Endogenous and exogenous fluorescence spectroscopy of gastrointestinal tumours – in vitro studies

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We present fluorescence data obtained from normal and cancerous gastrointestinal tissues – oesophageal and colon lesions, taken up to two hours after surgical removal with and without exogenous fluorescent markers applied. The major goal was to acquire information on the differences between the fluorescent spectra of normal and pathological tissues. Excitation-emission matrices in a broad spectral range (excitation from 280 to 450 nm, a step of 10 nm, and emission from 300 to 700 nm, a step of 1 nm) were detected and the major endogenous fluorophores for the normal and the diseased tissues were identified. The fluorescence signals detected during endoscopic observations can be used for initial diagnosis of gastrointestinal tract (GIT) cancer, as well as for fluorescent monitoring and mapping during open surgical procedures for removal of lower gastrointestinal tract tumours. The endogenous and exogenous fluorescent data were compared using the 5-ALA-mediated PpIX fluorophore as a contrast agent in view of evaluating the diagnostic applicability of these two fluorescent spectroscopic modalities and their feasibility for common clinical applications alone and in a combination.

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1. Introduction

In the last few decades, optical spectroscopic techniques have been intensively investigated and developed as supporting tools for improving the cancer diagnosis as of a great variety of lesions – skin, prostate, breast, bladder, brain, etc. Inproving the detection of gastrointestinal tract (GIT) tumours is one of the goals in the process of developing state-of-the-art, easy-to-use, objective and clinically feasible spectroscopic equipment.

GIT tumours have a major place in the statistics of newly developed cancers every year, colon cancer being on third place, stomach cancer, on fifth place, while oesophageal cancer is in the "top ten" of tumours according to the statistics of cancer incidence. The GIT tumours are usually detected in the advanced III and IV stages, where the prospects for the patients are quite poor [1, 2]. Despite the many technological advances that have taken place, based on improved detectors and image analysis of better discrimination, the conventional white-light GIT endoscopy is still suboptimal. The endoscopic observation is usually made on an advanced stage and detects lesions which already have symptoms of obstruction, bleeding and pain related to tumour growth. Experienced gastroenterologists only, with long years of practice in endoscopy observations, could be expected to discern the slight initial changes to dysplastic and neoplastic stages of oesophageal, stomach or colon mucosa. Inflammatory mucosa cannot be easily discriminated from an initial stage adenocarcinoma, which leads to a further decrease of the diagnostic accuracy [3]. The visualisation and discrimination problems during open surgical interventions on tumour excision procedures for lower GIT also create significant clinical problems and require the development of new tools and diagnostic and monitoring modalities that could improve the selectivity and accuracy of these procedures. The existing clinical limitations are one significant technical challenge provoking the implementation of new diagnostic modalities based on different spectral and optical techniques.

All optical diagnostic approaches are based on light-tissue interactions and the differences observed between the characteristics of normal and abnormal tissue sites. In gastroenterology, several optical methods have been applied recently, such as coherent optical tomography [4], chromo-endoscopy, confocal fluorescent microscopy [5, 6], Raman spectroscopy [7], reflectance spectroscopy [8] and laser- and light-induced fluorescence spectroscopy [9]. The optical spectroscopic diagnosis can provide both 2-D imaging and 1-D point measurement probe information; the techniques are divided into those that provide morphological data and those that have the potential for molecular and biochemical information. The techniques of optical coherence tomography, light scattering spectroscopy, and confocal microscopy provide *in vivo* histology by receiving morphological information about the tissue area investigated [4, 10]. Fluorescence imaging

and spectroscopy provide both morphological and biochemical data [11, 12]. Raman spectroscopy provides biochemical information based on molecular data [13].

Such advanced methods go beyond standard endoscopic techniques and, theoretically, could allow one to achieve better image resolution and contrast, higher sensitivity and tissue penetration, together with biochemical, structural and molecular data on the tissues investigated. One of the most sensitive optical detection approaches is the light-induced fluorescence spectroscopy (LIFS) of gastrointestinal mucosa for neoplasia detection. This technique is the most widely examined one among the spectroscopic techniques because of its fast and highly sensitive response to early biochemical and morphological changes in the tissues.

