RGB video microscopic system for *in vitro* monitoring of optical properties of hair shaft and follicle

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ABSTRACT

We presented RGB video microscopic system for *in vitro* monitoring of optical properties of hair shaft and follicle and experimental results of study of the hair optical and geometrical properties using RGB analysis technique.

Keywords: RGB analysis, optical properties, hair, *in vitro* measurements

1. INTRODUCTION

To study of hair pigmentation and morphology needs an objective and quantitative measure of its optical properties and geometry. Optical transmittance at the selected wavelengths and hair shaft diameter are the parameters, which being measured will satisfy many of medical and cosmetic applications, like laser hair removal,[1-4] studies of the hair phenotype or the hair-color mosaicism,[5] the internal structure examination,[6] or investigation of abnormal melanin aggregation into giant melanosomes for patient with Chediak Higashi syndrome.[7]

Melanin granules in the cortical layer of the hair shaft have a substantial effect on the optical properties of hair. The perceived color of hair depends not only on the type of melanin but also on the quantity, location, and shape of the granules in the cortex. Two types of melanin – eumelanin and pheomelanin produce a distinct pigmentation respectively for black - brown and red – blond hairs.[5, 8, 9] A relative deficiency of melanin granules results light colors such as blond and gray.

A few measuring systems for optical and morphological properties of hair fibers are described in literature.[4-7] Microspectrophotometry in a wide range of wavelengths is a very useful technique for melanins concentration monitoring, but it is a labor-content technique.[5] Low-coherence reflectometry is a very promising technique allows for high spatial resolution measurements across and along the hair shaft, but this technique also is a labor-content and costly.[6] The express analysis of the hair optical and geometrical properties, which is needed for medical and cosmetic practice, can be provided using color or black & white spectral CCD or tube cameras in transmittance or reflectance mode.[4, 7, 10, 11-15]

In this work a color-camera-based technique, so called RGB (red-green-blue) color measurements[10,14,15] was used for evaluation of spectral reflectance of the human hair shafts. Color-measurement technique, which analyzes the whole color spectrum, is suitable for this purpose because it perceives hair's color like the human eye; different standard color systems (coordinates) are available; when using a microscopy system the spatial resolution can be satisfactory well; measurements can be done quickly and automatically.

2. MATERIALS AND METHODS

Quantitative estimation of chromophores content in a scattering medium is based on the analysis of reflectance spectra in the selected spectral range. Optical density of turbid medium $D(\lambda)$ is introduced as[12]

$$D(\lambda) = -\log\left(R(\lambda)\right), \tag{1}$$

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where $R(\lambda)$ is the reflectance at the wavelength λ. Relation (1) allows for measure the relative changes of absorbing properties of a scattering medium. Spectrum $D(\lambda)$ for melanin has a linear approximation in spectral range from 620 to 720 *nm* and melanin content in tissue such as the human skin is determined as magnitude proportional to the linear slope of spectral dependence $D(\lambda)$ in this spectral range.[8, 13]

2.1 Experimental setup and measuring procedure

The experimental setup is shown in Fig. 1. The color imaging system is composed of a video-microscope (VHS color camera Panasonic NV-RX70EN, Tokyo, Japan (5) and microscope objective (4)) interfaced with a personal computer (6). The examined object – a plane plate with attached black and white test objects and hair shaft under study (1) – is illuminated by white light, which is guided to the object by an optical cable (2) from the light source (3) (halogen lamp). In order to avoid glares and to get smoothly illuminated high quality images the illuminating light was guided to the object under the angle of 30^0 along the hair shaft. The distance from the object surface to the objective (of magnification from 5^x to 8^x) was about 20 mm, and the dimensions of the illuminated object (8 x 6 mm) were much greater then the field of view of the imaging system (1.2 x 0.9 mm).

The image obtained provides a set of data which offers a spatial resolution of 647 (horizontal) x 485 (vertical) pixels and a color resolution of 256 levels for each band of red (*R*, $\lambda_{\max} = 600\ nm,\ \Delta\lambda = 51.4\ nm$), green (*G*, $\lambda_{\max} = 540\ nm,\ \Delta\lambda = 74.1\ nm$) and blue (*B*, $\lambda_{\max} = 460\ nm,\ \Delta\lambda = 47\ nm$), indicating that each band has an independent brightness in 256 gradation (0 – darkness; 255 - brightest).

