Supplementary Information for

A Droplet-Based Screen for Wavelength-Dependent Lipid Production in Algae

Steve C.C. Shih,[1, 2†] Nooman Mufti,[1,2] M. Dean Chamberlain,[1,2] Jihye Kim,[3] and Aaron R. Wheeler[1,2, 3 *]

[1] Institute for Biomaterials and Biomedical Engineering, University of Toronto, 164 College St., Toronto, ON, M5S 3G9, Canada

[2] Donnelly Centre for Cellular and Biomolecular Research, 160 College St., Toronto, ON, M5S 3E1, Canada

[3] Department of Chemistry, University of Toronto, 80 St George St., Toronto, ON, M5S 3H6, Canada

†Current affiliation: Joint Bioenergy Institute (JBEI), 5855 Hollis St. Emeryville, CA 94608, USA

* Corresponding Author
email: aaron.wheeler@utoronto.ca
tel: (416) 946 3864
fax: (416) 946 3865

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DMF device fabrication and operation

Devices were fabricated in the Toronto Nanofabrication Centre (TNFC) cleanroom facility. Fabrication reagents and supplies included Parylene-C dimer from Specialty Coating Systems (Indianapolis, IN, US), gold- and chromium-coated glass slides from Telic (Valencia, CA), indium tin oxide- (ITO) coated glass slides from Delta Technologies Ltd (Stillwater, MN), Teflon-AF from DuPont (Mississauga, ON), Shipley S1811 photoresist and MF-321 photoresist developer from Rohm and Haas (Marlborough, MA), KI/I_2 gold etchant from Sigma, CR-4 chromium etchant from OM Group (Cleveland, OH), and AZ-300T photoresist stripper from AZ Electronic Materials (Somerville, NJ).

DMF device bottom-plates bearing patterned electrodes and contact pads were formed using methods similar to those described previously.[1] Briefly, gold- and chromium-coated substrates were spin-coated with S1811 photoresist (3000 rpm, 30 s). The substrates were pre-baked on a hot-plate (100°C, 2 min) and exposed to UV through a photomask printed at Pacific Arts and Design (Markham, ON) for 10 s (30 mW cm^{-2}) and then developed by immersing in MF-321 for ~3 min. Substrates were then sequentially immersed in gold etchant (~5 min), CR-4 (~10 s), and AZ 300T (5 min), with a final rinse in DI water followed by drying under a stream of nitrogen. The substrates were then coated with 7 μm of Parylene-C and 50 nm of Teflon-AF. Parylene-C was applied using a vapor deposition instrument (Specialty Coating Systems), and Teflon-AF was spin-coated (1% wt/wt in Fluorinert FC-40, 1000 rpm, 30 s) followed by post-baking on a hot-plate (160ºC, 10 min). Dicing tape was placed on the electrode contact pads prior to parylene coating and was removed after Teflon-AF coating to facilitate electrical contact. For additional details, see the narrated video video by Jebrail et al.[2]

As depicted in Fig 1 in the main text, each bottom plate features an array of 204

electrodes, including two four-electrode active reservoirs (~324 mm^2/electrode ea.), eight bussed dye dispensing electrodes (~27.47 mm^2/electrode.), eight algae culture zone electrodes (~51.41 mm^2/electrode), eight absorbance detection electrodes (~2.25 mm^2/electrode), eight fluorescent detection electrodes (~11.19 mm^2/electrode), and eight waste reservoir electrodes (~9 mm^2/electrode). The electrode array has inter-electrode gaps of 30-100 μm, and each electrode is connected to an array of contact pads on the side of the device spaced appropriately to interface with a 40-pin connector (Compar Inc., Burlington ON). After coating, a 1 mm dia. through-hole was drilled in the center of each waste reservoir electrode.

DMF device top-plates were formed from ITO-glass substrates coated with 50 nm Teflon-AF using the same procedure described for bottom plates (as above). Devices were assembled with an ITO-glass top plate and a gold-on-glass bottom plate separated by a spacer formed from four pieces of double-sided tape (total thickness of 360 μm). Each 110 mm x 75 mm top plate was roughly aligned with the outer-edges of the reservoir electrodes on the bottom plate.

