IMAGING WORKBENCH

Software for Multichannel Dynamic Fluorescence Image Acquisition and Analysis

INDEC BioSystems
4701 Patrick Henry Dr., Bldg. 24
Santa Clara, CA 95054, USA
www.imagingworkbench.com
techsupport@imagingworkbench.com
Rev. 120112

Imaging Workbench Application Note 40 Rev. 1

Using the Rapp OptoElectronic UGA-40 with Imaging Workbench

Purpose

Imaging Workbench supports the use of the Rapp OptoElectronic UGA-40 High Speed Programmable Spot Illumination subsystem, when connected to a microscope and used with a supported camera.

This AppNote describes how to configure, test and use the UGA-40 subsystem.

Prerequisites

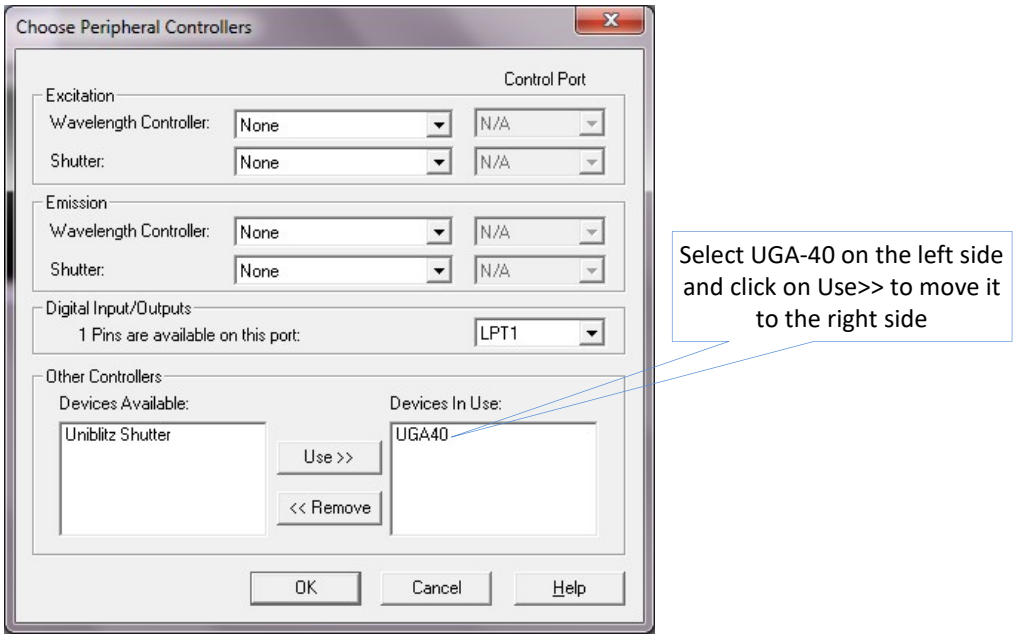
- A UGA-40 Control Unit with one or two lasers, and a Laser Controller for each laser. The laser(s) must be connected to a microscope adapter, as shown in Fig. 1.
- A PC-type computer running Windows XP or Windows 7 (32- or 64-bit).
- Imaging Workbench v6.0.28 or later.
- Suitable digital or video camera, controlled by IW.
- One parallel port on the computer, if pre-programmed triggering of the UGA-40 is desired during an acquisition session. If an INDEC BioSystems DIO-3 Cable Interface unit is present as part of the setup, the Trigger In connector on the UGA-40 should be connected to the Trigger Out connector on the DIO-3.
- One serial port through which IW communicates with the UGA-40 Control Unit. A USB port can be used along with a suitable USB-to-serial converter; please contact Rapp OptoElectronic or INDEC BioSystems for specific information.

IW can also control filter wheels, shutters, and other excitation and emission wavelength control devices, while controlling the UGA-40.

IW can also cooperate with the pClamp™ Software from Molecular Devices, LLCxxx, installed either in the same computer or in a second computer.

