Bi 227 Final Project

Building and Operating a Light Sheet Microscope

Joshua Brake

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1 Introduction

Light sheet microscopy is an exciting recent development in the world of microscopy. As any microscopist knows, when imaging a living sample there are continual tradeoffs that must be made between the illumination intensity, imaging speed, and resolution. One of the key limitations that must be considered when imaging biological specimens is the tendency for phototoxic effects or photobleaching of the fluorescent chromophores in the sample. When a molecule absorbs light and therefore rises to an excited energy state, it has several avenues for relaxation as shown in Fig. 1. In most cases, we hope that all of the excited molecules relax through the fluorescence pathway and emit fluorescent light. However, in practice, molecules also relax through the other pathways as well which end up damaging the sample of interest and also reducing the fluorescence intensity over time.

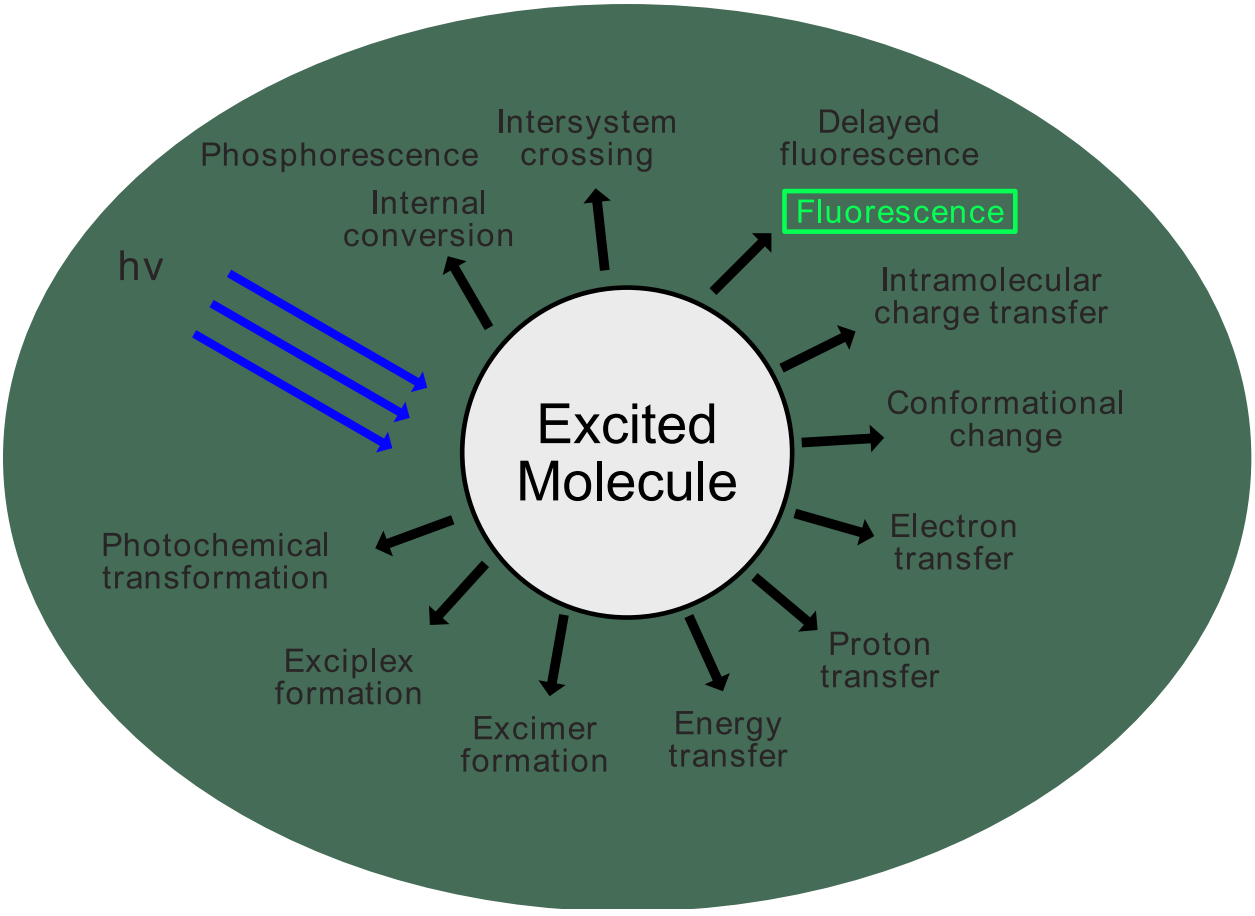

Figure 1: Excited molecule relaxation pathways. Adapted from: Valeur and Berberan-Santos "Molecular Fluorescence"

