KEY WORDS: confocal microscopy, FLIM, FRET, CARS, multi-photon excitation, tunable pulsed laser

A tunable laser would be perfect for exciting the multitude of fluorescent markers that are used in confocal microscopy. Thus far confocal microscopy has relied on diode and gas lasers, because they are compact, economical and easy to operate. Since these lasers have fixed wavelengths, microscopes are often equipped with more than one laser. Nevertheless, the user must always look for a fluorescent marker that fits one of the wavelengths available to him. With a tunable laser, the user can choose the marker that is preferred from a scientific point of view and then adjust the wavelength to it.

Recently, novel frequency conversion schemes have made it possible to turn a femtosecond fiber laser into a tunable laser that can be operated without laser expertise. The FemtoFiber Scientific (FFS) laser therefore appears to be ideally suited for confocal microscopy.

As the figure on the left shows, the FFS can be tuned continuously across a large range of the visible spectrum. The narrow bandwidth of less than 2 nm ensures that the fluorescence signal is easily distinguished spectrally from the scattered excitation light. Besides being a quasi-continuous wave laser source for confocal microscopy, the laser can equally well be applied to various other novel microscopy techniques. For example, with picosecond pulse durations at a repetition frequency of 80 MHz, the FFS can be used very well for lifetimes measurements as used in e.g. FRET and FLIM. Furthermore, when equipped with two independent but synchronously pulsed beams, the FFS can be applied to CARS. Finally, there is the possibility to generate femtosecond pulses at 775 and 1550 nm with high peak powers, such that they can be used for multiphoton excitation. This, added to all the new opportunities that a continuously tunable and narrow-band laser offers to confocal microscopy, makes the FFS probably the most universal laser for microscopy currently on the market.