

Boyden Lab Meeting

Quilee Simeon
12/3/2025

Progress report on rebuilding remote-scanning light-sheet microscope for fast whole-brain zebrafish imaging

Acknowledgments

Corban Swain



Zeguan Wang



Shahar Bracha



Ed Boyden



Fira Zainal (ordering)

& all the Admins

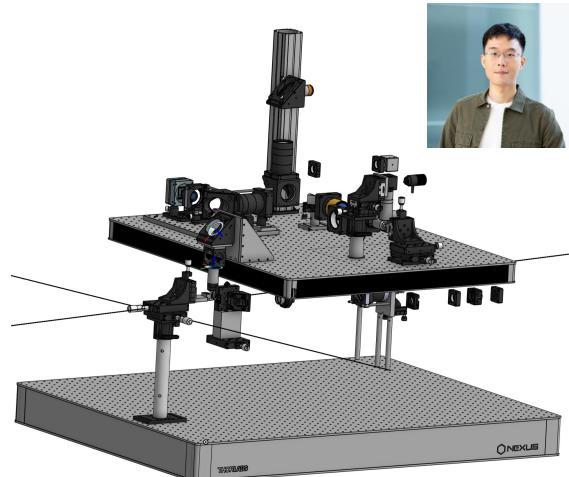
The K. Lisa Yang ICoN Graduate
Student Fellowship '25-'26



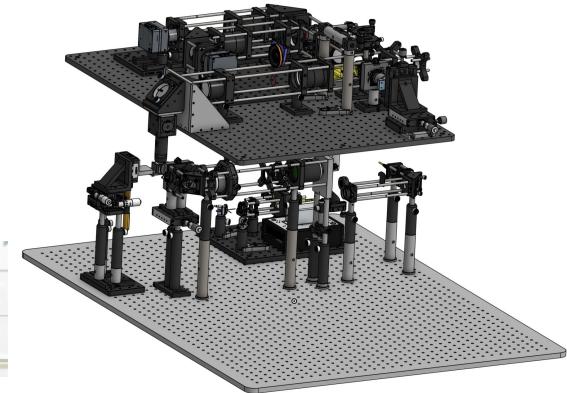
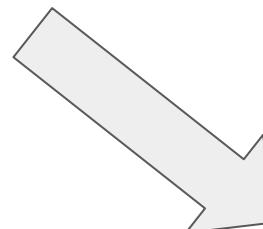
This project involves me learning to re-build a custom **remote-scanning light-sheet microscope** optimized for **voltage imaging** of neurons distributed across the entire larval zebrafish brain.

Original scope design and implementation is from:

Wang, Z. et al. *Imaging the voltage of neurons distributed across entire brains of larval zebrafish*. bioRxiv 2023.



original CAD (draft layout)



redesigned CAD (comprehensive)

Background

Light-sheet imaging of a whole brain

Understanding the brain requires taking precise measurements of neural activity at high spatial and temporal resolution.

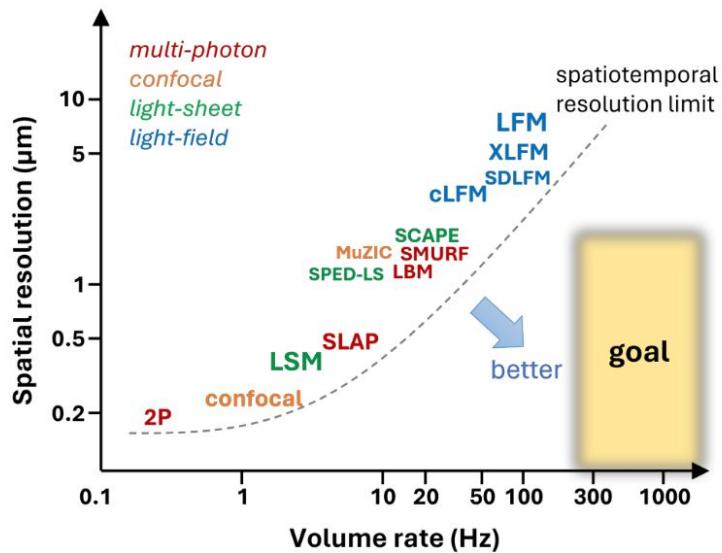


Illustration of the volume rate and spatial resolution of different light microscopy techniques.

The “goal” region indicates a rough estimate of the spatiotemporal resolution required for whole-brain voltage imaging. Techniques are classified into different categories marked in different colors.

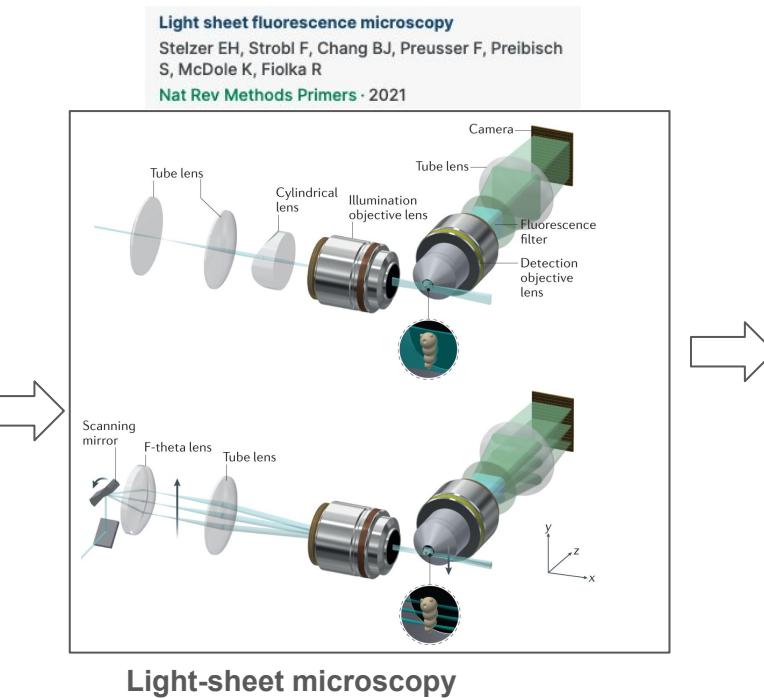
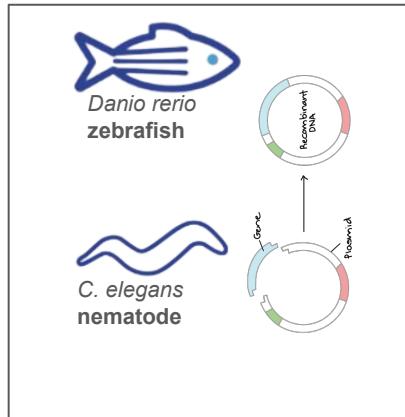
Sample of representative light microscopy techniques are:

- **2P**: two-photon microscopy;
- **confocal**: spinning disk confocal microscopy;
- **LSM**: conventional light-sheet microscopy;
- **SCAPE**: swept, confocally aligned planar excitation;
- **LFM**: light-field microscopy

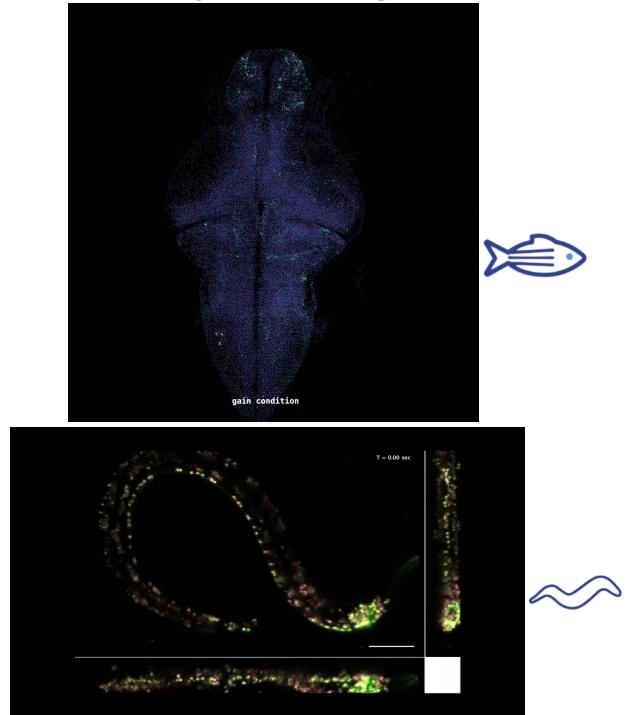
...

This is difficult to do in humans so in neuroscience we use small transparent animal models that allow us to apply cutting edge tools from many engineering disciplines (bio, optical, mechanical, electrical, software, etc.) to achieve this goal.

Transgenic model organisms

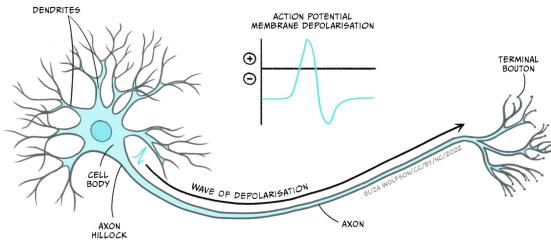


Data processing, analysis/modeling



Neurophysiology 101

Your brain is made up of millions of neurons (among other cells). **Neurons** are electrical conductors. They communicate by propagating electrical impulses called action potentials or spikes. Think of a spike as an all-or-none event that happens when the **voltage** of a neuron crosses a certain threshold.



Zebrafish TLDR

One model organism we use in neuroscience is **larval zebrafish**. At 5-6 days post-fertilization they ~5mm long and transparent.

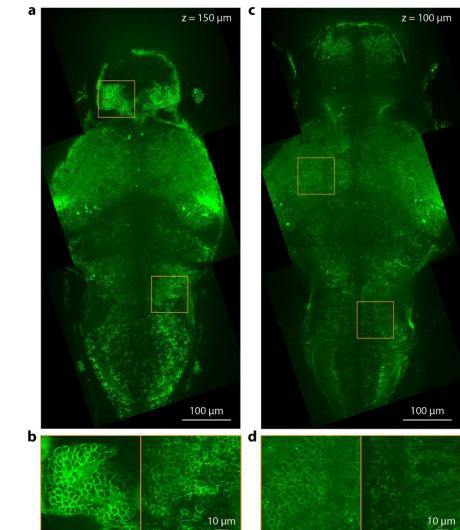
We can also bioengineer transgenic lines that express fluorescent **voltage indicators** in the brain! This allows to image the whole-brain (~100,000 neurons) using **light microscopy**.



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Imagining the Voltage of Neurons Distributed Across Entire Brains of Larval Zebrafish

Author(s):
Wang, Zeguan



Zeguan's original system

The screenshot shows a dark-themed digital repository interface. At the top left is the MIT Libraries logo and the text "DSpace@MIT". Below the header, a navigation bar includes links to "DSpace@MIT Home", "MIT Libraries", "MIT Theses", "Doctoral Theses", and "View Item". The main content area displays a thesis titled "Imagining the Voltage of Neurons Distributed Across Entire Brains of Larval Zebrafish" by Author(s) Wang, Zeguan.

DSpace@MIT Home » MIT Libraries » MIT Theses » Doctoral Theses » View Item

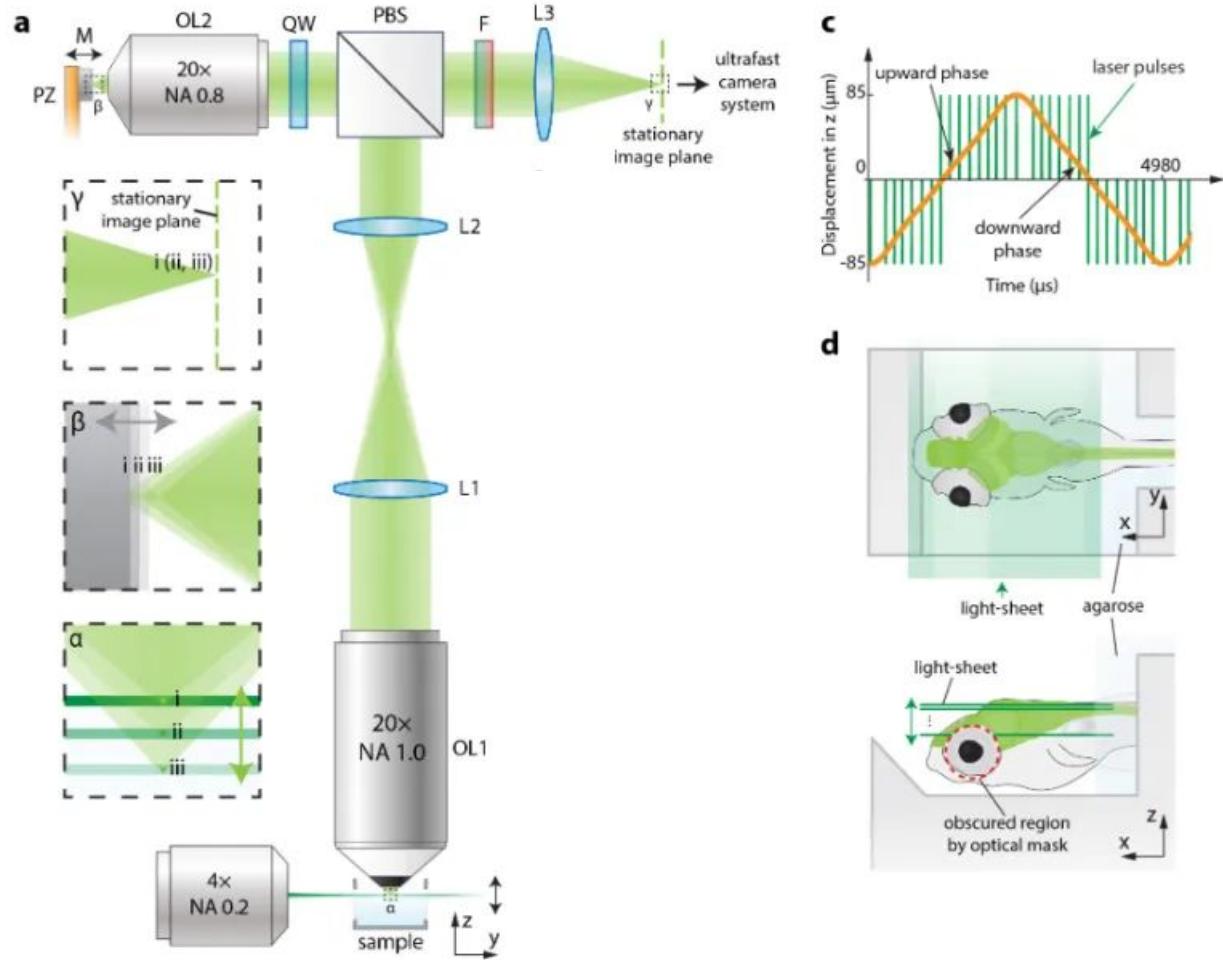
Imagining the Voltage of Neurons Distributed Across Entire Brains of Larval Zebrafish

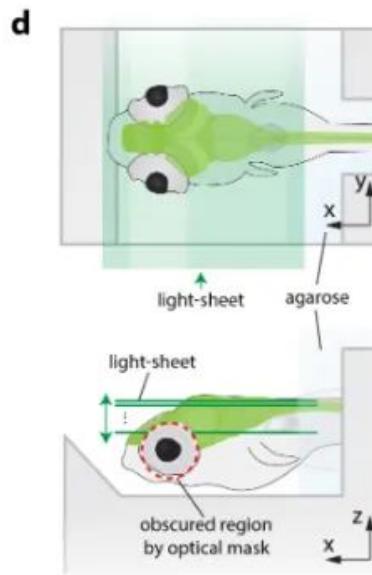
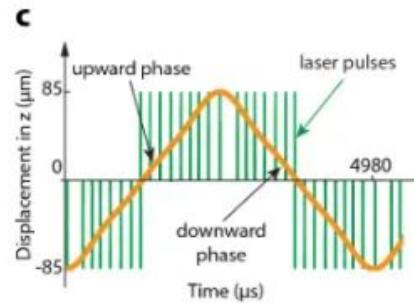
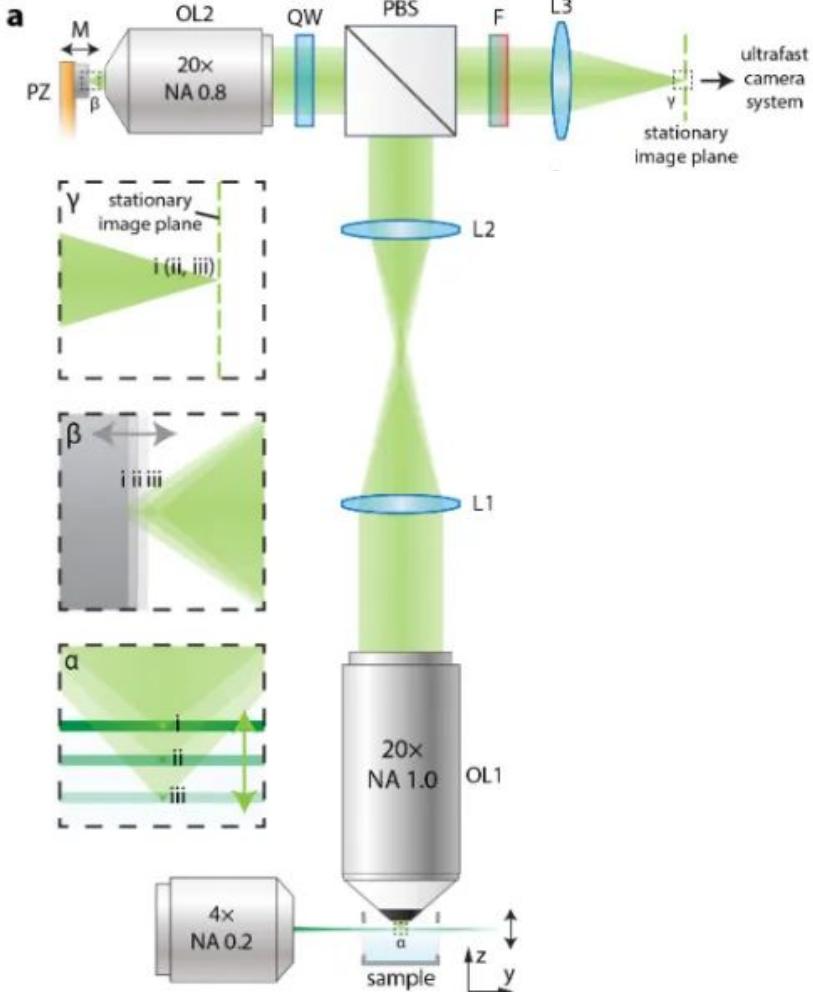
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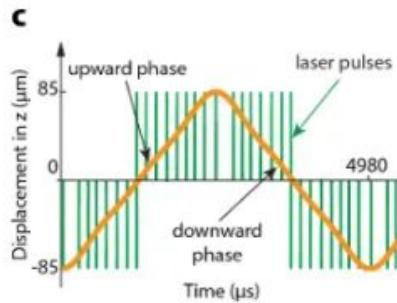
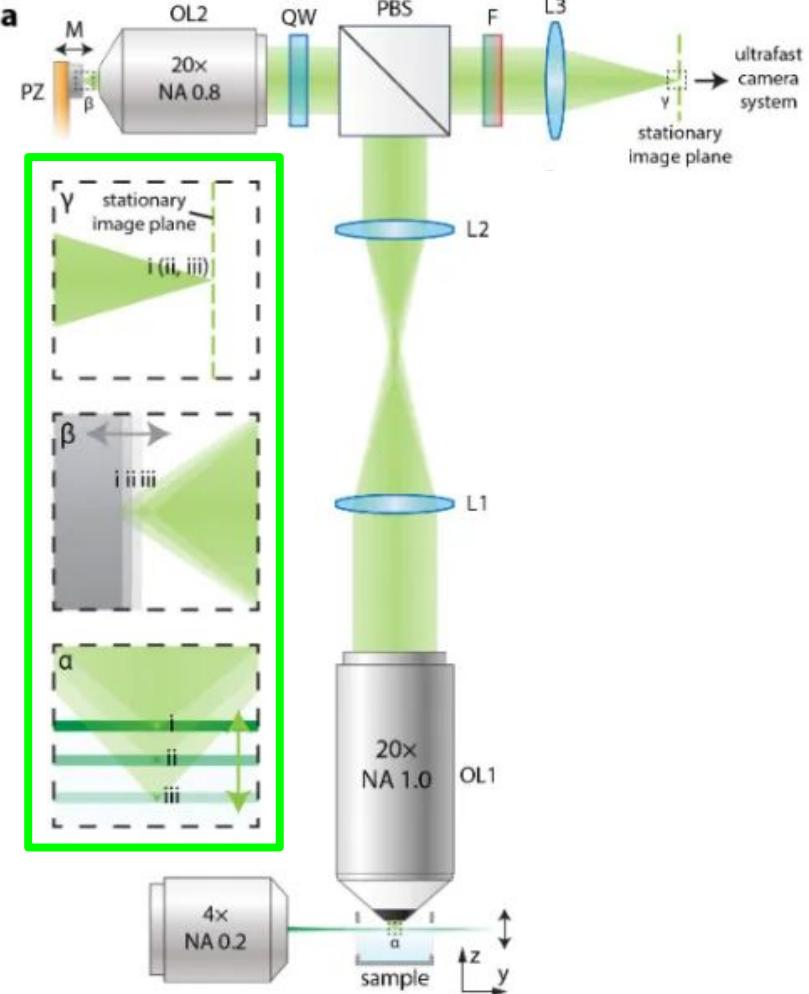
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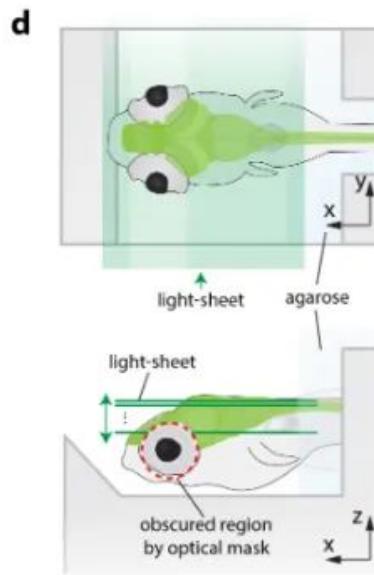


