

Development of a Bessel Beam Sheet Illumination Microscope for Large-Scale 3-D Neural Dynamics in Model Organisms

BLAKE MADRUGA, Paul Chin, William Chen, Edward Polanco, Christopher Carmona, Addam Hammond, Lindsay Ling, Katsushi Arisaka
UCLA, Department of Physics and Astronomy

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 detection 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 across multiple model organisms.

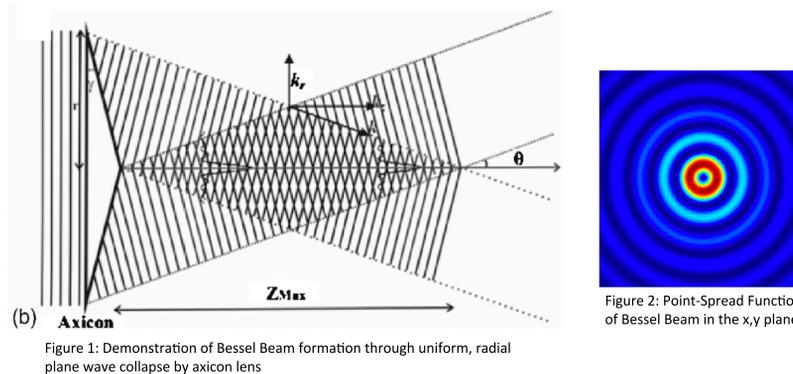
OBJECTIVES

- Design a microscope system capable of observing three dimensional neural dynamics in 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 in 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 a 40X/0.8NA water-dipping lens from Nikon, focused through a 200mm air-gap achromatic doublet tube lens, to be focused onto a Hamamatsu Flash 4.0 scientific CMOS camera. A custom-designed, translucent water-filled chamber is used to support the sample, and provide necessary conditions for the Zebrafish's survival. 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



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 1μm in width are possible, making them ideal for a line-based readout. In addition, the Bessel beam possesses 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. Under long-term imaging periods, such as in developmental studies, there exists a potential to introduce phototoxic effects on the sample. However, under experimental conditions, such as visual virtual reality stimulation, or short term behavior studies, we can easily observe several hundred neurons without introducing any phototoxic effect.

SPATIAL LIGHT MODULATOR MOTIVATIONS

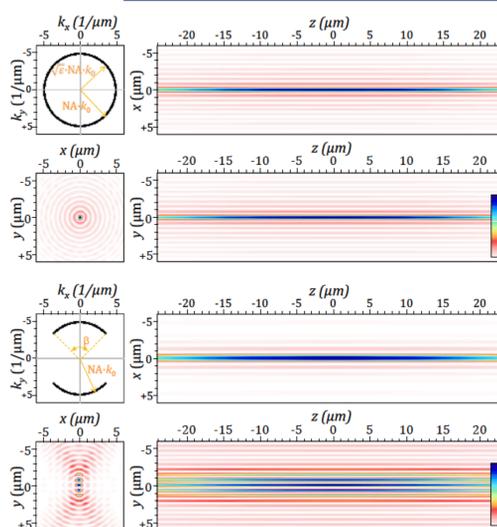


Figure 3: Two dimensional simulations of illumination Point-Spread Functions for traditional and sectioned Bessel beams in the x,y plane as well as along the illumination axis. Farbach 2013

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.

