

# Spatial-resolved Microspectrophotometry for Hair Optical Properties and Geometry Studies: CCD Hair Tester

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## ABSTRACT

Results of development of spectrophotometric technique for human hair testing in the laboratory and clinical conditions are presented. Hair tester, which can be used for measurements of optical parameters of in-vitro hair samples at three fixed wavelengths of illuminating light, is described. Usage of CCD sensor as photodetector allows one to provide spatial-resolved analysis of the hair optical properties and to measure geometric parameters of the hair sample. Experimental results obtained with designed instrumentation and technique are presented.

**Keywords:** transmittance, absorbance, CCD sensor, hair.

## 1. INTRODUCTION

To study the human hair pigmentation and morphology one 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<sup>1-4</sup>, studies of the hair phenotype or the hair-color mosaicism<sup>5</sup>, the internal structure examination<sup>6</sup>, or investigation of abnormal melanin aggregation into giant melanosomes for patients with Chediak Higashi syndrome<sup>7</sup>.

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<sup>5,8,9</sup>. A relative deficiency of melanin granules results in light colors such as blond and gray. A few measuring systems for optical and morphological properties of hair fibers are described in literature<sup>4-7</sup>.

Microspectrophotometry in a wide range of wavelengths is a very useful technique for melanins concentration monitoring, but it is a labor-content technique<sup>5</sup>. 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<sup>6</sup>. 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<sup>4,7,10,11</sup>.

This paper describes results of the development of spatial-resolved microspectrophotometric technique for human hair testing on the base of commercially available monochrome CCD camera. Designed instrument allows one to measure not only optical properties of the human hair for different wavelengths but also the hair shaft diameter for different cross-sections.

## 2. INSTRUMENT DESCRIPTION

The CCD hair tester is presented in Fig. 1. Object under study (human hair shaft in the immersion oil) is placed in the object plane of the imaging lens (microscope objective with magnification equal to 8 and numerical aperture equal to 0.20), and is illuminated by fiber-optic illuminator (6 mm in diameter) assembled with interference filters (the bandwidth of each was about 10 nm centered at 624, 700, and 800 nm, filter at 624 nm was combined from two filters). Prism is used to change the optical axis direction. Image of the part of hair under study is formed on the photosensitive area of the black & white

spectral CCD camera (Electrim 1000); used length of the microscope tube (125 mm) allows one to obtain linear magnification of the hair image approximately equal to  $8\times$ . For given number of pixels in rows and columns of CCD chip (192×165 for non-interlace mode) the number of pixels in the transverse direction of the hair image is approximately equal to 35 (for 50- $\mu\text{m}$  hair diameter). The special software developed by the camera producer (Electrim Inc.) supports camera operation. This software allows one to capture images of the object under study in 8-bit bitmap format.

To exclude CCD saturation at 624 nm a combination of two filters was used. The special MathCad (MathSoft Inc., USA) computer program was used to process the captured images. Number of pixels in the transverse direction of hair image allows one to evaluate the hair diameter; comparison of the pixel code in the central part of the hair image (averaged within 25-30 pixels across and 21 along hair shaft image) and for reference field out of hair image allows one to evaluate the hair transmittance for given wavelengths (624, 700 or 800 nm). Ratio of code values of pixels is calculated by taking into account the background signal caused by the dark current of CCD elements. To measure the hair diameter preliminary calibration by using precision 50- $\mu\text{m}$  grid on the glass substrate is applied.

