Identifying Isolated Glioblastoma Tissues in Human Patients through Their Optical and Spectral Properties

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Abstract: Survival rates and health-related quality of life of adult patients suffering from glioblastoma depend significantly on the extent (no residual tumor tissue) and precision (no collateral damage) of the surgical resection. Assistance in defining the borders of the infiltrating component of the glioblastoma would be valuable to improve outcomes. A tissue can be defined by its optical properties : absorption, scattering, intensity of fluorescence, that will give a unique signature. In this work we look at the absorption and scattering coefficients of glioblastoma and control tissues from adult patients using an integrating sphere, spectral measurements were also took on the samples using a fiber endoscope. The preliminary results show the potential of using endogenous fluorescence for intraoperative identification of residual glioblastoma tissue in the wall of the surgical cavity of resection.

1 INTRODUCTION

Glioblastoma (GBM) is the most common and most aggressive malignant primary brain tumor in adults. Following a magnetic resonance imaging (MRI) analysis, its oncological treatment comprises 1) a maximal safe resection encompassing the contrast-enhanced tumor tissue, whenever feasible, 2) an adjuvant treatment with combined radiotherapy and concomittant chemotherapy followed by adjuvant chemotherapy. The surgical resection technique is limited by the difficulty to discriminate intraoperatively between healthy tissue and tissue infiltrated by isolated glioblastoma cells, mainly in the wall of the surgical resection cavity, which contain the infiltrated boundaries of the tumor. The clinical concern is that, because glioblastoma infiltration is not completly resected, the tumor recure systematically and the patient will have to return for a new operation which could lead to more constraining side effects and reduce chances of survival. The median overall survival is less than 18 months.

Indeed, glioblastoma is one of the most infiltrating tumor, even if the solid tumor area is easily detectable on MRI as a contrast-enhanced tumor tissue, the infiltrated regions, which contains active and isolated glioblastoma cells, are not contrasted from healthy regions. These infiltrated area have the same visual appearance as the healthy ones, which makes them difficult to delineate.

Nowadays, the only technique that gives accurate information on infiltrated area is the histolopathogical analysis of a biopsy sample, which is done ex-vivo and takes several days.

Recently, several intraoperative techniques have been proposed to solve this problem, such as the linear endomicroscope commercialized by Mauna Kea technologies. This tool presents the advantages of in-vivo and real time imaging. However, this technique still not able to provide a multimodality of measurements, which seems necessary to discriminate healthy from tumoral tissues with a strong relialability.

Our team develops a non-limear optical endomicroscope, which is a new tool designed to the surgical room. This intraoperative system will allow

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the neurosurgeon to have a fast, reliable and reproducible response on the nature of surrounding tumor tissues, using several endogenous contrasts.
The multimodaly, regrouping Two Photons Excitation (TPEF), Second Harmonic Generation (SHG), spectral and fluorescence lifetime measurements, was already proved as essential in glioma tissue detection (Zanello et al., 2017).
In this context, it is necessary to identify all optical properties of glioblastoma tumor, and compare them with those of healthy tissues. In this work, many optical parameters of frozen samples were found, absorption and scattering coefficients using the integrated sphere technique under 430 nm. The spectral signature of endogenous fluorophores, using linear excitation at 375 nm and 405 nm excitation wavelength, were recorded.

2 MATERIALS AND METHODS

2.1 Samples

Samples were carried from the neurosurgery department to the neuropathology laboratory at Sainte-Anne hospital, Paris. The delay between the end of the resection operation and the reception of tissue was about fifteen minutes. After that, the samples were stored at -80°C for few days. Then they were cut at -18°C into 600 µm thickness sections using a cryostat (Leica Microsystems) and fixed with a 100% alcohol solution. Six glioblastomas and seven healthy cortex samples were analysed in this preliminary study.

2.2 Spectral, Transmittance and Reflectance Study

The set-up shown in figure 1, is placed in the neuropathology department at Sainte-Anne Hospital, Paris-France. The setup is based on a linear excitation fiber endoscope with three pulsed diode lasers.

For integrating sphere measurements, the pulsed diode laser emitting at 430 nm (Picoquant Germany, LDH-P-C-430B, FWHM 70 ps) excitation wavelength was used. Transmittance and reflectance measurements of each sample were achieved using an integrating sphere (Thorlabs, IS200-4). Its inner surface is covered by a 99% reflective teflon, it have four ports with a 12.7 mm diameter, and a fifth port with a 3mm diameter to transfer the signal from the integrating sphere to the spectrometer (Ocean optics, HR2000) using an optical fiber (Ocean optics).

