Developing ChemFin^{TM}, a Miniature Biogeochemical Sensor Payload for Gliders, Profilers, and other AUVs

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LONG-TERM GOALS

The first goal of this project involves the further development and transition of ChemFIN^{TM}, a prototype autonomous profiling sensor for chemicals and biomolecules, into a commercial product that can be readily deployed on fixed or mobile ocean observation platforms such as coastal gliders, profiling moorings, and propeller driven unmanned underwater vehicles (UUVs). The second goal of this project is to integrate a flow immunosensor technology (i.e. biosensor), developed by researchers at the Naval Research Laboratory, into ChemFIN for the detection of biomolecules of interest, such as specific biotoxins (i.e saxitoxin) that are released during harmful algal blooms (HABs). ChemFIN is being developed for sustained, autonomous ocean observations of specific chemical and biochemical distributions and spatial and temporal variability. ChemFIN is an evolving compact sensor payload, utilizing microfluidics, and is particularly designed for “low-power” underway measurements on gliders, propeller-driven autonomous underwater vehicles (AUVs) and autonomous profilers.

OBJECTIVES

The first objective is to use recent advances in micro-fluidics and optical detectors to improve the ChemFIN sensor. The technical improvements involve reducing sample flow rates and volumes and thus reagent and power consumption, extending the length of field deployments by developing new technologies to suppress bio-fouling, increasing the ease of use by simplifying operation, pre-packaging reagents and thoroughly documenting the performance by conducting demonstration experiments in coastal waters. The second objective is to adapt and integrate the flow immunosensor analytical technology, developed by NRL researchers, into the MarChem and ultimately the ChemFIN sensor payloads.

APPROACH AND WORKPLAN

These objectives are being achieved through a partnership between industry (Alfred Hanson, SubChem Systems, Inc.) and government (Anne Kusterbeck, NRL). The two partners have prior experience working together to develop and test new biological/chemical sensing and deployment systems. During this project, the industry partner are taking the lead in developing and testing the commercial versions of the MarChem and ChemFIN sensor payloads while the government partner are taking the lead in the development of alternative analytical technologies for the flow immunosensor application and assistance with the testing and performance evaluation.

WORK COMPLETED

Developmental testing was continued this year by SubChem Systems on their newest new submersible chemical analyzer. ChemFIN^{TM} is a small independent sensor payload, utilizing microfluidics, and is particularly designed for "low-power" underway measurements on gliders, propeller-driven AUVs and autonomous profilers. SubChem engineers completed the deign effort and fabricated a new "fourth generation" ChemFIN prototype (Figure 1). The ChemFIN Prototype 4 is configured for the measurement of nitrate and/or nitrite. Laboratory experiments were conducted to evaluate the analytical capability for the newest ChemFIN prototype to measure low concentration levels of nitrites.

Collaborative design work and info-exchange discussions between SubChem and NRL also continued on the integration of the NRL Flow Immunosensor technology into the MarChem Analyzer (Figure 2). NRL efforts focused this year on testing specialized microfluidic coupons for high sample throughput in the immunosensor payload. Work also continued at NRL to identify and synthesize the appropriate toxin molecules, fluorophore labeled conjugates and antibody combinations needed for the sensor assays (Figure 3). NRL scientists and SubChem Systems engineers and chemists spent a week working together in RI during August 2010, deploying the MarChem payload on the REMUS AUV in Narragansett Bay (Figure 4).

RESULTS

1. ***ChemFin Development:*** Based on the findings from testing previous ChemFIN prototypes, the improved fluidic system consists of a flow-through fluidic manifold with several external connections (Figure 1). Leading the system is a sample inlet, calibration standard addition, and two reagent inlets. A single sample pump resides at the end of the system to pull fluid through all the individual paths. The flow rates through the individual reagent and calibration inlets are metered with the use of narrow bore PEEK tubing. This prototype uses a custom sample pump. The wetted materials contained within the pump are resilient enough to withstand the mixed chemicals at the end of the system. Included in the design is a thin film resistive heater which is adhered to one side of the micro-fluidic manifold and used to heat the entire manifold as well as the fluid contained within. The theory is that the fluid moves slow enough that the manifold temperature would translate to the fluid. The results from the power study provided the information required to allow the ChemFIN design to be as low power as possible.

