Development of Optics with Micro-LED Arrays for Improved Opto-electronic Neural Stimulation

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Development of Optics with Micro-LED Arrays for Improved Opto-electronic Neural Stimulation

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ABSTRACT

The breakthrough discovery of a nanoscale optically gated ion channel protein, Channelrhodopsin 2 (ChR2), and its combination with a genetically expressed ion pump, Halorhodopsin, allowed the direct stimulation and inhibition of individual action potentials with light alone. This work reports developments of ultra-bright electronically controlled optical array sources with enhanced light gated ion channels and pumps for use in systems to further our understanding of both brain and visual function. This work is undertaken as part of the European project, OptoNeuro.

Micro-LED arrays permit spatio-temporal control of neuron stimulation on sub-millisecond timescales. However they are disadvantaged by their broad spatial light emission distribution and low fill factor. We present the design and implementation of a projection and micro-optics system for use with a micro-LED array consisting of a 16x16 matrix of 25 μm diameter micro-LEDs with 150 μm centre-to-centre spacing and an emission spectrum centred at 470 nm overlapping the peak sensitivity of ChR2 and its testing on biological samples. The projection system images the micro-LED array onto micro-optics to improve the fill-factor from $\sim$2% to more than 78% by capturing a larger fraction of the LED emission and directing it correctly to the sample plane. This approach allows low fill factor arrays to be used effectively, which in turn has benefits in terms of thermal management and electrical drive from CMOS backplane electronics. The entire projection system is integrated into a microscope prototype to provide stimulation spots at the same size as the neuron cell body ($\sim$10 μm).

Keywords: Optogenetic, micro-LED array, micro-optics, micro-lens array, ganglion cells, electrophysiology

1. INTRODUCTION

The Optoneuro project is part of a large multidisciplinary European project to develop opto-electronic neural stimulation technology. This is part of a new discipline called Optogenetics, which groups fields as diverse as optics, genetics and virology. It started with the discovery of an ion-gated protein, Channelrhodopsin 2 (ChR2), which can be activated by blue light. Combined with a light activated pump, like Halorhodopsin (NpHR), which can be activated by orange light, it allows to instantaneously activate and silence specific groups of neurons in cells of living tissues with a precision that cannot be achieved by other methods.[1] In this particular project, neurons linked to the photoreceptors in the eyes, called ganglion cells, are targeted.

The promising technology chosen for delivering the light to the cells of interest is Gallium-Nitride micro-LED arrays, which emit in the blue. They permit spatio-temporal control of neuron stimulation on sub-millisecond timescales. Our role is to develop and implement an optical system through modeling and experiments to deliver the light efficiently through an electrophysiological microscope platform from our partner, Scientifica[2] to illuminate a sample and stimulate the ganglion cells transfected with ChR2. It then activates transient electrical signals, which form the basis of neuronal communication.[3] Electrophysiology is therefore used to monitor these changes.

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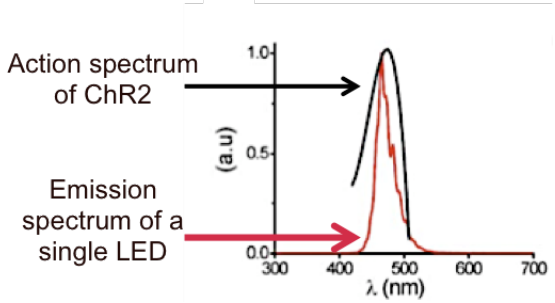

Figure 1. Micro-LED array emission spectrum and absorption spectrum of the light gated ion channel Channelrhodopsin 2 (ChR2)

2. METHODS

2.1 Light source

A micro-LED array (MLA), developed by TYNDALL, with 16 by 16 emitters, 150μm pitch and typically about 25μm diameter. Spatio-temporal control was provided by flip-chip bonding the MLA device to a CMOS back-plane, developed at Newcastle. Its emission spectrum is centered at 470 nm matching the action spectrum of the Channelrhodopsin 2 (ChR2) (see Figure 1). Several forms of MLA device were used including simple planar (Lambertian) emitters and others with etched parabolic back reflectors in order to improve the forward projection of light by reducing the divergence.[4]

In this project, the MLA was coupled to a conventional microscope to stimulate neurons transfected with ChR2. However, when connected directly in a conjugate image plane to the sample, a lot of light is lost at the tube lens and pupil of the microscope objective (see Figure 2). Another limitation of this simple direct projection arrangement is the low fill-factor of the illumination on the sample plane due to the construction of the MLA.

