RAMM Microscope Configuration Guide

ASI's RAMM frame microscopes are modular open frame alternative to conventional commercial microscopes. Using a RAMM frame may be especially appropriate for instruments dedicated to a specific task, or when the user needs easy access to the optical paths of the microscope for their own custom optical configurations. Here we describe some of the main variants of the RAMM frame and microscope system to aid in selecting the optimal configuration for a particular application.

Plain RAMM versus RAMM-DV frame

The original **RAMM** frame is based upon the **MIM-INVERTED-BASIC** microscope assembly where there is a right angle mirror directly under the objective and the main optical path is then horizontal. This means that complicated optical paths require more bench space, but not more height. As an option, this assembly can use the **C60-RA_2nd_PORT** to provide a second lower layer to the microscope. Frequently, this lower layer is used for the **CRISP** autofocus system.

The **RAMM-DV** frame can support the **MIM-FC** series of inverted microscopes that are based upon the **C60-BEAMSPLITTER** cubes placed directly below the objective. The main optical path for the **RAMM-DV** microscopes is vertical, since there need not be a mirror directly behind the objective. This arrangement has the advantage that filter cubes can be located very close to the objective to minimize collimated space vignetting. However, as systems get more complicated, they tend to get taller with several cubes in a row. Often this requires **RAMM-STILTS** to raise the system higher off of the table.

The **RAMM** and **RAMM-DV** frames differ in the main cross-arms and drop-arms that are used to support the microscope components. The original **RAMM** frame has fixed positions where the drop arms can be attached. The **RAMM-DV** frame utilizes a dovetail mounting method that allows for more flexible positioning of the drop arms. Mounting **MIM-FC** series inverted microscopes on the original **RAMM** frame will cause the optical axis of the microscope to be off-center by about 23 mm.

Figure 1: Basic RAMM inverted microscope with automated stage and trans-illumination.

Examples of RAMM frame microscopes

Typical basic RAMM frame microscopes are shown in Figure 1 and Figure 2. The photo in Figure 1 has the **MIM-INVERTED-BASIC** microscope, XY stage and top-side trans-illumination. The model drawing in Figure 2 shows the **MIM-INVERTED-BASIC** with the **C60-RA_2nd_PORT** option, which provides the lower layer optical path.

