

## TM006 - FocusTrack

## WiRE™ 5

This document aims to show the WiRE™ 5.0 user how to set up the FocusTrack option, to be used whilst collecting mapping data, such as point imaging or rapid line focus imaging. The focus track option allows the laser spot (or line) focus to be maintained by minimising its size as the sample position is changed.

**Note this function cannot be used with StreamLine or StreamLineHR imaging.**

### FocusTrack set-up

Initially the FocusTrack should be set-up to verify that the parameters to be used during the mapping experiment enable the laser focus to be adequately maintained for the extremes of the change in sample depth.

1. Focus on the sample with the objective to be used for the mapping experiment, set the

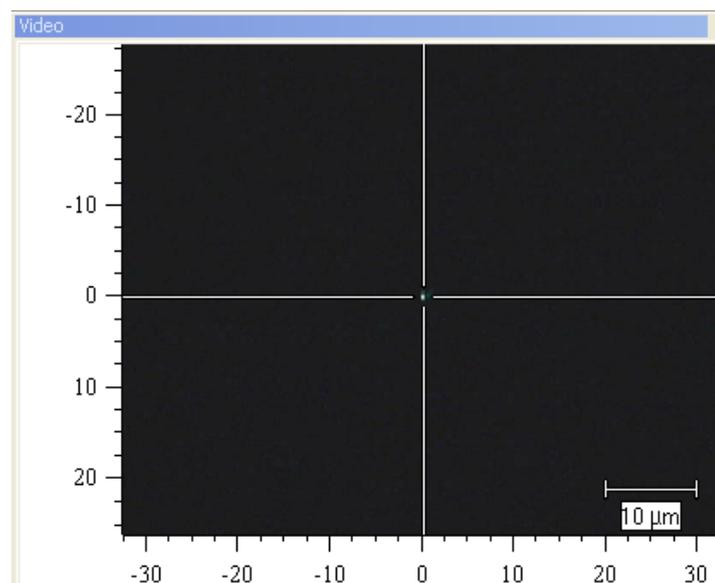
co-ordinates to zero using the  button.

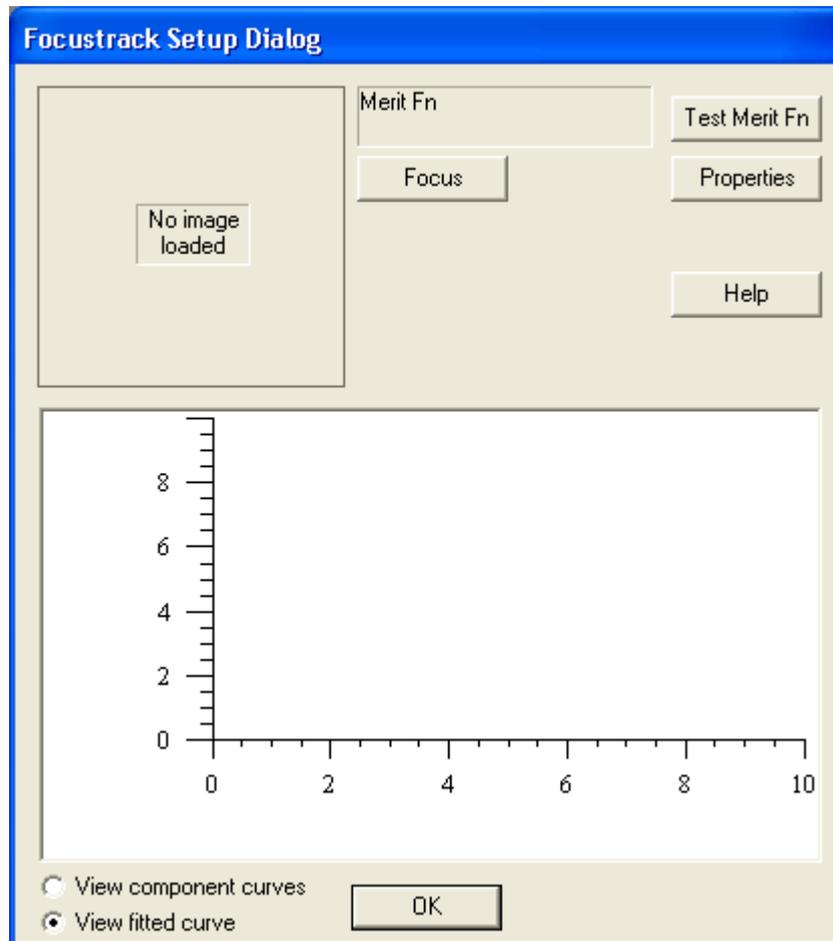
2. Ensure that the correct objective magnification is shown in the Sample Review tool (this is reflected in the scale bar of the Video viewer).

