ENDOGENOUS AND EXOGENOUS FLUORESCENCE OF GASTROINTESTINAL TUMORS – INITIAL CLINICAL OBSERVATIONS

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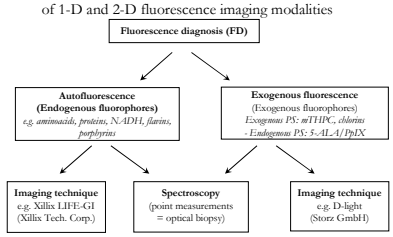
The limitations of standard endoscopy for detection and evaluation of cancerous changes in gastrointestinal tract (GIT) are significant challenge and initiate development of new diagnostic modalities. Therefore many spectral and optical techniques are applied recently into the clinical practice for obtaining qualitatively and quantitatively new data from gastrointestinal neoplasia with different level of clinical applicability and diagnostic success. One of the most promising approaches is fluorescence detection using naturally existing fluorescent molecules or added fluorescent markers.

PRINCIPLES AND TECHNIQUES

Fluorescence detection techniques – principal scheme of 1-D and 2-D fluorescence imaging modalities



Excitation and emission wavelengths of various exogenous photo-sensitizers and their gastrointestinal neoplasia diagnostic application

Photosensitizer	Excitation wavelength (nm)	Fluorescence wavelength peak (nm)	Investigated pathologies
5-ALA/ PpIX hematoporphyrin derivative (HpD)	405, 514, 630	635, 690, 704	Barrett esophagus, low- and high-grade colon dysplasia, esophageal squamous cell cancer adenocarcinoma, stomach carcinoma
chlorin (chlorin e6, mTHPC)	660	665	Esophageal squamous cell carcinoma
Phthalocyanines (PCs)	410, 530, 670	675-685, 740	Stomach carcinoma, esophageal adenocarcinoma

Excitation and emission wavelengths of various endogenous fluorophores in human tissues

Fluorophore	Origin	Optimal excitation wavelength (nm)	Peak of fluorescence emission (nm)
Tryptophan	amino acid	280, 305	340-350
Tyrosine	amino acid	275	300
Phenylalanine	amino acid	260	280
Collagen	structural protein	330-390	390-440
Elastin	structural protein	280, 360	350, 410
Protein cross-links	structural proteins	380-420	460-500
Pyrodixine	vitamin B6 compound	330-340	400
Ceroid, lipofuscin	lipo-pigment granules, oxidation products	340-395	430-460, 540-640
NADH	metabolic co-factor	340	450-470
FAD, Flavins	metabolic co-factor	420-460	500-520
Porphyrins	heme biosynthesis byproducts; bacterial flora	390-430, 630	635, 690

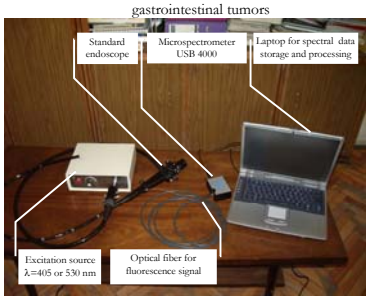
MATERIALS AND METHODS

Investigations presented are a part of our initial clinical experience during trial procedure for introduction of spectroscopic diagnostic system for gastrointestinal tumors in the clinical practice of University Hospital "Queen Giovanna - ISUL". For fluorescence measurements of gastrointestinal pathologies excitation sources at 405 and 530 nm are applied.

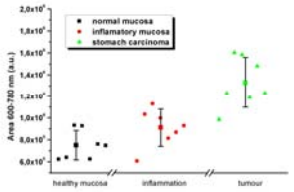
Optical fiber probe, consisting of 6 emitting fibers and one collecting fiber, is applied through instrumental channel of the endoscope for detection of tumors *in vivo*. When *in vitro* samples are investigated similar 7-fibers probe is applied on the samples excised during surgical procedures for tumors' removal. For all pathologies investigated using exogenous fluorescent detection, as a fluorescent marker is used Protoporphyrin IX. Initially 5-ALA is applied orally – as a water solution (20 mg/kg dose) for the gastrointestinal lesions. After 6 hours exogenous fluorescence detection of accumulated in the pathologies protoporphyrin IX was carried out.

Both kinds of spectra – autofluorescence signals and protoporphyrin IX signal are recorded and stored using a fiber-optic microspectrometer (USB4000, Ocean Optics, Dunedin, FL, USA). A personal computer is used to control the system and to store and display the data using the specialized microspectrometer software OOI Base ("Ocean Optics", Inc., Dunedin, FL, USA). Normal tissue fluorescence was used in both localizations as a basis for comparison with the pathologies observed.