2.2 Micro-optics principle

An approach to addressing fill factor issues is to use micro-lenses, and these are used regularly on CCD and CMOS detector arrays for instance for precisely this purpose. In particular this method permits the trade off of angular acceptance/emission for spatial extent in order to use the lost emission at high angles to fill the spaces between individual emitters. Here the MLA is positioned in the focal plane of the micro-lenses and aligned with it in order to fill each micro-lens completely with light. The micro-lenses are then imaged onto the sample, therefore increasing the apparent fill-factor from 2.2% with only the MLA to near 100% for gapless square micro-lenses. The higher NA of the micro-lens array, compared to the tube lens of the microscope, allows for more light to be collected from each LED by the optical system, thus increasing its optical efficiency. The combination of these two improvements achieves the same brightness at the sample as the direct projection method but does so with

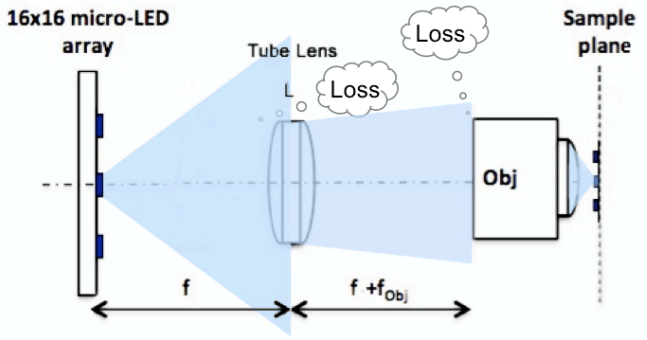

Figure 2. Schematic of a micro-LED array coupled to a conventional microscope

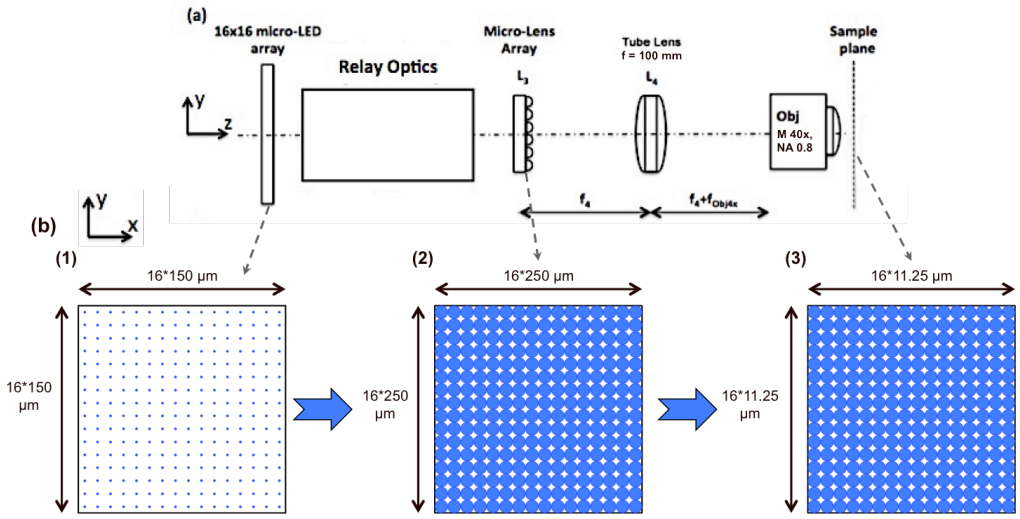

Figure 3. Projection optics principle: (a) Schematic of the illumination optics with the projection system and the microscope set-up with a modified tube lens (f = 100 mm), and a 40x and 0.8 NA microscope objective, (b) Illumination visible from different point in the optical set-up: (1) Micro-LED array (Object), (2) Image of the array just after the micro-lens array and (3) Image of the array onto the sample

a high fill factor using small emitters with big gaps, which in turn need less drive current, produce less heat, and by deduction cause less constraints on the CMOS backplane.

