NanoWizard® Series
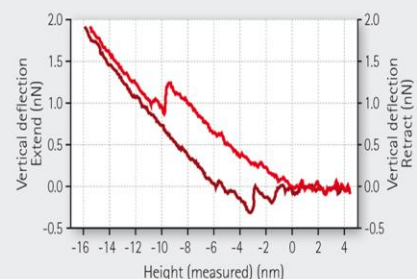## User Manual

SPM Software Release 6.0 – 11 / 2016

JPK
Instruments

Nanotechnology for Life Science

Table of contents

§ 0   General information

This manual covers the general installation steps and the operation of all systems of the NanoWizard® Series as well the functionality of the SPM control software.

All available NanoWizard® configurations show the same basic design, which is described in detail within this manual. Some of the optional systems contain additional components which are thoroughly described in respective manuals.

The SPM control software applies for all NanoWizard® applications as well as for optional modes and features (like QI™ Advanced or the DirectOverlay™ module) that can be purchased optionally depending on your system configuration.

Please Read carefully this manual and all additional manuals that apply specifically for your system configuration.

Please contact JPK for more information and assistance (++49 30 726243 500) support@jpk.com.

0.1   Explanation of symbols

Three main symbols appear in this manual to highlight accessory options, warnings and useful information:

Features and options marked with this sign are additional extensions and must be purchased separately.

Warning: Indicates potential sources of danger and gives hints to prevent any damage to the system or sample.

Note: Useful information and hints

§ 1 Safety instructions and warnings

1.1 Important safety information

Laser safety warnings:

Infra-red laser diode:

DANGER
INVISIBLE LASER RADIATION
AVOID EYE EXPOSURE
TO DIRECT OR SCATTERED RADIATION
DIODE LASER
1 mW MAX OUTPUT at 850 nm
CLASS III LASER PRODUCT

Most users have a JPK NanoWizard® equipped with a laser diode that emits **invisible near infrared** laser light (wavelength 750-1000 nm), with an emitted power of <5 mW. The laser belongs to **laser class 3R**. Be extremely careful with such lasers, since the blink reflex will not protect your eyes. The NanoWizard® head contains 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:

DANGER
LASER RADIATION
DO NOT STARE INTO BEAM
DIODE LASER
1 mW MAX OUTPUT at 670 nm
CLASS II LASER PRODUCT

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 mW. Even with the cantilever holder detached, the emitted power of the laser remains below 1 mW. 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 user's 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 and other purposes 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) supplied to the system may cause injury to the user.

Do not insert anything into 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.

Electrostatic discharge:

The AFM head is sensitive to 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 cantilever holder, 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 rubber membrane that protects the AFM head from underneath is not resistant to benzene and alcohol. Do not allow aggressive fluids such as acids and bases to come in contact with the membrane.

Our product is fully CE-approved.

1.2   Warnings for the Life Science version with optical microscopes

1.2.1   Prevention of condenser lens crash

Be careful not to hit the AFM head when lowering the condenser (illumination optics) of the optical microscope or when moving the head upwards using the stepper motors. If the condenser is going to hit the head, either raise the condenser position or lower the head on the motors first.

The condenser may hit and damage the AFM head if it is positioned far too low. Always move up the condenser completely and lower it carefully!

The AFM head may hit the condenser if the stepper motors are moved upwards in large steps. Always move up the head position in small steps.

1.2.2   Prevention of objective lens crash

The maximum load of the objective revolver is three objectives, arranged in a 120 degree angle, as shown in the picture on the left. Please check that the specific lenses used fit in this conformation. Bulky lenses may have to be used alone, or the objective lens holder must not be rotated.

Do not load more than three objectives. Always load the objectives with a 120 degrees angle. Otherwise the objectives as well as the AFM stage may be damaged.
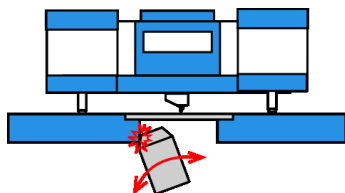
The objective revolver must only be rotated if it is completely retracted. This is particularly important if the optical microscope uses any automatic movement of objectives. Please ensure that the automatic switching between objectives makes a complete retract before the rotation. For the empty positions it is strongly advised to set the parfocality at the lowest position, so that when an empty position is accidentally selected the adjacent objectives are not driven into the stage. Please contact the supplier of your optical microscope for advice on how to set this.

Do not allow the objectives to crash into the AFM stage. Always lower the objective revolver completely before rotating it. Otherwise the objectives as well as the AFM stage may be damaged.

Please see **Section 2.3.1** for detailed cleaning instructions.

Do not touch the optical surfaces with your fingers, tweezers or any other material that could scratch the surface, and do not store the cantilever holder lying on these surfaces. The optical surfaces may be damaged and impair the function of the cantilever holder.

1.3.2 Sample stage and sample holder

The NanoWizard® AFM system is optimized for measuring immersed samples. For this purpose, JPK provides dedicated sample holders, such as the PetriDish™ Heater. Please use these sample holders for safe operation in liquid. Please contact JPK for more information and assistance (++49 30 726243 500) support@jpk.com.

Never immerse the sample holder or sample stage in liquid. Parts of the optical microscope or the sample holder/stage may be damaged. Always use dedicated sample holders from JPK for safe operation in liquid.

Do not allow the sample stage or sample holder to come into contact with acids, bases or other aggressive chemicals. The corresponding parts may be damaged.

§ 2   Installation

2.1   Components

The components provided depend on your system configuration and may vary. If a complete NanoWizard® system is ordered, the most important components are:

NanoWizard® AFM head

* Stepper motor housing

▲ Feet for positioning AFM head

Life Science Stage with sample holder for the use with inverted optical microscopes, if the **BioScience** version was ordered (see **Section 2.1.1**).

Standard stage if the **NanoScience** version was ordered (see **Section 2.1.1**).
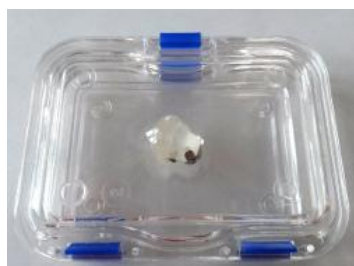
Cantilever holder

Cantilever changing stand

NanoWizard® controller with or without Signal Access Module (SAM). Please see **Section 11.1.1** for a description of the SAM.

PC (with monitor, mouse and keyboard)

All required cables to set up the system, such as the head cables (left-hand image) to connect the NanoWizard® controller to the NanoWizard® head, are provided with the system.

2.1.1 Fast Scanning Option

The NanoWizard ➕ **Fast Scanning Option** is available as an optional accessory and contains additional components:

The JPK **Fast Scanner** cantilever holder and the ➕ **Fast Imaging** software module (**Section 8.2**) are the main components of the Fast Scanning Option. Please read the **NanoWizard® ULTRA Speed Series & Fast Scanning Option User Manual** for a detailed description of the use of the Fast Scanner and related software settings.

2.1.2 BioScience and NanoScience systems

If the NanoWizard® **BioScience** version was ordered, then it can be installed on various inverted optical microscopes made by Zeiss, Leica, Nikon, or Olympus with an appropriate Life Science sample stage. The entire optical microscope and AFM should be placed on an active anti-vibration table or air table.

Note that the Life Science stages are specially shaped to fit a particular model of optical microscope. They are not interchangeable and should only be used on the type of microscope they are designed for. Optical microscopes from different manufacturers have different designs. 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 stages can be manual or motorized, depending on the type of stage.

The Life Science stages 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 TopViewOptics, as for the **NanoScience** system.

CCD camera

JPK Top View Optics™

In the **NanoScience** system, the JPK NanoWizard® AFM is usually used together with the JPK standard stage.

This is mainly interesting for non-transparent samples, where the inverted optical microscope is not suitable. The sample can be observed in reflected light using the CCD camera attached to the **TopViewOptics™**. The optical magnification is generally much lower in this configuration than on an inverted optical microscope due to physical limitations.

2.2   Assembly

2.2.1 Location – find a quiet place for the instrument

For highest resolution imaging and most sensitive force measurements, 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, ideally within an acoustic enclosure.

- Building vibrations are usually smallest in the basement; AFM labs on higher floors may have more noise problems. Mind the mechanical vibrations caused by lifts or other large machinery which may influence the measurements 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, noise or drift, so place the AFM system away from doors, windows and vents. Keep doors and windows shut. Air conditioning can cause noise and drift problems if it blows near the AFM system.

- Any loud noises or people passing by can disturb the AFM experiment, so small rooms with just a single instrument will generally give the best results.

The two AFM head cables can transmit unwanted vibrations to the AFM head. Therefore it is recommended to weigh down the cables to the vibration isolation table using the supplied weights. Adhesive tape may leave residues and the cables can become sticky.

2.2.2 The PC

The NanoWizard® is equipped with a controller and PC that are connected via Ethernet.

There are several USB sockets on the computer to connect keyboard, mouse, JPK accessories (such as the motorized precision stage or temperature devices) and any other USB devices.
The RS232/USB socket connects some temperature devices to the system.

The network plug labelled "LAN" can be used to connect to the internet/external network. The second network plug is dedicated for connecting to the controller.

There is one monitor cable, which plugs directly into the graphics card.

Firewire cameras connect directly to the Firewire (IEEE1394) connecting port (see **Section 4.1.1**).

For the PC as well as for the controller standard power cables for the correct voltage (230 or 115V, depending on the country) can be used.

2.2.3   The controller
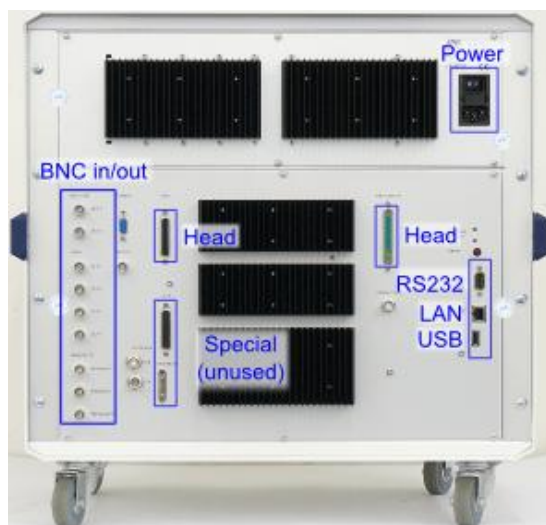
The connections for the head are found on the back of the controller box.

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).

First check that the main power switch of the controller is switched to "OFF" and then connect the black power supply cable for the controller. Connect the head cables to the NanoWizard® head (see below) and the network cable to the dedicated network plug of the computer (see picture above). Now the controller can be switched on.

There are additional (e.g. BNC) connections allowing the use of JPK accessories. Please read the corresponding manuals.

The controller has two power switches. The one at the back needs only to be used when disconnecting or reconnecting it to the mains supply. The power switch on the front of the controller is intended for more regular use.

Do not store heavy or electronic equipment on the controller. Otherwise the performance of the controller may be impaired.

2.2.4   AFM head connections

There are two dedicated cables connecting the AFM head to the controller.

Make sure that the controller is switched off before connecting or disconnecting the AFM head. Otherwise the AFM head and/or controller may be damaged.
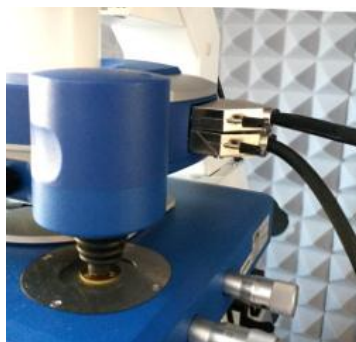
On the right hand side of the AFM head are the two sockets for the controller cables. Check the plugs and sockets carefully; there is only one way to connect them. Do not force the connectors!

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 to the controller. Use the weights provided to secure the two head cables to the anti-vibration table. Take care that no tension or torsion is transmitted to the head. The firm connection to the anti-vibration table removes differential movement between the head and the stage. See **Section 2.2.1** for more details.

Do not force the connectors and avoid any strain to the head cables. The AFM performance may be reduced or the AFM head and/or connectors may be damaged.

2.2.5   Power on

If all components, i.e. the head, controller and computer, are connected properly, switch on the main power switch on the back of the computer, and then switch on the computer at the front. The Linux Ubuntu system will start automatically (for more information about the operating system see **Section § 10**   ).

When the computer has booted, switch on the controller with the switch marked in the images below.

The upper image shows the front of the controller equipped with a Signal Access Module (SAM, see **Section 11.1.1**).

Controller with Signal Access Module

The lower image shows the controller without SAM.

Standard Controller
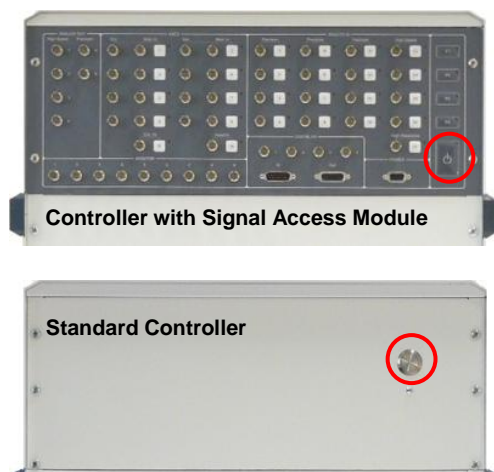
The blue LED on the front of the controller shows the status:

- Off (dark) – no power connection; or the main power switch at the back is off.

- Blinking (on/off) – the power is connected and the switch at the back is on, but the controller is still off.

- On (stays lit) – controller is switched on and can be used.

It is alright to leave the PC switched on all the time. When the SPM program is closed, you can switch the controller on/off as required.

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

2.3   Practical tips

2.3.1   Maintenance and Cleaning

Cantilever holder

After using the cantilever holder in liquid (such as aqueous solutions or buffer, e.g. phosphate buffered saline (PBS) or other protein solutions) rinse the holder with pure buffer solution, followed by ultrapure water. Finally, rinse with pure ethanol and blow dry with clean air.

After use in liquid clean the cantilever holder immediately. Otherwise salts, proteins or any other molecules (depending on the liquid) may precipitate on the polished surface and reduce the optical transparency of the cantilever holder.

Precipitation of proteins, salts, or fats onto the polished surfaces can dramatically decrease the sum signal for the AFM detection laser. To prevent and remove such contamination, consider the following hints (see also

www.zeiss.de/courses):

- Use only pure cotton swabs e.g. ITW Texwipe Clean Tips® or Eurotubo® swabs

- Use only Whatman lens Cleaning Tissue 105

- Do not immerse the cantilever holder in strong acids or bases, or organic solvents.

- Tests revealed that strong acids or bases (pH 4.6 or lower, pH 9 or higher) can lead to a firmly attached white layer (cloudy surface) within 10 to 60 min.

- Avoid fingerprints on the optically polished surfaces, remove fingerprints immediately with ethanol

- Clean the cantilever holder with a mild detergent (~pH 7), ethanol or 2-propanol (e.g. 70% pure grade)
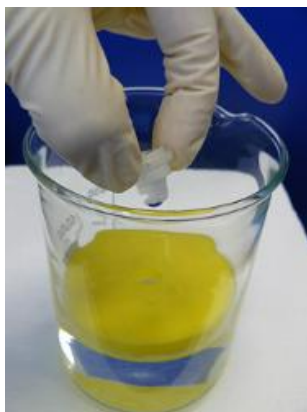
The cantilever holder can also be cleaned with an ultrasonic bath. If required, some detergent can be added to the water. Make sure that the cantilever holder is held using a soft support, such as the supplied swimmer (see corresponding manual). Do not ultrasonicate in a hard holder or glass beaker. Rinse the cantilever holder with ultrapure water afterwards and dry it in a stream of nitrogen. Try to avoid any contact with the optical planes. Please read the safety instructions given in **section 1.3.1**.

Do not touch the optical surfaces with your fingers or tweezers or any other material that could scratch the surface, and do not store the cantilever holder lying on these surfaces. The optical surfaces may be damaged and impair the function of the cantilever holder. Always store the cantilever holder in the supplied box or cantilever changing stand.

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.

2.3.2   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 by inappropriate scanning conditions:

- Too high gain parameters (IGain and PGain) may lead to oscillations and damage the cantilever tip.

- Too high setpoint values (high force) in contact mode may damage the tip.

- Too low setpoint values (high force) in AC mode may damage the tip.

2.3.3 Setting up 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 bottom of the AFM head is sealed to protect the scanning system from water, buffer or any other liquid whose vapor could damage the scanning system.

Always remove the cantilever holder immediately after experiments in liquids. Always remove the liquid from the cantilever holder immediately if the head is moved into an upright position.

§ 3   Software overview

3.1   SPM software introduction and index

The JPK NanoWizard® is delivered with the latest version of the SPM software, which controls the AFM operation. This chapter provides a quick overview of the software structure and main functions, and links to find more specific information on particular features from the menus or shortcut icons. More detailed instructions are given in the referenced sections later in the manual.

3.1.1   Starting the program

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

3.1.2   Software overview

The graphic below shows the initial software state. The SPM software provides a dark and a light theme (**Look&Feel**), i.e. it is possible to choose between a dark and a light software background (see figure below). In this manual most screenshots show the light theme.
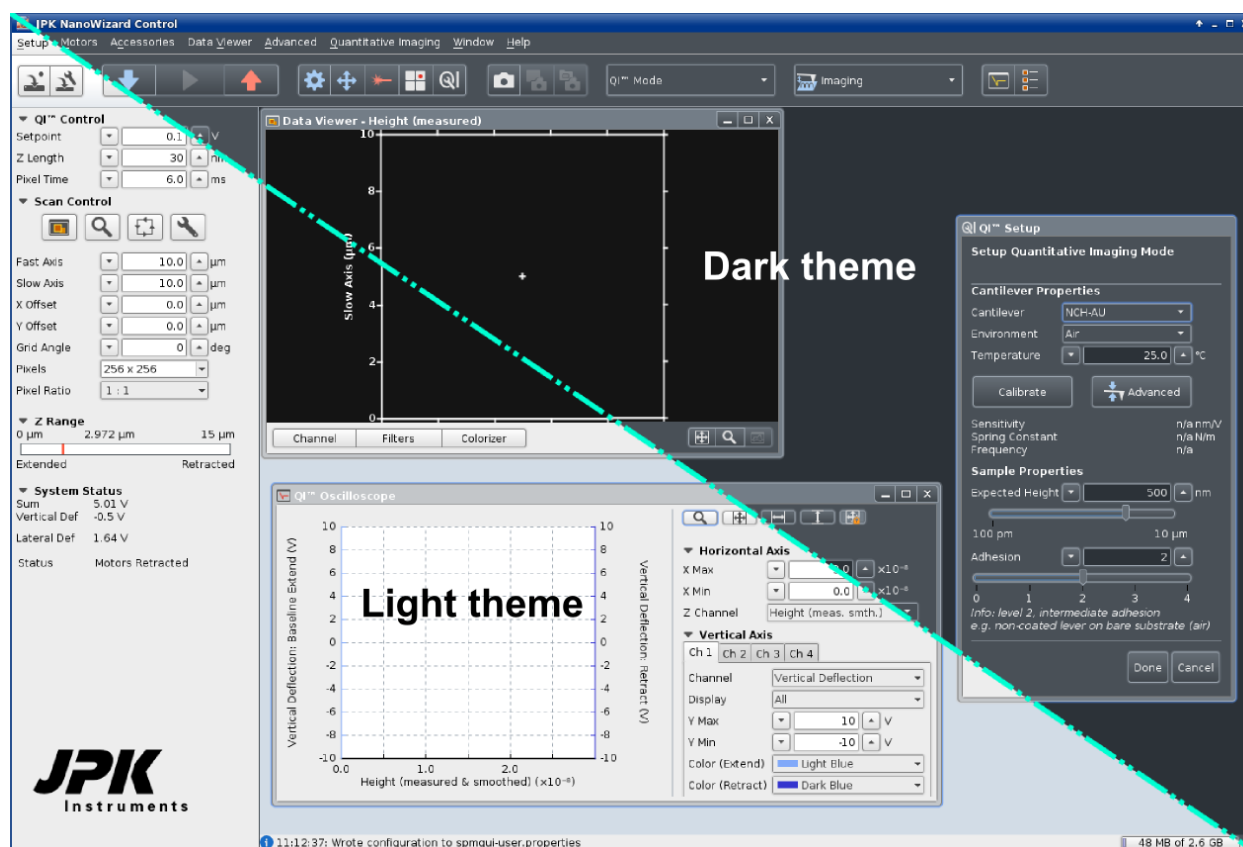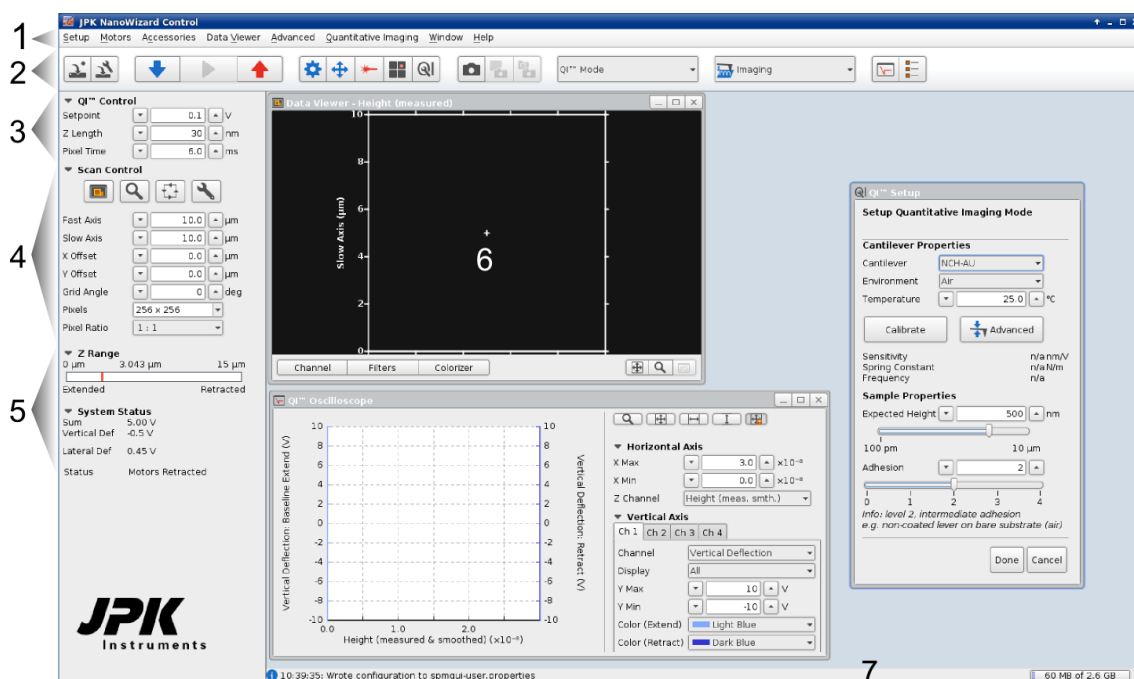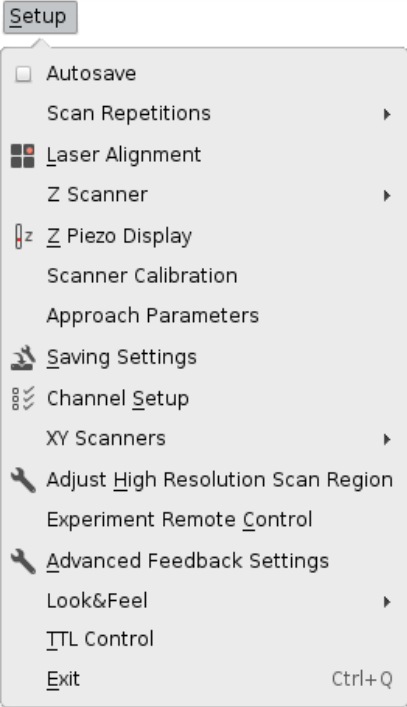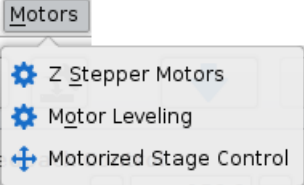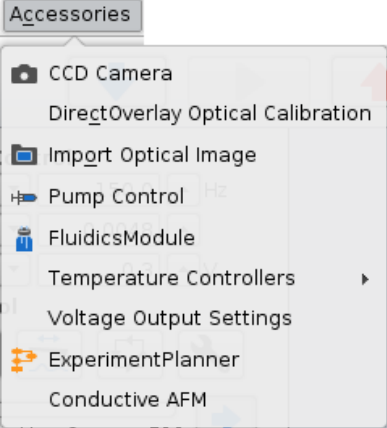
1. The menu bar runs along the top of the screen; the drop-down menu options are listed in **Section 3.1.3**.

2. The icon toolbar provides shortcuts for some of the most commonly used options, as listed in **Section 3.1.4**

3. The **QI™ Control/Feedback Control** panel can be seen on the left side of the screen. Here the parameters controlling the measurement can be set. The **QI™ Control** parameters are discussed in **Section 5.7.4**. In other feedback modes than QI™, the **Feedback Control** panel appears, which is briefly introduced in **Section 3.2.2** and more thoroughly discussed in **Section 5.2**

4. The **Scan Control** can be seen on the left side of the screen during any of the imaging modes. These settings are discussed in **Section 5.1.1**and **5.7.4** (QI™ mode).

5. The **Z Range** 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 **Data 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 3.2.4**.

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.

The **QI™ Oscilloscope** and **QI™ Setup** appear upon starting the software, as **QI™ Mode** is initially selected. Please read **Section 5.7** for a detailed description of their functionality.

3.1.3   The menu bar

Along the top line of the user interface you will find a drop-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.

Drop-down menu	Short explanation	Details in section

Setup

Autosave enables automatic saving of scans	**3.2.8**
Set **Scan Repetitions** (single/infinite scans)	**3.2.3**
Open the **Laser Alignment** window	**4.2.3**
Limit the **Z-Range** of the z piezo for better z resolution	**5.4.1**
Open the vertical **Z Piezo Display**	**5.4.1**
Open the conversion manager for Z **Scanner Calibration**	**7.1**
Change **Approach Parameters** and initial piezo position	**4.5.2**
Open the **Saving Settings** to manage data saving	**3.2.8**
Open the **Channel Setup** to manage channel storing	**3.2.7**
Adjust the **XY** scan range to improve resolution	**5.3**
Adjust the **High Resolution Scan Region**	**5.3**
Experiment Remote Control (see corresp. manual)	
Show and adjust **Advanced Feedback Settings**	**5.2.5**
Switch between the dark and light **Look&Feel** theme	
Open **TTL Control** to control and monitor TTL signals	**9.8**
Exit the SPM software	

Motors

Move the **Z Stepper Motors** to set the coarse height	**5.4.2**
Open the wizard for **Motor Leveling**	**5.4.3**
Motorized Stage Control (see corresponding manual)	

Accessories

Launch the CCD camera	**4.1.1**
DirectOverlay™ Optical Calibration in SPM	**8.2**
Calibrate and **Import Optical Image** into the data viewer	**8.4.4**
Pump Control for syringe pump	**8.8.2**
FluidicsModule (see corresp. manual)	
Temperature Control for JPK temperature devices	**8.8.1**
Voltage Output Settings for setting a voltage on a DAC	**9.4**
Experiment Planner (see corresp. manual)	
Conductive AFM (see corresp. manual)	

Data Viewer

Open a new Data Viewer	
Height (measured)	
Height	
☑ Synchronize Data Viewer Channels	
☑ Tile Data Viewers	
Default Colortable ▸	
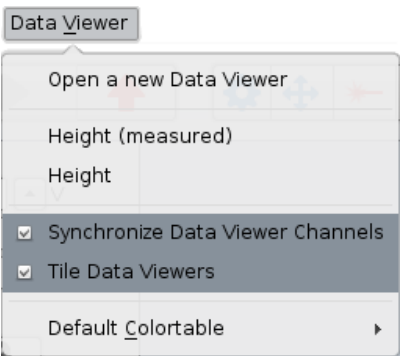
Open a new **Data Viewer** window **5.1.2**

List of active channels that can be displayed in the data viewer **3.2.4**

Display active channels in the currently opened Data Viewers

All Data Viewers are arranged on a grid and equally sized

Set the **Default Colortable** for image display

Advanced

- 🔲 Open Script
- JPK SPM Jython Console
- 🖼 Real Time Scan
- Logging Settings
- Advanced System Status
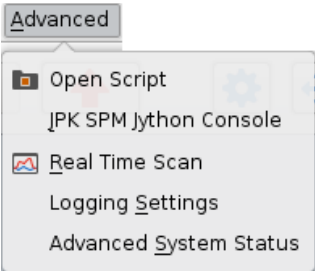
Open Script loads scripts for custom experiments **9.6**

Open **JPK SPM Jython Console** for running macros etc. **9.5**

Open the **Real Time Scan** oscilloscope window **9.2**

Manage the **Logging Settings** **9.3**

Open the **Advanced System Status** window **4.5.4**

Window

- ☒ Close All Frames
- ☑ Auto-save Window Layout
- Save Window Layout Now
- ☐ Use Window Layout
- Forget Saved Window Layout
- Data Viewer - Adhesion 1
- Data Viewer - Measure Slope 1
- Data Viewer - Height (measured)
- QI Setup
- Quantitative Imaging Oscilloscope
- Manipulation Pattern Manager
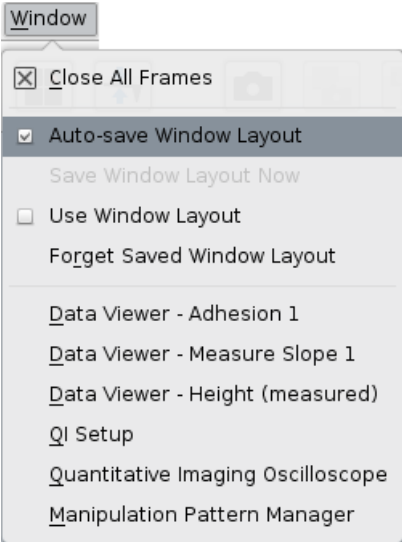
Auto-save Window Layout

Save current **Window Layout Now**

Forget deletes the personal settings

Select from the list of other currently open windows

Help
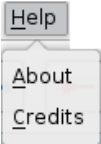
- About
- Credits

Information about the software version and related projects **3.3.1**

Some drop-down menus depend on the choice of Measurement mode. They provide access to the main windows necessary to manage data acquisition.

Open Saved Image: load AFM image into the scan list		**5.1.4**
Image Record List: manage data storage		**5.1.4**
Advanced Imaging Settings for scan speed and overscan		**5.6**
Hover Mode Settings		**5.9**
Open the **Oscilloscope** window		**5.5.1**
Open the frequency **Spectrum Analyzer** window		**9.1**
Open the **Calibration Manager** for cantilever calibration		**7.2**

Force Scan Series List: manage data storage		**6.6.2**
Open the **Force Spectroscopy Oscilloscope** window		**6.1.2**
Open the **Force Time Oscilloscope** window		**6.1.3**
Open the **Advanced Force Settings** window		**6.3**
Open the **Calibration Manager** for cantilever calibration		**7.2**

Image Record List: Manage data storage		**6.7.4**
Open the **Force Mapping Oscilloscope** window		**6.7.3**
Open the **Force Time Oscilloscope** window		**6.1.3**
Open the **Advanced Force Settings** window		**6.3**
Open the **Calibration Manager** for cantilever calibration		**7.2**

Image Record List: manage data storage		**5.7.6**
Open the **Quantitative Imaging Oscilloscope** window		**5.7.2**
Open the **Advanced QI™ Settings**		**5.7.5**
Open the **Calibration Manager** for cantilever calibration		**7.2**
Open the **QI™ Setup** to optimize QI™ settings		**5.7.3**

Software extension modules

➕ Some drop down menus only appear if the corresponding software extension modules have been purchased:

Open the **Manipulation Pattern Manager**		**8.7**
Import a **Manipulation Pattern**		**8.7.4**
Import a **Pattern for Background**		**8.7.6**
Save a **Manipulation Pattern**		**8.7**
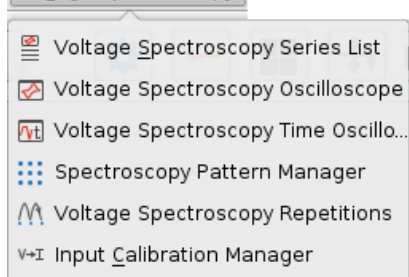
This mode is part of the ⊕ Conductive AFM module

Manage data storage in **Voltage Spectroscopy** mode
Open the **Voltage Spectroscopy Oscilloscope** window
Open the **Voltage Spectroscopy Time Oscilloscope**
Open the **Spectroscopy Pattern Manager**
Set **Voltage Spectroscopy Repetitions**
Open the **Input Calibration Manager**

⊕
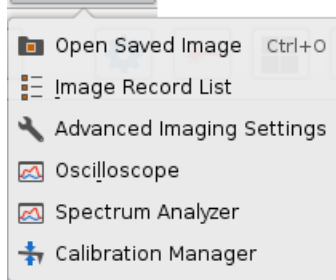
Open Saved Image: load AFM image into the scan list	**5.1**
Image Record List: manage data storage	**5.1.4**
Advanced Imaging Settings for scan speed and overscan	**5.6**
Open the **Oscilloscope** window	**5.5.1**
Open the frequency **Spectrum Analyzer** window	**9.1**
Open the **Calibration Manager** for cantilever calibration	**7.2**

3.1.4 The shortcut icon toolbar

Below the menu bar you can find icons for launching the most commonly used features. A short explanation for each icon is shown in the table below. Some icons or options only appear if software extension modules have been purchased.

The icons on the left hand side of the toolbar are always shown, regardless of the feedback or measurement modes that are selected.

Shortcut icon	Brief explanation	Details in Section:
	Autosave – this toggle button both shows the status and activates the **Autosave** function so that all scans are automatically saved.	3.2.8
	Saving Settings – this is used to set the name, location and content of data files saved from the software.	3.2.8
	Approach starts the movement of the cantilever towards the surface.	4.5.2
	Run starts a scan (in many modes this is only active if the cantilever is approached at the surface).	4.5.4
	Retract moves the cantilever away from the sample using the Z piezo or the Z stepper motors.	4.5.4
	The **Z Stepper Motor** window controls the 3 motors for coarse approach or retraction. They can also be moved independently to change the tilt of the head.	5.3

Open the ⊕ **Motorized Stage Control** to move the JPK Motorized Precision Stage (optional accessory, see the corresponding manual).

Shows the status and switches the **Laser** on/off (this is a toggle switch, and cannot be switched off during scanning or while approached).

Open the Calibration Manager to determine the spring constant and sensitivity of a cantilever.   **7.2**

Open the **Laser Alignment** window to adjust the laser beam onto the cantilever and the photodiode.   **4.2.3**

Start the **CCD**-camera window to see the image from the optical microscope or top-view optics.   **4.1.1**

Take a **Snapshot** and import a calibrated optical image using selected calibration (requires optional software module DirectOverlay). The button is only active when an optical image is selected in the scan list.   **8.4.4**

Import optical image loads an already existing optical image from disk   **8.4.4**
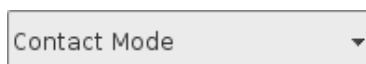
The **Feedback Mode** is set with this drop-down box. This defines how the Z-position of the cantilever is controlled during the measurements.   **4.4**

Contact Mode ▼

The list of available Feedback Modes depends on the controller type and also the software extension modules.
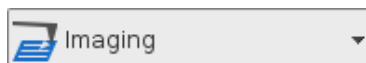
The **Measurement Mode** is set with the second drop-down box. This defines the kind of measurements or scans that will be made. The set of available measurement modes depends on the Feedback Mode.

Imaging ▼

Some icons only appear upon the selection of the corresponding measurement modes:

The **Cantilever Tuning** icon is only shown when dynamic feedback modes are selected. The tuning window is used to find the cantilever resonance.   **4.4.1**

The **Oscilloscope** shows the trace and retrace line cross sections of the current scan line.   **3.2.1**

The **Image Record List** gives an overview of the acquired images and allows sorting and saving of data.   **5.1.5**

The **Force Scan Series List** gives an overview of the acquired force curves and allows sorting and saving of data.   **6.6.2**

Open the **Force Spectroscopy** or **Force Mapping** or **Quantitative Imaging Oscilloscope** to display force distance curves.   **6.1.2**

	Open the **Force Time Oscilloscope** to display the force data plotted against time.	**6.1.3**

3.2   Introduction to the main controls

3.2.1   Oscilloscopes and the Oscilloscope Toolbar

Non-image data such as scan lines or force spectroscopy data are usually displayed in **Oscilloscope** windows. There are different kinds of Oscilloscopes for different measurement modes, but the display settings are common to all.
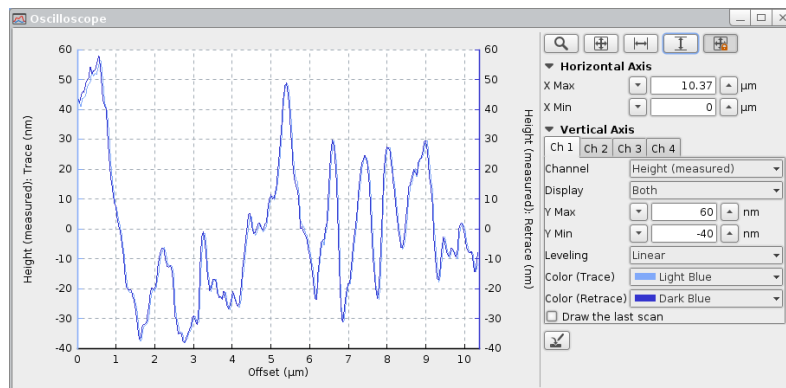
The plot area is usually on the left, with the display settings on the right. The scaling for both axes can be changed as well as the displayed data channels. Changing the display has no impact on data saving.

Vertical Axis: Use the different tabs (Ch1, Ch2...) to display several channels.

The Oscilloscope toolbar allows automatic scaling of the oscilloscope data display.

	Zoom –select a rectangular area with the left mouse button in the plot, which updates the axis scale.
	Full Range XY – sets X and Y axis to the full range of the current data
	Full Range X
	Full Range Y
	Autoscale XY – the X and Y axes are reset to the full range of each new data set as it arrives
	Save current data (e.g. scan lines in imaging modes or force curves in force spectroscopy based modes)

There are some useful mouse shortcuts for changing the Oscilloscope display region directly:

- Scroll the mouse wheel in the plot area to zoom in or out in X

- Shift + scrolling the mouse wheel in the plot area zooms in Y

- Click and drag with the mouse wheel in the plot area to shift the displayed region of the curve

- The tools **Zoom** and **Full Range XY** from the toolbar can be accessed in most oscilloscopes directly from the right mouse button menu in the plot area

3.2.2   Feedback Control

The **Feedback Control** appears in the top left position in all measurement modes, except QI™. These settings define how the Z-position of the cantilever is controlled during contact state. The Setpoint value represents the force applied to the sample. IGain and PGain are part of the PI (proportional-integral) feedback system.

The settings displayed here are explained in **Chapter § 5** . The **Setpoint** value represents the force applied to the sample, depending on the imaging mode. See **Section 5.2** for details on how to choose these parameters. In some feedback modes there are multiple gains and setpoints.

3.2.3   Scan Repetitions

Scan Repetitions allows switching between infinite data acquisition (Infinite Scans) and single measurements (Single Scan).

Choose **Scan Repetitions** in the **Setup** pull down menu to manage scan repetitions. The default is set to **Infinite Scans**. If **Single Scans** is enabled, the system stops scanning after one scan and rests in idle mode.

During scanning there is the possibility to select **Stop After Current Scan**, to prevent the system from scanning further images after the current scan.

3.2.4   The Data Viewer

The **Data Viewer** is designed for displaying data collected in **Imaging** modes. It is also useful in many other measurement modes, for displaying locations of point measurements (e.g. for Force Spectroscopy mode) or line measurements (e.g. for Manipulation mode) relative to previously scanned images or optical images. Therefore the Data Viewers are available in all measurement modes.
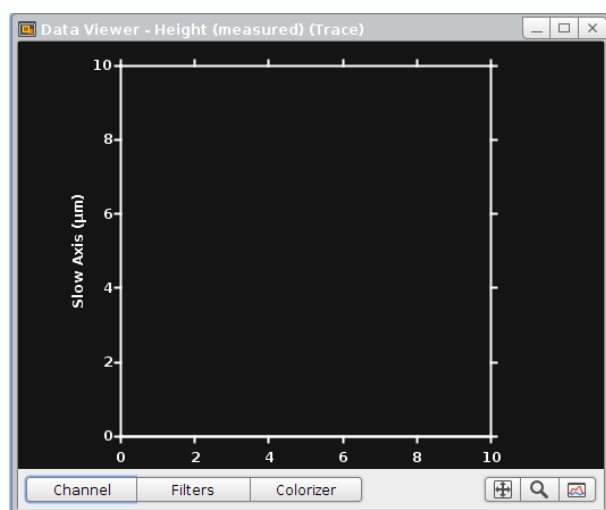
The **Data Viewer** displays images that are currently being scanned, and old scans from stored images in the Image **Record List** (see **Section 5.1.4**). Multiple windows can be opened to show data from different channels or with different settings.

The right mouse click menu allows moving of the scan region and controlling several display options (see **Section 5.1.3**). The controls on the bar at the bottom of the data viewer set the display options and are thoroughly described in **Section 5.1.2** as well.

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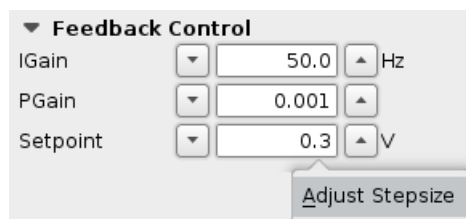
The SPM software contains many input fields, where values for the control parameters are shown and new values can be entered directly. The increment buttons (up and down arrows) are used to quickly increase or decrease the value of the input field by fixed steps using the mouse.

To adjust the step size for each input field (e.g. here the **Setpoint**), right-click with the mouse on one of the arrows, and select **Adjust Stepsize**.
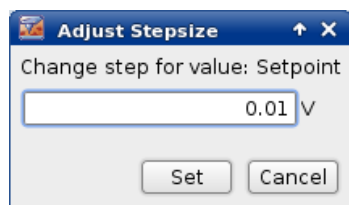
The value can then be changed.

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

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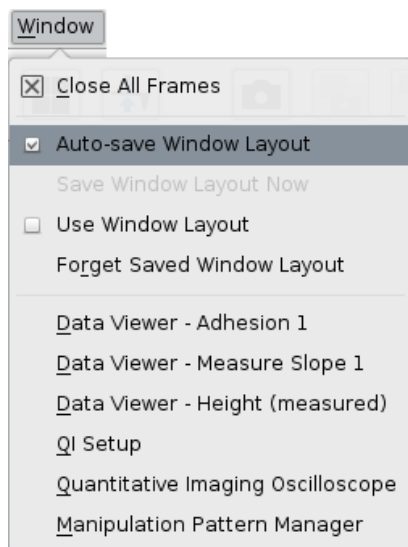
If **Auto-save window layout** is enabled (default is on), then the positions and sizes of the internal windows are remembered when they are closed so that they appear in the same place when they are opened again.

If it is off, the layout can be saved directly with **Save Window Layout Now**.

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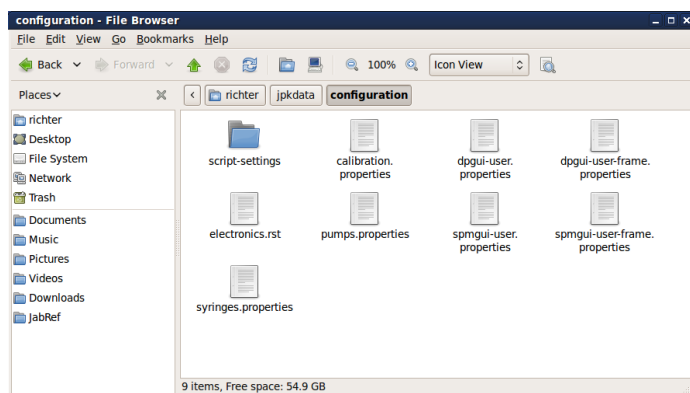
The personal display settings are saved for each login account under /home/username/jpkdata/configuration

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

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

Please close the SPM software before deleting any of these files.

3.2.7   Channel Setup

The **Channel Setup** lists all available data channels, which can be displayed and saved during AFM operation. The list of available channels depends on the type of controller, and also the feedback mode that is being used.

The channels to be displayed and acquired must be activated in the **Channel Setup** list. There is a preselection of standard channels, which are sufficient for the majority of experiments. Open the **Channel Setup** via the **Setup** drop-down meu to control channel activation or to enable additional channels.
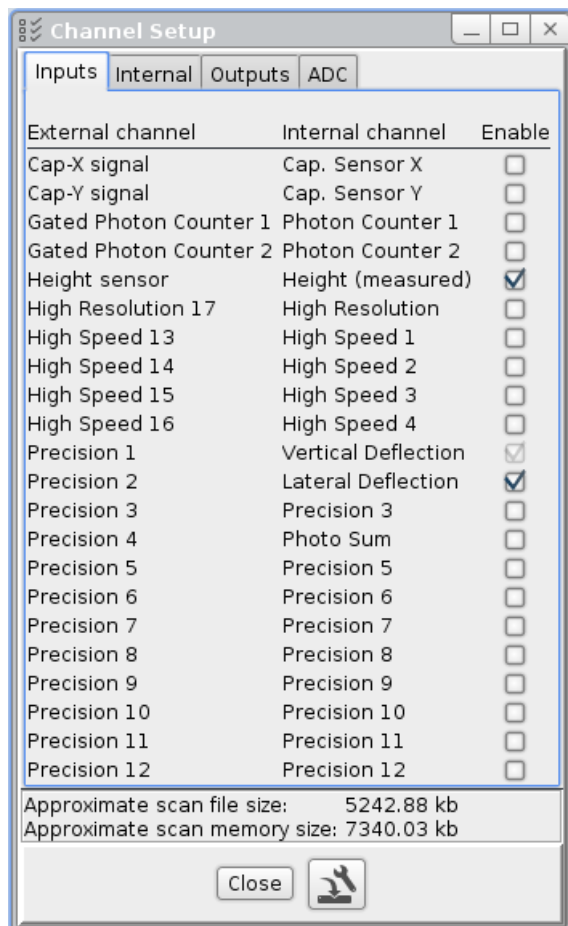
Enable the desired channels to make them available for data display (e.g. Data Viewer, **Section 3.2.4** or Real Time Oscilloscope **Section 9.2**) and saving (see next **Section 3.2.8**).

Open the Saving Settings (**Section 3.2.8**) to manage data saving.

External input channels from other devices or accessories connected to the **Signal Access Module** (see **Section 11.1.1**) must also be activated to make them available for data display and saving.

3.2.8   Saving Settings

The **Saving Settings** window is used to define the default file locations, filenames and text comments for data saving. The settings here also control the default list of data channels that will be saved in the different measurement modes. There is a preselection of standard channels, which are usually sufficient for the majority of experiments.

Open the **Saving Settings** via the **Setup** drop-down menu.
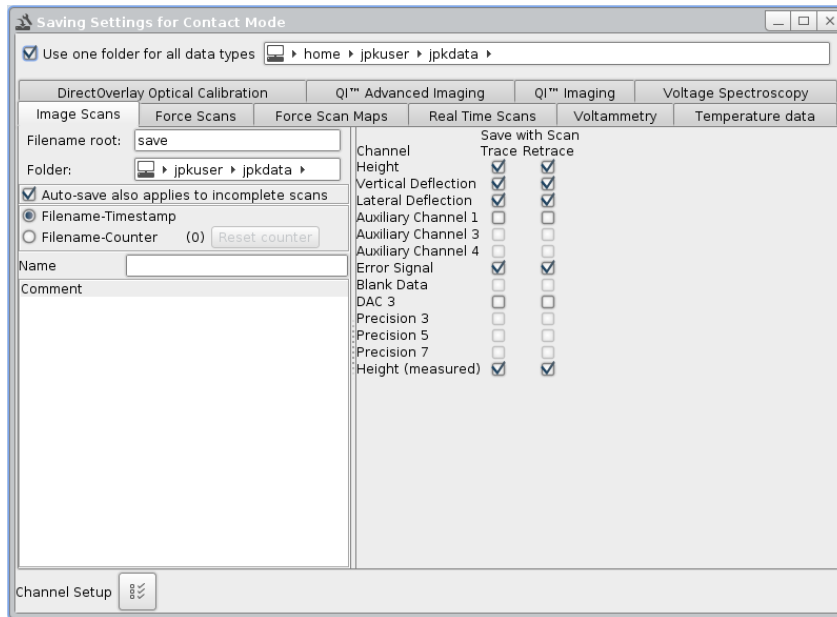
Select the channels to be saved within the register card of the corresponding measurement mode.

By default, the filename is composed of the **Filename Root** and the **Timestam**p (date and time). Type a filename root in the corresponding input field for individual data naming. Activate the **Filename Counter** to use numbering of the files instead of a timestamp and toggle **Reset Counter** to reset the counter to zero. Save additional information using the **Name** and **Comment** fields.

Type a desired **Folder** where to save all the data. The option **Use one folder for all data types** at the top is a useful shortcut to save all types of data, e.g. of each measurement mode, in one location.
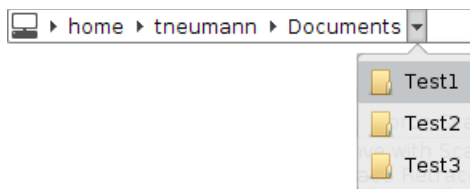
Browse the file system using the down buttons within the file path and choose the desired directory. Type a new directory by clicking directly into the file path.

Note that data channels must be activated in the **Channel Setup** (see **Section 3.2.7**) to make them available in the Saving Settings list. This also applies for external input channels from other devices or accessories connected to the **Signal Access Module** (see **Section 11.1.1**) In case of a missing channel, open the Channel Setup using the corresponding shortcut icon on the bottom of the Saving Settings window.

Autosave **ON**

Autosave **OFF**

Activate **Autosave** in the shortcut icon toolbar (see **Section 3.1.4**) to save all data by default. Enable the **Auto-save also applies to incomplete scans** tick box in the Saving Settings window to apply Autosave to incomplete scans as well. If this option is disabled, Autosaves only apply to completed scans. Alternatively, complete as well as incomplete scans can be saved manually using the Image Record List (see **Section 5.1.5**) for imaging modes and force mapping or the Force Scan Series List for Force Spectroscoy mode (see **Section 6.6.2**).

Not that the Autosave option for incomplete scans as well as the file naming and saving as well as additional information like Comment and Name must be defined for each measurement mode separately within the corresponding register cards.

3.3   Software versions and updates

The **SPM** control and **Data Processing** (DP) software are installed on an Ubuntu operating system based on Linux. The intuitive graphical user interface allows operating also by non-Linux specialists. Find some basic information about Ubuntu in **Section § 10** .

The **SPM** and **DP** software is developed by JPK. In case of any trouble or issues, please contact JPK for assistance (support@jpk.com / +49 30 726243 500).

The SPM and DP software undergo continuous development and regular updates (**releases**) are provided on the JPK customer website http://customers.jpk.com. Customers are informed of major new releases by email; in between there may be minor releases for particular features, which can be downloaded at any time. Please regularly check the customer website for new updates for download and installation.

The customer website is password-protected; please enter the login name and password. The login-name is your device number, e.g. *JPK00111*, which you can find on the instrument. The password is provided within a dedicated envelope with the AFM system at installation.

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. In brief, log-in with the administrator account **jpkroot** and copy the downloaded update installer to a local directory, e.g. to the Desktop. Run the installer from the Linux console using the following command line:

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

The full file path for the installation 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 if you have problems downloading or installing the update, please contact JPK for assistance (support@jpk.com / +49 30 726243 500).

3.3.1   SPM software versions

Open the **About** window using the **Help** drop-down menu to find information on the software version installed on your computer. Please note this number if you contact JPK for assistance.

The version number is located on the bottom left corner. Available Extensions are listed on the right hand side. This defines which options will be available in the software – controls will be enabled depending which modules are installed.

If there is any trouble with the software it helps to have as much information as possible. 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 when the software was last started, e.g. spm-2016.03.29-11.16.43.log. It will help us to respond quickly to your requests if you send us the file as an email attachment.

In case of any trouble or issues, please contact JPK for assistance (support@jpk.com / +49 30 726243 500).

§ 4   Setting up and approaching

This chapter describes the basic steps to prepare an AFM measurement. This covers the interplay with optical devices, mounting of the cantilever and set-up of the optical detection system, sample mounting as well as the final **Approach** to the sample surface.

4.1   Optical devices

Most NanoWizard® AFM systems are equipped with optical devices (e.g. inverted optical microscope or the JPK top view optics™) with a firewire camera mounted. The optics and camera help to align the laser spot position on the cantilever (see **Section 4.2.4**) and to find interesting scan regions on the sample for AFM analysis. The optional ⊕ DirectOverlay™ software module allows importing of optical images into the SPM software and to select a scan region directly within the optical image (see **Section 8.2**). The simultaneous use of advanced optical microscopy techniques (e.g. optical contrast techniques or fluorescence microscopy) provides complementary data for comprehensive sample analysis.

4.1.1   The CCD camera - JUnicam

The SPM software uses the **JUnicam** software to control camera devices. JPK supports several camera models from different manufacturers. Please contact JPK for more information on camera support (support@jpk.com / +49 30 726243 500).

 Make sure that JUnicam is closed. Connect the camera to the AFM computer, preferably to a port labelled **Video**. Start the JUnicam software using the camera icon at the shortcut icon toolbar.

Detailed information about JUnicam and software support for different camera families are given in a separate manual (**JPK Software Integration for Cameras**).

 Firewire is not a plug-and-play connection. Voltage pulses may destroy firewire cameras if plugged/unplugged during JUnicam operation. Close the JUnicam software before plugging/unplugging firewire cameras.

4.1.2   Optical imaging hardware - 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 can be adjusted by turning the wheel just below the steel ring that holds the camera.
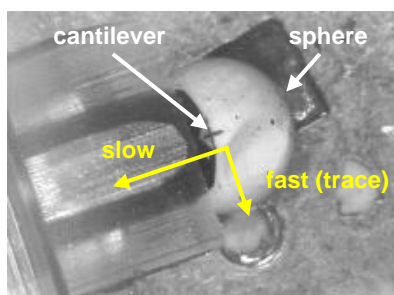
This is a CCD camera image (low magnification) of the cantilever and the sample taken with the AFM NanoWizard® in the standard version. In this case, the view of the sample and cantilever is *from above*.

The image shows the sample (a polymer sphere) and the approached cantilever from above. During the scanning process the cantilever moves as indicated (when the scan angle is set to the default).

4.1.3   Optical imaging hardware - inverted optical microscopes

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.*

Microscope alignment for Köhler illumination

Modern optical microscopes are equipped for Köhler illumination. This is important to achieve optimal contrast for transmission light techniques such as optical phase contrast or differential interference contrast (DIC). There are many websites with helpful tutorials and information on basic concepts such as Köhler illumination, phase contrast and DIC:

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

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

For inverted optical microscopes, the illumination light comes from above and passes through the AFM head before it reaches the sample. The optics within the AFM head influence the light path. Re-align the Köhler illumination upon mounting the AFM head.

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.

4.2   Set up the cantilever and optical detection system

4.2.1   Cantilever holder

The cantilever probe is fixed to a cantilever holder, which is locked into the AFM head for scanning. This cantilever holder consists of a transparent glass body and a holding mechanism to stably fix the cantilever. The top and bottom surfaces of the cantilever holder are polished to allow for transmission of the laser beam of the laser detection system to measure the cantilever deflection. The high transparency of the cantilever holder also enables the simultaneous use of transmission illumination for the application of contrast enhancing light microscopy techniques. The head is located between the top illumination of an inverted optical microscope and the sample, i.e. the light passes through the AFM head before it reaches the sample.

Cantilever holder with polished surfaces making it highly transparent. The cantilever is positioned directly over the polished surface to make it accessible to the laser detection system. The support area for the cantilever chip is tilted at 10 degrees to ensure that the tip at the end of the cantilever reaches the surface first.

The optical glass of the cantilever holder is resistant against most chemicals, but may be easily scratched. For cleaning advice see **Section 2.3.1.**

There are various shapes of the cantilever holder, but the base part that fits in the AFM head is identical in all cases. The alignment of the cantilever is also the same, since the central optical path is identical.

	Fixed spring cantilever holder.	The extra-long cantilever holder with angled faces and fixed cantilever spring is compatible with all JPK sample holders. Depending on the mounted cantilever, the spring implements an electrical tip connection.
	Straight-sided cantilever holder	The cantilever holder 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.
	Super-cut cantilever holder	The cantilever holder with the angled end provides more space for easier handling in enclosed liquid cells.
	Super-cut extended cantilever holder.	The extra-long cantilever holder with angled faces is designed for the use with the JPK PetriDishHeater™. This cantilever holder is compatible with all JPK sample holders.
	Side-view cantilever holder	This cantilever holder houses a mirror at a 45° angle, which allows for a side-view of the tip-sample interaction.
	HyperDrive™ cantilever holder	This cantilever holder houses a piezo ceramic incorporated into a PEEK composite. The cantilever can be driven directly using the optional ⊕JPK HyperDrive™ mode.
	Fast Scanner cantilever holder.	This cantilever holder houses a z-scanner incorporated into a PEEK composite for fast scanning or advanced dynamic modes like ⊕DirectDrive™ or ⊕HyperDrive™ mode.

4.2.2 Mounting the cantilever

Use the cantilever changing stand to mount or remove the cantilever chip from the cantilever holder. This tool secures the cantilever holder and helps to avoid damaging the cantilever holder when changing the cantilever. Additionally, the changing stand corrects for the tilt of the chip holding part of the cantilever holder to facilitate cantilever mounting.

Put the cantilever holder into the cantilever changing stand. The notches in the cantilever holder must be lined up with the metal tabs on the changing stand.

Turn the cantilever holder by 90 degrees into position ensuring that the clip side of the holder is nearest the highest part of the sloped tool. Turn the two finger grips on the rim of the steel disk anti-clockwise in order to lock the cantilever holder in place. This is basically the same mechanism as used to Mount the cantilever holder into the AFM head.

Mount the cantilever chip and clamp it to the cantilever holder as described below.

If the cantilever is mounted/unmounted to/from the cantilever without using the cantilever changing stand, the cantilever holder may slip sideways or fall down and break. Always use the cantilever changing stand to mount/unmount the cantilever.

Mounting the cantilever to the fixed spring cantilever holder

A

Cantilever
loose

C

Cantilever
fixed

B

untighten
phillips screw

tighten
phillips screw

Working with the **Fixed spring cantilever holder** a Phillips screw driver must be used to adjust the fixed spring by turning the Phillips screw (B). Turning the screw clockwise will tighten the clip onto the cantilever (C) and counterclockwise will release the cantilever (A).

S+

S-

S+   S-
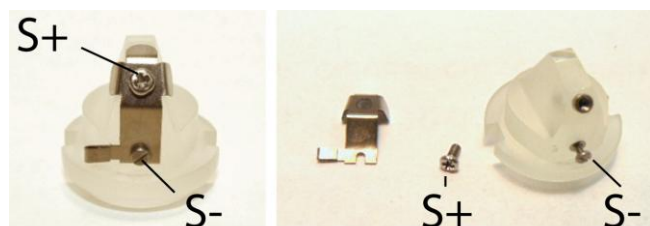
The fixed spring can be unmounted for replacement or cleaning by unscrewing the Phillips screw (S+) at the top and the slotted screw (S-) at the base of the cantilever holder.

Do not touch the optical surfaces with the screw driver or tweezers! This may scratch the optical surface and will reduce the transparency of the cantilever holder.
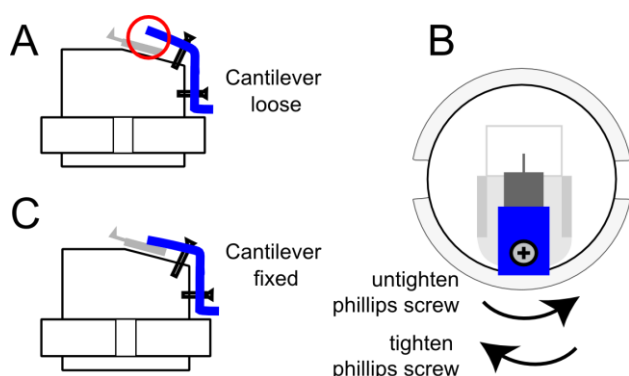
The use of inappropriate screw drivers may damage the Phillips screw or lead to slipping off with the screw driver and thus damaging the cantilever holder. Use only the supplied Phillips screw driver or equivalent! If in doubt please check with JPK

Mounting the cantilever to the cantilever holder using a loose spring
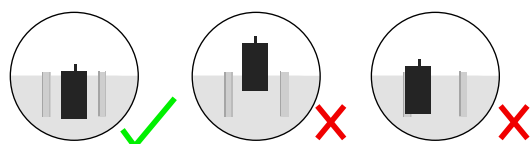
Place the cantilever chip onto the inclined part of the cantilever holder. The cantilever chip should be placed centrally between the two alignment marks (B), with the cantilever arm over the polished part. The cantilever itself should be in the center of the cantilever holder; do not move the cantilever substrate chip too far forwards.

