DBO-scope user manual

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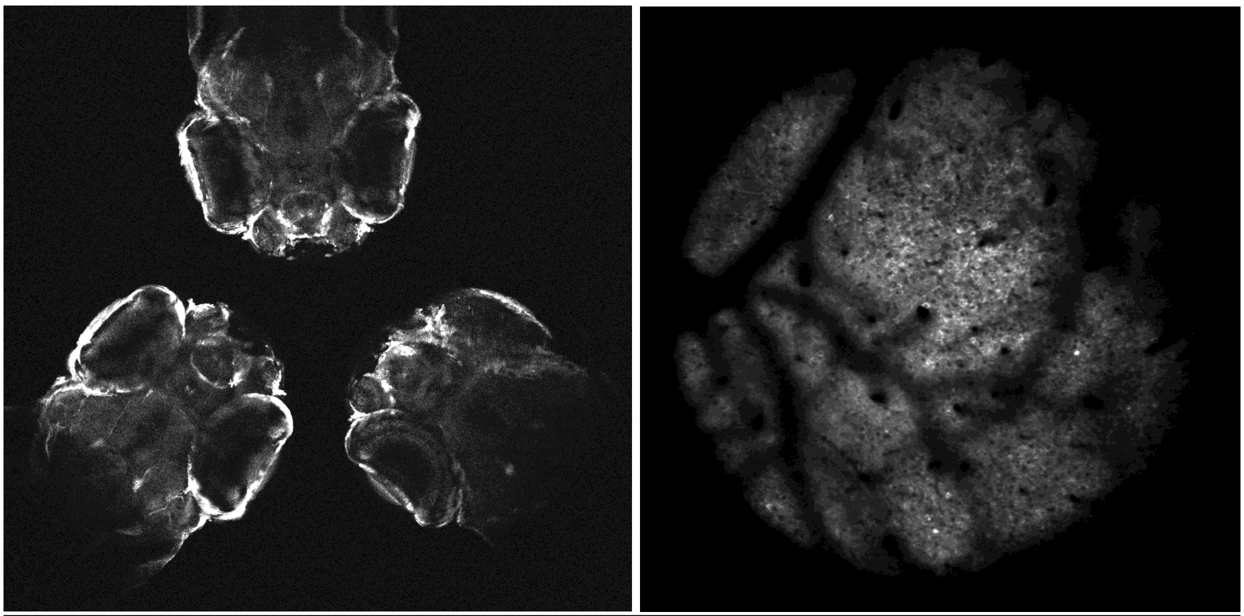
Repository: https://github.com/BadenLab/DBOscope



A note on safety

Remember to always wear safety googles when working with lasers. Take off all hand-worn jewellery and watches as these might reflect the beam into your or someone else's eyes. Always work at the lowest possible excitation laser power. And never, even with the safety glasses, look straight into the laser beam!

Useful tools to have at hand

Tape measure, fixed fluorescence sample (i.e. a test-slide), laser viewing cards

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Bill of Materials

Please see separate Excel file for now (cleaned up version coming soon)

Initial steps: Obtaining all the key numbers of your specific setup.

This section is intended to help you identify all key elements and your laser path between the scan mirrors and the sample plane, and to obtain concrete values for their relative distances and focal lengths etc..

1. Identify scan lens (SL). It should be the first lens ensemble after the scanning mirrors (from point of view of the laser source). Identify Tube Lens (TL), it should be the next one after SL. Measure a distance between them (D_2).

2. Carefully try to take out both lenses. Use gloves to avoid leaving smear-marks, and do not scratch them. Measure their focal distance of them (F_{SL},F_{TL}), and their dimensions ($Ø_{SL}$, $Ø_{TL}$), for example as demonstrated here: https://www.youtube.com/watch?v=joQw3_rM_jI

If you use a Sutter MOM setup, the SL model is VISIR 1534SPR136 from Leica (Fig. 1a), which is mounted in a WECO 77 tube (Fig. 1c). It has a focal length of 50 mm, and it is located 56.6 mm from second scan mirror. The thickness of the lens (which is made up of two biconvex lenses) is 43.1 mm. The External tube diameter is 38.2 mm, and the internal aperture diameter is 28.9 mm. It is easy to unscrew and take it out. (Fig. 1d). However, in the Sutter MOM the TL (MXA22018, Nikon) is less readily accessible (Figs. 1b,f). It has a 200 mm focal length (Fig. 1e) and is positioned at a distance of 250 mm from the SL. The length of the TL tube is 29 mm, and it has a 36 mm aperture.

