

Optical Touch Pointer for Fluorescence Guided Glioblastoma Resection Using 5-Aminolevulinic Acid

Neda Haj-Hosseini, MS,^{1*} Johan Richter, MD,^{1,2} Stefan Andersson-Engels, PhD,³ and Karin Wårdell, PhD¹

¹Department of Biomedical Engineering, Linköping University, Linköping 58185, Sweden

²Department of Neurosurgery, Linköping University Hospital, Linköping 58185, Sweden

³Department of Physics, Lund University, Lund 22100, Sweden

Background and Objective: Total tumor resection in patients with glioblastoma multiforme (GBM) is difficult to achieve due to the tumor's infiltrative way of growing and morphological similarity to the surrounding functioning brain tissue. The diagnosis is usually subjectively performed using a surgical microscope. The objective of this study was to develop and evaluate a hand-held optical touch pointer using a fluorescence spectroscopy system to quantitatively distinguish healthy from malignant brain tissue intraoperatively.

Study Design/Materials and Methods: A fluorescence spectroscopy system with pulsed modulation was designed considering optimum energy delivery to the tissue, minimal photobleaching of PpIX and omission of the ambient light background in the operating room (OR). 5-Aminolevulinic acid (5-ALA) of 5 mg/kg body weight was given to the patients with a presumed GBM prior to surgery. During the surgery a laser pulse at 405 nm was delivered to the tissue. PpIX in glioblastoma tumor cells assigned with peaks at 635 and 704 nm was detected using a fiber optical probe.

Results/Conclusion: By using the pulsed fluorescence spectroscopy, PpIX fluorescence is quantitatively detected in the GBM. An effective suppression of low power lamp background from the recorded spectra in addition to a significant reduction of high power surgical lights is achieved. *Lasers Surg. Med.* 42:9–14, 2010.

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Key words: background light suppression; fluorescence spectroscopy; glioblastoma multiforme; intraoperative

INTRODUCTION

The highly malignant brain tumor, glioblastoma multiforme (GBM) is difficult to fully resect under direct vision in the operating field. The challenge is due to GBM's infiltrative way of growing and its morphological similarities to the surrounding functioning brain tissue. A higher extent of tumor resection is reported to be associated with a significant survival advantage [1]. Magnetic resonance and/or computed tomography images are taken before the surgery for observing the location and form of the tumor but still the important task of identifying tumor margins is based on intraoperative tissue palpation and visual inspection under the surgical microscope.

Fluorescence guidance using endogenous [2,3] and exogenous [4,5] types of fluorophores for brain tumor detection have previously been reported. Stummer et al. [6,7] have evaluated a fluorescence microscopy system for guided resection of malignant gliomas. Protoporphyrin IX (PpIX) fluorescence induced by a dose (20 mg/kg body-weight) of 5-aminolevulinic acid (5-ALA) was used for intraoperative fluorescence microscopy guidance during the resection. However, utilization of fluorescence labeling and information is still far from the norm in clinical practice. Thus, further research and development of clinically suitable fluorescence methods is essential.

Our group previously used 5-ALA-induced fluorescence and MR spectroscopy during stereotactic biopsies of human glioblastomas [8] and recently a first prototype of a fiber-optic-based fluorescence spectroscopy system was introduced for guided neurosurgical tumor resection [9,10]. The significances of these studies are that the measurements are objective and a relatively low dose of ALA (5 mg/kg) provokes detectable fluorescence peaks related to the malignant tumor tissue. This drug passes the leaky blood–brain barrier (BBB) in the tumor but not the intact BBB in normal brain tissue and is converted to the fluorescence marker PpIX in the malignant cells [11,12]. PpIX is a natural substance in the heme cycle which is rapidly eliminated from the body. Laser light at 405 nm is absorbed by PpIX. The molecules of PpIX re-emit fluorescence with peaks at 635 and 704 nm.

A major issue with fluorescence monitoring is the photobleaching of PpIX under light exposure. Therefore, a separate study was undertaken where the measurement

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*Correspondence to: Neda Haj-Hosseini, MS, Linköping University Hospital, Linköping 58185, Sweden.

