

Electrochemical Biosensor for Pesticide Detection in Water

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Sensowaq Meeting

DTU Miljø

25 January 2012

Surface Engineering Group

DTU Nanotech

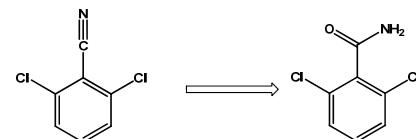
Department of Micro- and Nanotechnology



SENSOWAQ

Model System

2,6-dichlorobenzamide (BAM)



Dichlobenil

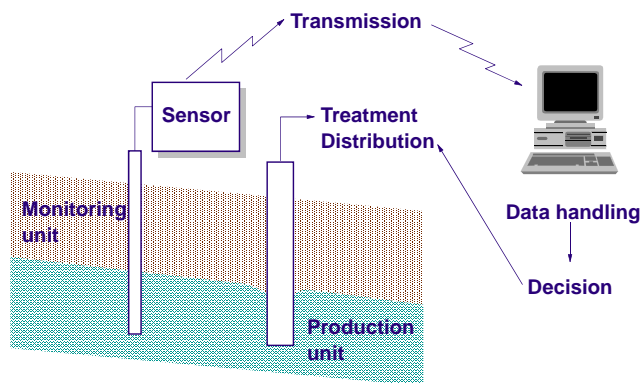
2,6-dichlorobenzamide (BAM)

- Highly resistant to further degradation
- Fairly soluble in water
- Found in Danish ground water resources

Anti-BAM monoclonal antibody and an inhibition assay are developed

Leif Bruun *et al.* *Journal of Immunological Methods* 240 (2000) 133–142

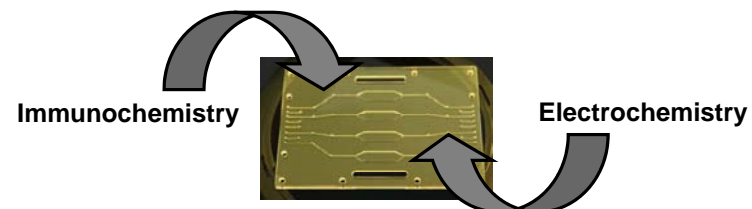
Project Concept



Courtesy to Claus Jørgensen, DHI

Goal of the Work

To develop an in-line sensor for on-site control of drinking water



Automation enables the sensor to be unattended for a few months

In-line sensor - Requirements



PHASE - I

The Task

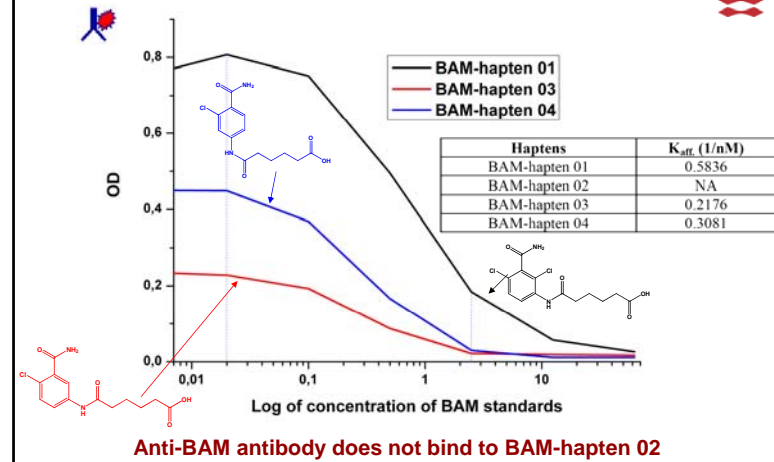
Bringing immunochemistry from ELISA to in-line sensing

- Repeatability
- Calibration
- Reproducibility
- Regeneration of the surface
- Antibody-conjugate stability ✓
- Substrate stability ✓
- Hapten coated surface stability ✓ ✓

