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

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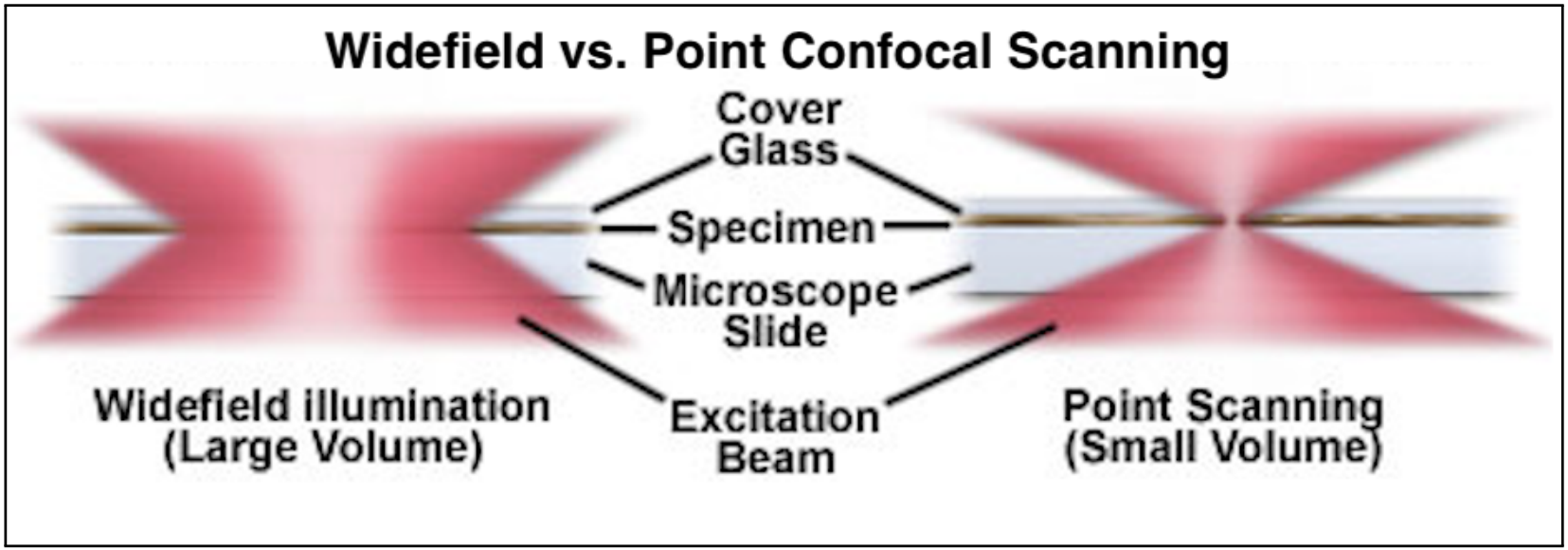
Motivation for Research

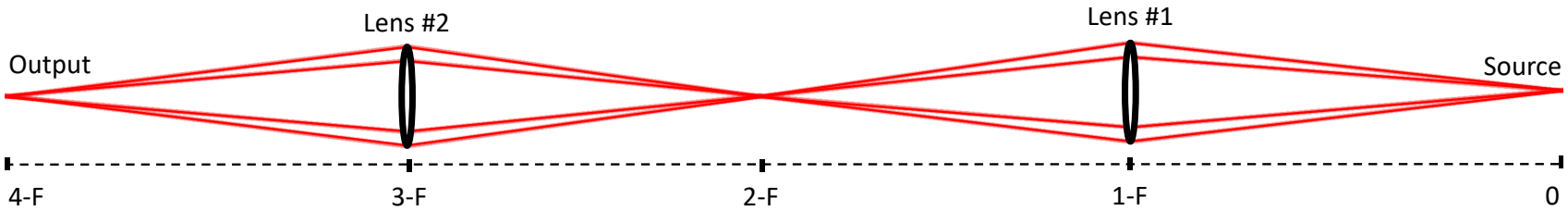
- Widefield fluorescence microscopy ("WFM") is limited
- Confocal microscopes provide numerous advantages over WFM
- Commercial research-grade confocals are very expensive (Leica, Nikon)
- A home-built confocal can overcome monetary limitations while still providing ample functionality
- We build a laser-scanning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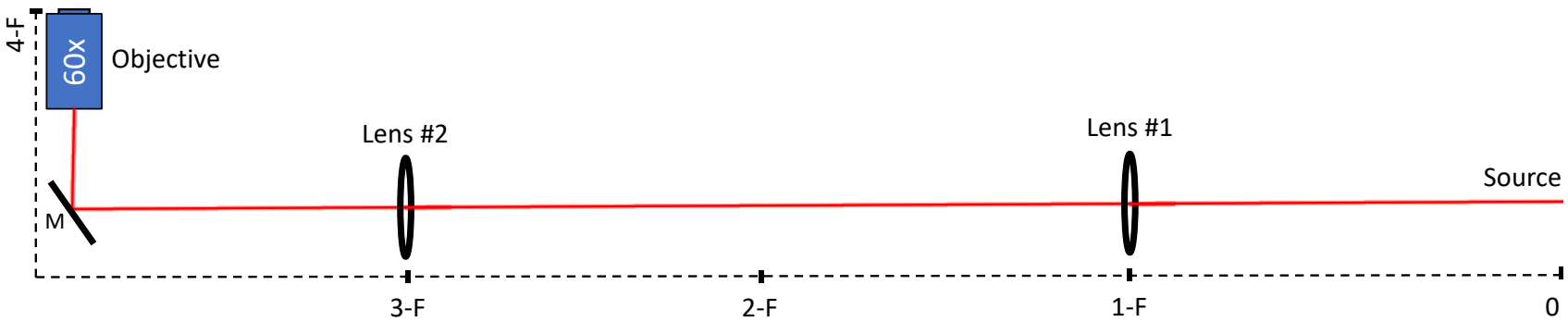

