

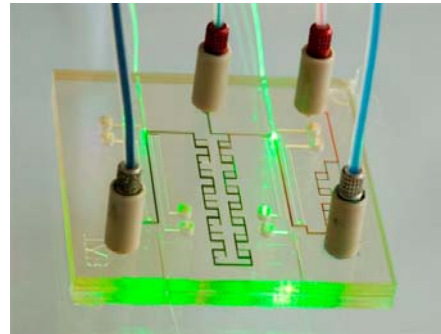
Challenger 2010: The 14th Biennial Challenger Conference for Marine Science

“Microfluidics and Sensor Technology for Oceanographic and Environmental Science Applications”



WORKSHOP
FRIDAY 10 SEPTEMBER 2010
Centre for Marine Microsystems, National Oceanography Centre,
Southampton, UK
www.soton.ac.uk/cmm

Current sensor systems for ocean biogeochemistry are mostly large expensive devices requiring expert operation and intervention. However, widespread multi-parametric measurements are often precluded due to their large size and high cost for mass production and deployment. Development of miniaturised multi-parametric metrology sensors for ocean deployment could thus make a significant impact in this field. Microfluidic devices provide unique opportunities for high resolution spatial and temporal analysis of ocean samples. For example, systems to measure nutrients, pH and key chemicals have been demonstrated. These systems can also deliver data describing the microbiology of the oceans and in related environmental and industrial applications. This workshop will provide an opportunity to learn about exciting developments in this field and awareness of how these technologies will impact future oceanographic research.



RS AQUA



Programme

- 9.00-9.10 Introduction: Prof. Hywel Morgan & Dr. Matthew Mowlem
Centre for Marine Microsystems, Southampton, UK
Ocean sensing and Integrated Systems
- 9.10-9.50 Plenary talk I: Dr. Chris Scholin
Remote Detection of Marine Microbes, Their Genes and Gene Products Using the Environmental Sample Processor (ESP)
Monterey Bay Aquarium Research Institute, USA
- 9.50-10.10 Invited talk I: Dr. Kim Lau
Microfluidics Based Phosphate Analyser for Water Quality Monitoring
National Centre for Sensor Research, Dublin City University, Ireland
- 10.10-10.30 Invited talk II: Dr. Vincent Sieben/Dr. Cedric Floquet
The Development of Autonomous Microfluidic Sensors for Nutrient and Trace Metal Detection
Centre for Marine Microsystems, Southampton, UK
- 10.30-11.00 Tea & biscuits
Sensors for Biological Applications
- 11.00-11.40 Plenary talk II: Prof. John H. Paul
Sensors for Detecting Microbial Targets in the Oceans
Marine Microbiology Group, University of South Florida, USA
- 11.40-12.00 Invited talk III: Prof. Andrew de Mello
High-Throughput Chemistry & Biology: Photons, Particles and Droplets
Department of Chemistry, Imperial College, London, UK
- 12.00-12.20 Invited talk IV: Dr. Greg Collins
Direct Injection of Seawater for the Analysis of Nitroaromatic Explosives by MEKC
Chemistry Division, Naval Research Laboratory, Washington, DC, USA
- 12.20-12.40 Invited talk V: Dr. Maria-Nefeli Tsaloglou
An integrated microfluidic system for cell concentration and lysis, nucleic acid purification and real-time quantitative detection of marine species
Centre for Marine Microsystems, Southampton, UK
- 12.40-13.45 Lunch
Wet Chemical Sensors for Metrology and Biogeochemistry
- 13.45-14.05 Invited talk VI: Prof. Gillian Greenway
New Approaches for Robust in situ Microfluidic Systems
Environmental Monitoring & School of Chemistry University of Hull, UK
- 14.05-14.25 Invited talk VII: Dr. Ralf Prien
Wet Chemical Sensors - Ready for Operational Application?
Leibniz-Institute for Baltic Sea Research, Rostock, Germany
- 14.25-14.45 Invited talk VIII: Prof. Peter Statham
A Biogeochemical Perspective on Sensors Systems
Centre for Marine Microsystems, Southampton, UK
- 14.45-15.40 Q&A and open discussion with all speakers
- 15.40-17.30 **Poster session £200 best poster award sponsored by Chelsea Technology Group**
Wine reception sponsored by Ocean Business 2011

Invited talks abstracts

Plenary talk I:

Remote Detection of Marine Microbes, Their Genes and Gene Products Using the Environmental Sample Processor (ESP)

Christopher Scholin

Monterey Bay Aquarium Research Institute, 7700 Sandholdt Road , Moss Landing, CA 95039

Application of molecular analytical techniques for identifying marine microbes, specific genes, and gene products currently demands collecting discrete samples, sometimes in liter quantities, and transporting samples to a laboratory for processing. This requirement typically results in delays ranging from many hours to days between collection of material and its analysis. Establishing new sample collection and processing paradigms is an essential step towards overcoming this impediment. We have approached this problem in an ocean observatory setting through development of the Environmental Sample Processor (ESP). The ESP is a field-deployable system that combines autonomous sample collection capability with molecular analytical detection functionality. Two-way communications with the device when it is deployed are currently achieved using a radio modem, but other modes of connectivity are also possible.

The presence and abundance of specific organisms, their genes and/or metabolites are presently assessed in near real-time using low-density DNA probe and protein arrays. Filter-based sandwich hybridization methodology enables direct detection of ribosomal RNA sequences diagnostic for groups of bacterioplankton, as well as a variety of invertebrates and harmful algal species. An antibody-based technique is used for detecting domoic acid, an algal biotoxin. Working closely with a team at the Lawrence Livermore National Laboratory, a 2-channel real-time PCR module has been incorporated. Users can configure this system to support a variety of PCR master mixes, primer/probe combinations and control templates. The ESP has been deployed on variety of platforms including moorings, remotely operated vehicles, coastal piers, and on a deep-sea cabled observatory. This presentation will highlight architecture of the ESP system, development of methods for use onboard the ESP, results of recent field trials and an outline of plans for future development of the instrument with an emphasis on microfluidic “analytical modules”.