E-mail: nedha@imt.liu.se, neda580@hotmail.com

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parameters were optimized for obtaining minimal photobleaching effect during fiber optical measurements of fluorescence spectra [13]. Another problem in many applications involving optical measurements in the operating room (OR) is the superimposition of surrounding light sources including surgical lamps and microscope on the collected signal. The aim of the present study is to introduce the concept of an optical touch pointer for fluorescence guided resection of brain tumors. The system performance, and possibility to reduce surrounding light by system modulation, has been evaluated in the OR during surgery.

MATERIALS AND METHODS

System Setup

An optical touch pointer for on-line fluorescence spectroscopy measurements was set up (Fig. 1a). The components comprise a near-UV laser module with the maximal excitation light at 405 nm and maximal power of 50 mW (Oxxius SA, France). The laser operates either in continuous or pulsed mode modulation. A spectrometer (EPP 2000, Stellarnet) with a 2,048 element CCD operating in the range of 240–850 nm wavelengths and with a resolution of 3 nm is used for light read out. To suppress the back-reflected light from reaching the spectrometer, a long pass, cutoff filter of 450 nm (Schott CG-GG-475-0.50-3, CVD) is fixed in front of the detector slit of the spectrometer.

Excitation light is brought to the tissue through a hand-held fiber optical probe ($l_{\text{probe}} = 12 \text{ cm}$, $\varnothing = 2 \text{ mm}$, $l_{\text{cable}} > 4 \text{ m}$) having one transmitting fiber ($\varnothing_{\text{core}} = 600 \mu\text{m}$,

$\varnothing_{\text{cladding}} = 640 \mu\text{m}$, numerical aperture = 0.37) and nine receiving fibers ($\varnothing_{\text{core}} = 200 \mu\text{m}$, $\varnothing_{\text{cladding}} = 240 \mu\text{m}$, numerical aperture = 0.22). The receiving fibers are matched to fit the slit configuration of the detector. A miniature micro-positioner (OFR, Inc., Caldwell, NJ) was mounted at the interface of laser and fiber probe in order to allow the alignment of the laser light towards the fiber probe. The laser effect, pulse generation and its synchronization are controlled by a DAQ-card (National Instruments, Inc.) and software developed in LabVIEW[®] (Version 8, National Instruments, Inc.). The different hardware components of the system are mounted in a compact box of $31 \times 25 \times 21 \text{ cm}^3$ which together with a laptop is easily carried on a trolley to the OR. The total weight of the box is 4.6 kg.

System Performance

The system was programmed to generate the pulses simultaneously with the spectrometer data collection. The laser pulse was set to have a constant duty cycle of 50% in order to enable a light spectrum collection followed by an equivalent dark spectrum collection. The dark spectrum is subtracted from the prior light spectrum giving a spectrum free from the background light effect (Equation 1)

$$\begin{aligned} \text{Compensated spectrum } (i) \\ = \text{Light spectrum } (i) - \text{Darks spectrum } (i) \end{aligned} \quad (1)$$

The captured spectra are presented on the monitor in real-time and saved for additional post-processing. The on-line presentation allows for an immediate control of the signal quality and possibilities for adjustments on site.

The laser pulse width, which is set to be the same as the integration time of the spectrometer, was chosen as a trade off between the detectable amount of collected light, the minimal energy given to the tissue and minimal photobleaching of PpIX. Laser power (0–30 mW), tissue exposure time (range 0.06–65.50 seconds) and the number of collected spectra can be changed through the software interface prior to initialization of a recording. Typical settings for a minimum of 2 mJ excitation at 405 nm allowing a distinguishable fluorescence signal during each spectrum, are 5 mW laser power and 0.4 seconds pulse width. Considering these time and power settings and that the probe is placed in contact with the tissue ($A_{\text{exposed tissue}} = 0.28 \text{ mm}^2$), the system gives an irradiance of 1.78 W/cm^2 and a fluence or dose of 0.71 J/cm^2 during each pulse.

The potential damage to the tissue by a laser beam occurs via tissue heating. Using Monte Carlo for light absorption and propagation, and a multiphysics program (COMSOL Multiphysics 3.4, Comsol AB, Sweden) for heat transfer simulation [14] the temperature increase was simulated using the optical and thermal parameters of brain [15,16]. The maximal temperature increase within each pulse (5 mW, 0.4 seconds) is estimated as 0.4°C .

