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Multi-angle projection imaging of short-wave infrared (SWIR) fluorescence for small animal optical tomography

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Abstract

Short-wave infrared imaging in tissue in the 1000–2000 nm range is characterized by reduced photon scatter and comparable or higher absorption compared to the NIR-I regime. These characteristics have implications for the performance of fluorescence molecular tomography (FMT) techniques, potentially improving the resolution of sub-surface structure, possibly at the expense of depth sensitivity. To examine these questions, we have developed a SWIR small animal fluorescence tomography system. This instrument acquires multi-angle SWIR projection images of a stationary platform through a rotating gantry technique. These images are then processed for tomographic reconstruction of the SWIR fluorescence activity. Herein, we describe the development of this system and show multi-angle images from a mouse carcass containing a SWIR-specific fluorophore inclusion.

Keywords

SWIR tomography; NIR II window; medical imaging; fluorescence image reconstruction

1. INTRODUCTION

Photons within the second infrared window (SWIR), generally considered between 1000–2000 nm, exhibit reduced photon scatter in biological tissue when compared to photon interactions in the NIR-I window.^{1–4} Recognizing the potential impact these properties could have on image resolution, research focused on imaging of SWIR fluorescence for biomedical applications has accelerated in recent years. Most of these efforts examined the use of SWIR imaging in an epi-illumination geometry for small animal or surgical guidance; however, imaging of SWIR fluorescence may also be exploited for tomographic reconstruction, analogous to the widely reported advances in fluorescence molecular tomographic (FMT) techniques in the NIR-I window.^{5,6} While FMT in the NIR-I is generally based off the Standard Diffusion approximation, photon propagation in the SWIR regime may not adhere to this approximation, requiring other modeling approaches, such as Monte Carlo. Early studies examining SWIR-FMT in a phantom have been reported previously.^{7,8} Herein, we report on a rotating-gantry style FMT system designed to image multiple projections of SWIR fluorescence from small animals to be used in SWIR-FMT. Projections and sinograms from a mouse carcass containing SWIR-specific fluorophores are presented and discussed.

2. METHODS AND MATERIALS

2.1 Multi-Angle SWIR Imaging System Specifications

A rotating-gantry approach was taken for the development of this SWIR tomographic imaging system. As seen in Figure 1, a gantry which included both the excitation source and the SWIR camera system were both mounted on a rotating table which was able to acquire projection images through a full 360 degree range. The table position was controlled by a rotary stage (Velmex Inc., Bloomfield, NY), a high-precision device with a large load capacity. The axis of this device is an open cylinder, allowing a stationary post to be mounted directly along the axis of rotation. In this configuration, the stage/table upon which the imaging system is mounted rotates around the stationary post. The excitation source and the SWIR camera were positioned at 90 degrees apart from one another, and this orientation was conserved as the gantry rotated through all angles.

The imaging target field was illuminated by a 40 degree con-shaped light field produced from a 785 nm laser (World Star Tech, Markham, Ontario, Canada) that was fiber-coupled to an achromatic lens (Thorlabs, Newton, NJ). The SWIR tomographic imaging system consisted of a Newport InstaSpec IR camera (Newport Corporations, Franklin, MA) cooled to -50°C . The camera's detector was a 1024×256 pixel InGaAs transferred-electron electron-bombarded charged coupled device (CCD). Emission light passed through an 1100 nm dichroic long pass filter (Thorlabs, Newton, NJ) positioned in a filter wheel (Finger Lakes Instrumentation, Lima, NY). For each projection, laser on and laser off SWIR fluorescence images were acquired, as well as excitation images and white light images using a secondary visible CMOS camera. In this experiment, we acquired projections in 1 degree increments and all SWIR images were acquired with an 80 ms exposure time.

As an imaging target, a deceased mouse carcass was mounted in the center of the rotating gantry and remained stationary throughout the data acquisition procedure (Figure 2). An $\frac{1}{8}$ inch diameter polyethylene tube was then filled with 1 mg/ml IR-m1050 (Nirmidas Biotech, Inc., Palo Alto, CA), a SWIR-specific fluorophore diluted in phosphate-buffered saline, and inserted into the throat of the carcass. As seen in Figure 2, the mouse was mounted onto a stationary bite bar mounted on an overhead bracket to minimize any occlusion of the imaging region of interest (i.e. the mouse throat). The tethering system placed below the upper arms of the mouse held the body in a rigid position throughout the image acquisition.

During the acquisition of each fluorescent image at every angle, a dark light image was also acquired and used for back subtraction in the fluorescence image. After a full rotation through 360 degrees to acquire both the fluorescence image and the excitation image, a flat field correction was also applied to the fluorescence image to compensate for the orthogonal direction of excitation light. As seen in Figure 3 below, by registering the fluorescence image with the excitation image then dividing the fluorescence image by this excitation image, it was possible to recover a fluorescence image of an fluorophore tube inclusion (1 mg/ml IR-m1050 diluted in PBS) submerged within an 1% intralipid PBS solution.

3. RESULTS AND CONCLUSIONS

Figure 4 shows representative projection images of the mouse phantom with the fluorophore tube inclusion. The $\frac{1}{8}$ th inch fluorophore tube inclusion can be seen with clear detail in Figure 4(b), while the fluorescence emission is mostly attenuated by the time it reaches the back surface through ~15 mm of tissue (Figure 4(d)). In the sagittal views, the fluorophore signal is brighter in Figure 4(c) with the tube closer to the imaged surface than in Figure 4(a) with the tube slightly further away to the imaged surface.

Sinograms of the projection data are provided in Fig. 5. Image reconstruction of SWIR fluorescence activity involves computing an excitation field using the diffusion equation and reconstructing the SWIR fluorescence yield. The latter must consider the sub-diffuse regime of photon propagation in the SWIR, and can be based on Monte-Carlo modeling or back-projection techniques with deblurring algorithms. Evaluation of these approaches is ongoing.

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a) Gantry Aerial View **b)** **Figure 1.**

(a) Aerial view and (b) photograph of SWIR tomography gantry set-up

Figure 2.
Mouse mounted on bite bar for minimal occlusion of the region of interest

Figure 3. Cylindrical phantom with tube inclusion demonstrates image correction process of dividing the fluorescence image by the excitation image to correct for non-uniform excitation of the imaging field.

Figure 4.
Corrected fluorescence projection images overlaid onto monochromatic images of mouse at varied angles of acquisition

Figure 5.

(a) Example SWIR fluorescence projection image and sinograms created from all projections of a selected row