AQU 04 Portable Algae μFlow Cytometer

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Overview

The portable algae μflow cytometer is a project that aims to expedite research in algae biology using microfluid-based and state-of-the-art detection technology. The project is a joint effort that will incorporate the expertise of two different groups, Dr. David Caron at USC and Dr. Yu-Chong Tai at Caltech. One main focus of the project is to develop a portable μflow cytometer that is suitable for on-field monitoring of algae population and reduce test time. The overwhelming growth of microalgal population, the microalgal bloom, has negative effects on marine ecosystems. The temporally-distinct increase in biomass results in a net loss of oxygen through respiratory and degradation process. The hypoxic event leads to the mortality of larger organisms including fish, shellfish and seagrasses. Further, many bloom-forming species are capable of toxin production which is harmful to the marine lives even affects human health. However, factors driving selective proliferations of microalgae and, especially, harmful species are still poorly understood. Part of the reason for this lack of knowledge is the time- and labor-intensive nature of analysis of water samples for specific bloom-causing organisms. Many of today's ocean-observing systems provide only rough proxies for algal biomass (e.g. chlorophyll fluorescence, absorption, or backscattering) and couldn't distinguish different species. To solve this problem we build a portable algae μflow cytometer system to provides a precise evaluation of the algae population. The μflow cytometer measures individual algae cells for their size, chlorophyll fluorescence and other biological properties, which is important to distinguish different species, especially to resolve the harmful ones among algae communities. Also, the portable system can be used for constant vigilance in the pre-bloom stage to tie down processes contributing to the increased growth of algae.

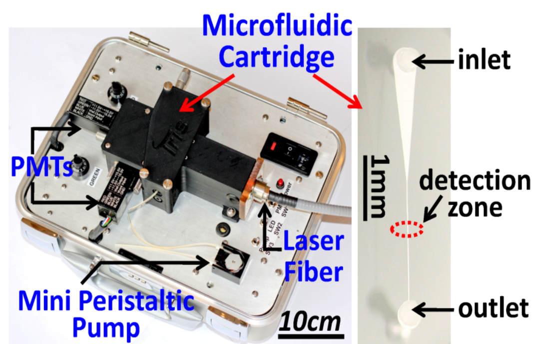


Figure 1. The portable algae μflow cytometer system (left) and the disposable μflow cell (right).

Approach

The portable system built at Caltech uses the microflow (μflow) cytometer technology. A disposable microfluidic chip is used as the flow cell of the cytometer to reduce the sample volume needed for each test. The dimension of the μflow cell can be optimized for different sizes of the target algae cells. Laser-induced-fluorescence measurement and light extinction measurement are used to evaluate the properties of the algae population such as the cell size, Chlorophyll-α fluorescence, viability, esterase activities, etc. Each algae cell is measured individually and several thousands of algae cells can be measured in a short period of time to achieve a precise evaluation of the algae properties. The portable algae μflow cytometer provides the on-field testing capability of the algae population and its biological properties, which is very useful for applications such as the on-field monitoring of the harmful algae bloom. It can also be used together with Algae Culture Chip we developed to further expand its functions such as controlling the number of algae cells being loaded into the culture chip by upstream test and evaluating the biological properties of the cultured algae cells by downstream test.

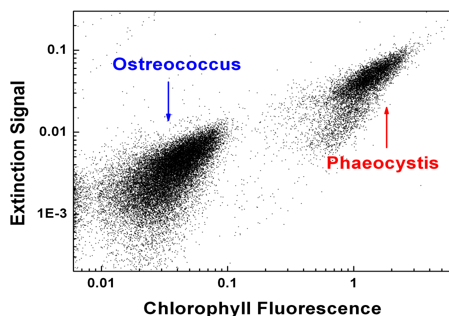


Figure 2. Scatter plot of the Chlorophyll-α fluorescence vs. extinction signal of algae cells, Ostreococcus and Phaeocystis.

System(s) Description and/or Experiments

A protocol of the portable system is built in Caltech with off-the-shelf components for facility demonstration (Fig.1). A microfluidic chip is used as the flow cell of the cytometer. The μflow cell, made by the standard soft lithography process, is disposable after each test. A blue (488nm) solid laser module is used as the excitation source, and the signals (extinction intensity, fluorescence intensity) are measured by two photomultiplier tubes (PMT). A mini peristaltic pump is used to draw the sample for test. Two-color fluorescence signals or one-color fluorescence signal and the extinction signal can be measured. The extinction measurement provides an estimation of the algae cell size. The fluorescence measurement can be used to evaluate the algae properties such as the Chlorophyll-α fluorescence, esterase activities (with fluorescein diacetate (FDA) staining), etc. The portable system is assembled in an aluminum case (12" x 9" x5"). It can be powered by the standard 110V AC or a 5V DC source. The testing data is read out through a USB port and can be visualized on a laptop computer or PDA device.

Algae cells are distinguished from other particles in water samples based on their intrinsic chlorophyll fluorescence. For example, in a mixture of two algae species, Ostreococcus and Phaeocystis, the number of algae cells are counted by the measured fluorescence peaks. Further, their fluorescence intensity and extinction signals allocate as two distinguished clusters in a scatter plot (Fig.2).

