the force curve on moving closer to the sample. This is usually the force when the tip snaps into contact with the sample.
    iv. Clicking **Calculate All** forces recalculation of all force curve analysis results using the above settings.
m. Once the initial scan settings have been entered, click the **Start** button in the Imaging section of the tool ribbon to begin the spectroscopy measurement.

Appendix E: Analysis Tools

1. **General Information**
   a. **General Workflow:** All of the tools below can be used to analyze a previously-captured image, or while a measurement is still being acquired. To use a quick evaluation tool:
      i. Click on the chart that you want to evaluate to activate it. It will be outlined in blue.
      ii. Select the desired tool using one of the following approaches:
         • Click on one of the tool buttons in the Analysis tab.
         • Select the tool from the Chart's context menu (right-click on the chart).
         Note: When a tool has been selected, the **Tool** panel moves to the top of the panel stack of the Info pane at the right of the screen.
      iii. Define the evaluation. **Note:** The procedure to define the evaluation is different for each tool. Depending on the selected chart type, some tools may be unavailable. Details can be found in the tool-specific instructions below.
   b. **Creating markers:**
      i. The first point of a line is defined by the mouse cursor position where the left mouse button is clicked, while the second point by the position where the button is released.
      ii. When the mouse is not moved between clicking and releasing, a line parallel to the X-axis is drawn.
      iii. The direction and length of selection markers can be adjusted by dragging the end markers (arrows).
      iv. Selection markers can be moved as a whole by dragging the center marker.
      v. All marker data will be displayed in the "Tool Status" section of the **Tool** panel (window at right).
   c. **Accessing Data:**
      i. All Calculated data will be displayed in the "Tool Result" section of the **Tool** panel.
      ii. Clicking "Store" in the "Tool Result" section records the data in the "Tool" data category of the **Data Info** panel (tab at right of .nid window) for the respective measurement document (as long as the tool is active when the document is stored).
      iii. For tools in the **Correction** and **Filter** groups, data in the image is changed. However, this is done to a copy of the original measurement document, so you won't lose any data and will always be able to access the original measurement data in addition to the corrected or filtered data. To get useful results, the Data filter option for the corrected image in the new document will be automatically set to "Raw data".

2. **The Measurement Group**
   a. **Measure Length:** Calculates the distance and signal difference between two points. The points are defined by drawing a line on the measurement chart.
   b. **Measure Distance:** Calculates the distance between two parallel lines. After drawing the first line, a second parallel line sticks to the mouse cursor that is released by clicking its desired position. The distance value only depends on the cursor positions, it does not on depend the displayed data values.
   c. **Measure Angle:** Calculates the angle between two lines. In Line graph-type displays, this tool can only be used when the chart displays data that has the unit "meters". After drawing the first line, the end of the second line sticks to the mouse pointer. The end of the second line is made by clicking its desired position. The angle can be changed by dragging the line end point markers or the corner mark.

3. **The Correction Group**
   a. **Correct Background:** Removes the effect of an ill-aligned scan plane when the line filter options (see below) do not give satisfactory results. This may be the case when the scan lines in different parts of the measurement have a different average height. An example of such a measurement is shown in Figure 1. To use the tool, select three points that should be on the same height. This is done in the same way as with the angle tool (see above).

