Label-Free Three-Photon Deep Imaging with High Power Femtosecond Yb-Fiber Laser

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Imaging biological samples with sub-cellular precision are important for our understanding of fundamental processes governing biological processes. While confocal imaging allows optical sectioning and thus to obtain high-resolution images at different depths, attenuations (due to absorption and scattering) limit high-quality imaging to superficial layers, in many samples, even to just the cells directly at the surface. Two-photon excited fluorescence microscopy [1] allows to increase the imaging depth. It has enabled imaging of cellular signals in animal brains at several 100 micrometers below the surface [2]. The main limitation in two-photon imaging is the loss of ballistic photons due to scattering, which in highly scattering samples again limits the imaging depth to just a few cell layers. In those cases, the scattering losses outweigh the intrinsic optical sectioning of the nonlinear (quadratic) response of the signal, and the non-specific signal at the top of the sample quickly overwhelms the specific signal from the focal spot. Those limitations are alleviated greatly by increasing the nonlinearity, for example, by using three-photon excited fluorescence [3].

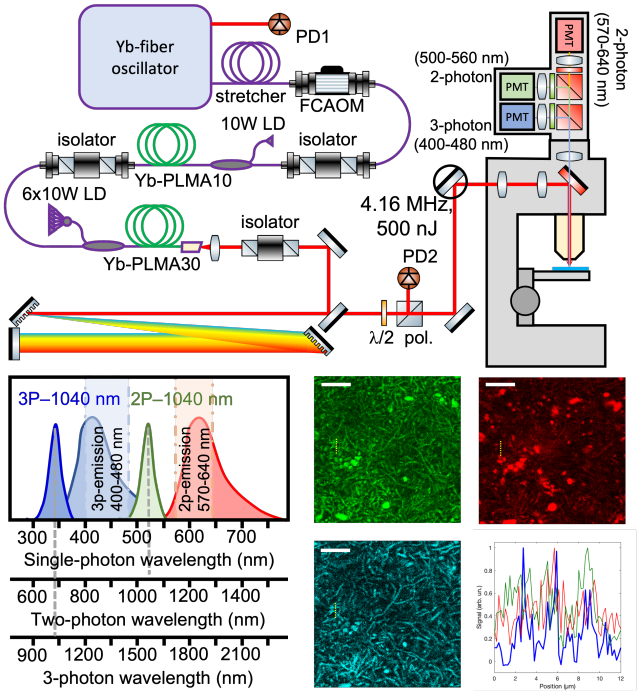

Figure 1: Two- and three-photon imaging of autofluorescence from a Streptomycin bacterial biofilm. The scale bar in the lower right images is 20 µm. Note the improved signal-to-background and signal-to-noise in the blue (3-photon) detection channel

I will present recent results we have obtained using label-free two- and three-photon imaging of biofilms formed by Streptomyces bacteria using our home-built high-power femtosecond Yb-fiber laser (see Fig. 1). Operating this laser at 4 MHz repetition rate allows us to effectively perform three-photon excited fluorescence microscopy, as a high peak intensity can be achieved at a sufficiently low average power to avoid sample damage, and the 1040 nm operation wavelength is optimal for 3-photon excitation of blue fluorescence. Three-photon imaging of Streptomyces allows us to visualize blue autofluorescence from linearmycins [4], which are produced by the bacteria and secreted in extracellular vesicles. The enhanced signal-to-background ratio enabled by three-photon imaging allows the detection of extracellular signals deep inside highly scattering biofilms.

References

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