

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 was 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 (Moore et al. 2009; Sosik et al. 2011). The intent of this project was twofold: to commercialize a field-proven state-of-the-art submersible imaging flow cytometer 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

We set out to develop a prototype commercial version of Imaging FlowCytobot (IFCB), reproducing its functions via a series of modular components whose integration would result in a simple and robust instrument that is both reliable and easy to manufacture. The first step involved a ground-up examination of an existing benchtop version of Imaging FlowCytobot. This examination established design goals for each functional module of the instrument (e.g., flow system, cell detector, imaging

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system, signal processing electronics, control system). The redesign process began with a mechanical backbone analogous to the optical breadboard now used, onto which have been designed core functional modules for cell detection and imaging, to establish a working imaging system that utilizes electronics and fluidics analogous to those in the research prototype Imaging FlowCytobot. This approach enabled us to compare performance of the commercial prototype to that of the original instrument, and to identify problems with components or integration (such as incorrect physical layout or optical components). We completed the first beta unit of the new design in July 2011 (Figs. 1, 2), and tested it during a several-week deployment off the WHOI dock. The results of this test were satisfactory in terms of image quality (Fig. 3) and general functioning of the instrument; several electronic and software faults were identified and corrected.

This project was conceived and funded as a collaboration between the WHOI researchers and Cytopeia, Inc., a well-respected manufacturer of high-speed sorting flow cytometers. Although the market for Cytopeia's products was the biomedical community, the founder/president had long been interested in marine applications of flow cytometry, and had sold several slightly-modified instruments (called Influx Marina) to oceanographic institutions for phytoplankton research. Cytopeia expressed interest in a partnership to develop IFCB into a commercially viable product (i.e., smaller, more robust, user-friendly, and manufacturable), and offered to contribute gratis a significant amount of engineering time and machining to the redesign effort.

However, soon after the project's start, Cytopeia was acquired by a much larger company, BD Biosciences, and it gradually became clear that under the new corporate structure IFCB redesign was not a high priority. In the 2nd year of the project, after many delays and relatively little contribution from the Cytopeia (now BD) engineers, we were told that only stock Influx parts could be used in the new IFCB. This development made it clear that the project had to take a new tack to succeed, so we began searching for a new commercial partner, while continuing our own redesign efforts at WHOI.

We approached McLane Research, Inc. as a potential commercial partner at the beginning of 2011. We knew of McLane through our WHOI Biology Department colleague Don Anderson, who was funded by NSF to purchase several Environmental Sample Processors (ESP), to be manufactured by McLane. The ESP was developed at MBARI by C. Scholin, in a process somewhat similar to ours with IFCB, and McLane is now successfully manufacturing these units. WHOI and McLane have entered into a formal licensing agreement to build IFCBs, and McLane is now working on their first IFCB.

WORK COMPLETED

We have achieved our design goals by utilizing smaller, more efficient components: 1) We replaced the camera and its 2-board framegrabber by a new, smaller GigE camera which requires no framegrabber; 2) We replaced the PC104+ computer by a new model based on the Intel Atom processor, which uses 1/3 the power of the old computer; 3) we replaced the flash lamp module and its power supply by a new model that is smaller, takes less power, and requires no separate power supply. We also designed our own opto-mechanical system and compact syringe pump, and (working with an outside consultant) have implemented custom electronics for instrument control and data acquisition that reduce the number of boards in the system. The same consultant has supplied new software. The current IFCB is half as heavy and uses 1/3 the power of the research instrument, and is more stable and simpler to use. The basic redesign has thus met our targets for instrument size and power consumption; optimizing for

manufacturability is now underway with our commercial partner, McLane Research, Inc. McLane engineers observed the construction by Olson of the a new IFCB beta unit.

RESULTS

The new design incorporates a compact and rigid optical structure, a new prototype syringe pump/distribution valve system, a new energy efficient computer and camera, and newly designed data acquisition and instrument control electronics. These changes combine to reduce the instrument's size and power requirement significantly, which enhance its utility for non-cabled platforms. We also obtained improved syringes and implemented a dual gear pump system to increase reliability and reduce maintenance costs.

We completed two beta units. One of these units is being used in the laboratory for testing and software development. The other was successfully deployed for several months in Spring 2012 in Salt Pond (Nauset, MA) to study a bloom of the toxic dinoflagellate *Alexandrium*, in collaboration with Dr. Mike Brosnahan at WHOI. This deployment, in a national park where it was not possible to lay a power cable, was facilitated by the reduced power consumption of the new design: IFCB was deployed from an independent mooring (Fig. 4) equipped with batteries which were automatically recharged when necessary by a gasoline-powered generator (which consumed 0.3 gal gasoline per day). Communication with shore for control and data transfer was via Ethernet radio. The instrument provided continuous data for *Alexandrium* and other phytoplankton species throughout the bloom period (Figs. 5 – 8); the data was downloaded hourly for archival and posting on a publicly-accessible web site (e.g., Fig. 6; Sosik and Futrelle 2012).

