

Module 1

The general layout and real setup of module 1 is shown in Figure 1 and it serves for 4 functions within the microscope: light-sheet scanning, fluorescent de-scanning, aligning laser enlargement and bright-field imaging. An aligning laser enlargement module is used so that its small laser spot could eventually overfill the back aperture of O2 in module 2 for easier alignment. Regular setup where aligning laser replaces O1 during the alignment would results in 0.83X demagnification of laser spot, compared with 4.16X magnification with enlargement module used.

For a perfect parallel light-sheet translation on the sample, the image point on galvo needs to be relayed on to the back focal plane within O1. Thus, a 4-f system needs to be formed between galvo mirror and O1. In addition, a well-aligned 4-f system also ensures proper de-scanning of the image. Otherwise, a linear translation of images will be observed on the camera. Finally, it should be noted that the collimation and divergence of the light-sheet would be different depends on angle of viewing as illustrated in Figure 2.

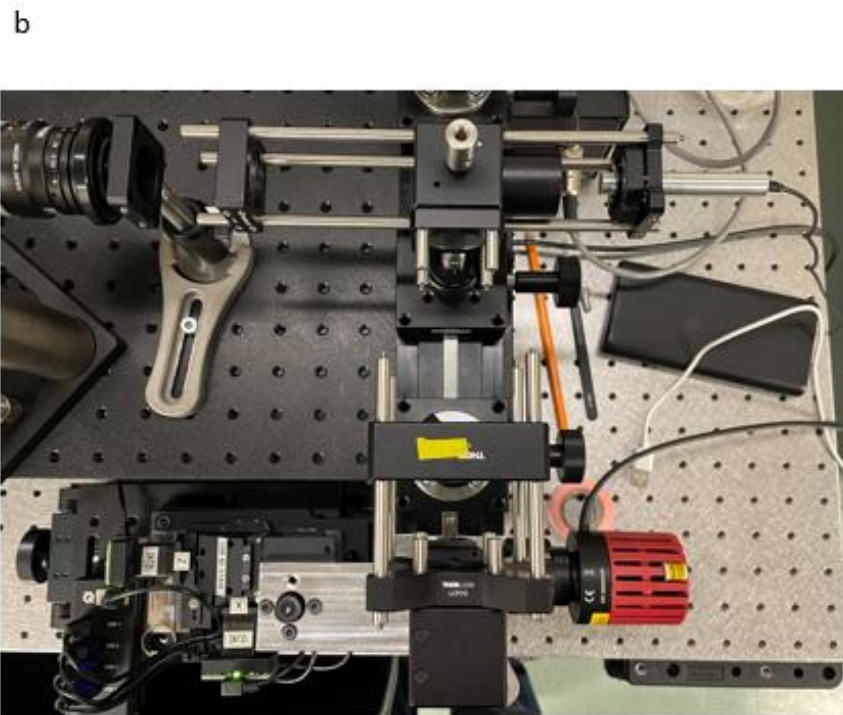
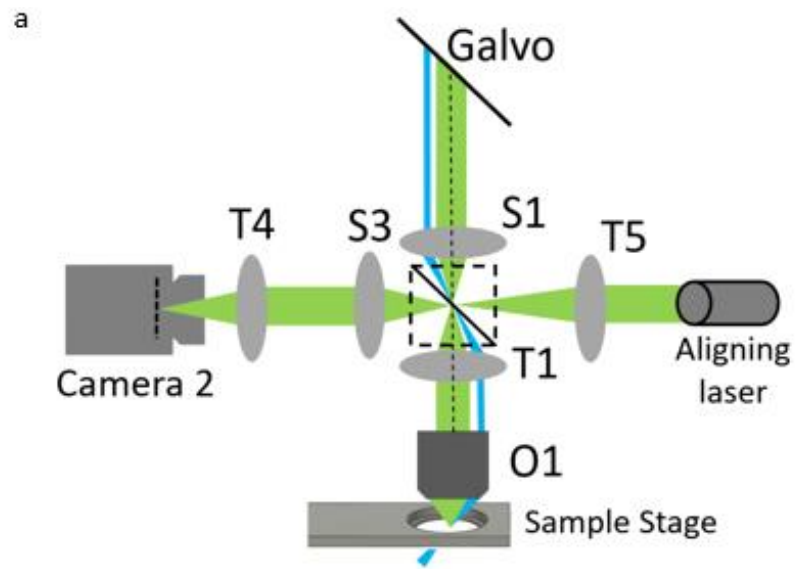


