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Development of a noncontact 3-D fluorescence tomography system for small animal *in vivo* imaging

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Abstract

Fluorescence imaging is an important tool for tracking molecular-targeting probes in preclinical studies. It offers high sensitivity, but nonetheless low spatial resolution compared to other leading imaging methods such as CT and MRI. We demonstrate our methodological development in small animal *in vivo* whole-body imaging using fluorescence tomography. We have implemented a noncontact fluid-free fluorescence diffuse optical tomography system that uses a raster-scanned continuous-wave diode laser as the light source and an intensified CCD camera as the photodetector. The specimen is positioned on a motorized rotation stage. Laser scanning, data acquisition, and stage rotation are controlled via LabVIEW applications. The forward problem in the heterogeneous medium is based on a normalized Born method, and the sensitivity function is determined using a Monte Carlo method. The inverse problem (image reconstruction) is performed using a regularized iterative algorithm, in which the cost function is defined as a weighted sum of the L-2 norms of the solution image, the residual error, and the image gradient. The relative weights are adjusted by two independent regularization parameters. Our initial tests of this imaging system were performed with an imaging phantom that consists of a translucent plastic cylinder filled with tissue-simulating liquid and two thin-wall glass tubes containing indocyanine green. The reconstruction is compared to the output of a finite element method-based software package NIRFAST and has produced promising results.

Keywords

Fluorescence diffuse optical tomography; noncontact; panoramic; small animal; *in vivo* imaging

1. INTRODUCTION

Fluorescence tomography is a rapidly expanding field of study in which traditional planar fluorescence imagery is being fused with many other modern technologies, among which target-specific probes and tomographical reconstruction methods are the most notable ones. Because of the highly scattering and absorbing nature of biological tissue, light propagation in the animal is dominated by diffusion, and as a result, tomographic fluorescence imaging is often termed fluorescence diffuse optical tomography (FDOT). Near infrared (NIR) light

sources are the primary choices in FDOT because of the relatively good penetration of NIR light in tissue as a result of the relatively low absorption coefficient of hemoglobin and water, and the relatively low autofluorescence compared to visible or ultraviolet light.

FDOT can noninvasively image labeled molecules in living organisms, which revolutionizes both basic research and clinical imaging. For example, it allows us to probe protease activity in animal preclinical imaging [1]—changes in protease activity are seen in a number of pathological states and diseases, including rheumatoid arthritis, cancer, atherosclerosis, angiogenesis, and cardiovascular disease.

A key to successful FDOT is computation. Light propagation in highly scattering heterogeneous media is well described by the radiative transport equation (RTE). Although it is complicated in its full form [2], the RTE reduces to a succinct form, known as the diffusion equation [3], for highly scattering media such as biological tissues. If the optical properties of an initial baseline condition are known, and if the deviation from this baseline is sufficiently small, gradient-driven perturbation approaches can be used to solve the diffusion equation. If we adopt the Born approximation, e.g., [4], the forward problem of FDOT can be expressed as

$$B(r_s, r_d) = \int_{\Omega} x(r) A_{r_s, r_d}(r) dr \quad (1)$$

where

$$x(r) = \frac{\eta(r)}{1 - j\omega\tau} \quad (2)$$

In this equation, B is the fluence rate (i.e., the optical signal) detected by the optical sensor, $A(r)$ is a sensitivity function for a given source-detector pair, Ω is the imaging volume, ω is the amplitude-modulation frequency of the excitation light, τ is the life-time of the fluorophore, and η is the product of concentration distribution and quantum efficiency of the fluorophore. Finally, r is position and the subscripts s and d represent the source and the detector, respectively. The discretized version of Eq. (1) for multiple fluorescence measurements is a set of simultaneous linear equations. The solution to this linear equation set is a fluorescence image, whose value is proportional to the distribution of fluorophore concentration, as defined in Eq. (2). A special case is when the light source is continuous-wave, where $\omega = 0$, and therefore $x = \eta$.

