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TM004 – Measurement set-up and data acquistion WiRE™ 5

The aim of this module is to detail the correct use of WiRE 5 to enable spectral data collection using the different measurement parameters available in conjunction with the inVia Raman microscope.

Defining the type of measurement

Measurements are used within the WiRE software to define the type of data collection. Several different types of measurement may be available to the user, depending on the exact configuration of the inVia Raman microscope. Measurements which are unavailable are greyed out.

New measurements are accessed using either the menu (**Measurement……New……**), or the toolbar arrow.

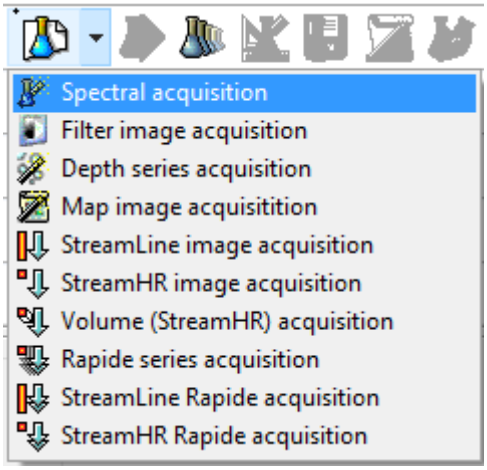


Figure 1 Toolbar new measurement access

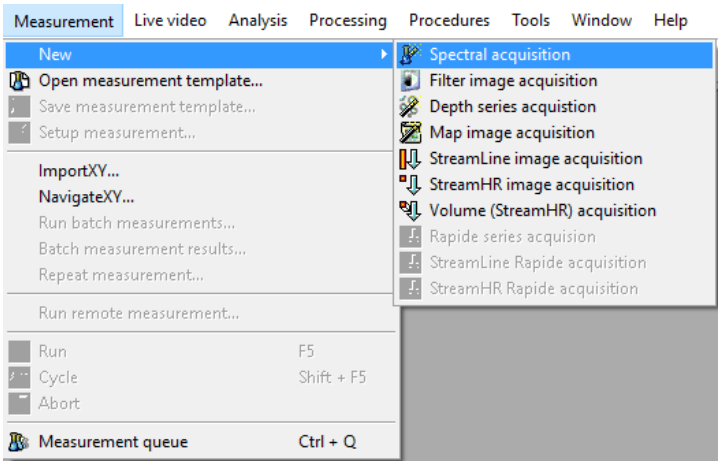


Figure 2 Menu new measurement access

The different types of measurements which may be available are:

- Spectral acquisition (standard spectral collection)
- Filter image acquisition (collection of filter spectra and filter images)
- Depth series acquisition (spectral collection at varying sample depths, Z only)
- Map image acquisition (spectral collection at varying lateral sample positions and depth slices)
- StreamLine image acquisition (high speed spectral collection at varying lateral sample positions with a minimised laser power density)
- StreamLineHR (high speed spectral collection at varying lateral sample positions)
- StreamLineHR 3D acquisition

When the appropriate measurement has been selected, the set-up of that measurement will be automatically displayed. This module details the standard set-up tabs, consistently used throughout the different measurement types. These tabs are *Range, Acquisition, File* and *Advanced.*

Range

The *Range* tab covers the basic settings for the scan such as the laser and grating to be used, and the type of scan to be performed.

