Alignment of the UltraMicroscope II with the alignment tool from LaVision

19 Jul 2020

Description of tool

The alignment tool is a Quartz cuvette filled with oil with refractive index that matches dibenzyl ether (1.561-2). The oil has a fluorescent compound that is excited effectively with 561nm laser power. In the cuvette there is a thin sheet of metal with an X-shaped hole, in the center of which is a 6µm diameter pinhole (Figure 1).

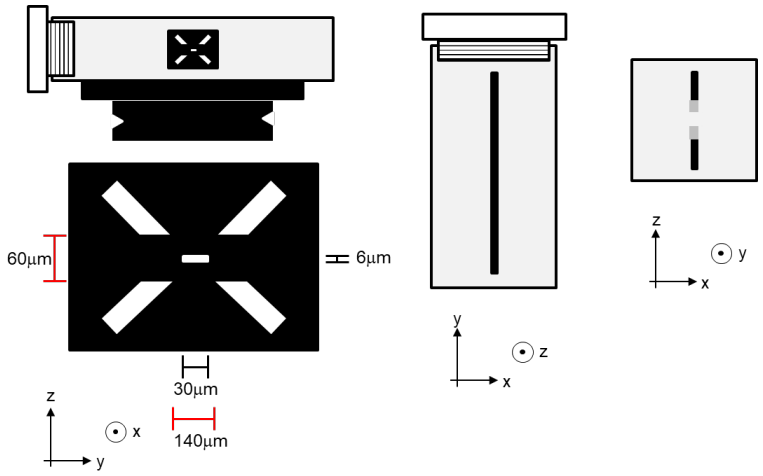


Figure 1. Alignment tool, not to scale.

The alignment tool simplifies the following:

- Alignment of the 3 light sheets on each side such that they are centered in Z and Y.
- Alignment of left and right light sheets to the same Z position
- Sheetmotor calibration such that the horizontal focus position indicated by the software corresponds to the horizontal focus position shown by the sample.

The basic idea is that because the light sheet width is 5µm, if all sheets are aligned and centered on the 6µm pinhole we will see 3 beams of light going from right to left (if the right sheet is on) and 3 beams of light going from left to right (if the left sheet is on).

Initial setup for alignment

Objective: get central light sheet from the right side through the pinhole and in focus on the camera.

1) Put in alignment tool into the reservoir filled with DBE
2) Turn on 561nm laser using the right light-sheet
3) Look into the reservoir and maneuver alignment tool until you can see the right light sheet going through the X-shaped target. It will look something like **Figure 2**

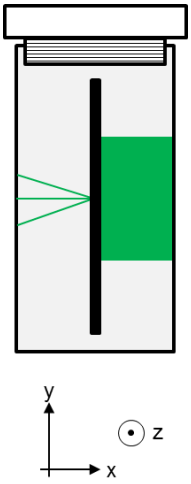


Figure 2. Right light sheet going through X-shaped target (view from top). There might be more than one set of lines coming through on the left hand side.

4) Set the zoom to the lowest setting on the microscope and in the software.
5) Set the light-sheet NA to the highest possible value.
6) Set the light-sheet width to the lowest possible value.
7) To start, use only the central light-sheet on the right side.
8) Move the focus knob on the objective until the sheet is visible.
9) Zoom in to around 3.2X on the knob to see the sheet more clearly.
10) Center the cuvette such that the pinhole is centered in X and Y. Put a crosshair on the image using the tools on the display window.
11) Adjust the horizontal focus such that it is aligned with the edge of the metal sheet inside the cuvette. Move the stage in Z until the central sheet goes through the pinhole. Adjust the horizontal focus further if need be to ensure the sheet is at its thinnest (in Z) at the pinhole. What you see will depend on whether the light sheet is going through the pinhole or through the arms of the X and whether it is at its thinnest horizontal focus position at the pinhole (Figures 4 and 5).
12) Once the right central light sheet is going through the pinhole on the alignment tool, it should look like Figure 3. If the sheetmotor calibration is correct, the horizontal focus should be perfect when it is aligned in the software to the position of the metal sheet. Once the alignment tool is properly positioned in X, Y and Z, the actual alignment can begin.

Figure 3. Correct alignment of right central light sheet in Z and horizontal focus. View from camera.