The sensitivity function can be computed analytically only for highly simplified geometries such as semi-infinite, multilayered, or cylindrical homogeneous media [3]. However, heterogeneous and more anatomically realistic geometries are required for FDOT in small animals and therefore more computationally challenging methods must be employed. Finite element methods and Monte Carlo simulations are the main approaches available, as reviewed in [5], both of which can be used with arbitrarily complex geometries.

The ill-posed and underdetermined characteristics of the inverse problem in F-DOT require regularized image reconstruction. Numerous studies have demonstrated that prior

information can significantly enhance reconstruction in diffusive optical imaging, e.g., [6–10]. Prior information not only reduces the number of unknowns in the inverse problem and thus makes it less underdetermined, but also gives the image reconstruction algorithm a mechanism to fine-tune the solution toward the optimum by inhibiting undesirable solution. From the mathematical modeling point of view, structural priors can also be used to precisely define the geometry of the medium. This is particularly important for small animal imaging because otherwise the modeling error can be disproportionately large. For example, the representative dimension of interest in small animals (e.g., 20–30 mm for the torso of the mouse) is only on the order of 10 times larger than the mean diffusion length of NIR light in tissue (~ 1 mm).

With the latest advances in photonics, it is now feasible to build bench-top fluorescence imaging systems and conduct whole-body imaging in live animals [11]. Recent results show that noncontact imaging techniques can produce better results [12]. Noncontact FDOT systems with animals positioned vertically on a rotation stage now exist [13, 14]. These studies show that animals can be imaged without immersion in index-matching fluid. Thus, the potential scope and impact of *in vivo* small animal F-DOT has improved dramatically.

The introduction of a fully noncontact imaging system is clearly an exciting advancement in FDOT hardware development because it allows dramatically increased sampling density as well as unprecedented flexibility in data sampling strategy compared to fiber-based systems. With the typical number of pixels available on a modern charge-coupled device (CCD) camera ($\sim 10^6$), a reasonable laser scanning density ($\sim 10^2$ – 10^4 points within an area of $\sim 25 \times 25$ mm), and the number of angles a rotation stage can provide ($\sim 10^2$), a noncontact FDOT system can potentially provide 10^{10} – 10^{12} measurements. Using this approach, the reconstruction problem in FDOT can easily become overdetermined, given that the usual upper limit of the field-of-view in small animal imaging ($30 \times 30 \times 60$ mm) contains only 5.4×10^4 of 1 mm^3 voxels, a typical resolution limit due to scattering in tissue. Furthermore, with a scanning laser and a CCD camera, the points of illumination and sensing can be virtually anywhere on the skin of the animal.

2. METHODS

The methodology of FDOT that has been developed in our lab will be described in terms of the system configuration, the forward problem, image reconstruction (the inverse problem), and digitization techniques, which includes 3-D surface rendering and image registration.

2.1 FDOT workbench

We employed a completely noncontact and panoramic imaging approach for the configuration of our FDOT workbench, which is similar to the one reported in [15]. The overall configuration of the workbench is schematically shown in Fig. 1.

The excitation light source is a continuous-wave laser diode (785 nm center wavelength, 50 mW optical output power, HL7851G, Opnext, Tokyo, Japan) as a part of an assembly that focuses and thermally/electronically stabilizes the laser (TCLDM9, LDC205C, and TED200C, Thorlabs, Newton, NJ). The optical sensor is an intensified CCD camera

(PicoStar HRI, LaVision, Goettingen, Germany). It consists of a cooled CCD camera (1376×1040 pixels with a pixel size of 6.45 μm) coupled to an image intensifier (photocathode S35 and phosphor P43) and equipped with a large-aperture lens (AF Nikkor 50 mm f/1.8D, Nikon, Melville, NY). The animal is vertically suspended in a rotation stage, which has two stepper motors to achieve simultaneous rotation of the fore- and hind-legs of the animal. During an experiment, the fore-legs of the animal are taped to a pair of vertical rods, which are in turn attached to the shaft of the upper motor via a horizontal bar. The hind-legs of the animal are fixed to the shaft of the lower motor using the same method. The animal's head is fixed using a bite-bar, which is aligned to the shaft of the upper motor. The excitation light is directed to the skin of the animal via a two-axis galvo-scanner (6230H, Cambridge Technology, Lexington, MA), which is a pair of perpendicularly positioned galvanometers each equipped with a high-reflectance mirror. The fluorophore is Indocyanine green (ICG or Cardiogreen, Sigma-Aldrich, St. Louis, MO), which has a maximum absorption wavelength at around 785 nm and the maximum fluorescent emission at approximately 830 nm in deionized water. Two interference band-pass filters (CVI Laser, Albuquerque, NM) are used at the excitation and the emission wavelengths (785 and 830 nm center wavelength, respectively, both 10 nm pass-band and 50 mm diameter). The fluorescent light emitted from the fluorophore is filtered and intensified before it generates a CCD image in the camera.

