Module 3

The general layout of module 3 is shown in Figure 1 and it mainly serves to reimage the aberration corrected but still tilted image onto the camera. It should be noticed that a right-angle turning mirror can be placed behind O3 if the length of optical table was not sufficient for the linear layout of module 3. In addition, 2 identical emission filters were used in series as the reflection of light-sheet could not be fully blocked with the use of one single filter. The choice of O3 dictates the overall magnification range of the system. A commercial photography zoom lens was chosen over microscopy camera lens with fixed focal distance so that it gives some flexibility in adjustment of magnification. Furthermore, both T3 and camera were mounted vertically for easier aligning. As a result, another right-angle turning mirror was used after emission filters to direct the light upward. Finally, the assembly and alignment of module 3 is rather simply compared to other modules as it does not require a 4f system setup. However, the alignment between module 2 and module 3 is the most important and hardest part of the entire system. Detailed step by step procedure will be presented in **Error! Reference source not found.**.

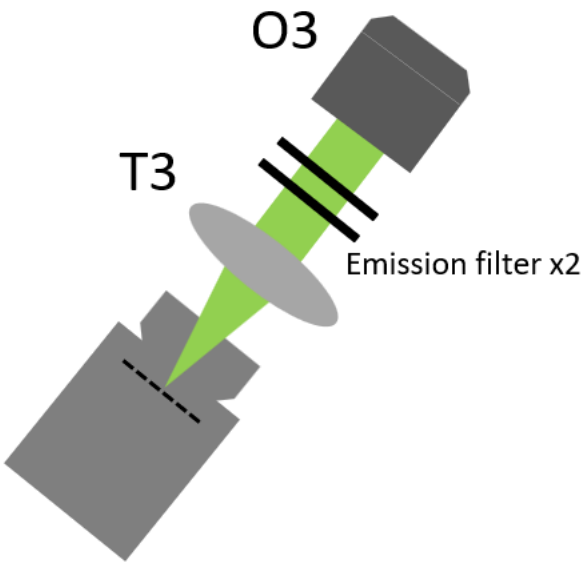

Figure 1 General layout of module 3

For major optical components used in module 3, a 20X, high NA dry objective (Edmund Optics, 58-373) was used. However, the choice of O3 is rather flexible and another 10X, high NA dry

objective (Nikon, CFI Plan Apo Lambda 10x/0.45 NA) can also be used as alternative. 2 sets of pre-mounted emission filters for GFP and Tritc (Thorlabs, MF525-39 and MF620-52) were used with 2 manual filter wheels (Thorlabs, CFW6). A commercial photography zoom lens (Canon, EF 70-200 f/2.8L IS III) was used as T3 for flexible magnification adjustment. Finally, a sCMOS camera (Andor Oxford instrument, Zyla 4.2+) was used to capture final images.

Alignment for module 3:

1. Using a C-SM1 adaptor to mount O3 onto a right-angle turning mirror cube. Then use a post, post holder, base adaptor and clamping fork to secure O3 onto a 4''×6'' breadboard
2. Using a set of cage rod to mount a cage compatible iris on the exiting side of the turning mirror cube
3. Turn on the aligning laser and place O3 head to head toward O2. The angle between O2 and O3 should be roughly 180° and the spacing should roughly be 15mm ($WD_{O2} + WD_{O3}$)
4. Adjust the height of O3 until the light from the backside of O3 passes through the center of iris. Tightly secure the side screw of post holder and use a post collar to further fix its height
5. Remove iris and mount 2 manual filter wheel in series on the exit side of the turning cube. It should be noticed that only bottom 2 cage rods should be used according to the instruction of the filter wheel
6. Slide in another 30mm cage plate with post after emission filters. Then mount a turning mirror onto the cage plate with top 2 cage rod threaded only
7. Machine the long travel vertical stage that it is securely connected to the tripod mount for T3
8. Thread an EOS-C mount adaptor onto T3 and carefully insert it into the vertically mounted tripod before connect it to the camera. Firmly secure the tripod
9. All components in module 3 is now set up. All components before T3 were mounted on one single breadboard and will be referred as "the breadboard plate" in section **Error! Reference source not found.**

Table 1 Part list for module 3

Optical Part	Part description	Part number (Vendor)

O3	EO HR 20x/0.60 NA	58-373 (Edmund Optics)
GFP emission filter ×2	CW=525, BW=39	MF525-39 (Thorlabs)
Tritc emission filter ×2	CW=620, BW=52	MF620-52 (Thorlabs)
T3	70-200mm, f/2.8 Zoom	EF 70-200 f/2.8L IS III (Canon)
Camera	sCMOS	Zyla 4.2+ (Andor Oxford Instruments)
Mounting	**Part description**	**Part number (Vendor)**
Breadboard	4"x6", tapped	MB4 (Thorlabs)
C mount to SM1 adaptor	C mount internal to SM1 external	SM1A10 (Thorlabs)
Turning mirror ×2	30mm cage turning mirror	CCMG01 (Thorlabs)
filter wheel ×2	30mm cage manual filter wheel	CFW6
EOS-C mount adaptor	C mount external, EOS internal	(Amazon)
Tripod	tripod for Canon 70-200 lens	(Amazon)
vertical translation stage	10" vertical travel	VAP10 (Thorlabs)
30mm cage plate	0.35" thick, SM1 threaded	CP33 (Thorlabs)
Cage rod set	Length varied depends on need	(Thorlabs)
1.5" post ×2	1/2" optical post	TR1.5 (Thorlabs)
1.5" post holder ×2	1/2" post holder	PH1.5 (Thorlabs)
Base adaptor ×2	1.25" pedestal base adaptor	BE1 (Thorlabs)
Table clamp ×2	1/4" 20 tapped	CL2 (Thorlabs)