Confocal Raman Configuration

This document describes the principles of Confocal Raman Microscopy followed by the WITec Control software configuration for Confocal Raman Microscopy . The beam path and the step by step alignment procedure can be found in Sections 3 and 4.

1 Principles of Confocal Raman Microscopy

1.1 Introduction

The Raman effect is an interaction process of electromagnetic waves (light) with matter in which a vibrational quantum is excited (Stokes Raman scattering) or annihilated (Anti-Stokes Raman scattering).

When light of a certain wavelength interacts with a molecule, most photons are elastically scattered and therefore have the same energy as the incident photons.

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 effect is called the Raman effect and was discovered by Sir Chandrasekhara Raman in 1928. Unlike today, he used a filtered beam of sunlight as excitation source and his eye as a detector for the frequency shifted light. This was long before the development of the first laser by Maiman in 1960. Raman was awarded the Nobel prize in 1932 for this discovery. The theory behind the Raman effect was derived five years earlier by A. Smekal (1923).

The tremendous importance of the Raman effect lies in the fact that the energy shift between the exciting and the Raman scattered photon is caused by the excitation (or annihilation) of a molecular vibration. This energy shift is characteristic and therefore a fingerprint for the type and coordination of the molecules involved in the scattering process.

The *alpha300/alpha500/alpha700* system with its Raman capability is not only the most sensitive instrument for this purpose, it combines this sensitivity also with an unrivalled spatial resolution down to the sub-micrometer regime.

1.2 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:

$$\Delta t \cdot \Delta E \geq \frac{\hbar}{2} \quad (1)$$

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.

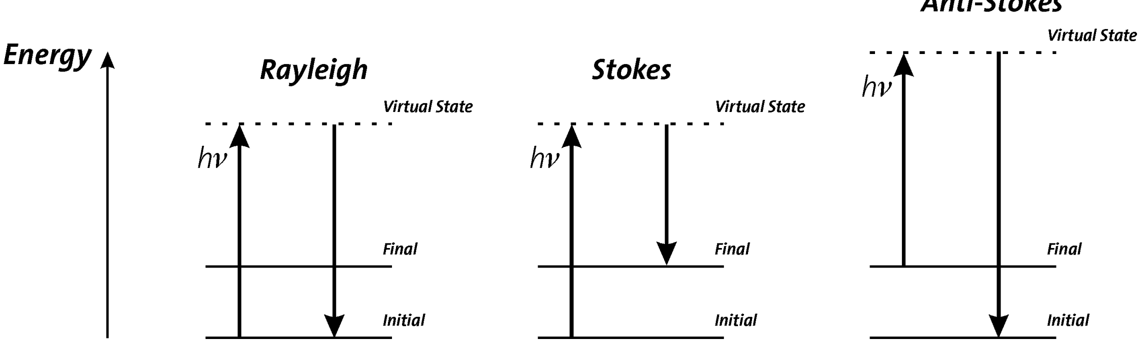


Fig. 1: Energy level diagram for Raman scattering

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:

$$\vec{P} = \vec{\vec{\alpha}} \cdot \vec{E} \quad (2)$$

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

$$\vec{E} = \vec{E_0}\cos(2\pi\nu t) \tag{3}$$

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

$$q_v = q_0 \cdot \cos(2\pi\nu_v t) \tag{4}$$

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

$$\alpha = \alpha_0 + \left(\frac{d\alpha}{dq_v}\right)_0 q_v + ... \tag{5}$$

$$\approx \alpha_0 + \left(\frac{d\alpha}{dq_v}\right)_0 q_0 cos(2\pi\nu_v t) := \alpha_0 + \alpha_1 q_v \tag{6}$$

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

$$\vec{P} = \alpha \cdot \vec{E} = (\alpha_0 + \alpha_1 q_v)\vec{E_0}\cos(2\pi\nu t) = (\alpha_0 + \alpha_1 q_0 cos(2\pi\nu_v t))\vec{E_0}\cos(2\pi\nu t) \tag{7}$$

$$= \alpha_0\vec{E_0}\cos(2\pi\nu t) + \alpha_1 q_0 \vec{E_0} cos(2\pi\nu_v t)\cos(2\pi\nu t) \tag{8}$$

$$= \underbrace{\alpha_0\vec{E_0}\cos(2\pi\nu t)}_{Rayleigh} + \underbrace{\frac{1}{2}\alpha_1 q_0\vec{E_0}cos(2\pi(\nu+\nu_v)t)}_{Anti-Stokes} + \underbrace{\frac{1}{2}\alpha_1 q_0\vec{E_0}cos(2\pi(\nu-\nu_v)t)}_{Stokes} \tag{9}$$

As one can see, besides the elastically scattered Rayleigh line, additional lines appear in the spectrum which are shifted $\pm\nu_v$ 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:

$$\bar{\nu} = \frac{1}{\lambda_{incident}} - \frac{1}{\lambda_{scattered}} \tag{10}$$

$\lambda_{incident}$ and $\lambda_{scattered}$ are the wavelengths (in cm) of the incident and Raman scattered photons, respectively.