The average laser power used for this study was around 5 mW. A diaphragm was placed after the laser source to reduce the beam diameter to 1 mm and the laser beam was pointed to five differents Region Of Interest (ROI) in each specimen. Transmittance and reflectance were measured for each ROI, and finally the average of these five values was calculated.

For spectral measurements, the excitation is performed with the two others pulsed diode lasers emitting at 405 nm (Picoquant-Germany, LDH-P-C-405B, FWHM 60 ps,) and 375 nm (PicoQuant-Germany, LDH-P-C-375B, FWHM 45ps) with a maximal power of 1.1 mW. These diodes were controlled with a driver (PicoQuant-Germany, PDL-808 “Sepia”).

A specific Photonic Crystal Double-Clad Fiber (PC-DCF) was used to excite and collect the fluorescence signal (Ibrahim et al., 2016a, 2016b). Collected signal is lead to the spectrometer (Ocean Optics, QEPro 6500) through a long pass filter (Semrock, SR420) to eliminate laser reflection.

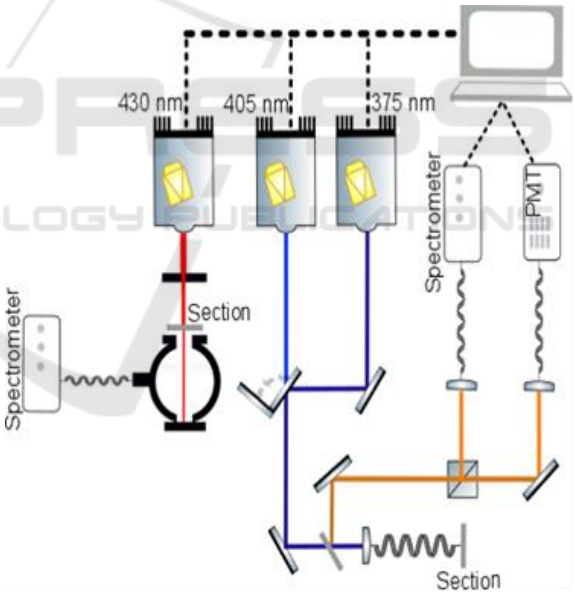

Figure 1: Schematic representing the implemented setup, including the integrating sphere and the spectral analysis.

3 DATA ANALYSIS

3.1 Optical Coefficients

The Inverse Adding Doubling (IAD) algorithm (S. A. Prahl et al., 1993) was used to find the absorption coefficient (μ_a) and the reduced scattering

coefficient (μ'_s) by refearing to the measured values of the transmittance and the reflectance.
The scattering coefficient μ_s is deduced using the equation (1)

$$\mu_s = \mu'_s/(1-g) \quad (1)$$

Where g is the anisotropy factor of the sample, so we consider that g=0.89 for glioblastoma samples and g=0.86 for cortex samples (Poulon et al., 2017). This algorithm solves iteratively the radiative transport equation until the numerical adjustment and the experimental values of reflectance and transmittance matches (Prahl et al., 1993). The refractive index of the samples was not measured: we consider that the refractive index n = 1.44 is the same for all samples examinated by refering to the literature (Bevilacqua et al., 1999).

3.2 Spectral Analysis

A Matlab code developed by our team and already used in previous publications (Haidar et al., 2015) (Zanello et al., 2017) was used to process spectral data.

During spectral measurements, we used two excitation wavelengths: (i) 405 nm to excite efficiently five different endogenous fluorophores: Nicotinamide Adenine Dinucleotide (NADH), Flavins (FAD), lipopigments, porphyrins and chlorines, and (ii) 375 nm to excite effectively the NADH and FAD.

4 RESULTS

4.1 Optical Coefficients

The distribution of all scattering coefficient μ_s values obtained in our measurements are shown in Figure 2. The glioblastoma samples presented a higher scattering coefficient than the cortex samples and the glioblastoma samples had a higher absorption coefficient than the cortex samples.