Fig. 2 shows a comparison between the four basic fluorescence microscopy techniques most commonly used today. This comparison helps to highlight the advantages of light sheet microscopy both from the perspectives of biology and optics.

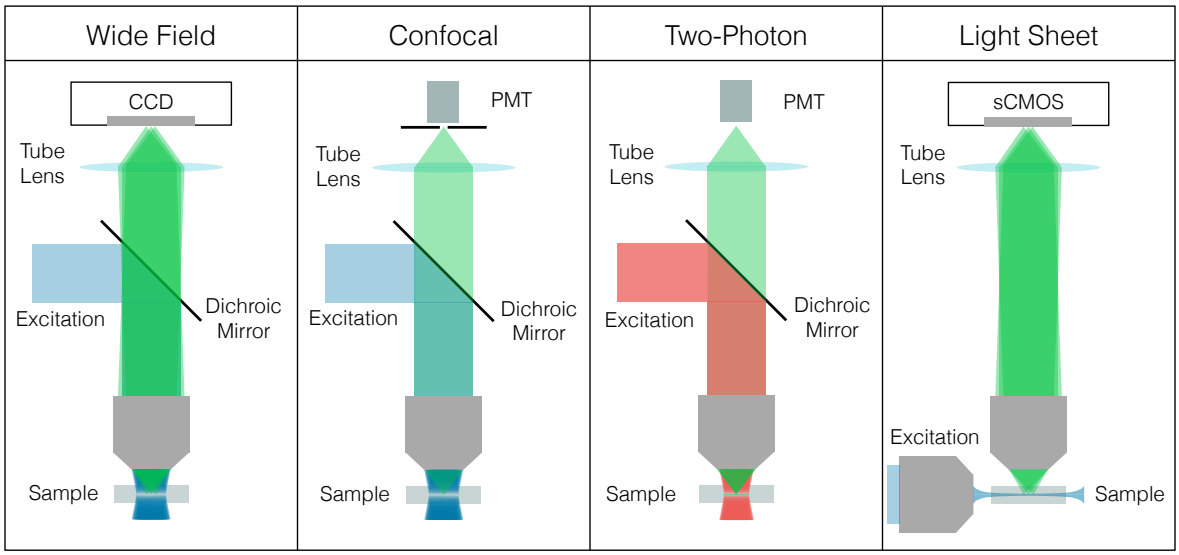

Figure 2: Comparison of the four major fluorescence microscopy techniques.

Some of the major criteria for analyzing a microscopy technique are the following:

- Speed: Widefield or scanning
- Resolution: Lateral and Axial
- Imaging Depth
- Excitation efficiency (how much of the excited fluorescence is considered "signal" from the point of interest?)

Technique	Speed	Resolution	Imaging Depth	Excitation Efficiency
Widefield	Fast (full frame)	$\frac{\lambda_{em}}{2\mathrm{NA}}$	100 µm	Medium
Confocal	Slow (scanning)	$\frac{\lambda_{ex}}{2\mathrm{NA}}$	100 µm	Medium
Two-Photon	Slow (scanning)	$\frac{\lambda_{ex}}{2\mathrm{NA}}$	1 mm	High
Light Sheet	Fast (full frame)	$\frac{\lambda_{em}}{2\mathrm{NA}}$	100 µm	High

The key advantage of the light sheet microscope over other fluorescence imaging modalities is that it combines good axial resolution with wide-field imaging speed. While the single photon operation does limit it to mostly transparent samples and the orthogonal illumination and detection arms make the setup more complex than a typical epi-fluorescence microscope, the fast imaging speed combined with minimal photobleaching is very advantageous for imaging living biological samples in particular.

