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# Two-dimensional simultaneous spatial and temporal focusing for two-photon excited fluorescence microscopy

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We utilized a cross disperser setup for two-photon excited fluorescence (TPEF) microscopy. The cross disperser was previously introduced and used for wavelength de-multiplexing in optical communication, wavelength-parallel polarization sensing, frequency-comb spectroscopy and two-dimensional (2-D) pulse shaper. By this setting, all the frequency components were spatially dispersed into a 2-D matrix at the Fourier plane and then were spatially and temporally focused at the focal plane of the objective lens. So we call this setup as 2-D simultaneous spatial and temporal focusing (SSTF) microscopy. Compared to one-dimensional (1-D) SSTF microscopy which has the capability of axial excitation confinement equivalent to a line-scanning system for two-photon excited fluorescence (TPEF) microscopy [1, 2], we improved the axial excitation confinement to a point-scanning system. On the other hand, 2-D SSTF microscopy as well as the 1-D SSTF microscopy is a widefield microcopy [3]. The pulses automatically scan in lateral domain with a scanning rate same as the repetition rate of the laser source. This makes frame rate higher than 100Hz possible.

We experimentally proved the improvement of axial excitation confinement ability by surface SHG measurement. 2-D SSTF microscopy showed around 1.7 times improvement of FWHM of the axial TPE intensity distribution compared to a 1-D SSTF microscopy. Furthermore, we used the sectioning images of fluorescent beads to verify the improvement of axial confinement ability.

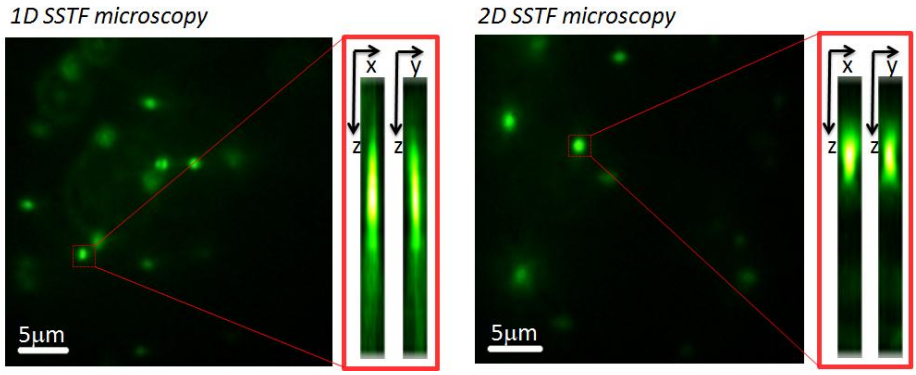


Fig.1 Comparing sectioning images of fluorescent beads and its axial cross section images of single bead (inside red box) from 1-D SSTF microscopy and 2-D SSTF microscopy