80-120 V_{RMS} droplet driving potentials were generated by amplifying the sine wave output of a function generator (Agilent Technologies, Santa Clara, CA) operating at 5-15 kHz. The application of driving potentials was managed using a home-built automated feedback control system for high-fidelity droplet manipulation[3] using hardware and methods described in detail elsewhere.[1, 4] Briefly, as shown in Figure S1, the top ITO-plate electrode is connected to an AD5206 digital potentiometer (Analog Devices, Brampton, ON, Canada). To move a droplet onto a given destination electrode on the bottom plate, a 200 ms pulse of driving potential is applied to the destination electrode relative to the top-plate electrode. During the final 5 ms of the voltage pulse, the potentiometer is triggered to deliver 5% of the voltage to the positive

terminal of a buffered op-amp (MCP6004, Microchip, Brampton, ON, Canada), the output of which is connected to the analog input of an RBBB Arduino microcontroller (Modern Device, Providence, RI). The magnitude of the output voltage, V_{sense}, is proportional to the impedance of the volume between the destination electrode on the bottom plate and the top plate electrode. The driving software compares the measured impedance to a threshold level associated with a droplet of a given liquid, and if the measured impedance is below the threshold, additional voltage pulses are triggered with +10 V_{RMS} higher magnitudes until the droplet completes the desired operation.

Optimization of Dispensing

Dispensers in DMF typically comprise three electrodes: a reservoir (R), a middle electrode (M), and a destination electrode (D). In most systems described in the literature, each combination of R, M, and D is individually addressable, such that if particular dispensing step is observed to fail, the operation can be replayed until it is successful. In the work described here, it was necessary to reduce complexity by "bussing" the dispensers (i.e., eight R electrodes, eight M electrodes, and eight D electrodes are each controlled with a single potential). Unfortunately, in initial trials with preliminary device designs, these bussed dispensers were particularly susceptible to failure – i.e., for a given dispensing operation, some droplets were successfully dispensed but some were not. Thus, building on designs described to enhance droplet necking,[5] we tested six different geometries to identify conditions useful for reliable dispensing for bussed electrodes.

Six different DMF device bottom plates bearing different dispenser designs (Table S1) were fabricated and assembled with top plates as described above. Aliquots of DI water (15 μL) were loaded into the reservoirs. The control system was programmed to dispense one droplet on

each device at constant voltage (80 V_{RMS}) and duration of driving pulse (200 ms). The outcome was observed and recorded, and a successful dispense was defined as cases in which a daughter droplet was created that covered fully the area of the D electrode. This process was repeated (loading a fresh aliquot into the reservoir for each replicate) ten times per device. The results are listed in the right column of Table S1. As shown, only design six (in the bottom row) was capable of perfect dispensing. From these results, we extrapolated a general guideline for designs capable of perfect dispensing: the ratio of the widths of middle to reservoir electrode (M:R) must be 0.26:1 (or less) and the ratio of the widths of middle to destination electrode (M:D) must be 0.68:1. This observation merits future work to determine comprehensive design rules, which likely are dependent on liquid surface tension and viscosity. But for the work described here, the general guideline was incorporated into the bussed dispensers included in the final device design used here (Figure 1 in the main text). Perfect dispensing of all eight droplets was observed for all replicates described in the main text.

Illumination Systems

Two custom illumination systems ("multicolour" and "blue/yellow") were formed for use with DMF screening experiments by soldering light emitting diodes (LEDs) to a printed circuit board. Each system contained eight illumination elements positioned 9 mm apart, and each element was formed from zero, one, or two LEDs. The multicolour system comprised seven one-LED elements and one zero-LED element. The LEDs were 5 mm dia. from Lumex Components Inc. (Palatine, IL) with the following colours: white, violet (peak wavelength: 410 nm), blue (470 nm), green (502 nm), yellow (585 nm), orange (610 nm), and red (635 nm). The blue/yellow system comprised six two-LED elements (one blue and one yellow) and two one-LED elements (one blue or one yellow LED); these LEDs were 3 mm dia. and were from Vishay

Semiconductor (Phoenix, AZ). The two systems were mounted in black, bottom-transparent 96-well well plates with one illumination element penetrating into each well.

In algae culture experiments, an illumination system was positioned ~2.5 cm above a DMF device in a darkened box (Fig. S2A), aligned such that each element was directly above one of the eight algae culture droplets. The timing and duration of the LED "on" and "off" states and the intensities of the "on" states were programmed and managed using a custom system developed for this work comprising an Arduino Mega 2560 (Sparkfun Electronics, Boulder, CO) controller connected to a TLC 5940 (Texas Instruments, Dallas, TX) constant-current sink LED driver with pulse width modulation (PWM) control (Fig. S2B). The intensity of each LED-containing element was measured using a LightScout Quantum meter (Spectrum Technologies Inc., Aurora, IL), and was tuned to 50 or 250 μmol m^{-2} s^{-1} by modulating the PWM values.

