Operation Guide Overview

Welcome to the WITec Operation Guide.

This guide explains how to perform measurements with a WITec microscope using the WITec Suite, especially WITec Control.

The WITec product portfolio includes imaging systems for Raman, AFM and SNOM analysis as single technique solutions as well as correlative imaging configurations. All WITec microscopes are high-quality modular systems. Therefore each system will be an individual solution to match the needs of each customer. Maybe not all of the described techniques will be available on your system. If you are interested in extending the capabilities of your system, please contact us.

WITec Control is equipped with predefined Configurations matching the setup of your system. A configuration determines which hardware should be used and which data channels are recorded during the measurement. This guide explains the differences between the configurations and how to use them.

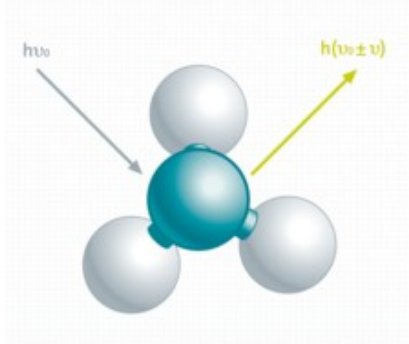
General

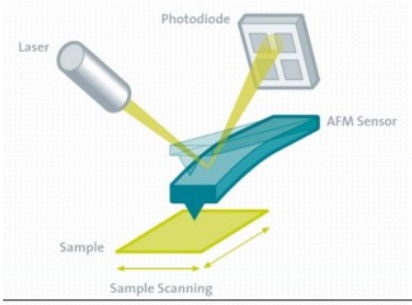
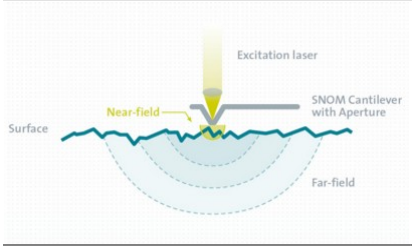
- Modularity
- Confocality
- Objectives

Common procedures:

- Power-up/-down the system
- Focus on sample
- Focus on sample from below

Techniques

	Raman	Raman spectroscopy, Photoluminescence spectroscopy

	AFM	Atomic force microscopy
	SNOM	Scanning near-field optical microscopy
	Confocal	Confocal microscopy and StrobeLock (time-resolved microscopy)
	Photocurrent	Analyze photosensitive devices
	Lithography	DaVinci nanolithography package
	Profilometer	TrueSurface sensor as profilometer

Modularity

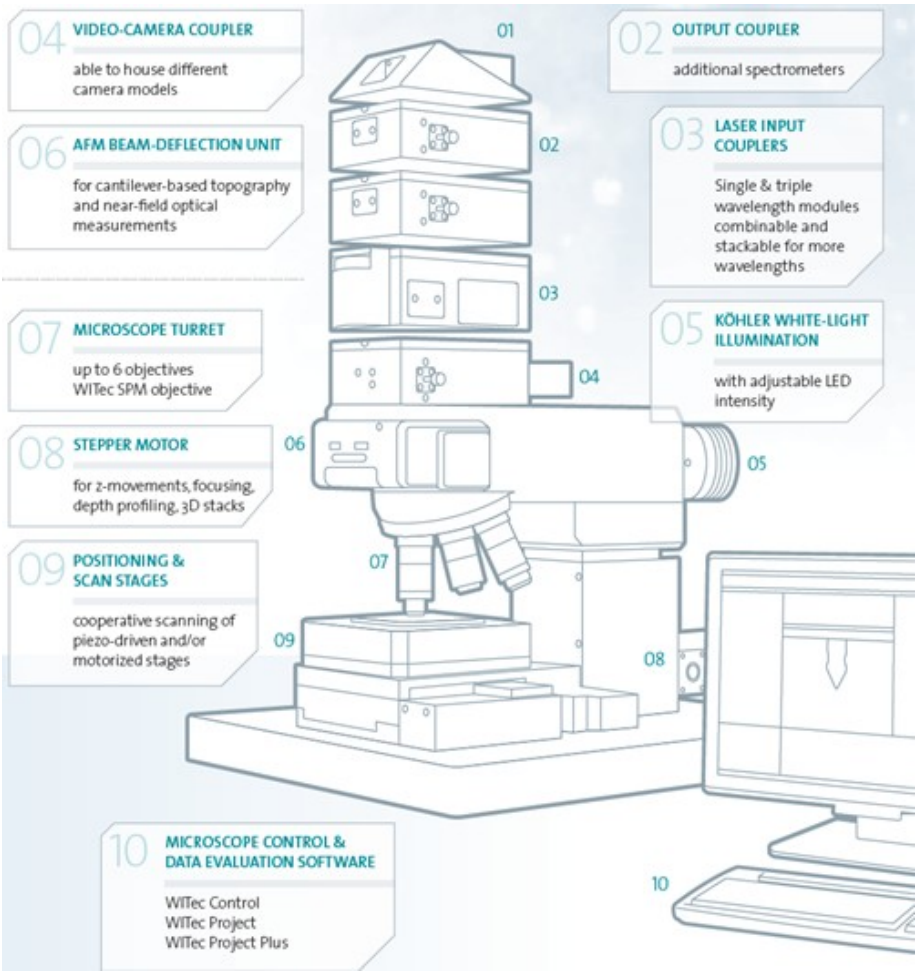


Figure 1: Sketch of the WITec alpha300 modules

All WITec microscopes are high-quality modular systems with exceptional optical throughput, unparalleled signal sensitivity and outstanding imaging capabilities. The common thread throughout is that all systems are based on the

same hardware architecture. Whenever required it is possible to simply upgrade any system, even the most basic, with additional features and equipment, allowing our customers to keep pace with future challenges.
The WITec system contains several components common to various configurations of the instrument (such as the AFM and the scanning near-field optical microscope configurations). Therefore, the system can be fully upgraded to include AFM or SNOM capabilities at any time. In particular, the combination of Raman microscopy with AFM allows the chemical information gained by confocal Raman microscopy to be linked directly with the ultra-high lateral and topographical resolution of an Atomic Force Microscope at the same sample position with just a rotation of the turret. SNOM allows for optical investigation of the sample beyond the diffraction limit.
For Raman systems it is always possible to add further excitation laser wavelengths, detectors, spectrometers, or advanced modules like TrueSurface for profilometric Raman measurements.
Contact WITec for more details.

Confocality

Confocal microscopy requires a point source (usually a laser), which is focused onto the sample. The reflected light (also Raman scattering or fluorescence) is collected with the same objective and focused through a pinhole in front of the detector (Fig. 1). This ensures that only light from the image focal plane can reach the detector, which greatly increases image contrast and with the proper selection of pinhole size, slightly increases resolution (max. gain in resolution: factor √2).

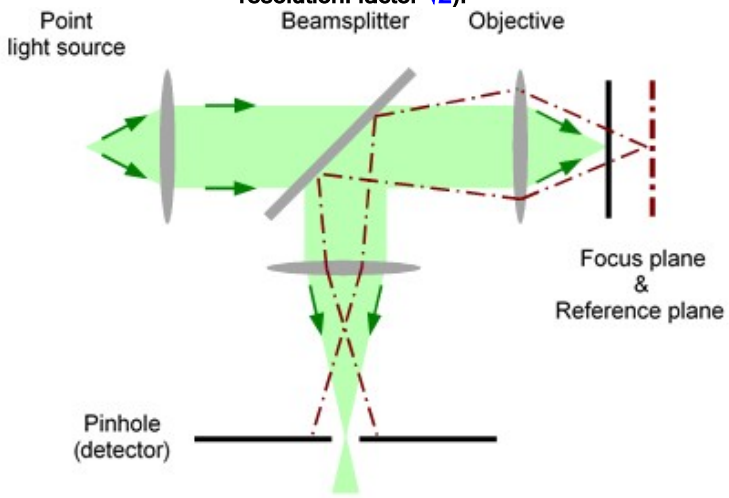


Fig. 1: Principal setup of a confocal microscope

In WITec systems, the laser light is delivered through a single-mode optical fiber. This type of fiber supports only a single transversal mode (LP01, Gaussian beam) which can be focused to a diffraction-limited spot. The light reflected by the sample is collected by the objective and is directed as a parallel beam toward the top of the microscope. Here, the light is focused onto a optical fiber. The core of this optical fiber acts as a pinhole for confocal microscopy or confocal Raman microscopy.
The fiber directs the beam to a photon counting device (for confocal microscopy) or a spectrometer equipped with a CCD camera (for confocal Raman microscopy). Using fibers for beam delivery and signal pick-up is very convenient because the excitation laser, the spectrometer, and the detectors do not need to be mounted on the microscope itself. They can be placed anywhere, far away from the microscope body.

Objectives

Objectives are among the most important components in a microscope. In WITec systems they define the magnification of the video image and the resolution of, for example, the Raman image. The objectives are infinitely corrected, meaning that the beam is parallel inside the microscope. Please ensure that the microscope objectives are used in the proper way. Most objectives are only intended for use in air. Some objectives are corrected for use with a cover slip and require an immersion medium to function properly.

Figure 1: Labeling of Zeiss objectives (Source: https://www.zeiss.com/microscopy/int/products/microscope-components/objectives.html)

The magnification is given for the image plane (at the position of the video camera or the collection fiber). The numerical aperture (NA = n · sin α) describes the resolving power of the objective. Additionally, the working distance (in mm) is printed on the objectives.

For instructions regarding the cleaning of objectives, please refer to the brochure, "The Clean Microscope" by ZEISS.

Power-up/-down

Power-up

1. Switch on the alphaControl and peripheral devices. This is usually done using the switch on the multi-plugs.
2. Power up the computer and wait until the pink H (service monitor) appears in the taskbar.
3. Start WITec Control.

There is no mandatory order for switching on devices. Even switching on or off the alphaControl while the computer is switched on is no problem as long as WITec Control is not running.

Power-down

1. Close WITec Control.
2. Shut down the computer.
3. If you do not use the system for several days, switch off everything using the switch on the multi-plugs.

Lasers

- Only switch on lasers if you want to use them, because the lifetime is limited.
- Switch off the lasers if you do not use them for more than one hour.
- Please refer to the lasers manual for specific information.

CCD cameras

- Switching off the CCD camera even when cooled down, does not harm the camera.

Focus on sample

The following steps explain how to easily focus on a sample and are common for all configurations. This applies not for use with the inverted objective (look here).

Procedure

1. Mount the sample on the microscope stage. Make sure it is fixed (i.e. with clamps) to avoid movements during the measurements.
2. If possible: Select an **objective** with low magnification (i.e. 10x).
3. For non-automated systems: Configure the beampath for video mode in brightfield illumination.
4. Use reflected light (top illumination) and make sure that the top camera is selected.
5. Close the field stop. For non-automated systems: Field stop is located on the right side of the microscope body marked with a "F".
6. Adjust the brightness to see at least some light, i.e. use Auto brightness.
7. Focus in the direction in which the image becomes brighter:
   - Use the Microscope-Z stage.
   - For RISE: Use the scan table z.
8. If the edges of the field stop are in focus, also the sample is in focus.
9. Open the field stop.
10. If necessary: Select an objective of higher magnification and repeat the steps starting with step 4.

Hints

- During focusing features can appear that are not the sample surface (originating from surfaces within the optical brightfield beampath). The edges of the field stop will not be in focus in that case. Slightly move the microscope stage, to see whether the features do move.
- For transparent samples there could be more than one focus plane.
- If you have difficulties to focus, it is also possible to focus using the laser spot at low laser power.
- For rough samples, maybe only parts of the field stop will be in focus.

The field stop is focusing in the focus plane because it is positioned at the back focal plane of the objective.

Focus on sample from below

The following steps explain how to focus on a transparent sample using the inverted objective. This applies not to the alpha300 Ri (look here).
If you have a non-transparent sample, do the focusing on a glass slide and change back to your sample afterwards.

If you want to do measurements in transmission setup (e.g. Confocal or SNOM), it is necessary to align the inverted objective for collection under the laser from above. Please follow also the steps marked with "for transmission" below.

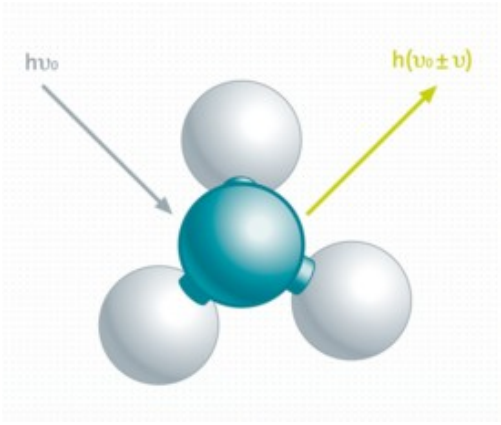
Procedure

1. **Focus on the sample** using the upright microscope with an objective not smaller than 20x and follow the steps until step 8.
2. Select the **bottom camera** and if necessary set the inverted beampath accordingly.
3. Use the coarse adjustment knob of the inverted objective to bring it close to the sample. (Take the working distance of the used objective into account.)
4. Focus on the sample using the control of the inverted objective.
5. For Transmission: Select the laser you want to use and configure the beampath accordingly to see the laser in the video image. Adjust it to low laser power (about 1 mW).
6. For Transmission: Move the inverted objective in x-y direction until the laser spot is centered in the green circle in the video image. Close the laser shutter.

Hints

- For transparent samples there could be more than one focus plane, make sure you focused on the intended one.
- If the sample has no visible features, focus on the field stop of the upright microscope. (It is not visible, if the magnification of the upright objective is too low.)
- If you have difficulties to focus, it is also possible to focus using the laser spot. If the laser is coming from above, make sure it is focused on the sample surface in the Top view.
- For Transmission: If you don't see the laser spot at step 5 and the top camera is mounted above the laser coupler in your system, check that the edge filter is removed.
- For 6.: Observe the laser using the bottom camera if the laser comes from above and the top camera if the laser comes from below.

Raman Overview



Quick Start:

- Signal optimization (Pinhole alignment)
- Setting up a Large Area Scan
- Change of Laser Wavelength

Raman spectroscopy is a method to observe vibrational modes of molecules which provides chemical and structural information about the sample. The needed excitation light source in the range from UV to NIR combined with the confocal light collection of the WITec microscope provides high spatial resolution. Furthermore the WITec UHTS spectrometers equipped with an optimized CCD camera allow Raman Imaging in high speeds and excellent spectral quality.

Topics:

- Introduction
- Theoretical Background
- Raman Configurations
- Spectrometer calibration
- Signal optimization (Pinhole alignment)
- Change of Laser Wavelength
- Setting up a Large Area Scan

Raman modes:

- Raman – standard Raman, refer to the Configurations section for more information
- Raman inverted – Raman using the inverted objective
- Raman-AFM – for simultaneous Raman and AFM refer to Raman-AFM

All Raman configurations can also be used for just doing normal spectroscopy like for Photoluminescence (PL) by changing the unit in the spectrograph settings to nm.

Measurement modes:

- Oscilloscope: Continuous readout of spectra e.g. for focusing.
- Single Spectrum: Acquisition of a spectrum at the current position.
- Spectral Stitching: Acquisition of a spectrum with extended spectral range by stitching spectra from different spectral positions.
- Fast Time Series: Continuous acquisition of spectra over time. (Can be used as alternative to single spectrum for saving each accumulation.)
- Slow Series: Intermittent time series, laser power series or polarizer series.
- Line Scan: Acquisition of spectra along a line in three dimensional space.
- Large Area Scan: 2D or 3D Raman Imaging using the motorized stage up to centimeter scale.

- Image Scan: 2D or 3D Raman Imaging using the piezo stage for highest resolution.
- Sample Raster: Automated single spectra or image scans on predefined points.

Advanced features:

- Manual Topography Correction: Enables to correct the tilt of the sample or a simple surface.
- TrueSurface Mk1 and Mk2: Learning the topography by a CCS and follow during the scan.
- TrueSurface Mk3: Live topography correction during the scan.
- Signal Stabilization: Compensates slow focus changes due to thermal drift.
- ParticleScout: Automated Raman analysis of optically identified particles.
- EMCCD camera: Ultra Fast Raman imaging
- InGaAs camera: Raman Imaging in the NIR

System requirements:

- Raman (M, R, RA, RAS and RS systems)
- access and RISE systems

Introduction

The Raman effect describes the interaction of electromagnetic waves (light) with matter in which a vibrational quantum is excited (Stokes Raman scattering) or annihilated (Anti-Stokes Raman scattering).
When light interacts with a molecule, most photons are elastically scattered and therefore retain the same energy as the incident photons. This is Rayleigh scattering, which is visible in the blue of the sky that results from sunlight scattered by water molecules.
However, a very small fraction (approximately 1 in 10^6 to 10^7 photons) is inelastically scattered, which means that the energy of the scattered photon is different (usually lower) than the energy of the incident photon. This is called the Raman effect and it was discovered by Chandrasekhara Venkata Raman in 1928. He used a filtered beam of sunlight for the excitation source and his eye to detect the frequency-shifted light as this was long before the development of the first laser by Maiman in 1960. Raman was awarded the Nobel Prize in Physics in 1932 for this discovery. The theory underlying the Raman effect had been published five years earlier by A. Smekal (1923).
The great utility of the Raman effect lies in the fact that the energy shift between the incident and the Raman-scattered photon is caused by the excitation (or annihilation) of a molecular vibration. Each molecule has several vibrational modes with defined energy shifts that are visible in its characteristic Raman spectrum. This serves as a fingerprint for the type and coordination of the molecule involved in the scattering process. Recording Raman spectra at a high spatial resolution enables the generation of clear and informative 2D and 3D Raman images.
WITec systems provide exceptional Raman imaging capability by combining spatial resolution down to the sub-micrometer regime with unrivalled sensitivity, simultaneously.

Knowledge Base

Answers to (almost) every question about Raman microscopy can be found in Knowledge Base.

The book for the system...

For further information, please refer to Confocal Raman Microscopy, edited by WITec scientists Olaf Hollricher, Thomas Dieing and Jan Toporski, It includes a comprehensive overview of the theoretical background, practical considerations and real-world applications of Raman microscopy along with sub-sections on instrument technology, novel materials, geosciences, life and pharmaceutical sciences, materials science and many other topics. It can be purchased in print or e-book formats directly from Springer or through online shops.

Figure 1: Confocal Raman Microscopy, 2nd edition, Editors: Jan Toporski, Thomas Dieing, Olaf Hollricher, ISBN: 978-3-319-75380-5

Theory

In quantum mechanics, the scattering process between a photon and a molecule is described as an excitation of a molecule to a virtual state lower in energy than a real electronic state and the (nearly immediate) de-excitation.
The lifetime of the virtual state is extremely short and can be calculated by the Heisenberg uncertainty relation:

$$\Delta t \cdot \Delta E \geq \frac{\hbar}{2}$$

With typical photon energies of 1-2 eV, the lifetime of the excited state is only about 10-15 s. After this extremely short time, the molecule falls back either to the vibrational ground state or to an excited state (Fig. 1). When the initial and final states are identical, the process is called Rayleigh scattering.

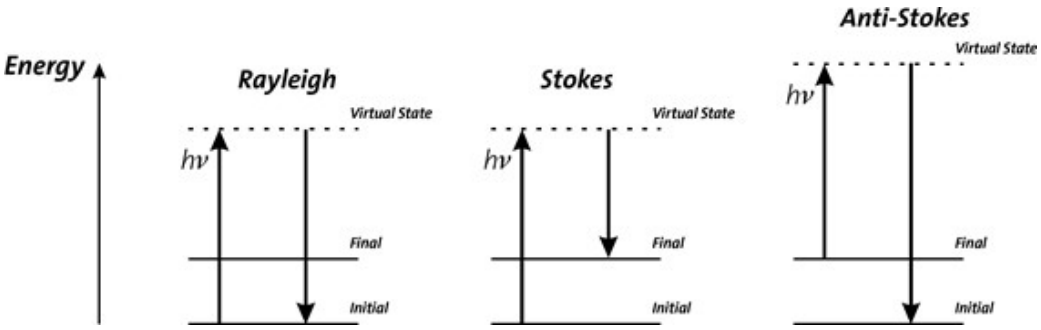


Fig. 1: Energy level diagram for Raman scattering

If the initial state is the ground and the final state a higher vibrational level, one refers to Stokes scattering, if the initial state is energetically higher than the final state, to Anti-Stokes scattering.
The difference in energy between the incident and the Raman scattered photon is equal to the energy of a vibration quantum of the scattering molecule. A plot of intensity of scattered light versus energy difference is called a Raman spectrum.

When a photon interacts with a molecule, the electrical field $\vec{E}$ induces a dipole moment $\vec{P}$ in the molecule:

$$\vec{P} = \vec{\vec{\alpha}} \cdot \vec{E}$$

The proportionality constant $\vec{\vec{\alpha}}$ is the polarizability tensor of the molecule and is a measure of the ease with which the electron cloud around a molecule can be distorted. In the case of an isotropic molecule, α reduces to a scalar.
The time dependence of the electromagnetic field is

$$\vec{E} = \vec{E}_0 \cos(2\pi\nu t)$$

If one takes a vibrating diatomic molecule as a model system, assuming a simple harmonic motion, its internuclear distance can be written in the form

$q_v = q_0 \cdot \cos(2\pi\nu_v t)$

The polarizability a is a function of internuclear distance. For an isotropic molecule, α can be expanded in a Taylor series

$$\alpha = \alpha_0 + \left(\frac{d\alpha}{dq_v}\right)_0 q_v + \cdots$$

$$\approx \alpha_0 + \left(\frac{d\alpha}{dq_v}\right)_0 q_0 \cos(2\pi\nu_v t) := \alpha_0 + \alpha_1 q_v$$

where higher than linear terms are neglected for small interatomic displacements.
If we now look at the molecule in the external electrical field, one finds

$$\vec{P} = \alpha \cdot \vec{E} = (\alpha_0 + \alpha_1 q_v)\vec{E}_0 \cos(2\pi\nu t) = (\alpha_0 + \alpha_1 q_0 \cos(2\pi\nu_v t))\vec{E}_0 \cos(2\pi\nu t)$$

$$= \alpha_0 \vec{E}_0 \cos(2\pi\nu t) + \alpha_1 q_0 \vec{E}_0 \cos(2\pi\nu_v t)\cos(2\pi\nu t)$$

$$= \underbrace{\alpha_0 \vec{E}_0 \cos(2\pi\nu t)}_{Rayleigh} + \underbrace{\frac{1}{2}\alpha_1 q_0 \vec{E}_0 \cos(2\pi(\nu+\nu_v)t)}_{Anti-Stokes} + \underbrace{\frac{1}{2}\alpha_1 q_0 \vec{E}_0 \cos(2\pi(\nu-\nu_v)t)}_{Stokes}$$

As one can see, besides the elastically scattered Rayleigh line, additional lines appear in the spectrum which are shifted ±νv relative to the excitation light.
The position of a Raman line is usually given in wavenumbers (1/cm), which is the energy shift, relative to the excitation line:

$$\bar{\nu} = \frac{1}{\lambda_{incident}} - \frac{1}{\lambda_{scattered}}$$

λincident and λscattered are the wavelengths (in cm) of the incident and Raman scattered photons, respectively.

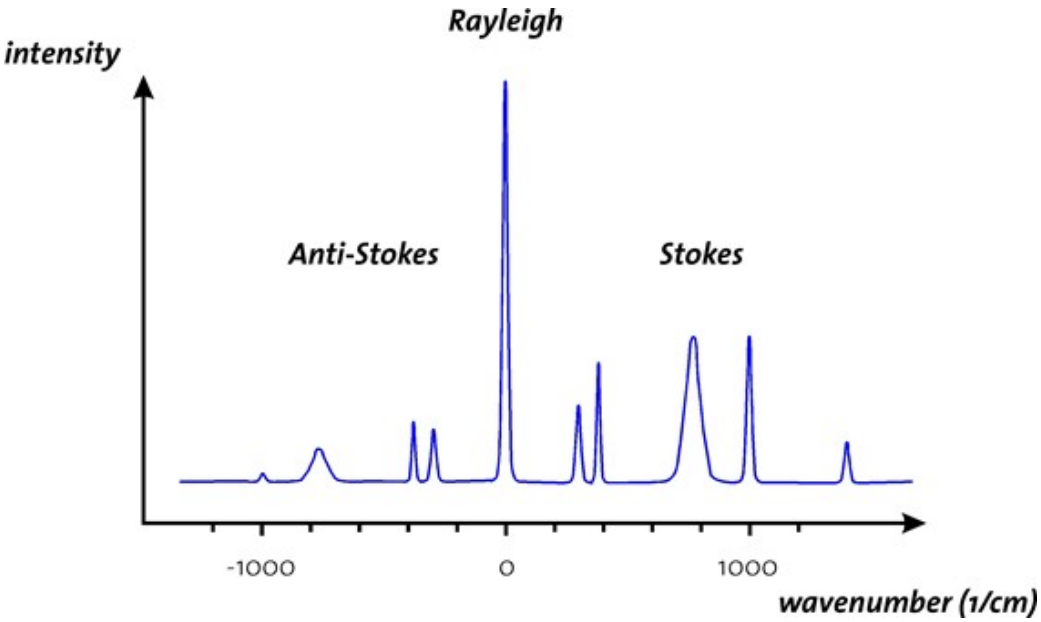


Fig. 2: Typical Raman spectrum

As can be seen in Fig. 2, a typical Raman spectrum is symmetric to the Rayleigh line and the Anti-Stokes lines are smaller than the Stokes shifted lines.
From classical scattering theory, one finds that the intensity I of scattered light is proportional to the 4th power of the excitation frequency ν.

$I \sim \nu^4$

Exciting a sample with blue light of 400 nm would therefore give a 16 times higher Raman signal than using red light of 800 nm.
The problem of using blue (or UV) excitation light is fluorescence. Most samples show fluorescence when they are excited with blue light. The Raman effect is extremely weak compared to fluorescence. If a sample shows fluorescence, obtaining a Raman spectrum is usually very difficult because of the strong fluorescence background. In the red (or even IR) part of the spectrum fluorescence is usually not a problem anymore, but the excitation intensity has to be much higher (I~ν4!). Another problem is, that Silicon detectors cannot be used above 1100 nm (bandgap energy of Si: 1.12 eV). Other IR detectors (like InGaAs) are extremely expensive, show much more thermal noise than Silicon and photon counting detectors with a reasonable dark count rate were not available up to now. In real experiments one must always find a compromise between detection efficiency and excitation power. From the above equation one would assume that

$I_{Stokes} \sim (\nu - \nu_v)^4$, and $I_{Anti-Stokes} \sim (\nu + \nu_v)^4$

and therefore IAnti-Stokes>IStokes. Experimentally, one finds the opposite: IAnti-Stokes<IStokes.
Here, quantum mechanics comes into play. For Anti-Stokes scattering, the molecule must already be in an excited vibrational state.
The Boltzmann distribution defines which portion of N molecules are thermally excited to an energy level Ej

$$N_j = N \cdot e^{-\frac{E_j}{k_B T}}$$

Using the energy of a harmonic oscillator, one gets

$$N_j = N \cdot e^{-\frac{\left(j+\frac{1}{2}\right)h\nu}{k_B T}}$$

The probability of finding a molecule in the ground state is much higher than finding it in an excited state. At room temperature, Stokes scattering is therefore much more effective than Anti-Stokes scattering. To calculate the relative intensity, the exponential function must be taken into account

$$\frac{I_{Anti-Stokes}}{I_{Stokes}} \sim e^{-\frac{h\nu_v}{k_B T}} \cdot \left(\frac{\nu + \nu_v}{\nu - \nu_v}\right)^4$$

Usually, the e-function will dominate this term, so that

$$I_{Stokes} > I_{Anti-Stokes}$$

If one measures the intensity ratio between Stokes and Anti-Stokes lines, one can determine the sample temperature.

Configurations

Different configurations are available for Raman measurements. The configuration defines the used CCD camera 1, 2 or 3 and thus the respective spectrometer that should be used for the measurement. The following list describes the purpose of each configuration. Only Configurations suitable for your system are installed by default.

Raman CCDX	Standard Raman configuration which uses microscope z
Raman without z-stepper CCDX	Is used for systems without microscope z e.g. RISE, z movement during measurement by piezo stage, Topography correction and signal stabilization are not available
Raman inverted CCDX	Is used for Raman from below for systems with additional inverted objective, z movement during measurement by piezo stage if equipped, otherwise no z movement possible, Topography correction and signal stabilization are not available
attoRaman CCDX	Raman Imaging in an attocube cryostat, no z movement during measurement

Calibration

Introduction

Any spectrograph used for spectroscopy experiments needs to be calibrated in order to obtain reliable measurement results. The calibration of any WITec system will have been performed at WITec before delivery of the system. However it may be desirable to recalibrate the spectrograph from time to time especially if it was subject to strong temperature changes.
In order to calibrate the spectrometer an Argon/Mercury calibration lamp is used. For an UHTS400S NIR InGaAs with an InGaAs camera a Xenon lamp is needed.
Once the calibration is started, the system will drive the selected grating to various positions and determine the position of certain Ar and Hg (or Xe) lines both on- and off-axis in order to determine the calibration parameters.
In order to check the calibration, just a verification can be performed without changing the current calibration.

Procedure

1. For non-automated systems: Configure the beampath for Calibration.
2. Click on Spectrograph Calibration under Additional Devices.
3. Select the Spectrograph you want to calibrate.
4. Optional: Activate **Verify only**, if you just want to check the calibration.
5. Click on **Calibrate all** or **Calibrate** for the respective grating.

Further information:

Spectrograph Calibration

Alignment

The entrance of the output fiber functions as a confocal pinhole. To optimize Raman signal and z-resolution the fiber has to be aligned with the laser spot. If the system has more than one spectrometer, the alignment has to be done with each output coupler.
This procedure is recommended after changing the laser wavelength or if you observe lower Raman signal than expected.

Required sample:
Silicon sample (preferably the one delivered with the system)

General steps

We recommend to perform the alignment of the pinhole on a silicon sample.

1. Focus on the surface of the silicon. Make sure that your laser position is on a clean and uniform area of your silicon sample.
2. For non-automated systems configure the beam path for Raman (choose the right laser wavelength and the output coupler accordingly, camera coupler and white light slider should be out). Adjust the laser to full power.
3. Start the oscilloscope to see the spectrum currently recorded by the spectrometer. Use a short integration time < 0.25 s to be able to see the effect of the subsequent alignment steps spontaneously.

If everything is configured correctly there should be at least a small peak at 520 rel. cm^{-1} (the first order of silicon). This is the basis for further adjustment. If you have no signal please refer to Hints and Troubleshooting at the bottom.

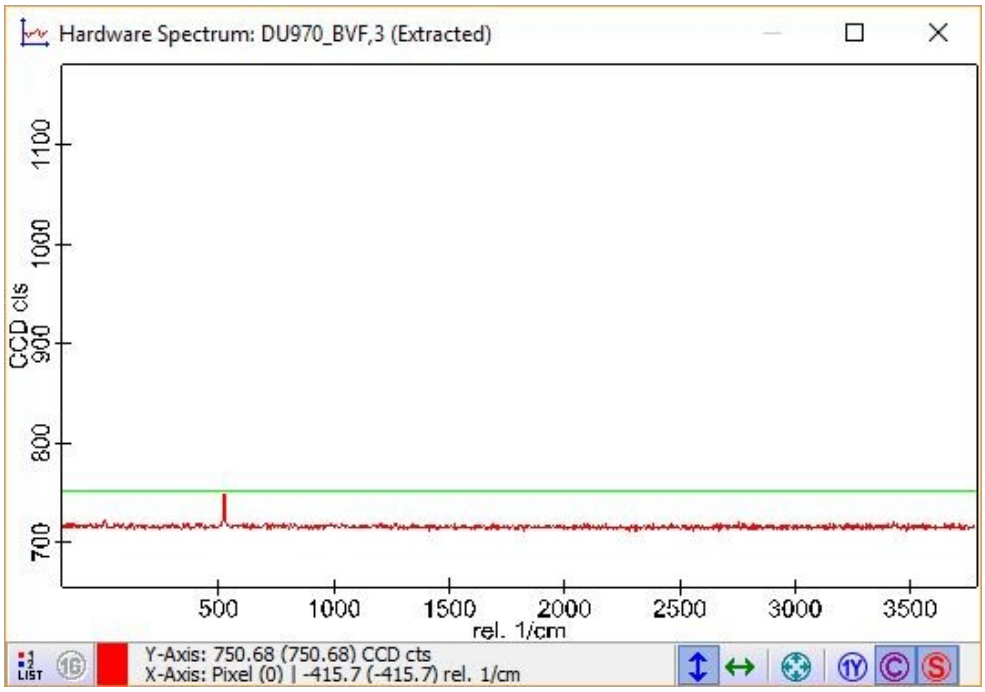


Figure 1: Low intensity silicon spectrum

If a signal similar to the one in Figure 1 is visible use the focus drive carefully to go in one direction. Check the signal intensity. Keep the direction if the signal gets higher, or try the other direction if it gets lower. Stop at the maximum signal.
If you have an apyron or RISE system refer to the automated output coupler section

Manual output coupler

On the backside of each output coupler (on the upper section of the microscope tower), you have two knobs for the alignment in x- and y-direction (Figure 2). If you turn the knobs, try to avoid applying force on the microscope tower, as this will lead to defocusing and a lower signal. After you turned a knob, remove your fingers before you check the signal strength.
If you have more than one output coupler, make sure you are touching the right knobs. Stop turning the knobs if you see no change in the intensity and check again that you turn the right knobs.

Figure 2: Fiber alignment screws

Automated output coupler

The alignment of automated output couplers could be done using the software. Click Output Alignment  to open the Output Alignment window. (You can either use the remote control or the mouse.)

Adjustment

The goal is to get the signal as high as possible. (You can mark the actual signal intensity in the graph viewer with the green line at the mouse position by moving the mouse sideward out of the window.) Concentrate on the first order of silicon as long as the signal is still weak.
Now try to increase the signal by changing the alignment in x and y direction.

1. Change the position in x-direction and check the signal strength. If the signal strength decreases, turn to the other direction. Otherwise, increase the signal to the maximum.
2. Use the y-direction to do the same. And try again with each of the directions, until you cannot increase the signal anymore.
3. Drive with z like before, to get the highest signal, you can reach.
4. If the signal is high enough, use the second order Raman line (around 950 rel. cm^{-1}) for further adjustment.
5. Start from step 1 again and repeat all the steps until you have found the absolute maximum of the signal.