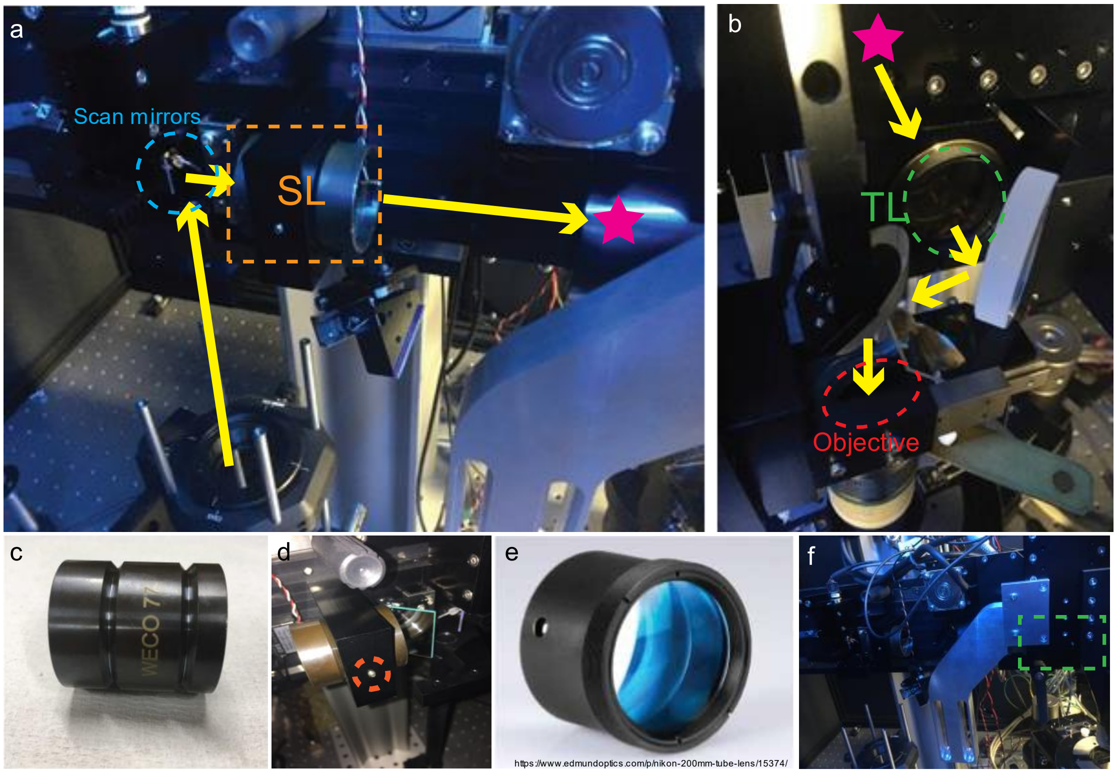

Figure 1.

3. For your objective, check the magnification, nominal working distance, and size of the back aperture ($Ø_{obj}$). Measure the total distance between the objective's back aperture and the SL.

Note: Each of the SL, TL and objective are actually not single lenses, but instead consist of multiple stacked lenses. In optics, such constituent lenses can be replaced by a single, ideal lens, and this is what we are doing in all calculations. All calculated distances are a distance between ideal lenses, which are "located" in the centre of the a given tube.

Note: Related to the above, for a thick lens (one which has a non-negligible thickness), or an imaging system consisting of several lenses or mirrors (e.g. a photographic lens or a telescope), the focal length is often called the <u>effective</u> focal length (<u>E</u>FL), to distinguish it from other commonly used terms.

If two thin lenses are separated in air by some distance *d*, the focal length for the combined system is given by

$$\frac{1}{f} = \frac{1}{f_1} + \frac{1}{f_2} - \frac{d}{f_1 f_2}.$$

The distance from the front focal point of the combined lenses to the first lens is called the *front focal length* (FFL):

$$\mathrm{FFL} = \frac{f_1 (f_2 - d)}{(f_1 + f_2) - d}.$$ [26]

Similarly, the distance from the second lens to the rear focal point of the combined system is the *back focal length* (BFL):

$$\mathrm{BFL} = \frac{f_2 (d - f_1)}{d - (f_1 + f_2)}.$$

As *d* tends to zero, the focal lengths tend to the value of *f* given for thin lenses in contact.

