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TECHNICAL INNOVATION

Optical microplates for high-throughput screening of photosynthesis in lipid-producing algae†‡

Meng Chen,[a] Taulant Mertiri,[b] Thomas Holland[a] and Amar S. Basu*[b]

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It is well known that biological systems respond to chemical signals as well as physical stimuli. The workhorses of high throughput screening, microplates and pipetting robots, are well suited for screening chemical stimuli; however, there are fewer options for screening physical stimuli, particularly those which involve temporal patterns. This paper presents an optical microplate for photonic high-throughput screening. The system provides addressable intensity and temporal control of LED light emission in each well, and operates on standard black-wall clear-bottom 96-well microplates, which prevent light spillover. Light intensity can be controlled to 7-bit resolution (128 levels), with a maximum intensity of 120 mE cm^{-2}. The temporal resolution, useful for studying dynamics of light-driven bioprocesses, can be as low as 10 μs. The microplate is used for high-throughput studies of light-dependent growth rates and photosynthetic efficiency in the model organism *Dunaliella tertiolecta*, a lipid-producing algae of interest in 2nd generation biofuels. By conducting 96 experiments in parallel, photoirradiance studies, which would require 2 years using conventional tools, can be completed in <2 weeks. In a 12 day culture, algal growth rates increase with total photon flux, as expected. Interestingly, the lipid production efficiency, defined as lipid production per unit photon flux per capita, increases nearly 5 fold at low light intensity (constant light) and at low duty cycle (pulsed light). High throughput protocols enabled by this system are conducive to systematic studies and discovery in the fields of photobiology and photochemistry.

Introduction

Cells and biological systems respond not only to chemical signals (such as drugs, signaling molecules, and pH), but also to physical stimuli including light, heat, radiation, electrical potentials, and mechanical stress.[1–3] Traditional high-throughput screening technology, based on microplates and pipetting robots, has generally focused on screening chemical stimuli in the fields of drug discovery, proteomics, and cytotoxicity. In a typical screen, a cell or biomolecule is assayed against a library of compounds using 96, 384, or 1,536 well plates.[4] Active, inactive, and/or toxic compounds can be identified based on ligand binding, enzymatic, or morphological assays recorded in each well. Another common screen, the serial dilution assay, systematically measures the response to compound concentration.

While chemical screening technology has matured over many years, there are fewer analogous technologies for screening physical stimuli. This represents a growing need in biological research, as many key bioprocesses and signalling cascades are triggered by environmental pressures.[5] Examples range from the cellular to the physiological level, including photosynthesis, heat shock, neurobiology, sensory networks (vision, olfaction), mechanobiology, circadian rhythms, and homeostasis.

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 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.[6]

Although the basic photosynthetic pathways have been well documented, the dynamics and regulation of light-to-biomass energy conversion pathways and their impact on conversion efficiency are still not comprehensively understood.[7] As one may expect, higher light intensity typically leads to higher lipid production; however, the conversion efficiency (lipid produced per unit light input) is low.[8] Even at moderate light levels, most of the light energy is wasted as heat or in non-photosynthetic processes. This is largely because chlorophyll absorbs energy at a faster rate than what can be utilized by the slower downstream processes of photosynthesis. Temporal variation in light can

[a]*Department of Immunobiology and Microbiology, Wayne State University School of Medicine, ,*
[b]*Electrical and Computer Engineering and Biomedical Engineering, Wayne State University College of Engineering, ECE Dept, 5050 Anthony Wayne Drive, Detroit MI 48202, USA. E-mail: abasu@eng.wayne.edu; Tel: +1-313-577-3990*

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significantly affect energy conversion efficiency.[9–11] It has been suggested that pulsed illumination can 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.[8]

