

## TM010 – StreamLine™ imaging

## WiRE™ 5

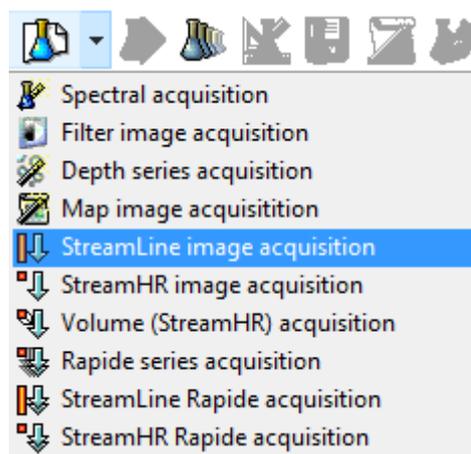
This document aims to show the WiRE™ 5 user how to set-up a StreamLine™ image acquisition within WiRE 5. It assumes that the user is familiar with the basic operation of the software and that the inVia instrument is correctly configured for StreamLine™ imaging including the StreamLine™ feature permission.

The following information provides a step guide on setting up and collecting StreamLine™ images using the Renishaw inVia Raman microscope. The steps involved in this process briefly comprise of the following:

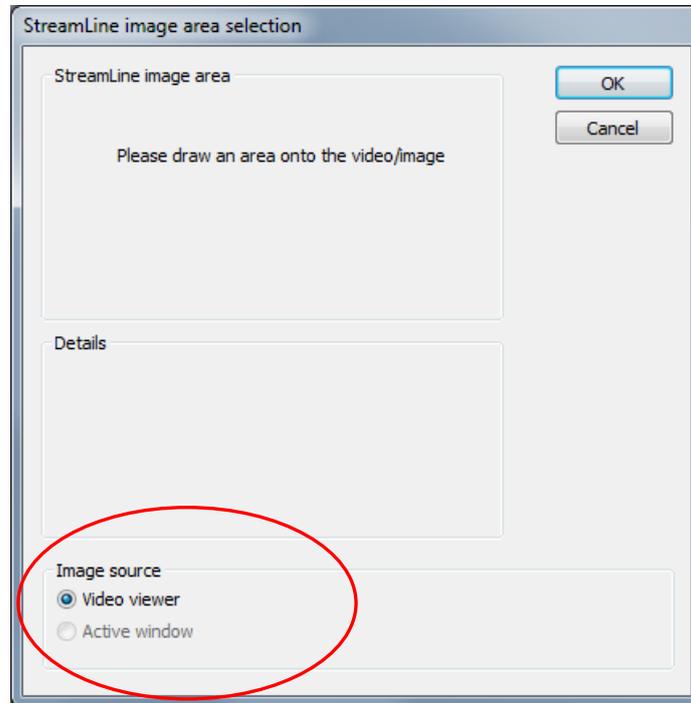
- Start either from a live video image or static image captured from video (montage)
- Choose 'New StreamLine image acquisition'
- Define the area to be analysed and click 'OK'
- Refine parameters within 'measurement set-up'
- Configure 'Live Imaging' if desired
- Run measurement

A detailed breakdown of each step is now provided with exact information on how to define the StreamLine™ image.