Invited talk I:

Microfluidics Based Phosphate Analyser for Water Quality Monitoring

Kim Lau and Dermot Diamond

National Centre for Sensor Research, Dublin City University, Ireland

Field deployable water quality sensors analysers face formidable challenges from all areas including technical capability, data reliability due to environmental impacts and energy demand. Microfluidic devices offer excellent opportunities to succeed maintenance free long-term data collection owing to the small sample reagent volume requirement, high sensitivity, and high throughput and low power consumption. We present a microfluidic based field deployable phosphate analyser for fresh/marine water quality monitoring. The design rationale and performance of the analyser will be discussed.

Invited talk II:

The Development of Autonomous Microfluidic Sensors for Nutrient and Trace Metal Detection

Vincent J. Sieben^{1*}, Cedric F.A. Floquet^{2*}, Samer Abi Kaed Bey², Iain R.G. Ogilvie², Edward M. Waugh², Alexander D. Beaton², Matthew C. Mowlem² and Hywel Morgan¹

¹ *Nanoscale systems integration group, University Of Southampton, UK and* ² *National Oceanography Centre, University Of Southampton, UK ; * co-first authorship*

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We present sensors that have been developed for analysis of Nitrite, Nitrate, Phosphate, Iron and Manganese. The application of microfluidics to in-situ colorimetric analysis provides several advantages over the traditional large-scale “macro” systems. These include a significant reduction in reagent consumption, smaller physical size, low power consumption, with significantly longer lifetime, and increased system integration. Such advantages enable both high-resolution temporal and spatial data sets that would be logistically impossible using traditional sampling techniques. Often, previously demonstrated microchip-based chemical analysers have required extensive support infrastructure thus making them unsuitable for remote and on-site deployment. Here we demonstrate an advanced level of integration to achieve stand-alone sensors that integrate all the sub-systems required to realise portable and on-site nutrient analysers based on microfluidics. The sensors operate using a common technology platform of measuring the optical absorption of a coloured reagent, where the adsorption is proportional to the concentration of the analyte. For example, nitrite is measured using an Azo dye, based on the Griess reaction, whilst nitrate is measured by reduction to nitrite using a miniature integrated copper cadmium column. The sensors have been tested with extensive temperature and pressure cycling, as well as at dock-side. In the near future, the sensor platform will be deployed on buoys and profiling floats (eg. ARGO floats) to monitor biogeochemical processes in ocean waters.

Plenary talk II:

Sensors for Detecting Microbial Targets in the Oceans

J.H. Paul and D. Fries

College of Marine Science, University of South Florida, St. Petersburg, FL 33701 USA

Rapid microbial detection, quantification, and identification is needed for detection of microbial pathogens and indicators, harmful algal bloom monitoring, detection of biowarfare agents, and point of care technologies for patient monitoring. The oceanic applications of microbial detection are further confounded (in most instances) by low concentrations of target organisms. This results in the need for nucleic acid amplification and target detection. Target detection is usually via fluorescence of specific probes. Although microfluidics have many applications in microbial detection and identification, fluorescence or colorimetric detection sensitivity is favoured by larger volumes (10 μ l). We have devised a series of target-specific microbial detection assays for several HABs, viruses, and microbial water quality indicators based on Nucleic Acid Sequence-Based Amplification (NASBA) and binding of Molecular Beacons. Our assays can be run in standard bench instrumentation (BioMerieux EasyQ) or our third generation handheld analyzer, licensed to Bioplex Inc. We have recently applied this technology for detecting illegal seafood substitutions (“grouper forensics”) in Florida. A multi-sample handheld analyzer is under development, as well as our totally automated platform, the AMG.

Invited talk III:

High-Throughput Chemistry & Biology: Photons, Particles and Droplets

Xavier Casadevall i Solvas and Prof. Andrew de Mello

Department of Chemistry, Imperial College London, Exhibition Road, London, SW7 2AZ, UK

Recent years have seen considerable progress in the development of microfabricated systems for use in the chemical and biological sciences. At a primary level, interest in miniaturized analytical systems has been stimulated by the fact that physical processes can be more easily controlled and harnessed when instrumental dimensions are reduced to the micron scale. For example, it is well recognized that when compared to macroscale instruments, microfluidic systems engender a number of distinct advantages with respect to speed, analytical throughput, reagent usage, process control, automation and operational and configurational flexibility.

In general terms, such systems define new operational paradigms and provide predictions about how molecular synthesis and analysis might be revolutionized in the coming years. My talk today will describe on-going work in the area of droplet-based microfluidics. Recent studies have exploited the formation of droplets in microfluidics systems to perform a variety of analytical processes. Of particular note are those that use flow instabilities between two immiscible fluids. Droplets can be formed spontaneously when multiple laminar streams of aqueous reagents are injected into an immiscible carrier fluid. These droplets define picoliter volumes, and because each droplet is isolated from channel surfaces and other droplets, each one acts as an individual reaction vessel. Variation of the cross-sectional dimensions of microchannels can be used to regulate droplet volumes, and flow rate variation allows control of reagent concentrations. Importantly, droplets can be generated at kHz frequencies, meaning that millions of individual reactions can be processed in very short times. In my talk I will report recent advances in this area with particular reference to high throughput reaction screening and cell-based assays.

Invited talk IV:

Direct Injection of Seawater for the Analysis of Nitroaromatic Explosives by MEKC

Braden C. Giordano, Dean S. Burgi and Greg E. Collins

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Methods for improving detection limits of nitroaromatic explosives and their degradation products in seawater continue to be an ongoing concern of the U.S. Navy. The presence of these materials in coastal waters may be indicative of underwater mines that are military, commercial and environmental threats to our coastal regions.

