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OPTOFLUIDIC CYTOMETRY ON A CHIP

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ABSTRACT

The effects of global warming, pollution in river effluents, and changing ocean currents can be studied by characterizing variations in phytoplankton populations. We demonstrate the design and fabrication of a microflow cytometer for characterization of phytoplankton. Guided by chevron-shaped grooves on the top and bottom of a microfluidic channel, two symmetric sheath streams wrap around a central sample stream and hydrodynamically focus it in the center of the channel. The lasers are carefully chosen to provide excitation light close to the maximum absorbance wavelengths for the intrinsic fluorophores chlorophyll and phycoerythrin, and the excitation light is coupled to the flow cytometer through the use of an optical fiber. Fluorescence and light scatter are collected using two multimode optical fibers placed at 90-degree angles with respect to the excitation fiber. Light emerging from these collection fibers is directed through optical bandpass filters into photomultiplier tubes. The cytometer measured the optical and side scatter properties of *Karenia b.*, *Synechococcus sp.*, *Pseudo-Nitzschia*, *Alexandrium*, *Nitzschia*, and *Thalassiosira pseudonana*. The microflow cytometer proved sensitive enough to detect and characterize picoplankton with diameter approximately 1 μm and larger phytoplankton of up to 80 μm in length. The wide range in size discrimination coupled with detection of intrinsic fluorescent pigments suggests that this microflow cytometer will be able to distinguish different populations of phytoplankton on unmanned underwater vehicles. We also studied the effect of the sheath-to-sample flow-rate ratio on the light scatter and fluorescence of these marine microorganisms.

Experimental Details

The microflow cytometer is made entirely of polydimethylsiloxane (PDMS). There are three inlets with the center inlet designated for introducing the sample fluid while the other two inlets offset by a certain angle relative to the sample inlet are for introducing the sheath fluid.

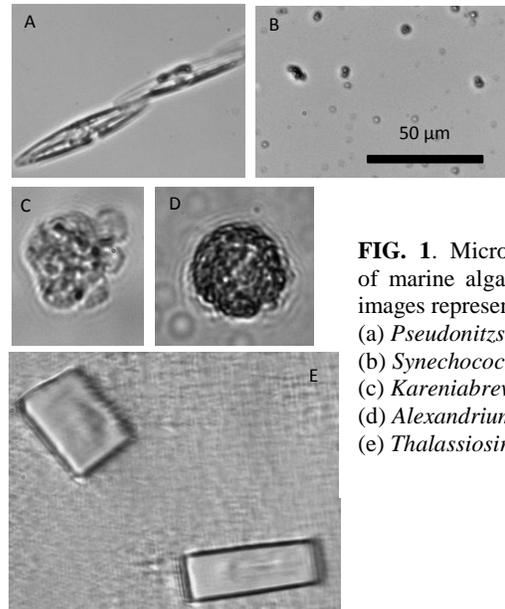


FIG. 1. Microscopic images of marine algae species. The images represent: (a) *Pseudonitzschia*., (b) *Synechococcus sp.*, (c) *Karenia brevis*, (d) *Alexandrium*, (e) *Thalassiosira pseudonana*.

The sample fluid and the sheath fluid are both introduced into the system using a syringe pump (CAVRO XE 1000, Tecan Systems, Inc., San Jose, CA) and a bidirectional peristaltic pump (P625/66.143, Instech Laboratories, Inc., Plymouth, PA). The sample inlet has the dimensions of 390 μm wide by 130 μm deep which is sufficiently large to accommodate a wide range of sizes of marine particles [1-3]. Two multimode optical fibers were installed perpendicular to the light source, each on the opposite sides of another, to collect laser-induced fluorescence signals and light scatter upon the impact of the excitation light on the particle in the frame at any instant. Each collection fiber was equipped with a bandpass filter (Omega Optical In., Brattleboro, VT), with a designated bandwidth.