The system is wavelength calibrated with the specific coefficients of the spectrometer provided by the manufacturer (Stellarnet) and checked against the room's fluorescent lamp with known wavelengths for registration of intensity versus

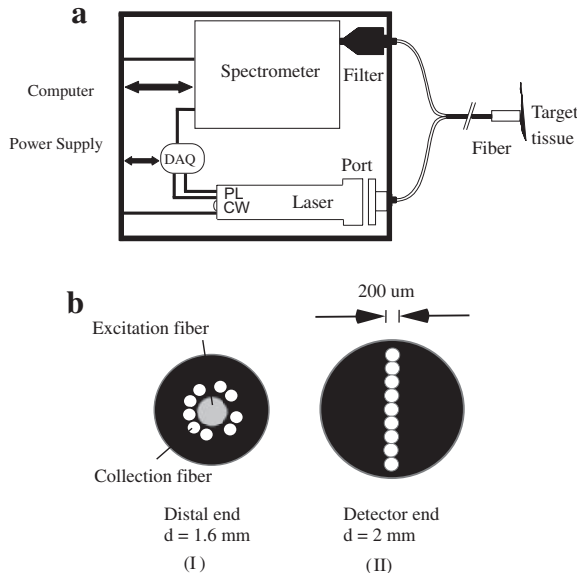


Fig. 1. **a**: Hardware design of fiber-optic based, pulsed system. **b**: Fiber probe configuration at (I) probe tip, and (II) detector end. The vacant parts are for the placement of fiber optics of different optical diagnostic applications. The excitation fiber and the surrounding collection fibers, including the cladding, have a diameter of 0.64 and 0.24 mm, respectively.

the correct wavelengths. An intensity calibration standard was produced from a sterilizable plastic material with very stable fluorescence properties. A fluorescence spectrum of this calibration standard was recorded before each set of measurements.

Evaluation of the System

Reduction of surrounding light. The suppression of background light was tested in the OR. The lamps in the OR are categorized into low power (conventional fluorescent tube) and high power (surgical lamps; Hanualux, Siemens AG, Germany, and surgical microscope; OPMI Carl Zeiss©, Oberkochen, Germany). Measurements were repeated ($n = 3$) with different pulse settings (0.1–0.5 seconds and 1–10 mW) on non-ALA treated human forearm skin on a volunteer and the fluorescent intensity standard. The distances between tissue and lamps were kept identical to the brain surgery circumstances. Five spectra were recorded with a pulse width of 0.4 seconds and excitation light of 5 mW using the fluorescent tube, surgical lamps, and surgical microscope as a background light illumination, respectively. The integration time is set long enough to average out any fluctuations due to the 50 Hz drive current for the lamps.

Measurements during tumor resection. Patients undergoing surgical resection of presumed glioblastoma were given a 5 mg/kg bodyweight of 5-ALA dissolved in orange juice 2 hours prior to skull opening. They were then anesthetized as a preparation for surgery. The fiber probe used was sterilized with the STERRAD® procedure. Measurements were approved by the local ethics committees (No: M139-07) and written informed consent was received from the patients. To allow direct comparability, measurements on one patient are presented in this study.

A measurement was performed on the calibration standard prior to the measurements on the brain as a primary check for the system operation. Measurements were then made on the surface of white, gray, and known tumor tissue. Further recordings were made 1–2 mm inside the tumor as well as before and after resection on the tumor margin. In the latter cases biopsies were taken for pathological examination.

At each measurement site five spectra were collected. This was to allow further signal analysis in the primary measurements. During the collection of the spectra, the surgical microscope was directed away from the measurement field or its light was obstructed by hand.

