CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	Colorectal cancer detection based on the extraction of scattering properties and biochemical concentrations from fluorescence spectroscopy measurements
Authors	Nogueira, Marcelo S.;Matthews, Robert;Killeen, Shane;Oâ Riordain, Micheal;Andersson-Engels, Stefan
Publication date	2022-04-01
Original Citation	Nogueira, M.S., Matthews, R., Killeen, S., O'Riordain, M. and Andersson-Engels, S. (2022) 'Colorectal cancer detection based on the extraction of scattering properties and biochemical concentrations from fluorescence spectroscopy measurements', in Biophotonics Congress: Biomedical Optics 2022 (Translational, Microscopy, OCT, OTS, BRAIN). Fort Lauderdale, Florida, 24-27 April, Optica Publishing Group, TS2B.5, (2 pp). https://doi.org/10.1364/TRANSLATIONAL.2022.TS2B.5.
Type of publication	Conference item
Link to publisher's version	Biophotonics Congress: Biomedical Optics 2022 (Translational, Microscopy, OCT, OTS, BRAIN) - 10.1364/TRANSLATIONAL.2022.TS2B.5.https://doi.org/10.1364/TRANSLATIONAL.2022.TS2B.5
Rights	© 2022 The Author(s). Published by Optical Publishing Group
Download date	2026-11-15 18:06:07
Item downloaded from	https://hdl.handle.net/10468/13747



University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Biophotonics Congress: Biomedical Optics (Translational, Microscopy, OCT, OTS, BRAIN) © Optica Publishing Group 2022

Colorectal cancer detection based on the extraction of scattering properties and biochemical concentrations from fluorescence spectroscopy measurements

Marcelo Saito Nogueira[1,2], Robert Matthews[1,2], Shane Killeen[1,3], Micheal O'Riordain[1,3], Stefan Andersson-Engels[1,2]
1.Tyndall National Institute, Lee Maltings, Dyke Parade, Cork, Ireland
2.Department of Physics, University College Cork, College Road, Cork, Ireland
3.Department of Surgery, Mercy University Hospital, Cork, Ireland

E-mail address: marcelosaitonogueira@gmail.com

Abstract: We report the first study estimating fluorophore and chromophore concentrations with scattering properties for colorectal cancer detection, as well as one of the first studies performing such estimation based on fluorescence spectra alone. © 2022 The Author(s)

1. Introduction

Colorectal cancer (CRC) is the 3rd most common and the 2nd most deadly type of cancer worldwide [1]. Late CRC detection leads to poor patient prognosis and remains a global challenge given the ineffectiveness of current screening methods. Early-stage CRC is typically detected by colonoscopy followed by relevant biopsies of suspected lesions as the current gold standard diagnostic test. However, early CRC detection and cancer-patient stratification still relies on various medical tests to observe tissue structural changes, which happen in late stages of CRC compared to molecular changes. Therefore, molecular-sensitive optical techniques capable of real-time tissue differentiation could benefit CRC patients with early CRC detection, improved treatment planning, and CRC delineation during surgery [1]. One of these techniques is fluorescence spectroscopy (FS).

FS uses one or more combinations of monochromatic light to excite specific molecules (fluorophores). The fluorescence emitted is dependent on the tissue composition in terms of fractions of collagen ($\boldsymbol{f_{coll}}$), elastin ($\boldsymbol{f_{elast}}$), flavins ($\boldsymbol{f_{flav}}$), lipopigments ($\boldsymbol{f_{lipop}}$), nicotinamide adenine dinucleotide ($\boldsymbol{f_{NADH}}$), piridoxine ($\boldsymbol{f_{pirod}}$), porphyrins ($\boldsymbol{f_{porp}}$), and tryptophan ($\boldsymbol{f_{trypt}}$). These fractions can be used to monitor cancer biomarkers including those related to changes in tissue metabolic rates and collagen matrix. Also, both fluorescence excitation and emission light are affected by tissue absorption associated with volume fraction (volume percent) of tissue chromophores ($f_{choromophore}$) such as bile (f_{bile}), oxyhemoglobin (f_{HbO2}), deoxyhemoglobin (f_{Hb}), methemoglobin (f_{metHb}), lipid (f_{lipid}), and water (f_{water}), as well as scattering properties such as the reduced scattering amplitude α', Mie scattering power b_{Mie}, and percentage contribution of Rayleigh scattering f_{Ray}. Scattering parameters describe average particle sizes and refractive index mismatches occurring in tissue due to the distribution tissue/cell micro- and nano-structures such as cell/organelle membranes, organelles (such as mitochondria), and collagen fibers and fibrils [2].

