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Development and Characterization of a Brain Tumor Mimicking Fluorescence Phantom

Neda Haj-Hosseini^{*a}, Benjamin Kistler^{a,b}, Karin Wårdell^a

^a Department of Biomedical Engineering, Linköping University, Linköping, Sweden,

^b Institute of Medical and Analytical Technologies, University of Applied Sciences Northwestern Switzerland, Switzerland

ABSTRACT

Fluorescence guidance using 5-aminolevulinic acid (5-ALA) for brain tumor resection is a recent technique applied to the highly malignant brain tumors. Five-ALA accumulates as protoporphyrin IX fluorophore in the tumor cells in different concentrations depending on the tumor environment and cell properties. Our group has developed a fluorescence spectroscopy system used with a hand-held probe intra-operatively. The system has shown improvement of fluorescence detection and allows quantification that preliminarily correlates with tumor malignancy grade during surgery. However, quantification of fluorescence is affected by several factors including the initial fluorophore concentration, photobleaching due to operating lamps and attenuation from the blood. Accordingly, an optical phantom was developed to enable controlled fluorescence measurements and evaluation of the system outside of the surgical procedure. The phantom mimicked the optical properties of glioma at the specific fluorescence excitation wavelength when different concentrations of the fluorophore were included in the phantom. To allow evaluation of photobleaching, kinetics of fluorophore molecules in the phantom was restricted by solidifying the phantoms. Moreover, a model for tissue autofluorescence was added. The fluorescence intensity's correlation with fluorophore concentration in addition to the photobleaching properties were investigated in the phantoms and were compared to the clinical data measured on the brain tumor.

Keywords: Protoporphyrine (PpIX), 5-aminolevulinic acid (5-ALA), autofluorescence, photobleaching, fluorescence guided resection, spectroscopy

1. INTRODUCTION

Fluorescence guidance using 5-aminolevulinic acid photosensitizer for resection of highly malignant brain tumors is an optical imaging method which has recently entered the clinical routine. Five-ALA, administered orally prior to surgery, accumulates in the tumor cells as protoporphyrin IX (PpIX) as a result of tumor's broken blood brain barrier and altered enzyme levels [1, 2]. The conventional guidance is performed through modified surgical microscopes [3], as a technical alternative for detection of fluorescence, hand-held fiber-optical probes have been suggested for their higher detection sensitivity, fluorescence quantification possibilities and navigational features [4-8].

In the attempt to quantify the fluorescence signals, methods have been proposed which combine other optical modalities, apply Monte Carlo simulations or use the tissue autofluorescence to calculate the amount of PpIX concentration in the tissue [9-12]. The quantification methods which are based on fluorescence signals alone have the advantage of offering more practical and real-time measurements feasible during surgery. Detection of PpIX fluorescence has the main challenges of being highly affected by blood and photobleaching when exposed to surgical lamps or blue excitation light. These factors when not eliminated significantly affect the diagnosis which is based on the intensity of the fluorescence, either visually observed or measured with a fiber-optical probe. To validate any of the quantification methods it is essential to apply optical phantoms which in addition to the controlled photosensitizer concentrations implement the tissue fluorescence (autofluorescence) and photobleaching properties. In this paper, we present a preliminary phantom model for PpIX-enhanced brain tumor in which photobleaching properties of tissue are considered and compared to the data obtained from brain tumors.

*neda.haj.hosseini@liu.se; phone 46 10 103 2488; fax 46 13 101902; imt.liu.se

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2. MATERIAL AND METHODS

2.1 Optical properties of the brain tumor

Optical properties of the brain tumor have been measured in several literature [13-15] according to the cited references are included in Table 1. Different malignancy grades demonstrate different optical properties from each other and from the normal brain tissue [14, 15]. The data provided by Gebhart [13] was adapted for the phantoms.

Table 1. Optical properties of glioma and normal brain tissue at 405 nm

	μ_a [mm ⁻¹]	μ_s [mm ⁻¹]	g	Reference
Glioma	1.3	26	0.85	[13]
Gray matter	0.2	15	0.86	[15]
White matter	0.3	40	0.75	[15]

2.2 Phantom composition

The phantoms were prepared to have the optical properties of the high grade brain tumor tissue [13]; i.e., added scattering ($\mu_s = 26$ mm⁻¹), absorption ($\mu_a = 4$ mm⁻¹) and autofluorescence. Intralipid 20% (Fresenius Kabi, Uppsala, Sweden) and black ink was used to induce scattering and absorption properties, respectively. A yellow fluorescent objective slide (Chroma Technology Corp, Bellow Falls, VT, USA) was used to produce autofluorescence. Protoporphyrin IX Disodium Salt (MP Biomedicals, France) dissolved in dimethyl sulfoxide (DMSO, Thermo Fisher Scientific Inc., France) was used in concentrations of 2, 6, 10, 14 and 18 mg l⁻¹. These concentrations were chosen according to the PpIX concentration in the glioma samples [16]. To obtain a solid platform for photobleaching measurements, agarose gel 0.6% [g ml⁻¹] was used to add identical mechanical properties of brain to the phantom (Young's modulus, E = 10 kPa) [17, 18].

