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Evaluation of a Fiber-Optic Fluorescence Spectroscopy System to Assist Neurosurgical Tumor Resections

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ABSTRACT

The highly malignant brain tumor, glioblastoma multiforme, is difficult to totally resect without aid due to its infiltrative way of growing and its morphological similarities to surrounding functioning brain under direct vision in the operating field. The need for an inexpensive and robust real-time visualizing system for resection guiding in neurosurgery has been formulated by research groups all over the world. The main goal is to develop a system that helps the neurosurgeon to make decisions during the surgical procedure.

A compact fiber optic system using fluorescence spectroscopy has been developed for guiding neurosurgical resections. The system is based on a high power light emitting diode at 395 nm and a spectrometer. A fiber bundle arrangement is used to guide the excitation light and fluorescence light between the instrument and the tissue target. The system is controlled through a computer interface and software package especially developed for the application. This robust and simple instrument has been evaluated in vivo both on healthy skin but also during a neurosurgical resection procedure. Before surgery the patient received orally a low dose of 5-aminolevulinic acid, converted to the fluorescence tumor marker protoporphyrin IX in the malignant cells. Preliminary 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.

Keywords: Fluorescence spectroscopy, brain tumor, 5-aminolevulinic acid, fiber optics, light emitting diode

1. INTRODUCTION

Optical biopsy is the generic term for techniques employing light and its interaction mechanisms with matter in order to evaluate a tissue in vivo[1]. The interest in novel instrumentation in this field has been increasing continuously, especially with regard to recent advances in optoelectronics. The development of light sources, light guides, optical filters and sensors has boosted optical biopsy techniques to diagnose diseases, direct surgical interventions and measuring tissue parameters[2]. The obvious advantages over traditional biopsy are that unnecessary tissue removal is superseded and that results are obtained in real time, both being important aspects of the used treatment procedures and thus the final clinical outcome.

In the specific case of the highly malignant brain tumor, glioblastoma multiforme (GBM), total resection without aid is almost impossible because of the tumor's infiltrative way of growing and its morphological similarities to surrounding healthy and functioning brain tissue. In spite of that, the important identification of the tumor margin is still based on visual inspection and palpation of tissue. Due to the difficulty of the task the failure percentage of total resection becomes overwhelming; over 80% of the resections have been reported to be incomplete[3]. At the same time, patients undergoing complete resection derive the most benefit from complementary oncological therapy in terms of longer recurrence free intervals[4, 5]. Hence, an accurate intraoperative system for resection guidance in neurosurgery becomes imperative.

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Previous studies have shown that fluorescence techniques using both endogenous and exogenously induced fluorophores can be used successfully during stereotactic biopsy[6] but may also assist the surgeon in the difficult evaluation of brain tissue[7-13]. The most promising results have been obtained when using administration of 5-aminolevulinic acid (ALA) in order to induce detectable fluorescent porphyrins. ALA is a natural biochemical precursor of haemoglobin that elicits synthesis and accumulation of protoporphyrin IX (PpIX) within malignant glioma tissue[14-16]. While some researchers have developed microscopy based systems for the investigation of suspect tissue without a strict quantification of fluorescence[17] others have built fiber based systems expressing an ambition to utilize the fluorescence spectral characteristics than merely intensity differences[7]. Furthermore, the need to evaluate tissue in the deeper brain is an important issue that has only recently been addressed in fluorescent studies[18]. The diagnostic and resection guidance potential of PpIX fluorescence spectroscopy has thus started to be acknowledged. Still, instrumentation tends to be complicated and/or expensive with little or no connection to clinically used imaging modalities and navigational systems.

The goal of this study is to develop an optical biopsy tool that is based on spectral recordings of PpIX fluorescence and that gathers the attractive features of robustness, cost effectiveness and simplicity of implementation.

2. APPARATUS

The fiber based fluorescence spectroscopy system consists of three distinct parts with corresponding interfaces (filtering) in between: the light source, transmission and detection part. Figure 1 presents a schematic drawing of the system, overviewing the light stages from the excitation source to the emission detection. Emphasis has been placed on constructing a robust, small-sized and low-cost system versatile with regard to system optimization e.g. by allowing interchangeable components. The current system weighs less than 3 kg with approximate outer dimensions of 25 cm (Width) × 30 cm (Depth) × 14 cm (Height), making its transport and handling manageable.

