PERFORMANCE AND LIMITATIONS OF MULTIPHOTON MICROSCOPY FOR IN VIVO OPTIMAL IMAGING OF OCULAR TISSUES

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Multiphoton (MP) microscopy imaging of living tissues has been a challenge during the last years. Although different studies have centred their efforts in brain imaging of in vivo animal models [1], ocular structures are also of particular interest for clinical diagnosis [2]. The visualization of biological features in thick tissues is limited by the presence of aberrations and scattering. In addition, living samples impose additional limits such as uncontrolled movements and the maximum permissible exposure (MPE). Here, we analyze the performance of a novel non-contact compact MP microscope able to acquire optimized images of both ex-vivo and in vivo ocular tissues [3]. Different combinations of recording parameters are explored to obtain the optimum MP image within the MPE.

The research MP microscope is controlled and synchronized through custom C++ software. This software allows modifying the recording parameters freely, to use an exposure time (ExT) that ensures image stability and safe acquisition for in vivo conditions. A number of parameters including detector sampling frequency (MHz), pixel integration (samples/px), image resolution (μm/px) and scanned/useful area (μm^2) are tested to measure their impact on the acquired MP images in terms of image quality, based on different objective metrics.

For femtosecond laser sources, the relationship between the MPE and the ExT is critical to establish the safety limits of the living human eye during image recording. However, the dependence between this ExT and the microscope acquisition parameters differs significantly: whereas this is linear for pixel integration, an exponential decay appears when increasing the detector sampling frequency. Moreover, the distortion of the image (i.e. sub-area where structural information is not available) is also closely related to the ExT. In general, when this time is in the range 0.11-3.43 s, the fraction of useful/non-distorted image is between 36 and 97 %. Detector sampling frequencies below 1 MHz produce noise levels leading to a significant decrease in the MP image quality. In addition, this image quality does not improve for pixel integration values higher than 10 samples/px. MP images of ocular tissues are of optimum quality to extract structural information parameters for an ExT of 0.90 s (200x200 μm^2, 0.7 μm/px, 9 samples/px and 1.2 MHz, 88% of useful image) which is well-below the human eye safety limits. Although this ExT might go down to 0.42 s by reducing the resolution, to gain image stability, the presence of additional noise will require extra image-processing procedures.

References

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