System for fluorescence spectroscopy of gastrointestinal tumors

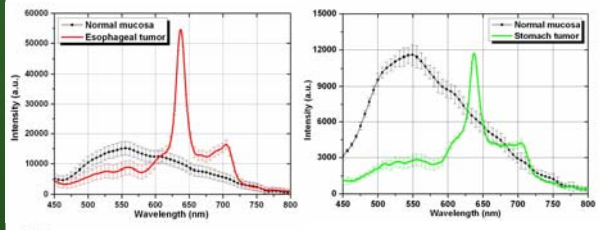


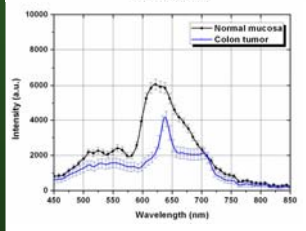
RESULTS AND DISCUSSION



Comparison of the integrated fluorescence signal for the region 600-780 nm, calculated for all cases detected from stomach normal mucosa, inflammation, and carcinoma. Lines represent the mean values of the areas calculated.

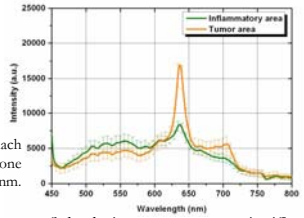
In the recent study 5-ALA/PpIX is applied for tumor detection in esophagus and stomach. A rapid lesions border determination from exogenous fluorescence signal is obtained in 1-D scanning spectroscopic mode using excitation at 405 nm. Our results from *in vivo* detection show very good differentiation between normal and abnormal tissues in 1-D spectroscopic regime, but moderate discrimination in 2-D imaging. In the case of 2-D video visualization the problem of relatively high levels of the autofluorescence signal in the red spectral region gives low contrast between normal and abnormal mucosa when standard CCD camera of the endoscope is applied. Similar effect of autofluorescence and exogenous fluorescence similar levels in the red spectral region is observed from other research groups too



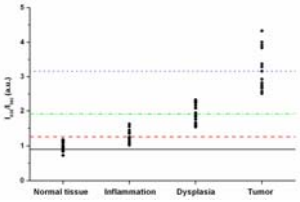


Fluorescence spectra of normal mucosa and tumor of patient with esophageal carcinoma, patient with stomach carcinoma and patient with colon adenocarcinoma at 405 nm excitation.

This problem could be solved partially by change of excitation wavelength applied using longer wavelengths for excitation of PpIX (absorption at 509 nm, 544 nm or 584 nm), where autofluorescence is not so strong factor, as well as back scattered excitation light from the mucosal surface does not lie in the spectral region of PpIX fluorescence itself. The evaluation of the influence of autofluorescence of normal and abnormal mucosa on the 2-D visualization of the tumor areas in the red spectral region is carried out. In general, the fluorescence detected from esophagus and stomach lesions consists from combined response of endogenous and exogenous fluorophores in the region of 500-750 nm. The emission signals are additionally altered from the re-absorption of the hemoglobin accumulated in the tissue investigated. Comparison of the spectra received from normal mucosa, inflammatory and tumor areas is also applied to evaluate the feasibility for development of simple but effective algorithms based on dimensionless ratio of the fluorescence signals, for differentiation of normal/abnormal gastrointestinal tissues.



Fluorescence spectra of stomach inflammation and tumor of one patient, using excitation at 405 nm.



Dimensionless ratio (•) $R=I_{635}/I_{560}$ calculated for all cases detected from stomach normal mucosa, inflammation, dysplasia and carcinoma. Lines represent the mean values of the ratio calculated.

In the case of esophageal and stomach tumors the autofluorescence spectra of the lesion area are not significantly different by shape and intensity from the surrounding normal mucosa. Some additional absorption of hemoglobin is observed in lesion' areas, but false-positive results in this case are observed when inflammatory tissues have place as well. Autofluorescence results gives high sensitivity (>90%), but extremely low specificity (~55-60%), which is not acceptable for a technique, which must improve the diagnostic accuracy of initial tumor detection and evaluation of tumor borders and spreading lesions in the mucosa investigated. These drawbacks are overloaded when exogenous fluorophore 5-ALA/PpIX is applied. Sensitivity and specificity observed for both localizations – esophageal and stomach carcinoma lesions exceed 90%, which make the exogenous fluorescence diagnosis of gastrointestinal tumors and useful tool for clinical practice.

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