Quantitative fluorescence spectroscopy of pigmented and non-pigmented tissues

Paszczka Pawel¹, Szczygieł Małgorzata², and Matuszak Zenon¹

¹Department of Medical Physics and Biophysics, Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, Al. Mickiewicza 30, 30-059 Krakow, Poland, e-mail: Zenon.Matuszak@fis.agh.edu.pl
²Department of Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, 7 Gronostajowa St., 30-387 Krakow, Poland

Photodynamic therapy (PDT) is based on the dye-sensitized photooxidation of biologically important cell structures. Direct cell kill is caused by damage to plasma membranes or mitochondria. In PDT, a tissue containing dye is exposed to light, mainly corresponding to wavelength at maximum of its absorption. The dye (PS-photosensitizer) is transformed from its ground state to excited triplet state, interacts with oxygen, transfers its energy directly to oxygen to form highly reactive singlet oxygen (type II reaction). This type of therapy has some advantages: PS is non-toxic for organism before irradiation, selectivity is obtained by localization of PS in tissue and photodynamic damage is restricted to irradiated tissue area. PS used in PDT are often fluorophores and monitoring of their fluorescence allows the localization of the tumor, determination of PS concentration, and definition of tissue margins for light delivery. However, with tissues being turbid media, their absorption and scattering properties influence strongly both excitation and emission photons transport, as a consequence — the intensity and shape of PS emission. Due to these complications, absolute quantification of drug fluorescence (and concentration in tissue) is complex [1]. We were particularly interested in quantitative PDT of melanoma, and how to predict concentration and shape of fluorescing dye in tissue. The aim of this study was to elaborate and test a simple method of quantification of PS (hematoporphyrin, eosin, fluorescein, rose bengal) in tissue mimicking phantoms using various detection configurations: absorption and fluorescence spectrometry with fiber optics detection and plate-reader. Non-pigmented tissues were simulated by Intralipid solutions, a good model of scattering properties of tissues. Pigmented tissues were mimicked by mixtures of Intralipid and synthetic melanin in various proportions. The Monte Carlo method (modified codes – MCML, MCVM) was used to quantify spatial distributions of excitations/emission photons in both non-pigmented and pigmented tumor tissues, true excitation/emission spectrum were simulated for each fluorophore for various experimental conditions. Reflectance spectra and visual appearance of pigmented tissue phantoms was simulated additionally. Optical properties of tissue phantoms used in simulations are based on measured and reported (literature) values. Volumetric distributions of both, fluorescence excitation and emission spectra were obtained as functions of tissue optical properties for different PS and melanin concentrations. It was demonstrated that:

1. emitted fluorescence intensity is a function of the depth of fluorescence generation,
2. strong influence of melanin content on the intensity and shape of PS emission (more than 10 times delay). Diffuse reflectance was calculated separately as a function of melanin concentration, and compared with skin appearance, giving visual criterion for estimation melanin content within tissues layers. It was demonstrated that the models used are able to predict the spatial distribution of the PS fluorescence excitation/emission pattern within skin phantoms as functions of PS and melanin concentrations. Only in the case of non-melanized tissues was it possible to predict distribution of PS, concentration and spectrum shape within tissue on the basis of its fluorescence intensity with high accuracy.

Keywords: photosensitizers, fluorescence, Monte Carlo simulation, melanin