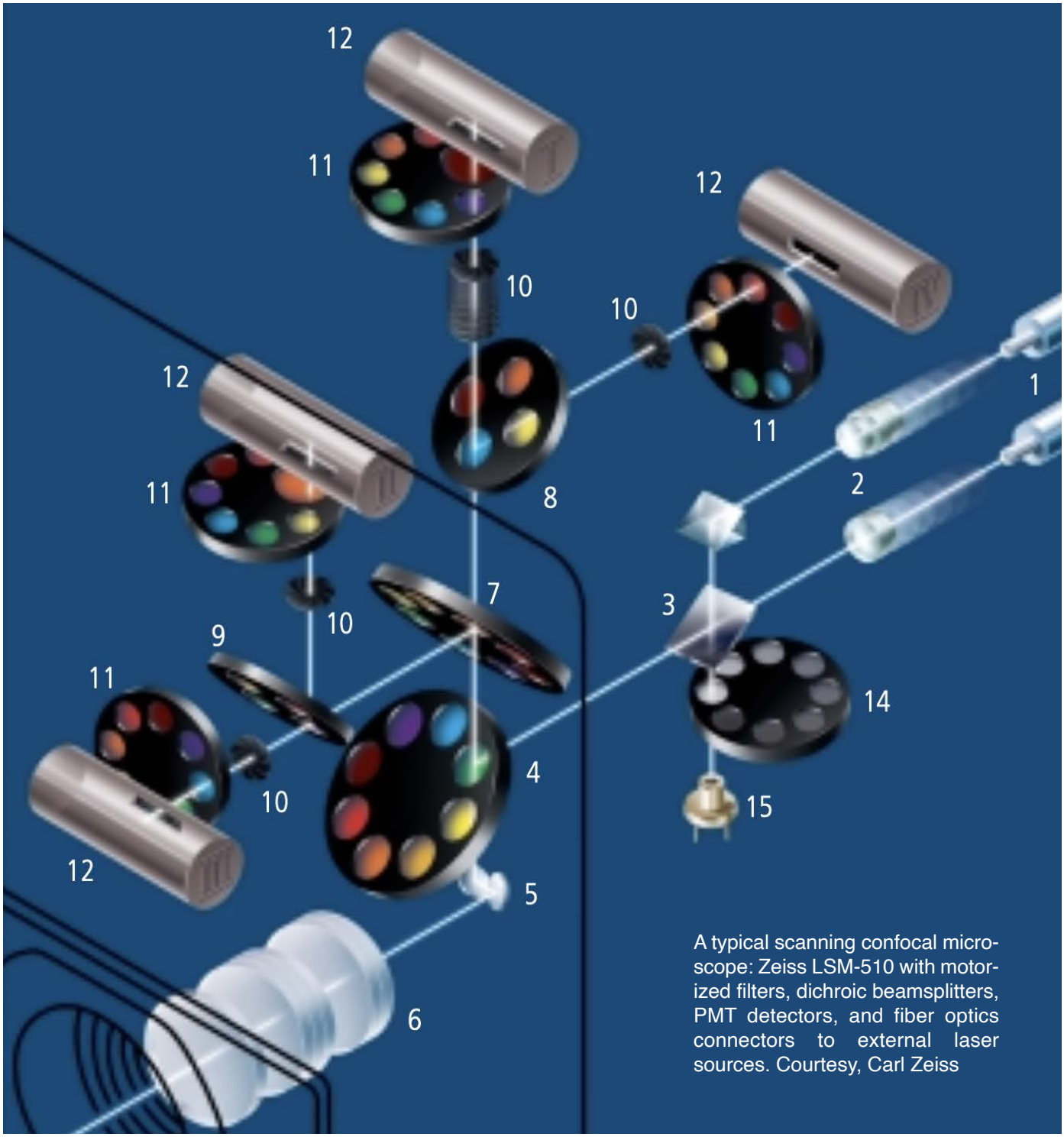
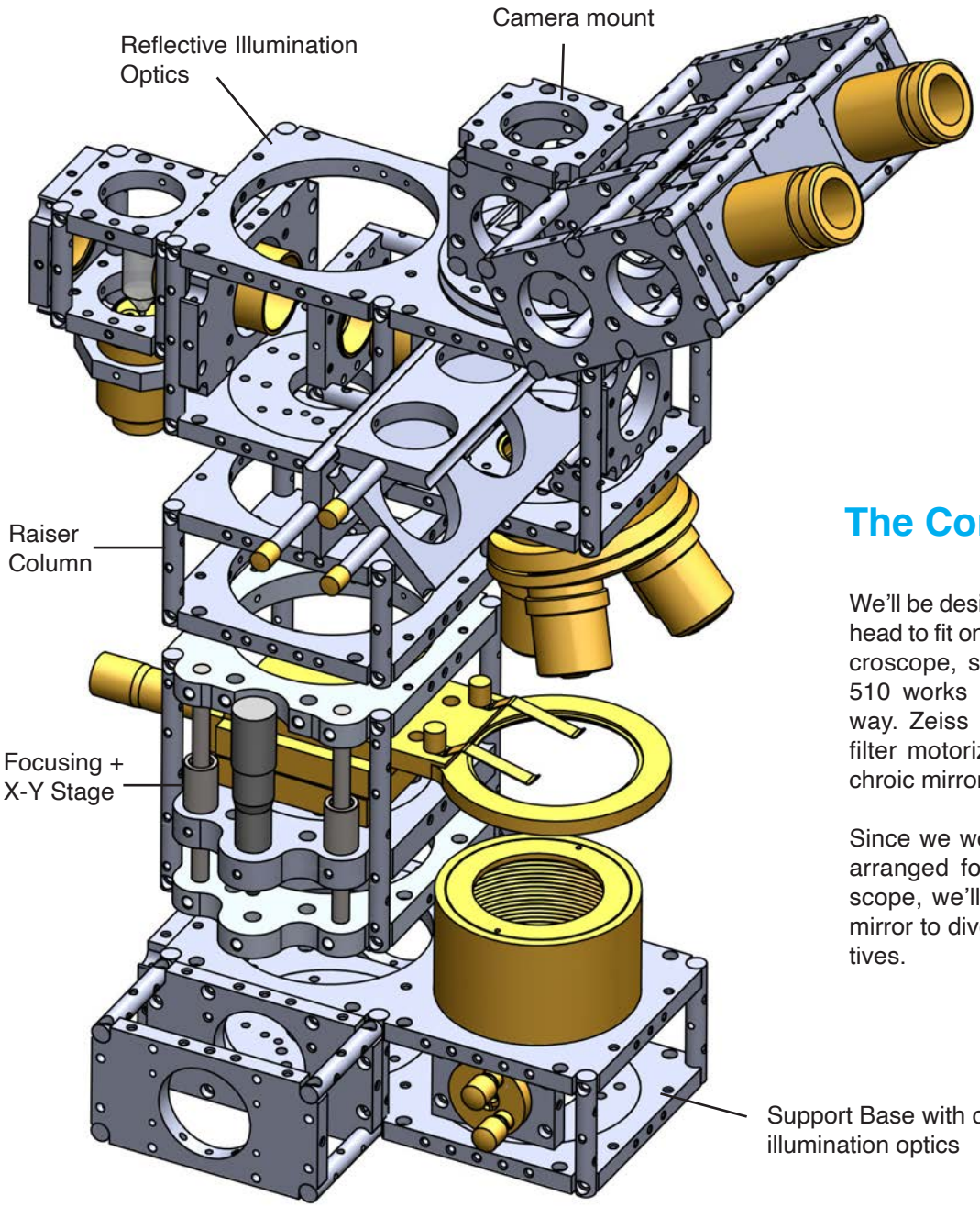
Designing the Confocal Head for Optoform Microsocpe

