Melanin spatial distribution in iris of human eye

Alexey N. Bashkatov[1], Elina A. Genina[1], Ekaterina V. Koblova[2], Tatyana V. Danilova[3], Leonid E. Dolotov[1], Yury P. Sinichkin[1], Olga A. Perepelitsina[1], Valery V. Tuchin[1], Tatyana G. Kamenskikh[2]

[1]Department of Optics and Biomedical Physics of Saratov State University, 83, Astrakhanskaya str., Saratov, 410012, Russia
[2]Ophthalmology Department of Saratov State Medical University, 112, B. Kazach'ya str., Saratov, 410012, Russia
[3]Saratov State Technical University, 77, Polytekhnicheskaya str., Saratov, 410054, Russia

ABSTRACT

Based on the experimental data obtained *in vivo* from digital analysis of color images of human irises, the mean melanin content in human eye irises has been estimated. For registration of the color images a digital camera Olympus C-5060 has been used. The images have been obtained from irises of healthy volunteers as well as from irises of patients with open-angle glaucoma. The computer program has been developed for digital analysis of the images. The result has been useful for development of novel and optimization of already existing methods of non-invasive glaucoma diagnostics.

Keywords: iris, melanin content, glaucoma, reflectance spectroscopy, digital image analysis

1. INTRODUCTION

Knowledge of tissue optical properties is important for development of theoretical models describing the light propagation within tissues (including a human eye iris). These models can be used when designing laser therapy and diagnostic techniques, or interpretation of the data of spectrophotometric measurements. There are numerous papers describing the methods of determining optical properties of many types of tissues.[1]

Recently some diagnostic criteria of dystrophic, degenerative and inflammatory diseases of an eye iris have been based on descriptive, relative and, in many respects, subjective criteria to which the discoloration of an iris of the eye is also refered.[2-7] Now the eye iris classification is based on the data of iridochromoscopy, iridochromophotography, and biomicroscopy in polarized light.[2-8] Objective criteria of inflammatory and degenerative changes can be obtained from the data of the fluorescent angiography. Unfortunately, the method is invasive and cannot be applied to all patients. Iris coloration depends mostly on the amount and depth of location of melanin located in the forefront mesodermal part of the iris.[4,5,7,8] The quantitative assessment of melanin content in an iris can be used as objective criterion in investigating the series of pathological conditions.[2,4,7,8] Besides, the objective data could be supplement to the existing classifications of iris types.

In this study, we present a method for estimation of melanin content in eye iris with digital analysis of color images of the eye iris.

2. STRUCTURE AND PROPERTIES OF THE HUMAN EYE IRIS

Figure 1 shows the transverse (horizontal) section of an eyeball. The eye iris is forefront of choroid of the eye. The iris has the form of a plate with lightly elliptical shape. Peripheral edge of the iris (its root) is merged with the ciliary body and the trabecular meshwork. The iris is 12-12.5 mm in diameter with a circumference of 36-37 mm.[4,9,10] The forefront surface of the iris can be divided into two zones: the pupil zone (1-2 mm wide) and the ciliary zone (3-4 mm wide). The separation line corresponds to plexus of small arteries, which form small arterial iris circle. The distance between the separation line and edge of eye pupil is about 1.5 mm.[9] Iris thickness is not everywhere equal. The thickness has maximal value (up to 480-550 μm) in the area of the small arterial circle, and minimal value was observed in ciliary zone

Saratov Fall Meeting 2005: Optical Technologies in Biophysics and Medicine VII,
edited by Valery V. Tuchin, Proc. of SPIE Vol. 6163, 616312, (2006)
1605-7422/06/$15 · doi: 10.1117/12.697309

- up to 350 μm.[11] Eye pupil has also very different size. For children the pupil diameter is minimal (up to 2 mm), in the young age the pupil diameter is maximal (about 4 mm), and in the old age the pupil diameter decreases again.

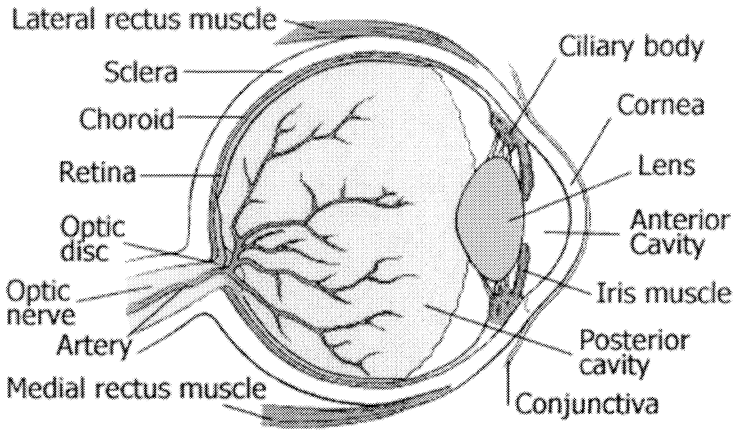


Figure 1: The transverse (horizontal) section of eyeball

Structurally, the iris contains two different layers. The iris thin innermost layer is called the iris pigment epithelium (IPE) and consists of a compact array of opaque cells. The outermost layer refers to the iris stroma, which contains more loosely arranged cells, including melanocytes that synthesize the pigment.[4-6,12,13] The iris structure is illustrated in figure 2. According to electronic microscopy, basic cellular elements of the stoma layer are fibroblasts, melanocytes and a network of collagen fibrils.[4-6,12] Diameter of collagen fibrils in iris stroma is 60 nm and its axial periodicity is 50-60 nm.[5] Size of the iris melanocytes is about 100 μm and the cells are filled with very small melanin particles.[5,11-13] Structurally, the stroma can be subdivided in two sublayers: the mesodermal upper and deep sublayers. In the upper sublayer of stroma (in ciliary zone) melanocyte conglomerations form the pigmentary spots, so-called nevuses.[4-6,12]