Configuring IW for Use with the UGA-40

1. Power on the UGA-40 Control Unit and the individual Laser Controllers. IW cannot communicate with the UGA-40 unless that unit has power on.
2. Start IW. In the Configure menu, choose Configure Wavelength Controllers. See Fig 2, and follow the instructions to bring the UGA-40 entry in the Devices in Use pane. Select the parallel port (e.g. LPT1) to be used in the group Digital Input/Outputs. Choose any other controllers you need, and click on OK.

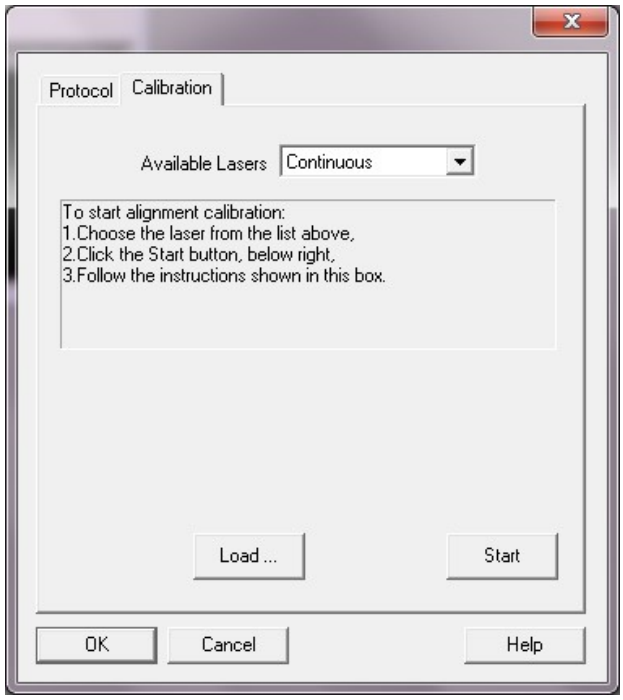


.

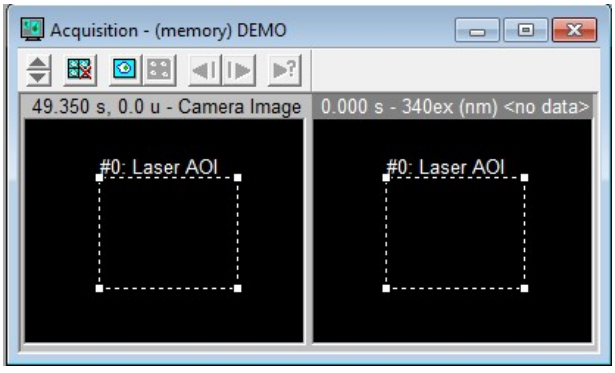
Calibrating the UGA-40 for Use with IW

The purpose of the calibration step is to relate the laser spot (x,y) location control to the corresponding (x,y) locations on the camera image. Once calibration is performed, the user can expect that specifying a particular pixel on the camera image will result in the laser spot being centered close to that pixel.

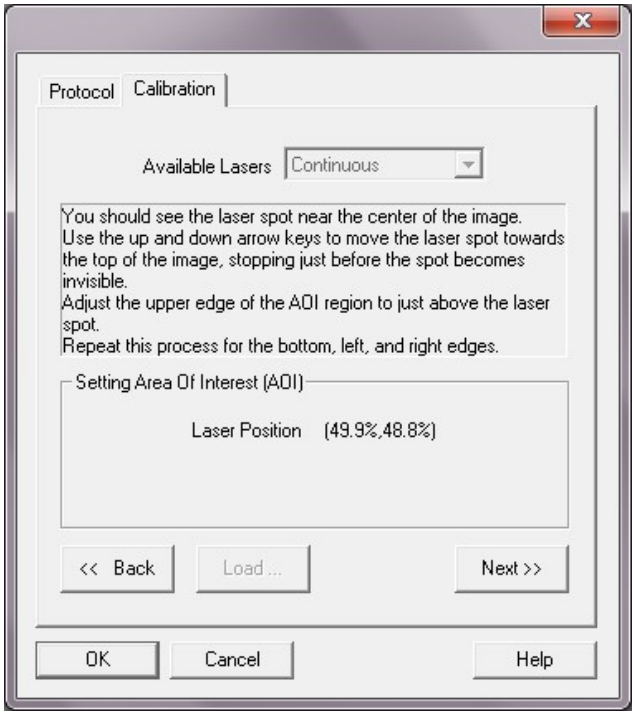
1. In the Configure menu, choose Wavelength Controllers; see the adjacent screenshot. Choose the Calibration tab, if you wish to calibrate the UGA-40 laser position. It is recommended to perform the calibration sufficiently frequently, such as once a day, until you are sure that the laser reaches the precise spots you specify. Follow the instructions shown in the box: choose the laser from the drop-down list and click on Start.



