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Application Note

Dispensing Microalgae

Introduction

Microalgae inhabit a wide variety of marine and freshwater habitats around the globe, and can additionally be cultured in a lab setting. Isolating individual cultured or natural microalgal cells has traditionally been reliant on conventional cell sorters. However, these high pressure systems (20-70 psi) can easily compromise the integrity of the sorted cells.

Namocell's single cell dispensing platform offers a gentle alternative to traditional cell sorters, with an operating pressure of <2 psi. The Namocell Single Cell Dispenser can detect multiple fluorescent channels, including FITC (533nm), FITC (533nm), PE (585nm) and PerCP (676nm). Green algae are abundant with chloroplasts containing chlorophyll pigments, which are responsible for their characteristic green appearance. These pigments absorb blue light and emit red light (680 nm), making them easily detectable by the PerCP channel. In this application note, the Namocell Single Cell Dispenser was used to isolate individual microalgae directly from pond water with minimal handling and preparation. In addition to utilizing the autofluorescence of green algal cells, the nucleic acid dye SYTO® 16 was used to distinguish DNA-containing cells from debris in the FITC detection channel.

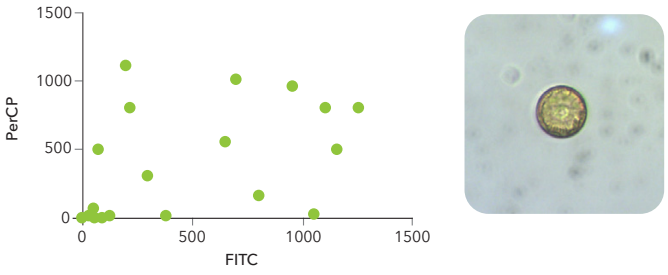
Methods

Two sets of algae samples were used in this study: cultured algae and algae collected from pond water at a site near Shanghai, China. Both samples were filtered (40 µm) to remove large debris and aggregates. Samples were stained with SYTO® 16 (0.4 nM, ThermoFisher S7578) and incubated for 10 minutes at room temperature. For each sample, a new microfluidic cell cartridge (Namocell) was used to prevent sample carryover. Samples were loaded into the Namocell dispenser and analyzed with the PerCP channel as the trigger. Gates for each fluorescent detection channel were set as follows: positive PerCP selection (100-4999), positive FITC selection (100-4000), and negative PE selection (0-10). Cells were dispensed onto glass microscope slides and single cell isolation was verified via light and fluorescence microscopy.

Results

For both sample types, the fluorescent signals from the FITC and PerCP channels produced a clear separation between target algal cells and background non-algal cells and debris (Fig. 1). Interestingly, the algae isolated from pond water showed a population of PerCP-positive algae (PerCP around 2000) as well as a distinct population with extremely bright PerCP fluorescence, saturating this detection channel with values over 4000 (Fig. 1B).

A. CULTURED ALGAE



B. POND WATER ALGAE

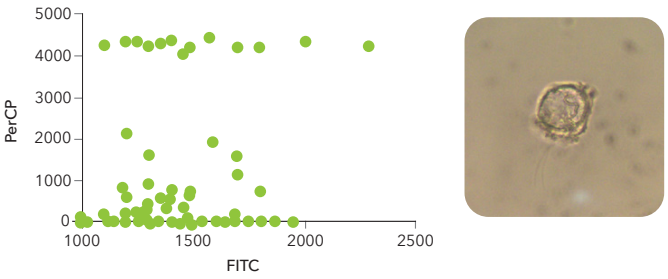


FIGURE 1. Analysis plots showing the FITC (533 nm) and PerCP (676 nm) detection values for both cultured (A) and pond water-collected (B) algal cells. Images on the right show individual algal cells isolated from each sample type and dispensed onto a microscope slide (brightfield).

Further imaging of single algal cells from both the high PerCP (Fig. 2A-C) and saturated PerCP (Fig. 2D-F) populations confirmed the presence of two distinct subtypes of microalgae collected from this site.

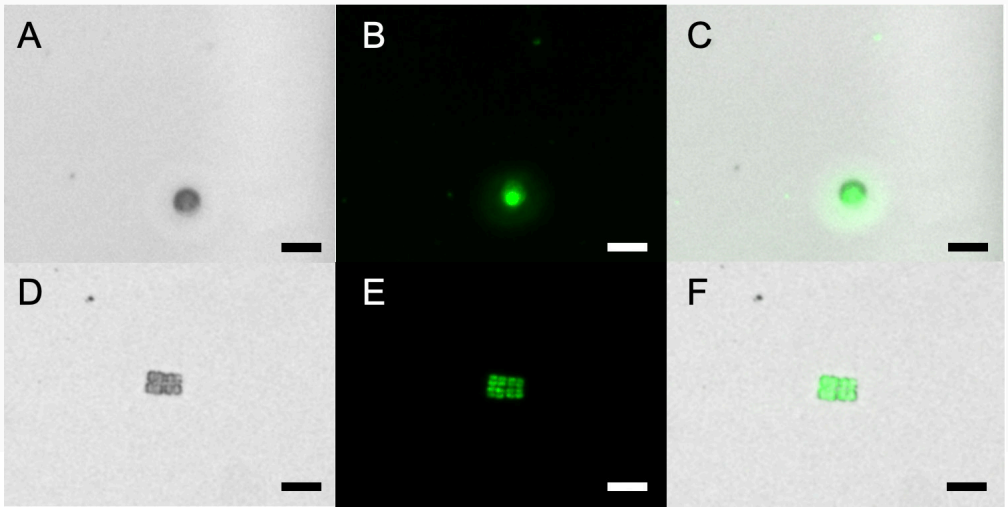


FIGURE 2. Brightfield (A, D), fluorescent (B,E) and merged (C,F) images of dispensed single algal cells from both the high PerCP (top panel) and saturated PerCP (bottom panel) cell populations. Scale bars 20 µm.

In this study, Syto 16-labeled green algal cells were sorted based on both the FITC and PerCP detection channels, resulting in successful isolation of the target cell types despite an abundance of FITC-positive non-target cells and debris. These results highlight the utility of the autofluorescence of green algae in the PerCP channel for robust isolation of these cells. Similar to green algae, red algae exhibit autofluorescence from an abundance of phycoerythrin (PE). Thus, red algae can be detected and sorted using a parallel approach with the PE detection channel as the trigger. Overall, the Namo dispenser offers a simple workflow for gentle single cell isolation that can be applied to a wide variety of autofluorescent cell types.

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