Title
Optical Detection of Domoic Acid: a major marine algal toxin

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Optical Detection of Domoic Acid: a Major Marine Algal Toxin

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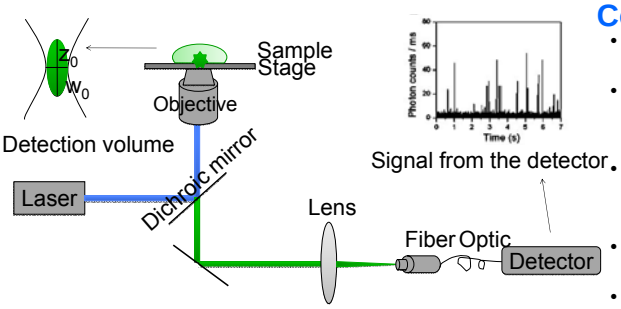
Introduction: Ultra sensitive optical detection principle



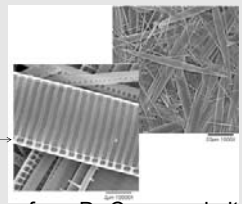
Confocal Laser Induced Fluorescent

- **Spectroscopic method used for detection of selected species down to single molecule.**
- **The sample is exited with a laser.**
  - Monochromatic excitation light makes the Rayleigh scattering easier to be filtered.
- **The excitation volume defined by the high powered objective is small (~1 fL).**
  - noise from fluorescent and spurious background is reduced.
- **Using a pinhole light coming from any plane other than the detection plane will not go to the detector.**
- **The sensitivity is improved by two orders of magnitude to 50 fM.**

Problem Description: Amnesic shellfish poisoning caused by Domoic Acid (DA)

• DA is produced by a number of marine algae, and is accumulated by shellfish filterfeeding during Pseudo-Nitzschia blooms.
• Ingestion of contaminated shellfish causes amnesic shellfish poisoning (ASP), which in severe cases leads to death in both animal and human consumers.
•The limit for DA in shellfish set by the method of *Iverson et all* (1994) is 20 µg/g of shellfish tissue.
•One of the current detection methods for DA is competitive ELISA.
•The sensitivity of competitive ELISA is 0.2 ng/mL for measuring the concentration of DA in organisms so this method is not sensitive enough.
• With the sensitivity of our sensor detection of DA is possible both in sea water and in shellfish tissue.

Pseudo-Nitzschia



Picture taken from Dr Caron website

Proposed Solution: Our optical sensor provides the sensitivity needed for DA detection

Optical DA Sensor

•Assay preparation is suggested by Professor David Caron's group in USC.

•The preparation is done in a chip made in Caltech.

•Sample is excited with laser.

• The laser light is redirected with a dichroic mirror and focused on sample through an objective.

• The same objective collects the light and after going through optics is focused on the fiber optic.

•Information is carried to the APD with the fiber optic.

•A photon burst is detected with the APD each time a fluorescent target molecule passes the detection volume.

•The number of peak points in a period of time is related to concentration of sample.

•The upper limit of detection is set by the size of detection volume and the fact that there should be one or less molecules in the detection volume at each time.

$$\frac{1 molecule}{10^{-15}\ liter} = \frac{1/6.022*10^{23}\ moles}{10^{-15}\ liter} = 1.67*10^{-9}\ Molars$$

•The lower limit is set by the level of noise.

•Peaks higher than noise level plus three standards of deviation of it are considered signals.

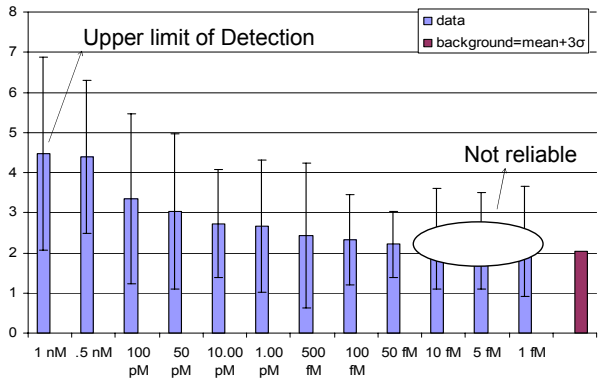
•Signal level drops directly with lowering sample concentration.

Current Results; Peak Counts Versus Concentration

•Test being done with Alexa Fluor 488 F(ab')2 fragment of goat anti mouse IgG (invitrogen) , with DI water as the buffer

•concentrations between 1 nM to 1 fM are tested.

•The sensitivity is improved by 2 orders of magnitude to 50 fM.



Number of the peaks reduces with lowering the sample concentration.

Future work

•Detection of DA in buffer and optimization of the detection process for DA detection in shellfish tissue.

•Detection and study of DA inside the Pseudo-Nitzschia cell.

•Integration of the microorganism analysis system chip made in Caltech for algae growth and DA production and our sensor detection of DA produced by algae.