

BSC PROJECT

IMPERIAL COLLEGE LONDON

DEPARTMENT OF PHYSICS: PHOTONICS GROUP

**Developing low-cost open source microscopes
for LMIC laboratories: 4-LED emission
source optical module integrated with the
 μ Manager software for epifluorescence
microscopy**

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1 Abstract

This project aimed to develop a low-cost and open-source module to be used for light microscopy in LMIC laboratories with applications in life sciences and histopathology, specifically for fluorescence microscopy. The module has 4 LUXEON Rebel LEDs mounted on a SinkPAD-II™23mm Quad LED Module that can be controlled via the μ Manager software through an Arduino Nano. The brightness of the LEDs is controlled by using an MCP4725 DAC to vary the potential across a 2100mA Dimmable BuckBlock LED Driver. The electronic design was designed as a PCB with a total cost of £155.40 for all the electronics. An optical system was also designed to enable the beams from the LED, which were offset from the principal axis by 7.1 ± 0.1 mm, to overlap using optical fibres and a 3D printed mount. The setup provided uniform illumination on the sample plane via Köhler illumination with a 200mm collector lens, a 206mm tube lens and a 10x objective. This setup was compared with an alternative setup which used a single on-axis optical fibre to transmit light from a single LED source and used a 32mm Fresnel lens for the collector lens - this did not achieve uniform illuminating at the sample plane. The overall percentage optical efficiency (from LED to sample plane) for the red, deep red, blue and green LEDs were $0.571 \pm 0.001\%$, $0.149 \pm 0.001\%$, 0% and $0.331 \pm 0.001\%$ respectively. This setup was used to perform epifluorescence microscopy to image pollen grains.

2 Introduction

Light microscopy^[1] is an essential element of histopathology^[2] and is used extensively for clinical diagnosis of a wide range of medical conditions^{[3][4]}. However, with the recent developments in new labelling^[5] and imaging techniques and photonic technologies^[6], it has become unsustainable to maintain such instrumentation for laboratories in Low- and Middle- Income Countries (LMIC), i.e. countries whose gross national income (GNI) is below US \$13,205 as classified by the World Bank^[7]. With technology rapidly advancing, expensive instruments are becoming obsolete and without service agreements for said obsolete instruments, it is not financially feasible for LMIC countries to contribute to research in growing research fields such as life sciences and histopathology.

openScopes^[8] is an organisation that allows such LMIC laboratories to assemble low-cost devices for microscopy with advanced capabilities. They offer modular components and open-source software which provide LMICs with access to advanced microscopy techniques such as super-resolution, 3D imaging etc. The openFrame^[9] microscope platform was designed with such intentions and it provides an open-source and modular approach to fluorescence microscopy. It was designed as a collaboration between the Photonics Group and Optomechanical Instrumentation Workshop in the Physics Department at Imperial College London, and Cairn Research Ltd.

This project involves developing a low-cost and open-source computer-controlled LED light source for fluorescence microscopy applications in life science and histopathology. It aims to be low maintenance, has automation capabilities and has a low power consumption.

2.1 Fluorescence Microscopy

Fluorescence microscopy^[10] is a common microscopy technique used in life science research. In contrast to traditional light microscopy, which uses visible light, fluorescence microscopy exploits the fluorescence properties of the sample itself to produce more detailed and reliable images^[11].

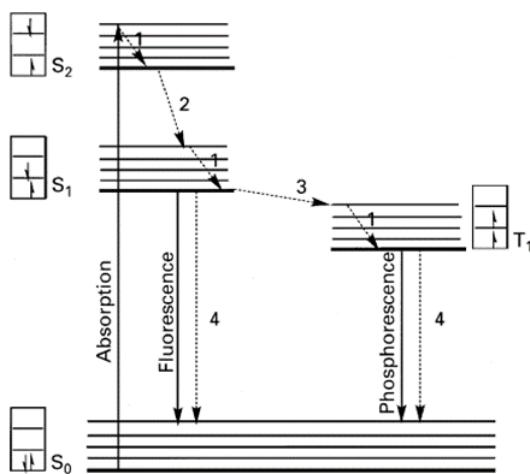


Figure 1: Jablonsky diagram for absorption, fluorescence and phosphorescence. S_0 is the ground state, S_1 and S_2 are the first and the second singlet states and T_1 is the first triplet state. Radiative and non-radiative processes are depicted by the solid and dashed lines respectively. Reproduced from^[12].

2.1.1 Fluorescence

Fluorescence is a form of luminescence where the substance absorbs light and remits it at a longer wavelength. The processes involved in fluorescence are excitation via absorption of a photon, relaxation processes to "relax" the molecule to the first singlet state (S_1) and stimulated emission of the fluorescence photon. The wavelengths of the photon observed during absorption and stimulated emission in fluorescence, are given by the equation $\lambda = \frac{\hbar c}{\Delta E}$, where λ is the wavelength of the photon, c is the speed of light and ΔE is the energy gap between the 1st singlet state and the ground state. Phosphorescence, which will not be discussed heavily in this report, is also a form of luminescence caused by the stimulated emission from the first triplet state to the ground state, but it lasts much longer than fluorescence.^[12]

2.1.2 Epifluorescence Microscopy

Epifluorescence microscopy is a technique commonly used in cell biology because the illumination beam can penetrate over $10\mu\text{m}$ through the sample. This method allows for imaging with multi-coloured labels on the same sample as the setup can be designed to accommodate multiple wavelengths for the excitation light and emission light.^[13]

Epifluorescence microscopy consists of five essential components:

- **Light source:** This is the emission source and determines the wavelength and intensity of the light entering the system.
- **Excitation filter:** This filter only allows the bands of wavelength that will be used to excite fluorophores in the sample to be transmitted.
- **Emission filter:** This filter only allows the bands of wavelength that will be emitted by the fluorophores by de-excitation to be transmitted.

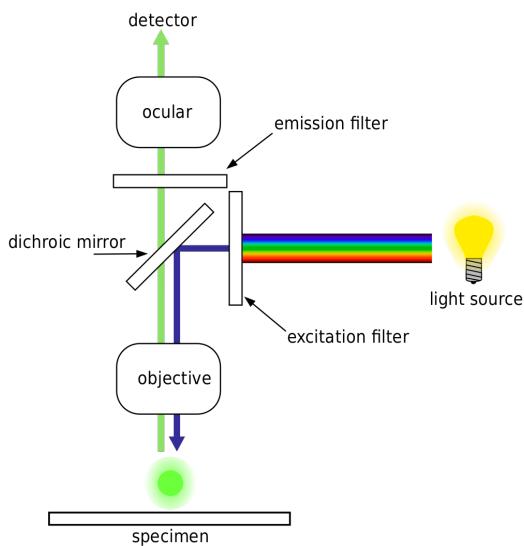


Figure 2: Diagram showing the setup for epifluorescence microscopy. The blue line shows the light path used for excitation. The green line shows the light path of the fluorescence. By derivative work: Henry Mühlfordt (talk)

- **Dichroic mirror:** This is essentially a beam splitter, but it allows specific wavelengths to be transmitted and the rest to be reflected. The light from the excitation filter is reflected and the light from the emission filter is transmitted.
- **CCD camera:** This is used to capture the image of the fluorescence from the fluorophores in the sample.

The light path and the setup of the components are shown in Fig(2). The intensity of the fluorescence emission is proportional to the intensity of the excitation light from the light source because more photons will be incident on the sample causing the probability of the fluorophore to fluoresce to increase.^[14]

2.1.3 Emission source

Typical light sources for epifluorescence microscopy are based on LEDs, arc lamps and lasers. Arc lamps and lasers are expensive to maintain so they are not viable for LMIC laboratories.^[15] LEDs offer a better range in terms of pricing, but the commercially available ones such as the CHLORIST™6-Wavelength High-Power LED Sources^[16] and CoolLED pE-4000^[17], which offer wavelength control, are quite expensive.

This project aims to design a low-cost, open-source and sustainable alternative to the currently available LED-based light sources with brightness and wavelength control.

2.1.4 Köhler illumination

Köhler illumination is used to achieve uniform illumination, without imposing the image of the light source, at the sample plane.^[18] This is a 4f optical system that uses a collector lens to first collimate the beam, then a field lens and a condenser lens to create a uniform distribution of light across the sample plane (see Fig(3)).

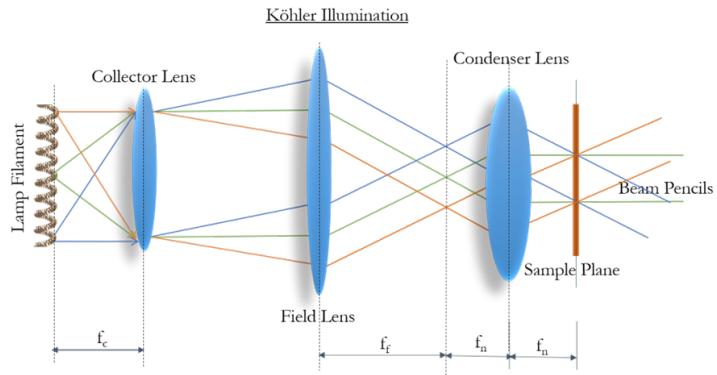


Figure 3: Köhler illumination of the light source with a collector lens, field lens and condenser lens with focal lengths of f_c , f_f and f_n for uniform illumination at the sample plane. Reproduced from^[19].

3 Declaration of work undertaken

This was our first project supervised by Professor Paul French. The project is divided into two parts: an electronics part for controlling the brightness and integration with μ Manager, and a photonics part for designing the coupling with the emission source and the microscope. The two parts were developed independently with the electronics part being developed by myself (Vamsikalyan Sridharbabu) and the photonics part developed by my project partner (Punveer Chima). The common element between the two parts was the SinkPAD-II™23mm Quad LED Module mounted with the 4 Rebel LEDs. The data acquisition, results and analysis were done collectively.

