

# Investigation of Relations Between Skin Cancer Lesions' Images and Their Reflectance and Fluorescent Spectra

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## 1. Introduction

Biomedical optics is one of the fastest growing areas of research. The non-ionizing nature of light applied for investigation and detection of abnormalities in human tissues make this area very attractive for development of new diagnostic techniques and modalities (Wang, 2007). The optical spectra provide biochemical and morphological information about the tissue under investigation based on its absorption, reflectance, fluorescence and elastic scattering properties (Wang, 2007; Tuchin, 2002). Many techniques based on the recent progress in optics have been developed for biomedical applications. Fluorescence, absorption and diffuse scattering spectroscopy have been widely applied as probes acquiring information about physical, chemical, or physiological processes in the tissues. These methods have been proposed to be used by the medical community in view of extending the capabilities of the standard diagnostic modalities that have already been introduced in the clinical practice, as x-ray, magnetic resonance and ultrasound imaging.

Laser-induced autofluorescence spectroscopy (LIAFS) could be utilized to quantify differences between normal and abnormal tissues *in vivo*, thus providing an appropriate method for detection of pathological lesions in real time. Diffuse reflectance spectroscopy (DRS) also allows distinguishing between pathological areas and normal tissue surroundings. In recent years, there has been growing interest in the combined use of laser-induced autofluorescence and reflectance spectroscopy to differentiate diseased from normal surrounding tissue - mainly for detection and differentiation of cancerous and pre-cancerous changes in human body.

Laser-induced autofluorescence spectroscopy is very promising from a technical point of view due to the easy coupling with optical fibres for delivering the excitation light to every part of the human body without significant losses of light power, as is the case of incoherent light sources, as well as because of the possibility to use information from naturally occurring endogenous fluorophores, without adding external fluorescent markers. LIAFS is also notable among the other non-invasive diagnostic techniques, as it offers real-time detection and differentiation of the lesion investigated with promising precision, selectivity

and specificity (Bigio, 1997; Bachmann, 2006). The fluorescent technique is widely applied to cutaneous lesions' investigations, including erythema (Sinichkin, 1998), psoriasis, vitiligo (Borisova, 2000), and skin cancer (Wang, 2006; Troyanova, 2006). This method yields information about the biochemical composition of the tissue under study.

On the other hand, diffuse reflectance spectroscopy is mainly providing morphological information concerning the tissues. The scattering intensity and spectral distribution of the signals detected could give information about the scatterers' size and distribution (cells, nuclei, etc.). Most of the tissue pathologies, including tumours, exhibit significant architecture changes on a cellular and sub-cellular level. The elastic scattering of light penetrating into the tissues depends on this alteration in their architecture, so that the back-scattered signals detected could be used for determining pathological structural changes occurring in the organ under study (Morant, 2003). As the diffuse reflectance signal detected is a superposition of diffuse scattering and absorption from tissues pigments, the resultant spectrum on the tissue surface reveals also information about the main absorbers in the biological tissues, such as haemoglobin and melanin in the skin and its pathologies. To benefit fully from reflectance spectroscopy's advantages, one needs to relate the spectral features with the morphology and biochemical composition of the tissue investigated. (Tuchin, 2002, Borisova, 2006).

Combination of fluorescence and reflectance optical techniques could rapidly improve the sensitivity and specificity of cutaneous tumours' diagnosis (Borisova, 2008). Using imaging analysis techniques in the processing of fluorescence and reflectance spectral data received from cutaneous pathologies could further improve the diagnostic accuracy (Borisova, 2008). Our work is a stage in the development of methods for skin cancer diagnostics on the basis of optical spectra. The preliminary analysis showed that the spectra of lesions differ from those obtained from normal tissue, with the differences observed depending on the type of pathology. A more "damaged" tissue changes the colouring more intensively. If a lesion' area could be localised correctly, it would provide more precise information about this lesion's structure. In addition, not only does the intensity value of the reflected/emitted light vary from patient to patient, but it also depends on the position of the tested area inside a given lesion. We proposed that the variations in the reflected/emitted value of the light could be caused by two possible reasons:

- different degree of "damaging" – lesion stage of development, and
- different thickness of the suspicious lesion area.

The specific features of the spectra are suitable for the development of a hybrid diagnostic algorithm (including both image and spectra) for detection and discrimination of different type of tissue damaging. We applied this approach to studying skin cancer in an attempt to separate the melanoma from benign and dysplastic melanin-pigmented lesions. The ultimate goal of the investigation is developing a reliable technique for predicting the type and level of skin damaging and the stage of its progress.

## 2. Basic theory

### 2.1 Image processing

The aim of image processing is obtaining an image with a value of the lightness of pixels, which depends on the saturation of the damaged tissue. CIE.Lab is a standard for estimating of colours using features of the illuminating light source.

The system presents the colours as relative lightness and chromaticity that consist of two components: a - a red- green axis, and b - a yellow-blue axis. The other parameter -

chroma, is calculated as the distance of the particular chromaticity from the centre of the area (Fig. 1) and corresponds to the visible saturation of the colours.

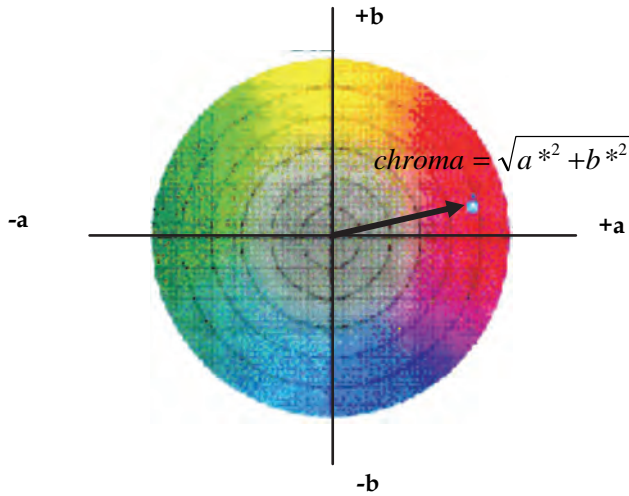


Fig. 1. Chromaticity diagram and chroma distance in the Lab system.

