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Fluorescence Spectroscopy Using Indocyanine Green for Lymph Node Mapping

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ABSTRACT

The principles of cancer treatment has for years been radical resection of the primary tumor. In the oncologic surgeries where the affected cancer site is close to the lymphatic system, it is as important to detect the draining lymph nodes for metastasis (lymph node mapping). As a replacement for conventional radioactive labeling, indocyanine green (ICG) has shown successful results in lymph node mapping; however, most of the ICG fluorescence detection techniques developed are based on camera imaging. In this work, fluorescence spectroscopy using a fiber-optical probe was evaluated on a tissue-like ICG phantom with ICG concentrations of 6-64 μM and on breast tissue from five patients. Fiber-optical based spectroscopy was able to detect ICG fluorescence at low intensities; therefore, it is expected to increase the detection threshold of the conventional imaging systems when used intraoperatively. The probe allows spectral characterization of the fluorescence and navigation in the tissue as opposed to camera imaging which is limited to the view on the surface of the tissue.

Keywords: Intra-operative optical imaging, near infrared fluorescence, breast cancer surgery, optical phantom, optimal ICG concentration, spectroscopy

1. INTRODUCTION

Cancer is a common cause of morbidity and mortality, and represents a major public health problem in many parts of the world [1]. Surgery is usually the first line of treatment against cancer. The great challenges for future personalized oncology are to explore improved methodology for the early detection of localized and disseminated tumor cells in patients which is important to the success of cancer therapy and improvement of patients' survival. Optical imaging approaches can provide molecular information of biological tissues and are related to the tumor anatomical structure as well as the tumor metabolism and biochemistry [2].

In breast cancer, sentinel lymph node biopsy (SNB), the first tumor draining lymph node, is a reliable predictor of axillary lymph node status and therefore it is used as a routine examination in breast surgery. Intra-operative visualization of the lymph nodes also referred to as *lymph node mapping* makes it easier for surgeons to localize the potential metastasis. Intra-operative visualization of the lymph nodes also referred to as *lymph node mapping* makes it easier for surgeons to localize the potential metastasis. Lymph node mapping is useful in several oncological surgeries including breast, head and neck, gynecologic, urologic and lung cancers [3].

Sentinel lymph node mapping in the breast cancer surgery is performed by using a combination of methylene blue dye (for visual identification) and radioisotope labelling using gamma probe. Gamma probe has a high detection rate compared to the lower detection rates achieved by visual blue dye inspection [4] but application of the gamma probe is limited by the availability of nuclear medicine at the county and university hospitals. As a less demanding alternative, optical imaging of fluorescent dyes such as ICG may be employed in lymph node mapping [5-7].

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ICG emits fluorescence in the optical near infrared region (NIR) and is widely used for angiography applications and monitoring of circulation in various organs of the body [8]. Lymph node mapping using NIR fluorescence provides a practical method with a high detection rate [9] while it circumvents the use of nuclear medicine which is available at a limited number of hospitals (about 4% of the total hospitals worldwide) [10, 11]. However, the currently available NIR fluorescence imaging systems are bulky and lack spectroscopic and navigational features [6, 7, 12]. In this paper we have evaluated application of fluorescence spectroscopy using a hand-held fiber-optic probe for detection of ICG in the phantoms and during breast cancer surgery.

2. MATERIALS AND METHODS

2.1 ICG optical properties

ICG is a dark green substance with molecular weight of 775 which emits fluorescence in the near infrared region when excited with light at an appropriate wavelength. Absorption and emission properties of ICG are shown in Figure 1. The maximum absorption of ICG occurs at the wavelength of 785 nm and the maximum emission can be detected at about 820-850 nm. The emission peak shifts depending on the chemical environment or ICG concentration.

Figure 1: Absorption and emission spectra of 64 μM ICG in DMSO solution. Data is normalized.

