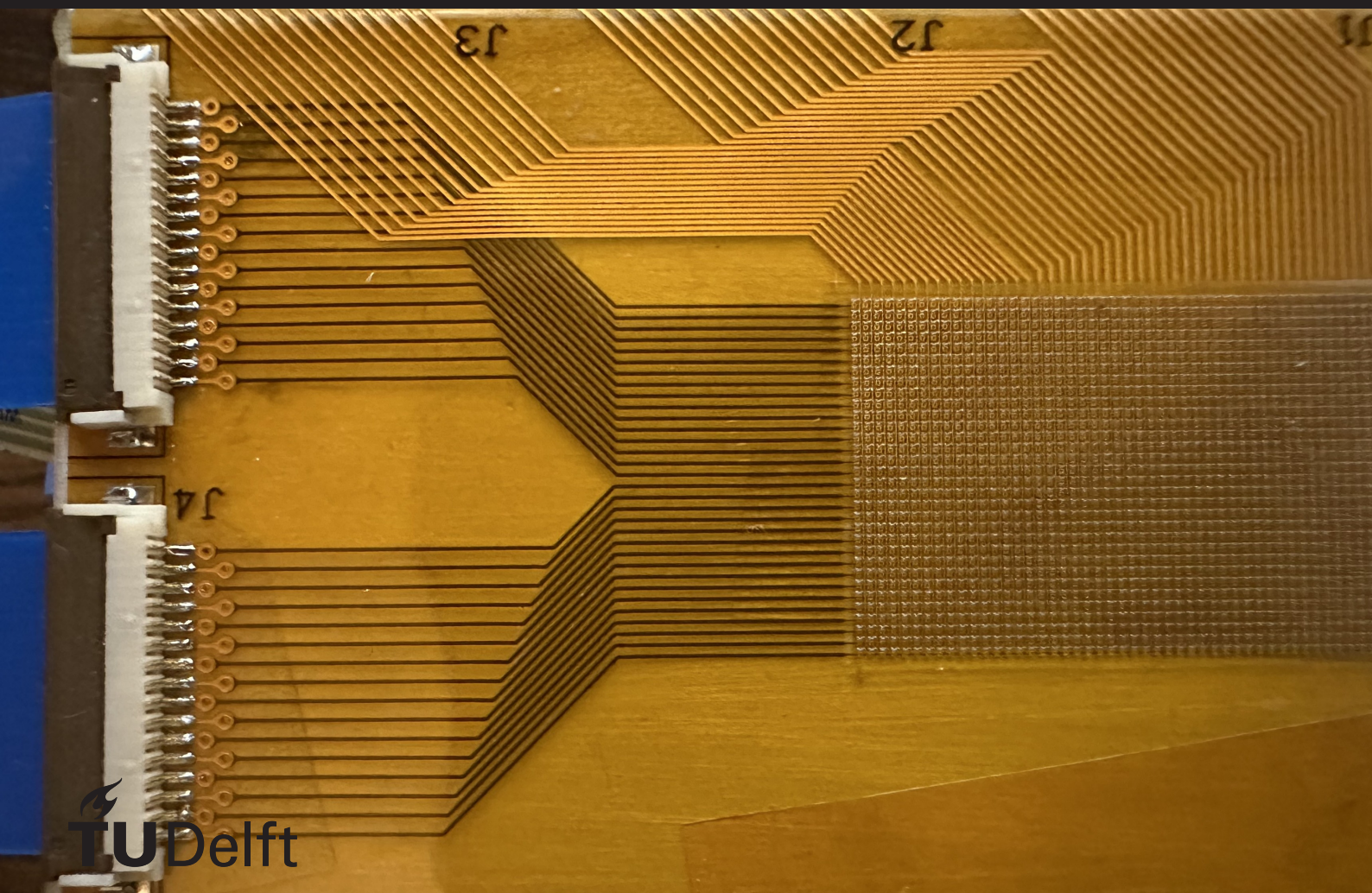


Mini LED Matrix System for Potential Biomedical Applications

From 48x32 to 160x160 mini LED Matrix Systems

Jiahui Zhang



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by

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An electronic version of this thesis is available at <http://repository.tudelft.nl/>.

Preface

This master's thesis represents the culmination of my academic journey, a journey that, although filled with ups and downs, has ultimately reached its conclusion. The path to completing this work has been challenging, with moments of uncertainty and obstacles that tested my resolve, but it has also been incredibly rewarding, bringing with it a deep sense of accomplishment.

This thesis would not have been possible without the support and guidance of several individuals to whom I owe my deepest gratitude. I would like to extend my sincere thanks to my thesis supervisor, Professor Guoqi Zhang, and a special shout-out to Shanliang Deng, whose expertise, encouragement, and constructive feedback have been invaluable throughout this journey. I am grateful to the faculty members and colleagues in the ECTM especially Zichuan Li for materials and lab support. Special thanks go to Remko Seijffers, Bram den ouden and Yunfan Niu.

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*Jiahui Zhang
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Abstract

The increasing demand for precise light control in biomedical research, especially in fields like optogenetics and liquid crystal elastomers (LCEs), has exposed the limitations of traditional light sources regarding precision, controllability, and size. To address these challenges, this research focuses on developing miniaturized LED matrix systems capable of producing high-precision, high-power customizable light patterns essential for complex biological experiments. Two mini LED matrix systems—a 48x32 matrix and a larger 160x160 matrix—were designed and developed to achieve precise modulation of light intensity and patterns. These systems were tailored to meet the rigorous requirements of optogenetic experiments, which demand precise control over cellular activities, and to explore the potential applications in light-responsive LCEs. Controlling the light-responsive behavior of LCEs is critical for advancements in soft robotics, artificial muscles, and other applications requiring adjustable light sources. The 48x32 mini LED matrix system was successfully applied to control cardiac activity through optogenetics, demonstrating its ability to modulate light for inducing and terminating arrhythmias precisely. Additionally, its integration into LCE experiments showed promise for broader biomedical applications. Building on this success, the development of a larger 160x160 mini LED matrix system extended the technology's applicability, supporting larger-scale experimental setups and human-sized models. By providing innovative solutions to the challenges of precise light control in biomedical research, this work opens new possibilities for experimental and clinical applications, offering significant contributions to the field.

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Introduction

In recent years, Light Emitting Diode (LED) technology has seen remarkable advancements, leading to widespread adoption in various fields, particularly where precise light control is required, such as in biomedical research. Among the notable innovations is the development of mini LEDs, which offer significant advantages due to their compact size, high brightness, and energy efficiency [1]. These features make mini LEDs an excellent choice for applications requiring precise and controlled pattern illumination, a critical component in advanced biomedical procedures [2]. Pattern illumination involves precisely projecting light patterns onto a target, allowing for localized and controlled light delivery. This technique is integral to biomedical fields such as optogenetics [3], where light is used to modulate cellular activity with high precision and in the activation of Liquid Crystal Elastomers (LCEs), which undergo shape changes in response to light, particularly in the development of soft robotics, artificial muscles, and responsive implants [4]. Mini LEDs in these applications provide a level of control and precision that is difficult to achieve with traditional light sources, opening up new avenues for biomedical research and therapeutic interventions.

1.1. Pattern illumination in biomedical fields

Pattern illumination has emerged as a significant technique in various biomedical applications, particularly due to its ability to spatially and temporally control light delivery. This technique is increasingly utilized in areas such as optogenetics and the manipulation of liquid crystal elastomers (LCEs), demonstrating profound implications for experimental and therapeutic processes.

Recent research has highlighted optogenetics with pattern illumination in experimental cardiology. According to recent studies, applying pattern illumination in optogenetics has shown high termination efficiency in cardiac arrhythmias [5]. The ability to precisely control the activation of specific cardiac tissues via light patterns opens new avenues for targeted therapeutic interventions, significantly improving the prospects for treating complex cardiac conditions.

In addition to its role in optogenetics, pattern illumination has also found applications in manipulating liquid crystal elastomers (LCEs). LCEs are smart materials that can change shape in response to external stimuli such as light. Recent advancements have demonstrated that pattern illumination can precisely control the deformation of LCEs, paving the way for their use in biomedical devices, such as soft actuators and adaptive scaffolds for tissue engineering [4][6]. This capability is precious in creating dynamic biomedical devices that respond to real-time physiological conditions, offering enhanced functionality and adaptability.

Besides, functional imaging, particularly in neuroscience and cardiovascular research, has benefited from pattern illumination by improving the signal-to-noise ratio and enabling more detailed observation of dynamic biological processes. A recent study from MIT introduced a novel technique for enhancing the detection of brain activity using patterned light in functional MRI (fMRI). The researchers developed a method called bioluminescence imaging using hemodynamics (BLUsH), which involves engineering blood vessels in the brain to become light-sensitive [7]. When light is emitted from genetically modified

cells, the changes in blood vessel dilation can be detected with fMRI, enabling precise brain activity mapping in response to specific stimuli. This breakthrough could significantly advance our understanding of brain function.

These advancements underscore the versatility and potential of pattern illumination in biomedical research and applications. Integrating these techniques into clinical practice may offer new solutions for various medical challenges as the field evolves.

1.2. Display technologies for pattern illumination in biomedical applications

The choice of display technology for pattern illumination can significantly impact biomedical devices' performance, resolution, and efficacy. This section reviews the state-of-the-art display technologies used for pattern illumination in biomedical applications, focusing on recent developments that justify the selection of mini LED matrix technology as the optimal choice.

1.2.1. Mini LEDs

Miniature LEDs, commonly called mini LEDs, have emerged as a pivotal development due to their compact size, high efficiency, and enhanced optical performance compared to traditional LEDs [1]. The evolution of LED (Light Emitting Diode) technology has seen significant advancements from traditional LEDs to the latest mini LEDs. Traditional LEDs, first introduced in the 1960s, were mainly used for indicator lights due to their low brightness [8]. Over the decades, materials, efficiency, and brightness improvements enabled LEDs to be used for more general lighting applications. The latest advancement, mini LED technology, uses smaller diodes to achieve higher contrast, better brightness control, and thinner displays, particularly in TVs and monitors [1]. This evolution is marked by significant research into semiconductor materials and light efficiency, enabling LEDs to dominate lighting and display applications. As research and industry collaboration progress, the integration of mini LEDs is expected to expand beyond displays, influencing areas such as general lighting, medical devices, and innovative communication systems.

Mini LED have gained attention but are still limited in biomedical fields [5]. They are mainly considered in applications where precise light control is essential for stimulating specific cells, such as optogenetics. Back in 2013, Clements et al. developed a mini 465 nm LED source for in vivo optogenetics experimentation [9]. Wu et al. reported an implantable probe in the brain with 12 mini LEDs providing up to 1 mW/mm^2 In 2015 [10]. Recently, in 2023, a 16×16 mini LED array platform for optogenetics actuation was discussed [11]. The light intensity of these mini LED matrices or arrays all reaches the threshold to stimulate optogenetic-modified cells but they are all limited in size, which means they are not suitable for experiments using petri dish [12]. While as for the mini LEDs application for large areas, it gives a large pitch resulting in a low light intensity around 3.4 mW/mm^2 [13].

1.2.2. Other display technologies

Digital Micromirror Devices (DMDs) are widely used in pattern illumination systems, especially in optical projection systems and structured light microscopy. DMDs consist of microscopic mirrors that can be individually controlled to reflect light and create specific patterns. While DMDs offer high speed, complex wavefront shaping and high light intensity typically ranging from 0.1 to 10 W/cm^2 depending on the light source used [14], they often require powerful external light sources, leading to significant heat generation. The chips heat up more quickly when increasing the frame, leading to pattern deformation [15], which prevents it from being used directly in biomedical applications without a designated cooling system. DMD technology is not flexible and can be limited in exploring wearable biomedical devices. In contrast, Mini LEDs can achieve high light intensity with lower power consumption, reducing heat output due to their inherent efficiency and eliminating the need for complex cooling systems.

Liquid Crystal on Silicon (LCoS) displays are used in biomedical applications for phase modulation and creating complex light patterns. LCoS operates by modulating the phase of light rather than its amplitude, making it suitable for holography and advanced microscopy. LCoS displays can operate with light intensities to 5 W/cm^2 , although their efficiency can drop due to light scattering and absorption

within the liquid crystal layers, particularly at higher light intensities [16]. It also needs a cooling system, as its operation temperature can rise to 50 °C or even higher [17]. Mini LEDs offer comparable or superior light intensity with better energy efficiency than LCoS, which suffers from light losses in its liquid crystal layers. Mini LEDs have less heat generation compared to LCoS displays. LCoS is rigid as well and not suitable for wearable biomedical devices.

OLED technology is used in biomedical applications that require flexible, thin, and high-contrast displays. OLEDs emit light directly from organic materials, allowing for the creation of light patterns without the need for a backlight. OLEDs typically provide lower light intensities and have a big pixel pitch size [18]. This is particularly notable in blue OLEDs, where efficiency and longevity are ongoing challenges. Both OLEDs and mini LEDs are efficient in heat management, but mini LEDs can achieve higher brightness without significant heat generation.

Mini LED matrix technology stands out as a versatile and efficient solution for pattern illumination in biomedical applications. Compared to other technologies such as DMDs, LCoS, OLEDs, and holographic systems, mini LEDs offer a superior balance of light intensity, heat management, cost, and flexibility. These advantages make mini LEDs an ideal choice for next-generation biomedical devices. As discussed in the mini LED section, existing mini LED matrices used in biomedical fields, particularly in optogenetics and LCE modulation, are generally limited by their size. The brightness of mini LED matrices typically used in displays is insufficient for these types of medical experiments. This limitation forms the basis for the system described in this thesis.

1.3. Case study: pattern illumination in optogenetics

1.3.1. Introduction to optogenetics

Optogenetics is a groundbreaking neuromodulation technique that uses light to control genetically modified neurons precisely. This method allows for the targeted activation or inhibition of specific cells, thanks to the genetic modification that makes them responsive to light. The foundation of optogenetics lies in discovering microbial opsins, proteins in algae that open or close ion channels when exposed to specific wavelengths of light, a process that mimics neuronal signalling [19]. Scientists can precisely control neuronal activity by introducing viral vectors carrying these opsins into neurons.

Channelrhodopsin-2 (ChR2) is pivotal in this technology, responding to blue (~470 nm) light as shown in Figure 1.1 [20]. These opsins allow for high temporal precision, with responses occurring on a millisecond scale. Optogenetics enables high specificity in targeting neurons and achieves temporal precision by varying the light's wavelength to depolarize or hyperpolarize the neurons.

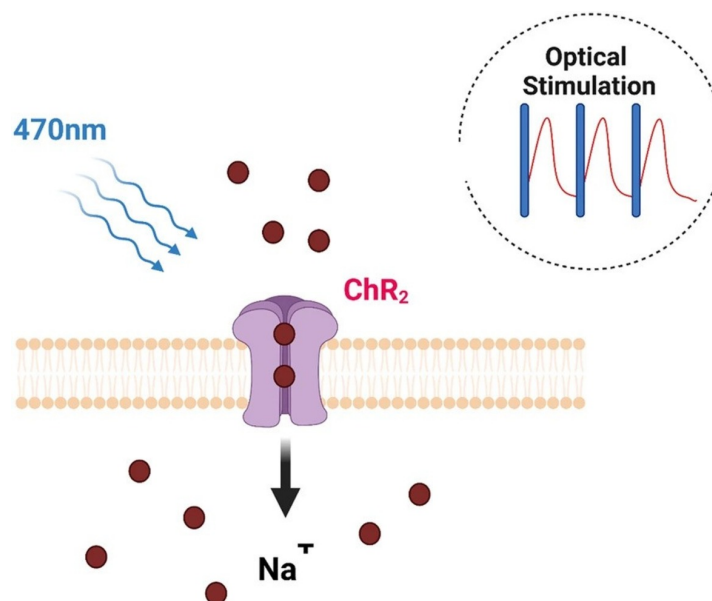


Figure 1.1: Light-gated ion channel modulation [20]

1.3.2. Pattern illumination in optogenetics

The application of pattern illumination in optogenetics has opened new avenues for exploring brain function and neural dynamics. This technique allows for the control of neural circuits with unprecedented precision, enabling researchers to manipulate neural activity to mimic natural brain processes. In addition to its applications in neuroscience, optogenetics combined with pattern illumination is being explored in other areas, such as cardiac research and the study of cellular signalling pathways. The ability to control cellular behaviour in a non-invasive and reversible manner has significant implications for both basic research and potential therapeutic interventions. Fundamental advances in this area include:

- Patterned illumination allows for the activation or inhibition of specific groups of neurons within a defined region. This is achieved by using techniques such as digital micromirror devices (DMDs) or liquid crystal spatial light modulators (SLMs) to project precise light patterns onto neural tissues [21][22]. This spatial control is crucial for studies involving mapping neural circuits and understanding the role of specific neurons in behaviour.
- In optogenetics, temporal precision is just as important as spatial control. Patterned illumination can be modulated at high speeds, allowing researchers to follow the natural dynamics of neuronal activity and manipulate them in real-time [23]. This temporal control is vital for investigating fast neural processes such as synaptic transmission and network oscillations.
- Advanced pattern illumination systems can use multiple wavelengths of light to control different optogenetic tools simultaneously. This multicolour approach enables the independent modulation of various neural populations within the same tissue, facilitating complex studies of neural interactions and network function [24].

1.3.3. Applications of pattern illumination in optogenetics

The combination of pattern illumination and optogenetics has led to numerous applications that are transforming neuroscience research: A 2022 study by Jin et al. highlighted the benefits of a beaded-ring light pattern in two-photon optogenetics, especially for high-resolution neuronal stimulation in vivo [25]. Additionally, recent research has shown that patterned light can control cardiac activity, improving atrial fibrillation treatments through rotor-guided ablation using 470 nm patterned light. [7].

The primary illumination sources used in optogenetics include lasers, fibre-optic systems, and traditional LEDs. While these systems have enabled groundbreaking research, they also present significant limitations. Lasers, for example, provide highly focused light but can cause photodamage and lack flexibility in targeting larger tissue areas [26]. These systems require the implantation of optical fibres into the brain, which can cause significant tissue damage due to the size and rigidity of the fibres. This invasiveness can lead to chronic inflammation and negatively impact the health of the subject [27]. Traditional LEDs, while less invasive, suffer from low spatial resolution, making it difficult to precisely target small cell populations or individual neurons [28].

