2021 43rd Annual International Conference of the
IEEE Engineering in Medicine & Biology Society (EMBC)
Oct 31 - Nov 4, 2021. Virtual Conference

FluoRa - a System for Combined Fluorescence and Microcirculation Measurements in Brain Tumor Surgery

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***Abstract* – In brain tumor surgery it is difficult to distinguish the marginal zone with the naked eye. Fluorescence techniques can help identifying tumor tissue in the zone during resection and biopsy procedures. In this paper a novel system for combined real-time measurements of PpIX-fluorescence, microcirculation and tissue grey-whiteness is presented and experimentally evaluated. The system consists of a fluorescence hardware with a sensitive CCD spectrometer for PpIX peak detection, a laser Doppler system, optical probes, and a LabView software. System evaluation was done on static fluorescing material, human skin, and brain tumor tissue. The static material indicates reproducibility, the skin measurements exemplify simultaneous fluorescence and microcirculation measurement in real-time, and the tumor tissue showed PpIX peaks. These decreased over time, as expected, due to photo bleaching. In addition, the system was prepared for clinical use and thus laser- and electrical safety issues were considered. In summary, a system for multiparameter measurements during neurosurgery was successfully evaluated in an experimental environment. As a next step the system will be applied in clinical brain tumor biopsies and resections.**

I. Introduction

Fluorescence guided resection [1] has become a routine method in many neurosurgical suits when removing malignant brain tumors under a blue-light microscope. In the case of highly malignant tissue, the perceived tissue color will shift to purple due to the administration of five-aminolaevulinic acid (5-ALA) and its transformation to protoporphyrin IX (PpIX). The Linköping group has previously developed a handheld probe [2] for fluorescence spectroscopy measurements. The probe enhances the fluorescence under the neurosurgical microscope [3] and makes the grading along the tumor marginal zone objective.

Brain tumor biopsies have a high risk of hemorrhage as the biopsy needle can puncture a vessel [4] and risk for low diagnostic yield as the actual tumor tissue can be missed due to a brain shift. The procedure is often time consuming as the samples must be sent for evaluation by a pathologist during surgery.

It has been suggested to use the blue-light microscope as intraoperative guide for fluorescing tissue [5]. An alternative is to redesign the hand-held probe to a stereotactic device with optical fibers for both fluorescence and microvascular measurements as presented by [6]. In this design, two separate systems with their own software were linked through the probe. It has been evaluated successfully during stereotactic brain tumor biopsies [7]. The added advantage by measuring the microcirculation is the possibility to warn for increased blood flow in front of the probe during insertion, i.e., it can act as a "vessel alarm" in a similar way as during deep brain stimulation implantations [8] and help minimizing the risk of hemorrhage. In the present paper we take this approach to the next level. A novel system combing these features as one concept is presented and evaluated experimentally.

II. System Set Up

A. System overview

The FluoRa 1.0 system consists of four parts, an inhouse built fluorescence module, a commercial laser Doppler flowmetry (LDF) system (PF 5000, Perimed AB, Sweden), LabView software and optical probes. The system is developed for use in stereotactic brain biopsy or tumor resection. An overview of the system setup and typical parameters recorded is presented in Fig. 1. The parts are presented in more detail below.

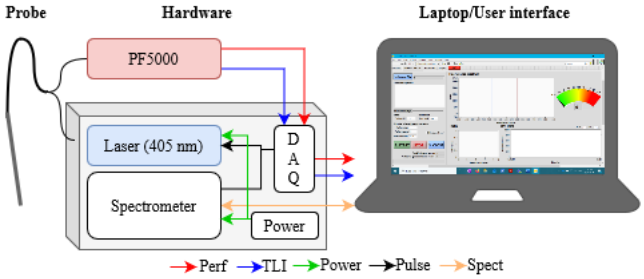

Figure 1. System overview with communication flow between fluorescence system, LDF, user interface and probe.

*Research supported by the Swedish Foundation for Strategic Research (RMX18-0056).

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B. Fluorescence system

The fluorescence module was built around a ZM18 laser (Z-laser GmbH, Germany) with a programable max output power of 40 mW at the blue wavelength (λ) of 405 nm. A CCD spectrometer (AVASpect-ULS2048L-EVO, Avantes BV, The Netherlands) with a bandwidth of 450-950 nm and resolution of 2.4 nm was used as the recording unit of the raw spectra. A collimator (F810SMA-405, Thorlabs AB, Sweden) with fiber connection was placed in front of the laser to focus the light and prevent stray light from entering the optical fiber. A filter (Longpass, cut-on 455 nm, Newport, Newport Corporation, USA) was placed in front of the spectrometer to filter out the remaining 405 nm laser light. A second filter (Shortpass, cut-off 750 nm, Edmund Optics, Edmund Optics Inc., USA) was positioned in front of the entry point to the spectrometer to filter out the LDF signal from the fluorescence spectrum.

