EFFECTS OF PULSE COMPRESSION IN MULTIPHOTON MICROSCOPY OF OCULAR TISSUES

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Multiphoton microscopy performance relies on the quality of the focal spot within the imaged sample. A number of experiments reported a significant image improvement when the spatial properties of the beam were optimized through the minimization of aberrations [1,2]. However, special attention must also be paid to the temporal properties of the illumination beam. When femtosecond pulses from a laser source travel through the microscope optics before reaching the sample, they are broadened in time due to optical dispersion. This imposes a limit on multiphoton imaging and reduces its optical sectioning capabilities. To counteract the effect of dispersion, it is possible to pre-compensate for the broadening of the laser pulse by introducing negative group velocity dispersion into the beam path. Here we report on the use of a pulse compressor (A.P.E femtocontrol, Germany) in the illumination pathway of a custom multiphoton microscope to improve the quality of images acquired at different depth locations. Autocorrelation measurements showed that the pulse duration at the microscope entrance was reduced from 400 to 180 fs. Multiphoton images of non-biological and biological (ocular tissues) thick samples were acquired for different pulse-compression states and depth locations. Both, two-photon excitation fluorescence (TPEF) and second harmonic generation (SHG) signals were analyzed.

Results showed that pulse compression significantly improved the recorded signal and more details were visible. Comparisons of multiphoton images before and after pulse compression showed up to 3-fold improvement, although this depended on the type of nonlinear signal and the depth location of the imaged plane. Figure 1 shows two examples. The pulse-compression state providing the best image was particular for each sample but kept constant with depth. 3D images reconstructed with the pulse-compressor in operation also showed a noticeable enhancement. 3D multiphoton microscopy techniques might significantly be improved with an accurate optimization of the pulse duration. Lower energy levels can then be used, what minimizes tissue damage and allows a more in-depth imaging, what is essential in the visualization and analysis of ocular tissues. The combination of pulse compression with adaptive optics may further improve the performance of the multiphoton microscope.

Figure 1: (a, b) TPEF (human epi-retinal membrane, 20-μm depth) and (c, d) SHG (rabbit cornea, 100-μm depth) images before (a, c) and after (b, d) pulse compression. Images subtend 180x180 μm.


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