OPTICAL BIOPSY - CLINICAL TOOL FOR INITIAL DIAGNOSIS AND THERAPEUTIC MONITORING OF CUTANEOUS TUMORS

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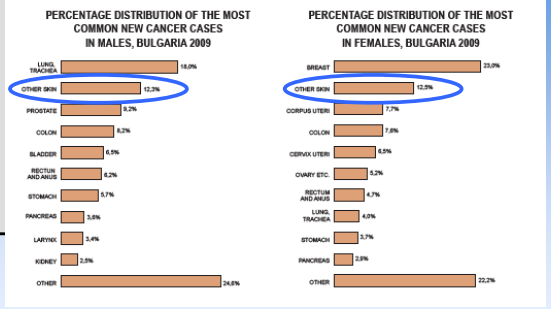
Why cutaneous tumor diagnosis is interesting?

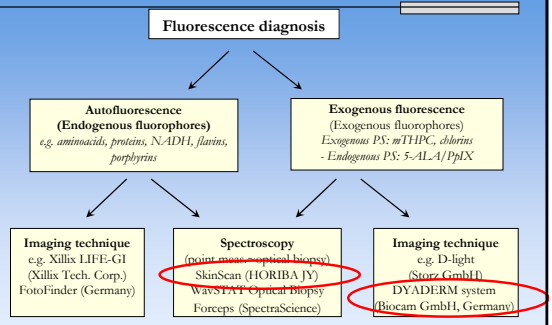


Optical spectroscopy of human skin

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

SOLUTION?

Fluorescence in clinics



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

Skin cancer

- **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.

Patients

Number of patients for the period February 2009-June 2012 – 516

Procedure

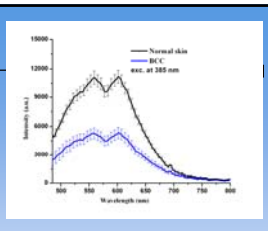
1) Clinical observation, and if case is appropriate: · · ⟶
2) Informed consent and authorization for investigation
3) Short questionary to the patient – ages, working conditions, skin phototype, medications used, other pathologies and/or diseases
4) Lesion image obtaining – standard, dermatoscopic
5) Fluorescence (365, 385, 405 nm exc.) and reflectance (400-900 nm) measurements
6) Sampling for histological analysis

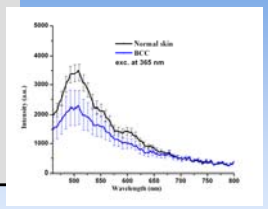
Diagnoses

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.

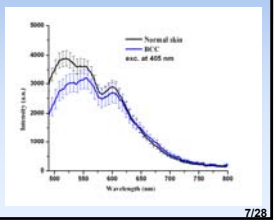
BCC – fluor. results

Exc. at 385 nm





Exc. at 365 nm



Exc. at 405 nm

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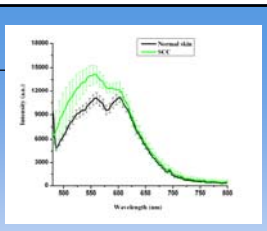
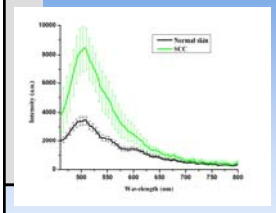
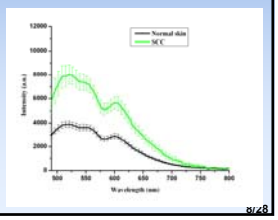
SCC – fluor. results