2.3 Projection optics principle

It was shown, in a previous study,[5] that the stimulation of a neuron is stronger when the main body of the neuron cell, the soma, is stimulated and optimal when its dendrites are as well. It was decided that the illumination spot should be roughly the same size as the soma, 10μm. We start from a fixed MLA size with a pitch of 150μm and aim to project an array of approximately 10μm pitch onto the sample plane of the standard Scientifica slicescope system that itself is based on Olympus 40x (NA=0.8) using a custom 100mm focal length tube lens (in place of the normal 180mm olympus tube lens). For reasons of ease of alignment we actually choose to use a relay optical system to project an $\times$1.667 magnified image of the MLA into the focal plane of a 250μm pitch square micro-lens array. This arrangement gives an 11.25μm pitch illumination pattern on the sample (see Figure 3).

The relay optics were designed and modelled using optical design software, Zemax,[6] and then tested. The quality of the optical set-up depends on the amount of the geometrical optical aberrations[7] introduced while maintaining a high numerical aperture. The goal is to obtain an image of the micro-lens array as homogeneous as possible on the sample, which means a similar intensity through the entire area of the illumination, and identical images of each of the micro-lenses at the centre or at the edge of the array. Field dependent aberrations such as field curvature, astigmatism, coma and distortion must therefore be minimised. Also in order to achieve uniform illumination within each micro-lens, spherical aberrations should be minimised. The micro-lens array itself adds nearly no aberrations because of the small size of each of the micro-lenses, and the already optimised microscope optics ensures that this is imaged well onto the sample plane.

2.4 Reading the outcome of an opto-electronic stimulation

Electrophysiology is used to monitor changes in membrane potential. It involves placing electrodes into various preparations of biological tissue. The approach, used here, is the patch-clamp technique, which allows the recording of the activity of an ion channel present in a patch of membrane, the cell- attached mode, or of the average response of all the ion channels in the entire cell membrane, the Whole-cell mode.[8] In the cell-attached mode, a micro-pipette, containing the electrode, is placed next to the neuron membrane. A gentle suction is applied through the micro-pipette to draw a piece of the membrane into the electrode tip. This forms a high

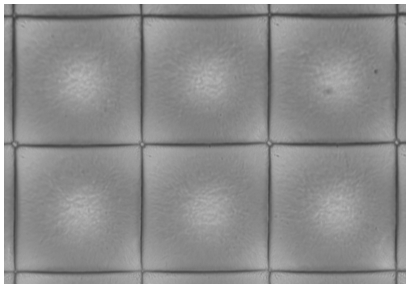

Figure 4. Photography of the micro-lens array from AOA with a microscope under white light

resistance seal with the cell membrane, allowing low current to be measured. In whole-cell mode, more suction is applied and the electrode is sealed to the rest of the cell). Fluorescence-based biosensors with a compatible excitation profile can be used to measure different cellular readouts. ChR2 protein can also be coupled to a fluorescent protein to decipher the functional connections in C.elegans neural circuits.[9]

3. RESULTS AND DISCUSSION

3.1 Projection optics development

The system was constructed based around a 250μm pitch micro-lens array with a numerical aperture (NA) of 0.125 manufactured by Adaptive Optics Associate (AOA)[10] (see Figure 4). This micro-lens array was made by replication and had a smooth profile. The micro-lens array is fabricated with a glass plate as the substrate, a master negative mold of the micro-lens array and a UV curable lacquer, which is dispensed between the two before being cured by UV light.[11] Nearly gapless parabolic shaped micro-lenses could therefore be obtained. For the design 25μm diameter Lambertian emitter micro-LEDs were assumed.