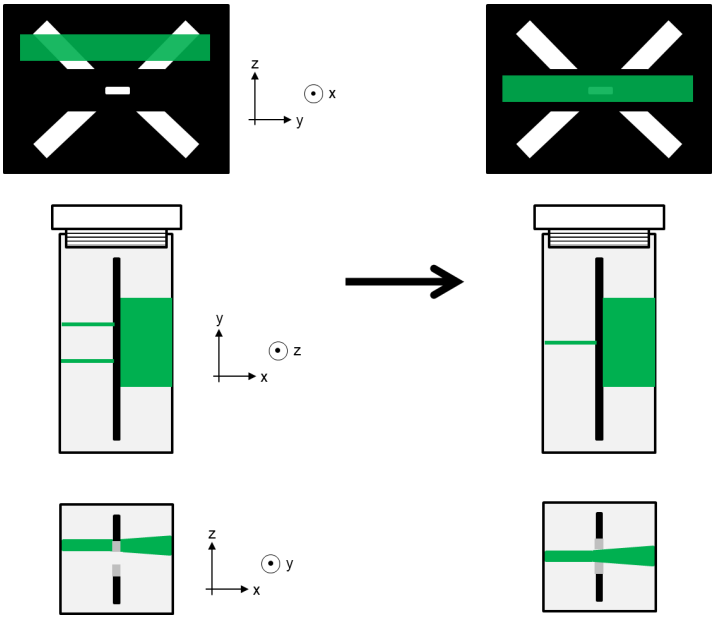


Figure 4. Effect of moving the right central light-sheet in Z relative to the pinhole. Note that for the central light-sheet on the right side, this should be done by moving the Z stage (moving the alignment tool up and down). All other light-sheets will be aligned to this one (if need be) by changing the Z position of each corresponding sheet by moving mirrors inside the microscope.

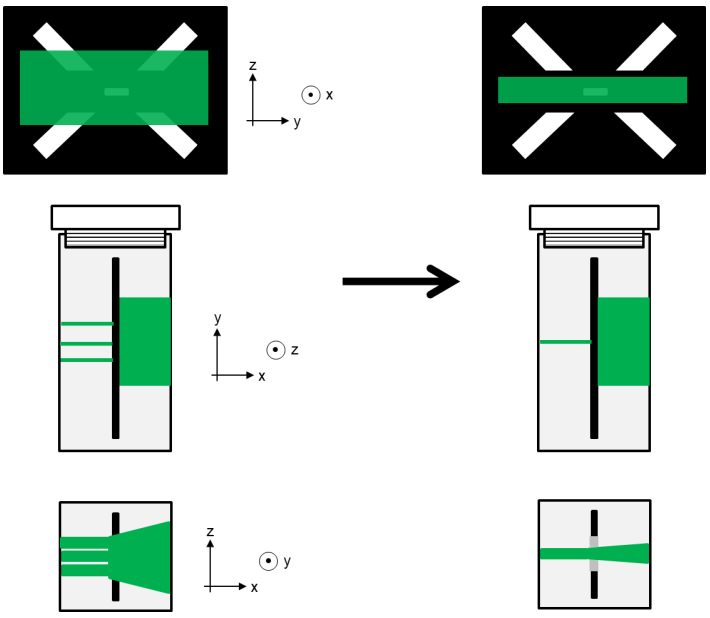


Figure 5. Effect of changing the horizontal focus of the right central light-sheet. On the left, the horizontal focus is set too far to the left. On the right it is perfectly adjusted.

Description of optical elements that are used to align

There are 4 mirrors on each side that control the alignment of the light sheets (Figure 6). Mirrors L_A and R_A control the Z and Y position of all light sheets on the left and right side. Mirrors L_B, L_C, R_B and R_C control the Z and Y position of the corresponding light sheets on the left and right side of the scope. By using the alignment tool, it is possible to determine if any of the sheets are misaligned in Z or Y. The Z screws can be accessed directly from outside the scope but to access the Y screws on the mirrors a side panel on the left (or right) side of the system's base needs to be removed. Depending on which sheet has problems, the proper mirror for adjustment can be selected. Each mirror has 3 adjustment screws, only 2 of which should be touched; those allow control of Z and Y positioning (Figure 7).

