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TM003 – Sample viewing and configuration change WiRE™ 5

This module details recommended procedures for:

1. Viewing different types of samples using the Renishaw video.
2. Selecting different laser / grating / CCD camera configurations within the inVia Raman microscope.
3. Selecting between multiple microscopes, where applicable.

Suitable knowledge of the WiRE 5 software is assumed in this document.

Sample viewing

inVia and inVia Reflex Raman microscopes typically consist of direct microscope sample viewing using eye pieces and/or a microscope video camera. Other options are available. Systems can be equipped with multiple microscopes, for example an AFM and associated interfacing may be fitted in addition to a standard microscope. In such cases, the user may automatically switch between microscope options in the software.

The Sample review is opened from the View menu or short cut button. 

For non-Reflex systems the Sample review contains:

- Objective selection (not motorised)
- Laser shutter control within inVia
- Laser power control for viewing
- Pinhole control (in or out)
- Selection control of Laser
- Selection control of grating

Where configured within inVia, additional controls for CCD detector selection, and microscope selection may be available.

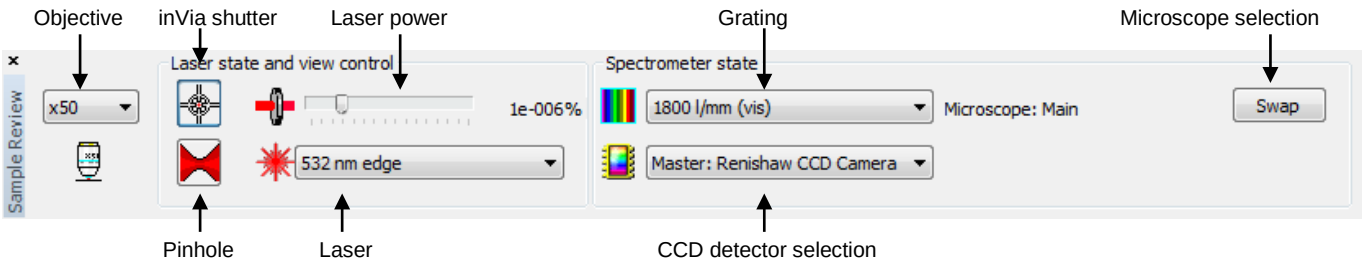
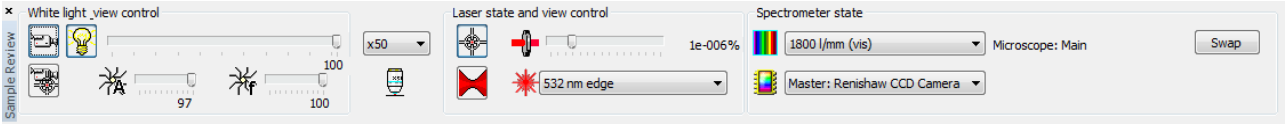


Figure 1. inVia sample review

In addition to these, the inVia Reflex Sample review contains:

- Viewing control of eye piece and video (white light only)
- Viewing control of video only (white light and laser)
- Illumination brightness (on/off and intensity)
- Aperture stop control
- Field stop control

For inVia non-Reflex systems, these options are all configured manually directly from the microscope.



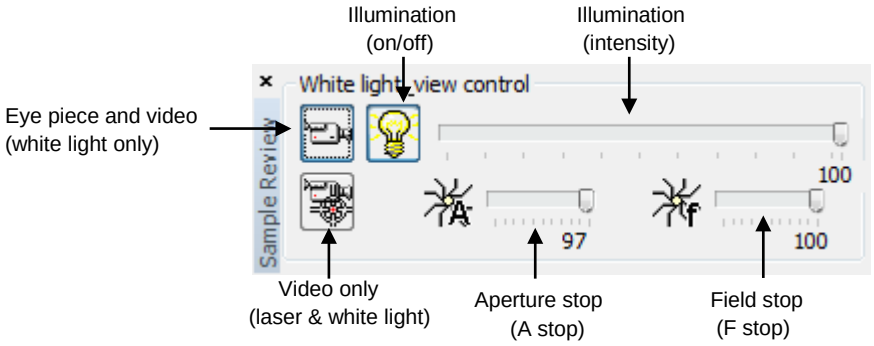


Figure 2. inVia Reflex sample review

Whether used manually or through the Sample review, the illumination control, A stop, F stop, and camera control are used together to aid focussing and sample viewing and generate high quality white light images of the sample. The figures associated with the illumination intensity, A stop and F stop sliders represent the percentage of the total range available being used for each in the current sample view.

