Spectroscopic fluorescence measurements as an intraoperative tool for glioma resection

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Context:

Gliomas account for 80% of malignant primitives tumors of the central nervous system. It is the most common primary brain tumor in adults, with a median age at diagnosis of 64 years. They are infiltrative cancers and are often not curable [1]. Surgery is the first step to treat gliomas and studies have shown that the survival rate is linked with the quality of the surgery [2],[3]. Thus, it is mandatory to get the most extended resection.

Today, 5-ALA-induced protoporphyrin IX (PpIX) fluorescence is widely used to help the surgeon distinguish infiltrative compound of gliomas. The current methods are based on intraoperative surgical fluorescence microscopy [4] or optical fiber systems that allow a local emission spectrum measurement [5],[6] or a quantification [7], [8] of 5-ALA-induced PpIX concentration. However, the accuracy of such technics remains limited because of a still low sensitivity [5],[7],[9] to evaluate infiltrative compound of gliomas.

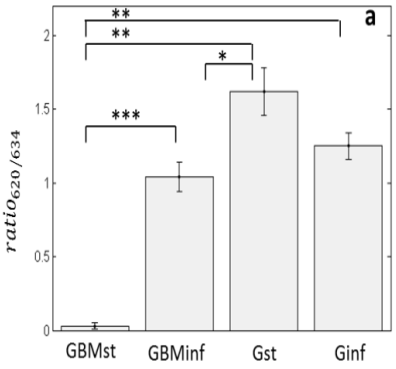


Figure 1: Extracted tumor tissues measurements of the $ratio_{620/634}$, for the 4 groups *GBMst* (n = 5), *GBMinf* (n = 16), *Gst* (n = 7) and *Ginf* (n = 7). Mean (grey column) and standard error mean are shown (error bar). *P < 0.05, **P < 0.01, ***P < 0.001. Two sample Kolmogorov-Smirnov test.

We demonstrated in a previous in vitro and ex vivo study that the PpIX spectrum is more complex than expected. Biopsies were from patients with glioblastomas (GBM), which are high grade gliomas. We showed that, added to the known peak at 634 nm (PpIX634), a second peak appears sometimes at 620 nm (PpIX620). We assumed that those two peaks correspond to two states of PpIX, which depend on the microenvironment. Knowing this, we proposed new measured parameters to distinguish the solid component of GBM from low grade gliomas and infiltrative component of GBM [10]. Figure 1 shows the ratio between the two states and discrimination of the solid component of GBM.

Our goal is then to create an intraoperative tool based on fluorescence spectroscopy to assist the surgeon during tumor's margins resection. This paper focuses on new instrumental development to distinguish the two states of PpIX, based on multi-wavelength excitation.

Materiel and Methods:

We assumed that the state of PpIX peaking at 620nm also has a shifted excitation spectrum. Then, the use of several excitation wavelengths would give us different ratios for the same tissue and this added information would help us to assess the concentration of both states of PpIX.

The set up (figure 2) uses three light-emitting diodes (LED) that sequentially illuminate the tissue at 385 nm, 405 nm and 420 nm. Then it gets the re-emitted spectrum though a probe set on the brain. A spectrometer measures the emitted spectrum and data are collected and analyzed on Matlab. The whole system is driven by Labview, software from National Instrument.

The spectrum is fitted with three reference spectra determined in preceding work, as explained in the preliminary ex vivo study [10]. Those references are the spectra of PpIX620 and PpIX634 and the spectrum of photoproducts. For ex vivo data, auto-fluorescence is fitted with a Gaussian centered around 595-600 nm and an exponential, as done in literature [11].

