CORRECTION OF DISCONTINUOUS MOVEMENTS FOR TWO-PHOTON IN-VIVO IMAGING OF MOUSE RETINA

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Using mouse-models for investigation on autoimmune diseases like Multiple Sklerosis, Diabetes or Uveitis has become a topic of main interest for medical and biological research. Especially, using the Nervus Opticus as direct interface to the Central Nervous System (CNS) to observe the pathology of neuro-inflammatory processes on the cellular level. This has now been shown in case of experimental autoimmune Uveitis (EAU) and experimental autoimmune Encephalomyelitis (EAE)[1].

Imaging deep into living tissue leads to photon losses and aberrations due to scattering and absorption. Applying multi-photon excitation allows to extend the penetration depth significantly and yields an inherent optical sectioning effect. To ensure a proper imaging, the mouse's eye has to be well aligned with the optical system. Even if the mouse itself is anesthetized optimally, it is impossible to avoid artifacts coming for example from spontaneous eye movements, constant influence of heartbeat, blood-flow, breathing and other muscular contractions. Hence, the image has to be corrected for a set of discontinuous movements - spontaneous 3D changes at any time during acquisition.

Our new approach applies a cross-correlation algorithm to first correct for lateral displacement between slices of the focal series. Afterwards, a line-wise cross-correlation in combination with a center-of-mass method is used to achieve a lateral line-wise dejittering. Finally, an appropriate deconvolution is applied to reconstruct the original object.

References:

[1] Bremer, D. *et al*. Longitudinal Intravital Imaging of the Retina Reveals Long-term Dynamics of Immune Infiltration and Its Effects on the Glial Network in Experimental Autoimmune Uveoretinitis, without Evident Signs of Neuronal Dysfunction in the Ganglion Cell Layer. *Frontiers in Immunology* 7, ISSN 1664-3224 (2016).