High-Speed Laser Line Scanning Confocal Microscope for 3D neural imaging In a freely behaving c.elegans under a localized thermal stimulation

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Introduction

The goal of the presented research is to study the perception and navigation through space of a freely behaving c. elegance under a localized thermal stimulus. We will investigate the ability of the AIY, a first layer interneuron and the only postsynaptic partner to AFD thermosensory neuron, to integrate thermal information to return a specific well-defined behavioral phenotypes.

This will be realized with a laser line scaning confocal microscope capable of a localized thermal stimulation, real time worm tracking and fast imaging. It will be capturing captures dynamic Ca+2 signals in Cameleon labeled neurons of a freeliy navigating c. elegans.

Prototype Epi-Fluorescence Microscope

The initial approach is to build a simple epi-fluorescent microscope and then gradually upgrade it to the laser line scanning confocal microscope.

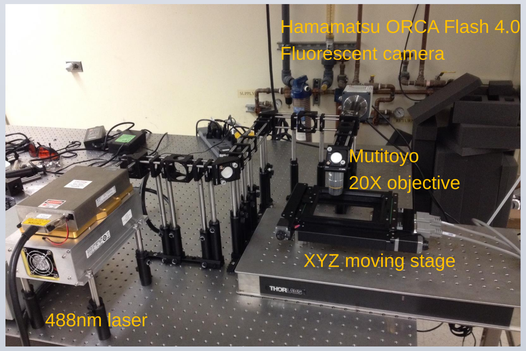

Figure 1: A photograph of the epi-fluorescent microscope.

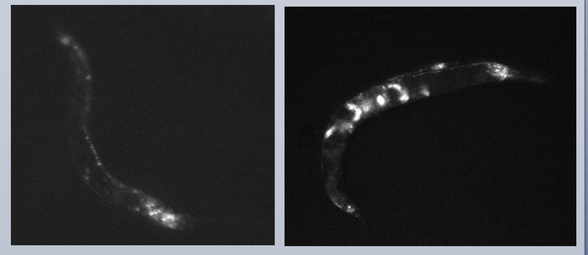

Figure 2: Images showing the GFP labeled sensory neurons in c.elegans taken with a 20X Plan Fluorite Nikon objective lens (left) and 10X APO Mutitoyo objective lens.

Design Concept

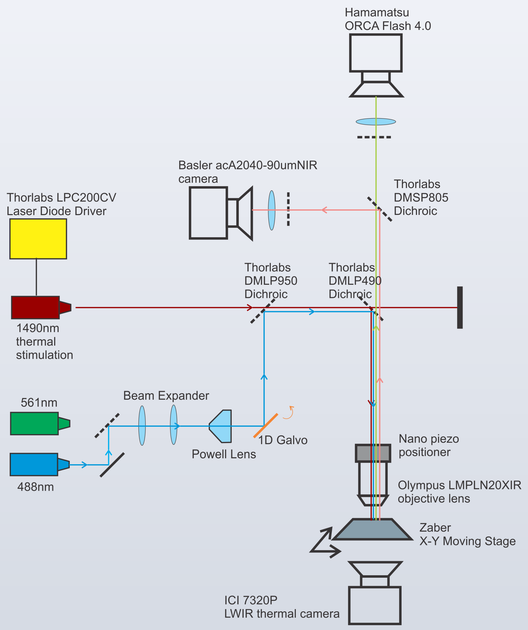

Figure 3: The proposed design of the line scanning confocal microscope for the fast 3D imaging of a freely behaving C. Elegans with a virtual reality thermal stimulation

The microscope will utilize the IR laser for a localized thermal stimulation of c.elegans. The position and the temperature of the "hot spot" will be monitored using a thermal camera. The microscope will be capable of a real-time worm tracking and fast data acquisition at more thanan 100fps.

Optical Simulations

Comprehensive optical simulations were performed using similar optical components as in the conceptual design.