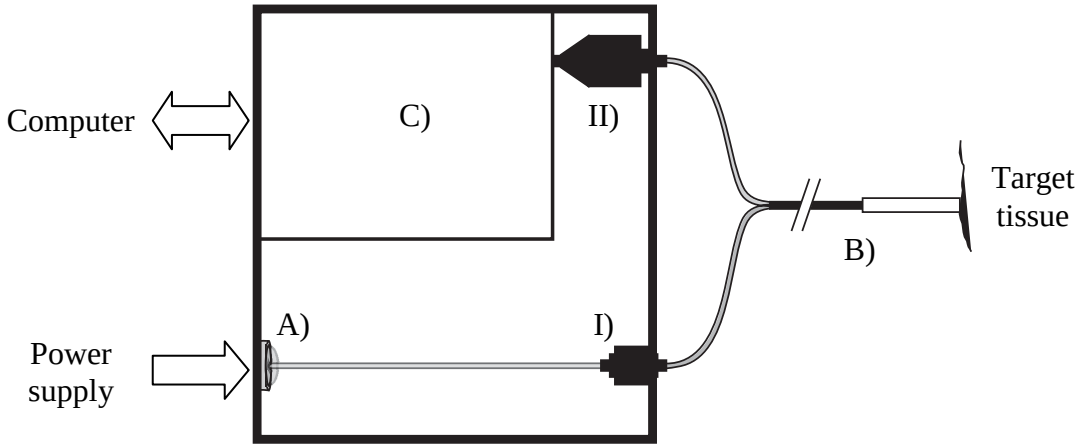

Figure 1: Schematic drawing of the apparatus parts (A-C) and their interfaces (I-II). A) Excitation source (LED). B) Light transmission (fiber bundle). C) Fluorescence detection (spectrometer). I) In-line filter holder with exciter filter. II) In-line filter system with emitter filter.

2.1 Excitation source

Recent developments in light-emitting diodes (LED) technology have resulted in high power optical outputs in the ultraviolet wavelength region. This progress is particularly interesting considering the low-cost, reduced size and ease of operation associated with LEDs making them attractive for use in medical fluorescence applications. The excitation source used in this setup comprises a semiconductor device emitting light with a peak intensity at 395 nm (Figure 2) and a radiant flux of 200 mW at a typical forward current of 350 mA (Cree® XLamp™ 7090 UVV LEDs, Cree Inc, USA). Tailor-made pigtailing of a fiber (fused silica, ∅ 600 µm, NA 0.37) to the diode minimized the connection losses;

passing through the lens of the LED and attaching the fiber end as close as possible to the light emitting chip gave the best coupling efficiency (Figure 2).

The introduction of LEDs in this context demands taking into account the incoherent nature of the emitted light that might otherwise interfere and complicate the interpretation of the fluorescence signal. Therefore, filtering of the diode light is performed by means of a band pass exciter filter (D395/30X, Chroma Technology Corp, USA) with a transmission of almost 80% in the waveband from 380 to 410 nm. The filter is incorporated in an in-line filter holder, i.e. a fiber-to-fiber connector with adapted quartz collimating lenses and SMA connectors on both ends (FH-Inline-UV/VIS-VAR, Avantes, The Netherlands). The filtered light reaches the target tissue via the source fiber with its distal end enclosed in a rigid metal tube defining the operative probe.

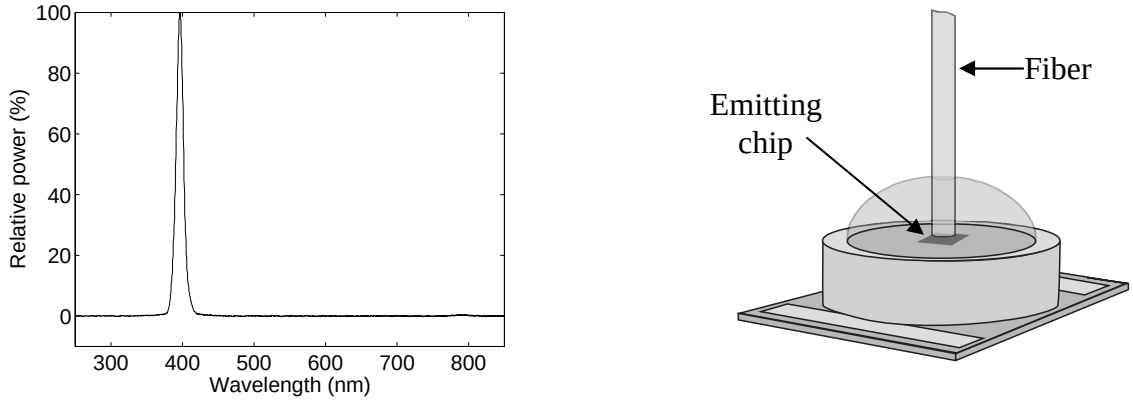

Figure 2: The relative emission spectrum of the Cree XLamp LED after filtering with the exciter filter to the left. The tailor-made pigtailing of the LED to the right. The fiber is brought in close contact to the emitting chip in the diode.