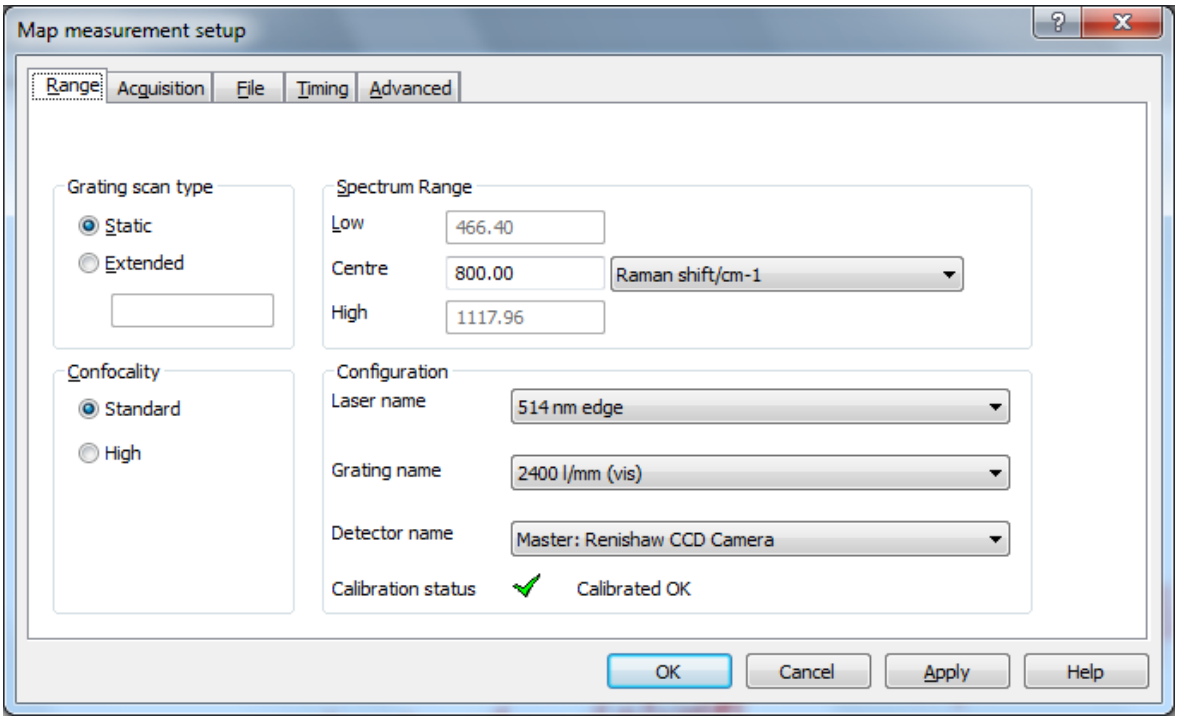


Figure 3 *Range* tab

1. *Grating scan type* gives the option for two types of scan.

    - *Static* covers a range of about 200 cm^{-1} to 500 cm^{-1} either side of the centre, depending on the wavelength and the grating used. The desired centre can be entered in the *Spectrum range* box. A static scan is quicker to perform than an extended scan, but only covers a limited range.

    - *Extended* (SynchroScan) scans between the upper and lower limits entered in *Spectrum range,* and is used when a static scan will not cover the required wavenumber range.

2. *Configuration* allows the user to select the laser, grating and detector to be used.

3. The *Confocality* box allows the user to choose between high and standard confocal performance. The confocality defines the sample volume that signal is collected from. Using the *High* confocality option reduces this volume increasing the spatial resolution but also reducing the total Raman signal

Note the instrument is always confocal due to the optical layout. High confocal mode is not available in line focus or StreamLine imaging configurations.

Acquisition

The *Acquisition* tab allows the user to alter scan conditions such as the exposure time and laser power to be used.