Exc. at 385 nm





Exc. at 365 nm



Exc. at 405 nm

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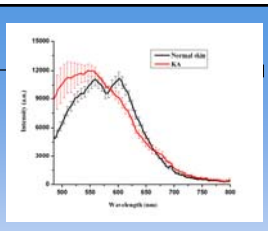
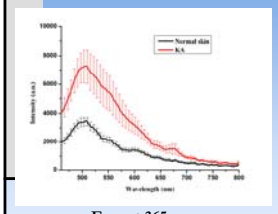
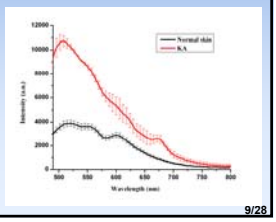
KA – fluor. results

Exc. at 385 nm





Exc. at 365 nm



Exc. at 405 nm

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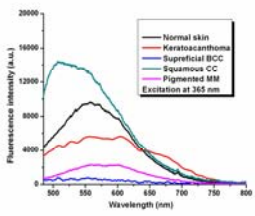
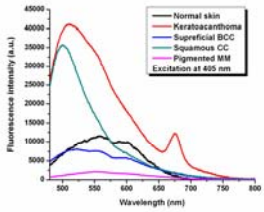
Comparison of malignant lesions' spectra





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

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Statistical values of LIAF spectroscopic diagnosis of skin lesions

Comparison	SN %	SP %	DA %
Normal skin/ Compound nevus	91,2	100	91,2
Compound nevus/ Dysplastic nevus	82,9	86,8	69,6
Dysplastic nevus/ Malignant melanoma	89,6	78,7	74,1
Normal skin/ Base-cell papilloma	89,4	99,6	87,5
Normal skin/ Base-cell carcinoma	91,0	97,4	82,2
Base-cell papilloma/Base-cell carcinoma	94,0	97,9	92,6

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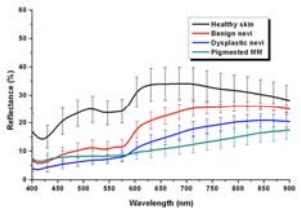
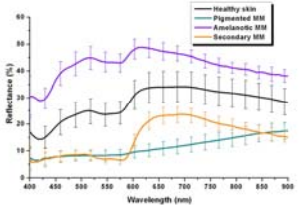
Reflectance of melanin-pigmented benign and malignant lesions

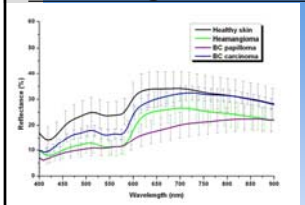


Reflectance spectra presented with their standard deviations of normal skin, compound nevus, dysplastic nevus and pigmented MM, averaged by lesion type for all patients



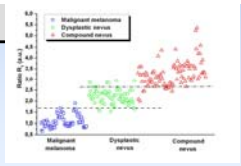
Reflectance spectra presented with their standard deviations of normal skin, pigmented, amelanotic and secondary melanoma lesions, averaged by lesion type for all patients

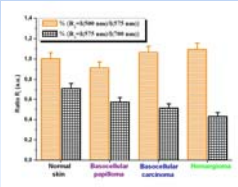
Reflectance of non-melanin-pigmented benign and malignant lesions



Reflectance spectra presented with their standard deviations of normal skin, compound nevus, dysplastic nevus and pigmented MM, averaged by lesion type for all patients

Ratios chosen for differentiation and valuation between normal skin, melanin-pigmented lesions, BC lesions and heamangioma - dimensionless ratios of the reflectance values at 500, 575 and 700 nm.





Statistical values of reflectance diagnosis of skin lesions

Comparison	SN %	SP %	DA %
Compound nevus/ Dysplastic nevus	82,1	77,6	61,8
Dysplastic nevus/ Malignant melanoma	92,4	77,6	75,3
Normal skin/ Base-cell papilloma	95,7	98,5	89,2
Normal skin/ Base-cell carcinoma	94,0	98,2	86,8
Base-cell papilloma/Base-cell carcinoma	91,0	87,2	83,8

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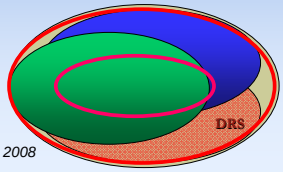
Combination - LIFS+DRS+colorimetry