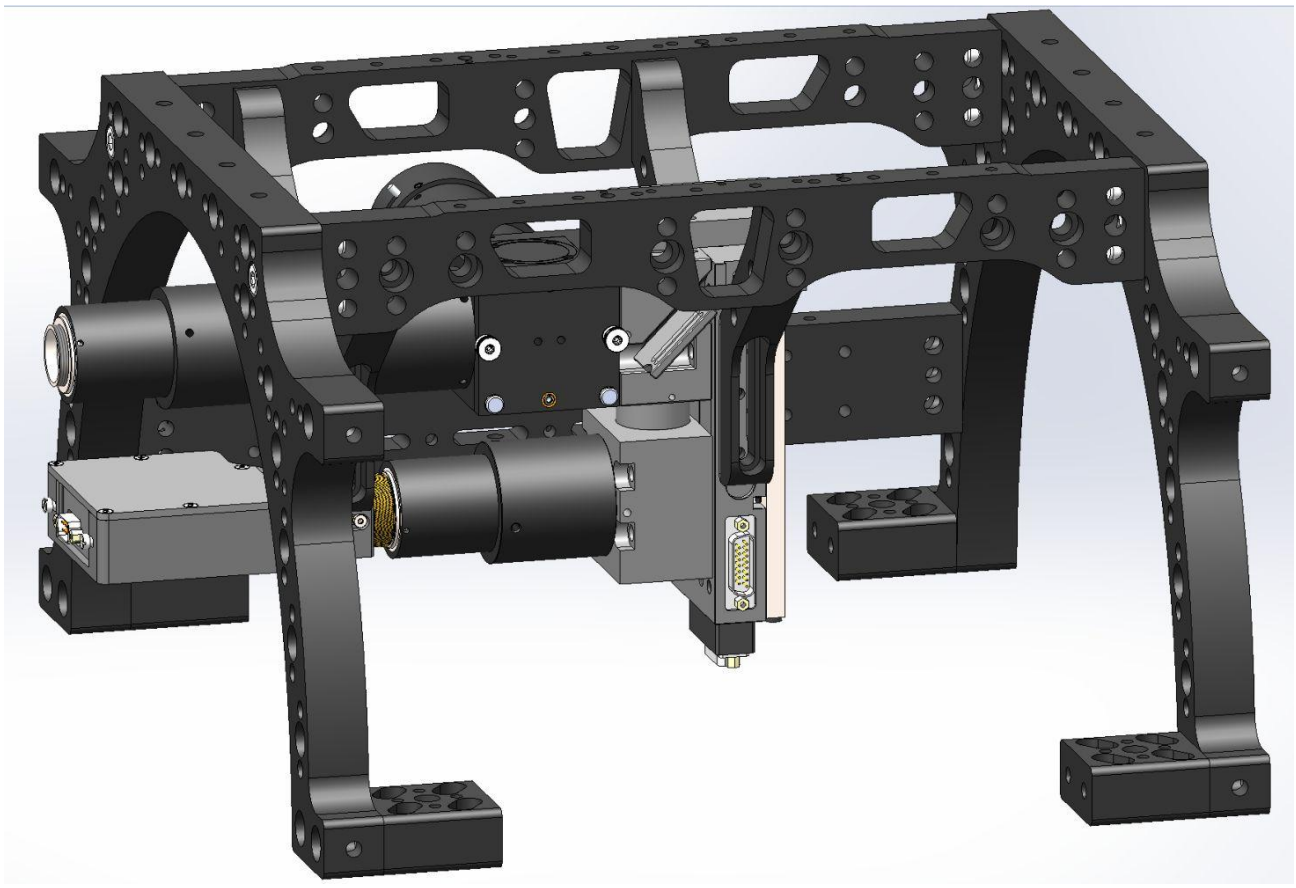

Figure 2: Basic RAMM frame with **C60-RA_2nd_PORT** option. Here the 2nd PORT dichroic is an imaging-flat IR long-pass mirror that allows the bottom layer of the microscope to be used for the CRISP autofocus system. To the left of the 2nd PORT cube is a **C60-BEAMSPLITTER-II** cube which contains the epi-fluorescence dichroic filter cube. The tube lens and camera port are to the left, while the epi-illumination condenser and port are out the back.

Examples of RAMM-DV frame microscopes

A couple of examples of RAMM-DV frame microscopes are shown in Figure 3and Figure 4 below. Note that these implementations tend to be taller. The example in Figure 4 required **RAMM-STILTS** to provide additional height to allow enough clearance for the lower camera.