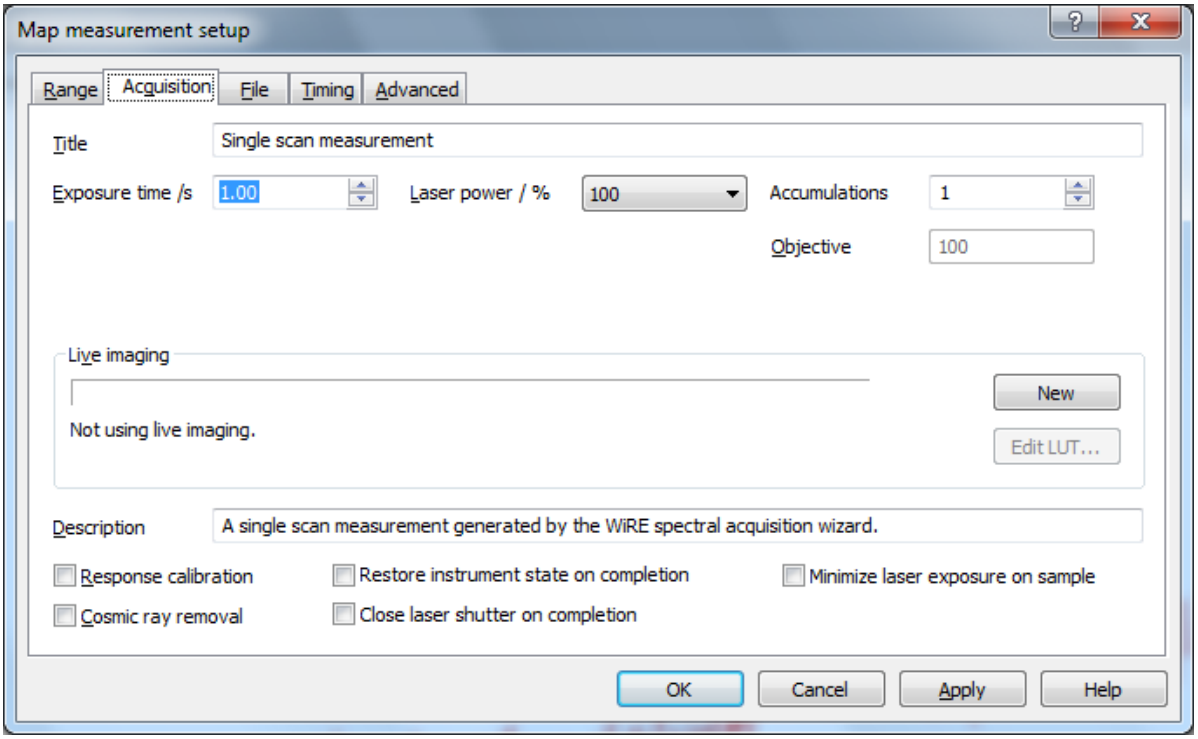


Figure 4 *Acquisition* tab

1. *Exposure time* is the time the detector is exposed to the Raman signal. Longer exposure times give a better signal-to-noise ratio in the spectra. The minimum exposure time for a static grating scan is 0.02 s. If the Extended option is selected in the Range tab, the exposure defaults to the minimum required: 10 s. There is no maximum exposure in either case.

2. *Laser Power* is the percentage of laser power that will be used for the scan. Higher power will give a better signal-to noise ratio, but can damage some samples, depending on the laser used.

3. *Accumulations* is the number of repetitions of the scan. The accumulations are automatically co-added, to produce spectra with better signal-to-noise ratios. Using several accumulations of a short scan can be preferable to performing one long scan. For example:

    - If the sample has a high fluorescence background, a long scan will saturate the detector, whereas several short scans will not. This allows an improvement in the single-to-noise ratio.
    - If cosmic ray removal is used, two extra accumulations are performed. So if the scan consists of 10 accumulations of 10 seconds, then two extra 10 second accumulations are performed. If the scan consists of one 100 second accumulation, then two extra 100 second accumulations will be performed, which is clearly more time consuming.

    Generally it is preferable to conduct longer exposures when possible as each accumulation adds readout noise from the CCD to the collected spectrum.

4. *Objective* indicates the magnification of the objective being used. Better signal-to-noise is usually obtained from higher magnification objectives, as they give a higher power density at the sample. The box is greyed-out. The value reflects the value set in the Sample Review window.

5. *Live imaging* allows Raman images to be defined and subsequently viewed during data collection. This feature is used in conjunction with *Map image acquisition* and *StreamLine/StreamLineHR image acquisition* measurements only. This option requires the user to know the expected changes within the Raman data or have pre-collected reference spectra of specific components. (PCA and MCR-ALS options are not possible with Live imaging).

6. *Cosmic ray removal* removes random sharp peaks due to cosmic background radiation. The cosmic rays are eliminated by automatically obtaining three spectra and taking the median average of the three.

7. *Close laser shutter on completion* forces the laser shutter to be closed after data collection, and will override the *Restore instrument state on completion* checkbox (recommended when performing imaging experiments).

8. *Restore instrument state* on completion is used to automatically restore the instrument to the state it was in prior to collection (as defined in the Sample Review). This function applies largely to inVia Reflex Raman microscopes where there is a greater degree of motorisation.

9. *Minimise laser exposure on sample* will close the laser shutter when data is not being collected during a single measurement (e.g. temperature ramp measurement)

10. *Response calibration* will collect data using a pre-defined transmission profile normalising the instrument response.

File

The *File* tab covers options for automatically saving the data. Either insert a filename or browse to a folder.