2.2 Transmission of light

While excitation light is guided to the tissue target through a 600 µm core fiber, the collection and transmission of fluorescence emission can be done in either of two collection fiber arrangements (see Figure 3A-B): A) Through one large core single fiber with the same characteristics as the excitation fiber (∅ 600 µm, NA 0.37) and B) Through multiple smaller core diameter fibers (∅ 200 µm, NA 0.22) arranged around the excitation fiber. The second alternative was suggested in an effort to increase the collection efficiency of fluorescence although it poses greater alignment demands on the other end towards the spectrometer. The 10 collection fibers formed a column enclosed in an SMA-connector to be coupled to the detection part of the system (Figure 3-II); the fiber column has to be aligned to the entrance slit of the spectrometer (200 µm wide slit).

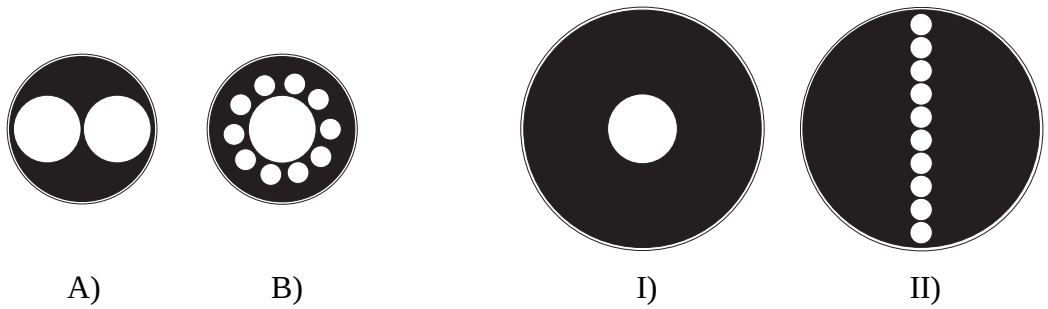

Figure 3: Excitation light is always transmitted from a large core single fiber, while fluorescence emission is collected by A) a single fiber or B) multiple smaller core fibers arranged around the emission fiber. At the detector end collection fibers were aligned to the slit entrance of the spectrometer which is more cumbersome in the case of a fiber column II).

At this stage of the project both collection solutions are followed up since they have different advantages over each other. In both cases, collection fibers were placed as close as possible to the emission fiber, minimizing separation

distance. Other investigational aspects considering illumination-collection geometry and its effect on detected photon pathlength in tissue are not included in this work.

The transmission part of the system is the only part in direct physical contact with the tissue target. This end of the fiber bundle is enclosed in a rigid metal tube in order to facilitate probe handling. Additionally, a black, non-transparent plastic funnel is attached to the distal end of the probe shielding off stray light and thus increasing purity of the fluorescence optical signal (see Figure 4). Finally, with a total fiber-bundle length of approximately 3 m and with the use of sterilized probe materials patient safety is assured with respect to electric hazard and bacterial infections respectively.

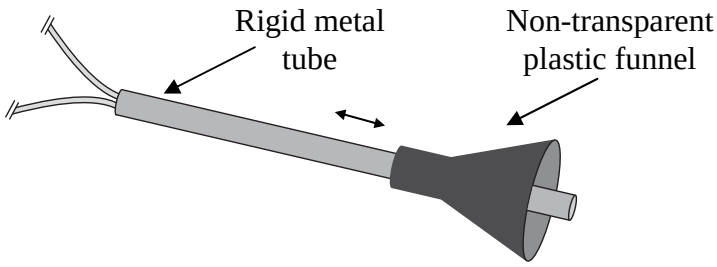

Figure 4: The fiber bundle is enclosed in a rigid metal tube coming in contact with the tissue target. The non-transparent plastic funnel can slide freely along the tube and shields off stray light.