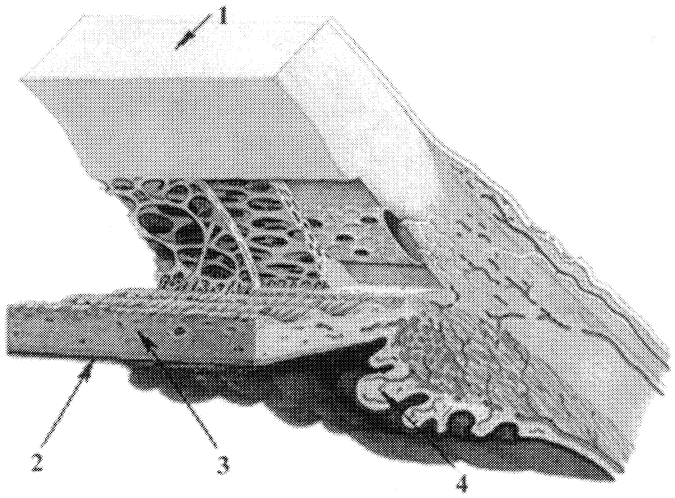


Figure 2: The eye iris structure: 1 - the cornea; 2 - the iris pigment epithelium (IPE); 3 - the iris stroma; 4 - the ciliary body

Iris vessels are localized within the iris stroma. The vessels include large vessels (arterioles and venules) and capillaries.[6] In the depth of the iris stroma, nearly the iris root (in area of the eye trabecular meshwork), iris arteries and venues form large arterial circle of the iris. From this circle, radial vessels with a diameter smaller than the diameter of the vessels of the large arterial circle go to the eye pupil.[9] Size of the large iris vessels (arterioles) is 50-100 μm. Precapillaries have

diameter 14-16 μm, and capillaries - 3-12 μm. Diameter of venules is 12-100 μm.[14] Unfortunately, blood content in eye iris has been investigated not enough, however, for choroid the volume fraction of blood are known. Hammer and Schweitzer[15] found the volume fraction as 20%, Delori and Pflibsen[16] estimated the fraction as 50%, and Preece and Claridge[17] reported that the choroid blood volume fraction ranged from 50 to 80%. Since iris is forefront of the eye choroid it is necessary to expect, that blood content in the iris can be taken from approximately 30 to 70%.

From the iris structure, there are three principal elements that contribute to its color.[18] One is the pigment in the IPE, which is black in irises of all colors. Another is the melanin content in the iris stroma, which is the primary cause of color variations among different irises.[17] Brown irises have a large amount of melanin, which absorb much of the incoming light especially at short wavelengths. For blue irises which have low melanin content in the stroma, long-wavelength light penetrates the stroma and is absorbed in the IPE, while the short-wavelength undergoes Rayleigh scattering and reflection. Green and hazel irises are products of moderate amounts of melanin. Thus, the spectrum of iris colors basically results from varying amounts of the stroma melanin. However, blood of the stoma vessels also contributes in forming iris color, especially in short-wavelength spectral range. The third structural component that influences color is the cellular density of the iris stroma. For example, in an area of low density, little light is reflected by the semi-transparent stroma, so it shows the black color of the IPE, and, on the contrary, in an area of high density light does not penetrate IPE and backreflected by the iris stroma.

Melanin synthesis occurs in special organoids - melanosomes, and then a transformation into enzymatic-inert pigmentary granules takes place. In accordance to its chemical composition the pigment is a combination of sulfur-containing pheomelanin and sulfur-free eumelanin, and the content of the pheomelanin produced by melanocytes at early and mature cells age is dominating (up to 99%). At cells ageing there is eumelanin contents dominating.[19-23] Melaninous granules in the residual bodies laying in the stroma's back part are identical in size and form the pigment granules of the pigmentary epithelium covering the iris back surface. It should be noted that melanin's granules size enclosed in the residual bodies located in forefront of iris stroma is equal to melanin granules size in melanocytes.[4-6] In Fig. 3 the extinction coefficient spectra[24] of pheomelanin and eumelanin are presented.