A relay optics (RA) was designed to fit the parameters of, a magnification of 1.667 and an NA of 0.125, on the image side, similar to the micro-lens array in order to fill each micro-lens with light to its edge. This means a NA at the entrance of at least 0.21 (1.667×0.125). The initial design is based on a pair of stock achromats (60mm and 100mm focal length) to give the required magnification and a pair of identical meniscus lenses in aplanatic conditions on each side of two stock achromatic doublets to increase the NA. The field dependent aberrations were reduced by optimising the positions of these elements to obtain the best compromise (see Figure 5).

Modelling and experiments gave similar results with the intensity at the center more than twice the intensity at the edge of the array (see Figure 6). More than 12 times more light than without any projection optics was measured as well as a fill-factor close to 100%.

3.2 MLA development and testing

Several type of micro-LED arrays were developed by our partner, Tyndall. The first approach had been to modify the shape of the reflective anode to decrease the angle of view of the emission from 60° for a Lambertian

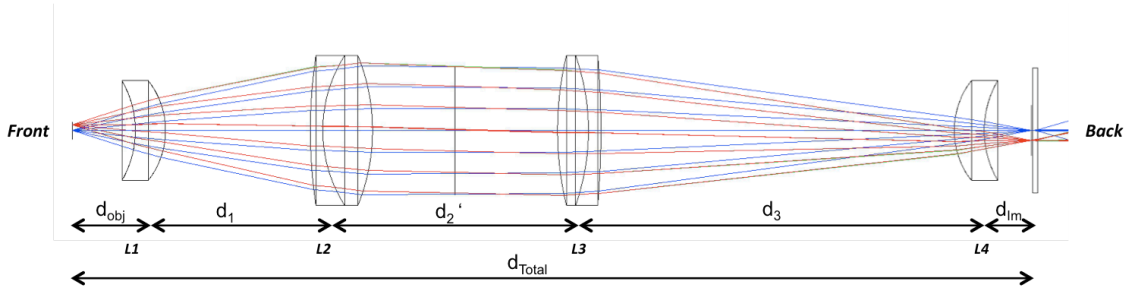

Figure 5. Optimised relay optics and micro-optics from the MLA (front) to the micro-lens array (back)

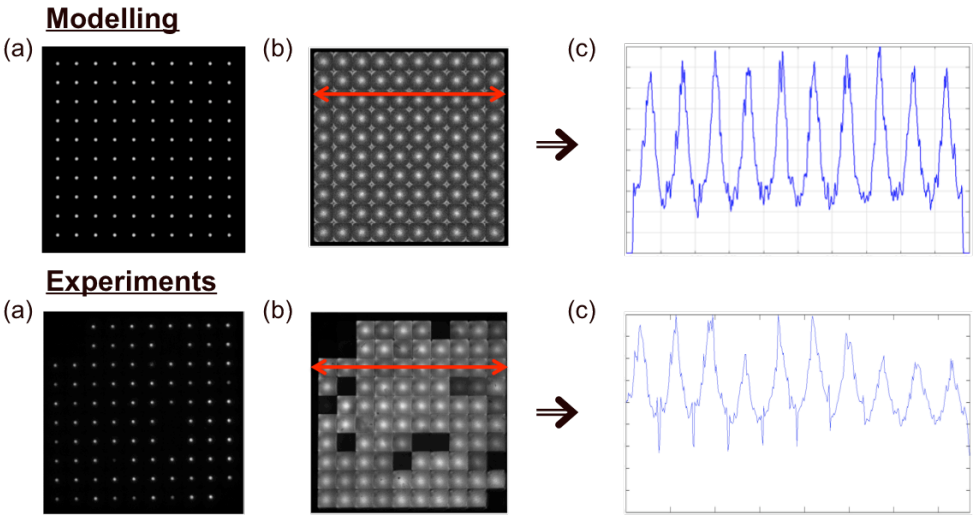

Figure 6. Modeling and Imaging without projection optics (a) (Image at the sample plane) and with it, (b) (Image at the sample plane) and (c) (Cross-section relative intensity measured at the red arrow)

emitter to below 30° and keeping the same emitter size. This approach allows for at least four times more light to be collected by the optics. The second approach was to increase the size of the Lambertian emitter from 25 μm to 90 μm to increase the fill-factor of the emitting area. The third approach was a cluster of slightly smaller emitters driven like one emitter, using the same shaped reflective anode as for the first approach and with therefore the same angle of view (see Figure 7).