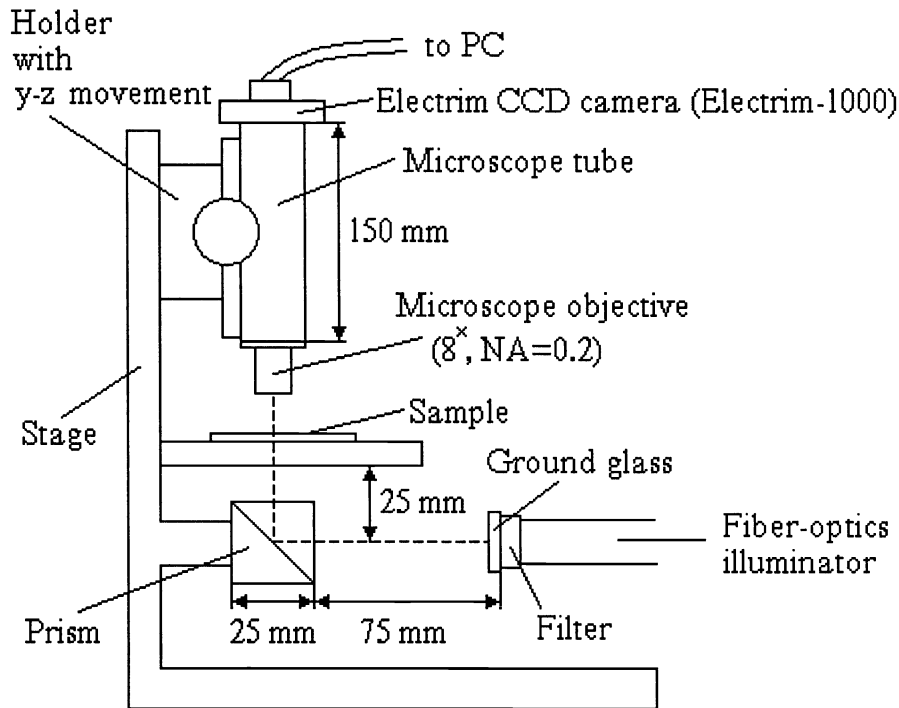


Fig.1. Optical scheme of CCD hair tester.

### 3. MEASUREMENT TECHNIQUE

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 hair shaft stored image at one of the wavelengths (624, 700, or 800 nm) was scanned across the hair shaft within the band of 21 pixels (10 pixels up and down of the preliminary chosen marker line) along the hair shaft. Averaging of measured transmittance values was done within this band. The choice of the scanning band position 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. It should be noted that usually about 25-30 measurements (pixels) of transmittance within hair shaft diameter were done.

2. To define transmittance of hair shaft we took the ratio of intensity of transmitted light in the area of hair image  $I_h$  to intensity of light transmitted out of the hair image,  $I_r$  - reference. In addition the background signal defined by the dark current of CCD elements  $I_b$  was subtracted from both hair shaft and reference signals. Thus transmittance was defined as

$$T = \frac{I_h - I_b}{I_r - I_b}, \quad (1)$$

where  $I_h$ ,  $I_b$ , and  $I_r$  are the transmitted intensity of hair shaft, background, and reference, respectively. Transmittance was measured for the central part of the hair shaft diameter, where the transmittance was minimal, averaging for 25-30 pixels around the minimal transmittance was done. The transmittance profile across the hair shaft looked like trapezium with a flat bottom within 25-30 pixels (the main part of hair shaft diameter around its center). That is why the averaging procedure did not reduce the mean value of transmittance. Using data for all three wavelengths we have obtained values of absorbance  $Abs$ , and attenuation (turbidity)  $\mu_t$  at three wavelengths 624, 700, and 800 nm.

3. To measure the hair shaft diameter a preliminary calibration of the hair CCD tester was done by using a precision 50- $\mu\text{m}$  grid on the glass substrate.

#### 4. RESULTS OF MEASUREMENTS

Results of measurements for two series of hair shafts obtained from Palomar Medical Technology Inc.(USA) are presented in Tables 1 and 2. The average values of transmission ( $T$ ), absorbance ( $Abs$ ), and attenuation coefficient ( $\mu_t$ ), as well as their mean square deviation ( $SD$ ) at the wavelengths 624, 700 and 800 nm for CCD tester measurements are shown. The standard deviation ( $SD$ ) of these measurements is defined by variations of hair transmittance and diameter within each series. The instrumental  $SD$  for both transmittance and diameter is about 2-3 %. For diameter measurements such error is defined by a discrete step of one pixel.

