

Live Spinning Disk Hardware Autofocus

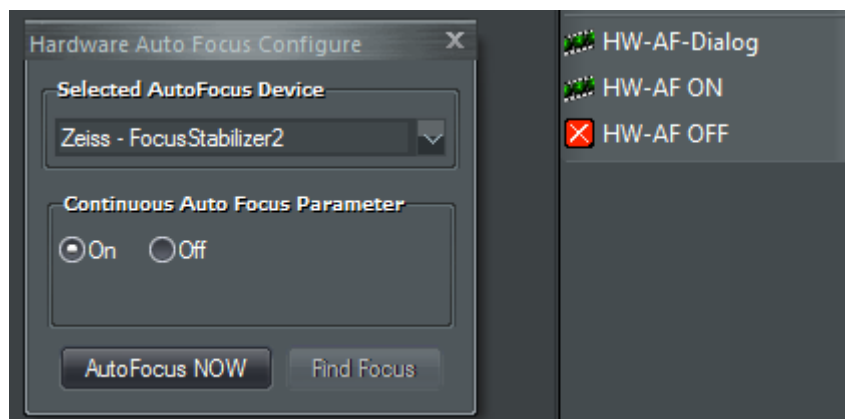
The hardware autofocus permits focus stabilization under non-bleaching conditions. It uses backreflection of grid pattern generated with a weak LED (850 nm) to measure the distance between the objective and the cover glass and to keep it constant. An aqueous medium (with a refractive index close to that of water) is required. It cannot be used with glycerol-based or hardening mounting media.

Objects above the cover glass/medium interface are not monitored. If objects of interest can change their focus position (e.g., cells rounding up during mitosis), the software autofocus should be used.

A. Continuous mode (constant offset)

This mode is convenient to keep the focus at a constant distance above the cover glass while moving the XY stage across large areas of the sample.

1. Click the button "HW-AF Dialog" in the toolbar on the right side of the application. Alternatively, the dialog can be opened with the menu command Configure → Autofocus.
2. Click on the "Find Focus" button. The device will try to find the z-position of the cover glass/medium interface
3. Focus on the desired height above the cover glass and click on the "On" radio button. Once the offset is determined, the "Find Focus" button is disabled.

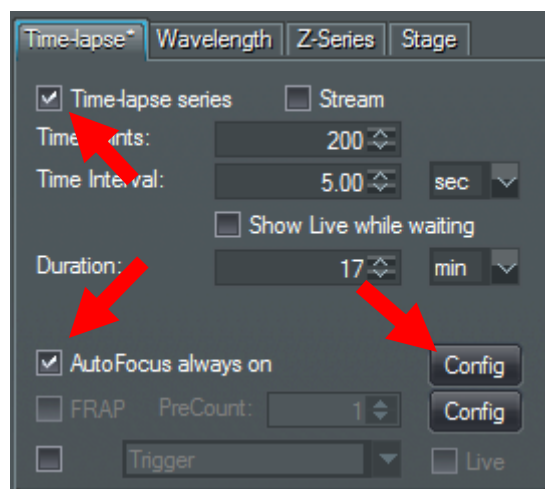


To use a different offset, turn off the autofocus, focus manually on a different position and turn it on again. For convenience, the toolbar also contains "HW-AF ON" and "OFF" buttons. The button "AutoFocus NOW" has no function (it works only with devices from other manufacturers).

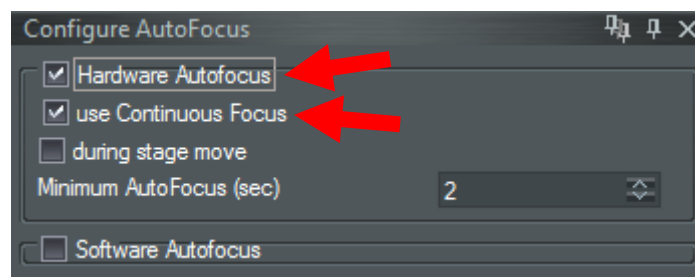
B. Stage positions with multiple offsets

The following procedure describes the setup of a multiposition time series with multiple offsets (i.e., positions may have different z-coordinates above the cover glass).

1. In the Time lapse tab, check "Time-lapse series" and the "Autofocus" option. Click the "Config" button right of the "Autofocus" option

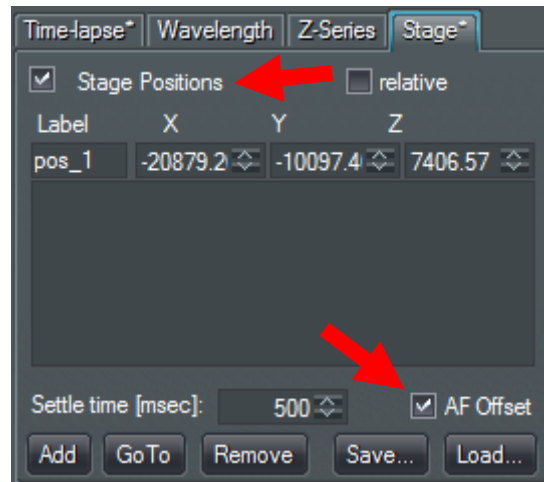


2. In the "Configure AutoFocus" dialog, check "Hardware Autofocus" and "use Continuous Focus". For very distant stage positions, additionally check "during stage move". Close the dialog ("x" on the right upper corner)

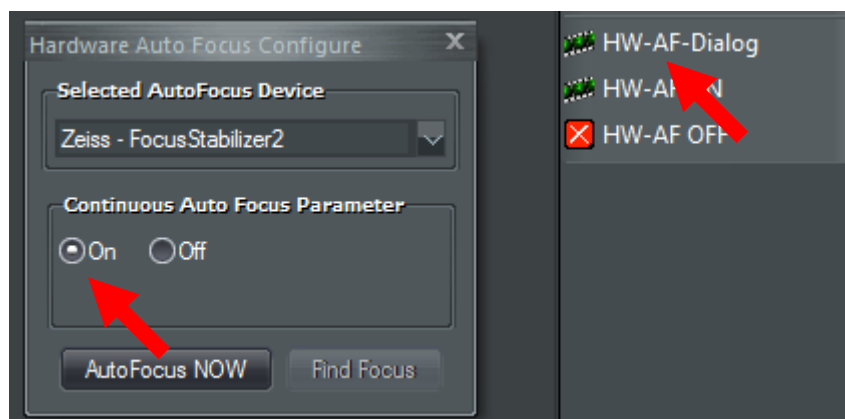


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3. In the Z-series tab, check "Stage Positions" and "AF Offset". For very distant positions, a settle time of 200-500 msec is recommended (to let the immersion oil film keep up with the XY stage movement)



4. Turn on hardware autofocus in continuous mode: in the toolbar (right side of the GUI), click "HW-AF Dialog" and then select "Continuous Auto Focus Parameter" = "On"



5. Move to the desired stage positions. In the "Stage" tab, add them by clicking the "Add" button. Coordinates added successfully with offset should display the suffix "a=Set". Start the sequence as usual.

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