Focussing the sample is aided by F stop, white light focus, and laser focus.

Typically the sample is initially viewed using a low magnification (e.g. 5×) objective. If the sample has discernible features these can be used to focus using the white light. Closing the F stop will reduce the field of view and produce an octagonal ring. When the edge of this ring is sharp, the sample is nominally in focus. This is of particular use for featureless samples.

The laser spot / line and white light are co-focal for most visible and near infra-red laser wavelengths. Therefore when the laser spot is in focus the sample is in focus with the white light.

The sample focus can be checked by moving sample position and seeing an equivalent change on the video. Progress through higher magnification objectives, refocusing each time, until the objective to be used for data collection is reached.

Achieving the best video image requires appropriate control of the illumination control and video settings for different types of sample (different colours and reflectivities).

A closed A stop produces high contrast images at the expense of reduced illumination. For most samples the A stop should be closed to achieve the best image quality (although the exact setting will depend on the objective used and the size of its back aperture). The properties of the video can be controlled by the user to optimise exposure, and gain. Depending on the sample reflectivity it may be appropriate to use auto settings to achieve the best quality (note that under these settings the frame rate may reduce the responsiveness of the video).

Hovering the mouse over the cog in the top right of the video viewer allows the user to adjust the exposure, gain, brightness and contrast directly from controls which overlay onto the video. The video viewer updates live.