A

Cantilever

Spring
groove

C

Cantilever
spring

B

Alignment marks
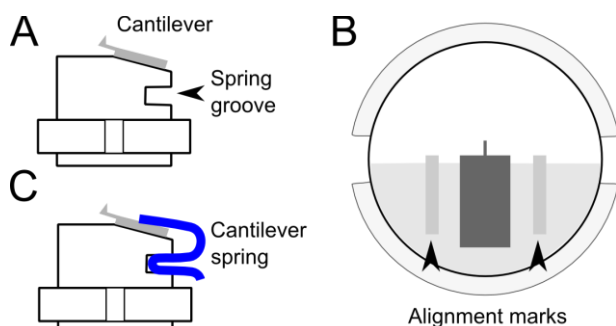
When the cantilever is in the right position (A, B), grip the bottom loops of the spring firmly with a pair of tweezers and squeeze to lift the front part. Slide the spring into the groove and release the loops to clamp the cantilever firmly (C). The position of the cantilever can be carefully adjusted using tweezers once the spring is in place, taking care not to allow the tweezers to contact the polished part of the cantilever holder.

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

Do not touch the optical surfaces with the screw driver or tweezers! This may scratch the optical surface and will reduce the transparency of the cantilever holder.

Mount the cantilever holder to the AFM head

Put the AFM head in the upright position to mount/unmount the cantilever holder.
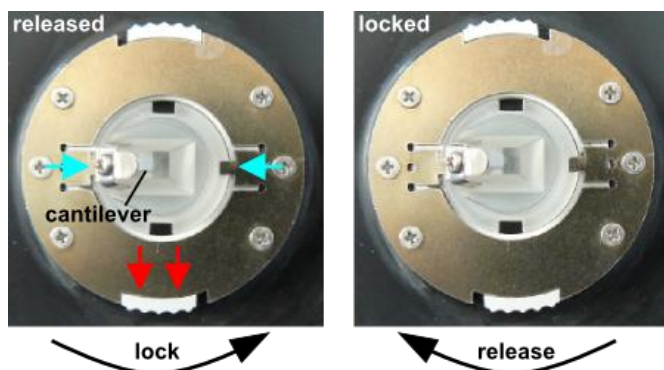
The cantilever holder is held in the AFM head with a clamp mechanism. Line up the notches in the canti-lever holder with the metal tabs on the AFM head to place the cantilever holder properly (blue arrows). Turn the cantilever holder by 90 degrees into posi-tion; the cantilever spring is on the left hand side. Lock the cantilever holder with the two finger grips on the rim of the steel disk (red arrows); a faint click should be felt as the spring locks.

In the mounted position, the cantilever chip must be positioned parallel to the desk, with the spring on the left hand side.

The cantilever holder may fall off the AFM head and break if the clamp mechanism is not locked properly. Make sure that the clamp mechanism is locked before putting the AFM head in place for scanning opera-tion.

4.2.3   Setting up the laser detection system

As the tip of the cantilever is scanned across the sample its deflection is detected by a laser beam that is focused onto and reflected by the cantilever. As the cantilever interacts with the sample, the reflection angle changes, which is de-tected by a four-segment photodiode. At zero deflection, the reflected laser spot must be in the center 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 cantilever holder, since the can-tilever will always be in a slightly different position.

Open the SPM software and click the icon on the shortcut icon toolbar to open the **Laser Alignment** win-dow.
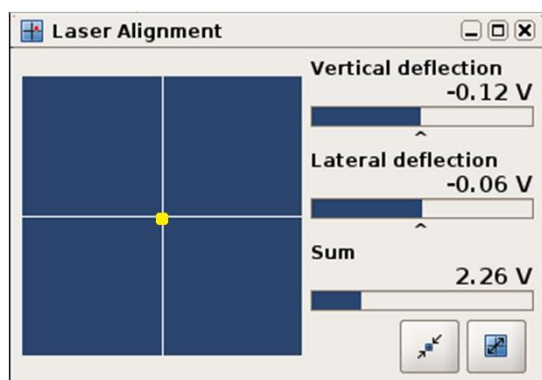
The **Laser Alignment** window gives a graphical representation of the detector signals for the adjustment process. The **Sum** value is the total signal from all four quadrants of the detector. If the laser beam does not fall onto the detector, the **Sum** will be 0 V and no yellow spot is displayed.

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

The shortcut icons allow the user to change 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.

The laser can be switched on and off manually. When the head is placed in the horizontal position ready for scanning, the laser is switched on by default.

Make sure that the laser is switched on before starting the alignment procedure. If the laser is switched on in the software, but the red diode in front of the AFM head is off, the laser safety tilt switch is activated. The laser will switch on when the head is placed in the horizontal position.

4.2.4 Adjust the laser beam onto the end of the cantilever

Use the optical microscope or the JPK TopViewOptics™ to visualize the cantilever with the CCD camera. The IR laser spot cannot 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. Decrease the illumination to find the laser spot as this makes scattered light far from the focus visible.

If the laser spot cannot be seen in the optical microscope, check for any safety or fluorescence filters in the optical path that may be cutting IR wavelengths.

Optical path schematic   AFM head adjustment   Optical microscope image
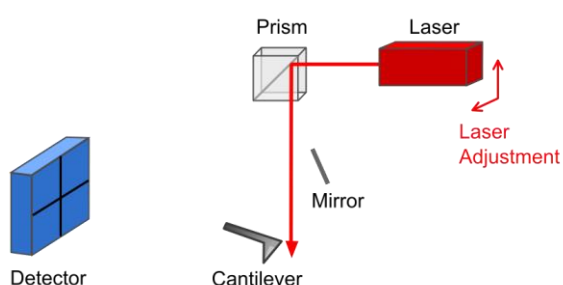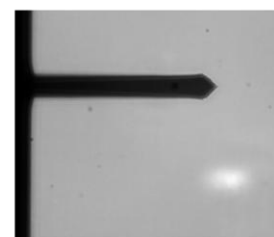
The laser position must be moved using the adjustment screws until the beam is directed onto the cantilever.

All four positioning screws have end stops in each direction. Do not over-wind the screws. This may destroy the positioning mechanism.

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.

4.2.5   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 center 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 upon proper alignment of the laser spot onto the cantilever. Otherwise the laser beam will not be reflected and further alignment is impossible.

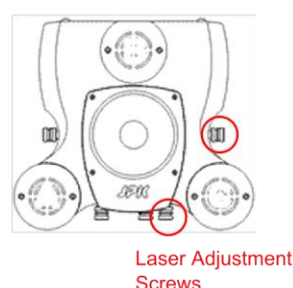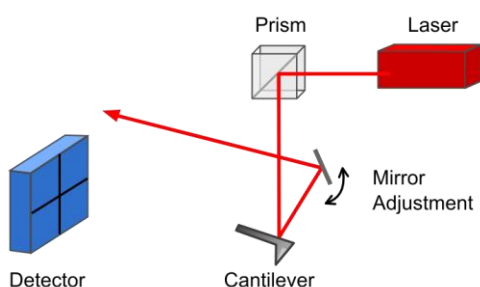If the laser spot is properly aligned on the cantilever, but the **Sum** is close to 0 V, the mirror needs adjusting. In order to minimize drift, there is a release mechanism that holds the positioning screw out of contact with the mirror when not in use. Push carefully on the finger grip and turn gently to make contact. It is easy to feel when the mechanism slots into place and the mirror can be turned. Only small movements are necessary, as small changes in the mirror angle result in relatively large changes in the optical path. Adjust the mirror until the **Sum** shows the maximum value achievable.

The maximum Sum value depends on the type of cantilever – both the shape and whether there is any metal coating on the back. Typically, values vary between around 1 V for non-coated silicon cantilevers and around 5 V for gold-coated silicon nitride cantilevers.

Setting up in liquid

When the bottom face of the cantilever holder and the cantilever are immersed in liquid the optical path of the laser beam reflected from the tip is changed due to the difference in refractive index between air and liquid. The mirror helps to correct for the difference in angle of the optical path.

The mirror will not always need readjustment every time a new measurement is made. Adjustments need to be made when changing from air to liquid operation or back again.

If 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.

The vertical deflection signal may drift continuously upon setting up in liquid. This is due to thermal effects, which result in bending of the cantilever. The drifting will reduce as the system reaches equilibrium. Wait until the drifting effect is reduced to a minimum before starting the measurement.

4.2.6 Adjust the spot onto the center of the detector

When the laser spot position and, if necessary, the mirror angle have been adjusted, the photo detector must be moved until the laser beam falls on the center.
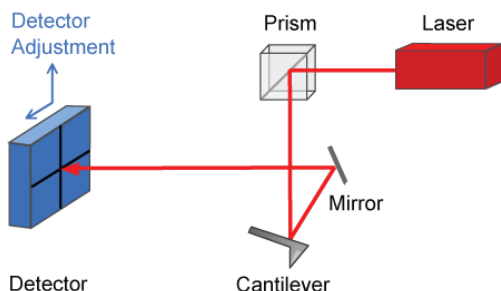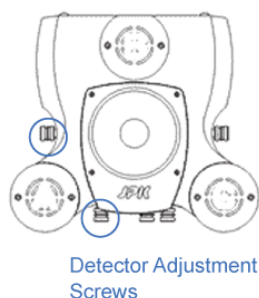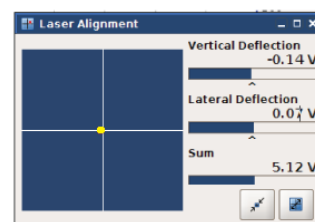
The **Laser Alignment** window in the SPM software shows the signal from the different quadrants of the detector graphically. Adjust the detector adjustment screws so that the spot is in the center of the detector in the **Laser Alignment** window. This means that equal intensity is reaching all four quadrants. Note that the spot in the blue region shows the center position of the laser beam relative to the four quadrants, not the representative size!
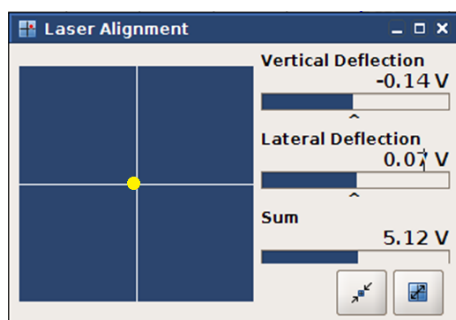
This is what the **Laser Alignment** should look like after successful adjustment:

1. **Sum** is at the maximum value for that cantilever.

2. The yellow spot is in the center of the photodiode.

3. **Vertical Def** and **Lateral Def** are close to zero.

4.2.7 Troubleshooting alignment problems

Not able to align the laser onto cantilever
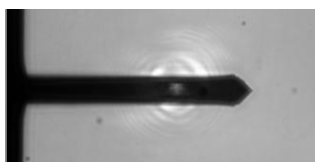
First check that the laser beam is correctly aligned on the cantilever as in the image here. If it is not possible to move the laser onto the cantilever, then probably the position of the cantilever or cantilever holder is wrong.
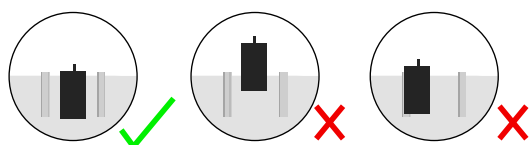
Check the cantilever position on the cantilever holder: it should be placed centrally between the two grooves, with the cantilever arm over the polished glass part, close to the inclined edge. If the cantilever is positioned too far forwards or to one side, this may cause alignment problems. Try repositioning of the cantilever.

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.

Not able to align the 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 values, 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.

If this procedure does not work for **Lateral deflection**, then the cantilever is probably damaged, or either it or the cantilever holder have been inserted at an angle. 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 in **Section 4.2.5.**

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 cantilever holder – this can also interfere with the optical path. Focus on the cantilever holder 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.

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 adjusting the laser again to the center of the detector.

There may also be problems in liquid, as the deflection of the cantilever is sensitive to many environmental effects. Air bubbles may stick to the cantilever and cause the vertical deflection to jump. Check the cantilever optically and try to remove the bubbles by lifting up the head. The resulting liquid-to-air-transition of the cantilever may help to tear off any bubbles. If this does not succeed, use lint-free tissue to remove the liquid from the cantilever. To prevent the formation of air bubbles pre-wet the cantilever with a drop of liquid using a pipette, when the cantilever is mounted in the AFM head in the upright position.

Aluminum-coated silicon cantilevers are not recommended for use in any liquid. The coating is used to increase the reflected laser signal and is useful 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. It is important to check the product codes when ordering silicon cantilevers. 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 aluminum 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 show 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 AC mode the amplitude is not as directly sensitive to these changes as the deflection in contact mode.

4.3 Mounting the sample

In AFM microscopy, it is crucial to avoid unwanted movements 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.

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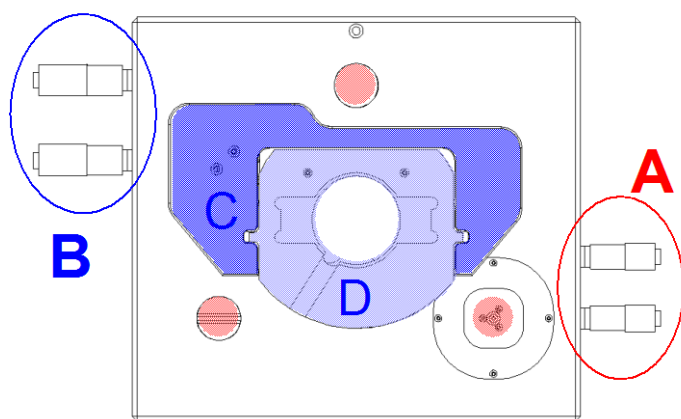
The positioning screws A move the AFM head. The three foot positions are marked in red. The combination of a point, a line and a flat surface ensures reliable positioning, so that the head will always fit stably into the same position. Always ensure that the feet slot firmly into position.

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 center part. The gap is shown here exaggerated, to visualize how the sample is pushed by the positioning arms.
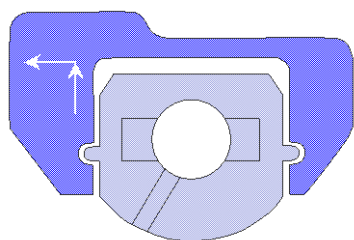
When the sample is in the correct position, the sample holder must be released by turning the positioning screws BACK one quarter turn. The samp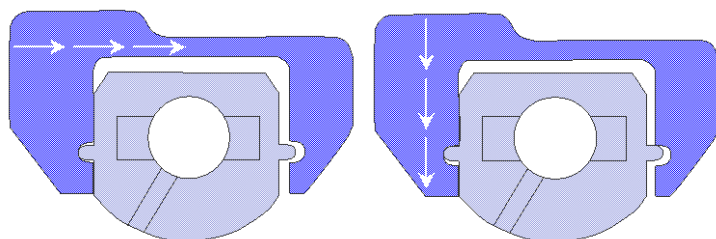le will not move during this **de-coupling**. The de-coupling releases the sample holder from the positioning arms, but it is still held firmly by the magnetic contacts underneath.

The release mechanism is required to remove the mechanical coupling between the sample and sample holder. This is very important to reduce mechanical noise to a minimum and to optimize the performance for high resolution imaging.

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.

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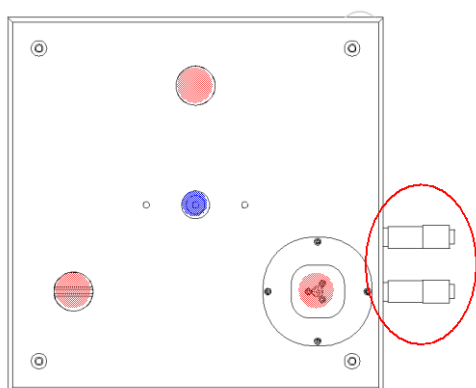
The positioning screws A move the AFM head. The three foot positions are marked in red, and are similar to the Life Science stage. The point, a line and flat surface ensures reliable positioning, so that the head will always fit stably into the same position. Always ensure that the feet slot firmly into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

4.4   Selection of feedback mode

When the sample is mounted and the cantilever aligned, the cantilever has to be **approached** to the sample until contacting with a defined **setpoint**. There are different feedback modes which differ in what it is about the cantilever that is being controlled (typ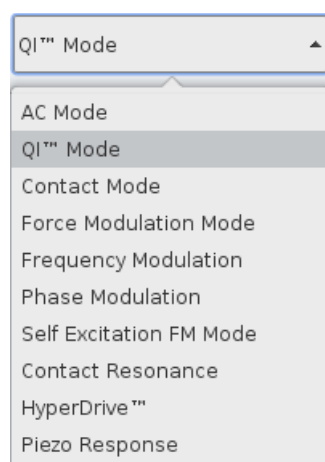ically its deflection or amplitude). Prior to approaching, the desired feedback-mode for imaging must be selected. Some of the listed feedback modes are optional and would need to be purchased separately. Please read the corresponding manual for detailed description.

There are several options for the NanoWizard® feedback modes, which are either based on the vertical deflection, such as **Contact Mode** (**Section 4.4.1**) or **QI™ Mode** (**Section 5.7**), or which are based on the resonance properties of the oscillating cantilever, such as **AC Mode** (**Section 4.4.1**), or **Phase Modulation** mode.

Select the desired feedback mode from the feedback drop-down list.

The feedback mode defines which signal is used for the main control of the Z position during imaging or other types of measurement. The aim is usually to control the force, or keep it constant during imaging for example. The simplest type of feedback mode is **Contact Mode**, where the direct deflection of the cantilever is used as the feedback signal. In this case, the **Vertical Deflection** value is used as the feedback channel. In modes using the vertical deflection as setpoint, like **QI™ Mode** or **Contact Mode**, no additional parameter adjustment is necessary for approaching.

The other main group of feedback modes uses some kind of cantilever oscillation, where the resonance properties of the cantilever are used for the feedback signal. The feedback channel could for instance be **amplitude, phase, frequency**, or some combination of different feedback loops can be used. For the NanoWizard®, the simplest version of this type of mode is called **AC Mode**. For approaching the cantilever in oscillation-based modes, the cantilever resonance must be found and the parameters for driving the cantilever oscillation and the setpoint values must be determined. This procedure is referred to as **Cantilever Tuning**.

4.4.1 QI™ Mode

In QI™ mode, a force curve with a defined setpoint force is acquired at each pixel of the scan region, and the piezo height at 80 % of the setpoint force is determined. Please read **Section 6.2** for basic information on force spectroscopy.
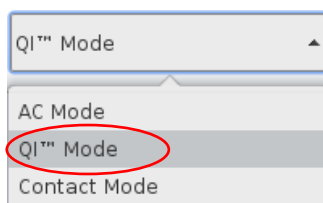
Choose **QI™ Mode** from the feedback-mode list in the shortcut icon toolbar to approach and perform experiments in QI™ mode.

The **QI™ Control** panel appears on the left hand side and the **QI™ Setup** will open.

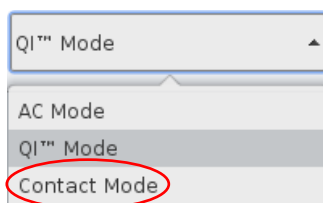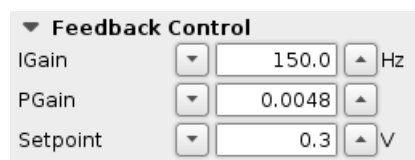The **Setpoint** for imaging can directly be typed into the corresponding input field in the **QI™ Control** panel. Please read **Section 5.7** for detailed information on how to perform the **QI™ Setup** and imaging in QI™ mode.

4.4.2 Contact Mode

In contact mode the direct vertical deflection of the cantilever is used as the feedback signal. An imaging setpoint (defined vertical deflection) is set, and the feedback-loop adjusts the piezo height to keep this setpoint deflection constant.

Choose **Contact Mode** from the feedback-mode list in the shortcut icon toolbar to approach and perform experiments in contact mode.

The **Feedback Control** panel appears on the left hand side. The **Setpoint** for imaging, which is also used for the approach, can directly be typed into the corresponding input field, as well as the imaging gains. Please read **Section 5.2.1** for detailed information on how to optimize imaging gains.

4.4.3 Cantilever Tuning - AC Mode

In AC mode the cantilever is oscillated with defined amplitude close to its resonance frequency. In basic AC mode, a setpoint amplitude is determined, and the feedback-loop adjusts the piezo height to keep the setpoint amplitude constant.

Choose an appropriate cantilever. Cantilevers for AC mode in air are rather stiff (> 30 N/m) with high resonance frequencies (~ 200-400 kHz). AC mode in liquid requires laterally stable cantilevers with relatively high resonance frequencies. But at the same time they often need to be reasonably soft not to damage delicate samples in liquid. Most cantilever manufacturers list their cantilevers by feedback mode, imaging condition and application to facilitate finding an appropriate cantilever. Find more information on AC mode and cantilever choice in the JPK AFM Handbook.

Choose **AC Mode** from the feedback-mode list in the shortcut icon toolbar to approach and perform experiments in AC mode.

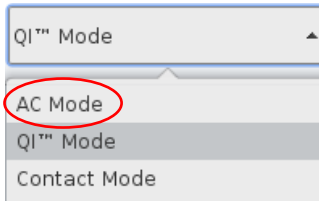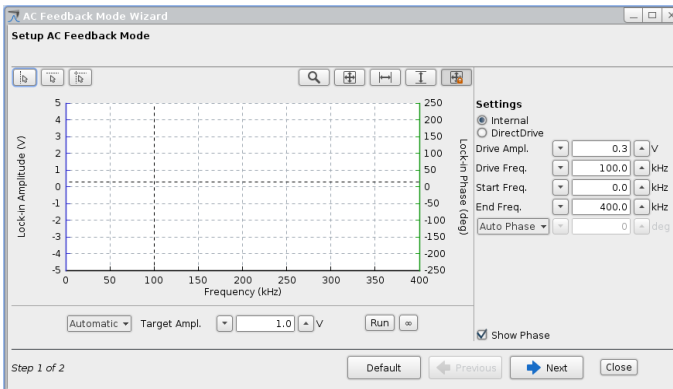The **AC Feedback Mode Wizard** will open. It leads through the cantilever tuning, i.e. to find the cantilever resonance and selecting an appropriate driving amplitude and frequency.

In AC feedback mode, the tuning window can always be opened manually to readjust the cantilever tuning using the Tuning button at the shortcut icon toolbar.

The default range for **Start Frequency** and **End Frequency** in AC mode is 0 - 400kHz (max. freq. 6 MHz on request). The input fields on the right part of the window control the display of the frequency range (**Start and End Freq.**) as well as the selection of the **Drive Amplitude**, **Drive Frequency** and **Phase** shift. Alternatively the frequency range can be adjusted by using the **mouse wheel**.

The cantilever tuning can be performed automatically or manually.

Some cantilever holders allow the use of **Direct Drive**, i.e. the oscillation is directly applied to the cantilever base. Contact JPK for detailed information (support@jpk.com, +49 30 726243 500).

Automatic cantilever tuning
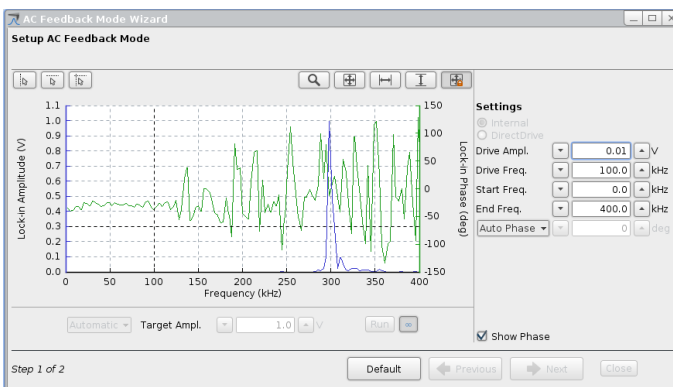
For Automatic cantilever tuning, the cantilever Driving Amplitude and gains are adjusted by the software in order to achieve the Target Amplitude that is set.

The Target Amplitude is set to 1 V by default, which is appropriate for most measurements. Adjust the value for the Target Amplitude if necessary.

Blue curve: Frequency. Green curve: Phase

Toggle the Run or Infinite icon to run a frequency sweep. **Run** records one frequency sweep, the **Infinite** icon performs continuous frequency sweeping.
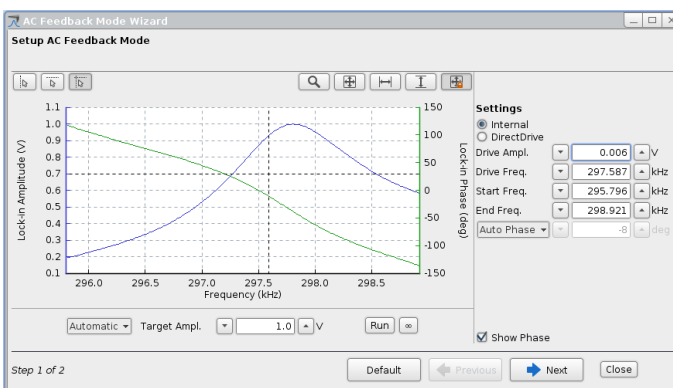
Use the mouse wheel in order to zoom into the resonance peak.

Auto Phase correction is enabled by default. This setting automatically shifts the phase inflection point (at 0 degrees) to the drive frequency, which is generally recommended to achieve optimal phase contrast. The Auto-Phase correction is calculated during frequency sweeping. Select **Fixed Offset** instead of Auto Phase to shift the phase manually.

Select the **Drive Frequency** and **Setpoint** using the corresponding shortcut icons in the upper left of the tuning window.
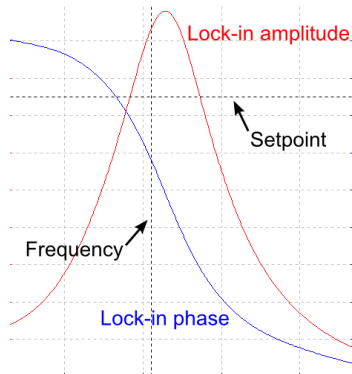
Click inside the amplitude/frequency plot area to get a cross hair of black dotted lines. Holding the mouse button down, move the cross hair to choose a suitable **Drive Frequency** and **Setpoint**, as in the example shown here.

The values for the **Drive Frequency** and **Setpoint Amplitude** depend on the sample and environment. The Drive Frequency is usually chosen on the left upper rising edge of the peak. The value of the Setpoint Amplitude must be lower than the chosen Target Amplitude (Lock-in amplitude). The Setpoint Amplitude is usually chosen around 70 – 80 % of the Target Amplitude at that frequency.

The free amplitude (Target Amplitude) is damped as the cantilever starts to contact the sample surface. The z piezo position is adjusted in order to reach and maintain the Setpoint Amplitude, which basically reflects a particular amount of damping. This means: in contrast to contact mode, the lower the setpoint amplitude, the higher the damping and finally the force applied to the sample.

Finish the tuning using the **Close** icon.

Manual cantilever tuning

Manual cantilever tuning allows setting the **Drive Amplitude** and **Gains** manually. The **Drive Amplitude** is the value of the alternating voltage that is applied to the piezo which drives the cantilever oscillation. The Drive Frequency should be set as described above for automatic tuning. In fluid a higher **Drive Amplitude** is required because of the increased damping of the oscillation.
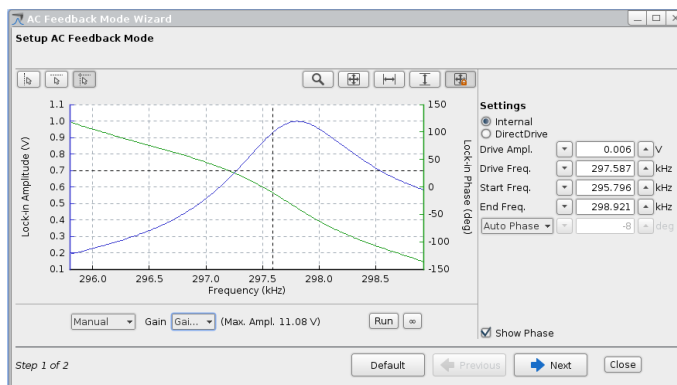
Enter a **Drive Amplitude** value.

Select the **Gain** for the expected range of amplitude:

Gain 1: lock-in amplitudes (**Max. Ampl.**) up to 11 V,

Gain 4: lock-in amplitudes (**Max. Ampl.**) up to 200 mV.

The values can be adjusted during continuous sweeping.

Select the **Drive Frequency** and **Setpoint** as described above for the automatic cantilever tuning.

Hints and tips

Variation of the drive amplitude

Tip-sample interactions can have a strong effect on the imaging conditions. If the imaging behavior and quality is not satisfactory although a good signal-to-noise ratio has been achieved, a higher drive amplitude (manual cantilever tuning) or target amplitude (automatic cantilever tuning) can help to improve imaging, as tip-sample interactions can be overcome (e.g. sticky samples).

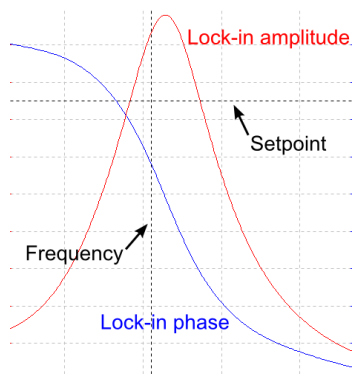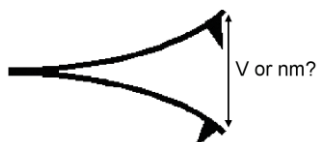The force applied to the sample depends on the relationship between the setpoint and the drive amplitude/target amplitude. If the setpoint is not changed, then the force applied to the sample is increased when the drive amplitude is increased. Therefore it is recommended to increase the setpoint manually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

Additional hint: In the cantilever tuning window the magnitude of the lock-in amplitude (Target Amplitude) is displayed in Volts. The cantilever must be calibrated to get the amplitude displayed in nanometers. See **Section 7.2** for details about sensitivity calibration.

Signal-to-noise-ratio

It is important to have a good signal-to-noise ratio, which 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 (manual cantilever tuning) or the Target Amplitude (automatic cantilever tuning) carefully. Try to keep the peak of the resonance curve below 2 V of amplitude.
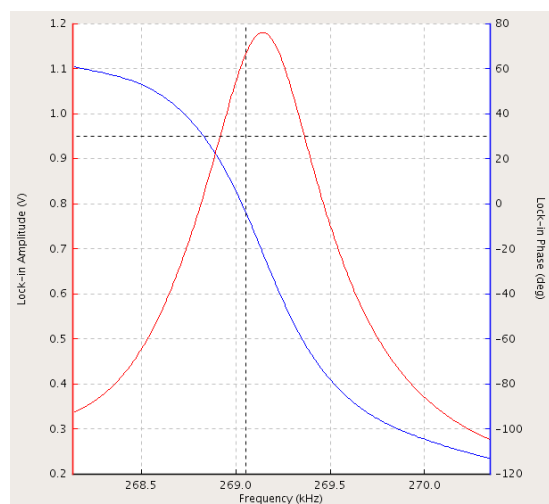
Possible reasons for badly shaped resonance curves:

- Contamination of the tip with adsorbed dirt

- Cantilever or tip are physically damaged

- Chip of the cantilever not tightly fixed to the cantilever holder

- The tip is making contact with the sample during the cantilever tuning

4.4.4 AC Mode in liquid

AC mode in liquid works basically the same way as in air. The main difference is the increased damping due to the high viscosity of the liquid and the requirements of the sample, which are often rather soft and delicate in liquid environment.

Choose an appropriate cantilever. AC mode in liquid requires laterally stable cantilevers with relatively high resonance frequencies. But they also need to be reasonably soft not to damage delicate samples in liquid. Most cantilever manufacturers list their cantilevers by feedback mode, imaging conditions and application to facilitate finding an appropriate cantilever. Find more information on AC mode and cantilever choice in the JPK AFM Handbook.

The free oscillation of the cantilever at a given Drive Amplitude is damped considerably as the cantilever is approached to the surface and a higher Drive Amplitude is needed to achieve the Target Amplitude. If the cantilever tuning was performed far away from the surface, it should be re-tuned upon approaching.

It may be difficult to approach in AC mode in liquid with extremely soft cantilevers as they are very suscepti-ble to oscillations. Choose **Contact Mode** for feedback to make a first **Approach**. Make a piezo retract (see Section 4.5.4), change to AC mode and perform the tuning procedure to image in AC mode.

4.4.5   Force Modulation Mode

Force Modulation Mode is a mixture between Contact mode and AC mode and can be thought of as a kind of Contact mode with an added vibration of the cantilever. The cantilever is in continuous contact with the sample while oscillating.
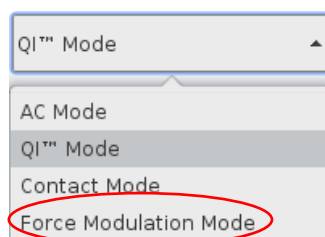
Choose **Force Modulation Mode** from the Feedback Mode drop-down menu.

The cantilever tuning for Force Modulation Mode is similar to the normal tuning procedure for AC mode. 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.

Find a closer description of the Force Modulation Mode in **Section 5.8**.

4.5   Approaching

4.5.1   Coarse approach

The whole NanoWizard® AFM head can be raised or lowered using the three stepper motors, which allows a wide range of sample heights to be measured. The automatic approach routine can 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 fast correction is generally known as the coarse approach, since there is no feedback on the cantilever signal. This can also be useful to move the cantilever into the range of the optical microscope objective in order to see the cantilever and laser spot for alignment.

Open the **Stepper Motor** window using the motor icon in the shortcut icon toolbar.
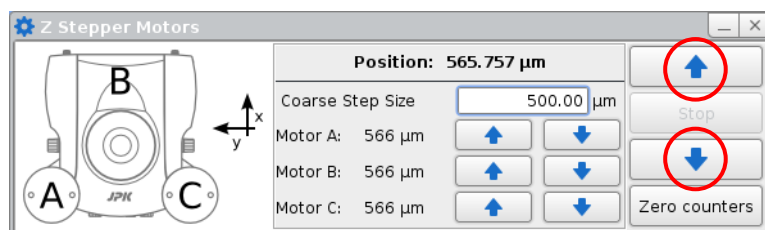
Set the **Coarse Step Size** in the **Stepper Motor** window. Toggle the **Up** arrow to move the head and cantilever away from the sam-ple, and the **Down** arrow to move the head and cantilever towards the sample.

There is no feedback on the cantilever deflection during the stepper motor movement. Moving large distances without feedback may result in crashing the cantilever holder and cantilever into the sample. Always control the cantilever-sample distance optically. Use small step sizes only.

The motors can also be moved independently (see **Section 5.3** for details), to remove any overall tilt between the sample and the AFM head.

4.5.2   Automatic approach

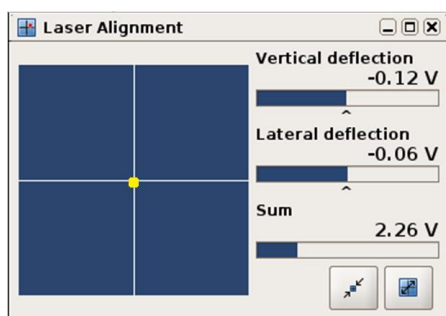During the automatic approach the cantilever is moved towards the sample surface using the vertical deflection signal as feedback. The z piezo and stepper motors are moved alternately until the cantilever deflects to the setpoint value, which reflects the surface position. When the surface is reached, the system is ready for scanning.

Before starting the approach, make sure that the laser is properly aligned on the cantilever and photodetector, as described in **Section 4.2.** The **Lateral** and **Vertical Deflection** should be near 0V.

Select the desired feedback and imaging mode using the corresponding drop-down boxes at the shortcut icon toolbar.
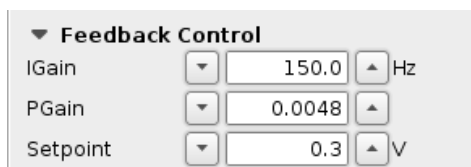
Set a suitable **Setpoint** value in the **Feedback Control** panel. In oscillation based modes (e.g. **AC mode**), the setpoint is chosen during cantilever tuning (**Section 4.4.1**). In **Contact mode**, the setpoint value should be chosen depending on the spring constant of the cantilever. The preset value is normally reasonable for soft cantilevers. In QI™ mode, the setpoint used for the automatic approach is coupled to the imaging setpoint, which can be set in the Feedback Control panel. The coupling of the approach setpoint can be edited or disabled in the **Advanced Feedback Settings** (**Section 5.7.4**). Normally, the preset coupling factor and therewith the approach setpoint is set to a reasonable value and doesn't need to be changed.

In oscillating modes, where the oscillation amplitude is used as feedback channel, a lower setpoint value means a higher imaging force, while in contact mode a lower setpoint value means a lower imaging force.

Click the **Approach** button in the shortcut icon toolbar to start the approach routine. You should hear the stepper motors moving the NanoWizard® head.
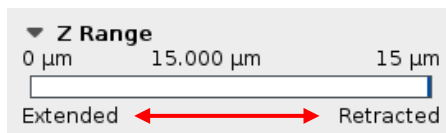
During the approach, the z piezo extension and retraction can be observed in the **Z Range** display on the left. The position of the blue line, which reflects the z piezo position, oscillates from the 0 to 15 µm position. At each step, the z piezo extends and the vertical deflection signal is checked. If there is no change in deflection, i.e. the surface is not reached, the piezo retracts and the stepper motors move the AFM head towards the sample for approximately one whole z piezo range. This procedure is repeated until the surface is detected, i.e. the setpoint is reached.

During the approach procedure, the **Approach Parameters** window is displayed. This window can also be opened from the **Setup** drop-down menu. Usually, the preset setting are reasonable for successful approach and do not need

any modifications.

There are two approach types: **Approach with feedback on** and **Approach with constant velocity**. Both approach types basically use the same approach routine; there are different ways in adjusting the approach:

Using **Approach with constant velocity**, it is possible to make very sensitive approaches to protect the cantilever. Either the **Extend Time** or the **Extend Velocity** can be set, depending on which option, **Constant Extend Time** or **Constant Extend Velocity**, is selected for **Preference**. **Constant Extend Time** updates the **Extend Velocity** when the **Extend Time** is changed, if the approach is made in **Constant Extend Velocity** mode, changing the **Extend Velocity** updates the **Extend Time**, vice versa.

The **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.

The gains must be adjusted in case that **Approach with feedback on** is selected as approach type. 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 AC 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 AC mode).

Timeout defines the time that may elapse until the z piezo has fully extended. The software aborts the approach if the setpoint has not been reached or the piezo has not fully extended after that period of time. Increase gains and/or setpoint (faster approach) or increase Timeout (slower approach) if that period of time is not sufficient for a full piezo extend.

There are two common problems approaching, which may occur in **Approach with feedback on**:

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 slower, 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 AC mode). If it is impossible to get a stable approach where the blue 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 over 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 AC mode). Furthermore the Timeout has to be increased.

The 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 (Idle mode).
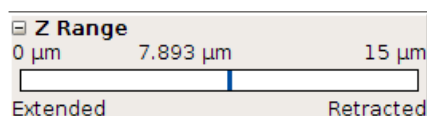
There is also an option in the Approach Parameters to approach using a variable setpoint using the **Baseline Adjust** – please see the following section.
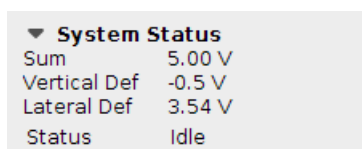
To confirm a successful approach, check the following settings:
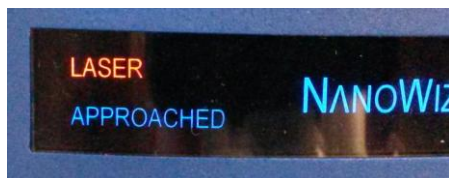
The blue **approached** LED is illuminated on the front plate of the AFM head.

The **Z Range** display shows the blue bar at the **Target Height**, which is in the center if the default approach settings are used.

The **System Status** panel at the bottom left hand corner shows the **Status** as **Idle**. This means that the cantilever is waiting at the surface and the system is ready to start a measurement.

If the System Status shows idle mode and the blue approached LED is on, but the Z Range display shows the blue bar at the end of the range (e.g. at 0 µm), or the blue line moves slowly away from the sample, the approach was not successful. Click the **Approach** button again start a new approach.

If it is not possible to achieve a successful approach, the setpoint must be adjusted (increased in contact mode, decreased in AC mode) to reach the surface properly. In AC mode, the oscillation amplitude and frequency may change close to the surface due to electrostatic interactions in air or hydrodynamic interactions (damping) in liquid. Retune the cantilever close to the surface.

4.5.3   Advanced approach using Baseline adjust

In the standard approach routine, the **Setpoint** value in the main Feedback Control window is used directly as the desired value for the adjustment of the feedback system.

The feedback signal with the free cantilever, i.e. the cantilever away from the sample is referred to as **Baseline**. During the approach or AFM operation, the Baseline may change due to thermal effects, electrostatic interactions close to the sample or any other effects. As the setpoint is a fixed value which remains constant for the whole approach, the actual force applied to the sample differs from the setpoint if the baseline changes.

Contact Mode and QI™

If the free **Vertical Deflection** (Baseline) on the photodiode is -0.52 V, and the **Setpoint** is set to 1.0 V, then the actual setpoint, which is just the difference between the baseline deflection and the final setpoint, is 1.52 V.

To correct for any changes of the baseline, the **Baseline Adjust** option is applied by default. It determines the current **Baseline** and calculates the vertical deflection that must be applied to reach the **Setpoint** value. I.e. another, relative setpoint is calculated based on the measured baseline and applied in order to reach the user defined Setpoint in the Feedback Control window.

The **Baseline Adjust** within the **Approach Parameters** window provides different options to determine and adjust the baseline.

Baseline update at start adjusts the vertical deflection baseline after each motor step, i.e. prior to each z piezo extension, to the current value of the feedback channel. The value is also updated whenever **Update** is clicked.

Dynamic baseline update detects and adjusts the vertical deflection baseline for a defined averaging time during the approach, i.e. during the z piezo extension.

If **No baseline adjust** is selected, the actually applied force corresponds to the difference of the baseline value and the Setpoint.

There is no need to adjust the **Setpoint** value in the **Feedback Parameters** manually. If one of the two **Baseline Adjust** options is active, the system automatically calculates and applies a relative setpoint to achieve the **Setpoint** value.

If **No baseline adjust** is selected, the user defined **Setpoint** value is used for the approach, no matter of the vertical deflection baseline. This may destroy your sample and/or cantilever tip if the baseline changes considerably. Always control the vertical deflection baseline and adjust the Setpoint or the position of the photodiode (see **Section 4.2.6**).

Example

If the **Vertical Deflection** on the photodiode far from the sample was -0.52 V 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.

AC Mode:

In AC mode the free amplitude is measured (**Lock-in Amplitude Baseline**) and used for baseline adjust. The **Approach Parameters** window offers the same options as for Contact mode.

When **Baseline update at start** or **Dynamic baseline update** is enabled, another **Setpoint** value, the **Relative Setpoint**, appears in the **Feedback Control** panel. **Relative Setpoint** means a defined percentage of the free amplitude, i.e. of the Lock-in Amplitude Baseline. In this example, the Relative Setpoint is selected as 70 % of the free amplitude. As the measured Lock-in Amplitude Baseline is 1 V, the absolute Setpoint is 0.7 V. If the Lock-in Amplitude was 0.8 V and the Relative Setpoint was still set to 70 %, the absolute Setpoint would be adjusted to 0,56 V automatically.

4.5.4   Retracting the tip from the sample

Toggle **Run** to start and stop most measurements. Click **Run** again during a measurement t⁰ stop the measurement at a convenient point (e.g. at the end of a scan line or on the completion of a force curve). The cantilever tip will remain approached on the surface, at the starting point of the scan area.

Toggle the **Retract** button once to retract the tip from the surface, and also to interrupt a scan as a kind of emergency stop. The measurement is stopped almost immediately, and the tip is moved to the fully retracted position.

Click **Approach** again, and the cantilever will return to exactly the same position, because there is no mechanical movement, i.e. no movement of the stepper motors. This is convenient for changing settings between scans or measurements. The actual distance from the sample depends on the z-range and the position of the sample, but it is not usually enough of a safety distance for any mechanical movement, for instance to lift up the AFM head.

Clicking the **Retract** button twice 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 motor retract, the cantilever will not return to exactly the same sample position when approached again (typically within a couple of microns). Moving the cantilever further away from the sample is convenient for adjusting the sample position (e.g. retracting 20 – 50 µm), or lifting up the AFM head (e.g. retracting 300 – 500 µm).

There are three possible cantilever states (**Status**) shown in the Status field of the **System Status** at the bottom left hand corner of the software.

- **Motor Retracted** means that the motors have been moved. The cantilever is far away from the sample.

- **Z Piezo Retracted** means the cantilever is retracted form the surface using the z-piezo. The motors have not been moved

- **Idle** means that the cantilever is approached and waiting at the surface, ready to start a measurement.
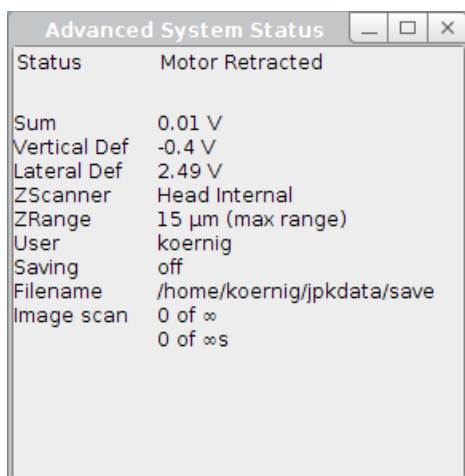
Find more information about the system status in the **Advanced System Status** window, which can be opened under the **Advanced** drop-down menu.

4.6   Starting a measurement

After successful approach the system is ready to operate in the selected **measurement mode**. There are several measurement modes available, depending on the selected feedback mode and software extension modules.

The measurement mode can be changed using the drop-down menu in the shortcut icon toolbar. **Imaging** is set by default.
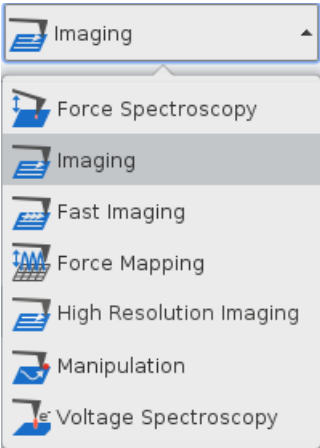
Measurement mode	Find in section:
Force Spectroscopy mode	**§ 6**
Imaging mode	**§ 5**
⊕ Fast Imaging mode	
Force Mapping mode	**6.7**
⊕ High Resolution Imaging	**8.3**
⊕ Manipulation mode	**8.7**
⊕ Voltage Spectroscopy	
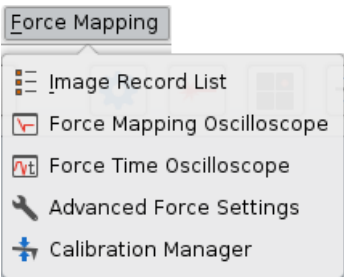
Depending on the **measurement mode**, an additional drop down menu appears in the menu bar, which provides access to the main relevant controls. This screenshot shows the **Force Mapping** drop down menu upon selecting Force Mapping mode.

Some feedback modes, like QI™, may show different measurement modes. However, the following sections describe the individual measurement modes in detail.

§ 5   Imaging

After successful approach, a measurement can be started with the desired feedback mode (**Section 4.4**). In the following sections, the imaging settings and feedback control are described for imaging in contact mode and AC mode. Please read **Section 5.7** for a detailed description of QI™ mode. During contact mode and AC mode imaging, the setpoint is kept constant while the x and y piezos scan the cantilever tip over the sample. The feedback loop continuously compares the actually applied force with the setpoint force and corrects the z piezo height in order to maintain the setpoint force. The result is a height image/topography of the sample. Depending on the feedback mode, there are additional channels, like the error signal, which is the difference between the actual and setpoint force, or the phase channel, which is specific for AC feedback mode.

5.1   Imaging settings

After a successful approach, the Run icon in the top shortcut menu bar becomes active. When the Run button is clicked, the scanner starts moving. By default, **Run** launches an infinite series of scans. If only a single scan is desired, adjust the **Scan Repetitions** under the **Setup** drop-down menu (see **Section 3.2.3**).


To stop the scan click **Run** again. This will stop the scan right after the current scan line. The tip will remain approached on the surface, at the starting point of the scan area. Or click the **Retract** button to retract the cantilever from the surface immediately (this can be used as a kind of emergency stop).

5.1.1   Image properties and the Scan Control panel
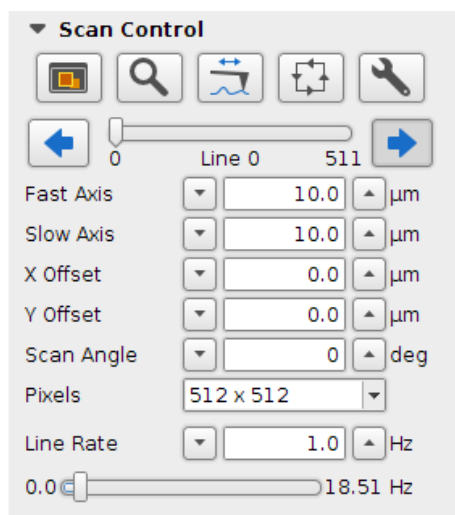
The main controls for scanning can be set in the **Scan Control** panel.

By default, the first image is started from the bottom of the **Data Viewer** window (as marked below). This is the case when the **right arrow** is active in the scan control box. 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.
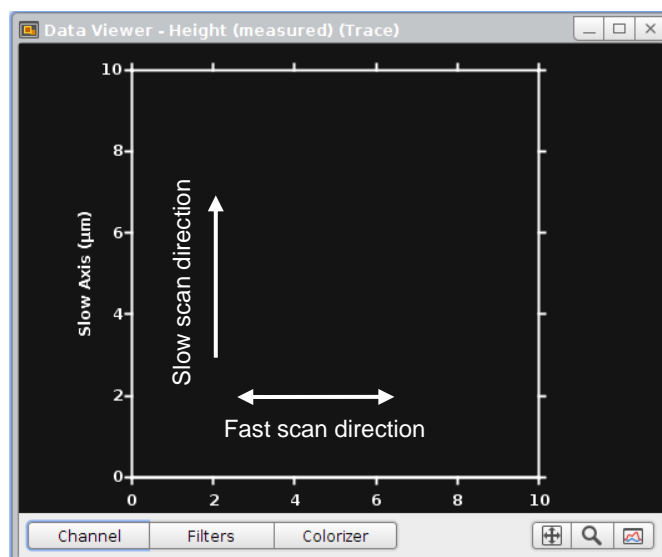
Scanning from **bottom to top** of the Data Viewer window means that during the scan the slow scan movement is in the direction towards the cantilever substrate.

Slow scan direction

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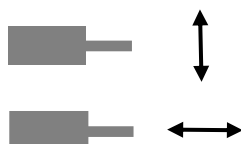

Fast scan direction

The image size can be set using the input fields for the **Fast** and **Slow Axis**, as well as by using the mouse in the **Data Viewer**. The default is for square scans, so if one axis is changed, the other one is automatically updated. This can be changed in the **Advanced Imaging Settings** panel (see **Section 5.6**).

The maximum scan region of the piezo is 100 x 100 microns. Within that range, smaller scans can be performed at different locations. Adjust the **X** and **Y Offset** to move the scan region, or use the mouse in the **Data Viewer** window (see **Section 5.1.2**). If the instrument is left on overnight or longer, set the scan offset close to zero to reduce strain on the piezo.

The **Scan Angle** is set to 0 degrees by default. Adjust the scan angle using the input field or using the mouse in the Data Viewer window.

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 resolution of the image is set to 512 x 512 pixels by default. Adjust the the number of **Pixels** to change the resolution. 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.

The **Line Rate** controls the scanning velocity. Adjust the Line Rate using the corresponding input field or the slider, which allows scan rates to a certain maximum value (displayed on the right side of the slider). This maximum value is limited by the maximum **Tip Velocity** that can be found in the Advanced Imaging Settings Window (see **Section 5.6**).

The maximum line rate changes with different scan sizes, as the tip velocity directly depends on the size of the scan region. If high line rates are used and a large scan region is set, the line rate will be corrected not to exceed the maximum tip velocity. Typically the line rate is set to values of several Hz. Choose generally lower scan rates if the sample is very rough or the scan size is big.

Note that the full range of values to control the scanning velocity is found in the **Advanced Imaging Settings** panel (see **Section 5.6**).

There are several shortcut icons on top of the **Scan Control** panel.

Open a new **Data Viewer** window, see **Section 5.1.2**.

Activate a new scan region that has been drawn in the **Data Viewer** using the mouse (see **Section 5.1.3**).

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 scanning parameters (see **Section 5.2.4**). The scan line is set using the **Line** slider in the **Scan Control** panel.
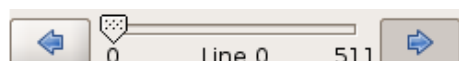
The slider displays the current scan line number, and can be used to move the tip over the surface to a particular position within the scan region (click and drag on the slider arrow). Move the slider by single steps using the arrow icons. Or activate the slider by clicking on it, and use the "left" and "right" arrow keys on the keyboard to move the slider. Once the slider is in the desired position, toggle **Run** to start a normal scan or to start **Line Scanning** if it is selected.
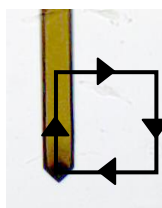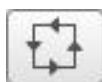
The **Outline** function makes the cantilever repeatedly perform a square or rectangle movement around the edge of the scan region that is currently set. During the outlining process the scanner is in piezo retracted mode. In conjunction with an optical microscope or top-view optics this feature helps to set a suitable size and position of the scan region prior to scanning.

Open the **Advanced Settings** window to set additional parameters such as Overscan or non-square scan regions (see **Section 5.6**).
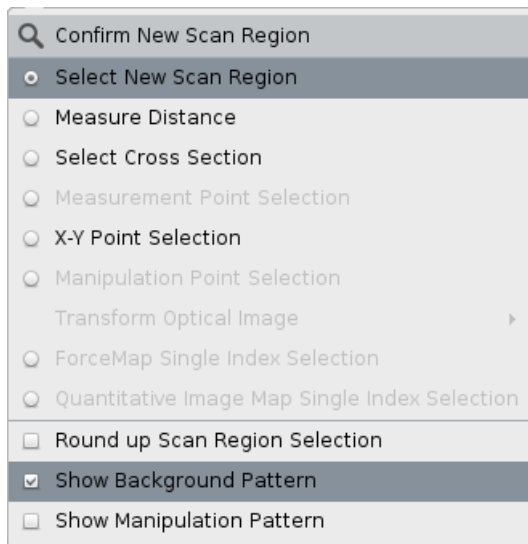
5.1.2   The Data Viewer window
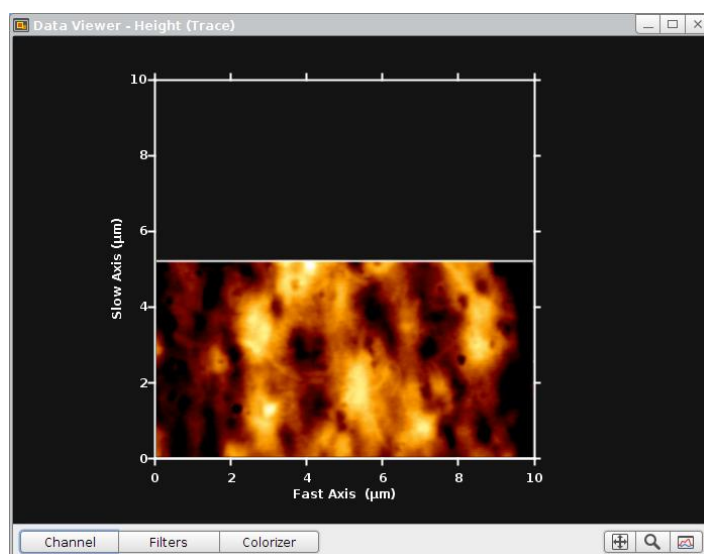
The **Data Viewer** displays real-time the image that is currently being scanned. Also old scans from saved images may be displayed using the **Image Record List** (see **Section 5.1.4)**.

Multiple Data Viewer windows can be opened simultaneously to show data from different channels or with different display settings (see below).

Open a new Data Viewer using the shortcut Icon in the **Scan Control** panel or via the **Data Viewer** drop-down menu at the top menu bar.

Right-click directly within the Data Viewer window to show and adjust the Data Viewer display options. Depending on the measurement mode, different options are active.

Activate the left mouse button for **Select a New Scan Region** to drag a new scan region directly into the Data Viewer window (see also **Section 5.1.3**).

Measure Distance allows drawing a measurement line into the Data Viewer window. Draw a cross section line using the **Select Cross Section** option. A cross section panel will open and displays the cross section profile.

Round up Scan Region Selection adjusts the scan size automatically to convenient values while dragging a new scan region.

Show or hide the **Background** or **Manipulation Pattern** by the respective entries.

Some useful mouse shortcuts allow for changing the Data Viewer display region directly:

- Scroll the mouse wheel in the image plot area to zoom in or out of that location

- Click and drag with the mouse wheel in the image plot area to shift the view region

- Toggle [Shift] on the keyboard and scroll the mouse wheel to rotate the view.

- Click [Control] on the keyboard and the left mouse button simultaneously to draw a measurement line into the Data Viewer window.

The controls on the bar at the bottom of the **Data Viewer** window provide additional display options:

Channel
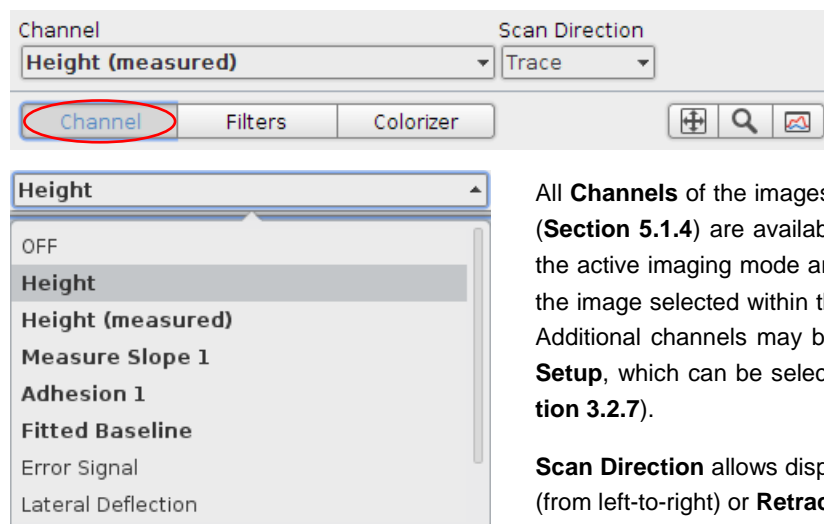
All **Channels** of the images that are stored in the **Image Record List** (**Section 5.1.4**) are available and can be displayed. The channels of the active imaging mode are highlighted in boldface. The channels of the image selected within the Image Record List are framed in green. Additional channels may be activated for display using the **Channel Setup**, which can be selected from the main **Setup** menu (see **Section 3.2.7**).

Scan Direction allows displaying the data acquired in **Trace** direction (from left-to-right) or **Retrace** direction (from right-to-left).

