FAST AND HIGH-RESOLUTION MESO-IMAGING WITH RE-SCANNED MULTIBEAM TWO-PHOTON MICROSCOPY

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Imaging biological tissue across scales is essential to understand how molecules, organelles, and cells self-organize into tissues, organs, and organisms. Multiscale, volumetric imaging requires (1) sufficient resolution to detect the smallest scale of interest, (2) high throughput to cover extensive lengths within a short timeframe, and (3) sectioning power and long penetration depth to enable imaging deep inside the tissue.

Two-photon laser scanning microscopy (2p-LSM) has become the modality of choice for imaging cellular and subcellular structures in thick and opaque tissues. However, 2p-LSM suffers from relatively low throughput due to raster-scanning. By implementing spatial multiplexing of 16 collinear laser beamlets combined with re-scanned fluorescence detection, we aim to increase the imaging throughput of 2p-LSM without sacrificing optical resolution, sectioning power, or penetration depth. Fluorescence is detected efficiently via a multianode linear-array photomultiplier under a non-descanned configuration. The fluorescence emission is re-scanned to keep the fluorescent spots stationary at each anode in the slow scanning direction, and therefore, to avoid optical loss in the inter-anode dead spaces [1]. In the fast axis, the fluorescent spots sweep across the stripe-shaped anodes as a resonant mirror raster-scans the excitation foci over the sample rapidly.

Combining in-situ serial sectioning [2] with resonant scanning and spatial multiplexing, this mesoscopy platform is designed to perform multichannel, centimeter-scale, volumetric imaging at sub-micron resolution in 1-2 days.