Through-skull vasculature assessment using fluorescence brain imaging on murine models at around 800 nm

Hanh N. D. Le, Yung-Tian A. Gau, Arman Rahmim, Dean F. Wong, Dwight E. Bergles and Jin U. Kang

Department of Electrical and Computer Engineering, Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218, USA;
The Solomon H Snyder Department of Neuroscience, Johns Hopkins University, 725 N Wolfe Street # 813, Baltimore, MD 21205, USA;
Department of Radiology, 601 North Caroline Street, Johns Hopkins University, Baltimore, MD 21287, USA.

ABSTRACT

We describe a scanning near-infrared fluorescence imager for through-skull non-invasive brain imaging on live murine models. The captured photoluminescence feature through scattering media was enhanced using a high sensitivity scientific CMOS sensor with the obtained spatial resolution of 15.63 µm, depth of field of 5 mm and an average local signal-to-noise ratio of 37.5 dB.

Keywords: Fluorescence imaging, vasculature monitoring, brain imaging.

1. INTRODUCTION

Understanding the vasculature dynamic within brain facilitates multiple essential validation of pharmaceutical research [1, 2] and in neuroscience application [3-5]. The phenomenon of photoluminescence is widely used to monitor the highlighted sample of interest [6-8]. In brain imaging, due to the strong absorption and scattering properties from scalp, cranium, dura matter and fluids, certain sample preparations such as craniotomy, cranial windows or skull-thinning procedures are used to minimize the effect. Moreover, to enhance the emission strength from the tagged fluoresce agent, certain efforts using carbon-nanotube and quantum dot fluorescent agents with longer near-infrared emission capturing windows has been used in through-skull imaging in murine and show the capability of reaching deeper penetration depths [9-12]. Here we contribute a near-infrared fluorescence-based scanning imager design for non-invasive brain imaging on live murine model that reduces toxicity of the illuminated light power via the scanning illumination schematic. The vasculature features in the brain are monitored via the inherent photoluminescence of the injected non-toxic indocyanine green fluorescence dye. The system performance was validated and compared image quality using a scientific CMOS (sCMOS) camera operating around 800 nm.

2. METHOD

2.1 Experimental setup

As indicated in Figure 1, the laser-scanning fluorescence imaging system consists of a 780-nm diode laser (70 mW) as the excitation source for the near infrared fluorescence dye. The laser is scanned in
horizontal and vertical directions using a 2D galvo system with two Galvo mirror GM1 and GM2 (GVSM002, Thorlabs, New Jersey, USA) and the scanned light is directed through a dichroic mirror (DM) with a cut-off wavelength at 806 nm (DMSP805R, Thorlabs, New Jersey, USA). The excited light absorbed by the target indocyanine green dye (ICG, Sigma Aldrich, Missouri, USA) creates the emittance of fluorescence signal at longer wavelength. This fluorescence at deeper layer in the sample is collected through an emission filter (EM, 850-nm long pass filter (FEL0850, Thorlabs, New Jersey, USA)) and the collected fluorescence is imaged onto a sCMOS sensor (Hamamatsu ORCA-Flash4.0 sCMOS camera) or a CMOS sensor (BasleracA2000-50gmNIR) through an imaging lens (IL). This long pass filter can be alternated with other long-pass filter or band pass filter with cut-on wavelength above 805 nm and covers the emission range of the prepared ICG solution for a desired target of interest. Study with different near-infrared windows for emission capturing at specific layer of interest through scattering media such as mouse brain have been proposed in [12]

![Near-infrared fluorescence imaging setup and its schematic.](image)

2.2 Sample preparation

a. *In vitro* experiment:
To validate our setup performance in capturing fluorescence through scattering medium, we designed several phantoms from mixtures of indocyanine green solution, ethanol and glue. The phantom is attached behind a human cadaver temporal bone with an average thickness of 1.75 mm as the fluorescence target for imaging.

b. *In vivo* experiment:
Experiment was carried out in strict accordance with protocol MO14M390 approved by the Animal Care and Use Committee at Johns Hopkins University. A C57BL/6N mouse aged P84 (Charles River) was anaesthetized by i.p. injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). The animal’s head is shaved and then placed in a stereotaxic frame on a heating blanket. The respiratory rate and body temperature are monitored every 15 minutes throughout the procedure. Sterile ointment is placed on both eyes. The surgical area is disinfected with three alternating swabs of alcohol and betadine. An incision is made along the midline of the scalp, subcutaneous tissue and periostium cleaned and removed and skull exposed for imaging. To identify blood vessel in mice brain, ICG powder is reconstituted in saline (10 mg/mL 0.9% NaCl). After the scalp incision, 50 µL of the prepared ICG solution is injected into the lateral tail vein of the animal. For the vascular
monitoring, top-view of the animal’s head is imaged right after the injection using the proposed near-infrared imaging setup.