2.2 Optical phantom

An optical phantom which mimicked optical properties of breast tissue at 785 nm ($\mu_s = 4.6 \text{ mm}^{-1}$, $\mu_a = 0.07 \text{ mm}^{-1}$) [13, 14] was prepared for performing controlled measurements in the laboratory. The phantom was made of intralipid 20% (Fresenius Kabi, Sweden), ICG (Pulsion Medical Systems SE, Feldkirchen, Germany) and agarose gel. Breast tissue is a scattering dominant medium ($\mu'_s \gg \mu_a$) and therefore only scattering properties were considered in the phantom. The scattering coefficient of intralipid at 785 nm was measured with a spectral collimated transmission setup (SCT) [15]. The scattering coefficient of intralipid, $\mu_{s(il)} [\text{mm}^{-1}]$, was related to the intralipid volume concentration, ρ_{il} , by Equation 1 where ρ_{il} is the volume percentage of intralipid 20% in the total phantom volume. As intralipid at higher volume concentrations does not follow the same linearity, Equation 1 is only valid for $1\% \leq \rho_{il} \leq 12\%$.

$$\mu_{s(il)} = 0.49 \rho_{il} \quad (1)$$

ICG powder dissolved in dimethyl sulfoxide (DMSO, Thermo Scientific, France) and diluted with distilled water was prepared to have concentrations of 6.4 - 645 μM in the total phantom volume. Agarose gel was added with a concentration of 1% g ml^{-1} to maintain the phantoms with the mechanical properties similar to that of breast tissue (Young's modulus = 25 kPa) [16, 17]. Solidification of the phantom is specifically of importance to restrict the molecular kinetics in the phantom for intended photobleaching measurements. Each phantom was prepared in a 2 ml volume. Attenuation coefficient of the DMSO, distilled water and agarose gel was 0.04 mm^{-1} in total. Absorption

properties of ICG was calculated by Equation 2 where $\mu_{a(\text{ICG})}$ is the absorption coefficient of ICG at 785 nm [mm^{-1}] and c_{ICG} is the concentration of ICG [μM].

$$\mu_{a(\text{icg})} = 0.05 c_{\text{ICG}} + 0.03 \quad (2)$$

2.3 Fluorescence spectroscopy equipment

An optical spectroscopy setup was developed for fluorescence excitation and detection. A laser (Z-laser Optoelektronik GMBH, Freiburg, Germany) with maximum emission of 80 mW at 785 nm was used as the excitation light source. A spectrometer (EPP 2000, Stellarnet, USA) with 240-880 nm range and 4096 photon counts was used for fluorescence detection. A fiber-optical probe (Avantes, Apeldoorn, Netherlands) appropriate for UV/NIR light was connected to the laser and spectrometer. The probe included a central collecting fiber and six surrounding fibers for excitation ($\varnothing_{\text{core}} = 400 \mu\text{m}$, numerical aperture = 0.22). The system was controlled by a LabVIEW[®] program. Surgical lamps in the operating room did not interfere with the fluorescence detection in the NIR region; therefore, no ambient light removal was considered in the measurement system.

2.4 Experiments on the phantom

Measurements were performed on the phantom to characterize the ICG fluorescence spectroscopy for clinical use. Additional measurements were performed on the phantom without agar and on pure ICG solution (without intralipid) to specify possible effects of agar and intralipid on the fluorescence emission. The ICG concentrations were considered regarding the injected ICG concentration during surgery (6.4 μM). Excitation light was applied for 0.2 s with a power of 4 mW for each measurement.

2.5 Data analysis

MATLAB version R2013a (The MathWorks, Inc., Natick, USA) was used to analyze the signals. The ICG signal was interpreted as the maximum fluorescence intensity in between 815-860 nm in the signals. The reflection of the laser light at these wavelengths was reduced from the ICG fluorescence intensity by taking the reflection amount at 740 nm as a symmetry for reflection at 830 nm. Data in Figure 2 are fitted using a linear polynomial curve.

2.6 Measurements on breast tissue

Measurements were performed on patients undergoing breast cancer surgery ($n = 5$) with received written consent. The study was approved by the local ethics committee (No. 2012/237-31). ICG (Pulsion Medical Systems SE, Feldkirchen, Germany) was injected in the tumor site with a concentration of 5 mg ml^{-1} (6.4 μM) prior to the measurements. Measurements were performed on breast lumps during surgery and ex vivo directly after excision. A spectrometer with higher sensitivity (AvaSpec 2048-2e, Avantes BV, Netherlands) and a probe with a central fiber for excitation ($\varnothing_{\text{core}} = 600 \mu\text{m}$, numerical aperture = 0.37) and surrounding fibers for fluorescence collection ($\varnothing_{\text{core}} = 200 \mu\text{m}$, numerical aperture = 0.22) was used for clinical measurements. Excitation light was applied for 0.4 s with excitation power of 40 mW for each measurement.

