

Detection and Identification of Marine Microorganisms

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Introduction: Detection of Marine Microorganisms and Identification of Important Species

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*.

Aureococcus anophagefferens (Brown Tide Alga)

• Brown Tides of the Mid-Atlantic Eastern US

- Recurrent discoloration of water off Mid-Atlantic coasts caused by *massive blooms* of *A. anophagefferens* ($>10^6$ cells/ml).
- Harmful to commercial shellfisheries, specifically scallops and hard clams
- Small (2-3 μm), non-descript morphology makes traditional counting techniques (e.g. light and epifluorescence microscopy) difficult

Problem Description: Application of New Technologies for Detection and Identification

Flow cytometry and ELISA

• Flow cytometry

- Utilizes fluor-labeled monoclonal antibody specific to *A. anophagefferens*.
- Combines fluorescent and *cell size* information.
- Allows for analysis of single sample in <5 minutes.
- Detects concentrations of 1000 cells/ml

• Enzyme-Linked ImmunoSorbent Assay (ELISA)

- Utilizes highly-specific monoclonal antibody developed against *A. anophagefferens*.
- Allows for analysis of 24 samples in *multiwell plate* format in 4-5 hours.
- Detects concentrations of 5000 cells/ml

QCM and AFM

• Quartz Crystal Microbalance (QCM)

- Utilizes quartz crystals functionalized with monoclonal antibody specific to *A. anophagefferens*.
- Detects adsorption of molecules to the crystal surface as changes in *resonance frequency*.
- Allows for theoretical detection of $\sim 5\text{ng}/\text{cm}^2$ (Q-Sense, Inc.)

• Atomic Force Microscopy (AFM)

- Utilizes silicon AFM tips coated with monoclonal antibody specific to *A. anophagefferens*.
- Images immobilized cells and measures *adhesion forces* between the MAb-functionalized tip and a surface containing target cells.
- Allows for theoretical detection of a single target molecule.

Proposed Solution: Immuno-based Detection Techniques: Flow Cytometry, AFM & QCM

Experimental Design

• Column Testbed

- A bloom of *A. anophagefferens* was stimulated in a 3-meter high glass column of nutrient-rich seawater.
- The growth of BT and distribution with depth was monitored over time using ELISA (Figure 1A).
- *Pedinella*, a known grazer of *A. anophagefferens* was added and the decline of the BT population with depth was monitored.

• Comparison of ELISA and flow cytometric enumeration techniques

- Cultures of *A. anophagefferens* were analyzed using both ELISA and flow cytometric techniques.
- In the absence of grazing, the techniques yielded comparable data (not shown); however, in the presence of grazing, the flow cytometric technique yielded lower, more accurate concentrations (Figure 1B).
- The flow cytometric technique incorporates size information, which ELISA does not, thus distinguishing between whole cells and fragments.

• Quartz Crystal Microbalance (QCM)

- 5 MHz quartz crystals with gold electrodes were functionalized with MAb using a variety of schemes. Following reaction with *A. anophagefferens* in the QCM, crystals were microscopically analyzed and the number of attached cells counted.
 - N-Succinimidyl 3-(2-pyridyldithio) propionate (*SPDP*, targeting amines on the MAb) and 3-(2-pyridyldithio) propionyl hydrazide (*PDPH*, targeting carbohydrates) have yielded the best results.
- Frequency curves, however, have shown only small changes in resonant frequency with the addition of BT cells (Figure 2).

• Atomic Force Microscopy

- BT cells have been immobilized on Si/SiO₂ surfaces using polyethylene imine (PEI, Figure 3B) silicon AFM tips have been functionalized with MAb using the scheme in Figure 3A.
- Force-distance analyses for antibody-coated tips and immobilized *A. anophagefferens* were performed on cell surfaces (Figure 3C top) and bare surfaces (Figure 3C bottom). There is a distinct difference between the force-distance curves; the forces on the cell surface are much higher on average than those on bare Si/SiO₂ surfaces

Experimental Results

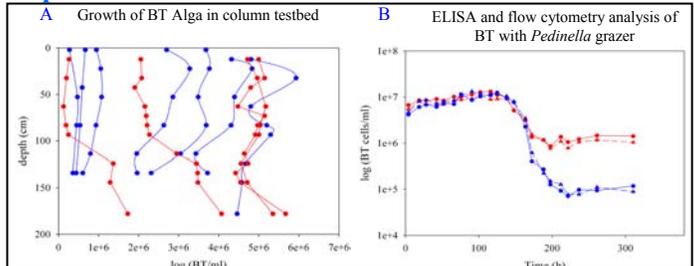


Figure 1: A) BT growth and decline, following addition of *Pedinella* grazer in testbed column. B) Enumeration of BT in culture with *Pedinella* using ELISA and flow cytometry

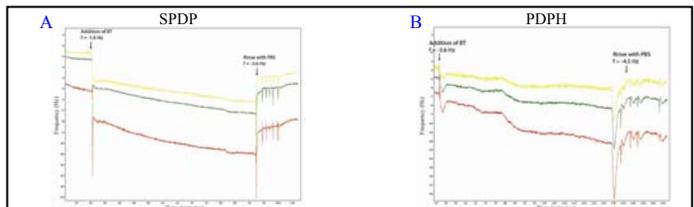


Figure 2: Frequency curves using crystals functionalized with SPDP (A) and PDPH (B). Yellow, green, and red curves are the 3rd, 5th, and 7th overtones, respectively.

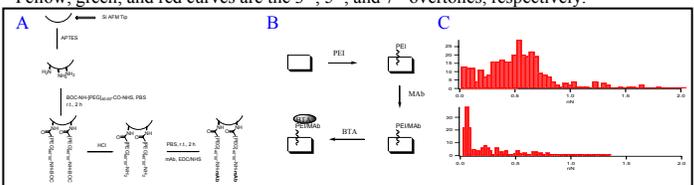


Figure 3: A & B) Functionalization schemes for Si AFM tips & Si/SiO₂ surface with BT cells. C) Force-distance analysis of MAb and BT cells (top: on cell surface; bottom: on bare surface).