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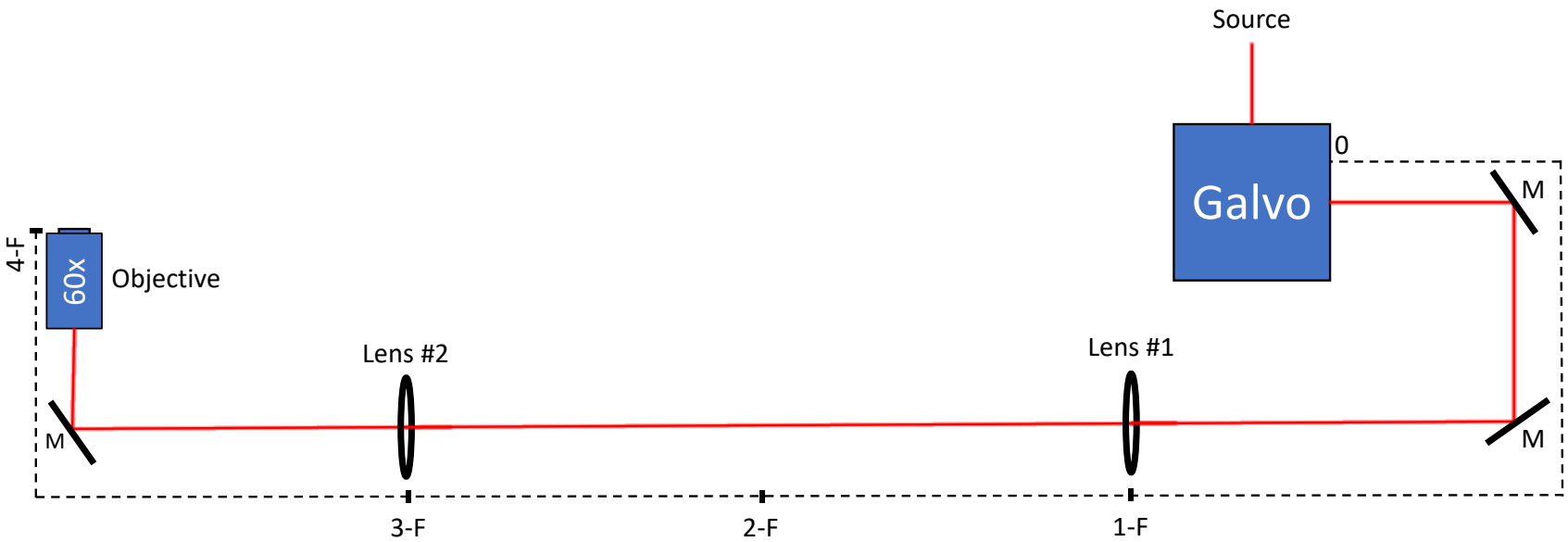

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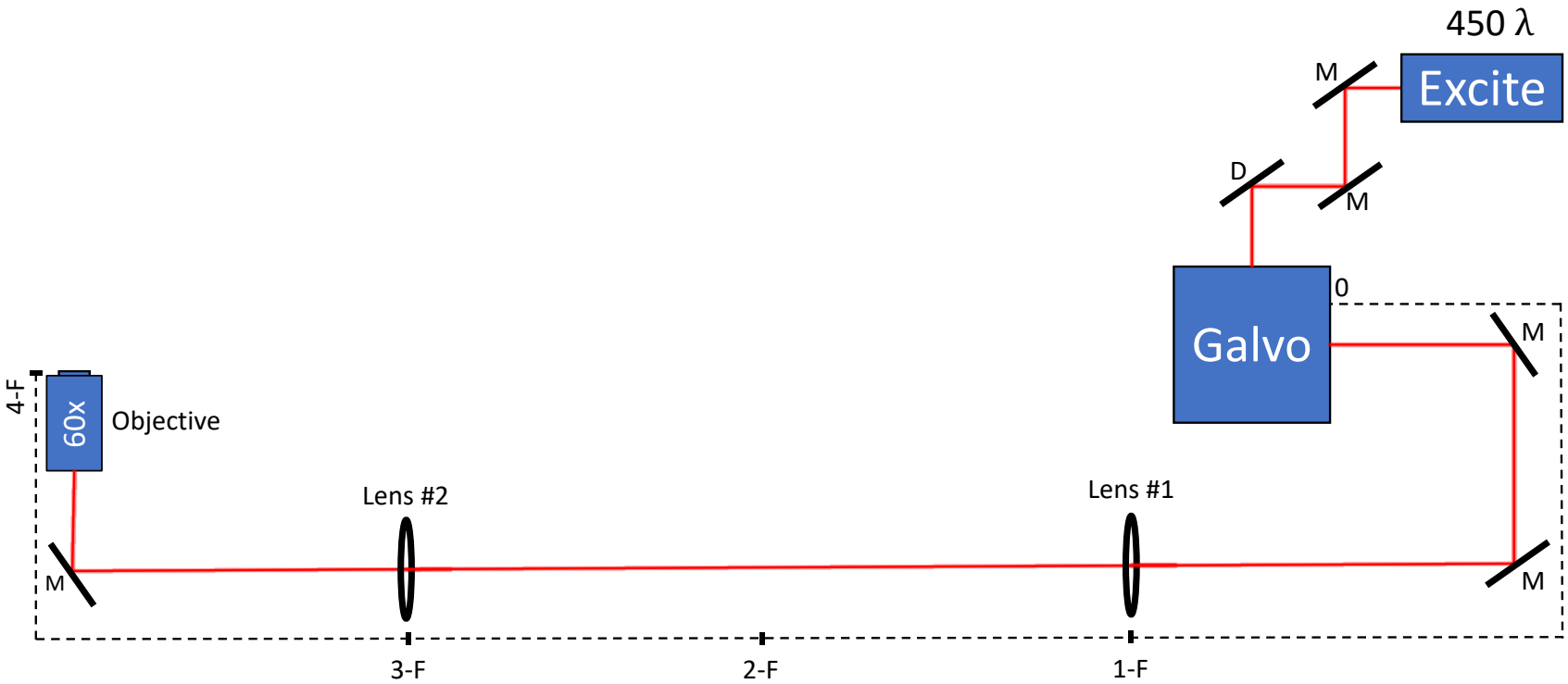

