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Optical Microplates for Photonic High Throughput Screening of Algal Photosynthesis and Biofuel Production

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***Abstract* — Biological systems respond not only to chemical stimuli (drugs, proteins) but also to physical stimuli (light, heat, stress). Though there are many high throughput tools for screening chemical stimuli, no such tool exists for screening of physical stimuli. This paper presents a novel instrument for photonic high throughput screening of photosynthesis, a light-driven bioprocess. The optical microplate has a footprint identical to a standard 96 well plate, and it provides temporal and intensity control of light in each individual well. Intensity control provides 128 dimming levels (7-bit resolution), with maximum intensity 120 mE/cm^2. Temporal modulation, used for studying dynamics and regulation of photosynthesis, can be as low as 10 µs. We used photonic screening for high throughput studies of algal growth rates and photosynthetic efficiency, using the model organism *Dunaliella tertiolecta*, a lipid producing algae of interest in biofuel production. Due to the ability to conduct 96 studies in parallel, experiments that would require 2 years using conventional tools can be completed in 1 week. This instrument opens up novel high throughput protocols for photobiology and the growing field of phenomics.**

I. Introduction

Cells and other biological systems respond not only to chemical signals (such as drugs, signaling molecules, pH), but also to physical stimuli such as light, heat, radiation, and mechanical stress [1]. Traditional high throughput screening technology (microplates and pipetting robots) has enabled researchers to perform large-scale screening of chemical stimuli. In a typical screen, a cell or biomolecule is serially tested against a library of compounds using 96, 384, or 1536 well microplates [2], and binding or phenotypic responses of each compound are measured. However, no similar technology exists to perform screening of environmental stimuli. This represents a limitation in biological research, since many key bioprocesses related to photobiology, energy conversion, homeostatasis, stress responses, and pathology are triggered by environmental pressures [3].

One of the timely examples is in photobiology, specifically the study of photosynthesis in algae. Societal challenges in energy sustainability have renewed interest in how nature utilizes photon energy to produce biomass. Producing lipid-based biodiesel from algae is highly regarded as one of the most efficient and environmentally sustainable methods of generating biofuels, and appears to be the only renewable source of oil that could meet the long term global demand for transport fuels [4].

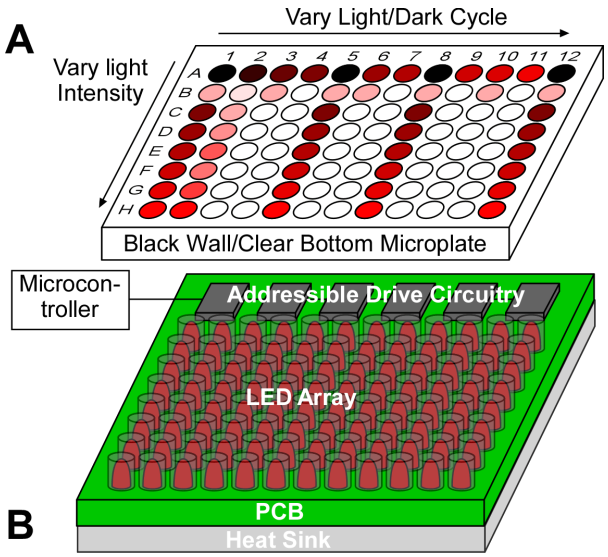

Figure 1: (A) Concept of photonic screening. Each well of the 96 well plate provides a different light intensity or duty cycle, enabling high throughput studies of photobiology. (B) Implemention of the optical microplate, using a 8x12 matrix of 650 nm LEDs controlled via individually addressable, serially controlled drive circuitry.

Although basic photosynthetic pathways have been well documented, the dynamics and regulation of energy conversion pathways, and its impact on energy conversion efficiency, are still not comprehensively understood [5]. For example, as one may expect, higher light intensity typically leads to higher lipid production; however, the conversion efficiency (lipid produced divided by the input power) is low [6]. Even at moderately high light levels, most of the light energy is wasted as heat or in other nonphotosynthetic processes, largely due to the ability of chlorophyll to absorb energy at a faster rate than what can be utilized by the slower downstream processes of photosynthesis. Temporal variation in light can significantly affect energy conversion efficiency [7-9]. It has been suggested that pulsed illumination would improve the efficiency of light energy utilization by allowing the energy absorbed by chlorophyll during a light pulse to be fully utilized for biomass production before another pulse is administered [6].