SPATIAL LIGHT MODULATOR CONCEPT

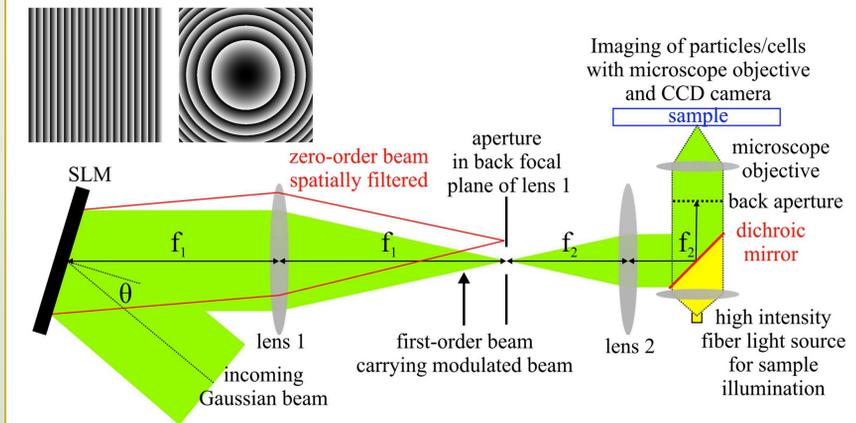


Figure 4: Schematic demonstrating the principle of spatial light modulation through filtering out lower-orders of diffraction

HARDWARE DEVELOPMENT AND FLEXIBILITY

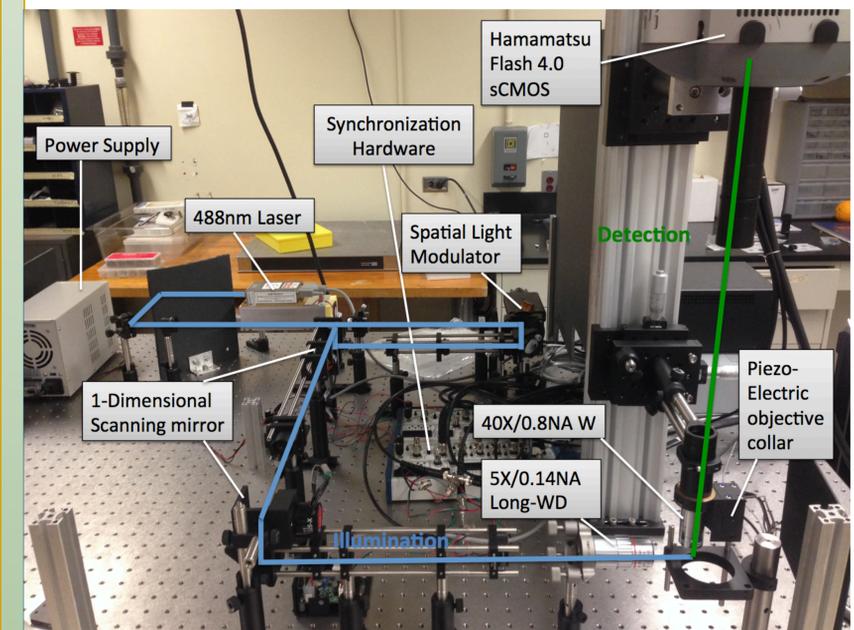


Figure 5: Hardware is developed, built and optically tuned at Knudsen Hall, B-171.

Perhaps the most important aspect of this research project is the development of a system capable of acquiring data from multiple model organisms, to answer any multitude of scientific questions.

- **Zebrafish**
 - Short-Term Three-Dimensional Neural Dynamics
 - Sectioned Bessel Beam Light Sheet
 - Up to 100 Frames/Second Frame Rate
 - 150μm deep scanning in depth
 - ~1000 Neuron Observation
 - Long Term Three-Dimensional Cellular Development Study
 - Low power Bessel Beam Light Sheet
 - Automated long-term data acquisition
- **C. Elegans**
 - Three-Dimensional Neural Network Studies
 - Whole-Worm Fluorescent 3-D imaging
 - ~100 Neuron Observation

RESOURCES

- Figures:
- 1) Anguiano-Morales, Marcelino. F2. Digital image. Conical Dynamics of Bessel Beams. SPIE, 2 July 2007. Web. 2 May 2015. <<http://opticalengineering.spiedigitallibrary.org/article.aspx?articleid=1088425>>
 - 2) Bouchal, Z. 1c. Digital image. NONDIFFRACTING PROPAGATION & SELF-RECONSTRUCTION OF LIGHT BEAMS. UPOL, n.d. Web. 3 May 2015. <http://thunder.upol.cz/optics/research/nondiffracting_beams/>
 - 3) Farbach, Florian O. "MICROSCOPY WITH SELF-RECONSTRUCTING BEAMS." Diss. Albert-Ludwigs-Universität, 2013. Print.
 - 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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