Chromacity™

Two-Photon Lightsheet Microscopy

using the Chromacity 1040

Lightsheet microscopy is a new paradigm in two-photon imaging. Sectioned images can be generated in real time, and a high-density 3-D image stack can be recorded in seconds, representing a major advantage over traditional confocal laser-scanning microscopy.

Fluorescence excitation and detection are split into two separate light paths. Illumination of the sample is perpendicular to the detection axis. This allows for detection of the fluorescence signal only at the in-focal plane without the need for a pinhole or image processing. Images can be generated significantly faster than conventional confocal systems.

This application note describes an easy-to-build lightsheet microscope able to image a zebrafish embryo using the Chromacity 1040.

Two-photon lightsheet microscope

The Chromacity 1040 has excellent beam quality and its high average power makes it ideal for two-photon lightsheet microscopy[1,2]. The experiment described here combines the Chromacity 1040 laser with an open-source design for a lightsheet microscope[3]. Illuminating samples perpendicular to the detection axis makes the technique very photon efficient. The lightsheet microscope generates photons only in the focal plane and not in other layers of the sample. The technique is ideal for imaging deep within transparent tissues, or within whole organisms. Deep penetration is possible even within scattering tissues, as the samples are exposed to only a thin plane of light (0.5-5 µm of a sample can be illuminated), in contrast to epifluorescence microscopy. This means photobleaching and phototoxicity are comparatively less than in confocal, wide-field fluorescence, or multiphoton microscopy, making it possible to perform more scans per specimen. The technique also delivers high speed imaging with image acquisition up to 1000 times faster than those offered by point-scanning methods.

Two-photon lightsheet microscopy is a powerful tool for imaging embryonic development. zebrafish are often used as an alternative to mammalian species as they are vertebrates with a high degree of similarly in their early development (with respect to many mammals, including humans) and the roles of many biological processes and cell development is homologous across species with vertebrae. They are also mostly transparent and thus enable lightsheet imaging through the whole embryo enabling a stack to be created at different image depths enabling a 3-D reconstruction of the embryo. The specimen for this demonstration was a zebrafish embryo stained with eosin and fixed in agar gel inside a capillary. In the leftmost image (Fig 1.), the zebrafish head and tail are in focus and generating the strongest two-photon fluorescence signal.

As the zebrafish is translated through the lightsheet, these features move out of the sheet and become dark, while the yolk sac moves into the sheet and emits strong two-photon fluorescence.

This image stack illustrates how easy it is to generate detailed information of the entire embryo using a fast scanning technique.

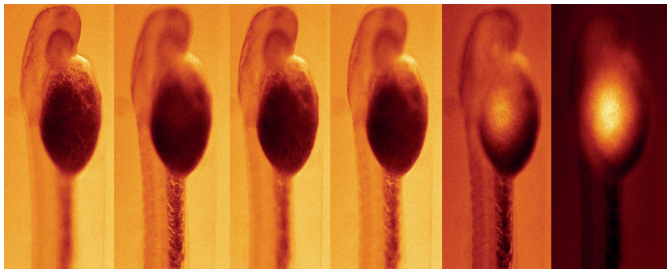

FIGURE 1. Zebrafish embryo image stack with 50-µm increments between the images.

Chromacity™

Two-Photon Lightsheet Microscopy

using the Chromacity 1040

Experimental setup

The sample was suspended in a glass capillary inside a cuvette on top of a stepper motor which allowed the sample to be rotated through 360°. The cuvette was fixed onto an optical rail, which also supported a x4 infinity corrected objective lens (Zeiss®) and a camera. A lens tube (Thorlabs®) was attached to the camera and used to support a 160 mm tube lens at its focal length from the camera sensor. This arrangement allows the camera to be moved freely to allow filters to be inserted between the tube lens and the 4x objective. An xyz stage (Newport®) with one axis motorised was used to suspend the stepper motor and sample combination.

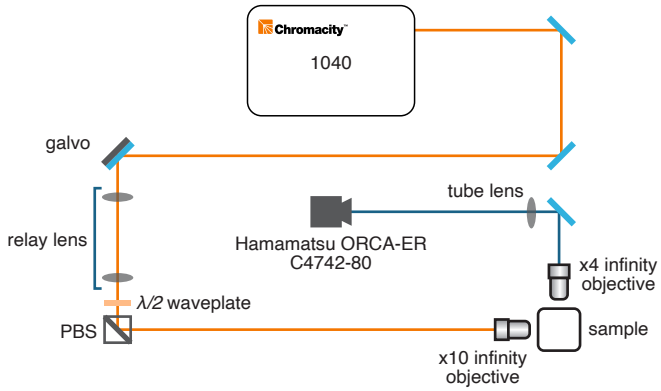

FIGURE 2. Two-photon lightsheet microscope layout.

Fig. 2 shows the layout, with the entire optical system on a 75 cm x 75 cm breadboard. Light from the laser is steered onto a galvanometer mirror (Thorlabs®) which is relay imaged using a pair of AR-coated lenses onto the pupil of a x10 infinity corrected objective lens (Mitutoyo®). Before entering the objective the light is attenuated using a half-wave plate and a polarizing beamsplitter, enabling adjustment of the illumination intensity to avoid camera saturation.

Summary

A two-photon lightsheet microscope is easily implemented using the Chromacity 1040 laser and readily available stock optical components.

The high stability and high average power of the laser means that two-photon lightsheet microscopy can be easily carried out using any fluorescent agents with some linear absorption in the 500 nm region, including RFP, YFP, GFP, Sytox green, fluorescein and many others.

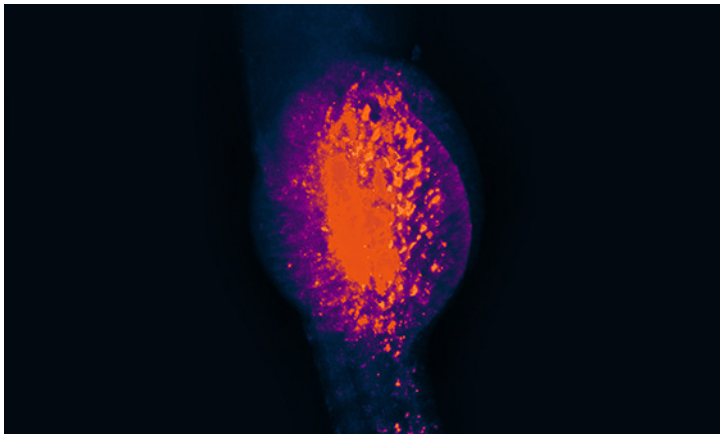

FIGURE 3. Image of zebrafish embryo when using a x4 objective.

Acknowledgements

We are grateful to the Institute of Genetics and Molecular Medicine at the University of Edinburgh for their collaboration in developing the lightsheet microscope.

References

1. T. V. Truong, et al, "Deep and fast live imaging with two-photon scanned light-sheet microscopy," Nat. Methods 8, 757 (2011).
2. O. E. Olarte et al, "Image formation by linear and nonlinear digital scanned light- sheet fluorescence microscopy with Gaussian and Bessel beam profiles," Biomed. Opt. Express 3, 1492 (2012).
3. OPEN-SPIN Microscopy: https://sites.google.com/site/openspinmicroscopy/

Learn how our ultrafast lasers can enable you to discover more.
For more information, email: sales@chromacitylasers.com

Discover More
chromacitylasers.com