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Toward in situ detection of algae species

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Abstract:

For the last few years there is a more and more pressing need to develop system for detecting HAB at their early stage. Researchers have then been working on new in situ sensor. In this paper a submarine transducer based on surface plasmon resonance is reported. It demonstrated a refractive index resolution of 3.10^{-6}, which is promising for later phytoplankton detection.

Keywords : Algae , Biosensors ; DNA ; Gold ; Plasmons ; Resonance ; Spectroscopy ; Surface waves ; Transducers ; Underwater vehicles

1. Introduction

Proliferation of some algal species in marine waters can give rise to massive fish kills, seafood contamination with toxins and ecosystem alterations. Although these events referred to as Harmful Algal Bloom (HAB) have always happened, clear evidences show that their impacts on human activities have dramatically increased for the last few years [1]. Then there is a more and more pressing need to develop tools to prevent health threats and economic losses they lead to.

Up to now, species involved in HAB are monitored by laboratory analysis of seawater samples: after a 24h decantation, the samples are observed with a light microscope to determine the cell concentration of each of the species of interest. It requires taxonomy experts, is time-consuming and then expensive.

For few years, researchers have worked on developing *in situ* detection and quantification systems for algae species ([2-4]). In previous work we described a laboratory assay for detecting *Alexandrium minutum* [5] based on PCR product detection by an SPR DNA biosensor. The whole protocol consists of four steps. First, the whole genomic DNA of phytoplankton is extracted from the sample. Second, a 677 base pair long sequence of the 28S ribosomal DNA is amplified by PCR. Third, the double stranded PCR product is digested by an λ-exonuclease to obtain a single strand DNA. Last, the single stranded PCR product is detected by hybridization on our biosensor.

In this paper, a submarine SPR sensor is reported. It was designed for being coupled to a sampling device capable of DNA extraction, PCR amplification and DNA denaturation.

1.1. Surface plasmon resonance

The biosensor is based on Surface Plasmon Resonance (SPR) spectroscopy.

Surface plasmon is an electromagnetic wave that propagates along a metal/dielectric interface. This mode can be excited with photons.

In the Kretschmann-Raether configuration a gold thin film of 50 nm thickness is deposited on a glass prism as shown in fig. 1. A polychromatic collimated light beam illuminates the glass/gold interface with an incidence angle θ. It can be shown [6] that for a given dielectric medium in contact with the metallic surface, the surface plasmon mode can be excited if:

$$\varepsilon_p(\lambda)\sin^2\theta = \mathrm{Re}\{\varepsilon_s(\lambda)\,\varepsilon_m(\lambda)/[\varepsilon_s(\lambda)+\varepsilon_m(\lambda)]\} \qquad (1)$$

with λ the wavelength, $\varepsilon_p(\lambda)$,$\varepsilon_s(\lambda)$ and $\varepsilon_m(\lambda)$ the permittivity of the prism, the dielectric medium and the metal respectively. Re{x} denotes the real part of x.

In this case, the photonic power is transferred to the gold film by resonant coupling. Then, the spectrum of the reflected light present a sharp absorption band, called a dip. This phenomenon only occurs for TM polarized light.
As ε_s varies, the dip shifts. It is then possible to monitor changes of the refracted index as small as 10^{-6} by measuring the spectrum of the reflected light [6]. This is the principle of surface plasmon resonance spectroscopy sensors.

1.2. Biosensors

Biosensors use biological molecules for specifically detecting target molecules. They consist of receptors such as antibodies, DNA, RNA or enzyme immobilized on a transducer, which detects the target fixation to the receptor.

The first SPR based biosensor was reported by Liedberg *et al* [7]. Since then, many systems have been developed. They can detect few tens of $pg.mm^{-2}$ of molecules on a surface in real-time without any labeling. This is a very versatile technique that has been used to detect different kind of molecules (nucleic acids, proteins, toxins) as well as micro-organisms [6].

2. Materials and method

2.1. Submarine system

The submarine system is an SPR transducer. It was designed to be versatile and can be used with different kinds of receptors (DNA, RNA, antibodies,…). The system was developedfor coastal applications down to 100 m.

It is composed of two modules: the analyzing and the sensitive module (Fig. 2).

The former contains a light source (HL-2000, Mikropack), a spectrometer (Jobin-Yvon), a numeric CCD camera (ST3200ME, SBIG), an embedded computer and some electronics. It is connected to the sea surface by marine cables for power supply and communication. Its housing is made of anodized aluminum
The sensitive module is called the optode. It contains optic components, a polarizer, a prism and a substrate. Its housing is of stainless steel. A fluidic system can be connected to this module.

The two modules are connected with optical fibers.

Fig. 3 presents the general architecture of the transducer. Light coming from the polychromatic lamp is launched into an optical fiber connected to a collimator. The output beam goes through a linear polarizer and gets into the prism. A substrate is coupled to the prism. It is a F2-glass disk of 1 mm thickness and 10 mm diameter. A gold thin film of 50 nm thickness is deposited on one side. A flow cell is mounted on the prism and the substrate so that the reaction chamber is in contact with the gold.

