Laser leap forward

Compact ultrafast lasers enable simultaneous 2- and 3-photon microscopy.

Oliver Prochnow

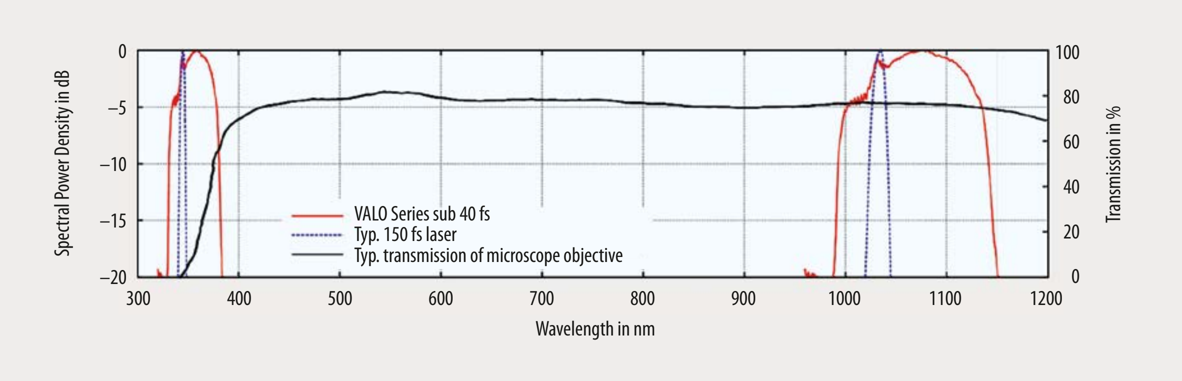

Fig. 1 Fundamental and third harmonic generation spectrum of a 30 fs, broadband fiber laser (red) compared with standard 150 fs lasers and typical transmission characteristics of a standard microscopy objective (black solid line). Only a THG spectrum generated from wavelengths of above 1080 nm will be transmitted.

For the first time a new generation of compact ultrafast fiber laser systems enable simultaneous two- and three-photon microscopy. These lasers feature a unique design that efficiently generates temporally clean sub-40 femtosecond (fs) pulses with a very broadband optical spectrum.

Compared to other laser systems, these ultra-short pulse lasers produce a broadband optical spectrum up to 1140 nm – that is much broader than common femtosecond lasers and much further into the near infrared (NIR, **Fig. 1**). In multiphoton microscopy applications, they can not only be used to excite two photon processes, for example Second Harmonic Generation (SHG) and Two Photon Excitation (2PE), in the way common femtosecond lasers at 1 µm wavelength currently do, they can also be used for Three Photon Excitation (3PE) and Third Harmonic Generation (THG). This is possible because the three photon signal generated from wavelengths above 1080 nm is not blocked by the UV absorption of standard microscope objectives. In contrast, traditional laser wavelengths around 1 µm produce a THG signal in the UV range, which is often too short to be transmitted through standard objectives. Quite simply, the very broad optical bandwidth of these new laser systems allow for simultaneous 2PE, SHG, THG and 3PE generation and detection.

With significantly shorter pulse durations than conventional systems, these new ultrafast lasers achieve significantly higher peak power while maintaining low average power. This results in a higher imaging contrast. By lowering the average power the heat exposure to the sample can be reduced by keeping the contrast high enough. Shorter pulse durations allow for a high imaging contrast while minimizing photodamage and photobleaching, making these lasers ideal for long-term studies of living cells and tissues (**Fig. 2**).

The high peak power from sub-40 fs pulses is essential for nonlinear optical processes such as 2PE, SHG and 3PE, THG, as their signals scale with the square and cube of the incident intensity. These advanced imaging methods allow precise, label-free visualization of molecular and structural details with exceptional clarity. SHG, a coherent nonlinear process, arises in non-centrosymmetric structures such as collagen fibrils, microtubules, and other ordered biological assemblies. This makes it particularly useful for imaging connective tissues, cytoskeletal structures, and fibrillar proteins with high contrast and specificity. On the other hand, THG occurs at interfaces with refractive index mismatches or in regions of material inhomogeneity, providing detailed imaging of cell membranes, lipid-rich structures, and organelle boundaries.