Micellar electrokinetic chromatography (MEKC) has been demonstrated to be a useful analytical tool in the analysis of nitroaromatics when using capillaries or lab on a chip based devices. Our group and others have demonstrated that MEKC-based separations not only allow for the resolution of the analytes of interest, but also preconcentration of the sample via sweeping or high-salt stacking. On-line preconcentration allows for longer injection volumes which improves both detection limits and resolving power due to analyte preconcentration at the interface between the sample zone and the micelles in the background electrolyte (BGE). The underlying challenge associated with nitroaromatic explosives detection in seawater is the presence of the matrix, itself, which has a basic

pH ~8, contains several organic materials, and bears a large number of charged ions at very high concentrations. The primary difficulty, from an electrophoresis standpoint, is the large difference in sample matrix conductivity relative to BGE conductivity that arises when a seawater sample is directly injected onto the capillary column either by hydrodynamic or electrokinetic means. Relatively high sample matrix conductivity can lead to broadening due to electrodispersion, joule heating, and disruption of the sample zone/BGE interface needed for sample preconcentration. In order to address the issue of sensitivity of neutral analytes in very high conductivity matrices, it is necessary to develop appropriate injection/separation schemes robust enough to tolerate the presence of the seawater matrix. This work presents practical considerations applicable to capillary and microchip devices when sampling directly from seawater matrices. The use of high surfactant concentrations and long electrokinetic injections allows for an order of magnitude improvement in detection limits for individual explosives in seawater over previous methods, and, additionally, permits the direct injection of seawater onto the capillary without any form of dilution. Sensitivity was enhanced by two mechanisms, improved stacking at the detector side of the sample plug and desorption of analyte from the capillary wall by surfactant-containing BGE from the inlet side of the sample plug. Calculated limits of detection ($S/N = 3$) for analytes prepared in pure seawater were 70-800 ppb with injection times varying from 5 to 100 seconds.

Invited talk V:

An integrated microfluidic system for cell concentration and lysis, nucleic acid purification and real-time quantitative detection of marine species

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Harmful marine microalgæ *Karenia brevis* form blooms with detrimental effects to affected ecosystems and major financial losses to local economies. Current methods of monitoring for harmful algal blooms (HABs) include pigment absorption study of satellite images, *in situ* hyperspectral measurements and *ex situ* cell and molecular biology analyses. An autonomous macro-scale microbial genosensor has been developed at the University of South Florida. A hand-held NASBA analyser has already been demonstrated for detection and identification of phytoplankton species *K. brevis*.

Our lab has already demonstrated on-chip dielectrophoretic concentration of *K. brevis* non-motile cells, followed by electric field mediated lysis for RNA extraction. We are developing a microfluidic system that has individual sub-systems for performing cell concentration and lysis, RNA extraction/purification using magnetic beads and real-time quantitative Nucleic acid sequence-based amplification (NASBA). This integrated system could potentially be used for the quantitative detection of any species with a known target nucleic acid sequence for *in situ* environmental monitoring or medical diagnostics.

Invited talk VI:

New Approaches for Robust *in situ* Microfluidic Systems

Gillian Greenway

Department of Chemistry, University of Hull, Cottingham Road, Kingston upon Hull, HU6 7RX, UK

Different approaches to developing reliable portable microfluidic systems will be discussed. Ways in which to simplify microfluidics systems are investigated including the use of monoliths for sample extraction, preconcentration, separation and electro-osmotic pumping. Ideas such as holding reagents in gels, using magnets and simple chemiluminescence and conductivity detectors will be discussed as well as the problems and challenges.

Invited talk VII:

Wet Chemical Sensors - Ready for Operational Application?

Ralf Prien

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Operational measurements of chemical parameters in automated systems are still rare, but these measurements are a necessary component (together with physical and biological parameters) to understand the processes within the ecosystem. Electrochemical and optical sensors for chemical parameters slowly find their way into observatories but are limited in the choice of target parameters and/or concentration ranges. Wet chemical measurement procedures have been developed and optimised over decades in the lab for a wide variety of parameters. The strengths and weaknesses of these methods are well known.

Technological advances make it feasible to build compact and robust wet chemical analysers that can be deployed in the field, use standard lab methods and at the same time minimise the risk of sample contamination. In the design of wet chemical analysers, however, knowing the requirements for the intended deployments is important to find the right balance of reagent and power consumption, response time and measurement uncertainty. A very important component of future developments will be the search for alternative wet chemical methods that are better suited for long term deployments in regard of chemical stability and/or preparation of reagents *in situ*.

This presentation will highlight some of these points in the ongoing development of a wet chemical analyser for Fe(II) and Mn(II), originally developed for hydrothermal exploratory work and now modified to be used for measurements in the redoxcline of the Gotland Basin, Baltic Sea.

Invited talk VIII:

A Biogeochemical Perspective on Sensors Systems

Peter Statham

School of Ocean and Earth Science, National Oceanography Centre, Southampton, SO14 3ZH, UK

This talk considers the use of sensor systems from a scientific "end user" perspective, with a focus on aquatic biogeochemistry. The rationale for development of *in situ* analytical systems is briefly considered and the requirement for multi parameter systems introduced. A range of remaining analytical challenges at this stage of development of *in situ* sensors is then covered including discriminating between the species of interest in aquatic systems.

Poster abstracts

No.	Title	Author
PO1	Development of Anti-fouling Strategies for Long-term Deployed <i>in situ</i> Sensors in Marine Environments	Alexandra Meier
PO2	Development of an Automated Total Alkalinity System for Long-term Ocean Acidification Studies	David Owsianka
PO3	Real-time NASBA and <i>nifH</i> Gene Expression in <i>Trichodesmium spp</i> from the Atlantic Meridional Transect	Sophie Richier
PO4	High-resolution Microfluidic Phosphate Sensor for In Situ Measurements	Francois-Eric Legiret
PO5	Extremely Sensitive Conduction-based Chemical Biosensors using Suspended Silicon Nanostructures	Mohammad Adel Ghiass
PO6	Solvent Processing of PMMA and COC chips for Bonding Devices with Optical Quality Surfaces	Iain Ogilvie
PO7	Solvent Processing of PMMA and COC chips for Bonding Devices with Optical Quality Surfaces	Cedric Floquet
PO8	The Lake Ellsworth Probe and Methods for Decontamination	Ross Arthurs
PO9	Sensing and <i>in situ</i> Detection of Harmful Algal Blooms using Molecular Biology Methods	Magdalena Bolesta
PO10	SeaMon-HC: A specific Real-time-Lightweight-moored Buoy Platform for Fast Hydrocarbon Detection	Carlos Barrera
PO11	"No bubbles no troubles" : Monitoring Techniques for CO ₂ Seepage in Aqueous Environments	Giorgio Caramanna
PO12	Microfluidic Colorimetric Nutrient Analyser Characterised under Deep-Sea Environmental Conditions	Alexander Beaton
PO13	CHEMINI CHEmical MINIaturized Analyser	BUCAS Karenn
PO14	Optodes for Aquatic O ₂ and pCO ₂ Measurements: Experiences and New Developments.	Anders Tengberg
PO15	Optofluidic Bragg Grating Sensors for Chemical Detection	Richard Parker
PO16	Miniaturisation of RNA Extraction and Purification	Ysobel Baker
PO17	Towards the Development of a Closed-Loop Semi-Biotic Device Prototype	Richard Wilson
PO18	Bioinspired Antifouling Using Natural Products against Marine Biofilms	Maria Salta
PO19	Micro-sensing Technology for in situ Low Level Detection of Fe and Mn in Seawater	Ambra Milani
PO20	Microdroplet Formation in a T-junction Microfluidic Device Using the Two Phase Level Set Method	Shazia Bashir
PO21	Designing a miniaturised ion chromatographic system for <i>in situ</i> water analysis	Amy Webster
PO22	Capacitively coupled contactless conductivity detector for microchip ion chromatography	Etienne Joly