During this project, we also constructed 2 additional IFCBs of the new design with funding from outside users; these efforts contributed to the development of the design and served as additional testing opportunities. The first instrument was built for Lisa Campbell (Texas A&M Univ.), who was using one of the original IFCB units to monitor toxic dinoflagellate blooms in Port Aransas, TX until that instrument was vandalized in Spring 2011; an insurance settlement supported construction of a new instrument. Data produced by the new unit at Texas A&M is also openly accessible at a new web site (http://toast.tamu.edu/ifcb7_new_data/web/dashboard). The second was for Yannick Huot (University of Sherbrook, Quebec, Canada); this unit was delivered and successfully tested in summer 2012. Feedback from both of these users is helping to identify and correct problems with software and operation of the instruments.

IMPACT/APPLICATIONS

National Security

There is potential for this application to be useful for detecting pathogens in water supplies.

Economic Development

The Imaging FlowCytobot represents a new product line with a varied market, since it has proven utility for 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 instruments, remotely and in near-real time, should contribute effective components of educational programs about the oceans, both in science curricula and for the general public. With assistance from COSEE, we have already produced a simple web-based introduction to phytoplankton built around Imaging FlowCytobot data (<http://ifcb-data.whoi.edu/about>).

TRANSITIONS

Quality of Life

Imaging FlowCytobot has already provided early warning of a toxic dinoflagellate blooms in the Gulf of Mexico (the first toxic *Dinophysis* bloom observed in Texas waters, and subsequent *Karenia brevis* blooms), allowing timely closure of shellfisheries that prevented human illnesses. It is now also contributing to new understanding of processes that contribute to toxic *Alexandrium* blooms in New England waters.

Science Education and Communication

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. New web-based accessibility of all images promises to expand these impacts (e.g., <http://ifcb-data.whoi.edu>).

RELATED PROJECTS

This project builds on previous projects in the Olson and Sosik laboratories. See <http://www.whoi.edu/sites/hsosik/> for more details.

PUBLICATIONS

- Moore, C., A. Barnard, P. Fietzek, M. R. Lewis, H. M. Sosik, S. White, and O. Zielinski. 2009. Optical tools for ocean monitoring and research. *Ocean Science* **5**: 661-684
- Sosik, H. M., and J. Futrelle. 2012. Informatics solutions for large ocean optics datasets, p. 1-7. *Proceedings of Ocean Optics XXI*.
- Sosik, H. M., R. J. Olson, and E. V. Armbrust. 2011. Flow cytometry in phytoplankton research, p. 171-185. *In* D. J. Suggett, O. Prasil and M. A. Borowitzka [eds.], *Chlorophyll a fluorescence in aquatic sciences: Methods and applications*. *Developments in Applied Phycology* 4. Springer

