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Enhanced collection of scattered photons in nonlinear fluorescence microscopy by extended epi-detection with a silicon photomultiplier array

Key words: Extended epi-detection; Enhanced collection; Nonlinear fluorescence microscopy; Silicon photomultiplier array

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Motivation

1. To maximize signal collection, non-descanned epi-detection is generally adopted for in vivo multiphoton fluorescence imaging.

2. Due to the limited collection capability of objectives, severe scattering leads to miss-detection of most fluorescence photons, as in the conventional non-descanned epi-detection scheme.

3. Extended detection has been demonstrated to enhance the collection of back-scattered pump/probe signals in stimulated Raman imaging. However, the extended detection of backscattered fluorescence signals has not been demonstrated in nonlinear fluorescence microscopy.

Main idea

1. A silicon photomultiplier (SiPM) array can be placed ahead of the front apertures of objectives to collect miss-detected fluorescence photons.

2. The effectiveness of the extended detection scheme can be validated through numerical simulations. Specifically, the bio-sample scattering can be modeled by the Henyey-Greenstein distribution, and the photon propagation can be simulated by Zemax (OpticStudio).

Method

1. We used the Henyey-Greenstein distribution to model bio-sample scattering, Zemax (OpticStudio) to simulate photon propagation, and custom MATLAB scripts for statistics.

2. To simulate the incoherent emission of fluorescence in multiphoton fluorescence microscopy, we first generated a large number (10^5) of rays from a point in the simulated bio-samples, and then calculated the spatial distribution of emitted light at the plane of the SiPM surface. Specifically, the collection numerical aperture (NA) of the objective was simulated by limiting the acceptance angle.

