Chromacity™ GMP

TWO-PHOTON FLUORESCENCE MICROSCOPY

with the Chromacity 1040

The Chromacity 1040 provides the perfect balance of peak-power, pulse duration and average power for generating images of biological samples through a two-photon non-linear process. In this application note, we illustrate the suitability of the Chromacity 1040 for two-photon microscopy, to image a wide range of cells with various labels.

Two-photon fluorescence microscopy

In recent years, two-photon fluorescence microscopy has become a powerful tool to study biological functions in vivo. In comparison to many standard imaging techniques it has become vital to optogenetic research as it has enabled deeper imaging in highly scattering brain tissue with reduced photobleaching and improved spatial resolution. The main advantages of two-photon fluorescence microscopy is that it enables excitation with a source which scatters less and only excites samples in the focal plane, thereby providing better spatial resolution. This requires a highly stable femtosecond laser, which provides the required combination of pulsewidth, pulse energy and average power to enable high-resolution imaging of biological samples. A range of samples have been imaged using the Chromacity 1040 to demonstrate its two-photon fluorescence microscopy capabilities.

Unlike solid-state lasers, which can produce beams with an elliptical cross-section, the Chromacity 1040's beam originates from a single-mode fibre, so it is perfectly symmetrical.

Imaging of invitrogen fluocells, kidney, liver and collagen

The Chromacity 1040 was used as the excitation laser to selectively highlight several different markers in a range of samples. Unlike solid-state lasers, which can produce beams with an elliptical cross-section, the Chromacity 1040's beam originates from a single-mode fibre, so it is perfectly symmetric. This makes the laser system ideally suited for coupling into commercial laser-scanning microscopes to provide excellent beam shape and high average powers at the sample plane.

Fig. 1 shows typical images acquired from the system when imaging; Fig. 1(a) - Invitrogen Fluocells - section of mouse intestine (cells approximately 5 µm in diameter) via excitation of SYTOX® Green; Fig. 1(b) demonstrates liver cells imaged using direct excitation of RFP, YFP and GFP using the same excitation laser (images overlaid); and Fig. 1(c) which shows kidney cells overlaid by collagen and demonstrate the ease with which samples can be differentiated using mT:mG as a marker and SHG from the collagen fibres (making it easy to differentiate between cell types).

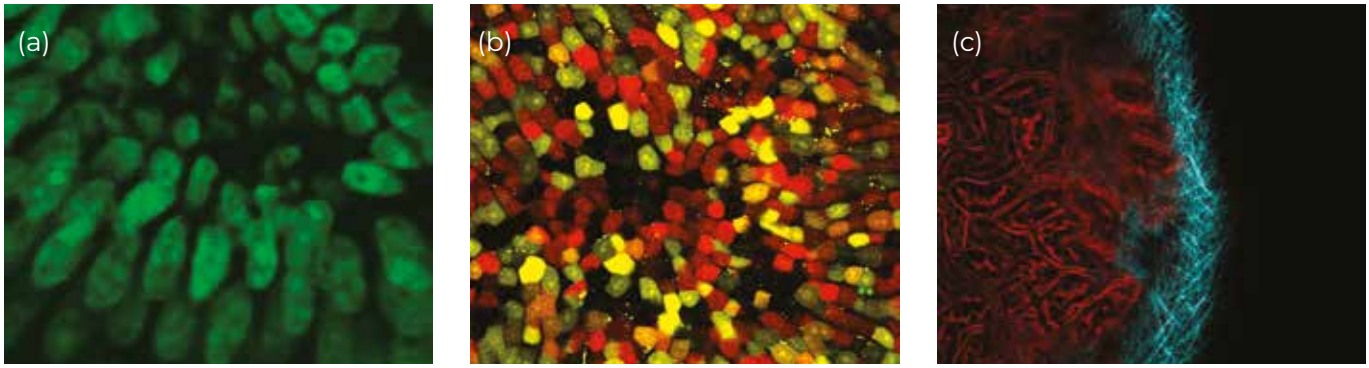

FIGURE 1. Two-photon fluorescence observed in (a) the nuclei of mouse intestine cells stained with SYTOX® Green, (b) liver cells visualized with RFP, YFP and GFP, and (c) kidney cells with mT:mG.

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The power levels from the Chromacity 1040 were more than adequate for generating these fluorescence images, which were recorded with only 150 mW incident on the galvo-scanning mirrors.

The Chromacity 1040 produces a free-space beam ideally suited for coupling into microscope systems. Additionally, the output is horizontally polarized, making it a perfect source for two-photon polarization microscopy (including second harmonic generation microscopy). Unlike other systems on the market, the Chromacity 1040 is insensitive to small amounts of light reflected back from the microscope, so an optical isolator is typically not required between the laser and the microscope, thus minimizing the losses within the optical system.

Experimental setup

The images were acquired using a Nikon microscope, into which the beam was introduced via a pair of galvo-scanning mirrors. A Nikon 60x Plan Apochromat oil immersion objective (NA 1.4, working distance 0.21 mm) was used to image the samples. A 520 nm notch filter (Semrock Brightline FF01-520/15-25) was used to filter out the two-photon fluorescence signal, which was collected using a standard photo-multiplier tube (PMT). Fig. 2. illustrates the setup used and the Chromacity 1040 system.

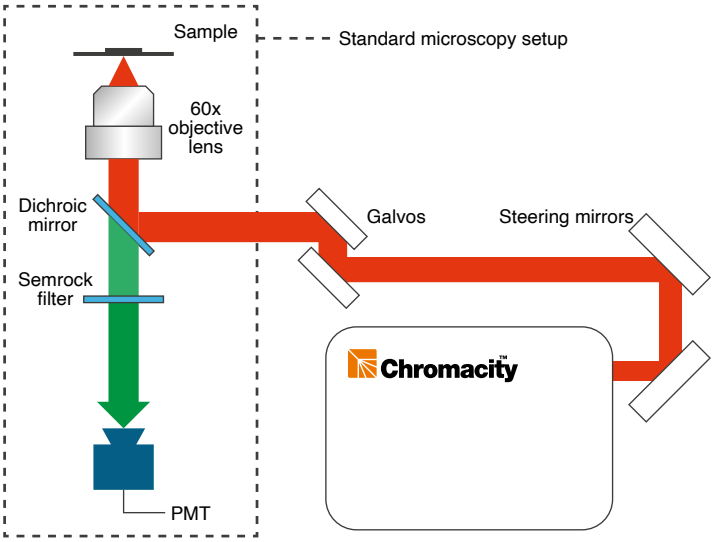

FIGURE 2.
Schematic representation of microscopy setup and the Chromacity 1040.

Summary

Including the Chromacity 1040 femtosecond laser as part of a multi-photon imaging system allows users to generate exceptionally clear, high-resolution images, as a result of its excellent beam quality, ultrafast pulses and high average power levels.

Acknowledgements

This work was carried out in partnership with ICFO - The Institute of Photonic Sciences in Barcelona and The Institute of Genetics and Molecular Medicine, at the University of Edinburgh.

The Chromacity 1040 is ideal for coupling into microscope systems, empowering users to produce clear and high-resolution images, as a result of its excellent beam quality, ultrafast pulses and high average power levels.

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