Improving biomedical diagnosis through light–based technologies and machine learning

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Abstract (for dissemination)	This deliverable reports the work and research carried out to develop a non-invasive and accurate method for retinal oximetry. We modified a multispectral imaging system capable of time-resolved fundus acquisition at selected wavelengths (660/955 nm and 598/660/955 nm), enabling temporally and spectrally resolved sequences from which pulsioximetric maps can be derived at every retinal location. These maps are expected to provide biomarkers relevant to multiple retinal diseases. Future work will integrate oximetry with other modalities (color fundus, reflectance data, optical coherence records) through multimodal deep learning, enabling advanced diagnosis, classification, and monitoring.
Keywords	Multispectral imaging, fundus, oximetry

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EXECUTIVE SUMMARY

This deliverable reports the work and research carried out to develop a non-invasive and accurate method for retinal oximetry. Over the past year, we refined a MultiSpectral Imaging (MSI) system capable of acquiring time-resolved fundus images at multiple wavelengths. The software was adapted to drive the existing LEDs closest to the recommended combinations: 660/955 nm for two-wavelength acquisition and 598/660/955 nm for three-wavelength acquisition. This setup enables temporally and spectrally resolved image sequences from which pulsioximetric measurements can be derived at every retinal location, providing potential biomarkers of various retinal diseases. Future work will be devoted to combine retinal oximetry with other imaging modalities (e.g., color fundus, MSI reflection-based images, Optical Coherence Tomography-OCT) using multimodal Deep-Learning (DL) methods to achieve a more complete characterization of ocular structure and function, supporting diagnosis, classification, and disease monitoring in both research and clinical practice.

INTRODUCTION

The motivation behind this project is to develop a non-invasive and accurate method for retinal oximetry measurements. Retinal oximetry is a technique used to measure the oxygen saturation levels in the retina, which can be an indicator of various retinal diseases. Current methods for retinal oximetry have limitations, such as requiring invasive procedures or using complex and expensive equipment. Our goal is to develop a multispectral camera system that can capture high-quality images of the retina and provide accurate oxygen saturation measurements in real time [1].

Over the past year, we have refined a MSI-based system capable of acquiring time-resolved fundus images at multiple wavelengths. This enables the generation of both temporally and spectrally resolved image sequences, which can then be used to derive pulsioximetric measurements at every retinal location with high efficiency.

Our future goal is to combine retinal oximetry with other imaging modalities (e.g., color fundus, MSI reflection-based images, OCT) using multimodal DL methods to achieve a more complete characterization of ocular structures and function, enabling for diagnosis, classification, and disease monitoring in clinical and research settings.

1 METHODS AND MATERIAL

1.1 Experimental setup

We have been working on a multispectral camera system in our lab, which was originally developed by one of our colleagues [2]. The camera system consists of two cameras: a high-resolution CMOS camera and an infrared InGaAs camera. The CMOS camera has a resolution of 2048 × 2048 pixels, with a pixel size of 6.5µm and 16-bit depth, while the InGaAs camera has a resolution of 640 × 512 pixels, with a pixel size of 20µm and 14-bit depth. The camera system is designed to capture multispectral fundus images, with the CMOS camera capturing 12 spectral images ranging from 416 to 955 nm, and the InGaAs camera capturing images in three bands from 1025 to 1213 nm.

The light source consists of multiple LED rings, each emitting light at a specific peak wavelength as mentioned. The LEDs are used to illuminate the retina, and the cameras capture images of the reflected light. In addition to that, there is a computer to control the cameras, illumination properties, and image acquisition (Fig. 1).