We will utilize the already developed central beamsplitter slider for Fluorescent microscope. Although Zeiss utilizes this scheme, their slider is a solid block which has to be pre-assembled before its insertion into the microscope. In our design, there are three sliders, that could be independently selected to get the desired emission/excitation/absorption effect. The standard filter size for Ziess is 25 mm, and the bemsplitter size is 25.5 x 36 mm. A high quality 3-filter/beamsplitter set will cost around $1,200 each, adding up to $3,600 just for filters.

Typical combinations that are available are: **546/ 580/ 590**, **485/ 510/ 515**, and **385/ 395/ 397** for Excitation/ Dichroic/ Emission filters respectively. As we see in LSM 510 (below), all the internal filters of Axiovert 2 microscope are bypassed, and the scan head has its own sets of filters, and beamsplitters. Looking back at our Optoform microscope, the placement of the Confocal head could be on the back side of 74-120 mount, behind the trinocular observation head we built in previous section.



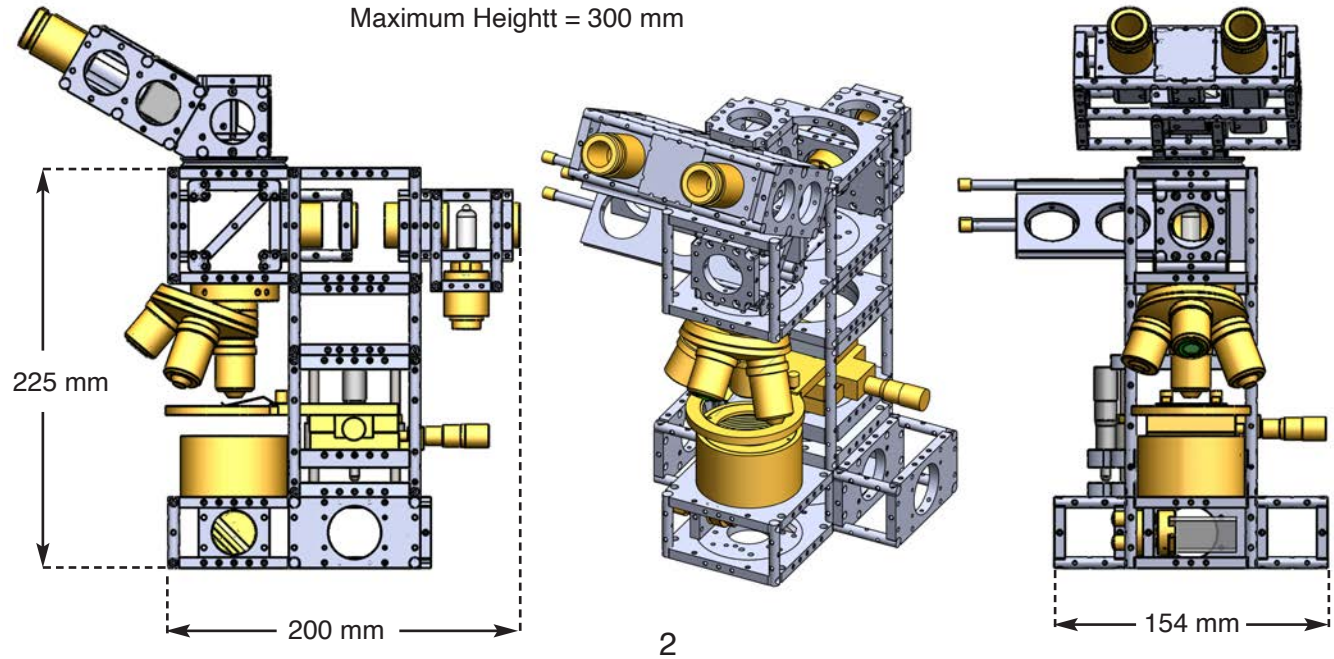
A typical scanning confocal microscope: Zeiss LSM-510 with motorized filters, dichroic beamsplitters, PMT detectors, and fiber optics connectors to external laser sources. Courtesy, Carl Zeiss