In recent developments, digital micromirror devices (DMDs) and spatial light modulators (SLMs) have been employed to project intricate light patterns that can activate or inhibit specific neuronal populations based on their spatial configuration. They are particularly useful for targeting small, well-defined neural populations in complex circuits. However, they require complex optics, are typically bulky, and consume significant power, which can limit their application in portable or implantable devices.

The miniLED matrix provides a more compact and power-efficient alternative to DMDs and SLMs, with the capability to deliver precise, high-intensity light patterns. Unlike DMDs, which use moving mirrors, or SLMs, which rely on liquid crystals, miniLED matrices offer faster switching speeds and higher durability due to their solid-state nature. The miniLED matrix also allows for direct light emission without the need for additional projection optics, simplifying the setup and reducing overall system size. This makes miniLED matrices particularly advantageous for in vivo optogenetics applications, especially in mobile or wearable devices, where space and power are at a premium.

1.4. Case study: pattern illumination in light-responsive LCEs

1.4.1. Introduction to LCEs

Liquid crystal elastomers (LCEs) are bio-compatible materials that combine the properties of liquid crystals and elastomers, exhibiting unique mechanical and optical characteristics that are highly responsive to external stimuli such as heat, light, and electric fields. When exposed to light, LCEs can undergo significant shape changes, making them ideal for applications in soft robotics, artificial muscles, and adaptive optics [29].

As shown in Figure 1.2, under illumination, Liquid Crystal Elastomers (LCEs) undergo a photomechanical response where the aligned liquid crystal molecules within the polymer matrix absorb light, causing them to change shape and reorient. This molecular reorientation leads to a macroscopic contraction of the material along the direction of the initial alignment. When the light is removed, the process is reversible, allowing the LCE to return to its original shape.

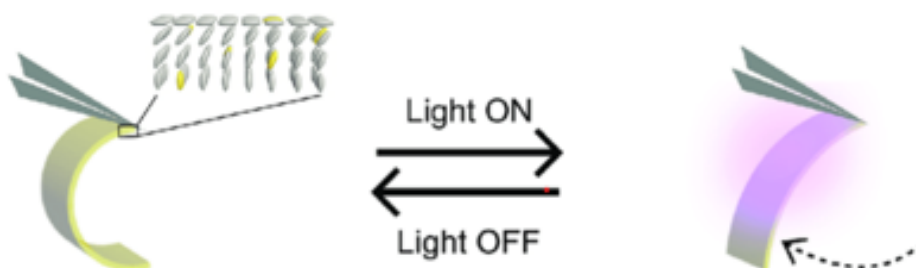


Figure 1.2: Contraction of the LCE under illumination [30]

Patterned LCEs take this concept a step further by introducing spatially controlled variations in the material's properties, enabling the creation of complex, programmable deformations. Pattern illumination plays a critical role in fabricating and activating patterned LCEs, allowing for precise control over their shape and function.

1.4.2. Fabrication and activation of patterned LCEs

The fabrication of patterned LCEs typically involves the use of photolithography or direct laser writing, where light is used to induce spatially controlled polymerization or crosslinking within the material. This process creates regions with distinct mechanical properties, enabling the LCE to undergo controlled deformations when exposed to external stimuli [31].

Pattern illumination is also used to activate these materials. By projecting specific light patterns onto a patterned LCE, researchers can induce localized heating or photoisomerization, leading to targeted shape changes. This capability is particularly useful in applications where precise control over the material's deformation is required, such as in developing microactuators or reconfigurable surfaces.

1.4.3. Applications of patterned LCEs

Researchers have used near-infrared (NIR) light to initiate photothermal effects within LCEs that have been doped with organic photothermal agents. This method allows for ultrafast and tunable shape changes, such as bending, twisting, and rolling, which can be precisely controlled by adjusting the light patterns and exposure time [32]. Another study highlights the use of light to create localized phase transitions within LCEs, resulting in reversible shape changes. These shape morphings are achieved by selectively heating specific regions of the LCE using NIR light, which causes a transition from the liquid crystalline phase to the isotropic phase in those areas, leading to localized contraction or expansion [33].

A study by Ferrantini et al. in 2019 proved the availability of developing light-responsive LCEs to assist cardiac contraction by using a green light laser ($0\text{--}200\text{ mW/mm}^2$) and a LED-light beam ($0\text{--}1.5\text{ mW/mm}^2$) on the LCE films were cut into strips ($200\text{--}400\text{ }\mu\text{m}$ width, $1\text{--}4\text{ mm}$ length) [34]. In 2022,

Vitale et al. demonstrated a system using two arrays of eight blue mini-LEDs of 470 nm increasing the light power from 0 to 22 mW on light-stimulated LCEs (8 mm length $\times$ 0.75 mm width) to mimic muscular function and potentiate cardiac contraction [6]. It allows for conducting more in-depth biomedical experiments using a larger mini LED matrix. At present, the use of miniLED matrix technology to control the contraction and expansion of Liquid Crystal Elastomers (LCEs) holds significant research value, particularly in applications such as regulating cardiomyocyte activity. This area of study is still relatively unexplored, offering ample opportunities for innovative research.

1.5. Outline of the thesis

Based on the current context, this thesis discusses the background and significance of pattern illumination in biomedical fields, along with the advanced applications of display technology for pattern illumination. It delves into more detailed studies in two specific areas: optogenetics and patterned LCE. By comparing existing technologies with mini LEDs, the thesis highlights the research potential and importance of mini LEDs in the biomedical domain, providing a background and reference for the subsequent content.

The main content begins with a macro system overview, followed by the design and fabrication of a 48x32 mini LED matrix. This includes the topology structure and the determination of the optical scheme through simulations to achieve good illumination uniformity. The development of matrix drivers and user interfaces enables a real-time interactive optoelectronic system. The entire process of creating a mini LED matrix system capable of precise optical stimulation is thoroughly detailed, with performance evaluations conducted in terms of matrix, optical, and thermal aspects. Another significant advancement is the scale-up from the 48x32 mini LED matrix system to a larger human-size 160x160 mini LED matrix system. This section covers the definition and fabrication of the large matrix, the design of suitable LED drivers and interfaces, and concludes with electrical, optical, and thermal evaluations.

Next, the thesis introduces the potential applicability of this system in biomedical experiments, describing its experimental application in the optogenetic control of cardiac activities and discussing its prospective use in patterned LCE applications.

Finally, the thesis concludes by summarizing all the content and proposing future improvements and further research directions.

48x32 Mini LED Matrix System

2.1. Introduction

This chapter discusses the development of a mini LED matrix system capable of real-time, flexible pattern and brightness modulation. The discussion begins with a general overview of the system, then divided into several blocks, starting with the details of the mini LEDs matrix, followed by the assembly of a 48x32 mini LED matrix. Subsequently, a driver for this matrix will be introduced to ensure stable output. Additionally, a user interface has been developed, allowing researchers to directly adjust and control the matrix's real-time images and brightness through clicks or inputs on a PC. After fabrication, the 48x32 mini LED matrix and its driver will undergo testing and analysis, focusing on optical and thermal characterization, to confirm its suitability for biological experiments.

2.2. Requirements

Designing, developing, and evaluating a mini LED matrix system capable of generating customizable illumination patterns for biomedical applications should meet the specific objectives:

- Mini LED technology can provide various wavelengths to meet the needs of various applications. In this design, blue light with a wavelength of 470 nm was chosen for implementation because the final validation applications were selected for optogenetics [20] and light-responsive LCEs experiments [30]. The 470 nm wavelength of blue light excites both.
- Ensure uniform illumination across the matrix to avoid introducing artefacts or uneven exposure in the target area.
- Incorporate precise intensity control for each LED to allow for adjustable brightness levels.
- The brightness of the mini LED matrix should be controllable to make the experiment repeatable and make the system suitable for various biomedical applications. The intensity of at least 50 mW/cm² was selected based on the need to balance effectiveness with safety and practicality. An intensity of 50 mW/cm² is sufficient to provide strong excitation of fluorophores or other light-sensitive biological molecules without the need for excessively long exposure times [35][36]. Also, in optogenetics, the maximum light intensity of the mini LED matrix should be at least 50 mW/cm² to activate the optical sensitive ion channels in cells fully [37]. This intensity is within safe limits for exposure to human tissues under controlled conditions, as stipulated by various safety standards(IEC 62471) as well [38].
- Develop a reliable driver circuit that can individually address each LED in the matrix, allowing for complex pattern generation. A 50 ms latency for image update is chosen because it provides a good balance between responsiveness, system complexity, and operational efficiency. It is fast enough to meet the needs of most biomedical applications, ensuring that updates are timely and imperceptible to human operators while also allowing the system to be cost-effective, stable,

and energy-efficient. This latency choice supports a wide range of applications, from imaging to therapy. Besides, a scanning speed of 200 Hz ensures that the image updates are frequent enough to avoid visible flicker [39].

- Design a user-friendly control interface allowing easy configuration of illumination patterns, intensity, and timing. It should have flexibility and be expandable to suit different matrix sizes.

2.3. System overview

Figure 2.1 illustrates the control system for managing the mini LED matrix. The system comprises three main components: a real-time illumination control user interface, a high-speed LED driver, and a mini LED matrix.

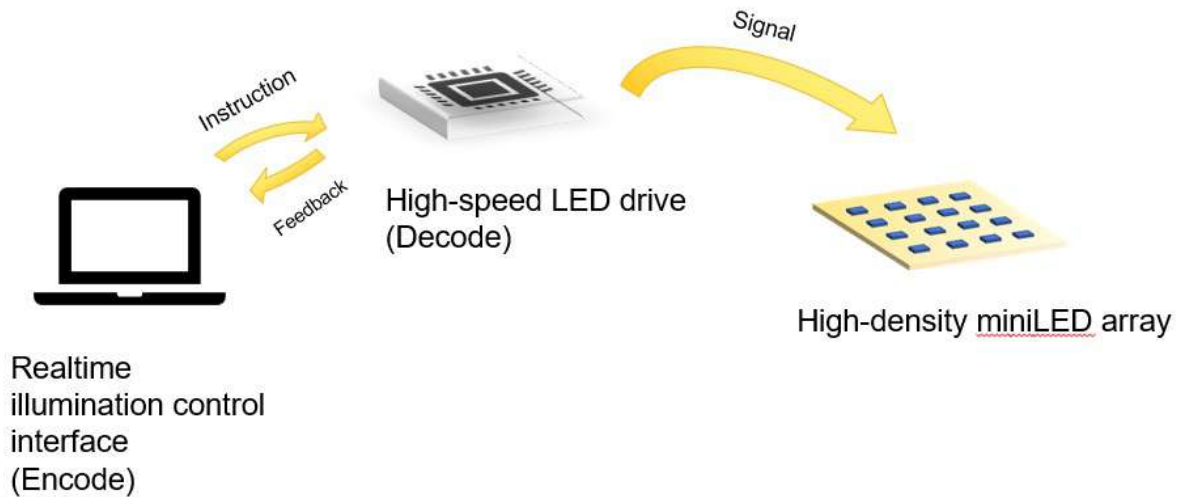


Figure 2.1: System operation process

- The system begins with a real-time illumination control interface encoding the control instructions. These instructions dictate how the mini LED matrix should behave regarding illumination intensity, pattern, and timing. The control interface is likely a software-based tool running on a computer, which allows the user to input and adjust these parameters in real time.
- The encoded instructions from the control interface are transmitted to a high-speed LED driver. This component acts as a decoder, interpreting the instructions and converting them into electrical signals, controlling the mini LED matrix. The driver's high speed is critical, ensuring that the mini LEDs can respond promptly to the instructions, allowing for precise illumination control.
- The final component in the system is the high-density mini LED matrix. This is the actual display or lighting unit where the mini LEDs are arranged in a matrix. The LED driver's signals dictate how each LED within the matrix is illuminated, enabling the display of complex patterns, high-resolution images, or specific lighting effects.
- The system also incorporates a feedback loop, where the LED driver sends feedback to the real-time control interface. This feedback likely contains information about the current state of the mini LED matrix, allowing for dynamic adjustments and ensuring that the output matches the desired performance as closely as possible.

2.4. Design and fabrication

2.4.1. Mini LED matrix

In the initial design phase, the mini LED interconnect topology was considered to drive the matrix. The topology choice greatly influences the mini LED system's performance, cost, complexity, and scalability.

Row-column scanning topology shown in Figure 2.2 was chosen as it is particularly advantageous for small mini LED matrices. It balances control complexity and cost well, making it an ideal choice when resources such as GPIO pins and PCB space are limited. The row-column scanning is a practical and efficient solution for maintaining sufficient brightness and minimising flicker for small matrices.

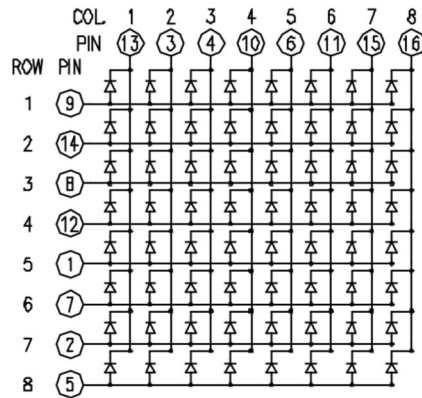


Figure 2.2: Row-column scanning

The mini LED matrix is a discrete light source, leading to non-uniform illumination on the receiving plane. A Lambertian light source model was used as an approximation for the mini LED, as it simplifies the calculation by assuming the light intensity radiates according to the cosine of the observation angle from the normal direction [40]. The Lambertian emission model describes a light source that emits light with intensity proportional to the cosine of the angle θ from the normal. The radiance $I(\theta)$ from a Lambertian emitter is given by: $I(\theta) = I_0 \cdot \cos(\theta)$, where I_0 is the intensity directly average to the surface and θ is the angle between the direction of emission and the normal to the surface, illustrated in Figure 2.3.

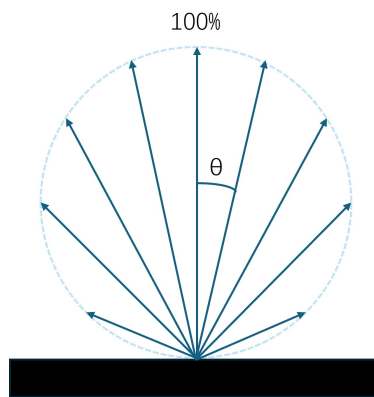


Figure 2.3: Light intensity with Lambertian emission model

For close viewing distance normally at the cellular level used in biomedical fields, a smaller pitch is necessary to avoid visible gaps between LEDs. The distance between the surface of the mini LED matrix and the observing plane was assumed to be less than 3 mm [12]. An array of 32 mini LED matrix with Lambertian emission with different pitches was simulated and shown in Figure 2.4. When Pitch is equal to 0.2 mm, the LEDs are closely packed, leading to a more uniform distribution of light across the observation plane. As the pitch is equal to 1 mm, the LEDs are spaced further apart, leading to a more distinct pattern with visible gaps between the intensity peaks corresponding to each LED. It illustrates that smaller pitches lead to a more uniform illumination.

To determine the pitch required to achieve 80% uniformity on the observation plane, we'll define the uniformity metric as $\text{uniformity} = I(\min)/I(\max)$, where $I(\min)$ is the minimum intensity on the observation

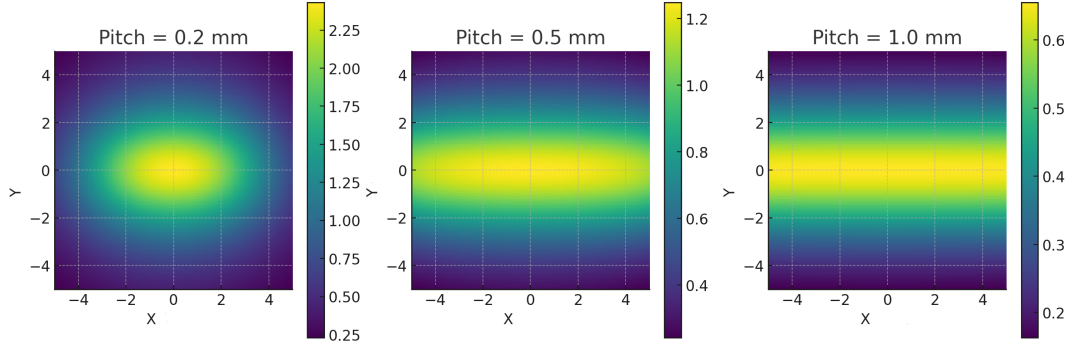


Figure 2.4: Light intensity distribution of an LED array based on Lambertian emission model with pitch=0.2 mm, 0.5 mm, 1.0 mm respectively

plane, and $I(\max)$ is the maximum intensity on the observation plane. As iterating with different pitches, the optical pitch value which gives the best balance should be around 0.8 mm.

To determine the appropriate mini LED chip and achieve a light intensity of at least 50 mW/cm^2 , considerations included that the size of the mini LED is proportional to its power rating [41] and in the matrix, the LEDs are not turned on simultaneously but refreshed row by row sequentially.

Assume the mini LED chip's power output is P , and its width and length calculate the area A it covers. P equals the product of the desired intensity I and the area covered. In practice, efficiency loss must be considered. After comparing different types of LED chips, the LED chip BFB0F11C, Aucksun, was chosen.

The chips were bonded on the substrate using flip-chip bonding, pick-and-place, and reflow soldering. The protection of the mini LED matrix from dust and damage was implemented by applying optical glue (NOA-61) cured by a UV light source on the surface.

The substrate for future implantable applications needs to be flexible and biocompatible. Among materials like PDMS, PI, PET, and Parylene C, Polyimide (PI) is the most suitable due to its stability and ability to withstand reflow temperatures up to 210°C [42][43][44]. Despite not being transparent, a white reflective layer can enhance light reflection. For row-column scanning, the anode and cathode of each mini LED must connect to specific row and column wires, avoiding short circuits. Wire crossing can be achieved through same-layer or different-layer methods, with the latter preferred to avoid weak points. The final substrate has a total thickness of $104 \mu\text{m}$. The interconnection traces must be no less than $180 \mu\text{m}$ on the substrate to supply adequate current to drive the mini LEDs according to the document for the design of printed circuit boards (PCBs) IPC-2221 [45]. They also require a sufficient width to prevent unwanted heating and voltage drops.

The final mini LED matrix, shown in Figure 2.5, was tested using two PI substrates. One substrate is yellow, and the other is white.

2.4.2. Mini LED matrix driver

When designing the driver for the 48x32 mini LED matrix, both hardware and software must be considered. The flexibility to accommodate different matrix sizes is essential as a foundation for future research.

