5. Conclusion

In this paper, we showed that using a homogeneous geometry assumption to approximate the depth-distribution of the collected fluorescence signal is not an accurate for vascular fluorescence measurements. A more complex model, involving the size, concentration and position of the microvasculature being imaged, the difference in absorption between the excitation and emission wavelengths, and the difference in absorption coefficients in the fluorescing region compared to the non-fluorescing region is required to provide a more accurate picture of depth penetration.

We also examined three illumination and detection schemes used in fluorescence imaging. The targeted and confocal illumination schemes offer a more focused signal distribution over wide field illumination. Using the confocal illumination and detection scheme in particular offers very strong localization of signal to the surface vasculature, while the wide-area detection in the targeted scheme can provide more depth-weighted signals. Using the camera scheme offers the greatest depth weighting of the three illumination and detection schemes we evaluated, but at the cost of including fluorescence from near the surface over the entire illumination region.

Lastly, we examined intra-vessel sampling and demonstrated that when using an excitation wavelength that is strongly absorbed by blood, our collected signal originates from only the top-surface of the targeted vessels. This may need to be taken into account if using a lifetime imaging method that will produce different results near the vessel walls–a wavelength which is absorbed by blood less may be more useful to provide a signal which is integrated across the vessel diameter.

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