Fluorescent technique can be applied to detecting and evaluating tumours in different localisations using endoscopic equipment. Endoscopic systems that combine white-light and fluorescent modes have already been developed and introduced to clinics for the needs of bronchoscopy and lung cancer diagnosis, e.g., the D-Light system of Karl Storz GmbH, the DAFE system (Diagnostic AutoFluorescence Endoscope) of Richard Wolf GmbH, the LIFE (Lung Fluorescence Endoscopy) system of Xillix Technologies Corp. [3, 9, 14, 15]. However, in what concerns the field of fluorescence gastro- and colon-endoscopy, just a few systems have only been reported to be in clinical use. The Olympus Evis Lucera family systems are digestive tract videoscopes used for observing blood vessels in mucous membranes under infrared light in the 790 – 820 nm and 905 – 970 nm ranges. Another system – a variant of the Xillix fluorescent endoscopic system for bronchi observation, is the Xillix-LIFE-GI. It is being applied to autofluorescence detection of stomach neoplasia and was approved for use in Japan and European countries. Several fluorescent endoscopy systems have also been developed and proposed for applications to the practice by different research teams; very good clinical results have been demonstrated [9, 16, 17] using autofluorescence or exogenous fluorescence detection of gastrointestinal neoplasia.

The fluorescent diagnosis of tumours of the upper part of the gastrointestinal tract continues to be of interest and is being the object of extensive research and development activities worldwide, as the systems mentioned above are still on the feasibility studies stage, or have not yet received all approvals needed for access to the broad clinical market. At present, detecting differences in the autofluorescence obtained by gastro-endoscopic equipment is still a relatively difficult task because of the faintness of the images produced. Real-time gastrointestinal fluorescence endoscopy is now based mainly on the use of exogenous fluorophores [3, 17-19], which increases the contrast, improves the endoscopic resolution and sampling, and can be used to achieve a better 2-D visualisation for the needs of clinicians. The need of exogenous fluorescence markers restricts the applicability of the fluorescence techniques for initial detection of GIT tumours, which is why scientists have sought ways to optimise the endogenous fluorescence detection. The requirements to the fluorescence approach are that it be suitable for clinical diagnosis of GIT in both endoscopic and open surgery modes for initial diagnosis, monitoring and mapping of the tumours [20-22].

Different sources of endogenous fluorescence signals have been reported, depending on the excitation sources applied by the different researchers. Table 1 presents the major endogenous fluorophores producing a fluorescent spectrum from gastrointestinal mucosa as per the data reported by different research groups [23-27].

Table 1. Excitation and Emission Maxima of Endogenous Fluorophores in GIT mucosa

Endogenous Fluorophores	**Excitation Maxima (nm)**	**Emission Maxima (nm)**
Amino acids		
Tryptophan	280	350
Tyrosine	275	300
Phenylalanine	260	280
Structural proteins		
Collagen	325, 360	400, 405
Elastin	290, 325	340, 400
Co-enzymes		
FAD, Flavins	450	535
NADH	290, 351	440, 460
NADPH	336	464
Vitamins		
Vitamin A	327	510
Vitamin K	335	480
Vitamin D	390	480
Vitamin B6 compounds		
Pyridoxine	332, 340	400
Pyridoxamine	335	400
Pyridoxal	330	385
Pyridoxic acid	315	425
Pyridoxal 5'-phosphte	330	400
Vitamin B12	275	305
Lipids		
Phospholipids	436	540, 500
Lipofuscin	340-395	540, 430-460
Ceroid	340-395	430-460, 540
Porphirines	400-450	630, 690

In the last decade, many feasibility studies have been reported for detection of GIT tumours in view of introducing new equipment based on optical and spectroscopic information detected from the tissues investigated; these have been with a different level of success, diagnostic accuracy achieved and clinical applicability. The fluorescence spectroscopy applied in such studies has been used in both its modalities – without and with exogenous fluorescent markers that are selectively accumulated into the tumour tissues of the GIT [3, 9, 12, 19, 28].

In the recent study presented here, we focused our attention on experiments on using autofluorescence and 5-ALA-mediated exogenous fluorescence in oesophageal, stomach and colon cancer diagnosis and differentiation with respect to the corresponding normal mucosa. We also report a feasibility analysis of the fluorescence spectroscopy techniques chosen as diagnostic tools for GIT tumours detection in endoscopic and open-surgery observations. Further, discussions are included on the advantages and drawbacks found in both modalities during the measurements.

In general, we are optimistic that the advances in the development of spectroscopic instruments could improve the role of imaging as a facilitator in interpreting the research results. Our results could assist in the development of tools for future *in vivo* quantification of tumour borders, types, and origin, as well for accelerating the transition from pre-clinical studies to early clinical trials and routine diagnostic practice.

2. Materials and methods

2.1. Clinical and endoscopic analysis and sampling

All samples investigated *in vitro* were obtained by surgical excision of the malignant oesophagus and colon tumours in such a way as to include tumour mass and surrounding normal mucosa areas. Initially, the endoscopic clinical observation and biopsy sampling for the following histological analysis were carried out in the clinic. The "gold standard" histological analysis proved the tumor type and stage of growth in all cases investigated by the spectroscopic techniques applied. Freshly excised tumors were placed in a liquid immediately after removal, thus keeping the vital properties of the samples up to 24 hours, and transported from the hospital to the spectroscopy laboratory in a standard Dewar vessel for the purposes of the subsequent fluorescent measurements, the latter being carried out up to two hours after tissue removal.