It is clear that light drives complex dynamics in photosynthesis; however, determining the optimal light conditions for maximum conversion efficiency is a laborious process. For example, in photoirradiance curves, a single data point requires one to measure growth rate of a cell culture over several days. Large scale screens would require several months of experiments. Therefore, a high throughput system to evaluate tens or hundreds of light conditions simultaneously would be a valuable tool for photosynthesis research. A photosynthetron described previously[12] provided 12 distinct light levels, but no temporal control. Toward this end, this work has developed an optical microplate which can provide 96 programmable light conditions (intensity as well as temporal variation) in the standard 96-well footprint (Fig. 1A). Design and fabrication is discussed in section II. Section III reports microplate characterization and calibration, followed by experimental studies on the growth rates and lipid production efficiency in *Dunaliella tertiolecta*, an algal species of interest in 2nd generation biofuels.[6,13]

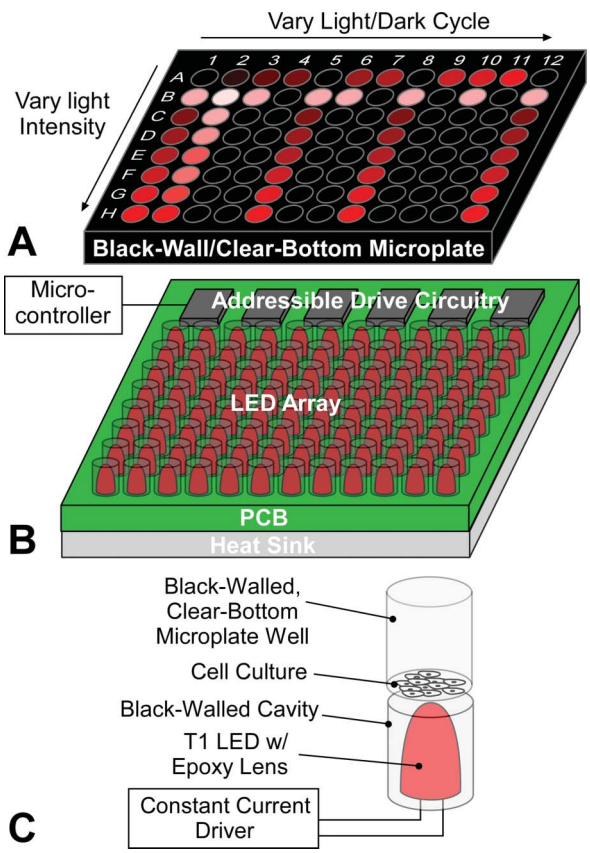

Fig. 1 (A) Photonic screening concept. Each well of the optical microplate can provide a different light intensity or duty cycle, enabling high throughput screening in photobiology. (B) Schematic of the electronics design. (C) Optical design. To prevent light spillover, the LEDs and cells are enclosed in stacked black-wall, clear-bottom 96 well microplates. Each LED is allocated a dedicated current driver.

Experimental

Microplate design and fabrication

The optical microplate system consists of an 8 × 12 matrix of LEDs, each of which can be controlled individually *via* a serial microcontroller (Fig. 1B). LEDs are efficient light sources for horticulture, and the 650 nm wavelength is chosen because it provides photosynthetic rates comparable to white light.[14] To prevent light spillover between adjacent wells, the LED matrix is encased in a standard black-wall, clear bottom 96 well microplate (Corning) with the bottom layer removed. A second microplate containing the algae culture is stacked directly above (Fig. 1C). The control electronics consist of 6 serial LED drivers (Texas Instruments), each with 16 programmable current drivers, for a total of 96 outputs. Each output provides 128 discrete current levels (7-bit resolution) to its respective LED. The range of current can be selected by choosing an appropriate bias resistor. The maximum current is 80 mA, which can drive LEDs with light intensity up to 1000 μE cm^{-2}. The light intensity and on/off state in each well are set using a high-speed serial peripheral interface (SPI) operating at up to 12 MHz. For pulsed illumination, refreshing the state of entire array requires n clock cycles, where n is the number of LEDs. In a 96-well plate, the hardware-limited refresh rate is 100 KHz, or 10 μs. This resolution is sufficient for studying the light/dark reaction pathways in photosynthesis.[9]