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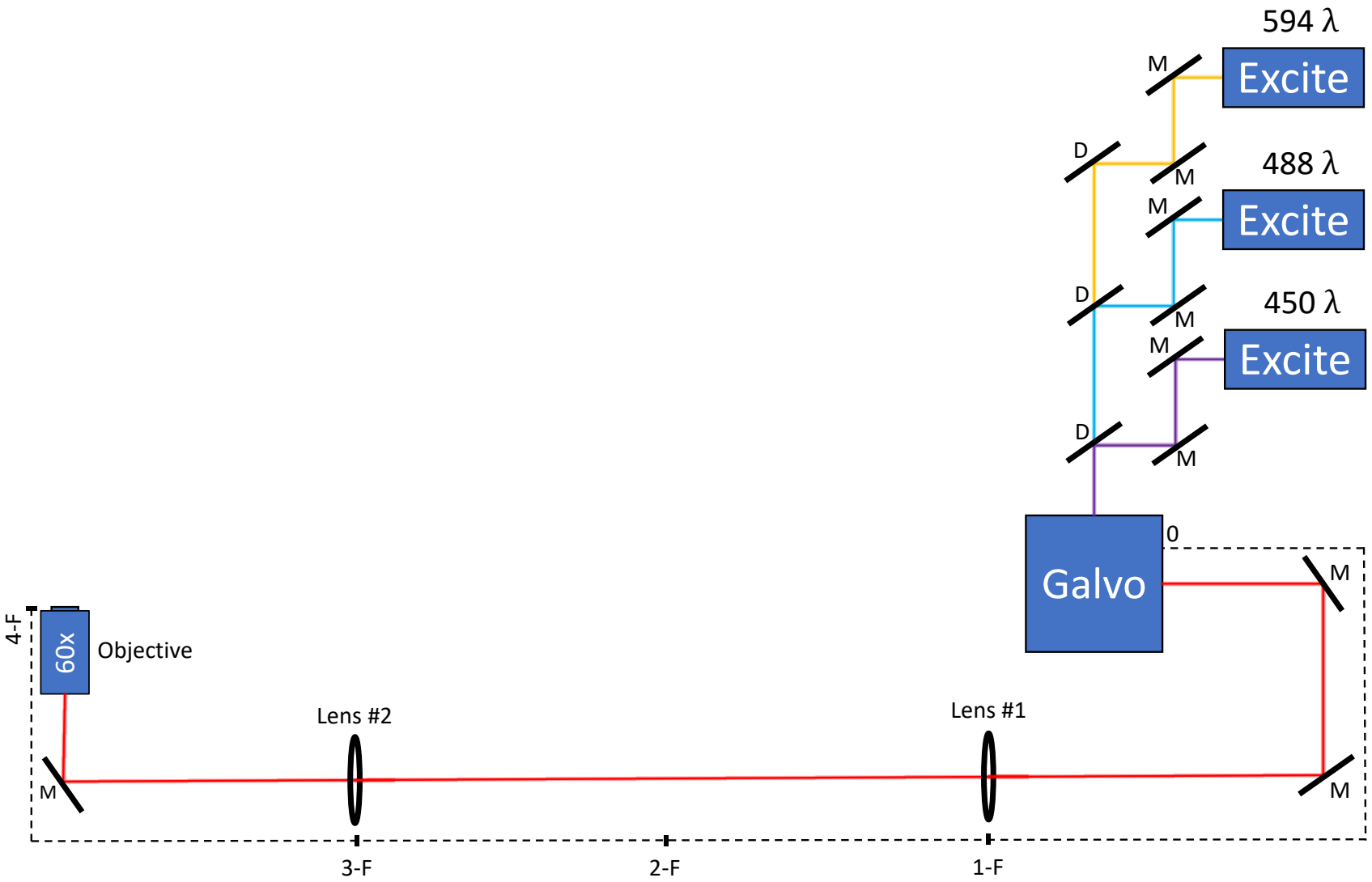

