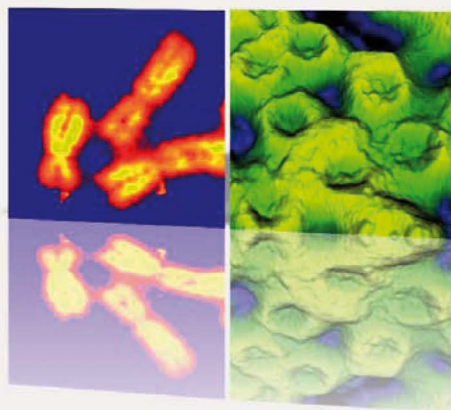


# JPK NanoWizard® II

## User Manual

SPM Software Release 3.3a  
04 / 2009



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## § 0 Safety instructions and warnings

### 0.1 Important safety information

#### Laser safety warnings:

##### Infra-red laser diode:



Most users have a JPK NanoWizard® equipped with a laser diode that emits **invisible infrared** laser light (wavelength 850nm). Be extremely careful with such lasers, since the blink reflex will not protect your eyes. The NanoWizard® head is provided with a tilt switch to turn off the laser automatically when the head is in an upright position.



Use laser filters in optical equipment

##### Red laser diode:



Some users have NanoWizard® AFMs containing a class 2 laser diode with emission in the (visible) red part of the spectrum. This may be harmful to your eyes.

Do not stare into the laser beam.

The emitted wavelength of the laser diode is 670 nm, with an emitted power of 0.5 milliwatts. Even with the glass block detached, the emitted power of the laser remains below 1 milliwatt. Thus the laser belongs to **laser class 2**.

##### Additional notice for users of the Life Science version:

Users with the NanoWizard® in the Life Science version **must have a laser filter in the optical microscope** to prevent the laser shining into the users eyes. The laser filter is fitted by JPK on installation and fixed in the housing of the binocular tube. The filter in the top of the AFM head is also provided for your safety. **Do not remove the filters in the AFM head or optical microscope.** Note also that the laser beam will be present in the optical path through the side ports of the optical microscope. Do not stare into the sideports of the optical microscope.

##### Switching off the laser using the software

For safety reasons, for both laser diodes the user can switch off the laser with the laser toggle button in the JPK SPM software if the AFM head is not in use. It is not possible to turn off the laser when the cantilever is approached on the sample surface and during scanning.



Laser on



Laser off



Laser disabled

#### Electric shock hazard:

Do not remove or open the cover of the AFM head or controller when it is plugged into the power supply. The voltage (115 or 230V only) supplied to the system may cause injury to the user.

Do not stick anything through the slots in the top of the AFM head or other open parts of the AFM head or controller.

Removal of covers or servicing parts is for trained JPK personnel only. There are no user serviceable parts or components inside. Ask JPK for assistance if any problem occurs.



#### Sensitive environments:

Our product is fully CE-approved. Do not use the AFM with its controller in an electromagnetically sensitive environment.



#### Electrostatic discharge:

The AFM head is sensitive against electrostatic discharge. Touch ground before you use it, or use a ground bracelet.



#### Prevention of damage to the AFM head:

Do not use the AFM head or any of its components under water or any other fluid (the only exception is the glass block, its spring and the attached cantilever, which can be immersed in water). Perform your experiments in a dry atmosphere.

Do not pour any fluid over your AFM head. The metal cover of the AFM head is made of aluminum. Be careful not to allow any contact with any aggressive acid or base.



The black membrane that protects the AFM head from underneath is not stable against benzene and alcohol. Do not allow aggressive fluids such as acids and bases to come in contact with the polymer.

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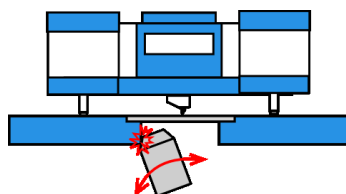
## 0.2 Warnings for the Life Science version with optical microscopes

### 0.2.1 Prevention of condenser lens crash

Be careful not to hit the AFM head when lowering the condenser (illumination optics) of the optical microscope. This is especially important for users of the NanoWizard® I microscope, and particularly when the motors are extended and the head is higher than normal. If the condenser is going to hit the head, lower the head on the motors first.

*Always lower the condenser carefully!*

### 0.2.2 Prevention of objective lens crash

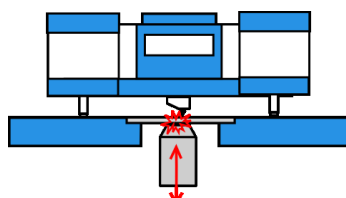


Do not allow the objectives to crash into the microscope stage. The maximum load of the objective revolver should be *three objectives* – arranged in a 120 degree arrangement, as shown in the pictures here. Please check that the specific lenses used fit in this conformation. It is possible that some lenses with an extra-large width may have to be used alone, or the objective lens holder must not be rotated.



Note: The revolver with objectives can only be rotated if it is *completely retracted*. This is particularly important if the optical microscope uses any automatic movement of the stage or objectives. Please ensure that the automatic switching between objectives makes a retract before the rotation

### 0.2.3 Prevention of sample crash



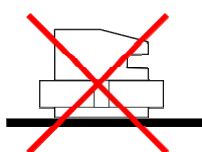
Be careful with objectives that have a short working distance. When the objective is moved upwards it may lift up the sample and damage the cantilever.

The sample holder has a groove on the left side that helps to observe the objective-sample distance during the movement.

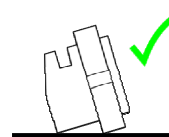
## 0.3 Other important information

### 0.3.1 Glass block cantilever holder

*Note: The flat top and bottom of the glass block are optically polished glass. Only place these planes on soft tissue paper. JPK recommends Kimberly Clark Kimwipes Lite 200 (green box). Do not touch the optical surfaces with tweezers or any other material that could scratch the surface, and do not store the glass block lying on these surfaces.*



When deposited this way the polished optical planes of the glass block can be damaged.



When not in use, and for cleaning in an ultrasonic bath, always set the glass block on its side.

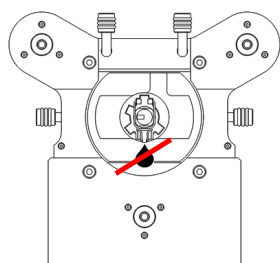
The glass block can be cleaned conveniently using an ultrasonic bath. However, please make sure that the glass block is held using a soft support or mesh. Do not ultrasonicate in a hard holder or glass beaker.

### 0.3.2 Sample stage and sample holder

Never immerse the sample holder or sample stage in liquid

Do not allow the sample stage or sample holder to come into contact with acids, bases or other aggressive chemicals.

### 0.3.3 Prevention of liquid spilling onto motor spindles



When the AFM head is put into an upright position after an experiment has been performed in liquid, sometimes some fluid drops may fall from the glass block onto the back spindle of the AFM head. This fluid may lead to corrosion, it is especially important for users of the NanoWizard® I microscope.

*Please remove the fluid from the glass block immediately after use with a soft piece of tissue paper.*

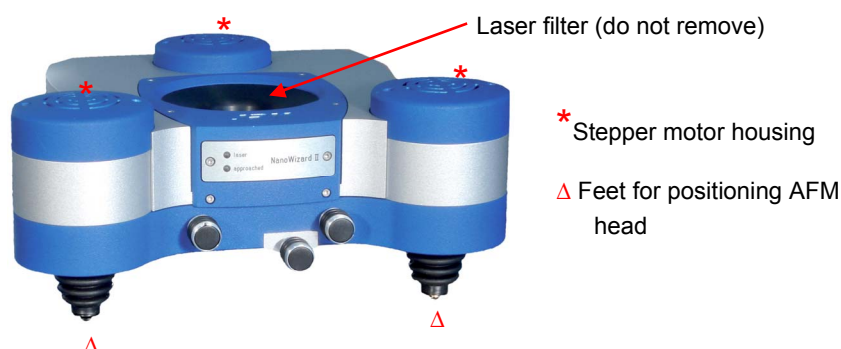
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# § 1 Installation

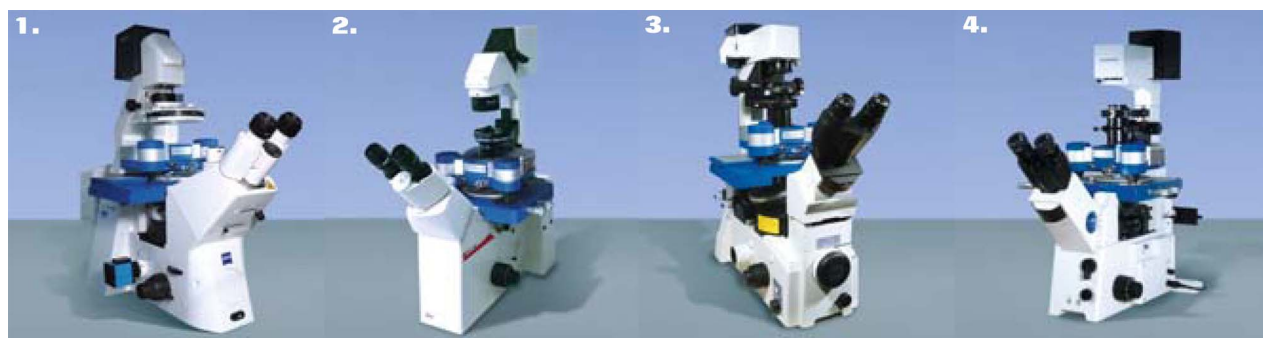
## 1.1 Components

### 1.1.1 NanoWizard® head and controller

The most important parts of your AFM system are the NanoWizard® AFM head and controller. Other components are different for the **Life Science** or **Standard** systems, but the AFM head and controller are the same for both.



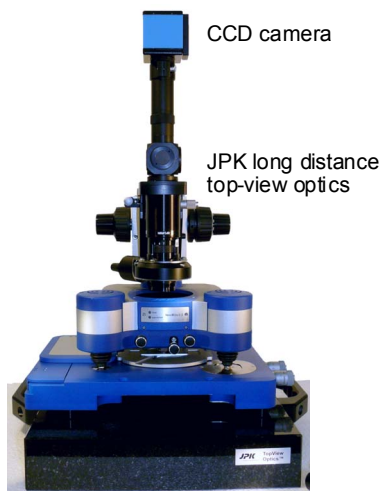
### 1.1.2 Life Science and Standard systems



If the NanoWizard® was ordered in the **Life Science Version**, then it can be installed on an inverted optical microscope made by Zeiss (1), Leica (2), Nikon (3), or Olympus (4). In this case the optical microscope must be equipped with a special AFM stage made by JPK. The images here show the NanoWizard® installed on the following inverted optical microscopes: Zeiss Axiovert 200 (1), Leica DMI (2), Nikon TE 2000 (3) and Olympus IX 71 (4). In each case, the NanoWizard® head and Life Science stage can be seen as the blue components installed in the optical microscopes. The entire optical microscope and AFM can also be placed on an active vibration table.

Note that the Life Science stages are specially shaped to fit the model of optical microscope they are ordered for. Optical microscopes from different manufacturers have different designs, so the stages are not interchangeable, and should only be used on the type of microscope they are designed for. In each case the stage fits directly onto the optical microscope body with several screws. These must be firmly attached to make a rigid connection between the optical microscope and the stage, and to prevent unwanted drift or vibrations.

The sample positioning for the **Life Science** stage can be manual or motorized. In both cases, the stage also can be removed from the optical microscope and placed directly on the anti-vibration table, so that the AFM can be used as a stand-alone system, or with top-view optics, as for the **Standard** system.



CCD camera

JPK long distance  
top-view optics

In the **Standard** version the JPK NanoWizard® AFM is installed on a special stage, designed to sit directly on a workbench or anti-vibration table.

This is the main option for non-transparent samples, where the inverted optical microscope is not suitable. The sample can be observed in reflected light using the CCD camera and long-distance top-view optics.

## 1.2 Assembly

### 1.2.1 Location – find a quiet place for the instrument

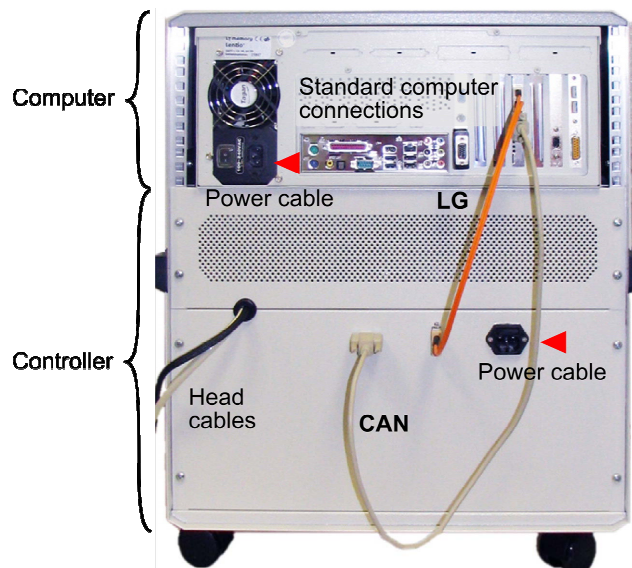
For highest resolution imaging and most sensitive force measurement, it is important to minimize vibrations and noise from the environment of the AFM. Reasonable results may be obtained even in non-ideal situations, but generally it is worth reducing external noise or isolating the AFM to obtain reliable results at higher resolution. The most important thing is to place the AFM system in a quiet room on a solid, stable base.

- Building vibrations are usually smallest in the basement; AFM labs on higher floors may have more noise problems. Remember the effects of lifts or other large machinery may be felt even some distance away.
- The temperature should also be stable, ideally the ambient temperature should not change by more than 0.5 °C per hour.
- Moving air will cause vibrations or noise, so place the AFM system away from doors, windows and vents. Keep doors and windows shut. Air conditioning can cause noise problems if it blows near the AFM system.
- Any loud noises or passing people can disturb the AFM experiment, so small rooms with just a single instrument will generally give the best results.

Good vibration isolation is always required for scanning probe microscopy experiments. The two AFM head cables can transmit unwanted vibrations to the AFM head. Therefore it is recommended that the cables are taped firmly to the vibration isolation table. Simple adhesive tape can be used - wide masking tape for marking areas for home decorating is particularly suitable, because the wide tape sticks well, but the adhesive is designed to not leave a residue, so the cables do not become sticky.



## 1.2.2 Controller connections



The connections for the AFM controller and computer are found on the back of the controller box.

There are separate power connections for the computer and controller. Standard power cables for the correct voltage (230 or 115V, depending on the country) can be used. The power supply socket of the controller is equipped with a fuse, which depends on the particular equipment. Please ask JPK for assistance, if required (email [support@jpk.com](mailto:support@jpk.com)).

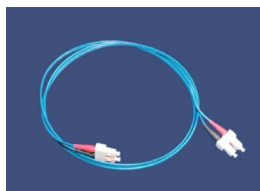
Note that the power switch for the controller is on the front of the controller box. First check that the computer and controller are both switched to "OFF" and then connect the 2 black power supply cables for the computer and controller.

The computer connections for the keyboard and mouse are as for a standard computer. Connect the green and pink PS-2 mouse and keyboard cables to the matching sockets. There are two monitor cables, which plug directly into the graphics card. These can be connected now along with the power supply for each screen.



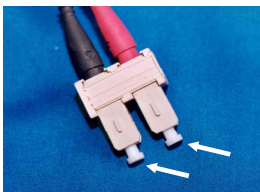
CAN bus

The CAN bus and light guide (LG) are the connections between the host computer and the controller electronics. If there are any problems with these connectors or cables, the connection between controller and computer will be lost and the SPM software will not start.

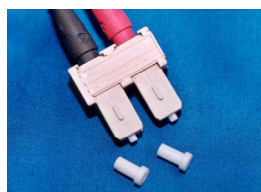


Light Guide

The proper connections are shown in the above picture for CAN and LG. Both connect from the same computer card to the controller panel.



Protection caps shown.



Protection caps removed

Note that the light guide is an optical cable, and is very sensitive to damage to the ends. The cable may also be damaged if it is bent too sharply. There are protection caps on the cable ends and on the computer and controller sockets. These must all be removed before the cable is connected. They should be stored nearby for using if the controller is moved or shipped. Without the caps and plugs, dust may enter the sockets or the cable ends may be scratched.

The CCD-camera (if included) can also be connected at this point. Firewire cameras connect directly with the Firewire connection on the back of the computer, using the special cable provided. See **Section 9.1** for more information on the CCD camera.

### 1.2.3 AFM head connections

In the picture above of the back of the controller box, two cables can be seen at the bottom left corner, which connect to the AFM head. These have a permanent connection to the controller unit – please do not strain on these cables.

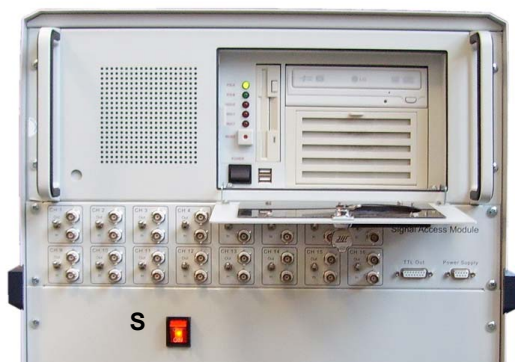


On the right hand side of the AFM head are the two sockets for the controller cables. Check the plugs and sockets carefully; there is only one possible way to connect them. Do not force the connectors!

*Note: Always check that the controller is switched OFF before connecting or disconnecting the AFM head!*

**Note:** the placement of the head cables can be very important for taking high resolution images. Try to eliminate all sources of vibration from the AFM head. Vibrations can be transmitted via air, via the table and via the two cables which connect the AFM head with the controller. We recommend taping the two head cables to fix them to the anti-vibration table. They should be loose, so no tension or torsion is transmitted to the head, and the cables' weight is not pulling on the head. The firm connection to the anti-vibration table removes differential movement between the head and the stage. See **Section 1.2.1** for more details.

### 1.2.4 Power On



Here the front of the controller is shown. First make sure that the power switch on the back of the computer is on.

1. Switch on the computer first (open the front computer panel), and the Linux system will start automatically.
2. When the computer has booted, switch on the controller with the switch S marked in the image.

**Note:** switch off in the reverse order, i.e. controller first and then computer.

It is alright to leave the PC switched on all the time. When the SPM-program is closed, you can easily switch on/off the controller. For more information about the LINUX operating system see **Section 10.1**.



At the log in screen, enter your user name and password. For a new system, a default account is already created, and the log-in details are sent separately. Once you have logged in with a valid user account, the JPK icon can be found on the desktop to start the SPM software.

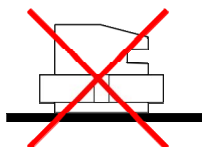
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## 1.3 Practical tips for using the NanoWizard®

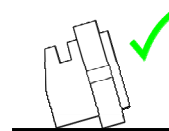
### 1.3.1 Cleaning and maintenance

#### 1.3.1.1 Glass block cleaning and storage

Note: The flat top and bottom of the glass block are optically polished glass. Only place these planes on soft tissue paper. JPK recommends Kimberly Clark Kimwipes Lite 200 (green box). Do not touch the optical surfaces with tweezers or any other material that could scratch the surface, and do not store the glass block lying on these surfaces.



When deposited this way the polished optical planes of the glass block can be damaged.



When not in use, and for cleaning in an ultrasonic bath, always set the glass block on its side.

The glass block can easily be cleaned with an ultrasonic bath. If required, some detergent can be added to the water. When the glass block is put into the bath, it should be set on its side, otherwise the optical planes of the glass block can be seriously damaged. Rinse the glass block with ultrapure water afterwards and dry it in a stream of nitrogen. Try to avoid any contact with the optical planes.

#### 1.3.1.2 AFM housing and filters

The NanoWizard® AFM head does not require much maintenance. From time to time clean the housing with a soft damp cloth. The yellow reflective laser filter in the top of the AFM housing can be cleaned with a soft piece of clean lens paper soaked with a few drops of pure alcohol.

### 1.3.2 Cantilever selection and handling

The cantilever and tip are both susceptible to damage. Cantilevers are expendable items and have to be replaced regularly. The lifetime of AFM cantilevers strongly depends on the way they are handled. If the tip is damaged, the tip radius generally increases. With worn or contaminated tips, the image resolution will be reduced and the images may have serious artifacts. Damage to the cantilever arm may also cause problems for imaging. See also the more general discussion about cantilever choices for different imaging modes in the NanoWizard AFM Handbook.

Handle the cantilever chips carefully:

- Do not touch the cantilevers with fingers. Use tweezers to handle them.
- Do not drop the cantilever chips. The cantilevers are delicate and may break off from the chip.
- Only open the cantilever package when necessary for taking out or inserting a cantilever, and always in a clean environment.

The tips may also be damaged through inappropriate scanning conditions:

- Too high gain parameters (IGain and PGain) may lead to a damage of the cantilever tip.
- Too high setpoint values in contact mode may damage the tip.
- Too low setpoint values in intermittent contact mode may damage the tip.



## § 2 Software overview

### 2.1 SPM software introduction and index

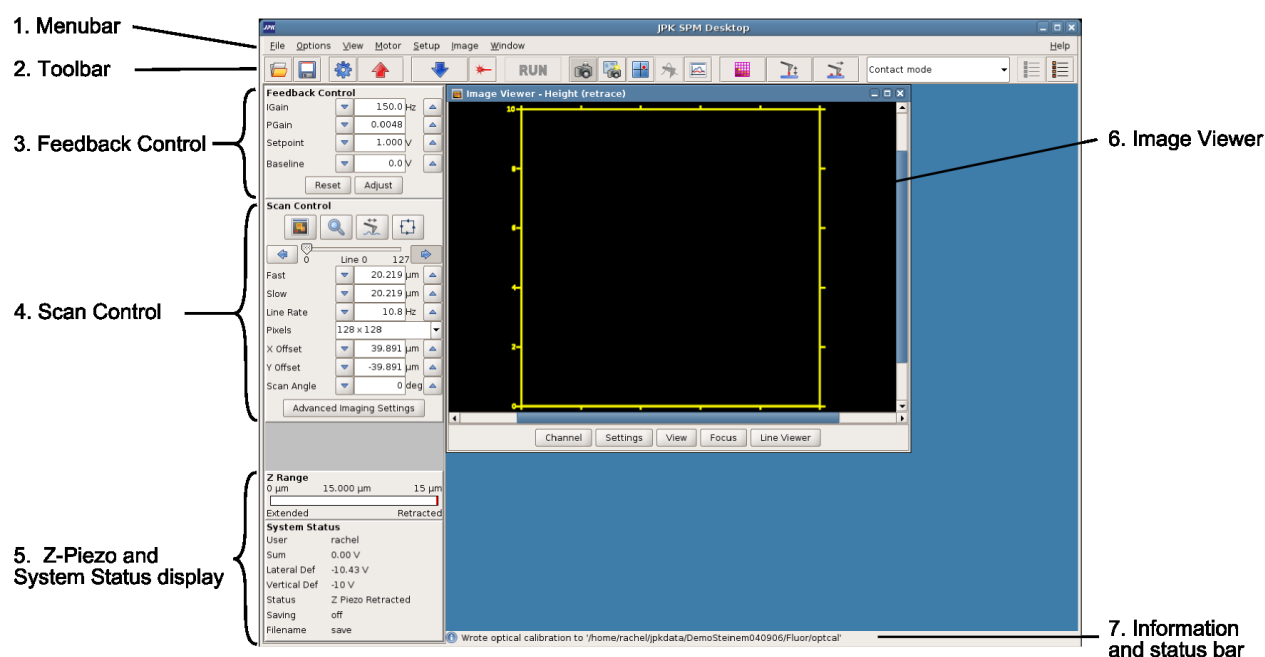
The JPK NanoWizard is delivered with the latest version of the JPK software. The software programs **SPM** is developed in-house by JPK. The information in this chapter provides an overview of the main components of the SPM software, and links to find more specific information on particular features from the menus or shortcut icons.

#### 2.1.1 Starting the program



Double-click the SPM icon on the desktop and the program will start.

#### 2.1.2 Software overview



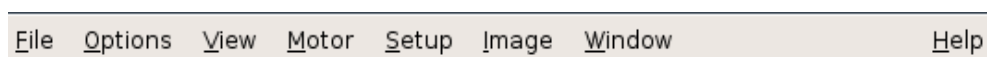
The graphic here shows the initial software state. The next sections of this manual introduce some of the software options and controls that can be seen in this screenshot. This part of the manual just provides quick overviews of the functions; more detailed instructions are given in the referenced sections later in the manual that describe approaching, imaging and the particular types of experiment that are possible.

1. The menu bar runs along the top of the screen; the pull-down menu options are listed in **Section 2.1.3**.
2. The icon toolbar provides shortcuts for some of the most commonly used options, as listed in **Section 2.1.4**.
3. The **Feedback control** panel can be seen on the left side of the screen. Here the feedback parameters can be set, these are briefly introduced in **Section 2.2.1** and more thoroughly discussed in **Section 5.1**.

4. The **Scan control** can be seen on the left side of the screen during imaging mode. These settings are briefly introduced in **Section 2.2.1** and more thoroughly discussed in **Section 4.2**.
5. The Z-piezo display and **System Status** panels display information about the current state of the AFM system, such as the current piezo position, and whether the instrument is approached on the surface.
6. The **Image Viewer** window displays the scan data (either from the current scan, or from previous data files). The options for setting the data and display settings are introduced in **Section 2.2.2**.
7. The status bar at the bottom of the software provides information about the most recent software actions. Confirmation of file saving, warnings and other information will be displayed here. It is normal that this is empty when the software is first started.

### 2.1.3 The menu bar

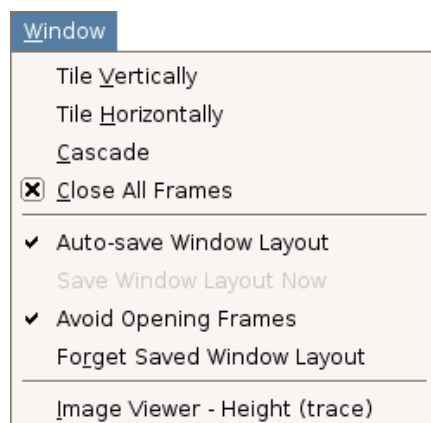
Along the top line of the user interface you will find a pull-down menu bar, as shown here. The table below lists the options available and gives references for more detailed explanations of their functions in imaging.



Pull down menu	Short explanation	Details in section:
	<b>File</b> <b>Open:</b> load AFM images into the scan list <b>Import manipulation pattern:</b> import scalable vector graphics patterns (SVG files) for manipulation <b>Import pattern for background:</b> import SVG background patterns for manipulation <b>Import optical image:</b> import a calibrated optical image into the image viewer. <b>Autosave</b> enables automatic saving of scans <b>Open script</b> loads scripts to perform custom experiments <b>Save manipulation pattern:</b> Save a hand-drawn or modified manipulation pattern as a SVG file. <b>Exit:</b> Close the SPM program	<b>4.3</b> <b>7.2</b> <b>7.2.4</b> <b>9.3</b> <b>4.3</b> <b>10.2.5</b> <b>7.2</b>
	<b>Options</b> <b>Single scans:</b> A single scan (image, force distance curve or force map) is performed, and the scanner is stopped <b>Infinite scans:</b> Continuous scans (images, force distance curves or force maps) are performed, one after another <b>GUI settings:</b> set display of errors and warnings. <b>JPK SPM Jython console</b> gives a direct command line for running macros etc.	<b>10.2.4</b>

<div>View</div> <div> <div>CCD Camera</div> <div> Laser Alignment</div> <div> Oscilloscope</div> <div> Spectroscopy</div> <div> Force Time Oscilloscope</div> <div> Force Mapping</div> <div> Manipulation</div> <div> Spectrum Analyzer</div> <div> Real Time Scan</div> <div> Z Piezo Movement</div> </div>	<div>View</div> <div>Launch the <b>CCD camera</b> 9.1</div> <div>Open the <b>Laser Alignment</b> window 3.1.2</div> <div>Open the <b>Oscilloscope</b> window 5.5.1</div> <div>Change to force <b>Spectroscopy</b> mode § 6</div> <div>In spectroscopy, open the <b>Force Time Oscilloscope</b> 6.1.3</div> <div>Change to force <b>Mapping</b> mode 7.1</div> <div>Change to <b>Manipulation</b> mode 7.2</div> <div>Open the frequency <b>Spectrum Analyzer</b> window 10.2.1</div> <div>Open the <b>Real-time</b> oscilloscope window 10.2.2</div> <div>Open the <b>Z Piezo Movement</b> vertical display 5.2</div>
<div>Motor</div> <div> <div> Stepper Motor</div> <div> Motor Leveling</div> </div>	<div>Motor</div> <div>Control the coarse height position using the <b>Stepper motors</b> 5.2.2</div> <div>Open the wizard for automatic <b>Motor Leveling</b> (if installed) 5.2.3</div>
<div>Setup</div> <div> <div> Calibration Manager</div> <div> <div>Z-Range ▶</div> <div>Approach Parameters</div> <div>Voltage Output Settings</div> </div> <div> Cantilever Tuning</div> <div> <div>Saving Settings</div> <div>Channel Setup</div> <div>Advanced Imaging Settings</div> </div> <div> Advanced Force Settings</div> <div> <div>Spectroscopy Grid Manager</div> <div>Optical Image Calibration</div> </div> <div> Pump Control</div> <div> <div>Temperature Controllers ▶</div> </div> </div>	<div>Setup</div> <div><b>Calibration Manager</b> – set calibration of height, vertical deflection and spring constant § 8</div> <div>Limit the <b>Z-Range</b> of the z-piezo for better z resolution 5.2.1</div> <div>Change <b>Approach</b> gains and initial piezo position 4.1</div> <div><b>Voltage Output Settings</b> for setting a voltage on a DAC 10.2.3</div> <div>Open <b>Cantilever tuning</b> window in intermittent contact mode 3.3.2</div> <div>Set the default <b>Saving settings</b> for automatic saving of data 4.3.2</div> <div>Choose the data <b>Channel setup</b> that are stored to memory 4.3.1</div> <div><b>Advanced Imaging Settings</b> for scan speed and overscan 5.6</div> <div><b>Advanced Force Settings</b> for spectroscopy rates and delays 6.6</div> <div><b>Spectroscopy Grid</b> to set points for spectroscopy 6.3.2</div> <div><b>Optical image calibration</b> for <b>DirectOverlay</b> in SPM 9.3</div> <div><b>Pump Control</b> for syringe pumps 7.4.2</div> <div><b>Temperature Controllers</b> opens controls for installed temperature accessories (see separate manuals) 7.4.1</div>
<div>Image</div> <div> <div>Height</div> <div>Height (measured)</div> <div>Vertical deflection</div> <div>Lateral deflection</div> <div>Error signal</div> </div> <div> <div>Colortable ▶</div> <div> Scan List</div> </div>	<div>Image</div> <div>List of active channels that can be displayed in the image viewer 4.3.1</div> <div><b>Colortable</b>: Choose the color to display the images</div> <div><b>Scan list</b>: manage holding and saving of scan data 4.3.3</div>





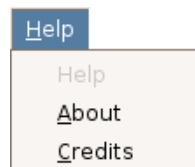
## Window

Settings to allow tidy alignment of open windows

If **Auto-save** is enabled, then sizes and positions of internal windows are remembered **2.2.4**

The personal settings can also be deleted with **Forget**

Select from the list of other currently open windows



## Help

Information about the software version and related projects. **2.3.2**










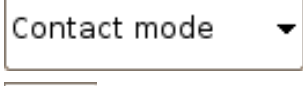



### 2.1.4 The shortcut icon bar

Below the menu bar you can find icons to launch the most commonly used features. A short explanation for each icon is shown in the table below.



Shortcut icon	Brief explanation	Details in Section:
	<b>Open</b> a file and load it into the scan list.	<b>4.3</b>
	Shows the status and activates the <b>Autosave</b> function so that all scans are automatically saved.	<b>4.3</b>
	Open the <b>Stepper Motor</b> window. The stepper motors are for coarse approach or retraction and can also be moved independently to change the tilt of the head.	<b>5.2</b>
	<b>Retract</b> the cantilever from the sample or move the AFM head up with stepper motors.	<b>4.2.4</b>
	Start an <b>Approach</b> and show the with controls in the <b>Approach</b> window.	<b>§ 4</b>
	Shows the status and switches the laser on/off (this is a toggle switch, and can not be switched off during scanning or while approached).	<b>3.1</b>



	Start a scan (only active if the cantilever is approached).	4.1
	Start the <b>CCD</b> -camera attached to the optical microscope or top-view optics.	9.1
	Take a <b>Snapshot</b> and import a calibrated optical image using selected calibration (optional extra software module, active when an optical image is selected)	9.3
	Open the <b>Laser Alignment</b> window to adjust the laser beam onto the cantilever and the photodiode.	3.1.2
	Open the <b>Cantilever Tuning</b> window to find the cantilever resonance for intermittent contact mode.	3.3.2
	Open the <b>Oscilloscope</b> window to observe data from the image channels during scanning (several channels can be viewed simultaneously).	5.5.1
	Change to <b>Force Mapping</b> mode (toggle to return to imaging mode).	7.1
	Open the <b>Spectroscopy</b> window to perform force-distance measurements (toggle to return to imaging mode).	§ 6
	Open <b>Manipulation</b> mode (toggle to return to imaging mode)	7.2
	Choose the feedback mode. The main options are <b>Contact mode</b> or <b>Intermittent contact mode</b> (air or fluid). Some users also have <b>Force modulation mode</b> .	3.3
	Open the <b>Scan List</b> , to view and save image scans.	4.3.3
	Open the <b>Map List</b> , to view and save force mapping data.	7.1.4
	Open the <b>Pump Control</b> interface for controlling syringe pumps.	7.4.2

## 2.2 Introduction to the main display controls

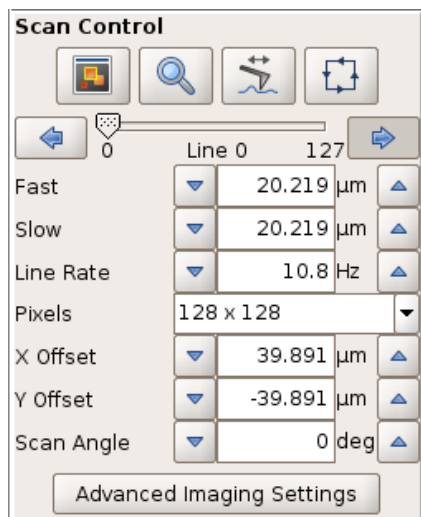
### 2.2.1 Feedback Control and Scan Control

The main parameters for imaging controls are set in the feedback and scan control panels, which are fixed on the left hand side of the SPM software window.

Feedback Control			
IGain	▼	150.0	▲ Hz
PGain	▼	0.005	▲
Setpoint	▼	1.000	▲ V

#### Feedback Control

The features displayed here are explained in **Chapter § 4** **IGain** and **PGain** are part of the PI (proportional-integral) control system. The **Setpoint** value represents the force applied to the sample, depending on the imaging mode. See **Section 5.1** for details on how to choose these parameters.



## Scan Control

The icons in the upper part of the window are introduced briefly below, and further discussed in **Section 4.2.1**.

The parameters displayed in the lower part of the scan control window are also explained in **Section 4.2.1**. They are used to change the scan size and position (offset), the speed of the tip over the surface and the number of pixels captured in the images.

The **Advanced imaging settings** panel can also be opened directly from here for more advanced imaging parameters. See **Section 5.6** for more information.



Open a new **Image Viewer** window, see **Section 2.2.2** and for more details **Section 4.2.2**.



The zoom symbol activates a new scan area that has been drawn in the **Image Viewer** using the right mouse button option **Select new scan region**. See **Section 4.2.3**.

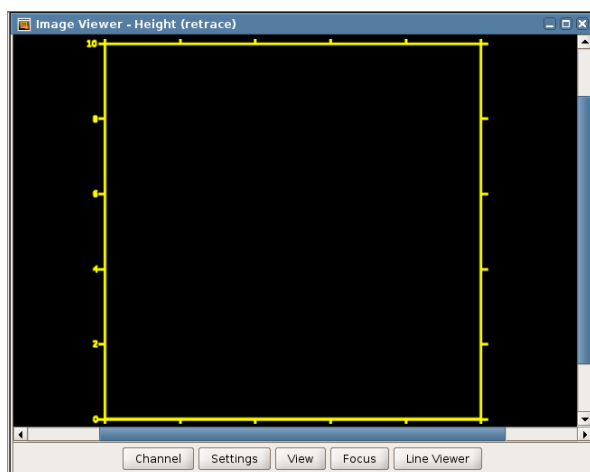


Switch between normal image scanning and **Line Scanning** mode. In **Line Scanning** mode, one scan line is scanned repeatedly, rather than moving to the next line to form an image. See **Section 4.2.1**.



Switch between normal image scanning and **Outline** scanning mode, to get an overview of the sample area using an optical microscope or top view optics. See **Section 4.2.1**.

## 2.2.2 The image viewer



The **Image Viewer** displays real-time the image that is currently being scanned, and also old scans from saved images using the **Scan List** (see **Section 4.3**). Multiple image viewer windows can be opened simultaneously to show data from different channels or with different settings.

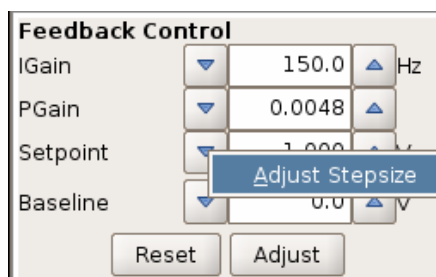
The controls on the bar at the bottom of the image viewer are described briefly below, and mainly control the display settings. The image viewer also allows many controls to be applied graphically, using the right mouse click menu. For more detailed information about using the **Image Viewer**, see **Section 4.2.2**.

### Channel

Many data channels can be collected and saved simultaneously, creating multiple images in one scan. The available channels depend on the imaging mode, but also on the **Channel Setup**, which can be selected from the main **Setup** pull-down menu (see **Section 4.3.1** for more information). Channels can be displayed in either the **trace** (left to right in the normal view) or **retrace** (right to left) scan direction.

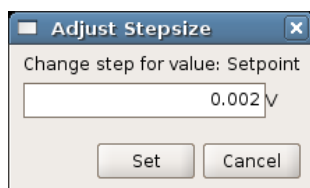
<b>Settings</b>	These settings control the pre-processing of the displayed data and also how the numerical values are converted to a color image.
<b>View</b>	The controls here can be used to rotate and zoom into parts of the image within the viewer. Neither function affects the scanning angle of the cantilever, only the data display. The scan angle can be changed if a new scan region is drawn at a particular angle and selected using the zoom magnifying glass icon. The view also can be changed at any time by using the mouse wheel in the image display area. Scroll the mouse wheel to zoom and click and drag with the mouse wheel to shift the view.
<b>Focus</b>	Rescales the currently scanned or selected image to the borders of the image viewer.
<b>Line viewer</b>	Displays the current line scan for each fast-scan line as it is acquired. The channel and leveling settings are used as for the main image viewer image.

### 2.2.3 Increasing and decreasing values with the increment buttons



The SPM software contains many text fields, where values for the control parameters are shown and new values can be directly entered. The increment buttons (up and down arrows) are used to quickly increase or decrease the value of the text field by fixed steps using the mouse.

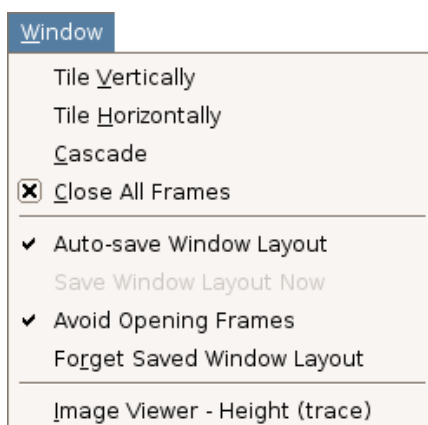
To adjust the step size for each text field (e.g. here the **Setpoint**), right-click with the mouse on one of the arrows, and select **Adjust Stepsize**.



The value can then be updated.

Note, the keyboard shortcuts page-up and page-down can also be used to quickly change a selected value using these increments.

### 2.2.4 Personal display settings



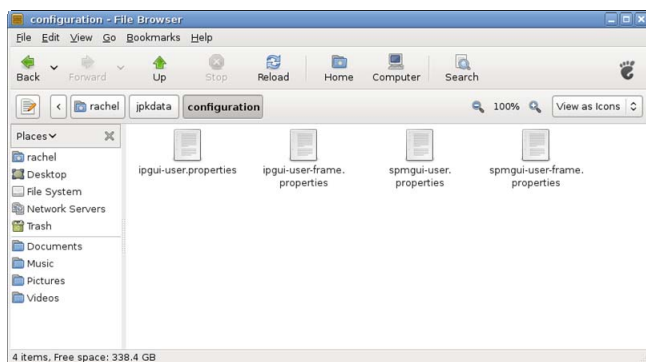
If **Auto-save window layout** is enabled (default is on), then the positions and sizes of the internal windows are remembered when they are closed so they appear the same when they are opened again.

If it is off, the layout can be saved directly with **Save window layout now**.

The default has **Avoid opening frames** turned on. This means that the positions of windows are remembered when they are opened by hand, but the windows are not automatically reopened when the software is started or the mode is changed.

The personal settings can also be deleted with **Forget saved window layout**. This resets all the saved settings, but does not change any windows that are currently open.

The list of windows at the bottom gives the choice to bring any of the currently open windows to the top level.



The personal display settings are saved for each login account under username/jpkdata/configuration

The files here store display and channel settings (saving settings selections etc.) for both SPM and IP.

To reset everything to default, these files can be deleted in the normal file browser *when SPM and IP are closed*. New files will automatically be created with the default values the next time SPM and IP are started.

## 2.3 Software versions and updates

The software programs for online control, **SPM**, and data or image Processing, **IP**, are developed by JPK. If you have any problems or suggestions for improvement, please send an email to [support@jpk.com](mailto:support@jpk.com). SPM and IP are generally installed on an Ubuntu system, but no specialist Linux knowledge is required.

### 2.3.1 Updating the software

Free software updates can be downloaded from <http://customers.jpk.com>. Customers are informed of major new releases by email; in between there may be minor updates for particular features and these can be downloaded at any time. This web page is password-protected; please enter the login name and password. The login-name is your device number, eg. *a0016*, which you can find on the sticker on your AFM head. The password is provided with the AFM system. Note that the website password is *not* the same as the "root" or "jpkroot" administrator password for the instrument computer. The full install instructions are given on the website link, here is just a short version.

To install the file on the instrument computer, log in with the administrator account jpkroot and copy the installer to a local directory, e.g. to the Desktop. The installer can be run from the linux console using this command:  

```
sudo sh "/home/jpkroot/Desktop/jpkspm-xxx.bin"
```

The full file path for the is required (with xxx substituted for the actual release number), and the jpkroot password must be confirmed. If you have lost or forgotten your password, or have problems downloading or installing the upgrade, please contact us for assistance. E-mail [support@jpk.com](mailto:support@jpk.com) or call our technical support line on +49 30 5331 12545.

### 2.3.2 SPM software versions

If you contact JPK for assistance concerning the software it is helpful to know which version of the SPM software you are using. From the main drop-down menu at the top of the window open **Help** → **Info** to see the version number (in this example Version 3.0.11).

If you have a software problem it helps to have as much information as possible about the circumstances. Any errors or warning messages are automatically written to a log file in the data directory, \user\jpkdata. The file name includes the date and time the software was last started, e.g. spm-2008.05.27-21.16.43.log. It will help us to respond quickly to your requests if you send us the file as an email attachment.

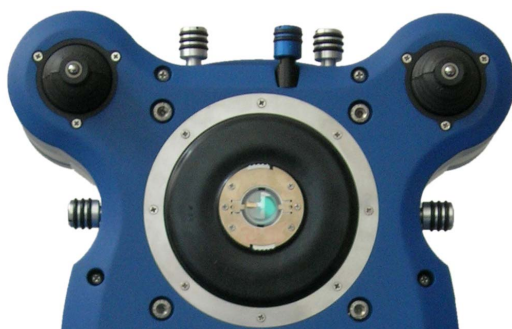


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## § 3 Setting up for imaging

### 3.1 Set up the cantilever and optical detection system

#### 3.1.1 Mount the cantilever



The cantilever is fixed to a glass block, which is locked into the AFM head during scanning. The optical glass is inert against most chemicals, but should be protected against scratches. For cleaning advice see **Section 1.3.1**.

The cantilever is first clamped to the glass block with the cantilever spring, and then held fixed in the AFM head ready for scanning. The glass block can be mounted/removed from the head when it is in the upright position, as shown in the schematic here.



Straight-sided glassblock for use with Top-view optics (Standard stage)

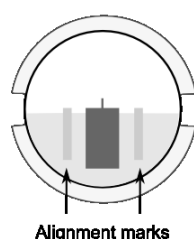


Side-cut glassblock for use with inverted optics (Life Science stage), especially for the BioCell and CoverslipHolder

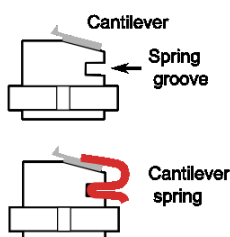
There are two shapes of the glass block, as shown here. The base part that fits in the AFM head is exactly the same in both cases. The alignment of the cantilever is also the same, since the central part of both the glass blocks are the same.

The glass block with straight cylindrical sides is designed for use with the top-view optics setup. It has a large polished region at the end that gives a large field of view for low-magnification optics.

The glass block with the angled end faces is for using with an inverted optical microscope. In this case the field of view is much smaller, and the end region has been cut down to give more space and easier handling in enclosed liquid cells.



Alignment marks

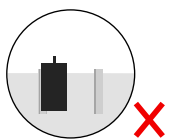
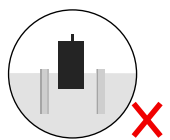
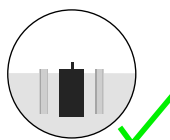


Cantilever

Spring groove

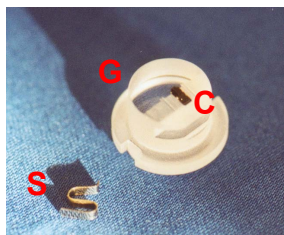
Cantilever spring

*Note that the polished top and bottom surfaces of the glass block must not be scratched! Always put the polished glass surface on a soft lens tissue free of dirt or dust.*



Place the substrate chip of the cantilever onto the inclined part of the glass block. This has an angle of 10 degrees to prevent any part of the mounting assembly from touching the sample during scanning. The cantilever should be placed centrally between the two grooves, with the cantilever arm over the polished glass part, close to the inclined edge. The cantilever itself should be in the centre of the glass block; do not move the cantilever substrate chip too far forwards.