3. Ensure the video displays a black background (white light off) and highly attenuated laser so that the spot or line can be just observed without saturating the video camera.



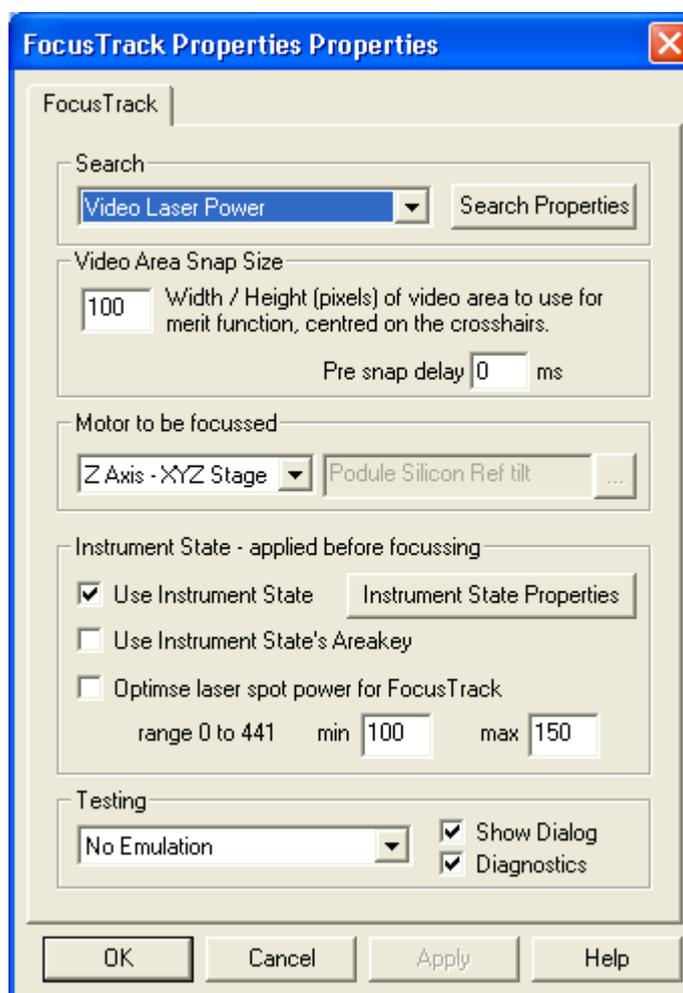
Objective magnification



4. Select **Live Video...FocusTrack...Set-up.**

The 'test merit function' button takes a snap of the video, and calculates the merit function value. It is suggested that a value of between 1000 and 1500 is achieved where possible. The attenuation can be varied with the 'FocusTrack Setup Dialog' window still open. Adjust the attenuation and recalculate the merit function. The optimum attenuation to be used with focus track can then be determined.