Major results

Enhanced collection via extended epi-detection in the human skin model

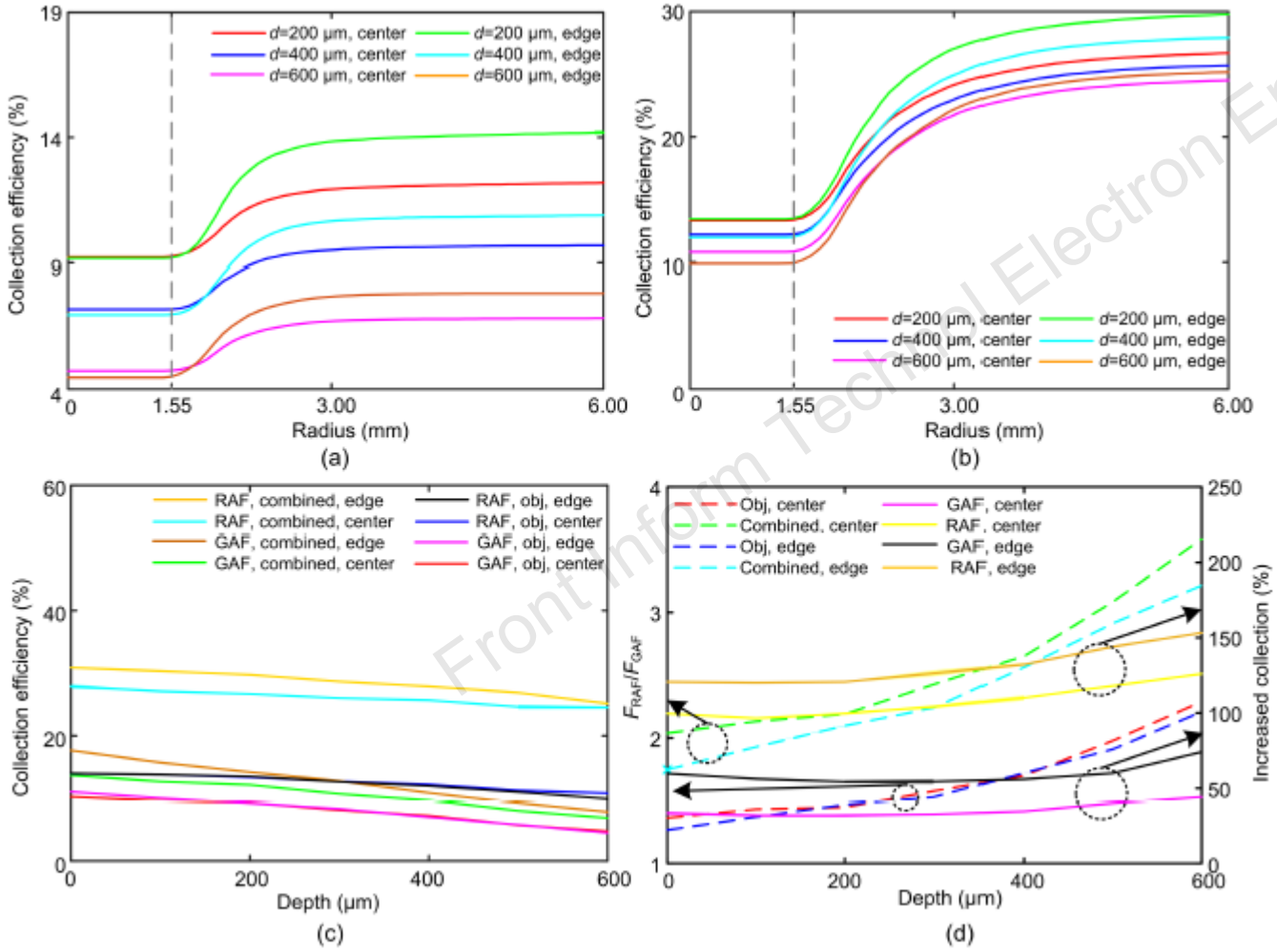


Fig. 2 Enhanced collection via extended epi-detection in the human skin model

Collection efficiency vs. detection radius at different imaging depths for GAF (a) and RAF (b); (c) Collection efficiency vs. imaging depth in the objective collection mode and combined collection mode; (d) Collection efficiency ratio of the RAF and GAF at different imaging depths in the objective collection mode and combined collection mode (left vertical axis), and the increased collection of GAF and RAF as a function of the imaging depth (right vertical axis)

Major results (Cont'd)

