MULTIMODAL COMPACT MULTIPHOTON MICROSCOPE FOR NANOPROCESSING AND NONLINEAR IMAGING AT VARIABLE TEMPERATURES

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Cell-biological application of multiphoton imaging is often hindered by fast metabolic processes not detectable normally by conventional scanning multiphoton microscopes. One solution is to slow down metabolic processes by cooling. We have implemented a cryo-multiphoton microscope not only capable of slowing down these metabolic processes and enhancing the fluorescence quantum yield by lowering the sample temperature but also to detect the direct dependency of cofactor activity on the temperature. [1,2] The temperature of the sample can be controlled from -196°C (77K) up to +600°C (873 K) with heating/freezing rates between 0.01 K / min and 150 K / min. With high temperature, especially apoptotic processes of the cells up to carbonization of tissue can be imaged.

We imaged the impact of freezing on collagen fibers in human and porcine corneas by comparing the SHG in forward and backward direction. In agreement with former studies the SHG was found to be reduced after freezing [3]. Water was extracted from the sample by osmosis to allow a recovery of low water content by rehydration. Freezing of the tissue with lower water content did show significant difference to untreated tissue as shown before on dried samples [4]. The effect of denaturation of collagen above temperatures of 60° C and the intensity of SHG at elevated temperatures has also been a target of our study. Furthermore, this microscope cannot only detect in epi-direction but also in transmission to image SHG from collagen in thin tissue samples.

An ultra-compact ultra-short femtosecond laser with pulse lengths down to <20 fs (at 800 nm) as nonlinear excitation source was employed in our multimodal compact multiphoton microscope (MPM*compact*, JenLab GmbH) to realize additional nanoprocessing by high-order multiphoton processes. First laser cutting and drilling experiments in dependence on temperature will be discussed.

References

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