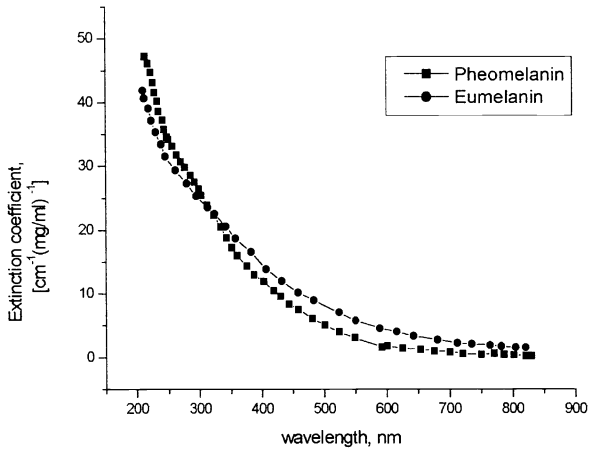


Figure 3: Extinction coefficient spectra of melanins[24]

Since structure and function of iris pigment epithelium is very similar to structure and function of retinal pigment epithelium we can assume that optical properties of these tissues are also very similar. Hammer et al.[25] measured the optical properties of retinal pigment epithelium early and we have used the data in our calculation. Figure 4 and 5 show the optical properties of retinal pigment epithelium obtained by digitization of the data presented by Hammer et al.[25]

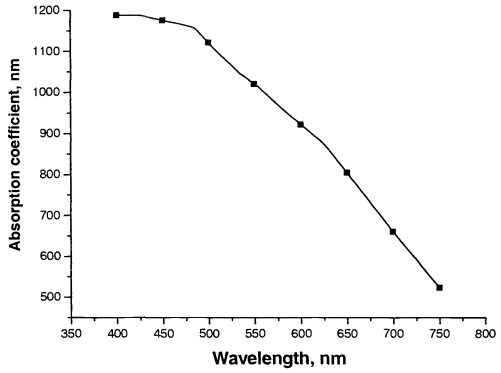


Figure 4: The absorption properties of retinal pigment epithelium[25]

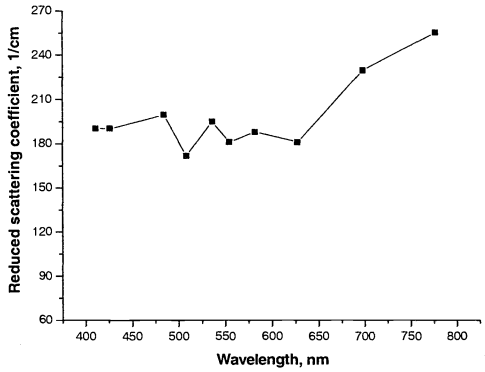


Figure 5: The scattering properties of retinal pigment epithelium[25]

3. MATERIALS AND METHODS

Estimation of melanin content in human and animal irises using experimental data obtained *in vitro* from reflectance measurements and *in vivo* from digital analysis of color images of irises has been carried out.

In this work the method of quantitative estimation of the human iris melanin content by a registration of reflectance spectra was used. As material the irises of 2 cadaver human eyes and 10 bovine eyes have been used. Time interval from post mortem to enucleating does not exceed 24 hours. After the enucleating all samples were kept in a normal saline solution (0.9% NaCl) where they were stored up for spectroscopic measurements. All measurements were performed at room temperature about 20°C.

Eyeballs were prepared as follows: a cornea was cut off on limbs, then an iris was adjoined from choroid of an eye. The bovine irises have dark brown or black color. Color of the human iris was more diverse: from light blue up to dark brown. Iris thickness and width were not measured, as they have constant parameters.

For estimation of iris hydration thirty iris samples have been obtained from rabbit's eye. The samples were placed in a desiccator and were dried over silica gel at room temperature. Constant dry weight was obtained after at least 2 weeks. The tissues were kept in desiccator until they were used. The tissue hydration (H) can be calculated from[26]

$$H = \frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}}.$$

For estimation of scattering properties of iris stroma, the reflectance measurements of rabbit's vitiligo irises with different blood content have been performed. The reflectance measurements were performed using commercially available optical multichannel spectrometer LESA-6med (BioSpec, Russia). Figure 6 shows a scheme of the experimental setup.

As a light source a 250 W xenon arc lamp with filtering of the radiation in the spectral range from 400 to 700 nm has been used in the measurements. Light was delivered to the iris and collected from the tissue using the originally designed optical probe, which consists of two optical fibers. Both fibers had 400 mm in core diameter and a numerical aperture of 0.2. The fibers have been enclosed in aluminum jacket (8-mm outer diameter) to provide a fixed distance between the fibers and the tissue surface. The central fiber has been placed in perpendicular to iris surface delivering incident light to the surface. Distance between the delivering fiber and iris surface is 12 mm. Diameter of the illuminated spot is about 5 mm. The collecting fiber is mounted at angle of 20° regarding to central fiber. Distance between tip of collecting fiber

and iris surface is 20 mm and, in this geometry, light has been collected from area with diameter about 8 mm. The spectrometer was calibrated using white slab $BaSO_4$ with a smooth surface.

