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RESEARCH ARTICLE

Excitation-emission matrices (EEMs) of colorectal tumors – tool for spectroscopic diagnostics of gastrointestinal neoplasia

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Abstract The autofluorescence spectroscopy of biological tissues is a powerful tool for non-invasive detection of tissue pathologies and evaluation of any biochemical and morphological changes arising during the lesions' growth. To obtain a full picture of the whole set of endogenous fluorophores appearing in the gastrointestinal (GI) tumors investigated, the technique of excitation-emission matrix (EEM) development was applied in a broad spectral region, covering the ultraviolet and visible spectral ranges. We could thus address a set of diagnostically-important chromophores and their alterations during tumor development, namely, collagen, elastin, nicotinamide adenine dinucleotide (NADH), flavins, porphyrins, while hemoglobin's absorption influence on the spectra obtained could be evaluated as well. Comparisons are presented between EEM data of normal mucosae, benign polyps and malignant carcinoma, and the origins are determined of the fluorescence signals forming these matrices.

Keywords autofluorescence spectroscopy, excitation-emission matrix (EEM), colon carcinoma, gastrointestinal (GI) tumours

1 Introduction

One of the most exciting potential growth areas of the biophotonics diagnostic methods could be found in the oncology research applications. The standard biopsy procedure for cancer detection is limited by the accuracy of the current diagnostic modalities, the latter relying almost entirely on the physician's experience and being limited by the high probability of miss rates and rigorous biopsy protocols, which are costly and time consuming [1]. Therefore, the necessity arises of finding new sensitive diagnostic modalities for analysis of biopsy tissue samples, or on site, *in vivo*, non-invasive tissue examination.

Gastrointestinal (GI) tumors have a major place in the cancer statistics of newly developed lesions every year, as the colon cancer is on third place, stomach cancer is on fifth place, and esophageal cancer is also in the top ten of tumors according to the statistics of neoplasia incidence [2]. Usually, the tumors are detected at the advanced III and IV stages, where the prospects for the patients are not very optimistic. The incidence of GI cancer is increasing, so that its accurate diagnostics and effective treatment are of primary public health concern [2]. Conducting more frequent screenings has resulted in a decline of the GI cancer mortality rate, but in order to achieve a further decrease, improvements of the diagnostic practice are needed [3]. The potential of the biomedical optical techniques for a detailed minimally- or non-invasive analysis of multi-component substances, such as biological tissues, is intensively researched for implementing in novel clinical diagnostic modalities for differentiation of cancerous and normal tissues with high sensitivity and accuracy.

The feasibility of different optical techniques as possible new GI cancer diagnostic modalities has been researched intensively in the past few decades. Several optical approaches could become appropriate answers in the tasks related to early detection of tumor tissues, as well as for monitoring the therapeutic treatment procedures, such as laser- or light-induced fluorescence spectroscopy (LIFS), Fourier-transform infrared reflectance spectroscopy (FTIR), laser-induced breakdown spectroscopy

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(LIBS), diffuse-reflectance spectroscopy (DRS), Raman spectroscopy, among others. All these optical techniques have their benefits and drawbacks, but have shown their capacity to be used as a complementary or even primary tool for non-invasive early detection and in real-time modes of detection in a broad range of preclinical investigations.

When initial *in vivo* diagnosis is the primary goal for the development of a new diagnostic modality, exogenous contrast agents or markers are not suitable to be applied. Therefore, when one focuses one's attention on the light-induced fluorescence spectroscopy approach, as one of the most sensitive optical spectroscopic techniques for detection and evaluation of tissue neoplasia, endogenous, or the "auto-" fluorescence spectroscopic technique, could only be used. Autofluorescence spectroscopy is one of the most intensively researched optical techniques for an add-on modality to endoscopic GI diagnostics in its steady-state regime of detection [4–8]. Cancerous tissue alterations affect the light propagation, the absorption properties, and the fluorophores' content in the tissues, to which parameters the fluorescence spectroscopy is sensitive. The fluorescence intensity and spectral shape, the emission peak positions of endogenous fluorophores, such as tryptophan, tyrosine, collagen, elastin, nicotinamide adenine dinucleotide (NADH) and flavine adenine dinucleotide (FAD), hold diagnostic values [9], whose better understanding and evaluation are necessary for successful implementation of the fluorescence diagnostic into the clinical practice. The steady-state autofluorescence spectroscopy technique (SS-AFS) could be implemented by relatively inexpensive continuous-wave (CW) excitation sources, based on laser or light-emitting diodes, and by sensitive detectors to which no requirements for fast optical response are imposed, in contrast with the case of time-resolved detection. Thus, steady-state autofluorescence detection of tumors could be introduced easily into the clinical practice.