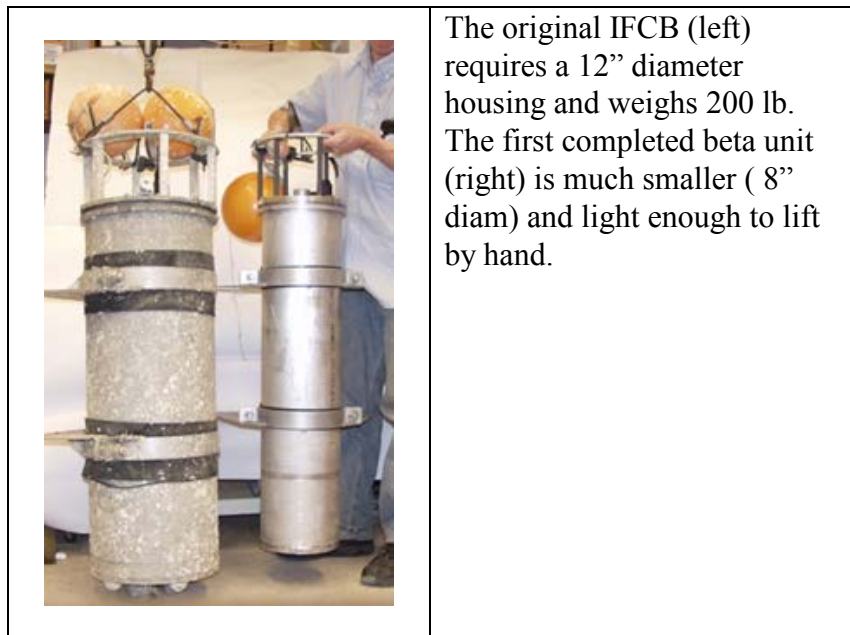


Fig. 1. Original IFCB and first beta unit

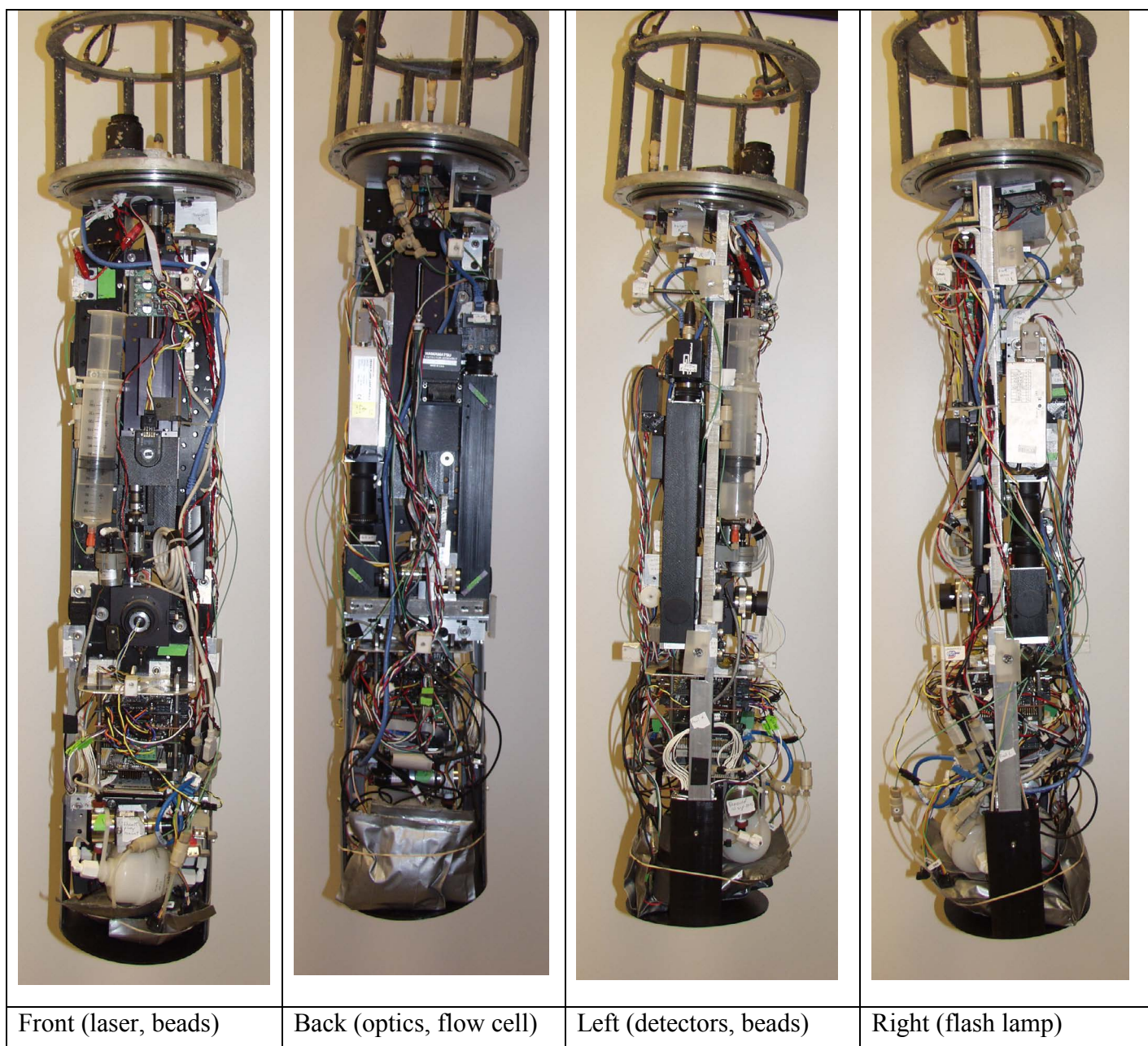


Fig. 2. First beta unit (out of its watertight housing). See figures below for details.