The design is implemented on a standard FR4 printed circuit board (PCB) and manufactured at a commercial foundry. Components are mounted using through-hole and surface mount soldering (Fig. 2). Passive aluminum heat sinks are attached to the board using a thermal adhesive in order to reduce the system temperature. A 10-pin ribbon cable connects the cascaded drivers to an SPI interface card (USB-8451, National Instruments) which controls the LEDs. Light patterns are programmed *via* a graphical user interface in Labview (National Instruments). Light levels are measured using a quantum light meter (LI-1400, LI-COR Biosciences) aligned manually above each well.

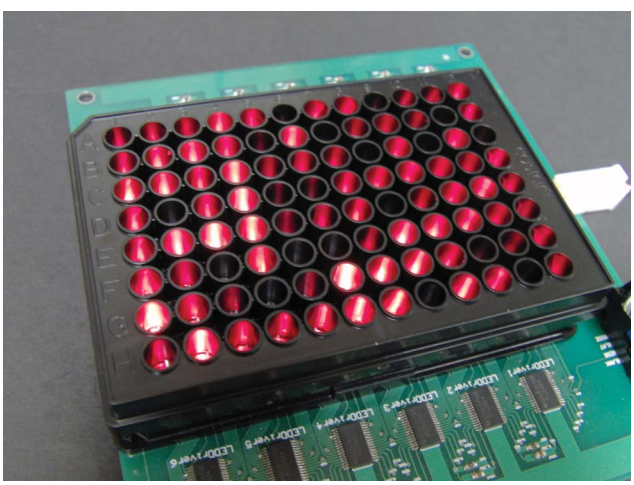

Fig. 2 Assembled optical microplate.

Algae culture and assay

Equal volumes of *Dunaliella tertiolecta* (200 μL) were cultured in all 96 wells of the optical microplate over 12 days. The culture medium and conditions are described in our previous study.[13] Growth rates were recorded daily by measuring optical absorbance at 405 nm in a commercial microplate reader. The initial culture was diluted to an optical density of 0.1. *Dunaliella* species are known to increase lipid production under nutrient-deficient conditions, in particular dissolved nitrates.[13] To maximize lipid production, the media was left unchanged, so that 90% nitrogen depletion occurred within 7 days. The optical microplate was placed in a laboratory incubator at 32 °C and 5% CO_2. The USB interface card and power supply was kept outside the incubator. Nile red stain was added to the culture at the end of the experiment to quantify lipid production. Nile red is a selective indicator for intracellular lipids,[15] and fluoresces only in a lipid environment. Fluorescence intensity was quantified daily using a microplate reader at 485 nm excitation/595 nm emission.

Results and discussion

Characterization of the optical microplate

The LED drivers employ a current mirror design which provides linearity and high precision in current control. The LED current *vs.* intensity setting (Fig. 3A) shows an R^2 value of 0.9992. Well-to-well variation was characterized by measuring LED currents for all 96 wells on an intensity setting of 20. The average current is 2.17 mA and the standard deviation is 0.039 mA (1.8%).

Well-to-well variation in light intensity (Fig. 3B) was measured by manually aligning the light meter above each well. The variation in light levels is 17%, presumably due to errors in manual alignment and manufacturing variation in the LEDs. The variation can be reduced to <10% through a one-time compensation where the intensity setting in each well is scaled by an appropriate calibration factor. The microplate refresh rate, controlled by the Labview software loop, is 1 KHz. Therefore, the smallest pulse width and the time resolution in sampling temporal light patterns are both 1 ms. This can be reduced by two orders of magnitude (10 μs) by using a hardware timed loop.