Development of Anti-fouling Strategies for Long-term Deployed *in situ* Sensors in Marine Environments

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Long-term monitoring of the environment is an essential part in understanding global processes such as global warming and its impact. The development of anti-fouling strategies for *in situ* sensors is critical for their functionality and requires a multidisciplinary approach. Biofouling occurs even after only a short deployment in the marine environment. Its effects range from measurement drift, which can be compensated, to blockage of channels, which render the sensor inoperative. The longer the deployment period the more severe the effects of the biofouling become, in general. For the development of anti-fouling strategies, first we need to understand the processes of fouling and identify targets for the research efforts. An understanding of the differences in the severity of the fouling in relation to the location of the sensors is also an important part on the development. Targets for antifouling research can be either the prevention of initial stages of biofouling or treatment and removal of biofilms. The prevention of biofouling can be achieved by identifying materials less susceptibility towards biofouling and surface modification sensor materials. The research in this area has been concentrated on the eutrophic zones of the oceans neglecting the deep sea. Conditions in the deep sea are extremely variable and have a huge impact on fouling. We have deployed short-term and long-term a variety of materials in an oligotrophic environment, the Cayman Trough, at a depth of 4, 500 m. Initial results from the short-term deployment show biofouling after 10 days (Fig. 1).

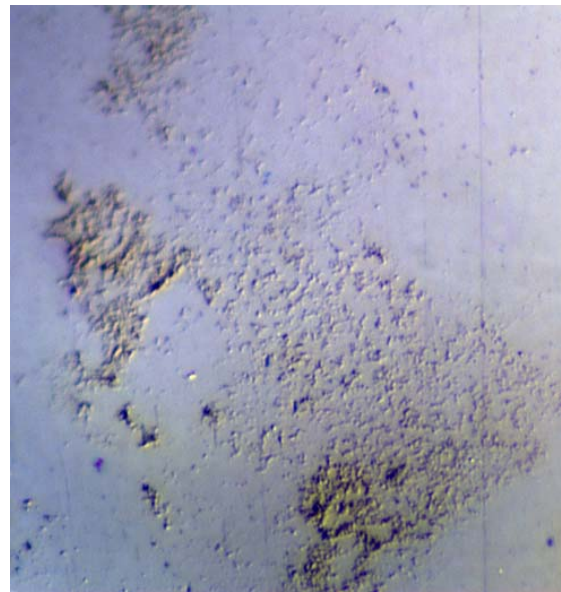


Figure 1- Glass slide deployed at 4, 500 m for 10 days shows the early stages of biofouling

Development of an Automated Total Alkalinity System for Long-term Ocean Acidification Studies

David Owsianka

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The carbon cycle in natural waters has become a high priority for scientific study particularly in relation to anthropogenic CO₂ uptake leading to ocean acidification. Increasingly acidic seawater regimes could have unpredictable and potentially damaging effects on some organisms, and there is therefore a desire to measure the extent and rate of ocean acidification, and its biological and ecological implications.

Measurement of more than just seawater pH is necessary to ascertain the effect of increased CO₂ uptake, as hydrogen ions are weakly buffered by dissolved carbonate (CO₃²⁻) and bicarbonate (HCO₃⁻) along with other alkali species. Direct measurement of these 2 main carbonate species is difficult, and so instead interpretation of a combination of 4 more easily measured parameters is used, namely pCO₂, dissolved inorganic carbon, pH and total alkalinity.

Conventional water sampling followed by laboratory analysis represents a costly and laborious approach to determining these parameters, often leading to under-sampling. Compact, sensitive, and robust systems capable of measuring total alkalinity autonomously could improve this, but commercial products are not readily available.

We present advances in the development of automated, miniaturised systems to measure total alkalinity (AT), through which we aim to deliver the precision required for long term ocean acidification studies. This approach has focused on the modification of analytical procedures to simplify the design characteristics, and makes use of novel technologies such as lab-on-a-chip (LOAC). At the core of the design is the ability to operate without user intervention for extended periods of time aboard a variety of platforms. A concept system has been built that is able to resolve large changes in HCO₃⁻ standard concentrations, and a comprehensive analysis of associated errors to direct further development has been undertaken.

PO3

Real-time NASBA and nifH Gene Expression in *Trichodesmium spp* from the Atlantic Meridional Transect

Sophie Richier, M. Nefeli Tsaloglou, C. Mark Moore, Matt Mowlem and Thomas S. Bibby
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Primary producers play important roles in controlling both the oceanic food chain and the overall biogeochemistry of the ocean. Open-ocean diazotrophic cyanobacteria, such as *Trichodesmium spp.* Are of particular interest to researchers studying global biogeochemical cycles, due to their contribution to both the carbon (C) cycle via primary production and to the nitrogen (N) cycle because of their ability to fix N₂. The 'new' N, N that has not been regenerated from degradation of organic matter in the mixed layer, produced by these cyanobacteria is vital to the N and C cycles on regional, and global scales, as well as potentially influencing CO₂ sequestration over geologic timescales.