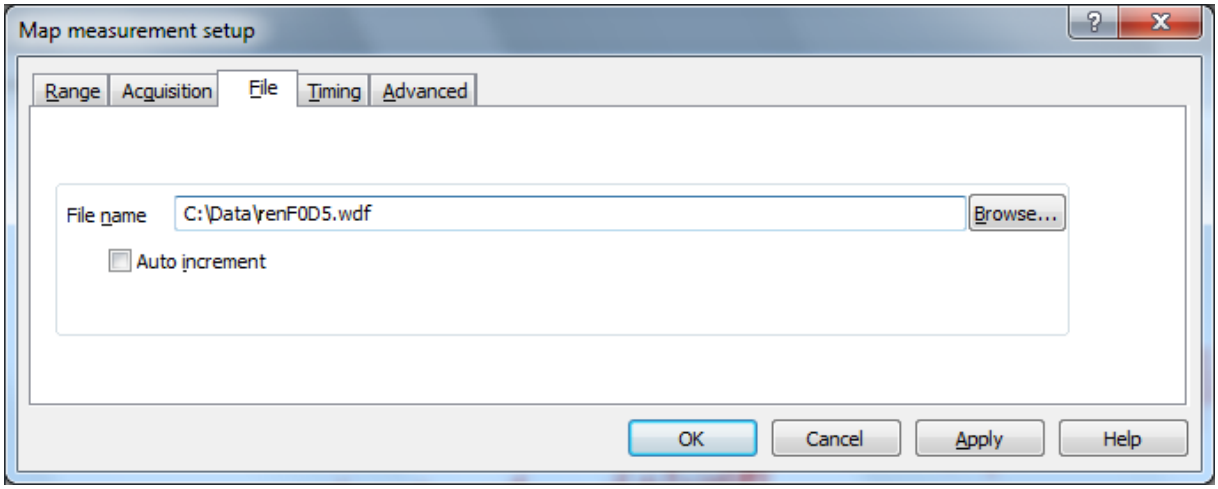


Figure 5 *File* tab

1. *Autosave file* saves the file to the file specified in *File name* directly after collection. Its use is recommended, as it removes the risk of losing data by forgetting to save it. The next dataset will overwrite the first unless the *Auto increment* checkbox is selected. Checking the Auto increment function will force the data to be saved each time this measurement is performed. The format will be filename, filename0, filename1, filename2…unless the original filename is appended numerically, e.g. filename1.

Timing

The Timing tab consists of two main functions: time series and sample bleaching measurements. The Time series measurement allows multiple spectra, with same instrument conditions, to be acquired with an identical period of time elapsing between each one. This function may be useful to monitor the lifetime of a biological sample, for example, by its Raman spectrum. Set the total number of spectra to be acquired in the first box ('Number of acquisitions') and the interval in the second ('Time series measurement settings'). A ‘profile’ can be created at the end of the sequence from the data. For example, the intensity at one frequency in the spectrum with acquisition number (time).