2 OpenSPIM Setup

2.1 Overview

The OpenSPIM system basic setup is well documented on their website (`www.openspim.org`) so in this section I will simply highlight the major concepts and and construction procedures. A schematic of the OpenSPIM system is shown below in Fig. 3.

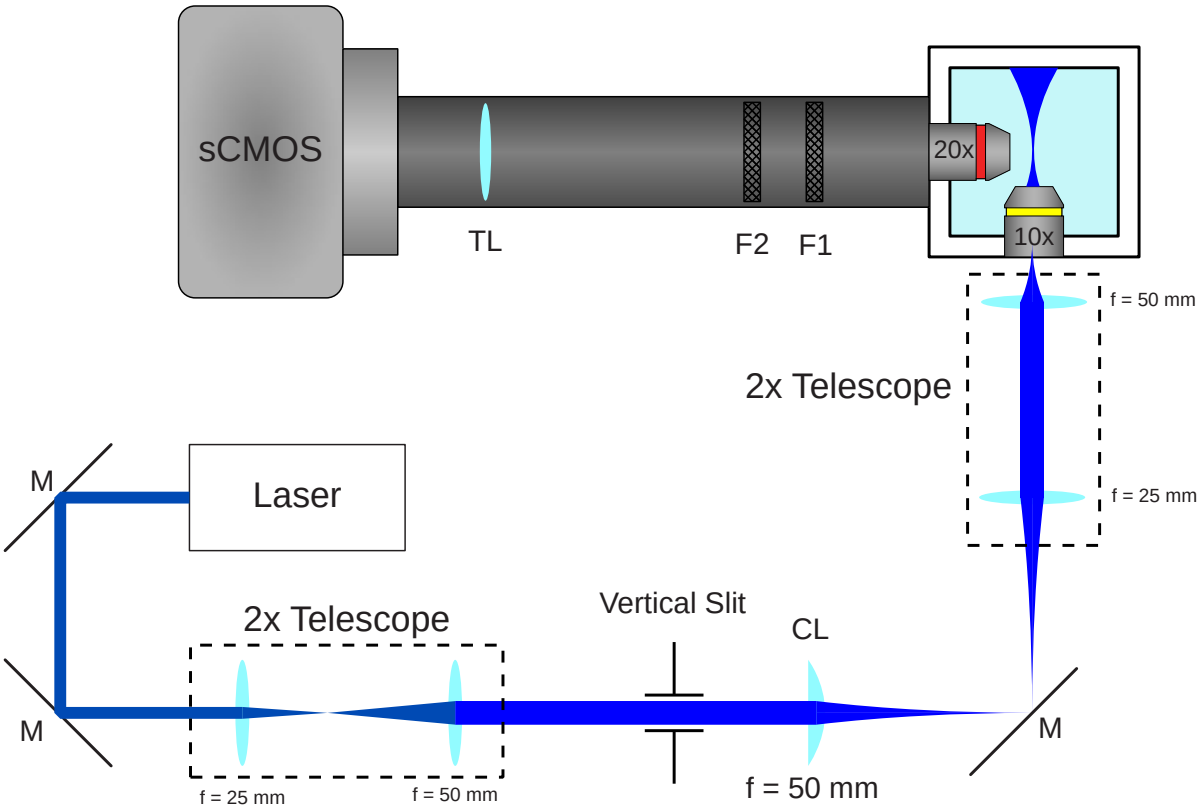

Figure 3: Schematic of the OpenSPIM system

The basic principle of the microscope is to first expand the beam from the laser using a 2x telescope. Then the vertical slit and cylindrical lens shape the beam into a variable thickness light sheet (N.B. as the slit closes further, the sheet gets thicker!). The second 2x telescope acts to further magnify the light sheet and to image the light sheet to the back focal plane of the illumination objective to fill as much of the NA as possible to achieve a thin light sheet. It is worth noting here that the schematic and instructions on the website for the OpenSPIM system seems to have the lenses in the second telescope swapped. This limits the thinness of the light sheet and so the arrangment shown in Fig. 3 has been adopted. A photo of the assembled system is shown below in Fig. 4.

