OPTICAL SPECTROSCOPY OF HUMAN SKIN - TOOL FOR CANCER DETECTION IN VIVO

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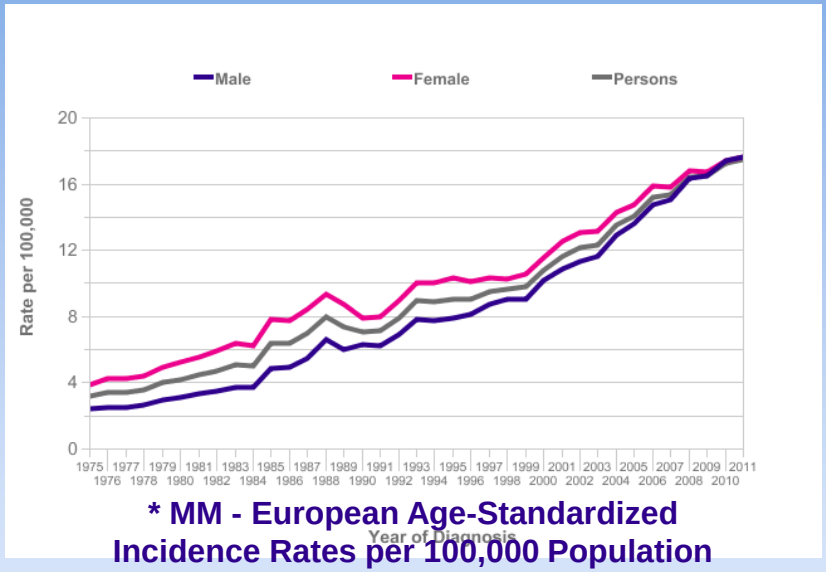
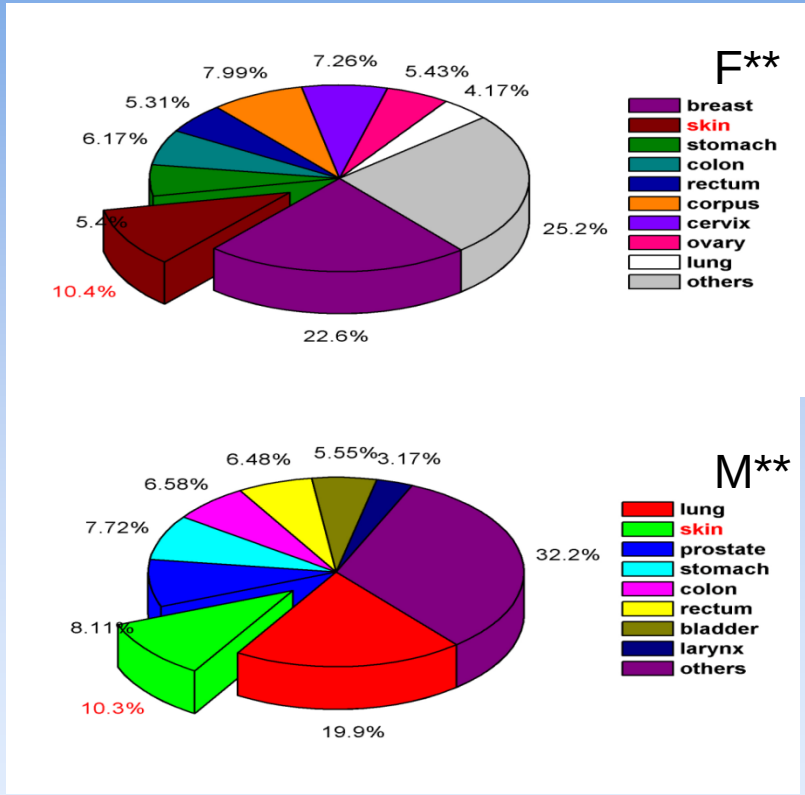
Statistics of skin cancer

- Skin cancer is the **second most common cancer** - approximately 10 % of new cases. MMs are about 10-12 % from all cites, and about 90 % of skin cancer mortality rate.
- According WHO - currently, between 2,5 and 3 million non-melanoma skin cancers and 130,000 melanoma skin cancers occur globally each year. One in every three cancers diagnosed is a skin cancer, according to Skin Cancer Foundation Statistics.



*** MM - European Age-Standardized Incidence Rates per 100,000 Population**



Individual risk factors for skin cancer

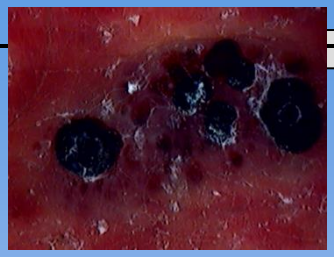
- fair skin
- blue, green or hazel eyes
- light-coloured hair
- tendency to burn rather than suntan
- history of severe sunburns
- many moles or/and freckles
- a family history of skin cancer

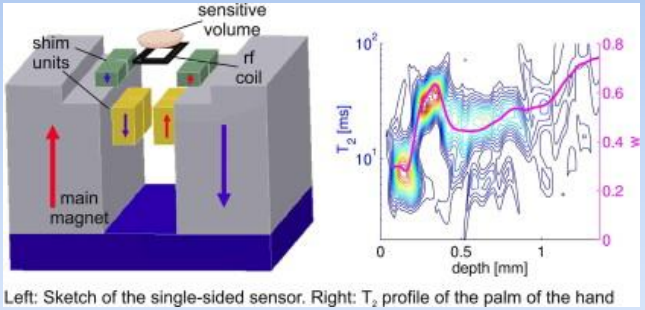
** Cancer Research UK -* http://info.cancerresearchuk.org/cancerstats/faqs/#How

*** ed. Sh.Danon, Zdr.Valerianova, Tzv.Ivanova, Cancer incidence in Bulgaria, Vol. XVI, 2013, National Oncological Centre, Bulgarian National Cancer Registry.*

Used 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





Left: Sketch of the single-sided sensor. Right: T_2 profile of the palm of the hand

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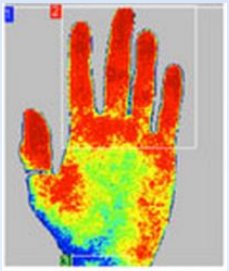

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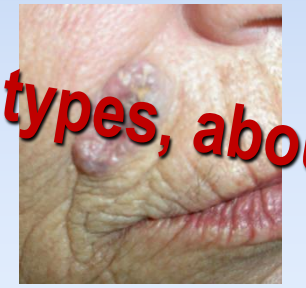
* *M. Landeghem et al., J MR, 215:74, 2012*

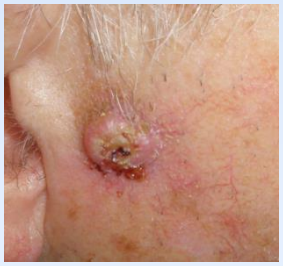
** http://us.moor.co.uk/

Skin cancer types

- **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.

3 major types, about 10 rare types, more than 20 sub-types





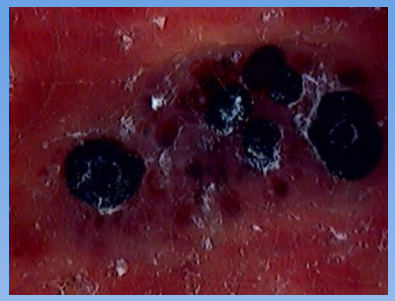
+ dysplasia

+ benign

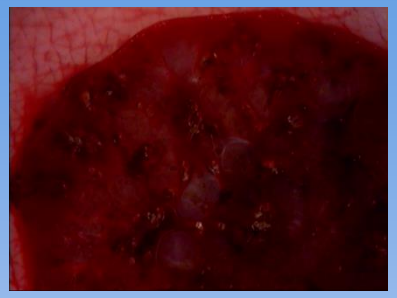
Skin tumours diagnosis – human factor

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



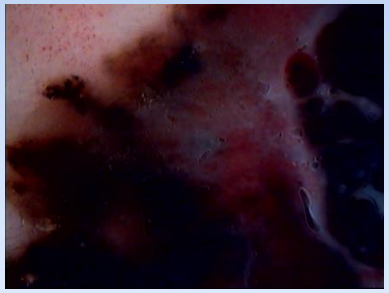
Heamangioma



Base-cell papilloma

Dermatoscopic pictures of different skin lesions, magnification x100

Pigmented BCC



Malignant melanoma

Comparison of Epiluminescence microscopy diagnoses before and after the training course

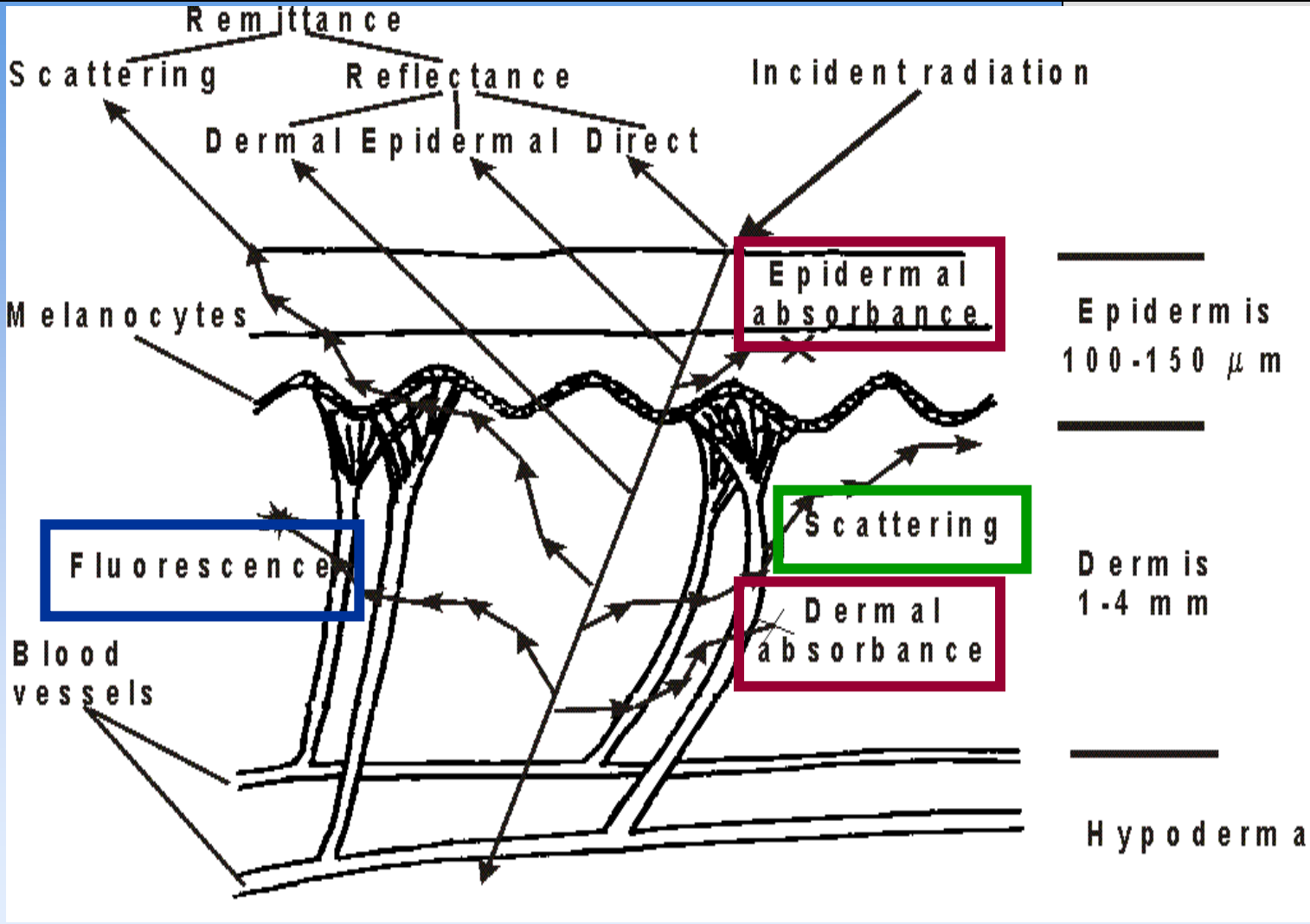
Diagnostic Indicator	Before	After
SENS (%)	75.31	89.69
SPEC (%)	83.44	83.12
DA (%)	62.92	77.74

Troyanova P., A beneficial effect of a short-term formal training course in epiluminescence microscopy on the diagnostic performance of dermatologists about cutaneous malignant melanoma, Skin Res Tech 2003; 9: 269-273.

