**Hotsko Oksana, Galaychuk Igor. Spectral Analysis of Digital Images as a New Method in Diagnosis of Malignant Melanoma. Journal of Education, Health and Sport. 2015;5(10):340-349. ISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.42599**
**http://ojs.ukw.edu.pl/index.php/johs/article/view/2015%3B5%2810%29%3A340-349**
**https://pbn.nauka.gov.pl/works/686454**
**Formerly Journal of Health Sciences. ISSN 1429-9623 / 2300-665X. Archives 2011–2014**
**http://journal.rsw.edu.pl/index.php/JHS/issue/archive**

**Deklaracja.**
**Specyfika i zawartość merytoryczna czasopisma nie ulega zmianie.**
**Zgodnie z informacją MNiSW z dnia 2 czerwca 2014 r., że w roku 2014 nie będzie przeprowadzana ocena czasopism naukowych; czasopismo o zmienionym tytule otrzymuje tyle samo punktów co na wykazie czasopism naukowych z dnia 31 grudnia 2014 r.**
**The journal has had 5 points in Ministry of Science and Higher Education of Poland parametric evaluation. Part B item 1089. (31.12.2014).**

**The authors declare that there is no conflict of interests regarding the publication of this paper.**
**Received: 25.09.2015. Revised 25.10.2015. Accepted: 31.10.2015.**

# Spectral Analysis of Digital Images as a New Method in Diagnosis of Malignant Melanoma

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## Summary

Spectrophotometric analysis (SPM) of the digital images of pigmented skin tumours was performed using ImageJ software. The following algorithm: selection of the area and segmentation of the tumours nevus image; decomposition of the image into primary colours (red, green and blue), and extraction of their histograms; histographic evaluation of the colour spectra using absolute and relative values and charts built using Microsoft Excel. To validate the method, the SPM values were correlated with the results of morphological study of the excised nevi.

We determined that the red color spectrogram is the main diagnostic measure. In it, we selected the following parameters: intensity of the red colour ($I_{start}$, $I_{max}$,) and $AUC_{128}$ the total area under the graph.

The cluster analysis of the spectrophotograms to determine thresholds of the intensity of the red colour needed to differentiate malignant melanoma and dysplastic nevi were done. Melanomas can be characterized using the following values: $I_{start} \leq 18.5$ units, $I_{max} \leq 115.5$ units, $AUC_{128} \geq 5442.1$. Opposite values are characteristic for dysplastic nevi.

**Keywords:** skin melanoma, melanocytic nevi, spectrophotometric analysis.

**Introduction.** The timely diagnosis of skin melanoma (SM) remains a pressing issue, since it determines the course, prognosis and results of the treatment [8]. Melanoma is among the most aggressive tumors and oncologic dermatology it is the cause of death in 80% of cases [5, 12]. This problem is particularly acute because pigmented lesions of the skin, nevi, which can mask developing melanoma, occur in almost 90% of population with white skin [7, 15].

In 2013 in Ukraine there was recorded 3,278 cases of SM, of them 1,117 resulted in death; the ratio of mortality to incidence rates was 0.34 [11]. In the USA this rate is 0.12 [19]. Prompt detection and radical removal of primary melanoma remain the main elements of successful outcome [13, 15, 20, 21].

Among the effective non-invasive methods on SM diagnosis are epiluminescence microscopy (dermatoscopy), SIA-scopy, confocal laser microscopy, high-frequency ultrasound, optical coherence tomography and others [1-7, 10, 14, 16-18, 20]. However,

neither of those methods is used in screening programs to detect melanocytic nevi and skin melanomas. Additionally, all these methods are based on personal contact with and require physical presence of the patient.

**Aim:** to evaluate distance spectrophotometry as a potential diagnostic method of melanomas and melanocytic nevi of the skin.