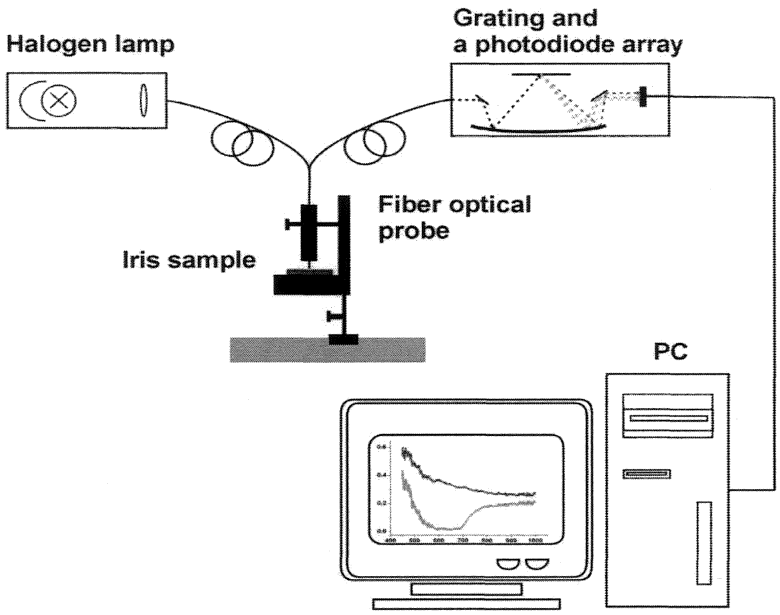


Figure 6: Experimental setup for in vitro measurements

Experimental setup for registration of digital images of human eye irises is presented in figure 7. For registration of the color images the digital camera Olympus C-5060 (Japan) was used. Its parameters: 5,100,000 effective pixels, Olympus lens from 5.7 mm to 22.9 mm. During the shooting the maximum rating aperture value equals f3.3. Focal length 11.5 was used. The distance between eye and camera objective was 3 cm.

Figure 7: Experimental setup for *in vivo* registration of digital images of human irises

For the investigations the special facial fixer (for a chin and a forehead) has been made. It included a mobile part, which supports a chin and moves up - downwards with manual screw rotation. Such equipment needs to be enforced by two lighting elements. Lighting elements were fixed on a movable basis that allowed adjusting the intensity of illumination during the measurements. Digital camera was attached to the vertical holder fixed to movable basis that could be described as a movable device with ability to move in all directions according to the tool stage. Camera can be shifted with fixed steps. It allowed us to carry out shooting each eye separately in super macro mode with manual focusing. Shooting was done at constant light exposure, which was registered by metering-tool. In fig. 8 digital images of healthy human iris and human eyes with primary open angle glaucoma are presented.

a)

b)

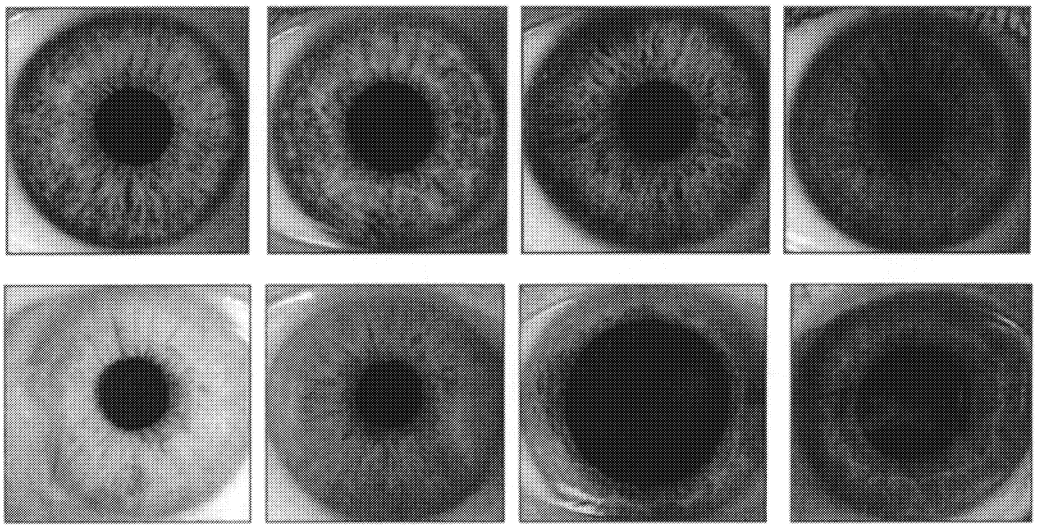
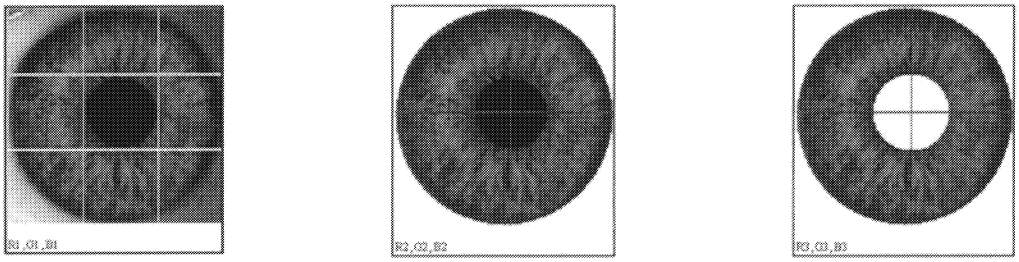


Figure 8: Digital *in vivo* images of (a) healthy and (b) primary open angle glaucoma human eyes

To process the images of the iris the special computer program has been developed. The base image was separated in three color matrixes of red, green, and blue components. As a result, the averaged scans of the iris image for separated color components (red, green, and blue) corresponding to three spectral ranges for reflectance measurements have been obtained. The brightness of images of the studied irises and test-objects are expressed in units from 0 to 256.