Hardware

The PCB was discussed first in the hardware design. As shown in Figure 2.6, considering the matrix's final integration and size, the mini LED matrix PCB driver was designed in an "L" shape surrounding the matrix. The PCB can be divided into major sections: row control, column control, and the micro-controller. The mini LED matrix and the PCB were connected by flat flex cable (FFC). FFCs are used for their flexibility, compactness, and ease of installation. They are ideal for space-constrained and movable electronic applications, offering cost-effective and reliable connections with improved signal integrity.

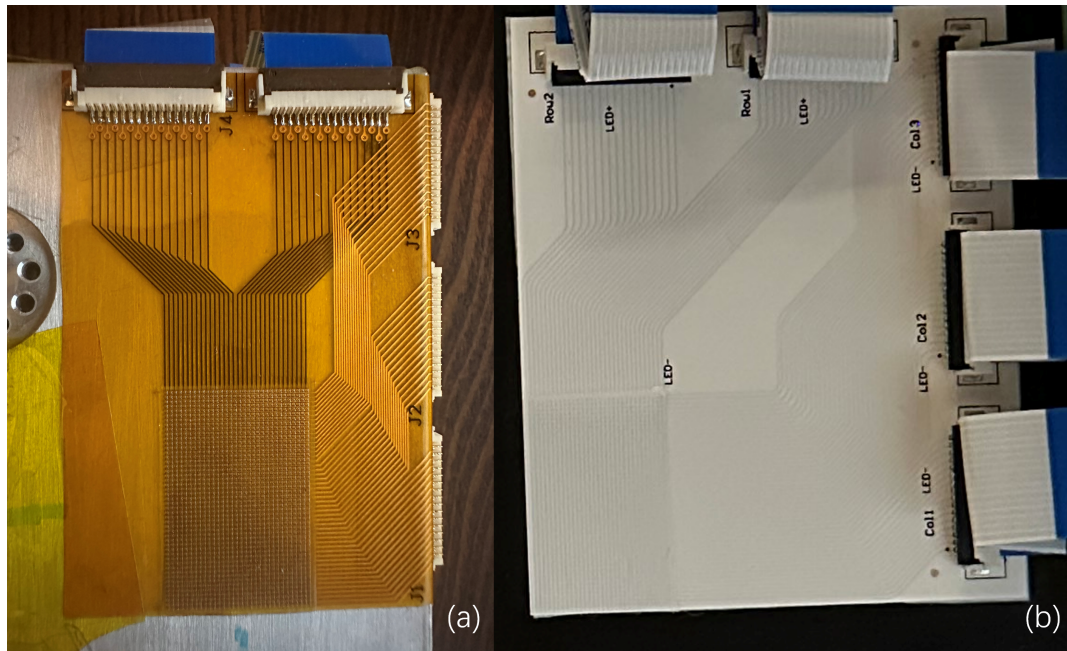


Figure 2.5: View of the 48x32 mini LED matrix with (a) yellow substrate and (b) white substrate

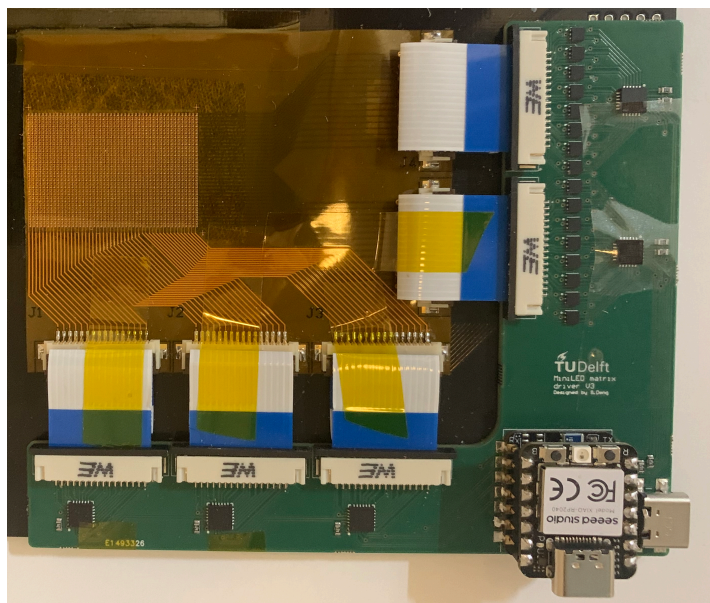


Figure 2.6: View of the mini LED matrix with yellow substrate with driver PCB

The row controller in the matrix was responsible for sequentially activating the rows of LEDs in a controlled manner to enable the proper display of images or patterns. This allowed for efficient multiplexing and synchronized operation with the column controller, ensuring a fast, flicker-free display of images or patterns across the entire LED matrix.

Row Selection Using Shift Registers

- The row controller used a series of shift registers (74HC164) to control the selection of each row in the mini LED matrix. A shift register is a type of sequential logic circuit that takes in serial input data and shifts it across its outputs with each clock pulse.
- Data Input: The microcontroller provided a serial data input (DAT) to the first shift register in the series. This data determines which row should be activated.

- Clock Signal (CLK): A clock signal was sent to all the shift registers to synchronize the data shifting across the register chain. Each rising edge of the clock pulse moves the data to the next stage in the shift register.

PMOS Transistor Array as Switches

- Each shift register output was connected to the gate of a PMOS transistor in a PMOS array. The PMOS transistors act as switches that control the power supply to the rows of LEDs.
- Gate Control: When the shift register outputted a low voltage (0-1 V), the corresponding PMOS transistor was turned on, allowing current to flow through the row, thereby activating it. Conversely, when the output was high (4.5-5 V), the PMOS transistor was turned off, disconnecting the row from the power supply.

Sequential Row Activation

- Sequential Switching: As the data was shifted through the shift registers, the corresponding rows were sequentially activated. This means only one row was active at any given moment, allowing the columns to control which specific LEDs in that row are lit.
- Multiplexing: The row controller effectively used multiplexing to control all 32 rows with only a few control signals. This method minimized the number of direct connections needed, reducing the complexity and cost of the circuit.

Synchronization with Column Control

- The row controller must be perfectly synchronized with the column controller. While the row controller activated a specific row, the column controller (using integrated current sources) determined which LEDs within that active row would be lit based on the image or pattern data provided by the microcontroller.

Fast Scanning to Avoid Flicker

- The row controller operated at high speed to scan through all rows quickly enough to create the illusion of a steady image. The scanning speed of the controller should be at least 200 Hz to avoid any unwanted action potential triggering [39].

Integration and Power Management

- The row controller's PMOS array was designed to handle the current required by the entire row of LEDs. Since multiple rows share the same power source, the row controller must efficiently manage the switching to avoid power spikes or drops, ensuring stable operation of the matrix.

The column controller in the mini LED matrix used CAT4016 LED drivers to manage the individual LEDs in each column. It employed a combination of shift registers and a latch mechanism to control LED states, regulated current via a feedback resistor, and used PWM for dimming. This setup ensured precise control over each LED, allowing for high-quality, flicker-free displays that can dynamically adjust brightness and pattern.

Use of CAT4016 LED Drivers

- The column controller utilized 16-channel programmable constant current LED drivers, CAT4016, to manage the LEDs in each column. Each CAT4016 chip can control 16 LEDs, storing their on/off states in a built-in 16-bit shift register.

Shift Register and Latch Mechanism

- The CAT4016's shift register received serial data from the microcontroller that indicates which LEDs should be on. The CAT4016 includes a latch mechanism, which made the data in the shift register not immediately affect the LEDs; instead, it remained stored until a latch signal (LAT) was received. When the latch signal was triggered, the stored data was transferred to the output, and the corresponding LEDs were turned on or off.

Current Regulation

- The CAT4016 featured a global current regulation system controlled by an external feedback resistor (Rfb). This resistor sets the current that flows through each LED. With an Rfb value of 600Ω, the current through each LED was regulated to 100mA, ensuring consistent brightness and preventing overdriving of the LEDs.

PWM Dimming

- To enable global dimming across the LED matrix, the column controller used Pulse Width Modulation (PWM). A PWM signal, operating at 1 MHz, was applied to the enable (EN) pin of the CAT4016 driver. By adjusting the duty cycle of the PWM signal, the overall brightness of the LEDs can be modulated, allowing for smooth dimming effects across the entire matrix.

Synchronization with Row Controller

- The column controller operated in tight synchronization with the row controller. As the row controller sequentially activated each row, the column controller updated the LED states in each column according to the data stored in the shift registers. This coordinated operation ensured that the correct LEDs were lit at the right time, producing the intended image or pattern on the display.

The RP2040 dual-core processor was chosen for this mini LED matrix project because of its powerful dual-core architecture, flexibility in I/O, cost-effectiveness, ample memory, and ease of programming. These features make it well-suited to efficiently manage the complex tasks of controlling a large LED matrix, ensuring smooth, high-quality performance while allowing for future scalability and customization.

Dual-Core Processing Power

- **Parallel Processing:** The RP2040 featured two cores, which allowed for parallel processing of tasks. The division of labour between Core 0 and Core 1 suggested that the system was designed for high efficiency and responsiveness. Core 0 focused entirely on updating the LED matrix without interruption from other tasks, while Core 1 handled command processing and communication, ensuring that the system could quickly respond to new inputs or changes.

High Performance and Flexibility

- **133 MHz Clock Speed:** With a clock speed of up to 133 MHz, the RP2040 offered ample processing power to handle complex control algorithms and high-speed data transfers, ensuring smooth operation of the LED matrix even at high refresh rates.
- **Flexible I/O:** The RP2040 provides 30 programmable I/O pins, allowing for a highly customizable interface with the row and column controllers, as well as other peripherals. This flexibility made it easier to adapt the microcontroller to different matrix configurations or additional features.

Large Memory Resources

- **264 KB SRAM:** The RP2040 was equipped with 264 KB of on-chip SRAM, providing ample memory for storing display data, control algorithms, and handling multiple buffers. This was essential for managing the high data throughput required by a large LED matrix.

USB-wired communication was chosen for this project. USB (Universal Serial Bus) provides a reliable and fast data transfer rate, with modern USB standards (USB 2.0 and above) capable of delivering data at speeds sufficient to maintain low-latency communication. This ensures that the time between sending data from the PC and the data being processed by the microcontroller is minimized, keeping the system's overall latency well below the 50 ms threshold. USB provides a stable, wired connection that is less prone to disruptions that can affect wireless communication, such as interference from other devices or physical obstacles. This stability is critical for maintaining the system's consistently low latency. It simplified the design as well. USB can carry both power and data over a single cable,

simplifying the system design. This is particularly useful for reducing clutter and improving reliability, as there are fewer points of failure.

Software

Regarding the software design, the hardware control part was stored and running in the microcontroller. It generated all timing signals for hardware operation and synchronized the row and column control parts. Serial Peripheral Interface (SPI) was used for serial communication protocol because of its high-speed data transfer, low latency, simplicity, scalability, and efficient use of pins.

As mentioned above, the system was implemented on a microcontroller RP2040 with dual-core. The LED matrix comprises 48 columns and 32 rows, with control logic distributed across the two cores. Core 1 is responsible for command processing, while Core 0 handles the real-time updates of the LED matrix display. The microcontroller interfaced with the LED matrix through various GPIO pins configured for data, clock, latch, and reset signals. Pulse-width modulation (PWM) was employed to control the brightness of the mini LEDs, with precise timing required to avoid visual artefacts.

Core Synchronization and Initialization

- Core 0's initialization focused on preparing the hardware for display control. This involved configuring the GPIO pins, initializing the SPI interface, and setting up the PWM parameters. The initialization sequence was carefully timed, with Core 0 waiting for Core 1 to signal readiness before proceeding to its main operation.
- Core 1 initialized the serial communication interface, which was used to receive commands from an external source. It then waited for Core 0 to complete its initialization. The synchronization mechanism ensured that both cores were in a known state before the system began its primary operation.

Communication Protocol

Commands sent via the USB serial interface followed a specific structure to ensure the microcontroller correctly interpreted them. The general structure of a command was as follows:

- Command Identifier (1 byte): The first byte of the command specifies the type of command. A single ASCII character (e.g., 'A', 'X', 'B') instructed the microcontroller on what action to perform.
- Optional Data Payload (variable length): Depending on the command, additional data might follow the command identifier. This could be a set of parameters, raw image data, or other relevant information.
- End Marker ('!'): The '!' character marked the end of the command. This was critical for the microcontroller to know when the command had been fully received and was ready for execution.

The following was a list of all the commands used in the communication protocol, along with their descriptions and potential payloads:

- 'A' Command: Purpose: This command was used to set all LEDs on; Payload: A single byte representing the value to which all LEDs should be set' Example: 'A0xFF!'.
 - 'X' Command: Purpose: This command was used to transfer an arbitrary amount of byte data, such as an image to be displayed on the matrix; Payload: A stream of bytes representing the image data. The size of the payload depended on the resolution and colour depth of the image'; Example: 'X<image data>!' - Transferred the image data to be displayed.
 - 'Z' Command: Purpose: This command was used to set all LEDs off; Payload: A single byte representing the value to which all LEDs should be set; Example: 'Z0x00!'.
 - 'P' Command: Purpose: Sets the PWM duty cycle, which controls the brightness of the entire matrix; Payload: A number representing the duty cycle, ranging from 0 to 100; Example: 'P80!' - Sets the PWM duty cycle to 80 %.

- 'R' Command: Purpose: Toggles a specific row of LEDs on or off; Payload: The row number to toggle; Example: 'R10!' - Toggles the LEDs in row 10.
- 'S' Command: Purpose: Toggles a specific mini LED at a particular row and column; Payload: The row and column numbers; Example: 'S5,10!' - Toggles the LED at row 5, column 10.
- 'C' Command: Purpose: Toggles a specific column of LEDs on or off; Payload: The column number to toggle; Example: 'C10!' - Toggles the LEDs in column 10.

Core 1 continuously listened for incoming commands via the serial interface within the `loop1()` function. The steps for processing the commands were as follows:

- Buffering: As data arrived, it was stored sequentially in a command buffer ('commandBuffer'). The buffer accumulated the data until the end marker ('!') was detected.
- End Marker Detection: The '!' character signified the end of a command. When this marker was detected, the system recognized that a complete command had been received and was ready for parsing.
- Parsing and Execution: The `parseCommand()` function was invoked to interpret the buffered command and execute the corresponding action. The system used a 'switch' statement to handle different command identifiers, executing the appropriate function based on the command type.

The communication protocol was designed to handle potential errors and ensure data integrity:

- Command Buffer Management: The command buffer was reset after each command was processed, preventing overflow and ensuring that each new command started at the beginning of the buffer.
- Timeouts and Invalid Commands: The system was designed to handle timeouts or invalid commands, where incomplete or malformed commands were discarded, and the system returned to a standby state.
- Synchronization with Core 0: After a command was processed, Core 1 communicated with Core 0 to update the LED matrix display as needed. This ensured that the system remained synchronized and responsive to new commands.

2.4.3. Graphic user interface for the mini LED matrix

This section presented the design and implementation of a graphical user interface (GUI) for controlling a 48x32 mini LED matrix. The interface shown in Figure 2.7 was built using Python and the Tkinter library, allowing users to interact with the LED matrix through various control mechanisms, including individual LED toggling, row and column operations, and PWM brightness control. Additionally, the interface supported automated control via script uploads and image display functionality. This section discusses the system architecture, the development process, and the key features of the interface.

System architecture

The system architecture comprised three main components: the user interface (UI), the communication module with the LED matrix hardware, and the control logic.

- User Interface (UI): The UI was constructed using the Tkinter library, which provided a simple and effective means of creating windows and graphical controls in Python. The UI was responsible for rendering the LED matrix on the screen and offering controls such as buttons, sliders, and text fields for user interaction.
- Communication Module: Communication with the LED matrix was handled via serial ports facilitated by the Communication module. This module refreshed the available serial ports, established connections, and sent commands to the matrix hardware.
- Control Logic: The control logic interpreted user inputs from the UI and translated them into commands that the LED matrix hardware could execute. This included toggling individual LEDs, entire rows or columns, managing PWM for brightness control and executing uploaded scripts.

User Interface Design

The user interface shown in Figure 2.7 was designed to focus on usability and functionality, allowing users to interact with the LED matrix intuitively. The main features of the UI are described below.

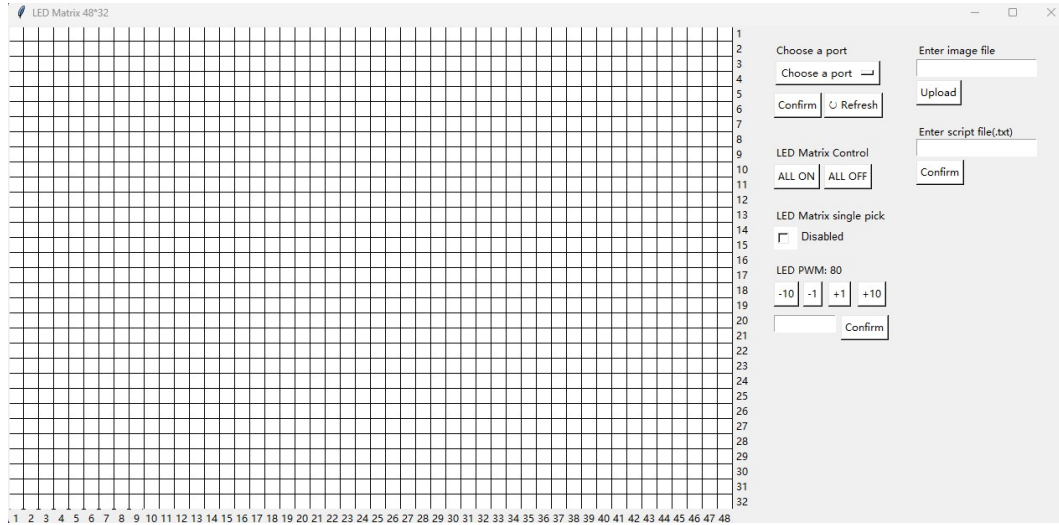


Figure 2.7: UI for the 48x32 mini LED matrix

LED Matrix Display

The LED matrix was visualized on the screen as a grid of rectangles, each corresponding to an individual LED. Users could click on any rectangle to toggle the state of the corresponding LED. The state was visually represented by the rectangle's colour—blue for "on" and white for "off." This provided an immediate visual representation of the LED matrix's current state.