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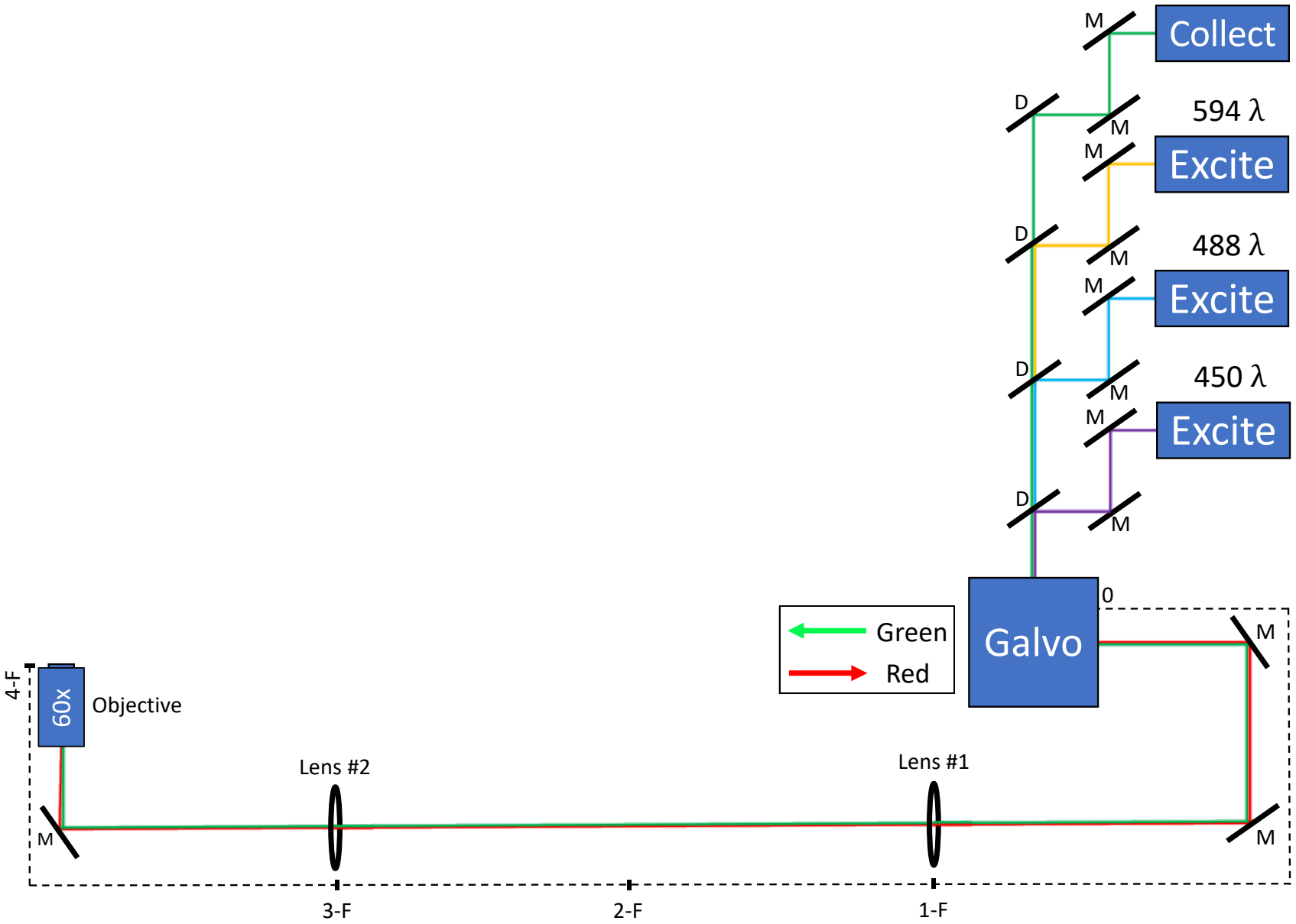

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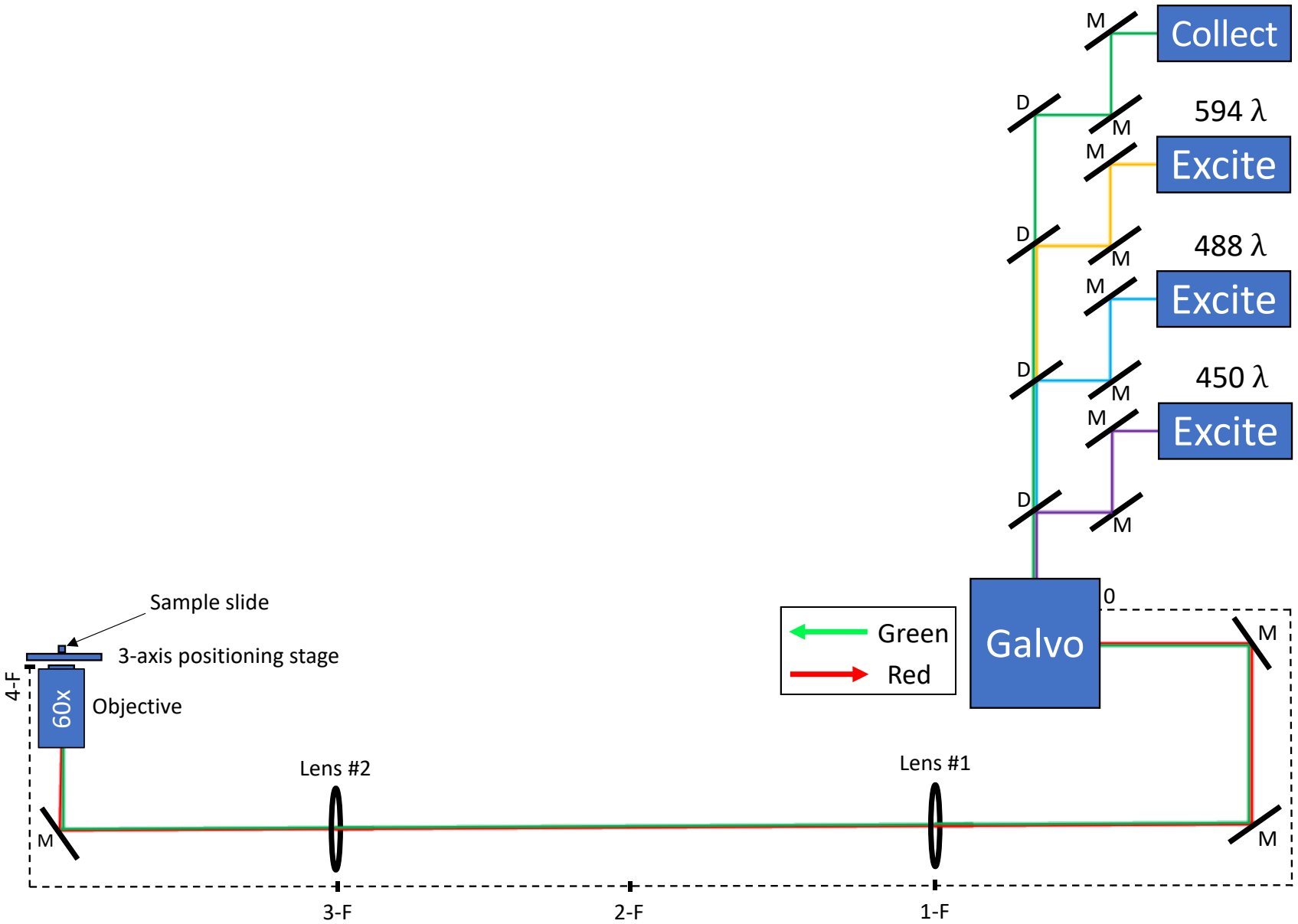