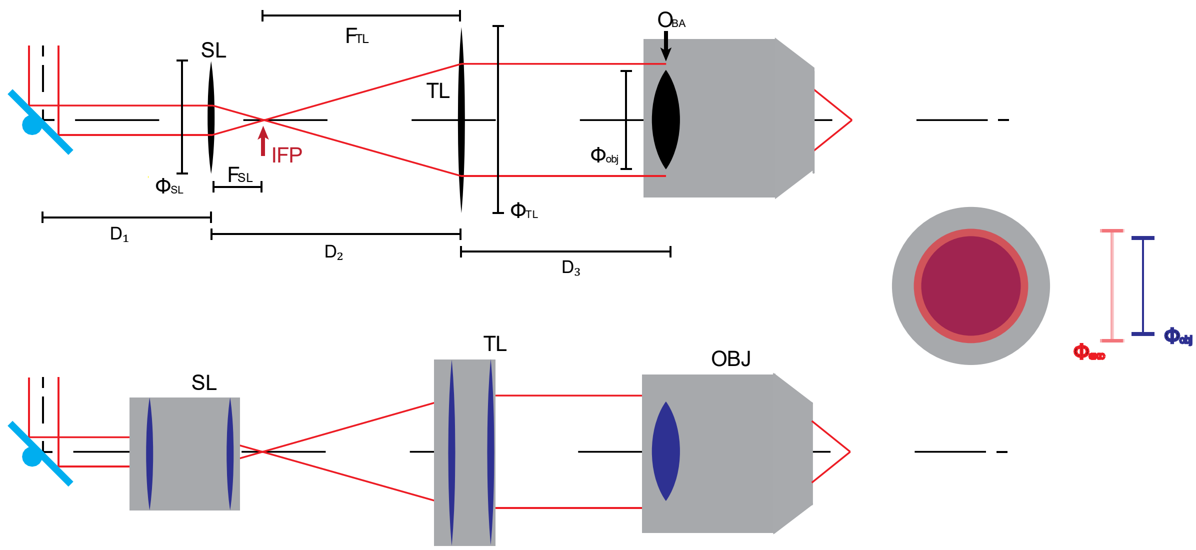

Figure 2.

Note: Microscope objectives are designed to correct for chromatic aberration at a specific distance D_3 between TL and its back aperture O_{BA}. For Zeiss objectives, $D_3 = 95$ mm. (Philbert S. Tsai and David Kleinfeld, 2009).

In our setup, we use a Zeiss Objective W "Plan-Apochromat" 20x/1.0, 1.8 mm working distance.

4. In a standard diffraction limited (DL) configuration, the distance between SL and TL is equal to the sum of their focal distances $D_2 = F_{SL} + F_{TL}$. D_3 is specified by the objective as discussed above.

5. Based on these numbers, calculate the distance D_1 between the second scan mirror pivot point and SL that minimizes motion of laser beam (intensity fluctuation) on objective back aperture:

$$D_1 = \frac{F_{SL}^{\ 2}}{F_{TL}} + F_{SL} - D_3 \left(\frac{F_{SL}}{F_{TL}} \right)^2$$

6. Check objective's back aperture (O_{BA}) filling factor (Øexc/Øobj). To do so, turn on a regular scan mode at "high zoom" (i.e. with minimal movement of scan mirrors) without the objective in place. Use a laser viewing card in the position where the objective's back aperture would be to assess the setup's fill factor (i.e. the size of the laser spot relative to the objective's back aperture). (Fig. 3). Alternatively, if your laser supports an alignment mode at a visible wavelength (typically low 700s of nm) you can also do this procedure with a piece of paper in place of the laser viewing card.