2.3 Image processing

Due to the nature of the scanning imagery and the emission signatures of the tested dye, fluorescence signal is collected and highlighted within the scanned regions under the light illuminated section. The scanning regions covers an area of 12 mm-by-12 mm with each scanning step of 0.63 mm. All scanning image frames are combined by summation to result in the full fluorescence frames as displayed in Figures 3-5. The signal-to-noise ratio (SNR) in dB is computed as the ratio between the difference of average signal value and the average background value with the root-mean-square of the noise level.

3. RESULTS

We first validate our optical imaging capability by testing the spatial and lateral resolution using a standard positive 1951 USAF test target (R3L3S1P, Thorlabs, New Jersey, USA) and a depth of field target (DOF 5-15, Edmund Optics Inc., New Jersey, USA), respectively. The horizontal and vertical line pattern from group 5, element 1 is recorded in Figure 2 indicating the resolved line width of 15.63 µm and the DOF of the setup is indicated as within 5 mm to 6 mm range (Figure 3).

Fig. 2. (a) Reflectance image of a USAF resolution target. The scale bar represents 2 mm; (b) The intensity profiles along the lines indicated in red box in (a).
Next, we experimented fluorescence imaging through a cadaver human temporal bone. Inasmuch as a high signal-to-noise ratio is essential in low light and strongly diffusive systems, such as through-bone imaging, we also conducted a comparison study between a standard and a highly sensitive CMOS sensor in detecting ICG signal through a human head bone (Figures 4 and 5). The results indicate apparent features of the ICG phantoms behind the bone for both sensor types. When the phantoms are placed behind the skull, due to the highly scattering nature of the skull, the images became highly diffusive. This effect can be enhanced by using an iterative procedure such as the Richardson–Lucy deconvolution or a highly sensitive sCMOS sensor improve the SNR of the image. The SNR captured using a CMOS and enhanced by the deconvolution method in Figure 4 was calculated as an increase from 18.55 dB to 28.48 dB; and the SNR improvement using a sCMOS camera as indicated in Figure 5 is increased from 22.45 dB to 37.37 dB.
Due to the outperformance of the sCMOS, we further performed in vivo fluorescence capturing of mice brain through skull bone using the above-mentioned sCMOS camera. We injected ICG into the tail vein of mice and concurrently imaged the exposed mice skull using the setup described in Fig. 1. As indicated in Figure 6, in reference to the white reflectance image, the collected fluorescence image successfully highlights the detailed vasculature surrounding the sagittal suture, the lambdoid suture and the lambda. The detailed vascular structures are observed clearly in two zoom-in ROIs illustrated in Figure 6e-f with an average SNR of 37.5 dB.
Fig. 6. Upper row: White reflectance images of (a) an exposed mice skull with indicated ROIs and (b, c) their corresponding zoom-in regions for ROI 1 and ROI 2. Lower row: Fluorescence images of mice vascular structures after ICG injection (d) corresponding to (a) and (e, f) corresponding to (b, c) for ROI 1 and ROI 2, respectively.

4. DISCUSSION AND CONCLUSION

Fluorescence imaging through scattering media such as skull bone successfully demonstrated using both in vitro and in vivo experiments on murine model. This confirms the ability to minimize the invasive sample preparations such as craniotomy, cranial windows or skull-thinning procedures while maintaining an adequate brain imaging quality. The proposed setup utilizing the scanning scheme of a laser diode, which induces a highly concentrated power of laser circular beam with diameter of 2 mm, however, prolongs the imaging collection time. Our future setup development will involve the use of a more uniformly distributed light source such as from a high power light emitting diode for a single-shot capturing of the fluorescence event, thus, assists the real-time monitoring of vasculature dynamic and neural circuit functions in the brain.

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REFERENCES


