Development of a Bessel Beam Sheet Illumination Microscope for Large, Multi-Volume, High Resolution Three Dimensional Imaging of Clarified Tissue

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Abstract

Sheet Illumination has recently gained a lot of attention as a technique, due to many benefits over standard microscopy methods. Decreased phototoxicity, increased signal to noise ratio, and higher photon efficiency are only a few of the reasons why many researchers are beginning to answer sensitive scientific questions with sheet illumination microscopy. Here, a bessel beam is formed through the use of an axicon lens, to generate a long, thin beam with deep-penetrating, self-reforming properties, which is then scanned to the sheet through the use of a galvano scanning mirror. Positioned at 90 degrees to the working distance of the illuminative lens, is a large working distance, refractively corrected detective objective, specifically designed for the observation of clarified tissue samples, such as CLARITY-treated neural tissue, along multiple wavelength channels. Utilizing several high accuracy linear stages, it is possible to take many data sets continuously, to acquire three dimensional information on the order of 1cm cubed, with sub-micron spatial resolution.

Objectives and Goals

- Develop a microscope system capable of scanning an entire clarified mouse brain volume in three dimensions without any physical slicing
- Cut down the time required to take a full brain volume by an order of magnitude
- Automate the system, and design user-friendly software so many researchers can take data
- Exploit the benefits of a Bessel Beam for long, narrow illumination path
- Utilize an electronic line confocality by rolling shutter

Materials and Methods

We utilized sheet illumination to prevent phototoxic damage to the sample over long periods of imaging. The bessel beam begins from a four channel (405/488/561/640nm) fiber-coupled Coherent OBIS laser system, which following a beam expander set, passes through an axicon lens to generate a bessel beam. The beam is then focused onto a scanning mirror, which scans along the focal plane of the camera, in this case the y-dimension. Illumination is provided by a long working distance Mitutoyo 10X/0.2NA objective lens, while detection is handled by a 25X/1.0NA High Index of refraction matched lens from Olympus. The CLARITY–treated mouse brain sample is moved through a custom-designed immersion chamber filled with matching liquid, with the use of a four dimensional zaber series stage (x,y,z,θ). Images are acquired following various emission filters onto a Hamamatsu Flash 4.0 sCMOS camera.

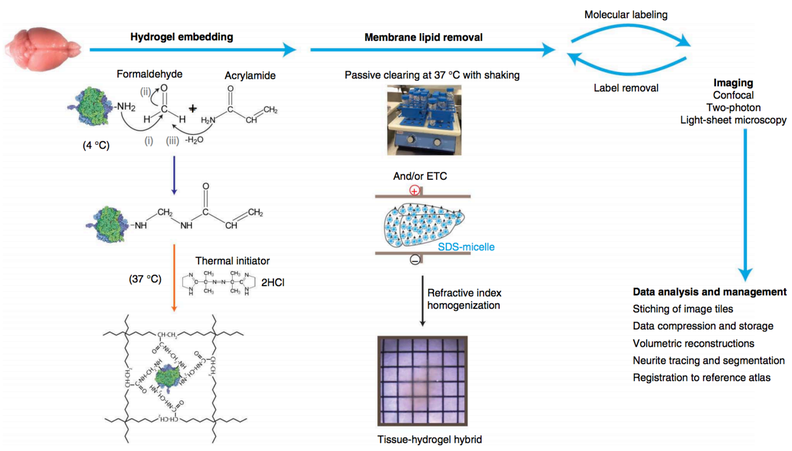
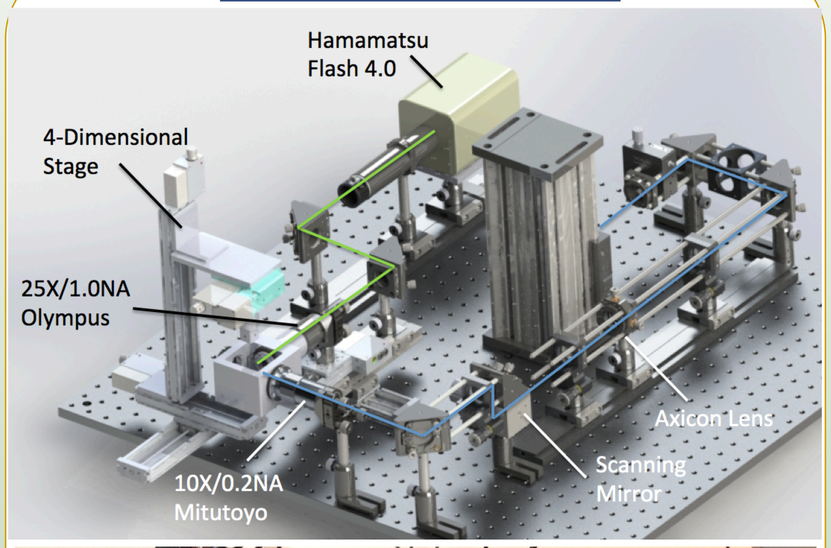