Optical spectroscopy of human skin

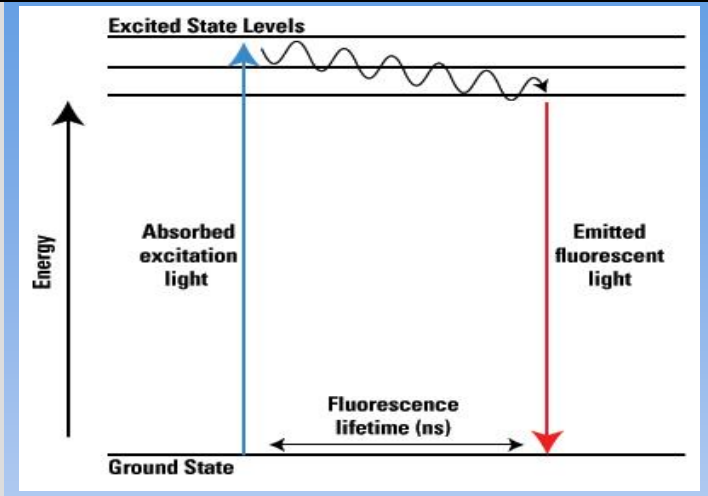
- Objective
- Real-time
- Non-invasive
- Highly sensitive
- Repeatable results
- Biochemical and morphological correlations
- Multiple applications
- Monitoring of therapeutic procedures
- High diagnostic accuracy
- Exclude human factor

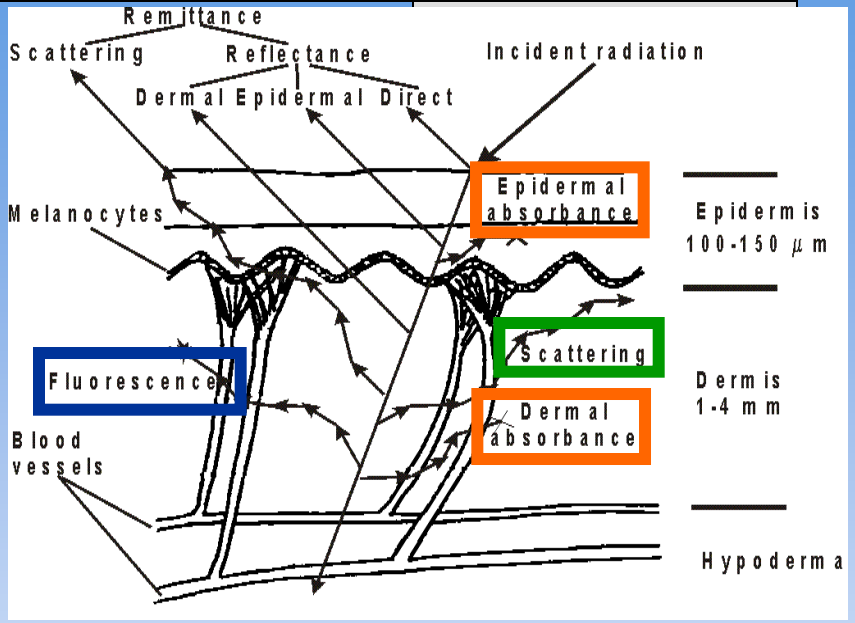
Light-skin interactions



Endogenous fluorophore	Excitation (nm)	Emission (nm)
Amino acids		
Phenylalanine	260	280
Tyrosine	275	300
Tryptophan	280	350
Structural proteins		
Collagen	280, 320-350, 390	370 - 440
Elastin	290 - 325	340, 400
Collagen cross-links	380 - 420	440 - 500
Elastin cross-links	320 - 360, 400	480 - 520
Keratin	380 - 400, 450 - 470	500 - 550
Enzymes and co-enzymes		
NADH	290, 350 - 370	440, 460
NADPH	340	460
FAD, Flavins	420 - 450	520 - 550
FMN	420 - 500	520 - 570
Vitamins		
Vitamin A	327	510
Vitamin K	335	480
Vitamin D	290, 350 - 390	400 - 480
Vitamin B6 compounds	315, 330, 340	385, 400, 425
Vitamin B12	275	305
Lipids		
Phospholipids	430	500, 540
Lipofuscin	340 - 395	430 - 460, 540
Ceroid	340 - 395	430 - 460, 540
Porphyrins	390 - 450, 630	600 - 710

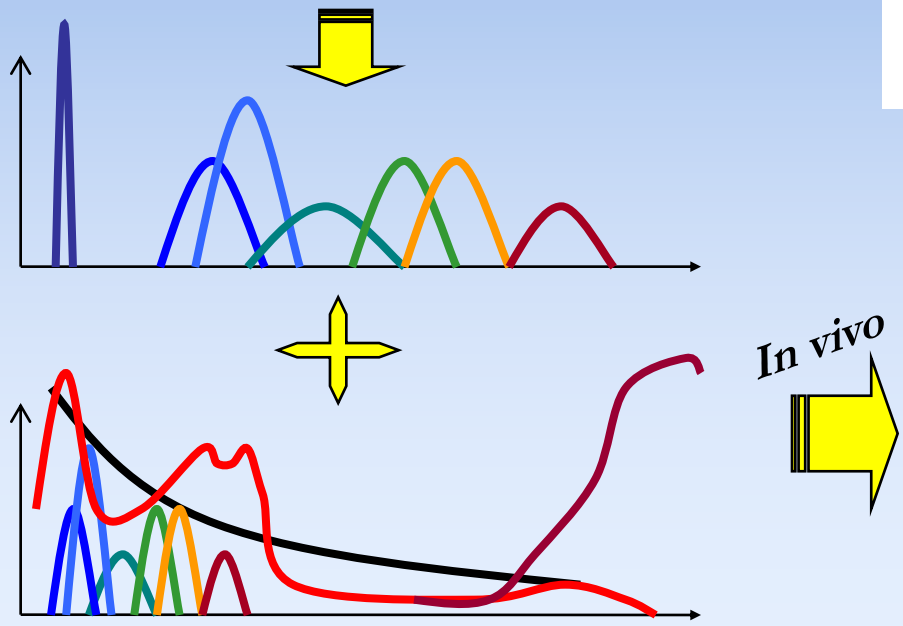
Chromophores in the skin tissues



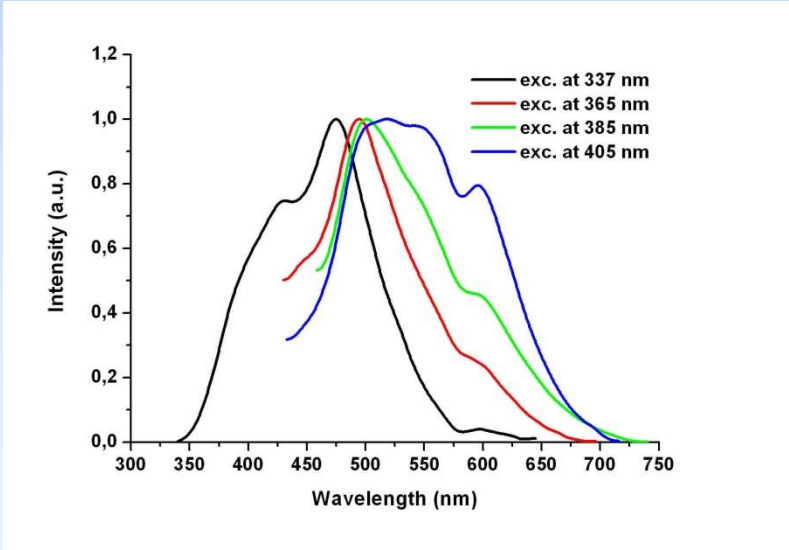


Fluorophores

Absorbers



In vivo



Requirements for in vivo measurements in clinics

- Noninvasive (intact lesion)
- Reliable
- User-friendly equipment
- Fast measurement ~ 1-2 min
  (EEM needs ~18-20 min.)
- Allows to reach high sensitivity
  (presence of lesion)
- Allows to reach high specificity
  (type and stage of lesion)
- Repeatable results
  (for therapy monitoring goals)
- Sensible level of the optical signal – need to combine optical techniques due to highly pigmented cutaneous lesions (**MM**, pigm BCC, dysplastic nevi, etc.)

Fluorescence

(+) Biochemical information
(+) High sensitivity
(+) Repeatable
(+) Noninvasive
(-) Moderate specificity
(-) Require sensitive detectors
(-) Signal complex analysis

Reflectance

(+) Morphological information
(+) Repeatable
(+) Noninvasive
(+) Not require sens. detectors
(+) Pigmentation sensitive
(-) Moderate sensitivity
(-) Moderate sensitivity

Procedure for excised samples studies

FluoroLog 3 system (HORIBA Jobin Yvon, France) It consists of Xe lamp (200-650 nm), scanning double monochromators, and PMT detector with high performance in the region 200-800 nm.

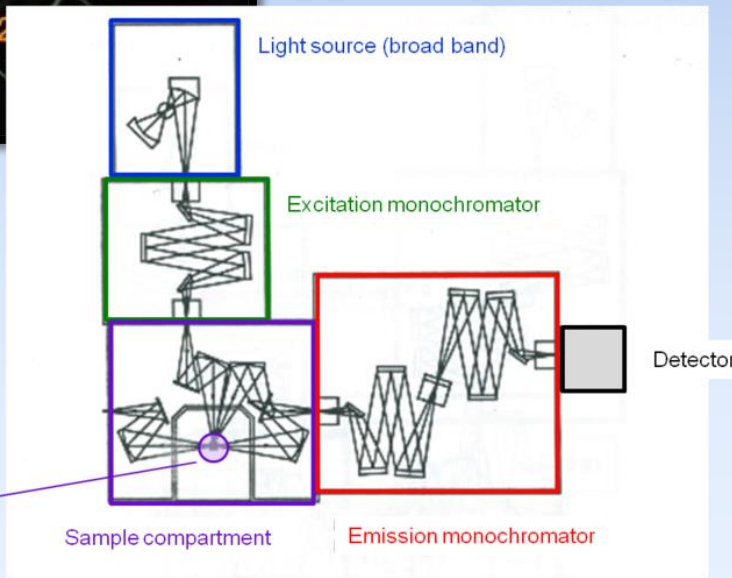
Ethical committee approval #286/24.07.2012

1. Initial clinical observation and diagnosis;
2. Histological/cytological analysis;
3. Clinical investigations (blood, scanner, etc.) – decision for surgical treatment
4. Excision of tumours – sampling for histology and for fluorescent spectroscopy detection
5. Transport from the hospital to spectral lab in isothermal conditions and safe-keeping solution
6. Fluorescence EEM measurements– up to 2 hours after surgical removal
7. Comparison of the spectral data b/n samples and histological diagnosis

Compound	Concentration [mg/500ml]
NaCl	3680
KCl	210
Glucose	1800
Taurine	1250
Hepes	1190
Piruvic acid	275

In volume 20ml physiological solution are added 100µl $CaCl_2 2H_2O$ at concentration 0,1mM. pH is fixed on 7,4.

Methods and materials



FluoroLog 3 (HORIBA Jobin Yvon, France) and F-3000 fiber-optic module
SFS regime
exciation – 280-440 nm,
Δλ = λem – λexc, 0 -200 nm, step 10 nm
emission – 300-800 nm, step 1 nm