To check which lasers are misaligned, the center or oblique lasers can be toggled on and off in the advanced tab. Alternatively, if there is any suspicion of a mismatch between the two oblique sheets on either side, the system's top covers can be removed and the corresponding lasers can be blocked individually with a business card.

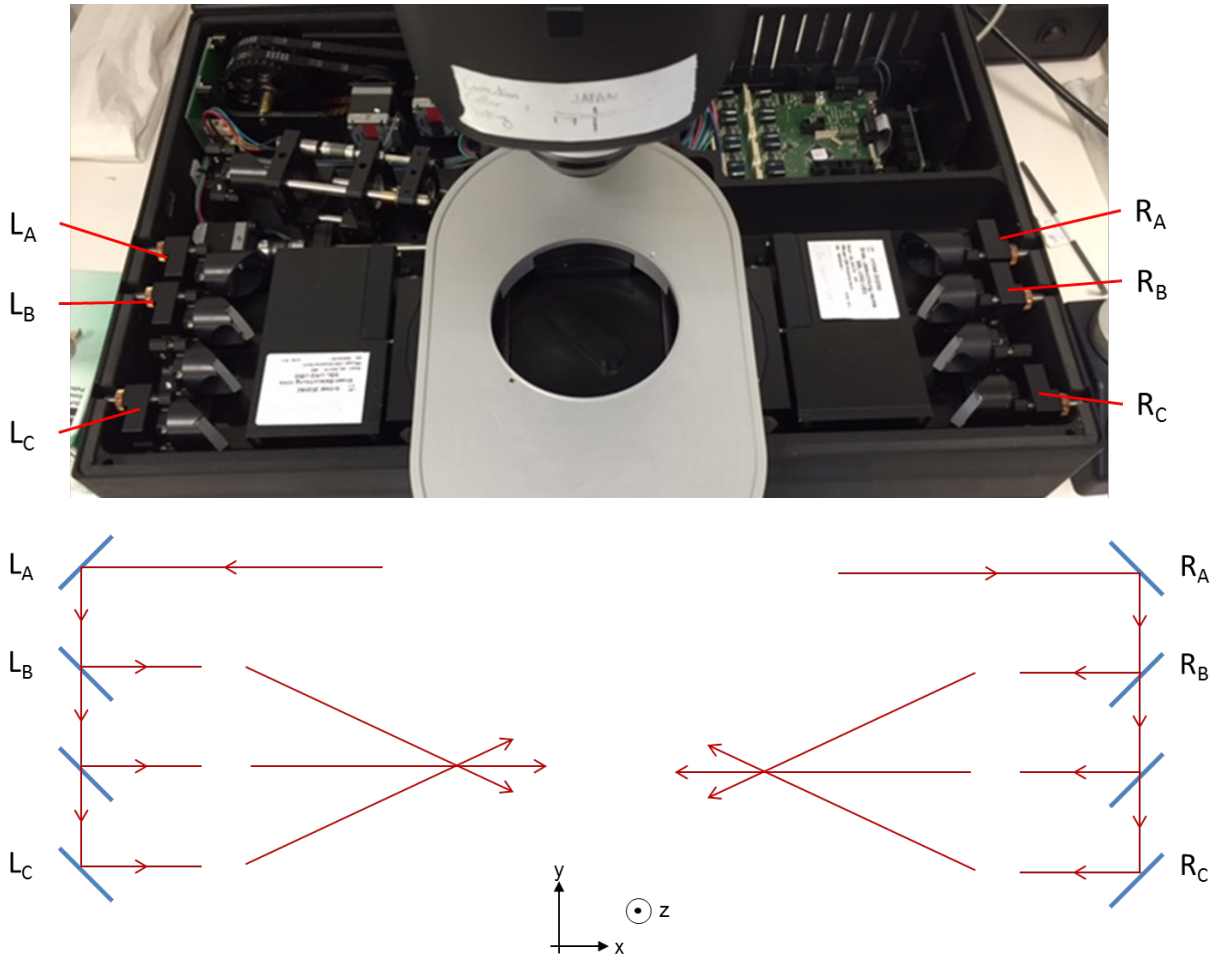


Figure 6. Mirrors that control light sheet alignment (view from top).

