GE Healthcare

Life Sciences

DeltaVision OMX™

Customer Instructions

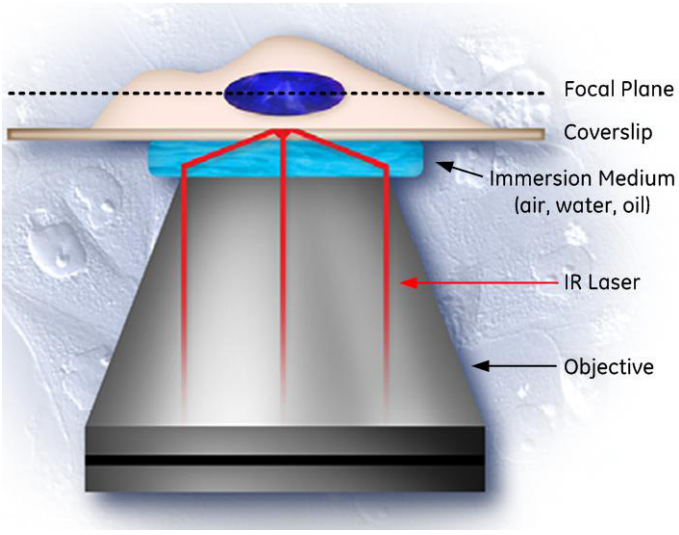
UltimateFocus™

DeltaVision OMX™ - UltimateFocus™

- *Describes the advantages of the UltimateFocus Module on the DeltaVision OMX system*
- *Describes where to find and how to activate the UltimateFocus fields in the software*

What is UltimateFocus?

UltimateFocus automatically and seamlessly identifies the focal plane of interest and maintains the sample Z position, regardless of the mechanical or thermal changes that occur on a DeltaVision OMX system. The patent-pending design detects the coverslip surface in real-time and maintains the sample Z position within 25nm (at 37°C with a 60X oil and 1.42NA objective) for unprecedented focus control. Continuous Z-position monitoring, with focus



corrections that occur in less than 200 milliseconds, enables accurate time-lapse data collection.

UltimateFocus is compatible with the air, water, and oil objectives used in DIC, widefield fluorescence, and Ring TIRF imaging. Z position is unaffected by the addition of reagents, eliminating additional focus corrections. Z focus is specific to the sample focal plane of each marked point and minimizes the interference of hardware in the field of view. Z focus is set when the point is marked, eliminating the need to create additional offsets.

UltimateFocus Fields

The three primary UltimateFocus fields are described briefly below:

- **Move threshold (nm).** Defines the minimum Z-position change from the point of origin. If the change in the Z position is less than the value indicated in the **Move threshold** field, the position of the stage will not change. If the change in position is greater than the **Move threshold** value, the stage will move to correct the Z position.
- **Maximum iterations.** Defines the number of stage corrections the UltimateFocus module will make using the **Move threshold** value. This field sets the upper limit for corrective moves allowed to the stage position at one time. Limiting the number of iterations increases the speed of your image acquisitions at the possible cost of focus accuracy. For fast imaging, it is recommended that the **Max iterations** value be set no greater than **1**.
- **Perform every ___ time points.** Defines the frequency of stage corrections. Entering a value of **1** tells the software to perform the UltimateFocus routine at every time point; entering **2** tells the software to perform the routine for every other time point, and so on. Higher values will increase the acquisition speed.

Using UltimateFocus

UltimateFocus functionality can be turned on with a simple click from several locations on the user interface:

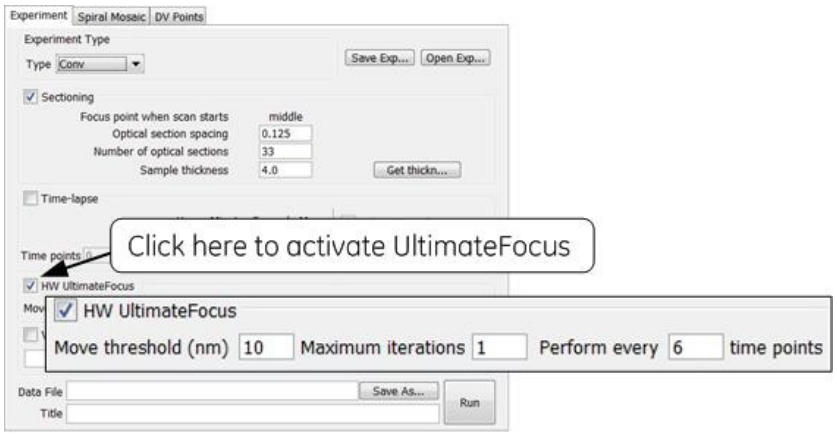
- During Experiment Design
- From Stage Tools
- While Marking and Visiting Points
- While Running Spiral Mosaic

During Experiment Design

Fields available for each experiment type on the Experiment tab include the **HW UltimateFocus** check box and related fields. Check this box to allow the system to automatically compensate for drift during an experiment.