3. RESULTS

3.1 ICG fluorescence in phantom

Measurements revealed that there is an optimal concentration for ICG fluorescence due to self-quenching effect. Concentrations lower and higher than this level emitted less fluorescence (Figure 2). The optimal concentration showed to be lower for ICG solution in intralipid (16 μM) than for pure ICG solution (64 μM <) while addition of agarose gel to the intralipid solution did not show any effect on the optimal concentration. Maximum emission peak occurred at higher wavelengths for higher ICG concentrations.

3.2 ICG photobleaching in the phantom

Measurement of ICG fluorescence over time in the phantom showed that decay behavior of ICG fluorescence (photobleaching) varied at different concentrations. ICG concentrations above the optimal concentration first had an increase in the fluorescence emission and thereafter their emission decreased. Figure 3 shows photobleaching of ICG for optical phantoms prepared with agarose gel.

3.3 ICG fluorescence in breast tissue

ICG signals were detectable in four out of five patients. Examples of the signals detected in the four patients are shown in Figure 4. The ICG peak emission was located between 820-855 nm.

Figure 2: ICG fluorescence intensity (a) and wavelength of the maximum emission peak (b) as a function of concentration in intralipid solution (circles) and in the intralipid phantom with agarose gel (triangles).

Figure 3: Photobleaching of ICG fluorescence in phantoms with ICG concentrations of 6, 16, 32 and 64 μ M.

Figure 4: ICG signal detected in the breast lymph nodes of four patients.

4. DISCUSSION AND CONCLUSION

In this study, application of a fluorescence spectroscopy system was investigated on a tissue mimicking optical phantom and during surgery. ICG fluorescence had the highest emission at an optimal concentration (Figure 2) due to self-quenching effect which has also been reported in other literature [18, 19]. Optimal concentration in breast tissue phantoms (ICG and agarose) is reported in the range of 10 -250 μM depending on the other chemical ingredients of the phantoms [19-21]. The concentration also influenced the wavelength at which the maximum emission occurred.

Scattering coefficient of intralipid reported in the literature has some variances, however, the values measured was in the same range as is the literature [22, 23]. The scattering coefficient of intralipid and breast tissue show lower scattering at the emission wavelengths of ICG compared to the excitation wavelength [14, 23]; therefore the phantom should provide similar optical properties to that of the breast tissue even at the ICG emission wavelengths. The ICG fluorescence from the phantom had a maximum emission peak close to the signals collected from the tissue. The excitation power was reduced in the laboratory to make the measurements comparable to the tissue measurement as the lymph nodes are covered by tissue layers and require a high power excitation to emit the same amount of fluorescence comparable to what is collected from the phantom.

Measurements in the operating room were unaffected by the interference from the surgical lamps or tissue chromophores such as blood compared to the visible range fluorescence measurements [24]. Signals were well detected only when the probe was close to the lymph nodes due to the low penetration depth of light in the tissue. A maximum detection depth of 3 mm in animal fat is reported by Mohs measured with a similar hand-held fiber optical probe [25].

Fluorescence of ICG at its optimal concentration and the applied settings reached its half life time within 10 min. Measurements during surgery were performed within a few seconds (several pulses of 0.4 s length); therefore, according to the phantom measurements, effect of photobleaching on the collected signals was considered to be negligible for the applied measurements. The surgical lamp used during breast cancer surgery emits light in the region below 700 nm (not included in the results) where ICG has a low absorption and is therefore not expected to bleach out the ICG fluorescence significantly (Figure 1).

In conclusion, fluorescence spectroscopy using a hand-held probe was evaluated for lymph node mapping during breast cancer surgery and on optical phantoms in the laboratory. The ICG concentration affected fluorescence intensity, wavelength at which the maximum fluorescence emission occurred and fluorescence decay behavior which may be useful in interpreting the clinical data. Future work will include further experimental and clinical evaluation of the spectroscopy data and combination with optical imaging.

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