If we use another coloured light to illuminate the object, all the visible colours would change. However, the calculated coordinates (eq. (1)) in the Lab chromaticity area will keep their position, since the colour of the source becomes achromatic.

$$\begin{aligned}
 L &= 116 \cdot f(Y / Y_n) - 16 \\
 a^* &= 500 \cdot [f(X / X_n) - f(Y / Y_n)] \\
 b^* &= 200 \cdot [f(Y / Y_n) - f(Z / Z_n)]
 \end{aligned}
 \tag{1}$$

$$chroma = \sqrt{a^{*2} + b^{*2}}$$

where  $f(t) = t^{1/3}$  for  $t > 0,008856$  and  $f(t) = 7,787 \cdot t + 16 / 116$  otherwise.

Here  $X_n$ ,  $Y_n$  and  $Z_n$  are the CIE XYZ three stimulus values of the reference white point.

If we use another colour as a “referent white”, all available colours’ coordinates would be changed, but the white point again will be at the centre of the chromaticity area with no saturation. Thus, if the healthy skin colour is used as a “referent white” point, all changes of the pigmentation could be described by chroma. Applying this to digital images, the colour of the tissues transforms to grey levels and the areas whose colour differs from the “healthy” one will occur with higher intensity. However, the images consist of pixels whose RGB signals measure relatively and reproduce the colorimetric features of the objects. Concerning equations (1), a transformation from RGB to XYZ is needed to obtain the Lab parameters and chroma. The relation depends on the chosen area of Locus that needs to be converted and are established for several more frequently used spaces – sRGB, AdobeRGB, ProPhoto etc. given as values of the coefficients  $c_{ij}$  in equation (2).

$$\begin{bmatrix} X \\ Y \\ Z \end{bmatrix} = \begin{bmatrix} c_{11} & c_{12} & c_{13} \\ c_{21} & c_{22} & c_{23} \\ c_{31} & c_{32} & c_{33} \end{bmatrix} \cdot \begin{bmatrix} R \\ G \\ B \end{bmatrix} \quad (2)$$

Applying these transformations to images of lesions we achieve a decrease in the saturation of the healthy skin surrounding the damaged one. The sequenced linear contrast stretching of the whole image improves the visibility of the tested area by highlighting the saturated pixels and allowing the lesion localization. The saturation could be imaged by transforming each pixel's chromaticity to a grey level presenting their chroma.

## 2.2 Spectra processing

The aim of the processing is to highlight the peculiarities of the distribution obtained from suspicious tissues. We used a simple technique developed for the reflected spectra also described in monograph about chromatic monitoring [ed Jones, 2008] that ensures a comparison between the healthy and the tested unhealthy tissues' spectra. We calculated the ratio "healthy skin" to "tested skin" spectrum (Fig. 2)

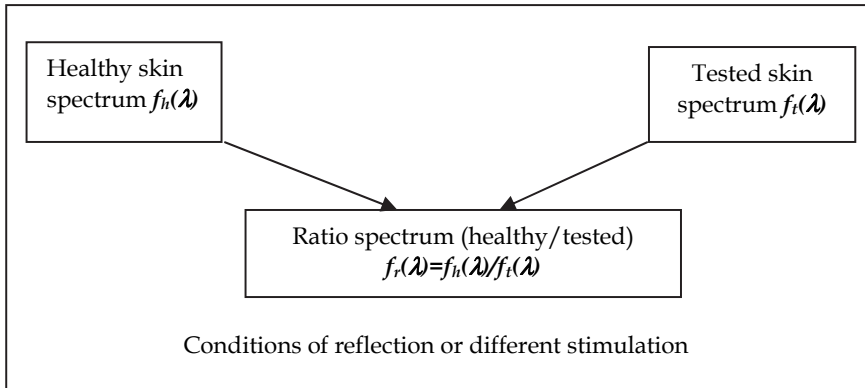


Fig. 2. Scheme of spectra processing.

The result is a new distribution, which is a straight line if the two spectra are proportional to each other, or otherwise may have any other shape. This gives rise to a possibility to overcome the ambiguities resulting from the different patients' states and to extract only the peculiarities common for the disease.

## 3. Experimental set up

In our investigation we used images and spectra of melanin-pigmented cutaneous benign lesions obtained from 30 patients, dysplastic nevi - obtained from 18 patients, and lesions of malignant melanoma obtained from 27 patients.

The images were obtained from simple photographs taken by a photo camera and processed by a specially prepared computer program that transforms the colour parameters by the sRGB standard. The fluorescent spectra were obtained using three different excitation sources with maxima of the emitted light at 365, 385, and 405 nm, respectively. The emitted fluorescence is registered by a fibre optic micro spectrometer (USB4000, "Ocean Optics,"

Inc., Dunedin, USA). A high-sensitivity linear CCD detected the reflected light dispersed by a grating with 600 lines/mm, with the total spectral resolution of the micro spectrometer being approximately 2 nm. The same device was used for registering the reflectance spectra. All spectra were limited into the range of visible light between 420 and 780 nm. The intensity and shape of the curve of reflected/emitted spectra in different conditions could be estimated in the cases of different patients and different points of obtaining the spectra inside of the lesion.

The results were analysed based on two criteria:

- Significant signal level dependence on the reflection and excitation light wavelength,
- Coincidence of the shape of the spectra ratios obtained from different patients and different points of registering depending on the diagnosis.

## 4. Results and discussion

### 4.1 Benign nevus

Figure 3 shows the initial image of a benign nevus lesion, the image of healthy tissue used for obtaining the values of  $X_n Y_n Z_n$  parameters, and the grey "chroma" image after processing. The damaged area is inhomogeneous. The pixels that pertain to it could be extracted by simply setting a threshold to the grayscale. One could expect that spectra registered from the central and the peripheral area of the spot should be similar in shape and level.

Figure 4 shows the reflected and emitted spectra of the central area obtained from three different patients denoted by P1, P2 and P3. The intensity of the reflected light changes from patient to patient, but, as it is seen, the curves have similar shape. In what concerns the intensity of the emitted spectra, a higher level is present in the case of stimulation with the wavelength of 385 nm. Figure 5 shows the reflected and fluorescent spectra of the central and peripheral areas and the spectra of the healthy areas. The comparison of the shapes of these spectra allows us to suggest that only 385 nm is suitable for seeking similarity. Concerning the reflected spectra, the shape of the curves does not depend on the positions. This fact is confirmed by the spectra ratio given in figure 6.