Fig. 2A: Front view

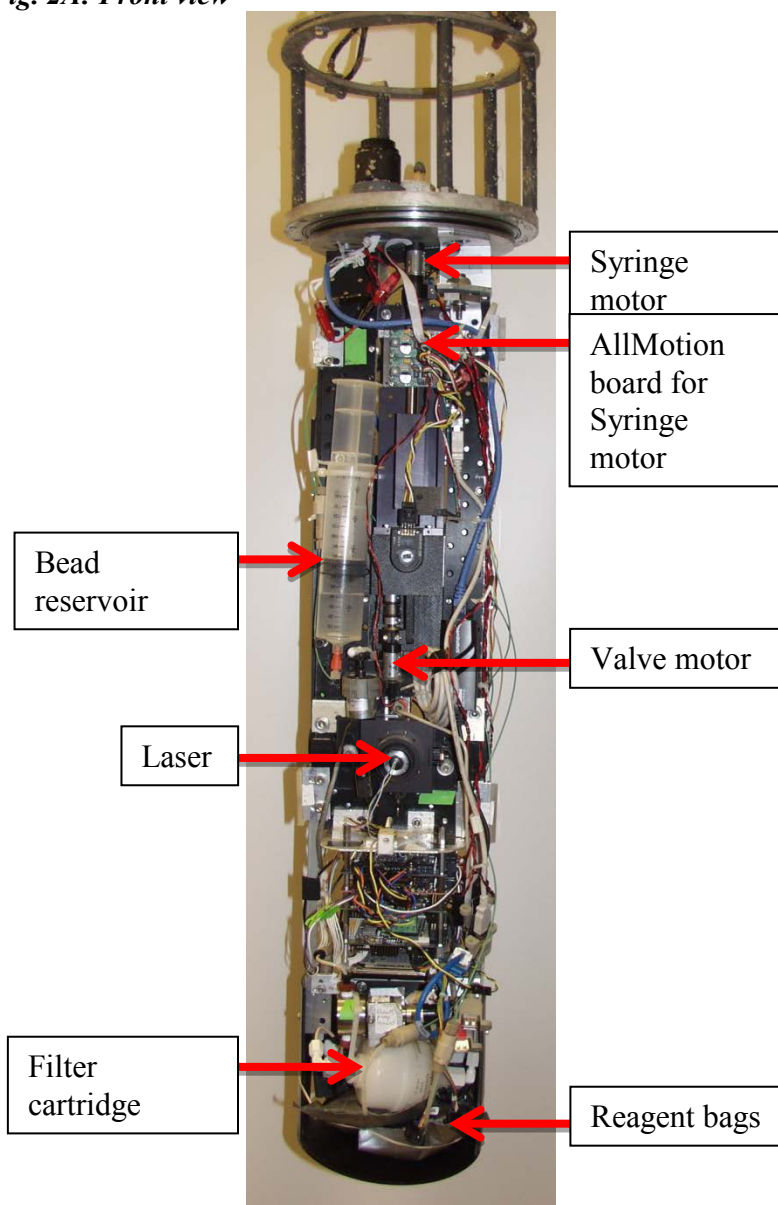


Fig. 2B: Back view

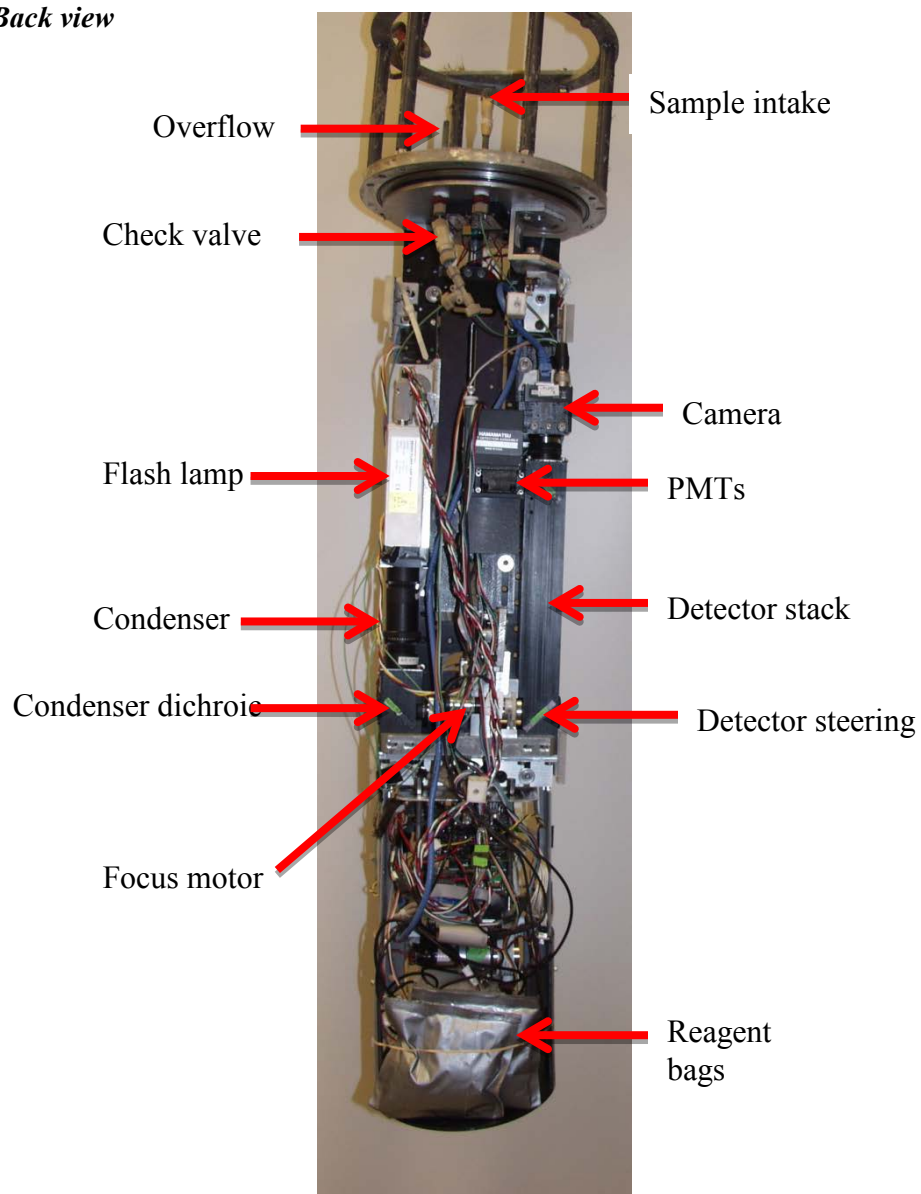


