Design of High-Speed Line Confocal 3D Imaging Microscope System

Timothy R. Sherry and Katsushi Arisaka

Elegant Mind Club, UCLA, Department of Physics and Astronomy

Introduction

- This project utilizes the basic construction of an epi-fluorescence microscope.
- The typical system uses:
  - high powered light or LED to illuminate the sample at the appropriate excitation wavelength for the fluorophore.
  - High NA Objective
  - Emission and Excitation filters
  - Dichroic Mirror
- How can this system obtain higher resolution?
- How can this system obtain higher speeds of read out?
- How can this system be used to obtain 3D images?

Hypothesis

- Increased resolution, higher speeds, and 3D images can be obtained by utilizing a galvanometer (galvo) to scan a high powered laser across the sample followed by de-scanning of the beam on to the sCMOS sensor using a rolling shutter mode to achieve confocality.

Proposed Line Confocal High Speed 3D Imaging System

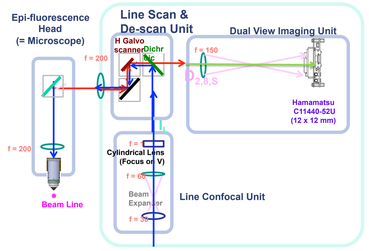

Figure 1: This is the initial blue print for the high speed, 3D imaging, line confocal microscope that utilizes a 40 x objective with the proposed Hamamatsu Flash 4.0 Orca sCMOS Camera.

Initial Approach

- Optical Simulations and a custom built epi-fluorescence microscope base system
- Illumination arm:
  - 470 nm LED
  - Focusing lens
  - 488 nm Excitation Filter
  - Objective (40x 0.17 mm WD, 0.65 NA, Air gap)
  - Objective (40x 3.5 mm WD, 0.8 NA, Water Immersion)
- Detection arm:
  - emission filter
  - Andor iXon EMCCD camera with focusing lens
  - Emission filter
- Sample was placed on a 3D manual stage allowing for micron resolution adjustments.

Optical Simulations

- We used Zemax optical software for modeling the system through ray tracing.
- We used all lenses with visible coatings, and put them together to generate the theoretical Point Spread Function (PSF) of the sheet of light.
- The PSF was calculated using the Huygens' approximations to get an accurate prediction for the beam.
- At Full Width Half Maximum (FWHM), the width of the sheet of light is .974 microns.
- The predicted sheet seen below has also been seen below in the prototype microscope in Figure 8.

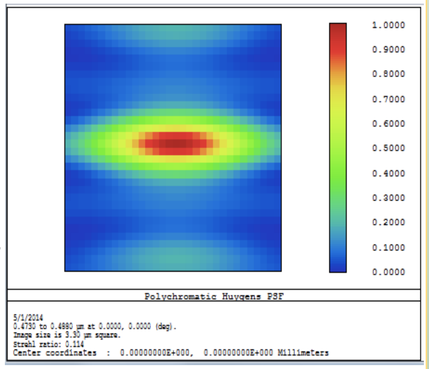

Figure 2: This is colorized Huygens' PSF formed through the 40x objective.

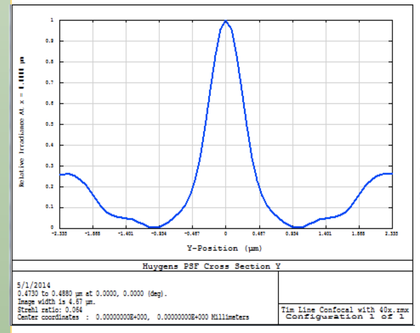

Figure 3: This is the beam PSF cross section as calculated with the Huygens' model.

Zemax Beam Shape Simulations

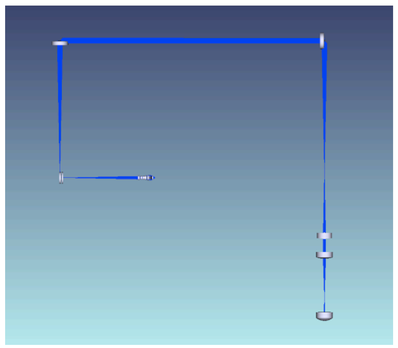

Figure 4: This is the model beam path from the first beam expander, through the cylindrical lens, past the second set of lenses to the 40x objective.