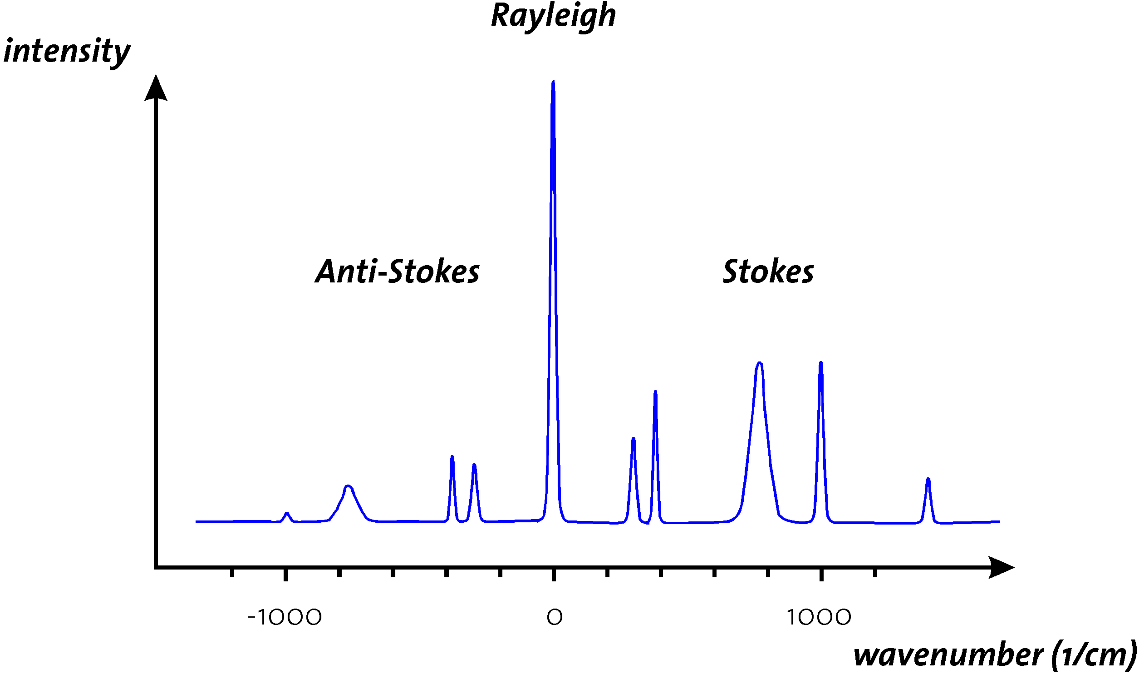


Fig. 2: Typical Raman spectrum

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

$$I \sim \nu^4 \tag{11}$$

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 impossible because of the strong fluorescence background. In the red (or even IR) part of the spectrum fluorescence is usually not a problem any more, but the excitation intensity has to be much higher (I$\sim \nu^4$!). Another problem is, that Silicon detectors can not 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

$$I_{Stokes} \sim (\nu - \nu_v)^4, \quad and \qquad I_{Anti-Stokes} \sim (\nu + \nu_v)^4 \tag{12}$$

and therefore $I_{Anti\text{-}Stokes} > I_{Stokes}$. Experimentally, one finds the opposite: $I_{Anti\text{-}Stokes} < I_{Stokes}$.

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 E_j

$$N_j = N \cdot e^{-\frac{E_j}{k_b T}} \tag{13}$$

Using the energy of a harmonic oscillator, one gets

$$N_j = N \cdot e^{-\frac{(j+1/2)h\nu}{k_b T}} \tag{14}$$

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

$$\frac{I_{Anti-Stokes}}{I_{Stokes}} \sim e^{-\frac{h\nu_v}{k_B T}} \cdot \left(\frac{\nu + \nu_v}{\nu - \nu_v}\right)^4 \tag{15}$$

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

$$I_{Stokes} > I_{Anti-Stokes} \tag{16}$$

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

1.3 The instrument

The *alpha300/alpha500/alpha700* system with its Raman capability combines a highly efficient Raman spectrometer with a high resolution confocal optical microscope. With this combination, it is not only possible to obtain a Raman spectrum of a sample but also to combine this chemical information with a lateral resolution in the sub-micrometer regime. Using green excitation light, resolution down to 220 nm is possible. The microscope, as well as the spectrometer and the detectors, are optimized for the highest throughput and efficiency which gives the *alpha300/alpha500/alpha700* an unrivalled sensitivity.

Using the *alpha300/alpha500/alpha700* system with its Raman capability, a variety of Raman modes are possible:

- **Collection of Raman spectra at selected sample areas (Single Spectrum):** Single Raman spectra can be collected at user-selectable sample areas with integration times ranging from a few ms to hours. The position of the collected spectrum can be fully controlled in 3D. The microscope Z stage (the focusing stage)

has a step size of 10 nm, while the capacitively controlled piezo-stage has a positioning accuracy of only 3 nm in the lateral directions. This ensures that the point of interest will remain fixed under the excitation focus. This is very important when spectra with longer integration times for the best quality and signal to noise ratio are to be obtained from extremely small sample volumes.

- **Collection of time series of Raman spectra at selected sample areas:**
  Time series of Raman spectra can be obtained to analyze dynamic sample properties. Time series of thousands of spectra can be obtained and analyzed with integration times ranging from a few ms to tens of seconds.

- **Raman fast imaging (optional and using a different software configuration):**
  This is the most sensitive mode of the *alpha300/alpha500/alpha700* system with its Raman capability. In this mode, complete images in the light of a single Raman line are obtained. The spectrometer is tuned to a selected Raman line and the light of this Raman line is directed to the photon counting APD. The result is an image showing the lateral distribution of the appropriate chemical species in the sample.

- **Raman spectral imaging:**
  This is the most versatile mode of the *alpha300/alpha500/alpha700* system with its Raman capability. In this mode, complete spectra are obtained at every image pixel. Images with a size of 512×512 (= 262144 spectra) or even more (limited only by the computer memory) can be acquired. Even during data acquisition, images can be calculated from the spectra by applying a large number of analyzing modes, such as integrating over certain areas, calculating the peak position or determining the peak width etc. in the spectra. As each spectrum is a fingerprint of the chemical species at a specific image point, the distribution of different materials or local properties of the same material (like crystallinity, and local stress) can be analyzed in 3D and with a spatial resolution down to 200 nm.

- **Collection of Raman spectra along a selected line (Line Spectrum):**
  If only the distribution along a certain line in a sample is of interest (cross section, depth profile etc.), Raman spectra can be obtained along a user selected line in three dimensional space. This reduces the number of spectra compared to the spectral imaging mode and allows longer integration times per spectrum.

Of course, the *alpha300/alpha500/alpha700* system with its Raman capability can be also used as a confocal microscope for reflection or fluorescence measurements giving unrivaled sensitivity and the best resolution achievable with c conventional microscopy.

1.4 Modularity

The *alpha300/alpha500/alpha700* 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 (only for the *alpha300* system) 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.