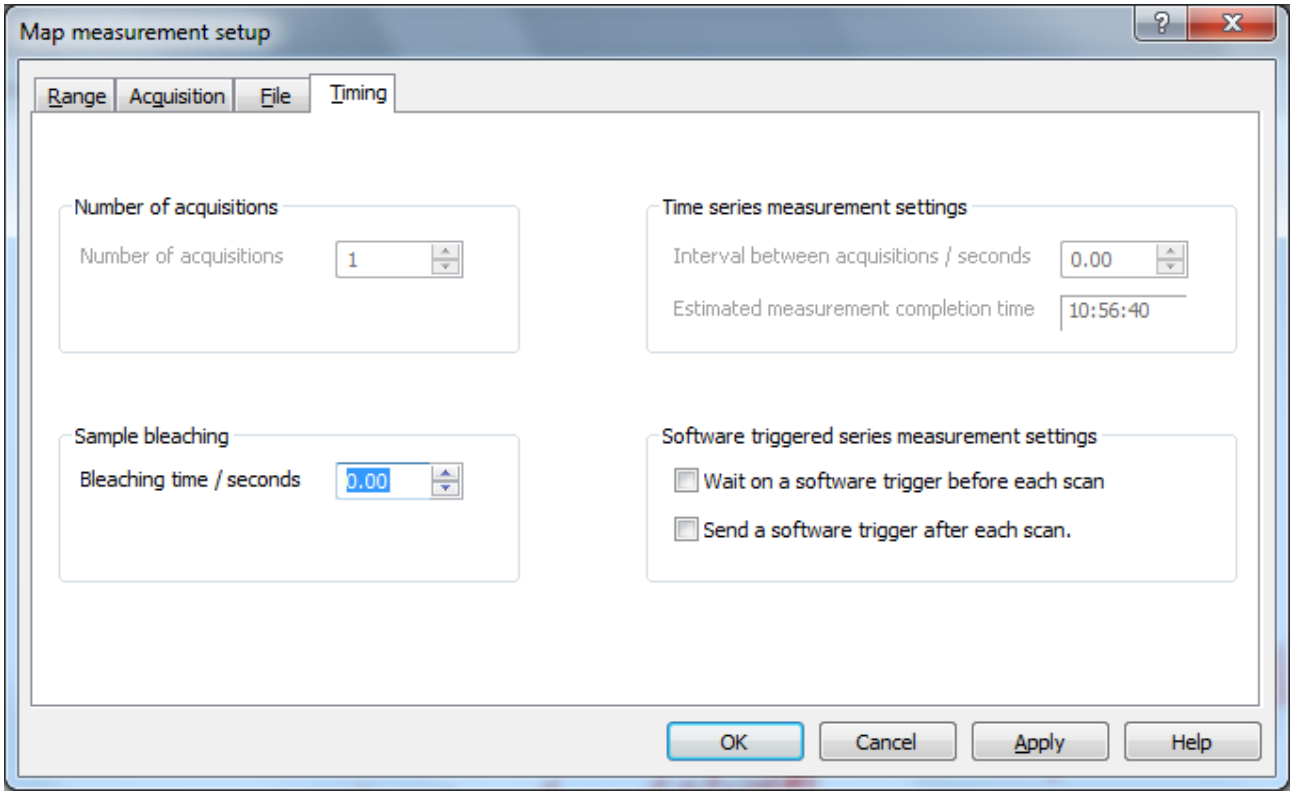


Figure 6 *Timing* tab

Sample bleaching, also called photobleaching or photoquenching, is a phenomenon whereby fluorescence is observed to decrease simply by the having the laser incident on the sample. There are various mechanisms that part contribute all or in part to this effect. Setting a value in the box exposes the sample with the laser for a set time before the spectrum is acquired. The period of time may range from seconds to tens of minutes and will be sample and laser dependent.

Software triggering is only required in special cases using external hardware.

Temperature

The temperature series measurement tab is only available when suitable heating/freezing temperature stages have been installed with the appropriate WiRE feature permission.

By default, the ‘use’ check box is unchecked. To activate the temperature series parameters, check the box.