Filters
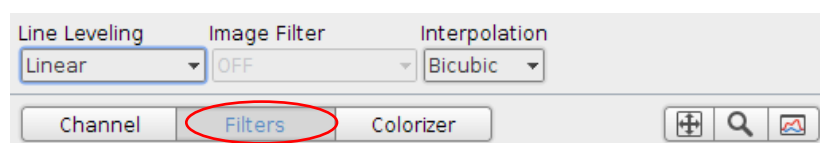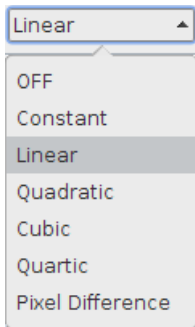
Line Leveling corrects any offset within the image line by line. Each scan line is fitted independently with a linear or polynomial fit, which is subtracted from the corresponding scan line. The higher the order, the more small features in the image will be highlighted. This only affects the online display – the raw image data is saved for later analysis. There is also the option **Pixel Difference**, which calculates the difference between adjacent pixels. Using **Pixel Difference**, height changes are highlighted and the image displays a high edge contrast.
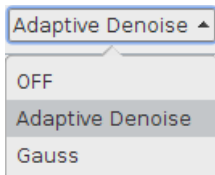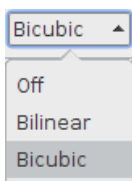
Image Filter provides **Gauss** smoothing with a width (sigma) of 3 pixels, or **Adaptive Denoise**. Adaptive Denoise combines two filters: a 2D Savitzky-Golay filter (fourth order, mask width: 7) and a filter replacing pixels that are outliers (more than 3.0 sigma deviation from the median) within a constricted-square mask of the form:

$$* * *$$
$$*$$
$$* * *$$

By default, the image data are displayed using **Bicubic Interpolation.** Choose **Bilinear** for bilinear interpolation or **Off** to display the raw data. Always the raw image data are saved; **Interpolation** is only a display setting.

Changing the colorizer settings, using leveling or data interpolation are only display options. Always the raw data are saved, independent of the display settings.

Colorizer
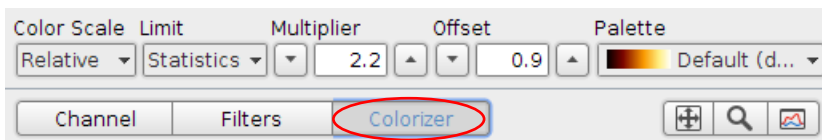
The **Color Scale** can be either displayed in **Absolute** values, which are directly measured by the instrument, or **Relative** can be used, to set the minimum value to zero.

Limit provides two different color scale settings: **Statistics** or **Min-Max**.
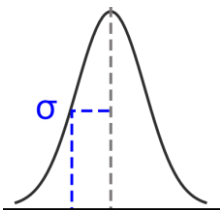
Statistics: Sigma is the full width at half maximum of the data range distribution over the whole image. Offset and Multiplier are dimensionless numbers in terms of sigma.

Multiplier is the factor that is applied to scale the width σ of the color scale, in terms of contrast.

Offset shifts the center position of the color table, in terms of brightness.
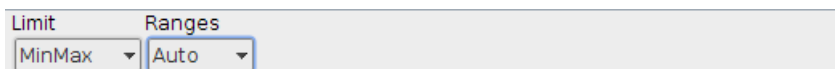
The **Limit** settings **MinMax Auto** finds the lowest and highest values automatically.
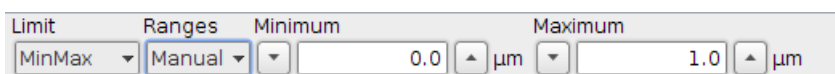
Set the **Minimum** and **Maximum** values manually using **Manual**.

Focus

Focus rescales the selected image to full size. The selected image is usually the current scan, but other images can be selected using the **Image Record List** (see **Section 5.1.4**).

Open Oscilloscope
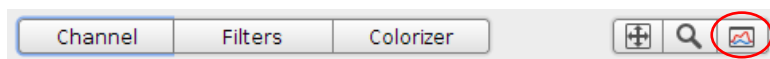
Open the **Oscilloscope** (see **Section 5.5.1**) using the respective shortcut button.

5.1.3   Selecting a new scan region

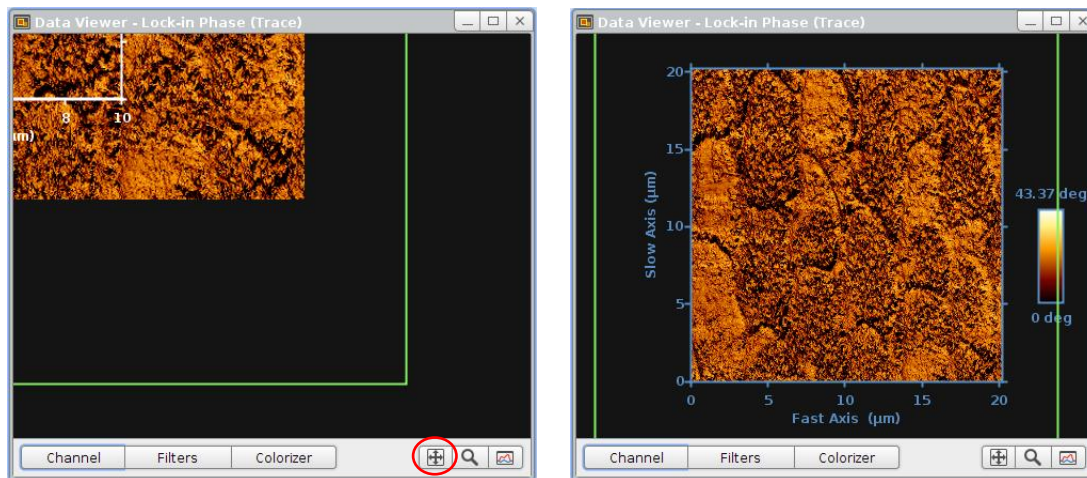The JPK NanoWizard® is equipped with state-of-the-art hardware-linearized xy piezos. This means that xy positioning is very precise and the piezos will accurately move to the newly selected scan region. The maximum scan region is generally 100 µm x 100 µm, indicated by a blue rectangle within the Data Viewer window. A new scan region can be selected using the **mouse** or the **Scan Control** panel.
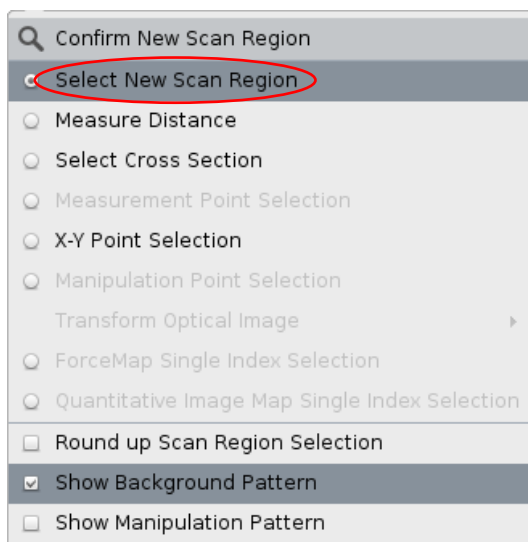
Open the right click drop-down menu in the **Data Viewer** window and enable **Select New Scan Region** to use the mouse in order to select a new scan region.

Nanotechnology for Life Science
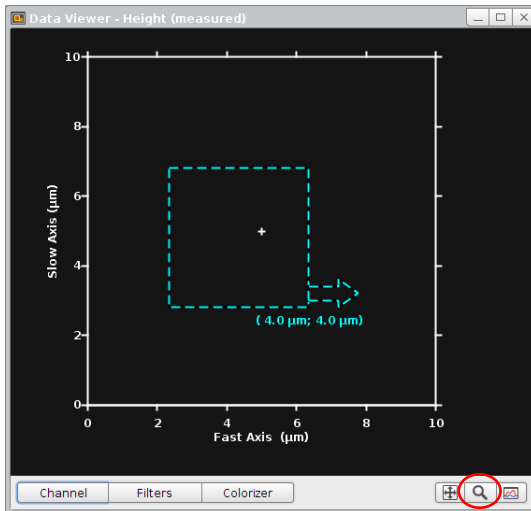
Click and drag in the **Data Viewer** to draw a cyan box defining the new scan region.

- Move the scan region in X and Y dimension by keeping the left mouse button pressed in the center.

- Adjust the size related to the center of the new scan region by moving the cursor to the center and scrolling the mouse wheel.

- Adjust the size by selecting any of the four sides of the new scan region.

- Use the arrow in the right bottom corner to rotate the new scan region.

Toggle the zoom icon to activate the new scan region. Now the scan region is surrounded in yellow.

A double click with the left mouse button into the recent scan generates a new scan region with the same parameters.

It is not necessary to retract the cantilever from the sample to select a new scan region. When the new scan region is activated, the cantilever is automatically retracted from the surface and moved to the new scan region.
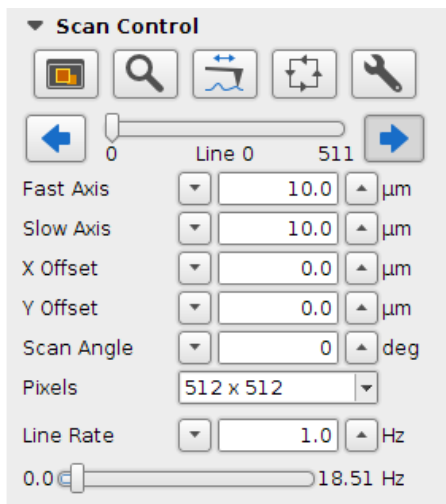
To select a new scan region using the **Scan Control** panel, type the relevant offset (**X/Y Offset**) and scan size (**Fast/Slow Axis**) values directly into the scan control panel.

Be aware of the maximum piezo range when selecting values directly in the **Scan Control** panel. The origin of the coordinate system is the center of the 100 x 100 μm scan region. Accordingly, the X and Y Offset have a range -50 to +50 μm. E.g. a scan size of 20 μm with a 40 μm offset is possible, whereas a scan size of 20 μm with a 50 μm offset is not possible, since the endpoint would be 60 μm away from the zero position.

5.1.4 Rectangular images
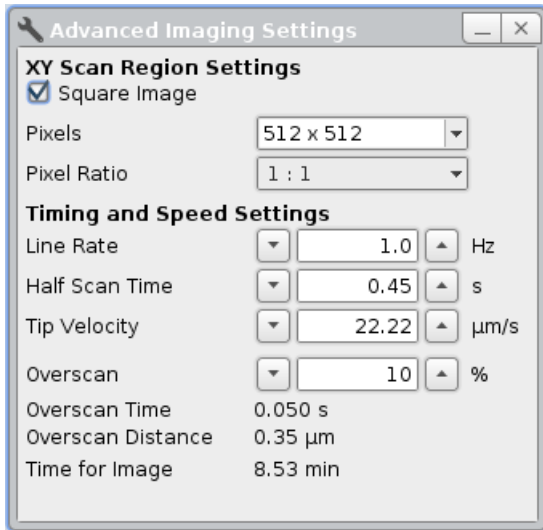
Square Image is enabled by default, i.e. new scan regions are always square shaped. Open the **Advanced Image Settings** using the **spanner** icon on the Scan Control panel or the **Imaging** drop-down menu and disable **Square Image** to allow for **rectangular image** regions. For rectangular images, the pixel number is automatically updated in order to keep the pixels square.

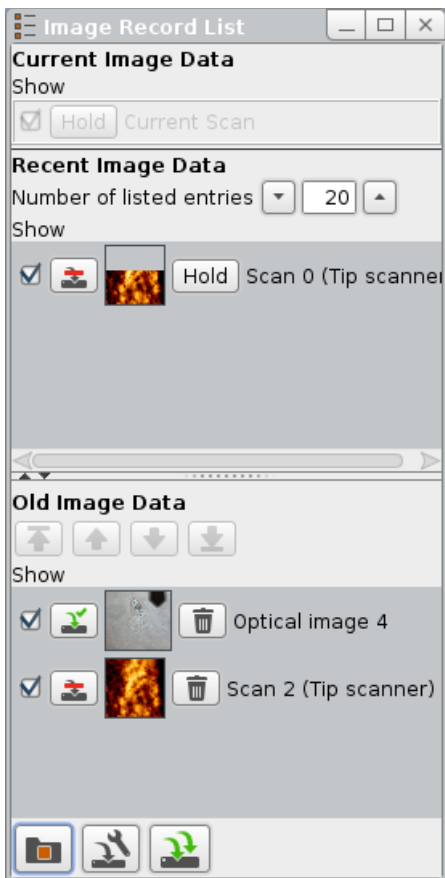Change the **Pixel Ratio** to allow for non-square pixels.

5.1.5 The Image Record List

The Image Record List gives an overview and allows saving or removing of the acquired image data. The image files are collected in three lists:

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

Recent Image Data: When an image scan has finished, the file is automatically moved to the **Recent Image Data** section. Only a limited number of scans are held here; older scans at the end of the list are removed automatically as new scans are finished. The default number of scans is 20, which can be increased using **Number of listed entries**. If the scans have been saved (either manually or with **Autosave** enabled), they are still stored on disk even if they are removed from the **Image Record List.** If they have not been saved, they are lost permanently if they reach the bottom of the **Recent Image Data** list.

Old Image Data: To store scans for reference during the session, they can be moved to the **Old Image Data** section by clicking **Hold**. This list keeps them until the software is closed or they are removed manually. There is no limitation regarding the number of scans in this list. Unsaved scans will get lost if the software is closed. If the Direct Overlay feature is installed, imported optical images also appear in the **Old Image Data** section.

Click **Hold** to move a scan into the **Old Image Data** list.

Click **Remove** to remove the image from the **Old Image Data** list.

Unsaved Images show this icon. They are lost if they reach the bottom of the **Recent Image Data** list or if the software is closed. Press this icon to save the corresponding image.

Images with this icon are permanently **saved** to the hard disk

Toggle the **Saving Setting** icon to change the saving parameters

Save all images of the **Old Image Data** list at once using the **Save all unsaved Images** icon

Load old scans using the **Open File** icon.

The more images are stored in the Image Record List, the more memory is used.

Unsaved images get lost when the software is closed, or if they reach the bottom of the Recent Image Data list. Save important data using the **Unsaved Images** icon or enable Autosave to save all images by default (see **Section 3.2.8**).
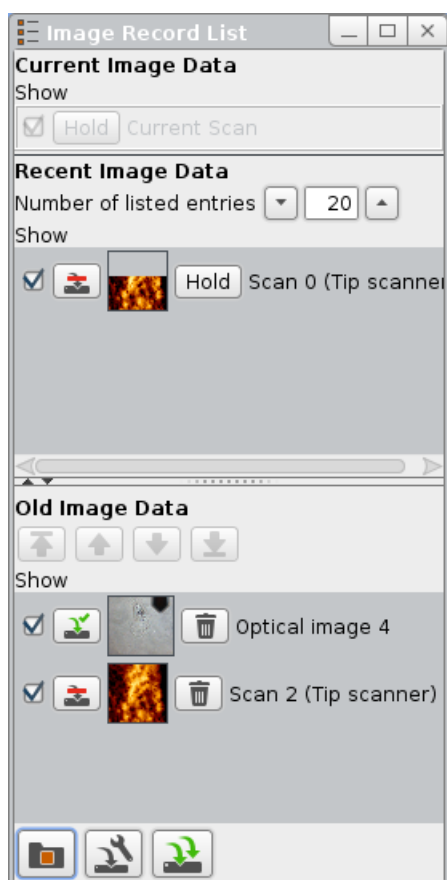
The **Show** tickbox controls which scans from the **Image Record List** are displayed in the **Data Viewer** window.

The order that the scans are plotted in the **Data Viewer** is set in the **Image Record 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 **Data Viewer** with green axes.

In **Old Image Data** the list order can be changed using the arrows. If a scan is selected, it can be moved up or down through the list using the arrows – top, up, down, bottom.
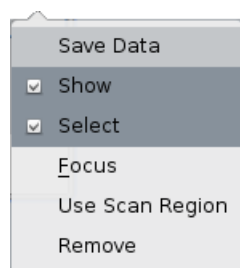
For more display options, right-click on the file entry and choose from the menu displayed:

Save Data stores the data on the hard drive

Show shows/hides the image in the data 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.

Use Scan Region imports the parameters of the selected scan and generates a new scan region with same parameters.

Remove removes the selected scan from the Image Record List.

5.2   Feedback Control for imaging in contact mode and AC mode

While the cantilever is moved over the sample in x/y direction, a feedback loop corrects the z piezo position in order to maintain the setpoint. The main feedback parameters are the gains and the setpoint, which can be optimized to achieve optimal imaging.

The gains control the reaction speed of the feedback loop. In the one hand, the gains should be sufficiently high, i.e. the feedback should be fast enough to be able to follow the sample topography. If the feedback is too slow, the z-scanner has a delayed reaction and cannot follow the sample features. On the other hand, if the feedback is too fast, the z-scanner tends to overcorrect and the cantilever starts to oscillate.

The setpoint should be optimized as well. For most applications the applied force should be as low as possible, not to modify the sample topography or to damage the tip or sample. At the same time, the applied force should be sufficiently high to follow the sample topography as accurate as possible and not to lose contact. The Feedback Control panel provides adjusting imaging gains and setpoint.

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 cantilever tip follows the surface more accurately, producing a better topography, and the tip re-acts quickly to any change of height, preventing damage to the tip or the sample. It is also often possible to reduce the imaging force (decrease setpoint in Contact mode, Increase Setpoint in AC mode) 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 (increased tip velocity)

- 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 case of 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 AC 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, see **Section 5.4.1**) 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 AC 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.

> The approach gains are set separately in the Approach Parameters window (see **Section 4.5**).

5.2.1   Feedback gains for Contact Mode imaging

IGain and **PGain** correspond to the integral and proportional gains for the adjustment of the height (z) feedback loop. The time constant, **IGain**, determines the integrator and the gain parameter **PGain** the proportional amplifier. The optimal gain values depend on the imaging mode, the sample environment (air, liquid, etc.) and topography, the scan size and scan rate, the setpoint, the selected z-range and the cantilever.

▼ Feedback Control		
IGain	▼ 150.0 ▲ Hz	
PGain	▼ 0.0048 ▲	
Setpoint	▼ 0.3 ▲ V	

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 input field, or the value can be changed in increments by clicking on the arrows.

5.2.2   Feedback gains for AC Mode imaging

| ▼ Feedback Control		
Gain	▼ 150.0 ▲	
Setpoint	▼ 0.7 ▲ V	
Rel. Setpoint	▼ 70.0 ▲ %	
Drive Ampl.	▼ 0.3 ▲ V	
Drive Freq.	▼ 305.0 ▲ kHz	
Phase Shift	▼ 0 ▲ deg	

In AC mode, **IGain** and **PGain** are combined to a single **Gain** parameter. Adjusting the main Gain value updates both **IGain** and **PGain** internally, depending on the value set as a **Transition Frequency**. If **IGain** and **PGain** should be varied independently, then this is possible using **Advanced Feedback Settings** (see **Section 5.2.5**). The **Transition Frequency** can also be set there; the default value is 10000, which is optimized for higher resonance frequencies (≥ 100 kHz).

5.2.3   Simple procedure to optimize gains

As described above, the objective is to increase the gains as much as possible in order to follow the sample topography accurately. The simplest procedure to set the maximal possible value is to increase the gains until the cantilever starts to overreact/oscillate, and then reducing the gains again.

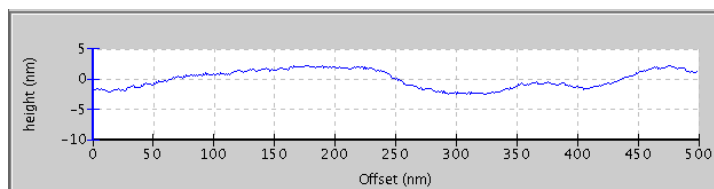| Channel	Filters	Colorizer		⊞ 🔍 🖼
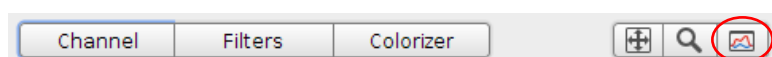
Open the **Oscilloscope** (see **Section 5.5.1** for detailed description) using the shortcut icon on the **Data Viewer** window or via the **Imaging** drop-down menu to display the height profile of the single scan lines.
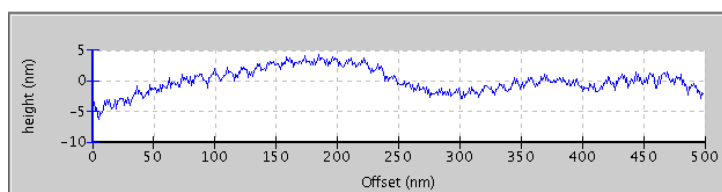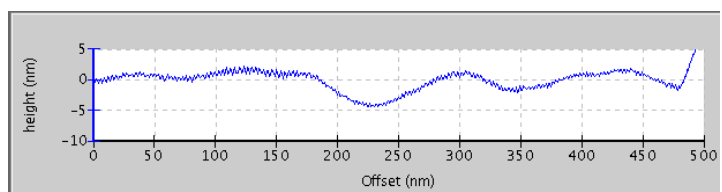

Increase I-Gain until the z-feedback loop starts to oscillate.
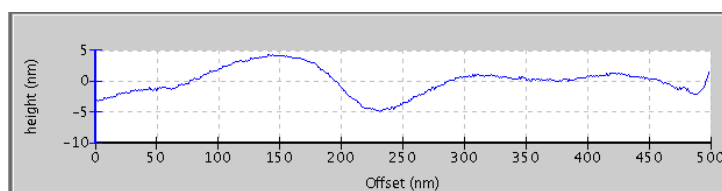

Reduce **IGain** slightly until the oscillations are eliminated.

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

Reduce PGain until the oscillation vanishes.

> In AC mode, only the main **Gain** parameter is available and can be adjusted if the standard **Feedback Control** panel is used. If necessary, open the **Advanced Feedback Settings** window under the **Setup** menu to adjust the **IGain**, **PGain** or **Transition Frequency** individually (see **Section 5.2.5**).

## 5.2.4   Scan speed and feedback adjustment

The scan parameters should be adjusted regarding the sample properties. Big scan regions with large height features require low scan speeds and sufficiently high gains to be able to follow the surface topography. Small and flat scans allow using higher scan speeds, but also sufficiently high gains are needed to image the surface accurately. Very soft samples, like living cells or hydrogels need low imaging forces (setpoint) not to be damaged. To achieve good results with low setpoints the gains and imaging speed must be adjusted properly to stay on the surface and follow the sample features.

The gains, line rate/scan speed, scan size and setpoint are interrelated and also need to be aligned in dependence on each other. E.g. if the scan size is increased while the line rate remains constant, the scan speed increases. If the scan speed is increased, the gains need adjustment accordingly. If low setpoints are used, the speed and gains must be sufficiently high to stay on and follow the sample surface.

Open the **Oscilloscope** window via the **Imaging** drop-down menu (see **Section 5.5.1**) to display the trace and retrace data of each scan line. Compare the trace and retrace data during the adjustment of the scan parameters – they should be in congruence. The imaging parameters can be optimized best by scanning directly on the sample features. The **Line Scanning** option allows repeated scanning of the same scan line (see **Section 5.1.1**) and helps to adjust the scan parameters directly on top of a sample feature.

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 (alt-

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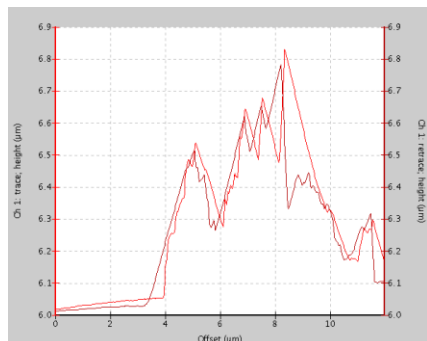
When the gains and/or imaging force are not high enough for the scan speed and topography, the scan lines tend to "trail" as the tip follows the surface downwards, and the trace and retrace lines do not agree with one another.

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.
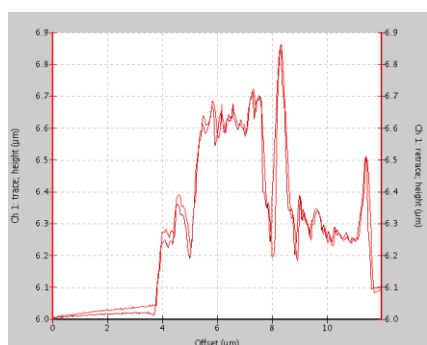
Increasing the gains and/or imaging force (decrease setpoint in AC mode/ increase setpoint in Contact mode) leads to better congruence of trace and retrace curves.

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.

Too high imaging forces (High setpoint in contact mode, low setpoint in AC mode) may damage or even detach delicate and loose samples. The trace and retrace scan lines will be shifted as the sample is moved in scan direction. The imaging force should be reduced to save the sample and the gains increased to maintain sample contact.

5.2.5   Advanced Feedback Settings

The **Advanced Feedback Settings** window can be opened via the **Setup** drop-down menu. This panel provides an extension to the parameters found in the **Feedback Control** panel.
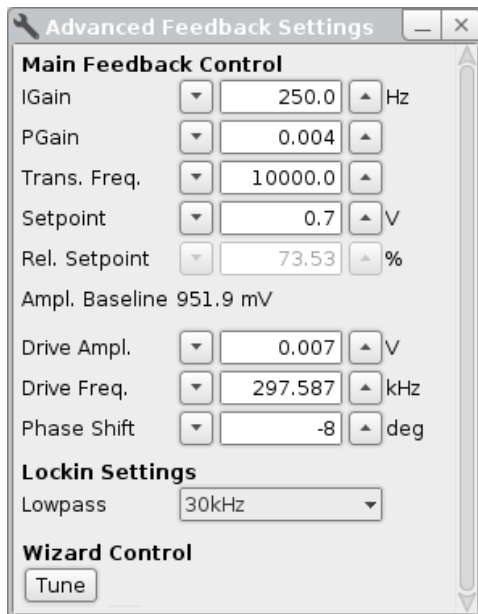
The **Advanced Feedback Settings** window allows access to all parameters that can be set during the measurements.

The **Main Feedback Control** Panel is required for adjusting parameters like **IGain, PGain** and **Transition Frequency**.

Various predefined Low pass filters between 1kHz and 100kHz can be selected under **Lockin Settings**.

Wizard Control offers short cut buttons. **Tune** opens the Tuning wizard.

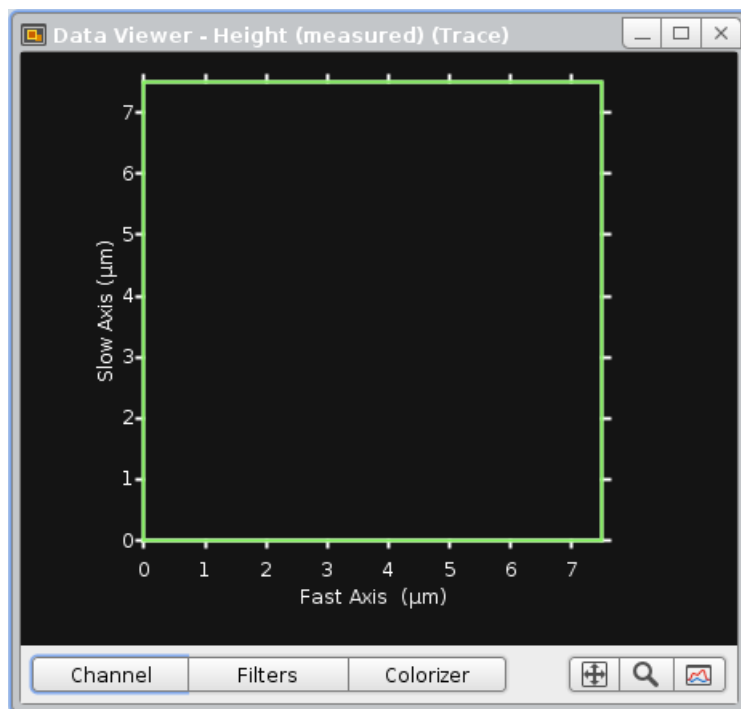## 5.3   Limit the lateral scan range for higher resolution

⊕ The lateral scan range can be limited in order to improve resolution.
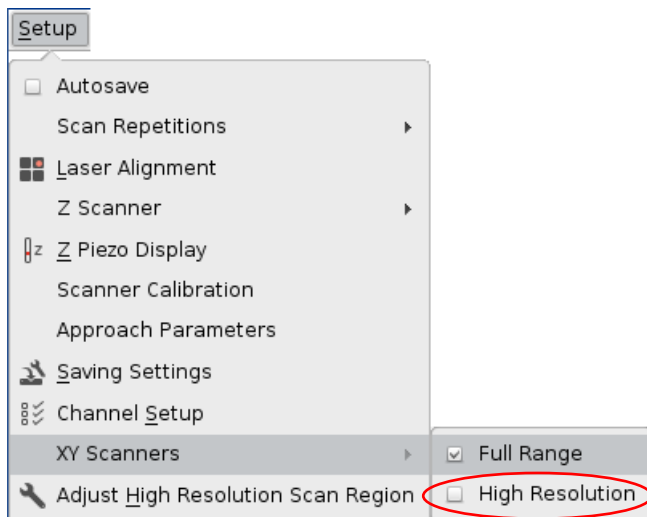
Select **XY Scanners** from the **Setup** drop-down menu und select **High Resolution**.
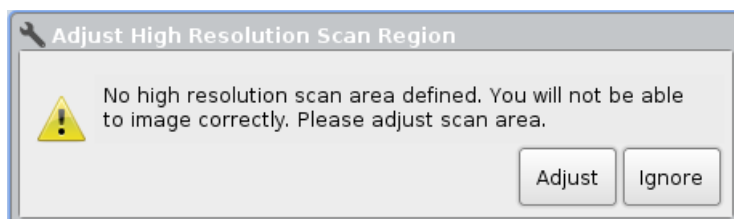
A message appears and requests to adjust the high resolution scan area. Click **Adjust** – the scan area is adjusted automatically. This may take several seconds.

The maximum lateral scan range will be limited to 7 x 7 µm^2. Now the system is ready for scanning.
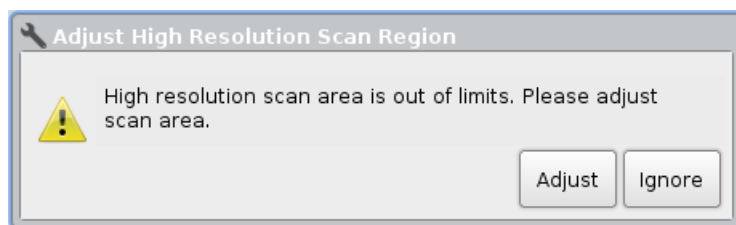
The scan area shifts slightly as soon as the head temperature changes. This will reduce the lateral movement. A warning will appear and request for scan area adjustment; click **Adjust**. The adjustment will retract the cantilever and stop scanning.

Click Ignore to adjust the scan region later, e.g. if a measurement is running.
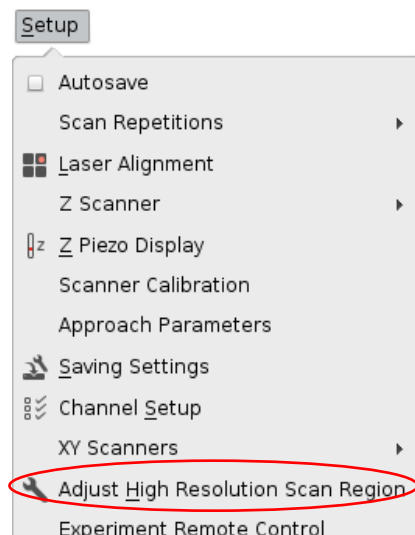
Select **Adjust High Resolution Scan Region** from the **Setup** drop-down menu to start the adjust procedure manually, e.g. if **Ignore** was clicked to finish a measurement.

5.4   Controlling the Z-piezo and stepper motors

5.4.1   Reducing the Z-Range for higher resolution

To optimize the range and resolution, the working range of the Z-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 in order to maximize the resolution. The resolution is increased for smaller Z-ranges because of the resolution of the AD-converter, and also the amplifier noise is lower.

The piezo cannot retract further than the currently set Z-Range! If the sample roughness exceeds the selected Z-Range, the tip will not be able to move sufficiently far away from the surface. Tip and/or sample damage may result. Adjust the Z-range corresponding to the sample roughness!

The Z-range covered by the piezo during a scan includes any sample tilt. If the sample tilt along the scan line exceeds the selected Z-range, the cantilever cannot be moved sufficiently far away from the sample. Tip and/or sample damage may result. Select a sufficiently high Z-range in case of any sample tilt or use Automatic Motor Leveling to compensate for any sample tilt (see **Section 5.4.3**)!

The height range being covered can also be seen on the Z-Range display (see below). If there is a significant sample tilt, it may be better to remove this first by adjusting the stepper motors (see **Section 5.4.2**) before reducing the Z-Range.

The scan area is slightly shifted upon stepper motor movement. It may not be possible to find exactly the same sample area upon stepper motor movement, unless it can be located optically.
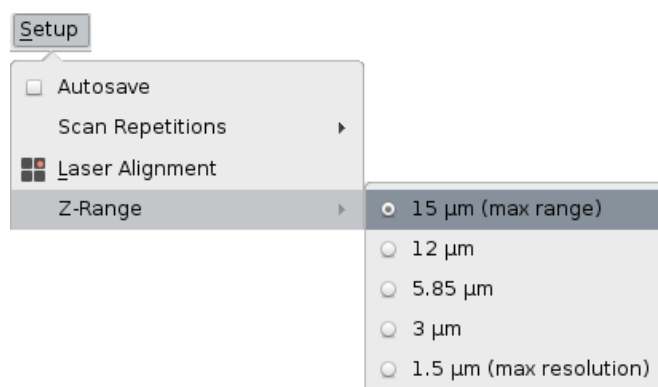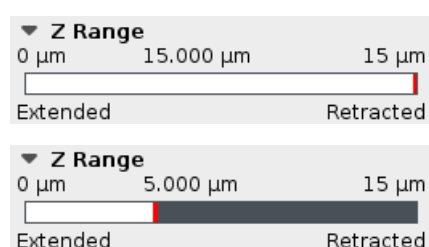
The **Z-range** can be set from the **Setup** drop-down menu. It can only be changed if the tip is retracted from the surface.

There are seven different z-ranges available that can be selected: 15, 10, 7.5, 5, 3, 1.5 or 0.75 μm

Always start with a large Z-Range for samples with unknown topography range! Take some test images after the first approach. Reduce the Z-range according to the sample roughness derived from the test images.

The Z-range display can be seen at the bottom left of the software, and the active z-range is highlighted in white. The current piezo position is marked as a red line, and the range covered within the current scan line is highlighted in grey.

0 – 15 μm (maximum range)

0 – 5 μm (useful range for small samples)
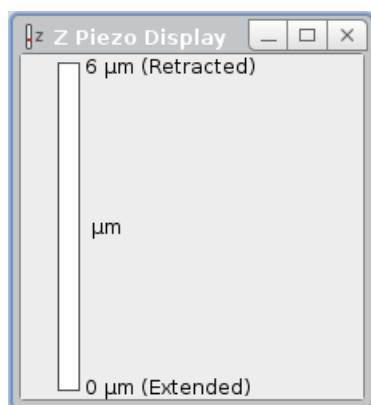
Open the **Z-Piezo Display** from the **Setup** drop-down menu to view the Z-piezo movement vertically.

5.4.2 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, which allows better imaging, particularly because the Z-Range may be reduced (see **Section 5.4.1**). Note that generally the exact location on the sample is changed when the stepper motors are moved independently of each other.

The currently selected scan region is shifted when the stepper motors are moved. It may not be possible to find exactly the same sample area upon stepper motor movement, unless it can be located optically.
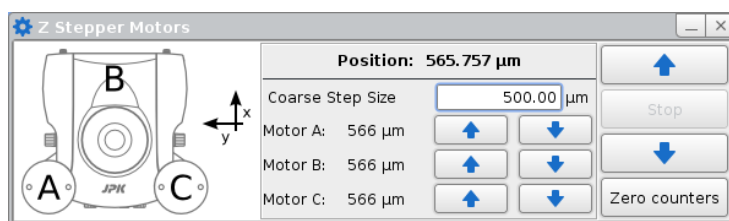
Open the **Z Stepper Motors** window to move the stepper motors independently. The diagram shows the direction of the main scan axes to help translate the scan to the movement of the motors.
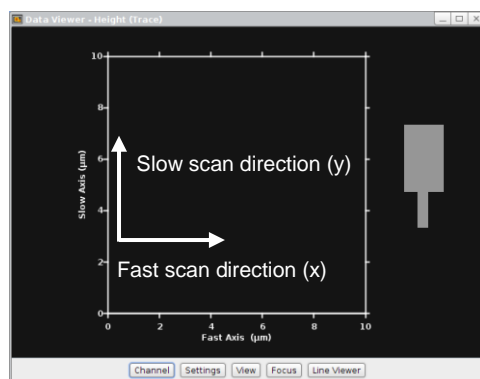
The down arrows of the stepper motors are inactive if the tip is approached. Retract the piezo before moving the stepper motors.
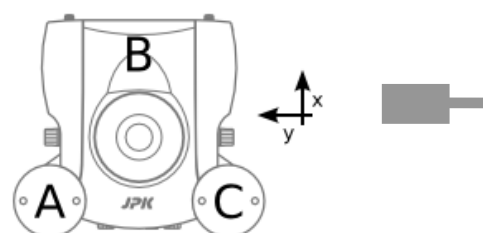
The scheme shows the orientation of the cantilever relative to the AFM head.

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

Open the **Oscilloscope** (see **Section 5.5.1**) to visualize the tilt of the individual scan lines and to decide if re-adjustment of the stepper motors is necessary.

The **Leveling** option must be set to OFF in the Data Viewer window (see **Section 5.1.2**) to see the overall tilt of the scan lines in the oscilloscope.
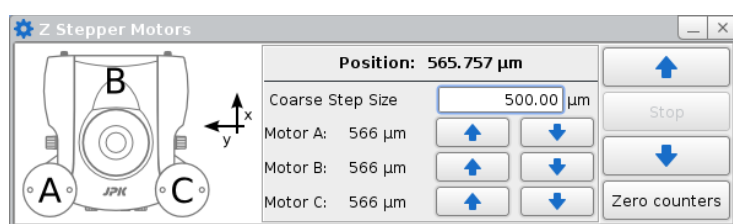
The position of the motors is shown on the **Stepper Motor** window. The main pair of up and down arrows on the right control all three motors together. The three pairs of arrows in the center control each motor independently.

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.

Toggle **Zero counters** to reset the counter at any position. It may be helpful to reset the counter at the sample surface, to provide a reference for the sample height and aid re-approaching later.

The Position numbers are no absolute values, and the motors can all read zero even though the head has a significant tilt. To remove any tilt of the head, move the head up as far as possible, until all three motors are at their highest position. **Zero counters** if all motors are extended to the maximum height. Now the counter is levelled with respect to the stage.
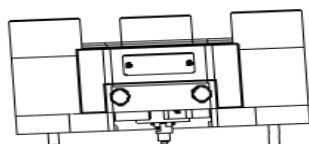
*Not recommended*
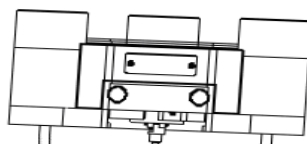
Better in this direction

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

Do not tilt the AFM head into the direction of the cantilever spring (left hand image above). The cantilever spring may touch the surface rather than the cantilever tip. E.g. there will be no cantilever deflection during the approach even though the spring and cantilever holder already touch the sample. Damage of the cantilever holder or sample may occur.

5.4.3 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 the plane tilt of a scanned image and carry out the compensatory movement automatically. 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.
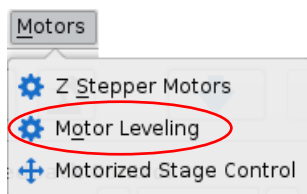
Open **Motor Leveling** from the main **Motor** drop-down menu. The Motor Leveling Window will open.
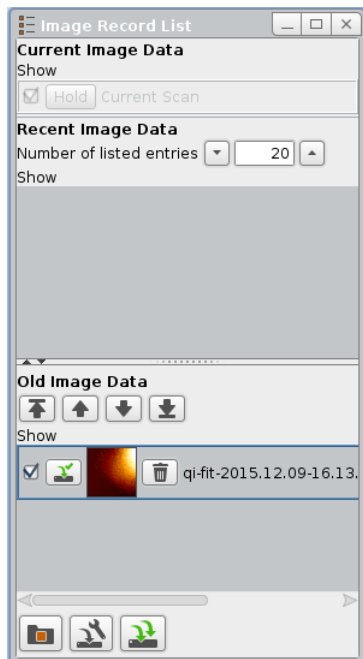
Open the **Image Record List** (**Section 5.1.5**) and select a scanned image. The image will be shown in the Motor Leveling window display.

Use large scans (> 10 microns) to determine the large-scale tilt of the sample. Use completed, square scans, so that the angle can be measured well in both scan directions. The pixel number or resolution is not important, so the scan can be done with fewer pixels if required for speed.

After the compensation movements of the motors, the center position will change laterally, so the tip will not land on exactly the same region of the sample.
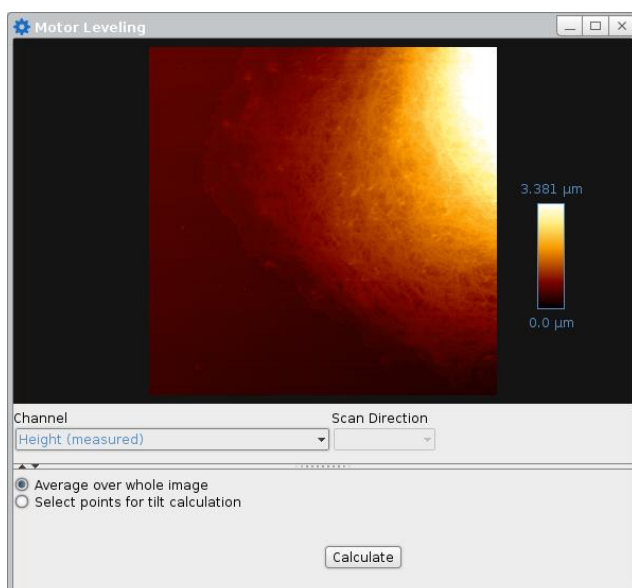
The **Motor Leveling** display shows the currently selected image from the **Image Record List**. The **Height (measured)** channel is generally used, as this has the best height accuracy. The image is shown without any **Leveling** (Section **5.1.2**), so it may look different in the **Data Viewer**, 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**.

Use **Select points for tilt calculations** for samples where there is a clearly defined background that should be used for calculation, and features that should be ignored, like cells as shown in the image here.
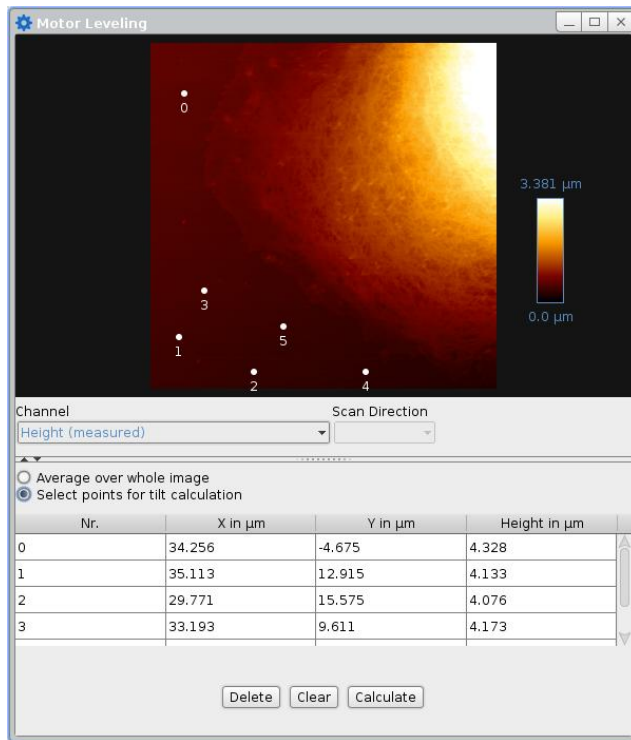
If **Select points for tilt correction** is used, click in the image display to set points. Three points is the minimum, but it is better to use more points, to ensure there is a good average.

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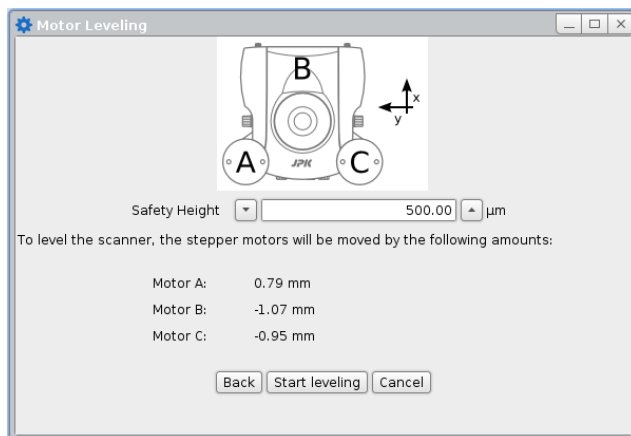
Once the background level has been set from the image, the calculated stepper motor movements are shown.

All stepper motors are additionally moved, so that the **Safety Height** is the final height of the tip above the surface.

Click **Start leveling**.

Re-approach to the surface to start imaging.

5.5   Tools for monitoring scanning

5.5.1   The Oscilloscope window

Click the **Oscilloscope** icon in the shortcut icon toolbar to open the oscilloscope window. It can also be opened from the **View** drop-down menu.

The **Oscilloscope** window can be used to display the current scan line of up to four channels simultaneously. Both trace and retrace data may be displayed together, which can help with optimizing the imaging settings (see **Section 5.2.4**). Any channel can be displayed, so long as it is switched on in the **Channel Setup** (see **Section 3.2.7**).
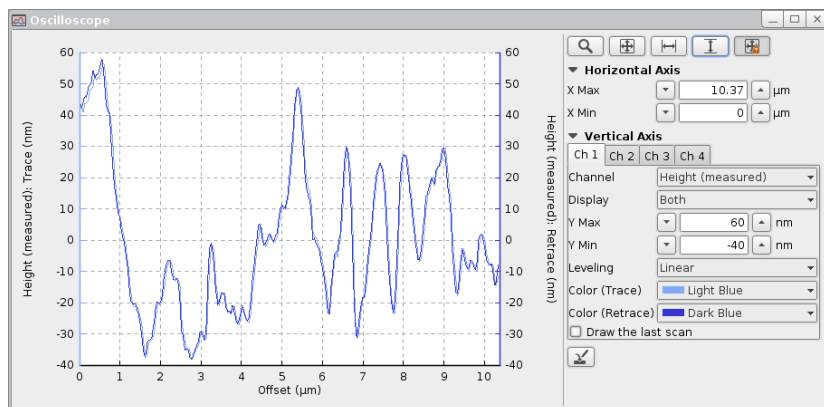
Use the mouse-wheel in the oscillo-scope window to zoom in/out the **Horizontal Axis**. Click and drag with the mouse wheel into the graph to move it in x and y.

Adjust the **Horizontal/Vertical Axis** using the corresponding input fields.

   Use the standard oscilloscope toolbar to show the full data range (see **Section 3.2.1**)

In **Contact** mode **Error signal** and **Vertical deflection** provide basically the same information, but for **Error Signal** the **Setpoint** is always subtracted. With a calibrated cantilever (see **Section 7.2**) the **Vertical deflection** signal is displayed in nm/nN.

In **AC** mode **Error signal** and **Lock-in amplitude** provide the same information, but for **Error Signal** the **Setpoint** is always subtracted. With a calibrated cantilever (see **Section 7.2**) the **Lock-in amplitude** signal is displayed in nm.

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5.6   Advanced Imaging Settings

This panel provides an extension to the parameters found in the **Scan Control** panel.

The **Advanced Imaging Settings** window can be opened directly from the **Scan Control** panel, or via the **Imaging** drop-down menu.
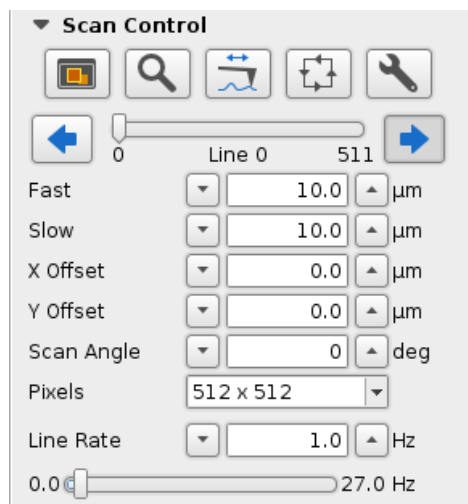
The speed for scanning is usually set in the main **Scan Control** panel, through the **Line Rate**. A line rate of 1Hz means that the tip makes the trace and retrace scan lines and returns to the starting position for the next scan line in a time of 1s.

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 in the **Advanced Imaging Settings**.
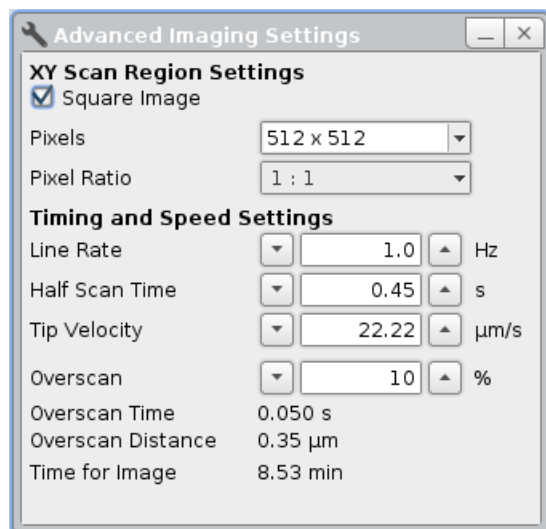
XY Scan Region Settings allows to disable **Square Images** in order to scan rectangular images (see **Section 5.1.4**).

The **Pixel Ratio** can be changed between predifined and not fixed values.

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

The total time for the image is also displayed, calculated for the overall set of parameters.
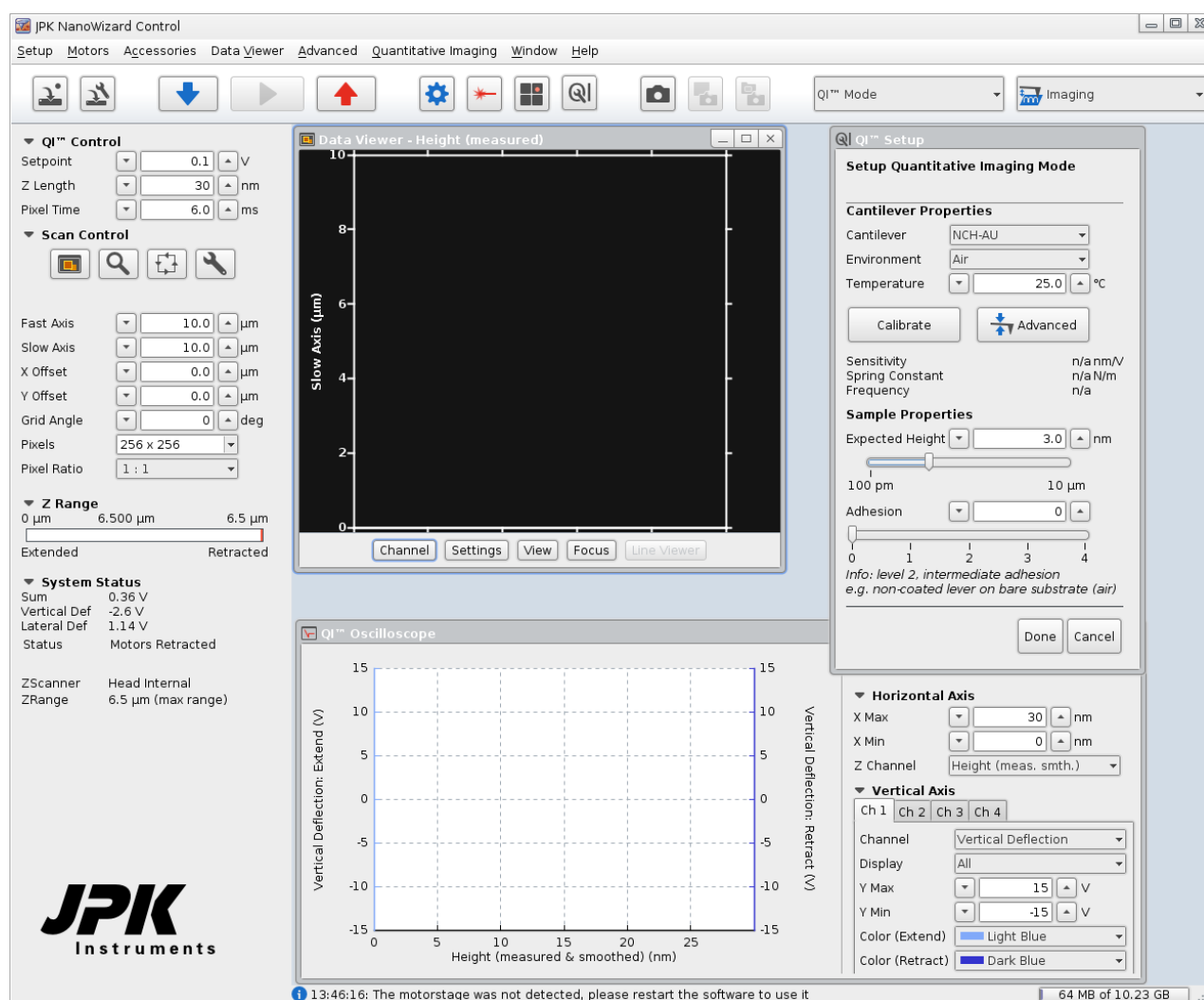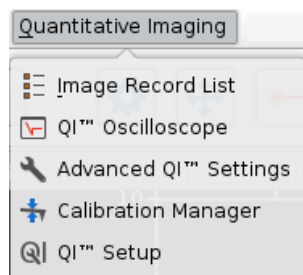
5.7   QI™ Mode

QI™ is a force spectroscopy based imaging mode. Please read **Chapter § 6** to get an overview of the basics of force spectroscopy mode and settings. Similar to Force Mapping (see **Section 6.7**), a whole force curve is measured at every pixel of the selected sample region. The main difference lies in the algorithm of the tip motion and the sample rate which both allow a higher imaging velocity. In the standard QI™ mode there are two data channels available, Height and Height (measured). These channels are calculated online from the force curves and saved as jpk-qi-images. The force curves are not saved in standard QI™ mode. To have full access to all force curves the software extension ⊕ **QI™ Advanced** is necessary (see **section 8.1**).
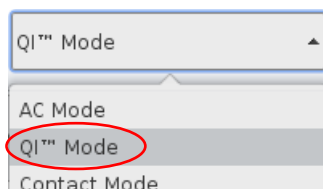
Select **QI™ Mode** in the Feedback Mode drop-down menu.

Upon changing to QI™ mode, the corresponding data viewers (**Data Viewer** and **QI™ Oscilloscope**), the **QI™ Control** panel and the **QI™ Setup** window open automatically.

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The **QI™** drop-down menu appears in the menu bar. All necessary windows concerning QI™ mode can be opened from this menu.

5.7.1 The QI™ Data Viewer
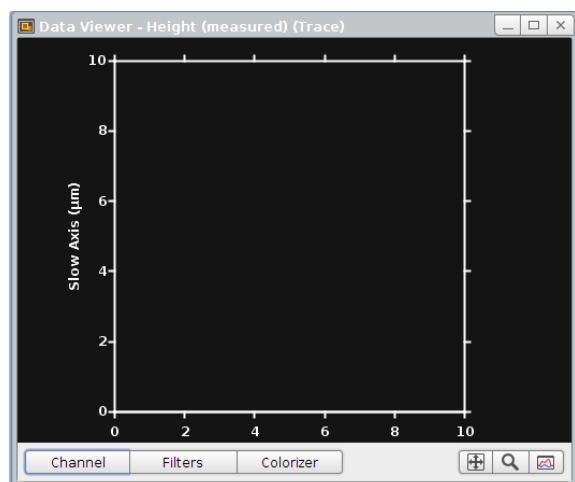
The **Data Viewer** has the same function as in all other imaging modes. Please read **Section 5.1.2** for detailed information.

The menu, which appears upon right-click within the **Data Viewer**, contains basically the same options as for all imaging modes (see **Section 5.1.2**).
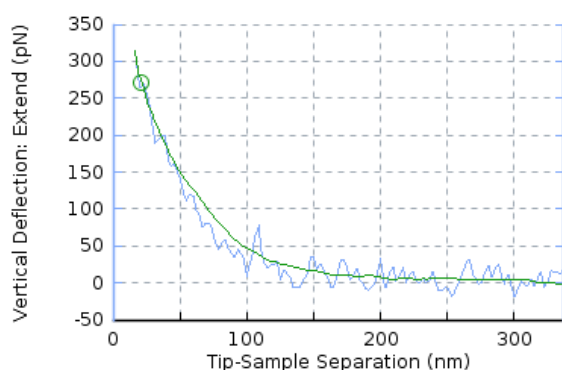
In QI™ mode, there are two channels available: **Height** and **Height (measured)**. The two height channels are calculated online from the extend curves, smoothened with a 40 pixels moving average. The height corresponds to the height at 80% of the setpoint force determined from the smoothened curves, as indicated by the green curve and circle in the left-hand image. Please find a close description of the so called **Reference Force Height** operation in **Section 6.1.2** and the **JPK Data Processing User Manual**.

5.7.2   The QI™ Oscilloscope
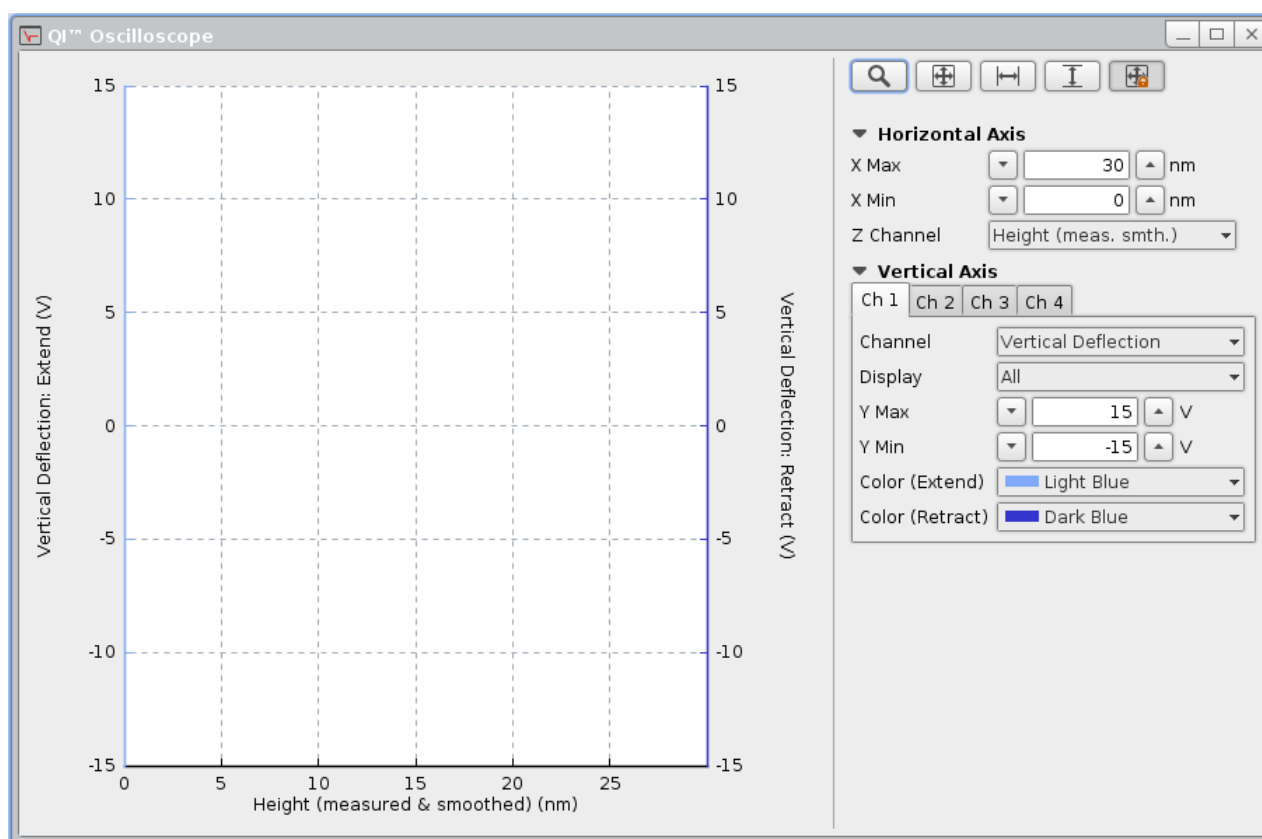
The **QI™ Oscilloscope** plots the force curve of the pixel currently measured and allows for adjustment of the display parameters. Please see **Section 3.2.1** for a closer description of the basic functionality.

