Detection and Identification of Aquatic Microorganisms

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Introduction: Understanding and observation of aquatic microbial populations

Ecologically important marine microorganisms

- **Harmful Algal Blooms**
  - Blooms that are toxic to marine life and harmful to human health are increasing nationally and globally.
  - Many bloom-forming algae are small in size and patchy in distribution, making detection and identification problematic.
  - The conditions under which blooms occur and subside are still poorly understood and require massive sampling efforts on both spatial and temporal scales.

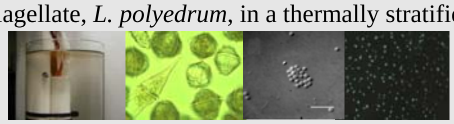
Model systems

- ***Aureococcus anophagefferens***
  - *A. anophagefferens* is a small (2-3 µm) spherical cell which is the cause of so-called ‘Brown Tides,’ discolorations of the waters off the Mid-Atlantic coast
  - Brown Tides have serious impacts on shellfisheries and water quality.
- ***Lingulodinium polyedrum***
  - *L. polyedrum* is a bloom-forming dinoflagellate that causes so-called ‘Red Tides’ off the California coast.
  - Blooms of *L. polyedrum* have been associated with fish and shellfish mortality events

Problem Description: Understanding aquatic microbial population development

Experimental studies in laboratory testbed

- Artificial stimulation of a ‘brown tide’ in a thermally stratified column with a demonstration of predation effects
- Monitoring of diel vertical migration of a red tide dinoflagellate, *L. polyedrum*, in a thermally stratified column



Microorganism sensing and identification

- Flow cytometric identification & enumeration using a fluorescently-labeled monoclonal antibody specific against *A. anophagefferens*
- Identification of single *A. anophagefferens* cells using atomic force microscopy (AFM) and antibody-functionalized tips
- Fabrication of nanowire and carbon nanotube sensors and demonstration of sensing principles for *A. anophagefferens*

Proposed Solution: Laboratory-based population studies and unique detection techniques

Approaches

- **Artificial stimulation of Brown Tide in column testbed**
  - A thermally stratified column was inoculated with a culture of *A. anophagefferens* and its growth was monitored over several weeks.
  - After maximum growth was attained, a predator, *Pedinella sp.*, was added to the column and the population dynamics were followed using flow cytometry and microscopical techniques.
- **Study of diel vertical migration of Red Tide dinoflagellate in column testbed**
  - A thermally stratified column was inoculated with a culture of *L. polyedrum* and its relative vertical position was studied over the course of a week.
  - *L. polyedrum* showed distinct vertical migration over a 24-hour period, accumulating in the surface waters early in the morning and dispersing throughout the column at night (Figure 1).
  - This experiment also allowed for direct comparison between a newly-developed QPCR technique and more classical microscopical techniques for enumeration of *L. polyedrum* cells.
- **Immuno-based flow cytometric approach for enumeration of *A. anophagefferens***
  - A fluorescently-labeled antibody specific to *A. anophagefferens* was used to detect cells in natural seawater samples.
  - This technique is now used for routine analysis of natural water samples (Figure 2A).
- **Detection of *A. anophagefferens* using AFM and antibody-functionalized tips**
  - *A. anophagefferens* cells were immobilized on an Si/SiO2 surface using PEI and the specific monoclonal antibody; AFM tips were functionalized with antibody using an ethanolamine approach.
  - Over 100 force-distance measurements were made of single cells on surfaces containing immobilized cells and surfaces with blocked, non-reactive cells. The f-d curves show a definite difference between these surfaces (Figure 2B).
- **Nanowire and carbon nanotube sensing of *A. anophagefferens***
  - Successfully synthesized single-walled carbon nanotubes and a variety of novel nanowires based on In_2O_3, SnO_2 and CdO.
  - Preliminary tests to confirm the capability of our nanotube transistors to detect *A. anophagefferens*, showing a drop in conductivity following the addition of cells (Figure 3)

Results

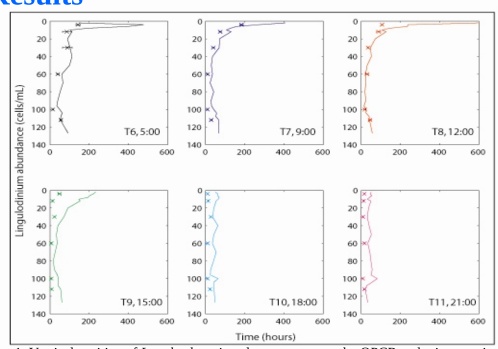


Figure 1: Vertical position of *L. polyedrum* in column as measure by QPCR and microscopical counts

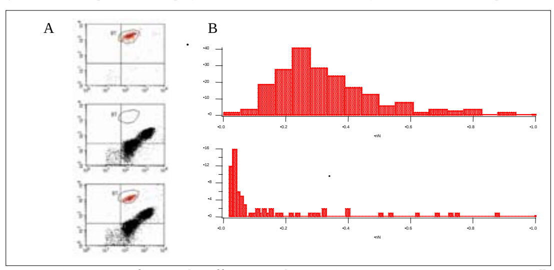


Figure 2: (A) Detection of A. anophagefferens in culture (top), in mixture not containing target cells (middle) and in mixed population containing target cells (bottom). (B) Detection of immobilized A. anophagefferens on a surface using AFM (top) compared to detection of a bare surface (bottom).

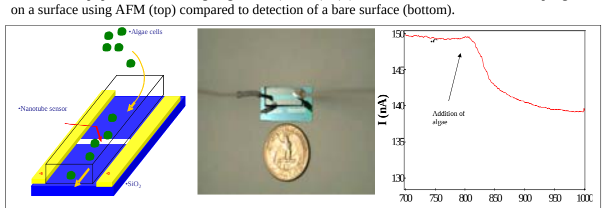


Figure 3: Schematic of carbon nanotube sensor (left) and conductivity profile of sensor following addition of algae (right).