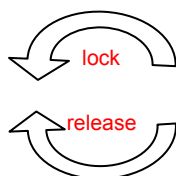
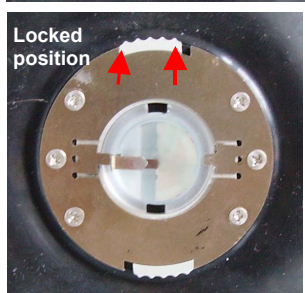
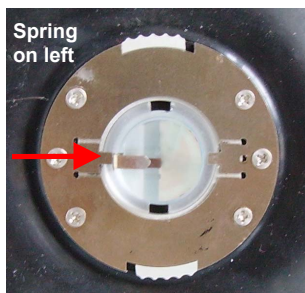


G glassblock  
C cantilever  
S spring



When the cantilever is in the right position, grip the bottom loops of the spring firmly with a pair of tweezers and squeeze to lift the front part. Slide the spring into the groove and release the loops to clamp the cantilever firmly. *Do not touch the optical surfaces with tweezers!*

The spring may gradually become weaker and must be replaced occasionally. Several springs are delivered with new instruments, more can be ordered from JPK if they need replacing or are lost.



The glass block is held in the AFM head with a locking mechanism. The notches in the glass block must be lined up with the metal tabs on the AFM head to place the glass block into the AFM head. The glass block should then be turned by 90 degrees into position.

*Make sure that the cantilever and spring are on the left.*

The glass block can now be locked in position with the two finger grips on the rim of the steel disk. Using the thumb and index finger, turn the knobs slightly to the left and a small click should be felt as the spring locks.

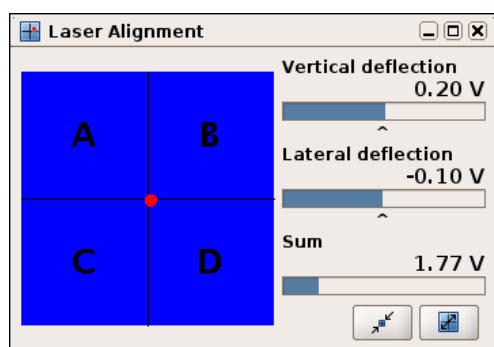
Summary: when the microscope is in its upright position the cantilever spring must be parallel to the desk. *The groove in the glass block the cantilever spring must be on the left.*

### 3.1.2 Setting up the laser detection system

As the tip of the cantilever is scanned across the sample its motion is detected by a laser beam that must be focused onto the cantilever. The angle of the reflected beam from the cantilever is detected by a four-segment photodiode. The reflected laser spot must be in the centre of the detector to give maximum sensitivity for imaging and force control. The adjustment of the laser beam on the top of the cantilever and on the photodiode must be repeated each time a new cantilever is mounted in the glass block, since the cantilever will always be in a slightly different position.



Open the SPM software and click the icon on the shortcut bar to open the **Laser Alignment** window.



The **Laser Alignment** window gives a graphical representation of the detector signals for the adjustment process. The **Sum** value is the total signal from all four quadrants of the detector. If the laser beam does not fall onto the detector, the **Sum** will be 0 V and no red spot is displayed.

If the reflected beam reaches the detector, then the position is represented by the red spot. The actual values of the vertical and lateral deflection are also given in Volts.



The shortcut icons allow the user to toggle quickly between a large and small view. The **maximize** icon enlarges the window, which is convenient for laser alignment during setup. The **minimize** icon returns the window to its smallest size for normal operation.



If there are any problems, check that the laser is switched on in the main SPM icon toolbar. If not, toggle the laser icon. If the laser is on in the software, but the red diode is off on the AFM head, then the laser safety tilt switch is activated. This switches off the laser when the head is upright to protect the operator. When the head is placed on the three motor feet, the laser will switch on again.



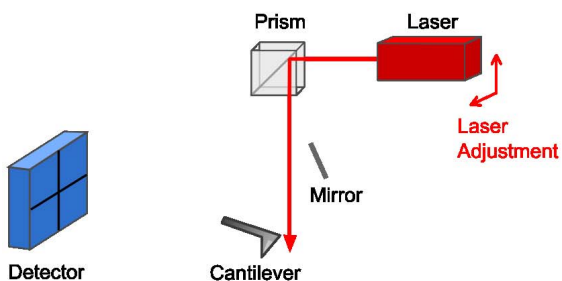
### 3.1.3 Adjust the laser beam onto the end of the cantilever

Use the microscope or top view optics to view the cantilever with the CCD-camera. The IR laser spot can not be seen with the human eye, but the CCD camera is sensitive to a wider range of wavelengths.

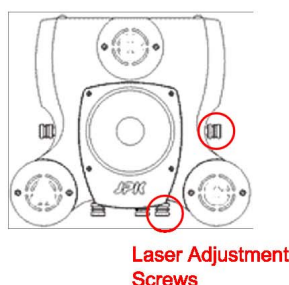
If an inverted optical microscope is used, a low magnification objective is recommended (e.g. 10x or 20x) to give a large field of view and make it easier to find the laser spot. Decreasing the illumination can help to find the laser spot, as scattered light even far from the focus is visible. If the laser spot can not be seen in the optical microscope, check for any filters in the fluorescence path or DIC slider that may be cutting IR wavelengths.

The optical microscope image here shows the laser spot in the field of view, but not yet adjusted onto the cantilever:

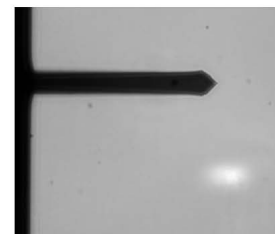
Optical path schematic



AFM head adjustment



Optical microscope image



The laser position must be moved using the adjustment screws until the beam is directed onto the cantilever. Note: All four positioning screws have stoppers in each direction. *Do not over-wind the screws!*

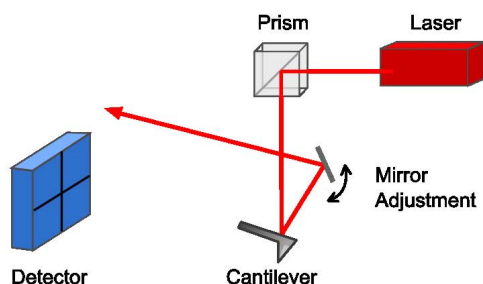
On an optical microscope, the cantilever is viewed from below while the laser beam comes from above. The intensity of the spot will drop as it is adjusted onto the cantilever, because the cantilever blocks some of the light. Soft contact mode cantilevers can be quite transparent, and the laser spot may still be seen through the cantilever.

### 3.1.4 Adjusting the mirror for large changes in deflection

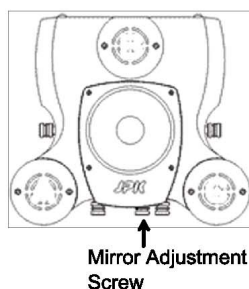
Once the optical image shows that the laser is directed onto the cantilever, the detection system must be adjusted so that the reflected beam falls onto the centre of the photodiode detector. A mirror can be tilted for coarse adjustment of the laser path, and positioning screws are used for fine adjustment.

The mirror and detector adjustment should only be made after the optical image of the cantilever shows the laser is aligned properly. Without proper alignment of the cantilever first, there is no reflection to direct onto the detector

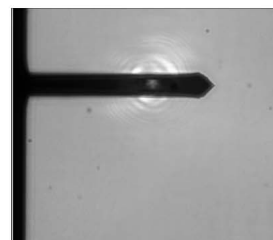
### Optical path schematic



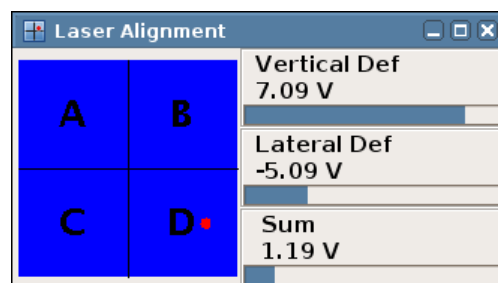
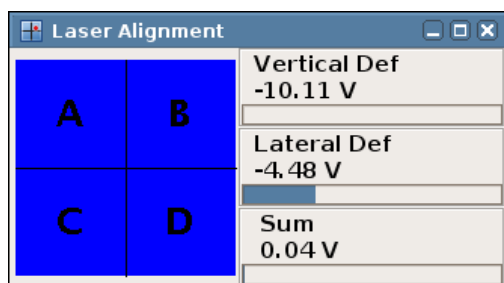
### AFM head adjustment



### Optical microscope image



If the laser spot can be seen on the cantilever, but the **Sum** is close to 0 V, then probably the mirror needs adjusting. A small change in the mirror angle makes relatively large change in the optical path, so the mirror is only used for coarse adjustment. Once the **Sum** is reasonable, the detector positioning screws can be used for fine alignment to maximize the **Sum** and center the spot. The two Laser Alignment screenshots here show the situation before and after adjustment of the mirror.

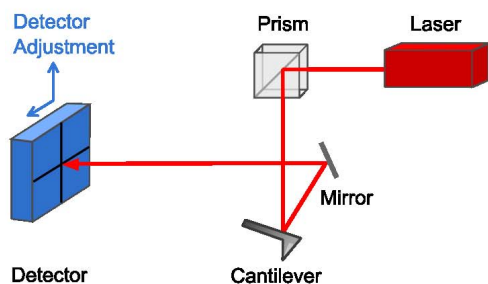


The mirror will not always need readjusting every time a new measurement is made. The mirror helps to correct for the difference in angle of the optical path when the cantilever is immersed in air or liquid. Adjustments need to be made when changing from air to liquid operation or back again. **Usually the mirror will not have to be readjusted for similar cantilevers, except on changing from air to liquid.**

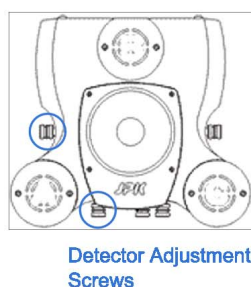
### 3.1.5 Adjust the spot onto the centre of the detector

Once there is a reflection from the back of the cantilever onto the photodiode, then the detector must be moved until the laser beam falls on the centre, using the adjustment screws.

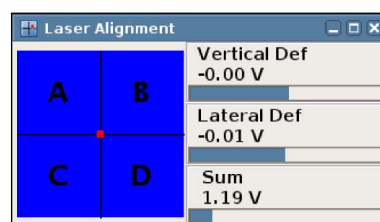
### Optical path schematic



### AFM head adjustment

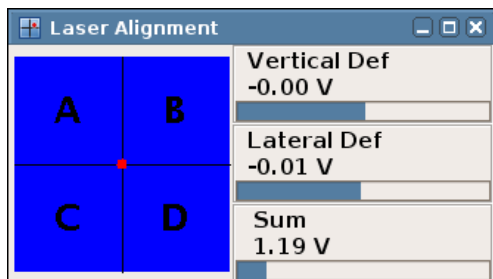


### Laser Alignment Window





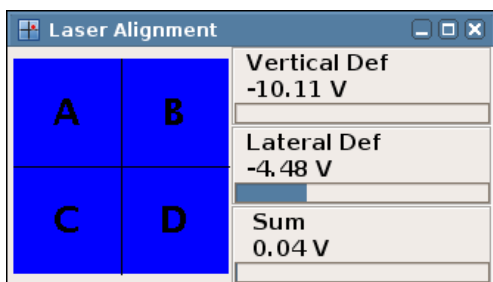
The **Laser Alignment** window in the SPM software shows graphically the signal from the different quadrants of the detector. Adjust the detector adjustment screws so that the red spot is in the centre of the detector in the **Laser Alignment** window. This means that equal intensity is reaching all four quadrants. Note that the spot in the blue region shows the centre position of the laser beam relative to the four quadrants, not the representative size!



This is how the **Laser Alignment** should look like after successful adjustment:

1. **Sum** is at the maximum value for that cantilever.
2. The red spot is in the middle of the crosshair.
3. **Vertical Def** and **Lateral Def** are close to zero.

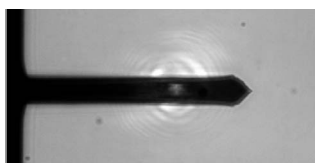
### 3.1.6 Troubleshooting alignment problems



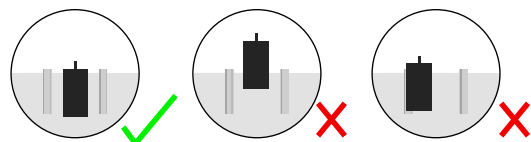
If there are problems aligning the optical system as described above, first check that the **Sum** value is reasonable. If **Sum** is near 0 V, then the optical system is not properly aligned, and **Vertical Def** and **Lateral Def** values are not reliable. The maximum **Sum** value depends on the type of cantilever – both the shape and whether there is any metal coating on the back side. Values vary between around 1.5 V for non-coated silicon cantilevers and around 5V for gold-coated silicon nitride cantilevers.

If the **Sum** is significantly below 1 V, then there is generally a problem and the system should definitely be readjusted.

#### 3.1.6.1 Not able to align laser onto cantilever



Check first that the laser beam is correctly aligned on the cantilever as in the image here. If it is not possible to move the laser onto the cantilever, then probably the position of the cantilever or glass block is wrong.



Check the cantilever position on the glass block: it should be placed centrally between the two grooves, with the cantilever arm over the polished glass part, close to the inclined edge, as shown here. If the cantilever is far forwards or to one side, this may cause alignment problems. Try repositioning the end of the cantilever nearer the centre of the glass block.

Make sure the spring and cantilever are on the left as you look at the AFM head from the front. If they are on the other side, the optical alignment will not be successful.

### 3.1.6.2 Not able to align detector with laser reflection

Sometimes the **Sum** value is reasonable, but **Vertical** or **Lateral deflection** are at the +/- 10 V position. In this case, turning the detector adjustment screws may not change the value, because they stay at the saturated end position. If so, watch the **Sum** in the **Laser Alignment** window as the adjustment screws are turned. When the detector is moving in the right direction, the **Sum** should gradually increase. In the wrong direction, the **Sum** will decrease. When the laser spot is fully on the detector, and the **Sum** value does not increase any further, the **Vertical** or **Lateral deflection** should reach the central 0 V position.

**Note - do not over-wind the screws for the detector or laser!** If this procedure does not work for **Lateral deflection**, then the cantilever is probably damaged. If this procedure does not work for **Vertical deflection**, first move the detector positioning screws back to the middle of the range, then adjust the mirror as described below and in **Section 3.1.4**.

Note also that if the cantilever is damaged or dirty, it may also prevent the reflected laser beam reaching the photodiode. If other changes do not work, try exchanging the cantilever for a new one of the same type. Check also for dirt or scratches on the surface of the glass block – this can also interfere with the optical path. Focus on the glass block surface with the top view optics or inverted optical microscope (in this case optical phase contrast can be very useful) to check for dirt or scratches above the cantilever.

### 3.1.6.3 Unstable Vertical or Lateral Deflection values

Sometimes the initial alignment seems successful, but afterwards there are problems that the vertical or lateral deflection values change a lot. In air there may be a systematic change as the cantilever approaches a surface because of electrostatic effects. This can be corrected by just adjusting the laser again to the centre of the detector.

In liquid there are more complicated problems, because the deflection of the cantilever is sensitive to many environmental effects. If the vertical deflection shows a large jump or sudden large changes, then always check the cantilever optically first. One common problem is that of air bubbles sticking to the cantilever, particularly if the buffer or medium contains a high protein concentration (e.g. cell medium with added serum/FCS). In experiments with cells or large objects, sometimes material from the sample can adsorb to the cantilever, causing a change in deflection.

One specific problem in liquid is when aluminium-coated silicon cantilevers are used. Soft silicon cantilevers can usually be bought either with or without aluminium coating on the back side. The metal coating is used to increase the reflected laser signal and is fine for use in air, but it is not stable in liquid. The coating may corrode, or even peel off the cantilever completely, causing unstable changes in the vertical deflection and sum values. JPK does not recommend using aluminium coated cantilevers for any experiments in liquid. It is important to check the product codes when ordering silicon cantilevers, since it is possible to order the coated ones without realizing it. Check the back side of the cantilever (the side that is usually hidden when lying face-up on a gel pack). Both sides of the cantilever should be the same shiny dark grey color. If one side is a bright silver color, this is usually the aluminium coating and the cantilever should not be used in liquid.

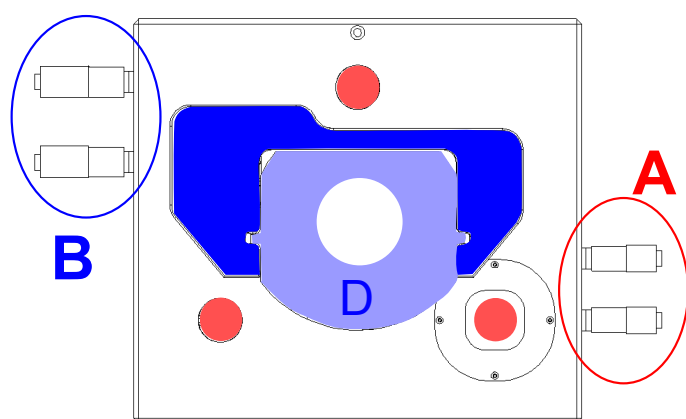
Silicon nitride cantilevers are almost always supplied with a gold back side coating, because uncoated silicon nitride cantilevers would have almost no laser reflection. In this case, the gold coating is stable in water-based liquids and there are no long-term problems with deterioration of the coating. There is, however, an increased temperature and pH or ionic strength sensitivity. The different surface materials react differently, causing a bending seen in the Vertical deflection signal. For soft silicon nitride cantilevers, it is important to reduce the variations in temperature and liquid conditions, especially in contact mode. Sometimes it can help to work in a liquid cell rather than a free droplet, for instance. In intermittent contact mode the amplitude is not as directly sensitive to these changes as the deflection in contact mode.

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## 3.2 Mounting the sample

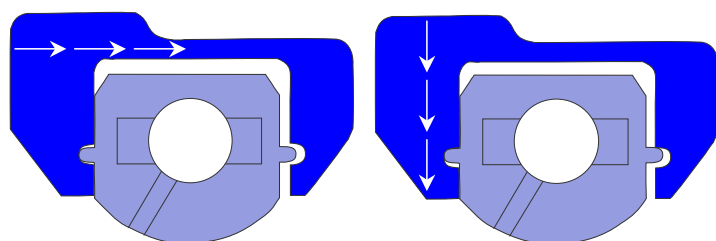
The most important thing in AFM microscopy is to avoid unwanted movement between the tip and the sample (such as vibration, drift or other movements due to unstable mounting of the sample). Therefore the sample must be properly mounted in order to reduce vibrations and drift.

### 3.2.1 Life Science stage

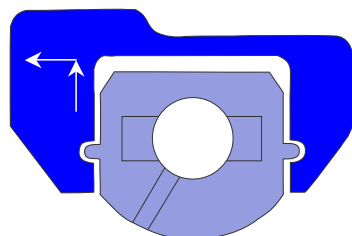


**The positioning screws A move the AFM head.** The three foot positions are marked in red. The combination of a point, a line and a flat surface ensures reliable positioning, so that the head will always fit stably into the same position. Always ensure that the feet slot firmly into position.

**The positioning screws B move the sample holder.** The sample is mounted on the inner sample holder D. The arms of C are moved by the positioning screws and push the inner sample holder.



The positioning arms do not grip the sample holder tightly. There is a small gap between the arms and the centre part. The gap is shown here exaggerated, to make it clear how the sample is pushed by the positioning arms.

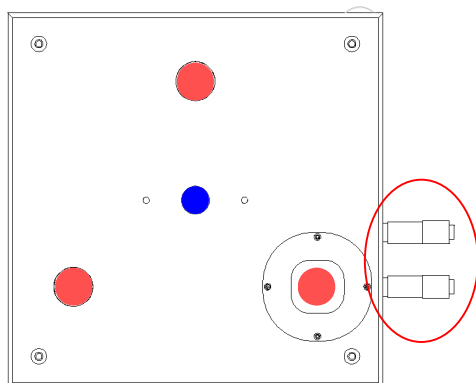


When the sample is in the correct position, the sample holder must be released by turning the positioning screws BACK one quarter turn. In the life science version, one notes that the sample does not move during this de-coupling. The sample holder is then free from contact with the positioning arms, as shown here, but still held firmly by the magnetic contacts underneath.

**The release mechanism is very important for high resolution imaging to remove the mechanical coupling between the sample and holder.**

The standard sample holder fits biological standard samples such as petri dishes (50 mm diameter, height below 10 mm) and microscope slides, as well as custom made fluid cells. Special sample holders are available from JPK, such as the BioCell and CoverslipHolder, which offer advanced performance such as temperature control, perfusion etc. Sample preparation information, including different suitable petri dishes, is given in the JPK AFM Handbook.

### 3.2.2 Standard stage



**The positioning screws A move the AFM head.** The three foot positions are marked in red, and are very similar to the Life Science stage. The point, a line and flat surface ensures reliable positioning, so that the head will always fit stably into the same position. Always ensure that the feet slot firmly into position.

**The sample position is fixed.** The central magnet can be used to hold samples mounted on standard holders such as magnetic steel stubs. Standard sizes are 12 mm diameter, 0.9 mm thickness. For imaging in air (where there is less drag from the head movement) samples can also be prepared on normal glass microscope slides. For imaging in liquid, a more firm mount is required, such as the magnetic attachment.

## 3.3 Selection of feedback mode

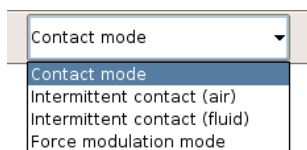
The two main imaging modes are **contact mode and intermittent contact mode**. In contact mode the cantilever deflection is used as the feedback signal, and a setpoint is chosen to work in constant force mode. In intermittent contact mode, the cantilever is oscillated and the oscillation amplitude is used as the feedback signal. See the JPK AFM Handbook for a fuller discussion of the different modes and their application to different types of imaging or samples.

### 3.3.1 Contact Mode

To image in contact mode, first attach a contact mode cantilever to the glass block and align the laser beam as described above. The currently selected imaging mode is shown in the shortcut bar across the top of the SPM software, and contact mode is the default imaging mode when the SPM program is launched:



### 3.3.2 Intermittent Contact Mode



To perform experiments in intermittent contact mode the correct option should be selected from the feedback mode list in the shortcut bar.

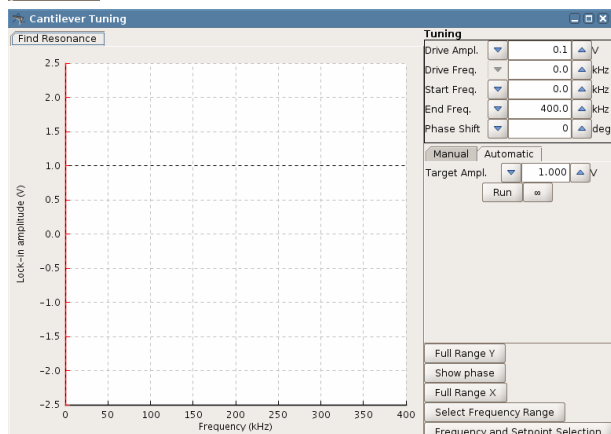
Choose:

- **Intermittent contact mode (air)** for oscillating the cantilever in air. A non-contact cantilever is usually used in this case (generally a stiff cantilever, with a high resonant frequency, 200-400kHz).
- **Intermittent contact mode (fluid)** for oscillating the cantilever in water or other fluid. Triangular silicon nitride cantilevers with a spring constant around 0.3 N/m are suitable for this type of application.

For more information on intermittent contact mode and cantilever choice see the JPK AFM Handbook. The resonance of the cantilever must be found, and the drive amplitude set before an appropriate setpoint can be chosen for imaging.



Make sure that the cantilever is retracted from the sample, and click the **Tune** icon to open the **Cantilever Tuning** window.

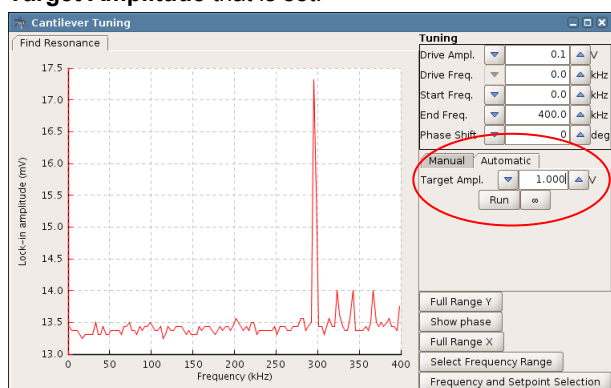


The default values for **Start Frequency** and **End Frequency** are set differently for intermittent contact mode in air or fluid. In air, the default range is 0 - 400kHz (max. freq. 2 MHz), and in fluid it is 0 - 50kHz. The frequency range can be set by entering values in the text boxes at the top of the window.

The user has the choice between **Manual** or **Automatic** tuning, which is selected using the register cards in the middle of the window. The buttons in the lower part of the window control the display of the frequency curves and the selection of the drive frequency and setpoint.

### Automatic cantilever tuning:

For **Automatic** cantilever tuning, the values of the drive and gains are chosen by the software to try and achieve the **Target Amplitude** that is set.

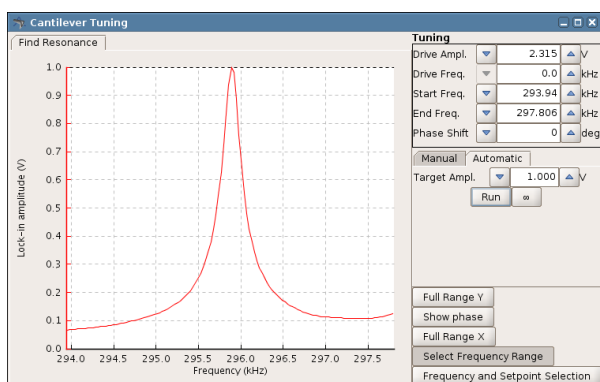


Select a value of the **Target amplitude** (default is 1 V).



Click the infinity symbol to run continuously (repeatedly perform sweep curves). Click **Full Range Y** for a spectrum overview.

**Red curve:** frequency. **Blue curve:** phase (only plotted if the **Show phase** button is activated).



Click **Select frequency range** to zoom in around the resonance peak. Click on the left of the peak and drag the cursor to the right of the peak to define the range to display.

Typical resonance frequencies:

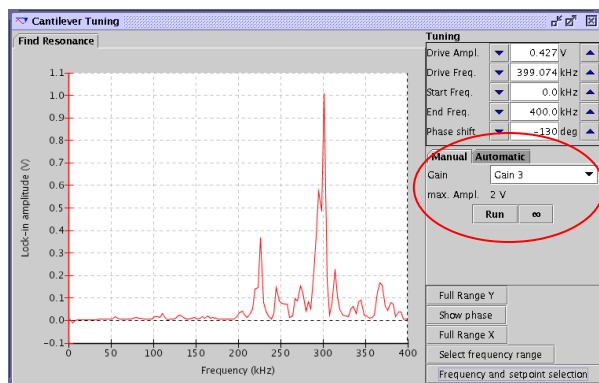
Stiff silicon cantilevers (in air) 100 – 400 kHz,

Soft silicon or silicon nitride cantilevers (in liquid) 1 – 50 kHz

Follow the step **Frequency and setpoint selection** described below. (**Section 3.3.2.1**)

### Manual cantilever tuning:

For **Manual** cantilever tuning, the user sets their own values of the drive amplitude and gains. The frequency range should be set as above (the default values are the same as for Automatic tuning).

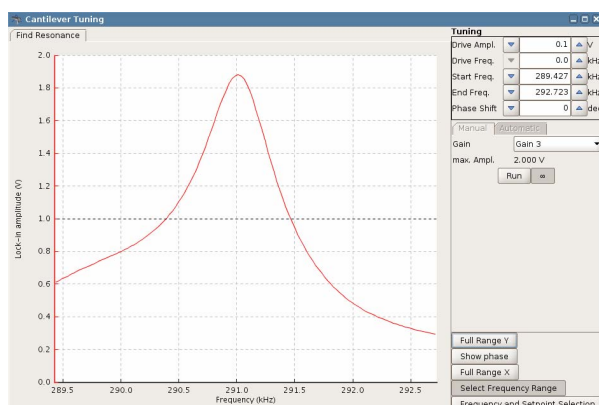


Enter a **Drive Amplitude** value. In fluid a higher drive amplitude is required because of the extra damping from the water or other liquid.

Select the **Gain** for the expected range of amplitude: e.g. select **Gain 1** for lock-in amplitudes up to 8 V, select **Gain 8** for lock-in amplitudes up to 62.5 mV.



Click the infinity symbol to run continuously – the instrument will repeatedly perform sweep curves, while the values can be adjusted.



The display of the frequency curve is as above for automatic tuning. Again, click **Full Range Y** for a spectrum overview and click and drag using **Select frequency range** to zoom into the resonance peak.

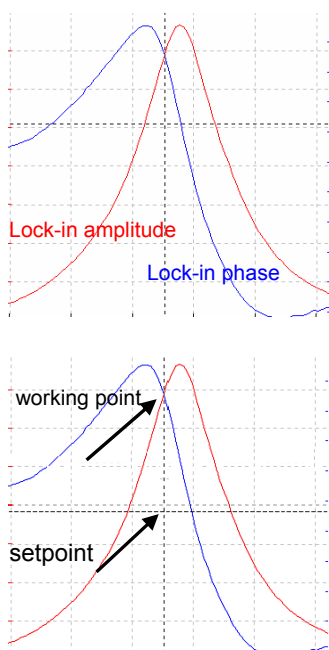
Adjust the value of the **Drive Amplitude** get a good signal to noise ratio of 6:1 or better for the cantilever resonance peak.

In this example Gain 3 has been chosen and a lock-in amplitude of just below 2 V has been reached.

### 3.3.2.1 Frequency and setpoint selection

#### Frequency and Setpoint Selection

The frequency and setpoint selection button enables the selection of the drive frequency and setpoint for imaging using the mouse.



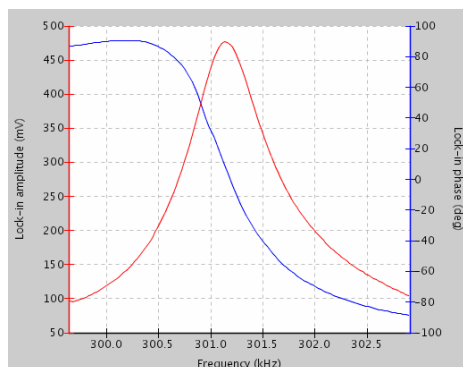
Click inside the amplitude/frequency plot area to get a cross hair of black dotted lines. Holding the mouse button down, move the cross hair to choose a suitable frequency and setpoint, as in the example shown here.

The **Drive Frequency** is usually chosen on the left upper rising edge of the peak. The value of the **Setpoint** must be lower than the chosen lock-in-amplitude in the **Cantilever Tuning** window. The setpoint is usually chosen around 70 – 80 % of the lock-in amplitude at that frequency, although the actual numbers depend on the sample and conditions.

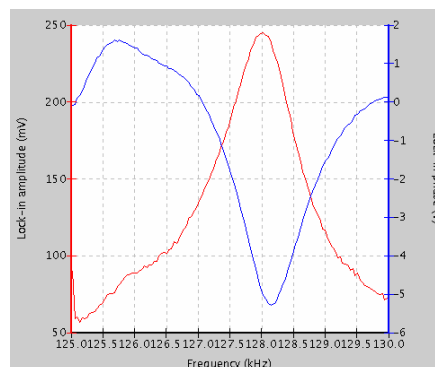
If the setpoint is set too low, as in the example here, the cantilever imaging forces may be too high and damage the sample or the tip. In liquid, however, the free amplitude may decrease between the retracted position where the cantilever tuning is performed, and the surface. In this case, a lower value of the setpoint will be required to reach the surface. It is safer to start with a higher value and then reduce it if necessary, rather than risk damaging the tip or sample.

### 3.3.2.2 Oscillation amplitude and resonance curves

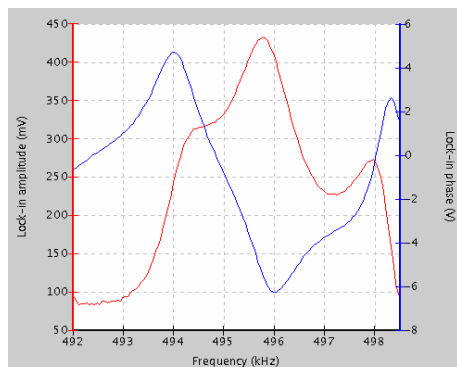
It is important to have a good signal-to-noise ratio. This means that the resonance curve must be well-shaped and its peak must be high enough above the background noise. Signal-to-noise at about 6:1 or better is reasonable. The signal-to-noise ratio can be improved by increasing the drive amplitude, but do not increase it too much. Try to keep the peak of the resonance curve below 2 V lock-in amplitude.



Well-shaped resonance curve, good signal-to-noise ratio.



Critical signal-to-noise ratio (approx. 4:1)

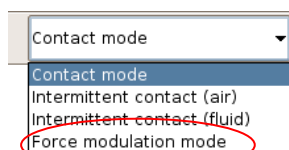


Badly-shaped resonance curve.

Possible reasons for badly shaped resonance curves:

- Contamination of the tip with adsorbed dirt
- Cantilever or tip physically damaged
- Chip of the cantilever not tightly fixed to the glass block
- The tip is making contact with the sample during the cantilever tuning

### 3.3.3 Force modulation mode



Force modulation mode is not available for all of our customers. This mode must be specially requested, since it depends on the design of the z-amplifier in the controller.

In the graphical user interface **Force modulation mode** can be found in the drop-down menu with contact and intermittent contact mode. Force modulation mode is something of a mixture between contact mode and intermittent contact mode and can be thought of as a kind of contact mode with an added vibration of the cantilever. See **Section 7.3** for more details. The cantilever tuning for Force Modulation mode is similar to the normal tuning procedure for intermittent contact mode. For imaging, the setpoint value is the average vertical deflection, and the feedback control is therefore similar to contact mode. The oscillation of the cantilever provides extra amplitude and phase channels to observe differences in mechanical properties of the sample.



## 3.4 Measurements in liquid

The NanoWizard® AFM is optimized for performing experiments on samples in a liquid environment. The instrument is designed to protect sensitive parts of the microscope, but particular care is still needed when working in liquid.

The glass cantilever holder is easy to clean and chemically inert and well-suited to working in buffer solutions as well as in low-concentration bases and acids. The cantilever holder spring is made of medical steel, or on request a gold-coated spring can also be supplied. The glass block can be immersed up to the upper edge of the groove that holds the spring. This is a maximum immersion depth of approximately 4-5 mm.

The bottom of the AFM head is sealed against vapor to protect the scanning system from water, buffer or any other liquid whose vapor could damage the scanning system. For tips on cleaning the glass block see **Section 1.3.1.1**. *Always remove the glass block immediately after your experiment and clean it.*

### 3.4.1 Setting up in liquid

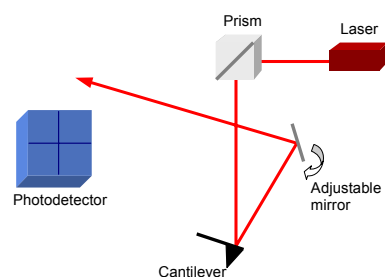
When the bottom face of the glass block and the cantilever are immersed in liquid the optical path of the laser beam reflected from the tip is changed because of the difference in refractive index between air and the liquid. Therefore you will need to correct the optical path with the adjustment mirror when you immerse the cantilever in liquid. See **Section 3.1.4** for more details.

**Tip:** When the system was set up for experiments in air and should now be adjusted for liquid, turn the mirror a few degrees to the right. Turn it to the left when you want to scan in air after an experiment in liquid.



For the NanoWizard®I, the mirror adjustment is on the front of the AFM head in a relatively deep recess. The screw is marked by a red circle on the image here. Insert a small screwdriver carefully in the black plastic housing, and turn very gently. The full turning angle of the screw is only approximately 100 degrees with stoppers at both ends. *Do not over wind the screw!*

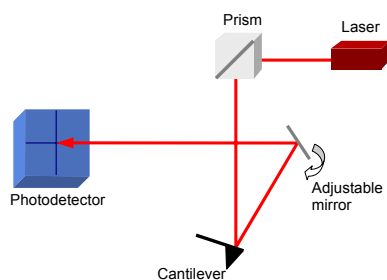
On the NanoWizard®II head the mirror is adjusted using a screw that looks like the laser and detector positioning screws. This is the central screw at the front of the head (marked by a red circle in the image). There is a release mechanism so that the positioning screw is not permanently in contact with the mirror, to minimize drift. Push carefully on the finger grip and turn gently to make contact. It is easy to feel when the mechanism slots into place and the mirror can be turned. Again, the range of movement is quite small. *Do not force the mirror!*



The mirror helps to correct for the difference in angle of the optical path when the cantilever is immersed in air or liquid. Adjustments need to be made when changing from air to liquid operation or back again.

A small change in the mirror angle leads to a relatively large change in the optical path, so the mirror is only used for coarse adjustment. Once the **Sum** is reasonable, the detector positioning screws can be used for fine alignment to maximize the **Sum** and center the spot.





See **Section 3.1.4** for more details.

The screw movement for the mirror adjustment is very stiff. This is to prevent drift in the system; turning carefully will move the mirror the small distance needed.

### 3.4.2 Using intermittent contact mode in liquid

Load an appropriate cantilever (spring constant around 0.3 N/m, often a silicon nitride triangular cantilever, see our User Handbook for more details) and use the steps above to align the laser detection path. For optimal conditions, the oscillation of the cantilever should be adjusted when the tip is relatively close to the surface. Therefore we recommend approaching the surface in contact mode, then going to piezo retracted mode by clicking the retract icon only once, to set up the parameters for the intermittent contact mode imaging close to the surface.

1. Start with **Contact mode** as the feedback mode.
2. **Approach** until the cantilever is in contact with the sample.
3. Click **Retract** once to put the instrument in piezo retracted mode (indicated in the system status panel)
4. Change to **Intermittent contact mode (liquid)** as the imaging mode.
5. Perform a sweep curve in the **Cantilever Tuning** window, as described previously for IC mode in air. The resonant frequency of the cantilever will be much lower in liquid, because water is moved with the cantilever.
6. **Approach** again, to image as normal in intermittent contact mode.

Resonance frequency values of about 8 - 12 kHz are typical for the silicon nitride probes around 100  $\mu\text{m}$  long, with spring constant around 0.3 N/m. The resonance frequency of the cantilever depends on several parameters, including:

- The total amount of fluid and the depth of the cantilever in the liquid
- The properties of the cantilever itself
- The geometry of the vessel/fluid cell
- The viscosity of the liquid

If the cantilever tuning is performed far from the surface, the resonance frequency may have moved significantly by the time the cantilever reaches the surface. Therefore the setpoint may need to be much lower than expected to reach the surface. If the cantilever tuning has been performed far from the surface, then it is recommended to retract using the piezo only, by clicking the retract button once, as described above to re-tune the cantilever near the surface.

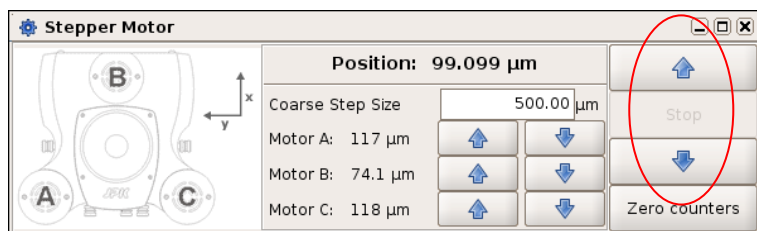
It can be helpful to compare the tuning frequency peaks with the natural frequency response of the cantilever. This can be done by looking at the thermal noise spectrum. See Section 8.3 for more information. For a simple frequency comparison, the sensitivity measurement is not necessary, and a typical value can just be entered in the text field without making the force spectroscopy measurements first.

## 3.5 Coarse approach

The head can be raised or lowered using the stepper motors. This allows the head to be moved up for higher samples, or moved down to be closer to lower samples. The approach routine will take a long time if the cantilever is far from the sample surface, so usually the distance is initially reduced using the stepper motors. This is generally known as the coarse approach, and the controlled approach using the feedback is known as the fine approach.



The motor icon in the shortcut menu bar opens the **Stepper Motor** window.



The coarse step size can be set in the Stepper Motor window.

The **Up** arrows move the cantilever away from the sample, and the **Down** arrows move the head towards the sample.

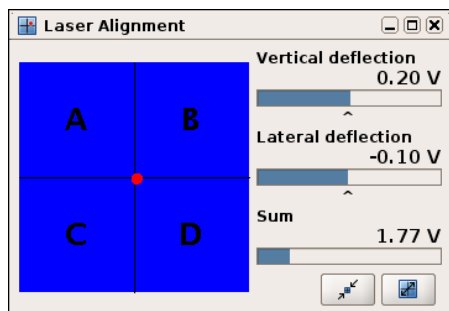
**Warning:** If in doubt about the tip-sample separation, start with relatively small step sizes. During the coarse approach, watch the movement of the glass block.

*Do not crash the glass block and cantilever into the sample!*

The motors can also be moved independently (see **Section 5.2** for details), to remove any overall tilt between the sample and the AFM head.

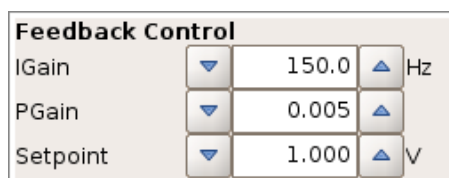
## § 4 Approaching and imaging

### 4.1 Approaching



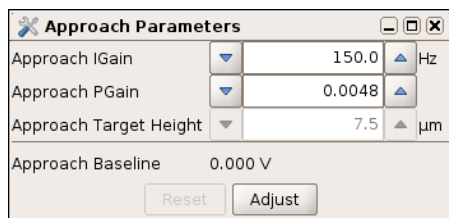
Before trying to approach, make sure that the laser is properly aligned on the cantilever and photodetector, as described in **Section 3.1**. The **Sum** signal should be around 1V or above, and the **Lateral** and **Vertical Deflection** should be near 0V.

The correct imaging mode should be selected on the shortcut bar at the top of the window (see notes for using intermittent contact mode in liquid, **Section 3.4.2**).



A suitable setpoint value should be set in the **Feedback Control** panel. In **Intermittent contact** mode, this is chosen using the **Cantilever Tuning** window, as described in **Section 3.3.2**. In **Contact mode**, a value should be chosen depending on the vertical deflection on the photodiode and the spring constant of the cantilever. A value of around 1V should be reasonable for a soft cantilever, if the vertical deflection is around 0V.

*Note that in intermittent contact mode, a lower setpoint value means a higher imaging force, while in contact mode, a lower setpoint value means a lower imaging force!*



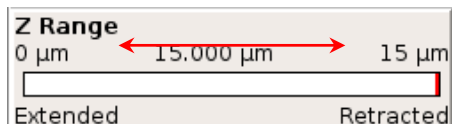
Choosing **Setup** → **Approach Parameter** from the main pull-down menu allows you to set the gains for the approach. The default values are **Approach IGain** 150Hz and **Approach PGain** 0.0048. Note that these gains only affect the approach. Change the values in the **Feedback Control** panel to adjust the gains used for scanning and waiting on the surface.

The **Approach Target Height** sets the z-piezo height on the surface at the end of the approach. The default puts the piezo in the middle of the current z-range, to give maximum piezo range above and below the starting value. Approaching onto glass beside a cell, for example, the **Target Height** should be set around 2-3 microns, to give maximum range above the glass for the cell.

There is also an advanced option in the software to approach using a variable setpoint using the **Approach Baseline** setting – see **Section 5.4**.

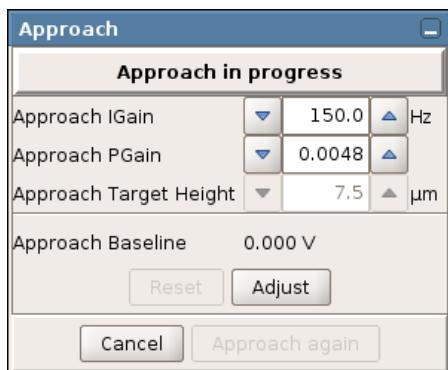


Click the **Approach** button in the icon bar to start the approach routine, and you should hear the stepper motors move the NanoWizard head.



During the approach, the z-piezo extension and retraction can be observed with the **Z Range** display on the left. The position of the red line should oscillate from right to left as it checks for the sample surface at each step.

*If the red line does not cover the full range of the piezo, then the approach will probably be unsuccessful!*

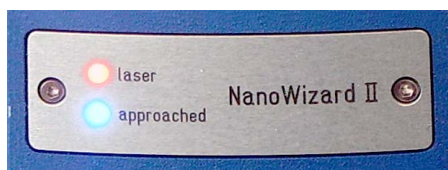


The **Approach** window is displayed during the motion, and the approach parameters can be altered during the approach.

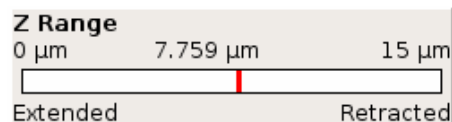
To make a slower approach (for instance to approach more carefully on a delicate sample or with an extra-sharp cantilever), reduce the gains in the approach window. This can also help if the feedback rings during the approach. Alternatively, reduce the setpoint force (decrease the setpoint in contact mode, or increase it in intermittent contact mode).

To make a faster approach, the gains in the approach window can be increased. Alternatively, the setpoint force can be increased (increase the setpoint in contact mode, or decrease it in intermittent contact mode).

To confirm a successful approach, check the following settings:



The blue **approached** LED should be illuminated on the front plate of the AFM head.



The software panel **Z Range** should show the red bar in the centre, as shown here (or at the **Target Height** set in the **Approach Parameter** window, if that is different).

Sometimes the blue light is illuminated on the front plate of the AFM head but for some physical reason the cantilever is not in real contact with the surface. If the **Z-Range** shows the red bar at the ends of the range (i.e. 15 microns, fully retracted, or 0 microns, fully extended), the approach has been unsuccessful. Sometimes the routine may find the surface if **Approach again** is clicked, but sometimes the value of the setpoint must be adjusted to reach the surface.

There are two common problems approaching:

1. The feedback is too sensitive, causing oscillations in the vertical deflection, and possibly an audible ringing tone. In this case, follow the instructions to make a more slow, careful approach. Decrease the approach gains (especially **Approach IGain**) and/or reduce the setpoint force (decrease the setpoint in contact mode, or increase it in intermittent contact mode). If it is impossible to get a stable approach where the red bar covers the whole z-piezo, sometimes it is helpful to reduce the z-range slightly, for instance to 5.85 or 12 microns.
2. The feedback is too slow, meaning that the approach routine is not searching the full z-range. This can lead to an over-slow approach, to false/unsuccessful approaches, or to the head moving too far away from the sample. Increase the approach gains (especially **Approach IGain**) and/or increase the setpoint force (increase the setpoint in contact mode, or decrease it in intermittent contact mode).

