

# Multispectral Retinography in Healthy Adult Population

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**Abstract:** Spectral reflectance of the eye fundus was evaluated in adult healthy patients through a fast visible and near-infrared multispectral fundus camera. Spectral signatures were analyzed for different ocular structures of the retina and the choroid. © 2021 The Author(s)

## 1. Introduction

Retinographs are widely used nowadays in ophthalmology to acquire images of the retina, thus making easier the diagnosis of diseases affecting this ocular structure in comparison to traditional ophthalmoscopes. Conventional color fundus cameras offer high spatial resolution, but their spectral sampling is limited as they only evaluate three broad-band channels (RGB). Therefore, metamerism might prevent differentiation between structures and limit the diagnostic power of such devices. Moreover, since their sensitivity is restricted to the visible (VIS) range of the electromagnetic spectrum, layers behind the retina such as the choroid are hardly observable due to the high absorption of light in the biological tissue, and especially in the first layers of the retina, at these wavelengths. From the morphological point of view, other technologies such as the optical coherence tomography (OCT) allow deeper structures to be imaged with good axial and lateral spatial resolutions; nevertheless, they neither offer color nor spectrometric information, which might be relevant for medical diagnosis. Spectral imaging has recently shown a great potential on the assessment of eye fundus as it offers high spatial and spectral information altogether [1,2]. Even the first attempts carried out provide accurate spectral information from the fundus through several narrow-band channels, they do not have sensitivity in the near-infrared (NIR) so that spectral information from deeper layers of the fundus is not yet available.

Spectral features of structures of the ocular fundus have been analyzed by several authors, revealing traits that can be used to discriminate them. The retinal nerve fiber layer and the optic disc show high reflectance at short visible wavelengths (460 nm – 560 nm) [3] and, only in the case of the optic disc, it intensely reflects light up to 1000 nm due to the myelin surrounding the fibers at this area [4] and because of the high reflectivity of the lamina cribosa [5]. Arteries (oxygenated blood) show a local maximum in terms of reflectance around 560 nm, whereas for veins (deoxygenated blood) it corresponds to a local minimum value, thus allowing to discriminate them, as it also happens beyond 600 nm, where they show maximum spectral differences. In fact, it is known that an isosbestic point exists around 800 nm, where the absorbances of de- and oxygenated blood switch [6,7]. The retinal pigment epithelium (RPE) and the choroid have a high concentration of melanin, absorbing strongly the ultraviolet (UV) and the VIS and up to 600 nm [6,8]. The macular pigments in the fovea have maximum absorption at 460 nm, acting as a blue filter [8]. The overall reddish appearance of eye fundus is due to the pronounced reflectance at long wavelengths [6].

In this context, the goal of this work is to study the spectral reflectance of eye fundus structures in healthy subjects by means of a fast visible and extended infrared multispectral fundus camera (400 nm – 1300 nm) with high spectral and spatial resolution, including the relatively unexplored range beyond 900 nm [9], which has been recently developed at the Center for Sensors, Instruments and Systems Development (CD6) of the Universitat Politècnica de Catalunya (UPC).

## 2. Material and Methods

### 2.1. Subjects

This study was conducted on healthy subjects at the University Vision Center (CUV) of the UPC. A total of 126 eyes from 81 subjects (54.4% females and 45.6% males) with a mean  $\pm$  standard deviation in age of  $47.9 \pm 17.4$  years (range: 19 to 81 years) was evaluated. The inclusion criteria were: best-corrected visual acuity equal to or higher than 0.9 in decimal units, intraocular pressure equal to or lower than 21 mmHg, normal fundus and no history of any ocular condition or trauma. Ethical committee approval was obtained and all patients provided written informed consent before any examination. The Declaration of Helsinki tenets of 1975 (as revised in Tokyo in 2004) were followed throughout the study.

## 2.2. Multispectral Fundus Camera

The developed multispectral fundus camera [9] integrates two detection arms, one for the spectral range from 400 nm to 950 nm (VIS-NIR) and another for the NIR range from 960 nm to 1300 nm. The illumination system comprises three light emitting diode (LED) rings that cover 15 spectral bands with peak wavelengths of 416 nm, 450 nm, 471 nm, 494 nm, 524 nm, 595 nm, 598 nm, 624 nm, 660 nm, 732 nm, 865 nm, 955 nm, 1025 nm, 1096 nm, and 1213 nm. The system is non-mydratiac (no need for pupil dilation), and features an angular field of view of 30°. The prototype also allows the compensation of the patients' spherical refraction (maximum correction  $\pm 15D$ ). Although 15 images are acquired for each measurement, one for each spectral band, the acquisition only takes 613 ms (220 ms corresponding to the VIS-NIR spectral bands and 393 ms to the NIR ones), thus avoiding imaging problems caused by eye movements and blinking.

## 2.3. Image Processing and Spectral Reflectance Analysis

Before retrieving the reflectance from the fundus images, they were processed in order to remove artifacts caused by reflections and non-uniformities of the illumination. Spectral reflectance was firstly quantified in terms of pixel intensity after calibration with a calibrated reference white (BN-R98-SQC, Gigahertz-Optik GmbH, Germany), leading to an absolute reflectance parameter. However, this value did not allow comparing among patients, even healthy, since light reflected by the fundus is also affected by the intrinsic transmittance of the preceding ocular media in each individual. To solve this, a relative reflectance parameter was also computed by dividing the absolute reflectance of each structure by the mean absolute reflectance of the entire fundus image. Images with artifacts that could not be properly removed were not included in the analysis (14 eyes).