Figure 1: CLARITY treatment of the mouse brain through cellular stabilizing and membrane removal to achieve optical transparency without compromising cellular structure, composition, and dendritic integrity.

Three-Dimensional Visualization



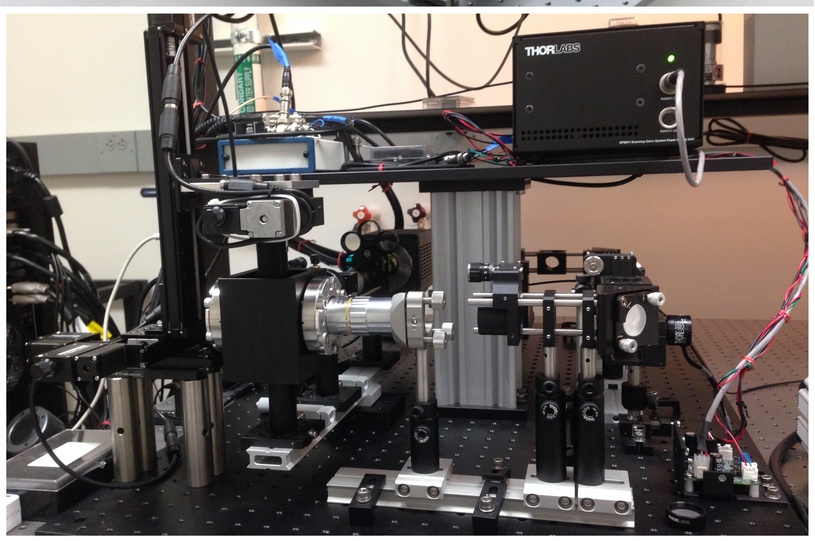


Figure 1: Three Dimensional reconstruction and beam diagram, designed in SolidWorks 2014 and completed system at CNSI user facility

Z-Projection Image

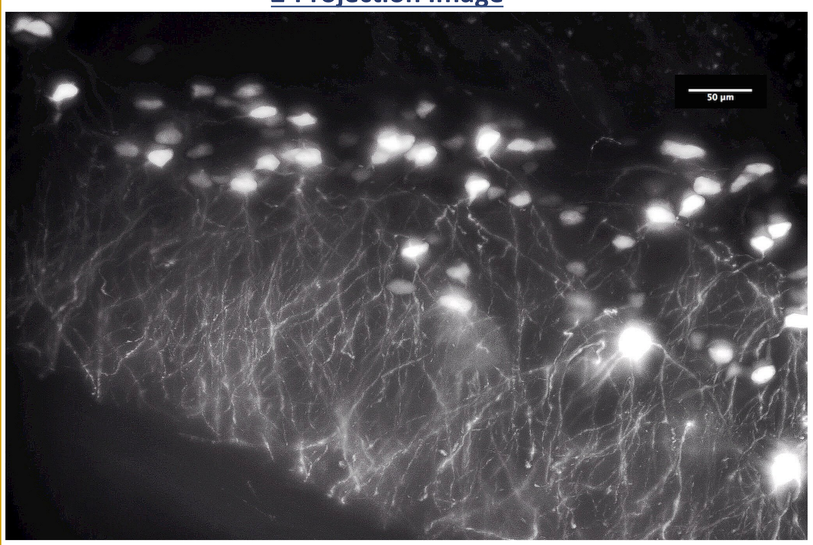


Figure 2: 480 um x 480 um x 1 mm deep of an intact CLARITY treated mouse brain (unsliced). Detecting the fluorescence of ALEXA555 using an emission filter and 2mW of 561nm laser illumination. Images were acquired every 1um in depth, and the image stack was compressed to a single maximum z-projection image over the 1mm volume.