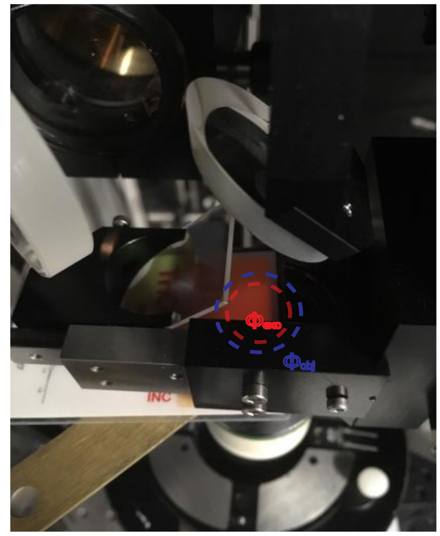

Figure 3

In a DL configuration, overfilling O_{BA} by the excitation laser beam minimizes the lateral and axial size of the excitation spot (point spread function, *psf*), but also decreases effective excitation power delivered by the objective (Helmchen & Denk, 2005; Philbert S. Tsai and David Kleinfeld, 2009). .

Based on your data, check and calculate if your setup operates on DL settings.

We present two modifications to obtain a divergent excitation 2-photon setup leading to an expanded field of view (FOV) and excitation spot (point spread function, *psf*).

Throughout, we will be focussing on two parameters: Intermediary focal point (IFP) and the objective's back-filling factor:

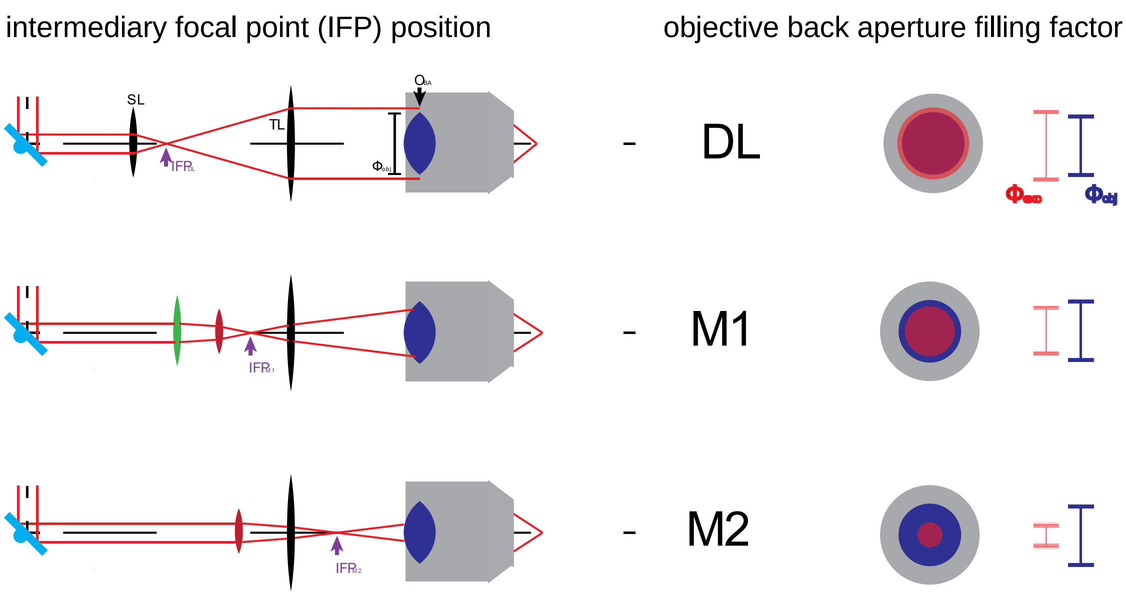

Figure 4

DBO_1 configuration

1. For "Divergent Beam Optics modification 1" (DBO_1), the goal is to move the IFP closer to the TL, yet without passing it, while simultaneously underfilling O_{BA} (Fig. 5).

2. To measure scale of the changes, use a fixed fluorescence sample. Prepare your setup for a standard experiment with DL configuration. Using lowest possible power find a suitable sample plane (one with obvious structure), and determine the original maximal FOV and working and working distance.

3. Prepare setup to measure (Initial steps, point 5) objective back aperture (O_{BA}) filling factor (Øexc/Øobj).

4. Completely remove SL from the system. **Before implementing any modifications, remember to switch off laser!**

Sidenote: Why not simply shift the original SL? We can of course move the IPF closer to TL by simply shifting forward the SL. However, this will <u>increase</u> overfilling O_{BA} (we are hoping to decrease this) which in turn will decrease effective excitation power on the sample plane (we are hoping to increase this).

5. Insert new lenses in place of the SL. In our tested DBO_1 solution for the Sutter MOM, we use 2 lenses in place of the SL: L1 and L2, with focal lengths 190 and 175 mm, respectively.