Contact WITec for more details.

1.5 Confocal Raman Microscopy

Confocal microscopy requires a point source (usually a laser), which is focused onto the sample. The reflected light (Raman, fluorescence) is collected with the same objective and focused through a pinhole at the front of the detector (Fig. 3). 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 $\sqrt{2}$).

For Raman microscopy, the enhancement of image contrast and depth resolution is very important. An enhancement of the lateral resolution in confocal microscopy requires extremely small pinhole diameters and will therefore decrease the detection efficiency to a level usually unacceptable in most experiments (Fig. 4).

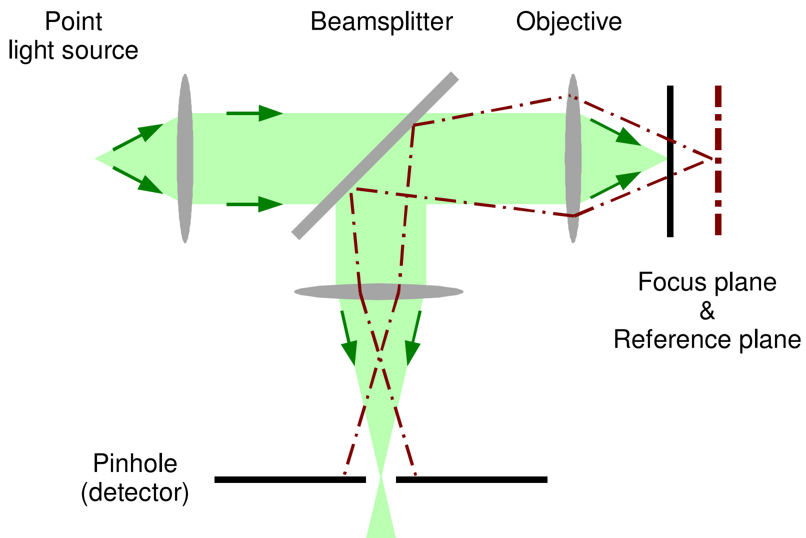


Fig. 3: Principal setup of a confocal microscope

In *alpha300/alpha500/alpha700* systems, the laser light is delivered through a single-mode optical fiber. This type of fiber supports only a single transversal mode (LP_{01}, Gaussian beam) which can be focused to a diffraction-limited spot. The reflected (Raman scattered) light is collected with the same objective and is focused into a multi-mode fiber, which directs the beam to a spectrometer equipped with a CCD camera (Fig. 8) and if the fast Raman imaging option is included, a photon counting APD. The core of the multi-mode optical fiber acts as a pinhole for confocal microscopy. The laser is focused onto the sample and the image is acquired by scanning the sample in X and Y. Scanning is performed with a capacitively controlled piezo-electric scan table with 3nm positioning accuracy in the lateral direction. This system is superior to laser scanning devices, especially for the low scanning speeds necessary for Raman imaging.

Using fibers for beam delivery and signal pick-up is very convenient because the excitation laser, the spectrometer, and the detectors need not to be mounted on the microscope itself. They can be placed anywhere, far away from the microscope body.

In the next section, a simple formula to calculate and choose the correct pinhole size (multi-mode fiber) is given. For a deeper understanding the reader is referred to the tutorial *High Resolution Optical Microscopy* also included with the documentation of the *alpha300/alpha500/alpha700* system.

1.5.1 Pinhole size

The binocular of the microscope has a second output port which is equipped with an adjustable fiber coupler for multi-mode fibers. The core of the multi-mode fiber acts as a pinhole for confocal microscopy. The fiber is mounted in the image plane of the microscope and can be adjusted laterally so that maximum collection efficiency is achieved.

The fiber is protected against mechanical strain and shielded against room light. With the fiber it is simple to direct the light to any detector, e.g. a PMT or a spectrometer.

The lateral position of the pinhole must be adjusted with micrometer precision (typical pinhole size 10-100 μm) while the focus position is not as critical, since the focal depth is quadratic in the magnification M.

The choice of the pinhole size is important because on one hand the signal should be as high as possible, while on the other hand the image should be as confocal as possible (highest depth resolution).

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) can be easily changed. As standard, the *alpha300/alpha500/alpha700* comes with three fibers of 100 μm, 50 μm and 25 μm core diameter. The degree of confocality can be chosen through these fibers. Always use the largest fiber (100 μm) initially, because it gives the maximum Raman signal.

The size of the pinhole, in optical coordinates, should not exceed $v_{P_{max}} = 2.5$. This will avoid a loss in depth resolution. To obtain the highest lateral resolution, the pinhole size should be below $v_{P_{max}} = 0.5$.

In practice, the pinhole size can be up to $v_{P_{max}} = 4$ without significantly changing depth resolution and up to $v_{P_{max}} = 2$ without significantly changing lateral resolution. As shown in the tutorial *High Resolution Optical Microscopy*, if $v_{P_{max}} > 4$, at least the resolution of a conventional microscope remains. This is due to the fact that for a large detector the resolution is always determined by the diameter of the exciting laser spot. Only the depth resolution (and therefore contrast for a thick sample) is lost in this case.

For the experiment, the relation

$$\frac{M}{NA} \geq \frac{\pi d_0}{v_{P_{max}} \lambda}$$

must be fulfilled, where M is the magnification, d_0 the diameter of the pinhole and NA the numerical aperture of the objective. The left side of this equation is defined by the objective and the beam path. In Tab. 1, the parameter $\frac{M}{N_A}$ is calculated for several typical objectives.

objective	10/0.25	20/0.4	40/0.6	60/0.8	100/0.9	100/1.25	100/1.4
M/NA	53	50	67	75	111	80	71

Tab. 1: M/NA for different objectives.

The right side of Equ. 1.5.1 is defined by the wavelength and the pinhole size itself (Tab. 2).

wavelength (nm)	440	488	532	633	785
$d_0 = 10\mu m$	29	26	24	20	16
$d_0 = 25\mu m$	71	64	59	50	40
$d_0 = 50\mu m$	142	129	118	99	80
$d_0 = 100\mu m$	286	258	236	199	160
$d_0 = 200\mu m$	571	515	472	397	320

Tab. 2: $\frac{\pi d_0}{2.5\lambda}$ for typical wavelengths and pinhole sizes.