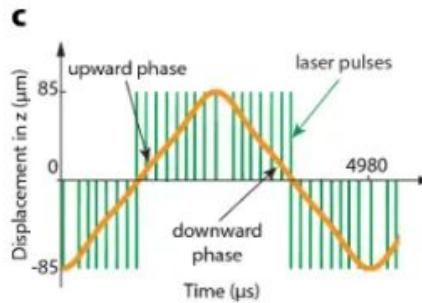
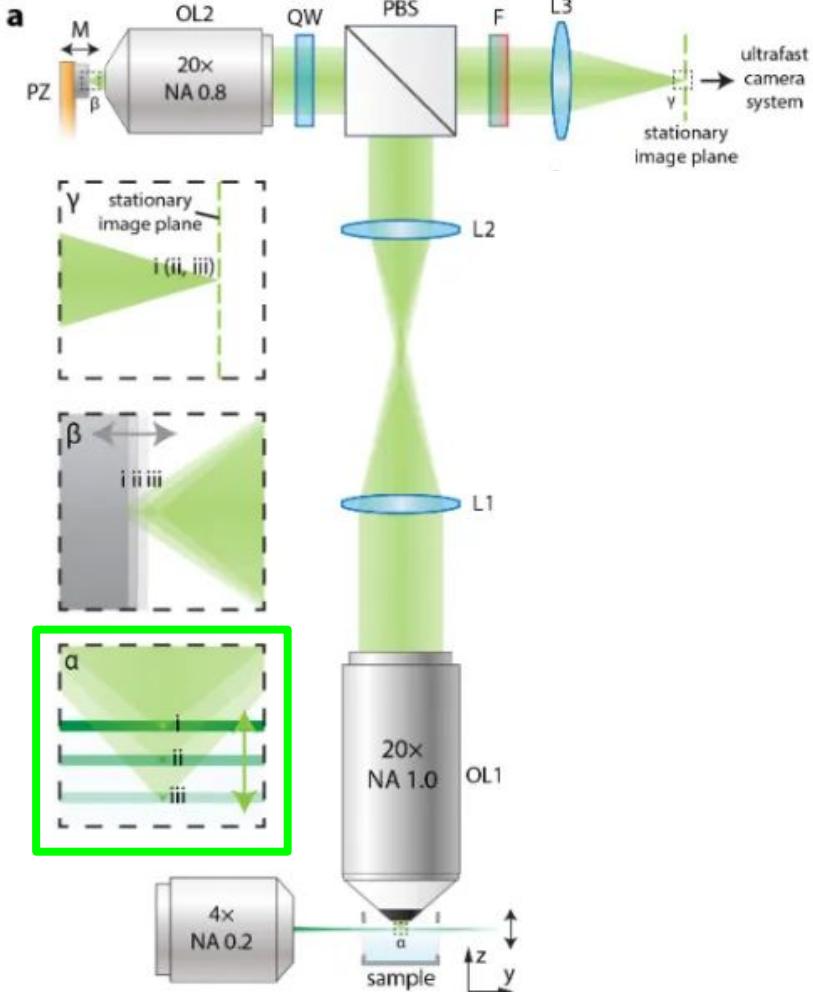
Remote-scanning light-sheet microscopy optimized for voltage imaging of neurons distributed across the entire larval zebrafish brain.



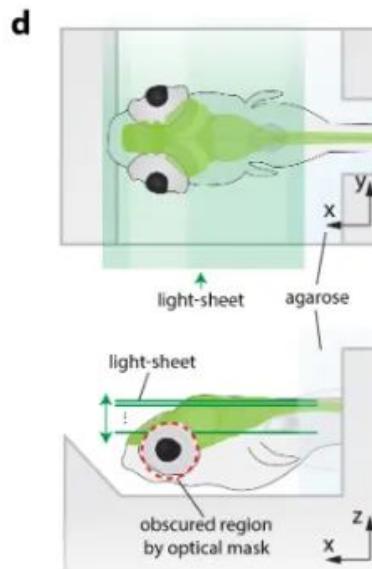
Remote-scanning light-sheet microscopy optimized for voltage imaging of neurons distributed across the entire larval zebrafish brain.

Small views at the sample (dashed box α), the remote mirror (dashed box β), and the focused images at the focal plane of tube lens L3 (dashed box γ) are enlarged and shown.

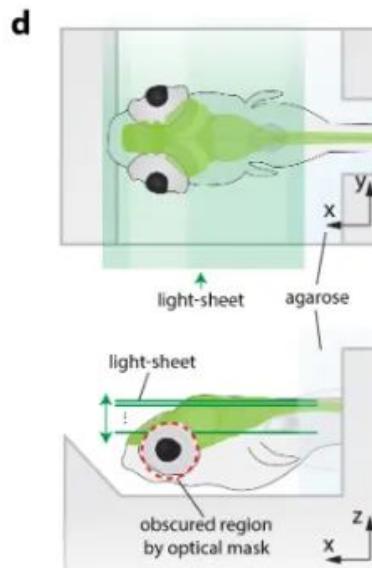
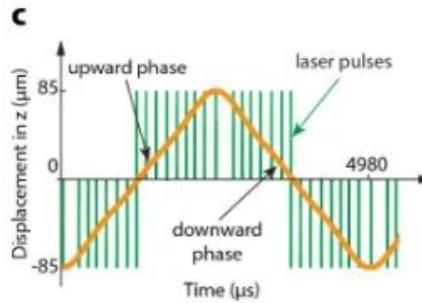
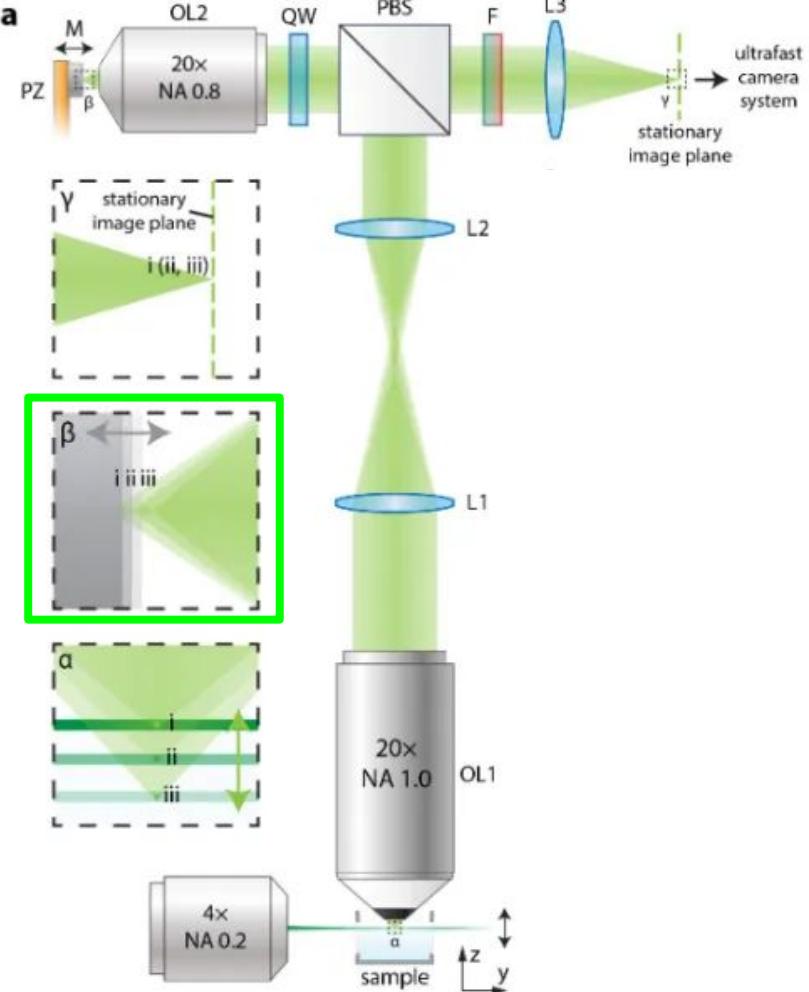




Remote-scanning light-sheet microscopy optimized for voltage imaging of neurons distributed across the entire larval zebrafish brain.



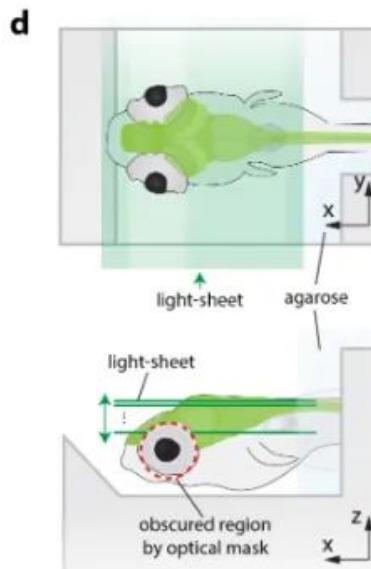
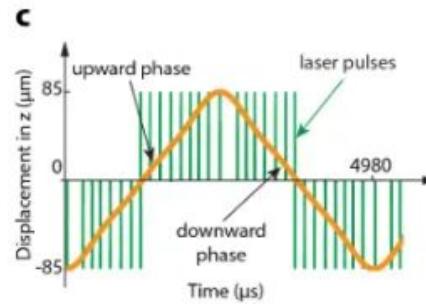
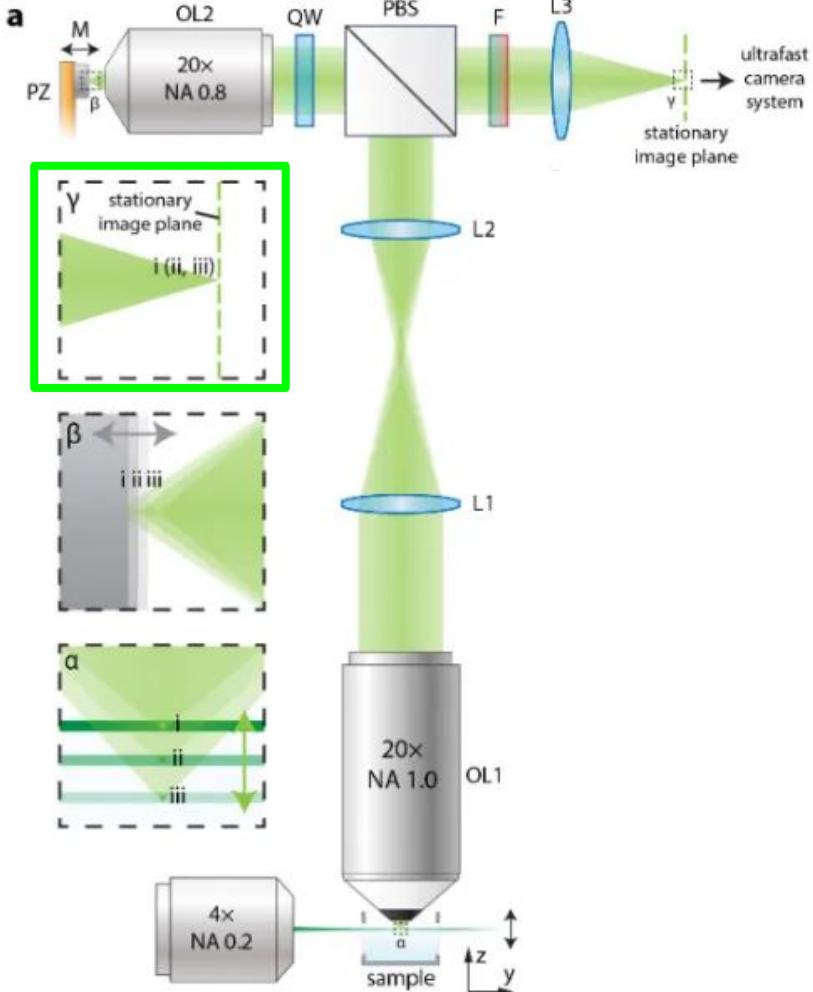
Dashed box α shows that, at the three time points (i, ii, iii), the light sheet excites an infinitely small fluorescent bead (green dots) at three different z locations, and the emitted fluorescence is collected by OL1.



Remote-scanning light-sheet microscopy optimized for voltage imaging of neurons distributed across the entire larval zebrafish brain.

Dashed box β shows that, at different time points (i, ii, iii), the remote mirror translates and reflects the fluorescence light (green cones) back to OL2. The mirror's translational motion is synchronized with the light sheet scanning.

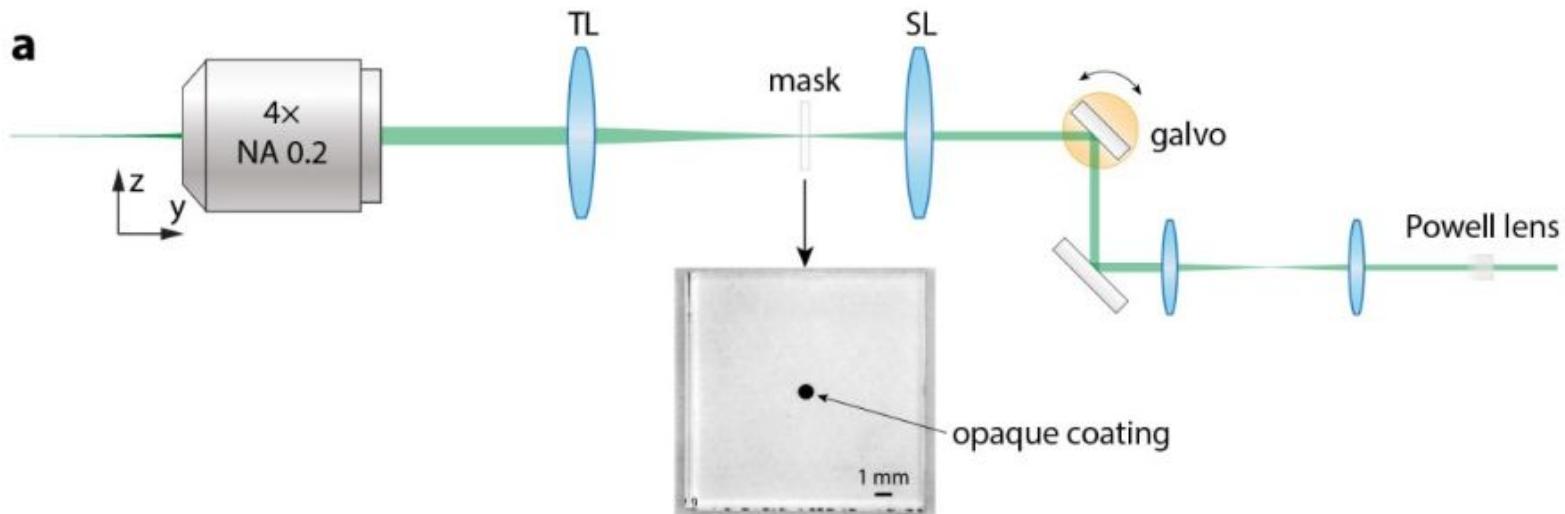
The mirror's translational motion is synchronized with



Remote-scanning light-sheet microscopy optimized for voltage imaging of neurons distributed across the entire larval zebrafish brain.

different time points (i, ii, iii) is focused on the same image plane (dashed green line).

Illumination Path

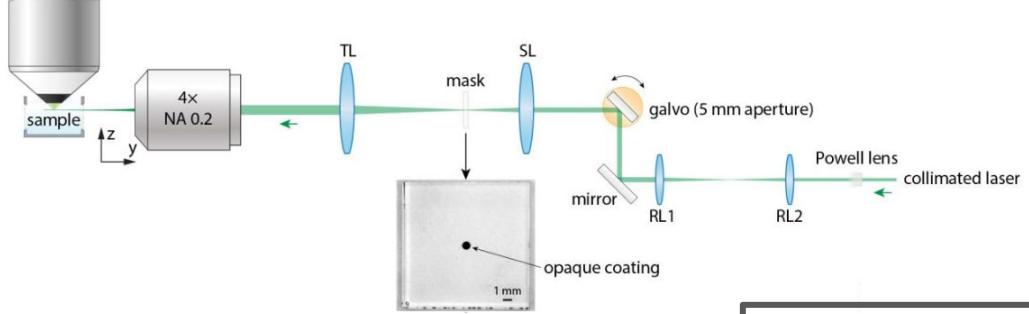


The light path for light-sheet illumination and measurement of the light sheet profile.

(a) Schematic diagram of the light-sheet illumination arm. Collimated laser (from the right side) first passes through a Powell lens, a 4F system, then reflects on a galvo mirror scanner, passes through a scan lens (SL), a tube lens (TL), and finally illuminates on the sample from a 4 \times objective lens. An optical mask is placed at the sample's image plane to prevent direct light entering the fish's eye.