The laser and the camera are arranged in a transillumination configuration. During an experiment, the galvo-scanner moves the laser beam in a raster pattern in small steps and creates a discrete 2-D grid of illumination source positions across the surface of the animal. For each source position, the ICCD camera is activated to take a fluorescence CCD image (with the 830 nm filter). After the galvo-scanner completes a scan and resumes its initial state, the animal is turned a small angle by the rotation stage, followed by another laser scan by the galvo-scanner. This process repeats until the phantom returns to its initial angular position. Afterwards, the above imaging procedure is repeated at the excitation wavelength (with the 785 nm filter) to obtain the distribution of excitation light, which is used to normalize the previously acquired fluorescence signal. The activation of the ICCD camera, the actuation of the rotation stage, and the control of the galvo-scanner are implemented using our lab-developed LabVIEW (version 8.5, National Instruments, Austin, TX) applications and auxiliary computer hardware. All surfaces of the components on the optical workbench are either black anodized or covered with black velvet. The entire optical workbench is also covered with light absorbing/blocking material.

2.2 Forward problem

The forward problem is solved following a perturbation approach based on the Born solution to the diffusion equation [3], which is re-written here as the basis of further derivation

$$B(r_s, r_d) = \int_{\Omega} x(r) U_0(r_s, r) G_0(r, r_d) dr \quad (3)$$

where $B(r_s, r_d)$ is the fluorescence signal excited by a light source at r_s and measured by a photodetector at the boundary position r_d , $x(r)$ is the spatially dependent fluorophore concentration multiplied by the fluorescent yield, $U_0(r_s, r)$ is the excitation fluence rate at r

irradiated from the light source at r_s , and $G_0(r, r_d)$ is the Green function evaluated at r and r_d . Note that in a perturbation approach, $U_0(r_s, r)$ and $G_0(r, r_d)$ are computed under the baseline conditions, i.e., using the optical properties at the excitation wavelength. The integration domain Ω is defined as the entire imaging volume.

The fluorescent fluence rate is multiplied by multiple factors, which are dictated by the components of the imaging instrument before it is digitized and recorded by the computer. If one assumes that these factors are identical for the excitation and emission wavelengths, the overall effect of these factors can be eliminated by normalizing Eq. (3) using the excitation fluence rate and its counterpart in the forward model

$$\frac{B(r_s, r_d)}{B_0(r_s, r_d)} = \frac{1}{U_0(r_s, r_d)} \int_{\Omega} x(r) U_0(r_s, r) G_0(r, r_d) dr \quad (4)$$

where $B_0(r_s, r_d)$ is the optical signal at the excitation wavelength measured at the boundary position r_d irradiated from the source at r_s and $U_0(r_s, r_d)$ is the computed excitation fluence rate at r_d irradiated from the light source at r_s .

The product of the excitation fluence rate and the Green function $U_0(r_s, r)G_0(r, r_d)$ in Eq. (3) is termed sensitivity function because it is a representation of the contribution of each unit volume to the fluorescence signal. We compute the sensitivity function using a photon-tracing method based on Monte Carlo simulations [16]. Using this method, we can compute the sensitivity function efficiently because the individual fields of $U_0(r_s, r)$ and $G_0(r, r_d)$ are not computed explicitly. Note that we assume the excitation and emission wavelengths are sufficiently close so that the absorption and scattering coefficients can be considered identical.

