Fluorescence and SHG imaging with a single-spinning disk two-photon excitation microscope

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Confocal microscopes have been the workhorses of 3-D biological imaging, but they are slow, offer limited depth penetration and collect only ballistic photons. With their inefficient use of excitation photons they expose biological samples to an often intolerably high light burden. The speed limitation and photo-bleaching risk can be somewhat relaxed in a spinning-disk geometry, due to shorter pixel dwell times and rapid re-scans during image capture. Alternatively, light-sheet microscopes rapidly image large volumes of transparent or chemically cleared samples. Finally, with infrared excitation and efficient scattered-light collection, 2-photon microscopy allows deep-tissue imaging, but it remains slow. Here, we describe a new optical scheme that borrows the best from three different worlds: the speed and direct-view from a spinning-disk confocal, deep tissue-penetration and intrinsic optical sectioning from 2-photon excitation, and a large field of view and a low invasiveness of a selective-plane illumination microscope – all with a single objective lens. We validate the performance of our 2-photon spinning-disk microscope in various applications that have in common to simultaneously require a large depth penetration, high speed and larger fields of view. Beyond biological fluorescence, we demonstrate an application in material science, imaging coherent non-linear scattering from a 3-D nano-porous network.