# Customer Needs Statement

UF Integrative Mechanobiology Lab

Imaging Chamber & Laser Path for Zebrafish
Brain Functional Imaging Microscopy

*Updated June 28, 2022*

**Overview**

Functional imaging is an advanced biomedical technique to probe physiological activity in living tissues by visualizing spatial-temporal distribution of chemical-physical tracers within an organism. In humans, for example, functional magnetic resonance imaging (MRI) detects brain injuries otherwise invisible to conventional structural imaging techniques. Our customer, UF's Integrative Mechanobiology Lab, seeks EML4501 mechanical engineers to design a new biomedical product: a high-throughput Selective Plane Illumination Microscopy (SPIM) functional imaging system for in-vivo studies. **A working prototype of this system exists at UF in NEB 148, and our goal is to draw insight from this prototype to develop a commercial Zebrafish SPIM Functional Imager capable of being used reliably and reputably by a lab technician for deployment by companies and research labs.** Ultimate applications include measuring relationships between neuron activity and animal behavior to understand the electrochemical function of new therapies and drugs. Early adopters of this product include microscope companies who will market the SPIM as an add-on to their platforms as well as drug companies and research institutions that conduct therapy and drug discovery research and development.

The animal at the heart of this product is the zebrafish, a common animal model for some human disease. Like humans, zebrafish have two eyes, a mouth, brain, spinal cord, intestine, pancreas, liver, bile ducts, kidney, esophagus, heart, ear, nose, muscle, blood, bone, cartilage, and teeth. Moreover, humans and zebrafish share over 70% of the same genome. Many genes and critical pathways required to produce physiological development and diseases are conserved between humans and zebrafish. Thus, many diseases that causes changes in these body parts in humans can be potentially modeled in zebrafish. [1]

The desired product is a commercial add-on kit that integrates a 488 nm wavelength laser (supplied by the customer) and a zebrafish environmental habitat (part of the kit) into an existing microscope (supplied by the customer). The 488 nm laser enables (1) functional calcium imaging of the whole live zebrafish brain and (2) high-resolution patterned optogenetic stimulation of action potential in targeted neurons. The 488 nm laser path must coordinate with a 639 nm laser path that enables parallel zebrafish brain voltage imaging. In addition, the 488 nm laser must be electromechanically switchable between the calcium imaging illumination path and the optogenetics stimulation path, which involve filtering, shaping, and splitting the beam through different optical elements (supplied by the customer).

While being imaged, the zebrafish resides in an environmental habitat (i.e., imaging chamber + fish holder), which is another essential part of the product that maintains appropriate liquid water

volume, temperature, pressure, and chemical balance to ensure zebrafish survival and comfort during the experiment. The imaging chamber and holder must provide optical access to the zebrafish's brain and eyes.

The mechanical layout of the laser path (including optical element alignment) will be performed by EML4501 engineers. The design of the zebrafish environmental habitat / imaging chamber + fish holder platform will also be performed by EML4501 engineers. The design must integrate the 488 nm laser path controller and the zebrafish imaging chamber + holder into the customer's SPIM system. Optical design of the 488 nm laser path elements (e.g., specifying optical components) is outside the scope of this product development process.

**Customer Requirements**

EML4501 engineers will design a product kit with the following four elements and capabilities:

A) An optically accessible zebrafish environmental habitat (i.e., imaging chamber + fish holder),
B) The mounting and support system for a series of precisely placed customer-specified optical elements,
C) A mirror mounted on a magnetic base that changes the 488nm laser's path, enabling it to switch between two unique functions without interrupting the path of an existing 639 nm laser, and
D) An overall system configuration accessible to all human users (5% female to 95% male) that can be set up and operated by a lab technician with no additional oversight.

*For the 488 nm wavelength laser path and controller:*

1. **Resolution Preservation:** The 488 nm laser path must not interfere with the existing 639 nm laser illumination path and imaging path.
2. **Laser Optogenetics Stimulation Path:** The 488 nm laser must be directed along an optogenetics stimulation path that uses customer-specified optical elements to form high-resolution light patterns onto the zebrafish sample.
3. **Light Sheet for Calcium Imaging:** The 488 nm laser beam should be guided by a switchable mirror into the 639 nm light sheet illumination path to be compressed after size adjustment and angle alignment. The final light sheet requires the following nominal specifications: 46 µm Rayleigh length, 6.1 µm thickness at focus, and 708 µm width (all values as $1/e^2$).
4. **Patterned Stimulation:** In the other path, the 488 nm laser beam should be resized and guided to a digital mirror device (DMD) to form high-resolution (1920 x 1080-pixel aspect ratio or greater), computer-controllable light patterns onto the sample.
5. **Alignment:** The optical elements must be well aligned along the laser optic axis to avoid any aberration and distortion of the laser beam.
6. **Sample Targeted:** The 488 nm laser must be finally guided to the sample (5–7-day old zebrafish or other living matter).

*For the imaging chamber + fish holder:*

7. **Size:** Chamber: 76.2mm (L) × 76.2mm (W) × 38.1mm (H).
8. **Transparency:** The imaging chamber and zebrafish holder platform must be optically transparent for visual stimulation, quantitative functional measurement, and behavior recording of the zebrafish.

