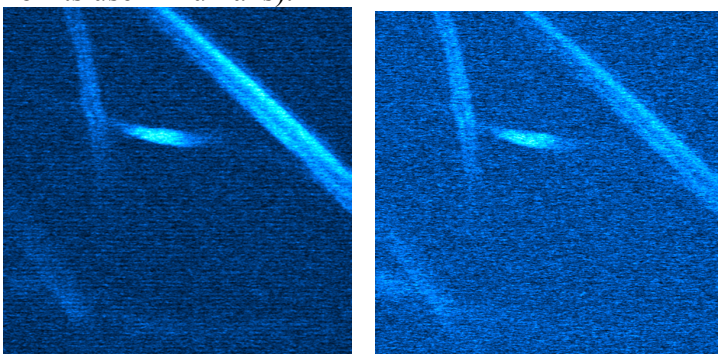
O-68

TOWARDS TWO-PHOTON ENDOMICORSCOPY FOR APPLICATIONS IN HUMANS: CHARACTERIZATION AND OPTIMIZATION OF A FS LASER SOURCE IN A FIBER BUNDLE.

Omar E. Olarte[1], Domenico Demasi[1], Katharina Thomsen, Sunil Kumar[1], Rodrigo Avilés-Espinoza[1], Heinz Huber , Ronald Sroka, David Artigas[1], Pablo Loza-Alvarez[1]

[1] ICFO-The Institute of Photonic Sciences, Av. Carl Friedrich Gauss, 3, 08860 Castelldefels (Barcelona), Spain.
[2] Laser-Forschungslabor, LIFE-Zentrum, Klinikum der Universität München, 81377 (München) Germany.
[3] High Q Laser Innovation GmbH, Am Feldgut 9, 6830 Rankweil, Austria

Endomicroscopy enables cellular resolution and is therefore a helpful tool for precise identification of malignant tissue layers. In confocal endomicroscopy only cell layers at tissue depths of about 100 µm can be imaged, because of the limited penetration depth of the light. Two-photon excited fluorescence endomicroscopy (2p-EM) allows reaching deeper cell layers up to tissue depths of 500 µm. In primary clinical application of TPEM this would allow, for example, the investigation of bladder cancer (CIS), which develops in the superficial tissue layers. By means of TPEM it may become possible to get a microscopic view of the morphology of cell structures up to a depth of 500µm, thus the complete thickness of the epithelial layer down to the basement membrane could be investigated with a result of improving the clinical in-vivo diagnosis by optical biopsy. Thereafter other clinical applications may profit from this first stage.
In here we use an ultrashort pulsed laser going through a fiber bundle to show its feasibility as a 2p-EM excitation source. To show that, the pulse distortion within a fiber bundle was characterized. As expected the pulse duration increased from 250fs to more than 6 ps. Analogously, pulse bandwidth increased from 3nm to more than 200nm. Characterization of pulses after propagating in a fibre bundle was performed using standard autocorrelation techniques. To adequately pre-compress pulse chirp using standard pairs of i) prisms and ii) gratings. After optimum pre-compensation, the output of the fibre was sent to a standard multiphoton microscope. With the complete setup it was possible to produce images (two-photon excited fluorescence) of a test samples stained with cresyl violet (an approved fluorescent marker for its use in humans).



Images of test samples taken with the fibre bundle with two different setting of the dispersion compensation unit compressed. Dye is Cresyl Violet. 130 cm prism separation gives the best pulse compression.

In conclusion, we have demonstrated the feasibility of delivering ultrashort pulsed lasers through a fiber bundle for 2p-EM. We show that, despite pulse distortion, it is possible to excite the Cresyl Violet dye in an efficient way. Future studies will be performed in human samples and towards capturing the excited fluorescence using the same fiber bundle.