ACOUSTO-OPTIC RANDOM-ACCESS LASER-SCANNING MICROSCOPY WITH SINGLE- AND MULTI-PHOTON EXCITATION FOR FAST FUNCTIONAL IMAGING OF LIVING NERVE CELLS

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Laser-scanning microscopy (LSM) commonly employs galvanometer-driven mirrors for beam steering. To achieve frame rates sufficient to capture time-dependent signals in functional imaging applications, these inertia-limited components often restrict scan patterns to line-scans. This has several disadvantages: 1) frame rates are limited to ~100Hz, despite the sacrifice of one scan dimension; 2) signal integration time at sites of interest is reduced by time spent illuminating sites of no interest along the scan-line; 3) line-scans cannot match the shape of biological structures; and 4) line-scan orientation is not always easily adjustable.

An alternative scheme uses acousto-optic deflectors (AODs) to steer a laser beam by a sound wave, acting as a diffraction grating that can be rapidly tuned to reposition the beam (~1µs). Two orthogonal AODs form a 2-dimensional scanner, allowing user-selected sites of interest to be random-accessed at high frame rates (>1kHz). Functional parameters, such as intracellular \([\text{Ca}^{2+}]\) and membrane potential, can be concurrently recorded at multiple sites in neuronal dendrites, as demonstrated with single-photon excitation in cultured neurons loaded with fluorescent indicators [1,2]. Employing AO scanning together with multi-photon excitation (MPE), such studies can be extended to more physiologically realistic living brain slices, which are significantly more light-scattering. To date, 2-dimensional AO scanning has not been integrated with multi-photon LSM, because AODs induce significant temporal and spatial dispersion of the ultra-fast laser pulses needed for MPE (but see [3] for dispersion compensation of a single AOD in a hybrid mirror/AOD scanner). Temporal dispersion increases the pulse-width, decreasing MPE efficacy. Spatial dispersion results in larger focal volumes, reducing both MPE efficacy and spatial resolution. We have developed compensation schemes for AOD application in multi-photon LSM [4]. For temporal dispersion compensation, we developed a pre-chirper with ~50% reduced footprint (see [5] for discussion on pre-chirping). For spatial dispersion compensation, we developed a scheme based on a single diffraction grating per AOD and documented the importance of such compensation for achieving nearly diffraction-limited resolution.

We will demonstrate that spatial dispersion compensation is paramount for maximal performance of a multi-photon LSM, presenting the spatial resolution obtained with high-resolution TeO2 AODs and a Ti:S laser. We will show pulse durations measured at the object plane, demonstrating temporal dispersion compensation for an entire multi-photon LSM.