4 Method

4.1 The LED Module

Controlling the wavelength and the brightness of the light source is incredibly important for fluorescence microscopy. In cell biology, each part of the sample is usually labelled with a different fluorophore which has different excitation levels, meaning wavelength control would allow the user to choose which labelling fluorophore would fluoresce, and thus be detected. It is not possible to manufacture LEDs with varying wavelengths throughout the visible range, so we have to use multiple LEDs with different wavelengths to achieve this control. Brightness control is also essential as it would allow us to control the intensity of the fluorescence emission. Control over the intensity of the detected light will also be useful to combat CCD saturation, where the pixels of the CCD would become saturated^[20] i.e. the pixel cannot collect more charge, this limit is known as the saturation charge level and exceeding this can result in blooming artefacts in the captured images.

4.1.1 The SinkPAD-II™23mm Quad LED Module

Both parts of this project are designed to be compatible with the SinkPAD-II™23mm Quad LED Module^[21], see Fig(4.a). This module has 4 LUXEON Rebel LEDs^[22] mounted $7.1 \pm 0.1\text{mm}$ ^[21] from the centre, the assembly of the SinkPAD-II™is shown in Fig(4.b).

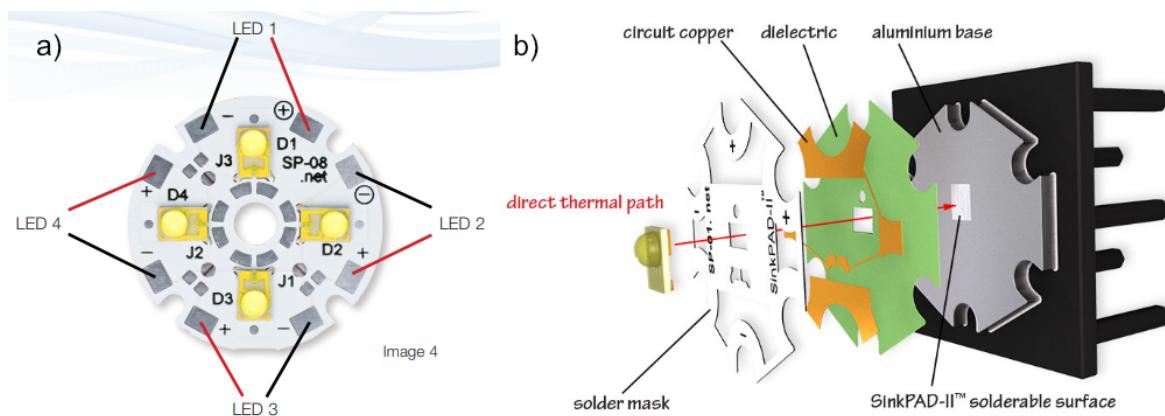


Figure 4: (a) Image of the SinkPAD-II™23mm Quad LED Module. (b) The assembly drawing of the SinkPAD-II™Technology. Reproduced from^[21].

We chose Deep Red, Red, Green and Royal Blue for the configuration of the LED module. The LEDs have a beam angle of 125° and the dimensions are $4.5 \times 3 \times 2\text{mm}$. The product attributes of the individual LEDs are shown in Table 1.

The wavelengths of the LEDs were chosen to cover a good amount of the visible range. We ensured this by measuring the wavelength spectra of the 4 LEDs using the ThorLabs PM100D with an 18mm by 18mm Microscope Slide Photodiode Power Sensor. The measured wavelength spectra are shown in Fig(5).

The cost of each LED is listed in Table 1. With the added cost of US \$5.25 for the SinkPAD-II mounting, the total cost of the chosen configuration amounts to US \$29.79. This is considerably cheaper compared to any mainline CoolLED fluorescence light sources, which means it will be cheaper to maintain and replace in LMIC laboratories.

Product attributes of the LUXEON Rebel LEDs					
LED colour	Peak wavelength (nm)	Wavelength range (nm)	Max drive current (mA)	Cost (US \$)	
Deep Red	655	650 - 670	700	4.08	
Red	627	620 - 645	700	5.82	
Green	530	520 - 540	1000	5.48	
Royal Blue	447	440 - 460	1000	9.16	

Table 1: Table listing the useful product attributes of the LUXEON Rebel LEDs.

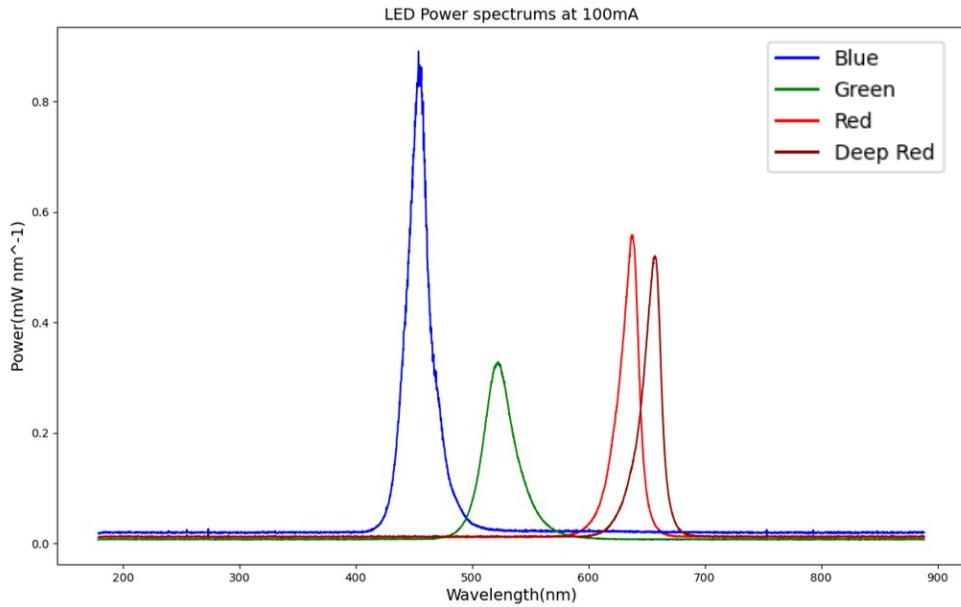


Figure 5: The wavelength spectra of the 4 LUXEON Rebel LEDs mounted on the SinkPAD-II 23mm Quad LED Module measured using ThorLabs PM100D with an 18mm by 18mm Microscope Slide Photodiode Power Sensor.

4.1.2 2100mA Dimmable BuckBlock LED Driver

Controlling the brightness of an LED is achievable by varying the current going through the LED. This can be achieved using a constant current supply, such as the 2100mA Dimmable BuckBlock LED Driver.^[23]

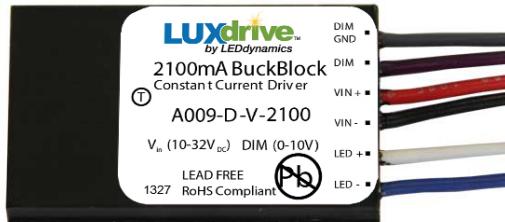


Figure 6: Image of a 2100mA Dimmable BuckBlock LED Driver. The component has six terminals which are labelled DIM GND, DIM, VIN +, VIN -, LED + and LED -. Reproduced from^[23].

The BuckBlock is shown in Fig(6). The device is powered by a potential difference across VIN + and VIN -. The LED is connected to LED + and LED -. The ratio of potential across DIM and DIM GND to 10V scales the current going into the LED.

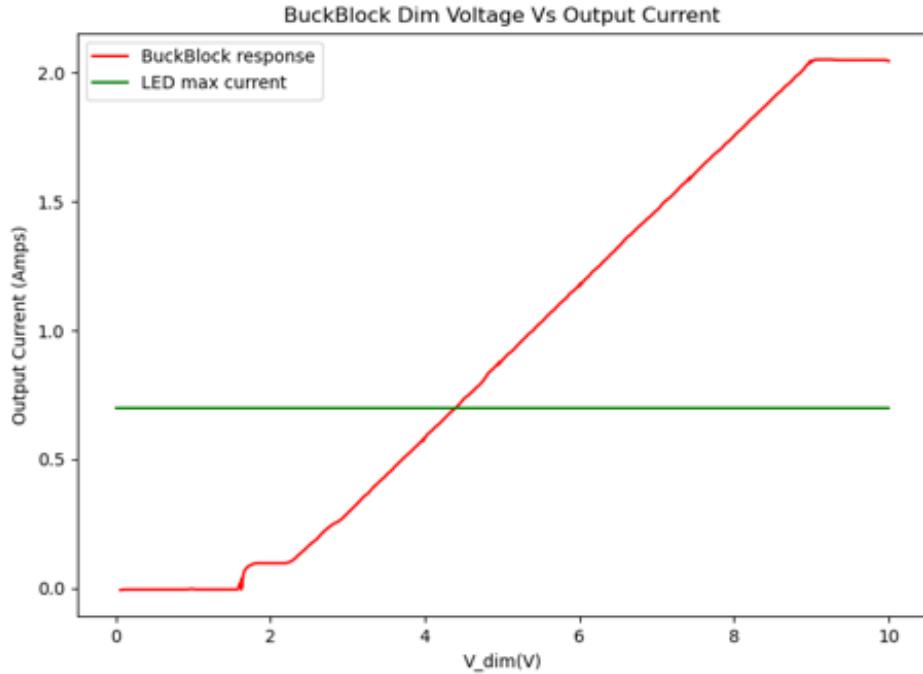


Figure 7: (red) The current through the LED terminals of the 2100mA Dimmable BuckBlock LED Driver depends on the potential across the DIM terminals. (green) The maximum current of 700mA corresponds to the lowest max driving current of the SinkPAD-II™23mm Quad LED Module - Red and Deep Red LEDs.