The Confocal Scheme

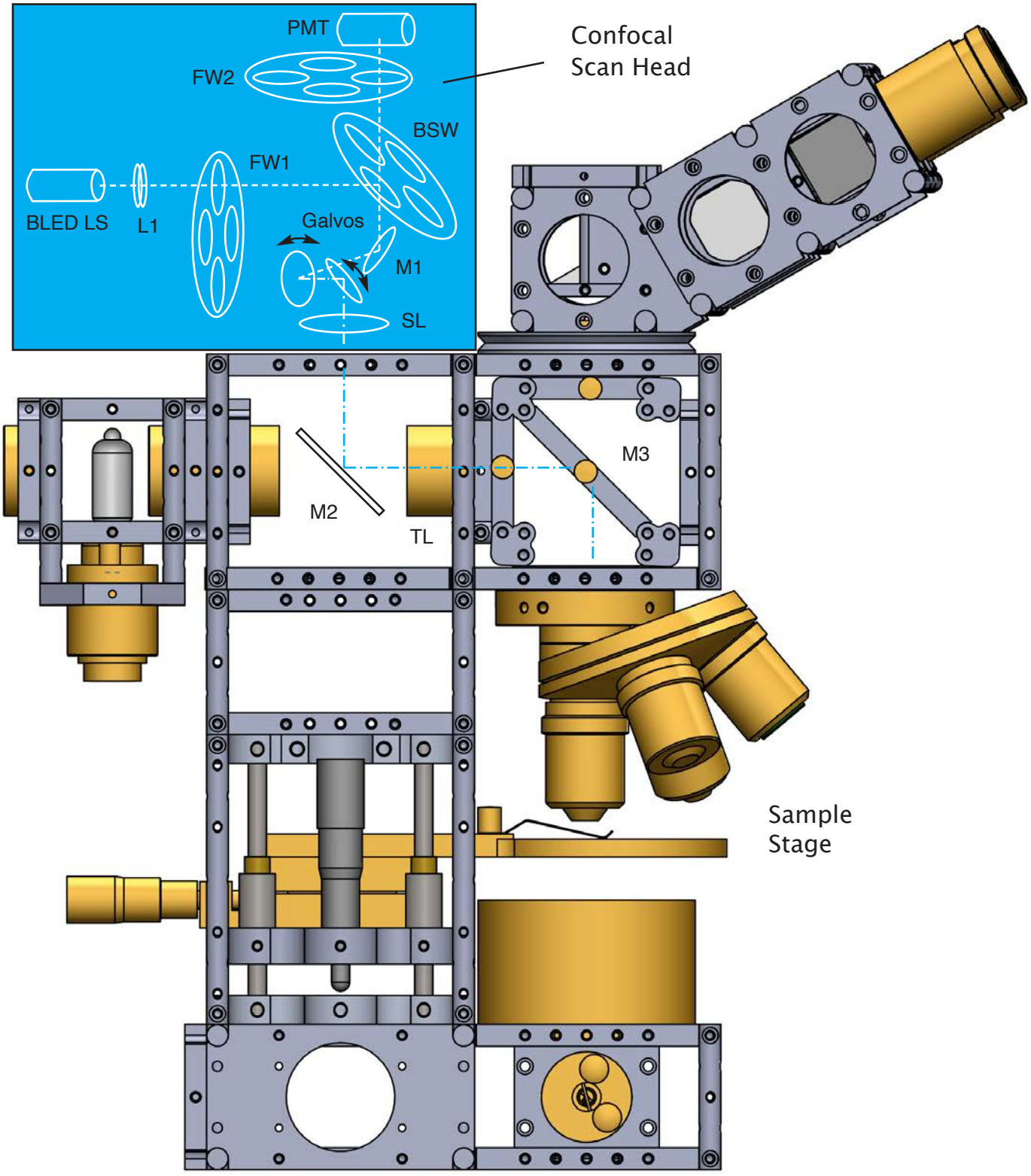
We'll be designing a confocal scanning head to fit on top of the Fluorescent microscope, similar to how Zeiss LSM 510 works but in a much simplified way. Zeiss utilizes an assortment of filter motorized filter wheels, and dichroic mirrors.

