Next generation two-photon microscopy using the FemtoFiber ultra 920 fiber laser

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Two-photon fluorescence microscopy has become a key technology in biological imaging enabling three-dimensional, noninvasive studies of biological tissue on the submicron scale. To boost the usability of this method and to provide an ultracompact, turn-key laser source, TOPTICA Photonics is proud to introduce the new FemtoFiber ultra 920, the latest member of their successful femtosecond fiber laser family. With its robust and compact design, the FemtoFiber ultra 920 is an easy to operate and maintenance-free laser system. The novel concept of the laser features a pulse duration <100 fs with a center wavelength of 920 nm and 1.5 Watt of average power (18.5 nJ at 80 MHz repetition rate). The unmatched temporal and spatial beam characteristics of the laser are fully tailored for deep-tissue nonlinear microscopy, providing excellent optical contrast and signal-to-noise.

Keywords: femtosecond lasers, ultrafast lasers, fiber lasers, neuroscience, laser synchronization, microscopy, spectroscopy, GFP, multi-photon microscopy, fluorescence microscopy, nonlinear microscopy

Figure 1: Picture of the new FemtoFiber ultra 920. The control and supply unit is integrated into a 19”-type standard rack which is connected via a detachable fiber to the laser head for straightforward OEM integration. The laser system and its unique characteristics is the perfect choice for nonlinear microscopy like two-photon excitation of fluorescent proteins.

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Introduction

Understanding the structure and function of the constituents of organic matter at the cellular level has always been a major goal in optical microscopy. For this application, linear, one-photon microscopy, like fluorescence microscopy, has traditionally been used for contrast generation. However, due to the light absorption and scattering, this technique is limited to near-surface imaging of tissue and suffers from strong degradation in optical resolution with increasing probing depth.

A milestone to resolve this limitation has been the development of nonlinear optical microscopy, in particular two-photon microscopy and second-harmonic generation (SHG) microscopy. As compared to linear microscopy, the nonlinear character of two-photon microscopy features several huge advantages with respect to deep-tissue imaging, signal detection, photodamage, and in-vivo imaging.

With the availability of turn-key, robust, and cost-efficient femtosecond laser sources based on fiber-laser technology, nonlinear optical microscopy is now widely available for everyone to use without large complexity.

Two-Photon Microscopy

Conventional fluorescence microscopy is based on the absorption of a single photon that promotes an electron within a fluorescent molecule into an excited state. Upon vibrational relaxation, the excited electron decays back to its ground state by emitting a single, Stokes-shifted photon (Fig.2 a). Using epi-microscopy schemes, images of the fluorophore distribution at the surface of the sample can be recorded. Due to the one-photon excitation in conventional fluorescence microscopy, this approach suffers from strong background radiation leading to a blurring of the image (Fig.2 b). To increase the spatial resolution and to suppress unwanted fluorescence from areas outside the focal plane, a confocal microscope is used. Due to the aperture-based reduction of unwanted background radiation, three-dimensional tissue imaging can also be performed with a probing depth of up to 100 µm, mainly limited by the absorption and the scattering of the excitation light.

A more sophisticated approach towards high resolution deep-tissue fluorescence microscopy is the use of two-photon microscopy. Here, two photons with

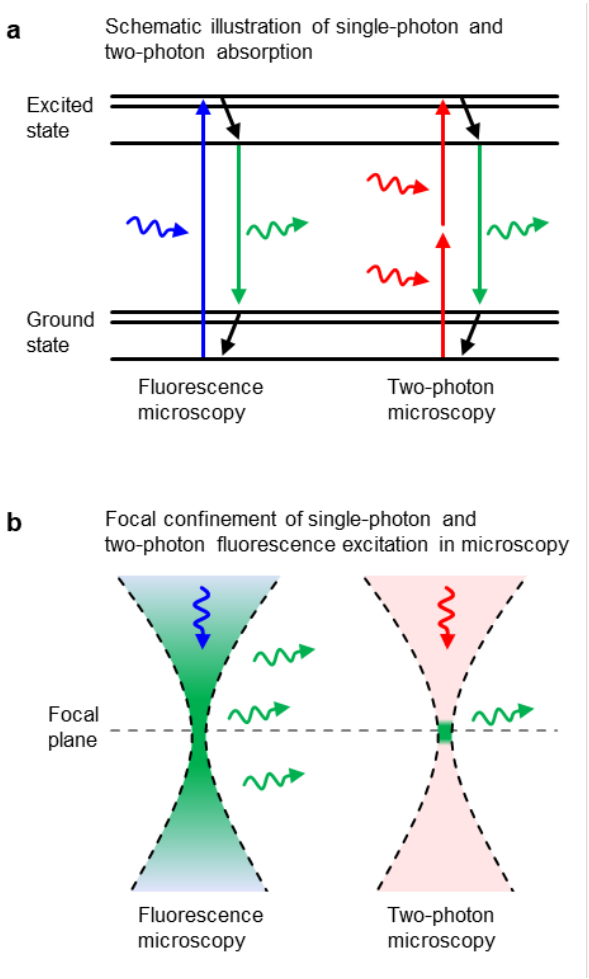

Figure 2: **a,** Illustration of the single-photon and two-photon absorption process for fluorescence excitation. **b,** Spatial confinement of fluorescence signal generation in conventional fluorescence microscopy and two-photon microscopy.

a photon energy approximately half of the photon energy for conventional fluorescence microscopy are used to excite a molecule.

