Optical and Mechanical Adjustments and Alignments on RAMM Frame Microscopes

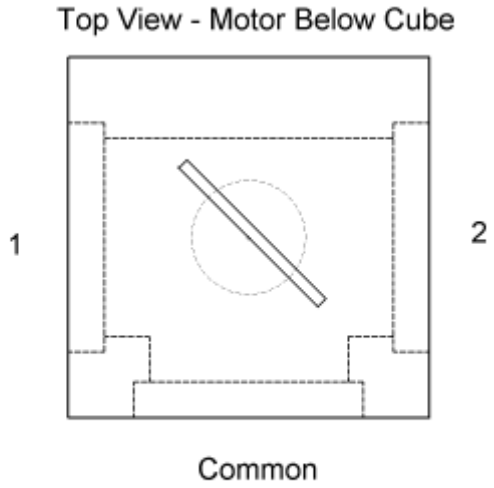
Correct adjustment of the illumination optics will depend upon the particular configuration of the RAMM/MIM microscope. Here we provide general guidelines for a strategy to accomplish proper alignment on systems with multiple optical paths as well as specific instructions for the particular illumination devices. As an example system we will go through the procedure for a RAMM/MIM system with both a TIRF illuminator and multi-LED illuminator with a C60-PORT-SWITCH to select the illumination port.

General adjustment strategy

1. Align TIRF laser when in the “centered” position of the TIRF angle adjuster to the objective Back Focal Plane (BFP).
    1. Establish the correct zero position for the Port Switch mirror and other clocked devices.
    2. Center TIRF beam with adjusters on the main dichroic CUBE-III
    3. Focus TIRF beam roughly in BFP using the lens in the Cage assembly.
2. Align and focus LED illuminator LEDs at the objective BFP.
3. Center camera view on the epi-illumination field stop.

Setting the C60-Port_Switch Zero Position

This is usually the first adjustment for a newly assembled system. For best results, first pre-align illuminators and/or mirrors in the optical path to their nominally centered state. Remove the objective and place a witness target across the objective hole opening. A suitable target can just be a piece of semi-transparent “Scotch” tape across the objective adapter threads. The Port Switch is usually used with condenser lenses on each port that are designed to focus light into the objective BFP. With a TIRF illuminator, the laser should be focused there. For a wide field epi-illumination source, the light guide tip or the LED emitting die should be focused at the BFP. The C60-PORT_SWITCH moves a mirror to select either port 1 or port 2 to be reflected to the common port. The geometry is defined by the figure below.



To set the mirror location for port 1 we need to have the illumination source connected to port 1 turned on and roughly focused at the objective BFP. Determine the name of the Port_Switch axis. For the TIGER controller the WHO or BU X commands will reveal what is in the controller box. Below is an example where the port switch is named 'M' and is on card #2.

who
At 30: Comm v3.17 TIGER_COMM Jun 01 2017:12:50:01
At 31: X:XYMotor,Y:XYMotor v3.15 STD_XY_LED Sep 27 2016:10:26:01
At 32: Z:ZMotor,**M:Switch** v3.18 STD_Z_SWITCH_SLOW Aug 09 2017:16:19:05
At 33: O:Tur,T:Motor v3.18 TURRET_TIRF Aug 09 2017:16:30:11
At 37: L:Leds v3.16 TGLED_4 Oct 25 2016:12:09:03

bu x
TIGER_COMM
Motor Axes: X Y Z **M** O T L
Axis Types: x x z **h** o l i
Axis Addr: 1 1 2 **2** 3 3 7
Hex Addr: 31 31 32 32 33 33 37
Axis Props: 74 10 2 2 0 0 0

For the MS2000 controller the name is revealed by the BU X command, in this case called the 'S' axis.

bu x
STD_XYZ_PSWTCH
Motor Axes: X Y Z **S**
Axis Types: x x z **h**
CMDS: XYZSFTR
BootLdr V:1
Hdwr REV.E
LL COMMANDS
RING BUFFER 50
SEARCH INDEX
CLOCKED POSITIONS
IN0_INT

We want to be able to manually rotate the mirror to the best position. To do that we need to connect the Port-Switch motor to the controller control knob using the "JOYSTICK" or "J" command.