Trichodesmium spp. contributes to 25-50% of the geochemically derived rates of N₂ fixation in various ocean basins especially in the oligotrophic tropical and subtropical oceans and stimulates the biogeochemical cycling of carbon and nitrogen in an area corresponding to almost half of the Earth's surface. Our study aims at monitoring the N₂ fixation in *Trichodesmium* colonies collected as part of the Atlantic Meridional Transect (AMT19) cruise (Falmouth, UK; Punta Arenas, Chile), using the state of the art technique NASBA (Nucleic acid sequence-based amplification). NASBA is an isothermal method able to quantify target RNA. This latter method, combined with molecular beacon probes, becomes a real-time analysis tool that offers faster results than quantitative PCR (QPCR).

In the present work, we are targeting the nifH gene involved in N₂ fixation in order to monitor *Trichodesmium spp.* gene expression in response to a nutrient limited environment. Our preliminary results have shown successful real-time NASBA and detection of the nifH gene from samples collected between 31°25'74N and 01°16'N for the first time in the literature. Further work will include validation of the results using a NASBA microfluidic setup with potential use on the field.

High-resolution Microfluidic Phosphate Sensor for *in situ* Measurements

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Climate change is one of the more important environmental problems future generations will face. The warming of the oceans and consequent further stratification has significant consequences for ecosystem functioning and carbon sequestration.

Nutrients play a controlling role in microbial ecosystems. However, surface water nutrient concentrations in large regions of the global ocean are depleted to levels below the detection limit of conventional analytical techniques due to the biological uptake. Moreover, these oligotrophic ocean regions will increase in size as a consequence of global warming. This strengthens the requirement for techniques with low limits of detection in these regions where nitrate and phosphate control primary production. Considering phosphorus in the marine environment, dissolved orthophosphate is the key factor since it is taken up by photosynthetic organisms at the base of the marine food web.

Gathering nutrient data is vital for understanding biogeochemical changes with location and time and responses of ecosystems in an increasingly stratified future ocean.

In recent years, a number of techniques have been developed for shipboard low-nutrient analysis [1] with a high sample throughput. These traditional techniques are not suitable for autonomous deployment in oceans under long-term observing [2]. Microsystem technology or, more specifically, microfluidic technology enables minimization of reagent and power consumption for *in situ* deployment of wet-chemical methods which provide accurate results with low limits of detection and high spatial and temporal resolution. We present a stopped-flow microfluidic sensor based on the vanadomolybdate method for the determination of dissolved reactive phosphorus.

[1]Patey, M. et al. (2008). "Determination of nitrate and phosphate in seawater at nanomolar concentrations." *TrAC Trends in Analytical Chemistry* 27(2): 169-182.

[2]Adornato, L. et al. (2007). "High-resolution *In situ* analysis of nitrate and phosphate in the oligotrophic ocean." *Environmental Science & Technology* 41(11): 4045-4052.

PO5

Extremely Sensitive Conduction-Based chemical Biosensors Using Suspended Silicon Nanostructures

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This work reports on design and implementation of suspended silicon nanostructures as sensing element of extremely sensitive and fast chemical/biosensors, which can be used in a vast verity of applications including medical and environmental science. Development of nanotechnology made it possible to scale the sensing element down to dimensions of chemical and biological target species. This makes the sensors more susceptible. Silicon nanostructures such as nanowires can be used efficiently in the application due to their high surface to volume ratio, well-known surface chemistry, and compatibility with complementary metal oxide semiconductor technology.

In this work, three different structures were designed and fabricated to be used in a charge-base sensing approach. In fact, the charge transfer of nanostructure is influenced by adsorption of target molecules on its functionalised surface. The surface of sensing element is selectively functionalised utilising a combination of Joule heating and architectural optimisation. Besides, a novel sensing mechanism providing sensitivities towards single molecules was proposed. The method uses an advanced suspended silicon nanowire containing a single-electron transistor. Hybrid simulations were used to investigate and confirm the sensing of a 12-mer single-strand DNA. Finally, the promising achievements will be used to realise a new generation of ultra-sensitive smart sensors.

Solvent processing of PMMA and COC chips for bonding devices with optical quality surfaces

I R G Ogilvie*, V J Sieben**, C F A Floquet**, R Zmijan*, M C Mowlem** and H Morgan*

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Many prototype microfluidic devices are manufactured by some form of micromachining or injection moulding which often leaves poor quality surface. This work presents a simple method that both significantly reduces surface roughness of chips and at the same time is used to bond the device. The method has been tested on devices made from poly(methyl methacrylate) (PMMA) and cyclic olefin copolymer (COC). The technique uses a solvent vapour exposure process which creates an irreversible bond between two substrates. It also re-flows the material, producing a surface with optical quality. Many different methods have been presented for bonding microfluidic devices from PMMA and COC; however none have addressed the issue of surface quality. Rapid prototyping tools often create a surface roughness in the region of hundreds of nanometers making them unsuitable for manufacturing integrated optical components such as lenses. Our work provides a low-cost method of surface smoothing (giving <15nm surface roughness) and irreversible bonding in one simple process. We have performed further extensive characterisation with SEM and AFM, and optimisation of process parameters to ensure maximum bond strength, minimising channel collapse and reflowing the polymer to give maximum bond uniformity. The method will enable further use of rapid prototyping systems to fabricated micro-devices with integrated optical components.

PO7

Nanomolar detection with high sensitivity microfluidic absorption cells manufactured in tinted PMMA for chemical analysis

Cedric F.A. Floquet**, Vincent J. Sieben*, Ambra. Milani**, Etienne. P. Joly**, Iain R.G. Ogilvie*, Hywel Morgan*, and Matthew C. Mowlem**

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Abstract content embargoed for IP reasons.

PO8

The Lake Ellsworth Probe and Methods for Decontamination

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Lake Ellsworth is a subglacial lake in Antarctica just west of the Ellsworth Mountains. The lake is at a depth of approximately 3.4 km beneath the ice, with a length of 10km [1]. Lake Ellsworth is a prime candidate for in situ exploration. Using a remote probe, the lake water will be sampled in order to try learn about the conditions and determine whether or not any microbial life exists in Antarctic subglacial lakes [2].

The water sampling probe will be equipped with an array of sensors including, pH, temperature, oxygen concentration, redox, sound velocity and conductivity sensors, as well as a video camera and sonar apparatus. A corer measuring 60 cm will also be included in order to get sediment samples from the bottom of the lake. The probe will also house 24 pressure and temperature tolerant bottles for water samples.