This resulted in the development and implementation of autofluorescence spectroscopy and imaging in the traditional white-light endoscopy as a standard procedure for GI cancer detection [10,11]. Although the benefits of the autofluorescence techniques have been presented and discussed in many investigations [12,13], this modality has not found the broad use it deserves, leaving room for improvement. The latter has so far mostly consisted in improving the equipment and evaluating the parameters in view of achieving better accuracy [14–16].

Some specific techniques for better evaluation of the endogenous fluorophores' content could be applied, such as synchronous fluorescence spectroscopy, or excitation-emission matrix (EEM) development. EEM measurements are based on a broad spectral scanning of the excitation using a specific step between the excitation wavelengths applied (10 – 20 nm), and polychromatic spectral detection of the fluorescence signals obtained from the tissues. Two-dimensional spectral maps are constructed, where the iso-lines are the ones where autofluorescence signals of equal intensity are detected for a given wavelength, and the X and Y axes are respectively the emission and excitation wavelengths of these signals. Every endogenous fluorophore is described by a unique couple of excitation and emission wavelengths for which it has a maximally efficient fluorescence emission – these "islands" are used in the EEM as fingerprints of every endogenous fluorophore existing in the tissue investigated.

As the concentration and state of the endogenous fluorophores are altered in neoplastic tissues vs. normal ones, the differences observed in their spectral EEM "islands" – their size, intensity, appearance or, on the contrary, disappearance, are useful and important markers of tissue alterations and pathology growth.

We present below our results from using the EEM technique for detection and discrimination of colorectal neoplasia in a broad spectral range, covering all endogenous autofluorescence sources in gastrointestinal tract (GIT) tissues. The specific origins of the signals are discussed and their diagnostic value are assessed. Comparisons between EEM data for normal mucosae, benign polyps and malignant carcinoma colorectal lesions are presented. In addition, the influence of the lesion's stage of growth on the autofluorescence signal detected is discussed and presented with examples obtained from our experimental and pre-clinical spectroscopic practice. These results could be used for further development of discrimination algorithms in *in vivo* autofluorescence detection during surgical interventions to evaluate the tissue's state and the lesion's borders.

2 Materials and methods

Our studies are based on *ex vivo* measurements of excitation-emission matrices (EEM) of lower gastrointestinal neoplasia to evaluate the autofluorescence technique applicability to *in vivo* clinical observations of lower GIT tumors. A fluorescence signal from the surgically excised samples was obtained in the 3D modality of spectral data organization, the so-called EEM scan. The EEMs are graphically represented with the excitation wavelength on one axis, the emission wavelength on the second, while the fluorescence intensity forms the third axis and is presented as iso-lines with the same fluorescence intensities level. This method for three-dimensional fluorescence spectroscopy provides enough information about the fluorescence spectra of biological tissue samples to enable one to determine the excitation wavelengths that produce emission fluorescence spectra containing the most diagnostic meaning for clinical diagnostic analysis. EEMs were performed on pairs consisting of a cancerous tissue and a healthy tissue from the lower GIT from 15 different patients.

The procedure of obtaining the samples investigated included their excision during surgery for removal of GIT neoplasia lesions. After the surgery, the removed biological samples were transported under isothermal conditions in a safe-keeping solution (water solution of NaCl, KCl, glucose, taurine, HEPES, piruvic acid, fixed pH = 7,4) from the hospital to the spectral laboratory, where their fluorescence was studied. All patients were given and signed written informed consent; also, this research was approved by the Ethics Committee of the Tsaritsa Yoanna University Hospital, Sofia. All lesions removed surgically were also prepared for histological analysis, as part of the standard clinical protocol; the histological diagnoses provided by the specialist-histopathologist were used as a "gold standard" for comparison with the spectral data obtained.

