Plankton Analysis by Automated Submersible Imaging Flow Cytometry: Transforming a Specialized Research Instrument into a Broadly Accessible Tool and Extending its Target Size Range

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LONG-TERM GOALS

Detailed knowledge of the composition and characteristics of the particles suspended in seawater is crucial to an understanding of the biology, optics and geochemistry of the oceans. The composition and size distribution of the phytoplankton community, for example, help determine the flow of carbon and nutrients through an ecosystem and can be important indicators of how coastal environments respond to anthropogenic disturbances such as nutrient loading and pollution. Our goal is to provide researchers with instruments to continuously monitor phytoplankton community structure and investigate questions about the world's ocean ecosystems.

OBJECTIVES

Flow cytometry is one of the most promising technologies for studies of the microscopic constituents of marine ecosystems. The intent of this proposal is twofold: to commercialize a field-proven state-of-the-art submersible imaging flow cytometer (Fig. 1; Olson and Sosik 2008; Sosik and Olson 2008) for nano- and microplankton so that other researchers can utilize this exciting new technology, and to develop a next generation of the instrument with extended measurement range, capable of analyzing cells from pico- to microplankton.

APPROACH AND WORK PLAN

Proposed approach - We will develop a prototype commercial version of Imaging FlowCytobot in a close collaboration between the WHOI developer/users and Cytopeia engineers, reproducing its functions via a series of modular components whose integration will result in a simple and robust instrument that is both reliable and easy to manufacture. The first step will involve a ground-up examination of an existing benchtop version of Imaging FlowCytobot by the Cytopeia engineering team (at WHOI). This examination will establish design goals for each functional module of the instrument (e.g., flow system, cell detector, imaging system, signal processing electronics, control system). The redesign process will begin with a mechanical backbone analogous to the optical breadboard now used, onto which will be designed core functional modules for cell detection and imaging, to establish a working imaging system that utilizes electronics and fluidics similar to those in the present Imaging FlowCytobot. This approach will enable us to begin to compare performance of

the commercial prototype to that of the original instrument at an early stage of development. Problems with modules or integration (such as incorrect physical layout or optical components) will be corrected by consultation between WHOI researchers and Cytopeia engineers, followed by redesign and fabrication of new modeules. After the image quality of this core system is shown to be satisfactory, we will continue with redesign and evaluation of the other aspects of the instrument. When the benchtop unit upgrade has been satisfactorily tested in the laboratory, we will construct a pressure housing for it and conduct field tests.

Key individuals - WHOI PIs Olson and Sosik, who built and operate the prototype Imaging FlowCytobot, will be responsible for project management and reporting, and will advise Cytopeia, Inc., led by Ger van den Engh, in the flow cytometer transition efforts. Cytopeia engineers (T. Petersen, W. Stodkjke, D. Horner, and B. Loeding) will consult with Olson and Sosik during design of the prototype commercial instrument; lab and field testing of the new design, including side-by-side comparisons with Imaging FlowCytobot, will be carried out at WHOI. Sosik will be primarily responsible for software and data analysis aspects of the project; Olson will be primarily responsible for hardware, deployment, and operational aspects. S. Laney, WHOI Postdoctoral Investigator, will contribute to integration of hardware and software, and user interface development. They will be assisted by Research Associate A. Shalapyonok, who is a WHOI-certified diver and has extensive experience operating and maintaining Imaging FlowCytobot, and Research Assistant E. Crockford, who will carry out supervised monitoring and testing. At University of Washington, E. Armbrust's laboratory will investigate approaches to efficiently obtain both large- and small-dimension laser spots (dual beam), for simultaneous detection of pico- and microphytoplankton, using a newly-developed position sensitive detector.

Work plan - In the coming year, WHOI researchers and Cytopeia engineers will carry out a detailed review of each function of Imaging FlowCytobot (in person and via Internet) and finalize a plan for development. Cytopeia will fabricate a mechanical frame (analogous to the optical breadboard now used) and flow cell/laser and condenser/detector modules that will mate to this frame. These will enable the researchers at WHOI to assemble a working imaging system, utilizing electronics and fluidics similar to those in the existing Imaging FlowCytobot. This system will be used to begin an iterative process of module implementation and evaluation at WHOI to guide the optimization of these modules (Cytopeia). Development of the data acquisition and processing software will be an ongoing component of the project development cycle. First stage development will focus on adding image capture capability to existing acquisition software for the Cytopeia inFlux. The UW developers of the position sensitive detector will investigate approaches to efficiently obtain both large- and small-dimension laser spots (dual beam), for simultaneous detection of pico- and microphytoplankton.

WORK COMPLETED

Project PIs and senior personnel met at WHOI (in October 2008) to outline the strategy for transitioning imaging flow cytometery to a prototype commercial product. Target goals for system improvement were identified along with methods for achieving payload, power, and cost savings. We also discussed preliminary ideas for integrating the picoplankton (position sensitive) detector into the Imaging FlowCytobot design. At WHOI, investigations have begun into replacement of the power-hungry syringe pump by a custom design, replacement of the xenon flash lamp by LEDs, substitution of a new syringe design that should have greater endurance, and substitution of the miniature gear

pump for sheath flow by one with greater endurance. We have also identified a new low-power computer system to investigate. At Cytopeia, electronic and opto-mechanical re-designs are underway.

