ARBITRARY INCLINATION, TWO-PHOTON LIGHT-SHEET MICROSCOPY

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ABSTRACT

An ideal system for the study of the dynamics of excitable tissues, such as the heart or the brain, should provide complete three-dimensional data sets in times commensurate with the time scale of the events being observed.
Camera technology has advanced the available frame rates to hundreds of fps with megapixel resolutions. Light sheet microscopes (LSMs) [1] are capable of taking advantage of these speeds, using parallel excitation of the entire field of view by means of lateral illumination with a planar light beam or a quickly scanned pencil beam (DS-LSM) [2].
Acquisition of 3D data sets as sequences of two-dimensional images is the most common and reliable strategy, but it requires a close match between the sample and the microscope geometry, and while precise physical reorientation of either of those is possible with current technology, it allows only slow reconfiguration and brings the risk of disturbing the sample. Quicker, non-invasive alternatives exist today: illumination fields of arbitrary geometry can be generated by the optical path [3] and sample perturbation can be avoided by imaging a conjugate copy of the sample relayed by fixed optics (remote focusing, RF [4]).
Here, we propose a solution able to provide efficient 3D imaging of excitable tissues dynamics, a two-photon DS-LSM platform with RF capabilities. 2D lateral scanning of an infrared pencil beam provides arbitrary inclination of the excitation plane respect to the objective focal plane, increasing the efficiency and flexibility of stack acquisition. On-the-fly correction of aberrations along the pencil beam is provided by the RF module, synchronized with the scanning setup. We will show the scheme and the optical setup of the machine, together with calibrations on test fluorescent samples.

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