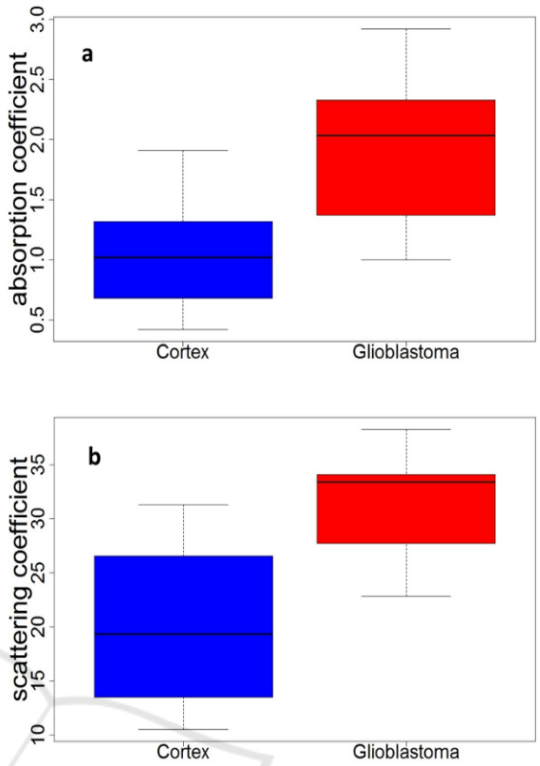

Figure 2: Distribution of the scattering coefficient values (mm^{-1})(a) and the absorption coefficient values (mm^{-1}) (b) for glioblastoma and cortex tissues excited with 430 nm.

4.2 Spectral Measurements

In Figure 3, we compared the total fluorescence intensity of glioblastoma and cortex samples, excited with 405 nm (a) and 375 nm (b).

The difference in the spectral intensity was well underlined between glioblastoma and cortex samples. The emitted fluorescence from cortex samples was 2.5 times higher than for glioblastoma samples using a 405 nm excitation wavelength, and 2 times higher using 375 nm. We observed that using 375 nm excitation wavelength we optimized the NADH excitation and so the emitted fluorescence signal was higher at this wavelength. Using 405 excitation molecule we excited five endogenous molecules (Zanello et al., 2016) as shown in the spectral shape of figure 3.a.

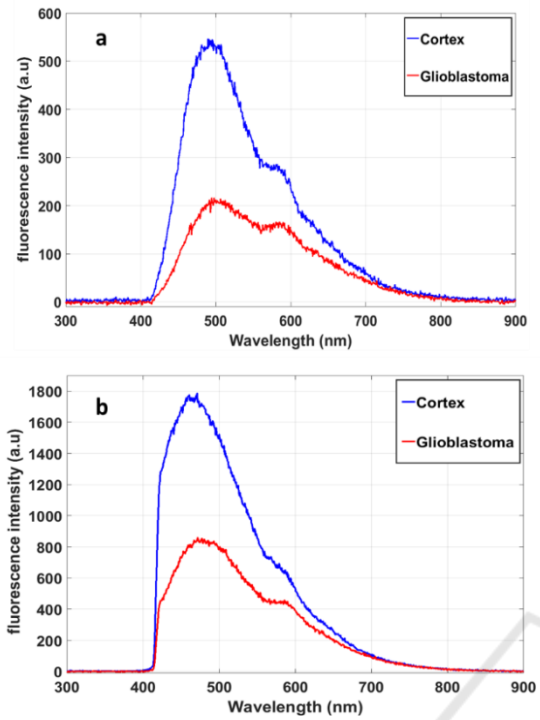

Figure 3: Fluorescence spectra of glioblastoma and cortex tissues excited with 405 nm (a) and 375nm (b) wavelength.

5 DISCUSSION

In this preliminary study, we compared fixed cortex and glioblastoma samples from adult patients using two types of measurements. First, we observed that the scattering and absorption coefficient are higher in glioblastoma samples than in cortex samples. This could be related to the fact that glioblastoma tissues have denser vascularization and more collagen fibres than cortex. This vascularization could be the source of light scattering in such tissues. Added to the fact that glioblastoma tissues contain neovascularization which could affect the absorption coefficient.

By referring to our previous spectral studies on rats brain tumors and healthy tissues (Haidar et al., 2015) and on human brain tissues (Zanello et al., 2017) using 405 nm excitation wavelength, the five excited fluorophores are more concentrated in the healthy tissues than in the glioblastoma tissues. For that, the total fluorescence signal in cortex tissue is higher than in glioblastoma. The same trend was shown using 375 nm excitation. At this excitation wavelength the absorption efficient section of NADH and the FAD is higher than at 405nm excitation wavelength. So, we optimise the efficiency of excitation of this two endogenous molecules and specially the excitation of NADH. We can show also that endogenous fluorescence from healthy tissues is higher than glioblastoma tissues. This observation is of paramount important as spectral response can discriminate healthy from glioblastoma tissues. This is in accordance with the literature of spectral analysis on freshly resected tissues (Zanello et al., 2017).

Here, we show bimodal quantitative measurements on the same tissues. These measurements prove the power of optical detection, based on endogenous fluorescence of frozen tissues to discriminate healthy from tumoral tissues.
This opens a promising door in the detection of tumors margins. It proved also how important is the multimodality to detect with reliability the nature tissues area, highlighting the importance of our optical endomicroscope.

In the future studies, we will extend our cohort by examining more types of human brain tumors and also extend our study to freshly extracted samples.

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