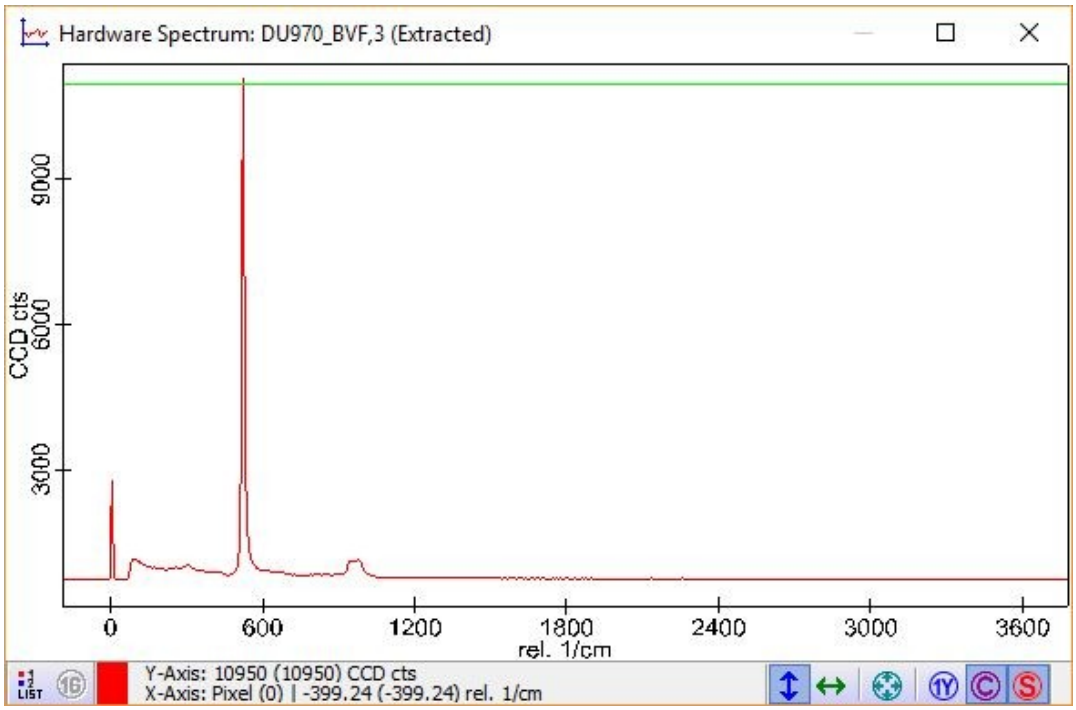


Figure 3: Silicon spectrum with optimized fiber position

Hints and Troubleshooting

If it is not possible to get any Raman signal, check the following points:

1. For non-automated systems, check if the beam path is set up correctly for Raman measurements and if you have selected the correct configuration in the software for the spectrometer you want to use.
2. Check if the right grating is chosen and if the center wavelength is configured correctly: A smaller grating is recommended with a spectral range to cover the Rayleigh peak at 0 rel. cm^{-1} the 1st order silicon band at about 520 rel. cm^{-1} and also the 2nd order at about 950 rel. cm^{-1}.
3. Check the laser power if possible.
4. Increase the integration time to several seconds.

If you have an apyron or RISE system, you could try a Video alignment (see below).

If all other things are excluded, the fiber might be at a completely wrong position.

Please ask WITec support for your further advice.

Video Alignment

The automated output coupler is equipped with an adjustment camera, showing the laser beam position.

Activate it by clicking on Video in the Output Alignment window. You should see the laser spot and the probe position (red circle). Decrease the laser power until you can just clearly see a small spot.

The goal is to get back the laser beam position to the center of the red circle (compare with Figure 4). You can move the laser spot like described above with the remote control or the software buttons. Once the laser beam is back in this circle, it should be possible to get at least a small signal in Raman mode. This will be the base for further adjustment like described above.

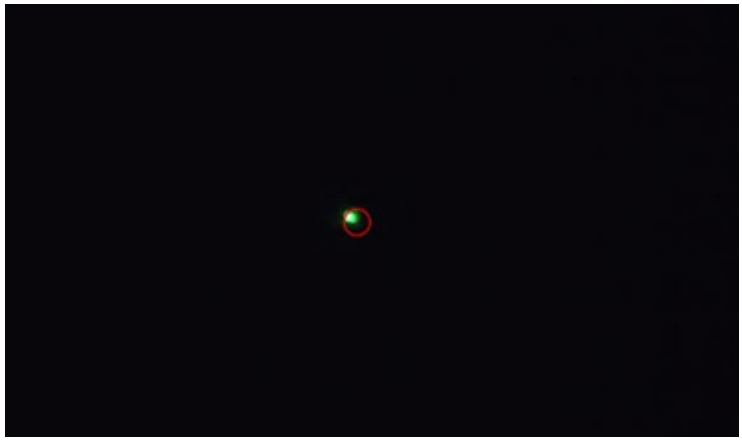


Figure 4: Output Alignment window (left) and view of the adjustment camera (right)

Change Laser Wavelength

If the laser you want use implies a spectrometer change, refer to the first section. If not, you can jump to the second section.

Change the spectrometer

1. Select the appropriate configuration for the spectrometer you want to use.
2. For non-automated systems: Configure the beampath for the use of this spectrometer.
3. The laser used with the spectrometer the last time is automatically selected.
4. For non-automated systems: Configure the beampath for this laser.
5. If you want to use a different laser, refer to the next section.

Change the laser

1. Select the laser you want to use.
2. For non-automated systems: Configure the beampath for the laser.
3. If necessary: Select an appropriate grating (in the spectrograph section).
4. Adjust the spectral center (in the spectrograph section).

After changing the spectrometer or laser, check the alignment.

Large Area Scan

The Large Area Scan is using the motorized stage for Raman Imaging. Even large regions from tens of microns to centimeters can be measured.

System requirements:

- **motorized stage (+ system)**
- **For access+ only Stepwise Raster is possible (and Area if licensed)**
- **Not possible with RISE and access**

License requirements:

- **CrossTableScanning for continuous measurement modes**

Procedure

1. **Select the Scan Method:**
   a. **Stepwise Raster (no continuous movement, slow mode, single spectra)**
   b. **Area (Imaging in the x-y-plane)**

   c. Depth (Imaging in z-direction)
   d. Stack (3D-Imaging)
2. Define the Geometry of your measurement (the center position defines the center of your measurement also in z-direction.)
   a. By mouse with the Listen Position/Area function (consider to correct Width and Height to round values). Define the Depth for Depth Scan or Stack Scan.
   b. By directly typing in the values for Width, Height and/or Depth. The Center Position could be set by directly typing in the position or by pressing Center at Current Pos. to use the current position.
3. Optional: Change the Gamma value to adjust the angle of the measurement in the x-y-plane. (Select 90° to make the y-axis the first moving direction, if Area is the selected Scan Method.)
4. Define the number of pixels for the Image with Points per Line and Lines per Image (and Layers per Scan for Stack Scan) (This defines the resolution of your image in conjunction with the Width, Height and/or Depth parameter.)
5. Set the integration time:
   a. For Stepwise Raster define it in the Single Spectrum section.
   b. For all continuous measurement modes use the Integration Time parameter in the Large Area Scan section.
6. For non-automated systems: Change the beam path for Raman measurements.

Z-coordinates used for the geometry definition of measurements are always Software-z-coordinates. This enables focusing without changing the z-coordinate of the measurement, because usually User-z (U:) is selected and the Software-z remains at zero when changing Microscope Z.

7. Adjust the focus for your measurement using the applicable option:
   a. For Stepwise Raster and Area: Optimize the focus for your measurement by using the Oscilloscope. (Best position is probably the center of your measurement area.)
   b. For Depth and Stack use one of these options:
      i. Focus in the middle between highest and lowest point of your measurement.
      ii. Focus on the sample surface (Software-z should be zero) and change the Center (z) parameter to negative values to shift the measurement downwards or to positive values to shift the measurement upwards (i.e. for a depth of 10 µm use -4 µm as Center (z) value to measure from 1 µm above the surface to -9 µm below the surface).
8. Click at Start Large Area Scan to start the measurement.

Further information:

Large Area Scan, 3D Data Analysis

Hints

- The smallest step for the motorized stage is:
  - 100 nm for alphaControl with a serial number 120-1050-XXX (Marvin 4b) or lower
  - 25 nm for alphaControl with a serial number 120-1060-XXX (Marvin 5) or higher
- Try to use multiples of the minimum step size as resolution for the x-y-plane. Lower values can lead to artifacts in the image.
- Use the oscilloscope mode to check the necessary integration time.
- Consider to use a lower laser power for focusing to prevent damage from your sample. For scanning a higher laser power is possible in most cases.
- Make sure that User-z is selected while adjusting the focus, otherwise changing the focus has no effect on the measurement position.
- Consider using the Signal Stabilization function for measurements of several hours.

Image Scan

The Image Scan is using the piezo stage for Raman Imaging within its range.

System requirements:

- Piezo stage

Procedure

1. Select the Scan Method:
   a. Area (Imaging in the x-y-plane)

   b. Depth (Imaging in z-direction)
   c. Stack (3D-Imaging)

2. Define the Geometry of your measurement (the center position defines the center of your measurement also in z-direction.)
   a. By mouse with the Listen Position/Area function (consider to correct Width and Height to round values). Define the Depth for Depth Scan or Stack Scan.
   b. By directly typing in the values for Width, Height and/or Depth. The Center Position could be set by directly typing in the position or by pressing Center at Current Pos. to use the current position.
3. Optional: Change the Gamma value to adjust the angle of the measurement in the x-y-plane. (Select 90° to make the y-axis the first moving direction, if Area is the selected Scan Method.) (Even rotating the measurement area along the other axes is possible by the hidden parameters Alpha and Beta.)
4. Define the number of pixels for the Image with Points per Line and Lines per Image (and Layers per Scan for Stack Scan) (This defines the resolution of your image in conjunction with the Width, Height and/or Depth parameter.)
5. Set the integration time.
6. For non-automated systems: Change the beam path for Raman measurements.

Z-coordinates used for the geometry definition of measurements are always Software-z-coordinates. This enables focusing without changing the z-coordinate of the measurement, because usually User-z (U:) is selected and the Software-z remains at zero when changing Microscope Z.

7. Adjust the focus for your measurement using the applicable option:
   a. For Area: Optimize the focus for your measurement by using the Oscilloscope. (Best position is probably the center of your measurement area.)
   b. For Depth and Stack use one of these options:
      i. Focus in the middle between highest and lowest point of your measurement.
      ii. Focus on the sample surface (Software-z should be zero) and change the Center (z) parameter to negative values to shift the measurement downwards or to positive values to shift the measurement upwards (i.e. for a depth of 10 µm use -4 µm as Center (z) value to measure from 1 µm above the surface to -9 µm below the surface).
8. Click at Start Scan to start the measurement.

Further information:

Image Scan, 3D Data Analysis

Hints

- Consider to use a lower laser power for focusing to prevent damage of your sample. For scanning a higher laser power is possible in most cases.
- Image Scan is using the microscope z for moving in z direction for Depth and Stack scan. Exception: If the configuration is using piezo z.
- Use the oscilloscope mode to check the necessary integration time.
- Make sure that User-z is selected while adjusting the focus, otherwise changing the focus has no effect on the measurement position.
- Consider using the Signal Stabilization function for measurements of several hours.

Slow Series

The slow series offers several options:

- Slow time series - Intermittent time series with fixed time interval
- Laser power series - spectra at linearly varying laser power (only for TruePower laser)
- Polarizer series - spectra at linearly varying polarizer angle (only with automated polarizer)
- Analyzer series - spectra at linearly varying analyzer angle (only with automated analyzer)
- External triggered series - spectra on external triggered events (with COM Automation interface and e.g. LabView)

Laser power series

Keep Dose constant option:

- Keep Dose constant reduces the integration in the amount the laser power is raised.
- The integration time defines the maximum integration time.
- If the chosen integration time is too short, it is maybe not possible to reduce it enough at higher laser powers.
- Even with keep dose constant a small effect on the intensity will be visible especially at very short integration times, because the integration cannot be determined 100 % exactly from the CCD camera.

Polarizer/Analyzer series

If you perform a Polarizer or Analyzer series the angle between polarizer and analyzer can be fixed with the synchronize angle option.

Data evaluation

You will get several data objects as result (at least two). One contains the spectra, others contain the elapsed time, laser power etc. All have their data points plotted against P, the number of the data point. Also if you use e.g. the filter view to get a intensity distribution from the Raman spectra, it will be plotted against P. Refer to the parametric view to create graphs e.g. intensity against time or intensity against laser power.

Further information:
Series Slow

Sample Raster

Sample Raster enables processing a script at a predefined list of up to several thousand points.

The following tasks are possible for Raman:

- Auto Focus
- Single spectrum
- Image scan

It is not possible to use TrueSuface or other Topography correction for Sample Raster.

For automatic measurements of optical visible particles please refer to the ParticleScout.

For more sophisticated automation projects please refer to our COM Automation interface and e.g. LabView.

System requirements:

- motorized stage (+ system)
- Piezo stage for Image scans
- Not possible with RISE and access

Procedure

The following steps describe how to set up a Sample Raster measurement in an easy way doing an autofocus and taking a single spectrum.

1. Focus on your sample.
2. Click on Point List Editor.
3. Activate the Move Sample to Mouse Position.
4. Select a point by clicking in the video image or in a recorded image.
5. Click on Take current point as new point in the Point viewer.
6. Repeat points 4 and 5 until all desired points are in the list.
7. Enter autofocus; singlespectrum in the Command line parameter.
8. Define the parameters in the Autofocus and Single spectrum section.
9. For non-automated systems: Configure the beampath for Raman and open the laser shutter.
10. Optional: Click on Start Script to test the parameters at the current position.
11. Click on Start Raster to start the measurement.

Further information:
Sample Raster, Single spectrum, Image scan, Auto Focus

Manual Topography Correction

The purpose of the Manual Learned Topography Correction is to measure along simple sample surfaces without having the TrueSurface hardware. There is the possibility to compensate the tilt of the sample by using the 3 point plain correction or even more complex surfaces by the 5 x 5 option.

System requirements:

- **motorized stage (+ system)**
- **Not possible with R, RISE and access**

Possible for Large Area Scan and Line Scan.

Procedure

1. **Define your** Geometry **parameters for the Large Area Scan.**
2. **Go to** Manual Learning **in the** Topography Correction **section and select either** Learn Plane (3 Pts) **or** Learn Surface (5x5 Pts) **by clicking on the respective button.**
3. **The software now starts the oscilloscope to enable focusing using the Raman spectrum (For non-apyron systems: Configure the beam path for Raman).**
   Alternative: It is also possible to switch back to video modus **and use the video image for focusing.**
4. **Optional: It is possible to slightly change the current position, when it is not suitable for focusing.**
5. **If the height difference of the sample is more than 200 µm, the Software-Z travel range needs to be extended. Refer to the** microscope-z section.
6. **Click on** Next Step.
7. **Repeat the steps 3 to 5 until all points are focused.**
8. **Optional: Switch to** Software-z **by clicking** S: **in the Microscope Z area of the Video Control Window. (Prevents changing z-position of the surfaces relative to the sample by moving Microscope Z.)**
9. **Optional: Move to a point on your sample within the measurement area and click** Goto Surface**. Now it is possible to refocus the surface on the Raman spectrum by using the Oscilloscope (For non-apyron systems: Configure the beam path for Raman). (Make sure** Software-z **is switched to** U:**)**
10. **Switch** Topography Correction **to True Surface.**
11. **For non-automated systems: Configure the beam path for Raman (insert Output and Laser coupler, remove video and brightfield coupler).**
12. **Click on** Start Large Area Scan.

Further information:
Topography Correction

TrueSurface Mk1/2 Overview

The TrueSurface Microscopy option enables confocal Raman imaging following the surface topography. Therefore, the topography is recorded in a first step with a Confocal Chromatic Sensor (CCS).

TrueSurface Mark I:

The first generation of TrueSurface was only available for the alpha500. The CCS is installed close to the turret and has an offset of several centimeter.

TrueSurface Mark II:

In the second generation of TrueSurface the CCS is integrated as objective in the turret. The first version of this objective has a longer black body, the second version has a shorter metallic body.

Topics:
- Calibration
- Setting up a measurement

System requirements:

- **TrueSurface Mark I or Mark II**
- Not possible with RISE and access

Possible for following measurement modes:
- Large Area Scans
- Line Scans

Calibration

The precondition to get good results with TrueSurface is that the offset between the objective and the Confocal Chromatic Sensor (CCS) has to be corrected. This is accomplished by the objective compensation.

If you have TrueSurface Mark I please refer to Mk1 Calibration. If you have TrueSurface Mark II please follow the next steps.

Sample for calibration:
- silicon (i.e. silicon with cross)

1. The CSS needs to be calibrated like any other objective (only necessary if not calibrated before):
   - use the piece of silicon with cross (metallic CCS)
   - if contrast is to weak, use the edge of silicon (black CCS)
2. Start the Objective Compensation Wizard (located in the Menu in the Video Control Window).
3. Follow the instructions for TrueSurface Mk2 found by clicking the question mark.

Hints

- Check that the appropriate TSMO2 objective is installed in the slot marked with TS.
- Make sure that the objective you want to use for the measurement is also compensated. Check in the Objective Compensation Wizard (located in the Menu in the Video Control Window).

Calibration Mk1

The following instructions apply only to alpha500 systems with TrueSurface Mark I.

Sample for calibration:
- pinhole probe with 50 µm hole

Recording a profilometer image of the pinhole
1. Switch to the Profilometer configuration (in the section Confocal).
2. Mount the pinhole probe (with adhesive tape) to the True Surface area of the cross table. (The dashed line is marking the allowed area.)
3. Locate the pinhole in the video image using a small magnification objective and switch to the 50x objective. Put the pinhole into the center of the video image.
4. Optional: Click the Set Zero button of the Sample positioner, to easily find back to this position.
5. Save a video image of the pinhole.

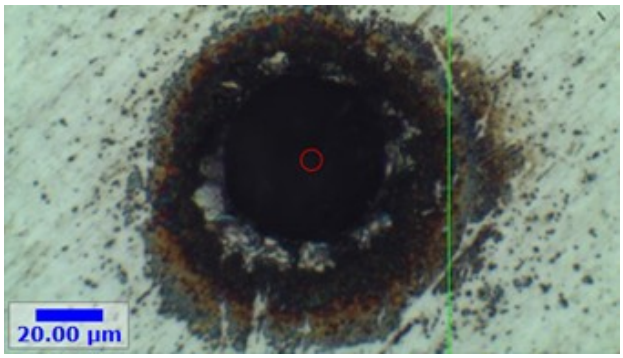


Figure 1: Video image of the pinhole

6. Switch to the TSMO1 objective (has to be Slot 7).
7. Use the remote control to drive with the pinhole probe to the Confocal Chromatic Sensor (CCS) (roughly [-90 mm; -1 mm] from the video center). Try to get the light beam as close as possible to the middle of the pinhole probe.
8. Define a Large Area Scan of about 5000 µm x 5000 µm to find the pinhole. Click on Center at current position and start the scan.
9. You should see the pinhole as a black dot in the resulting images. Decrease the area of the Large Area Scan (e.g. select with Listen Area) until you get pictures like in Figure 2. Keep both images open.

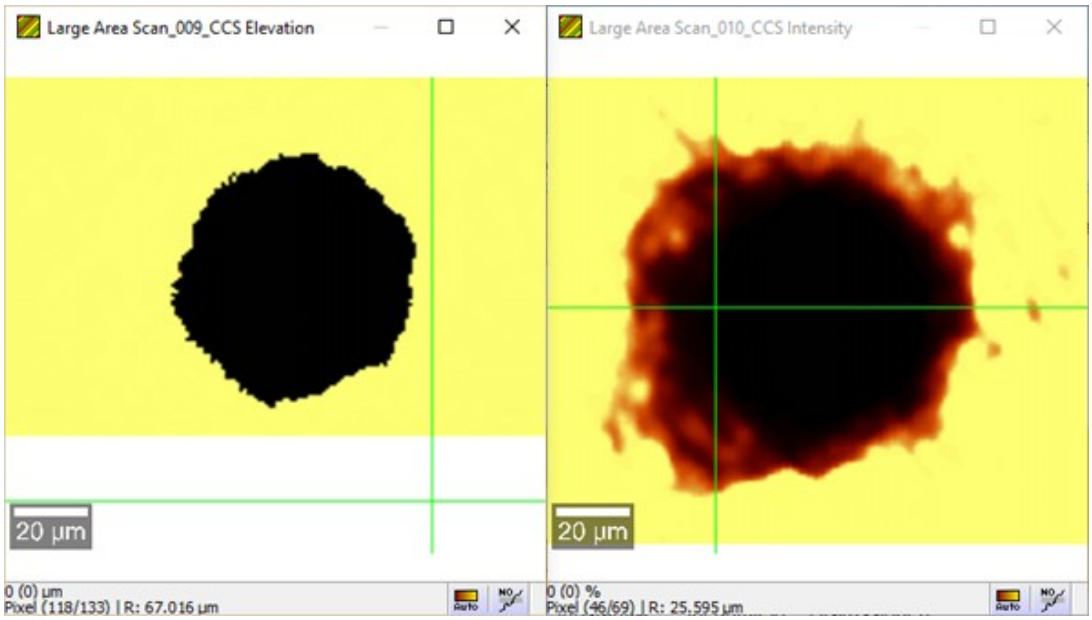


Figure 2: Pinhole with CCS sensor

10. Switch to the 50x objective, activate the Move Sample to Mouse Position and click in the previously recorded video image to move the pinhole back under the 50x objective.

Objective Compensation

1. Start the Objective Compensation Wizard (located in the Menu in the Video Control Window).
2. Move the center of the pinhole under the cross. Make sure the flat area around the pinhole is in focus.
3. Click on Start Calibration.
4. Select the TSMO1 objective and acknowledge the appearing window.
5. Click on the flat part around the pinhole in one of the two profilometer images.

6. Adjust the focus until the CCS Elevation [µm] in the status is half of the sensor range (i.e. 1500 µm for 3 mm).
7. Click in the center of the pinhole in one of the two profilometer images as exact as possible.
8. Click on Stop Calibration and close the wizard.

Check the compensation

1. Put the pinhole in the center of the video image using the 50x objective and save an image.
2. Select the TSMO1 objective.
3. Start a Large Area Scan with 200 µm x 200 µm and Center at current position using the Profilometer configuration.
4. Select the 50x objective after the measurement.
5. Profilometer image and video image should match. (Check by moving through the image with mouse button pressed or use the Overlay tool.)
6. If you are not satisfied, check the hints section and redo the objective compensation using the new images.

Hints

- Check that the appropriate TSMO1 objective is installed in Slot 7.
- Make sure that the objective you want to use for the measurement is also compensated. Check in the Objective Compensation Wizard (located in the Menu in the Video Control Window).
- Fix the pinhole as good as possible, any shift of the probe will cause an offset in between the surface and the Raman map later on.
- Delete the compensation of the TSMO1 objective, if the stage goes to a completely wrong position, when you select the objective.

Procedure

1. Define your Geometry parameters for the Large Area Scan.
2. Go to a point on your sample within the measurement area, change to the CCS objective.
3. Only Mk2: Click on TS in the Video Control Window and for non-automated systems: Configure the beam path for TrueSurface (insert TrueSurface coupler, remove video and brightfield coupler).
4. Set the parameters of the Optical Distance Sensor. (Reduce Sampling Rate, if the signal is too weak. Move arround on the sample to check at different positions.)
5. Set Image SizeX [Pixels] and Image SizeY [Pixels] under Learn By CCS LA-Scan in the Topography Correction section.
6. Click on Learn By CCS LA-Scan.
7. After the scan click on Edit Surface Scan.

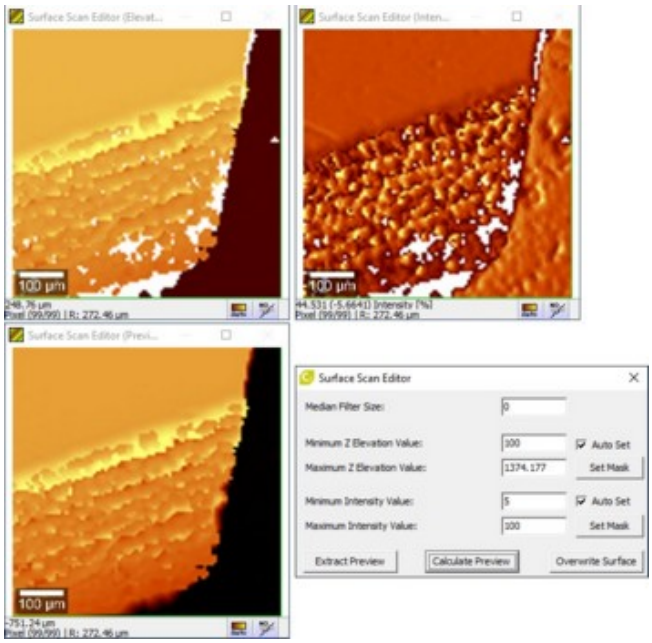


Figure 1: Surface Scan Editor

8. Surface points with a Z Elevation below zero or too low intensity have to be removed. Change the filter settings and click on Calculate Preview. (Figure 1)
9. Click on Overwrite Surface (Figure 1)
10. Optional: Click on Extract Preview (Figure 1) (Image could be used for navigation in 14.)
11. Optional: Switch to Software-z by clicking S: in the Microscope Z area of the Video Control Window. (Prevents accidentally changing z-position of the surfaces relative to the sample by moving Microscope Z.)
12. If the roughness of the sample is greater than 200 µm, the Z Travel has to be adjusted. Extend the Software-z travel range that is fitting the roughness of your sample.

If the Extended Z Travel is to small the Geometry parameters in the Large Area Scan become red and the measurement will not start.

13. Switch to the objective, which should be used for the measurement.
14. Optional: Move to a point on your sample within the measurement area and click Goto Surface in Topography Correction. Now it is possible to refocus the surface on the Raman spectrum by using the Oscilloscope (For non-automated systems: Configure the beam path for Raman). (Make sure Microscope Z is switched to U:.)
15. Switch Topography Correction to On in **Large Area Scan**.
16. For non-automated systems: Configure the beam path for Raman (insert Output and Laser coupler, remove video and brightfield coupler).
17. Click on Start Large Area Scan.

Further information:
Topography Correction, Optical Distance Sensor, Large Area Scan

Hints

- Less pixels can be used for the CCS scan compared to the Large Area Scan.

TrueSurface Mk3 Overview

WITec's TrueSurface Microscopy option enables confocal Raman imaging guided by surface topography. In its 3rd generation, topographic Raman imaging uses an advanced optical profilometer integrated within the instrument to provide one-pass simultaneous operation.

Topics:

- Choice of Objective
- Adjustment and Hints

System requirements:

- alphaControl with a serial number 120-1050-XXX (Marvin 4b) or higher
- Not possible with RISE and access
- Objective with magnification of 20x or higher

Possible for all measurement modes besides:

- Sample Raster

Introduction

TrueSurface (TS) enables real-time large area topographic and Raman imaging within the scan range of the motorized positioning device even for rough or inclined samples. The effect is demonstrated in Figure 1 on a silicon lens. The image on the left shows the topography of the sample surface. Without TS, only one plane is in focus during a measurement, resulting in a ring of strong Raman signal (middle). The image on the right shows the same area measured with TS active, which allows the acquisition of a Raman map with a consistently strong signal.

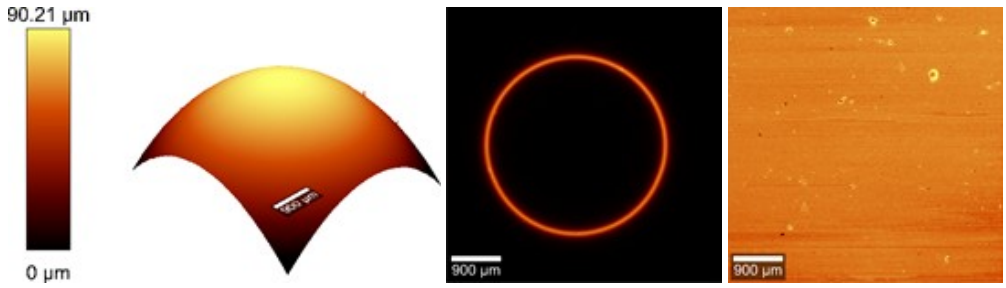


Figure 1: Si lens. Left: Topography. Middle: Only one depth level is in focus with the standard confocal Raman microscope. Right: The same image as in the middle, but with TrueSurface activated the Si signal is constant from all depths as the sample surface is always kept in focus.

Technique Principles

TrueSurface focuses light onto the same spot on the sample at which the excitation laser for Raman measurements is focused. This establishes the distance between the objective and the sample. As reflected light is measured, a different index of refraction at the sample surface is required. A feedback loop allows the system to keep the distance constant and thus the sample surface always stays within the Raman measurement's focus. This enables simultaneous measurement of the sample's topography and Raman signal.

Choice of Objective

Many objectives are suitable for TS measurements, but they differ in their properties and not all objectives are recommended in combination with all samples. Several objectives have already been tested and recommended for operation with TS. A warning message indicates if any untested objective is selected, nevertheless it may be possible to use it. Objectives with a magnification of less than 20x are not suitable.

For a proper choice of objective, the following aspects should be considered:

- The objective's **transmission** properties for TS differ. Some objectives thus give a low TS signal.
- The TS principle requires reflected light from the sample surface to be measured. Edges or strongly inclined areas can scatter or reflect the light beyond the collecting angle of the of the objective, which results in lower TS signal, especially for objectives of low **numerical aperture** (NA). Figure 1 shows video images of a silicon step sample. In the image on the left, acquired with a 20x/0.5 NA objective, the edges appear black because less light from there is collected by the objective. In the image on the right, the 100x/0.9 NA objective could collect light reflected from the edges due to the higher NA and the respective larger collecting angle.

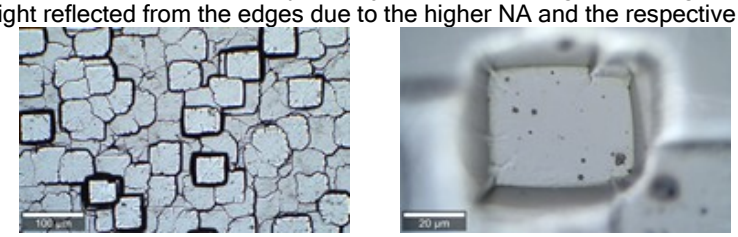


Figure 1: Video image with 20x/0.5 NA objective (left) and video image with 100x/0.9 NA objective (right)

- The roughness of the **sample surface** is important, as the reflected light captured from out-of-focus areas after a large step might not be strong enough for a continuous feedback. Measurements on samples with large height variations may be easier with low magnification objectives because a small magnification is

accompanied by a large focal range, while high magnification correlates to a small focal range.
The following step heights are typically manageable for standard objectives:

100x objektive: +/-4 µm

50x objektive: +/-16 µm

20x objektive: +/-100 µm

Objectives with a small magnification are therefore better suited, if the sample features high steps, while a high NA is required to measure sharp edges.

Adjustment

Procedure

1. Focus on your sample **with the objective that should be used for the measurement.**
2. **Move the microscope focus position in the middle between highest and lowest point within your measurement area.**
3. **Set the** Software-z **to zero.**
4. **If necessary: Extend the Software-z** travel range **so that the highest and lowest point could be reached. Please also mind the objective's working distance.**
5. **For non-automated system: Configure the beampath for TrueSurface.**
6. **Turn on TS in the** TrueSurface section **and click on** Start**.**
7. **Adjust the signal intensity to about 50 %.**
8. **Move around within measurement area and check whether TS follows the topography. If not please refer to the hints section. Check the signal intensity during the movement.**
9. **Start the** Oscilloscope **mode.**
10. **For non-automated systems: Configure the beampath for Raman.**
11. **Use the** Focus Shift **value to maximize the Raman signal or to adjust a certain z offset. (The value is not in µm. Please refer to the** software-z **to observe the change in µm.)**
12. **Now the Raman measurement could be set up in the same way like without TS.**

Hints

If the feedback does not work well, the following parameters should be reconsidered:

- Objective (Magnification, NA)
- P-Gain
- I-Gain (usually zero)
- Min. Value (%)
- TS Light Intensity
- Scan Speed
- Scan Area
- Time for Retrace

Adjustment of gains: Typically, higher P-gains are more effective for rough topographic features. The I-gain might be helpful on very flat samples that are tilted or bent. You can hear a shrill sound if the gains are too high. In that case lower the gains or change the position on the sample a bit if the topography causes the feedback problem (e.g. at a steep edge).
The parameter Min. Time for Retrace [s] for Large Area and Image Scans should be long enough to allow the feedback to follow the sample surface during the turnaround movement. A good value to start with is several seconds.

Signal Stabilization Overview

The purpose of the Signal Stabilization is to compensate focus drifts in long term (several hours) measurements due to changes of the ambient temperature.