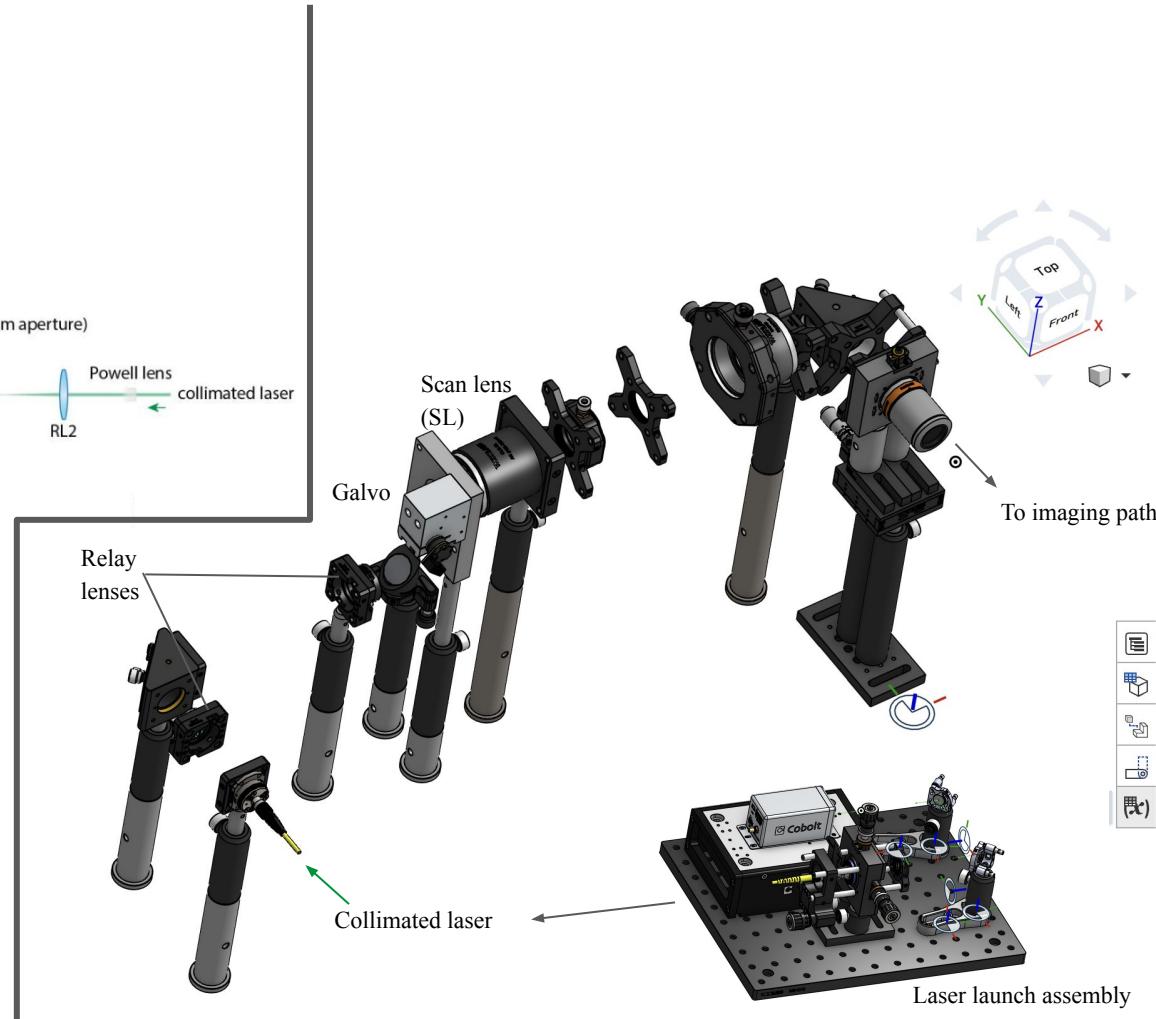
Illumination Path

a



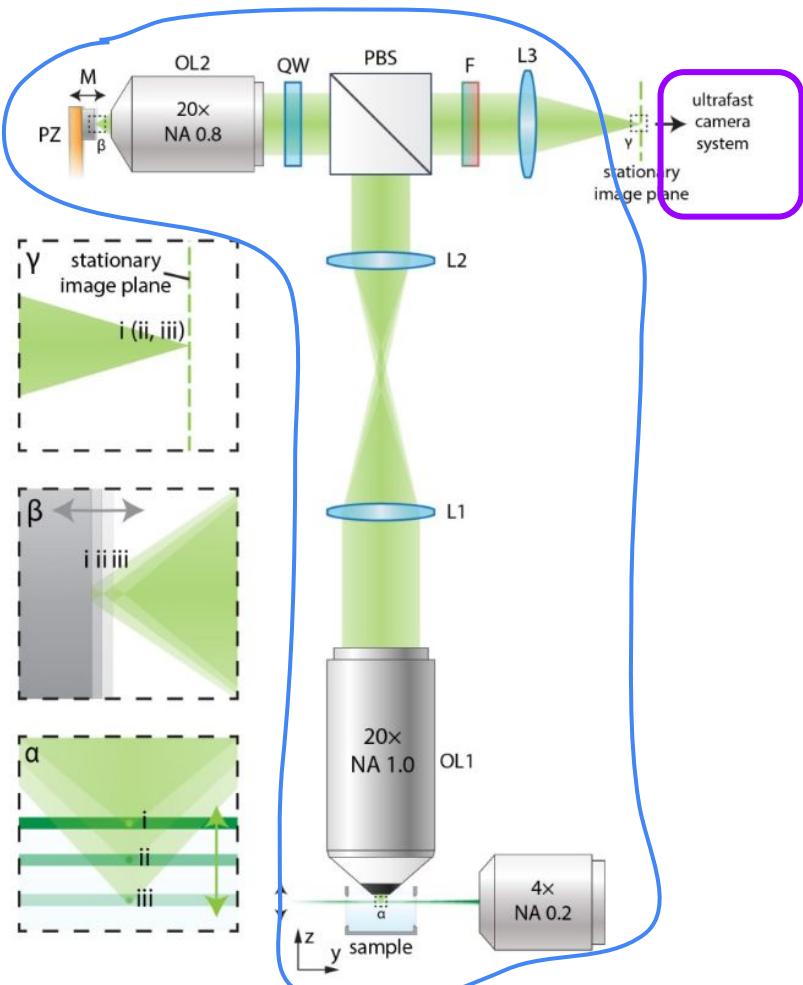
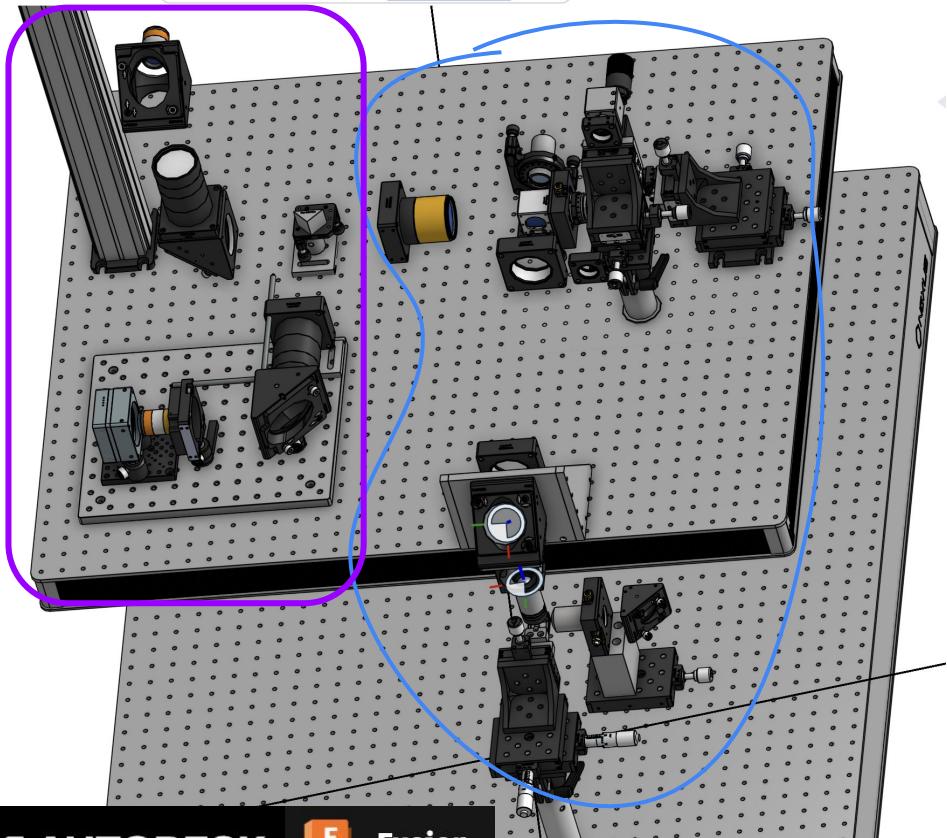
Schematic diagram of the excitation light path.

(a) Excitation light path without light-sheet pivoting capability. A collimated laser illuminated from the right side, first passing through a Powell lens and then a pair of relay lenses (RL1, RL2). After being deflected by the galvo scanner, the excitation light passed through a scan lens (SL) to form an enlarged light sheet near its focal plane. This enlarged light sheet finally passed through a $0.25 \times$ microscope composed of a tube lens (TL) and a 4 \times objective lens, to illuminate the sample. We placed an optical mask at the scan lens's focal plane to prevent the excitation light from directly entering the fish's eye.



Imaging Path

original CAD (draft layout)



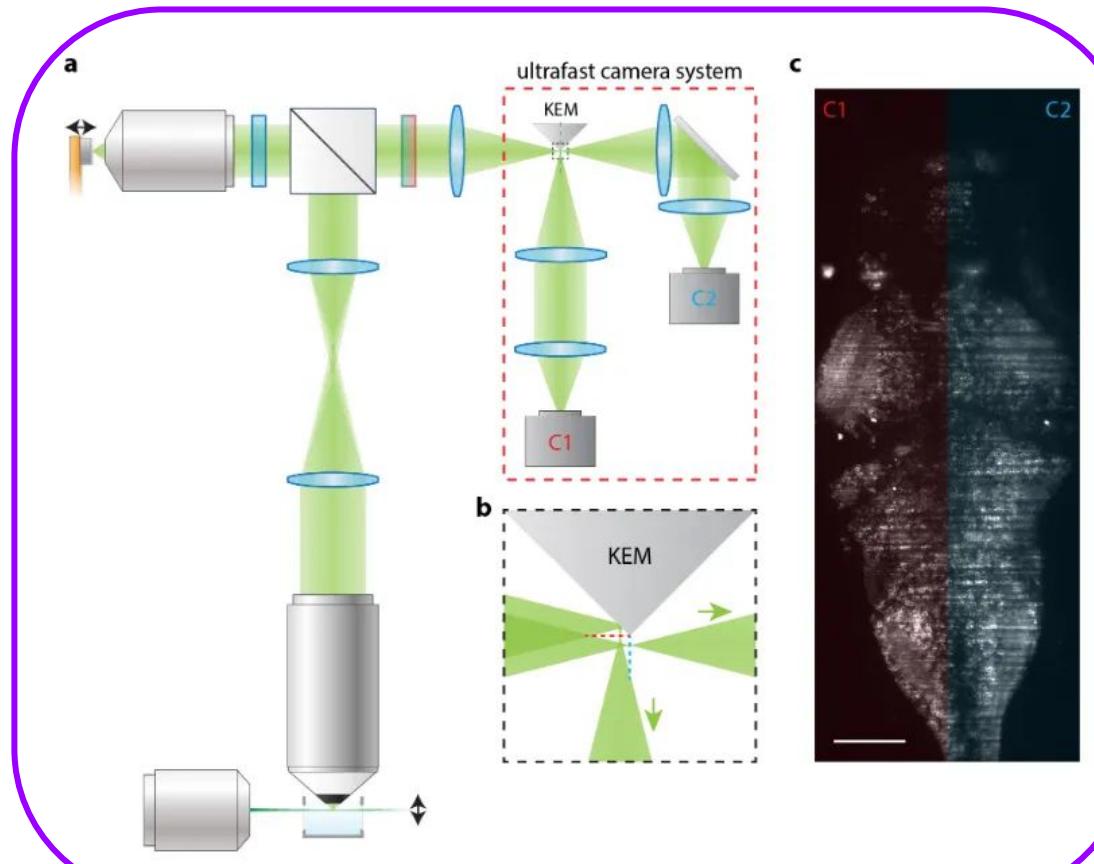
Imaging Path

Overview of the ultrafast dual camera system.

(a) Images from the high-speed light-sheet microscope are recorded by an ultrafast camera system (red dashed line box) consisting of an image splitter and two ultrafast cameras. Focused images from the microscope are divided by a knife-edge mirror (KEM) into two halves. These split images are then relayed through two identical lens pairs to the ultrafast cameras (C1 and C2) for recording.

(b) Enlarged view of the areas in the black dashed box in (a). The KEM splits an image by deflecting only its upper half to a different light path.

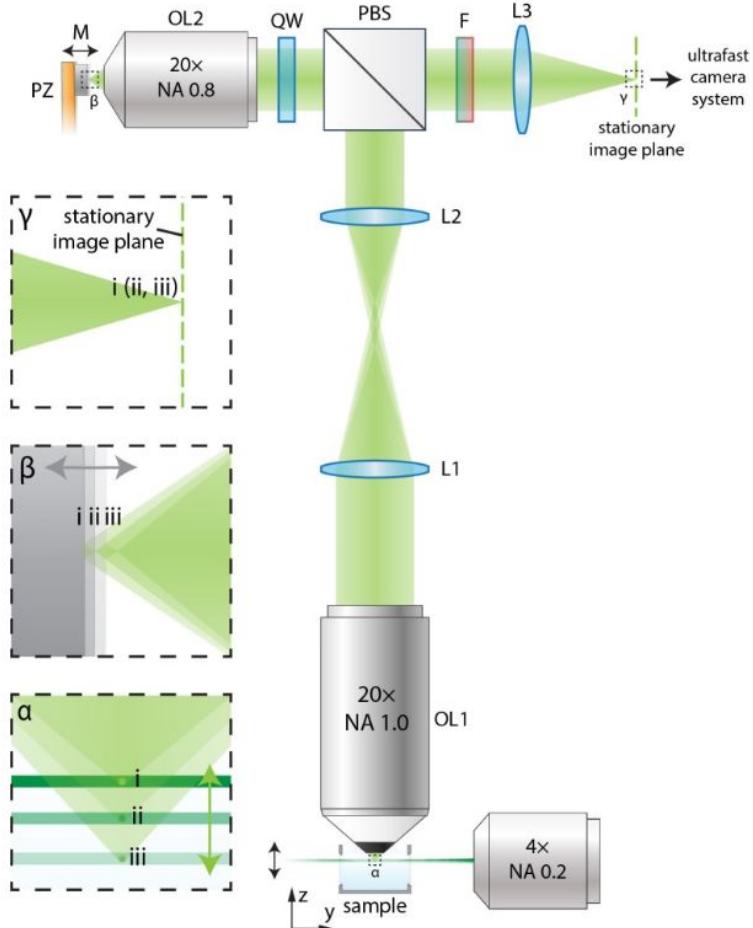
(c) Stitching raw images from cameras C1 and C2 produces a full section image of the zebrafish brain.



Imaging Path

Overview and operational principles of the whole-brain, voltage imaging-optimized, remote scanning light-sheet microscope design.

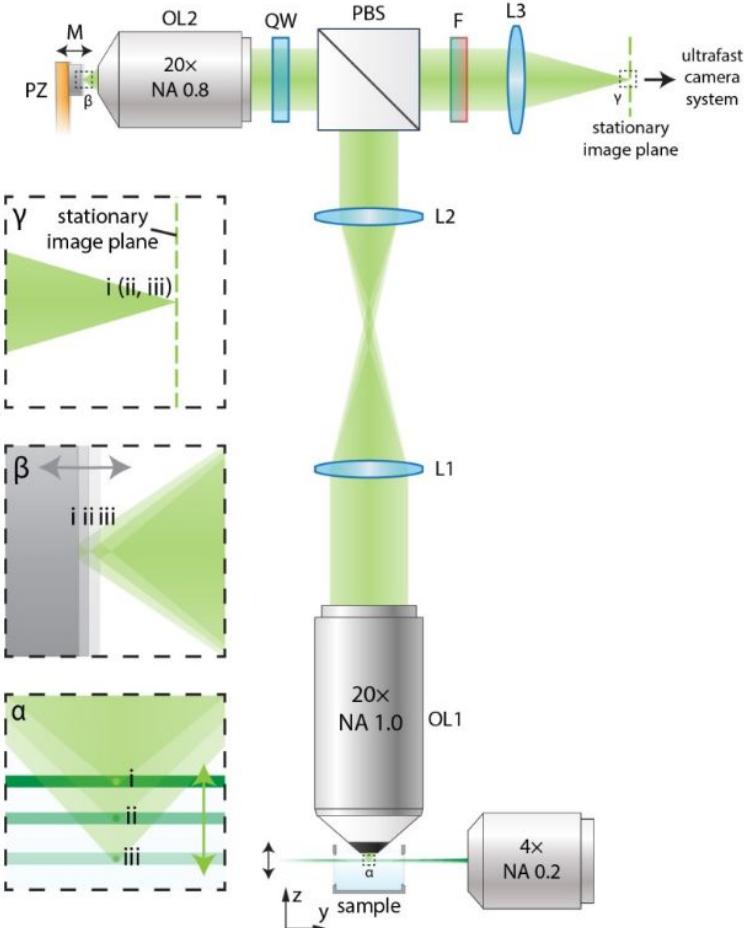
- At the bottom, a sample in a water chamber is illuminated from one side by a rapidly scanning light sheet from an excitation objective lens.



Imaging Path

Overview and operational principles of the whole-brain, voltage imaging-optimized, remote scanning light-sheet microscope design.

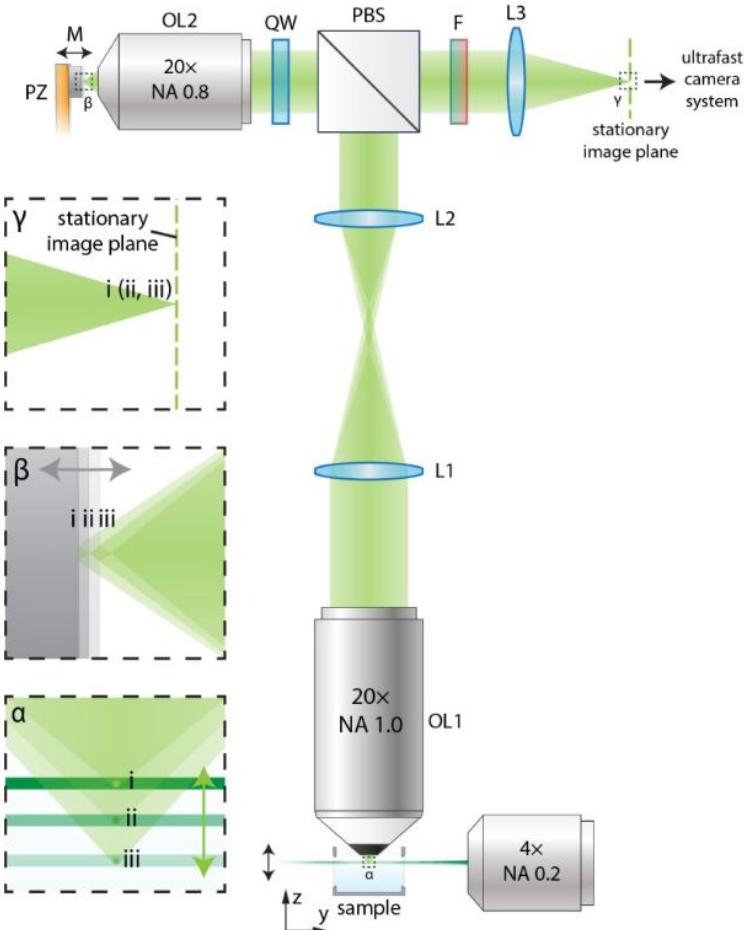
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- The fluorescence excited by the light sheet is collected orthogonally through a 20 \times high numerical aperture water-immersion objective lens (OL1).