Fluorescent observations and measurements *in vivo* were applied as well in the case of exogenous fluorescence detection of tumours and then compared with the fluorescence data obtained from excised (*in vitro*) samples. The tissues containing the exogenous fluorophore protoporphyrin IX (PpIX) for the *in vitro* fluorescence measurements were taken from the tumours observed *in vivo* using endoscopic pinch biopsy sampling. However, we will only discuss here the data obtained from *in vitro* fluorescence measurements, as the *in vivo* studies were presented elsewhere [29, 30].

The samples without exogenous fluorophores applied for the needs of autofluorescence measurements were obtained after surgical removal of proved tumours during surgical excision of the lesions in open surgical procedures. The samples prepared for the fluorescence analysis were with a diameter of 1,5 cm and were taken from the border areas of the tumours excised. Thus, the samples contained a tumour area, a normal mucosa area and a transient border zone, which allowed us to form a more precise and complete picture of the possible spectroscopic differences in these three areas of diagnostic interest.

In total, twelve oesophageal and seven colon carcinoma lesions, as well as five benign colon polyps, were detected using fluorescence spectroscopy *in vivo* endoscopically and *in vitro* on surgically excised tissue samples. In all the cases, fluorescence of the cancerous and healthy mucosa were detected and normal tissue fluorescence was used as a basis for comparison with the pathology fluorescence signal.

All procedures for the *in vivo* endoscopic measurements were developed after an approval for the protocol of exogenous fluorescence diagnostics modality verification was issued by the local ethical committee under the framework of project #VU-L-01/2005. All procedures for performing the *in vitro* measurements were developed after approval #286/24.07.2012 for the protocol of autofluorescence diagnostic modality verification was issued by the local ethical committee under project #MU-03-46/2011. The patients underwent initial clinical endoscopic observation and diagnosis, followed by histological analysis and clinical studies (blood, scanner, etc.), which were taken into account when the decision for surgical treatment was made.

2.2. Autofluorescence measurements

We measured excitation-emission matrices for normal and tumourous GIT mucosa samples obtained by surgical excision during procedures for removal of GIT neoplasia lesions. For this purpose, we used a FluoroLog 3 spectrofluorimeter (HORIBA Jobin Yvon, France). This is a modular system of high sensitivity allowing one to measure steady-state or time-resolved fluorescence of liquid or solid samples in a time-correlated single photon counting mode in the 200 – 650 nm range for excitation and the 220 – 800 nm range for fluorescence detection. An additional F-3000 fiber-optic module with a 1950-1M fiber-optic probe allowed us to measure the fluorescent properties of samples that cannot be placed in a standard cuvette, such as the tissue samples the studies of which are reported here. Using this system, we could perform measurements of the excitation and fluorescence spectra and excitation-emission matrices of biological tissues *in vivo* and *in vitro.*

After surgical removal, the biological samples were transported under isothermal conditions from the hospital to our laboratory; during the experiments they were held at a constant temperature. After the spectroscopic measurement procedure, the samples were kept in a formalin solution. The point-by-point measurements were taken from the excised tumour lesions and outwards from the normal surrounding mucosa, which is a part of the safety area excised during the tumour removal. Autofluorescence signals using different excitation wavelengths for differentiation between tumour and healthy tissue were detected, thus forming an excitation-emission matrix of data. The excitation applied was in the 280 – 440 nm region, while the fluorescence emission was measured between 300 nm and 800 nm. The last step of the study consisted in a comparison between the histological analysis and the spectral data obtained in order to verify the spectral results and address the most significant diagnostic features observed.

2.3. 5-ALA mediated fluorescence measurements

The measurements were conducted by using the exogenous photosensitzer 5-ALA/PpIX as a fluorescent marker for tumour detection during the endoscopic procedures for initial observation and diagnosis. A fiber bundle was inserted through the instrumental channel of the endoscope (Olimpus Inc.); the excitation light was delivered through the central fiber and the fluorescence response of the tissue was collected by the surrounding six fibers and fed to a microspectrometer (USB4000, OceanOptics Inc., Dunedin, USA. The excitation source was an AFS-405 diode (Polironik Ltd., Moscow, Russia) emitting at 405 nm with an output power of 25 mW at the end of the fiber tip. The 1-D spectroscopic measurements with the fiber bundle placed in the instrumental endoscopic channel exhibited good contrast due to the high sensitivity of the microspectrometer.