Topics:

- Setting it up
- Hints and Troubleshooting

System requirements:

- Not possible with RISE and access
- High magnification objective > 20x

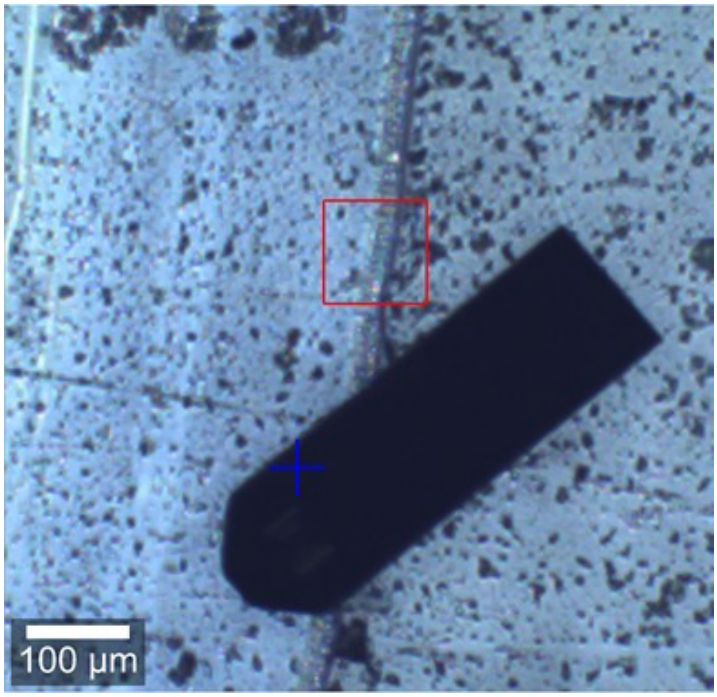
Possible for all continuous measurement modes of Image Scan and Large Area Scan:

- Images
- Depth Scans
- Stacks

- including Topography Correction for the Large Area Scan

A reference point at the sample is needed i.e.:

- sample itself (sufficient Raman signal needed)
- silicon (i.e. Cantilever (Figure 1))
- adhesive strip



Procedure

1. Define your measurement parameters for the Image Scan or Large Area Scan (at least the geometry and integration time).
2. Choose the appropriate **Stabilization Mode** (Peak for samples giving just a signal at the surface or Positive Edge for sample giving a signal also under the surface (Figure 1))

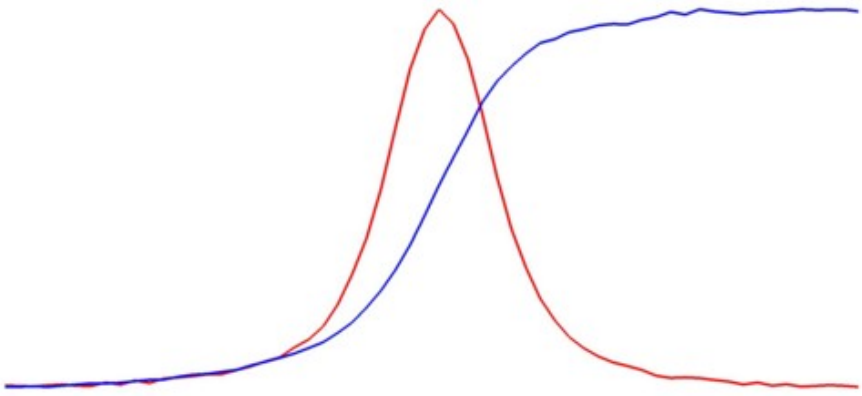


Figure 1: Depth profile of silicon (red) and adhesive strip (blue)

3. Choose the **Actuator for Compensation**. If possible use the Scan Table for the best results because of the better linearity for very small steps.
4. Define your reference point using **Listen Stabilization** or typing the coordinates. (Refer to Hints points a to f)
5. Choose the **Signal Range** for the Stabilization by selecting **Listen** at Once and then mark the region to use in a spectrum. (If necessary start the Oscilloscope mode.)
6. Click **Start Stabilization** to check the performance and success in the Messages window. (Change the beampath to Raman before.)
7. If the stabilization failed, focus on maximum spectral intensity for peak mode or on half of maximum for edge mode using the Oscilloscope. (User-z (U:) has to be selected.)

8. Repeat clicking on **Start Stabilization** several times until the stabilization height shown in the Messages window is nearly zero (Figure 2).

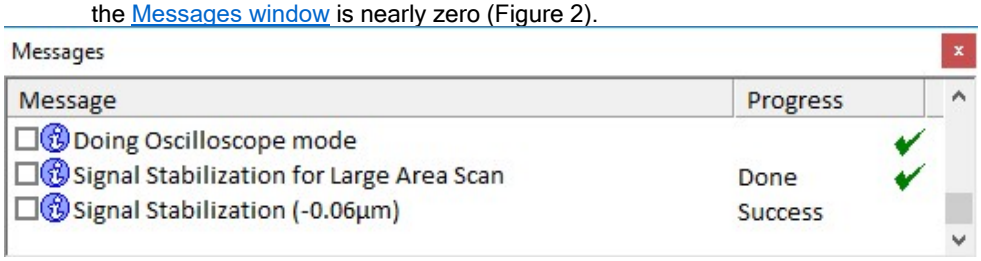


Figure 2: Messages window

9. Adjust the focus for your measurement using one of the two following options:
   a. Optimize focus within the measurement area and change the Center (Z) value to the current Software-z value or optimize focus in the center of the measurement area and click on Center at Current Pos.
   b. Use topography correction (Manual or with CCS-Scanner). After the topography was read in, choose the appropriate of the two following options:
      i. If you are doing manual topography correction by Raman spectrum, you do not need to do something additionally.
      ii. If you are doing manual topography correction by Video image or using the CCS-Scanner, go to a point within your measurement, click on Goto Surface, keep the current Software-z in mind, optimize the focus, calculate the difference between the current Software-z and the value before optimizing the focus, enter this value at Z-Shift and finally check this value at several points.
10. Click at Start Large Area Scan or Start Image Scan to start the measurement.
11. You can observe the progress of the Signal Stabilization in the messages window. (Figure 3) If it fails at one time, it will try again at each following stabilization step. (Pointing with the mouse at the message gets you a hint, why it did not work.)

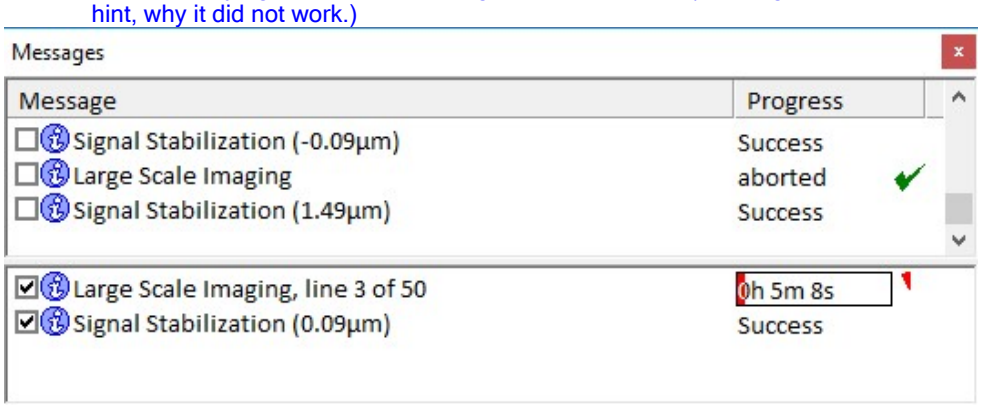


Figure 3: Messages window

Further information:

Signal Stabilization, Large Area Scan, Image Scan

Hints and Troubleshooting

Preconditions for choosing a reference point:

a. Enough Raman intensity under measurement conditions (Stabilization uses the same integration time as the measurement)
b. The area around the reference point should be flat (otherwise slight drifts in x-y-plane could affect the focus)
c. The reference point should be stable for the selected laser power.
d. The focus at the reference point should drift in the same way like the sample.
e. It is possible to choose a reference point within your measurement area, but this could cause a modification of the sample at this point, i.e. photobleaching.
f. Multilayer material could disturb the stabilization. Select a spectral range which is unique for the layer you want to use for stabilization. Maybe check it by doing a depth scan at the reference point.

If you followed the procedure, but the signal stabilization is still not working, check the following points:

1. Move the mouse to the message in the message window and read the hint.

2. Did you select the right objective?
3. Did you select an appropriate spectral range?
4. Have you burned your reference point?
5. Maybe the spectral quality is too bad. Check the signal to noise ratio while testing the stabilization. Try a higher**Number of Accumulations**, a longer integration time for the measurement or use a better Raman scatterer as reference point.
6. Are there unique bands for the layer you want to use for stabilization, in case of a multilayer material?
7. Have you focused to the maximum signal at the reference point for peak mode or to the half of the maximum for edge mode with User-z (**U:**) selected?
8. Make a depth scan at the reference point and check the profile whether you selected the right **Stabilization Mode**. (compare with this figure)
9. Have a look at the signal intensity while you are testing the stabilization. For peak mode it should go down to the half of the intensity. If the signal does not reduce that much due to a bad z-resolution (i.e. laser with high wavelength or 100 µm fiber), try a higher **Step Size Multiplier** i.e. 1.2. If the signal reduces more than this (i.e. 355 nm laser), try a lower **Step Size Multiplier** i.e. 0.9.
10. The Signal Stabilization could only work for slow drifts, if the sample surface is moving too fast, it will not work. TrueSurface Mark 3 could be a solution in this case.

If you want to stop the Signal Stabilization during the measurement change **Stabilization Enable** to No.

EMCCD Camera

This section gives a brief introduction and some background information spectroscopic electron multiplying CCD (EMCCD) cameras. The key advantage of using an electron multiplying CCD (EMCCD) camera is the improvement in the signal to noise ratio for fast measurements. Therefore, the sources of noise within the CCD camera are analyzed first in the section below, before the EMCCD technology and its advantages in the signal-to-noise ratio (S/N) are introduced. Following this, the examination of a contaminated PMMA sample on a glass slide will be presented as an example to illustrate the performance of the system in combination with the EMCCD camera.

System requirements:

- EMCCD camera (Newton)

Background: Sources of noise within a CCD camera

The back-illuminated CCD cameras typically used in confocal Raman setups have a quantum efficiency of more than 90 %. Therefore, most of the photons arriving at the detector are converted into electrons. This means that when optimizing the S/N, the signal can hardly be improved further and thus the noise needs to be reduced in order to produce an improvement.

The photons detected by the CCD camera underlie the Poisson statistic. For any given signal, the noise associated with it will be the square root of the signal itself. This noise is called the photon shot noise. For a signal of 100 electrons, the detection uncertainty is 10 electrons and therefore the maximum S/N is 10. Other sources of noise are mainly thermal noise (dark noise) and readout noise. As the shot noise sets the upper S/N limit, the goal must be to minimize all other sources of noise.

Thermally generated carriers within the CCD chip are responsible for the dark noise. These can be reduced through efficient cooling and high quality CCD cameras will have a thermal dark current below 0.01 electrons/pixel/s at -60 °C. This cooling is sufficient for integration times of several seconds and becomes irrelevant in confocal Raman imaging where typical integration times are below 100 ms.

The electrons generated through the photons are converted to digital counts by the readout amplifier. This conversion process is the source of the readout noise. It is directly linked to the quality of the readout amplifier and the readout speed used. Typical values are a noise of 5-10 electrons for a 50 kHz readout rate and about 30 electrons at 2.5 MHz.

The aim of any spectroscopic experiment is to achieve a shot noise-limited signal. This is the case if the shot noise is the dominant source of noise. If single spectra are recorded, this can simply be achieved by increasing the integration time. However, as stated earlier, in confocal Raman imaging, short integration times and thus fast readout of the detected data are essential. Unfortunately, the faster the readout of the detector is, the higher the readout noise of the amplifier becomes. A 1024x128 pixel CCD equipped with a 50 kHz readout amplifier can be read out in about 22 ms, which is also the shortest possible integration time. If a readout noise of 10 electrons is assumed, every signal below

100 electrons/pixel will be readout limited (Poisson noise ≥ readout noise). If a fast readout amplifier is used which displays a readout noise of 30 electrons, even a signal of 900 electrons (1000 photons/pixel on the detector) will be readout limited.

S/N improvements using an EMCCD camera

An EMCCD is a normal CCD with an additional readout register which is driven with a much higher clock voltage than a normal CCD readout register. Due to this high clock voltage, an electron multiplication through impact ionization is achieved with an adjustable total amplification of the signal of up to 1000 times. Since this amplification occurs before the readout amplifier, it is always possible to amplify the signal above the readout noise. The S/N ratio is then always limited by the Poisson noise of the signal, even if a very fast readout amplifier is used. As an example, a 1600×200 pixel EMCCD with a 2.5 MHz readout amplifier can be read out in about 2 ms.

The following calculations show the improvement in S/N that can be expected for different signals. It is assumed that the quantum efficiency (QE) of the CCD is 90 % and that a 2.5 MHz readout amplifier is used. This amplifier has an associated readout noise of 30 electrons. The A/D conversion is generally set by the manufacturer so that this noise corresponds to 1 A/D count (30 electrons = 1 A/D count).

If 100 photons reach the detector in standard CCD mode, 90 will be registered which equals 3 A/D counts. The Poisson noise will be 9.5 electrons or 0.3 A/D counts and the readout noise 1 A/D count. Therefore the S/N will be about 2.9.

For an EMCCD, the signal is amplified before it reaches the A/D converter. While this amplification is variable, the maximum amplification of 1000 is used for illustration in this example. In this case, the 90 electrons detected will be amplified to 90,000 electrons, resulting in 3000 A/D counts. The Poisson noise will be amplified to 9500 electrons, which translates to 317 A/D counts. The electron multiplication process adds another noise factor of 1.4 (the so-called excess noise factor). Thus, the total noise including this factor and the 1 A/D count due to the A/D conversion is 443 A/D counts. Therefore, the S/N for the EMCCD in this mode is 6.8, which is an improvement of a factor of 2.4 compared to the “normal” CCD camera.

This improvement depends strongly on the amount of photons hitting the CCD camera and is much more prominent for small signals than for large signals. For higher signals, in which the signal intensity is no longer readout limited, the excess noise factor of the EM process reduces the S/N ratio of an EMCCD to below that of a normal CCD. In this case, the EM register can be switched off and then the “normal” readout register is used. Thus, the EMCCD behaves just as a standard back-illuminated CCD.

Figure 1 shows the dependence of the S/N of a “normal” CCD camera as well as that of the EMCCD camera on the signal reaching the detector. Additionally, the ratio of these two S/N ratios is shown.

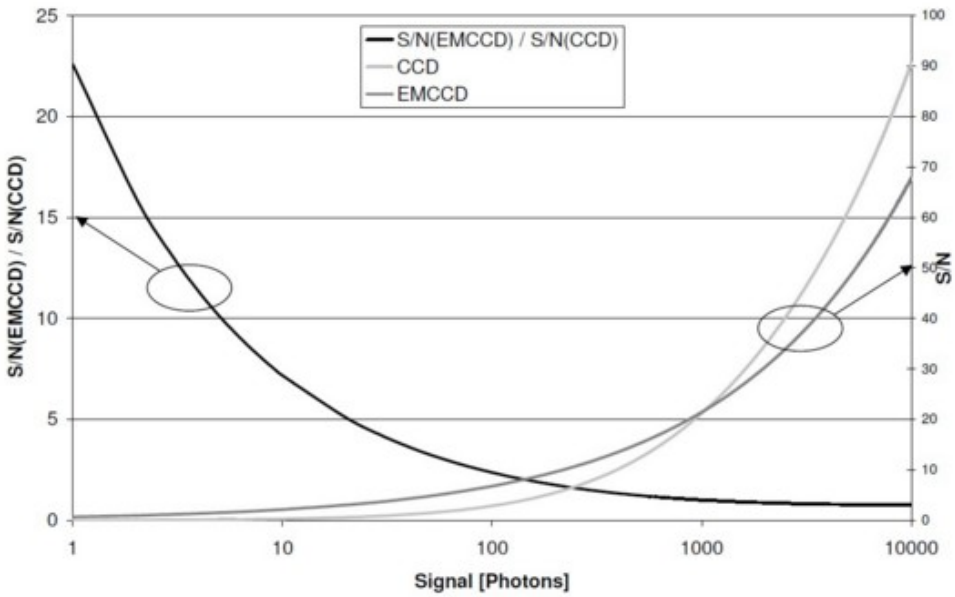


Figure 1: The theoretical S/N for a “normal” CCD camera and an EMCCD camera as a function of the signal reaching the detector. The ratio of the two S/N ratios is shown as well.

It can be seen that the advantage of the EMCCD camera clearly lies in the region where only a faint signal can be detected. Above approximately 1100 photons reaching the detector, the “normal” CCD camera mode is preferable. Due to the fact that the EMCCD camera with a gain of 1000 would be saturated at about 1900 photons, its operation up to about 1000 photons is not a problem. For small signals, as are present in ultra fast confocal Raman imaging measurements, the S/N of the EMCCD camera can be as much as 20 times higher than a “normal” CCD camera.

Experimental Setup and Results

The sample for the tests presented in the following was an ultra-thin poly(methyl methacylate) (PMMA) film spin-coated onto a glass substrate. The layer was scratched and the PMMA removed in parts of the sample (in the center of the figures below). Here the height of the sample was determined by AFM to be 7.1 nm. Additionally, a needle-shaped contamination was found on the surface with a thickness of 4.2 nm. The origin and material composition of this contamination layer was not known initially, but could be determined by the confocal measurements.

The results presented in Figure 2 were obtained using a WITec system equipped with a UHTS 300 spectrometer and an EMCCD detector. The images were obtained by acquiring 200×200 Raman spectra in a 50×50 µm scan range and integrating the intensity of the CH2 stretching band of PMMA at around 3000 cm-1. Excitation power was 20 mW at 532 nm using a 100x, NA=0.9 objective.

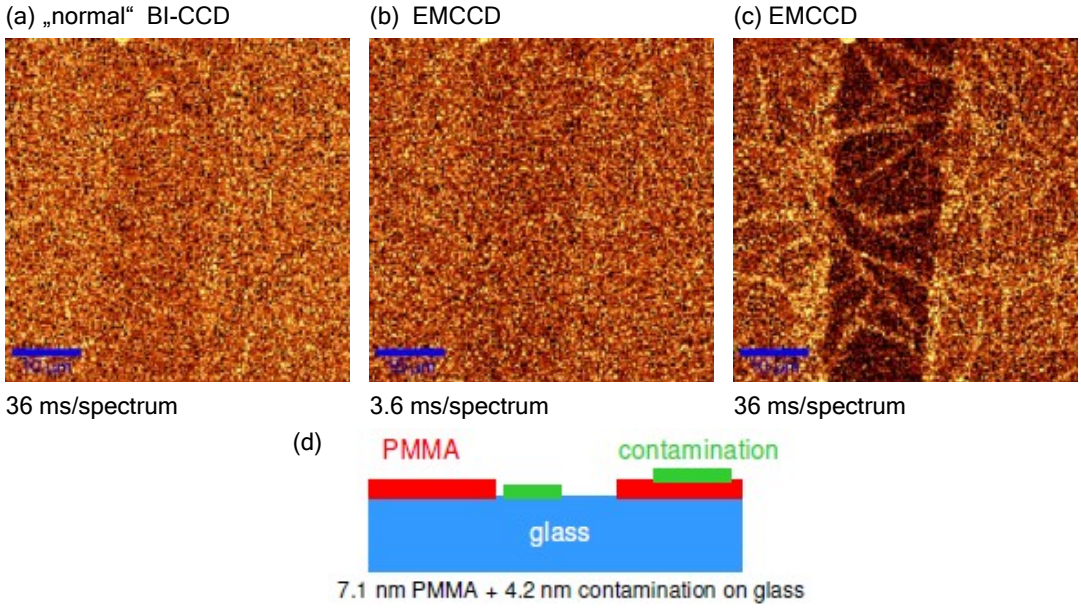


Figure 2: Confocal Raman Images of a 7.1 nm PMMA layer on glass obtained in the CH_2 stretching band around 2950 cm^{-1}: (a) back-illuminated CCD, (b,c) EMCCD. Scale bar: 10µm. (d) Schematic of the sample.

Figure 2 (a) was obtained with a standard back-illuminated (BI) CCD using a 62 kHz readout amplifier and 36 ms integration time per spectrum. With a little imagination, the scratch in the center of the image is just visible, but the S/N ratio is much smaller than 1. Figure 2 (b) shows the same part of the sample imaged with an EMCCD with a gain of about 250. The image shows nearly the same S/N, but now the integration time was only 3.6 ms, 10 times faster than in Figure 2 (a). The complete image acquisition took 25 minutes for Figure 2 (a), but only 3.4 minutes for Figure 2 (b). Figure 2 (c) was taken with the EMCCD, though now with the same integration time as in Figure 2 (a). One can not only clearly see the scratch, but also the contamination in the form of a needle-like structure across the PMMA and glass surface. Figure 2 (d) shows a sketch of the sample.

Further information:

Spec Camera

Further Reading

1. T. Dieing, O. Hollricher, High-resolution, high-speed confocal Raman imaging. Vibrational Spectroscopy 48, 22-27 (2008). doi:10.1016/j.vibspec.2008.03.004

InGaAs Camera

For recording spectra in the NIR, InGaAs array detectors are the best choice. Dependent on the ratio of the elements the bandgap of the material can be shifted. Usually sensors are sensitive up to 1.7 µm or with extended range up to 2.2 µm. Silicon-based sensors have a bad sensitivity in the NIR region especially above 1000 nm.

A significant difference between a silicon-based CCD and an InGaAs array is how the readout is accomplished. In a CCD the electric charge of each line is shifted to a readout register. From there it is shifted pixel by pixel to an amplifier and an A/D converter. The resulting data has a high homogeneity because each pixel is treated in the same way. InGaAs arrays are a kind of active-pixel sensor (APS) like CMOS sensors where each pixel has its own electronics. Some arrays also treat even and odd pixels with different electronics. This results in some effects that has

to be taken into account:

1. Each pixel has a different offset.
2. Each pixel has a different gain.
3. The gain changes with intensity and is also dependent on the position where the light hits the pixel.
4. The dark current is very high.
5. Each pixel has a different dark current rate.
6. Gain, offset and dark current depend on detector and scene (spectrograph) temperature.

Therefore a correction of the raw data is necessary. Three routines are available in WITec Control.

At least offset and dark current correction are highly recommended.

Offset Calibration

The offset of each pixel is determined using shortest integration time and without light falling on the detector. The recorded average spectrum is then subtracted from all measured spectra and an offset of 2000 is added (see Fig. 1).

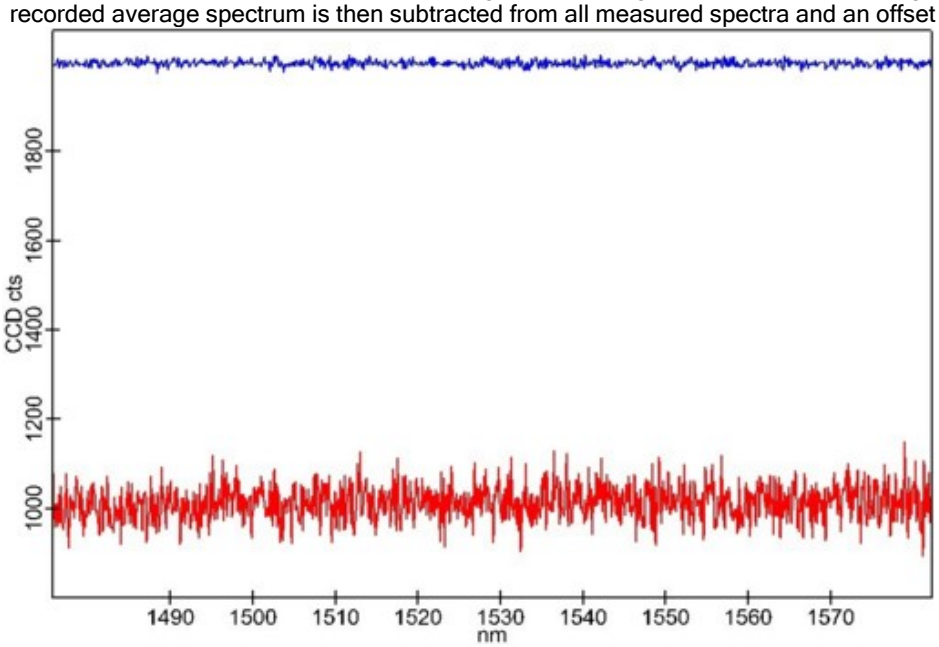


Fig. 1: Single spectra without (red) and with (blue) offset correction.

Intensity Correction

In order to calibrate the gain of each pixel, a spectrum with a smooth distribution is needed (see Fig. 2).

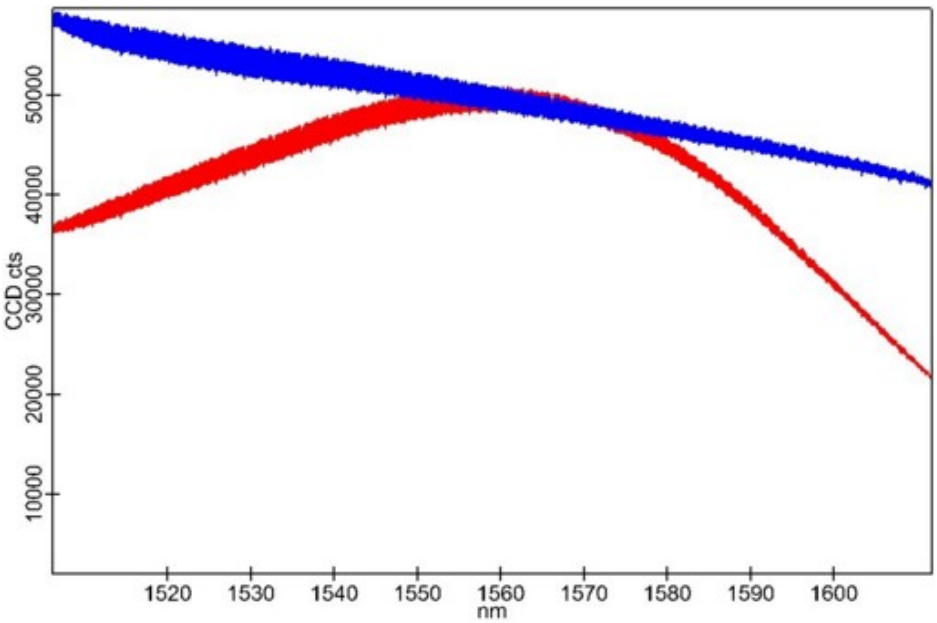


Fig. 2: Spectra of a LED (red) and tungsten halogen lamp (blue) with 600 g/mm@1560nm grating.

For the intensity correction a grating with a high groove density (e.g. 600 g/mm@1560nm) in combination with a tungsten halogen lamp with big filament is recommended to get a smooth spectrum. Using a broader spectral range the spectrum is not smooth due to light absorption of water and other gases and cannot be used for the gain intensity calibration. Using a lamp with a small tungsten filament can produce oscillations in the spectrum.

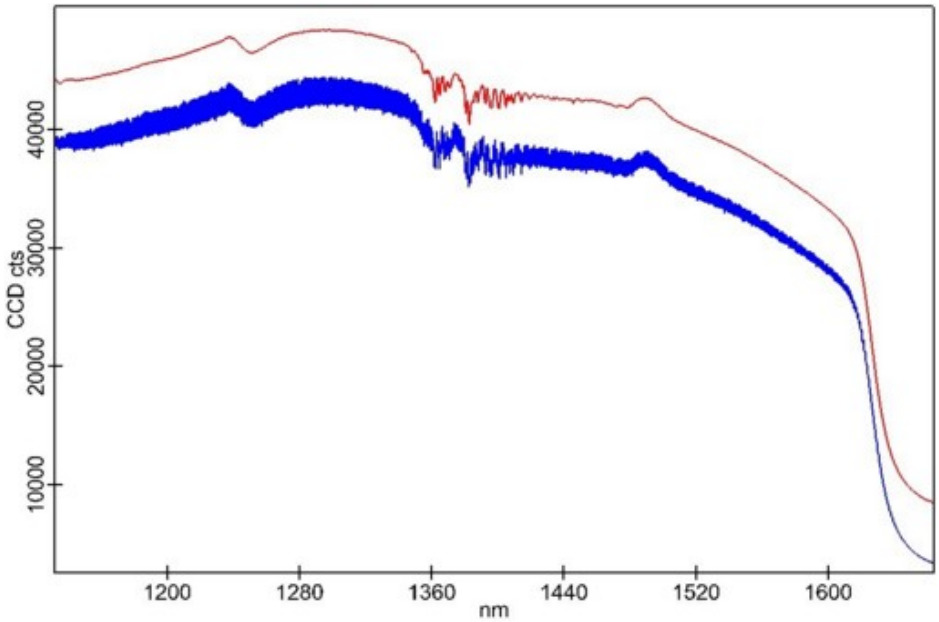


Fig. 3: Spectrum of a tungsten halogen lamp with (red) and without (blue) intensity calibration

Dark Current Correction

Background signal is coming from dark current of the detector as well as from the temperature radiation of the surrounding (scene). If the detector and the scene temperature is constant, each pixel has an individual but constant dark signal rate. The measured signal is proportional to the integration time.

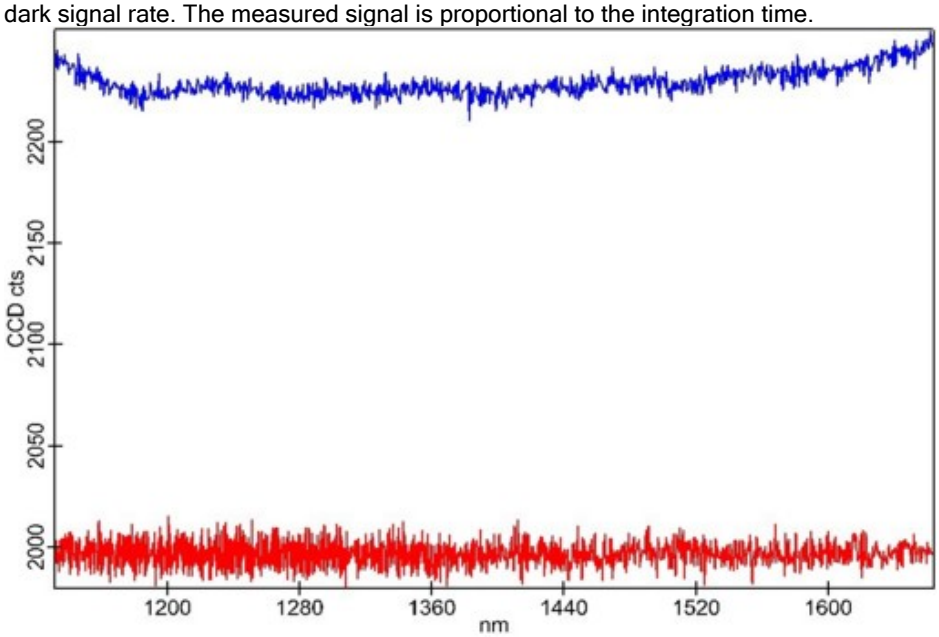


Fig. 4: Single spectra without (blue) and with (red) dark current correction (0.5 s integration time).

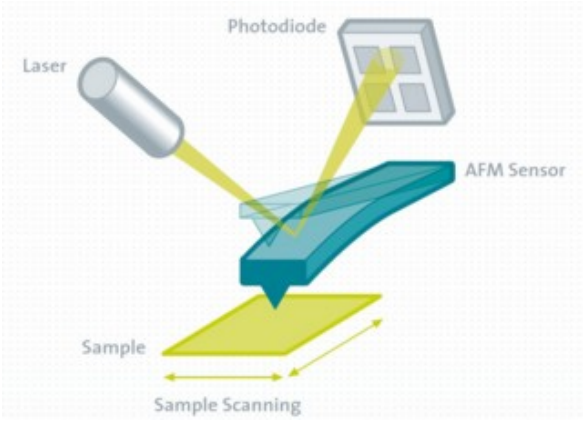
Spectral Stitching

Spectral stitching can be done, but artifacts in the stitched spectrum are possible.

Further information:

Spec Camera

AFM Overview



Atomic force microscopy (AFM) is a type of scanning probe microscopy (SPM), where the sample is scanned mechanically on the nanometer scale. Besides the sample topography additional properties can be determined with special AFM modes.

Topics:

- Introduction
- Setting up a measurement
- Hints

AFM modes:

- Contact Mode - standard AFM mode with constant force
- AC Mode (Tapping Mode) - for soft samples, material contrast by phase image
- DPFM (Digital Pulsed Force Mode) - provides information about adhesion and stiffness
- AC Lift Mode - for MFM or EFM, magnetic or electric forces can be observed
- KPFM (Kelvin Probe Force Microscopy) - work function is observed (surface potential)
- PRFM (Piezoresponse Force Microscopy) - image domains of piezoelectric or ferroelectric materials
- Raman-AFM - Contact or AC Mode combined with Raman

System requirements:

- AFM (A, RA, RAS and AS systems)

Introduction

Since the invention of atomic force microscopy (AFM) in 1986 by Binning, Quate and Gerber [G.Binning, C.F.Quate and Ch.Gerber; Phys.Rev.Lett. 56, 930 (1986)], AFM has rapidly developed into a powerful and invaluable surface analysis technique on micro- and nanoscales and even on atomic and molecular scales. Using AFM, it is possible to image surfaces in real-space with a resolution down to the level of molecular structures. In addition to the imaging of small topographic features on surfaces, AFM is also used to image additional surface properties such as adhesion, stiffness, magnetic properties, conductivity and many more.
Over the past decades, a large number of additional AFM imaging modes were developed for a variety of applications. AFM can be used on any kind of sample. Therefore the number of publications in materials science, life science, and related industries has increased tremendously since its invention.

Setting up a measurement

Sample mounting and focusing

1. Select the appropriate AFM configuration.
2. Make sure the magnetically fixed cantilever arm is removed.
3. **Focus on the sample** using the AFM objective and search for the region of interest.
4. Optional: Set the Microscope-Z user position to zero for your reference.

Cantilever mounting

5. Move the microscope up at least 1500 µm.
6. Attach a cantilever (glued on a washer) to the cantilever arm and mount it to the inertial drive. (If necessary: Connect the cable of the cantilever arm.)
7. Position the cantilever manually by gently moving the bottom part of the inertial drive in X, Y and Z until the

cantilever is somewhat visible in the video image.

8. Move the microscope down not closer than 100 µm above the sample surface. (The cantilever must be within 2000 µm above the sample for the automatic tip-approach.)

Adjustment

9. Click on Start Adjustment in the section Adjustment and follow the steps displayed in the **Message window**:
   a. Position the cantilever under the beam like in Fig. 1:
      - The Cantilever Control is opened automatically.
      - Activate Show Cantilever Position and put the cross to the assumed tip position by selecting Set Probe position in the Menu. Acknowledge the compensation.
      - If the beam is not visible the blue circle marks its position. Maybe the illumination needs to be reduced in order to see the beam.
      - if the cantilever is tilted more than approximately 10°, the cantilever should be re-mounted.
   b. Center the beam spot on the four quadrant diode:
      - Use the T-B and L-R adjustment screws at the beam deflection unit.
      - Observe the changes on the green spot in the AFM Status.
      - If the spot is on one edge, observe the Sum signal (bar on the right). It will increase if the screw is turned in the correct direction.
   c. Further adjustment is explained for the respective AFM mode.
   d. Check the P-Gain and I-Gain. (5 is a good starting value for both.)

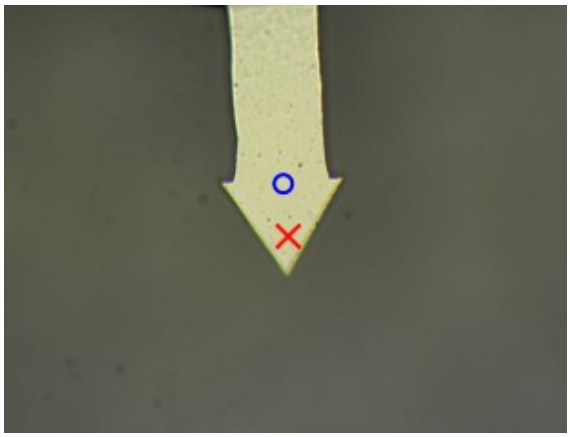


Fig. 1: Cantilever in the video image at appropriate position.