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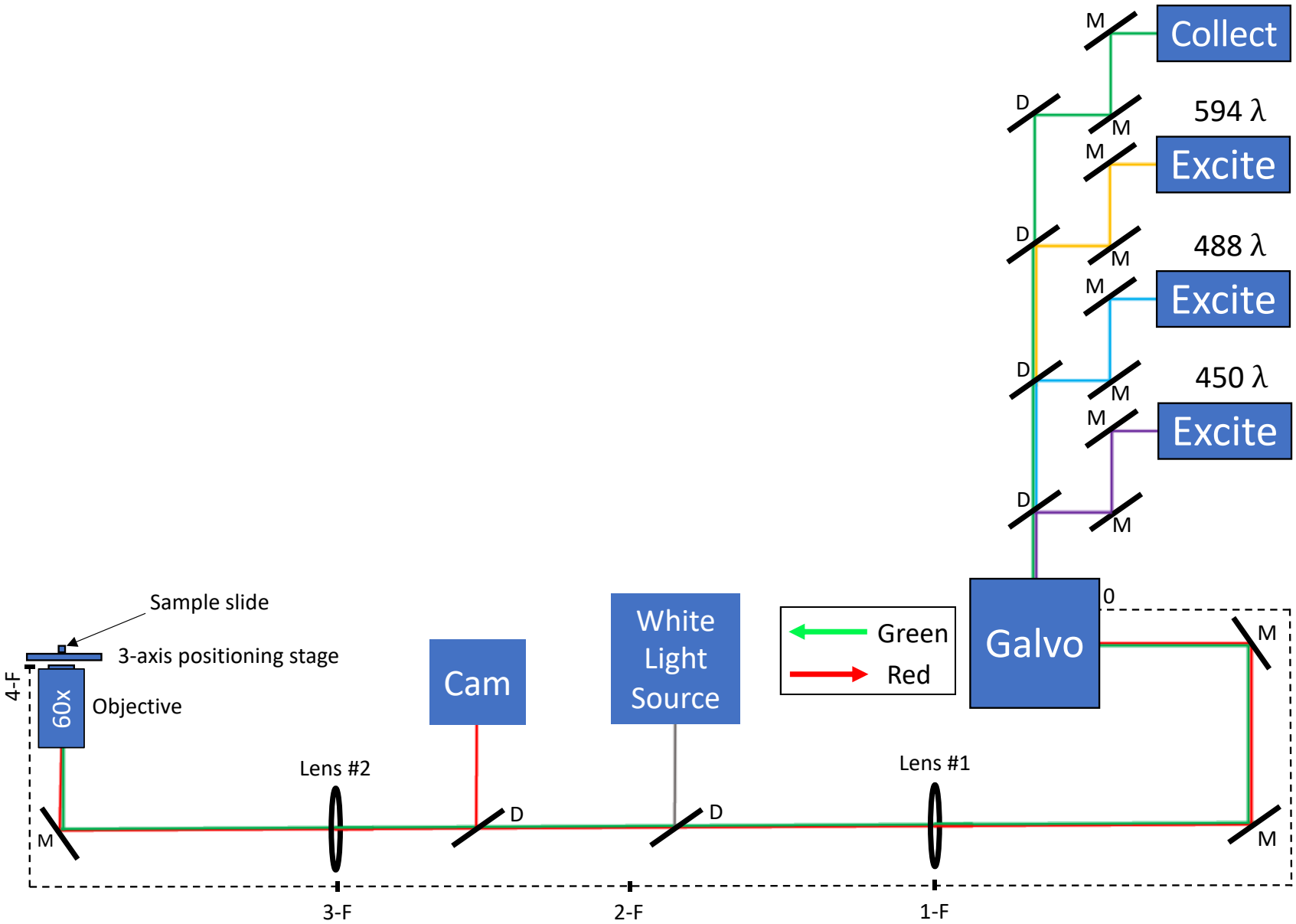

