

Improving a Multispectral Camera for Simultaneous Retinal Oximetry and Multispectral Image Acquisition

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Retinal oximetry is a non-invasive, in-vivo technique that quantifies the oxygen saturation of hemoglobin within the retinal blood vessels. This measurement of retinal blood oxygenation plays a crucial role in the diagnosis and monitoring of various systemic and ocular conditions, including hypoxia, vascular endothelial growth factor, and diabetic retinopathy¹. However, a significant limitation of existing retinal oximetry systems is their inability to provide spectral information about the retina, which could offer valuable insights into retinal health and disease.

Several methods have been developed to assess retinal oxygenation. One approach utilizes single-wavelength imaging to detect blood flow dynamics over a brief period, typically a few seconds. This technique allows for the observation and detection of heart pulse-related fluctuations in blood flow, capitalizing on the normal human heart rate of 60 to 120 beats per minute. Optical Coherence Tomography Angiography (OCT-A) offers a three-dimensional visualization of the retinal vasculature, providing detailed structural information. However, OCT-A's temporal resolution limitations necessitate alternative methods for capturing dynamic blood flow changes. Some techniques employ specialized cameras and laser light sources to analyze blood cell movement based on speckle patterns, enabling the assessment of blood flow velocity².

Another approach focuses on multi-wavelength imaging, where oxygen saturation is calculated from a single capture. These methods capture retinal images at oxygen-sensitive and non-oxygen-sensitive wavelengths simultaneously from the same retinal field of view. By applying the Beer-Lambert law, the oxygen saturation within the retinal vessels can be calculated³. A significant challenge in these methods is the potential influence of Retinal Pigment (RP) layer on the measurements. RP variations can significantly affect reflectance from retinal veins, although the effect on arterioles is generally considered negligible. Traditional methods of estimating RP levels based on patient ethnicity have been proven unreliable³. To overcome this, researchers are exploring deep learning techniques to predict oxygenation levels directly from retinal images, potentially mitigating the need for separate RP calibration.

Our research utilizes multispectral retinal imaging, a technique providing rich spectral information crucial for detecting subtle pathological changes and biomarkers often missed by traditional methods. The foundation of our work is a multispectral camera initially developed by Alterini et al.⁴

Building upon previous work conducted by our research group (Burgos-Fernández et al.⁵, 2022), where the fundus reflectance was analyzed across various ocular conditions and retinal sections, we applied phasor analysis to extract notable features from the multispectral fundus images. Phasor analysis effectively reduces the dimensionality of data from numerous spectral bands (e.g., >12) into an intuitive 2D phasor plot (see Figure 1 for illustration), simplifying interpretation. Using features derived from this analysis, we successfully trained machine learning classifiers to differentiate between healthy and diseased retinas.

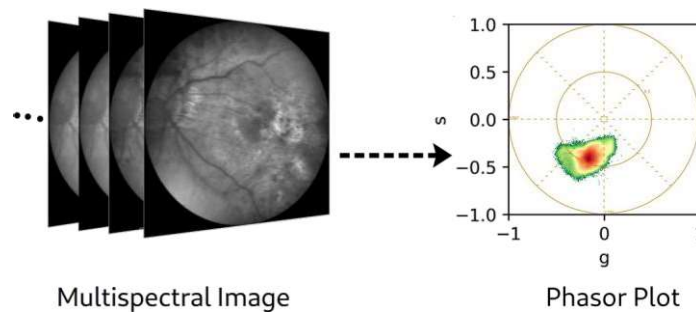


Figure 1: Every multispectral image consists of different bands, each representing a specific wavelength. By applying phasor analysis, the complex data from the multispectral image (known as a multispectral cube) can be simplified and converted into a 2D plot.

To further enhance the diagnostic potential of this approach, we are now reconfiguring the imaging system to incorporate retinal oximetry measurements. The device is capable of capturing images at multiple, distinct wavelengths necessary for this purpose. This modification will enable the acquisition of retinal vessel oxygen saturation data, allowing for the generation of oximetry maps. This oximetry information will then be fused with the multispectral retinal images. We hypothesize that the resulting fused 'multispectral-oximetry' data will provide more comprehensive information, significantly improving the ability to distinguish subtle disease-related biomarkers and spectral signatures that are difficult for the human eye to comprehend.

Generating retinal oximetry maps with this system encounters several key challenges. A primary concern arises from the sequential illumination of the retina using various LEDs, which introduces eye movement artifacts and a slight temporal delay in the acquisition of images at different wavelengths, potentially necessitating additional processing. Furthermore, retinal oximetry measurements, potentially lasting several seconds, necessitate specific wavelengths that might be in the visible spectrum leading to pupil's constriction. This, combined with the low reflectance of the retina, requires high-intensity LED illumination, which could lead to patient discomfort. To mitigate this issue while meeting oximetry requirements, we will prioritize the use of wavelengths at 620nm and above.

The multispectral-oximetry fusion process will be implemented using a two-step deep learning methodology. First, multiple oximetry related images will be fused into a single composite image. Second, this composite oximetry map will be fused with the multispectral retinal image to facilitate disease diagnosis.

ACKNOWLEDGEMENTS: Funded by European Union (HORIZON-MSCA-2022-DN, GA n°101119924-BE-LIGHT) and by MCIU/AEI/10.13039/501100011033 and FEDER, EU (Grant PID2023-147541OB-I00).

¹ E. Stefánsson, et al.; Retinal oximetry: metabolic imaging for diseases of the retina and brain. *Progress in retinal and eye research*, **70**, 1 (2019).

² K.A. Cho, et al.; Portable, non-invasive video imaging of retinal blood flow dynamics. *Scientific reports*, **10**, 20236 (2020).

³ K. Hirsch, R. P. Cubbidge, and R. Heitmar; Dual wavelength retinal vessel oximetry—influence of fundus pigmentation. *Eye*, **37**, 2246 (2023).

⁴ T. Alterini, et al.; Fast visible and extended near-infrared multispectral fundus camera. *Journal of biomedical optics*, **24**, 096007 (2019).

⁵ F.J. Burgos-Fernández, et al.; Reflectance evaluation of eye fundus structures with a visible and near-infrared multispectral camera. *Biomedical optics express*, **13**, 3504 (2022).