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THE NEW INDUSTRY

ARTICLE

New Horizons in Two-Photon Microscopy

The SCH is a new class of femtosecond fiber laser for two-photon microscopy. It is a new proposal, it is differential. Powered at the core by FYLA´s proprietary technology, it enables the simultaneous excitation of the largest variety of fluorescent probes. It provides images with higher brightness. It introduces a new era of simplicity and cost.

Differential

· Simultaneous excitation of distant fluorescent probes

· Higher brightness

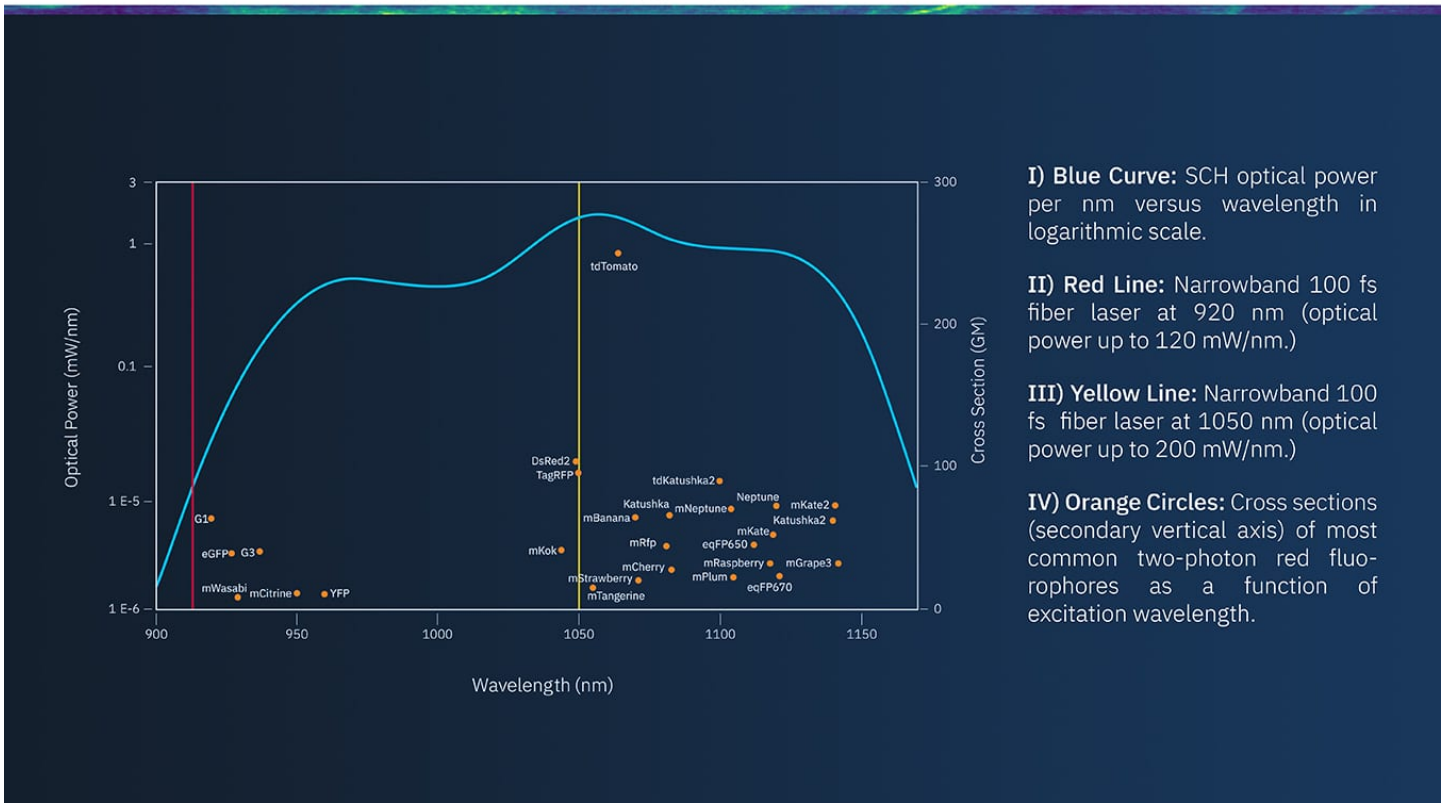
· Simpler and cost-effective

FYLA´s SCH provides an extremely wide spectral bandwidth which

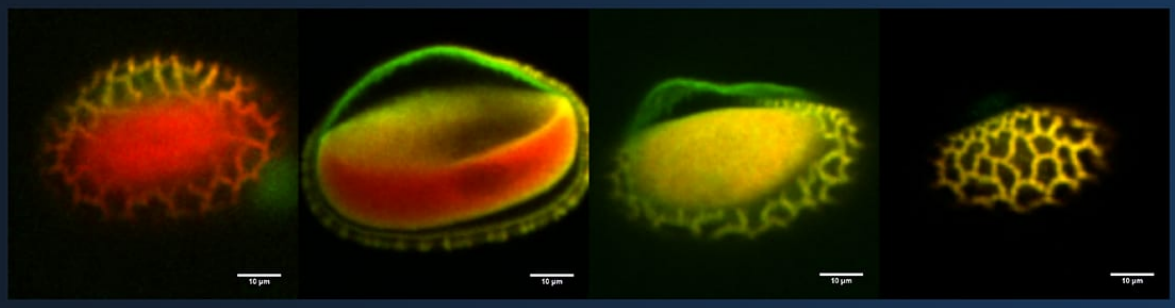