The beam reflected on the gold film is coupled into the entrance optical fiber of the spectrometer. The spectrum is recorded by the CCD camera, driven by the embedded computer.

The spectrum of the reflected light is processed to measure the dip shift in the near infra-red range (700-950 nm). The spectroscopy system has a resolution of about 0.3 nm. An algorithm based on centroïd calculation [8] was chosen because of its relative insensitivity to noise. It can detect dip shift smaller than the spectrometer resolution.

The transducer can analyze as many signals as the spectrometer can. At the present time, the system presents two analyzing channels but it can be extended if necessary.

2.2. Experiments

The transducer was characterized with standard solutions for measuring its sensitivity to refractive index changes. Sucrose solutions were chosen because their refractive indexes are proportional to their mass concentration [9].

Solutions of increasing mass concentration (0.05%, 0.1%, 0.2%, 0.5%; 1%) were injected in the flow cell, distilled deionized water being used for rinsing in between.

For this experiment, the solutions circulate in channel 1 first and then in channel 2.

3. Results and discussion

The dip shift of the two channels with regard to time is plotted in fig. 4. At t=0, sterile water flows in the reaction chamber. Sucrose solutions are then injected. As the sucrose solution penetrates in the reaction chamber, the dip shifts to higher wavelength, meaning an increase of the refractive index. When the chamber is rinsed, the dip wavelength goes back to its initial value.

The two channels showed similar sensitivity. The graph in fig. 5 represents the dip wavelength shift with regard to the refractive index of the sucrose solution. As expected, the graph is a straight line.

The sensitivity can be estimated to 4100 nm/RUI^{-1}, which is 30% smaller than state of art sensor [10]. Indeed, the submarine system works at short wavelengths (the dip wavelength in distilled deionized water is 620 nm), which results on a decrease in sensitivity [6]. The spectral range of the whole system is limited to 950 nm by the CCD camera. Then, a shorter working wavelength leads to a greater dynamic range. On the one hand it is less sensitive, on the other hand it is more versatile.

Taking three times the standard deviation of the noise as the resolution limit, the refractive index resolution is about 3.10^{-6}, which is consistent with previous works [6].

4. Conclusion

The submarine system was characterized in laboratory. This series of experiments showed a resolution on the refractive index of about 3.10^{-6}. This value is comparable to the resolution of laboratory sensor we used for detecting PCR product of *Alexandrium minutum* [5]. These first results are then promising for *in situ* detection. *In situ* validations are now necessary for a complete evaluation.

Its use as a DNA biosensor requires an automatic sampling device capable of extracting DNA, amplifying it by PCR and denaturating the product. Further developments are necessary for detecting and quantifying algae *in situ*.

Acknowledgement

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References

[1] A. McMin, H. Heijnis, A. Murray, G. Hallegraeff, “Diatom and dinoflagellate assemblages of the Hawkesbury River, NSW, over the last two centuries: evidence for changes in hydrology”, *Alcheringa*, Vol. 28, pp. 505-514, 2004.

[2] L. Campbell, J. N. Walpert, and N. L. Guinasso, “A new buoy-based in situ optical early warning system for harmful algal blooms in the Gulf of Mexico,” *Nova Hedwigia*, pp. 161-+, 2008.

[3] S. Diercks, K. Metfies, F. Schroder, L. K. Medlin and F. Colijn, "Detection of phytoplankton with nucleic acid sensors.", *Algal Toxins: Nature, Occurrence, Effect and Detection,* V. Evangelista, L. Barsanti, A. M. Frassanito, V. Passarelli and P. Gualtieri, Eds. Springer Netherlands, 2008, pp. 285-299.

[4] C. Scholin, S. Jensen, B. Roman, S. Jensen, E. Massion, B. Roman, J. Feldman, C. A. Scholin, “The environmental sample processor (ESP) - An autonomous robotic device for detecting microorganisms remotely using molecular probe technology,” *Oceans 2006, Vols. 1-4*, pp. 1179-1182, 2006.
[5] S.Laurent, F. Colas, M. Hamelin, M.-. Crassous, E. Antoine, M. Lehaitre, C. Compere “Toward Detection of Harmful Algae Blooms by in situ Surface Plasmon Resonance Spectroscopy”, *Sensors Applications Symposium*, pp. 29-33, 2009.
[6] J. Homola, *Surface plasmon resonance based sensors*, Berlin : Springer, 2006.
[7] B. Liedberg, C. Nylander, I. Lundström, „Surface plasmon resonance for gas detection and biosensing”, *Sens. Actuators,* Vol 4, pp.299-304, 1983.
[8] T. M. Chinowsky, L. S. Jung, and S. S. Yee, “Optimal linear data analysis for surface plasmon resonance biosensors,” *Sensors and Actuators B-Chemical*, vol. 54, no. 1-2, pp. 89-97, Jan, 1999.
[9] D. R. Lide, *CRC Handbook of chemistry and physics*, 88th ed, Boca Rato, FL: CRC Press, 2008.
[10] S. D. Soelberg, T. Chinowsky, G. Geiss, C.B. Spinelli, R. Stevens, S. Near, P. Kauffman, S. Yee, C.E. Furlong,“A portable surface plasmon resonance sensor system for real-time monitoring of small to large analytes”, *Journal of industrial microbiology and biotechnology*, Vol. 32, pp. 669-674, 2005.