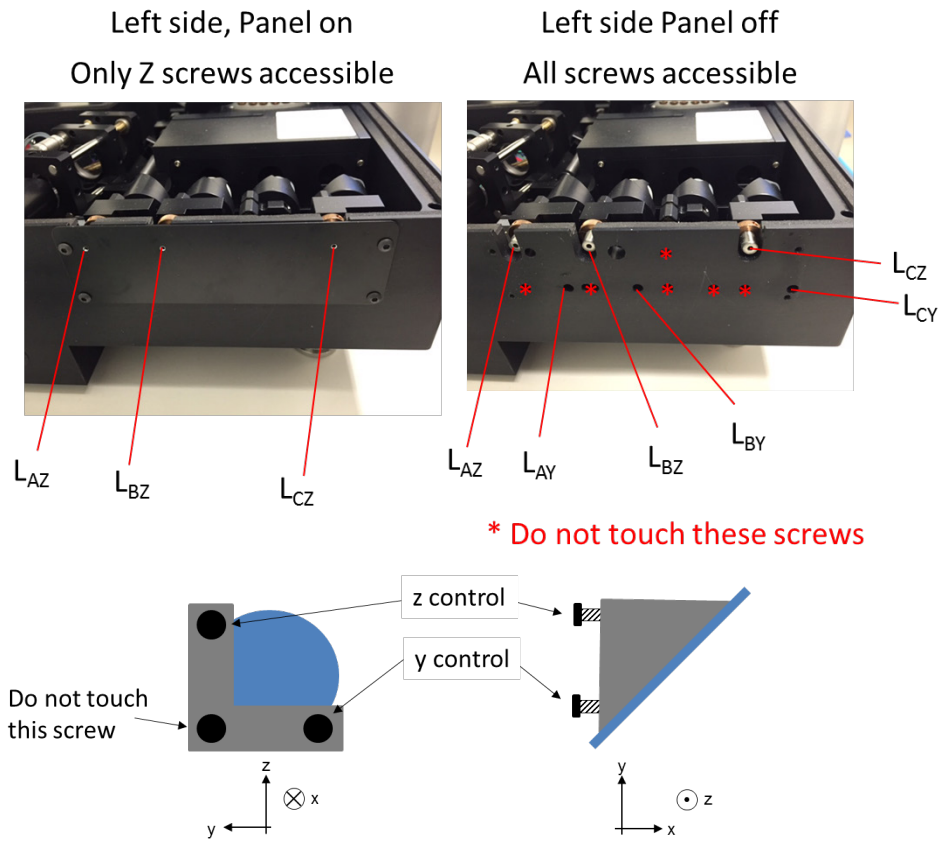


Figure 7. Screws that adjust mirror position and corresponding light sheet position in Z and Y.

All alignments should be performed with the 561nm laser, since that is the best laser to excite the fluorophores inside the cuvette. The other lasers are coaligned to this one and will only vary slightly in the central position of their horizontal focus.

Alignment procedure

ALWAYS START WITH RIGHT SHEET

Right-central light sheet in Y

If the right central light sheet is not aligned in Y, adjust screw R_{AY} (Figure 8). Supposedly this should only rarely be necessary.

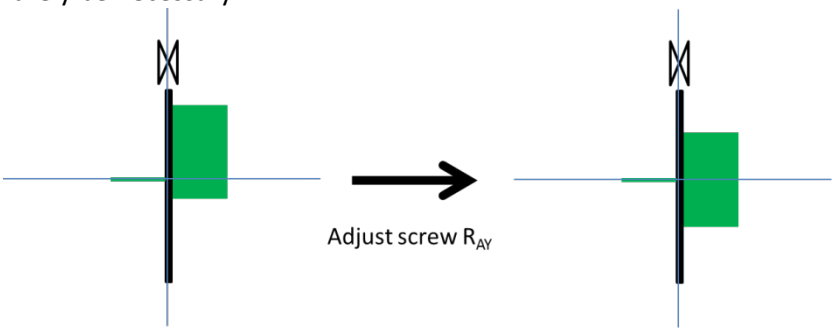


Figure 8. Example of right central light sheet misaligned in Y, and its adjustment. The image shown assumes the alignment in Z is perfect.

Right-oblique light sheets in Y

If either of the oblique right light sheets are not aligned in Y, adjust screws R_{BY} or R_{CY}, depending on which sheet needs alignment (Figure 9).