For calibration of experimental setup on the wavelength dependence we have used the next procedure. The reflectance of white and laser (632.8 nm and 488.0 nm) light was provided from a white screen. Dispersion of the reflected light was done by the diffraction grating spectrometer. This white light source was used for hair shaft optical density measurements. Standard procedure of RGB filtering on the base of Mathcad (Mathsoft, USA) software was used for the wavelength dispersion of preliminary spatially-dispersed white light. These three spectra were used as calibration spectra defining intensity of white light source at *R* (red), *G* (green) and *B* (blue) coordinates (wavelengths), their wavelengths, and wavelength broadening (width). Results of such calibration is presented in Fig. 2.

The brightness level and dynamic range of brightness were tested by using of two test-objects (black and white) disposed at the examined object together with the hair shaft under study. Prior the starting of each series of measurements, the electronic white balance adjuster of Panasonic camera was used to improve the mean brightness of the image of a white test-object to be constant.

Regions of the interest can be selected from the total image for *R*, *G* and *B* bands separately using Mathcad (Mathsoft, USA) software. Using the mean brightness values of the white standard (white test-object) (R_{test}, G_{test} and B_{test}) and those of hair tested (R_{hair}, G_{hair} and B_{hair}), the integrated reflectance (R_{R}, R_{G} and R_{B}) in each band was defined as follows:

$$R_R = R_{hair}/R_{test},\quad R_G = G_{hair}/G_{test},\quad R_B = B_{hair}/B_{test}, \tag{2}$$

Optical density of the hair shaft in each band was defined as follows:

$$D_R = -\log(R_R),\ D_G = -\log(R_G),\ D_B = -\log(R_B). \tag{3}$$

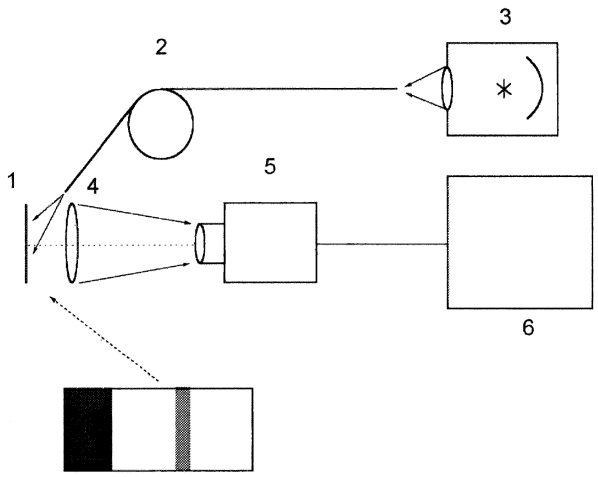

Figure 1. Experimental setup for RGB measurements: 1 – object – a plane plate with attached black and white test objects and hair shaft under study, 2 - light guide, 3 - halogen lamp, 4 - objective, 5 – VHS color RGB-camera, 6 - PC, 7 - black test-object, 8 - white test-object, 9 – hair shaft.