The dependence of the current through the LED terminals of the 2100mA Dimmable BuckBlock LED Driver to the potential across the DIM terminals is shown in Fig(7). The cost of the 2100mA Dimmable BuckBlock LED Driver is US \$20.49.

4.2 Electronics: Luminosity Control System

The electronics were designed to be controlled by an Arduino Nano^[24] through the μ Manager software^[25]. μ Manager is open-source software designed for the control and automation of microscope hardware. Integrating the product with μ Manager will benefit the usability and automation of imaging and data acquisition with the multiple LEDs.

4.2.1 Arduino Nano integrated with μ Manager

Arduino Nano (Nano) is an incredibly low-cost microcontroller (US \$23.37), which makes it accessible to LMIC laboratories. It is an extremely versatile device with 22 I/O pins (of which 6 are analogue), 30 kilo-bytes of programmable flash memory (this stores the program), 16MHz clock

speed and it is also capable of both SPI and I2C communication (these will be explained further in this chapter). The pinout of the Arduino Nano is shown in Fig(8).

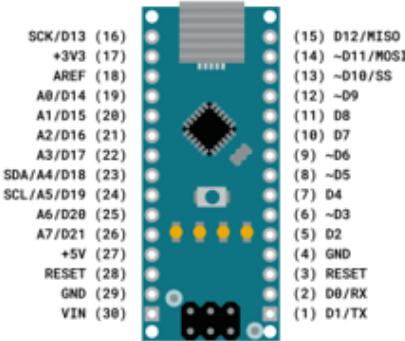


Figure 8: Pinout diagram of the Arduino Nano. Reproduced from^[26].

The Arduino Nano can be connected to a PC using a Mini-B USB cable. I used the Arduino Integrated Development Environment (IDE) to write the C++ code for the Arduino Nano. The code is compiled and transferred to the Arduino Nano's flash memory automatically by the IDE.

The Arduino firmware can be found here: <https://valemab4.ucsf.edu/svn/micromanager2/trunk/DeviceAdapters/Arduino/AOTFcontroller/AOTFcontroller.ino>

The Arduino firmware for communicating with μ Manager was uploaded to the Arduino Nano. Then I set up the Arduino Hub device adapter with a BaudRate of 57600 as specified on the μ Manager documentation. Updating the DAC1/DAC2 slider (range of 0 to 5) in μ Manager's Device Property Browser calls the **analogueOut()** function in the firmware source code with the parameters of the channel number (0 or 1) corresponding to DAC1/DAC2 and the lower and upper bytes of the 12-bit integer, between 0 to 4095, corresponding to the slider value.

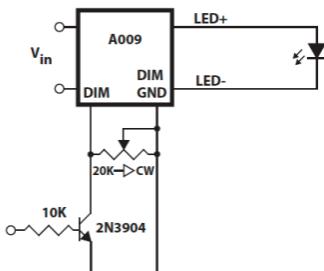


Figure 9: External dimming control with ON/OFF control. The dimming is controlled using a potentiometer across DIM and DIM GND.^[23]

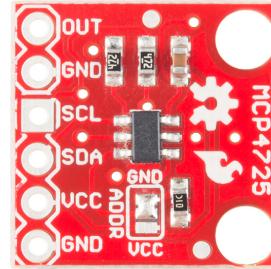


Figure 10: Image of the SparkFun I2C DAC Breakout - MCP4725. The pins are two GND pins, VCC, SCL, SDA and OUT.^[27]

4.2.2 Dimming Control

This part of the electronic part was initially done by both me and my project partner when we were testing a potential divider circuit to control the potential difference across the DIM terminals of the BuckBlock using the MCP4151-103E/P DigiPot^[28]. I, then replaced this circuit with a digital-to-analogue converter (DAC) based design using the MCP4725^[29] since the DigiPot design was quite

unreliable in switching the potential difference. Although the DigiPot design was unreliable, it is worth discussing the design. The design was inspired by a standard configuration in the BuckBlock's documentation, see Fig(9). The potentiometer in the circuit was replaced by the MCP4151-103E/P DigiPot, which used SPI communication to vary the resistance from 0Ω to $10k\Omega$, with a step size of about 40Ω . When this circuit was implemented, the DigiPot struggled to shift the resistance reliably and it was contributing noise into the system. This setup was abandoned, however, it would have worked with a better potentiometer.

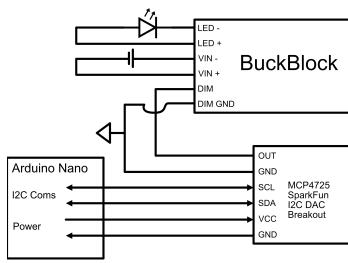


Figure 11: Block diagram of the single LED dimming circuit. The BuckBlock, MCP4725 and Arduino Nano are connected to control the potential across DIM and DIM GND using I2C communication.

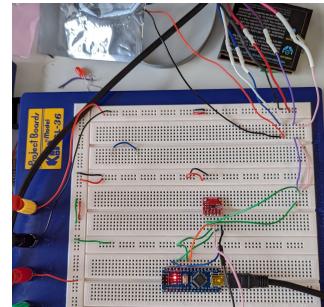


Figure 12: Physical circuit on a breadboard of the single LED dimming circuit, LED is inside a box for safety.

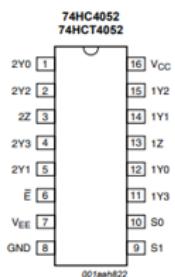


Figure 13: Pinout diagram of the 74HC4052 4-channel analog-multiplexed/demultiplexer. Reproduced from^[30].

Channel selection of the 74HC4052		
S0	S1	n
0	0	0
1	0	1
0	1	2
1	1	3

Table 2: Table showing the S0 and S1 values (0 - digital low, 1 - digital high) and the corresponding channel number (n) for the 74HC4052 4-channel analog-multiplexed/demultiplexer.

The dimming circuit was redesigned to use the MCP4725 DAC to control the potential difference across the DIM and DIM GND of the BuckBlock. A DAC varies the voltage between the ground and the VCC depending on the digital value input. The MCP4725 uses I2C communication^[31], which is controlled by the A4/SCL and the A5/SDA pins on the Arduino Nano. This circuit was tested with the SparkFun I2C DAC Breakout board^[27] for the MCP4725 chip (see Fig(10)). Both GND pins were grounded and the Arduino Nano's 5V was used to power the chip. The block diagram of the signal dimming setup is shown in Fig(11). I2C is a two-way communication between the controller (Arduino Nano) and the peripheral (MCP4725). SCL is the clock and SDA is for the data packets. The peripheral is detected I2C by an address to allow for multi-device communication. The MCP4725's A0 pin can be set to a digital low (0V) or a digital high (5V) to change the address^[29]. The chip is available to be manufactured in 4 different address configurations, thus allowing 8 different addresses for the MCP4725. The MCP4725 takes 12-bit input on the SDA, which maps perfectly to the 12-bit

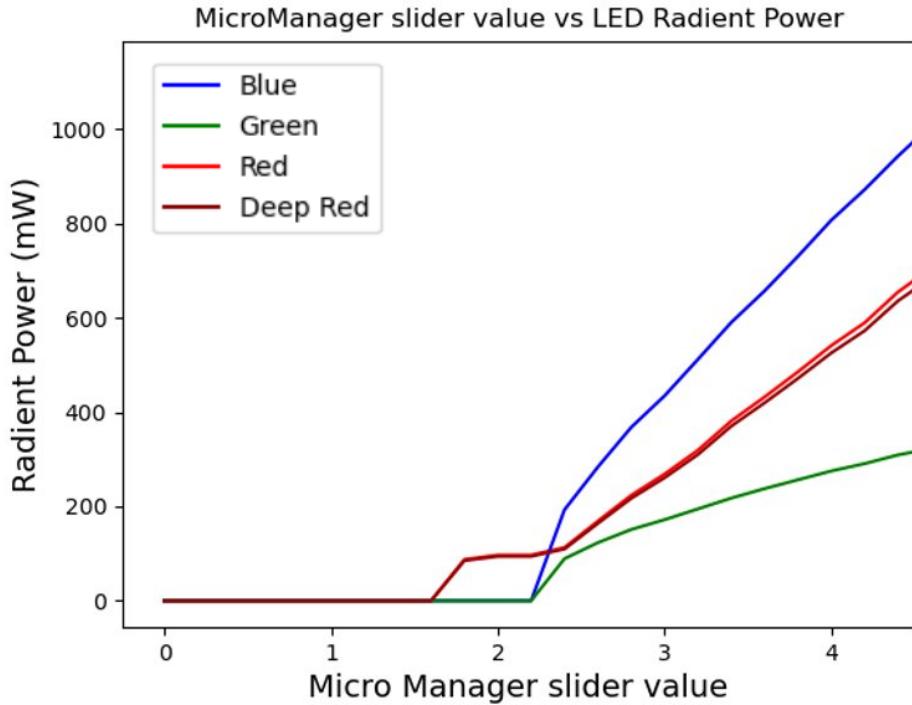


Figure 14: Relative luminous flux of the LUXEON Rebel LEDs vs the value along μ Manager's slider.

values from μ Manager's sliders. The I2C is implemented using the Wire.h from the Arduino library. The response of the LEDs' power output to the slider value is plotted in Fig(14).

4.2.3 4-LED Control System

The 4-LED control system sets up 4 MCP4725-based dimming circuits running in parallel. The SparkFun breakout board only used the 0x60/0x61 MCP4725 configuration^[27], which is not enough addresses for 4 DACs on the same 12C channel. To combat this, a 74HC4052 4-channel analog-multiplexer/demultiplexer^[30] (see Fig(13)) was used to allow two-way communication, which allows us to select one of the four electric pathways for the SCL and SDA signals to flow through.