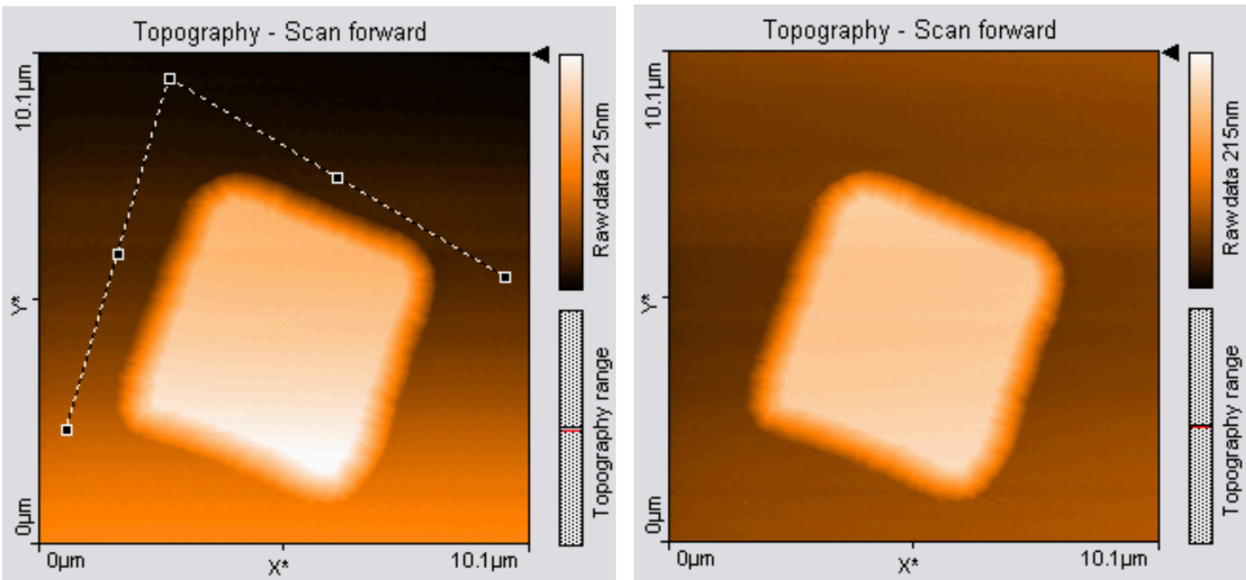


Figure 1: Correct Background. (Left) Uncorrected image; the end points of the selection marker have been moved to points that should have the same height. (Right) Corrected image.

   b. **Correct Scan Line Levels:** Removes the effect of drift when the line filter options (see below) do not give satisfactory results. This may occur when the scan lines in different parts of the measurement have a different average height. An example of such a measurement is shown in Figure 2. To use the tool, draw a line through points that should have the same height in the same way as with the Measure Length tool (above).

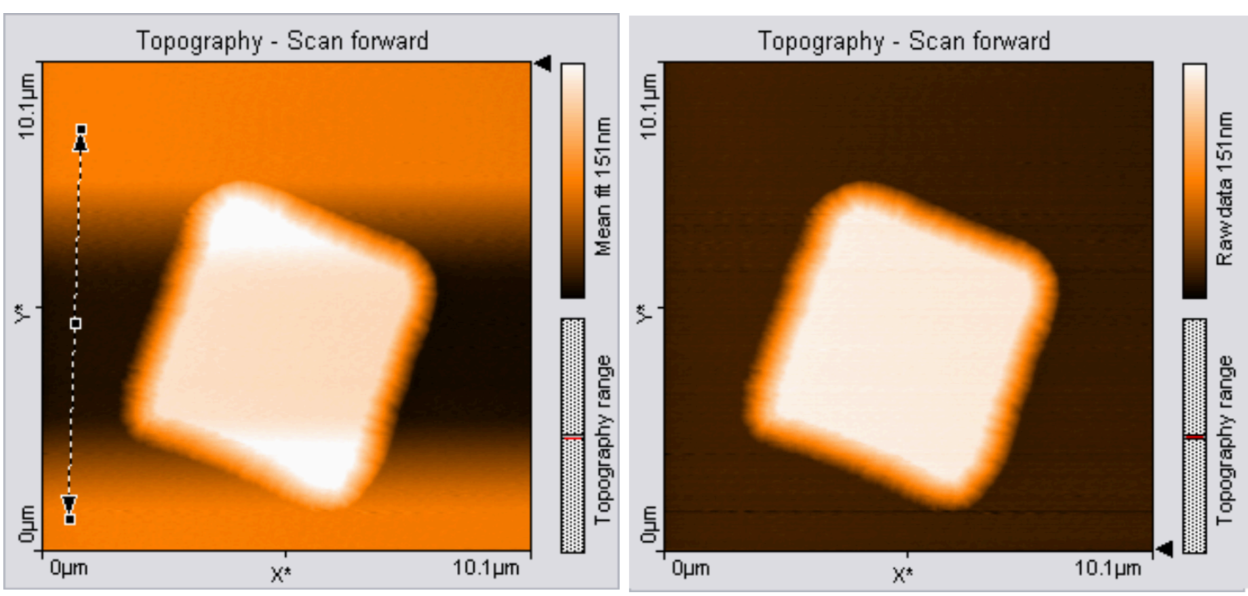
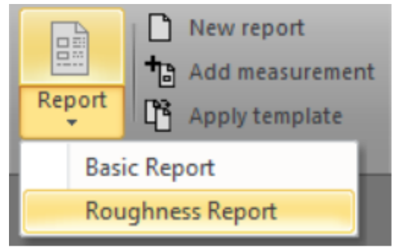


Figure 2: Correct scan line levels. (Left) Uncorrected image with a selection marker through points that should be at the same height. (Right) Corrected image.