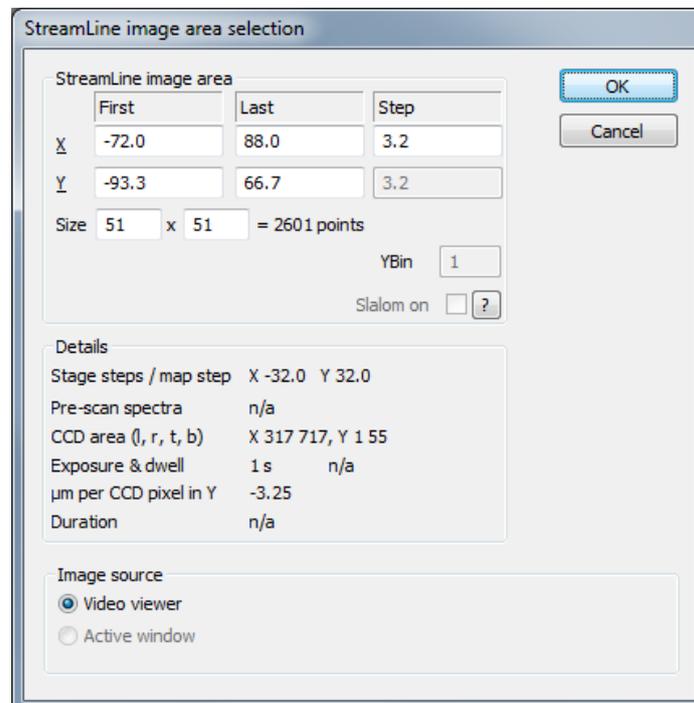
1. Ensure the correct objective is set within the sample review and any white light montages have been collected, if desired. It is crucial that the correct objective is selected; failure to do so will result in erroneous images.
2. Select 'Measurement....New measurement.....StreamLine image acquisition' or 'StreamLine image acquisition' from the drop down menu.  
(This will be greyed out unless either the video viewer or still image viewer are displayed)



3. Ensure the video or white light image option is correctly set depending on which window is to be used to define the area to be imaged (the option for still image viewer is greyed out if video snapshot / montage has not been collected)