Approach and measurement

10. Click on Start Approach.
11. Start the measurement.

Further information:

Adjustment, **Feedback settings**, **Tip approach**

Hints

- If you hear a noise from the stage during contact, the feedback is too aggressive. In this case, reduce the I and P-Gain until the noise stops.

Contact mode Overview

In this imaging mode, the tip is always in contact with the surface under a constant force.

Topics:

- Theoretical Background
- Setting up a measurement

Measurement modes:

- Image Scan: acquisition of AFM contact mode images
- Line Scan: acquisition of force-distance curves along a line
- Distance Curve: acquisition of force-distance curves at the current position

Recommended Cantilevers:

- Contact Mode cantilevers with a spring constant of k = 0.2 N/m (at least below 1 N/m) are recommended.

For samples that are either soft or weakly bound to the substrate, AC mode, which operates in the intermittent contact regime, provides better results. Because in contact mode lateral forces might lead to the dragging of particles weakly bound to the substrate, thus resulting in blurred images.

Theory

The operating principle of an AFM is rather simple. A probe is scanned over a sample and various interactions between tip and sample are used as feedback mechanisms to trace the surface topography (Fig. 1).

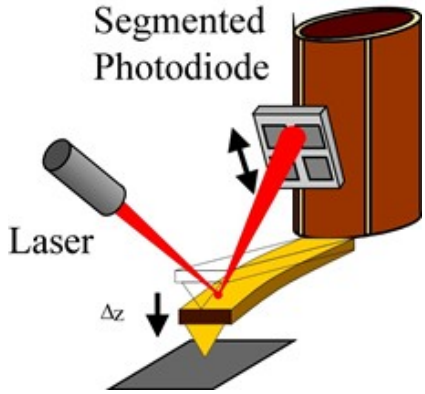


Fig. 1: Principle of AFM operation.

At the free end of a cantilever (typically 100 to 200 µm long) a sharp tip (less than 10 nm across) is mounted. This tip is brought into contact with the sample while the repulsive force between tip and sample bends the cantilever. The bending of the cantilever is measured using a highly focused beam deflection system as shown in Fig. 1. By keeping the bending of the cantilever constant, a constant force is applied to the sample while scanning the tip across the surface. The WITec microscopes are sample scanning systems, where the cantilever remains at a constant position and the sample is scanned precisely underneath it. This setup has the advantage of fixed optical beam-paths, which eliminates the requirement of tracing the beam deflection system. The vertical movement of the scanner follows the surface profile and is recorded as the surface topography.

Several forces typically contribute to the bending of the AFM cantilever. The force most commonly associated with scanning force microscopy is the interatomic van der Waals force. The dependence of the van der Waals force upon the distance between tip and sample is described by a Lennard Jones potential V, which can be written as

$$V_{sample}(z) = Az^{-12} - Bz^{-6}$$

.

Here z denotes the tip sample distance and A and B are interaction parameters. As the tip is approaching the surface, attractive forces act between the tip and sample before repulsive forces start to dominate. In an AFM, the tip is attached to a flexible cantilever which is subject to Hook's law

$$V_{canitlever}(z) = k\frac{(z - z_0)^2}{2}$$

,

where k is the spring constant of the cantilever and z0 is the tip-sample distance for an unbent cantilever. An example of a resulting force distance curve of this coupled system is shown in Fig. 2.

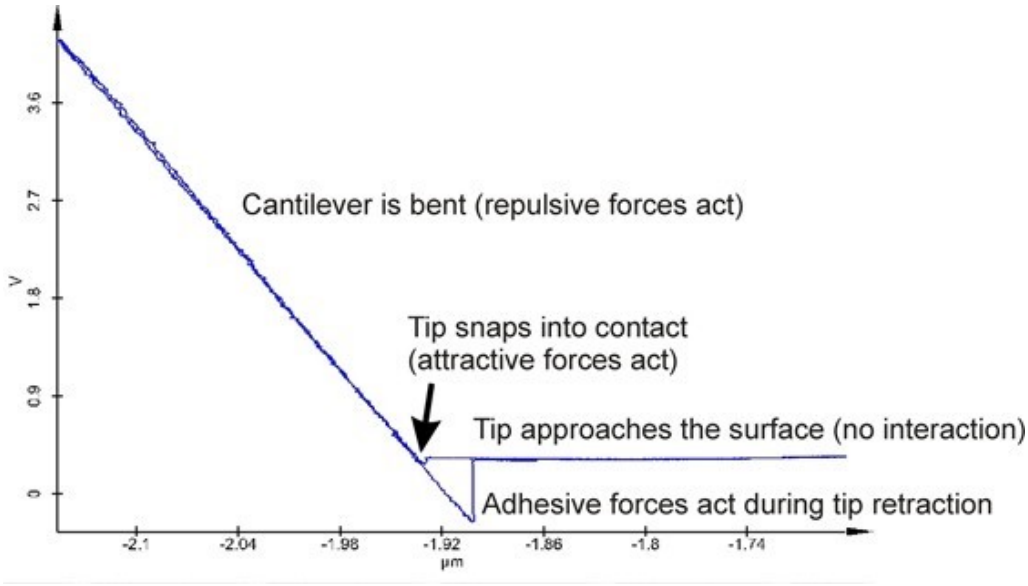


Fig. 2: Force distance curve in an AFM measurement.

These curves contain all tip-sample interactions, enabling the mapping of material properties on the nanometer scale. Different mechanical properties of the surface can be evaluated from the friction images. In this case the torsion of the cantilever is recorded. The operating principle of friction mode is shown in Fig. 3.

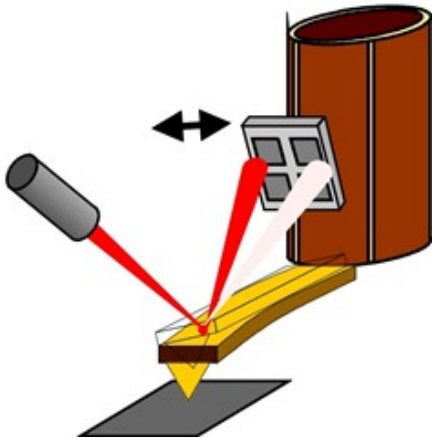


Fig. 3: Principle of AFM friction measurements.

Parameters

Follow the steps in the general procedure. Further adjustment (10. c) for Contact mode is explained in the following:

Setpoint

The recommended value for the Setpoint is 0.5 V.

The feedback parameter in AFM contact mode is the bending of the cantilever, which ensures a constant force between tip and sample. The up and down movement of the scan stage is recorded as surface topography. Any deviation from the constant bending of the cantilever, measured on the photo-detector, is represented in the deflection image. These images highlight the edges of various topographic levels.

High setpoint values correspond to high tip-sample interaction forces.

The force distance curve shown in Fig. 1 displays the initial T-B value and the range within which the setpoint should be selected for contact mode measurements. The feedback loop maintains a constant bending of the cantilever at the selected setpoint.

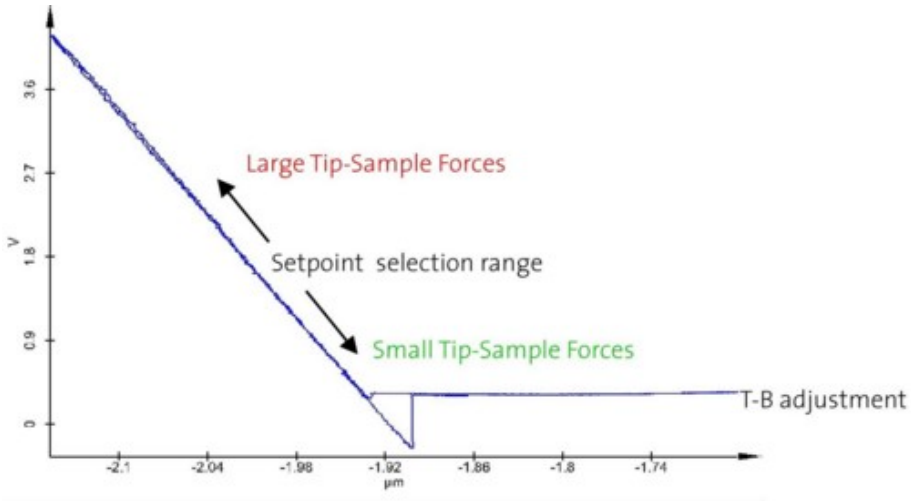


Fig. 1: Typical force-distance curve showing the setpoint selection for AFM contact mode measurements.

Recorded Channels

Image Scan

Each data object in a AFM image scan is created twice marked with [F] for data aquired during the forward movement and [B] for data aquired during the backward movement.

Distance Curve

Opening the recorded data channels will show them separately. To get the view shown when recorded use the parametric view.

Further information:
Feedback settings, Data channels (T-B, L-R, Topography, Feedback)

AC mode Overview

In Acoustic (AC) mode, also known as Tapping mode, the cantilever is oscillated at its resonant frequency which results in an intermittent contact mode.

Topics:

- Theoretical Background
- Setting up a measurement

Measurement modes:

- Image Scan: acquisition of AFM AC mode images,
- Line Scan: acquisition of amplitude- & phase-distance curves along a line,
- Distance Curve: acquisition of amplitude- & phase-distance curves at the current position.

Recommended Cantilevers:

- Force Modulation (FM) cantilevers with resonance frequencies in the range of 65−85 kHz and a spring constant of k = 2.8 N/m. These cantilever are recommended for the imaging of delicate samples where small tip sample interaction forces are required.
- Non-Contact (NC) cantilevers with resonance frequencies of 230−300 kHz and spring constants of k = 42 N/m, are recommended for very soft samples (e.g. gels, plastic foils) and for samples in which the phase contrast within the material is the highest priority.

Theory

In Acoustic (AC) mode, also known as Tapping mode, the cantilever is oscillated at its resonant frequency with a free amplitude **A0**. While the cantilever is approaching the surface, the oscillating amplitude is damped to a value **A**, which depends on the distance to the surface (see also blue curve in Fig. 1). The ratio

$$r = \frac{A}{A_0}$$

defines the damping of the amplitude while the tip is in contact with the surface and is proportional to the applied force. By keeping the damping of the amplitude constant, the surface topography can be imaged. The interaction between the tip and the sample is predominately vertical, though negligible lateral forces are encountered. Consequently, AC mode AFM does not suffer from the tip or sample degradation effects that are observed in contact mode AFM, and is therefore a suitable technique for imaging soft samples.
Phase images can be recorded simultaneously along with the surface topography. In this image, the phase shift between the free oscillation and the oscillation while the tip is in contact with the surface is recorded. Since the phase shift depends on the viscoelastic properties of the sample as well as on the adhesive potential between the sample and the tip, the phase image outlines domains of varying material properties without describing the nature of the properties themselves. Nevertheless, phase images are often used to characterize soft samples at high resolution.

Parameters

Follow the steps in the general procedure. Further adjustment (10. c) for AC mode is explained in the following:

1. Enter the driving amplitude (Driving Amp. pk-pk [V]) in the in the frequency sweep section.

A typical starting value is 0.05 V (FM) to 1 V (NC) depending on the type of the cantilever. For golden KPFM cantilever arms use 1 V for FM cantilevers.

2. Auto-Resonance is performed. Check if the automatically selected resonance frequency is within the cantilever range. If this is not the case check the hints section.
3. Adjust the free cantilever oscillation amplitude displayed in the status section by increasing or decreasing the Driving Amp. pk-pk [V].

Cantilever oscillation amplitude: FM cantilever 1-2 V and NC cantilever 1-1.5 V.

4. Optional: Lower the starting value for the setpoint (damped amplitude) to about 75 % of the free cantilever oscillation amplitude.

The setpoint is automatically reduced during approach.

Setpoint

The setpoint in this imaging mode is the damped amplitude **A** (equation) and should be selected at a lower value than the free amplitude **A0**. The amplitude distance curve shown in Fig. 1 illustrates the correlation between the free amplitude and the range within which the setpoint should be selected in an AC mode measurement.
Low setpoint values correspond to high tip-sample interaction forces.
When cantilevers with relatively low spring constants are used for AC Mode imaging (e.g. 2.8 N/m), the free amplitude of the cantilever decreases slightly before the tip touches the surface due to the air compression between the lever and the surface as shown in Fig. 1.

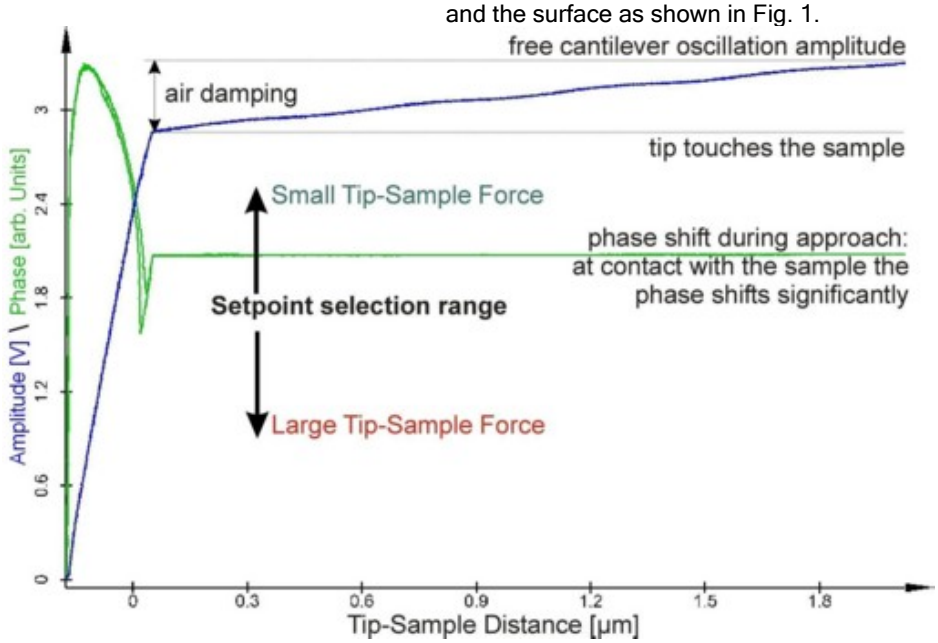


Fig. 1: Phase (green) and Amplitude (blue) as a function of tip-sample distance. The effect of the setpoint selection is also indicated.

Further information:
Feedback settings, Frequency sweep, Data channels (Lock-in R, Lock-in Phi, Topography, Feedback)

Hints

- A driving frequency slightly below the resonance frequency of the cantilever can improve the sensitivity of the measured phase. The damping lowers the resonance frequency when the tip is in contact. Therefore a lower driving frequency at about 30° depending on the sample using i.e. the Listen option after the Auto-Resonance.

If the resonance frequency of the cantilever is not found correctly during Auto-Resonance, check the following points:

- The cantilever is not properly fixed to the cantilever holder, frequencies other than the cantilever resonance frequency might then be more pronounced and therefore automatically selected. In this case, remove the cantilever from the holder, check for dirt particles on the cantilever holder or the cantilever-washer and repeat the step by step adjustment procedure.
- Perform a manual resonance sweep by selecting the start and end frequencies in the frequency sweep section of the Control Window. The selected range should include ±50 kHz of the cantilever resonance frequency. Once the sweep is completed and the resonance curve is displayed in the graph window, set the driving frequency to the resonance frequency found. (The numerical value of the resonance frequency can either be manually entered or the listen option can be used to select the value in the graph window by mouse.)

DPFM Overview

The Digital Pulsed Force Mode (DPFM) is a non-resonant, intermediate contact mode which extends the capabilities to aquire additional surface properties such as local stiffness and adhesion with high lateral resolution.

Topics:

- Theoretical Background
- Setting up a measurement
- Data evaluation

Measurement modes:

- Image Scan: acquisition of AFM DPFM mode images.

Required License feature:

- PFM Mode

Recommended Cantilevers:

- Contact Mode cantilevers with a low spring constant of k = 0.2 N/m are recommended for imaging soft polymers and biological samples which are not sticky. The contrast in the stiffness image is enhanced using these cantilevers though the user must ensure that the cantilever still snaps out of contact.
- Force Modulation (FM) cantilevers with a high spring constant of k = 2.8 N/m are recommended for the imaging of sticky samples. These cantilevers are stiff enough to overcome the higher adhesion forces of these samples at the cost of some of the contrast achievable in the stiffness image.

Chemically modified cantilever may improve the material contrast and enhance tip-sample interactions.

Theory

The Digital Pulsed Force Mode (DPFM) is a non-resonant, intermediate contact mode which extends the capabilities of an AFM beyond simply measuring topography. It allows the acquisition of additional surface properties such as local stiffness and adhesion with high lateral resolution at normal scan-speeds. It also avoids surface damage due to lateral shear forces and a controlled normal force is used as the feedback signal. In this imaging mode, the alphaControl introduces a sinusoidal modulation on the cantilever with an amplitude of between 10−500 nm at a user-selectable frequency. Typical frequencies range from 1−10000 Hz, which is usually well below the resonance frequency of the cantilever. The amplitude of the signal is adjusted so that the tip snaps in and out of contact during each period. Therefore, a complete pulsed force curve as shown in Fig. 1 is measured during each cycle.
The pulsed force curve shown in Fig. 1 displays the same tip-sample interaction features as a force-distance curve. (By using the distance information instead of time as the X-axis, the DPFM curve is actually transformed into a force-distance curve.) During the DPFM modulation cycle, the AFM tip is initially well above the sample surface. Moving closer to the surface, the tip snaps into contact due to the attractive force between the tip and sample surface. As the piezo pushes the tip further toward the sample, the repulsive force reaches a maximum (Fmax). The piezo then pulls back and consequently the repulsive force decreases and the force signal changes sign from repulsive to attractive. Finally, the tip loses contact to the surface when the force on the cantilever is larger than the attractive force between tip and sample (adhesion peak). The subsequent free oscillation of the cantilever around the baseline is damped. The cycle is then restarted.

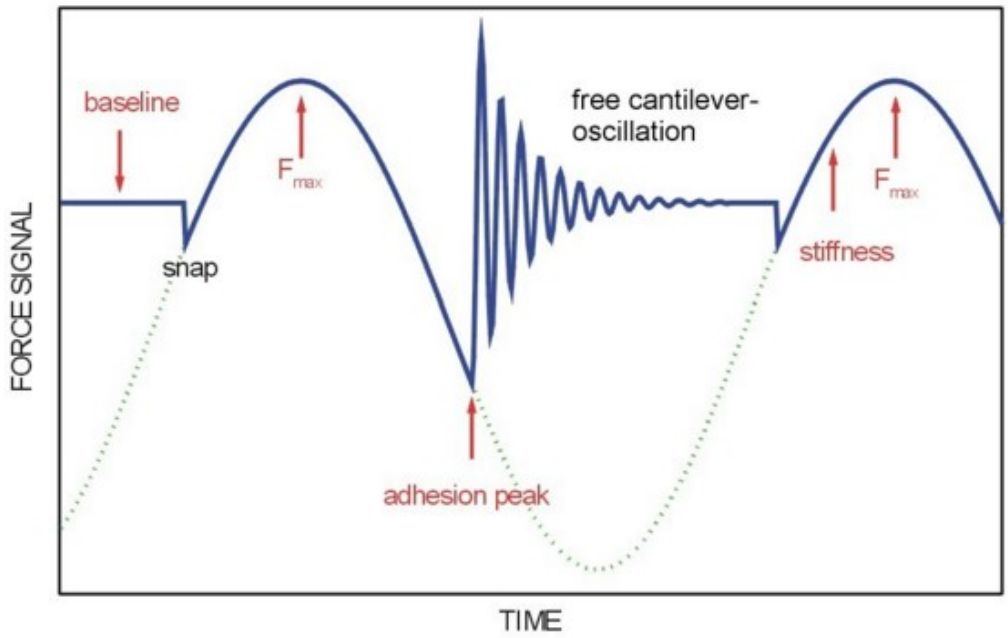


Fig. 1: Schematic DPFM curve with sinusoidal modulation. The parameters of interest are marked.

The high speed control electronics allow the digitization of each curve at a rate of 5 MHz. The force signal of the AFM is monitored in a graph window where it is presented in an oscilloscope-like mode. With this, it is possible to set evaluation areas, from which different features of the force signal are extracted and converted into an image. The online processable features are:

Maximum Force

This value is determined from each pulsed force curve and is used by the alphaControl as the PI controlled channel (see WITec Control manual Section 3.4.5). Therefore, in DPFM operation, the maximum repulsive force acting between tip and sample is kept constant. The value of Fmax is determined by the setpoint setting. The up and down movement of the scan stage to maintain a constant Fmax is recorded as surface topography.

Adhesion

The maximum adhesion force is determined from the adhesion peak of the DPFM curve. This signal forms the adhesion image of the sample.

Stiffness

The gradient of the rising slope of the repulsive force signal (see Fig. 1) is determined during each cycle of the pulsed force curve. This gradient is related to the local stiffness of the sample. On soft parts of the surface, this gradient is smaller than on hard parts. This signal thus forms an image of the sample stiffness.

Parameters

Follow the steps in the general procedure. Further adjustment (10. c) for DPFM is explained in the following:

1. The recommended value for the Setpoint is 0.5 V.
2. Check the P-Gain and I-Gain. (5 is a good starting value for both.)
3. Enter a Driving Amplitude pk-pk [V] of about 2 V (depends on cantilever and adhesion of sample).
4. As initial value for the approach the Fmax window should cover the entire range. Set the Fmax Window Width [°] to 360 and the Fmax Window Start [°] to 0.

Approach

5. Click on Start Approach and wait until it is finished.

DPFM Parameters

The maximum force between tip and sample acts at the maximum of the sinusoidal curve, which is also the point at which the cantilever starts to retract from the sample.

After the approach the graph window will either show a sinusoidal curve as shown in Fig. 1 or a pulsed force curve similar to the one in Fig. 2. In the first case, the modulation of the cantilever is too small to completely detach the cantilever from the sample, refer to step 8.

6. Change the Excitation Phase parameter of the PFM Control until the maximum force is in the first half of the oscilloscope graph window (as shown in Fig. 1).
7. Select the Fmax window as shown in Fig. 1 using the corresponding Listen parameter from the PFM Control mark the range by mouse.

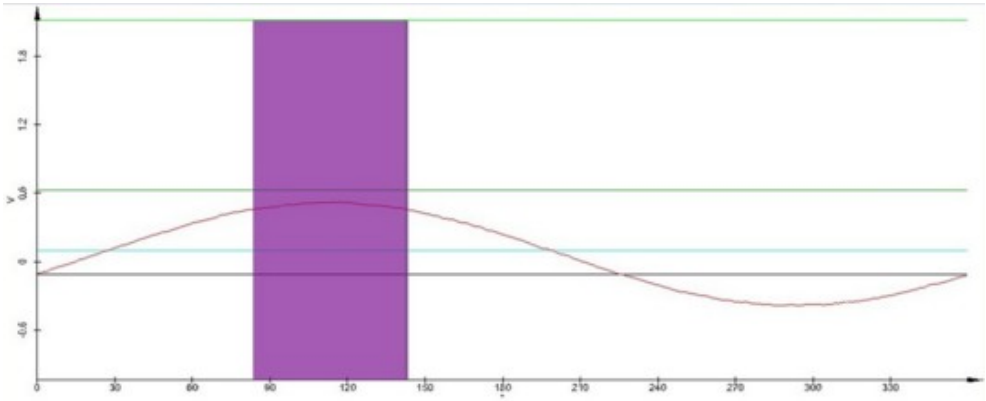


Fig. 1: Sinusoidal curve with Fmax search window

8. Increase the Driving Amplitude pk-pk [V] until a pulsed force curve is displayed in the graph window as shown in Fig. 2.

9. Select the search windows for adhesion and stiffness as shown in Fig. 2 using the corresponding Listen function.

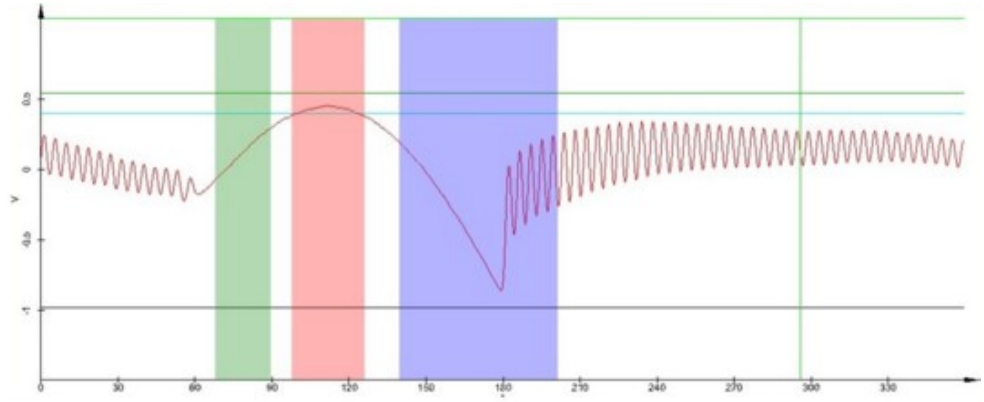


Fig. 2: Pulsed force curve with Fmax (red), Adhesion (blue) and Stiffness (green) search window

Measurement

10. Define the parameters for the Image Scan.
11. Optional: Select Store Data in order to save pulsed force curves in a .wsd file. The user is prompted to enter a file name before the measurement starts.
The size of this additional file can be very large (up to several GB).

12. Click on Start Scan.

Further information:
Feedback settings, PFM Control, Data channels (Adhesion, Stiffness, Topography, Feedback)

Hints

- For high sensitivity in the adhesion image use a low setpoint.
- For high sensitivity in the stiffness image use a high setpoint.

Theory

The aquired data of the DPFM measurement, i.e. the adhesion and stiffness images, have voltage as unit. For a mathematical conversion of the measured volts into physical units, such as N for the adhesion image or N/m for the stiffness image, further information related to the experimental conditions is required. The following section describes the required formulas and parameters to convert the voltage values into the appropriate physical values.
The nature of the interacting forces however is not taken into account. The obtained physical values can nevertheless be used for modelling of the tip sample interface.
Please note that the extraction of quantitative values from DPFM measurements depends strongly on environmental conditions such as temperature and humidity. In addition, no changes of the tip during measurements are taken into account in the procedure described in the following.

Adhesion

A calibration of the adhesion force is possible according to the following formula:

$$Adhesion = V_{adhesion} \cdot k \cdot S \quad (1)$$

with the measured voltage VAdhesion, the spring constant k of the cantilever and the sensitivity S of the beam deflection system.

VAdhesion is the voltage determined by the negative value of the adhesion peak.

The approximate spring constant can be found in the cantilever's datasheet. Another approach is the cantilever spring constant calibration using the Sader method. Further information and an online calculation form can be found under http://www.ampc.ms.unimelb.edu.au/afm/calibration.html. The frequency can be determined by a frequency sweep. The quality factor Q is calculated using the cantilever frequency f and and FWHM of the frequency peak:

$$Q = \frac{f}{FWHM}$$

The sensitivity S can be determined by performing force distance curves on a surface which is stiff in comparison to the cantilever, such as a silicon wafer. The sensitivity is shown as the inverted slope of the force curve in the repulsive area (thus having the units nm/V). The values are highly dependent on the cantilever type (particularly its length) and the alignment of the laser spot on the segmented photo diode. Therefore it is necessary to recalculate S every time the cantilever is changed.

Stiffness

To calibrate the stiffness knowledge about some additional quantities is required. These quantities must be determined before measurements are taken. An important measure in this case is the modulation amplitude M of the tip itself. If a direct observation of that quantity is not possible (by an interference method, for example), the modulation amplitude can be estimated from a linear relation between the applied driving amplitude to the modulation piezo and response amplitude of the photo detector.

For this purpose a preliminary measurement on a hard Si wafer, similar to the determination of the cantilever sensitivity S, is required. By applying a small driving amplitude ads to the modulation piezo while the cantilever is in contact with the Si wafer, a sinusoidal modulation aosc is expected as response from the photo detector. The ratio U of the amplitude of these two modulations should not change as long as the cantilever is not detaching from the sample and can be used to determine the modulation M during DPFM measurements.

$$U = \frac{a_{osc}}{a_{ds}} \quad (2)$$

$$M = U \cdot S \cdot A \quad (3)$$

with A denoting the driving amplitude applied during DPFM imaging and S the sensitivity.

Using the width of the stiffness search window, the angle σ, the penetration depth Δz can be determined:

$$\Delta z = M \cdot \left(1 - \cos\left(\frac{\sigma}{180°} \cdot \pi\right)\right) - V_{stiffness} \cdot S \quad (4)$$

VStiffness is the voltage measured in the stiffness image.

If the local stiffness of the sample is defined as:

$$Stiffness = \frac{Force}{penetration\ depth} \quad (5)$$

then it is:

$$Stiffness = \frac{V_{stiffness} kS}{\Delta z} \quad (6)$$

This relation allows the DPFM data to be calibrated in the same manner as the adhesion. Since the conversion of the stiffness and adhesion data into physical units is repetitious, it can be achieved by using the calculator function of WITec Project.

An example on how to convert the voltages supplied by the DPFM measurements to physical units is described on the next pages.

Further Reading

1. B. Hopp et al., Investigation of incubation in ArF excimer laser irradiated poly(methyl-methacrylate) using pulsed force mode atomic force microscopy. Journal of Applied Physics 96, 5548 (2004).

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13. U. Schmidt, Multimodal Imaging of Polymeric Materials and Coatings. Physics Best, (2013).

Preliminary measurements

Parameters like the sensitivity **S** and the modulation factor **U** have to be determined by additional measurements. Please note that these additional measurements have to be performed with the same cantilever as the DPFM measurements, as well as with the same alignment of the cantilever and of the beam deflection system.
For the example measurement an arrow contact mode cantilever (Nanosensors) was used with a nominal spring constant k:

k = 0.2 N/m

Force Distance Measurements

As described in the theory section, the sensitivity **S** is required for further calculations. This sensitivity describes the calibration of the laser detection system. With the initial alignment of the segmented photo detector, the laser spot is adjusted to the center of the four quadrant diode (maximum sum signal, top minus bottom (T-B) signal to 0 and left minus right (L-R) signal to 0). As soon as the cantilever touches the sample, the cantilever bends and the laser spot is displaced from the center position. This results in a T-B signal different from zero, which increases with increasing bending of the cantilever. In a force-distance curve the bending of the cantilever as a function of the piezo z displacement is recorded. For the calibration of the photo detector it is recommended to record force-distance curves on a stiff sample (e.g. Si wafer or glass cover slip). Under these conditions the elastic properties of the sample can be neglected and the displacement of the laser spot on the photo detector is only a result of the cantilever bending.

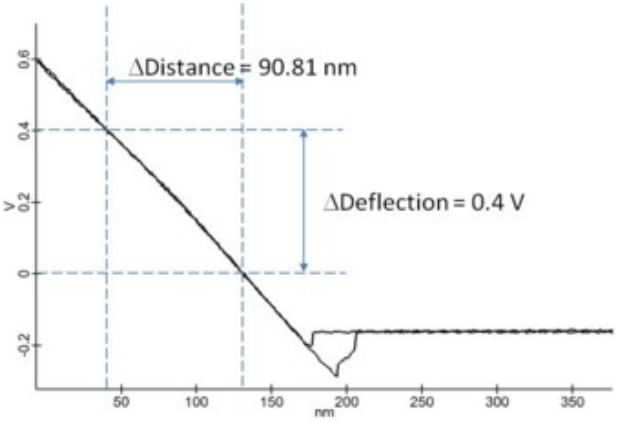


Fig. 1: Force-distance curve measured in contact mode on a Si wafer.

The rising slope of the force distance curve is calculated as:

$$Slope = \frac{\Delta Deflection}{\Delta Distance} \quad (7)$$

For the example shown in Fig. 5 the result is:

$$Slope = \frac{0.4\ V}{90.81\ nm} = 0.0044048\ \frac{V}{nm} \quad (8)$$

With this value it is then possible to convert the voltage change of the photo detector into distances. Multiplying the sensitivity **S** with the measured voltage (T-B signal) converts it into distance:

$$S = \frac{1}{Slope} \quad (9)$$

For the above mentioned measurement, the sensitivity is:

S = 227 nm/V

Modulation factor U

For calculations of the stiffness, the modulation amplitude of the tip is required. If interference methods are not integrated in the AFM used, the modulation factor U can be determined from a preliminary measurement described in the following:

1. Approach the cantilever under DPFM control to a stiff surface (e.g. Si wafer).
2. Select a small driving amplitude **a**ds in the PFM control parameter group such that the tip is always in contact with the surface. On the oscilloscope a sinusoidal modulation signal will be visible (Fig. 2 (a)).
3. Measure the peak-to-peak amplitude of this sinusoidal modulation **a**osc from the oscilloscope display.
4. Increase the modulation amplitude slightly but stay in the range where the cantilever is not detached from the sample and measure the peak to peak value on the oscilloscope again (Fig. 2 (b) and (c)). An example of the curve shape if the applied voltage is too high is shown in Fig. 2 (d).

With equation (2) the modulation factor **U** for the specific experiment can then be calculated.

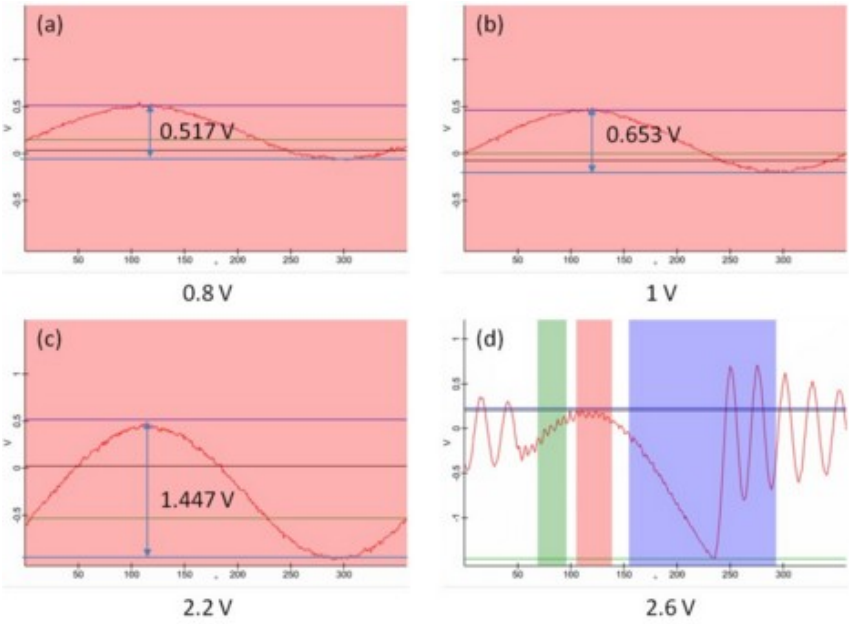


Fig. 2: Illustration of the oscilloscope window while measuring the modulation factor on a Si wafer at various driving amplitudes of (a) 0.8 V, (b) 1 V, (c) 2.2 V and (d) 2.6 V (where the cantilever is already detaching from the sample).