With the special markers the square form area is cut out from the received image (containing an iris of the eye) in which the circle is entered. Then nearby sclera sites are cut, the markers determine the center pupil area, which are deleted. Fig. 9 shows the main steps of image processing.



Step 1 Step 2 Step 3

Figure 9: The main steps of image processing

The image decomposed into R, G, B color coordinates in each pixel. As a reference a white-test object has been used. For each pixel measured R, G, B values have been normalized to RGB values of the test object. The obtained reflectance values have been averaged for the whole investigated area. See fig. 9 step 3.

For estimation of melanin content in an iris of human eye the algorithm based on inverse Monte Carlo technique has been developed. In the framework of the algorithm the following assumptions have been made:

1) The iris is presented as a two-layer plane-parallel slab with thickness 460 mm. The deep layer is IPE (10 μm thick) and the upper layer is the iris stroma (450 μm thick).
2) The optical properties of the IPE and melanin is presented above.
3) Optical properties of iris stroma (for vitiligo samples) have been estimated using inverse Monte Carlo technique and the result has been presented below.
4) Melanin concentration is the variable.

Based on the presented two-layer iris model, the iris diffuse reflectance has been calculated by Monte Carlo technique in the spectral range 400-750 nm. Then the spectrum has been recalculated in color coordinates with the relations

$$R = \int \bar{r}(\lambda) P_0(\lambda) R(\lambda) d\lambda$$

$$G = \int \bar{g}(\lambda) P_0(\lambda) R(\lambda) d\lambda$$

$$B = \int \bar{b}(\lambda) P_0(\lambda) R(\lambda) d\lambda$$

where $R(\lambda)$ is the iris reflectance, and $r(\lambda)$, $g(\lambda)$, $b(\lambda)$ are specific color coordinates, $P_0(\lambda)$ is spectrum of light source. The calculations have been performed for each pixel. Melanin concentration varies until color coordinates obtained from the calculations, and color decomposition of experimentally obtained iris images has not been matched. Due to the complex structure of the investigated tissue and simplicity of the presented model, melanin concentration obtained for each spatial coordinate has been averaged throughout iris area.

4. RESULTS AND DISCUSSION

Results of the measurements of iris hydration are presented in table 1. The results have shown the iris hydration, iris density and volume fraction of iris scatterers. For estimation of volume fraction of iris scatterers the following relation is used, $\varphi = V_c / V$, where V_c is volume of the iris scatterers (the collagen fibers and melanocytes), and V is volume of the tissue sample. In its turn the volume can be calculated as $V = V_c + V_{H_2O}$, where V_{H_2O} is volume of water in the sample. Volume of the iris scatterers can be calculated as $V_c = M_a / \rho_c$, where M_a is iris weight after drying, and $\rho_c = 1.41$ g/ml is density of the dry collagen.[26] The $V_{H_2O} = (M_b - M_a) / \rho_{H_2O}$, where M_b is iris weight before drying and $\rho_{H_2O} = 1$ g/ml is water density. The iris density has been calculated as relation of iris weight before drying to the iris volume.

Table 1. The iris hydration, iris density and volume fraction of iris scatterers

Sample number	Iris weight before drying, mg	Iris weight after drying, mg	Solid, %	Hydration	Iris density, g/ml	Scatterers volume fraction
1	61	8	13.1	6.625	1.04	0.097
2	128	12	14.1	9.967	1.028	0.068
3	85	12	14.1	6.083	1.043	0.104
4	83	12	14.5	5.917	1.044	0.107
5	105	11	10.5	8.545	1.031	0.077
6	126	10	8.0	11.6	1.026	0.058
7	106	9	8.5	10.778	1.025	0.062
8	93	8	8.6	10.625	1.026	0.063
9	125	9	7.2	12.889	1.021	0.052

10	140	14	10.0	9	1.03	0.073
11	109	9	8.3	11.111	1.025	0.06
12	155	13	8.4	10.923	1.025	0.061
13	113	9	8.0	11.556	1.024	0.058
14	115	9	7.8	11.778	1.023	0.057
15	104	8	7.7	12	1.023	0.056
16	117	10	8.5	10.7	1.025	0.062
17	121	11	9.0	10	1.027	0.066
18	124	9	7.3	12.778	1.022	0.053
19	130	10	7.7	12	1.023	0.056
20	98	7	7.1	13	1.021	0.052
21	101	9	8.9	10.222	1.027	0.065
22	112	10	8.9	10.2	1.024	0.065
23	105	9	8.6	10.667	1.026	0.062
24	90	8	8.9	10.25	1.027	0.065
25	112	11	9.8	9.182	1.029	0.072
26	91	9	9.9	9.111	1.03	0.072
27	91	8	8.8	10.375	1.026	0.064
28	110	9	8.2	11.222	1.022	0.059
29	109	9	8.3	11.111	1.025	0.06
30	112	9	8.0	11.444	1.024	0.058
Mean value			9.22±2.06	10.389±1.798	1.027±0.006	0.066±0.014

Figure 10 shows reflectance spectra of rabbit irises.