The input pin 1Z outputs to the four 1Yn pins and the input pin 2Z outputs to the four 2Yn pins. The value of n is determined by the 2-bit integer formed by the S0 and S1 pins by setting either pin to a digital high/low, as shown in Table 2. The SCL and SDA pins of the Arduino Nano were wired to the 1Z and 2Z pins respectively. The S0 and S1 pins were connected to the D4 and D5 digital I/O pins of the Arduino Nano. The 1Yn and 2Yn pins were connected to the SCL and SDA pins of their respective MCP4725 DACs. The \bar{E} , V_{EE} and GND pins are all connected to the Arduino Nano's ground.

The Arduino device adapter of the μ Manager software was modified to allow for 4 sliders to control the 4 DACs (see Fig(15)). All 4 sliders have a range from 0 to 5.

Tweaking a slider sends its corresponding channel number (0,1,2,3) to the **analogueOut()** function, described in Fig(16). This then changes the values of S0 and S1 to change the pathway from the 1Z and 2Z pins to the corresponding 1Yn and 2Yn pins, allowing I2C communication between the

Arduino Nano and the corresponding DAC.

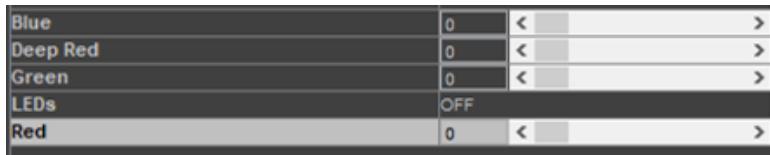


Figure 15: 4 sliders corresponding to the DAC of each LED (Blue, Deep Red, Green, Red) on μ Manager's Device Property Browser. The LEDs button allows the user to set all sliders to 0.

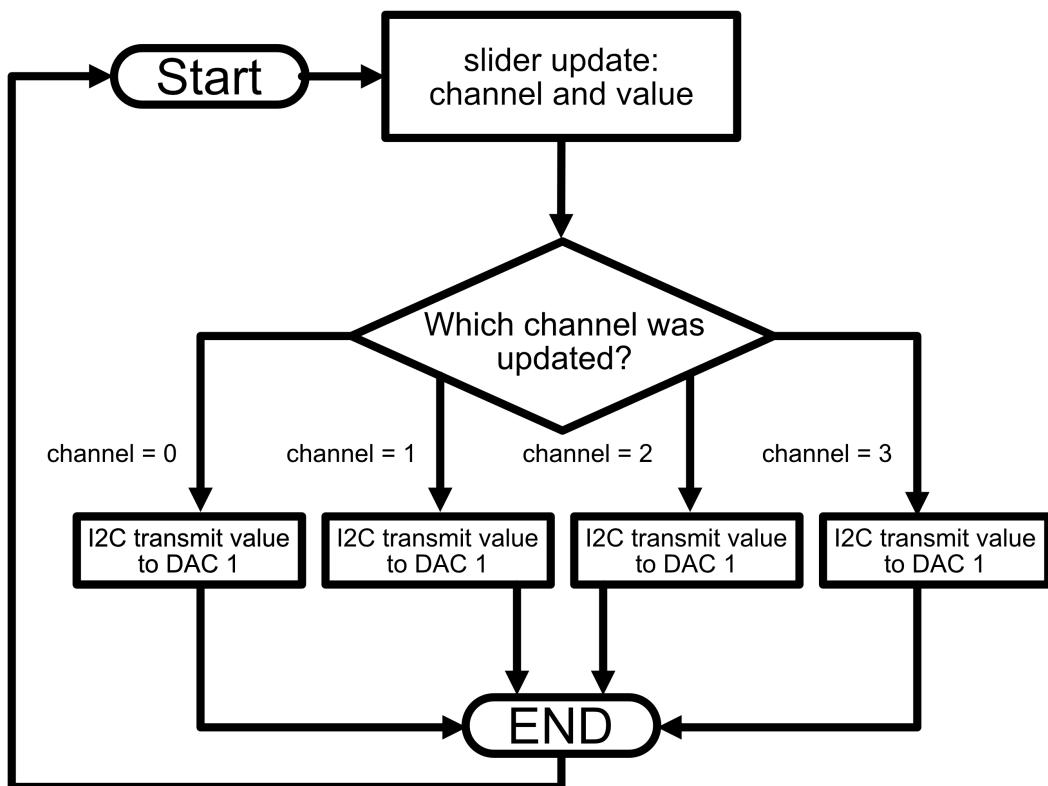


Figure 16: Flowchart showing the logic of the `analogueOut()` function.

The schematic of the final circuit for the 4-LED dimming control system is shown in Fig(17). The final circuit was built and tested on a breadboard. This is shown in Fig(18). Looking at Fig(18.e), you can see that the wiring resembles a bird's nest - this is the reason why a printed circuit board (PCB) is necessary for easier usage and debugging.

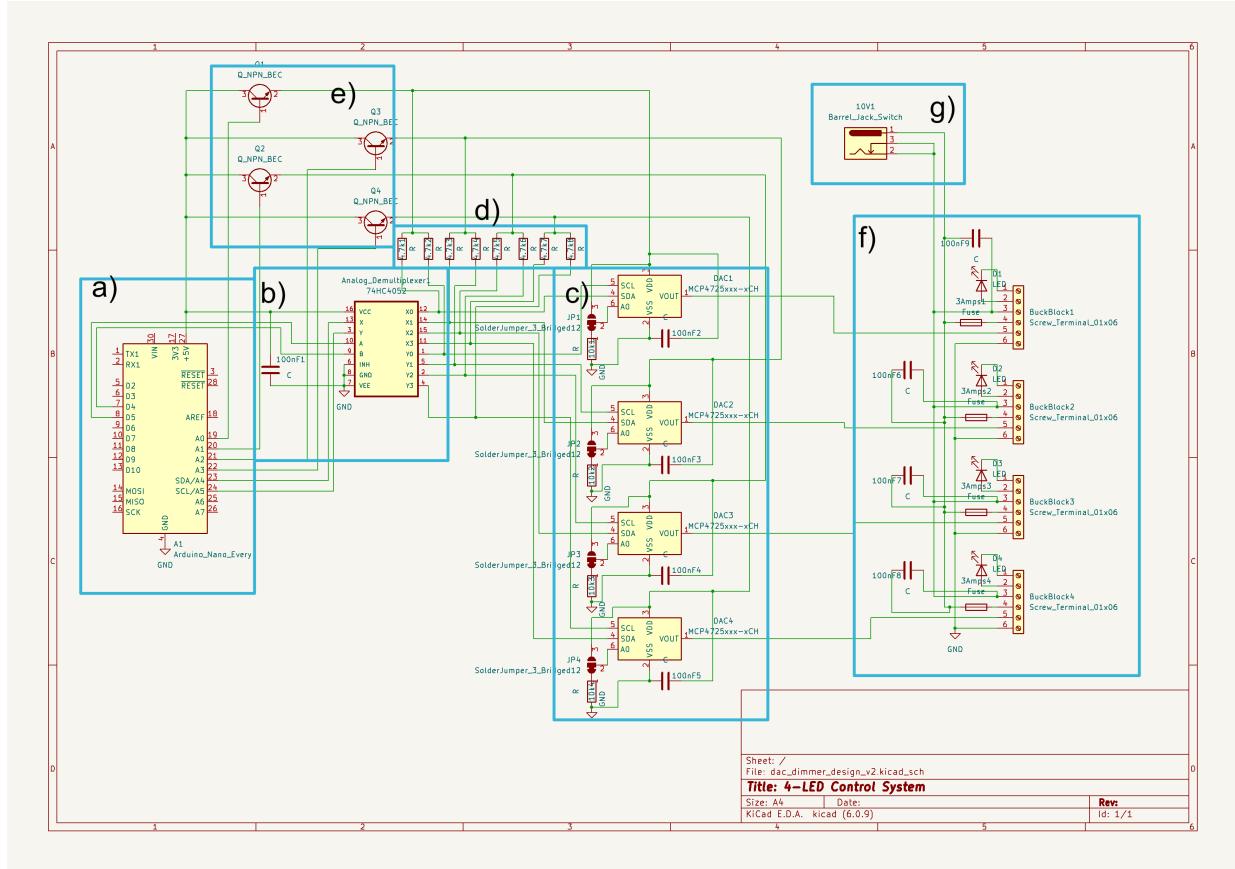


Figure 17: Full schematic of the 4-LED control system designed using KiCAD 6.0. a) The Arduino Nano, b) 74HC4052 4-channel analog-multiplexer/demultiplexer, c) the schematic of the SparkFun I2C DAC Breakout - MCP4725 (without the pull-up resistors on the SCL and SDA), d) the pull-up resistors for the SCL and SDA, e) NPN bipolar transistors connecting to the VCC of the MCP4725, f) The BuckBlocks connections with the LEDs, and g) barrel jack connector to connect to the mains power source.