For this example the parameters listed in table 1 apply:

Table 1: Example of measured voltages for the determination of the modulation factor U.

Source	ads [V]	aosc [V]	U
Fig. 6 (a)	0.8	0.517	0.646
Fig. 6 (b)	1.0	0.653	0.659
Fig. 6 (c)	2.2	1.447	0.657
Average Modulation Factor U:			0.654

The force distance curves and the modulation factor U have to be measured again, as soon as the cantilever is changed or the laser spot is readjusted on the cantilever or on the segmented photo detector.

The results obtained from the preliminary experiments and settings are summarized in table 2.

Table 2: Summary of the data required for DPFM data evaluation from preliminary measurements on a Si wafer.

k [N/m]	S [nm/V]	U
0.2	227	0.65

DPFM Images

In the next two sections data evaluation is described on the basis of an example DPFM measurement. The sample used here is a polymer blend consisting of PS (polystyrene), SBR (styrene-butadiene rubber), and EHA (ethyl-hexyl-acrylate). In this section only the acquired images are considered for evaluation. The second section will focus on the evaluation based on the acquired pulsed force curves.

Fig. 1 shows the topography, adhesion and stiffness images as measured using the parameter listed in table 1. A typical pulsed force curve from this dataset shows the window selection for stiffness, Fmax, and adhesion.

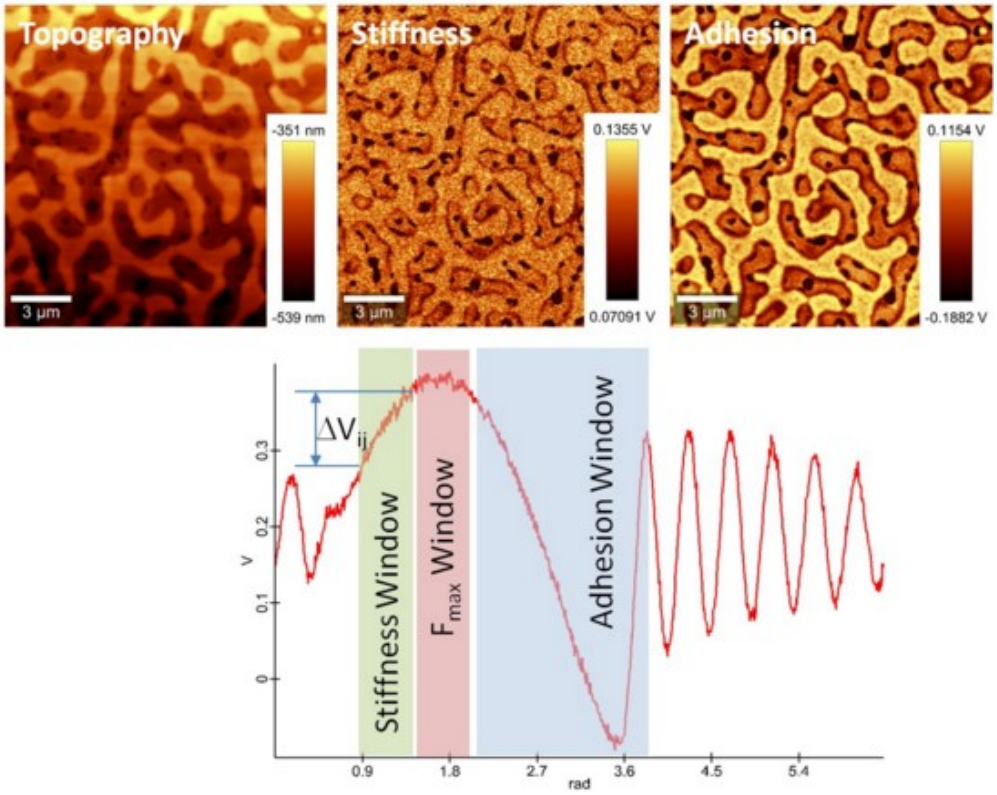


Fig. 1: Results of the DPFM measurement on the polymer blend

Remarks:

- the shown topography, adhesion and stiffness images are raw data with the corresponding z-color scale in nanometer and volts respectively.
- The stiffness image displays the changes in ΔVij as shown in the pulsed-force curve in Fig. 1.
- The adhesion image displays large adhesion in dark color because the adhesion values are negative values in the DPFM curve.
- The DPFM curve is acquired at a certain offset, indicating a small drift in the T-B signal during the approach or the measurements. This is not unusual with soft cantilevers. As a result of this offset, the measured voltages in the adhesion image vary from -0.1882 V to 0.1152 V even though the theory predicts that they should always be negative.

Table 1: Parameters of the example measurement (important information is highlighted in green and red)

Image Scan:	
Points per Line:	256
Lines per Image:	256
Scan Width [µm]:	16.000
Scan Height [µm]:	16.000
Scan Speed [s/Line]:	1.000
Retrace Speed [s/Line]:	1.000
P-I Controller:	
Setpoint [V]:	0.40000001
P-Gain [%]:	6
I-Gain [%]:	8
Controlled Channel:	Fmax
PFM-Control:	
Driving Amplitude [Vpp]:	2.5999997
Driving Frequency [Hz]:	1000
Sampling Rate [Hz]:	999998.31
Excitation Phase [°]:	0
Fmax Window [°]:	(Start = 87.114723, Width = 33.543232)
Fmax Window [µs]:	(Start = 241.99, Width = 93.176)

Adhesion Window [°]:	(Start = 139.38011, Width = 143.92336)
Adhesion Window [µs]:	(Start = 387.17, Width = 399.79)
Stiffness Window [°]:	(Start = 56.406136, Width = 27.873955)
Stiffness Window [µs]:	(Start = 156.68, Width = 77.428)

Adhesion

For the calculation of the adhesion, the adhesion image VAdhesion has to be converted into a force unit. The voltages are represented in the measured adhesion image as color scale, where dark areas correspond to higher adhesion than bright areas.
When determining voltages from an image, ensure that no average or background subtraction was applied to the image.

Using the equation (1), with k = 0.2 N/m and S = 227 nm/V in this specific case, it is possible to calculate the adhesion values measured at each image pixel using the relation (10) in the calculator tool (Fig. 2).

$$Adhesion\ [nN] = 0.2\left[\frac{N}{m}\right] \cdot 227\left[\frac{nm}{V}\right] \cdot -V_{adhesion}\ [V] = 45.4\left[\frac{nN}{V}\right] \cdot -V_{adhesion}\ [V] \qquad (10)$$

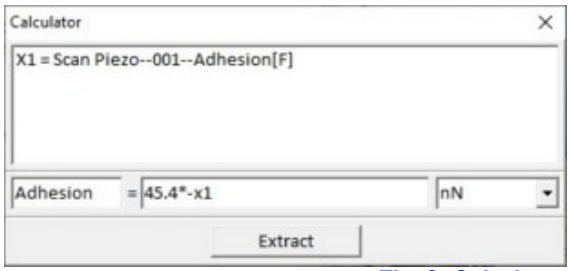


Fig. 2: Calculator tool with equation (10) applied

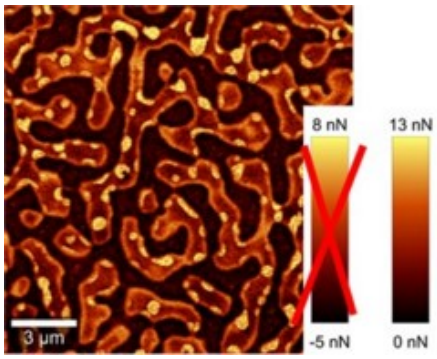


Fig. 3: Adhesion image with converted force units (bright color shows high adhesion)

The result of the calculation varies from negative to positive values, which has no physical meaning. These negative values arise from the offset of the pulsed force curves shown in Fig. 1. In DPFM measurements only relative adhesion forces can be determined and as color scale a leveled color scale can be exported as shown in Fig. 3. The color scale of the adhesion image is now converted into the proper force unit.

Stiffness

The local stiffness is defined by equations (5) and (6), where the nature of forces which contribute to the stiffness measurements are neglected. The resulting local stiffness values obtained from these calculations can be used for i.e. modelling of the tip-sample interface.
The stiffness image recorded in Fig. 1 shows a contrast between the PS and EHA. Again, dark colors correspond to lower stiffness values than bright colors. In order to calculate the stiffness for these two areas, the calculation of the penetration depth Δz is required, which also implies the calculation of the cantilever modulation **M**.

The modulation **M** is calculated using equation (3). The parameters for this example are summarized in Table 2 (Preliminary measurements). The voltage **A** applied to the cantilever is marked green in Table 1.

$$M = U \cdot S \cdot A = 0.65 \cdot 227 \left[\frac{nm}{V}\right] \cdot 2.6[V] = 383.6\ [nm] \quad (12)$$

With the collected data (modulation **M** from equation (12), sensitivity **S** from Table 2 (Preliminary measurements), σ is the red value in Table 1), it is now possible to calculate the penetration depth Δz of the cantilever using equation (4):

$$\Delta z = 383.6[nm] \cdot \left(1 - \cos\left(\frac{27.874[°]}{180[°]} \cdot \pi\right)\right) - V_{stiffness}[V] \cdot 227\ \left[\frac{nm}{V}\right]$$

$$\Delta z = 44.5[nm] - V_{stiffness}[V] \cdot 227\ \left[\frac{nm}{V}\right] \quad (13)$$

The parameter X1 in the calculator (Fig. 4 (c)) is the initial measured stiffness image (Fig. 4 (a)). The resulting image is shown in Fig. 4 (b).

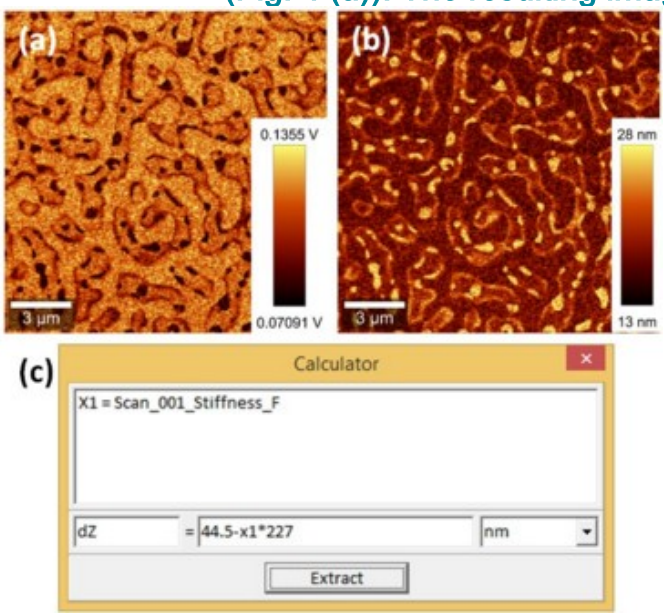


Fig. 4: Measured stiffness image (a), evaluated penetration depth (b) using equation (19) and the calculator tool (c)

The stiffness image is obtained by using equation (6), with the appropriate parameters for this example measurement:

$$Stiffness\left[\frac{N}{m}\right] = \frac{0.2\left[\frac{N}{m}\right] \cdot 227\left[\frac{nm}{V}\right] \cdot V_{stiffness}\ [V]}{\Delta z\ [nm]} \quad (14)$$

Equation 14 can be used directly in conjunction with the calculator tool as shown in Fig. 5 (d).

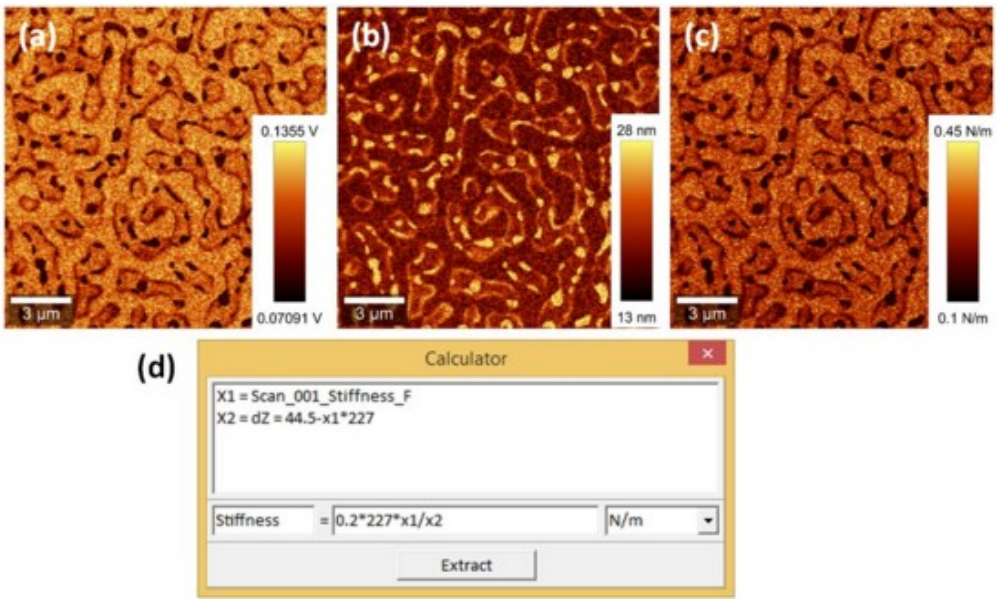


Fig. 5: Measured stiffness image (a), penetration depth (b), stiffness image evaluated using equation (14) and the calculator tool (d)

On samples with high topographical features a high contribution to adhesion and stiffness measurements results from changes of the contact area between tip and sample. Adhesion measurements are also sensitive to capillary forces interacting with the cantilever. These forces are due to water contamination on the sample from air environment. To overcome capillary forces, higher modulation amplitudes are recommended.

DPFM Curves

Import of DPFM data

The pulsed force curves are stored in a .wsd file, if selected before starting the measurement. For the analysis of the data it is necessary to import it into the project using File > Import > DPFM Data**. After selecting the appropriate .wsd file, the menu shown in Fig. 1 opens. Select the T-B Channel without any data reduction (enter 1 for** Data Reduction**) and click** Extract All**.**

The imported pulsed force curves appears as new graph data object and can be treated similar to spectral data arrays.

The new .wip file can be very large and you may run out of memory. Check your Memory options**.**

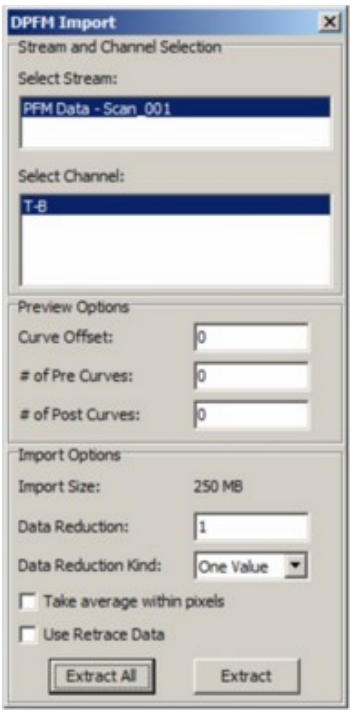


Fig. 1: DPFM import window.

Offset correction

As first step a background subtraction should be performed on the imported pulsed force curves. A slight drift of the T-B signal during approach or the measurement can affect the adhesion values. The background correction eliminates this error. Use a zero order polynom (Fig. 2 (a)) over the range highlighted as blue area on the curve (Fig. 2 (b)). The result of the background subtraction is shown in Fig. 3 (blue curve).

Change the x axis unit of the curves from deg to rad using the Graph Viewer Context Menu (Double-click on the data object to open the data in the graph viewer).

All the following operations are performed using the background corrected curves in rad.

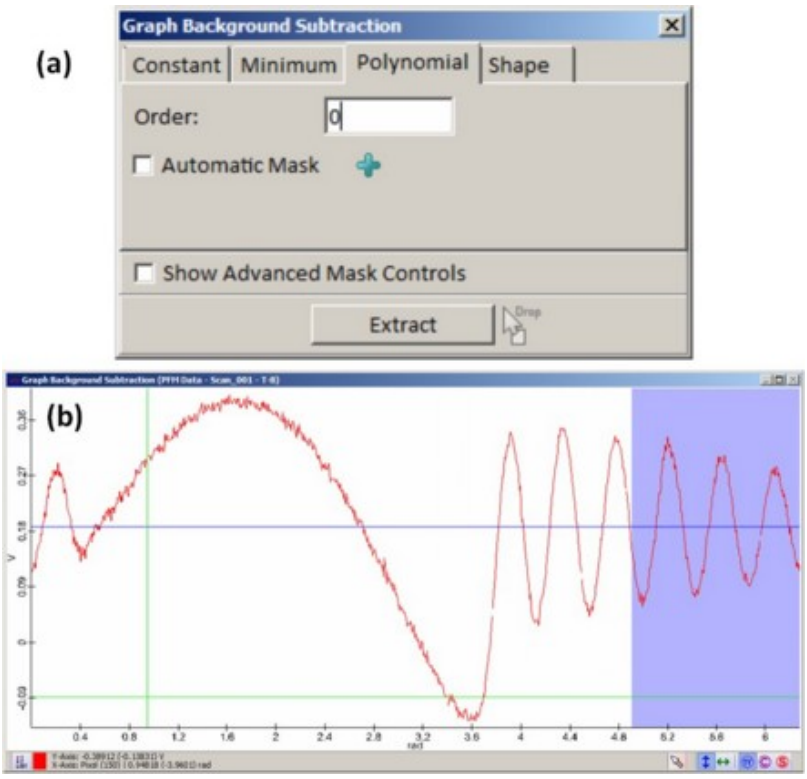


Fig. 2: Graph background subtraction menu (a) and example for range selection (b).

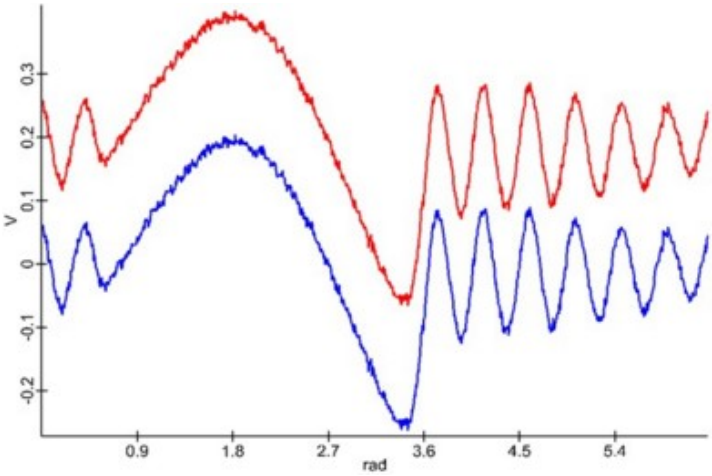


Fig. 3: Result of the background subtraction: original curve (red) and corrected curve (blue)

Evaluation of adhesion and stiffness from the DPFM curves

Adhesion

In order to create the adhesion image use the Filter Viewer **with a minimum filter. Select the appropriate range for this filter (Fig. 4 (a)). Based on the resulting image in V (Fig. 4 (b)) the adhesion image in nN (Fig. 4 (c)) can be calculated following the steps in the** DFPM Images **section.**

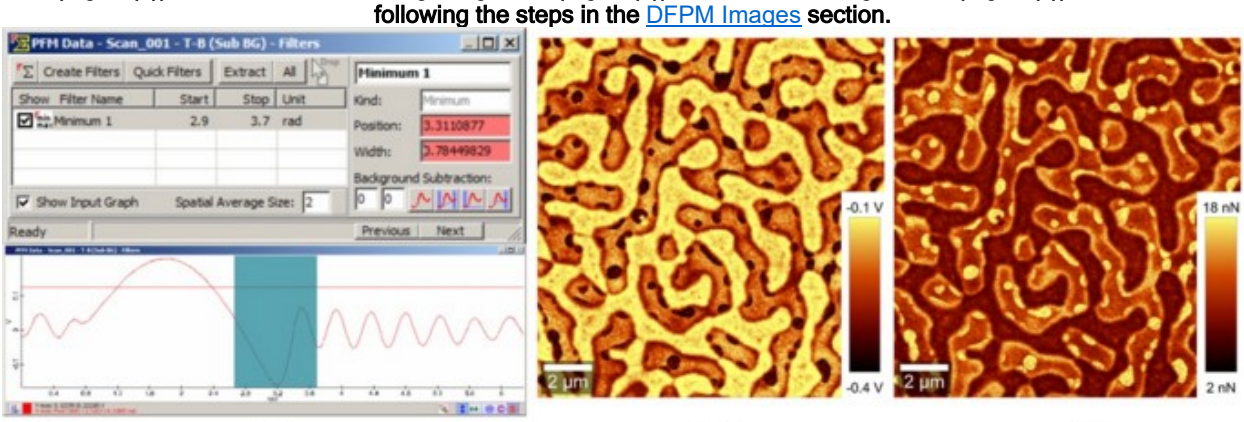


Fig. 4: Evaluation of adhesion from the PFM curves using the filter manager of WITecProject in conjunction with the Minimum filter and the appropriate filter selection range (a), extracted image from the filter manager (b) and the evaluated adhesion image (c).

Stiffness

The used Fit and Extract All **of the Advanced Fitting Tool is a** Plus feature**.**

The stiffness is defined by the slope of the rising part of the DPFM curve. By using the Advanced Fitting Tool**, a 1st order polynomial can be fitted to the curves in every image pixel.**

1. **Select Polynomial as the category and 1 as the Polynom Order (see Fig. 5 (a)).**
2. **Select the fit range as shown in Fig. 5 (c) and note the width of the selected window** Δrad.
3. **Click on** Fit and Extract All**.**
4. **The slope** a1 **is the data object ending with (Fit Polynom: a_1) in V/rad.**

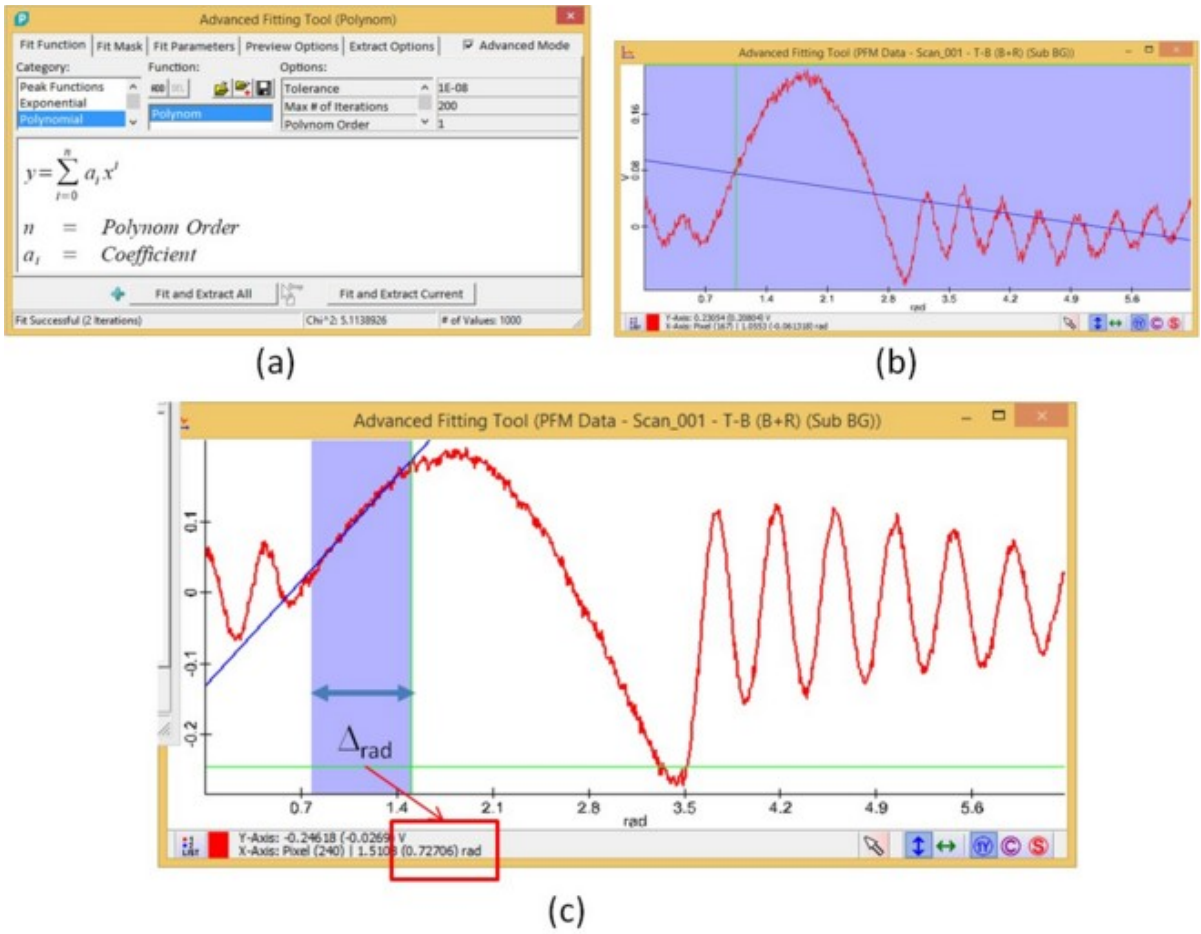


Fig. 5: Advanced fitting tool for 1st order polynomial fit (a), graph viewer which opens together with the fitting tool (b) and graph window showing the selected range of interest together with the blue fitting curve (c).

The slope image can now be used to determine the penetration depth in nm and consequently the corresponding stiffness image using equation (6) and also according to the DPFM Images section. The example for this specific measurement is shown in the two equations below and the resulting images in Fig. 6.

$$\Delta z = M \cdot (1 - \cos(\Delta_{rad})) - a_1 \cdot \Delta_{rad} \cdot S \quad (15)$$

$$\Delta z = 383.6[nm] \cdot (1 - \cos(0.727[rad])) - a_1 \left[\frac{V}{rad}\right] \cdot 0.727[rad] \cdot 227 \left[\frac{nm}{V}\right]$$

$$Stiffness = \frac{a_1 \cdot \Delta_{rad} \cdot k \cdot S}{\Delta z} \quad (16)$$

$$Stiffness \left[\frac{N}{m}\right] = \frac{a_1 \left[\frac{V}{rad}\right] \cdot 0.727\ [rad] \cdot 0.2 \left[\frac{N}{m}\right] \cdot 227 \left[\frac{nm}{V}\right]}{\Delta z\ [nm]}$$

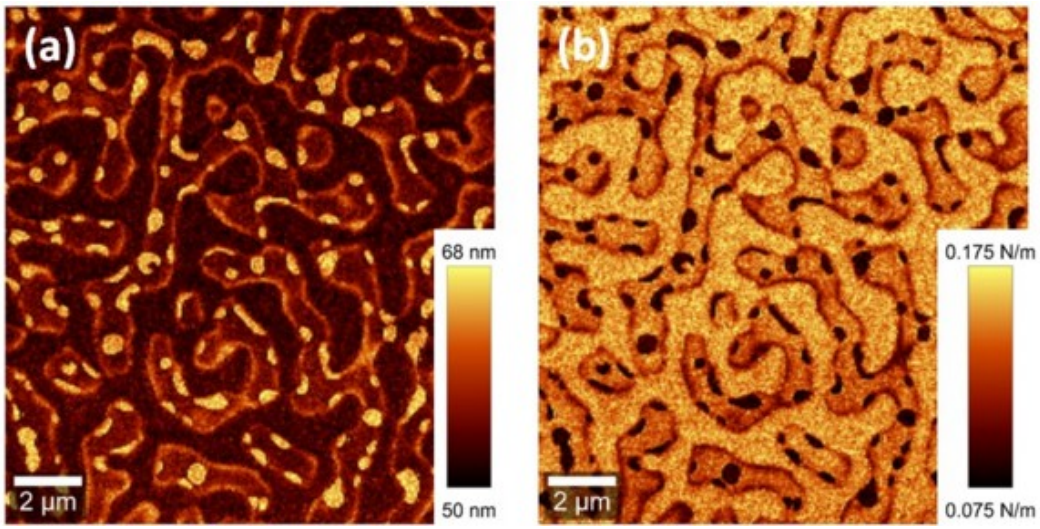
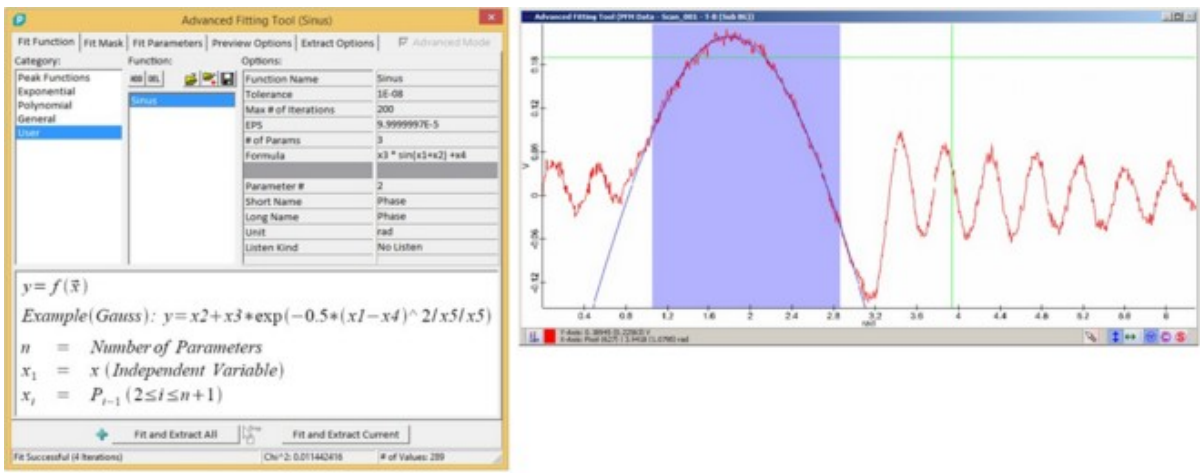


Fig. 6: Penetration depth (a) and stiffness (b) images evaluated using the advanced fitting tools and equations 15 and 16.

Force Distance curves from DPFM Measurements

The DPFM curves can be displayed as force-distance curves:

1. **Fit a sinus to the Fmax region of the DPFM curves.**
   a. **Click on** Load user fit functions from file **in the** Advanced Fitting Tool **and select** 04 Sine.wps **in the Examples folder.**
   b. **Select the appropriate range in the Fmax region (Fig. 7 (b))**
   c. **Activate** Extract Fit Curve with Input Data Supporting Points **and** Extract Fit Curves **in the** Extract Options **tab.**
   d. **Click** Fit and Extract All
2. **Multiply the resulting** fsinus **function (the data object ending with (Fit Sine: Curves)) with the** modulation M **to get a distance vs. time function (**fmodulation-distance**).**
3. **Open the DPFM curves and the** fmodulation-distance **curves in one graph viewer like shown in Fig. 9 (a). (Mark both and press** Enter**)**
4. **Switch to the** parametric view **to show force-distance curves of all measured DPFM curves (Fig. 9 (b)). In this view higher distance values correspond to a position closer to the sample.**



(a) (b)

Fig. 7: Example of sinus fitting function in the advanced fitting tools (a) and region selection in a PFM curve for the fit (b).

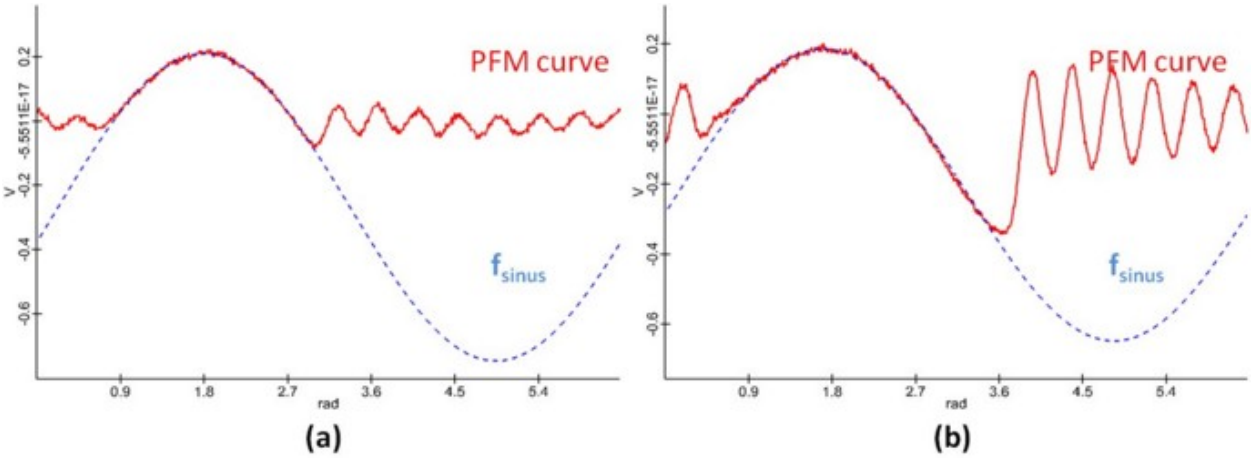


Fig. 8: PFM and f_{sinus} curves from a stiff (a) and soft (b) measuring point represented with the same y-axis.

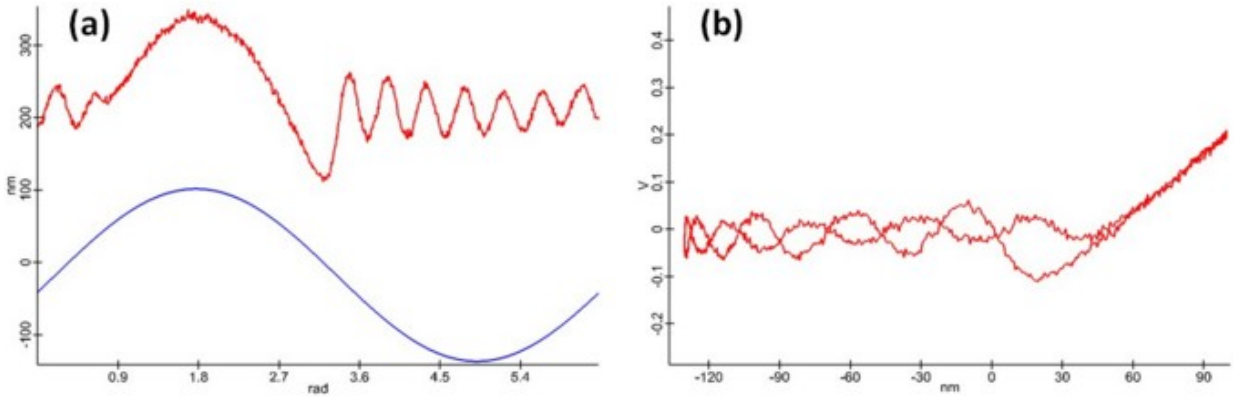


Fig. 9: Example of DPFM and $f_{modulation-distance}$ curve displayed in one graph viewer (a) and force-distance curve obtained from a DPFM measurement (b).