In theory, without projection optics, the size of the emitter should not have a big impact on the quantity of light collected and illuminating the sample apart from the effect on the fill-factor. However, as soon as the projection optics are used, any increase in the size of the emitter beyond a certain diameter has no benefit because the micro-LED emitters are imaged at the entrance pupil of the microscope objective, which has a limited aperture. It means that above a certain diameter, part of the light won't go through. For a 40x microscope objective with an NA of 0.8, the maximum size of emitter that can be used before clipping is seen at the objective aperture is 43 μm (see Figure 8). The first approach seems to be the best when combined with the benefit of the projection optics.

The system was then integrated into an olympus head, which was used in a modified upright microscope platform, "Slicescope", developed by our partner, Scientifica. The quality of the illumination was then observed using a fluorescence slide and sample (see Figure 9) using parabolic reflector LEDs.

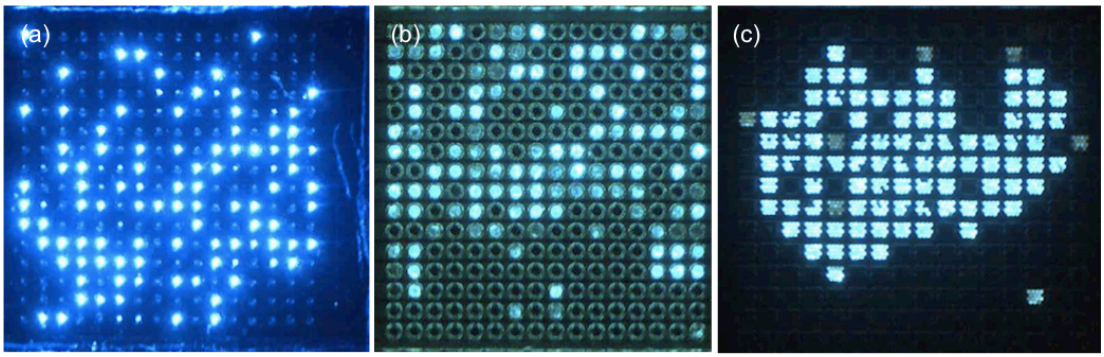

Figure 7. MLAs with (a) 25m emitters and shaped reector, (b) Larger Lambertian emitters and(c) a cluster of micro-emitters with shaped reflector on the same pitch

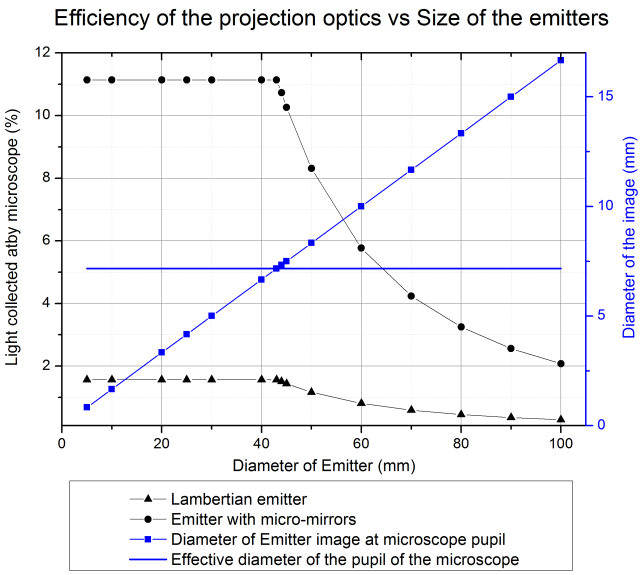

Figure 8. Diagram of the evolution of the efficiency of the projection optics with the size of the emitters in the MLA for emitters without and with shaped reflective anode (micro-mirrors) and variation of the effective diameter of the image at the pupil of the microscope objective

3.3 Opto-electronic neural stimulation

The optical set-up was tested on the experimental electro-physiological microscope platform of our partner from Newcastle University. A micro-LED array with micro-mirrors within each micro-emitter and therefore a more directional light emission was used. The collection efficiency was increased from ∼0.1% to more than 10% with a power per unit area only decreased by a factor of ∼4 on the entire area compared to the power per unit area of the area illuminated before without any projection optics and therefore with a low fill-factor. Fluorescence imaging was tested and works effectively (see Figure 10).