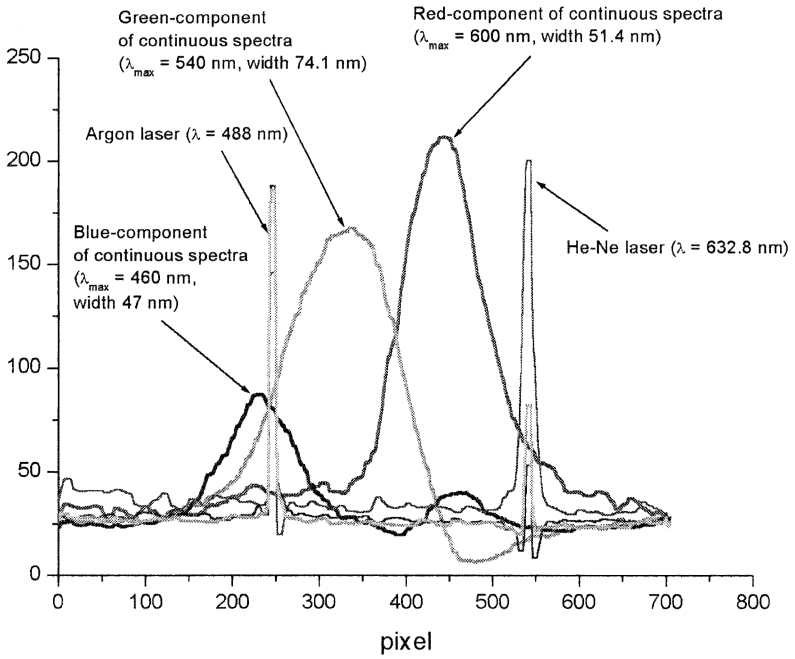

Figure 2. Laser light calibration of VHS color CCD camera, used in RGB measurements.

2.2 Method for processing of measurements of hair shaft optical properties and diameter

To process hair images we have developed the special computer program with using Mathcad software (MathSoft Inc., USA). Algorithm of the program includes the following steps:

1. The base image (photo) was separated in three color matrixes of red, green, or blue components by intrinsic function of Mathcad system. Figure 3 demonstrates one of the typical images.

2. The marker (continuos line which transverses this photo) allows for making a choice of image scanning area across the hair shaft. The width of the scanning band along the hair shaft was 21 pixels (10 pixels up and down of the marker). Averaging of the measured values was done within this band. The choice of the scanning band was defined visually (glares, dust particles, particles of glue, and other external optical inhomogeneities were avoided). Furthermore, obtained curves were smoothed to remove the noise. Result of this procedure is shown in Fig. 4. Squares accord to distribution of red component along the band, circles and up triangles accords to distribution of green and blue components, respectively. It should be noted that usually about 30-50 measurements (pixels) of reflectance within hair shaft diameter were done.
3. To define reflectance of hair shaft we took the ratio of hair brightness (reflected intensity) I_h to reference brightness (white paper) I_w for each color component. In addition the background signal from black paper I_b was subtracted from both hair shaft and reference brightnesses (see, Fig. 4). Thus reflectance R was defined as

$$R = \frac{I_h - I_b}{I_w - I_b} \tag{4}$$

where I_h, I_b, and I_w are the brightness (reflected intensity) of hair shaft, background, and reference, respectively. Reflectance was measured for the central part of the hair shaft diameter, where the reflectance was minimal. Averaging for 10-20 pixels around the minimal reflectance was done, that allowed us to receive more smooth data, but caused of about 15-20% lower optical density in comparison with the using of the absolute minimum of reflectance. Using data for all color components, we have obtained values of optical density D for three wavelengths 600 (red), 540 (green), and 460 (blue) *nm*

$$\begin{aligned} D_{red} &= -\log R_{red} \\ D_{green} &= -\log R_{green} \\ D_{blue} &= -\log R_{blue} \end{aligned} \tag{5}$$

4. To obtain the hair diameter its diameter in pixels at it's half of height (see, Fig. 4) was estimated, then using the spatial dispersion of the imaging system (see, Fig. 1), which was equal to 521 pixel/mm, diameter was calculated in micrometers. It should be noted that such definition of the hair shaft diameter can causes some underestimation of the hair shaft diameter.

