ENHANCING FLUORESCENCE COLLECTION EFFICIENCY FOR MULTIPHOTON MICROSCOPY BY QUARTER ELLIPSOIDAL REFELCTOR WITH HEMISPHERICAL LENS

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Multiphoton microscopy is a powerful technique for neural scientists to obtain tiny fluorescence sparking from excited neurons. Usually multiphoton microscopy utilizing a 800nm Ti:Sapphire femtosecond laser has capabilities of imaging live bio-tissue as deep as ~500um. Fluorescence emitted below this depth is too weak due to limited collection efficiency. A commonly used 40X water immersion objective lens has only 10% geometrical collection efficiency of fluorescence in turbid media [1]. A variety of methods has been developed to collect “escaped” fluorescence photons out of an objective lens. For example it is quite effective to add external light reflectors or collectors, such as parabolic reflectors [1] and ring of fibers [2].

In this paper we will present a quarter ellipsoidal reflector and a hemispherical lens based method for fluorescence collection efficiency enhancement. A quarter ellipsoidal reflector collaborates a hemispherical lens to focus fluorescence into an external PMT. Aforementioned devices work on a Nikon two-photon microscope. Fluorescence photons from turbid media are collected by the objective lens, the quarter ellipsoidal reflector and the hemispherical lens separately. Fluorescence enhanced images are formed by precisely tracking scan traces of an optical scanner of the microscope. Performance of this method is evaluated to be 16-fold improvement at 700um by Monte Carlo simulation. Imaging tests using artifact scatter and brain tissue of live GFP transgenic mouse are also done. Both imaging speed and penetration depth could benefit from enhanced fluorescence collection efficiency by using this method.

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