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Multi-spectral vascular oximetry of rat dorsal spinal cord

Victor Ochoa-Gutierrez[a,b], Pavan C. Konda[a], Sara Motaghian[a], Julien Reboud[b], Jonathan M. Cooper[b], and Andrew R. Harvey[a]

[a]School of Physics and Astronomy, University of Glasgow, Glasgow, UK
[b]James Watt School of Engineering, University of Glasgow, Glasgow, UK

ABSTRACT

We describe a visible-light multi-spectral system for vascular oximetry studies that can be implemented in low- and middle-income countries, using a low-cost electronics and optical elements, for instance a Raspberry Pi, a Pi camera under a resolution of 5-megapixel, and four light sources at 480nm, 532nm, 593nm and 610nm. It is designed to quantify the vascular oxygen saturation of the rat dorsal spinal cord, Python custom application synchronizes all elements to execute the imaging process in one system. The device is suitable offers a replacement for high cost bulky systems in drug discovery, tracking disease progression and understanding of progressive and degenerative diseases.

Keywords: Multispectral Imaging, Oximetry, Imaging System, Gradient-Index Lenses, Low-resource Settings, Biomedical Engineering.

1. INTRODUCTION

The aim of this visible-light multi-spectral system is to achieve a solution capable of multispectral imaging analysis in real time that potentially can be adapted or modified according the especial needs of a particular research and researchers in low- and middle-income countries or remote places, by simply using a rechargeable battery pack to power the portable imaging system. In-vivo microscopic imaging techniques [1,2], *i.e.* oximetry on mice and rats is widespread and increasingly animal models are used in biomedicine, for drug discovery and tracking disease progression. The number of animals used for experimental purposes continues to rise every year, in 2018 an estimated of 3.6 million procedures were made using mice and rats [3]. We propose an in-vivo imaging system that has the promise to reduce the number of animals by collecting of high-resolution imaging data in longitudinal repeated-measures during one single study especially the oxygen consumption in deep tissue that has been proved that has a relationship to different diseases.[4–9]

2. LOW-COST SYSTEM OVERVIEW

An imaging system was assembled and designed using; red LED (625nm), amber LED (590nm), green LED (525nm) and blue LED (475nm), dichroic optical blocks (Thorlabs - CM1-DCH/M), longpass dichroic mirrors (Thorlabs), focusing lenses, heat sinks (Figs. 1, 2 and 3). Universal port that holds a GRIN lens (GRINtech), structured illumination is used by a fiber-array adapted to Raspberry Pi camera, these elements were used to build a low-cost system for imaging.[10] In addition, the system is capable of directing the illumination into an annular illumination pattern, allowing a single pass illumination of the vasculature,[7] *i.e.* reducing the complexity of the oxygenation measurement in deep tissue and blood vessels for a better understanding of axonal remyelination, multiple sclerosis research, immune cells in lymph nodes and towards a cure for rheumatoid arthritis. The universal imaging sensor; is constituted by a Pi camera (resolution 5-megapixel, 2592x1944-pixel static images) and 3D printed holder for the GRIN lens and the illumination fibers, with a locking mechanism to improve coupling between the camera and the GRIN lens, see Fig. 1. The system uses a Python custom application to control the imaging and illumination elements synchronizing all elements at once for oximetry measurement prospectuses.

Further author information: (Send correspondence to A.R.H.)
A.R.H.: E-mail: Andy.Harvey@glasgow.ac.uk

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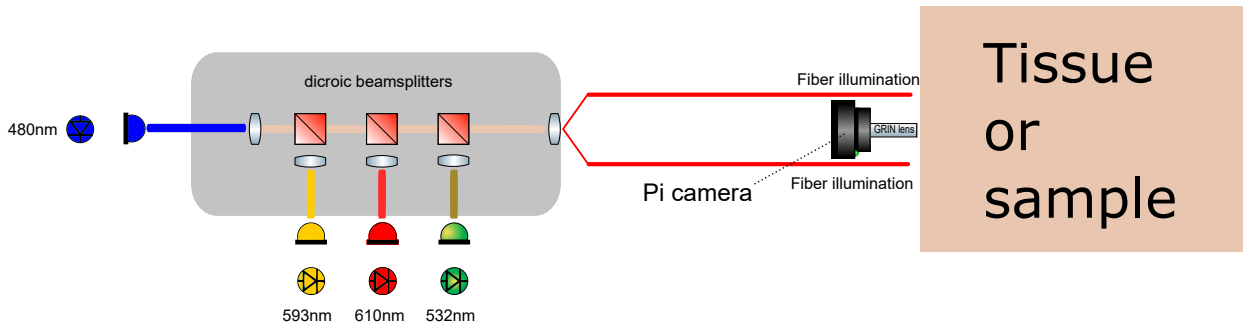

Figure 1. Principal imaging system elements; optical illumination, image acquisition and optical magnification using a GRIN lens, tissue representation.

Figure 2. Optical block for structured illumination, dichroic mirrors in position (Thorlabs).