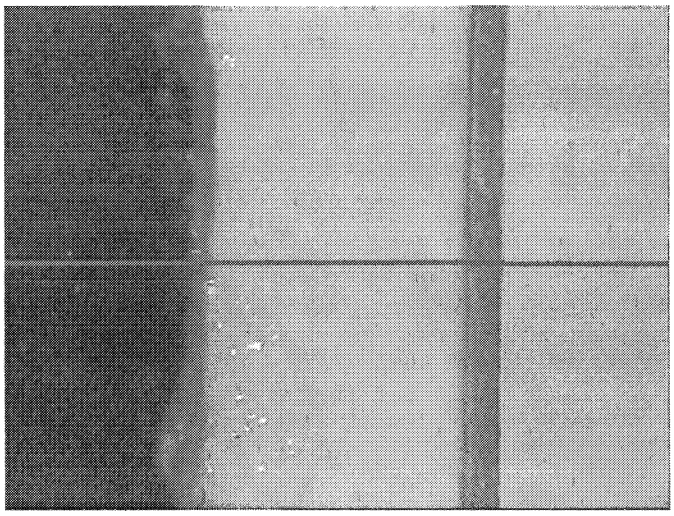

Figure 3. The typical image of the studied sample (see, Fig. 1); from the right to the left: black-test, white-test, hair shaft, again white test; red line shows a scanning line, 21 scans at and around this line was provided, the typical averaged (for 21 scans) scans are presented in Fig. 4.

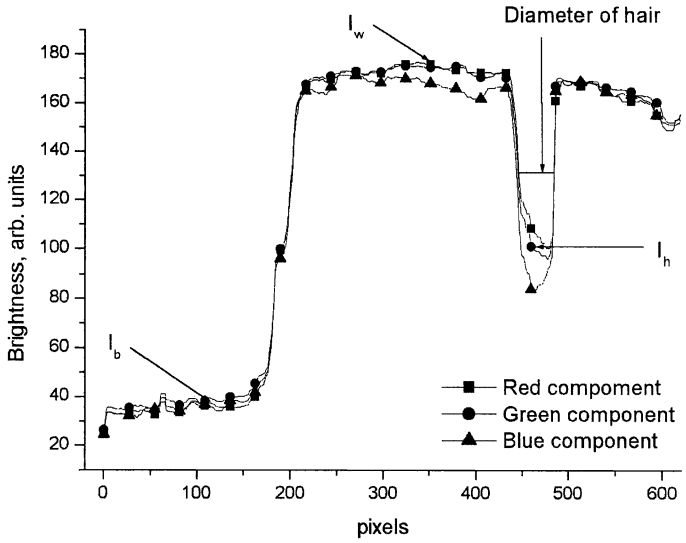

Figure 4. The typical averaged scans for already separated spectral components. Not all measured values (pixels) are shown; I_h, I_b, and I_w are the brightness (reflected intensity) of hair shaft, background, and reference, respectively.

2.4 Estimation of melanin concentration

The dependence of optical density (1) in the wavelength range above 600 *nm* can be presented as a straight line.[2] Such linear dependence was used for estimation of melanin concentration in the human skin in the terms of *DOPA* melanin.[2] Using the absorbance measurements for *DOPA* melanin solutions with various concentrations the following linear approximation in the range 620-720 *nm* was received:[2]

$$Absorbance = -\ln(T) = 2.3 \cdot D = C_1 - C_2 \cdot 10^{-3} \cdot \lambda \tag{6}$$

where T is the transmittance of *DOPA* melanin solution, λ is the wavelength in *nm*;

$$C_1 = 0.0688 + 0.103 \cdot C \tag{7}$$

$$C_2 = 0.0794 + 0.124 \cdot C \tag{8}$$

C is the *DOPA* melanin concentration in *mg/ml.*

From this we can take that $C_1 = 0.84\ C_2$ [8] and

$$Abs(\lambda) = C_2\left(0.84 - \lambda \cdot 10^{-3}\right) = C_1\left(1 - \frac{\lambda \cdot 10^{-3}}{0.84}\right) \tag{9}$$

From Eq. (9) we have

$$C_1 = \frac{Abs(\lambda)}{1 - \frac{\lambda \cdot 10^{-3}}{0.84}} \qquad and \qquad C_2 = \frac{Abs(\lambda)}{0.84 - \lambda \cdot 10^{-3}} \tag{10}$$

From Eqs. (7) and (8) we have

$$C = \frac{C_1 - 0.0688}{0.103} = \frac{C_2 - 0.0794}{0.124} \tag{11}$$

For performing of measurements in reflectance mode (RGB analysis technique): $Abs(\lambda) = -\ln(R)$, where R is reflectance for selected wavelength.