## 4.2 Imaging

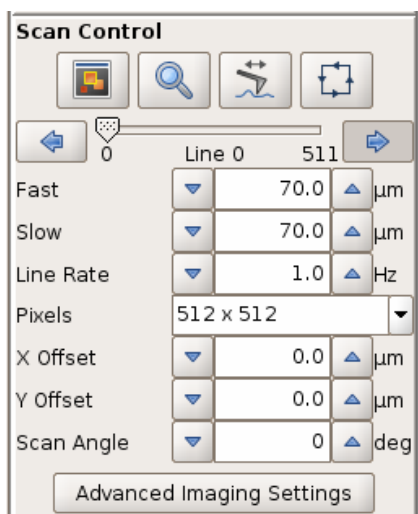


After a successful approach, the Run icon in the top shortcut menu bar will be active. When **Run** is clicked, the scanner will start moving. By default, **Run** launches an infinite series of scans. If only a single scan is desired, this can be changed under the main menu **Options**.



To stop the scan click **Run** again, or click the retract button to leave the surface (this can be used as a kind of emergency stop, it will interrupt the movement if the scan has been started with inappropriate settings).

### 4.2.1 Image properties and the Scan Control panel



The main controls for scanning are found in the **Scan Control** panel.

During a scan, most of the options (except the **Line Rate** and feedback settings) in the **Scan Control** panel become inactive. The feedback parameters and scan speed can be altered during a scan, but the size, shape or offset of the scan can not be changed. To change these settings, stop the scan (by toggling **Run**) and restart with the new values.

Further parameters can be set using the **Advanced Imaging Settings** panel, which can be opened through the **Setup** menu or directly from the **Scan Control** panel. Here more complex parameters can be set, such as the duty cycle or overscan, or non-square scan areas can be enabled. See **Section 5.6** for details.



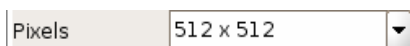
The image size can be set using the text fields for the **Fast** and **Slow** scan sizes, as well as by using the mouse in the **Image Viewer**. The default is for square scans, so if one size is changed, the other one is automatically updated. This can be disabled in the **Advanced Imaging Settings** panel.



The **Line Rate** controls the speed of the scan. Typically the line rate is set to values between 0.5 and 2.0 Hz. Choose generally lower scan rates if:

- the sample is rough/strongly corrugated
- the scan size is high
- intermittent contact is selected as the imaging mode

Note that the full range of values to control the speed of the tip over the surface are found in the **Advanced Imaging Settings** panel.



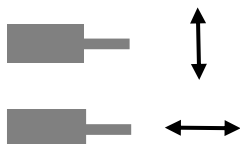
The resolution of the image can be set with the number of **Pixels**. Normally it is set to 512 x 512. The higher the pixel number, the better the resolution for a given image size, but the longer the scan will take, because there will be more scan lines.

X Offset	<input type="text" value="0.0"/>	<input type="text" value="μm"/>
Y Offset	<input type="text" value="0.0"/>	<input type="text" value="μm"/>

The total scan region of the piezo is 100 x 100 microns. Within that range, smaller scans can be performed in different locations. The **X** or **Y Offset** can be entered directly in this text field, or set using the mouse in the **Image Viewer**. If the instrument is left on over a night or weekend, then the scan offset should be set close to zero to reduce strain on the piezo. Likewise, for very prolonged scanning it is better to offset the sample than to scan a small region right on the edge of the piezo range.

Scan Angle	<input type="text" value="0"/>	<input type="text" value="deg"/>
------------	--------------------------------	----------------------------------

The Scan Angle can also be set graphically or using the text field. By default, the scan angle is set to 0 degrees.



At values of 0 or 180 degrees, the fast scan direction is perpendicular to the cantilever.



At values of 90 deg/270 deg the fast scan direction is parallel to the cantilever.

The icons in the **Scan Control** panel allow you to move around the sample surface, together with the **Image Viewer**:



Open a new **Image Viewer** window, see **Section 4.2.2**.



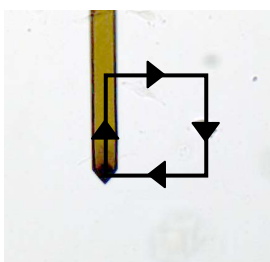
The zoom symbol activates a new scan area that has been drawn in the **Image Viewer** using the right mouse button option **Select new scan region**. See **Section 4.2.3**.



Switch between normal image scanning and **Line Scanning** mode. In **Line Scanning** mode, one scan line is scanned repeatedly, rather than moving to the next line to form an image. This can be useful for adjusting settings, or for flat samples that do not show surface features on every part of the sample. The scan line is set using the **Line** slider in the **Scan Control** panel.

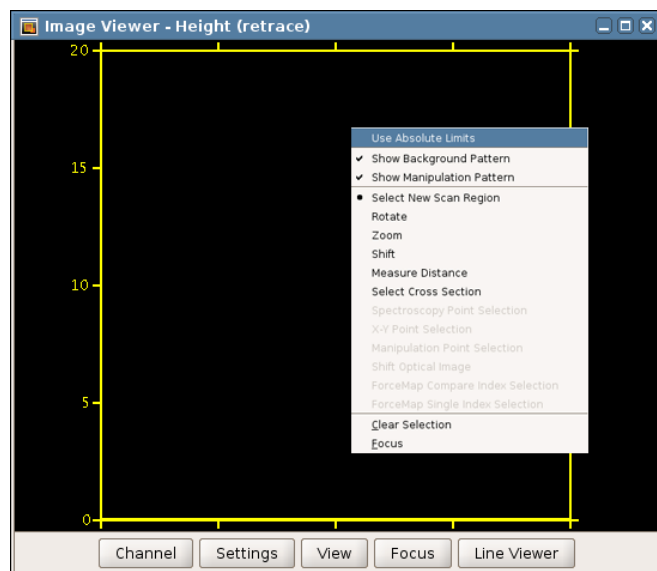
<input type="button" value="←"/>	<input type="text" value="0"/>	Line 0	<input type="text" value="511"/>	<input type="button" value="→"/>
----------------------------------	--------------------------------	--------	----------------------------------	----------------------------------

The slider displays the current scan line number, and can be used to move the tip over the surface to a particular part of the sample (click and drag on the slider arrow). It is possible to move the slider by single steps by clicking on the background of the slider to the left or right of the "handle", or by activating the slider by clicking on it, then using the "left" and "right" arrow keys on the keyboard. Once the slider is in the chosen position, a normal scan can be started using **Run**, or **Line Scanning** can be started with **Run** if it is selected. The repeated scan lines do not form an image, so the **Line Viewer** (**Section 4.2.2**) or **Oscilloscope** (**Section 5.5.1**) should be used to display the height profile.



The **Outline** function makes the cantilever repeatedly perform a square or rectangle movement around the edge of the scan region that is currently set (during the outlining process the scanner is in piezo retracted mode). In conjunction with an optical microscope or top-view optics this feature helps to set a suitable area prior to scanning, and the user can decide if the selected scan region is the right size or in the right location before approaching and imaging.

## 4.2.2 The Image Viewer window



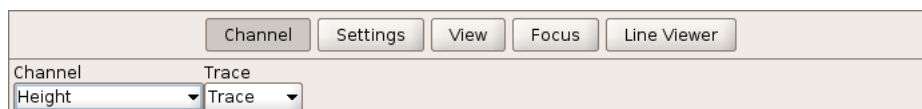
The **Image Viewer** displays real-time the image that is currently being scanned, and also old scans from saved images using the **Scan List** (see **Section 4.3** Managing and saving images).

Multiple image viewer windows can be opened simultaneously to show data from different channels or with different settings.

More options for moving the scan region and displaying information can be found in the menu that appears when you right-click with the mouse within the image viewer window (see also **Section 4.2.3**).

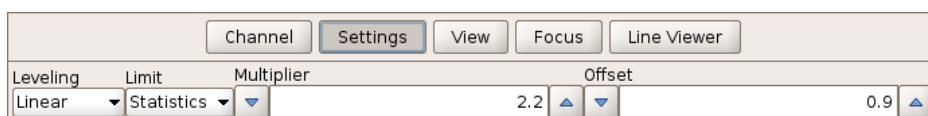
The controls on the bar at the bottom of the **Image Viewer** are used to set the selected channel, the scale and orientation of the image view and the colorizer settings.

### Channel



Other channels apart from the height can also be displayed. The channels available depend on the imaging mode, and on the **Channel Setup**, which can be selected from the main **Setup** menu. Each channel can be displayed as either trace or retrace.

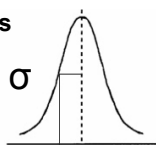
### Settings



**Leveling:** The image can be leveled by subtracting the background from each scan line: none, linear, quadratic (2<sup>nd</sup> order), cubic (3<sup>rd</sup>) or quadratic (4<sup>th</sup>). The average value or curve is subtracted from each line independently; the higher the order, the more small features in the image will be highlighted. This only affects the online display – the full image information is always saved for later analysis.

**Limit:** Allows the user to vary the color scale settings, through a choice between **Statistics** or **Min-Max**.

#### Statistics



Sigma is the statistical width of the image range of data values. This is the "unit" for the automatic colorizer settings.

**Multiplier** is the factor that is applied to scale the width  $\sigma$  of the color scale.

**Offset** gives a shift in the centre position.

#### Min-Max

#### Auto

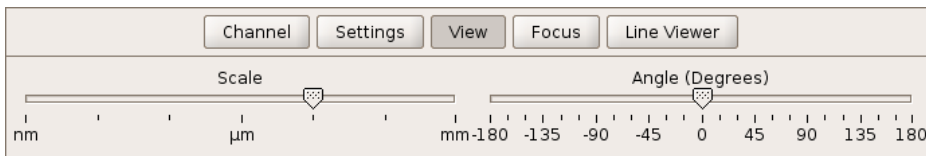
The lowest and highest values are read automatically.

#### Manual

The lowest and highest values can be set manually.



## View

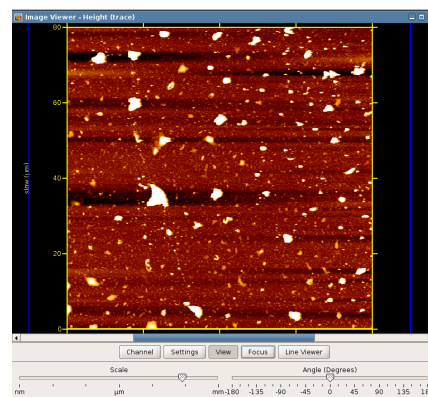
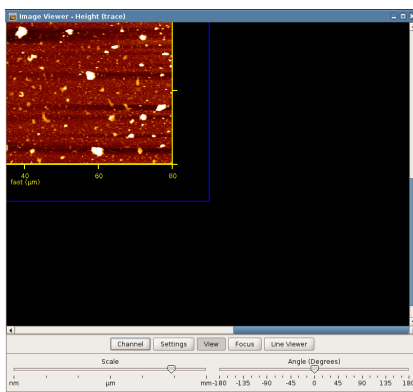


The display can zoom into parts of the image in the viewer using the slider **Scale**, or turn the image with the slider **Angle**. Using the right-click menu in the **Image Viewer**, the view can also be shifted and zoomed using the mouse.

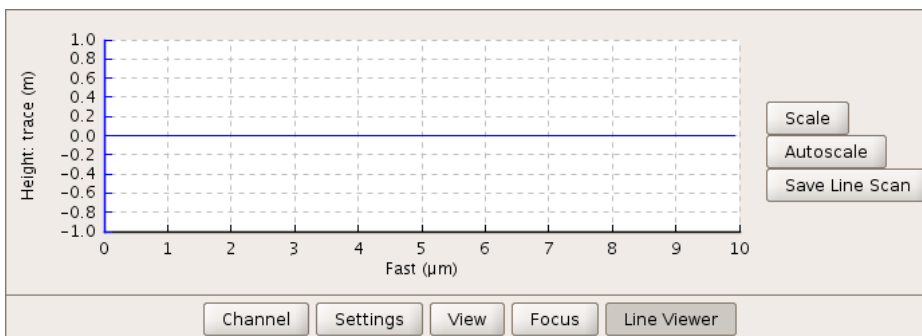
*Note: Neither function affects the motion or scanning angle of the cantilever, only the data display.*

## Focus

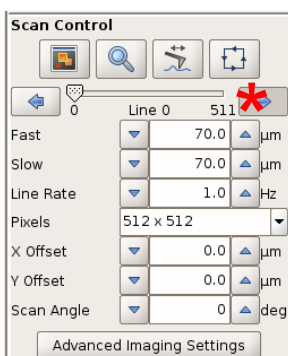
Rescales the selected image to full size. The selected image is usually the current scan, but other images can be selected using the **Scan List**. This quickly brings a selected image into view, as in the before and after screenclips below.



## Line Viewer



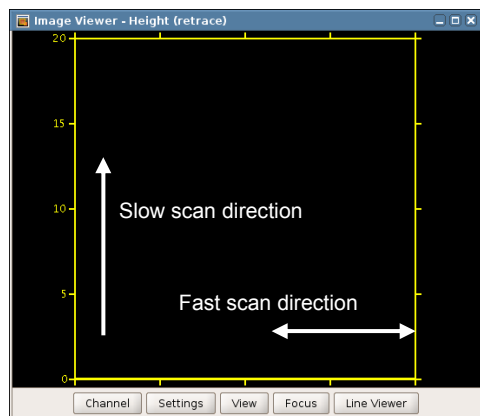
The **Line Viewer** displays the current line scan as it is acquired. The channel and leveling settings are taken from the image viewer. The buttons on the right hand side can be used to adjust the scaling, or to save a single line scan.



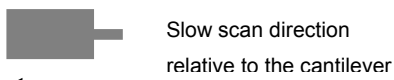
By default, the first image is started from the bottom of the **Image Viewer** window (as marked below). This is the case when the **right arrow** is active in the scan control box, as marked with the asterisk here on the left. If the **left arrow** is active, the image is scanned from top to bottom. If the software is set to perform repeated scans, then the second scan starts from the top position where the previous one finished.



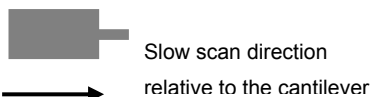
To start the first scan from the top of the viewer, move the slider to the end position (here 511) and select the left arrow.



Scanning from *bottom to top* of the Image Viewer window means that during the scan the slow scan movement is in the direction towards the cantilever substrate.



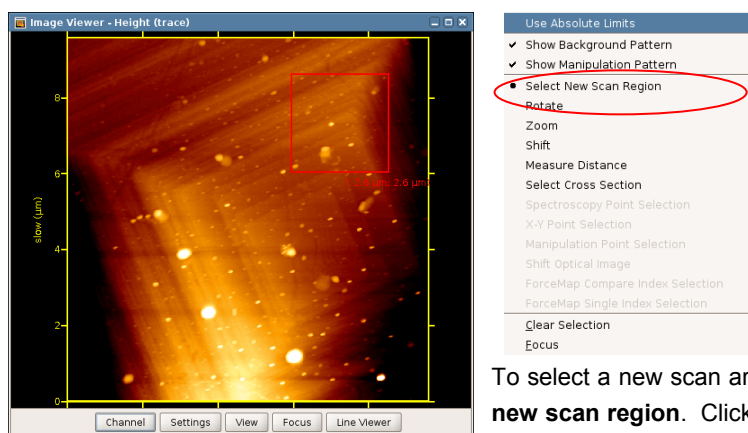
Scanning from *top to bottom* of the Image Viewer window means the slow scan movement is in the direction of the cantilever tip.



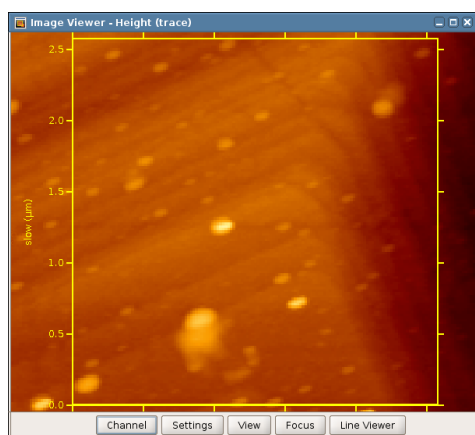
### 4.2.3 Selecting a new scan region (zooming)

The JPK NanoWizard is equipped with state-of-the-art hardware-linearized xy piezos. This means that xy positioning is very precise and that when a new scan region is selected the piezos will accurately move to the new region. Selecting a new scan region and zooming in or out of images can be done by selecting an area with the mouse in the image viewer, or by typing the relevant offset and scan size values directly into the scan control panel.

**Note:** It is not necessary to retract the cantilever from the sample when you select a new scan range. The cantilever is automatically retracted from the surface during the move to the new scan region. A new region can not be activated during a scan, however: **Run** must be toggled to allow the new region to be set.



To select a new scan area, right-click with the mouse and choose **Select new scan region**. Click and drag in the **Image Viewer** to draw a red box defining the new scan region.

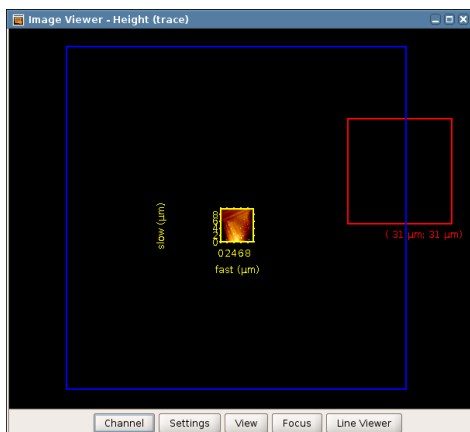


Nothing happens immediately when a new region is drawn. The region can be redrawn as many times as required until the region is correct, then it must be activated.



To activate the new scan region, click the zoom icon in the Scan Control panel. The new scan area should now be surrounded by yellow axes.

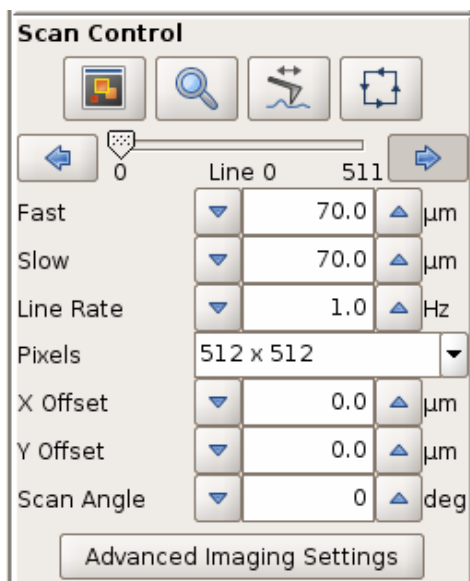
The old scan image is still shown in the background, with the pixels shown with the real size. Start the new scan with **Run**.



It is also possible to select a new scan region completely outside the previous image. To zoom in or out:

- Select **View** in the Image Viewer control and adjust **Scale**
- Select **Zoom** in the right mouse button menu, click and drag
- Scroll the mouse wheel

The scanner in the AFM head has a maximum range of 100 x 100 µm in xy. This maximum scan range is indicated with a blue frame in the **Image Viewer**. It is not possible to choose the new scan region shown here in red, because it is partly outside the 100 µm piezo range. The region will be automatically reset inside the piezo range.



It is important to remember the piezo range when selecting values directly in the **Scan Control** panel. The scan area is defined by the x- and y-offsets and the scan size.

The origin of the coordinate system is the center of the 100 x 100 µm region, so x and y have the range -50 to +50 µm.

e.g. Scan size 20 µm and offset 40 µm is possible.

Scan size 20 µm and offset 50 µm is not possible, since the endpoint would be 60 µm away from the zero position.

Rectangular (non-square) scan regions can be set if the **Square image** option is unchecked in the **Advanced Imaging Settings**. For a rectangular image, the pixel number is automatically updated in order to keep the pixels square.

#### 4.2.4 Retracting the tip from the sample



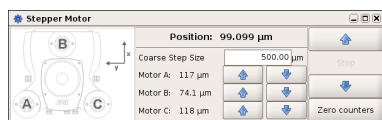
To stop a normal scan, click **Run** again and the scan will stop. The cantilever tip will remain approached on the surface, at the starting point of the scan.



The **Retract** button can be used to retract the tip from the surface, and also to interrupt a scan as a kind of emergency stop. It can be used during scanning, or also when the tip is just sitting in "idle" mode on the surface.



Clicking the **Retract** button once retracts the cantilever from the sample using only the z-piezo. The final distance from the sample depends on the selected z-range and the position of the sample within that range and can vary from a few nm up to about 14 µm. If **Approach** is clicked again, the cantilever will return to exactly the same position, because there is no mechanical movement. This is good for changing settings between scans, e.g. for cantilever tuning in IC mode, or for measuring thermal noise. This distance is not usually enough of a safety distance for any mechanical movement, for instance to lift up the AFM head.



System Status	
User	kate
Sum	2.21 V
Lateral Def	-0.44 V
Vertical Def	502 mV
Status	Idle
Saving	off

System Status	
User	rachel
Sum	0.00 V
Lateral Def	-10.43 V
Vertical Def	-10 V
Status	Z Piezo Retracted
Saving	off

Clicking the **Retract** button again (second and subsequent clicks) means the cantilever is retracted from the sample using the stepper motors. The distance is the amount set in the **Stepper Motor** window. After a stepper motor retract, the cantilever will not return to exactly the same sample location, because the mechanical movement always has some backlash. Moving the cantilever further away from the sample is good for adjusting the sample position (e.g. retracting 20 or 50  $\mu\text{m}$ ), or lifting up the AFM head (e.g. retracting 300-500  $\mu\text{m}$ ).

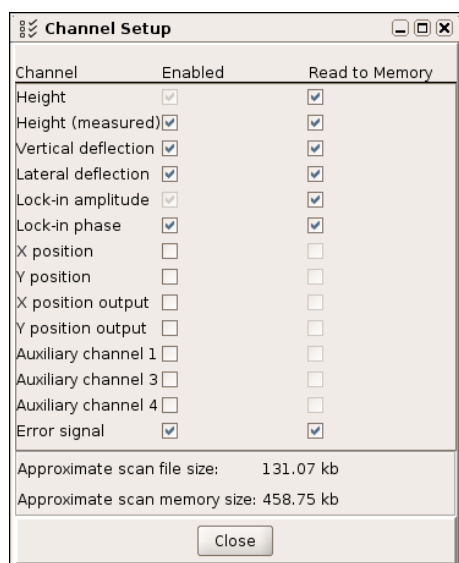
The instrument status is displayed on the **System Status** panel at the bottom of the left hand side of the software window. The **Status** will show **Idle** when the tip is approached but not scanning, and **Image Scanning** during imaging. Note that the piezo display may show the cantilever at the fully retracted position even when the instrument is approached or scanning, if the setpoint is set too high in intermittent contact mode or too low in contact mode. If the setpoint is adjusted, the cantilever will return to the surface. The actual status can be seen in the **System Status** panel.

When the piezo is retracted the first time using the **Retract** button, the **Status** will display **Z Piezo Retracted**. When the cantilever is retracted again, using the stepper motors, the status will show **Scanner Retracted**. When leaving the instrument for a period of time, it is often better to retract from the surface, so any disturbance does not cause tip or sample damage.

The **Autosave** status is also displayed here; **Saving** is **off** in these examples.

## 4.3 Managing and saving images

### 4.3.1 Channel setup



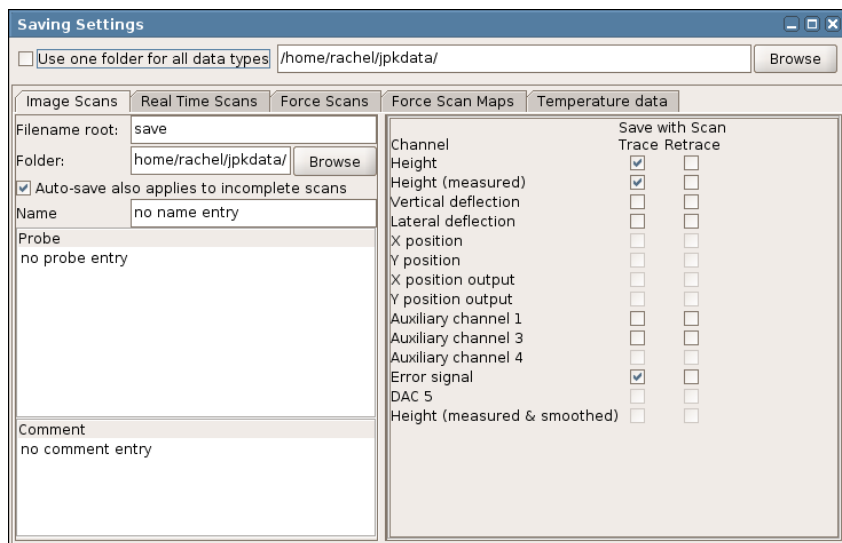
The height is only one of many data channels that can be acquired during AFM imaging. Depending on the imaging mode (contact mode or intermittent contact mode) other information can also be collected and displayed. See the table in **Section 5.5.1** for more details on available signals. External input channels from other devices can also be recorded, using the **Signal Access Module** (see **Section 10.3**) and the **Aux** channels.

The user can decide which channels will be collected during scanning. The channel setup can be selected in **Setup → Channel Setup**.

**Enabled:** these channels can be shown in the image viewer.

**Read to memory:** These channels can be saved to memory.

### 4.3.2 Saving Settings



The **Saving settings** window is used to define the default file locations, filenames and text comments for the different types of data files that can be collected using the SPM software.

This is opened by choosing **Setup** → **Saving settings** from the main menu.

Once a channel is enabled in the **Channel Setup**, the **Saving Settings** panel can be used to specify whether it should be saved in different types of data files. The more channels that are saved, the larger the data files, so there is a complete freedom to enable or disable different channels for different types of experiment.

The **Filename root** is a prefix for the filename; the time and date are added to automatically generate file names. This is convenient if the computer clock is set at the correct time.

Files are saved by default in the chosen **Directory**. **Browse** can be used to select an existing directory or to create a new directory. In addition to the data channels it is possible to add comments, such as notes on the sample or the probe into the stored file. The filename and comments can also be changed in the **Approach** window.



Autosave ON



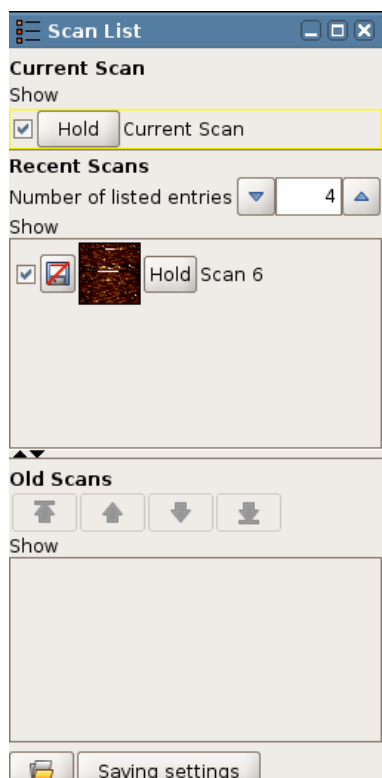
Autosave OFF

To avoid losing any images, the **Autosave** function can be activated on the shortcut toolbar. If **Autosave** is not active, then any images must be saved manually. There are two options – either Autosave saves only the full (completed) images, or it saves every partial image, even if only one data line is collected. This is controlled by the tickbox option “**Auto-save applies also to incomplete images**” in the **Saving Settings** window. In some cases it is important not to lose any data, and in other cases it is better to reduce the amount of saved data by only automatically saving the complete scans. Interesting partial scans can still be saved manually.

### 4.3.3 Scan List



There are often a large number of interrupted scans that would fill up the memory over time. The **Scan List** manages the saving and removal of images. The image files are held in three levels:

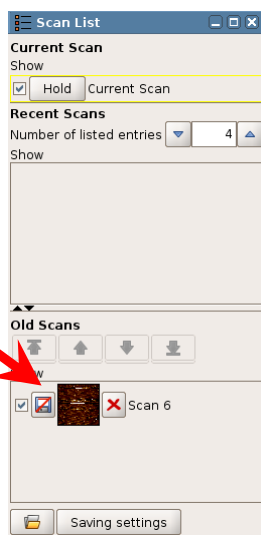
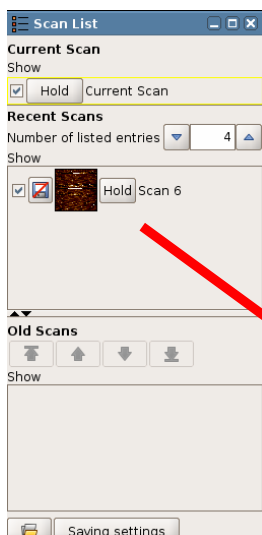


**Current Scan:** The scan that is still in progress is held in **Current Scan**. This is the only scan with a yellow outline in the **Image Viewer**.

**Recent Scans:** When an image scan has finished, the file is automatically moved to the **Recent Scans** section of the **Scan List**. Only a limited number of scans are held here; older scans are removed automatically as new scans are finished. The default number of scans is four, but this can be increased using **Number of listed entries**. If the scans have been saved (either manually or with **Autosave**), they are still stored on disk even if they are removed from the **Scan List**. If they have not been saved, they are lost permanently if they reach the bottom of **Recent Scans**.

**Old Scans:** To store good scans for reference during the session, they should be moved to the **Old Scans** section by clicking **Hold**. Here they are kept until the software is closed or they are removed manually, they will not fall out of the list. Unsaved scans will still be lost if the software is closed. An image will be sent straight to the **Old Scans** list if **Hold** is clicked while it is still scanning.

If the Direct Overlay feature is installed, then imported optical images also appear in the **Old Scans** section of the scan list.



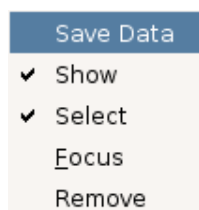
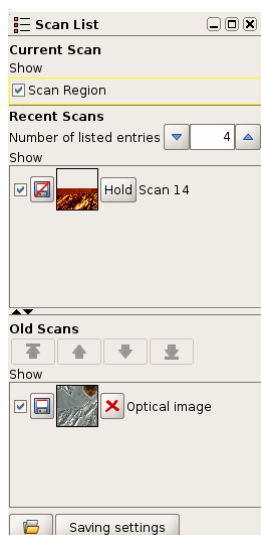
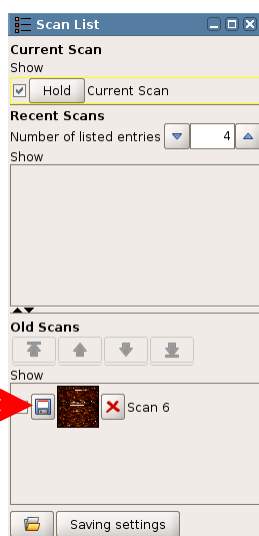
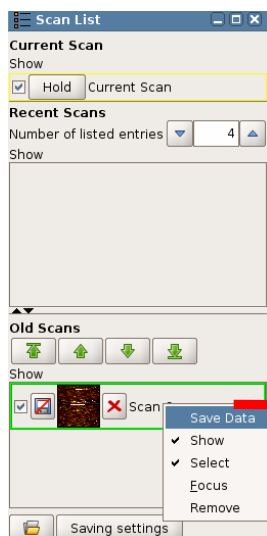
Click **Hold** to store a good scan.

The images are displayed with a thumbnail image and the scan number.



Note: The **Remove** icon just clears the image from the software memory in **Old Scans**. It does NOT remove it from the hard disk if the file has been saved. However, if the file has not been saved, it is impossible to recover it once it has been removed.

Note that the more images you store in **Scan List**, the more memory is used.



Files with this symbol are not yet permanently saved to the hard disk. At any time in **Recent Scans** or **Old Scans** the image can be saved clicking directly on the icon, or with the right mouse button on the scan name/number and selecting **Save data**.



Once the file has been saved, it will be displayed with this icon.



The **Open file** icon can be used to reload old scans from former imaging sessions.

If the DirectOverlay option is installed, optical images can be opened and will appear in the **Old Scans** list.

The **Show** tickbox controls which scans from the **Scan List** are displayed in the **Image Viewer** window.

The order that the scans are plotted in the **Image Viewer** is set in the **Scan List**. Scans at the top of the list are plotted over scans further down the list.

The **Select** function can be used to bring one of the scans temporarily into the foreground by clicking on the scan number or filename. The scan entry will now be outlined in green, and the image is shown in the **Image Viewer** with green axes.

In **Old Scans** the list order can be changed using the arrows. If no scan is selected the movement arrows are inactive (grey). If a scan is selected, it can be moved up or down through the list using the arrows – top, up, down, bottom.

For more display options, right-click on the file entry and choose from the menu displayed:

- ☒ **Show** shows/hides the image in the image viewer#
- ☒ **Select** displays green axes and selects the image for other functions, such as cross-section measurement or shift optical image for DirectOverlay.
- ☒ **Focus** centers the viewer on the selected image.

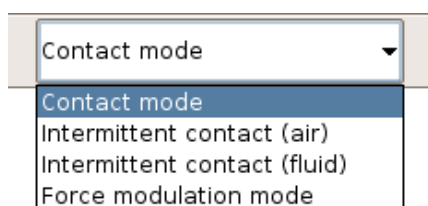


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## § 5 Optimizing the imaging settings

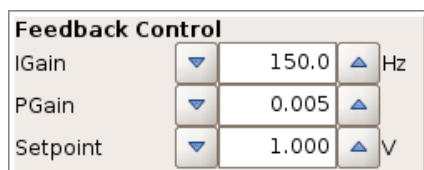
### 5.1 Feedback adjustment for imaging: the feedback control panel

#### 5.1.1 Feedback gains for imaging: IGain and PGain



When the feedback mode is changed in the drop-down menu on the shortcut toolbar (see **Section 3.3**), the JPK software automatically updates the value of the gains **IGain** and **PGain** in the **Feedback Control** panel to appropriate default values for that imaging mode. Generally the initial parameters will need optimizing, however, to obtain the best images of each sample.

**IGain** and **PGain** correspond to the integral and proportional gains for the adjustment of the height (z) feedback loop, so the optimal value for the gains depends not only on the imaging mode, but also on the sample environment (air, liquid, etc.), the scan size and scan rate, the choice of setpoint and the topography of the surface, and the selected z-range. The time constant, **IGain**, determines the integrator and the gain parameter **PGain** the proportional amplifier.



Both gains can be adjusted at any time (including during imaging) and can be set independently from each other. The numbers can be entered directly in the text field, or the value can be changed in increments by clicking on the arrows. (Note: the approach gains are set separately in the approach window)

In the simplest explanation, the gains control the reaction speed of the feedback loop. If the feedback control is too slow, the z-scanner always has a delayed reaction to the topography. If the feedback control is too fast, the z-scanner tends to overcorrect, and may start oscillating.

As a general rule it is best to increase the gains as much as possible. Using higher gains means the feedback reacts more quickly and the maximum force is reduced. The cantilever tip follows the surface more accurately, producing a better topography, and the tip reacts quickly to any change of height, preventing damage to the tip or the sample. It is also often possible to reduce the setpoint imaging force when higher gains are used. If the gains are set too high, however, the feedback may oscillate and they will need to be decreased again.

In general, the gains will usually need to be *higher* for:

- Faster scanning (increasing the Line Rate)
- Larger scan region (faster tip speed over the surface in microns/second)
- Samples with a large topography range (more z-height adjustment in the same time)
- Samples with very sharp changes in topography (the feedback needs to react very quickly at the edges)
- Low force scanning (to give fast enough correction even for a small setpoint change)
- Decreased Z-range (lower z amplification)

In the opposite cases (small, slow or flat scans), the gains do not need to be so high. The feedback response depends on the setpoint, since the gains control the amount of correction for a certain difference between the setpoint and the

current feedback channel value. Therefore, for low-force scanning (high setpoint in IC mode, low setpoint in contact mode) the gains need to be set higher to react properly to very small changes in the setpoint value.

In some situations there is a large range of reasonable values that give very similar results; for robust samples the feedback only has to react fast enough to follow the topography. Scanning medium size regions in air, for instance, it is often possible to use the default values over a wide range of conditions, particularly in contact mode. If the sample is delicate then the feedback parameters really need to be optimized and it may be necessary to adjust all the parameters many times during different scans.

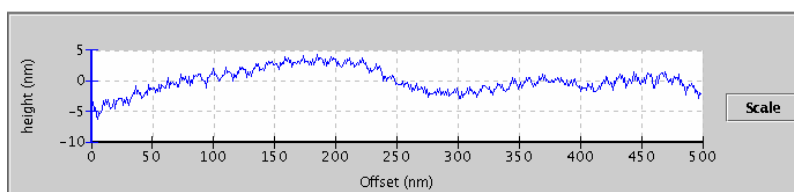
In the SPM software the gains are applied relative to the current piezo range. Therefore when the piezo range is reduced (e.g. from the maximum 15 microns down to the 3 microns range) the gains need to be increased to give the same response. The features will usually be smaller, since the reduced range is chosen for very flat samples, but for scanning at low force the gains can be increased a lot.

The **Height** image is obviously the first channel to use in setting the imaging parameters; the quality of the scan may be obvious from the plain height image. The **Error Signal** image (**Vertical Deflection** in contact mode and **Amplitude** in IC mode) is also vital for adjusting the feedback settings; this shows the correction signal that the feedback is using to adjust the height. If there are dark and bright patches of almost black and white around features, then the feedback loop is not correcting fast enough and the error is large at these points. If the image shows a more softly shaded "shadowed" image of the height, then the feedback loop is adjusting the height within a reasonable time.

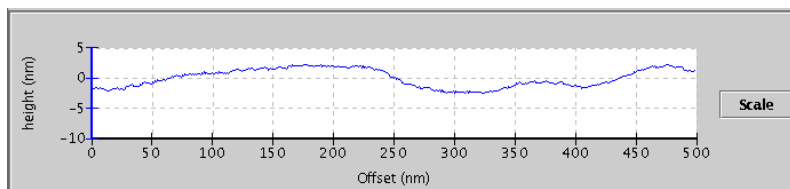
### 5.1.2 Simple procedure to optimize IGain and PGain

It is best to be able to see the current line scan in order to judge the effect of adjusting the gains or setpoint values on the imaging. Therefore, the **Line Viewer** in the Image Viewer window (see **Section 4.2.2**) or **Oscilloscope** window (see **Section 5.5.1**) should be opened, so that the height profile for each line can be seen.

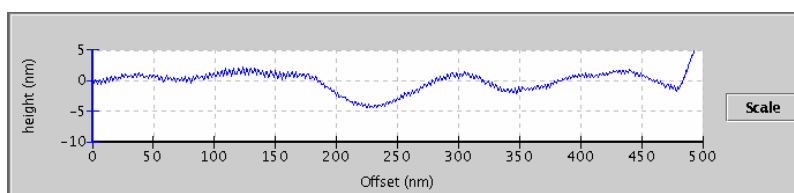
In this example, the Line Viewer in the Image Viewer window is used to show the effect of adjusting the gains.



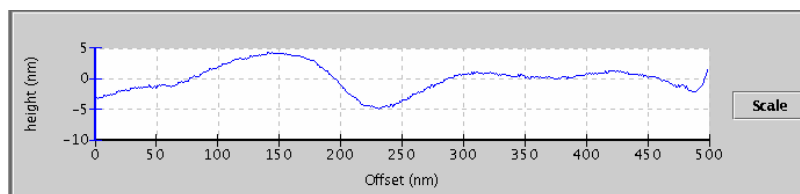
**Increase** I-Gain until the z-feedback loop starts to oscillate.



**Reduce** IGain slightly until the oscillations are eliminated.



**Increase** PGain until z-feedback oscillations are observed again.



**Reduce PGain** until the oscillation vanishes.

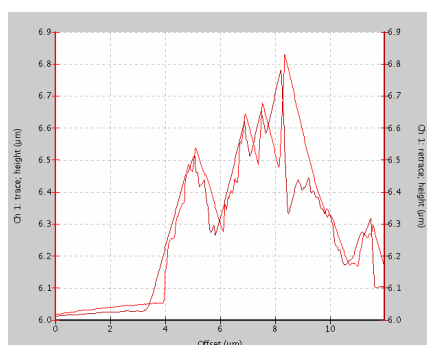
### 5.1.3 Scan speed and feedback adjustment

Generally you can achieve better results with lower scan rates in these cases:

- The sample is rough or strongly corrugated (a large z piezo range is used in scanning)
- The scan size is large (a larger x-y range is used for scanning)
- The scan is made in intermittent contact mode in liquid
- The sample is very soft and easy to damage

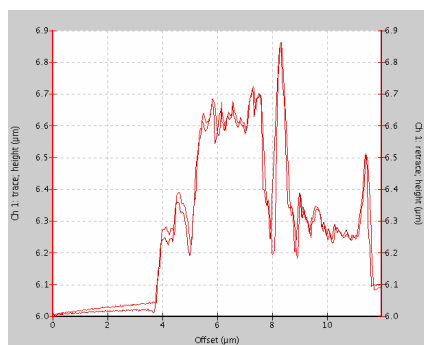
A combination of several of these features means that the scan should definitely be in the lower end of the range. For example, when scanning living cells in liquid there is a very soft sample that has a large height range, and is often scanned with x-y scan sizes up to 100  $\mu\text{m}$ . In this case, the speed should be around 0.3 - 0.5 Hz. For smaller scans, e.g. for zooming into particular features on the cells, the speed can be increased. When scanning a relatively flat, hard sample in air then the scan speed can often be increased to 2 - 3 Hz (although note that the feedback gain values should also be increased so that the feedback reacts quickly enough). For scanning small flat areas in contact mode for high resolution imaging, for example when imaging bacteriorhodopsin protein crystals, the scan rate should be increased further, usually in the range 4 - 8 Hz. Note that the full range of values to control the speed of the tip over the surface are found in the **Advanced Imaging Settings** panel.

It is useful to open the **Oscilloscope** window during scanning (see **Section 5.5.1**), to show the trace and retrace data together. On regions where the height of the sample increases, the difference between the setpoint and the measured value of the feedback channel can become quite large, so the feedback follows the surface even if the gains are too low (although high forces may be applied if the gains are too low). On regions where the height of the sample is decreasing, the difference between the setpoint and the measured value of the feedback channel is limited by the free state of the cantilever, and the feedback responds more slowly to the changes. This leads to a difference between the "uphill" and "downhill" parts of the scan, and this can be seen clearly by comparing trace and retrace. Regions where the height increases on the trace scan line correspond to regions where the height decreases on the retrace scan line.



When the gains are not high enough for the scan speed and topography, the scan lines tend to "trail" as the tip follows the surface downwards, and the trace and retrace lines do not agree with one another.

In this example, the trace and retrace curves of the height channel are displayed. On parts of the lines where the topography height is increasing, the line follows the surface corrugations. On parts of the lines where the height is decreasing, the tip follows a smooth downwards curve and does not follow the surface details.



*Increasing the I-gain leads to better congruence of trace and retrace curves.*

*Decreasing the scan rate also leads to better congruence of trace and retrace.*

When the feedback parameters are set appropriately for the scan speed and topography, the trace and retrace lines both follow the surface topography and there are only small differences between them.

## 5.2 Controlling the piezo and stepper motors

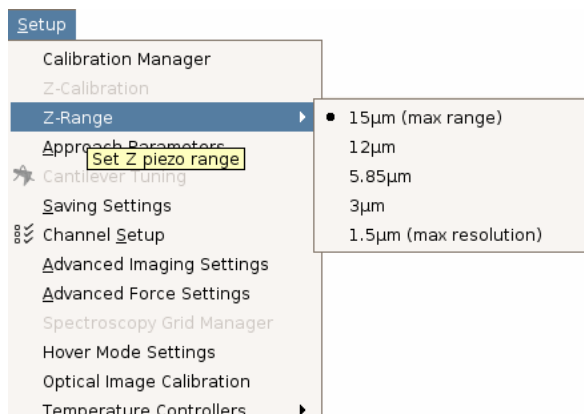
### 5.2.1 Reducing Z-Range for higher resolution

To optimize the range and resolution, the working range of the cantilever piezo can be set to different values. The z-range that is most suitable for a particular experiment depends on the sample and type of measurement. For flat samples with a height range of less than 500 nm it is recommended to select a smaller z-range to maximize the resolution. The resolution is increased for smaller z-ranges because of the 16 bit resolution of the AD-converter, and also the amplifier noise is lower. The 16 bit resolution means  $2^{16} = 65,536$  digits available over the full selected Z-range:

Z-range	15 µm	1.5 µm
Resolution	0.2 nm per digit	0.02 nm per digit

*Note: the piezo can not retract further than the currently set **Z-Range**!* Therefore, if the sample topography differences are larger than the chosen Z-Range, the tip will not be able to move far enough away from the surface, and tip or sample damage can result.

Note also that the Z-range covered by the piezo during a scan includes any sample tilt, as well as the roughness of the sample over a scan line. Therefore, even if the sample is quite flat, a relatively large Z-range will be needed if there is a significant tilt angle between the sample and the AFM head. It is important to consider the height differences without any leveling procedure being applied to the scan lines. The height range being covered can also be seen on the Z-Range piezo display, as below. If there is a significant sample tilt, it may be better to remove this first by adjusting the stepper motors (see **Section 5.2.2**) before reducing the Z-Range. Note that you may then be scanning a different area of the sample, unless you can find the same location again optically.

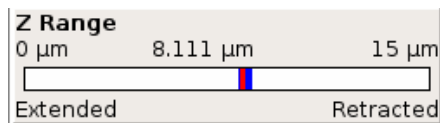


The **Z-Range** can be set from the **Setup** menu on the main pull-down menu bar.

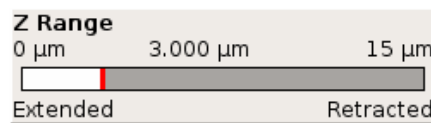
There are five different z-ranges available that can be selected: 15, 12, 5.85, 3 or 1.5 µm.

Always start with a larger Z-Range for samples with an unknown topography range! It can then be reduced if needed, according to the sample image. The approach will also be faster for a larger z-range, then the range can be decreased at the surface just before imaging.

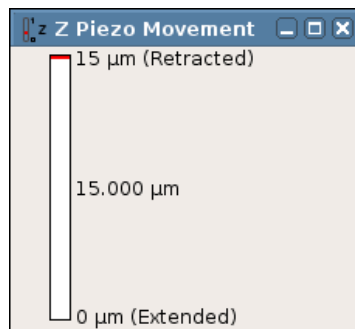
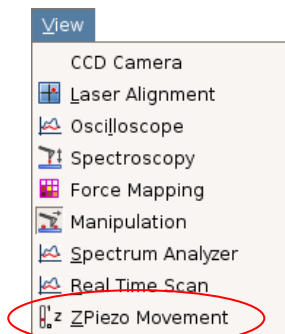
The z-range panel can be seen at the bottom left of the user interface, and the active z-range is marked in this panel, as the white part of the piezo diagram. The current piezo position is marked as a red line, and the recent range covered within the scan line is marked in blue.



**0 – 15 μm** (maximum range)



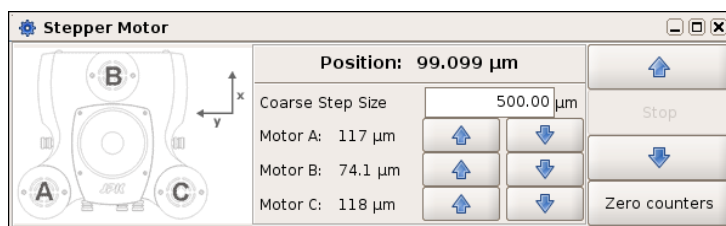
**0 – 3 μm** (useful range for small samples)



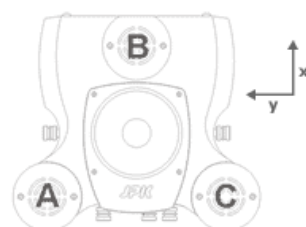
It is also possible to view the Z Piezo display vertically, which is more intuitive. This extra panel is opened from the **View** menu.