The probe ends were screwed to the collimator and the spectrometer respectively through mechanical fixations using SMA connectors. This assured that the light had the best entry into the optical fibers and could be repeatedly connected without distortions. With this system a spectrum in the range 450-750 nm is used for visualization. In the presence of *PpIX,* a peak is found at 635 nm in the spectra. In brain tissue this peak is found in high grade tumor a few hours after oral intake of 5-ALA, and thus a prominent tumor marker [3, 9].

C. Laser Doppler flowmetry

The LDF system was previously adapted for use during neurosurgery [10]. Low power laser light (2 mW, λ = 780 nm) is used to record two signals, the *Perfusion* (i.e., microcirculation) which can act as a "vessel alarm" [8] and *Total Light Intensity (TLI)* which in the brain can distinguish between grey and white matter as previously shown [11]. The perfusion is presented in the range of 0-1000 arbitrary units [a.u.] and the TLI between 0-10 a.u.

D. Software

LabView (Professional Development System, Version 2019, NI Inc, USA) was used to program the signal control, sampling, data storage and the interface for presentation of the captured information. For the fluorescence part, the software controlled the signal flow between the laser and spectrometer, a user set integration time, number of spectra and selection of laser input voltage, which relates to the output effect. The laser is only turned on when measuring, but as an extra precaution, a LabView controlled shutter also blocks the blue laser light when no measurement is initiated.

Three measurement modes were implemented: fluorescence, combined LDF and fluorescence, and LDF. In the combined mode the LDF signals are streamed in parallel to the sampling of a preset number of fluorescence spectra. A fluorescence ratio (FL-R) [2] is calculated between 635 nm (*PpIX peak)* and the autofluorescence at approximately 510 nm. This is updated in real-time for each captured spectrum and displayed both in a speedometer and as a scalar. A user-controlled threshold can be set, which gives a sound signal if FL-R precedes the threshold. The user interface from the fluorescence mode is shown in Fig. 2.

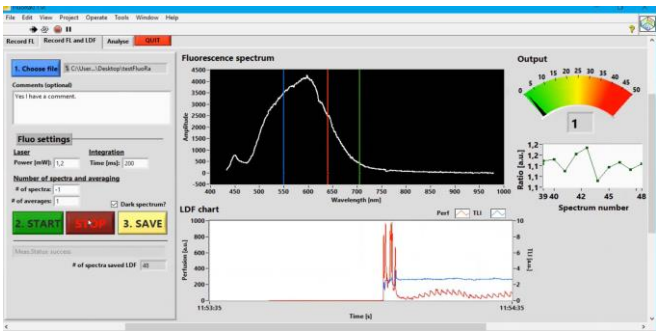

Figure 2. System interface with settings, spectrum, speedometer, and scalar indication of the fluorescence ratio.

E. Optical probe

A forward-looking optical probe developed for stereotactic brain biopsy were used for evaluation of the system performance. The probe was designed to comply with Leksell Stereotactic System (LSS, Elekta Instrument AB) and thus has a length of 190 mm and a width 2.2 mm with optical fibers placed in the space between the inner and outer stainless-steel tubes. The fibers were fixated with biocompatible glue and the probe tip polished to a rounded tip.

III. Experimental Evaluation

A. Experimental system tests

The system's performance was tested with the probe connected to both the LDF and the fluorescence module, first evaluating each mode at a time, then in combination. Fluorescence spectra were recorded from a red plastic material with static fluorescence. The laser power was increased stepwise (range 1.0-2.0 V), and the output laser effect was measured for each step at the laser and at the probe end. The relation between voltage, power and the number of spectrometer counts were plotted to find a suitable signal-to-noise level at the same time as the output power was as low as possible.

The LDF tests were done positioning the probe in a standard microsphere solution (PF1001 Motility, Perimed AB, Sweden). To investigate potential interference between the two systems, LDF measurements were also performed in the microsphere solution with and without the fluorescence recording active.

