Optimization of Multi-Photon Excitation Microscopy by Total Emission Detection (TED) Using a Parabolic Light Reflector

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One of the benefits of multi-photon excitation microscopy is the inherent optical sectioning that occurs during excitation at the diffraction-limited spot. Fluorescence generated at the focal spot propagates in all directions. We have constructed a device that maximizes the probability of collecting all of the scattered and ballistic light generated at the focal spot of multi-photon excited emissions (MPE) to optimize the signal-to-noise ratio (SNR) for micro-imaging. This was accomplished by optically coupling a parabolic reflector (that surrounds the sample and top of the objective) to a pair of collimating lenses (above the sample) that redirects emitted light to a separate detector. These additional optics, combined with the objective, allow the total emission detection (TED) condition to be approached. This scheme was tested on both a fluorescent beads (embedded in an agarose gel that mimicked the optical properties of brain) and a mouse brain slice (approximately 250\,$\mu$m thick) that contained non-muscle myosin labeled with GFP and compared to images generated through the traditional (objective collected) non-descanned emission pathway. Numerical simulations for a 20x (0.75 NA) objective suggest a >10 fold increase in SNR is possible using the TED device. The results show that TED increased SNR by a factor of up to 8.9 in a mouse brain slice (Fig.1) and showed a similar relationship in the bead embedded agarose sample. While the improvement is less than predicted by theoretical calculations of an ideal sample emitting light in all directions (likely due to non-ideal optical coating), this increase in SNR can be used to improve time resolution, reduce laser power requirements/photodynamic damage, and, in certain cases, detection depth, for MPE imaging techniques.