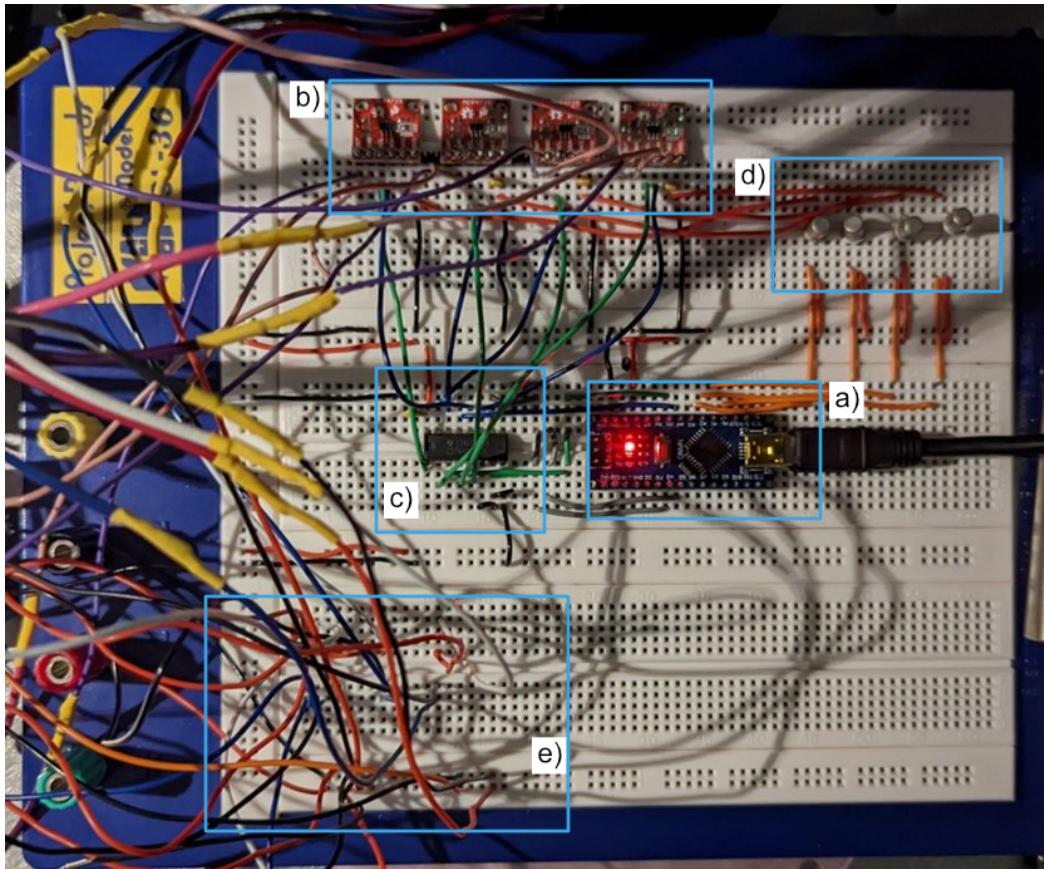


Figure 18: Physical circuit of the 4-LED dimming circuit assembled on a breadboard. a) Arduino Nano, b) SparkFun I2C DAC Breakout - MCP4725, c) 74HC4052 4-channel analog-multiplexer/demultiplexer BC108 NPN bipolar single transistors and e) wiring mess of the LED and BuckBlock connections and the mains power supply.

4.2.4 PCB Design

It is not a viable solution to have LMIC laboratories assemble the circuit on a breadboard since it would be non-standardised and time-consuming for the user to debug and build the circuit. The design was translated into a printed circuit board (PCB) to make the 4-LED control system a standardised part for microscopy applications in LMIC laboratories.

The PCB was designed to meet the following conditions:

- The PCB needs to occupy a small workspace footprint.
- The MCP4725 DACs need to be incorporated without the need for the SparkFun I2C DAC Breakout - MCP4725.
- Electrically safe and easy to debug and test.

The design of the PCB was created using KiCAD 6.0^[32] and it is shown in Fig(19). It was manufactured by JLCPCB^[33], and the assembled PCB is shown in Fig(20). The cost of 1 assembled PCB is £45.30 (US \$55.60). So the overall electronic component is US \$190.72 or £155.40, excluding the 10V power brick. The pathways going to the LED pins are too thin, which would need to be increased to thicker pathways in future iterations in consideration to electrical safety.

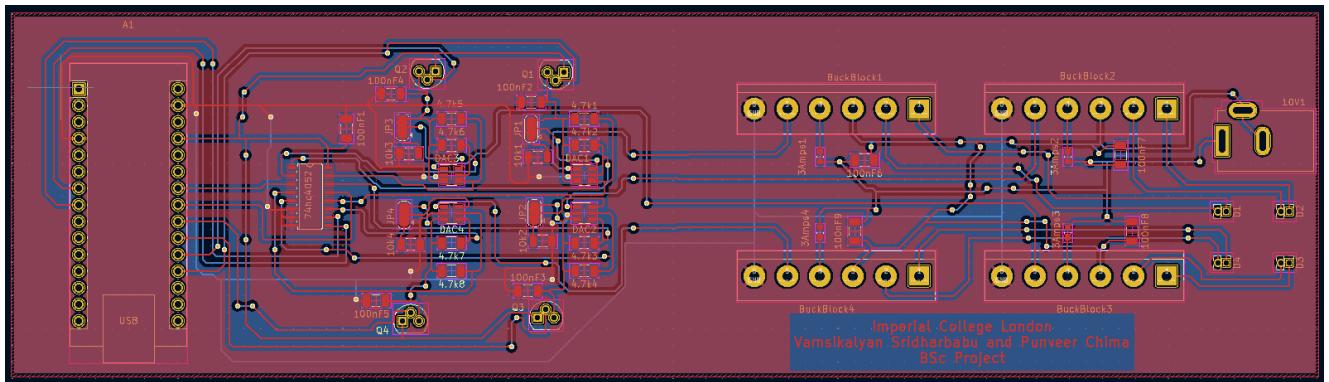


Figure 19: PCB Design in KiCAD 6.0

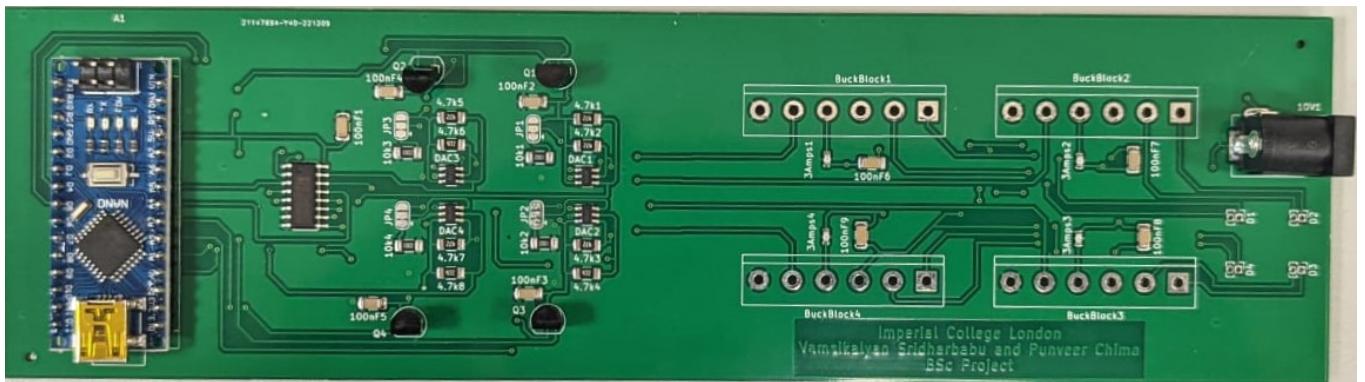


Figure 20: PCB manufactured from JLCPCB

4.3 Photonics: Uniform Illumination

The SinkPAD-II™23mm Quad LED Module causes the light incident onto the Köhler illumination setup to be offset from the principal axis, as shown in Fig(21). The beam produced from the LEDs has a finite size, which means that after collimation from the collector lens, the two beams may not overlap depending on the beam divergence ($\Phi = \frac{D}{f_1}$, D is the diameter of the beam in rear focal plane). This would cause the light illuminating the sample plane to not overlap, but be offset from the centre of the sample.

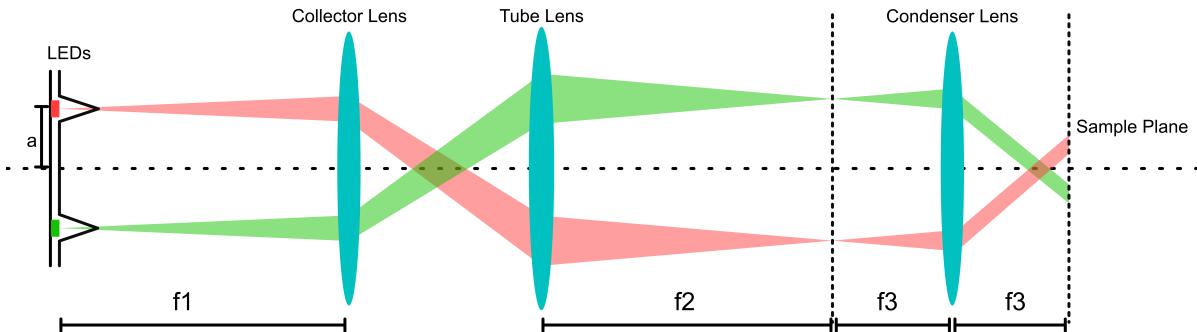


Figure 21: Köhler illumination of LEDs displaced from the optical axis, with an optic that produces a beam with a small divergence (red and green). The setup incorporates a collector lens, a tube lens and a condenser lens with focal lengths of f_1 , f_2 and f_3 . The image is focused on the sample place.

Using a large focal length collector lens will reduce the beam divergence and allow the two sources to partially overlap, thus increasing the probability of the light overlapping in the sample plane. My project partner, Punveer Chima, did the majority of the work in this section.

