DEEP TISSUE CONFOCAL MICROSCOPY THROUGH PUPIL FUNCTION ENGINEERING

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Fluorescence microscopy has revolutionized biological research during the past few decades, because it can provide details about tissue’s structural, functional and molecular information. However, one of its major limitation is the shallow imaging penetration depth. To improve imaging performance in deep tissue, a lot of methods have been proposed. One of the powerful tools is to adopt adaptive optics to achieve better imaging results [1, 2]. Others are either to use 1.3-1.4 μm near-infrared window [3], or speckle correlations [4].

Here we report a new strategy to enhance the background rejection capability of a confocal microscopy by modifying transmittance amplitude of the illumination pupil function, thus deep imaging penetration can be achieved. Theoretical analysis shows that the signal generated by a defocused plane decays extremely fast as the defocus distance increase, shown in the figure 1. By combining this method with angular gating technique or focal modulation techniques [5], we can further improve its deep tissue imaging performance, while not reduce its imaging acquisition speed. The strategy can be used not only in confocal microscopy but also other microscopy, such as two-photon microscopy. It could lead to further advances in in vivo optical imaging.