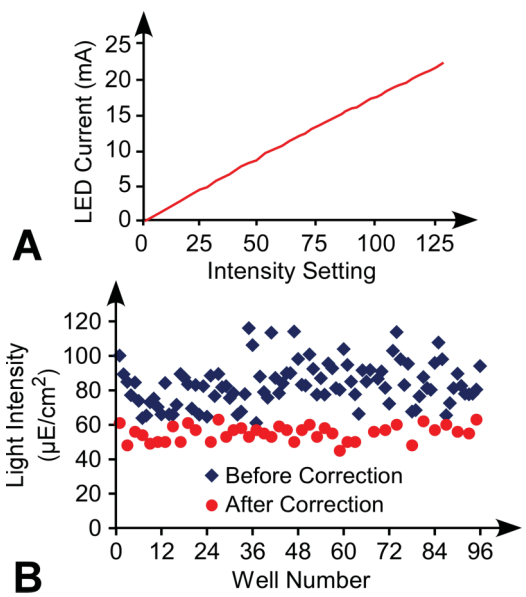

Fig. 3 (A) LED current *vs.* Intensity setting for a selected LED. (B) Well to well variation in light intensity before and after calibration.

Photonic screening of algae growth curves

We utilize the optical microplate for photobiology studies in *Dunaliella tertiolecta*, where both intensity and duty cycle of light play an important role in the efficiency of photosynthetic conversion of light to energy storage lipids.[9–11] The screening protocols are used to optimize light conditions to maximize lipid synthesis as well as the conversion efficiency. The first study is a photoirradiance curve which characterizes the growth rate of algae under 6-fold variation in light intensity. In this study, algae were cultured in 6 groups of 8 wells. The 8-fold replication helped to compensate for well-to-well experimental variation. Each group was assigned a light intensity ranging from 10 to 60 μE cm^{-2}. Daily averages of optical density are shown in Fig. 4. As expected, cultures receiving higher light have a faster doubling time and reach the stationary phase earlier than those receiving less light. This is due to the dependence of metabolic rates and reproduction on light intensity.[13] Microscope observation reveals that the seeded cells form a single layer, but they do not attach to the plate. Over time, they may become multilayers, in which case shadowing can potentially limit exponential growth rates. Cells under strong light conditions (>40 μE cm^{-2}) reach a maximum density by day 8, and subsequently begin to die in days 8–12 due to the depletion of nutrients in the well. Upon death, their color changes from green to white, causing the optical density to decrease. By contrast, cultures receiving less light tend

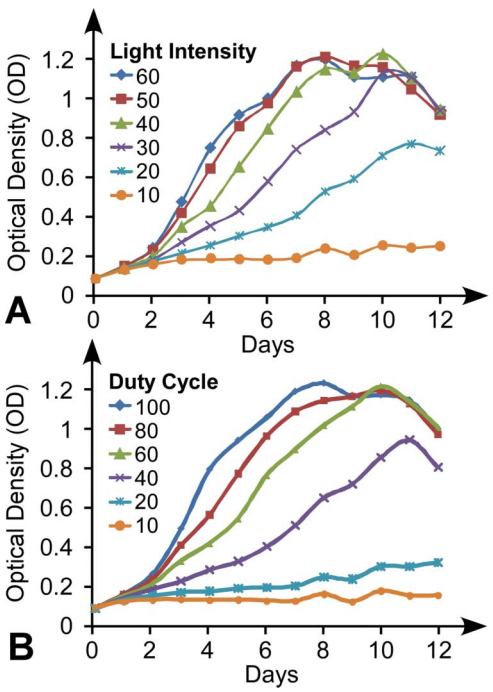

Fig. 4 Optical density of *Dunaliella tertiolecta* microplate cell cultures over 12 days at different settings of (A) light intensities and (B) duty cycle. The results illustrate the sensitivity of growth rates to light intensity.

to remain in a low metabolic state where minimal replication occurs. Due to the reduced nutrient consumption, these cells appear to remain viable well beyond day 8.