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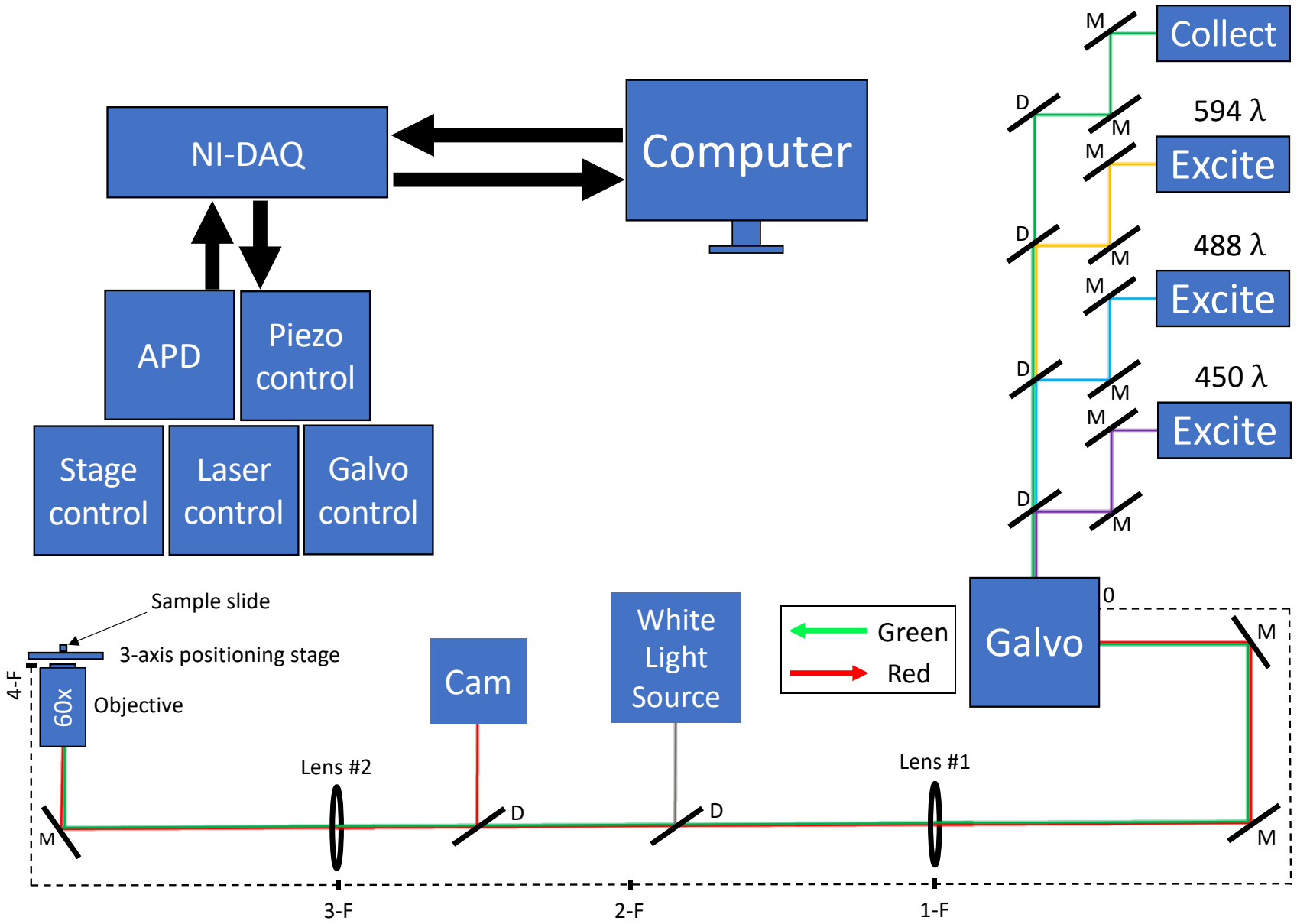
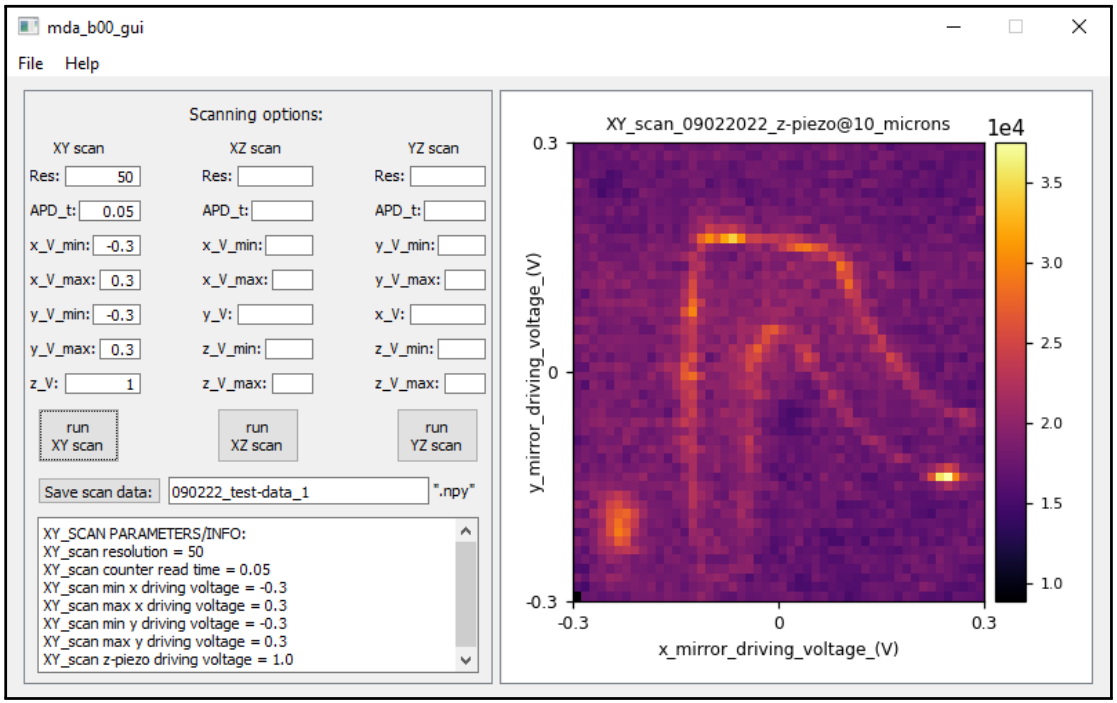