The fluence rate measured at a position r is the number of photons per unit time injected from a source at r_s which pass through a unit volume centered at r . Because the Green function can be interpreted as the impulse-response function of the medium, and since the fluence rate is defined as the number of photons per unit area per unit time, the product of the fluence rate and the Green function represents the number of photons injected from the light source at r_s which subsequently pass a given voxel at r , and eventually reach the detector at r_d . If the light source is amplitude modulated, the intensity of the detected light is complex-valued, as is the sensitivity function. If the light is continuous-wave, as is the case in this article, the sensitivity function is real-valued. If the migration of a very large number of photons in the medium is simulated, the average of these photon paths weighted by their fluence rate seen by the detector is precisely the sensitivity function. Following the above analysis and the formula reported in [17], the frequency-domain sensitivity function based on photon-tracing in Monte Carlo simulations is given by

$$U_0(r_s, r) G_0(r, r_d) = \left[\sum_p \exp \left(\sum_m K_m L_{p,m} \right) \right]_r \quad (5)$$

where p is the index of the detected photons that passes the position r , m is the index of tissue types, $L_{p,m}$ is the total path length of photon p in medium m , which is given by the simulation software, and K is a complex wave vector given by

$$K_m = -\mu_{a(m)} + i \left(2\pi f \frac{n_m}{c_0} \right) \quad (6)$$

where μ_a , f , n , and c_0 are the absorption coefficient, modulation frequency, refractive index, and speed of light in vacuum, respectively.

The normalization factor $U_0(r_s, r_d)$ in Eq. (4) is the excitation fluence rate in Monte Carlo simulations, which is given by

$$U_0(r_s, r_d) = \frac{1}{N_s} \sum_p \exp \left(\sum_m K_m L_{p,m} \right) \quad (7)$$

where N_s is the total number of photons injected from the source in the simulations. Overall, the discretized version of Eq. (4) can be written as

$$\frac{B(r_s, r_d)}{B_0(r_s, r_d)} = \frac{N_s}{\sum_p \exp \left(\sum_m K_m L_{p,m} \right)} \sum_r \left\{ x(r) \left[\sum_p \exp \left(\sum_m K_m L_{p,m} \right) \right]_r \right\} \quad (8)$$

Using the algorithm formulated by Eq. (8), a partial Jacobian matrix for a given source/detector combination (a.k.a., a measurement channel) is computed from all the photon paths that initiate at the given source and terminate at the given detector. This procedure is repeated for all measurement channels until the full Jacobian matrix is generated.

Overall, the forward problem can be expressed in a vectorized form

$$AX = \mathbf{b} \quad (9)$$

where $\mathbf{b}$ is the normalized measurement vector, A is the Jacobian matrix of Eq. (8), and X is the fluorescence image. This is the basis to solve the inverse problem in FDOT.

2.3 Photobleaching correction

It is well known that the illuminated fluorophore gradually loses its fluorescence over time, known as photobleaching. The rate of photobleaching is dependent on the energy levels characteristic to the molecular structure of the fluorophore and is also a function of concentration and light intensity [18–21]. In this article, we assume the photobleaching process occurs at a linear rate within a short period of time and globally reduces the yield of the fluorophore proportional to its concentration distribution. This is a reasonable simplification because the elapsed time between consecutive source positions is ~ 1 s, which is very short compared to the time course of the fluorescence data acquisition (~ 10 min), during which the signal intensity reduces by approximately 10% under our experimental conditions. Therefore, the effect of photobleaching on signal intensity can be written as

$$q_{m,n}(i) = q_{m,n}(0) [q_{m,n}(N)/q_{m,n}(0)]^{i/N} = q_{m,n}(0)^{1-i/N} q_{m,n}(N)^{i/N} \quad (10)$$

where $q_{m,n}(i)$ is the fractional signal intensity measured for the m -th source and the n -th detector positions at the i -th rotation angle ($0 \leq i \leq N$), and N is the total number of rotation angles. Note that the index of rotation angle is used to model photo-bleaching instead of using the exposure time of the fluorophore to the laser because the laser scans at a constant time interval. The effect of photobleaching on measurements at the excitation wavelength is ignored because of the relatively small fraction of contribution of the fluorophore to absorption at this wavelength compared to the background medium. To correct for the photobleaching effect, the fluorescence signal B in Eq. (8) is divided by the factor q given by Eq. (10).