Reference data – endogenous fluorophores

Endogenous fluorophores	Excitation (nm)	Emission (nm)
Amino acids		
Phenylalanine	260	280
Tyrosine	275	300
Tryptophan	280	350
Structural proteins		
Collagen	280, 320-350, 390	370 – 440
Elastin	290 - 325	340, 400
Collagen cross-links	380 - 420	440 - 500
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Flavins	420 - 450	520 - 550
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Vitamin B6 compounds	315, 330, 340	385, 400, 425
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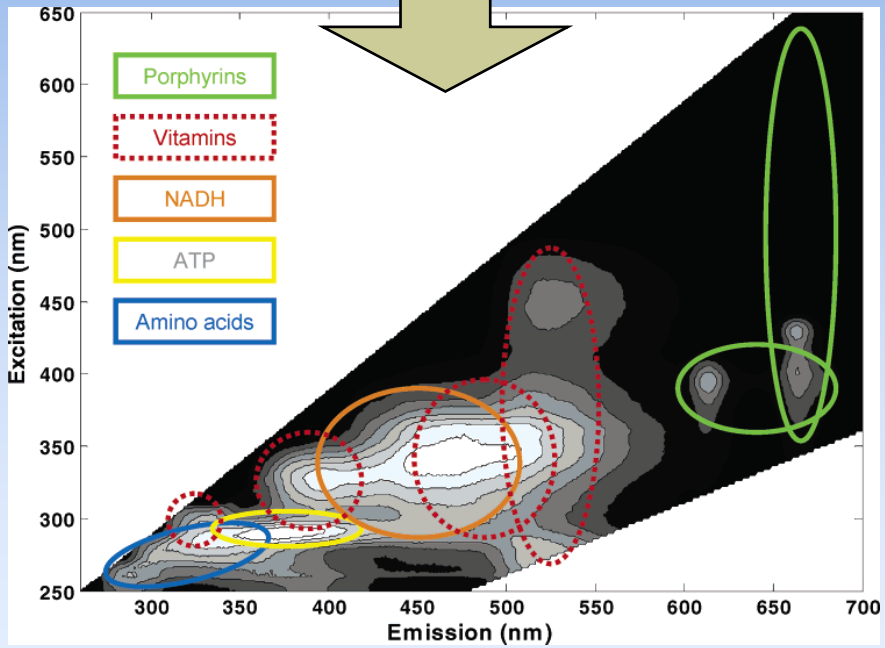
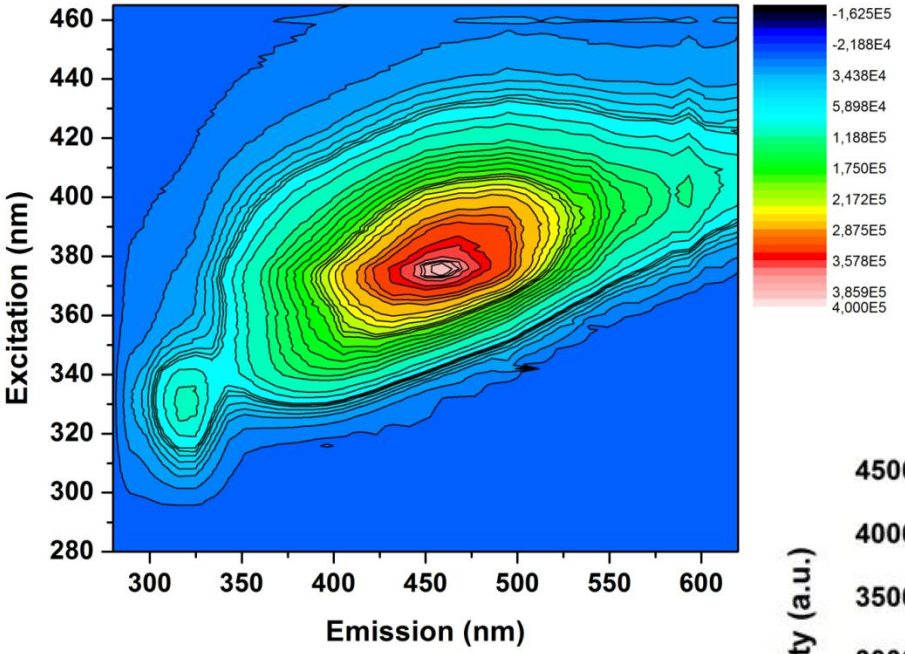
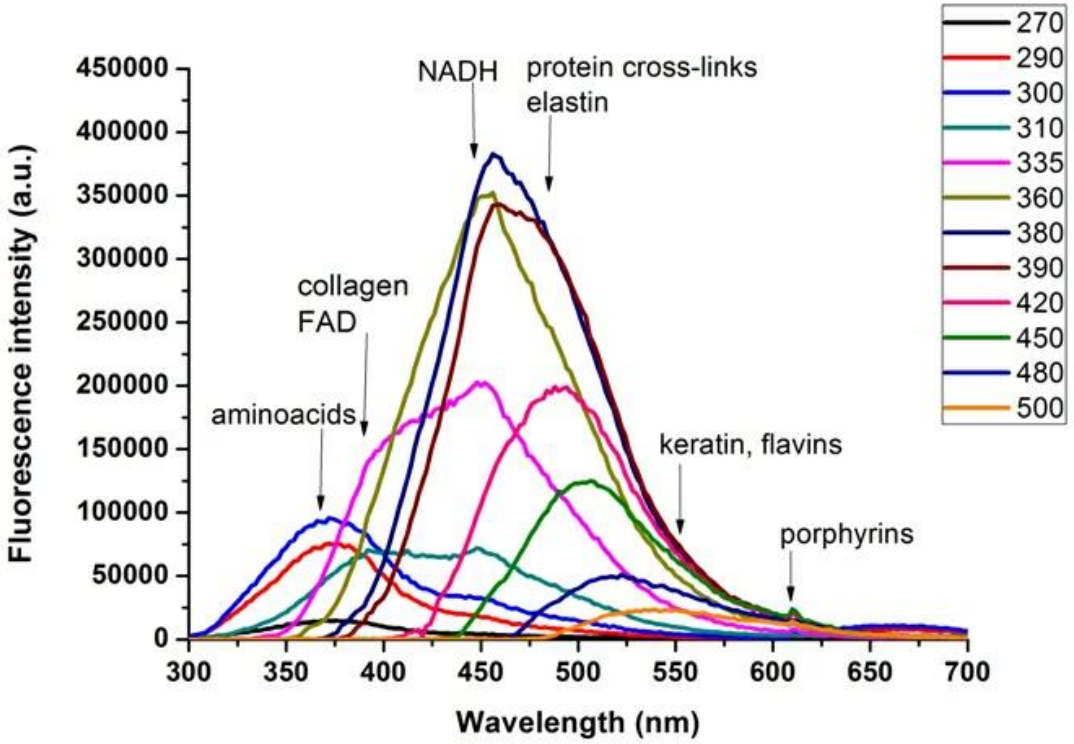


Skin EEM data – in vivo



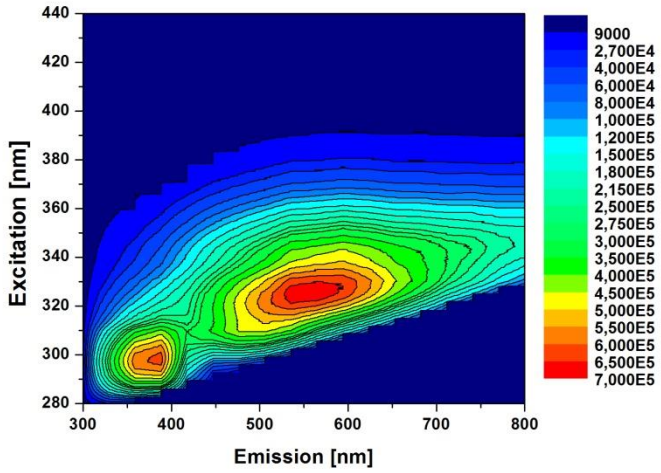
Normal skin phototype II autofluorescence – excitation - emission matrix

