

Building a Confocal Laser-Scanning Microscope (CSLM) and Writing GUI-Based Control Software for Biological Imaging Purposes

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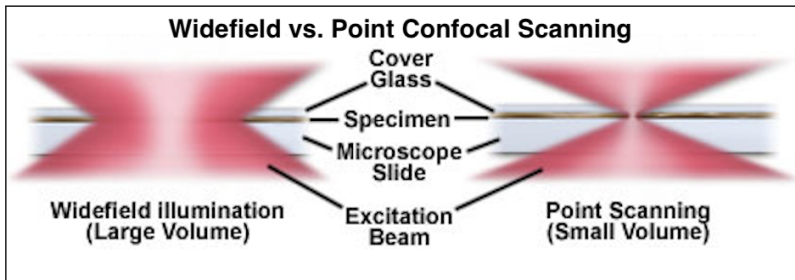
Motivation for Research

- Widefield fluorescence microscopy ("WFM") is limited
- Confocal microscopes provide numerous advantages over WFM
- Commercial research-grade confocals are very expensive (Leica, Nikon)
- A home-built confocal can overcome monetary limitations while still providing ample functionality
- We build a laser-scanning confocal microscope from scratch

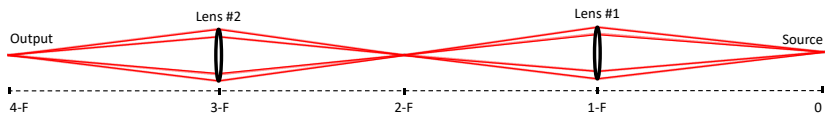


Widefield Fluorescence Microscopy (WFM) Background

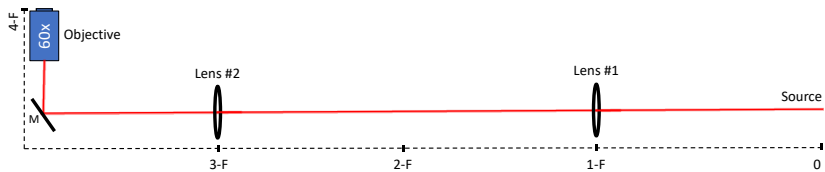
- For in-vivo biological imaging, WFM has been paramount
- It is limited in resolution, specifically for thick samples
- CSLM was invented by Marvin Minsky in 1955
- Designed to overcome WFM limitations by eliminating secondary fluorescence