5.7.3 The QI™ Setup

Before starting a QI™ measurement, the spectroscopy and imaging settings should be adjusted corresponding to cantilever and sample. The **QI™ Setup** appears upon QI™ Mode selection. It allows for setting the **Cantilever Properties** in order to calibrate the cantilever using the Contact-free method (see **Section 7.4**) as well as some **Sample Properties** to calculate reasonable imaging parameters.

The **Cantilever Properties** allow quick and automatic calibration of the cantilever with the **Contact-free** method (see **Section 7.4**). Select the corresponding cantilever from the **Cantilever** drop-down list as well as the **Environment**, and type the **Temperature** used for the measurement. If your cantilever or environment is not available, select **User defined** from the drop-down lists. The Calibration Manager opens and allows for adding cantilevers and environments (see **Section 7.4**).

Click **Calibrate**; the software automatically retracts the cantilever using the stepper motors, measures the thermal noise and calculates the spring constant. Finally, the Vertical Deflection is displayed in units of Force (nN).

Click **Advanced** if additional parameter adjustment is required (e.g. correction factors) or to use the **Contact-based** instead of the Contact-free method. The **Calibration Manager** will open and provide all parameters and settings (see **Section 7.2**).

The calibration can also be skipped if not desired.

The **Sample Properties** automatically calculate reasonable imaging parameters depending on the sample height and adhesive properties.

Type the **Expected Height** of the sample as well as the expected **Adhesion** within an arbitrary scale of 0 to 4. The imaging parameters will be adjusted to reasonable starting values.

Click **Done** if all parameters are properly set; the **QI™ Setup** will close.

The **QI™ Setup** can be opened at any time from the **Quantitative Imaging** drop-down menu.

5.7.4   The QI™ Control and Scan Control panel

The **QI™ Control** panel on the left-hand side contains all main parameters to define the shape of the force curves taken at each pixel. If the QI™ Setup is completed properly, the preset values are usually appropriate for image acquisition.
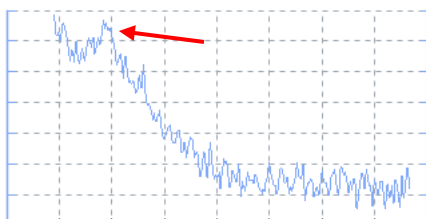
As in force spectroscopy mode (see **Section § 6**), the **Setpoint** means the maximum force applied, and the **Z length** is the distance covered by the z-piezo during a force curve.

Have a close look at the force curves, especially at the extend curves (see below), shown in the Quantitative Imaging Oscilloscope during imaging to adjust the parameters properly if necessary.

Decrease the setpoint, if the sample is fragile and e.g. break-through events are visible in the curves (red arrow in the extend curve at the left).
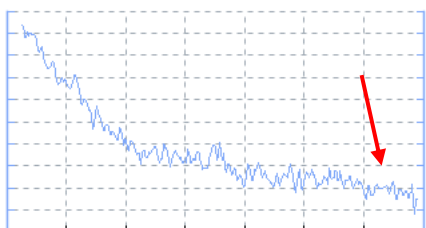
Ensure that the Z Length is sufficiently high to separate the cantilever completely from the sample between pixels in case of high adhesive interactions or large height changes between adjacent pixels. The baseline of the extend curve will be clearly tilted upon uncomplete separation due to adhesive interactions (red arrow in extend curve on the left).
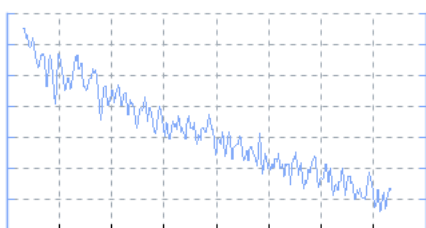
If the height changes between adjacent pixels are much higher than the Z Length, the repulsive contact already starts at the beginning of the force curve, i.e. the whole curve is tilted and there is no baseline visible. The software automatically applies an **Additional Retract** (see scheme of the QI™ movement in **Section 5.7.5**) in between pixels, but the Z Length must be sufficiently high to acquire complete force distance curves for the calculation of the height channel. On the other hand, the Z Length should be reduced as much as possible to minimize the acquisition time.

The **Pixel Time** determines the duration of one pixel, including vertical (force curves) and horizontal (pixel-to-pixel) tip

motion. Upon changing the Pixel Time, the software automatically adjusts the single parameters that constitute the pixel time to reasonable values. Decrease the Pixel Time to speed up force curve acquisition and scan speed. Increase the Pixel Time if the tip motion is too fast for the sample or type of cantilever. The softer the cantilever, the stronger it tends to oscillate at shorter Pixel Times.

Open the Advanced Settings using the shortcut icon in the **Scan Control** panel below to show and adjust the advanced scan parameters determining the pixel time manually (see **Section 5.7.5** below).

By default, the approach setpoint in QI™ Mode is coupled to the QI™ imaging setpoint by a multiplier of 2. Open the **Advanced Feedback Settings** window via the Settings drop-down menu to change the coupling multiplier or to switch off the coupling.
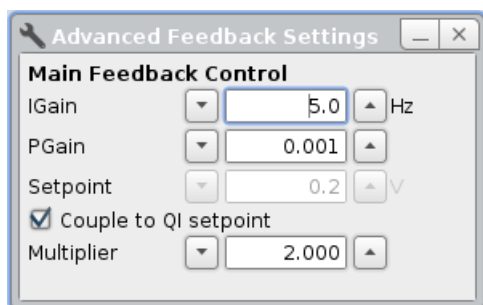
Open the **Advanced Feedback Settings** window via the Settings drop-down menu. **Couple to QI setpoint** couples the approach **Setpoint** to the QI™ imaging setpoint:

Setpoint = **Multiplier** * QI™ imaging setpoint

Untick **Couple to QI setpoint** in order to switch the coupling off and set manually the desired setpoint for approaching.
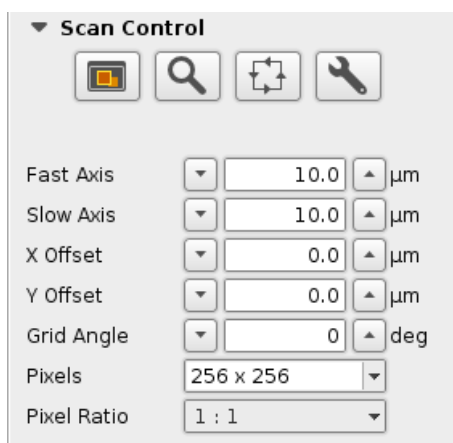
The **Scan Control** panel defines the size, position and resolution of the scan area (see also **Scan Control**, **Section 5.1.3**).

The shortcut icons on top allow for opening new Data Viewers (see **Section 5.7.1**), activating new scan regions (see **Section 5.1.3**), outlining the scan region (see **Section 5.1.1**) and opening the Advanced Settings window (see **Section 5.7.5**).
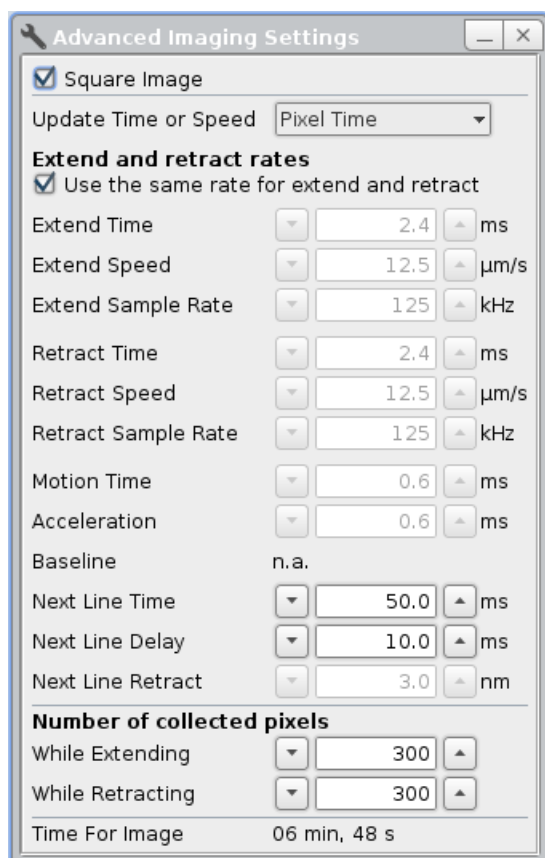
Pixel Ratio enables the usage of non-square pixels.

5.7.5 Advanced Imaging Settings

Open the **Advanced Imaging Settings** window using the **QI™** drop-down menu or via the shortcut button in the **QI™ Control** panel.

The standard settings meet the requirements of a wide range of samples. However, very delicate and challenging samples may need further adjustment of the force curves themselves or the pixel to pixel movement.

Advanced Imaging Settings

☑ Square Image

Update Time or Speed | Pixel Time ▼

Extend and retract rates
☑ Use the same rate for extend and retract

Extend Time	▼	2.4	▲	ms
Extend Speed	▼	12.5	▲	µm/s
Extend Sample Rate	▼	125	▲	kHz
Retract Time	▼	2.4	▲	ms
Retract Speed	▼	12.5	▲	µm/s
Retract Sample Rate	▼	125	▲	kHz
Motion Time	▼	0.6	▲	ms
Acceleration	▼	0.6	▲	ms
Baseline	n.a.			
Next Line Time	▼	50.0	▲	ms
Next Line Delay	▼	10.0	▲	ms
Next Line Retract	▼	3.0	▲	nm

Number of collected pixels
While Extending	▼	300	▲
While Retracting	▼	300	▲
Time For Image	06 min, 48 s		

By default, the selected scan regions are automatically adjusted to squares. Deselect **Square Image** to allow rectangular scan regions.

Update Time or Speed provides parameter adjustment using different dependencies of time, speed and sample rate. **Pixel Time** is set by default, i.e. the software calculates the timing settings automatically.

Only the time to move the cantilever to the next scan line (**Next Line Time**) and the time the cantilever waits at the starting point of the new scan line (**Next Line Delay**) can be adjusted using Pixel Time. Increase these timings if the image shows artifacts on the left, e.g. to allow soft cantilevers to recover from the next line motion (Next Line Delay) and to prevent oscillations due to a too fast next line motion (Next Line Time).

Additional information about the **Number of collected pixels** and **Time for Image** is shown at the bottom.
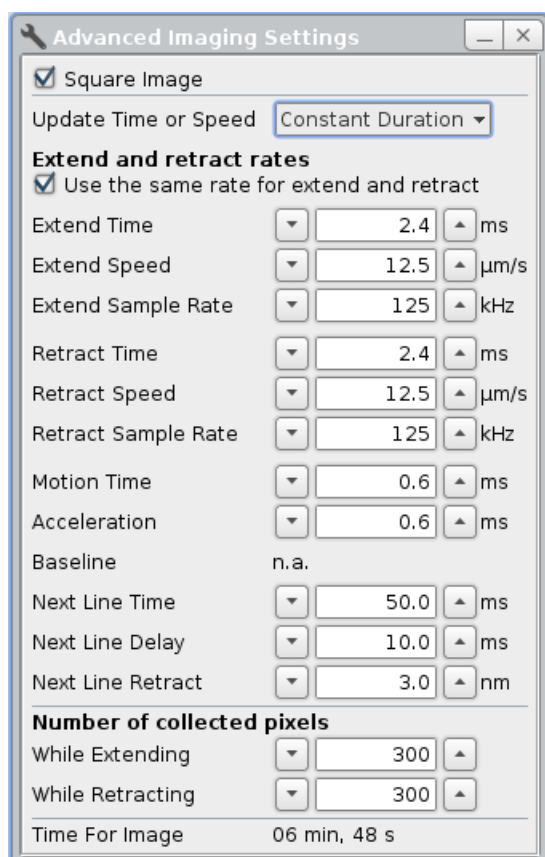
Besides **Pixel Time**, **Constant Duration** or **Constant Speed** can be selected under **Update Time or Speed**.

If **Use the same rate for extend and retract** is enabled, the **Retract Sample Rate** automatically updates if the **Extend Sample Rate** is changed and vice versa.

Extend/Retract Speed, **Z Length** (**QI™ Control** Panel) and **Extend/Retract Time** depend on each other. **Constant Duration** helps to maintain the time of a segment if the speed or z length is changed, **Constant Speed** maintains the speed if the duration or z length is changed. The **Sample Rate** determines the number of collected pixels per second. It should be sufficiently high to detect all features within the force curves.

The relevance of **Motion Time** and **Acceleration** is explained by the drawing below.

The **Baseline** function automatically corrects for vertical drift. It is measured at the beginning of each scan line.
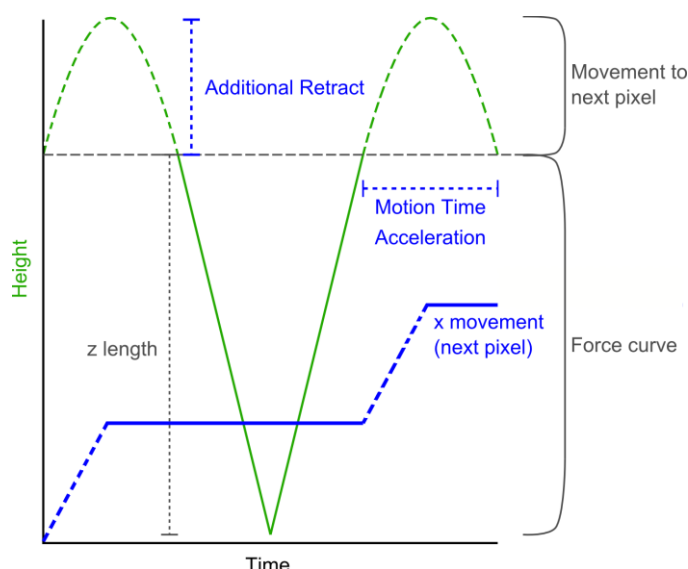
This sketch shows a plot of the piezo height (green) and x movement of the piezo/cantilever (blue) over time during a QI™ measurement. The round caps in the height signal between two force curves represent schematically the x movement from pixel to pixel. The shape of these caps can be adjusted manually by **Motion Time** and **Acceleration**. Reducing these values increases the scan rate. Generally, the scan rate concerning the x direction is limited by the height contrast of the sample and the mechanical properties of cantilever (spring constant, resonance) and sample (stiffness, slackness, stickiness, e.g. probe-sample interaction).

5.7.6 QI™ data and file saving

All QI™ data can be found in the **Image Record List**. Open the Image Record List using the corresponding icon at the shortcut icon toolbar on top of the SPM software. Managing, saving and removal of images using the **Image Record List** is described in **Section 5.1.5.**

Open the **Saving Settings** menu via the **Setup** drop-down menu or the shortcut icon in the toolbar. Data saving is described in **Section 3.2.8**. Choose the register card **QI™ Imaging** to set the channels for file saving.
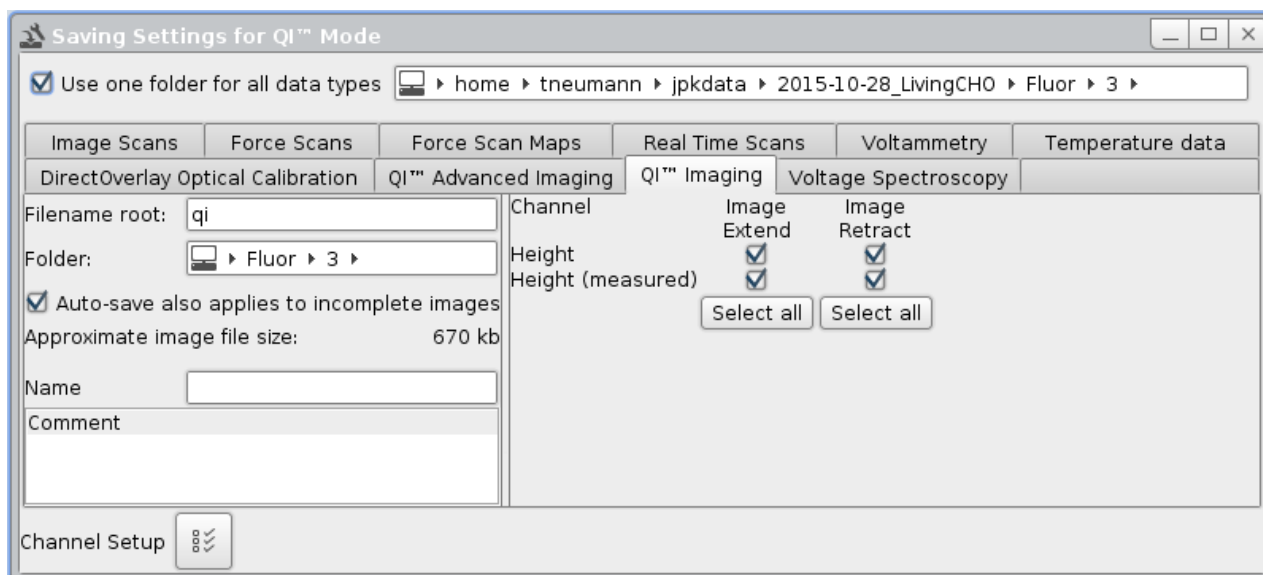
The QI™ Imaging mode provides pure image files. These contain the results of online height data analysis. The information is saved in **jpk-qi-image** files.

5.7.7 Cantilever recommendation

The choice of cantilever is important for the performance of QI™ and mainly affects the maximum achievable imaging velocity and the minimal possible setpoint. This means that the cantilever needs to be as short as possible, to reduce oscillations at high imaging speeds, but appropriately soft for fragile samples. In liquid environment, these requirements

are met, for instance, by the qp-BioAC cantilevers (http://www.nanosensors.com) or Biolever mini (http://probe.olympus-global.com). For QI™ mode in air, e.g. force modulation cantilevers, as the Multi 75 (http://www.tedpella.com) perform well.

5.8   Force Modulation Mode

Force Modulation Mode can be used to show material contrast, e.g. materials with different stiffness within one sample, such as polymer blends. This mode is something of a mixture between Contact mode and AC 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 is in contact with the sample, it does not lift off the surface as in AC 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.
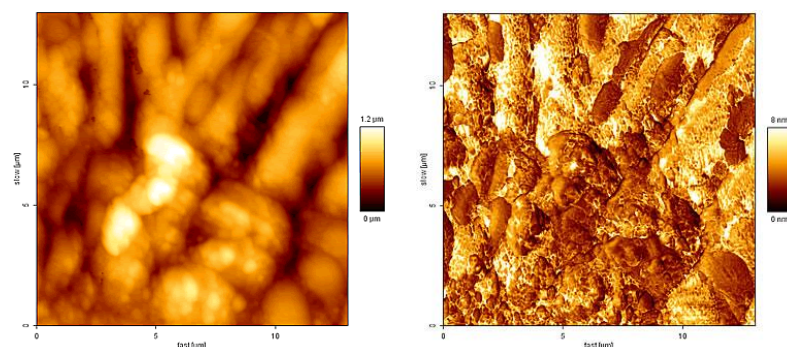
Height image   Amplitude image

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 resonance 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.
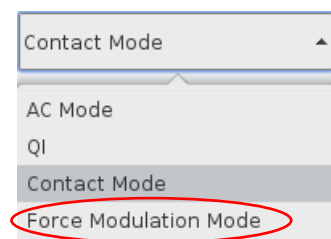
Select **Force Modulation Mode** from the feedback mode drop-down menu at the top of the software.

5.8.1   Off-resonance cantilever tuning

Upon selecting **Force Modulation Mode**, the cantilever tuning window will open. The **Cantilever Tuning** routine in **Force Modulation Mode** is basically the same as for the normal procedure for AC mode (see **Section 4.4.1**). In this case, however, the aim is not to find a resonance of the cantilever or system.
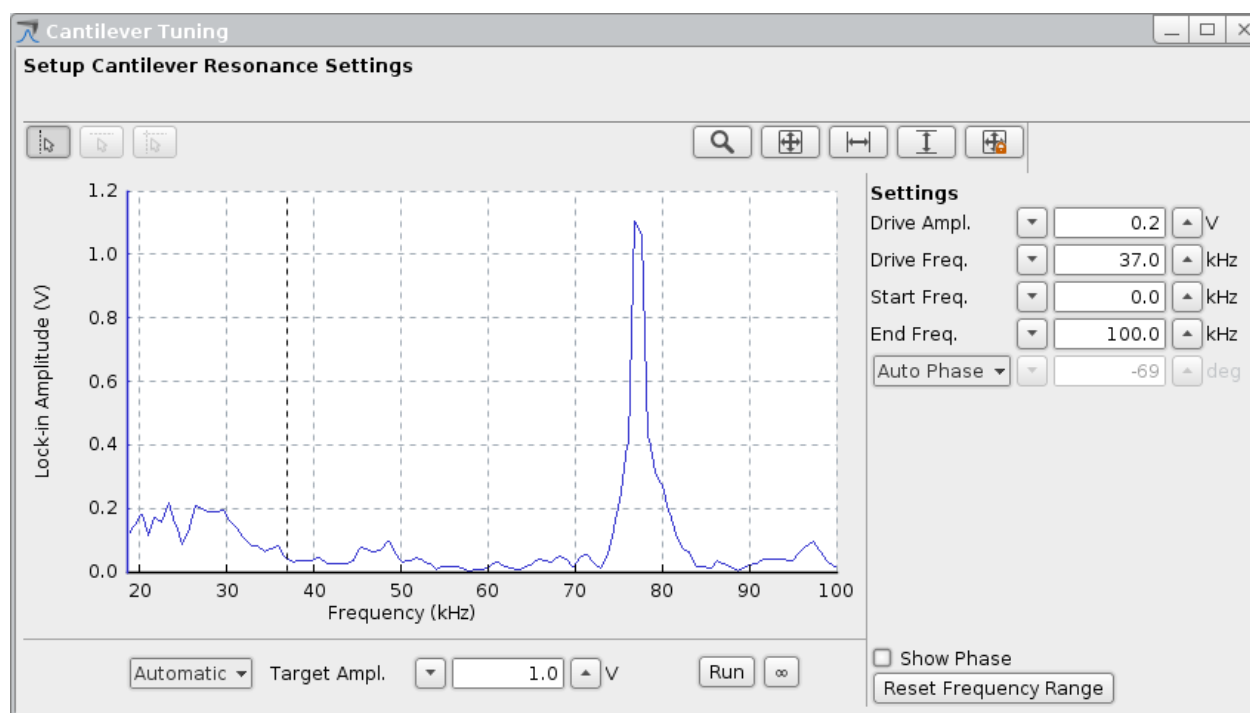
The cantilever is modulated at a relatively low frequency, well below its resonance frequency. There are special "force modulation" cantilevers available. The resonance frequency is around 75 kHz in air (k = 1-5 N/m). In Force modulation mode a typical drive frequency would therefore be 30 - 40 kHz. The force constant bridges the gap between contact and non-contact cantilevers. AC 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 to try another cantilever with a higher stiffness if there is no surface contrast.

5.8.2 Typical starting values

For the imaging, the **Setpoint** value is the average vertical deflection, so the feedback gains settings are 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)

5.9   Hover Mode

In **Hover Mode**, during a first pass (trace) over the sample the topography is measured, and on the return pass (retrace), this height information is used to maintain the cantilever at a constant offset height above the surface.
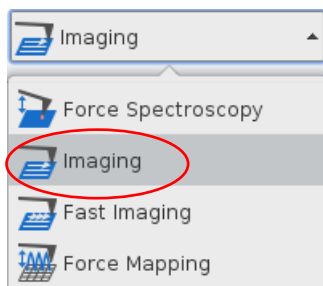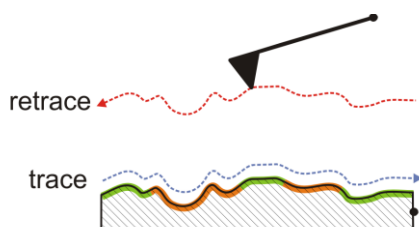

Hover Mode can be used in **Contact** or **AC** feedback mode. Select **Imaging** from the Measurement Mode drop-down menu.
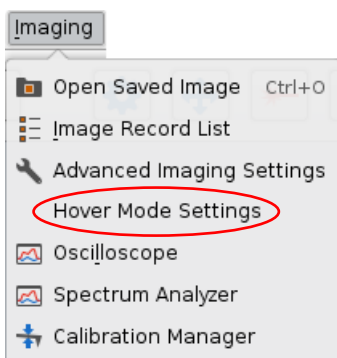
Open the **Hover Mode Settings** via the **Imaging** drop-down menu.

5.9.1   Hover Mode for Contact mode

During **Hover Mode** for **Contact Mode** the tip is following the measured height profile (trace). The retrace movement is accomplished without any excitation of the cantilever.
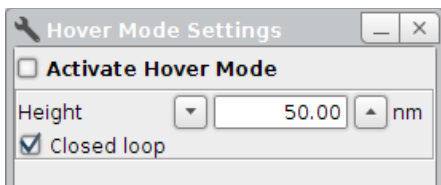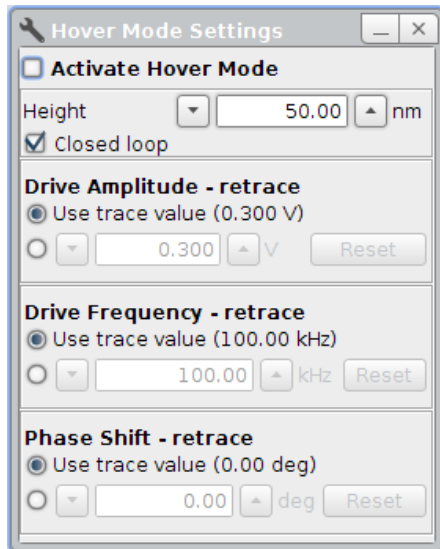

Hover Mode can be activated by a simple tick and the offset height for retrace can be customized within the **Hover Mode Settings** window.

5.9.2 Hover Mode for AC Mode
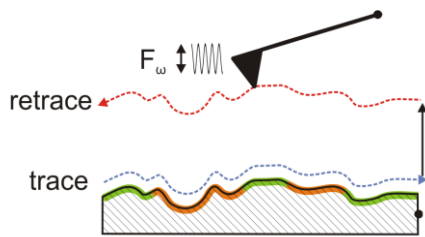
In contrast to Hover Mode for Contact Mode, **Hover Mode** for **AC Mode** contains an excitation of the cantilever.

The **Hover Mode Settings** window can be used for activating the Hover Mode and for adjusting the height offset.

The excitation of the cantilever for the retrace movement is per default the same as for trace. If needed it is possible to modify the **Drive Amplitude – retrace**, the **Drive Frequency – retrace** and the **Phase Shift – retrace** separately from each other.
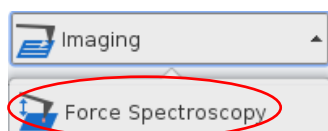
§ 6   Force Spectroscopy

The **Force Spectroscopy** mode performs force-distance measurements. 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. The cantilever tip moves vertically towards and away from the surface. As the tip is pushed against the sample surface, elasticity values can be measured from indentation. Moving away from the surface, adhesion can be measured, or the response can be measured for material or molecules stretched between tip and surface. The absolute forces can be measured if the cantilever is calibrated, i.e. the spring constant has been measured (see **Section 7.2**).

A simple way to become familiar with the **Force 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.
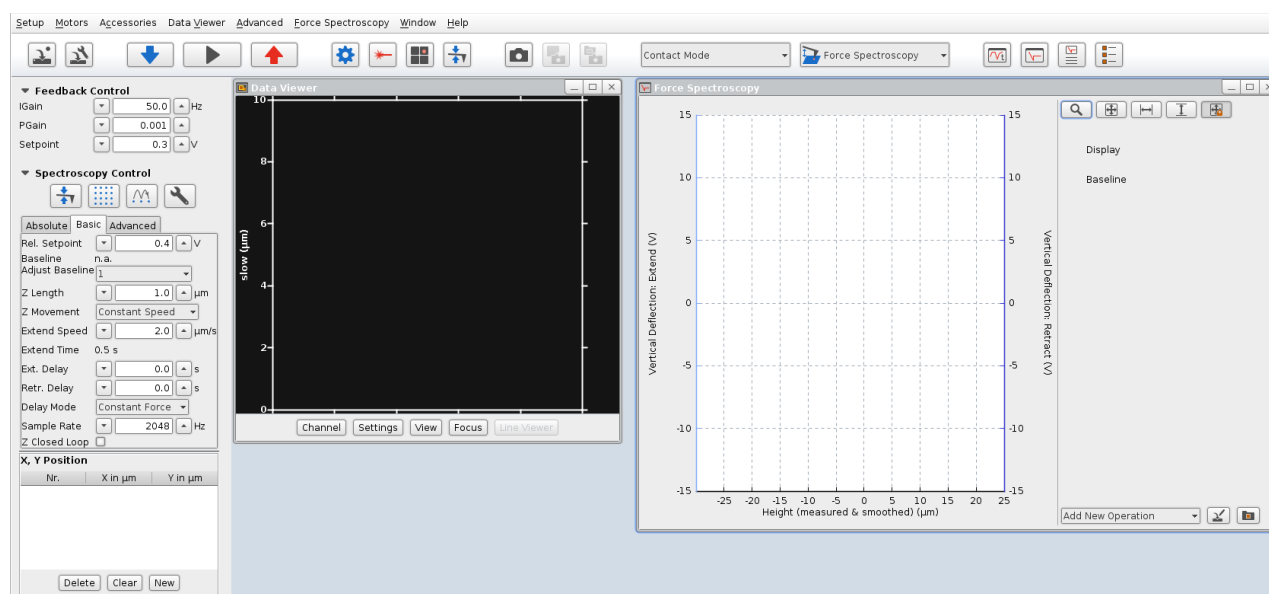
6.1   Overview of Force Spectroscopy Mode
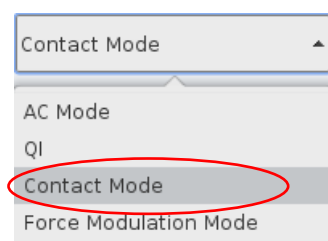
Select **Contact Mode** from the feedback mode drop-down menu at the top of the software.
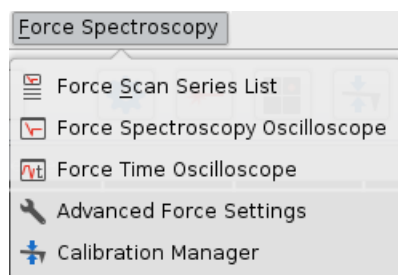
Select **Force Spectroscopy** in the **Measurement Mode** drop-down list.

On the left side of the main SPM window, the **Scan Control** panel is replaced by the **Spectroscopy Control** panel with

the parameters to control the tip movement during force spectroscopy experiments. The **Force Spectroscopy Oscillo-scope** which displays the force curves opens automatically.

The **Force Spectroscopy** drop-down menu appears in the menu bar. All necessary windows concerning Force Spectroscopy mode can be opened from this menu.

The shortcut icons at the toolbar on top allow for quick opening of the **Force Spectroscopy** (see **Section 6.1.2**) and **Force Time Oscilloscope** (see **Section 6.1.3**)to display the acquired force curves, as well as the **Force Scan Series List** to manage data saving (see **Section 6.6.2**).

6.1.1   Introduction to the Force Spectroscopy Control
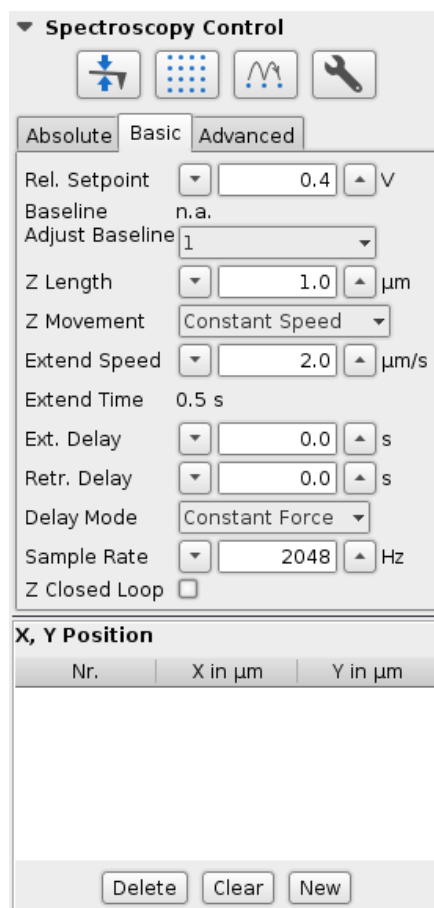
The shortcut icons at the top can be used to open the **Calibration Manager** (see **Section 7.2**), the **Spectroscopy Pattern Manager** (see **Section 6.4.2**), the **Force Scan Repetitions** (see **Section 6.4.1)** window or the **Advanced Force Settings** (see **Section 6.3**).

Tabs for ⊕ **Absolute**, **Basic** and ⊕ **Advanced** mode offer different control settings for the force scan movement. These **Basic** settings are explained in **Section 6.2** and provide an easy way to perform force curves including variable approach, delay and retract settings.

The **Advanced** mode is an optional software extension and is not included in the Basic software version. These **Advanced** mode settings are explained in **Section 8.6** and allow more complex force scans. Different types of force segments can be freely combined, such as length segments, relative force movement, force clamp and delays. Custom settings can also be saved and reloaded.

The **Absolute** mode (see **Section 8.5**) allows for driving absolute distances with the Z piezo and comes with the ⊕ **JPK ForceWheel™**.

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 6.4.2**). Alternatively the force experiment can be controlled as **Force Mapping** (see **Section 6.7**).

6.1.2   The Force Spectroscopy Oscilloscope

The **Force Spectroscopy Oscilloscope** opens automatically. The current force-distance curve is plotted in the main area of the window.
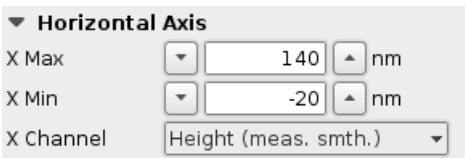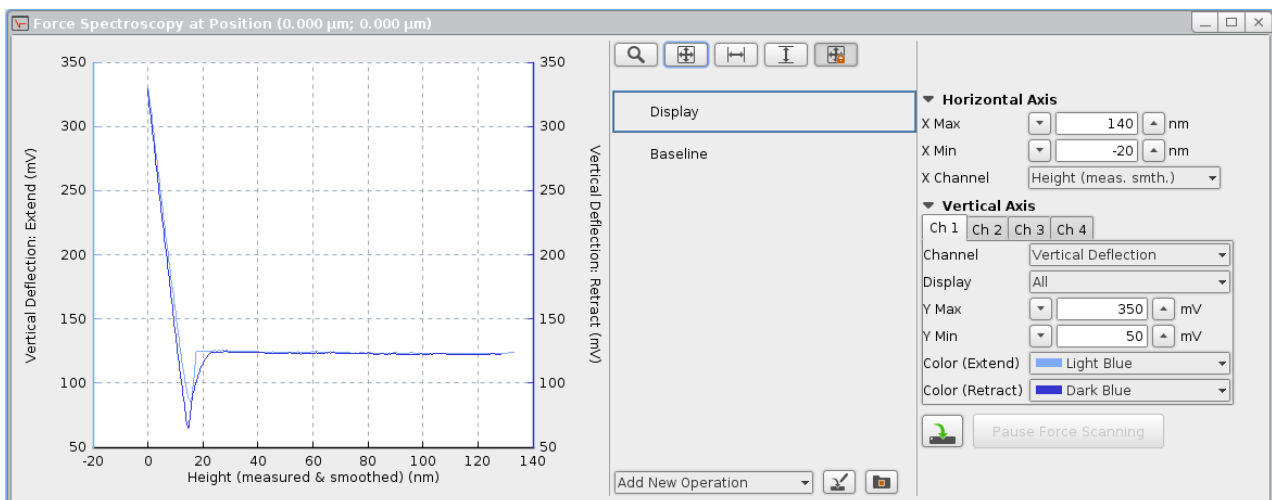
Use the shortcut button at the toolbar on top to open the **Force Spectroscopy Oscilloscope** if necessary. The oscilloscope can also be opened via the **Force Spectroscopy** drop-down menu.
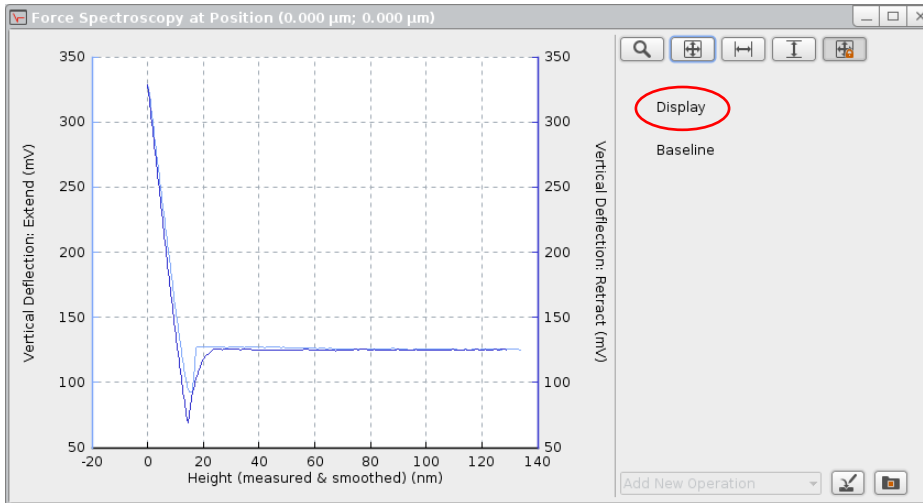
The standard oscilloscope toolbar on top can be used to modify the display settings, see (**Section 3.2.1**).

Click **Display** to show and adjust the display settings more specifically.

The standard **X 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 (see **Section 7.1.2**). This channel is used for the **Horizontal Axis** of the force-distance display.
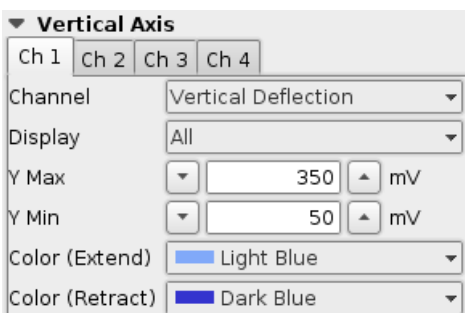
In contact mode the **Vertical Deflection** is directly related to the force, and this is used for the **Vertical Axis** of the force-distance display. Always make sure that the **Vertical Deflection** channel is enabled (default setting). If it is not available, see **Section 3.2.7** for details of the **Channel Setup** and **Saving Settings** controls. For force spectroscopy in other feedback modes, such as AC mode (see **Section 4.4.1**), the feedback channel is usually used.
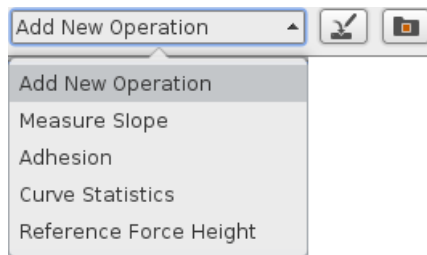
The force oscilloscope provides online analysis in terms of operations which can be chosen using the **Add New Operation** drop-down menu at the bottom of the Force Spectroscopy Oscilloscope. These operations are based on the JPK Data Processing software; please read the JPK Data Processing User Manual to learn more about parameter adjustment. All operations can be saved for loading into later sessions using the corresponding buttons at the right. They can also be generated or loaded and used in the JPK Data Processing software.

New operations appear in the tab in the middle. Click on the added operation to open and adjust the corresponding parameters:
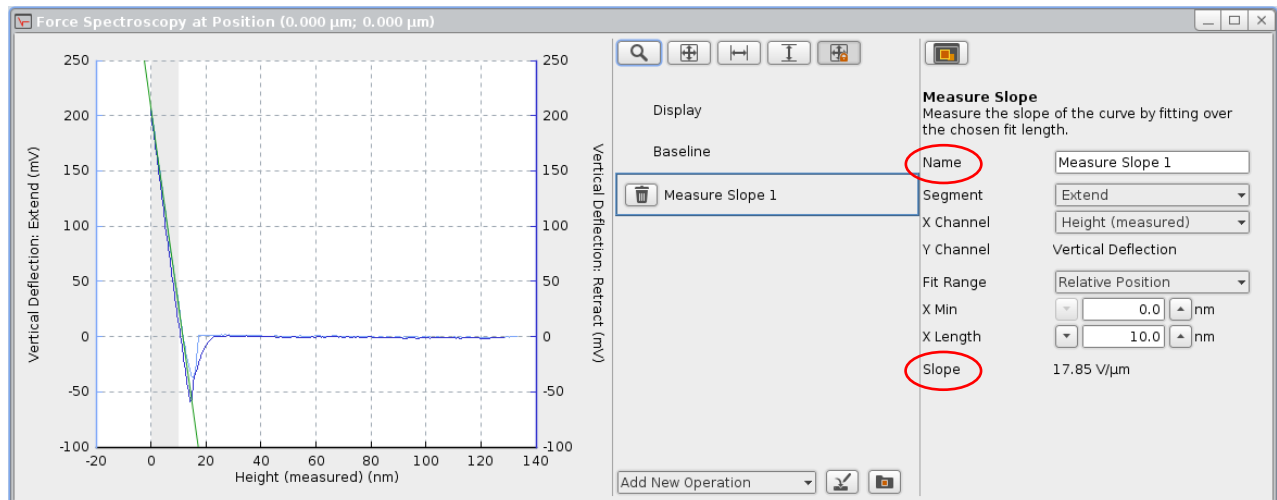
The parameter settings as well as the **Name** of new operations can be customized. The result is shown at the bottom of the operation settings panel (here **Slope**).
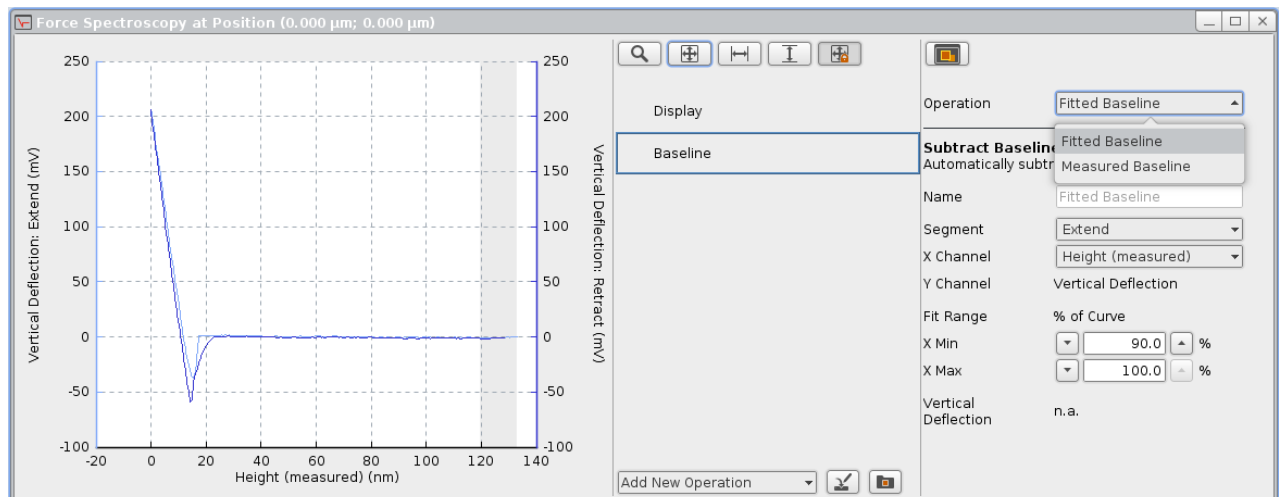
All online operations are only display-options and are not saved with the force file. Customized operations can be saved using the corresponding save button and opened/applied in the JPK Data Processing software.

You can find a short description of the selected operation on top of the operation settings panel.

The following operations are available:

Baseline

The **Baseline** offset is obligatory for most of the available operations to yield reasonable results. For this reason, the **Baseline** operation appears by default before any other operation can be applied. The **Fitted Baseline** operation is shown by default and the corresponding settings and results open in the right-hand panel upon selecting **Baseline**. Adjust the settings (**Fit Range**, marked in grey) using the **X Min**/**X Max** input fields or drag the fit range directly into the force curve display. The baseline is set to zero (y-offset) and used for subsequent operations. Also the **Measured Baseline** can be used for subsequent operations. This is the baseline, i.e. free vertical deflection, measured at the beginning of the force curve, which is also used for the **Adjust Baseline** feature (see **Section 6.2.1**).

Measure Slope
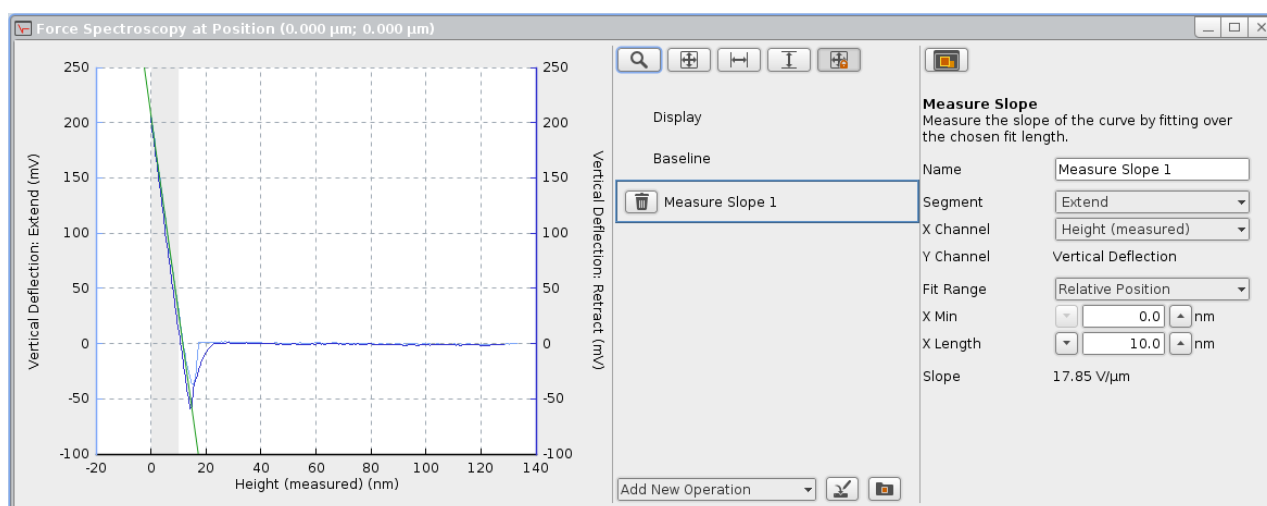
The **Slope** value is fitted with a linear fit (green line) over a defined **Fit Range** (marked in grey), starting, by default, from the end of the force curve at the sample. This can be changed by varying the **X Min** parameter. The **X Length** should be chosen to be shorter than the steep straight part of the force curve. The **Fit Range** can also be drawn directly within the force distance plot.

Adhesion
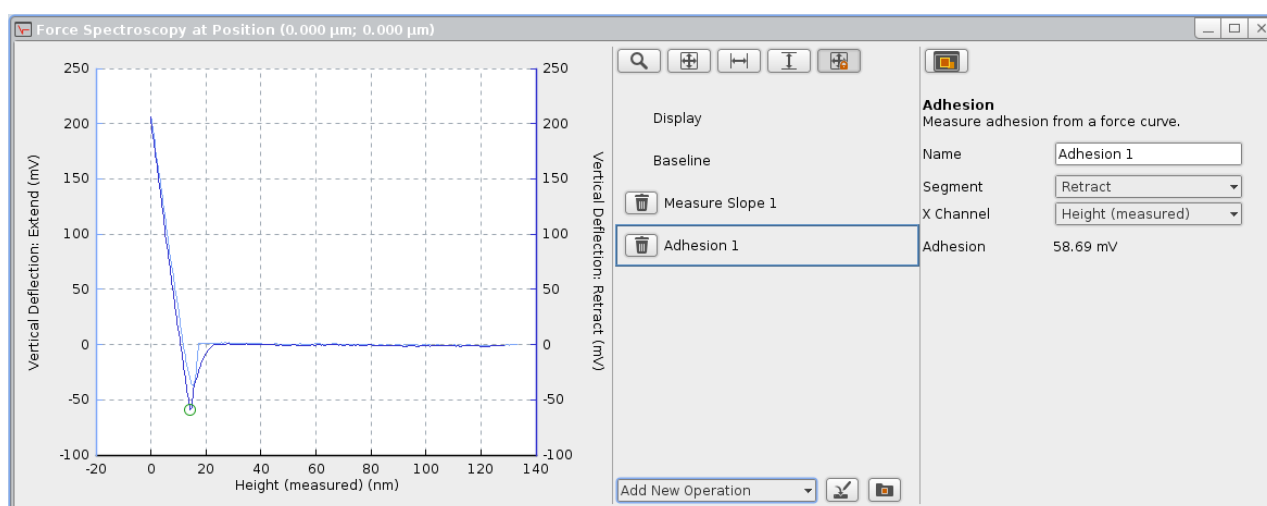
The **Adhesion** is measured as a single value, the difference between the lowest data point (green circle) and the base-line.

Reference Force Height
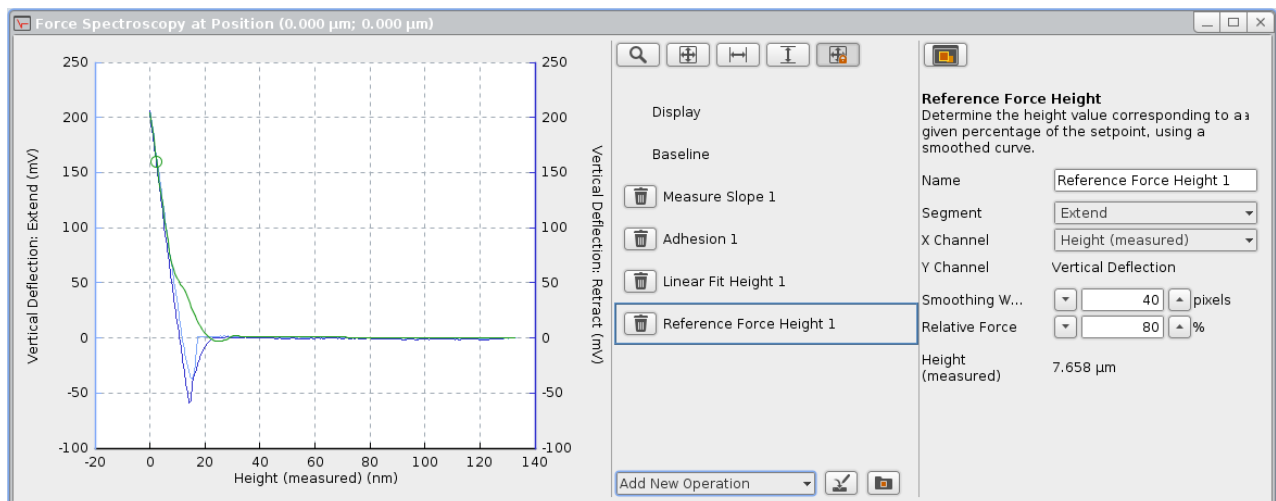
The **Reference Force Height** operation determines the height value at a particular percentage of the applied setpoint force. Therefore the raw curve is smoothened (green curve) by a moving average using the **Smoothing Width**, and the **Height** at the **Relative Force** (green circle) is determined. The height channel selected for **X Channel** is used for this calculation.

Curve Statistics
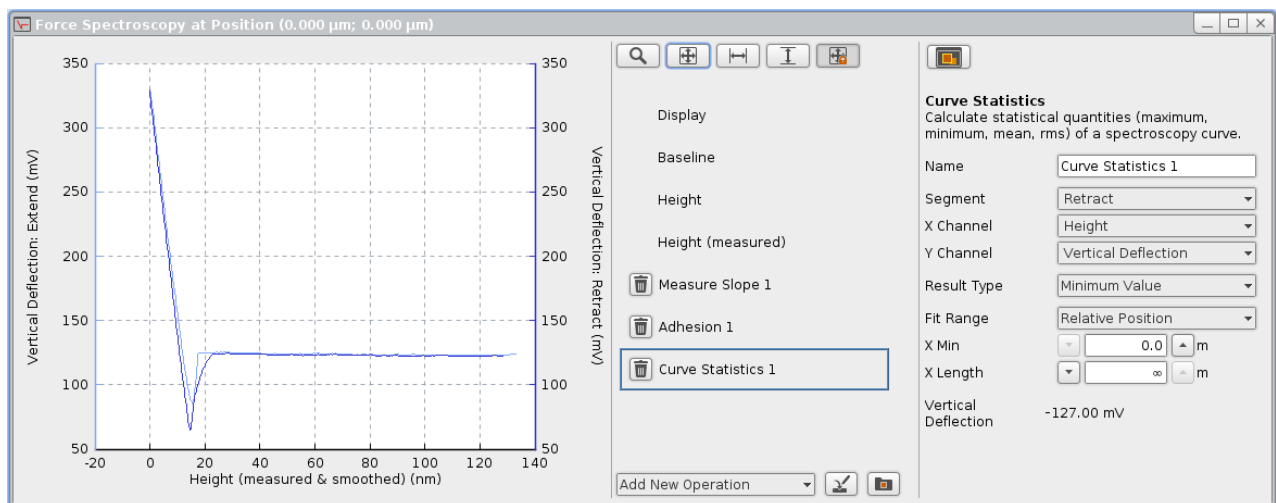
The **Curve Statistics** operation calculates common statistical values of the force curve: The **Minimum** and **Maximum Value** along with their **Position** on the X axis, the **Mean Value** and the **RMS Value**. Choose the desired **Result Type** to show the corresponding result at the bottom. By default, all data of the selected **Segment** are used for statistical analysis. Adjust the **Fit Range** by drawing into the force data plot or using the **X Min** and **X Length** input fields.

Invalid operation warning
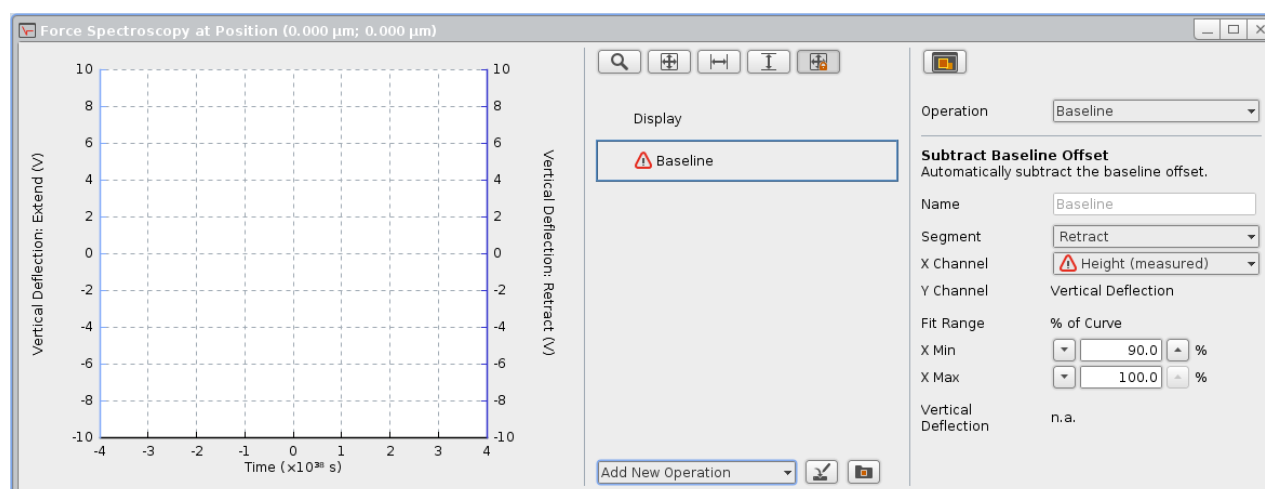
Active operations might become invalid if they lose their channels or segments (e.g. in Advanced Spectroscopy mode, **Section 8.6**). This fact is indicated by a small warning icon. In this example, the Baseline operation is invalid because the Height (measured) channel is missing. In case of invalid channels open the **Channel Setup** (**Section 3.2.7**) and check if the corresponding channel is activated.

⚠ Always check whether all required channels are activated in the **Channel Setup** (**Section 3.2.7**) when the invalid operation warning icon appears for any operation. If the required channels are not activated, i.e. no data are collected, the corresponding operation is invalid and cannot analyze or show any data!
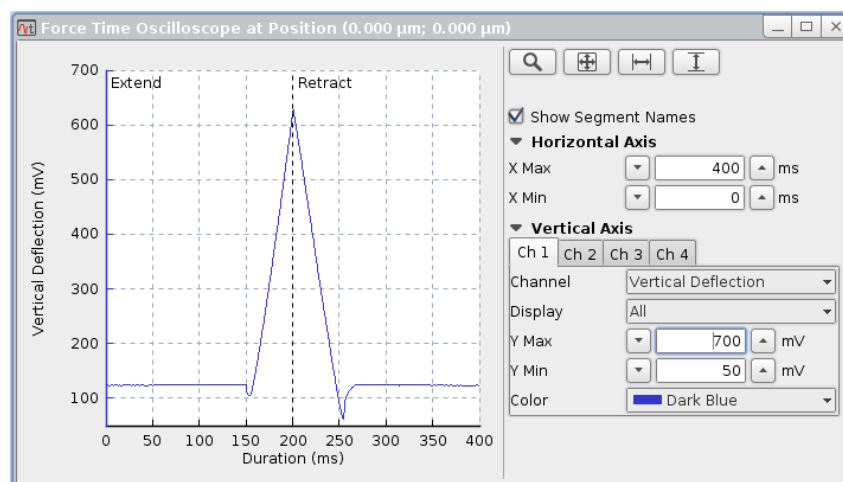
6.1.3   The Force Time Oscilloscope

Open the **Force Time Oscilloscope** using the corresponding icon in the shortcut icon toolbar when force spectroscopy mode is active. Alternatively, the oscilloscope can be opened via the Force Spectroscopy drop-down menu.

The main **Force Spectroscopy Oscilloscope** described 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 height) is plotted against time.

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

Choose the desired **Channel** to be displayed over time (**Duration**).

Adjust the view using the standard oscilloscope toolbar (see **Section 3.2.1**) or the **X/Y Max/Min** input fields.
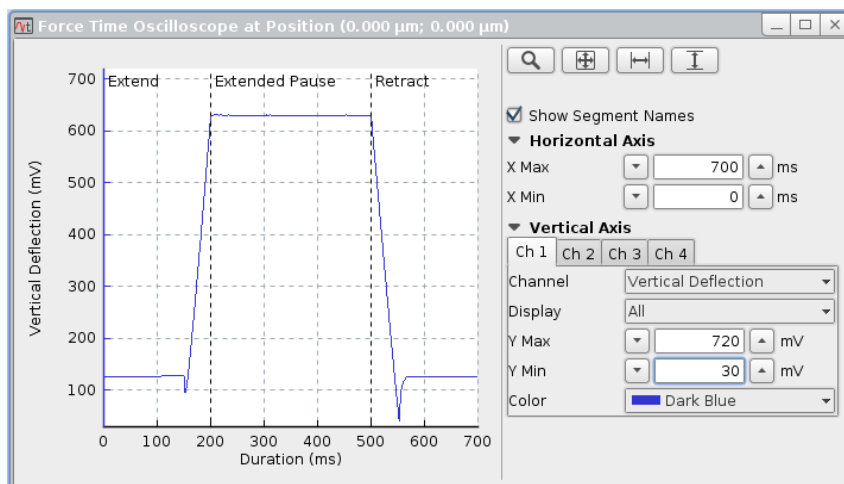
The **Force Time** display also includes the data collected during pause or delay segments, while the oscilloscope in **Section 6.1.2** only displays the extend and retract movements. The example here shows the data curve for a movement with 300 ms **Extended Pause**, (see **Section 6.2.2** for details).

The time data are saved by default and can be displayed within the JPK Data Processing software as well.

6.2   Basic Force Spectroscopy Mode

In **Basic** mode, the surface position is defined by the **Relative Setpoint** value of the feedback channel (i.e. **Vertical deflection** in contact mode), i.e. the cantilever is moved towards the surface until the Relative Setpoint is reached. The piezo movement **Z length** is performed relative to this position. This is particularly helpful when the lateral position changes between curves, and the surface height is different from point to point.

