The overarching theme of the Center's Aquatic application area continues to be the creation and application of a new genre of wireless sensing systems that will provide real-time monitoring capabilities of chemical, physical and biological parameters in freshwater and coastal ecosystems. Temporal and spatial measurements at high-resolution are essential for understanding the highly dynamic nature of aquatic ecosystems and the rapid response of microbial communities to environmental driving forces. Our unique approach to aquatic sensing and sampling, Networked Aquatic Microbial Observing Systems (NAMOS), employs coordinated measurements between stationary sensing nodes and robotic vehicles (surface robotic boats and autonomous underwater vehicles) to provide in-situ, real-time presence for observing plankton dynamics (e.g. chlorophyll concentration, dissolved oxygen), and linking them to pertinent environmental variables (e.g. temperature, light, nutrients, etc.). Sensing and sampling capabilities of the autonomous vehicles are carried out through the development of adaptive protocols, directed through the network. These systems enable the generation and testing of novel hypotheses regarding the processes that control the distribution, growth and demise of aquatic microbial populations.

The primary research foci (Figure 4) within the Aquatic Application during the past year have been: (1) participation in two major collaborative field expeditions to characterize and study harmful algal blooms within the southern California Bight region (Bight ’08 Study; with the Southern California Coastal Water Research Project) and Monterey Bay off the central California coast (CANON: Controlled, Agile, and Novel Observing Network, with Monterey Bay Aquarium Research Institute); (2) characterization and analysis of fine- to micro-scale temporal and spatial distributions of chemical and physical parameters in protected embayments of southern California for the purpose of defining the factors controlling the appearance and distribution of algal blooms; (3) establishment of a collaborative regional-scale program for monitoring the occurrence of harmful algal species in coastal waters off southern California; (4) the development of a portable algae μflow cytometer to expedite research in algal studies using microfluid-based and state-of-the-art detection technology, and (5) a risk analysis-based approach to the design of safe paths for Autonomous Underwater Vehicles (AUVs).

Research to examine the fine- to micro-scale forcing factors affecting microalgal bloom development in protected embayments have focused on the deployment of sensor networks in marinas within the cities of Redondo Beach and Marina Del Rey. These coastal municipalities struggle to maintain high levels of coastal water quality in the face of an increasing chemical and biological contaminants originating from the activities within their own communities, or via the transport of contaminants from inland sources. Responsible environmental stewardship by these municipalities requires an understanding of the factors affecting local water quality data. Our research efforts in these harbors have involved the deployment of sensing capabilities to support observational and experimental studies of the harmful algal blooms in the harbors, and to examine the complex interactions between physical forcing (e.g. tidal movement, light availability) and microalgal physiology, ecology, and behavior (e.g. photoadaptation, vertical migratory behavior) within these harbors.

Our sensor network research in the open coastal ocean represents a larger-scale implementation of a distributed sensing system to study the environmental factors leading to toxic algal blooms caused by phytoplankton species that produce the powerful neurotoxin domoic acid. This component of the Aquatic Application is being conducted in...
Knowledge transfer and outreach activities of the Aquatic Application research group have been accomplished through partnerships with local municipalities (Cities of Redondo Beach, Los Angeles and San Diego), regional water districts (West Basin Municipal Water District, Orange County Sanitation District, Los Angeles County Sanitation District), and universities and joint powers agencies concerned with water quality in the southern California Bight. Collaboration with the Southern California Coastal Water Research Project and Southern California Coastal Ocean Observing System (a regional component of the national Ocean Observing System) are being conducted in Spring 2010 as part of an extensive investigation of water quality within the southern California Bight. Our partnership with local municipalities provides these cities with vital information to aid decision making for preventing harmful algal blooms or ameliorating their impact. Finally, we have worked closely with marine animal rescue and care centers (Fort McArthur Marine Mammal Care Center, San Pedro CA; Pacific Marine Mammal Center, Laguna Beach, CA; International Bird Rescue Research Center, San Pedro, CA; Wetlands and Wildlife Care Center, Huntington Beach, CA; Whale Rescue Team, South Bay, CA) to examine linkages between harmful algal blooms in coastal waters and mass stranding events of local marine animal populations. In this role we have provided numerous interviews to newspapers, radio and television, as well as scientific lectures, on the causes and impacts of harmful algal blooms in the southern California region.

