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Evaluation of a Fiber-Optic Based Pulsed Laser System for Fluorescence Spectroscopy

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Abstract — **A fiber optic based continous wave laser setup has been developed to record the 5-aminolevulinic (5-ALA) induced Protoporfyrin IX (PpIX) fluorescence signals from cerebral gliomas. To reduce the energy delivered to the tissue as well as suppression of the ambient lamp artifact from the recorded spectra, a pulsed laser setup has been developed and evaluated. This setup has been calibrated and first evaluations were performed on the 5-ALA treated skin showing PpIX fluorescence peaks from the ALA treated skin at 635 and 704 nm wavelengths. The system controls laser pulses through a computer interface and labview software package. Pulses as short as 50 ms over a period time of 500 ms are generated and optimally detected. The results from primary measurements on skin show an effective suppression of room fluorescent lamp artifact from the recorded spectra.**

Keywords — **Fluorescence spectroscopy, Pulsed laser modulation, 5-aminolevulinic acid induced fluorescence, Glioma resection, Intraoperative optical guide**

I. Introduction

The highly malignant brain tumor, glioblastoma multiforme, is difficult to totally resect due to its infiltrative way of growing and its morphological similarities to surrounding functioning brain under direct vision in the operating field. MR and/or CT images are taken before and after surgery for observing the location and form of the tumor but still the important task of identifying tumor margins is based on visual inspection and palpation of tissue.

Optical measurements may offer a precise, safe and spatially beneficial option for intraoperative measurements. Thus the goal of this study has been to develop a system that assists the neurosurgeons with delineating the border between normal and malignant tissue during tumor resection. Although some research groups [1,2,3,4] have reported competitive studies of cerebral tumor demarcation using different detection methods of endogeneous and exogeneous fluorescence, quantitative tracing of 5-ALA induced fluorescence in cerebral tumors have remained unexplored.

The general idea is that about 3 hours prior to surgery the patient receives orally a low dose of 5-aminolevulinic acid (ALA) which passes the defect blood-brain barrier in the tumor and is converted to the fluorescence tumor marker protoporphyrin IX (PpIX) in the malignant cells to facilitate tumor demarcation via the tissue fluorescence signal. Laser light at 405 nm is absorbed by the PpIX and a fluorescence emission spectrum with peaks at 635 nm and 704 nm can be collected. PpIX is a natural substance in the haem cycle which is rapidly eliminated from the body. Stummer et al [5] have conducted extensive research on detection of cerebral glioma through oral administration of ALA reporting a higher resection rate by using PpIX fluorescence microscopy. The inspection done through a microscope, is based on visual judgement of the surgeon.

A study has been previously conducted using a compact fiber optic based fluorescence spectroscopy system using an LED at 395 nm both on skin and during neurosurgical resection procedure [6]. Results indicate that PpIX fluorescence and brain tissue autofluorescence can be recorded with the help of the developed system intraoperatively during resection of glioblastoma multiforme. To omit the undesired effect of superimposed unsuppressed operating lamp noise on the recorded spectra, a non-transparent funnel had been previously used in the measurements. Though this funnel effectively restricts the ambient light artifact, it blocks the view of the surgeon which is of a great disadvantage during operation.

To restrict the sensitivity of the collected fluorescence to the ambient light and to avoid excessive bleaching of the protoporphyrin IX marker under exposure to laser as well as reducing the measurement time, a system based on a pulsed mode laser at 405 nm (50 mW peak power) and a spectrometer has been developed and evaluated on skin prior to clinical measurements.

II. System and setup

The different components of the system, as described below, are mounted in a compact box of 31×25×21 cm which is easily carried on a trolley to the operation theater (Fig 1). The total weight of the box is 4.6 kg.