As a fluorescent marker for dysplasia and tumour detection in the oesophagus, stomach and colon we used delta-aminolevulinic acid/Protoporphyrin IX (ALASENS, NIOPIK JSCo, Russia). The δ-ALA was administered per os six hours before the measurements at a dose of 20 mg/kg weight in accordance with the clinical experience on gastrointestinal diagnostic applications of δ-ALA/PpIX fluorescence [9, 31]. The 5-ALA/PpIX-mediated fluorescence diagnosis of GIT lesions were performed during standard endoscopic examinations of patients in the Gastroenterology Department of Tsaritsa Yoanna-ISUL University Hospital.

Following a diagnostic decision about the existence of suspicious tumourous mucosal area based on the clinical endoscopic observations of the gastroenterologists and on the high-level detected of PpIX fluorescence, tissue sampling was carried out for biopsy and for *in vitro* fluorescent diagnostic procedures. Five to seven points were measured from every section and averaged spectra were used to evaluate their state. The resultant spectra were smoothed using the Savitzky-Golay algorithm to reduce the instrumental noise of the spectrometric system used.

3. Results and discussion

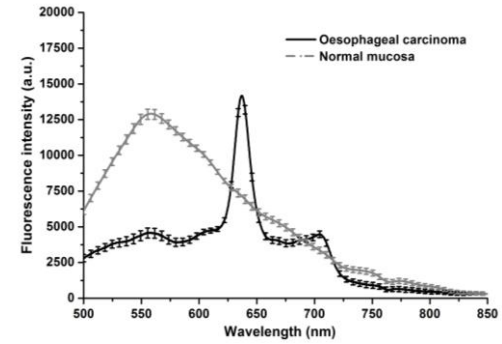
The autofluorescence spectroscopy of gastrointestinal tissues after their surgical removal can provide information on intrinsic sources of fluorescence that correlate with biochemical and morphological changes occurring in tumours in comparison with normal mucosa. We evaluated the background autofluorescence originating from the physiological solution where the tissue samples were kept during the measurements; the signal was found negligible in comparison with the fluorescence intensities detected from tissue samples for all excitation wavelengths applied in our investigations.

The exogenous fluorescence spectroscopy of GIT tissues *in vivo* and after excision can provide information on and validation of the level of exogenous fluorophore accumulated (in our case PpIX) in the tumour and in the surrounding normal mucosa. The contrast ratio "normal/abnormal" fluorescence intensities at 635 nm (the maximum of the PpIX fluorescent signal) allows one to evaluate the fluorescence levels needed for proper diagnosis and to eliminate the false-positive red fluorescence that could originate from inflamed or from normal mucosa GIT tissues.

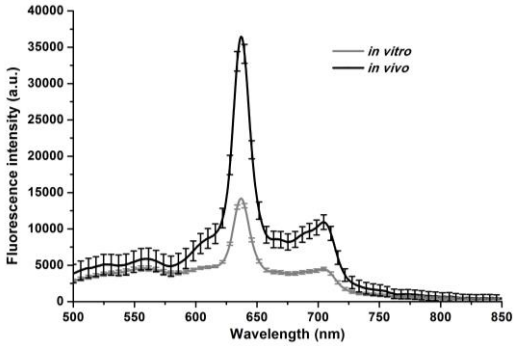
Fig. 1a presents fluorescence spectra of a freshly excised oesophageal carcinoma and of the surrounding normal mucosa excited at 405 nm where PpIX has significant absorption (Soret band) for a patient to whom a 5-ALA-mediated fluorescence diagnosis was applied. Fig. 1b presents a comparison of the fluorescence spectra obtained from the tumour during the *in vivo* endoscopic observation procedure and when the excised lesion was investigated spectroscopically. All spectra were averaged by successive measurements made in the areas investigated; the error bars are shown in the figures.

The autofluorescence of a normal tissue (fig. 1a) has a maximum in the 520 – 580 nm range. In the same spectral region in the case of a tumour, we observed a significant distortion due to the re-absorption of the autofluorescence signal by the haemoglobin – with well-pronounced minima within 540 – 575 nm. Such minima were not observed in the cases investigated of the oesophagus and the healthy stomach mucosa areas, but were well-pronounced in the healthy colon mucosa (see fig.2); therefore, this feature could not be used as a universal indication of tumour growth and the related neovascularisation in GIT mucosa. If darker areas (related to the haemoglobin absorption) are observed in the blue-green autofluorescence images, they are considered as being diagnostic predictors of a tumour neo-vascularisation process [26, 28].

Two typical maxima – at 635 and 704 nm, were detected in the red fluorescent signal from the tumour, which originated from the protoporphyrin IX accumulated.



a)



(b)

Fig.1. (a) Comparison of the fluorescence spectra from normal oesophageal mucosa and from carcinoma; (b) Comparison of the fluorescence spectra from oesophageal malignancy measured in vivo endoscopically and in vitro on a surgically excised tumour; the fluorescence spectra were obtained using excitation at 405 nm.