The Challenge

System Regeneration

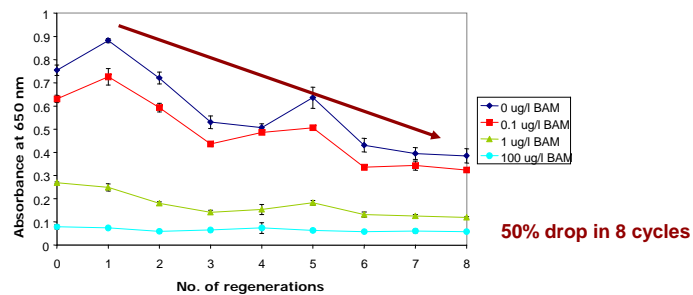
Standard Curves



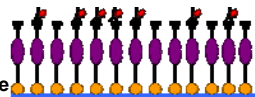
System Regeneration



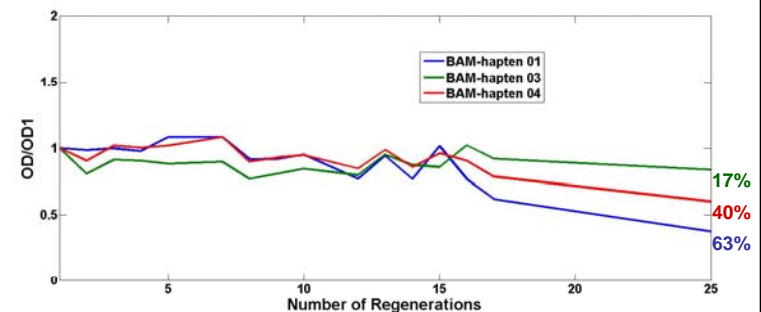
Different concentrations of BAM standards were used



- The signal drops down with no. of regeneration
- The signal drop is prominent for more bound surface



Regeneration Curves



- BAM-hapten 01 shows poor regeneration and better signal
- BAM-hapten 03 shows better regeneration and a very poor signal
- BAM-hapten 04 shows better regeneration and reasonably good signal

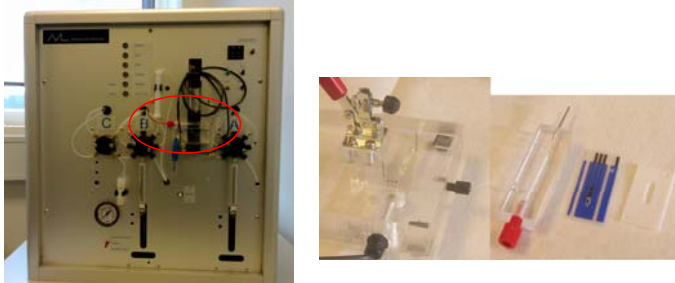
Design of Microfluidic Platform



PHASE - II

The Task

Incorporating the optimized immunochemistry with detection system



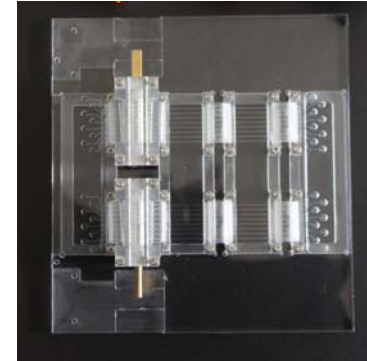
The Challenge

Leak-proof, easy-handling microfluidic system

MainStream



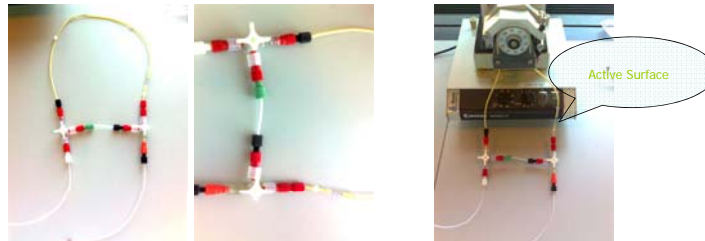
FAST group Fluidic Array Systems and Technology



- Miniaturized peristaltic pump
- Miniaturized valves
- Monolithic PDMS inlays
- Multichannel
- PMMA and PC
- Low cost, stand-alone
- $\mu\text{L min}^{-1}$

P. Skaft-Pedersen, D. Sabourin, M. Dufva,
D. Snakenborg, *Lab Chip* 9, 3003 (2009)

The Beginning!!!

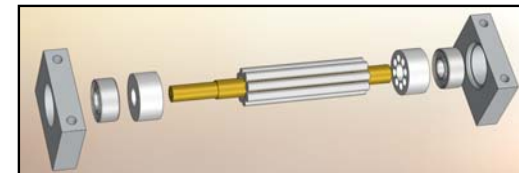


- The immunochemistry worked well
- The protocol could not be standardized

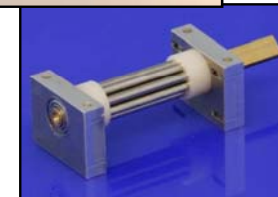
Miniaturized Peristaltic Pump



Multi-Roller



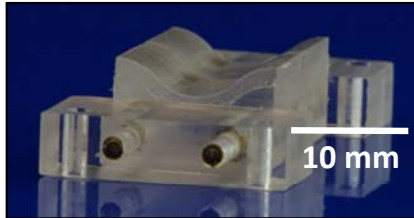
Central brass rod,
8 steel pins,
ball bearing holders