2. MarChem NRL sensor prototype development and testing in the laboratory: Significant collaborative progress was made this year in testing and demonstrating the high throughput microfluidic coupon engineered in FY09 to reduce band broadening via an increased flow rate. Shown in Figure 2 is the fluorescence response versus time for the successive injection of five 100 µl samples of Cy-5 dye ranging in concentration from 25 to 250 ppb through the displacement –based immunoassay system. In this case an evaluation of peak broadening i.e. longitudinal diffusion as a function of the flow rate was targeted, thusly no capture antibodies were present on the channel walls. The injections were performed at a constant flow rate of 0.5 ml/min in the graph below.

Peak resolution of sensor - In order to demonstrate improved performance with the high throughput (HT) chip, several flow rates were evaluated. These data, shown in Table 1, comprise injections performed in both the conventional serpentine microfluidic devices and the high-throughput 39 channel device at the indicated flow rates in the column headers. By increasing the flow rate 20-fold and employing HT microfluidics, a 10-fold reduction in peak width was achieved. The variance in the average FWHM values was less than 5% at each flow rate in all determinations. The overall results demonstrate an improved sensor response time, with results seen in under a minute, and baseline resolution for sample peaks, resulting in better signal to noise ratios. The new format also provides broad operational parameters that allow the sensor to be tuned as needed for different analytes or detection scenarios.

Table 1 – Evaluation of peak width at Half Max (FWHM) for various flow rates

Flow Rate, ml/min	**0.1**	**0.5**	**1.0**	**2.0**
Serpentine	2.084	0.953	0.348	0.172
39-channel	0.836	0.332	0.161	0.106

3. NRL Assay development for Toxins: Domoic Acid – Due to difficulties isolating the single domain antibodies for saxitoxin originally reported in the FY09 Annual Report, an alternative toxin was identified and binding elements were isolated for domoic acid (DA). As shown in the graphs (Figure 3), DA competitive assay were performed using both a biotin labeled llama polyclonal IgG and a polyclonal rabbit anti-DA antibody. A higher titer was observed for the DA than for the Llama and there was also has less background noise. In addition, though the llama titer appears lower, this binding elements responds over a greater range, suggesting that there is a mix of both higher and lower affinity binders present, while the rabbit antibodies seem to have a more narrow range of medium affinity binders.

4. Field demo of NRL/MarChem sensor payload on REMUS: Field trials to demonstrate the sensor payload operation on a REMUS were conducted by SubChem and NRL in August, 2010 during a deployment off the coast of Narraganset RI. For these tests, a standard Venturi apparatus was mounted to the nose of REMUS and served as the plume source for the analyte simulant. The Venturi was used to deliver the analyte at concentrations of 0-220 ppb (estimated), corresponding linearly to flow rates ranging from 0-9 ml/min, respectively. Sampling and recording were continuous over the course of the REMUS mission and could be monitored withweb accessible PCs and cell phones. Shown in the Figure 4 below is the fluorescent response of the sensor with the HT microfluidic coupon as it detects the presence of the analyte in seawater while the REMUS is underway. The survey map corresponds to a composite of the REMUS mission track and the analyte concentration.

IMPACT/APPLICATIONS

The oceanographic community does not currently have the capability to make routine and sustained biogeochemical measurements, *in situ* and autonomously, at the same space and time scales that are possible for temperature, salinity, oxygen, and chlorophyll fluorescence. In recent years, though, there has been significant progress in the development and application of reagent-based optical chemical sensors. The on-going research for this NOPP project is giving us the opportunity to further develop, improve and demonstrate these autonomous chemical sensing technologies. These efforts represent substantial advancements in the development of this technology and bring us much closer to a demonstrated capability for sustained, autonomous ocean observations of biogeochemical distributions and variability.

RELATED PROJECTS

SubChem Systems recently completed their effort on an FY09 Phase 1 SBIR Contract from NAVSEA (N6553809M0064) for Topic N091-46 “Compact, Lightweight Chemical Sensor for Underwater Explosive Ordnance (EOD) Application”. WET Labs, Inc., SubChem Systems, Inc. and other partners also have a FY08 NOPP project “Long-term in situ chemical sensors for monitoring nutrients: phosphate sensor commercialization and ammonium sensor development”.