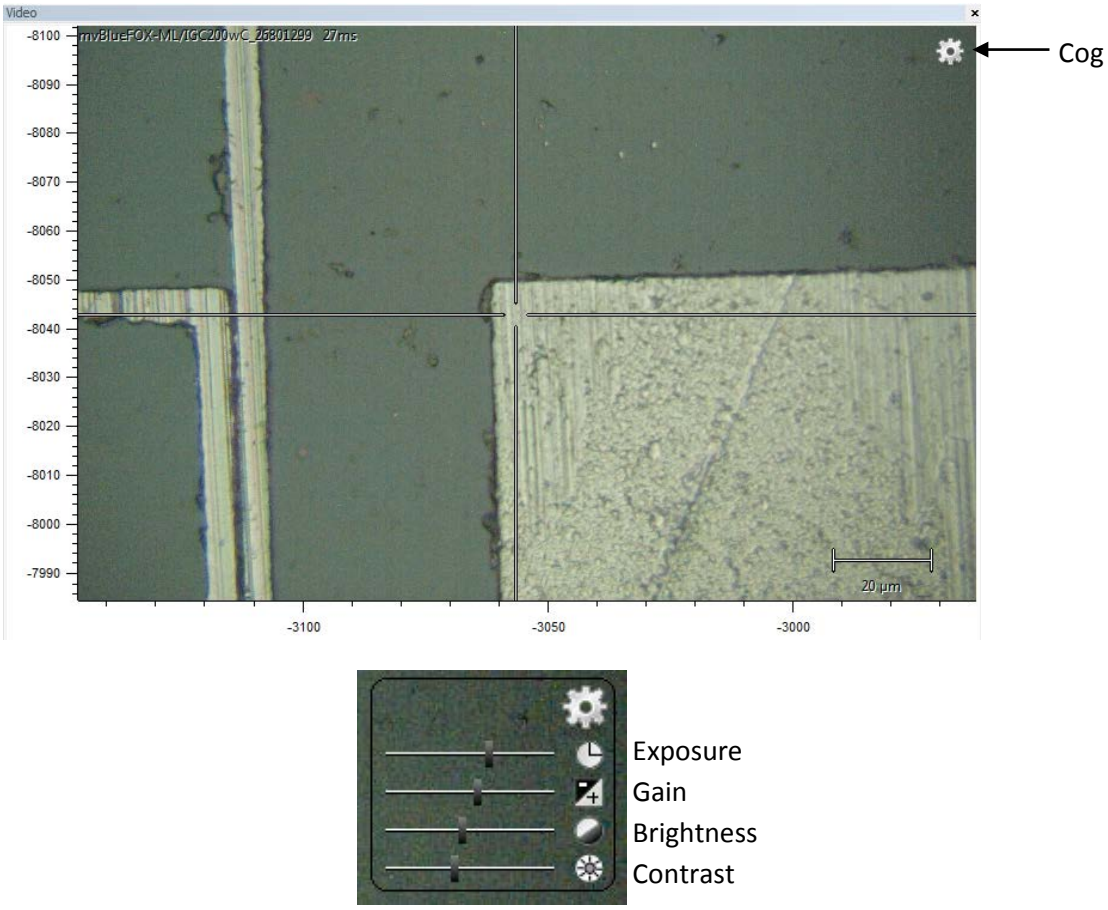


Figure 3. Video properties

Clicking on the exposure or gain icon will select the auto mode, this will grey out the relevant slider and prevent adjustment. Click on the icon again to turn off auto mode.

To change the video settings click directly on the cog (Figure 5).

For additional options right click on the video image and select Video properties.

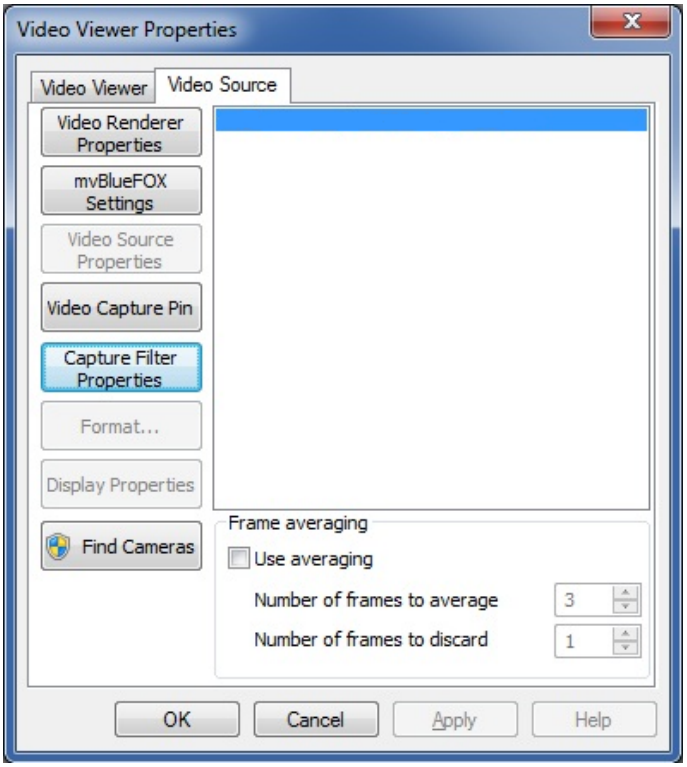


Figure 4. Video properties

Under the main property page the user can apply image averaging to the video. This can reduce noise for dark/low contrast samples but may affect video responsiveness.

The Capture filter properties contain the different camera settings. Note that a left-click on the cog within the video viewer acts as a shortcut to the Capture Filter Properties dialogue box.

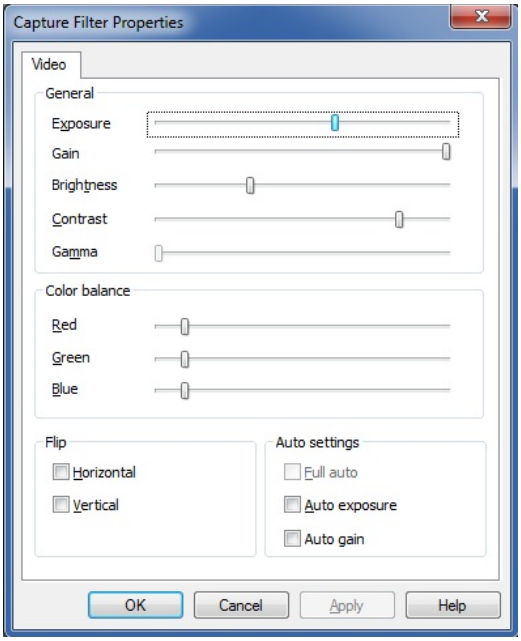


Figure 5. Video property settings

Using auto exposure adapts the exposure based on the image contrast and brightness but may not be appropriate for all sample types, where the F stop is closed, or where auto mode continually hunts (does not produce a single stable exposure value). In these scenarios turn off auto exposure from this dialogue.