expands in the NIR across the 900-1200nm spectrum. This overlaps with the two-photon excitation spectra of most green and red-shifted fluorescent labels, including eGFP, mRFP and DsRED. This remarkably exceeds the range of fluorescent labels that can be simultaneously excited with conventional femtosecond lasers, including broadly tunable lasers and single-line femtosecond fiber lasers.

FYLA´s SCH offers a highly flexible and versatile solution or two-photon excitation fluorescence microscopy, enhancing the features that can be imaged simultaneously on a sample, of particular importance for in-vivo and ex-vivo microscopy.

A Rich Variety of Imaging Probes



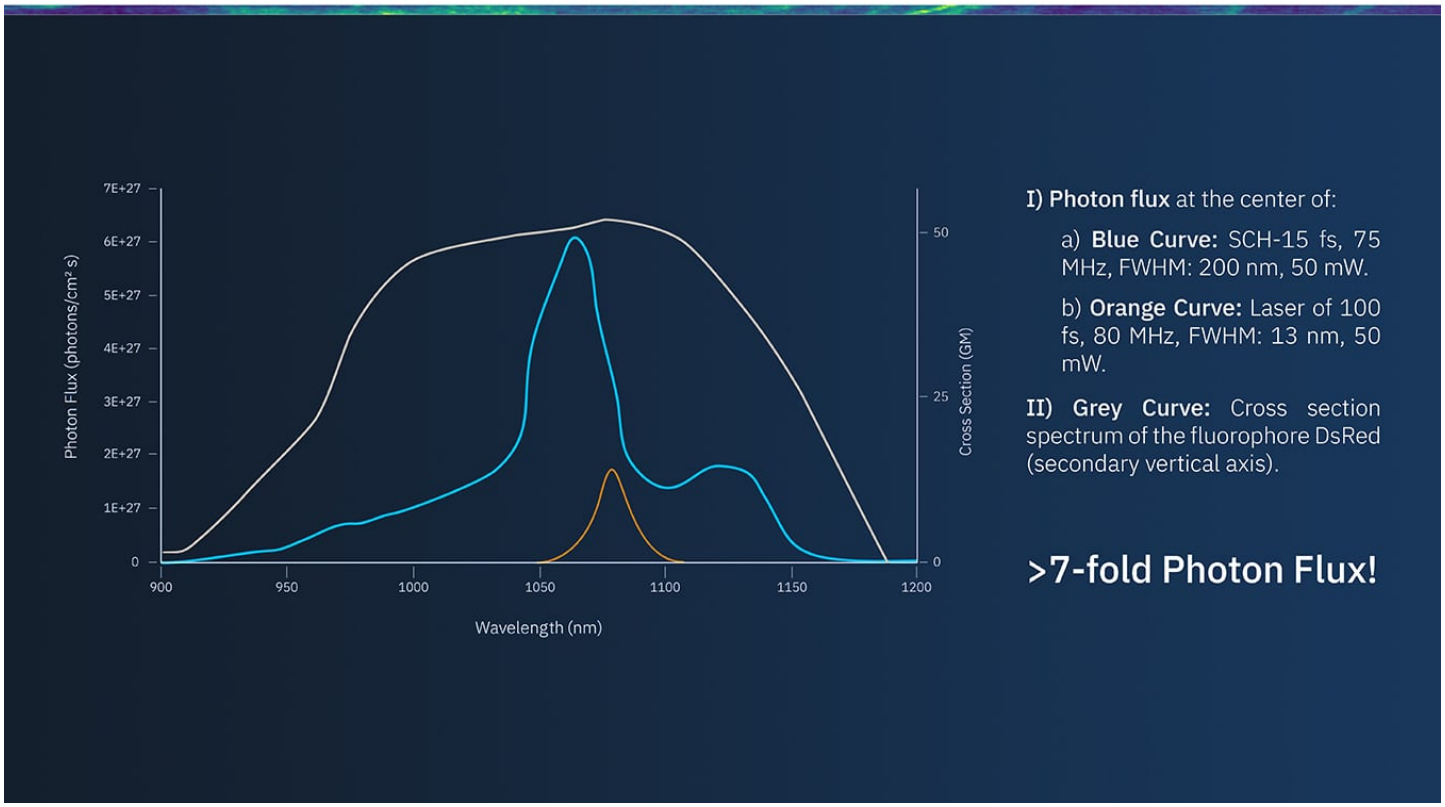
An image of a pollen grain using the pollen autofluorescence excited by the SCH laser shows the great image quality and simultaneous excitation at different spectral channels.



