Combined hemoglobin and fluorescence diffuse optical tomography for breast tumor diagnosis: a pilot study on time-domain methodology

Wei Zhang,1 Linhui Wu,1 Jiao Li,1 Xi Yi,1 Xin Wang,1 Yiming Lu,1 Weiting Chen,1 Zhongxing Zhou,1,2 Limin Zhang,1,2 Huijuan Zhao,1,2 and Feng Gao1,2,*

1College of Precision Instrument and Optoelectronics Engineering, Tianjin University, Tianjin 300072, China
2Tianjin Key Laboratory of Biomedical Detecting Techniques and Instruments, Tianjin 300072, China
*gaofeng@tju.edu.cn

Abstract: A combined time-domain fluorescence and hemoglobin diffuse optical tomography (DOT) system and the image reconstruction methods are proposed for enhancing the reliability of breast-dedicated optical measurement. The system equipped with two pulsed laser diodes at wavelengths of 780 nm and 830 nm that are specific to the peak excitation and emission of the FDA-approved ICG agent, and works with a 4-channel time-correlated single photon counting device to acquire the time-resolved distributions of the light re-emissions at 32 boundary sites of tissues in a tandem serial-to-parallel mode. The simultaneous reconstruction of the two optical (absorption and scattering) and two fluorescent (yield and lifetime) properties are achieved with the respective featured-data algorithms based on the generalized pulse spectrum technique. The performances of the methodology are experimentally assessed on breast-mimicking phantoms for hemoglobin- and fluorescence-DOT alone, as well as for fluorescence-guided hemoglobin-DOT. The results demonstrate the efficacy of improving the accuracy of hemoglobin-DOT based on a priori fluorescence localization.

OCIS codes: (170.3880) Medical and biological imaging; (170.6960) Tomography; (170.6920) Time-resolved imaging; (170.3010) Image reconstruction techniques.

References and links
1. T. Durduran, R. Choe, W. B. Baker, and A. G. Yodh, “Diffuse optics for tissue monitoring and tomography,” Rep. Prog. Phys. 73(7), 076701 (2010).
2. A. P. Gibson, J. C. Hebden, and S. R. Arridge, “Recent advances in diffuse optical imaging,” Phys. Med. Biol. 50(4), R1–R43 (2005).
3. D. R. Leff, O. J. Warren, L. C. Enfield, A. P. Gibson, T. Athanasiou, D. K. Patten, J. C. Hebden, G. Z. Yang, and A. Darzi, “Diffuse optical imaging of the healthy and diseased breast: a systematic review,” Breast Cancer Res. Treat. 108(1), 9–22 (2008).
4. B. J. Tromberg, B. W. Pogue, K. D. Paulsen, A. G. Yodh, D. A. Boas, and A. E. Cerussi, “Assessing the future of diffuse optical imaging technologies for breast cancer management,” Med. Phys. 35(6), 2443–2451 (2008).
5. S. G. Demos, A. J. Vogel, and A. H. Gandjbakhche, “Advances in optical spectroscopy and imaging of breast lesions,” J. Mammary Gland Biol. Neoplasia 11(2), 165–181 (2006).
6. X. Intes, “Time-domain optical mammography SoftScan: initial results,” Acad. Radiol. 12(8), 934–947 (2005).
7. S. Fantini and A. Sassaroli, “Near-infrared optical mammography for breast cancer detection with intrinsic contrast,” Ann. Biomed. Eng. 40(2), 398–407 (2012).
8. S. M. W. Y. van de Ven, S. G. Elias, A. J. Wiethoff, M. van der Voort, T. Nielsen, B. Brendel, C. Bontus, F. Uhlemann, R. Nachabe, R. Harbers, M. van Beek, L. Bakker, M. B. van der Mark, P. Luijten, and W. P. Mali, “Diffuse optical tomography of the breast: preliminary findings of a new prototype and comparison with magnetic resonance imaging,” J. Biomed. Opt. 9, 1108–1113 (2009).
9. G. Gulsen, B. Xiong, O. Birgul, and O. Nalcioglu, “Design and implementation of a multifrequency near-infrared diffuse optical tomography system,” J. Biomed. Opt. 11(1), 014020 (2006).
10. L. Spinelli, A. Torricelli, A. Pifferi, P. Taroni, G. M. Danesini, and R. Cubeddu, “Bulk optical properties and tissue components in the female breast from multiwavelength time-resolved optical mammography,” J. Biomed. Opt. 9(6), 1137–1142 (2004).
11. H. Jiang, Y. Xu, N. Ifrimia, J. Eggert, K. Klove, L. Baron, and L. Fajardo, “Three-dimensional optical tomographic imaging of breast in a human subject,” IEEE Trans. Med. Imaging 20(12), 1334–1340 (2001).
12. L. C. Enfield, A. P. Gibson, N. L. Everdell, D. T. Delpy, M. Schweiger, S. R. Arridge, C. Richardson, M. Keshtgar, M. Douek, and J. C. Hebdon, “Three-dimensional time-resolved optical mammography of the uncompressed breast,” Appl. Opt. 46(17), 3628–3638 (2007).
13. B. Chance, S. Nioka, J. Zhang, E. F. Conant, E. Hwang, S. Brist, S. G. Orel, M. D. Schnall, and B. J. Czernecki, “Breast cancer detection based on incremental biochemical and physiological properties of breast cancers: a six-year, two-site study,” Acad. Radiol. 12(8), 925–933 (2005).
14. S. Srinivasan, B. W. Pogue, C. Carpenter, S. Jiang, W. A. Wells, S. P. Poplack, P. A. Kaufman, and K. D. Paulsen, “Developments in quantitative oxygen-saturation imaging of breast tissue in vivo using multispectral near-infrared tomography,” Antioxid. Redox Signal. 9(8), 1143–1156 (2007).
15. D. Grosenick, H. Wabnitz, K. T. Moesta, J. Mucke, P. M. Schlag, and H. Rinneberg, “Time-domain scanning optical mammography: II. Optical properties and tissue parameters of 87 carcinomas,” Phys. Med. Biol. 50(11), 2451–2468 (2005).
16. C. Li, S. R. Grobmyer, N. Massol, X. Liang, Q. Zhang, L. Chen, L. L. Fajardo, and H. B. Jiang, “Noninvasive in vivo tomographic optical imaging of cellular morphology in the breast: possible convergence of microscopic pathology and macroscopic radiology,” Med. Phys. 35(6), 2493–2501 (2008).
17. V. Ntziachristos, A. G. Yodh, M. D. Schnall, and B. Chance, “MRI-guided diffuse optical spectroscopy of malignant and benign breast lesions,” Neoplasia 4(4), 347–354 (2002a).
18. C. M. Carpenter, S. Srinivasan, B. W. Pogue, and K. D. Paulsen, “Methodology development for three-dimensional MR-guided near infrared spectroscopy of breast tumors,” Opt. Express 16(22), 17903–17914 (2008).
19. Q. Fang, S. A. Carp, J. Selb, G. Boverman, Q. Zhang, D. B. Kopans, R. H. Moore, E. L. Miller, D. H. Brooks, and D. A. Boas, “Combined optical imaging and mammography of the healthy breast: optical contrast derived from breast structure and compression,” IEEE Trans. Med. Imaging 28(1), 30–42 (2009).
20. Q. Zhu, S. H. Kuritzkes, P. Hegde, S. Tannenbaum, M. Kane, M. Huang, N. G. Chen, B. Jagiavan, and K. Zarfoss, “Utilizing optical tomography with ultrasound localization to image heterogeneous hemoglobin distribution in large breast cancers,” Neoplasia 7(3), 263–270 (2005).
21. V. Ntziachristos, A. G. Yodh, M. Schnall, and B. Chance, “Concurrent MRI and diffuse optical tomography of breast after indocyanine green enhancement,” Proc. Natl. Acad. Sci. U.S.A. 97(6), 2767–2772 (2000).
22. A. Godavarty, M. J. Eppstein, C. Zhang, S. Nioka, A. G. Yodh, and B. Chance, “In vivo continuous-wave optical breast imaging enhanced with Indocyanine Green,” Med. Phys. 30(6), 1039–1047 (2003).
23. A. Corlu, R. Choe, T. Durduran, M. A. Rosen, M. Schweiger, S. R. Arridge, M. D. Schnall, and A. G. Yodh, “Three-dimensional in vivo fluorescence diffuse optical tomography of breast cancer in humans,” Opt. Express 15(11), 6696–6716 (2007).
24. S. M. W. Y. van de Ven, A. J. Wiethoff, T. Nielsen, B. Brendel, M. van der Voort, R. Nachabe, M. Van der Maas, M. Van Beek, L. Bakker, L. Fels, S. Elias, P. Luijten, and W. Mali, “A novel fluorescent imaging agent for diffuse optical tomography of the breast: first clinical experience in patients,” Mol. Imaging Biol. 12(3), 343–348 (2010).
25. V. Ntziachristos, J. Ripoll, L. V. Wang, and R. Weissleder, “Looking and listening to light: the evolution of whole-body photonic imaging,” Nat. Biotechnol. 23(3), 313–320 (2005).
26. E. Kwana and E. M. Sevick-Muraca, “Fluorescence lifetime spectroscopy for pH sensing in scattering media,” Anal. Chem. 75(16), 4325–4329 (2003).
27. A. Godavarty, M. J. Eppstein, C. Zhang, S. Theru, A. B. Thompson, M. Gurfinkel, and E. M. Sevick-Muraca, “Fluorescence-enhanced optical imaging in large tissue volumes using a gain-modulated ICCD camera,” Phys. Med. Biol. 48(12), 1701–1720 (2003).
28. A. Hagen, D. Grosenick, R. Macdonald, H. Rinneberg, S. Burock, P. Warnick, A. Poellinger, and P. M. Schlag, “Late-fluorescence mammography assesses tumor capillary permeability and differentiates malignant from benign lesions,” Opt. Express 17(19), 17016–17033 (2009).
29. A. Leproux, M. van der Voort, M. B. van der Mark, R. Harbers, S. M. van de Ven, and T. G. van Leeuwen, “Optical mammography combined with fluorescence imaging: lesion detection using scatterplots,” Biomed. Opt. Express 2(4), 1007–1020 (2011).
30. S. Achilleu, R. B. Dorshow, J. E. Bugaj, and R. Rajagopalan, “Novel receptor-targeted fluorescent contrast agents for in vivo tumor imaging,” Invest. Radiol. 35(8), 479–485 (2000).
31. F. Gao, J. Li, L. M. Zhang, P. Poulet, H. J. Zhao, and Y. Yamada, “Simultaneous fluorescence yield and lifetime tomography from time-resolved transmittances of small-animal-sized phantom,” Appl. Opt. 49(16), 3163–3172 (2010).
32. E. M. C. Hillman, J. C. Hebden, F. E. W. Schmidt, S. R. Arridge, M. Schweiger, H. Dehghani, and D. T. Deeply, “Calibration techniques and datatype extraction for time-resolved optical tomography,” Rev. Sci. Instrum. 71(9), 3415–3427 (2000).
33. W. Becker, Advanced Time-Correlated Single Photon Counting Techniques (Springer-Verlag, Berlin, 2005).
34. V. Ntziachristos, C. H. Tung, C. Bremer, and R. Weissleder, “Fluorescence molecular tomography resolves protease activity in vivo,” Nat. Med. 8(7), 757–761 (2002B).
35. F. Gao, H. J. Zhao, Y. Tanikawa, and Y. Yamada, “Time-resolved diffuse optical tomography using a modified generalized pulse spectrum technique,” IEICE Trans. Inf. Syst 85-D, 133–142 (2002).
36. H. J. Zhao, F. Gao, Y. Tanikawa, and Y. Yamada, “Time-resolved diffuse optical tomography and its application to in vitro and in vivo imaging,” J. Biomed. Opt. 12(6), 062107 (2007).
37. K. Furutsu and Y. Yamada, “Diffusion approximation for a dissipative random medium and the applications,” Phys. Rev. E Stat. Phys. Plasmas Fluids Relat. Interdiscip. Topics 56(5), 3634–3640 (1994).