B. Skin measurements

To investigate the *PpIX*, *Perfusion* and *TLI* simultaneously, measurements were done on the forearm of a healthy volunteer (Ethics approval: M139-07/T83-09) after topical application of Metylaminolevulinat cream (METVIX® 160 mg/g, Photocure ASA, Norway). Aminolaevulinic acids in the cream converts to PpIX. Four hours prior to the measurements the skin was stripped with tape and the cream applied in a circular area with a diameter of approximately 1.5 cm. To not induce photobleaching from surrounding light the skin was covered with a dressing (Tegaderm™, 3M Health Care, Germany) and a non-transparent tape. For more details regarding the methodology, see [12, 13]. Fluorescence measurements were done at 5, 10, 15 and 20 mW and pulse widths of 200 and 400 ms. Ten

spectra were captured for each setting and skin position simultaneously with the microcirculation (*Perfusion* and *TLI*) recorded with LDF.

C. *Brain tissue measurements*

Fluorescence recordings were also done on fresh brain tumor tissue excised during total gross resection using a fluorescence guided blue-light microscope. The patient received a standard oral dose of 20 mg/kg 5-ALA (Gliolan®, Medac GmbH, Germany) prior to surgery. Informed written consent was received from the patient (Ethics approval: EPN-2020-01404). The probe was moved to different sites of the tissue slice while the peak spectra and bleaching process were studied. Different integration times and laser power were tested.

IV. Results

A. *Experimental evaluation*

It was found that the relation between output power and voltage was nonlinear for low voltage (< 1.3 V) at the laser end (Fig. 3).

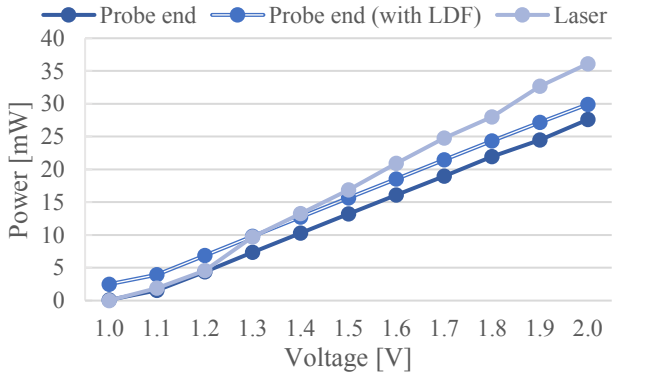

Figure 3. Output power as a function of input voltage for 11 voltages (1.0-2.0 V) at two locations: at the fluorescence laser and at the probe end. At the probe end measurements were taken for only fluorescence, and in combination with LDF.

A software programmed upper limit was set to 1.76 V to limit the fluorescence laser power to 20 mW.

Measurements on the static fluorescing material is presented in Fig. 4 for the 5, 10, 15 and 20 mW output power. The shape was the same, but the maximum number of counts increased proportionally. This was about one third (34%) of the maximum no of counts for the spectrometer.

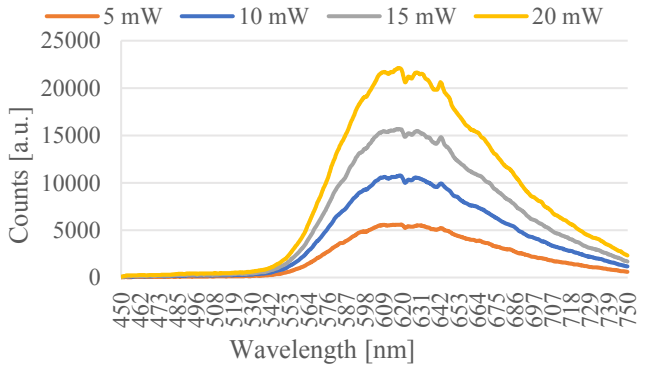

Figure 4. Counts as a function of wavelength for the static fluorescing material. The curves represent 5, 10, 15 and 20 mW laser output power.

B. *Measurement of PpIX on the skin*

The same laser output power (5, 10, 15 and 20 mW) was tested on a skin prepared with METVIX®, see Fig. 5. The counts for autofluorescence and 635 nm peak were related in descending order of laser power. The corresponding FL-R were 0.27, 0.24, 0.34, and 0.17 for increasing laser power.