The deep penetration of the bessel beam translates to high image quality even near the center of the brain, sufficient enough to discern axons, dendrites, and cellular bodies with ease.

Programming and Image Processing

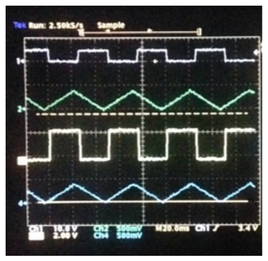


Figure 3: Waveform Diagram

Hardware is synchronized and automated through the use of LabView Software, developed by National Instruments. A TTL external trigger is used to synchronize the camera readout to the scanning of the mirror, and movement of the sample in three dimensions.

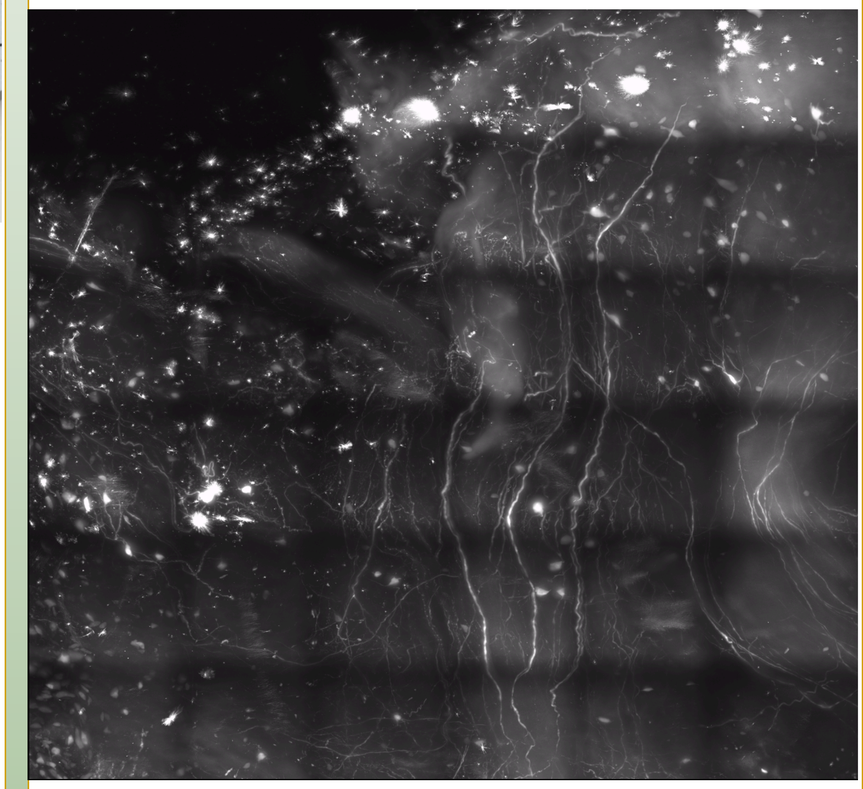


Figure 4: Multi-image acquisition and visualization over a 2.4 mm x 2.4 mm x 2mm volume.

Image volumes are aligned in a 6 x 6 grid, using a 20% overlap between frames. Programs are being developed by the UCLA Brain Mapping Center and the Shattuck Lab to easily visualize datasets which will eventually span 10's of terabytes in total size for a single brain. Eventually, such volumes will be easily viewable in three dimensions, as a powerful research tool for neuroscientists.

Conclusions

The Bessel Beam Sheet Illumination Microscope System has been developed, and in it's preliminary phases is operational. More development and tuning will be required to perfect the system's speed and image quality in the future.

Resources

Figure 1: Diesseroth, Karl. Figure 1. Digital image. Advanced CLARITY for Rapid and High-resolution Imaging of Intact Tissues. Nature Methods, 19 June 2014. Web. 7 May 2015. <http://web.stanford.edu/group/dlab/media/papers/Tomer_2014.pdf>.

Acknowledgements

California NanoSystems Institute Light Microscopy Division: Laurnet Bentollia
Geschwind Laboratory: Luis De la Torre, and Jason Stein
UCLA Brain Mapping Center: David Shattuck
UCLA Department of Physics and Astronomy: Katsushi Arisaka
NSF IDBR and NIH Brain Initiative for support