A grain of pollen taken with the SCH laser. MAX projection of a Z-stack 2P fluorescence images of a pollen grain corresponding to 60um. Autofluorescence has been splitted with two fluorescence filters, FITC filter (green) and TRITC filter (red).

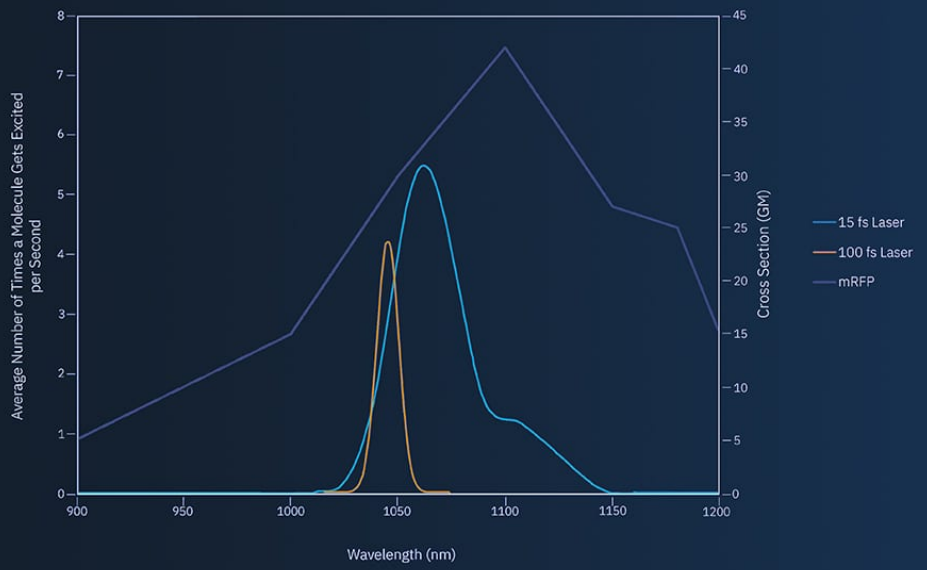
Not only the spectrum is broader but the SCH also delivers shorter pulses. Combined with a dedicated state-of-the-art dispersion pre-compensator, pulses of the order of 15-20fs can be delivered on the microscope sample plane. This leads to an extraordinary peak power and an unparalleled photon flux at the sample plane, reaching more than 7-fold the photon flux of conventional femtosecond lasers with pulses in the range of 100-200fs.