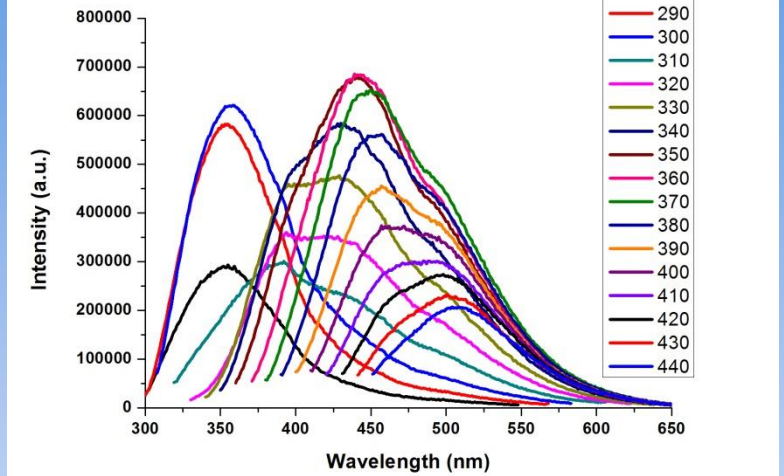
Origins of the AF signal

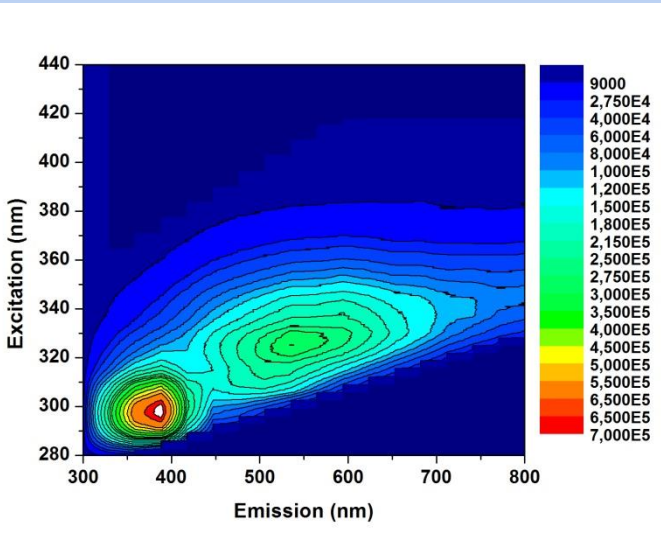


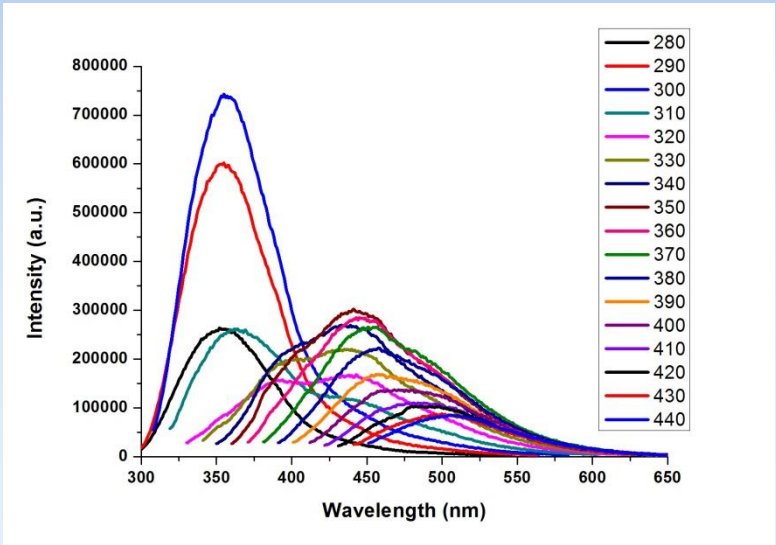
Results - EEM

EEM and steady-state autofluorescence spectra of normal tissue



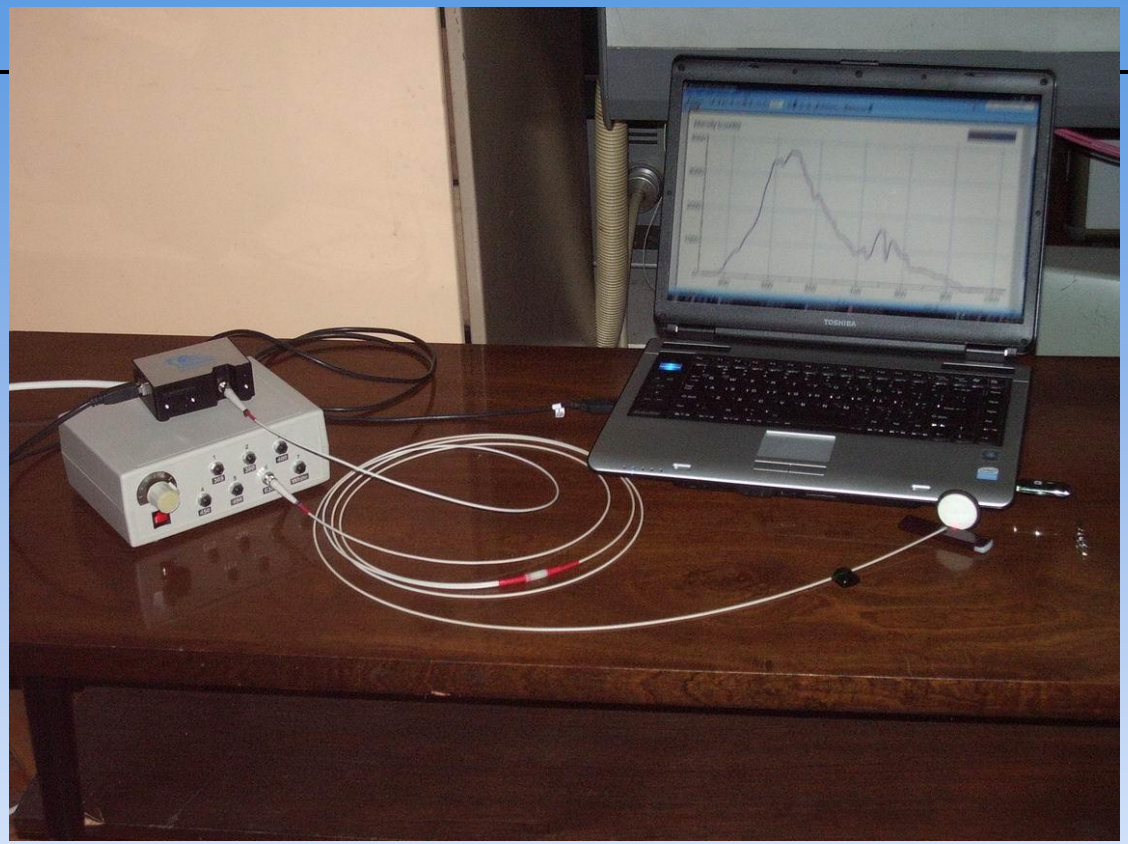






EEM and steady-state autofluorescence spectra of cancerous tissue - BCC

Materials and methods – in clinics



Set-up used for the initial clinical investigations

-Halogen lamp (400-900 nm) for diffuse reflectance spectroscopy

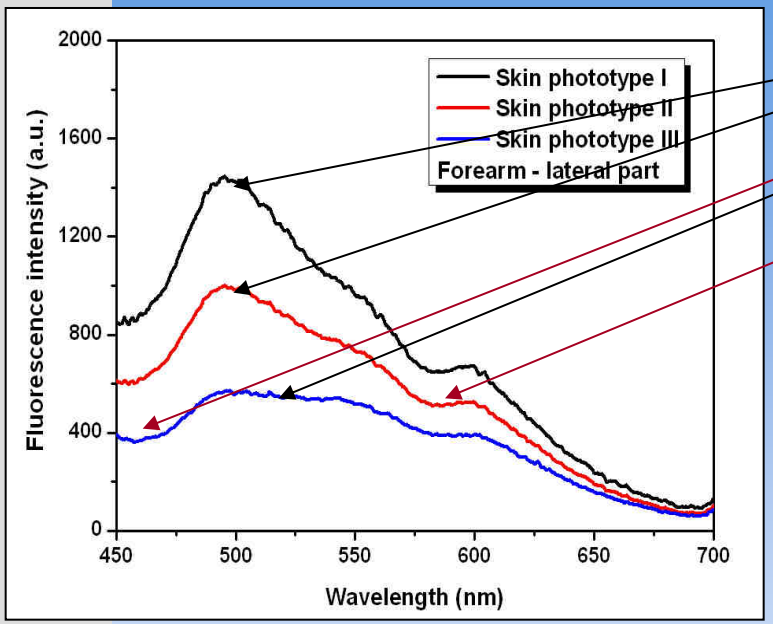
-LEDs - emission max at 365, 385, and 405 nm, 10-15 nm FWHM, 3-5 mW optical power – as excitation sources for autofluorescence spectroscopy

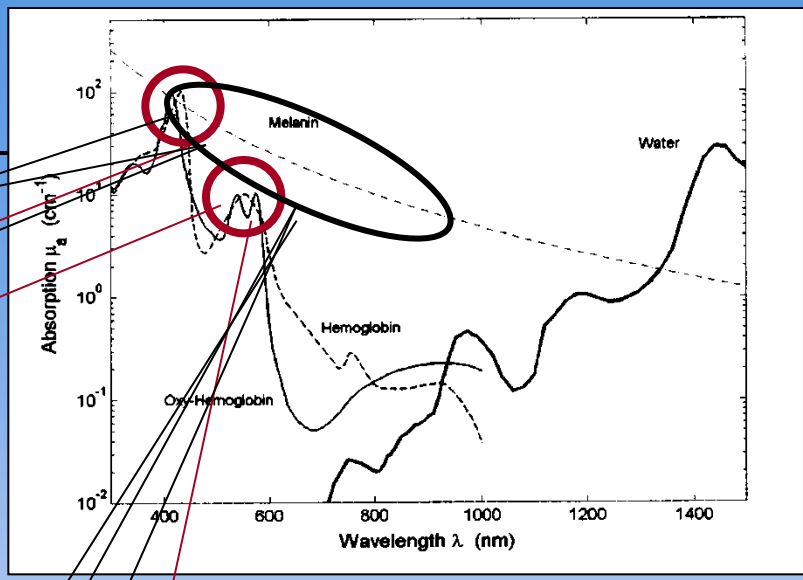
Y-fiber bundle with 6 fibers for excitation light and 1- central fiber – as optical fiber probe

USB4000 microspectrometer (USB4000, OceanOptics Inc.) 350-1000 nm detection range

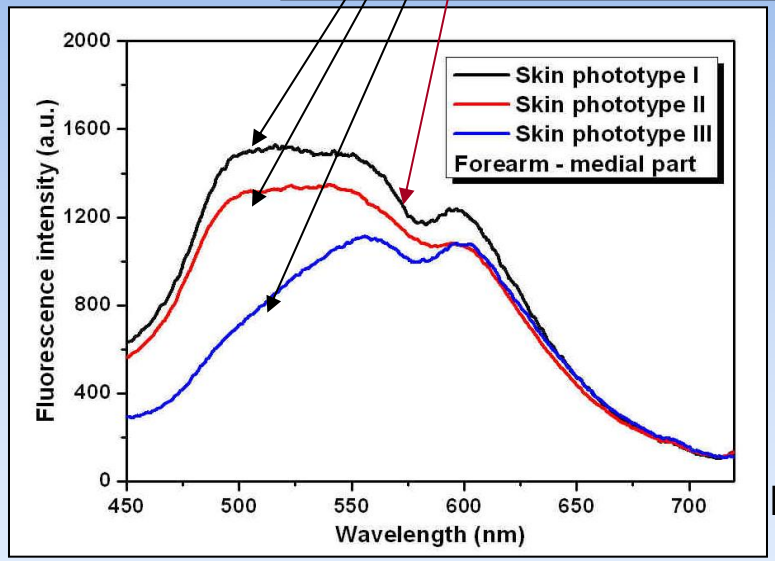
PC - storage and visualization of the spectral data

Influences

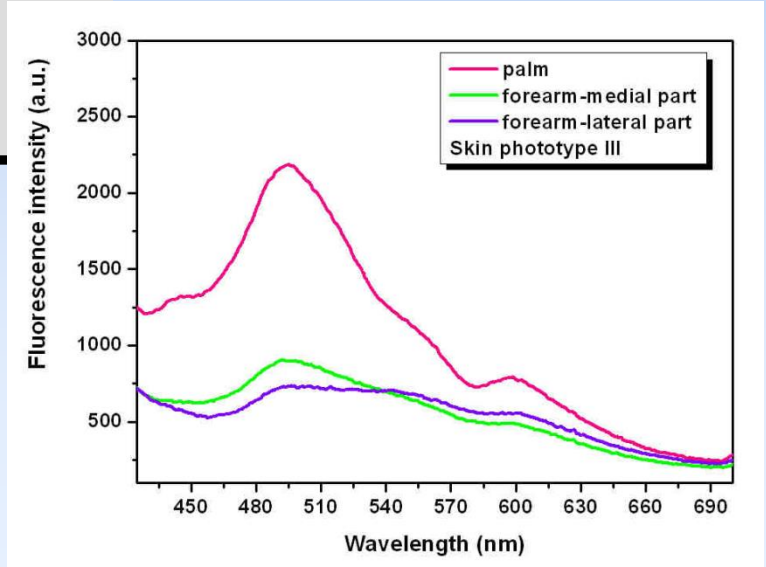




Exc. at 365 nm

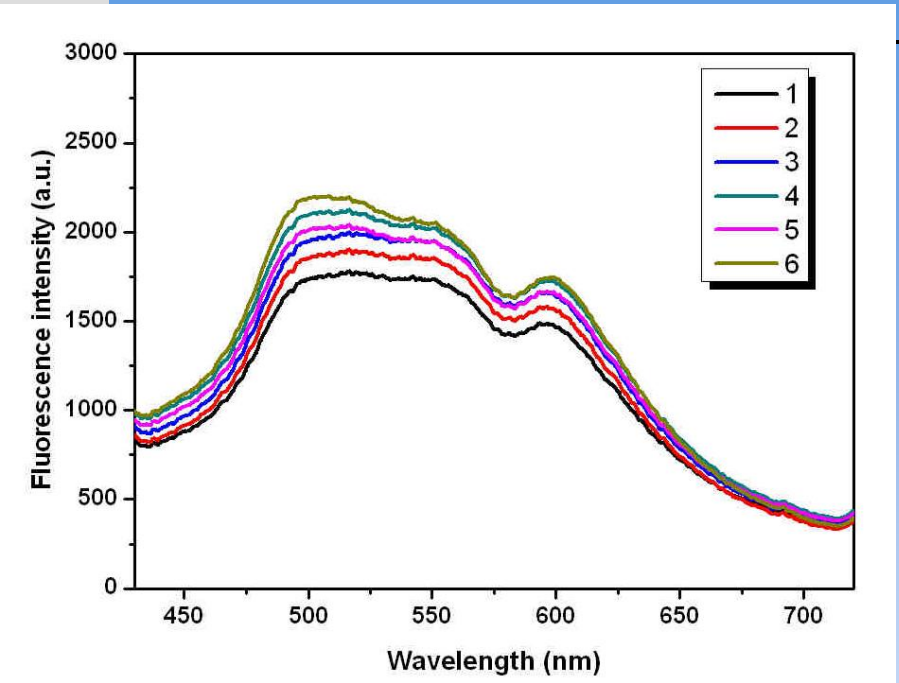


Exc. at 385 nm

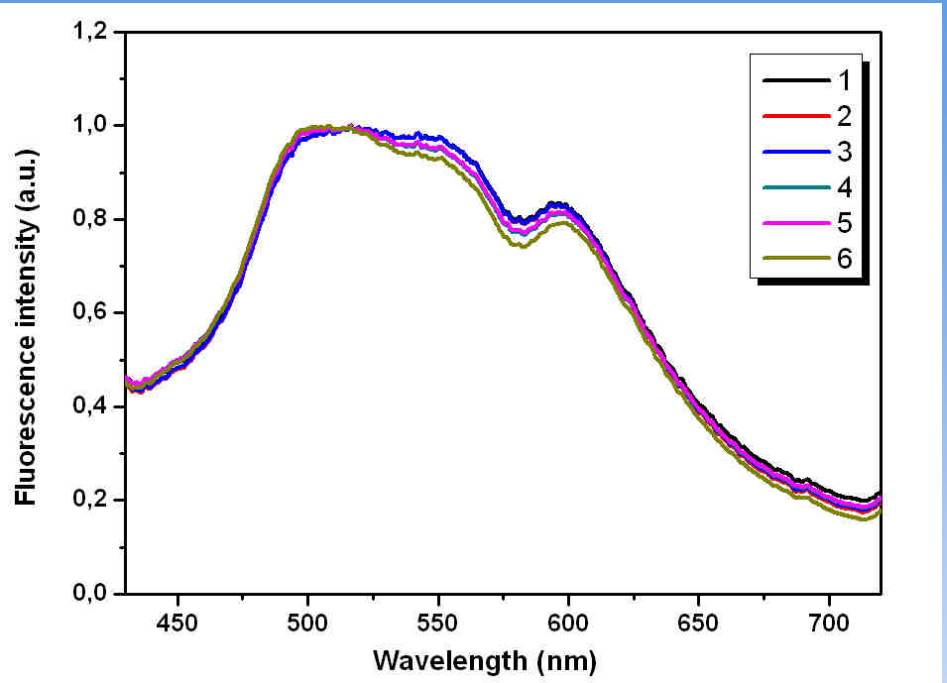


- anatomic place
- skin phototype
- excitation wavelength

Results - repeatability



unnormalized with respect to max

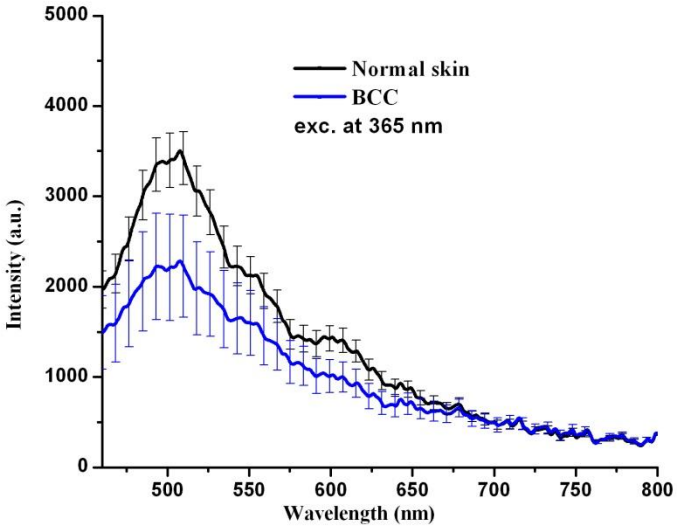


normalized with respect to max

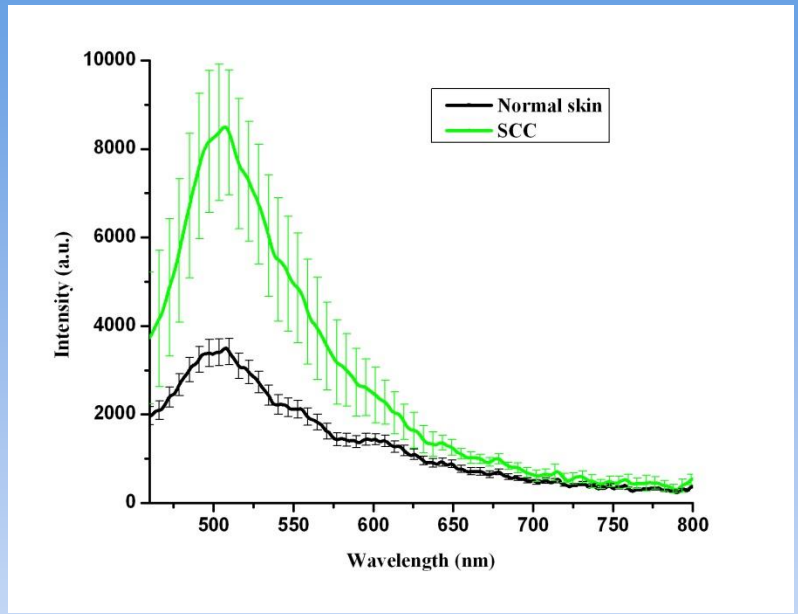
Comparison of the fluorescence spectra obtained from lateral part of the forearm of six volunteers with phototype I, excitation used at 385 nm

