

Real-Time Observation of Biological Processes using Light-sheet Microscopy

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Introduction

In current research, observing a biological process in real time is undoubtedly one of the most powerful approaches and fluorescence microscopy is one of the best techniques for this task. However, one should consider the fact that is often overlooked, or deliberately ignored.

The fact is, light is not harmless, and can cause damage to the sample being studied. Due to this light-induced damage to cells, known as photodamage, observing without disturbing is not possible in fluorescence microscopy.

Photodamage to a developing sample is a major limitation in live imaging and various other traditional microscopy techniques. Scientific journals are dedicating more editorial space to the risks of phototoxicity.

Controls are increasingly demanded by peer reviewers to ensure that the described behavior is not principally photo-induced. Over the years, many traditional fluorescence microscopes have been shown to use illumination at cell-damaging levels, thus providing inconsistent and even misleading results.

However, with careful light exposure, it is possible to reduce photodamage to physiologically manageable levels, thereby ensuring that the behavior of an organism is normal and can be observed for extended periods.

These reasons are the major driving factors behind the ever-increasing demand for microscopy techniques that avoid photodamage. One such technique is Light-sheet Fluorescence Microscopy, also called selective plane illumination microscopy (SPIM).

Illuminating one plane at a time

Light-sheet fluorescence microscopy produces the least photodamage compared to other fluorescence techniques. The modern form of SPIM has been in use for just over a decade. During this time, this breakthrough technique has enabled spectacular real-time imaging of developing organisms.

Simple uncoupling of illumination and detection path is the novel feature of light-sheet microscopy. The same path is used in traditional techniques, but the light is brought in from the side in light-sheet microscopy.

SPIM is less photodamaging in nature owing to the fact that only the focal plane is illuminated. On the other hand, in traditional techniques, multiple focal depths are illuminated for each frame acquired. This feature of SPIM has two advantages:

1. There is no (to very little) out-of-focus light to blur the image.
2. 3D samples can be acquired with significantly lower light exposure.

In other words, SPIM combines the best features of the two most commonly used traditional techniques: The sensitivity of widefield fluorescence microscopy and the optical sectioning of confocal microscopy. The combination of these two capabilities makes the SPIM to have the lowest photodamage compared to any other technique.

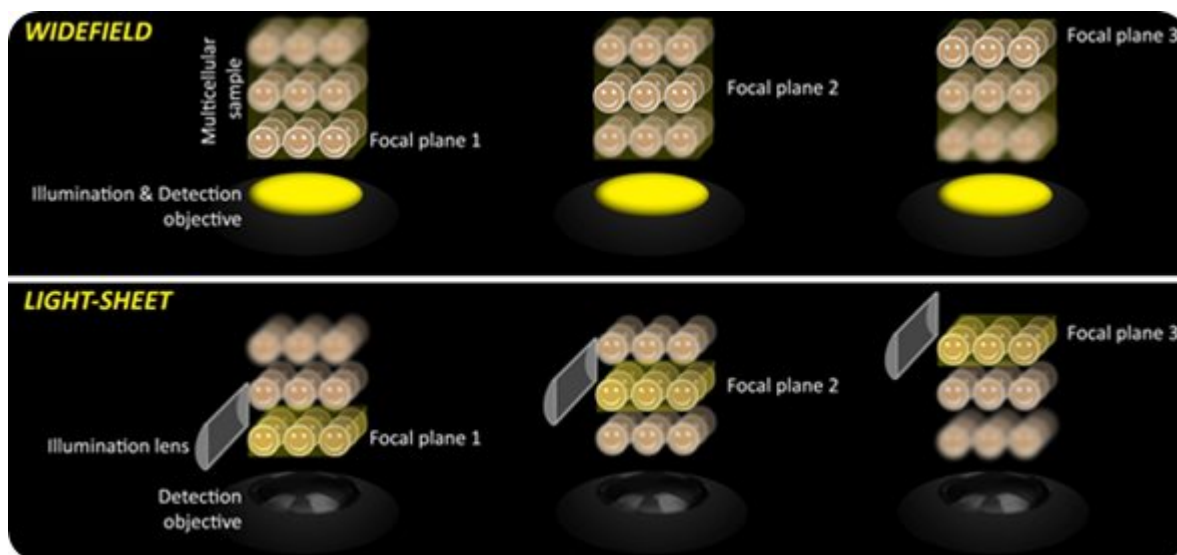


Figure 1. Comparing conventional and light-sheet configurations. Top row: Conventional approach, where for every focal plane the whole sample is illuminated, since the illumination and the detection objective are the same. Bottom row: In light-sheet microscopy, illumination is brought in from the side (here using a cylindrical lens), illuminating only one focal plane at a time. In this example, light exposure in the light-sheet setup is three times less compared to the conventional approach. The more time points and focal planes are used, the larger the difference in light exposure becomes.