By combining these nonlinear optical techniques, researchers can extract a lot of simultaneous information from biological samples,

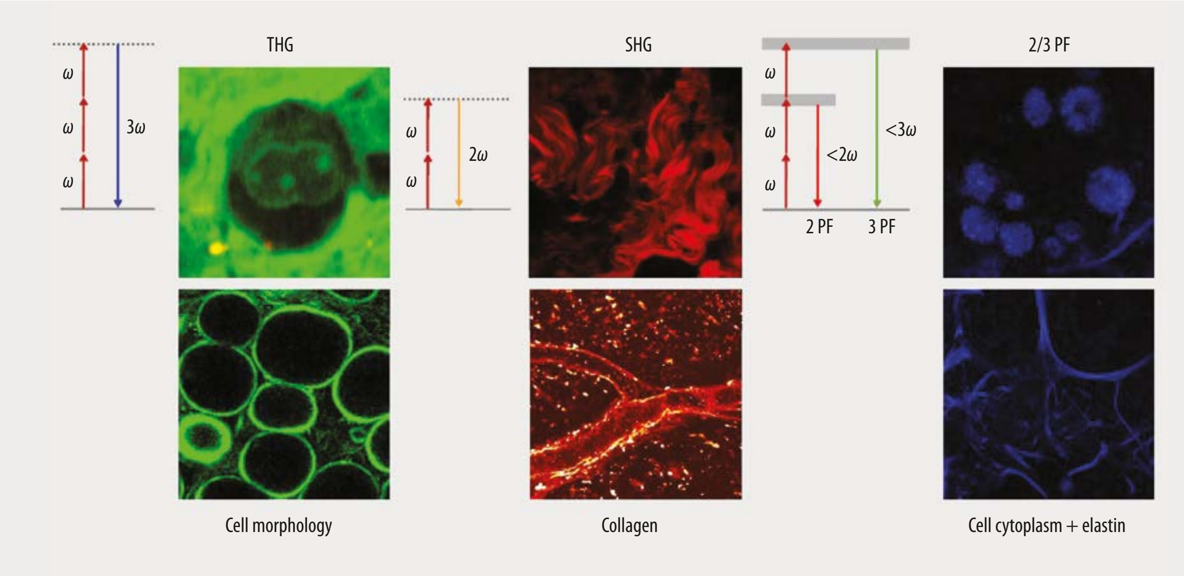

Fig. 2 Example of THG, SHG and 2 and 3 Photon Fluorescence, simultaneously detected in different channels, thereby allowing rapid visualization of molecular and structural details in unprocessed samples.

which is now being explored for applications in for example cancer diagnosis and personalized medicine.

Ideal Lasers

Beyond their scientific advantages, these ultrafast laser systems are compact, easy to operate, robust, and do not require water cooling (**Fig. 3**). Their small footprint and durable design make them ideal for integration into both laboratory and industrial settings and means they have huge potential, especially in simultaneous nonlinear optical processes for long-term studies of living cells and tissues.

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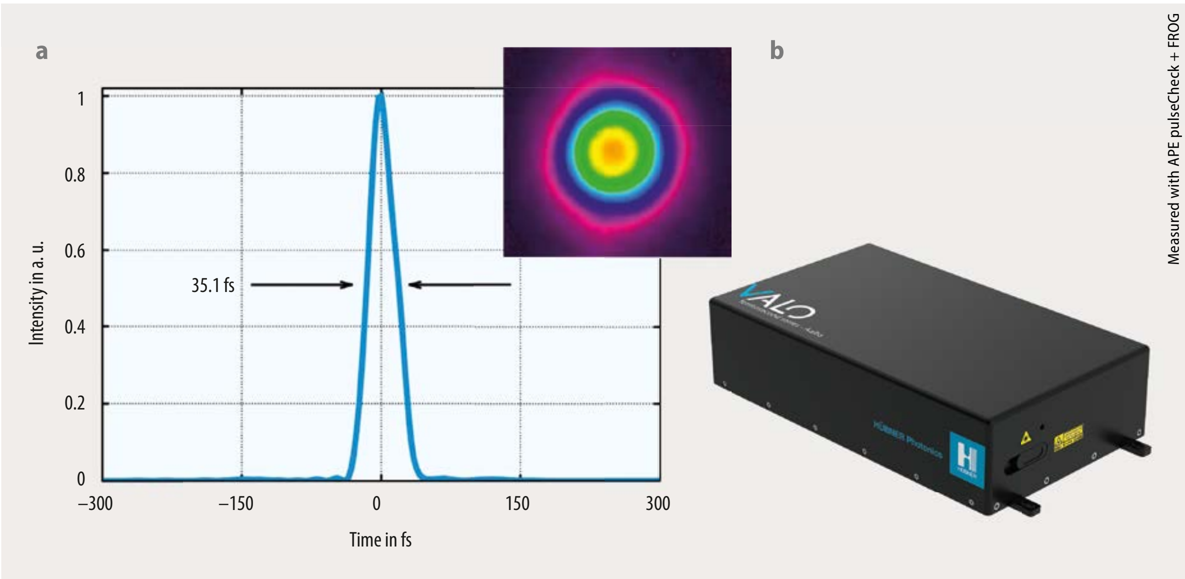

Fig. 3 Typical temporal pulse profile highlighting the sub 50 fs pulse duration (a) with very low pulse pedestal with inset showing the typical beam profile of HÜBNER Photonics VALO Femtosecond Series lasers (b).