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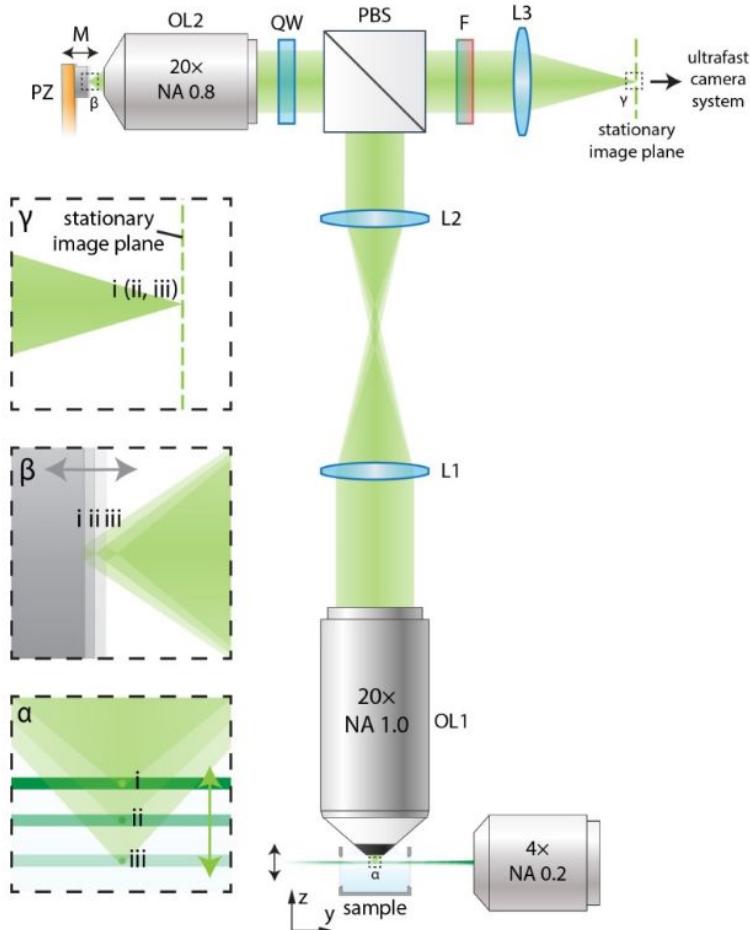
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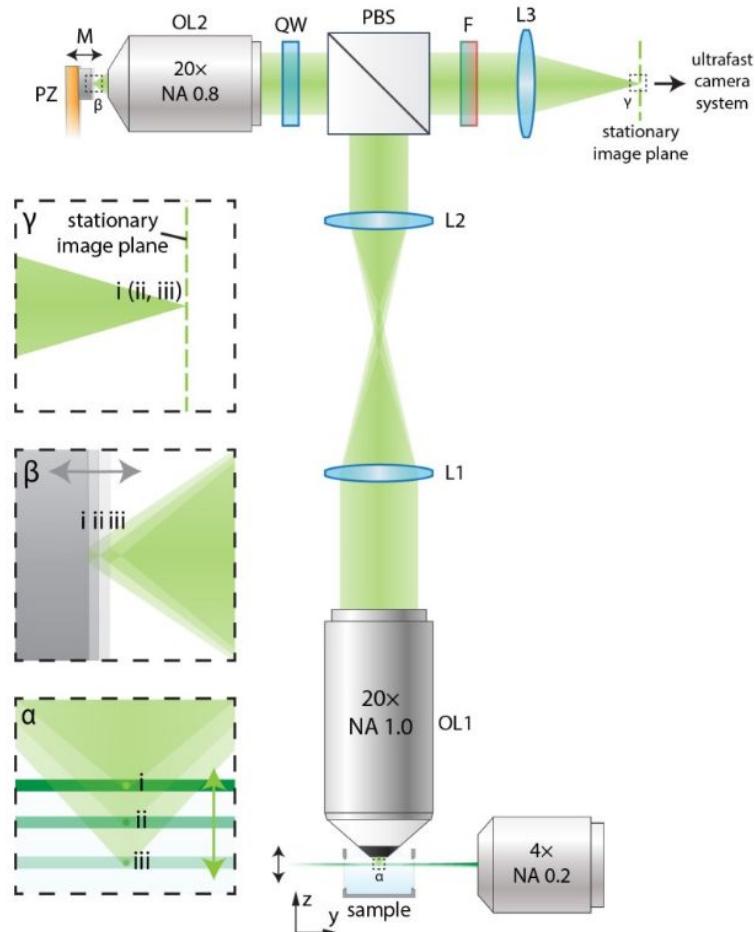
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- The PBS deflects fluorescence of particular polarization into a quarter wave plate (QW) and a remote objective lens (OL2).



Imaging Path

Overview and operational principles of the whole-brain, voltage imaging-optimized, remote scanning light-sheet microscope design.

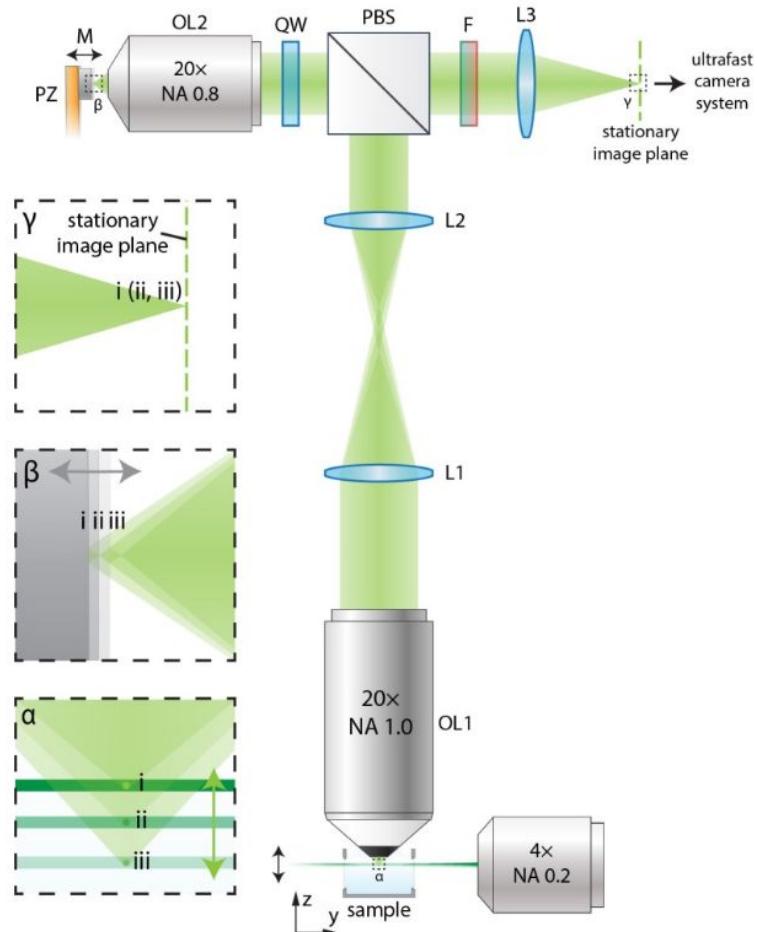
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- The PBS deflects fluorescence of particular polarization into a quarter wave plate (QW) and a remote objective lens (OL2).
- The remote objective then focuses the fluorescence into real images. These images are reflected by a mirror (M) that is translated by a piezo (PZ), and re-imaged by the remote objective.



Imaging Path

Overview and operational principles of the whole-brain, voltage imaging-optimized, remote scanning light-sheet microscope design.

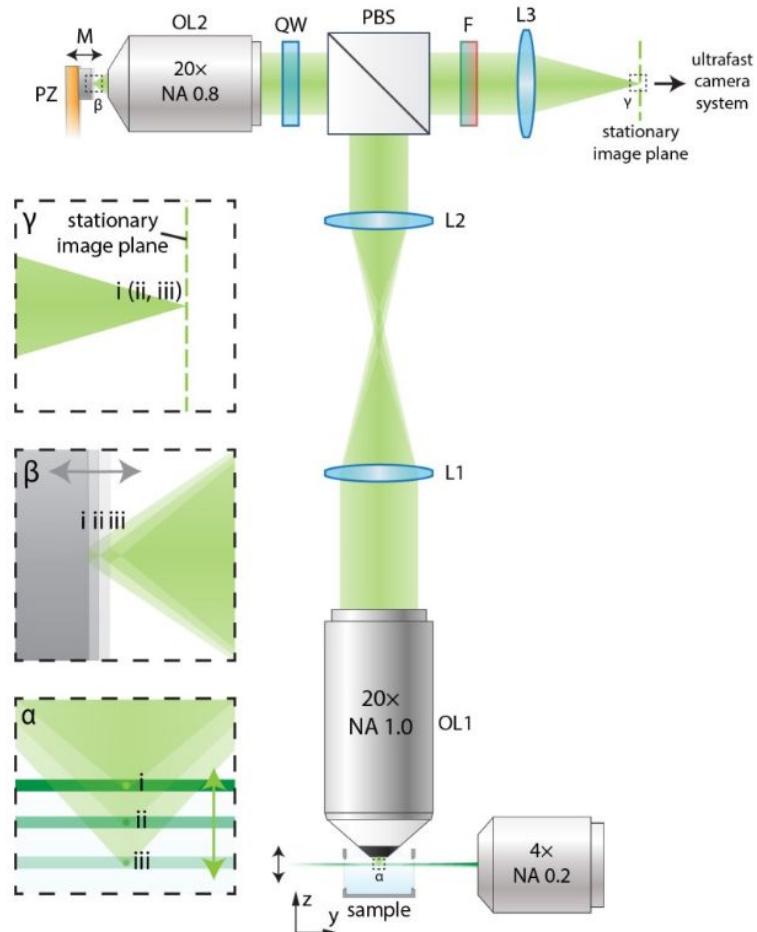
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- The remote objective then focuses the fluorescence into real images. These images are reflected by a mirror (M) that is translated by a piezo (PZ), and re-imaged by the remote objective.
- After transmitting through the quarter wave plate again, the fluorescence rotates polarization by 90 degrees. The fluorescence then passes through the polarized beam splitter and an emission filter (F), before being refracted by a tube lens (L3) into real images.



Imaging Path

Overview and operational principles of the whole-brain, voltage imaging-optimized, remote scanning light-sheet microscope design.

- At the bottom, a sample in a water chamber is illuminated from one side by a rapidly scanning light sheet from an excitation objective lens.
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- The fluorescent light passes through a 4F imaging system composed of two tube lenses (L1 and L2) before entering a polarized beam splitter (PBS).
- The PBS deflects fluorescence of particular polarization into a quarter wave plate (QW) and a remote objective lens (OL2).
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- After transmitting through the quarter wave plate again, the fluorescence rotates polarization by 90 degrees. The fluorescence then passes through the polarized beam splitter and an emission filter (F), before being refracted by a tube lens (L3) into real images.
- The remote piezo actuator (PZ) moves the mirror in synchrony with the light sheet, ensuring images of different z planes.



Sample holder (3d printed)

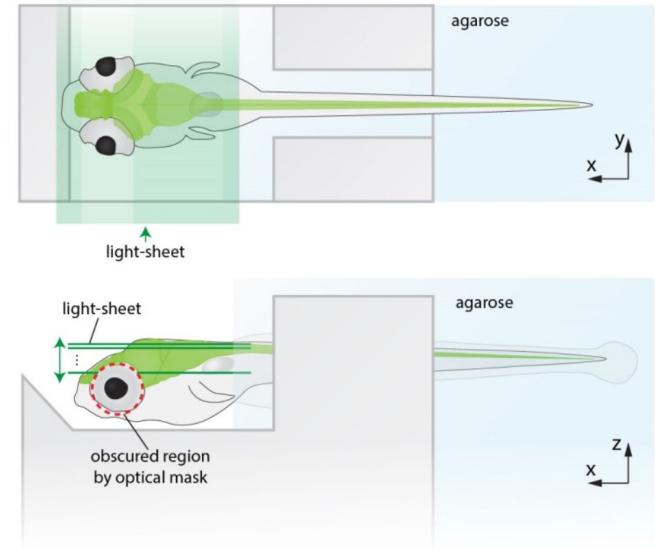
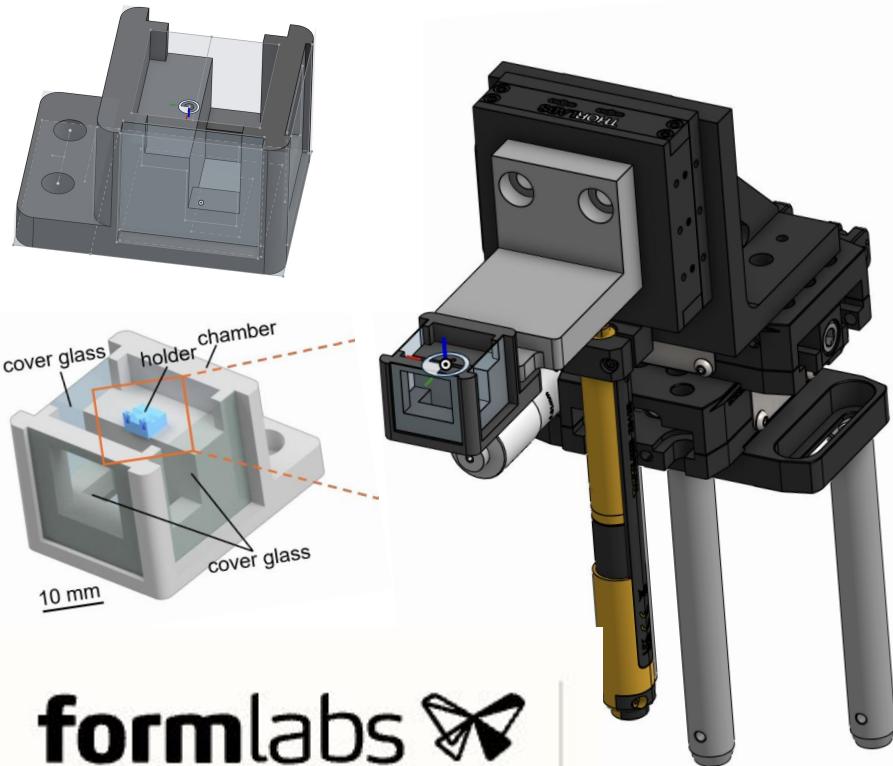


Illustration of a mounted larval zebrafish for light-sheet imaging.
A larval zebrafish with pan-neuronal labeling is immobilized on a 3D printed holder, with its body restrained in agarose gel and its head exposed. A light sheet, illuminated from one side, scans the fish's brain along the z-axis. Excitation light at the fish's eye areas is obscured using a circular opaque optical mask (red dashed line). The top view (top of the panel) and the side view (bottom of the panel) of the fish are shown.

Redesigning the system with



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voltage-fish-light-sheet | Docs +

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voltage-fish-light-sheet

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zeguan-original-designs	4:25 PM Nov 11	Corban Swain	Corban Swai

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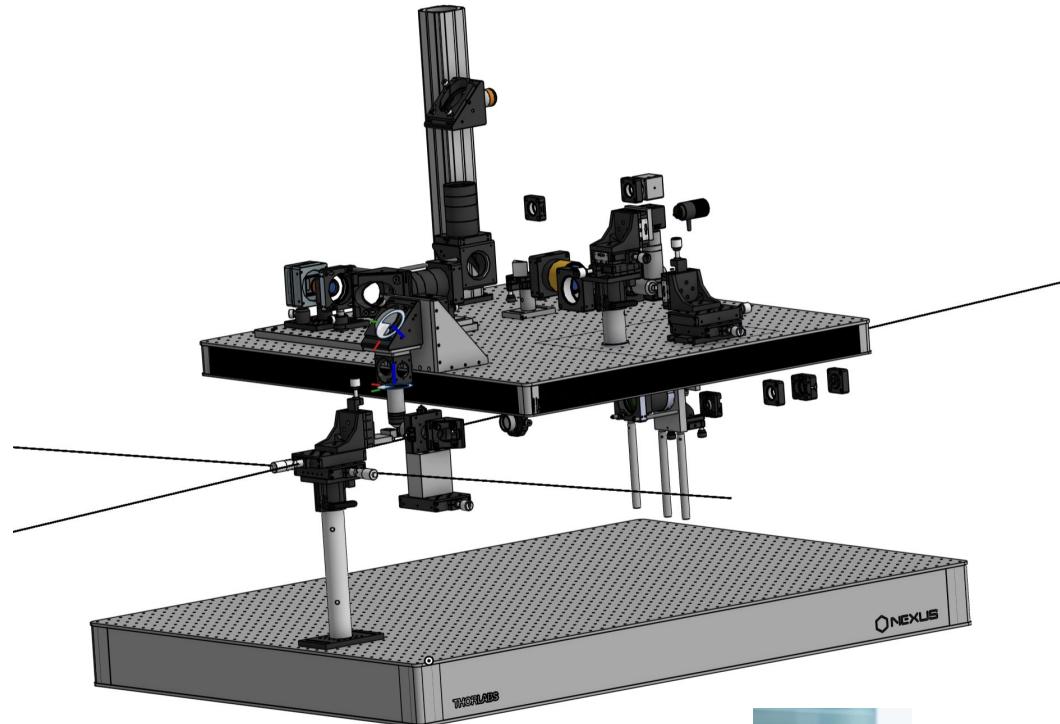
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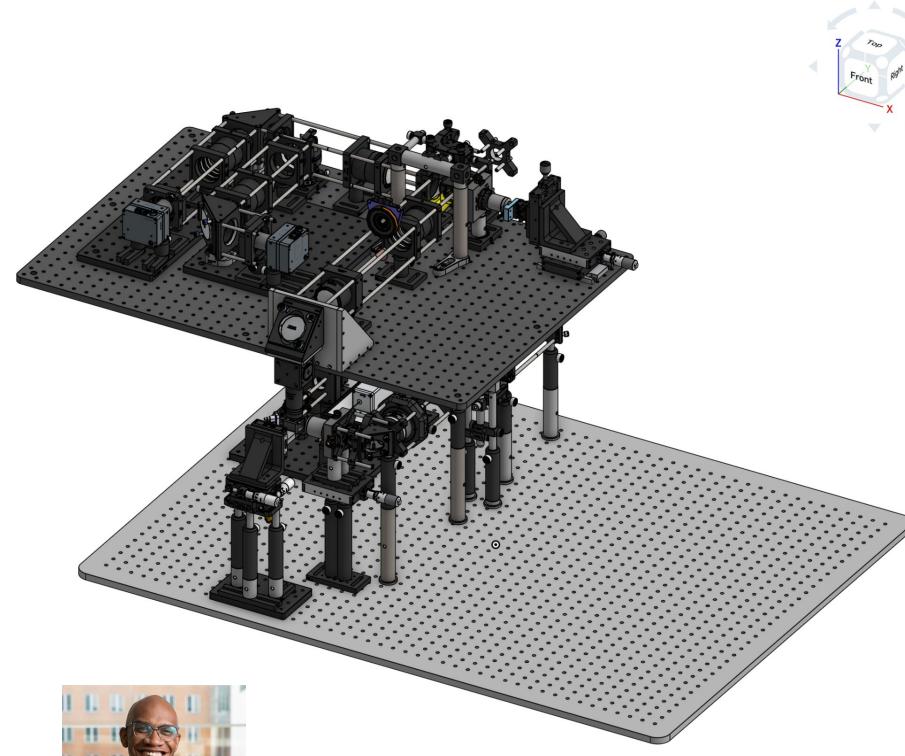
Subscription: Student

