Fluorescence spectroscopy and microscopy of cutaneous tumours – correlation between micro- and macro- spectral measurements

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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


** [http://us.moor.co.uk/](http://us.moor.co.uk/)
Skin cancer 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 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???

Dermatoscopic pictures of different skin lesions, magnification x100

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>
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<tbody>
<tr>
<td>SENS (%)</td>
<td>65.00</td>
<td>71.56</td>
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<tr>
<td>SPEC (%)</td>
<td>80.93</td>
<td>79.69</td>
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<tr>
<td>DA (%)</td>
<td>54.59</td>
<td>59.48</td>
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Comparison of Epiluminescence 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>75.31</td>
<td>89.69</td>
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<tr>
<td>SPEC (%)</td>
<td>83.44</td>
<td>83.12</td>
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<tr>
<td>DA (%)</td>
<td>62.92</td>
<td>77.74</td>
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</tbody>
</table>

Fluorescence detection - chromophores in skin

Excited State Levels

Absorbed excitation light

Emission level

Fluorescence lifetime (ns)

Ground State

Energy

Absorbed fluorescent light

Scattering

Reflectance

Incident radiation

Remittance

Dermal Eidermal Direct

Epidermal absorbance

Eidermis 100-150 μm

Dermis 1.4 mm

Hypodermis

Fluorophores

Absorbers

In vivo

Absorption spectra for different wavelengths:
- exc. at 337 nm
- exc. at 365 nm
- exc. at 385 nm
- exc. at 405 nm

Wavelength (nm)

Intensity (a.u.)
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-March 2015 – 672

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, hemangioma, angioma, compound nevus, atypical nevus, verruca seborea, actinic keratosis, blue nevus, Sutton nevus, etc.
Skin EEM data – in vivo

Normal skin phototype II
autofluorescence – excitation - emission matrix

Origins of the AF signal
Confocal fluorescent microscopy

- Samples – hemoxylin – stained histology samples from variety of cutaneous benign, dysplastic and malignant tissues
- Microscopy system: Leica TCS SP confocal scanning microscope
- Excitation wavelength applied – 405 nm; images detected in the regions 415-500 nm, 500-600 nm, and 600-700 nm.
Results – Basal cell carcinoma

- Fluorescence intensity is lower for the lesion vs. surrounding normal tissue
- Excitation at 405 nm
BCC confocal fluorescence microscopy for three spectral regions – 415-500 nm (a), 500-600 nm (b) and 600-700 nm (c). Excitation applied is 405 nm.

Most pronounced fluorescence signal is observed for keratin and protein cross-links in the green spectral region. Low level fluorescence of endogenous porphyrins also is observed in the spectral region 630-700 nm.
Results – Squamous cell carcinoma

Fluorescence intensity is higher for the lesion vs. surrounding normal tissue.

Excitation at 405 nm.
Spectral comparison BCC vs. SCC

![Spectral comparison graph showing intensity vs. wavelength for normal skin, SCC, and BCC.](image-url)
Results – Malignant melanoma

- Not well-defined borders of the lesion
- Lower fluorescence intensity
- Tissue structure demolition
- Going deeper in dermal layer
Results – dysplastic nevus

- Well defined borders of the lesion
- Lower fluorescence intensity
- Situated on epidermal-dermal junction
Malignant Melanoma

Normal skin

Malignant Melanoma
Spectral comparison of melanoma vs. nevi

Significant fluorescence intensity decrease, correlated with the type of pigment lesion, was observed for all lesions. The fluorescence intensities of the maximum of the two kinds of nevi investigated are very close one to another.

The malignant melanoma fluorescence intensity is much lower than that of normal skin and nevi and could be separated from pigmented nevi spots. Using only intensity criteria for differentiation we obtain sensitivity higher than 80% for discrimination between nevi and MM lesions.
Comparison of malignant lesions’ AF spectra

Fluorescence spectra of the common 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
MM lesions have low fluorescence but no significant spectral shape changes vs. dysplastic nevi – low specificity of diagnostics based only on fluorescence data

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.
Thank you very much for your attention!

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