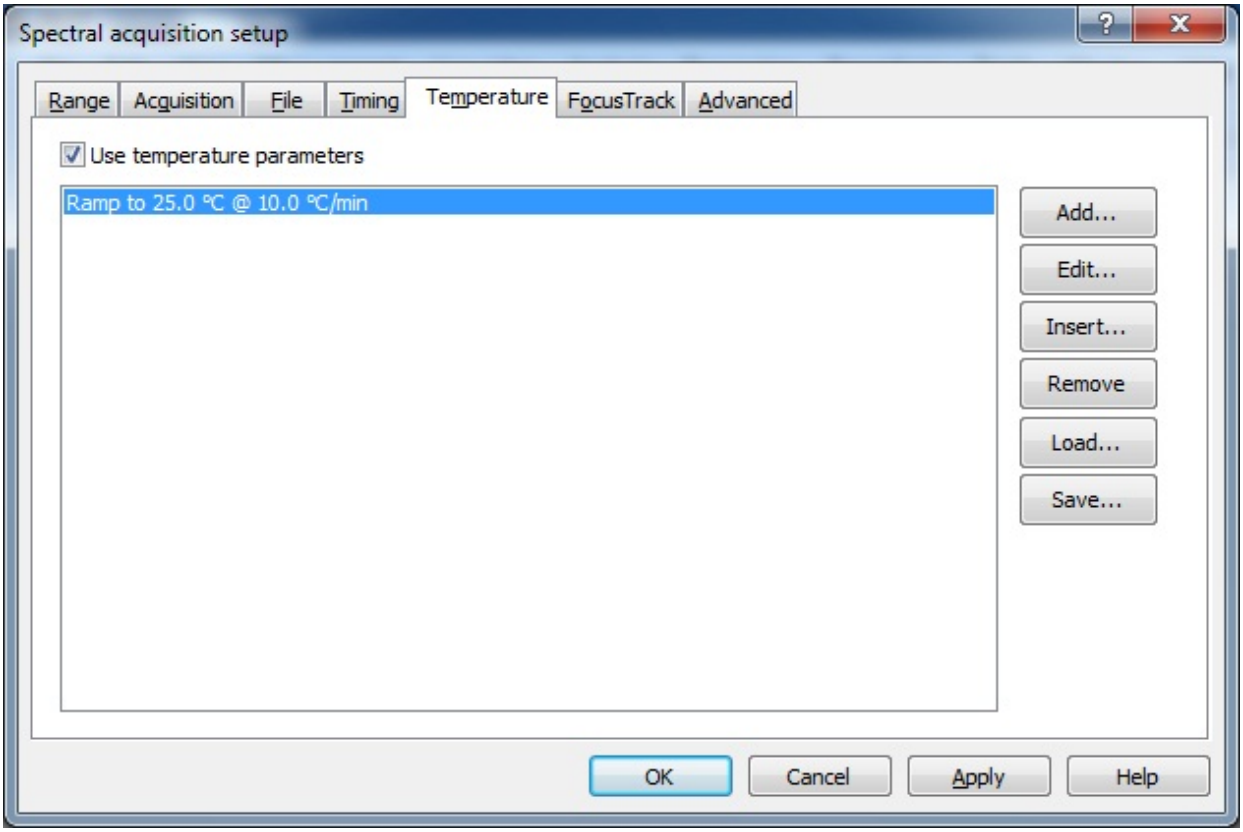


Figure 7 *Temperature* tab

See module TM22 for instructions on the set up of temperature series measurements.

FocusTrack

To maintain the laser focus for spectral acquisition, for example, during time, temperature and mapping measurements, you may use the FocusTrack function. Refer to module TM6 for guidance notes. The 'Focustrack' tab allows the user to enable this function and specify how often it is used during the measurement.

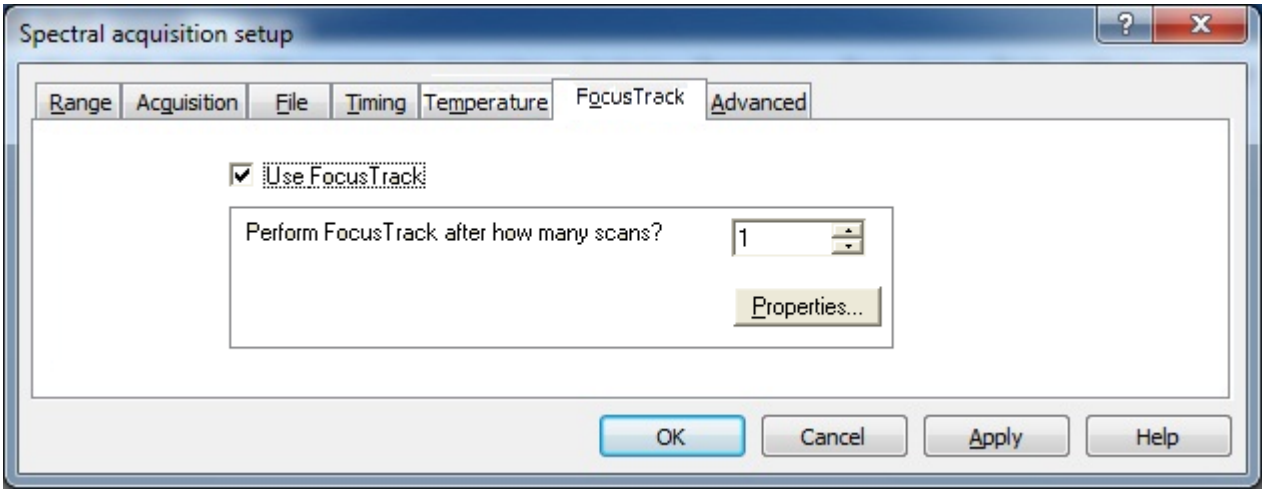


Figure 8 *FocusTrack* tab

Advanced

The advanced tab covers more specialised options for the measurement.

