Development of a user-configurable SLM-based sheet illumination microscope for rapid, large-scale, video-rate 3-D neurodynamic observation in multiple model systems.

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ABSTRACT

Sheet Illumination microscopy has made a large impact on the microscopy community due to its many inherent advantages. Increased photonic 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 such technique, a Spatial Light Modulator (SLM) is used to generate a deep-penetrating, extremely long and narrow Bessel beam interference pattern. Using an SLM, one can easily modulate the phase characteristics of an illumination beam in real time. This property enables greater flexibility and aberration compensation at the sample. The Bessel beams are generated by displaying the beam's modulation transfer function on the SLM with a prism phase rotation to spatially separate inherent diffractive orders. The beams are then mapped onto the readout of two scientific CMOS cameras for rapid multi-channel imaging. A piezoelectric objective collar is used to enable z-scanning, thereby creating 4D image volumes with adequate time resolution to characterize and observe active neural dynamics. A long working distance, high numerical aperture, refractive-corrected objective lens is used to study neurobiology and participate in ratiometric calcium imaging. Tools of such flexibility will enable the study of whole-brain-scale neuronal activity and structure in many model organisms, under various controlled conditions over multiple temporal and spatial scales.

INTRODUCTION

To a large extent, the form a microscope system takes is largely dependent on the scientific aims of the research to be conducted. Typically, this fact results in systems that are highly optimized for capturing single type of target data. Our aim is to develop a highly versatile imaging platform that is capable of imaging various model organisms in numerous spatial and temporal resolutions without inter-trial hardware modifications. As such, we created a scanned light sheet system for many of its inherent benefits over traditional epi-fluorescent geometries.

OBJECTIVES

- Design an optical 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 photonic efficiency
- Configure software tunable Bessel Beam illumination for deep penetration and a long, thin beam optimized to each scientific specification.

MATERIALS & METHODS

A Bessel beam is generated from a linearly-polarized 350mW 488nm Coherent Sapphire laser reflecting off a Holoeye Phase-Only PLUTO spatial light modulator displaying custom-generated phase masks. 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 tracking and observation, a 25X/0.95NA water-dipping lens is more tailored to zebrafish, and a 25X/1.0NA lens for clarified neural tissue. 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 physiological conditions for the organism's survival. Because the beam is scanning in two dimensions, a PI-725 piezoelectric objective collar is used to physically move the detection lens' focal plane 400µm through the sample in depth, enabling the acquisition of highly-accurate three dimensional volumes at high temporal resolution.

BEAM SHAPING AND OPTICAL CORRECTIONS

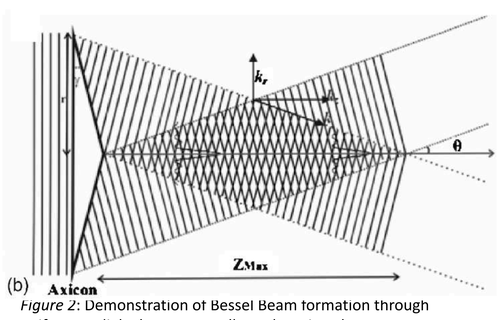


Figure 2: Demonstration of Bessel Beam formation through uniform, radial plane wave collapse by axicon lens

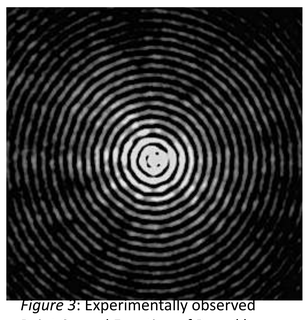


Figure 3: Experimentally observed Point-Spread Function of Bessel beam

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.