Table 1. Transmission ( $T$ ), absorbance ( $Abs$ ) and attenuation coefficient ( $\mu_t$ ) for hair shafts of first series, and their mean square deviations ( $SD$ ); averaged for  $n$  samples. Attenuation coefficient ( $\mu_t$ ) was calculated for diameter  $d=50 \mu\text{m}$ .

Parameter	CCD, $\lambda=624 \text{ nm}$	CCD, $\lambda=700 \text{ nm}$	CCD, $\lambda=800 \text{ nm}$
$n$	40	40	40
$T, \%$	$62.7 \pm 14.2$	$64.9 \pm 15.0$	$68.6 \pm 16.3$
$Abs$	$0.47 \pm 0.21$	$0.43 \pm 0.21$	$0.38 \pm 0.22$
$\mu_t, \text{cm}^{-1}$	$94 \pm 42$	$86 \pm 42$	$76 \pm 44$

Table 2. Transmission ( $T$ ), absorbance ( $Abs$ ) and attenuation coefficient ( $\mu_t$ ) for hair shafts of second series, and their mean square deviations ( $SD$ ); averaged for  $n$  samples. Attenuation coefficient ( $\mu_t$ ) was calculated for diameter  $d=50 \mu\text{m}$ .

Parameter	CCD, $\lambda=624 \text{ nm}$	CCD, $\lambda=700 \text{ nm}$	CCD, $\lambda=800 \text{ nm}$
$n$	27	27	27
$T, \%$	$55.9 \pm 8.8$	$60.6 \pm 6.5$	$71.6 \pm 10.6$
$Abs$	$0.61 \pm 0.16$	$0.50 \pm 0.10$	$0.33 \pm 0.08$
$\mu_t, \text{cm}^{-1}$	$122 \pm 42$	$100 \pm 37$	$66 \pm 25$

Table 3 presents results of measurements of optical parameters and diameter for third group of hair shafts with a small number of samples, taken from Palomar collection.

Table 3. The mean diameter ( $d$ ), transmission ( $T$ ), absorbance ( $Abs$ ), and attenuation coefficient  $\mu_t$  for hair shafts of various series of Palomar collection with a small number of hair samples, and their standard deviations ( $SD$ ) at the wavelengths 624, 700 and 800 nm; averaged data for  $n$  samples of each series  $N$  ( $N$  is the series number in Palomar notation).

$N$ ( $n$ )	$d, \mu\text{m}$	$\lambda = 624 \text{ nm}$			$\lambda = 700 \text{ nm}$			$\lambda = 800 \text{ nm}$		
		$T, \%$	$Abs$	$\mu_t, \text{cm}^{-1}$	$T, \%$	$Abs$	$\mu_t, \text{cm}^{-1}$	$T, \%$	$Abs$	$\mu_t, \text{cm}^{-1}$
5 (4)	66.7± 16.0	57.8± 5.6	0.55± 0.10	82±27	64.7± 4.8	0.44± 0.04	68±22	67.6± 12.0	0.40± 0.14	60±15
6 (4)	61.4± 42.0	65.5± 10.2	0.42± 0.04	68±25	65.8± 5.8	0.42± 0.09	67±20	69.5± 5.6	0.37± 0.08	59±18
10 (7)	80.1± 20.0	44.9± 11.8	0.81± 0.12	101±33	51.8± 10.8	0.66± 0.11	82±27	54.0± 11.0	0.62± 0.10	77±19
23 (4)	73.5± 13.6	63.2± 12.0	0.47± 0.20	64±27	66.6± 10.0	0.42± 0.15	57±21	74.5± 9.4	0.30± 0.12	41±17
25 (6)	102.0± 29.0	57.1± 20.6	0.56± 0.07	55±14	60.1± 17.8	0.51± 0.06	50±12	71.6± 12.0	0.34± 0.16	33±16
39 (4)	70.1± 39.0	54.6± 5.0	0.61± 0.09	86±12	56.1± 12.0	0.58± 0.21	83±15	64.1± 9.0	0.45± 0.14	63±21
42 (13)	53.8± 24.0	61.0± 10.2	0.51± 0.16	93±31	64.8± 14.0	0.47± 0.15	88±28	69.6± 15.1	0.36± 0.12	68±22
M21 (10)	76.7± 26.0	56.5± 14.2	0.58± 0.19	72±26	59.8± 10.2	0.52± 0.09	67±19	68.3± 10.1	0.38± 0.14	50±10
M22 (9)	55.1± 26.0	60.0± 14.2	0.52± 0.19	94±23	63.2± 14.1	0.47± 0.18	84±23	67.3± 14.4	0.40± 0.15	72±19