**Research methods:** Spectrophotometry (SPM), clinical, morphological and statistical methods.

**Materials and Methods.** In this study we used 180 digital images of nevus skin lesions of patients that had been previously surgically treated. All removed lesions underwent histological study. Spectrophotometric analysis of digital images was performed using ImageJ software. This software was used to produce histograms of red (R), green (G) and blue (B) colours. We then used Microsoft Excel to create RGB-spectrophotograms in grayscale. We evaluated R-G-B colours graph curves relative to the x-axis (in the units of grey colour scale) and y-axis (amplitude height in %). These measures were selected as a basis to differentiate between malignant and benign skin nevi. To validate this methodology, we correlated data generated by SPM and the results of morphometric study.

Images of the lesions were evaluated visually using the ABCD guidelines.

Comparative SPM analysis of nevus lesions was performed in three groups of patients: 45 suffering from skin melanoma, 44 with melanocytic nevi and 91 with common skin nevi (Table 1).

Table 1

**Age distributions of the patient with melanoma and nevi**

| Patient group | Age interval (in years) | | | Total |
|---|---|---|---|---|
| | 18–40 | 41–60 | 61–70 | |
| 1 | 2 | 3 | 4 | 5 |
| Skin melanoma, n / % | 6<br>13,3 % | 25<br>55,6 % | 14<br>31,1 % | 45<br>100,0 % |
| Dysplastic nevus, n / % | 31<br>70,5 % | 11<br>25,0 % | 2<br>4,5 % | 44<br>100,0 % |
| Pigmented nevus, n / % | 70<br>76,9 % | 17<br>18,7 % | 4<br>4,4 % | 91<br>100,0 % |

SPM analysis of digital images was performed in ImageJ using the following sequence of steps:

- selection of the borders and segmentation of the image of the;
- decomposition of the image into primary colours (red, green, and blue) and creation of the histogram;
- histographic evaluation of the spectral colours (red, green and blue) using relative and absolute values and construction of corresponding graphs in Microsoft Excel.

Then, we created R-G-B-spektrophotograms using Microsoft Excel on the grey scale ranging from 0 to 255 (Figure 1).

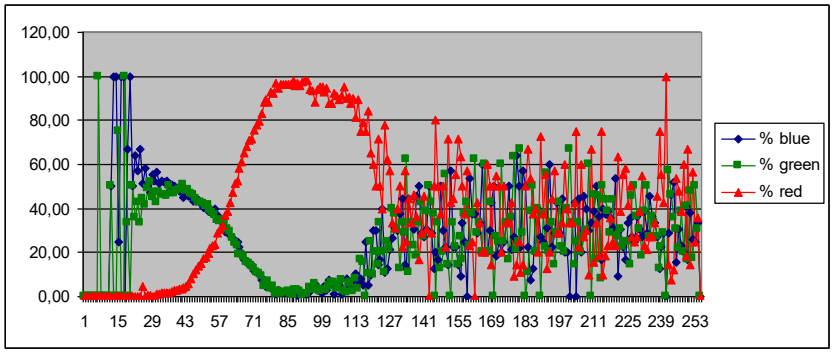


Figure 1. R-GB spectral diagram of pigmented lesion of the skin.

We evaluated R-G-B colour curves due to the abscissa axis (in units of grey scale) and the axis of coordinates (amplitude height in %) – these criteria were basic for differential diagnosis of pigmented lesion of the skin of various genesis [9].

Red (R) spektrophotograms with the following options were the main diagnostic criterion:

—The beginning of the diagram on abscissa axis of the grey scale is 5% colour intensity ($I_{start}$);

—colour intensity where maximum amplitude of the diagram graph with the corresponding number of points ($I_{max}$) on the abscissa axis is observed;

—area under the curve ($AUC_{128}$) to the intensity limit of 128 units.

**Research results and discussion.** We have studied the results of spectrophotometric diagnostics of 180 digital images of pigmented skin lesions of different localization in 85 patients of 18 to 70 years old who received surgical treatment in Ternopil Regional Oncologic Hospital.

We found out some differences in R-G-B-histogram of digital images of melanoma and melanocytic (dysplastic) nevi by direct vision. The most significant changes were defined in red spectrum diagrams, which were basic for differential diagnosis.