AC Lift Mode Overview

Lift Mode is a two-pass technique that interleaves AC mode scan lines at the sample surface with scan lines at a designated height above the surface. There forces on the cantilever can be measured excluding the influence of the topography.

Topics:

- Magnetic Force Microscopy (MFM)
- Electric Force Microscopy (EFM)
- Setting up a measurement
- Hints section

Measurement modes:

- Image Scan (Multi Pass): acquisition of AFM AC Lift mode images
- Image Scan: acquisition of AFM AC mode images (only for optimizing parameters)

System Requirements:

- alphaControl with a serial number 120-1030-XXX (Marvin 3b) or higher

Required License feature:

- Lift Mode

Parameters

Follow the steps in the general procedure. For further information refer to the AC mode adjustment.

The Z Offset depends on the chosen free amplitude and the forces between tip and sample. Always work exactly on the resonance frequency of the cantilever for highest sensitivity, because there is no damping of the cantilever during the second pass.

After tip approach:

1. Set the Time / Line (Trace) [s] under Image Scan.
2. Click on Start Scan.
3. Optimize the Feedback settings.
4. Stop the Image Scan. (Data can be deleted.)
5. Adjust the Z Offset for Measurement at the section Image Scan (Multi Pass).
6. Click on Start Multi Pass Scan.

Each data object in a Multi Pass scan is created twice marked with [L] or [M]. The data objects marked with [L], which stands for Learn, is measured during the first pass at the surface. The data objects marked with [M], which stands for Measure, are recorded during the second pass above the surface. By default, both Phase and Topography images with the according graphs are opened (Figure 2).

Contrasts due to changing forces between tip and sample can be observed in the measured Phase image (Phase [M]).

Further information:

Image Scan (Multi Pass), Image Scan

Hints

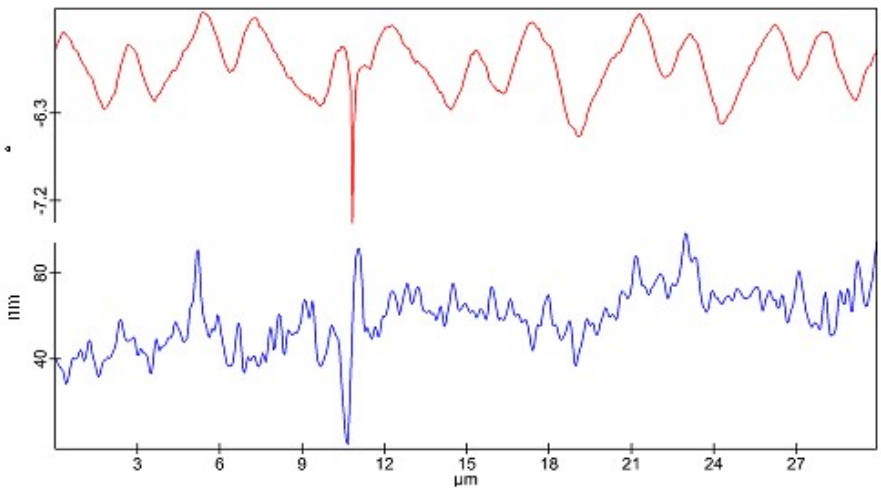


Figure 1: Phase [M] (red) with artifact and corresponding Topography [L] (blue)

If the cantilever collides with the sample during the second pass an artifact will be created in the measurement. It can be seen in Figure 1 that the anomaly at around 10.5 µm in the Phase of the second pass correlates with a big step in the topography. If this happens, a higher z-offset can be chosen. This is also necessary for a high topography in general. A z-offset that is too high, however, will lead to lower contrast because most of the forces are stronger close to sample. Therefore, an alternative is to use the **Look Ahead** value, which leads to reaction on the topography before it is really reached.
To speed up the measurement the **Min. Time for Retrace** parameter can be reduced. This could also lead to more stress for the tip on the other hand. So, the appropriate compromise is important.
Further information:

Overview

In Magnetic Force Microscopy (MFM) the magnetic force gradient along the sample surface is measured using the Lift mode. It can be used to investigate e.g. magnetic recording materials, superconductors, magnetic nanoparticles, etc.

Topics:

Recommended cantilevers:
For MFM special cantilevers with magnetic coated tips are needed.

Reference sample

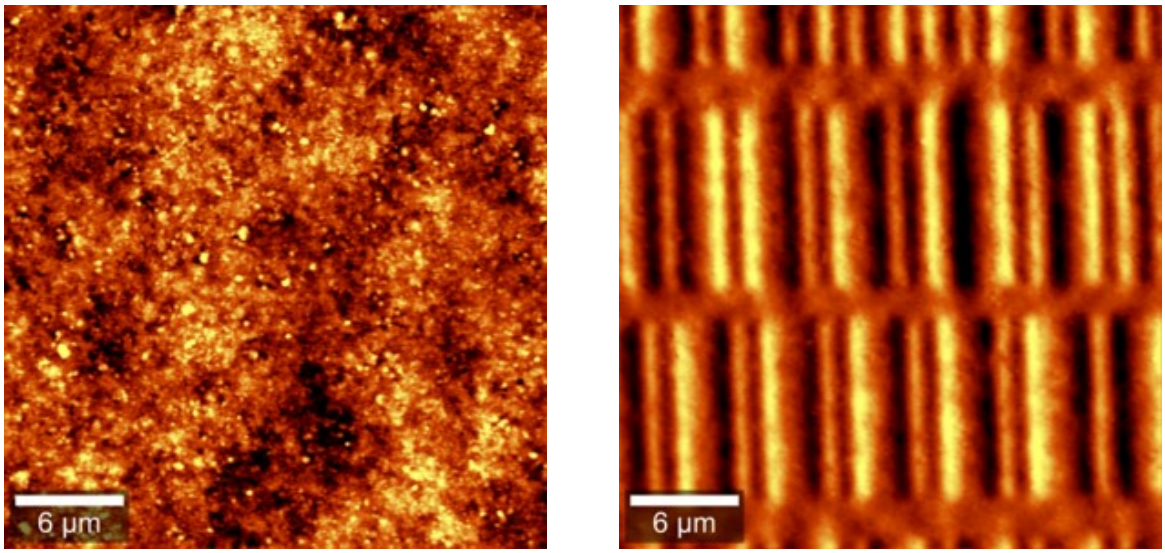


Figure 1: Topography (left) and MFM (right) image of a floppy disc

Besides commercial MFM samples a part of a floppy disc or hard disc platter is a good sample to test and practice MFM. The MFM image in Figure 4 shows the single bits on different tracks.

Table 1: Exemplary parameters for the above measurement

Parameter	Value
Size [µm]	5 x 5
Pixel	512 x 512
Time per Line [s]	1
Z Offset [nm]	200
Look Ahead [%]	0

For MFM a high z-offset is necessary in order to see a contrast and really detach the tip from the sample surface.

Overview

Electric Force Microscopy (EFM) measures electric field gradient distribution along the sample surface using the Lift mode. EFM is used e.g. for electrical failure analysis, detecting trapped charges, mapping electric polarization, and performing electrical read/write.

Topics:

- Example measurement
- EFM hints section
- Lift Mode hints section

System requirements:

- EFM package with EFM arm and clamps

Required License feature:

- EFM Mode

Recommended Cantilevers:

The cantilever needs to be electrically connected to the mounting ring. Therefore, a silver conductive paint is used (Figure 1). A metal coated cantilever is recommended.

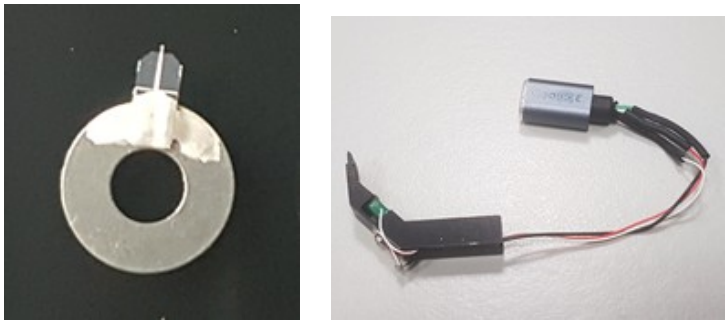


Figure 1: Cantilever connected with silver conductive paint (left) and EFM arm with electric connection (right)

Voltage Supply

Connecting the cantilever:

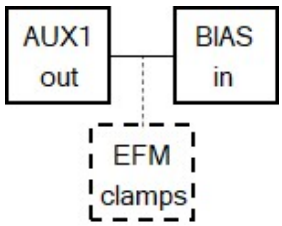


Figure 1: Wiring diagram

- Make sure BIAS-in connector of the alphaControl electronics is connected with AUX1-out via a BNC cable (Figure 1).
- If you want to use the EFM-clamps, connect them over a T-piece adapter with BIAS-in and AUX1-out (Figure 1).
- The sample has to be connected to ground over the black EFM-clamp or by an external potential.
- Make sure the red clamp has no contact to ground.
- Mount a contacted cantilever with the EFM cantilever arm.

EFM Control:

The section EFM Control is used to apply a voltage between tip and sample. Select Aux1 DAC as Output DAC and switch EFM Output to Enabled. Now, the voltage can be adjusted at DC Component. Setup these settings before starting the EFM measurement.

For optimization the voltage can be changed or switched on/off also during the measurement.

Hints

- If the contrast in the Phase image is too low, check the electric connections:
  - connection to the cantilever
  - connection of the sample
  - To check the connection the EFM Control could be used to apply a DC voltage between tip and sample
- Check if the used cantilever is connected properly using silver conductive paint.
- Try another cantilever, if the result should not improve.

Reference sample

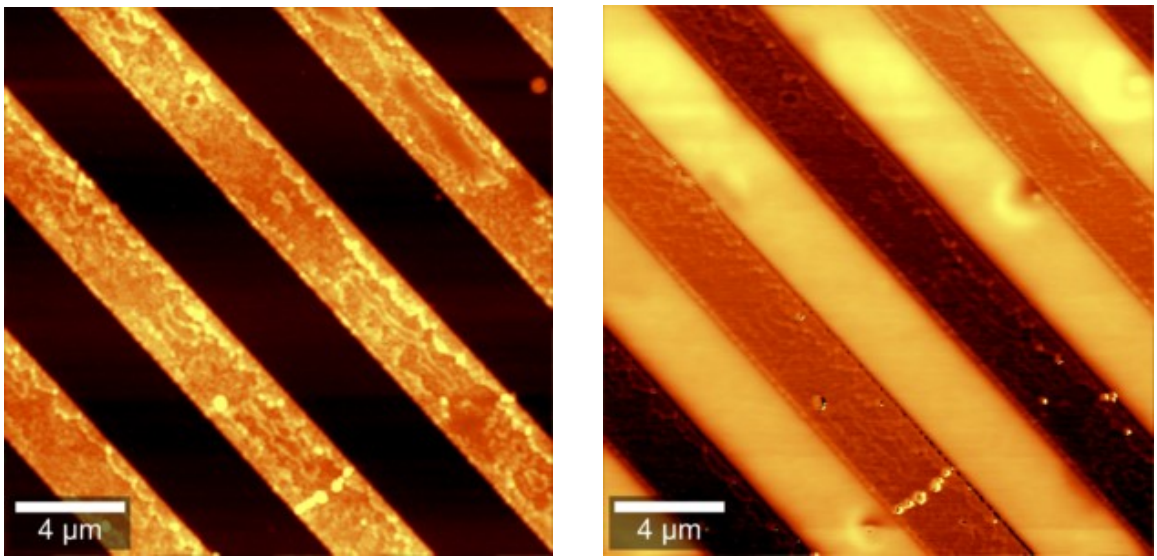


Figure 1: Topography (left) and EFM (right) image of electric traces

For EFM, reference samples are available (e.g. from WITec) with electric traces that can be put on different potentials. The EFM image in Figure 1 shows the traces where potentials of 0 V and 1 V are applied. The isolating material can also be clearly distinguished (dark stripes in the topography, bright stripes in the EFM image).

Table 1: Exemplary parameters for the above measurement

Parameter	Value
Size [µm]	20 x 20
Pixel	512 x 512
Time per Line [s]	1
Z Offset [nm]	50
Look Ahead [%]	10
EFM voltage [V]	4
Sample potential [V]	0 and 1

KPFM Overview

Kelvin probe force microscopy (KPFM) is an AFM mode to determine the work function of surfaces at the nano scale.

Topics:

- Theory

- WITec implementation
- Sample mounting
- Setting up a measurement
- Example measurement
- Interpretation of values

Measurement modes:

- Image Scan (Multi Pass): **acquisition of AFM AC Lift mode images**
- Image Scan: **acquisition of AFM AC mode images (only for optimizing parameters)**

System requirements:

- alphaControl with a serial number 120-1030-XXX (Marvin 3b) or higher
- EFM package with EFM arm and clamps

Required License feature:

- Kelvin probe

Recommended Cantilevers:

The geometry of the cantilever and the tip is a critical factor defining resolution and accuracy of the acquired KPFM images. Long and slender but slightly blunt tips on cantilevers of minimal width and surface area are the best choice.

The cantilever needs to be electrically connected to the mounting ring. Therefore, a silver paint is used (Figure 1). As cantilever a metal coated one (like for EFM) or one made of highly doped silicon (FM cantilevers) can be used.

Metal coats have a poor stability. The tip electrode often loses parts of its coating during scanning. As a result, the tip electrode will act as an unstable reference since its surface potential distribution is changing during the measurement.

Figure 1: Cantilever connected with silver paint

Theory

Kelvin probe force microscopy (KPFM) is an AFM mode to determine the work function of surfaces at the nano scale. The technique uses an electric field between the sample surface and the probe. The voltage ΔVsp needs to be adjusted to a value such that the gradient Φ is flat and the electric field is dissolved (Figure 1). In this case the following equation is valid, where Ws is the work function of the sample and Wp is the work function of the probe.

$$\Delta W = W_s - W_p = e\Delta V_{sp}$$

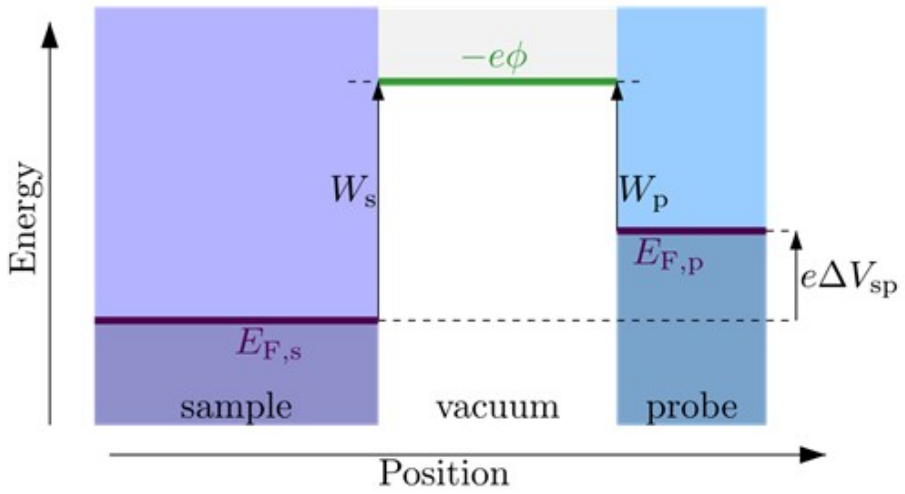


Figure 1: Energy diagram (source: https://en.wikipedia.org/wiki/Work_function)

The difference between the work functions is also denoted like this, where VCPD is called the contact potential difference:

$$\Delta W = -eV_{CPD}$$

To measure the work function using an AFM tip as a probe a voltage is applied between tip and sample. This voltage consists of a DC-bias VDC and an AC-voltage VAC sin(ωt) of frequency ω. The total potential difference between tip and sample is

$$\Delta V = V_{DC} + V_{AC}\sin(\omega t)$$

The electrostatic force in a capacitor may be found by differentiating the energy function with respect to the separation of the elements and can be written as

$$F = -\frac{1}{2}\frac{\partial C}{\partial z}(\Delta V)^2$$

where C is the capacitance, z is the separation, and V is the voltage, each between tip and surface. Substituting the previous formula for voltage (V) shows that the electrostatic force can be split up into three contributions, as the total electrostatic force F acting on the tip then has spectral components at the frequencies ω and 2ω.

$$F = F_{DC} + F_{\omega} + F_{2\omega}$$

$$F_{DC} = \frac{\partial C}{\partial z}\left(\frac{1}{2}(V_{DC} - V_{CPD})^2 + \frac{1}{4}V_{AC}^2\right)$$

$$F_{\omega} = \frac{\partial C}{\partial z}(V_{DC} - V_{CPD})V_{AC}\sin(\omega t)$$

$$F_{2\omega} = -\frac{1}{4}\frac{\partial C}{\partial z}V_{AC}^2\cos(2\omega t)$$

The DC component, FDC, contributes to the topographical signal, the term Fω at the characteristic frequency ω is used to measure the contact potential and the contribution F2ω can be used for capacitance microscopy. The formulae are valid if the voltage is applied to the tip. If the voltage is applied to the sample, VCPD is added not subtracted.

Further information

- https://en.wikipedia.org/wiki/Work_function
- https://en.wikipedia.org/wiki/Kelvin_probe_force_microscope

Implementation

The KPFM mode is accomplished as a two-pass measurement to minimize crosstalk using the lift mode like for MFM or EFM measurements. In the first pass the topography is recorded using Tapping mode and the Primary Lock-in. In the second pass the cantilever is not mechanically stimulated for a vibration anymore. The AC voltage $v_{AC(\omega t)}$ is applied between sample and tip. The cantilever starts to vibrate with ω due to the electric forces and the signal is analyzed by the Secondary Lock-in, which delivers the amplitude. To search for the value of v_{DC} where the minimum amplitude of the cantilever occurs, the v_{DC} voltage is modulated with 100 Hz (by default).

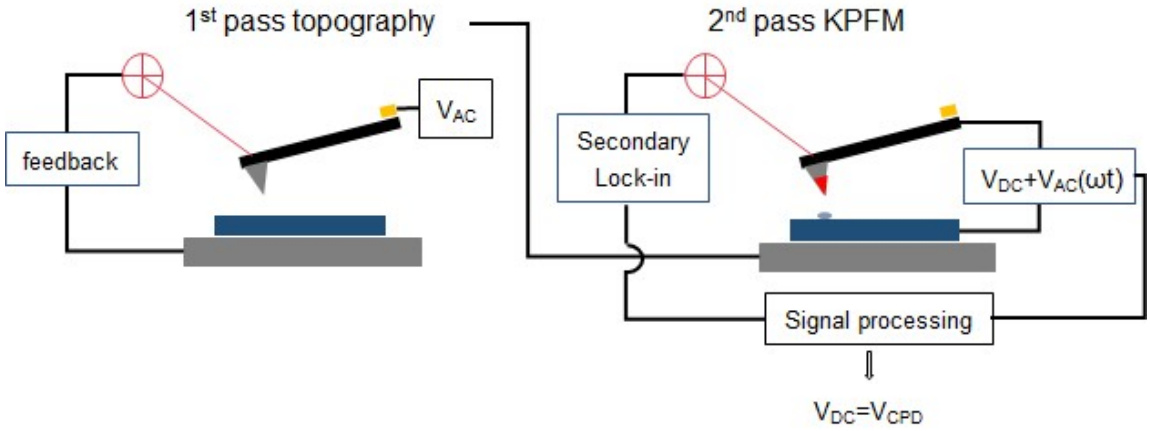


Figure 1: KPFM measurement principle

This kind of implementation is also called KPFM-AM. The advantage of KPFM-AM is a higher sensitivity but compromises the achievable resolution. This is due to the fact that not only the tip contributes to the measurement but also the whole cantilever.

Sample Mounting

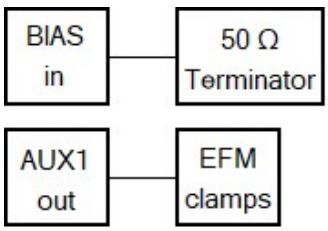


Figure 1: Wiring diagram

- **Terminate BIAS-in with a 50 Ω Resistor (grounds the cantilever).**
- **Connect the EFM-clamps with AUX1-out.**
- **Make sure the sample is isolated and not connected to ground.**
- **Connect the red EFM-clamp over the sample clamp and put it on the sample surface.**
- **Mount a contacted Cantilever with the EFM cantilever arm.**

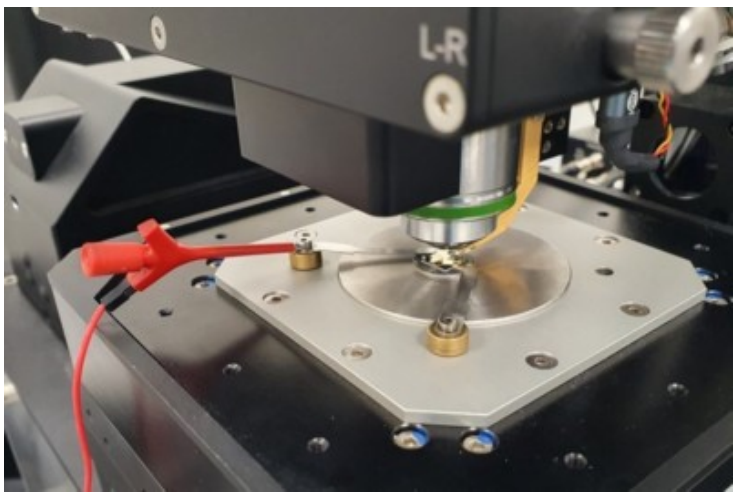


Figure 2: Reference sample mounted on the microscope

Parameters

Follow the steps in the Lift mode procedure.

The Time / Line (Trace) [s] should be set dependent of the Points per Line value. Allow at least 0.01 s for each pixel to prevent digitizing (2.56 s for 256 points).
The Z Offset for Measurement should be negative (e.g. -150 nm) in most cases because the amplitude of the cantilever is much smaller in the second pass.
CPD shows the KPFM image and curve.

Figure 1 shows the PFM Control window during the second pass of a KPFM measurement. The red line shows the T-B signal, the blue line is the smoothed amplitude of the T-B signal (the phase shift is due to filtering), the red area is the search range for the minimum, the black line (by default it is white) shows the VDC. The value of VDC at the minimum value of the T-B signal is taken as value for the CPD image. The blue line should show two sharp minima. If not check the sample mounting and connections.

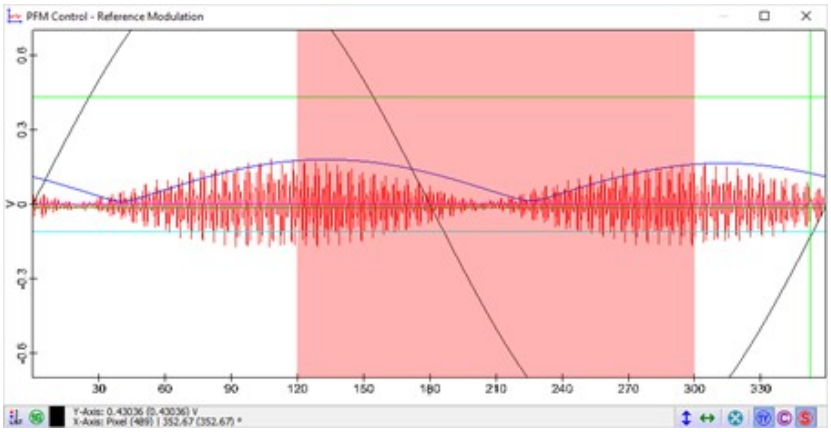


Figure 1: PFM Control window

All settings related to VAC and VDC are set up automatically when the measurement starts. Nevertheless, it is possible to change settings after the measurement started. All usefull parameters are summarized in the Kelvin Probe Control section.

Hints

- **Before measuring a sample, the performance of the cantilever should be checked with the reference sample.**
- **If the sensitivity is too small check the electric connections:**
  - **connection of the cantilever to ground.**
  - **connection of the sample to Aux 1 Out.**
  - **To check the connection the EFM Control could be used which makes it possible to apply a DC voltage between tip and sample like for EFM using Aux 1 Out.**
- **If the KPFM curve shows digitizing check that the Time per Line fits to the VDC Driving Amplitude. (i.e. 2.56 s for 256 points @ 100 Hz)**
- **If the resolution is too low check the Z offset (needs to be negative).**
- **Check if the used cantilever is connected with silver paint.**
- **Try another cantilever if the result should not improve.**
- **Compare your results only with such measured under ambient condition, not in ultra-high vacuum.**

Reference Sample

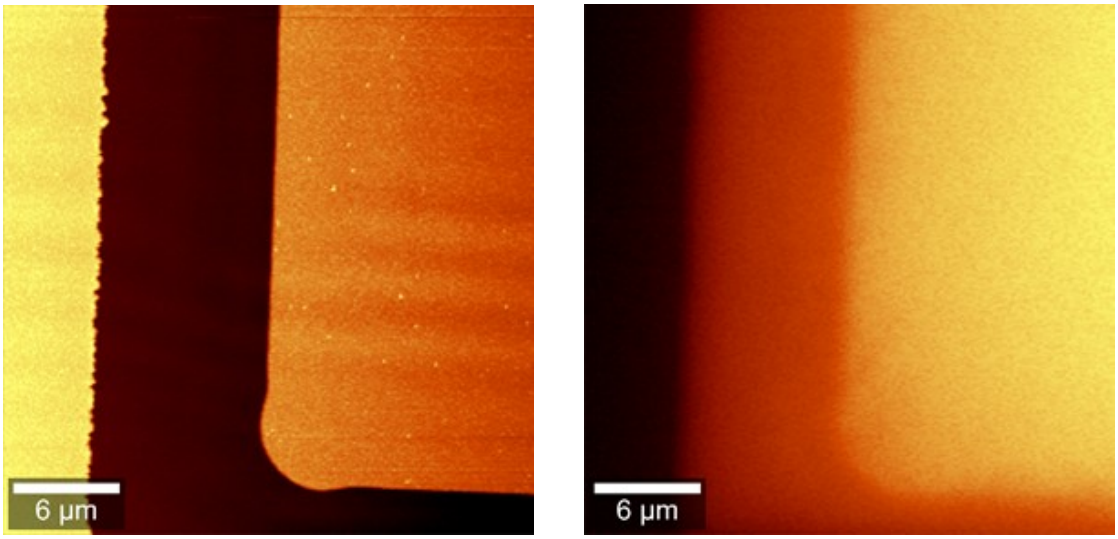


Figure 1: Topography (left) and CPD (right) image of the reference sample
A good reference sample for KPFM measurements is the PFKPFM-SMPL from Bruker.

Table 1: Exemplary parameters for the above measurement

Frequency sweep	
Driving Amp. pk-pk [V]	0.040
Driving Frequency [Hz]	50917.28
Feedback settings	
Setpoint [V]	0.60
P-Gain [%]	8
I-Gain [%]	8
Image Scan (Multi Pass)	
Z Offset for Measurement [nm]	30
Look Ahead (X-Axis) [%]	0.0
Image Scan	
Points per Line	256
Lines per Image	256
Width [µm]	30
Height [µm]	30
Time / Line (Trace) [s]	2.56
Min. Time for Retrace [s]	0.5

Interpretation of Values

The VDC values measured during the KPFM measurement cannot be equaled to VCPD, because they are offset affected (due to the phase shift) and inverted. It is also important to note that these values are always a weighted average, and all surface elements of the tip and the sample affect them. The main idea of the measurement is to see contrasts between materials or areas with different work function. To measure quantitively a system which is capable to work in ultra-high vacuum is needed.

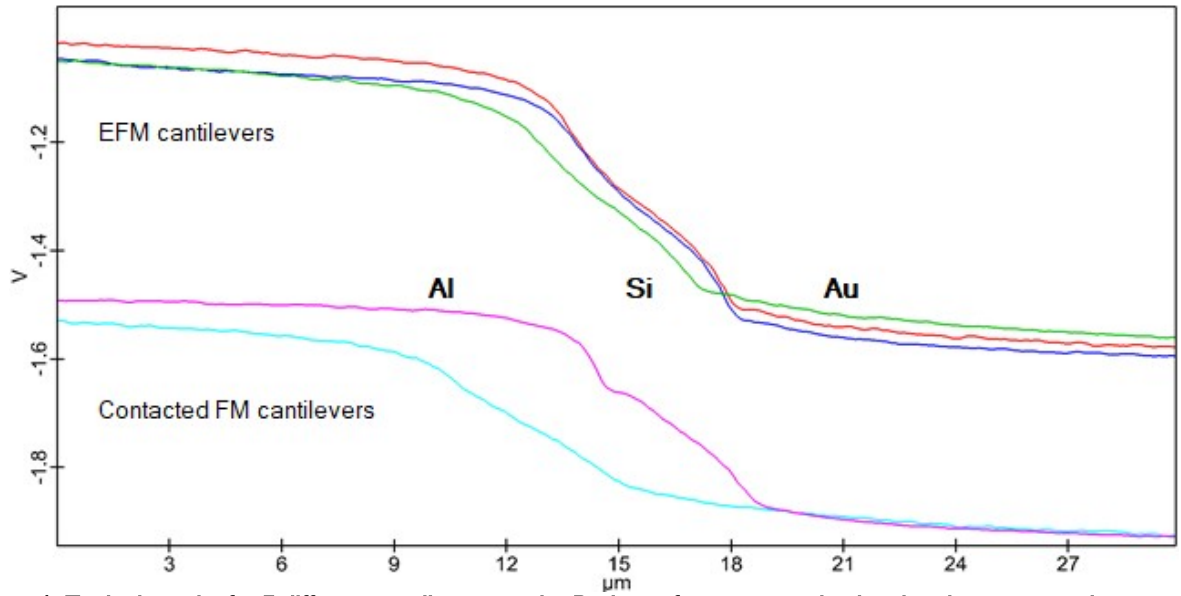


Figure 1: Typical results for 5 different cantilevers on the Bruker reference sample showing the cross section over the edge from Al over Si to Au

To get a more useful value the following equation can be used by determine Voffset against a reference sample with the used cantilever.

$$V_{CPD} = -V_{DC} + V_{offset}$$

Nevertheless, if values for the work function should be calculated a calibration on a reference sample is necessary before a sample can be measured. This has to be done for every time the cantilever is exchanged. Values for the work function are needed from literature for the reference material. In order do to do a linear calibration at least two different materials are needed. To calculate values of the work function from the measured VDC the following equation is needed:

$$\frac{W_s}{e} = sV_{DC} + V_{offset}$$

The values of s and Voffset are calculated from the reference values and the measured values on this material:

$$s = \frac{\frac{W_{Au}}{e} - \frac{W_{Al}}{e}}{V_{DC,Au} - V_{DC,Al}}$$

$$V_{offset} = \frac{\frac{W_{Al}}{e} V_{DC,Au} - \frac{W_{Au}}{e} V_{DC,Al}}{V_{DC,Au} - V_{DC,Al}}$$

$$\frac{W_{Al}}{e} \approx 4.2V; \frac{W_{Au}}{e} \approx 5.3V$$

Using the Calculator Drop Action it is possible to calculate values of the work function for each point of a KPFM image or cross section (Figure 2).

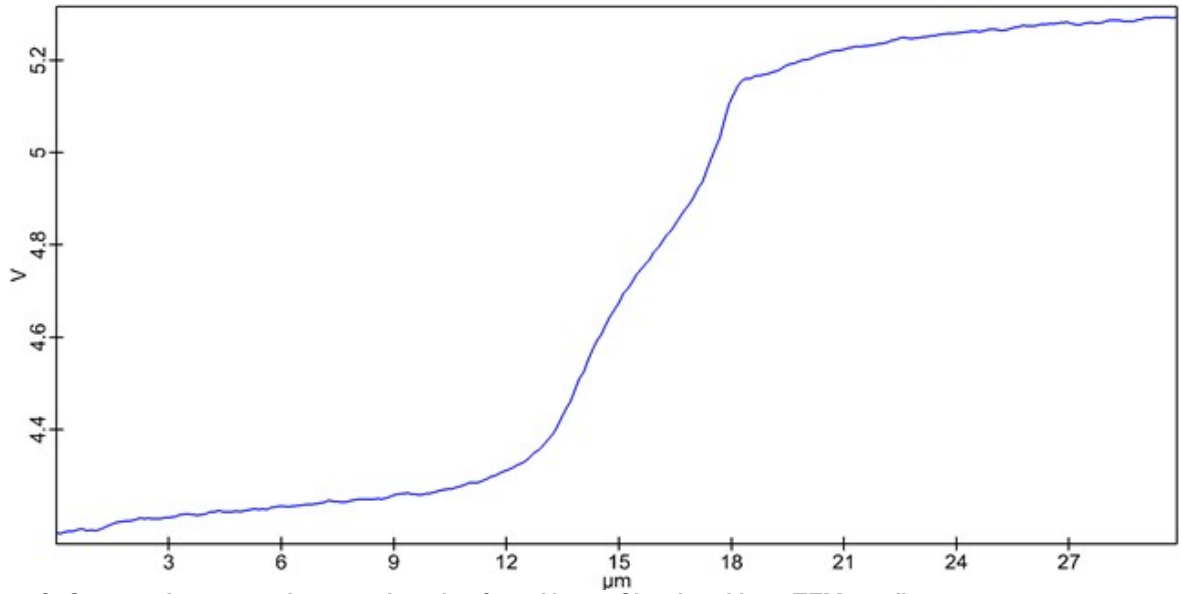


Figure 2: Corrected cross section over the edge from Al over Si to Au with an EFM cantilever

Figure 3: Corrected KPFM image over the edge from Al over Si to Au with an EFM cantilever

The measured values are strongly dependent on the resolution of the method. As already mentioned, the resolution of KPFM-AM is limited. This means that measured values for small spots are always averaged with the surrounding material. Furthermore, the cantilever changes during the measurement, which will also affect the calibration. To get more reliable results, the measurement should be repeated with another cantilever.