This method offers significant advantages:

(i) Due to the lower photon energy, the absorption and the scattering of the excitation radiation is strongly reduced, leading to a much larger probing depth in deep-tissue imaging of up to 1 mm.

(ii) The signal detection does not require spatial filtering as in confocal microscopy, since the fluorescence excitation is limited to the focal plane of the microscope (Fig.2 b). In addition, due to the dramatically different excitation and detection wavelength, no optical filtering is necessary.

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(iii) The wavelength shift towards the near-infrared directly results in a reduction of photodamage within the sample that can be induced by high-energy photons.
(iv) The lower photon energy of the excitation light reduces the phototoxicity and is therefore highly suitable for in-vivo imaging.

Since nonlinear two-photon microscopy requires a high photon density to be efficient, typically intense femtosecond lasers with a pulse duration below 100 fs are required. The laser features repetition rates of 80 MHz with a pulse energy of several nJ.
Although many nonlinear spectroscopy and microscopy techniques have used titanium-sapphire lasers in the past, recent breakthroughs in laser technology have made fiber lasers equal alternatives. Fiber lasers provide unmatched flexibility and modularity, combined with robust and reliable operation that lend themselves to the usage in medical or biological laboratories.

FemtoFiber ultra 920

Up to now, wavelengths around 920 nm for addressing green fluorescent proteins (GFP) and other fluorophores has been a challenge for fiber lasers. To resolve this limitation, TOPTICA Photonics has now developed the new FemtoFiber ultra 920, the latest addition to TOPTICA's successful industrial high-power fiber lasers at 780 nm and 1050 nm.

Optimized for in-vivo, deep-tissue imaging, the laser features a center wavelength of 920 nm (Fig. 3a) to efficiently excite GFP and other fluorophores. The unique combination of <100 fs pulse duration with an output power of >1.5 W at a repetition rate of 80 MHz results in an unmatched peak power of >185 kW as compared to other fiber lasers in this wavelength range.

FemtoFiber ultra 920 at a glance:

- Wavelength: 920 nm
- Output power: >1.5 W
- Pulse energy: >18.5 nJ
- Pulse duration: <100 fs
- Peak power: >185 kW
- Repetition rate: 80 MHz
- Beam quality M^2: <1.2 (typ. <1.1)

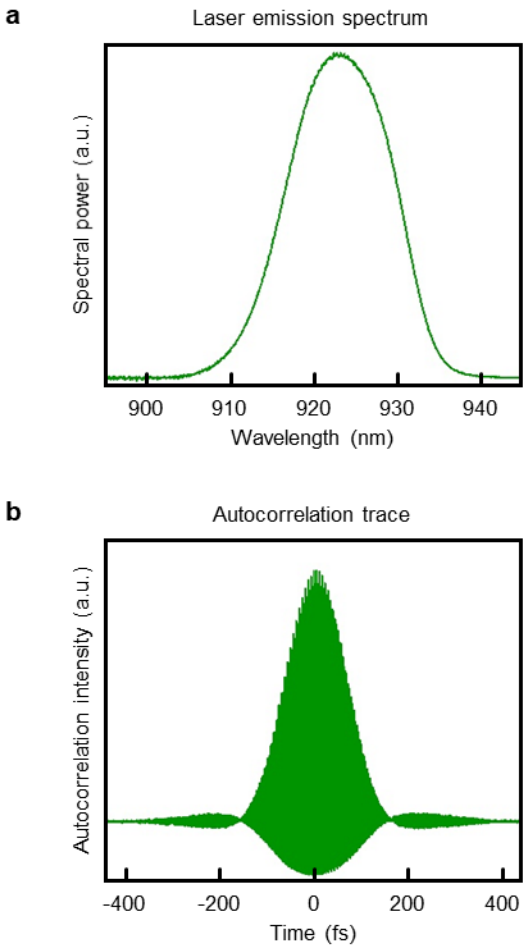

Figure 3: **a,** Laser emission spectrum of the FemtoFiber ultra 920 centered at a wavelength of 922 nm with a FWHM bandwidth of 15 nm. **b,** Autocorrelation trace of the FemtoFiber ultra 920 featuring a pulse duration of <100 fs with 99% of the pulse energy within the main pulse.

