Ultrafast Fiber Lasers for Multiphoton Microscopy

High-resolution microscopy using ultrafast fiber lasers

Pascal Dupriez

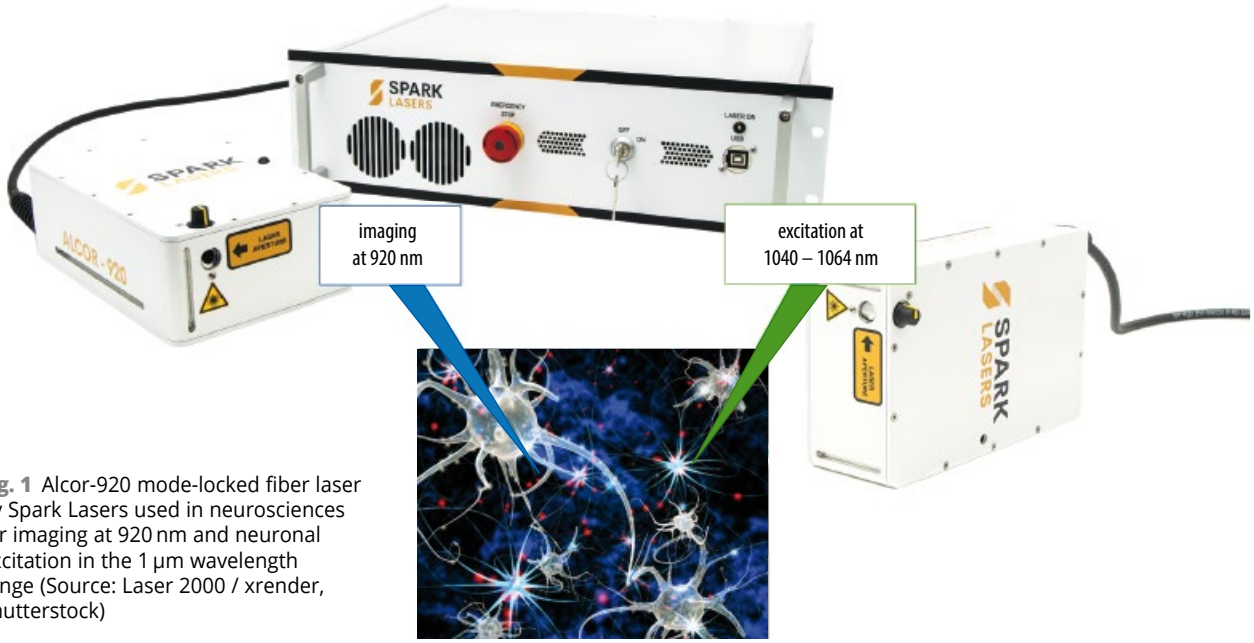

Fig. 1 Alcor-920 mode-locked fiber laser by Spark Lasers used in neurosciences for imaging at 920 nm and neuronal excitation in the 1 µm wavelength range (Source: Laser 2000 / xrender, Shutterstock)

Multiphoton excitation with ultrashort laser pulses has enabled high-resolution imaging in biomedical applications, specifically for in-vivo analyses in neurosciences. With the growth of microscopy techniques, laser technology has evolved. A new generation of femtosecond fiber lasers has recently emerged, outclassing conventional solid-state lasers, owing to their remarkable high performance, small size, low cost, and high reliability. The attractive features have proved to be key benefits in a various number of imaging applications, particularly in neurosciences.

Since its advent in the 1990s, multiphoton microscopy (MPM) has been developing in an increasing number of applications in the field of life sciences as a result of outstanding properties. Two-photon microscopy relies on nonlinear effects happening in a molecule which simultaneously absorbs two photons; the excited molecule releases energy by emitting a photon which produces the two-photon fluorescence signal. This technique offers many benefits compared to conventional one-photon fluorescence methods making it very attractive for biological imaging applications and particularly neurosciences.

The key benefit of two-photon microscopy relies on confinement of the excited volume as fluorescence occurs only at the focal point which increases the three-dimensional spatial resolution significantly. Another advantage is the near infrared excitation wavelength located in the 800 – 1100 nm range providing higher transparency and lower diffusion in biological tissues than visible or UV wavelengths used in one-photon excitation. This offers the ability to achieve deeper 3D imaging in tissues. The combination of long wavelengths and interaction of intense light in a reduced volume also diminishes photodamaging effects and thus maintains the quality of the sample.

For a given molecule, high efficiency of two-photon absorption requires high peak power produced by the excitation laser. For this reason, mode-locked lasers which produce ultra-short pulses (on the order of 100 fs) at high repetition rates have become the standard excitation source for two-photon microscopy.