With the help of these tables, the correct pinhole size can be determined for any experiment. If an objective with a magnification of 100x and a numerical aperture of 0.9 is used at a wavelength of 532 nm, the optimum pinhole size would be 50 μm for maximum depth resolution and 10 μm for maximum lateral resolution.

In actual experiments, one usually has to find a compromise between the highest resolution and collection efficiency. This is very important in Raman microscopy because Raman is an extremely weak effect. Typically only 1 in 10^6 photons focused on the sample is Raman scattered and can be used for image generation. When a very small pinhole is used, the collection efficiency is strongly reduced. This is plotted in Fig. 4.

This graphic shows the intensity on the detector as a function of pinhole size, normalized to the total intensity in the image plane. One can see that the collection efficiency is about 75% for maximum depth resolution (v_P=2.5), but only 6% for maximum lateral resolution v_P=0.5.

Using the fibers delivered with the instrument, it is always possible to obtain maximum depth resolution. As one can see, about 75% of the Raman scattered signal in the focal plane is collected by the fiber. If smaller fibers are needed for higher resolution (such as the 10 μm fiber in the example above), they can be ordered separately.

For more information, see the tutorial *High Resolution Optical Microscopy*.

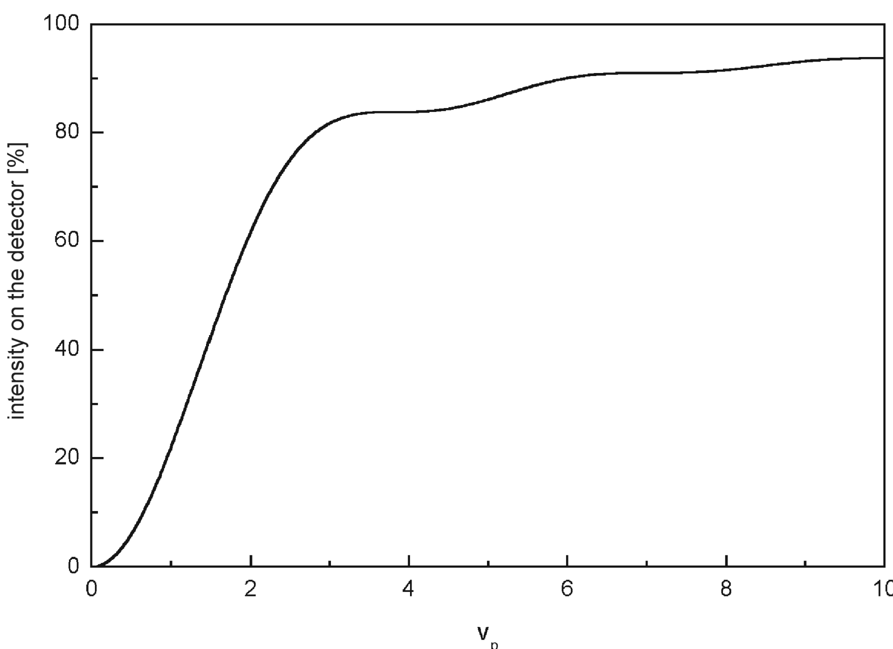


Fig. 4: Collection efficiency as a function of pinhole size normalized to the total power in the image plane.

1.5.2 Objectives and resolution

Make sure that the microscope objectives are used in the proper way. Some of the objectives supplied may be corrected for use with a cover slip. This means they will give best results only when a cover slip of 0.17 mm thickness is placed between the objective and the sample.

Usually, there are four numbers printed on each objective. The first number gives the magnification in the image plane (at the position of the color video camera (U6 in Fig. 8) or the multi-mode fiber(U8 in Fig. 8)). The second number is the numerical aperture (NA) which describes the resolving power of the objective. The third number indicates the distance between objective and image plane. The sign ∞ indicates that the objectives are infinitely corrected (the beam is parallel inside the microscope). The fourth number gives the cover glass thickness that the objectives are corrected for.

The magnification printed on the objective is of minor importance. In the *alpha300/alpha500/alpha700* system, the sample is scanned with respect to the (fixed) optical axis of the microscope. Therefore, the range of the scan stage (200×200 μm) determines the maximum image size, independent of the magnification of the objective. The important number is the numerical aperture, which (together with the excitation wavelength) determines the lateral resolution of the objective. The magnification is only important for the choice of the pinhole size.

The maximum resolution of a classical microscope is given by the Rayleigh criterion

$$\Delta x = \frac{0.61\lambda}{NA} \tag{17}$$

where Δ x is the smallest distance between two point objects that will appear separated in the image plane, λ is the wavelength of the excitation light and NA is the numerical aperture of the microscope objective. In this case, the image of two point objects will look like Fig. 5.

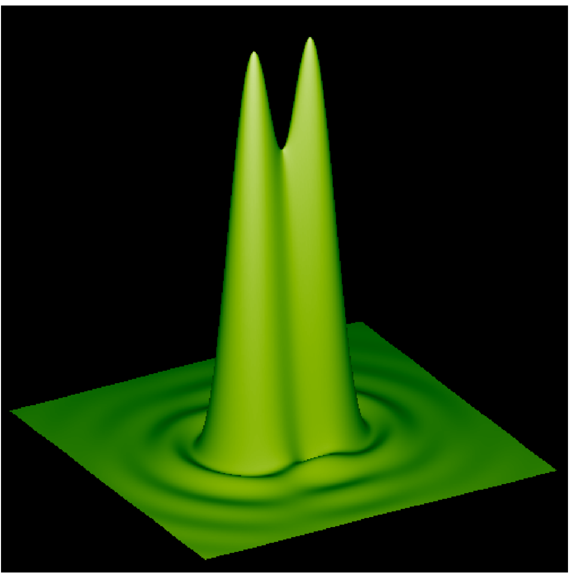


Fig. 5: Rayleigh criterion

2 WITec Control Raman configuration description

This description is intended to be used in combination with the WITec Control manual. The WITec Control manual contains the description of the full functionality of WITec Control , whereas this section indicates only which functionalities are included in this configuration. Please refer to the WITec Control manual for further details. The configuration-specific speed buttons, as well as the layout of the Control Window, will also be illustrated below.

