

Introduction: The effects of global warming, pollution in river effluents, and changing ocean currents can be studied by characterizing variations in phytoplankton populations. We demonstrate design and fabrication of a microflow cytometer for characterization of phytoplankton. Guided by chevron-shaped grooves on a microfluidic channel, two symmetric sheath streams wrap around a central sample stream and hydrodynamically focus it in the center of the channel. Correspondingly, the main purpose of these chevron-shaped grooves is to create a flow path that would allow the surrounding flow to completely ensheath the core flow. This allows accurate collection of data corresponding to an event as it potentially eliminates the issue of having a cluster of particles passing through the detection region at a given time. The lasers 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. Light emerging from these collection fibers is directed through optical bandpass filters into photomultiplier tubes. We successfully characterized different population of phytoplankton using this optofluidic approach.

Experimental setup: Optical excitation of individual phytoplankton passing through the interrogation region produces the light scatter and fluorescence signals. The resolution of this device for discriminating different populations of phytoplankton has been demonstrated using a 488 nm argon laser for direct comparison to a commercial benchtop cytometer. Three PMTs were used to collect data for phycoerythrin (orange fluorescence), chlorophyll (red fluorescence) and light scatter. Four different populations of phytoplankton with different shapes and sizes were used for this study.

Results and Discussion: Finite element analysis was used to investigate the influence of the proportional flow rates (sheath to sample) on the size of the core flow by solving the Navier-Stokes equations and diffusive transport in the microchannel [3]. We have investigated the effects of different core flow rates and the amount of light sources on chlorophyll fluorescence, phycoerythrin fluorescence, and light scatter for different types of algae. Fig. 1 shows the schematic of the whole setup and the microchannel. *Karenia b.*, *Alexandrium*, *Synechococcus* sp. and *Pseudo-Nitzschia* showed chlorophyll peaks at 10^2 , 10^2 , 10^1 , and 10^{-1} fluorescence units respectively.

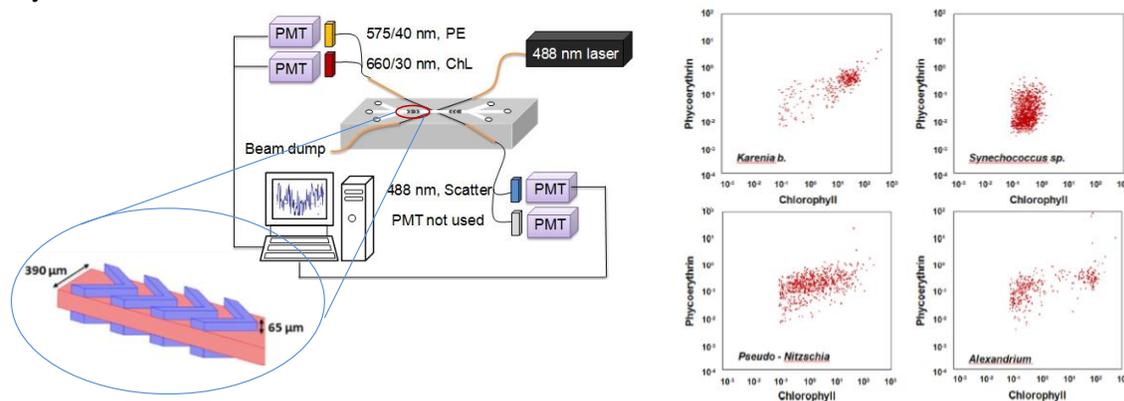


Fig 1. Schematic of the experimental setup and chevron-shaped grooves; Phycoerythrin fluorescence emission vs. chlorophyll fluorescence emission scatter plots

Conclusion: The microflow cytometer is sensitive enough to detect and characterize picoplankton with diameter approximately $1 \mu\text{m}$ and larger phytoplankton of up to $80 \mu\text{m}$ 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.

References:

[1] Hashemi, N, et al. "Optofluidic characterization of marine algae using a microflow cytometer" *Biomicrofluidics*, 5, 032009 (2011)
 [2] Hashemi, N, et al. "Microflow Cytometer for optical analysis of phytoplankton" *Biosensors and Bioelectronics*, 26, 4263-4269 (2011)
 [3] Hashemi, N, et al. "Dynamic Reversibility of Hydrodynamic Focusing for Recycling Sheath Fluid" *Lab on a Chip*, 10, 1952-1959 (2010)