

# Fiber-optic based fluorescence spectroscopy for glioblastoma demarcation using 5-aminolevulinic acid

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## 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 endogenous and exogenous 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, the ambient light had to be mechanically suppressed. Though this suppression 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 photobleaching 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 patients with glioblastoma multiforme.

## II. MATERIALS AND METHODS

### A. System

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. The laser light output power was measured prior to recording with a laser power meter (Ophir-Spiricon, Ophir Optonics Ltd., Israel), while the detection unit of the system was calibrated with well characterized light sources.

The excitation light is delivered by a violet laser diode module emitting at 405 nm with a maximum power of 50 mW (Oxxius SA, France). The laser functions in pulsed and continuous wave modes. A spectrometer (EPP 2000, Stellarnet, USA) with 2048 element CCD capturing light in the range of 248-850 nm is used for recording the laser-induced fluorescence emitted by the tissue. The spectral resolution of the recorded spectra is about 3 nm. A long pass filter (Schott, CVI, USA) eliminates the laser reflection light at 400 nm from entering the spectrometer.

A hand held fiber probe is used as the interface between tissue and the system. An optical fiber in this probe with core diameter of 600  $\mu\text{m}$  and numerical aperture of 0.37 brings the excitation light from laser to the tissue. This excitation fiber is surrounded by receiving fibers which collect the light from the measurement site and transfer it to the spectrometer. The receiving fibers were of 200  $\mu\text{m}$  core diameter and numerical aperture of 0.22. The third probe included two receiving fibers of 600  $\mu\text{m}$  and 0.37 numerical aperture. The optical fibers at the detector end were arranged to match the slit configuration of the spectrometer to the possible extent. The probe in total has a diameter of 2 mm. A fiber port (OFR Inc., Caldwell, NJ, USA) was used at the interface of the probe and laser for light alignment.

The system operates both in the continuous and pulsed modes and is controlled via a software interface (LabVIEW®, National Instruments Inc., USA) and a hardware interface (National Instruments Inc., USA). The system is chosen to operate at  $17.8 \text{ mW/mm}^2$  excitation light power density at the tissue surface. In continuous mode, the laser irradiates continuously at this power density while the spectrometer collects light. A pulsed mode is added to the system to optimize the energy delivery and to omit the background light.

#### *B. Measurements on glioblastoma multiforme*

Patients undergoing surgical resection of 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 probes used were sterilized with the STERRAD® procedure. Measurements were approved by the local ethics committees (No: M139-07) and written informed consent was received from all the patients.

### III. RESULTS

Typical fluorescence spectra of malignant tissue compared to gray matter from a patient diagnosed with malignant glioblastoma, Fig. 1, are presented. PpIX fluorescence peaks at 635 and 704 nm are apparent in the malignant tumor. Signals recorded from gray matter of the cortex show no PpIX peaks. Experimentally, a minimum of 2 mJ excitation energy gives an acceptable signal level when measuring in brain. Under a fluence rate of  $17.8 \text{ mW/mm}^2$  (5mW laser power), PpIX in brain photobleaches within one minute of 405 nm light exposure. This amount is reduced to about half a minute when pulsed energy is used [7].

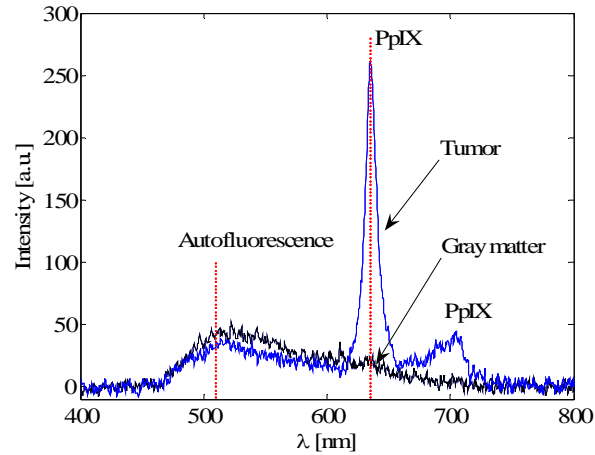


Fig. 1. Fluorescence signal recorded from a patient with glioblasoma multiforme. [7]

#### IV. CONCLUSION

Using the developed fiber optic based fluorescence spectroscopy system, it has been shown that PpIX fluorescence signals can be quantitatively detected on the glioblastoma and the artifact of the ambient fluorescent lamps are omitted using the pulsed system. However, further clinical studies are needed to investigate the sensitivity and specificity of the method at the tumor margin.

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