Automation of a Biosensor for High Resolution Observation of Marine Phytoplankton

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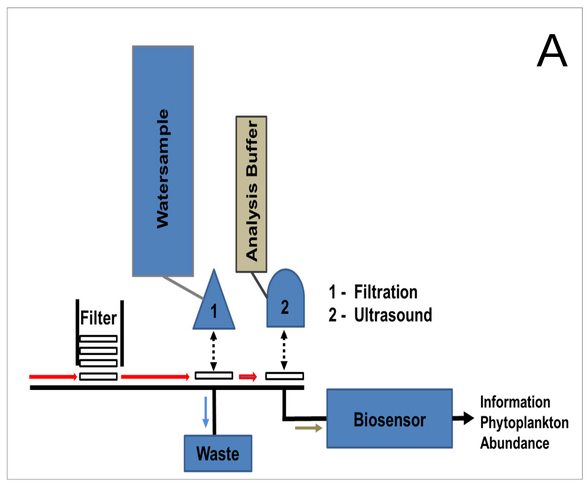
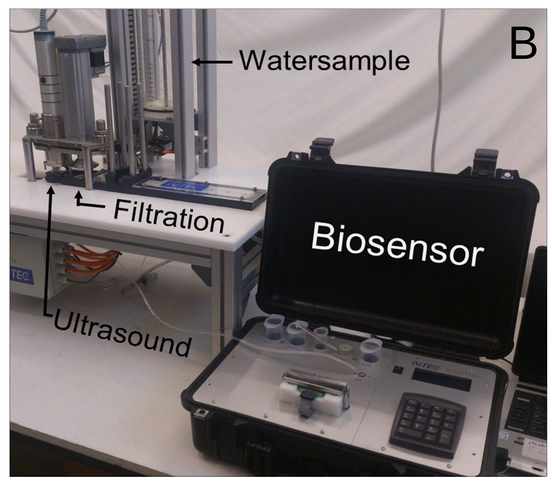
Introduction

Challenges and Mission Statement

Present changes in North Sea plankton communities identify this area as an important site to further assess the impact of climate change on the base of the marine food web – the phytoplankton. Regular phytoplankton assessments including nano- and pico-eukaryotes require new approaches and methods that provide high resolution information on the phytoplankton occurrence and cut down the costs and effort related to phytoplankton observations. Molecular methods have the potential to serve these needs. They are independent of taxonomic features or cell size and molecular analyses can be automated.
In this COSYNA project we aim to develop an autonomous nucleic acid biosensor system that can be applied on board ships. Such a system will provide information on the biogeography, diversity and succession of North Sea phytoplankton species with high spatio-temporal resolution.

Automated Nucleic Acid Biosensor

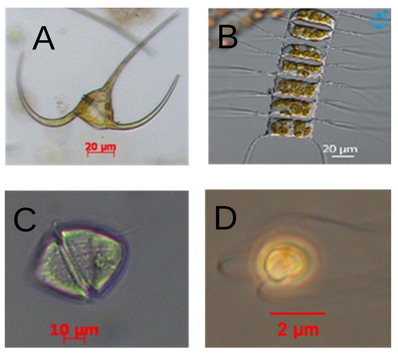




A: Schematic drawing of the automated biosensor system. Filters are stored in a reservoir and pushed by a bar (red arrow) under the filtration cap. Here POM (particulate organic material) is collected on the filter. Subsequent to filtration the filter is pushed further to the next position for application of analysis buffer and ultrasound treatment. The cells are lysed via ultrasound. After the treatment rRNA is dissolved in the analysis buffer and gets transferred to the biosensor. Molecular detection is automated in the biosensor. **B:** Prototype of the automated biosensor system including automated sampling, filtration, sample preparation and detection of target species.

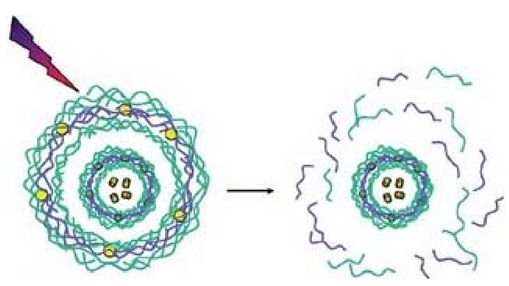
Analysis Procedure

Phytoplankton Species in a Community



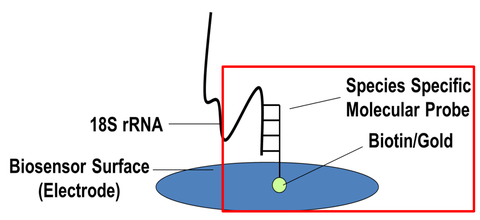
Typical phytoplankton types (groups) present in natural assemblages with different abundances