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. **Z-Length** defines the range of the force curve. **Z movement** can be used to choose between **Constant Duration** and **Constant Speed** for the tip movement. Depending on the selected setting it is possible to adjust the **Extend Time** or the **Extend Speed**.

The bottom part of the panel has the main parameters to control the timing of the force curves (see **Section 6.2.2**).

The **Relative Setpoint** is independent from the **Setpoint** of the **Feedback Control**. The Feedback Control parameters do not influence the motion during a force spectroscopy experiment.
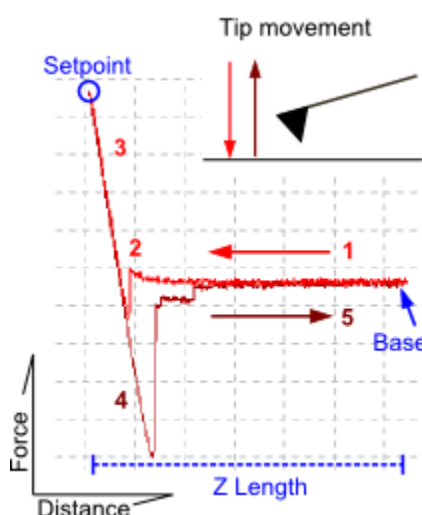
The z piezo extends, moving the cantilever towards the surface until the setpoint is reached and retracts it again.

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 in very close contact with the sample. The movement stops when the vertical deflection reaches the **Relative Setpoint** value (blue circle).

4. Retraction of the cantilever while the tip is still in contact (adhesion).

5. Tip is pulled free from the surface. The movement stops after a total retraction of **Z Length**.

For the extend part of the motion, the surface position is not necessarily known in advance. The piezo extends, moving the cantilever towards the surface, and data is collected continuously. Once the surface position is found, the data for the last **Z Length** of piezo movement is stored as the extend part of the curve. For the next force curve, the expected starting position is used from the previous curve, plus an extra safety distance.

6.2.1   The Baseline function

In contact mode, the **Baseline** (see also drawing above) can be used 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 absolute value of the **Vertical deflection**.

The **Adjust Baseline** is set to 1 by default, i.e. the baseline is measured and used to calculate the Relative Setpoint before every force curve. The interval can be increased if fast spectroscopy measurements are being made, or if the environmental changes are small. For each **Baseline** measurement, 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**.

In this example, the baseline is being adjusted for each spectroscopy curve. The **Baseline** from the last curve has been measured as 125.3 mV. If the **Relative Setpoint** is set as 0.2 V, then the force curve will use the **Vertical deflection** value of 0.3253 V as the turnaround point.

6.2.2   Timing settings

Extend Time/Speed controls the speed of the movement on both extend and retract parts of the force spectroscopy experiment, as they are the same in Basic force spectroscopy mode.

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. This is always at constant height (Z-position).

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

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

The **Delay Mode** sets the feedback during the Extended Delay:

For **Constant Height** the piezo height is held constant at the surface position.

For **Constant Force** the piezo height is adjusted to keep the cantilever deflection at the setpoint value.

The Sample Rate is the rate at which data values are stored through the whole force curve. Combined with the times for each part of the movement, this defines the sample number for each segment of the force spectroscopy cycle.

A higher sample rate better resolution but a larger data file. If the rate is too low, particularly for a long **Z length** value, it

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may affect some control options, (e.g. **Tip Saver** or **Relative Setpoint**). There must be enough data samples so the software can react to limit the deflection.

6.2.3   Z closed loop

If **Z 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. Since the movement is relative to the surface in Basic mode, the absolute position is not set in advance. In open loop, e.g. **Z Closed Loop** disabled, the piezo voltage is increased and decreased and the collected data is displayed against **Height (measured)** at the end, so there are no errors in the position information in the final force curve.

It is critical to use **Z Closed Loop**, however, where the speed of the motion is important. In closed loop, the **Height (measured)** position at each point of the force curve is used to correct the piezo voltage, and the force curve has a constant speed. Closed loop also becomes most important for larger Z lengths, where the piezo nonlinearity is more significant. **Z Closed Loop** can introduce a small amount of noise, as there is an extra feedback. This is not usually significant for the low-noise Z sensors of the NanoWizard® AFM heads.

6.3   Advanced Force Settings

The **Advanced force Settings** window can be opened via the **Force Spectroscopy** drop-down menu in the main menu list or using the corresponding shortcut icon in the **Force Spectroscopy Control** panel. The **Advanced force Settings** provide extra parameters in addition to those found in the **Spectroscopy Control** panel.
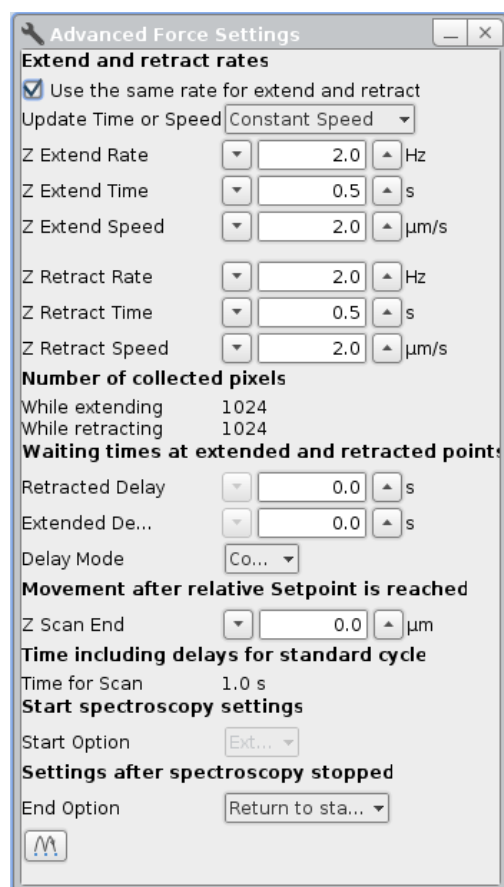
In the **Advanced Force Settings** window the **Z Extend Rate** and **Z Retract Rate** can be set to different values, if the **Use the same rate for extend and retract** box is unticked.

Z Speed, **Z Length** and **Z Time** for extend or retract segments depend on each other. **Update time or speed** helps to either maintain the duration of a segment (**Constant Duration**), or to maintain the Z Speed (**Constant Speed**).

The sampling rate is displayed for extend and retract segments.

The delay parameters can be adjusted as well as the **Delay Mode**.

There are different **Settings** available **after spectroscopy is stopped**. **Return to starting mode** is selected by default. **Constant height mode** maintains the end position of the last force curve segment. **Retracted piezo mode** and **Idle mode** turn the piezo into retracted and approached mode respectively.

6.4   Selecting spectroscopy points

By default the force spectroscopy experiments are performed in the center of the currently selected scan area. The measurement points can also be freely selected within the whole scan area, either as single points or a defined pattern.

6.4.1   Point selection and the Position list
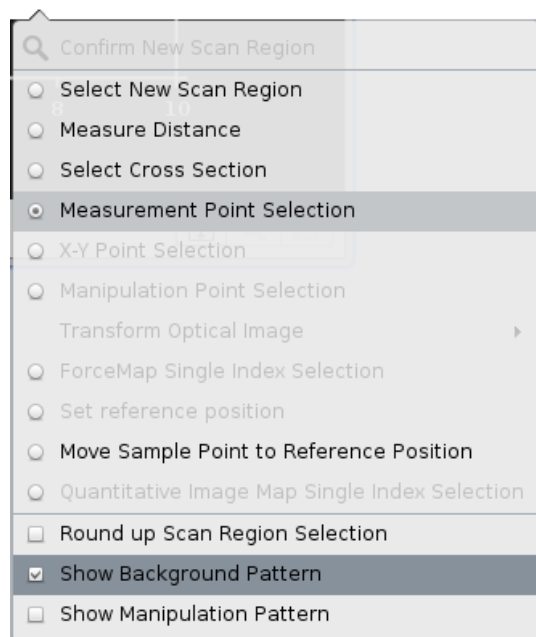
When **Force Spectroscopy** mode is active, measurement points can be selected directly in the **Data Viewer** window.

Right-click in the **Data Viewer** window and choose **Measurement Point Selection**. Select the desired measurement position in the Data Viewer window using the left mouse button. The coordinates of the measurement position appear in the **X,Y Position** list of the **Spectroscopy Control** window (see below). The coordinates are relative to the center of the 100 x 100 µm scanner range (not necessarily the current scan region).

If an image has been made with the same cantilever, it is easy to select particular points within a scanned image.

Several measurement positions can be selected and activated by clicking on them in the **X, Y Position** list of the control panel. The selected spectroscopy point is highlighted in green in the **Data Viewer**, and is highlighted in the **X, Y Position** list.

Double-click on the table entry to type new values to set an absolute position.

Delete removes the currently selected position. **New** adds a new position; type the coordinated manually into the corresponding input fields. Use **Clear** to delete all positions.

By default, the position of the tip is only changed when another point in the list is selected, **Run** will repeat the movement indefinitely at the current point. To move automatically from point to point, open the **Force Scan Repetition** settings using the shortcut icon on top of the **Spectroscopy Control** panel.
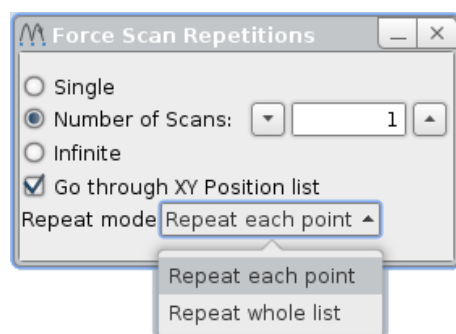
Single scans, an arbitrary **Number of Scans** or **Infinite** scans can be recorded when the **Run** button is clicked.

Go through XY Position list goes through all the positions in the list. If **Number of Scans** is active and greater than 1, there are two settings for the measurement sequence. **Repeat each point** makes the set number of scans at each point before moving. **Repeat whole list** makes one force measurement at each point before moving, and goes through the point list the set number of times.

6.4.2 Spectroscopy Pattern Manager

Open the **Spectroscopy Pattern Manager** using the shortcut icon on top of the **Spectroscopy Control** panel to create grids of spectroscopy points.
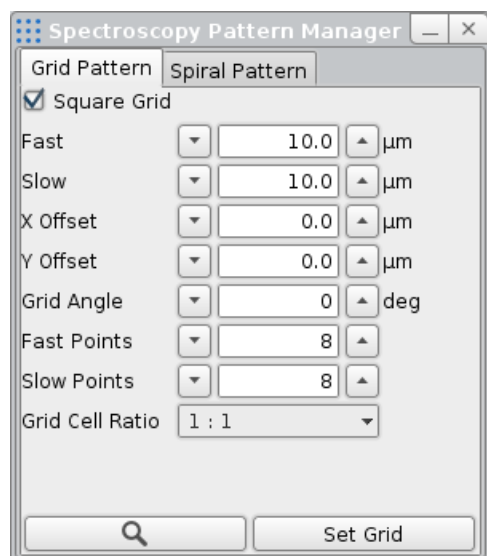
The **Spectroscopy Pattern Manager** allows a grid of spectroscopy points to be generated automatically. This is similar to performing a **Mapping** experiment (see **Section 6.7**), but the points can be measured independently and the force spectroscopy files are handled separately. The settings in the **Spectroscopy Pattern Manager** have the same definitions as for selecting scan regions. The values are automatically initialized from the current scan area, but new values can be set anywhere within the full piezo range.

Update Position takes the **X** and **Y Offset** position from the current cantilever position. The sizes and offsets can be typed directly in the input fields, and the grid will be updated when **Set Grid** is clicked.
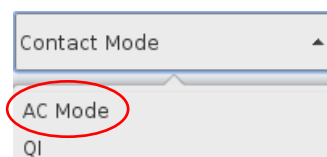
The spectroscopy points list in the **Spectroscopy Control** panel automatically updates when the grid is set, and points can be added or moved as normal. If any positions have been changed, the **Spectroscopy Pattern Manager** will be greyed out. The last grid can be restored using **Set Grid**.
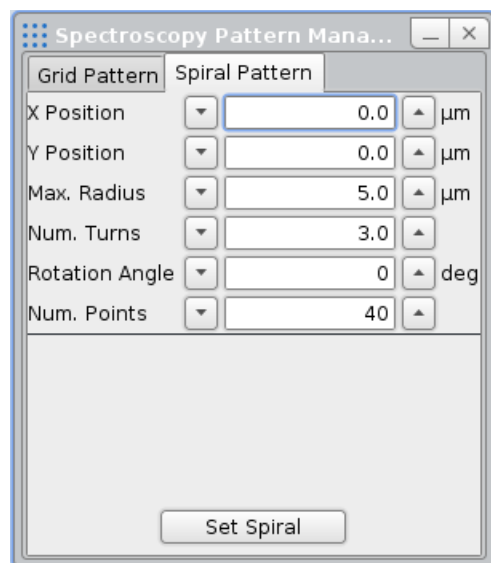
Also **Spiral Patterns** can be generated. Choose the corresponding tab and define the desired position, size and **Number of Points**.
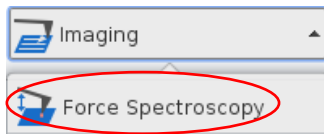
6.5   Force Spectroscopy in AC Mode

Typically, force measurements are performed in contact mode. Force-distance experiments can also be performed in AC modes, however, measuring the amplitude or phase of the cantilever during the force spectroscopy cycle.

Select **AC Mode** from the feedback mode drop-down menu at the top of the software.

The **AC Feedback Mode Wizard** will open. Perform the cantilever tuning as described in **Section 4.4.1**.

Select **Force Spectroscopy** in the measurement mode drop-down list.

Like in contact mode based force spectroscopy, the **Scan Control** panel is replaced by the **Spectroscopy Control** panel and the **Force Spectroscopy Oscilloscope** which displays the force curves opens automatically. Also the **Force Spectroscopy** drop-down menu appears in the menu bar as well as the force spectroscopy related shortcut icons. Please read **Section 6.1** for details.
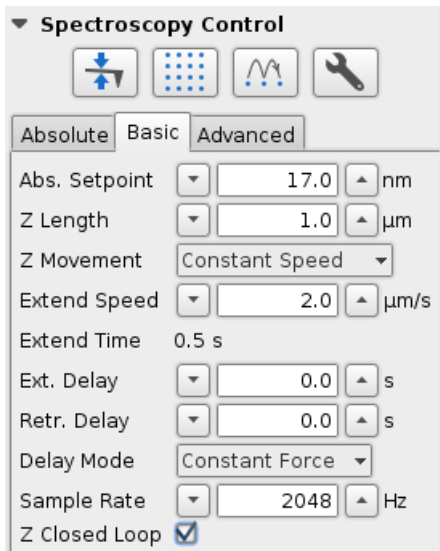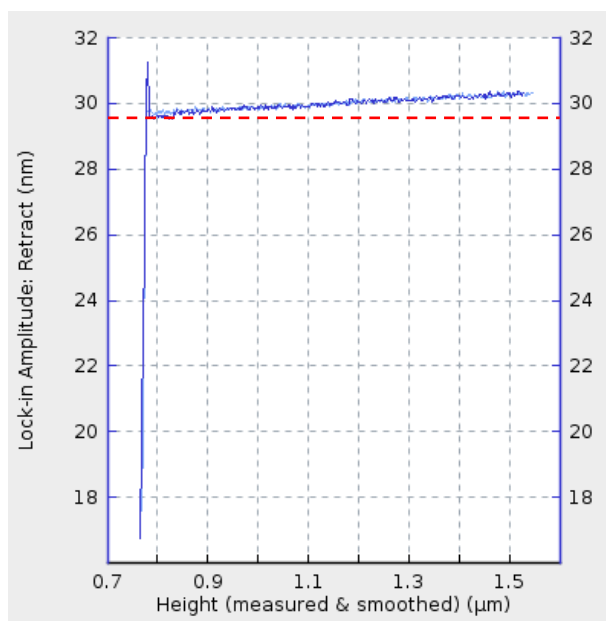
In AC mode based force spectroscopy, the surface position is defined by the **Absolute Setpoint** value of the feedback channel (**Lock-in Amplitude**), i.e. the cantilever is moved towards the surface until the Absolute Setpoint is reached.

Please read **Section 6.2** for a detailed description of the Spectroscopy Control settings.

When the **Force Spectroscopy Oscilloscope** window is opened in AC mode, the default channel is **Lock-in Amplitude**. Close to the surface, the Lock-in Amplitude decreases from the free value (on the right) as the cantilever approaches the surface (towards the left).
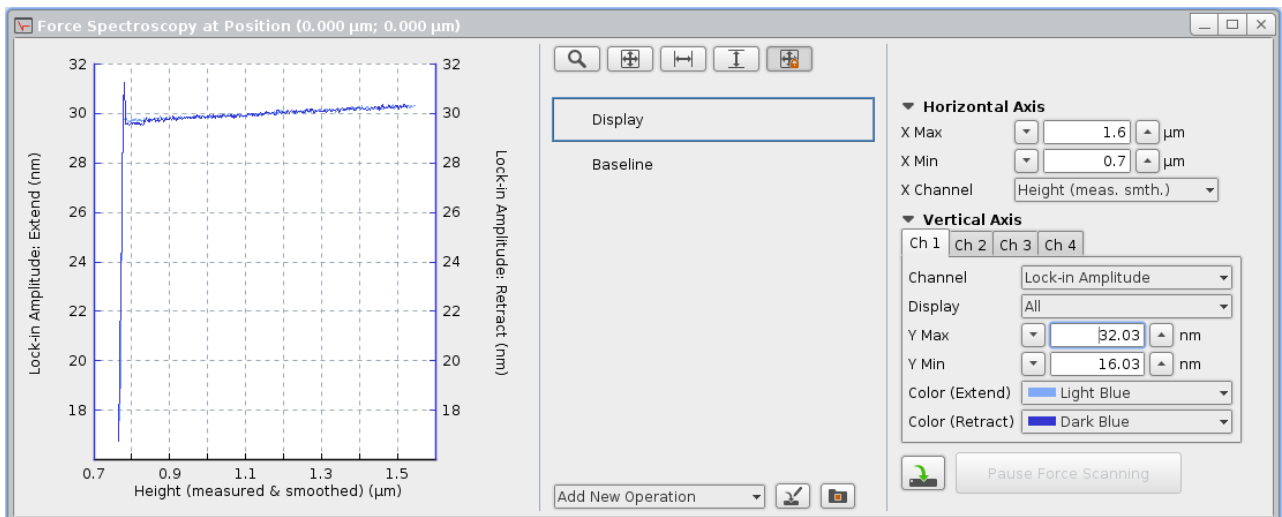
Other channels, including the **Phase** can also be selected for **Vertical axis** on the right of the **Force Spectroscopy** oscilloscope. Select the **Display** Tab and choose a different Channel, e.g. Ch 2 to display the Phase deflection simultaneously with the Lock-in Amplitude.

Force spectroscopy in AC mode can help to find a suitable imaging setpoint for AC Mode imaging. Strong tip sample interactions or measuring in liquid result in amplitude changes/damping, even though the surface has not been reached (amplitude regime above the red line). This unstable behavior makes it difficult to find a reasonable imaging setpoint. If an amplitude distance curve is recorded, the regime where the amplitude starts to decrease linearly (below the red line) becomes visible and allows for setting the setpoint appropriately (e.g. somewhere below the red line).

6.6   Managing and saving spectroscopy curves

6.6.1   File saving

After obtaining a force spectroscopy curve, save the data using the **Save Force Scan** shortcut icon in the **Spectroscopy Oscilloscope**.


Enable the **Autosave** function at the shortcut icon toolbar to save each completed force distance curve automatically (see **Section 3.2.8**).

The force curves produced during force 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. Force curves can be converted into simple text using the JPK **Data Processing** software, either individually or as a batch for a whole folder of files. Alternatively, a script can be used to convert a folder of force curves to this text format.

All parts of a force curve (trace and retrace segments as well as time delays) can be saved in the .jpk-force force curve format. The only part of a force curve that is not saved is the retracted pause between one curve and the next.

Open the **Saving Settings** via the **Setup** drop-down menu to define the settings for all file formats. Choose the **Force Scans** tab and adjust the naming, storage location and channels to be saved as described in **Section 3.2.8**.
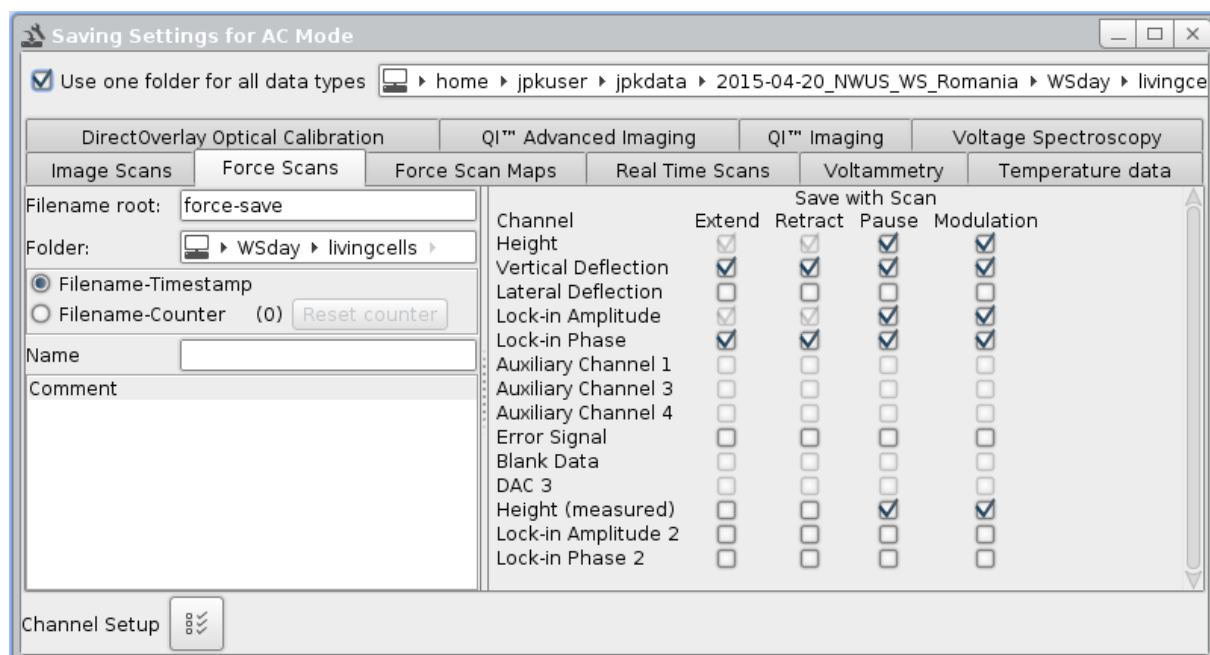
Extend and **Retract** of **Height** and **Vertical deflection** channels are required data for collection. Other channels can be enabled if required for the experiment.
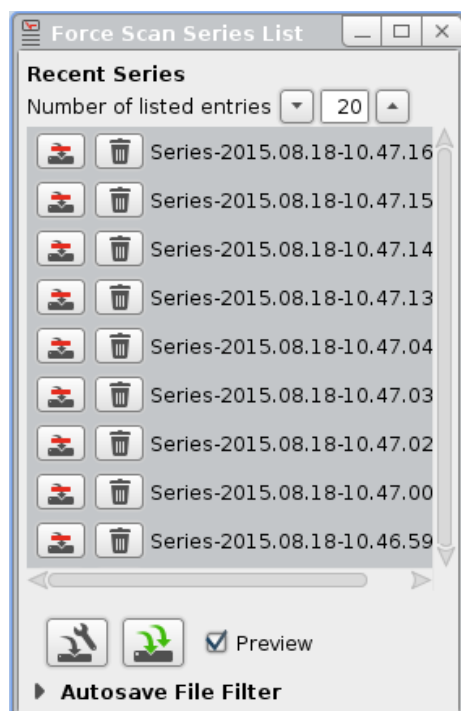
 During force scanning, the keyboard shortcut **Ctrl-F** can be used at any time to save the last recorded force curve. The name and curve settings are all taken from the **Saving Settings** panel.

6.6.2 Force Scan Series List

 The **Force Scan Series List** manages the saving and removal of force spectroscopy scans.

When a scan has finished, the file is automatically shown in the **Force Scan Series List**. Only a limited number of scans are held; older scans are removed automatically as new scans appear. The number can be changed using **Number of listed entries**. Note that the more scans you store in **Recent Series** list, the more memory is used.

 Files with this symbol are not yet permanently saved to the hard disk. At any time the scan can be saved clicking directly on the icon, or with the right mouse button on the scan name/number and selecting **Save data.**
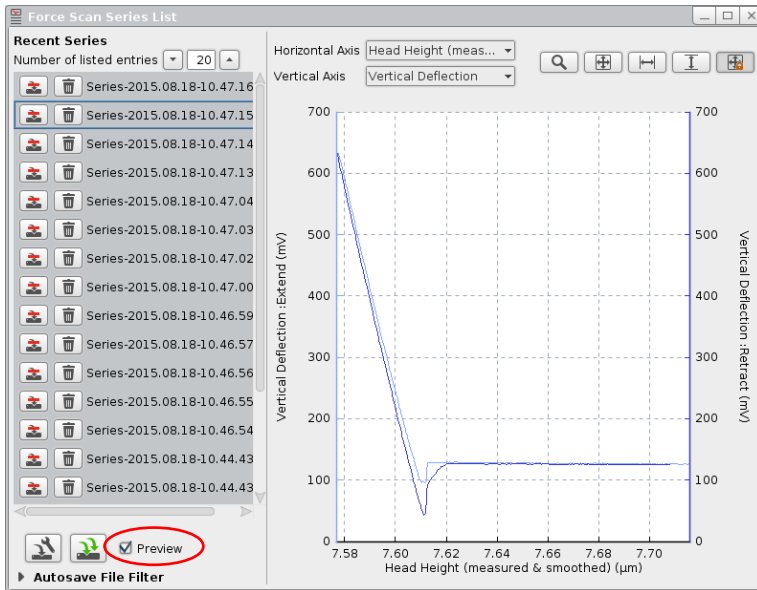
 Once the file has been saved, it will be displayed with this icon.

 The **Remove** icon removes the image from the list.

If the scans have been saved (either manually or with **Autosave**), they are still stored on disk even if they are removed from the list, either by clicking on **Remove** or by letting them reach the end of the list**.** If they have not been saved, they are lost permanently.
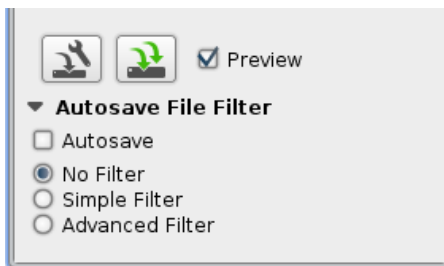
Enable **Preview** to show the force scan in the scan viewer (select the scan in the list and the name is highlighted with a blue box).

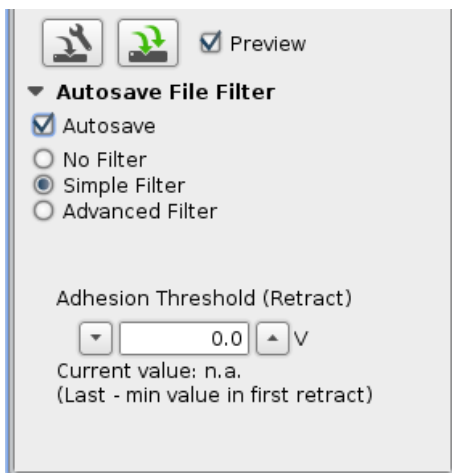**Saving settings** opens the window for setting the saving directory and channels

**Save all** saves all the current files in the list

The **Autosave File Filter** automatically manages where and whether the files are saved. **Autosave** must be switched on for the filter to be used.

If **No Filter** is active, all force scans are saved using the default settings.

The **Simple Filter** uses the value of **Adhesion** that can be calculated for all force curves (see 6.1.2). If the **Adhesion Threshold** is not reached during retraction, the curve is not saved. Note that the threshold value set here is an absolute value, even though the adhesion has actually a negative sign in the retract curve.
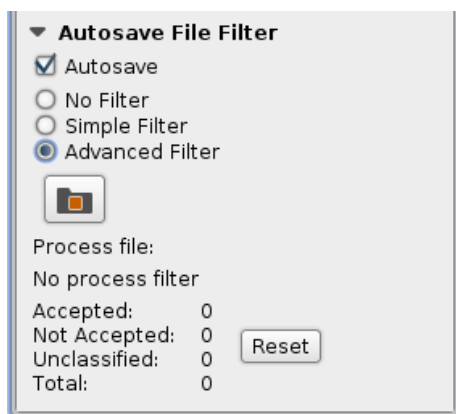
The **Advanced Filter** allows very complex filtering operations, as the full potential of the JPK **Data Processing** software is used. The analysis must first be set up in the **Data Processing** software and saved as a process file. Any combination of operations can be used for the analysis, but the process must include one filter operation. The filtering can be on the results of any operation, or a combination of them. The process must be saved in the normal way, as if for batch processing, this creates a file with the extension *.jpk-proc-force. Please read the **Data Processing** manual to see in detail how a process file can be created and saved.

Open a Data Processing file for filtering.
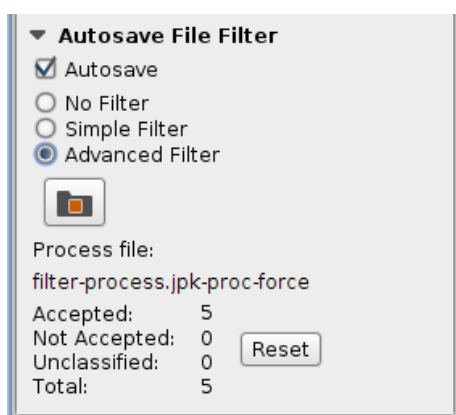
Once the process has been selected, the **Process file** name is shown in the panel. Each force curve is processed in the background, and only the results of the filter are shown:

Accepted is the number of files that have a filter result 1 or true.

Not Accepted is the number of files that have a filter result 0 or false.

Unclassified is the number of files that produced an invalid filter result.

Total is the total number of curves saved since **Reset** was clicked.

All the files are saved. The filtering is complex and happens in the background, so it is a safeguard that the files can be recovered later if required. The files are saved in subfolders named for the filter results. The typical case is that two sub-folders are created with the names **1** and **0**, where the accepted and not accepted force curves are saved.

If there is a folder **unclassified**, then there were unexpected results from the filter (fit did not converge, missing channels etc.). Open one of these curves in the **Data Processing** software and apply the saved process file to see what the problem was. Please note that **Reset** just sets the counters in the filter panel to zero. It does not create new folders or change the saved data.

6.7   Force Mapping

6.7.1   Introduction to Force Mapping

Force Mapping is an extension of the **Force Spectroscopy** mode. Please read **Section 6.1** and **6.2** for a close description of Force Spectroscopy mode and its functionality. The tip movement in Force Mapping is similar to normal force spectroscopy, but the measurements are performed over a grid, and the force curves are analyzed online to give a value at each point. These values are displayed as Force Mapping images in the Data Viewer window.
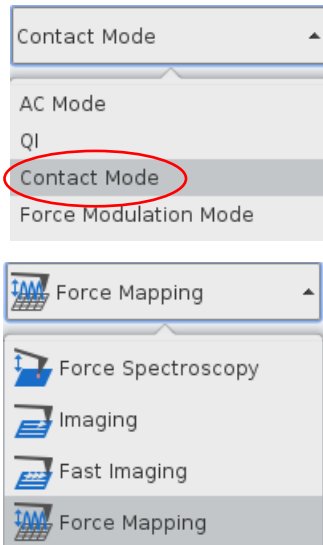
Select the desired feedback mode, normally **Contact Mode**, from the feedback mode drop-down menu at the top of the software.

To perform Force Mapping in AC Mode, please read Section 6.5 for a further description of Force Spectroscopy in AC Mode.

Select **Force Mapping** from the **Measurement Mode** drop-down box.

On the left side of the main SPM window, the **Scan Control** panel is replaced by the **Force Mapping Control** panel with the parameters to control the tip movement during force mapping experiments. The **Force Scan Map Oscilloscope** which displays the force curves opens automatically as well as 3 **Data Viewers** displaying the results of the online analysis.
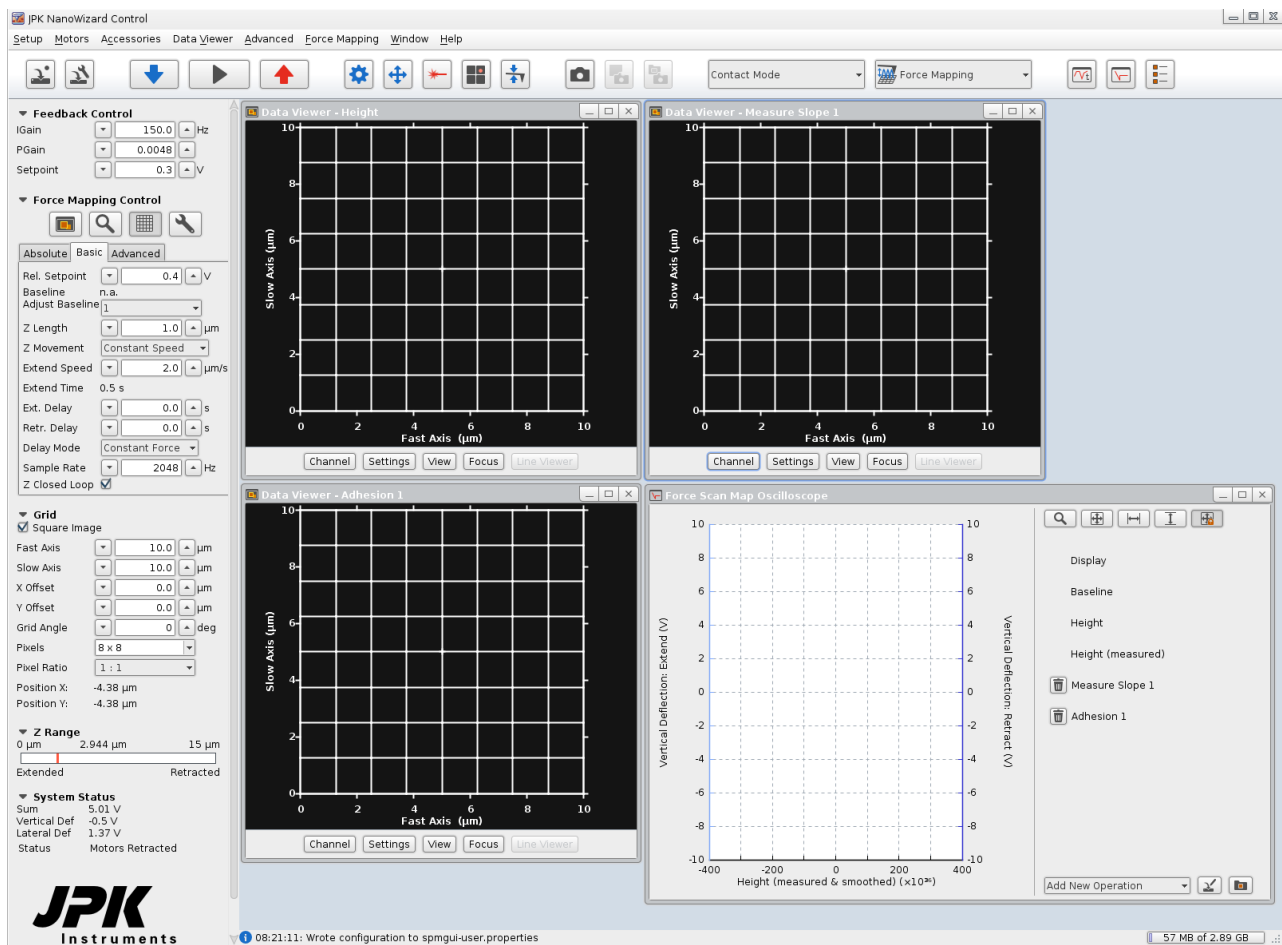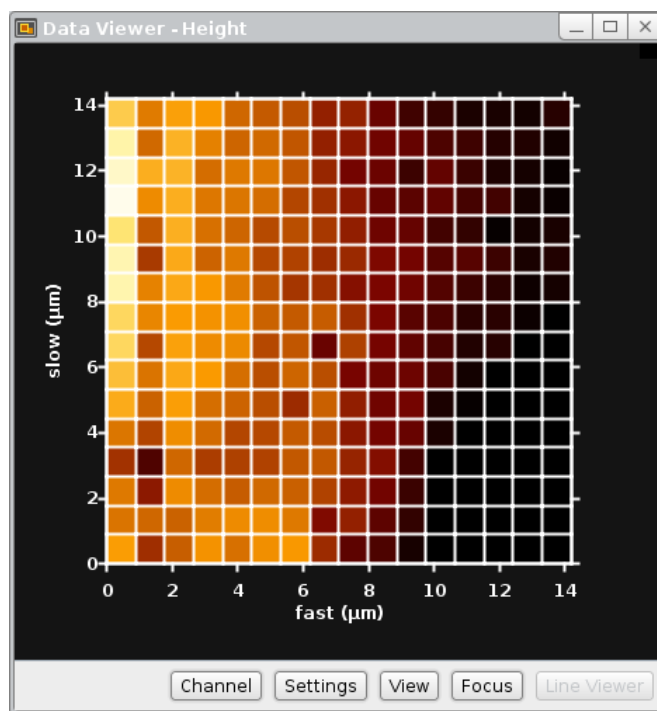
The map in the **Data Viewer** shows a grid of filled pixels, whereas a single force spectroscopy curve is acquired at the center of every pixel.

Values for **Slope**, **Adhesion** and **Height/Height (measured)** are calculated automatically from each force curve (see **Section 6.1.2**), and these values are used to generate the images seen in the Data Viewer. The height channels correspond to the reference force height described in **Section 6.1.2**. In brief, the height is calculated online as the height at 80% of the setpoint force, determined from the extend curves, smoothened with a 40 pixels moving average. The preset values of 40 pixels smoothing and 80 % of setpoint force can be changed in the Force Scan Map Oscilloscope (see **Section 6.7.3**).

The tip movement is not the same as the trace and retrace movement in Contact Mode imaging. The force mapping experiment is started at index 0 (bottom left 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 blue frame.

6.7.2 The Force Mapping Control panel

The **Force Mapping Control** panel contains the main settings for the shape the single force curves.
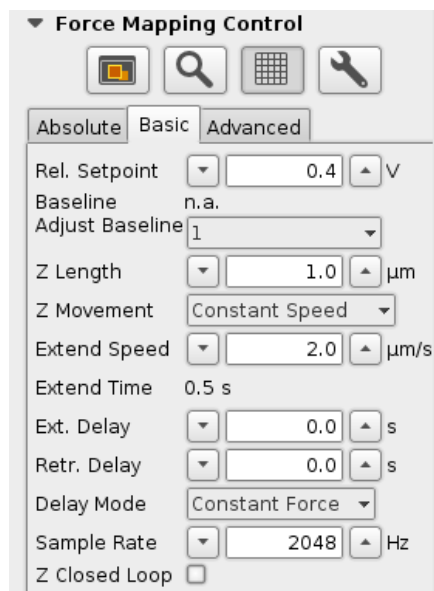
The shortcut icons on top allow opening a new Data Viewer window, to confirm a new scan region (see **Section 5.1.3**), to show/hide the grid frame or to open the Advanced Force Settings (see **Section 6.3**).

The **Basic** settings are used as default; please find a close description in **Section 6.2.** For starting with Force Spectroscopy Maps, **Basic** offers an easy tool to perform Force Mapping including variable approach, delay and retract settings.

⊕ **Absolute** and ⊕ **Advanced** are optional modes and offer different control settings for the force scan movement (see **Section 6.1.1**, **8.5** and **8.6**). Using **Advanced** mode, more complex force spectroscopy curves can be performed, e.g. different types of force segments can be strung together.

Grid panel

Grid
☑ Square Image

Fast Axis	▼ 10.0 ▲	µm
Slow Axis	▼ 10.0 ▲	µm
X Offset	▼ 0.0 ▲	µm
Y Offset	▼ 0.0 ▲	µm
Grid Angle	▼ 0 ▲	deg
Pixels	8 x 8 ▼	
Pixel Ratio	1 : 1 ▼	
Position X:	-4.38 µm	
Position Y:	-4.38 µm	

The **Grid** panel provides the parameters to set the size (**Fast/Slow Axis**), and position (**X/Y Offset**) of the scan region. Alternatively, click and drag a new scan region directly within the **Data Viewer** as described in **Section 5.1.3**. Disable **Square Image** in order to acquire rectangular force maps.

The desired resolution of the grid can be set by selecting the number of **Pixels**.

Pixel Ratio allows acquiring force maps with non-square pixels.

Position X/Y gives the current tip position, i.e. changes with ongoing measurement.

6.7.3   The Force Scan Map Oscilloscope

Please read **Section 6.1.2** for a close description of the **Display** settings and application of **Operations** in the **Force Scan Map Oscilloscope**.
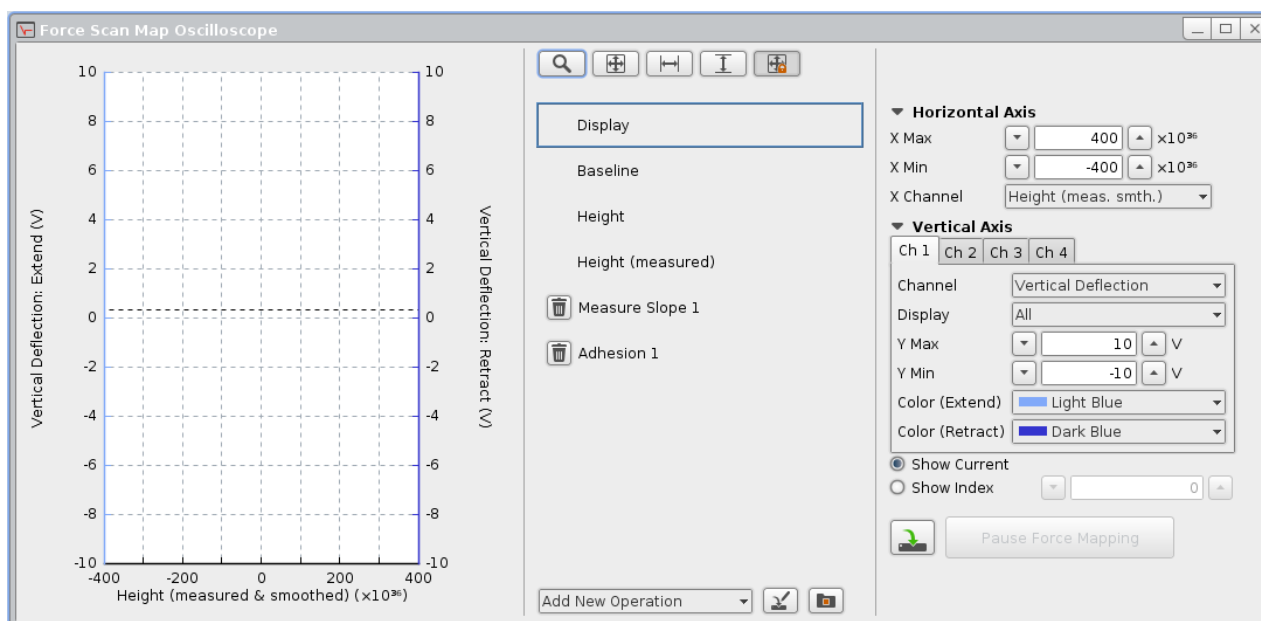
The **Force Scan Map Oscilloscope** displays spectroscopy curves from either the current measurement (**Show Current**) or a given index (**Show Index**). If Show Index is selected, type the desired index into the input field or move the mouse over the map and click on any pixel to show the corresponding curve.

ℹ️ To show the force curves of a completed force map, the corresponding map must be selected in the **Image Record List**: Open the **Image Record List**, select the desired force map and click **Focus** in the Data Viewer window. Now the curves of this map can be shown.

⚠️ Always check whether all required channels are activated in the **Channel Setup** (**Section 3.2.7**) when the invalid operation warning icon appears for any operation. If the required channels are not activated, i.e. no data are collected, the corresponding operation is invalid and cannot analyze, show or save any data! (see **Section 6.1.2**)

### 6.7.4   Data types and file saving

Click the corresponding icon at the shortcut icon toolbar to open the **Image Record List**. All Force Mapping data can be found in this list.
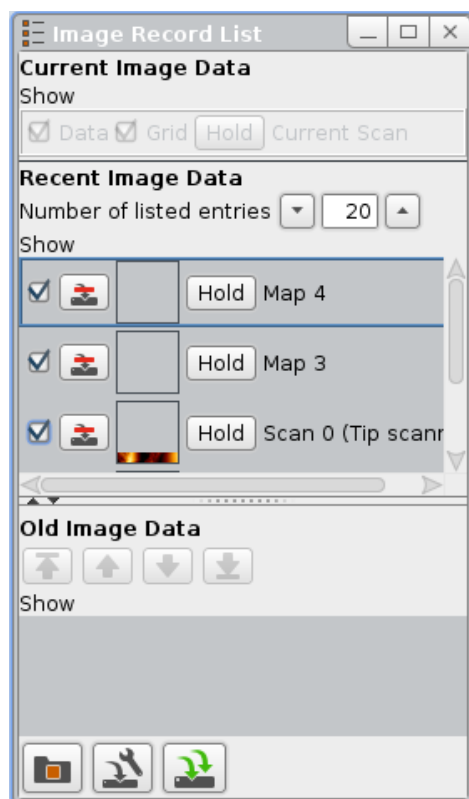
Use the **Image Record List** for saving or removing of acquired force map data.

Please read **Section 5.1.5** for a detailed description of the Image Record List.

Open the **Saving Settings** via the **Setup** drop-down menu to define the settings for all file formats. Choose the **Force Scan Maps** tab and adjust the naming, storage location and channels to be saved as described in **Section 3.2.8**
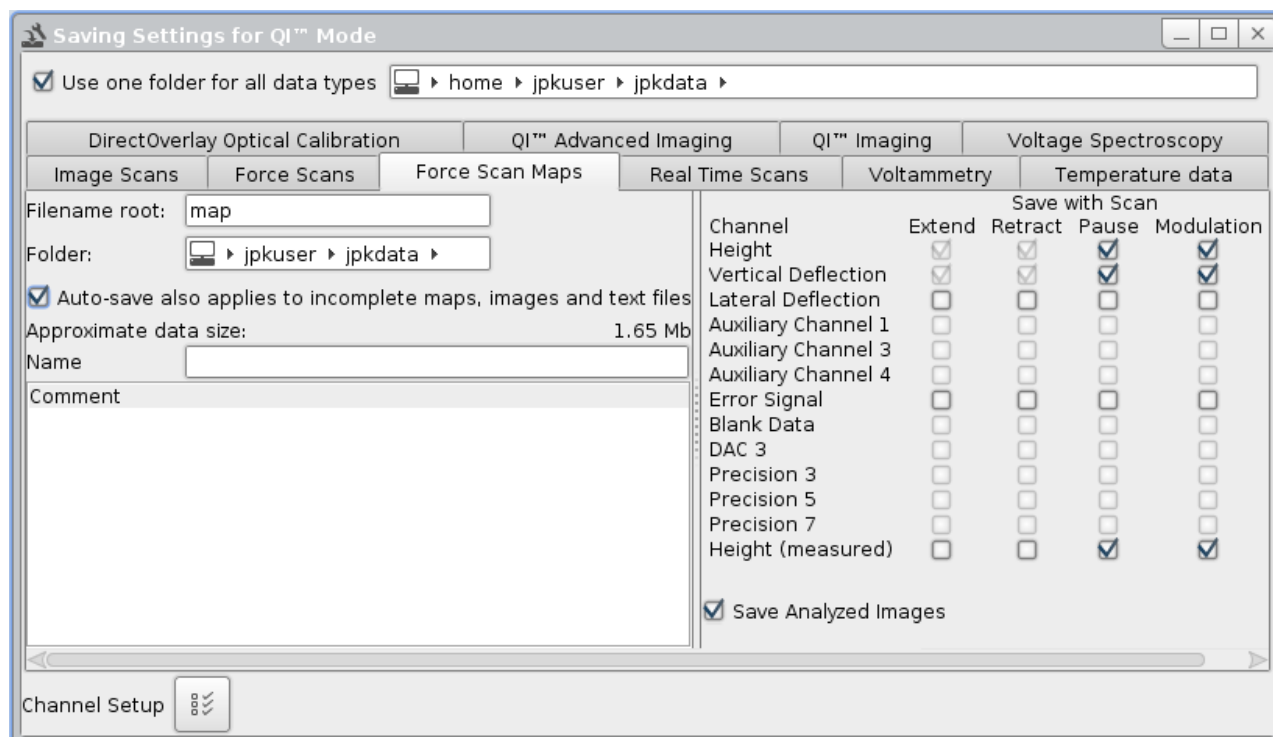
Several formats for saving force mapping data are availbale; the full set of force spectroscopy curves along with their

index/position are saved as one file (*.jpk-force-map) by default. Enable **Save Analyzed Images** at the bottom of the Saving Settings window to save the analyzed images which are also shown in the Data Viewer Window.

§ 7   Calibration

## 7.1   Height calibration

7.1.1   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. The Z linearization (see **Section 7.1.2**) provides a height channel (**Height measured**), 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. Due to aging of the piezo material the z-calibration should be repeated around every 6 months.

First image the calibration sample and measure the step height in the **Height** image using the cross section feature (see **Section 5.1.2**). This value can then be used to calculate the **Multiplier** for the calibration:

$$\frac{Known\ step\ height}{Measured\ step\ Height} * old\ Multiplier\ =\ new\ Multiplier$$

Disable any line leveling in the **Filter** control bar at the bottom of the **Data Viewer** window (see **Section 5.1.2**). Line leveling fits each scan line independently with a linear or polynomial fit, which is subtracted from the corresponding scan line, i.e. the height values are modified and unsuitable for calibration.
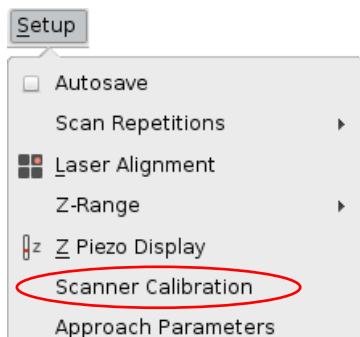
Open **Scanner Calibration** via the Setup drop-down menu.
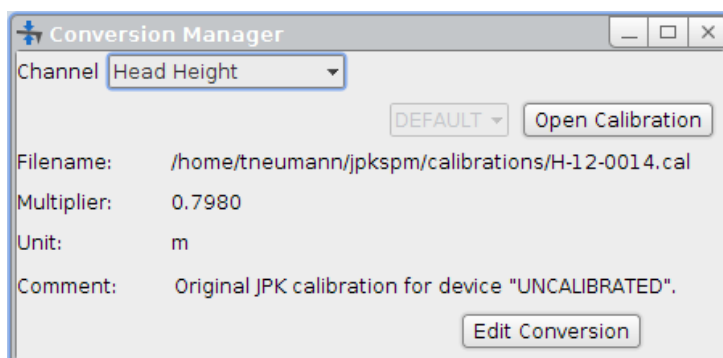
If existing, old calibrations can be selected from the drop-down box (here showing **DEFAULT**). The details of the selected file are shown in the main panel.

Also a file chooser can be opened using **Open Calibration**, which allows 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.
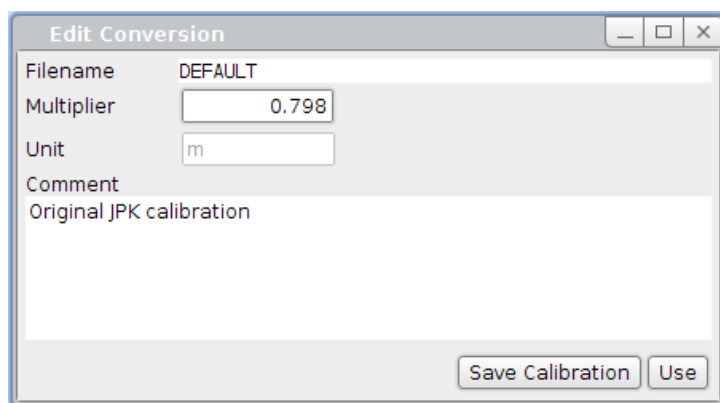
In **Edit Conversion**, the details can be changed. Enter the new value of **Multiplier**.

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.

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

The new calibration file is only used for the user account, where it was created. Give the new Multiplier value or calibration file to other users who want to use it.

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, it should be calibrated with the smallest available calibration standard.

7.1.2   Hardware z-linearization – Height (measured)

Generally the JPK NanoWizard® is equipped with a z-linearization. A sensor continuously measures the length of the z piezo, which is referred to as **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 Z sensor measures the actual current height of the piezo and this value is shown in the **Height (measured)** channel. The calibration of the Z sensor 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 around every six months (see **Section 7.1.1**).

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 more precise over larger z-ranges, because it is linear, but over very small ranges the noise of the Z sensor can be 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. Choose **Height (measured)** or (**Height measured & smoothed)** as the X axis in:

- Spectroscopy mode

- Force Mapping mode

- Sensitivity calibration

7.2   Spring constant calibration

The deflection of the cantilever is measured by a laser detection system using a four quadrants photodiode. The natural output of the photodetector is a Voltage, i.e. the vertical and lateral deflection is displayed in Volts. The **Calibration Manager** allows for the conversion of the vertical deflection into units of length or forces by determining the **sensitivity** and **spring constant** of the cantilever. The conversion into units of length is, for instance, relevant for dynamic modes, if it is necessary to adjust the oscillation amplitude to a defined size. For some experiments it is relevant to apply a defined force, e.g. to measure the adhesion or mechanical properties of a sample.

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). As the spring constant is very sensitive to the thickness of the cantilever, these quoted values are not very reliable. The range of values quoted by the suppliers should give some idea of the variability, but generally the spring constant is not known within a factor of 2 or 3.

The SPM software provides two methods to calibrate cantilevers, i.e. to determine the **sensitivity** (conversion into units of length) and the **spring constant** (conversion into units of force) of an arbitrary cantilever:

The **Contact-based** method comprises two steps: 1) the acquisition of a force distance curve on a hard substrate and deriving the sensitivity of the repulsive part of this curve and 2) a thermal noise measurement to calculate the spring constant.

The **Contact-free** method is based on the geometry of the cantilever and the physical properties of the environment/medium – it does not require a preceding acquisition of a force curve and is thus a more gentle way to determine the spring constant and sensitivity of a cantilever. If the different properties are known they can be used, along with a thermal noise measurement, to calculate the spring constant as well as the sensitivity of the cantilever.

> The thermal noise measurement is very susceptible to acoustic noise: provide a quiet environment for the thermal noise measurement.

> The cantilever may be susceptible to illumination of different sources: switch of any direct light sources (i.e. microscope illumination).

7.3   Cantilever calibration using the Contact-based method

7.3.1   Measuring the sensitivity using a force curve

The sensitivity can be measured by doing a force curve on a hard surface, and looking at the deflection of the cantilever when it is in repulsive contact with the sample. In this region of the force curve, the movement of the piezo should correspond to the deflection of the cantilever. The plot of the deflection versus distance should have a slope of 1 in this region, and the piezo movement is used to calibrate the measured deflection. Since for this sensitivity measurement relatively high contact forces are required, this method has the potential to damage sharpened and sensitive tips. Another, more gentle way to determine the sensitivity without the need of taking a force curve is described in **Section 7.4**.

> The sensitivity determination must be repeated after each re-alignment of the laser spot and each time a cantilever is mounted or if the position of the cantilever on the cantilever holder was changed. Different media (e.g. water, air) have different refractive indices, so changing the medium also requires re-alignment, especially of the mirror (**Section 4.2.3 et seq.**)
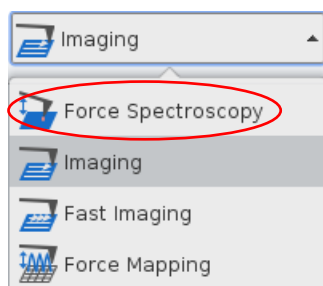
To measure the cantilever sensitivity using the **Contact-based** method, it is necessary to acquire a force distance curve on a hard clean surface, such as glass or mica.

Select **Force Spectroscopy** from the **Feedback Mode** drop-down menu.

Approach onto the surface and perform single or multiple force-distance curves as described in **Chapter § 6** .
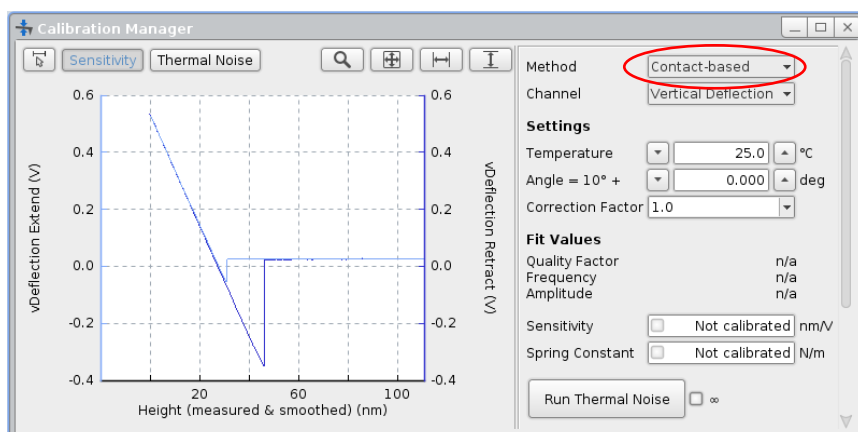
Open the **Calibration Manager** using the shortcut icon in the toolbar or via the **Force Spectroscopy** drop-down menu. Select **Contact-based** in the **Method** drop-down menu. The latest force distance curve is loaded.

Adjust the display using the Oscilloscope toolbar (see **Section 3.2.1**) or the mouse if necessary.
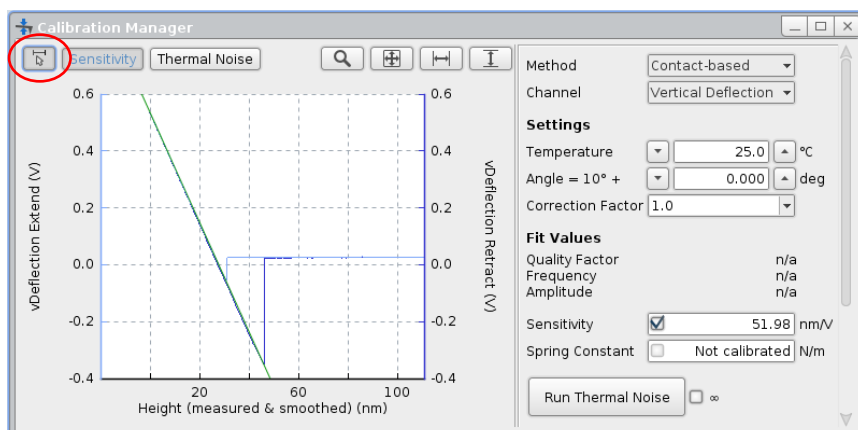
Only the linear repulsive part of the force curve is used to fit the sensitivity.

Click the **Select Fit Range** button and drag the fit range directly into the linear repulsive part of the force curve. The fit appears as a green line. The slope of the fit is automatically transferred to the **Sensitivity** box.

When the checkbox is enabled, the sensitivity is applied to the Vertical Deflection signal, which is then displayed in units of length (m). When the checkbox is unticked, the Vertical Deflection value is displayed in Volts, but the sensitivity value is still stored and saved with the files.

The Sensitivity checkbox must be ticked to proceed to the thermal noise measurement of the cantilever spring constant.

7.3.2   Spring constant calibration using the thermal noise

Using the Contact-based method, the cantilever spring constant is measured using the thermal noise method along with the sensitivity value derived from a preceding force distance measurement. The thermal noise method relies on measuring the free fluctuations 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. This method is based on the approach suggested by J.L. Hutter and J.

Bechhoefer 1993, "Calibration of atomic-force microscope tips" *Rev. Sci. Instrum.* **64**:1868-1873.

For the spring constant calculation using the method of Hutter and Bechhoefer the cantilever deflection must be given in units of length. It is thus necessary to measure the **Sensitivity** as described in **Section 7.2**.