A similar result is obtained when duty cycle is varied at a fixed frequency. In this experiment, the wells were again split into 6 groups of 8. This time, the intensity was fixed at 60 μE cm^{-2}, the frequency was fixed at 10 Hz, and the duty cycle was set between 10 and 100%. Both sets of experimental results support the expected relationship that algal growth rate scales with the total photon flux. With this instrument, the photoirradiance growth curves can be generated in <2 weeks.

High-throughput studies of lipid conversion efficiency

The objective of the last study is to understand the lipid conversion efficiency of *Dunaliella tertiolecta* under different conditions of light intensity (Fig. 5A) and duty cycle (Fig. 5B). The experimental conditions were the same as those used in Fig. 4A–B, respectively. The fluorescence intensity of Nile red was measured in each case. The conversion efficiency was calculated by dividing the fluorescence intensity (reflecting lipid content) by the product of optical density (to normalize by the cell count) and the electrical power provided to the LEDs.

The gross lipid production (data not shown) is found to increase with increasing photon flux, as expected. However, the lipid conversion efficiency (Fig. 5) shows a substantial increase at low light intensity and low duty cycle. A nearly 5 fold increase is observed at the smallest light intensity in the case of fixed light, and the smallest duty cycle in the case of pulsed light.

The underlying mechanism is believed to be due to the coupling between the two reaction cycles involved in photosynthesis.[16] The light-dependent reactions generate energy and reducing power *via* ATP and NADPH intermediates while the dark reactions (*i.e.* the Calvin cycle) consume these molecules along with CO_2 to produce glucose. The former process occurs at timescales <1 ms, while the latter occurs over seconds. The dark reactions are therefore rate limiting, causing photosynthesis to saturate at high light intensity. Conversely, at low light levels, the majority of light can be fully utilized, resulting in improved efficiency.[8] Pulsed illumination gives a similar effect by allowing the intermediates produced by light reactions to be fully consumed by the dark reactions before the next pulse arrives. The results in Fig. 5 correlate with prior studies[10] which describe an improvement in photosynthetic efficiency when exposed to alternating light/dark cycles at low pulse rates. The advantage of pulsed illumination is that the organism can maintain high efficiency at higher intensity light pulses, resulting in high bioproductivity.[8] It is clear that the combined parameters of light intensity, light cycle time, and dark cycle time work together to determine photosynthetic efficiency. The system and experiments presented here suggest that the optical microplate can be useful for systematic studies investigating such complex interplays in photobiology.

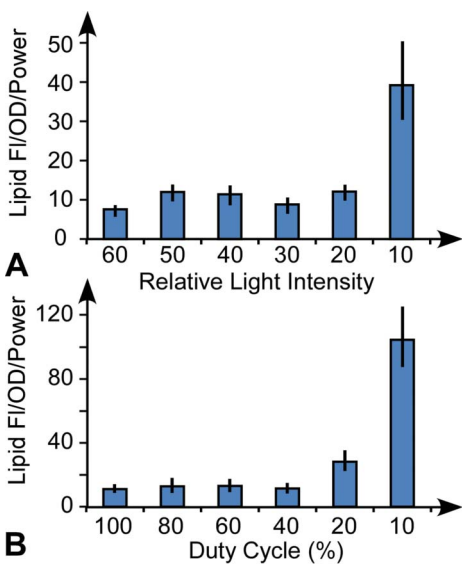

Fig. 5 Lipid conversion efficiency as a function of (A) light intensity and (B) duty cycle. Experimental conditions are described in the text. In each case, the measurement was averaged over 8 wells.

Conclusions

This work has described the design and application of an optical microplate for high-throughput screening in photobiology. In algae photosynthesis studies, parallel screening of light intensity can save substantial time in generating photoirradiance curves and optimizing growth conditions. From a broader perspective, this system can be useful for studying other light-driven bioprocesses, including photoreceptors, phototherapy, photoacclimation, circadian rhythms, and optogenetics.[3] In such examples, systematic screens of light intensity and temporal variation can help reveal the properties and dynamics of photobiological processes and regulatory networks.[2]

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