Light-sheets in the UK

The third public Light-sheet Fluorescence Microscopy conference ended in Sheffield last month (31 August - 3 September 2016). This was the first time the event was held in the UK. It was incredible to see how light-sheets are employed in many model systems, ranging from zebrafish to zebrafinch, to obtain answers to dynamic biological questions that otherwise would have been left unanswered with other live imaging techniques.

This is mainly attributed to the low photodamage generated by the light-sheet microscopy. The assessment and reporting of photodamage was one of the popular round table discussions. No microscopy technique is immune to photodamage and one can also overdo it with light-sheets.

One of the main sponsors of this conference was Cairn Research, who showcased its lightsheet illumination module, including its open-source version, in this vibrant meeting. Cairn Research is currently the only manufacturer providing lightsheet solutions in the UK.

The instrument is only one of its kind in using very low illumination intensity. Also, it is the only commercially available instrument which from the start was considered to be a low-cost, highly adaptable system to different experimental setups, and open-source.

The instrument is independent of software and microscope brand. In the OpenSPIM spirit, blueprints, parts list, building instructions and image acquisition protocols will be released and openly available soon.

The ability to offer turn-key solutions is vital because building one's own instrument is a challenging task for most biologists. This open and flexible approach shows promise for the widespread adoption of the L-SPI into many research laboratories, helping to spread the brilliant technique of SPIM.

The L-shaped single plane illuminator (L-SPI)

The L-SPI, a flexible, low-cost illumination module for large and photosensitive specimens, is equipped with a stage-mounted, fiber-coupled illuminator head that generates a wide, uniform lightsheet from two orthogonal directions (Figure 2A). It is possible to adjust the intensity, height, and angle of each light-sheet individually.

[Click here to learn more about the L-shaped single plane illuminator](#)

Standard light-sheet thickness (waist) is 20 μ m and light-sheets with a thicker or thinner waist can be generated using different collimators and cylindrical lenses. The sample is positioned on a sub-stage (Figure 2B) and moved through the illuminated focal plane with the help of an integrated long travel (21mm) piezo stage, generating a 3D dataset. Four-sided illumination can be achieved using a second L-SPI (Figure 2C).

It is possible to mount the L-SPI on any upright or inverted micro- or macroscope and hook it up to any software already used in the laboratory. An example is shown in Figure 2D. Post-processing for the fusion of viewpoints is not required, thereby saving on computing power and storage space.

The compact size, modularity of optical components, and large freedom of access (for example, for positioning micro-electrodes) of the L-SPI allow it to be adapted to a wide variety of experimental purposes.