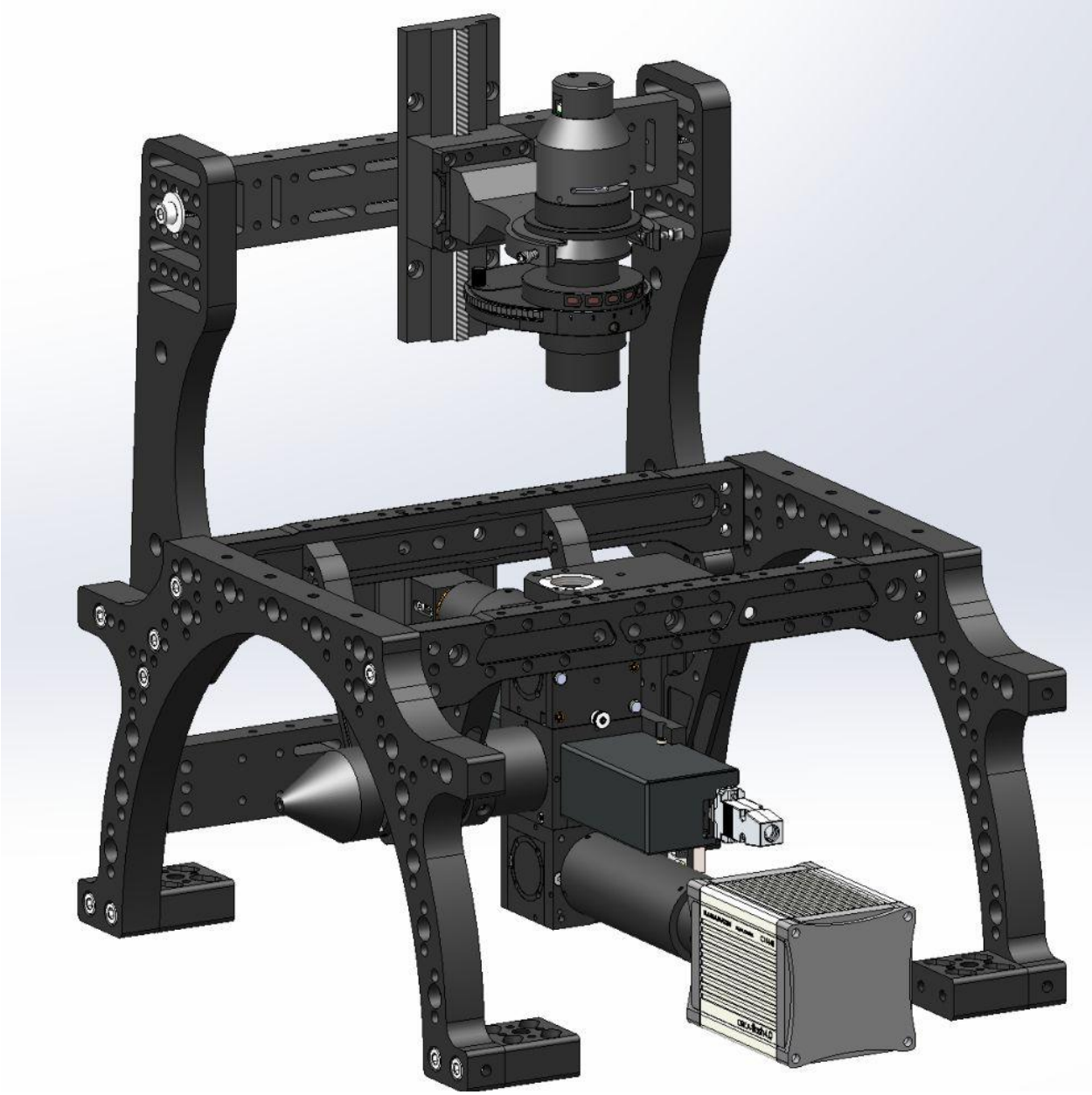

Figure 3: RAMM-DV frame microscope for single objective with three vertical cubes. Top cube is for CRISP autofocus, second cube is a four-position automated filter slider and bottom cube has a mirror for the imaging path.

Figure 4: RAMM-DV frame microscope with three vertical cubes and a manual nosepiece. Below the nosepiece is a four-position automated filter slider, below which are cubes for two camera ports. This system has a transmitted light condenser and another microscope mounted to a top-side slider. Either the condenser or the microscope can be place directly over the bottom-side objective.

Configuration Decisions and Trade-offs

Single objective, manual, or automated nosepiece
The original **RAMM** microscope was envisioned with a single objective in mind. This results in the most stable and compact microscope; a single objective is recommended when possible. We can also supply either Nikon or Olympus manual nosepieces, or a six-position automated Olympus nosepiece. The larger Nikon nosepiece requires a larger stage to make room for the turret to turn without hitting the stage plates. Heavy nosepieces are more subject to vibration than is the single objective. Air tables are more important.

Automated filter slider or just a single cube
The automated four-position filter slider works best on the **RAMM-DV** system. Experiments that require changing dichroic filters in real time may require an automated slider. However, routine experiments where a different filter set may be desired can often be accommodated with just the **C60-BEAMSPLITTER-II** cubes. These cubes have quick change magnetic latches that make swapping cubes fairly painless. If you don't need the automated slider, then the ordinary **RAMM** frame scopes are often the best choice.