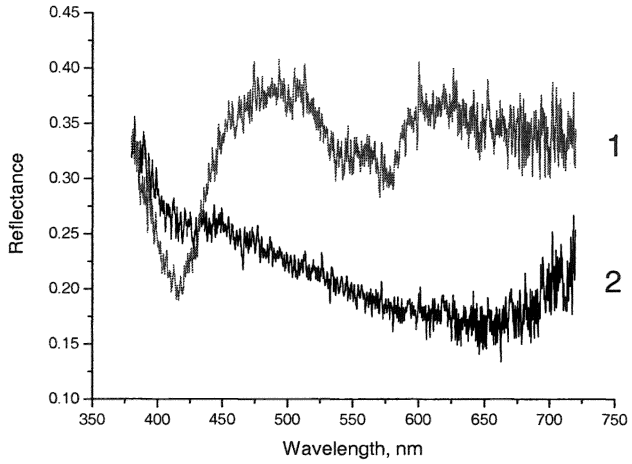


Figure 10: The typical reflectance spectra of rabbit irises. 1 - the reflectance spectrum of vitiligo iris; 2 - the reflectance spectrum of brown iris of a rabbit.

Using inverse Monte Carlo technique and taking into account geometry of the measurement optical properties of bloodless vitiligo iris stroma have been estimated. The result is presented in figures 11 and 12. Proceeding from the given technique based on measurement of reflectance, it is possible to estimate the melanin content in an iris of an eye.

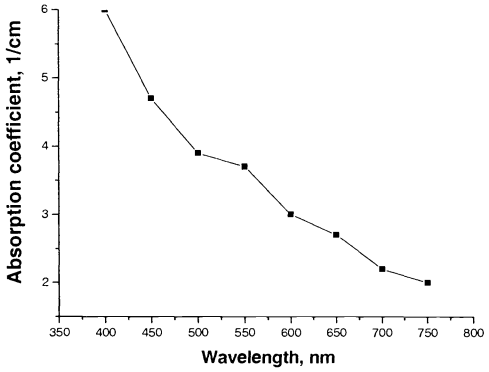


Figure 11: The absorption properties of bloodless vitiligo iris stroma

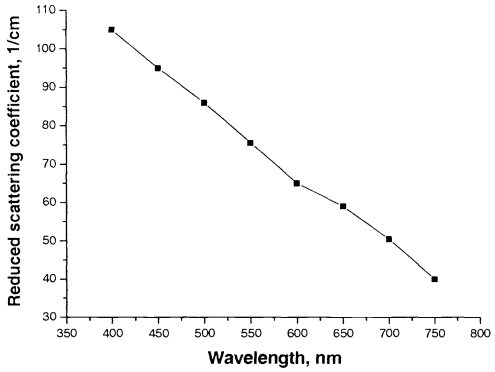


Figure 12: The scattering properties of bloodless vitiligo iris stroma

In figure 13 the reflectance spectra of brown human iris measured *in vitro* are presented.

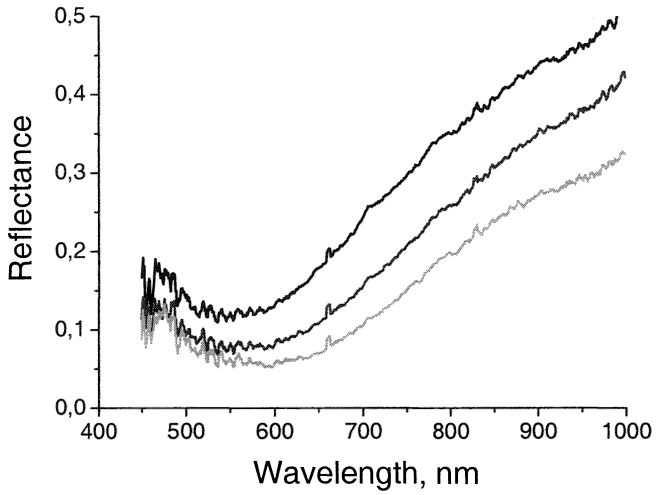


Figure 13: The reflectance spectra of brown human iris measured *in vitro* in different points of the sample

In table 2 the mean melanin concentration values obtained from the processing of the spectral dependence of iris reflectance of bovine irises measured *in vitro* are presented.

Table 2. Mean melanin concentration values obtained from the *in vitro* measured reflectance of the bovine irises

Sample	Average melanin concentration in an iris, mg/ml	Sample	Average melanin concentration in an iris, mg/ml
1	30.36	6	62.57
2	31.18	7	16.07
3	31.81	8	18.56
4	65.50	9	61.12
5	56.41	10	21.42

Basing on the results the average melanin concentration in the bovine iris has been estimated as 39.5 ± 6.2 mg/cm^3. At examination of human iris samples, the melanin concentration has been estimated as 44.9 ± 2.1 and 46.7 ± 2.2 mg/cm^3, respectively. Thus, the mean melanin concentration obtained from the human irises has been estimated as 45.8 ± 1.2 mg/cm^3.

