ENHANCEMENT OF RESOLUTION AND CONTRAST IN TWO PHOTON FLUORESCENCE MICROSOPY

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Two-photon microscopy (TPM) have a deep penetration depth and thus has an important role in biomedical research. The simultaneous absorption of two photons needs high photon intensity and thus has few excitations out of the focal plane which assures a better optical sectioning ability. But the resolution of TPM decreases compared to confocal microscopy because of the longer wavelength utilized. As for the excitation efficiency of TPM is low, the contrast of the image deduces. Two-photon fluorescence emission differential (FED) microscopy has been proposed to enhance the resolution and contrast of TPM for better applications. But because that the fluorescence intensity is linearly quadratic of the excitation intensity within certain range [1], the central region of hallow focal spot is broadened while the solid focal spot is shrunken which combined undermines the resolution enhancement and brings about more negative values in subtraction.

Here, we demonstrate for the first time that the saturated TPE FED combined with ratio concerned quadratic intensity weighted subtraction (RQIWS) method to enhance the resolution and contras. We take advantage of the saturation effect of fluorescence imaging. The dark region of the hollow focal spot is shrunken and the solid focal spot is broadened deducing the negative values in the subtraction process and with RQIWS we can enhance the resolution and contrast considerably. The RQIWS method is a modified method of intensity weighted subtraction method [2]. The subtraction coefficient is a matrix consisting of the ratio of the maximum intensity of the two images and the intensity difference of every pixel instead of a constant value. Furthermore, we verified the feasibility of pixel reassignment in TPM for enhancement in resolution and contrast. An ICCD replaces the PMT as the detector for latter pixel reassignment and we get an obvious enhancement in resolution and contrast. Our methods and findings further enhance the performance of TPM and will broaden its applications in biomedical research significantly.

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