In our implementation, L1 is a modified Sutter MOM SL - VISIR 1534SPR136, Leica (Fig. 6b), and L2 is LA1229 from Thorlabs (Fig. 6c). This specific choice for L1 is one of convenience, but it can be equally be replaced by a new 190 mm lens.

DBO_1 can also be achieved using a single lens. However, in this case, the O_{BA} filling factor will be much smaller, and the psf will disproportionately inflate already for a small FOV increase.

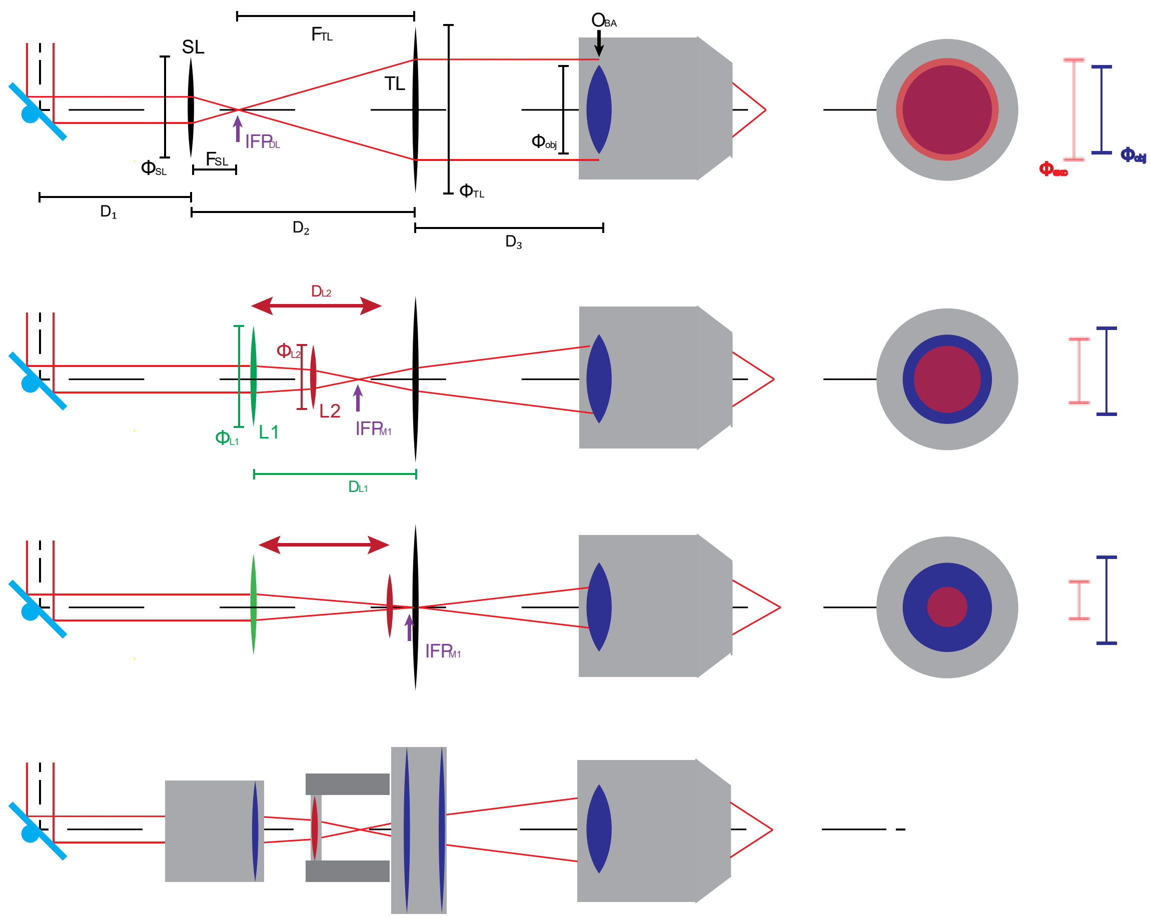

Figure 5

For *mounting L1, we use the original SL mount (as our L1 is a modified SL). For assembling and shifting L2, we use a 3D printed lens holder that fits into the existing tube that also holds the TL at its end (Fig. 6a).*