## 3. Results and Discussion

Figures 1a and 1b show the absolute and relative mean reflectance curves of different eye fundus structures, respectively, for subjects aged from 19 to 69 years old. It is to be noted that older patients (70 to 81 years) have not been included in the plots as they showed slightly higher reflectance curves than younger ones because all of them had an intraocular lens implanted after cataract surgery. Apart from this particular condition, no remarkable differences were found when comparing the mean reflectance curves of eye fundus structures among different age ranges.

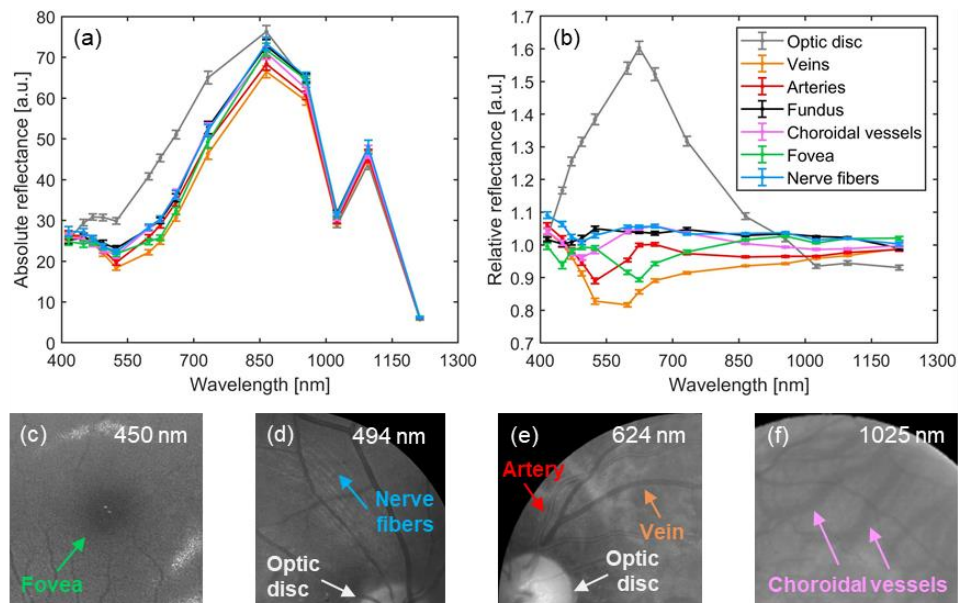


Fig. 1. Absolute (a) and relative (b) mean reflectance ( $\pm$  standard error) for different eye fundus structures. From (c) to (f) different eye fundus structures are visible at specific wavelengths.

As formerly mentioned, the reflectance computed in absolute terms (Figure 1a) did not offer enough discrimination among structures, except for the optic disc that presents a high reflectance along all VIS wavelengths. The relative reflectance was the parameter that distinguished the most among structures, as expected.

As reported in the literature, the retinal nerve fiber layer and the optic disc were easily observable at short wavelengths, with common higher reflectance than the other structures around 500 nm (Figure 1d) and as in [3]. The optic nerve showed an even higher reflectance and was clearly visible almost up to 1000 nm as in [4,5]. Arteries and veins showed the largest differences in relative reflectance around 600 nm as seen in Figure 1b and 1e. At this spectral range, the deoxygenated blood inside the veins is still absorbing light, while the oxygenated blood in the arteries presents an absorption close to zero [7], thus veins were seen darker than arteries in those images. The absorption of melanin in the RPE and in the choroid decreases progressively from 600 nm [6,8], letting light to reach the sclera and be reflected by it; due to this back-illumination, choroidal vessels were seen dark at NIR wavelengths (Figure 1f). The absorption of blue light by the macular pigment in the fovea [8] can be observed as a local minimum in Figure 1b around 450 nm and in Figure 1c as the fovea appears darker the surrounding tissue. The regions of fundus without structures showed higher reflectance values at the end of the VIS range (i.e. 732 nm) [6] and at the beginning of the NIR up to 955 nm, also caused by the light reflected by the sclera.

#### 4. Conclusions

The reflectance analysis performed by means of the fast visible and extended infrared multispectral fundus camera [9] provides a new methodology to spectrally analyze the main structures in the eye fundus, supporting the findings made by other authors. The NIR region entails relevant spectroscopic information that is commonly missed by traditional techniques, such as that related with the choroid. The precise characterization of the spectral reflectance of eye fundus in healthy population will be very helpful to detect any alteration that could lead to a pathology, especially if it affects deep layers. Thus, this spectral fundus imaging and the associated spectroscopic information could be used in the early diagnosis of retinal diseases, which is crucial to avoid vision loss.

#### 5. Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Marie Skłodowska-Curie grant agreement No. 801342 (Tecniospring INDUSTRY) and the Government of Catalonia's Agency for Business Competitiveness (ACCIÓ). This research was also supported by the Ministerio de Economía, Industria y Competitividad (MINECO), the Agencia Estatal de Investigación (AEI) and the European Regional Development Fund (FEDER) under the grant DPI2017-89414-R.

#### 6. Disclaimer

This work only expresses the opinion of the authors and neither the European Union nor ACCIÓ are liable for the use made of the information provided.

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