Multimodal Imaging: Combining Fixed Wavelength Lasers and Tunable Ti: Sapphire Lasers for Expanded Capability

Two-photon (2P) microscopy based on tunable ultrafast lasers has become a widespread tool for 3D imaging with sub-cellular resolution in living tissues. In recent years fixed-wavelength, ultrafast Yb lasers have gained increasing interest for multiphoton microscopy due to their considerable average and peak power and infrared (IR) wavelength. Red fluorescent proteins (RFPs) that can be excited at a 1 µm wavelength are becoming increasingly available for practical use. At these longer excitation wavelengths, deeper imaging penetration can be achieved due to lower optical scattering in the tissue. Spectra-Physics' HighQ-2™ is a cost-effective, fixed wavelength ultrafast Yb laser that delivers high peak power at 1045 nm from an ultracompact package.

An initial test was performed to demonstrate the imaging capability of the HighQ-2 with RFPs. The sample was a GAD2cre/Ai9 transgenic mouse line, in which the GABAergic cells express the red fluorescent protein TdTomato. This RFP has its 2P excitation peak at 554 nm and maximum emission at 581 nm. As indicated by Figure 1, the HighQ-2 laser is ideal for two photon imaging of this molecule and generates high-contrast images up to a tissue depth of about 500 µm.

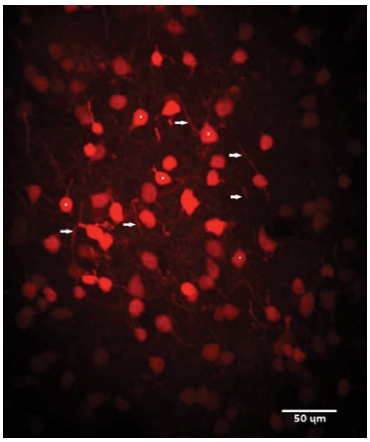

Figure 1: Representative photomicrograph of GABAergic cells in the neocortex of GADcre/Ai9 mouse in vivo. The maximal intensity projection of a 10 µm (z axis) imaged area captured 200 µm under the brain surface. Maximum imaging depth was found to be 500 µm. The somas (marked with white asterisks (*)) and main dendrites (marked with white arrows) are clearly visible. RFP Td Tomato was visualized by a 2P microscope equipped with a HighQ-2 fs-laser source. Image courtesy of Gaspar Olah, Gabor Molnar and Gabor Tamas, University of Szeged.

To extend the functionality of existing 2P microscopes equipped with tunable Ti:sapphire lasers, the straightforward addition of a HighQ-2 laser can enable dual wavelength excitation. The images in Figure 2 show excitation of 3T3 transgenic mice cells with a Ti:Sapphire laser at a center wavelength of 940 nm, excitation with the HighQ-2 at 1045 nm, and then excitation with both simultaneously. The combination of the two laser systems allows for the simultaneous excitation of a broader range of fluorophores, especially ones with 2P excitation wavelength in the longer IR wavelengths.

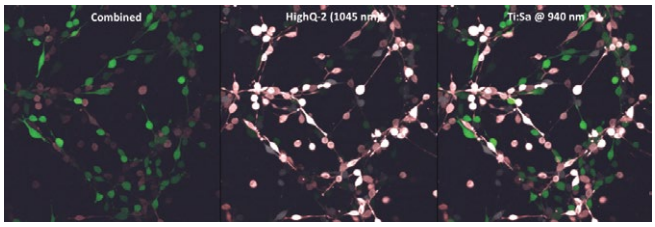

Figure 2: Multimodal imaging combining YFP excitation (Green) by a Ti:Sapphire laser at 940 nm with dsRed excitation (red) and mKate2 (gray) at 1045 nm. Images are of 3T3 cell lines that had been transfected with modified versions of the MIGR1 retroviral vector, such that either YFP, dsRed, or mKate2 was inserted in place of GFP expression. The individual cell lines were then co-cultured together after transfection and imaged on a LaVision Biotec TriM Scope II with a Ti:Sapphire laser tuned to a center wavelength of 940 nm and HighQ-2 at 1045 nm. The filter sets on the system to separate out the emission were 525/50 (YFP signal), 605/60 (YFP/dsRed signal), and 665/70 (dsRed and mKate2 signal). Images courtesy of David Gonzalez, Ann Haberman, Yale University.