A third LED illumination system was developed for macro-scale culture, comprising two LED bulbs (one yellow and one blue) with a power rating of 1.3 W (1000Bulbs, Garland, TX). These LEDs were positioned 10-15 cm away from 200 mL Pyrex culture flasks and were managed using the control system described above.

Supplementary References

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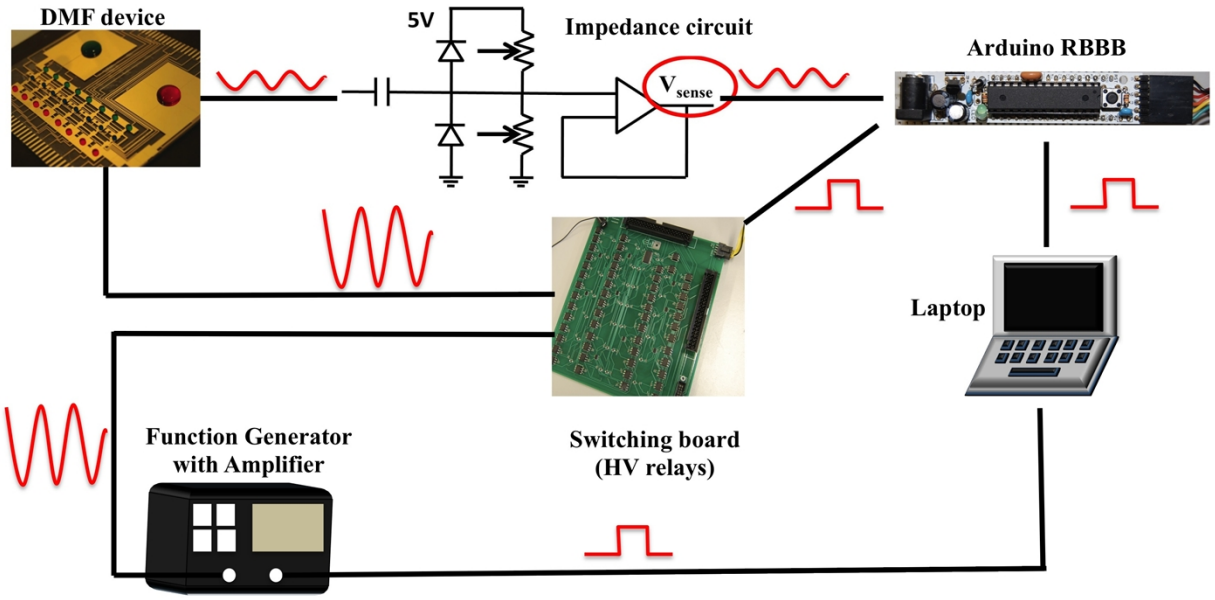


Figure S1. Schematic of droplet control system. The function generator with amplifier sends high-voltage signals to the switching board which actuates the droplets on the DMF device. Droplet position is detected by the impedance circuit and a decision is made by the Arduino RBBB to continue actuating the activated electrode (if the droplet movement was not successful) or to start the next step in the sequence (if the droplet movement was successful). The result of this decision is transmitted to the laptop which triggers the function generator and amplifier. The red signal traces depict the type of signal (analog as sine waves or digital as square waves) and the relative amplitudes of the signal that are delivered between components.