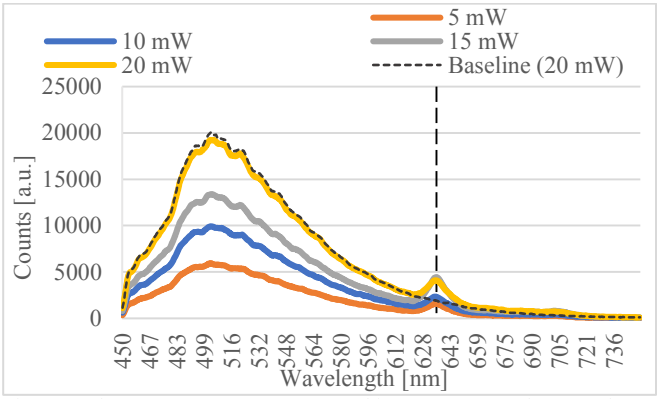

Figure 5. Fluorescence measurements on skin at 5, 10, 15 and 20 mW laser power, and baseline spectrum at 20 mW (no fluorescence). The PpIX-wavelength at 635 nm is marked with a dotted line.

C. *PpIX measurements in brain tumour tissue*

Four measurements at the same site in the tissue sample illustrate the bleaching process of (mainly) the 635 nm peak. The first spectrum, here called baseline, has a peak of 6700 counts. The following spectra were measured after 2.4 s, 4.8 s and 9.6 s continuous laser light exposure. The spectra were collected at a laser output power of 10 mW and integration time of 800 ms. The corresponding FL-R were 5.0, 3.1, 2.6, and 2.0. Thus after 5 s of laser exposure, the FL-R was almost halved.

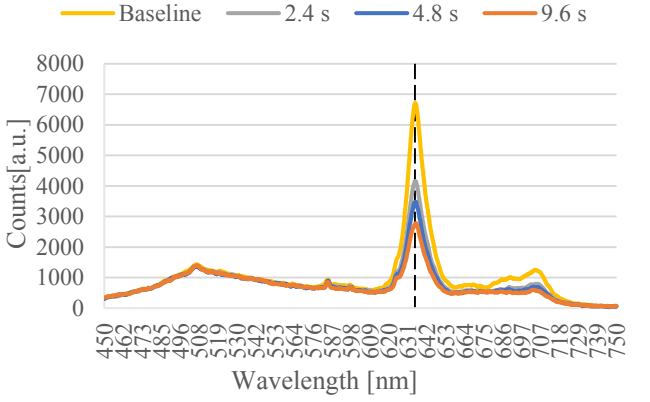

Figure 6. Brain tissue sample fluorescence spectra at four exposure times, baseline, 2.4 s, 4.8 s and 9.6 s.

V. DISCUSSION

A novel fluorescence hardware system has been designed for detection of *PpIX* during brain tumor biopsies and resection. It is directly linked to a PF 5000 LDF device through the probe and the LabView software. Compared to previous designs [2, 6], the spectrometer is more sensitive. This opens for probe design with thinner optical fibers and thus more standardized manufacturing. FluoRa was successfully evaluated during in-vitro experiments on both skin and brain tumor tissue with 5-ALA. This gave an opportunity to investigate the system's dynamics for various pulse lengths and integration times. Several safety aspects were considered in the FluoRa design in order to prepare the system for clinical use [14]. This included automatic blockage of the laser beam, a pre-set maximum laser output, optical isolation through the fibers, and medical grade isolation transformer. This makes all electrical signals isolated from the patient and the clinical staff. Another advantage is the interface which controls and displays both the fluorescence and LDF signals and give auditory feedback. In a previous study of 28 stereotactic brain biopsies we used two separate systems [7], which was complex to handle in the operating room. With the new design, the surgeon will receive direct visual and sound feedback, indicating malignant tissue and increased microvascular blood flow [11] i.e. acting as a "vessel alarm" [8].

Other groups have also presented combined measurements between different optical features and fluorescence, but to our knowledge no groups have combined PpIX fluorescence with LDF [15]. As PpIX fluorescence is limited to high-grade brain tumors our next step is to investigate if Raman spectroscopy can be integrated with fluorescence and LDF. This would increase the possibility to also study low-grade brain tumors. Preliminary Raman data captured from brain tumor tissue show promising results [16]. Other prospects are to adapt the probes to frameless navigation systems as these are becoming more common during biopsies than the stereotactic frame approach.

In summary, the combined fluorescence and LDF system using a forward-looking probe is intended to provide real-time information of high-grade brain tumor tissue *(PpIX)*, identify grey-white matter (*TLI*), and to act as a "vessel-alarm" through microcirculation (*Perfusion*) recordings. As a next step, the system will be used for in-vivo measurements during resection and biopsies in neurosurgery.

ACKNOWLEDGMENT

The authors are thankful for the skillful construction of the probe by Research Engineer Håkan Rohman. KW and JR have shares in FluoLink AB.

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