Previous FS studies have shown FS has potential for CRC detection, as classification performance metrics achieved include sensitivities and specificity of up to 100%. However, these studies have focused on differentiating tissues with multivariate analysis methods instead of extracting tissue biochemical parameters based on fluorophore concentrations. To the best of my knowledge, methods extracting interpretable parameters from fluorescence spectra alone (without combination with other optical methods to correct for the effect of absorption and scattering) do not currently exist for CRC applications. In this study, we report the first set of absorption, scattering and fluorescence parameters extracted from CRC and normal mucosa based on a spectral fitting algorithm to fit fluorescence spectra with no correction by other techniques.

2. Material and methods

Our study included 6 patients undergoing bowel resection for CRC. A total of 318 fluorescence spectra were collected from normal mucosa and 364 for cancer tissues. Measurements were collected from about 7 normal mucosa and 7 tumor locations over an area of approximately 100 cm^2. Measurement locations were identified by experience surgeons and the ground truth was confirmed by histopathological analysis. Contact measurements were performed by using an FS system containing a 340-nm LED for fluorescence excitation, illumination and collection of backscattered light through the same 600-µm-core optical fiber, a dichroic mirror, a 370-nm long-pass optical filter, and a spectrometer to measure fluorescence between 380-700 nm. The study was performed under the approval of the Clinical Research Ethics Committee of University College Cork, following methods according to the relevant guidelines and regulations. Spectral fitting parameters obtained in this study was based on a dataset of 223 fluorescence spectra from fresh ex vivo tissue samples from 1 subject. All analyses were performed by using a spectral fitting algorithm based on a lookup table (LUT) of fluorescence emission intensity values $F^{theorectical}(\mu_a^{ex}, \mu_s'^{ex}, \mu_a^{em}, \mu_s'^{em})$ as a function of the tissue absorption coefficient at the excitation and emission wavelengths (μ_a^{ex} and μ_a^{em}, respectively) and reduced scattering coefficient for the same wavelengths ($\mu_s'^{ex}$ and $\mu_s'^{em}$, respectively). $F^{theorectical}$ was obtained via integration of photon hitting density (PHD) maps for each combination of $\mu_a^{ex}, \mu_s'^{ex}, \mu_a^{em}, \mu_s'^{em}$. PHD maps were obtained via convolution between fluence rate profiles generated from Monte Carlo

(MC) simulations of light propagation in complex media. By associating combinations of $\mu_a^{ex}(\lambda), \mu_s'^{ex}(\lambda), \mu_a^{em}(\lambda), \mu_s'^{em}(\lambda)$ set by chromophore and fluorophore concentrations as well as scattering properties referred to in the introduction section, we calculated $F^{theorectical}(\lambda)$. At each iteration of the fitting process, $F^{theoretical}(\lambda)$ is compared with the experimental fluorescence spectra $F^{experimental}(\lambda)$ until the error is within the tolerance limit. Spectral fitting was performed for 3 spectral regions: 470-490 nm, 490-600 nm and 600-700 nm.

3. Results

A number of trends can be observed for differences between normal mucosa and tumor tissues, as well as in the data from superficial or deeper tissue layers (probed by shorter or longer wavelengths, respectively). In general, normal mucosa tends to have lower $\boldsymbol{\alpha'}$, $\boldsymbol{b^{Mie}}$, and higher $\boldsymbol{f_{bile}}$ for superficial tissues (470-490 nm), which corroborates with our previous diffuse reflectance spectroscopy study [3], including data of the same patient whose was analyzed in this study.

Table 1: Spectral fitting parameters of normal mucosa and tumor tissues. High confidence fitted values are shown in green fields, whereas low confidence values are shown in red fields. Parameters associated with absorption of a certain chromophore were considered low confidence when the maximum μ_a^{chrom} at a specific wavelength range $\boldsymbol{\lambda^{min} - \lambda^{max}}$ is <5% *of the* maximum μ_a^{chrom} between 400-700 nm, whereas low confidence fluorophore concentrations were determined by wavelength ranges where the maximum fluorescence emission is <1% *of* the maximum emission between 400-700 nm of the fluorophore in question.