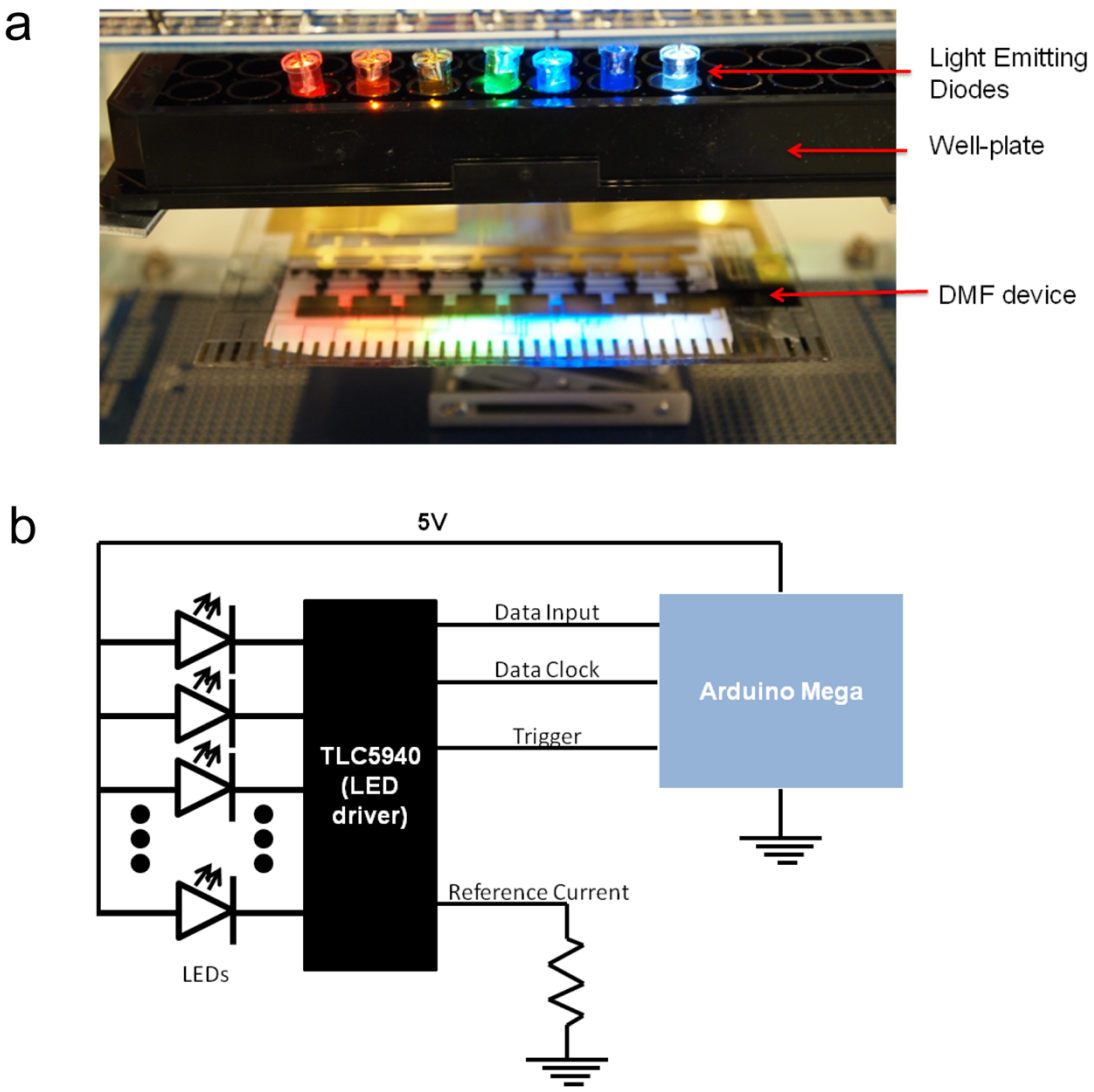


Figure S2. LED illumination system. (a) Picture of the multicolour system mounted above a digital microfluidic device. For screening experiments, this system was positioned in a darkened box containing Kimwipes moistened with deionized water for various incubation times, after which absorbance and fluorescence measurements were collected as described in the main text. (b) Schematic of the circuit used to program and control LED states ("on" or "off") and intensities as a function of time.