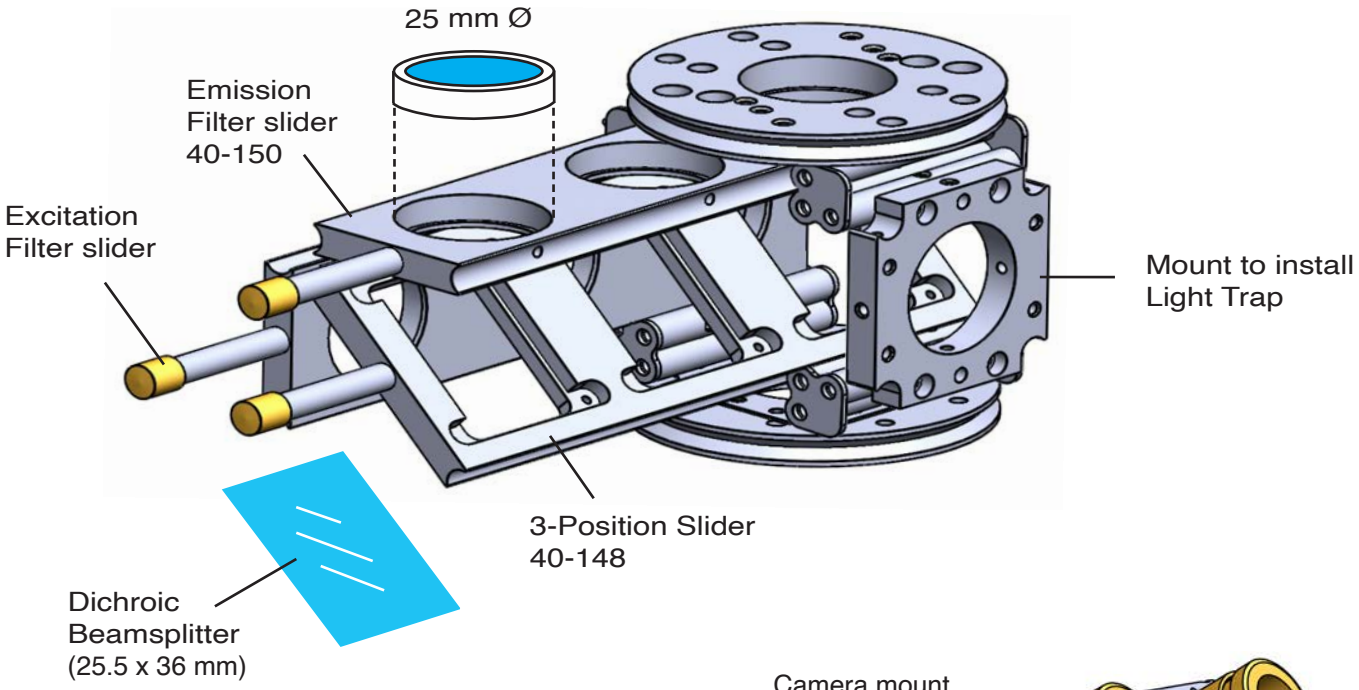
Since we won't be needing the filters arranged for the Fluorescent microscope, we'll replace them with a 45º mirror to divert the beam to the objectives.