4. CONCLUSIONS

In conclusion, we demonstrated the advantages of the use of micro-optics with a micro-LED array by increasing the fill-factor of the illumination on the sample from ∼2% to more than 80% and at the same time improving the collection efficiency from ∼0.1% to more than 10% in the last experiment with a micro-LED array with a

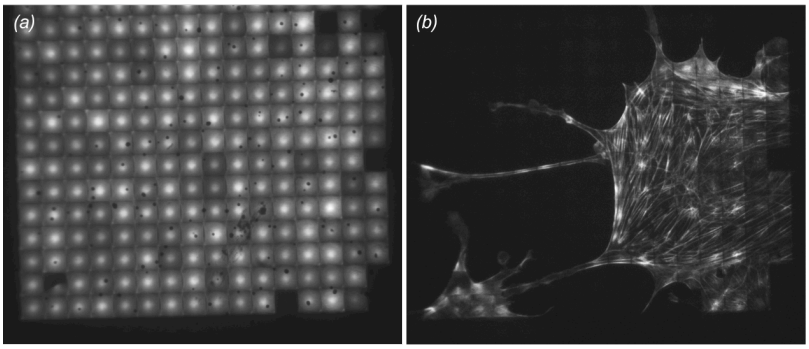

Figure 9. Fluorescence image of (a) an illuminated uniform fluorescent sheet with a microscope objective 20x NA 0.4, LED pixel size, 22.5 μm at sample and 360 μ field of view, and (b) a biological sample (stained cells) with a microscope objective 40x NA 0.75, LED pixel size 11.25 μm at sample and 180 μm field of view)

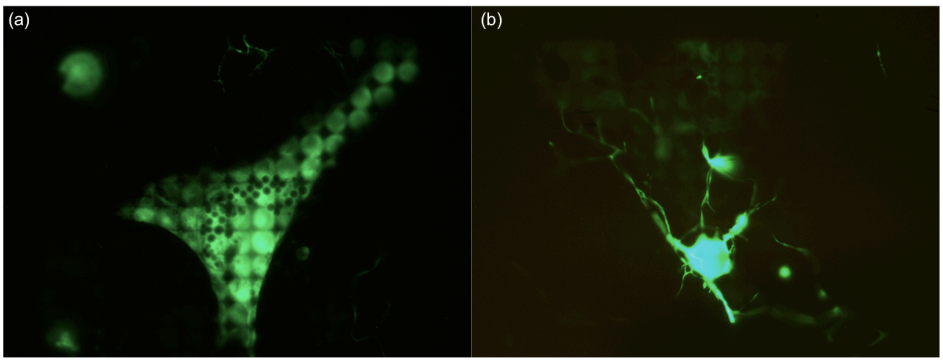

Figure 10. GFP stimulation and imaging with a 60x NA1.0 microscope objective and a blue micro-LED array: (a) Astrocyte and (b) Neuron

single 25μm diameter emitter and a micro-mirror back electrode allowing a more directional light emission. This is allowing the use of smaller emitters with bigger gaps, which need less current to be driven, produce less heat, and by deduction cause less constraints on the CMOS backplane. This European project is multidisciplinary, allowing us to work alongside companies and laboratories to develop and implement the projection optics into electrophysiological microscope platforms following their specific requirements. Testing will be done soon to verify its advantages for neuronal stimulation. In the near future, we expect to improve the projections optics to obtain a more homogeneous illumination and we are planning to integrate the micro-optics directly onto the micro-LED array. Ultimately, our aim is to demonstrate the applicability of that system to retinal stimulation and assess its feasibility for use in visual prostheses.

ACKNOWLEDGMENTS

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