Standard Epi-Flourescence Microscope

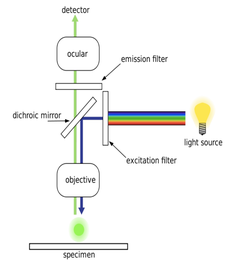

Figure 5: This is the standard configuration for any epi-fluorescence microscope. It forms the base parts for our new 3D imaging line confocal microscope.

Prototype Epi-Fluorescence Microscope

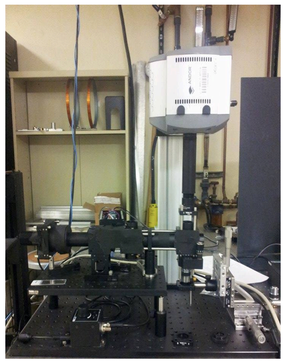

Figure 6: This is the initial prototype that serves as the base epi-flourescence microscope that will be converted to the new experimental line confocal design.

Initial imaging

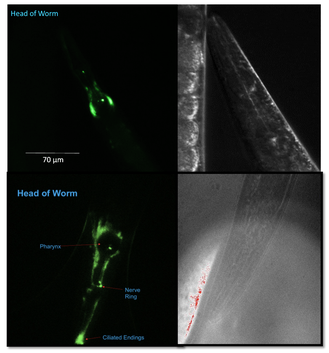

Figure 7

- Upper left is *C. elegans* with GFP in about 40 neurons, most of which are ciliated sensory neurons (Strain SX620).
- Upper right is the non-filtered version of the upper left image.
- Bottom left is another *C. elegans* with GFP labeled sensory neurons (Strain SX620).
- Lower right is the non-filtered version of the bottom left image
- Both samples were prepared on slides and transferred using M9 buffer with no agarose on the slide.
- Both images are 210 x 210 microns, and the scale bar is 70 µm.
- Top two images taken with WD Nikon 40x NA=0.8
- Bottom two images taken with standard 40x NA=0.65

Full Prototype Development

- We mounted the laser to a heat sink and set up the optical rails to allow for easy alignment of the different optical components.
- Each optic was aligned one piece at a time to ensure that the laser followed the beam path seen in Figure 4.
- The laser was focused through the cylindrical lens onto the galvo to allow for smooth scanning of the beam and future de-scanning as well.
- The light sheet was then focused onto the back focal plane of the 40x objective.
- We then used a labview driver to send voltage signals to the galvo to achieve beam scanning.

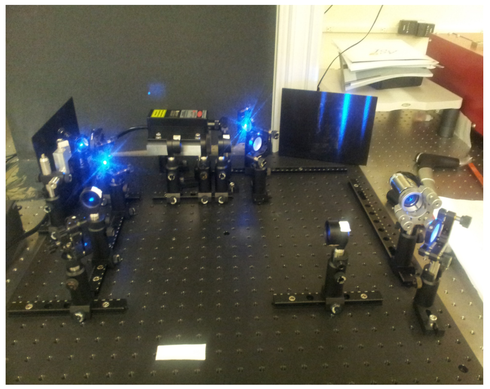

Figure 8: This is the first prototype of the line confocal system set up. It is utilizing a 470 nm laser for alignment purposes with a Cambridge Technologies galvo, and a 40x Water Dipping NA=0.8 Nikon Objective.

Future Directions

- Replace the LED illumination on the epi-flourescence microscope with the line confocal system.
- We will then attach the faster camera and the dichroic mirror with filters.
- We then need to sync up the Flash 4.0 Orca camera's rolling shutter with the scanning galvo using micromanager to control both pieces of hardware.
- Then to achieve 3D images, we will attach a PI Piezo scanner to allow the system to take Z-stacks through the worms at high speeds and precision.
- Once these systems are synced up, we will be able to image more live samples and also use it to observe C. elegans, Drosophila larvae, and slides of tissues.
- We will also incorporate a DMD module to allow for optogenetic stimulation of the samples for future studies.

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