Supplementary Material (ESI) for Lab on a Chip

Microfluidic cytometer for high-throughput measurement of photosynthetic characteristics and lipid accumulation in individual algal cells

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Supplementary Materials and Methods

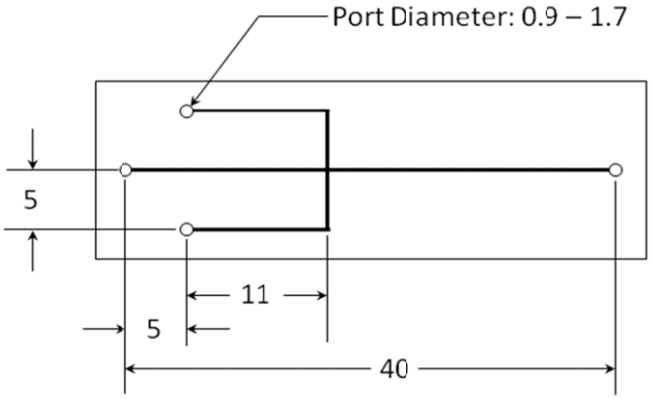

Fig. S1 Microfluidic chip configuration. Material: fused silica. Dimensions are in mm. Overall dimensions 45 X 15 X 1.8. Channels are 180 µm wide X 90 µm deep.

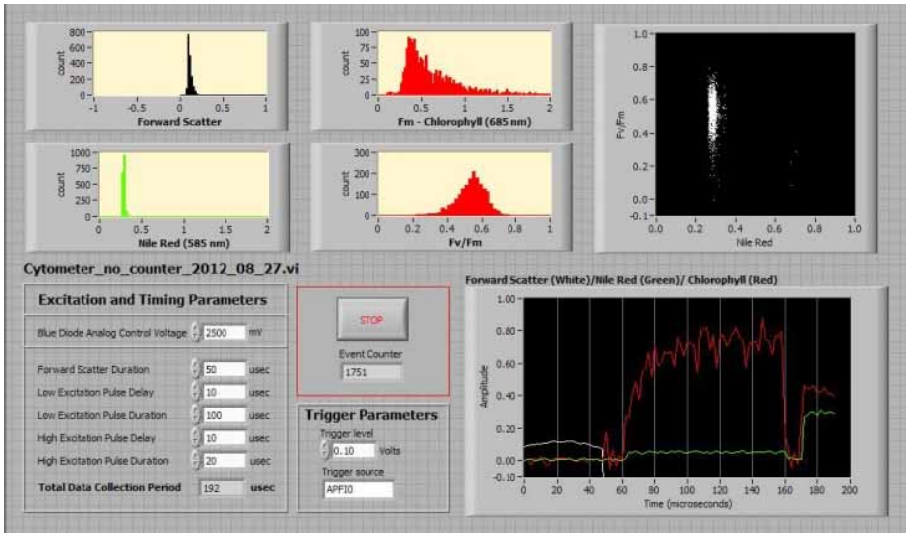

Fig. S2 Control panel showing operator controls and real time data display. Histograms for FS, F_m, NR fluorescence and F_v/F_m are shown in the upper left. A scatter plot of F_v/F_m versus NR fluorescence is shown in the upper right. A graph (shown on the lower right) is updated after each event and shows signals for FS, induced Chl fluorescence and NR fluorescence for the interrogation time period. Operator adjustable controls for Chl excitation intensity and interrogation timing are shown on the lower left.

Supplementary Optics

An aspheric collimator lens (Lightspeed Technologies, Campbell, CA) is used to shape the Lambertian radiation pattern of the LED. A bandpass filter (460 nm center wavelength, 60 nm FWHM, Semrock FF460/60-25) removes long wavelength spectral components from the LED output which is then homogenized by a holographic beam shaper/diffuser (5 degree divergence, Edmund Optics). The excitation beam fills the entire entrance pupil of the objective. By measuring the beam intensity with a CMOS camera (MCN-B013U, Mightex) placed at the base of the objective it is found that the excitation intensity has a 2.5% coefficient of variation (CV) within a 5 mm diameter region. A 20X planapochromatic (numerical aperture 0.75) infinity-corrected objective lens (UPLSAPO 20X, Olympus) collects cellular fluorescence, which is split into three channels by dichroic filters (FF650-Di01; FF740-Di01; FF510-Di01, Semrock) and detected by separate photomultiplier tubes (PMTs) with bandpass filters for the Chl (775 nm center wavelength, 46 nm FWHM; Semrock FF01-775/46-25) and NR (583 nm center wavelength, 120 nm FWHM; Semrock FF01-583/120-25) emission channels. An infrared-extended, multialkali PMT (H7422-20, Hamamatsu) is used to detect Chl fluorescence and a multialkali PMT (H7583-20, Hamamatsu) is used to detect NR fluorescence.

Supplementary Electronics

In contrast to the Chl fluorescence channel, the excitation LED was operated in a digital mode to create a higher, but shorter, current pulse. Though this higher excitation intensity would result in an unacceptably fast rise time if used to measure induced Chl fluorescence, it is desirable in the NR fluorescence channel to improve the signal-to-background ratio (S/B). In the work of Banerjee *et al.,* experiments (using a microfluidic device with a 50 μm channel height, organic LED excitation (peak λ = 530 nm), and Rhodamine 6G dye) demonstrated that 10 – 100-fold increases in excitation power lead to 2 - 4-fold improvements in S/B.[1] They additionally demonstrated that improved S/Bs, in conjunction with low signal-to-noise ratios, resulted in better limits of fluorescence detection.

To prevent damage to the Chl PMT during NR fluorescence excitation, a custom-built gating circuit was implemented to disable the PMT by shorting the first dynode and the cathode through an FET (field effect transistor) switch. The PMT is enabled by restoring the operating voltage between this dynode and the cathode with another FET switch. Full PMT gain is then reinstated within microseconds.[2]

Supplementary References

1. A. Banerjee, Y. Shuai, R. Dixit, I. Papautsky and D. Klotzkin, *Journal of Luminescence*, 2010, **130**, 1095-1100.
2. D. J. Creasey, P. A. Halford-Maw, D. E. Heard, J. E. Spence and B. J. Whitaker, in *Review of Scientific Instruments*, 1998, vol. 69, pp. 4068-4073.