To validate this technique, the SPM pre-surgery findings were compared with the results of morphological studies. This comparison was conducted for two groups of pigmented lesions. During pathohistological examination melanoma was found in 45 cases and dysplastic nevi – in 44 cases, pigmented nevus – in 91 cases. In these groups, the analysis of red spektrophotograms was performed: Istart, Imax and $AUC_{128}$ (Table 2).

Table 2

**The results of red spektrophotograms study in cases of skin melanoma, melanocytic and normal nevi**

| Measurement | Lesion | n | Average | Median | Standard deviation | Minimum | Maximum |
|---|---|---|---|---|---|---|---|
| **$I_{start}$** | Melanoma | 42 | 27,3 | 28,5 | 15,1 | 4,0 | 54,0 |
| | Melanocytic | 47 | 37,7 | 38,0 | 13,8 | 11,0 | 68,0 |
| | Nevus | 91 | 86,6 | 82,0 | 27,4 | 3,0 | 160,0 |
| **$I_{max}$** | Melanoma | 42 | 74,6 | 83,5 | 35,5 | 7,0 | 123,0 |
| | Melanocytic | 47 | 122,4 | 118,0 | 25,0 | 77,0 | 195,0 |
| | Nevus | 91 | 156,5 | 158,0 | 27,2 | 61,0 | 229,0 |
| **$AUC_{128}$** | Melanoma | 42 | 5939,2 | 5808,6 | 1155,2 | 3751,5 | 8443,3 |
| | Melanocytic | 47 | 4849,5 | 5064,2 | 1032,8 | 2605,5 | 6239,4 |
| | Nevus | 91 | 2002,0 | 1782,4 | 1334,3 | 0 | 4868,9 |

To evaluate the differences between the groups, we performed an analysis of variance (ANOVA) according to model: dependent variable – the analysed measurement (melanoma or dysplastic nevi) for further paired comparison of Tukey Multiple Comparison test groups. We used Shapiro-Wilk test to check restrictions of ANOVA against normality. We studied the ranges to determine the variable $I_{start}$ and found out statistically significant differences between the groups for each of these measurements ($I_{start}$, $I_{max}$ and $AUC_{128}$).

We used cluster analysis on two variables to set the limit values for group separation (melanoma and dysplastic nevi). The variable ‘group’ was the first. The analyses of related measurements ($I_{start}$, $I_{max}$ and $AUC_{128}$) was the second. We used χí-square statistic, adjusted by Yates corrected chi-square, as the metrics to estimate the distance between the clusters. Step by step we set the limit value and processed the quantitative variable into categorical one for this.

To evaluate the significant differences between the groups we performed the analysis of variance (ANOVA) for further paired comparison of Tukey Multiple Comparison test groups. The test assumptions were made using criteria Shapiro-Wilk, we also held rank analysis. We used Shapiro-Wilk test to check limited value and also studied the ranges.

The search for limited mathematical value for differentiation of lesions due to certain measurements was performed in two phases. First, we determined if the pigmented lesion relates to the group 'Melanoma/melanocytic nevi' or to the group 'normal nevi'. During the second phase we differentiated melanoma and melanocytic nevi. We searched for limited value to divide into the groups using cluster analysis on two variables using Pearson's chi-square test with Yates' correction and odds ratio of 95% confidence interval.

Due to the results of the first stage we determined that the limited value for pigmented lesions division into groups 'Melanoma'/'melanocytic nevi' and 'normal nevi' are: to $I_{start}$ = 56.5 units (sensitivity 95.5%, specificity 92.3%); for $I_{max}$ = 125.5 units (sensitivity 77.5%, specificity 93.4%); for $AUC_{128}$ = 3706.8 (sensitivity 93.3%, specificity 86.8%). So, if the red spectrum diagram $I_{start} \leq 56{,}5$ units $I_{max} \leq 125{,}5$ units, and $AUC_{128} \geq 3706{,}8$, a pigmented lesion refers to the group 'Melanoma'/'melanocytic nevi', if the other measurements – to the group 'nevi'.