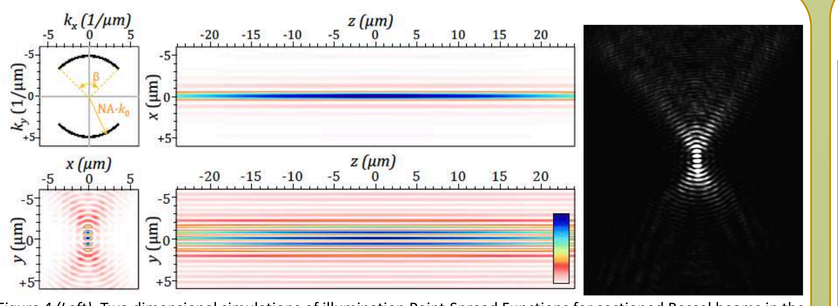


Figure 4 (Left): Two dimensional simulations of illumination Point-Spread Functions for sectioned Bessel beams in the x,y plane as well as along the illumination axis. Farbach 2013 *Figure 4 (Right)*: Experimentally measured sectioned BB

SLMs enable 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. A spatial light modulator also enables the pre-distortion of the wave-front prior to illumination objective, allowing for video-rate aberration correction.

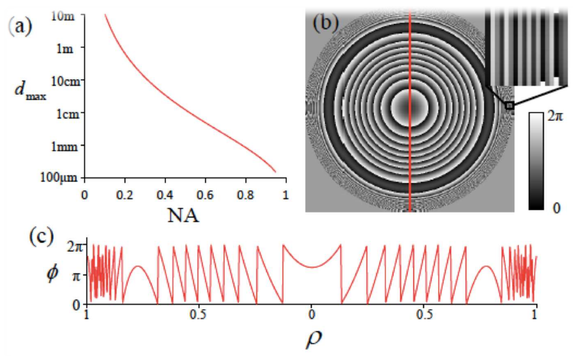


Figure 5: Compensation of third and fifth order spherical aberration through pre-distortion pattern generation. These phase profiles apply an equal and opposite wave-front modification to correct for refractive-mismatch induced losses in resolution. This modification will restore the PSF of the illumination beam to its original state, and maintain diffraction limited beam widths across hundreds of microns.

HARDWARE DESCRIPTION AND BEAM PATH

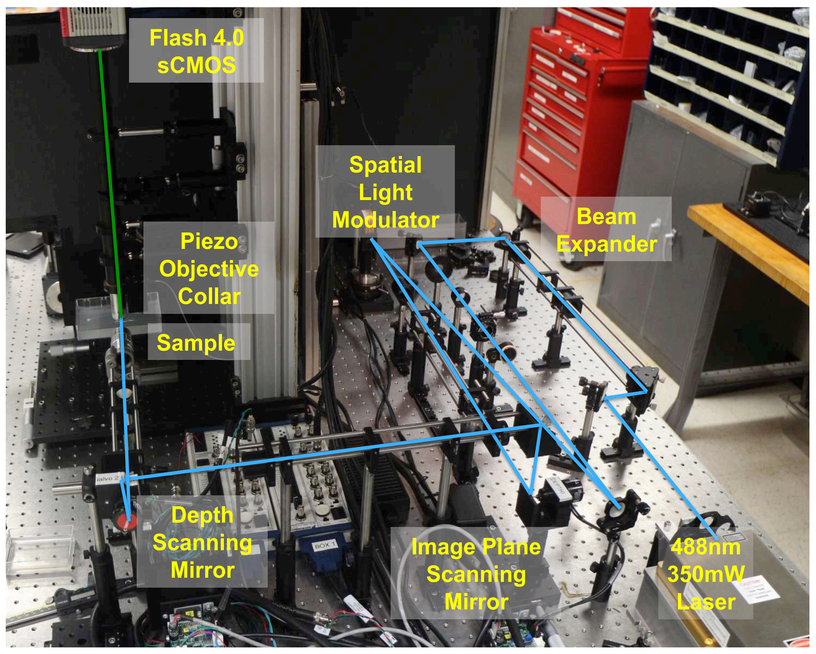


Figure 1: Conceptual design of the SLM-BB system's illumination and detective paths. Please note that the expanded view is the detection path, and it is portrayed in a rotated fashion to more clearly demonstrate the system's operation and geometry.