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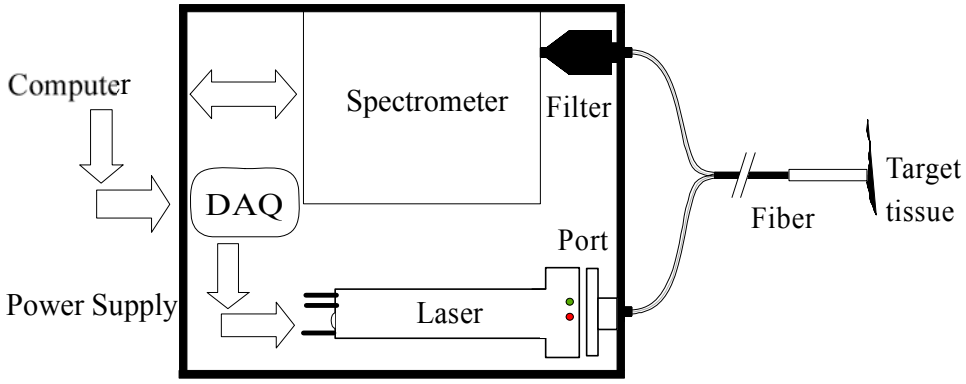

Fig. 1 Fiber optic based pulsed system design.

A. System components

Laser: Near-UV laser diode module with the maximum excitation light at 405 nm and maximum power of 50 mW was acquired (Oxxius, Inc., CA). The laser operates both in modes of continous wave and pulsed modulation. Shortest generated pulse may have a width of 7 µs. Relatively small mechanical dimensions make the packaging of this laser very handy.

Spectrometer: The spectrometer (EPP 2000, Stellarnet) uses 2048 element CCD in the range of 200-850 nm wavelengths with a practical resolution of 2 nm, though the theoretical resolution is higher. The minimum reliable integration time of the spectrometer is 30 ms.

Filter: As the reflection from the laser light saturates the spectrometer, a long pass cut off filter of 450 nm (Schott CG-GG-475-0.50-3, CVI, USA) is fixed before the detector slit of the spectrometer.

Fiber optical probe: Excitation light is brought to the tissue through a hand-held optical fiber probe with core and total diameter of 600 and 950 µm and numerical aperture of 0.37. This excitation fiber is surrounded by 9 other fibers of 200 µm core diameter and numerical aperture of 0.22 which collect the light from the measurement site and transfer it to the spectrometer (Fig. 2 a). The optical fibers are arranged to match the slit configuration of the spectrometer at the detector end (Fig. 2 b and c). However, some light at the upper and lower fibers at the detector end are lost (Fig. 2 b).

Fiberport: A miniature micropositioner (OFR Inc., Caldwell, NJ) has been mounted at the interface of laser and fiber probe to allow the alignment of the laser light. The maximum attenuation of light through the fiberport is 25%.

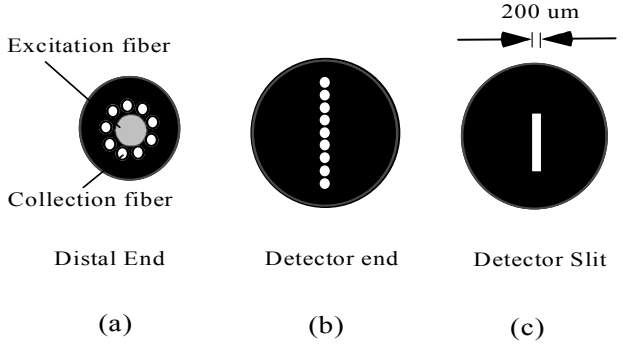

Fig. 2 Fiber probes (a) distal end of the fiber probe (b) detector end connected to the spectrometer and (c) detector slit.

B. Pulse generation and spectrum collection

The system controls generation of laser light pulses and the spectrometer through a computer interface and labview software package. The concept is to have a simultanous pulse generation and spectrum collection. The pulse and laser pulsewidth should be a trade off of the integration time of the spectrometer, minimum energy given to the tissue and the time needed to keep the ambient light sensitivity at minimum while collecting detectable amount of light. The software is programmed such that pulses are generated as a function of given spectrometer integration time and period time. However, the execution time of the loops in the software package do not allow setting of the period time below a certain level with the current design.

C. Calibration

Calibration: The system is calibrated with the coefficients specific of the spectrometer against the fluorescent lamp to make sure of the validity of the recorded wavelengths. Before each set of measurements a dark spectrum is recorded to measure the background level of the system without light input. All measured spectra are corrected for this background. The spectral sensitivity of the system needs to be taken into account in subsequent data analysis

by recording the spectrum from a National Institute of Standards and Technology (NIST) traceable stable calibration light source.