If you are performing a Conventional, Structured Illumination, or Localization experiment, the **HW UltimateFocus** check box and related fields will appear in the Experiment tab in a format similar to that shown in the following figure.

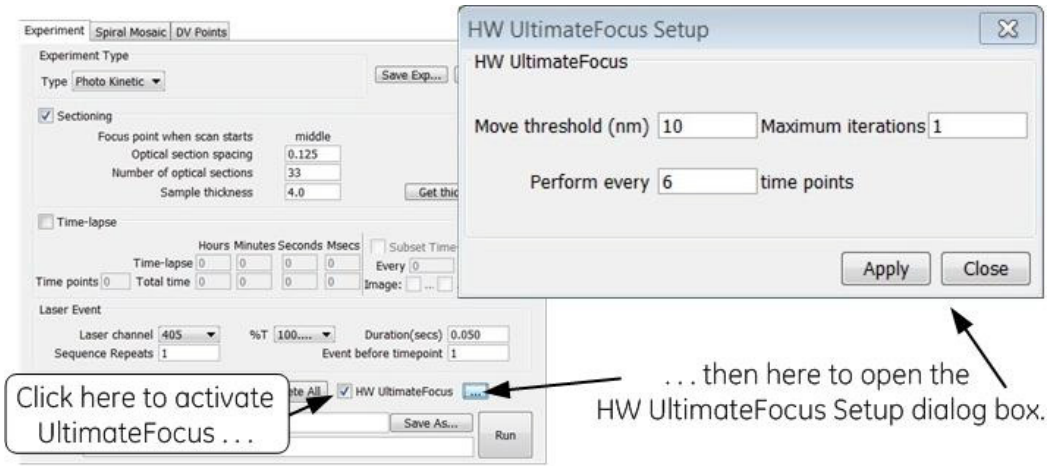
Figure 1. Activating UltimateFocus for Most Experiment Types



NOTE If no point visiting is selected, a Hardware AutoFocus calibration will be performed at the start of the experiment and that calibration will be used throughout the experiment. If point visiting is selected, points that have been calibrated will use their individual calibrations, while uncalibrated points will use a default calibration taken at the beginning of the experiment.

If you are performing a PhotoKinetic experiment, the UltimateFocus check box and related fields will appear as shown in the following figure.

Figure 2. Activating UltimateFocus for PhotoKinetic Experiments



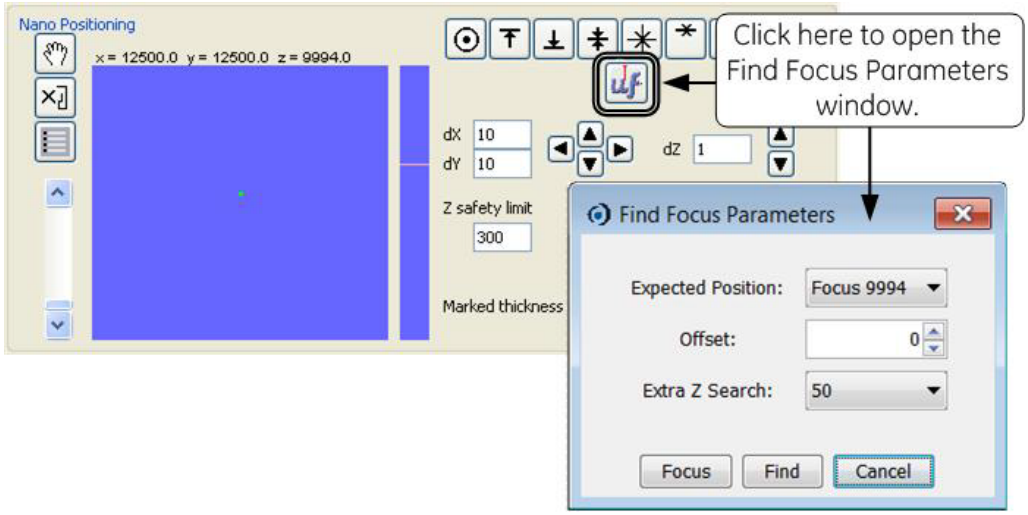
From Stage Tools

To activate UltimateFocus functionality from the Nano Positioning section of the main program window, simply click the UltimateFocus button to display the Find Focus Parameters window. This window contains the following fields and commands and can be useful for finding an initial point of focus on new samples:

- **Expected Position.** Displays the touchdowns defined using the **Z touchdowns** field (also located in the Nano Positioning section of the main program window). Select the value that most closely matches the expected position of your sample.