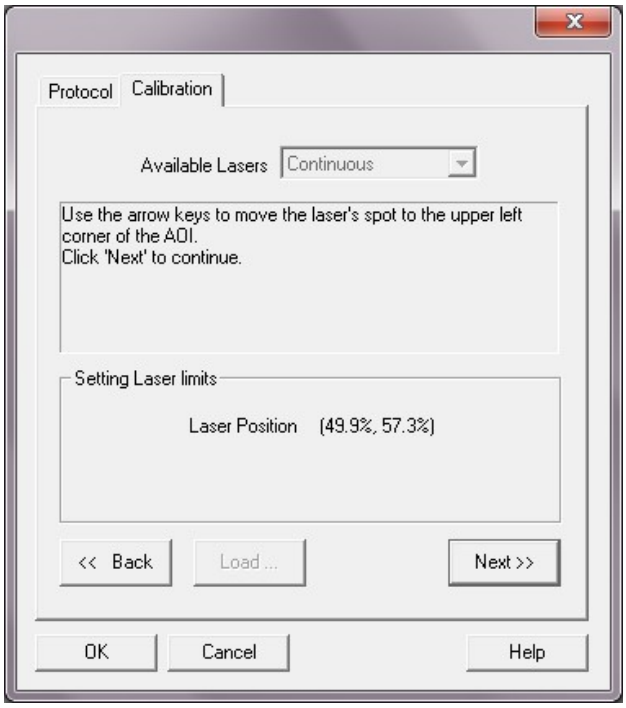
2. An acquisition window opens in the IW main window, as shown in the adjacent screenshot. The acquisition window shows a rectangular Region of Interest, labeled #0 and Laser AOI in the Figure. Live camera acquisition is started, and the laser is turned on.



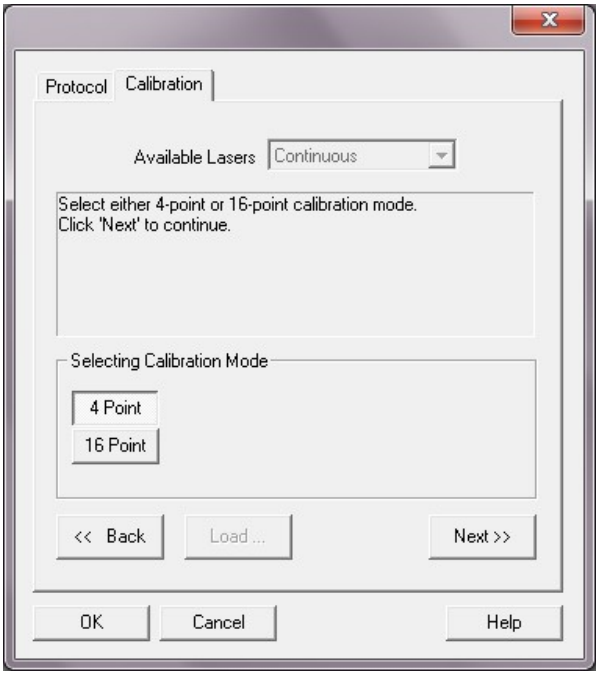
3. The Laser AOI is used to show the area over which the laser spot appears undistorted and at the precise location requested. As indicated in the text box in the screenshot, use the up and down arrow keys on the keyboard to move the laser spot towards the top of the camera image, to a point just before the spot becomes distorted or invisible. Then adjust the top line of the Laser AOI rectangle to just above the spot location. The laser position is shown in the text box once you start moving the spot using the arrow keys. Click on Next once you have defined the top limit for the laser spot.



