Estimation of Fluorescence Contributions using Multiple-Wavelength Excitation: the example of PpIX for improving Glioma Classification

Arthur Gautheron^{1,2}, Michael Sdika¹, Mathieu Hébert², Bruno Montcel¹

 ¹ Université de Lyon, INSA-Lyon, Université Claude Bernard Lyon 1, UJM Saint Etienne, CNRS, Inserm, CREATIS UMR 5220, U1206, F-69100, LYON, France
² Université de Lyon, Université Jean Monnet de Saint Etienne, Institut d'Optique Graduate School, CNRS UMR5516 Laboratoire Hubert Curien,F 42000 Saint Etienne, France

gautheron@creatis.insa-lyon.fr

Protoporphyrin IX (PpIX) is a fluorophore that can be used to identify tumoral tissues [1]. Usually, the tissue is excited at one wavelength, e.g., 405 nm, and the amount of PpIX is estimated from the fluorescence signal generated by this molecule and other fluorophores (baseline). However, one observes that fluorophores too close to PpIX impair the specificity of the estimation methods. We thus propose to cope this issue with an efficient multi-excitation wavelengths method, free from any a priori on the baseline.

<u>Method</u>: In contrast with method from the state of the art, we use several excitation wavelengths. We wrote the model has a system of non-linear equation whose variables are the PpIX contribution, the baseline signal and the baseline quantum yield. As the baseline is also estimated, our method is free from a priori on the baseline spectral curve. Additionally, we were able to derive an analytical solution for all the variables of our model. This yields a computationally efficient method, free from any tuned parameter or iterative estimation procedure.

<u>Experiments</u>: We used a digital phantom of PpIX fluorescence at two excitation wavelengths, parameterized from experimental data[2,3], with endogenous fluorophores. We compared the parameter values estimated by each model with a ground truth value. In addition, we measured the impact of the estimation error on the pathological status prediction, by means of a bayesian classifier applied on data whose tumoral categories are known [3]. We set parasitic fluorophore amplitude *A* to 50% of the PpIX amplitude in healthy tissues as inspired by previous works carried out in vivo [3].

<u>Results and discussion</u>: We have simulated the image of a high-grade glioma, a tumor that is rich in PpIX. In Figure 1, we notice that specificity between healthy and cancerous tissues of existing models (BF_1 , GB_1 , BF_2 and GB_2) drops to 0% when the amplitude A is increased by a factor 10 (like the evolution of lipofuscin with age). In contrast, our method (EB_2) keeps a specificity of 87% while the specificity of the ground truth (GT) reaches 88%.



Figure 1 – Simulated maps of tissues in a high grade glioma, as retrieved by various models and ours.

Références

[1] T. Ando *et al.*, « Precise comparison of protoporphyrin IX fluorescence spectra with pathological results for brain tumor tissue identification », *Brain Tumor Pathol.*, vol. 28, p. 43-51, févr. 2011, doi: 10.1007/s10014-010-0002-4.

[2] C. Jonin *et al.*, « Two photon excited fluorescence and hyper Rayleigh scattering of Protoporphyrin IX », *J. Photochem. Photobiol. Chem.*, vol. 402, p. 112812, nov. 2020, doi: 10.1016/j.jphotochem.2020.112812.

[3] L. Alston *et al.*, « Spectral complexity of 5-ALA induced PpIX fluorescence in guided surgery: a clinical study towards the discrimination of healthy tissue and margin boundaries in high and low grade gliomas », *Biomed. Opt. Express*, vol. 10, n° 5, p. 2478-2492, mai 2019, doi: 10.1364/BOE.10.002478.