The *Confocal Raman* configuration is used to perform confocal Raman measurements. During a confocal Raman measurement, data can be acquired using the following sequencers:

- Image Scan: acquisition mode for two dimensional Raman imaging.
- Line Scan: acquisition of Raman spectra along a line.
- Oscilloscope: displays the complete spectrum as read from the spectral camera in a graph viewer. The display is updated as soon as the next spectrum is completed.
- Single Spectrum: acquisition of a single Raman spectrum at the current position.
- Time Series: allows the collection of a series of Raman spectra over time at the same position to determine, for example, dynamic changes in the sample.

The characteristics of the Raman configuration are described in the following sections. The typical layout of the Raman configuration is shown in Fig. 6.

2.1 Speed Buttons

The main menu contains, in addition to the standard WITec Project speed buttons, several speed buttons which provide quick access to microscope and controller functions used in this configuration. A short description of these speed buttons is given below.

Stop

This speed button is used to stop any sequencer.

Start Scan

With this button, the *Image Scan* sequencer is started.

Single Spectrum

With this button, the *Single Spectrum* sequencer is started. It uses the parameters of this sequencer as defined in the Control Window.

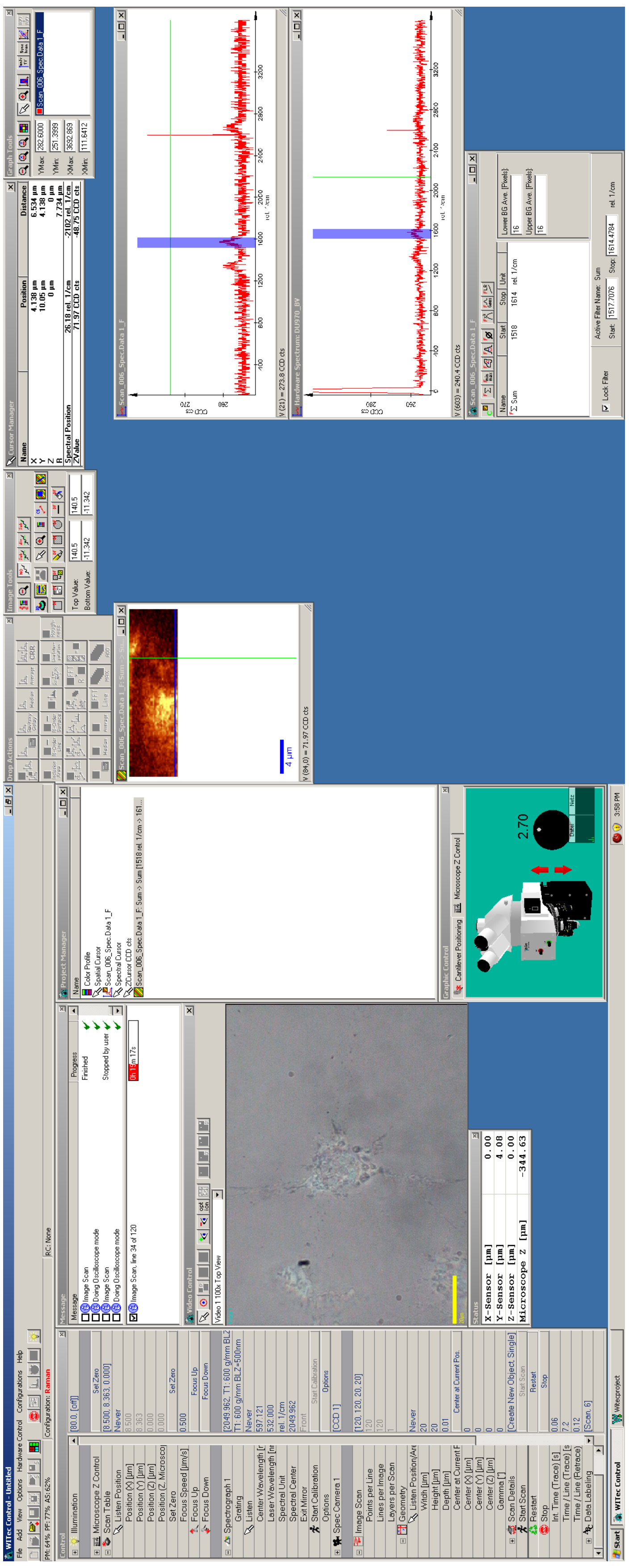


Fig. 6: Typical layout of the Confocal Raman configuration.

Time Series (Fast)

With this button the *Time Series (Fast)* sequencer is started. It uses the parameters of this sequencer as defined in the Control Window.

Oscilloscope

Clicking with the mouse on this speed button starts the oscilloscope sequencer (WITec Control manual Section 3.5.11). A graph viewer showing the variation of the selected data source (channel) as a function of time is displayed.
In confocal Raman measurements, the readout of the spectral camera is typically displayed in a graph viewer. This signal is mainly used for the alignment of the system.

Illumination

With this speed button, the white light illumination can be switched on and off. The brightness of the illumination is defined in the Control Window using the illumination device.

2.2 Control Window

The Control Window described in Chapter 3 of the WITec Control manual is customized for confocal Raman measurements. The reduced tree structure of the Control Window in the confocal Raman configuration is shown in Fig. 7. Devices and sequencers are grouped in this configuration based on functionality. Each device and sequencer contains the list of parameters which are used during a confocal Raman measurement. The following devices and sequencers are accessible in the confocal Raman configuration.

2.2.1 Setup and Control

In this section, the software controls for the hardware components (called devices in WITec Control) and the software controls for data acquisition processes (called sequencers) are listed and briefly described. These controls are used for microscope setup and measurement control.