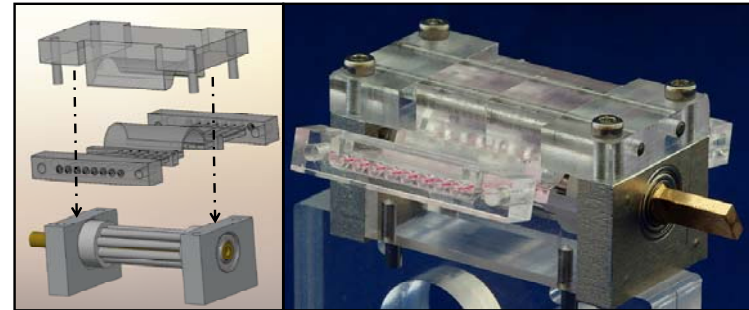
Miniaturized Peristaltic Pump



Rotor Bed (Rigid Support)



Miniaturized Peristaltic Pump



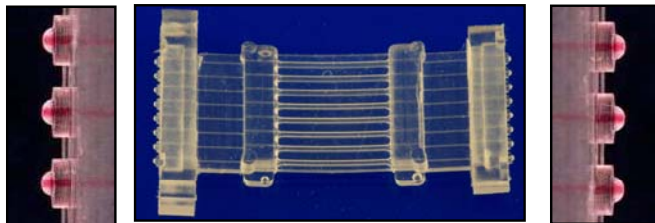
8-CHANNEL PUMP
40 mm (L) x 20 mm (W) x 20 mm (H)

Miniaturized Peristaltic Pump



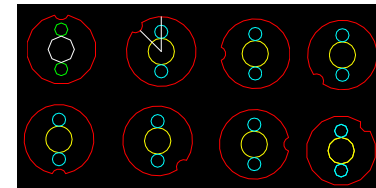
Microfluidic Ribbon (μ FR)

- Integrated Ball-End Joints
- Eight 240 μ m Channels

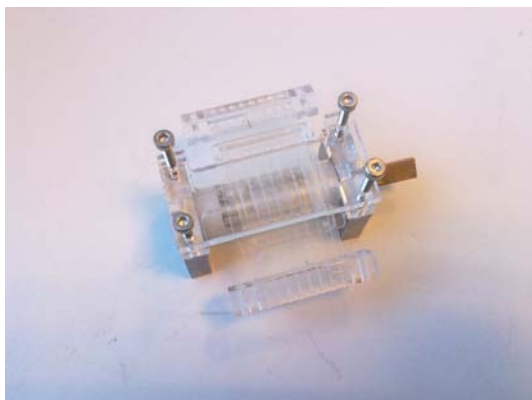


48 mm (L) x 30 mm (W) x 5 mm (H)

Miniaturized Valves



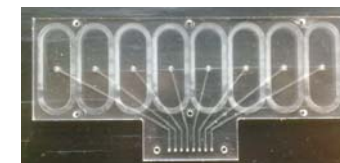
Miniaturized Valves



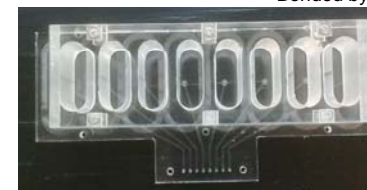
Reservoirs and Reservoir chip



Made of Polycarbonate (1cm thick)
Each chamber has 3 mL capacity

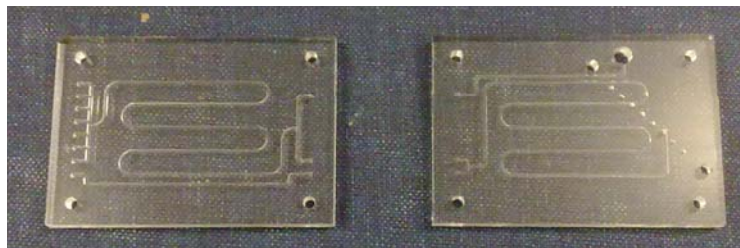


Made of PMMA
Bonded by hot press bonding



Pressed together with a PDMS gasket in between

Chips

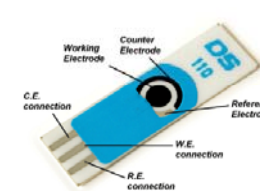


Reaction Chip

Mixing Chip

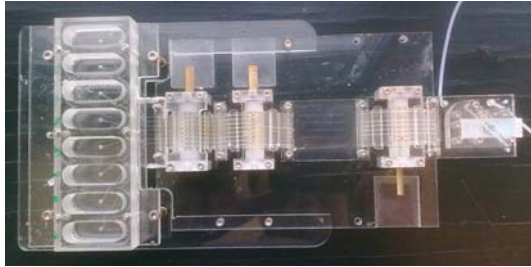
- Made of PMMA
- Bonded with lids by hot press – bonding technique
- 85 degree Celsius, 7 kN for 40 min
- 30 μ L capacity