## 5. Select **Properties**.



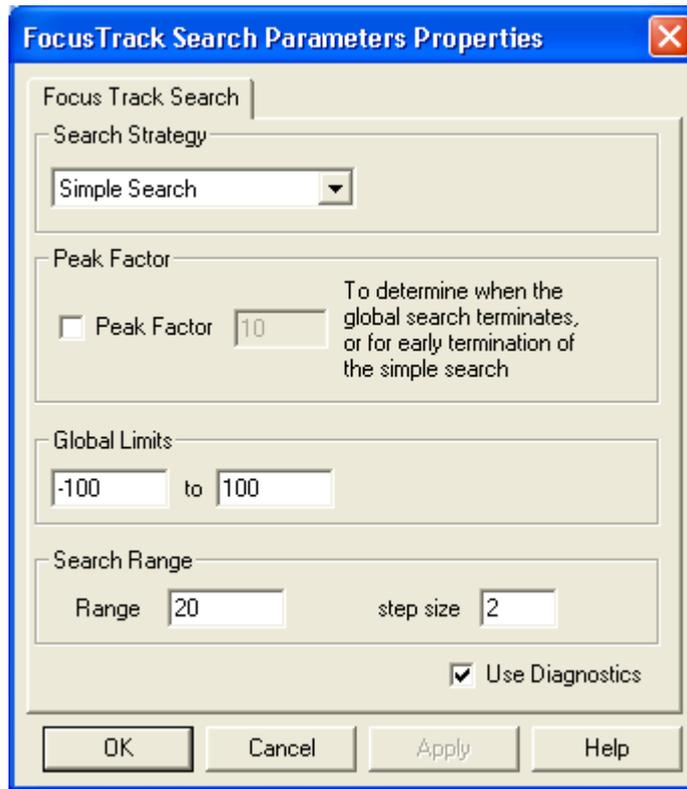
Firstly the correct search type is required. 'Video Laser Power' for spot lasers, 'Video Laser Power (Line)' for line lasers or lasers using a line focus lens.

## 6. Select **Search Properties**.

The search strategy allows the user to access different search algorithms (Simple Search recommended). The Global Limits act as safety limits to ensure objectives with small working distances are not exposed to possible contact with the sample. The upper and lower limit should therefore be set to a sensible number within which the maximum and minimum sample positions can be reached without risk of objective damage.

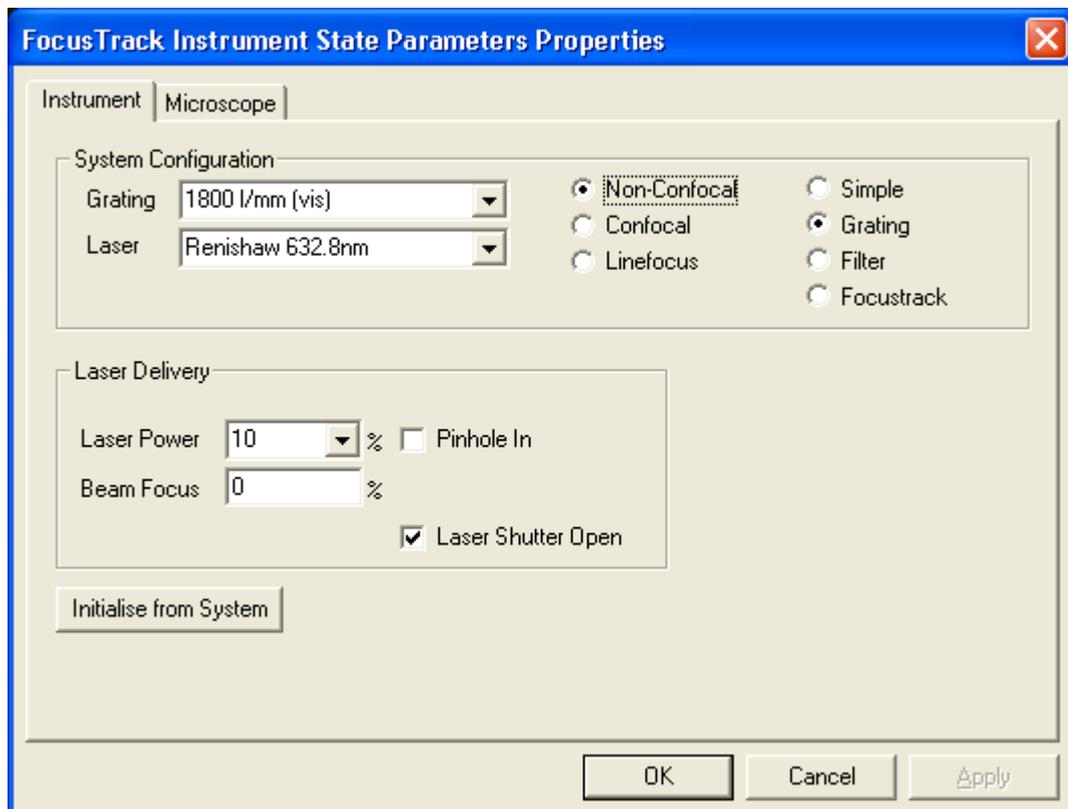
The Search Range dictates the magnitude of the focus search at any single point. Therefore point to point variations in height which are large would require a greater search range, whilst balancing the potential increase in analysis time which this would give.