Special care has also been used while designing the excellent temporal and spatial beam parameters of the FemtoFiber ultra 920. Typically 99% of the pulse energy is temporally compressed in the main pulse (Fig. 3b), which results in very effective usage of every photon for two-photon fluorescence excitation reducing unwanted thermal load on the sample. In addition, the spatial beam quality shows excellent characteristics of $M^2 < 1.2$ (typ. <1.1) leading to a diffraction limited minimal focal spot size in the experiment.
As the two-photon fluorescence excitation efficiency is proportional to the square of the laser intensity in the focal plane, the impressive combination of peak power, temporal pulse shape, and spatial beam quality makes the FemtoFiber ultra 920 the ultimate choice for two-photon microscopy.

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The FemtoFiber ultra 920 comes with an ultracompact cold laser head with a footprint of only 23 x 15.5 cm^2 (Fig. 1). The laser head is designed to ensure minimum heat dissipation to its environment, providing highest stability with respect to beam pointing. The design of the laser head also allows mounting under various orientations such as vertically or horizontally. The control and supply unit of the laser system is integrated into a 19"-type standard rack (3 units height), which is connected via a detachable fiber and electronic lines of 2 meters length to the laser head. No water-cooling is required, convection cooling of the control/supply unit is sufficient for stable operation of the system. In addition to manual operation, the laser system can also be controlled remotely via Ethernet or USB. A simple graphics interface enables user friendly access to all laser parameters.

FemtoFiber ultra 920 key features:

- AOM and GDD options available
- Detachable, ultracompact, cold laser head, optimized for OEM integration
- Excellent beam pointing stability guaranteed by all fiber design
- Polarization maintaining fibers only
- Variable, scalable output power 1-100%
- Multi-color laser solution available (e.g. 780 nm, 1050 nm)
- 19" rack based air-cooled laser controller
- 24 V DC, <150 W power consumption

FemtoFiber ultra 920 for Two-Photon Microscopy:

The excellent performance of the FemtoFiber ultra 920 for two-photon microscopy is demonstrated in a proof of concept experiment by the research group of Prof. Thomas Hellerer at the University of Applied Science in Munich. Using the FemtoFiber ultra 920, high-resolution two-photon microscopy images of a human stem cell expressing GFP attached to its actin network are recorded, clearly resolving the actin network within the cell (Fig. 4a). In addition, the FemtoFiber ultra 920 can be used for two-photon microscopy of e.g. human stem cells with ATTO594 labeled actin and a DAPI labeled nucleus (Fig. 4b), *S.pneumoniae* bacteria labeled with ATTO425 (Fig. 4c), or other fluorophores suited for two-photon excitation at 920 nm.

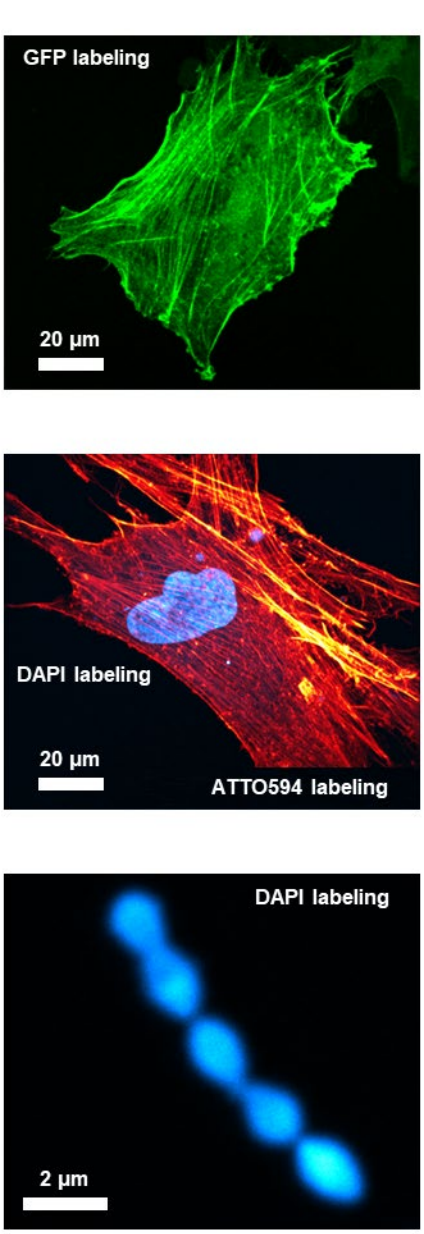

Figure 4: Two-photon microscopy images using the FemtoFiber ultra 920 for fluorescence excitation. **a,** Image of a human stem cell expressing GFP attached to its actin network. **b,** Human stem cell with ATTO594 labeling of the actin network and DAPI labeling of the cell nucleus. **c,** *S.pneumoniae* bacteria labeled with DAPI.

All images have been recorded by the research group of Prof. Thomas Hellerer at the University of Applied Sciences in Munich.

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