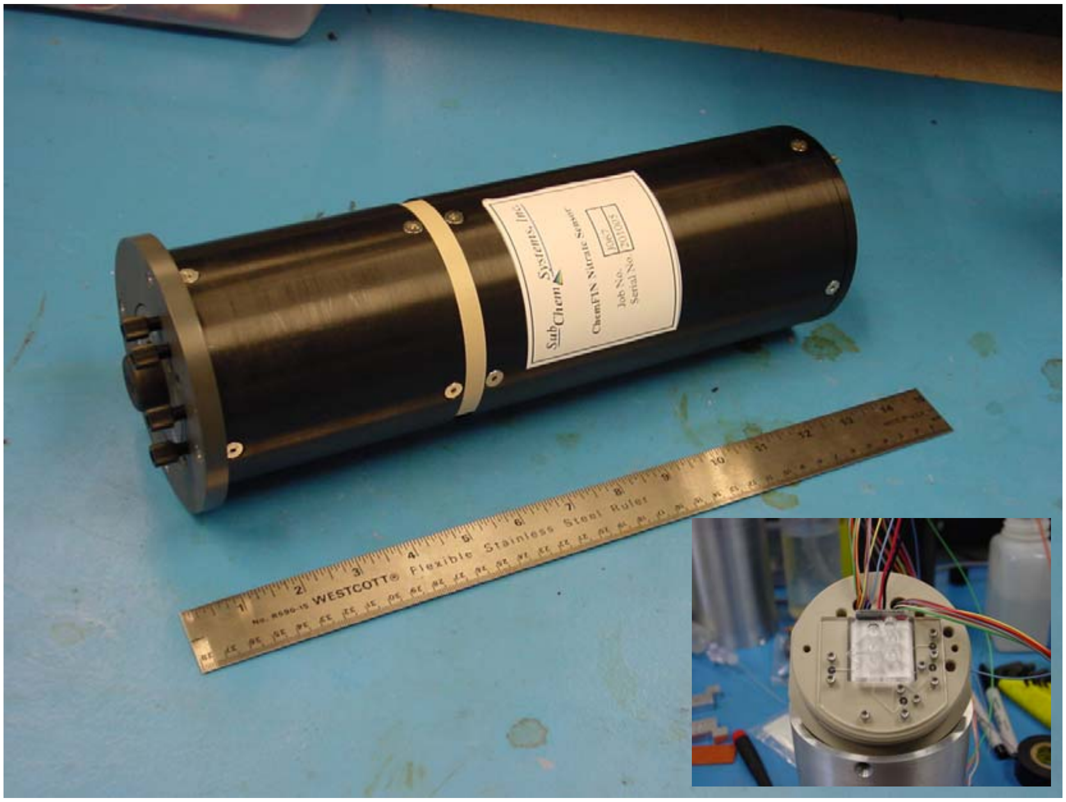

Figure 1. SubChem Systems's compact design for ChemFIN^{TM} the next generation chemical sensing payload for AUVs, Gliders and Profilers. The ChemFIN compact microfluidics module is also shown (inset).

[The ChemFIN^{TM} is designed as an independent compact payload containing a micro-fluidic chemical analyzer that minimizes the power and space demands on the AUV platform.]