PRFM Overview

Piezoresponse force microscopy (PRFM) is an AFM mode to image domains of piezoelectric or rather ferroelectric materials. In order to induce vibrations to the material an AC current is applied to the sample by a conductive AFM tip. The resulting very weak response of the material is than demodulated by a lock-in amplifier. By analyzing either the T-B or the L-R signal, vertical or lateral movements of the material can be detected. With this technique it is possible to image the topography and piezoelectric or ferroelectric domains at the same time.

Topics:

- Connecting devices
- Sample mounting
- Setting up a measurement
- Example measurement

Measurement modes:

- Image Scan: **acquisition of PRFM images.**

System requirements:

- alphaControl with a serial number 120-1050-XXX (Marvin 4b) or higher
- EFM package with EFM arm and clamps
- Piezoresponse components

Recommended cantilevers:

The cantilever needs to be electrically connected to the mounting ring. Therefore, a silver conductive paint is used. A metal coated cantilever is recommended.

Connecting devices

Transformer

- The transformer increases the voltage by a factor of 4.5 (i.e. from 20 Vpp to 90 Vpp). The EFM clamp is not affected, if connected correctly.

1. Connect the transformer BIAS-out with BIAS-in using the angle piece.
2. Connect the T-piece from AUX1-out and the EFM-clamps with AUX-in of the transformer.

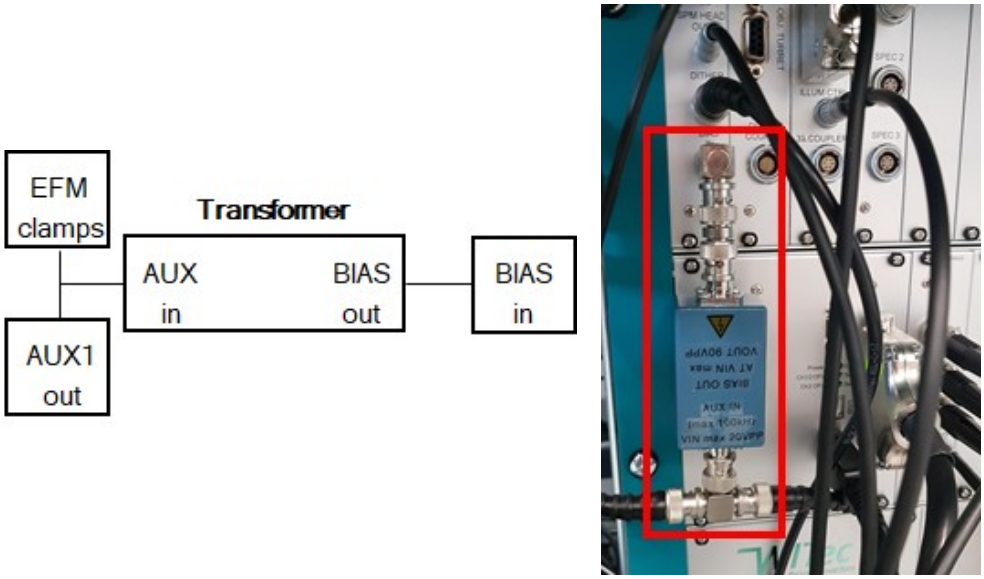


Figure 1: Wiring diagram (left) and picture (right)

Make sure that the transformer is only connected to the BIAS in. Electronic boards or even the whole electronics could be destroyed!

Be careful with the higher voltage at the cantilever!

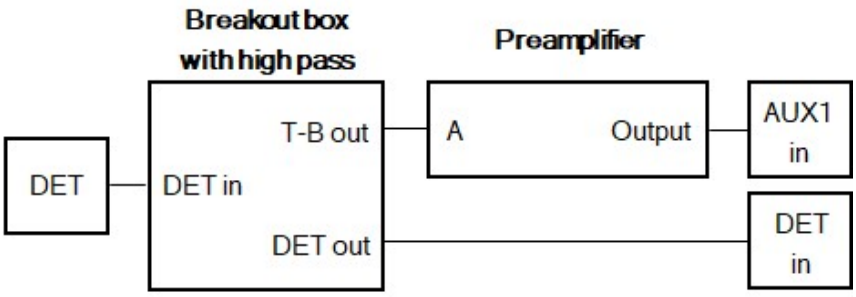
Remove the transformer before using EFM or KPFM mode.

Preamplifier

- The high pass filter removes the DC-offset from the T-B signal.
- The preamplifier amplifies the weak signal. If the amplification is too low, digitalization steps become visible in the signal.

alphaControl with a serial number 120-1060-XXX (Marvin 5) and above:

1. Connect the DET cable with DET-in of the breakout box.
2. Connect DET-out of the breakout box with DET-in of the controller.
3. Connect T-B-out of the breakout box with A of the preamplifier.



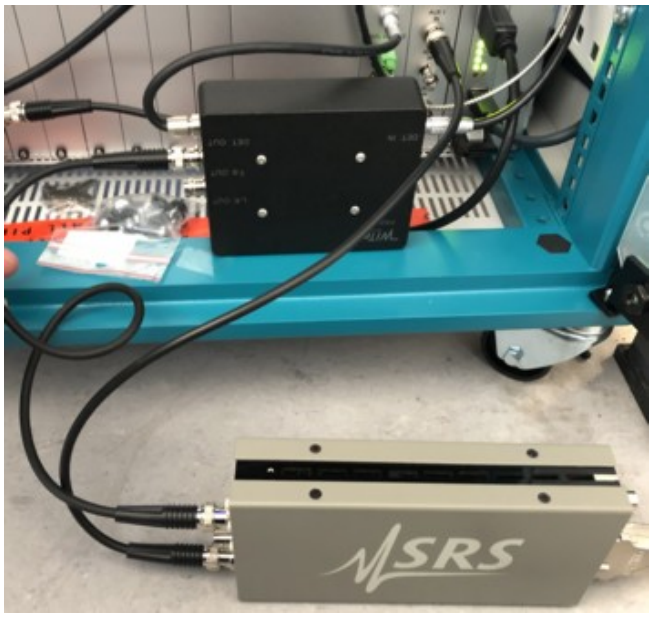
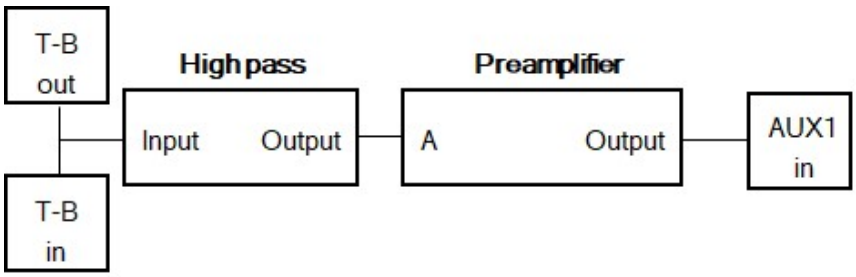


Figure 2: Wiring diagram (top) and installed components (bottom)

alphaControl with a serial number 120-1050-XXX (Marvin 4b):

1. Connect the T-Piece with T-B-in and with the high pass filter (Figure 3).
2. Connect the high pass module with A of the preamplifier.



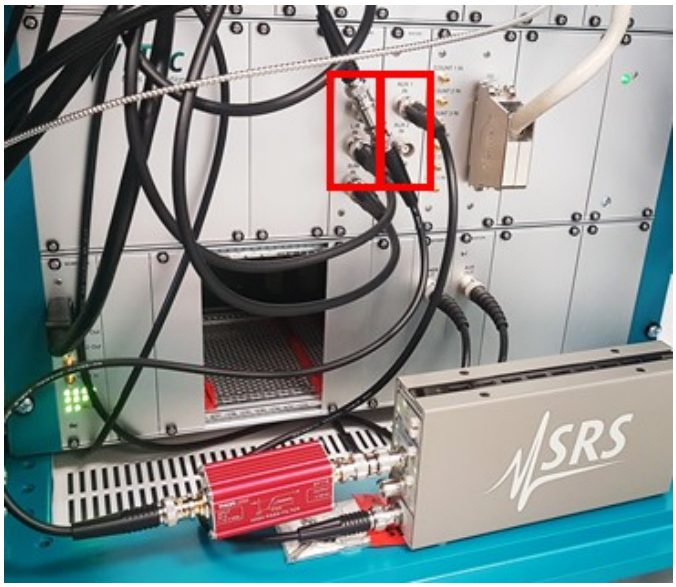


Figure 3: Wiring diagram (top) and installed components (bottom)

Both versions:

1. Connect Output of the preamplifier with AUX1-in. (Figure 2 and 3)
2. Connect the preamplifier with its power supply.
3. Switch the preamplifier gain to 100x and the Input to A and AC (Figure 4).

Figure 4: Preamplifier settings

Sample Mounting

- The sample backside must be connected to ground.
- Ground the sample with the black EFM-clamp over the sample clamp. Make sure the red clamp is not connected with something and has no contact to ground.
- Mount a contacted cantilever with the EFM cantilever arm.

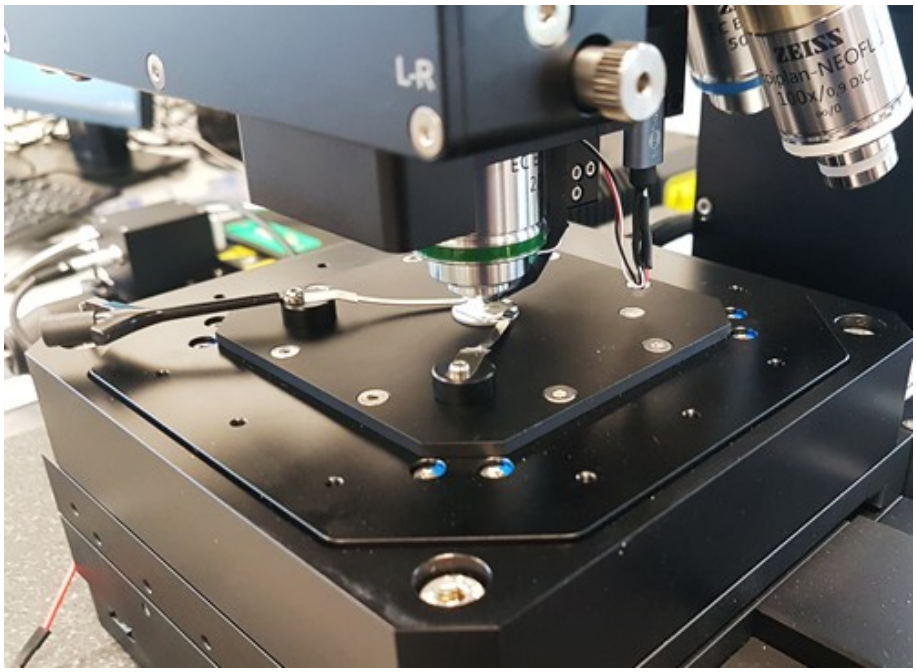


Figure 1: Reference sample mounted on the microscope

Parameters

Follow the steps in the contact mode procedure.

- PRFM is a contact mode configuration, using an AC voltage on the tip for electrical stimulation of the piezoactive material and a lock-in for analyzing the signal.
- For the Piezoresponse configuration the low pass filter of the T-B signal is deactivated.
- Refer to Piezoresponse Control
- Filter frequency is a parameter of the lock-in
    - High values result in more noise and a quick signal response.
    - Low values result in a smooth signal, but a greater time delay.
    - This creates a shift between forward and backward signal and softer edges.
- Low I-Gain is recommended.
- The driving frequency has virtually no influence in the result.

Reference sample

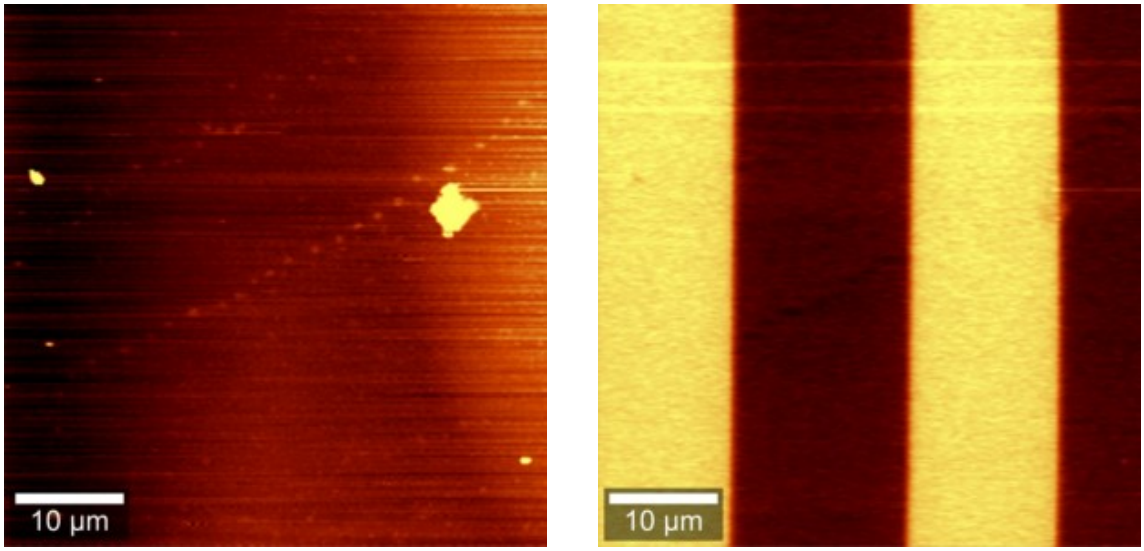


Figure 1: Topography (left) and Phase (right) image of the reference sample

A recommended reference sample to test the appropriate PRFM settings is the PFM-SMPL of Bruker, which consists of periodically poled $LiNbO_3$. The piezoactive structures are present in the center of the sample. Do not measure parallel to the structure. Try to change the scan gamma angle to achieve this.

Table 1: Exemplary parameters for the above measurement

Parameter	Value
Size [µm]	**50 x 50**
Pixel	**256 x 256**
Time per Line [s]	**3**
Setpoint [V]	**0.5**
Driving Amplitude [V]	**20**
Driving Frequency [Hz]	**35000**
Filter Frequency [Hz]	**10**

Raman AFM Overview

This imaging mode enables simultaneous Raman and AFM measurements in contact or AC mode.

Topics:

- Setting up a measurement

Configurations:

The following configurations are available for all CCD cameras:

- Raman AFM Contact CCDX
- Raman AFM AC CCDX

Measurement modes:

- Image Scan: acquisition of Raman and AFM contact or AC mode images

System Requirements:

- Raman (RA and RAS systems)

Advanced experiments:

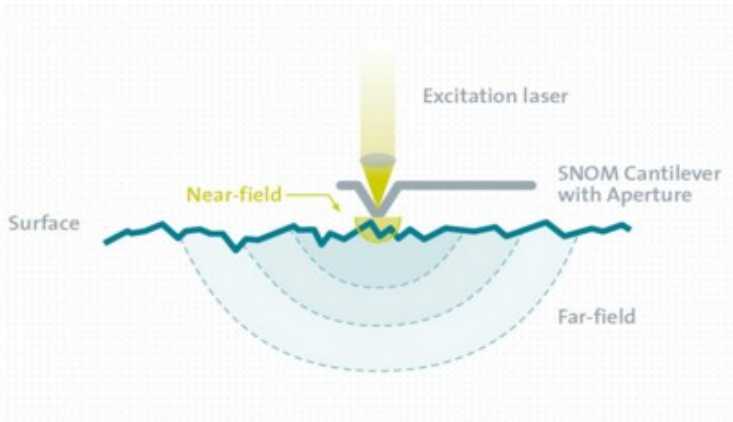
Since this configuration just combines the AFM with the spectroscopy capabilities more experiments are possible besides just doing Raman-AFM.

- AFM-PL
- Tip-enhanced Raman spectroscopy (TERS)
- SNOM-PL
- SNOM-Raman (in most cases signal intensity will be too low)

Parameters

Follow the steps in the general procedure. Further information can be found in the **Contact mode** or AC mode section depending on the used configuration. For information about the spectroscopy part refer to Raman.

SNOM Overview



Scanning Near-field Optical Microscopy (SNOM) is a technique to achieve lateral resolution below the defraction limit using an aperture and a point detektor.

Topics:

- Theoretical Background
- Setting up a measurement

- Setting up a measurement in Pick-Up mode
- Setting up a measurement in reflection mode
- Example measurement

SNOM modes:

- Contact Mode – standard SNOM mode
- AC Mode – for soft samples, material contrast by phase image
- DPFM – distance depended SNOM using an analog PMT
- SNOM-PL or SNOM-Raman – for combination of SNOM with spectroscopy refer to Raman-AFM

Configurations:

The following configurations are available depending on the equipped photon counting device:

- SNOM Contact PMT/APD
- SNOM AC PMT/APD

Measurement modes:

- Oscilloscope: displays the output channel of a photon counting device as a function of time similar to the display of an oscilloscope.
- Image Scan: acquisition of SNOM images
- Line Scan: acquisition of distance curves along a line
- Distance Curve: acquisition of distance curves at the current position

Recommended Cantilevers:

SNOM cantilevers feature a hollow pyramid as the aperture for SNOM measurements and can be purchased from WITec with apertures of < 150, < 90 and < 60 nm.

System requirements:

- SNOM (S, AS and RAS systems)

Theory

The resolution in classical (as well as confocal) microscopy is limited by the wavelength of the excitation light. This was investigated in detail by ABBE around 1890. According to him, at least the first diffracted order of an object (e.g. a grating) has to be captured by the lens system to resolve the object in image space. This is the reason for the importance of the numerical aperture for the resolution of an optical system.
In a perfect lens system with circular aperture, the image of a point object will be an Airy-pattern. On integrating the irradiance, one finds that 84 % of the light arrives within the central spot and 91 % within the bounds of the second dark ring.
If one brings two point objects close together, so that the maximum of the first Airy pattern is at the first minimum of the second, we are still able to resolve the two spots. This is the Rayleigh limit and the resolution defined by this (arbitrary) criterion is:

$$\Delta x = 0.61 \cdot \frac{\lambda}{NA}$$

where **λ** is the wavelength of the light and **NA = n · sin α**, the numerical aperture of the lens system, while **n** is the index of refraction of the surrounding medium.
The maximum NA for commercially available objectives is about 0.95 when working in air and about 1.4 for oil immersion objectives (sample immersed in oil).
From this formula, one can see that the maximum resolution for conventional microscopy is

- **2/3 · λ** in air and
- **1/2 · λ** using immersion oil.

Using confocal microscopy this resolution can be further improved to

- **1/2 · λ** in air and
- **1/3 · λ** using immersion oil.

These values are only valid for optimum conditions, e.g. thin samples.
The only way to overcome the diffraction limit is to observe very close to the sample in the near-field regime (observer-sample distance **λ/5**).
The idea was first proposed by SYNGE in 1928. He suggested using a metal plate with holes much smaller than the wavelength of light, to illuminate this with light from the back side and then scan this plate in close proximity across a sample. If the plate-sample distance is much smaller than the diameter of the holes, the resolution is limited by the diameter of the light source (holes) and not by the wavelength of light.
It was not possible at that time to prove this idea and it took until 1972 before ASH and NICOLLS could verify the theory with electromagnetic waves in the microwave range (3 cm wavelength, 0.5 mm resolution ⇒ **λ/60**). The first results in the optical regime where obtained by POHL and BETZIG (1986), who used pulled optical fibers and shear-force feedback for distance control.
The WITec SNOM solution goes a step further and uses microstructured, cantilever sensors and beam deflection feedback (well known from AFM) for distance control. To take advantage of the high resolution obtainable with SNOM, one should consider the following points:

The maximum resolution is given by the aperture of the sensor, but this resolution is only possible in the near-field. Therefore, the distance between tip and sample should be less than the radius of the aperture (< 50 nm for a 100 nm aperture), otherwise the resolution decreases. This resolution can only be obtained at the surface of a sample (one can not look inside the sample as in confocal microscopy).
As a result of the small distance between tip and sample there is always an interaction between topographic features and light emitted by the aperture, which gives rise to artifacts. The user should always be aware that these artifacts might be present. On the other hand, the importance of these artifacts should not be exaggerated.
A careful comparison of the optical image with the simultaneously obtained topographic image can help to identify artifacts. If one finds an optical feature at exactly the same position as a topographic step, one should be skeptical. Particularly if the change in optical contrast is only a few percent of the overall intensity.
If there is a topographic step, the distance between tip and sample changes as does the coupling of the electrical field (near-field) between them, which may cause a change in detected intensity.
Usually, the optical aperture is slightly shifted (50-150 nm) from the point of contact. This is due to the fact that the mechanical contact is somewhere at the rim of the optical aperture. The hollow aperture is surrounded by a 100-150 nm thick layer of aluminum providing the mechanical contact. Therefore, a genuine optical contrast should show a shift between the optical and topographic images of 50-150 nm.
The preferable mode for near-field optical microscopy is transmission. In this mode, the light transmitted through the transparent (or fluorescing/luminescing) sample is collected by the detector and topographic artifacts are usually a minor problem.
In reflection mode, the light transmitted through the aperture and reflected from the sample surface is collected with auxiliary optics. In this mode, topographyinduced artifacts are most likely and can even dominate the image. The reflection mode is most often used when the sample does not transmit the excitation light. In this case, the light transmitted through the aperture must cross the extremely small gap between tip and sample. If the tip is scanned across a topographic step, it is very likely that the detected light intensity would change and give rise to topography-induced artifacts. Here again, a careful comparison between topography and optical images is very helpful in distinguishing actual sample features from artifacts.
The following near-field optical modes are possible with the WITec system:

- near-field transmission
- near-field fluorescence/luminescence transmission (using additional filters)
- near-field reflection (with the reflection mode upgrade)
- near-field reflection fluorescence/luminescence (with the reflection mode upgrade and additional filters)

Setting up a measurement

This section describes how to start a SNOM measurement in transmission.

Sample mounting and focusing

1. Select the appropriate SNOM configuration.
2. Make sure the magnetically fixed cantilever arm is removed.
3. **Focus on the sample** using the AFM objective and search for the region of interest.
4. Optional: Set the Microscope-Z user position to zero for your reference.
5. Focus on the sample using the inverted objective including the steps marked with "For transmission".

Coarse alignment

6. Follow the steps in the **general procedure** for AFM of the sections Cantilever mounting and Adjustment. Ignore the second hint in article 9.a. in the general procedure and regard to the following points:
   - Position the cantilever like shown in Fig. 1 so that the laser is focused into the aperture. Open the laser shutter for that and activate the Laser Shutter Lock (for TruePower).
   - For contact mode: 1 V is a good starting value for the setpoint.

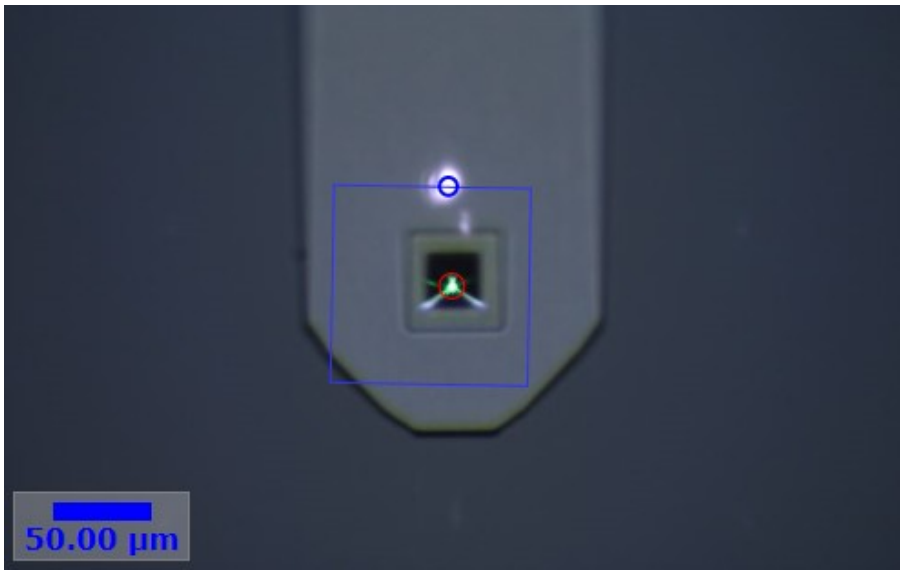


Fig. 1: SNOM cantilever with laser focused into the aperture (top view)

Fine alignment

7. Check that the beam spot is centered on the four quadrant diode.
8. Click on **Start Approach**.
9. Optional: As soon as the tip is in contact with the surface (T-B signal reaches the setpoint and the scan table starts to retract) the approach can be aborted by clicking **Stop**.
10. After double-checking that the Retract Distance [µm] is set to 50 µm, press **Retract Tip**.
11. Select the bottom camera increase the top illumination and/or use the bottom illumination, if present.
12. Move up the inverted objective until the tip of the pyramid is in focus (about 50 µm). (Refer to the hints section.)

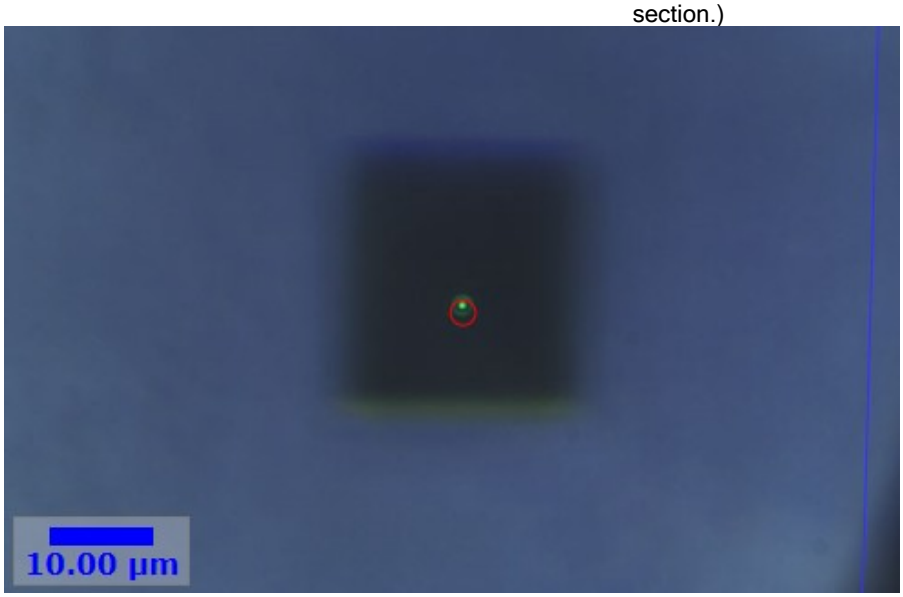


Fig. 2: SNOM cantilever with transmitted light (bottom view)

13. Switch to Raman Mode. For non-automated systems: Configure the upright beampath for Raman.
14. Increase the brightness of the video image and if necessary the laser power until you see some laser light. (Refer to the hints section.)
15. If there is no light spot visible at the aperture like in Fig. 2, slightly change the cantilever position in the x-y direction until you see it.
16. Focus the inverted objective on this spot.
17. Fine-adjust the cantilever position in order to maximize the throughput through the cantilever.

Approach and measurement

18. Check that the beam spot is centered on the four quadrant diode.
19. Click on **Start Approach**.
20. Move the inverted objective down to focus on the tip of the pyramid and the transmitted light again. (Refer to

the hints section.)
21. Move the inverted objective in the x-y direction until the laser spot is in the green circle.
22. Reduce the laser power until it is barely visible.
23. If necessary: Set up the inverted beampath for measurement.
24. Click on **Start Oscilloscope**. (Refer to the hints section.)
25. Maximize the signal by moving the inverted objective in x, y and z direction.
26. Adjust the laser power for an appropriate signal intensity.
27. **Stop** the Oscilloscope.
28. Start the measurement.

Further information:
Feedback settings, Detection, AFM Contact mode, Data channels (Topography, Count Rate)

Hints

Always double-check that you selected the appropriate device before you move anything. In case of the cantilever position or the inverted objective the corresponding window needs to be open.

- For 12.: If you are not able to see the tip of the pyramid, focus on the edge of the cantilever and then move the inverted microscope 8-10 µm down.
- For 14.: The new user might have difficulties in positioning the cantilever in order to obtain light through the cantilever. The following steps can be performed if no light can be obtained through the cantilever:
  - The laser intensity can be increased. This is only possible up to a certain point since the scattered light will start to dominate the image if the laser intensity is too high. Note also, that by increasing the laser power the power dissipated into the cantilever increases. Since it acts similar to a bi-metal strip this will change the bending of the cantilever. Again move the cantilever position in X and Y in order to find the transmitted light.
  - Follow the hint for 12. This ensures the proper focusing onto the tip of the pyramid. Again move the cantilever position in X and Y in order to find the transmitted light.
  - Move the cantilever to the side in order to allow the laser beam to hit the inverted objective directly. You most likely will have to decrease the laser intensity in order to locate the exact position of the laser spot. Then verify that this position is where the red circle on the video screen is. Adjust the position of the inverted microscope in X and Y if necessary. Once this is optimized, move the cantilever back and move the cantilever in X and Y in order to find the transmitted light.
- For 20.: You need to go down at least 50 µm plus half of the scan table z range. You can follow the tip with the inverted objective already during approach.
- For 24.: Make sure the detector is not overloaded. Refer to Detection.

Pick-Up mode

System requirements:

- Additional filter for blocking the beam deflection laser before detection

Setting up Pick-Up mode

1. Configure the beampath for excitation from below and detection from above. Switch to laser from below in the **inverted objective control**.
2. Follow the **instructions for the standard measurement** for sample mounting and focusing and regard to the following points:
   - For focusing the laser on the sample use the Confocal configuration and adjust the piezo stage z-position to 0. (This ensures that the laser is in focus after tip approach.)
   - Change back to the appropriate SNOM configuration afterwards.

Coarse alignment

3. Follow the steps in the **general procedure** for AFM of the sections Cantilever mounting and Adjustment. Ignore the second hint in article 9.a. in the general procedure and regard to the following points:
   - Position the cantilever like shown in Fig. 1. The aperture needs to be within the green circle and it needs to be focused inside.
   - For contact mode: 1 V is a good starting value for the setpoint.

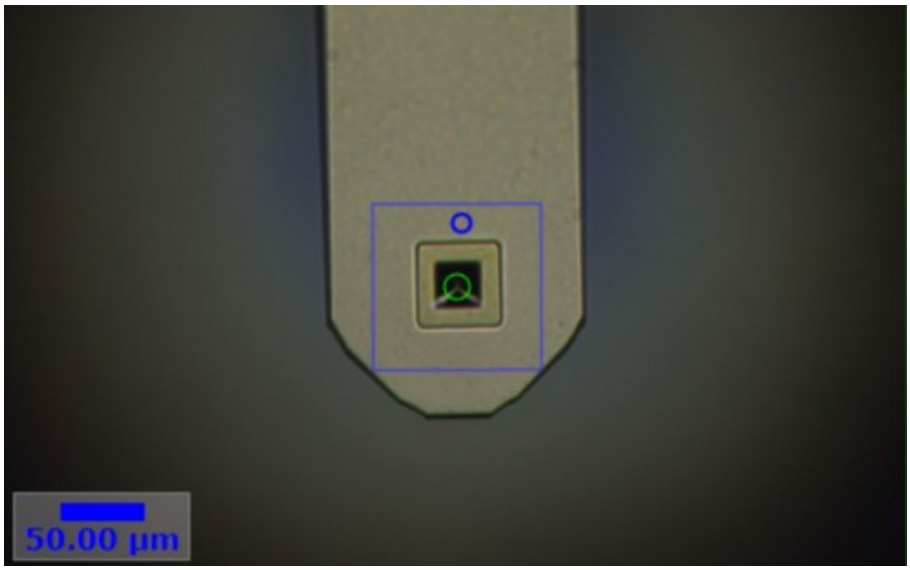


Fig. 1: SNOM cantilever focused into the aperture (top view)

Fine alignment

4. Check that the beam spot is centered on the four quadrant diode.
5. Click on Start Approach.
6. After successful approach adjust the setpoint to -10 V. (This retracts the piezo stage and the cantilever is about 10 µm above the surface.)
7. Switch off the illumination. For non-automated systems: Put the withelight slider to the empty or darkfield position.
8. Open the Laser Shutter.
9. Increase the brightness of the video image and, if necessary, the laser power until you see some laser light.
10. Slightly change the cantilever position to maximize the intensity of the light spot like in Fig. 2. Start with the z-axis.

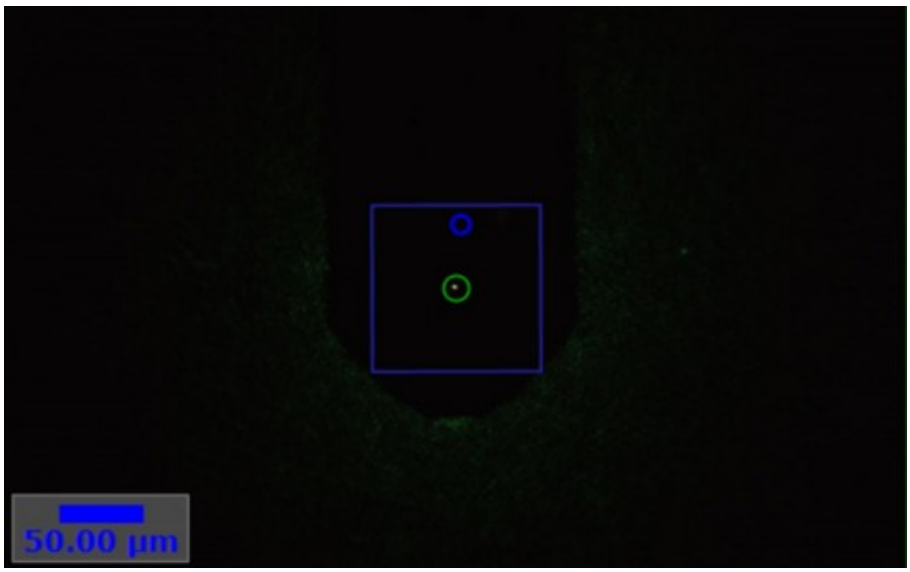


Fig. 2: SNOM cantilever with transmitted light (top view)

Measurement

11. Switch to Raman Mode. For non-automated systems: Remove also the top camera from the beampath.
12. Check that the beam spot is centered on the four quadrant diode.
13. Put the setpoint back to the initial value. (The piezo stage moves up until the cantilever is back in contact.)
14. Reduce the laser power until it is barely visible.
15. Click on Start Oscilloscope.
16. Adjust the laser power for an appropriate signal intensity.
17. Stop the Oscilloscope.
18. Start the measurement.

