Fluorescence spectroscopy for skin cancer diagnosis - clinical trial

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Statistics of skin lesions

- Skin cancer is the second common cancer in Bulgaria—approximately 10% of new cancer cases.
- The “most malignant” skin cancer—malignant melanoma cases are about 10-12% from all skin cancer cites, and about 90% of skin cancer mortality rate, SCC—other 10% of MR.

Structure of cancer incidence in Bulgaria*

Female

Male

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.
Non-optical diagnostic modalities

- **Dermatoscopy** – combined *in vivo* microscopic investigation with optical clearing of the epidermis

- **Ultrasound** – evaluation of lesion thickness and structures of tumors and foreign bodies

- **NMR** – information about tissue metabolism – intracellular pH, biochemical changes in cutaneous layers, hydrogenation, skin aging

- **Doppler diagnostics** – monitoring of vascular changes during pathology development, UV-radiation, vaso-active drugs and cosmetic products
Skin benign and malignant lesions - confusions

Comparison of Surface microscopy diagnoses before and after the training course

<table>
<thead>
<tr>
<th>Diagnostic Indicator</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>SENS (%)</td>
<td>65.00</td>
<td>71.56</td>
</tr>
<tr>
<td>SPEC (%)</td>
<td>80.93</td>
<td>79.69</td>
</tr>
<tr>
<td>DA (%)</td>
<td>54.59</td>
<td>59.48</td>
</tr>
</tbody>
</table>

Dermatoscopic pictures of different skin lesions, magnification x100

Comparison of Epiluminescence microscopy diagnoses before and after the training course

<table>
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<tr>
<th>Diagnostic Indicator</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>SENS (%)</td>
<td>75.31</td>
<td>89.69</td>
</tr>
<tr>
<td>SPEC (%)</td>
<td>83.44</td>
<td>83.12</td>
</tr>
<tr>
<td>DA (%)</td>
<td>62.92</td>
<td>77.74</td>
</tr>
</tbody>
</table>

Light-skin interactions
Skin absorbers and fluorophores

Absorption spectral regions of main tissue chromophores

Main cutaneous fluorophores emission spectral regions

Fluorescence spectroscopy of human skin for cancer diagnostic applications - references

Differences - intensities
- Comparison normal/abnormal skin – tumors usually are less fluorescent
- Comparison between tumor types - BCC fluorescence signal is lower, SCC – higher vs. normal skin

Differences - spectral shape
- Higher/lower level of NADH fluorescence – metabolic activity of tumor cells
- Neovascularization – re-absorption from blood hemoglobin
- Pigmentation – reduction and distortion of fluorescence signal
- Appearance/decrease of specific maxima in the fluorescence signals – untargeted changes in the most of the cases observed

Differences – benign lesions
- Broad survey is almost impossible due to variety of benign and malignant forms
- Specific comparisons are made – spectral shape and intensity changes are observed - benign vs. malignant, benign/malignant vs. normal skin

SENS >90 %, SPEC – from 60-90 %
Materials and methods

Principal set-up used for initial clinical investigations:

LEDs with emission maxima at UV-VIS region are used as excitation sources, 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 - May 2012 – 464

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.
Normal skin AF – influence of different excitation $\lambda$

- appearance of new emission maxima
- changes in fluorescence intensity, depending on absorption for given excitation wavelength of exact fluorophore
Comparison of fluorescence spectra from different anatomic areas, using exc. at 365 nm

phototype I

phototype III
**BCC - results**

- Exc. at 365 nm
  - Intensity vs. Wavelength
    - Normal skin
    - BCC
    - Exc. at 385 nm

- Exc. at 365 nm
  - Intensity vs. Wavelength

- Exc. at 405 nm
  - Intensity vs. Wavelength
SCC - results

Exc. at 385 nm

Exc. at 365 nm

Exc. at 405 nm
KA - results

Exc. at 385 nm

Exc. at 365 nm

Exc. at 405 nm
Comparison of malignant lesions’ spectra

Fluorescence spectra of the most often lesions observed, compared for two different excitation sources.
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.
Optical biopsy – to be or not to be....
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

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

- Porphyrin-like signal from advanced lesions
**Tumor type evaluation – metastatic/ non-metastatic**

-differences in the fluorescent signal intensity vs. normal skin

![Graphs comparing fluorescent signal intensity of normal skin, non-metastatic BCC, and metastatic SCC](image)

**Non-metastatic BCC**

**Metastatic SCC**
Mixed tumor – therapy failure

- 78 – years old patient
- tumor size 5 x 6 cm on the head
- failed Ro-therapy
Tumor type evaluation – dysplastic/malignant

- fluorescence spectral shape differences

Dysplastic lesion - KA

Malignant lesion - SCC
### Skin pathologies differentiation

<table>
<thead>
<tr>
<th>No</th>
<th>COMPARISON</th>
<th>Diagnostic accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sound skin / Compound nevus</td>
<td>91.1 %</td>
</tr>
<tr>
<td>2</td>
<td>Compound nevus / Displastic nevus</td>
<td>69.6 %</td>
</tr>
<tr>
<td>3</td>
<td>Displastic nevus / Malignant melanoma</td>
<td>74.1 %</td>
</tr>
<tr>
<td>4</td>
<td>Sound skin / Base cellular pапyloma</td>
<td>87.5 %</td>
</tr>
<tr>
<td>5</td>
<td>Sound skin / Base cellular carcinoma</td>
<td>82.2 %</td>
</tr>
<tr>
<td>6</td>
<td>Base cellular pапyloma / Base cellular carcinoma</td>
<td>92.6 %</td>
</tr>
</tbody>
</table>

\[
DA = \frac{TP}{TP + FP + FN} \times 100\% 
\]
ALA-sensitized photo diagnosis
The image presents a slide discussing ALA-sensitized PDT (Photodynamic Therapy). The slide features two images: one of a person's hand under a light, likely undergoing treatment, and another of a dry skin condition. Additionally, there is a before-and-after comparison, showing a before and after photos of a basal cell carcinoma (BCC) lesion.

The text reads:

“MediRay PDT” – implemented in MC of integrative medicine

The images and text together illustrate the application of Photodynamic Therapy and its potential outcomes in the treatment of skin conditions like BCC.
Comparison with “gold standard” – histology for all cases – benign and malignant lesions

Clinical trial in National Oncological Medical Center – 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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