We are working on the development of a to portable µflow cytometer that is suitable for in-field monitoring of algal population and reduce test time. Many of today’s ocean-observing systems provide only rough proxies for algal biomass (e.g. chlorophyll fluorescence, absorption, or backscattering) and cannot distinguish different species. To solve this problem we build a portable algae µflow cytometer system to provide a precise evaluation of the algal population. The µflow cytometer measures individual algal cells for their size, chlorophyll fluorescence and other biological properties, which is important to distinguish different species, especially to resolve the harmful ones among algal 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.

The Southern California Bight (SCB) region is home to the ports of Los Angeles and Long Beach which collectively handle approximately 40% of all US container traffic. This large shipping traffic along with a significant presence of smaller crafts in the ocean necessitates careful path-planning to avoid risking collisions with ships while the vehicle is at the surface. All container ships as well as commercial passenger craft are mandated to transmit their locations to VTS terminals nearby to indicate their location, speed and other parameters using the Automatic Information System (AIS). We have analyzed AIS information from 2009 and 2010 for the SCB and use this processed data along with path-planning algorithms to plan missions for the gliders which reduce risk while traveling between way-points chosen for the scientific mission. Finally, we have been working on AUV-based observation in a Lagrangian frame of reference moving with a feature of interest. Often, the only way to understand an ocean process is to acquire measurements at sufficient spatial and temporal resolution within a specific feature while it is evolving. Examples of coastal ocean features whose study requires such techniques include concentrated patches of toxic algal blooms or anoxic patches of low-oxygen that may cause marine life mortality. To study and track such phenomenon, drifters are often used as proxies which are in turn tracked by robotic vehicles such as Autonomous Underwater Vehicles (AUVs). In collaboration with MBARI’s CANON initiative we have performed a series of Lagrangian survey experiments carried out with drifter relative AUV surveys.
**Overview**

The overarching theme of the Center’s Aquatic application area continues to be the creation and application of a new genre of wireless sensing systems that provide real-time monitoring capabilities of chemical, physical and biological parameters in freshwater and marine coastal ecosystems. Temporal and spatial measurements at high-resolution are essential for understanding the highly dynamic nature of aquatic ecosystems and the rapid response of microbial communities to environmental driving forces. Wireless, networked sensors provide in-situ presence for real-time monitoring of microbial populations, and information that will improve predictive models of community response to changing environmental conditions.

**Approach**

Our networked Aquatic Microbial Observing Systems employ coordinated measurements between stationary sensing nodes and robotic vehicles (surface robotic boats and autonomous underwater vehicles). These activities provide:

1. In situ, real-time ‘presence’ for monitoring environmental variables that are affected by microbial abundances and activities (e.g. chlorophyll and dissolved oxygen concentrations);
2. Linkages between biological activities and pertinent environmental parameters (e.g. temperature, light, salinity, etc.);
3. Improved decisional capabilities for water sample collection; and
4. Information for retrospective modeling of biological events. Sensing and sampling capabilities of the autonomous vehicles are carried out through the development of adaptive protocols, directed to optimize the monitoring of microbial populations and their response to environmental changes.

**Figure 1.** CINAPS (right panel) is the web portal for the observing systems used for the Aquatic Microbial Observing Systems application. Contour plots (4 upper left panels) display a time series of depth-resolved chemical parameters along the coast. Wavelet power spectrum analysis of a tidal record (lower panels) reveals a strong daily and semi-daily periodicity (red bands; bottom panel). Physical forcing transports cells, and also affects light and nutrient availability.
through the network. These systems enable the generation and testing of novel hypotheses regarding the processes that control the distribution, growth and demise of aquatic microbial populations.

**Field Observations and Experiments**

The primary research foci during the past year have been: (1) participation in two major collaborative field expeditions to characterize and study harmful algal blooms within the southern California Bight region (Bight ’08 Study; with the Southern California Coastal Water Research Project) and Monterey Bay off the central California coast (CANON: Controlled, Agile, and Novel Observing Network, with Monterey Bay Aquarium Research Institute); (2) characterization and analysis of fine- to micro-scale temporal and spatial distributions of chemical and physical parameters in protected embayments of southern California for the purpose of defining the factors controlling the appearance and distribution of algal blooms; (3) establishment of a collaborative regional-scale program for monitoring the occurrence of harmful algal species in coastal waters off southern California.

