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. elegans 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 scanning confocal microscope capable of a localized thermal stimulation, real time worm tracking and fast imaging. It will be capturing dynamic Ca²⁺ signals in Cameleon labeled neurons of a freely 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.

Design Concept

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 than 100fps.

Optical Simulations

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

Simulation Results

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.

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

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

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 6: Simulated fluorescent image of the 3um 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.

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