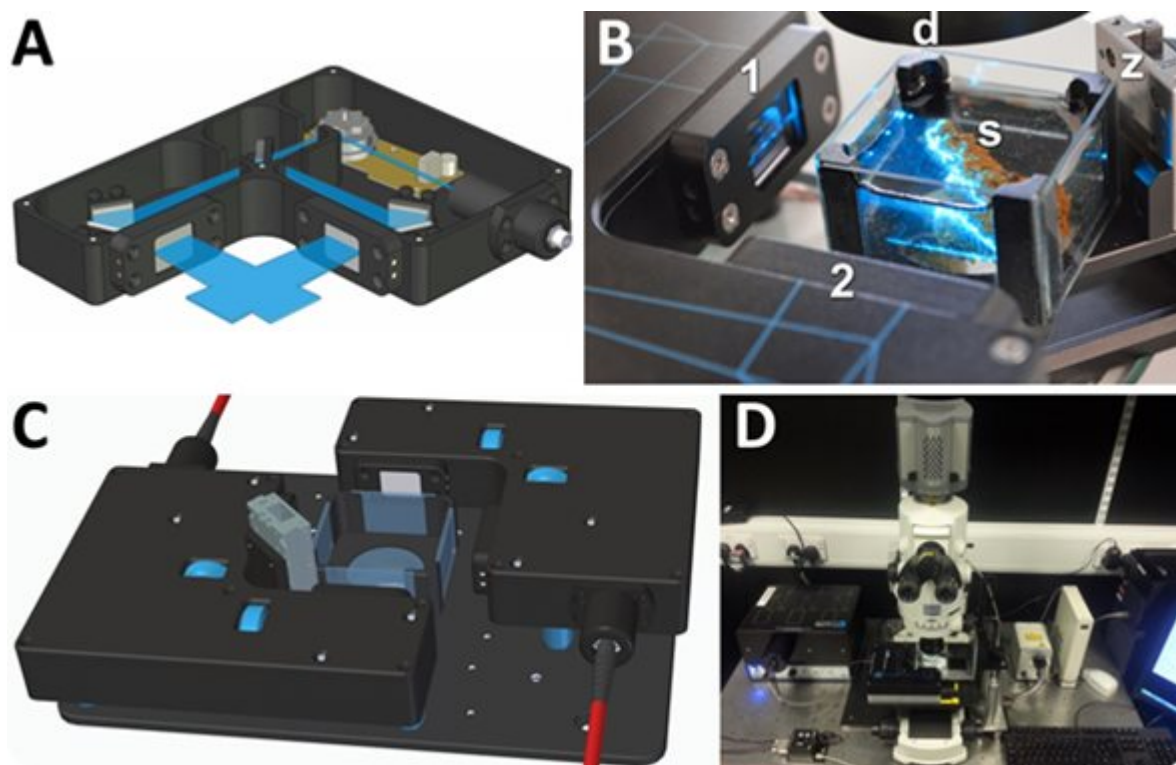


Figure 2. A) CAD drawing of the L-SPI, showing the generation of the two wide, perpendicular lightsheets (blue) recombined orthogonally. B) L-SPI detail, sample holder and z-stage. Lightsheets emerge from two orthogonally arranged windows (1 and 2) with the cylindrical lenses, and focus on the sample (s), which is moved through the lightsheets by a fast, precise z-motor (z). The fluorescence is detected by a macro- or microscope of choice (d). C) Setup with two L-SPIs, providing four-sided illumination. D) Lower-cost experimental setup with L-SPI, Thorlabs z-stage and Nikon AZ100 macroscope.

The light-sheet is continuously swept in the focal plane, offering two key features to the L-SPI. Generating a uniform light-sheet with orders of magnitude wider (20mm) than that of a standing (non-swept) light-sheet (\sim 1mm), as widely used in SPIM setups (Figure 3A) is one feature, which makes the L-SPI suitable to image larger samples (20 x 20 x 21mm) than any other commercially available system.

The second feature is that sweeping, results in breaks in illumination, thereby further reducing the risk of photodamage. As a result, time-lapse imaging of highly photosensitive samples can be captured with minimal to no measurable photodamage. The development of photosensitive larvae of the reef-building coral *Favia fragum* has been observed using autofluorescence of the organism.

It is also possible to use the L-SPI with reflectance, where fluorescence labeling is impossible. Such a system was used for taxonomy to image an unlabelled daisy flower (Figure 3C, *Bellis perennis*) and ghost moth (Figure 3D, *Trioda sylvina* with short lamellate antennae and missing proboscis).

Using two L-SPIs, a buff-tailed bumblebee (*Bombus terrestris*) was also imaged (Figure 3E and also Figure 2C). Although the major applications are of larger samples, cellular and subcellular structures as tiny as 5 μ m, such as

nuclei in the endothelial cells of tailfin blood vessels in zebrafish and endosymbiotic algal cells in corals, were also resolved.