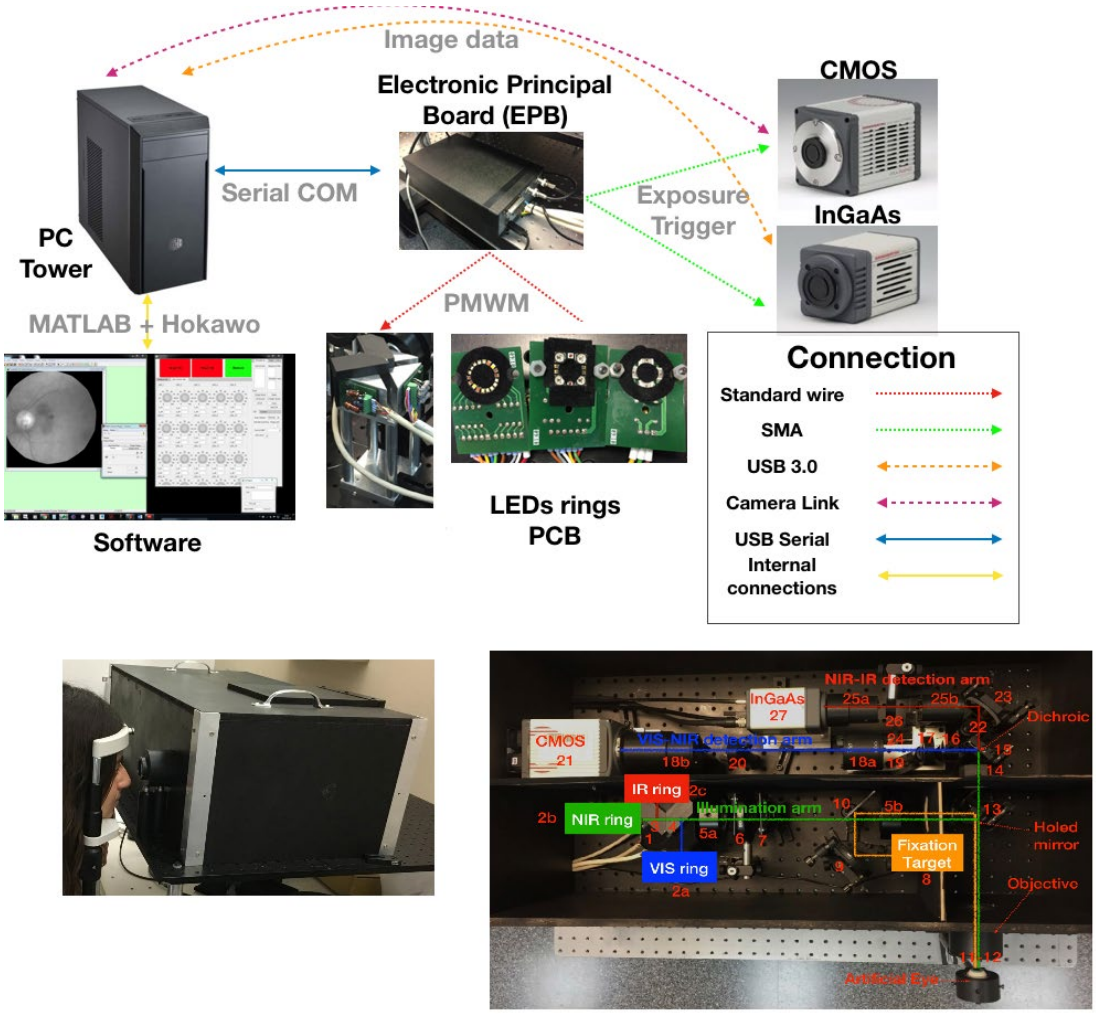

Fig. 1. Top: Optical components (LEDs rings and cameras), electronics and software included in the multispectral fundus camera system. Bottom: Lateral external (left) and top internal (right) views of the multispectral camera system.

1.2 Software and Measurements

To achieve non-invasive, time-resolved spectral records that allowed us to perform retinal oximetry measurements, we needed to modify the software of the multispectral fundus camera to capture images at specific wavelengths over time. We reviewed multiple studies that suggested different wavelength combinations for retinal oximetry, such as (532nm, 633nm), (635nm, 965nm), (520 nm, 546 nm, and 555 nm), (488 nm, 635 nm, & 905 nm), and (600 nm, 635 nm, & 905 nm) [3], [4]. However, we did not want to modify the LEDs to exactly match these wavelength combinations, as this could compromise the camera's ability to capture multispectral images.

Instead, we modified the software to use the existing LEDs with close wavelengths to the suggested combinations (Fig. 2). For two-wavelength combinations, we chose 660nm and 955nm, and for three-wavelength combinations, we chose 598nm, 660nm, and 955nm.

We also modified the software to reduce LED intensity and to repeat the wavelength combinations multiple times over a few seconds, enabling the acquisition of retinal image sequences suitable for recording pulsioximetric data. This was necessary because, unlike multispectral images, these images would take a few seconds to complete, and we wanted to minimize the impact of patient movement on the images. We also wanted to diminish the discomfort of the patient who was being analysed.

The total acquisition time and the frame duration is variable and under investigation. Initial findings indicate that at least 10ms are required for each shot to achieve a satisfactory image intensity. Allowing for a time-off between shots is necessary to prevent image capture and computer saving issues. In total, a frame duration of 20ms per wavelength is currently considered optimal, but this can be optimized further by adjusting camera settings. The total frame rate for image acquisition depends on the number of wavelengths used. For example, using two wavelengths yields a frame duration of 40ms, and frame rate of 25 frames per second (fps), while three wavelengths result in a frame duration of 60ms, and frame rate of almost 16 fps. The total acquisition time is also being investigated, with scenarios including 1s, 1.5s, 2s, 2.5s, and 3s.