If the sensitivity has been measured, click the **Thermal Noise** button.
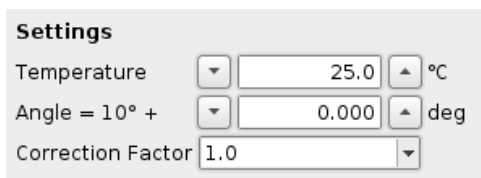
There are several Settings which are part of the calculation of the spring constant and may need adjustment.

The thermal noise depends on temperature. Type the current ambient **Temperature** under **Settings**.

Also the tilt **Angle** of the cantilever is important. The SPM software takes account of the normal 10 degree tilt (see **Section 4.2.2**) and calculates the vertical component of the spring constant as a **Vertical k** for the experiment. Type any additional **Angle** if necessary.

The sensitivity measured by the force curve on a hard surface provides a large static deflection of the cantilever. The cantilever bending shape during dynamic fluctuations (thermal noise) is different from the bending shape of the cantilever being in contact with the sample (one fixed and one free end against two fixed ends). And since the detection system is primarily sensitive to angular deflections, it has a slightly different sensitivity for the measurement of the thermal noise. **Correction factors** have been calculated by Butt and Jaschke (1995, Nanotechnology 6: 1-7) to take account of the difference between z-deflection and angular deflection for the different bending modes of the cantilever.

It is recommended to use the first eigenmode along with the corresponding correction factor calculated by Butt and Jaschke (0.817, see table below). Please read the publication by Butt and Jaschke (1995, Nanotechnology 6:1-7).

By default, no Correction Factor is used, i.e. Correction Factor = 1. Adjust the Correction Factor if necessary.

Usually the first resonance is used for the spring constant calculation, as this has the largest amplitude, and therefore the best signal to noise ratio for accurate measurements. For very soft cantilevers in liquid, the first resonance may be unsuitable. In this case the second resonance can give more reliable results. The second and higher resonances have different relations between z-deflection and angular deflection at the tip, so different correction factors are needed.

| Peak	Correction factor	Comments
1	0.817	Generally used
2	0.251	Used when first resonance frequency is too low
3	0.0863	Not generally used

Example correction factors for rectangular cantilevers calculated by Butt and Jaschke (1995, Nanotechnology 6:1-7).

The correction factors calculated by Butt and Jaschke are only valid for rectangular cantilevers. Correction factors for triangular cantilevers are discussed by Stark et al. (Stark et al., 2001 Ultramicroscopy 86: 207-215).

Note that these correction factors are only valid when the laser spot is positioned on the cantilever tip. The correction factors and sensitivities change if the laser spot is moved towards the cantilever chip. Especially for higher modes (2nd and higher peaks), the calculated spring constant is changing drastically by moving the laser spot along the cantilever.
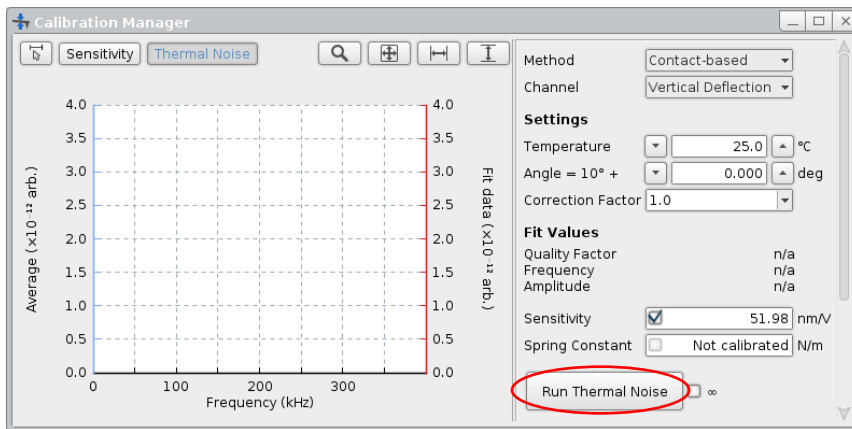
Click **Run Thermal Noise** to start the thermal noise measurement. If the infinity checkbox is unticked, the software collects 25 spectra, which is usually sufficient. If the infinity checkbox is ticked, continuous spectra are collected until **Stop** is clicked again. The more spectra collected, the smoother is the result.

The cantilever must be retracted from the surface for this measurement! If the **Run Thermal Noise** button is inactive, check that the piezo is retracted.
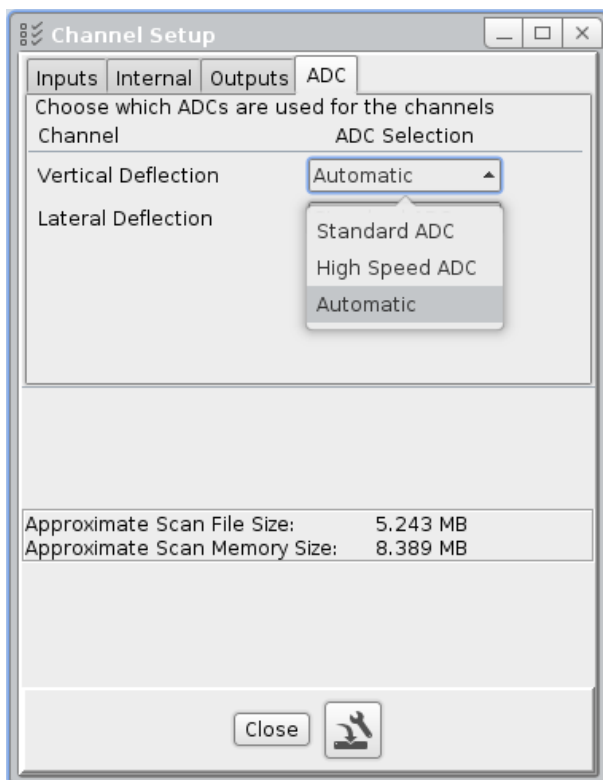
If a higher frequency range is needed, e.g. for very stiff cantilevers or to detect higher modes, **High Speed ADC Settings** can be used. Open the **Channel Setup** window via the **Setup** drop-down menu or right-click with the mouse into the thermal noise plot area. Select the panel **ADC** and enable **High Speed ADC** for **Vertical Deflection** to extend the thermal noise frequency range.
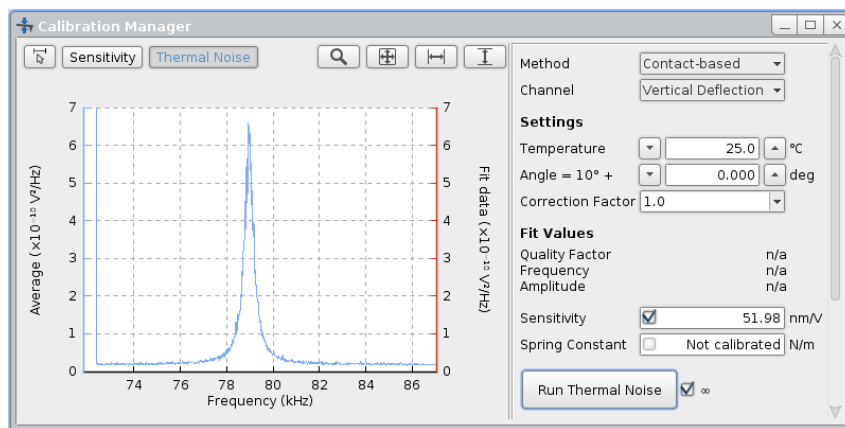
The resulting frequency spectrum should show a peak at the cantilever resonance frequency.

The thermal noise data are saved automatically in ascii format as *.tnd file in the jpkdata directory.

If necessary, zoom into the peak using the display settings as described in **Section 3.2.1**.
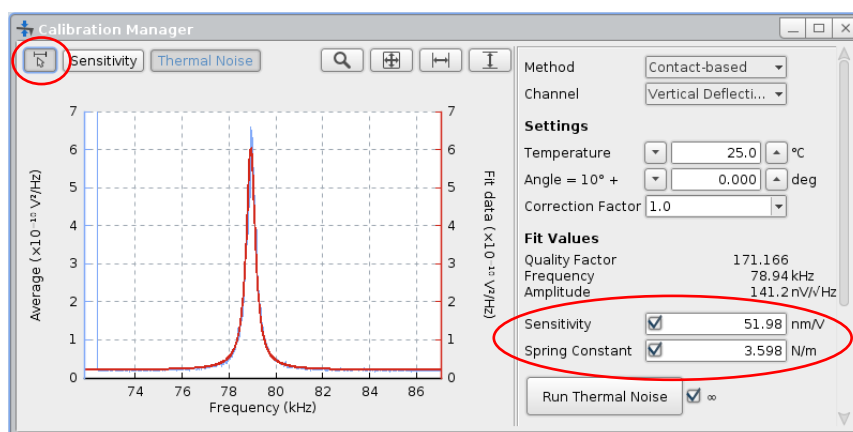
The resonance must be fit with a Lorentz curve. Select the resonance using the **Select Fit Range** button. Make sure that resonance and Lorentz fit match properly.

The measured resonance frequency and the calculated spring constant of the cantilever should be in the range of the nominal value that is quoted by manufacturers.

If the checkboxes are active, the spring constant is applied by the software in all feedback modes, i.e. the vertical deflection is now displayed in units of force (nN) or length in oscillating modes.

Besides the spring constant, the fitting algorithm also calculates the resonance **Frequency**, the integrated **Amplitude** of the resonance and the **Quality Factor** (Q-factor), which can be found under the **Fit Values** in the Calibration Manager Window. The Q-factor is a measure for the width of the fitted resonance peak. Typical values for the Q-factor are a few hundred in air, and around 1-3 in liquid. Generally speaking, the narrower the resonance peak, the higher the Q-factor.

7.3.3 Using thermal noise to calibrate soft cantilevers in fluid

The most accurate spring constant determination using the thermal noise method requires that the calibration is performed in air. The thermal noise detection method can also be used in fluid, but there may be additional complications, particularly with very soft cantilevers.
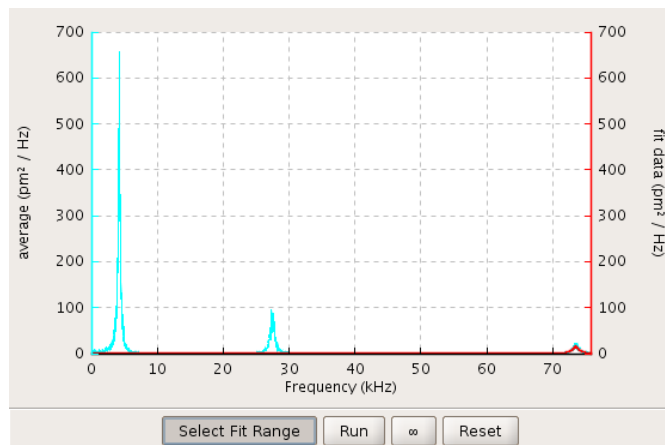
This spectrum is representative for a soft cantilever (0.02 N/m) in air. There are three peaks visible, corresponding to the resonance (at around 4 kHz) and the first and second overtone.
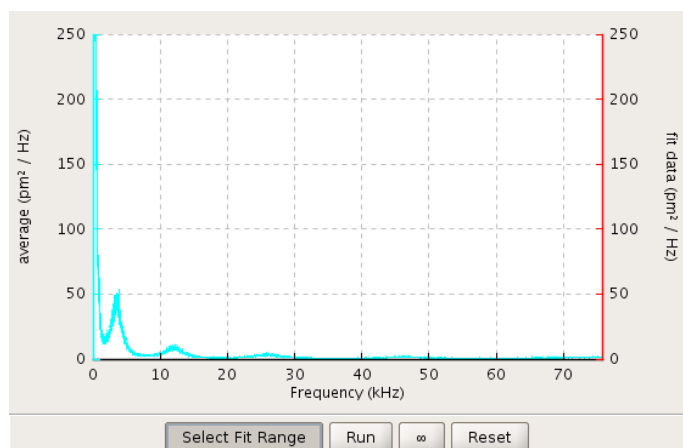
When the same cantilever is immersed in a fluid each peak is damped, reducing both amplitude and frequency. The resonance of the cantilever is now extremely low and more susceptible to noise, which makes it difficult to fit it properly. In this case the second peak (first overtone) could give a more reliable fit. In this case a correction factor must be used (see **Section 7.3.2**).

The desired **Correction Factor** can be typed into or selected in the corresponding input field. Then the determined spring constant is multiplied with the correction factor in order to calculate the corrected spring constant.

7.4   Cantilever calibration using the Contact-free method

7.4.1   General information

The **Contact-free** method for cantilever calibration does not require preceding force distance curve acquisition to determine the sensitivity. It is therefore particularly suited for calibration of cantilevers with very sharp and sensitive tips, which could be damaged by force spectroscopy measurements on hard substrates.

For this method, the plan view dimensions of the cantilever (length and width) and the physical properties of the environment/medium (density and viscosity) must be known. Along with the quality factor Q and the resonance frequency, both derived from the thermal noise measurement, the spring constant k as well as the sensitivity of the cantilever can be calculated. This method is only valid for rectangular cantilevers and is based on the calculations of J.E. Sader et al. 1999, Rev. Sci. Instrum. 70:3967. Please read this article for a closer description of the calibration method.

> ⚠️   This calibration method is only valid for rectangular cantilevers.

Basically, the main assumptions are:

- The length (L) of the cantilever must be much larger than the width (b) (e.g. L/b > 3), which must be much larger than its thickness.

- Q must be much larger than 1 (so that the damping is independent of frequency over the width of the resonance, also for linear harmonic oscillator)

- Γ(ω) is the complex hydrodynamic function, analytical expression to be found in J.E. Sader 1998, J. Appl. Phys. 84(1):64-76

- The calculation of the hydrodynamic functions requires that the cantilever is far from the surface. If comparison measurements were made "near" a surface, the additional damping from the confined fluid would artificially underestimate the spring constant; The critical parameter is the cantilever width - measurements should be made several widths away from the surface

The spring constant is finally calculated from the density of surrounding fluid (ρ), cantilever width (b) and length (L), fitted Q and ω_R (cantilever resonance frequency of the fundamental mode) and the gamma-function (Γ_i) at this value:

$$k = 0,1906 \rho b^2 L Q \Gamma_i (\omega_R) \omega_R^2$$

7.4.2   Calibration procedure

Please note that the cantilever width and length as well as the density and viscosity of the environment must be known for the calibration. Make sure that the cantilever is far away from the surface (e.g. 150 µm) or enable the **Automatic motor retract** checkbox (see below).
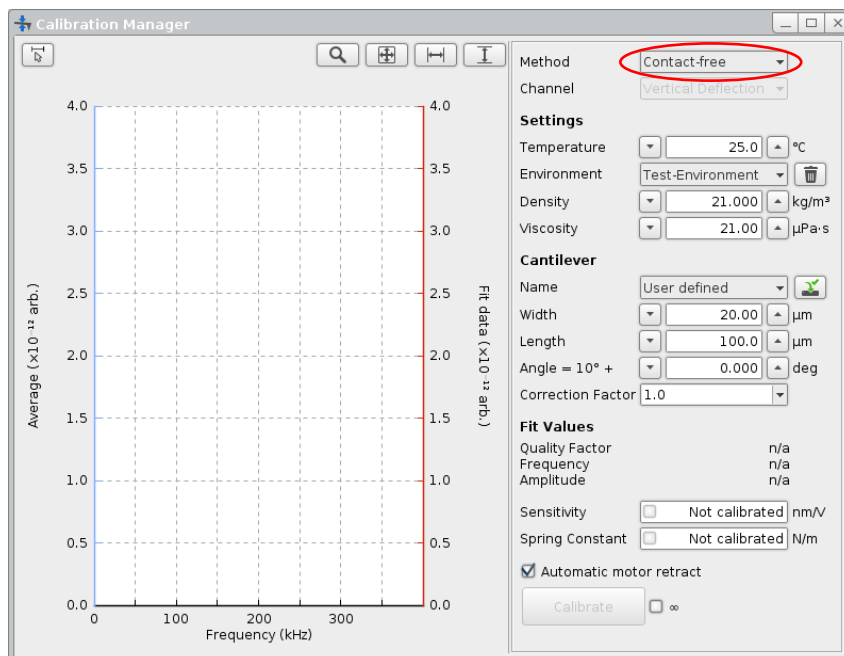
Open the **Calibration Manager** and select **Contact-free**.

The **Settings** and **Cantilever** must be selected/typed corresponding to the environment and cantilever used.
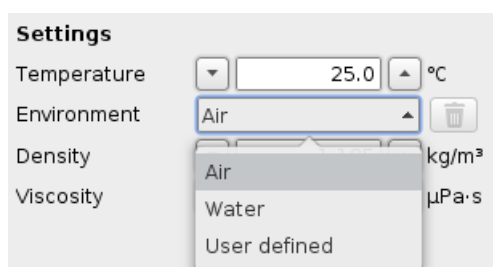
The **Settings** provide **Air** and **Water** for **Environment** with corresponding **Density** and **Viscosity**. The density and viscosity change with temperature. Adjust the temperature if necessary; the density and viscosity of the predefined environments air and water will be adjusted automatically.

Choose **User defined** to type a **Density** and **Viscosity** manually if another medium is used. Consider the temperature used for the experiment and type the corresponding values valid for this temperature.

The density and viscosity of liquids change with temperature. The Calibration Manager only adjusts the density and viscosity for the predefined environments **Air** and **Water**. If another medium is used, determine the density and viscosity for exactly the temperature which is used during the experiment and also type the corresponding temperature. Otherwise the determined spring constant and sensitivity may be wrong.
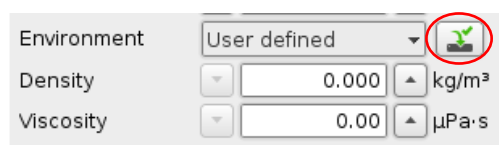
The user defined Environment can be saved for the corresponding temperature. Click the **Save** button which appears upon selecting **User defined**; The **Save Environment** dialog will open.
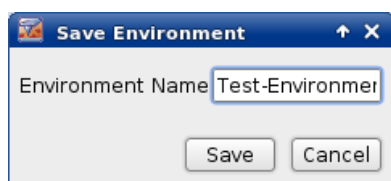
Type the desired **Environment Name** and save it for later use. It will appear in the **Environment** drop-down list.

The **Cantilever** panel provides several common cantilevers with the geometry given by the manufacturers. Select the corresponding cantilever or choose **User defined** if your cantilever doesn't appear in the list.

Type the cantilever **Width** and the **Length** as well as any additional **Angle** or **Correction Factor** if applied. The 10 degrees angle due to the cantilever holder (see **Section 4.2.2**) is applied automatically.

Click the **Save** button to open the **Save Cantilever** dialog.

Type a **Cantilever Name** and the **Resonance Frequency** given by the manufacturer. This resonance frequency is only a starting point for the fitting algorithm. The actual resonance frequency will be fitted from the thermal noise spectrum.

The calibration manager will switch to High Speed ADC Settings automatically if a cantilever with high resonance frequency (higher than 280 kHz) is selected.

It is recommended to use the first resonance along with the corresponding correction factor calculated by Butt and Jaschke (0.817, see **Section 7.3.2**). Please note that, according to J.R. Lozano et al. (2010, Nantechnology 21:465502), the Sader method "is poorly suited for the calibration of higher eigenmodes".

Automatic motor retract is enabled by default. The stepper motors retract for 150 microns before thermal noise acquisition starts and re-approach for 140 microns after acquisition.

If measuring close to the surface, the additional damping from any confined fluid or electrostatic tip-sample interactions in air would influence the thermal noise and result in a wrong spring constant. Always make sure that the cantilever is retracted several widths away from the surface using the stepper motors or enable the **Automatic motor retract.**
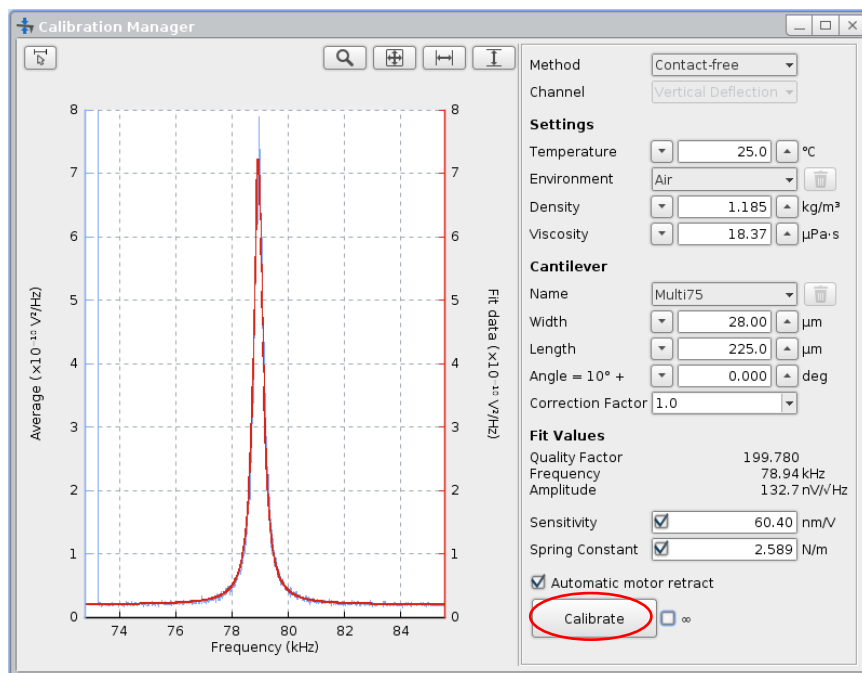
Click **Calibrate** to start the thermal noise measurement. If the infinity checkbox is unticked, the software collects 25 spectra, which is usually sufficient. If the infinity checkbox is ticked, continuous spectra are collected until **Calibrate** is clicked again. The more spectra collected, the more reliable is the result.

The thermal noise data are saved automatically in ascii format as *.tnd file in the jpkdata directory.

 If the fit fails or another peak of the thermal noise spectrum is supposed to be fitted, click the **Select fit range** button in the top left of the Calibration Manager and fit the resonance manually.

Besides the **Spring Constant** and the **Sensitivity**, the fitting algorithm also calculates the resonance **Frequency**, the integrated **Amplitude** of the resonance and the **Quality Factor**, which can be found under the **Fit Values** in the Calibration Manager Window. The Quality Factor describes the quality of the fit and is correlated to its width. Typical values for the Q-factor are a few hundred in air, and around 1-3 in liquid. Generally speaking, the narrower the resonance peak, the higher the Q-factor.

If the checkboxes for **Sensitivity** and **Spring Constant** are active, they are both applied by the software in all feedback modes, i.e. the vertical deflection is now displayed in units of force (nN) or length in oscillating modes.

§ 8   Available software extensions

The standard software version can be extended by a variety of extensions in order to enable specific measurements. The **About JPK NanoWizard Control** window lists all extensions included in your personal SPM software. Open the **About** window from the **Help** drop-down menu at the top of the software. The most common available extensions are explained in this chapter. Some of them are add-ons or accessories to the **Measurement** modes, but there are also additional **Feedback Modes** available.

⊕ Features and options marked with this sign are additional extensions and must be purchased separately. Please contact JPK for more information and assistance (++49 30 726243 500) support@jpk.com.

8.1   QI™ Advanced Imaging

⊕ **QI™ Advanced Imaging** works similar to the basic QI™ Imaging mode but provides a whole force curve for each pixel as well as variety of image channels which can be calculated online. All curves and image channels can be loaded into the JPK Data Processing software and additional channels may be calculated.
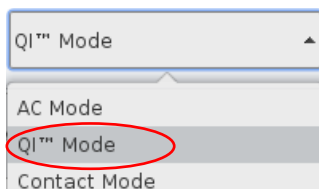
Select **QI™ Mode** in the **Feedback Mode** drop-down menu.
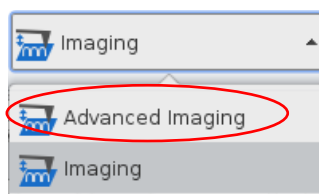
Select **Advanced Imaging** in the **Measurement** drop-down menu.

Like in **QI™ Imaging** mode, the corresponding data viewers (**Data Viewer** and **QI™ Advanced Oscilloscope**) as well as the **QI™ Control** panel and the **QI™ Setup** window open automatically. A **Quantitative Imaging** drop-down menu appears in the main bar on top of the software. Please read **Section 5.7** for detailed information on the basic operation of QI™ mode. The main differences between **QI™ Imaging** and **QI™ Advanced Imaging** are the **QI™ Advanced Oscilloscope** and the available channels in the **Data Viewer** window and will be explained in the following sections.

8.1.1   The QI™ Advanced Oscilloscope
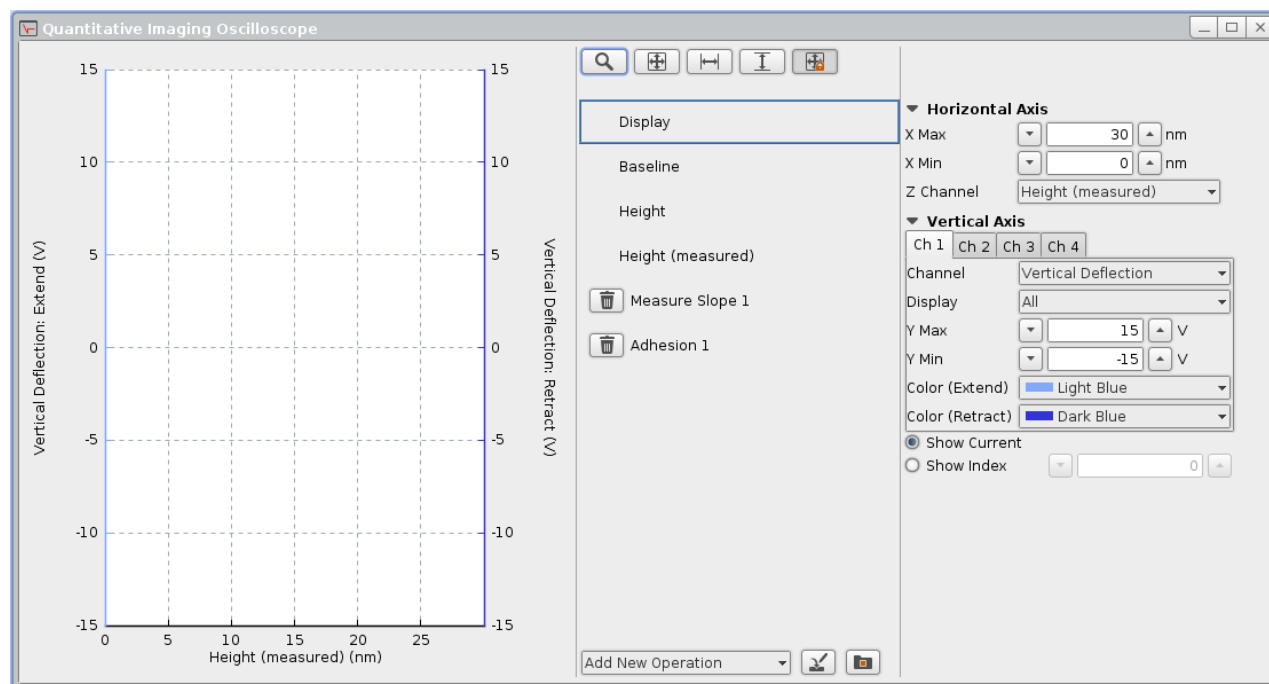
The **QI™ Advanced Oscilloscope** displays spectroscopy curves from either the currently measured pixel (**Show Current**) or a given index (**Show Index**). If Show Index is selected, type the desired index into the input field or move the mouse over the image in the Data Viewer and click on any pixel to show the corresponding curve.

> To show the force curves of a completed image, the corresponding image must be selected in the **Image Record List** (**Section 5.1.5**): Open the **Image Record List**, select the desired image and click **Focus** in the Data Viewer window. Now the curves of this image can be shown.

The **Display** panel allows the adjustment of the force curve display. Select the individual **Operations** to open the corresponding parameter panels. Please read **Section 6.1.2** for a detailed description of the functionality of the **Display** panel and the available **Operations**.

The **Baseline** operation appears by default before any other operation can be applied, since it is obligatory for most operations. The Height and Height (measured) operations, as well as Measure Slope and Adhesion are preset operations and the corresponding channels are shown in the Data Viewer window. All operations can be adjusted or new operations can be added corresponding to the description provided in **Section 6.1.2**.

The results of all operations selected in the **QI™ Advanced Oscilloscope** appear as **Channels** in the **Data Viewer** window and can be displayed as images.

> Always check whether all channels required for the desired operation are activated in the **Channel Setup** (**Section 3.2.7**) when the invalid operation warning icon appears for any operation. If the required channels are not activated, i.e. no data are collected, the corresponding operation is invalid and cannot analyze, show or save any data (see **Section 6.1.2**)!

8.1.2   The QI™ Advanced Imaging Data Viewer

The **Data Viewer** has the same function as in all other imaging modes. Please read **Section 5.1.2** for a detailed description.
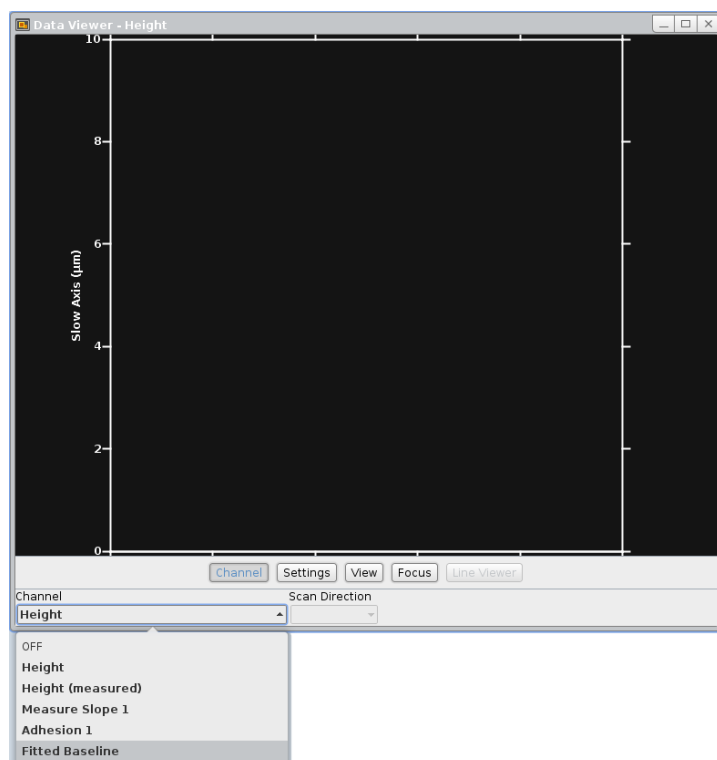
Like in **QI™ Imaging** mode (see **Section 5.7.1**) the **Height** and **Height (measured)** channels correspond to the **Reference Force Height** (see **Section 6.1.2**) at 80% of the setpoint force, determined from the smoothened extend curve (40 pixels, moving average).

The results of all operations selected in the **QI™ Advanced Oscilloscope** appear as **Channels** in the **Data Viewer** window and can be displayed as images.
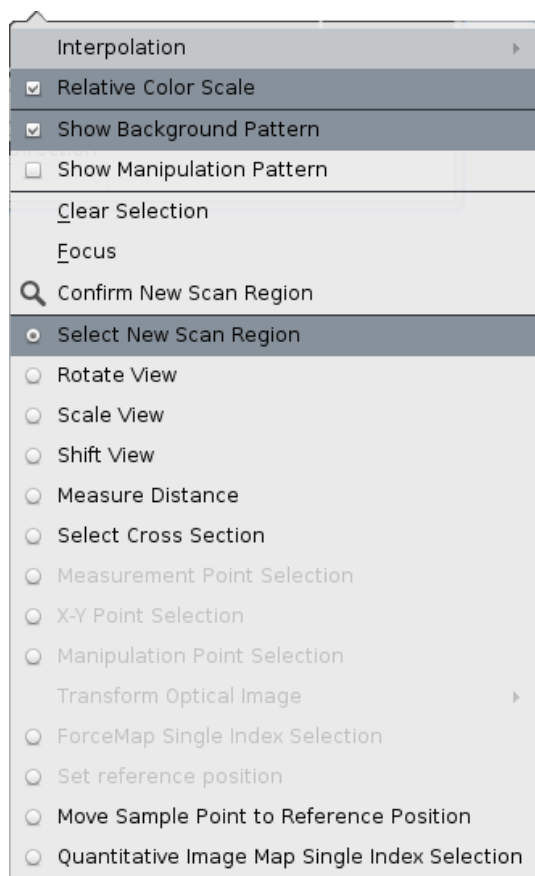
The menu, which appears upon right-click within the **Data Viewer**, contains basically the same options as for all imaging modes (see **Section 5.1.2**).

QI™ Advanced Imaging additionally provides the option **Quantitative Image Map Single Index Selection**. This option allows a display of an arbitrary force curve of a pixel which can be selected by mouse click directly into the active image (see **Section 8.1.1**). This is normally valid for the current scan region. Another, previous image can be activated by clicking on the scan in the **Image Record List**.

8.1.3 QI™ Advanced data types and file saving

All QI™ data can be found in the **Image Record List**. Open the Image Record List using the corresponding icon at the shortcut icon toolbar on top of the SPM software. Managing, saving and removal of images using the Image Record List is described in **Section 5.1.5.**

Open the **Saving Settings** menu via the **Setup** drop-down menu or the shortcut icon in the toolbar. Data saving is described in **Section 3.2.8**. Choose the register card **QI™ Advanced Imaging** to set the channels for file saving.

QI™ Advanced Imaging mode provides two data types. The raw files, containing all force curves, are saved as *.**jpk-qi-data** files. The force curve channels **Height**, **Vertical Deflection** and **Height (measured)** are essential for data collection and are saved by default. Other channels can be enabled if required. The mere image files, containing the results of online data analysis (the results of all operations applied in the QI™ Advance Oscilloscope, such as Height/Height (measured), Adhesion and Slope), are saved as **jpk-qi-image** files.
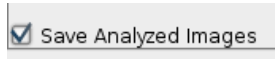
Enable the **Save Analyzed Images** tickbox at the bottom of the QI™ Advanced Images register card (scroll down with the scroll bar) to save/discard the analyzed images.

8.2   Fast Imaging mode

⊕ **Fast Imaging** mode is available for **Contact Mode** and **AC Mode**. The lateral scanning movement of the tip is improved for higher scan speeds. This Imaging mode is also a component of the **Fast Scanning** option for the new generation of the NanoWizard® Series devices (see also **Section 2.1.1**).
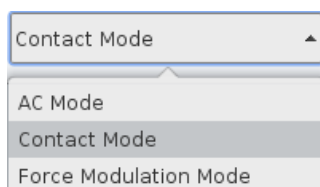
Select **AC Mode** or **Contact Mode** in the **Feedback Mode** drop-down menu.
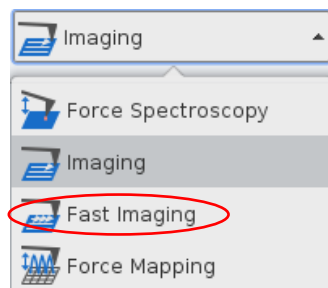
Select **Fast Imaging** in the **Measurement** drop-down menu.

When Fast Imaging mode is selected, the same windows and panels will open as for the corresponding feedback mode in standard imaging mode. The imaging settings and parameters apply in the same way. Due to the improved lateral movement, higher scan velocities are possible.

8.3   High Resolution Imaging

⊕ **High Resolution Imaging** mode is available for **Contact Mode** and **AC Mode**. The lateral scan range is limited and the scan motion is improved for higher resolution.
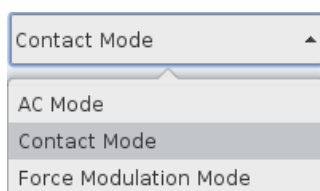
Select **AC Mode** or **Contact Mode** in the **Feedback Mode** drop-down menu.
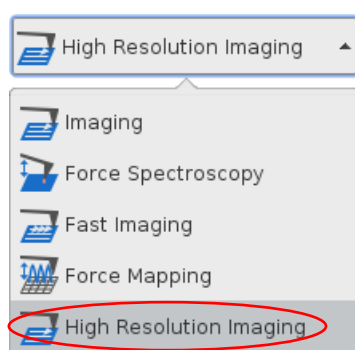
Select **High Resolution Imaging** in the **Measurement** drop-down menu.

The new (limited) scan range must be adjusted. Therefore a message appears right upon selecting **High Resolution Imaging** and requests for adjusting. Please read **Section 5.3** for more details on adjustment.

When High Resolution Imaging is selected, the same windows and panels are available as for the corresponding feedback mode in standard imaging mode. The imaging settings and parameters apply in the same way.

8.4   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 exactly the 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 in x (Fast

Axis) and y (Slow Axis). The difference between the two images is due to aberrations arising from the lenses and mirrors of the light microscope. ➕ The **DirectOverlay™** software uses the automatically recognized cantilever position to map the optical image and calibrate it before importing into the SPM software. Using this kind of calibration, AFM scan regions can be selected directly within the acquired optical image. The generated calibration files and optical images can further be used for offline overlay using the JPK Data Processing software.

The SPM software uses **JUnicam** to integrate and operate the supplied Imaging Source CCD camera. Please read the **JPK Software Integration for Cameras** user manual for a detailed description of the JUnicam camera software.

> Several types of Andor, Jenoptik and Imaging Source cameras are supported, i.e. may be operated directly via the SPM software. Please contact JPK for information: support@jpk.com, +49 30 726243 500

8.4.1   Image focus for optimal tip location

Recognition of the cantilever position is used in the optical image calibration procedure. 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 co-localization 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, are calculated solely from the cantilever images.

The cantilever is automatically recognized from each optical image, without needing to 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 in order to enhance the contrast for the cantilever before starting the calibration. This can be easily done by changing the condenser illumination settings to bright field illumination (as shown below). The illumination can be changed back at the end of the optical calibration procedure to acquire an image with good sample contrast for importing.
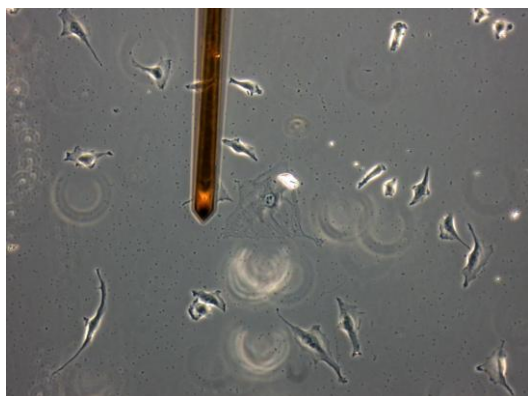
Phase contrast illumination
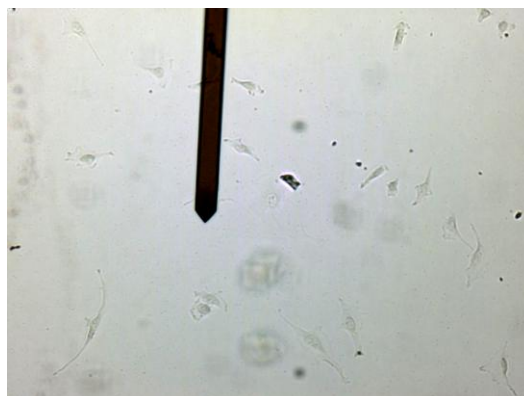
Bright field illumination – same region with enhanced contrast for optical calibration

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 a few microns back to the sample level to take a sharp sample image for import.

8.4.2   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 μm x 100 μm (the piezo range of the NanoWizard®). The optical image is extrapolated to 50% larger than the calibration area, i.e. maximal region 150 μm x 150 μm. When using higher magnification objectives (63x or 100x) it will be necessary to reduce the size of the area to be calibrated below 100 μm, since part of the AFM region may be outside the optical field of view.

Before optical calibration, make sure that the AFM scan region is aligned with the desired sample area visible within the optical field of view:

If a SPM supported FireWire camera is being used, start **JUnicam** 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.

Approach to the surface as normal and use the optical microscope to find the region of interest for scanning.

Retract the tip from the sample. The safety distance between the tip and the surface depends on the **Target Height** (see **Section 4.5.2**). Make sure there is enough safety distance for the sample or tip movement. If necessary, move the stepper motors for some additional distance (10-20 microns) to prevent cantilever/sample crash.

Use the **Outline** tool (see **Section 5.1.1**) can be helpful to show the scan area and move either the sample or the cantilever until the scan area is within the desired region of the optical image.

Make sure that the cantilever is not in contact with surface. Check the system status window at the bottom left corner. It indicates 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 retract the cantilever.
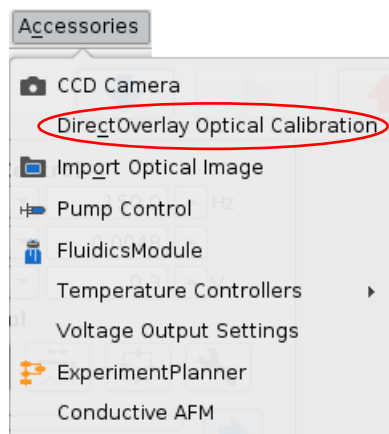
Open the **DirectOverlay™ Optical Calibration** via the **Accessories** drop-down menu at the top of the SPM software. The calibration window will open and lead through the calibration procedure.

If one of the JPK supported firewire cameras is used with the JUnicam 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 regular graphic files (TIFF, JPG, BMP or PNG) can be used for the calibration, manually transferring the images to the correct folder on the AFM computer. See **Section 8.4.5** for the manual calibration procedure.

8.4.3 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 8.4.1**) before starting.
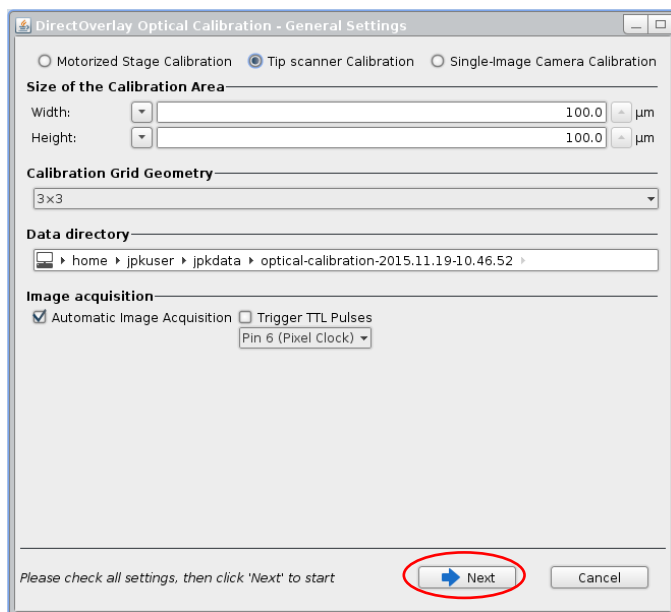
Adjust the **Size of the Calibration Area** if necessary (default full piezo range). Reduce it for high magnification objectives. The calibration region is always centered within the xy piezo range.

Calibration Grid Geometry determines the number (3x3 or 5x5) of cantilever positions used for the calibration.

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

Use **Automatic Image Acquisition** (default) for cameras with the **JUnicam** software. Switch off for external cameras with separate software - see **Section 8.4.5** for manual calibration.

Click **Next** to start image acquisition.
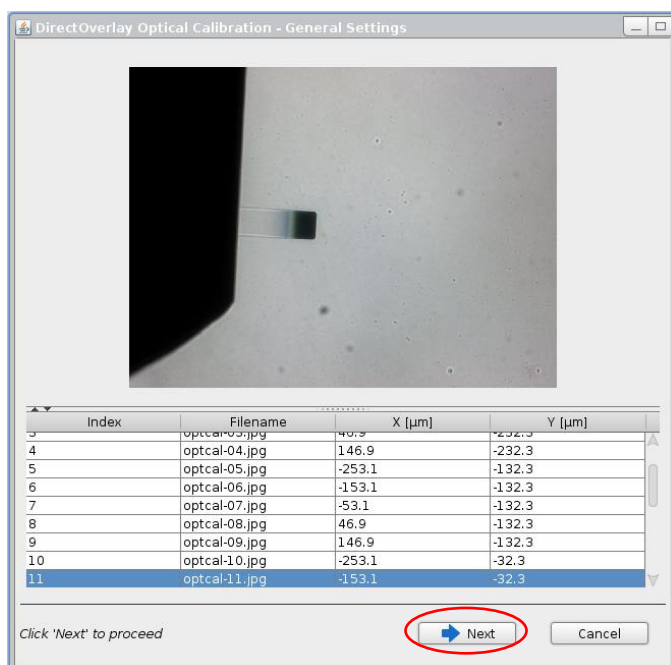
This panel needs no input when Automatic Image Acquisition is being used. In this case, the tip moves automatically to each of the e.g. 25 grid points, and an image is transferred automatically from the camera. The size of this grid corresponds to the size of the Calibration Grid Geometry selected in the first step of the DirectOverlay™ calibration.

Since each image is saved, it is displayed in the calibration window and the **Filename** appears in the list. These files are automatically saved in the **Data Directory** chosen in the first step.

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

Wait until all the images have been collected and click **Next.**
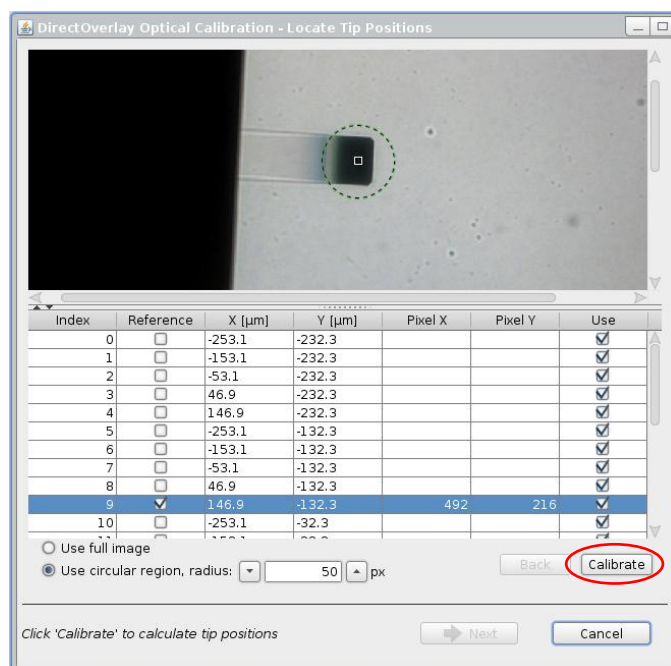
The next step is to locate the pixel position of the cantilever tip in one reference image. Select an image from the list, and enable the **Reference** tick box.

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 corresponding pixel, which also appears as **Pixel X/Y** in the image list. Enlarge the image if necessary using the mouse wheel to make this step more precise.

Either the whole image can be used for the calibration, or a circular region with defined radius. **Use a circular region** is set by default. The tip detection algorithm is applied in this circular region only, which makes it less susceptible to mismatches due to any other features in the image. Select the radius sufficiently large, i.e. the circular region should contain the cantilever edge with the tip and some bright background (as shown in the left image). **Use full image** to apply the tip detection algorithm to the whole image, if there are no disturbing features at all.

Click on **Calibrate** at the bottom of the panel.
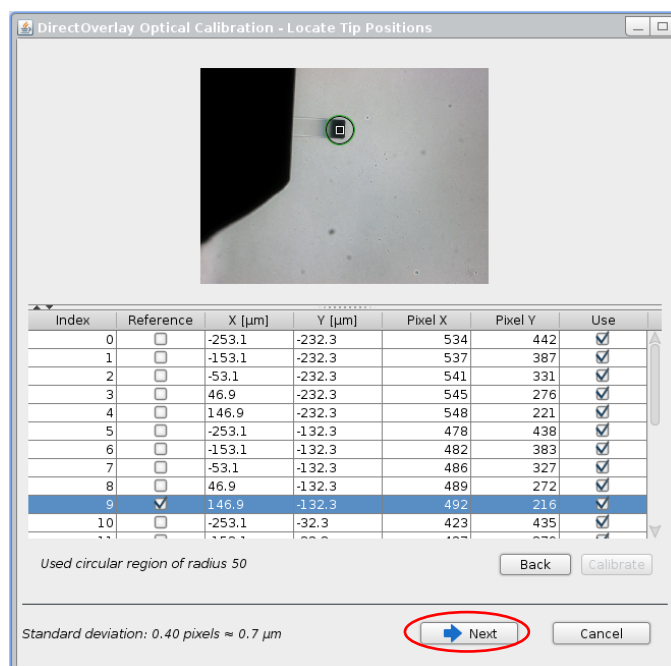
The software calculates the corresponding tip position in the other 24 images. This progress can be monitored as the values appear in the **Pixel X** and **Pixel Y** columns.

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. When one of the images is selected in the list, 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 visible in the selected image, the calculation may be inaccurate. Images with an incorrect tip location can be deselected with the **Use** tick box. The deselection of some points will not disrupt the calibration. Some images may have been automatically deselected.

When satisfied with the calibration points, click **Next.**
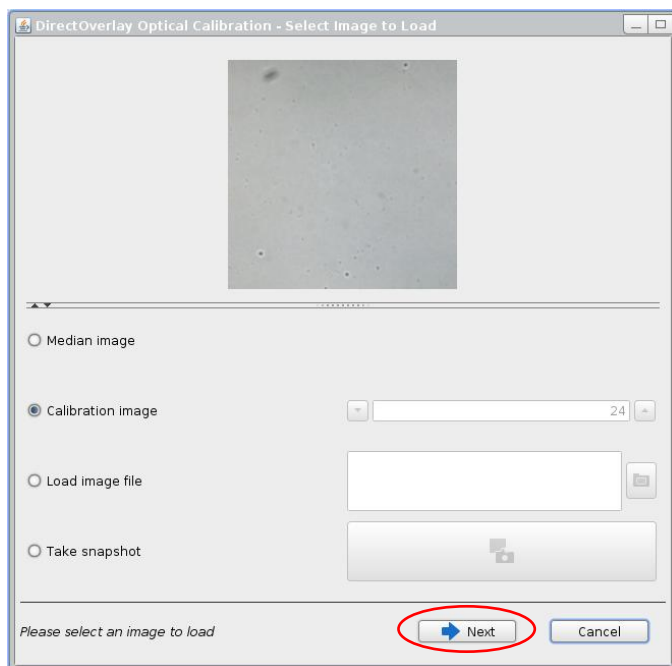
At this point the calibration is finished and the user should select an image to be imported into the background of the SPM program.

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 one of the **Calibration images** or a pre-existing image (taken before the calibration) using **Load Image file** can be loaded.

Take snapshot allows acquiring another sample image. Refocus the microscope, adjust the contrast and click the camera icon to take an image of current field of view. The file is saved in the calibration **Data Directory** with a filename **Snapshot**. The cantilever does not obscure the field of view as it is still positioned over the final point of the calibration grid.

When the correct image has been selected click **Next** to import the calibrated image into SPM.

8.4.4   Managing and adjusting imported images in SPM
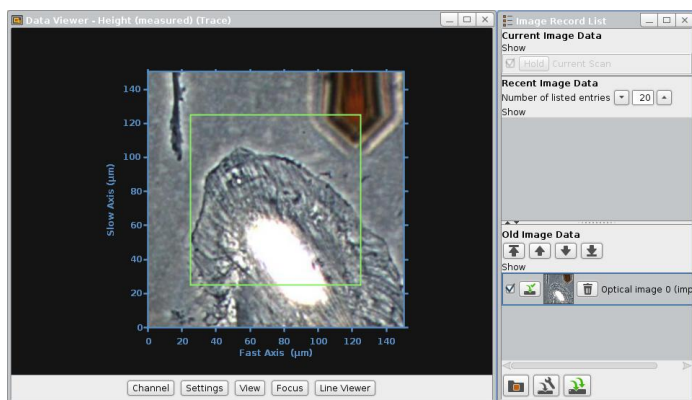
At the end of automatic or manual calibration, the final image is automatically calibrated and imported into the SPM software. It appears in the background of all **Data Viewer** windows and in the **Old Image Data** section of the **Image Record List**.

In the **Image Record List** the image behaves like AFM images, i.e. the image can be displayed or hidden by clicking on the **Show** toggle.

Click the **Remove** button to remove the image from the **Data Viewer**.
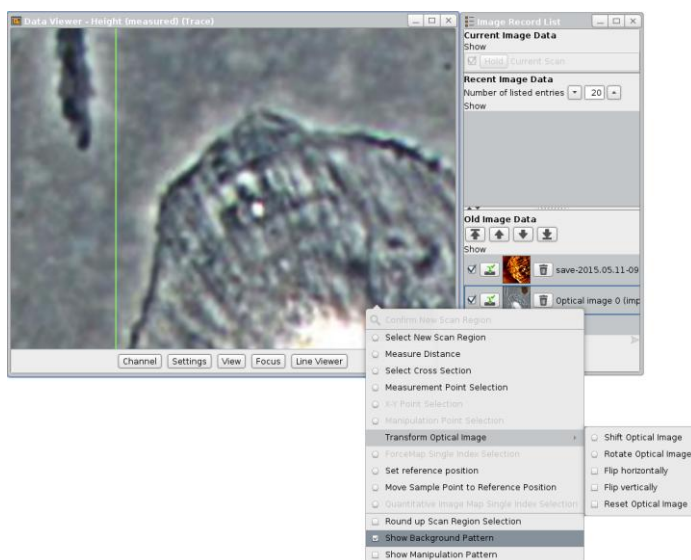
An AFM scan area can now be selected directly in the optical image. However, as the initial selection of the tip location may be slightly incorrect it may be necessary to shift the optical image slightly to correct for the actual tip position.

Start an AFM scan; a low resolution is sufficient. Select the optical image in the **Image Record List**. The optical image is now highlighted in green in the **Image Record List** and plotted on top of all the images within the **Data Viewer**.

Click the right mouse button within the **Data Viewer** and select **Transform Optical Image**. **Shift Optical Image** enables an offset correction and will make the optical image semitransparent.
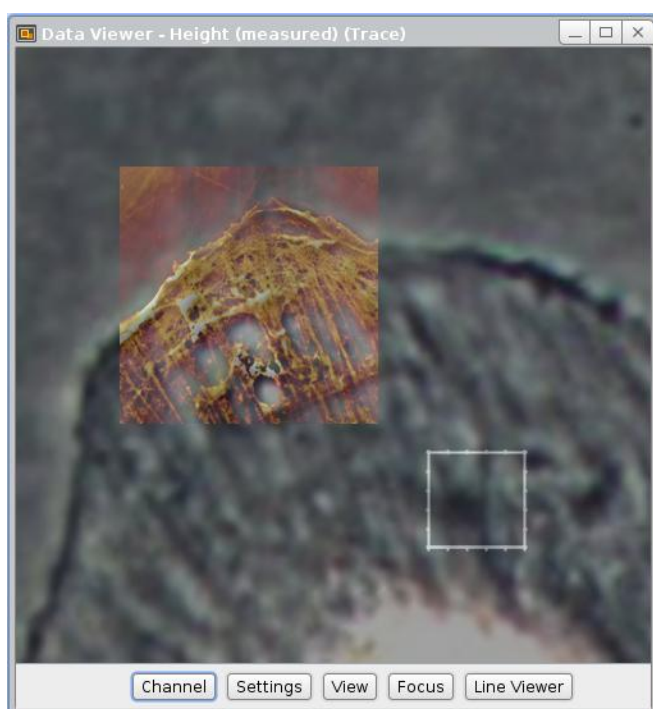
The semi-transparent optical image can now be shifted using click and drag with the mouse. Align the features of the two images. To go back to the normal view of the AFM image, go to the **Image Record List** and click on the current scan.

After transformation/shifting, the optical image is labeled unsaved. Click the unsaved button to save the transformation offset. A new calibration file with the extension "transformed" is created.

The **Snapshot** function can be used to acquire and import additional optical images using the current optical calibration. First select an optical image in the **Image Record List** to define the optical calibration for import. Click on the **Snapshot** icon to acquire an image from the **JUnicam** software and import it into the SPM software. The **Snapshot** icon is only active when the JUnicam 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.
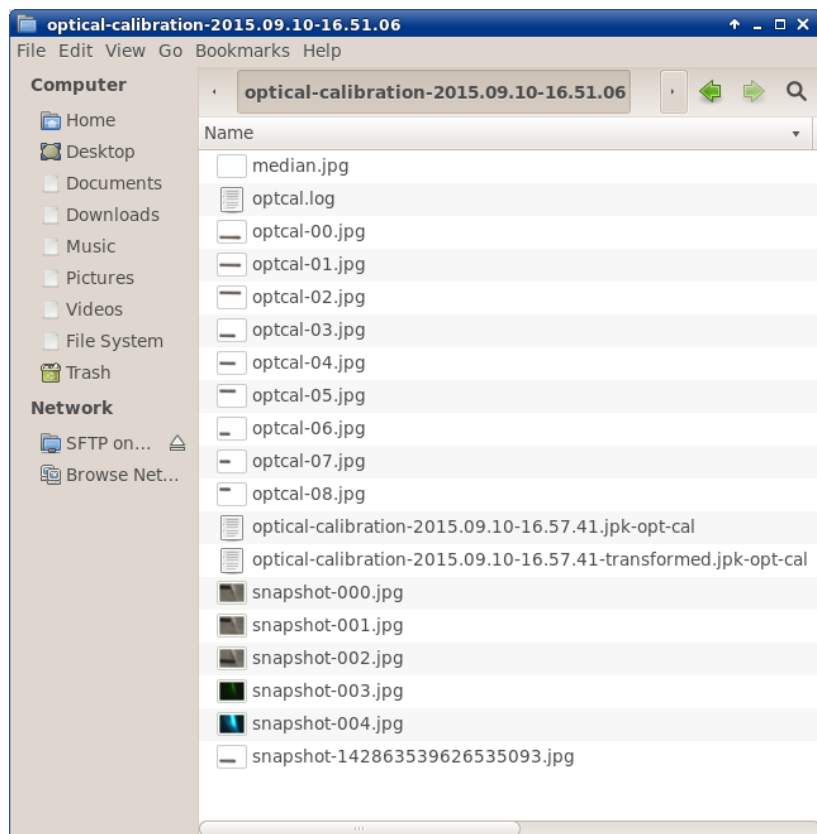
The calibration folder that was set at the beginning of the procedure contains a number of files of different types.

The 25 **calibration images** (optcal*.jpg) are stored along with the calculated **median image**.

Both the original optical calibration file (***.jpk-opt-cal**) and the transformed calibration file with the user determined offset **(*-transformed.jpk-opt-cal**) are located in this folder.

Any snapshots taken while this calibration is active are stored in this folder as well.
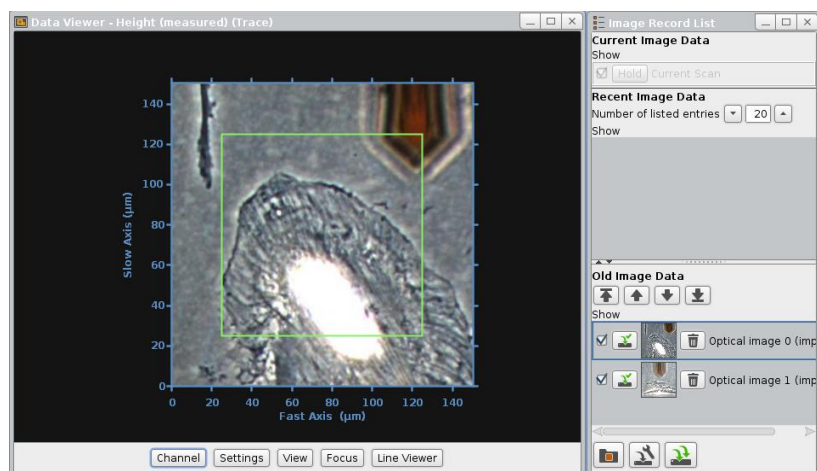
If there are more than one optical image in the Image Record List, then they will both cover the whole displayed area. The only visible image will be the one at the top of the list.