CRISP autofocus location
It is nice to have the CRISP infrared (IR) beam splitter right behind the objective. This way the visible imaging and epi-illumination filters do not need to accommodate the CRISP's IR LED. With the **MIM-INVERTED-BASIC** using the **C60-RA_2nd_PORT,** the beam splitter in the **2nd_PORT** can be an imaging-flat mirror with IR long pass coating. Adding CRISP this way doesn't increase the length of the collimated path compared to the **MIM-INVERTED-BASIC** without the **2nd_PORT**. On the **RAMM-DV**, with **C60-BEAMSPLITTER** cubes, CRISP can be place off of the top cube, in which case the IR dichroic will be a short-pass version. Using CRISP this way on the **RAMM-DV** systems will increase the distance between the objective and the epi-fluorescence filter cube.

Field of view and vignetting
With today's large format cameras and with high numerical aperture objectives (especially low at magnification) one needs a pretty big light pipe or you can bump into vignetting problems. Frequently, it is the clear diameter of the emission filter in the filter cube that is the offending aperture. Minimizing the distance between the objective and the filter cube will maximize the field of view possible without vignetting. Please see the appendix for further information.

Epi-fluorescence illumination
Many modern light sources can be coupled into the microscope using a liquid or fiber light guide. Modular microscope can be built with condenser assemblies that supply either Kohler or critical illumination from a light guide. ASI also has high intensity LEDs that can be used for simple, electronically controlled fluorescent illumination. See the document "*Epi-illumination on the RAMM"* for further information.

Filter Wheels
Filter wheels can be added to imaging or illumination paths either in collimated space or near the camera C-mount. It is often easier to fit the filter wheel near the camera, but placing the filter

wheel in collimated space may be best optically, since dust on the filters will not be seen in the image and there will be fewer aberrations induced by glass in collimated space rather than in converging space.

Controllers

The choice of controller will depend upon the complexity of the system. Relatively simple systems that consist of the microscope with and XY stage and perhaps one other motorized device can be controlled with the MS2000 controller. Systems that have additional motorized components should use the expandable TIGER controller.

Controller	Number Motor Axes	Number Piezo Axes	Filter Wheel	LCD display	Manual control	Serial Communication Interface
MS2000	4	1	No	Yes	Built in: XY joystick; one knob	USB & RS-232
RM4-FW	4	1	Yes	Yes	External pod: XY joystick; two knobs	USB & RS232 for stage 2nd RS-232 for FW
TIGER-8	Up to 14	Up to 3	Yes	No		USB
TIGER-16	Up to 30	Up to 7	Yes	No		USB

Besides motor control, the TIGER controller also supports ASI's MicroMirror light sheet scanners and inter-card and external TTL coordination of events on multiple cards via TTL logic cards.

Figure 5: Tiger Controller

Figure 6: MS2000 Controller

Software

ASI's controller family is supported in many third party software packages. Both MetaMorph and MicroManager support all of our controllers.