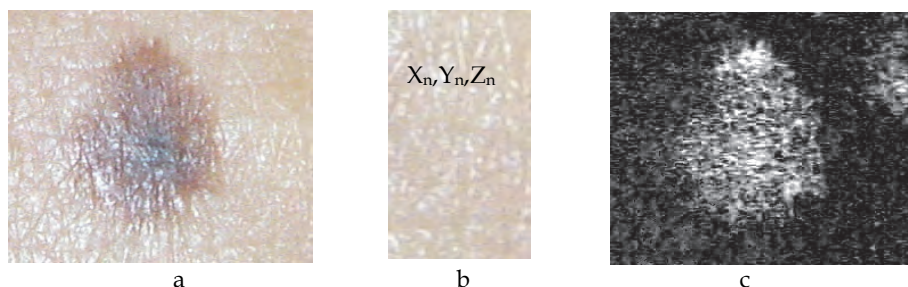


Fig. 3. Benign nevus. Initial image-a, healthy area-b and the "chroma" image after processing- c.

The suggestion that the spectra do not depend on the position inside of the lesion is valid for the intensity in practically all cases of fluorescence; this holds true for the shapes only if stimulation is carried out at 385 nm. Similarity is present in the shapes of the reflected spectra only, but not in their intensity.

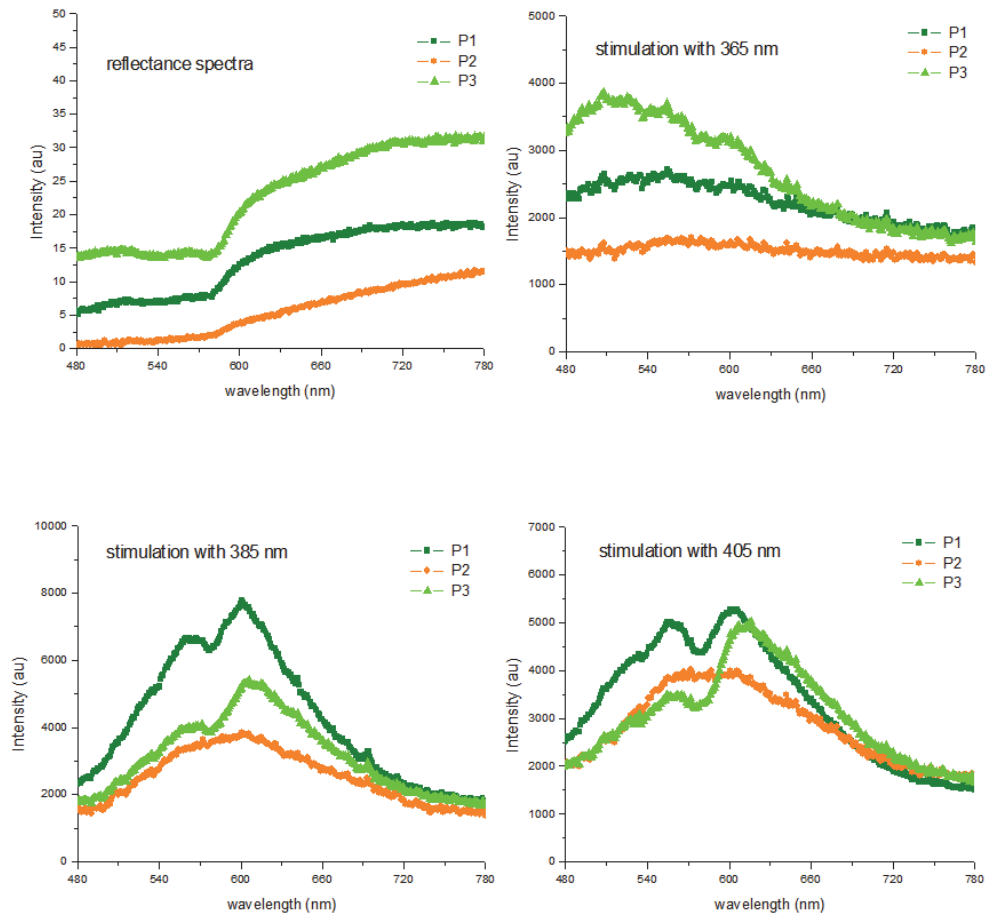


Fig. 4. Reflected and fluorescent spectra of the central area obtained from different patients in dependence on the wavelength of stimulation for benign nevus diagnose