Fig. 2C: Left side view

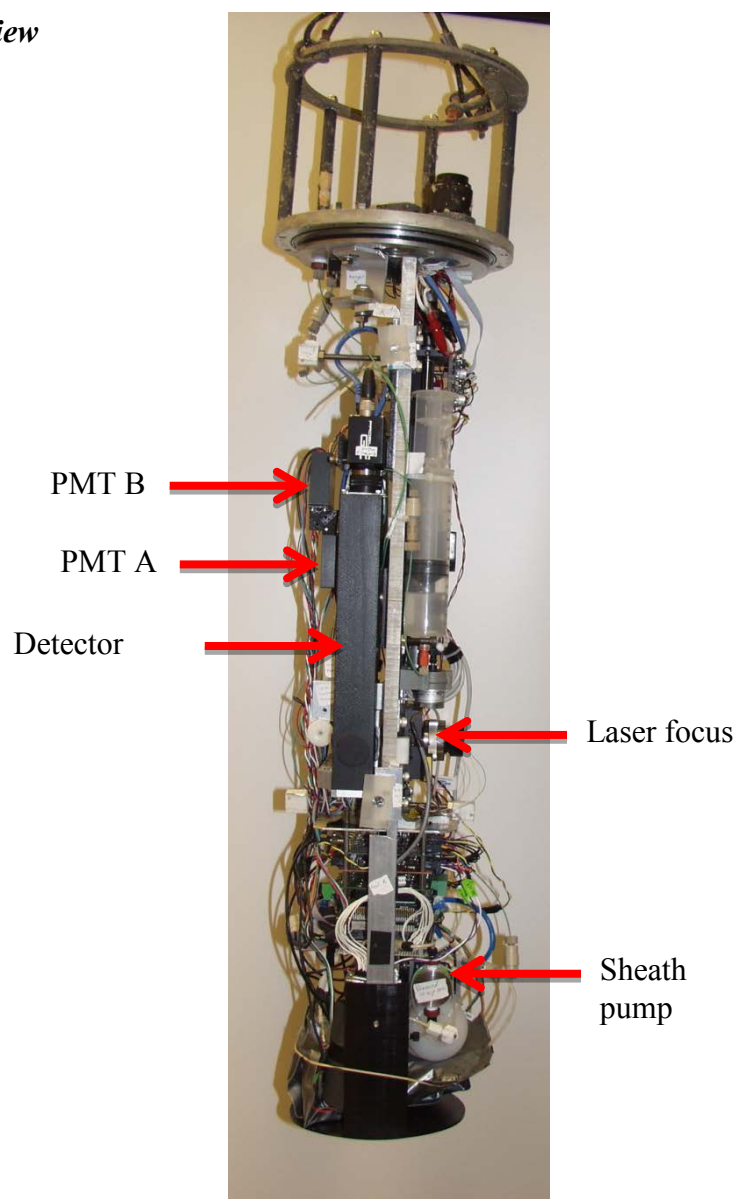
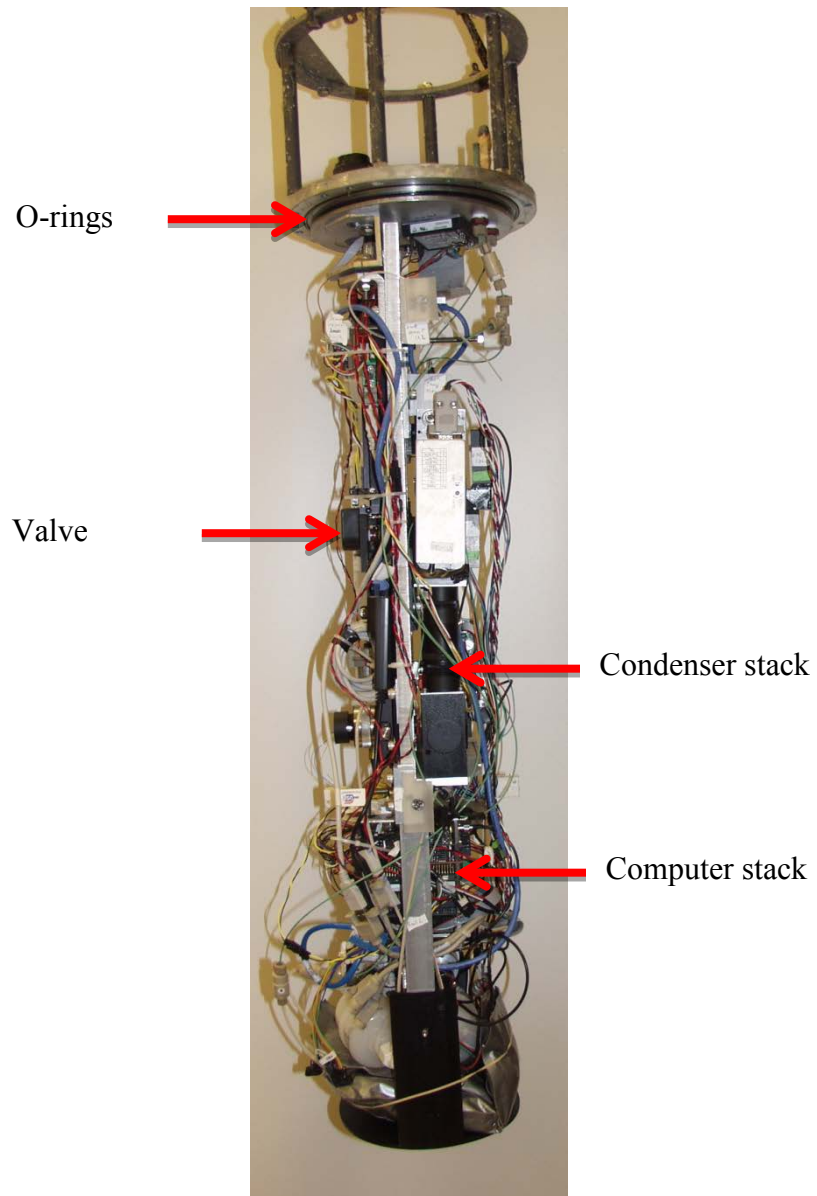