The fluorescence measurements were conducted on a FluoroLog 3 spectrofluorimeter (HORIBA Jobin Yvon, France). This system is equipped with a xenon lamp as a light source (power of 300 W, emission range of 200 – 650 nm) and a PMT detector (range of 220 – 800 nm) for fluorescence detection. Since our samples varied in shape and size, their fluorescence was studied by the Fluorolog 3 additional fiberoptic module F-3000, which allows placing the samples outside the sample chamber. We detected spectral data from 15 normal colon mucosa samples, 3 benign colon polyps, 4 rectum carcinomas, and 12 colon carcinomas.

The fluorescence signals measurements of the different tissue samples obtained as EEMs were performed with excitation applied in the 280 – 440 nm spectral region. The step used for wavelength variation was 10 nm. The emission was observed between 300 and 800 nm, with a scanning step of 1 nm. All measurements were performed in the same spectral ranges of excitation and emission light for all types of tissue samples – normal, benign and cancerous. After the spectral measurements, the samples were stored in formalin solution and archived.

3 Results and discussion

Using the spectral data observed, we determined several endogenous fluorophores, as follows: tyrosine and tryptophan, which are amino acids, the co-enzymes NADH and FAD, and the structural proteins collagen and elastin and their cross-links. To process the signals, we applied comparisons with the maximal number of spectral parameters, including excitation and emission wavelengths and the reference fluorescence characteristics of the main endogenous fluorophores, as presented in the work of Ramanujam [9].

The main differences observed between the fluorescence spectra of healthy and cancerous tissues were in the intensity levels of the fluorescence originating from the amino acids (tyrosine and tryptophan), the enzymes and coenzymes (NADH and FAD), and from the structural proteins elastin and collagen.

The increase observed in the intensity of the fluorescence of the amino acids tyrosine and tryptophan arises from the extensive production of proteins, built of amino acids, due to the higher metabolic rate of cancerous cells. One of the functions of the coenzyme NADH is to be an electron carrier in cellular respiration process, which is altered in cancerous cells; this could be one of the reasons for the lower intensity observed of the fluorescence maxima of NADH in these cells.

Other fluorophores, whose fluorescence intensity as part of the cancerous cells' overall fluorescence is reduced, are the structural proteins collagen and elastin. The most likely reason for this reduction is the abnormal oversized growth of cancerous cells, which causes disruption of the extracellular matrix, built by structural proteins and their cross-links, and the metalloproteinase enzymes secreted by cancer cells to break the surrounding extracellular matrix to accommodate the tumors' need of a new vascularization [17,18].

To evaluate the EEM's suitability for tissue differentiation, we displayed the results as 2D color maps with two axes presenting the excitation and emission wavelengths during the scan, and a color contour map scheme illustrating the intensity of the fluorescence observed (Figs. 1(a) – 1(d)). In view of achieving a better comparison between the fluorescence signals arising from the different tissue types, the spectral EEM data shown were averaged by lesion type and normalized with respect to the area under the fluorescence curve.

A fluorescence intensity reduction in the 450 – 500 nm is well seen in the normal-benign-malignant tissue line; also, the signals variability is higher for the abnormal tissues in comparison with the normal and benign ones. This signal variation from lesion to lesion could be related to personal differences between the patients and differences in the tumor-growth level and lesion stage. All tissue samples investigated were at the III and IV stages of growth, but variations in their size, thickness and lesion state existed, which affected the fluorescence signals observed in the separate cases.

Figures 2 (a) – 2(d) present autofluorescence signals obtained using excitation in the 280 – 440 nm range with a step of 20 nm and normalized with respect to the spectral curves' area, which allowed for a better comparison of the spectral shape changes in the fluorescent data detected when specific excitation was applied.

In general, the whole set of endogenous fluorophores is present in the different types of GIT tissues; their concentrations vary somewhat depending on the tissues' general condition. The exceptions are the endogenous porphyrins, which are salient in the malignant pathologies only, as is clearly seen when comparing the fluorescence signals excited by the wavelength of 420 nm (Fig. 3).