38. F. Gao, H. J. Zhao, Y. Tanikawa, and Y. Yamada, “A linear, featured-data scheme for image reconstruction in time-domain fluorescence molecular tomography,” Opt. Express 14(16), 7109–7124 (2006).

39. S. R. Arridge, “Optical tomography in medical imaging,” Inverse Probl. 15(2), R41–R93 (1999).

40. X. Intes, V. Ntziachristos, J. P. Culver, A. Yodh, and B. Chance, “Projection access order in algebraic reconstruction technique for diffuse optical tomography,” Phys. Med. Biol. 47(1), N1–N10 (2002).

41. H. Dehghani, S. Srinivasan, B. W. Pogue, and A. P. Gibson, “Numerical modeling and imaging reconstruction in diffuse optical tomography,” Phil. Trans. R. Soc. A Math. Phys. Eng. Sci. 367(1900), 3073–3095 (2009).

42. G. R. Walsh, Methods of Optimization (Wiley, New York, 1975).

43. D. Qin, H. J. Zhao, Y. Tanikawa, and F. Gao, “Experimental determination of optical properties in turbid medium by TCSPC technique,” Proc. SPIE 6434, 64342E–10 (2007).

44. F. S. Azar and X. Intes, Translational Multimodality Optical Imaging (Arttech House, Boston, 2008), Chap. 8.

45. F. Gao, H. J. Zhao, Y. Tanikawa, K. Homma, and Y. Yamada, “Influences of target size and contrast on near infrared diffuse optical tomography—a comparison between featured-data and full time-resolved scheme,” Opt. Quantum Electron. 37(13-15), 1287–1304 (2005).

46. D. D. Noltling, J. C. Gore, and W. Pham, “Near-infrared dyes: probe development and applications in optical molecular imaging,” Curr Org Synth 8(4), 521–534 (2011).

47. M. E. Kilner, E. L. Miller, A. Barbaro, and D. A. Boss, “Three-dimensional shape-based imaging of absorption perturbation for diffuse optical tomography,” Appl. Opt. 42(16), 3129–3144 (2003).

48. M. Zacharopoulos, M. Schweiger, V. Kolehmainen, and S. Arridge, “3D shape based reconstruction of experimental data in diffuse optical tomography,” Opt. Express 17(21), 18940–18956 (2009).

1. Introduction

Breast tumor diagnosis is widely regarded as one of the most applicable areas of diffuse optical tomography (DOT), which aims at reconstructing blood concentration and oxygenation images of the breast by spatially and spectrally resolving its tissue optical properties [1–5]. This endogenous-contrast-based imaging modality, referred to as hemoglobin-DOT, has potentials to compensate the limitations of the established modalities, such as X-ray mammography, ultrasound (US) and magnetic resonance imaging (MRI), in the safety, sensitivity and specificity, particularly for screening young women under 35 years of age who have dense breasts [1,4,5], and allows for improved discrimination between malignant and benign lesions [3,5–7]. Breast hemoglobin-DOT has been widely explored with three distinct technologies, following the same evolitional track as the universal diffuse light imaging: continuous-wave [8], frequency-domain [9], and time-domain (TD) [6,10]. Many groups have reported high total hemoglobin concentration in malignant tumors as compared to normal tissues - an expectable contrasting mechanism due to the sustained angiogenesis occurring in tumors [3,11,12]. Despite of unapparent manifestation in hemoglobin-DOT, some groups have observed a decrease of the oxygen saturation (StO2) in the tumor regions [12–14]. In addition, some researchers have described tumor-to-normal contrast in tissue scattering parameter, ascribed to morphologic changes at a cellular level as well as changes in size and density of subcellular organelles in tumors [15,16]. However, it has been argued that, hemoglobin-DOT with no a priori information, termed standalone hemoglobin-DOT, does not have sufficient sensitivity for early lesion detection, and might be readily corrupted by the breast and its periphery structures, such as the blood vessels, glands, nipple, and chest wall etc., due in part to the moderate tumor-to-normal hemoglobin contrast of about 1.5-2.5 [3], as well as the limited quantitative accuracy and spatial resolution of the inversion [1]. To overcome these intrinsic shortcomings, multi-modal approaches that incorporate the hemoglobin-DOT with other imaging techniques have been intensively investigated, where the feasible-region information provided by the other modalities is normally used to reasonably regularize the inverse problem of the hemoglobin-DOT. Thus far, advances in multi-modal approaches using standard imaging modality of considerably high spatial resolution have been made in three fronts: concurrent optical measurements with MRI [17,18], X-ray mammography [19], and US [20].