Control Elements

The UI included several control elements to interact with the LED matrix:

- **Communication Control Buttons:** The desired USB port connected to the matrix could be selected.
- **All On/Off Buttons:** These buttons allow users to turn all LEDs on or off with a single click. The commands were sent to the matrix hardware, and the visual representation on the UI was updated accordingly. A 2D list was used to store the state of each LED in the matrix. This data structure allowed for efficient access and modification of LED states. Each list element corresponded to an LED, storing a boolean value indicating whether the LED was on or off. The grid of rectangles in the UI was programmatically linked to this data structure, ensuring that the visual representation was always in sync with the actual state of the LED matrix.
- **PWM Control:** The brightness of the LEDs was controlled through PWM. The UI included buttons for fine-tuning the PWM value in increments of 1 or 10 and an entry field for direct input. The current PWM value was displayed on the UI to provide real-time feedback. PWM control was implemented to allow the user to adjust the brightness of the LEDs. The UI provided feedback on the current PWM setting, which was calculated and displayed based on user input. The system ensured that the PWM value remained within the valid range (0-100) and adjusted the LED brightness accordingly.
- **Image Upload:** Users could upload an image file in jpg format to be displayed on the LED matrix. The uploaded image was processed and rendered on the matrix. The UI provided the necessary controls to select and upload the image file. The 'ConvertImg' module handled the processing of images uploaded by the user. Images were converted into a binary format compatible with the LED matrix, resized to fit the matrix dimensions, and rendered on the matrix. The UI was updated to display the image on the virtual LED grid, providing a real-time preview of the matrix output.

- **Script Upload:** The UI supported uploading script files containing a sequence of commands to automate the control of the LED matrix. This feature was handy for executing predefined patterns or sequences. Each command in the script was executed sequentially, allowing for complex control patterns and automation. The control logic parsed the script file, interpreted the commands, and sent them to the matrix hardware in real time. The list of commands is as follows:
 - 'ON' Command: Purpose: Turn on all LEDs.
 - 'OFF' Command: Purpose: Turn off all LEDs.
 - 'C' Command: Purpose: toggle single column; Example: 'C10'.
 - 'R' Command: Purpose: toggle single row; Example: 'R10'.
 - 'S' Command: Purpose: toggle single LED; Example: 'S10,10'.
 - 'P' Command: Purpose: change PWM (light intensity); Example: 'P10'.
 - 'D' Command: Purpose: wait for some time in milliseconds; Example: '100'.
 - 'X' Command: upload an image as a pattern; Example: '/img/logo.jpg'.

Interactive row and column labels

Interactive labels for each row and column were provided alongside the LED matrix display. Users could toggle an entire row or column of LEDs by clicking on these labels. This feature allowed for quick and efficient control of large matrix sections. The function and the single LED toggle could be implemented as the 'Enable' was toggled to avoid miss-clicking.

Communication with the mini LED matrix driver

Serial communication was used between the UI and the LED matrix driver. The 'Communication' module managed the connection to the hardware and transmitted the appropriate commands.

- **Command Structure:** Each command sent to the LED matrix driver followed a specific structure, such as '!S0,row,col!' to toggle an individual LED at the specified row and column. The command structure was designed to be straightforward, ensuring reliable communication between the UI and the hardware.
- **Error Handling:** The communication module included error-handling mechanisms to ensure the system responded appropriately to invalid commands or communication errors. If an error was detected, it was reported back to the UI, where an error message was displayed to the user.

Results and Evaluation

The mini LED matrix control system was tested extensively to evaluate its performance and usability. The system successfully managed the mini LED matrix, providing real-time feedback and control with minimal latency. The PWM brightness control was found to be responsive and precise, allowing for fine adjustments to mini LED brightness. Image uploads were processed efficiently, with the matrix displaying the uploaded images accurately.

User testing indicated that the UI was intuitive and easy to use. The ability to upload scripts was particularly appreciated by users who needed to automate repetitive tasks or display complex patterns. The system's error-handling mechanisms effectively caught and reported errors, contributing to a robust and reliable user experience.

2.5. Characterization methods

2.5.1. Wavelength

The mini LED chip's spectrum was measured by mounting in front of the FOIS-1 integrating sphere, which was connected to a flame spectrometer (Ocean Optics) for spectrum measurement. The spectrometer was calibrated with an HG-1 wavelength calibration lamp (Ocean Optics). A source meter (Keithley 2450) provided currents from 1 mA to 20 mA. The input current increased by 1 mA steps. The result shows an average peak wavelength of 453 nm. The peak wavelength shift was within 2 nm, and the FWHM was under 26 nm. These results confirm that the mini LED has a stable wavelength.

2.5.2. Driver characterization

The goal of the driver measurements by observing the timing of control signals within the driver module is to assess the stability of the driver module and quantify its inherent delay.

Measurements

Figure 2.8 illustrates the detailed process of a CCS shift register data write cycle. It corresponds to a row pattern where the 9th, 16th, 26th, 36th, and 43rd LEDs in a row are lit. Each time the rising edge of the DCLK (light blue) signal is reached, pixel data is written from the RDAT signal. In this driver implementation, the SPI communication speed is set to the adjustable maximum of 4 MHz to optimize the matrix's refresh rate.

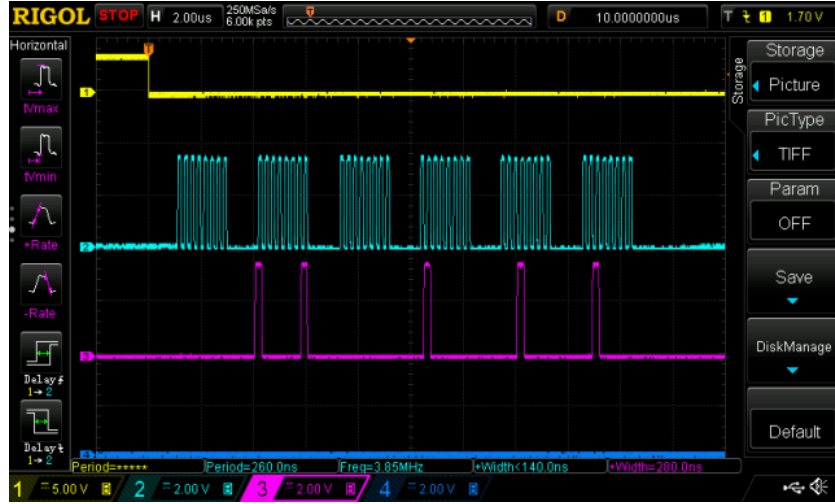


Figure 2.8: CCS shift register timing signal for specific row pattern.

As shown in Figure 2.9, to avoid row overlapping when writing new line data to the shift register, all mini LEDs were turned off by pulling up BL at first. Then, the RCLK generated a pulse to switch to the next row line. The LAT then gave a pulse to push new row data into CCS. Finally, BL was pulled down to turn on the mini LEDs.

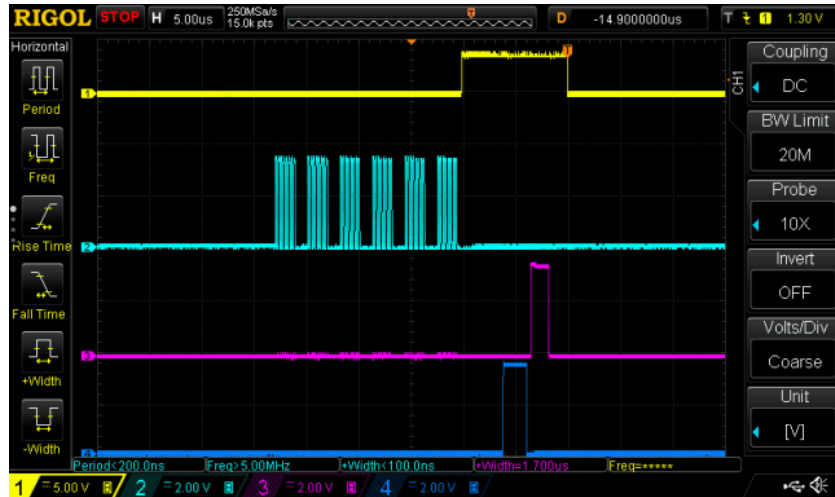


Figure 2.9: CCS latch Timing signal, BL (yellow), DCLK (light blue), LAT (pink) and RCLK (blue)

Figure 2.10 illustrates the timing signal measured at initiating a new scan. After the falling edge of the GPIO0 signal, the RDAT line is pulled low until the first RCLK signal is transmitted, resulting in the PMOS controlling the first row being turned on. After each scan, a blank interval is maintained

to provide a margin for the next scan. The refresh rate of the mini LED matrix was determined to be 333 Hz as shown in Figure 2.10.



Figure 2.10: Scanning timing signal measured at GPIO0 (yellow), DCLK (light blue), RDAT (pink) and RCLK (Blue)

2.5.3. Optical characterization

To ensure that the 48x32 mini LED matrix met the requirements to activate the light-sensitive ion channels in cells and activate LCE film efficiently, the mini LED matrix should provide a maximum light intensity of at least 50 mW/cm^2 , with the ability to adjust the intensity continuously and linearly. This experiment tests whether the 48x32 mini LED matrix can fit the requirements.

Experimental setup

The mini LED matrix consisted of 1536 individual LED elements arranged in a 48x32 grid. The following equipment was used to characterize the optical properties as shown in Figure 2.11: Thorlabs S142C Integrating Sphere Photodiode Power Sensor and Thorlabs PM400 Optical Power Meter.

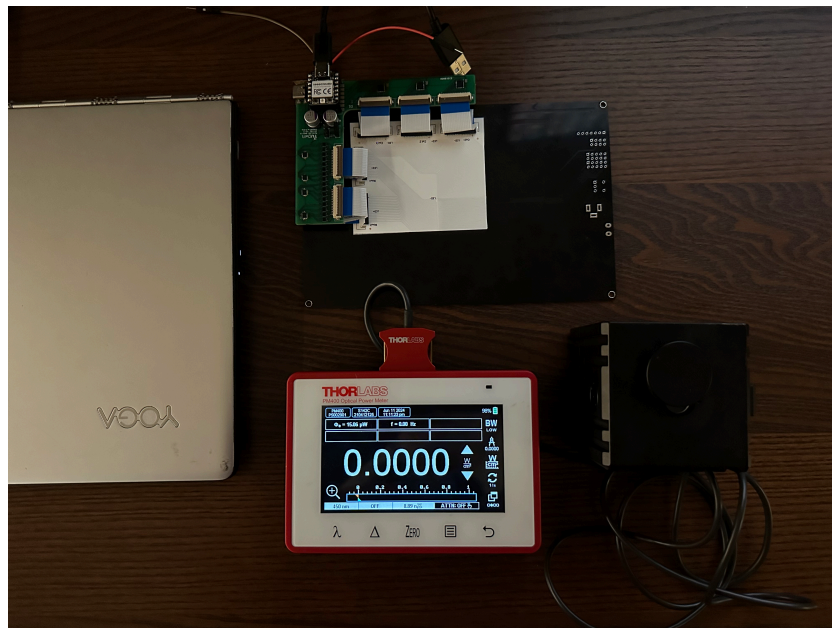


Figure 2.11: The light intensity experimental setup of 48x32 mini LED matrix with white substrate

Measurement of Light Intensity

The power meter was positioned at the closest distance from the mini LED matrix to determine the light intensity across the matrix. By changing the PWM value of the matrix through the user interface and driver from 0 to 99, the mini LED matrix changes from switching off to fully on, which means it provides a range from minimum to maximum light intensity.

Figure 2.12(a) indicates that the maximum light intensity of the matrix with the yellow substrate was recorded at 140.2 mW/cm^2 . Moreover, the light intensity decreases to below 50 mW/cm^2 when the PWM value is less than 32. From the graph, we can infer that the light intensity changes linearly. The results indicated that the matrix can provide sufficient intensity while maintaining a relatively uniform distribution across the matrix.

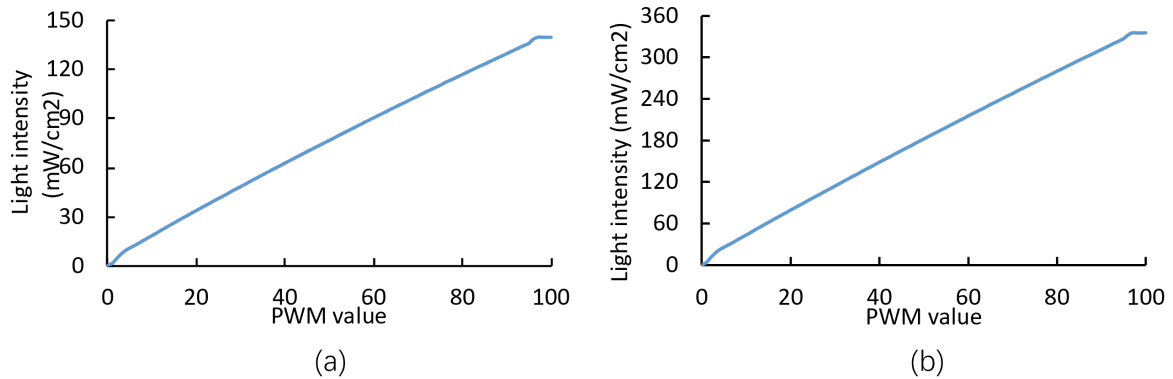


Figure 2.12: The light intensity of 48x32 mini LED matrix with (a) yellow substrate and (b) white substrate

The light intensity of another sample matrix with a white substrate was measured following the same procedure. Figure 2.12(b) shows that the maximum light intensity for the matrix with the white substrate was recorded at 335.8 mW/cm^2 , more than twice that of the matrix with a yellow substrate. Additionally, the light intensity drops below 50 mW/cm^2 when the PWM value falls below 12. The graph also suggests a linear relationship in the change of light intensity.

Both the mini LED matrix with yellow substrate and white substrate can deliver a peak light intensity exceeding 50 mW/cm^2 . The one with a white substrate gives better results, which indicates that the material can be selected for future work.

2.5.4. Thermal characterization

Thermal characterization was performed to evaluate the matrix's thermal performance under various operating conditions to ensure the safe use of a mini LED matrix for biomedical experiments. Proper thermal management is essential to prevent overheating, which can damage biological tissues and compromise experimental results.

To preserve cell viability and ensure accurate experimentation, the matrix must maintain a surface temperature that does not exceed 41°C [46], under varying patterns or intensity changes when operating at the physiological temperature of 37°C .

Experimental setup

The full experiment setup is shown in Figure 2.13. The experiment utilized the following equipment:

- A multi-channel stimulus (MCS Stimulus Generator STG4002 Series) to generate pulses for test
- A thermal meter (Extech 4-channel temperature meter SDL200) to ensure it provides accurate temperature and heat measurements in practical applications.
- Thermocouples were strategically placed at various points on the matrix to continuously monitor localized temperature changes.

- A thermal camera (VarioCAM HD S/N:1266322) with a resolution of 1024x768 was utilized to capture high-resolution thermal images, providing a visual representation of the temperature distribution across the matrix.
- A heating plate was maintained at 37 °C to replicate physiological conditions.
- A thermal pad to improve heat transfer between the heating plate and the mini LED matrix, ensuring efficient thermal management by filling air gaps and reducing thermal resistance.



Figure 2.13: The thermal characterization experimental setup for 48x32 mini LED matrix with white substrate

Measurement

To ensure accurate thermal performance of the mini LED matrix during operation, a thermal calibration process was carried out using the Extech 4-channel temperature meter SDL200 and the thermocouples. It involved placing the mini LED matrix on the heating plate set to 37 °C to simulate physiological conditions. The thermal meter, calibrated against a certified reference standard, was used to measure the surface temperature of the matrix as it reached thermal equilibrium. Thermocouples were strategically placed at the centre point of the matrix to monitor localized temperature variations. The experimental setup is shown in Figure 2.14. Additionally, the laboratory room temperature was 21 °C.

The temperatures were recorded as the adjustment file in APPENDIX A and B for reference by the infrared camera.

The thermal characterization was performed under two different pacing tests on the 48x32 mini LED matrix with yellow substrate and white substrate: 500 ms on, 500 ms off, for a total of 5 cycles, and 5 seconds on, 15 seconds off, for one cycle. The first pacing experiment was chosen because the optical termination protocol, which consisted of up to three 500 ms pulses with a 500 ms interval, has already been successfully validated in optogenetic experiments. The 1 Hz light frequency was also confirmed in the light-responsive LCE experiment [34]. The second experiment represents a relatively long time usage.

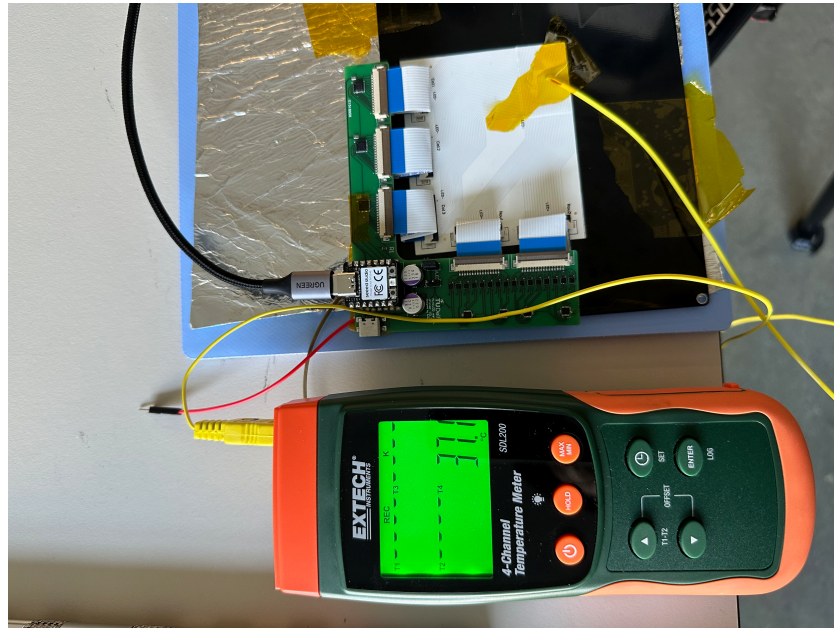


Figure 2.14: The thermal calibration experimental setup of 48x32 mini LED matrix

Under different pacing tests, the temperature changes were measured on the mini LED matrix with different configurations: an entire line lit, a single point lit, and the entire matrix lit. These tests were conducted at various output intensities controlled by PWM values ranging from 0 to 99, with steps of 10. The results for temperature rise regarding different PWM values are shown in Figure 2.15, 2.16, 2.17 and 2.18.