## 5.2.2 Independent movement of the stepper motors

Most samples are not mounted exactly parallel to the sample stage, and sometimes the interesting region of a sample is at an angle to the AFM head. In this case, the feedback has to correct for this general sample tilt as well as the local changes in height due to the roughness of the sample. If the sample is parallel to the AFM head over the region that is being scanned, the feedback only has to correct for the roughness of the sample, and this can lead to better imaging, particularly if it allows the Z-Range to be reduced (see **Section 5.2.1**). Note that generally the exact location on the sample is changed when the stepper motors are moved independently of each other. Therefore it may not be possible to find exactly the same area of the sample, unless it can be located optically.



Open the **Stepper Motor** window to move the stepper motors independently. The diagram shows the direction of the main scan axes to help translate the scan to the movement of the motors. Note that if the tip is approached, the “down” arrows of the stepper motor are inactive.

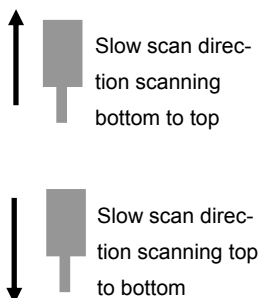
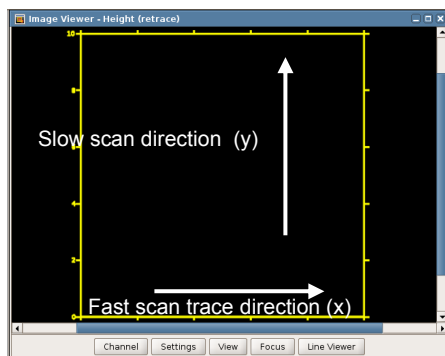


View of the AFM head from the top.

Orientation of the cantilever in the AFM head

For a zero scan angle, the fast scan direction (x) is front-to-back relative to the head, and the slow scan axis (y) is right-to-left.

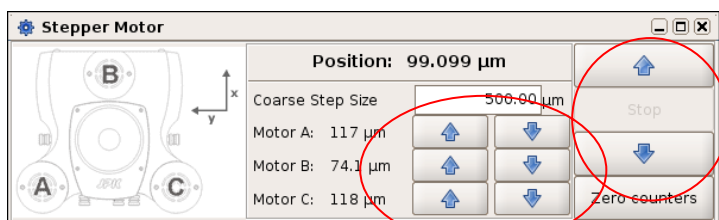
The orientation of the cantilever relative to the AFM head is shown here.



The movement of the fast and slow scan directions relative to the **Image Viewer** window and the cantilever is shown here.

Looking at the tilt of the scan lines in the oscilloscope is useful to see if it is necessary to re-adjust the stepper motors.

*Note that the leveling must be set to OFF to see the overall tilt of the curves.*

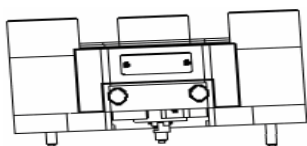


The position of the motors is shown on the **Stepper Motor** window. The main pair of up and down arrows on the right control all three motors together. The three pairs of arrows in the centre control each motor independently.

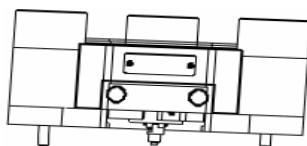
Increasing numbers in the display represent the approach of the AFM head towards the sample, decreasing numbers represent the movement of the AFM head away from the sample.

The counter can be reset at any position, for example they can be set to zero at the sample surface, to provide a reference for the sample height and aid re-approaching later. Note however, that this means they are not an absolute value, and the motors can all read zero when the head has a significant tilt, for example. To obtain reference values, move the head up as far as possible, until all three motors are at their highest point, and then zero the counters. The head is now level, so if all the numbers are equal the head should be level with respect to the stage.

*Note, for the NanoWizard I heads, never move the motors down to their lowest point! In this case the motor spindle may stick inside the head.*



Not recommended

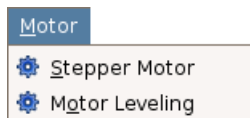


Better in this direction

If the AFM head has to be tilted it is better to tilt it as shown in the right image. This is to avoid the cantilever spring touching the sample as the head approaches.

### 5.2.3 Automatic Motor Leveling

As described above, it is possible to adjust the AFM head manually to take account of some sample tilt by moving the three head motors independently. It is also possible to calculate and carry out the movement automatically, based on the tilt of a scanned image. Remember though that the tilt range is rather limited by the geometry. This correction is effective in correcting for small angle differences from sample mounting, but not intended to apply large tilt angles to the scan head.

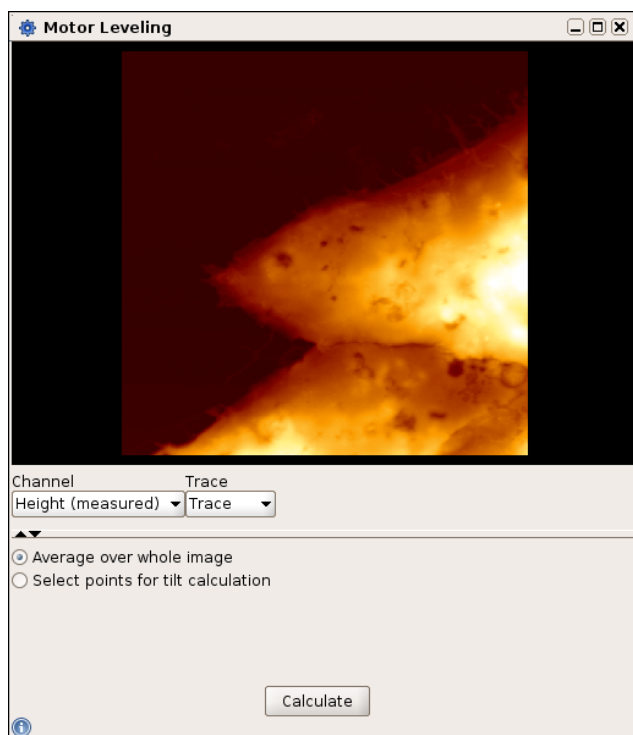


**Motor Leveling** can be opened from the main **Motor** menu.



The movement should be calculated from an image that has just been scanned. This should be selected in the **Scan List**, and will be shown in the **Motor Leveling** window display. Check that the correct image is selected, and shown with a green border.

The tilt angle can be determined better from larger images. Therefore larger scans (more than 10 microns) are recommended, and it is better to finish the scan (so that the angle is measured well in both scan directions). The pixel number or resolution is not so important, so the scan can be done with fewer pixels if required for speed. If very small scans are used, the error will be higher, and there is more chance that the angle is only local, and not representative of the whole surface. After the independent movement of the motors, the center position will change laterally, so the tip will not land on exactly the same region of the sample. So it is important to measure the large-scale tilt of the sample and not some local feature.

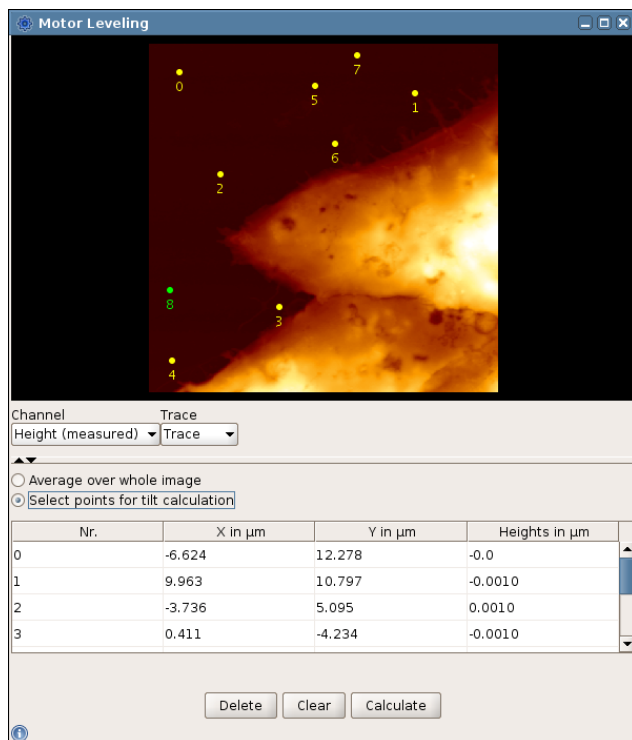


The **Motor Leveling** display shows the currently selected image from the **Scan List**. The **Height (measured)** channel is generally used, as this has the best height accuracy, and the display here always shows the display **leveling off** (so it may look different in the **Image Viewer**, depending on the display settings there).

The default setting for the angle calculation is **Average over whole image**. This is appropriate where the surface is very flat, for example single molecules on mica, and the large-scale height differences are dominated by the sample tilt. In this case, proceed to the next step with **Calculate**.

For samples where there is a clearly defined “background” that should be used for calculation, and features that should be ignored, this is not appropriate. For the image shown, it is better to choose **Select points for tilt correction**.





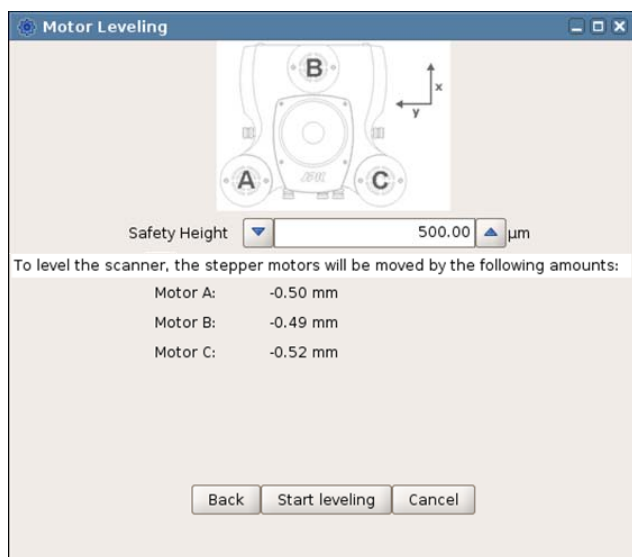
If **Select points for tilt correction** has been chosen, click in the image display to set points. Three points is the minimum, but it is better to use rather more points than needed, to ensure there is a good average.

Choose points all over the region of the background level of the image. The listed points will appear in the table below.

**Delete** deletes the currently selected point.

**Clear** deletes all the points in the list.

When the points are set reasonably, click **Calculate** to proceed to the next step.



Once the background level has been set from an image, the calculated stepper motor movements are shown.

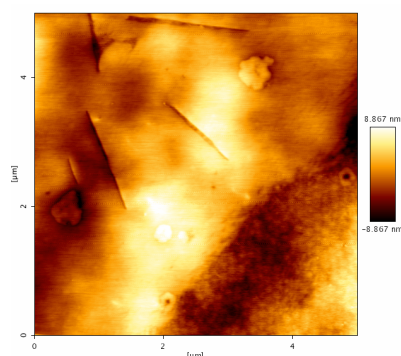
The **Safety Height** is added to all the stepper motors, and should be the final height above the surface at the end of the movement.

Check the calculated motor movements to see that they are sensible before clicking **Start leveling**. *Always try to avoid the stepper motors reaching the end of their range!*

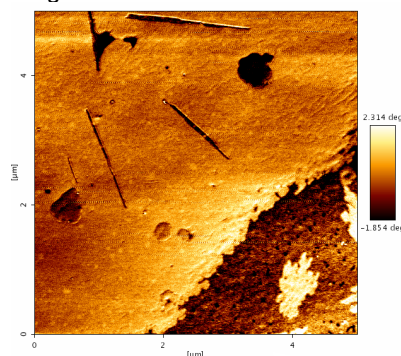
Once **Start leveling** is clicked, the movement will start – first the motors moving away from the surface, followed by the motors moving towards the surface. After the movement, re-approach to the surface to start imaging.

## 5.3 Intermittent contact mode

### 5.3.1 Phase contrast



Height



Phase

During an AFM experiment in intermittent contact mode the cantilever is driven at a specific resonance frequency. Depending on the conditions of the sample interaction, there will be some phase shift between the drive amplitude (applied to the piezos) and the lock-in-amplitude (measured from the detector). Most contributions to this phase shift are independent of the sample position, and will not change over a scanned image.

The part of the phase shift that is due to the tip-sample interaction can show contrast between different parts of the sample. This phase shift can be measured and displayed in a phase image. The height and phase images are collected simultaneously.

Sometimes in the phase images two different components within a topographically flat sample can be distinguished, as in the example shown here.

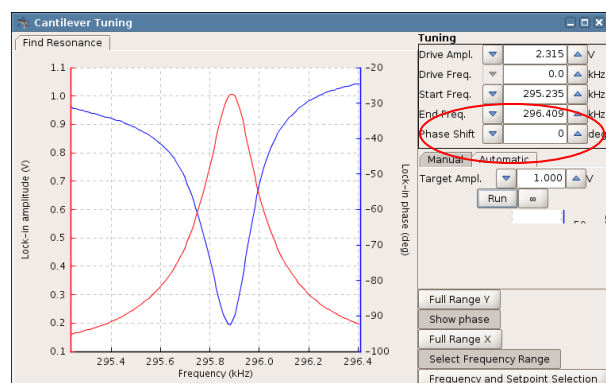
The quality of phase images is generally strongly influenced by varying the setpoint. Often the phase contrast is lower in intermittent contact mode imaging in liquid, since the width of the resonance peak is greater.

The phase contrast also depends on any static offset between the drive amplitude and cantilever response, so the offset can be changed, as described below, to bring the phase in line with the amplitude resonance.

### 5.3.2 Adjusting the phase shift to improve phase contrast

To get the best quality phase images, the phase curve generally has to be shifted using the **Cantilever Tuning** window, as explained below. The **Phase Shift** is used to compensate for the uninteresting contributions to the phase difference between drive amplitude and the measured cantilever lock-in amplitude, so the images show the best contrast for the part that depends on the tip-sample interaction.

The plots of the **lock-in amplitude** (red) and the **lock-in phase** (blue) are displayed for each value of the **Phase shift**.

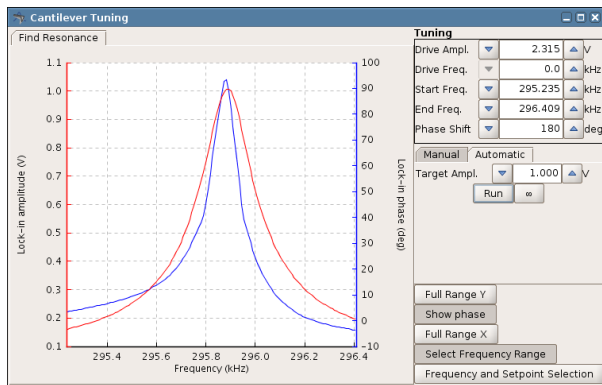


Initial phase and amplitude – **Phase shift** is 0 deg.

In this example, the drive frequency is 295.8 kHz, and the target amplitude is 1.0 volt. To image the phase contrast from the sample sensitively the inflection point of the phase curve must be at the drive frequency.

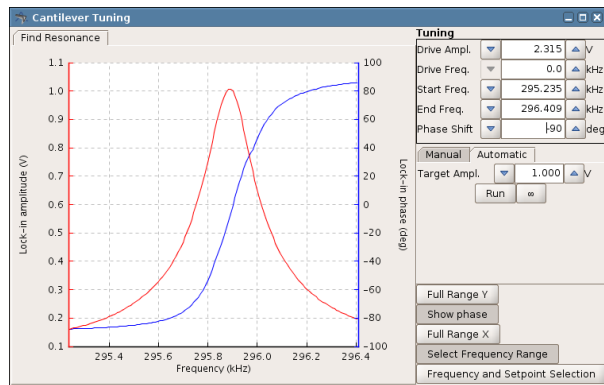
The phase curve can be shifted manually by adding a shift (offset) to the phase. Enter a value between  $-180$  and  $+180$  deg in the textbox **Phase shift**.

By toggling **Run**, or continuous (the infinity symbol), the new resonance curve can be seen.



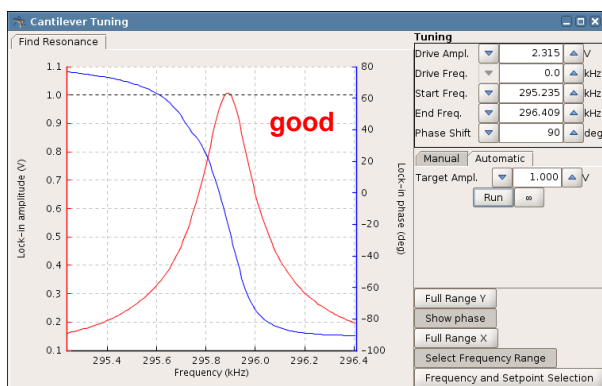
Enter "180" deg and **Run**

Result: bad, inflection point does not hit drive frequency.



Enter "-90" deg and **Run**

Result: Inverted phase curve. The color scale in the resulting phase image would be inverted. Change the phase shift by +/-180 deg. (to -130 deg) instead.



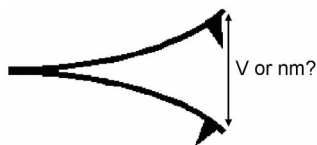
Enter "90" deg and **Run**

Result: good, inflection point hits drive frequency.

### 5.3.3 Variation of the drive amplitude

In intermittent contact mode the drive amplitude can also have an effect on the imaging conditions. The drive amplitude is the value of the alternating voltage that is applied to the piezo which drives the cantilever oscillation. The drive amplitude is not related to the drive frequency. For a higher drive amplitude, the oscillation amplitude of the cantilever is also increased, which can be helpful on some sticky samples.

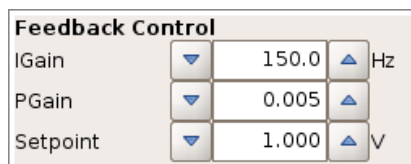
The force applied to the sample depends on the relationship between the setpoint and the drive amplitude. If the setpoint is not changed, then the force applied to the sample is increased when the drive amplitude is increased. Therefore it is recommended to increase the setpoint manually while increasing the drive amplitude.



Additional hint: In the cantilever tuning window the magnitude of the lock-in amplitude is displayed in Volts. The cantilever must be calibrated to get the amplitude displayed in nanometers. The sensitivity of the cantilever has to be determined. See **Section 8.2** for details about sensitivity calibration.

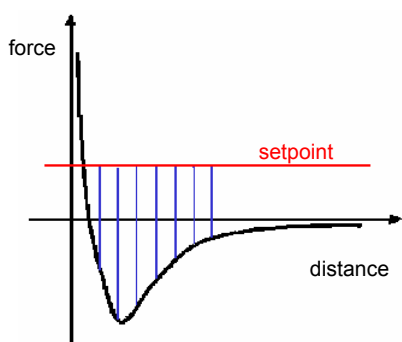
## 5.4 Advanced approach in contact mode

For users who wish to have a more sensitive approach in contact mode, there is a special feature available in the software for approaching with a variable setpoint.



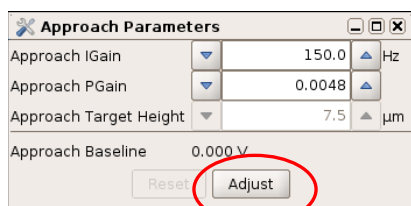
“Normal” approach routine:

The default setting for approaching the surface in contact mode is with a fixed setpoint. The **Setpoint** value in the main **Feedback Control** is used directly as the desired value for the adjustment of the **Vertical deflection** of the cantilever (whether this is set in nm or V).



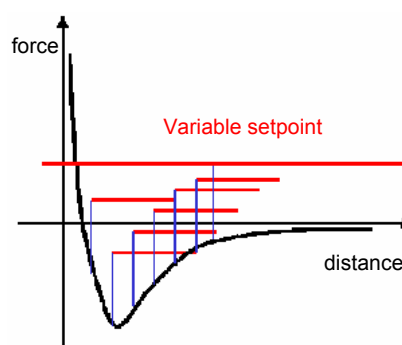
The setpoint is a defined value that remains constant for the whole approach. The setpoint is chosen relatively far from the surface, however, and as the tip approaches the forces between the tip and sample change the deflection of the cantilever. The standard routine does not take account of this change in deflection value, and only looks for the point where the absolute **Vertical deflection** becomes more than the **Setpoint** value.

If the **Vertical deflection** on the photodiode far from the sample was -0.52 V, for example, and the **Setpoint** in the **Feedback Control** panel was 1.0 V, then the *effective* setpoint (the difference between the initial deflection and the final setpoint) would actually be 1.52 V at the surface.



“Advanced” approach with **Adjust** enabled:

When the **Approach Baseline** feature is used in the **Approach Parameters** window, it is possible to perform the approach with a relative setpoint instead of a fixed one. When **Adjust** is active, then the number in the **Setpoint** text field is used as the *difference* between the offset value of **Vertical deflection**, and the actual setpoint used for the approach. The **Approach Baseline** is adjusted after each motor step to the current value of the **Vertical deflection**. The value is also updated whenever Reset is clicked.



In this case, the setpoint is varied during the approach. The blue bars that are shown in the diagram are calculated by adjusting the setpoint for the local vertical deflection. In the user interface, the **Setpoint** value stays constant, and the **Approach Baseline** value is updated to take account of the deflection changes. The actual value of the vertical deflection being used as the current setpoint by the feedback can be calculated by adding the **Approach Baseline** number to the **Setpoint**.

If the **Vertical deflection** on the photodiode far from the sample was -0.52 V, for example, and the **Setpoint** in the **Feedback Control** panel was 1.0 V, then the feedback routine would seek to achieve a *difference* (relative setpoint) of 1V between the actual deflection and the **Approach Baseline** value (here automatically initialized to -0.52 V), and start the approach with a “real deflection” setpoint of 0.48 V.

## 5.5 Tools for monitoring scanning

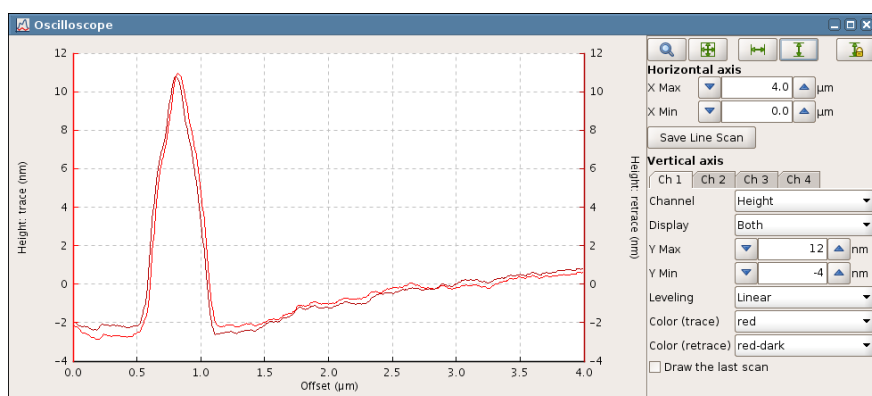
### 5.5.1 The Oscilloscope window



The **Oscilloscope** icon in the shortcut bar opens the oscilloscope window directly. It can also be opened from the pull-down menu **View** in the menu bar.

The **Oscilloscope** window can be used to display the present line scan of several channels simultaneously. Both trace and retrace information can be displayed together, which can help with optimizing the imaging settings (see **Section 5.1**, for example). Up to four channels can be displayed in the same window, all with both trace and retrace. Any channel can be displayed, so long as it is switched on in the **Channel Setup** (see **Section 4.3.1**).

The **Line Viewer** that is located in the **Image Viewer** window (see **Section 4.2.2**) gives a simplified oscilloscope function. In the **Line Viewer**, the leveling settings are taken from the **Image Viewer**, but in the **Oscilloscope** the leveling can be set independently. The same scan lines may look different in the oscilloscope and line viewer, if different leveling settings are chosen.



It is also possible to zoom into a smaller range to take a closer look at the curves. The display range can be set using the text fields for the horizontal and vertical axis ranges.



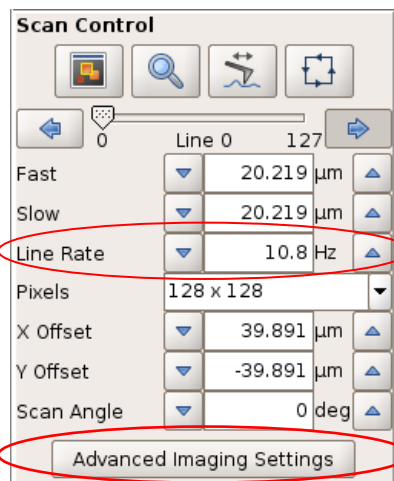
The standard oscilloscope toolbar can also be used to set the view ranges

Note: In **Contact** mode **Error signal** and **Vertical deflection** provide basically the same information, but for **Error Signal** the **Setpoint** is always subtracted. Also, with a calibrated cantilever (known sensitivity) the **Vertical deflection** signal is displayed in nm.

In **Intermittent contact** mode **Error signal** and **Lock-in amplitude** provide the same information, but for **Error Signal** the **Setpoint** is always subtracted, and with a calibrated cantilever (known sensitivity) the **Lock-in amplitude** signal is displayed in nm.

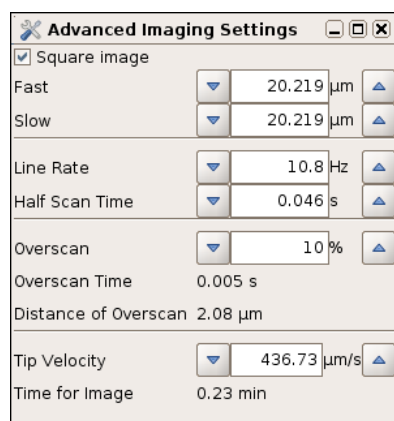
## 5.6 Advanced imaging settings

The **Advanced Imaging Settings** window can be opened directly from the **Scan Control** panel, or under the **Setup** menu in the main menu list. This panel provides an extension to the parameters found in the **Scan Control** panel.



The speed for scanning is usually set in the main **Scan Control** panel, through the **Line Rate**. A line rate of 1Hz means that the tip makes the trace and retrace scan lines and returns to the starting position for the next scan line in a time of 1s.

To have a constant scan speed across the surface while collecting data points, however, the tip must scan a slightly larger area to give room for slowing down and changing direction. The relationship between the movement of the tip used for imaging, and this extra movement to change direction is normally handled using default values from the software, so the user only has to choose one number for the **Line Rate**. For advanced applications, the user can choose particular values to control the other parts of the tip movement if desired.



The scan speed can now be set either as a **Line Rate** (1/ time for both trace and retrace), or as a **Half Scan Time** (the time for either the trace or retrace part of the scan line). The numbers are always updated to be consistent.

The value set for **Overscan** controls the extra movement of the tip as it turns around at the ends of the scan lines. This is set as a percentage of the half scan time. *(Note that this option replaces the "Duty cycle" option from old versions of the software.)*

The **Tip Velocity** takes account of the physical scan size, and can be used to see the tip velocity for particular settings, or as an alternative way to set the scan speed. The total time for the image is also displayed, calculated for the overall set of parameters.





## § 6 Spectroscopy

The **Spectroscopy** mode performs force-distance measurements, also known as force spectroscopy. In imaging mode, the force is held constant while the cantilever is scanned laterally over the surface. In force spectroscopy measurements, the lateral position is set at a fixed point, and the z position of the cantilever is scanned.

As the z-position of the cantilever is scanned, the tip moves towards and away from the surface. As the tip is pushed against the sample surface, elasticity values can be measured for indentation. Moving away from the surface, molecules attached to the cantilever tip can be stretched. The forces can be measured if the cantilever is calibrated, i.e. the spring constant has been measured (see **Section 8.3**).

A simple way to become familiar with the **Spectroscopy** features is to perform force-distance curves on a clean glass slide or mica surface in air, using a contact mode cantilever with a moderate spring constant (around 0.1 - 0.5 N/m). These samples are hydrophilic, so the tip and sample are usually covered with a thin layer of water. When the two surfaces are brought close together, the water layers can form a capillary neck and there is strong attractive force. These classic force-distance curves show the attractive adhesion as the surfaces come together or are separated, and also the strong repulsion as the tip is pushed against the hard surface.




### 6.1 Overview of Spectroscopy mode

#### 6.1.1 Introduction to the spectroscopy settings



Change to **Spectroscopy** mode using the icon in the main shortcut bar. The **Run** command starts an continuous series of spectroscopy curves.

**Spectroscopy Control**

**Absolute** **Relative** **Clamp**

Rel. Setpoint  V

Baseline n.a.

Adjust Baseline

Z Length   $\mu\text{m}$

Z Closed Loop ☐

Extend Time  s

Retract Time  s

Retr. Delay  s

Ext. Delay  s

Delay Mode

Pixel Rate  Hz

**X, Y Position**

Nr.	X in $\mu\text{m}$	Y in $\mu\text{m}$
0	-0.49	-0.373

On the left side of the main SPM window, the **Scan Control** panel is replaced by the **Spectroscopy Control** panel. Here are the parameters to control the tip movement.

The top part of the panel has shortcut icons for several useful settings (see directly below for details). The top set of parameters control the distances moved by the z-piezo, and the features for limiting the repulsive forces between the tip and sample. Register cards for **Absolute**, **Relative** or **Clamp** mode offer different control settings for the force scan movement. These settings are explained in **Section 6.2**.

The middle part of the panel has the main parameters to control the timing of the force curves. The full settings for rates to approach and retract and how to control the height position during the waiting times can be found in the **Advanced Force Settings** panel (see **Section 6.6**).

The lower part of the panel has the list of (x,y) positions where the force curves will be performed. These can be selected by hand or set automatically as a grid (see **Section 6.3**). Alternatively the force experiment can be controlled as **Force Mapping** (see **Section 7.1**).



Shortcut to open the **Calibration Manager** window, to calibrate the sensitivity and spring constant. See **Chapter § 8** for more information.

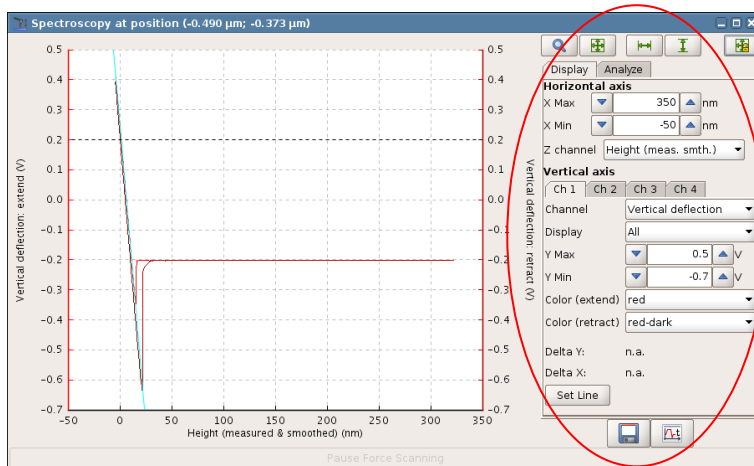


Shortcut to open the **Spectroscopy Grid Manager** to create grids of spectroscopy points. See **Section 6.3.2** for more information.

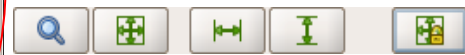


Shortcut to open the **Advanced force settings**, to set advanced options for rates, waiting times and repeats of force curves. See **Section 6.6** for more information.

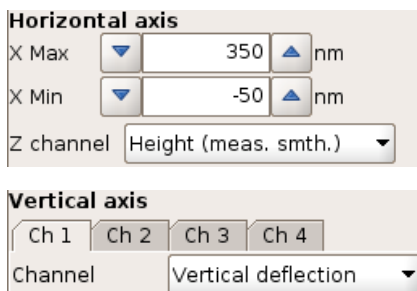
## 6.1.2 The Spectroscopy Oscilloscope



The **Spectroscopy** oscilloscope opens automatically. The force-distance curves will be plotted in the main area of the window. The **Display** panel on the right hand side allows the view to be modified, the settings do not change the experiment parameters.



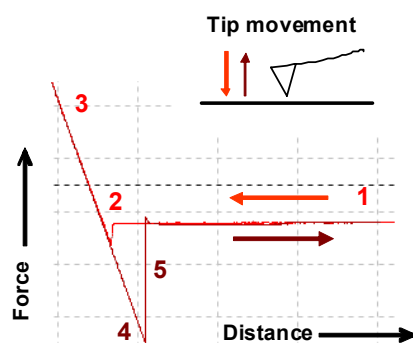
The standard oscilloscope toolbar can also be used to modify the display settings (see **Section** ).



The normal **Z Channel** for displaying the force-distance data is **Height (measured and smoothed)**. This uses the linearized sensor channel **Height (measured)** rather than the simple piezo voltage **Height**, so there is no hysteresis or piezo creep. This channel is used for the **Horizontal axis** of the force-distance display.

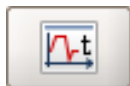
In contact mode the **Vertical Deflection** is directly related to the force, and this is used for the **Vertical axis** of the force-distance display. It may be in Volts, or calibrated in nm or N, see **Section 8.3**. Always make sure that the **Vertical Deflection** is enabled (this is the default setting). If it is not available, see **Section 4.3.1** for details of the **Channel Setup** and **Saving Settings** controls. For force spectroscopy in IC mode, the amplitude is used.

The z-piezo extends, moving the cantilever towards the surface and retracting it again.

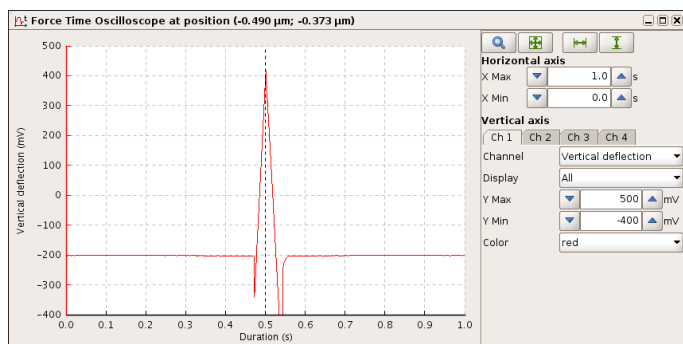


1. Approach of the tip from far distance
2. Tip snaps to the surface (jump to contact)
3. Increase of the repulsive force when the tip is in very close contact with the sample.
4. Retraction of the cantilever while the tip is still in contact (adhesion)
5. Tip is pulled free from the surface.

### 6.1.3 Force Time Oscilloscope

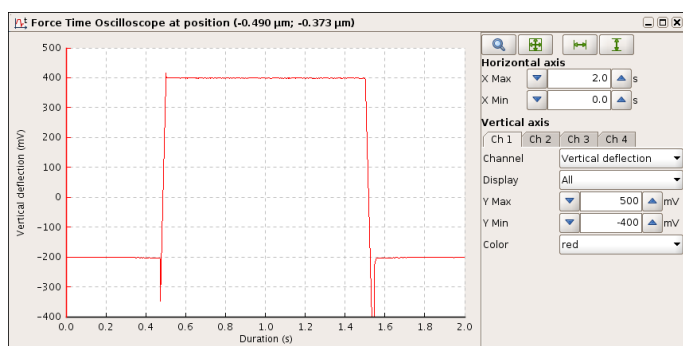


The **Force Time Oscilloscope** can be opened using this button in the **Spectroscopy** oscilloscope, or through the main pull-down **View** menu.



The **Spectroscopy** oscilloscope shown in **Section 6.1.2** shows the conventional force-distance plot of the spectroscopy data.

The **Force Time Oscilloscope** shows an alternative view of the same data. Here, the force data (or other channel, including the piezo height) is plotted against time. The data here is the same as that shown in **Section 6.1.2**, showing the similarities and differences between the display modes.



Apart from the horizontal axis of the plot, there are two important differences between the two displays:

The **Force Time** display is updated continuously during the movement, while the oscilloscope in **Section 6.1.2** is only updated once at the end of the cycle.

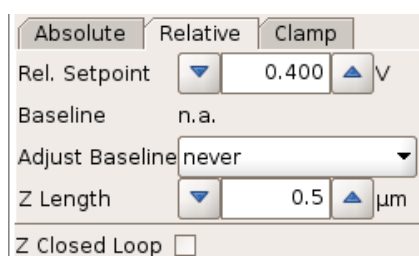
The **Force Time** display also includes the data collected during pause or delay segments, while the oscilloscope in **Section 6.1.2** only displays the extend and retract movements. The example here shows the data curve for a movement with 1s extended delay, (see **Section 6.2.4** for details).

## 6.2 Spectroscopy Control panel

The **Spectroscopy Control** panel contains the numbers that control the actual cantilever movement, for instance the vertical range of the movement, the x,y position of the tip and the maximum force.

There are three main ways to define the movement in the software. **Absolute** mode uses absolute start and end positions of the z-piezo. In both **Relative** and **Clamp** modes, the surface position is defined by a feedback channel setpoint (generally Vertical deflection in contact mode), and the piezo movement is relative to this position. The **Clamp** mode is an extension to **Relative** mode, with an extra segment to hold a molecule or cell in tension between the tip and surface. The force **Clamp** means that the position is controlled to maintain the same force. **Relative** spectroscopy mode is the most commonly used option, and the best place to start testing **Spectroscopy** mode for new users.

### 6.2.1 Relative spectroscopy mode



In **Relative** mode, the surface position is defined by a setpoint value (i.e. **Vertical deflection** in contact mode, or **Amplitude** in IC mode). The piezo movement is performed relative to this position. This is particularly helpful when the (x,y) position changes between curves, as in force mapping, so the surface height is unknown from point to point.

**Z-Length** defines the range of the force curve.

The piezo extends, moving the cantilever towards the surface, until the **Relative Setpoint** is reached. This is taken as the surface position. As this position is not known in advance, data is collected continuously, and when the surface is reached the data for the last **Z Length** of piezo movement is kept as the extend part of the curve. The retract movement is then made and data is collected also for **Z Length**.

*Note: The **Relative Setpoint** is independent from the “normal” feedback setpoint. The setpoint in the **Feedback Control** panel with the parameters **IGain** and **PGain** does not influence the motion during a force spectroscopy experiment.*

## 6.2.2 Relative spectroscopy mode using the Baseline function

In contact mode, there is an additional option to use the **Baseline** feature to correct for changes in the vertical deflection of the cantilever over time. The cantilever can bend due to changes in the environment, such as temperature, pH or ionic strength in the liquid. When **Adjust Baseline** is not being used (set to **never**), then the **Relative Setpoint** corresponds to the actual value of the **Vertical deflection** that is used for the turnaround point.

When the **Baseline** is being used, then the **Vertical deflection** value is measured at the start of the force curve (the furthest point from the sample). The **Relative Setpoint** is now used as the *change* in **Vertical deflection** to define the surface or turnaround position.

The number in the **Adjust Baseline** list sets the interval for how often the baseline should be measured. If **never** is selected, it is switched off and no baseline is used. If **1** is used it is measured before every force curve. The interval can be increased if fast spectroscopy measurements are being made, or if the environmental changes are small.

In this example, the baseline is being adjusted for each spectroscopy curve. The **Baseline** from the last curve has been measured as 263.7 mV, and the **Relative Setpoint** is set as 0.2 V. Therefore the force curve will use the **Vertical deflection** value of 0.4637 as the turnaround point.

If **Closed Loop** is enabled ☒, the piezo movement during the experiment is defined by the linearized value of **Height (measured)** rather than the piezo voltage value **Height**, so the nonlinearity and hysteresis of the piezo is corrected during the movement.

## 6.2.3 Absolute spectroscopy mode

In **Absolute** mode, the absolute piezo position defines the tip movement. **Tip Saver** can be used to stop the movement early if the deflection value is reached.

**Z length** is the piezo movement range, and **Z Scan End** is the piezo position closest to the sample. The piezo starts at position **Z length + Z Scan End**, moves towards the sample to position **Z Scan End** and back again. If the surface position has been measured, it is used for the initial **Z Scan End** value.

Z Closed Loop ☐

If **Closed Loop** is enabled ☒, the piezo movement during the experiment is defined by the **Height (measured)** channel rather than the piezo voltage **Height**, as for **Relative** mode. Additionally, in **Absolute** mode, **Z Scan End** is updated from the current piezo surface position in the relevant channel to prevent high forces from the different values of **Height (measured)** and **Height**.

With **Tip Saver** enabled ☒, a maximum cantilever deflection can be defined (or minimum amplitude in IC mode). The piezo begins extending to the **Z Scan End** position. If the **Tip Saver** setpoint is reached first, the piezo movement is stopped early and the retract curve started, so the force curve will have an actual range smaller than the set **Z length**. If the **Tip Saver** setpoint is not reached during the approach, the force curve proceeds as normal and the range is equal to **Z length**. When **Tip Saver** is disabled, the piezo moves to **Z Scan End** regardless of the cantilever deflection.

*Note: The **Tip Saver Setpoint** is independent from the “normal” feedback setpoint. The setpoint in the **Feedback Control** panel with the parameters **IGain** and **PGain** does not influence the motion during a force spectroscopy experiment.*

The **Force Fishing** button is only relevant for customer with a force wheel device –see the accessories section.

## 6.2.4 Timing settings for Absolute and Relative modes

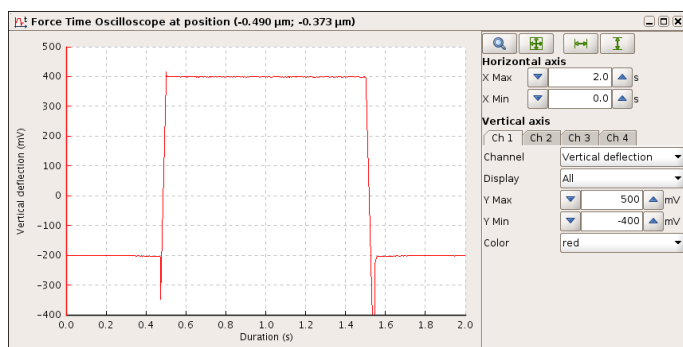
Both **Absolute** and **Relative** modes have the same basic movement sequence, the difference is the way the start and end points are defined.

Extend Time	<input type="button" value="v"/>	<input type="text" value="1.0"/>	<input type="button" value="^"/>	s
Retract Time	<input type="button" value="v"/>	<input type="text" value="1.0"/>	<input type="button" value="^"/>	s
Retr. Delay	<input type="button" value="v"/>	<input type="text" value="0.0"/>	<input type="button" value="^"/>	s
Ext. Delay	<input type="button" value="v"/>	<input type="text" value="1.0"/>	<input type="button" value="^"/>	s
Delay Mode	Constant Force			
Pixel Rate	<input type="button" value="v"/>	<input type="text" value="2048"/>	<input type="button" value="^"/>	Hz

**Extend Time** and **Retract Time** control the speed of the movement on the extend and retract parts of the force spectroscopy experiment.

The **Retracted Delay** is a waiting time at the retracted position (far from the surface) e.g. to allow the sample to recover, or to hold stretched molecules between the tip and surface.

The **Extended Delay** is a waiting time at the fully extended position (near the surface), e.g. to wait for sample molecules to adsorb to the tip.



The **Force Time Oscilloscope** (see **Section 6.1.3**) is useful to see the movement during the force curve. Here, an example is shown with the **Extend time** and **Retract Time** set to 0.5s, and an **Extended Delay** of 1s. The delay mode is **Constant Force**, which can be seen in the Vertical deflection plot shown here.

The **Retracted Delay** takes place between consecutive force curves, and is not shown in the **Force Time Oscilloscope** or saved with the data.

Pixel Rate	▼	2048	▲	Hz
------------	---	------	---	----

The **Pixel Rate** is the rate at which pixels are stored through the whole force curve. Combined with the times for **Extend**, **Retract**, and **Extended Delay**, this defines the pixel number for each segment of the force spectroscopy cycle.

A higher number produces better resolution but a larger data file. If the rate is too low, particularly for a long **Z length** value, it may affect some control options, (e.g. **Tip Saver** or **Relative Setpoint**). There must be enough pixels so the software can react to limit the deflection.



The full options for movement speeds (including setting extend and retract rates independently) can be found in the **Advanced Force Settings** panel (see **Section 6.6**), which can be opened directly with this button. The actual pixel numbers for each segment are also displayed.

## 6.2.5 Force Clamp spectroscopy mode

In **Clamp** mode, the surface position is defined in the same way as in **Relative** spectroscopy, and the initial part of the movement is the same – the piezo is extended until the setpoint is reached. On the retract part of the motion, however, there are extra control segments that allow a force clamp with the sample under tension. It is best to become familiar with **Relative** mode before starting **Clamp** experiments, as the movement is more complicated. The **Clamp** experiments are only meaningful if there is something to be stretched between the tip and sample, for instance for single molecule unfolding.

Absolute	Relative	Clamp
Indent. Setp...	▼	0.400 ▲ V
Clamp Setpoint	▼	-0.100 ▲ V
Baseline	n.a.	
Adjust Baseline	never ▼	
Z Length	▼	0.2 ▲ μm
Initial Retract	▼	10.0 ▲ nm

There are two different setpoints for this mode:

The **Indentation Setpoint** is used to define the surface position on the **Extend** movement (like the **Relative Setpoint** in **Relative** mode). The use of the **Baseline** is exactly the same as in **Relative** mode (see **Section 6.2.2**). **Z Length** defines the movement length up to this point.

The **Clamp Setpoint** is the force during the clamp segment, and is usually tension (a force below the baseline), hence the negative default value. Often there is some non-specific adhesion to the surface, so there is usually an **Initial Retract** before the **Clamp** segment starts. This is usually a short distance, to free the tip from the surface before starting the force control. If a molecule is being stretched, the **Initial Retract** should be set shorter than the molecule length, so that it is not overstretched before the **Clamp** starts.

Z Closed Loop	<input type="checkbox"/>		
Extend Time	▼	1.0	▲ s
Clamp Time	▼	2.0	▲ s
Retr. Delay	▼	0.0	▲ s
Ext. Delay	▼	1.0	▲ s
Delay Mode	Constant Force ▼		
Clamp Mode	Constant Force ▼		
Pixel Rate	▼	2048	▲ Hz

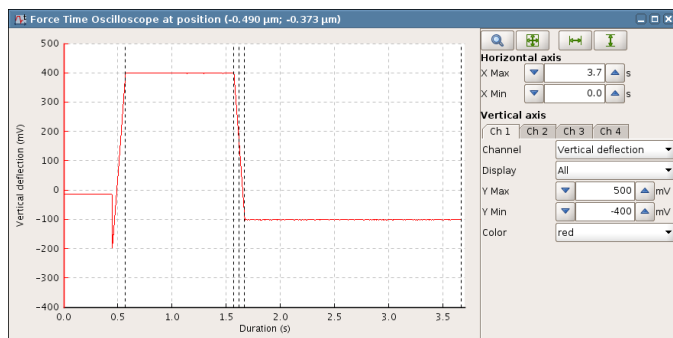
The **Extend Time**, **Extended Delay** and **Retracted Delay** are exactly the same as for **Relative** mode (see **Section 6.2.4**). The **Clamp Time** is the waiting time during the **Clamp** segment.