Electrode and Electrode Chamber



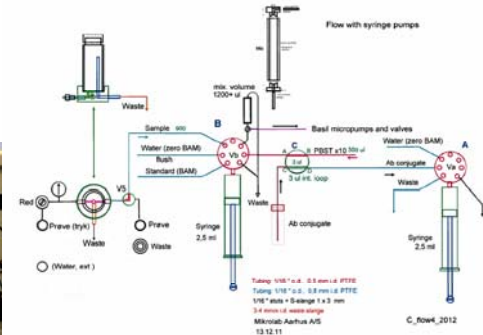
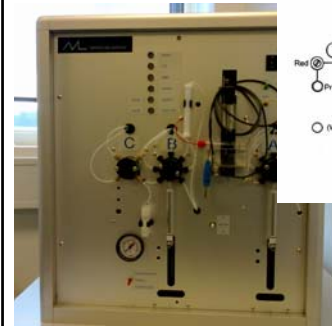
Made of PMMA and screwed together
with PDMS gasket in between

The Complete System

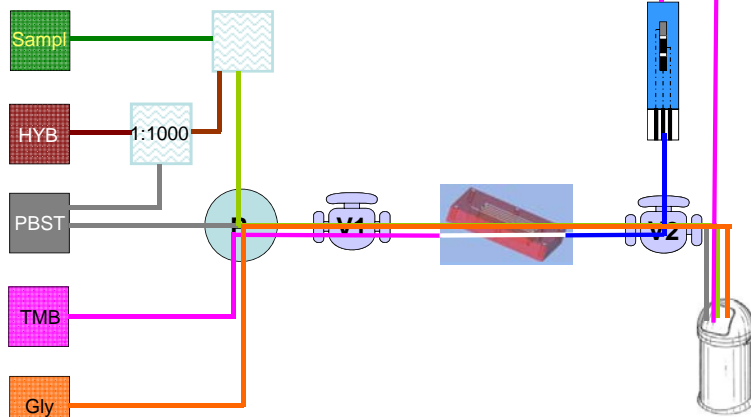


All the parts are screwed onto a base plate
Tubing is used only for waste outlets

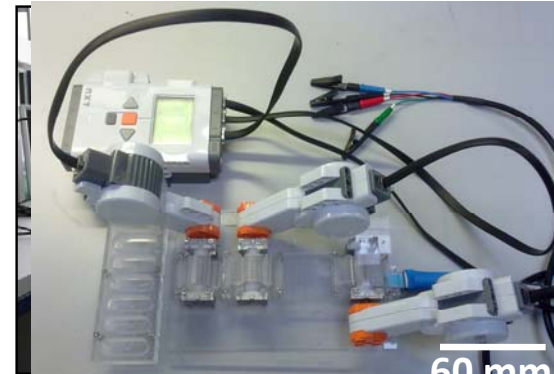
Interface Between Macro- and Micro- Worlds



Flow Sequence



Advantages of Microfluidic System

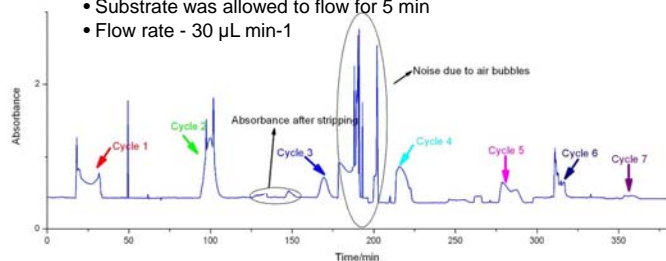


Portability, Compatibility with Microscopy/Detection
Easy Automation
Tubing: Dead volumes, Compliance, Hard to handle conveniently

Flow experiment with UV-Vis



- Original BAM hapten is immobilized
- Zero BAM in sample
- 15 min incubation with Antibody
- Substrate was allowed to flow for 5 min
- Flow rate - 30 $\mu\text{L min}^{-1}$

