A Place in the Sun:
Creating bespoke spectral recipes using a simple LED Array

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Team

Susan Duncan – *Post Doc, Hall Group, Earlham Institute, Norwich.*

Susan will manage the project and provide circadian imaging expertise.

Hannah Rees – *PhD Student, Hall Group, Earlham Institute, Norwich.*
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Hannah will work with Susan to set up the prototype systems at EI. She has experience of micro-manager coding and luciferase imaging.

Mark Greenwood – *PhD Student, Locke Group, Sainsbury Laboratory Cambridge.*
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Mark will help Susan and Hannah with scripting. He has programming and luciferase imaging expertise.

James Locke – *PI, Sainsbury Laboratory, Cambridge.* James.Locke@slcu.ac.uk

James will provide programming expertise and supervisory support.

Anthony Hall – *PI, Earlham Institute, Norwich.* Anthony.Hall@earlham.ac.uk

Anthony will provide hardware/coding/imaging expertise and supervisory support.

Summary
Experimental protocols for growth of plants typically use square-wave white light regimes which do not reflect the natural diurnal light patterns. Our aim is to build an LED lighting array system and write software that can enable researchers to explore a range of physiological responses under more natural dawn and dusk lighting transitions. We plan to use this equipment to compare the phase and amplitude of clock gene expression (via expression luciferase image analysis) of plants grown under our simulated natural lighting conditions and a standard square wave light regime.

Proposal
Understanding how plants integrate and respond to environmental cues is a central goal in biology. Considerable progress has been made toward this aim through experiments carried out in plant growth cabinets with classic square wave white light (on/off) and constant temperature settings. Discussions about plant wavelength absorption often mention classic *in vitro* experiments that show very little absorbance of green 500-600nm light by chlorophyll. However, fewer people are aware that significantly more green-light absorption has been reported for intact leaves (Moss and Loomis, 1952). In addition, *all* wavelengths between 400-700nm have been shown to drive photosynthesis for a wide range of plant species in a field experiments (McCree 1972). As LED prices have fallen, researchers have become interested in creating increasingly complex spectral conditions and exploring the impact of more natural light fluctuations. Recently exposure to natural dynamic light cues has been shown to produce thinner leaves, reduced light absorption and reduced photosynthetic capacity in Arabidopsis (Vialet-Chabrand et al., 2017). It is unclear how many other fundamental phenotypes are being skewed by experimental conditions that incorporate unnatural square wave lighting regimes.

In plants, the circadian clock is vital for measuring day length and aligning development with the changing seasons. Circadian gene regulation has been associated with many agronomic traits including flowering time, dormancy, water use efficiency, nitrogen metabolism and vegetative yield. Recent work also points to correlations between stress and plant/pathogen interactions and the clock. Mutant screens and studies over the last twenty years have revealed that the plant circadian clock is regulated by a small set of genes that act as a core oscillator. They are embedded within a complex gene network that constitutes a series of feedback loops. But our understanding of circadian gene regulation, as for most areas of plant biology, is based on data generated from plants grown with binary white light settings - on or off. We propose the development of a low-cost open source LED lighting rig and bespoke code that will allow us to generate highly-tunable sinusoidal spectral recipes to simulate dawn and dusk in a growth cabinet. Through the analysis of luciferase reporter lines, we will be able for the first time to investigate circadian gene induction under more natural light transitions.

Biological systems
In the first instance, we will determine circadian clock parameters (i.e. period, phase and amplitude) under our sinusoidal mixed spectral lighting regime using an existing Arabidopsis Col-0 lines carrying a luciferase construct driven by the CAB promoter. Imaging will be carried out using existing equipment at EI.

Hardware design goals
1. Couple a Raspberry PI to an LED array and write code to allow the array to be switched on and off via micro-manager.
2. Develop code to vary colour across the RGB LED array temporally
3. Include ability to also alter light intensity temporally

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4. Optimize acquisition loops to perfect image capture with an existing luciferase camera

Project implementation
1. Set up a commercially produced LED array: 3x 8x8 Unicorn HAT (Susan and Hannah at EI)
2. Create a spectral mixing script that can be integrated with existing image acquisition software (Mark at SLC, Susan and Hannah at EI)
3. Configure hardware and software (Susan, Hannah and Mark at EI)
4. Conduct luciferase imaging experiment (Susan and Hannah at EI)

Outcomes and benefits
This project will enable the creation of an open-source, low-cost system that will provide lighting conditions that more closely simulate natural lighting conditions. This will not only enable circadian biologists to improve our understanding of circadian gene regulation in a more natural context, but also provide a useful low cost option for any researcher wishing to create laboratory conditions that are more akin to field lighting conditions.

Costings for LED BioMaker Project	
Low spec laptop	£350.00
3x Unicorn HAT 8x8 RGB LED arrays (Part No. PIM054, CPC Order Code SC13913 (£21.00 ea)	£63.00
Adafruit ARDX - v1.3 Experimentation Kit for Arduino (Uno R3) (Product Number 170)	£63.00
1x Adafruit Raspberry Pi 3 Starter Kit (Product 3058)	£70.00
Soldering iron	£25.00
Misc, Travel	£179.00
TOTAL	**£750.00**

References

McCree, K.J. (1972) Action Spectrum, Absorptance and Quantum Yield of Photosynthesis in Crop Plants. *Agricultural Meteorology*. 9, 191-216.
Moss, R.A., Loomis, W.E., (1952) Absorption Spectra of Leaves. The Visible Spectrum. *Plant Physiology*. 27(2), 370-391.

Vialet-Charand et al., (2017) Importance of Fluctuations in Light on Plant Photosynthesis Acclimation. *Plant Physiology*. 173(4), 2163-2179.