Due to the results of the second phase of cluster analysis, to find out the limited values for the differentiation of melanoma and melanocytic nevi, the following measurements were defined: for $I_{start}$ = 18.5 units (sensitivity 33.3%, specificity 95.7%); for $I_{max}$ = 115.5 units (sensitivity 90.5%, specificity 61.7%); for $AUC_{128}$ = 5442.1 (sensitivity 71.4%, specificity 72.3%).

So, if at the red spectrogram the pigment lesion is $I_{start} \leq 18{,}5$ units, $I_{max} \leq 115{,}5$ units, $AUC_{128} \geq 5442.1$ it relates to the group 'melanoma', if the other measurements – to the group 'melanocytic nevi'.

**Examples of clinical use of spectrophotometric diagnosis of pigmented lesions of skin**

*Case history No 1.* A 58-year-old patient R., pigmented lesion of the skin at left shin (Figure 2). Antecedent anamnesis: it appeared about five years ago, the lesion's size was increasing. According to clinical criteria ABCD: A +, B +, C +, D +.

Figure 2. Patient R.: digital image of pigmented lesion of skin.

We performed a SP study of pigment lesion digital image. We obtained red (R), green (G) and blue (B) spectrograms. Red spectrum begins to increase at 7 units of the abscissa axis ($I_{start}$) and reaches a maximum at a point of 104 units. ($I_{max}$) with a maximum amplitude of 90% (Figure 3). Green and blue spectra have low spectral activity. In this case $I_{start}$ = 7 units, which is less than the limit value $I_{start}$ (18.5 units), $I_{max}$ = 104 units, which is also less than the limit value $I_{max}$ (115.5 units.), $AUC_{128}$ – 7373,94. Such red diagram spectrum indicates a high probability of malignant disorders, such as melanoma.

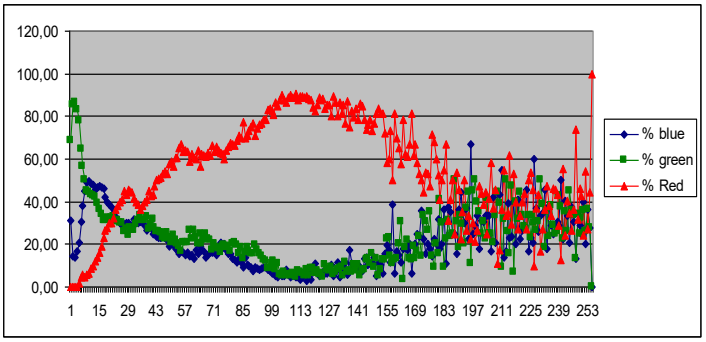


Figure 3. R-G-B spectrogram of pigmented lesion.

Cytological examination – melanoma cells. Surgery – wide lesion resection with skin grafting of the wound. Histopathological conclusion № 12222-6 – nevus melanoma, 1.3 mm Breslow thickness, the third Clark level of invasion.

Final diagnosis: Melanoma of the skin of the upper third of left shin posterior surface pT2aN0M0, stage IA.

So, the SPM diagnostics results of melanoma coincided with morphological examinations of the lesion after surgery.

*Case history No 2.*

A 19-year-old patient L. (case history No 1988) was examined on January 29, 2015. Antecedent anamnesis: pigmented skin lesion was increasing for one year.

Dermatological status. Skin photo type II due to Fitzpatrick classification. A lesion of the skin 11 × 8 × 2 mm on abdominal wall with clear contours was observed (Figure 3). Uneven pigmentation: dark brown with black area. Regional lymphatic nodes were not palpable.

Due to the ABCD rule of visual examination the coincidence of all the criteria was defined A+, B+, C+, D+. According to the Glasgow test system 4 of 7 criteria coincided: changes 1 – in size, 2 – in contours, 3 – in colour, 4 – the biggest size (more than 7 mm.).

