A handheld fluorescence molecular tomography system for intraoperative optical imaging of tumor margins

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Purpose: Accurate identification of tumor margins presents a major challenge in the surgical treatment of human cancers. Inability of complete removal of tumor lesions after surgery causes local recurrence and increases the incidence of developing tumor metastasis. It is clear that novel approaches that allow defining tumor margins intraoperatively for removal of small tumor lesions in the surgical cavity is critical for improving prognosis of cancer patients. To facilitate image-guided surgery using targeted optical imaging probes, we have developed a reflection-mode fluorescence molecular tomography (FMT) system with a handheld probe that is able to provide three-dimensional tumor margin information.

Methods: The imaging method and system were validated using both simulated and phantom experiments. We further examined the accuracy of the handheld FMT system in an orthotopic mouse mammary tumor model following systemic delivery of near-infrared (NIR) dye-labeled and urokinase plasminogen activator receptor targeted magnet iron oxide nanoparticles.

Results: Our results show that when the targets are located within 5 mm beneath the surface of the media, fluorescent images can be reliably detected and reconstructed with an average positional error of 0.5 mm laterally and 1.5 mm axially. For *in vivo* imaging in the mouse tumor model, the location and size of the tumor detected by FMT correlated well with that measured by the magnetic resonance imaging (MRI).

Conclusions: Our system can three-dimensionally image targets located at a depth of up to 7 mm. The *in vivo* results suggest that in combination with targeted optical imaging probes, this handheld FMT system can be potentially used as an intraoperative tool for the detection of tumor margins and for image-guided surgery. © 2011 American Association of Physicists in Medicine. [DOI: 10.1118/1.3641877]

Key words: fluorescence molecular tomography, intraoperative optical imaging, mouse mammary tumor model, targeted optical nanoparticle imaging probes.

I. INTRODUCTION

Surgical resection plays a critical role in treatment of most human cancers. The key aspect for the postoperative prognosis of cancer is the complete tumor resection including the primary tumor, small metastatic tumors, and sentinel nodes that may contain tumor cells. In current practice, intraoperative assessment of tumor-free margin is dependent on visual appearance and palpation of the tumor. Recent advances in computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), and x-ray have greatly improved tumor detection. Each of these modalities has its own advantages and limitations. PET can map tumor metabolism at an early stage, but it involves the use of expensive positron-emitting radio-pharmaca and has a limited spatial resolution.^{1,2} x-ray, CT, and MRI provide high spatial resolution. However, they are highly prone to shift of tissue,

provide only structural information and are generally not appropriate for real-time intraoperative assistance.³⁻⁶

Optical-based imaging technologies appear to be ideal for intraoperative imaging as they can be miniaturized, and are inexpensive, fast, and sensitive.⁷ While to date most of the optical studies conducted focused on the intrinsic fluorescence of malignant tissues,⁸⁻¹⁴ the high heterogeneity of malignant and benign tissues in their molecular and cellular compositions often leads to unacceptable false-positive rates for benign tissues and unacceptable false-negative rates for malignant tissues using these autofluorescence and intrinsic measurements.¹⁵ A large number of studies using nontargeted exogenous fluorescent markers (e.g., indocyanine green (ICG) and ALA-induced PpIX) have shown improved tumor detection.¹³⁻¹⁷ Recent development of new near-infrared (NIR) fluorophores and nanomaterials has led to a revolution in fluorescence molecular imaging since

considerable enhancement in specificity and sensitivity for tumor imaging can be achieved by using targeted contrast agents.^{18–20} In these agents, NIR fluorophores are conjugated to a specific targeting ligand, allowing excellent signal to background ratio to be obtained in whole animals.