The fieldwork needs to be completed in a clean and environmentally friendly way in accordance with the Scientific Committee on Antarctic Research (SCAR). This means that the probes to be sent down into the lake, need to be completely clean and rid of any microbial life that could contaminate this pristine environment (and samples collected). The probes will be have to be cleaned and sterilised in a manner that will ensure no contamination takes place and also retains the properties of the materials used on the probes.

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Sensing and in situ detection of harmful algal blooms using molecular biology methods

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Karenia brevis is a toxic marine dinoflagellate species, which is common along the Gulf Coast of Florida, causing Harmful Algal Blooms (HABs). A typical *K. brevis* cell has a size between 18-45 µm and show a characteristic straight, non-curved groove on its dorsal side (Steidinger, 2009). The life cycle of *K. brevis* includes: asexual division, a sexual cycle and resting stages. Growth can be influenced by a variety of factors, for example sunlight, temperature, salinity and nutrients availability in the environment. *Karenia brevis* produce a harmful toxin, called brevetoxin which causes neurotoxic shellfish poisoning and high mortality in marine animals and respiratory irritation to humans (Steidinger, 2009).

Our lab developing an *in situ* biosensing microfluidic integrated system which uses Nucleic Acid Sequence-Based Amplification (NASBA) for detection. This isothermal amplification method is optimal for RNA and is targeting the *rbcl* gene of *Karenia brevis* (Casper et al, 2007).

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SeaMon-HC: A specific real-time-lightweight-moored buoy platform for fast hydrocarbon detection

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According with the main rules and protocols established by GOOS (Global Ocean Observing System) through its coastal ecosystems component COOP (Coastal Ocean Observations Panel), the present work describes the design, last development stages and the derived results from a specific buoy platform for fast hydrocarbon detection in seawater.

Under the name of SeaMon-HC (Patent No. P200302219/8), the buoy represents a very chief tool for coastal monitoring, mainly surrounding areas with a high oil-spill risk level, like harbours, off-shore fish farming, beaches and so on. Nowadays, the Macaronesian area has nine units working in real-time, under the frame of the Red ACOMAR Network.



Figure 1. SeaMon-Hc buoy moored nowadays in a marine reserve area in Girona (Spain)



Figure 2. Specific real-time GIS data-viewer application.

The main innovative aspect from this buoy is the detection system. It's based in polymer technology, working as a resistance, who increase its value when the pollutant on water surface is detected. The response time from the sensor is a direct function of the hydrocarbon volatility level. For hydrocarbons with high volatility levels (like petrol), the sensor needs less time (around 3 minutes) than others with less volatility such as oils.

SeaMon-HC is an autonomous, modular, reusable and a very low-cost development integrated by four subsystems (SS): SS-Flotation (different materials and shapes available); SS-Sensors (hydrocarbon detector and additional sensors –up to 15-, to solve specific sensor configuration requirements); SS-Power Supply (equipped in its basic configuration with a couple of solar modules and two 12V batteries) and the SS-Communication (based on a RF or GSM/GPRS modem technology, with a selectable communication frequency).

All SeaMon-HC units, as well the rest of the ODAS buoys who joint together the Red ACOMAR Network, works in real-time, sending the collected information to the control centre that manages the communications, providing data, in a useful form (as a web site), to diverse socio-economic important sectors which make an exhaustive use of the littoral in the Macaronesian region. The access to the information by the users is done through a specific GIS software application.

PO11

“No bubbles no troubles” : Monitoring Techniques for CO₂ Seepage in Aqueous Environments

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Carbon sequestration in sub-seafloor consists in the storage of CO₂ inside geological trapping structures below the seafloor. One of the main concerns is related to the possibility of leakage from the storage sites and the consequences on the marine environment. In order to develop safe and reliable methods for CO₂ monitoring, field studies were conducted in a natural analogue at an area where there is a natural release of carbon dioxide from the seafloor. Sampling procedure for of free and dissolved gas and measuring techniques of the main physical and chemical parameters were developed for use both from the surface and directly underwater by scientific SCUBA divers. The first results of the research indicate that high levels of CO₂ released in the marine realm strongly affect the local environmental conditions with a generalized acidification of the seawater. The experience gained in this study allows further development of a monitoring suite that will integrate multi-parametric sensors.

PO12

Microfluidic colourimetric nutrient analyser characterised under deep-sea environmental conditions

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We characterise the performance of a microfluidic wet-chemical nutrient sensor when subjected to environmental conditions similar to that of the deep-ocean. Individual components and subsystems of the nitrite/nitrate analyser developed at the National Oceanography Centre in Southampton are tested over the full ocean pressure and temperature range, and this is linked to the impact on the full system performance.

Microfluidic wet-chemical sensors represent a promising advance towards high-resolution long-term temporal and spatial monitoring of the world's oceans. This new generation of micro-sensors (based on lab-on-a-chip technology) offer reduced reagent consumption, smaller physical size and enhanced temporal response compared with traditional wet-chemical seawater analysis methods, making them particularly suitable for deployment on a range of oceanographic sensor platforms (e.g. AUVs, ROVs, Argo floats and gliders).

When deployed on such platforms, microfluidic sensors will be subjected to extreme pressure and temperature cycling, which has the potential to alter, for example, reaction kinetics, fluid flow rates, mixing dynamics, sub-component alignment and the behaviour of optical components. It is essential that the effects of these environmental perturbations on the overall system performance are well understood and characterised.

The research presented enables high-performance colourimetric microfluidic sensors that are robust over a wide range of environmental conditions, paving the way towards widespread deployment of microfluidic sensors in the oceans, as well as in more extreme settings such as subglacial environments.

Keywords: microfluidics, sensors, ocean monitoring, Argo floats

PO13

CHEMINI CHEMical MINIaturized analyser

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Since 2004, a new generation of in situ analyzers (CHEMINI) is developed by Ifremer to measure seawater chemical parameters. Technical choices were made during the development of CHEMINI according to several criteria : reliability, analytical performance, miniaturization to easily implement CHEMINI on deep submersibles, consumption (energy and reagents), and costs.