DOT with the use of exogenous near-infrared dyes, referred to as fluorescence-DOT, was pioneered by Dr. Chance group for in vivo reconstruction of Indocyanine Green (ICG) uptake in the breast [21,22]. This modality has been attracting considerable attentions for recent years [23,24], in part because of its successes in molecular imaging regime of small animals [25],

```
and in part due to its contrast enhancement mechanism of significantly improving detection sensitivity as well as its potential to simultaneously access tissue functionality and micro-environmental indices such as pO2, pH etc. [26]. Currently, ICG, with its peak excitation and emission wavelengths around 780 nm and 830 nm, respectively, is the only fluorescent agent approved by the Food and Drug Administration (FDA) for human use. Godavarty et al. [27] have demonstrated use of ICG-fluorescence imaging in both canine breast cancers and phantoms. Corlu et al. [23] have demonstrated the first three-dimensional (3-D) ICG-fluorescence-DOT of in vivo human breast cancer. Other groups have also reported more cases of in vivo fluorescence-DOT for human breast applications [28,29]. These preliminary studies have observed a marked fluorescence contrast between the tumors and normal tissues that originates from the agent accumulation in leaky malignant tissues [30], especially during the late-fluorescence phase [28]. Nevertheless, the characteristics of ICG are suboptimal for fluorescence-DOT of human breast: the low quantum yield often leads to increasingly long measuring time and auto-fluorescence that is clinically difficult to suppress - a challenge to instrument sensitivity and to early tumor diagnosis. Accordingly, a novel fluorescent agent with higher quantum yield has been under investigation by Philips since 2002 [24].

While fluorescence-DOT can markedly enhance detection sensitivity of breast tumor imaging and is reliable in tumor localization, it relies on the uptake level of the extrinsic agent into the tissue that is strongly subjected to physiological and physical status of the human body, and lacks in quantitative merits. Therefore, from specificity point of view, hemoglobin-DOT that objectively discloses the pathological and physiological abnormalities of in vivo tissues by measuring its hemodynamic patterns is more preferred for differentiation between benign and malignant lesions as well as for tumor staging. To cope with both the limitations of hemoglobin-DOT in sensitivity and fluorescence-DOT in specificity, the combination of both approaches is pursued, by which the quantitative accuracy of hemoglobin-DOT is improved using the location a priori information provided the fluorescence-DOT, referred to as fluorescence-guided hemoglobin-DOT. Compared with the other multi-modality methods, this hybrid scheme of fluorescence and hemoglobin imaging has the potential of cost-effectiveness of instrumentation and an enhanced capability of detecting early lesions.

For the above aim, we herein have developed a combined TD fluorescence- and hemoglobin-DOT methodology, including a 32-channel time-resolved system based on the time-correlated single photon counting (TCSPC) technique and a fluorescence-guided hemoglobin image reconstruction scheme within the framework of the generalized pulse spectrum technique (GPST). The performance of the methodology was evaluated using phantom experiments. We first assessed the linearity and sensitivity of the system responses to optical and fluorescence inclusions of various concentrations using two-dimensional (2-D) scheme, respectively. Then, the feasibility of 3-D fluorescence- and hemoglobin-DOT was justified using a realistic layered 2-D measuring and full 3-D reconstructing strategy. Finally, we performed a fluorescence-guided hemoglobin-DOT experiment and demonstrated that the reconstruction accuracy in hemoglobin-DOT could be significantly improved by the regularization of a priori fluorescence location.

2. Methods

2.1. Instrumentation

Figure 1 illustrates the schematic of the developed hybrid TD fluorescence- and hemoglobin-DOT system. In this setup, a picosecond (ps) pulsed laser system with a multi-channel controller (PDL-828“Sepia II”, PicoQuant, Germany) driving 2 fiber-coupled laser diode (LD) heads (LDH-P-780 and LDH-P-830, PicoQuant, Germany) at wavelengths of \( \lambda_1 = 780 \text{ nm} \) and \( \lambda_2 = 830 \text{ nm} \) is employed as the source. The two LD-wavelengths are chosen according to the peak excitation and emission wavelengths of ICG agent, respectively, as well as to be within the optical spectral range optimized for quantifying the tissue hemoglobin indexes. The controller is programmed to drive the two LD-heads in a temporally
Fig. 1. Schematic diagram of the developed hybrid TD fluorescence- and hemoglobin-DOT system for breast tumor diagnosis. The system uses two ps-pulsed LDs at 780 nm and 830 nm driven in a temporally multiplexing mode with an interval of 25 ns for the light delivery, and works with 4 PMT-TCSPC channels in a tandem serial-to-parallel scheme for the time-resolved detection.

multiplexing mode with the interval of 25 ns, delivering a sequence of the alternatively pulsed \( \lambda_1 \) - and \( \lambda_2 \)-wavelength light with a repetition rate of 20 MHz, a power of >5 mW, and a pulse width of <70 ps for each wavelength. The two LD-beams are combined using a custom-made wave-division-multiplexer (OZ Optics, Canada) and delivered to the input fiber of a programmable \( 1 \times 32 \)-fiber-optic-switch (FSW1 \( 1 \times 32 \)-MM-C, Gulin Institute of Optical Communications, China) that then sequentially directs the light to each of \( S (=32) \) source-fibers with a core diameter of 62.5 µm and a numerical aperture \((NA) = 0.37\). For the signal collection, \( D (=32) \) detection-fibers of 1000 µm in core diameter and \( NA = 0.22\), are connected to four \( 8 \times 1\)-fiber-optic-switches (FSW1 \( 8 \times 1 \)-MM-S, Gulin Institute of Optical Communications, China), whose 4 outputs are collimated and then routed to 4 PMT photon counting heads (PMC-100, Becker & Hickl, Germany) coupled to a 4-channel TCSPC module (SPC-134, Becker & Hickl, Germany) to acquire the 2-wavelength outward time-resolved flux, i.e. the temporal point spread function (TPSF), at 4 detector-fiber locations in parallel. Prior to each PMT detector is mounted a 6-hole motorized filter-wheel (FW102B, Thorlabs, USA) that houses 4 neutral density (ND) filters with the optical density \((OD) = 1.0\) to 4.0, respectively, and a stack of a band-pass (BP) interference filter (ICG-A Emitter, Semrock, USA) with transmission of >93% over the bandwidth from 813.5 nm to 850.5 nm and the rejection ratio of \( OD = 6.0 \) at 20 nm away from the central wavelength of 8.32 nm and a long-pass (LP) absorption-layered interference filter (GCC-202006, Daheng Optics, China) with the transmission of >85% at \( \lambda = 820 \) nm and the rejection ratio of \( OD > 4.0 \) at \( \lambda < 785 \) nm. The physical temporal-resolution of the whole system is about 250–320 ps, primarily depending on the transient time spread of the PMT heads that is about 180 ps and the pulse width of the LDs. The output-ends of the 32 source-fibers and the input-ends of the 32 detection-fibers are paired with a side-by-side alignment to construct 32 bifurcated-fiber optodes.

As depicted in Fig. 2, a cylindrical chamber with an inner diameter of 100 mm and an inner height of 80 mm was fabricated, within which the breast is freely suspended in the matching fluid. For the optode-settings, 64 holes were drilled perpendicular to the wall of the chamber at 4 imaging planes (named Imaging Plane #1-#4) that are evenly distributed along the height at an interval of 16 mm, with the 16 holes at each plane equally-spaced. The inner walls of the chambers are blackened to suppress the light reflection.

2.2. Measurement protocols

The fluorescence emission and excitation signals are detected with and without the fluorescence filter-stacks in place, respectively. For the TCSPC module, the time-to-amplitude