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Although it is known that temporal and intensity variation in light drives complex dynamics in photosynthesis, determining the optimal light conditions for maximum conversion efficiency is a laborious process. For example, a single data point in a photoirradiance curve requires one to measure growth rate of an algal culture over several days. Large scale screens, therefore, would require weeks or months of experiments to complete. Providing a high throughput means to evaluate tens or hundreds of light conditions simultaneously would be a valuable tool for photosynthesis research. Towards this end, this research has developed an optical microplate which can provide 96 user programmable lighting conditions (intensity as well as temporal variation) in the standard 96-well footprint (**Fig. 1**). The design and fabrication of the microplate is discussed in section II. Section III discusses calibration results and the use of the plate in studying growth rates and lipid production of *Dunaliella tertiolecta*, an algal species of interest in 2nd generation biofuels.

II. Experimental

A. *Microplate Design and Fabrication*

The optical microplate system consists of an 8x12 matrix of LEDs, each of which can be controlled individually using a serial microcontroller (Fig. 1B). In general, LED's are efficient light sources for horticulture, and the wavelength of 650 nm is chosen because it provides photosynthesis rates comparable to white light [10]. The LED matrix is covered with a black walled 96 well microplate (Corning) to ensure no light spillover between neighboring wells. A second identical microplate placed above this contains the algae culture. (Fig. 2). The control board (Fig.1B, Fig.4) consists of serial 6 LED drivers (Texas Instruments), each with 16 constant current outputs (for a total of 96 outputs). Each output can provide 128 analog current levels (7 bit resolution) to its respective LED. The range of current can be selected by choosing an appropriate bias resistor, and the maximum range is 0-80 mA, which is suitable for standard and intermediate power LEDs which can provide up to 1000 $\mu E/cm^2$. The light intensity and on/off state in each well is set using a high-speed serial peripheral interface (SPI) operating at up to 12 MHz. For pulsed and DC illumination, refreshing the state of entire array requires *n* clock cycles, where *n* is the number of LEDs. Therefore, in a 96-well plate, the hardware-limited refresh rate of the array is 100 KHz, or 10 µsec. This time resolution is sufficient to study the impact of light/dark reaction pathways in photosynthesis [7].

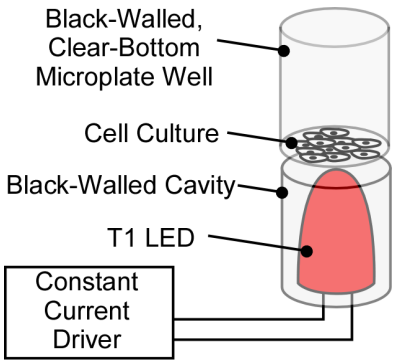

Figure 3: Optical design. To prevent light spillover, LEDs and cells are enclosed in black-wall, clear-bottom 96 well plates.

The design is implemented on a standard FR4 circuit board and fabricated at a commercial foundry. The LEDs and microplate are assembled using standard through hole and surface mount connections (Fig. 3). Heat sinks, attached to the drivers and/or the board using a thermal compound, reduce the overall temperature of the drivers. A 10 pin ribbon cable connects the cascaded drivers to a USB serial interface card (USB-8451, National Instruments) which controls the light intensities. The card is controlled via Labview software on a standard desktop computer. Light levels were measured using a quantum light meter (LI-1400, LI-COR Biosciences) aligned manually above each well.

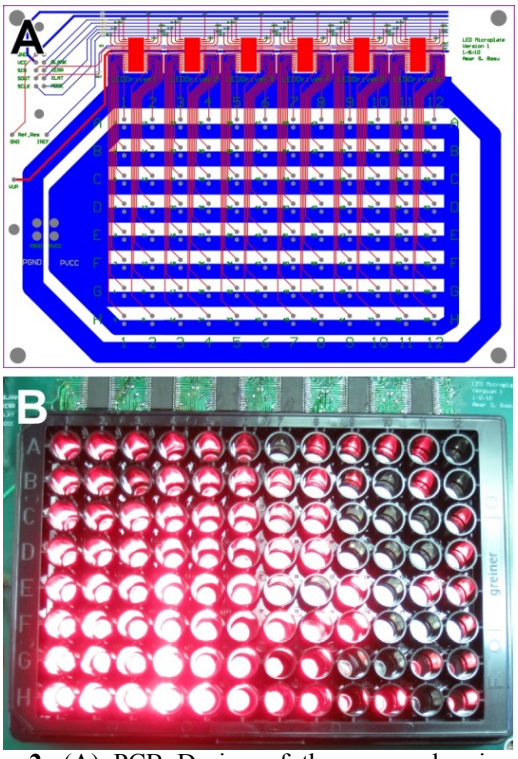

Figure 2: (A) PCB Design of the array, showing chip placement and routing. (B) Assembled PCB, LED array and black well microplate.