Accomplishments

In last report period, we demonstrated that our platform can be used to measure the esterase activity of individual algae cells and to conduct statistic analysis of the whole population. The esterase activity of the algae cells are evaluated by a fluorescent probe, Fluorescein Diacetate (FDA). Cells with normal esterase activity show strong green fluorescence from FDA staining, while cells with inhibited esterase activity show weaker green fluorescence (Fig.3). Our platform is successfully used to provide a quantitative evaluation of this difference. As an example, the algae cells, Dunaliella, are exposed to a toxic Cu2+ solution, and the change of their esterase activity vs. different exposure time can be monitored as shown in Fig.4. The results can be used to study the algae cells' biological responses to toxic water contamination. Two students from this project group lead a student team to explore the idea of using this platform and algae as bio-indicator for early alert of heavy metal pollutions in water, and the entry won the 2nd prize in 2010 IEEE President's Change the World Competition.

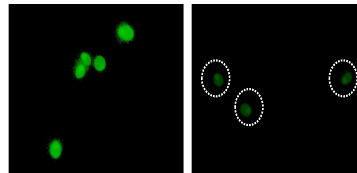


Fig. 3. Fluorescent microscope pictures of algae, Dunaliella, with (left) normal vs. (right) inhibited esterase activity.

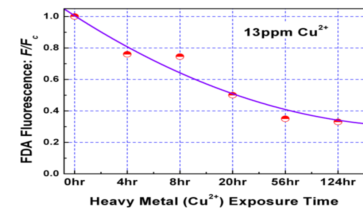


Fig. 4. FDA fluorescence intensity of algae cells vs. exposure time of Cu2+ contaminated water.

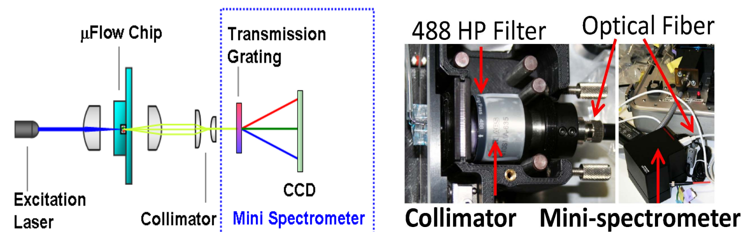


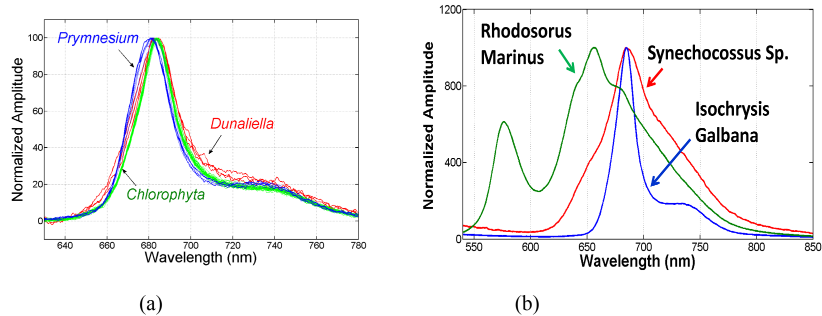
Fig.5. The configuration of the spectrum detection.

In another perspective, we explored the capability of our portable μflow cytometer to study the fluorescence spectrum of individual algae cell. A compact spectrometer, mini-spectrometer C10083CA (Hamamatsu), is used to replace the detection end of the original platform (Fig.5). With the 488nm blue laser excitation, the whole emission spectrum (488nm and above) of individual algae cell can be detected in flow.

Fig.6(a) show the measured fluorescence spectrums of three type of algae cells, Prymnesium, Dunaliella and Chlorophyta. The measured spectrums show close consistence. In one application, the fluorescence spectrum of algae cells can be used to provide further differentiation among different algae species. As an example, Fig.6(b) shows the measured fluorescence spectrums of three types of algae cells, Synechocossus Sp., Isochrysis Galbana, and Rhodosorus Marinus. Their fluorescence spectrums show clearly distinguishable features from each other.

Future Directions

In the coming year, we will further explore the feasibility of using fluorescence spectrum features to differentiate algae species. As the first step, more sophisticated microfluidic fluidic design will be employed to improve the detection sensitivity of cell spectrum. For algae cells with relatively low fluorescence intensity, fluidic channel will be design to slow the flow velocity of the cells and elongate the integration time of each detection. As the second step, we will look into the spectrum features which can be used for differentiation of algae species. A combination of the extinction



(a) (b)

Fig.6. Measured spectrum of individual algae cells. (a) Prymnesium, Dunaliella and Chlorophyta. Spectrum measured in flow. (b) Isochrysis Galbana, Synechocossus Sp. and Rhodosorus Marinus. Spectrum measured in static.

signal (cell size), chlorophyll fluorescence intensity, and the spectrum features could provide useful tags for differentiation in flow, and further be used as tools to study the algae cells' responses to environment. As the third step, we will explore the on-chip algae analysis, where the sample collection, µflow cytometer analysis and waste collection could be integrated on one single chip to largely simplify the analysis procedure.