Lysis of Cells and Release of ribosomal RNA (rRNA)



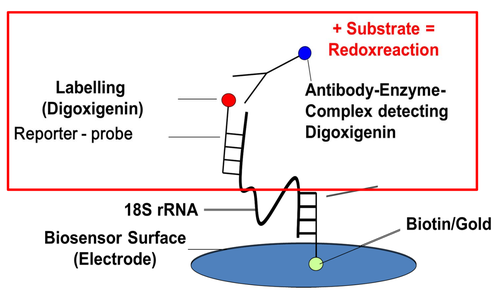
The cell wall and the cell membranes are disintegrated with chemical detergents or ultrasound and rRNA is released

Hybridization of rRNA to Species Specific Molecular Probes



The rRNA is a conserved molecule, but it contains highly species specific regions that can be used for an identification of species by hybridization to complementary molecular probes

Detection Hybridization



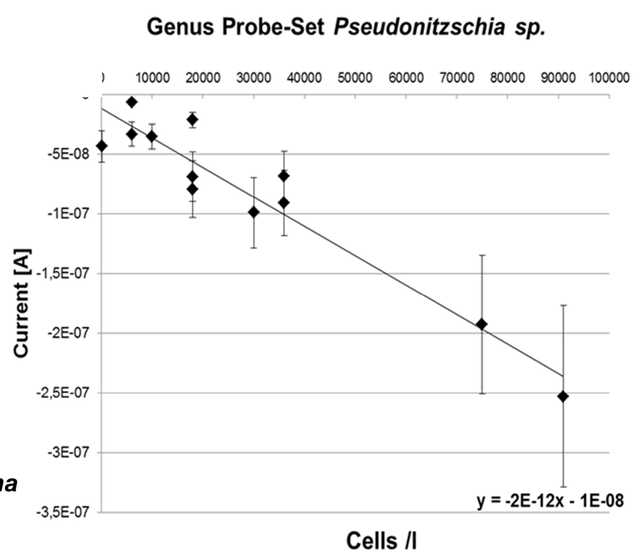
The hybridization is detected by an electrochemical reaction in a sandwich hybridization

Ongoing Work

New molecular probes are evaluated for their specificity and subsequently calibrated in the biosensor system

Target Species:

1. ***Chaetoceros calcitrans***
2. ***Chaetoceros debilis***
3. ***Chaetoceros socialis***
4. *Ceratium furca*
5. *Ceratium fusus*
6. *Leptocylindrus danicus*
7. *Leptocylindrus minimus*
8. ***Odontella aurita***
9. ***Odontella sinensis***
10. *Paralia sulcata*
11. ***Prorocentrum micans***
12. ***Pseudonitzschia seriata***
13. ***Pseudoditzschia delicatissima***
14. ***Pseudonitzschia pseudodelicatissima***

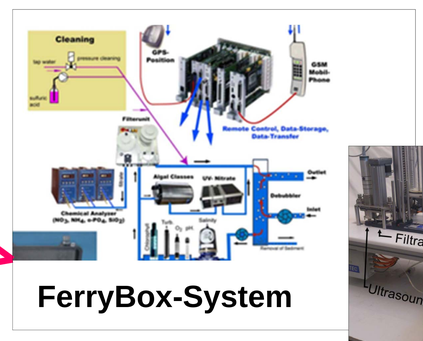


Specific probe sets for North Sea key species are evaluated

The signal intensity of the electrochemical detection is proportional to the cell number in the assay (*Pseudonitzschia sp.*)

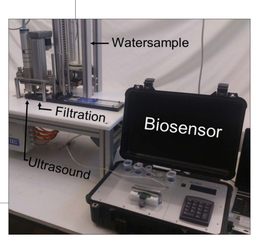
Conclusions and Future Work

The biosensor is currently suited for automated taxon specific identification of marine algae. Autonomous operation of the device will be possible after automation of the sensor chip exchange in a final step of the biosensor development.
As an autonomous device, it can be operated independent of laboratory equipment on board ships or in the field. It could be part of a smart *in situ* observation strategy, e.g. in combination with the FerryBox-system



FerryBox-System

Autonomous Phytoplankton Observations



Biosensor -System

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