Enhanced collection via extended epi-detection in the mouse brain model

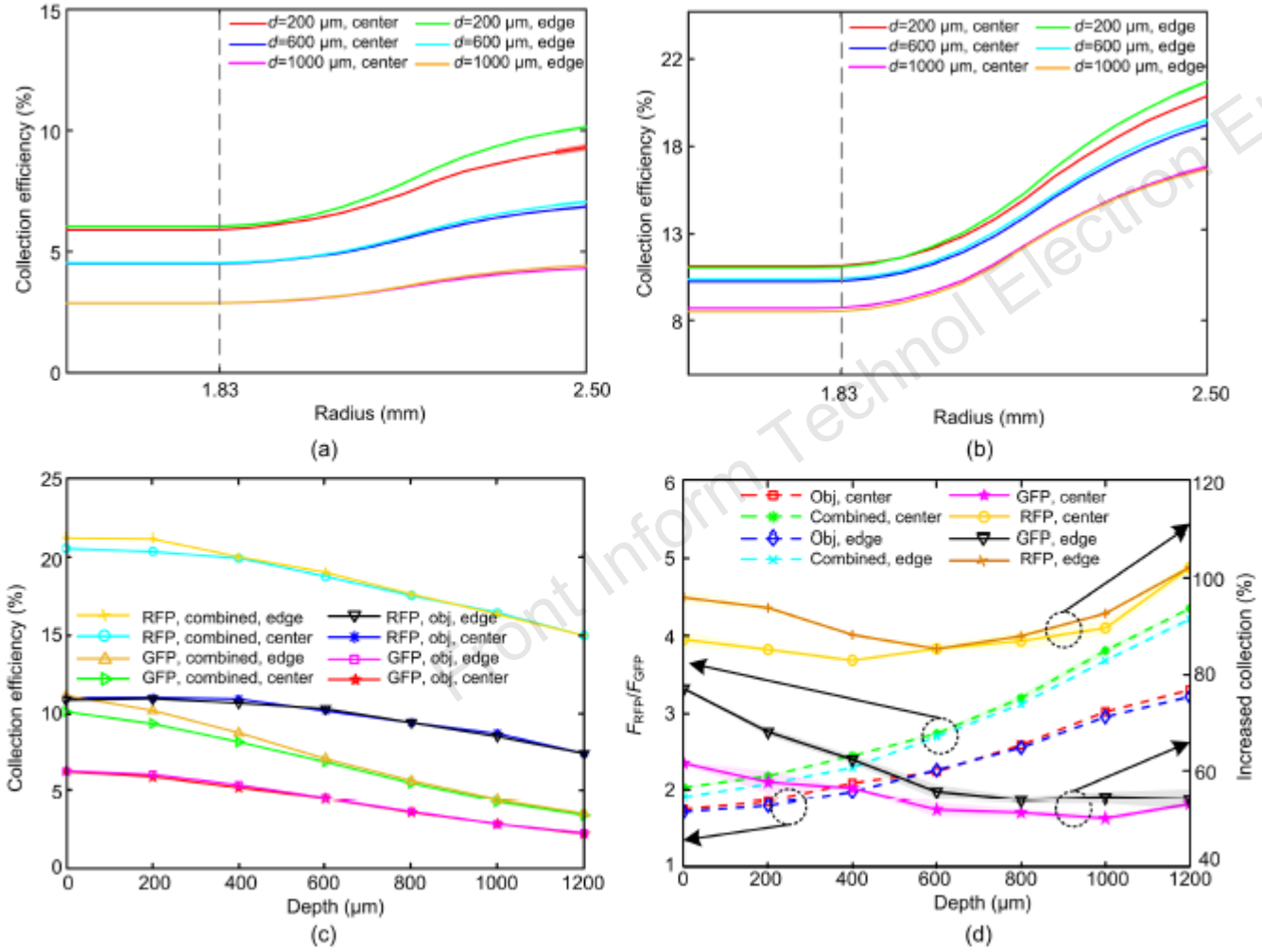


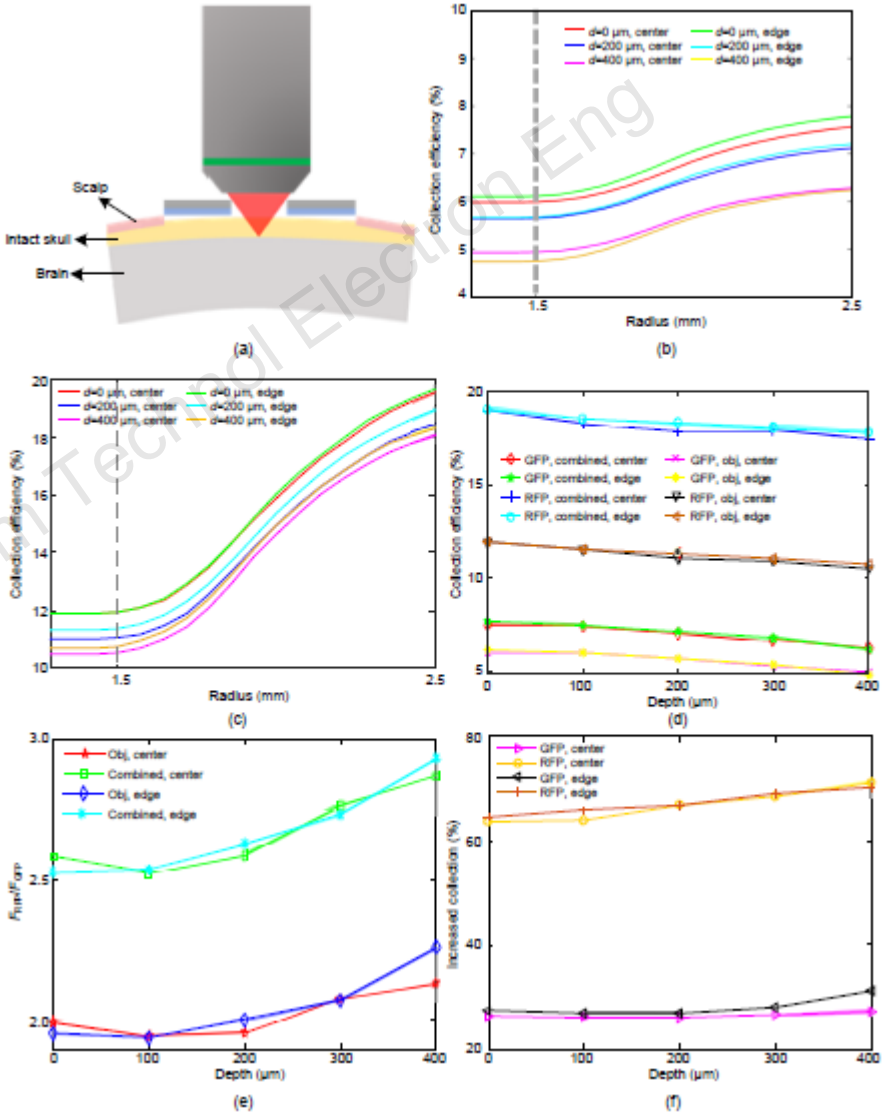
Fig. 3 Enhanced collection via extended epi-detection in the mouse brain model