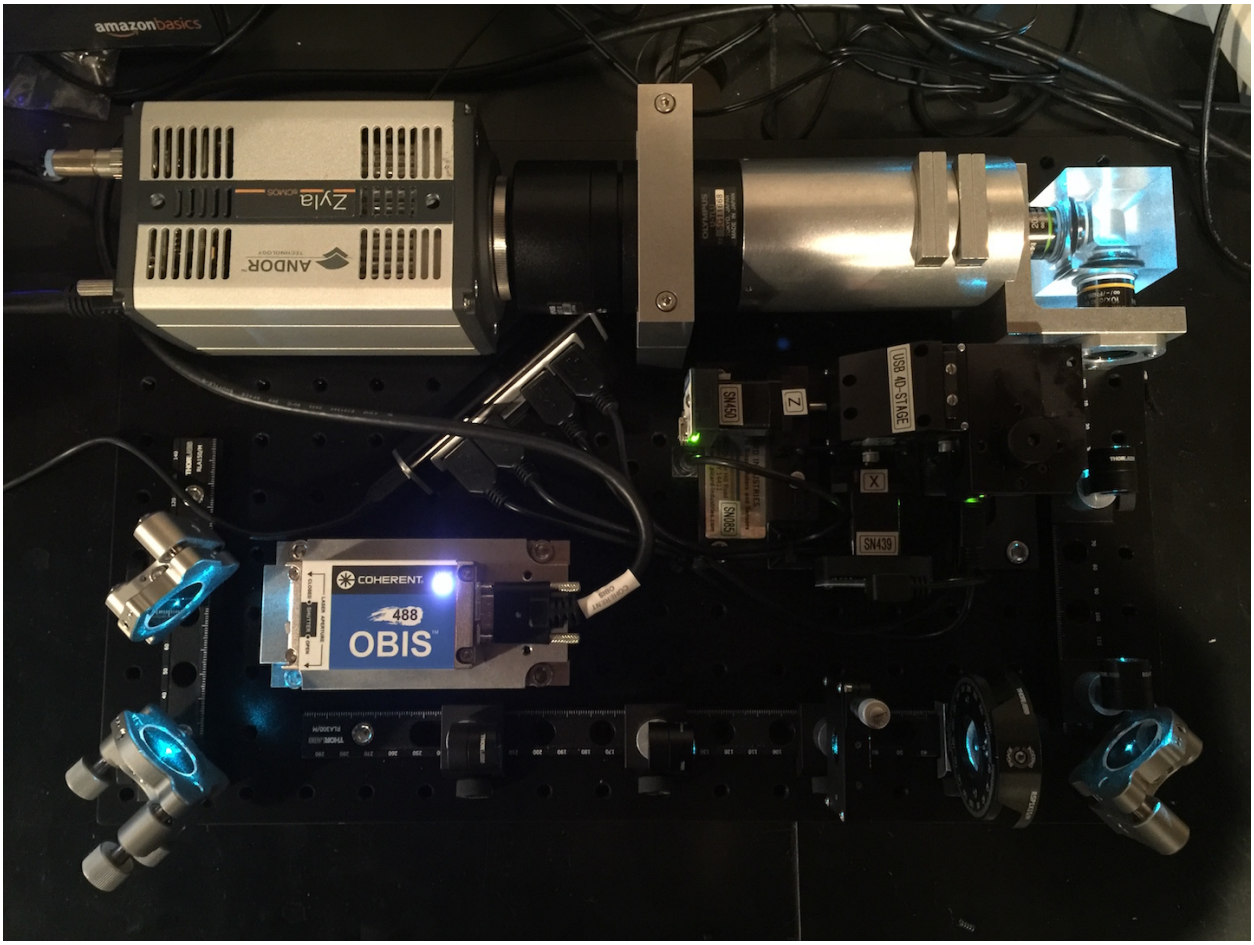

Figure 4: Photo of the Bi 227 OpenSPIM system.