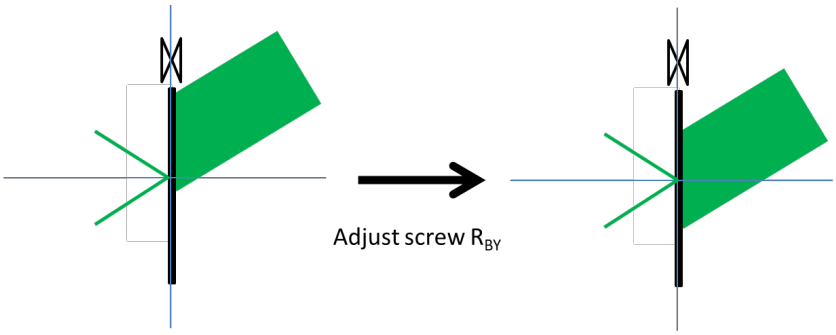


Figure 9. Example of a right oblique sheet misaligned in Y, and its adjustment. The image shown assumes the alignment in Z is perfect.

Right-oblique light sheets in Z

If either of the oblique right light sheets are not aligned in Z, adjust screws R_{BZ} or R_{CZ}, depending on which sheet needs alignment (Figure 10).

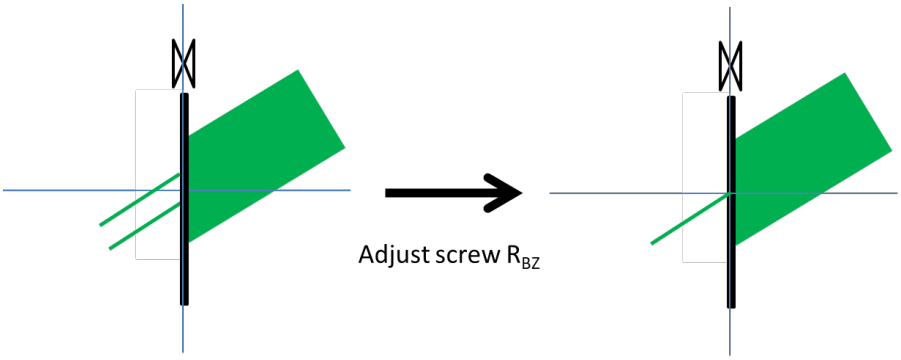


Figure 10. Example of a right oblique sheet misaligned in Z, and its adjustment. The image shown assumes the alignment in Y is perfect.

Note that the horizontal focus point of oblique light sheets is not always perfectly at the same position as that for the central sheet. So the lateral sheets might not be at their brightest point. The imprecision is around 100-300um and is almost impossible to improve much further.

Once all right sheets are aligned in Z, the image on the camera will look like Figure 11.

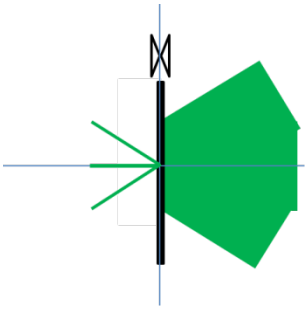
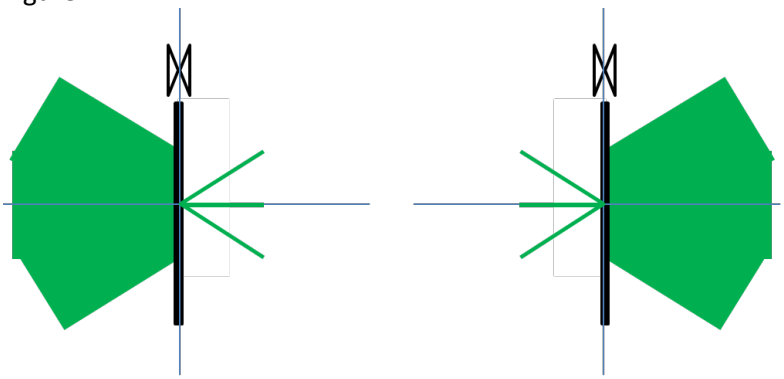


Figure 11. Perfect alignment of all right sheets.