3. RESULTS AND DISCUSSION

For testing of RGB video microscopic system we used two series of the hairs. The first series contained 51 samples of the hairs and the second series contained 30 samples of the hairs.

Using procedure described in Sections 2.1 and 2.2 we have obtained the following values of optical density for the first series of the hairs (averaged for 51 samples):

$$\begin{aligned} D_{600} &= 0.254 \pm 0.073 \ \ (sd) \\ D_{540} &= 0.305 \pm 0.093 \ \ (sd) \\ D_{460} &= 0.386 \pm 0.123 \ \ (sd) \end{aligned} \tag{12}$$

where sd is the standard deviation.

For the second series (containing 30 hairs samples):

$$\begin{aligned} D_{600} &= 0.443 \pm 0.200 \ \ (sd) \\ D_{540} &= 0.522 \pm 0.226 \ \ (sd) \\ D_{460} &= 0.630 \pm 0.274 \ \ (sd) \end{aligned} \tag{13}$$

The mean absorbance (*Abs*) for 51 samples of hair shafts of the first series and for 30 samples of the second series of the hairs measured using RGB reflectance technique for three colors: blue (460 nm), green (540 nm), and red (600 nm) are presented in Table 1.

Table 1. The mean absorbance (*Abs*) of *n* samples of hair shafts of first and second series of hairs measured using RGB reflectance technique for three colors: blue (460 *nm*), green (540 *nm*), and red (600 *nm*). Standard deviation (*sd*) values also presented. For the first (51 hair samples) series – optical system (OS) was slightly unfocused, for second (30 hair samples) series – OS was sharply focused.

Name of the series	*n*	*Abs* 460 *nm*	*Abs* 540 *nm*	*Abs* 600 *nm*
first	51	0.90±0.28	0.69±0.21	0.58±0.16
second	30	1.45±0.63	1.20±0.52	1.02±0.46

The mean diameter of the hairs from the first series, averaged for 51 samples is equal to

$$d_{mean} = 60.94 \pm 15.74 \ \ (sd); \qquad [d_{mean}] = \mu m \tag{14}$$

The mean diameter of the hairs from the second series, averaged for 30 samples is equal to

$$d_{mean} = 58.0 \pm 10.0 \ \ (sd); \qquad [d_{mean}] = \mu m \tag{15}$$

However, RGB color reflectance measurements give about 20% bigger mean diameter than measurement performed with micrometer measurements. For studied hair shafts the mean diameter is equal to 50 μm. Probably, such difference is explained by the influence of light scattering within hair shaft, when about 1.2-1.7 bigger mean pathlength in a scattering media can lead to a thicker image.

For D (600 *nm*) = 0.25, *Abs* (600 *nm*) = 0.58, using relationship $C_1 = 0.84\ C_2$ [8] for solutions of *DOPA* melanin, we can found that C = 21 *mg/ml* for *DOPA* melanin taken as a reference for hair shafts of the first series (51 hairs samples). For the second series we have calculated C = 33.6 *mg/ml* for *DOPA* melanin taken as a reference for our experimental data. Thus, we can conclude that studied hair shafts have melanin concentration on the level of a mean and highly pigmented human skin.[8]

4. CONCLUSION

RGB technique on the basis of VHS color CCD camera is a useful technique for express estimation of the optical density and diameter of the hair shaft and estimation of the equivalent melanin concentration (in *DOPA* melanin units). RGB technique due to usage of visible light spectral lines is very sensitive to melanines content in the hair shaft. The advantages of technique are: simple instrumentation, available standard software, and the possibility of making measurements in reflectance mode without any immersion of the hair shaft.

The disadvantages are: only visible wavelength range is available; the broad band equivalent light sources and corresponding averaging of the optical density (absorbance) values for the wavelength range of about 50-70 *nm*. For absolute determination of melanin concentration (absorption coefficient) on the basis of RGB color-resolved and spatially-resolved measurements the inverse scattering problem should be solved; the inverse Monte Carlo technique is the most useful to solve such inverse problem due to complex light-source-sample-detector geometry.

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