Under the 1 Hz pacing with 5 pulses test, when the 48x32 mini LED matrix with the yellow substrate was full-on, Table 2.1 indicates the matrix's highest temperature is 43.28 °C. The highest temperature for the white substrate is a bit higher, which is 44.68 °C in Table 2.2. Despite the highest temperature, the highest temperature rise is almost 7 °C, as stated in Figure 2.15 and 2.16, which exceeds the 4 °C limitation. It can be considered to be used in full-on mode when no more than three pulses and low brightness are required.

Meanwhile, both the matrix with yellow substrate and the white state are safe to use in single-line on mode and single-dot on mode as both the highest temperature and temperature rise meet the requirement for both tests. For the 5 s on 15 s off test, when the mini LED matrix was set to full-on, high temperatures for both the yellow and white substrate matrices were above 50°C in Table 2.3 and Table 2.4. It is not recommended for a long-time whole matrix lighting.

48x32 mini LED matrix with yellow substrate	Highest temperature(°C)
Full on	43.28
Single line	36.75
Single dot	35.87

Table 2.1: The highest temperatures for 48x32 mini LED matrix with yellow substrate as the matrix under full-on, single-line on and single-dot on, 1 Hz 5pulses

48x32 mini LED matrix with white substrate	Highest temperature(°C)
Full on	44.68
Single line	36.92
Single dot	35.43

Table 2.2: The highest temperatures for 48x32 mini LED matrix with white substrate as the matrix under full-on, single-line on and single-dot on, 1 Hz 5pulses

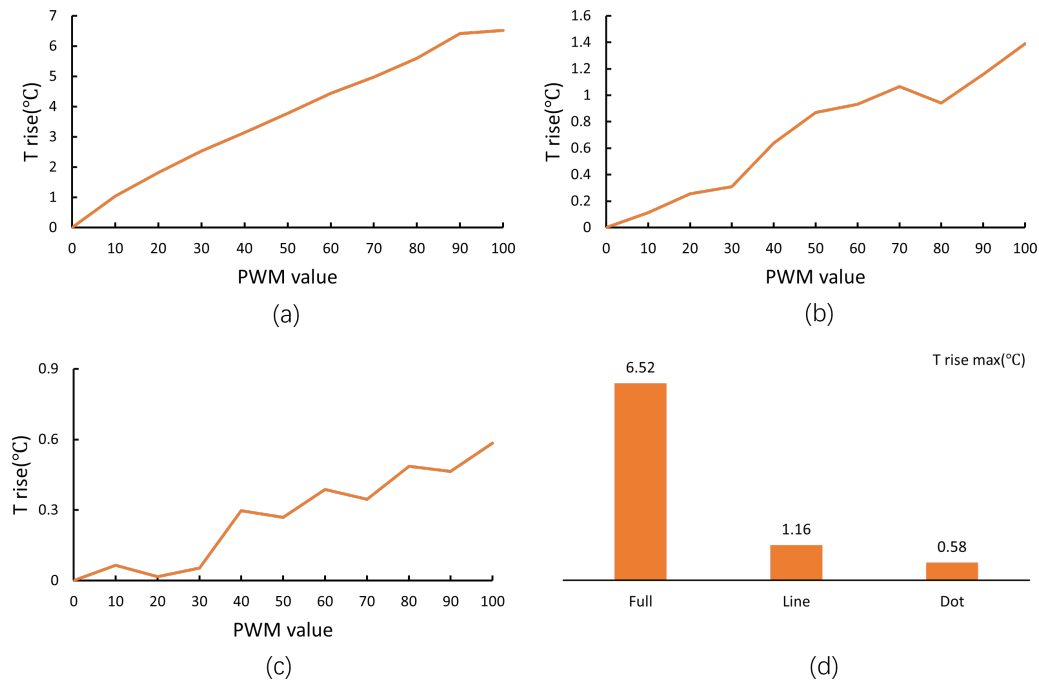


Figure 2.15: The temperature rise of 48x32 mini LED matrix with yellow substrate under 1 Hz pacing 5 pulses test as (a)full-on; (b)single-line on; (c)single-pixel on

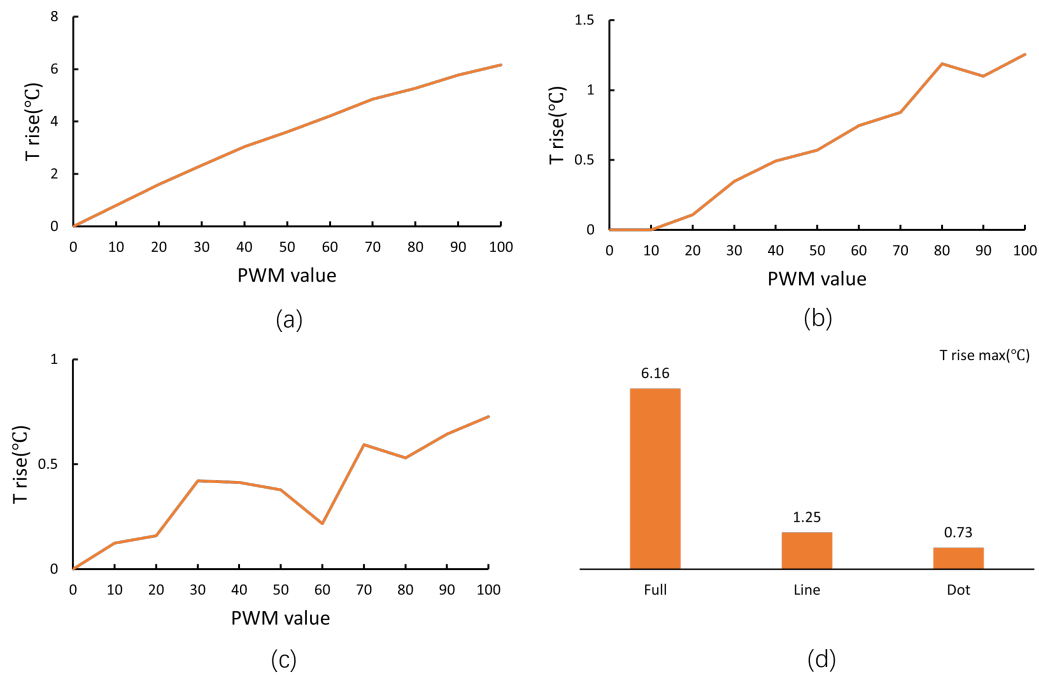


Figure 2.16: The temperature rise of 48x32 mini LED matrix with white substrate under 1 Hz pacing 5 pulses test as (a)full-on; (b)single-line on; (c)single-pixel on

2.6. Conclusion and discussion

In this chapter, we discuss and develop a mini LED matrix system designed for use in optogenetics research and experiments. From the design and implementation of the matrix, driver, and user interface to the driver timing characterization and optical and thermal characterization, we have examined and

48x32 mini LED matrix with yellow substrate	Highest temperature(°C)
Full on	51.87
Single line	38.67
Single dot	38.07

Table 2.3: The highest temperatures for 48x32 mini LED matrix with yellow substrate as the matrix under full-on, single-line on and single-dot on, 5 s on 15 s off

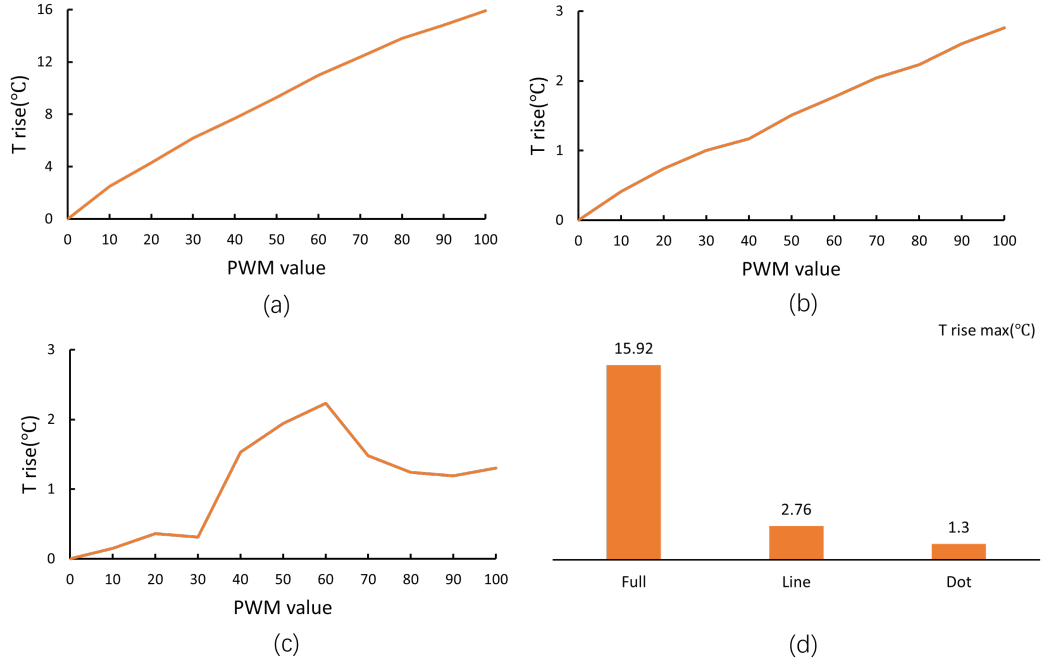


Figure 2.17: The temperature rise of 48x32 mini LED matrix with yellow substrate under 5s on 15s off test as (a)full-on; (b)single-line on; (c)single-pixel on

48x32 mini LED matrix with yellow substrate	Highest temperature(°C)
Full on	52.93
Single line	35.93
Single dot	35.23

Table 2.4: The highest temperatures for 48x32 mini LED matrix with white substrate as the matrix under full-on, single-line on and single-dot on, 5s on 15s off

validated this 48x32 mini LED matrix system's usability, stability, and safety in practical optogenetic experiments. This matrix has a stable wavelength at around 453nm. When on full power, the one with a white substrate can emit more than $300mW/cm^2$ light. In terms of luminance, the mini LED matrix with a white substrate demonstrates superior performance. For future research, it may be beneficial to further explore the use of white substrate materials. Under full operation, both types of materials exhibit similar thermal performance. However, when operating at full capacity for extended periods, the surface temperature of both materials rises above $50^{\circ}C$. This issue should be considered for improvement in subsequent work.

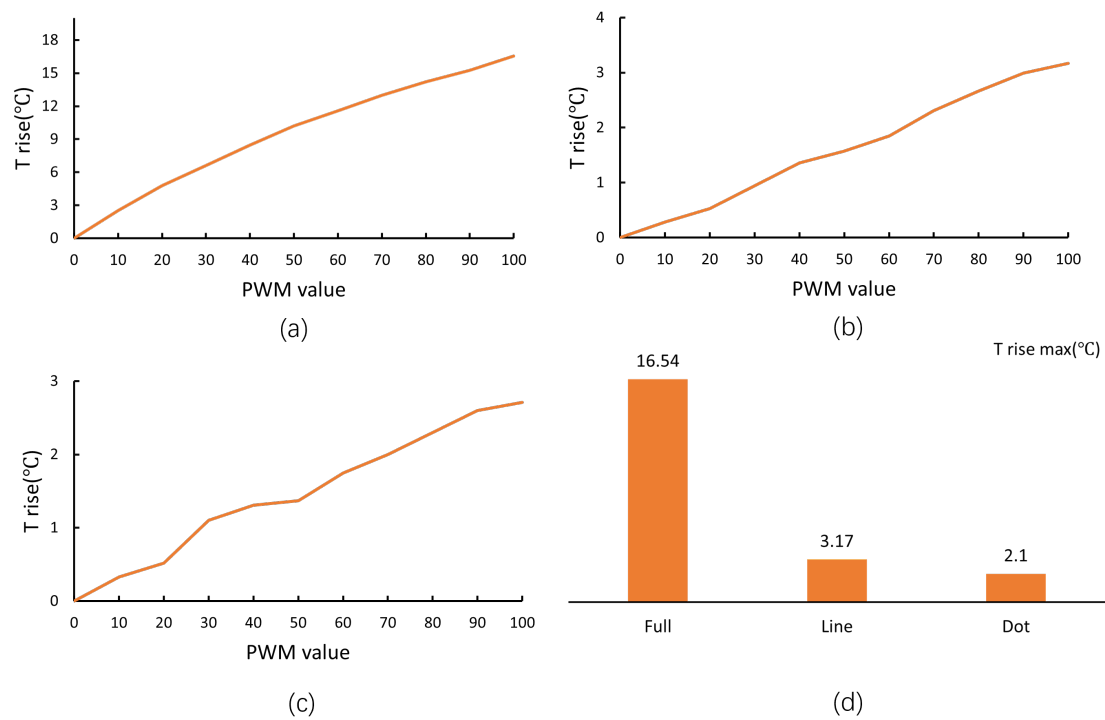


Figure 2.18: The temperature rise of 48x32 mini LED matrix with white substrate under 5s on 15s off test as (a)full-on; (b)single-line on; (c)single-pixel on

Applications with 48x32 Mini LED Matrix System

A validated mini LED matrix system should be used for experimental verification in biomedical research and experiments. The 48x32 mini LED matrix was applied in an in-vivo optogenetics experiment modified human immortalized atrial myocytes (hiAM) in 6-well size format and a mechanical-electrical characterization experiment of hiAM monolayers implemented by patterned LCEs.

3.1. Optogenetics control of cardiac activities

Optogenetics in the heart typically involves genetically modifying cardiac cells to express light-sensitive ion channels. When these channels are exposed to specific wavelengths of light, they open, allowing ions to flow across the cell membrane. This ion flow can depolarize or hyperpolarize the cardiac cells, effectively controlling their electrical activity. In this experiment, the primary focus was using a mini LED matrix to generate different spatial and temporal illumination patterns on cardiomyocytes modified with the light-sensitive protein to develop new strategies for terminating cardiac arrhythmias.

3.1.1. Experiment setup

In this experiment, a monolayer of human immortalized atrial myocytes (hiAM) embedded with the optogenetic tool CheRiff, which is sensitive to blue light, was used for research. During cell preparation, to culture and seed an adequate number of cells, a standard 6-well plate (or a culture plate of equivalent size) with a surface area of 9.5 cm^2 , pre-coated with fibronectin, was used. This plate was suitable for seeding 3.75 million cells at a density of 395 K/cm^2 . A heat plate was placed under the culture plate to maintain the cells at a temperature of 37°C . The mini LED matrix was positioned between the heat plate and the cell culture plate. As shown in the figure 3.1, besides an OVM camera, an optical filter box with 730 nm wavelength and the excitation light source LXZ1-PH01 for the voltage-sensitive dye Di4-anbdqbs were arranged directly above the cells to not interfere with the opt tool spectrum. A computer was connected to the OVM system, and the mini LED matrix was used to control and display data in real time.

3.1.2. Measurements

In this experiment, to induce AF, the S1S2 protocol described in [7] was used to induce a single rotor at the centre of the monolayer. A small area 10 ms , 470 nm light pulse was first applied at the boundary using a mini LED matrix, enabling normal pacing of the hiAM monolayer and initiating wave propagation from the stimulated region. After around 20 ms , a patterned pulse (20 ms , 470 nm) covering half of the monolayer was delivered, perpendicular to the previous repolarization front. This pattern created a wave break at the interface between the illuminated and non-illuminated regions, with only the non-illuminated area remaining excitable. This approach successfully induced a rotor. The method significantly improved both efficiency and immediacy. The rotor was illustrated in Figure 3.2(a)(b). Figure

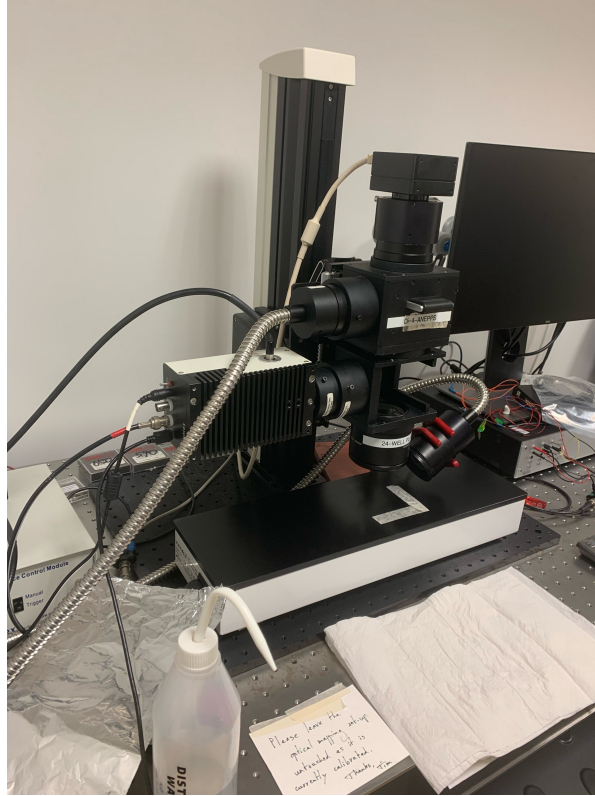


Figure 3.1: The setup includes an OVM camera, an optical filter box with a wavelength of 730 nm, and the excitation light source LXZ1-PH01 from top to bottom

3.2(c) illustrates the rotor's generation through the fluorescent signal waveform.

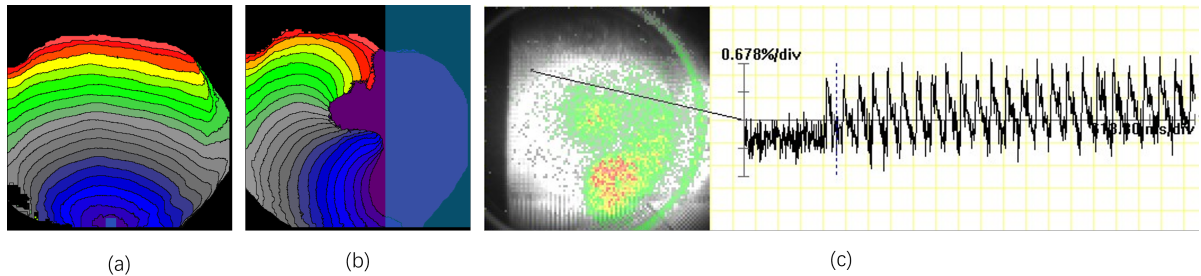


Figure 3.2: Rotor generated when inducing AF

After the arrhythmias were generated, the mini LED matrix generated a single line connecting the core and the nearest unexcitable boundary to terminate the AF. The light pattern generated by the mini LED blocked the propagation of the rotor wave and forced its front to gradually move toward the boundary of the culture dish until it dissipated. The process is shown in Figure 3.3. Figure 3.4 shows the fluorescent signal during the termination.