2.3 Fluorescence detection

The interface between the transmission part and the detection part consists of a long pass filter (coloured glass, GG 475, Schott AG, Germany) rejecting mainly backscattered excitation light and thus protecting the spectroscope's charged coupled device (CCD) sensor from saturation and subsequent pixel blooming. Relying on absorption to achieve attenuation this filter might give rise to autofluorescence when blocking the backscattered excitation light. However, this autofluorescence was found to be within acceptable limits, especially considering the negligible interference with the characteristic emission spectrum of PpIX. The filter is mounted in an in-line filter holder (Inline Filter System, m·u·t GMBH, Germany) with a male SMA connector on one side allowing connection to the fluorescence collection fibers. On the other side a collimator with a female SMA connector made it possible to couple to the spectrometer.

The latter is a portable spectrometer (EPP2000C UV-VIS, StellarNet Inc, USA) connected to a notebook computer via a USB-2 port. Given the 200 µm wide slit, the line-width resolution of the spectrometer is approximately 6 nm (measured at HeNe lines of 632.8 and 546 nm) while the wavelength range of detection is from 250 to 850 nm. The spectroscope sensor comprises a CCD with 2048 pixels (ILX511 Sony Electronics Inc, USA) and the final output is a 12 bit signal yielding a dynamic range of 1 to 4096 counts. The operation of the spectroscope and basic handling (storage, presentation etc) of raw data is done by means of dedicated software (Labview 8.0, National Instruments, USA). Intrinsic spectral characteristics of the system (i.e. spectrometer CCD spectral sensitivity, transmission of emitter filter and fibers) were accounted for by calibration against a standard lamp with a known spectral emission.

3. SYSTEM PERFORMANCE MEASUREMENTS

The LED based fluorescence spectroscopy system has been employed in a number of experiments and measurements both in laboratory and in clinical settings. Firstly, the signal-to-noise-ratio (SNR) of the system has to be controlled on a regular basis as well as before and after every measurement series, in order to assure performance stability over time. A piece of plastic, possible to sterilize for use in the clinical settings, serves as a reference fluorescent sample, yielding consistent and reproducible results as long as the system is intact. Data from measurements on the fluorescent reference are used to determine the noise of the system by calculating the standard deviation in a wavelength region where the signal is negligible after subtraction of the dark background (e.g. 760 to 860 nm). Given this definition of noise, these repeated measurements show an SNR of about 1300 for the double-fiber probe and about 1500 for the multi-fiber probe, in the peak of the fluorescence signal (~510 nm). Integration time for those measurements is chosen so that the signal peak is just below the detector's overload level.

3.1 Suppression of ambient light

The benefit of adding the non-transparent plastic funnel to the system probe was demonstrated by measuring skin autofluorescence. Several measurements were performed in a room with lit fluorescent lamps on the ceiling, resembling the light conditions in the operating theatre. Finally, a funnel with a base diameter of 3 cm was tested and yielded a clear suppression of the characteristic peaks of the fluorescent lamps spectrum, see Figure 5.

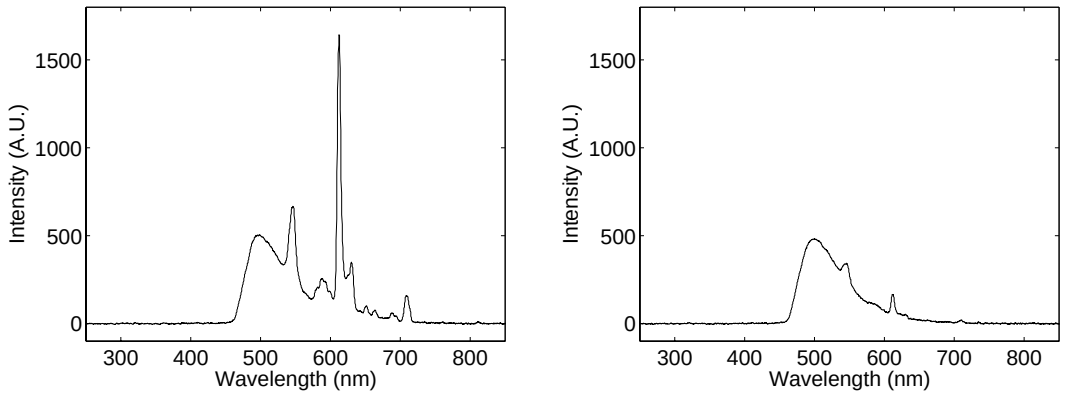

Figure 5: The left panel shows an autofluorescence spectrum of skin with the stray light from the fluorescent tubes substantially degrading the signal. The right panel shows the same autofluorescence spectrum recorded while having shielded the measurement site with the help of the plastic funnel, all other measurement settings were identical. The peaks at 546 and 612 nm are still visible, but greatly suppressed. Measurements were performed with the multi-fiber probe.

