Deeper penetration depth can be achieved with two-photon microscopy [1-3]. Application of focal modulation microscopy [4] to 2-photon fluorescence microscopy (to give 2PFMM) is described. Image formation in a turbid medium is investigated theoretically for a system based on annular illumination [5]. The ballistic signal is

\[ I_{2PFMM}^{b} = (h_{a}h_{b}^{*} + h_{b}^{*}h_{a}) \left( |h_{a}|^{2} + |h_{b}|^{2} \right) \exp(-2\alpha z), \]

where \( h_{a,b} \) are the point spread functions for the two illumination pupils, while the background is

\[ I_{2PFMM} = I_{s} \left( h_{a}h_{b}^{*} + h_{b}^{*}h_{a} \right) \exp(-\alpha z), \]

where \( \alpha = 1/l_{s} \) is the scattering coefficient and \( I_{s} \) is the intensity of the scattered light [6, 7]. The results show that compared with conventional two-photon fluorescence microscopy (2PM), the ballistic excitation of 2PFMM is concentrated in a much smaller region around the focal point and decays more rapidly outside the focal volume. The scattered excitation is largely suppressed, so that comparison of signal to background ratio between 2PM and 2PFMM suggests that given the same excitation intensity, 2PFMM can provide a three-fold increase in the imaging penetration depth.

![Figure 1. The signal to background ratio of 2PFMM and 2PM with different anisotropy factors g as a function of focus depth z. Scattering mean free path \( l_{s} = 200\mu m \), \( n = 1.33 \), NA = 0.56, and \( \lambda = 0.9\mu m \).](image)

References