A Boundless Flux of Photons



The larger photon flux associated to the superior SCH peak power leads to an increased number of photons that reach the sample per area and time. This enhances the two-photon excitation efficiency up to a 49-fold when compared with conventional fixed-wavelength or broadly tunable lasers with pulse durations in the range of 100-200 fs. When used with fluorescent label DsRED, over a 50% efficiency is achieved.

Extremely Efficient



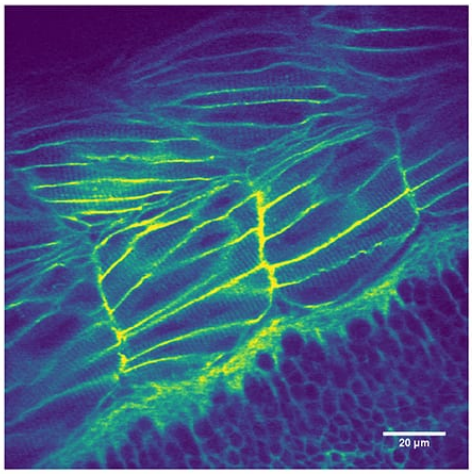
Average number of times a molecule of mRFP fluorophore gets excited by a pulse of:

a) Blue Curve: SCH-15 fs, 75 MHz, 50 mW, FWHM: 200 nm.

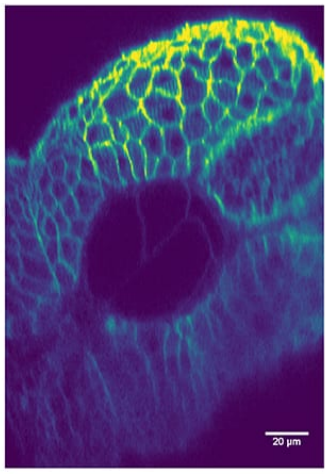
b) Orange Curve: Laser of 100 fs, 80 MHz, 50 mW, FWHM: 13 nm.

The red-shifted NIR wavelengths of FYLA´s SCH laser combined with the increased excitation efficiency leads to better image brightness and deeper penetration. A 200 microns deep sample of a zebra fish is simply imaged by the SCH laser.