converter (TAC) range is set to 70 ns and the biased amplifier gain to 1. Since the TAC output is resolved into 4096 time-bins by a 12-bit analog-to-digital converter, the above settings lead to a bin-width, \( i.e., \) sampling time interval, of \( \Delta t \approx 17.1 \text{ ps} \).

Prior to the measurements, the realistic transmittances of the ND filters and fluorescence filter stacks in the 4 filter wheels are measured for the data calibration. For the former one a relative calibration method is adopted to preclude the influence of the dark counts on the measurement accuracy, where the ratio of the transmittance of the \( OD = n \) filter to that of the \( OD = n - 1 \) filter is measured for the 4 filter wheels all under the proper photon counts, and then the absolute transmittance of each filter calculated. The latter one uses the LD at \( \lambda \) -wavelength as the source to obtain the transmittances of the fluorescence filter stacks in the 4 filter wheels, at the maximum ICG emission wavelength.

The proposed ratio-formulated schemes of fluorescence- and hemoglobin-DOT, as will be described later, does not require the determination of the time-origins of the system channels, yet, it is still significantly beneficial to analyzing the sequencing of the measurements among the channels for an optimal setting of the TCSPC range as well as for reasonably choosing an informative and noise-robust transform-factor pair [31]. We measure approximately the time-origins of the system channels using the double-calibration procedure similar to that proposed by Hillman et al. [32]: (1) a source-calibration that collects the instrumental response functions (IRFs) by the first detector from the \( s \)th source (\( s = 1, 2, \ldots, S \)), with their mean flight time denoted by \( \langle t \rangle_{s,1} \); (2) a detector-calibration that acquires the IRFs by the \( d \)th detector from the first source, with \( \langle t \rangle_{1,d} \) representing their mean flight time. With these two calibrating steps involving only 64 IRF measurements, the mean flight time of the IRFs for any source-detector pairs that is taken as the time-origin is calculated with a simple arithmetic: \( \langle t \rangle_{s,d} = \langle t \rangle_{s,1} + \langle t \rangle_{1,d} - \langle t \rangle_{1,1} \). The calibration method and tool designed for the time-origin determination has been described by Gao et al. [31].

The workflow of the complete hybrid fluorescence- and hemoglobin-DOT process is shown in Fig. 3. Rigorously, it consists of five phases, referred to as ND-filter determination, dual-wavelength hemoglobin-DOT, fluorescence-DOT, extraction of the region of interest (ROI), and fluorescence-guided hemoglobin-DOT:

**Phase #1**: a dual-wavelength DOT measurement is performed in the temporal multiplexing mode, as outlined in Sec. 2.1, with the matching fluid filled to adjust the ND filters for each of the used source-detector pairs so that the photon counting rate reaches the maximum below 1/20 of the LD repetition rate, \( i.e., \) 1 MHz. This upper limit for the photon-counting-rate can reliably prevents the “pile-up” effect of the TCSPC system during the successive hemoglobin-DOT measurement [33]. The phase ends with a record of the OD.
Phase #1: ND-filter determination

Reference-DOT data

Phase #2: Dual-wavelength hemoglobin-DOT

Hemoglobin-DOT data at $\lambda_1$

Phase #3: Fluorescence-DOT

Yield Image

Images of the optical properties at $\lambda_1$

Phase #4: Extraction of the region of interest (ROI)

ROI mask

Phase #5: Fluorescence-guided hemoglobin-DOT

Fig. 3. Workflow of a complete hybrid fluorescence- and hemoglobin-DOT process, where the supporting information required by a phase is explained beside the associated arrows.

indices of the ND filters, and a set of TPSFs simultaneously acquired at $\lambda_1$ and $\lambda_2$ for all the source-detector pairs, referred to as the reference-DOT data set.

Phase #2: With the subject in place and the ND filters fixed, a hemoglobin-DOT measurement is performed in the temporal multiplexing mode, to simultaneously acquire the TPSF data sets (hemoglobin data set) at the two wavelengths, as shown in Fig. 4(a). From the ratio of the hemoglobin (task) data set to the reference one, the background absorption and scattering images of the subject at the dual-wavelengths are then reconstructed for the successive fluorescence-DOT imaging.

Phase #3: the fluorescence-DOT measurement is performed in the similar way to that in Phase #2, except that the $\lambda_2$-LD is powered off and the fluorescence filter-stacks are in place. The $\lambda_1$-LD serves as the excitation source to the ICG targets and the fluorescence TPSF data set around the peak emission-wavelength of ICG ($\lambda_2$) is acquired, as shown in Fig. 4(b). The obtained fluorescence-DOT data and the hemoglobin-DOT data at $\lambda_1$ acquired in Phase #2 are used to calculate the Born-ratio with considering the actual integration times, the ODs of the used ND filters as well as the transmittances of the fluorescence BP-filter-stacks in the TCSPC channels, and then the yield- and lifetime-images of the fluorescence-DOT are reconstructed with the support of the background optical properties at $\lambda_2$.

Phase #4: the fluorescence-yield image is segmented to extract the ROI.

Fig. 4. Normalized TPSFs acquired in parallel by Detection-fiber #5-#8 for the illumination of Source-fiber #1 in (a) 2-D hemoglobin-DOT experiment with the reference scenario, as described in Sec. 3.1, and (b) 2-D fluorescence-DOT experiment with a target ICG concentration of 250 nM, as described in Sec. 3.2.
Phase #5: the image reconstruction of the dual-wavelength DOT is re-performed with the regularization of the ROI priors, starting from the images of the optical properties obtained from the dual-wavelength hemoglobin-DOT in Phase #2. The same task-to-reference ratio data-set as in Phase #2 is used.

According to the optode configuration of the cylindrical imaging chamber, the measurement can be performed in a 2-D way, where, for each source illumination on an imaging plane, two groups of the 4 detection fibers on the same plane are switched successively to the 4 parallel TCSPC channels for the photon collection. With a TCSPC integration time of $T_i$, this configuration leads to a maximum data-acquisition time of about $32 \times 2 \times T_i$. The measured data set can then be organized either in a 2-D way for reconstructing the sliced images of the fluorescent and optical properties on the 4 imaging planes, or in a full 3-D way for producing the volume images within the whole chamber. The whole setup is placed in a dark box to shield the ambient light.

A counting threshold strategy is adopted to further optimize the measurement performance, where a low limit is set to the counted photon number so that the 4-channel parallel TCSPC process continues until the low limit is reached in the channel with the minimum photon-counting-rate or the integration time spent. Then the actual integration time used by this 4-channel parallel detection is recorded for the later data calibration. By deploying the 4 optodes for each parallel detection group on the geometrically symmetric sites, this strategy can essentially reduce the data-acquisition time.

2.3. Data preprocessing

Prior to the image reconstruction of the fluorescence- and hemoglobin-DOT, two procedures are required for the pre-processing of the raw data. Firstly, the raw data is filtered by a 5-point median filter following by a 5-point average filter, and the dark count of each measurement, which is calculated as the mean of a TPSF section before the calibrated time origin, is deducted the filtered curves. Then, for fluorescence-DOT, the ratio of the Laplace-transformed ICG-fluorescence TPSF signal, $\hat{I}_{ICG}(\xi_d, \zeta_s, \beta)$, to the transformed excitation one, $\hat{I}_h(\xi_d, \zeta_s, \beta)$, referred to as the Born ratio [34], is calculated as the data type

$$\hat{I}_{ab}(\xi_d, \zeta_s, \beta) = \Theta(d,s) \frac{\hat{I}_{ICG}(\xi_d, \zeta_s, \beta)}{\hat{I}_h(\xi_d, \zeta_s, \beta)}$$