B. *Algae culture and Assay*

Equal volumes of *Dunaliella tertiolecta* (200 µL) were cultured in the 96 well microplate over 12 days. The cells and the microplate system were placed in a laboratory incubator at 37°C and 5% CO_2. The microplate was placed on an aluminum block which acted as a heat sink. An extended ribbon cable provided connection to the DAQ card and power supply placed outside the incubator.

Growth rates were recorded daily by measuring absorbance at 405 nm in a standard microplate reader. Nile red stain was added to the culture at the end of the

experiment to measure the lipid production. Nile red is a selective indicator for intracellular lipids [11], and fluoresces only in a lipid environment. Fluorescence intensity was quantified daily using a microplate reader at 450 nm excitation/530 nm emission.

III. Results And Discussion

A. *Characterization of the Optical Microplate*

The LED drivers are based on a current mirror configuration which provides a high degree of linearity and precision in current control. Current vs. intensity setting (Fig. 4) shows an R^2 value of 0.9992. Well-to-well variation in current was characterized by measuring LED currents for all 96 wells on an intensity setting of 20. Out of 96 wells, the average current was 2.17 mA and the standard deviation was 0.039 mA, giving a variance of 1.8%.

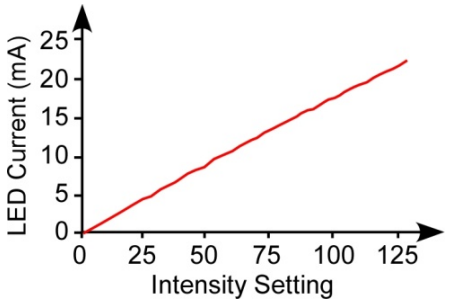

Fig. 4: Current vs. Intensity setting for a selected LED.

Well-to-well variation in light intensity (Fig. 5A) was measured by manually aligning the light meter to the wells of the plate. Light levels have a variance of 17%, presumably due to errors in aligning the light meter to each well, and possibly due to manufacturing variation in the LEDs. The variation in light levels can be reduced to <10% through a one-time calibration, where the intensity settings are scaled by an appropriate calibration factor (Fig. 5B).

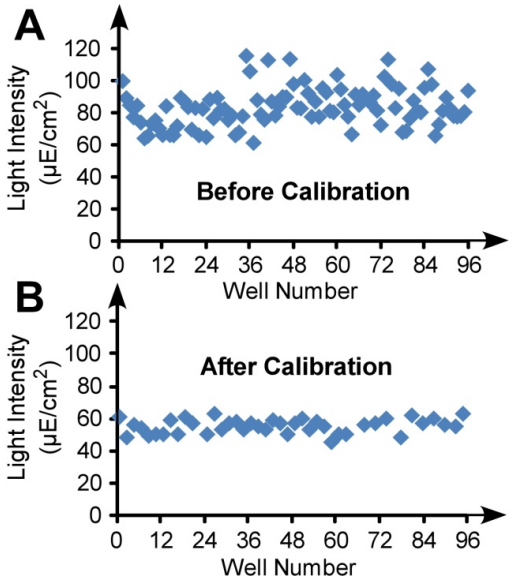

Fig. 5: Well to well variation in light levels (A) before and (B) after calibration.

The microplate refresh rates, which are controlled by using a Labview software control loop, can provide as little as 1 ms pulse width. This can be reduced by 2 orders of magnitude by using a hardware timed solution.

B. *Photonic Screening of Algae Growth Curves*

The utility of the device is demonstrated in algal photobiology, where both intensity and duty cycle play role in the efficiency of photosynthetic conversion. We are currently using the optical microplate for photonic screening of *Dunaliella tertiolecta*. Photonic high throughput screening is used to optimize the light conditions to maximize the synthesis of lipids, which can be used as biofuels [5]. The first study is a photoirradiance curve which characterizes the growth rate of algae under 6-fold variation in light intensity. Having a large number of wells allows each experiment to be replicated in 8 wells. The daily averages are shown in Fig. 5. As expected, cultures receiving higher light intensity reach stationary phase faster than those receiving less light. This is due to the dependence of metabolic rates and reproduction on light intensity. Microscope observation revealed that cells under strong light conditions (intensity setting >40) reached 100% confluence by day 8. Subsequently, these cells began to die in days 8-12 due to the depletion of nutrients in the microplate well. When algal cells die, their color changes