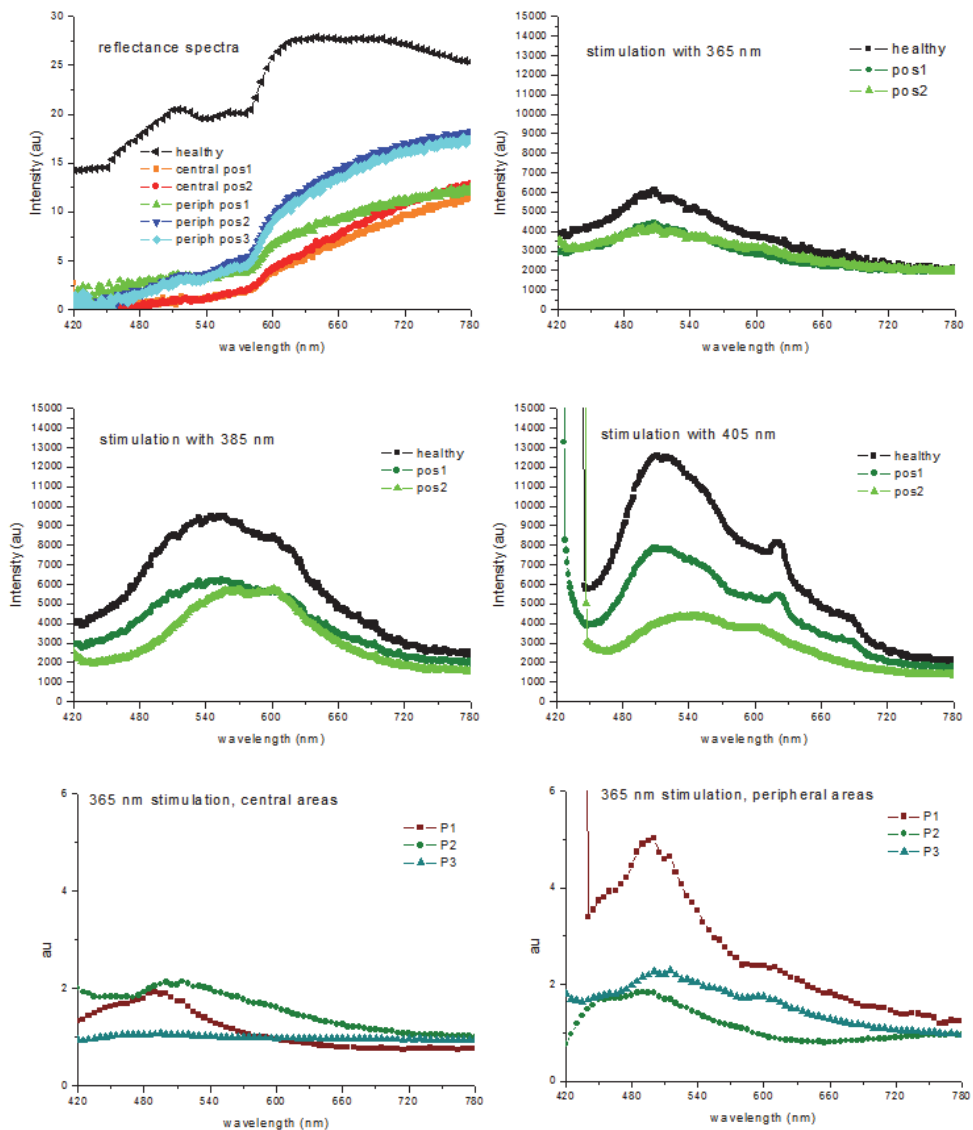


Fig. 5. Reflected and fluorescent spectra of several points in dependence on the wavelength of stimulation for benign nevus diagnose.

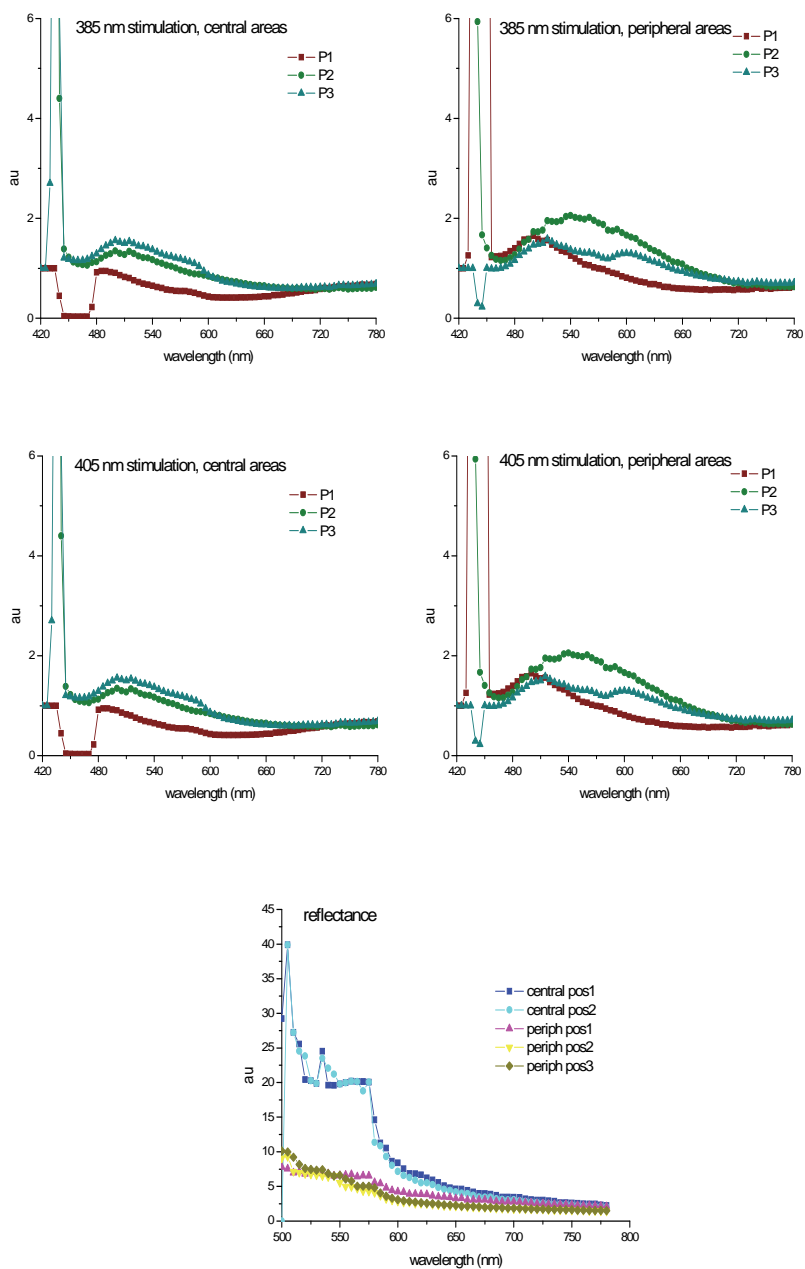


Fig. 6. Reflected and fluorescence spectra ratio of several points in dependence on the wavelength of stimulation for benign nevus diagnose.



### 4.2 Dysplastic nevus

Figure 7 illustrates the results of applying the techniques discussed to the image of a dysplastic nevus. The damaged area has clear outlined borders and could be easily extracted again by setting a threshold to the greyscale. The internal structure shows a low-density area concentrated into the centre of the lesion, and higher density near the borders. It could be assumed that the spectra would have different shape and intensity in dependence on the position of registering.

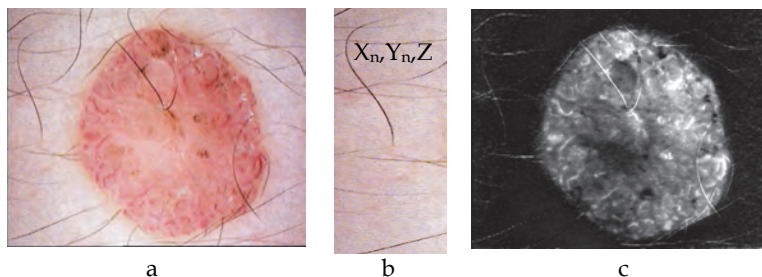


Fig. 7. Dysplastic nevus. Initial image - a, healthy area - b and the “chroma” image after processing - c.

