Simplified alignment procedures for the ALBA system

The ISS-ALBA system uses the very sensitive APD’s detectors from Perkin Elmer. These detectors have a small active area, of approximately 200 um in diameter. These detectors act as their own pinhole, so that the pinhole assembly of the ALBA unit is not necessary. Of course it is necessary for other PMT detectors. There are only three degrees of freedom for the alignment of the detectors, in the x, y and z directions.

For the alignment in the x and y direction, place a slide containing beads (the source of fluorescence must be a point!) at the sample position and search using the x-y-z lens motor the position for the best intensity. This is done in the ALBA motors page using the commands highlighted below.

The position in the x-y plane is set at the factory and should require only very small alignments. Mainly the required alignments are for the channel 2 positions when the filter wheel is changing position. To use the explorer tool, select the motor (x, y or z) and press the explore button. Then move the vertical line cursor in the plot to the maximum of the intensity, press “move to cursor position” and repeat until the largest intensity is found. Repeat for both channels. Remember to select the channel you want to align in the group below the motor selection.

For the z-alignment, you must make it coincident with the microscope focus. First focus the bead using the wide field of view and then align the z-position of the lens.

For the x-y position, since the alignment is done in the de-scanned part of the optical path, the coincidence with the cross-hair of the microscope viewer cannot be obtained with the x-y lens commands. Instead, you could use the scanning lens mirrors to make the cross-hair and the image as seen by the scanning system coincident. Also for this alignment, first focus the bead at the center of the wide field and exactly at the cross-hair. Set the x-y offset of the scanner to be at zero. Then start image acquisition. While the image is being acquired, move the scanning lens motor to bring the image at the center of the field. The scanning lens motors are accessed by the “edit motor” button in the ALBA.
motor page and selecting motors 15 and 17 for the scanning lens. Use only small movements. The system should have been set at the factory to be pre-aligned, so that you should only need very small corrections.

In general, the alignment is quite straightforward and should not be needed except when changes in the optical system have been done.