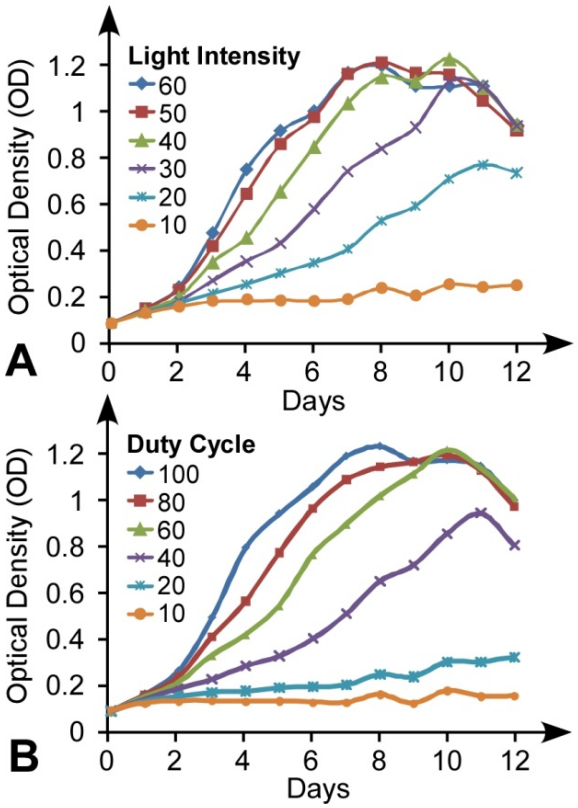

Fig. 6: Optical density of *Dunaliella tertiolecta* microplate cell cultures over 12 days at different settings of (A) light intensities and (B) duty cycle. The results illustrate the sensitivity of growth rates to light intensity.

from green to white, dramatically decreasing the optical density as shown in Fig. 6B. Cultures receiving less light tend to remain in a low metabolic state where minimal replication occurs. A similar graph is obtained when duty cycle is varied at a fixed frequency (10 Hz). Both experimental results support the expected relationship that algal growth rate scales with the total photon flux. With this instrument, photoirradiance growth curves can be generated in 1 week, as compared to the 2 months it would require using conventional instruments.

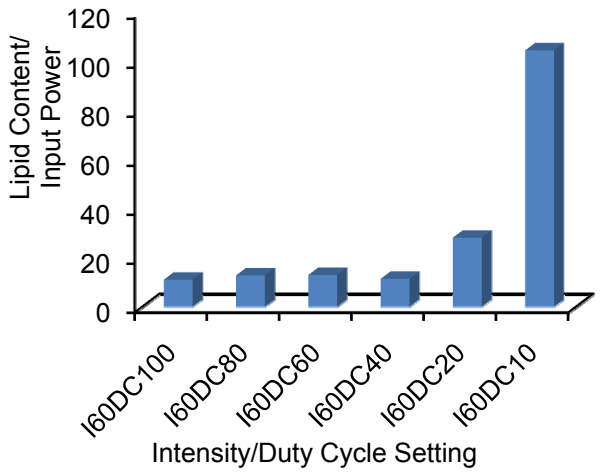

Fig. 7: Lipid conversion efficiency as a function of duty cycle at a fixed intensity setting of 60. Conversion efficiency is calculated as the lipid fluorescence intensity divided by the total input power. The results indicate the lipid production efficiency increases at low duty cycle.

Lipid production efficiency shows a surprising increase at low duty cycles. Fluorescence intensity of nile red was measured for screens over duty cycles ranging from 10 to 100%. Conversion efficiency is found by dividing the fluorescence intensity by the total integrated photon flux (or alternatively, the total electrical energy) provided to the cell culture. It is found that efficiency peaks at low duty cycle. These results correlate with prior studies [8] which describe an improvement of photosynthetic efficiency when exposed to alternating light/dark cycles at low pulse rates. Future work will include additional experiments to clarify the impact of duty cycle and light intensity on total lipid production efficiency as well as total lipid production.

IV. Conclusions

This paper has demonstrated the design and application of an optical microplate for photonic screening in algal photosynthesis. While some of the screening data is as expected, the increase in efficiency at low duty cycles is an example of an unexpected finding that can occur in a high throughput approach. This highlights the ability of a photonic screening technique to drive biological discovery and the development of new hypotheses in photosynthesis and photobiology in general. Future work will examine additional synthesis pathways to determine optimal light conditions, as well as the mechanisms of energy conversion efficiency.

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