For TIGER J M=22 will connect the right knob on the joypod to the M axis.

For MS2000 J S=4 will connect the knob on the MS2000 to the S axis.

At this point you will be able to manually steer the port switch mirror using the knob on the controller. Use the mirror to bring the illumination spot to the center of the witness target over the objective thread hole. To lock in the zero position for Port 1 of the port switch, simply reset the controller or controller card that is turning the mirror.

Tiger	**MS2000**	
2RESET	RESET	will reset card #2 or controller that has the port switch control.

Check your work. Be sure you can now command the port switch to move the second position and back and that if comes back where you expect it to.

TIGER	**MS2000**	
M M=2	M S=2	should move to the second port.
M M=1	M S=1	should move back and illumination should in the correct location.

Setting the Automated Objective Turret and/or Automated Cube Slider Zero Position

The same procedures outlined above applies to setting the home/first position for other ASI clocked devices such as the automated objective turret or the automated filter cube slider. In the previous TIGER example, you may notice the WHO and BU X commands show that the Tiger controller also has an automated objective turret with axis name 'O' on card #3.

For the objective turret it is best just to turn off the controller and manually move the nosepiece so that the first position is in place and the mechanism is centered on the detent for that position. Then turn on the controller and issue the reset command for the card controlling the objective to lock in the correct #1 position.

TIGER	**MS2000**	
3RESET	RESET	will reset card #3 or controller that has the port switch control.

Use the same procedure for the automated filter cube slider. Turn off the controller and manually slide the cube slider to the 1st position. There is a white engraved mark on the slide that should be lined up with the side of the cube housing to accurately position the slider correctly. Turn back on the controller and again, issue a RESET command to the card controlling the slider.

Index line marked

Steering the Beam by Adjusting the Dichroic Mirror

The procedure above can only adjust the beam position in the axis that the mirror movement allows. To perfectly center the laser beam in the center of the objective aperture, you may wish to adjust the tilt of the dichroic mirror slightly. Use the three adjusting screws on the CUBE-III or CUBE-II to move the beam to the center of the aperture.

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Click to Enlarge

Newer CUBEs use thumb screw adjusters, older CUBEs use a 3/32 Allen driver. Adjust the mirror to bring the beam to the center of the objective hole as shown 1n the picture above.

Focusing the TIRF Laser

The TIRF laser is focused at the objective BFP by sliding the condenser lens on the cage assembly.

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Rough focus of the laser at the objective hole witness target is good enough for initial alignment. However, for proper TIRF alignment, the TIRF objective should be installed in the microscope and the laser beam should be focused to a collimated spot above the microscope on the ceiling of the room.

Aligning the Multi-LED Illuminator

On systems that include a port switch and TIRF illuminator, setting the port switch home position and adjusting the tilt of the dichroic mirror should be done before aligning the LED illuminator. On systems with only the LED illuminator for epi-illumination, one can use the tilt adjustment of the dichroic mirror to roughly center the LED emitters in the objective back focal plane at the objective thread mount. In both cases, fine adjustment of each of the LED emitters should be done, one at a time, to best center and focus the emitter on the witness target at the objective aperture. See the LED command for the syntax for turning on/off the LEDs. In our example with card #7 the LED control card,

7LED x=0 y=0 z=0 f=0 will turn them all off.
7LED y=50 will turn on the ‘Y’ LED at 50% intensity.

LED Focus

The LED focus I achieved using the helical focus on the individual LED illuminators.

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There are six set screws that hold the focus assembly, three in the helical grooves and three outside the grooves. Loosen the three set screws **outside** of the grooves but leave the three set screws that are in the grooves as they are. The assembly will now twist and will allow you to focus the LED emitting die on the target over the objective hole. You know the die is in focus when you can see the structure of the emitting element.

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LED Centering

Once the LED is in focus, you can also center the emitter on the objective opening.

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Loosen the three LED board mount screws and manipulate the LED board using the bolt heads of the mounting screws and the cable connector. Carefully center the LED emitter on the witness target at the objective hole and retighten the three screws. Repeat the LED focus and centering adjustments with each of the LEDs in the multi-LED illuminator.

manual, ramm, led