RESULTS

The first months of this project, which started in July 2008, were mostly devoted to planning. We have identified ways to reduce the instrument's power requirement by nearly half, which will significantly enhance its utility for non-cabled platforms. Likewise, we have identified improved syringes and gear pumps that will allow longer deployments, and an improved opto-mechanical design that will be more rugged and user-friendly.

IMPACT AND APPLICATIONS

Economic Development

The imaging flow cytometer represents a potential new product line, since it has utility for plankton ecologists studying plankton processes (including effects of pollution and climate change), and also for water resource managers (as a means to monitor harmful algal species).

Quality of Life

Species-level information is critical for such societally important problems as understanding the regulation and fate of regional harmful algal blooms. At the global scale, it is becoming increasingly evident that simple nutrient-phytoplankton-zooplankton models are inadequate for predicting effects of environmental change and that biogeochemical functional groups such as nitrogen fixers, silicifiers, and calcifiers need be resolved. We presently lack observational capabilities to provide data for building and evaluating models, as well as for developing new approaches such as satellite-based remote sensing approaches to monitor functional group distributions. Widespread availability of instruments such as Imaging FlowCytobot will be an important step to overcoming present observational limitations.

Science Education and Communication

The images of individual plankton cells provided by these instrument, remotely and in near-real time, should comprise effective components of educational programs about the oceans, both in science curricula and for the general public.

TRANSITIONS

Quality of Life

A prototype Imaging FlowCytobot has already provided early warning of a toxic dinoflagellate bloom in the Gulf of Mexico (the first toxic *Dinophysis* bloom observed in Texas waters), allowing timely closure of shellfisheries that prevented human illnesses (Campbell et al. 2008).

Science Education and Communication (Delete this section if there are none)

Images from a prototype Imaging FlowCytobot have been circulated to plankton experts via the Internet, allowing species identification and better interpretation of potential processes behind bloom dynamics.

RELATED PROJECTS

A Submersible Imaging-in-Flow Instrument to Monitor Nano- and Microplankton (NSF Grant No. OCE-0525700, 08/01/2005 - 7/31/2009, $393,140). This project was to complete development and testing of the prototype Imaging FlowCytobot (the subject of the present commercialization project).

REFERENCES (*DELETE THIS SECTION IF THERE ARE NONE)*

Campbell, L., R. J. Olson, and H. M. Sosik. 2008. First toxic Dinophysis bloom observed in the Gulf of Mexico, USA. Harmful Algae News 36: 10-11.

Olson, R. J., and H. M. Sosik. 2007. A submersible imaging-in-flow instrument to analyze nano- and microplankton: Imaging FlowCytobot. Limnol. Oceanogr. Methods 5: 195-203.

Sosik, H. M., and R. J. Olson. 2007. Automated taxonomic classification of phytoplankton sampled with imaging-in-flow cytometry. Limnol. Oceanogr. Methods 5: 204-216.

PUBLICATIONS (*DELETE THIS SECTION IF THERE ARE NONE)*

Sosik, H. M., R. J. Olson, and E. V. Armbrust. 2008. Flow cytometry in plankton research, p. submitted. In D. J. Suggett, O. Prasil and M. A. Borowitzka [eds.], Chlorophyll a fluorescence in aquatic sciences: methods and applications. Springer. In Revision.

Moore, C., A. Barnard, P. Fietzek, M. R. Lewis, H. M. Sosik, S. White, and O. Zielinski. 2008. Optical tools for ocean monitoring and research. Ocean Science. Published for discussion: http://www.ocean-sci-discuss.net/special_issue22.html.

PATENTS

Non-provisional patent application number 11,978,246: Systems and methods for submersible imaging flow apparatus.

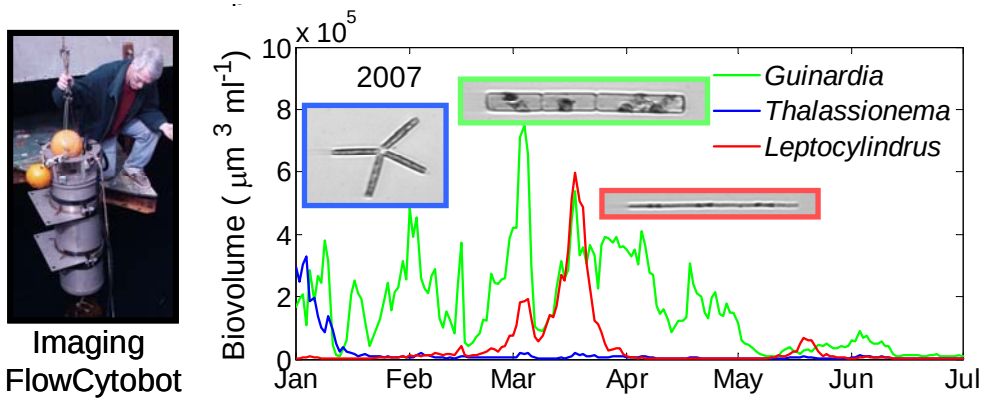

Figure 1. Automated submersible imaging flow cytometry generates high resolution time series of the phytoplankton community at the Martha's Vineyard Coastal Observatory. The left panel shows Imaging FlowCytobot, and the right panel shows daily resolved fluctuations in selected diatom genera that can be quantified with automated image processing and classification of Imaging FlowCytobot observations (Sosik & Olson, 2007); insets show example cell images from the data set for these taxa. Overall in this temperate shelf environment picoplankton dominate in summer and diatoms are important during winter and early spring bloom events, but submersible flow cytometry emphasizes that taxon-specific bloom patterns are extremely variable.