Excellent Image Quality and Depth



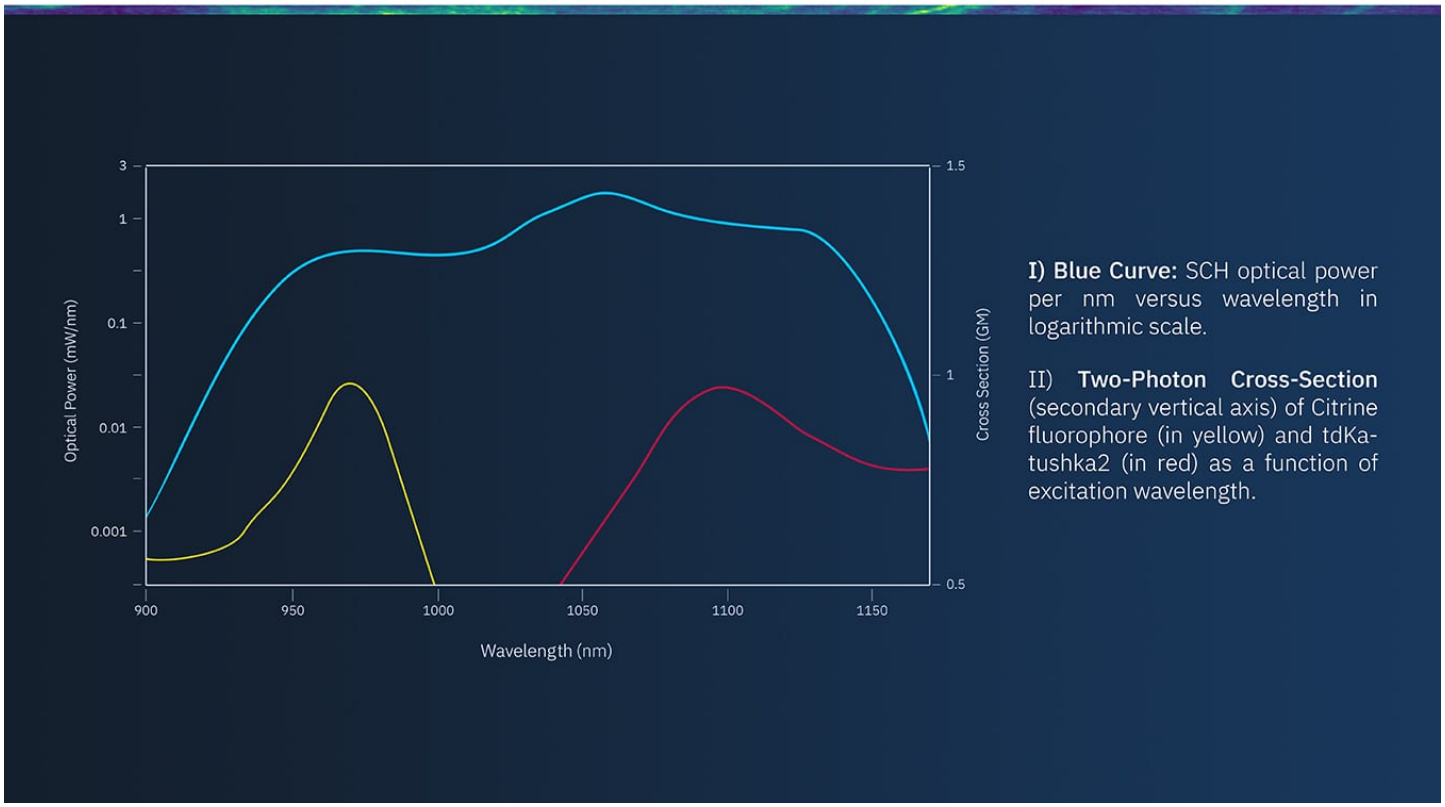
2P fluorescence microscopy images of the tail of a 2-days-old transgenic line zebrafish embryo (Caax-GFP) expressing GFP in all membranes.



Reslice of a 200 um Z-stack of 2P fluorescence microscopy images of the tail of a 2-days-old transgenic line zebrafish embryo (Caax-GFP) expressing GFP in all membranes.

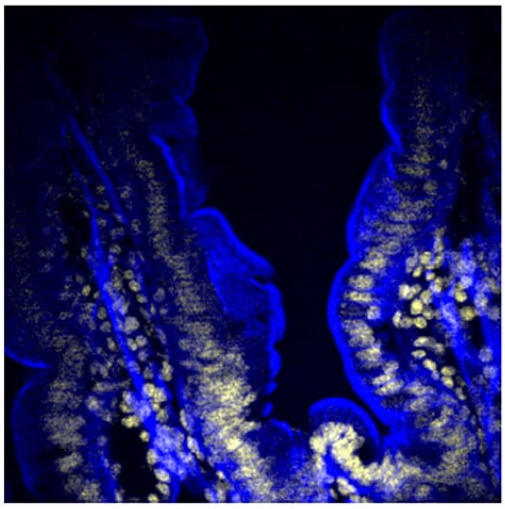
The SCH broad spectral bandwidth not only enables the excitation of a large range of indicators but it also permits the multicolor excitation across the 900-1200 nm range with a single scan, making simultaneous excitation of different probes possible. This eliminates microscope alignment issues associated to broadly tunable lasers.