CHEMINI is a mono-parameter *in situ* chemical analyzer based on FIA (Flow Injection Analysis) coupled at fluorimetric or colorimetric detection. Several advantages result from this concept such as the ability to make simultaneous measurements, the independence of each measurement, increased reliability, the possible use of different detection methods, and the modularity of the system (one or more parameters). The analyzers can be set serially on a sampling line and operated by a single controller. Calibration of the analytical methods is performed *in situ*. Two versions are available: a Coastal version and a Deep-Sea version.

The Coastal version can be implanted on a buoy system (MOLIT buoy) or in a shellfish farming experimental station (Argenton Ifremer) to a long-term monitoring as in Vilaine's Bay in south Britain.

The Deep-Sea version can be implanted on ROV (Victor), inhabited submersibles (Nautile and Alvin) or seabed stations. For example, CHEMINI was deployed for Iron and Sulphide analysis in hydrothermal vents at Mid Atlantic Ridges, East Pacific Ridges and Guaymas Bay.

Several new parameters are in development as the measurement of pH, pCO₂, Sulphates and nanomolar Iron.

Keywords : *In situ* chemical analyzer, FIA, seawater chemical parameters, coastal, deep-sea, monitoring.

PO14

Optodes for aquatic O₂ and pCO₂ measurements: experiences and new developments.

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Commercially available oxygen optodes for oceanographic application were introduced in 2002. The long-term stability (years) and reliability of these sensors have revolutionized oxygen measurements and several thousand are in use in applications ranging from streams to the deep sea (6000 m), from fish farms to waste water, from polar ice to hydrothermal vents. The aim of this poster is to demonstrate the possibilities and limitations of this technology using field data. Recently PCO₂ optodes based on similar principles have been developed and submitted to a first series of field tests. Results from these tests will be presented and the challenges of measuring pCO₂ in different aquatic applications will be briefly described.

PO15

Optofluidic Bragg Grating Sensors for Chemical Detection

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Optical sensors are used to measure changes in physical and chemical properties in environmental processes. Operating at well established telecoms wavelengths, these sensors benefit from using relatively cheap, highly developed telecoms components and consequently have received much attention. They demonstrate many benefits over electronic sensors including immunity to electromagnetic interference, presenting no spark risk in flammable environments and remote interrogation of large arrays over many tens or even hundreds of kilometres. Combining integrated planar optics with microfluidic systems to form "lab-on-a-chip" optofluidic sensor devices enables fluorescent or refractive index sensing of chemical analytes with precise small scale fluid control. We present here a novel optofluidic refractive index sensor for chemical detection and sensing. Optical waveguides can be written with a UV-laser into a photosensitive planar glass layer to produce a wide range of optical devices. One such device is the Bragg grating; an optical device that reflects at one particular wavelength of light and transmits all others. The wavelength reflected by a Bragg grating is dependent on the refractive index it is exposed to, an inherent property of a material. Etching away the surface exposes these Bragg gratings to their surroundings with the corresponding observed shift in Bragg wavelength used to detect subtle changes in the refractive index of this environment. Encapsulation of these sensors within a microfluidic network allows for autonomous, continuous, real-time monitoring of a fluid flow system. Further, chemical modification of the sensor surface introduces specific chemical interactions that enhance the chemical specificity of the sensor.

We shall discuss the development of this proof-of-concept chemical sensor, focusing on the selective detection of copper and sodium cations in solution. Further, we shall present our latest results toward fabricating a sensor for monitoring gaseous and dissolved oxygen concentrations.

PO16

Miniaturisation of RNA Extraction and Purification

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Nucleic Acid Sequence-Based Amplification (NASBA) of ribosomal RNA extracted from samples can be used for sequence (species) specific detection of Algae before harmful blooms occur. The aim of this work is to miniaturise the extraction process resulting in a portable device that can extract and purify RNA from water samples. This will enable reliable results to be obtained quickly as the conventional methods for extraction and purification of RNA samples are time consuming requiring both toxic substances and laboratory equipment. Preliminary results show that successful extraction of RNA from concentrated cell samples can be achieved using a chip using bioMérieux miniMAG extraction kits; based on the BOOM method of RNA extraction with ferromagnetic silica beads. A second chip is in development that incorporates a filter preventing the need for cell concentration. The study explains and provides a discussion relating to the different extraction methods and chips available.

PO17

Towards the Development of a Closed-Loop Semi-Biotic Device Prototype

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Semi-biotic systems are hybrid systems incorporating biologically derived components integrated with synthetic components. Often, these hybrid systems can be developed to produce a device, or 'semi-biotic machine', that fulfils a particular function, usually of significant biological or medical function. Hybrid devices utilising semi-biotic systems are attractive as they are 'designed' and so can be made to exhibit specific traits, such as higher degrees of adaptability, something that is restricted with non-hybrid biological systems.

Under the NEONUCLEI research programme, an initial semi-biotic transcription device was developed, using transcription of linearised T7-luciferase from DNA partitioned into the inverse hexagonal phase of 1,2-dioleoyl-3-*sn*-glycerophosphoethanolamine (DOPE). From the first rudimentary tests performed upon a prototype microfluidic device, mRNA was produced using the DNA lipoplex as the DNA template for transcription.

Through the initial development and implementation of a transcription-capable semi-biotic device, and then a transcription-translation-capable device, it is expected that proof of concept will be established for an intelligent closed-loop semi-biotic device.

Poster title: "Developing a Continuous-Flow Transcription Device using DNA Lipoplexes"

PO18

Bioinspired Antifouling Using Natural Products against Marine Biofilms

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Biofouling is the accumulation of marine organisms on underwater surfaces. In the marine environment, all submerged surfaces are affected by the attachment of fouling organisms, such as bacteria, diatoms, algae and invertebrates causing increased hydrodynamic drag, resulting in higher fuel consumption and decreased speed and range. Specifically, a 14 % increase in fuel cost results from the formation of diatom-dominated biofilm on ocean going vessels. Generally, past solutions to antifouling have used toxic paints or coatings that severely affected marine life worldwide. The prohibited use of these antifoulants has led to the search for biologically inspired antifouling strategies. Most algal species have evolved antifouling strategies, such as natural products (NP) and surface texturing, to deter predation and epidermal fouling. Biomimetic approaches such as, the use of NPs as antifouling agents is attractive from an environmental point of view, since these compounds occur naturally in the environment. Significant effort has previously been directed towards surface topography, however, ultimately a combination of surface features and chemistry will lead to greater AF performance.