-spectral shape is similar for all cases
-slight differences in max intensity, related to pigmentation and blood content deviations

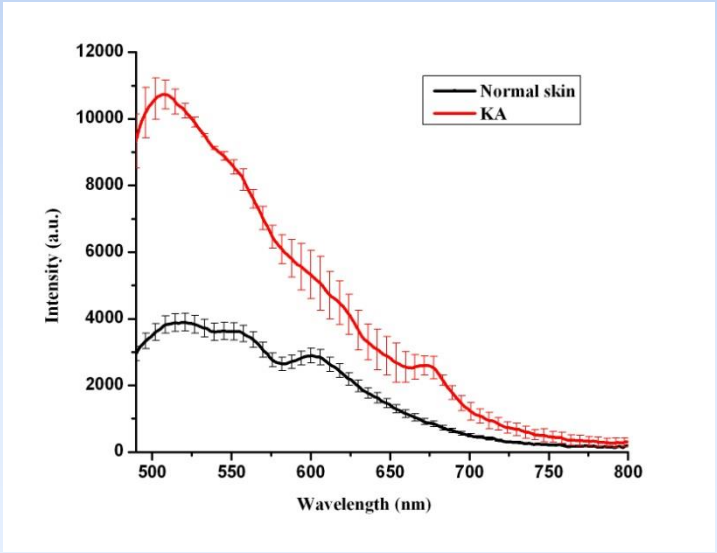
Non-melanoma tumors - results



BCC - Exc. at 365 nm

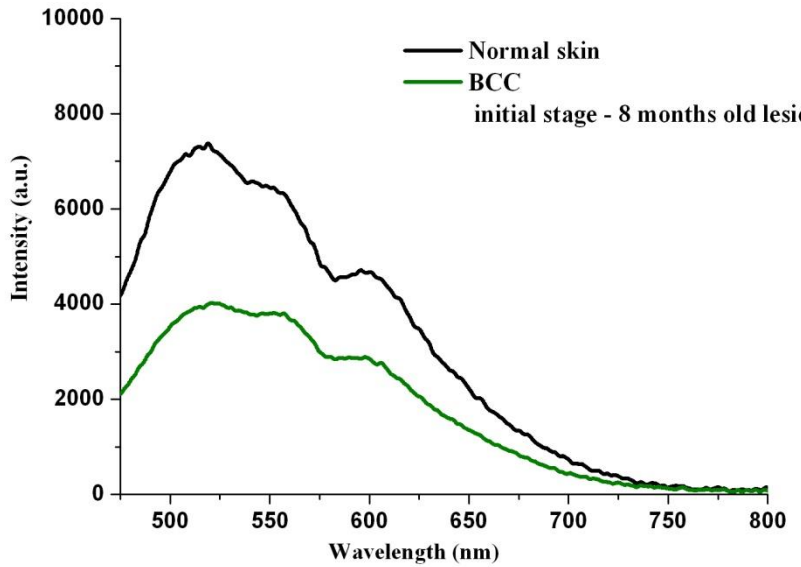


SCC - Exc. at 365 nm



KA – Exc. at 405 nm

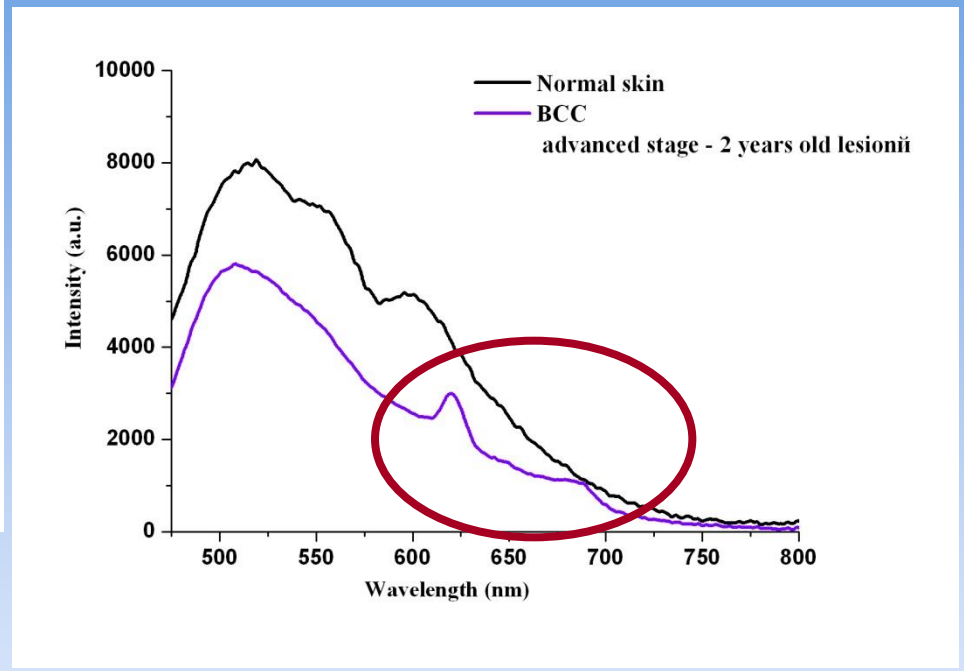
Tumor stage evaluation – initial vs. advanced



Initial BCC

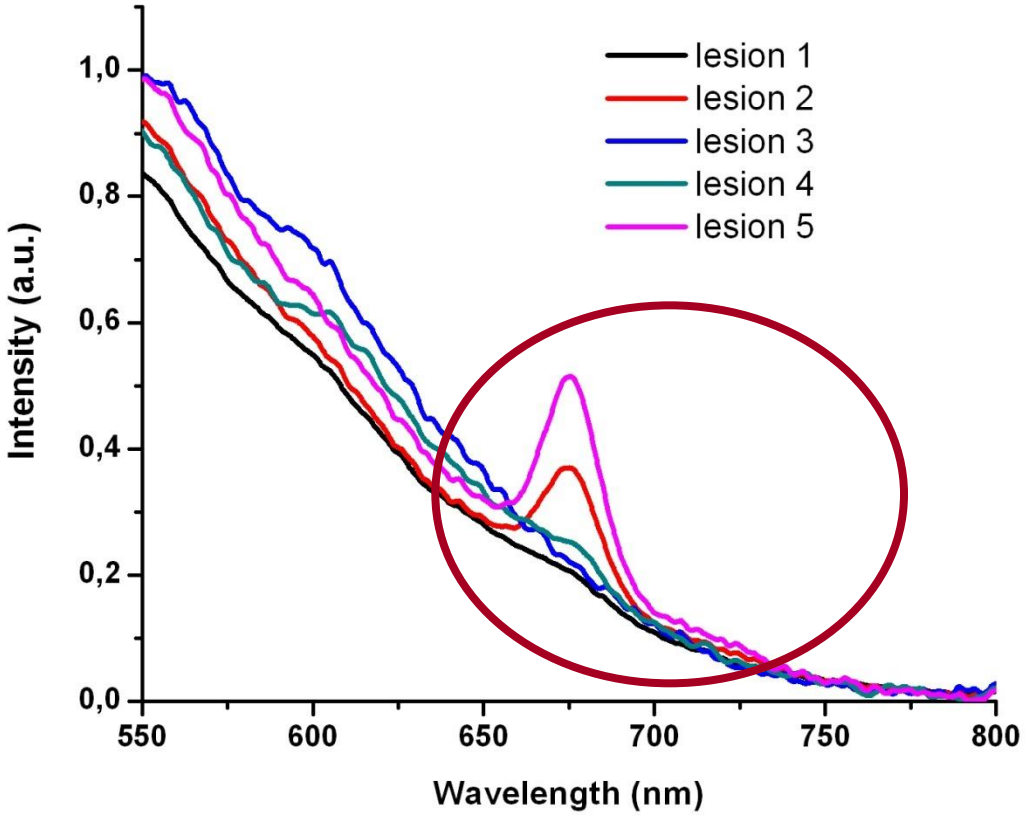
Excitation at 405 nm

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

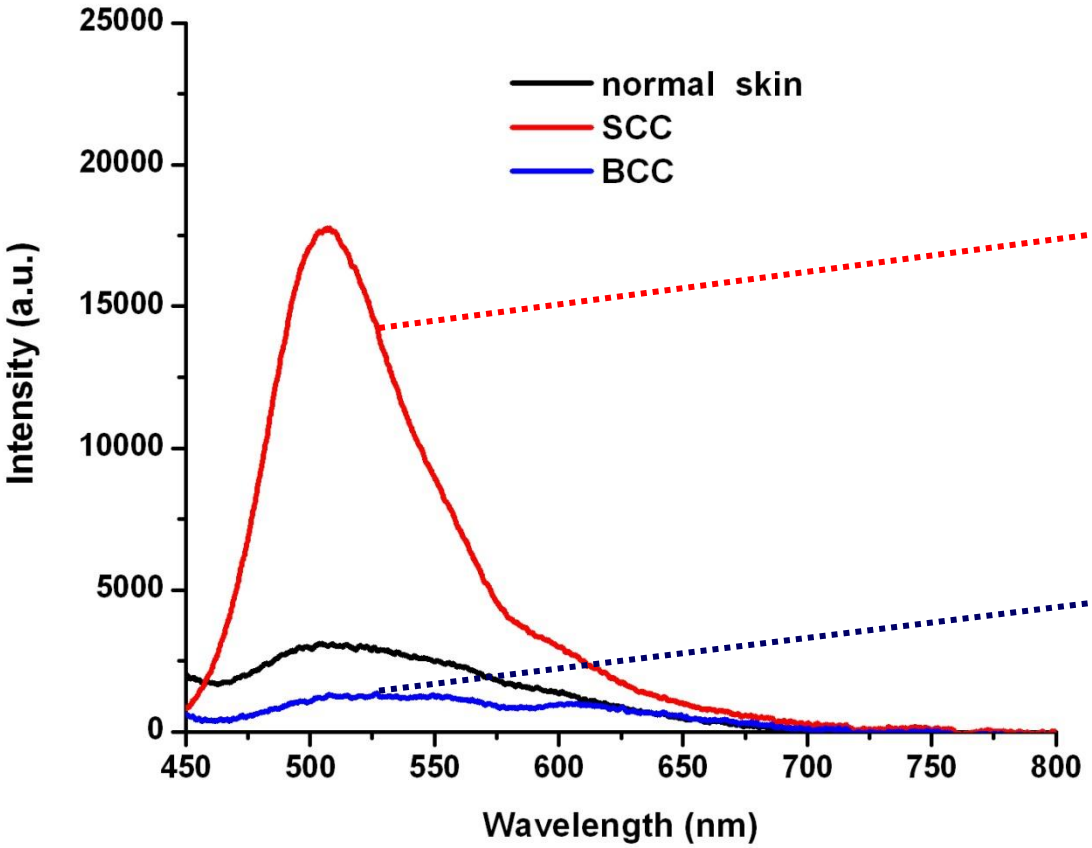
One patient, 69 years-old - 5 BCC lesions on different stage of growth, similar size (1x1cm area) and clinical picture

