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Multiphoton Mesoscope



- Subcellular-Level Resolution over a Ø5 mm Field of View
- High-Speed Functional Imaging Across Several Brain Regions Operating in Concert

Features

- Enables Functional Imaging within a Ø5 mm Field of View
- Scans can be Configured over Whole Field of View or over Multiple Non-Contiguous Regions
- Microscope Body Enables ±20° Rotation Around Sample and Fine XYZ Motion
- Remote Focusing Mirror for Fast Axial Control over 1 mm Travel Range
- Enclosure Provides Large Working Volume for Specimen and Experimental Apparatus
- Field of View can Move While Specimen Remains Fixed
- Technology Used Under License from HHMI's Janelia Research Campus
- Volume Imaging Technique Using Bessel Beams and Dual-Plane Imaging Add-Ons Available; See the *Applications* Tab

Range of Motion

- -20° to +20° Rotation About the Objective Focus
- 2" of Fine X Motion
- 6" of Fine Y Motion
- 2" of Fine Z Motion
- X, Y, and Z Axes Rotate with the Objective
- Remote Focusing Mirror Enables Fast Focusing Adjustments over 1 mm Range During Scans

Thorlabs' 2-Photon Random Access Mesoscope (2p-RAM, US Patent 10,295,811 and 10,901,194) provides subcellular resolution over an exceptionally large Ø5 mm field of view. Developed and commercialized in collaboration with Karel Svoboda's research laboratory at HHMI's Janelia Research Campus, this multiphoton mesoscope is designed for *in vivo* functional imaging of multiple spatially separated brain regions operating in concert. When imaging across user-defined, non-contiguous regions of interest within the field, near-video frame rates are possible; see the video to the right and the *Applications* tab.

Our 2p-RAM is capable of two-photon random access scanning; see the image to the upper right. This system features a built-in remote focusing unit, which translates the focal plane over a 1 mm range. The remote focusing unit can be coordinated with the lateral scan unit,

which is comprised of virtually conjugated mirrors and a resonant scanner, to enable both lateral and axial translation of the field during the measurement. The lateral scan unit can direct the excitation beam from region to region within the Ø5 mm field of view in ~6 ms. We offer a 2.7 mm WD objective which provides large excitation and collection NAs of 0.6 and 1.0, respectively. The scan path wavelength range of 900 - 1070 nm was chosen for optimal two-photon excitation of GFP and red fluorescent proteins, and is compatible with any tunable Ti:sapphire laser designed for multiphoton microscopy, such as Thorlabs' Tiberius® laser.

The mesoscope features motion control systems that permit the mesoscope body to move while the specimen remains fixed. The mesoscope body allows -20° to +20° rotation for the objective, as well as 2" of fine X motion, 6" of fine Y motion, and 2" of fine Z motion; just as with Thorlabs' Bergamo® II multiphoton microscope, X, Y, and Z rotate along with the objective. A multi-jointed periscope maintains the laser alignment over the entire range of motion. Since the study of awake, behaving specimens benefits from large working spaces, the mesoscope's enclosure leaves the surface of the optical workstation free for the experimental apparatus.

Mesoscope Specifications

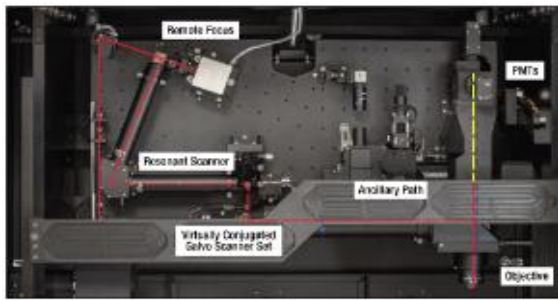
Scan Path	900 - 1070 nm	
Wavelength Range		
Field of View	Ø5 mm	
Objective	Excitation NA ^a	0.6
	Collection NA ^a	1.0
	Working Distance (Minimum) ^b	2.7 mm
Lateral Scan Unit	12 kHz Resonant Scanner + Virtually Conjugated Galvo Scanner Set	
	Field of View is Divided into 608-µm-Wide Vertical Stripes; Time per Scan Line is 42 µs	
	Scan Speed = 42 µs × Number of Stripes × Number of Scan Lines	
Scan Speed	Low Resolution, Full Field of View:	
	4.3 Frames per Second (6.3 mm × 5.4 mm Area, 500 Scan Lines, 9 Stripes)	
	Examples ^c	High Resolution, Multiple Regions of Interest:
	9.5 Frames per Second (Four 600 µm × 600 µm Areas, 512 Scan Lines per Area, 1 Stripe per Area)	
Epi-Detection	Two Ultrasensitive GaAsP PMTs	
Range of Motion		
Objective Rotation	-20° to +20° Around Objective Focus; 0.1° Encoder Resolution	
X	2" (50.8 mm); 0.5 µm Encoder Resolution	
Y	6" (152.4 mm); 0.5 µm Encoder Resolution	
Z	Stepper Motor	2" (50.8 mm), 0.1 µm Encoder Resolution
	Remote Focusing Mirror	1 mm Travel Range