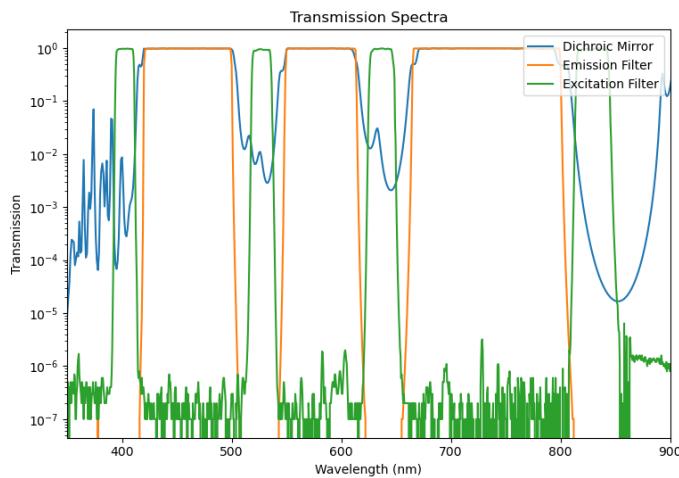


Figure 22: Transmission curves for the dichroic (ZT405-528-640-830rpc), the emission filter (ZET405-528-640-830m) and the excitation filter (ZET405-528-640-830x).

4.3.1 Epifluorescence microscope using the openFrame microscope

The dichroic (ZT405-528-640-830rpc), the emission filter (ZET405-528-640-830m) and the excitation filter (ZET405-528-640-830x) were fitted into the openFrame microscope for epifluorescence microscopy between the tube lens and the objective.

The LEDs were encased in a 3D printed casing (see Fig(23)) to allow the optical fibres to be mounted flush on top of the LEDs.

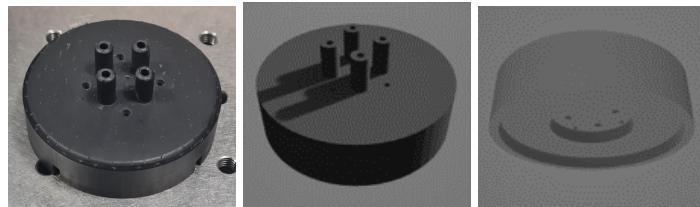


Figure 23: (left) 3D printed casing for optical fibre and LED coupling. (middle) CAD model from the top. (right) CAD model from the bottom.

4.3.2 Optical Fibres

The easiest solution to combat the optical problem is to manually bring the beams closer to the principal axis, as this would increase the amount of overlap before it is collimated by the collector lens.

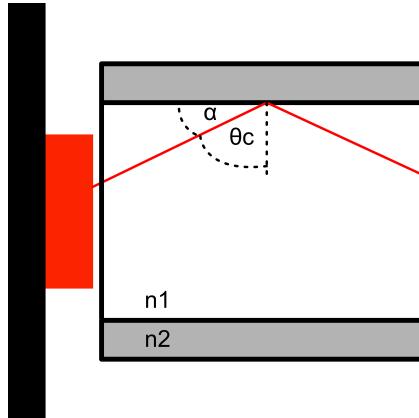


Figure 24: Ray transmitted through an optical fibre. The refractive index of the core and the cladding are n_1 and n_2 . TIR at the critical angle, θ_c and angle to the surface is α .

Optical fibres are used to transmit light via total internal reflection between the core and the cladding which have refractive indices of n_1 and n_2 respectively, see Fig(24). Total internal reflection (TIR) occurs when the angle of incidence of the incident ray is greater than the critical angle, θ_c , which is given by $\sin(\theta_c) = \frac{n_2}{n_1}$.

4.3.3 The Single Fibre Setup

The single fibre setup uses a 3D printed mount to individually have the selected LED emit light from the principal axis. The diagram of the setup is shown in Fig(25). This method effectively ignores the displacement of the LEDs. The epifluorescence microscope was set up using the openFrame. The mount was designed to be used with the ThorLabs caging system. This is shown in Fig(26).

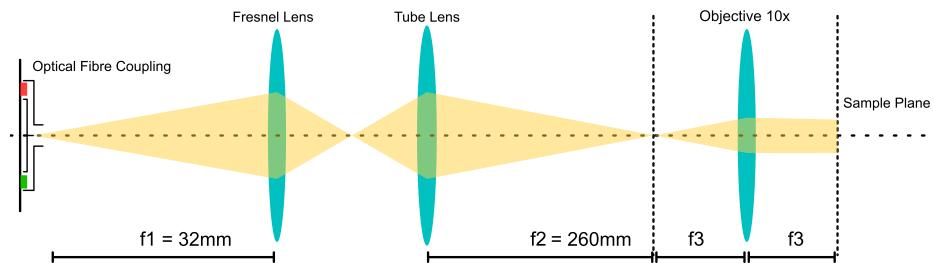


Figure 25: Köhler illumination of the single fibre setup using an optical fibre coupling. A Fresnel lens (32mm focal length) is used as the collector. 206mm focal length tube lens and a 10x objective .

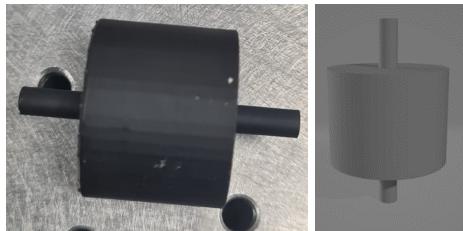


Figure 26: (left) 3D printed single fibre mount, (right) CAD model

4.3.4 The Mirror Setup

The mirror setup uses a prism of angled mirrors in front of the collector lens to alter the refracted angle of the beam going into the tube lens. The theoretical setup is shown in Fig(27). This was not constructed because we did not have time to test it.

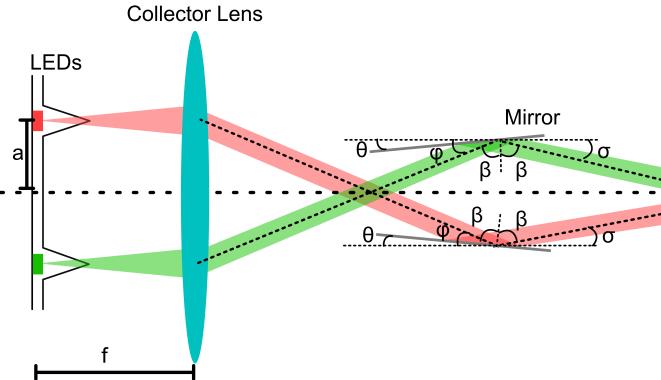


Figure 27: Using a mirror prism to bring the beams closer together. θ is the mirror angle, β is the angle of reflection, ϕ is the angle of the incident ray to the principal axis and σ is the angle of the output ray to the principal axis.

This setup alters the beam so that the angle of the beam to the principal axis (σ) is given by Eq(1).

$$(\beta + \phi - \theta = \frac{\pi}{2}) - (\beta + \phi + \theta = \frac{\pi}{2}) = (\sigma = \phi - 2\theta) \quad (1)$$

This would be a feasible alternative to the optical fibres as it would allow better control over the light beam going into the tube lens.

4.3.5 The 4 Optical Fibre Setup

The 4 optical fibre setup was designed to allow multi-colour imaging. This uses a 4-fibre 3D printed mount (see Fig(28)).

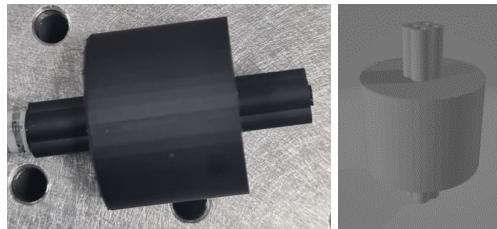


Figure 28: (right) 3D printed 4-fibre mount. (left) CAD model of the 4-fibre mount

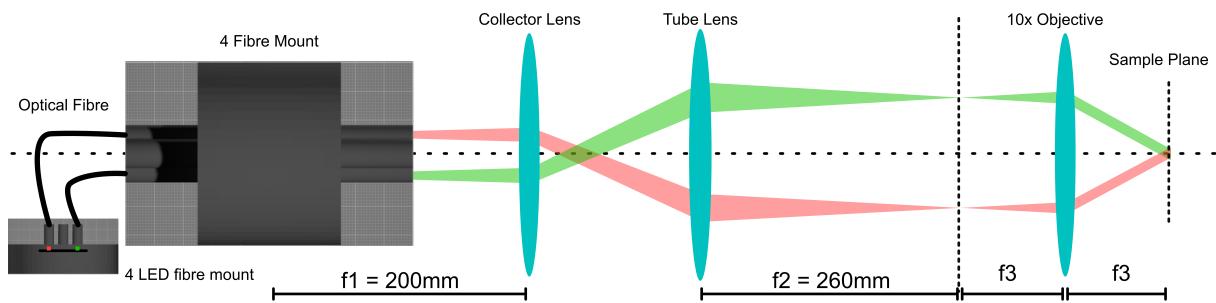


Figure 29: 4 Optical Fibre Setup. The LEDs are routed to the 4 fibre mount from the 4 LED fibre mount via optical fibres. The focal lengths of the condenser and tube lens are 200mm and 206mm respectively. A 10x objective is used for the condenser lens.

The 4 optical fibre setup is our finalised setup, the diagram in Fig(29) shows how it is set up with the Köhler illumination optics. The physical setup is shown in Fig(30).