4. **The Roughness Group**
   Note: The roughness values in the "Tool Results" section of the **Tool** panel depend on the line Filter option (see below) that is applied to the chart, because they are calculated from the filtered data.
   a. **Calculate Line Roughness:** Calculates several roughness parameters from the data at points along a selected line. The line is selected in the same way as with the Measure length tool (see above).
   b. **Calculate Area Roughness:** Calculates several roughness parameters from the data points in a selected area. The area is selected in the same way as with the Cut out Area tool (see below). **Note:** The Area Roughness tool can be used to determine the mean height difference between two plateaus with more accuracy than with the Measure Distance tool (see above). To determine the mean height difference, select an area on each plateau, and calculate the difference between their Sm-values.
5. **The Filter Group**
   a. **Glitch Filter:** The Glitch Filter removes the effect of small defects in the image such as single short glitches in the scan where the tip lost contact with the sample. Compared to the Noise Filter (see below), it has the advantage of not reducing resolution on step edges. The glitch filter is implemented as a Median filter on a 3×3 pixel matrix. To apply the filter, activate the color map chart that is to be filtered, then click the "Glitch Filter" button. A new Measurement document with the filtered data is created.
   b. Noise Filter: The Noise filter removes high frequency noise from the image, but applying the filter will also decrease the resolution of the image. The Noise Filter is implemented as a convolution with a 3×3 pixel Gaussian kernel function. To apply the filter, activate the color map chart that is to be filtered, then click the "Noise Filter" button in the Tools bar. A new measurement document with the filtered data is created.
6. **The Tools Group**
   a. Create Cross-Section: Creates a new (profile) measurement document containing a line cross-section of a Color map or Line View display. The line is defined by drawing a selection arrow. The arrow points toward the forward direction of the line. Double-clicking the graph, or clicking the "Cut out line"-button in the Tool Status section of the **Tool** panel creates a new document that contains the line section. The Tool Chart section of the **Tool Results** panel displays a preview chart of the selected line. The Tool Status section of the **Tool Results** panel displays the calculated "Length" and "DeltaZ" of the selected line.
   b. Cut Out Area: Creates a new (zoomed or resized) measurement document containing a subsection of an existing measurement. One corner of the (square) area is defined by the mouse cursor position where the left mouse button is clicked, the opposite corner by the position where the button is released. When the mouse is not moved between clicking and releasing, an area is defined that has a size of 33% of the current measurement, and is centered on the clicked location. Once an area is defined, it can be resized by dragging one of its corners, and moved as a whole by dragging its center point. Pressing the "Shift" key while dragging a corner defines a rectangular area. Double-clicking the graph, or clicking the "Cut out area" button in the **Tool Results** panel creates a new measurement document that contains the selected area. The Tool status section of the **Tool Results** panel displays the calculated "Size" or "Width" and "Height" of the selected area.

7. **The Report Group** (**Note**: MountainsSPIP is not currently installed. The section below is not currently applicable.)



   a. **Report:** The “Report” button starts the MountainsSPIP software from within the C3000 control software. When a measurement is opened by the MountainsSPIP software, it will import all measurement channels that are displayed in the current measurement document. By default, a Basic Report is generated. Other Report styles can be chosen from the “Report” button’s drop-down-menu (see figure at right). This drop-down menu lists templates stored in a template folder. The Menu Item’s name is equal to the template name without the extension (*.mnt) and is sorted alphabetically. This standard folder is configured by the Report Template File path (see Reporting (page 280)).
   b. **New report:** An empty report is opened.
   c. **Add measurement:** The currently active measurement is added to the currently opened report.
   d. **Apply template:** Opens a dialog that allows you to select a template that is applied to the currently active measurement. If selected, a menu item the template is applied to the current selected measurement document.

8. **The Scripting Group** (**Note**: Scripting is not currently allowed by KUNF users.)
   The scripting interface is an optional component for creating user defined scripts (software components) to add new features or automating tasks. For details, see the “Script Programmer’s Manual”.
   a. **Script:** This button opens the Script editor dialog. In this dialog, a script source code can be loaded, edited, saved and run directly.
   b. **The script button drop-down menu:** Accessed by clicking the arrow head at the bottom part of the Script button. In this drop-down list, scripts from the standard scripting folder are displayed and can be started by selecting the corresponding menu item. The menu item's name is equal to the script’s name without the script extension (*.vbs) and is sorted alphabetically. The standard folder is configured through the Scripting acquisition and Analysis file paths (accessed via “File” > “Options” > “Scripting”).
      At the bottom of the drop-down menu, the “Run from File…” menu item is displayed. It allows selection and execution of a script file anywhere on your hard disk (or other storage media). With the selection of this menu item, a file open dialog is displayed and a script file can be manually selected. This script file is executed directly after “Open” is selected.