- These NAs are valid over the entire scan path wavelength range.
- The mesoscope's remote focusing mirror can be used to translate the focal plane over a 1 mm range without movement of the objective or the specimen, allowing the specimen to be placed farther from the objective than its working distance.
- Both examples described here are shown in the Calcium Imaging video in the *Applications* tab.

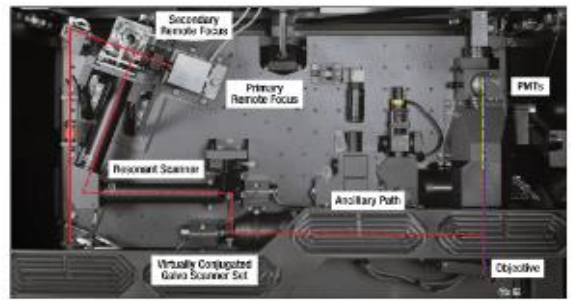
Configurations: 2p-RAM vs 2p-RAM with Dual-Plane Imaging Add-On

The 2p-RAM (lower left) contains many optical systems that are specifically optimized to work together, including a built-in remote focusing mirror, which translates the focal plane over a 1 mm range; a lateral scan unit, which comprises virtually conjugated mirrors and a resonant

scanner; a multi-jointed periscope that maintains the laser alignment over the entire range of motion; an ancillary path for one-photon imaging and photostimulation; and a custom large-NA objective. The 2p-RAM equipped with the dual-plane imaging add-on (lower right) includes all the same optical systems in addition to a secondary remote focusing module. For more details on this add-on, please see the *Applications* tab.



Click to Enlarge
2p-RAM



Click to Enlarge
2p-RAM with Dual-Plane Imaging Add-On

Applications

- Calcium Imaging
- Dual-Plane Imaging
- Volumetric Imaging Using Bessel Beams

Calcium Imaging

Developed and commercialized in collaboration with Karel Svoboda's research laboratory at HHMI's Janelia Research Campus, our 2-Photon Random Access Mesoscope (2p-RAM) is able to capture the activity of neurons across multiple regions of the brain with calcium imaging. Calcium imaging is a common technique used for tracking populations of neurons with calcium indicators. Unlike widefield microscopy, which has high light scattering and low contrast, two-photon microscopy provides the high resolution and improved contrast needed for *in vivo* calcium imaging.

A low-magnification image from layer 2/3 cortex expressing GCaMP6f under the *thy-1* promoter (GP 5.17 line), followed by four fields of view acquired at a higher resolution and frame rate. (Courtesy of Nicholas James Sofroniew, Daniel Flickinger, Jonathan King, and Karel Svoboda; Janelia Research Campus and Vidrio Technologies, Virginia, USA.)

Using the 2p-RAM, Svoboda's research team has demonstrated *in vivo* imaging with a specimen expressing the GCaMP6f calcium indicator. As shown in the video to the right and in the image below, the multiphoton mesoscope can image across user-defined, non-contiguous regions of interest within the field at near-video frame rates. For more details, please see the complete research paper.

Source: Sofroniew NJ, Flickinger D, King J, and Svoboda K. "A large field of view two-photon mesoscope with subcellular resolution for *in vivo* imaging." *ELife*. 2016 Jun. 14; 14472.

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