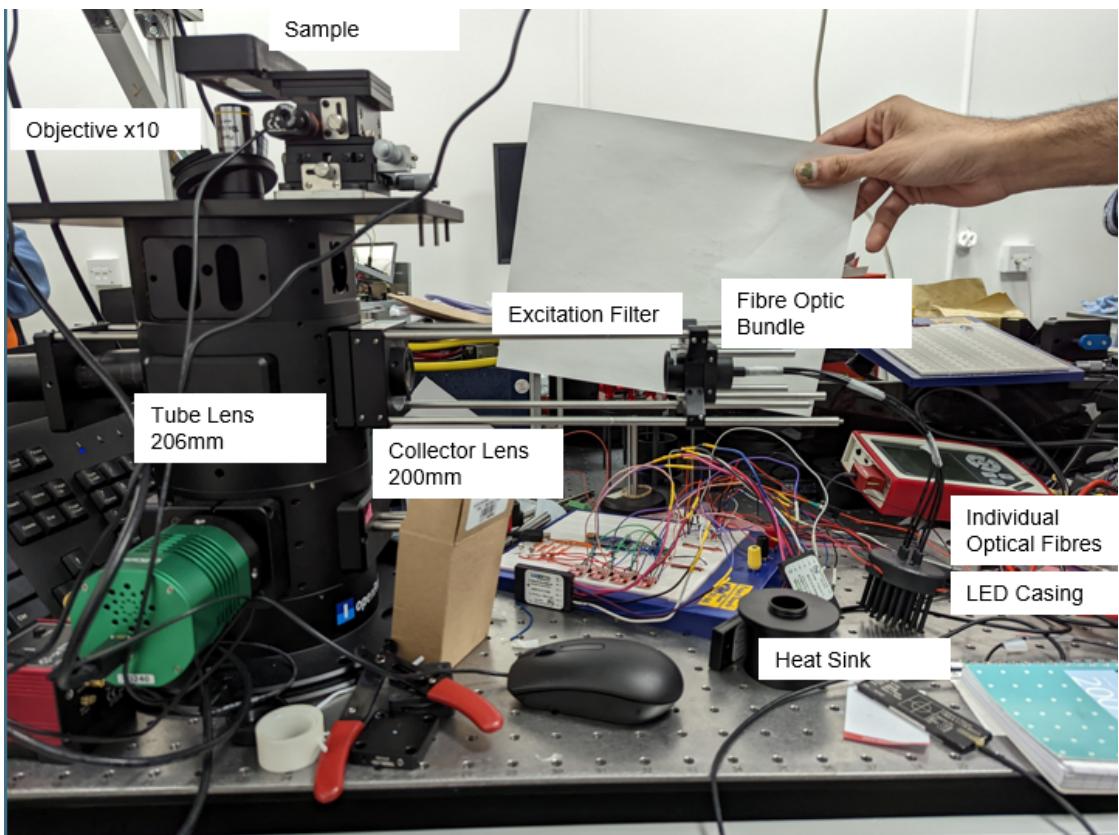


Figure 30: The physical setup of the 4 optical fibre setup with the openFrame microscope.

5 Setting up the data acquisition system

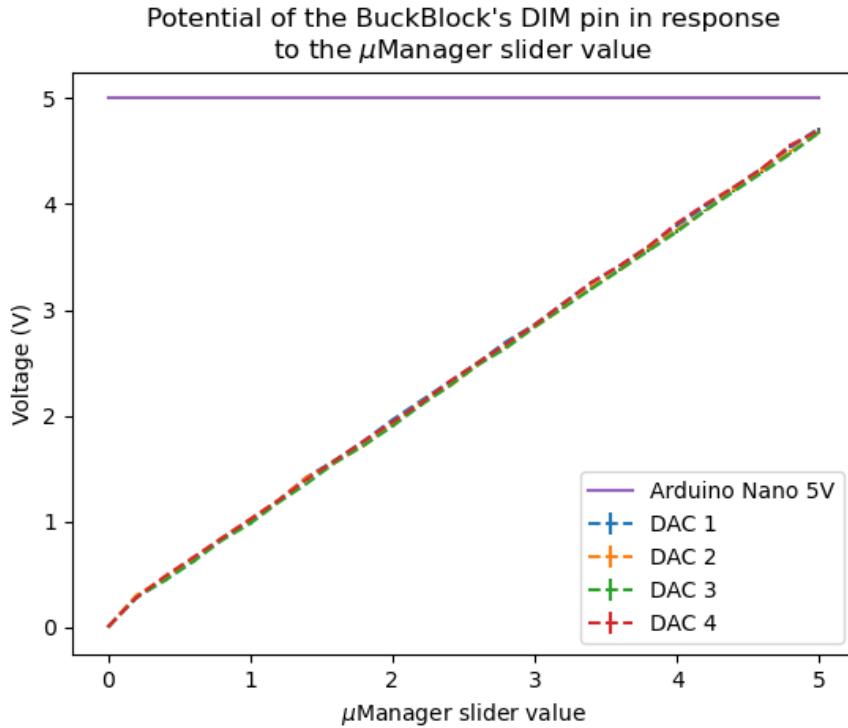


Figure 31: The potential difference of the DIM pin of each BuckBlock caused by their respective DACs in response to the μ Manager slider.

This section was done by both me and my project partner.

We first measured the response of the DACs/BuckBlock's DIM pin to μ Manager sliders (see Fig(31)). We can see that value of 5 does not correspond to 5V, but instead to 4.69 ± 0.01 which caused due to a voltage drop over the transistors. For future iterations it would be beneficial to remove the transistors as their ON/OFF function is redundant.

We imaged two samples: a slide coloured using a fluorescent marker and a slide of pollen grains. We tested the single optical fibre setup and the 4 optical fibre setup using the 4-LED control system PCB with control from μ Manager. We also measured the efficiency of both setups. The imaging was done using a CellCam Kikker 100MT microscope camera^[34] via the Multi-Dimensional Acquisition feature in μ Manager, settings are shown in Fig(32). We used the red and green LEDs to acquire an RGB image (the blue and deep red were negligible due to the filters) with slider values set to 3.4 corresponding to a current of about 400mA. We tested different exposure times (the amount of time the photons are collected by the CCD) and the binning of the imaging. The binning count was set to additive, so a binning of 3 would sum up the intensity of every 3-by-3 pixel grid which leads to a decrease in the resolution but results in a brighter image.

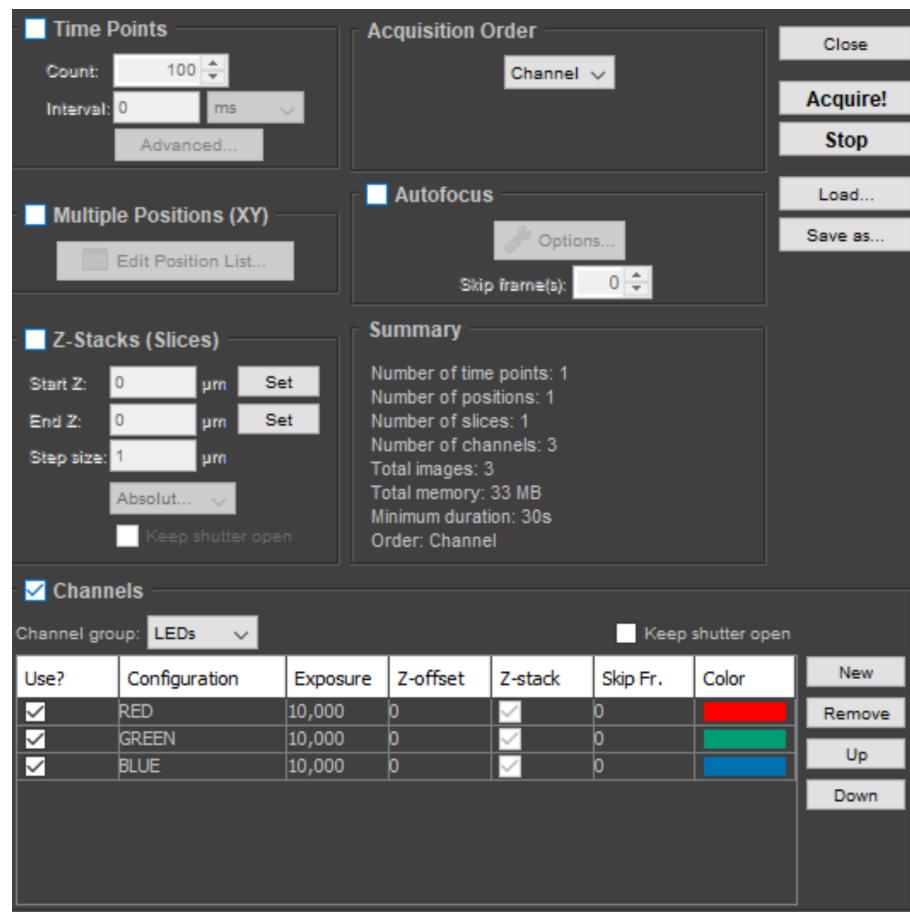


Figure 32: Multi-Dimensional Acquisition GUI on μ Manager.

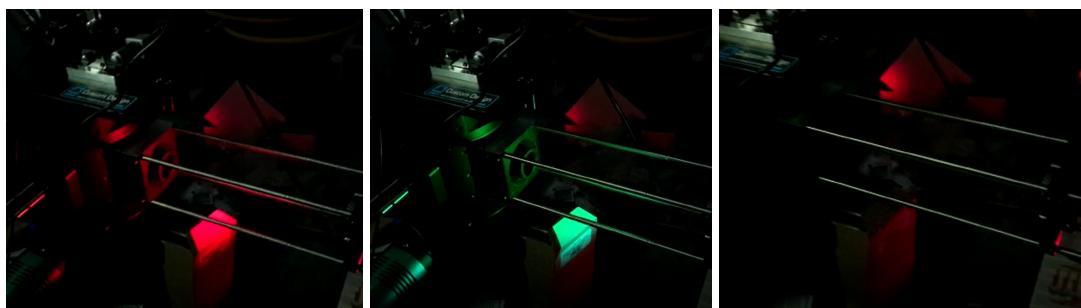


Figure 33: (left) Red light, (middle) green light, (right) blue light - all incident on the tube lens.