where $\zeta_s$ and $\xi_d$ denote the locations of the $s$th source and the $d$th detector, respectively; $\beta$ is the transform-factor; $\Theta(d,s)$ is the gain factor of the PMT-TCSPC channel associated with the $s$th source and the $d$th detector, determined by the actual integration time for hemoglobin-DOT $T_h(d,s)$, and the used transmittance of the ND filter $\Gamma_{ND}(d,s)$, as well as the actual integration time for the fluorescence-DOT $T_m(d,s)$ and the used transmittance of the fluorescence BP-filter stacks $\Gamma_{BP}(d,s)$

$$\Theta(d,s) = \frac{T_h(d,s) \cdot \Gamma_{ND}(d,s)}{T_m(d,s) \cdot \Gamma_{BP}(d,s)}$$

For hemoglobin-DOT, the ratio of the Laplace-transformed TPSF for the hemoglobin-DOT, $\hat{I}_{h}^{(\text{hp})}(\xi_d, \zeta_s, \beta)$, to that for the reference-DOT, $\hat{I}_h^{(\text{h})}(\xi_d, \zeta_s, \beta)$, referred to as the task-to-reference ratio, is used as the data type

$$\Theta(d,s) = \frac{T_h(d,s) \cdot \Gamma_{ND}(d,s)}{T_m(d,s) \cdot \Gamma_{BP}(d,s)}$$

#182189 - $15.00 USD Received 21 Dec 2012; revised 21 Jan 2013; accepted 23 Jan 2013; published 25 Jan 2013
(C) 2013 OSA 1 February 2013 / Vol. 4, No. 2 / BIOMEDICAL OPTICS EXPRESS 338
\[
\hat{I}_h(\xi, \zeta, \beta) = \frac{\hat{I}^{(m)}_h(\xi, \zeta, \beta)}{\hat{I}^{(ref)}_h(\xi, \zeta, \beta)}
\]  

(3)

Since the ND filters are fixed and the same integration time is used for the reference- and hemoglobin-DOT measurements, the above task-to-reference ratio, analogous to the Born ratio in fluorescence-DOT, can substantially cancel out the influence of the IRFs of the TCSPC channels on the reconstruction accuracy and mitigate any inconsistencies between the measured data and their model-predicted noiseless counterpart at high signal-to-noise ratio (SNR) [31].

2.4. Image reconstruction algorithms

For TD hemoglobin-DOT regime with incidence of a fiber-coupled ps-pulsed laser, as adopted in the proposed system, the physical process of the photon migration in tissue domain \( \Omega \) is commonly described by the time-dependent diffusion equation (DE) with an isotropic point source in both time and space (\( \delta \)-source), and its Laplace-transformed form can be written as [35,36]

\[
\left[ -\nabla \cdot \kappa (r, \lambda_i) \nabla + \left( \mu_a (r, \lambda_i) c + \beta \right) \right] \Phi_{h_i}(r, \xi, \zeta, \beta) = \delta_\lambda (r - \xi), \quad i=1, 2
\]  

(4)

where \( \xi \) is now located at a depth of one scattering length beneath the surface; \( \mu_a (r, \lambda_i) \) is the wavelength-dependent absorption, and \( \kappa (r, \lambda_i) = \frac{1}{\left[ \frac{3}{4} \mu'_a (r, \lambda_i) \right] } \) is the diffusion coefficients with \( \mu'_a (r, \lambda_i) \) being the reduced scattering coefficient [37]; \( \Phi_{h_i}(r, \xi, \zeta, \beta) \) is the Laplace transform of the time-dependent photon-density \( \Phi_{h_i}(r, \xi, \zeta, t) \), in response to the \( \lambda_i \)-wavelength \( \delta \)-source at \( \zeta \); \( c \) is the velocity of light in tissue.

In TD fluorescence-DOT, migration of the excitation photons follows the similar way as in TD hemoglobin-DOT, with the exception that the absorption coefficient is constituted of the intrinsic portion contributed by the tissue chromophores, and the extrinsic one originated from the injected ICG-agent, i.e., \( \mu_a (r, \lambda_{ICG}) = \mu_{a,intrinsic} (r, \lambda_i) + \mu_{ICG} (r, \lambda_{ICG}) \), while the Laplace-transformed fluorescence photon density, \( \Phi_{ICG}(r, \xi, \zeta, \beta) \), is governed by the following coupled diffusion equation [38]

\[
\left[ -\nabla \cdot \kappa (r, \lambda_{ICG}) \nabla + \left( \mu_a (r, \lambda_{ICG}) c + \beta \right) \right] \Phi_{ICG}(r, \xi, \zeta, \beta) = c \Phi_{ICG}(r, \xi, \zeta, \beta) \frac{y_{ICG}(r, \lambda_{ICG})}{1 + \beta r_{ICG}(r, \lambda_{ICG})}
\]  

(5)

where \( y_{ICG}(r, \lambda_{ICG}) = \eta_{ICG}(r, \lambda_{ICG}) \mu_{ICG} (r, \lambda_{ICG}) \) is referred to as the yield of the ICG-agent, and \( \eta_{ICG}(r, \lambda_{ICG}) \) and \( r_{ICG}(r, \lambda_{ICG}) \) are the quantum efficiency and lifetime of the agent, respectively; \( c \) is the velocity of light in tissue.

The image reconstruction in hemoglobin-DOT uses a modified GPST-based nonlinear scheme. In this approach, a linearized formulation with regard to recovering the intermediate image, \( P_h^{(k)} (r, \beta) = c \delta \mu_a^{(k)} (r, \lambda_i) + \mu_a^{(k)} (r, \lambda_i) c + \beta \right] \delta \mu_a^{(k)} (r, \lambda_i) \), is firstly given by the iteration [35]

\[
I_h(\xi, \zeta, \beta, \mu_a^{(k)}, \mu_a^{(k)}) = \int_{\Omega} G_h(\xi, \zeta, \beta, \mu_a^{(k)}, \mu_a^{(k)}) \Phi_{h_i}(r, \xi, \zeta, \beta, \mu_a^{(k)}, \mu_a^{(k)}) P_h^{(k)} (r, \beta) d\mathbf{r}
\]  

(6)
where $\mu_{a}^{(k)}$ and $\mu_{s}^{(k)}$ are the absorption and reduced scattering coefficients at the $k$th iteration, respectively, with $\mu_{a}^{(0)} = \mu_{a}^{(0)}$ and $\mu_{s}^{(0)} = \mu_{s}^{(0)}$ denoting the mean background absorption and reduced scattering coefficients, and $\delta \mu_{a}^{(k)}$ and $\delta \mu_{s}^{(k)}$ the perturbations to $\mu_{a}^{(k)}$ and $\mu_{s}^{(k)}$; $I_{a}(\xi_{d}, \zeta, \beta, \mu_{a}^{(k)}, \mu_{s}^{(k)})$ is the model-predicted flux of the hemoglobin-DOT at the $d$th detection site $\xi_{d}$ and for the $s$th source irradiation at $\zeta_{s}$; $\Phi_{a}(r, \zeta, \beta, \mu_{a}^{(k)}, \mu_{s}^{(k)})$ is the photon density for the $s$th source irradiation at $\zeta_{s}$; $G_{a}(\xi_{d}, r, \beta, \mu_{a}^{(k)}, \mu_{s}^{(k)})$ is the photon density for the $s$th source irradiation at $\zeta_{s}$; $\Phi_{v}(\zeta_{s}, \beta, \mu_{a}^{(k)}, \mu_{s}^{(k)})$ the mean background absorption and reduced scattering coefficients, and $\delta \mu_{a}^{(k)}$ and $\delta \mu_{s}^{(k)}$ the perturbations to $\mu_{a}^{(k)}$ and $\mu_{s}^{(k)}$; $I_{a}(\xi_{d}, \zeta, \beta, \mu_{a}^{(k)}, \mu_{s}^{(k)})$ is the model-predicted flux of the hemoglobin-DOT at the $d$th detection site $\xi_{d}$ and for the $s$th source irradiation at $\zeta_{s}$; $\Phi_{a}(r, \zeta, \beta, \mu_{a}^{(k)}, \mu_{s}^{(k)})$ is the photon density for the $s$th source irradiation at $\zeta_{s}$; $G_{a}(\xi_{d}, r, \beta, \mu_{a}^{(k)}, \mu_{s}^{(k)})$ is the photon density for the $s$th source irradiation at $\zeta_{s}$.

Then, $P_{a}^{(k)}(r, \beta)$ is reconstructed by inverting Eq. (6) with two distinct $\beta$-wavelength dependent transforming-factors, $\beta_{a}(\lambda)$ and $\beta_{s}(\lambda)$, to separate the perturbation quantities, $\delta \mu_{a}^{(k)}(r, \lambda)$ and $\delta \mu_{s}^{(k)}(r, \lambda)$

$$
\left[ \begin{array}{l}
\delta \mu_{a}^{(k)}(r, \lambda) \\
\delta \mu_{s}^{(k)}(r, \lambda)
\end{array} \right] = \left[ \begin{array}{l}
\mu_{a}^{(k)}(r, \lambda) + \beta_{a}(\lambda)
\mu_{s}^{(k)}(r, \lambda) + \beta_{s}(\lambda)
\end{array} \right] P_{a}^{(k)}(r, \beta, \lambda) - \left[ \begin{array}{l}
\mu_{a}^{(k)}(r, \lambda) + \beta_{a}(\lambda)
\mu_{s}^{(k)}(r, \lambda) + \beta_{s}(\lambda)
\end{array} \right] P_{a}^{(k)}(r, \beta, \lambda) \left[ \begin{array}{c}
\beta_{a}(\lambda) - \beta_{a}(\lambda)
\beta_{s}(\lambda) - \beta_{s}(\lambda)
\end{array} \right]
\right]
$$

For TD fluorescence-DOT, a GPST-based linear scheme can be directly established with regard to the intermediate quantity, $F_{ICG}(r, \beta) = y_{ICG}(r)[1 + \beta \tau_{ICG}(r)]$, on the assumption that the influence of the agent absorption on the photon migration can be neglected [38]

$$
\hat{I}_{ab}(\xi_{d}, \zeta, \beta) = \int cG_{a}(\xi_{d}, r, \beta)\Phi_{a}(r, \zeta, \beta)F_{ICG}(r, \beta)dr
$$

where $I_{ab}(\xi_{d}, \zeta, \beta)$ is the boundary flux of excitation light, i.e., the $\lambda$-wavelength flux in the hemoglobin-DOT, at the detection site at $\xi_{d}$ and for the source at $\zeta_{s}$; $\Phi_{a}(r, \zeta, \beta)$ is the $\lambda$-wavelength photon density for the source at $\zeta_{s}$, while $G_{a}(\xi_{d}, r, \beta)$ the $\lambda$-wavelength flux-regarding Green’s function for the detector at $\xi_{d}$.

Analogously, the yield and lifetime images of the ICG agent can be separated through reconstructing $F_{ICG}(r, \beta)$ with a pair of the transforming-factors

$$