The size of the video window can be changed to balance the desired image resolution and image size. Select the Video capture pin button and choose a display resolution. Ensure the aspect ratio of the video is kept the same as this will otherwise affect the calibration of the video.

Figure 6. Renishaw video option

Configuration selection

The sample review shows the current instrument configuration (laser/grating/microscope/detector) and also allows the configuration to be changed.

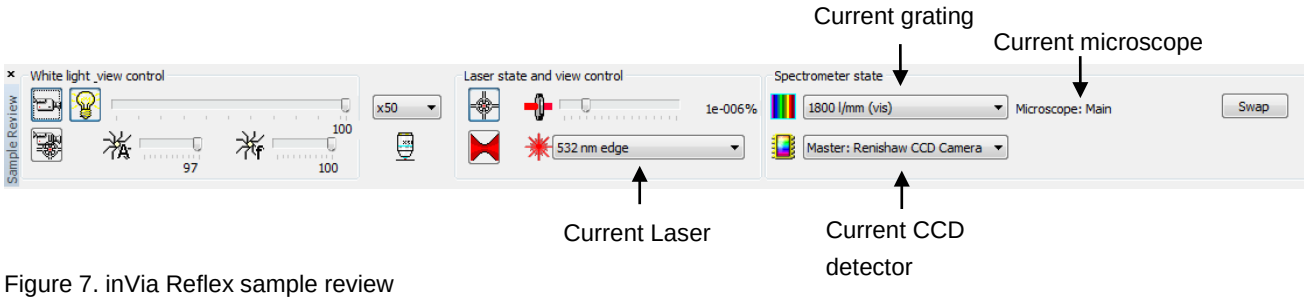


Figure 7. inVia Reflex sample review

Changing configuration

To change configuration the user must know the desired laser, grating, detector, and microscope they intend to use for their analysis. Some combinations are not appropriate, and when attempting to collect data a message will appear to inform the user that this configuration is not calibrated (and therefore should not be used).

If the system has been set up with multiple microscopes, the user can switch between them by clicking the 'Swap' button in the Spectrometer state section within sample review. This will open a dialogue box with a drop down list containing all available microscopes. Selecting the desired microscope and clicking 'Swap microscope' will make the selected microscope current within inVia. The video and current microscope field will automatically update. The user will need to select either the eyepiece and video or video and laser button in sample review to view the sample, regardless of what mode inVia was in prior to microscope swap. If different, the laser/grating/detector configuration and the objective will need to be updated by the user in sample review.

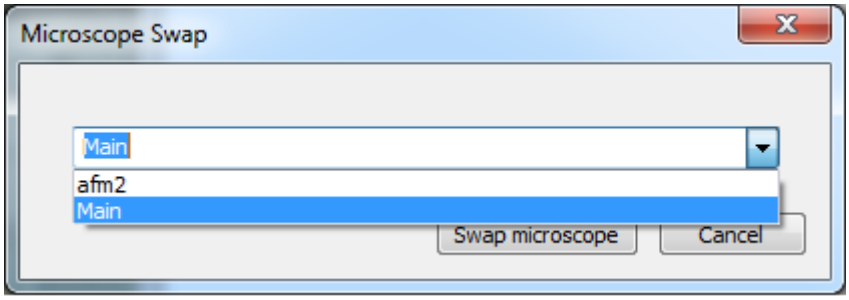


Figure 8. inVia Reflex microscope swap

When the 'Laser' is changed, several or all of the following will occur immediately on selection dependent on the system type:

- Motorised beamsteer autochange (only if the motorised beamsteer mirrors are installed)
- Motorised Rayleigh filter change (only if the motorised Rayleigh filter change is installed)

- Beam expander autochange (all, although UV lasers are sometimes used with no beam expander)
- Internal laser shutter autochange (all)
- Internal silicon reference re-focus (Reflex only)

The flexibility and upgradability of the Renishaw inVia microscope is such that the degree of automation desired can be gained from any previous configuration. Manual, partial automation, full automation, and full auto validation options are available.

For inVia instruments without motorised Rayleigh rejection filter change, the user must open the instrument door (see instructions below for safe operation of the instrument door and laser interlock) and manually swap the Rayleigh filter for the new wavelength. Some instruments with motorised filter change may require manual filter change if all four positions on the mount are occupied and the new laser's filter is not currently fitted.