Z. Wang Original Design and Draft Parts List

Category	Part name in this paper (if applicable)	Corresponding figure number	Part description	Part number	Vendor/Source
Imaging path	OL1	Fig. 1	Imaging objective lens	XLUMPLFLN20XW	Olympus
	L1		Tube lens	TTL200MP	Thorlabs
	L2		Tube lens	TTL200-A + 2x AC508-750-A	Thorlabs
	PBS		Polarized beam splitter	PBS251	Thorlabs
	QW		Quarter wave plate	AQWP10M-580	Thorlabs
	F		Emission filter	FF01-571/72-25	IDEX/Semrock
	L3		Tube lens	TTL200MP + AC508-1000-A	Thorlabs
	OL2		Remote objective lens	UPLXAPO20X	Olympus
	PZ		Piezo scanner	PB4VB2S	Thorlabs
	M		Remote mirror	Customized 2x2x1 mm ³ mirror	Chroma
	KEM	Fig. S5	Knife edge mirror	MRAK25-P01	Thorlabs
			Camera relay lens 1	ACT508-1000-A + 3x ACT508-750-A	Thorlabs
			Camera relay lens 2	TL4X-SAP or CFI Plan Apo 4x	Thorlabs or Nikon
	C1		Camera 1	CB024MG-GP-X8G3	Ximea
	C2		Camera 2	CB024MG-GP-X8G3	Ximea
Illumination path (configuration 1, without RS)		Fig. S17a	Illumination objective lens	Plan Apo Lambda 4x	Nikon
	TL		Tube lens	ITL200	Thorlabs
	SL		Scan lens	CLS-SL	Thorlabs
	Mask		Optical mask	PhotomaskPOR-TAL	
			Galvo mirror	Saturn 5B	ScannerMAX
	RL1		Relay lens	AC127-075-A	Thorlabs
	RL2		Relay lens	AC127-050-A	Thorlabs
			Powell lens	LOCP-8.9R05-1.4	Laserline Optics
			Illumination laser	Cobolt 06-MLD 515nm	HÜBNER Photonics



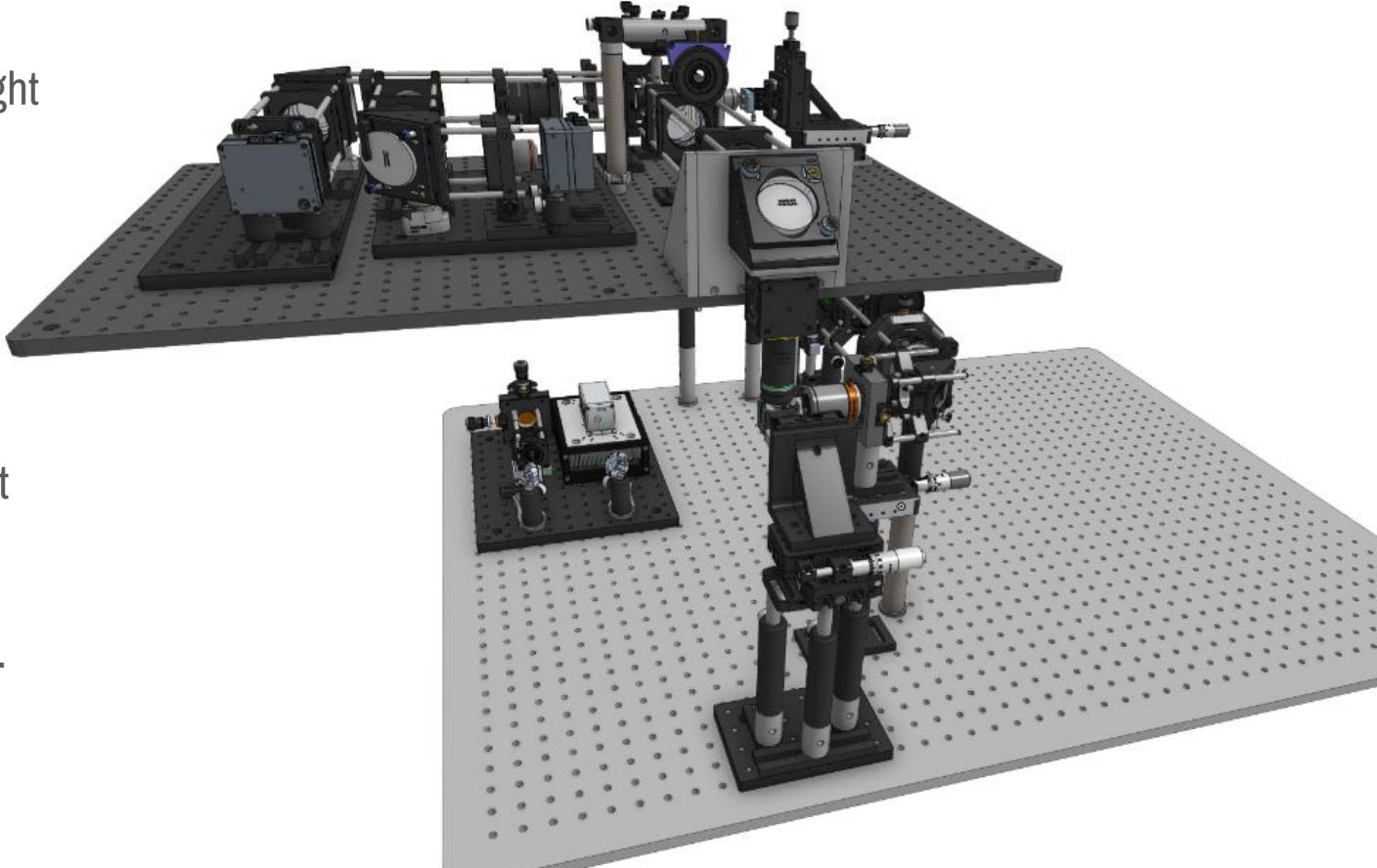
Our Improved Z. Wang Design and Detailed Parts List



Layout of Light Sheet Voltage Imaging System (Improved Z. Wang Design)

Design Modules:

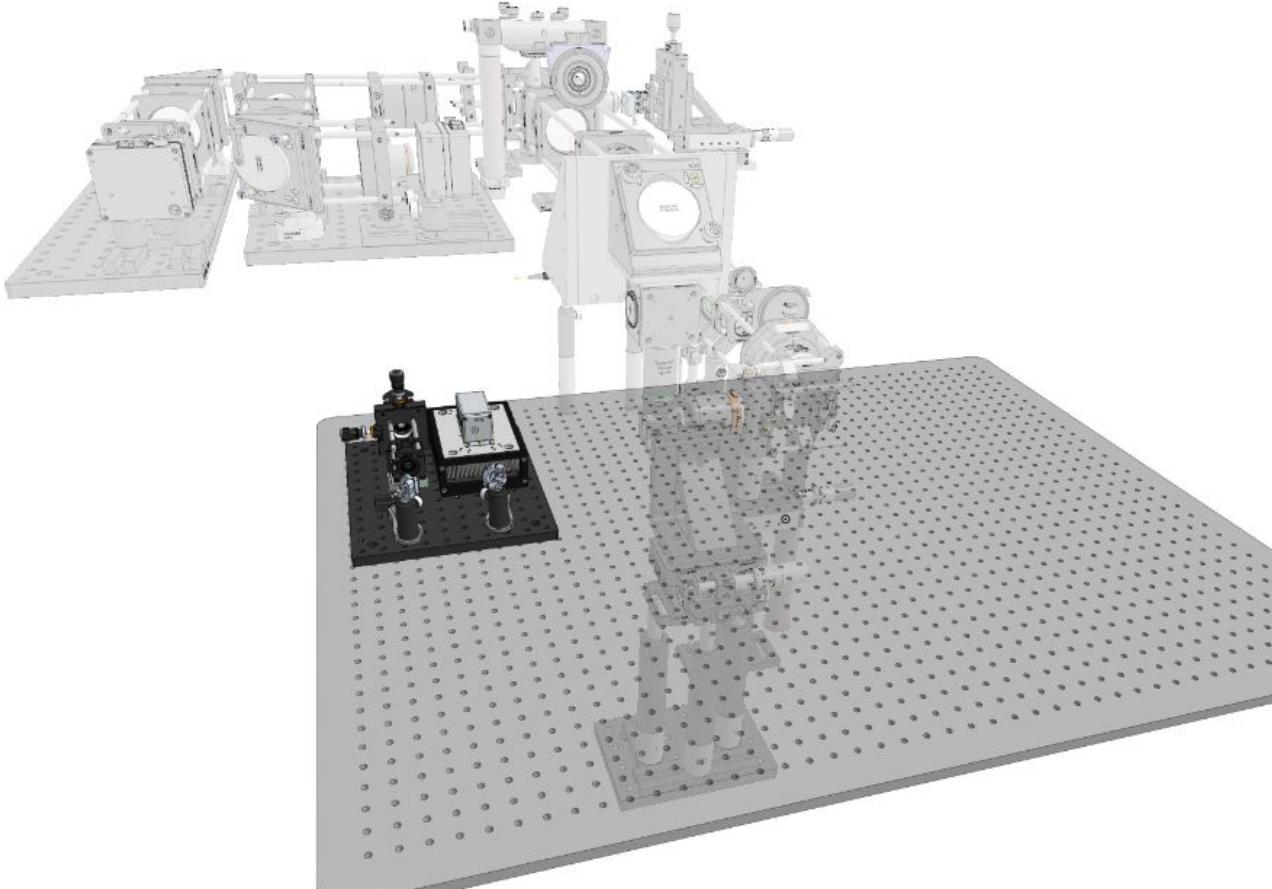
1. Launch excitation light into fiber.
2. Form lightsheet through scanning galvos.
3. Position sample in lightsheet path.
4. Image emission light through high-speed remote refocusing & dual camera system.



Layout of Light Sheet Voltage Imaging System (Improved Z. Wang Design)

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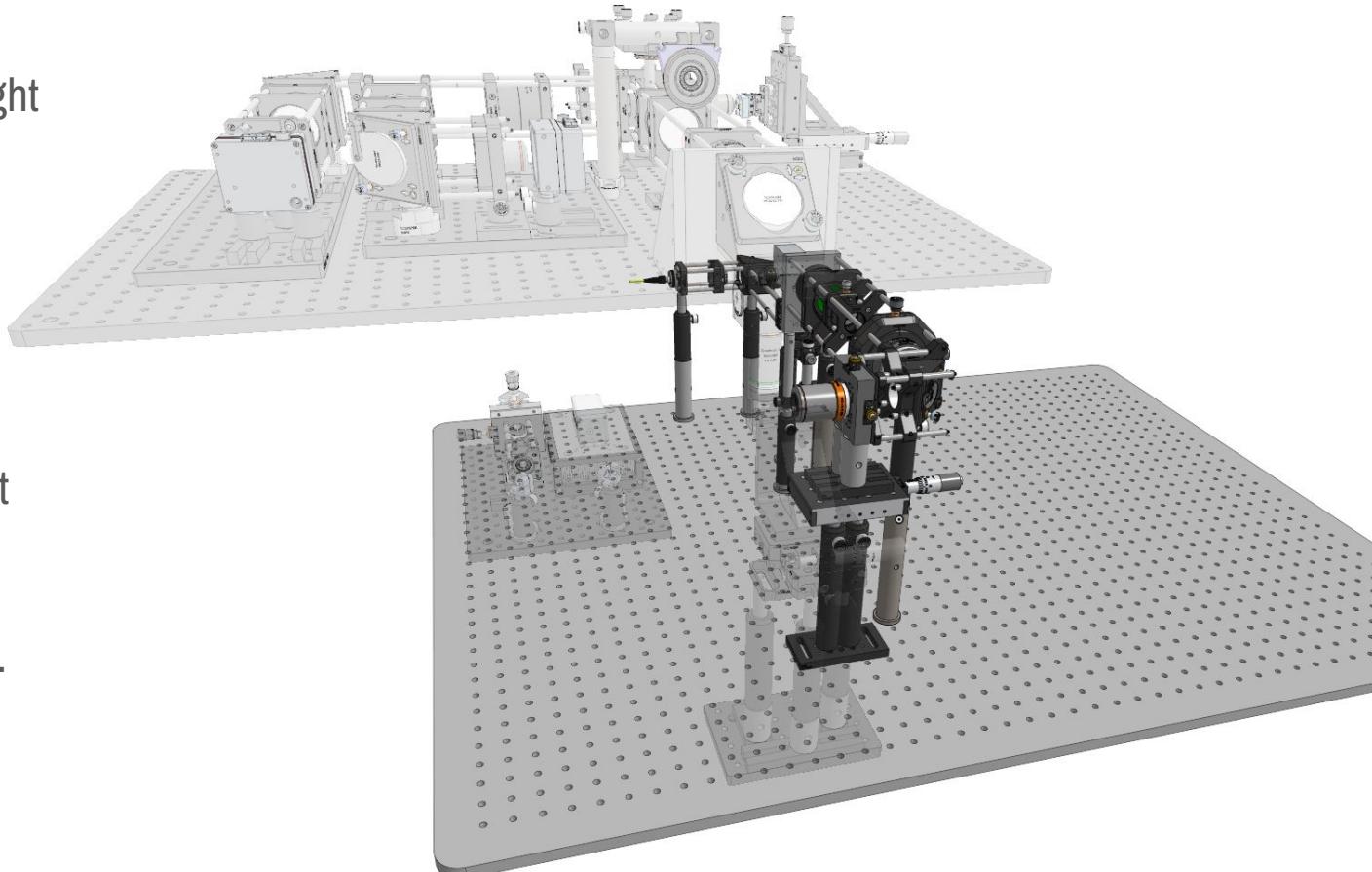
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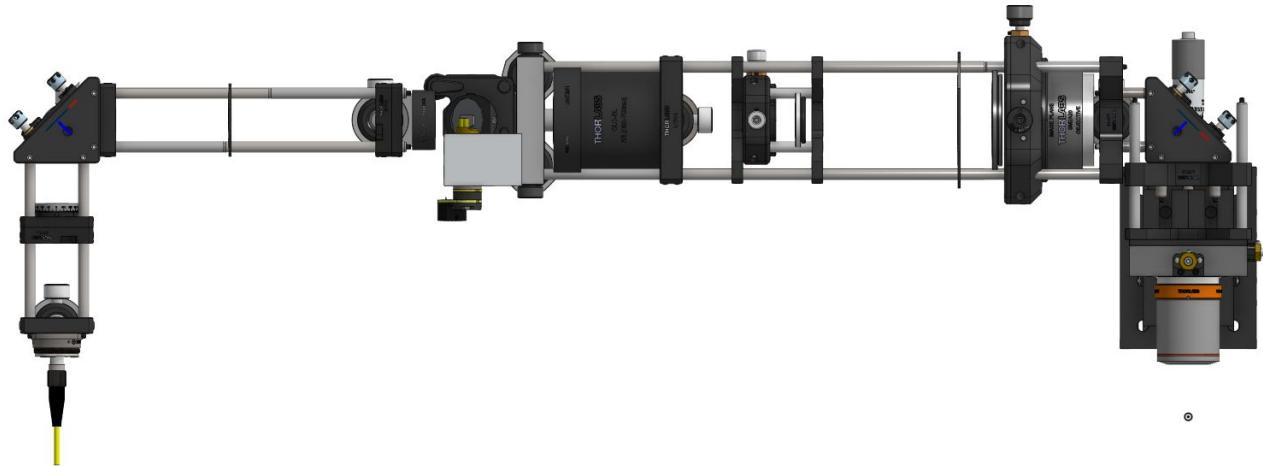
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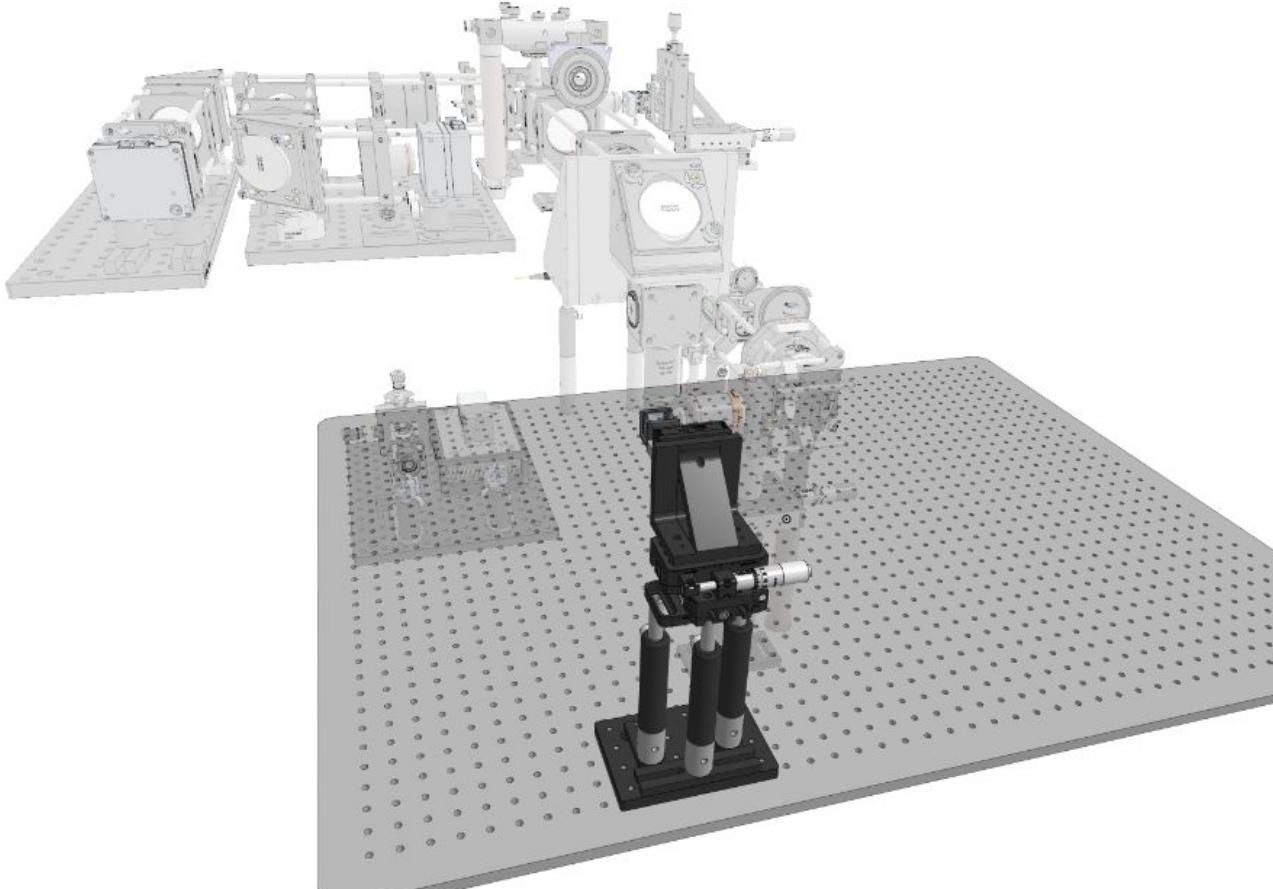
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Layout of Light Sheet Voltage Imaging System (Improved Z. Wang Design)