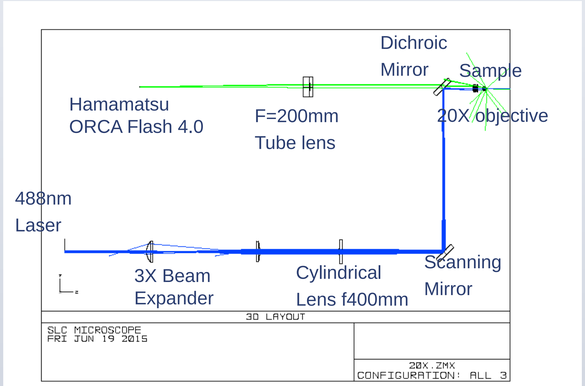

Figure 4: Laser line scanning confocal microscope layout for optical simulations. Blue is the laser excitation beam, green is the fluorescent light.

Simulation Results

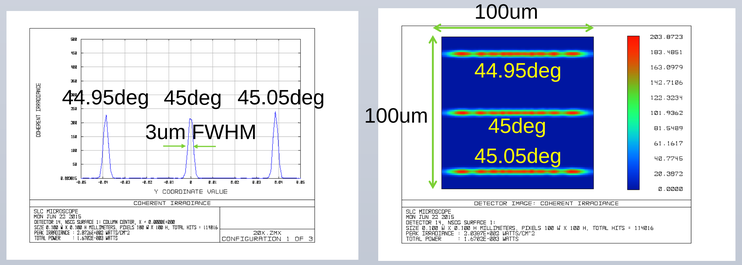

Figure 5: Simulated image of the laser line at the objective focal plane at various angular position of the scanning mirror (right). The intensity profile of the laser line is shown on the left.

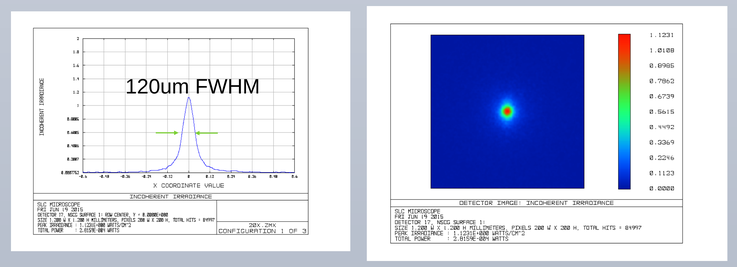

Figure 5: Simulated fluorescent image of the 5um fluorescent sphere placed at the objective focal plane taken with the camera sensor (right). The Point Spread Function of the sphere is shown on the left.

3D imaging with the epi-fluorescent microscope

We attempted for a 3D reconstruction of the c. elegans neurons by scanning the worm in z-coordinate. We took several images at various z-position of the stage with a step of 5um which were stacked for the 3D image reconstruction.

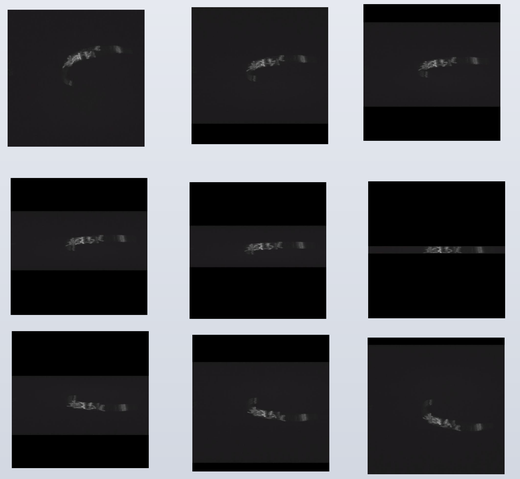

Figure 7 A 3D reconstruction of the GFP labeled sensory neurons in c.elegans. The image was reconstructed from a stack of images taken at various z-position of the stage scanning across c. elegans.

Laser Line Scanning confocal Microscope status

- All the components have been purchased
- Worm tracking Labview software for the X-Y stage manipulation synchronized with the IR camera is complete

Future Directions

- Complete the installation of the optical components
- Complete writing the Labview routines for laser line scanning and the thermal stimulation laser system
- First results are expected by the August 2015

Acknowledgments

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http://www.elegantmind.org