Fig. 2D: Right side view



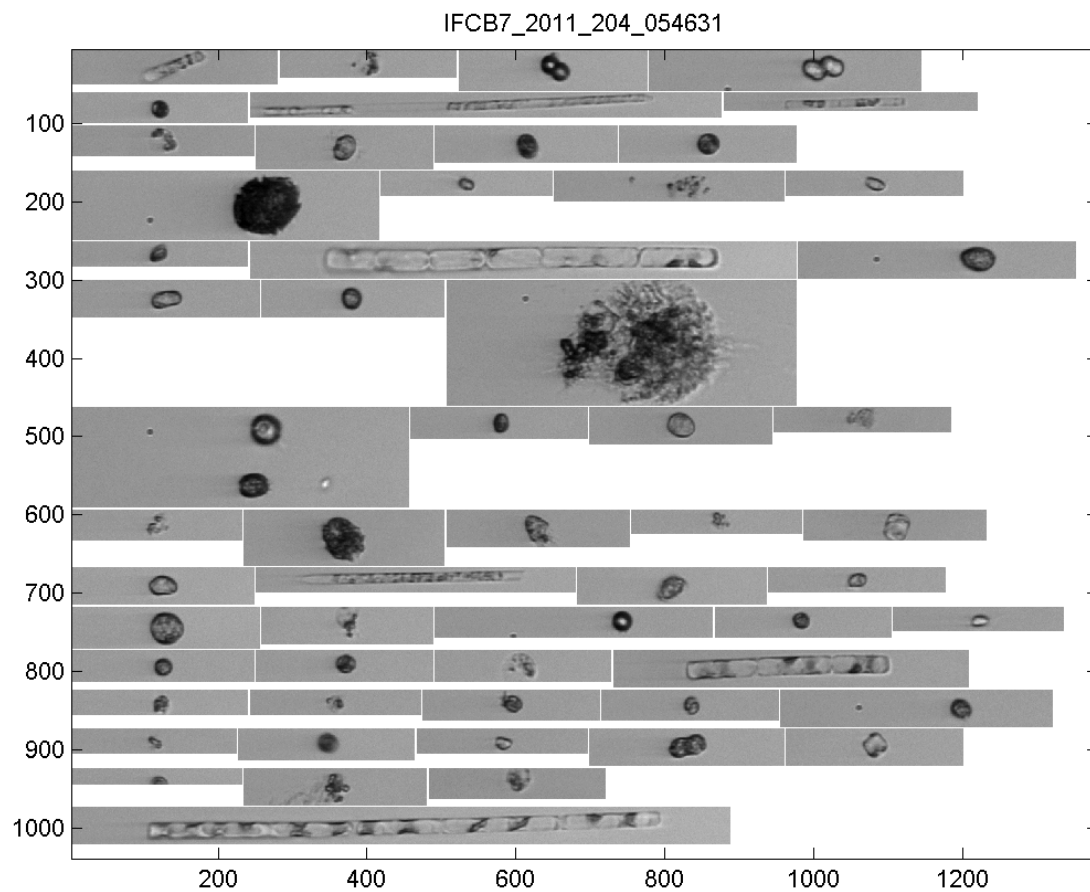


Fig. 3. Typical images from first beta unit during test deployment off WHOI dock, July 2011. Dinoflagellates and chain diatoms dominate the larger phytoplankton in July.



Fig. 5. Deployment of an IFCB beta unit on a cable-free mooring in Salt Pond (Nauset, MA) (Feb – June 2012). The box contains the batteries, generator, control electronics, and Ethernet radio, while the IFCB is suspended below the raft at 4 m depth. The IFCB is connected to the internet via a radio link in the Salt Pond Visitor Center (in background).

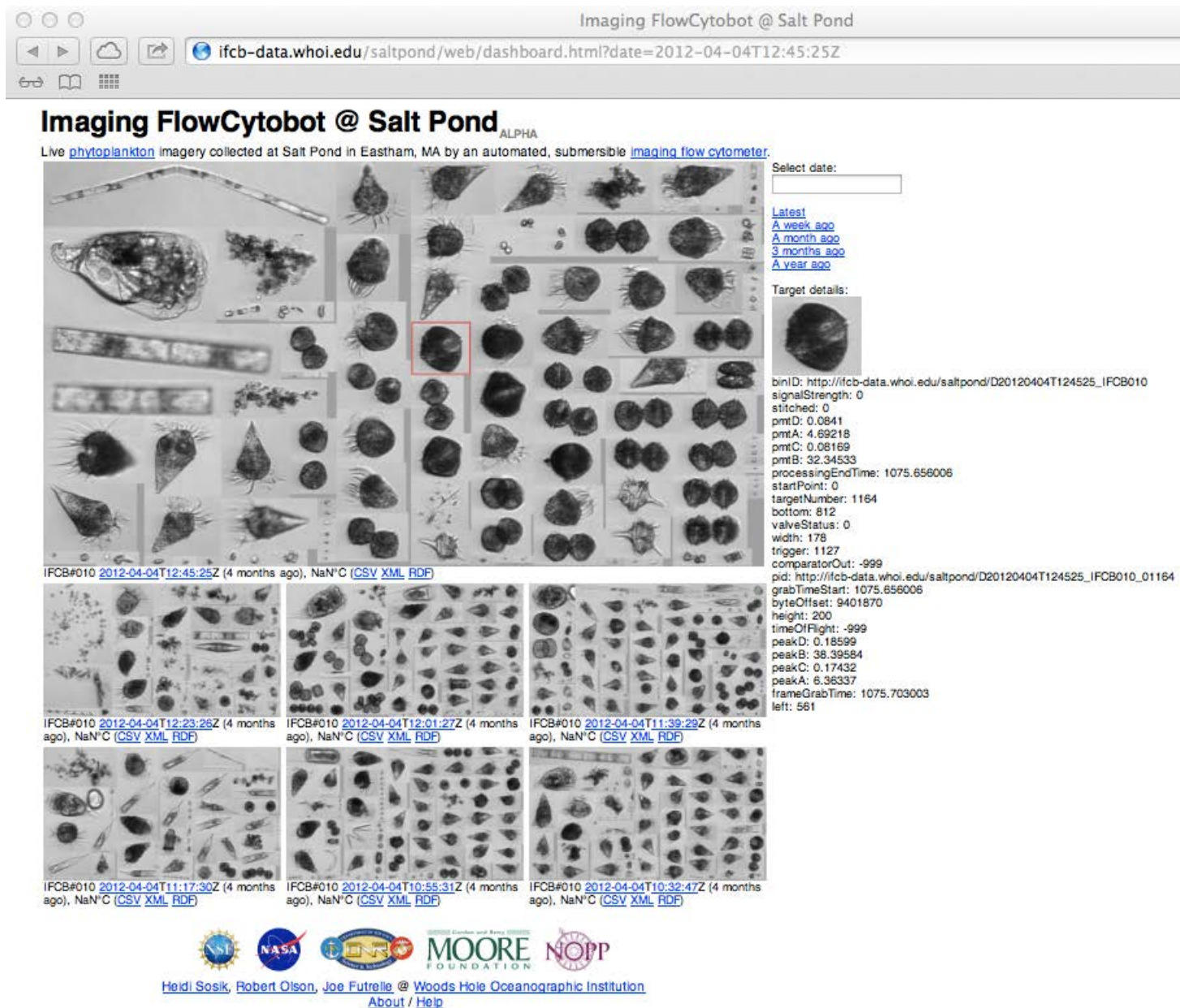
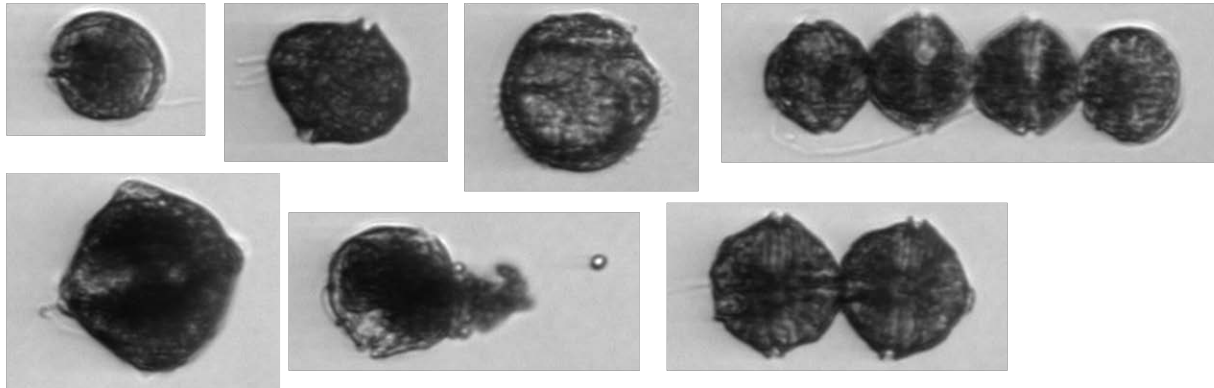