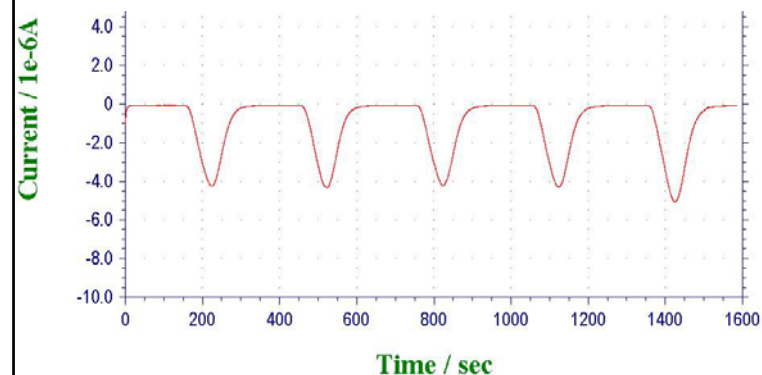


Signal falls down with cycles

Electrochemical Characterization



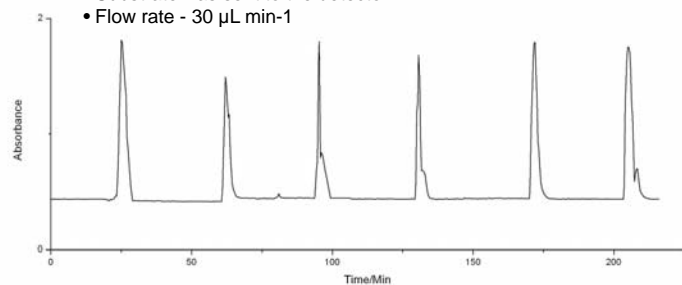
- 0.01 M Potassium Ferricyanide + 0.1 M KCl
- $E = -200 \text{ mV}$
- Potassium Ferricyanide was flown for 30 s @ 5 min interval



Flow experiment with UV-Vis



- Newly synthesized hapten is immobilized
- Zero BAM in sample
- 15 min incubation with Antibody
- 15 min incubation time with substrate
- Substrate was sent to the detector in FIA
- Flow rate - 30 $\mu\text{L min}^{-1}$



Signal is better in terms of cycles
Noises due to air bubbles are removed

Conclusions



Goals

- Optimization of existing Elisa
- Regeneration of assay surface
- From microtitre plates to micro-fluidics
- Electrochemical detection
- Interface between micro and macro worlds

Results

- Achieved
- Achieved
- Achieved
- Positively progressing
- Positively progressing



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SPEAKERS

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Francisco Chamela, Cyto SA, P
Mike Eaton, University of Nottingham, UK
Andreas Engel, University of Basel, CH
Mauro Ferrari, University of Texas, Health Science Center, US
Henk Gardeniers, MESA+, University of Twente, NL
Randy Goodall, NanoMedical Systems Inc, US

Andje Graber, Risk Dialogue Foundation, CH
Andreas Jacobs, European Institute of Molecular Imaging, Münster, D
Jürgen Kasper, RWTH, CH
Michael H. Kuhn, Philipps Heutheues, D
Christoph Michael, Leibniz Universität, D
Christian Strohriegl, University of Münster/CeNTECH, D
Marcus Texter, ETH Zurich, CH

Objectives

Nanobiotechnology as one of today's most fascinating and challenging fields of research is a multidisciplinary scientific field.

After five successful congresses in Münster, Grenoble and Barcelona taking place at yearly intervals **NanoBio-Europe** will once again be hosted in Münster.

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Webinar
Simplifying nanoLCMS workflows and increasing throughput using chip-based columns
24 January 2012 9:30 GMT / 10:30 CET

- 1 Optimization of an immunoassay of 2,6-dichlorobenzamide (BAM) and
- 2 development of regenerative surfaces by surface modification with newly
- 3 synthesised BAM hapten library
- 4
- 5 Basil Uthuppu^a, Jens Aamand^b, Claus Jorgensen^c, Spire M. Kiersgaard^d,
- 6 Natalie Kosterha^a, Mogens Havsteen Jakobsen^a

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SAS 2011

IEEE Sensors Applications Symposium

February 22 - 24, 2011 | Hyatt Regency San Antonio | San Antonio, TX

Optimization of Immunochemistry for Sensing Techniques to Detect Pesticide Residues in Water

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Oral Presentation

INASCON 2010, 20th – 22nd August
Baarlo, The Netherlands

Danish Water Forum and DWRP
4th Annual Meeting, 28 – 29 Jan 2010
GEUS, Copenhagen

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Thank You

Questions