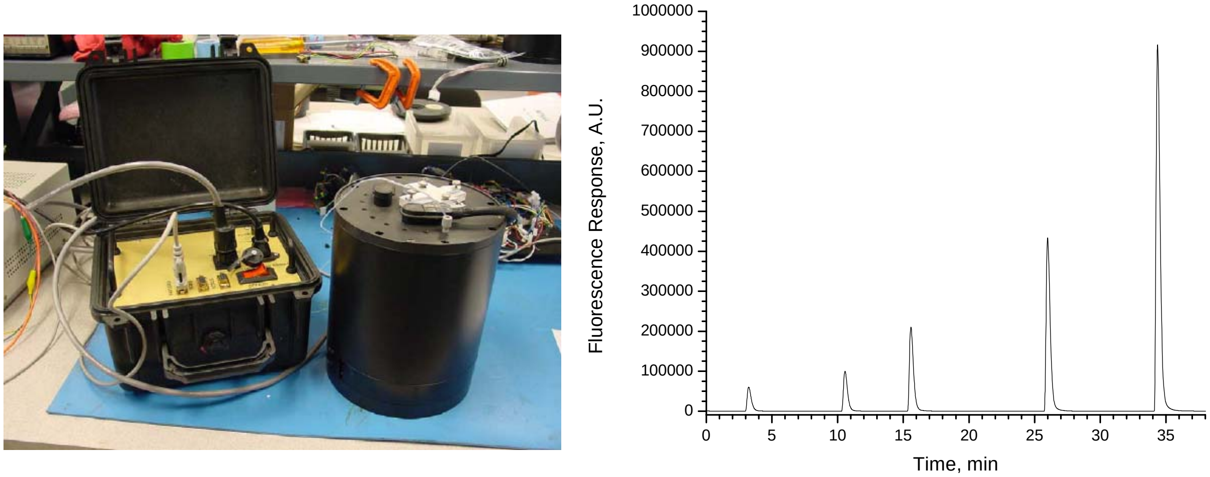

Figure 2. The MarChem Analyzer (left) configured for NRL displacement – based immunoassay with the external integrated face sealing coupon holder and the laser-based fluorescence detection of the CY-5 fluorophore. Shown on the right is the fluorescence response versus time for the successive injection of five 100 µl samples of Cy-5 dye ranging in concentration from 25 to 250 ppb through the system.