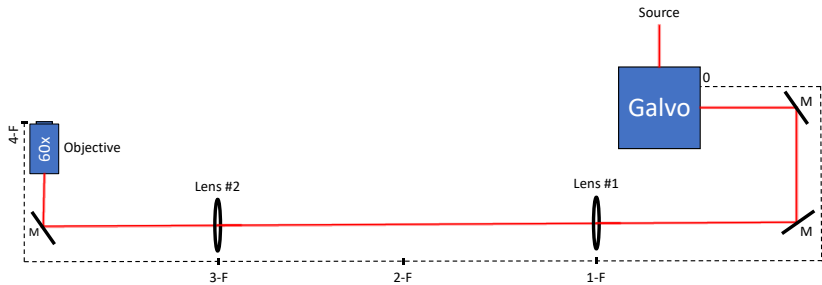
Optical Setup



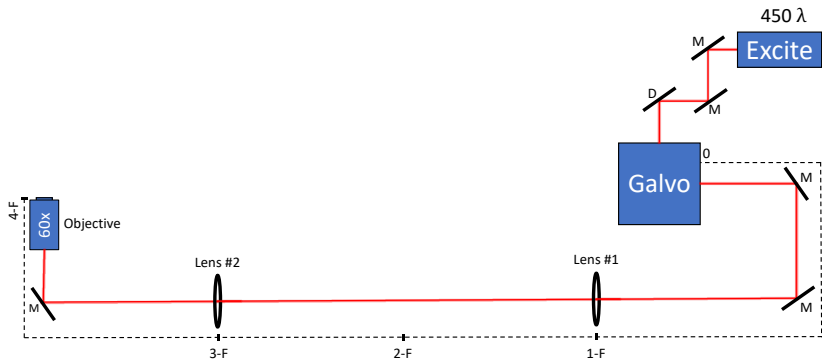
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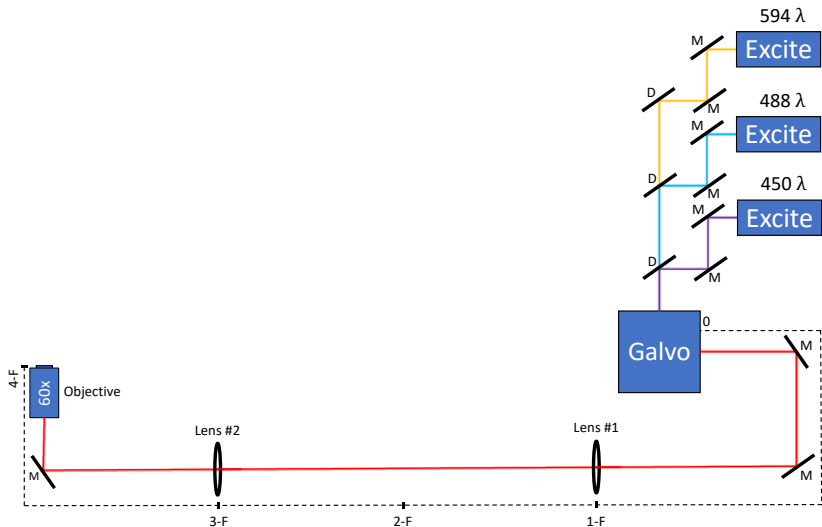
Optical Setup



