Development of a SLM-Based Bessel Beam Sheet Illumination Microscope for Large-Scale 3-D Neural Dynamics Observation in C. Elegans and other Model Organisms

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ABSTRACT

Sheet Illumination microscopy has made a large impact on the microscopy community due to its many advantages. Increased photon efficiency allows for lower power light sources, which in turn reduce phototoxic damage to the sample, while providing an increased signal to noise ratio. To take advantage of this technique, a type of phase modulator, known as a Spatial Light Modulator (SLM) is used to generate a deep-penetrating, extremely long and narrow Bessel beam pattern. Through the use of an SLM, one can easily modulate multiple characteristics of the illuminative beam in real time, enabling greater flexibility, ensuring high resolution across multiple scientific applications. A piezoelectric objective collar is used along the detective axis, to enable fast z-dimensional scanning in depth, thereby creating three-dimensional volumes with adequate time resolution to characterize and observe active neural dynamics in several hundred neurons. Such tools will enable the study of large-scale neuronal activity under controlled or experimental conditions in C. Elegans and other model organisms.

OBJECTIVES

- Design a microscope system capable of observing three dimensional neural dynamics in many model organisms
- Utilize the benefits of Sheet Illumination Microscopy to prevent phototoxic effects to the sample, and increase photon efficiency
- Use Bessel beam illumination for deep penetration and a long, thin beam profile.

MATERIALS AND METHODS

A Bessel beam is generated from a linearly-polarized LP 488nm Coherent Sapphire laser reflecting off a Holoeye Phase-Only PLUTO spatial light modulator displaying an axicon pattern. The beam is translated onto two, single-dimension galvano scanning mirrors through the use of 100mm relay lens sets, positioned at twice their focal length. After the second scanning mirror, the beam is focused onto the back focal plane of the illumination 5X/0.2NA Mitutoyo long-WD objective lens. Detection is provided through one of several lenses dependent on application. A 10X/0.3NA air-gap objective from Nikon is well suited to C.Elegans observation, while a 40X/0.8NA water-dipping lens is more tailored to zebrafish observation. The image will then be focused through a 200mm air-spaced achromatic doublet tube lens, to be focused onto a Hamamatsu Flash 4.0 scientific CMOS camera. A custom-designed, translucent sample chamber is used to support the sample, and provide necessary conditions for the organism's survival, dependant on physiology. Because the beam is scanning in two dimensions, a PI-722 piezoelectric objective collar is used to physically move the detection lens' focal plane through the sample in depth, enabling the acquisition of highly-accurate three dimensional volumes at high temporal resolution.

BESSEL BEAM CONSIDERATIONS

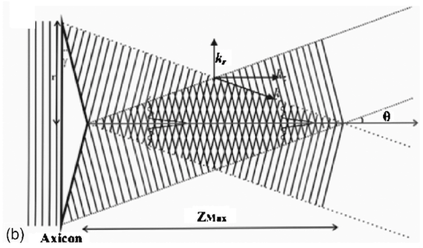


Figure 1: Demonstration of Bessel Beam formation through uniform, radial plane wave collapse by axicon lens

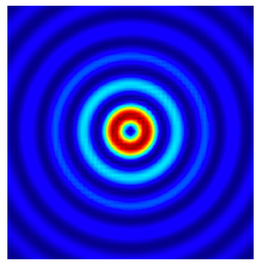
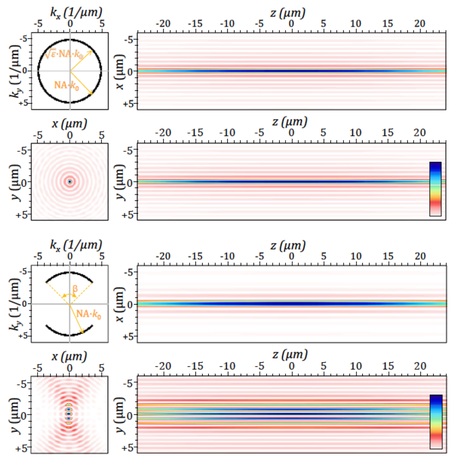


Figure 2: Point-Spread Function of Bessel Beam in the x,y plane

The bessel beam has many beneficial aspects when compared to the traditional Gaussian beam formed through the use of a spherical lens. Bessel beams are formed by the collision of a plane wave radially, resulting in an interference pattern, with a central mode which is far longer, and narrower than that of a standard spherical lens. Illumination paths of up to 1mm in length, and approximately 1um in width are possible, making them ideal for a line-based readout. In addition, the bessel beam posses a self-recovering wave-front, allowing for deep tissue penetration in depth.