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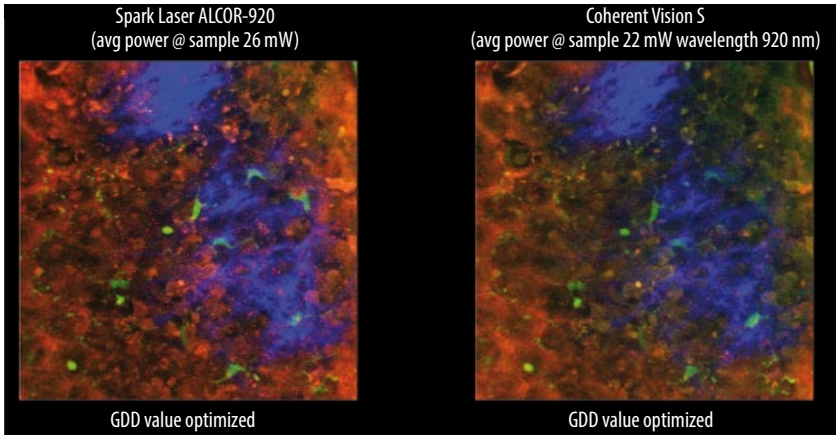

Fig. 2 Comparison of images of skin tissue (CX3CR1-GFP, β-actin-DsRed, SHG) obtained with fiber laser Alcor 920-2 (left image) and Ti:Sapphire laser Coherent Chameleon Vision S (right image). Courtesy of Dr. Yoonha Hwang & Dr. Howon Seo at IVIM Technology and KAIST.

Ti:sapphire lasers are conventionally used in these scientific applications thanks to combined ultrashort femtosecond pulses and wide wavelengths tunability (from ~680 to about 1080 nm), making it a remarkable scientific tool for multiphoton excitations (MPE) experiments.

However, Ti:Sapphire lasers have one major drawback: The peak lasing efficiency of Ti:Sapphire is around 780 nm while the majority of fluorescent proteins of interest to biologists have a two-photon absorption band primarily in the 900 – 1100 nm range, as shown in Fig. 2. Additionally, it has been shown that there is a significant decrease in damage to living tissue by tuning the laser from 780 – 920 nm [1]. As a result, in most two-photon microscopes the laser is tuned to 920 nm where, unfortunately, Ti:Sapphire lasers are far less efficient. For applications requiring longer wavelengths in the range of 1000 – 1100 nm, the historical Ti:Sapphire is even less efficient and ytterbium-based fiber lasers have become the best solution because of their well known benefits such as high efficiency, excellent beam quality, compactness, and no maintenance.

Consequently, in recent years, there have been rapid developments in the field of mode-locked fiber lasers emitting in the 1030 – 1070 nm wavelength range. That results in a wide variety of lower cost, fit for purpose fiber lasers sources available on the market.

Recently Spark Lasers from Bordeaux, France, developed a unique fiber laser source specifically tailored to the needs of two-photon microscopy applications.

The Alcor laser series, shown in Fig. 1, is a mode-locked fiber laser with an M^2 of < 1.2, producing ultrashort (<100 fs) pulses with 80 MHz pulse repetition rate at 920 nm and 1064 nm. Alcor is capable of producing up to 2 W average power both at 920 nm and 1064 nm.

Benefits of ultrafast fiber lasers in the context of multiphoton excitation microscopy

920 nm is a preferred wavelength for two-photon microscopy due to its high compatibility with most fluorescent proteins and reduced diffusion and absorption in tissue. Wavelengths around one micron (1030 – 1070 nm) are relevant for well-known red proteins (such as tdTomato, mCherry, mKate2) for excitation and light stimulation of different cellular areas. The focus on these wavelengths is essential to understand the fundamentals of neurotransmitters, neurological dysfunctions leading to disorders, pathologies, and diseases.

In optogenetics, laser sources at around one micron offer deeper penetration into cellular tissues allowing for excitation and activation of cells easing the understanding of neuronal subcellular activities.

While Ti:sapphire laser technology can provide decent performance

Companies

Spark Lasers

Spark Lasers cumulates more than twenty years of experience in developing compact picosecond and femtosecond lasers dedicated to micromachining and biophotonics applications by providing innovative technology, high performance, and industrial-grade reliability to best serve highly demanding industrial companies and scientific researchers. All ultrafast products are designed, developed, and manufactured in France according to the highest EU quality standards.