Illumination

This device is used to control the white light illumination of the tip/sample video image. A detailed description of the parameters used to control the white light illumination is given in the WITec Control manual Section 3.4.2. The illumination speed button located in the main menu uses the brightness parameter set here.

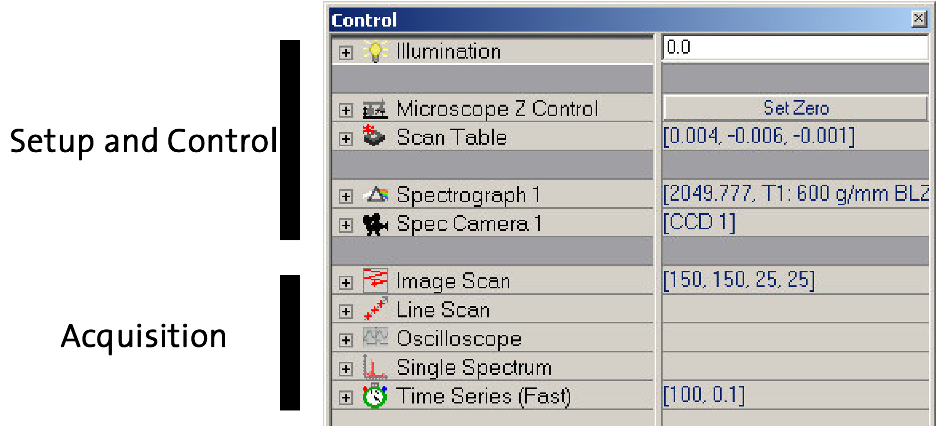


Fig. 7: Control window for the Raman configuration.

Microscope Z Control

The microscope Z control is described in WITec Control manual Section 3.4.3 as part of the scan table device. In this configuration, the *Move Mode* is automatically set to *Z by Microscope*. The microscope Z-stage is used as the Z axis of the internal coordinate system. Depth scans of up to $200\ \mu m$ can be performed in this mode by changing the Z position of the microscope stage from line to line or image to image.

HINT The microscope Z-stage can be controlled via the remote control (seeWITec Control manual Section 3.4.1), the Graphic Control Window (seeWITec Control manual Chapter 8) or using the parameter *Speed*, *Move Up* or *Move Down* listed in the Control Window.

Scan Table

If the microscope is operated in this configuration, the scan table can be moved in all three directions, allowing precise positioning within the scan-range. For a detailed description of the scan table, please see also WITec Control manual Section 3.4.3.

Spectrograph

The parameters required to control the spectrometer connected to the alpha-Control are listed in this group. A detailed description of each parameter can be found in the WITec Control manual Section 3.4.11.
The calibration of the spectrometer is performed at WITec and stored in the hardware of the spectrograph.

Before re-calibrating the spectrometer, please carefully read the section *Start Calibration* of the WITec Control manual Section 3.4.11 and ensure that the correct calibration lamp is used.

Spec Camera
The parameters required to control the spectral camera connected to the alphaControl are listed in this group. A detailed description of each parameter can be found in the WITec Control manual Section 3.4.12.

2.2.2 Acquisition

The sequencers used to acquire data in this configuration are listed and briefly described below.

Image Scan
The parameters used to perform an image scan are described in WITec Control manual Section 3.5.2. In this configuration, all parameters required to perform an image scan in confocal Raman mode are listed.

Line Scan
The parameters used to perform a line scan are described in WITec Control manual Section 3.5.3. In confocal Raman mode, Raman spectra can be recorded along a line. The parameters used to record each Raman spectrum are the actual parameters defined in the single spectrum sequencer (see below).

Oscilloscope
The parameters used to display measured values in a similar way as is done through an oscilloscope are described in WITec Control manual Section 3.5.11. In confocal Raman measurements, the readout of the spectral camera is typically displayed in a graph viewer. The graph viewer is updated upon completion of each measured Raman spectrum.

Single Spectrum The parameters used to record single spectra using the CCD camera are described in the WITec Control manual Section 3.5.12. In this configuration, all parameters required for the acquisition of single Raman spectra are listed.

2.3 Data Sources and Status Window

The data sources (WITec Control manual Section 3.6) used during a confocal Raman measurement are summarized in Tab. 3 along with the default data labeling of the

acquired channel. The data sources used for adjustment and control of the measurement are displayed in the Status Window (WITec Control manual Chapter 5).

Channel	Image Scan	Line Scan	Time Series	Oscillos-cope	Channel Caption	Unit	Display
Spec. Camera	✓	✓	✓	✓	Spec Data 1	$[CCDcts]$	
X-Sensor						$[\mu m]$	Status Window
Y-Sensor						$[\mu m]$	Status Window
Z-Sensor						$[\mu m]$	Status Window
Microscope Z						$[\mu m]$	Status Window

Table 3: Output channels and their sequencers in the confocal Raman configuration.

3 Beam Path

In this section, the beam path is illustrated schematically. The parts common to all instruments are displayed in dark grey. Parts not common to all instruments, but necessary for the described measurement mode are indicated in blue. Optional parts which may be present with the instrument, but are neither common to all instruments nor are necessary for the described measurement mode are displayed in light grey. The parts indicated are listed below the figure where an *alpha300* system is shown exemplarily. Detailed descriptions of the parts indicated can be found in the *alpha300/alpha500/alpha700* system description. The beam path is indicated in green in Fig. 8.