9. **Laser Accessible:** The chamber should be laterally accessible by both the 639 nm and 488 nm laser light sheets. A laser window (much thinner than other parts) is needed on the surface of the chamber facing the light sheet laser beam.
10. **Geometry:** The chamber should be a 5-sided rectangular hollow monolith without a top to enable optogenetics stimulation and imaging via a microscope objective above the sample. The bottom surface of the chamber should have at least four 8-32 tapped holes at the corners for mounting purpose.
11. **Zebrafish attachment:** The zebrafish should be mounted in clear agarose gel, which will be attached to the fish holder platform. The platform material selection and manufacturing method must preserve compatibility between the agarose gel, the zebrafish, and the holder platform.
12. **Zebrafish holder platform:** dimensions must adhere to the enclosed reference drawing and be mounted onto a vertical translator (Thorlabs LT1). Dimensions for fish-holding surface are : 38.1mm (L) × 12.7mm (W).
13. **Sterilizable:** With common laboratory methods and UF animal compliance policy.
14. **Bio-Compatible:** The material of the chamber and holder should not cause any harm to the zebrafish or other living matter placed therein.
15. **Environmental Regulation:** The chamber is watertight and maintains appropriate liquid water level, pressure, and chemical balance to ensure zebrafish comfort during the experiment.

*For both:*

16. **Mounting:** The laser, optical elements, and the imaging chamber must be mounted on a standard optical table where the SPIM system is constructed in New Engineering Building, Room 148.
17. **Accessibility:** The overall system configuration is accessible to all human users (5% female to 95% male) that can be set up and operated by a lab technician with no additional oversight.
18. **Biosafety level:** The items will be operating in a biosafety clean-room environment (BSL-1).
19. **Prototype Cost:** the combined costs for functional prototypes of the laser path configuration and imaging chamber cannot exceed $300, which includes a) materials, b) energy, c) fabrication labor, and d) assembly labor.
20. **Production Product Cost:** the customer expects to sell 100 units per year as an add-on microscope feature. This product's market price point given a production run of 100 units cannot exceed $200, which includes a) materials, b) energy, c) fabrication labor, d) assembly labor, and e) profit. Design to maximize profit.

## Customer Questions & Answers

*For Customer Need 1. Crosstalk Free: The 488 nm laser path must not interfere with the existing 639 nm laser illumination path and imaging path.*

Do the laser beams need to physically intersect to create crosstalk, or is it enough that they just be in close proximity? How close is too close? How is crosstalk measured? If beams are "too close" and yet the CMOS sensor at the end of the path registers no cross talk, does that geometry meet the requirements?

Basically, the 488 nm laser will be switching between 2 paths: (1) light-sheet illumination path and (2) optogenetic stimulation path. In the first path, the 488 laser will be guided to the existing

639 nm light-sheet path, and spatially "overlap" with the 639 path. However, when doing the imaging, we will let the 488 and 639 lasers on alternately so that on the CMOS sensor, they will not be overlapped or cause crosstalk. Here in the Customer Need 1, I do want to emphasize that when guiding the 488 nm laser to align the light sheet, all the optic tools (lens, mirrors, etc.) in the original 639 path should not be interfered or disturbed.

In the second path, the 488 stimulation path will also partially overlap with the imaging path. In that configuration, the 488 laser will not be used for imaging, but the same, we should avoid any disturbance to the items on the existing imaging path when doing the alignment.

*For Customer Need 3. Laser Calcium Imaging Path: the 488 nm laser must be directed along a calcium imaging illumination path that uses customer-specified optical elements to compresses the laser beam into a light sheet.*

Can you please provide a list of all the components that are customer specified and an example of how they might be laid out to produce a desired laser sheet?

I would not worry too much about the components here. All the optic tools in the 488 Calcium imaging path will be provided and already aligned to generate the light sheet path. The beam sizes of the 488 and 639 lasers are very close, so no need to do the realignment. For the 488 laser, students will need to (1) guide the laser to the existing light sheet path and adjust the incident angle and position to finally generate a light sheet with correct size and position, and (2) set up the optogenetic stimulation path. The first purpose is relatively easy to achieve.

An example of the components, as well as the core of the light sheet path, is a cylindrical lens we used to compress a gaussian laser beam in one direction to generate the light sheet. Other components, like pairs of spherical lens, are used to adjust the laser beam size and therefore the geometric size of the final light sheet.

*For Customer Need 5. Switchable: The 488 nm laser must be easily user-switchable between the calcium imaging illumination path and the optogenetics stimulation path.*

Are there any ways of switching the laser path that are not permissible? For example, can we physically move the laser? Can we put a mirror or beam splitter in front of it? Can we use a prism or lens to deflect it? Can we heat the air around the beam to change refractive index to bend light like a mirage?

Physically moving the laser is not desired since that will mess up the already aligned optic path. All the other suggestions are great and interesting. The easiest way should be using a mirror mounted on a magnetic base plate that can switch its position (in the optic path or not) to switch the direction of laser beam. We will provide those tools as well.

*For Customer Need 6. Light Sheet: The 488 nm laser beam should be guided into the 639 nm light sheet illumination path to be compressed after size adjustment. The final light sheet requires the following nominal specifications: 46 µm Rayleigh length, 6.1 µm thickness at focus, and 708 µm width (all values as 1/e2).*

Does a laser sheet of rectangular cross section 6.1 μm thick by 708 μm wide pass through the zebrafish? If not, where in the system must the dimensions in Customer Need 6 be achieved?

Exactly yes!

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1 E. Burke, "Why Use Zebrafish to Study Human Diseases?" I Am Intramural Blog, *Intramural Research Program, U.S. National Institutes of Health*, August 9, 2016. URL: https://irp.nih.gov/blog/post/2016/08/why-use-zebrafish-to-study-human-diseases