The step size dictates the magnitude of each step.



When suitable values have been added / amended **OK** the dialogue.

7. Select **Instrument state properties**



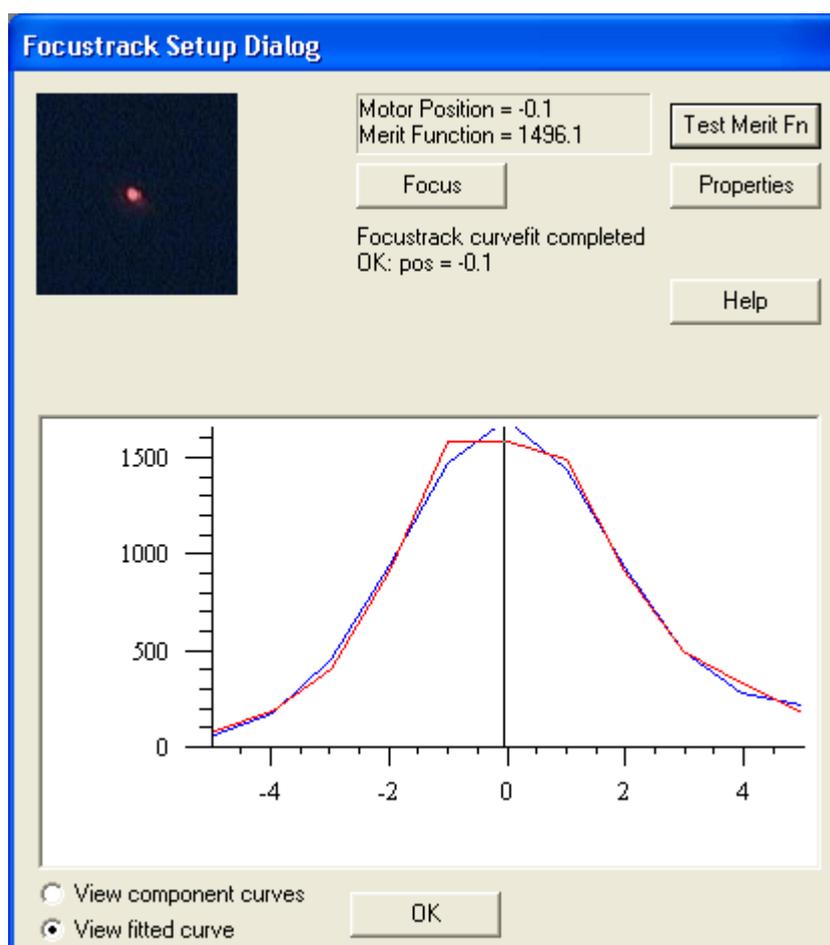
Select 'Initialise from System'. This will update the current video viewer settings (microscope configuration, laser power) to the FocusTrack set-up.

8. The 'optimise laser spot power for FocusTrack' option will automatically calculate the correct laser attenuation to apply. This will be calculated for every FocusTrack scan, and therefore will add time to the experiment. It is advantageous to determine the optimum attenuation prior to collecting the data (detailed in step 4.).

### FocusTrack check

When the parameters detailed previously have been set-up, a trial FocusTrack can be performed to ascertain the performance. This is achieved by selecting **Focus**, or  button.

The 'Detail' button will display a graph on completion of the FocusTrack to allow a visual representation of the change in merit function (spot intensity) with sample depth. The optimum focus position is determined by an automatic curve fit of this graph. It is therefore clear that a meaningful change in merit function as the focus position is approached is required. For lower magnification objectives (where small focus changes do not significantly affect the spot size) larger FocusTrack step sizes are therefore required.



The vertical line confirms the centre position of the curve fit.

### FocusTrack use in Map image acquisitions

The use of FocusTrack within maps can be easily set from the FocusTrack tab, available from the Map image acquisition set-up. To use FocusTrack simply tick the box. The 'Properties' button can be used to confirm the values previously determined. In addition the periodicity of the FocusTrack use in the map can be set. This value should relate to the rate of change of the sample surface position with mapping step size. Where the sample is generally quite flat, the FocusTrack is required infrequently, whereas rapidly changing sample surfaces require a frequent FocusTrack.