3.2 Skin measurement

The first in vivo measurement with the system was done on human skin in order to demonstrate the system's ability to detect tissue autofluorescence and exogenously derived fluorescence, in this case after application of 16% methyl 5-aminolevulinc acid (Metvix, Photocure ASA, Norway). The ALA-cream was applied in a thick layer (2-3 mm) over an area of 4 cm^2 and remained on the skin under occlusion (Tegaderm, 3M Health Care, USA) during 4 hours. Fluorescent measurements were done both on untreated and treated skin sites, with the latter sites revealing a concentration of PpIX since fluorescence spectra contained the characteristic peaks of PpIX emission at 636 and 704 nm (Figure 6). Skin autofluorescence is still intense, which is expected by normal, healthy tissue.

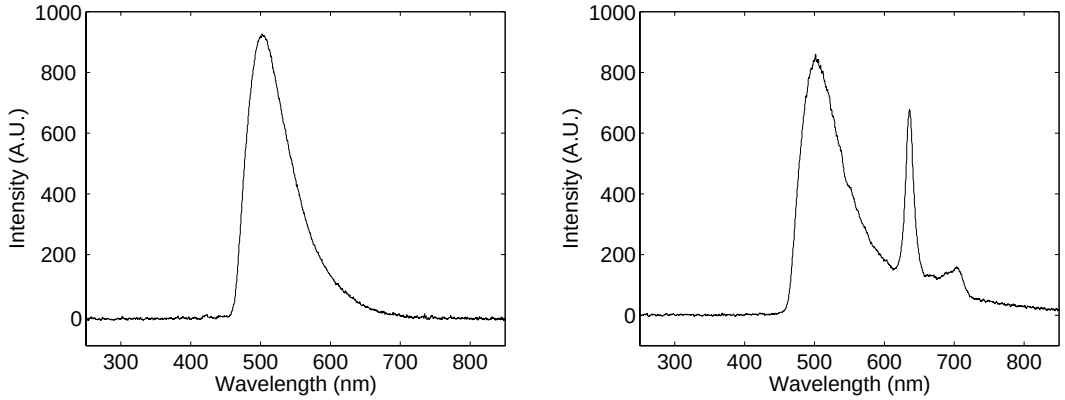

Figure 6: The left panel shows untreated skin, while the right panel shows skin pre-treated with ALA-cream (4 h application). Both spectra are recorded with an integration time of 6 seconds. The uptake and transition of ALA to PpIX in the pre-treated skin area is revealed by the characteristic fluorescence PpIX peaks. Fluorescence recordings were done with the multi-fiber probe.

3.3 Brain measurement

The system's performance was also evaluated on a patient referred to the Neurosurgery clinic at the University Hospital in Linköping for tumor surgery. The measurement procedure was approved by the local ethics committee (#94269) and

with the fully informed consent of the patient. An oral dose (5 mg/kg body weight) of 5-aminolevulinic acid was dissolved in a glass of orange juice and administered before surgery. Approximately 3 hours after administration, spectra were captured in tumor as well as in normal brain tissue.

Figure 7 shows two fluorescence spectra, the one to the left derived from normal brain tissue (white matter) and the other one from tumor tissue. The judgement of tissue type and localisation was done by the surgeon (author J.R.) based on direct vision (both with and without microscope) in situ, as well as on preoperative MR imaging. The two measurement sites were chosen to be in close vicinity, one in the tumor and the other one just outside the tumor. Characteristic differences between the two spectra can be observed, mainly consisting of the PpIX peaks (~636 and ~704 nm) dominating only the tumor spectrum and the more intense autofluorescence peak (around 500 nm) of the normal tissue. Biopsy confirmed a tumor diagnosis of glioblastoma multiforme[19].