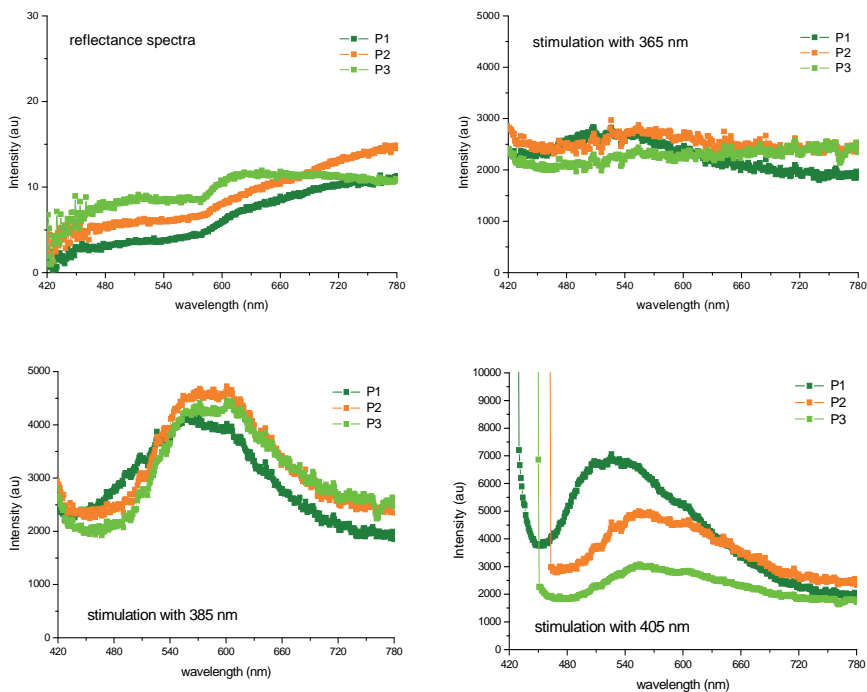


Fig. 8. Reflected and fluorescent spectra of the central area obtained from different patients in dependence on the wavelength of stimulation for dysplastic nevus diagnose.

Figure 8 shows reflected and fluorescent spectra of the central area obtained from three patients denoted again by P1, P2 and P3. The intensity of the reflected light is almost similar for the different patients, but the shape of the curves is different. Concerning the emitted spectra intensity, a higher level is seen again in case of stimulation at 385 nm. The next figure 9 also shows the reflected and fluorescent spectra of the central and peripheral areas and the spectra of the healthy areas. It could be seen that the shape of the fluorescent spectra does not depend on the position in all wavelengths stimulations tested. However, only the stimulation with 385 nm gives simultaneously a similarity between the spectra ratios and sufficient differences from the other spectra ratio (figure 10) in the cases of stimulations at 365 and 405 nm.

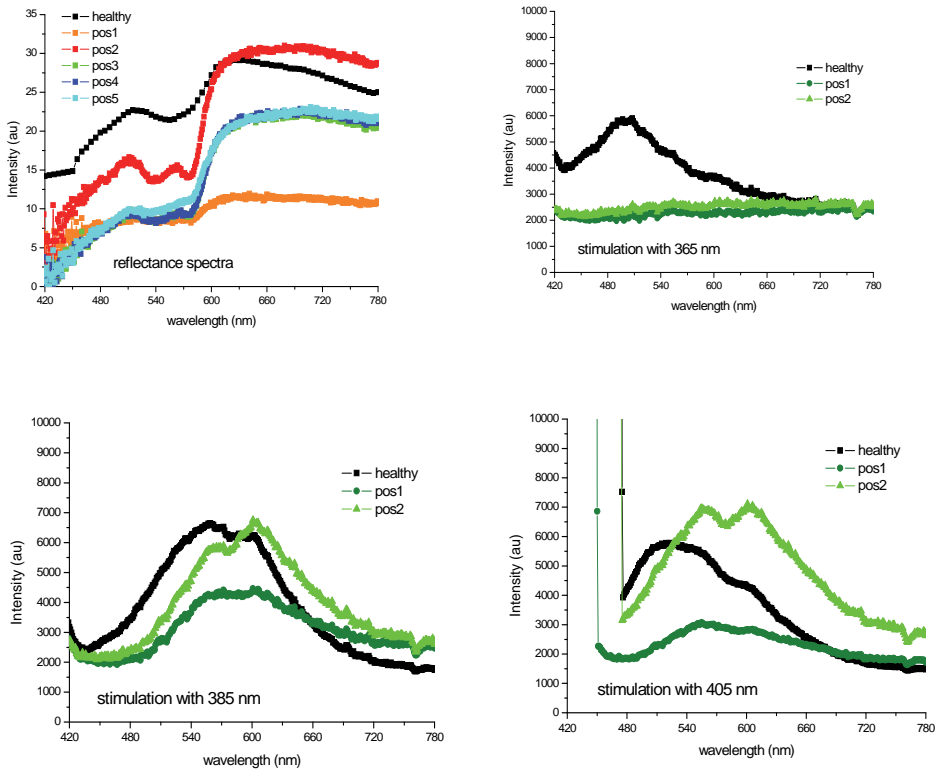


Fig. 9. Reflected and fluorescent spectra of several points in dependence on the wavelength of stimulation for dysplastic nevus diagnose.

