SPIRIT: A Portable Biosensor for the Early Detection of Harmful Algal Blooms.

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Background and Significance

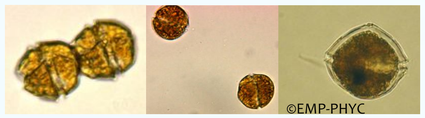


Fig. 1 *Alexandrium catenella, A. tamarense, A. ostenfeldii*

✧Paralytic shellfish poisoning (PSP), caused by consumption of shellfish that have fed on toxic algae, is a major health issue worldwide.[1]
✧Dinoflagellates of the genus *Alexandrium* can produce dangerous amounts of toxins responsible for PSP, even at very low cell densities.
✧Species that produce toxins are difficult to distinguish morphologically from non-toxin producing species (Fig. 1).
✧Current identification methods are expensive, time-consuming, and require special training.[2]
✧Development of a rapid, low-cost, easy-to-use device for detection and monitoring would be an important advancement for human health and prevent costly and unnecessary closures for the shellfish industry.
✧We have developed a biosensor assay for the detection of Harmful Algal Blooms that shows great promise as a rapid field-based assay.

Methods

Surface Plasmon Resonance (SPR)

✧A label-free, optical detection method.
✧Measures the change in refractive index after binding (hybridization) of target to probe on a surface (Fig. 2).[3]

Portable SPR Instrument: SPIRIT

✧SPIRIT was developed with collaborators at the University of Washington (Fig. 3).
✧It employs a miniature sensor module containing a light source, array detector, and modifiable gold-coated surface (Spreeta, Icx Nomadics; Fig. 4).
✧Species-specific probes[4,5] are covalently bound to the gold sensor surfaces.
✧Liquid samples containing RNA from *Alexandrium* cells are flowed over the sensor surface.
✧Assay results are obtained in 10 minutes or less to distinguish between toxic and non-toxic species.

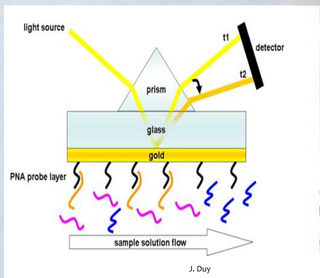


Fig. 2 SPR detection

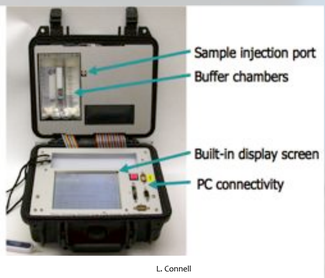


Fig. 3 Custom built field SPR instrument (SPIRIT)

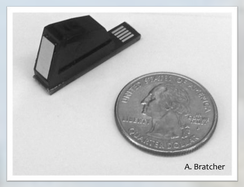


Fig. 4 Spreeta sensor

Results

Validation against Biacore SPR instrument

✧Because the SPIRIT instrument is custom-built, we conducted comparison assays on the industry standard benchtop SPR instrument, Biacore Q (Fig. 8) at Queen's University Belfast, UK.

✧Results indicate that SPIRIT shows higher SPR responses than Biacore (Fig. 6).

Fig. 5 Biacore Q

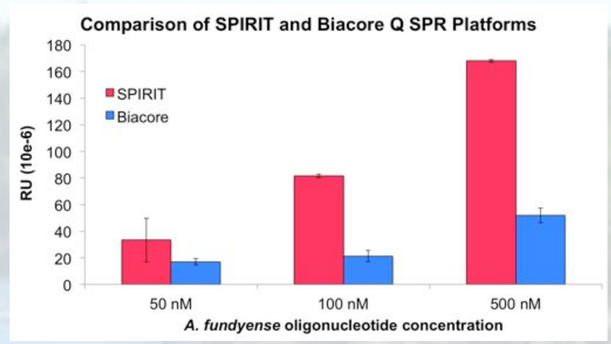
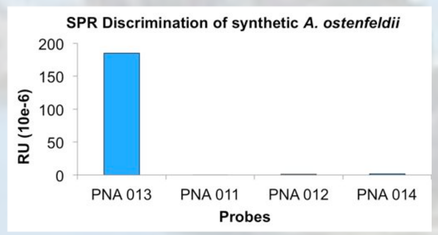


Fig. 6 SPIRIT shows higher SPR responses than Biacore to identical samples of synthetic *A. fundyense* RNA at multiple concentrations.

Specificity

✧Using synthetic DNA from two different species of *Alexandrium* we have confirmed that each probe only detects its target species (Figs. 7a, 7b).

Fig. 7a Probe PNA 013 designed for *A. ostenfeldii* detected *A. ostenfeldii*, with no detection by probes designed for *A. fundyense* or other negative control probes.



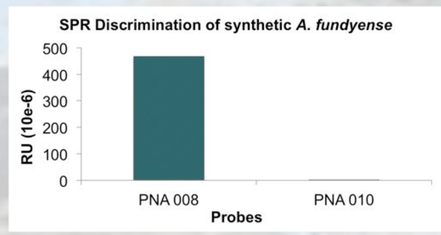
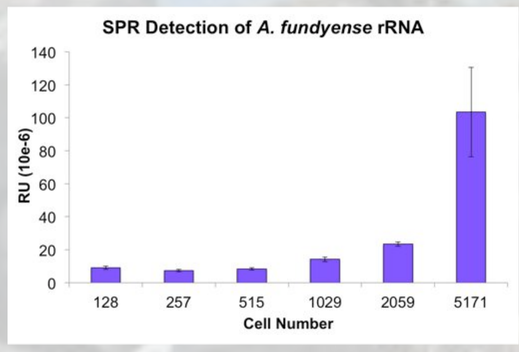


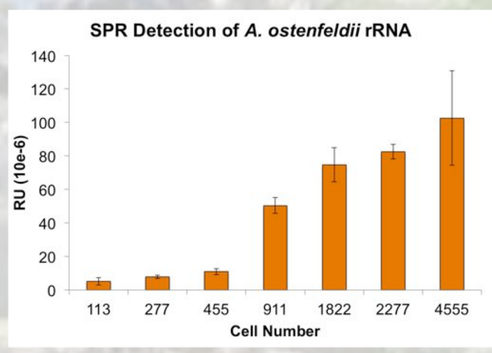
Fig. 7b Probe PNA 008 designed for *A. fundyense* detected *A. fundyense*, with no detection by a negative control probe.

RNA Detection

✧Results from *A. fundyense* and *A. ostenfeldii* grown in culture show the ability of SPIRIT to detect RNA extracted from less than 200 cells, which is the minimum usually required to produce the high toxicity levels that prohibit shellfish harvesting (Figs. 8a, 8b).

Figs. 8a, 8b Mean SPR signal with standard error (background removed) obtained from RNA samples extracted separately from cell cultures of *A. fundyense* (8a) and *A. ostenfeldii* (8b) at varying cell densities.





Conclusions

- **SPR can differentiate between genetically similar species that are difficult to distinguish by morphology.**
- **SPIRIT provides reliable data and is more sensitive than the current industry standard in SPR detection.**
- **SPIRIT can detect fewer than 200 *Alexandrium* cells.**
- **This method shows great promise as a rapid assay for on-site bloom detection.**

Future Work

✧Summer 2012-Test method using field samples from the Gulf of Maine containing *Alexandrium.*

Literature Cited

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[3]E. Kretschmann. Zeitschrift Fur Physik. 241(4) (1971).
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