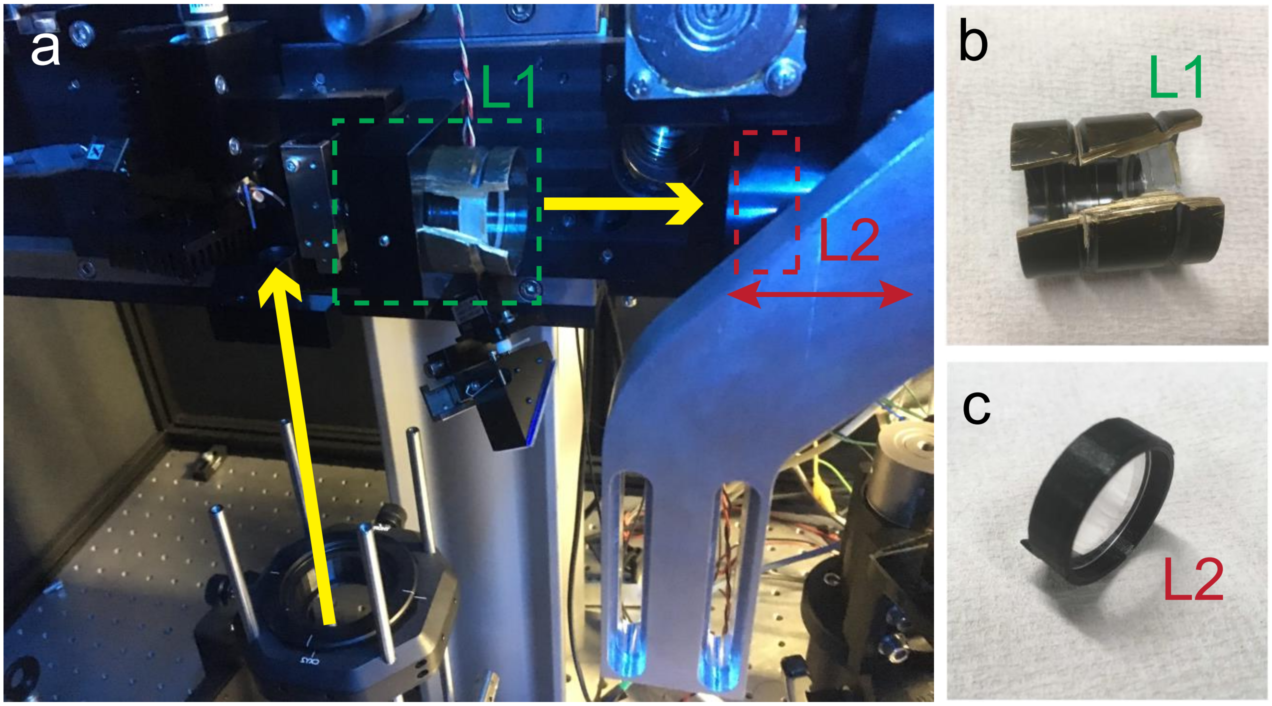

Figure 6.

6. **Select the "right" L1 and L2 lenses for your setup.**

Sketch out how you want the optical configuration of your setup to look like. Note original SL, TL and their apertures. Note existing holders for lenses and the physical constraints imposed by your setup's mechanics to position and shift L2.

For L1, use a lens with a focal distance $F_{L1} = D_{L1}$, such that by itself, L1 sets up an IFP exactly at the centre of the TL. We use a 190 mm lens, positioned 190 mm in front of the TL.

If desired, you can also use an L1 with shorter or longer focal distance than D_{L1}. However, if the focal distance is shorter, the range of FOV changes generated by shifting of L2 position decreases. In contrast, of L1 focal distance is longer than D_{L1}, it will bring the IFP behind the TL, effectively resulting in DBO_2 (discussed below). In this case we can shift between modification 1 and 2 mode by change a position of L2.

Make sure that the aperture (diameter) of L1 is suitable. It should be in similar range as the original SL ($Ø_{L1} \approx Ø_{SL}$) to make sure the incoming laser beam does not get clipped on the edges during scanning.

For L2, the focal length (F_{L2}) should be in the range between $F_{L2 \text{ and }} F_{L2}/2$. F_{L2} shortening introduce an overfilling of O_{BA}, or even TL aperture, resulting in power lost and fluctuation of excitation intensity on FOV. F_{L2} extending decrease range of IFP and (Øexc/Øobj) changes.

On our setup, we use a 175 mm F_{L2}, which is shifted along the laser path between 100 and 5 mm from TL.