2.4 Image reconstruction

Because the forward problem is formulated as a linear equation using the Born approximation, the process of image reconstruction is to solve the linear equation set given in Eq. (9).

The fluorescent image is reconstructed using an iterative reconstruction algorithm, which uses Tikhonov regularization algorithm [22] to recover the fluorescence pattern. The fluorescence pattern, denoted by X in Eq. (9) is obtained by solving the following unconditioned minimization problem

$$X = \arg \min_X [H(X)] \quad (11)$$

In the above equation, the cost function $H(X)$ is defined as

$$H(X) = \underbrace{\|AX - b\|_2^2}_{\text{data consistency}} + \underbrace{\alpha \int_{\Omega} \|X\|_2^2 dr}_{\text{intensity penalty}} + \underbrace{\beta \int_{\Omega} \|\nabla X\|_2^2 dr}_{\text{smoothness penalty}} \quad (12)$$

where $\|k\|_2$ is the L -2 norm of a vector k , Ω is the region of interest, and r is position. The three terms of the cost function penalize the residual error, intensity, and smoothness of the solution, respectively, whose weights are independently adjusted by the user-specified factors α and β .

We solve the minimization problem given by Eq. (11) and Eq. (12) using the conjugate gradient optimization algorithm [23]. All data processing and image reconstruction algorithms are implemented on the scientific/engineering programming platform MATLAB (R2008a, The MathWorks, Natick, MA).

2.5 Digitization

Computing the precise surface contour of the animal is essential to solving the forward problem and accurate image registration. We use a white light-emitting diode (LED) light source equipped with a ground glass diffuser (LIU004 and DG20-120, Thorlabs, Newton, NJ) to illuminate the animal while taking a white light CCD image at each rotation angle.

Because this very diffusive LED light source is mounted just above the camera lens and also because it is far away from the animal compared to the dimensions of the animal, the animal can be considered as being illuminated by a parallel light source. These images are segmented using thresholding and converted into a set of binary images. These binary images that contain the profiles of the animal at different angles are equivalent to the back-illuminated parallel projection images. The relation between a 3-D volume and its 2-D projections is well modeled by Radon transform.

Because the rotation center of the animal does not necessarily coincide with the center of the CCD image, each CCD image is corrected for the horizontal displacement (horizontal shift). This is achieved by comparing each pair of images taken at the opposite angles and finding a displacement so that they become closest mirror images to each other in terms of the smallest root-mean-square (RMS) difference. For each horizontal line in this series of corrected binary images, inverse Radon transform is applied to produce a 2-D axial slice containing the surface contour of the animal. The 3-D surface contour is obtained by stacking all axial slices. This surface contour is the geometrical basis for computing the Jacobian matrix and image registration.

With the 3-D surface contour available, the positions of the illumination sources on the surface of the animal can be computed using the origin and the direction of the laser, which is known during experiment setup. The detector positions are chosen on the CCD image and mapped onto the surface contour using the same method.

2.6 Experiment

We used an imaging phantom to test and validate our newly developed FDOT system and data processing algorithms. The imaging phantom consists of a plastic cylinder (Nylon 6/6, off the shelf) with 23 mm outer diameter (1 mm wall thickness) and is filled with a mixture of Intralipid (Fresenius Kabi, Sweden) and India ink (off the shelf) in deionized water to simulate the optical properties of the mouse. The phantom has two fluorescent inclusions, which are made of thin-wall glass tubes (3 and 4 mm outer diameter, 0.3 and 0.4 mm wall thickness, respectively, Wilmad LabGlass, Buena, NJ) both filled with 10 μM indocyanine green in deionized water. The clear distance between the two glass tubes is 5 mm. The geometry of the imaging phantom is shown in Fig. 2. The absorption and scattering coefficients were measured at the excitation wavelength (Intralipid/ink mixture $\mu_a = 0.01$ and $\mu'_s = 1.0 \text{ mm}^{-1}$, Nylon 6/6 $\mu_a < 10^{-4}$ and $\mu'_s = 0.39 \text{ mm}^{-1}$). The refractive indices of the materials were taken from typical values in the literature (Intralipid/ink mixture $n = 1.4$, Nylon 6/6 $n = 1.5$). The phantom was mounted on the rotation stage to simulate the torso of the mouse. Using the rotation stage, full-angle imaging of the phantom is achieved—the entire panoramic view is divided into 40 rotation angles (9° per angular sample). For each rotation angle, 10 source positions and 20 detector positions are used, which equidistantly span arcs of 100° and 140° on the surface of the phantom along the circumference, respectively.