Data Analysis

The data were analyzed using MATLAB 7.0.1 (Math Works™, Inc.). As the PpIX photobleaches quickly and considerably between the first recorded spectra [13], the averaging of the signals has been avoided. The distance and angle of the probe relative to the tissue are not perfectly reproducible in different measurements, in addition to the fact that the source power might be varied during different measurements. To overcome this challenge, the autofluor-

escence of each spectrum (510 nm) was taken as the reference level for the corresponding PpIX peak and a ratio of intensities, *ratio number*, was calculated for each compensated spectrum as explained by Equation (2) [17].

$$\text{Ratio number of compensated spectrum } (i) = \left(\frac{I_{\text{PpIX}} - I_{\text{base}}}{I_{\text{autofl}}} \right)_i \quad (2)$$

where I_{PpIX} is the PpIX fluorescence peak at 635 nm, I_{autofl} is an average of the recorded signal around the peak which represents the peak for tissue autofluorescence, I_{base} is the autofluorescence at 635 nm and i denotes the sequence number of spectrum (Fig. 2). Compensated spectrum (i) is obtained by subtracting dark spectrum (i) from the prior light spectrum (i), see Equation (1)

RESULTS

Reduction of Surrounding Light

The measurements on non-ALA treated forearm skin in the OR with the background effect from fluorescent tubes, surgical lamps, and the surgical microscope are presented in Figure 3. The influence from the fluorescent tubes is effectively subtracted from the spectra (Fig. 3a). The influence from surgical lamps (Fig. 3b) and the surgical microscope (Fig. 3c) are reduced to a great extent.

Measurements During Tumor Resection

Measurements performed in healthy brain tissue, either white or gray, did not show any detectable sign of PpIX (Fig. 4a). Measurements performed on the tumor tissue showed a clear PpIX which is a sign of PpIX accumulation in the cancerous cells. Figure 4b shows a site measured inside the tumor where a high-grade glioma was pathologically diagnosed. The ratio number for this spectrum is 6.0. Figure 4c shows the signal from a site before and after resection. The surgeon did not review the spectroscopy results before announcing the diagnosis.

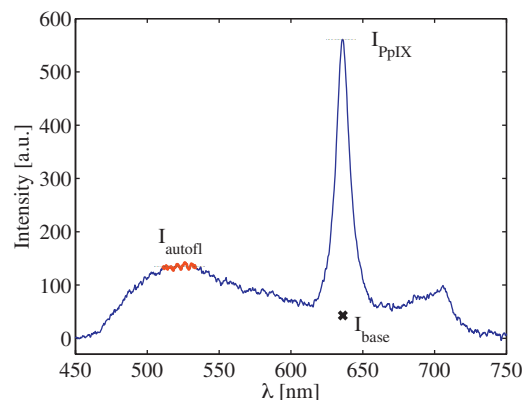


Fig. 2. A typical compensated fluorescence spectrum measured on tumor illustrating fluorescence intensity in arbitrary units versus wavelength. Peaks at 635 and 704 nm are representative of PpIX. The peak at 510 nm is due to the brain