Design Modules:

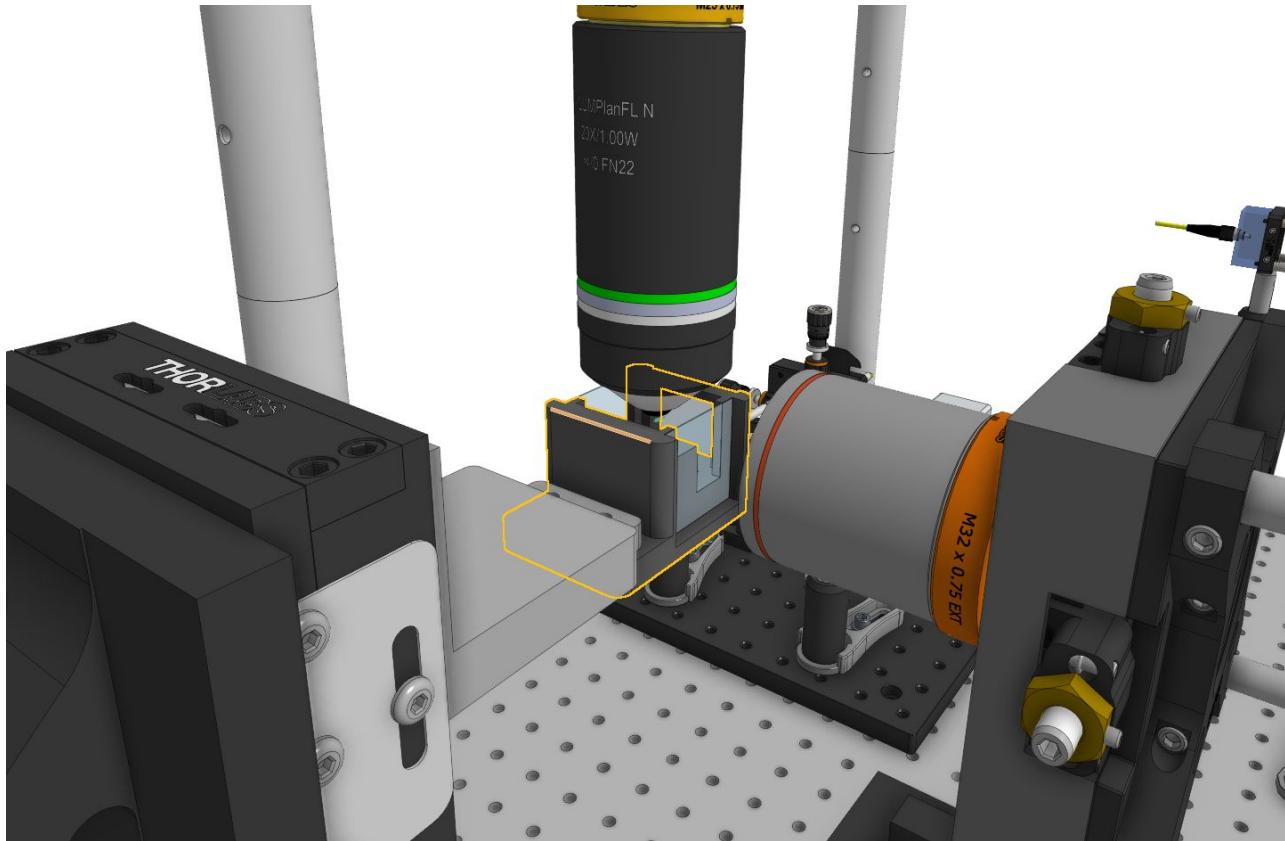
1. Launch excitation light into fiber.
2. Form lightsheet through scanning galvos.
3. Position sample in lightsheet path.
4. Image emission light through high-speed remote refocusing & dual camera system.



Layout of Light Sheet Voltage Imaging System (Improved Z. Wang Design)

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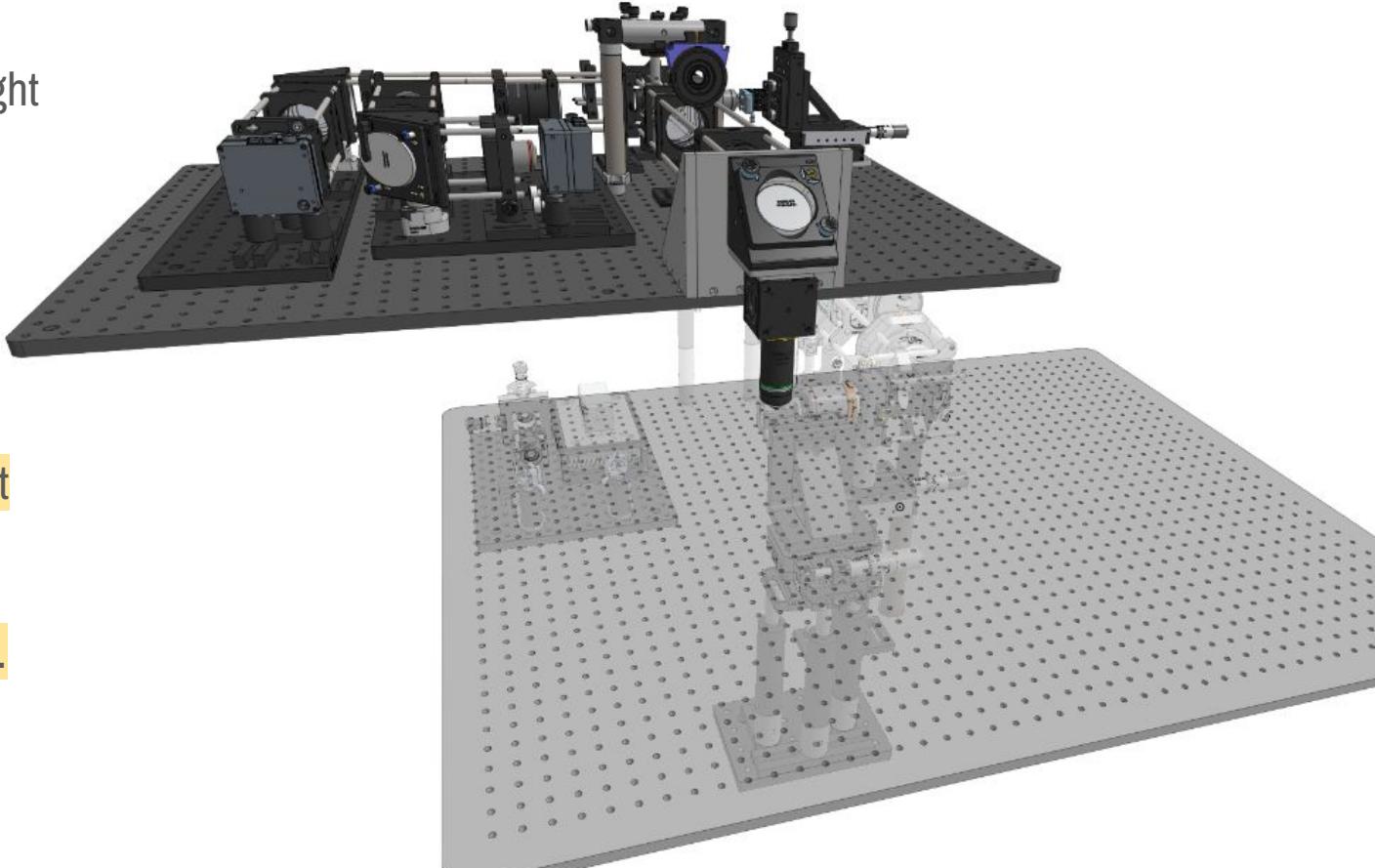
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Layout of Light Sheet Voltage Imaging System (Improved Z. Wang Design)

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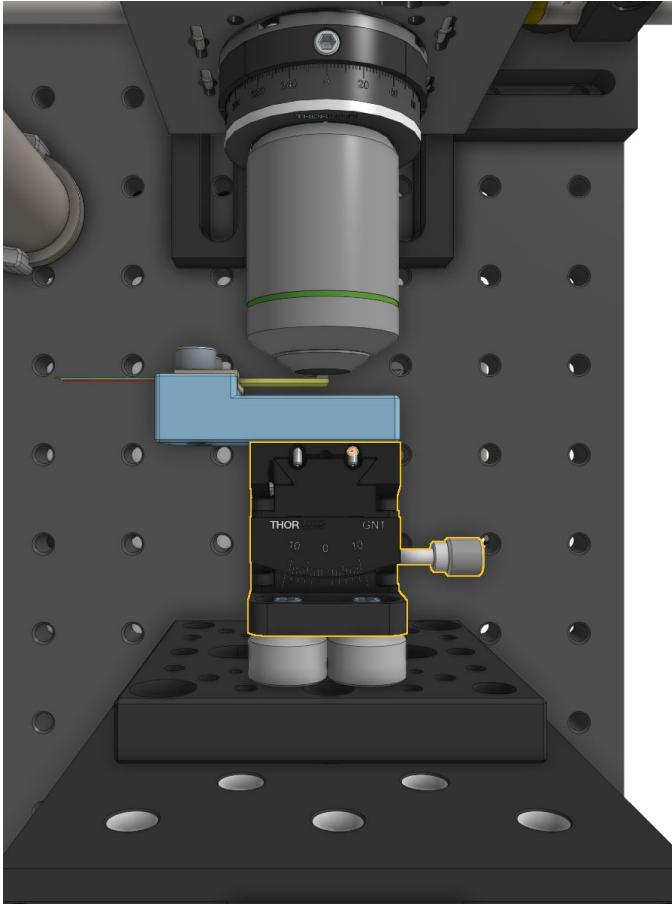
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Layout of Light Sheet Voltage Imaging System (Improved Z. Wang Design)

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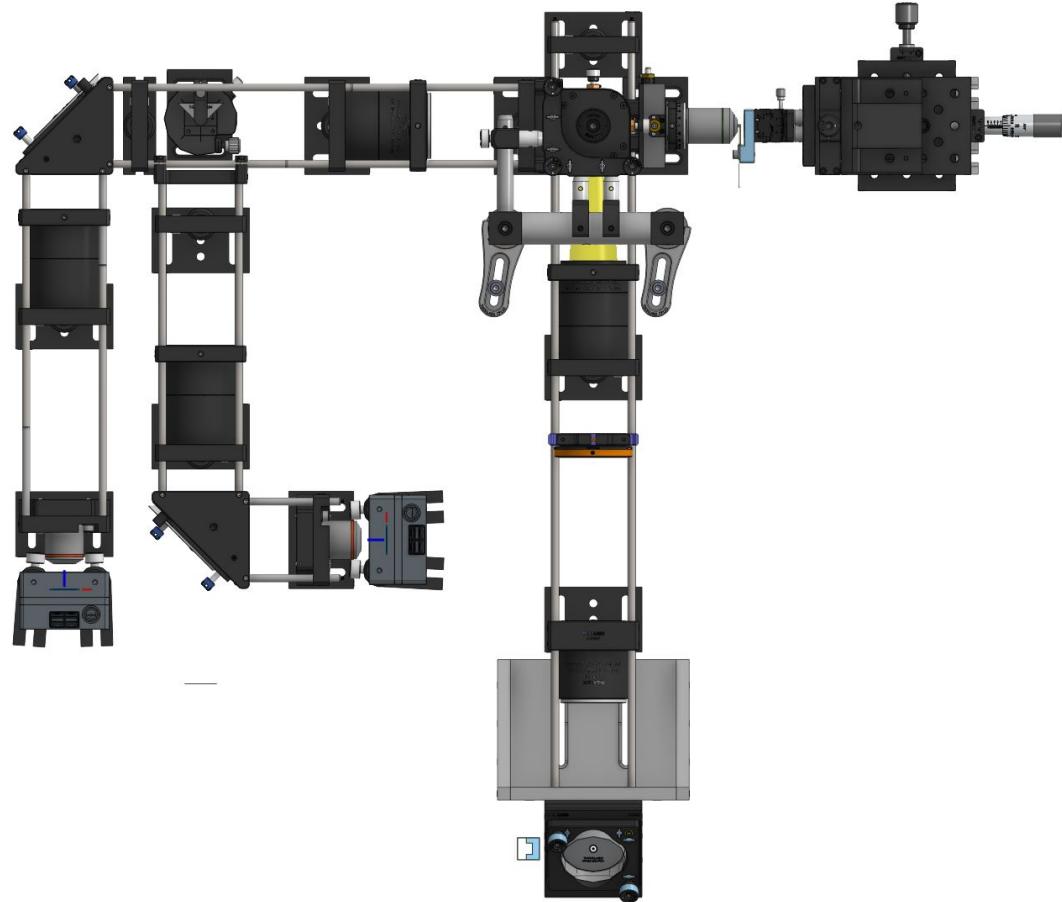
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Layout of Light Sheet Voltage Imaging System (Improved Z. Wang Design)

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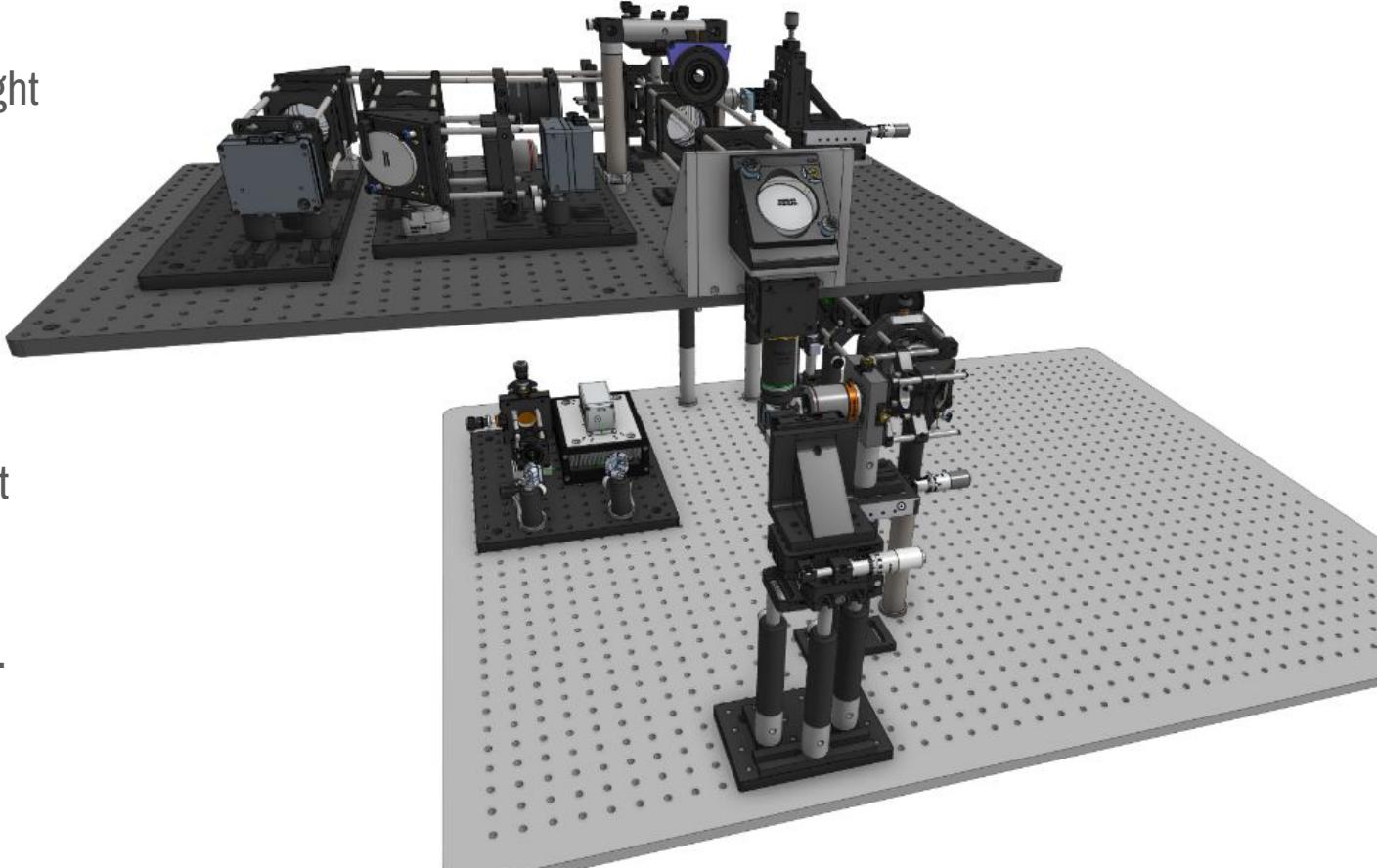
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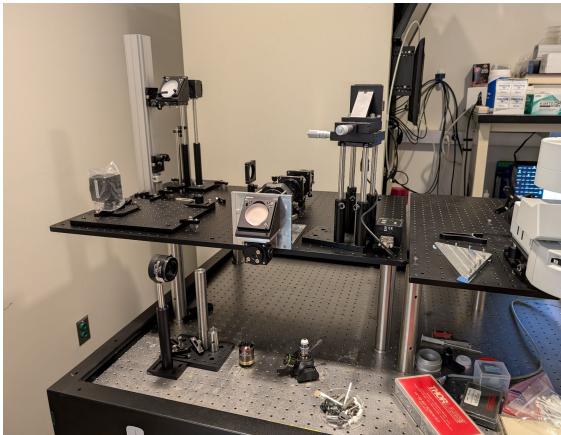
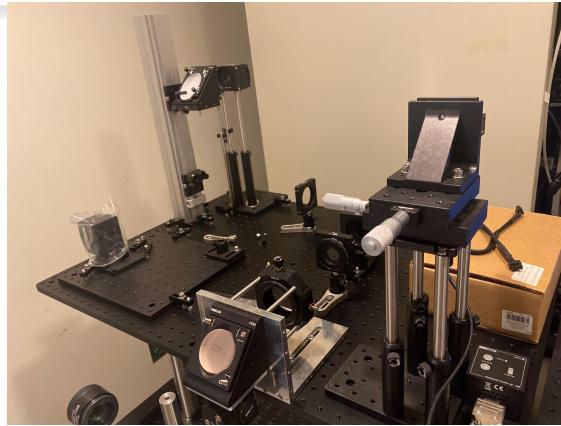
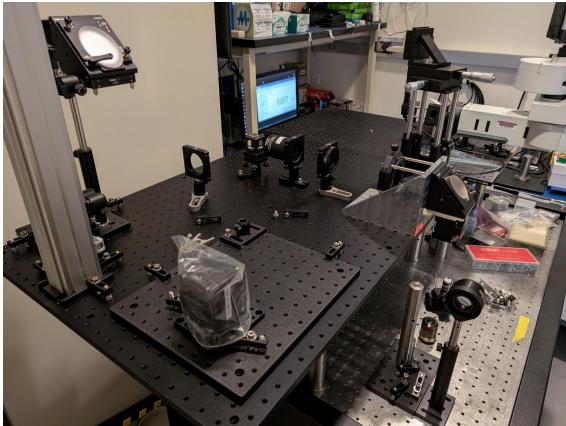
Design Modules:

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Initial build with

THORLABS

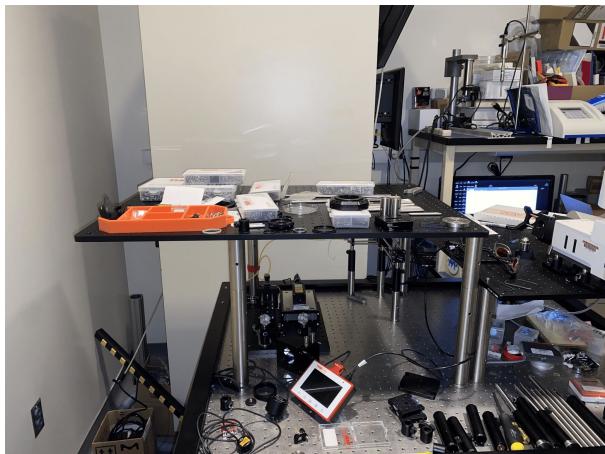
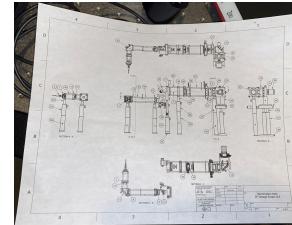
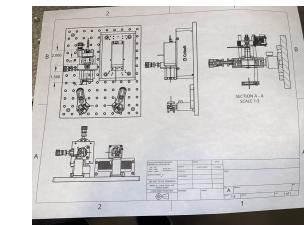
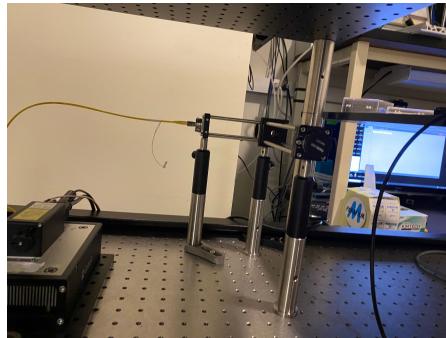
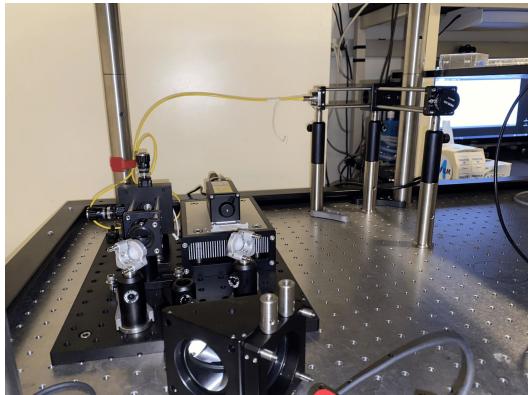


What we started with

- Old design pattern
- Missing many parts
- Not a clear plan for building



Initial build with



Where we are now

- Built and aligned the laser launch
- Started building out the illumination path
- Actively building based on clear design plan

Expenses

This stuff is very expensive. This is breakdown of our spending so far.

