Short Manual on CBIS diSPIM Image Acquisition

1. Overview of fine alignment steps:

    A. Objectives: co-focus, 3D space, two black bushing, and screw knob on right objective
    B. Adjust tilt of Dichroic mirrors (DM) so that the laser coming out the objective is straight
    C. Adjust tilt of the mirror for sending image to the camera

    It is a spiral adjustment on objective, DM and mirror.

2. Coarse alignment for beams and objectives: get the beam coming straight out of the objective.
    a. Fluorescence business card
    b. Open laser shutter
    c. Path B, beam -> laser coming out from right objective
    d. Path A, beam -> laser coming out from left objective
    e. Adjust DM by three screws on DM CUBE

3. Align the beams
    a. Fluorescing water
    b. "SPIM Z Drive": Lower SPIM head till objectives merged into water
    c. "Capture": "Open Alt" to Open laser shutter
    d. "Sheet Control" : "Neutral All" to return slice position to its neutral position.
    e. "Path Control": check "beam" for both Path A and B, not sheet.
    f. "Capture": Select same channel for A and B. Click on "Go" for both A and B.
    g. "Capture": Check "calibration dual camera" to see both beams.
    h. "Capture": click on "Open Alt" and click on "Live". You are able to see path A and B beam. Red: Path A; Green: Path B.
    i. If you don't see beam: 1) laser coming out the objectives are not straight 2) the two objectives focus are so far off. To adjust objective to co-focus using its black bushing and lateral adjust knob of right objective.
    j. Find beam image on screen.
    k. Uncheck "calibration dual camera". Select "Channel A" "Go"
    l. Adjust right objective bushing till the beam image is more in focus. Move the beam position on the screen (up and down) by adjust left objective (may still slant).
    m. Repeat k-l for channel B.
    n. Requires iterating adjustment for two objectives till co-focus: both beams look focus

4. Adjusting the Dichroic mirrors: get the beam come straight from the objective and beam not tilted to focal plane. If the beam tilt on the way right, when moving imaging objective to change focus, the beam will be coming in and out of focus uniformly.
    a. When one changes piezo of imaging objective and finds focus of the beam shifting laterally, one needs to change the tilt angle of the beam to the focus plane. → Change screw "A" of the DM cube.
    b. Make beam horizontal on the camera (DM cube screw "B" and "C").
    c. Adjust path A and B until when change right wheal of the remote joystick control, the beam looks shifting laterally and the beam is in focus and horizontal on the screen when the sheet control is on "Neutral All" position.

5. Adjusting the cameras:
    a. "Path Control": Check "laser switch", so you see both pencil beam and its epi image on the screen.
    b. "Capture": Check "Calibration dual camera", "grid" and "live", select "512" for camera chip.
    c. Adjust three screws on the mirror cube to bring the epi beam into center of the camera.

6. Spiraling in repeat all adjustments again (step 3-5) till beams are well aligned.

7. Piezo & Scanner Cross Calibration:
    a. Path A, B, "beam" on. (in case using samples, → sheet)
    b. "Neutral All"
    c. "Channel A" -> "Go". "Live"
    d. "Imaging setup": click "Go to" for "Calibration Start". Change right focus wheel till beam image is focus. Click on "set".
    e. "Imaging Piezo A": click on "Center"
    f. "Imaging Setup": click on "adjust" to bring piezo A to 0.
    g. Complete c-f for path B.
    h. Test calibration in sheet mode by moving Z in the Imaging Setup group. The sample should stay in focus when the Z coordinate is changed. If it is not, need to re-do calibration. If it is uniformly not in focus, need to restart from step 3.
    i. Slop generally is very stable. Offset is affected by temperature and RI of medium. Need to adjust if there any changes on these two parameters.
    j. To verify piezo and scanner calibration (when you move piezo, light sheet in focus), set step size e.g. 5um, click on "Step up" /"step down" to move piezo and scanner simultaneously. When image beads, there is not so much change on images. But when image biological samples, different structure will be shown.
    k. The structures should be in focus on different step position. If it is not, need to re-do calibration. If it is uniformly not in focus, need to repeat step 3 – 7.

8. Adjust light sheet to match image acquisition field. You may replace fluorescent water with your sample at this moment.
    a. Path A, B "beam" and "sheet" on.
    b. Channel A, "Go", "Open Alt", "Live".
    c. "Imaging Setup": adjust sheet width and sheet offset to set the lightsheet to cover the acquisition field.

9. Check your sample using eyepiece before you lower two SPIM objectives to touch your sample.
    a. SB/Focus to open "Focus Window".
    b. "Objective": 10x or 40x
    c. "Emission Selection:: Camera + Video
    d. "Camera": Eyepieces
    e. "Filter Set": Widefield and Dapi/GFP/RFP.
    f. "Open Fluor": Yes
    g. Microscope body left side: "FL on/off".
    h. LED light remote control: emission on.
    i. Move sample/interested imaging area into center of the view.
    j. To capture an image for widefield:
    k. "Camera": Andor camera 1
    l. "Microscope body right side: press the 3rd button (eye/camera interchange) till "Emission Selection" set to "camera" only.
    m. Adjust exposure time and "Snap".

10. Lower the SPIM Head to the Coverslip.
    a. Check beam/sheet, "Calibrate dual camera", "Open Alt", "live" (use laser power as low as possible)

b. Change focus down at small step size by using “SPIM Z Drive” or left wheel of remote controller, until you see fluorescent signal from sample.
c. If the focus lost suddenly, it may be due to the objective hits the side wall of the chamber. To confirm the position of objective to the coverslip, one may kick off the chamber by touching bottom of the chamber. Move the objective away from the side wall of chamber. Find beads in focus, move stage down till find the coverslip and light sheet interface.

11. For slice scan, move left wheel of stage remote control to define the imaging middle Z position, enter “Step size” and “Number of planes” to match the final imaging volume you need. (Slice scan uses piezo Z and maximum travel range is 150um).
12. For stage scan, define the stage position for “left” and “Right” by clicking “Set”. Enter “Step Size”.
13. Click on “Advanced” to call window for multiple fluorescence channel image. Set “3000” for “delay shooting” if you use stage scan.
14. “Capture”: enter “Time points” and “Interval” respectively if you need to do time lapse experiment.
15. “Execute” to start imaging and save file once the “saving” window is prompted out.