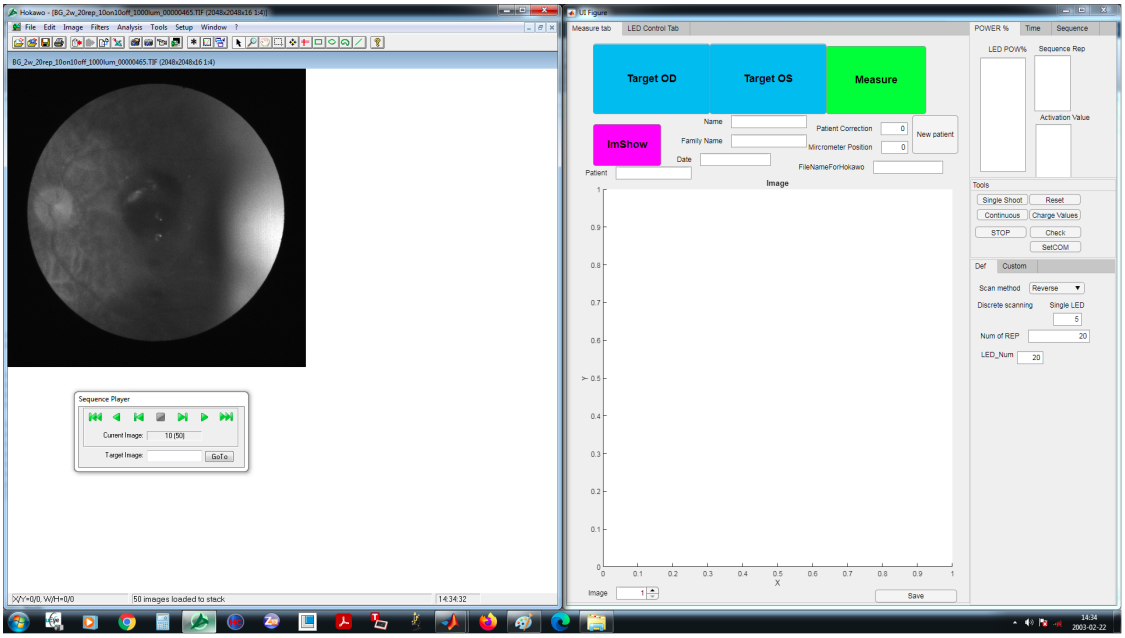

Fig. 2. Screenshot of the software which has been modified to allow for retinal oximetry measurements through the acquisition of spectral images over time at: two-wavelength combinations (660nm and 955nm) and three-wavelength combinations (598nm, 660nm, and 955nm).

Using the modified software, we captured multiple images from patients using both two- and three-wavelength combinations. However, we realized that the images were

not perfectly aligned due to small patient movement during the capture process. To address this issue, we are currently working on developing a software tool to register each image with each other, which will enable us to remove eye movements and perform further pulse oximetry analysis.

After capturing the images, we performed image processing to enhance the quality and remove noise. We used various techniques, such as filtering and normalization, to improve the quality and ensure that the images were suitable for analysis.

In particular, to improve image quality, the following steps are applied. The image is converted from 16-bit to float format. It is then normalized by subtracting the minimum value and dividing by a constant value chosen based on other images in the sequence captured with the same wavelength. This is done to prevent unwanted artifacts that could arise from choosing a maximum value based on each shot individually. Currently, the constant value is chosen manually to achieve the best visualization, but in the future, this process will be automated. After normalization, two Gaussian filters are applied to enhance the details of the image. A 40 pixels × 40 pixels Gaussian filter is used to blur the original image, and then this blurred image is subtracted from the original one, resulting in a dark image with enhanced edges. Another Gaussian filter with a size of 100 pixels × 100 pixels is then applied to the original image, creating a second blurred image. The second blurred image is added to the dark-edge image, resulting in a final image with enhanced details (Fig. 3).

