Quantitative and Depth-Resolved Fluorescence Techniques for Intraoperative Guidance of Brain Tumor Resection Surgery

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Abstract: We have developed a handheld fiberoptic probe for tissue fluorescence quantification and a technique to produce depth-resolved maps of sub-surface tumor fluorescence, to elaborate upon intraoperative fluorescence guided resection of brain tumor.

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1. Introduction
The clinical management of malignant glioma continues to be a challenge. The prognosis is poor—for glioblastoma multiforme, the most virulent of these brain cancers, median survival is 1 year [1]. Surgical resection of the tumor is the first line of defense. There is a significant survival advantage in patients who undergo >98% removal of the tumor, relative to those with a resection <98% [2]. Achieving complete resection is limited by the difficulty of visualizing residual tumor after de-bulking. Moreover, aggressive resection at the tumor margin must be tempered by the need to preserve normal brain tissue. Intraoperative fluorescence guidance is a promising candidate to better visualize residual tumor cell nests that are occult under white light. The most clinically developed form uses protoporphyrin IX (PpIX) fluorescence, the precursor to heme in its biosynthesis which preferentially accumulates in tumor cells with the administration of δ-aminolevulinic acid (ALA). PpIX-mediated fluorescence guidance has been shown to lead to an increase in tumor resection completeness when compared with standard white light resection [3]. Challenges remain in quantitatively assessing the fluorescence to reduce variability of outcome and potentially improve tumor detection specificity, and in observing tumor fluorescence lying beneath the tissue surface to improve tumor resection completeness. To these ends, our group is developing intraoperative techniques to 1) quantify the local fluorescence signal using a handheld fiberoptic probe and 2) improve detection by reconstructing the depth-resolved, sub-surface tumor fluorescence topography using multispectral excitation imaging. This paper reviews our work-to-date on these two different but related techniques.

2. Methods

**Handheld fiberoptic probe for fluorescence quantification:** Fluorescence quantification is achieved using a sterilizable, custom-built, handheld fiberoptic probe (Fig. 1A) designed to capture tissue fluorescence and white light reflectance spectra. In this work, the fluorescence spectrum is obtained at \( r = 260 \) µm source-collector distance. White light reflectance is obtained at \( r = 260 \) and 520 µm, and used to extract tissue optical absorption and elastic scattering properties using a spectrally-constrained diffuse reflectance method. A fluorescence model based on diffusion theory was used to correct for the distortions in the detected fluorescence emission caused by variation in the tissue optical properties. The resulting corrected fluorescence spectrum is directly proportional to the fluorophore concentration. The details of the fluorescence model and technical development of the probe will be elucidated in detail in an upcoming full article.

The probe tip geometry is shown in Fig. 1B. The fibers are epoxied into a stainless steel tube and ground flat. The tube is affixed to a stainless steel handle with the fiberoptic lead extending 3 m away to a control system that directs the optical signals. Inside the control system, LEDs (LEDengin, Inc.) provide the white light sources for the reflectance measurements and the 405 nm source for fluorescence excitation. The detector fiber in the probe is connected to a spectrometer (USB2000+: Ocean Optics, Inc.) to acquire the tissue fluorescence and reflectance. The control system consists of these elements in an enclosure (see Fig. 1C). A custom Labview (National Instruments Corp.) application is used to drive the control system. A measurement consists of a fluorescence spectrum, white light reflectance spectrum (450-720 nm) and background spectrum taken in sequence, with an acquisition time of ~0.5 s. The instrument met electrical requirements for the Canadian Standards Association.

Phantom experiments were performed for quantitative fluorescence validation. Intralipid fluid was used to provide scattering for the phantoms, yellow food coloring was used as an absorber and PpIX was used as the
fluorophore. A set of 9 phantoms were formulated, giving a range of optical absorption and scattering properties. PpIX was mixed in 6 concentrations (5, 2.5, ..., 0.15625 μg/mL) for each set of 9 phantoms, for a total of 54 phantoms. Probe measurements were taken in each phantom and the quantification algorithm applied to extract the PpIX concentration.