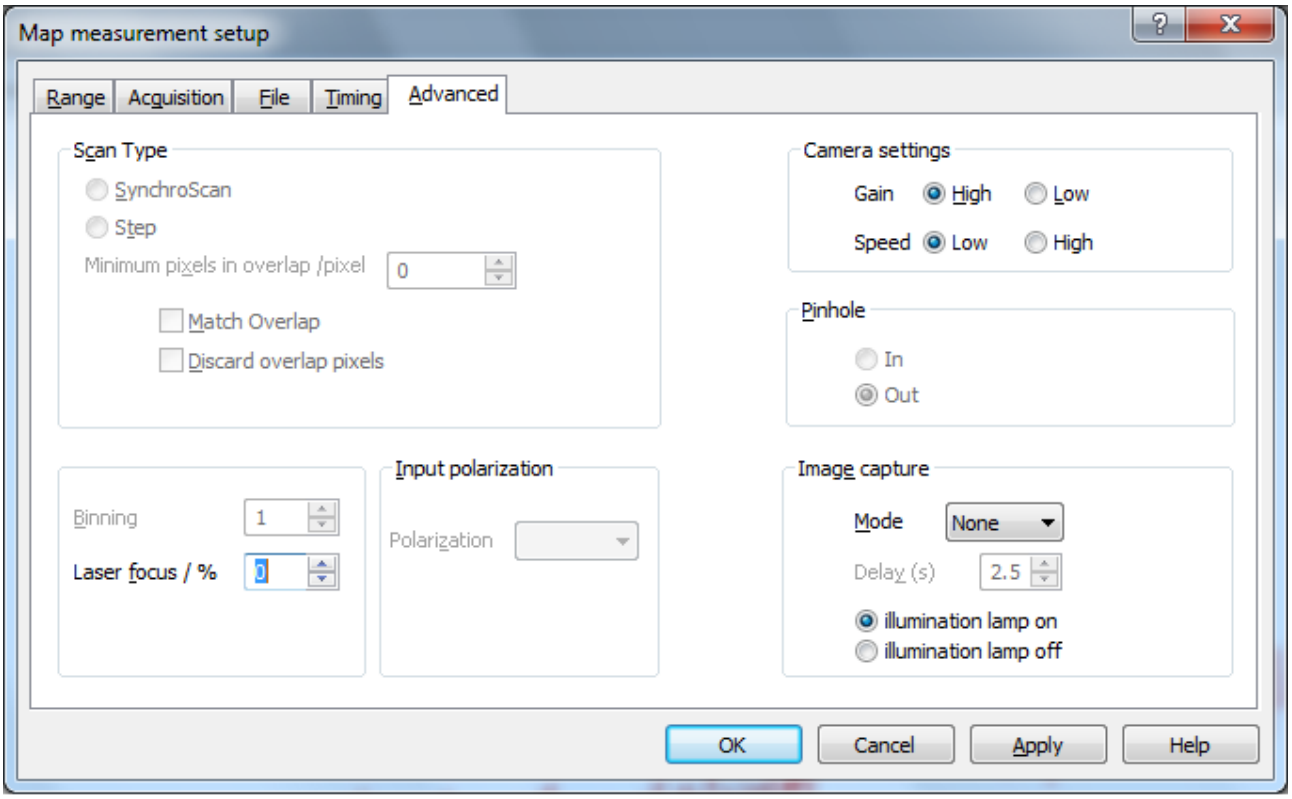


Figure 9 *Advanced* tab

1. *Scan type* is used when *Extended* scan is selected in the *Range* tab to select the type of extended scan to use. *SynchroScan* is recommended for most applications, as it does not contain the artefacts present in stitched scans. The *Step* option is included for samples that are very strong Raman scatters and might saturate the detector if the *SynchroScan* option, which requires a minimum 10 second exposure, is used.

2. *Camera Gain* switches between the sensitivity settings of the detector, and should usually be set to high.

   Camera Speed should be set to low

3. *Pinhole* allows the user to set whether the pinhole is *In* or *Out.* The pinhole may improve the beam profile. The pinhole can be used to convert a line laser into a spot laser. This function only operates on systems with a motorised pinhole. Its primary uses are in those instruments with True Raman imaging and where the automated alignment functions are set up.

