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Two photon spectral imaging and tomography: Applications in biomedical science

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The implementation of femtosecond pulsed NIR lasers for two photon excitation in laser scanning microscopy has overcome the limited observation depth in biological tissues due to absorption and scattering phenomena. This enables true three dimensional fluorescence tomography up to the objective's working distance. Furthermore photo-damaging by one photon excitation in the focus and the entire focal cone are no more interfering since NIR at 800nm does virtually not interact with biological matter and a significant two photon absorption only occurs within the sub-femtoliter focal volume.

Resolving the collected emission light in the spectral domain yields information about the chemical assembly of cells and tissues by endogenous luminescent compounds as well as physical and chemical properties by exogenous fluorescent probes. Diverse examples are presented for the application of spectral tomography in biological matter.