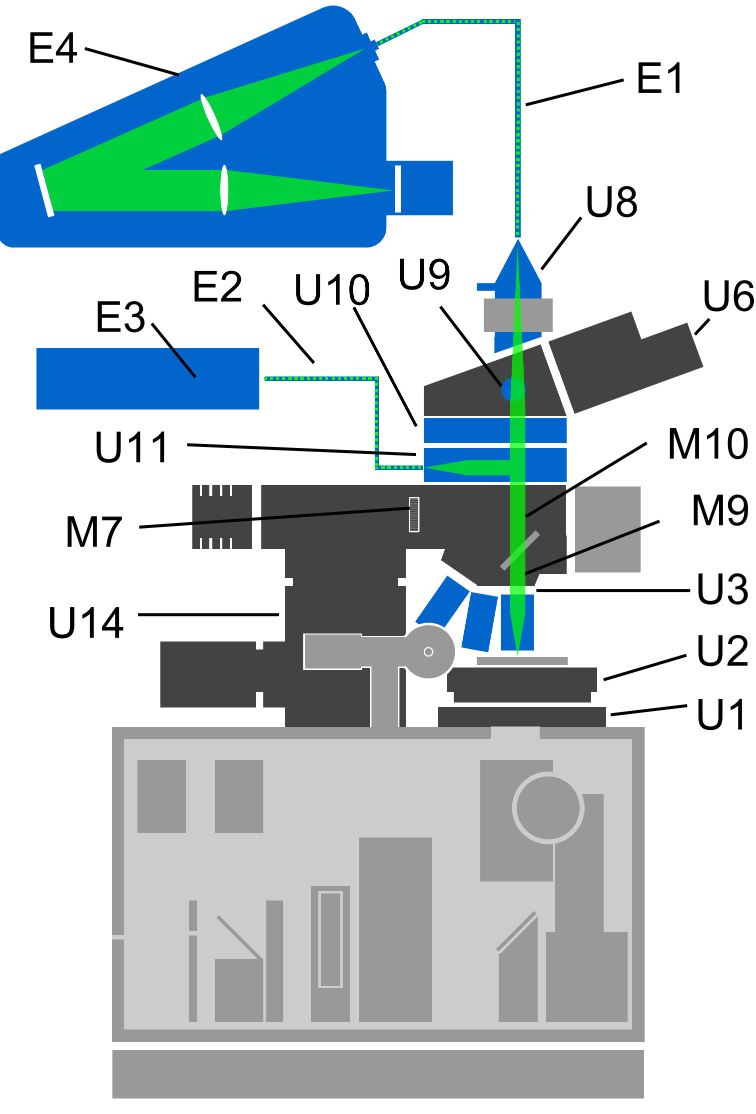


Fig. 8: Schematic illustration of the beam path for confocal Raman microscopy.

U1 XY positioner

U2 Scan stage

U3 Objective turret with objectives

U6 Binocular tube with ocular camera

U8 Fiber coupling unit optical output

U9 Pushrod

U10 Filter slider unit

U11 Laser coupling unit optical input

U14 Microscope Z stage with stepper motor

M7 Field stop diaphragm

M9 Objective turret

M10 Reflector slider.

E1 Multi-mode optical fiber (25, 50 or 100 μm core diameter included as standard)

E2 Single-mode optical fiber

E3 Laser

E4 Spectrometer

4 Step by step alignment

The following listing describes the alignment of the *alpha300/alpha500/alpha700* system in order to obtain a confocal Raman image, perform a line scan, a time series or capture a single Raman spectrum.

The first of the following steps describe the procedure for focusing the microscope on the sample using white light illumination followed by the focusing of laser radiation. Then, the optimization of the position of the SMA fiber connector, the focus and the collimation with the aid of the Raman spectrum obtained from the Si test sample is described.

1. Switch on the alphaControl. This is usually done using the switch on the multi-plug.

2. Power up the computer and start WITec Control . Select the Raman mode from the Configurations -menu. The CCD camera should be cooled to its minimum stable temperature automatically (to change the temperature go to: Control Window → Spec Camera).

3. Mount the Silicon test sample on the scanning stage.

4. Rotate the microscope turret until an appropriate objective is in the working position. Make sure you use the microscope objective in the proper way (e.g. use a cover slip if a cover slip corrected objective is used).

5. Push in the pushrod (U9) of the sliding prism and direct the beam to the eyepiece color video camera (U6).

6. Move the reflector slider (M10) to the illumination position (M11; see the *alpha300/alpha500/alpha700* system description for details). The beam splitter is mounted in the left position of the three position reflector slider. Therefore, move the reflector slider to the right.

7. Adjust the illumination to the required level using the illumination menu item in the Control Window.

8. Observe the image of the eyepiece color video camera on the computer monitor using the Video Control Window in the WITec Control software. Using the drop down menu in the Video Control Window, select the appropriate view (top view with the correct objective).

9. Focus on the surface of your sample with the Microscope Z stage (U14). This can be done in three different ways:

   - Using the Z microscope control in the Graphic Control Window. Clicking on the arrows will start the movement in the indicated direction and using the virtual potentiometer, the speed can be adjusted from 0.01 to 500 μm/s.
   - Using the remote control with the Z Microscope selected as the controlled device. Using the +Z and -Z buttons, the Z-focusing-stage can be moved up or down and the potentiometer allows the selection of speeds between 0.01 and 500 μm/s.

   HINT The remote control needs to be activated if the controlled device has changed or if it was idle for more than two minutes. To activate the remote control turn the potentiometer fully anticlockwise.

   - Using the Move Up and Move Down buttons, which can be found in the Microscope Z Control menu of the Control Window. The speed can be adjusted between the minimum and maximum values using the corresponding field.

If possible, move the objective initially away from the sample to avoid a collision between the objective and the sample.

It can sometimes be very difficult to focus on flat and clean surfaces. The field stop diaphragm (M7) can help to overcome these difficulties as outlined in the following. To focus, close the field stop diaphragm (M7) to a value of 1-3. Make sure to move in the 50:50 beam splitter (M11) using the reflector slider (M10) to illuminate the sample. Approach the sample until the edge of the field stop appears focused. At this point, the sample is also in focus. This is due to the fact that the field stop is positioned at the back focal plane of the objective. If the field stop is not in the middle of the field of view, move it to this position with the centering screws (M7; see the *alpha300/alpha500/alpha700* system description for details).

