ENDOGENOUS AND EXOGENOUS FLUORESCENCE OF BIOLOGICAL TISSUES FOR CLINICAL APPLICATIONS

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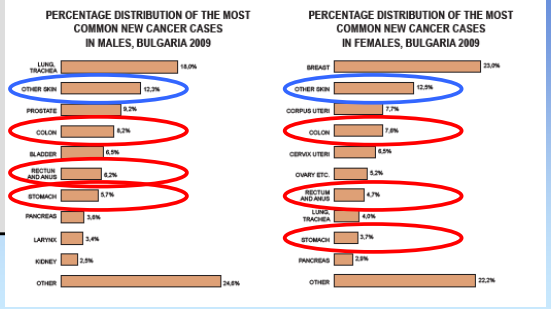
Why tumour fluorescence is interesting?



Fluorescence in clinics

Fluorescence diagnosis

Autofluorescence (Endogenous fluorophores)
e.g. aminoacids, proteins, NADH, flavins, porphyrins

Exogenous fluorescence
(Exogenous fluorophores)
Exogenous PS: mTHPC, chlorins
- Endogenous PS: 5-ALA/PpIX

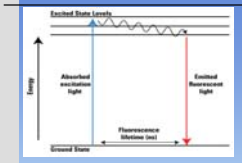
Imaging technique
e.g. Xillix LIFE-GI (Xillix Tech. Corp.)
FotoFinder (Germany)

Spectroscopy
(point meas.~optical biopsy)
SkinScan (HORIBA JY)
WavSTAT Optical Biopsy
Forceps (SpectraScience)

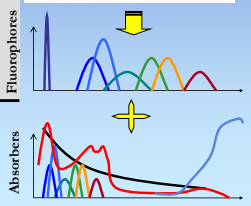
Imaging technique
e.g. D-light (Storz GmbH)

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

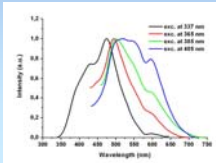
Fluorescence of tissues



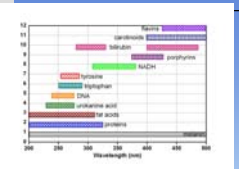
- Objective
- Real-time
- Non-invasive
- Highly sensitive
- Repeatable results
- Biochemical and morphological correlations
- Multiple applications
- Monitoring of therapeutic procedures
- High diagnostic accuracy
- Exclude human factor



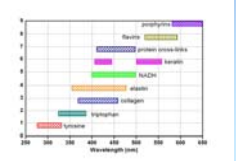
In vivo



Tissue absorbers and fluorophores



Absorption spectral regions of main tissue chromophores



Normal skin phototype II autofluorescence – excitation – emission matrix

Main cutaneous fluorophores emission spectral regions*

**Utz S., Skin optics, in Low-level laser therapy, ed. S. Moskvin, V. Builin, Moscow, TOO "Tehnika" 2000, pp. 58-71.*

Tumours' variety

- **Basal cell carcinoma (BCC)** ~75 % of the cases – more than 10 subtypes - nodular, cystic, morpheaform, infiltrative, micronodular, superficial, pigmented, polypoid, pore-like, aberrant BCC
- **Squamous cell carcinoma (SCC)** ~ 15 % of the cases – several subtypes – Signet-ring cell, Clear cell, Adenoid, Basaloid SCC
  - **Keratoacanthoma (KA)** – several subtypes - giant, multiple, generalized eruptive, subungual keratoacanthoma, and keratoacanthoma centrifugum marginatum
- **Melanoma** ~ 10% of the cases
- Uncommon kinds of skin cancer - dermatofibrosarcoma protuberans, Merkel cell carcinoma, Kaposi's sarcoma, spindle cell tumors, sebaceous carcinomas, microcystic adnexal carcinoma, atypical fibroxanthoma, etc.

Benign and malignant lesions – diagnosis???

Comparison of Surface microscopy diagnoses before and after the training course

Diagnostic Indicator	Before	After
SENS (%)	65.00	71.56
SPEC (%)	80.93	79.69
DA (%)	54.59	59.48

Heamangioma

Base-cell papilloma

OBJECTIVITY PROBLEM

Dermatoscopic pictures of different skin lesions, magnification x100

Pigmented BCC

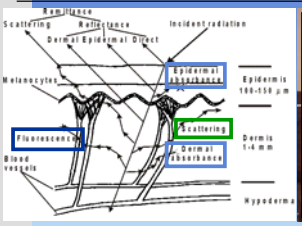
Malignant melanoma

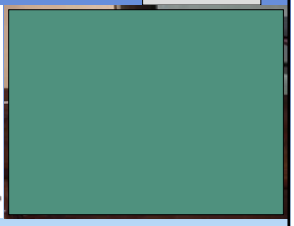
Comparison of Epiluminescence microscopy diagnoses before and after the training course

Diagnostic Indicator	Before	After
SENS (%)	75.31	89.69
SPEC (%)	83.44	83.12
DA (%)	62.92	77.74

Troyanova P., A beneficial effect of a short-term formal training course in epiluminescence microscopy on the diagnostic performance of dermatologists in cutaneous oncology, Folia Med Tomsp, 2003; 9: 269-273.