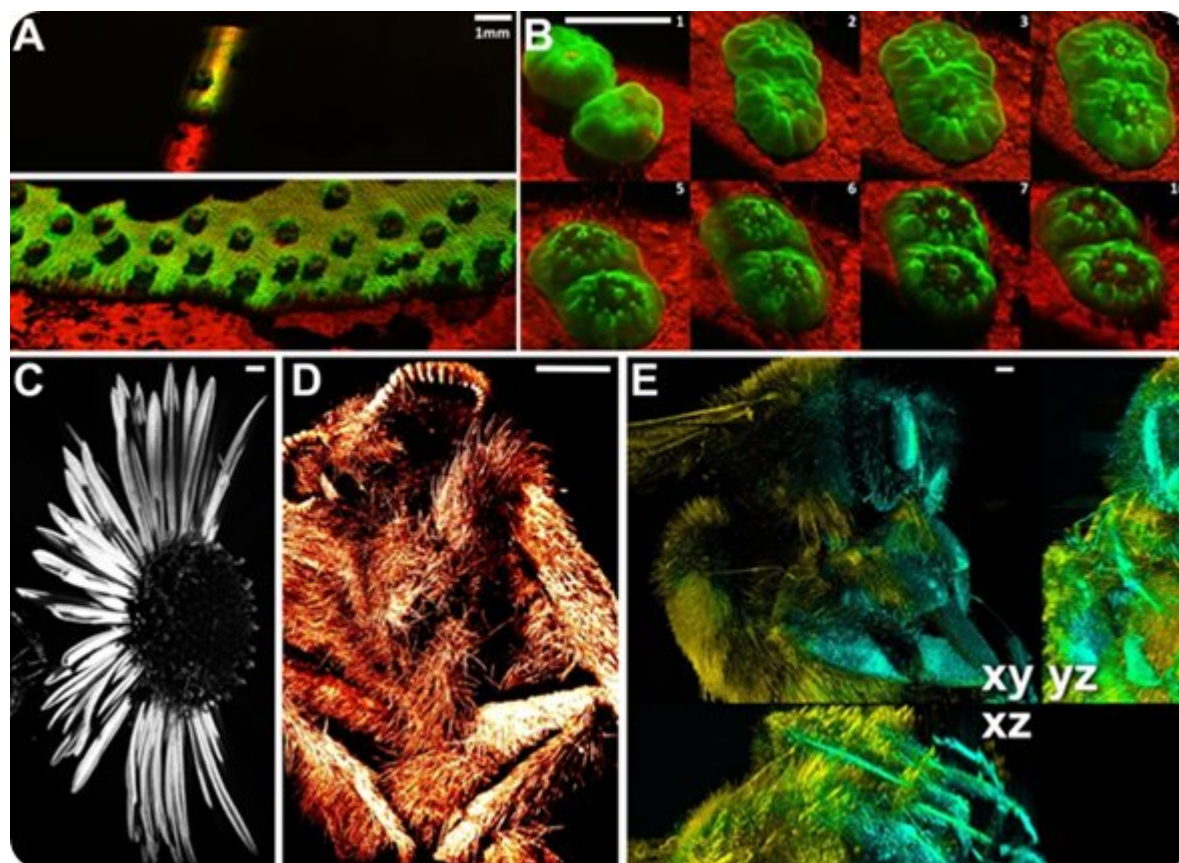


Figure 3. All scalebars are 1mm. A) Comparing the width of a standing light-sheet (top row) with the swept one of the L-SPI (bottom row), demonstrating why it is suitable for large samples. B) Early development of photosensitive coral larva of *Favia fragum*. Days after settlement are indicated by numbers. C) Reflectance imaging of daisy flower. D) Reflectance imaging of ghost moth. E) False-colored, three-dimensional image of bumblebee. Two L-SPIs were used, and color-separated into yellow and cyan.

Acknowledgement

Produced from materials originally authored by Pierre Philippe Laissue, who is director of the bioimaging facility and bioimaging senior research officer at Laissue School of Biological Sciences, University of Essex and a Royal Society Industry Fellow at Cairn Research Ltd.

Funding from the Royal Society and the University of Essex has enabled the development of a prototype and its conversion to the L-SPI. Laissue is currently a Royal Society Industry Fellow (IF150018).

About Cairn Research



[Cairn Research Ltd.](http://www.cairnresearch.com) is an independent scientific instruments manufacturer based in Faversham, Kent, in the UK. They mainly produce instrumentation for fast, sensitive fluorescence measurements, and related optical techniques such as flash photolysis and luminescence.

Their customers are typically bioscience researchers in prestigious institutions, and their input has encouraged their expansion into experimentally related areas, such as electrophysiology, with their innovative Optopatch patch clamp

amplifier. In addition to manufacturing their own proprietary research equipment they are experienced in providing complete integrated systems based around their hardware and high quality components from other suppliers.

They have good working relationships with the top manufacturers of research microscopes, and are happy to supply and install microscopes from Olympus, Nikon and Zeiss. In the fluorescence imaging field they work with companies including Andor Biosciences, Chroma Inc., Roper Scientific, Hamamatsu, IonOptix and RedShirt Imaging to provide comprehensive solutions.

They guarantee that whether you choose to buy a single component or an entire system from Cairn, you will get the same high level of service and support.

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