In patients with both malignancies and a benign pathology, such as a colon polyp, this fluorescent signal was observed in the benign areas as well, but with a lower intensity of the main maximum at 635 nm (fig. 2).

When *in vivo* endoscopic observations are conducted, this red fluorescence signal can give rise to false-positive results in lesion determination. However, when a comparison of normal/malignant lesions is carried out, a rapid determination of the lesion border can be obtained from the exogenous fluorescence signal in a 1-D scanning spectroscopic mode using excitation at 405 nm. To improve the contrast between benign and malignant lesions, a simple dimensionless ratio between the maxima of the exogenous fluorophore signal and the endogenous fluorescence can be calculated – $R = I_{635}/I_{560}$,

An assessment of the feasibility of such a ratio-based algorithm for differentiating between normal tissue, inflammation, benign and malignant lesions in the gastrointestinal tract was presented in our previous works [30, 32], which allows for a sensitive differentiation of the lesion type.

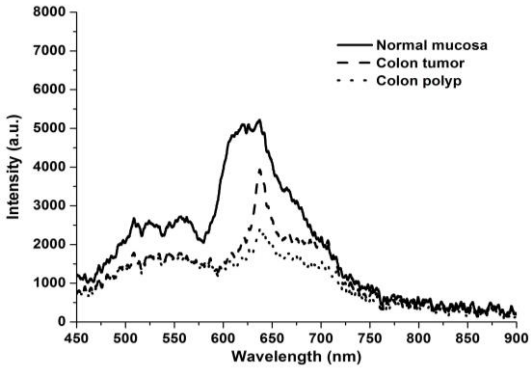
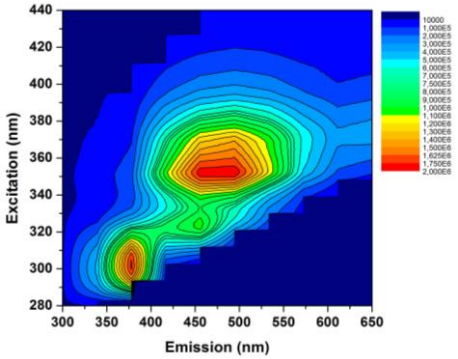
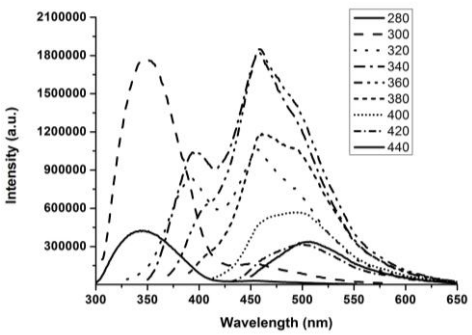


Fig.2. Fluorescence spectra of a colon carcinoma and a benign polyp with 5-ALA/PpIX used as exogenous fluorophore with excitation at 405 nm.

Fig. 3a and 3b present an excitation-emission matrix (EEM) and steady-state autofluorescence data from normal colon mucosa. Fig 4a and 4b show an EEM and autofluorescence spectra for different excitation wavelengths used to study a colon carcinoma. Several major autofluorescence sources in the tissues investigated could be identified. The amino acids tryptophan and tyrosine were excited by deep UV (280 – 300 nm) and emitted in the 320 – 360 nm range. The structural proteins elastin and collagen were excited between 320 – 360 nm and emitted at 400 nm and within the 460 – 500 nm interval; their protein cross-links were excited by light in the 360 – 400 nm range and emitted with a maximum at the region of 480 – 500 nm. Fast degradation of the NADH and flavins was observed in autofluorescence from excised tissue samples due to the degradation of the co-enzymes in such tissues. We could only detect this in freshly excised samples with excitation in the 340 – 380 nm range and emission within the 440 – 480 nm range for NADH, and with 340 – 400 nm excitation and 500 – 530 nm emission for flavins.



(a)

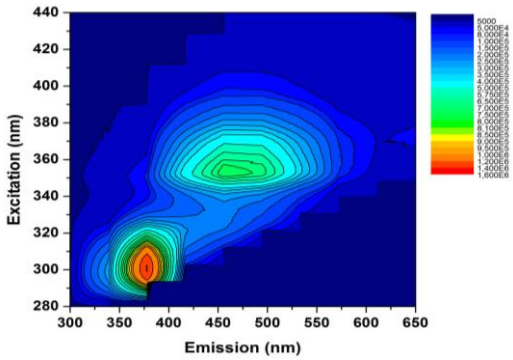


(b)

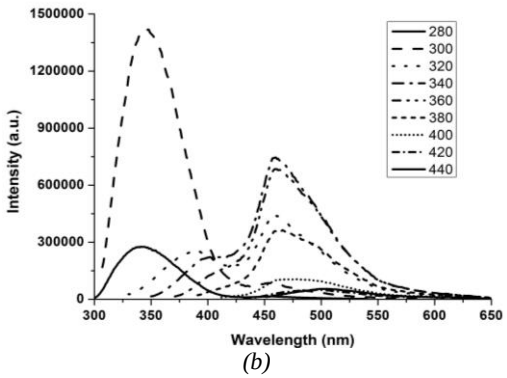
Fig.3. (a) EEM and (b) steady-state autofluorescence spectra of excised normal colon mucosa.