The goal of this work is to demonstrate the use of a novel near-infrared dye NIR-830 labeled urokinase plasminogen activator receptor (uPAR) targeted-nanoparticle probe²¹ for intraoperative tumor imaging using a fiber optic based handheld three-dimensional (3D) fluorescence molecular tomography (FMT) system. The portable handheld probe developed is highly suitable for the detection of small tumor lesions in the surgical cavity and tumors involved lymph nodes. Such a small probe can also be used in mouse animal tumor models for preclinical studies. Additionally, the conventional planar fluorescence optical imaging has a low spatial resolution and unable to provide precise information on tumor location. Tumor lesions and tumor cell involved lymph nodes are surrounded by normal tissues and located in various depths inside surgical cavity. The major advantages of intraoperative imaging using 3D-FMT lays its excellent spatial resolution and quantification ability that allow accurate detection of tumor margin and measurement of the depth and size of small tumor lesions. We demonstrate this system in tissue-mimicking phantoms and in the mice bearing mammary tumors after systemic administration of the novel uPAR targeted NIR fluorescent probes. Some of the FMT images obtained are compared with the MRI images of the same mouse.

II. INSTRUMENTATION

The fiber optic based handheld FMT system is depicted in Fig. 1. A continuous-wave (CW, 730 nm) diode laser (Model HL7301MG, Openxt, Japan) was mounted on a linear stage (Model 17AMA045, CVI Melles Griot). The excitation light beam from the diode laser was sequentially delivered to each fiber in the source fiber array through a convex lens. For each source position, the emission light from the detection fiber array was collected by a 1024 × 1024 pixel CCD camera (Princeton Instrument, Trenton, NJ) coupled with an 830 ± 2 nm band-pass filter (Thin Film Imaging Technologies, MA). For each experiment, the exposure time of the CCD camera was different and a binning of 4 × 4 pixels was used to improve the signal-to-noise ratio (SNR). A Visual c++ program was used to control the entire data acquisition.

In our study, we used 10 source and 15 detector positions (a total of 150 measurements) covering an area of 20 × 20 mm². The adjacent source/detector optic fibers were spatially distributed 4 mm apart (see Fig. 1).

III. RECONSTRUCTION ALGORITHM

The fluorescence images obtained were reconstructed using an iterative finite element based algorithm that was described in detail previously.^{22–24} Here, we outline the algorithm based on the following coupled diffusion equations that describe the propagation of excitation and emission light in tissue:

FIG. 1. Photograph of the fluorescence molecular tomography system consisting of a laser diode, linear stage, source, and detection fiber optic array, CCD camera, and handheld probe. In the insert, the source and detector positions are indicated by circles.

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

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

where $\varphi_{x,m}$ is the photon density for excitation (subscript x) or emission light (subscript m); $D_{x,m} = 1/3(\mu_{a_{x,m}}(r) + \mu'_{s_{x,m}}(r))$ is the diffusion coefficient. $\mu_{a_{x,m}}$ and $\mu'_{s_{x,m}}$ represent the absorption coefficient and the reduced scattering coefficient for excitation and emission, respectively. In this work we used quantum yield 0.016 and 0.034 for ICG (Ref. 25) and NIR 830 dye, respectively. $S_x(r) = S_0 \delta(r - r_0)$ is the excitation source term for a point source, where S_0 is the source strength and $\delta(r - r_0)$ is the Dirac-delta function for a source centered at r_0 . The nonzero photon density or type-III boundary condition was applied. $-D_{x,m} \nabla \varphi_{x,m} \cdot \hat{n} = \alpha \varphi_{x,m}$, where $\hat{n}$ is the unit normal vector to the boundary surface, and α is the coefficient related to the internal reflection at boundary.

Making use of finite element discretization, we obtain the matrix representations of Eqs. (1) and (2), which lead 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 $\eta \mu_{a_{x-m}}$; $J_{x,m}$ is the Jacobian matrix consisting of the derivatives of $\varphi_{x,m}$ with respect to χ at each boundary observation node. $\Delta \chi$ is the update vectors for the optical and fluorescent property profiles; I is identity matrix; λ may be a scale or a diagonal matrix; $\varphi_{x,m}^0$ 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.

It is known that proper calibration methods are necessary for high quality image reconstruction. In our study, we used a subtraction-based postprocessing technique to reduce the background noise, i.e., optical measurements from a homogeneous phantom were subtracted from the heterogeneous phantom measurements. In addition, we adopted the calibration method previously described in Ref. 26. Briefly, a calibration matrix was obtained from a homogeneous phantom measurement together with the forward calculation from a homogeneous medium. Applying this matrix to the measurement data from the heterogeneous medium of interest allowed us to remove errors from model mismatch, fiber optic positioning, and numerical noises in the inverse computation involved.