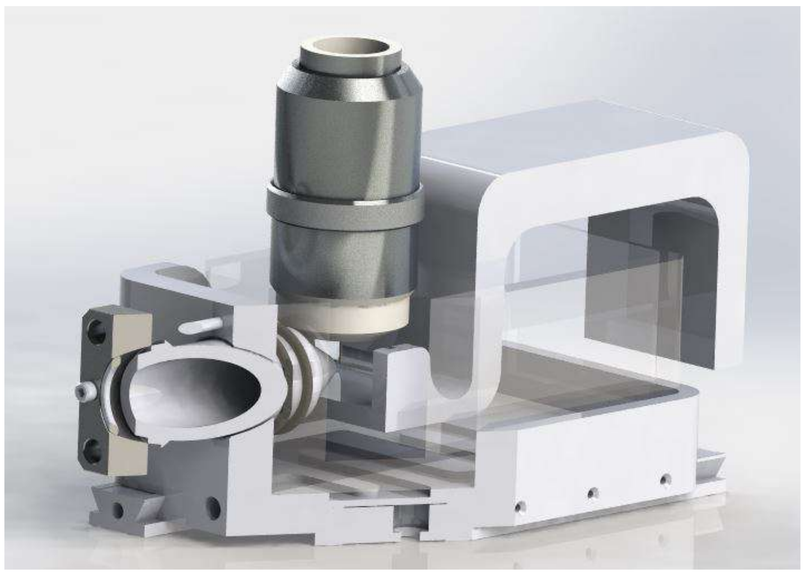


Figure 3: 3D Solidworks rendering of the sample chamber, illuminative and detection optics, along with supporting sample arm and dovetail optical alignment rails.

PRELIMINARY DATA

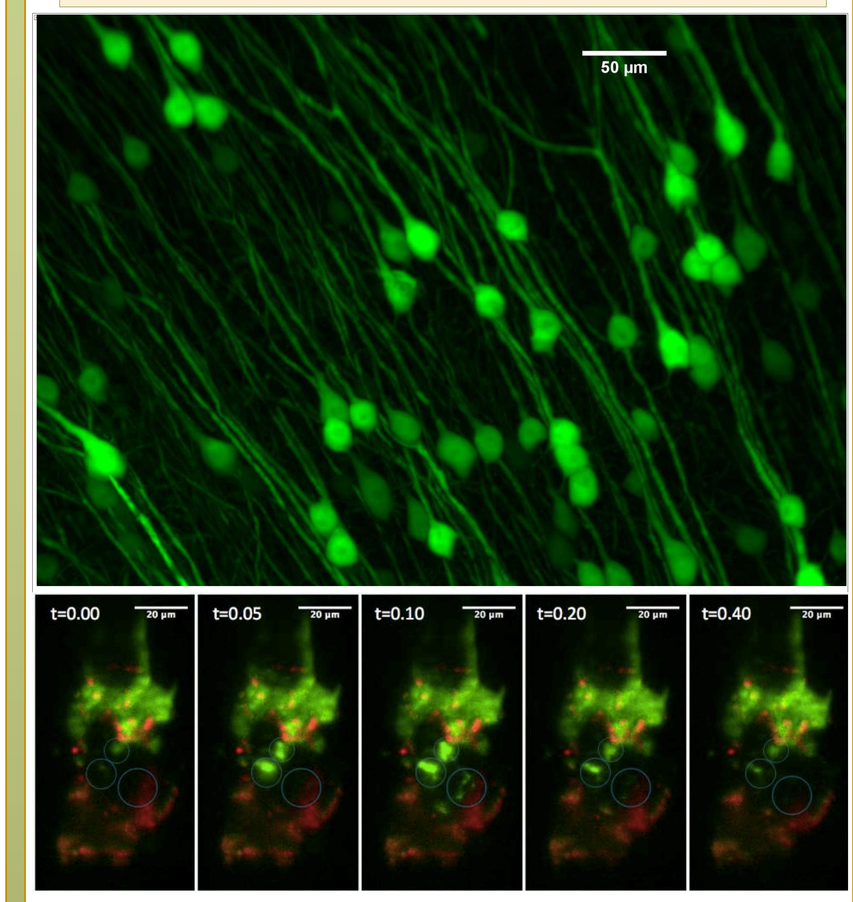
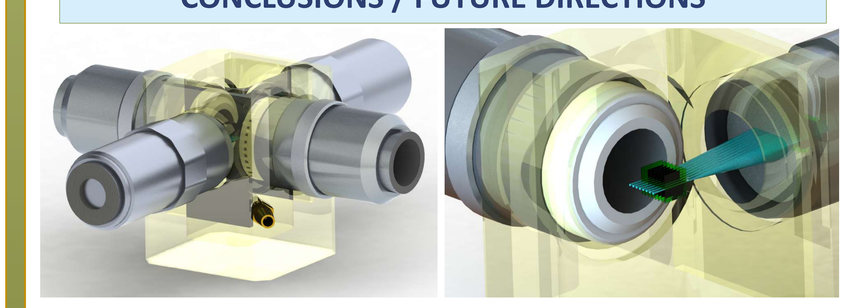