Software / GUI Application

- 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



Software Goal / Model

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

Options:

Microscope control

Multi-spectral aquisition

3D-sampling

Spectrum collection

Dye finder

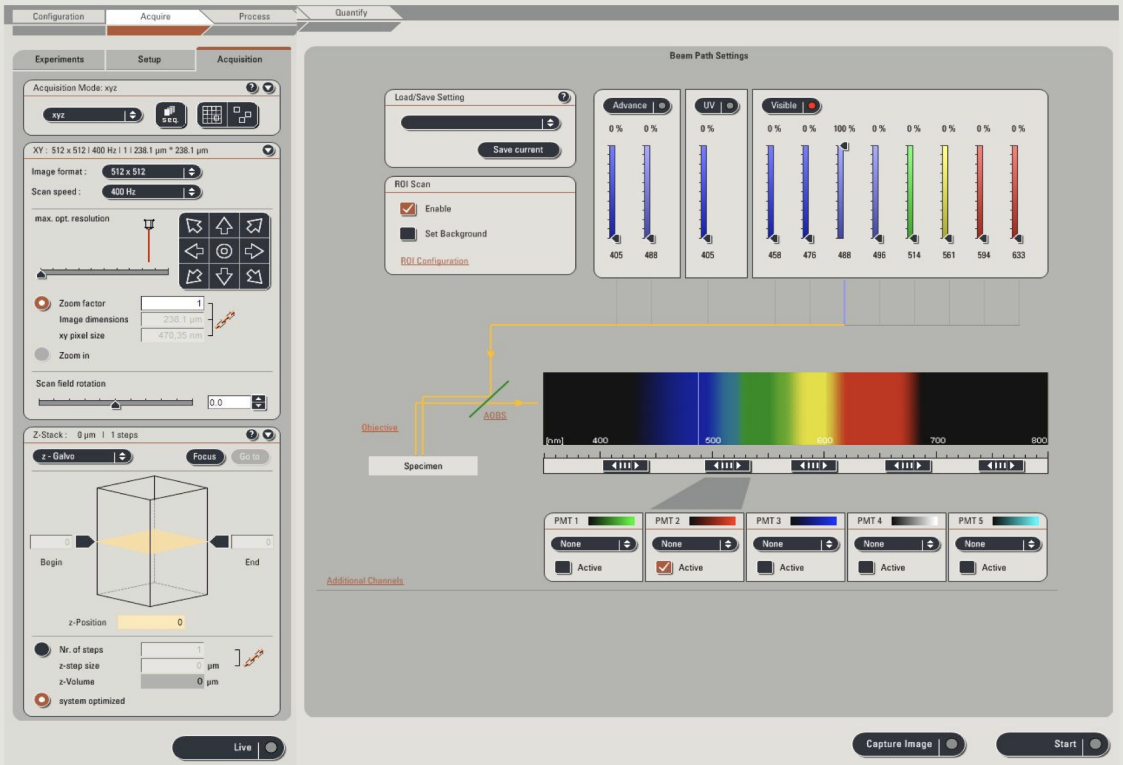
Quantification

Time laps

Image processing

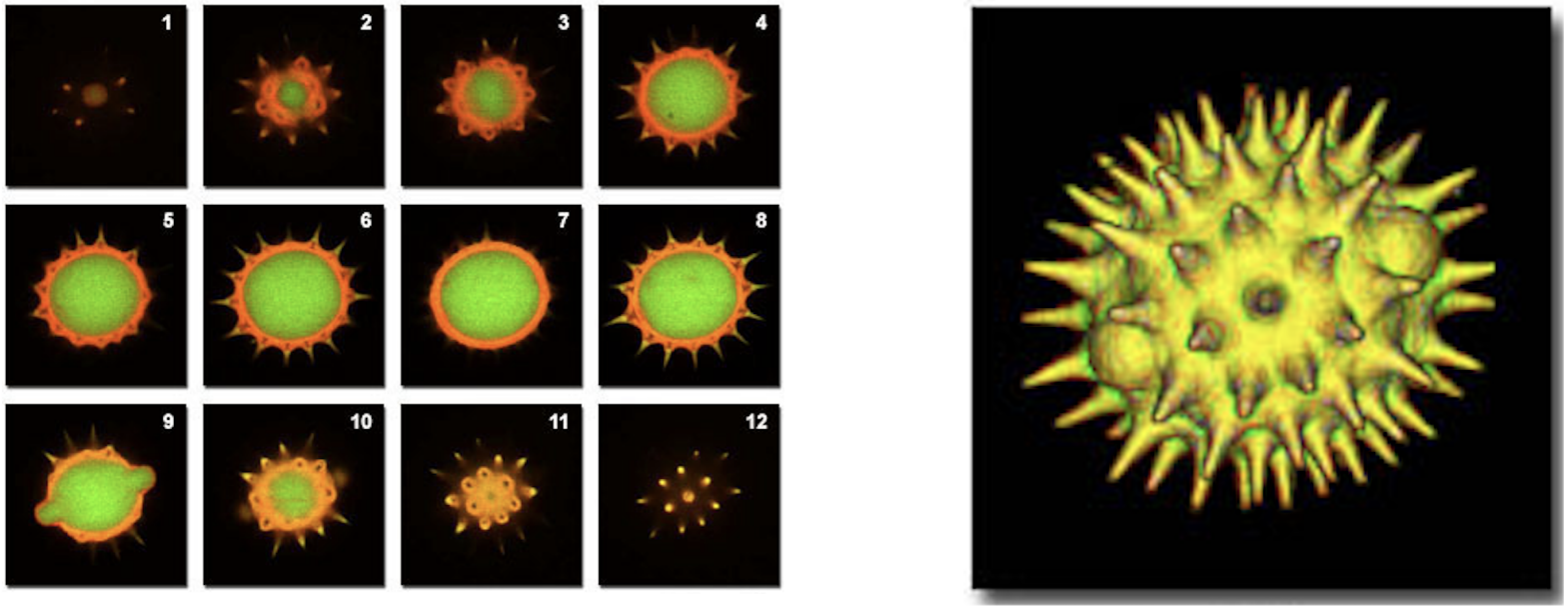
Multi-position imaging

Modules for FRET,FRAP, FLIM