Results and Discussion

For the single light source system with a core sample flow rate of 200 $\mu\text{L}/\text{min}$, scatter plots such as phycoerythrin fluorescence vs. chlorophyll fluorescence, chlorophyll vs. side scatter, and phycoerythrin vs. side scatter were generated. To reiterate, this particular system only dealt with *Synechococcus sp.*, *Nitzschia*, and *Thalassiosira p.*, and all the data pertinent to the three species were combined and plotted on the same graph as shown in Fig. 2. According to the phycoerythrin vs. chlorophyll scatter plot in Fig. 2, the least intense phycoerythrin fluorescence was observed in the *Thalassiosira p.* population indicated by purple dots, which was at the order of 10^{-1} . Conversely, the *Nitzschia* population indicated by green dots emitted the largest magnitude of fluorescence and was at the order of approximately 10^1 . Overall, *Nitzschia* and *Synechococcus sp.* (indicated by red dots) demonstrated fairly similar magnitude of chlorophyll fluorescent emission which was at approximately an average of 10 fluorescence units while it was slightly higher in *Thalassiosira p.*

For the two light source system with a core sample flow rate of 10 $\mu\text{L}/\text{min}$, the same scatter plots were generated, however the data was represented individually because it was analyzing four different species of algae which were *Synechococcus sp.*, *Nitzschia*, *Karenia brevis*, and *Alexandrium*. Chlorophyll fluorescence signal was particularly strong at approximately 10^2 fluorescence units in both *Karenia brevis* and *Alexandrium* population. However, the presence of phycoerythrin fluorescence signal was less predominant in all species. For example, according to the phycoerythrin vs. side scatter and phycoerythrin vs. chlorophyll scatter, the average maximum detectable phycoerythrin fluorescence signal was approximately 10^{-1} fluorescence units. Furthermore, light scatter data was most prevalent in both *Nitzschia* and *Karenia brevis* population, and least profound in *Synechococcus sp.* Both *Nitzschia* and *Karenia brevis* approximately approached side scatter magnitudes of 10^3 whereas *Synechococcus sp.* with a side scatter magnitude barely reaching 10^1 .

Conclusions

The results demonstrated that for the two light source systems, slightly higher fluorescence signals for both phycoerythrin and chlorophyll were observed. One possible explanation is that since the two light source system has a relatively low sample volumetric flow rate at 10 $\mu\text{L}/\text{min}$, the core stream ultimately appeared more refined thus making data acquisition more accurate. Additionally, the other reason is that

two light sources were used and each of them possessed the characteristics to adequately excite the elements of interests. For instance, the 532nm laser was capable of triggering phycoerythrin fluorescence response and the 404nm laser maximally excited chlorophyll fluorescence. Unlike the two light source system, the one light source system was only equipped with 488nm light source and was more capable of exciting one element than the other.

Ultimately, although both light systems have shown to be able to trigger responses in various sizes of marine algae species using microflow cytometer, the two light source system with an adjusted and refined core volumetric flow rate is likely to be more accurate than the single light source system in performing the same task. Consequently, this might suggest that the two light source system would be a more appropriate choice for future applications as it is more likely to yield reliable results.

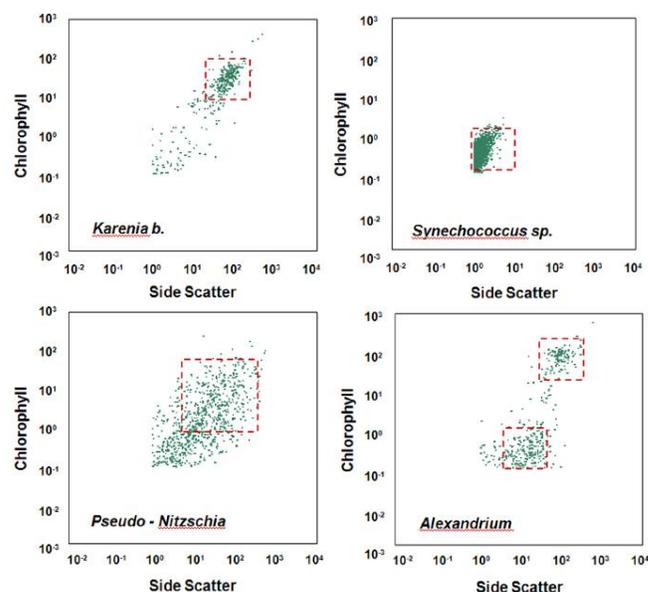


FIG. 2. The scatter plot illustrates the chlorophyll vs. side scatter plot for the two light source system with a compressed core flow rate of 10 $\mu\text{L}/\text{min}$. This image represents the scatter plots for *Karenia brevis* and *Pseudo-Nitzschia*., *Synechococcus sp.*, and *Alexandrium*.

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