

# OPTIMIZING MULTI-PHOTON FLUORESCENCE MICROSCOPY LIGHT COLLECTION FROM LIVING TISSUE BY NON-CONTACT TOTAL EMISSION DETECTION (epiTED)

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A key benefit of multi-photon fluorescence microscopy is the inherent optical sectioning that occurs during excitation at the diffraction-limited spot. The scanned collection of fluorescence emission is incoherent; i.e., no real image needs to be formed on the detector plane. The isotropic emission of fluorescence excited at the focal spot allows for new detection schemes that efficiently funnel all attainable photons to detector(s). We previously showed [1] that parabolic mirrors and condensers could be combined to collect the

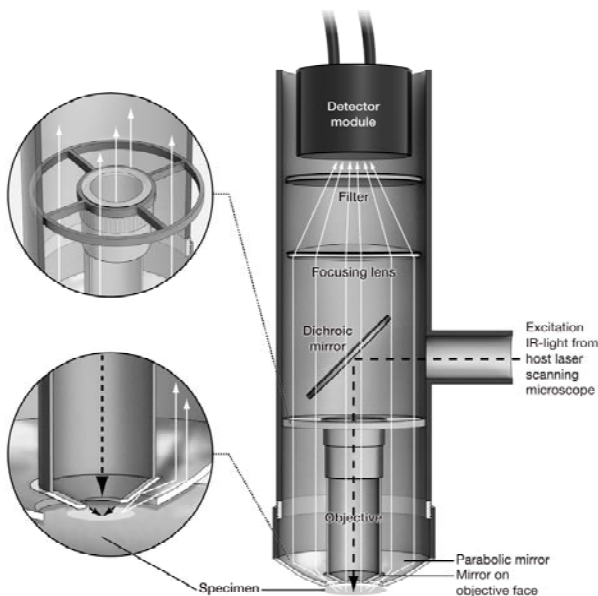


Figure 1: Schematic illustration of the epiTED device

totality of solid angle around the spot for tissue blocks, leading to ~8-fold signal gain. We have developed an *in vivo* Total Emission Detection (epiTED) instrument modified to make non-contact images from living tissue (Figure 1). Simulations suggest that a ~4-6 fold enhancement is possible with this approach depending on objective characteristics, imaging depth and the optical properties of the sample being imaged. In general, two fold improvements were demonstrated in the mouse brain and skeletal muscle as well as the rat kidney using a variety of fluorophores with no compromise of spatial resolution. These results show that epiTED emission detection *in vivo* that can maintain image SNR at greater than two times the scan rate or an more than 30% reduction in laser excitation

power to minimize photo-damage. These results will be discussed in relation to other multi-photon emission detection enhancement devices.

- [1] Combs, C. A., Smirnov, A. V., Riley, J. D., Gandjbakhche, A. H., Knutson, J. R. and Balaban, R. S.. Optimization of multiphoton excitation microscopy by total emission detection using a parabolic light reflector. *J Microsc* 228, 330-7 (2007).