New Approach For The Detection Of Toxic Algae Species

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Novel sensor

Up to now, toxic species are monitored by expensive and time-consuming laboratory analysis of seawater samples: after a 24h decantation, the samples are observed with an optical microscope and the cell concentration per species is determined. In this context, systems able to detect HAB species directly underwater would be of great help. An assay based on an SPR biosensor was then developed for detecting *Alexandrium minutum*.

The developed system is based on SPR spectroscopy [1]. Its sensitivity was characterized underwater with sucrose solutions as illustrated on figure 2. It showed similar sensitivity at 0, 10 and 20m. It can detect changes of refractive index of the order of magnitude of 10^{-6}, which corresponds to few tens of $pg.mm^{-2}$ of biomolecules on the sensor surface without labeling.

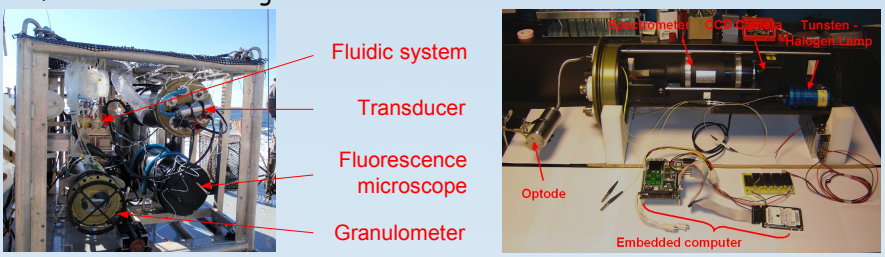

Figure 1: Submarine system

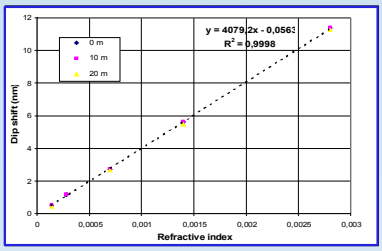

Figure 2: Sensitivity measurement

The SPR system was used to specifically detect DNA of *Alexandrium minutum*. Prior to any experiment, the sensor surface was functionnalized by a 25-mer synthetic nucleic probe (5'-AAGCCCTTACACATCAGTGCTGGCA-3') *via* thiol chemistry [2] .

Then, the DNA biosensor was characterized with synthetic oligonucleotides. Sensorgrams of the hybridization kinetics for different concentrations of 25-mer oligonucleotides can be seen on figure 3. The biosensor showed a linear response for concentrations between 20 and 100nM.

Then the biosensor was used for specifically detecting PCR product of an *A. minutum* strain (AM89BM, Bay of Morlaix, France) coming from Ifremer Centre de Brest Collection. The protocol is schemed on figure 4. The PCR primers were designed for amplifying a specific sequence of *A. minutum* of 677-mer. The sensorgrams of the hybridizations are shown on figure 5 and 6.

The results on figure 5 show that the biosensor can quantitatively detect PCR product in the range 25nM to 100nM with a detection limit of 12.5nM.

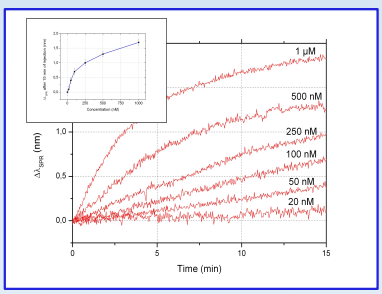

Figure 3: Hybridization of 25-mer oligonucleotides

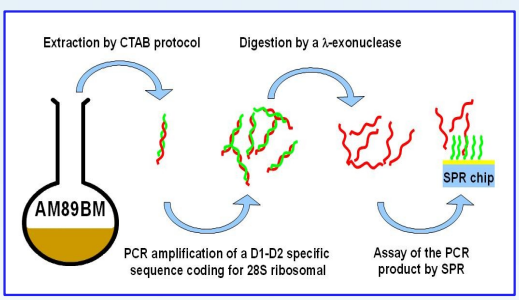

Figure 4: Detection protocol

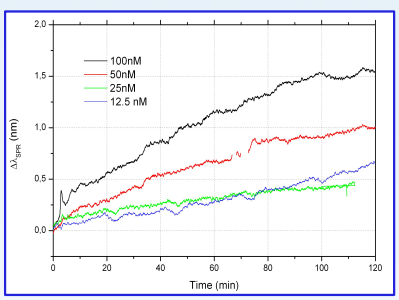

Figure 5: Kinetics of hybridization of PCR products

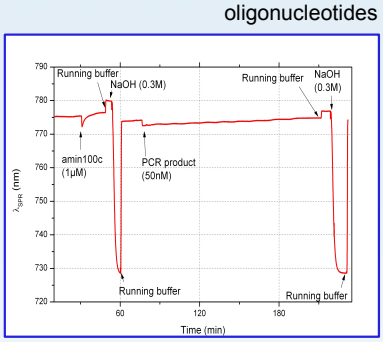

Figure 6: Kinetics of hybridization of synthetic 100-mer oligonucleotides and then PCR products. A regeneration of the biosensor is shown between these two injections

An underwater transducer based on SPR was developed and characterized. This system was then used in the laboratory for detecting PCR products of *Alexandrium minutum*. It was then demonstrated that this biosensor can quantitatively detect PCR product in the range 25nM to 100nM. During this set of experiments, the biosensor was regenerated about 20 times without significant loss of sensitivity.

An automated system for sampling seawater, extracting and amplifying the DNA is currently being developed. It will be coupled to the SPR biosensor.

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[1] F. Colas, S. Laurent, M. Lehaitre, Optode pour transducteur optique, patent 09/51751
[2] S.Laurent, F. Colas, M. Hamelin, M.-P. Crassous, E. Antoine, M. Lehaitre, C. Compère, Toward Detection of Harmful Algae Blooms by in situ Surface Plasmon Resonance Spectroscopy. Sensors Applications Symposium Proceedings, pp. 29-33, 2009.