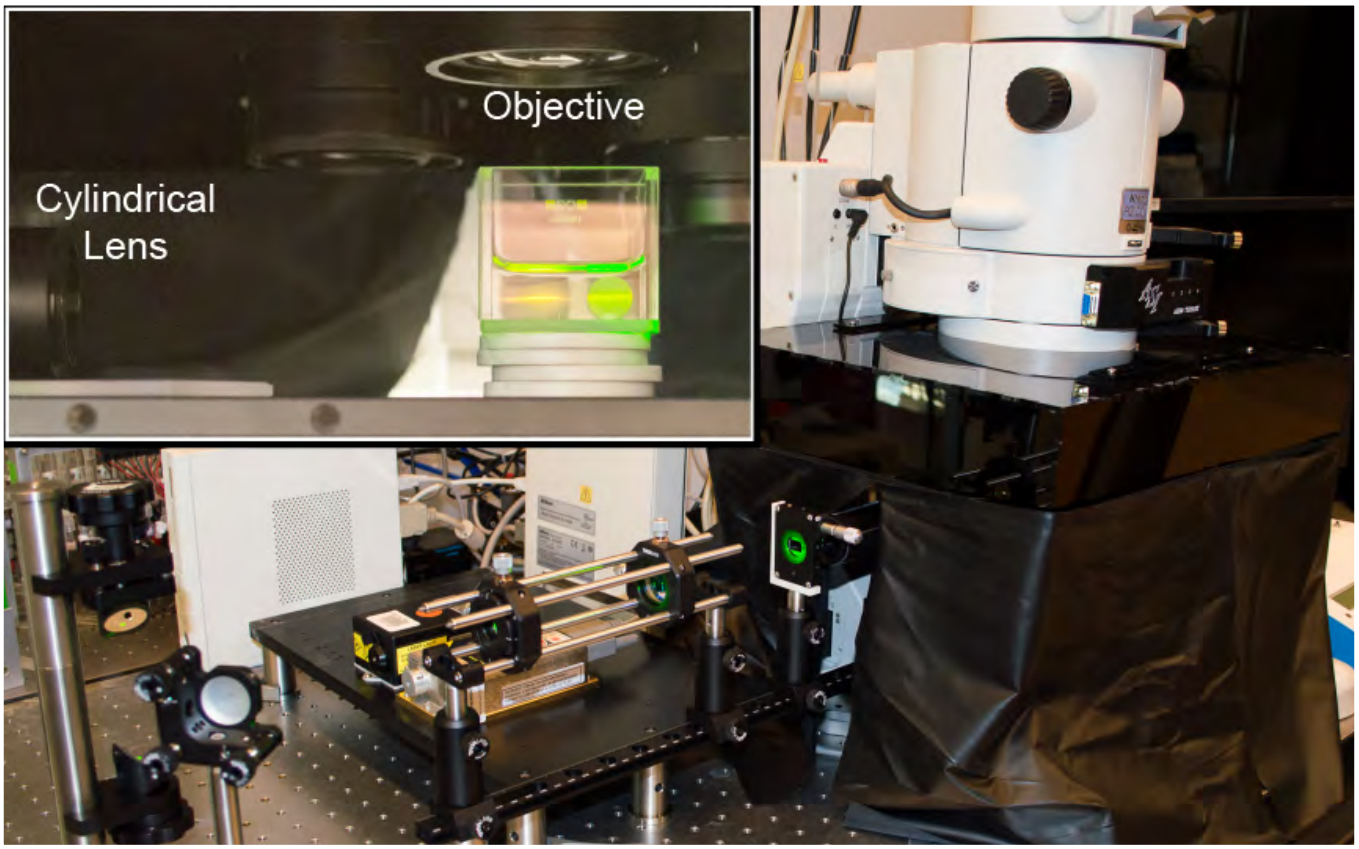
Building a light sheet microscope around a Nikon AZ100, Part 1

