In this study, we used the same averaged values of optical properties for both the excitation and emission wavelengths using our Monte Carlo software. Note that in conventional Monte Carlo methods, the Jacobian matrix was constructed by taking the product of the fields of photon density waves irradiated from the sources and the detectors at the excitation and emission wavelengths, respectively. In our preliminary comparison, we found that our method produced better image quality (data not shown). We hypothesized that it was because our Monte Carlo algorithm more closely represented the physical process of photon migration from the sources to the detectors within the medium. Further study is underway to better investigate our findings.

Resolution is the ability of an imaging system to resolve two distinct objects, which is highly subjective to the viewer. In this study, resolution was defined as the center of a Gaussian peak coincided with the half-maximum width of another identical Gaussian peak, which translated to a threshold hold values of 85%. Note that different criteria and peak profiles can result in more or less different definition of resolution, e.g., 80% threshold if using the Rayleigh criterion and 95% using Dawes' limit for Airy diffraction patterns.

One limitation of this FDOT study was the reconstruction artifact shown in Fig. 3. This artifact was likely due to the inaccurately known in vivo optical properties, especially in the lung area where the background signal was relatively high. Nonetheless, in preclinical applications, the disease model is typically known, which assists the interpretation of imaging results and avoids misjudgment. Note that under comparatively more extreme experimental conditions, such as the case in this study, imaging artifact tend to manifest more severely. It is certainly a direction for us to continuously work on to improve reconstruction quality in future.

Another limitation was the lack of quantitative results. Although in this study, it was feasible to calibrate the scaling factor γ in Eq. (1) using another QD solution with known concentration, we noticed that this calibration technique could not be readily generalized to *in vivo* animal experiments, where this calibration technique would become impractical because creating a fluorescent inclusion in the thorax of the animal is difficult, and is also problematic for preclinical studies. Another concern for this calibration technique was the use of the regularization method in reconstruction due to the ill-posed inverse problem. In addition, it is known that regularization in reconstruction affects the quantitative result in diffuse optical imaging [40]. The values of the regularization parameters (0.1 for both) indicated that the reconstruction was non-negligibly influenced by the second and third terms of the cost function defined in Eq. (3). With improved experimental control, signal acquisition, and imaging apparatus, we anticipate a decreased noise level in the optical signal and, as a result, significantly less regularization will be required in the reconstruction. In addition, more accurate optical properties will likely reduce the systematic error in the forward model. Under these circumstances, it may be feasible to use this calibration technique to produce reliable quantitative FDOT reconstruction for *in vivo* studies.

In conclusion, we demonstrated a new FDOT method consisting of our newly developed parallel Monte Carlo software and anatomically guided sampling strategy for preclinical imaging. The Monte Carlo software fully utilized the power of parallel supercomputing and thus is better suited for handing large data sets in preclinical imaging. The anatomically guided sampling strategy significantly improved fidelity of data acquisition and subsequently the quality of reconstruction. Using the proposed method, the new FDOT system achieved significantly better resolution and sensitivity compared to our previous system, which consisted of essentially the same hardware. This method can be applied to any FDOT system adopting similar imaging principles to improve resolution and sensitivity.

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