**Accomplishments**

Our sensor network research in the coastal ocean represents an implementation of a distributed sensing system to study the environmental factors leading to toxic algal blooms caused by phytoplankton species that produce powerful neurotoxins. The regional-scale programs (Bight ’08 Study: http://www.sccwrp.org/Meetings/Bight08.aspx, and CANON: http://www.mbari.org/canon/) have been conducted in collaboration with a number of universities, research institutions, regional water districts, animal rescue groups and other entities (see Partnerships). Establishment of a regional harmful algal monitoring and alert system has been performed in collaboration with the Southern California Coastal Ocean Observing System (SCCOOS) (http://www.sccoos.org/data/habs/index.php). These application areas have employed CENS hardware, software, and overall approaches to characterize these toxic algal events, investigate environmental forcing factors leading to toxic algal events, develop predictive models that will establish a causal relationship between environmental conditions and toxin outbreaks, and relate toxic events to mass strandings of marine mammals and sea birds in the region. These projects entail a network of coastal sensor buoys and autonomous underwater vehicles. Advancements in vehicle control accomplished through CENS constitute major improvements in our ability to characterize rapidly evolving biological events in aquatic ecosystems. The work includes the development of algorithms and approaches for transmitting sensed information to shore-based facilities, assimilation the information into predictive models of coastal ocean physics, and use the resulting predictions of feature dynamics to retask the underwater vehicles to optimize their activities (setting new tasks, way points, etc.).

**Outreach and Education**

Education and outreach activities continue to focus on partnerships with local municipalities, regional water districts, marine animal rescue and care centers, as well as sharing of information with collaborators at other universities, research institutions and joint powers agencies concerned with water quality in the southern California Bight (see Partnerships).

**Web Page Development**

We have established a web-based portal (Center for Integrated Networked Aquatic PlatformS; CINAPS: http://cinaps.usc.edu/) for disseminating information collected through our CENS research and collaborative and complementary research programs.

Research to examine the fine- to micro-scale forcing factors affecting microalgal bloom development in protected embayments have focused on the deployment of sensor networks in marinas within the cities of Redondo Beach (King Harbor) and Marina Del Rey. Research efforts in these harbors involve the deployment of senors to support observational and experimental studies of the harmful algal blooms in the harbors, and to examine the interactions between physical forcing (e.g. tidal movement), as well as microalgal physiology and behavior (e.g. photoadaptation, vertical migratory behavior). Datasets generated by CENS and other research programs are also publicly available for viewing and download.

**Future Directions**

Acquisition of new instrumentation (buoys and autonomous vehicles) to expand the activities of the AMOS application has been accomplished through research grants supplemental to our CENS work. Two new Webb gliders, a powered autonomous vehicle (EcoMapper) and two buoys have increased the mobile and stationary nodes comprising our coastal observatory. A major focus of research for the coming year will be collaborative studies with MBARI, JPL, SCCOOS and UCSC to examine the environmental causes of toxic algal blooms in coastal waters of southern and central California, and to develop predictive models of these phenomena. Comparative studies of the regional similarity and differences of toxic algal blooms in these regions should provide insight into the causes of these events, as well as significant improvements in monitoring for their occurrence.
AQU 02 Coordinated Sampling using a Drifter and an Autonomous Underwater Vehicle

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Overview
Studying ocean processes often requires observations made in a Lagrangian frame of reference moving with a feature of interest. Often, the only way to understand a process is to acquire measurements at sufficient spatial and temporal resolution within a specific feature while it is evolving. Examples of coastal ocean features whose study requires such techniques include concentrated patches of toxic algal blooms or anoxic patches of low-oxygen that may cause marine life mortality. To study and track such phenomenon, drifters are often used as proxies (Fig. 1), which are in turn tracked by robotic vehicles such as Autonomous Underwater Vehicles (AUVs). We describe a series of Lagrangian survey experiments carried out in collaboration with the Monterey Bay Aquarium Research Institute (MBARI), with drifter relative AUV surveys.