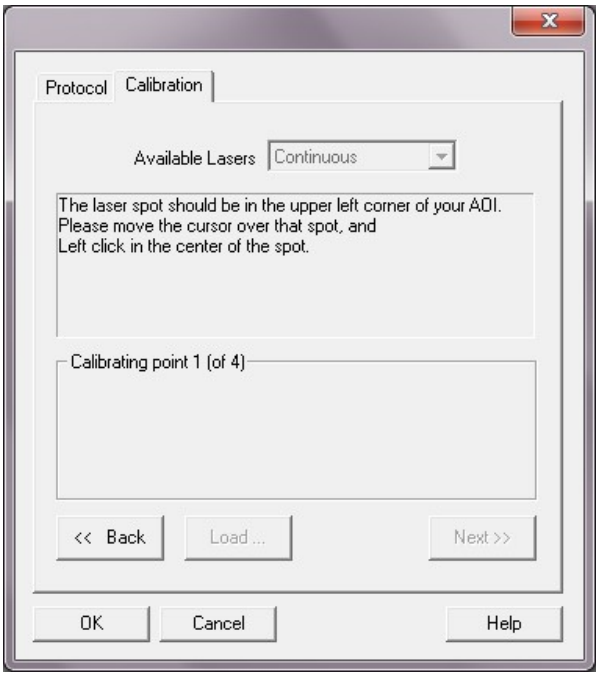
4. Repeat the step above for the bottom, left and right limit edges of the Laser AOI. The screenshot shows the instructions for the upper left limit.



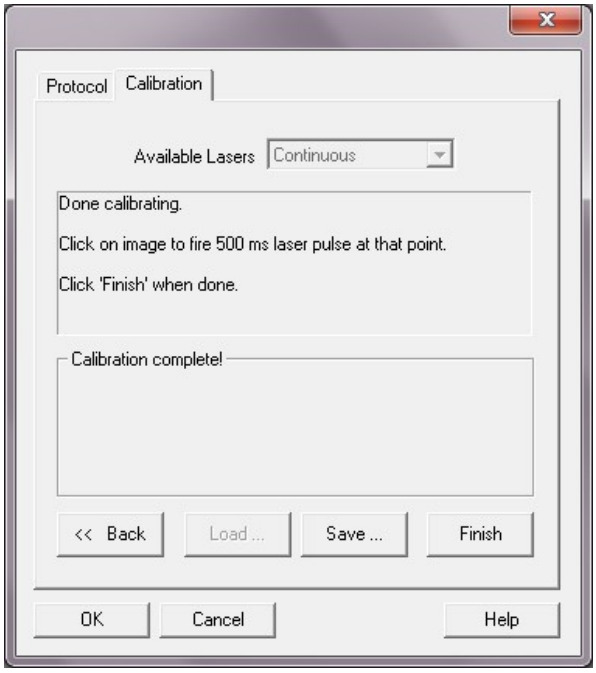
5. Once all four edges are defined, the next step begins. In the Dialog box, choose whether a 4-point or a 16-point calibration is desired. In general, a 16-point calibration results in more reliable results. For this example, a 4-point calibration is illustrated; a 16-point calibration is analogous.



6. For the first point, the laser spot is placed at the upper left corner of the Laser AOI. Move the mouse cursor to the center of that spot and left-click there.



7. Once the calibration procedure is complete, you may click anywhere inside the Laser AOI, and a laser spot will illuminate that location for 500 ms, allowing the user to verify that the spot is accurately .positioned.



8. Stop the Live Video acquisition. This ends the calibration procedure.

Using the UGA-40 with IW

The purpose of the calibration step is to relate the laser spot (x,y) location control to the corresponding (x,y) locations on the camera image. Once calibration is performed, the user can expect that specifying a particular pixel on the camera image will result in the laser spot being centered close to that pixel.

1. In the Configure menu, choose Wavelength Controllers, then the Protocol tab. The adjacent screenshot shows you the dialog box IW uses to specify and run spot illumination protocols.