4. Use the left mouse button to draw the desired rectangular area (click and drag).

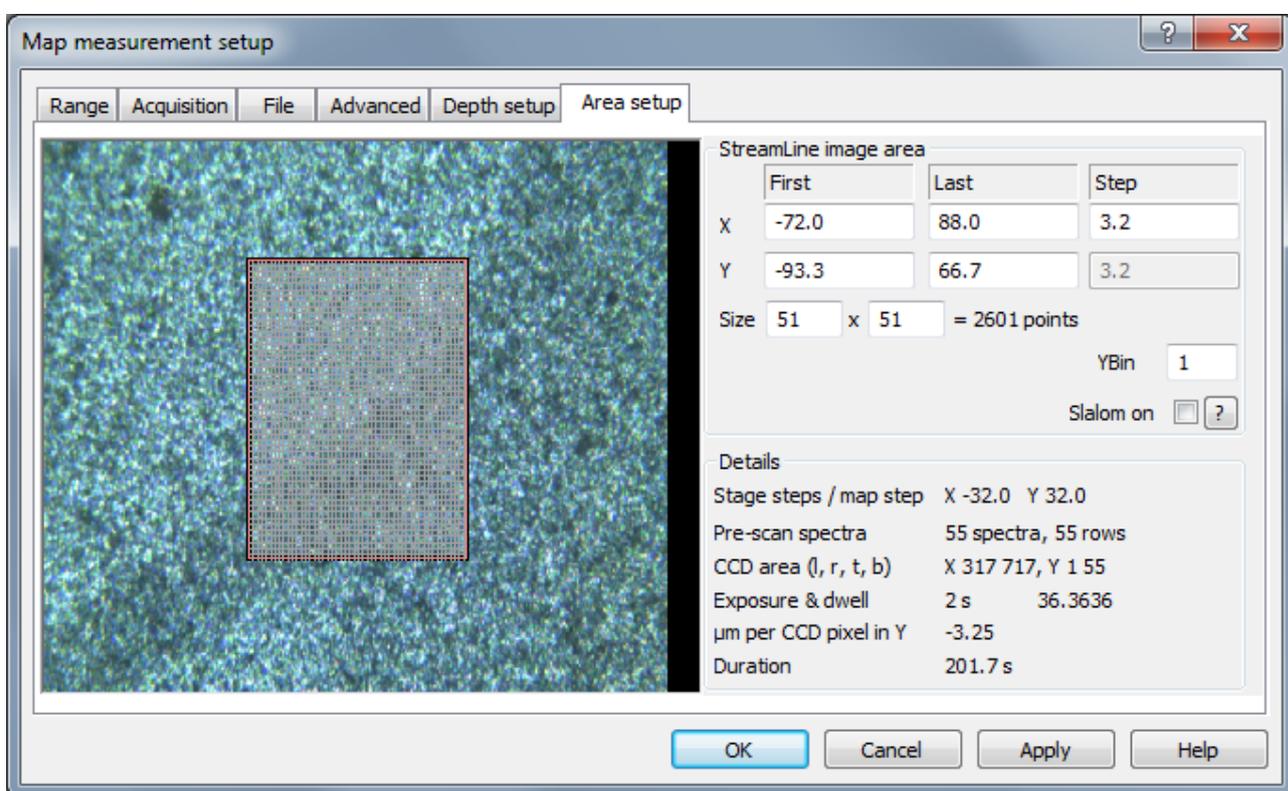


Note: StreamLine imaging speed is improved when the image is larger in Y compared to X.

5. Change only the image area in X and Y, if necessary (i.e. **not** the step size). These parameters can still be changed after this dialog.
6. Click 'OK' to launch the measurement setup
7. Go to the Area setup tab and change the Y bin to obtain the desired Y spatial resolution (multiple of the native Y spatial resolution).

Note: the minimum exposure time per CCD row (defined in stage 9) can be reduced as the Y binning is increased (the exact value depends on several factors including the defined CCD height)

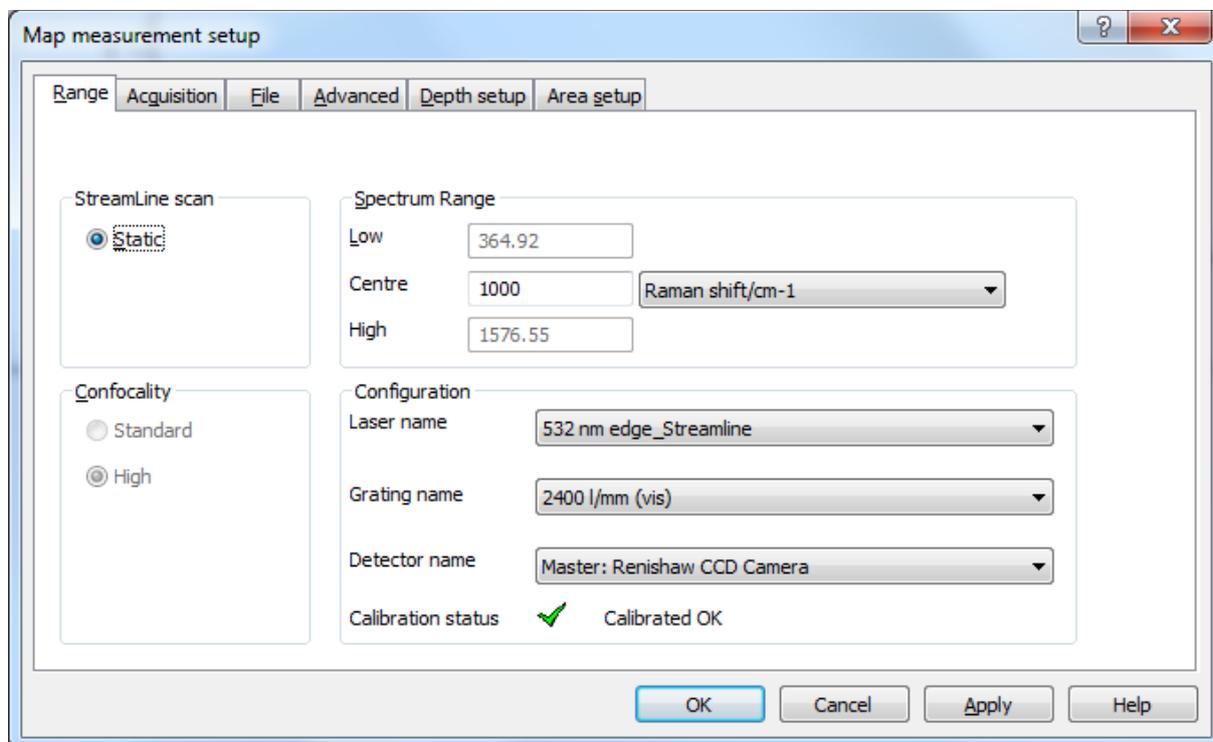
Now change the X step size to match the Y spatial resolution (different X and Y spatial resolutions can be used but are **not** recommended)