Technique	SN %	SP %	PPV %	NPV %	IS %	DA %
LIAFS + DRS	100	93,3	90	100	111,1	90
DRS + SH∑	88,9	93,3	88,9	93,3	100	80
LIAFS + DRS + SH∑ (3/3)	55,6	93,3	83,3	77,8	66,7	50
LIAFS + DRS + SH∑ (2/3)	77,8	93,3	87,5	87,5	88,9	70
LIAFS + DRS + SH∑ (1/3)	100	93,3	90	100	111,1	90

LIAFS + DRS + SH∑ 1/3

LIAFS + DRS + SH∑ 3/3



Borisova et al., QE 38(6), p. 597-605, 2008

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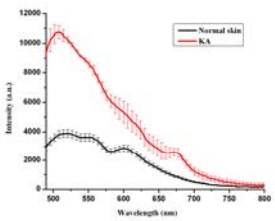
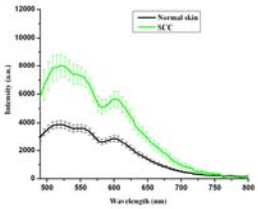
Tumor type evaluation – dysplastic/malignant

-fluorescence spectral shape differences



Dysplastic lesion- KA



Malignant lesion - SCC

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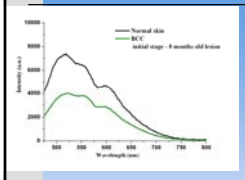
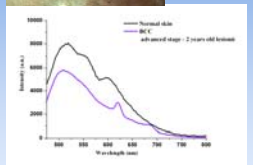
Tumor stage evaluation – initial and advanced



Initial BCC

Excitation at 405 nm



Advanced BCC

2-D picture of advanced BCC High-pass filter >590 nm applied

- 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.

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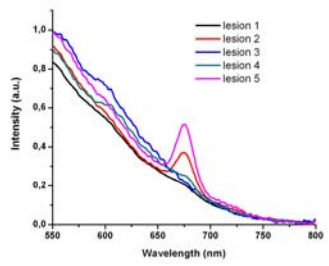
Multiple lesions – treatment planning



- 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

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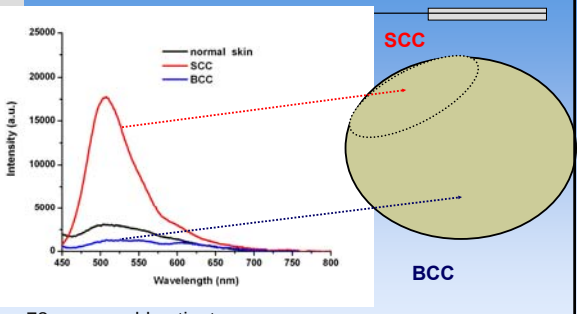
Mixed tumor – therapy failure



- 78 – years old patient
- tumor size 5 x 6 cm on the head
- failed Ro-therapy

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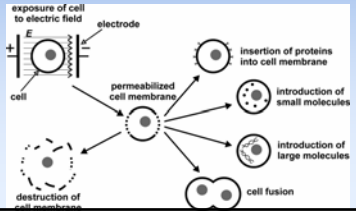
Monitoring of novel therapeutic procedure – electrochemotherapy of cutaneous tumors

- Electrochemotherapy combines chemotherapy and electroporation to increase locally cytostatic drug delivery in the cancer cells.
- The electroporation creates aqueous pathways in the cell membrane as a result of applied short intensive electric field usually generated by high voltage electrical pulses.



