Volumetric super-resolution microscopy approaches for investigating synaptic connectivity in the mammalian visual system

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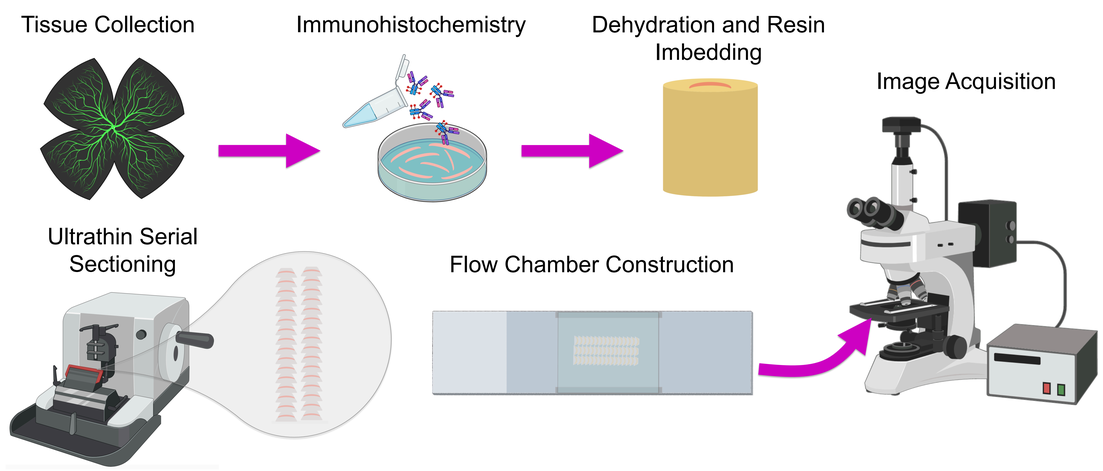
Introduction

Imaging synapses in the brain is difficult due to the diffraction limit of light microscopy, which limits image resolution to ~200nm laterally and ~600nm axially. Super-resolution fluorescence microscopy techniques circumvent this problem, allowing us to visualize subsynaptic molecular interactions. Stochastic Optical Reconstruction Microscopy (STORM) is a single molecule imaging technique that relies on stochastic photoswitching of organic dyes between fluorescent and non-fluorescent to states to produce a resolution of ~20nm laterally and ~50nm axially. Together with ultra-thin serial sectioning, this approach allows for the collection of volumetric super-resolution data. Expansion Microscopy (ExM), on the other hand, is a different super-resolution approach that does not rely on special dyes or instruments. ExM achieves sub-diffraction-limit image resolution by physically expanding the specimen within a swellable polyacrylamide matrix. This technique is exciting in that it offers a simple, fast, and inexpensive method of achieving high image resolution.

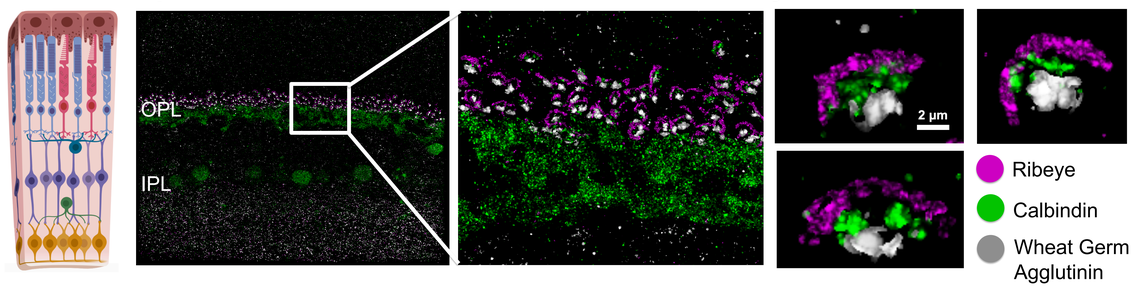
Projects goals:

- Perform volumetric STochastic Optica Reconstruction Microscopy (STORM) imaging and use this approach to test new immunohistochemical markers for visualizing excitatory synapses in the mouse retina
- Develop an assay for performing Expansion Microscopy (ExM) in the mouse retina and brain
- Develop experience in advanced sample preparation including preparation of custom antibody probes

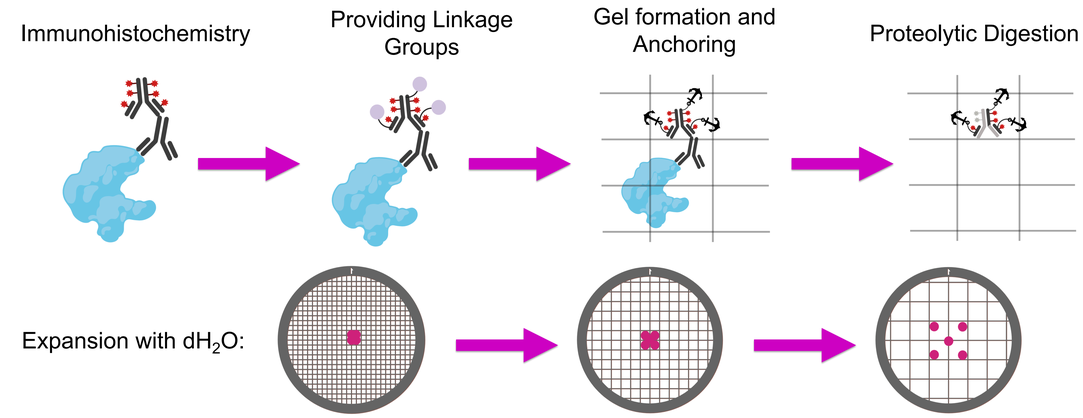
Volumetric STochastic Optical Reconstruction Microscopy (STORM) Methods



Rob Bipolar Connections in the Outer Plexiform Layer of the Mouse Retina

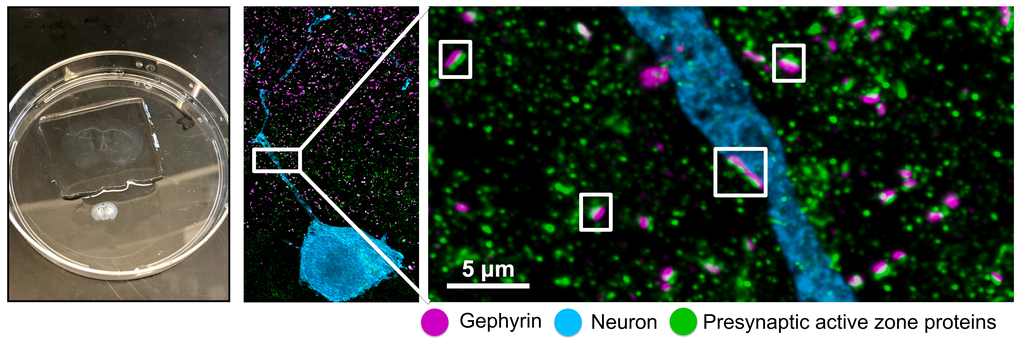


Expansion Microscopy (ExM) Methods



Expansion Microscopy Imaging of Inhibitory Synapses in the Mouse Cortex

Brain tissue expanded yielding ~4x isotropic expansion. Expanded tissue placed above pre-expanded.



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Citations

Chozinski, T. J., Halpern, A. R., Okawa, H., Kim, H. J., Tremel, G. J., Wong, R. O., & Vaughan, J. C. (2016). Expansion microscopy with conventional antibodies and fluorescent proteins. *Nature methods*, *13*(6), 485–488. https://doi.org/10.1038/nmeth.3833

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