Skin cancer autofluorescence detection





Principal set-up used for initial clinical investigations:

LEDs with emission maxima at UV-VIS region are used as excitation sources (360-440 nm), Y-fiber bundle with 6 fibers for excitation light and 1- central fiber - for emission light USB4000 microspectrometer and PC for storage and visualization of spectral data

Patients

Number of patients for the period February 2009-July 2012 – 528

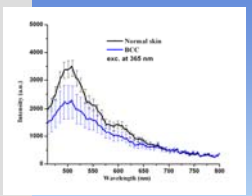
Procedure

1) Clinical observation, and if case is appropriate · · ⟶
2) Informed consent and authorization for investigation
3) Short questionary to the patient – ages, working conditions, skin phototype, medications used, other pathologies and/or diseases
4) Lesion image obtaining
3) Fluorescence spectroscopic measurements
4) Sampling for histological analysis

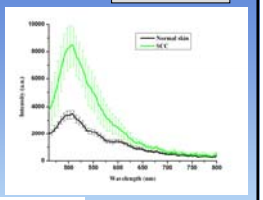
Diagnoses

1) Malignant: basal cell carcinoma (BCC), squamous cell carcinoma (SCC); Malignant melanoma – pigmented and amelanotic; Sebaceous carcinoma, Bowen's disease
2) Dysplastic: Keratoacantoma (KA); Dysplastic nevus
3) Benign: BC papilloma, fibroma, atheroma, heamangioma, angioma, compound nevus, atypical nevus, verruca seborea, actinic keratosis, blue nevus, Sutton nevus, etc.

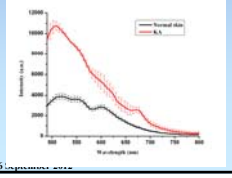
Non-melanoma tumors - results



BCC - Exc. at 365 nm

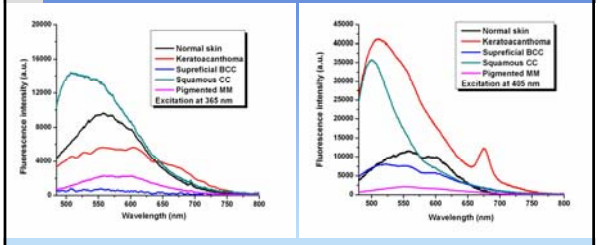


SCC - Exc. at 365 nm



KA – Exc. at 405 nm

Comparison of malignant lesions' AF spectra

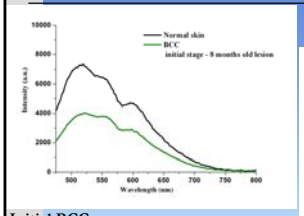


Fluorescence spectra of the most often lesions observed, compared for two different excitation sources

Trends….

- BCC lesions have lower fluorescence than normal skin
- SCC lesions have higher fluorescence than normal skin
- KA lesions have strong keratin fluorescence signal in green spectral region
- Compounds, which fluoresce are collagen type I – at 400-405 nm; its cross-links – at 460-490 nm; elastin – with maxima at 400-420, 460 nm; elastin cross-links – about 500 nm; NADH – at 440-470 nm; keratin – at 430-460, and around 500-520 nm, and flavins.
- In several patients red fluorescence, related to endogenous porphyrins accumulation in the lesions is also observed for advanced stage lesions.
- Influence of the hemoglobin and melanin pigments is well pronounced in the received in vivo fluorescence spectra related to relative decrease of the short-wavelength vs. long-wavelength intensity, as well as appearance of minima at 420, 543 and 575 nm respectively.

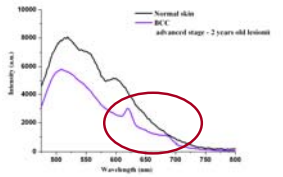
Tumor stage evaluation – initial vs. advanced



Initial BCC

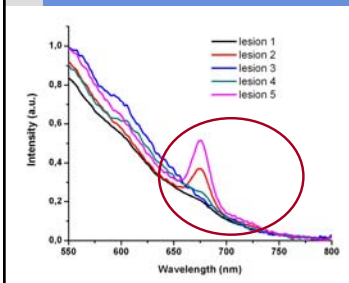
Excitation at 405 nm

Advanced BCC



- Two BCC lesions of one patient, one of the lesions has appeared about two years before the observation; second has appeared about eight months before the light-induced fluorescence measurements carried out.
- No needs for compensation related to inter-patients differences. Intra-patient differences could be taken as negligible, due the fact that both pathologies were nearby.