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Portable electroporator

It was developed with battery charging supplied with QRS synchronizer and equipment for recording of pulse parameters during treatment of surface tumors

- Chemipulse III coupled with the module for recording of pulse parameters during treatment of surface tumors (A) and (B) QRS synchronizer (pulses generation immediately after the QRS detection is the most reasonable approach)

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Procedure for monitoring and treatment

1) Initial clinical evaluation;
2) Fluorescence detection and evaluation of lesion' type before treatment – normal skin is used as a reference signal;
3) All patients were treated under local anesthesia (lidocain 1%) with anticancer drug - Bleomycin, (MW 1 416)
4) The electro-treatment is done by 8-16 biphasic rectangular pulses each of them 50+50 μs duration with 20 μs interval between both phases and pause between bipolar pulses of 880 μs
5) Fluorescence detection imediatelly after procedure of electrochemo-therapy
6) Fluorescence monitoring – 1, 4, 12 week and if needed – repeat the therapeutic procedure

A pair of parallel flat electrodes with the adjustable inter electrode distance (calliper type) in the range 5-30 mm are used. Good contact between electrodes and the skin is ensured by a conductive gel.

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Patients

Ca Basocellulare		Kaposi sarkoma		Ca Spinocellulare	
Patients number	Lesions number	Patients number	Lesions number	Patients number	Lesions number
32	41	4	5	1	1
Retreated					
3	3	-	-	-	-

Total 37 patients with 47 lesions of Ca basocellulare, Ca spinocellulare and Kaposi Sarcoma were treated with electrochemotherapy using intralesional application of bleomycin and new developed equipment *Chemipulse III.*

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Electrochemotherapy procedure

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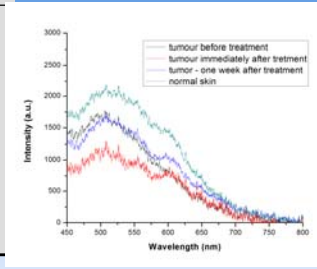
54 years old woman 1.5 ml bleomycin and 1000 V
A)before treatment,
B)one week after treatment,
C)one month after electrochemotherapy

82 years old woman 2 ml intralesional bleomycin and 1000 V
D) before treatment,
E) one week later,
F) one month follows up

57 years old man 1 ml bleomycin and 900 V
G) before treatment,
H) 24h after treatment-edema at the site of the treatment,
I) 12 months follows up

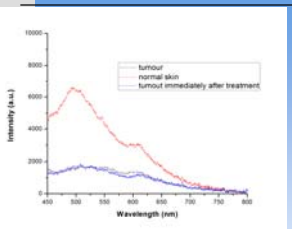
Results of fluorescence monitoring

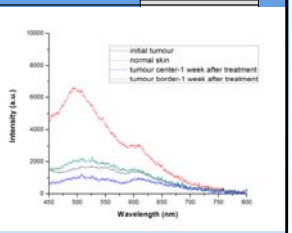


- Increased hemoglobin locally is observed immediately after electro-treatment
- Inflammatory reaction (healing effect) is clearly observed in the spectrum on 1 week
- Inflammatory reaction usually disappear after 2-3 weeks

It is clearly observed immediate reaction after therapeutic procedure application – appearance of specific minima at 543 and 575 nm, related to increased hemoglobin absorption. One week later the fluorescence intensity of the lesion area is higher and approach to the "normal skin" spectral shape, which is indication for successful treatment of the tumour.

Re-treatment needs – fluorescence monitoring decisions





- For this patient improvement in the tumor center was not observed and after 1 month – new procedure for electro-treatment was carried out
- Fluorescence diagnostic procedure allow not only initial diagnosis, but a monitoring of the novel therapies as well and planning of repeating of the therapeutic procedures

Comparison with "gold standard" – histology for all our cases – benign and malignant lesions

Clinical trial in National Oncological Center and University Hospital "Queen Giovanna"– Bulgaria is currently under implementation (2009-2012) and with broadening of the database with fluorescence spectra of major skin benign and malignant pathologies we expect to receive objective tool for detection and evaluation of skin tumors' types.

Thank you very much for your attention!

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