ICG solution is prepared and injected into the glass tubes in the phantom just before data acquisition starts in order to minimize photobleaching due to room light. A fluorescence

image is taken for each of the source positions and for each of the rotation angles. The time delay between consecutive source positions is 1.3 s. An additional time delay of 1 s is used for each rotation. The exposure time of the ICCD camera is 20 ms per each measurement. Afterwards, this imaging procedure is repeated while excitation CCD images are taken. Finally, the phantom is replaced by a lab-made calibration target to compute the source positions. The calibration target is made of a thin and rigid, translucent yet highly attenuating plastic sheet, on which a grid is marked to show the scale in the CCD image. This calibration target is placed perpendicular to the camera-galvo axis and at the location where the phantom was centered. Another series of images (one image per source position) is taken to record the locations where the laser intersects the target for different source positions. Computing the illumination source positions on the surface of the phantom is trivial based on this calibration information. The detector positions are determined easily by comparing the calibration and the white light images. For each detector position, the signal intensity is an average over a small cluster of adjacent pixels (5×5 in this study) to increase the signal-to-noise ratio, which corresponds to an area of approximately 0.3×0.3 mm on the projected 2-D surface of the imaging phantom.

In the Monte Carlo simulations for the forward model, the medium consists of two components—the Intralipid/ink mixture (21 mm diameter cylinder) and Nylon 6/6 (inner/outer diameters 21/23 mm). The entire field-of-view is 30×30×60 mm with an isotropic voxel size of 0.1 mm. For each illumination source, 10 million photons are used. The reconstruction algorithm uses a matrix size of 23×23×15 with a pixel size of 1 mm. Because of the symmetry of a cylindrical structure about its axis, which is also the rotation axis of the phantom, Monte Carlo simulations and subsequent construction of the Jacobian matrix are only performed for one rotation angle. During FDOT reconstruction, the Jacobian matrices for other rotation angles are generated by geometrically rotating the original Jacobian matrix by the corresponding rotation angles using a geometrical transformation

$$J_{\theta} = J_0 R_{\theta} \quad (13)$$

where J_{θ} is the 3-D Jacobian matrix for a given measurement channel at the rotation angle θ , J_0 is the corresponding original Jacobian matrix previously computed from Monte Carlo simulations, and R_{θ} is the well-known geometrical transformation matrix (for a rotation of an angle θ) given by

$$R_{\theta} = \begin{bmatrix} \cos(\theta) & \sin(\theta) & 0 \\ -\sin(\theta) & \cos(\theta) & 0 \\ 0 & 0 & 1 \end{bmatrix} \quad (14)$$

The termination criterion for the iterative FDOT reconstruction algorithm is when the value of the cost function defined in Eq. (12) is less than 10^{-6} , in which optimal regularization parameters α and β (10^{-3} and 10^{-4} , respectively) are determined empirically.

The reconstructed raw fluorescence image is further processed for properly identifying the fluorescent structures in the image: only the center slice where the sources and detectors are located is retained due to the symmetry of light propagation about this plane; the

background noise is removed using a threshold such that two disconnected cluster of pixels remain; and the reconstructed fluorescent structures are defined as the clusters of pixels having values greater than half of the local maxima. This image processing procedure was programmed using MATLAB and without further human intervention.

