AIRYSCAN DETECTION IN MULTIPHOTON MICROSCOPY

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The advantages of Airyscan detection in confocal microscopy rely on the descanned detection scheme and the a priori knowledge of the detection point spread function. In contrast, multiphoton imaging at greater depth usually employs non-descanned detectors (NDDs) maximizing the number of photons collected from the scattering tissue. Thus, sectioning and resolution solely stem from the nonlinear excitation point spread function.
Since in highly scattering tissue also the excitation point spread function experiences scattering, the intensity of the excitation usually needs to be increased significantly with increasing depth. Thus, the limiting factor for depth penetration becomes the out of focus two photon excitation reducing sectioning and signal to noise.
So far image scanning microscopy with two photon excitation has been shown to improve resolution and image quality [1-4] and increase the depth at which super resolution is achieved [2-4] compared with single photon excitation. However, a lot of the neuroscience in vivo applications such as cranial window experiments, but also new applications using cleared tissue or whole organs require penetration depths beyond 100 microns (typical limt for the single photon case).Deep tissue imaging, especially in comparison to detection with NDDs, has yet not been investigated with multiphoton image scanning microscopes.
We will present data from a descanned Airyscan detection combined with multiphoton excitation. We demonstrate how image scanning microscopy in scattering media such as brain tissue improves image quality and resolution even at large depth (300µ) compared to the non descanned detection scheme.

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