Example RAMM Configurations for Special Purpose Microscopes

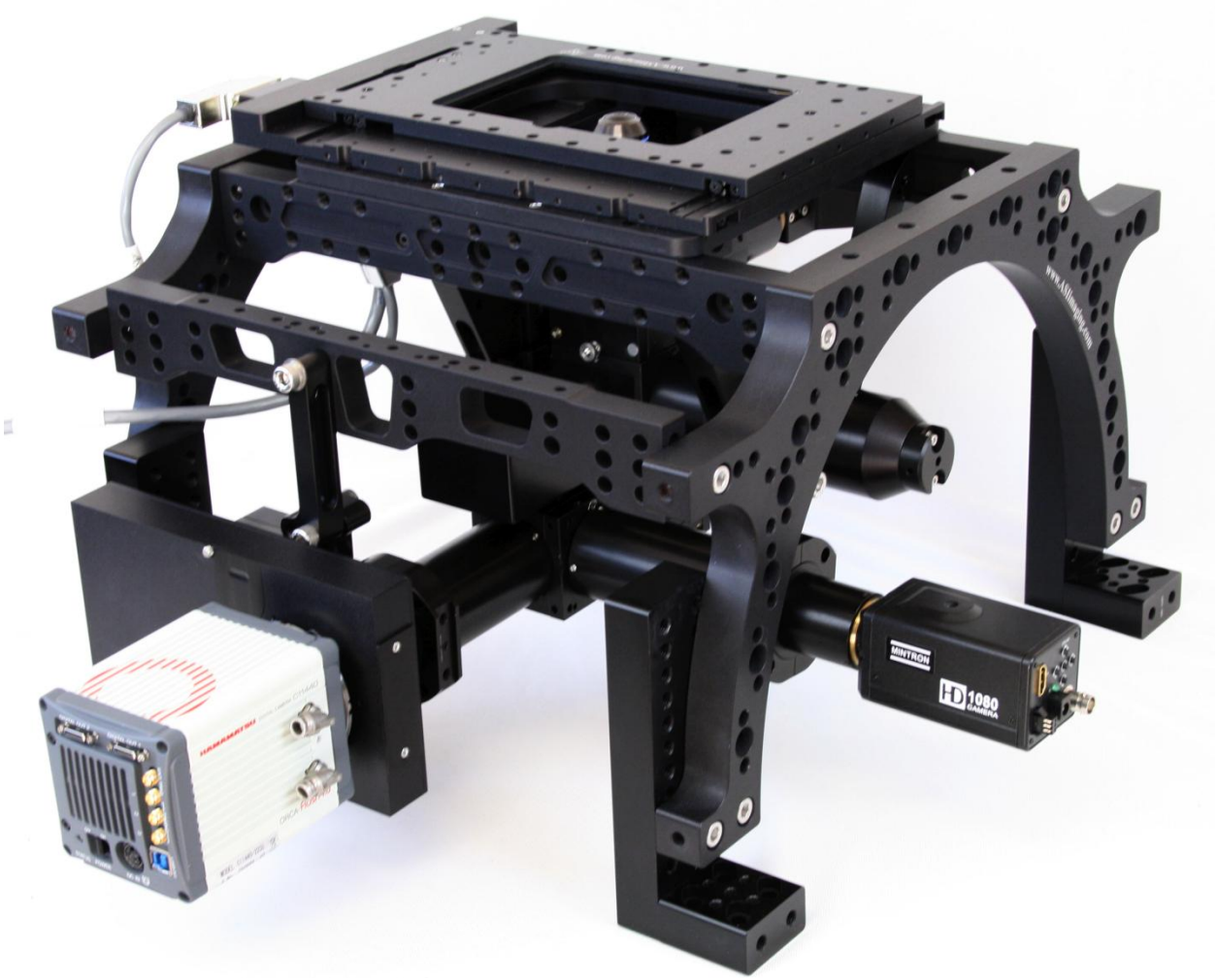

Figure 7: **RAMM-DV** system on low **STILTS** set up for two cameras, one with a filter wheel. The single objective system also has the four-position filter slider and CRISP autofocus.

Figure 8: Dual Camera **RAMM** system with large stage and Phototrack PMT.