To adjust the left light sheets, the procedure is analogous. Proceed in the following order:

1) Turn on only the central left light sheet to start.
2) Check the Y alignment, adjust with screw L_{AY}, if necessary
3) Check the Z alignment. The left light sheet should be aligned at the same Z plane as the right light sheet, so if the right light sheets are going through the pinhole, the central left light sheet should as well. If that is not the case, adjust screw L_{AZ}.
4) Turn on the oblique light sheets on the left side and adjust Z and Y with screws L_{BZ}, L_{CZ}, L_{BY} and L_{CY}, as needed.

Once the procedure is done, toggling between right and left sheets should give the images shown in Figure 12.



Left sheet on Right sheet on

Figure 12. Perfect alignment of all sheets in Z, Y and horizontal focus.

Summary of screw adjustments

- Do not adjust screw R_{AZ}
- If the right light sheets are not centered in Y, adjust screw R_{AY}.
- If any of the two oblique light sheets on the right are not centered in Y, but the central sheet from the right is, adjust screws R_{BY} and/or R_{CY}.
- If any of the two oblique light sheets on the right are not at the same Z position as the central light sheet from the right, adjust screws R_{BZ} and/or R_{CZ}.
- If the left light sheets are not centered in Y, adjust screw L_{AY}.
- If any of the two oblique light sheets on the left are not centered in Y, but the central sheet from the left is, adjust screws L_{BY} and/or L_{CY}.
- If the central light sheet on the left is not at the same Z position as the central light sheet from the right, adjust screw L_{AZ}.
- If any of the two oblique light sheets on the right are not at the same Z position as the central light sheet from the left (and right), adjust screws L_{BZ} and/or L_{CZ}.

Note that the middle mirror (between positions B and C) cannot be adjusted. To adjust the central light sheet from left or right, screws L_{AY}, L_{AZ} and R_{AY} (do not touch R_{AZ}).

Rotational alignment of camera

The objective of this final aspect of the alignment is to ensure the camera X and Y axis match the X and Y axis of the stage. To test this, use the crosshair tool in the display, and center a clear feature at the center of the crosshair. This can be the intersection of the middle light-sheet with the alignment tool's pinhole, but it could potentially be a clearly identifiable feature in any biological sample. Once the feature of interest is centered, move the stage up and down in the Y dimension. If the camera is rotationally aligned, the feature should move along the vertical line of the crosshair. If not, it will deviate from that line, systematically drifting to one side or the other above and below the center of the crosshair. If the camera is not rotationally aligned, loosen its holding screw, rotate it slightly and recheck the alignment, iterating until the best solution is reached. See Figure 13.

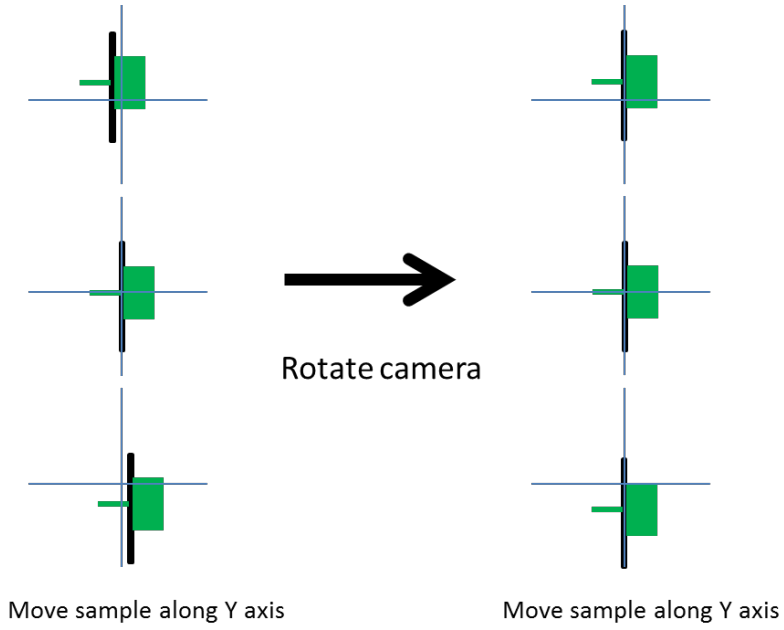


Figure 13. Incorrect (left) and correct (right) rotational alignment.