A Continuum of Multicoloured Excitation

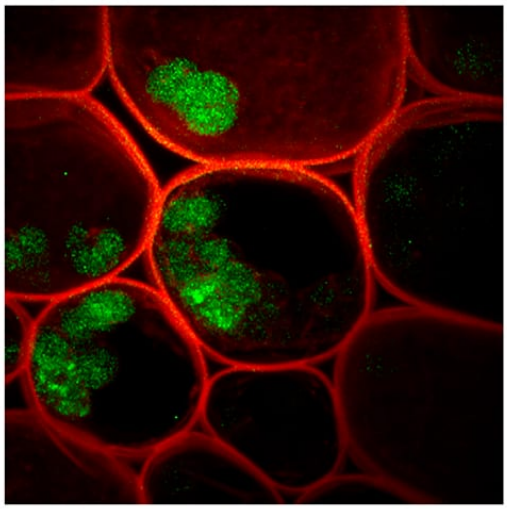


Images of a mouse intestine and a convallaria illustrate the great image quality that can be achieved when illuminating these samples with the broad bandwidth of the SCH laser.

Two-Photon Microscopy Images with FYLA SCH

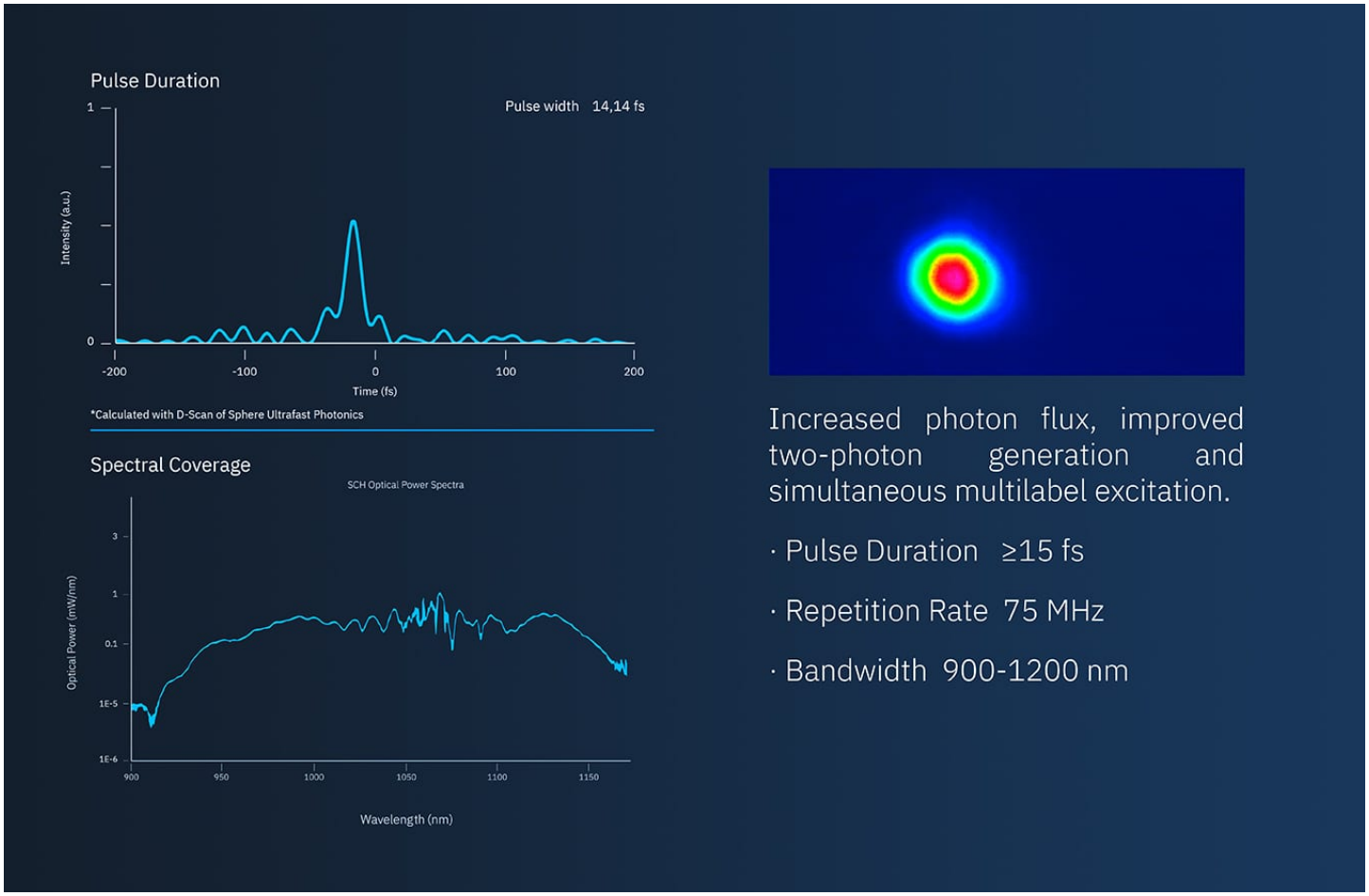


Fluo4: 2P fluorescence microscopy images of a mouse intestine section stained with SYTOX Green labelling the nuclei (magenta/yellow), and Alexa Fluor 568 phalloidin labelling the actin filaments (green/blue).



Convallaria: Autofluorescence taken with two channels (membrane and nuclei).