The **Initial Retract** does not have a separate timing setting. The same piezo speed is used as for the **Extend** segment, and the time is adjusted automatically for the length that is set.

The **Delay Mode** is used for the **Extended Delay**, and the **Clamp Mode** is used for the **Clamp** segment. Normally both are set to **Constant Force**.

The **Pixel Rate** is used for the whole force curve.



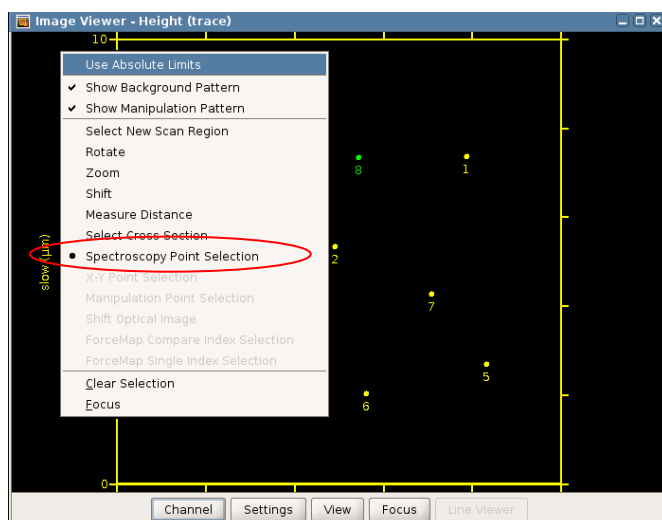


The **Force Time Oscilloscope** can be used to see the whole movement during the clamp experiment.

## 6.3 Selecting spectroscopy points

By default the force spectroscopy experiments are performed in the middle of the currently selected scan area. It is often useful to be able to perform spectroscopy experiments at particular defined locations.

### 6.3.1 Manual point selection



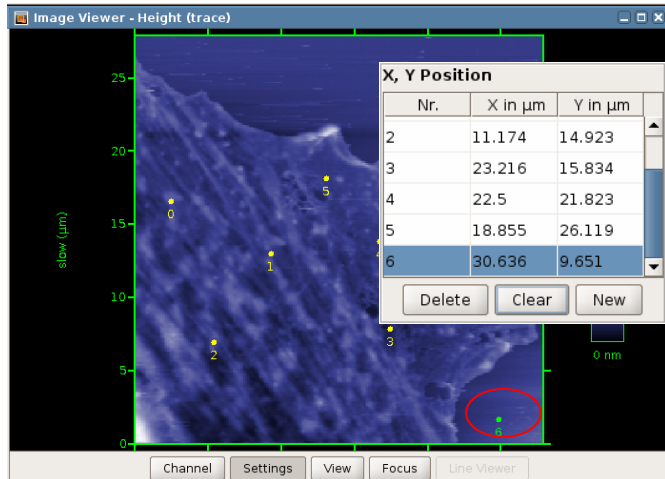
When **Spectroscopy** mode is active, points can be selected directly in the **Image Viewer** window.

Right-click in the **Image Viewer** window and choose **Spectroscopy point selection**. The normal left mouse button can then be used to select points in the image window where spectroscopy curves will be performed.

The coordinates of the points are shown in the **X,Y position** panel of the **Spectroscopy Control** window. The coordinates are relative to the center of the 100 x 100  $\mu\text{m}$  scanner range (not necessarily the current scan region).

X, Y Position		
Nr.	X in $\mu\text{m}$	Y in $\mu\text{m}$
0	-2.687	3.507
1	2.942	3.571
2	0.576	-0.693
<div> Delete Clear New </div>		





If the cantilever is not changed between imaging and spectroscopy, it is easy to select particular points within a scanned image. The Spectroscopy icon can be toggled to switch between image and spectroscopy views.

Spectroscopy points can be activated by clicking on them in the **X,Y Position** window. Activated spectroscopy points are green in the image viewer, and highlighted in the **X,Y Position** list. If you double-click on the table entry then you can type new values.

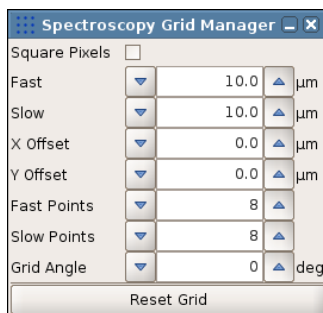
Note that if **Save analyzed** is chosen when a list of points has been selected, slope and adhesion values for the whole set of points are saved in a single file.

### 6.3.2 Spectroscopy Grid Manager

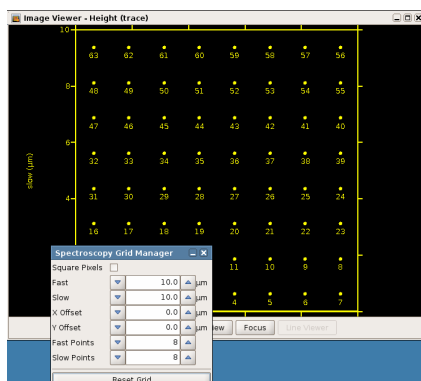


The **Spectroscopy Grid Manager** allows a grid of spectroscopy points to be generated automatically. This is similar to performing a **Mapping** experiment (see **Section 7.1**), but the points are handled independently rather than automatically going from one point to the next. Various scripts are available for custom movements from one point to another, including repetitions or other special settings. Ask JPK for more specific information.

The settings in the **Spectroscopy Grid Manager** are the same as the text fields for selecting scan regions or mapping grids. The values are automatically initialized from the current scan area, but new values can be set anywhere within the full piezo range. The sizes and offsets can be typed directly in the text fields, and the grid will be updated when **Set Grid** is clicked.



The spectroscopy points list in the Scan Control panel is automatically updated with the new points when they are set, and points can be selected and moved just as for manually set points. When the points are changed, the **Spectroscopy Grid Manager** will be greyed out. The last grid can be reset using **Set Grid** again.



The position of the tip is only updated when the next point in the list is selected, otherwise **Run** continuously will repeat the movement indefinitely at the current point.

X, Y Position		
Nr.	X in $\mu\text{m}$	Y in $\mu\text{m}$
0	-4.375	-4.375
1	-3.125	-4.375
2	-1.875	-4.375
3	-0.625	-4.375
4	0.625	-4.375

## 6.4 Other Features

### 6.4.1 Adhesion and slope

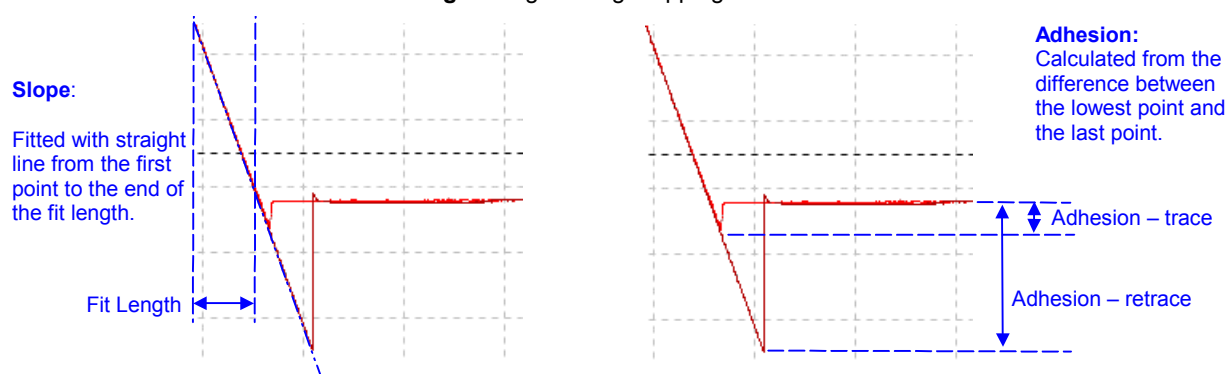
Slope - Linear fit	
Fit Length	50 nm
Slope (extend)	12.81 V/μm
Slope (retract)	12.93 V/μm
<input type="checkbox"/> Show extend fit	
<input checked="" type="checkbox"/> Show retract fit	
Adhesion	
Adhesion (extend)	1.631 V
Adhesion (retract)	1.489 V
Relative Setpoint Values	
Height (extend)	3.481 μm
Height (retract)	3.495 μm
Height (measured) (ext...)	6.427 μm
Height (measured) (retr...)	6.419 μm

In the right hand panel of the Spectroscopy oscilloscope display there are also options to perform simple online analysis using the **Analyze** panel. For some force curves, the adhesion and slope can be calculated as in the diagram below, as a linear slope and a single value for the maximum adhesion. Slope and adhesion values can be calculated from either the extend or retract curves.

The **Slope** value is fitted with a linear fit over a fixed range (software versions 2.3 and above), so it is no longer a simple subtraction. The single fit parameter is the **Fit length**, which must be shorter than the steep straight part of the force curve.

*Note that this analysis is not valid for more complex force curves!* In general, force curve analysis depends strongly on the type of sample or interaction being studied, and particular models should be chosen and fitted separately for the particular type of data.

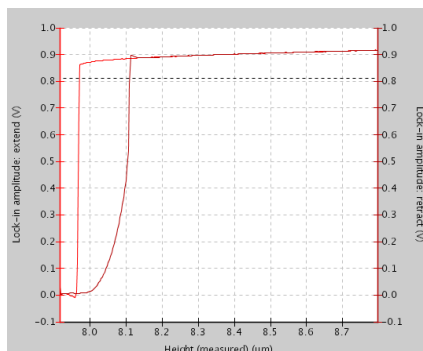
In **Relative** force mode, the **Height** value when the relative setpoint is reached is also displayed. These values can be used offline with other software to calculate sample volume, for instance, and are also used in the **Image Viewer** to form a **Height** image during mapping.



The display of the fit line can be turned on or off using the **Show fit** tickboxes in the **Analyze** panel. The fit lines give a quick guide to show whether the **Fit length** is set appropriately; if there is a significant difference between the line and the force curve, the **Fit length** should generally be reduced.

**Save Force Scan** saves complete force scans (i.e. one full trace and retrace curve) at each spectroscopy point. **Save Analyzed** only saves the slope and adhesion values (i.e. two simple numbers) at each spectroscopy point.

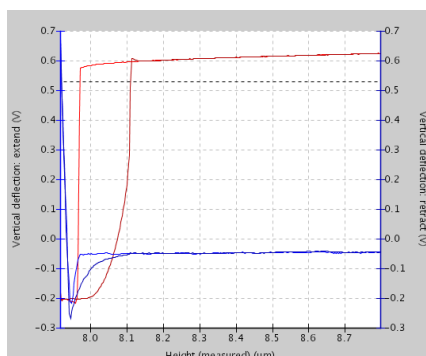
## 6.4.2 Force spectroscopy in Intermittent Contact mode



Typically force measurements are performed in contact mode. Force-distance experiments can also be performed in intermittent contact mode, however, measuring the vibrating cantilever during the force spectroscopy cycle.

When the spectroscopy window is opened in IC mode, the default channel is **Lock-in amplitude**. The amplitude of the cantilever decreases from the free value (on the right) as the cantilever approaches the surface (towards the left).

Other channels, including the **Vertical deflection** (here in blue) can also be plotted using the register cards on the left of the spectroscopy oscilloscope.



Near the surface, the vertical deflection increases as the lock-in amplitude (red) decreases because of the damping of the cantilever oscillations. The hysteresis in the amplitude curve can be seen from the vertical deflection channel to be caused by the adhesion of the tip.

Note – the **Slope** and **Adhesion** values are calculated from the feedback channel. In IC mode this is the amplitude, so the **Analysis** function is switched off for the IC mode force curves as the numbers are not relevant.

## 6.5 Managing spectroscopy curves

### 6.5.1 File saving



After obtaining a single spectroscopy curve, the full curve data can be saved using **Save scan** in the panel of the spectroscopy curve window. **Slope** and **Adhesion** values can be saved with **Save analyzed**, as described in **Section 6.4**. Using **Autosave**, each complete force distance curves is saved automatically

The force curves produced during normal spectroscopy mode are written in a compressed binary file format (file names "filename.jpk-force"), so they cannot be read into normal mathematical or spreadsheet processing software programs. They contain the data of the trace and retrace segments as well as time delays or force clamp steps. Force curves can be converted into simple text using the JPK IP processing software. Alternatively, a script can be used to convert a folder of force curves.

Under the **Setup** menu option, **Saving Settings** defines the settings for all file formats. Choose the **Force scans** register card, and the name, directory and channels can be set for saved force curves.

All parts of a force curve can be saved in the .jpg-force force curve format, including extended pauses and clamp segments. The only part of a force curve that is not saved is the retracted pause between one curve and the next.

Channel	Extend	Extended	Pause	Retract	Clamp	Pause on Tipsaver
Height	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Height (measured)	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Vertical deflection	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Lateral deflection	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
X position	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Y position	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
X position output	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Y position output	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Auxiliary channel 1	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Auxiliary channel 3	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Auxiliary channel 4	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Error signal	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
DAC 5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Height (measured & smoothed)	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

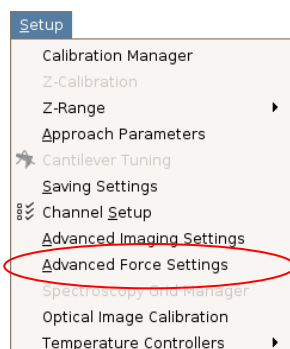
The **Filename root** adds the same prefix to every force spec file of this session.

Files are saved into the chosen **Folder**.

Extend and Retract of **Height** and **Vertical deflection** channels are required data for collection. Other channels can be enabled if required for the experiment.

**Note:** during force scanning, the keyboard shortcut **Ctrl-F** can be used at any time to save the last recorded force curve. The name and curve settings are all taken from the **Saving Settings** panel.

## 6.6 Advanced Force Settings



The **Advanced Force Settings** allow full control of the speed and waiting times during the force spectroscopy experiment and can be opened directly from the **Spectroscopy Control** or by selecting **Setup** → **Advanced Force Settings**.

The scan rates for extending and retracting can be set as a rate, as a time or as a speed of the tip movement. Changing the value of a parameter in one box updates the other text fields for the same parameter. If the tickbox is selected for using the same rate for approach and retract, then changing the numbers in one section automatically updates the other section.

**Advanced Force Settings**

**Extend and retract rates**

☒ Use the same rate for extend and retract

Update time or speed: Constant Time

Z Extend Rate: 1.0 Hz

Z Extend Time: 1.0 s

Z Extend Speed: 0.5  $\mu\text{m/s}$

Z Retract Rate: 1.0 Hz

Z Retract Time: 1.0 s

Z Retract Speed: 0.5  $\mu\text{m/s}$

**Number of collected pixels**

While extending: 2048

While retracting: 2048

**Waiting times at extended and retracted points**

Retracted Delay: 0.0 s

Extended Delay: 1.0 s

Delay Mode: Constant Force

**Movement after Relative Setpoint is reached**

Z Scan End: 0.0  $\mu\text{m}$

**Start spectroscopy settings**

Start option: Extend first

Extend first

Retract first

**Start spectroscopy settings**

Start option: Extend first

**Settings after spectroscopy stopped**

End option: return to starting mode

return to starting mode

constant height mode

retracted piezo mode

idle mode (approached)

**Force Scan Repetitions**

☐ Number of Scans: 1

☐ Infinite

☐ Go through all points in xy position list

Repeat mode: Repeat each point

In the **Advanced Force Settings** panel, there are further options for setting the extend or retract rates. The movement can be set as a rate, a time or a speed; these values are updated automatically when one is altered. The tickbox can be deselected when the extend and retract rates should be different.

The **Pixel Rate** is the setting in the main **Spectroscopy Control** panel, which is valid for all segments of the force curve. The **Number of collected pixels** is displayed here for the current timing settings.

The waiting times at the retracted or extended points can also be set. There are two options for the control system during these waiting times. If **Constant Height** is selected, then the piezo height is held constant during the waiting time. If **Constant Force** is selected, then the height is adjusted to keep the setpoint at the value it had at the beginning of the waiting time.

In **Relative** mode, there is the option here to set an extra **Movement after the Relative Setpoint is reached**. The setpoint can then be set to a small value, to just contact the surface, and the distance here can be thought of as an extra piezo movement to indent the sample.

In **Absolute** mode, the beginning of the force spectroscopy movement can start in either direction. The standard movement is to extend first (the piezo moves the cantilever towards the sample). It is also possible to set Retract first. In this case, the piezo retracts the cantilever away from the sample and then back again. In both cases, the spectroscopy movement is made and saved in pairs of extend and retract movements.

This setting is disabled in **Relative** and **Clamp** mode.

There are also settings for the piezo control at the end of the movement. By default, the instrument returns to the same state after the spectroscopy that it was in before. This option is called **return to starting mode**, i.e. if spectroscopy was started from the retracted position it returns there, and if it was started from the approached position (the **idle** state) it returns to the surface. It is also possible to force the spectroscopy to finish in a particular state, regardless how it was started. In addition to **retracted** and **approached**, it is also possible to end in **constant height** mode, where it waits at the end position of the spectroscopy movement.

The force scan can be repeated a defined number of times, using the options at the bottom of each panel. If all the points in the list are used, then the movement can be repeated a certain number of times at each point, or the whole list can be repeated a certain number of times.

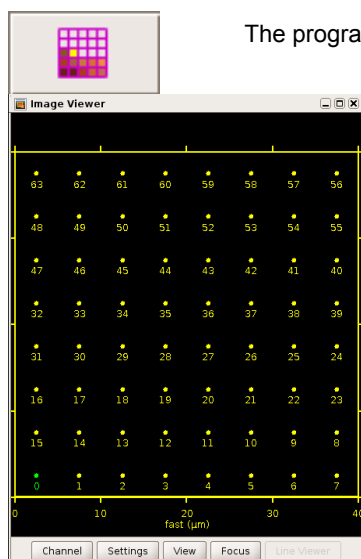
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## § 7 Other modes and features

### 7.1 Force mapping

#### 7.1.1 Introduction to force mapping

The **Force Mapping** feature is an extension of the **Spectroscopy** mode. Please first read **Chapter § 6** to have an overview of the basic **Spectroscopy** features. In the **Force Mapping** mode a defined number of spectroscopy experiments are performed in a given square or rectangular pattern. In most cases the user first scans an image and then overlays a grid of spectroscopy points.



The program is opened with the icon **Map**

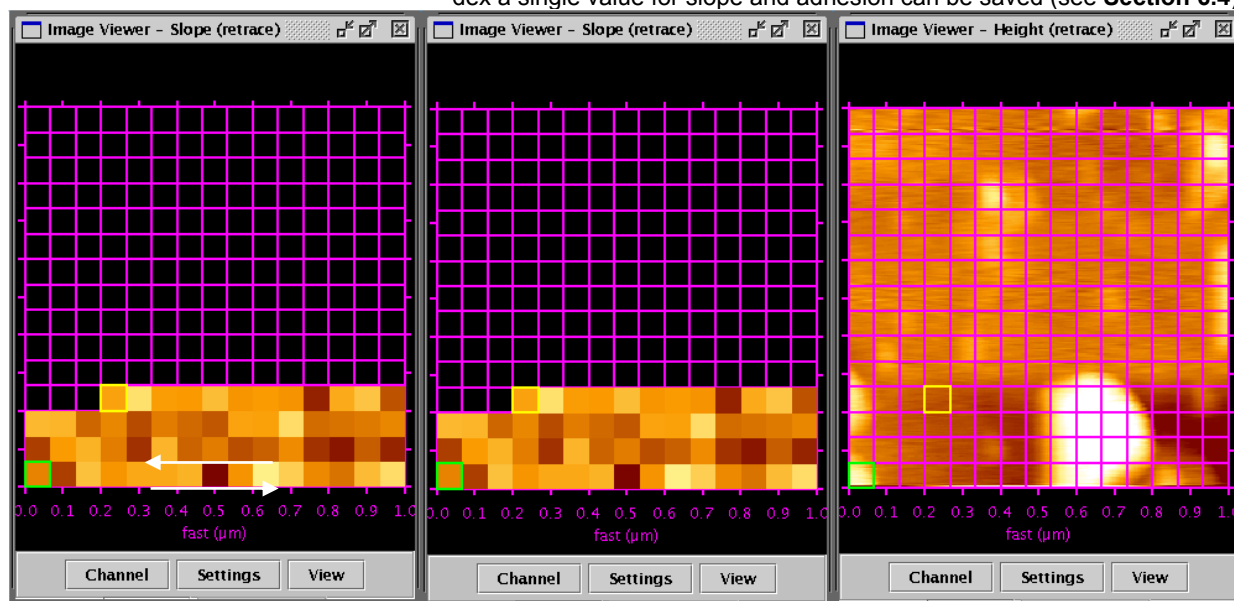
The Force mapping is similar to normal spectroscopy, at specially selected points (see **Section 6.3**). Although the display in force mapping mode normally shows a grid of filled points, the scanner performs a single spectroscopy experiment (force-distance curve) in the centre of every cell.

Minimum size of a grid is 8 x 8 cells.

Maximum size of a grid is 128 x 128 cells.

Rectangular (non-square) grids are allowed. The total number of pixels should be considered, i.e. not just the grid points but also the number of data points per force curve.

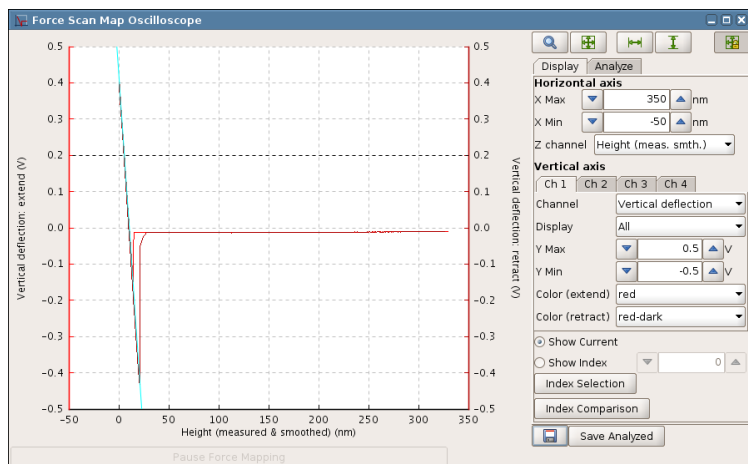
Each spectroscopy point is called an “index”. For every index a single force spectroscopy curve can be saved. Alternatively or additionally, for every index a single value for slope and adhesion can be saved (see **Section 6.4**).





In the user interface the values for **Slope** and **Adhesion** are displayed in **Image Viewer** windows (and can also be saved as tiff images) The values are calculated from the force curves, as described for the standard force spectroscopy in **Section 6.4**. Note that the adhesion and slope information is calculated from either trace or retract, so check that the channel in the **Image Viewer** is set correctly for force mapping images, or the image will appear blank.

The force mapping experiment is started at index 0 (bottom left hand corner) and proceeds “back-and-forth” i.e. left to right on the first row, right to left on the second etc. The current spectroscopy point is marked by a yellow frame.

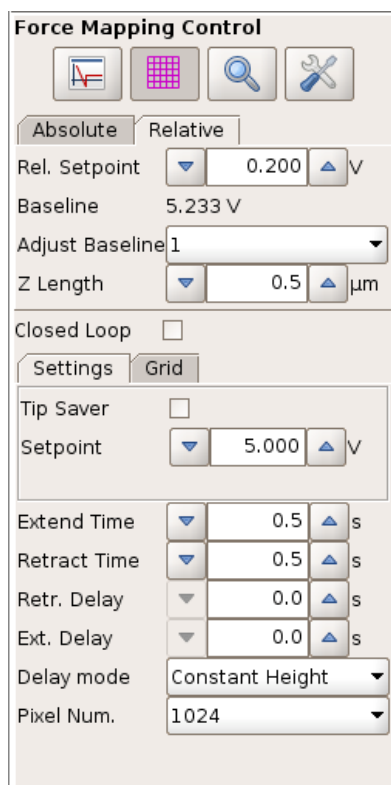


The force curves are also displayed individually in the **Force Scan Map Oscilloscope**.

The force curve for each index can be observed during the scanning. Index points can also be chosen from old force mapping scans, and the data displayed if the full force curves have been saved.

*Note: The map must be selected in the **Map List** for the data to be displayed here! If no data is seen in the map oscilloscope, then check that a map is selected in the **Map List** (the selected map is outlined in green)*

## 7.1.2 The Force Mapping Control panel



The **Force Mapping Control** panel contains the main settings for this mode.



The spectroscopy plot can be opened from the Mapping control panel. The current spectroscopy data is displayed while the mapping experiment is in progress, or selected data from old scans can be displayed.



The grid can be shown or hidden during the experiment.



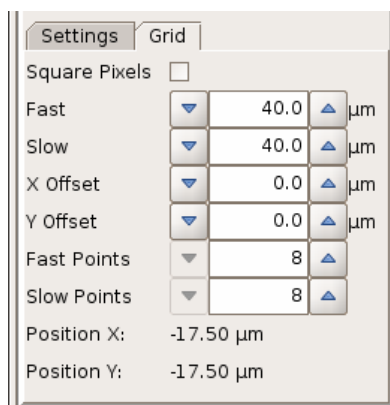
The area for the Force Mapping experiment can be updated using the **Image Viewer** window, as for normal images (see **Section 4.2.3**).



Shortcut to open the **Advanced force settings**, to set advanced options for rates, waiting times and repeats of force curves. See **Section 6.6** for more information.

As with normal force spectroscopy, **Absolute** and **Relative** modes are possible, see **Section 6.2** for details. The Baseline can be used as described in **Section 6.2.2**. The timing and pixel **Settings** have the same options as for spectroscopy mode; see **Section 6.2.4**



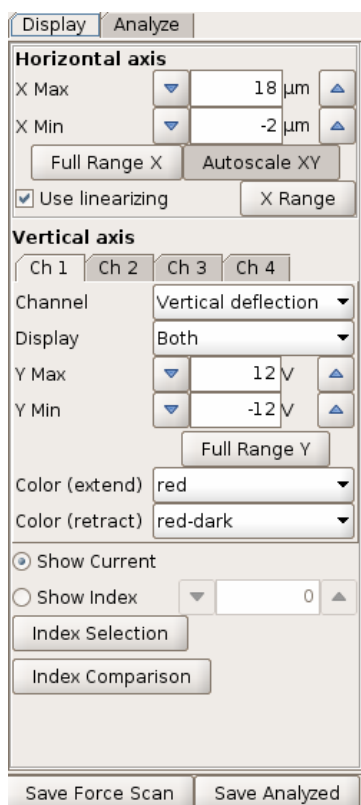


### Grid

The scan region can also be set here, using the **Fast** and **Slow** axis sizes and offsets as for images. Usually, however, the region is set using the mouse in the **Image Viewer**, as for image scans. The number of points in the grid can also be set. The minimum size is 8 x 8 spectroscopy points and the maximum size is 64 x 64 spectroscopy points.

Rectangular grids, and grids with different spacings in the fast and slow scan directions are also allowed, depending whether **Square Pixels** is enabled.

## 7.1.3 The Force Map Oscilloscope



In the **Force Map Oscilloscope**, spectroscopy curves from either the current measurement or a given index can be displayed. Chose the index (cell) number and the spectroscopy experiment is displayed in the oscilloscope. Click **Full range X** or **Autoscale XY** to get the force curve properly displayed.

**Show current:** current spectroscopy data are displayed as the map progresses

**Show index:** spectroscopy data from other points are displayed.

With **Index Selection**, the mouse can be used to click on a pixel in the image viewer to display the corresponding force curve.

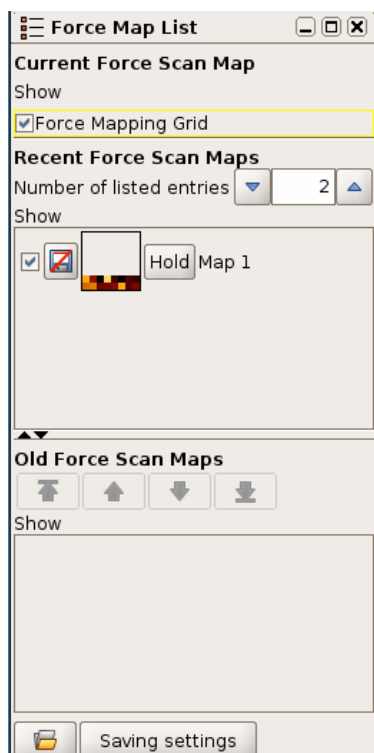
The **Index Comparison** button in this panel opens a special oscilloscope window where the data from several pixels within a map can be compared.

*Note that the force scan map file must be selected and focused in the **Map List** (shown with a green frame) to show the force distance curves from completed map images!*

## 7.1.4 Data types and file saving



The **Map List** icon can be found on the main shortcut bar across the top of the SPM software. The **Map List** is a tool for administering and saving Force Mapping files, and is similar to the **Scan List** for normal images.



As with the Scan List ( see **Section 4.3.3** for more details), there are three sections for the Map List.

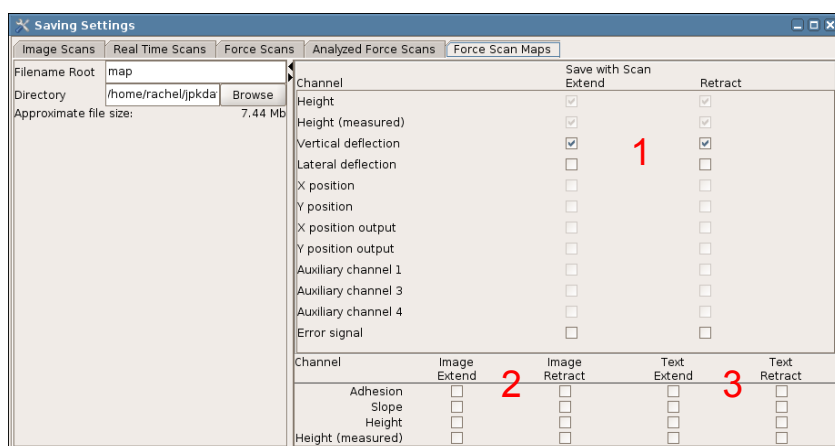
The **Current Force Scan Map** is held at the top, until it is finished. It is then sent to the **Recent Force Scan Maps** section. Files here are only held temporarily; the number of files to hold is set in the text field.

If the **Hold** function is used, the scans are sent to the **Old Force Scan Maps** section, where they will be held while the software is open. At any time in the Recent or Old sections, they can be saved by clicking the disk symbol. If the disk symbol has a red line through it, the files are not yet saved permanently. If Autosave is enabled, the files are all saved.

By right-clicking on a particular entry, the file can be selected, saved, or displayed on the Image Viewer. Files are saved in ascii-format storing all the force curves in a single file, with the extension \*.map\_txt



Old scans can be re-opened from the disk. When SPM loads the map file it calculates the slope and adhesion values to redisplay a force map image.



Under the **Setup** menu option, **Saving Settings** controls the default settings for file saving in all formats. Choose the **Force scan maps** option to set the channels for saving in force maps, along with the default directory and filename.

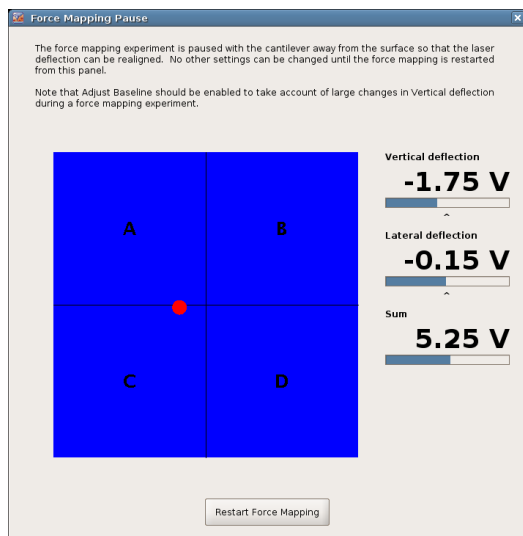
Several formats for saving data are possible for each force map – the full force spectroscopy curves, or selected images or text values from analysis channels.

1. Save the whole set of force map curves. Files are stored as a \*.map\_txt file and are in ascii format. This is recommended, since no data is lost, but the file size can be quite large.
2. Save the force map images (**Height, Slope and Adhesion**). Files are stored as a \*.force file and can be read with JPK image processing software IP.
3. The slope and adhesion data (two or four numbers per curve, depending if extend and/or retract are chosen). Files are stored as \*.out and are in ascii format.

---

## 7.1.5 Pausing force mapping

### Pause Force Scanning



In the **Force Map Oscilloscope**, below the spectroscopy curve display region, there is a tab button for pausing the force movement during a map.

The vertical deflection signal from soft, gold-coated cantilevers tends to change over time, as the cantilevers bend in response to any environmental changes. This should generally be corrected for by using the **Adjust baseline** feature (see **Section 6.2.2**) to adjust the **Relative Setpoint** during the map. For long maps with many pixels, the cantilever changes could be enough to move away from the center of the detector. The **Pause Force Scanning** feature allows the map to be interrupted, so that the detector signal can be returned to the center. Always make sure that **Adjust baseline** is enabled first, so that the new value of Vertical deflection will be accounted for in the **Relative Setpoint**.

The software will complete the current force curve movement and then wait in constant height mode. Check that the piezo is not moving, and then adjust the Vertical deflection using the normal positioning screws.

While the pause window is open, nothing in the software can be changed, everything is paused waiting for the laser adjustment. When **Resume Force Mapping** is clicked, the map will proceed as normal.

## 7.2 Manipulation and lithography



Manipulation allows the user the flexibility to define paths over the sample surface that the tip will follow. In imaging mode, the tip moves back and forth in straight lines across the sample, and in force spectroscopy the tip moves vertically over a specified point. In manipulation mode, the tip moves freely across the surface, along user-specified paths. Between the paths the tip is lifted from the surface so a series of disconnected lines can be drawn.

The manipulation mode can be used for two main kinds of experiments:

- The tip can be used to move particles or molecules around on the surface (nano-manipulation).
- The tip can be used to draw a pattern across the sample, e.g. scratching with a higher force (nano-scribing).

During manipulation, it may be useful to calculate the applied forces, to choose suitable values for manipulation of the surface or objects lying on the surface. Often stiff cantilevers may be used, in which case the thermal noise calculation is not appropriate, but the spring constant supplied by the manufacturer should be sufficient for a general estimate of the applied forces.

Typical forces used in nano-manipulation	$< 1$ nN in any case $< 500$ pN to move molecules in case of H-bonds between molecule and its support $\sim 100$ pN to move molecules
Nano-scribing	$\sim 5$ nN, depending on the material

Other manipulation experiments can involve applying a voltage to the tip (e.g. local oxidation) or using a functionalized tip to create some surface modification along the manipulation paths. In this case the manipulation force will probably be kept to a minimum, as for imaging, since some other mechanism will create the surface pattern or modification.

### 7.2.1 Paths and points

**Manipulation Control**

Velocity:   $\mu\text{m/s}$

Setpoint:  V

IGain:  Hz

PGain:

Path 1

Delete Clear New

**X, Y Position**

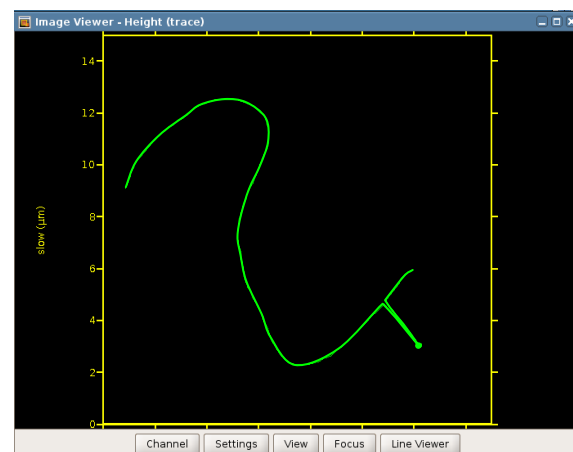
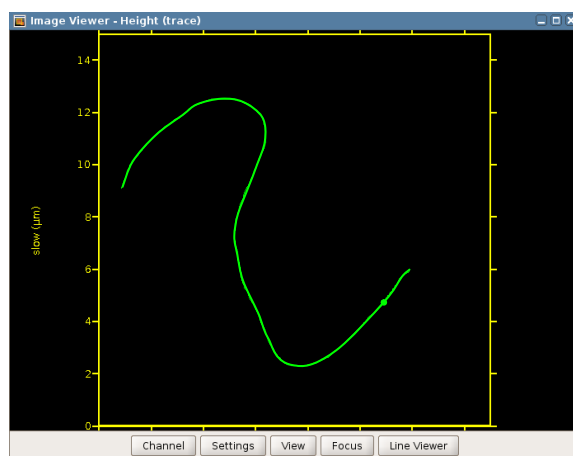
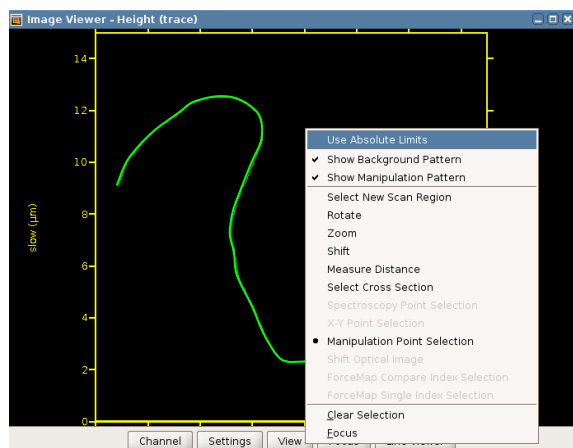
Nr.	X in $\mu\text{m}$	Y in $\mu\text{m}$
0	-17.18	6.945
1	-16.762	7.258
2	-16.136	7.676
3	-14.674	8.512
4	-13.943	9.034

Delete Clear New

In the SPM software the whole set of movements for the manipulation is divided into paths and points. Each path is a set of (x,y) points, and the tip moves from one point to the next with a constant **Velocity** over the sample surface. During the movement along each path, the **Setpoint** is held at the value in the **Manipulation Control** panel, and controlled using the **IGain** and **PGain** feedback values in the **Manipulation Control** panel. These values are independent of the normal imaging setpoint and feedback gains set in the imaging **Feedback Control** panel. Often the setpoint for the manipulation is set at a higher force than for imaging, so that objects can be moved or the surface modified.

During the manipulation, the tip moves along the list of specified paths in order. Between the paths the tip is lifted from the surface, forming a series of disconnected lines.

The paths for manipulation can be drawn freehand in the software, or loaded as a scalable vector graphics (.svg) file created by typical vector graphics software, such as Illustrator or CorelDraw. The path is defined as straight lines joining the points, so a curved line will have many points very close together, and for a completely straight line only two points are required.



In **Manipulation** mode, the default mouse action is to draw manipulation paths in the **Image Viewer**. Click and drag with the mouse and the movement will automatically be converted into a set of points in the **Manipulation Control** panel. When the mouse is released, then the next click and drag is saved as a new set of points in the next path.

The example here shows a single freehand path, the currently selected path is always displayed in green.

The mouse function can be changed with the right-click menu to change the view in the Image Viewer.

When a point in the path is selected in the **Position** list, then it is shown highlighted as a green dot in the **Image Viewer**. The selected point may be moved around using the mouse (click and drag).

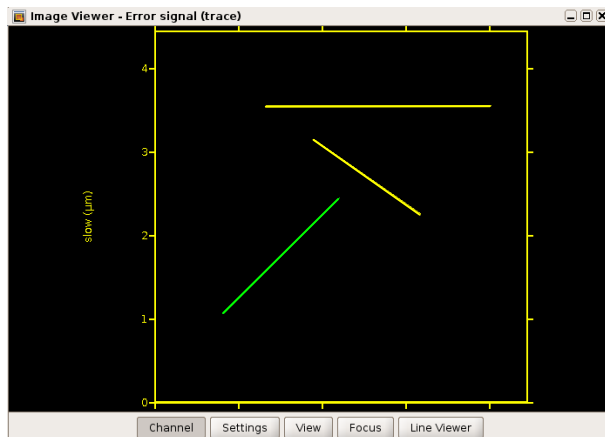
X, Y Position		
Nr.	X in $\mu\text{m}$	Y in $\mu\text{m}$
220	3.192	-2.563
221	3.224	-2.5
222	3.255	-2.437
223	3.287	-2.405
224	3.319	-2.342

Delete Clear New

The single point is moved, and the lines connecting it with the adjacent points can now be seen clearly.

X, Y Position		
Nr.	X in $\mu\text{m}$	Y in $\mu\text{m}$
220	3.192	-2.563
221	3.224	-2.5
222	4.613	-4.11
223	3.287	-2.405
224	3.319	-2.342

Delete Clear New



More than one path can be drawn – this example shows three paths. A path can be selected from the pull down menu in the panel to the left of the screen. The currently selected path is displayed in green and other paths are displayed in yellow.

Path 3

Delete Clear New

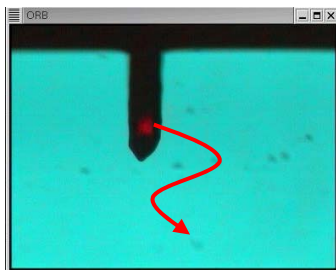
X, Y Position

Nr.	X in µm	Y in µm
0	34.415	46.06
1	33.046	44.691

Delete Clear New

The buttons **Delete**, **Clear** and **New** at the top apply to the whole paths. **Delete** removes the currently selected path, and **Clear** removes all stored paths.

The buttons **Delete**, **Clear** and **New** at the bottom apply to individual points within the selected path. **Delete** removes the currently selected point, and **Clear** removes all the points in the current path.

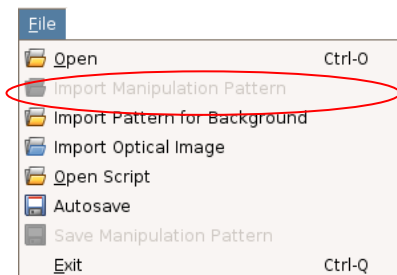


After the manipulation paths have been defined, clicking **Run** performs the entire manipulation movement (all set paths) once, after which the cantilever is left in the retracted position.

If an optical view of the cantilever is available then the movement of the cantilever can be observed during the experiment.

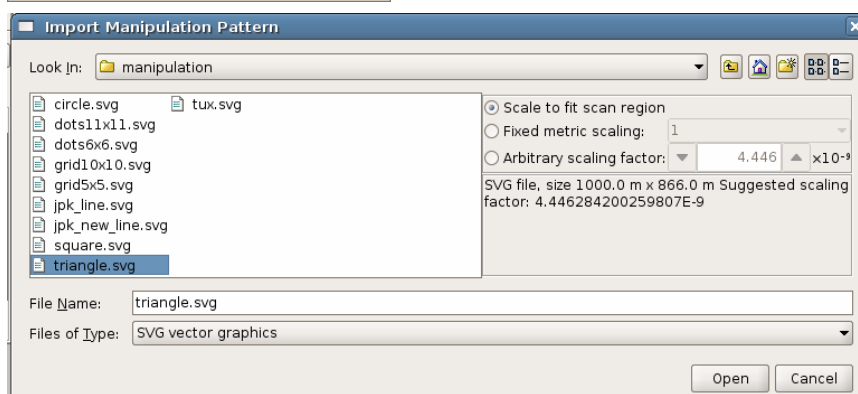
The current point is shown in red in the **Image Viewer** during the movement.

## 7.2.2 Importing and exporting scalable vector graphics files



Often manipulation patterns are required that have fixed shapes or distances, and are more complicated than is practical by hand drawing.

In the main menu the option **File -- Import SVG** can be used to import a pre-saved file, the format used is "scalable vector graphics", file.svg.



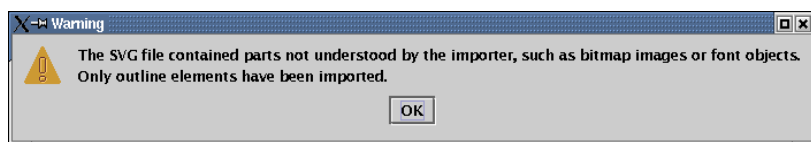
There are two standard scaling choices:

**Scale to fit scan region** (best for vector graphics created by external software packages)

**Fixed metric scaling** (factor 1.0), to import the pattern at the same size it was saved (best for paths exported from the SPM software).

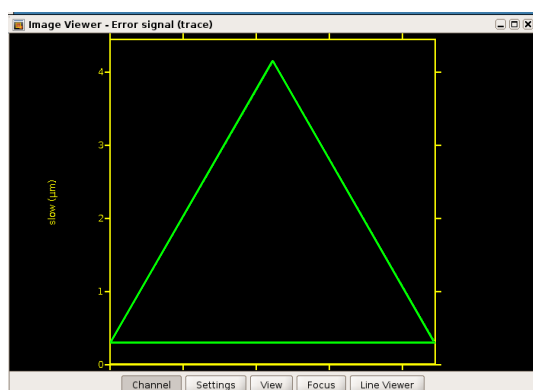
Note: manipulation paths exported from SPM are always defined over the full 100 x 100 micron scan area. Re-opening them using "scale to fit scan region" will generally reduce the size (e.g. if the scan area is 10 microns, the size will be x 10 smaller).

Additional **Fixed metric scaling** options allow factors of  $10^{-3}$ ,  $10^{-6}$  and  $10^{-9}$  to be quickly selected, or any arbitrary number can be entered in the text box, using the format 1.0e-6. The **Suggested scaling factor** in the information panel would be the scale factor used to fit the manipulation pattern to the scan area, so this can be used as a guide for appropriate scaling of the stored file. The **Arbitrary scaling factor** default value is the value to fit in the scan region.



Note – the SVG files must contain only outline objects. Other elements, e.g. font objects, filled or patterned objects, will be ignored by the import filter.

Text may be used, but must first be converted by the vector graphics software so that it is stored as a set of the outline paths rather than as characters in a font. The exact export option will depend on the vector graphics software, but should be in the "Text" menu and be described as something like "Convert text to outline" or "Convert to outline path".



This example shows one of the example manipulation paths which can be found under:  
`/opt/jpkspm/data/manipulation/*.svg`

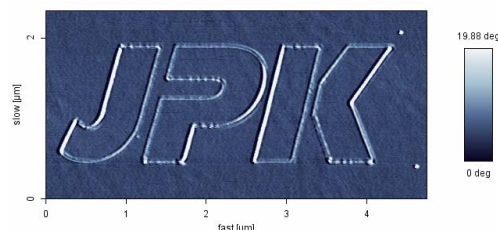
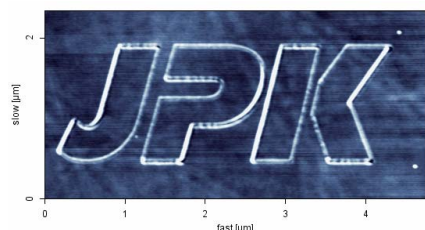
There are several basic examples supplied with the JPK SPM software, such as arrays of lines or points and simple geometrical shapes. These can also be modified in SPM and resaved.