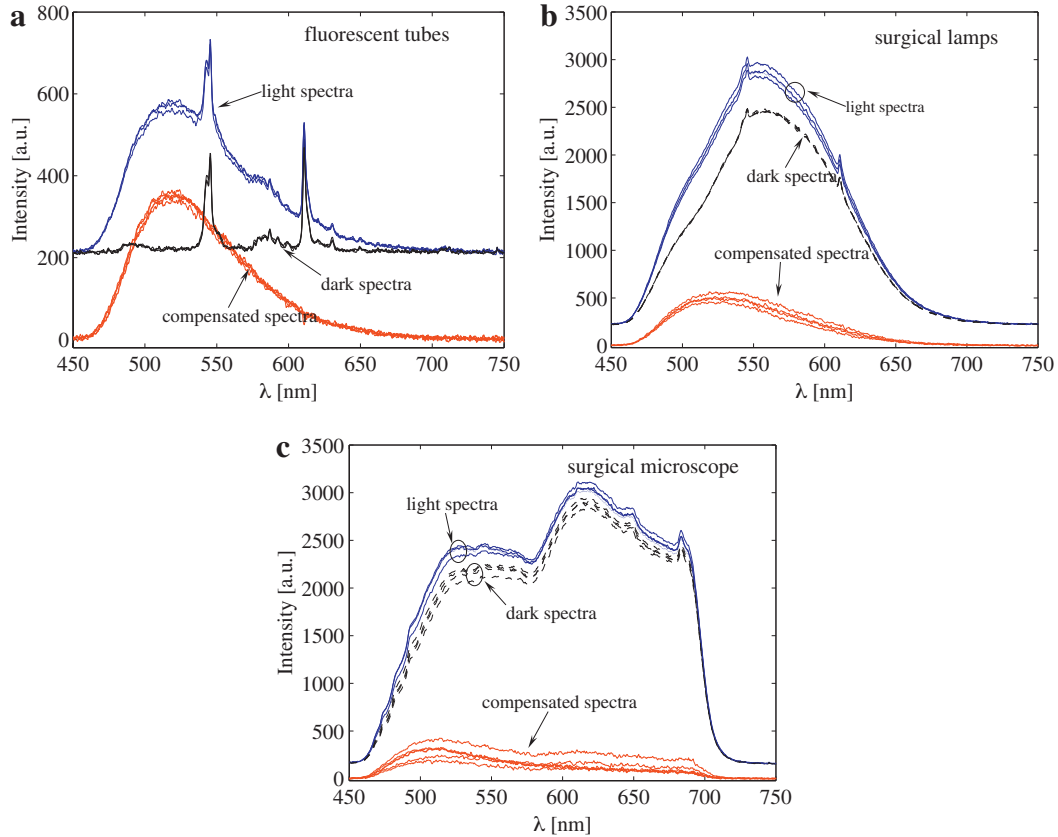


Fig. 3. Light, dark and the compensated spectra from measurements under lamps in the operating room. All measurements were performed on skin under 5 mW laser power and 0.4 seconds exposure time.

Pathological biopsy at this location showed a grade III glioma and the ratio number was calculated to 2.1. After resection no PpIX fluorescence was recorded at this site. Blood at the probe interface creates a dip in the spectra at 580 nm; however, a considerable amount of blood can block the total light propagation between the probe and the brain. In this case the probe was rinsed and the measurement was repeated. All the measurements presented in Figure 4 were performed on the same patient.

DISCUSSION

An optical touch pointer using a fiber-optic-based fluorescence spectroscopy system with continuous and pulsed modulation was developed to detect the 5-ALA-induced PpIX fluorescence in GBM. The method and the developed technique are proven to optimally detect malignant tumor tissue in a short measurement time (<1 seconds for each measurement point). Conventional biopsies with examination conducted by a specialist in neuropathology are used as the gold standard for evaluation of the fluorescence spectroscopy diagnosis at specific measurement sites.

We have been able to effectively omit the interfering light from the fluorescence tubes in the OR by using the pulsed

modulation to subsequently detect light plus background and background only, allowing the background light to be accurately subtracted. The influence of the surgical lamp and microscope background was reduced to a great extent; however, as these lamps are of high power, a slight difference between the recording of the light and dark spectra due to both pulse width differences and probe movement may result in inaccurate spectra compensation. Such effects could be avoided in the recordings by directing the high power surgical light away from the measured tissue precisely before the measurement acquisition. All other light in the OR could remain the same without influencing the measurements in any critical way. Regarding the simulated temperature increase (0.4°C) of exposed brain tissue during each pulse, it is not expected that in this specific application any clinical complication will arise due to laser heating. However, a long exposure time should be avoided.

What brings the use of this technique into clinical interest is its capability to quantitatively differentiate the malignant tissue from healthy tissue at the tumor margin. The device is easy to use and its design and construction are robust and easy to handle for the surgeon as well as for the nurses. In comparison to the well-established ALA-based tumor detection system as developed by Stummer et al.

