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Towards supraresolution 2-photon laser scanning microscopy

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ABSTRACT OF THE TALK

Two-photon laser scanning microscopy (2PLSM) has allowed unprecedented fluorescent imaging of neuronal structure and function deep within neural tissue. However, due to the near infrared (NIR) photons necessary for deep tissue penetration and excitation in 2PLSM, the resolution of this approach is poor compared to that of conventional confocal microscopy. Here we demonstrate supraresolution fluorescence imaging with 2PLSM deep within brain slices. Supraresolution imaging is accomplished by using NIR lasers for both pulsed 2-photon excitation and continuous wave 1-photon stimulation emission depletion (STED). Furthermore, we demonstrate that Alexa Fluor-594, a bright fluorophore commonly used in both live and fixed tissue fluorescence imaging, is suitable for combined 2PLSM/CW-STED. We demonstrate combined 2PLSM/CW-STED supraresolution microscopy with approximately 3 fold improvement in resolution in the radial direction over conventional 2PLSM, revealing greater detail in images of live neuronal dendritic spines. Further significant improvements are expected with higher local intensity of the stimulating beam.