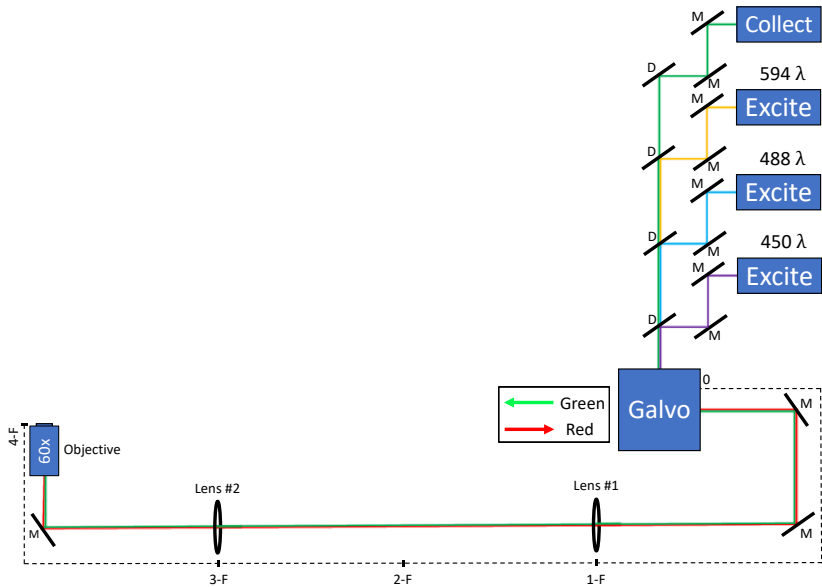
Optical Setup



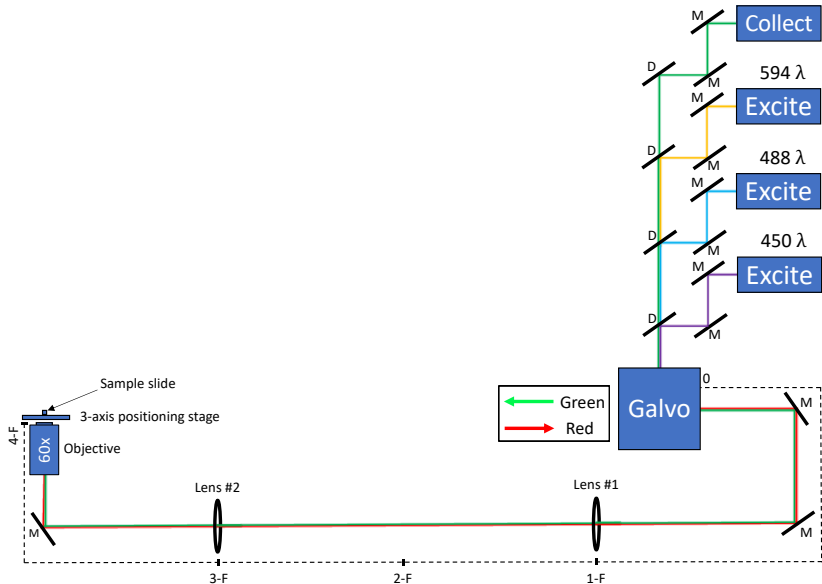
Optical Setup



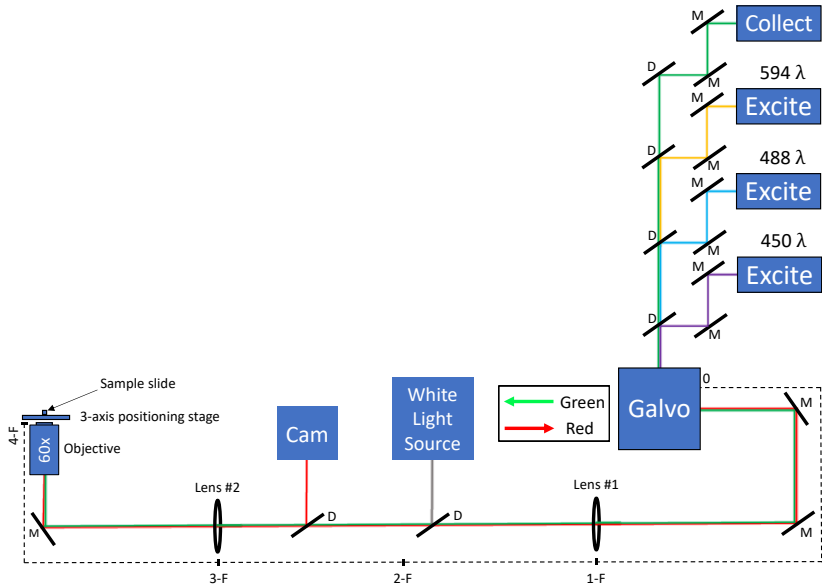
Optical Setup



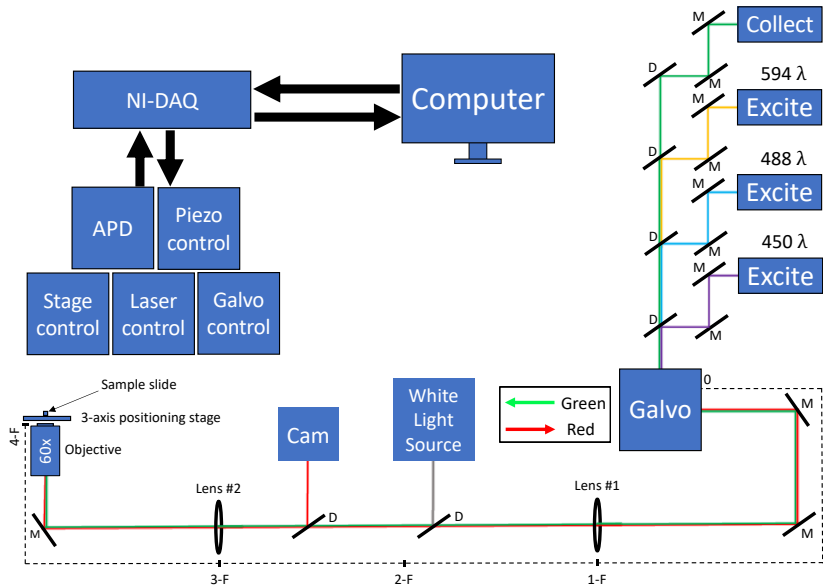
Optical Setup



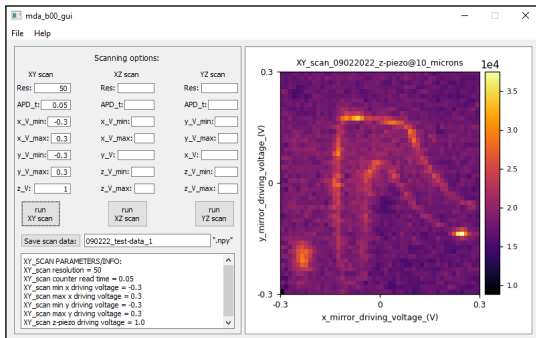
Optical Setup



Optical Setup



- Previous summer work included writing software package for CSLM with different applications (2D materials)
- National Instrument's API ("NI-DAQmx")
- PyQt5/6 Library
- Additional ThorLabs APIs



- Given complete hardware functionality potential, software is being written modeled after Leica Microsystems for ease of use

Options:

- Microscope control
- Multi-spectral acquisition
- 3D-sampling
- Spectrum collection
- Dye finder
- Quantification
- Time laps
- Image processing
- Multi-position imaging
- Modules for FRET,FRAP, FLIM

