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Full density fluorescence molecular tomography (FD-FMT) based on a dichroic mirror

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Abstract

We present a novel method called full density fluorescence molecular tomography (FD-FMT) that can considerably improve the performance of conventional FMT. By converting each source (or detector) to a detector (or source) through the use of a dichroic mirror, FD-FMT not only increases the amount of optical projections by more than fourfold (compared to conventional FMT) to achieve high-resolution image reconstruction, but also offers the possibility to realize miniaturized FMT systems.

Keywords

(300.2530) Fluorescence, laser-induced; (110.6955) Tomographic imaging; (110.2350) Fiber optics imaging; (170.3010) Image reconstruction techniques

1. INTRODUCTION

In the past decades, the increasing use of nontargeted or targeted exogenous fluorescence makers in clinical and biological research has prompted the development of several fluorescence imaging approaches [1–3]. One common fluorescence imaging method is the planar fluorescence reflectance imaging, which is commonly used for *in vivo* imaging in animal models of various diseases [4,5]. Despite its simplicity of operation, the applicability of this technique is currently limited to the investigations of phenomena at the surface and subsurface depths of a few millimeters because the recorded images are a superposition of fluorescence signals from multiple depths and do not represent true fluorochrome distribution. To overcome these limitations, near-infrared fluorescence molecular tomography (FMT) has been developed as an important molecular imaging tool [6,7]. FMT can provide accurate visualization and quantification of the three-dimensional (3D) distribution of fluorescent targets in deep turbid tissue. This convenient and cost-effective optical imaging modality has been widely used in biomedical research and clinical studies

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including cancer imaging, drug delivery, stem cell trafficking, and enzyme activity monitoring [8–10].

FMT recovers the internal fluorophore distribution and concentration by minimizing the discrepancy between boundary measurements and predicted values from a photon transport model typically through a regularized least-squares method. Due to the strong scattering and absorption of photons in biological tissue, FMT reconstruction is usually highly ill-posed and ill-conditioned. Therefore, the quality and accuracy of the reconstructed images are highly dependent on the number of measurements (or number of source–detector pairs). High-density configuration, which simply increases the number of sources and detectors in a given area, has proven to be a useful method that dramatically improves imaging resolution and sensitivity. Several studies have investigated the high-density source–detector configurations for other imaging modalities, such as diffuse optical tomography (DOT) and electroencephalography [11–13].

An alternative strategy for increasing the total number of optical projections for image reconstruction involves a special bifurcated optical fiber bundle in which each optical fiber bundle can be used as source and detector simultaneously. This method was applied to several optical fiber-based imaging systems, such as DOT and Raman tomography [14,15]. However, the obvious disadvantage is that the large size of this special optical fiber bundle limits efforts to miniaturize the imaging system.

Another way to alleviate the ill-conditioning and improve the image quality, involves integrating FMT with other imaging technologies into a hybrid modality. The benefits of this approach have been demonstrated in recent studies for modalities such as CT, MRI, and ultrasound [16–21]. Obviously, a miniaturized FMT system offers the potential to combine it with other imaging modalities, especially in the design of miniaturized imaging devices such as endoscopes. However, miniaturization is a big challenge in the imaging system design.

Here we propose a novel optical fiber-based FMT system called full density FMT (FD-FMT), which uses a dichroic mirror. Our objective is to realize high-density sampling without an increase in optical fibers and to improve miniaturization for potential combination with other imaging modalities. Several phantom experiments were performed to validate this new method and to demonstrate its advantages and potentials.

2. RECONSTRUCTION ALGORITHM

The fluorescence images obtained were reconstructed using an iterative finite-element-based algorithm that was described in detail previously [22]. Here, we outline the algorithm based on the following coupled diffusion equations that describe the propagation of excitation and emission light in tissue:

$$\nabla \cdot [D_x(r) \nabla \varphi_x(r)] - \mu_{ax}(r) \varphi_x(r) + S_x(r) = 0, \quad (1)$$

$$\nabla \cdot [D_m(r) \nabla \varphi_m(r)] - \mu_{am}(r) \varphi_m(r) + \eta \mu_{a_{x-m}}(r) \varphi_x(r) = 0, \quad (2)$$

where $\varphi_{x,m}$ is the photon density for excitation (subscript x) or emission light (subscript m), and $D_{x,m}$ is the diffusion coefficient. In this work the quantum yield = 0.016 for indocyanine green (ICG) [23]. $S_x(r)$ is the excitation source term for a point source. The nonzero photon density or Type III boundary condition was applied in this study.