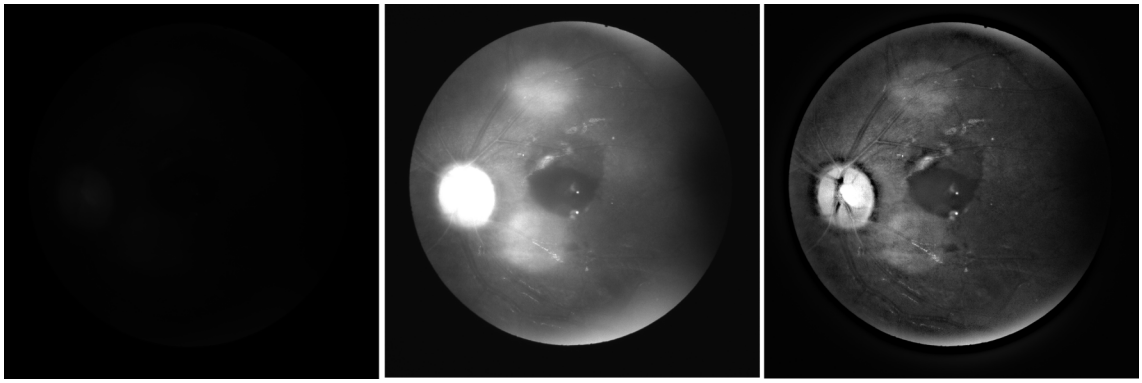

Fig. 3. The process of image quality enhancement on a single shot (frame). From left to right: a 16-bit raw image without normalization, an image normalized with a constant maximum value, and an enhanced image using Gaussian filters.

2 RESULTS

Fig. 4 and Fig. 5 show examples of spectral images taken at several wavelengths, which lasts 80 mseconds, and a time-sequence image acquisition taken during one second, respectively.

A calibration method is being developed using a finger pulse oximeter to validate the retina oximetry results. During image acquisition, data from the finger pulse oximeter is saved and will be used to compare the outcome of retina oximetry results with a commercial device used in clinics and hospitals. This will enable the establishment of a calibration curve for the developed retina oximetry algorithm, allowing for accurate measurements of oxygenation levels in the retinal vessels. It should be mentioned, however, that this pulse oximeter only allows for averaged measurements, whereas with

the MSI-based camera we can perform measurements locally since we have spatial resolution. In fact, Fig. 4 clearly shows the contrast between veins and arteries, stemming from the different oxygenation levels of the blood in each structure and the resulting differences in light absorption across wavelengths.

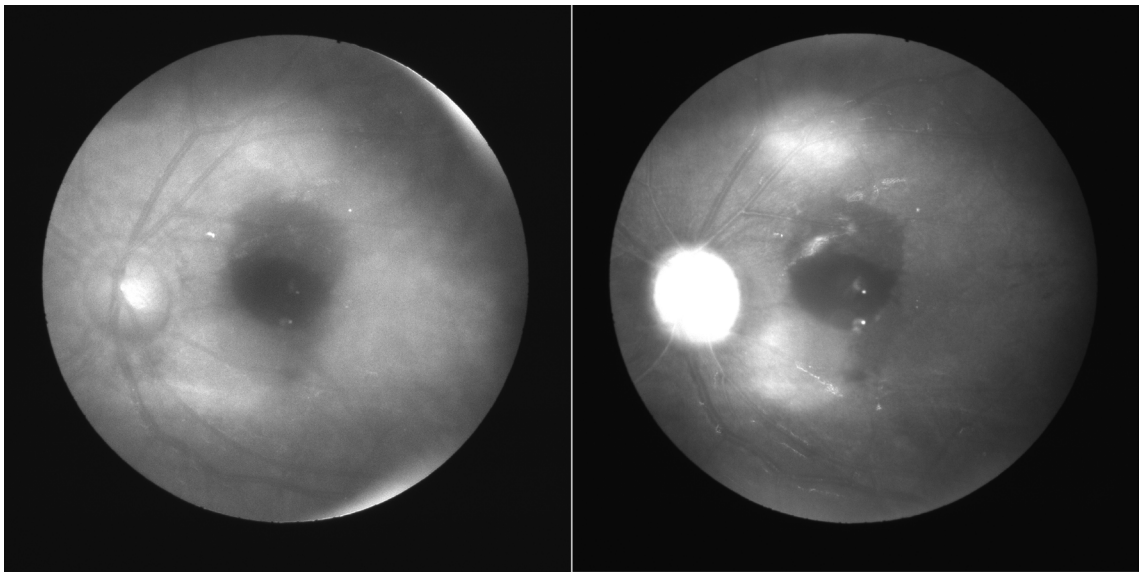

Fig. 4. Comparison of retinal images captured at two different wavelengths: 955nm and 660nm, shown from left to right. The difference in absorption between oxyhemoglobin and deoxyhemoglobin is clearly pronounced at 955 nm, whereas at 660 nm the two structures exhibit more similar absorption.

