

**NONLINEAR CONFOCAL MICROSCOPY
WITH NOVEL FEMTOSECOND LASER SOURCES**

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Microscopy based on nonlinear optical processes has emerged as a powerful tool to image biological specimens at high resolution. This includes both the imaging of fluorescently labeled specimens by multiphoton fluorescence microscopy as well as the visualization of biological structures in the absence of an exogenous label by second and third harmonic generation (SHG and THG) and coherent anti-Stokes Raman scattering microscopy (CARS).

The key advantages of these imaging techniques compared to linear confocal microscopy are the deeper penetration into the sample and the relatively low level of photodamage caused. These beneficial effects rely mainly on the long wavelengths used for excitation, typically above 800 nm, as their reduced scattering allows brighter images to be obtained from deep within biological samples (up to 600 μm). Additionally, excitation is restricted to the focal point as the place of highest photon density. This provides intrinsic optical sectioning and minimizes photobleaching and phototoxicity outside the focal plane. Nonlinear techniques rely on ultrashort pulsed lasers as illumination sources. Ti:sapphire based femtosecond systems with restricted tunability around 800 nm, which are costly and often difficult to handle, are mostly used for this purpose. This situation has hampered widespread application of nonlinear LSCM in the past. Femtosecond fiber lasers may represent an attractive alternative that may overcome the limitations of the conventional Ti:sapphire system. We are presently developing such an Er: fiber laser for bioimaging applications. This laser is widely tunable in the visible and near infrared, extremely stable and easy to operate ("turn-key"). In addition, the long wavelengths favored for deep tissue imaging and optical harmonics generation, as well as the two-color option for CARS are features inherently connected to our Er: fiber femtosecond technology.

The aim of this project is the development of a multiphoton confocal microscope equipped with such a compact and cost-effective fiber laser source and its application to the imaging of different types of biological specimen. The construction of the microscope will proceed by adapting a commercially available instrument. The resulting microscope will display significant advantages as compared to the currently available systems: it will operate at wavelengths above 1 μm , which experience less scattering, are less destructive and best suited for label-free microscopy via optical harmonics techniques. Further it will be easy to handle and cost-effective.

The microscope will be employed to investigate topics from different areas of biology ranging from neuronal development to cell death in intact tissues.