Making use of finite element discretization, we obtain the matrix representations of Eqs. (1) and (2), which leads to a set of equations capable of solving the inverse problem:

$$[A_{x,m}] \{\varphi_{x,m}\} = \{b_{x,m}\}, \quad (3)$$

$$[A_{x,m}] \left\{ \frac{\partial \varphi_{x,m}}{\partial \chi} \right\} = \left\{ \frac{\partial b_{x,m}}{\partial \chi} \right\} - \left[\frac{\partial A_{x,m}}{\partial \chi} \right] \{\varphi_{x,m}\}, \quad (4)$$

$$(J_{x,m}^T J_{x,m} + \lambda I) \Delta \chi = J_{x,m}^T (\varphi_{x,m}^0 - \varphi_{x,m}^c), \quad (5)$$

where the elements of matrix $[A_{x,m}]$ and the entries in column vectors $b_{x,m}$ can be expressed by a set of spatially varying Lagrangian basis functions; χ expresses D_x , μ_{a_x} , or $\eta \mu_{a_{x-m}}$ and $J_{x,m}$ is the Jacobian matrix consisting of the derivatives of $\varphi_{x,m}$ with respect to χ at each boundary observation node. $\Delta \chi$ are the update vectors for the optical and fluorescent property profiles; I is identity matrix; λ may be a scale or a diagonal matrix; and $\varphi_{x,m}^0$ and $\varphi_{x,m}^c$ are the observed and the computed excitation or emission photon density, respectively. Optical and fluorescent images are formed by iteratively solving Eqs. (3)–(5) and updating the optical (D_x and μ_{a_x}) and fluorescent ($\eta \mu_{a_{x-m}}$) property distributions from presumably uniform initial estimates of these properties.

3. EXPERIMENTAL

The schematic of our FD-FMT system is shown in Fig. 1(a). An optical fiber-based handheld probe, which was described in detail in our previous work [24], was used in this system. This handheld probe consisted of 25 optical fibers distributed 4 mm apart and covered an area of 20 mm × 20 mm. Similar to the settings of a conventional FMT system, we used a continuous-wave 780 nm diode laser (Openxt, HL7301MG, Japan) as the light source, a 1024 × 1024 pixel deep-cooled CCD camera (Princeton Instrument, NJ) coupled with an 830 nm bandpass filter with 32 nm bandwidth (Edmund Optics, NJ) to collect the emission fluorescence light from the optical fiber array, and two linear stages (Model

17AMA045, CVI Melles Griot) to perform 2D scanning to deliver laser beam to each optical fiber.

The key part of the FD-FMT system is highlighted with the black dashed square shown in Fig. 1(a). A dichroic mirror (Edmund Optics) was placed in front of the laser diode with 45 deg angle and between the optical fiber array and CCD camera, which spectrally separates light by transmitting the fluorescence and reflecting excitation light simultaneously. Thus, each optical fiber can be used as a source and as a detector. By using this setting, the number of projections for image reconstruction increases without adding more optical fibers or making alterations to them. A photograph of this arrangement in Fig. 1(b) shows the dichroic mirror, optical fiber array, laser diode, and linear stages. Figure 1(c) shows the absorption (red) and emission (green) spectrum of ICG and the transmission spectrum (gray) of the long-pass dichroic mirror with a cutoff wavelength at 801 nm. The dichroic mirror is highly reflective at the peak absorption wavelength of ICG at ~ 785 nm and highly transmissive at the peak emission wavelength at ~ 830 nm. Since a dichroic mirror is a common optical component offered in several different cutoff wavelengths ranging from 425 to 1800 nm, a proper dichroic mirror can be easily found for the spectrum of dye agents we use.

To demonstrate the advantages of FD-FMT against FMT, phantom experiments using ICG as the fluorescent marker were performed. A cylindrical background phantom (30 cm in diameter and 20 cm in height), consisting of TiO_2 solution, India ink, and 2% agar powder, was used and had an absorption coefficient of 0.01 mm^{-1} and a reduced scattering coefficient of 1.0 mm^{-1} . One or two small ICG-containing cylinders were placed at different depths to the background phantom to simulate the targets. The ICG concentration in targets used in the study was $1 \mu\text{M}$, and no ICG was added to the background. For each experiment, the exposure time of the CCD camera was different depending on the intensity of the fluorescence from the target, and a binning of 4×4 pixels was used to improve the signal-to-noise ratio. In addition, a calibration method based on homogeneous phantom was used to reduce the system noise [25]. A LabVIEW program was used to control the data acquisition.