Collection efficiency vs. detection radius for GFP (a) and RFP (b); (c) Collection efficiency in the objective collection mode and combined collection mode; (d) Collection efficiency ratio of GFP and RFP (left vertical axis), and the increased collection of GFP and RFP (right vertical axis)

Major results (Cont'd)

Enhanced collection via extended epi-detection in a hybrid model of mouse skull and brain

(a) A hybrid model of mouse skull and brain; Collection efficiency vs. detection radius for GFP (b) and RFP (c); (d) Collection efficiency in the objective collection mode and combined collection mode; (e) Collection efficiency ratio of RFP and GFP at different imaging depths; (f) Increased collection of GFP and RFP as a function of the imaging depth



Major results (Cont'd)

Performance enhancements with different objectives in the human skin model

Increased collection with different objectives for fluorescent sources in the center (a) and at the edge (b) of the FOV

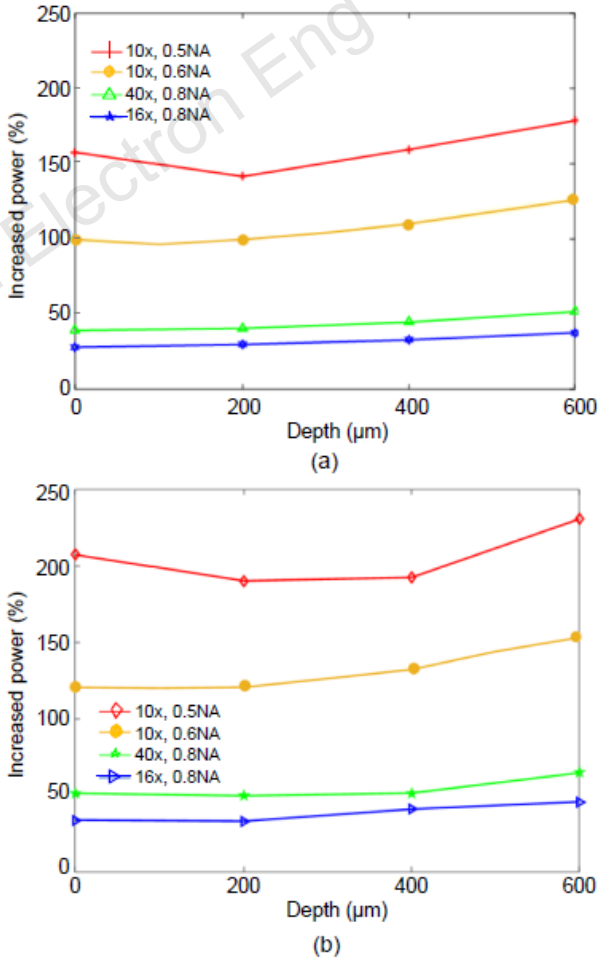


Fig. 5 Performance enhancements with different objectives in the human skin model

Conclusions

1. Through simulations of a human skin model, a mouse brain model, and a hybrid model of mouse skull and brain, we have shown that the extended epi-detection scheme is effective in deep tissue imaging.

2. Choosing fluorescence proteins of larger emission wavelength is also beneficial.

3. For objectives of the same magnification, the enhanced performance is poorer for those with a higher NA.

4. For objectives of the same NA, the enhanced performance is poorer for those with a lower magnification.

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