Ultimately, the exact position vale of F_{L2} as well as the position of L2 relative to L1 and TL will dictate the position of the IFP, and thereby set the (Øexc/Øobj) factor, FOV and the size of the excitation psf.

If possible, choose an aperture of L2 that is similar to the one for L1 ($Ø_{L1} \approx Ø_{L2}$). *In our case it was not possible to use the exact same size, so we used the largest possible L2 diameter (25,5 mm) which just about fit into the 3D printed holder that was slotted into the tube that also held the TL.*

7. Test the result of your changes and establish the range of FOV shifts achievable by shifting L2 up and down the laser path. Start by setting L2 as close as it is possible to L1 and take a scan of your fixed sample. Likely, working distance will be longer than in the original DL configuration so you will need to refocus. Note the difference of a working distance and check the maximal FOV size. Also, zoom in to assess spatial resolution. Now, shift L2 closer to TL and again check FOV and working distance. Both should increase. Note the range of changes that can be achieved with this configuration.

Remember to shift L2 only when you are not scanning and your laser is disabled!

If you find that working distance and FOV starts to decrease again at some point when shifting L2 closer to TL, it means that L2 is further away from L1 compared to L1's focal distance. In this case, put L1 closer to TL or exchange it for the one with longer working distance.

8. As for 7, also check the objective filling factor (Øexc/Øobj) for a different positions of L2 (see initial steps paragraph). Shifting of L2 towards TL will increase underfilling (i.e. will make the laser spot smaller at the level of the objective's back aperture), and should result in an expansion of the *psf*.

If desired, you can try to keep a (Øexc/Øobj) constant even with increasing FOV by exchanging of L2 for one with shorter working distance as you shift it closer to TL. This effectively allows setting FOV somewhat independently of psf expansion (up to a point).

Sidenote: Remember that overfilling of O_{BA} will reduce effective excitation power, and can also lead to uneven excitation power across the FOV.

9. As desired, also directly measure the excitation psf, for example by imaging fluorescent beads embedded in agarose. When doing so, remember to keep laser wavelength and excitation power at the sample plane approximately constant to ensure fair comparison (measured psf dimensions strongly depend on these factors). Note that excitation power at the sample plane should also increase as you decrease Øexc/Øobj, so it is best to directly measure this on the sample plane using a power meter.

DBO_2 configuration

1. In DBO_2, goal is to move the IFP beyond the TL (but still in front of O_{BA}), again while ensuring underfilling of O_{BA} (Fig. 7). Most steps to set-up DBO_2 are very similar to those required to set up DBO_1. Therefore, first consult the description in DBO_1. From here, implementation DBO_2 will be straightforward:

2. Starting from a DBO_1 configuration, remove L1 from the setup and replace L2 with L2*. (If you start from a DL setup, simply remove the SL entirely, and find a way to mount L2/L2* in front of the TL).

3. **How to select L2***? The goal of L2*, depending on its position in front of TL, is to shift the IFP anywhere between the TL and O_{BA}. *In our setup we use a 200 mm focal length $Ø_1$ plano convex lens (LA1708, Thorlabs) which is probably a good starting point for most optical configurations. L2* is assembled exactly like L2 in DBO_1*, using a *3D-printed holder. In our configuration we can slide it anywhere between just next to the TL and up to 10 cm in front of it.*

Side note: If you remove all lenses except the TL and Objective (i.e. remove SL/L1/L2$^{(*)}$), your parallel beam coming from the scan mirrors should come into focus beyond the objective back aperture (F_{TL} is always bigger than D_3). Accordingly, O_{BA} will be illuminated by <u>convergent</u> beam. However, we want the beam to <u>diverge</u>, so you will need a L2 lens that is powerful enough to generate an IFP (just) before the laser beam reaches the objective.*

Check always that the beam reaching the O_{BA} is divergent, not convergent (for example with a laser viewing card).

Throughout, when choosing new lenses, make sure they have high transmission efficiency in the infrared range such that the laser beam does not get attenuated too much.

(Coming soon: details on 3d printed lens holder)

Electrically Tunable Lens

(coming soon)

- *Optics*
- *Driver Hardware*
- *Software control*

Pockels Cell

(coming soon)

- *Driver Hardware*
- *Software control*

ETL driver schematic (full description and files coming soon)