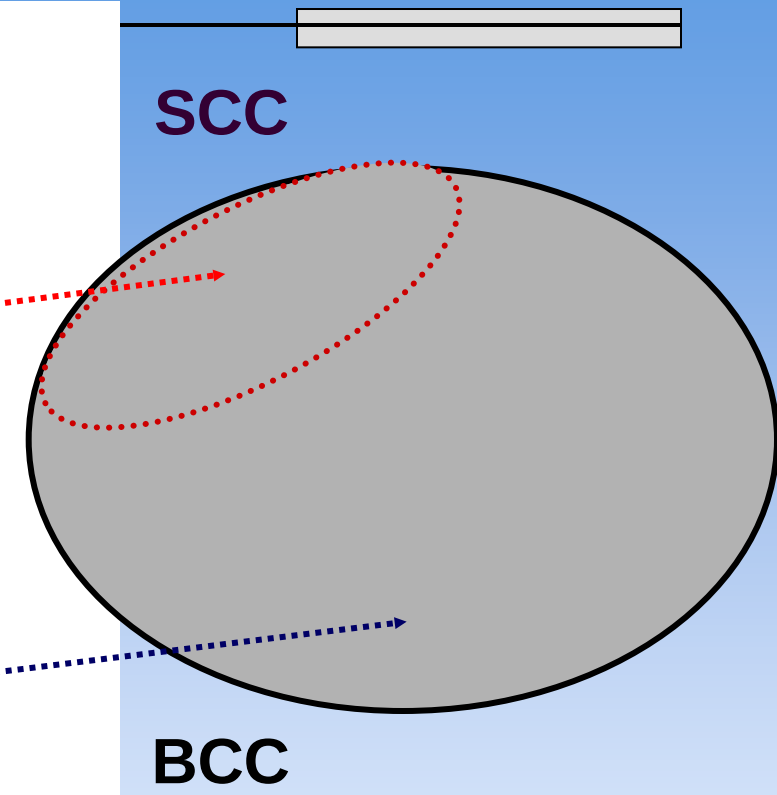
Treatment decision:
Lesions 2 and 5 – advanced stage – surgical removal and chemotherapy

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

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

Mixed tumor – therapy failure

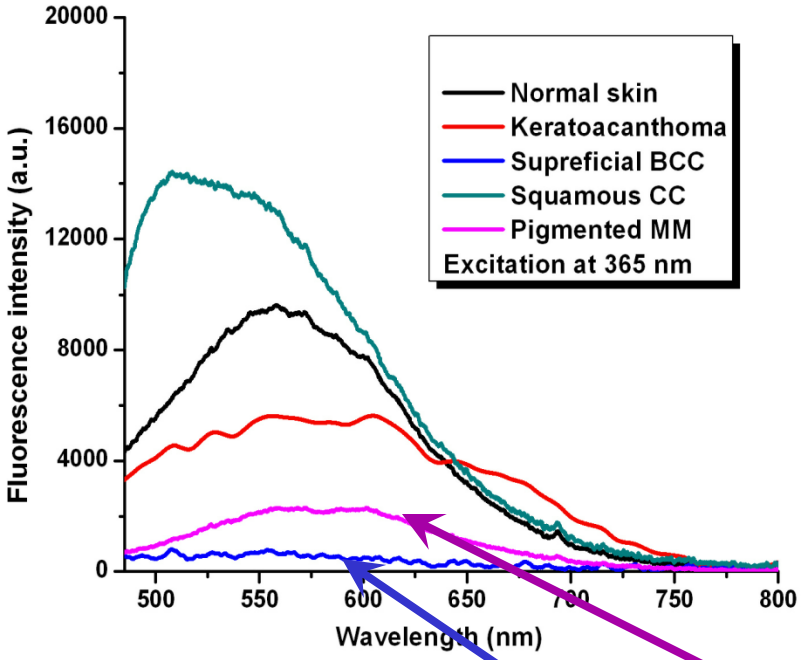


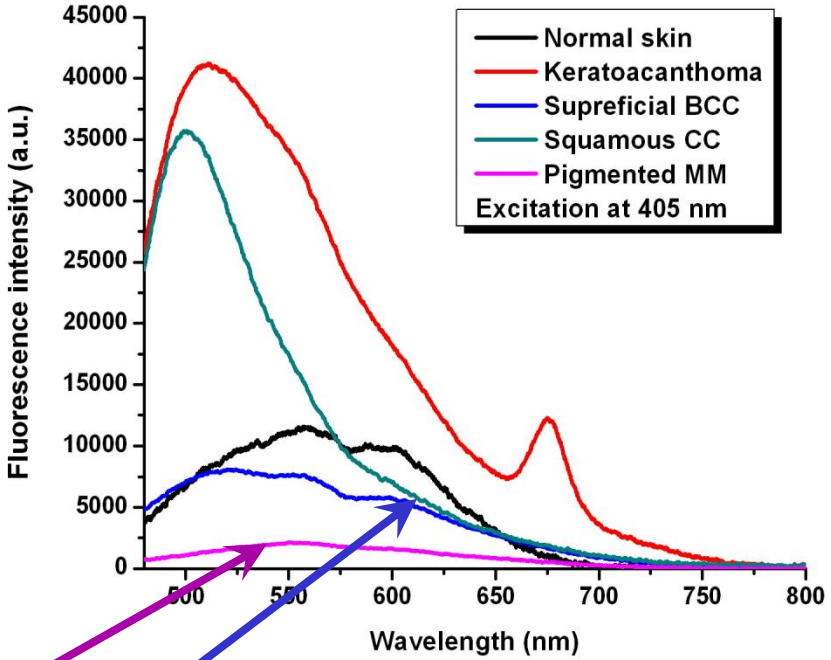


- 78 – years old patient
- tumor size 5 x 6 cm on the forehead
- failed Ro-therapy

Comparison of malignant lesions' AF spectra

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

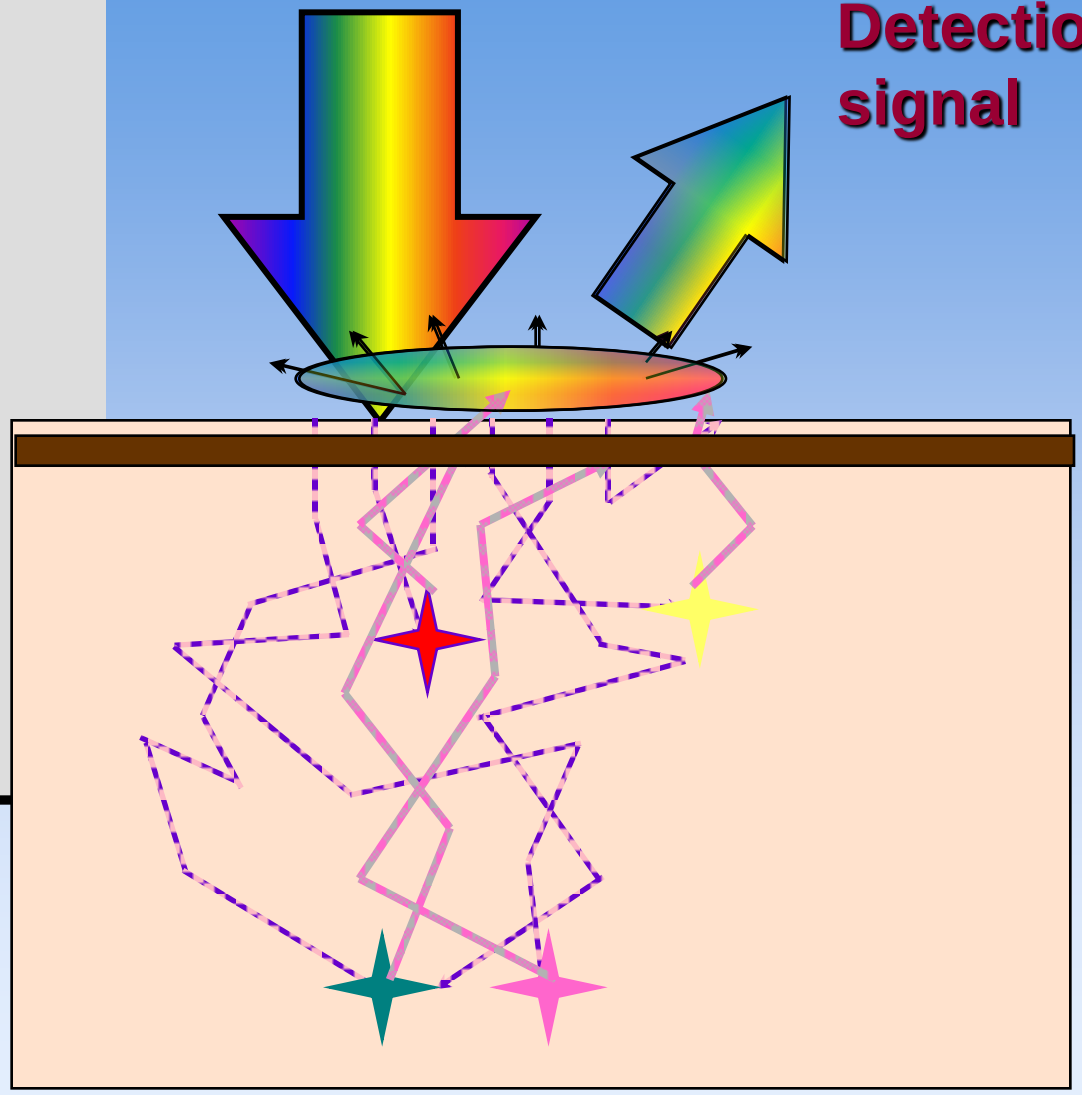




MM

Pigm. BCC

Skin reflectance



Detection of reflectance signal

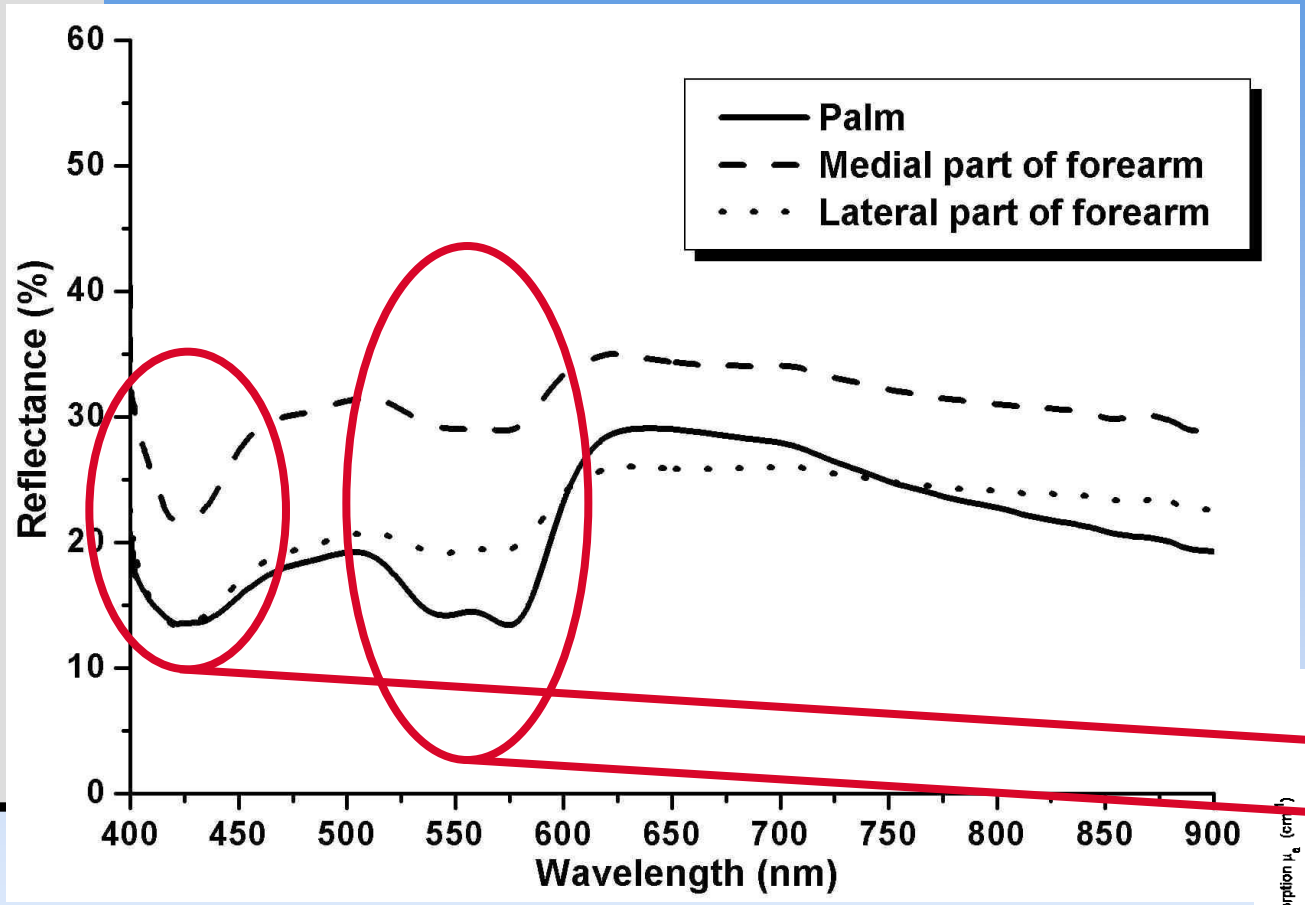
1) Light in broad spectral region penetrates in the skin