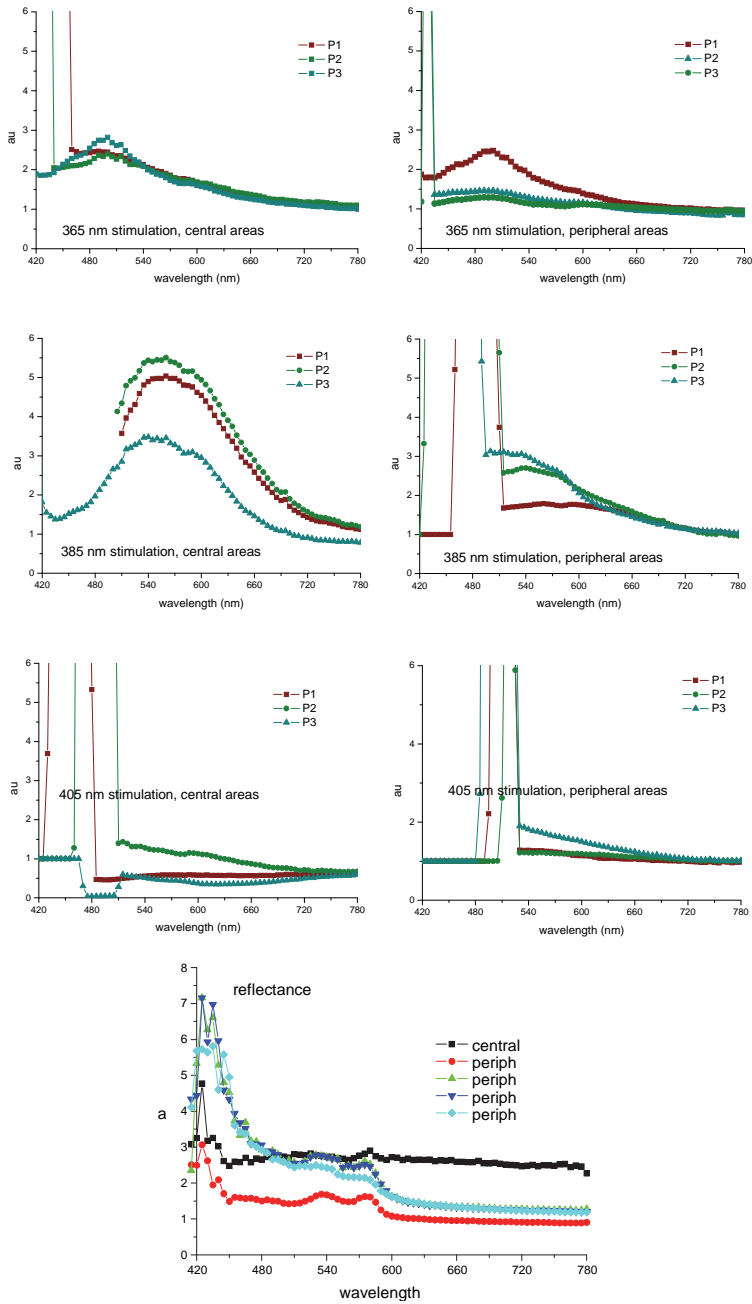


Fig. 10. Reflected and fluorescent spectra ratios of several points in dependence on the wavelength of stimulation for dysplastic nevus diagnose

**4.3 Malignant melanoma**

A malignant melanoma lesion is presented in fig. 11 by images resulting from two different processing: the one described above, the other using the program of MoleMax system. The latter uses ABCD criteria to set a diagnose by image analyses. The technique of greyscale „chroma“ image shows better visibility of the differences inside the lesions than the method used in the MoleMax program. The density spreading implies the absence of a relation between the position (centred or near borders) and the shape and intensity of the spectrum.

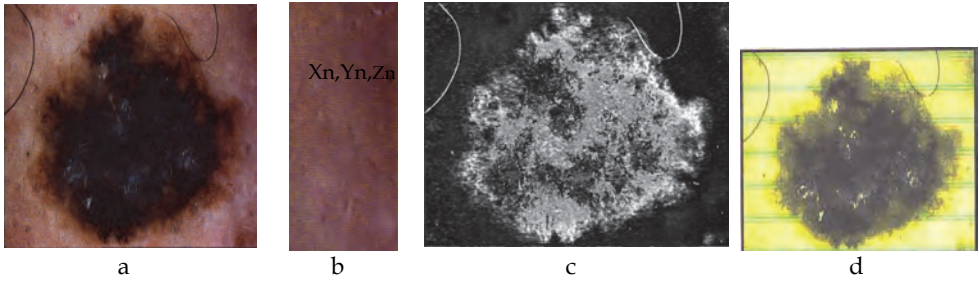


Fig. 11. Malignant melanoma: initial image-a, healthy area b, the “chroma” image -c, and image after MoleMax system processing-d.

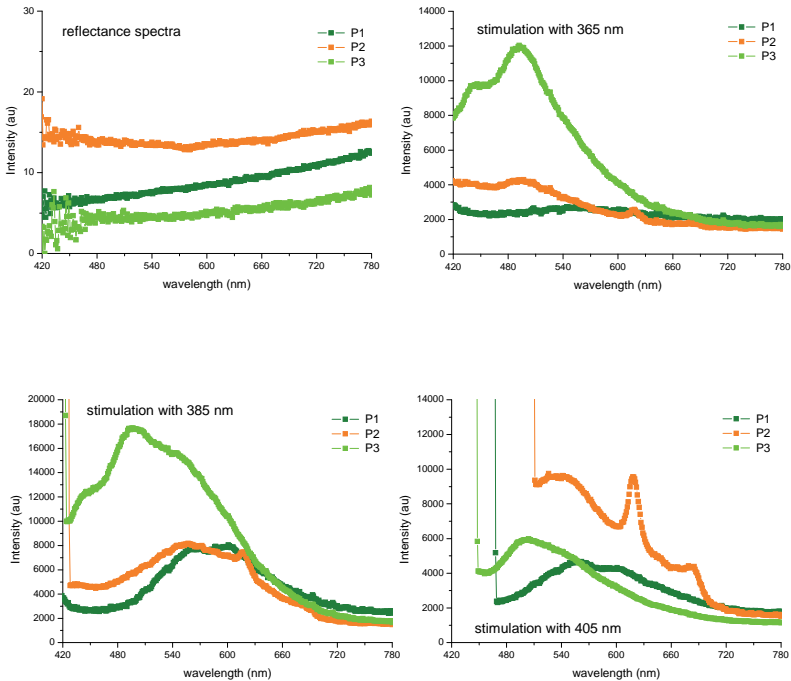


Fig. 12. Reflected and fluorescent spectra of the central area obtained from different patients in dependence on the wavelength of stimulation for malignant melanoma disease.

Figure 12 shows reflected and fluorescent spectra of the central area obtained from three patients denoted by P1, P2 and P3. The intensity of the reflected spectra varies from patient to patient. The same is valid for emitted spectra independently of the stimulation wavelength. The shapes of the spectra obtained from different areas of one patient are similar, but those of the spectra from different patients are different (figure 13). The spectra ratio given in figure 14 show different intensity, but the shape obtained by stimulation at 365 and 385 nm of peripheral areas are similar. The same applies to the reflected light spectra for.