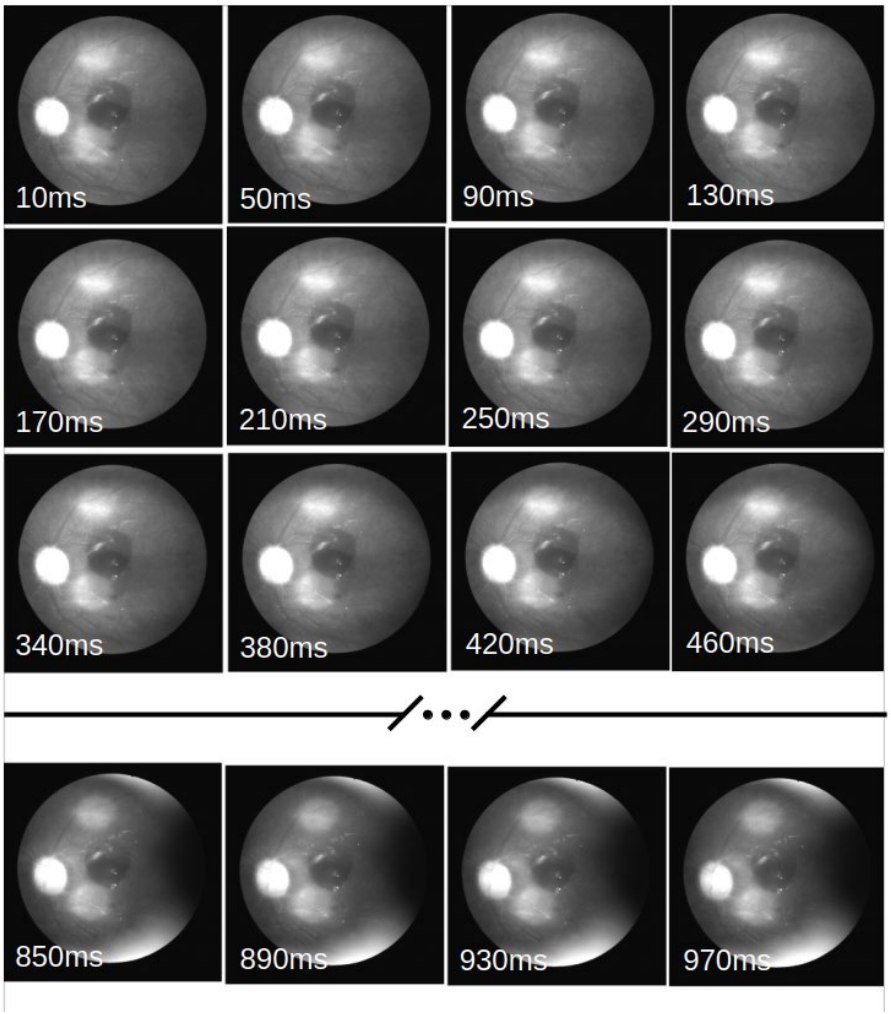

Fig. 5. Sixteen snapshots taken at 660nm, with timestamps for each image. The bottom row displays the last four images before the acquisition ended.

Ongoing work is devoted to automate the image enhancement process and apply a registration algorithm to keep each image at the same location. A region of interest will be chosen in the images to analyze the vessels, primarily those connected to the optic disk, as they are more visible than other vessels. By following the time changes in the images, it is expected to find the pulse inside the vessels. Then, by identifying pulses and changes in the intensity of the image, the oxygenation levels inside the retina vessels will be estimated.

3 SUMMARY

We have introduced several modifications to the experimental system described in [2], which is a multispectral fundus camera with sensitivity in the visible and near-infrared ranges (416–1213 nm) across 15 spectral bands. These modifications include a slight adjustment of the selected spectral bands and an increase in the temporal acquisition frequency (frame rate), allowing the system to capture not only spectral information but also time-resolved data. In parallel, custom algorithms are being developed to extract oximetric information from the multispectral channels and from temporal dynamics, enabling the generation of near real-time in vivo retinal oximetry maps. These maps

support the assessment of oxygenation levels in various ocular fundus structures, including arteries, veins, and others.

Our long-term objective is to combine oximetry metrics with additional modalities (e.g., color fundus photography, MSI reflection data, OCT) and to integrate them through multimodal and DL-based algorithms. By fusing complementary imaging sources, we aim to obtain a more comprehensive characterization of ocular structures and function. This enriched information will support the development of diagnostic and therapeutic tools based on Artificial Intelligence (AI) that can distinguish between healthy and diseased eyes, classify multiple fundus pathologies, improve understanding of disease progression, and ultimately provide valuable assistance in clinical and research settings.

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