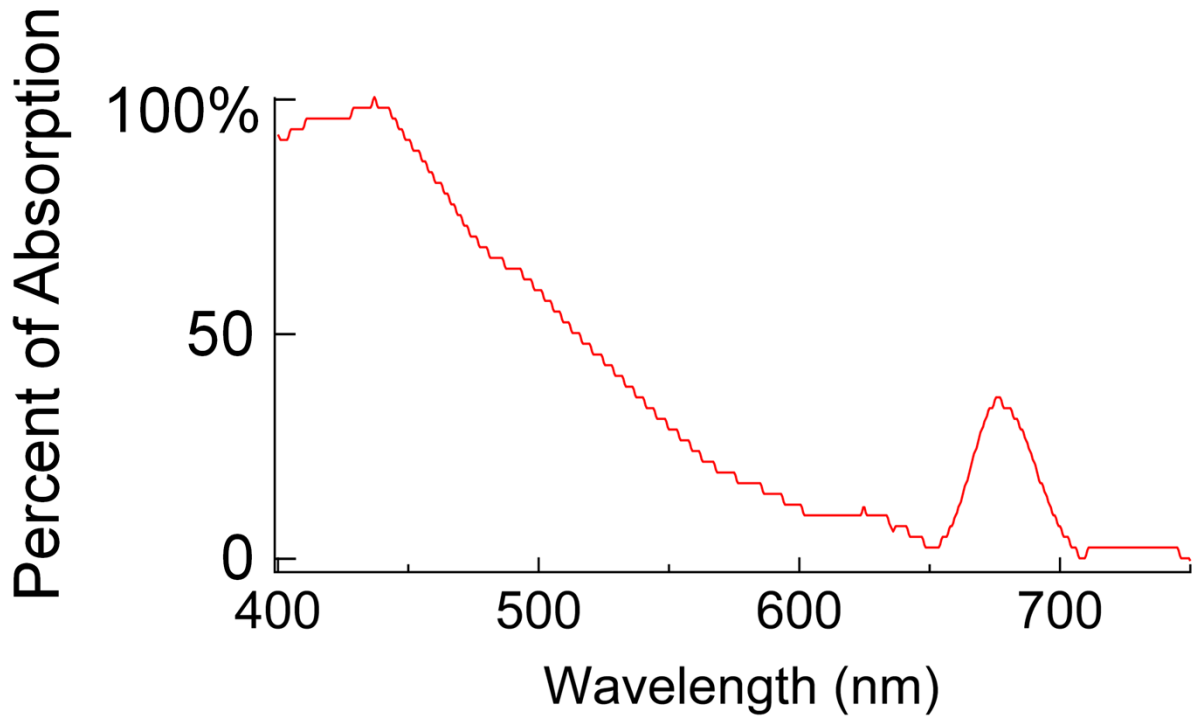


Figure S3. Absorbance spectrum for *C.cryptica* showing two maxima peaks at 465 nm (blue) and 675 nm (red).

Table S1: Summary of the challenges encountered and eventual solutions developed in creating a digital microfluidic device capable of long-term multiplexed culture and analysis of algae.

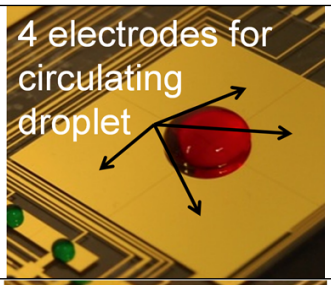
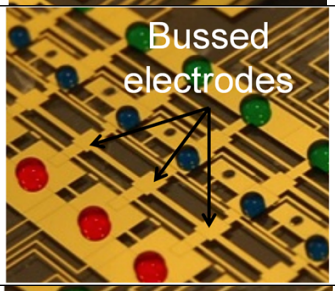
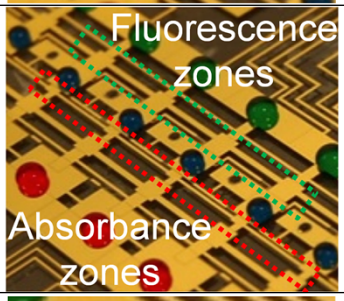
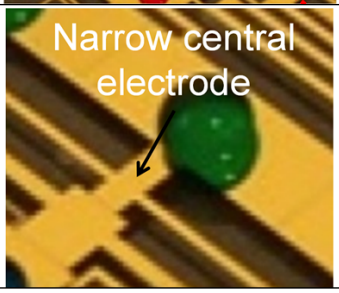
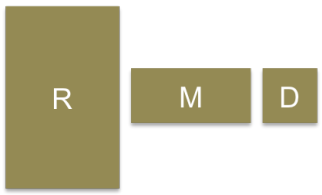
Challenge	Solution	Picture of solution
Uneven distribution of cells in sub-droplets	4-electrode reservoir structure that allows circulation of stock algae culture droplets until sub-droplets are dispensed	
Simultaneous control of >100 droplets for a wide range of volumes (2-150 μL)	Multi-sized electrodes, some of which are "bussed" (i.e., lines of analogous electrodes are connected and controlled by one source)	
Accumulation of waste products	Vertical through-holes allowing for waste products to wick into absorptive material below the device	
Multiplexed absorbance and fluorescence detection	Array of detection zones (incl. transparent windows for transmission)	
Reliable sub-droplet droplet dispensing in parallel	Narrow central electrode for dispensing	

Table S2: Dispensing reproducibility for six DMF electrode designs with reservoir (R), middle electrode (M), and destination electrode (D).

Design	Dimensions (mm)	Successful dispensing percentage (out of 10 trials)
	R: 3.6 square M: 2.25 square D: 2.25 square	0%
	R: 3.6 square M: 2.25 x 3 D: 2.25 square	30%
	R: 3.6 square M: 1.15 x 3 D: 2.25 square	50%
	R: 9 x 3.1 M: 2.25 square D: 2.25 square	40%
	R: 9 x 3.1 M: 2.25 x 3 D: 2.25 square	70%
	R: 3.6 square M: 1.15 x 3 D: 2.25 square	100%