One main negative quality of the Bessel beam is the fact that the majority of the energy from the beam itself exists outside of the first central mode. However, due to sheet illumination's improvements in photonic efficiency, only very low laser powers on the order of 5mW are required for adequate signal generation. Only under long-term imaging periods, such as in day-long developmental studies, there exists a potential to introduce phototoxic effects on the sample. However, under experimental conditions, such as chemosensory, thermosensory, or photonic stimulation, we can easily observe C.Elegans' 302 neurons without introducing any phototoxic effect.

SPATIAL LIGHT MODULATOR MOTIVATIONS



Using a spatial light modulator enables researchers to selectively create beam patterns of an arbitrary spatial intensity. Using such a device makes the creation of so-called sectioned Bessel beams possible, in order to maintain the desired long, thin, self-reforming properties while increasing the signal to noise ratio significantly, thereby improving overall image resolution. Sectioned Bessel beams are particularly well-adapted to light sheet microscopy due to the fact that the light intensity is limited before and after the focal plane.

Figure 3: Two dimensional simulations of illumination Point-Spread Functions for traditional and sectioned bessel beams in the x,y plane as well as along the illumination axis. Farbach 2013

SPATIAL LIGHT MODULATOR CONCEPT

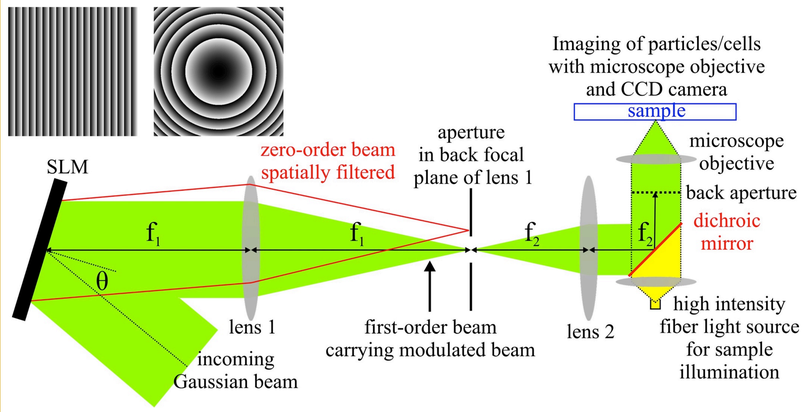


Figure 4: Schematic demonstrating the principle of spatial light modulation through spatial filtering of lower-orders of diffraction by implementation of a superimposed prism grating