Month	Focus Area	Monthly Total
Sep 2025	Core Optics & Controllers <i>(Piezo, Scan Lens, Water Obj)</i>	\$22,795.43
Oct 2025	Mounting Hardware <i>(Basic tubes & posts)</i>	\$203.34
Nov 2025	Precision Alignment <i>(FiberPort, XY Stages, Waveplates)</i>	\$5,823.52

Expenses

This stuff is very expensive.

This is breakdown of our spending so far. TOTAL: ~\$28,822

Timeline of Expenditures

September 2025: Core Optical Engine

- September 18, 2025 – Thorlabs Inc.
 - Cost: \$9,474.13
 - (Updated from previous estimate using Official Quote TQ0614494-1)
 - Key Items:
 - Piezo Controller (MDT693B): \$2,445.82 (Essential for Z-axis control).
 - Scan Lens (CLS-SL): \$3,148.66 (Large Field of View).
 - Tube Lens (TTL200MP): \$1,570.33 (Multi-Photon Telecentric).
 - Optics: Achromatic Wave Plate (\$1,093.70), Piezo Bender (\$111.31), and various Achromatic Doublets.
- September 22, 2025 – Spach Optics Inc.
 - Cost: \$12,760.00
 - Key Items: High-end objectives including the Olympus 20X Water Objective (\$8,500) and Olympus 20X Extended Apochromat (\$3,600).
- September 23, 2025 – IDEX Health & Science LLC
 - Cost: \$426.30
 - Items: 571/72 nm Bandpass Filter.
- September 23, 2025 – Edmund Optics Inc.
 - Cost: \$135.00
 - Items: Power Supply for ScannerMAX.

October 2025: Initial Structural Hardware

- October 31, 2025 – Thorlabs Inc.
 - Cost: \$203.34
 - Items: SM2 Lens Tubes, Lens Mounts (LMR2), and Optical Posts.

November 2025: Precision Alignment & Fiber Coupling

- November 6, 2025 – Newport Corporation
 - Cost: \$257.45
 - Items: Lens Spacers and Washers for fine tuning.
- November 18, 2025 – Edmund Optics Inc.
 - Cost: \$1,075.90
 - Items: λ/4 Achromatic Waveplate (450–650nm).
- November 18, 2025 – McMaster-Carr
 - Cost: \$6.00
 - Items: Stainless Steel Socket Head Screws.
- November 19, 2025 – Thorlabs Inc.
 - Cost: \$4,484.17
 - Items: FiberPort (PAF2-A7A), XY Translators, and Cage System components for the illumination path.

Expenses

The projection of our final order to get all the remaining parts. TOTAL: ~\$8,618

1. Imaging Arm – Thorlabs Order #103

Status: Cart Forwarded (Dec 2, 2025)

Total Cost: \$8,577.72

This order is the largest single expense remaining. It secures the detection path optics, the 60mm cage structural framework, and high-precision motion controls.

High-Value Optical & Optomechanical Components

Item	Description	Cost	Notes
TTL200MP2	Scanning Tube Lens (\$f=200\$ mm)	\$1,575.71	Critical for laser scanning path
ST1XY-A	XY Translator (100 TPI Drives)	\$416.67	High-precision beam/sample adjustment
GN2	Dual-Axis Goniometer	\$368.37	Angular alignment
K6XS	6-Axis Locking Kinematic Mount	\$321.50	For Ø1" Optics
M595L4	595 nm Mounted LED	\$293.65	Illumination source
KCB2EC	Right-Angle	\$286.62	60mm Cage
	Kinematic Mirror Mount		System compatible
BBE2-E02	2" Broadband Elliptical Mirror	\$267.69	400-750 nm range
PT1B	1" Translation Stage	\$268.20	Z-axis or focus adjustment
PL201	Compact Laser Module (520 nm)	\$199.63	Alignment/Excitation laser

Structural & Cage System Hardware

- Cage Plates:** Significant investment in 60mm cage plates (LCP08, LCP36) totaling over \$800 to ensure rigid optical alignment.
- Rods & Posts:** Heavy-duty rods (ER24, ER10) and optical posts (RS and TR series) to mount the arm to the breadboard.
- Breadboards:** Two aluminum breadboards (MB1012, MB618) included for sub-assembly mounting.

This report covers the **Imaging Arm (Order 103)**, the **Illumination/Sample Holding (Order 102)**, and necessary **Assembly Hardware**.

Executive Summary

- Date:** December 3, 2025
- Project Phase:** Final System Completion (Imaging Arm & Illumination Rebuild)
- Total Confirmed Cost:** \$8,617.50 (Order 103 + McMaster)
- Pending Estimates:** Thorlabs Order 102 (Pricing TBD)

2. Illumination & Sample Holding – Thorlabs Order #102

Status: BOM Finalized / Quote Pending

Estimated Cost: Quote Required (Not included in confirmed total)

This order addresses the "Voltage Lightsheet Rebuild" and sample positioning. While we have the item list, the specific pricing was not in the forwarded text. Based on the components, this will likely add \$2,000 - \$4,000 to the total budget.

- Key Components:**
 - FiberPort (PAF2-A7A):** Precision fiber coupling¹.
 - Translators:** ST1XY-A and ST1XY-D for sample positioning²².
 - Optics:** AC254-100-A-ML (Achromatic Doublet)³.
 - Mounting:** Large quantity of TR posts and PH holders, as lab stock was confirmed empty by Zeguan Wang on Nov 18⁴⁴⁴.

3. Assembly Hardware – McMaster-Carr

Status: Requisition Ready (Dec 2, 2025)

Total Cost: \$39.78

Essential consumables for the physical assembly of the scope re-build.

- Fasteners:** 300+ Stainless Steel Socket Head Screws (M4, M3, 6-32).
- Alignment:** 50 Alloy Steel Dowel Pins (1/8") for precision keying.
- Tools:** Bulb-Dropper Bottle for solvent/cleaning.

Timeline

End of December 2025

- Completed building the illumination path
- Ordered parts for and start build of imaging path

January 2026

- Complete building the imaging path
- Set up workstations (computers)
- Final alignments

February 2026

- Collect some data
- Refine

March 2026

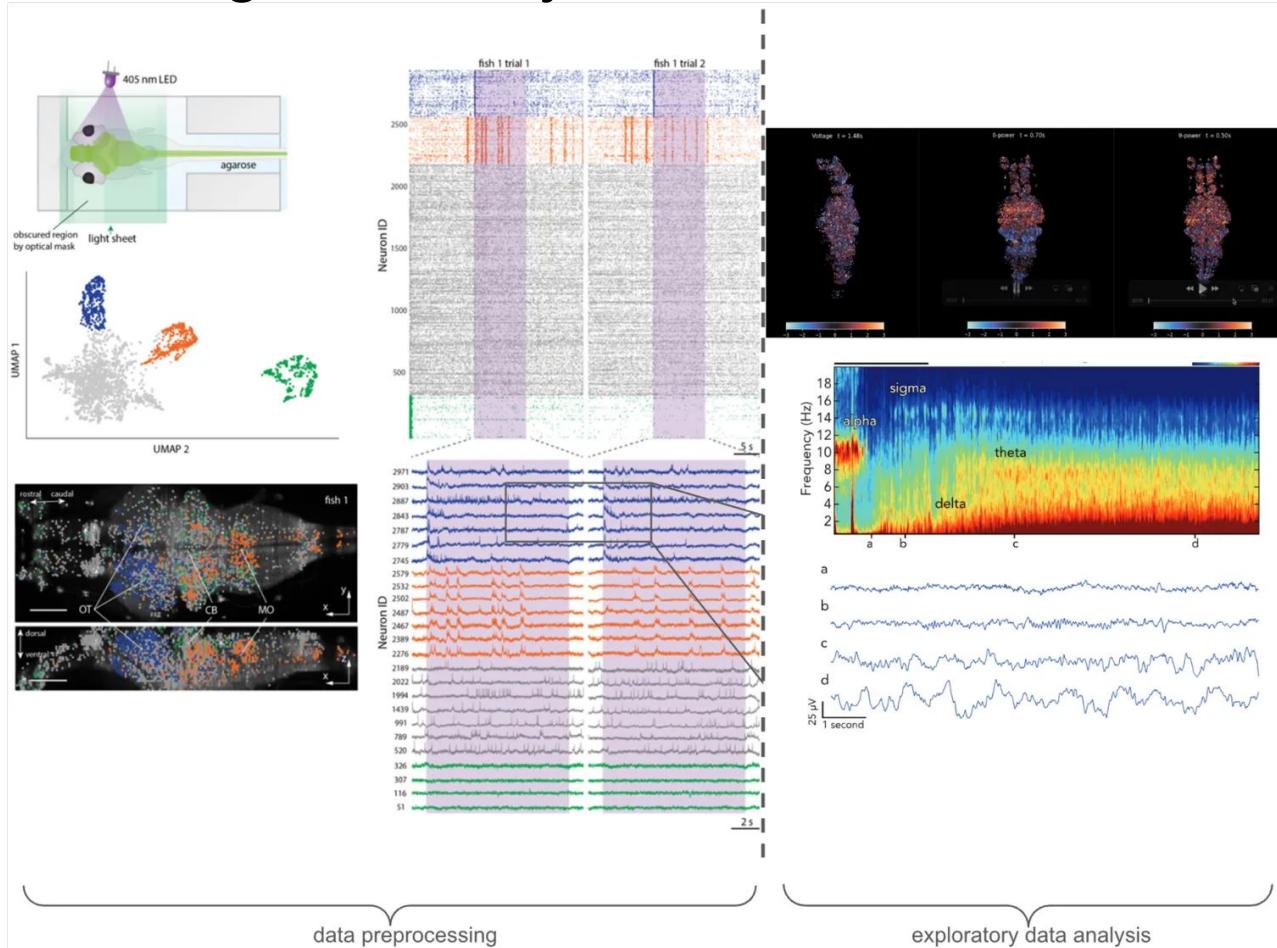
- Collect x zebrafish imaging datasets
- Data processing and analysis

Behavior sequence

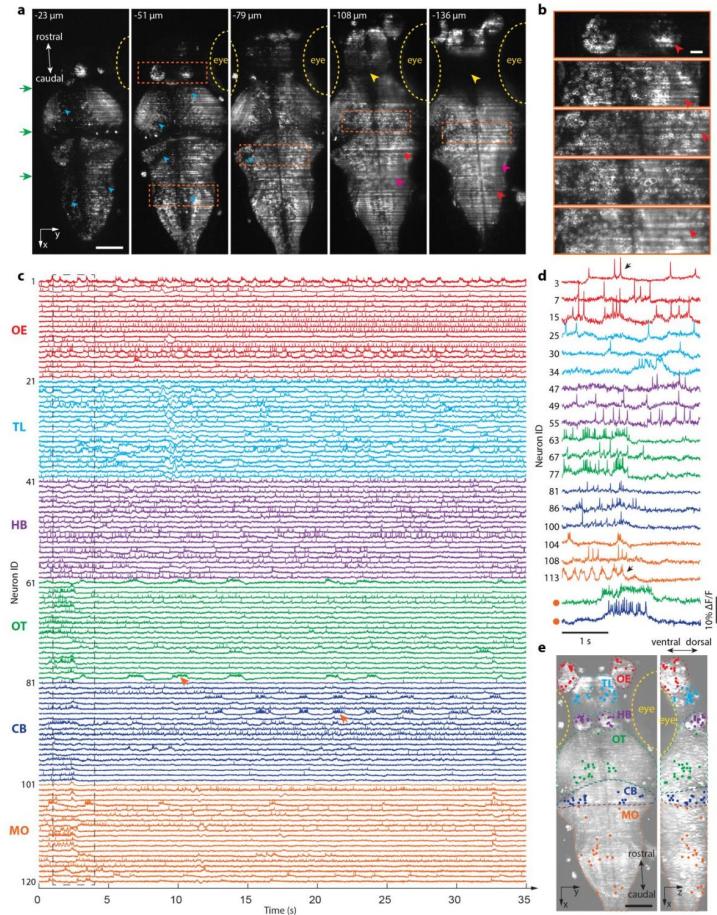
Lase

Data processing and analysis after build

Data processing and analysis



Data processing and analysis



Imaging the voltage of neurons across the entire larval zebrafish brain (a) Stitched raw images from a brain-wide **at cellular resolution**.

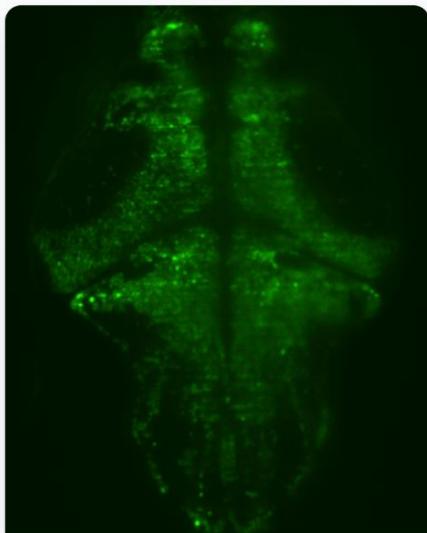
voltage imaging experiment showing 5 zebrafish brain sections out of a total of 30. Each section was imaged with 40-µs excitation time at a 200.8 Hz imaging rate. From left to right, green arrows indicate the direction of light-sheet illumination, cyan arrows show examples of unlabeled hollow regions, yellow dashed lines show contours of fish's eyes, yellow arrows indicate brain regions that are shadowed by the eyes, red arrows highlight "stripe" patterns of light-sheet illumination, magenta arrows indicate blurred regions. Scale bar: 100 µm. (b) Enlarged views of the areas highlighted by dashed rectangles in (a). Single neurons are visually distinguishable in these areas, showcasing the microscope's cellular resolution at various depths in the zebrafish brain. Scale bar: 20 µm. (c) Spontaneous activity traces of 120 exemplar neurons from 6 brain regions. Different colors signify traces from various regions (referred to in panel e), with olfactory epithelium (OE) in red, telencephalon (TL) in cyan, habenula (HB) in purple, optic tectum (OT) in green, cerebellum (CB) in blue, and medulla oblongata (MO) in orange. Orange arrows indicate two randomly selected examples of burst activity. (d) Zoom-in views of selected neurons (colored dots) whose marked with the traces enclosed by the dashed rectangle and the two burst examples in (c). (e) Locations of the putative activity traces are shown in (c), superimposed on the dorsal (left) and lateral (right) maximum intensity projections (MIPs) of the imaged brain. The putative neurons' locations are same colors as those of their corresponding activity traces in (c). Brain regions are annotated using dashed lines of different colors, indicated as in (c). Scale bar: 100 µm.

Preprocessing: VolPy + Cellpose → motion-corrected, segmented voltage traces.

Spectral & synchrony: Multitaper spectrograms.

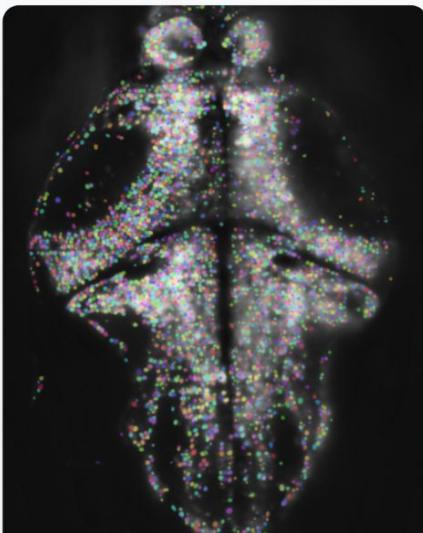
Dynamics & predictability: Low-dimensional embeddings of population trajectories.

Preprocessing



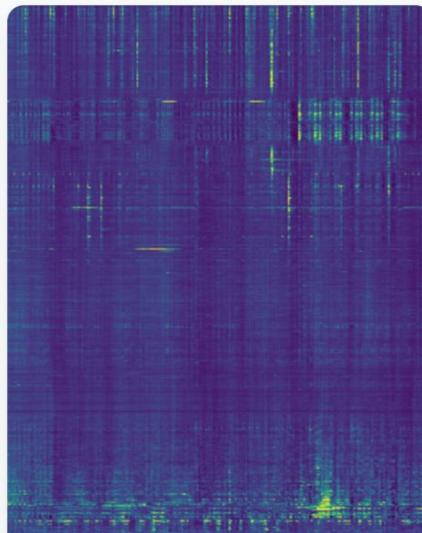
Volumetric Activity

Whole-brain calcium activity after elastic alignment, acquired by light-sheet imaging.



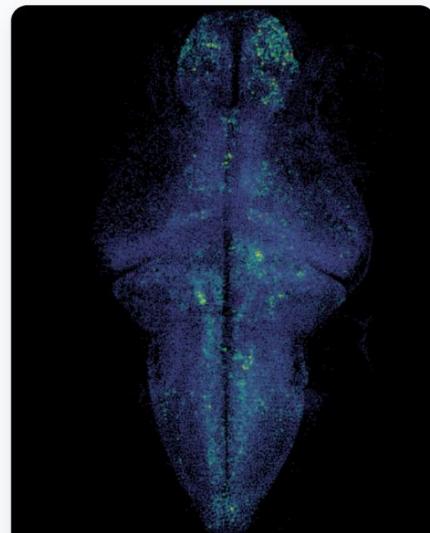
Segmentation

Segmentation masks for ~70,000 neurons obtained by training one-shot flood-filling networks.



