

# **Application of multiphoton autofluorescence imaging (MAI) and second harmonic generation (SHG) excited by near-infrared femtosecond nanojoule laser pulses in intrastromal surgery with rabbits**

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## **Abstract**

The intratissue autofluorescence imaging and second harmonic generation based on nonlinear absorption of femtosecond nanojoule laser pulses at wave length of 750-850 nm emitted from solid-state Titanium: Sapphire FemtoCut system have been used as a highly precise non-invasive monitoring means to visualise and inspect the intrastromal nanosurgery performed with the same laser system. Compared with photodisruptive surgical effects occurring at TW/cm<sup>2</sup> light intensity, imaging based on multiphoton-excited autofluorescence and SHG formation of collagen fibers come into being at GW/cm<sup>2</sup> photon intensity in a sub-femtoliter intrastromal volume obtained by diffraction-limited focussing with 40x objective (N.A.1.3 oil). The multiphoton-excited autofluorescence imaging (MAI) and SHG acting as a novel diagnostic system for corneal optical tomography with submicron resolution proved to be an essential tool to determine the interest of region before intrastromal treatment, visualize and verify the outcomes of the in-vivo intrastromal refractive surgery after operation. Additionally, we have observed the activated keratocytes in-vivo 48 hours after laser operation. The optical tomography realized the differentiation of the five layers of cornea according to the conventional histology.