Figure 6 (Top): Preliminary image collected from a 5 day old, GCaMP5 labeled zebrafish cerebellum. A laser power of 15mW was used, for a 20ms exposure time on the Hamamatsu Flash 4.0 sCMOS camera. The FOV of this image has been cropped to 300um x 120um. Each neural soma in the adolescent zebrafish measures between 6-7 microns in diameter, and are visualized with high resolution. *Figure 6 (middle)*: Region of the hippocampus taken from an in-tact GFP expressing RIMS clarified brain. This image is a maximum projection stack of one thousand images in depth, each with a pitch of 1um. Somas are clearly visible, as well as processes and dendritic growths. *Figure 6 (bottom):* 3D, Two color neurodynamic imaging of C. Elegans, strain QW1217. Cytosol-expressed GCaMP6 and nucleus-expressed RFP taken at a rate of 50 image volumes / second. These images demonstrate the flexibility of the SLM-BB system.

CONCLUSIONS / FUTURE DIRECTIONS



The SLM-BB system has performed well in numerous model organisms over a multitude of temporal and spatial requirements. Methods involving spatiotemporal multiplexing and improved volumetric sampling rates are being developed currently, to resolve rapid neuro-dynamic events *in vivo*.

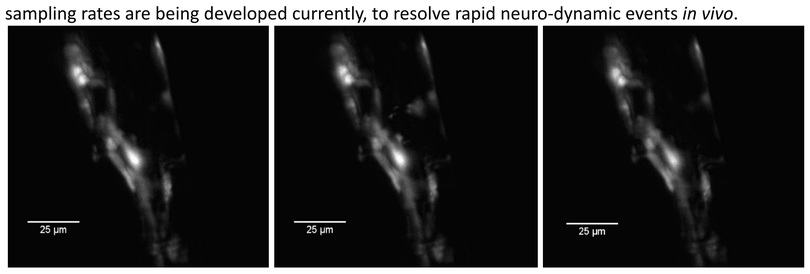


Figure 8(top): SolidWorks design of the 3D-tracking, three lens illumination system currently in development.
Figure 8(bottom): Preliminary dynamic maximum-intensity projection Ca2+ images of C. Elegans taken at 40Hz volume rate, 50um in depth, with the newly-machined sample chamber. Data processing and de-convolution methods are in progressed.

REFERENCES & ACKNOWLEDGEMENTS

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