TWO PHOTON FOCAL MODULATION MICROSCOPY FOR HIGH-RESOLUTION IMAGING IN DEEP TISSUE

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Two photon microscopy (2PM) is one of the most widely used tools for in vivo deep tissue imaging. However, it is difficult to achieve diffraction-limited resolution with nonlinear imaging depth increasing in turbid tissues. The main reasons lie in two facts: first, the ballistic component of the excitation light used for imaging is exponentially decayed with penetration depth increasing; second, refractive index heterogeneities prevent the surviving ballistic light from focusing a diffraction-limited spot. Single-photon focal modulation microscopy has been proposed and demonstrated to effectively reject the out-of-focus fluorescence[1]. Later, we proposed the extension of the focal modulation technique to two-photon fluorescence microscopy [2].

Here we analyze theoretically image formation in two-photon focal modulation microscopy with two different geometries: D-shaped pupils (2PDFMM) and a combination of one annular and one circular pupil (2PAFMM), respectively. Two kinds of shape patterns are applied to the spatial phase modulator. After phase modulation, the two half beams are coupled into a microscope. Lock-in techniques are used to demodulate the two-photon excited fluorescence. Thus only the ballistic photons contribute to modulated excitation intensity, while the scattered light is filtered out. Figures. 1(a), (b), (c), (d) show the resolution improvement and better capabilities of inhibiting background noise of 2PDFMM and 2PAFMM compared with 2PM by image result of sparsely distributed beads in transverse plane.

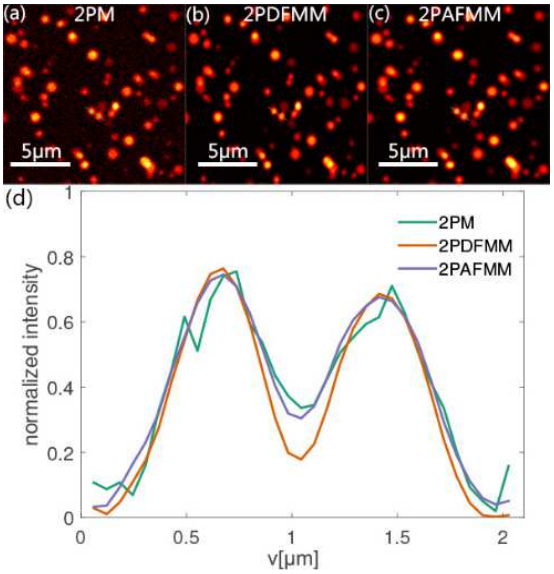

Figure. 1 Images of fluorescent beads in the transverse plane.

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