The 'Slalom' option can be used to ensure the laser covers the complete image area even when the Y step size (Y bin) and X step size are larger than the highest spatial resolution achievable (before deconvolution) with the current instrument set-up. If this option is not selected, vertical regions of the image area may be deliberately missed.

- Slalom ensures complete area coverage but will result in spectra which may be more averaged.
- No slalom means some parts of the image area are missed but the spectra are more pure.

8. Go to the 'Range' tab and set the centre position of the scan (the spectral range and spectral resolution will depend on the laser wavelength, grating groove density and detector type).



9. Go to the 'Acquisition' tab and select the time and laser power to be used with the scan.

StreamLine has a minimum exposure time which can be used.

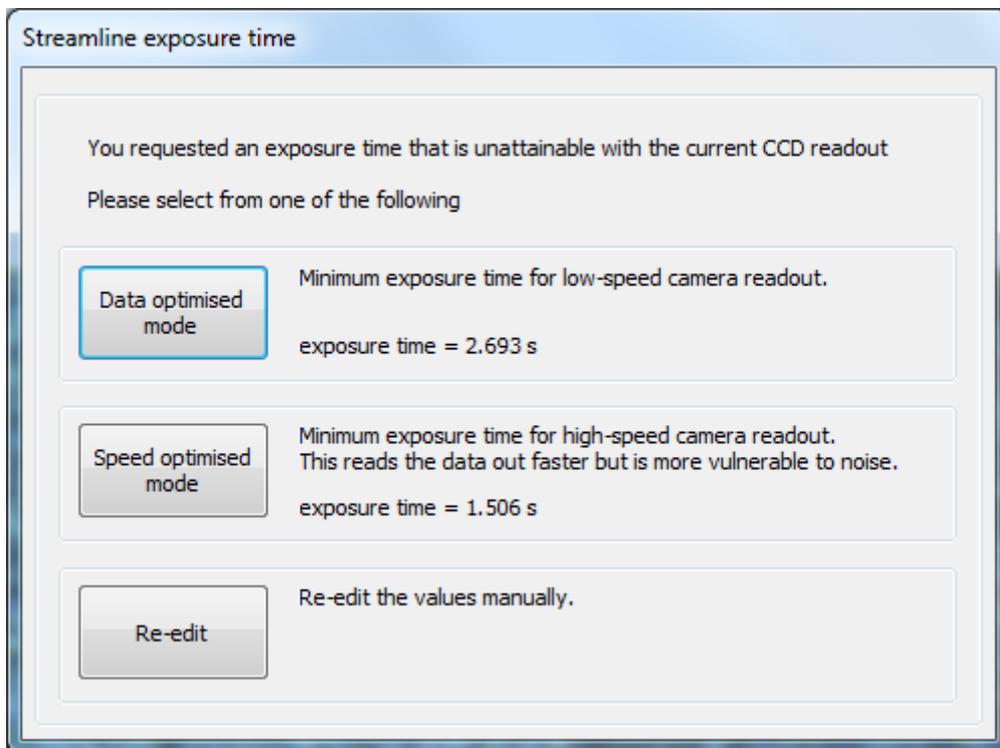
Two different modes are available depending on the strength of the Raman information required:

- Speed optimised mode (SO) uses faster readout of the detector to enable faster collection of Raman images.
- Data optimised mode (DO) uses slower readout of the detector but produces higher data quality.