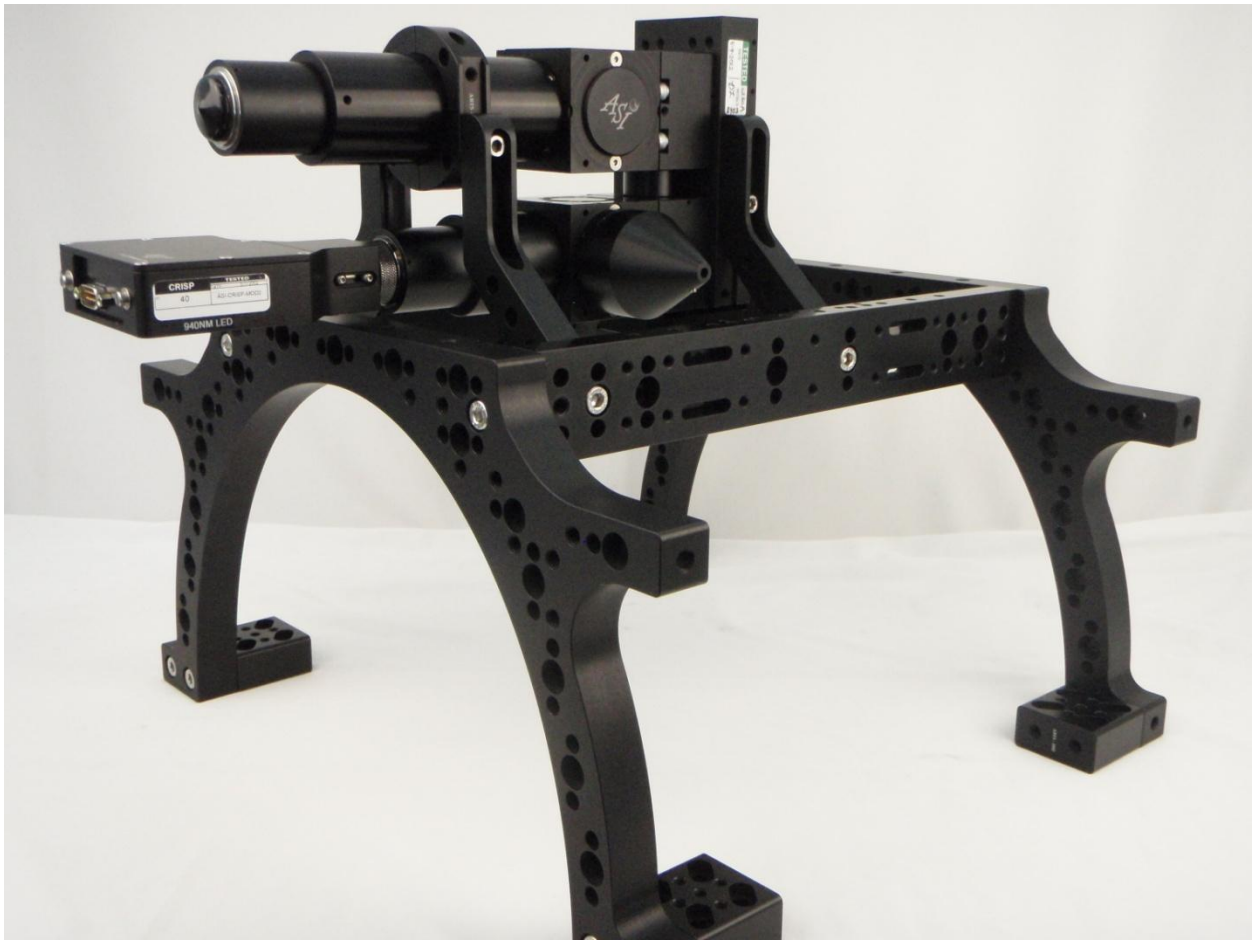

Figure 9: Inverted microscope inverted on older **RAMM** frame!

Figure 10: Inverted microscope inverted with long-travel stage and double objective slide on **RAMM-DV** frame.

Figure 11: Double microscope with piezo-Z stage CRISP and filter wheel on a **RAMM-DV** frame with **STILTS.**

Appendix 1: Infinity Space Limitations

The flexibility afforded by the infinity corrected microscope allows one to assemble an optical train with several beam splitters, mirrors, or focus devices in the infinity space region without substantially changing the optical characteristics of the system. However, there are limits to how far you can spread the distance from the objective to the tube lens before vignetting of the image will occur. The length of the infinity space, $L_{Infinity,}$ must be kept less than

$$L_{Infinity} \leq (D_{Tube} - D_{OBJ}) \times F_{Tube} / D_{Sensor}$$

where D_{Tube} is the diameter of the tube lens, D_{OBJ} is the diameter of the objective pupil, D_{Sensor} is the diagonal length of the sensor, and F_{Tube} is the focal length of the tube lens. We can use values for the MIM tube lens and consider a few examples for various objectives and sensor formats.