Reflection mode

System requirements:

- Reflection mode module

Setting up reflection mode

1. Check that the reflection microscope is connected with the photon counting device.
2. Follow the instructions for the transmission mode for sample mounting and focusing (skip 5.) and coarse alignment.
3. Select the Rear camera.
4. Move the reflection microscope using their positioning stage until you see the cantilever from the backside and try to focus on the tip using the forward/backward screw. (Fig. 1)

Fig. 1: SNOM cantilever in rear view with reflection on the sample surface.

Fine alignment

5. Switch to Raman Mode. For non-automated systems: Configure the upright beampath for Raman.
6. Place the stray light protection around the sample or close the front door of the enclosure to reduce ambient light on the sample.
7. Increase the brightness of the video image and if necessary the laser power until you see a small laser spot at the tip. (Refer to the hints section.)
8. Fine-adjust the cantilever position in order to maximize the throughput through the cantilever.

Approach and measurement

9. Check that the beam spot is centered on the four quadrant diode.
10. Click on Start Approach
11. Adjust the reflection microscope (using the left/right and up/down screw) until the light emitted from the cantilever is in the green circle of the video image. (If this is the case the light is hitting the fiber correctly.)

Do NOT alter the adjustment screws of the fiber coupling unit.

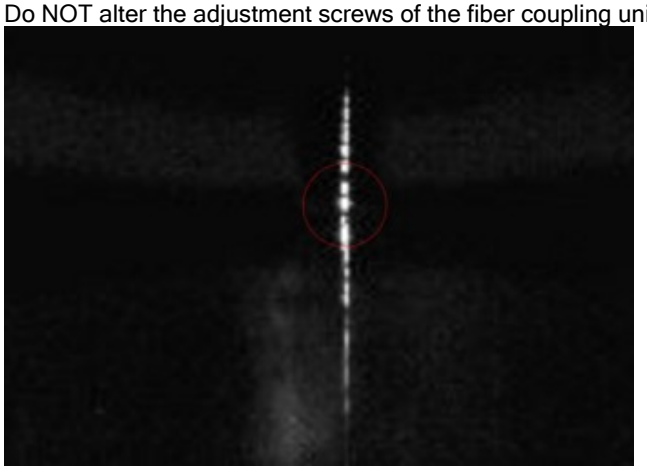


Fig 2: Light spot from the tip and reflections on cantilever and sample.

12. Click on Start Oscilloscope.
13. Optimize the signal by slightly moving the reflection microscope in all three direction. (Refer to the hints section.)

When the tip is in contact, try to avoid any vibrations of the microscope body.

14. Adjust the laser power for an appropriate signal intensity.

15. Stop the Oscilloscope.
16. Start the measurement.

Hints

- Don't be confused by reflections on the sample surface.
- Changing the laser power may also change the bending of the cantilever (acts similar to a bi-metal strip). This can cause the tip to loose the sample contact. If so, readjust the beam spot on the four quadrant diode while the tip is retracted.
- For 7.: If you are unable to observe the light from the cantilever aperture:
  - Try to repeat the coarse alignment.
  - Align the cantilever in transmission mode including the fine alignment (until 17.) using a 0.17 mm glass slide as a sample. Then move the microscope up using the microscope z control, replace the cover glass with your sample and move the microscope down again.
- For 13.: Reflections close to the tip can even have higher intensity then the near-field signal.
- For 13.: If the sample completely blocks the aperture, you will maybe observe no signal. Refer to the next hint.
- For 15.: If you don't observe the structure of your sample carefully adjust the reflection microscope while doing an image scan until you observe the near-field signal.
- If you observe the near-field signal outside of the red circle, you can reposition the red circle (Probe position) in the Menu.

SNOM AC Overview

SNOM AC mode measurements are especially useful for eliminating topography induced artifacts in reflection mode measurements. Additionally, it may be beneficial for samples that are either soft or weakly bound to the substrate. For these samples, the operation in the intermediate contact regime can be preferable because the AC mode reduces the effect of tip induced sample damage and the dragging of particles over the surface.
One should notice however, that the oscillation of the cantilever can have an influence on the contrast of the near field measurements. If a high oscillation amplitude of the cantilever is chosen, the tip will, on average, be further from the surface. Due to the fact that the contrast in SNOM measurements is strongly correlated with the tip-sample distance, it will decease with increasing amplitude.

System requirements:

- AFM (AS and RAS systems)

Recommended Cantilevers:
SNOM AC mode cantilevers are shorter, stiffer and feature a higher resonance frequency (≈ 45 kHz) compared to SNOM contact cantilevers and can be purchased from WITec.

Parameters

Follow the steps in the general procedure. For information about the AC mode adjustment process refer to AFM AC mode.

Setpoint

In comparison to AFM AC mode measurements, one should note that SNOM AC cantilevers are longer and significantly wider than AFM AC mode cantilevers. This results in a significantly stronger air damping of the oscillation amplitude than is the case for AFM AC mode cantilevers (see Fig. 1).

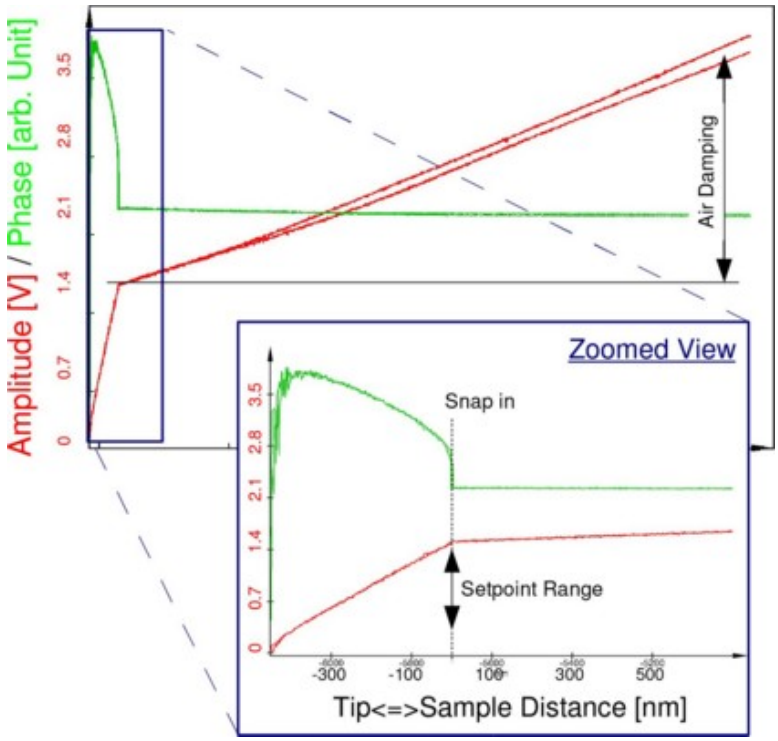


Fig. 1: Typical curves for phase (green) and amplitude (red) as a function of tip? sample distance. The usable setpoint range is indicated in the zoomed view.

The setpoint should be a significantly lower value (e.g. 1/4) than the free amplitude. This is necessary due to the heavy air damping which can be seen from the amplitude- and phase-distance curves in Fig. 1. The total tip-sample distance covered in the curve displayed in Fig. 1 exceeded 10 µm and the oscillation was still damped due to the air cushion between the tip and the sample. It is therefore important to move the tip far enough away from the sample surface (e.g. 100 µm) when determining the free oscillation amplitude.

The setpoint for SNOM AC measurements can be chosen in the range as indicated in the zoomed view of Fig. 1. (A higher setpoint results in a weaker sample-tip interaction.)

Confocal Overview

Confocal Microscopy is using a pinhole to reject out-of-focus light to improve the optical resolution and forms an image by scanning with a point detector.

Topics:

- Theoretical Background
- Setting up a measurement
- Example measurement

Confocal modes:

Depending on the system configuration the following confocal modes are possible with a WITec system:

- **Confocal microscopy in reflection and transmission**
- **Confocal fluorescence microscopy in reflection and transmission**
- StrobeLock **– Time-resolved microscopy**

Configurations:

The following configurations are available depending on the equipped photon counting device:

- Confocal PMT/APD
- Confocal Transmission APD/APD (z movement during measurement by piezo stage)

Measurement modes:

- Oscilloscope: displays the output channel of a photon counting device as a function of time similar to the display of an oscilloscope.
- Image Scan: acquisition of confocal images using the piezo scanner
- Large Area Scan: acquisition of confocal images using the cross-table

System requirements:

- systems equipped with a photon counting device

- inverted objective for transmission microscopy

Theory

The principles of confocal microscopy can be found in the general section.
To take full advantage of the lateral and depth resolution possible with confocal microscopy, the size of the pinhole (core diameter of the multi-mode fiber for detection) must be properly chosen.
The optimum pinhole diameter depends on the optical properties of the microscope objective along with the wavelength employed and can be calculated using the following formula:

$$D \leq \frac{\lambda \cdot v \cdot M}{NA \cdot \pi}$$

where **λ** is the wavelength of the laser, **M** is the magnification and **NA** is the numerical aperture of the microscope objective.
The property **v** is given in optical coordinates and should be 2.5 for the best depth resolution and 0.5 for maximum lateral resolution. If **v** < 0.5 is chosen, the lateral resolution will be $\sqrt{2} \approx 1.4$ times better than for conventional microscopy. However, in this case most of the light reflected from the sample does not reach the detector, so one sacrifices efficiency.

Procedure

Remarks

- For confocal fluorescence microscopy additional filters are needed to block the light of the laser.
- Reflection mode: For newer systems the edge filter is maybe not removable, so the signal will be very low, if there is no Raman or fluorescence from the sample. Remove the filter from the beampath if you just want to do confocal microscopy and if possible.
- Check that you are use an appropriate fiber (refer to Theory)

Before starting the oscilloscope ensure that the laser power is adjusted to very low power. Although the count rate of the photon counting devices (APD or PMT) is constantly monitored to avoid overexposure, a strong and abrupt rise of it, can destroy the unit.

Procedure

Choose an appropriate Confocal configuration.

Alignment for reflection mode

1. Focus on a piece of silicon.
2. For non-automated systems: configure the beampath for confocal.
3. Adjust the laser to very low power.
4. Click on Start Oscilloscope.
5. Rise the laser power until you see a signal.
6. Optimize the signal (compare to Raman).

Alignment for transmission mode

1. Focus on a cover glass using the inverted objective including the steps marked with "For transmission".
2. For non-automated systems: configure the upright and inverted beampath for measurement.
3. Adjust the laser to very low power.
4. Click on Start Oscilloscope.
5. Optimize the signal using the inverted objective.

Measurement

1. If the system is aligned, insert your sample focus on it (for transmission mode from both

sides).

2. Click on Start Oscilloscope.
3. Adjust the laser power.
4. Click on Stop.
5. Start your measurement.

Hints

- Make sure the detector is not overloaded. Refer to Detection.

StrobeLock Overview

StrobeLock is an extension for WITec microscope systems that enables fluorescence lifetime measurements. The available measuring modes include Fluorescence Lifetime Spectroscopy, Fluorescence Lifetime Imaging as well as Time-Resolved Luminescence Microscopy.

Topics:

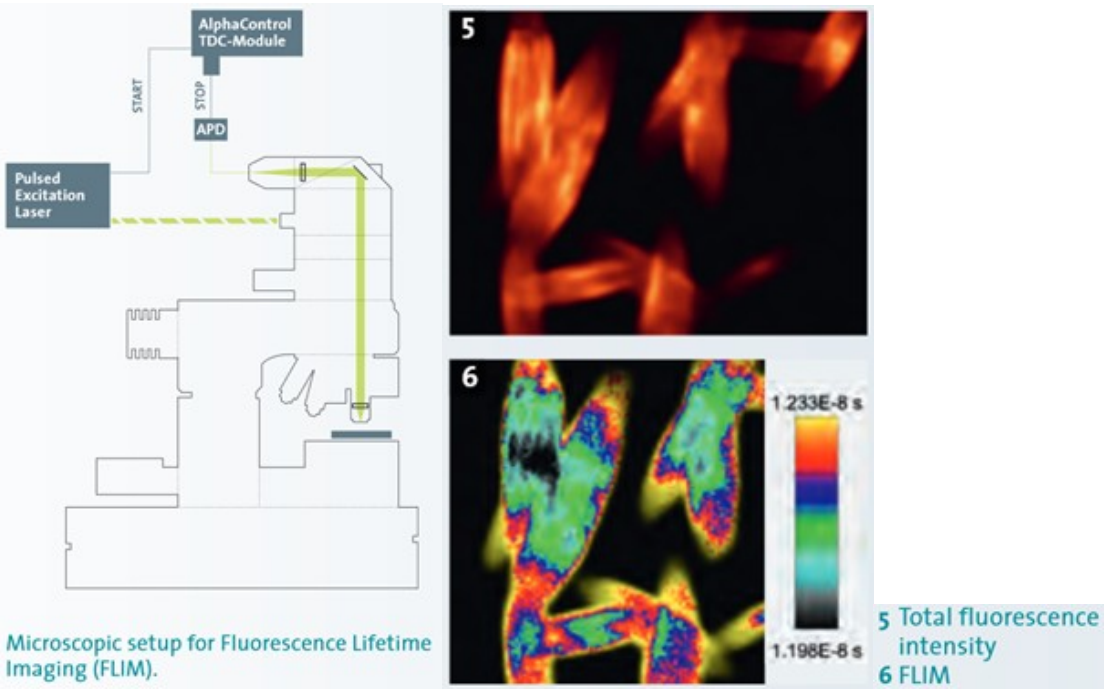
- Theoretical Background
- Laser settings – for PicoQuant LDH Laser only
- Setting up a measurement
- Example measurement
- Data evaluation

Measurement modes:

- Oscilloscope: Displays the output channel of a APD as a function of time.
- Single Spectrum: Acquisition of a time-spectrum at the current position.
- Fast Time Series: Continuous acquisition of time-spectra over time.
- Slow Series: Intermittent time series.
- Line Scan: Acquisition of time-spectra along a line in three dimensional space.
- Large Area Scan: 2D or 3D Imaging using the motorized stage up to centimeter scale.
- Image Scan: 2D or 3D Imaging using the piezo stage for highest resolution.

System requirements:

- an Avalanche Photo Diode (APD) which is a single-photon sensitive detector
- an appropriate pulsed laser for the optical excitation of the sample
- a Time-Correlated Single-Photon Counting electronic module (TCSPC) included in the alphaControl controller



Microscopic setup for Fluorescence Lifetime Imaging (FLIM).

5 Total fluorescence intensity
6 FLIM

Theory

To understand the principle of fluorescence lifetime measurements some theoretical considerations are helpful. A fluorescent sample can be regarded as an ensemble of single fluorescing objects. An experiment on such an ensemble shows the same characteristics as a measurement on a single one of these objects performed very often. The simplest model for describing the fluorescence behavior of such an emitter is a two-energy-level system:

- It exhibits a ground state and an excited state.
- It can be brought from the ground state to the excited state by energy absorption.
- The relaxation from the excited state back to the ground state happens spontaneously and the energy difference is emitted in form of a fluorescence photon.

If the emitter was brought to the excited state at **t** = 0, the fluorescence intensity at a later time **t** can be described by

$$I(t) = I_0 \cdot e^{-\frac{t}{\tau}},$$

where **τ** is the characteristic lifetime of the excited state and **I0** is a constant factor.

If the emitter exhibits not only one but **n** possible paths for absorption and subsequent emission of a fluorescence photon, this intensity becomes:

$$I(t) = \sum_{i=0}^{n} A_i \cdot e^{-\frac{t}{\tau_i}},$$

where **Ai** describe the relative probabilities of the different paths and **τi** represent the characteristic lifetimes of the different excited states.

In Fluorescence Lifetime measurements this characteristic temporal behavior of a sample's emitted fluorescence is determined:

- The sample is excited in a pulsed way. This can happen e.g. by a pulsed laser or electrically.
- A detector that is sensitive for very small amounts of light emitted by the sample.
- An electronic device that records the time differences between excitation events and fluorescence photon emission.
- By measuring these time-differences very often a histogram is recorded – in WITec Control it is called Time-Spectrum.
- Such a time-spectrum can be described by the equations above and the characteristic parameters like the fluorescence-lifetime **t** can be determined by exponential fits.

Laser settings

The following section is only relevant if a pulsed laser of the type **PicoQuant LDH** is used for excitation of the sample.

1. Start the laser by the power switch on the backside and the key switch on the frontside of the laser driving electronics (figure 1).
2. Choose the desired laser pulse repetition rate by using the TRIGGER and REP. FREQUENCY adjustment knobs:
   a. Check the manual of the PicoQuant LDH laser for the appropriate combination of both settings.
   b. For **20 MHz**, which is a good starting point, use:
      i. **TRIGGER: INT 1**
      ii. **REP. FREQUENCY: 4**
   c. For continuous wave operation set TRIGGER to CW.

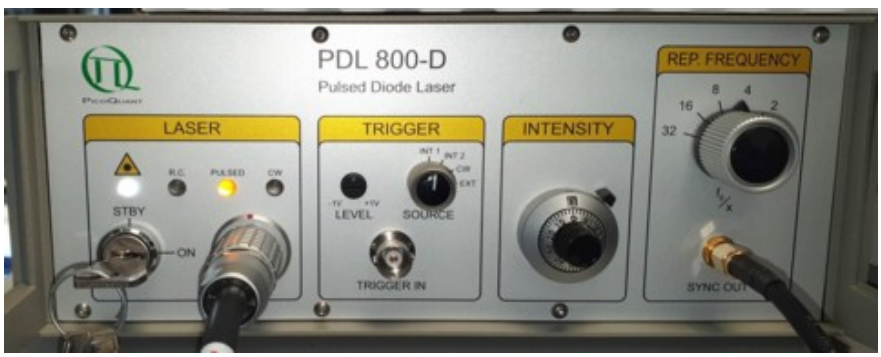


Figure 1: PDL 800-D driving electronics for PicoQuant LDH laser

3. Find out the appropriate diode current setting:
   a. Observe the emitted laser power by one of the following possibilities:
      i. using a power meter
      ii. observing the laser spot on the video image
      iii. measuring the count rate from a fluorescing sample
   b. Open the manual laser power adjustment screw at the laser head (Figure 2).
   c. Increase the diode current using the INTENSITY potentiometer screw (Figure 1). As long as the laser

threshold is not reached, the emitted laser power will not increase at all or only slowly. As soon as the laser threshold is reached, the increase will be significantly faster. Note the corresponding screw position.

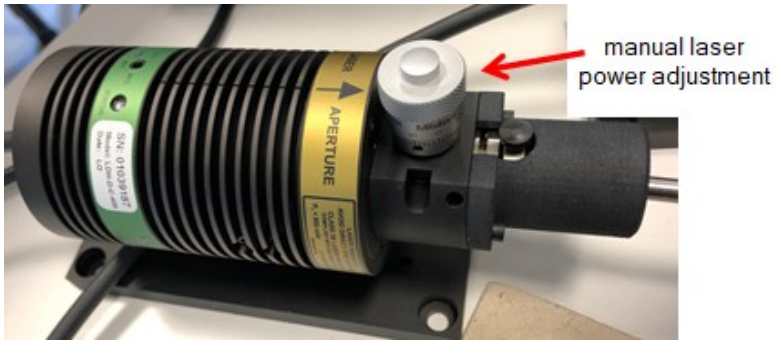


Figure 2: PicoQuant LDH series laser head

d. For the best performance in time resolved measurements (like FLIM), the excitation laser pulses should be as short as possible in general.
In case of the PicoQuant LDH this can be achieved, if the **diode current is as small as possible** (see also the PicoQuant laser's data sheets) – **but still above the laser threshold**! A potentiometer setting of 10 % above the threshold value determined in the step before is a good starting point (adjust e.g. 7.7, if the laser threshold screw position was 7.0).

4. The diode current setting can be kept constant from now on – **change the emitted laser power by the micrometer screw at the laser head only**! Touching the INTENSITY screw is only necessary if a higher maximum laser emission power is required.

Parameters

Mount your sample or use a reference sample for the adjustment and follow the steps in Focus on sample.

The appropriate excitation laser power

1. Select the configuration Time Resolved Microscopy.
2. Close the manual laser attenuator at the laser head completely.
3. Start the oscilloscope and have a look on the graph showing the count rate; at least the dark count rate of the APD should be measured with small fluctuations.
4. Switch the laser to pulsed operation – 20 MHz is recommended.
5. Start the oscilloscope and increase the laser power slowly until a significant count rate is measured (status window or graph showing the fluorescence count rate).
6. Increase the laser power until the appropriate fluorescence count rate is reached:
To prevent over-representing short-time photon-counting events, the fluorescence count rate should not exceed 1 % of the laser repetition rate, e.g. 200 kHz count rate for a laser repetition rate of 20 MHz.
7. In the Time Spectrum window a decreasing exponential decay curve similar to the one shown in Figure 2 should be visible now.

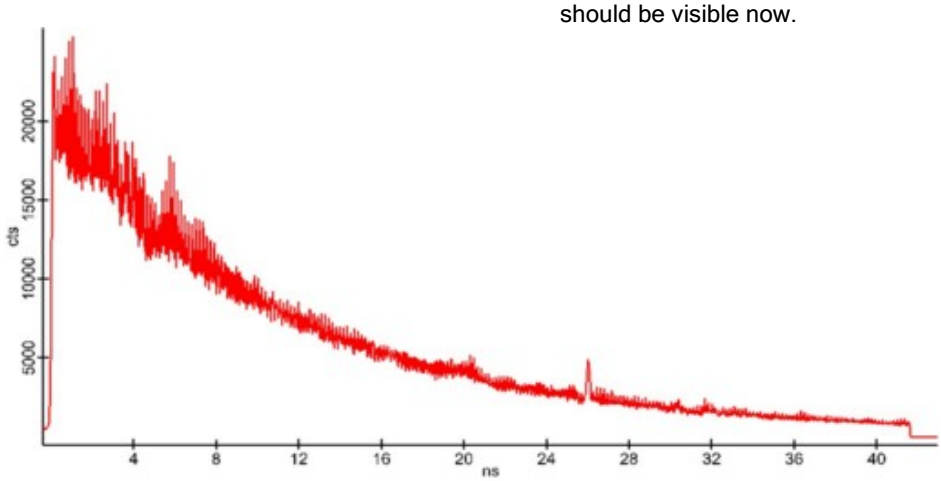


Figure 1: Example for "uncorrected" Time Spectrum from reference sample

Time Spectrograph Parameters

All parameters relevant for the recording of time spectra are stored in the corresponding WITec Control configuration. If they are not set correctly, it might happen that:

1. only a part of the curve above can be seen
2. the time scale is shifted
3. there are short-time oscillations in the spectrum (as visible in the spectrum above)
4. there is a small peak-artefact (in the spectrum above at around 26 ns)

If you see one of the things above, you have to optimize the parameters in the Time Spectrograph parameter group:

1. Laser Repetition Rate has to correspond to the repetition rate of the excitation laser.
2. With the parameters Time Bins and Time Binning the recording time and the timing resolution of the time-spectra can be defined. Recommended starting values for 20 MHz laser repetition rate:

Time Bins: 1800
Time Binning: 1

3. Most probably the visible time spectrum will be shifted to the right compared to the one shown above. This difference can be corrected with the following two parameters:
    a. Adjust Time Offset until the zero of the x-axis corresponds to the the steep increase of the time-spectrum.
    b. If desired: Shift the whole time-spectrum to the left edge by changing Start Time.
4. Record a single spectrum e.g. with 1 s integration time and 10 accumulations. Probably, two artefacts will be visible (compare to the spectrum above):
    a. A small peak (in Figure 1 at 26 ns): Such a peak occurs if the specified laser repetition rate is not 100 % correct. To correct for that:
        i. Change Laser Repetition Rate slightly (e.g. from 20 MHz to 19.95 MHz).
        ii. Record a single spectrum again and check if the peak changes.
        iii. Proceed optimizing Laser Repetition Rate until the peak disappears (almost) completely.
    b. Oscillations on a sub-ns scale: They are caused by the non-linearity of the TDC board time bins. To remove them, the readout of the TDC board must be calibrated:
        i. Close the manual attenuator of the laser completely.
        ii. Change the microscope to get white light on the sample.
        iii. Minimize the intensity of the white light source.
        iv. Start Oscilloscope and switch on the white light source.
        v. Adjust the illumination intensity to achieve a count rate corresponding to 1 % of the laser repetition rate.
        vi. Stop the oscilloscope.
        vii. Press **Calibrate**.
        viii. Observe the recorded time spectrum using the y-axis auto-scale.
        ix. If the spectrum is not changing qualitatively anymore, stop the measurement. The calibration is finished now.
        x. Save the calibration using Save Calibration.
5. If a single spectrum is recorded again, all anomalies should be removed (as in the spectrum in Figure 2).

If one of the parameters in the WITec Control section Time Spectrograph is changed, it might be necessary to repeat the calibration of the previous step completely.

It makes sense to store dedicated configurations and calibrations for all the different settings that are used frequently (e.g. for different relevant repetition rates).

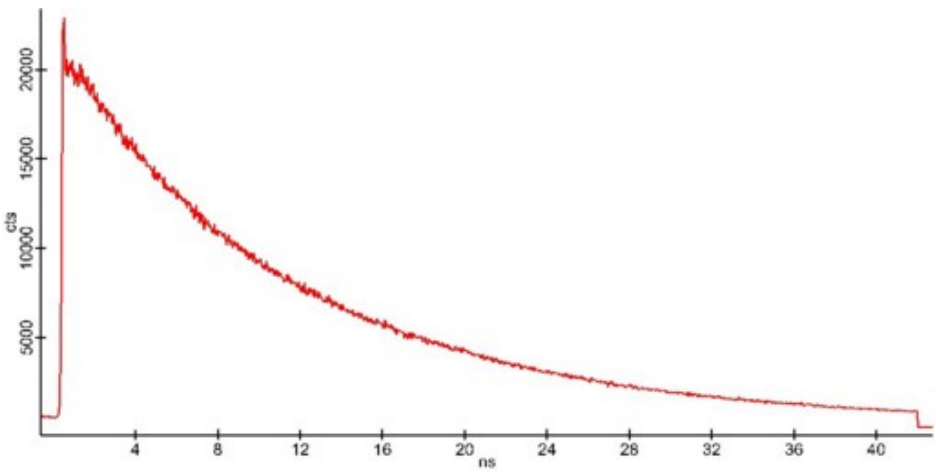


Figure 2: Corrected Time spectrum

Further information:
Time Spectrograph, Detection

Reference sample

Appropriate reference samples:

- For excitation wavelengths between 450 and 630 nm: The diamond reference sample can be used.
- For other wavelengths: A marker pen on a flat surface (e.g. glass) is feasible; yellow and red colored pens work

the best.

It is recommended to check the fluorescence spectrum of the reference sample:

1. Choose an appropriate Raman configuration. Use a small grating and set the Units to nm in the Spectrograph parameter group. Record a fluorescence spectrum.
2. If possible: Switch the laser to continuous wave operation.
3. Focus the laser on the reference sample and check if a reasonable fluorescence spectrum can be detected. In Figure 1, the fluorescence spectrum of the diamond reference sample recorded with a Time-Bandwidth Lynx laser is shown. Using a PicoQuant LDH the Raman lines would not be visible.

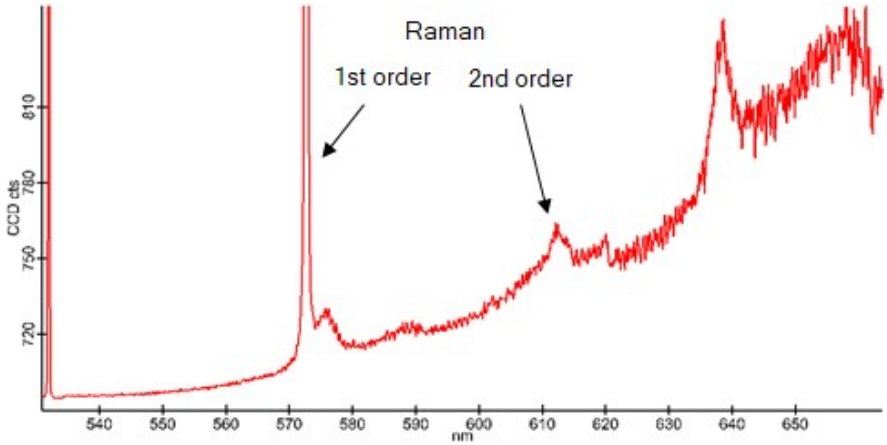


Figure 1: Fluorescence spectrum of diamond reference sample recorded with Time-Bandwidth Lynx laser.

Data evaluation

- The fluorescence lifetime measurements basically work the same as all other spectral measurements with the WITec instrument. The only difference: time-spectra are recorded instead of e.g. Raman or fluorescence spectra.
- A FLIM-measurement is a 2D- or even 3D-scan where the fluorescence lifetime(s) is/are displayed instead of Raman intensities or similar.
- Even all other routines that are known from confocal Raman spectroscopy and imaging are also possible in FLIM: Single Spectra as well as Line Scans, Image Scans and Large Area Scans also in 3D.
- As mentioned above the parameter visualized in FLIM images is/are the decay time(s) from the equations in the Theory section. This/these time(s) can be yielded by using the Advanced Fitting tool and choosing a single- (or multi-) exponential function to describe the measured time spectra.

Photocurrent Overview

Photocurrent is the result of light exposure to a photosensitive device, like a photodiode.

Topics:

- Setting up a measurement
- Example measurement

Photcurrent modes:

Depending on the system configuration the following photocurrent modes are possible with a WITec system:

- **Photocurrent**
- **Photocurrent with laser shopper and external lock-in**
- **Photocurrent combined with Raman or even SNOM**

Configurations:

The following configurations are available depending on the used data channel:

- Photocurrent AUX1/EXT1

Measurement modes:

- Oscilloscope: displays the current output from the amplifier
- Image Scan: acquisition of photocurrent images using the piezo scanner
- Large Area Scan: acquisition of photocurrent images using the cross-table

System requirements:

- Raman or SNOM (M+, R, RA, RS, RAS or S system)

- Extension or AUX A/D card for the alphaControl
- Low Noise Current Amplifier (e.g. femto)
- Optional: AUX Out card for the alphaControl for bias voltage supply.
- Optional: laser shopper and external lock-in

Procedure

1. Make sure the current amplifier or external lock-in is connected to either EXT 1 or AUX 1, depending on your photocurrent configuration.
2. Select your photocurrent configuration.
3. Focus on your sample.
4. Adjust the laser to low laser power suitable for your sample.
5. For non-automated systems: Configure the beampath for Raman and open the laser shutter.
6. Start the oscilloscope and check the signal. Adjust the current amplifier amplification to get a signal between between 0.01 V and 1 V.
7. Start your measurement.

Further information:

Data channels (Aux 1, Ext 1)

Hints

- If the amplification is too low, digitalization steps become visible in the signal.
- Controlling the bias over the Aux out channel is accomplished over the EFM Control.

Data evaluation

The measured data is in V. In order to calculate the photocurrent in A, divide the data by the amplification factor of the current amplifier. E.g. the setting in fig. 1 shows an amplification of 104. Drop the measured data on the calculator tool. If the amplification is 104 type X1/10 behind the equal sign and mA as unit.

Fig. 1: Example of the amplification setting of the femto current amplifier.

Reference sample

Sample: WITec Photocurrent reference sample

Ensure that the following settings are correctly applied:

- Femto amplifier gain to 10⁴ (small switch to “L“ position)
- Other Switches: GND/Bias to GND, 10Hz/FBW to FBW, AC/DC to DC
- 532 nm laser with approx. 7 mW
- 20x objective

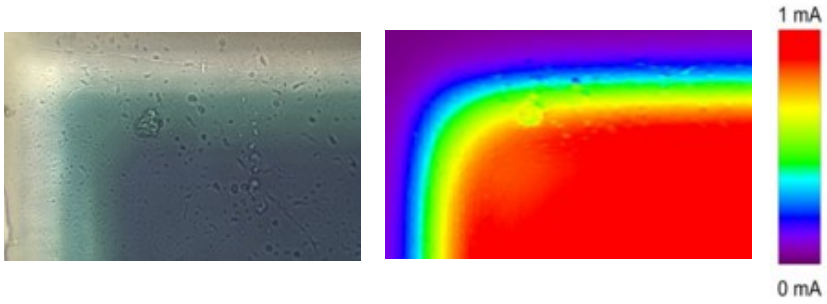


Fig. 1: Left: Video image, Right: current distribution

- The active zone (dark), a transition zone and the frame (bright) can clearly be identified in the video image.
- According to these zones, the measured photo current shows the expected behavior. 0 mA at the frame, up to approx. 1 mA in the center of the active zone. Fig. 1 shows an example of the upper left edge.
  - Remark: The photo diode consists of a transparent coating with approx. 170 µm and the silicon layer beneath, focus plane is not that critical.

Lithography

The lithography module can be used for sample manipulations on the nanometer and micrometer scale using an AFM tip or a laser.

Useable Configurations:
- AFM Contact and AFM AC for using an AFM tip or even a SNOM cantilever
- Confocal for using the laser

System requirements:
- AFM (A, RA or RAS system) for using an AFM tip
- Raman or SNOM (M+, R, RA, RS, RAS or S system) for using the laser

Required License feature:
- Lithography

Creating a script:
- List of commands
- Refer to the several example scripts in the "UserLithography" folder, which is the default folder for choosing a file.

Procedure:
1. Select one of the lithography configurations (see above).
2. Focus on the sample for laser lithography or follow the general procedure for AFM.
3. Choose the file in the Lithography section in the Control tree.
4. Optional: Click on Preview to check.
5. For laser lithography: Configure the beampath for Raman.
6. Click on Start Lithography.

Further information:
Lithography

Hints:
- Include DrawActive.txt and DrawInactive.txt in your script instead of hardcoding i.e. SetLaserShutter. This enables you to easily change the script from laser lithography to AFM or to change setpoint or retract values without changing it in every line.
- Refer to the DrawActive.txt file in the "include" subfolder of the "UserLithography" folder for further information about how to start and stop

the writing.

Profilometer

Profilometer is a configuration to use the Confocal Chromatic Sensor of TrueSurface Mk1 or Mk2 just for recording the topography. It is also possible to use this configuration with TrueSurface Mk3.

Measurement modes:

- Oscilloscope: displays the current value
- Image Scan: acquisition of profilometer scans using the piezo scanner
- Large Area Scan: acquisition of profilometer scans using the cross-table

System requirements:

- TrueSurface Mk1 or Mk2

Procedure:

1. Focus **between highest and lowest point of your sample.**
2. Change **to the TrueSurface objective.**
3. **For non-automated systems: Configure the beampath for TrueSurface.**
4. **Define and start the Large Area Scan or Image Scan.**