4. RESULTS AND DISCUSSION

We first demonstrate the improvement of imaging quality and resolution by utilizing FD-FMT coupled with the handheld probe. Figure 2(a) shows the pattern of the source and detector positions of FMT (left) and FD-FMT (right), in which 10 were used as the source (red) and 15 as the detector (green) in FMT, and all of them (25) as both the source and detector (blue) simultaneously in FD-FMT. Therefore, FD-FMT provided $25 \times 25 = 625$ projections, more than 4 times of the number of projections in FMT ($10 \times 25 = 125$). Figure 2(b) shows the FMT and FD-FMT reconstructed tomographic fluorescence images (along the transverse and sagittal planes) of two cylindrical targets (3 mm in diameter and 2 mm in height) located at $(-4, -4)$ and $(4, 4)$ in the XY plane at two different depths (5 and 7 mm). The black dashed circle or square in each image indicates the exact target location. We can see that both FMT and FD-FMT reconstructed the two targets well at the depth of 4 mm. The recovered target size is more accurate using the FD-FMT method. Significant improvement of image quality was observed for the deeper target depth (7 mm). Although it

is difficult to identify the target in the images with FMT, both targets were well reconstructed in the FD-FMT method. To further quantitatively analyze the improvement provided by FD-FMT, we plotted the recovered the fluorescent concentration values, based on FMT (blue) and FD-FMT (red), respectively, along the white dashed line shown in Fig. 2(b) crossing each center of the targets located at the depth of 5 mm in Fig. 2(c) and 7 mm in Fig. 2(d). As Figs. 2(c) and 2(d) show, FD-FMT improves the image quality and resolution to a great extent, especially when the targets were located at deeper depth. These results show that FD-FMT improves the image quality and resolution by providing more projections for image reconstruction than FMT.

To further demonstrate the advantages of FD-FMT, we performed several sets of phantom experiments using fewer optical fiber bundles. Figure 3(a) shows the pattern of the source and detector positions of FMT (left) and FD-FMT (right) with only nine optical fibers in total, in which four were used as the source (red) and five as the detector (green) in FMT, but all nine (blue) were used as the source and detector (blue) simultaneously in FD-FMT. By using FD-FMT, the total number of optical projections increases from $4 \times 5 = 20$ in FMT to $9 \times 9 = 81$ in FD-FMT. Figure 3(b) shows the reconstructed tomographic fluorescence images (along the transverse and sagittal planes) of a cylindrical target (2 mm in diameter and 2 mm in height) at depths of 3, 4, and 5 mm using FMT and FD-FMT, respectively. The black dashed circle or square in each image indicates the target outline. As Fig. 3(b) shows, the target cannot be recovered using FMT due to the limited number of projections. For the FD-FMT method, however, the target was well recovered even at the 5 mm depth. This significant improvement shows that FD-FMT offers more potential to perform 3D computed tomographic fluorescence imaging using fewer optical fibers. Since this method does not require the special bifurcated optical fiber bundle, which is typically large, the FD-FMT method provides greater potential to further miniaturize the FMT system, especially in the design of an FMT endoscope. Also, miniaturized hybrid modalities of FMT with other imaging modalities can be conveniently realized.

5. CONCLUSION

In summary, we have described and examined a novel method, which we call FD-FMT, to perform FMT using a dichroic mirror. The phantom results indicated that FD-FMT significantly improves the imaging quality and resolution due to the increased optical projections for image reconstruction. It is simple and economical to adapt similar conventional FMT systems to improve imaging quality with the FD-FMT method. FD-FMT shows greater potential for further miniaturizing FMT systems and which could then be combined with other imaging modalities.

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Fig. 1.
 (a) Schematic of the FD-FMT imaging system. (b) Photograph of the FD-FMT system. (c) The absorption (red) and emission spectrum (green) of indocyanine green (ICG), and the transmission spectrum (gray) of the dichroic mirror.

Fig. 2. Comparison of FMT to FD-FMT image quality and resolution. (a) Pattern of source and detector positions of FMT and FD-FMT. (b) Reconstructed tomographic fluorescence images for targets at depths of 5 and 7 mm using FMT and FD-FMT, respectively. Recovered $\eta\mu_{a_{x \rightarrow m}}$ values plotted along the white dashed line shown in (b) crossing each center of the target at the depths of (c) 5 mm and (d) 7 mm, respectively.

Fig. 3. Comparison of FMT to FD-FMT image quality and resolution using nine optical bundles. (a) Pattern of source and detector positions of FMT and FD-FMT. (b) Reconstructed tomographic fluorescence images for a target at depths of 3, 4, and 5 mm using FMT and FD-FMT, respectively.