2) Tissue absorbers selectively absorb light on the different wavelengths

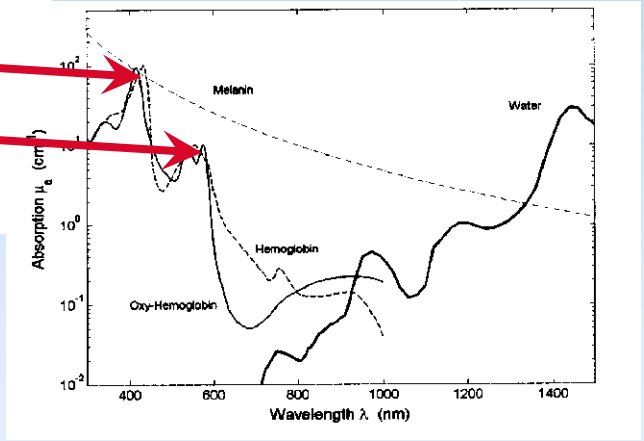
3) Light is scattered in the tissue volume and part of it scattered back to the surface

4) Reflectance signal consisted from specular reflectance component and diffuse back-scattered signal form the tissue volume could be detected by spectrometer

Normal skin reflectance spectra

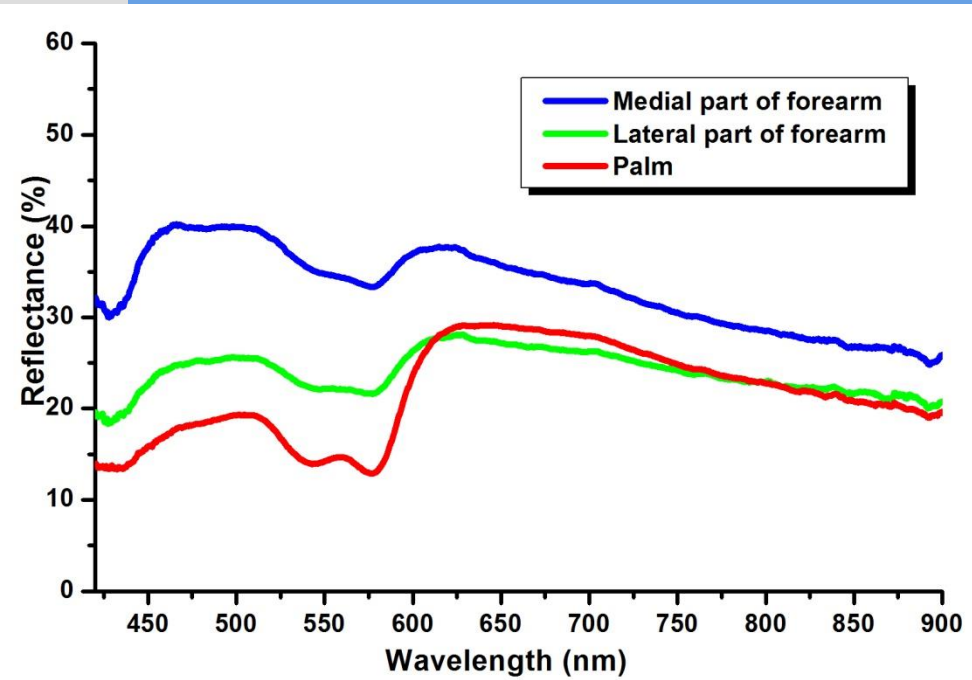


Main absorbers in human tissues



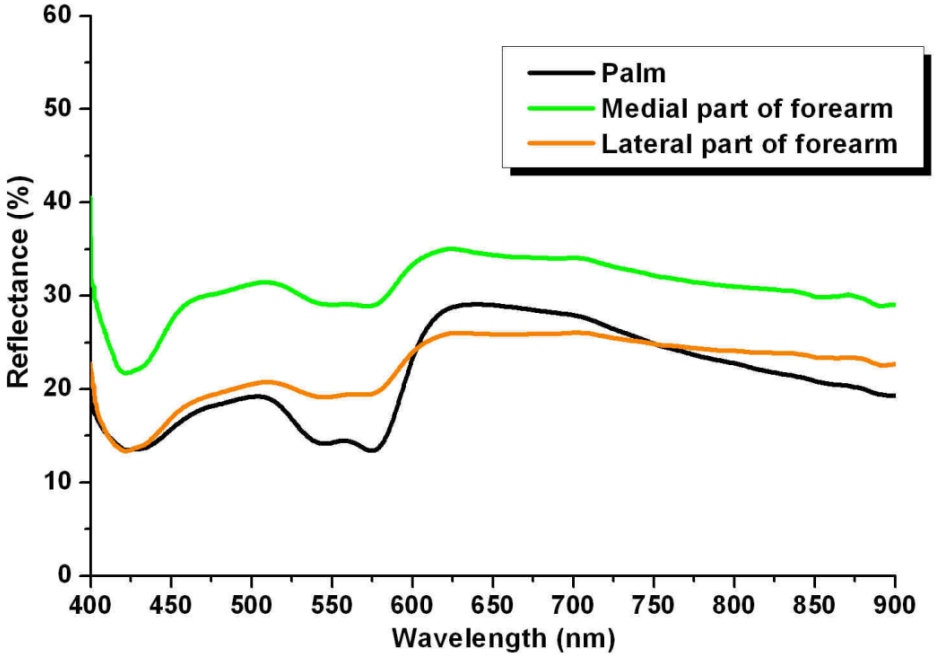
Normal skin reflectance spectra from the medial and lateral parts of the forearm and the palm for one patient

Normal skin reflectance

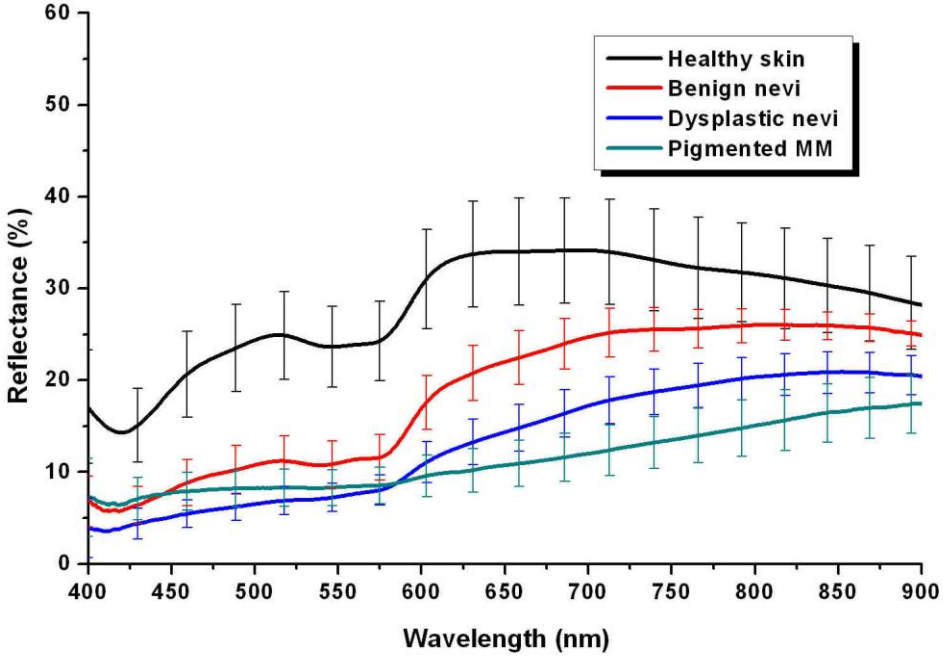


Skin phototype I

Skin phototype II

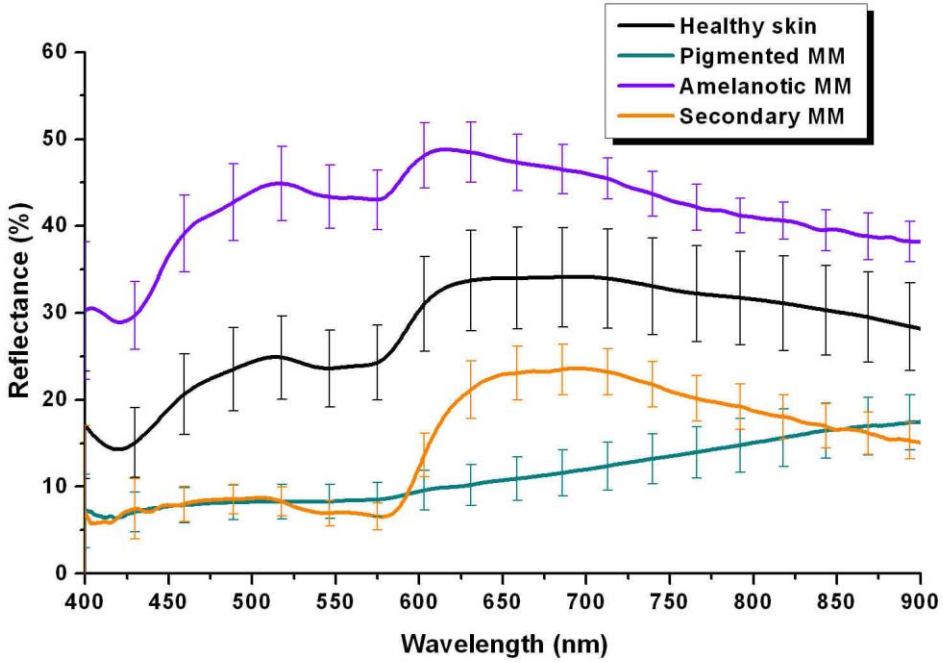


Reflectance of melanin-pigmented benign and malignant lesions

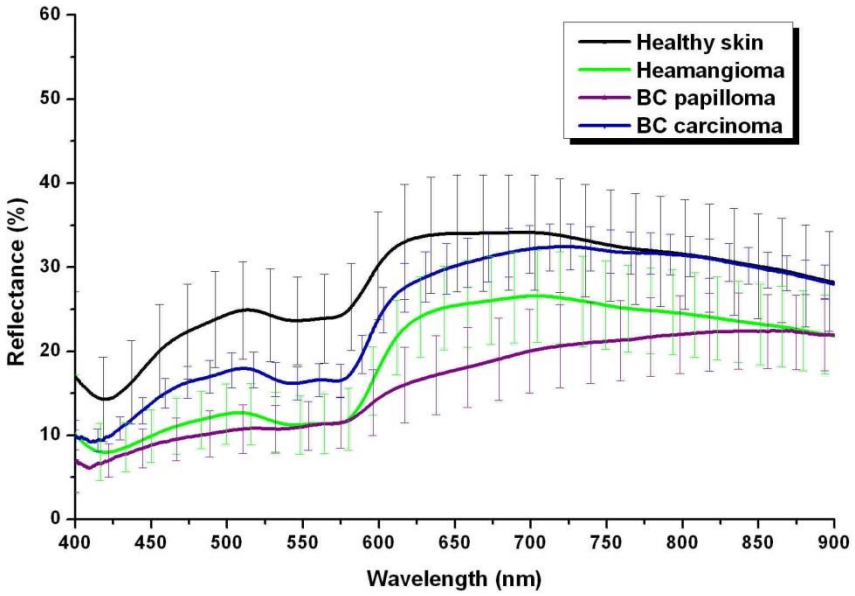


Reflectance spectra presented with their standard deviations of normal skin, compound nevus, dysplastic nevus and pigmented MM, averaged by lesion type for all patients

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

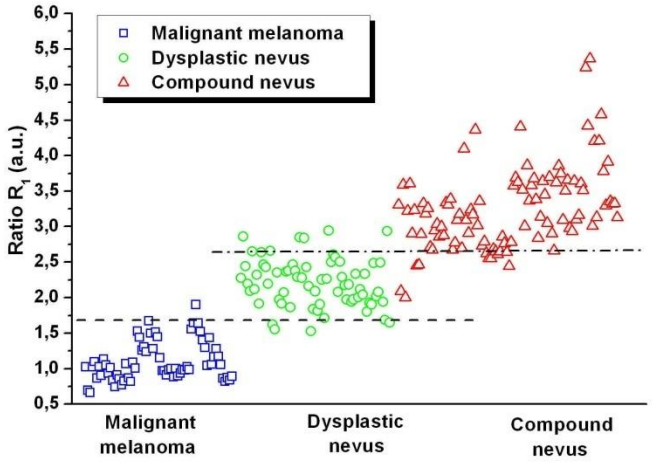


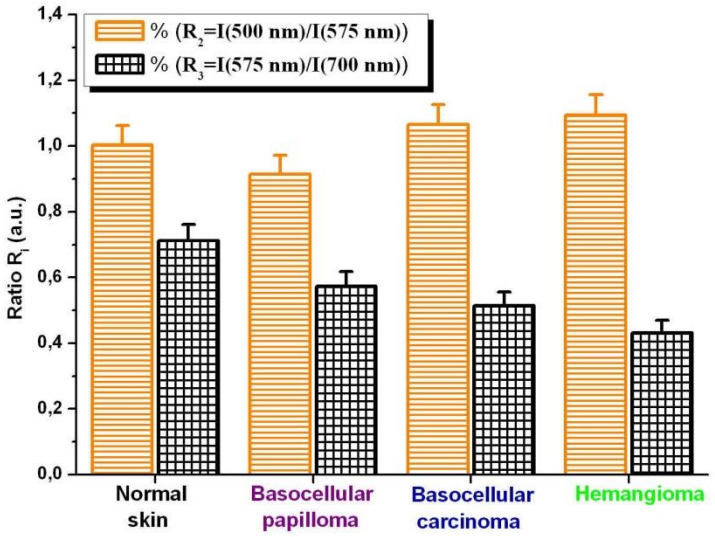
Reflectance of non-melanin-pigmented benign and malignant lesions



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.