Traces

Extracted activity traces per segmented neuron, sorted by similarity.

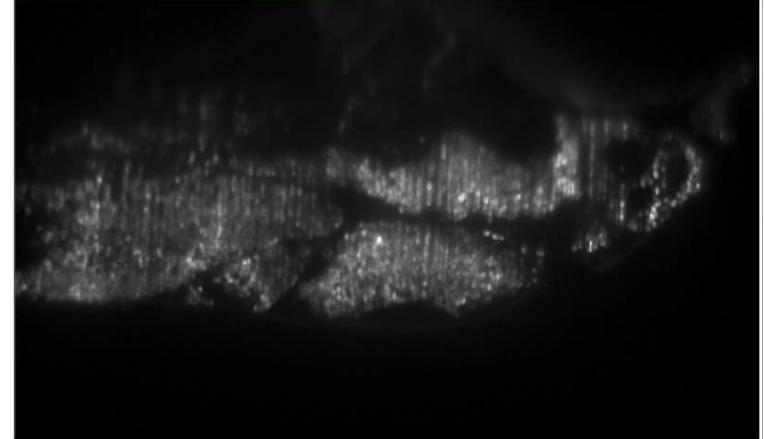


Cell Activity

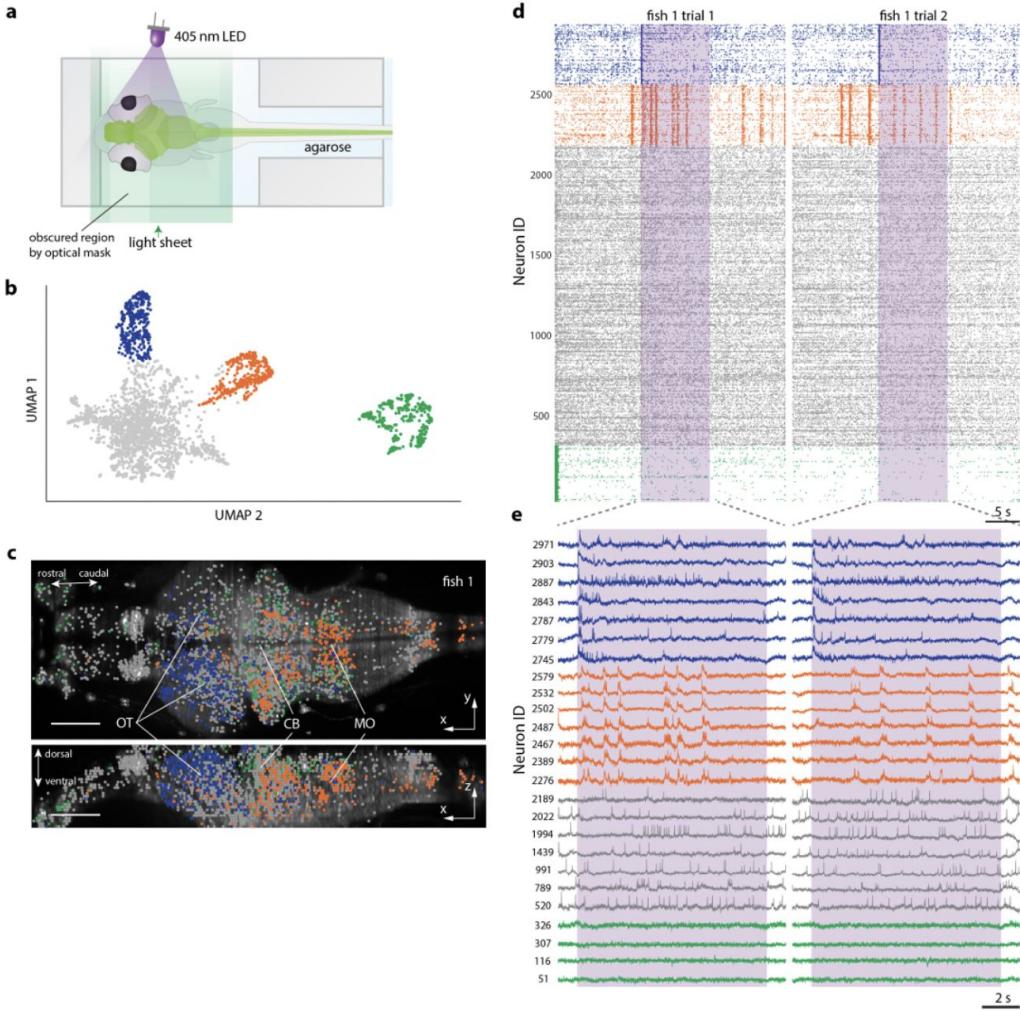
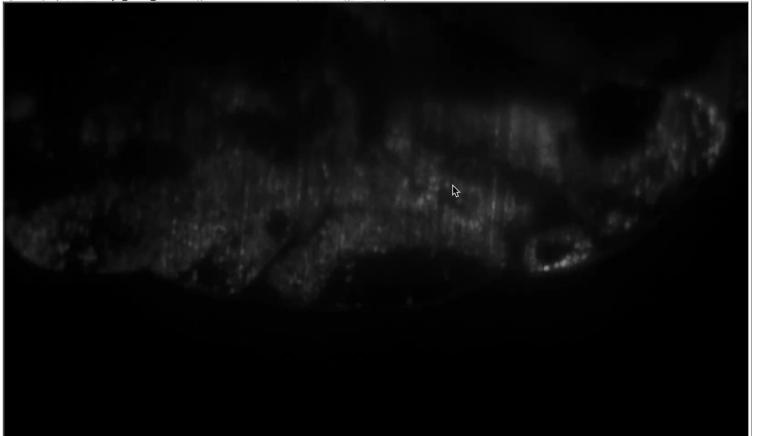
Rendering of activity traces to visualize spatial relationships.

Preprocessing

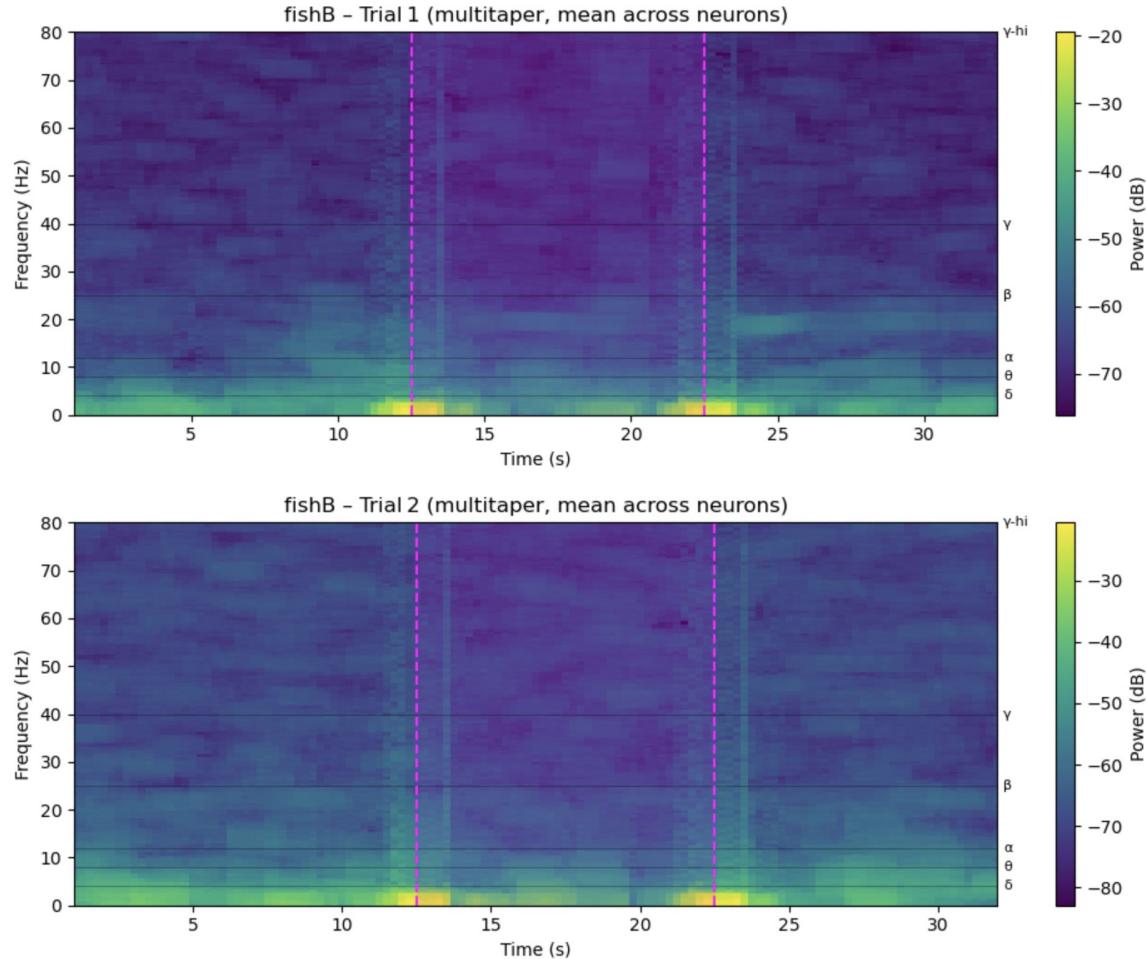
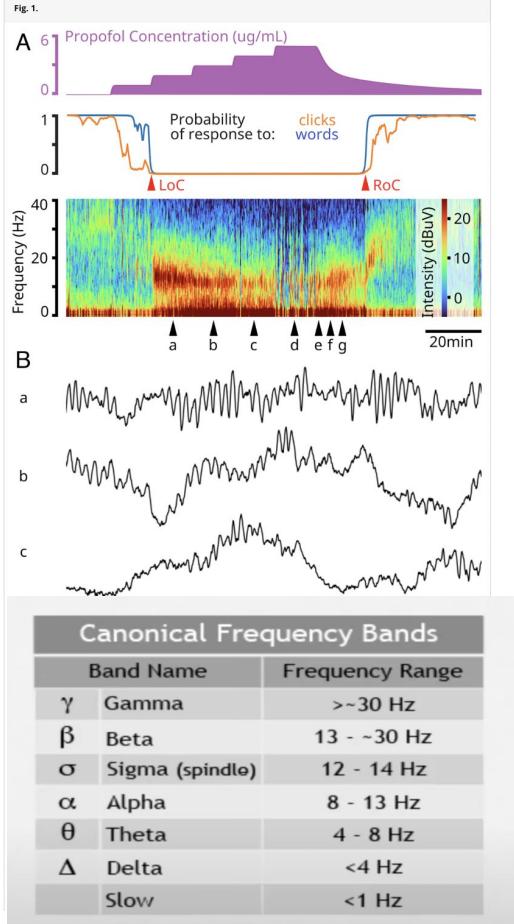
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z:25/42 t:23/81 (slice:949); 0.00x0.00 microns (1024x608); 16-bit; 3.9GB



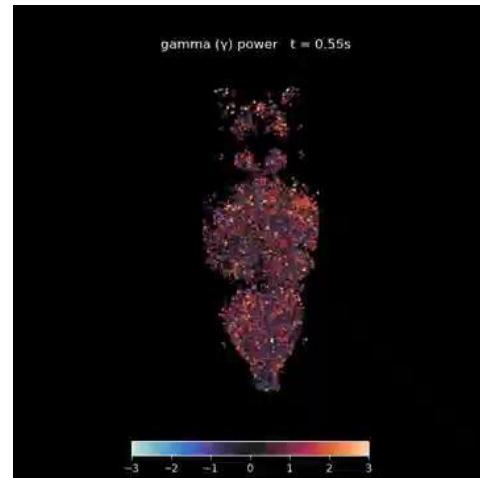
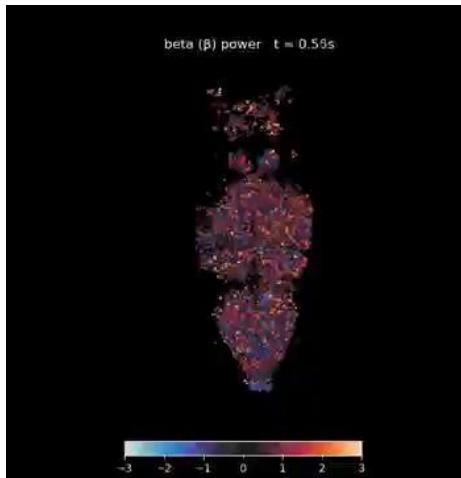
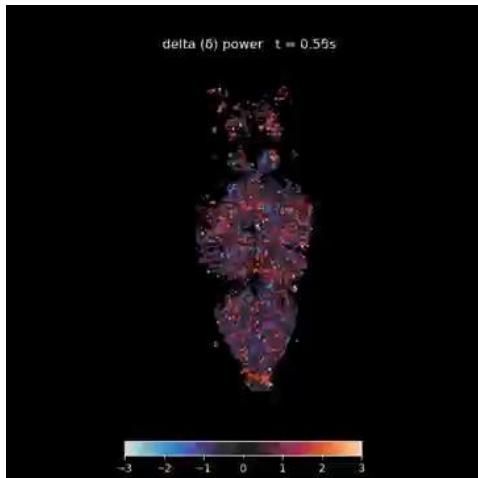
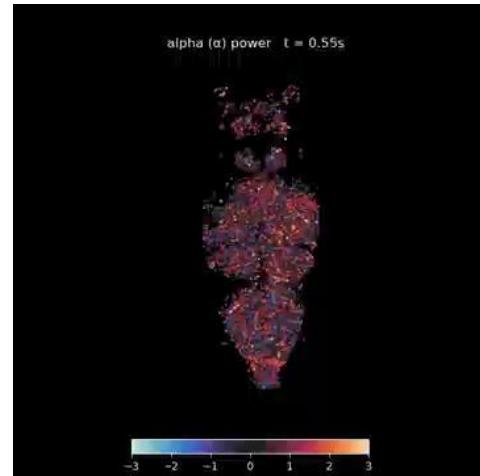
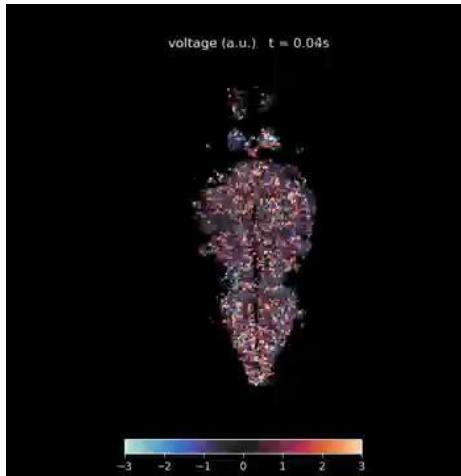
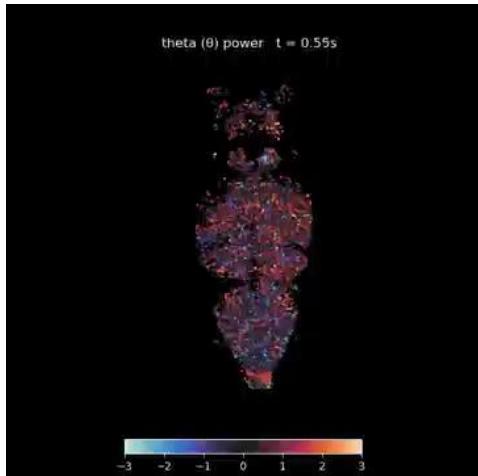
1/3440 (t:1/3440 - scape_fish2_vols.ome); 0.00x0.00 microns (1024x608); 16-bit; 4GB

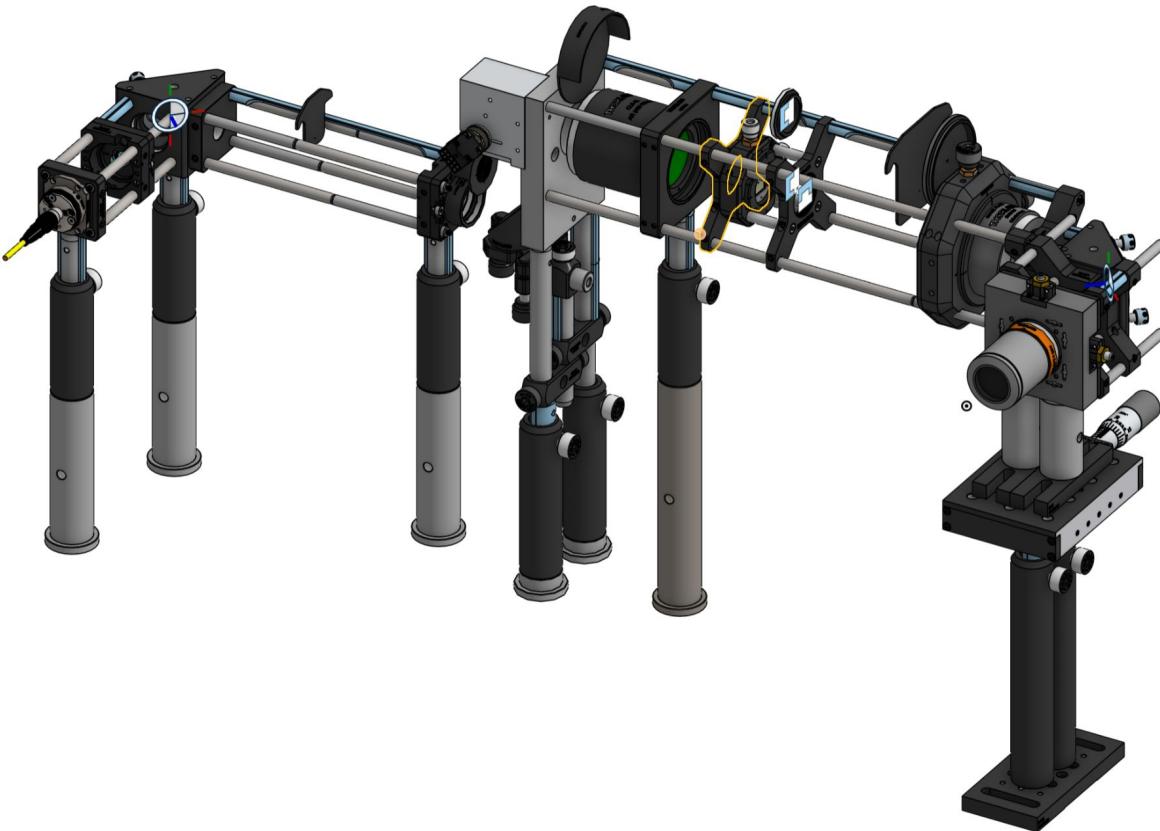


Spectral analysis



Preliminary results





) (

Goal is at the focal plane of the objective to have a sheet of light. Based on the shape of the light sheet you can calculate what you need to input into the back aperture of the objective.

Angles at the back aperture are converted into points at the sample plane.

In order to get the shape we have to put in a fan of angle that all focus to a line of points.

f_{obj} is the focal length of the objective.
Calculate Divide the design tube lens focal length by the magnification.

But we also need to scan z for the volume.

Powell lens only modifies light along one direction. It is only cylindrically symmetric.

First relay system focuses onto a new pupil (that of the Galvo)

into the page