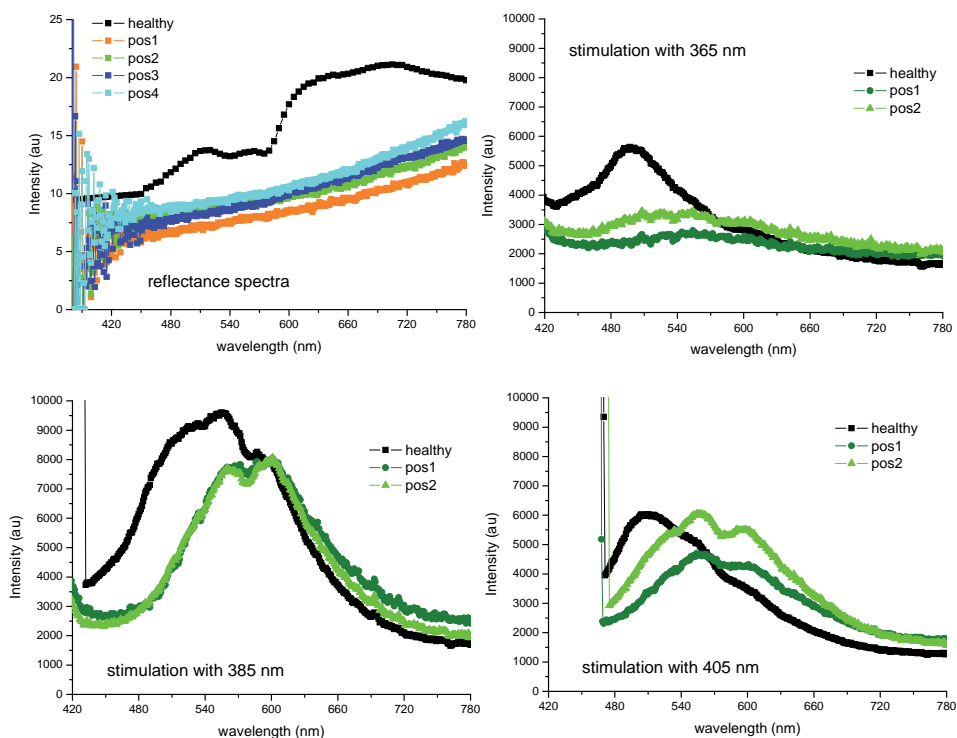


Fig. 13. Reflected and fluorescence spectra of several points in dependence on the wavelength of stimulation for malignant melanoma disease.

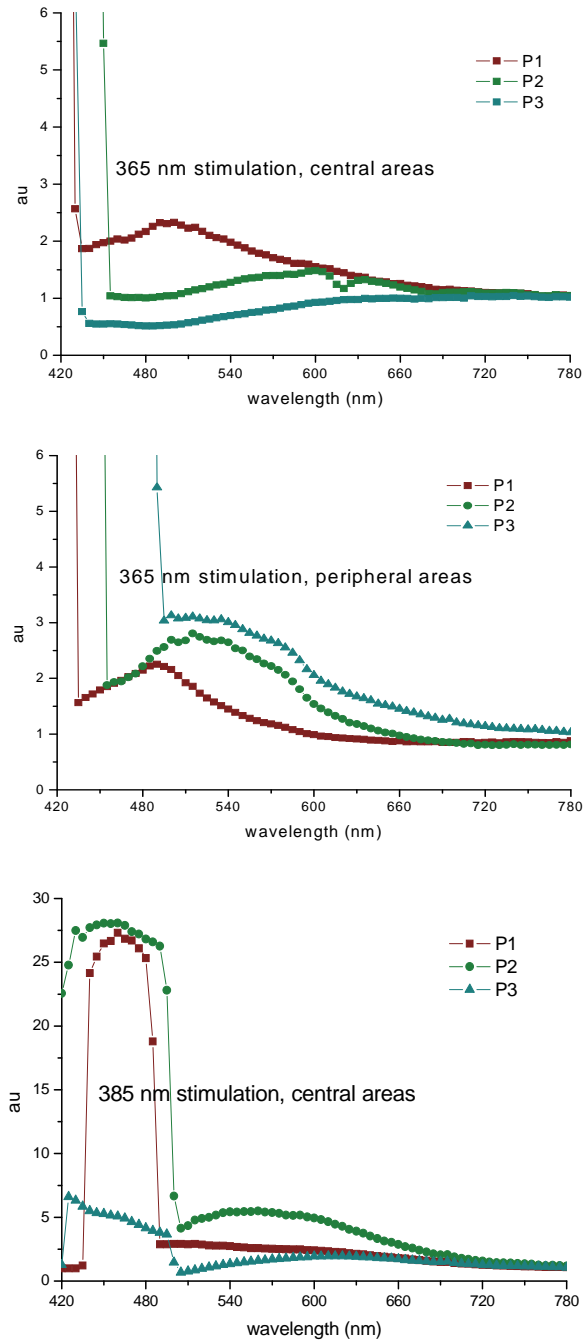


Fig. 14. (a)