6 Results

6.1 Epifluorescence Microscopy Images

Using the multi-dimensional acquisition system, we imaged a slide of pollen grains. Fig(34) shows the image taken using the single optical fibre setup with a 5000ms exposure time and a binning of 1. Fig(35) and Fig(36) were taken using the 4 optical fibre setup with a 5000ms and 10000ms exposure times and a binning of 3 and 1 respectively. The binning mode is set to additive.

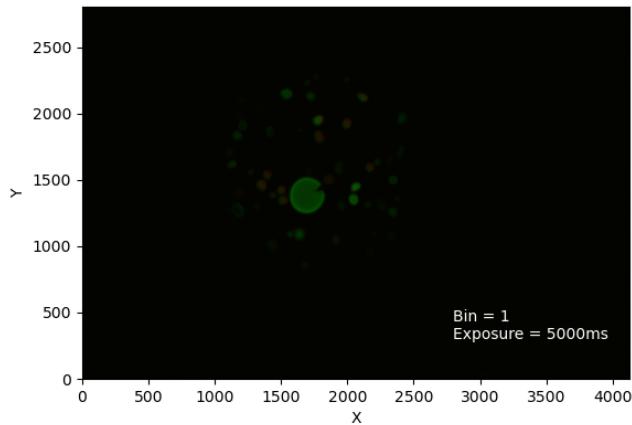


Figure 34: Image of pollen grains taken using the single optical fibre setup using a bin of 1 and an exposure time of 5000ms.

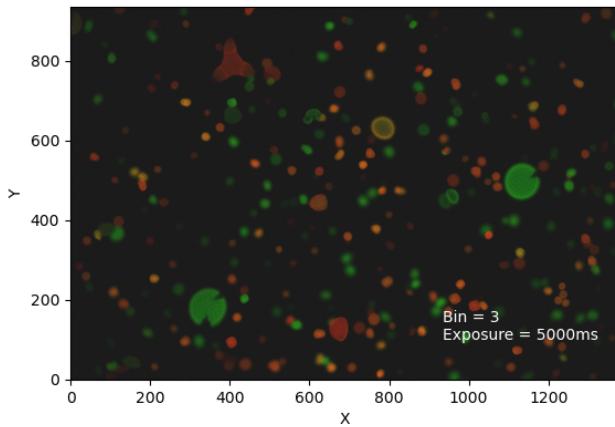


Figure 35: Image of pollen grains taken using the 4 optical fibre setup using a bin of 3 and an exposure time of 5000ms.

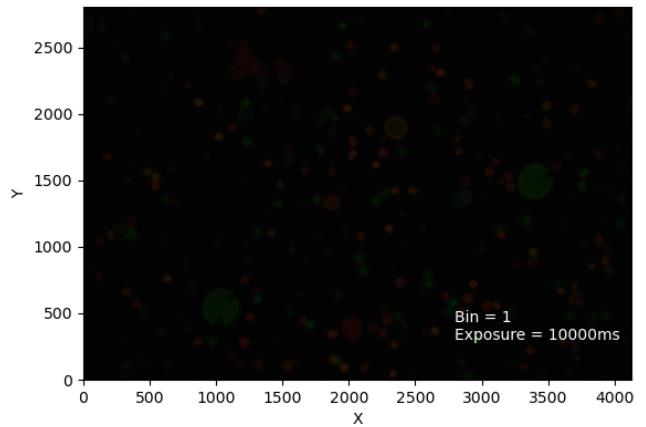


Figure 36: Image of pollen grains taken using the 4 optical fibre setup using a bin of 1 and an exposure time of 10000ms.

We also measured the uniformity of the illumination at the sample plane for both setups. We used a fluorescent control slide which was a slide coloured using a fluorescent marker to achieve a uniform

spread of fluorophores. We then used the ImageJ software to measure the relative brightness of each pixel. The uniformity of the single optical fibre setup and the 4 optical fibre setup are shown in Fig(37) and Fig(38) respectively. Both images are the same physical size. The 4 optical fibre setup appears brighter and smaller due to the summative binning of 3.

The single optical fibre setup does not achieve complete uniform illumination of the sample plane, instead, it is focused on a specific region. The 4 optical fibre was able to achieve uniform illumination of the sample plane. This shows that the 200mm focal length lens was a better fit than the 32mm Fresnel lens to the optical setup inside the openFrame microscope. Based on these results, we decided that the 4 optical fibre setup is best suited for our final product.

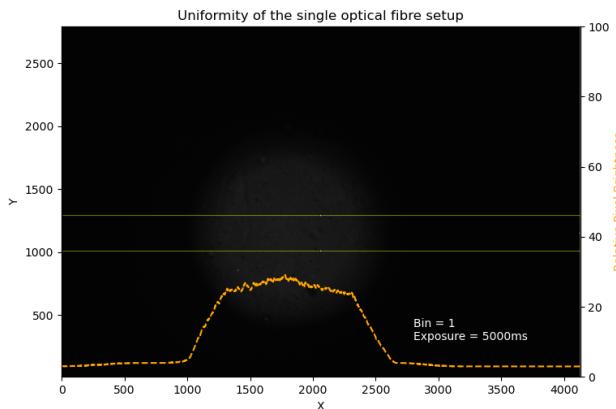


Figure 37: Plot showing the pixel brightness uniformity using ImageJ overlayed on an image of the fluorescent control slide using the single optical fibre setup using a bin of 1 and an exposure time of 5000ms.

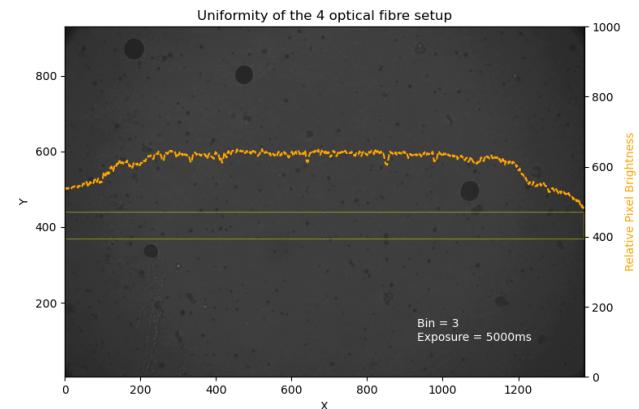


Figure 38: Plot showing the pixel brightness uniformity using ImageJ overlayed on an image of the fluorescent control slide using the 4 optical fibre setup using a bin of 3 and an exposure time of 5000ms.

6.2 Efficiency

The power outputs of the 4 LEDs through the 4 optical fibre setups are shown in Table 3 and their respective efficiencies in Table 4. We measured a background of less than $1\mu\text{W}$ in all parts of the system. The blue LED is not present after the tube lens due to it being almost completely blocked by the emission filter and loss in power due to the divergence of the beam between the emission filter and the tube lens. The red, deep red and green LEDs had a total efficiency of $0.571 \pm 0.001\%$, $0.149 \pm 0.001\%$ and $0.331 \pm 0.001\%$ respectively.

Power Output (mW)					
LED colour	LED (± 0.1)	Fibre (± 0.01)	Emission filter(± 0.001)	Tube lens(± 0.0001)	Sample plane(± 0.00001)
Red	103.1	0.95	0.454	0.0105	0.00542
Green	99.6	1.36	0.381	0.0097	0.00451
Royal Blue	113.5	1.9	0.0025	×	×
Deep Red	105.5	2.53	0.24	0.011	0.00378

Table 3: Power output through the 4 optical fibre setup

Efficiency with uncertainty of (± 0.00001)					
LED colour	Fibre	Emission Filter	Tube lens	Sample Plane	Total Effi- ciency
Red	0.00921	0.47823	0.02312	0.51611	0.00571
Green	0.01365	0.28020	0.02558	0.46213	0.00331
Royal Blue	0.01674	0.00131	0	0	0
Deep Red	0.02398	0.09486	0.04679	0.33616	0.00149

Table 4: Optical efficiency through each component in the 4 optical fibre setup.

7 Conclusion

The full 4 LED emission source achieved its main function of automatic and manual control over the brightness/power output and the wavelength of the light source. The PCB is based around the Arduino Nano microcontroller, and it was designed to control the LUXEON Rebel LEDs using a 2100mA Dimmable BuckBlock LED Driver by varying the potential on the DIM terminal using an MCP4725 DAC. A 74HC4052 analog multiplexer/demultiplexer was used to control the I2C communication pathway between the 4 DACs. The PCB allows the user to vary the power output using the LED-specific sliders on μ Manager, which can also be configured for multi-dimensional acquisition to cycle through all the available wavelengths. The SinkPAD-II™23mm Quad LED Module introduced an LED offset of 7.1 ± 0.1 mm from the principal axis. We tested two optical configurations for achieving uniform illumination at the sample plane via Köhler illumination using the openFrame microscope platform while combatting the offset. The single optical fibre setup had a much narrower beam on the sample plane (see Fig(37)), which was due to the Fresnel lens, compared to the 4 optical fibre setups which achieved a uniform illumination across the sample plane (see Fig(38)). The 4 optical fibre setup was chosen as the more suitable option. The optical efficiency through the chosen setup, since a lot of light, was lost via divergence between the emission filter and the tube lens. We achieved overall efficiency from LED to sample plane for the red, deep red and green LEDs as being $0.571 \pm 0.001\%$, $0.149 \pm 0.001\%$ and $0.331 \pm 0.001\%$ respectively, with almost no light from the blue LED entering the openFrame microscope. The total cost of the device amounts to around £155.40, which quite inexpensive compared to the modern LED sources. Although the efficiency is quite low and the capabilities do not add up to the industry standard, it is still a useful device for research in LMIC laboratories.

Overall, we gained a lot of experience with electronics, photonics and research skills throughout this project. It has given an insight into the organisation and time-management required to perform research, especially in an applied field such as photonics.

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