The spectroscopic results presented were obtained

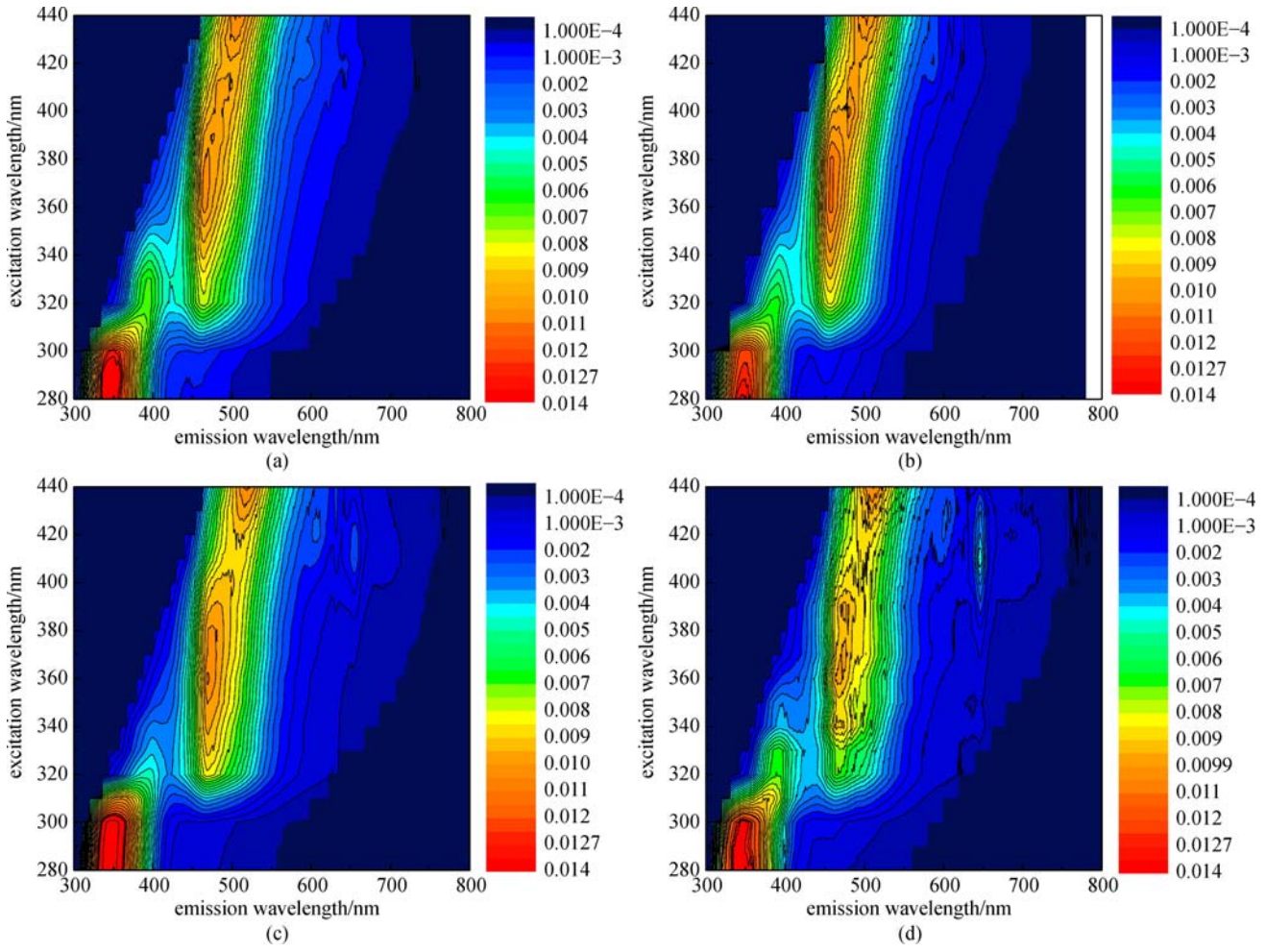

Fig. 1 Excitation-emission matrices of (a) normal mucosa, (b) benign colon polyp, (c) colon carcinoma and (d) rectum carcinoma for the 280–440 nm excitation wavelength range and emission detected in the 300–800 nm wavelength range. The EEM data presented are the resulting averaged spectrum of all tissue samples of a given type detected *ex vivo*

during *ex vivo* tissue investigations; their application to *in vivo* tumor differentiation would benefit from a few significant remarks:

- The hemoglobin's contribution to the fluorescence signals received, which causes a minimum in the fluorescence spectra due to re-absorption at about 425 and 540 – 575 nm, would be more prominent in *in vivo* tissue measurements;
- The NADH fluorescence emission is expected to be of a higher intensity in *in vivo* measurements, since this co-enzyme's fluorescence fades rapidly once the tissue is removed from the body;
- The effect of the temperature of an *ex vivo* sample, detected at room temperature, which is lower than the body temperature at the moment of measurement, could also alter significantly the spectra detected from normal and abnormal tissue sites.