This study has assessed the antifouling performance of NPs, both crude extracts and isolated compounds, from marine sources (*Chondrus crispus*) and a purified furan derivative from a terrestrial source, against biofouling organisms which included marine bacteria (*Pseudoalteromonas sp. Cobetia marina* ATCC 25374, and *Marinobacter hydrocarbonoclasticus* ATCC 49840). The biofilm growth and adhesion kinetics were quantified using a multidetection microplate reader utilising nucleic acid based viability staining and natural bioluminescence. These bioassays were corroborated using a novel application of the imaging capability of the microplate reader, to quantify biofouling in situ. Confocal laser scanning microscopy was used to compare biofilm structures in the presence and absence of the NPs. Also, we are currently using flow cells to accommodate various bulk materials, coatings and NPs which can be tested over a wide range of Reynolds numbers. The purified furan compound gave the best performance in terms of inhibiting biofilm growth and attachment for the selected marine bacteria, however, the crude *C. crispus* extract showed inhibitory effects in specific bacteria assays. We are developing these novel techniques to be used in combination with traditional methods, such as EC50 and LD50, in order to gain greater insight into the processes of marine biofouling and to allow us to assess the efficacy of our various AF strategies.

Micro-sensing Technology for *in situ* Low Level Detection of Fe and Mn in Seawater

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In situ sensors are crucially important for understanding the physico-chemical processes that occur in the ocean environments. Nowadays laboratory methods cannot provide the temporal and spatial resolution which would be required to characterize the chemical parameters of the marine ecosystems because sampling is both expensive and time consuming. Moreover, off site analysis techniques are often affected by artefacts due to sample handling such as contamination or chemical changes in the sample. *In-situ* sensors minimize these drawbacks and provide a tool to obtain long term data banks which will allow a more synoptic interpretation of oceans biogeochemical cycles.

Trace elements are some of the most crucial parameters that require long term observations. Among those, iron and manganese are of particular interest because of the essential role they play in deep ocean processes and in controlling phytoplankton growth. We developed a sensor for the detection of either Fe(II) or Mn(II) in deep waters at nM levels. The spectrophotometric sensor is based on Lab-on-Chip technology and wet chemistry and uses robust chemical techniques to enhance the accuracy and precision of the measurements. Onboard standards allow for in-situ calibration to be performed, compensating for instrumental drift. It is designed to be deployed at over 1600 m depth and embarks enough reagent and standards to work for at least 60 days taking hourly measurements. In measurement mode, the sensor requires 3W and less than 1 mW while in sleeping mode. A Fe(II) and Mn(II) versions of the sensor will be deployed *in-situ* near the Tour Eiffel hydrothermal edifice in the Lucky Strike vents field during the MoMAR-D cruise to the Azores Triple Junction in October 2010.

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PO20

Microdroplet Formation in a T-junction Microfluidic Device Using the Two Phase Level Set Method

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Microdroplet formation is an emerging area of research due to its wide-ranging applications within microfluidic based lab-on-a-chip devices. The two-phase level set method which is ideally suited for tracking the interfaces between two immiscible fluids has been used to perform the numerical simulations. Our goal is to understand the dynamics of droplet formation in a microfluidic T-junction in order to optimise the operation of the microfluidic device. Numerical results compare well with experimental observations. The influence of different parameters such as flow rate ratio, capillary number, contact angle, viscosity ratio and the interfacial tension between the two immiscible fluids, were investigated systematically. It was found that the droplet size increases with flow rate in the squeezing regime where breakup occurs due to the pressure drop across the emerging droplet. Droplet size decreases rapidly with capillary number as we move from squeezing to shearing regime. The transition from squeezing to shearing was observed at critical Ca of 0.019. Increasing hydrophobicity and reducing interfacial tension between the two liquids produced smaller droplets, especially when Ca was small. Understanding of this phenomenon forms the basis of many potential applications: synthesis of new materials, formulation of products in pharmaceutical, cosmetics and food industries.

PO21

Designing a miniaturised ion chromatographic system for *in situ* water analysis

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With increasing demand for high quality water comes the need for predictive models capable of anticipating the impact factors associated with urban expansion and climate change will have river systems. For these models to predict with greater certainty more frequent chemical measurements need to be made. At present, the automatic river water monitoring systems available for chemical measurements tend to involve cumbersome equipment which requires considerable servicing. The equipment has to be housed in purpose built huts and the water has to be pumped to the equipment.

The objective of this project is to design and manufacture a miniaturised system, which can be used to automatically perform analysis of a range of analytes; Na^+ , K^+ , Ca^{2+} , Mg^{2+} , NH_4^+ , NO_3^- , NO_2^- , SO_4^{3-} , PO_4^{3-} and Cl^- down to 0.01 mg/L. In this multidisciplinary project a range of ions will be separated and detected in a purpose built system designed in conjunction with engineers. Separation will occur in a monolithic column with conductivity detection and where possible the need for mechanical pumping will be avoided.

The system has proved successful so far with both anions and cations being detected after migration through a gel filled capillary with no volumetric movement.

PO22

Capacitively coupled conductivity detection (C⁴D) for a miniaturised separation system for in situ water monitoring

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The aim of this project is to develop a miniaturised separation system for in-situ water monitoring. The detection system is being developed by the Department of Engineering. The principle of the detection has been chosen according to the existing commonly used techniques in ion chromatography (conductivity). Capacitively coupled conductivity detection (C⁴D) is a contactless, inexpensive technique which provides accurate analytical results. These advantages of C⁴D make it a preferred technique when a sensitive, low cost conductivity measurement is required.

Several approaches for on-capillary conductivity detection using different electrode configurations have been evaluated. For application with the microchip a two electrodes plug version was selected for further investigation. These plug were manufactured from a photopolymer using 3D printing, the electrode surface was achieved by the end of the plug with silver conducting paint. Electrical conduct was achieved using tinned copper wire and the whole plug was covered with insulating varnish. This device could be introduced into the microchip (preferably near a ground electrode) by the mean of wells drilled into the top plate.

The current system gives convincing conductivity measurement results with fairly linear calibration curve and a limit of detection down to the ppm level for ions of interest (K⁺, Na⁺, Cl⁻). Future work will focus on the preservation of these characteristics while coupling the detection system to the separation module.

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