In tables 3 and 4 the results of digital image analysis using the designed computer program are respectively presented for healthy volunteers and for volunteers with primary open angle glaucoma.

Table 3. Results of digital image analysis of healthy volunteers

Volunteer (iris color)	R_{RGB}	G_{RGB}	B_{RGB}	Melanin concentration in an iris, mg/ml
0 (blue)	136 (40.8)	112.6 (31)	84.3 (22.3)	21.9 (10.1)
1 (brown)	126.6 (39.3)	62.6 (18.8)	15.5 (2.7)	24.7 (10.9)
2 (blue)	121.4 (14.9)	84.4 (8.7)	44.6 (2.9)	24.7 (4.3)
3 (green)	106.1 (26.5)	74.2 (20)	30.9 (17.9)	30 (8.5)
4 (blue)	114 (5.1)	102.3 (3.8)	90.3 (4.1)	26.7 (1.5)
5 (blue)	99.8 (5.6)	81.3 (6)	67.2 (7.8)	31.3 (1.9)
6 (green)	88.7 (3.7)	76.8 (4.3)	65.8 (8.8)	35.2 (1.4)
7 (brown)	71 (4.9)	43.9 (1.7)	19.4 (1.4)	42.8 (2.4)
8 (brown)	177.7 (7.1)	101.1 (3.4)	26.1 (7.8)	11.6 (1.3)
9 (blue)	112.9 (34.1)	85.4 (25.3)	53.5 (17.8)	28.2 (10.4)
10 (green)	118.8 (24.6)	83.5 (15.6)	30.4 (10.9)	25.8 (6.3)
11 (brown)	116.3 (8.3)	71.3 (7.3)	12.8 (5.4)	26.1 (2.4)
12 (blue)	105.4 (17.4)	84.6 (12.9)	58.3 (10.7)	29.8 (6)
13 (green)	110.8 (5.7)	70.7 (3.6)	20.3 (4)	27.7 (1.7)
14 (brown)	77.6 (6.4)	39.7 (3.1)	8.6 (2.6)	39.8 (2.7)
15 (blue)	124.4 (17.7)	105.6 (17.5)	86.2 (18)	24 (5.4)
16 (blue)	128.9 (16.1)	111.8 (12.9)	95.8 (9.1)	22.7 (4.4)
17 (brown)	87 (24.4)	47.7 (10.6)	8.4 (1.4)	36.6 (9.6)
18 (green)	146.8 (28.1)	88.7 (18.4)	22 (9.8)	18.6 (6.6)
19 (green)	112.7 (16.2)	71.6 (5.7)	22 (3.7)	27.3 (4.9)

Table 4. Results of digital image analysis of patients with open angle glaucoma

Patient (iris color)	R_{RGB}	G_{RGB}	B_{RGB}	Melanin concentration in an iris, mg/ml
1 (brown)	129 (11.2)	59 (6.1)	6 (1.4)	22.6 (2.9)
2 (blue)	116.3 (7.6)	71.5 (5.9)	20.4 (9)	26.1 (2.2)
3 (blue)	122.9 (16.2)	76.3 (10.7)	14.9 (5.8)	24.4 (4.8)
4 (brown)	130.5 (14.5)	59.8 (8.6)	6 (2.3)	22.3 (3.9)
5 (blue)	119.6 (7.6)	74 (10.1)	26.9 (23.9)	25.1 (2.2)
6 (blue)	128.6 (10.7)	81.7 (7.3)	30.4 (12.7)	22.7 (2.8)
7 (blue)	136.4 (8.5)	115.4 (8.1)	98.6 (9.3)	20.7 (2.1)
8 (green)	137.2 (18.5)	83 (6.9)	24.4 (9.6)	20.6 (4.3)
9 (green)	181.7 (39.4)	117.7 (36.9)	48.7 (34.8)	11.5 (7.5)
10 (blue)	98.3 (15.3)	81.5 (14.4)	66.5 (14.9)	32.1 (5.4)
11 (blue)	113.3 (14.2)	97.6 (11.8)	85.3 (13.2)	27.1 (4.2)
12 (brown)	128.8 (11.8)	58.3 (6.5)	9.5 (3)	22.7 (3.2)

In tables 5 and 6 data, averaged from tables 3 and 4 are presented. From table 5 it is seen that maximal melanin concentration has been obtained for brown eyes. For blue and green eyes the melanin content is smaller.

Table 5. Mean melanin concentration in healthy human iris measured *in vivo* (from digital analysis of color images)

Color of eyes	Melanin concentration, mg/ml
blue eyes	26.2±3.4
brown eyes	30.3±11.7
green eyes	27.4±5.4

Table 6. Mean melanin concentration in human iris with glaucoma measured *in vivo* (from digital analysis of color images)

Color of eyes	Melanin concentration, mg/ml
blue eyes	25.5±3.6
brown eyes	22.5±0.2
green eyes	16.1±6.4

From table 6 it is seen that maximal melanin concentration is observed for blue eyes in contrast to data of table 5. So, melanin content in human and animal irises using experimental data obtained *in vitro* from reflectance measurements and *in vivo* from digital analysis of color images of irises was estimated. In irises of eyes with glaucoma the mean content of melanin is less in comparison with one in irises of healthy eyes. The major difference was obtained for brown and green color eyes.