'SO' enables a lower minimum exposure time than 'DO,' but at the expense of data quality. 'SO' should only be used for extremely strong Raman scatterers (such as silicon and diamond) or strong photoluminescent bands (such as R1 and R2 PL bands from Cr<sup>3+</sup>)

To access the 'SO' or 'DO' time options the user is required to enter a low exposure time such as 0.01 s and select 'Apply'.

The following dialog appears:

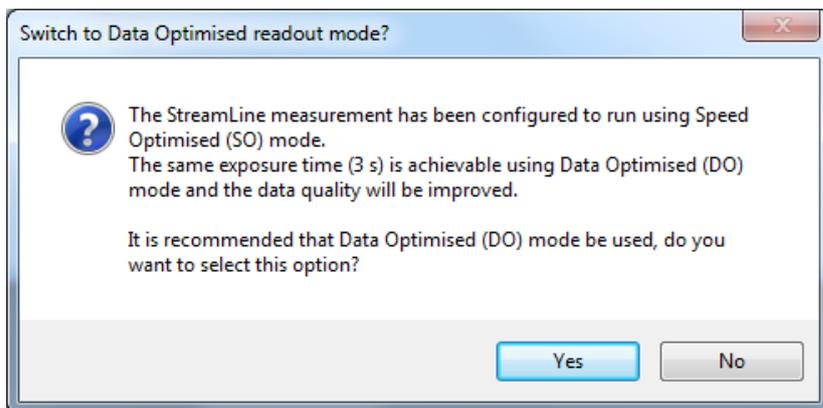


The minimum exposure times are calculated and the user has the option of selecting either 'Data optimised mode', or 'Speed optimised mode'.

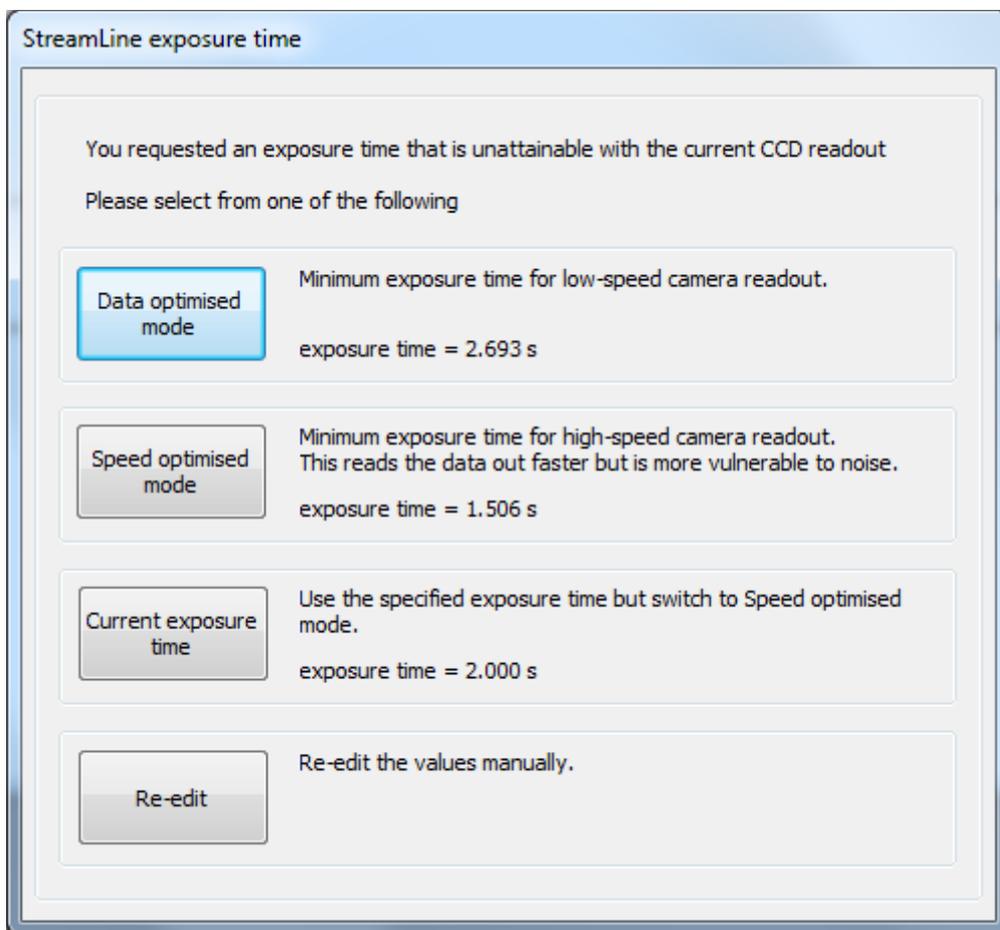
The 'SO' mode allows an exposure time approximately half that of 'DO'. However, the data quality will be worse than this time reduction alone due to the readout mechanism. 'SO' should be used for very strong scatterers only.