Figures

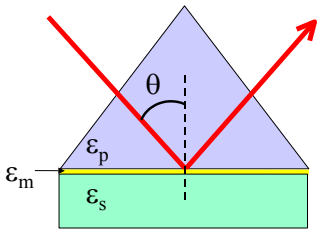

Figure 1: Kretschmann-Raether configuration

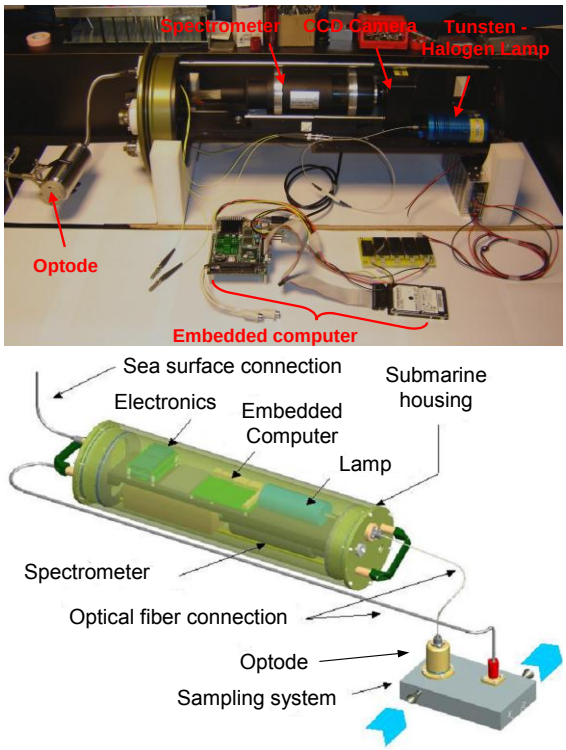

Figure 2: Submarine transducer

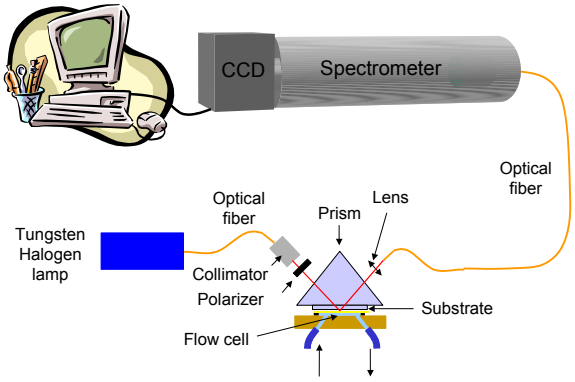

Figure 3: SPR transducer

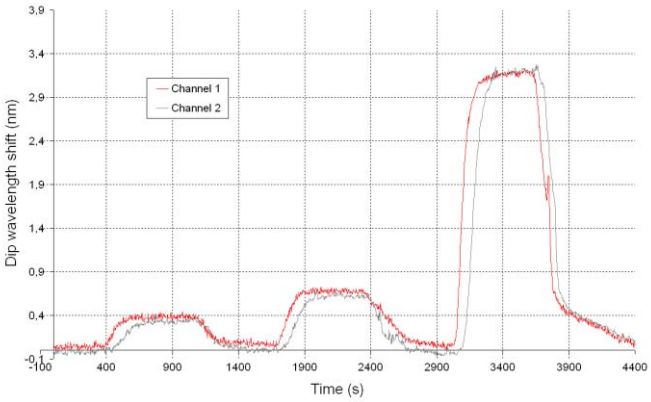

Figure 4: Dip shift with regard to time

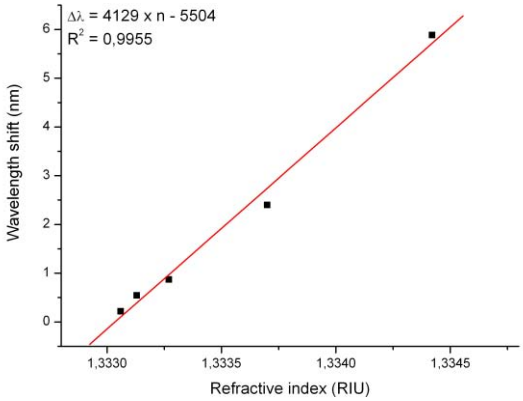

Figure 5: Dip shift with regard to refractive index of solutions