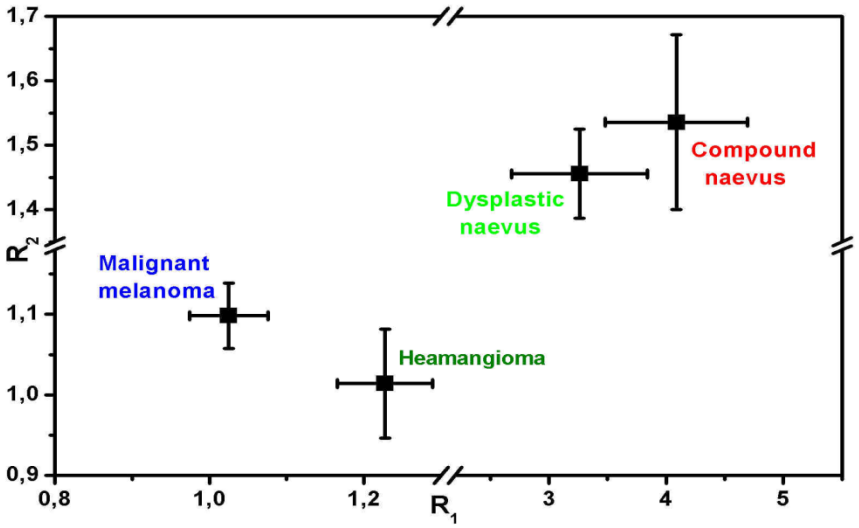


Comparison	SN %	SP %	DA %
Compound nevus/ Dysplastic nevus	82,1	77,6	61,8
Dysplastic nevus/ Malignant melanoma	92,4	77,6	75,3
Normal skin/ BC papilloma	95,7	98,5	89,2
Normal skin/ BCC	94,0	98,2	86,8
BC papilloma/BCC	91,0	87,2	83,8

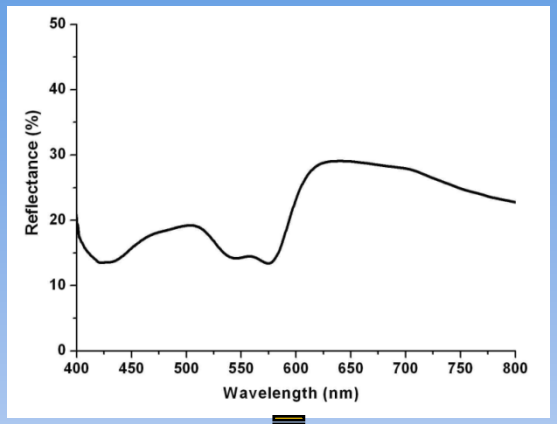
Statistical values of reflectance diagnosis of skin lesions

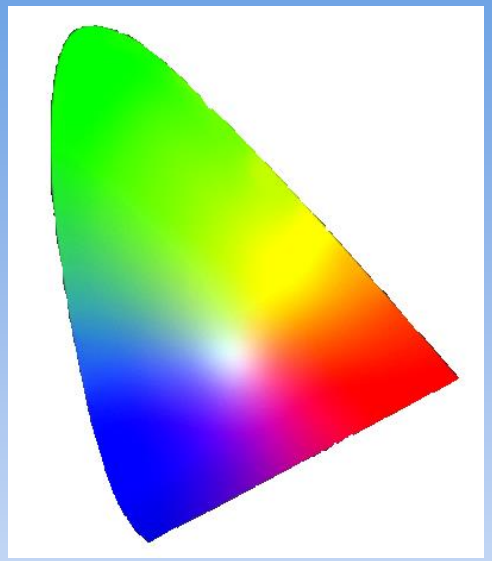
Algorithm for differentiation

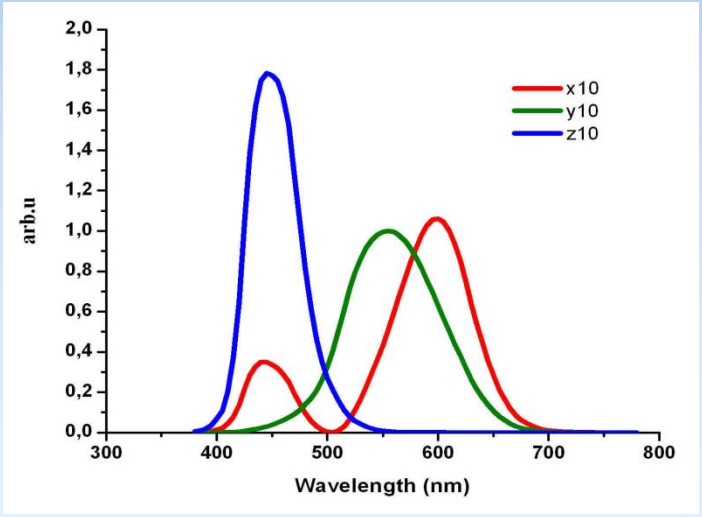
R_1=*Inorm(500)*Ipigm(700)/Inorm(700)*Ipigm(500)*
R_2=*Inorm(500)*Ipigm(575)/Inorm(575)*Ipigm(500)*

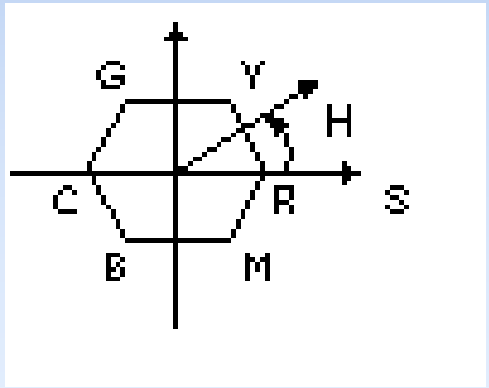


Automation of cancer diagnosis based on colorimetric transformation of cutaneous reflectance spectra









Transformations: spectrum–CIEXYZ -HS

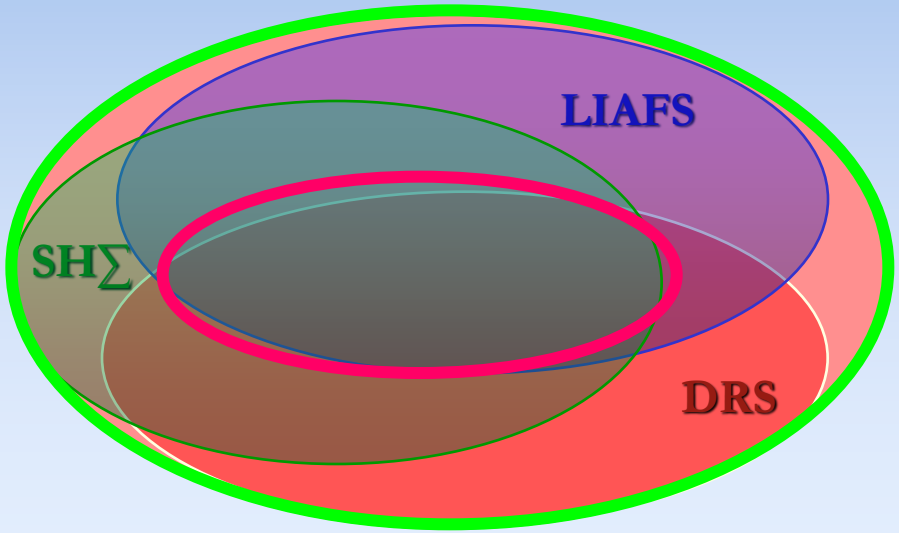
Combination - LIFS+DRS+colorimetry

Technique	SN %	SP %	PPV %	NPV %	IS %	DA %
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

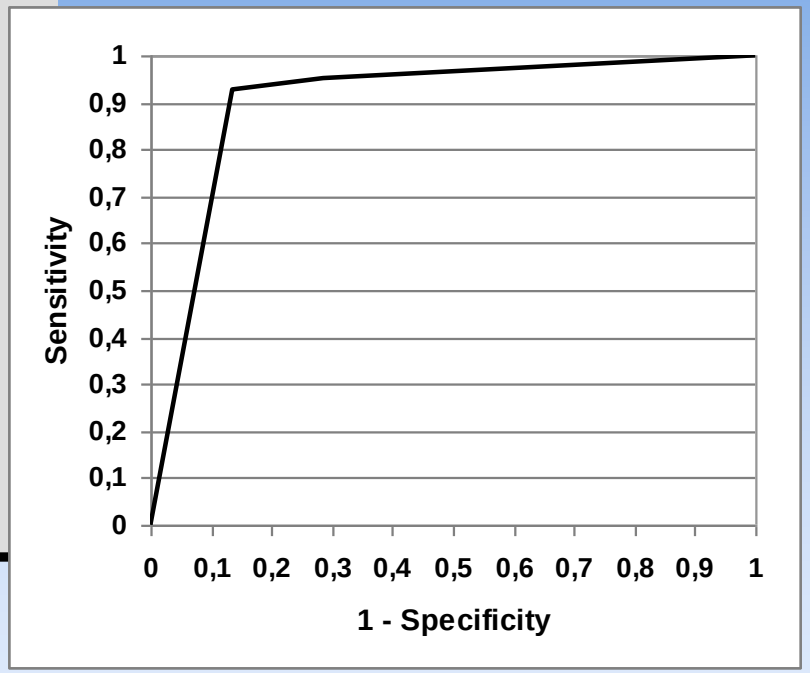
24 MM, DN and BN patients



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

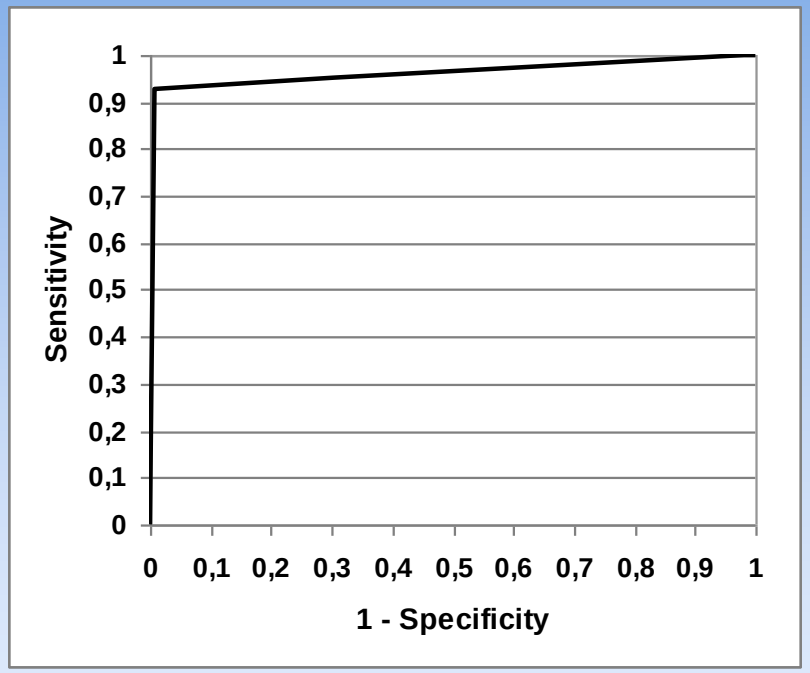
Diagnostic accuracy in clinical investigation for optical biopsy skin cancer detection

ROC – differentiation of malignant melanoma vs. melanocitic nevi



Area	p	95% CI	
		Lower	Upper
0,901	< 0,0001	0,840	0,962

ROC – differentiation of malignant melanoma vs. non-melanoma malignancies (BCC, SCC, KA, Bowen, etc.)



Area	p	95% CI	
		Lower	Upper
0,961	< 0,0001	0,915	1,007

Method and compared lesions	SE, %	SP, %	PPV, %	NPV, %	DA, %
AF – NMSC vs. benign	100	99,2	99,4	100	99,3
AF – MM vs. benign	100	99,2	97,6	100	98,4
AF – MM vs. NMSC	99,1	90,5	98,4	95,1	93,6
RS – NMSC vs. benign	86,6	41,3	68,8	67,4	69,4
RS – MM vs. benign	92,7	85,3	77,6	95,5	90,1
RS – MM vs. NMSC	92,7	99,1	97,4	97,4	96,1

Thank you very much for your attention!