**'DO' mode should be routinely used.** Longer times can be entered by the user and will use 'DO'.

If, having collected a StreamLine image using 'SO' mode, the user wishes to increase the time to a value which can be used in 'DO' mode, then the following dialog will appear;



The user should always use DO mode if presented with this option. If, having collected a StreamLine image using 'DO' mode, the user decreases the time to a value which can be used in 'SO' mode, but is greater than the minimum 'SO' time, then the following dialog will appear:



The user can select the fastest time for either 'SO' mode or 'DO' mode or can choose to use the current time in 'SO' mode.

The defined time is per CCD row. Therefore, as the Y binning is increased, the minimum time which can be used will reduce, but the exposure time experienced by each final spectrum will be the same or longer.

Example parameters for a 20x objective (785 nm) are shown below:

Y bin	Y step size (pixel size) (20x)	Minimum time / CCD row	Number of spectra added (Y)	Minimum time / spectrum
1	~ 3 µm	~ 1.34 s	1	1.34 s
2	~ 6 µm	~ 0.67 s	2	1.34 s
5	~15 µm	~ 0.27 s	5	1.34 s
10	~ 30 µm	~ 0.13 s	10	1.34 s

With the Y bin set to 1, the Y spatial resolution is determined by the objective used. Higher magnification will produce a higher spatial resolution.

There is no maximum time limit. It is also worth remembering that the total image time will be much lower than the time per spectrum multiplied by the number of spectra due to the fundamental StreamLine™ method.

10. Select the 'close laser shutter on completion' option.
11. If desired select the 'Live imaging' option (see module TM14 or TM15) to create Raman images during data collection.
12. Go to the 'File' tab and define the filename and location. Select the 'Auto increment' option to ensure the data cannot be overwritten.
13. Select 'OK' on the Map measurement dialogue to complete the set-up. Then 'run' the measurement. Go to the 'Area setup' tab if an accurate duration estimate is desired. The duration estimate is displayed and updated during data collection

Note: This process can be repeated for multiple measurements to 'queue' data acquisition which will then be collected consecutively without user intervention (Reflex model only). Please contact Renishaw ([raman.support@renishaw.com](mailto:raman.support@renishaw.com)) if a non-Reflex model is owned and the user wishes to 'queue' StreamLine measurements.

To minimise the total measurement time for the image, remember the following:

- The pre-scan overhead is per column of data (i.e. per Y section of the image), so longer and fewer columns will provide a faster total time.
- Start by trying the minimum 'DO' time. The data collection will be fast, and the acquisition time can be increased easily if higher data quality is necessary.
- Try the component analysis (DCLS) image creation method (see module M10). This requires reference spectra and can produce high quality images from low signal to noise spectral data.
- Data quality can be improved after data collection using the noise filtering method (see module TM13)