$$D_{Tube} = 32 \text{ mm} \quad F_{Tube} = 200 \text{ mm}$$

$$L_{Infinity} \leq (32 \text{ mm} - D_{OBJ}) \times 200 \text{ mm} / D_{Sensor}$$

Approximate pupil diameter for a few objectives and the size of a few CCD sensors:

20X N.A. 0.5	10mm	1" CCD	16mm
20X N.A. 0.75	15mm	2/3" CCD	11mm
10X N.A. 0.45	18mm	½" CCD	8 mm
40X N.A. 0.95	9.5mm	sCMOS 13.2x13.2	19mm
60X N.A. 1.45 oil	6.4mm		

So if we consider nominal objectives with 12mm pupil and a 2/3" CC camera, we find

$$L_{Infinity} \leq (32 \text{ mm} - 12 \text{ mm}) \times 200 \text{ mm} / 11 \text{ mm} = 363 \text{ mm}$$

For a fast objective and a 1" CCD camera, we find

$$L_{Infinity} \leq (32 \text{ mm} - 20 \text{ mm}) \times 200 \text{ mm} / 16 \text{ mm} = 150 \text{ mm}$$

So in general, large camera sensors with N.A. objectives impose the limit on how many beam splitter cubes you can stack up before running into problems. The minimum-length epi-fluorescent inverted configuration, with one C60-BEAMSPLITTER cube is about 130 mm long. The exact length of the infinity region can be determined from the tables below that list the infinity space length of various MIM components and can be used to determine if a particular configuration will result in collimated-space vignetting or not.

Table 1: Infinity-Space Length of MIM Components

Part Number	Description	Infinity-Space Length (mm)
C60-BEAMSPLITTER	Beam splitter cube mount	60
C60-RA_MIRROR	Right Angle mirror focus section	52
C60-RA_OBJ_MNT	Objective holder – variable focus	12 + focus range
C60-OBJ_MNT	Objective holder – fixed	18
C60_TUBE_B	Tube lens assembly 200 mm f.l.	6
U-R156M5	Automated 6-position objective turret	35
C60-RA_DOVE	Dovetail mount for automated turret	12 + focus range
C60-RA-2nd-PORT	Added distance to lower tier port	75
FW-1000 w/ Adapters	Filter-wheel with infinity path mounts	43

		12.5mm Radius FN 25		**15.2mm square chip FN 22**		**13.2mm square chip - FN 19**		**One inch chip FN 16**		**2/3 inch chip FN 11**	
Objective Example	**Objective Beam Diameter (mm)**	22mm aperture Free Length (mm)	29 mm aperture Free Length (mm)	22mm aperture Free Length (mm)	29 mm aperture Free Length (mm)	22mm aperture Free Length (mm)	29 mm aperture Free Length (mm)	22mm aperture Free Length (mm)	29 mm aperture Free Length (mm)	22mm aperture Free Length (mm)	29 mm aperture Free Length (mm)
60X NA 1.42	6	143	197	165	232	188	265	212	301	306	433
20X NA 0.6	12	95	149	108	176	122	200	138	227	197	324
10X NA 0.45	18	47	101	52	120	58	136	64	153	88	215

Collimated-Space MIM Configuration	**Name**	**Single Objective**	**C60-Duplex Objective**	**Olympus Turret**
C60-CUBE	MIM FC Mot	77	124	112
C60-CUBE; RA-Mirror	MIM Inv. Basic	129	176	164
C60-CUBE; FW1000		120	167	155
(2) C60-CUBES		137	189	180
(2) C60-CUBES; FW1000		180	227	215
(3) C60-CUBES		197	249	240
(3) C60-CUBES; FW1000		240	287	275

Avoid vignetting in collimated space be ensuring your configurations does not exceed the Free Length allowed by the camera chip size and objective beam diameter. Critical apertures are assumed to be near the tube lens and have either 22mm or 29mm clear aperture, as would be typical of 25 or 32 mm filters. When possible, place 25mm filters as close to the objective as possible. Mild collimated-space vignetting will appear as dimming in the corners of the image.