Paths or patterns can also be created using standard vector graphics software such as Corel Draw or Illustrator. Free software is available to create vector graphics on [www.inkscape.org](http://www.inkscape.org)

## 7.2.3 Simple manipulation examples

### 7.2.3.1 Lithography

For test purposes, a nano-scribing experiment can be performed onto the polycarbonate polymer surface of a commercially available compact disc. In this example an intermittent contact mode cantilever with a spring constant of around 40 N/m was used. Imaging in intermittent contact mode did not cause any changes to the surface of the polymer, but using a contact mode setpoint of around 0.3 V produced a sharp line engraved in the polymer. Material from the central depression is moved to the edges, which become raised. The overall topography of the scratch was a few nm. Deeper lines could be produced by applying higher forces. The images below show height and phase images (5.0 x 2.2 µm).

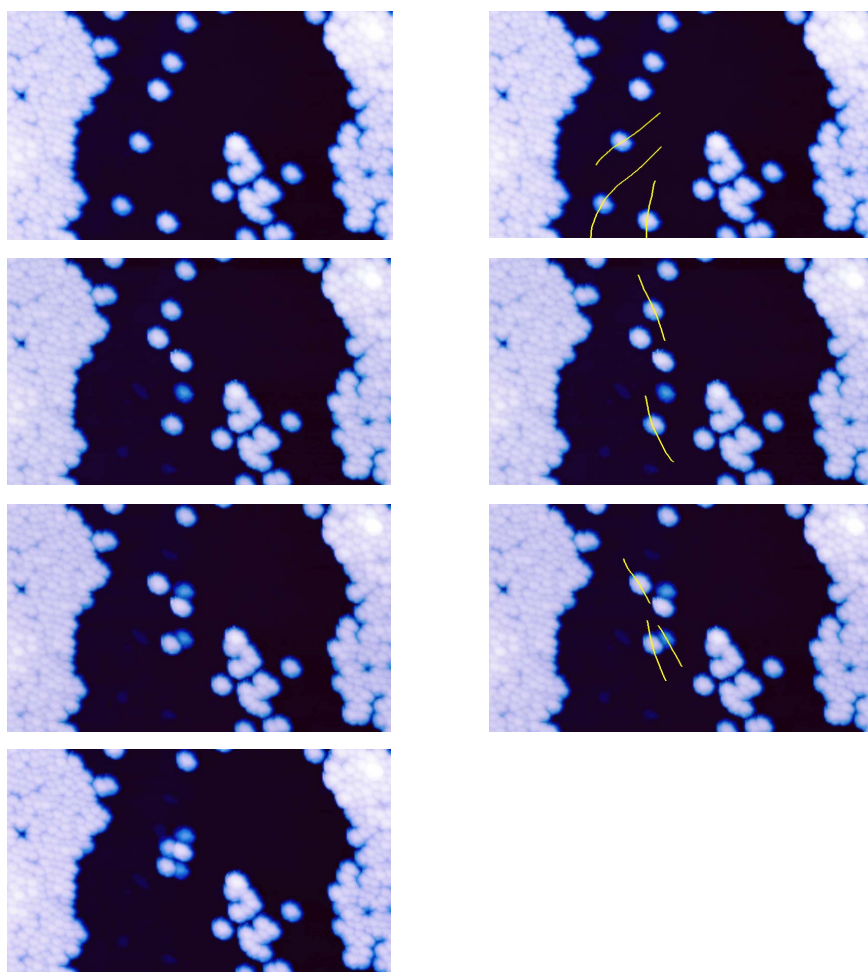




### 7.2.3.2 Moving spheres

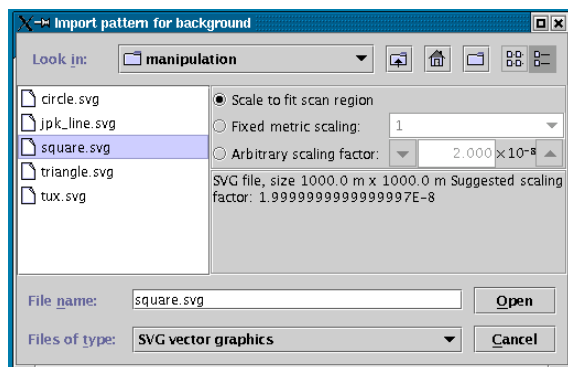
The freehand drawing of manipulation paths is useful for moving objects around on the surface, and switching between manipulation and imaging modes can be used to check where the objects have been moved to and adjust the manipulation accordingly. In this example, 120nm diameter polymer spheres were dried onto a glass slide, and manipulated using the AFM tip. The imaging was performed in intermittent contact mode in air, which did not disturb the spheres, and manipulation could then be performed using a very small setpoint in contact mode (around 0.05V higher than the free vertical deflection value).

This series of images show the spheres along with the manipulation paths drawn to produce the observed motion. All images are 5 x 3 microns, height scale 165 nm.



### 7.2.4 Background patterns

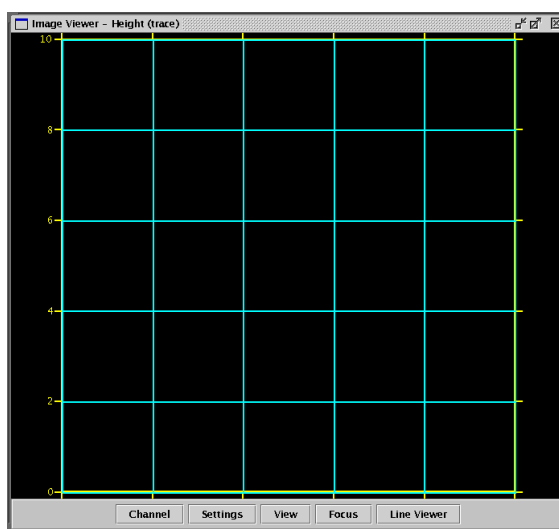
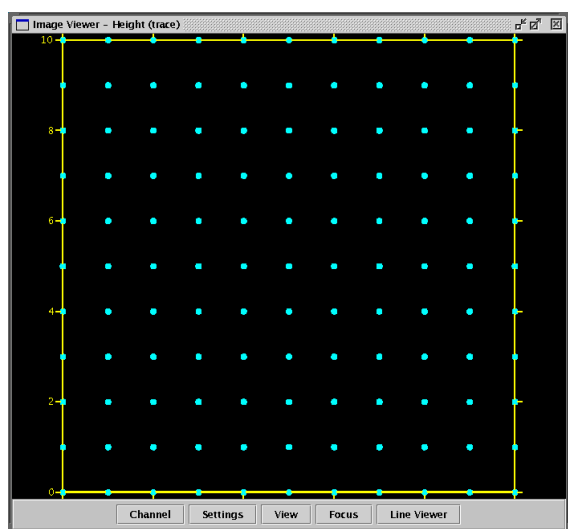
In some cases, for instance the example above moving spheres, it is useful to have a background pattern for aligning the objects. Arrays of lines or points, or even geometrical figures, can be useful for aligning the particles or other objects on the surface. For this purpose, patterns with the same format as the manipulation paths can be imported in a way that they remain visible in the software but are not used for the cantilever tip movement. The tip path can be drawn over the top to move the particles to the right positions on the background pattern.



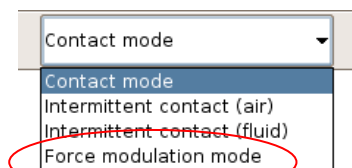
The import dialog is very similar to the manipulation path import dialog (see **Section 7.2.2** for details of the options).

Below are two of the standard examples in the directory `/opt/jpkspm/data/manipulation/*.svg`

The background pattern is always shown in blue, and can be turned off using the right-mouse click menu in the Image Viewer. The manipulation path can be drawn over the top, the background pattern has no influence on the tip movement.



## 7.3 Force modulation mode

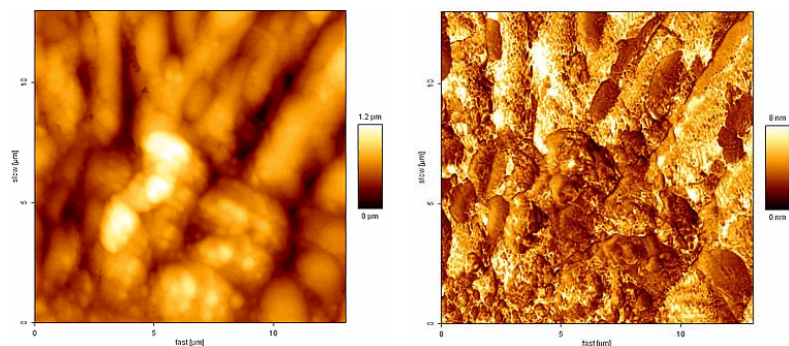


Force modulation mode is not available for all of our customers, it is a specially requested hardware feature. In the software, Force modulation is another feedback mode, like contact or intermittent contact (IC) modes, which can be used for imaging samples such as polymer blends to show materials with different stiffness. In the user interface, **Force modulation mode** can be found in the drop-down menu at the top, along with contact and IC mode.

### 7.3.1 Material contrast

Force modulation mode is something of a mixture between contact mode and IC mode, basically contact mode with an added oscillation of the cantilever. This provides qualitative data about sample stiffness alongside the normal information about sample topography. In Force modulation mode, the cantilever is modulated at a relatively low frequency during imaging using the average **Vertical Deflection** as the feedback signal and **Setpoint** (as in contact mode). The cantilever is always in contact with the sample, it does not lift off the surface as in IC (intermittent contact) mode. The force between force and sample is continuously varied by the drive amplitude, and the lock-in amplitude shows the cantilever/sample response.

On softer features the cantilever will indent further into the sample leading to a small amplitude (corresponding to a low slope in a force distance curve). Hard features only allow little indentation and lead to larger deflection amplitudes (corresponding to a high slope in a force distance curve). In principle, force modulation mode can be seen as a continuous force spectroscopy experiment in the repulsive regime during a contact mode imaging experiment. During imaging the system effectively measures the slope of the force distance curve (cantilever **Amplitude**) at a given contact force (**Setpoint**) over the image.



Height image

Amplitude image

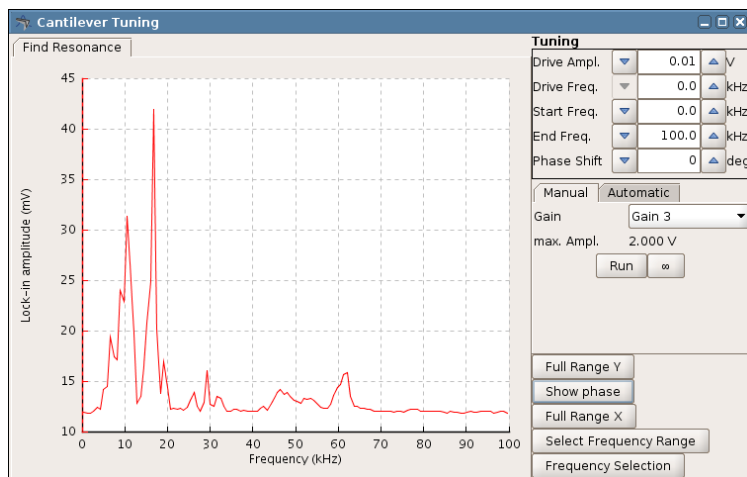
The pair of images here show an example of height and amplitude images of a polymer blend, imaged in force modulation mode in air.

The bright parts of the amplitude image have a higher cantilever deflection range, and hence represent a harder surface.

The drive is below the resonant frequency, so the measured lock-in amplitude of the cantilever will never be more than the drive amplitude. On a hard surface, the extra deflection of the cantilever (the amplitude) is exactly the same as the drive oscillation. On an ideally "soft" surface, the tip would just sink into the sample, and there would be no extra deflection at the tip, so no lock-in amplitude signal.

### 7.3.2 Off-resonance cantilever tuning

The software for the **Cantilever Tuning** for **Force Modulation mode** is basically the same as for the normal procedure for intermittent contact mode (see **Section 3.3.2**). In this case, however, the aim is not to find a resonance of the cantilever or system.



The cantilever is modulated at a relatively low frequency, well below its resonance frequency. For instance, a frequency for a typical contact mode cantilever in air would be around 5 kHz.

There are also special "force modulation" cantilevers available. The resonant frequency is around 75 kHz in air ( $k = 1\text{-}5\text{ N/m}$ ). In Force modulation mode a typical drive frequency would therefore be 30 - 40 kHz. The force constant bridges the gap between contact and non-contact cantilevers. IC mode is also possible with these cantilevers, but it may not be stable in air because of the surface adhesion.

For the choice of cantilever, it is important to match the spring constant to the particular sample. "Hard" cantilevers (i.e. cantilevers with a high spring constant) can indent both soft and hard surface material, thus reducing the contrast and potentially leading to artifacts from having the imaging force too high. "Soft" cantilevers may not be able to indent either harder or softer features on the surface. In general it is better to start with "normal" contact mode cantilevers (spring constant around 0.2 N/m) and try another cantilever with a higher stiffness if there is no surface contrast.

### 7.3.3 Typical starting values

Feedback Control		
IGain	150.0	Hz
PGain	0.0048	
Setpoint	1.000	V
Baseline	0.0	V
<input type="button" value="Reset"/> <input type="button" value="Adjust"/>		
Drive Ampl.	0.01	V
Drive Freq.	0.0	kHz
Phase shift	0	deg

For the imaging, the **Setpoint** value is the average vertical deflection, so the feedback gains settings is therefore similar to contact mode. The oscillation of the cantilever provides extra amplitude and phase channels to observe differences in mechanical properties of the sample, but is not used for feedback.

A high setpoint corresponds to a high tracking force (like contact mode imaging). Start with a setpoint of 1 V. The higher the setpoint is the smaller the drive amplitude should be to avoid artifacts. Start with a drive amplitude of 0.1 V.

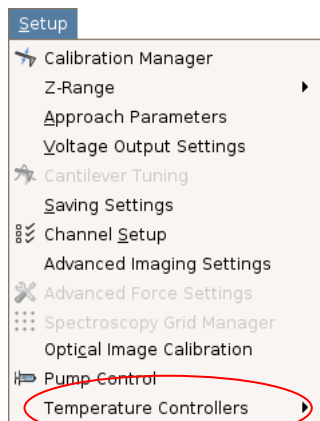
The image quality can be optimized by adjusting the drive amplitude: changing the force modulation drive amplitude during the imaging will influence the image quality of both the height and the amplitude images. As a rule of thumb, increasing the drive amplitude will lead to a bigger contrast in the amplitude image. However, sometimes, a too high drive amplitude will lead to more artifacts. Note that when the drive amplitude is changed it also might be necessary to readjust the IGain and PGain values.

This paper is one of the first where force modulation was presented, it can be used as a reference for finding further papers and information about force modulation mode:

"Using force modulation to image surface elasticities with the atomic force microscope" P.Maivald, H.J. Butt, C.B. Prater, B. Drake, J.A. Gurley, V.B. Elings and P.K. Hansma. Nanotechnology 2: 103-105 (1991)

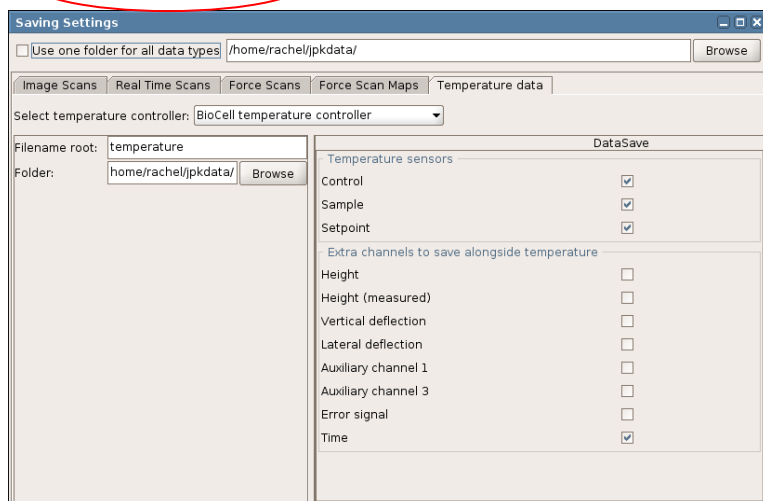
## 7.4 Environmental control for experiments

### 7.4.1 Temperature control and data saving



Many different temperature control accessories are available from JPK, which can be used to heat or cool the sample region. The details of these accessories are covered in their separated manuals.

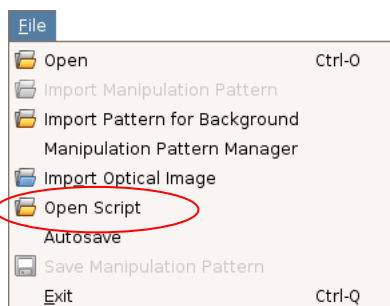
There is a general interface for controlling the temperature from the SPM software. The list of installed **Temperature Controllers** is found in the **Setup** menu. When the temperature controller is opened, the temperature is displayed and controlled from the SPM software. The temperature data can also be saved during the course of an experiment.



The **Temperature data** tab in the **Saving Settings** panel provides the settings for the temperature saving.

The saving data usually includes the **Control** and **Sample** temperatures, but other channels can also be saved.

The values of all the selected channels are saved every time a new temperature value is sent from the controller. Therefore, if the temperature is very stable, the values may only be saved occasionally.



The temperature is usually a direct control, so that the value entered in the panel is immediately used by the temperature device.

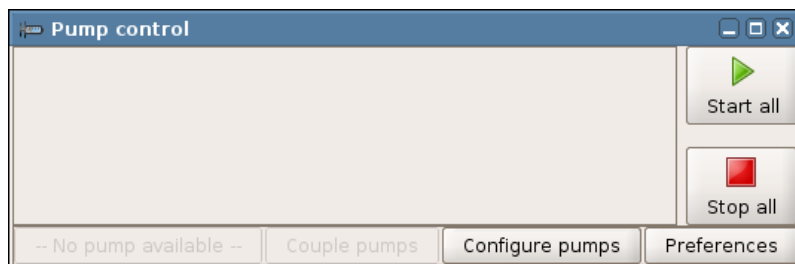
It is also possible to vary the temperature over time in a controlled way using scripts. These can be used for instance to run a temperature ramp over minutes or overnight.

### 7.4.2 Pump control for syringe pumps

Many different liquid cell accessories are available from JPK, most can be used with perfusion either by hand or using a syringe pump. Often it is convenient to control the syringe pump directly from the SPM software. This is possible for several supported models, such as the KD Scientific Model 200 Series, as well as various models from WPI.



The interface for controlling syringe pumps can be opened with this icon from the main toolbar. The pump must first be connected to the power and to the SPM computer (serial connection).



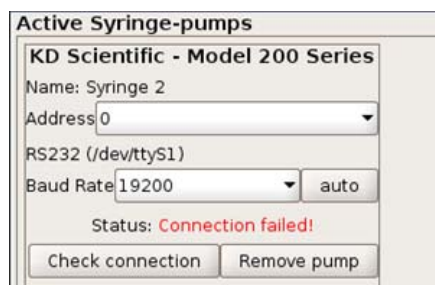
Before the pumps can be controlled, the communication must be set up.

Use **Configure pumps** at the beginning to configure the hardware settings for control.

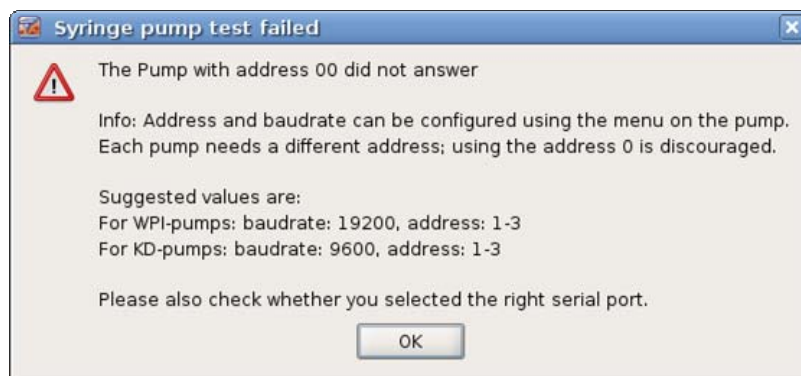


Select the model to be used from the list, under **Add syringe pump**.

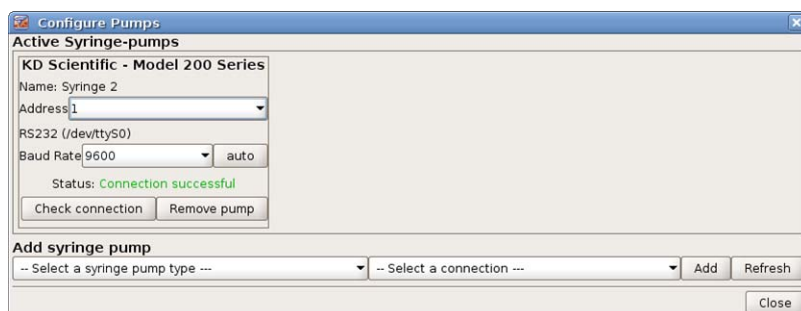
Select the serial port in the next combo box, from the list of available ports. Click **Add** to try the connection. A new syringe pump will be added in the **Active Syringe Pumps** section, even if the settings are not yet correct.



**Check connection** can be used to try the settings, or auto to try some default settings. Some of the communication settings should be first read from the syringe pump itself, such as the **Baud Rate** and **Address** (see the manufacturer's manual for details).



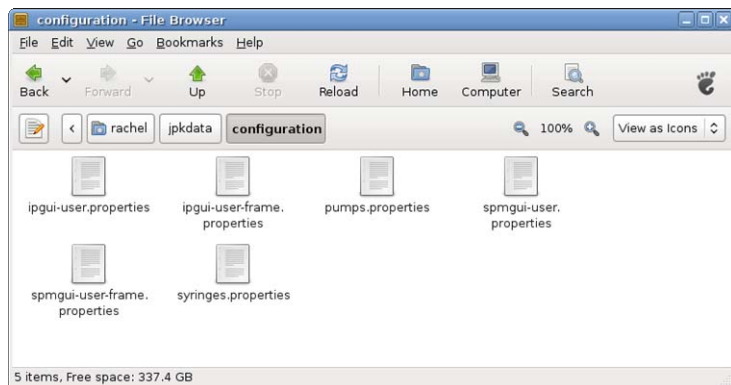
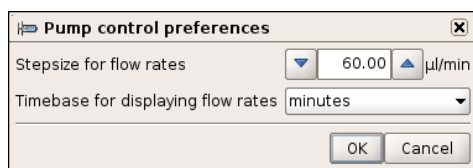
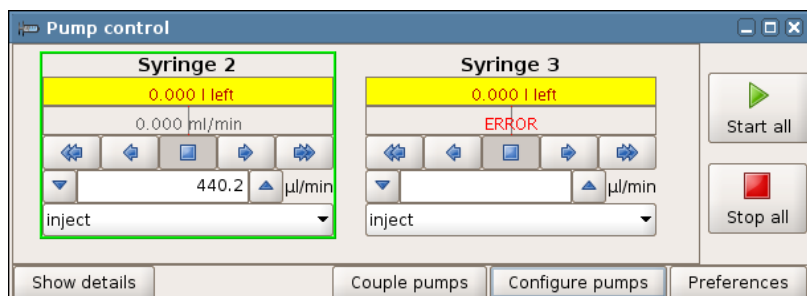
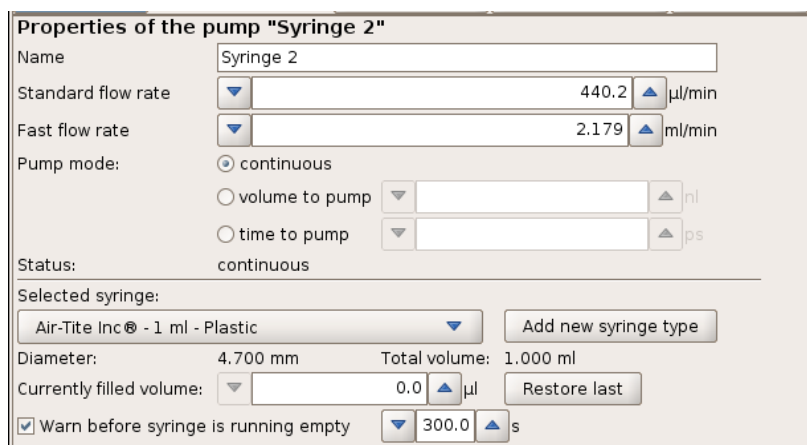
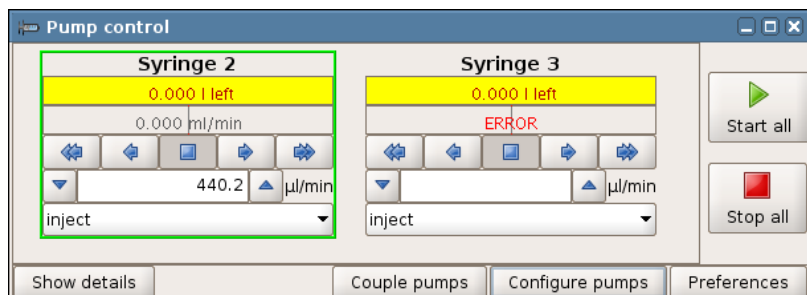
If the connection fails, the information about default settings can be helpful. The information here only covers general cases, as it is possible to change parameters such as the **Baud Rate** on the syringe pump itself. The settings for the pump and SPM software have to be identical before the communication can be successful.



In general, try combinations of serial port (/dev/ttyS0, S1, S2), Address and Baud Rate until **Connection successful** is shown. Additional pumps can also be added.

Once the configuration has been set, the information will be saved for the next time the SPM software is used.





The configured pumps will now be shown in the **Pump Control** section.

All the pumps can be controlled with **Start all** and **Stop all**. Individual pumps are controlled with their button bar



Clicking on a pump in the list will select it and it is shown with a green border. The toggle button **Show details** and **Hide details** shows the full list of settings for the currently selected pump.

The **Standard flow rate** and **Fast flow rate** are the speeds used for the single and double arrows in the button bar.

The **Selected syringe** settings are used for the conversion from distance to volume. Custom settings are saved.

If more than one pump is installed, then the option **Couple pumps** can be used to start and stop flow from all the pumps simultaneously. This is useful for push-pull systems, where the same volume should be injected with one pump and withdrawn using another pump. The pump speeds are adjusted so that the total volume is constant.

The **Preferences** settings are interface controls that are used for all the pumps.

The settings for the pump control are saved in `/home/user/jpkdata/configuration`, along with the general display settings for SPM and IP. The connection and syringe settings are remembered as long as these files are not deleted or overwritten by a new software installation.



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## § 8 Calibration

### 8.1 Height calibration

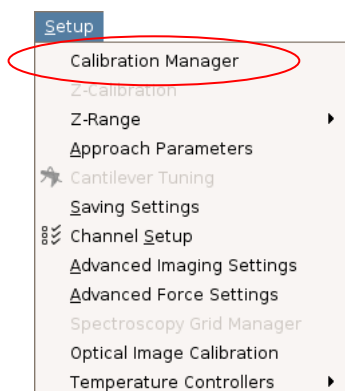
#### 8.1.1 Calibration procedure

Every z-piezo needs calibrating. This is necessary because the relationship between the applied voltage and the piezo length is not linear and suffers from hysteresis. Users with z-linearization have a separate **Height measured** channel, which does not suffer from these problems. Even so, on flat samples it can be helpful to image with a calibrated z-piezo, using the channel **Height** instead of the **Height measured** channel. For height calibration, you need a standard sample with features of a known height. These can often be purchased from cantilever manufacturers; a range of different step heights are available. It is recommended that a single user performs the calibration experiment and gives the calibration value to the others. Due to aging of the piezo material the z-calibration should be repeated around every 6 months.

First the calibration sample should be scanned, and the step heights measured using the channel **Height**. These values can then be used to calculate the scaling factor for the calibration:

$$\frac{\text{Known height of the sample}}{\text{Measured height of the sample}} = \text{Multiplier}$$

Note – it is important to know the calibration used to make the scan of the calibration grid as described above. Generally the image height channel will include the old calibration, so the Multiplier value above will be a *correction factor* (value should be around 1) that should be used to modify the old calibration.



The height calibration menu can be found in **Setup** → **Calibration Manager**. In the **Calibration Manager**, the two main panels available are **Height** and **Vertical Deflection**. Choose **Height**.

Old calibrations can be selected from the dropdown box (here showing DEFAULT).



The details of the selected file are shown in the main panel. A calibration file can be opened using **Open Calibration**, which will allow you to find a calibration file saved on disk.

Generally, values are updated using **Edit Conversion**, to create a new file from the old one.

**Edit Conversion**

filename: DEFAULT

Multiplier: 0.61939

Unit: m

Comment: Original JPK calibration for device "C6".

Save Calibration

In **Edit Conversion**, the details can be changed. Enter the new value of **Multiplier**.

*Note – the value of Multiplier here should always be around 0.6. If it is significantly different from 0.6, probably the calculation was wrong!*

*If the Multiplier value is around 1.0, then it is probably the correction factor that should be applied to the old calibration value.*

Comments, such as the date and user, or height of the calibration grid can be entered. After clicking **Save Calibration**, you will be prompted for a new filename for the edited file.

### 8.1.2 Hardware z-linearization – Height (Measured)

Generally the JPK NanoWizard® is equipped with a z-linearization. A strain gauge continuously measures the length of the z-piezo. The strain gauge can be read-out on auxiliary channel 2 and is called **Height (measured)**.

The movement of piezo material when a voltage is applied always suffers from some nonlinearity and hysteresis. Normally, the height of the piezo is taken directly from the voltage applied to extend it; this is the normal **Height** channel. When z-linearization is enabled, the strain gauges measure the actual current height of the piezo, and this value is shown in the **Height measured** channel. The calibration of the strain gauges for the **Height measured** channel is carried out by JPK, and does not normally have to be repeated by the user. The calibration of the Height channel varies with the ageing of the piezo and should be repeated every six months or so (see **Section 8.1.1**). Note that the piezo should be calibrated over the height range that will be most used for imaging; so when the **Height** channel is used for small z-ranges, as discussed below, it should be calibrated with the smallest available calibration standard.

Over small height ranges, the nonlinearity of the piezo material is not very noticeable; nonlinearity is more of a problem in the **Height** channel when the range is a significant fraction of the full piezo range. The **Height measured** channel is better over larger z-ranges, because it is linear, but over very small ranges the noise of the strain gauges is noticeable, and may be seen in very flat images. So overall, the choice of which channel to use depends on the height range of the sample topography:

- *For flat samples with height features in the range of a few nm* it is recommended to choose the **Height** channel with a manually calibrated z-piezo, which will have a lower noise.
- *For rougher surfaces with height features over 100 nm or in the micron range* it is better to use the **Height measured** channel to get linear operation over the greater height range.

For non-imaging applications where the piezo makes large movements, it is strongly recommended to use the z-linearization, since the piezo movements may be in the micron range. *Make sure that the linearization is enabled in:*

- Spectroscopy mode
- Force Mapping mode
- Sensitivity calibration

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## 8.2 Sensitivity calibration

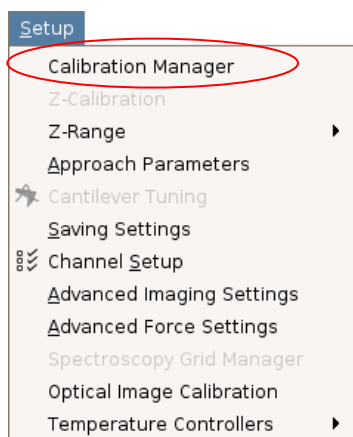
The movement of the cantilever is measured by the deflection of the reflected spot position on the photodiode. This **Vertical Deflection** value is usually displayed in Volts, and is the difference in voltage between the different sections of the photodiode. Often this value is sufficient, since the conversion from Volts to an actual deflection distance is relatively linear across the centre of the photodiode. The deflection value in Volts can be used for all the normal AFM modes and applications; imaging, force spectroscopy etc. For some measurements, however, it is important to know the deflection of the cantilever in units of length. The most common case of this is for calibrated force measurements, when both the deflection and spring constant of the cantilever must be known. This may be for force spectroscopy, to measure the forces of adhesion or mechanical properties of the sample, or it may be for contact mode imaging, to know the exact interaction forces during imaging. In intermittent contact mode this conversion from Volts to nm can also be important, if it is necessary to know the physical size of the oscillation amplitude.

The conversion factor from Volts to nm for the deflection of the cantilever is usually called the **sensitivity**. It depends on the type of cantilever and how it is mounted, so the measurements must be repeated each time a cantilever is mounted or re-mounted. The values will usually be similar, however, for identical cantilevers mounted a similar way.

The sensitivity is measured by doing a force curve on a hard surface, and looking at the deflection of the cantilever when it is in hard contact with the sample. In this region of the force curve, the movement of the piezo should correspond with the deflection of the cantilever, since the tip and sample move together. The plot of the deflection versus distance should have a gradient of 1 in this region, and the piezo movement is used to calibrate the measured deflection. These force curves in hard contact with the surface have the potential to damage the tip, however, so this procedure is often carried out at the end of an imaging experiment.



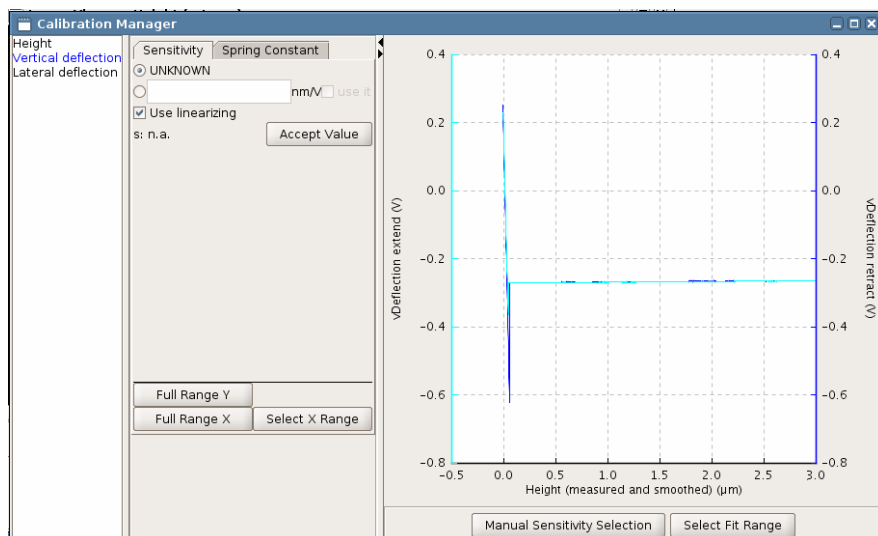
To measure the cantilever sensitivity, it is necessary to first perform a force distance experiment on a hard clean surface, such as clean glass or mica.



Select **Spectroscopy** (make sure that z-linearization is enabled).

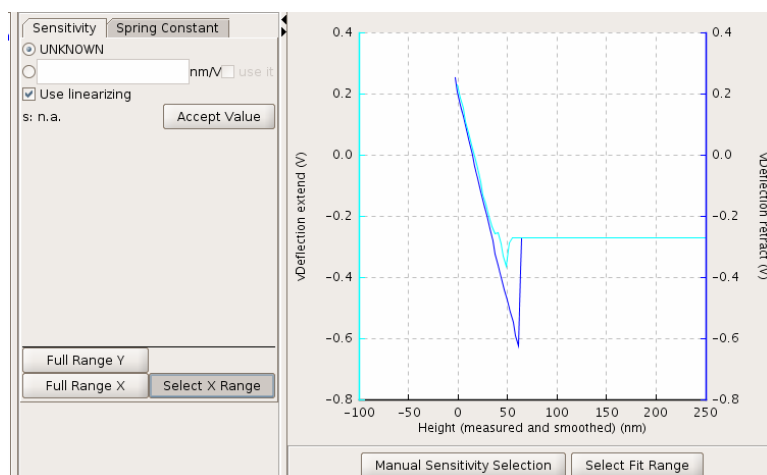
- Approach the cantilever onto the hard surface (using contact mode).
- Perform single or multiple force-distance curves as in **Chapter § 6**

The height calibration menu can be found in **Setup** → **Calibration Manager**. The panel **Vertical Deflection** should be selected by clicking on the far left of the window. The register card **Sensitivity** should be displayed by default. If the sensitivity has not yet been measured, the Sensitivity value is set as **UNKNOWN**.



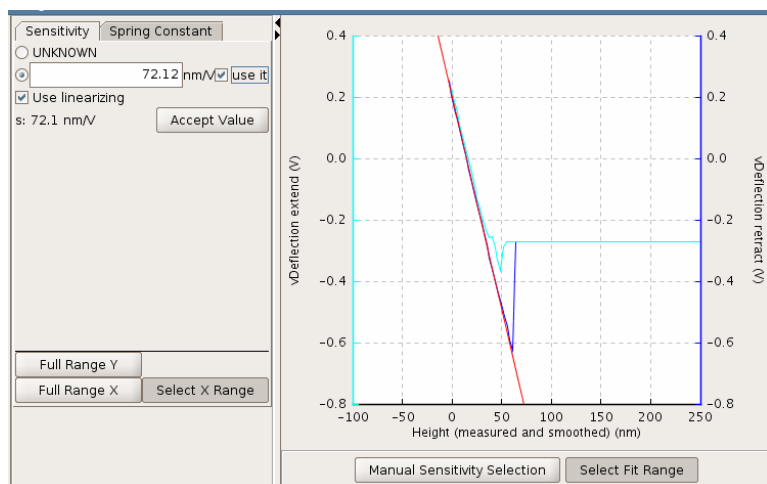
When the **Calibration Manager** opens, the latest force-distance curve is automatically loaded. The trace value of the **Vertical Deflection** is plotted in red and retrace in blue. The full curve can be displayed in the plot window using **Full Range X** and **Full Range Y**.

It is often helpful to zoom into the steep repulsive part of the curve using **Select X Range**.



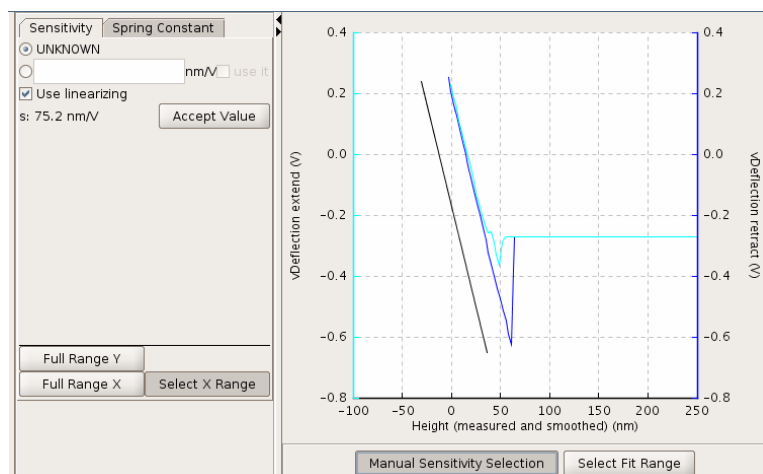
Only the steep repulsive part of the force curve is used for the sensitivity measurement. A larger scale can help with choosing a good sensitivity value. For **Select X range**, the new range is defined by clicking and dragging in the plot area with the mouse.

Once the display is convenient for the particular force curve, use **Select Fit Range** and click and drag on the plot to set a region for a linear fit of the retrace curve. These options are also available in the right-click mouse menu.



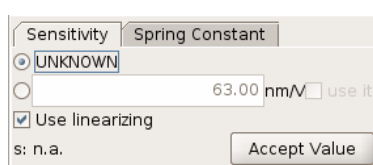
Only the linear part of the curve should be chosen for the sensitivity fit. Check that the red fit line accurately represents the steepest linear region of the force curve, or change the fit region to improve the results. The gradient of the line is automatically transferred to the left hand part of the panel, but is only permanently stored once **Accept Value** has been selected.

If the force curve is very linear, as in the example here, fitting should be the best way to measure the sensitivity.

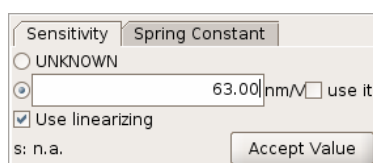


It is also possible to manually select a sensitivity using the **Manual Sensitivity Selection** button. This may be more helpful if the curve is less "ideal" and does not have such a clear linear region.

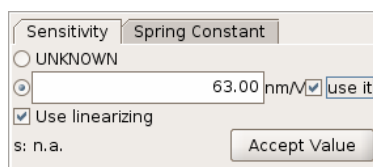
In this case, when the mouse is used to click and drag on the plot area, a straight line is plotted between the points (black line in left panel). The gradient of this line is used for the sensitivity. Select a best fit straight line through the steepest region of the repulsive part of the curve.



When a line is drawn or fitted, the gradient is used to calculate the sensitivity value shown in the left hand part of the window (here,  $s = 34.1 \text{ nm/V}$ ). The value is not used immediately, since the user may wish to redraw the line. When you are happy with the straight line fit, click **Accept Value** to store and be able to use the sensitivity value.



A sensitivity value can be stored with the scan or force data without actually being used directly in the SPM software to display the deflection values in units of length rather than Volts. When **use it** ☒ is ticked, then wherever the vertical deflection is used in the software (contact mode setpoint, intermittent contact mode amplitude, force spectroscopy axes etc) the value is now displayed as nm rather than V. When **use it** is unticked, these values are all displayed in Volts, but the sensitivity value is still stored for use later, and saved with the files. *Note: use it must be ticked to proceed to the thermal noise measurement of the cantilever spring constant*, as it is important to know the deflection in nm for this measurement.



If for some reason you want to discard the measurement (e.g. after changing the cantilever), **UNKNOWN** can be reselected, and the values will be disregarded.

Typical sensitivity values are in the range 30 – 100 nm/V. If the value lies significantly outside this range, it may be worth checking that everything is set up alright (see **Section 3.1** for details of adjusting the optical detection path).

The **Sensitivity** measurement is the first stage in the thermal noise measurement of the cantilever **Spring Constant**.

## 8.3 Spring constant calibration (thermal noise measurement)

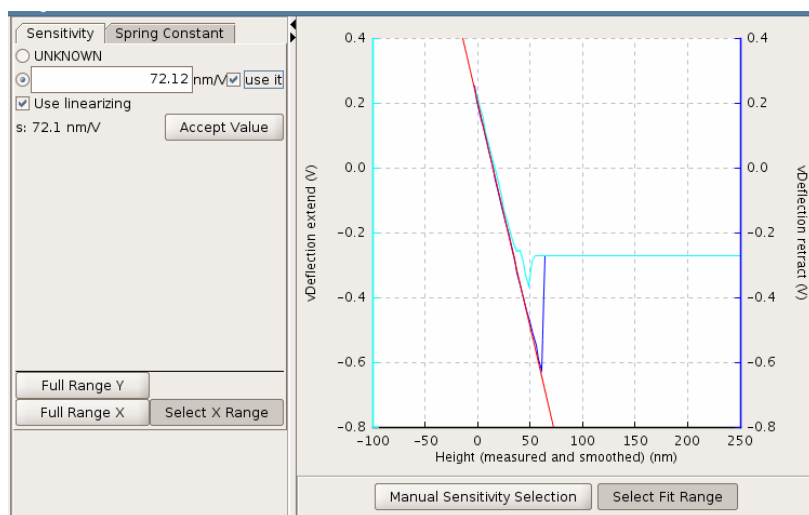
### 8.3.1 General information

Most suppliers of cantilevers deliver data sheets with their cantilevers that state the approximate spring constant. Usually this has been calculated from the cantilever shape (length, width, thickness). The spring constant is very sensitive to the thickness of the cantilever, however, and these quoted values are usually not very reliable. The range of values quoted by the supplier should give some idea of the variability, but generally the spring constant is not known within a factor of 2 or 3.

If you wish to know the spring constant more accurately, for quantitative force measurements, it should be measured in some way. Within the JPK SPM software there is the option to measure the spring constant for softer cantilevers using the thermal noise method. This allows an automatic measurement and calculation for cantilevers with resonant frequencies up to 70 kHz, which covers most “contact mode” cantilevers used for force spectroscopy. The method used is based on this paper: Hutter, JL and Bechhoefer, J. “Calibration of atomic-force microscope tips” *Rev. Sci. Instrum.* **64**: 1868-1873 (1993). For more information about cantilever spring constant calculation see the NanoWizard AFM Handbook, for example for information on using a reference cantilever to calculate the spring constant.

### 8.3.2 Spring constant measurement

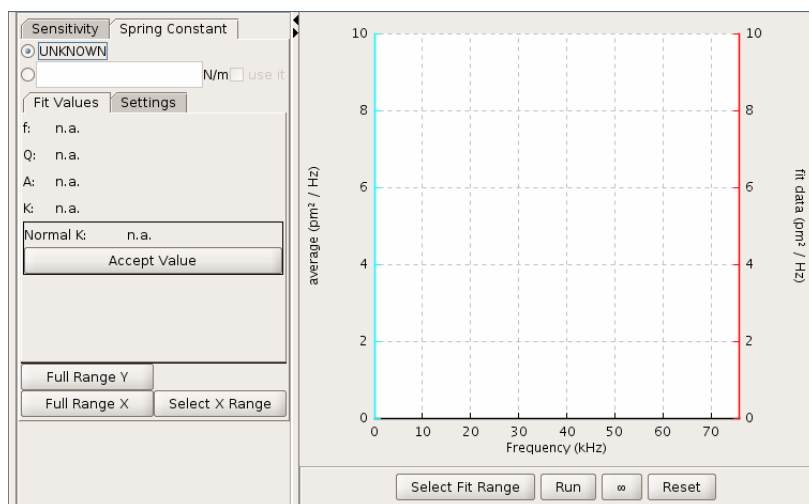
The thermal noise method relies on measuring the thermal fluctuations in the deflection of the cantilever, and using the equipartition theorem to relate this to the spring constant. Essentially, the thermal energy calculated from the absolute temperature should be equal to the energy measured from the oscillation of the cantilever spring. See the Hutter and Bechhoefer paper for the assumptions and conditions of these calculations. Since the deflection must be measured in units of length, the **Sensitivity** must first be measured, as described in **Section 8.2**.



If the sensitivity has just been measured, the **Calibration Manager** should be open on the **Sensitivity** panel.

