Namocell
The Fastest Way to a Single Cell

# Sorting Microalgae from Pond Water with the Namo Single Cell Dispenser

## Introduction

In order to investigate the microalgae flora composition, each individual alga cell must be isolated. Sorting individual cells directly from the source has been challenged by the limitations of technologies. For example, the high pressure of traditional sorters can easily compromise the integrity of the sorted cells. Herein, the Namo Single Cell Dispenser is used to isolate individual microalgae cells directly from the water source with minimal handling and preparation at a low pressure (less than 2 psi). The Namo Single Cell Dispenser has up to three channels for fluorescence detection: FITC, PE, and PerCP.

Green algae are abundant in pigments chlorophyll a and b in chloroplasts, which results in their bright green characteristic color. These pigments absorb blue light and emit red light (680 nm). The emitting red light is used as a positive label that is detectable by the PerCP channel of the Namo Single Cell Dispenser. In addition to utilizing the autofluorescence of green algae cells, SYTO® 16 was used to stain the DNA and aided in the detection with the FITC channel. Single double positive (FITC and PerCP) cells were dispensed onto glass slides and verified with microscopy.

## Experimental

Two sets of algae samples were used for this study: from culture and from pond water of a site near Shanghai, China. Both samples were filtered (40 µm) to remove large debris and aggregates. Samples were stained with 0.4 nM working concentration of SYTO® 16 (ThermoFisher S7578) and incubated for 10 minutes in room temperature. The algae cells within the samples were indistinguishable and not able to be counted visually. A single Cell Cartridge (Namocell) per sample was used to prevent sample crossover. Cell Cartridge with sample was loaded into the Namo Single Cell Dispenser III and analyzed with PerCP as the trigger. The channel gates on the Namo software was set for positive PerCP (100-4999), positive FITC (100-4000), and negative PE (0-10). Cells were dispensed onto glass slides and verified via light and fluorescence microscopy.

## Results and Conclusion

Analyses of samples (**figure 1**) illustrated a clear separation of the double positive algae in contrast to the background in both samples. **Figure 1b** from pond water had signals with high PerCP (up to 2000) and saturated PerCP (above 4000). Further imaging of a single alga cell with high PerCP (**figure 2a-c**) and saturated PerCP (**figure 2d-f**) revealed two different types of alga cells.

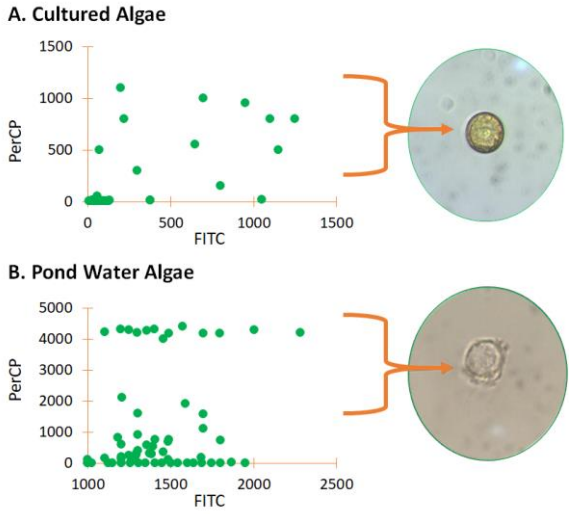


**Figure 1** Scatter plots from algae in culture (**a**) and pond water (**b**). Inset images of dispensed alga cells.

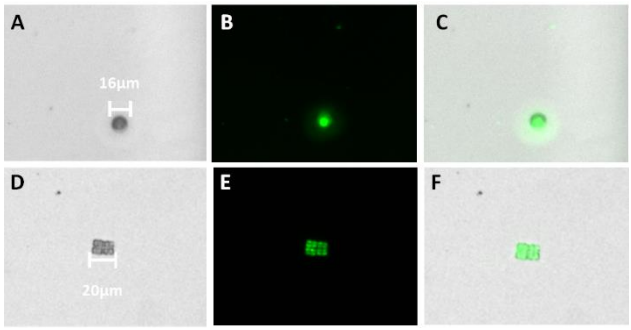


**Figure 2** Images of dispensed single alga cell from pond water with high PerCP (**a-c**) and saturated PerCP (**d-f**) include brightfield (**a, d**), fluorescence (**b, e**), and stacked (**c, f**).

The PerCP trigger was used to identify the autofluorescence of green algae cells. FITC was not used as the trigger as the pond water contained other microorganisms that gave false positive signals when using the FITC alone. Therefore, double positive gating with positive FITC and PerCP resulted in the successful isolation of single green alga cells. Similar to green algae, red algae exhibit autofluorescence from an abundance of phycoerythrin (PE). As a result, red algae can be detected and sorted with the PE channel. This simple workflow can be applicable to other samples with autofluorescence for gentle single cell isolation by the Namo Single Cell Dispenser.