\left[ \begin{array}{c}
y_{ICG}(r) \\
\tau_{ICG}(r)
\end{array} \right] = \left[ \begin{array}{c}
\beta_{a}(\lambda) - \beta_{a}(\lambda)
\beta_{s}(\lambda) - \beta_{s}(\lambda)
\end{array} \right] P_{a}^{(k)}(r, \beta_{a}(\lambda)) - \left[ \begin{array}{c}
\beta_{a}(\lambda) - \beta_{a}(\lambda)
\beta_{s}(\lambda) - \beta_{s}(\lambda)
\end{array} \right] P_{a}^{(k)}(r, \beta_{s}(\lambda)) \left[ \begin{array}{c}
\beta_{a}(\lambda) - \beta_{a}(\lambda)
\beta_{s}(\lambda) - \beta_{s}(\lambda)
\end{array} \right]
$$

For implementation, a finite-element-method (FEM) is developed under the Robin boundary condition for numerically solving the forward problems defined in Eqs. (4) and (5), from which the outward flux is proportionally associated with the boundary photon density according to the Fick’s law [39]. With the same mesh as in the FEM forward solver, Eqs. (6) and (8) are discretized into linear systems, and solved using the algebraic reconstruction technique (ART) with the random projection access order [40], respectively, for its modest
memory requirement and the attained high robustness for large-scale, ill-posed linear inversion.

As aforementioned, the fluorescence-DOT can provide a significantly enhanced contrast of 2–4 times over the hemoglobin-DOT. It is possible to segment the high-contrast yield image to extract the prior information on the ROI geometry, which can then be used to restrain the image reconstruction of the hemoglobin-DOT from the whole-region to one according to ROI. This “hard prior” scheme of the fluorescence-guided hemoglobin-DOT dramatically reduces the total number of the unknowns to be reconstructed, making the inverse problem better posed [41].

For the hemoglobin-DOT with the “hard prior” regularization strategy, the iterative linearization scheme corresponding to Eq. (6) can be expressed by the FEM-discretization in the following matrix notation

\[
\begin{align*}
\mathbf{J}^{(k)} &= \mathbf{J}^{(k)} \mathbf{T} \\
\mathbf{M}^{(k)} &= \mathbf{J}^{(k)} \mathbf{P}^{(k)} \\
\mathbf{P}^{(k+1)} &= \mathbf{T} \mathbf{P}^{(k)} + \mathbf{P}^{(k)}
\end{align*}
\]

where \( \mathbf{P}^{(k)} \) and \( \mathbf{P}^{(k)} \) are the column vectors that numerate the optical parameter \( P^{(k)}_a(\mathbf{r}, \beta) \) at the \( N \) global nodes and \( NR \) local ROI nodes, respectively; \( \mathbf{M}^{(k)} \) numerates \( I^a\Delta^a (\xi_j, \zeta_j, \beta, \mu^a_\alpha, \mu^a_\beta) - \tilde{I}^a\Delta^a (\xi_j, \zeta_j, \beta, \mu^a_\alpha, \mu^a_\beta) \) for all the source-detector combinations; \( \mathbf{J}^{(k)} \) and \( \tilde{\mathbf{J}}^{(k)} \) are the global and ROI Jacobian matrix at the \( k \)th iteration, respectively, with its computation found in [35,38] for hemoglobin- and fluorescence-DOT, respectively; \( \mathbf{T} \) is an association matrix that maps the locally indexed ROI nodes to the global ones

\[
\mathbf{T} = \begin{bmatrix}
T(\mathbf{r}_1, \mathbf{r}_1') & T(\mathbf{r}_1, \mathbf{r}_2') & \cdots & T(\mathbf{r}_1, \mathbf{r}_{NR}') \\
T(\mathbf{r}_2, \mathbf{r}_1') & T(\mathbf{r}_2, \mathbf{r}_2') & \cdots & T(\mathbf{r}_2, \mathbf{r}_{NR}') \\
\vdots & \vdots & \ddots & \vdots \\
T(\mathbf{r}_N, \mathbf{r}_1') & T(\mathbf{r}_N, \mathbf{r}_2') & \cdots & T(\mathbf{r}_N, \mathbf{r}_{NR}')
\end{bmatrix}, \quad \text{where} \quad T(\mathbf{r}_i, \mathbf{r}_j') = \begin{cases}
1, & \mathbf{r}_i = \mathbf{r}_j' \\
0, & \mathbf{r}_i \neq \mathbf{r}_j'
\end{cases}
\]

where \( \mathbf{r}_i \) and \( \mathbf{r}_j' \) are the positional vectors of the \( i \)th global node and the \( j \)th ROI node, respectively. Owing to the high contrast nature of the fluorescence targets, ROIs can be simply extracted by segmenting the reconstructed ICG-yield image with a threshold

\[
Y_{th} = \overline{Y}_{ICG} + \rho \sigma_{ICG}
\]

where \( \overline{Y}_{ICG} = \frac{1}{N} \sum_{i=1}^{N} y_{ICG}(\mathbf{r}_i) \) and \( \sigma_{ICG} = \sqrt{\frac{1}{N-1} \sum_{i=1}^{N} [y_{ICG}(\mathbf{r}_i) - \overline{Y}_{ICG}]^2} \) are the mean and variance of the yield image, respectively; \( \rho \) is an attending factor that is empirically selected at first and then can be automatically optimized following a criterion

\[
\min_{\rho} \left\{ \sum_{\mathbf{r}_i \in \text{ROI}} \left[ y_{ICG}(\mathbf{r}_i) - \max_{\mathbf{r}_j} y_{ICG}(\mathbf{r}_j) \right] + \sum_{\mathbf{r}_i \notin \text{ROI}} \left[ y_{ICG}(\mathbf{r}_i) - \min_{\mathbf{r}_j} y_{ICG}(\mathbf{r}_j) \right] \right\}
\]

Since the derivative of the objective function is analytically unavailable with regard to \( \rho \), a direct line-search strategy, such as the Powell method (i.e., the second-order interpolation method), can be used to solve the above one-dimensional optimization issue for its computational simplicity and reasonable convergence-rate [42].
3. Experiments

For experimental validations of the methods, a cylindrical solid phantom with a length of $L = 100$ mm and a radius of $R = 40$ mm was made of epoxy resin (Araldite GY257, Huntsman, USA) mixed with TiO$_2$ particles (Ti-Pure R-902+, Dupont, USA) and NIR dye (NIRD-09, Liaonin Huahao-Lanfan Chem.Tech. Co., Ltd., China), as shown in Fig. 5(a). The background optical properties of the phantom were measured to be $\mu_a^{(b)}/\mu_s^{(b)}(\lambda) = 0.0033/1.09$ mm$^{-1}$ and $\mu_a^{(b)}/\mu_s^{(b)}(\lambda) = 0.0029/0.87$ mm$^{-1}$, using a time-resolved spectroscopic (TRS) technique [43]. These values approximate to those of the normal breast tissue published in literatures [44]. For emulating breast lesions, two cylindrical holes, referred to as Hole #1 and Hole #2, with the same radius of $R_1 = R_2 = 7.5$ mm and different heights of $L_1 = 70$ mm and $L_2 = 65$ mm were drilled at $(r_1 = 15$ mm, $\theta_1 = 0^\circ)$ and $(r_2 = 20$ mm, $\theta_2 = 135^\circ)$ along the z-axis, respectively. For the convenience of visualization, two sets of Cartesian coordinates are set with X- and X'-axes intersecting the axes of Hole #1 and Hole #2, respectively. To achieve a 2-D scenario, Hole #1 were fully filled with the contrasting solution, and Hole #2 inserted with a homogeneous rod that has the same optical properties as the background, while, for 3-D case, the two holes were partially filled and then inserted the homogeneous rods to form two targets with lengths of $L'_1 \approx 16$ mm and $L'_2 \approx 16$ mm at different center heights of $Z = 43$ mm and $Z = 53$ mm, respectively. Figure 5(b) illustrates the photograph of the 2-D experimental setup, where 16 optodes were placed at Imaging Plane # 4, i.e., $Z = 64$ mm, and Fig. 5(c) demonstrates the 3-D optode configuration, where all the 32 optodes were installed on the 4 imaging planes with each holding 8 located at $Z = 16$, 32, 48 and 64 mm, respectively, as indicated in Fig. 5(a).

We first performed 2-wavelength hemoglobin-DOT and ICG fluorescence-DOT experiments in the 2-D geometry with emphasis on their sensitivity, and then evaluated the efficacy of the fluorescence-guided hemoglobin-DOT scheme for improving the quantitative performance of the DOT image reconstruction, using the 3-D geometry.
3.1. Hemoglobin-DOT

The optical-contrasting solution was prepared from mixing Intralipid-10% and India-ink with their concentrations controlled for the specific absorption and scattering contrasts in this study, and its optical properties determined by the TRS method. The experiments were performed for the 2-D scenario, where the absorption coefficient of the target was varied with the values of $\mu_a^{(T)} \approx 0.012, 0.010, 0.008, 0.006 \text{ mm}^{-1}$ while the scattering coefficient was kept at a constant of $\mu_s^{(T)} \approx 2.0 \text{ mm}^{-1}$.

After performing Phase #1 and #2 in the workflow described in Sec. 2.2, we firstly acquired the dual-wavelength reference-DOT measurement and then the hemoglobin-DOT (target) measurement, from which the ratio of the target data to the reference one finally is calculated. Because of the possibility of the failure of the diffusion approximation for the source-detector pairs with short spacing as well as of the influence of the source reflection from the inner wall of the chamber on the closely-located detectors, the data from the 9 detectors that are opposite to the each illuminating source were used for the image reconstruction, leading to a $16 \times 9$-dimensional data set for each of the wavelengths. With an integration time of 10 s and a low counting limit of $10^4$, one acquisition takes a maximum of 640 s, ignoring the time for the fiber switching.

