

## MULTI-PHOTON MICROSCOPY OF LIVING NERVE CELLS USING ACOUSTO-OPTIC LASER-SCANNING

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Laser-scanning microscopy (LSM) commonly employs mechano-optical schemes for beam steering, such as galvanometer-driven mirrors. These two-dimensional scanning schemes allow for accessing 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 is usually causing no limitation for structural imaging, where full field scans are performed and frame rates are less critical. However, when functional imaging requires high frame rates, small area-scans or line-scans have to be performed. In the latter case, despite the sacrifice of one scan dimension, frame rates are 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 shape of biological structures cannot be matched.

We had previously developed an alternative scanning scheme for a LSM that uses acousto-optic deflectors (AODs) to rapidly position a laser beam. This inertia-free scheme provides maximal versatile scan patterns, removing the above-mentioned limitations of mechano-optical schemes [1]. Using single-photon excitation with continuous wave lasers in the visible range (VIS), we performed multi-site non-confocal measurements with fluorescent voltage- and calcium-sensitive indicators at frame rates >1kHz at user-selected subcellular structures of mammalian nerve cells in culture [1, 2].

We have now extended this scanning scheme to multiphoton 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 pulsed laser in the near infrared range (NIR). We have demonstrated the compensation of temporal and spatial dispersion, which can reduce the spatial resolution of AODs with ultrafast lasers [3]. Both structural and functional imaging can be performed at optimal spatio-temporal resolution. We will discuss the present state of our system.

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