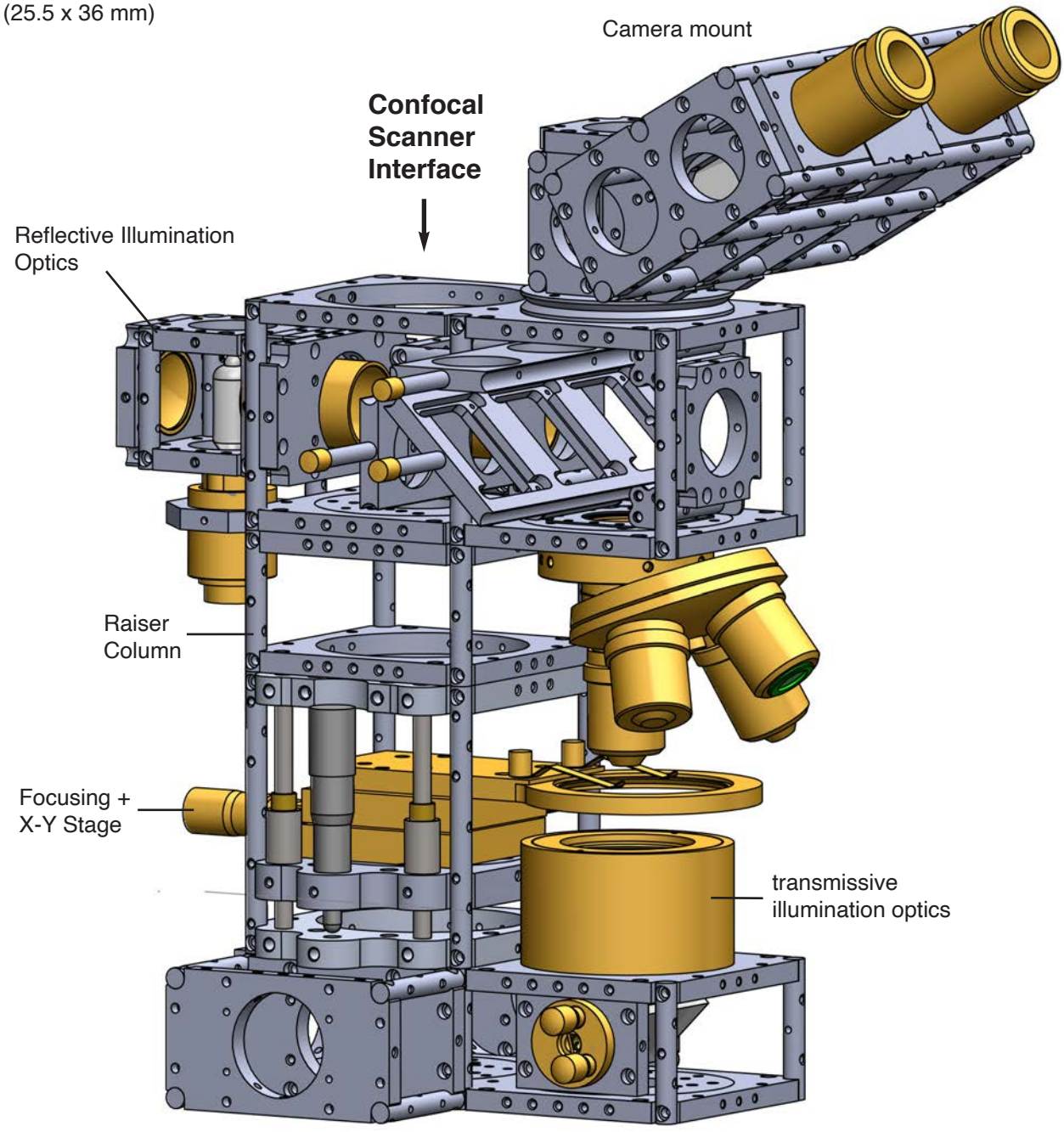


Optoform Version of Confocal Scan Head

Lets now begin designing a Confocal Scan Head with Optoform. At system level, if we were to copy Zeiss LSM 510 Confocal scanner, we'll have the simplified arrangement below: Light originates from a bright LED light source and collimation optics L1, goes through excitation filter set FW1, reflects off of beasplitter wheel BSW, reflects off of X-Y galvos, then through scan lens SL, and enters the light path of microscope through mirror M2, and M3, finally focused by an objective on the sample. The fluorescent light emitted from the sample goes through the objective lens, then reflects off of mirror M3, then M2, and goes through the galvos, passes through dichroic beamsplitter BSW, emission filter set FW2, and to the PMT detector. To start with, the filter wheels could be replaced with a particular filter set, and a dichroic beamsplitter. We don't know yet where to find the elliptical dichroic beamsplitters (Like those found in LSM 150). There is a better approach we could take to avoid Zeiss' spaghetti optical design, and this is how:




25 mm Ø
Emission
Filter slider
40-150
Excitation
Filter slider
Mount to install
Light Trap
3-Position Slider
40-148
Dichroic
Beamsplitter
(25.5 x 36 mm)


Camera mount
Confocal
Scanner
Interface
Reflective Illumination
Optics
Raiser
Column
Focusing +
X-Y Stage
transmissive
illumination optics

The Simplified Layout

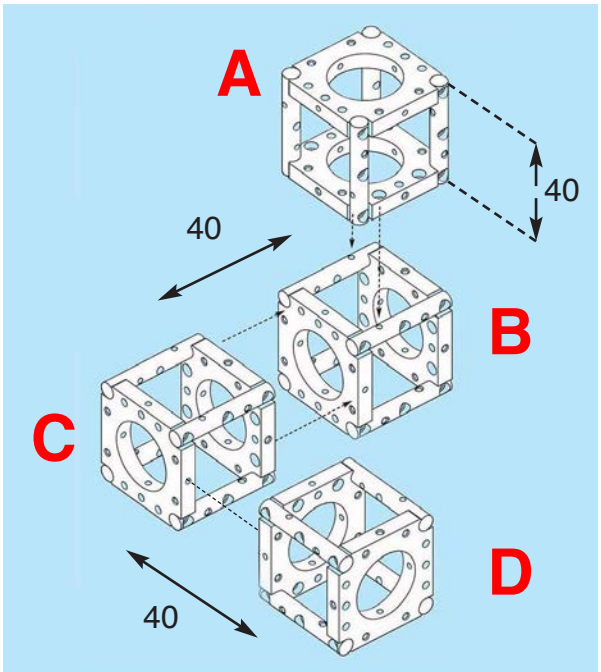
Instead of filter wheels, why not utilize the same assembly we built for fluorescent microscopy? If we decided to motorize the system, we could devise linear actuators instead of a rotary filter wheel drive. So here is a basic design for the system. For PMT housing we could utilize the same module we developed for Strategene and was made available in classical Optoform catalog. It is made of black Derlyn, and has a rectangular lens mount on its front face for mounting cylindrical optics.