HARDWARE DEVELOPMENT AND FLEXABILITY

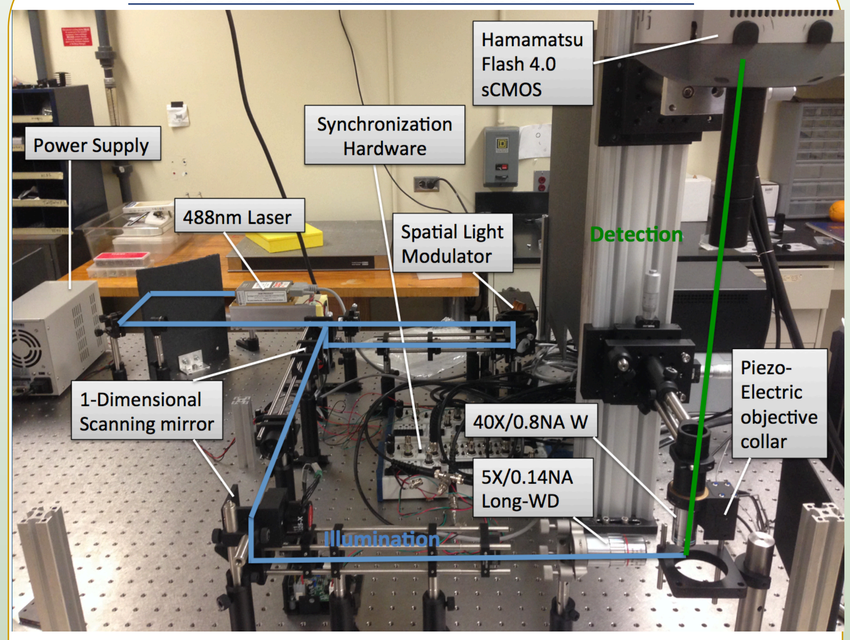


Figure 5: Hardware is developed, built and optically tuned at UCLA department of Physics and Astronomy

PRELIMINARY DATA ACROSS MODEL ORGANISMS

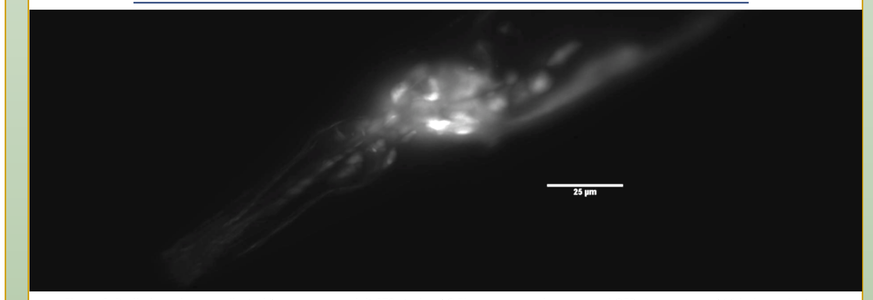


Figure 6: Preliminary image collected from a young adult ST2 strain of C.Elegans expressing neuronal GFP. A sequence of 11 independent 20ms exposures were collected, each approximately 1.5um apart in depth, and projected on top of one another to a single two dimensional field. A laser power of 5mW was used with a 1um Bessel beam phase mask on the SLM.

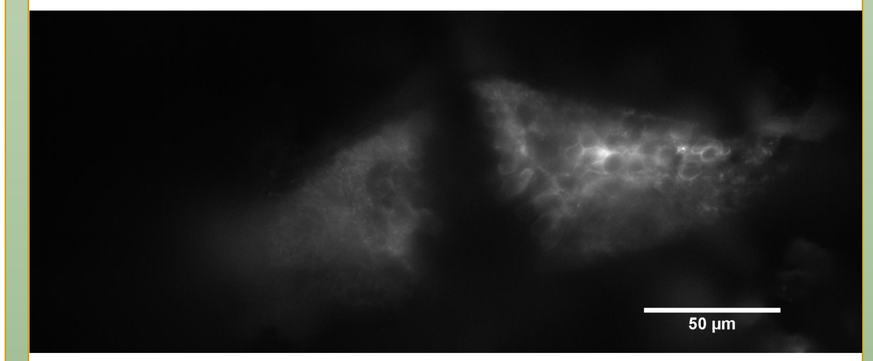


Figure 7: Preliminary image collected from a 5 day old, GCaMP5 labeled zebrafish cerebellum. A laser power of 15mW was used, for a 20ms exposure time on the Hamamatsu Flash 4.0 sCMOS camera. The FOV of this image has been cropped to 300um x 120um. Each neural soma in the adolescent zebrafish measures between 6-7 microns in diameter, and are visualized with high resolution.

RESOURCES

Figures:

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4) Kishan, Dholakia. Figure 1. Digital image. Spatial Light Modulators. Photonics4Life, 2010. Web. 7 May 2015. <http://www.photonics4life.eu/Consortium/P4L-DB/All-items/Spatial-light-modulators>

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