A fluorescent plastic is used for fluorescence intensity calibration to check the absolute level of the recorded spectra before each set of measurements.

III. System Evaluation

Undesired spectra from the continous wave laser mode: The background superimposed on the measured spectra due to the room fluorescent lamps during measurements with the 405 nm laser in continous mode is shown in Fig. 3.

Evaluation on skin: Metylaminolevulinat (METVIX® 160 mg/g, Photocure ASA, Norway) cream was applied on the skin 3-4 hours prior to the measurements. The skin was covered to avoid exposure to the light and thus prevent bleaching of the fluorophores. The aminolevulinic acid in this cream changes to PpIX in the skin and exhibits fluorescent peaks at 635 and 704 nm wavelengths. Measurements were performed on the untreated and ALA treated skin. Fig. 4 shows the fluorescence recorded from the untreated skin and the fluorescence from the treated skin, using the developed pulsed system. Pulses of 50 ms long with a duty cycle of 0.1 have been chosen and evaluated. The power was set to 1 mW. These values were chosen as an obtained compromise between the desired features mentioned earlier.

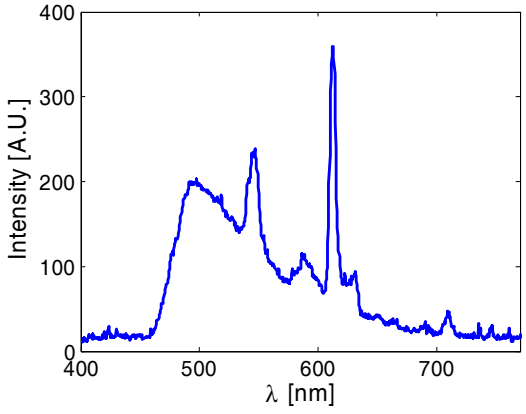

Fig. 3 Spectrum recorded with no mechanical suppression of room fluorescent tube lamp using the continous wave laser (405 nm) system. The artifact is superimposed on the tissue autofluorescence.

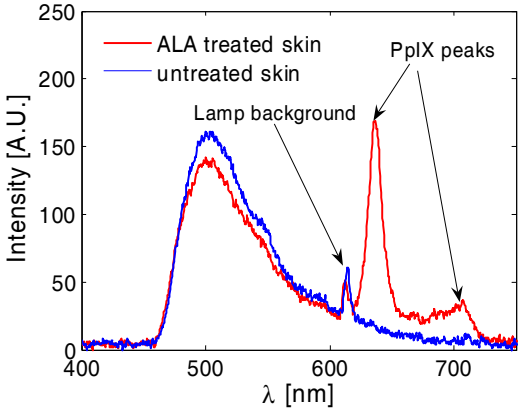

Fig. 4 Fluorescence signals detected from the ALA treated and untreated skin with pulsed mode laser system with the room lamps on. Pulse width of 50 ms over a duty cycle of 0.1 was applied.

IV. Discussion

The system offers a quantitative measurement method of the fluorescence in malignant glioma brain tissue compared to the microscope based inspection reported by Stummer et al. [5].

We have observed negligible traces of ambient light in the collected fluorescence spectra. This helps the visual supervision of the surgeon to a great extend compared to the previous measurements with continous wave excitation laser. In the continous wave mode the measurement site has to be covered with a non-transparent funnel and the lamps should be directed away from the surgery site. As the operation lamp and microscope lamp also exist in addition to the fluorescence lamp tubes in the operation theater, the sensitivity of the system to the background light of the mentioned lamps should be considered in the next step of the system performance evaluation.

Further work will include optimization of pulse generation and detection at the software level as well as averaging of the collected pulses for visualization of a more stable fluorescence spectra intensity. Clinical measurements on brain gliomas would follow the completion of the system.

V. Conclusion

Using the developed fiber optic based pulsed fluorescence spectroscopy system, it has been shown that PpIX fluorescence signals can be quantitatively detected on ALA treated skin and the artifact of the ambient fluorescent lamps are effectively omitted. However, further optimization and evaluation of the pulsed fluorescence detection system is necessary before clinical trials.

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