However, even based on *ex vivo* studies, specific and repeatable fluorescence particularities were observed when comparing all spectral features of the GIT normal mucosae and lesions investigated; these could be helpful diagnostic indicators in differentiation algorithms used as detection tools, namely:

- An increase in the amino acids tyrosine and tryptophan's UV fluorescence due to extensive protein synthesis, related to the higher division rate of cancerous cells vs. normal mucosae;
- Fading in the structural proteins collagen and elastin's fluorescence due to alteration (thickening) in the mucosal layer that screens and reduces the fluorescence from sub-mucosal layers;
- A decrease in the collagen cross-links' fluorescence, related to the destruction of the extracellular matrix in lesions in general;
- A general decrease in the NADH fluorescence in tumors vs. normal mucosae, due to the NADH transformation to its oxidized non-fluorescent form (NAD +) in cancer cells;
- Lack of a fluorescence maximum in the 630-640-nm range in normal and benign GIT mucosae, which is related

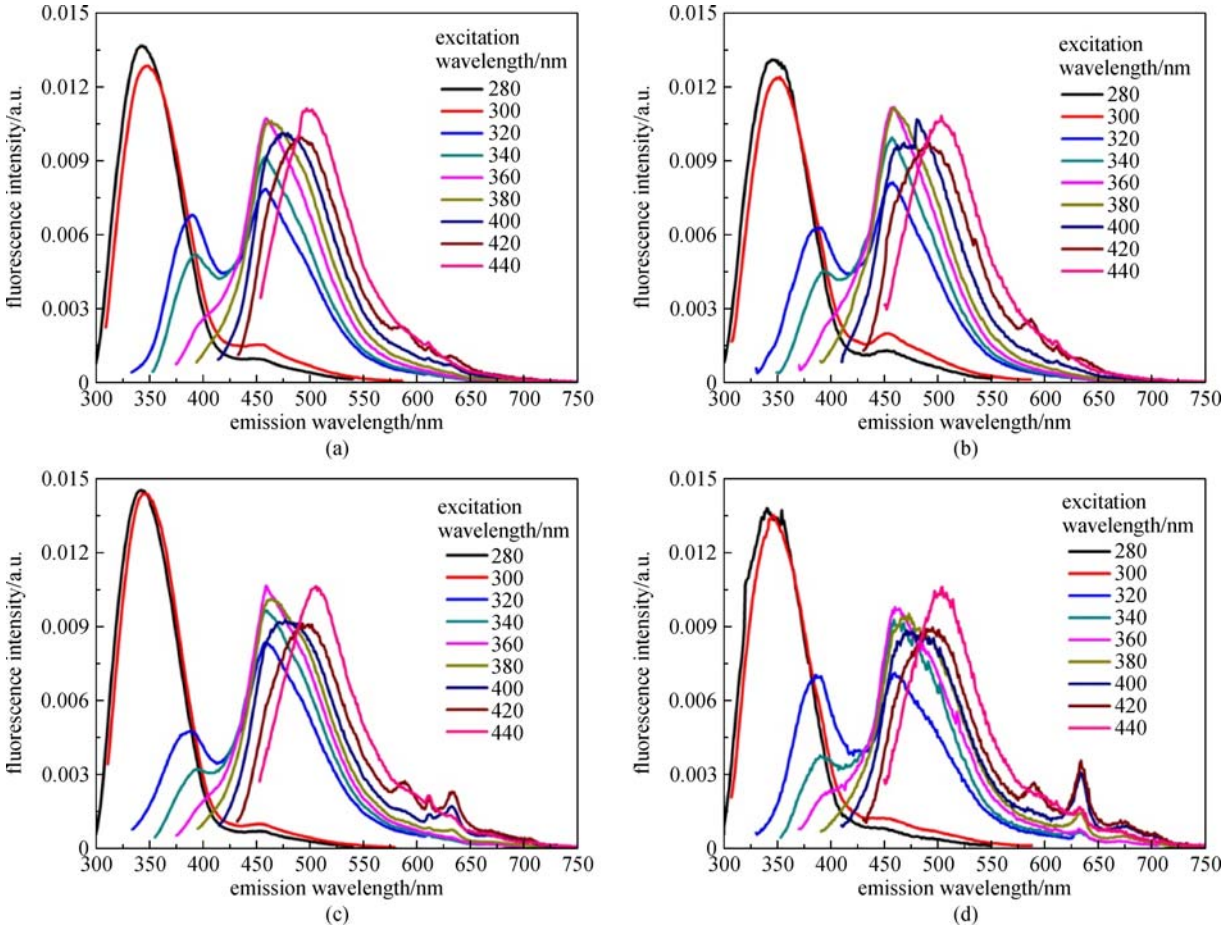

Fig. 2 Autofluorescence spectra of (a) normal mucosa, (b) benign colon polyp, (c) colon carcinoma and (d) rectum carcinoma for different excitation wavelengths applied in the 280–440 nm region, normalized with respect to the integral intensity (spectral curve area)

to the absence of endogenous porphyrins in these types of tissues;

- Appearance of endogenous porphyrins in the advanced stages of tumor growth because of metabolic changes occurring in the neoplastic cells, especially pronounced at the III and higher stages of lesion development.

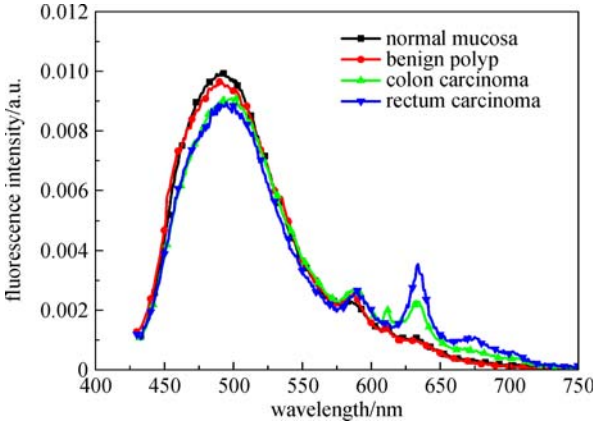

Fig. 3 Autofluorescence spectra of normal, benign and malignant gastrointestinal tissues using excitation at 420 nm. The spectra are normalized with respect to the integral intensity of the fluorescence emission