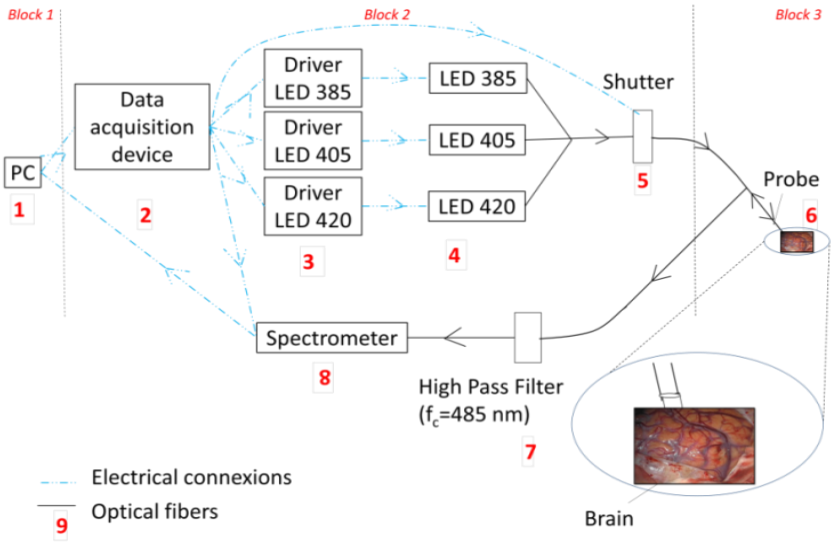
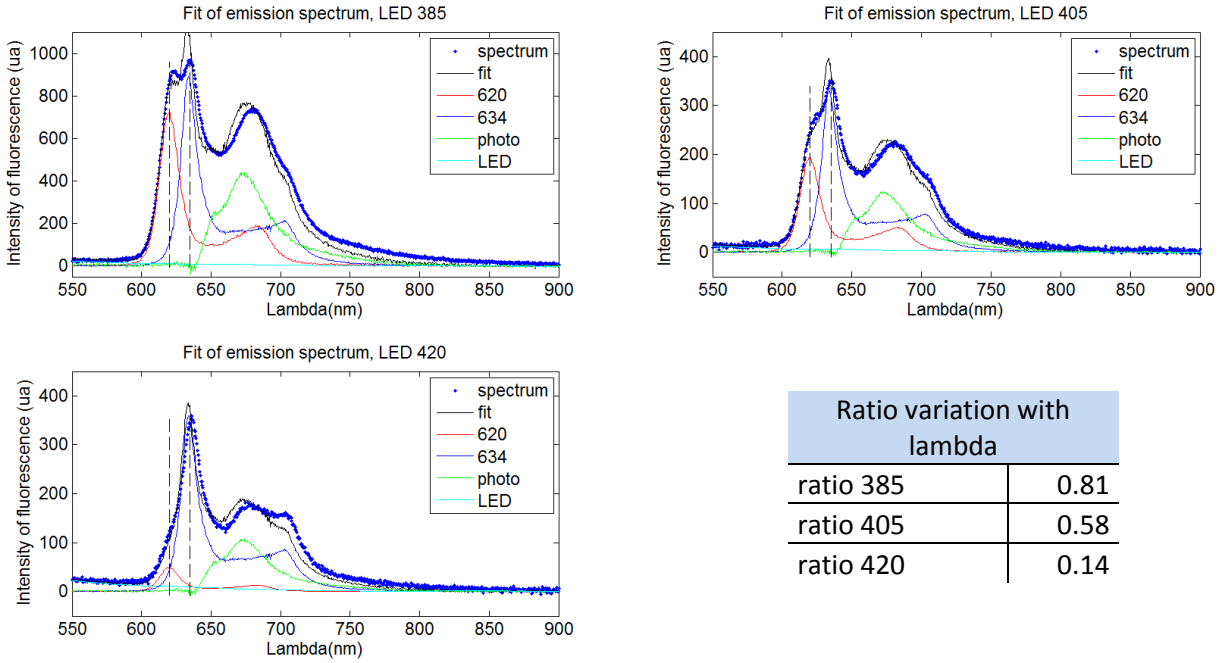


Figure 2 : drawing of the new device

In vitro experiments were made with solutions of PpIX in different micro-environment to validate the new setup. Then, biopsies measurements were realized to validate ex vivo the hypothesis of shifted excitation spectrum of PpIX620. Patients were undergoing surgery for gliomas and biopsies were samples for validation.

Results and discussion

In vitro experiments confirmed the shifted excitation spectrum of PpIX620 and showed that, for a given solution, the Ratio decreases when the excitation wavelength increases (figure 3). Ex vivo experiments seems to show a better sensibility of spectroscopy against microscopy, since we observed a spectrum of fluorescence in every situation, even when the surgeon qualifies the tissue as “non-fluorescent”. The ex vivo spectra are more complex than the in vitro ones because of autofluorescence. However results show the same trend. More detailed results will be presented and discussed at the conference. This study confirms the usefulness of multi-wavelength excitation for distinguishing both states of PpIX.



Ratio variation with lambda	
ratio 385	0.81
ratio 405	0.58
ratio 420	0.14

Figure 3 : variations in the ratio of the two states of PpIX. The solution is kept identical while the excitation wavelength is changed

Acknowledgement

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