Development of custom, low-cost multiscale microscopic imaging systems for neuroscience applications

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Introduction

- It is now possible to image brain cell activity in awake behaving animals; however, commercial systems are expensive, prohibiting their widespread use.
- To overcome this, we are developing cost-effective and easily customizable imaging tools (mesoscope and two-photon microscope) to visualize structural and functional details of the brain.
- Mesoscope - fluorescence imaging for studying meso-scale activity from diverse regions of the brain for understanding the functional properties and correlations of widely separated neurons in the brain.
- Two-photon microscope (TPM) - high-resolution imaging using ultra short pulses from a passively mode-locked fibre laser for studying micro-scale activity from individual neurons.

Theory and Design

- One-photon excitation: a single photon excites the fluorophore.
- Two-photon excitation: two low-energy photons combine to excite the fluorophore.
  - The average rate of two photon excitation per molecule :

$$\boldsymbol{Rate} \equiv \frac{1}{2}\delta \langle I^2 \rangle$$

where, δ is two photon absorption cross section and $< I^2 >$ is the time average of second order local laser intensity

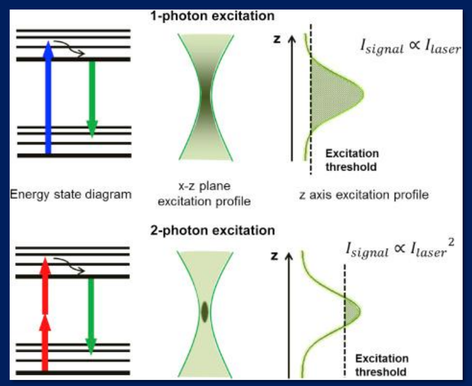
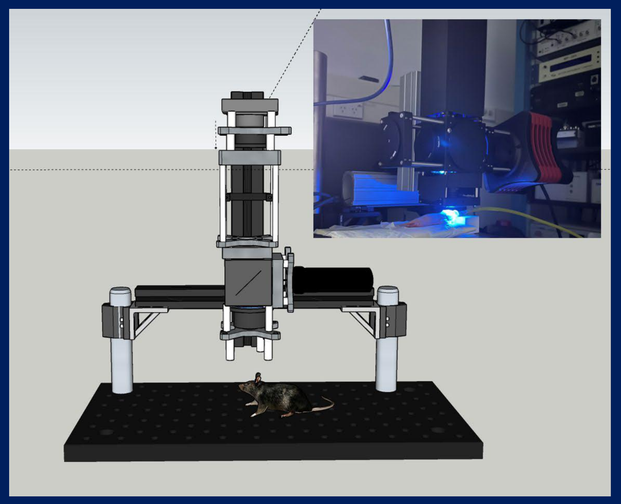


Fig 1. Principle of single-photon and two-photon excitation [3].

Why Mesoscope?
Large Field of View
Image entire mouse cortex
Examine multiple cortical areas simultaneously

Why TPM?
Localized excitation/reduced phototoxicity
Good depth penetration
Excellent resolution (subcellular)



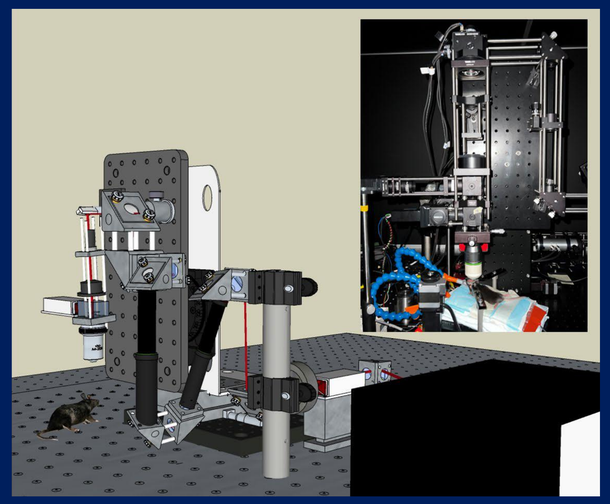


Fig 2.The 3D SketchUp designs of the experimental setup for a) the Mesoscope and b) the Two-Photon Microscope (with photo inset).

Current Directions

- Optimization of the mesoscope for *in vivo* fluorescence imaging in the entire cortical region of the mouse brain.
- Calibrating the TPM for *in vivo* fluorescence imaging in the auditory cortex of mouse brain
- Imaging response to sound in the auditory cortex of ASD mice compared to wild type.
- Genetically encoded Ca^{2+} indicators act as neuronal activity reporters - GCaMP for mesoscope and r-GECO for TPM.

	TPM	Mesoscope
Excitation	Mode locked pulsed laser Wavelength - 1030nm	LED source Wavelength - 470nm
FOV	<1mm x 1mm	>10mm x 10mm
Detection	Photo multiplier tube	CMOS camera

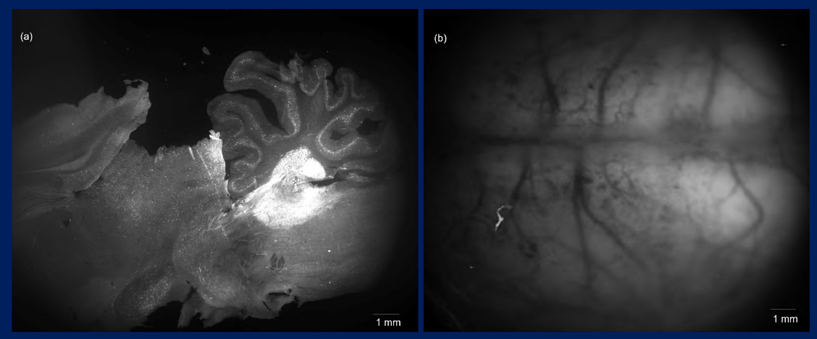


Fig 3. Images acquired using mesoscope (a) *In vitro* calcium imaging of brain slices (b) *In vivo* calcium imaging of neonate rat brain.

Conclusion

- We have built a fully customized TPM with 3D movement and rotation, that has given promising results for *in vitro* calcium imaging of brain slices.
- We have developed a low-cost, reverse tandem lens mesoscope for imaging cortical region of the mouse brain.
- These techniques will be combined to better understand neuronal circuit changes in rodent models of disease, including Autism Spectrum Disorder (ASD) and Spinal Cord Injury.

References

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