To use all capabilities of the SS-AFS modality as a diagnostic tool, spectroscopic data needs to be acquired about the entire set of endogenous fluorophores present in the tissues investigated. This means usage of a broad spectral range of excitation – to cover all absorption bands of the endogenous fluorophores, which lie in the ultraviolet and short-wavelengths range of the visible part of the electromagnetic spectrum. The detectors applied also should allow detection in a broad spectral region, as the emission range of endogenous compounds with diagnostic significance is situated in the end of UV and visible spectral regions. Thus, amino acids, such as tyrosine and tryptophan, emit in the 300–350 nm range, while endogenous porphyrins emit in the red visible spectral region of 630–700 nm. There exists in the tissues investigated a multi-compound mixture of endogenous fluorophores having different excitation and emission properties, different fluorescence quantum efficiencies, and different concentrations in unit volume. This is why

the analysis of the spectra of such complex, heterogeneous media presents extraordinary challenges to the researchers.

4 Conclusions

Our fluorescence measurements of gastrointestinal neoplasia demonstrate their particular sensitivity to structural and morphological changes related to the tissues' condition. The correlation between small alterations having to do with the structure and integrity of the extracellular matrix are more pronounced in the case of colorectal lesions, where the matrix is partially destroyed due to the lesion's growth. The EEM fluorescence measurements may provide a non-invasive method for cancer detection covering a broad spectral range and revealing the whole set of endogenous fluorophores' signals.

Although challenging for the clinical practice, applying the autofluorescence EEM technique can be highly beneficial in accumulating statistics on characteristic spectral patterns of cancerous tissues. This non-invasive technique can be applied to endoscopic fluorescence observation to detect tissue alterations. Moreover, scanning the tissue surface could offer a possibility for a further step in the development of the multispectral and hyperspectral imaging techniques for early detection and evaluation of tissue neoplasia.

