FAST THREE-DIMENSIONAL LASER-SCANNING SCHEME FOR MULTI-PHOTON MICROSCOPY

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Laser-scanning microscopy (LSM) commonly employs mechano-optical schemes for beam steering, such as galvanometer-driven mirrors. These 2D scanning schemes enable to access every point in the object plane of a microscope; however, their inertia-limited acceleration restricts possible scan patterns to those obtained by systematic raster scanning. This usually causes no limitation for structural imaging, where full field scans are performed and frame rates are less critical. However, in functional imaging applications which require high frame rates, scanning is often restricted to either small areas or even single lines. In the latter case, despite the sacrifice of one scan dimension, frame rates remain limited to ~100Hz, signal integration time at sites of interest is reduced by time spent illuminating sites of no interest along the scan-line, and the complex shape of biological structures cannot be matched.

We had previously developed an alternative scanning scheme to rapidly position a laser beam. This inertia-free scheme uses acousto-optic deflectors (AODs), providing versatile scan patterns to overcome the above-mentioned limitations of mechano-optical schemes [1]. This AO scanner was used for concurrent multi-site non-confocal imaging at frame rates >1kHz in cultured nerve cells with single-photon excitation of fluorescent voltage and calcium indicators [1, 2]. Recently, we have extended this scanning scheme to multi-photon microscopy, which allows us to fully utilize the flexibility of AOD-based scanning in light-scattering specimen such as live brain tissue. For efficient multi-photon excitation, we use a near infrared laser and have developed means to compensate for temporal and spatial dispersion of ultra-fast laser pulses, which can significantly reduce the spatial resolution of AO scanners [3]. However, even with this advanced scanning scheme, only fast 2D scan patterns are possible, and 3D imaging requires a mechano-optic change of focal plane, which severely limits the effective volume-of-interest frame rate.

We have now demonstrated a novel all-AOD-based scanning scheme which permits fast random-access 3D positioning of a laser beam without any mechano-optic interaction [4]. Its inherent self-compensation of spatial dispersion makes this scheme uniquely suited for multi-photon excitation of fluorescent indicators with ultra-fast laser pulses. The novel scanning scheme supports fast functional imaging of complex structures within a specimen volume, e.g. activity patterns of neuronal dendrites in live brain tissue. We will discuss the present state of our project.

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[4] Patent pending.

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