Besides, different line patterns, as shown in Figure 3.5, were tested to check the efficiency for different line parameters such as pixel density and light intensity. By identifying and comparing the minimum power required to terminate AF, the result was obtained when the LED spacing was 0.9 mm:

- (a) pitch = 0.6 mm, minimum light intensity to terminate AF = 24 mW/cm², power = 43.7 mW;
- (b) pitch = 0.84 mm, minimum light intensity to terminate AF = 29 mW/cm², power = 37.9 mW;
- (c) pitch = 1.2 mm, minimum light intensity to terminate AF = 42 mW/cm², power = 43.7 mW;

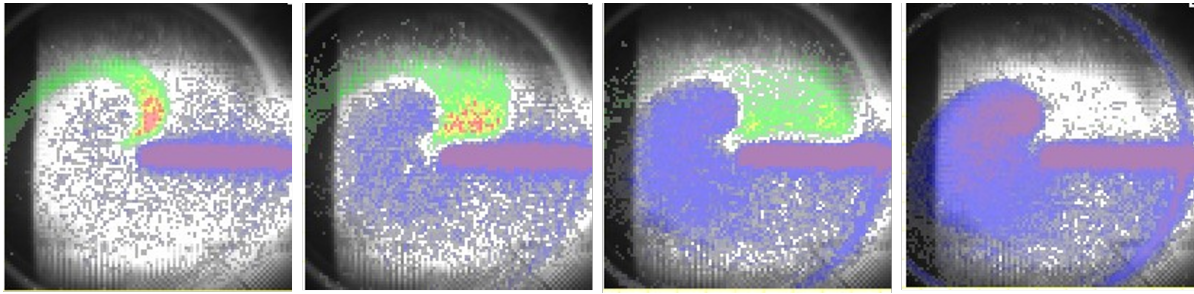


Figure 3.3: The process of AF termination

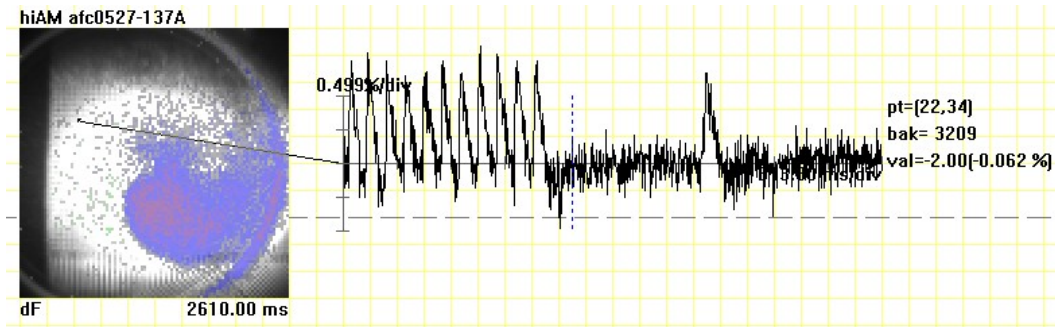


Figure 3.4: The fluorescent signal waveform when terminating AF

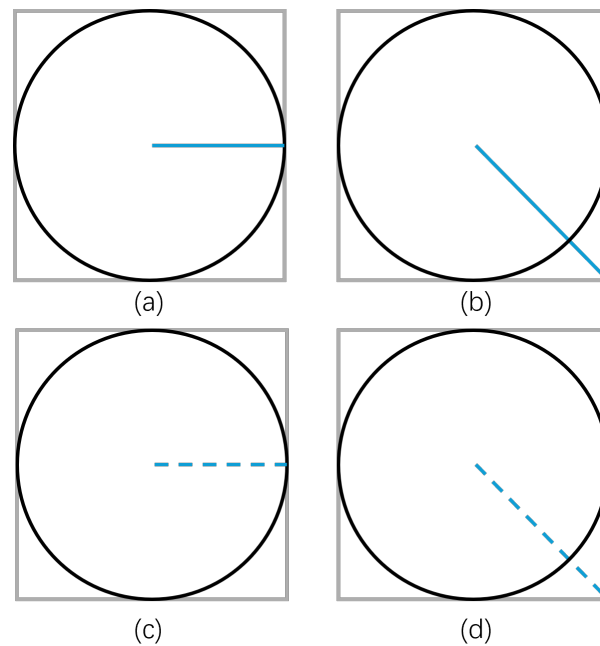


Figure 3.5: A light line block (a)horizontal line pattern; (b)diagonal line pattern; (c)horizontal lines with every other LED illuminated; (d)diagonal lines with every other LED illuminated

- (d) pitch = 1.68 mm, minimum light intensity to terminate AF = 68 mW/cm^2 , power = 54.7 mW;

3.1.3. Conclusion

The 48x32 mini LED demonstrated the ability to be used in optogenetics for cardiac activity control experiments, including arrhythmia induction and arrhythmia termination. The light intensity, heat generation and driver fulfilled the requirements. The light intensity was beyond the requirement. Thus, more efficient adjustments on the pitch and other aspects can be made for future work on optogenetics

in cardiac control using the mini LED matrix.

3.2. Characterization of hiAM monolayers implemented by light-driven LCEs

In a typical procedure for controlling cardiomyocytes, such as human-induced pluripotent stem cell-derived atrial myocytes (hiAMs), based on the principles of optogenetics, the normal process involves providing light stimulation to genetically modified cells. This stimulation induces an electric response in the cell membrane, leading to depolarization and hyperpolarization, which triggers a mechanical response such as cardiac contraction and relaxation.

In this experiment, the first step was to verify whether the hiAM monolayer could be successfully cultured on the LCE film. The objective next was to investigate whether light stimulation could induce the deformation of an LCE (Liquid Crystal Elastomer) membrane, which would then cause the cells to first undergo contraction and relaxation, followed by generating electrical potential changes. This represents a shift in the typical sequence of events, where the mechanical response precedes the electrical response rather than vice versa.

3.2.1. Experiment setup

The LCE film was fabricated by mixing 4 monomers [6]: 10 %mol/mol RM257, 88 %mol/mol C6BP, 1 %mol/mol Disperse Red 1 Acrylate and 1 %mol/mol Irgacure 369 and melting at 55 °C. The whole fabrication process is shown in Figure 3.6. A tool was created by moulding 2 PVA rubbed glasses in opposite with a 10 μ m/20 μ m metal foil in between. The mixing liquid was poured in by capillarity. Then, it was cooled down to 45 °C and irradiated for 10 minutes. The final step was heating it up to 65 °C for 10 minutes for post-cure. The final LCE strip is demonstrated in Figure 3.6 as well. Then, they were cut into different sizes to fit in the wells.

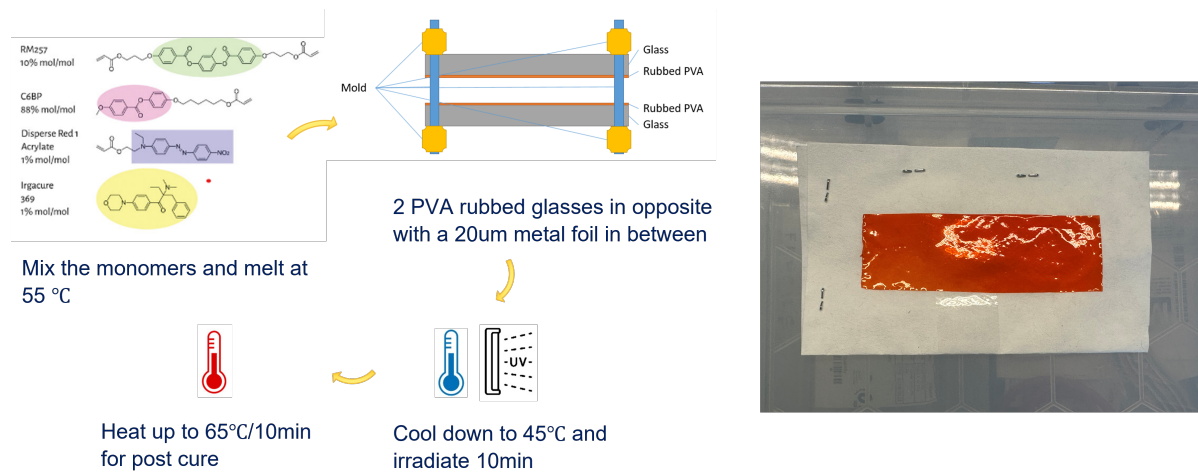


Figure 3.6: Overview of LCEs fabrication process(on the left) and the LCE strip(on the right)

In this experiment, human-immortalized atrial myocytes (hiAM) were used for research. The hiAM cells were cultured in custom wells, as shown in Figure 3.7. In the experiment, wells labelled 3 and 4 contain LCE with thicknesses of 10 μ m and 20 μ m, respectively, to observe whether the increase in LCE thickness results in a proportional increase in the degree of stretching and contraction. Sample 2 does not use a PDMS substrate; instead, the LCE directly adheres to a PMMA well using a two-component adhesive to culture cells on the LCE directly. Sample 1 serves as the control group, containing only a layer of PDMS, to eliminate any potential effects of PDMS under light exposure, ensuring that any observed phenomena are due to the LCE. The structure of the well consists of a 10 μ m/20 μ m thick LCE film sandwiched at the bottom of a specially designed clamp-shaped container with a diameter of 3 cm. Due to voids created by the insufficiency in LCE manufacturing, an additional 10 μ m thick PDMS layer of the same size was placed underneath to ensure the cells would not leak. Below this

setup, a heating plate was used to maintain the cells at a temperature of 37 °C. The mini LED matrix was positioned between the heating plate and the LCE clamp. An OVM camera was placed above the setup. The computer was connected to the OVM system, and the mini LED matrix was used for real-time control and data display.



Figure 3.7: The well used in the test

To create a hiAM monolayer for OVM, enough cells need to be seeded in the wells at good uniformity. Each well is suitable for 1.239×10^6 cells at a density of 395 cm^2 . The custom wells were pre-coated with fibronectin for reliable attachment of cells.

3.2.2. Measurements

The light-driven contraction of LCE at the environment temperature of 37 °C is shown in Figure 3.8. It was measured with the mini LED matrix and DMA Clamps. The mini LED matrix can be successfully used to drive the deformation of LCE films, but it is worth noting that it also results in a relatively high-temperature increase. LCE deformation can be stimulated at 37 hours. As the light intensity increases, the deformation brightness increases, but not linearly.

The monolayer was observed under the microscope during the proliferation period and before the experiment. The cells were well-seeded on both the PDMS membrane and the LCE membrane and were differentiated well into cardiomyocytes in a long stripe shape.

In this experiment, the traditional arrhythmia induction protocol was employed, which involves stimulating the cells with rapid and continuous electrical pulses, leveraging their refractory period and repolarization properties. Subsequent experiments were conducted using the designed miniLED matrix to apply different light patterns, including full illumination, half illumination, and line illumination. Observations were made using OVM; however, no significant arrhythmia termination was observed during the experiments.

3.2.3. Conclusion

Although this experiment did not achieve effective arrhythmia termination, improvements in various aspects could allow for further experimentation. First, the experiment was conducted using only one formulation of LCE, with a single fabrication process that resulted in the observation of pores. Future work could focus on adjusting and improving both the LCE fabrication process and its formulation. Second, the use of PDMS as a substrate beneath the LCE in the experiment may have counteracted

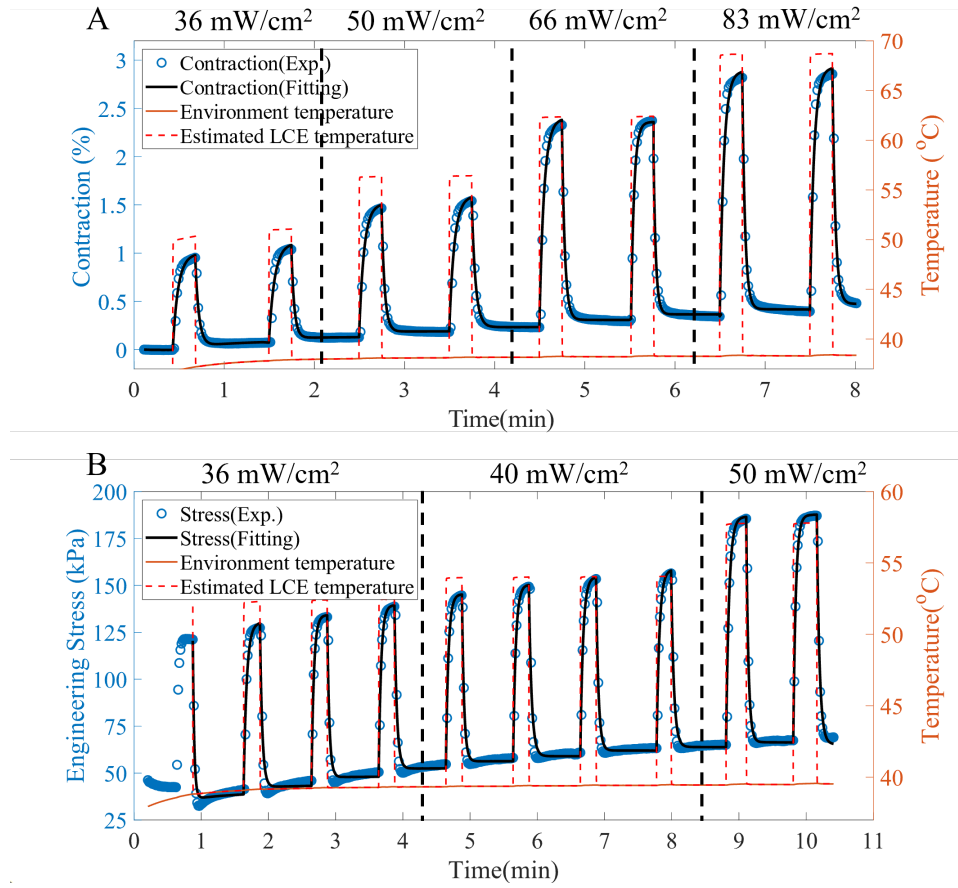


Figure 3.8: Light-driven contraction of LCE at the environment temperature of 210 °C (A) Stress-controlled deformation curve of LCE. (B) Stress out-put of LCE with both sides fixed.[47]

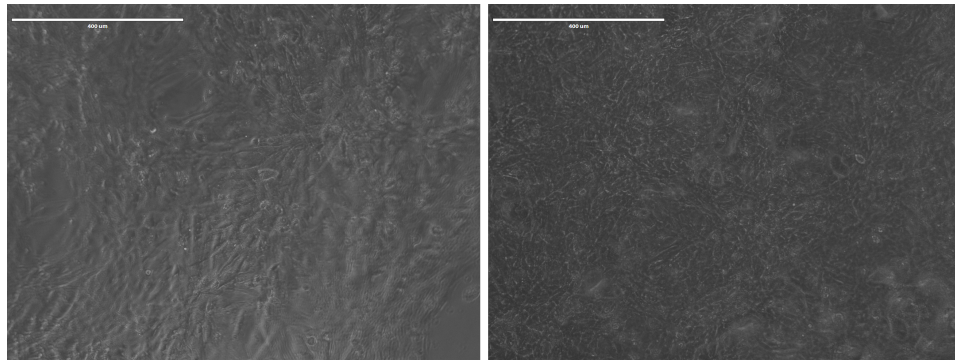
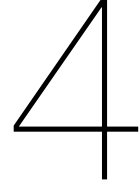


Figure 3.9: Microscope image of differentiated hiAM monolayer on PDMS and LCE

the intended contraction and relaxation of the LCE driven by the light source due to the toughness of the PDMS, rendering the LCE ineffective.



160x160 MiniLED Matrix System

4.1. Introduction

Based on the characterization of the small matrix discussed in Chapter 2 and its successful application in optogenetics for cardiac activity control, as detailed in Chapter 3, the small matrix can be further optimized for use in optogenetics experiments. According to the experimental results, the pitch of the matrix has been adjusted to 0.9 mm to achieve the best power efficiency. The 48x32 miniLED matrix is insufficient to cover the size of the human atrium. To further explore the application of miniLED matrices in the human body, this chapter discusses the design, fabrication, and characterization of a 160x160 miniLED matrix system with an area of 180 cm², capable of fully encompassing the size of the human atrium (as the range of the human atrium is typically from 55 to 143 cm² [48]). It can also fit the culture dish with a diameter of 13.5 cm, which is used in optogenetics experiments.

The new version of the mini LED matrix needs to be developed due to the goal of a larger matrix while still meeting the following requirements:

- Each LED was capable of emitting blue light with a peak wavelength of 470 nm, which is optimal for activating channelrhodopsin-2 (ChR2), a widely used optogenetic actuator [30].
- The input image update rate was required to be below 50 ms.
- The maximum temperature had to remain below 41 °C [46], with a temperature rise of no more than 4 °C under object temperature of 37 °C.
- The controller's scanning speed needed to exceed 200 Hz [39].

Besides, the matrix driver and the user interface on the PC should be changed accordingly.

4.2. Design and fabrication

4.2.1. MiniLED matrix

Similar to the 48x32 mini LED matrix discussed in Chapter 2, polyimide (PI), which is used for the small matrix with the white substrate, was used as the material for its good performance. The substrate thickness of 104 μm and interconnection traces of at least 180 μm wide were kept to prevent overheating. The LED matrix can reach a light intensity of more than 50 mW/cm² after pitch adjustment to 0.9 mm for optimal power efficiency, focusing on the optogenetics of the cardiac activity control test. The blue LED chip (BFB0F11C) was kept, emitting light around 470 nm, suitable for activating optogenetic actuators. The LEDs are bonded using flip-chip bonding, pick-and-place, and reflow soldering. However, the following points will be subject to change for the upscaled mini LED matrix shown in Figure 4.1.