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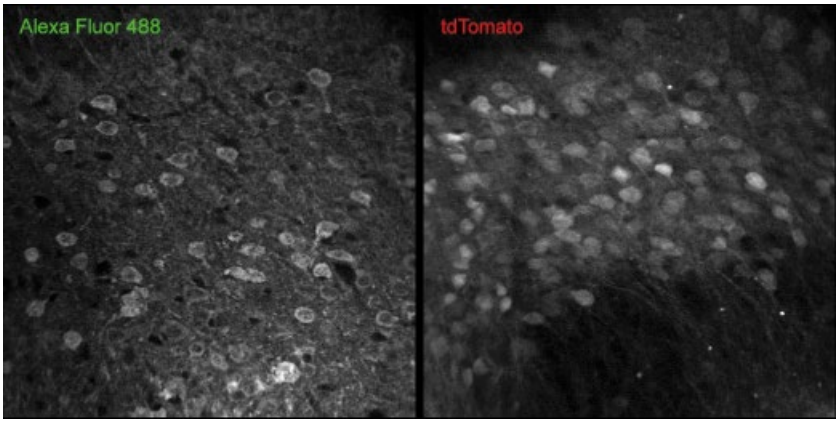

Fig. 3 Two-photon imaging of a mouse brain slide (sagittal section). Two channel acquisition. Left: Cortical neurons expressing GCaMP stained with Alexa Fluor 488. Right: Neurons in the same imaging field of view as on the left expressing tdTomato (raw fluorescence). Courtesy of Kavli Institute for Systems Neuroscience, Trondheim, Norway, Moser Group, Obenhaus.

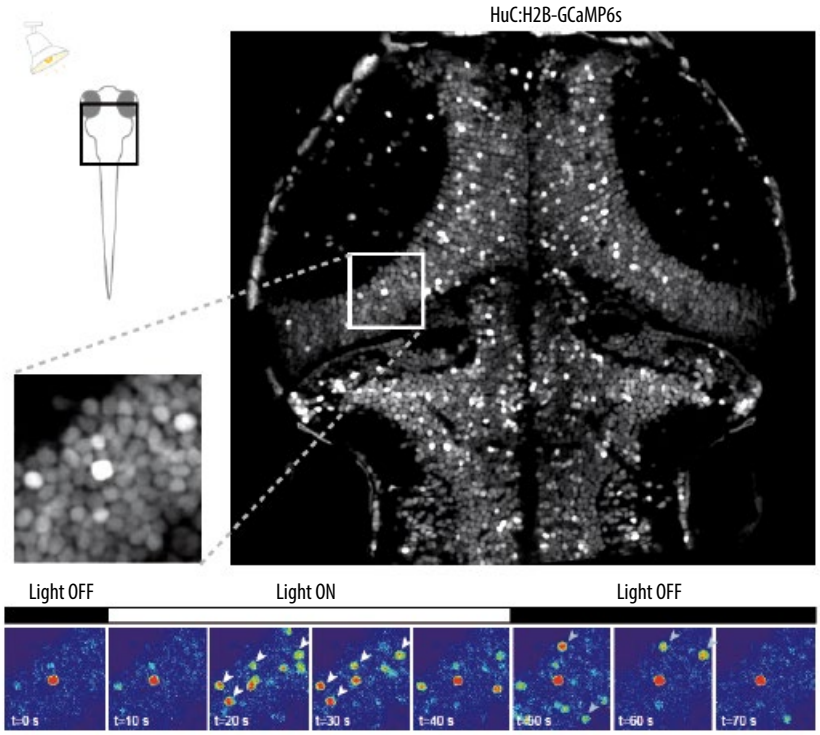

Fig. 4 In vivo 2-photon calcium imaging using Alcor 920. Top: HuC:H2B-GCaMP6s transgenic Zebrafish larvae (6 days post fertilization) was imaged while light stimulus was presented. Bottom: Upon light illumination, neurons in the optic tectum show increased activity in response to a decrease of light level (arrows). Courtesy of Dr. Kubo, National Institute of Genetics, Mishima, Japan.

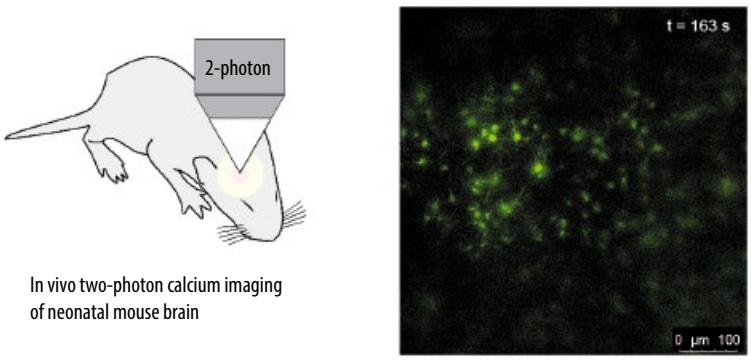

Fig. 5 Recording of temporal changes of neuronal activity in the neonatal mouse cerebral cortex using GCaMP6s and Alcor 920 2 W. Courtesy of Dr. Mizuno, Kumamoto University, Kumamoto, Japan [2].

in the 800 – 950 nm range, its lower gain around 1 µm greatly limits average power at these wavelengths to levels not exploitable for most applications. Fiber laser technology is more suitable in the 1 µm range offering much higher average power as well as higher peak power and pulse energy than traditional Ti:Sapphire lasers with all the benefits of fiber lasers.