- **Offset.** Defines the value used to compensate for the difference between the sample's actual Z position and the Z position as determined by the system.
- **Extra Z Search.** Defines the distance below the **Expected Position** to search.
- **Focus.** Performs a short range scan to optimize the focal plane.
- **Find.** When finding the initial focal plane, performs a long range scan based on the Z touchdown value (may take about one minute).
- **Cancel.** Closes the Find Focus Parameters window.

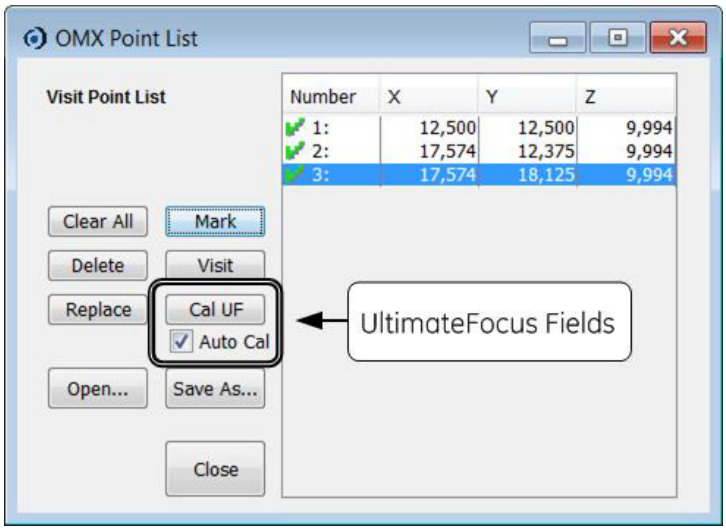
Figure 3. UltimateFocus in the Nano Positioning Tools



While Marking and Visiting Points

UltimateFocus is also helpful while marking and visiting points. In the OMX Point List dialog box, click the **Cal uF** button to run an UltimateFocus calibration on the selected point. If checked, the **Auto Cal** feature will run an UltimateFocus calibration on each new point that is marked.

Figure 4. UltimateFocus Fields in the Point List Dialog Box

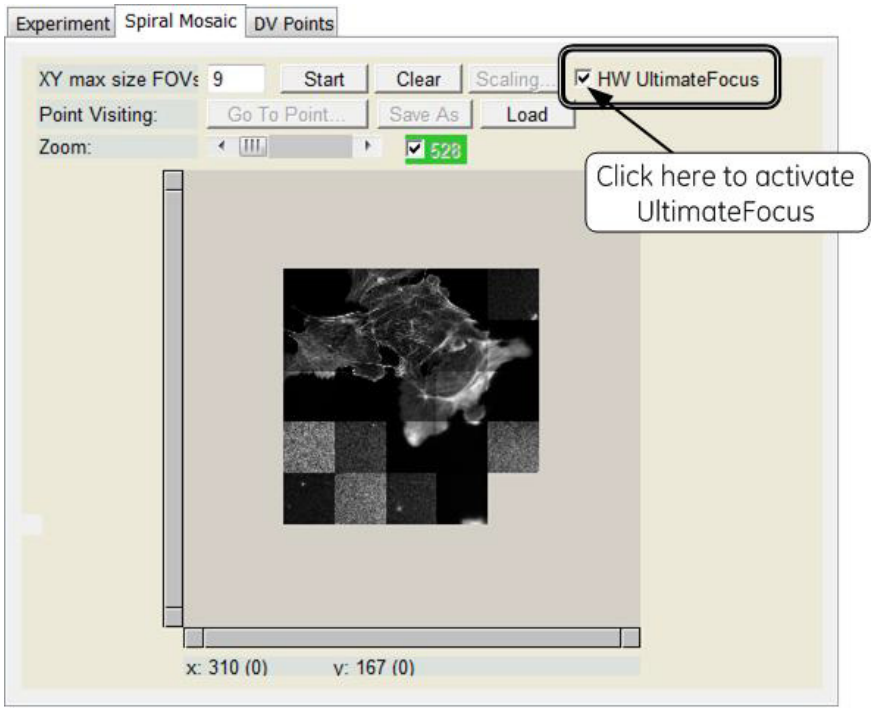


NOTE For best results, all points that will be visited during an experiment should be calibrated. Any uncalibrated point will use a default calibration which may be less accurate.

While Running Spiral Mosaic

When activated, the UltimateFocus module adjusts the Z position to keep the focal plane the same as the Spiral Mosaic scan progresses, correcting for tilt of the coverslip.

Figure 5. UltimateFocus and Spiral Mosaic



Notice to purchaser
DeltaVision OMX Imaging Systems are for research use only – not for use in diagnostic procedures.

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