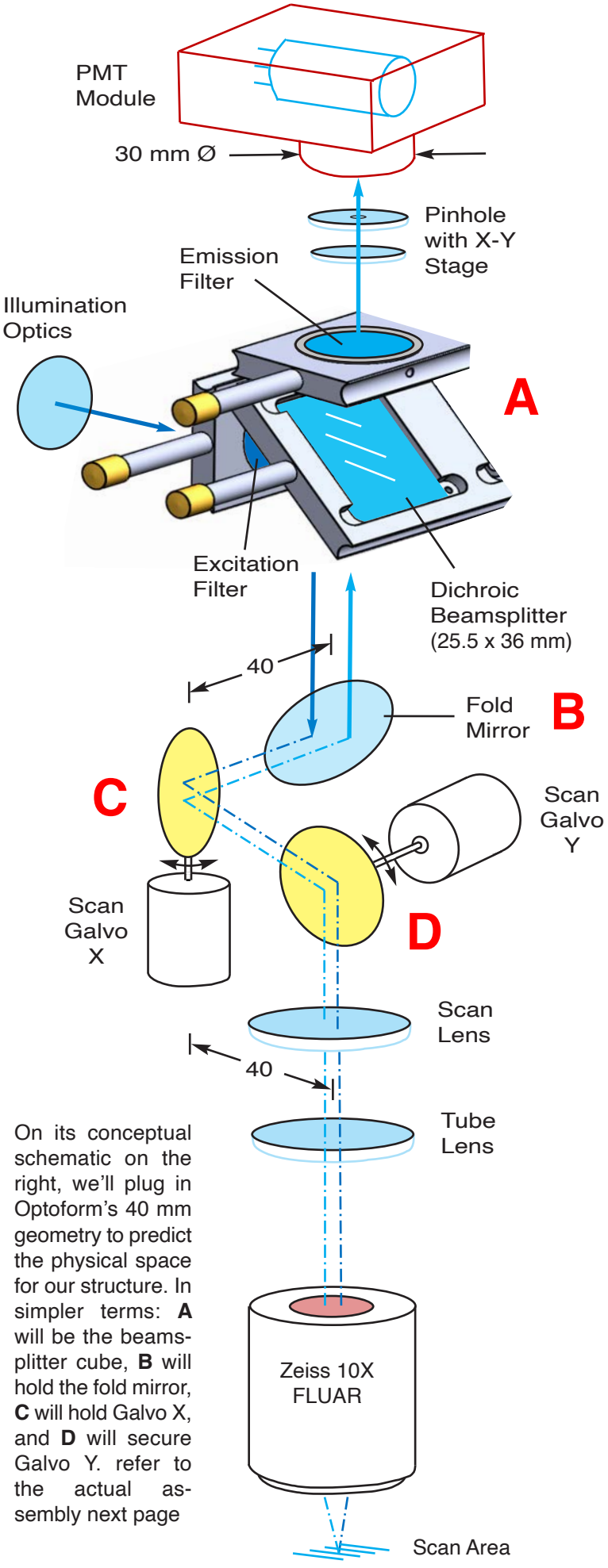
The detection side needs more work because the scan area is supposed to be a pinhole. So we'll need to have an X-Y stage like we saw in Zeiss LSM 510 to align the beam from the spot on the sample onto the PMT. In Zeiss design, there had to be compensation for a shift by the scan head laying on top of microscope. We'll also have the same challenge if this design is intended to be an add on module.

Integrating the Modules

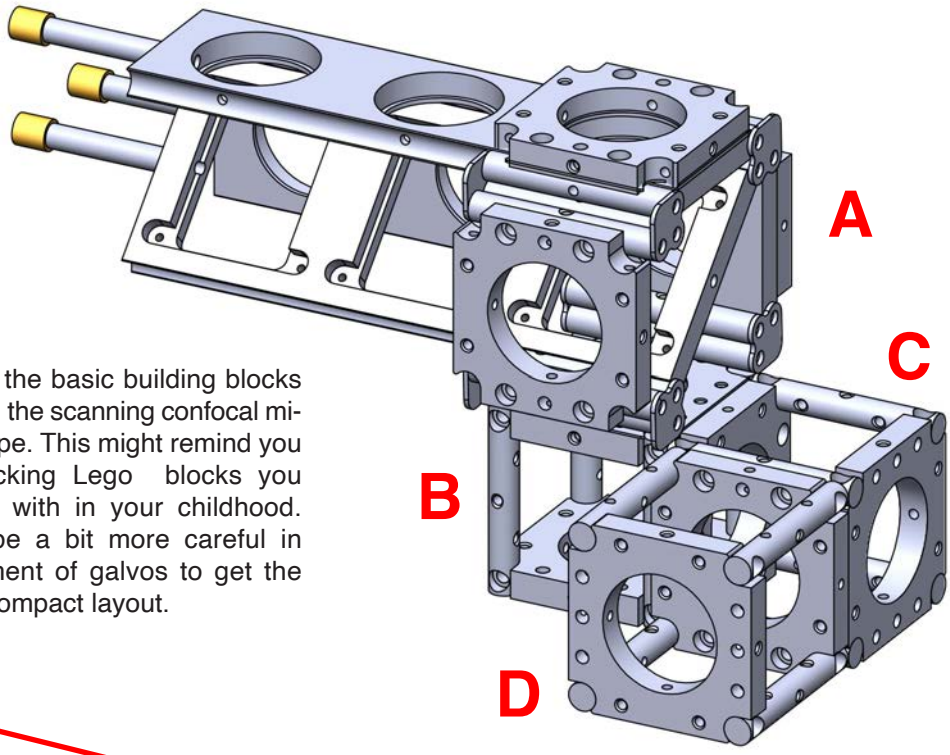
So here it is, put together by connecting cubes A, B, C, and D (right), and after the installation of the necessary hardware: The galvo cubes, the beamsplitters, and filters, fold mirror, PMT tube, the pinhole, and the light source.