The intensity of the autofluorescence of the tumourous area was much lower than that of the normal mucosa. For a given patient tissue sample contained a normal mucosa and a carcinoma area, the signal from the tumourous part was usually twice as low as that from the normal tissue.

Similar observations of a decrease in the fluorescence signal have been reported by different research groups and have been proposed to be used as an indication of a tumour lesion presence when autofluorescent topography of GIT is carried out. However, we observed changes not only in the intensity, but in the spectra's shape as well, which we assigned to changes in the signal intensity originating from collagen fibers and from collagen cross-links. In the case of a tumour lesion, the intercellular matrix is relatively loosened due to the increased tumour cells size and the general reduction in the collagen and elastin concentration in a unit volume. The fluorescence signal of structural proteins was also reduced due to the thickening of the mucosal layer which screens and reduces the 480 – 500 nm fluorescence from the submucosa layer. Another change observed was the general decrease of the NADH fluorescence vs. that of normal mucosa, because in tumor cells NADH undergoes a transition to its oxidized non-fluorescent form NAD^+ (fig.4a and 4b).



(a)



(b)

Fig.4. (a) EEM and (b) steady-state autofluorescence spectra of excised colon carcinoma.

The most significant differences in the fluorescence spectra were observed when excitation at wavelengths in the region of 300 –360 nm was used. We compared the spectral shape changes in normalised signals from normal mucosa, polyp and carcinoma lesions of a given patient. For the other excitation wavelengths applied in the 280 – 300 and 370 – 440 nm ranges, fluorescence intensity changes were only detected for all samples investigated. In two of the seven colon cancer samples, the appearance was observed of a weak fluorescence peak in the red spectral region. It was assigned to the endogenous porphyrins that could accumulate in the tumour tissues in amount sufficient to be detected during the fluorescence measurements carried out. An example of such fluorescence detected in samples with no 5-ALA applied preliminarily is shown in fig. 5, where porphyrins fluorescence appeared in the 620 – 650 nm region. Absorption in the 540 – 575 nm spectral region was also observed distorting the autofluorescence signal of the cancerous tissue, which we related with the increased vascularisation of the pathology vs. normal colon.

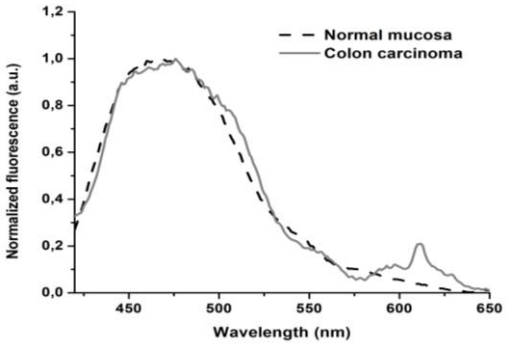


Fig.5. Autofluorescence spectra of normal and cancerous colon mucosa using 400 nm excitation. The spectra are normalised with respect to the maximum.

Fig. 6 presents spectra normalised with respect to the fluorescence signal maximum of normal mucosa, colon polyp and carcinoma lesion for one patient at several different excitation wavelengths, namely, 300, 320, 340, 360,380, and 400 nm. The most prominent differences observed were in the already-mentioned 320 – 360 nm range of excitation. These, therefore, were the wavelengths proposed to be used for the *in vivo* autofluorescence diagnosis of GIT tumours and 2-D visualisation for the needs of clinical observations.

This spectral region of excitation, where the most significant alterations in the autofluorescence of normal/abnormal GIT tissues appeared, is suboptimal from the point of view of its technical applicability into the clinical practice. When 1-D spectroscopic fiber probes are used, the sensitivity of the spectrometers allows one to obtain results suitable for diagnostic needs.

However, if a 2-D visualisation for the clinical needs is to be achieved, the UV excitation applied (320 – 360 nm) is not preferred by the clinicians.

For an appropriate 2-D visualisation of the autofluorescence, longer wavelengths are typically used (380 – 460 nm), where the most significant changes are observed.

