Turning Galvo scanning upside down for rapid remote focusing

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Various optical techniques exist to scan a laser beam laterally at high speed (kHz to MHz), which form the basis for modern confocal and multi-photon microscopes. However, axial scanning is traditionally performed by translating either the objective or the sample, which is limited by inertia to much slower rates (10Hz). This ultimately limits the rate at which 3D volumetric imaging can be performed.

Recently, innovations in remote focusing techniques, i.e. forming a focus outside of the nominal focal plane, have been widely applied to perform axial scanning. By conjugating either a movable-mirror to the sample plane or an electrotuneable lens (ETL) to the back-focal plane respectively, a laser focus can be rapidly scanned in the axial dimension. The mechanical actuation of the mirror however limits the rate at which axial scanning can be performed (usually only 10-100 Hz) while the usage of ETLs introduce spherical and higher order aberrations, thereby preventing high resolution imaging.

Here we introduce a novel optical design that can render a rapid lateral-scan motion into an aberration-free, high-resolution axial scan. Using a galvo mirror, we scan a laser beam laterally in the remote-focusing arm, which is back-reflected at different heights from a staircase-shaped mirror. On the way back, the laser light is descanned by the same galvo mirror, which removes the lateral scan motion and only the axial component remains. The resulting axial scan is only limited by the operational frequency of the galvo-scanning-mirror, which can reach 10kHz for resonant galvos and potentially faster using electro-optical modulators or acousto-optics. Using a staircase mirror, only a discrete number of axial steps can be produced. However, we will also discuss an implementation with a tilted mirror that allows continuous tuning of the z-position (see Figure 1). By applying this technology to axially swept light-sheet microscopy (ASLM), we achieve 20-fold faster framerates than previous implementations. We will present biological imaging with this fast version of ASLM and discuss applications of the novel scan technology for multiphoton raster-scanning microscopes.

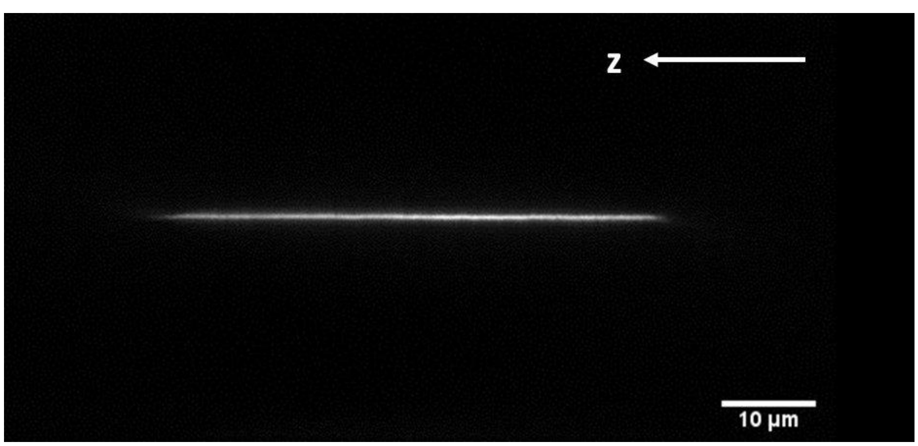

Figure 1: Axial and linear scanning of a laser focus (Numerical Aperture 0.8, 488nm wavelength).