Spectroscopic fluorescence measurements as an intraoperative tool for glioma resection
Laure Alston, Laurent Mahieu-Williame, Xavier Armoiry, David Meyronet, Mathieu Hebert, David Rousseau, Jacques Guyotat, Bruno Montcel

To cite this version:
Laure Alston, Laurent Mahieu-Williame, Xavier Armoiry, David Meyronet, Mathieu Hebert, et al.. Spectroscopic fluorescence measurements as an intraoperative tool for glioma resection. European Conferences on Biomedical Optic 2015, OSA/SPIE, Jun 2015, Munich, Germany. hal-01180039

HAL Id: hal-01180039
https://hal.archives-ouvertes.fr/hal-01180039
Submitted on 24 Jul 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Spectroscopic fluorescence measurements as an intraoperative tool for glioma resection

Laure Alston¹, Laurent Mahieu-Williame¹, Xavier Armoiry³, David Meyronet⁴, Mathieu Hebert², David Rousseau¹, Jacques Guyotat⁵, Bruno Montcel¹.

¹CREATIS ; Université de Lyon ; Université Lyon1 ; CNRS UMR5220 ; INSERM U1044 ; INSA Lyon, Villeurbanne, France.
²Laboratoire Hubert Curien ; Université Jean-Monnet, Saint-Étienne ; UMR CNRS 5516
³Pharmacy department / Cellule Innovation ; Groupement Hospitalier Est ; Hospices Civils de Lyon, Bron, France.
⁴Centre de Pathologie et Neuropathologie Est ; Hospices Civils de Lyon, Bron, France.
⁵Service de Neurochirurgie D ; Hospices Civils de Lyon, Bron, France

Laure.Alston@creatis.insa-lyon.fr

Context:

Gliomas account for 80% of malignant primitive 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.

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.

Context:

Gliomas account for 80% of malignant primitive 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.

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].

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

Acknowledgement

This work was supported by the LABEX PRIMES (ANR-11-LABX-0063) of Université de Lyon, within the program "Investissements d'Avenir" (ANR-11-IDEX-0007) operated by the French National Research Agency (ANR) and by the Cancéropôle Lyon Auvergne Rhône Alpes (CLARA) within the program « OncoStarter ».

References