Figure 1 a) Schematic layout of module 1; b) Real setup of module 1

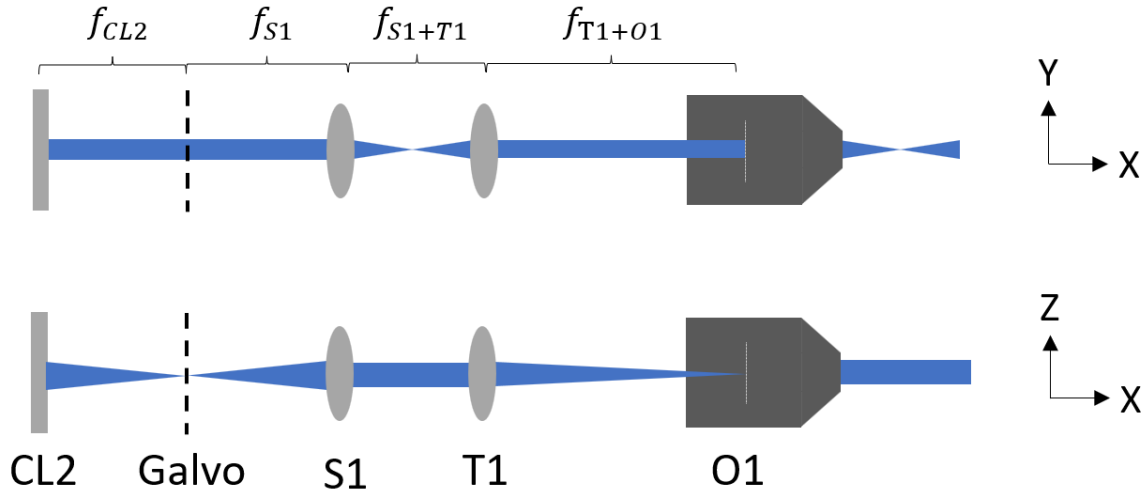


Figure 2 Collimation and divergence of light-sheet viewing from different axis

For major components used for light-sheet microscope, one 12inch travel rack and 3 pinion stages (Edmund Optics, 59-334 and 59-331) are required and a 1D large beam galvo-mirror (Thorlabs, GVS011) is needed to avoid image clipping. Both S1 and T1 are mounted using 60mm cage plate, ½” post and post holder. The high numerical aperture water immersion objective O1 (Olympus, XLUMPLFLN20XW) was connected with T1 through a 30mm-60mm cage adaptor (Thorlabs, LCP33). After that, a 30mm cage compatible turning cube (Thorlabs, CCM-G01) was used to turn the scanning light-sheet downwards for easier water immersion during the imaging process.

For major components used for bright-field imaging, a rectangular mirror (Thorlabs, FM03R) is housed using a 30mm cage cube (Thorlabs, CM1-DCH). The cage cube was mounted upside down so that its base where the mirror is attached could be removed freely to enable or disable the function of bright-field imaging. A 75mm achromatic doublets (Thorlabs, AC254-075-A) was mounted using lens tube as S3 and a 35mm machine vision camera lens (Thorlabs, MVL35M1) was used as T4. As the bright-field imaging only serves for easier sample location, a light weight, compact scientific camera (Thorlabs, CS165MU) was used. In addition, two more mounting adaptors (Thorlabs, SM1A57; Edmund Optics, 12484) were used to fit the camera into a 30mm cage plate. Finally, a mounted LED (Thorlabs, M450LP2) attached with collimator (Thorlabs, SM1U25A) was used as the light source for bright-field microscope.

