IN VIVO HIGH RESOLUTION NONLINEAR IMAGING USING FOCAL MODULATION TECHNIQUE

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KEY WORDS: Two photon imaging, in vivo, high resolution, deep penetration depth, thick tissue.

Two-photon excitation processes were first introduced by Göppert-Mayer in 1931 [1]. About two decades ago, multiphoton microscopy (MPM) was invented [2]. Despite the development of tomography [3] and hybrid imaging technologies [4-6], MPM remains the most powerful and widely used technique for high resolution deep tissue imaging [7]. However, as the imaging depth increases the images are increasingly degraded, due to inhomogeneous optics properties of biological samples. In recent years, several methods are demonstrated to recover nearly-diffraction-limited performance in deep tissue two-photon imaging by using adaptive optics [8, 9]. However, these methods are realized with the cost of additional imaging acquisition time.

Here we present an in vivo high resolution two-photon microscopy with the combination of modulation and demodulation techniques [10]. By using D-shaped pupils (2PDM) or one annular one circular pupil (2PAM), we can simultaneously improve the transverse and axial resolution of conventional two-photon microscopy (2PM) in deep tissue imaging, shown in the figure 1.