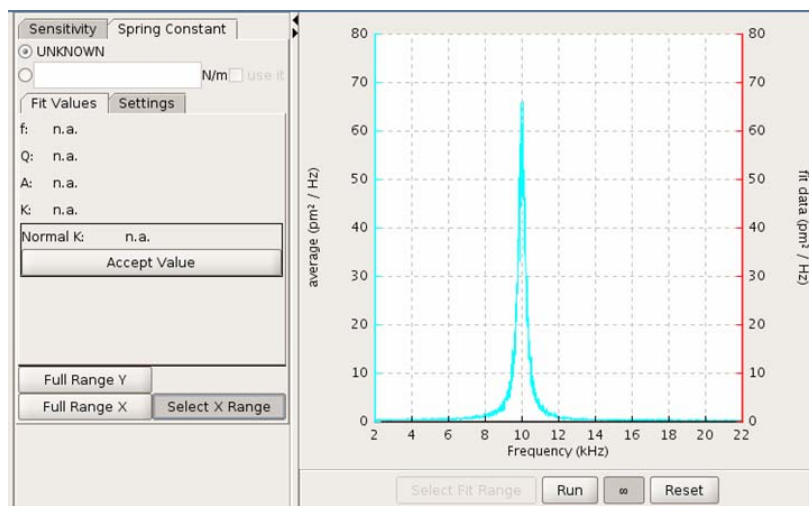
Check that the value is displayed in the text box, and **use it** ☒ is selected.

Then click on the register card **Spring Constant**.



Click **Run** or continuous (infinity symbol) to start the measurement. If continuous is chosen, each data packet is added together until it is clicked again to stop. If **Run** or continuous are started again, the data packets are added. **Reset** erases all the stored cycles from the memory to start a new measurement.

*Note: the cantilever must be retracted from the surface for this measurement!*  
If the **Run** buttons are grey and inactive, check that the piezo is retracted.



The result should be a frequency spectrum like this one. Click **Full Range Y** to see the whole spectrum. The spectrum should show a peak at the cantilever resonance frequency.

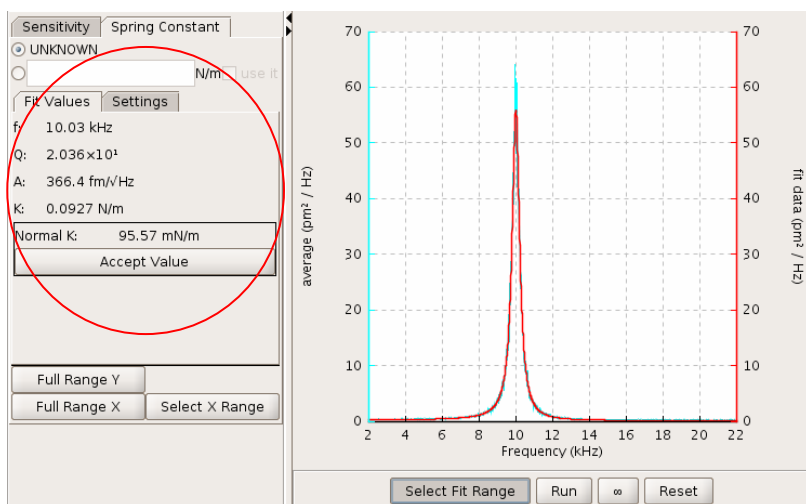
It is possible to save the frequency spectrum, using the right mouse button and selecting **Save**. Data are saved in ascii-format as \*.tnd file.

If necessary, zoom into the peak by right-clicking in the plot area and choosing **Set X range**. The new range is defined using click and drag.

The resonance must be fit with a Lorentz curve. The range for the fit can be defined using right-click and **Select fit range**. Click and drag in the plot area to define the data range for fitting.

The curve calculated for the fit is plotted in red, so you can check that the curves agree well.

In this example, the fit algorithm has found the resonant frequency of the cantilever and calculated its spring constant to be  $k = 0.09 \text{ N/m}$ .



Several other values are used for the calculation, they can be found in the **Settings** panel:

- **Temperature** (note that ambient temperature differences of a few degrees do not strongly affect the spring constant calculation).

The tilt **angle** of the cantilever is also important. SPM takes account of the normal 10 degree tilt (see **Section 3.1.1**). If an additional tilt angle is known it should be entered in **Cantilever Angle**.

As for the Sensitivity (see **Section 8.2**),  $k$  must be accepted before it is used by the software.

The round radio buttons enable the user to use or “forget” a spring constant measurement, as for the sensitivity. When **UNKNOWN** is selected, any value in the text box is disregarded. When the text box is selected, the calculated spring constant value can be used (with **Accept Value**), or another number entered manually. If for some reason you want to discard the measurement (after changing the cantilever, for example), **UNKNOWN** can be reselected, and the values will not be used.

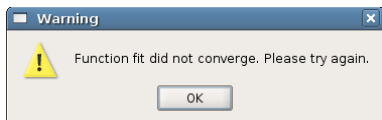
If the fit is reasonable, as in the above example, click **Accept value** and activate the tick button ☒ **Use it**. The spring constant is now applied in the rest of the software. In the **Spectroscopy** window the y-axis (vertical deflection) will now be displayed in units of force (nN). In **Contact mode** the value of the **Setpoint** is also displayed as a force.

Besides  $k$  (the spring constant), the fitting algorithm also calculates some other useful values:  $f$  is the resonance frequency,  $Q$  is the quality factor (Q-factor),  $A$  is the integrated amplitude of the resonance.

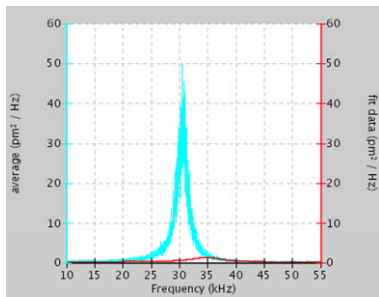
Typical values for the Q-factor are a few hundred in air, and around 1-3 in liquid.



Note that there are differences in the way the “spring constant”, or  $k$ , is calculated or measured, which are beyond the scope of this manual to discuss in detail. One difference is between the dynamic value calculated here using the thermal noise, and the static value such as when a large-range force curve is performed. Another difference is between the spring constant quoted by manufacturers, which is measured or calculated perpendicular to the cantilever, and the value **Spring Constant** calculated here, which takes account of the fact that the cantilever is mounted in the AFM head at a 10 degree angle to the sample.



Sometimes when the fit range has been selected, a red curve is not seen and this warning message may occur. This means that the frequency peak or choice of fit range was not appropriate, and either the measurement or the fit must be repeated.

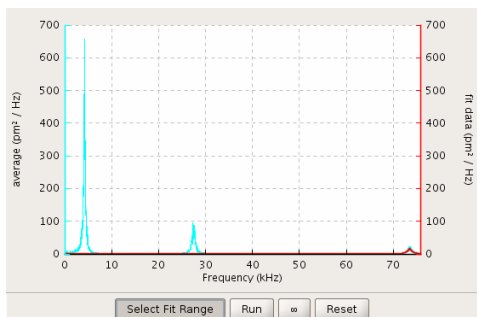


**Note:** caution must be used when fitting values for the spring constant, and the fit must be compared with the curve to check it is appropriate. The example here shows a bad fit curve that would not give a warning message, but where the fit algorithm has found another peak that does not represent the cantilever resonant frequency.

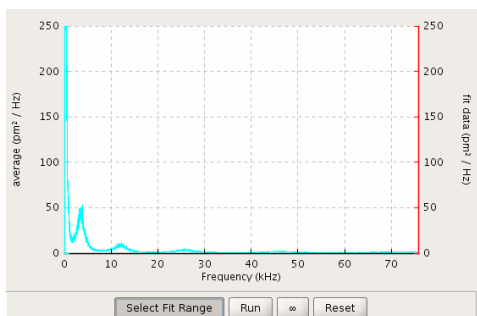
In this case you must select a better fit range.

### 8.3.3 Using thermal noise to calibrate soft cantilevers in fluid

The most accurate spring constant determination using the thermal noise method requires that the calibration be done in air. However, this will not always be optimal. The thermal noise detection method can also be used in fluid, but there may be additional complications, particularly with very soft cantilevers.



The spectrum to the left is representative of a soft (0.02 N/m) cantilever in air. There are three visible peaks in this spectrum, corresponding to the resonance (at around 4 kHz) and the first and second harmonic.



When the same cantilever is submerged in fluid each peak is damped, reducing both amplitude and frequency. The resonance of the cantilever is now extremely low, and it can be difficult to fit the curve properly. At lower frequencies these curves are also more susceptible to noise. In this case the user may wish to fit the second peak (or first harmonic) to get a better fit. However, if this is done a correction factor must be used. In this case the spring constant would be correction factor\*spring constant determined using the fit in the SPM software.

For rectangular cantilevers the correction factors have been described by Butt and Jaschke (Butt and Jaschke, 1995 Nanotechnology **6**: 1-7).

And for triangular (MLCT type cantilevers) by Stark et al. (Stark et al., 2001 Ultramicroscopy **86**: 207-215)

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## § 9 Optical images and integration

### 9.1 The CCD camera

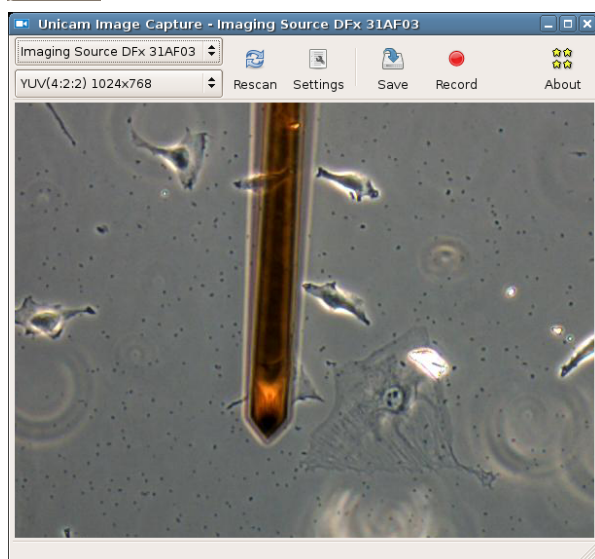
#### 9.1.1 Unicam



Recent installations of the NanoWizard® AFM system are equipped with a Firewire camera. The same camera can be used with either an inverted optical microscope (Life Science system) or the top view optics (standard system). If using an inverted optical microscope with the optional DirectOverlay feature, the optical image can be imported into SPM (see **Section 9.3**).

Simply connect the Firewire cable from the AFM computer to the camera. The connection will automatically be recognized by the software.

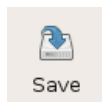
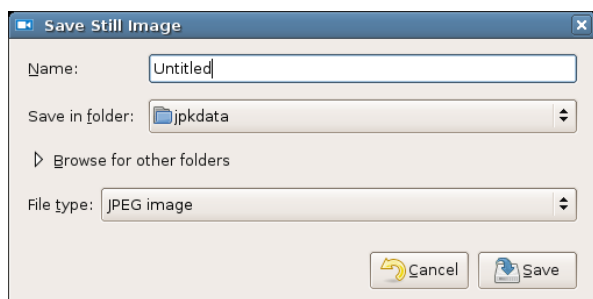
Start the camera viewer with the **CCD** button from within the JPK SPM software icon bar. The software for the optical camera is called **unicam**.



The software should automatically recognize the camera type and settings (this may take 1-2 seconds). The camera window will automatically display the live camera image.

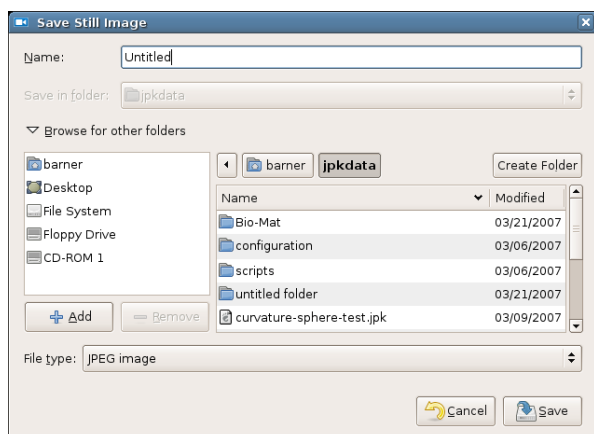
Generally no further settings are required – the camera electronics will make an automatic adjustment of gains and exposure time that will display a reasonable image in most circumstances.

Note that the default size of the camera window is smaller in pixels than the camera chip size. This is for convenience, so it does not cover too much of the screen. The window can be increased up to the size of the chip, thereafter the image panel will not increase in size. The saved images always have the full chip size, regardless of the display size.



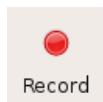
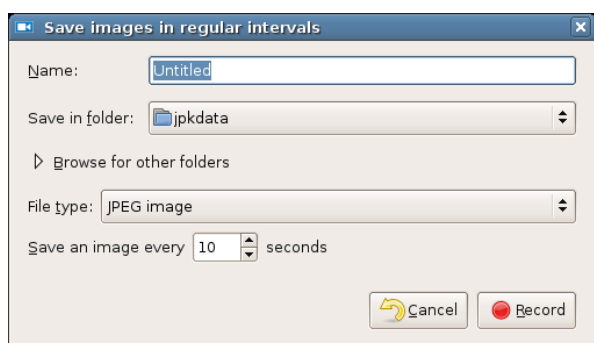
The most common function is to save a single image. When **Save** is selected, the save dialog appears where the name and location of the file can be set.

There is a shortlist of common locations in the drop down menu, under **Save in folder**.



A full list can be seen if **Browse for other folders** is chosen.

Now a file browser appears inside the save dialog window, and folders can be selected or created.

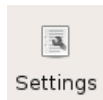
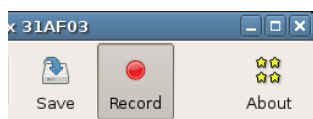


Record

It is also possible to **Record** a continuous series of images, for instance to observe changes over time. The images are saved separately, not as a compressed movie file, but the conversion is straightforward with a variety of commercial and free software programs.

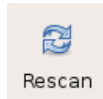
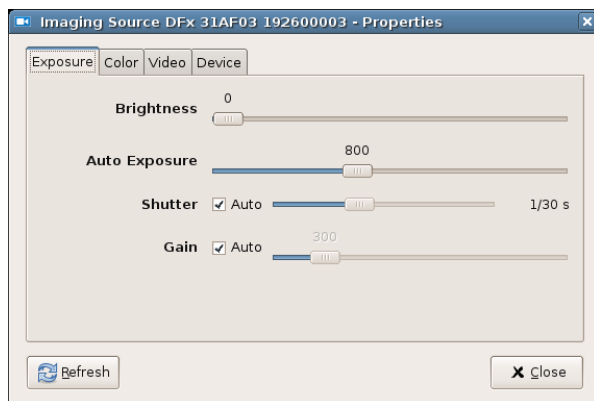
Again, there are the same options for the file location, and the time between saved images can also be set here.

Once the settings have been confirmed, the image window will show the **Record** toggle selected. Simply click on the same button a second time to stop the image recording. The images will be saved at regular time intervals until **Record** is switched off.



Settings

Generally the **Settings** do not need adjusting – everything is set automatically. However, it can be useful to have manual settings for instance to increase contrast in a particular image region (dark/light) or to improve performance at low light levels. Deselect **Auto** to move the sliders for **Shutter** or **Gain**, for example.



Rescan

Rescan will search again for the camera, if for instance it has been disconnected. Again, this usually happens automatically and does not generally need to be done manually.

### 9.1.2 Unicam and coriander

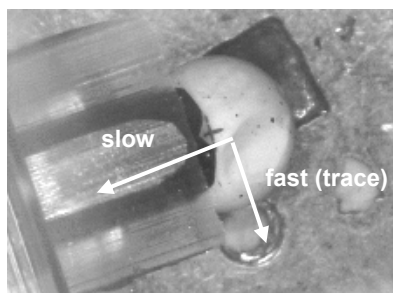
For all NanoWizard® AFM systems equipped with a Firewire camera, the current recommended software for the optical camera is called unicam. For customers who were previously working with coriander, this must be installed separately. The software can be downloaded from the main software page where the JPK and other software components (such as Gwyddion) can be found. If you see coriander when you start the CCD function, check the software updates. Unicam has many advantages over coriander, which is no longer supported by JPK.

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## 9.2 Optical imaging hardware

### 9.2.1 Top View Optics

The top view optical device for the NanoWizard® standard version allows you to adjust the laser spot on top of the cantilever, as well as helping you to navigate around your sample. Start the camera viewer with the **CCD** button or Linux command, as above. The magnification by turning the wheel just below the steel ring that holds the camera.



This is a CCD camera image (low magnification) of the cantilever and the sample taken with the AFM NanoWizard® in the standard version. In this case, the view of the sample and cantilever is *from above*.

The image shows the sample (a polymer sphere) and the approached cantilever from above. During the scanning process the cantilever moves as indicated.

### 9.2.2 Inverted optical microscope

In the NanoWizard® Life Science version the AFM is placed on an inverted optical microscope. The scan area can be selected by either looking directly through the eyepieces or using the CCD image displayed on the computer screen. The inverted optical microscope shows the sample and the cantilever *from below*.

#### 9.2.2.1 Microscope alignment for Köhler illumination

Modern optical microscopes are equipped for Köhler illumination. This is important to get good contrast for advanced optical imaging techniques such as optical phase contrast or differential interference contrast (DIC). Note that if the microscope is aligned for Köhler illumination and then the NanoWizard® is put in place afterwards, the Köhler illumination must be re-aligned.

It is worth reading some background information about optical microscope adjustment and optimization, since simple adjustments can make a large difference in the optical image quality and contrast. There are many websites with helpful tutorials and information on basic concepts such as Köhler illumination, phase contrast and DIC: Here are a few examples from some large optical microscope manufacturers:

Olympus: <http://www.olympusmicro.com/primer/anatomy/kohler.html>

Nikon: <http://www.microscopyu.com/tutorials/java/kohler/index.html>

#### 9.2.2.2 Interference of the optical microscope with the AFM system

When the optical microscope illumination is switched on during the AFM experiment there might be some perturbations visible on the AFM image. On flat samples especially, perturbations that appear as narrow lines visible parallel to the slow scan direction may be observed (typical frequency 300Hz). This is due to the rectifier in the illumination power supply. Therefore it is recommended to switch off the microscope illumination during measurements of small samples such as single molecules if this interference is seen.

## 9.3 DirectOverlay™ - importing calibrated optical images

The direct comparison of images acquired with AFM and light microscopy shows that features in the two types of images do not have the exact same dimensions. In the case of images acquired with the JPK NanoWizard®, one can be sure that the dimensions are correct, due to the precise positioning enabled by the use of linearized piezos. The difference between the two images is due to aberrations arising from the lenses and mirrors of the light microscope. With this in mind, the DirectOverlay™ software uses the automatically recognized cantilever position to map the optical image and calibrate it before importing the calibrated image into the SPM software. This optional feature can be used for online comparison of AFM and light microscopy images during imaging, or for precise offline overlays.

The DirectOverlay™ feature must be specially purchased. If you are interested in the DirectOverlay™ feature but it is not available on your instrument please contact our sales team at [sales@jpk.com](mailto:sales@jpk.com)

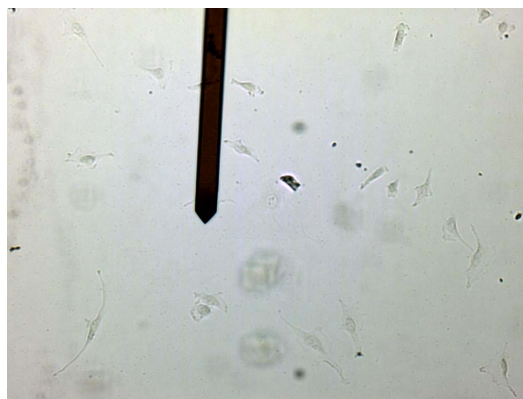
### 9.3.1 Image focus for optimal tip location

The optical calibration procedure uses the cantilever position to make the optical image calibration. This has a strong advantage that the transformation between optical image pixel coordinates and AFM scan coordinates is calculated using only the cantilever images, so the colocalization of the optical and AFM sample features is independent information. The only exception is for the final shift of the optical image to correct for the unknown cantilever tip position. All the magnification, rotation, stretching and nonlinearity, however, is calculated solely from the cantilever images.

The cantilever is automatically recognized from each optical image, without needing input on cantilever angle, shape or magnification. This requires that there is a good contrast for the optical image of the cantilever. Under normal imaging conditions the optical contrast is enhanced to give the best sample image, so the automatic routine could be confused by sample features and not properly recognize the cantilever. Therefore it is best to optimize the illumination and contrast for the cantilever before starting the calibration. This can be easily done by changing the condenser illumination settings, e.g. in optical phase contrast, change to bright field illumination (as shown below), or for DIC mode, slide the polarizer out of the illumination pathway. These changes do not affect the optical imaging path, and hence the calibration is unchanged, but the cantilever contrast is enhanced relative to the sample. The illumination can be changed back at the end of the optical calibration procedure to give an image with good sample contrast for importing.



Phase contrast illumination



Bright field illumination – same region, contrast is better for optical calibration

A slight movement of the focus position can also be helpful. Adjust the fine focus so that the tip of the cantilever is sharp. The focus can be moved the few microns back to the sample level to take a sharp sample image for import.

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### 9.3.2 Coarse alignment with optical image

The Direct Overlay feature is enabled by the linearized x and y piezos. As such, the calibration procedure can be carried out on an area up to 100  $\mu\text{m}$  x 100  $\mu\text{m}$  (the piezo range of the NanoWizard). The optical image is extrapolated to 50% larger than the calibration area, i.e. maximal region 150  $\mu\text{m}$  x 150  $\mu\text{m}$ . When using higher magnification objectives (63x and 100x) it will be necessary to reduce the size of the area to be calibrated below 100  $\mu\text{m}$ , since part of the AFM region may be outside the optical field of view.

To ensure that the appropriate area is calibrated, the user should initially make a coarse alignment of the area to be scanned using the AFM with an appropriate area within the optical field of view. Approach to the surface as normal, and use the optical microscope to find the region of interest for scanning.



If the JPK Firewire camera is being used, start **unicam** by clicking on the camera icon. If another camera (e.g. specialized fluorescence camera with separate software) is being used, start its software to get a live image of the optical field of view.



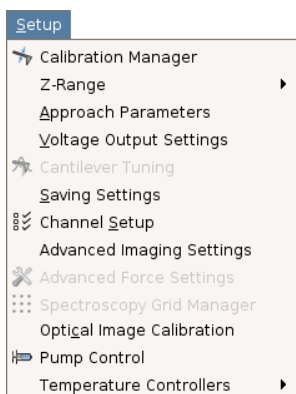
The **Outline** tool can be helpful to show the scan area and move either the sample or the cantilever until the scan area is within the interesting region of the optical image.



The safety distance between the tip and the surface depends on the approach **Target Height** (see **Section 4.1**). Make sure there is enough safety distance for the sample or tip movement. If necessary, move the motors up say 10-20  $\mu\text{m}$  to make a large movement, and then re-approach before performing the optical calibration.

System Status	
User	kate
Sum	1.37 V
Lateral Def	-0.14 V
Vertical Def	640 mV
Status	Z Piezo Retracted
Saving	off

To check that the instrument is in the right mode check the system status window. It will indicate whether the system is in **Z Piezo Retracted** mode. If the system is in **Imaging** or **Idle** mode (in contact with the surface), just click retract once to sit at the top of the piezo.



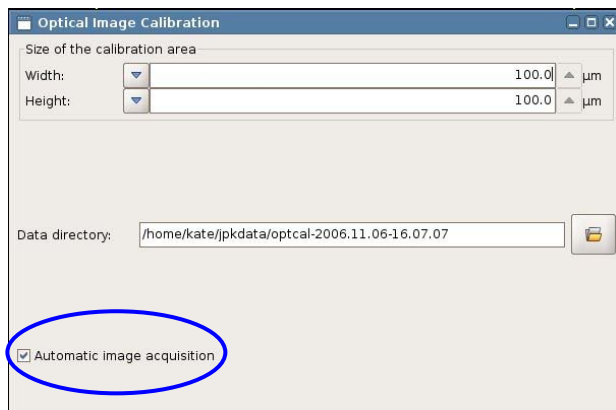
To start the calibration procedure, select the **Setup** pull-down menu at the top of the screen, then click on **Optical image calibration**. There are a number of panels to lead the user through the procedure.

If one of the JPK supported Firewire cameras is used with the unicom software, then the images are automatically transferred from the camera to the SPM software, which is the most convenient method. Other advanced cameras, which have their own software and computer, can also be used. Any system that can acquire cantilever images and export them as simple graphic files (TIFF, JPG, BMP or PNG) can be used for the calibration, but the images must be manually transferred to the correct folder on the AFM computer. See **Section 9.3.5** for the manual calibration procedure.

### 9.3.3 Automatic calibration

Once the image collection is started, all the images will be saved automatically. Therefore it is important to adjust the illumination and focus (see **Section 9.3.1**) before starting.



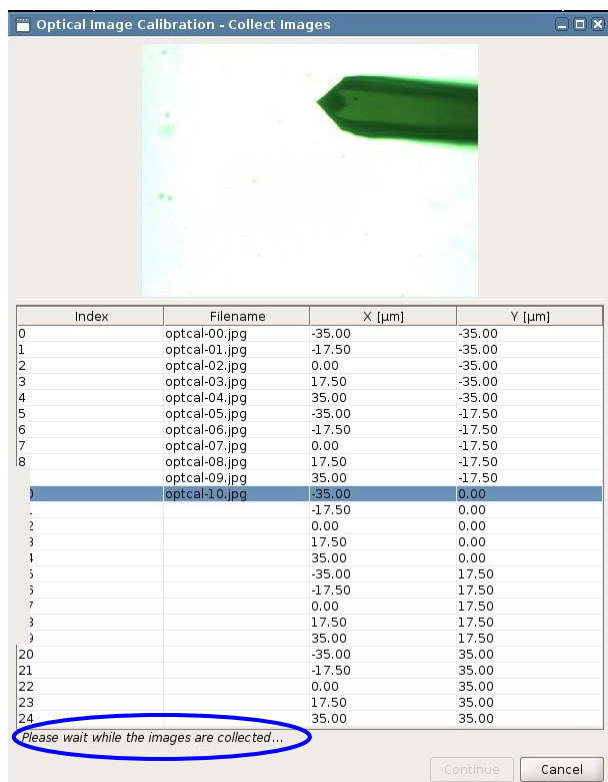


The initial panel allows the user to select the **Size of the calibration area** (default full piezo range). This should be reduced for high magnification objectives. The calibration region is always centered within the xy piezo range.

The **Data directory** for the calibration images and data is automatically generated, with a date-time stamp. This can also be edited.

**Automatic image acquisition** can be used for cameras with the **unicam** software. This should be switched off for external cameras with separate software - see **Section 9.3.5** for manual calibration.

Click **Continue** to start image collection.



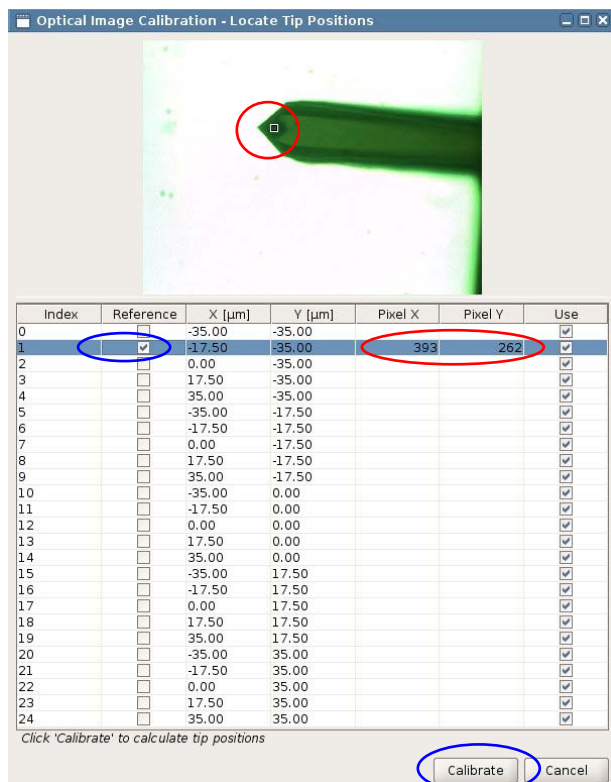
This panel in the needs no input from the user when automatic calibration is being used. In this case, the tip moves automatically to each of the 25 grid points, and an image is transferred automatically from the camera. The size of this grid corresponds to the size of the calibration area selected in the first step of the procedure.

As each image is saved it is displayed in the calibration window and the **Filename** will appear in the list. These files are automatically saved in the **Data directory**.

The **X [μm]** and **Y [μm]** values correspond to the cantilever position, where (0,0) is the centre of the X,Y range.

While the images are still being collected there is a message at the bottom of the panel. Once all the images have been collected, click **Continue**.



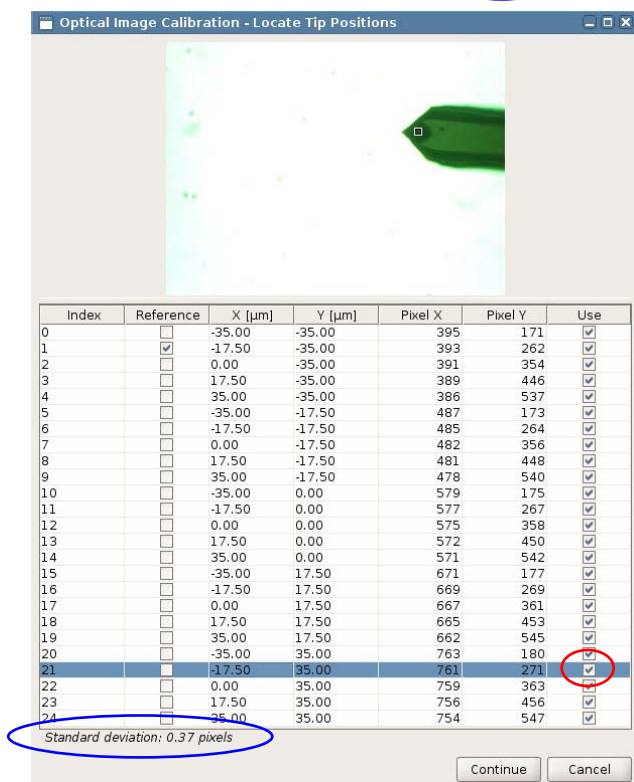


The next step is to locate the cantilever tip position in one reference image. Select an image from the list, and enable the **Reference** tickbox.

The image will be displayed in the top panel. Click in the image on the point corresponding to the tip location. A white square will appear in the image, indicating the designated tip location. Note that the display panel showing the image can be enlarged to make this step more precise.

When the tip location has been selected in this image, a **Pixel X** and **Pixel Y** value will appear for the chosen point.

When the selected tip location in the reference image is reasonable, click on **Calibrate** at the bottom of the panel.

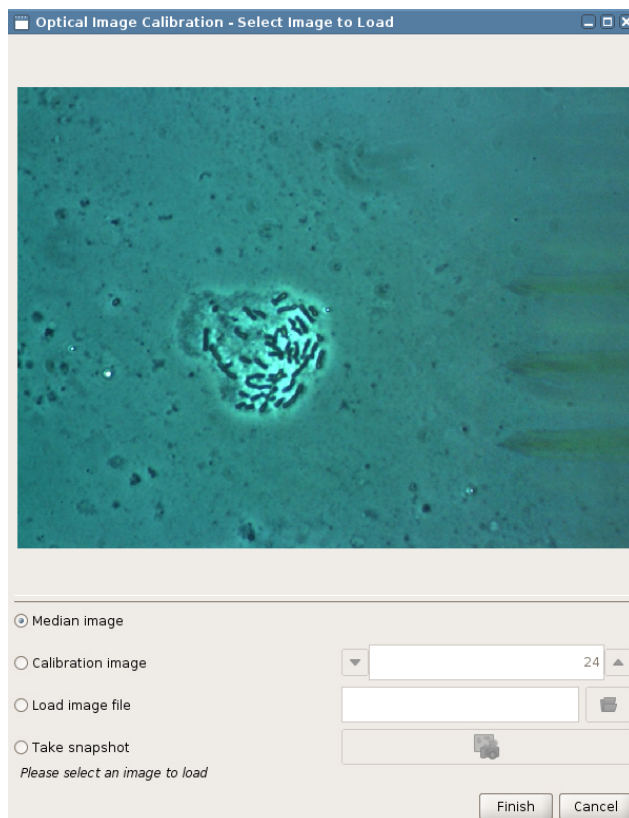


The software will now calculate the corresponding tip position in the other 24 images. The user must wait for all the values to be generated. The progress can be monitored as the values appear in the **Pixel X** and **Pixel Y** columns.

Check the accuracy of the automatic tip location at the bottom of the panel. The **Standard deviation** should be less than 1 pixel if the calibration is reasonable.

Scroll through the list using the up and down arrow keys. As each image is selected it appears in the top panel with the calculated tip location marked. If the contrast is poor, or if only a small part of the cantilever is still visible in the selected image, the calculation may be inaccurate. Images with an incorrect tip location can be deselected with the **Use** tickbox. There are an excess of points used for the calibration procedure, so the deselection of points will not disrupt the calibration. Some images may have been automatically deselected.

When satisfied with the calibration points, click **Continue**.



At this point the calibration is finished and the user should select an image to be imported into the background of the SPM program.

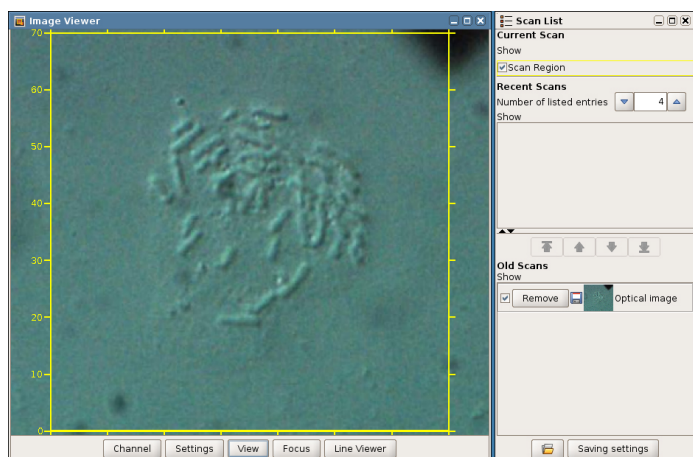
The **Median image** is calculated from all 25 calibration images. When the size of the calibration grid is large enough, this generates an image without the cantilever. This median image is displayed on the final panel of the calibration procedure by default.

Alternatively the user can select one of the **Calibration images** taken already, or a pre-existing image (taken before the calibration) using **Load Image file**.

The final option is **Take snapshot**. This allows the user to refocus the microscope, adjust the contrast, and then take an image to be imported. The cantilever does not obscure the field of view as it is still positioned over the final point of the calibration grid. The file is saved in the calibration **Data directory** with a filename **Snapshot**.

When the correct image has been selected click **Finish** to import the calibrated image into SPM.

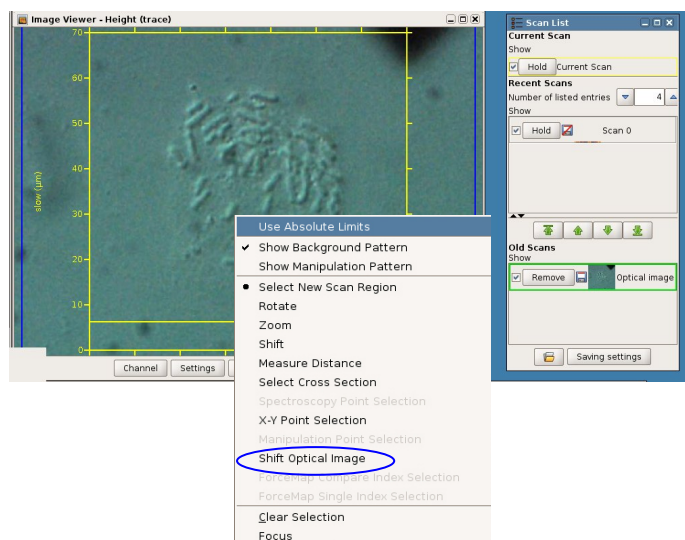
### 9.3.4 Managing and adjusting imported images in SPM



At the end of automatic or manual calibration, the final image is automatically calibrated and imported into the SPM software. It will appear in the background of all **Image Viewer** windows and in the **Old Scans** section of the **Scan List**.

In the **Scan List** the image behaves like AFM images, i.e. the image can be displayed or hidden by clicking on the **Show** toggle.

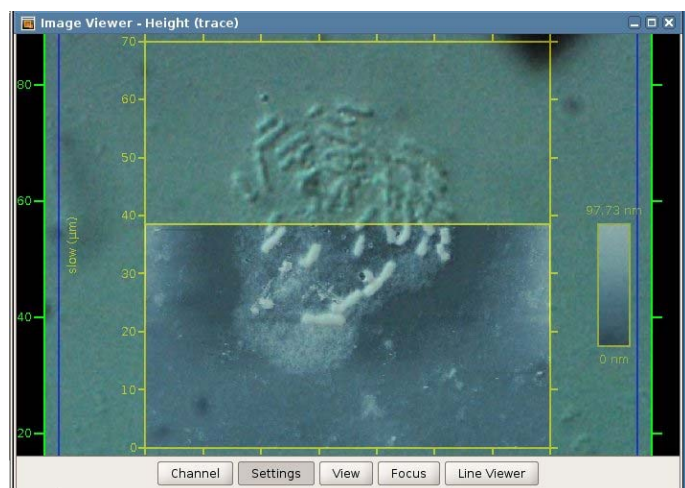
Clicking the **Remove** button will remove the image from the **Image Viewer**.



An AFM scan area can now be selected directly in the optical image. However, as the initial selection of the tip location may be slightly incorrect it may be necessary to shift the optical image slightly to correct for the actual tip position.

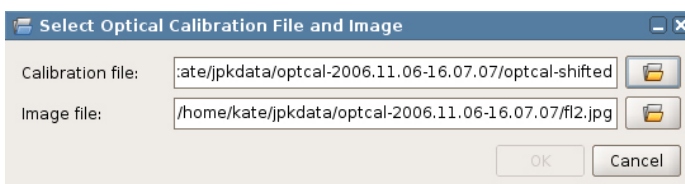
Start an AFM scan, low resolution (256 x 256 pixels) is sufficient. Select the optical image in the **Scan List**. The optical image is now highlighted in green in the **Scan List** and plotted on top of all the images within the **Image Viewer**.

Click the *right mouse button* within the **Image Viewer** and select **Shift Optical Image**. This will make the optical image transparent.



The semi-transparent optical image can now be shifted using click and drag with the mouse. Align the features of the two images. To go back to the normal view of the AFM image, click the right mouse button in the **Image Viewer** and deselect **Shift Optical Image**, then go to the **Scan List** and click on the current scan.

After shifting, the optical image should be resaved. This will adjust the original calibration file to contain the offset that was applied during the manual adjustment of the image position.



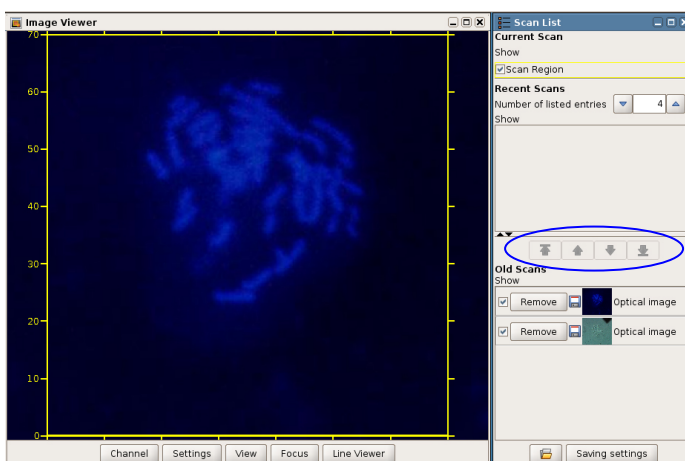
Additional images can be imported using the same optical calibration file, with the offset that was applied if the optical image had to be shifted.

By clicking on **File** at the top of the screen and then **Import Optical Image**, the user can select the appropriate calibration file and image.

If more than one optical image is in the **Scan List**, then they will generally both cover the whole displayed area. The only visible image will be the one at the top of the list.

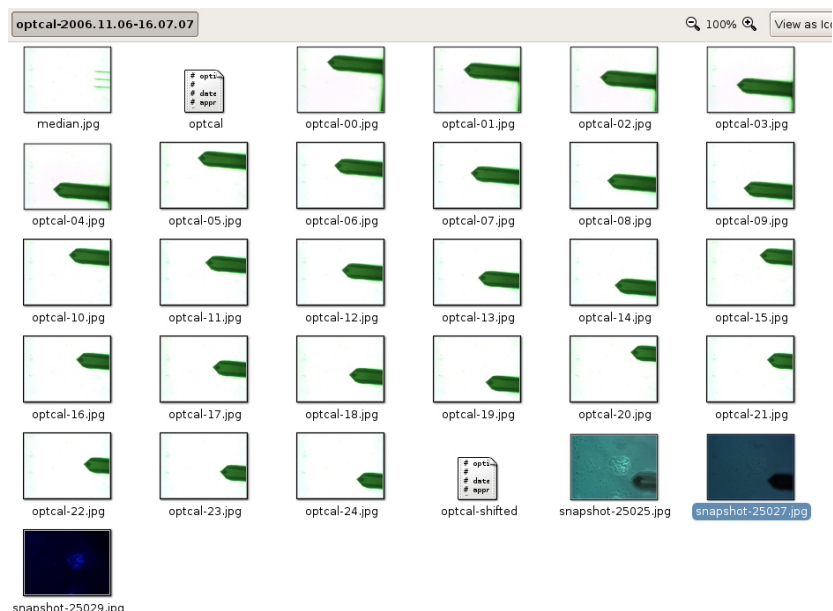
The up and down arrows in the Scan List can be used to change the order of the images

Note: do not open too many optical images at once, as the software may slow down.





Additionally, to import an updated optical image, the **Snapshot** function can be used. *First select an optical image in the **Scan List** to define the optical calibration for import.* Click on the **Snapshot** icon to acquire an image from the **unicam** software and import it into the SPM software. The **Snapshot** icon is only active when the unicam software is open and an optical calibration is defined (optical image is selected in the scan list). The snapshot image is saved into the folder with the selected calibration.



In the calibration folder that was set at the beginning of the procedure there are now many different types of files.

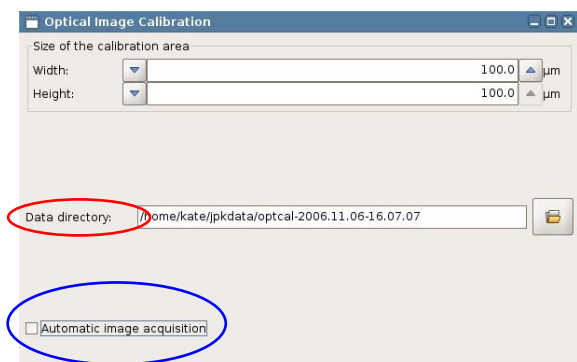
The 25 **calibration images** are stored here, along with the calculated **median image**.

Both the original optical calibration file (**optcal**) and the adjusted calibration file with the user determined offset (**optcal-shifted**) are found in this folder.

Lastly any snapshots taken while this calibration is active should be stored also in this folder.

### 9.3.5 Manual calibration

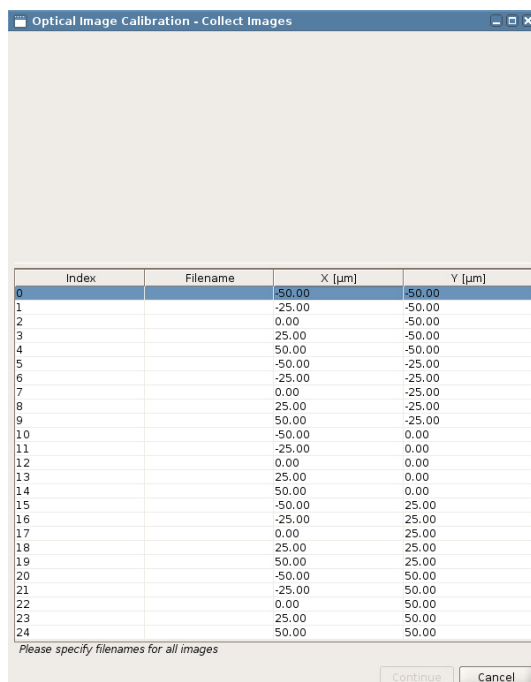
For some optical applications, the user may wish to use a different CCD camera, or an alternate imaging device. If the imaging apparatus can acquire images of the cantilever and then provide these images as either TIFF, JPG or PNG files then the calibration procedure can still be used, however, the acquisition of images for the calibration can no longer be automatically acquired.



For the manual calibration procedure, using external image acquisition software **Automatic image acquisition** must be *deselected*.

The manually saved optical images must be put in a known location. The default name can be used, or a special name set, but make sure the folder that is set in the **Data directory** panel is known.

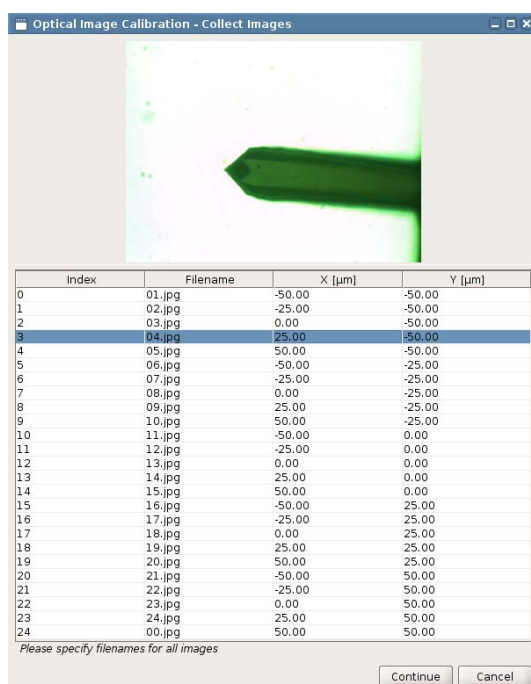




The second panel of the calibration procedure is the point at which the images must be manually acquired. The calibration grid points are listed and the up and down arrow keys scroll through the list. As each entry in the list is selected, the cantilever automatically moves to that position.

Images should be acquired at each point and saved with a sequential file name (start with file names of the form 01, 02 to ensure that the files are kept in the correct order when uploaded, as the index counts up to 25). Any names can be used, the only requirement is that they must have the correct alphabetical or alphanumeric sequence for the 25 AFM positions.

Most fluorescence camera or confocal software has the option for collecting a time series. This can be a convenient way to save and manage all the images. Set the time series running (e.g. set 25 images at 2-3 seconds time interval). As each image is acquired on the camera, move to the next position in the list to put the cantilever in position for the next image.



Once the files have been collected, they have to be transferred to the **Data directory** chosen at the start of the calibration. The SPM software will recognize any sequentially named images in a basic graphics format (TIF (8 bit), JPG, BMP or PNG).

Note that if a time series is used in the fluorescence camera software, it is often possible to export all the images automatically with a certain format and sequential names. The files can be transferred to the AFM computer in any normal way, for example using an ethernet connection or USB device. WinSCP is a useful free tool that can help make the transfer convenient.

Once there are suitable files in the **Data directory**, the SPM software will automatically shows the names in the list. The list display is updated every time a new position is chosen with the mouse or arrow keys.