IV. ANIMAL TUMOR MODELS

A mouse mammary tumor model was established using a luciferase gene stable transfected mouse mammary 4T1 tumor cell line. 4T1 cells (2×10^6) were injected either into the mammary fat pad or subcutaneously into the back flank area of the female Balb/c mice. The tumor grew to 8–10 mm in 2 weeks after the cell injection.

Recombinant mouse amino terminal fragment (ATF) peptides were produced from pET101/D-TOPO expression vector containing a mouse ATF of the receptor binding domain of uPAR cDNA sequences and expressed in E. coli BL21 (Invitrogen). ATF peptides were purified from bacterial extracts under native conditions using Ni2+NTA-agarose columns (Qiagen, Valencia, CA) and an established protocol.²⁰

Near-infrared dye, NIR-830 maleimide, was synthesized from IR-783 (Sigma-Aldrich, St. Louis, MO) in two steps in a manner described before.^{27,28} Mouse ATF peptides were labeled with the NIR-830 dye through free thiol groups on the peptides. NIR-830-dye-labeled peptides were then conjugated to amphiphilic polymer-coated and 10 nm core size magnetic

iron oxide nanoparticles (IONPs) via cross-linking of carboxyl groups of the amphiphilic polymer to the amino side groups of the peptides. Unconjugated peptides were removed by washing with 100 k spin columns for three times.

V. RESULTS AND DISCUSSION

V.A. Simulations

Numerical simulations were performed to test the performance of our reconstruction method under an ideal condition (i.e., no experimental noise involved). The 3D finite element mesh used, consisting of 1485 nodes and 6652 tetrahedron elements, had a dimension of $20 \times 20 \times 10 \text{ mm}^3$. In the simulations, 10 source and 15 detector positions were used. Time needed for image reconstruction was less than 1 min per iteration with a 3.00 GHz Intel Pentium 4 CPU computer. Two cylindrical targets (2mm in diameter and 2 mm in height each) were located at $(-4, -4)$ and $(4, 4)$ in XY at different depths (3, 5, and 7 mm). The absorption and scattering coefficients used for both the targets and background were 0.01 and 0.7 mm^{-1} , respectively. $\mu_{a_{x-m}}$ for the targets and background were 0.15 and 0.015 mm^{-1} (i.e., a contrast of 10:1), respectively. Reconstructed XZ slices with $Y=4$ and $Y=-4$ are presented in the first and second rows in Fig. 2. The exact location and shape of the targets are indicated by the boxes for easy comparison.

We found that the two targets can be reconstructed reliably for all the three cases. The errors of the recovered images along X-Y plane are less than 0.5 mm at all depths. The reconstructed targets are slightly shifted away from the actual positions, which are most likely the result of the limited angle and number of source/detector positions given the reflection-mode used. The average position error in Z direction is less than 1 mm.

V.B. Phantom experiments

Heterogeneous phantom experiments were performed to demonstrate the feasibility of our handheld FMT system. The

FIG. 2. Reconstructed XZ slices with $Y=4$ and $Y=-4$ are presented in the first row and second row for target depth of (a) 3 mm, (b) 5 mm, and (c) 7 mm. The empty boxes indicate the size and shape of the targets.

FIG. 3. Reconstructed tomographic fluorescence images from phantom data for target depth of 1.5 (first row), 3 (second row), 5 (third row), and 7 mm (fourth row). The empty circles and boxes indicate the exact target position.

background phantom with a volume $20 \times 20 \times 10 \text{ mm}^3$ was composed of 0.7% Intralipid, 0.034% India ink and 2% Agar powder providing $\mu_a = 0.01/\text{mm}$ and $\mu'_s = 0.7/\text{mm}$.^{29,30} Two cylindrical solid targets with a diameter of 3 mm and a height of 3 mm each were embedded in the cubic background phantom. The targets contained 1 μM of ICG dye (peak emission at 830 nm) while no ICG was added to the background. Four target depths (1.5, 3, 5, and 7 mm) were examined. The two targets were centered at $(-4, -4)$ mm and $(4, 4)$ mm along

XY mm with about 11 mm apart. The targets had the same scattering coefficient as the background and their absorption coefficient was contributed by both ink and $\mu_{a_{\text{ICG}}}$. The integral time used for CCD exposure changed from 100 to 600 ms to allow the largest SNR for each experiment while avoiding saturation of CCD camera.

