ARTICLE

# Multiphoton Microscopy III: A Novel Concept in Multiphoton Microscopy

EXCITE THEM ALL!

Imagine simultaneously imaging a wide range of fluorophores without having to think about selecting the optimum excitation wavelength of your laser. In two-photon microscopy, this is often a complex and sometimes an impossible task when using traditional 100fs excitation lasers.

Transform-limited 100fs solid-state lasers emitting in the IR have a spectrum in the range of 10 – 20nm and as a result, they can only simultaneously excite fluorophores whose excitation spectra falls within this 10-20nm spectrum.

To simultaneously excite a larger variety of fluorophores with a single laser, lasers with broader bandwidths and shorter pulse durations are required.

For example, a laser with 15 fs pulses, such as [FYLA´s SCH laser](#), centered at 1050nm, delivers 15fs pulses and a bandwidth of 200nm. All green and red fluorophores within this bandwidth (which extends across 900 to 1200nm) can be excited simultaneously by such laser.
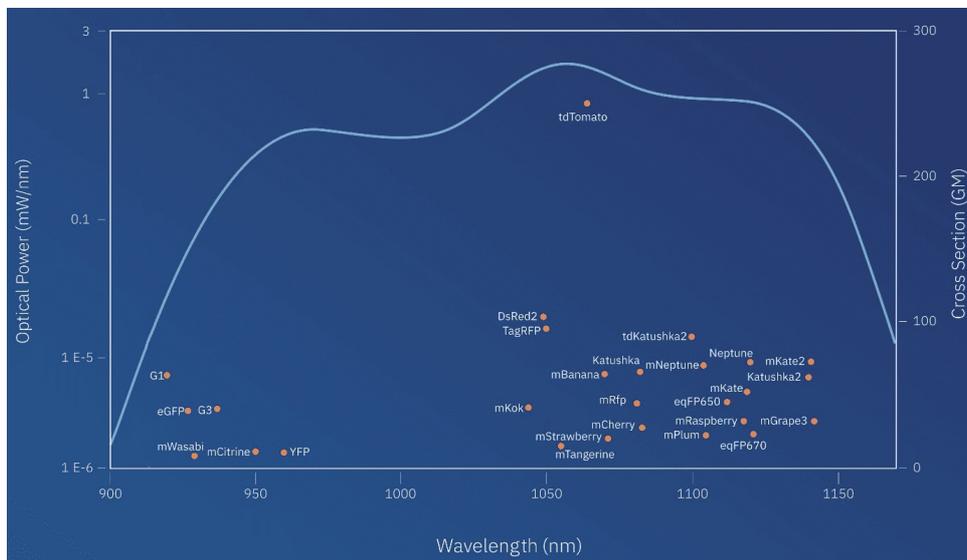
This makes simultaneous imaging of multiple fluorophores a viable, practical and simple alternative for two-photon microscopy.
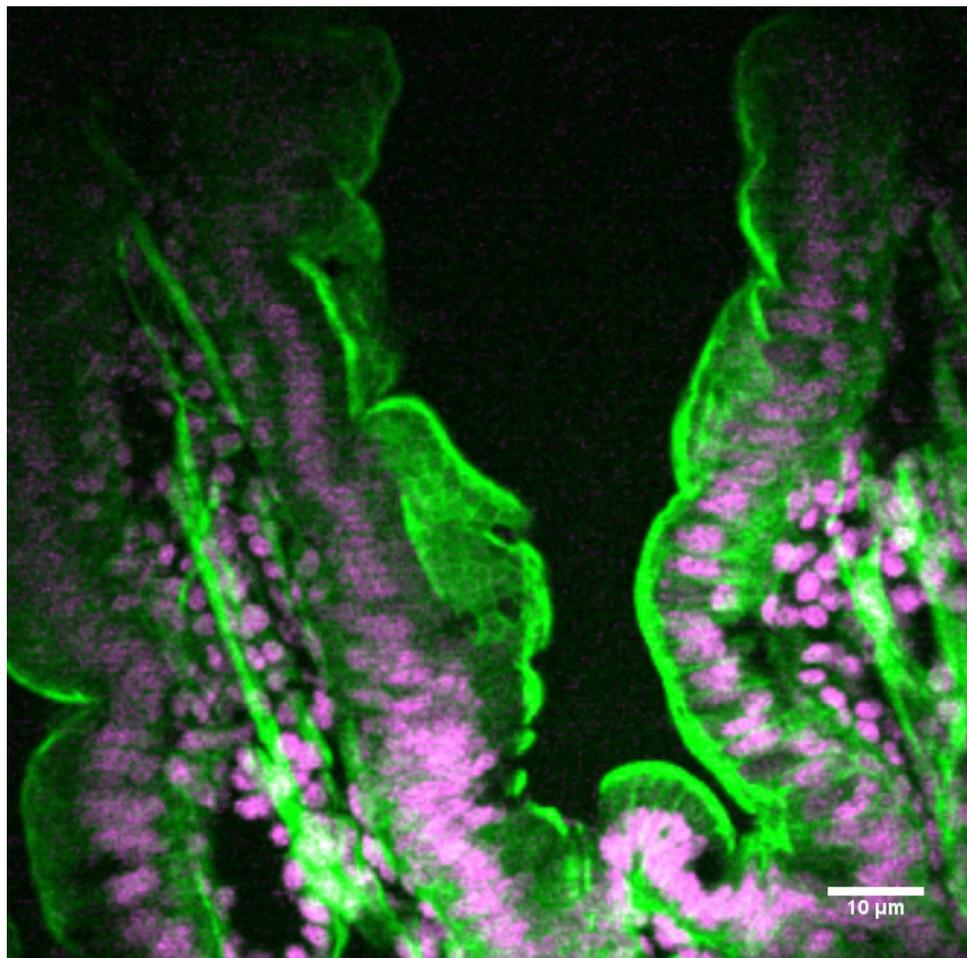
Two-Photon Fluorescence Microscopy Image of a Mouse Intestine.

Section stained with
Sytox Green: labelling the nuclei (magenta). FITC Filter
Alexa Fluor 568 Phaloidin: labelling the actin filaments (green). TRITC Filter

Both fluorescent markers were simultaneously excited with FYLA´s

SCH **and fluorescence was filtered with Nikon´s fluorescence cubes.**
Image taken at ICFO-SLN the Super-Resolution Light Microscopy at ICFO-Institute of Photonics Sciences, Barcelona, Spain.
Acknowedgements to Sphere Photonics for the D-SCAN pre-compressor

You can find the presentation of SPAOM2020, about this topic here.