For the setup of elevated breadboard, 2 separate board with size 12×18inch and 24×24inch (Thorlabs, MB1218 and MB2424) were used for illumination module and the rest of microscope system. One tapped hole at each corner of the breadboard was machined into a clearance hole so it can be aligned with the optical table using 1.5'' posts.

Setting up reference points:

1. Mount both galvo mirror and S1 using ½'' post and post mount, both their x-y location and height does not need to be precise
2. Turn on illumination laser and set it to the minimal power
3. Bring the galvo mirror in front of the laser. Adjust the height of ½'' post to make sure laser centers on galvo mirror's z-axis. Depends on the manufacturer of the galvo mirror, its rotation center may not be the same as its mounting center. A customized plate may be required to fix it.
4. Tighten the set screw and use a post collar on galvo mirror's post to mark and fix its height
5. Mount the post holder of S1 onto the front most manual stage on the translation rack
6. Use 2 sets of cage rod and a 30-60mm cage adaptor to connect an iris to the back of S1
7. Bring the entire rack with S1 attached in front of the laser. Adjust both position of the rack and height of ½'' post to make sure laser passing through the iris hole after S1
8. Tighten the set screw and use a post collar on S1's post to mark and fix its height. This will be the primary height reference for the rest of microscope system

Setting up O1 objective:

1. Use a C-SM1 mounting adaptor to connect O1 objective onto a 30mm cage plate
2. Use 1 set of cage rod to connect the cage plate onto a cubic turning mirror, add another set of cage rod on the other side of the cubic turning mirror for future mounting. Making sure the additional set of cage rod used are short enough
3. Mount T1 using a 60mm cage plate and use 1 set of cage rod to connect it with a 30-60mm cage adaptor
4. Remove screws from 1 set of cage rod and slide them into aligning laser's cage plate
5. Connect screwless cage rod with the rod used in step 2 so that aligning laser and front-end of the objective are face-to-face

6. Slide the additional set of cage rod from step 2 into the cage adaptor. Making sure it slides all the way to the end before tightening (figure)
7. Put on laser safety glass and turn on aligning laser, a spherical laser spot should be observed behind the T1
8. Slightly loosen the set screw on the outer holes of 30-60mm cage adaptor to slide the cage adaptor back and forth
9. Place the shear plate in the center of the laser spot. Keep adjusting the position of the cage adaptor until the shearing pattern is parallel to the reference line
10. Tighten the set screw on cage adaptor and remove the aligning laser by unscrewing the rod connected in step 5. The relayed system between T1 and O1 is now formed

Setting up 4f system:

1. Mount the translation rack on the right side of the breadboard, making sure its back end (opposite to the side with S1 mounted) is aligned with the edge of breadboard
2. Insert aligning laser in in cage adaptor connected to S1.
3. Place 2 post mountable iris in front of S1, making sure they are not too close with each other. Use the aligning laser coming out of S1 to adjust the height of iris
4. Once the height of irises are fixed, loosen the set screw on S1 and rotate it until the aligning laser passing through the center of both iris
5. Remove aligning laser from S1 and use it to replace the cubic turning mirror that slides into T1's cage adaptor
6. Mount T1 on the back most stage on the translation rack, leaving the middle one for bright-field imaging system
7. Turn on the aligning laser and adjust both the height and rotation of T1 until the light coming after S1 passing through the center of both iris.
8. Tighten the set screw and use a post collar to fix the height of T1
9. Remove irises and move the aligning laser back to the cage adaptor on S1
10. Mount galvo mirror at top right corner of the breadboard. Making sure to use the closest hole available in front of the S1. By using tapped holes available on breadboard, the center of galvo mirror and S1 should be auto-aligned

11. Using the knob on the translation stage to adjust the position of S1 until the laser spot is focused on the galvo mirror. Use the set screw on the stage to fix its position
 12. Move the aligning laser to the cage adaptor on T1 and adjust its position using the knob
 13. Place the shear plate in the center of laser reflected by galvo mirror and adjust the position of T1 until the shear pattern is parallel to the reference line
 14. Remove the aligning laser and put back the cubic turning mirror attached with O1 objective.
- The 4f system in module 1 is setup