2.3 Spectral collimated transmission

The scattering and absorption properties of the phantom ingredients were measured using a spectral collimated transmission setup (SCT) [19]. For obtaining the broad spectrum a white light (AvaLight-Hal-S, Avantes, Apeldoorn, Netherlands) and for obtaining optical properties at 405 nm, a blue laser light was used. Attenuation of the samples were measured and calculated using Beer-Lambert Law assuming intralipid as a scattering dominant medium and ink as an absorption dominant medium. Measurements were performed on three samples at each concentration.

2.4 Fluorescence spectroscopy instrument

The phantom was evaluated using a fluorescence spectroscopy system with a fiber-optical hand-held probe with which guided brain tumor resection is performed [4]. The probe used in the clinical measurements had a central fiber for excitation ($\varnothing_{\text{core}} = 600$ μm , numerical aperture = 0.37) and surrounding fibers for fluorescence collection ($\varnothing_{\text{core}} = 200$ μm , numerical aperture = 0.22). An identical probe was used on phantoms to avoid chemical contamination of the clinical probes. The probe composed of a central collecting fiber and six surrounding fibers for excitation ($\varnothing_{\text{core}} = 400$ μm , numerical aperture = 0.22). The laser and spectrometer were synchronized meaning that the integration time of the spectrometer was equal to the pulse width of laser light delivery.

2.5 Experimental settings

A multi-well sample holder was used to hold phantoms ($\varnothing = 6.6$ mm). Each well was filled with 60 μl of the phantom solution which gave a 1.7 mm thickness to the phantoms. The surrounding wells of each phantom well were filled with food color to avoid exposure of the phantoms to the excitation light (Figure 1). The fiber-optical probe was fixated on a micro-positioner for exact positioning above each phantom. The yellow fluorescent objective slide was placed behind the phantom sample holder as a model for tissue autofluorescence. A black plate was positioned behind the fluorescent slide to avoid reflection from the background surface.

Phantoms were prepared to have PpIX concentration of 2, 6, 10, 14 and 18 mg l⁻¹. Experiments on the phantoms were performed using excitation powers of 5, 7.5 and 10 mW with integration time of 0.2, 0.4, 0.6 and 1 s to allow evaluation of the quantification method using different power settings. All the measurements were performed using continuous

excitation. Signals with a weak autofluorescence below the system noise (20 photon counts [a.u.]) were excluded. Quantification was done by dividing the intensity at 634 nm by the maximum autofluorescence intensity (Figure 4b). The presented data from brain tumor correspond to an ALA dose of 5 mg/kg bodyweight [5]. The clinical measurements were approved by the local ethics committee (No: M139-07) with received written consent from the patients.

Figure 1: Experimental setup for the phantoms

2.6 Analysis

MATLAB version R2013a (The MathWorks, Inc., Natick, USA) was used to analyze the signals. Minitab software (Minitab, Inc, UK) was used for statistical analysis. Photobleaching data presented in Figure 5 are the curve-fits of the measurement points [20]. Pearson’s linear correlation was used for correlation analysis between two groups (Figure 4) and Kruskal-Wallis test was used to analyse significant difference among groups presented in Figure 4b.

3. RESULTS

3.1 Phantom composition

The amount of the phantom ingredients and the induced properties are summarized in Table 2. The volume concentration, ρ , of ink and intralipid may be calculated from Equations 1 and 2. $\mu_a (ink)$ is the absorption coefficient of ink [mm⁻¹] and $\mu_{s(il)}$ is the scattering coefficient of intralipid [mm⁻¹] at 405 nm measured using SCT setup. Absorption and fluorescence emission bands of PpIX are illustrated in Figure 2. Absorption coefficient of PpIX at 405 nm, $\mu_{a(PpIX)}$ [mm⁻¹], was a linear function of PpIX concentration, c_{PpIX} [mg l⁻¹] (Equation 3).

$$\mu_a (ink) = 146.6 \rho_{ink} \tag{1}$$

$$\mu_{s(il)} = 222.1 \rho_{il} \tag{2}$$

$$\mu_{a(PpIX)} = 0.3 c_{PpIX} \tag{3}$$

Table 2. Amount of the ingredients used in the phantom and the induced properties

Substance	Amount	property
Intralipid 20%	11.8 % in total volume	$\mu_s=26 \text{ mm}^{-1}$
Black ink	1.1 % in total volume	$\mu_a=1.3 \text{ mm}^{-1}$
Agarose gel	0.6% g ml ⁻¹	E = 10 kPa

Figure 2: Absorption and emission spectra of PpIX at concentration of 14 mg l^{-1} dissolved in DMSO.