Tissue ($\lambda^{min} - \lambda^{max}$ range) / **Parameters**	**Mucosa (470-490 nm)**	**Mucosa (490-600 nm)**	**Mucosa (600-700 nm)**	**Tumor Border (470-490 nm)**	**Tumor Border (490-600 nm)**	**Tumor Border (600-700 nm)**	**Tumor Center (470-490 nm)**	**Tumor Center (490-600 nm)**	**Tumor Center (600-700 nm)**
R (μm)	10 ± 10	30 ± 60	510 ± 270	30 ± 70	20 ± 60	460 ± 290	20 ± 10	20 ± 30	390 ± 230
f_{blood} **(%)**	5 ± 2	4 ± 5	0 ± 1	2 ± 3	5 ± 10	1 ± 2	2 ± 2	4 ± 3	0 ± 0
StO_2 (%)	69 ± 22	32 ± 28	54 ± 26	53 ± 33	43 ± 32	50 ± 28	63 ± 29	61 ± 27	49 ± 27
f_{water} **(%)**	16 ± 34	27 ± 39	69 ± 27	59 ± 44	26 ± 41	55 ± 27	45 ± 42	11 ± 27	61 ± 13
f_{lipid} **(%)**	84 ± 34	73 ± 39	31 ± 27	41 ± 44	74 ± 41	45 ± 27	55 ± 42	89 ± 27	39 ± 13
f_{bile} **(%)**	7 ± 4	41 ± 80	0 ± 2	3 ± 6	66 ± 100	3 ± 14	3 ± 4	157 ± 130	0 ± 0
f_{methb} **(%)**	1 ± 1	1 ± 2	0 ± 0	1 ± 2	2 ± 3	0 ± 1	2 ± 3	3 ± 3	0 ± 0
α' **(cm^{-1})**	57 ± 24	82 ± 23	98 ± 10	86 ± 22	81 ± 24	92 ± 20	90 ± 13	68 ± 26	100 ± 0
f_{Ray} **(%)**	52 ± 22	42 ± 27	1 ± 6	54 ± 36	41 ± 31	6 ± 15	60 ± 38	41 ± 24	0 ± 0
b^{Mie}	1.74 ± 1.67	1.85 ± 1.5	0.01 ± 0.06	2.96 ± 1.54	1.03 ± 1.22	0.07 ± 0.21	3.94 ± 0.16	0.99 ± 1.1	0 ± 0
f_{coll} **(%)**	0 ± 0	0.3 ± 0.5	12.6 ± 8.9	0 ± 0	0.2 ± 0.2	12.7 ± 9.9	0 ± 0	0.2 ± 0.2	8.4 ± 9
f_{elast}**(%)**	3.5 ± 2.3	1 ± 5.5	0.6 ± 1	0.4 ± 0.7	0.5 ± 0.7	1 ± 1.3	0 ± 0.1	1.4 ± 1.2	0.7 ± 0.7
f_{flav} **(%)**	0.2 ± 0.4	0.2 ± 0.2	1.1 ± 0.5	12.7 ± 16.6	0.3 ± 0.7	1.3 ± 0.9	52.8 ± 26.2	0.3 ± 0.4	1.2 ± 0.1
f_{lipop} **(%)**	0 ± 0	0.1 ± 0.3	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0
f_{NADH}**(%)**	0.3 ± 0.4	0.7 ± 1.2	60.4 ± 27.2	0.3 ± 0.3	0.5 ± 0.8	59.6 ± 30.3	0.3 ± 0.1	1.2 ± 1.1	73 ± 27.8
f_{pirod} **(%)**	0 ± 0	4.5 ± 8.7	12.7 ± 8.9	0 ± 0	1.9 ± 2.4	12.7 ± 9.9	0 ± 0	6.4 ± 10.2	8.4 ± 9.1
f_{porp} **(%)**	48 ± 1	3.7 ± 3.9	0 ± 0	43.3 ± 8.3	3 ± 3	0 ± 0	23.4 ± 13.2	3.1 ± 2.7	0 ± 0
f_{trypt} **(%)**	48 ± 1	90 ± 14	13 ± 9	43 ± 8	94 ± 5	13 ± 10	23 ± 13	87 ± 11	8 ± 9

4. Conclusions

In this study, we evaluated tissue microstructural and biochemical parameters retrieved from FS measurements directly. Results are preliminary and may be applied to CRC detection, delineation during surgery, and stratification of CRC patients by using physiological parameters associated with extracted parameters.

Funding

This research was financially supported by Science Foundation Ireland (SFI Professorship award SFI/15/RP/2828).

5. References

[1] M. S. Nogueira, S. Maryam, M. Amissah, H. Lu, N. Lynch, S. Killeen, M. O'Riordain, and S. Andersson-Engels, "Evaluation of wavelength ranges and tissue depth probed by diffuse reflectance spectroscopy for colorectal cancer detection," Sci. Rep. **11**, 1–17 (2021).

[2] M. S. Nogueira, M. Raju, J. Gunther, K. Grygoryev, K. Komolibus, H. Lu, and S. Andersson-Engels, "Diffuse reflectance spectroscopy for determination of optical properties and chromophore concentrations of mice internal organs in the range of 350 nm to 1860 nm," in Proceedings of SPIE - Biophotonics: Photonic Solutions for Better Health Care VI (2018), Vol. 10685, p. 106853G.

[3] M. S. Nogueira, M. Raju, J. Gunther, S. Maryam, M. Amissah, H. Lu, S. Killeen, M. O'Riordain, and S. Andersson-Engels, "Tissue biomolecular and microstructure profiles in optical colorectal cancer delineation," J. Phys. D. Appl. Phys. **54**, 454002 (2021).