Configuration Acquire Process Identify

Experiments Setup Acquisition

Acquisition Mode: spt

Image format: 32x32

Scan speed: 400 Hz

Zoom factor: 1

Image dimensions: 1280x1024

Scan field rotation: 0.0

3D visualization: Begin, End

Slit/FWHM Settings

Channel 1: 9% 9% 9%

Channel 2: 9% 9%

Channel 3: 9% 9% 9% 9% 9% 9% 9% 9%

Spectrum

Specimen

Additional Channels

PART 1: Name, Active

PART 2: Name, Active

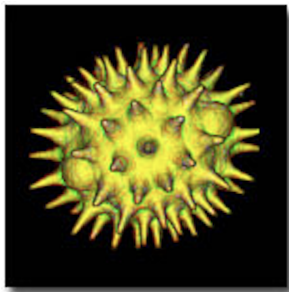
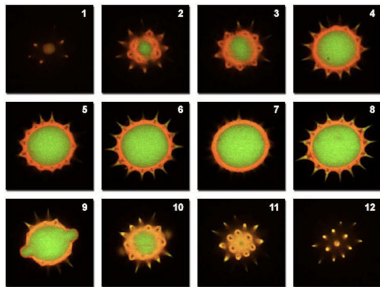
PART 3: Name, Active

PART 4: Name, Active

PART 5: Name, Active

Capture Image Start

- Multi-plane imaging (XY, XZ, and YZ)
- In biological imaging, open opportunity for 3D model reconstruction by serial optical sections



Pollen grain sections (left) and constructed 3D model (right)

Further Goals

- Time-resolved imaging
- Image processing (rasterizing, pseudocolors, correction)
- Auto-alignment of sample by brightness / focus
- z-stacking
- Beam-parking / region-of-interest ("ROI")
- Tiling

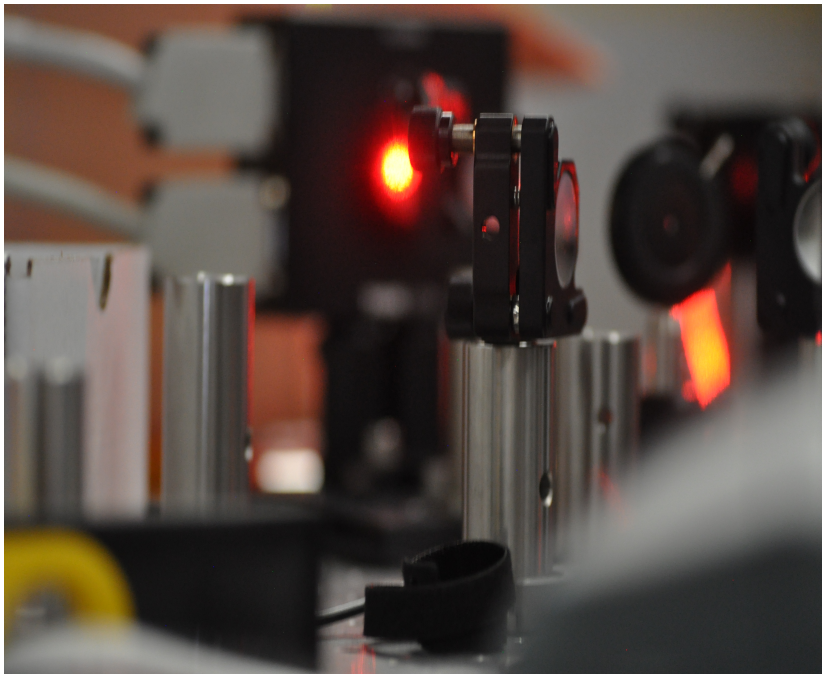


The image shows a screenshot of a code editor with two panels. The left panel displays a large block of code, most of which is commented out with '//' and '/* */' markers. The right panel shows a smaller section of code with several lines of active code, some of which are highlighted in blue. The code appears to be a mix of C++ and assembly-like instructions, possibly related to image processing or hardware control. The editor interface includes a file explorer on the left and a search bar at the bottom right.

Current Progress

- Setting up a new lab room after a move to a new science building
- All part orders planned (ThorLabs, Edmund Optics, 80/20, McMaster-Carr)
- Waiting on several items
- Strong foundation of hardware layout
- Setup alignment in-progress
- Implementing new improvements to existing software package
- Can follow on GitHub





-  Introduction to Widefield Microscopy (Leica)
Leica.
-  Introduction to Confocal Laser Scanning Microscopy (Leica)
Confocal Explanation.