Spark Lasers' Alcor dual answers these challenges, providing femtosecond pulses at 920 and 1064 nm from two separate laser heads for optimal flexibility and ease of use as illustrated in Fig. 1:

- A 920 nm wavelength with up to 2W average power is used for neural response detection and imaging.
- A longer wavelength between 1030 and 1070 nm, provides photoactivation in optogenetics applications.

With short pulse duration of less than 200 fs required for 2-photon microscopy, pulse duration may increase significantly after propagation through complex optical systems. The induced group delay dispersion (GDD) degrades image quality considerably. Therefore, ultrashort pulses must be prepared before exiting the laser. This is done by the right amount of GDD which compensates exactly dispersion induced by the optical system. This dispersion pre-compensation scheme that ensures pulse duration is the shortest on the sample allowing for optimal image quality.

Applications of femtosecond fiber lasers

Ultrafast solid-state laser technology has been a standard in scientific applications. Over many years, they have proved their ability to perform in a wide range of applications. After many years of development, ultrafast fiber technology is now challenging historical lasers in most applications and especially in the field of 2-photon microscopy. Today a fixed wavelength fiber laser perfectly suits these applications. Fig. 2 illustrates this comparing images of skin tissue taken using Alcor-920 and a Ti:Sapphire laser under identical conditions. In this example, the Ti:Sapphire laser was set to 920 nm and GDD was optimized using features included in each laser. It clearly shows that the Alcor fiber laser produces higher contrast confirming that a compact laser can

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outperform well established historical laser solutions.

Neuroscience is the new challenging area of research as researchers are trying to understand the complexity of neuronal network to then understand pathologies such as neurodegenerative diseases. With their high performance and small size, ultrafast fiber lasers offer many benefits to researchers who see their lab space occupied by an increasing number of complex equipment including large lasers. Fig. 3 presents typical two photon imaging of mouse brain slides obtained with the new generation of 920 nm femtosecond fiber laser Alcor. The high contrast and high resolution achieved with this technique are suitable for advanced research in neurosciences.

As brain and vision are strongly linked and involve very complex interactions, researchers are currently analyzing brain activity on well adopted living samples such as zebra fish. Fig. 4 reports in-vivo calcium imaging using Alcor 920. As light is turned on or off, various zones are activated allowing precise localization of specific neurons. This work is achieved thanks to the remarkable contrast, high resolution, and fast acquisition time.

Deep brain imaging is the current challenge in neurosciences as researchers need to observe neuronal activity in the largest possible volume. Increasing depth in microscopy is challenging as scattering and absorption severely reduce image quality as depth is increased. Using lasers with watt level optical power can push this limit but compromises on photodamages of the living samples. As fiber laser technology is power scalable, ultrashort fiber lasers are also highly suitable for deep brain imaging. For these experiments neuronal activity is often analyzed in-vivo in mice as shown in Fig. 5 where neurons are imaged using GCaMP6s excited by femtosecond pulses emitted at 920 nm by Alcor 920 2 W.

Conclusion

Multiphoton excitation microscopy has emerged in the last decade as the technique of choice for challenging applications such as neurosciences. Ultrashort pulsed fiber lasers are becoming the ideal tool in this field owing to their high performance, small size, and high reliability. Spark Lasers have developed lasers specifically designed for microscopy and in-vivo excitation offering two fixed wavelengths (920 and 1040 or 1064 nm) at the same time, exiting from two individually controlled and compact laser heads. Individual control of GDD pre-compensation ensures optimal performance even in the most complex optical systems.

This new generation of ultrafast fiber lasers constitutes a breakthrough in laser technology dedicated to two-photon imaging and neural excitation. The remarkable tool for neuroscientists is offering new possibilities to push boundaries and increase the understanding of the brain to help find new protocols to cure neurodegenerative disorders such as epilepsy, or diseases like Alzheimer's and Parkinson.

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[1] *K. Koenig*: Multiphoton microscopy in life sciences", Journal of Microscopy **200** (2000) Pt 2, 83 -104.

[2] *Mizuno* et al.: Neuron **82** (2014) 365-379; Cell Reports **22** (2018) 123-135

Author

Pascal Dupriez founded Spark Lasers in August 2015 after having served as R&D manager at Fianium Ltd., UK for five years after his doctorate at the University of Southampton, and also holding other positions at Waveguide Solutions Inc., Charlotte, NC, USA, as senior optical engineer, and at Corning Inc., Corning, NY, USA, as senior market development engineer.

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