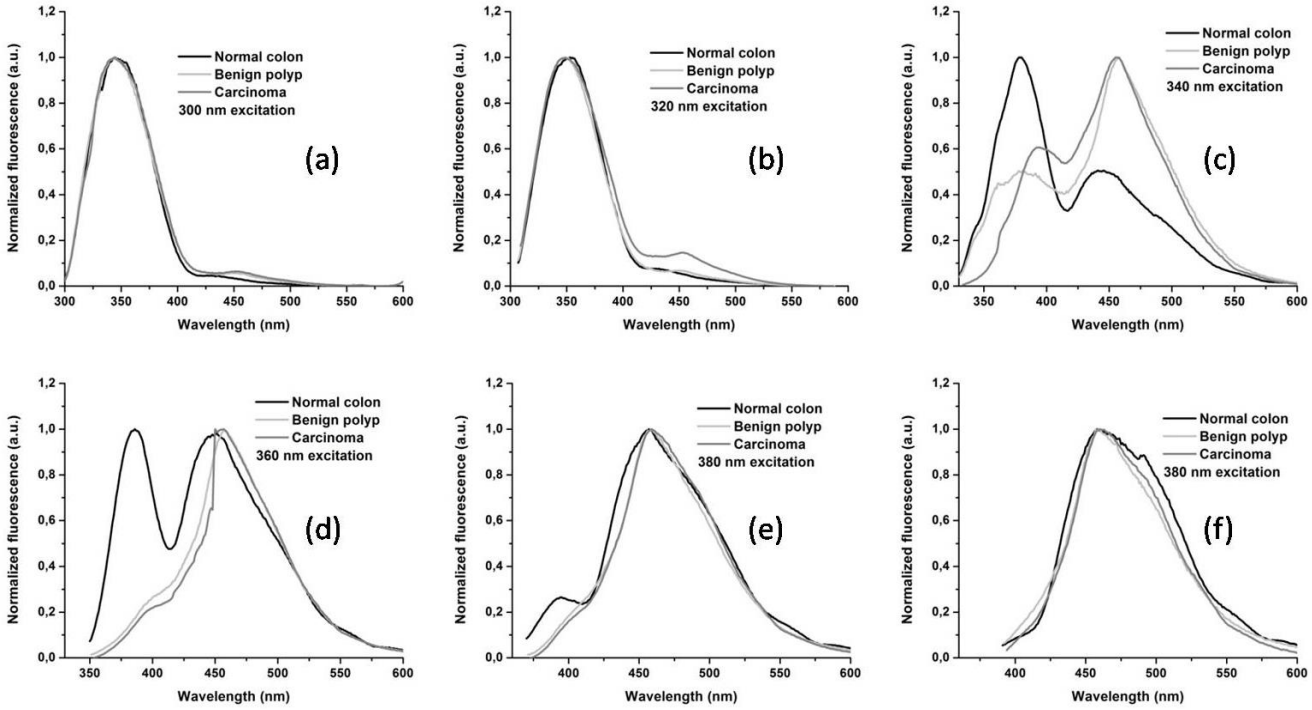


Fig.6. Autofluorescence spectra normalised with respect to the maximum of normal mucosa, polyp and carcinoma of colon obtained at different excitation wavelengths (a) 300 nm, (b) 320 nm, (c) 340 nm, (d) 360 nm, (e) 380 nm, (f) 400 nm.

When the spectra of normal and diseased mucosa are compared based on fluorescence intensity changes, tumours exhibit much lower autofluorescence and are seen as dark spots during endoscopic *in vivo* observations [12, 23, 28].

In general, when a normal tissue is excited by light in the end of the UV and blue spectral ranges, the autofluorescence spectrum consists mainly of fluorescence signals arising from collagen, elastin, protein cross-links, NADH and flavins. These compounds change their concentration or redox-state in the case of tumour growth, so that the correlated changes in their fluorescence intensities can be used as a sign of a development of malignancy. The problems related to the false-positive diagnoses due to the changes observed not only in malignant tissues, but also in polyps, inflammations and other benign tissues, exist in both modalities – in endogenous and exogenous fluorescence diagnosis of GIT tumours. Nevertheless, as one can see in fig. 6, if one applies multiple wavelength excitation, this problem can be overcome and the autofluorescence specificity becomes suitable for clinical application. The problem when the autofluorescence mode is solely applied is that the signal obtained is too faint and the image received is suboptimal for detection and differentiation of dysplastic lesions from neoplasia. This mode, therefore, cannot be used in 2-D visualisation of the lesions in endoscopic or in open surgical observations that are required in the clinical practice. At present, an improvement in the diagnostic accuracy and a better visualisation on can only be reached by applying exogenous contrast fluorophores, such as 5-ALA/PpIX [19, 20, 28].

The application of exogenous fluorophores in the GIT fluorescence diagnosis is still the more appropriate and preferred choice for the researchers working with this kind of spectral diagnosis. Very optimistic results have been reported by different research groups that prove the feasibility of the 5-ALA-PpIX exogenous fluorescence for GIT tumour detection and clinical diagnostics. Some of them are summarised in table 2 from the point of view of the pathological condition investigated, the excitation wavelengths applied and emission maxima detected, the technique and dose of fluorescent drug administration, the number of patients investigated and the sensitivity and specificity of the exogenous fluorescent tumour diagnosis achieved.