If the file path is correct and the files are in the correct format, the names will appear in the **Filename** column and scrolling through the list will display each calibration image in the top panel.

From this point the procedure for selecting the tip reference position and calculating the values from the other files is the same as described in **Section 9.3.3** for the **automatic** calibration. See **Section 9.3.4** for information on using the optical images in SPM. The only difference with an external camera is that the **Snapshot** function can not be used as this is dependent on **unicam**. The user must remember to transfer the files to the LINUX computer and then they can be imported as described above using **File - Import Optical Image**.

### 9.3.6 Offline Direct Overlay

Optical images can be opened in the JPK Image Processing (IP) program, to allow offline overlays and the export of calibrated optical images that correspond to particular AFM scans. The JPK Image Processing program is not de-

signed to allow full image processing capabilities for the optical images, instead functioning as a first step in offline image processing. See the separate IP Manual for details on importing and managing optical images in IP.

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## § 10 Advanced information

### 10.1 Ubuntu Linux information

The Ubuntu Linux operating system is relatively intuitive to use, and many features and settings can be found easily by people used to the Windows operating system.

#### 10.1.1 Basic tools and programs



The taskbar along the lower left hand side of the desktop contains most of the shortcuts that are regularly required.



Internet browser (usually Firefox)



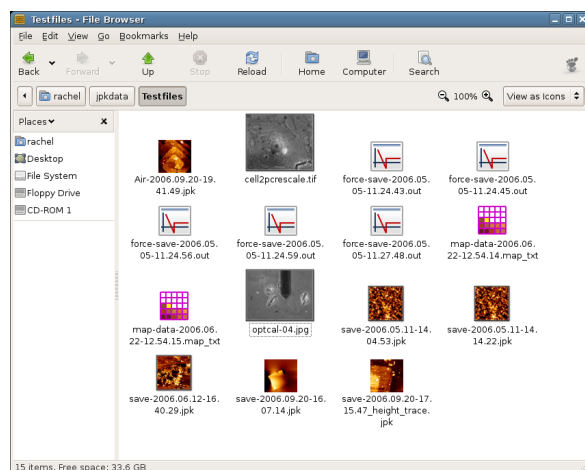
Console (terminal) window. Here many different commands can be entered directly.



Open a file browser for local folders (on this computer)



Open a file browser for remote folders (over the network)



In all the file browser windows there is an option on the right hand side **View as icons**.

If this is selected, then a thumbnail preview of each file is shown. The system can recognise normal image files, and also our jpk format. So this is useful for having a quick preview of which images are in a certain folder.



The JPK logo starts the main menu system for the Ubuntu operating system.

At the bottom, there are some commonly used options:

**Places** to open a file browser

**System** to find settings for user **Preferences** or system **Administration**

**Quit...** to log out

In the other menu options are program lists and small tools or accessories.



Sometimes it is useful to take a screenshot from the current screen. This can be done quite straightforwardly using the standard **Print Screen** key (for the whole double-monitor desktop) or the key combination **Alt + Print Screen** (to make a screenshot of only the active software window), as in Windows software.

A dialog appears showing a miniature version of the screenshot, with options to set the name and location to save the file. The default format is .png, which uses lossless compression and can be opened in most software like jpg.

In the terminal (console) window, the screenshot can be started directly from a command. This has the advantage that more advanced options can be added (e.g. a time delay). The basic command is: `gnome-screenshot`

To get the list of different advanced options, enter this: `gnome-screenshot --help-gnome-screenshot`

### 10.1.2 Use JPK-scripts with Linux console

There are several JPK-scripts, which can be applied to the data. "Splitforcefile", for instance, separates a force file into trace and retrace curve. "Splitmapfile" splits force maps into separate force files. There are two scripts to convert force files of the jpk-force format into other file formats. The script "jpk-force-legacy-export" creates .out files, and "jpk-force-export" creates text dump files.



To run a script, open the Linux terminal window.

The terminal window automatically opens the user specific jpkdata-directory. To apply a script the directory containing the script must be selected ("`cd /opt/jpkspm/bin`") and finally the script ("`./scriptname`") and the corresponding data, which are to be processed by the script, along with the storage directory, are to be specified ("`~/jpkdata/directory/filename`"). The script then finally processes the file and stores the new file in the same folder. In case that all force files of the file name root "Force...jpk-force", located in the directory jpkdata/Test, are to be converted into out files, the corresponding commands are to be entered:

```
tneumann@pcc13: /opt/jpkspm/bin
File Edit View Terminal Tabs Help
tneumann@pcc13:~$ cd /opt/jpkspm/bin
tneumann@pcc13:/opt/jpkspm/bin$ ./jpk-force-legacy-export ~/jpkdata/Test/Force*.jpk-force
```

If all jpk-force files of a directory (e.g. Test) are to be converted, either "\*.jpk-force" (i.e. without any filename root) is to be entered, i.e. all files of the jpk-force type are processed, or only the directory containing the files is specified with no particular reference to files:

```
tneumann@pcc13: /opt/jpkspm/bin
File Edit View Terminal Tabs Help
tneumann@pcc13:~$ cd /opt/jpkspm/bin
tneumann@pcc13:/opt/jpkspm/bin$ ./jpk-force-legacy-export ~/jpkdata/Test
```

In case that reference to the files is used to convert files (i.e. "\*.jpk-force" or "filename\*.jpk-force" is entered), there is one problem that can occur if a huge batch of curves is to be processed: The filename wildcards are expanded before the command is executed. That means, even if "\*.file extension" is entered (which means all files in the given directory with exactly this extension are processed), the script appends one file with the complete filename after the other. And since there is a limit on the allowed length of the expanded command line the script will abort if this limit is exceeded. In such case the directory alone should be specified rather than the filenames.

`./jpk-legacy-export ~/jpkdata/experiment/*.jpk-force` → wildcards limitation

`./jpk-legacy-export ~/jpkdata/experiment/` → unlimited number of files

For more information about these kinds of scripts please contact [support@jpk.com](mailto:support@jpk.com) .

### 10.1.3 User account administration

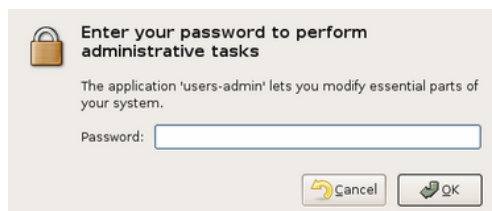
The main administration account in the Ubuntu AFM systems is **jpkroot**. The password is delivered with the AFM installation papers, and this account can be used to set up other standard accounts for all the individual AFM users.



To perform administration tasks, such as installing new software versions and creating new user accounts, log in as **jpkroot**. User accounts created using the standard settings do not have administrator rights – this is more secure, especially when the AFM computer is connected to the internet.

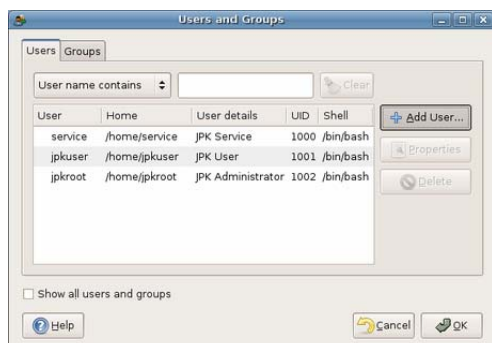
Use the main system menu (started from the JPK icon in the taskbar at the bottom left of the software) to choose:

#### System – Administration – Users and Groups



For security reasons, you need to confirm the account password again when starting administration tasks.

Enter the password for the **jpkroot** account.



The dialog window **Users and Groups** shows a list of the user accounts. Note, make sure that the option **Show all users and groups** is **OFF**, otherwise there will be a long list of internal groups and categories. When this option is off, only main user accounts are shown.

Click on the button **Add User** to create a new user account.



The **User Account Editor** window is shown with the **Account** tab selected. It contains the following sections:

- Basic Settings:**
  - Username: jpktest
  - Real name: JPK Testuser
- Contact Information:**
  - Office location: (empty)
  - Work phone: (empty)
  - Home phone: (empty)
- Password:**
  - ☒ Set password by hand
    - User password: (masked with asterisks)
    - Confirmation: (masked with asterisks)
  - ☐ Generate random password
    - Password set to: (empty)
    - Generate button

Buttons at the bottom: Help, Cancel, OK.

The **User Account Editor** lets you set up the particular options for the new account.

The first tab panel, **Account** has the basic details for the user account.

The **Username** will be the log in name for the new account. The **Contact Information** is optional, for a small group of users this is probably not required.

The **Password** section is very important. *Please choose secure passwords, especially if the computer will be connected to the internet!*



The **User Account Editor** window is shown with the **Advanced** tab selected. It contains the following sections:

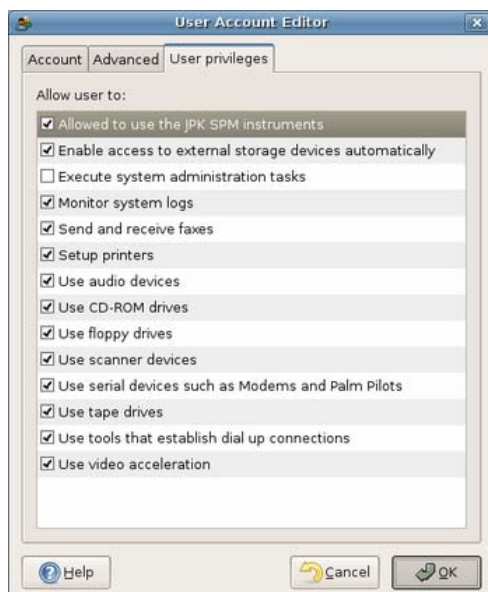
- User Profile:**
  - Profile: Desktop (selected from a dropdown menu)
  - Edit User Profiles... button
- Advanced Settings:**
  - Main group: \$user
  - Shell: /bin/bash
  - Home directory: /home/\$user
  - User ID: 1003

Buttons at the bottom: Help, Cancel, OK.

The next tab, **Advanced**, should be checked to see that the **User Profile** is correct.

The **User Profile** should be set to **Desktop**. This means that the new account will have normal user rights, but not administrator privileges. This is more secure, particularly when the computer is connected to the internet.

The **Advanced Settings** should not be changed.



The **User Account Editor** window is shown with the **User privileges** tab selected. It contains the following sections:

- Allow user to:**
  - ☒ Allowed to use the JPK SPM instruments
  - ☒ Enable access to external storage devices automatically
  - ☐ Execute system administration tasks
  - ☒ Monitor system logs
  - ☒ Send and receive faxes
  - ☒ Setup printers
  - ☒ Use audio devices
  - ☒ Use CD-ROM drives
  - ☒ Use floppy drives
  - ☒ Use scanner devices
  - ☒ Use serial devices such as Modems and Palm Pilots
  - ☒ Use tape drives
  - ☒ Use tools that establish dial up connections
  - ☒ Use video acceleration

Buttons at the bottom: Help, Cancel, OK.

The final tab, **User Privileges** is important to make sure that the new account will have access to the SPM software.

Make sure that **Allowed to use the JPK SPM instruments** option is **ON**. The other settings can be chosen as required, depending what is installed on the instrument computer.

At the end, click **OK** to save the details of the new account.

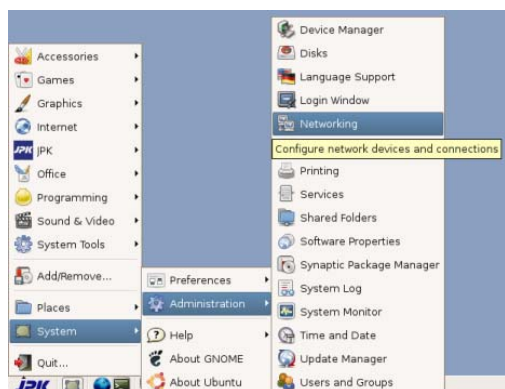


Click **OK** again to exit the **Users and Groups** settings and save the current status.

Remember to log out of the **jpkroot** account after the administration tasks are finished.

The new user will then be able to log on with their new account.

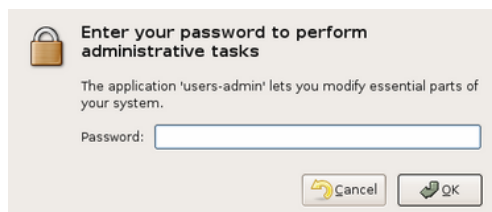
### 10.1.4 Network settings



To perform administration tasks, such as network configuration, log in as user **jpkroot**.

Use the main system menu (started from the JPK icon in the taskbar at the bottom left of the software) to choose:

#### System – Administration – Networking



For security reasons, you need to confirm the account password again when starting administration tasks.

Enter the password for the **jpkroot** account.



The Network Settings dialog contains all the settings for the network configuration.

In the first tab, **Connections**, select **Ethernet connection** to set up the eth0 or eth1 connection. Note, if this is *already active* then click **Deactivate** so that the properties can be set.

Click on **Properties** to configure the interface properties.

There are two types of network configuration – dynamic IP address allocation (DHCP) or static. This is decided by the type of local network. Please contact your network administrator for advice on the particular settings required for your network.

#### 10.1.4.1 DHCP (dynamic IP address allocation)



For **DHCP** (dynamic IP address allocation):

In the **Interface properties** panel:

- Select **Enable this connection**
- Choose **Configuration: DHCP**

Close the dialog with **OK** to save the settings and return to the **Network settings** dialog.



In the **Network Settings** dialog, on the first tab **Connections**, check that **Ethernet connection** is still selected and click **Activate**.

Click **OK** to save the new settings and exit the Network Settings dialog.

Remember to log out of the **jpkrout** account after the administration tasks are finished.

#### 10.1.4.2 Static IP address allocation



For static IP address allocation:

In the **Interface properties** panel:

- Select **Enable this connection**
- Choose **Configuration: Static IP address**

Now the **IP address**, **Subnet mask** and **Gateway address** from your network administrator should be entered in the relevant text fields.

Close the dialog with **OK** to save the settings and return to the **Network settings** dialog.



In the **Network Settings** dialog, on the second tab **General**.

The **Hostname** is the name of the computer. The **Domain name** is usually not required.



Still in the **Network Settings** dialog, click on the third tab **DNS**.

Use **Add** to add the required **DNS Servers** or **Search Domains** (from your network administrator).



Finally, click on the first tab **Connections**, check that **Ethernet connection** is still selected and click **Activate**.

Click **OK** to save the new settings and exit the **Network Settings** dialog.

Remember to log out of the **jdkroot** account after the administration tasks are finished.

### 10.1.5 Timer

A simple electronic timer can sometimes be useful during experiments. First this small tool must be installed with administrator rights. Log in as **jdkroot**, open a terminal window and type the following command:

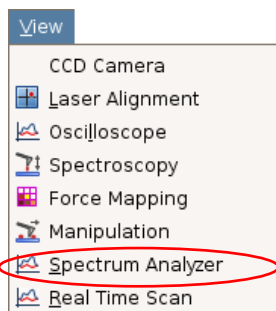
```
apt-get install timer-applet
```

The timer can then be added to the toolbar of each user individually. Click on the desktop with the right mouse button and choose the option "Add to panel". Select the tool "Timer". This now adds a small icon to the taskbar at the bottom of the Ubuntu desktop. This opens a timer with simple count-down settings.



## 10.2 Advanced SPM software options

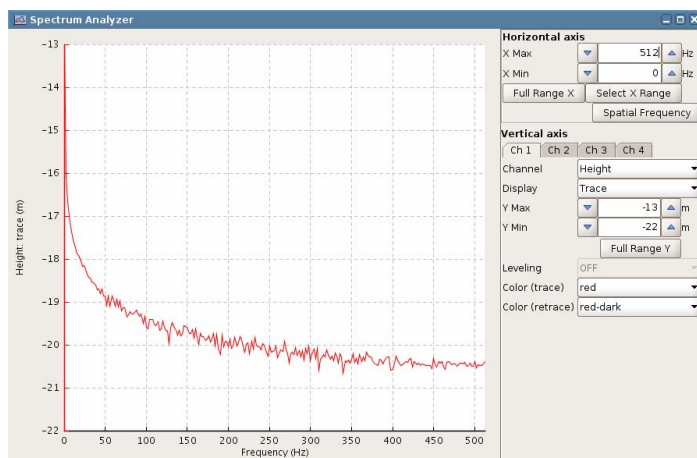
### 10.2.1 Spectrum Analyzer



The spectrum analyzer is opened using **View** → **Spectrum Analyzer**

With the help of the spectrum analyzer, acoustic or electronic noise on the cantilever can be detected. The calculation is made using an FFT of each scan line and combining the data from several consecutive scan lines to give an average noise spectrum.

The maximum frequency is limited by the so-called Nyquist-Frequency which depends on the scan rate, duty cycle, image size and pixel number.

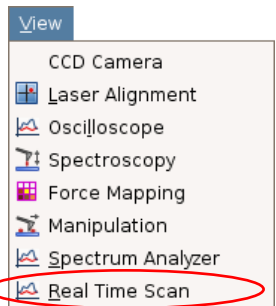


All the available channels (depending on the imaging mode) can be displayed as a function of temporal frequencies (1/time, as normal) or as spatial frequencies (1/distance) if **Spatial frequency** is selected.

The controls for the display in the **Spectrum Analyzer** are similar to the **Oscilloscope** window (see **Section 5.5.1**).

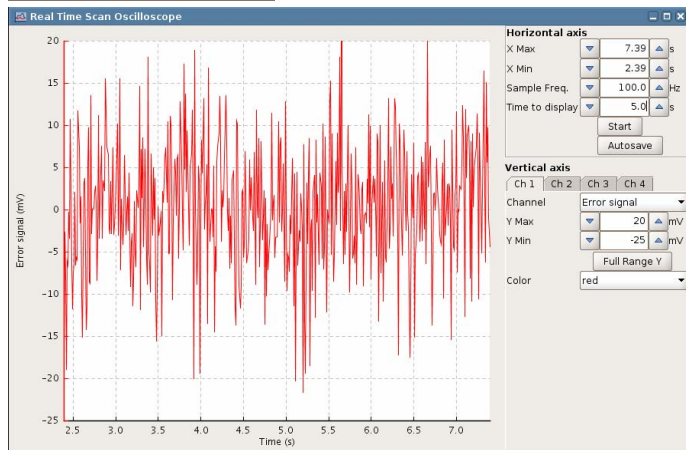
Multiple channels can be selected using the register cards in the lower part of the panel.

### 10.2.2 Real-time oscilloscope



The time-based oscilloscope is opened via **View** → **Real time scan**

In contrast to the normal **Oscilloscope** window described in **Section 5.5.1**, this displays the channels continuously as a function of time, rather than separately for each scan line. The display is therefore more similar to the output from a conventional oscilloscope connected to the particular channel.

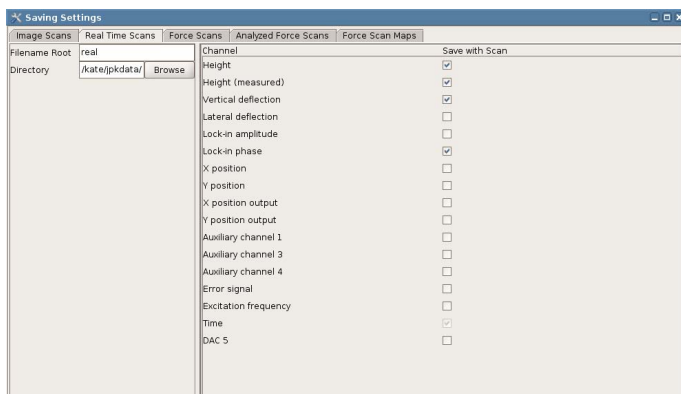


The **Sample Freq** is an important parameter, as it defined the data collection rate. If the data is collected very quickly, the performance may start to suffer. Check that only the required channels are being read (open the **Channel Setup**) to optimize the high-speed performance.

The data collection for the real-time oscilloscope is started with **Start**.

The data can be saved if the **Autosave** button is selected *before* the **Start** is clicked.

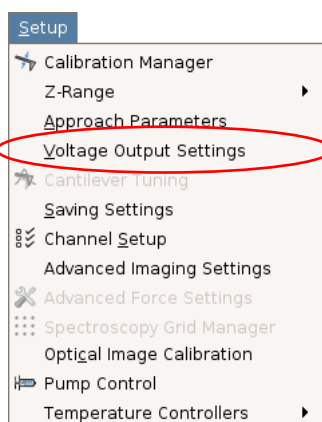




The settings for saving data can be chosen using **Setup** → **Saving Settings**, and choosing the register card for **real time scans**. Here the file name, saving directory and channels to save can all be selected. The date and time is added to the File-name root, so the files are named by default as: real-year.month.day-hrs.min.sec

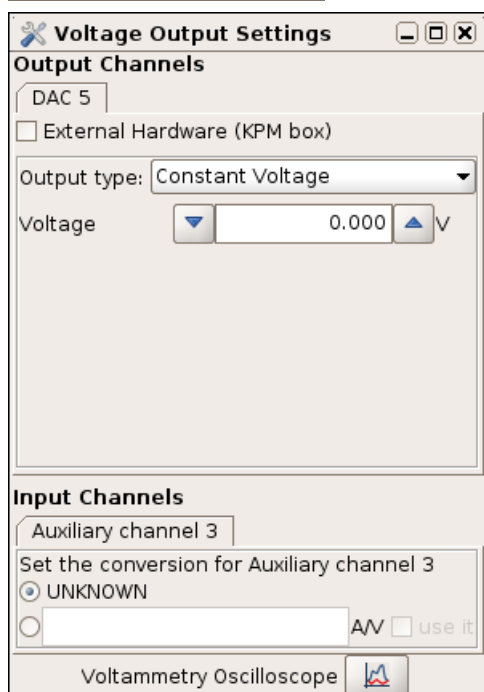
*Note – the saved data depends only on the **Saving Settings**, not the channels that are displayed during the real-time scan operation!*

### 10.2.3 Voltage Output Settings



This feature is started using **Setup** → **Voltage Output Settings**

The settings here allow a voltage to be sent out to a free DAC on the **Signal Access Module** (see **Section 10.3**). This is useful for some electrical measurements, to set a variable voltage without using an external power supply, for instance for oxidation lithography, conductive AFM or electrochemistry measurements.



The **Output Channels** settings are stored in the property file for the instrument, and depend on the controller type. Typically DAC3 or DAC5 would be used, see the channel assignment list with the controller information for the Channel number of the BNC connection.

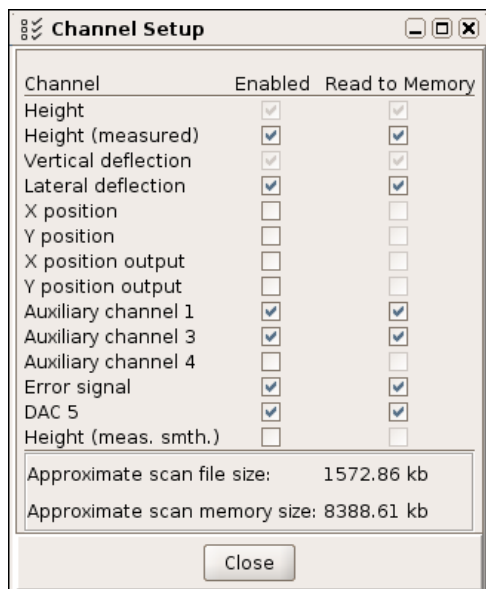
**External Hardware** is a switch that changes between the default 0 – 10V range (direct from the Signal Access Module) and -10V – +10V range (only available if the KPM box is connected inbetween, this is a separate optional electronics module).

If **Output Type** is set to **Constant Voltage**, then the output is taken directly from the **Voltage** text field, and updated whenever this is changed.

For many applications such as electrochemistry or conductive AFM, then another signal, such as the current, is used as an **Input Channel**.



There is a built-in **Voltammetry Oscilloscope** for these applications using **Auxiliary Channel 3** as the default input.



**Channel Setup**

Channel	Enabled	Read to Memory
Height	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Height (measured)	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Vertical deflection	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Lateral deflection	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
X position	<input type="checkbox"/>	<input type="checkbox"/>
Y position	<input type="checkbox"/>	<input type="checkbox"/>
X position output	<input type="checkbox"/>	<input type="checkbox"/>
Y position output	<input type="checkbox"/>	<input type="checkbox"/>
Auxiliary channel 1	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Auxiliary channel 3	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Auxiliary channel 4	<input type="checkbox"/>	<input type="checkbox"/>
Error signal	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
DAC 5	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Height (meas. smth.)	<input type="checkbox"/>	<input type="checkbox"/>

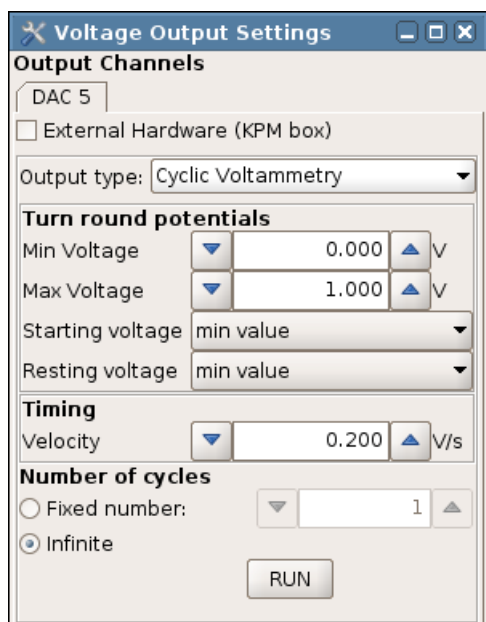
Approximate scan file size: 1572.86 kb  
Approximate scan memory size: 8388.61 kb

Close

Before starting measurements, check that the main **Channel Setup** has both the selected **Input Channel** and **Output Channel** switched on. Otherwise data from these channels will not be seen in the software, and hence can not be displayed in the oscilloscopes.

Usually this means that **Auxiliary channel 3** and either **DAC3** or **DAC5** should be selected.

Since the channel setup for the real time scan is handled separately, then the channels for real time scan capture are also important, if the **Voltammetry Oscilloscope** is used (see below).



**Voltage Output Settings**

**Output Channels**

DAC 5

☐ External Hardware (KPM box)

Output type: **Cyclic Voltammetry**

**Turn round potentials**

Min Voltage: 0.000 V

Max Voltage: 1.000 V

Starting voltage: min value

Resting voltage: min value

**Timing**

Velocity: 0.200 V/s

**Number of cycles**

☐ Fixed number: 1

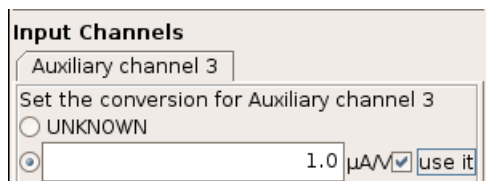
☒ Infinite

RUN

If the settings are changed to **Cyclic Voltammetry**, then the output will produce a triangular / saw tooth output with the settings.

The cycles can start and end at either the **Min Voltage** or the **Max Voltage**. The time for the cycle is set implicitly through a **Velocity** in V/s.

When **RUN** is clicked, then either continuous cycles or a **Fixed number** of cycles will be started. When **RUN** is not active (between cycles), the voltage stays at the **Resting voltage**.



**Input Channels**

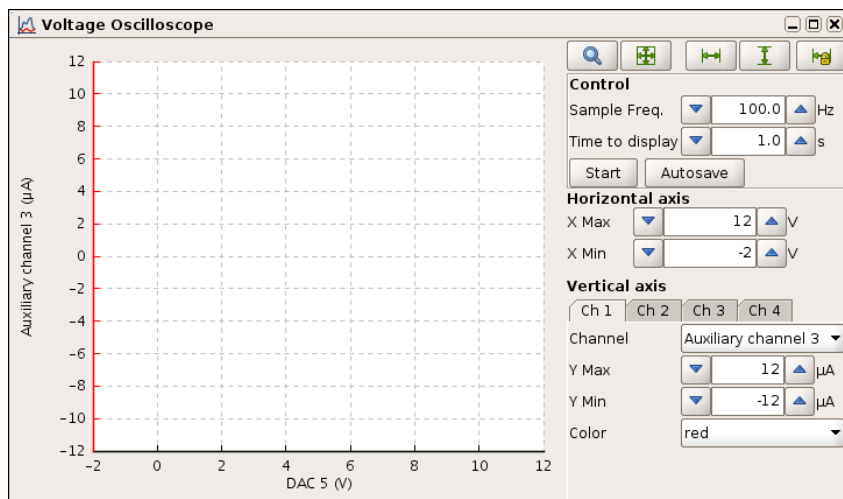
Auxiliary channel 3

Set the conversion for Auxiliary channel 3

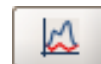
☐ UNKNOWN

☒ 1.0  $\mu\text{A/V}$  ☒ use it

The current-voltage amplification will generally be done with some external hardware, and the **Conversion** factor can be entered here. For instance, if the gain is set to 1 microAmpere per Volt, the conversion can be typed in as 1e-6. This will automatically be updated to show the current in the correct units if **use it** is ticked.



The **Voltammetry Oscilloscope** or **Voltage Oscilloscope** is opened from the settings panel,



If the **Conversion** is set and used, then **Aux 3** will be calibrated.

This is an analogue of the **Real Time Scan** (see **Section 10.2.2**), designed to plot channels against each other as a function of time.

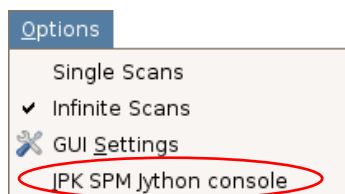
## 10.2.4 Python and macros

There is a very good online tutorial on [www.python.org](http://www.python.org). Within very short time this language can be learned and used together with our software documentation on the AFM computer: `file:/opt/jpkspm/doc/javadoc/index.html`

As a next step the jython tutorial at [www.jython.org](http://www.jython.org) can be useful. For a deeper insight these two books can be recommended:

Markus Lutz, Programming Python, ISBN 1565921976.

Robert W. Bill - Jython for Java Programmers, ISBN 073571119, (recommended for those with experience of Java programming).



The Jython console is opened via **Options** → **JPK SPM Jython console**.

The Jython console can be used to run pre-programmed macros from JPK, so a detailed knowledge of Jython is not required for many operations.

To load the full set of macros provided, enter the following command:



```
>>> from com.jpk.tools import macros
```

This command imports the file `macros.py` from the directory `com/jpk/tools`. The currently loaded modules can be displayed using the command:

```
>>> dir()
```

Any macros copied to the file `".spmmacros.py"` in the users home directory are automatically loaded when the Jython console is opened. (Note the first `"."` is part of the name, and files starting with a `"."` are hidden files in Linux.

There are many macros available for different applications; if you have a particular request, please contact us on [support@jpk.com](mailto:support@jpk.com) and we may be able to supply a macro for your needs. Only a few examples are listed here for the more commonly used macros.

## Fake approach

There is a macro to “fake” a successful approach, so the instrument goes into approached mode, regardless of the approach setpoint or current position:

```
>>> instrument.fakeSuccessfulApproach=1
```

this command also can be abbreviated by

```
>>> macros.fake()
```

## Save a frequency sweep

The data from a frequency sweep can be saved as a text file. Note: open the Jython console and import the macros, before performing the sweep. Perform the sweep, then save it using the command in the Jython console.

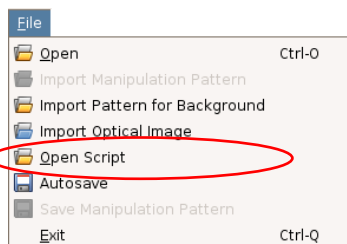
```
>>> macros.saveSweep(filename="test.dat")
```

The sweep time (t) and number of pixels (n) can be set before performing the sweep, or the default values used.

```
>>> macros.set_sweep_time(t)
```

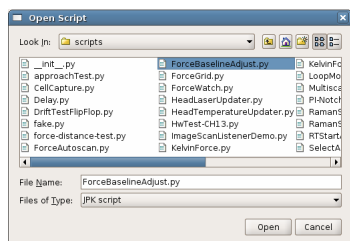
```
>>> macros.set_sweep_pixels(n)
```

## 10.2.5 JPK scripts



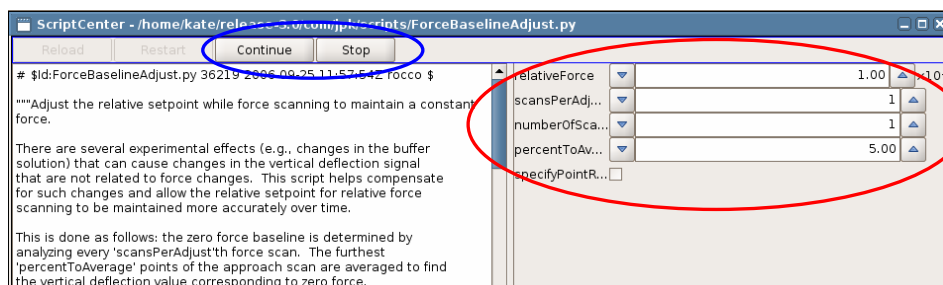
Scripting for your own applications is also available, and the window can be opened using **File** → **Open script**. This enables Jython programs to run on a separate thread. Contact [support@jpk.com](mailto:support@jpk.com) if you have a particular request or question.

There are many pre-programmed scripts that can be used for special experiments.



After selecting **Open script** from the **File** menu, a panel of the files in the scripts folder is displayed.

Select the correct script and click **Open**.



The script window will open, containing a description of the script, followed by the script text itself on the left and on the right a panel where parameters are entered. *Always read the script description for instructions!*

Continue

When the parameters have been set, usually it is necessary to click **Continue** to update parameters or begin to acquire data. *Usually start and stop from the script, not using the **Run** button!*

## 10.3 Signal access module



The controller unit for the JPK NanoWizard® is generally equipped with a signal access box. This allows electronic access or modification to all the software channels.

Each channel has two BNC connectors (IN and OUT) and a switch that controls how the signals are used:

BNC socket OUT always allows monitoring of the signal for both switch settings (switch either up or down).

BNC socket IN allows voltage/signals supplied to that channel *only* if the switch setting is also “IN” (i.e. switch down).

*Note: If no other instrument is connected to the signal access box, then all switches must be in the **up** position!*

This table lists the general software channels and Signal Access Module channels. Individual controllers may vary, depending on customer-specific settings. Please check the version in your installation papers for the definitive version.

Channel	In	Out
CH 1	vertical deflection	vertical deflection
CH 2	lateral deflection	lateral deflection
CH 3	auxiliary channel 3	X-sensor
CH 4	auxiliary channel 4	Y-sensor
CH 5	auxiliary channel 1	photo-diode sum signal
CH 6	auxiliary channel 2	Z-sensor
CH 7	/	/
CH 8	16Bit-DAC	/
CH 9	/	/
CH 10	Intermittent contact-excitation	Intermittent contact-excitation
CH 11	X-control	X-control
CH 12	Y-control	Y-control
CH 13	Z-control	Z-control
CH 14	/	/
CH 15	/	/
CH 16	Z-high voltage up to 120 V	Z-high voltage up to 120 V



**Danger, high voltage!**

## 10.4 Q-control with the NanoWizard® controller

Q-control devices help to increase or decrease the Q-factor of the cantilever resonance. For measurements in liquids it is sometimes useful to increase this value, and sharpen the resonance. For measurement in vacuum, where the Q values are generally very high, the imaging may be improved if the Q-factor is decreased.



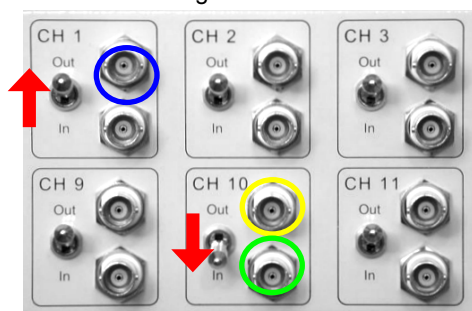
The Q-control box recommended by JPK Instruments AG is the Infinitesima ActivResonance Controller®.

The following information is to help to connect the Q-box with the NanoWizard® controller. See the Infinitesima ActivResonance user manual for details of Q-control operation.

Three BNC connector cables are required to connect the Q-box with the NanoWizard® controller:

NanoWizard® signal access box

Back of the ActivResonance Controller.



CH 1  
Switch Out

CH 10  
Switch In



Connect the BNC sockets shown marked with the same colors between the NanoWizard signal access box and the ActivResonance Controller.

**Note:** Set the switch on the ActivResonance Controller to **Sum In** when the ActivResonance Controller is used together with the NanoWizard® controller.

## 10.5 JPK data formats

The SPM software generates images with a \*.jpk file extension. JPK files are a form of the \*.tif-format. They contain one thumbnail, image data for each channel that has been stored, and a list of scan parameters.

A more detailed description about the jpk-files can be found on under `/opt/jpkspm/doc/TifSpec.sxw`  
This sxw file can be opened with "OpenOffice", which should already be installed on the instrument computer.

\*.jpk files are designed to be read by the JPK Image Processing software, IP. After opening an image in IP, it can be processed and either saved in the same data format or exported as a pure image in a graphics format, for example for import into a word processing or presentation software. The other data formats can be managed in a similar way.

Formats	JPK formats	Export formats	
Save option	Save Manager in Scan List (SPM) Save data or Save with JPK (IP)	Export as text	Export as picture
Format	*.jpk (image format) *.force (force map slope/adhesion, image format) *.jpk-force (force spectroscopy) *.jpk-force-map (force map curves) *.tnd (frequency spectrum in thermal noise, ascii) *.cal (height calibration file, ascii)	*.out *.txt *.map_txt	*.tif *.png *.jpg

---

File contains:	Full collected data, scan parameters and calibration conversions.	Limited information, specific to export format.	Single image without additional information.
Is reload in SPM/IP possible?	Yes (except ASCII formats .tnd or .dat)	No	No
File can be read by general software e.g. Powerpoint?	No (for most JPK formats) Yes (ASCII formats *.out, *.tnd, *.dat)	Yes	Yes
File can be read by SPIP AFM processing software?	Some (e.g. *.jpk)	Yes	Yes



## § 11 NanoWizard® I information

There are two different versions of the NanoWizard® AFM system, they can be easily recognized from the AFM head.



NanoWizard® I AFM head



NanoWizard® II AFM head

Most of the information in this manual is relevant to the NanoWizard® II AFM or common to both systems.

In this chapter is some specific information that is only relevant to the NanoWizard® I AFM system

### 11.1 RedHat Linux

The computer operating system for most NanoWizard® I AFM systems is RedHat Linux. In this section, the information for user accounts, data transfer and helpful accessory programs is explained. It may be that the computer has been upgraded or was originally installed with Ubuntu Linux, in which case the information in **Section 10.1** is relevant.

RedHat Linux can be operated with two desktop managers: KDE or Gnome. The choice between KDE and Gnome is made as a user logs in. Most accounts are set up with KDE as default, and the choice is made automatically on login. All the information here relates to the default KDE interface.

#### Shell console



Many functions can be carried out by typing a command into the shell console or terminal window. This is similar to the old DOS command interface that may be known by Windows users, and can be opened with the icon shown here. The following table has a few basic commands:

<code>spm</code>	Starts JPK SPM software
<code>ip</code>	Starts JPK IP software
<code>Ctrl-c</code>	Interrupts previous command (e.g. force exit from SPM software after it is started from the console)
<code>ls</code>	Lists the components of a directory
<code>cd ..</code>	Change directory up one level
<code>cd newdir</code>	Change directory to "newdir" (must be within the current directory)
<code>mkdir</code>	Make directory (create a new directory)
<code>man</code>	Opens the linux manual/online help

#### Mounting CD and floppy drives

CD and floppy drives have to be explicitly mounted as folders/directories prior to use. The CD or floppy-disk drives can easily be mounted or unmounted using the explorer-type programs Konqueror (in KDE) or Nautilus (in Gnome) by clicking the right mouse button onto the CD-drive or floppy-disk drive icons and selecting the appropriate option.

A CD can also be mounted from the console window with the following commands (insert the CD first):

```
>>mount /mnt/cdrom
```

---

The drive is then added to the directory structure and the local directory is named /mnt/cdrom. Before removing the CD or floppy-disk from the drive, the drive must be unmounted. This can also be done from the terminal window:

```
>>umount /mnt/cdrom
```

On newer systems there is a "stick" icon on the desktop for mounting and unmounting USB sticks. First insert the USB stick, then click the icon. There will be an extra green triangle when the USB stick is mounted. Right-click the icon and select unmount to finish using the USB stick.

During use, the data can be transferred to the local directory /mnt/ext1 or /mnt/ext2. Note that the files are only written as the USB stick is unmounted. Therefore the unmount process can take some time. Do not remove the USB stick until the process is finished. The progress bar may not display the correct remaining time, but it will disappear when the process is complete. *Do not remove the stick while the progress bar is displayed!*

### Checking network settings

The current network settings can be seen from a console window by typing:

```
>>/sbin/ifconfig
```

To change the network settings, long in as root and use the program "neat".

### Accessing other computers on the local area network

To get access to windows-based computers in your LAN from the Linux computer the following two options are recommended:

1. Start the Nautilus program (this is a Gnome program but should also run under KDE). As the address, type "smb://computername" (to show everything that is shared by the "computername" computer).
2. In Konqueror, type "smb://computername" as the address. This shows you everything that is shared on the "computername" computer. Konqueror lets you read from the windows computer but not write to it.

### Choosing a new password

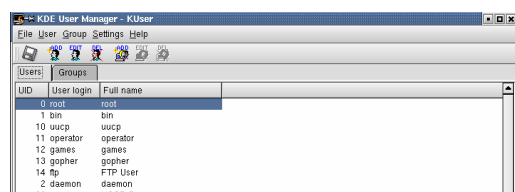
To change your Linux user password, open a console window and type:

```
>>passwd
```

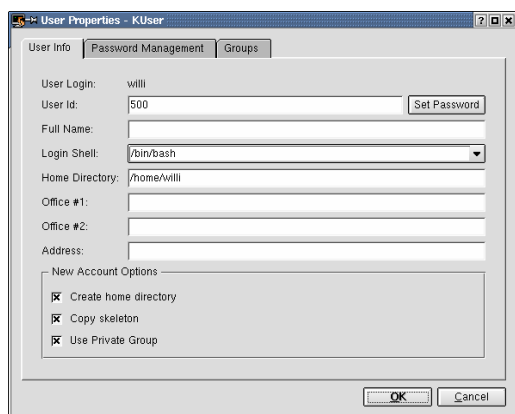
### Add a new user with KUser

Linux is a multi-user system which allows the superuser "root" to manage the user accounts. The program kuser is helpful for adding or managing user accounts. Log in as "root" and type "kuser" in the konsole.

```
>>kuser
```

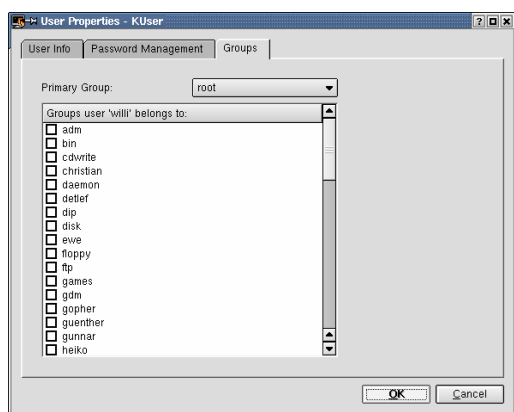


Click the button **Add** to add a new user name.



Use the button **Set Password** to input a password for the new user.

Most settings can be left as the default choices.



In the **Groups** tab you can see all groups which exist in the system. From the following list only group “spmusers” **MUST** be enabled.

☒ **spmusers (required)**

☒ disk (optional)

☒ floppy (optional)

☒ cdwrite (optional)

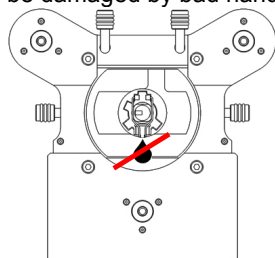
☒ local (optional)

☒ wheel (optional)

If a local z-calibration is used, then it must be set in the SPM program for a new user. See **Section 8.1** for details.

## 11.2 Taking care of the NanoWizard® I hardware

When using the NanoWizard® I AFM system, it is important to take care of the motor spindles. The three motors that move the head up and down extend under the AFM head, and the spindle thread is exposed. This means that it can be damaged by bad handling. This is particularly the case with salt buffers causing corrosion of the spindle thread.

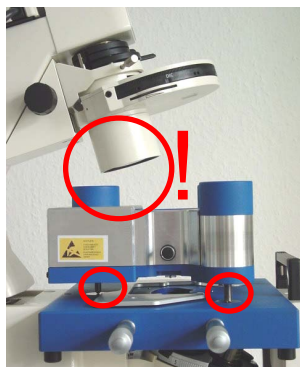


When the NanoWizard® I AFM head is moved into its upright position after an experiment has been performed in liquid, sometimes some fluid may drop from the glass block onto the back spindle of the AFM head. This fluid may lead to corrosion.

*Please remove the fluid from the glass block with a soft piece of tissue paper!*

With the NanoWizard® II the back spindle is protected by a rubber coating.

Also very important – *never move the head down to the end of the motor ranges!* The motor spindle can become stuck at the lowest point, and it is not possible to move the head back up again. For normal use with standard samples, this will not occur, because the AFM cantilever will reach the surface well before the motor spindle reaches the end of its range. If the motors have been moved independently, however, and then an approach is made, all the motors are moved together, and the tilt angle is preserved. Therefore, if one motor was at a much lower position than the others, then it is possible that it will reach the end of its range before the head reaches the surface. Avoid large tilt angles and very low samples (below the foot positions).



The image shows the NanoWizard® AFM in the Life Science version installed on a Zeiss Axiovert 200 optical microscope. If the three stands (spindles) of the AFM head are fully extended it is impossible to place the condenser over the AFM head.

*Always lower the condenser carefully!*

Lower the AFM head on its spindles before placing the condenser over it to avoid the condenser crashing into the AFM head.

## 11.3 Analogue cameras (xawtv)

Many RedHat systems have a Firewire camera. This is able to use the standard software unicam, as described in **Section 9.1**. The information here is only for those systems that have the older analogue camera.

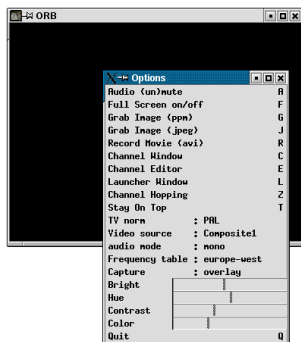


Start the camera viewer with the **CCD** button.



The camera software can also be started with a command from the Linux console: `xawtv`

If there are problems with the software, it may be useful to type: `xawtv -noxv`



Depending on the version of the program, it might be necessary to type the following:

Click **OK** in the welcome window

Right click with the mouse on the window named ???

Choose **Video source** → **television** → **svideo**.

The option **TV norm** must also sometimes be reset.

The screenshot here shows the menu when the mouse is right-clicked in the CCD window. The option **Stay On Top** keeps the image window on top of the SPM program, which can help when setting up and using the Laser Alignment window.



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Note: All trademarked names mentioned in this manual remain the exclusive property of their respective owners.

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