3. RESEARCH APPLICATIONS FOR IMAGING TECHNIQUES IN LOW-RESOURCE SETTINGS

The developed system will enable a longitudinal vascular oximetry study using a structured illumination technique on a single animal over a period of four or six weeks. An important contribution is the reduction of use of rodents by a factor four or five. a second important benefit is the reduction of variability inherent in longitudinal study of one single animal, this will enable high quality data related to the degenerative diseases reducing the uncertainty of using more rodents in short periods of time.

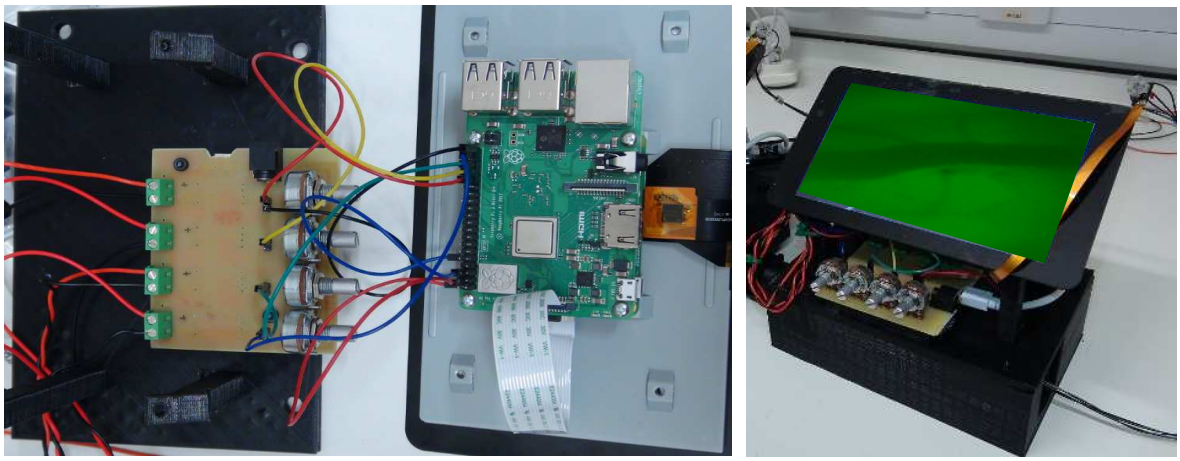

Figure 3. Imaging System completely assembled and operational for research studies.

4. INITIAL DEMONSTRATIONS

Recent evidence has implicated mitochondrial dysfunction, and hypoxia, in the aetiology and pathology of multiple sclerosis (MS) and experimental autoimmune encephalomyelitis, an animal model of MS.[6,7] The role of these pathological changes in neurological dysfunction, lesion formation, and disease progression remains unclear. However, obvious differences in the vasculature (such as diameter, and oximetry variations, especially hypoxia patterns) have been observed that correlate with measures of disease severity and neurological dysfunction in rats with experimental autoimmune encephalomyelitis (EAE) during the early stages of disease. For preliminary purposes images were recorded from a hyperspectral image sequence of the exposed rat spinal cord shown in Fig. 4. This initial demonstration shows the optical and imaging performance of the system, that provides the vital information for the oximetry signal extraction.

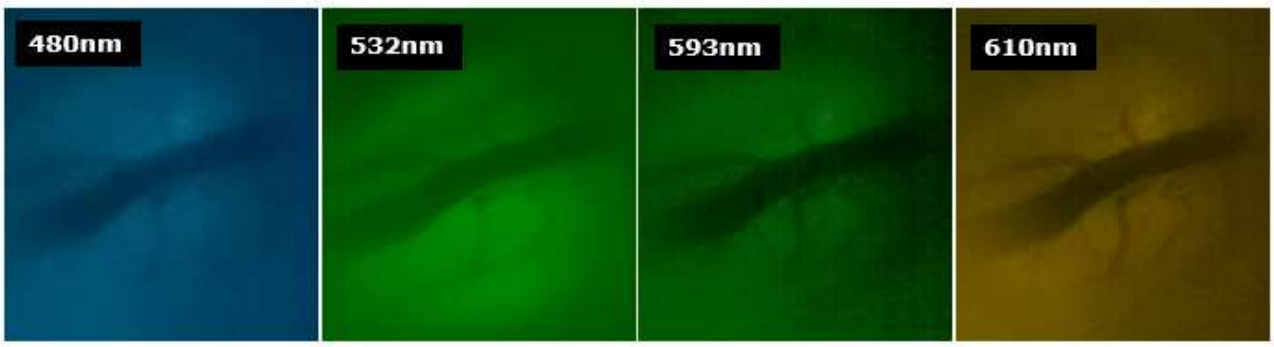

Figure 4. Rat spinal cord vasculature imaged at 480nm, 532nm, 593nm and 610nm. Oximetry is based on varying intensity of blood vessels with wavelength where the changes on oxi-haemoglobin can be measure.

5. CONCLUSION

We described a visible-light multi-spectral microscope system for longitudinal oximetry studies in small animals. This device will continue to be improved and continue tested in the field, that includes modifications at the mathematical model establish by [7,11] for oximetry calculations, avoiding some errors when using two oximetry wavelengths [12,13] for multi imaging vasculature studies. Enabling a low-resource settings tool that will lead to better understanding of the progression of degenerative diseases based on the oxygenation levels, especially during the absence of oxygenation or hypoxia stages [14] .

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