The up and down arrows in the Scan List can be used to change the order of the images

8.4.5   Manual Calibration

If other optical devices, e.g. a different CCD camera or a confocal laser scanning microscope, are used to acquire optical images, the DirectOverlay™ calibration can be used as a kind of manual calibration procedure. Therefore the calibration images are taken with the desired optical device (TIFF, BMP, JPG or PNG files) and transferred to the optical calibration folder.
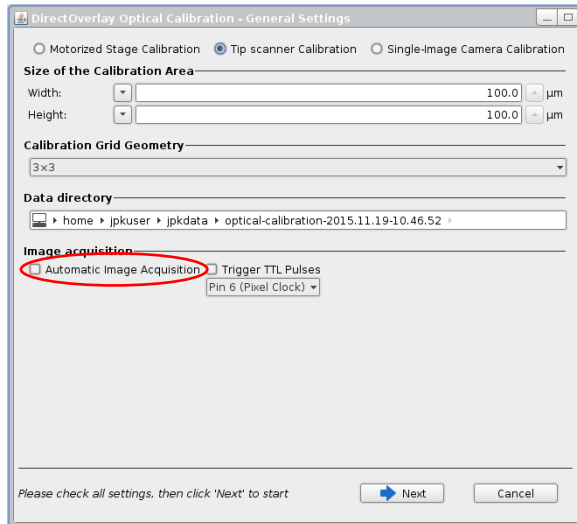
For the manual calibration using external image acquisition software **Automatic Image Acquisition** must be deselected.

Click Next to acquire the calibration images manually.

The manually saved optical images must be transferred to the **Data Directory**. The default name can be used, or a special name set.
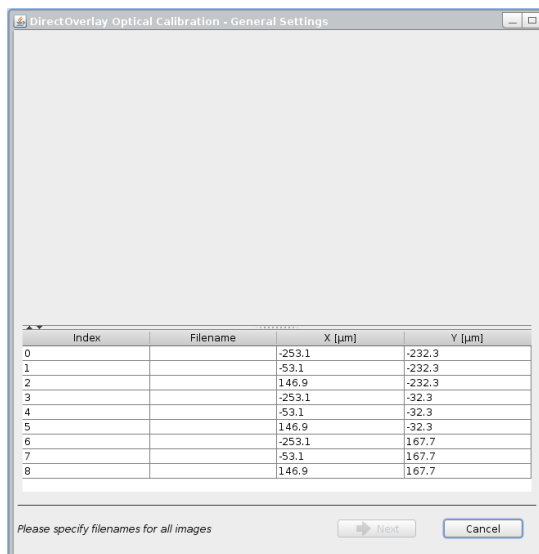
The 25 calibration piezo coordinates are listed and the up and down arrow keys can be used to scroll through the list. As each entry in the list is selected, the cantilever automatically moves to that position.

Acquire an image at each point and save the 25 images with sequential file names (such as 01, 02 etc.). Make sure 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 cameras or confocal software packages have 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 25 images at 2-3 seconds time interval. As each image is acquired, move to the next position in the list to put the cantilever in position for the next image.
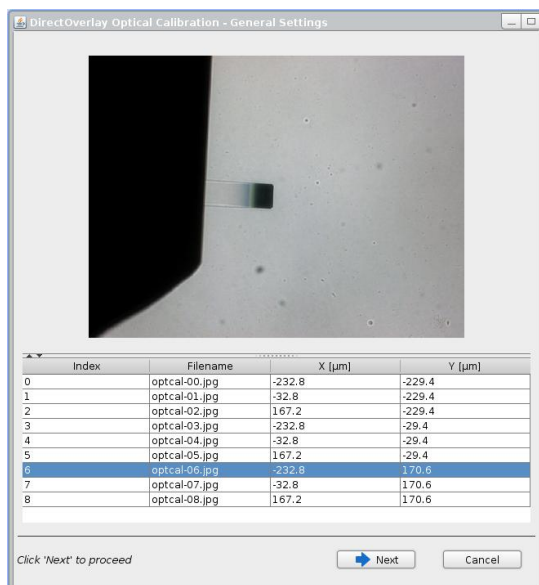
Once the files have been collected, they have to be transferred to the **Data Directory** chosen at the start of the calibration. The SPM software recognizes any sequentially named images in a basic graphics format (TIFF (8 bit), JPG, BMP or PNG).

Once there are suitable files in the **Data Directory**, the SPM software will automatically show the **Filenames** in the list. Scroll through the list to 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 image files is the same as described in **Section 8.4.3** for the **Automatic Calibration**. The only difference with an external camera is that the **Snapshot** function cannot be used. Acquire the desired snapshot with the external device and transfer it to the LINUX computer to import it as described in **Section 8.4.4**.

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.

8.4.6   Trigger TTL Pulses

Trigger TTL Pulses is an additional option for optical calibration with cameras/optical devices which are not supported by JUnicam. The SPM controller provides a TTL output, which can be connected to the camera trigger input. Each time the cantilever moves to the next calibration position, a trigger is sent to the camera. This option can be used with automatic and manual image acquisition, respectively.
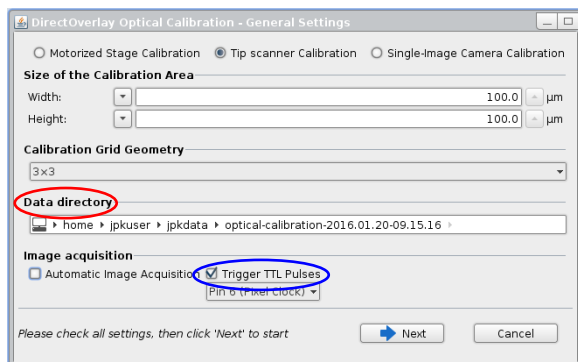
Trigger TTL Pulses has to be selected. Typically, the manual calibration procedure is used, and therefore **Automatic Image Acquisition** is disabled.

The manually saved optical images must be transferred to the **Data Directory**.

Here, the calibration procedure with an Imaging Source camera is described exemplary. Please read the **JPK Software Integration for Cameras** user manual (**§8**) for detailed information.
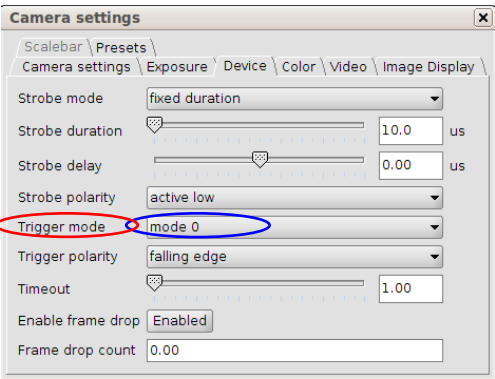
Adjust the camera on **Trigger mode**, and **Image series** mode.

Select the corresponding **Data Directory** for saving of the acquired images. The images should be saved with sequential file names (alphanumeric or alphabetical sequence) for the 25 cantilever positions.
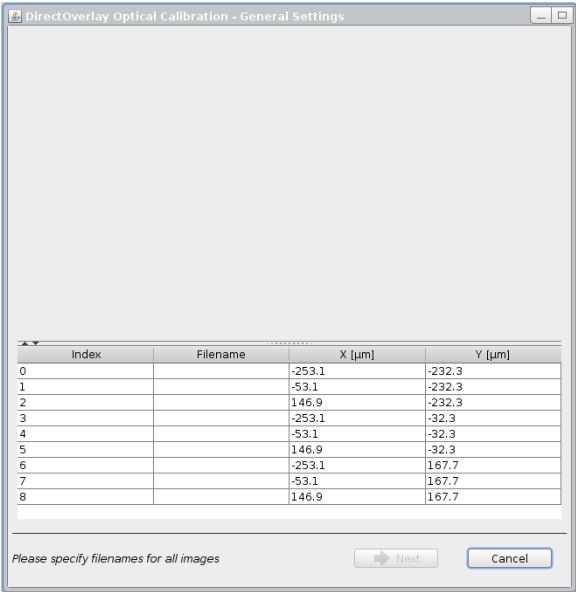
Scroll through the cantilever positioning list starting at **Index 0** using the down arrow key or by mouse click. A TTL-trigger will be induced for each cantilever position and the corresponding image acquired.

.
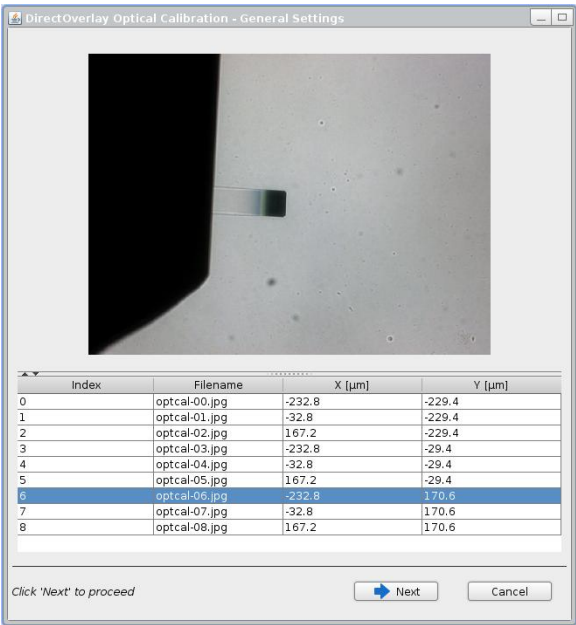
Once the images have been collected, they have to be transferred to the **Data Directory** chosen at the start of the calibration. The SPM software will recognize any sequentially named images in a basic graphics format (TIFF (8 bit), JPG, BMP or PNG).

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 8.4.3** for the **Automatic** calibration.

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8.4.7   Import Optical Images

Already existing optical images and calibration files can be imported together in the SPM software.
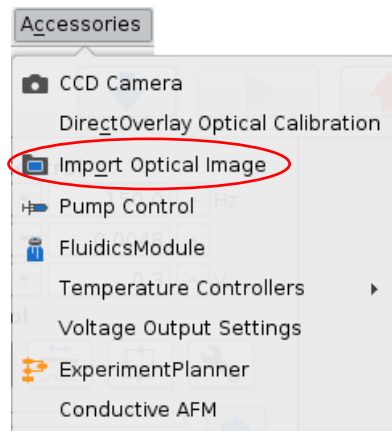
Choose **Import Optical Image** in the **Accessories** drop-down menu.
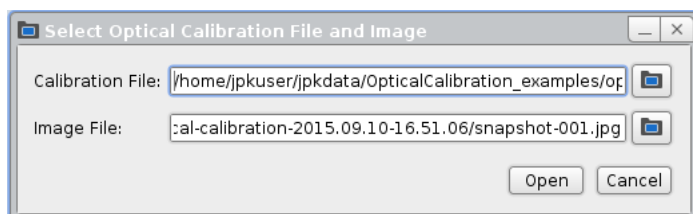
Select the desired **Calibration** and **Image File** in the file browser to open.

Optical images can also be opened in the **JPK Data Processing** (DP) program, to allow offline overlays and the export of calibrated optical images that correspond to particular AFM scans. The JPK Data Processing program is not designed to allow full image processing capabilities for the optical images, instead functioning as a first step in offline image processing. See the separate DP Manual for details on importing and managing optical images in DP.

8.5   Absolute Force Spectroscopy Mode

In ⊕ Absolute Force Spectroscopy mode, the z piezo is moved for absolute, defined distances, independent of the vertical deflection of the cantilever. I.e. there is no setpoint that defines the turnaround point of the force-spectroscopy movement, but the z piezo moves for an absolute distance towards and back from the sample.

The general functionalities, such as tip positioning or selection of force spectroscopy points, are the same as in **Basic** force spectroscopy mode. Please read **Section § 6**   for a detailed description of the basic functionalities.
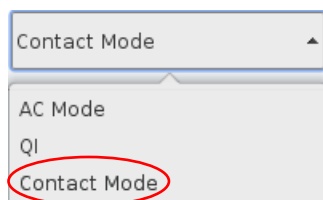
Select **Contact Mode** in the **Feedback Mode** drop-down menu.
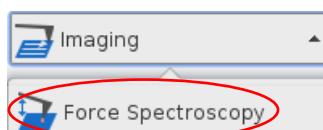
Select **Force Spectroscopy** in the **Measurement** drop-down menu.
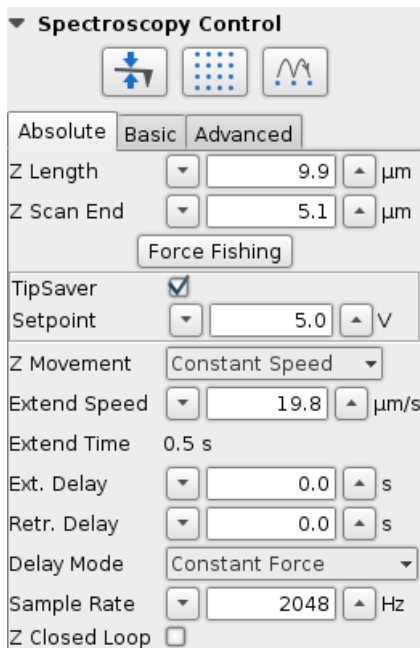
Select the **Absolute** tab from the Spectroscopy Control panel.

Set the absolute **Z Length** of the piezo movement range. **Z Scan End** defines the position of the turnaround point relative to the total piezo range.

Force Fishing is a special mode for the ⊕ **JPK Force Wheel** and is only active if this accessory is plugged-in. Please see the JPK Force Wheel user manual for a detailed description of this mode.

It is possible to set a maximum cantilever deflection value at which the piezo movement stops. Enable the **TipSaver** and define the **Setpoint** in terms of the maximum cantilever deflection. If the TipSaver Setpoint is reached during the force measurement, the piezo movement stops and starts the retrace segment, i.e. the resulting **Z Length** is smaller than set value.

The settings to control the speed, timing and resolution of the force curves are described in **Section 6.2**.

8.6 Advanced Spectroscopy Mode and Force Ramp Designer™

The ⊕ **Force Ramp Designer™** provides several segments that can be combined to force ramps, i.e. individual force distance experiments can be designed. A detailed description of Force Spectroscopy mode and the basic functionality can be found in **Section § 6** .
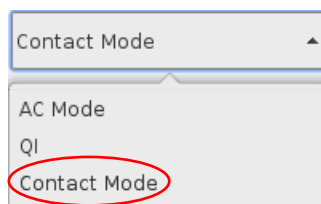
Select the desired feedback mode, usually **Contact Mode**, in the **Feedback Mode** drop-down menu.
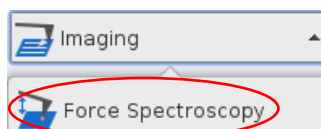
Select **Force Spectroscopy** in the **Measurement** drop-down menu.
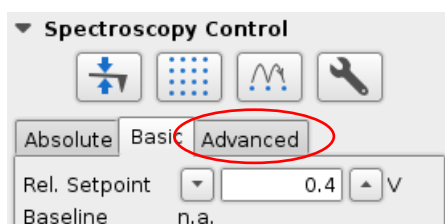
If **Advanced** mode is selected in the **Spectroscopy Control** panel, the **Force Ramp Designer™** opens automatically.
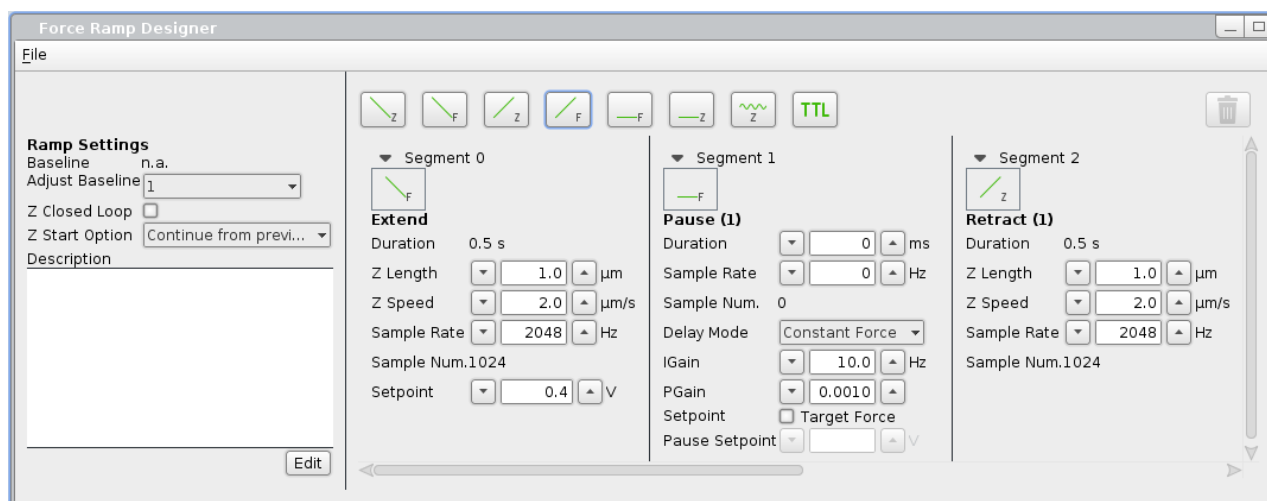
The Force Ramp Designer™ allows for the assembly of individual force spectroscopy experiments by sequencing different force segments.

Two extend and retract segments, in absolute (Z) and relative (F) mode.

Two pause segments in constant force (F) and constant height (Z) mode.

Sine modulation segment for microrheology measurements (see **Section 8.6.3**)

TTL Level/ Pulse segment to control TTL signals within a force distance curve (see **Section 9.8.3**)

Removes the selected segment from the force ramp.
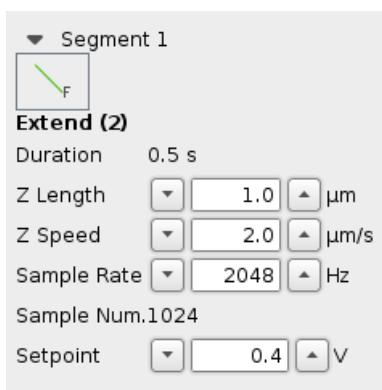
In **F Extend** and **F Retract** segments, the piezo moves for the distance **Z Length** but stops if the **Setpoint** is reached. If the **Setpoint** is not reached during **Z Length**, the piezo continues to extend/retract until it is reached. So **Z Length** is an expected value that helps to set parameters like **Z Speed** or **Sample Rate**.

The **Z** segments extend/retract the piezo for the distance **Z length**, independent of the vertical deflection. A **TipSaver** may be used in order to stop the motion if the **TipSaver Setpoint** is reached.

By default, the **F Pause** (constant force) maintains the **Setpoint** force of the previous segment for a defined **Duration** by adjusting the piezo height.

The **IGain** and **PGain** determine the reaction speed of this height adjustment (feedback loop) in order to keep the force constant. The higher the gains, the faster the feedback and correction. If the feedback is too fast, the cantilever may start to oscillate (depends on the cantilever properties).

Instead of the Setpoint of the previous segment, the **Setpoint** can also be set to a different value. Enable the **Target Force** tickbox and define the **Pause Setpoint** used for the pause segment.

The **Z Pause** (constant height) maintains the piezo end position of the previous segment for a defined **Duration**.

The segments can be sequenced as required for the experiment. The default sequence, which appears when the Force Ramp Designer™ is opened, produces a standard force curve with the same settings as in **Basic** mode.
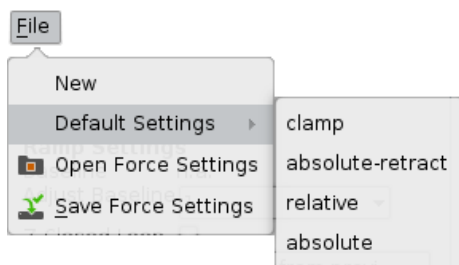
The File drop-down menu provides several pre-set force ramps for standard applications like force clamp (**Default Settings**), as well as opening/saving of self-designed force ramps (**Open/Save Force Settings**). Click **New** to remove all segments and design a new force ramp.

The experiment **Description** is saved along with the force ramp settings.

Click **Edit** to open the **Edit Force Settings Description** window.

Type any comments and confirm with **OK**.

8.6.1 Ramp Settings

The **Ramps Settings** provide automatic **Baseline Adjustment** (**Section 6.2.1**), Z Closed Loop (**Section 6.2.3**) as well as different **Z Start Options** that define the piezo position at the beginning of the force curve.

If **Piezo approach** is selected, each force curve of the experiment starts in idle mode, i.e. the cantilever is approached to the surface with the approach setpoint.

In **Continue from previous** mode the force curve is repeated right after the last force curve segment without any special starting position.

Piezo retract starts each curve in piezo retracted mode. The cantilever is moved with **Velocity** until the first segment begins.

It is also possible to start each force curve from a defined **Distance from surface**. Beginning from **Height** relative to the measured surface, the piezo extends with **Velocity** to the first force curve segment.

Similar to **Distance from Surface**, the **Absolute Z position** option starts from a defined, but absolute piezo position, i.e. there is no correlation to the surface determined by the precedent approach or F extend segment.

8.6.2 Advanced Spectroscopy Control
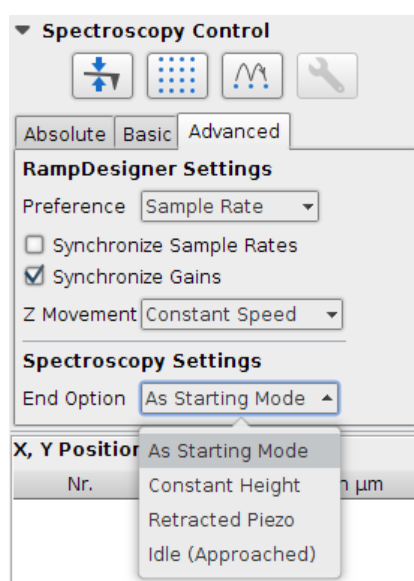
The **Advanced** panel in the **Spectroscopy Control** window provides general settings that are valid for all segments.

Preference allows typing the force curve resolution as **Sample rate** (in Hertz) or as **Sample number** (in data points per segment). Correspondingly, editable input fields appear in the force curve segments.

It is also possible to **Synchronize sample rates** and to **Synchronize gains** along all force segments in order to keep the bandwidth constant.

Z Speed, Z Length and Duration (see extend/retract segments) depend on each other. **Z movement** helps to either maintain the duration of a segment (**Constant Duration**) if Z Speed or Z Length is changed, or to maintain the Z Speed (**Constant Speed**) if Duration or Z Length is changed.

There are different modes available after spectroscopy is stopped. The first is to **Return to starting mode**. **Constant height mode** maintains the end position of the last force curve segment. **Retracted piezo mode** and **Idle mode** turn the piezo into retracted and approached mode respectively.

8.6.3 Sine Modulation

For microrheology measurements a **Sine Modulation** segment is available as a software add-on.
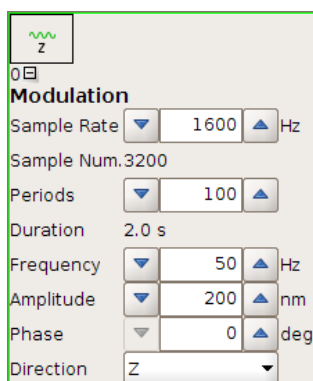
For each **Sine Modulation** segment the **Sample Rate** can be adjusted.

Depending on the measurement the number of **Periods** can be set between 1 and 10000.

Frequency and **Amplitude** of the modulation can be modified.

The starting position of the sinusoidal movement can be changed by adjusting the **Phase** value.

For the **Direction** of the movement the **Z** as well as **X** dimension can be selected.

8.6.4   Display force ramp data

As in Basic force spectroscopy based mode, the **Force Spectroscopy Oscilloscope** opens automatically upon select-ing **Advanced** mode. Please see **Section 6.1.2** for a detailed description of the functionality of the oscilloscope.
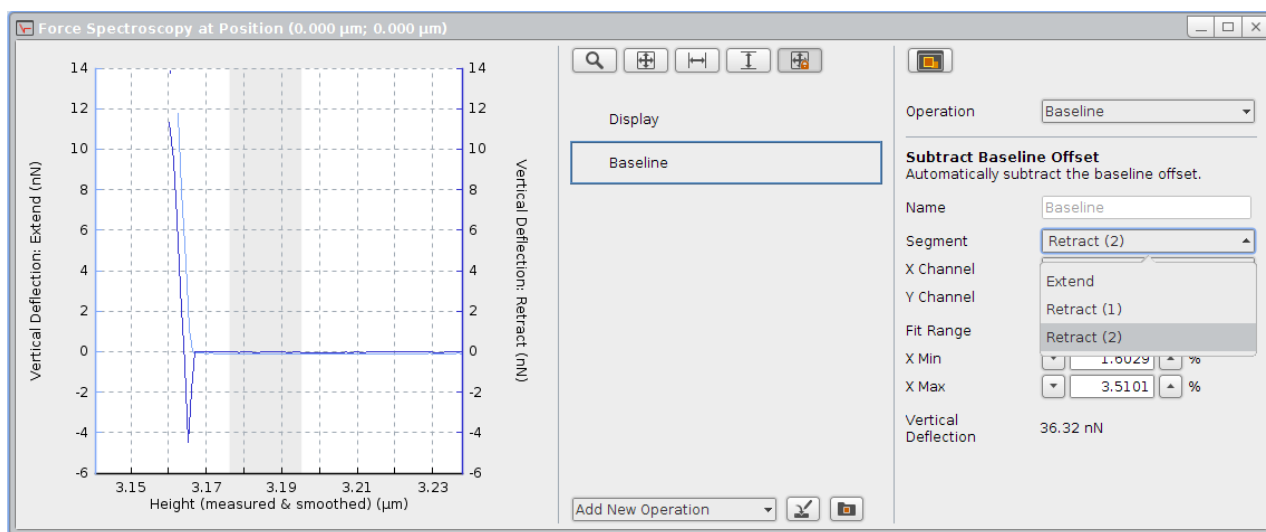
Operations can be added and applied to any segment. Select the desired segment in the **Segment** chooser.
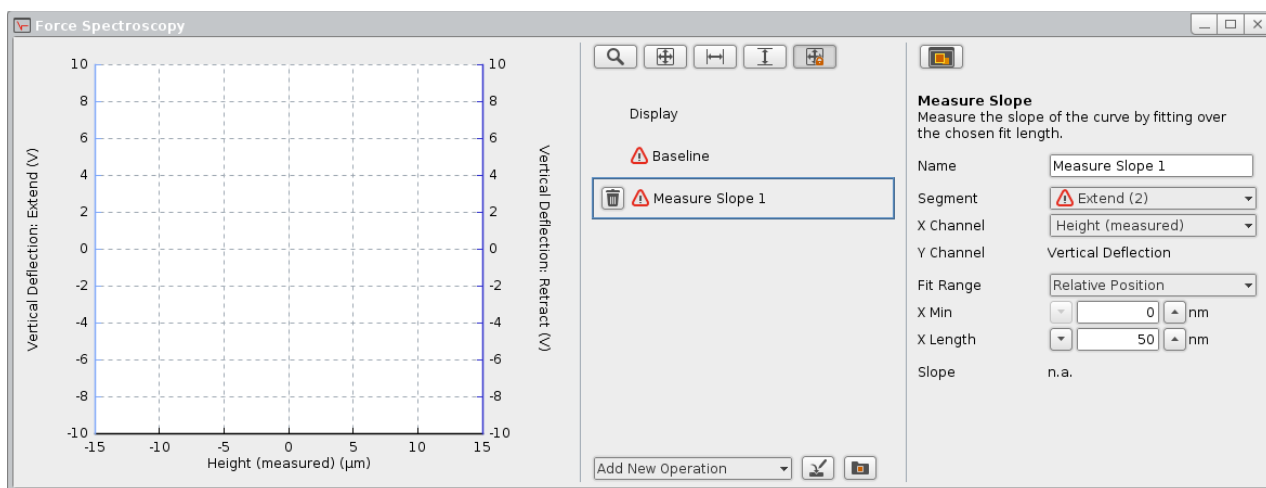
Invalid operation warning icons may come up if segments chosen for the corresponding operation are removed. Add-ing segments may result in the renaming of existing segments, which causes invalid operations if the renamed segment is chosen for any of the operations. E.g. if only one extend segment exists, its name is **Extend**. If another extend seg-ment is added, the Extend segment is renamed to **Extend (1)** or **Extend (2)**, depending on the segment order. The invalid operation waring icon will disappear as soon as a valid segment is selected in the **Segment** chooser.

Invalid operation warnings may occur if segments chosen for the corresponding operation are removed or renamed, or if the required channels are not activated in the Channel Setup (see **Section 6.1.2**). Always check whether all required channels are activated in the Channel Setup (**Section 3.2.7**) when the invalid operation warning icon appears for any operation.

To display the desired channel in dependence on time and to visualize pause times, open the **Force Time Oscilloscope**. Please see **Section 6.1.3** for details.

8.7 Manipulation and lithography

⊕ **Manipulation** allows defining paths over the sample surface that the tip will follow. In Imaging mode, the tip moves back and forth in scan lines across the sample, and in Force Spectroscopy mode, 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; a series of disconnected lines can be drawn. 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). Or the tip can be used to draw a pattern across the sample, e.g. scratching with a higher force (nano-scribing).
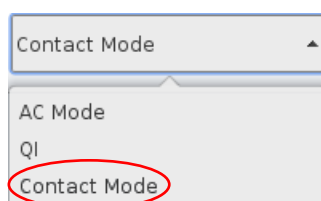
Select **Contact Mode** in the **Feedback** Mode drop-down menu.
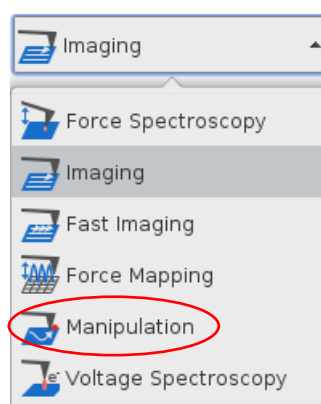
Select **Force Spectroscopy** in the **Measurement** drop-down menu.

8.7.1 Manipulation Control

At the beginning, it is useful to calculate the **Setpoint** force applied in order to choose suitable values for manipulation of the surface or objects lying on the surface.

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
	~ 100 pN to move molecules
Nano-scribing	~ 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.

Manipulation Control

Velocity	1.0	µm/s
Setpoint	0.2	V
IGain	150.0	Hz
PGain	0.0048	

Path 1

[Delete] [Clear] [New]

X, Y Position

Nr.	X in µm	Y in µm
0	-5.0	-4.33
1	0.0	4.33
2	5.0	-4.33
3	-5.0	-4.33

[Delete] [Clear] [New]

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**. During the movement along each path, the **Setpoint** is held 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 **Data Viewer**, 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.

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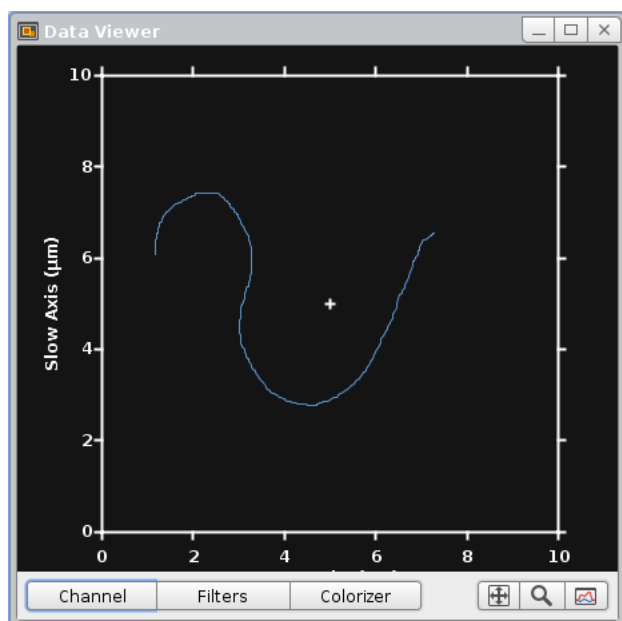
In **Manipulation** mode, the default mouse action is to draw manipulation paths in the **Data Viewer**. Click and drag with the mouse and the movement will automatically be converted into a set of points in the **Manipulation Control** panel. When the mouse is released, then the next click and drag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

The mouse function can be changed with the right-click menu to change the view in the Data Viewer.
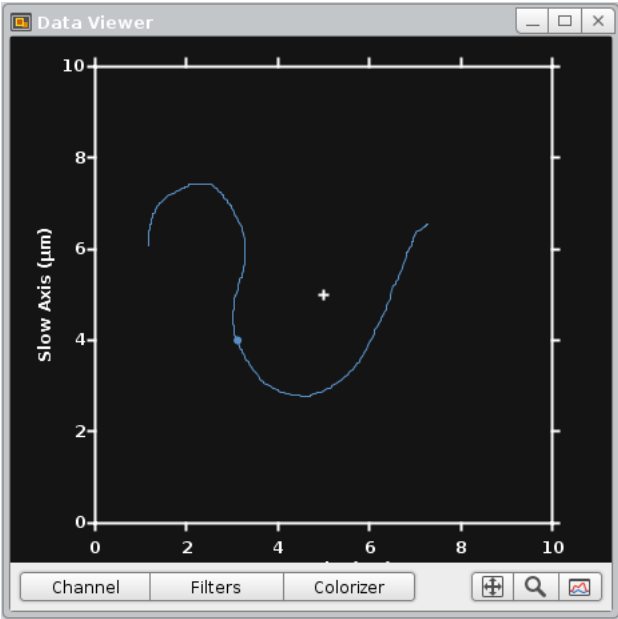
When a point in the path is selected in the **X,Y Position** list, then it is shown highlighted as a blue dot in the **Data Viewer**. The selected point may be moved around using the mouse (click and drag).

For drawing straight lines press the control key "Ctrl" and define the start and end position with the left mouse button
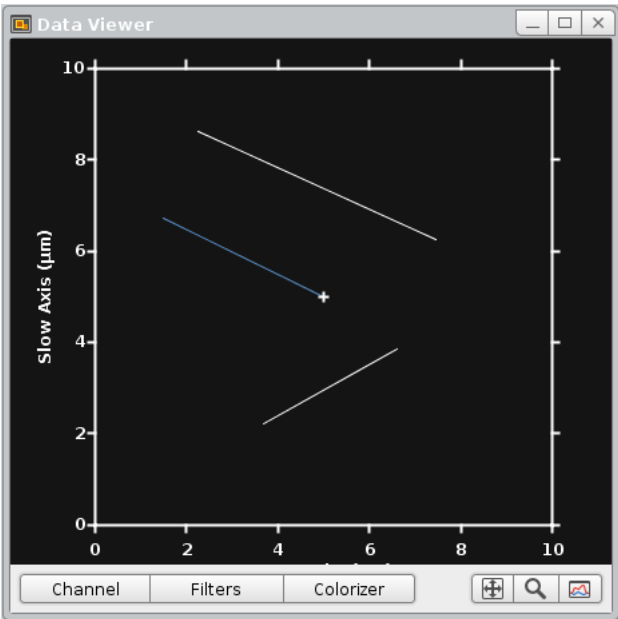
More than one path can be drawn – this example shows three paths. A path can be selected from the drop-down menu in the **Manipulation Control** panel. The currently selected path is displayed in blue.

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 of the current path.
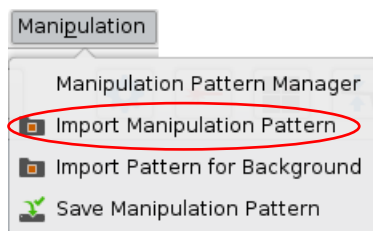
8.7.3   Run Manipulation
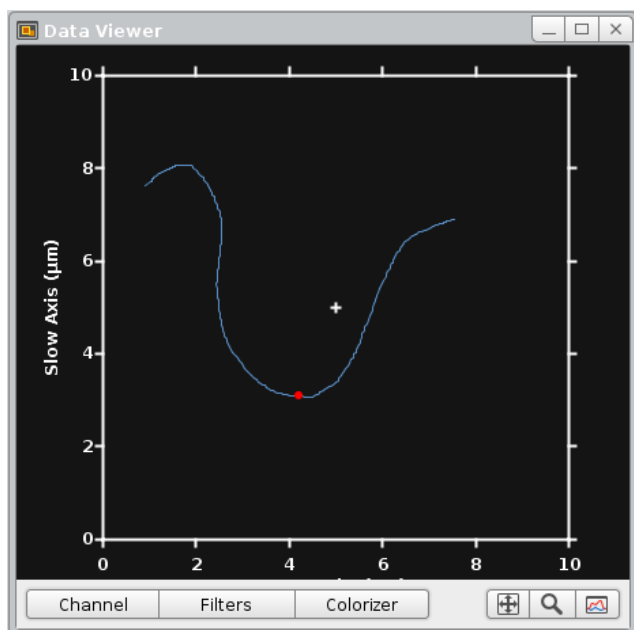
Define a manipulation pattern/path and click **Run** to perform the entire manipulation movement (all set paths). The current point is shown in red in the **Data Viewer** during the movement.

When all paths are performed, the cantilever is left in the retracted position.

8.7.4   Importing and exporting scalable vector graphics files

Manipulation mode allows importing manipulation patterns/path of the scalable vector graphics format. Prepare your manipulation pattern in any corresponding graphics software and export the pattern as *.svg file.

Select **Import Manipulation Pattern** from the **Manipulation** drop-down menu on top.
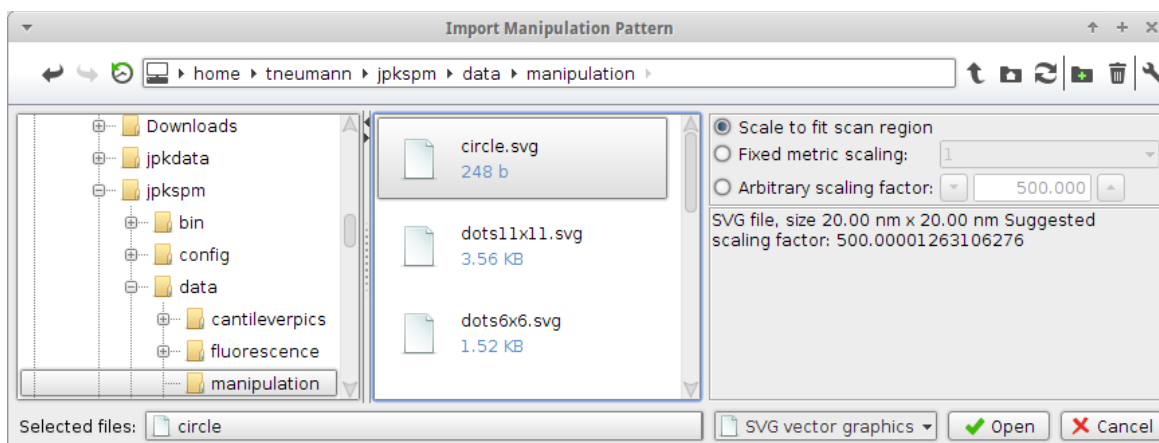
Choose the desired file and set the scaling:

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).

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 input field, 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.

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 depends 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".
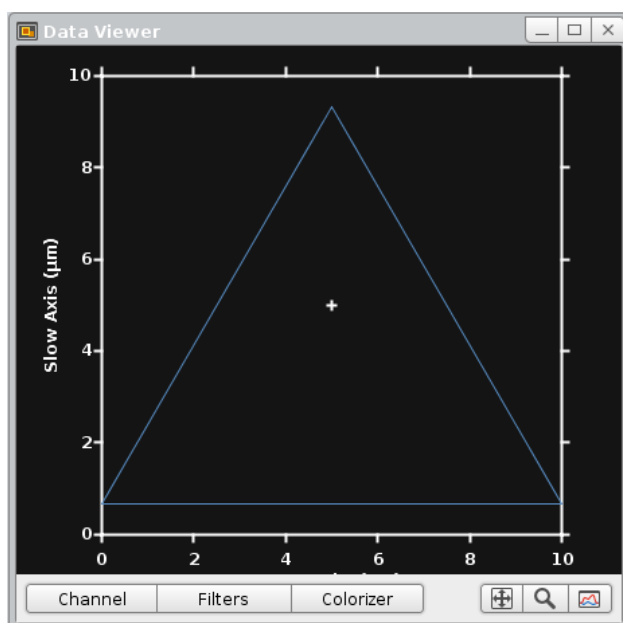
This example shows one of the example manipulation paths which can be found under:

/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

### 8.7.5   Simple manipulation examples

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 AC mode cantilever with a spring constant of around 40 N/m was used. Imaging in AC 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 image below shows a height image (5.0 x 2.2 µm).
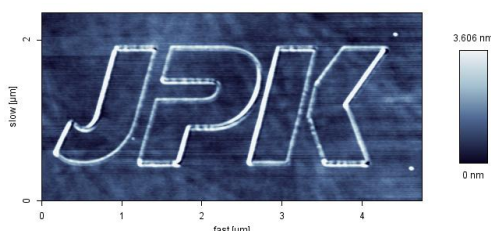
Moving spheres

The freehand drawing of manipulation paths is useful for moving objects around on the surface, and switching between manipultaion 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 AC mode in air, which did not disturb the spheres, and manipulation could then be performed using a very small setpoint in contact mode (around 0.05 V 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.
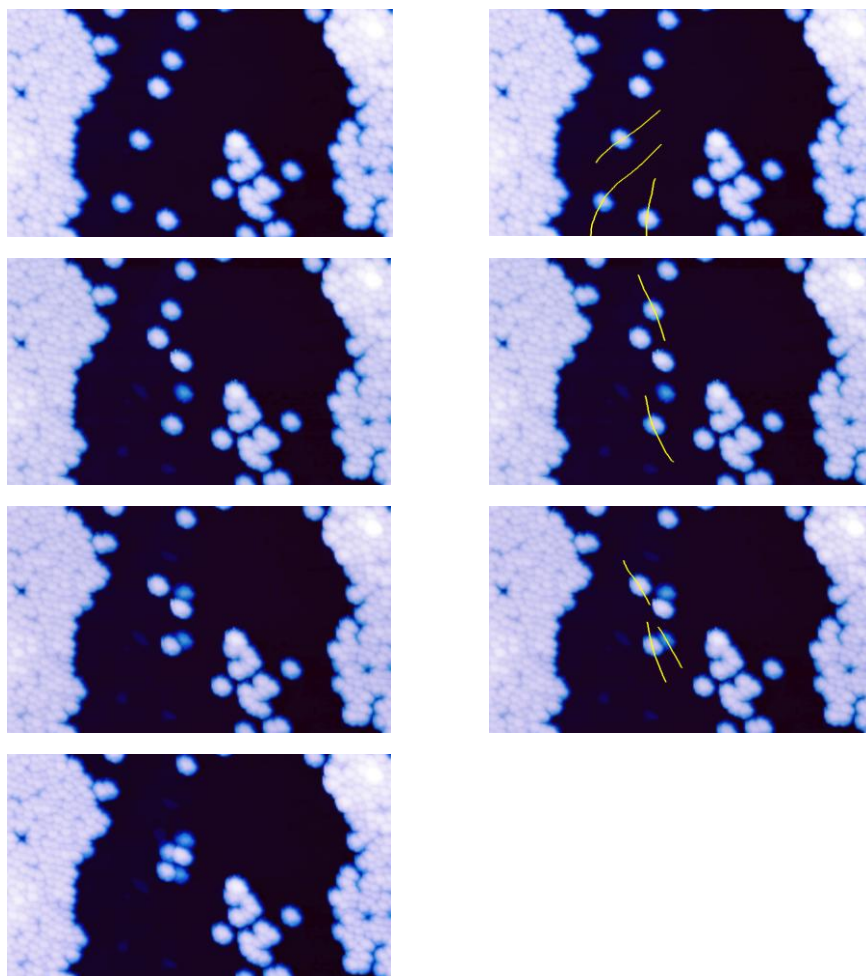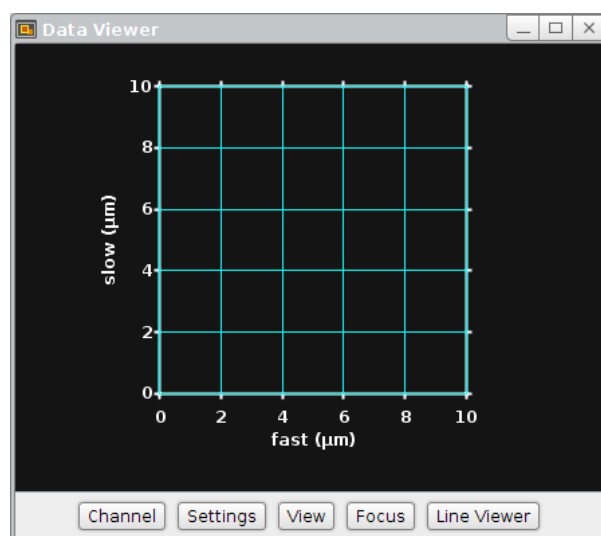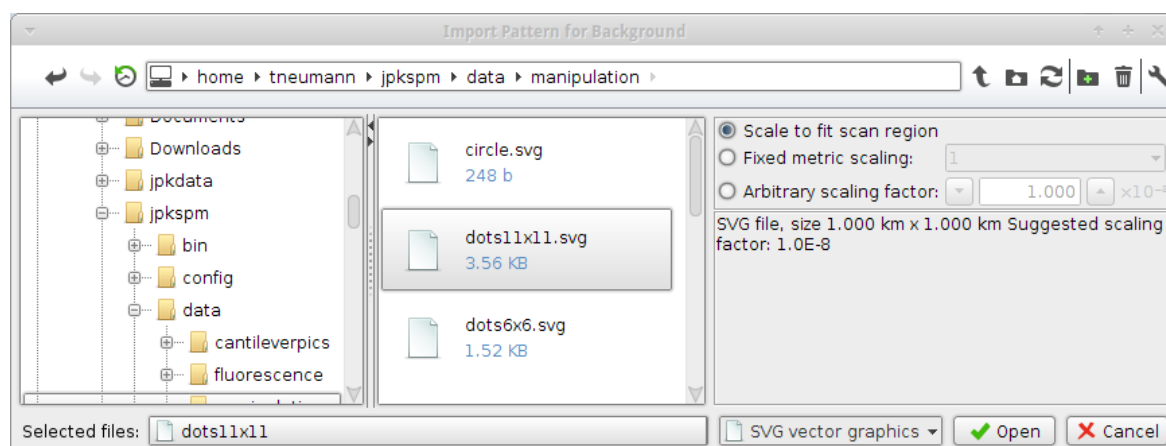

8.7.6   Background patterns

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.
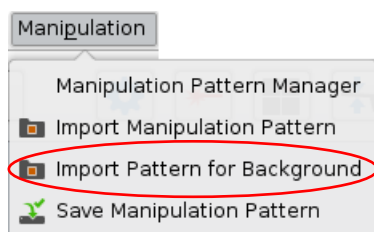
Select **Import Manipulation Pattern** from the **Manipulation** drop-down menu on top.

The import dialog is very similar to the manipulation path import dialog (see **Section 8.7.4** for details of the options). Patterns can be found under /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 Data Viewer. The manipulation path can be drawn over the top, the background pattern has no influence on the tip movement.

# 8.8   Environmental control for experiments

8.8.1   Temperature control and data saving

Many different temperature control accessories are available from JPK, which can be used to heat or cool the sample. There is a general interface for controlling the temperature from the SPM software. This Temperature Controller interface allows displaying and controlling the temperature as well as saving the temperature profile of an experiment.

The corresponding temperature control accessory must be connected to the PC using the dedicated USB port. Please find a close description of your temperature controller in the specified user manual.
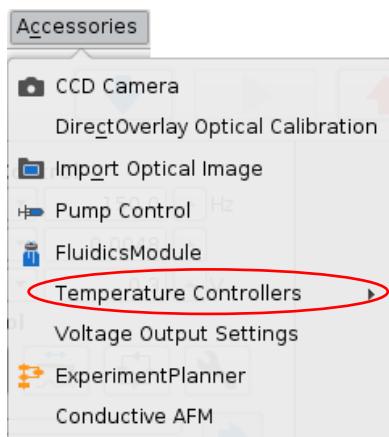
Open the list of installed ⊕ **Temperature Controllers** and select the corresponding device from the **Accessories** drop-down menu.
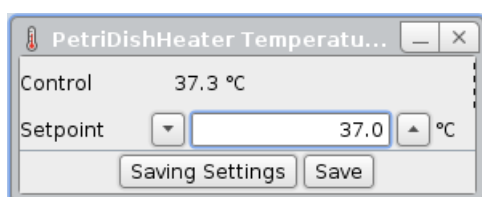
The Temperature Control panel for the chosen device shows the **Control** temperature, which gives the actual temperature of the sample holder. Type the desired **Setpoint** temperature.

Save allows saving of the temperature data along with the time as well as other channels in the form of a text file. **Select Saving Settings** to set the channels to be saved.
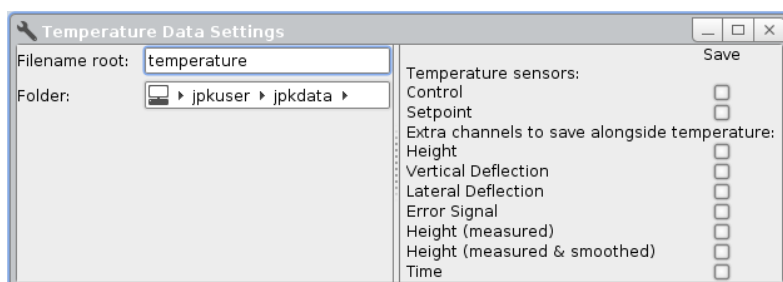
The **Temperature Data Settings** panel provides several data channels like the **Setpoint**, the **Control** and **Sample** temperatures.

The temperature text file is automatically updated as the temperature changes, with more data points being taken when the temperature changes more rapidly.
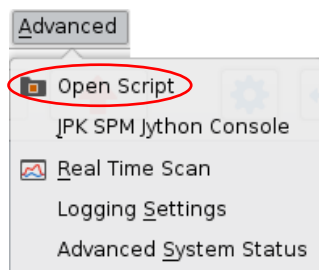
The temperature is usually a direct control, so that the value entered in the panel is immediately used by the temperature device.

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. Please read Section **9.6** for more information about JPK scripts.

### 8.8.2   Pump control for syringe pumps

⊕ Many different liquid cell accessories are available from JPK; most can be used with perfusion either manually or using a syringe pump. The SPM software provides control and monitoring of several syringe pump models, such as the KD Scientific Model 200 Series as well as various models from WPI.
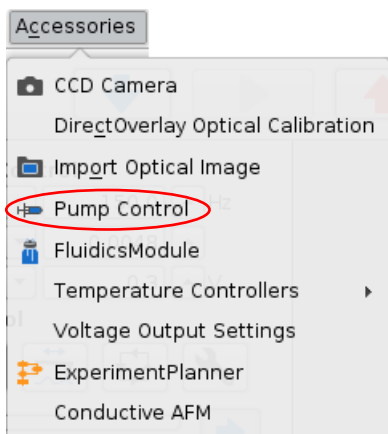
Open the **Pump Control** window from the **Accessories** drop-down menu. The pump must first be connected to the power supply and to the SPM computer (serial connection).
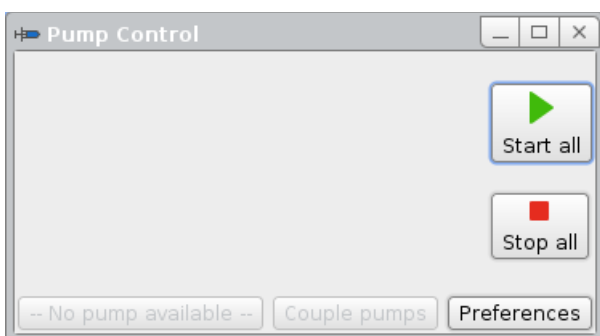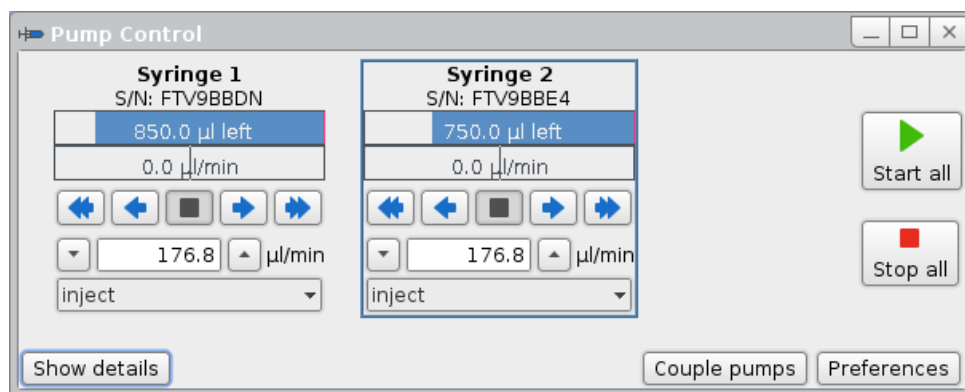
If no pumps are connected and configured, the main **Pump control** panel opens as shown here.
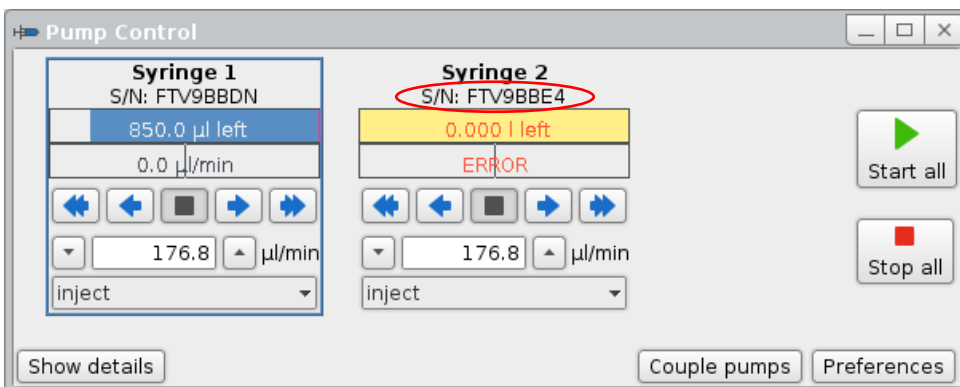
The Aladdin pumps interface via USB and are automatically recognized when connected, even if the pump itself is switched off. The serial number of the connected pump is indicated.
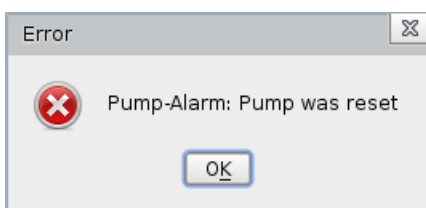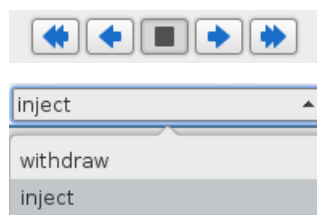
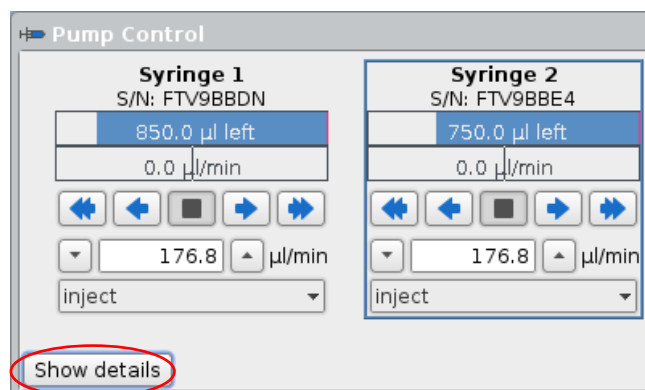Switch the pump on; a popup will show to indicate the pump reset.

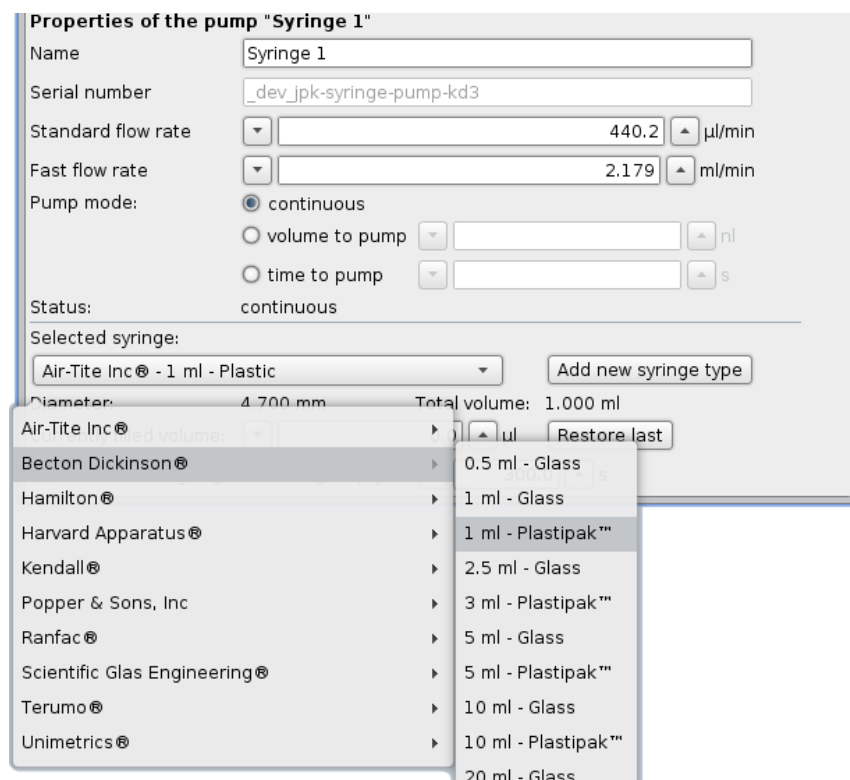When the connected pump is switched on, the pump settings can be adjusted.

All pumps can be controlled with **Start all** and **Stop all**.

Individual pumps are controlled with their button bar, and can pump to both, inject or withdraw direction.

Click on **Show details** to open the list of full settings for the pump currently selected.

It can be useful to enter a sensible **Name** for the pump to reflect its contents.

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 pumped volume. It is important to enter the used syringe type in order to obtain a properly calibrated flow speed. Custom syringes may be added and saved.

If more than one pump is available, then the option **Couple pumps** can be used for push-pull systems, where the same volume should be injected with one or more pumps and withdrawn using another pump. The pump speeds are adjusted so that the net rate is constant.
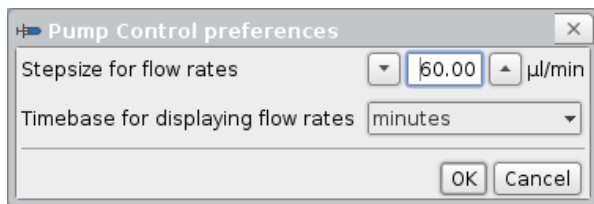
The settings in the **Preferences** panel affect all pumps at once.

The step-size for flow rate settings can be changed, as well as the time unit for flow rates.

The settings for the pump control are saved in /home/<username>/jpkdata/configuration, along with the general settings for the NanoWizard™ software. The connection and syringe settings are remembered as long as these files are not deleted or overwritten by a new software installation.

§ 9   Advanced SPM software options

9.1   Spectrum Analyzer

Using 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.
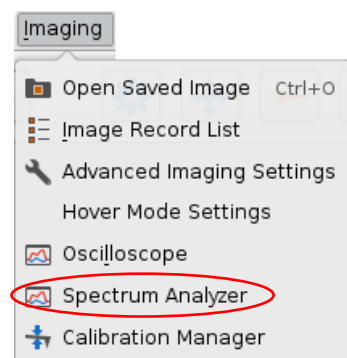
Open the **Spectrum Analyzer** via the **Imaging** drop-down menu.
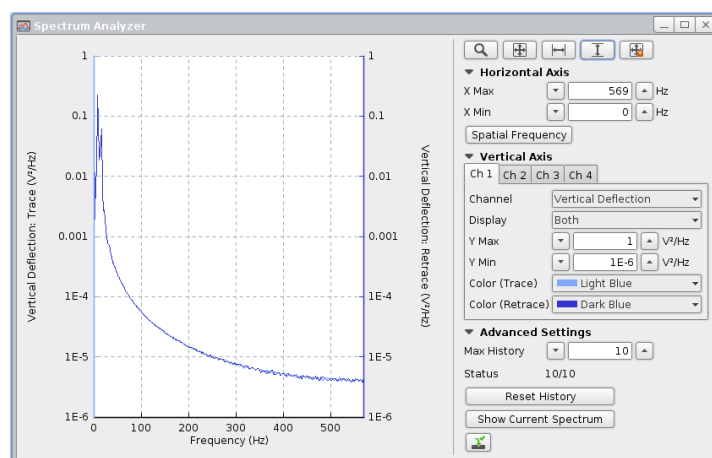
The main display controls are similar to the **Oscilloscope** window (see **Section 5.5.1**).

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.