10. Move the laser blocking filter out using the filter sliding unit (U10).

11. Switch on the excitation laser at low power.

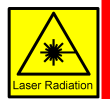
12. Turn off the white light illumination (using the speed button or the Illumination On/Off button), move out the 50:50 beam splitter (move the reflector slider (M10) into its middle position) and fine focus the microscope until the excitation laser is focused on the sample. You will observe several spots on the video screen, but only one of them changes during focusing. This is the spot that hits the sample while the other spots are reflections inside the microscope. Make sure that the other spots are as small as possible. The video camera is in the focal plane of the tube lens, so these spots will be minimized if the laser beam is collimated and therefore parallel inside the microscope. If they appear out of focus, rotate the micrometer screw at the laser coupling unit optical input (U11) until they are perfectly focused.

HINT If the video screen over-saturates (i.e. displays only white) reduce the laser power further.

13. If you performed a complete alignment recently, you can jump to point 19.

14. Close the laser shutter, remove the multi-mode fiber (E1), place a white card (e.g. a business card) a few millimeters above the SMA connector (U8) and re-open the laser shutter.

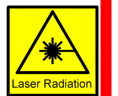
15. Pull out the pushrod (U9) to direct the beam to the SMA connector. Try to localize the laser beam on the name card by altering the position of the SMA connector using the micrometers attached to it. If necessary, increase the laser intensity.



DO NOT LOOK INTO THE SMA CONNECTOR.

16. Adjust the micrometers to center the beam in the SMA connector. You might recognize several laser spots, but only one of them changes during focusing. This is the spot that hits the sample while the other spots are reflections inside the microscope. Close the laser shutter and reinsert the multi-mode fiber. Disconnect it at the spectrometer (E4) and reopen the laser shutter.

17. Try to see the laser beam at the end of the multi-mode fiber. Use the name card again. Increase the laser intensity if necessary.



DO NOT LOOK INTO THE FIBER.

18. Adjust the micrometer screws at the SMA connector to maximize the laser power at the multi-mode fiber.

19. Place the shutter into the laser beam path, move the laser blocking filter into the beam path again (U10) and connect the multi-mode fiber to the Spectrometer (E4). Ensure that the CCD camera is cooled to the appropriate operating temperature.

20. Select an appropriate grating through the Spectrograph menu. Start with a low dispersion grating (150 l/mm or 600 l/mm) that covers a large frequency range and move it to an appropriate wavenumber position (e.g. using a 532nm laser and the 600 l/mm grating, using 2050 rel. 1/cm as the spectral center is a good start).

21. Before opening the laser shutter again set the laser power to its maximum because the Silicon test sample should always be checked with maximum laser power. Ensure that the laser blocking filter is in place. Do not allow Rayleigh scattered light to enter the spectrometer because this high intensity laser radiation may harm the CCD camera.

    Then open the laser shutter.

22. Start the oscilloscope in WITec Control using either the speed button or the Start Oscilloscope button in the oscilloscope menu of the Control Window. The integration time can be adjusted in the oscilloscope menu of the Control Window and should initially be set to 0.1-1s. You should now see a Raman spectrum of Silicon with the characteristic 1st order Si line at $\approx$520 cm^{-1}, updated every 0.1 s to 1 s depending on the selected exposure time.

23. Watch the oscilloscope reading on the monitor and make fine corrections with the micrometers at the SMA fiber connector (U8) until the maximum signal is achieved.

24. Adjust the focus of the microscope using the microscope Z stage (as described in point 9) to maximize the signal.

25. Using the micrometer on the back of the laser coupling unit optical input (U11) adjust the collimation for optimum intensity.

26. Repeat points 23 to 25 several times to optimize the signal.

27. Once the optimum signal intensity is achieved, stop the oscilloscope with any Stop button or icon.

28. Close the laser shutter.

29. Move the Z microscope up as described in step 9.

30. Exchange the Silicon for your sample.

31. Focus on your sample using white light as described in steps 4 to 9.

32. Move the reflector slider (M10) into the middle position.

33. Open the laser shutter. Make sure to start with low laser power since the sample might be altered or destroyed otherwise. The stability of your sample depends on various factors such as absorption, thermal conductivity and laser spot size (which is determined by the laser wavelength and the NA of the objective used for the experiment).

34. Start the oscilloscope and optimize the focus for maximum intensity as in step 24.
    At this point, do not alter the position of the SMA fiber connector or the collimation anymore.

35. Once the optimum count rate is found, stop the Oscilloscope with any Stop button or icon and close the laser shutter.

36. The parameters for an image scan, line scan, a single spectrum or a time series should then be defined.

    If an image scan is performed, the measurement parameters in the image scan menu of the Control Window should be adjusted (see WITec Control manual Section 3.5.2 for a description of the individual parameters).
    If the *(X)*, *(Y)* and *(Z,Microscope)* positions indicated in the scan table menu of the Control Window differ from the position entered as *Center (X)*, *Center (Y)* and *Center (Z)* in the image scan menu, the scan table will move to a different position before starting the scan. This might then lead to data acquisition at the wrong position/area. Use the Center at Current Pos. button in the image scan menu of the Control Window to avoid this displacement.

HINT Typical values for a confocal Raman imaging measurement are:
Points per line: 100-200
Lines per image: 100-200
Width: 10-50 μm
Height: 10-50 μm
Integration Time: 20-100 ms

37. You may now begin your measurement by opening the laser shutter and clicking on the corresponding Start button.

38. If an Image Scan is performed, you will see a FILTER MANAGER and two GRAPH windows: One of these will display the spectrum of your sample updated depending on the selected integration time.

39. A variety of images can be calculated from the spectra and displayed during the scan, without affecting the spectra acquisition. This is done by creating a new filter in the FILTER MANAGER window and selecting the appropriate spectral range in a displayed spectrum. Please refer to the WITec Project user manual (the functionality in WITec Control is identical) for more information about the variety of data evaluation tools available in this powerful software. Through the definition of filters in the FILTER MANGER, image data objects will be created which can be displayed in image windows. These image windows will display the calculated value of the Raman signal of your sample at each pixel. Clicking on any position in this image will display the spectrum obtained at this specific position in the second GRAPH window. This action does not effect the data acquisition.