ACKNOWLEDGMENTS

The research described in this publication has been made possible, in part, by grant PG05-006-2 of U.S. Civilian Research and Development Foundation for the Independent States of the Former Soviet Union (CRDF) and the Russian Ministry of Science and Education, grant RFBR 07-05-90394, grants RUB1-570-SA-04 and BRHE Award Annex 07 Appendix 11 of CRDF, and grant of the Russian Federal Agency of Education Russian Federation 1.4.06. The authors thank Dr. S.V. Eremina (Department of English and Intercultural Communication of Saratov State University) for the help in manuscript translation to English.

REFERENCES

1. V.V. Tuchin, *Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis*, SPIE Press, TT38, Bellingham, USA, 2000.
2. N.B. Shulpina, *Biomicroscopy of the eye*, Moscow, 1972.
3. M.L. Berliner, *Biomicroscopy of the eye,* in *Slit-lamp microscopy of the living eye,* New York, p .9-14, 1949.
4. M Zaltsmann, *Anatomy and histology of the human eye in normal, its development and withering*, Moscow, 1913.
5. E.S. Velhover, V.F. Ananin, *Clinical iridology*, Moscow, 1992.
6. E.S. Velhover, *Iridology*, Moscow, 1992.
7. A.M. Vodovozov, A.A. Ribnikov, *Estimation iris of the eye in transformed light, Moscow*, 1992.
8. V.V. Konovalov, A.A. Antonov, *Practical iridology*, 1990.
9. A.J. Samoilov, Iris of an eye, in Big Medical Encyclopedia, **27**, 842-849, 1962.
10. A.J. Samoilov, Choroid of an eye, in Big Medical Encyclopedia, **30**, 953-956, 1963.
11. D.A. Enikeev, S.A. Lobanov, *Iridoalloplastic*, 1996.
12. E.V. Bobrova, "Ultrastructure of human eye dilator," in *Some questions of experimental and clinical medicine*, 22-25, Moscow, 1977.
13. E.V. Bobrova, A.V. Petrov, "Ultrastructure of the front layers and pupil dilator of an human eye iris," *Bullen of Ophthalmology*, **4**, 33-36, 1978.

14. V.N. Alekseew, I.A. Samusenko, "Clinical-morphological changes in the forward piece of the eye at experimental glaucoma," *Glaucoma*, **1**, a80, 2004.
15. M. Hammer, D. Schweitzer, "Quantitative reflection spectroscopy at the human ocular fundus," *Phys. Med. Biol.*, **47**, 179-191, 2002.
16. F.C. Delori, K.P. Pflibsen, "Spectral reflectance of the human ocular fundus," *Appl. Opt.*, **28**, 1061-1077, 1989.
17. S.L. Preece, E. Claridge, "Monte Carlo modelling of the spectral reflectance of the human eye," *Phys. Med. Biol.*, **47**, 2863-2877, 2002.
18. P.D. Imesch, I.H.L. Wallow, D.M. Albert, "The color of the human eye: A review of morphologic correlates and of some conditions that affect iridial pigmentation," *Survey of Ophthalmol.*, **41**, 117-123, 1997.
19. T.P. Dryja, M. O'Neil-Dryja, D.M. Albert, "Elemental analysis of melanins from bovine hair, iris, choroid, and retinal pigment epithelium," *Invest. Ophthalmol. Visual Sci.*, **18**, 231–236, 1979.
20. E. Buszman, R. Rozanska, "Interaction of thioridazine with ocular melanin *in vitro*," *Acta Pol Pharm.*, **60**(4), 257-61, 2003.
21. M. Hammer, D. Schweitzer, E. Thamm, A. Kolb, "Non-invasive measurement of the concentration of melanin, xanthophyll, and hemoglobin in single fundus layers in vivo by fundus reflectometry," *Int. Ophthalmol.*, **23** (4-6), 279-89, 2001.
22. S. Peters, U. Schraermeyer, "Characteristics and functions of melanin in retinal pigment epithelium," *Ophthalmology,* **98**(12), 1181-5, 2001.
23. M. Braun, A. Kage, K. Heimann, U. Schraermeyer, "Retinal pigment epithelial cells from Royal College of Surgeons dystrophic rats can take up melanin granules," *Graefes Arch Clin Exp Ophthalmol.*, **237**(1), 67-71, 1999.
24. S. Jacques, Published on the personal web-site: www.omlc.ogi.edu.
25. M. Hammer, A. Roggan, D. Schweitzer, G. Muller, "Optical properties of ocular fundus tissues – an *in vitro* study using the double-integrating-sphere technique and inverse Monte Carlo simulation," *Phys. Med. Biol.*, **40**, 963-978, 1995.
26. Y. Huang, K.M. Meek, "Swelling studies on the cornea and sclera: the effects of pH and ionic strength," *Biophysical J.*, **77**, 1655-1665, 1999.