2.2 Assembly Procedure

The assembly of the microscope system can be decomposed as follows:

- Assemble and install mounts and rails
- Align laser beam
- Install lenses
- Install detection arm
- Align light sheet

The first four steps are relatively well documented on the website and easy to complete. However, the most challenging part of putting the light sheet microscope together is aligning the light sheet with the focal plane of the detection objective. Fig. 5 shows several of the potential misalignment cases. In short, the goal is to have the light sheet centered laterally so that the thinnest portion of the sheet is at the center of the field of view and also axially located at the focal plane of the detection objective.

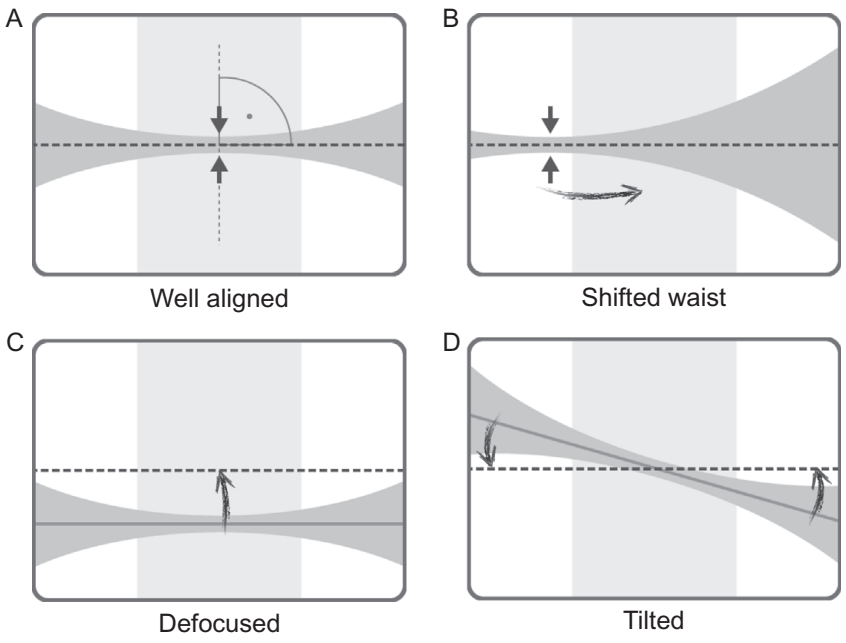

Figure 5: Light sheet alignment. (A) Correctly aligned light sheet. (B) Lateral misalignment. (C) Axial misalignment. (D) Tilt misalignment. Source: Weber *et. al.* "Light Sheet Microscopy" doi:10.1016/B978-0-12-420138-5.00011-2

The easiest way to do this alignment is the following:

1. Prepare a sample of fluorescent beads in agar.
2. Turn off the laser, remove the filters from the detection arem, and use an LED to illuminate the sample.
3. Using this brightfield illumination condition, focus the sample by moving the stage so that two beads on opposite sides of the field of view are both in focus.
4. Reinstall the filters and turn on the laser.
5. Adjust the light sheet position by tuning the corner mirror (which is located between the cylindrical lens and the first lens of the second telescope) until both of the beads you focused in the brightfield case are at their maximum brightness.

3 System Operation

The system operation is relatively well documented on the OpenSPIM website, so again I will simply list an abbreviated version of the operating procedure. Screenshots of the operation procedure are shown in Appendix A.

- Turn on camera and laser
- Open OpenSPIM version of ImageJ
- Open Micro-Manager from Plugins > Micro-Manager > Micro-Manager Studio

- Load configuration file (if first time, create configuration file using Tools > Hardware Configuration Wizard from Micro-Manager to add the laser, camera, and stage.)
- Open Plugins > Acquire SPIM image and live preview window
- Set the acquisition parameters and capture the data set.

4 Conclusion

The OpenSPIM system demonstrates that light sheet microscopy is accessible to nearly anyone given enough time and ample resources to purchase the components necessary ($\sim$ \$20k). While the fine alignment of the light sheet with the detection objective presents a challenge, it can be conquered with patience and determination. In the years to come it will be exciting to see the developments that come from the high spatio-temporal resolution enabled by light sheet microscopy.

Appendix A: Program Screenshots