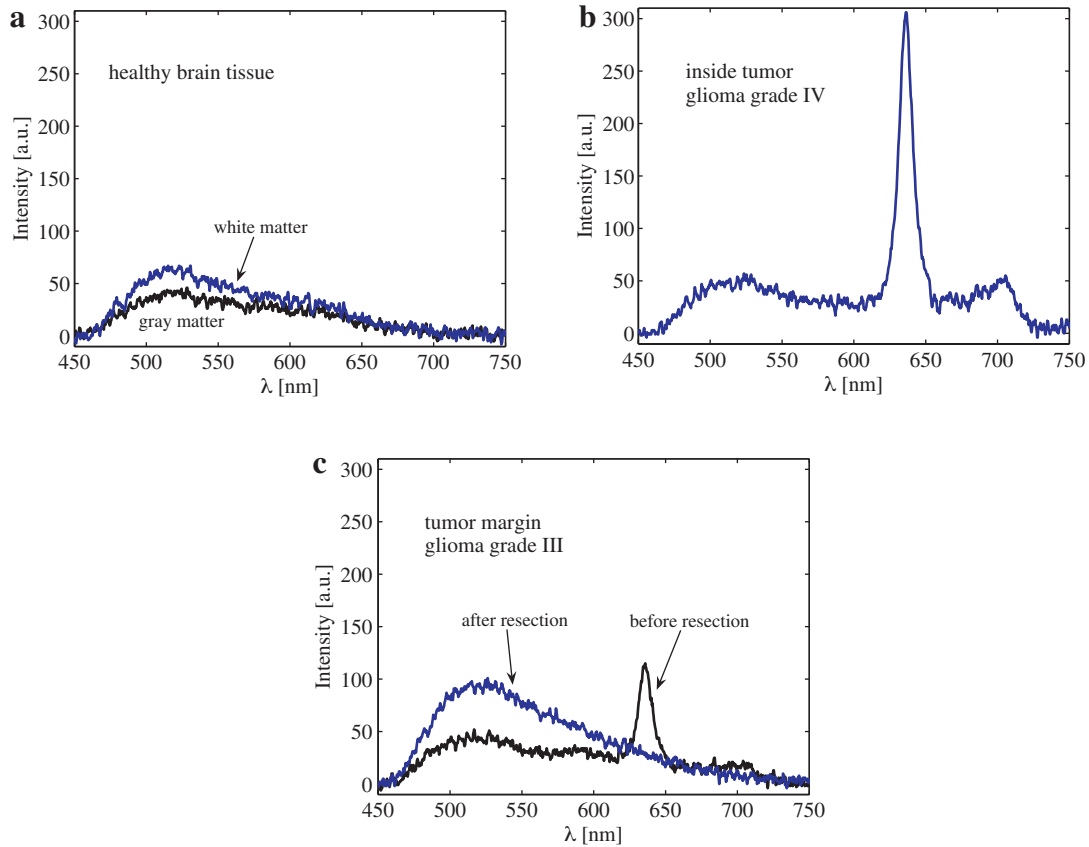


Fig. 4. Measurements performed with the pulsed system using a pulse width of 0.4 seconds and 5 mW power at 405 nm on one patient with glioblastoma multiforme. Fluorescence spectra from (a) healthy white and gray matter in vicinity of the tumor, (b) inside the tumor where pathological biopsy showed glioma grade IV, and (c) before and after resection of a site at tumor margin where pathological biopsy showed a glioma grade III. Ratio numbers for (b) and (c) are 6.0 and 2.1, respectively.

(with a filter integrated in operational microscopes), a disadvantage could be the fact that our device cannot give an overall view of the operational field. It can be discussed to what extent this is necessary, as we do not intend to reinvent the basic neurosurgical operational techniques for these specific tumor resections. However, we believe that the optical probe could be a valuable complementary method to the microscope-based system. By using this technique, even low intensities of PpIX fluorescence can be detected. Also the probe can be inserted in the tumor to investigate the tumor's extent in depth.

An objective of future clinical studies is the correlation of the PpIX fluorescence intensity with the degree of tumor malignancy. Intensity of the PpIX fluorescence is primarily evaluated by the commonly used ratio of PpIX fluorescence to autofluorescence (ratio number). Although of diagnostic value, the ratio number is accompanied by a certain amount of error. Thus, further research into alternative analysis methods would be beneficial. A more precise control over the detection resolution by performing simulations and design of an optimum probe configuration is also of interest.

Moreover, further research will include investigating PpIX uptake on the cellular level and estimation of sensitivity and specificity of the method within a larger group of patients.

In conclusion, a dual mode (continuous and pulsed) fiber-optic-based fluorescence spectroscopy system with the excitation light at 405 nm was developed. We were able to effectively detect 5-ALA-induced PpIX fluorescence in the GBM during surgical resection. The pulsed modulation of the system keeps the energy exposure to the tissue and the total measurement time short enough by synchronizing light delivery and fluorescence collection. A further benefit of this system is the omission of the background light from the lamps in the OR.

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