Multispectral excitation imaging for depth-resolved fluorescence: Our group is also developing a technique to recover depth-resolved maps of sub-surface fluorescing tumor by using the endogenous tissue absorption contrast that is dominated by hemoglobin. Different colors of light penetrate to varying depths due to the wavelength-dependence of tissue absorption. We exploit this to map the contour of the closest surface of a buried fluorescent object, reconstructing what we refer to as the sub-surface fluorescence topography of the tumor. This is achieved by capturing fluorescence images at different excitation wavelengths and fitting the data to a diffusion theory model of light transport in tissue. The details of the physical modeling and the depth-extraction technique will be presented in a forthcoming full article.

The proof-of-concept setup consists of an epifluorescence microscope (MZ FLIII: Leica Microsystems) custom-fitted with a filter wheel that filters a white light source for the fluorescence excitation. Excitation bandpass filters were fitted into the filter wheel with central wavelengths at 405, 495, 546 and 625 nm, approximately corresponding to PpIX absorption peaks. A cooled CCD camera (CoolSnap K4: Photometrics) mounted on the microscope imaged the fluorescence emission. A 700 nm bandpass filter was used to block the excitation light and pass the fluorescence emission to the camera. A custom Labview program was developed to automatically select the excitation filters and acquire the corresponding images. In order to test the technique in vivo, female Lewis rats were used, under institutional ethics approval (University Health Network, Toronto). Intracranial brain tumors were induced by injection of 1.5x10^5 CNS-1 cells through a burr hole in the left hemisphere. Tumors were allowed to grow for 7-10 days. On the day of surgery, ALA was injected i.p. at 100 mg/kg, with imaging scheduled 3.5-4 hours later. The animal was brought under general anesthesia and a 1 cm dia. craniotomy was performed, exposing both hemispheres, including the tumor cell injection site. Multispectral excitation images were taken in vivo. After sacrifice by anesthetic overdose, the brain was removed intact and coronal H&E histology sections were prepared.

3. Results

Handheld fiberoptic probe for fluorescence quantification: The quantitative fluorescence model was applied to the data from the 54 phantoms, with the results plotted against PpIX concentration. Fig. 2A shows the uncorrected fluorescence intensities at 635 nm, compared to the estimated PpIX concentration shown in Fig. 2B, calculated using the fluorescence model, removing the effects of optical property variation.

![Fig. 1. (A) Photograph of the fiberoptic probe; (B) tip geometry; (C) control system, probe and data acquisition computer](image)

![Fig. 2. (A) Measured fluorescence, F_{x,m}, at the 635 nm peak of PpIX emission and (B) estimated PpIX concentration using the fluorescence model for phantoms A-I, indicated by the symbol legend. The excitation optical properties are shown in the legend (x denotes excitation). The dashed line in (A) represents the best straight line fit through the origin; the dashed line in (B) is the unity line. R^2 values are likewise calculated from (A) the best fit and (B) the unity line.](image)
We are working with colleagues at the Dartmouth-Hitchcock Medical Center (Lebanon, NH, USA) to translate these and additional novel diagnostic techniques into the operating room. The fiberoptic probe is in active clinical use in brain tumor resection clinical trials currently underway to evaluate the probe as an intraoperative aid for delineating tumor margins.

**Multispectral excitation imaging for depth-resolved fluorescence**: Fig. 3 shows results from two animals, in the first of which the tumor was fully sub-surface, while in the second it was largely located on the cortical surface. Depth-resolved topographic images were derived and are shown in panels B and E, with the corresponding white light images in panels A and D. The corresponding H&E-stained histology sections in panels C and F illustrate the location of the tumors relative to the tissue surface. Phantom experiments were also carried out (to be presented in the forthcoming full article) to quantify accuracy of the technique.

![Fig. 3. Images of rat brain tumor in vivo](image)

**4. Summary and Future Directions**
We report progress-to-date of two techniques to elaborate upon the current state-of-the-art of fluorescence guided resection: 1) quantitative fluorescence using a handheld fiberoptic probe and 2) a depth-resolved technique that we refer to as sub-surface fluorescence topography. The fiberoptic probe is in active clinical use, with studies underway to evaluate the fiberoptic probe as an intraoperative tool for delineating tumor margins. The depth-resolved technique is emerging from the proof-of-concept phase, with further validation and designs for an intraoperative prototype underway.

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**6. References**