Topology

It was crucial to maintain the same illumination uniformity as discussed in Chapter 2 for the design of the large-scale mini LED matrix. The target was to design a matrix with a new size of at least 13.5 cm

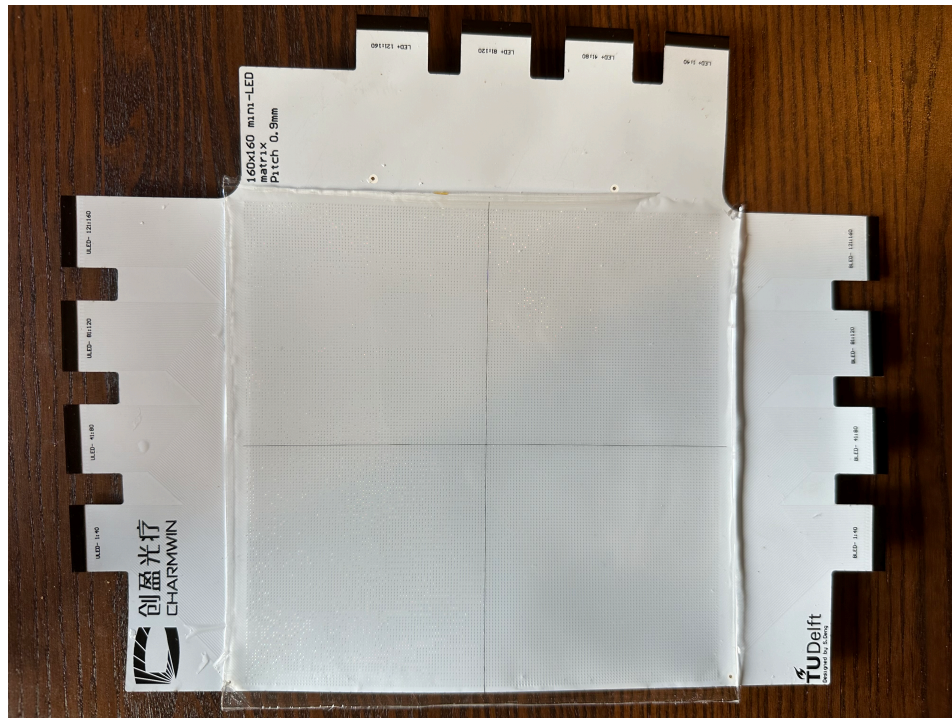


Figure 4.1: 160x160 mini LED matrix

while ensuring compatibility with the previous hardware design. As the pitch is 0.9 mm, a 160x160 mini LED matrix was chosen for implementation.

The increase in matrix size and the corresponding increase in the number of rows and columns resulted in a proportional rise in the refresh time. For the 48x32 matrix, as mentioned in Chapter 2, the refresh time was 1/333 Hz, equivalent to 3 ms. With the matrix's row count increasing fivefold, the refresh time increased to 15 ms. This extended refresh time would lead to more pronounced flickering if the original topology were to be used, which could negatively impact optogenetic experiments due to the sensitivity of these experiments to timing and flicker.

Given the smaller matrix's successful testing and performance, it was essential to maintain consistency in the design approach. Therefore, the final decision was to retain the row-column scanning topology. However, to mitigate the increased refresh time, the 160x160 matrix was divided into two sections, each functioning as a 160x80 mini LED matrix. This division effectively reduced the refresh time to 7.5 ms, within the acceptable range for optogenetic experiments and ensuring minimal flickering during operation.

Protection layer

Given the matrix's large size, the previously employed coating method was no longer applicable. Instead, a two-component epoxy resin was utilized to form the protective layer on the large mini LED matrix. The epoxy resin and hardener were mixed in a 1:1 ratio by slowly pouring both components into a container and stirring them in the same direction for over 10 minutes to ensure thorough mixing. Following this, the mixture was allowed to stand for at least 10 minutes to eliminate any air bubbles generated during the mixing process.

Before applying the resin, the surface of the mini LED matrix was meticulously cleaned using acetone and lint-free paper. Wells were then marked and placed 7.5 cm from the centre point on each side to contain the resin and prevent it from flowing out during application. The prepared epoxy resin was carefully poured onto the matrix surface. Given the resin's density of 1.1 g/cm², calculations based on weight difference and surface area were performed to achieve a protective layer that adequately covered the LEDs while maintaining a thickness of less than 1 mm.

If any air bubbles persisted after application, a heating gun set to 70 °C was used at a height of approx-

imately 10 cm 10 cm with low airflow to dissipate them. The resin's inherent flow properties and gravity created a uniform layer. This protective layer solidified after 24 hours while retaining the necessary flexibility for the application.

Connector

In the 160x160 mini LED matrix, the connection to the driver no longer utilized FFC cables. Due to the large size of the matrix, with 160 lines on both the top and bottom edges that required connection, using FFC would have made the structure overly complex. Instead, FPC was employed to streamline the approach. In this large matrix, the 160 lines on each edge were divided into four groups, with each group of 40 lines forming an individual FPC with a line pitch of 0.5 mm. Additionally, as mentioned in the topology, the matrix was segmented into a 160x80 configuration, resulting in 12 FPCs being used.

4.2.2. MiniLED matrix driver and graphic user interface

The driver and UI introduced in Chapter 2 can be directly utilized for this new matrix. The driver refresh rate and the matrix's row and column configuration were modified accordingly.

Given that the large matrix is approximately five times the size of the smaller matrix and utilizes a split control structure for the top and bottom zones, it is hard to place row and column control on a single PCB. Thus, the large matrix design incorporates four PCBs: two for controlling the rows and two for controlling the columns, as shown in Figure 4.2. The connection between the row and column controllers is achieved using FFC cables. The microcontroller is placed on the column control PCB. Each column control PCB has connectors on both the top and bottom sides to facilitate easy connection to the matrix.

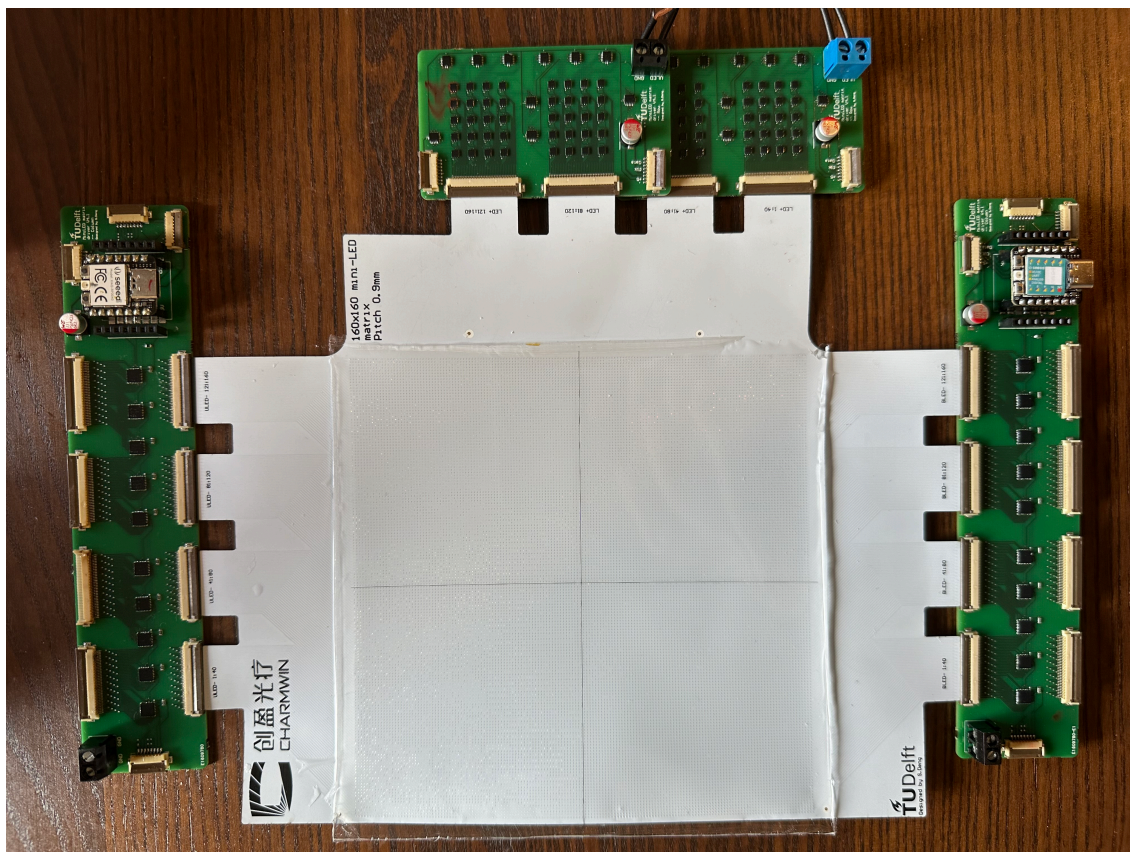


Figure 4.2: 160x160 mini LED matrix with driver PCB

Due to the use of a dual-zone control method, two microcontrollers are employed simultaneously. The time logic control within the microcontrollers remains unchanged, but the user interface has been modified for integration. Instead of selecting from a single port, the user now selects from two ports. The

primary functionality remains largely the same; however, due to the increased size of the matrix, a precise selection of individual LEDs or single rows/columns, as was possible with the 48x32 matrix, is no longer feasible. To address this, a direct input feature has been added to the user interface. Additionally, adjustments have been made to the functionality for lighting single columns and uploading images due to the mirrored PCBs in the top and bottom sections of the matrix. The new UI for the upscaled matrix is shown in Figure 4.3

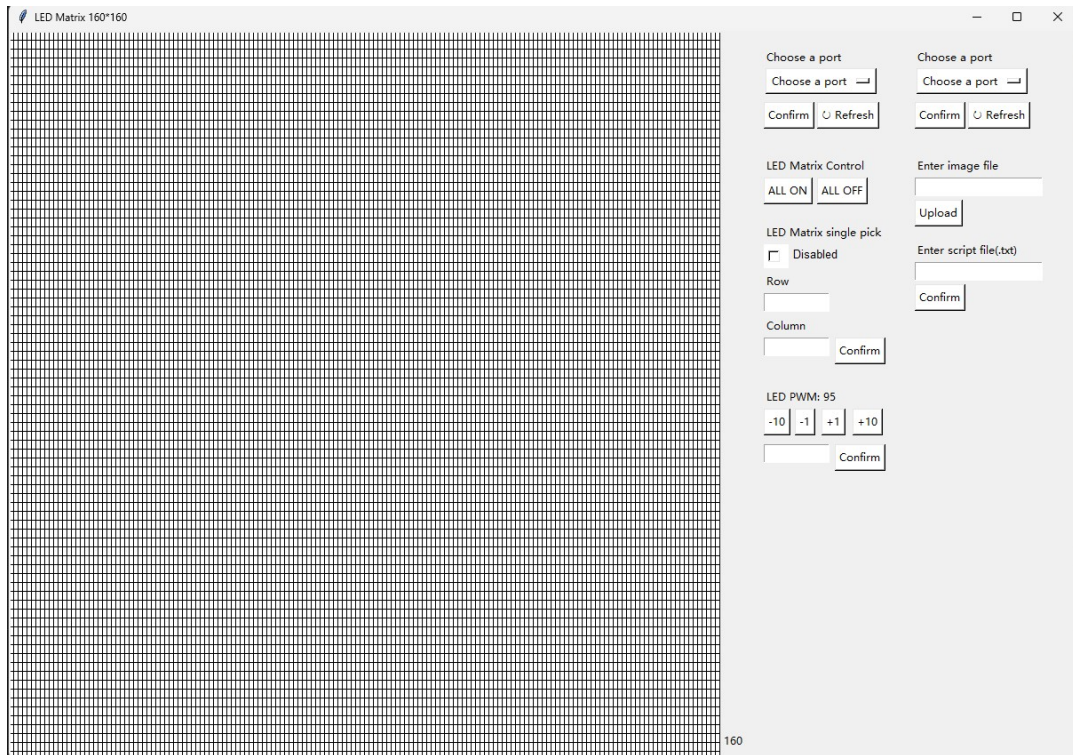


Figure 4.3: User interface for the 160x160 mini LED matrix

4.3. Characterization methods

4.3.1. Driver characterization

The same driver characterization was performed for the 160x160 mini LED matrix as in Chapter 2. The stability and required refresh rate can be verified by observing the timing signals.

Measurements

Figure 4.4 illustrates the detailed process of a CCS shift register data write cycle. The SPI communication speed is set to the adjustable maximum of 20 MHz to optimize the matrix's refresh rate.

As shown in Figure 4.5, to avoid row overlapping when writing new line data to the shift register, all mini LEDs were turned off by pulling up BL at first. Then, the RCLK generated a pulse to switch to the next row line. The LAT then gave a pulse to push new row data into CCS. Finally, BL was pulled down to turn on the mini LEDs.

Figure 4.6 illustrates the timing signal measured at initiating a new scan. After the falling edge of the GPIO0 signal, the RDAT line is pulled low until the first RCLK signal is transmitted, resulting in the PMOS controlling the first row being turned on. After each scan, a blank interval is maintained to provide a margin for the next scan. The refresh rate of the mini LED matrix was determined to be 250 Hz.

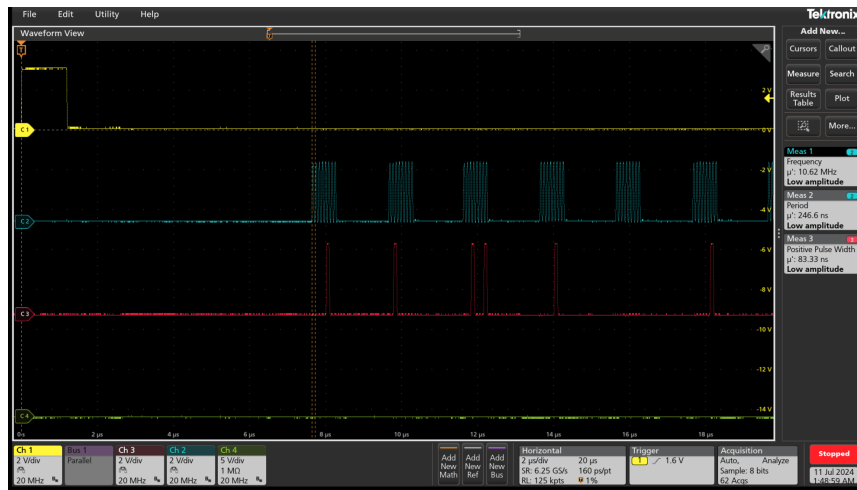


Figure 4.4: CCS shift register timing signal for specific row pattern.

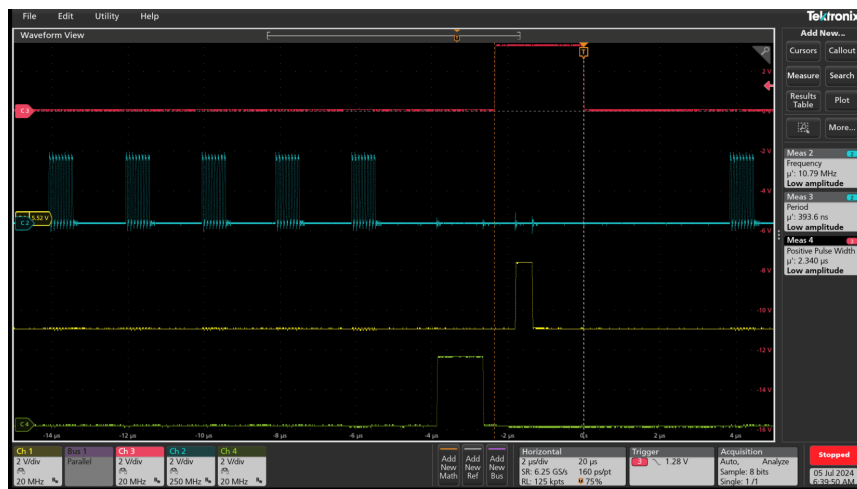


Figure 4.5: CCS latch Timing signal, BL (red), DCLK (blue), LAT (yellow) and RCLK (green)

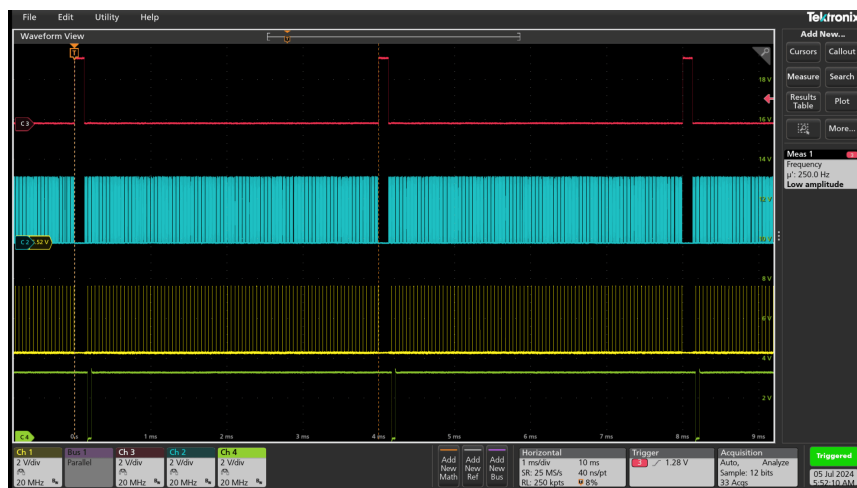


Figure 4.6: Scanning timing signal measured at GPIO0 (red), DCLK (blue), RCLK (yellow) and RDAT (green)

4.3.2. Optical characterization

To meet the stringent requirements for efficiently activating light-sensitive ion channels in cells, the 160x160 mini LED matrix needed to deliver a maximum light intensity of at least 50 mW/cm², capable

of continuous and linear intensity adjustment. This experiment assessed whether the 160x160 mini LED matrix met these critical specifications.

Experimental setup

The experiment setup and equipment used are the same as the characterization done in Chapter 2: Thorlabs S142C Integrating Sphere Photodiode Power Sensor and Thorlabs PM400 Optical Power Meter.

Measurements

To evaluate the light intensity distribution across the upscaled mini LED matrix, a power meter was positioned as close as possible to the matrix. The light intensity was controlled by varying the PWM value through the user interface and driver, ranging from 0 to 99. This adjustment allowed the mini LED matrix to transition from being completely off to reaching its maximum light output. The corresponding light intensity measurements across this range are illustrated in Figure 4.7.