The nonlinear reconstruction process employed a 2-D mesh of 2400 triangular elements connected at $N = 1261$ nodes, and was terminated after 20 iterations after which no evident improvement in the measurement error (i.e., the difference between the measured data and the calculated one) would be further achieved. The $\lambda_i$-wavelength transform-factors are empirically set to $\beta_i(\lambda_i) = \pm 0.25 \mu_a^{(0)}(\lambda_i) e$. To suppress the artifacts, a median filter operating on the adjacent nodes was employed after each updating stage. Figure 6(a) illustrates the 2-D absorption ($\mu_a$)- and scattering ($\mu_s$)-images reconstructed at 780 nm and 830 nm, respectively, with a series of the aforementioned target contrasts, and Fig. 6(b) shows their profiles along the X-axis, referred to as the X-profiles.

Fig. 6. 2-D standalone hemoglobin-DOT reconstructions of a single target with varying target absorption coefficient of $\mu_a^{(T)} \approx 0.012, 0.010, 0.008, \text{ and } 0.006 \text{ mm}^{-1}$, but a constant target scattering coefficient of $\mu_s^{(T)} \approx 2.0 \text{ mm}^{-1}$: (a) The $\mu_a$- and $\mu_s$-images reconstructed at 780-nm and 830-nm wavelengths, respectively; (b) the X-profiles in the $\mu_a$- and $\mu_s$-images at
both wavelengths, respectively. The circles in the images indicate the correct position and size of the target.

3.2. Fluorescence-DOT

The performance of fluorescence-DOT was experimentally assessed with the 2-D optode configuration. To mimic the fluorescing targets, a mixture solution of Intralipid-1% and ICG-dye (Cardiogreen-I2633, Sigma-Aldrich, USA) was made, with the controlled ICG concentrations of 250 nM, 125 nM, 62.5 nM, and 30 nM, leading to a decreasing fluorescent yield of $y_{ICG}^{(r)} = 1.2 \times 10^{-4}$ mm$^{-1}$, $6.0 \times 10^{-5}$ mm$^{-1}$, $3.0 \times 10^{-5}$ mm$^{-1}$ and $1.44 \times 10^{-5}$ mm$^{-1}$, respectively, and with a Intralipid concentration for $\beta_x^{(r)} (\lambda) = 2.0$ mm$^{-1}$.

![Fig. 7. 2-D fluorescence-DOT reconstructions of the single target with varying ICG-dye concentration of 250 nM, 125 nM, 62.5 nM, and 30 nM: (a) The reconstructed $y_{xy}$ - and $\tau_{xy}$ - images; (b) the X-profiles in the $y_{xy}$ - and $\tau_{xy}$ -images, respectively. The circles in the images indicate the correct position and size of the target.](image)

We performed firstly the reference-DOT measurement and then the fluorescence-DOT one, from which the normalized Born data were calculated. The same 2-D FEM meshing strategy as in the above 2-D hemoglobin-DOT was used for image reconstruction. To achieve a reasonable balance between the measurement SNR and the data-acquisition time, an integration time of $T_i = 20$ s and a low counting limit of $5 \times 10^3$ were set for the fluorescence-DOT measurement, while the same settings as the previous Subsection 3.1 was used for the hemoglobin-DOT measurement. Figure 7 shows the 2-D yield ($y_{xy}$) - and lifetime ($\tau_{ICG}$) -images, as well as their X-profiles reconstructed with a transform-factor pair of $\beta_x = \pm 0.25 \mu_x^{(a)} (\lambda) c$.

3.3. Fluorescence guided hemoglobin-DOT

We used the 3-D configuration to demonstrate the performance of the combined diffuse fluorescence and optical tomography. The inversion process employed a 3-D mesh that contains 19200 hexahedral elements joined at $N = 11349$ nodes, and adopted the same transform-factor pairs as those in the above 2-D assessments for the fluorescence and optical reconstructions, respectively. To simultaneously mimic the endogenous optical contrast due to tumor vasculature, and the exogenous fluorescence contrast due to ICG-administration in breast lesions, we prepared a hybrid fluorescence- and optical-contrasting solution by mixing (diluted) Intralipid-10% with India-ink and ICG with a controlled India-ink concentration of $\sim 0.30$ mg/100 ml and a ICG concentration of $\sim 125$ nM. This recipe finally results in a target optical contrast of $\mu_x^{(r)}/\mu_{ICG}^{(r)} = 0.005 / 1.5$ mm$^{-1}$ (at both $\lambda_1$ - and $\lambda_2$ - wavelengths) and a target fluorescence contrast of $y_{ICG}^{(r)} = 6 \times 10^{-5}$ mm$^{-1}$.
Fig. 8. 3-D fluorescence-DOT reconstruction of the dual targets from the fluorescence-guided hemoglobin-DOT experiment: (a) The reconstructed $y^{ICG}$- and $\tau^{ICG}$-images of the sectional slice at $Z = 38$ mm and the coronal slice at $Y = 0$ mm, all across the center of Target #1, as well as of the sectional slice at $Z = 48$ mm and the coronal slice at $Y = 0$ mm, all across the center of Target #2; (b) the X-profiles in the $y^{ICG}$- and $\tau^{ICG}$-images of the sectional slice at $Z = 38$ mm and the X'-profiles in the $y^{ICG}$- and $\tau^{ICG}$-images of the sectional slice at $Z = 48$ mm, respectively. The circles and rectangles in the images indicate the correct positions and sizes of the targets; (c) The ROI masks of the two sectional slices at $Z = 38$ mm and $Z = 48$ mm, as well as the two coronal slices at $Y = 0$ mm and $Y = 0$ mm. The ROI were extracted from the $y^{ICG}$-image of the 3-D fluorescence-DOT reconstruction with the threshold method using a attending factor of $\rho = 0.86$.

Following Phase #1 to #3 of the workflow described in Fig. 3, we performed firstly the fluorescence-DOT reconstruction, from which the ROIs were then extracted by segmenting the $y^{ICG}$-image by the threshold strategy with a manually optimized $\rho(=0.86)$ from the one-step Powell line search. Figure 8(a) illustrates the reconstructed $y^{ICG}$- and $\tau^{ICG}$-images of the sectional slice at $Z = 38$ mm and coronal slice at $Y = 0$ mm, all across the center of Target #1, as well as of the sectional slice at $Z = 48$ mm and coronal slice at $Y = 0$ mm, all across the center of Target #2. To facilitate the performance evaluation, the X(X')-profiles with respect to the above two sectional slices are plotted vs. the original ones in Fig. 8(b). Figure 8(c) shows the ROI masks of the two sectional and two coronal slices across the target centers. Finally, the hemoglobin-DOT reconstruction was performed with and without the ROI regularization for a comparison, with the reconstructed $\mu_s$- and $\mu'_s$-images of the two sectional and two coronal slices across the target centers shown in Fig. 9(a) and Fig. 9(b), respectively, and the X(X')-profiles with respect to the two sectional slices plotted vs. the original ones in Fig. 9(c).
Fig. 9. (a) 3-D fluorescence-guided and (b) standalone hemoglobin-DOT reconstruction of the dual targets from the fluorescence-guided hemoglobin-DOT experiment: The $\mu$ and $\mu'$ images of two sectional slices at $Z = 38$ mm and $Z = 48$ mm and as well as of two coronal slices at $Y = 0$ mm and $Y' = 0$ mm, reconstructed at 780-nm and 830-nm wavelengths, respectively. The circles and rectangles in the images indicate the original positions and sizes of the targets; (c) The X- and X'-profiles in the $\mu$ and $\mu'$-images of the two sectional slices at $Z = 38$ mm and $Z = 48$ mm, reconstructed from both the 3-D standalone and fluorescence-guided hemoglobin-DOT at 780-nm and 830-nm wavelengths, respectively. The profiles are plotted vs. their respective original ones for a comparison.

4. Discussion

Hemoglobin-DOT reconstruction without the support of a priori information has been reported to be quantitatively size- and contrast-dependent [45]. According to Fig. 6(b) in Subsection 3.1, the $\mu_s$-images reconstructed with the 2-D standalone hemoglobin-DOT exhibit a decreasing quantitative accuracy (defined as a ratio of the peak value in the reconstructed incremental target absorption or scattering to the original one) of 52%(39%), 34%(27%), 25%(21%) and 21%(20%) at $\lambda_1$ ($\lambda_2$)-wavelength for an increasing target absorption contrast of 0.006, 0.008, 0.010 and 0.012 mm$^{-1}$, respectively, demonstrating a saturating trend that is consistent with the previous observations in DOT investigations. In breast tumor diagnosis scenarios, the vascular-abnormality in cancerous regions often provides an absorption contrast of about 1.5-2.5, i.e., $\mu_0^{(r)} = 0.004-0.009$ mm$^{-1}$, depending on