Let's now go back to the discussion we had about the chassis. We have the option of fitting this assembly inside a symmetrical housing (with the scan lens centered in the middle of the box, or

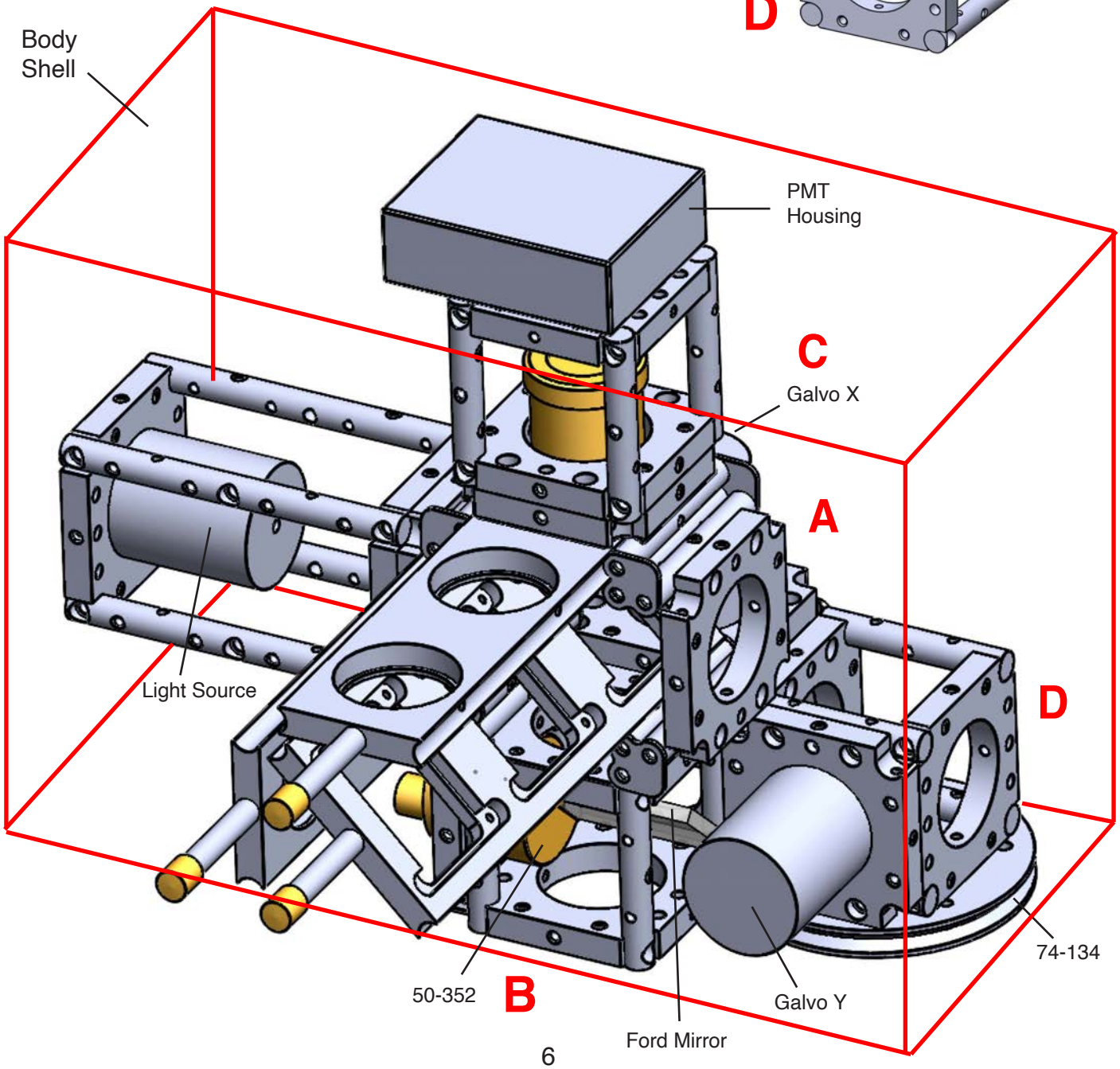


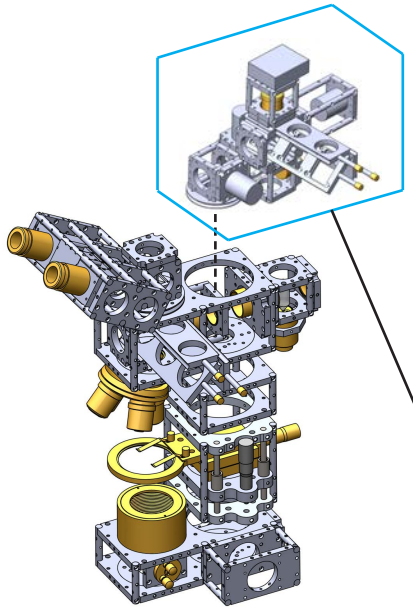


On its conceptual schematic on the right, we'll plug in Optoform's 40 mm geometry to predict the physical space for our structure. In simpler terms: **A** will be the beamsplitter cube, **B** will hold the fold mirror, **C** will hold Galvo X, and **D** will secure Galvo Y. refer to the actual assembly next page



Here's the basic building blocks to build the scanning confocal microscope. This might remind you of stacking Lego blocks you played with in your childhood. We'll be a bit more careful in placement of galvos to get the most compact layout.