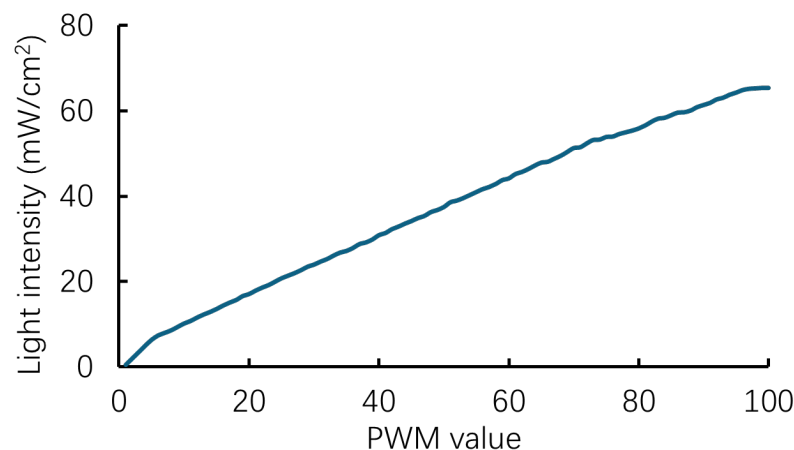


Figure 4.7: The light intensity of 160x160 mini LED matrix

The maximum light intensity given in Figure 4.7 is 65.4 mW/cm^2 and the light intensity decreased to below 50 mW/cm^2 when the PWM value is less than 32. The variation in light intensity, as depicted in the figure, can be considered linear. The results demonstrate that the matrix can deliver sufficient intensity for optogenetic activation. It is also evident that, under the same driver conditions, the maximum illumination of the large matrix is significantly lower than that of the small matrix. While this outcome was anticipated, it suggests that the power output may be substantially reduced.

4.3.3. Thermal characterization

A thorough thermal characterization was conducted to assess the matrix's thermal behaviour under different operational scenarios to guarantee the safe application of a mini LED matrix during extended optogenetic stimulation sessions. Effective thermal management is crucial to avoid overheating, which could damage biological tissues and potentially compromise the integrity of experimental outcomes.

To maintain cell viability and ensure the reliability of experimental procedures, the matrix's surface temperature must remain below 41°C , even when subjected to varying patterns or changes in intensity while operating at the physiological temperature of 37°C . Furthermore, the increase in temperature should be restricted to a maximum of 4°C . The objective was to assess the temperature distribution and identify potential hotspots that could affect the matrix's performance.

Experimental setup

The experiment utilized the following equipment and the experiment setup in Figure 4.8:

- A multi-channel stimulus (MCS Stimulus Generator STG4002 Series) to generate pulses for test
- A thermal meter (Extech 4-channel temperature meter SDL200) to ensure it provides accurate temperature and heat measurements in practical applications.



Figure 4.8: The experimental setup for thermal measurement of 160x160 mini LED matrix

- Thermocouples were strategically placed at various points on the matrix to monitor localized temperature changes continuously.
- A thermal camera (VarioCAM HD S/N:1266322) with a resolution of 1024x768 was utilized to capture high-resolution thermal images, visually representing the temperature distribution across the matrix.
- A heating plate was maintained at 37 °C to replicate physiological conditions.
- A thermal pad to improve heat transfer between the heating plate and the mini LED matrix, ensuring efficient thermal management by filling air gaps and reducing thermal resistance.

Measurement

A thermal calibration process was implemented using the Extech 4-channel temperature meter SDL200 and carefully positioned thermocouples to achieve precise thermal performance measurements of the mini LED matrix during operation. The mini LED matrix was placed on a heating plate set to 41 °C to simulate physiological conditions, keeping the temperature of the mini matrix around 37 °C. The thermal meter, calibrated with a certified reference standard, was used to monitor the surface temperature of the matrix as it stabilized at thermal equilibrium. Thermocouples were strategically located at the matrix's centre to detect localized temperature changes. The experimental setup is depicted in Figure 4.8. The ambient temperature in the laboratory was maintained at 23.5 °C throughout the process.

The temperatures were recorded as the adjustment file in Appendix C for reference by the infrared camera.

The thermal characterization was performed under two different pacing tests on the 160x160 mini LED matrix: 500 ms on, 500 ms off, for a total of 5 cycles, and 5 s on, 15 s off, for one cycle.

Under different pacing tests, the temperature changes were measured on the mini LED matrix with different configurations: an entire row lit, an entire row lit, a single point lit, and the whole matrix lit. These tests were conducted at various output intensities controlled by PWM values ranging from 0 to 99, with steps of 10. This experiment tested both scenarios of fully illuminating entire rows and columns. This approach was taken into account due to the significant increase in matrix size and the use of a multi-zone topology.

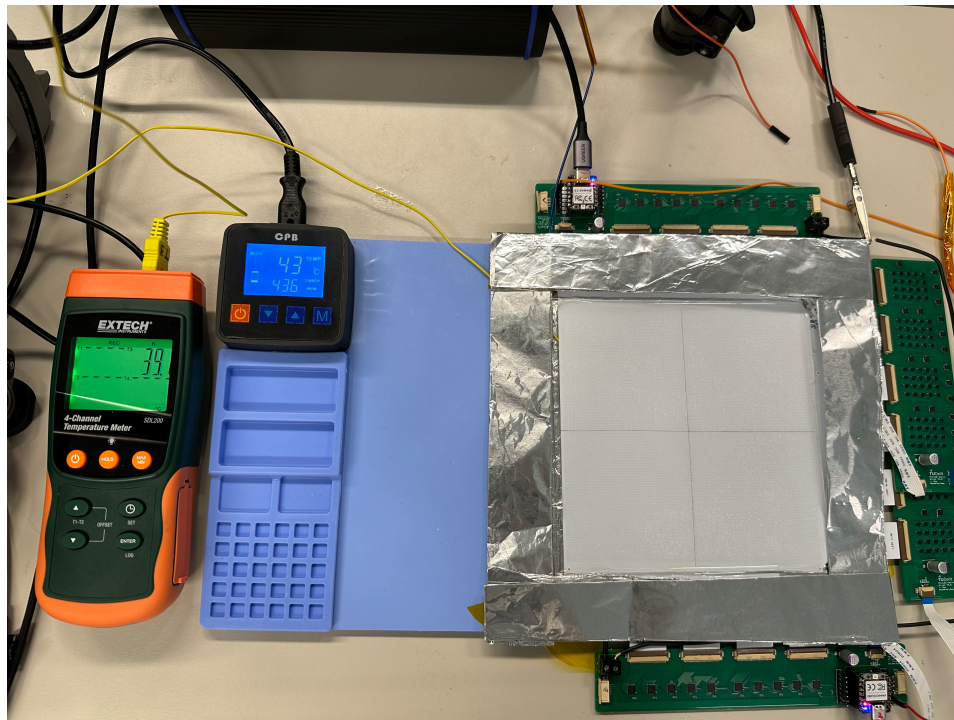


Figure 4.9: The Thermal calibration experimental setup for 160x160 mini LED matrix

Due to the use of epoxy resin coating, the heat dissipation on the matrix surface was slowed down. As a result, during the 1 Hz pacing experiment with five pulses, it was impossible to distinguish the temperature changes for each pulse as with the smaller matrix. Therefore, only a single value is reported without separately listing the temperature rise for each pulse.

160x160 mini LED matrix	Highest temperature(°C) under 1 Hz pacing test
Full on	38.06
Single row	35.56
Single column	35.78
Single dot	35.73

Table 4.1: The highest temperatures for 160x160 mini LED matrix as the matrix under 1 Hz, full-on, single-row on, single-column on and single-dot on

Under the 1Hz pacing with five pulses test, when the 160x160 mini LED matrix was full-on, Table 3.1 indicates the matrix's highest temperature is 38.06, less than the limit.

The maximum temperature rise and its trend are shown in Figure 4.10 when the mini matrix is full-on. The maximum temperature increase is observed to be 2 °C. The temperature increase trend can be considered linear.

Under the 1 Hz pacing test with 5 pulses, due to the heating plate's temperature maintenance error of ± 0.2 °C, it can be assumed that there was no significant temperature fluctuation on the surface in the cases of single row lit, single column lit, and single dot lit. This outcome was anticipated due to the reduced power density.

Under the 5 s on and 15 s off, when the 160x160 mini LED matrix was full-on, Table 3.2 indicates the matrix's highest temperature was 44.4 °C.

Figure 4.11(a) shows the maximum temperature increase and its trend of full-on under 5s on and 15s off. The maximum temperature increase is observed to be above 7 °C. The temperature increase trend can be considered linear. Figure 4.11(b) shows the temperature increase and its trend of single-column on under 5 s on and 15 s off. The maximum temperature increase is observed to be around 0.6 °C. The

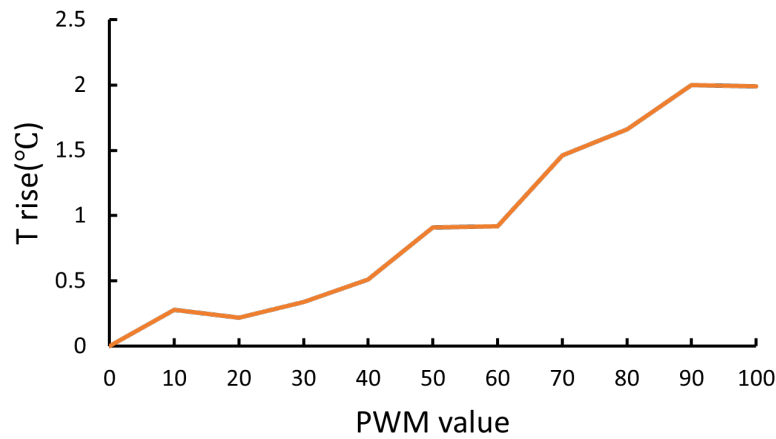


Figure 4.10: The temperature rise for 160x160 mini LED matrix on full-on under 1hz pacing, 5 pulses

160x160 mini LED matrix	Highest temperature(°C) under 5 s on and 15 s off
Full on	44.40
Single row	35.77
Single column	35.49
Single dot	35.73

Table 4.2: The highest temperatures for 160x160 mini LED matrix as the matrix under full-on, single-row on, single-column on and single-dot on under 5 s on and 15 s off

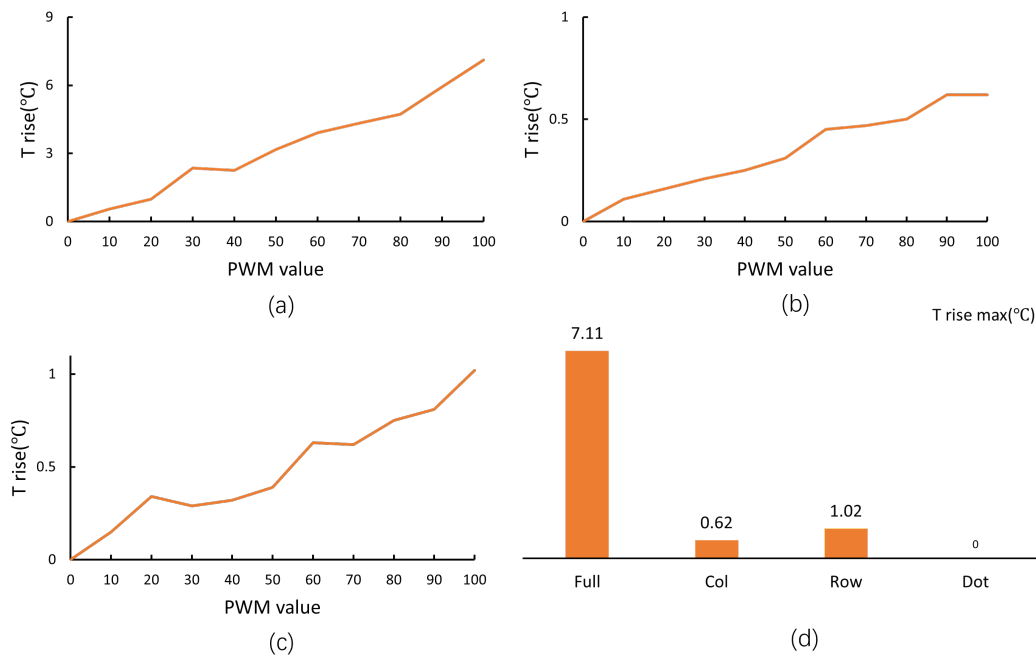


Figure 4.11: The temperature rise for 160x160 mini LED matrix under 5s on and 15s off on (a)full-on (b)single-column on, (c)single-row on

temperature increase trend can be considered linear. Figure 4.11(c) shows the temperature increase and its trend of single-row on under 5 s on and 15 s off. The maximum temperature increase is slightly above 1 °C. The temperature increase trend can be considered linear. No temperature increase was observed when using the infrared camera (VarioCAM HD S/N:1266322) with a resolution of 1024x768

as a single pixel dimming.

The temperature increase in the 1 Hz pacing test with five pulses was always less than 4 °C. In the 5 s on and 15 s off tests, when all lights were on, and the PWM value exceeded 63, the temperature increase surpassed 4 °C. Compared with the small matrix analyzed in Chapter 2, the large matrix performs well on thermal concentration.

4.4. Conclusion

In this chapter, we discuss and develop a human-size mini LED matrix system that focuses on optogenetics research and cardiac activity control experiments. From designing and implementing the matrix, driver, and user interface to the driver timing and optical and thermal characterization, we have examined and validated this 160x160 mini LED matrix system's usability, stability, and safety in practical optogenetic experiments. This human-sized matrix has laid the groundwork for future applications in human studies. Moving forward, exploring ways to enhance the efficiency, convenience, and safety of its use in practical applications will be essential. In the actual experiment, considering that the large matrix has a side length of 14 cm, which far exceeds the diameter of the currently used OVM, a new experimental method for observing cells should be proposed. The light uniformity still needs to be experimentally verified for this large matrix.

Conclusions and Future Work

5.1. Summary of research

This thesis has presented the design, fabrication, and characterization of mini LED matrix systems with potential applications in biomedical research, particularly optogenetics. The research was driven by the need to develop customizable illumination tools capable of precise light delivery with thermal control.

The research began with the development of a 48x32 mini LED matrix system, which demonstrated the ability to modulate light patterns in real-time with sufficient intensity and stability for biological applications. The system's performance was validated through driver timing and optical and thermal characterization, ensuring its suitability for safe use in experiments. This system was then applied in an in-vivo optogenetic experiment targeting cardiac activity control, specifically arrhythmia induction and termination, confirming its effectiveness further. The matrix was also implemented in LCE manipulation. Although the results obtained so far have not been ideal, this research has laid a solid foundation for future improvements and experiments. These efforts and findings provide important references and directions for future studies, allowing subsequent experiments to optimize better and address existing challenges, thereby gradually achieving the research objectives.

Building on the success of the optogenetics experiment, the research was extended to design and develop a larger human-size 160x160 mini LED matrix system. This system was intended to cover an area of at least 13.5 cm^2 , suitable for more extensive biological tissues, such as the human atrium, and to support more complex experiments. The design and fabrication processes were carefully considered to maintain the optical and thermal performance achieved with the smaller matrix while scaling up the size. Characterization of this larger system confirmed its ability to meet the stringent requirements necessary for optogenetic applications, including maintaining safe operating temperatures and delivering consistent light intensity.

The work presented in this thesis represents a significant step forward in the development of optogenetic tools, demonstrating the feasibility and potential of mini LED matrices for precise control and manipulation of biological processes through light.

5.2. Future work

While the research has successfully developed and validated two mini LED matrix systems, several areas of improvement and further exploration have been identified, which can be pursued in future work.

Improvement of the Mini Matrices

The current systems have shown promising results, but there is room for optimizing light uniformity across the entire matrix, especially in larger matrices like the 160x160 version. Future work could focus on enhancing the optical design, potentially through advanced simulations or incorporating additional optical elements to improve light distribution. Furthermore, more precise control over light intensity, possibly on a per-LED basis, could be beneficial for certain applications. The coating method used as

a protective layer on the large matrix can be further investigated, as the current resin used has shown suboptimal thermal dissipation performance in thermal testing.

Integration with Biological Experiments

While the mini LED matrix systems have been successfully applied in cardiac optogenetic experiments, their integration with other types of biological studies could be explored. For example, investigating their use in neural studies, where precise spatial and temporal light control is critical, could open new avenues for research. Additionally, exploring their potential in studies involving other light-sensitive proteins or in applications such as tissue engineering could provide valuable insights.

Development of User Interfaces and Software

The user interfaces developed for controlling the mini LED matrices have been effective but could be further refined. Future developments could focus on making the interfaces more intuitive and accessible, potentially incorporating more advanced features such as automated pattern generation, real-time feedback, or integration with other experimental systems while also considering AI. Enhancing the software to support more complex experiments, such as multi-wavelength stimulation or synchronized control with other devices, could significantly broaden the systems' applicability.

Miniaturization and Portability

The current mini LED matrix systems are relatively compact, but further miniaturization could make them more suitable for portable or implantable devices. Research into reducing the size and power consumption of control electronics and exploring alternative materials for the LED matrix could lead to the development of even more versatile optogenetic tools.

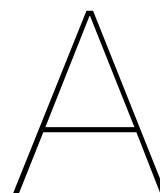
In Patterned LCE Experiment

Future work could focus on improving the LCE material properties, such as increasing the responsiveness to light, enhancing mechanical durability, and optimizing the phase transition characteristics. Additionally, experimenting with different doping agents, composite materials, or new fabrication methods could lead to better performance and new functionalities.

In Vivo and Clinical Applications

As the technology matures, the ultimate goal would be to transition from in vitro experiments to in vivo studies and, eventually, clinical applications. Future work could explore the biocompatibility of the materials used in the mini LED matrices and their performance in more complex biological environments. Additionally, the development of systems tailored for specific clinical applications, such as targeted therapies or diagnostic tools, could represent a significant advancement in the field.

In conclusion, this thesis has laid the groundwork for the future development of mini LED matrix systems for optogenetic and other biomedical applications. The continued refinement and expansion of this technology hold great promise for advancing our ability to control and study biological processes with unprecedented precision.



Thermal calibration file yellow substrate

[Ident] Ident=edf

[Values] eps1=0.82 TempK1=313.15

eps2=0.77 TempK2=323.15

eps3=0.74 TempK3=333.15

eps4=0.725 TempK4=343.15

eps5=0.71 TempK5=353.15

eps6=0.70 TempK6=363.15

eps7=0.69 TempK7=373.15

B

Thermal calibration file white substrate

[Ident] Ident=edf

[Values]

eps1=0.85 TempK1=313.15

eps2=0.80 TempK2=323.15

eps3=0.80 TempK3=333.15

eps4=0.79 TempK4=343.15

eps5=0.79 TempK5=353.15

eps6=0.79 TempK6=363.15

eps7=0.79 TempK7=373.15

C

Thermal calibration file 160x160 matrix

[Ident] Ident=edf

[Values]

eps1=0.93 TempK1=310.15

eps2=0.86 TempK2=312.45

eps3=0.78 TempK3=317.15

eps4=0.80 TempK4=321.15

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