Approach
Two initial science goals were targeted for Lagrangian patch studies. First to monitor nutrient budget through measurement of flux along the perimeter of a patch (Fig. 2), and second, mapping the entire patch to understand its spatio-temporal dynamics. Motivated by the above goals, we devised approaches that differ only in the survey patterns used. For nutrient flux, we use surveys that focus on patch perimeter; for patch mapping, we use templates that sample in the interior of a patch. Specifically, in this study we extended commonly used oceanographic survey templates with different sampling characteristics to the task of Lagrangian observation studies. We started our study by performing a desired survey pattern (e.g lawnmower) relative to the world frame, centered at the initial observed patch center. Once complete, the survey is repeated at the new patch center (Fig. 3). The results from this approach led us to design a fully Lagrangian survey where the survey pattern is implemented in the frame of reference of the drifter so that the survey pattern is performed relative to the drifter at all times (Fig. 4).

Experiments
Two sets of field trials were carried out. A two-day pilot experiment June 2010 in Monterey Bay, and a ten-day off-shore experiment with science crews on two vessels following a drifter with an genomic-sensor in September 2010.

June 2010 field trials
These were the first trials for the Lagrangian observation studies, and an important goal was to ensure the methodologies described worked smoothly within the logistical constraints of AUV operations. The approach with repeated fixed surveys was tried out.
September field trials

Based on the results of the June 2010 experiments, a ten-day field experiment was carried out in September 2010 a hundred nautical miles off the California coast (Fig. 5) as part of MBARI’s five-year initiative the Controlled, Agile, and Novel Observing Network or CANON. CANON is a science and technology program aiming towards developing a comprehensive set of computational and robotic tools to observe, track, and sample dynamic coastal ocean features. This experiment had multiple goals spread across crews on two vessels, the R/V Western Flyer, and R/V Zephyr. The Western Flyer visited the drifter every four hours to carry out a series of ship-based sampling experiments and lab analysis on water samples. This was done primarily to ground-truth the observations from the drifter's genomic sensor, as well as those observed by the performing Lagrangian observations directed from the Zephyr (Fig. 6). A number of logistical issues were kept in mind while designing and executing the experiment: each iteration began with the latest drifter update (position and velocity) which was transmitted to the AUV for in-situ adaptation. An onboard planning engine synthesized waypoints of the box survey autonomously. These waypoints were not recomputed in-situ so that the ship crew was situationally aware of the robots which was in close proximity to the drifter as well as the support vessels. The survey plan was hence known in advance for the duration of each survey lasting (1 to 1:30 hrs). Fig. 7 shows the box pattern performed by the AUV relative to the drifter for one day of the five-day experiment.

Accomplishments

These experiments were coordinated by an inter-disciplinary team of biological and physical oceanographers, technologists as well as operations personnel. The former articulated the designphilosophy as well as helped formulate the appropriate templates shown in this work. The latter designed the onboard control algorithms and aided in formulating protocols for multi-ship operation in with the close proximity of an unmanned robot and a drifter in the presence of manned vessels. Experimental design was therefore involved an involved effort to meet science, engineering and logistical goals. The work was accepted for publication and presentation in a peer reviewed conference - International Symposium on Experimental Robotics (ISER) 2010. CENS team members played a critical part in the technology development as well as in formulating the experiment design while closely working with MBARI technologists and oceanographers.
Future Directions

Experiments are being planned for March and April 2011 where the level of autonomy will be increased by enabling the AUV to receive drifter locations directly to plan each iteration; one is to ensure that the experiment occurs sufficiently offshore to discount any possibility of drifting towards shallow waters. Second, we are now considering more refined surveys where the AUV is able to compensate for drifter locations during the survey. Currently, the AUV plans a survey in advance, and no adaptation is performed during an iteration.

Figure 7. AUV trajectories in the drifter frame of reference for Lagrangian box surveys during the September 2010 experiment, Day 4. The plot shows nine iterations carried out that day.
AQU 03 A Planning Framework for the Efficient Operation of Slocum Gliders in Coastal Regions

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Overview
We have been involved in collaborative research that uses Slocum gliders to study Harmful Algal Blooms in the Southern California Bight region. The Southern California Bight (SCB) region is home to the ports of Los Angeles and Long Beach which collectively handle approximately 40% of all US container traffic. This large shipping traffic along with a significant presence of smaller crafts in the ocean necessitates careful path-planning to avoid risking collisions with ships while the vehicle is at the surface. All container ships as well as commercial passenger craft are mandated to transmit their locations to VTS terminals nearby to indicate their location, speed and so on using the Automatic Information System (AIS). We have analyzed AIS information from 2009 and 2010 for the SCB (see Figure 1), and use this processed data along with well-established path-planning algorithms to plan missions for the gliders which reduce risk while going between way-points chosen for the scientific mission.