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References

1. Young P E, Womeldorph C M. Colonoscopy for colorectal cancer screening. Journal of Cancer, 2013, 4(3): 217–226
2. Jemal A, Bray F, Center M M, Ferlay J, Ward E, Forman D. Global cancer statistics. CA: a Cancer Journal for Clinicians, 2011, 61(2): 69–90
3. Benson A B 3rd. Epidemiology, disease progression, and economic burden of colorectal cancer. Journal of Managed Care Pharmacy: JMCP, 2007, 13(6 Suppl C): 5–18
4. Song M, Ang T.Early detection of early gastric cancer using image-enhanced endoscopy: current trends. Gastrointestinal Intervention, 2014, 3(1): 1–7
5. Fujiya M, Kohgo Y. Image-enhanced endoscopy for the diagnosis of colon neoplasms. Gastrointestinal Endoscopy, 2013, 77(1): 111–118.e5
6. Johansson A, Kromer K, Sroka R, Stepp H. Clinical optical diagnostics: Status and perspectives. Medical Laser Application, 2008, 23(4): 155–174
7. Hasan M, Wallace M. Image-enhanced endoscopy. Clinical Update, 2009, 16(4): 1–5
8. Subramanian V, Ragunath K. Advanced endoscopic imaging: a review of commercially available technologies. Clinical Gastroenterology and Hepatology : the Official Clinical Practice Journal of the American Gastroenterological Association, 2014, 12(3): 368–76.e1
9. Ramanujam N.Fluorescence spectroscopy of neoplastic and non-neoplastic tissues. Neoplasia, 2000, 2(1- 2): 89–117
10. Wong KeeSong L M, Banerjee S, Desilets D, Diehl D L, Farraye F A, Kaul V, Kethu S R, Kwon R S, Mamula P, Pedrosa M C, Rodriguez S A, Tierney W M. Autofluorescence imaging. Gastrointestinal Endoscopy, 2011, 73(4): 647–650
11. Prosst R L, Gahlen J. Fluorescence diagnosis of colorectal neoplasms: a review of clinical applications. International Journal of Colorectal Disease, 2002, 17(1): 1–10
12. Ferreira D S, Henriques M, Oliveira R, Correia J H, Minas G. Autofluorescence spectroscopy of a human gastrointestinal carcinoma cell line: design of optical sensors for the detection of early stage cancer. In: Proceedings of the International Conference on Biomedical Electronics and Devices 2009, Porto, Portugal. 2009: 61–66
13. Van Putten P, Ramsoekh D, Haringsma J, Poley J W, Van Dekken H, Steyerberg E, Van M E, Kuipers E J. Autofluorescence endoscopy allows better differentiation than white light video colonoscopy in classifying adenomatous and non-adenomatous colorectal polyps. Gastrointestinal Endoscopy, 2009, 69(5): AB290
14. Luo X J, Zhang B, Li J G, Luo X A, Yang L F. Autofluorescence spectroscopy for evaluating dysplasia in colorectal tissues. Zeitschrift fur Medizinische Physik, 2012, 22(1): 40–47
15. Tajiri H. Autofluorescence endoscopy for the gastrointestinal tract. Proceedings of the Japan Academy Series B, Physical and biological sciences, 2007, 83(8): 248–255
16. Aihara H, Sumiyama K, Saito S, Tajiri H, Ikegami M. Numerical analysis of the autofluorescence intensity of neoplastic and non-neoplastic colorectal lesions by using a novel videoendoscopy system. Gastrointestinal Endoscopy, 2009, 69(3): 726–733
17. Hirayama A, Kami K, Sugimoto M, Sugawara M, Toki N, Onozuka H, Kinoshita T, Saito N, Ochiai A, Tomita M, Esumi H, Soga T. Quantitative metabolome profiling of colon and stomach cancer microenvironment by capillary electrophoresis time-of-flight mass spectrometry. Cancer Research, 2009, 69(11): 4918–4925
18. Lunt S Y, Vander Heiden M G. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annual Review of Cell and Developmental Biology, 2011, 27(1): 441–464

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