By admin · October 25, 2016

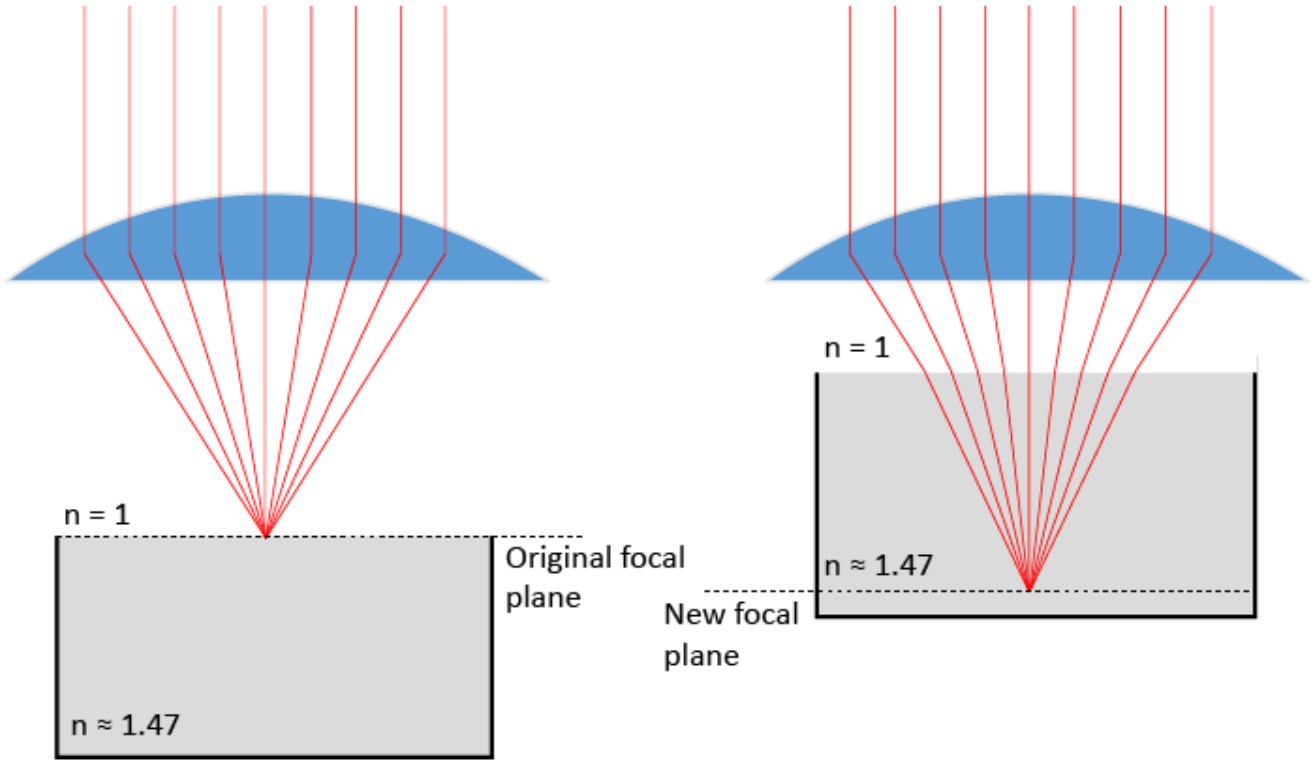
A few years ago we got a Nikon AZ100 microscope on indefinite loan from a lab here that no longer was using. The AZ100 is an interesting microscope – it has low magnification objectives with relatively high numerical apertures (we have 1x / 0.1, 2x / 0.2, and 5x / 0.5 objectives) combined with a 1x – 8x optical zoom system to allow both large field-of-view imaging and high resolution imaging of the same sample. I initially set this up for routine fluorescence imaging, but it didn't fill a useful niche and so largely went unused.

As groups on campus began testing various tissue clearing methods (CLARITY [1], PACT [2], iDISCO [3], …), I realized that this would make a good base for a simple "Ultramicroscope"-style [4] light sheet microscope. This is about the simplest kind of light sheet microscope you can build; you simply use a cylindrical lens to reshape an expanded laser beam to a sheet that propagates perpendicular to the optical axis of the microscope. We had an old 561 nm Coherent Sapphire laser sitting around from a rebuild of the laser launch on our spinning disk confocal, so a few hundred dollars in Thorlabs parts sufficed to set up a demo system. The sample is placed in a cuvette on the microscope stage, illuminated with the light sheet from the side, and imaged with the objective from above.



The initial light sheet test system. The laser is mounted on the black table; to the left you can see the mirrors used to direct the beam to propagate through the image plane, perpendicular to the optical axis. The cage system holds a Galilean beam expander and a slit; the cylindrical lens sits inside the dark enclosure. In the inset you can see the cylindrical lens and fluorescence excited in an agarose cylinder doped with fluorescent beads.

The initial results from this test system were quite promising – we were easily able to image through a 1 cm cleared mouse embryo and record cellular detail. However, a problem immediately became apparent: we are imaging into a high-refractive index solution (n = 1.47 for RIMS) so when we move the cuvette to change the focal plane, refraction at the air / RIMS interface changes the position of the imaging plane so that it no longer coincides with the the illuminated plane.



Changing the focus by moving the cuvette causes the focal plane to move from the original light sheet position.

Fortunately, the AZ100 allows you to focus both the objective position and the stage (cuvette) position, so we can adjust the objective position to focus on the sample plane illuminated by the light sheet every time we move the cuvette. The amount by which we need to move the objective focus is linearly related to the amount we moved the cuvette , so it's straightforward to optimize the objective focus at a few different cuvette positions and then fit the linear relationship between the cuvette and objective position, so that for any cuvette position we know where to focus the objective. The relationship changes depending on the refractive index of the sample immersion medium and its height in the cuvette, so it needs to be measured for each new sample, but this only takes a few minutes.

For our initial work, we just defined start and end positions for both the cuvette and objective and then linearly ramped between those positions. This worked well, but to make the process more robust we worked with the Micro-manager developers to build a driver that allows us to drive both cuvette and objecive as a single virtual stage where the position of the objective is linearly related to the position of the cuvette. We then wrote a plugin that allows the user to specify pairs of cuvette and objective position and then determines the best fit line to all the points. This makes the process more robust and error tolerant.

This initial system worked well enough to produce images like the vasculature in this mouse embryo. In later posts, I'll explain how we extended this system to multicolor imaging, optimization and measurement of the sheet thickness, and some peculiarities of the objective resolution as a function of the

AZ100 zoom.

References

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