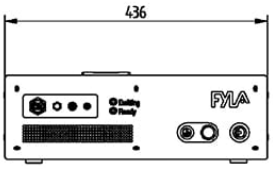
FYLA´s SCH delivers a bandwidth of 200 nm, across the 900-1200nm spectral range, with pulses of 15 fs and a repetition rate of 75MHz, enhancing two-photon excitation and enabling the individual or simultaneous excitation of bountiful indicators.

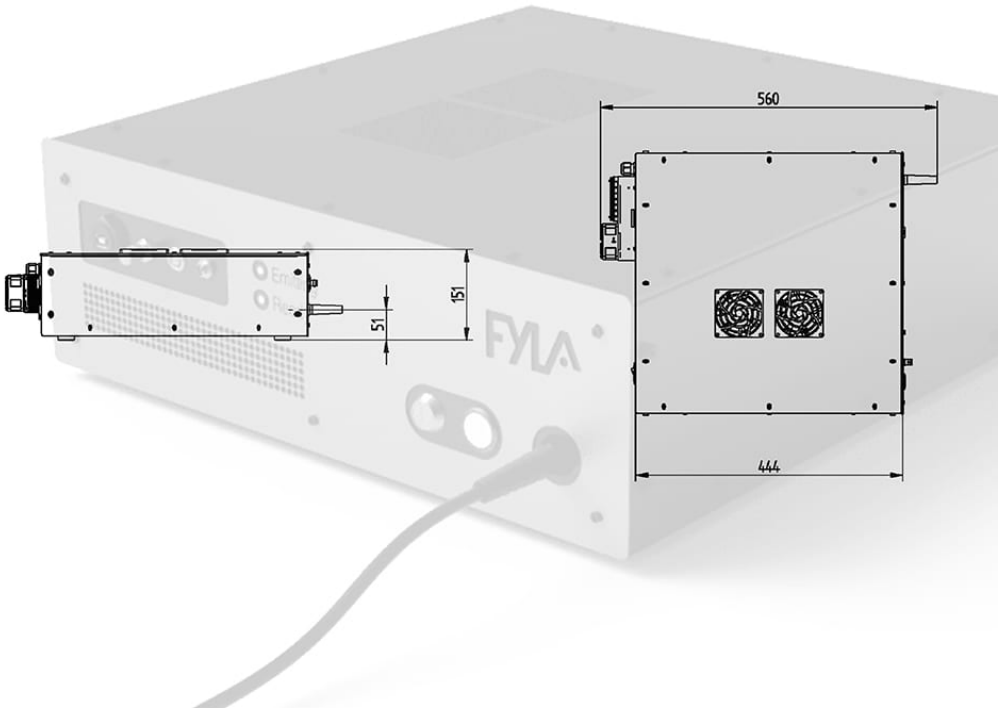


It is an all-fiber laser which is extremely compact, robust, and cost-effective.

Compact, Robust, Simple

Dimensions in mm





Contact us if you would like to find out more about how this laser could enhance your two-photon microscopy images and advance your research.

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