Setting up bright-field imaging system and laser enlargement:

1. Ensure all above alignment procedures are complete and place the aligning laser on T1 by switching off O1 objectives
2. Place 2 post mount iris after galvo mirror, adjust their height to the same level of reflected beam from galvo mirror
3. Use Matlab to control the angle of galvo mirror (details can be found in [control section](#)) until the laser beam passes the center of both iris
4. Thread T4 onto the camera and then use both M35.5-T mount adaptor and T-SM1 mount adaptor to mount front-end of T4 onto a 30mm cage plate
5. Use 2 separate lens tube to mount both S3 and T5. Then mount S3 onto a 30mm cage plate and T5 directly onto the cage cube. 2 different side of the cage cube that are opposite to each other should be used
6. Remove aligning laser from T1, then use 2 sets of cage rods to mount camera, S3 and aligning laser onto the cage tube. The positioning does not need to be precise
7. Mount the cage cube (with mirror attached to the base) upside down onto the middle stage on the track and turn on the aligning laser
8. Adjust the rotation and height of the cage cube mount until the laser passes the center of both iris setup in step 2
9. Tighten the mount of cage cube and adjust the location of the manual stage using the knob until the laser reflected from galvo is collimated (use shear plate for confirmation)
10. Switch the aligning laser back onto T1 and adjust the position of S3 until the light coming out from it is collimated

11. Put the aligning laser back onto the cage cube and place back the O1 objective. Both bright-field imaging system and laser enlargement is now set up. The bright-field imaging system can be turned on or off by simply keep or remove the mirror in the cage cube

Table 1 Part list for module 1

Optical Part	Part description	Part number (Vendor)
Galvo mirror	1D large beam scanner	GVS011 (Thorlabs)
S1	75mm achromat	49-292 (Edmund Optics)
T1	150mm achromat	49-285 (Edmund Optics)
O1	XLUMPLFLN 20x/1.0NA W	XLUMPLFLN20XW (Olympus)
T5	30mm mounted achromatic doublet	AC254-030-A-ML (Thorlabs)
S3	75mm achromatic doublet	AC254-075-A (Thorlabs)
T4	35 mm EFL, f/1.4	MVL35M1 (Thorlabs)
Removable mirror	25×36mm visible cold mirror	FM03R (Thorlabs)
Bright-field camera	1.6MP monochrome CMOS camera	CS165MU (Thorlabs)
Mounting part	Part description	Part number (Vendor)
Cage Cube	30mm cage cube for rectangular Optics	CM1-DCH (Thorlabs)
Travel track	12" pinion track	59-334 (Edmund Optics)
Manual track stage ×3	1 Knob stage	59-331 (Edmund Optics)
60mm cage plate ×2	SM2 threaded, 0.9" thick	LCP34T (Thorlabs)
30-60mm cage adaptor ×2	0.5" thick	LCP33 (Thorlabs)
Turning mirror	30mm cage turning mirror	CCMG01 (Thorlabs)
Lens tube ×2	1" lens tube, SM1 threaded	SM1L10 (Thorlabs)
30mm cage plate ×2	0.35" thick, SM1 threaded	CP33 (Thorlabs)
M35.5 to T mount adaptor	M35.5 external and T mount internal	12484 (Edmund Optics)
T mount to SM1 adaptor	T mount external and SM1 internal	SM1A57 (Thorlabs)
1.5" post ×2	1/2" optical post	TR1.5 (Thorlabs)
0.75" post ×2	1/2" optical post	TR075 (Thorlabs)
Cage rod set ×5	Length varied depends actual need	(Thorlabs)
1" post holder ×2	1/2" post holder	PH1 (Thorlabs)

1.5" post holder ×2	1/2" post holder	PH1.5 (Thorlabs)
Post collar ×3	Thin slip-on post collar	R2T (Thorlabs)