Applications / Expectations

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



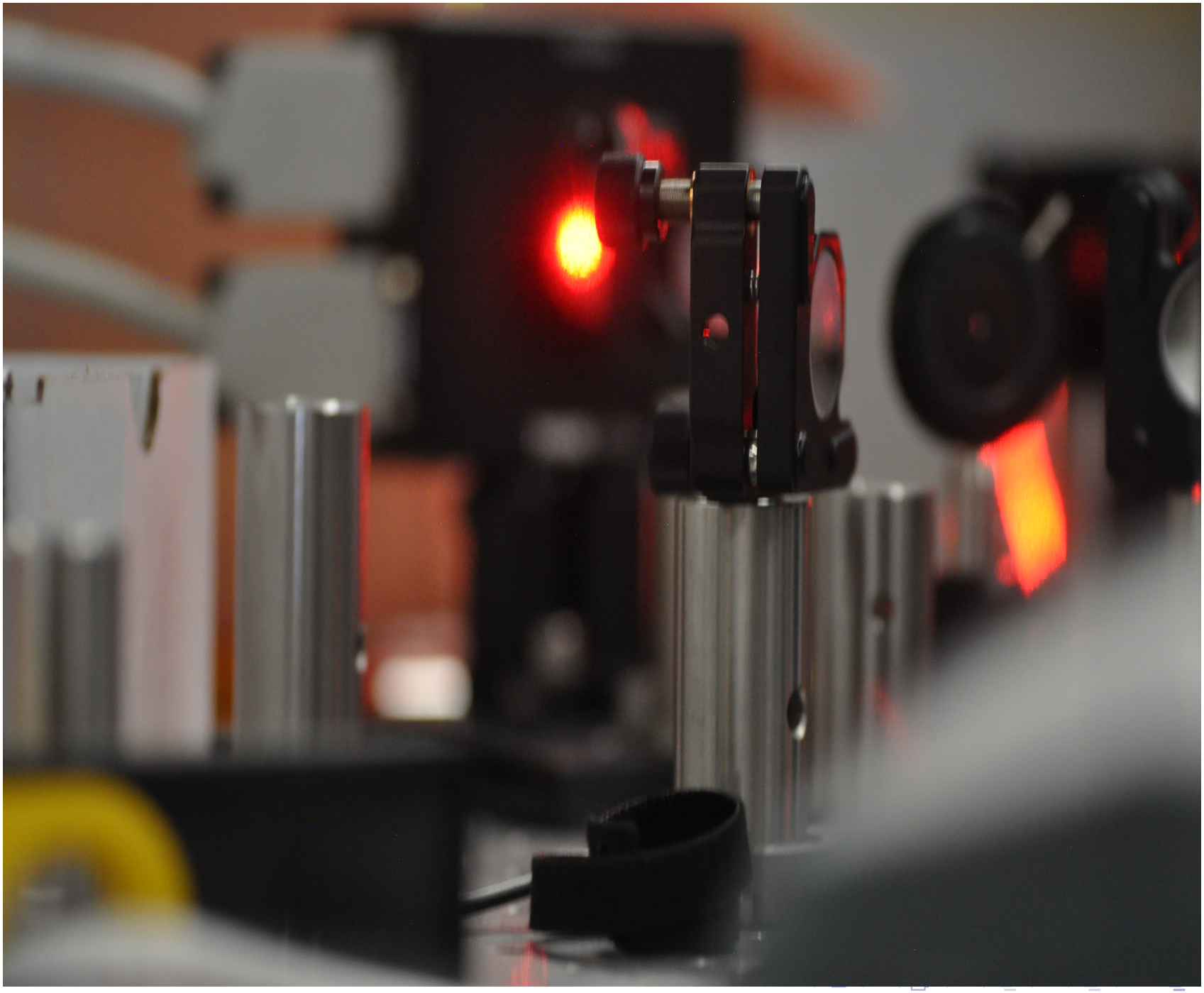
Pollen grain sections (left) and constructed 3D model (right)

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

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



References


Introduction to Widefield Microscopy (Leica)
Leica.


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