Fixed wavelength IR lasers are also well suited for producing label-free second harmonic generation (SHG) and third harmonic generation (THG) signals. A key benefit is that the resulting SHG and THG wavelengths (522 and 348 nm, respectively) are easy to detect due to detector sensitivity and good transmission by readily available optics. SHG emission occurs in non-centrosymmetric media, for instance regular patterns of anisotropic molecules in elastin- or collagen-rich tissues or in muscle tissue. Figure 3 shows SHG imaging of a cornea sample with a HighQ-2 laser. THG can occur when the polarization symmetry is violated in z-direction within the focal region, for example by refractive index mismatch. This enables 3D-imaging of tissues. Moreover, colorants like hemoglobin can cause resonance enhancement leading to a strong THG signal. Figure 4 shows 3D slices from THG imaging of an oil drop in water.

Spectra-Physics®
A Newport Company

Multimodal Imaging: Combining Fixed Wavelength Lasers and Tunable Ti:Sapphire Lasers for Expanded Capability

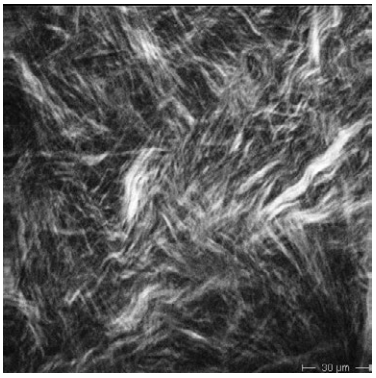

Figure 3: SHG imaging of a cornea sample using HighQ-2 laser at 1045 nm. The collagen fibers generate a strong SHG signal. Image courtesy of Hans Georg Breunig, University of Saarland, Germany

In summary, HighQ-2 is a cost-effective ultrafast laser source for multiphoton microscopy as a stand-alone source for RFP excitation, or alternatively to extend the infrared capabilities of a microscope powered by a tunable Ti:Sapphire laser. HighQ-2 is also suitable for label-free imaging of biological samples via SHG or THG.

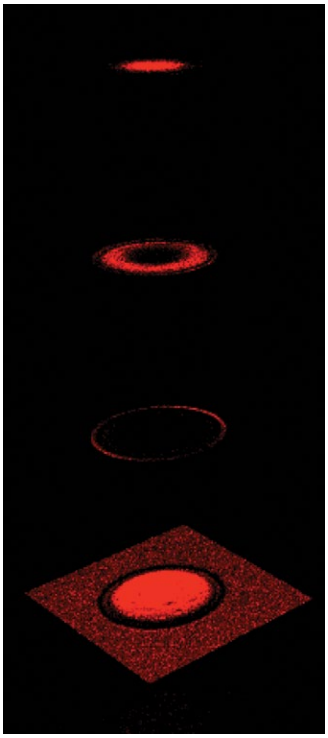

Figure 4: THG imaging of an oil drop in water on a glass substrate. The slices are 5 µm apart from each other, forming a 3D stack. Image courtesy of Mahesh Namboodiri, Chalmers University, Sweden.

PRODUCTS: *HighQ-2, HighQ-2-SHG*

	HighQ-2	HighQ-2-SHG
Output Characteristics	IR	SHG
Wavelength	1045 ± 5.0 nm	522 ± 3.0 nm
Power	>1.5 W	>0.65 W
Repetition Rate	63 MHz	
Pulse Width	<250 fs	

Spectra-Physics®
A Newport Company

www.spectra-physics.com

3635 Peterson Way, Santa Clara, CA 95054, USA
PHONE: 1-800-775-5273 1-408-980-4300 FAX: 1-408-980-6921 EMAIL: sales@spectra-physics.com

Belgium	+32-(0)0800-11 257	belgium@newport.com	Korea	+82-31-8069-2401	korea@spectra-physics.com
China	+86-10-6267-0065	info@spectra-physics.com.cn	Netherlands	+31-(0)30 6592111	netherlands@newport.com
France	+33-(0)1-60-91-68-68	france@newport.com	Singapore	+65-6664-0040	sales.sg@newport.com
Germany / Austria / Switzerland	+49-(0)6151-708-0	germany@newport.com	Taiwan	+886 -(0)2-2508-4977	sales@newport.com.tw
Japan	+81-3-3794-5511	spectra-physics@splasers.co.jp	United Kingdom	+44-1235-432-710	uk@newport.com

SP-AP-20161212-33