In order to validate our data processing and image reconstruction algorithm, we compared the reconstruction result with a well tested and documented software package NIRFAST [24], which is based on a finite-element method. It iteratively updates the fluorescence image and subsequently solves the forward FDOT problem in each iteration until the simulation matches the experimental data within the error tolerance. Tetrahedral meshes are generated using a lab-developed MATLAB program based on the phantom geometry and are subsequently converted to the appropriate data format, along with other necessary information such as the source/detector positions and experimental data. The termination criterion used in NIRFAST is a relative error of 0.1% between consecutive iterations.

3. RESULTS AND DISCUSSION

The fluorescence images reconstructed from our initial phantom experimental data are shown in Fig. 3. For our experimental configuration (10 sources, 20 detectors, and 40 angles), it took approximately 6 hours to finish the Monte Carlo simulations, 30 min to generate the Jacobian matrix, and 12 min to reconstruct the fluorescence image on a Dell personal computer (Optiplex 745, Dell Computers, Round Rock, Texas) equipped with a Intel Core 2 Duo 2.4 GHz dual-core processor (Intel, Santa Clara, CA) and 4 GB of computer memory. The intensity of the reconstructed images was normalized to their maximum values for comparison. Fig. 3(a) and (b) show the gray-scale and segmented images of the reconstruction, respectively, using our Monte Carlo simulation-based method. Comparing Fig. 3(b) and (d), the two fluorescent structures are correctly localized. Furthermore, the two structures are clearly distinguished from each other although both appear notably larger than their actual dimensions. The dark circle around the outer edge of the phantom in Fig. 3(a) consists of pixels with negative values (marked by the arrow), which we theorize as due to breakdown of the diffusion approximation near the boundaries where the optical properties at the air-plastic and plastic-liquid interfaces discontinue.

It took NIRFAST approximately 6 hours to reconstruct the fluorescence image, as shown in Fig. 3(c), on a Dell computer workstation equipped with dual Intel Xeon 2.83 GHz tetra-core processors and 32 GB of memory. It should be noted that in our method, the forward problem was solved only once for one rotation angle, during which the Jacobian matrix was computed, while in NIRFAST, the full forward problem was solved in every iteration. Compared to the result shown in Fig. 3(b), the reconstruction shown in Fig. 3(c) using NIRFAST matches the true image in Fig. 3(d) better in terms of the dimensions of the two fluorescent structures. However, the reconstructed structures in Fig. 3(c) appear to be slightly tilted and closer to each other than their actual locations. It should also be noted that the negative values (marked by the arrow) near the larger structure (positive valued) in Fig. 3(c) is an imaging artifact.

4. CONCLUSIONS

In this article, we describe our current methodological development in FDOT for small animal *in vivo* imaging. This FDOT system uses raster-scanned laser and CCD camera to achieve fully noncontact imaging without optical matching-fluid; it achieves panoramic imaging using a rotation stage on which the animal is vertically positioned. The entire system is controlled via our lab-developed LabVIEW program, which allows very fast and highly repeatable data acquisition. The forward problem solver is based on the Born solution to the diffusion equation, in which the Jacobian is computed efficiently using our Monte Carlo simulation-based algorithm. The inverse problem is solved iteratively using Tikhonov regularization method and the conjugate gradient algorithm. The cost function of the inverse problem solver accounts for data consistency, image intensity, and image smoothness, which are adjusted independently by two regularization parameters. Our initial test with our imaging system and data processing algorithms was performed in a phantom experiment using ICG as fluorophore. The reconstruction was compared against the output of a finite element method-based software package NIRFAST and has produced promising results.

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Fig. 1. Hardware configuration of the FDOT workbench: (1) CCD camera, (2) image intensifier, (3) lens, (4) filter wheel, (5) stepper motors, (6) experiment animal, (7) galvo-scanner, and (8) laser diode assembly.

Fig. 2.
Geometry of the imaging phantom.

Fig. 3.

Comparison of reconstruction results using our Monte Carlo based method: (a) gray-scale reconstruction result, (b) the segmented binary image, (c) result using the FEM software package NIRFAST, and (d) the true image. These axial slices are taken at the central plane where the sources and detectors are located. The areas marked by arrows have negative values.