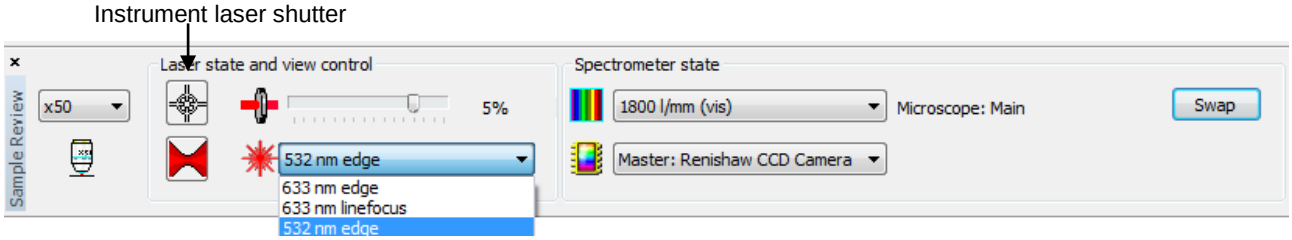
When the 'Grating' is changed, the relevant software changes are implemented, but no immediate mechanical change takes place. The grating used will affect the spectral range and the spectral resolution. The grating change procedure is identical for all inVia Raman microscope models. For instruments with more than two gratings, the user may have to manually remove and replace a grating to obtain the required configuration. Gratings may be mounted back-to-back and care should be taken in separating 'pairs' of gratings. Grating mounts are designed such that any one grating can only be mounted in one of the two positions (set during the system build phase). To manually add/replace a grating: remove the spectrograph cover plate, attach the grating dust covers, remove the gratings, separate, fit the new grating, remove dust cover and replace the spectrograph cover plate.

<u>Configuration change protocol</u>

1. Ensure all files and windows are closed (checking that no unsaved files are still required).

2. Decide on the desired configuration.

3. Change the 'Laser'. (Note the laser change does not only necessarily change the laser wavelength, but is also used to select for example different Rayleigh filter types of the same wavelength, and the use of line focus/StreamLine imaging with the same wavelength)

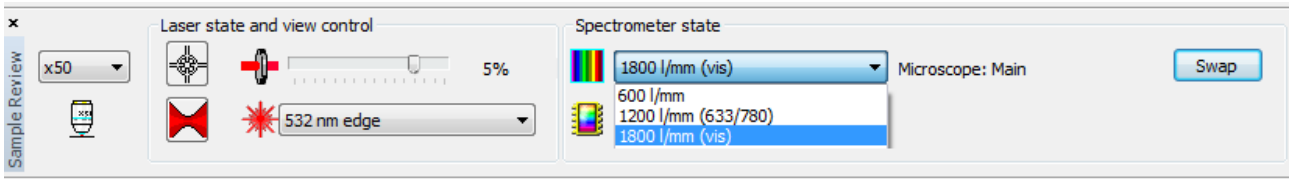


4. On changing the laser a dialogue will appear prompting the user to change the relevant spectrometer lenses (if change is required). If this dialogue appears, open the instrument

(the instrument laser shutter will be automatically closed), and carefully remove the appropriate lenses from their kinematic mounts. Replace with the new lenses ensuring that each is correctly seated. Close and re-lock the instrument door.

Note: under standard use, opening the instrument door will trip all laser interlocks **unless** the instrument laser shutter, accessed from the sample review, is closed. If the interlock circuit is broken, close and re-lock the instrument door and reapply the laser interlock (**Tools….Interlock…..Reset**).

5. Change the ‘Grating’. (Note that this should not prompt a lens set change, unless multiple gratings are configured for the same ‘Laser’).



Of course, if the configuration already set is the same as that desired, then no configuration change is needed, and the above protocol can be skipped.

Configuration change as part of a measurement

The configuration can also be changed by editing the current measurement. This is useful when the user requires analysis from the same region of the sample but with different excitation.

Use the Setup measurement button  to edit the laser and/or grating in the Acquisition tab (the laser power, exposure time and accumulations may also need to be adjusted for the new laser). Running this edited measurement may prompt the user for a lens change, if motorised lenses are not available.