Approach
We construct an occupancy grid map (Figure 4) with a grid size of 500m x 500m. This is the highest resolution that accommodates the largest ships currently operational, while also being comparable to the navigational accuracy of a glider. By this we mean that the, co-existence of a glider (at the surface) and a ship in the same cell on this map is highly undesirable even though it might not necessarily result in a collision. Our goal is to be able to plan paths that reduce the possibility of the occurrences of gliders surfacing in risky regions on the map as far as possible. At the same time we also want to plan paths for the gliders that are useful to scientists running their scientific experiments in the given region. The path-planning algorithm we want to devise should be able to take as inputs a start and end location, as well as a budget on the amount of time the vehicle has to traverse this path, and it should be able to plan feasible paths that try to go between the given locations while sticking to the risk and budget constraints. To construct
the occupancy grid we apply the following equation where \( O(x,y,t) \) is 1 when a given cell was occupied by a ship in the given time segment \( t \). We use \( \gamma \) to weight new observations higher than past observations, where \( 0 < \gamma \leq 1 \) and

\[
\gamma = \frac{\sum_{t'} \gamma O(x,y,t)}{\sum_{t'} \gamma'}
\]

(1)

System Description
We employ two types of path-planning approaches to find paths that satisfy the budgetary, bathymetric and risk conditions. The first is to find the shortest path on the occupancy grid that satisfies the constraints imposed. To write this more formally, the problem we are solving deals with finding a path \( P \) consisting of waypoints \((w_1, w_2, w_3 \ldots w_N)\) where \( w_1 \) and \( w_N \) are given as the start and end waypoints, along with the maximum time \( T_{\text{max}} \), and a maximum allowable risk \( R_{\text{max}} \). The algorithm returns a feasible path \( P \) that satisfies the given constraints.

\[
\text{Time}(P) < T_{\text{max}} \quad (2)
\]

and

\[
\text{Risk}(P) < R_{\text{max}} \quad (3)
\]

where Time and Risk are the expected Time taken by the glider to travel along the path \( P \) while the Risk is a function that determnes the maximum risk for each waypoint along the path.

We employ two methods to find feasible paths. The first involves doing a search for the shortest path between the start and goal waypoints that satisfies equations (2) and (3) using A* search using the heuristic of euclidean distance between waypoints. The second method involves randomly sampling waypoints and considering paths that satisfy the constraints listed above. Here we use two approaches, Probabistic Random Maps (PRMs) and Rapidly-exploring Randomized Trees (RRTs). These methods do not produce guarrante producing shortest paths, but they allow us to quickly sample the space for feasible paths. The PRM method allows us to generate a mesh-like set of highways connecting waypoints in regions with low-risk and then testing these for constraint-satisfaction.

Figure 3: Intermediate output from the PRM planner showing the randomly selected points connecting that can be used to select safe paths. The planner will then choose the nearest nodes and search the graph for the shortest path satisfying the desired constraints.

Figure 4: Occupancy Grid generated for the Southern California Bight region showing the AIS data. Riskier areas which have higher chances of being occupied by ships are shown in white. The official shipping lanes are clearly visible.
the start and end locations that allow safe traversal between the start and end locations. It will keep resampling the space to find a better path that meets the specified constraints. The paths found by the A* algorithm are the shortest paths between the waypoints which satisfies the risk and time-constraints. A* being an exhaustive search takes longer than the sampling-based methods, but seems to find a solution quickly enough to be practically applicable. We also perform A* on a combined cost-function of risk and distance, and try to minimize both subject to the constraint of total allowable time for paths.

Future Directions
A further extension of this problem relates to sequential decision making wherein the path is modified based upon information about ships at the surface. To do this, we plan to use models for ship-movement learnt from prior data to predict likely locations for the same ships into the future and to incorporate these to modify a previous plan for the glider to further reduce the expected risk it might face in the future. Here, the risk-map is probabilistic, dynamic and time-dependent which makes for a more interesting problem.

Publications
The work conducted over the past year, has not been published so far, but will be submitted to the Intelligent Robots and Systems (IROS) conference, 2011.
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.

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

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-a fluorescence, esterase activities (with fluorescein diacetate (FDA) staining), etc. The portable system is assembled in an aluminum case (12” x 9” x 5”). 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.

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, Synechococcus 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
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.

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