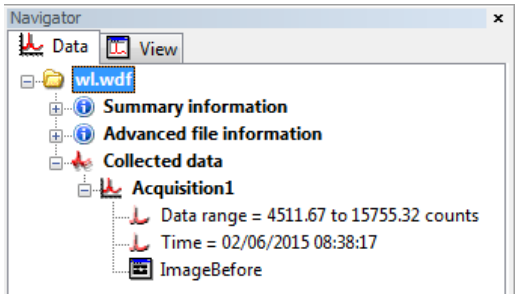
4. *Binning* allows the co-addition of adjacent signal from pixels on the detector to improve the signal-to-noise ratio in extended scans only. However, excessive binning reduces the spectral resolution. Use values of 2 or 3 unless the Raman bands are naturally broad when larger values can be used. The default value is 1.

5. *Laser focus* controls the use of the beam expander. 0% indicates the laser is tightly focussed, while 100 % indicates it is completely defocused by the beam expander. Defocusing reduces the power density at the sample, and so can reduce sample damage in sensitive samples, but reduces spectral resolution. Values greater than 0% are used for True Raman imaging measurements.

6. *Input polarisation* is used in instruments with polarising filters to select the polarisation of the laser beam. The different Raman scattering response of a sample to different laser polarisation can be useful in assigning the symmetry of the vibrational modes involved.

7. *Image capture* allows the user to specify the capture of a white light image of the sample before or after, or both for single acquisitions, time, temperature or mapping measurements by using the *Mode* drop-down menu. *Delay* sets the time the camera is allowed to adjust its settings to the conditions, so that a good image is obtained. The default time is 2.5 seconds. The image capture feature applies to both inVia and inVia Reflex models. On the former, the user is prompted to switch the optics such that an image can be collected, then back again such that a spectrum can be acquired.

   The image can be viewed or saved from the spectrum file using the Navigator



   Select and then right click on the image icon to load the white light image into the same window as the data. Right click on the image to save, show axes, show scale bar etc.

When reviewing one-dimensional datasets (time and temperature series), the acquired video images are displayed in the top right frame of the Map Review window. If images were acquired (before and/or after) spectral acquisition, these images may be toggled / selected from the right-click context menu (Show video image…).

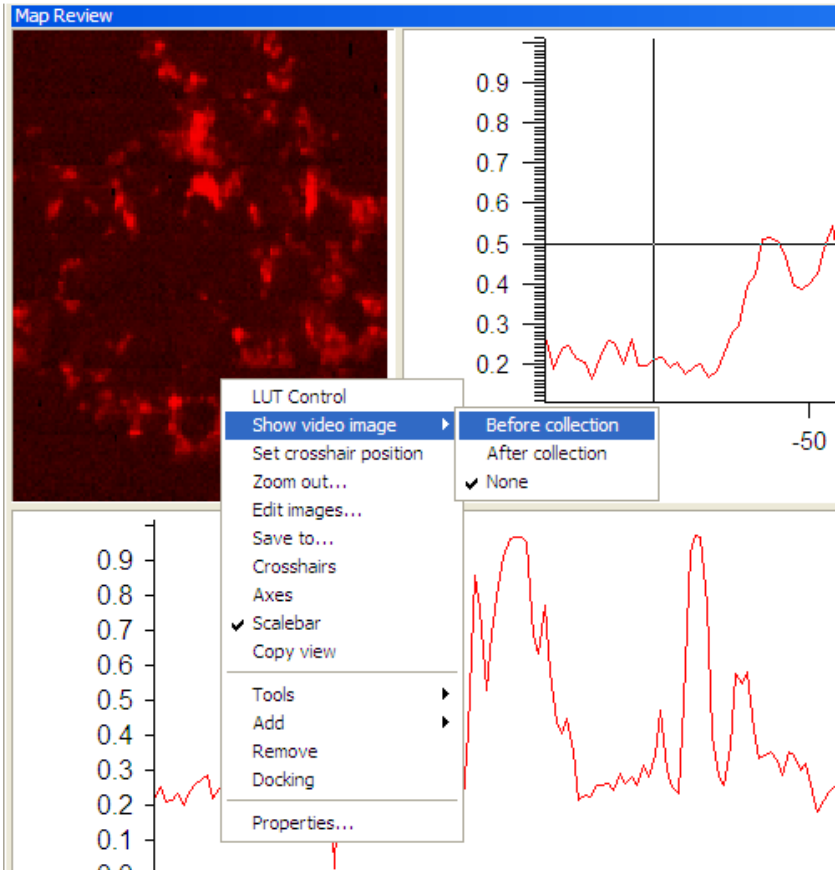


Figure 10 *Map Review*