SPM (Figure 4): the red diagram $I_{start}$ = 31 units. The abscissae axis, the curve reaches maximum at a point of 133 units. ($I_{max}$) with a maximum amplitude of 100%. In this case $I_{start}$ is more than limit value (18.5 units), also $I_{max}$ is more than limit value (115.5 units), $AUC_{128}$ – 1699,03. Such red spectrum diagram indicates no malignant disorders.

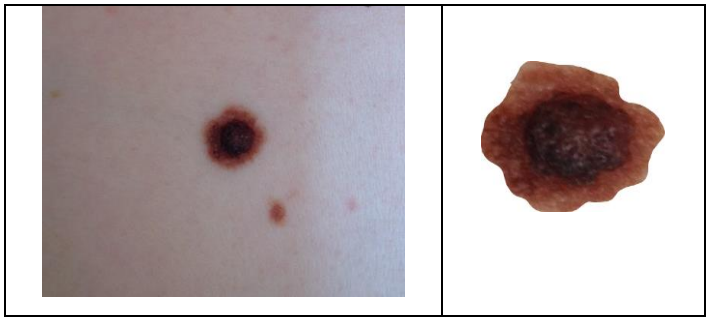

Figure 3. Digital clinical photo of pigmented lesion of skin and its 'cut' image for SP.

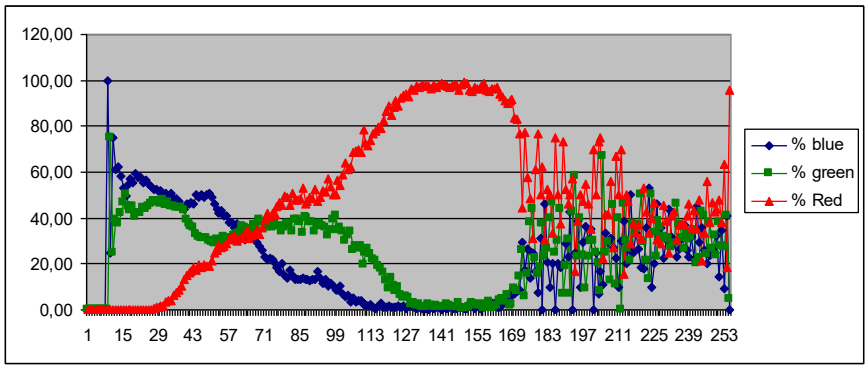


Figure 4. R-G-B spektrophotogram of pigmented lesion.

The surgery was performed under regional anaesthesia. Histopathological examination of the lesion No 1719-21 from February 06, 2015 proved border-line pigmented nevus.

So, presurgical SP diagnostics showed no malignant disorder, and postsurgical morphological examination proved it.

According to the results of the research we can determine the right diagnosis due to the spectrophotometric study of digital images of pigmented lesions of the skin, despite their morphological diversity, which can be used in telemedicine health care consultations on melanoma and melanocytic nevi of skin.

All sufficient methods of non-invasive diagnostics, despite the high sensitivity and accuracy rates, require contact with a patient that is a patient's presence during the examination. That is why these non-invasive contact methods cannot be used for mass screening of the population. We proposed non-contact spectrophotometric diagnostics of digital images at a distance that can be used as a screening test for pigmented disorders of skin.

**Conclusions.** Spectrophotometric diagnostics of melanoma and melanocytic nevi is based on specific characteristic of red, green and blue spectral diagrams for malignant and benign pigmented lesions of skin.

Red spektrophotograms are the main diagnostic criteria. The following measurements among them are defined: $I_{start}$ is the beginning of red diagram of 5% intensity, $I_{max}$ – the highest amplitude of the red diagram, $AUC_{128}$ – the area under the red diagram curve.

Comparison of histological, SPM and ABCD diagnostic criteria proves that the study of digital images of lesions using the software ImageJ improves significantly the diagnostics of melanocytic lesions of skin even during the prehospital period.

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