Multiple lesions – treatment planning



- Porphyrin-like signal from advanced lesions

One patient, 69 years-old - 5 BCC lesions on different stage of growth, similar size (1x1cm area) and clinical picture

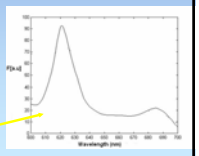
Treatment decision:
Lesions 2 and 5 – advanced stage – surgical removal and chemotherapy

Lesion 4 – intermediate stage – chemo- and radiotherapy, 2 months later

Lesions 1 and 3 – initial stage – local chemotherapy, 3 months later

Exogenous fluorescence of skin tumors

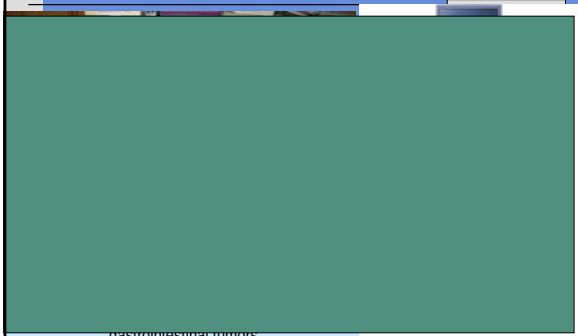
-5 –ALA/PpIX
- multiple BCC lesions
- 405 nm excitation
>590 nm filter for observation



- main tumour
- additional tumours
- benign tissues

δ-ALA – photodiagnosis and PDT of BCC

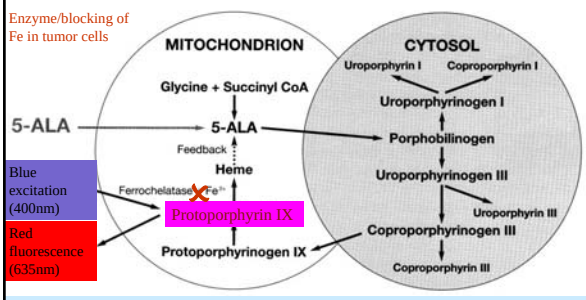
GIT cancer fluorescence detection

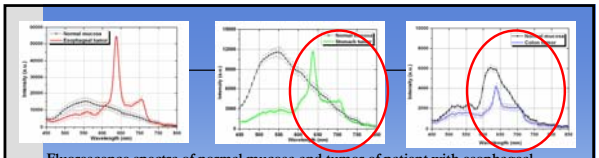


gastrointestinal tumors

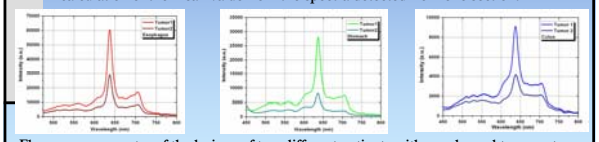
Olympus video-endoscope

Metabolism of δ-ALA in the cells



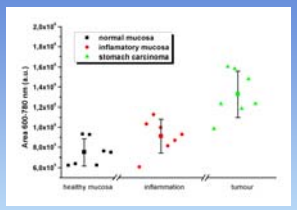


Fluorescence spectra of normal mucosa and tumor of patient with esophageal carcinoma, patient with stomach carcinoma and patient with colon adenocarcinoma at 405 nm excitation. Spectral are presented with their standard deviation, resulting of calculation of the mean value from the spectra detected from one section.



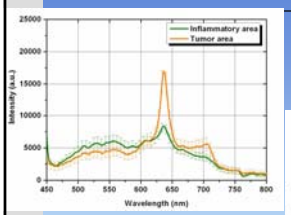
Fluorescence spectra of the lesions of two different patients with esophageal tumors, two patients with stomach tumors and two – with colon tumor lesions at 405 nm excitation. Spectra presented outlying cases of the highest ("Tumor 1") and the lowest ("Tumor 2") signals received from tumors in all localizations in different patients.

2-D fluorescence visualization of esophageal carcinoma using 5-ALA/PpIX



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.

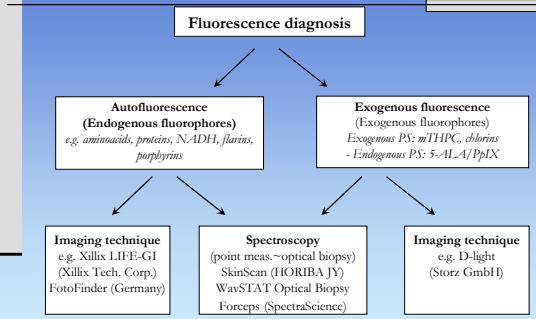
False positive results - inflammatory area



Conclusions

- A rapid lesions border determination from exogenous fluorescence signal is obtained in 1-D scanning spectroscopic mode using excitation at 405 nm. *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.
- 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.
- 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%, when spectral data in 1-D are taken into account.

Fluorescence in clinics



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

Thank you very much for your attention!

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