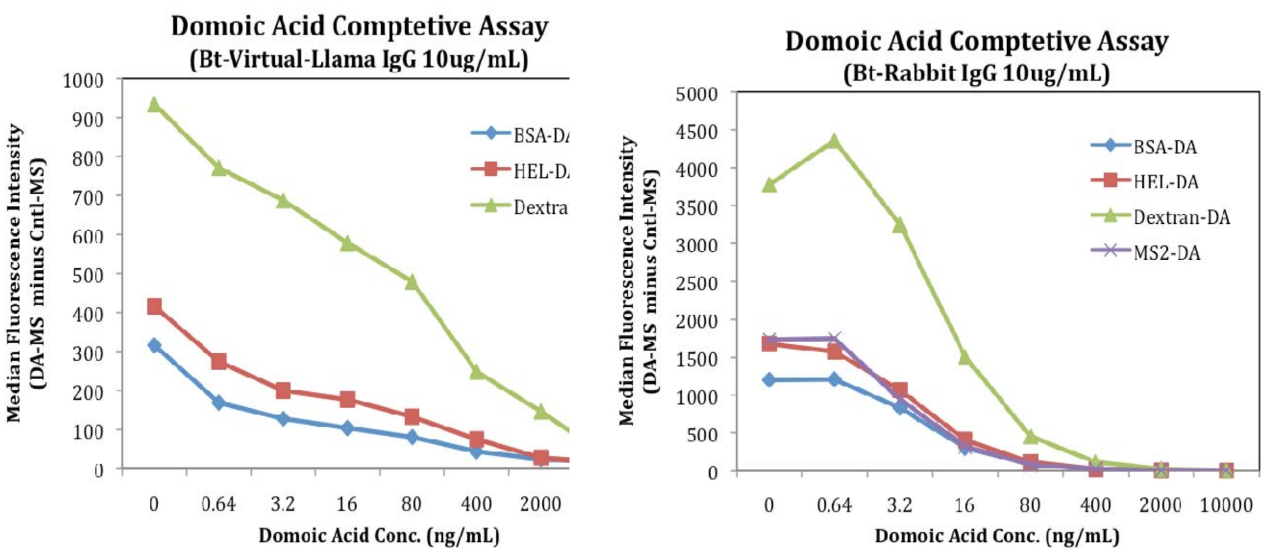

Figure 3. NRL's Domoic Acid Competitive Assay Experiments

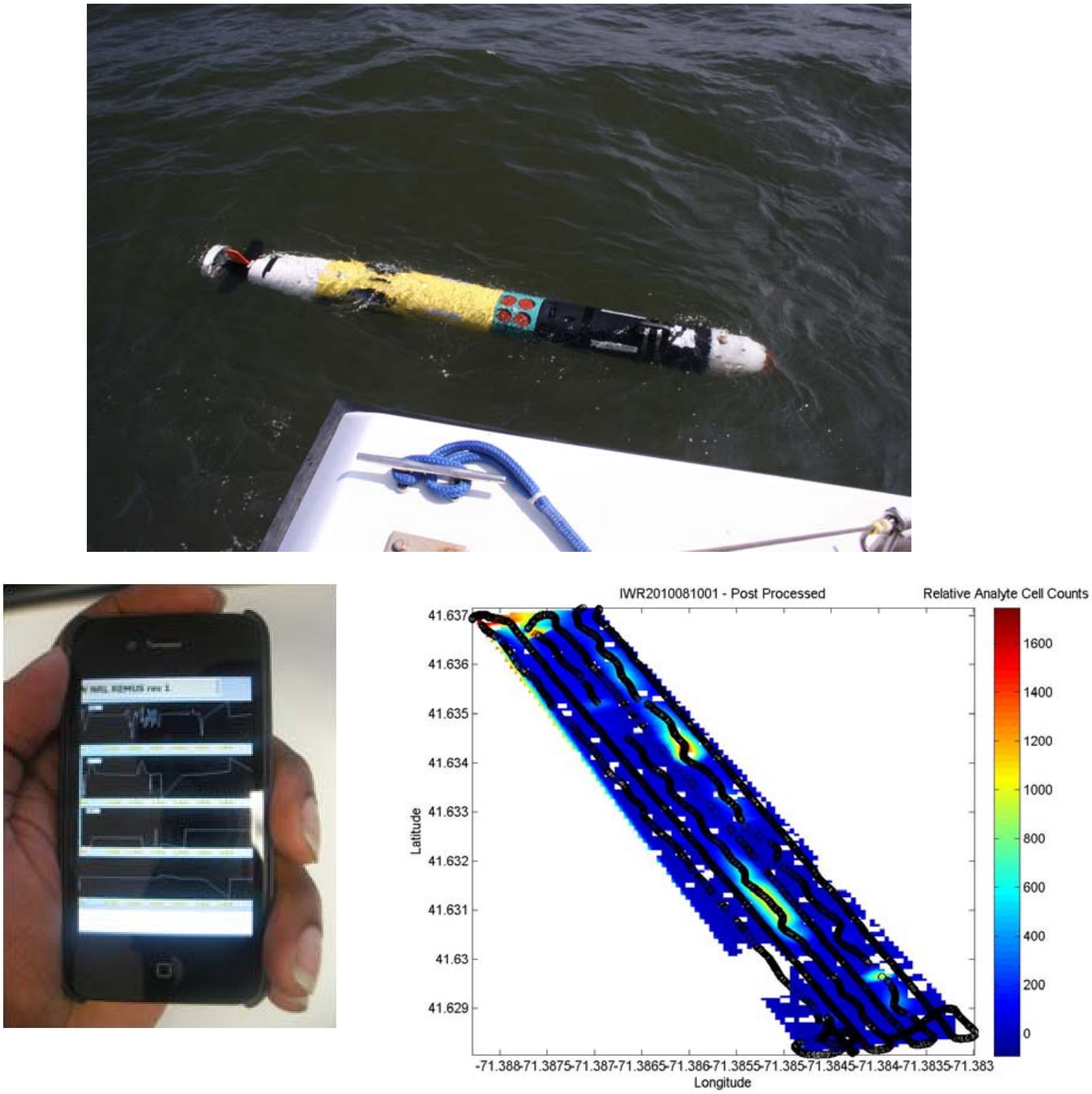

Figure 4. The "Gen 4" Prototype MarChem Analyzer (top photo), configured for NRL displacement –based immunoassay, deployed in Narragansett Bay on the REMUS 100 AUV. A survey map is shown on the lower right. Tracking AUV/MarChem sensor data in real time on a cell phone (lower left).

[The MarChem housing is specifically designed as a compact sensor payload for the REMUS AUV. The plot (lower right) corresponds to a composite of the REMUS mission track and detected analyte concentration.