## 5. CONCLUSIONS

CCD technique (CCD hair shaft optical tester) is a robust technique that allows fast measurements of the hair shaft diameter and its optical transmittance, and calculations of absorbance and attenuation coefficient. The advantages of technique and instrument are: fast measurements of hair diameter and light transmittance from the same image at 624, 700, or 800 nm; the possibility to make many measurements for a short period; relatively small instrumental random error (2-3%). The disadvantages are: the usage of immersion liquid is needed; high sensitivity to focusing and sample illumination of both measured parameters – transmittance and diameter.

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## 7. REFERENCES

1. G.B. Altshuler, H.H. Zenzie, A.V. Erofeev, M.Z. Smirnov, R.R. Anderson, and C. Diericks, Contact cooling of the skin, *Phys. Med. Biol.*, **44**, 1003-1023, 1999.
2. R.G. Wheeland, Laser-assisted hair removal, *Lasers in Dermatology*, **15**, 469-477, 1997.
3. Palomar Delivers E2000 Second-Generation Ruby Laser, *Med. Laser Insight*, Ed. M. Moretti, Suppl., Nov 1999.
4. T.-Y.D. Lin, C.C. Dierickx, V.B. Campos, et al., Reduction of regrowing hair shaft size and pigmentation after ruby and diode laser treatment, *Arch. Dermatol. Res.*, **136**, 00-00, 2000.

5. E.M. Nicholis, Microspectrophotometry in the study of red hair, *Ann. Hum. Genet., Lond.*, **32**, 15-26, 1968.
6. X.J. Wang, N.E. Milner, R.P. Dhond et al., Characterization of human scalp hairs by optical low-coherence reflectometry, *Opt. Lett.*, **20**, 524-526, 1995.
7. N.V. Joshi and J.Goyo-Rivas, Optical and morphological investigation of hair of patients of Chediak Higashi syndrome, *Proc. SPIE*, **3251**, 229-234, 1998.
8. N. Kollias, A.H. Baqer, On the assessment of melanin in human skin *in vivo*, *Photoch. Photobiol.*, **43**, 49-54, 1986.
9. I. A. Menon, S. Persad, H.F. Haberman, and C.J. Kurian, A comparative study of the physical and chemical properties of melanins isolated from human black and red hair, *J. Invest. Dermatol.*, **80**, 202-206, 1983.
10. H. Takiwaki, S. Shirai, Y. Kanno, Y. Watanabe, and S. Arase, Quantification of erythema and pigmentation using a videomicroscope and a computer, *British J. Dermatol.*, **131**, 85-92, 1994.
11. I. A. Watson, H.C. Ngiap, R. K. Wang, and G. Ward, Accurate counting of colony forming units with imaging processing systems, *OSA TOPS on Medical and Biological Applications*, **6**, R.Cubeddu (ed.), 68-71, 1996.