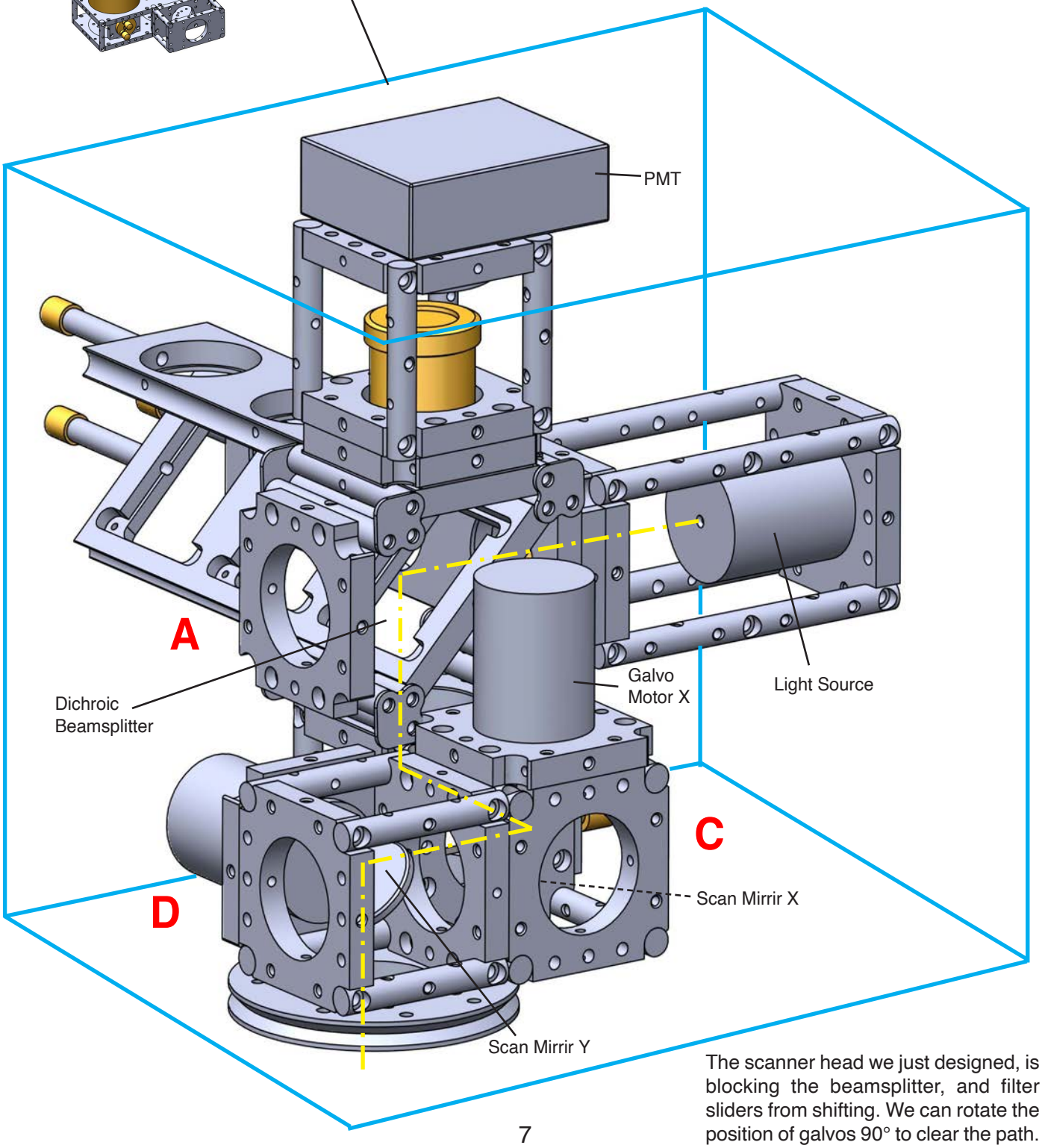




Confcal
Scanner
Module

Designing the Chassis

Designing the housing requires two parallel walls that could be connected by rods, and would also accept standard mounts 40, and 74 to house the inner modules (below). Optoform II is good at this because its support rods are secured on the outer edges of the mounts. So, the two walls could be any shape, and form. Like any other Optoform assembly, the chassis would turn out to be a light weight skeleton structure to gain its structural rigidity from thin Aluminum covering. Being light tight is also crucial in this application because the PMT may work under extremely dim lighting conditions. I hope by now, we have a better understanding of Optoform's design philosophy.



The scanner head we just designed, is blocking the beamsplitter, and filter sliders from shifting. We can rotate the position of galvos 90° to clear the path.