the malignancy stage and the wavelength. This means that the standalone hemoglobin-DOT method can only achieve a quantitative accuracy of about 30%-50%, in terms of the above 2-D absorption reconstructions. For the 3-D reconstructions, as illustrated in Fig. 9, this quantitative accuracy is even further degraded due to smaller target-to-background volume ratio. It is worth noting that, regardless of the target absorption contrast, the reconstructed 2-D $\mu'_{\lambda}$-images achieve a nearly invariant quantitative accuracy around ~40% and ~30% for $\lambda_1$- and $\lambda_2$-wavelengths, respectively, indicating slight crosstalk between the two parameters.

It is well known that the ICG lifetime is about 650 ps in aqueous solution. Our previous investigations have observed a slight drop of the ICG lifetime to about 550 ps in Intralipid solution [31]. Since the background fluorescence yield is essentially null in the used phantom, the assumption on the background lifetime $\tau^{(B)}$ could be arbitrary from mathematical point of view. For the fluorescence-DOT reconstruction here, it seems by our attempts that an empirical value of $\tau^{(B)} = 1000$ ps leads to a robust and acceptable solution. As a result, both the 2-D and 3-D experimental results in Fig. 7 and Fig. 8 show that, despite of evident artifacts observed in the images due to the uncertain target-to-background contrast, the reconstructed lifetime roughly localizes the target but shows smaller value than the expected. In contrast to the lifetime reconstruction, the reconstructed yield-image correctly discloses the target size and location with high sensitivity (down to 30 nM for the 2-D assessments shown in Fig. 7), and thus can be reliably employed for the ROI extraction.

As qualitatively demonstrated in Fig. 9(a) and Fig. 9(b), the images reconstructed in the hemoglobin-DOT has been significantly enhanced by means of the fluorescence-guided strategy. Furthermore, it is quantified from the X-profiles in Fig. 9(c) that, with the "hard-prior" regularization scheme, the quantitative accuracy of the reconstructed target is on average increased from 24.0% to 71.5% for the $\mu_\tau$-reconstruction and from 22.6% to 53.4% for the $\mu'_{\lambda}$-reconstruction, showing about 3:1 and 2.4:1 improvement respectively as compared to the standalone hemoglobin-DOT. Table 1 summarizes the quantitative accuracy of the reconstructed targets. In addition to the improvement in the quantitative accuracy, the ability for the proposed time-domain detection scheme to effectively distinguish between the absorption and scattering further enables optimal selection of a tissue optical-index that is derived from the hemoglobin concentrations and the reduced scattering coefficient to best differentiate the tissue pathological states for tumor diagnosis [7].

| Table 1. Quantitative accuracy of the reconstructed targets: fluorescence-guided hemoglobin-DOT vs. standalone hemoglobin-DOT |
|---------------------------------------------------------------|
| **Target #1 (\(\mu_\tau, \mu'_\lambda\))** | **Target #2 (\(\mu_\tau, \mu'_\lambda\))** |
| $\lambda_1 = 780$ nm | $\lambda_1 = 780$ nm |
| $\lambda_2 = 830$ nm | $\lambda_2 = 830$ nm |
| Standalone | (23.5%, 31.0%) | (19.1%, 17.2%) |
| Fluo. guided | (76.6%, 86.2%) | (66.7%, 47.8%) |
| | (66.7%, 47.8%) | (75.3%, 52.3%) |
| | (67.5%, 27.4%) | (67.5%, 27.4%) |

With an aim at demonstrating the efficacy of fluorescence-guided hemoglobin-DOT, we have just explored in the study the "hard-prior" regularization scheme for improving hemoglobin-DOT performance, which is intuitive to implement. The notable advantage of using a hard prior is that the total number of unknowns is dramatically reduced, making the inversion better-posed and thereby significantly enhancing the reconstruction quality, as shown in the above experiments. However, two major drawbacks of such a scheme are that its stability is critically dependent on the accuracy of the ROI a priori and the background optical structure (i.e., the absolute optical property distributions) is needed to prevent the reconstruction from being biased. Although the reliable ROI a priori is normally obtainable in phantom experiments thanks to the extremely high target-to-background yield contrast and the preset background fluorescence properties, this condition might not be assured in realistic situations due to the incomplete clearance and non-specific binding effect of the administrated agent [46]. Therefore, further fundamental studies are necessary for developing a "soft-prior"
regularization scheme for fluorescence-guided hemoglobin-DOT that is much more robust and unbiased in the presence of uncertainty in prior information [40]. In order to achieve acquisition of the background optical structure, some strategies must be worked out to cope with the highly ill-posed nature of the conventional pixel-based DOT, which has been used so far to map only the incremental optical properties over some baseline (reference-status), as noted in the context. It has been shown that, with the support of multi-modal structural images, the so-called “coarse-grained” DOT methods that are based on either region labeling or shape parameterization could provide promising solutions to the issue [47,48].

Comparing the images reconstructed in the 2-D imaging geometry (as shown in Fig. 6 and Fig. 7), it seems that the spatial resolution of fluorescence-DOT, even at the highest ICG concentration, is somewhat inferior to that of hemoglobin-DOT. This degradation in image quality might be primarily due to both the intrinsic low-SNR nature of the fluorescence signals and the measurement inaccuracy of the gain factors $\theta(d,s)$ in the Born-ratio data set. Nevertheless, fluorescence-DOT working with effectively-enhanced tumor-to-normal contrast would in principle provide reliable tumor localization for quantitative improvement of hemoglobin-DOT, provided the system could be well calibrated and the measurement phase be optimized.

5. Conclusions

We have presented a combined time-domain hemoglobin- and fluorescence-DOT system based on PMT-TCSPC technique. To balance instrumental complexity and measurement speed, the system employs the 780 nm / 830 nm -wavelength division multiplexing, 32×1 fiber-switching serial excitation and the 32×4 fiber-switching serial-to-parallel data acquisition. In practice, three primary conclusions might be obtained from developing this system. Firstly, fluorescence-DOT might take advantage of exogenously enhanced contrast to reliably profile the suspect regions. Secondly, the hemoglobin-DOT provides useful information on the background optical properties of the breast to improve the fluorescence-DOT accuracy. Thirdly, the information on the lesion localization provided by fluorescence-DOT is used to guide hemoglobin-DOT for enhanced characterization and quantitative assessment of the lesion. The experiments on a breast-mimicking phantom have validated the performances of the proposed methodology, and shown that the time-domain fluorescence-guided hemoglobin-DOT scheme presents a cost-effective and easy-to-operate way of markedly extending the capability of the optical mammography within the same optical radiation physics. Clinical evaluations of the proposed methodology are going on and the results will be reported in successive papers.

Acknowledgments

The authors acknowledge the funding support from the National Natural Science Foundation of China (30970775, 81101106, 61108081, 81271618), Chinese National Programs for High Technology Research and Development (2009AA02Z413), Research Fund for the Doctoral Program of Higher Education of China (2012032110056), and Tianjin Municipal Government of China (10JCZDJC17300).