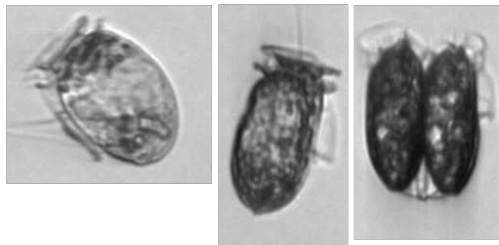
Fig. 6. Data from IFCB in Salt Pond was downloaded hourly and posted on a freely-accessible web page (<http://ifcb-data.whoi.edu/saltpond/>) (Sosik and Futrelle 2012).

Harmful algal bloom species in Salt Pond

Alexandrium fundyense



Dinophysis acuminata



Pseudo-nitzschia spp.

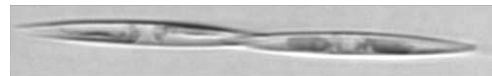


Fig. 7. Images of toxic phytoplankton from the Salt Pond deployment.

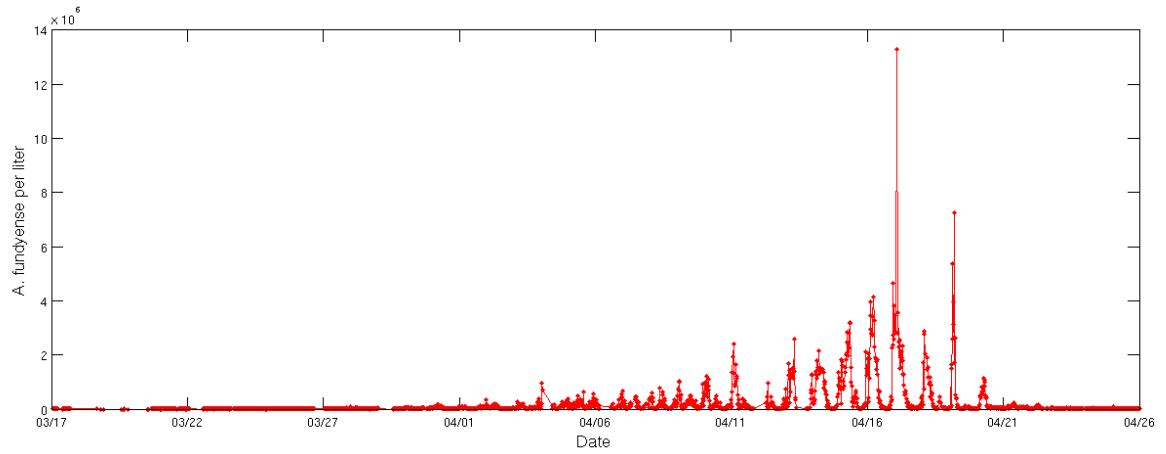


Fig. 8. Time course of the Alexandrium fundyense bloom in Salt Pond in 2012. The diurnal depth migration behavior of this species caused the cells to be sampled for only part of each day by the IFCB at 4 m.