The reconstructed images along the X-Y plane for the different target depths are shown in the first column of Fig. 3. The circles in the first column indicate the exact position of

FIG. 4. (a) X-ray/planner fluorescence image of a mammary tumor in the mouse using the Kodak Fx *in vivo* imaging system 48 h after systemic administration of NIR-830-ATF-IONP probes and (b) Photograph of the tumor in the mouse and a transverse slice of the recovered tomographic fluorescence image.

FIG. 5. MRI coronal images (top row, a–f) and reconstructed transverse slices of tomographic fluorescence images (bottom row, a–f).

targets. For target depths of 1.5, 3, and 5 mm, the two targets can be clearly imaged along the X - Y plane. Furthermore, the X - Y positions of the targets are accurate at the 1.5, 3, and 5 mm target depths. For target depth of 7 mm, the edges of the two targets were merged together while the centers of the two targets are still separable.

The second and third columns of Fig. 3 give the recovered images along the X - Z ($Y = 4$ mm) and Y - Z ($X = -4$ mm) planes, respectively. For target depth of 1.5 mm, the depths of reconstructed targets are accurate. With increased target depth, the error of the recovered images in depth direction becomes larger. We found that these errors were less than 1 mm for target depths of 3 and 5 mm, while it was ~ 5 mm for the 7 mm depth case.

V.C. Mouse experiments

To determine the accuracy in imaging tumor margins *in vivo* using the handheld 3D-FMT system, we used a mouse mammary tumor model. The Balb/c mice bearing orthotopic 4T1 mouse mammary tumors received a tail vein injection of 100 pmol of NIR-830-ATF-IONPs. Twenty-four hours after injection, planar fluorescence imaging was performed on the mice using Kodak FX *in vivo* imaging system. The mammary tumor showed strong a NIR signal [Fig. 4(a)].

The hair of the mouse was then removed using hair removal cream. The time for a full set of data collection for FMT was about 30 s (2.5 s CCD exposure time for each source position). MRI coronal images obtained from T_2 -weighted MRI using a 4.7 T MRI scanner are used to validate our reconstructed FMT images.

Figure 4(b) shows the photograph of the mouse and the recovered tomographic image along the skin of the mouse where the black empty box indicates the imaging area (20×20 mm²) and the tumor (the dark region). The image shown to the right of the box is the reconstructed FMT image at $Z = 0$ mm. We found that reconstructed FMT images correlated with the shape, position and size of the tumor very well.

Figure 5 presents the FMT images in transverse slices (bottom row), in comparison with the coronal MRI images of the same mouse (top row, T_2 -weighted images with a slice thickness of 1 mm) where the bright lines in the MRI images indicate the tumor margin. As shown in Fig. 5, the FMT images agree well with the MRI images. The MR images show a tumor size of 11 mm along X direction, while the

FMT images give a tumor size of 10.5 mm in the same direction. We also note that the size and shape of the tumor at each slice of the MRI and FMT images vary consistently. Since the tumor was compressed slightly during the FMT exam, some discrepancy in tumor shape is observed between the MRI and FMT images. For the purpose of comparison, we calculated the recovered whole volume of the tumor using both modalities. MRI gives a tumor volume of ~ 332 mm³, while FMT measures a tumor volume of ~ 285 mm³.

VI. CONCLUSIONS

In summary, we have described and examined a handheld FMT system for intraoperative tumor imaging. The simulation and phantom results indicate that our system can accurately reconstruct targets with a depth of 5 mm. The *in vivo* results with the novel uPAR targeted NIR probes agree well with the MR images. We are conducting a series of experiments to verify the sensitivity and resolution of the proposed system by varying the size and contrast of the targets. While the results shown are encouraging, improvements are certainly needed for better performance. First, more optic fibers can be added to the handheld probe to improve the depth resolution of reconstructed images. Second, a flexible probe would fit the surgical areas better. This can be realized by using flexible material to hold the optical fibers, such as the materials used in clinical endoscopes. Finally, real-time imaging can be achieved by high speed data acquisition and processing which makes the clinical application of a handheld system practical.

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