The National Center of Biomedical Photonics of the Institute of Electronics-BAS collaborates with Tsaritsa Yoanna - ISUL University Hospital in the process of development of clinically feasible fluorescence diagnostics applications. The studies presented here are a part of a clinical trial for introducing such a spectroscopic diagnostic endoscopy system and a system for fluorescence image-guided surgery for GIT pathologies in the clinical practice of the University Hospital.

The main aim of our studies is to compile a significant database for the main spectral characteristics of GIT neoplasia and to develop algorithms for benign/malignant tissue differentiation using autofluorescence and exogenous fluorescence tumour detection. Our role is to find the optimal modes and regimes of work using fluorescent spectroscopy techniques for GIT tumours detection that will be useful for and helpful to the clinicians.

Table 2. 5-ALA gastrointestinal neoplasia diagnostic applications.

Disease	λ excitation (nm)	λ emission (nm)	SENS [%]	SPEC [%]	Drug administration technique	Photo-sensitiser dose applied	Accumulation time	Number of lesions/ patients	Ref.
Barrett's oesophagus	505 nm	635 nm, 699 nm	76%	63%	sprayed on the mucosa	aqueous solution of 0.5g of 5-ALA in 50ml of $NaHCO_3$ 8.4% or 50ml NaCl -0.9%	60-120 min	53 patients	33
Barrett's oesophagus-low and high grade dysplasia (HGD)	380-440 nm	635 nm	60-80%-local, 100%-systemic	70%-local, 27-56%-system	orally or locally by spraying the mucosa via a catheter	5, 10, 20, 30 mg/kg 500–1000 mg	4–6 h after systemic 1–2 h after local sensitisation	47 patients with Barrett's oesophagus	34
Barrett's oesophagus-HGD	400 nm	635 nm	77%	71%	orally	10 mg/kg	3h	20 patients	35
Small GIT tumours-primer + peritoneal metastasis	405 nm	625-725 nm	81% peritoneal carc., 60% - gastric cancer	N/A	orally	20 mg/kg	6 h	6 patients 32 lesions	36
GIT tumours					orally	30-60 mg/kg	1-24h	26 patients, oesophagus, duodenum and colon	37
Low-grade and high-grade colon dysplasia	390-405 nm	635 nm	100% (spray catheter)	62% (spray catheter)	orally, using spray catheter and enema	20 mg/kg	1-2/ 4-6h	12 patients	38
Adenomatous and hyperplastic polyps	405/436 nm		89%/ 86%	94%/ 100%	orally			adenomas (n=32), hyperplasia (n=14)	39
Colon carcinoma and polyps	632,8 nm	635-700 nm	94,9%	62,5%	systematic	20-30 mg/kg	3 h	78 patients	19
Premalignant and maligmant oesophageal lesions		630 nm	85%	53%	orally	15 mg/kg	6-7h	22 patients	40
Esophagus and stomach tumours	405 nm	635 nm, 705 nm			orally	20 mg/kg	6 h	12 oesophagus, 15 stomach	29
Barrett's oesophagus			85%	70%		10 mg/kg	3h	35 patients	41

4. Conclusions

Diagnostic techniques based on fluorescence spectroscopy have the potential to correlate the biochemical and morphologic properties of tissues with the individual patient care. Fluorescence topography is a method for determining the site of the lesions, their borders and size. We hope that the systems and methods developed for diagnosis and monitoring will be introduced into the practice at the Tsaritsa Yoanna-ISUL University Hospital, as they could open new dimensions in diagnostics and in real-time monitoring of resections. These methods will make the entire procedure more personal, patient friendly and effective and will assist in the further understanding of the tumours' nature and in improving the patients' quality of live.

Based on the experience accumulated up to now, we believe that the 1-D fiber probe autofluorescence measurement is the most appropriate and useful mode for the needs of initial diagnosis of GIT tumours during an endoscopic observation. It avoids the use of exogenous drugs, while using excitation within the 320 – 360 nm wavelengths region one could reach very high sensitivity and specificity in such a primary diagnostic evaluation.

However, when a video visualisation is clinically required – for mapping of the lesions, precise border evaluation, searching of close metastases, evaluation of safety surgical areas, etc., the exogenous fluorophores are preferable, as they improve the fluorescent contrast of the tumour vs. surrounding inflammatory, benign and normal GIT tissues.

The way between the laboratory and the clinical environment in terms of transferring the research results to clinical equipment and applications is long and arduous. The results presented here can be taken into account when decision about the fluorescence mode of diagnosis need to be made.

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