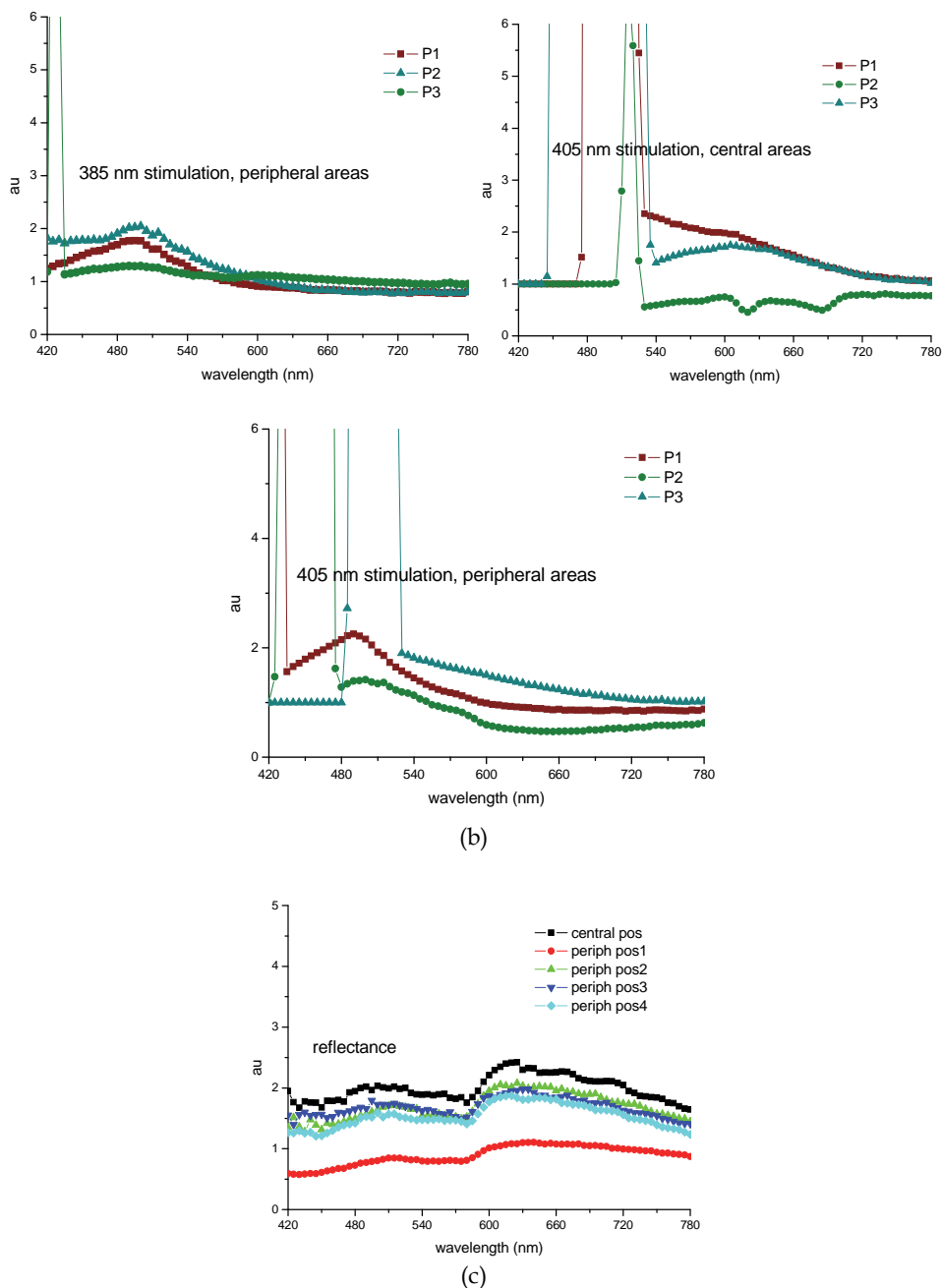


Fig. 14. Reflected and fluorescence spectra ratio of several points in dependence on the wavelength of stimulation for malignant melanoma disease.

Many technical advances and new optical methodologies for early detection of cancer have been recently developed, but there still exist considerable challenges concerning the precise diagnosis and differentiation of cutaneous malignant lesions. Most dermatologists still rely on their practical experience in visual evaluation of pigmented lesions. It is well known that the diagnostic accuracy depends on the clinical experience of the specialist (Morton and Mackie (1998)). In general practice, where such experience is low, diagnostic accuracy is quite bad (Bedlow 1995). In the early stages of the disease, the diagnosis is difficult even for experienced clinicians.

Within the wavelength range 400 – 800 nm, which we used in our observations, the stratum corneum, epidermis and dermis have relatively high scattering coefficients and lower absorption coefficients, particularly at longer wavelengths. Consequently, the spectrum of white light passing through such a tissue can be affected by the various components and thus become biased towards the longer wavelengths (Jones, 2000).

To benefit fully from optical spectroscopy's advantages, one needs to relate the spectral features with the morphology and biochemical composition of the tissue investigated. Account needs to be taken of various other skin tissue characteristics, such as melanin content in the epidermal layer, haemoglobin derivatives in the dermis and their influence on the detected spectra, so adding to the complexity of the conditions to be monitored.

Chromatic techniques have the potential for providing a means for quantifying the tissue spectral characteristics selectively under such conditions and for producing an assistive automated diagnosis means. When a combination of fluorescence, reflectance and chromatic techniques for analysis is applied, the diagnostic accuracy for malignant melanoma detection reaches 90 % (Borisova, 2008). In the situation when only one of these analytic techniques is applied, the diagnostic accuracies achieved are very similar for all three methods – about 70%.

Such an increase in diagnostic accuracy (by 20 %) demonstrates that applying chromatic techniques for monitoring biological tissues via the fluorescence, reflectance, absorption and scattering of polychromatic light could be extremely useful in tumour detection and evaluation. Therefore, unification of spectral and chromatic techniques in a common diagnostic tool is a very promising future step in the development of medical diagnostic systems, as it leads to increasing the diagnostic accuracy (ed. Jones, 2008, Borisova 2008) and allows one to develop computerized automatic systems for diagnostics decisions when one investigates cutaneous pathologies.

## 5. Conclusion

There are two common problems that impede one in obtaining optimal results.

- First, the greyscale “chroma” image resulting from the image processing depends on the healthy area; thus, any sparkles are able to produce incorrect chroma spreading inside the lesion. The problem could be avoided by controlling the image registration.
- The second problem is related to the wavelength interval of visibility of the stimulating laser pulse. In fact, the interval under 480 nm becomes useless.

Overcoming these problems is a task of data registration and do not have relation to the processing mentioned above.

Final greyscale images yield enough information about the density spreading inside one lesion. It makes it possible to control precisely the position of obtaining the spectrum.

Calculated spectra ratios emphasize the differences resulting from skin damages independently of the patients. A simple analysis shows that the data from emitted spectra after stimulation at 385 nm are suitable for setting a diagnose. For a benign nevus, the data



could be extracted from the any part of the lesion; for the dysplastic nevus, from the centre of the area; and from the peripheral area for the malignant melanoma.

The spectra ratios from reflectance calculated for a benign nevus could be distinguished from the spectra ratios for a dysplastic nevus, while the same spectra ratios for malignant melanoma clearly differ from the others.

Summarising the test results, we can conclude that the techniques described above form a good basis for the development a non-invasive method for estimating the type of skin damage.

## 6. Acknowledgements

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