# OpenSPIM

## Introduction

Conventional fluorescence microscopy uses high intensity light to illuminate the sample but this excites all fluorophores in the light path, not just the plane of interest. The result is that light emitted from outside the focal plane contributes to the image. Confocal microscopy overcomes this problem by using pinholes to selectively collect light only from the plane of interest. However, high intensity light still penetrates through the entire sample which causes photobleaching and photodamage.

Light Sheet Fluorescence Microscopy (LSFM) or Selective Plane Illumination Microscopy (SPIM) only illuminates the plane of interest which allows us to collect information from a single plane while also minimizing photobleaching and photodamage to the rest of the sample. By eliminating out-of-focus light in this way, lower light intensities can be used to excite the sample which further contributes to the reduction in photobleaching and photodamage, allowing us to image for extended periods of time. More exposures can therefore be taken using LSFM than any other form of fluorescence microscopy.

The difficulty of LSFM is knowing whether it's the best technique to image the sample of interest. Commercial systems can be expensive and may not fit the exact needs of the researcher. In an effort to make light sheet technology available to a larger group of researchers, the OpenSPIM platform was created to provide a maximally cost-effective solution that allows anyone to build an entry level system and further tweak it for the specific imaging needs.

## OpenSPIM principle

The OpenSPIM platform was created by the group of Pavel Tomancak, especially Peter Pitrone and Johannes Schindelin at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden (Germany) and the Laboratory for Optical and Computational Instrumentation from University of Wisconsin–Madison (WI, USA). As one might deduce from the name, it is completely open source. Via the OpenSPIM homepage (openspim.org) scientists can learn everything about the basics of light sheet microscopy, get access to a parts list for their own home-built system, read a detailed and animated manual and connect with other scientists who have already built successful OpenSPIM systems.

The system is aimed at scientists who want to get into light sheet microscopy and due to limited budget can't afford a commercially available system or want to figure out first whether light sheet technology is useful to solve their scientific question. Without a deep knowledge of optics even beginners can set up a functional system within a short amount of time.

 

## Building an OpenSPIM system

The parts list on the OpenSPIM website contains all the components required to build an L-SPIM layout (Figure 1) using two water immersion objectives, a 10x/0.3NA for the illumination path and a 20x/0.5NA for imaging. Many of the other required components such as parts for the lightpath and its alignment can be found at popular home builders' resources such as Thorlabs (thorlabs.com).

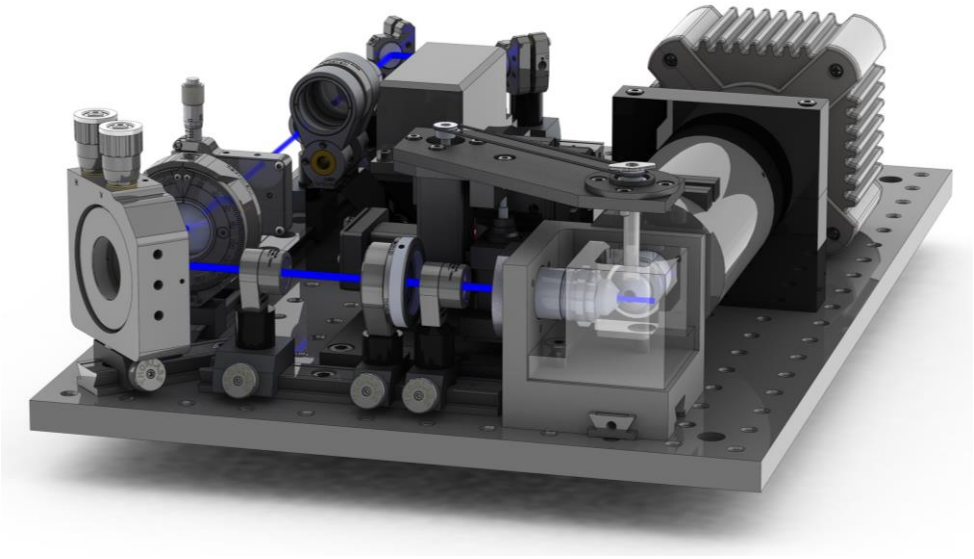

**Figure 1:** L-SPIM layout of an OpenSPIM system (openspim.org)

Picard Industries (picard-industries.com/) have put together a 4D stage which allows the user to move the sample in x, y, z and even contains a rotating actuator to obtain image stacks from multiple angles. A limitation for this system is that it requires access to a mechanical workshop which is useful to modify purchased parts for optimal use, but crucial to produce the sample chamber from plastic or aluminium and an infinity space. To circumvent this requirement people have used 3D printers and even Lego parts which have yielded very good results.

The beam path consists of a diode laser which runs through a beam expander, a slit aperture (to adjust the beam characteristics), a cylindrical lens and a telescope, which creates a standing sheet formed by a Gaussian beam.

The system is controlled is by an OpenSPIM plugin for Micromanager which gives the user access to the stage control and imaging parameters. Successful attempts have been made to include features such as shutter controllers via an Arduino board. All components can easily be controlled by a well-specked laptop.

## OpenSPIM advantages

One of the benefits of the open source nature of the project is the highly active online community where questions by users will be swiftly resolved with the help of other users and developers via a mailing list.

The OpenSPIM is the entry-level home-builders' choice with a high degree of flexibility and upgradability. Modifications involve dual sided illumination, as well as dual sided detection and sample chambers specifically matched to cleared tissue samples with dedicated lenses.

The versatility of the system and the attractive price point make the OpenSPIM system ideal for any scientists interested in implementing a light sheet microscope.

## Cameras for OpenSPIM

Another advantage of the OpenSPIM system is that any camera can be used but we believe that the best performance can be achieved with most modern sCMOS devices.

Light sheet microscopy is typically performed with low magnification objectives, such as the 20x detection objective recommended for the OpenSPIM system. This is to provide a large enough working distance to fit with the light sheet geometry and to have a field of view big enough for imaging large samples.

To reach the highest possible resolution with lower magnification objectives, a camera with a smaller pixel size is recommended. However, a smaller pixel camera will have a lower sensitivity than a larger pixel camera. Higher sensitivity may sometimes be necessary to allow for shorter exposure times which would increase imaging speed and minimize the photodamaging effect of light on samples.

A larger field of view camera may also be advantageous as it would allow larger samples to be imaged without needing to stitch multiple images together. Alternatively, for smaller samples, the large field of view could be split to perform multi-channel imaging easily on the same sensor.

Depending on the specific application and sample type, the researcher should consider all of these factors and choose a camera with specifications that works best for them.

## References

Peter G Pitrone, Johannes Schindelin, Luke Stuyvenberg, Stephan Preibisch, Michael Weber,Kevin W Eliceiri, Jan Huisken & Pavel Tomancak. OpenSPIM: an open-access light-sheet microscopy platform. Nature Methods 10, 598–599 (2013) doi:10.1038/nmeth.2507

Openspim.org

 