3.2 Fluorescence spectra and quantification

Fluorescence signals collected from the phantoms with c_{PpIX} of 2 and 18 mg l^{-1} (the lowest and highest concentration used), excited with two different powers of 5 and 10 mW are illustrated in Figure 3. The phantoms showed comparable spectra with those collected from the brain tumor.

Figure 3: Fluorescence spectra collected on the phantoms with c_{PpIX} of 2 and 18 mg l^{-1} and one example from brain tissue. All of the above spectra are collected with 0.4 s integration time.

Figure 4: PpIX fluorescence intensity as a function of its concentration (a) and ratio of PpIX fluorescence at 634 nm by maximum emission of autofluorescence measured on the optical phantom (b).

Figure 4 (a) shows correlation ($R^2 > 0.98$, $p < 0.01$) of PpIX fluorescence intensity with PpIX concentration using the same excitation settings. For measurements performed with various laser powers and integration times, ratio of PpIX fluorescence over autofluorescence showed a statistically significant difference ($p < 0.001$) among different concentrations. Regression analysis showed a linear correlation between the PpIX concentration and the fluorescence ratio ($R^2 = 0.79$, $p < 0.001$). The linearity was lower when PpIX fluorescence alone was correlated to the PpIX concentration ($R^2 = 0.67$, $p < 0.001$).

3.3 Photobleaching

Decay of fluorescence (photobleaching) in phantoms with different concentrations is shown in Figure 5. An example of photobleaching in brain tumor is shown by the measured points and the curve fitting. Figure 6 shows photobleaching in terms of the time at which only 37% of the initial fluorescence is left ($t_{37\%}$).

Figure 5: Photobleaching of PpIX fluorescence in phantoms with PpIX concentrations of 2, 10 and 14 μM . An example of fluorescence decay measurement points (dots) and curve-fit (dashed line) from brain tumor is added.

Figure 6 Photobleaching affected by the excitation power and PpIX concentration. The y axis shows the time at which only 37% of the initial fluorescence is left.

4. DISCUSSION AND CONCLUSION

A preliminary optical phantom model that mimicked brain tumor was characterized and compared to the data collected on the brain tumor [4, 5]. The phantom experiments (Figure 4) showed that the PpIX fluorescence was highly correlated to the PpIX concentration ($R^2 > 0.98$) but when excitation settings were varied ratio of PpIX fluorescence over autofluorescence showed a higher correlation to the PpIX concentration than to the PpIX fluorescence. The linearity of the PpIX fluorescence and concentration has also been reported by Johansson et al ($R^2 = 0.99$) and Kim et al ($R^2 = 0.64$) [11, 16].

Autofluorescence in brain tumor is emitted from within the tissue sampling volume. This might be more appropriately modelled in the future phantoms by adding corresponding fluorophores to the phantom. Effect of chemical environment of the phantom, although known to influence the PpIX emission [21], was not investigated; however, the emission peak of PpIX in the phantom located at 634 nm which is 1 nm blue-shifted from what is measured in the brain tumor, indicated a similar chemical environment in that of brain tumor. PpIX in DMSO solution alone had its emission peak at 627 nm.

Mean and standard deviation of $t_{37\%}$ measured on the brain tumor was 42 ± 24 [s] and 26 ± 5 [s] for excitation powers of 5 and 10 mW, respectively [22]. It is therefore concluded that the realized phantom with identical mechanical properties to that of brain (0.6% agarose gel) offers a platform comparable to brain tumor for photobleaching measurements. Photobleaching measurements were not possible in the liquid phantom due to the molecular kinetics. It was however not tested whether higher or lower gel consistencies would lead to different photobleaching results.

In conclusion, the phantom provided a brain-tissue-comparable platform for investigating PpIX fluorescence and photobleaching behavior in relation to its concentration. In the phantoms, the ratio of PpIX fluorescence over autofluorescence showed a higher correlation than the PpIX fluorescence alone using different excitation light settings. Photobleaching of the PpIX was affected by the PpIX concentration where a higher concentration resulted in a faster photobleaching.

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