Multiple channels can be selected using the channel tabs for the Vertical **Axis**.
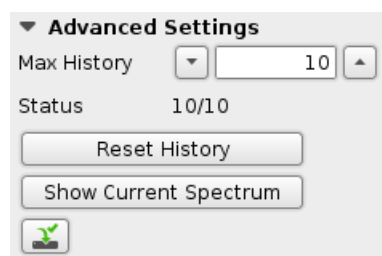
The **Max History** input field of the **Advanced Settings** allows for settings the number of scan lines used to calculate the average spectrum. The Status box shows the number of scan lines being used to calculate the spectrum. Click Reset History to remove the old scan lines from the calculation. **Show Current Spectrum** displays the spectrum calculated from the current scan line independently of previous scan lines. The save icon on the bottom allows saving of the current spectrum.

9.2   Real Time Scan
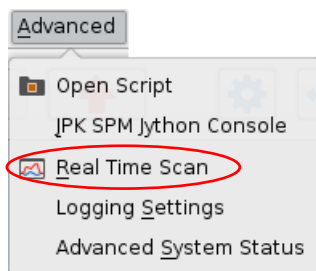
Open the **Real Time Scan** via the **Advanced** drop-down menu.

The Real Time Scan oscilloscope displays the channels data continuously as a function of time. The display is similar to the output from a conventional oscilloscope connected to the particular channel.
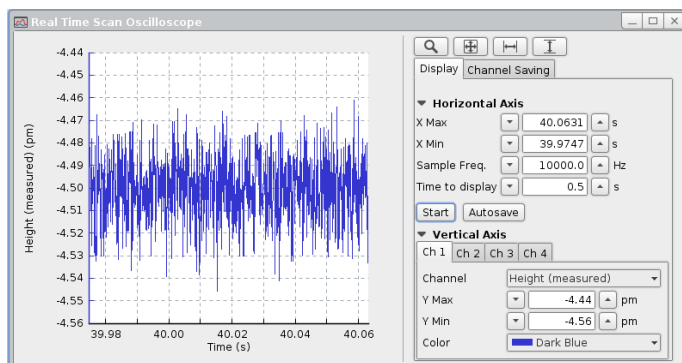
The main display controls are similar to the **Oscilloscope** window (see **Section 5.5.1**).

The **Sample Frequency** defines the data sampling rate. If the data is sampled with high frequency, the performance may start to suffer. Check that only the required channels are being collected (see the **Channel Saving** tab) to optimize the high-speed performance.

Click **Start** to collect and display the real time data. To save the data, enable the **Autosave** button before **Start** is clicked.
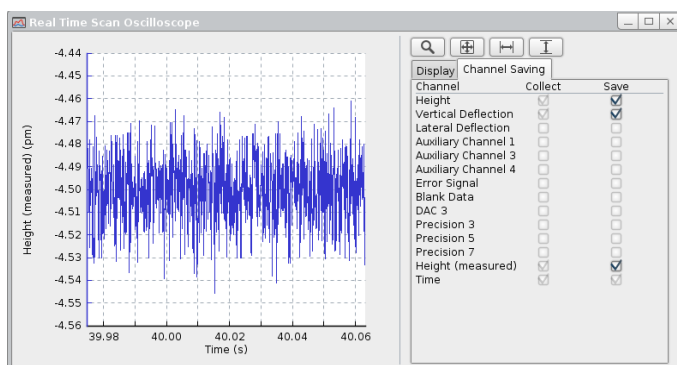
Select the **Channel Saving** tab to select the channels to be collected and saved. **Collect** means the data is collected for being displayed in the Real Time Scan. All channels with the **Save** tickbox enabled are saved when **Autosave** is enabled.
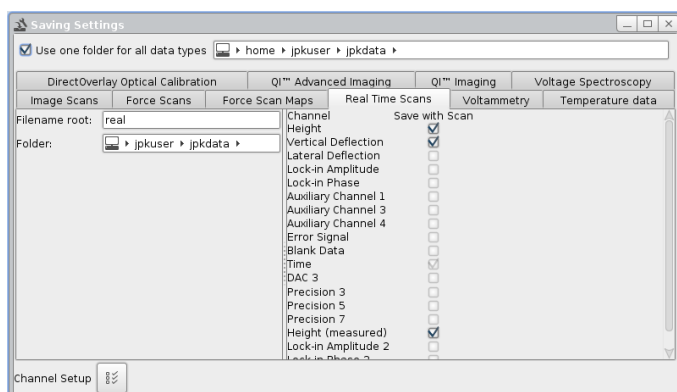
The channels to be saved can also be selected in the **Real Time Scan** tab of the **Saving Settings** window (see **Section 3.2.8**). Here additional settings concerning the file name and location can be set as well.

⚠ Enable the **Save** tickbox for all channels to be saved. If only **Collect** is enabled but not **Save**, the corresponding channel data can be displayed, but are not saved. Open the **Channel Saving** tab of the **Real Time Scan Oscilloscope** and tick the **Save** tickbox for the channels to be saved.

9.3   Logging Settings
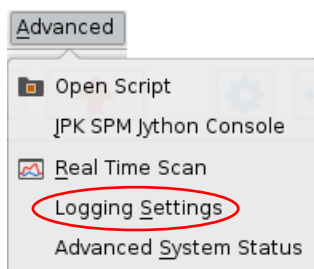
Open the **Logging Settings** via the **Advanced** drop-down menu to adjust the settings for error logging.

The **Loggers** determine where relevant messages, errors and warnings are displayed:

- **Log-File:** information written in the log file, which is automatically saved in the directory "/jpkdata/*.log".
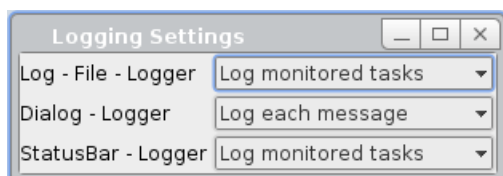
- **Dialog:** information displayed in pop-up dialogs.

- **Status Bar:** information appearing in the lower status bar of the SPM software
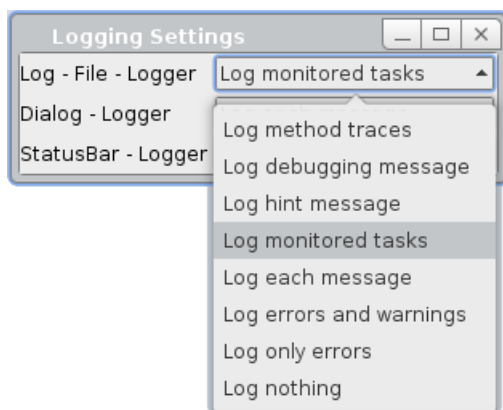
Each **Logger** can be adjusted separately by setting what is to be logged. Warnings and errors can give useful information about the status of the AFM and may be a guide to the source of a problem.

Before deactivating all loggers, please keep in mind that these loggers are used to track unforeseeable errors that might occur. Especially the log files can be used for later bug fixing.

9.4   Voltage Output Settings

The Voltage Output Settings allow sending a variable voltage to a free DAC on the **Signal Access Module** (see **Section 11.1.1**). This enables e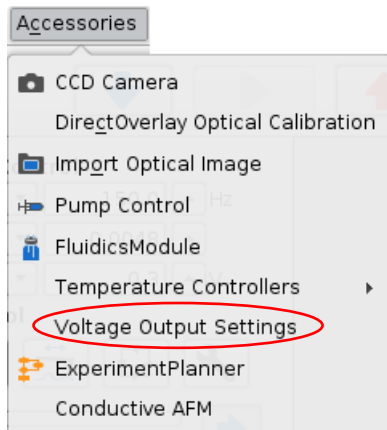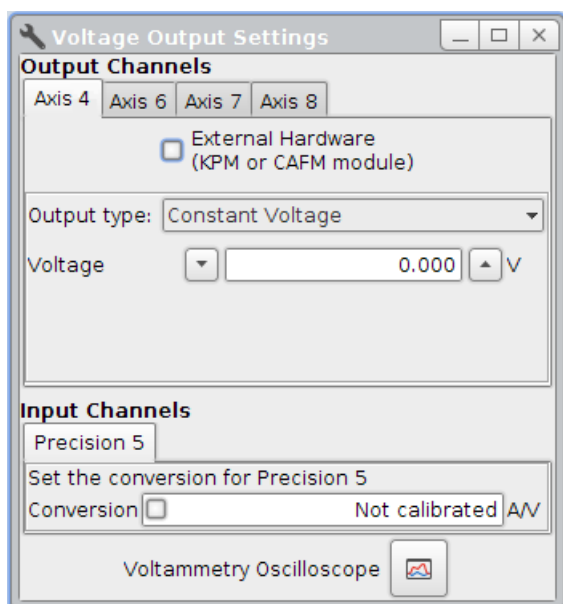lectrical measurements without using an external power supply, such as oxidation lithography, conductive AFM or electrochemistry measurements.

Open the **Voltage Output Settings** via the **Accessories** drop-down menu.

The **Output Channels** settings are stored in the property file for the instrument, and depend on the controller type. For the standard SPM controller, typically Axis 4 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 (directly from the Signal Access Module) and -10V – +10V range (only available if a ⊕ KPM box is connected in between).

If **Output Type** is set to **Constant Voltage**, then the output is taken directly from the **Voltage** input 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 such applications using the corresponding Axis as the default input.
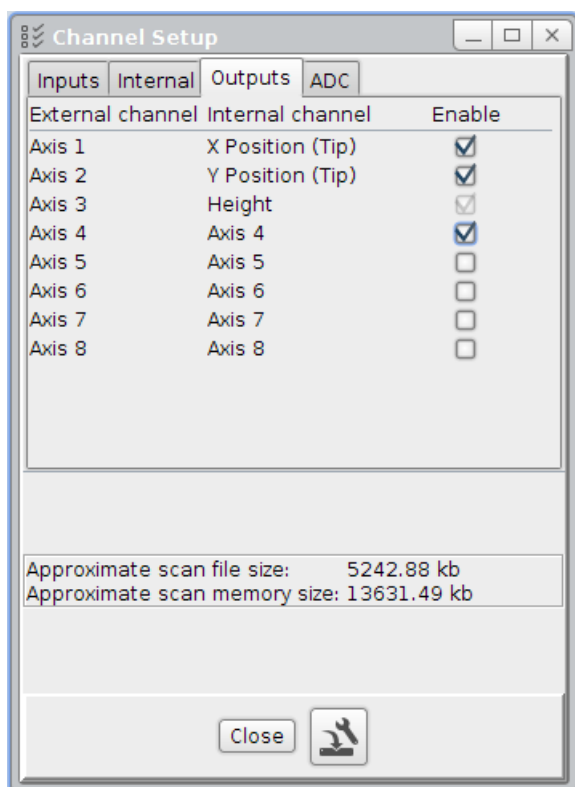
Before starting measurements, check that the selected **Input** and **Output Channel** are enabled in the main **Channel Set-up** (see **Section 3.2.7**). Otherwise data from these channels will not be seen in the software, and hence cannot be displayed in the oscilloscopes.

Usually this means that **Axis 4** and either **Precision 5** should be selected.

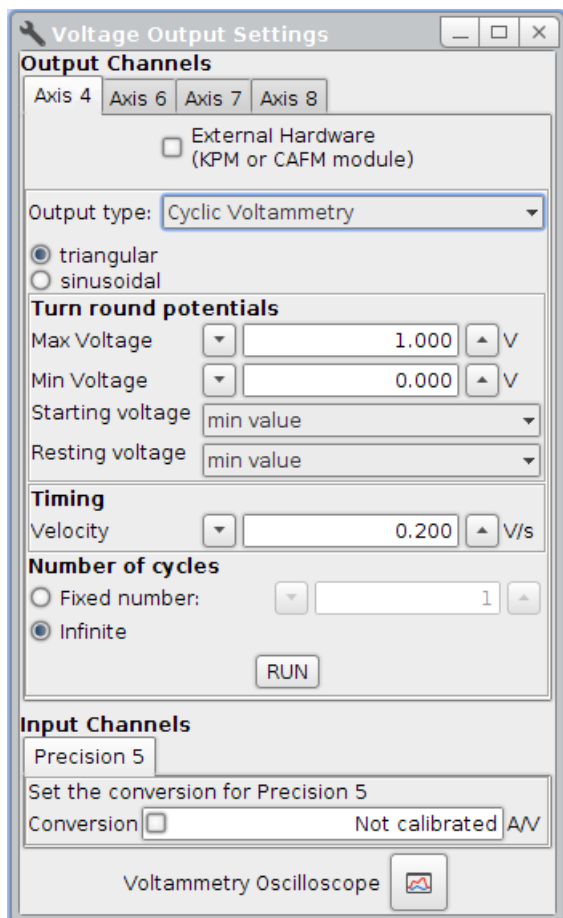The channel saving for data display is handled separately in the corresponding oscilloscope (see below). Make sure that the desired channels are enabled in the **Channel Saving** tab of the **Voltammetry Oscilloscope** (see below).

If the settings are changed to ⊕ **Cyclic Voltammetry**, then the output will produce a triangular / saw tooth output with the corresponding settings.

The cycles can start and end at either the **Min Voltage** or the **Max Voltage**.

The time for the cycle is set implicitly by 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**.

The current-voltage amplification will generally be provided by some external hardware, and the **Conversion** factor can be entered here. For instance, if the gain is set to 1 micro Ampere per Volt, the conversion can be typed in as 1e-6. This will automatically be updated to show the current in the correct unit.

Open the **Voltage Oscilloscope** using the corresponding shortcut icon.
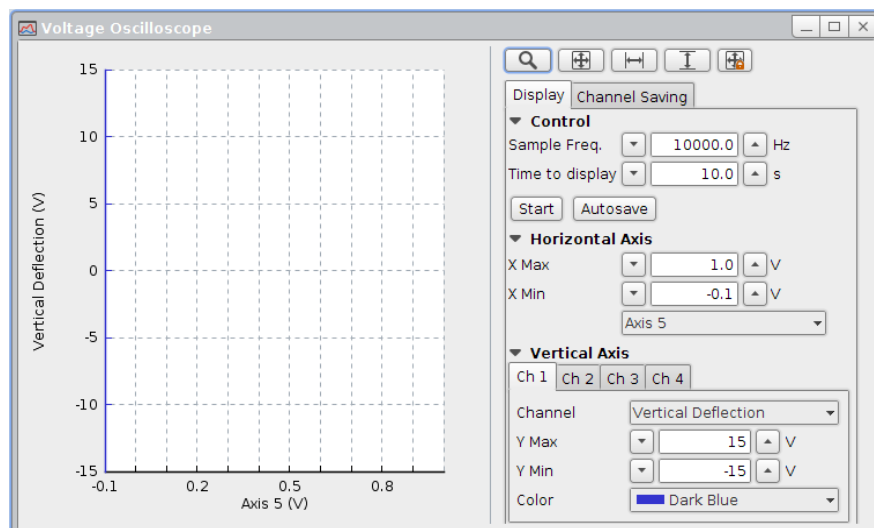
If the **Conversion** is set, then **Axis 4** will be calibrated.

Please read **Section 5.5.1** for a close description of the **Display** and **Channel Saving** settings in the **Voltage Oscilloscope**.

9.5   Python and macros

Python is a high level, dynamic, object-oriented programming language. SPM provides the Jython console, a Java implementation of Python, which allows running of individually programmed Python scripts. The Jython console can also be used to run pre-programmed macros from JPK, so a detailed knowledge of Jython is not required for many operations.

There is an online tutorial available on 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 can be useful. For a deeper insight these two books are recommended:

Markus Lutz, Programming Python, ISBN 1565921976.

Robert W. Bill - Jython for Java Programmers, ISBN 073571119, (recommended for those with experience of Java programming).
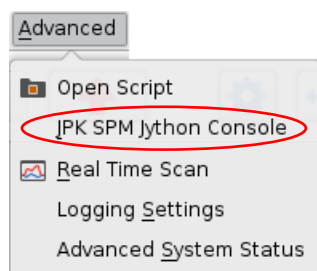
Open the **JPK SPM Jython Console** via the **Advanced** drop-down menu.
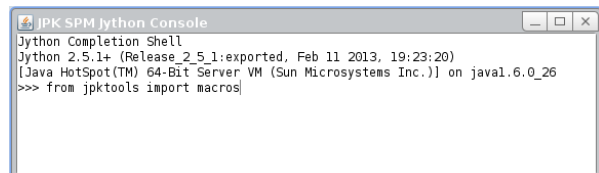
To load the full set of macros provided, enter the following command:

```
>>> from jpktools 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. 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. Contact support@jpk.com if you have any particular request.

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: open the Jython console and import the macros, before performing the sweep. Perform the sweep, and then save it using the following 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)
```

9.6   JPK scripts

The JPK Script Center allows for scripting individual applications. This enables Jython programs to run on a separate thread. Contact support@jpk.com if you have a particular request or question. There are many pre-programmed scripts that can be used for special experiments.
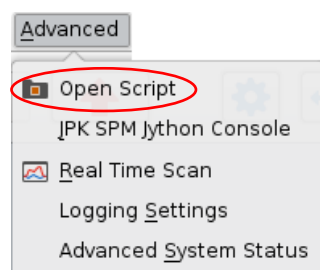
Selecting **Open Script** in the **Advanced** drop-down menu opens the **Open Script** file chooser.

Choose the desired script and click **Open**. The two folder icons on the right-hand side allow direct jumping to the **jkpdata** and **jpkscripts** directories.
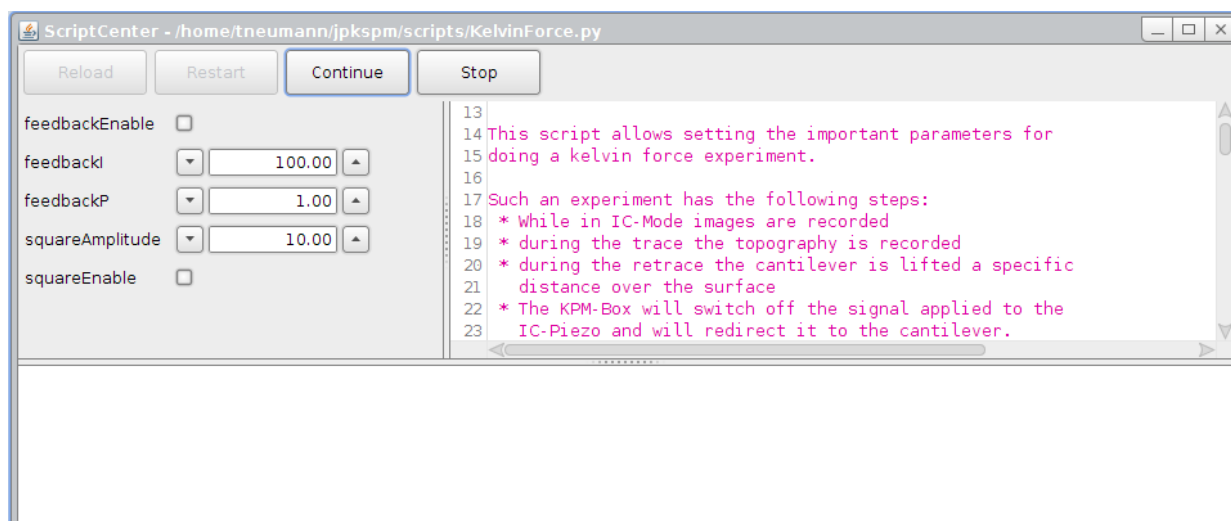
The **Script Center** window opens, containing a description of the script, followed by the script text itself on the right-hand side, and a panel with the required parameters on the left-hand side. The current status is displayed in the bottom window, e.g. Image Scan Started or Done....

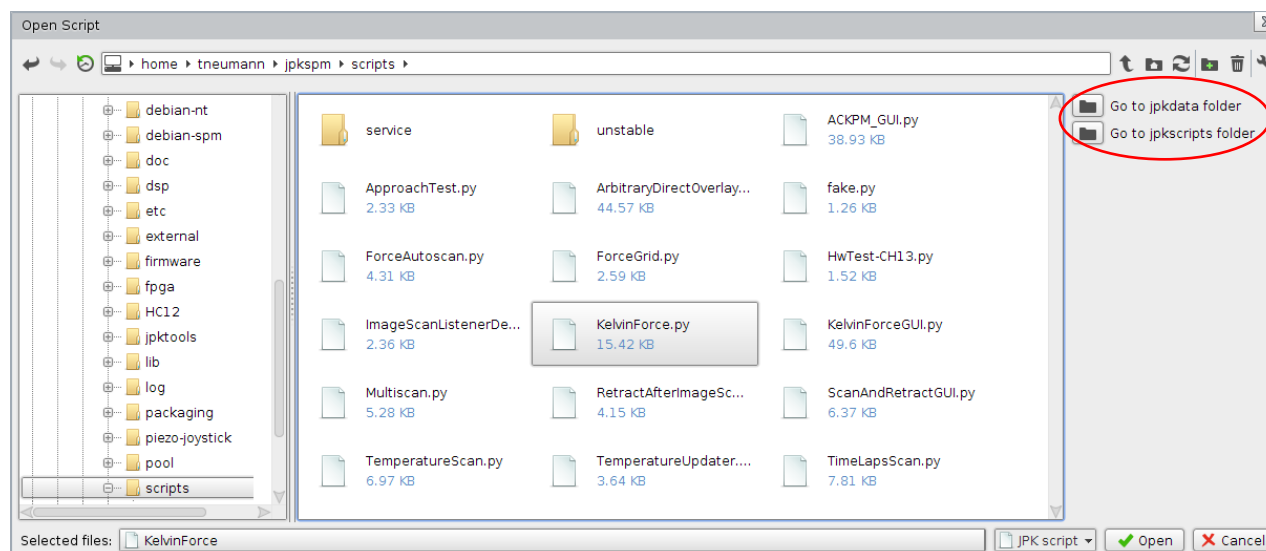Set the parameters as desired and click **Continue** to apply the parameters and acquire data. **Stop** allows aborting data acquisition. Click **Restart** to reload the set parameters in order to start a new measurement. **Reload** allows returning to the initial settings, i.e. the source code.

Always use the **Continue** button to start measurements using JPK scripts. Do not use the Run button (red arrow in the shortcut icon toolbar).

9.7   JPK data formats

Depending on the measurement mode, the SPM software generates different file types. E.g. Contact Mode or AC Mode imaging creates files with the extension "*.jpk"; Force Spectroscopy creates files with the extension "*.jpk-force". JPK image files (*.jpk) 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 of the *.jpk-files can be found in the directory   "/opt/jpkspm/doc/TifSpec.sxw".

This sxw file can be opened with "OpenOffice", which should already be installed on the AFM computer.

JPK data files are designed to be read and processed by the JPK Data Processing software (DP). Processed files can either be saved in the original data format or exported as text or pictures in a standard graphics format (e.g. *.jpeg).

	JPK formats	Export formats	
		Export as text	Export as picture
Format	*.jpk (image format) *.force (force map image) *.jpk-force-map (force map curves) *.jpk-force (force spectroscopy) *.jpk-qi-data (QI™ data file) *jpk-qi-image (QI™ image file) *.tnd (frequency spectrum in thermal noise, ascii) *.cal (height calibration file, ascii)	*.out *.txt *.map_txt	*.tif *.bmp *.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/DP 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

9.8   TTL Control

TTL (transistor-transistor logic) devices can be used to synchronize the NanoWizard® AFM with external hardware components like cameras and spectrometers. A particular characteristic of TTL signals is the so-called level change, the switching between a low state (below 1 V for a digital 0) and a high state (above typically 3.3 V for a digital 1) in a high switching speed. A combination of a fast switch between low and high state leads to a pulse segment.

9.8.1   Hardware configuration

The NanoWizard® controller can be equipped with and without a Signal Access Module (SAM, see **Section 11.1.1**). The full range of TTL control elements can only be used with the Signal Access Module.

NanoWizard® controller without SAM

NanoWizard® controller with SAM

TTL Signal without SAM
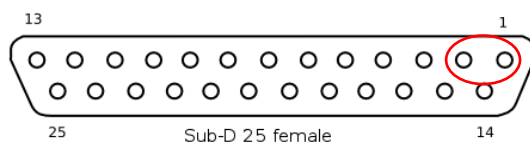
The access to the TTL signal is given at the back of the controller via a Sub-D 25 female pin assignment (marked in green). The standard controller without Signal Access Module offers the possibly to use two TTL outputs (marked in red).

The TTL outputs (Pin 1/2) can switch between high and low state (level changer), but there is no possibility to generate automatic pulses. This is only possible with the SAM (see below).

TTL Signal with SAM

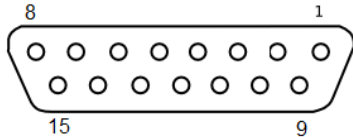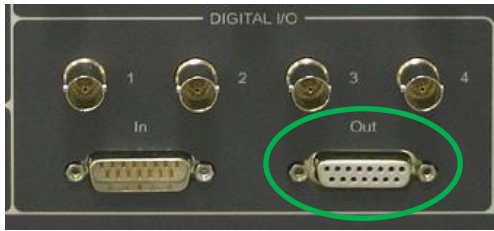The access to the TTL signal is given at the Signal Access Module of the controller via a Sub-D 15 female pin assignment (marked in green). Besides switching between high and low state, automatic pulses can be generated. Furthermore TTL pulses can be synchronized with AFM measurements through pixel, line and frame clocks.

Pin Assignment:

1-2	ground (no TTL output)
3	TTL (level change, pulse)
4	TTL (level change, pulse, frame clock)
5	TTL (level change, pulse, line clock)
6	TTL (level change, pulse, pixel clock)
7-9	ground (no TTL output)
9-11	TTL (level change)
12	ground (no TTL output)
13-15	TTL (level change)

9.8.2   TTL Control Panel

The **TTL Control** panel offers the possibility of controlling and monitoring TTL signals independently from the chosen mode (imaging or Force spectroscopy).
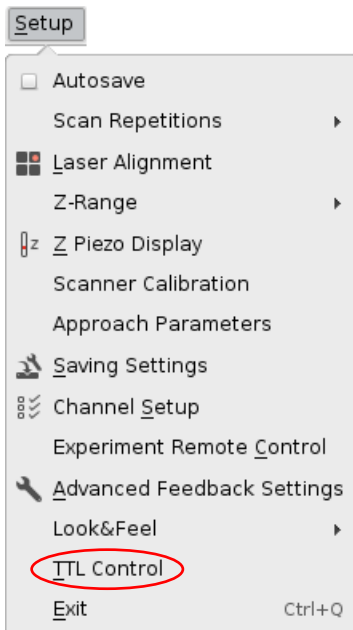
Open **TTL Control** via the **Setup** drop-down menu.

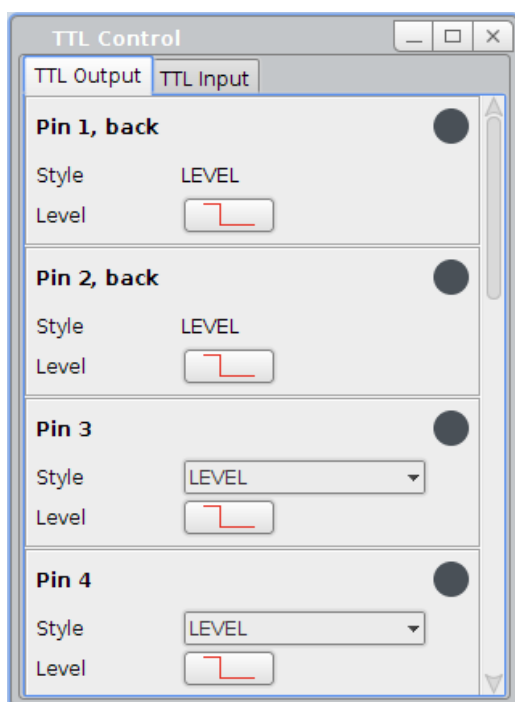The **TTL Control** panel provides an overview about all available TTL outputs.

Pin 1 (back) and **Pin 2** (back) are at the rear side of the controller (Sub-D 25 female pin assignment). These two Pins can be controlled with and without the Signal Access Module. Pin 1 and Pin 2 are level changer.

All other Pins can be accessed via the Digital out Sub-D 15 female pin assignment at the Signal Access Module.

Pin 3 - **Pin 6** are able to change between high and low state and send pulses. The Style can be switched between **LEVEL** and **PULSE**.

Pin 6 offers furthermore the option to start a pixel clock via the TTL Control Panel. During imaging and force spectroscopy a TTL pulse is sent at each tip position corresponding to a pixel.
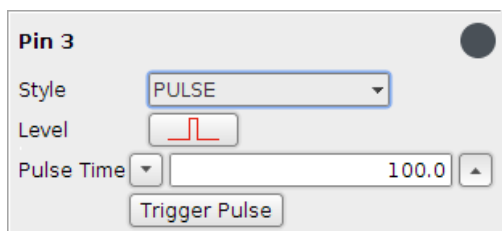
Pin 9 - **Pin 11** and **Pin 13** - **Pin15** are able to change the Level and can be accessed at the Signal Access Module.
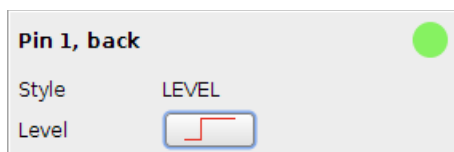
The **Style LEVEL** offers the possibility to switch between high and low state.

Whenever the Level-button is pressed a level change is done.

The **Style PULSE** offers the possibility to set pulses. Two sorts of pulses are available. The **Pulse Time** can be controlled.

Trigger Pulse sends a pulse with the defined **Pulse Time**.

TTL signal pulses can be synchronized with AFM measurements via **TTL clocks**. Three different sorts of TTL clocks are available:

Pin 6: **Pixel clock**　　A TTL pulse is generated for each pixel (trace and/or retrace)

Pin 5: **Line clock**　　A TTL pulse is generated for every line (trace and/or retrace)

Pin 4: **Frame clock**　　A TTL pulse is generated for every frame

The general Pulse settings have to be set at the **TTL Control** panel. Therefore, the Style **PULSE** has to be chosen and the **Pulse Time** has to be defined. For activation of the TTL clocks the **JPK SPM Jython Console** (see **Section 9.5**) has to be used.
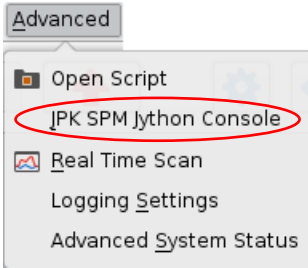
Open the Jython console via the **Advanced** drop-down menu.

The Jython console can be used to run pre-programmed macros like TTL clocks from JPK, so a detailed knowledge of Jython is not required for such operation.

To activate TTL clocks the following commands have to be used:

Pin 6: **Pixel clock**   `dspManager.setPixelClockEnabled(true,true)`

Pin 5: **Line clock**   `dspManager.setLineClockEnabled(true,true)`

Pin 4: **Frame clock**   `dspManager.setFrameClockEnabled(true)`

For a Line clock as well as a Pixel clock a pulse routine can be defined:

- Pulse only in trace (extend) = (true, false)

- Pulse only in retrace (retrace) = (false, true) or

- Pulses in trace(extend) and retrace(retrace) = (true, true)

TTL clocks synchronize AFM measurements and TTL Pulses. A TTL clock starts when the AFM measurement is started by pressing the red arrow on the shortcut icon tollbar on top of the SPM software.
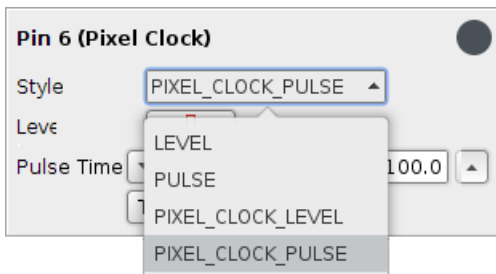
The Pixel Clock is the only TTL clock that can also be activated directly by using the TTL Control Panel

9.8.3 TTL Control with Force Ramp Designer™

The Force Ramp Designer™ (see **Section 8.5**) provides a TTL segment allowing for the use and controlling of TTL signals in force spectroscopy mode.
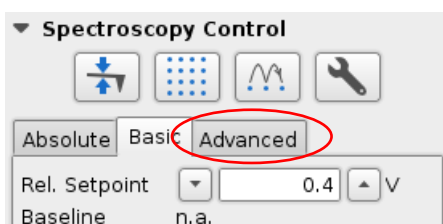
If **Advanced** mode is selected in the **Spectroscopy Control** panel, the **Force Ramp Designer™** opens automatically (see **Section 8.6**).

Use the **TTL Level/ Pulse** segment to control TTL signals within a force distance experiment.

TTL Output lists all available TTL Outputs. Pin 3 – Pin 6 can be used to switch between high and low state and to control TTL pulses. All other available Pins are limited to a level change. Pin 1 (back) and Pin 2 (back) can be addressed at the back of the controller. The other Pins are located at the Signal Access Module (see **Section 9.8.1**).
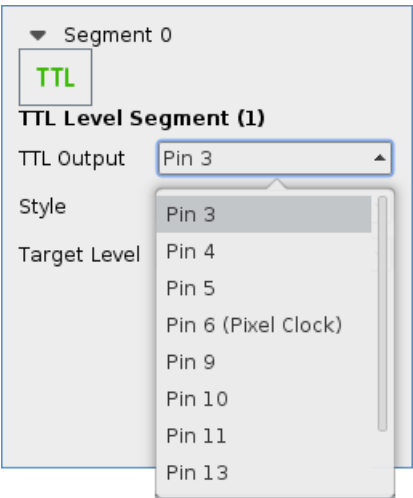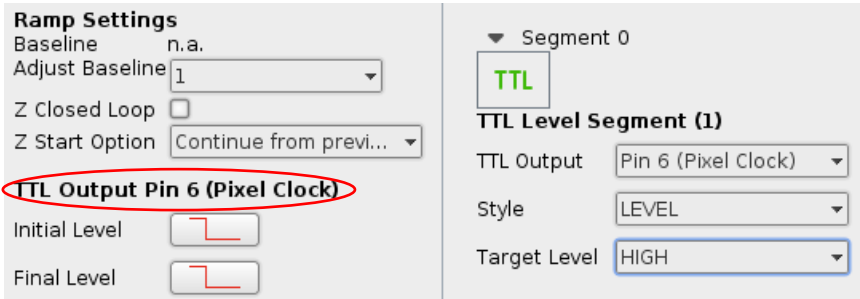
The Style **LEVEL** offers a switch between LOW and HIGH state. Therefore the **Initial Level** and the **Final Level** can be set at the **TTL Output** (marked in red). These values define the level state at the beginning and end of a force distance curve. The **TTL Level Segment** itself leads to a change to the chosen **Target Level**.
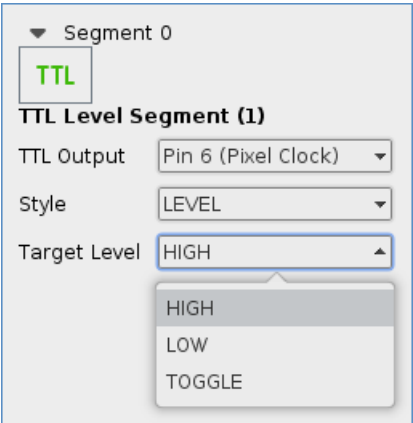
The **Target Level** can be **HIGH**, **LOW** or **TOGGLE**. The current level state will be detected and compared with the Target Level. In cases that the Target Level (HIGH/ LOW) is already reached there is no level change. The TOGGLE option leads to a level change independently of the current level state.
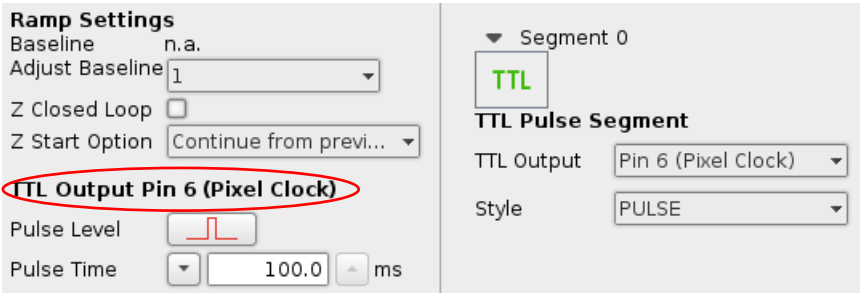
The Style **PULSE** offers the possibility to set pulses. The **Pulse Level** and **Pulse Time** can be set at the **TTL Output** (marked in red).

§ 10   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.

Ubuntu Linux update:

> ⚠ The Ubuntu operation system is modified for the AFM operation.
>
> Please note that a simple Ubuntu update is not possible. Contact us for assistance if you plan to update. E-mail support@jpk.com or call +49 30 726243 500 for assistance

10.1.1 Ubuntu updates

When logged in as jpkroot your Ubuntu operating system will frequently remember you to make Ubuntu updates. Making security updates is ok, but please refrain from making so-called LTS updates until further notice. LTS updates are clearly labeled as such in the Ubuntu update manager.

> ⚠ Do not perform LTS updates. It may happen that SPM and DP cannot be started anymore.

10.1.2 Basic tools and programs

The taskbar along the lower left hand side of the desktop contains most of the shortcuts that are regularly required.
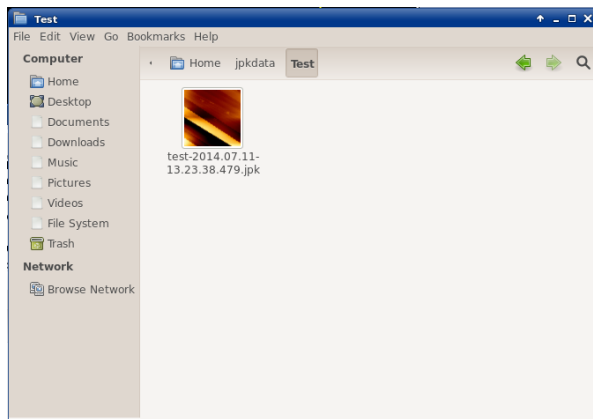
Internet browser (usually Firefox)

Console (terminal) window. Here many different commands can be entered directly.

Open a file browser for local folders (on this computer)

The file browser window is designed similar to Microsoft Windows, the data directory is displayed on the left hand side and the selected folders shown on the right hand side. The drop-down menus on top allow for various editinglike changing of properties (**File** menu), display options (**View** menu) or setting bookmarks (**Bookmarks**). More information is available under the Help menu - All Topics.
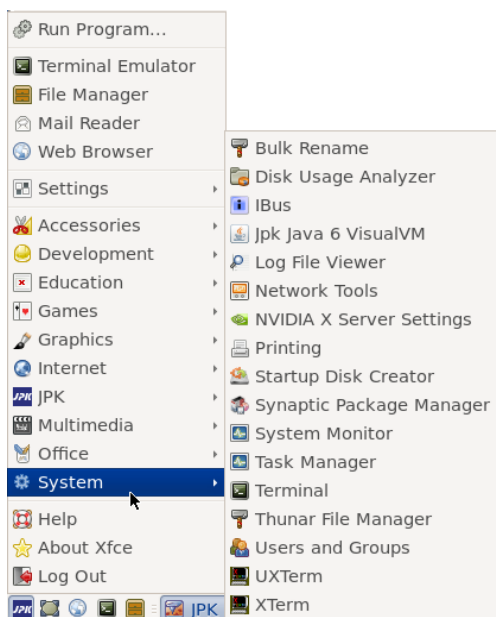
The JPK logo starts the main menu system for the Ubuntu operating system.

There are some commonly used options:

Settings to adjust e.g. display or network settings

System to find system settings

The other menu options contain program lists and small tools or accessories.

Open the **Help** option to find more information.

Screenshots of the whole monitor can be taken using the standard **Print Screen** key. The key combination **Alt + Print Screen** takes a screenshot of only the active software window.

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 programs.

In the terminal (console) window, the screenshot can be started directly from a command. Furthermore a small program to take screenshots can be open via **Accessories - Take screenshot**. This provides additional options (e.g. a time delay). The basic command is: `gnome-screenshot`

To get the list of available advanced options, type: `gnome-screenshot --help-gnome-screenshot`

10.1.3 Use JPK scripts with the Linux console
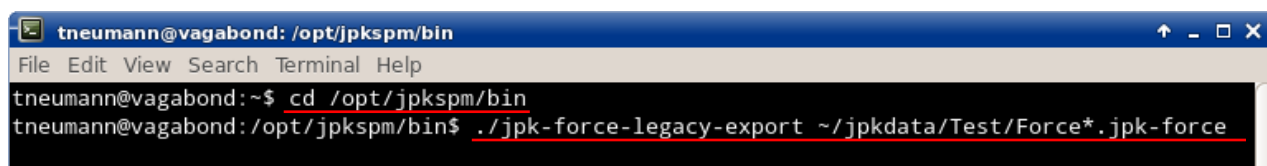
There are several JPK-scripts, which can be applied to the data. "Splitforcefile", for instance, separates a force file into trace and retrace curve. "Splitmapfile" splits force maps into separate force files. There are two scripts to convert force files of the jpk-force format into other file formats. 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:
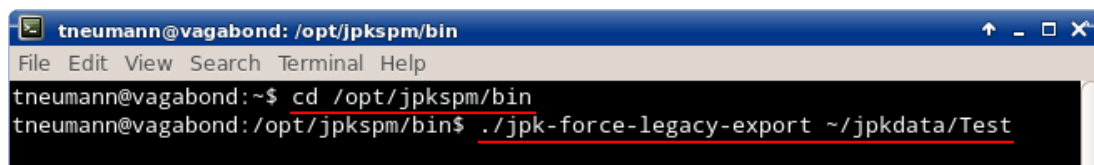
```
tneumann@vagabond: /opt/jpkspm/bin                                ↑ _ □ ×
File  Edit  View  Search  Terminal  Help
tneumann@vagabond:~$ cd /opt/jpkspm/bin
tneumann@vagabond:/opt/jpkspm/bin$ ./jpk-force-legacy-export ~/jpkdata/Test/Force*.jpk-force
```

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@vagabond: /opt/jpkspm/bin                                ↑ _ □ ×
File  Edit  View  Search  Terminal  Help
tneumann@vagabond:~$ cd /opt/jpkspm/bin
tneumann@vagabond:/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 "filenameroot*.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 .
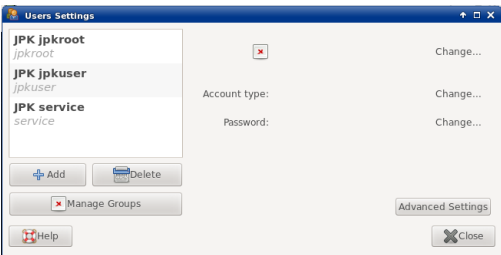
10.1.4 User account administration

The main administration account in the Ubuntu AFM systems is **jpkroot**. The password is delivered with the AFM instal-
lation papers, and this account can be used to set up other standard accounts for all the individual AFM users.
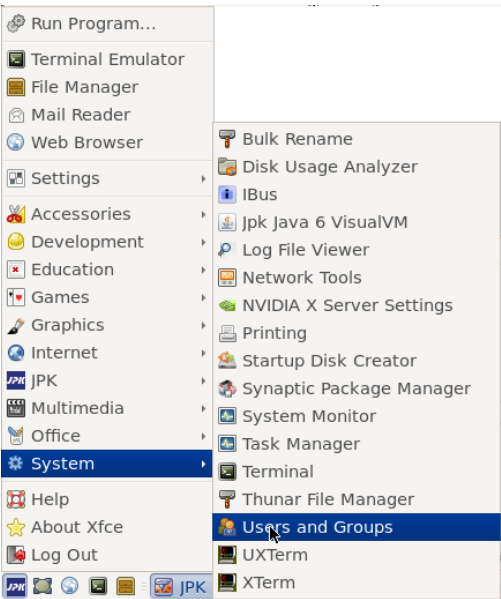
To create new user accounts, log in as **jpkroot**. User accounts creat-
ed using the standard settings do not have administrator rights – this
is more secure, especially when the AFM computer is connected to
the internet.

Use the main system menu (started from the JPK icon in the taskbar
at the bottom left of the software) to choose:

System –Users and Groups

The dialog window **Users Settings** shows a list of the user accounts.

Click on the button **Add** to create a new user account.
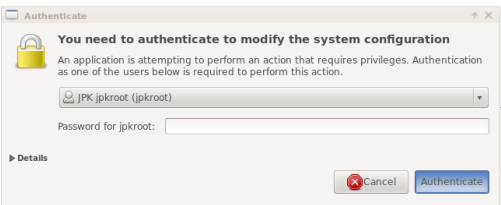
For security reasons, you need to confirm the account password
again when starting administration tasks.

Enter the password for the **jpkroot** account.

The **Create New User Editor** lets you set up the particular options for
the new account.

The first tab panel creates the name for a user account.

The **Name** will be the log in name for the new account.

The **Password** section is very important. *Please choose secure passwords, especially if the computer will be connected to the internet!*
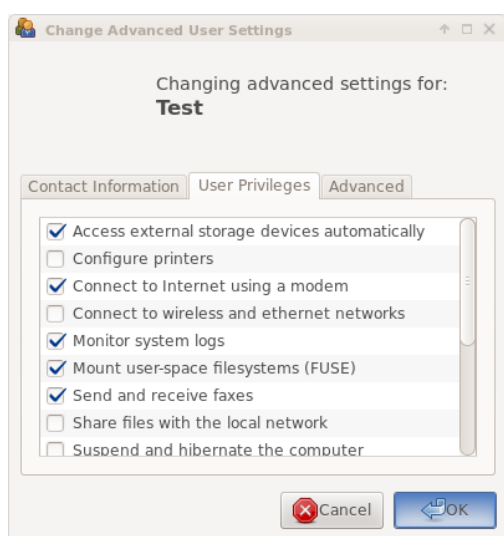
After the creation of the user profile it is required to click on the button **Advanced Setting** in the **Users Settings** main window, to check that the **User Privileges** are correct. New user accounts have not the privilege to administer the system. That means that the new account will have normal user rights, but not administrator privileges. This is more secure, particularly when the computer is connected to the internet.

The **Contact Information** is optional, for a small group of users this is probably not required.

The **Advanced Settings** should not be changed.

It is possible to control for each user the groups settings by using the option **Manage Groups**

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 **Close** to save the details of the new account.

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.

10.1.5 Network settings

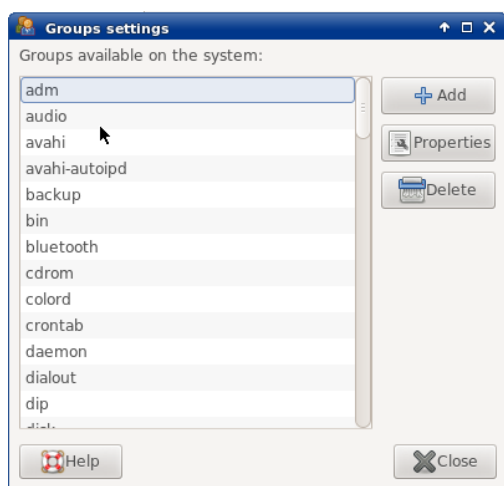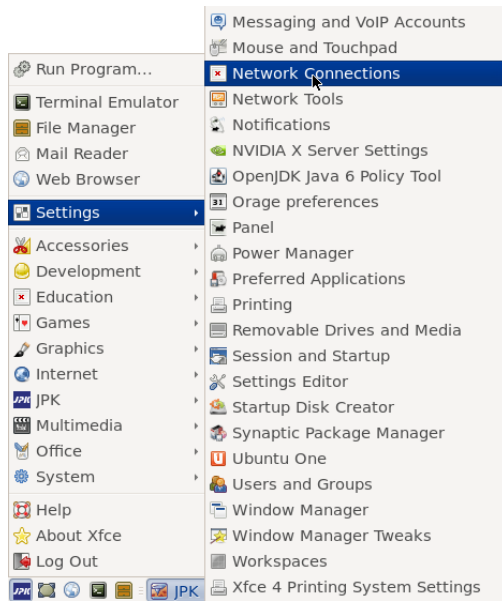To perform administration tasks, such as network configuration, log in as user **jpkroot**.

Use the main system menu (started from the JPK icon in the taskbar at the bottom left of the software) to choose:

Settings – Network Connections
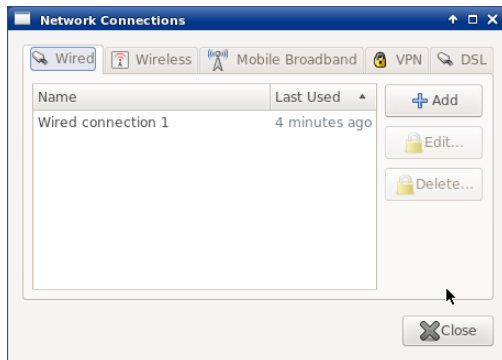
The **Network Connections** dialog contains all the settings for the network configuration.
Click on **Wired Connections** and Edit to configure the network connections.
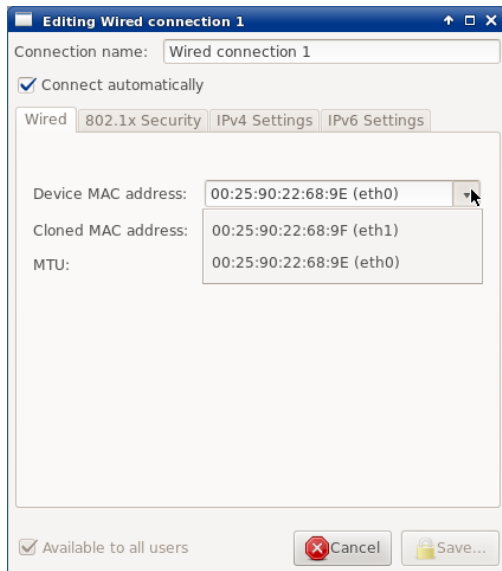
In the Editing Wired connection 1 tab, **Wired Connections** can be set up (eth0 or eth1 connection).

Do not modify the eth1 connection! eth1 is the Ethernet connection between the SPM controller and the computer.

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.

DHCP (dynamic IP address allocation)
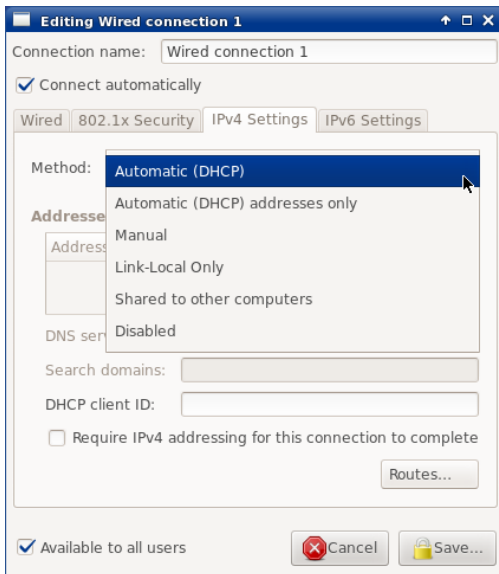
For **DHCP** (dynamic IP address allocation):

In the **IPv4 Setting** panel:

- Select the method **Automatic (DHCP)**

Close the dialog with **Save** to save the settings and return to the **Network Connections** dialog.

Static IP address allocation
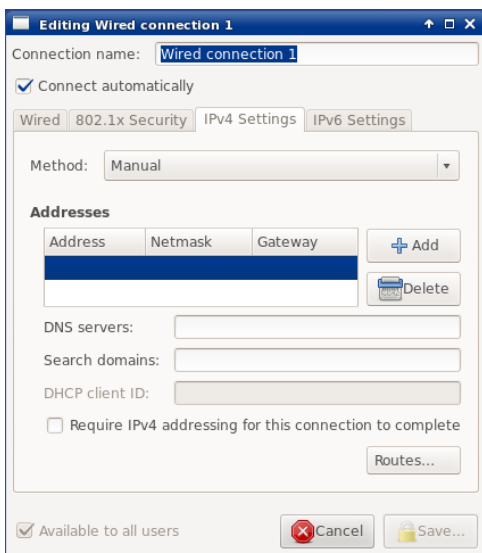
For static IP address allocation:

In the **IPv4 Setting** panel:

- Select the method **Manual**

- Choose **Add** to set up the Addresses

Now the **IP address**, **Netmask** and **Gateway address** from your network administrator should be entered in the relevant input fields.

The required **DNS Servers** or **Search Domains** have to be added (from your network administrator).

Close the dialog with **Save** to save the settings and return to the **Network Connections** dialog.

10.1.6 Timer

A simple electronic timer can sometimes be useful during experiments. First this small tool must be installed with administrator rights. Log in as jpkroot, 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.

§ 11 Specifications and Support

11.1 Technical specifications of the NanoWizard® controller

Dimensions:	approx. W 52 cm x H 50 cm x L 46 cm
Power Input:	88 – 240 V AC, 60 Watt
Weight:	approx. 30 kg
Fuses:	2 micro fuses, 20 mm x 5 mm, 2.5 A, 250 V slow blow
Environment:	15°C – 30°C
	Maximum relative humidity: 80%, non-condensing conditions

11.1.1 Signal Access Module (SAM)

The NanoWizard® controller can be equipped with a Signal Access Module (SAM). The SAM allows electronic access or modification to most of the analog signal channels that appear in the software — both input and output. In addition, several digital input and output channels are provided.

NanoWizard® controller without SAM

NanoWizard® controller with SAM
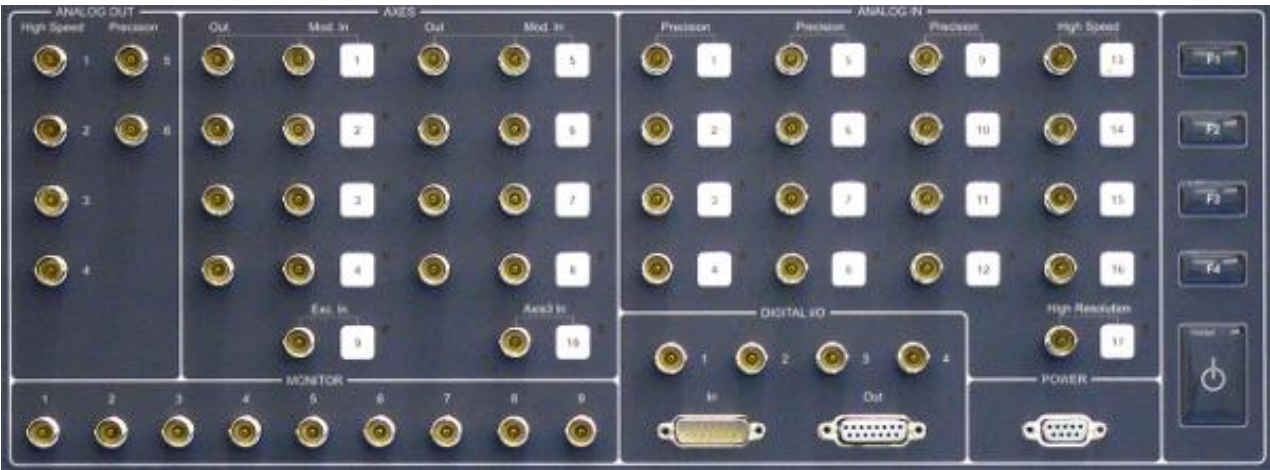
The SAM is divided into several logical blocks.

- The **MONITOR** connectors are analog channels that directly output the unprocessed electronic signals coming from the NanoWizard® head. These can be used to monitor signals by means of external equipment, and employed for feedback, triggering, etc.

- The **ANALOG OUT** connectors provide a few analog output (DAC) channels that can be programmed from the SPM software. A direct digital synthesizer (DDS) is also provided.

- The **AXES** connectors output the analog signals (DAC) that drive the xyz piezos. These signals are wired internally to the NanoWizard® head; the outputs can be used for monitoring or feedback purposes. These channels have a **modulation input** that can be activated using the tip button to the right. The tip buttons switch the modulation inputs in a three-way cyclic manner indicated by an LED:

  - **off** – no modulation (default);

  - **red** – *internal* modulation (do not use, or ask JPK for assistance);

  - **green** – *external* modulation: the signal provided on the **Mod. In** input is *added* to the internal, software-controlled signal. For example, a signal from a function generator can be used to move a scanner.

- The **ANALOG IN** connectors can be used to modify the internally wired channels to read in *externally supplied analog signals* instead. Like the AXES connectors, these connectors have tip button to switch between the internal signal channel (LED **off**; default) and the externally supplied one (LED **green**). Note that in this case the channel data read into the software for this channel will be *overwritten* by the external signal. A total of twelve 18-bit channels (#1-12) sampled at 800 kHz ('Precision'), four 16-bit channels (#13-16) sampled at 60 MHz ('High Speed'), and one 24-bit channel (#17) sampled at 2.5 MHz ('High Resolution') are available.

- The **DIGITAL I/O** connectors provide a multitude of digital (TTL) inputs and outputs. TTL out is used to trigger external JPK modules like the CellHesion® Module. Contact JPK for assistance if required, including pin assignments of the 2 D-sub 15 input and output connectors. The digital counter inputs at the 4 BNC connectors may be used for e.g. photon counting. They have a programmable trigger level.

- The **POWER** connector outputs –15 V, –5V, +5 V, +15 V and GND on the various pins of a D-sub 9 connector. Contact JPK for pin assignments or further assistance.

- The function tip buttons **F1-F4** on the far right of the SAM can be used to set individual configuration patterns/channel assignments. Please contact JPK for assistance.

The table below summarizes the designation of the various analog inputs/output on the SAM.

Connector	Designation		Connector	Designation
Monitor 1	Vertical deflection (unprocessed)		Axes 7 Out	external z DAC (400 kHz, 24 bit)
Monitor 2	Lateral deflection (unprocessed)		Axes 8 Out	Not assigned

Connector	Designation		Connector	Designation
Monitor 3	Not assigned		Axes 9 Exc. In	AC excitation
Monitor 4	Photosum (unprocessed)		Axes 10 Axis 3	z piezo modulation
Monitor 5	Not assigned		Analog In 1	Vertical deflection *or* external
Monitor 6	Not assigned		Analog In 2	Lateral deflection *or* external
Monitor 7	AC excitation 1 ($\Delta\varphi$ = 0 deg)		Analog In 3	External
Monitor 8	AC excitation 2 ($\Delta\varphi$ = 180 deg)		Analog In 4	Photosum *or* external
Monitor 9	AC excitation (unprocessed)		Analog In 5	Not assigned
Analog Out 1	High-speed DAC 1 (120 MHz, 14 bit)		Analog In 6	Not assigned
Analog Out 2	High-speed DAC 2 (120 MHz, 14 bit)		Analog In 7	Not assigned
Analog Out 3	High-speed DAC 3 (120 MHz, 14 bit)		Analog In 8	Not assigned
Analog Out 4	Direct Digital Synthesizer (DDS; 120 MHz, 14 bit)		Analog In 9	Not assigned
Analog Out 5	Precision DAC 1 (1.6 MHz, 16 bit)		Analog In 10	Not assigned
Analog Out 6	Precision DAC 2 (1.6 MHz, 16 bit)		Analog In 11	Not assigned
Axes 1 Out	Piezo stage X axis DAC (400 kHz, 24 bit)		Analog In 12	Not assigned
Axes 2 Out	Piezo stage Y axis DAC (400 kHz, 24 bit)		Analog In 13	Vertical deflection (high speed) or external
Axes 3 Out	Piezo stage Z axis DAC (400 kHz, 24 bit)		Analog In 14	Lateral deflection (high speed) or external
Axes 4 Out	Piezo stage Z axis DAC (400 kHz, 24 bit)		Analog In 15	External (high speed)
Axes 5 Out	external x DAC (400 kHz, 24 bit)		Analog In 16	Photosum (high speed) or external
Axes 6 Out	external y DAC (400 kHz, 24 bit)		Analog In 17	Vertical deflection (high resolution) or external

For applications that require any of the advanced functions of the Signal Access Module, please contact JPK at sup-port@jpk.com or call +49 30 726243 500 for assistance.

11.2 Technical specifications of the PC

Dimensions:	approx. W 21 cm x H 47 cm x L 50 cm
Power input:	100 – 240 V AC, 230VA (550 Watt max.)
Weight:	approx. 15kg
Environment:	15°C – 30°C
	Maximum relative humidity: 80%, non-condensing conditions

11.3 Technical specifications of the NanoWizard® head

Dimensions:	approx. W 22 cm x H 10 cm x L 21 cm
Power input:	The NanoWizard® head directly connects to the NanoWizard® controller
Weight:	approx. 3.0 kg
Environment:	15°C – 30°C
	Maximum relative humidity: 80%, non-condensing conditions

11.4 Support

For more information please contact:

E-mail: support@jpk.com or visit   www.jpk.com

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Note: All trademarked names mentioned in this manual remain the exclusive property of their respective owners.

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