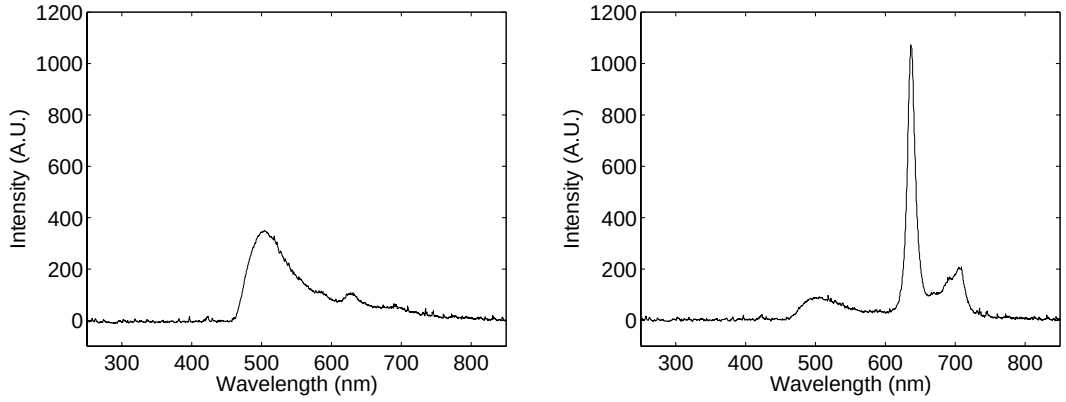

Figure 7: The left panel shows the fluorescence spectrum of normal brain tissue while the right panel shows the spectrum of glioblastoma tissue (glioma grade IV). Integration time equalled 4 seconds and the double fiber probe was used in both cases.

4. DISCUSSION

This study demonstrates the performance of a fluorescence spectroscopy system during in-vivo measurements of PpIX fluorescence both in laboratory and clinical settings. The initial aim of developing a robust and cost effective tool was prioritized over shorter integration times and more sophisticated means of suppressing ambient light. Thus, one of the main challenges has been to successfully incorporate a light-emitting diode as the excitation source something that was achieved and contributed significantly to a simple design. For the same reason, continuous wave operating mode was preferred which made system versatility regarding interchangeable parts easier and thus optimization of the used components more straightforward. At this stage of the project the developed prototype serves our goal of obtaining intraoperative fluorescence spectra during neurosurgery with reasonable effort from the surgical team.

4.1 Suppression of ambient light

The non-transparent plastic funnel used to shield off ambient light was easy managed and tolerated by the surgeon, although it still demanded avoidance of direct ambient light on the target tissue to fully accomplish its purpose. Thus operation and microscopy lamps were faced away from the open surgery area. In the future, the obvious choice would be to implement a pulsed gated mode system that besides suppression of ambient light would also allow time-resolved recordings of fluorescence. Such recordings have previously been shown to yield different results depending on the nature of the detected fluorescence (endogenous or exogenous) as well as on the biochemical environment of the tissue under examination[20-22].

4.2 Transmission of light

The fiber bundles employed to guide light between the fluoresensor and the target tissue did not reveal the expected degree of increase, in favour of the multi-fiber probe, regarding collection efficiency of fluorescence. Construction and alignment difficulties are thought to be the main reasons for the limited benefit of multiple fibers that were only used in laboratory settings when time for alignment is not a crucial factor. Fiber geometry in general needs to be reconsidered in

regard to probing depth and resolution. The latter factors may promote the use of a spliced fiber instead, in order to achieve guidance of the excitation and the fluorescence light through the same channel.

4.3 Advantages over other approaches

Other research teams concentrate on developing imaging modalities in order to enhance the surgeon's vision and thus discriminatory capacity[23]. However, this approach suffers the limitations of the absence of detailed spectral information of the detected fluorescence and of the inability to visualize tumors deeper in the brain. Fluorescence spectra may prove to be invaluable in the difficult characterisation of the diffuse border between normal tissue and tumor. This is possible since spectral data can be analysed with sophisticated algorithms, allowing for the full exploitation of the carried information, e.g. by the use of multivariate analysis that has been shown to be applicable in the discrimination of laryngeal lesions from normal tissue[24]. At the same time fluorescence signals are able to be recorded from tumors localized in the deeper brain, something that is assured by the physical design of the fiber bundle probe.

The presented prototype features the attractive qualities of robustness, mobility, low cost, ease-of-use and maintenance. Although the performance of the system is hampered by long integration times and special handling of ambient light, its ability to record fluorescence spectra of PpIX in vivo has been demonstrated. Hence, the system can be used for the purpose of collecting spectra for further analysis, a task that has until now been overshadowed by the technical complexity of early fluorosensors.

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