Development of optical sensor technology for

environmental applications of nutrients, bacteria and algal toxins.

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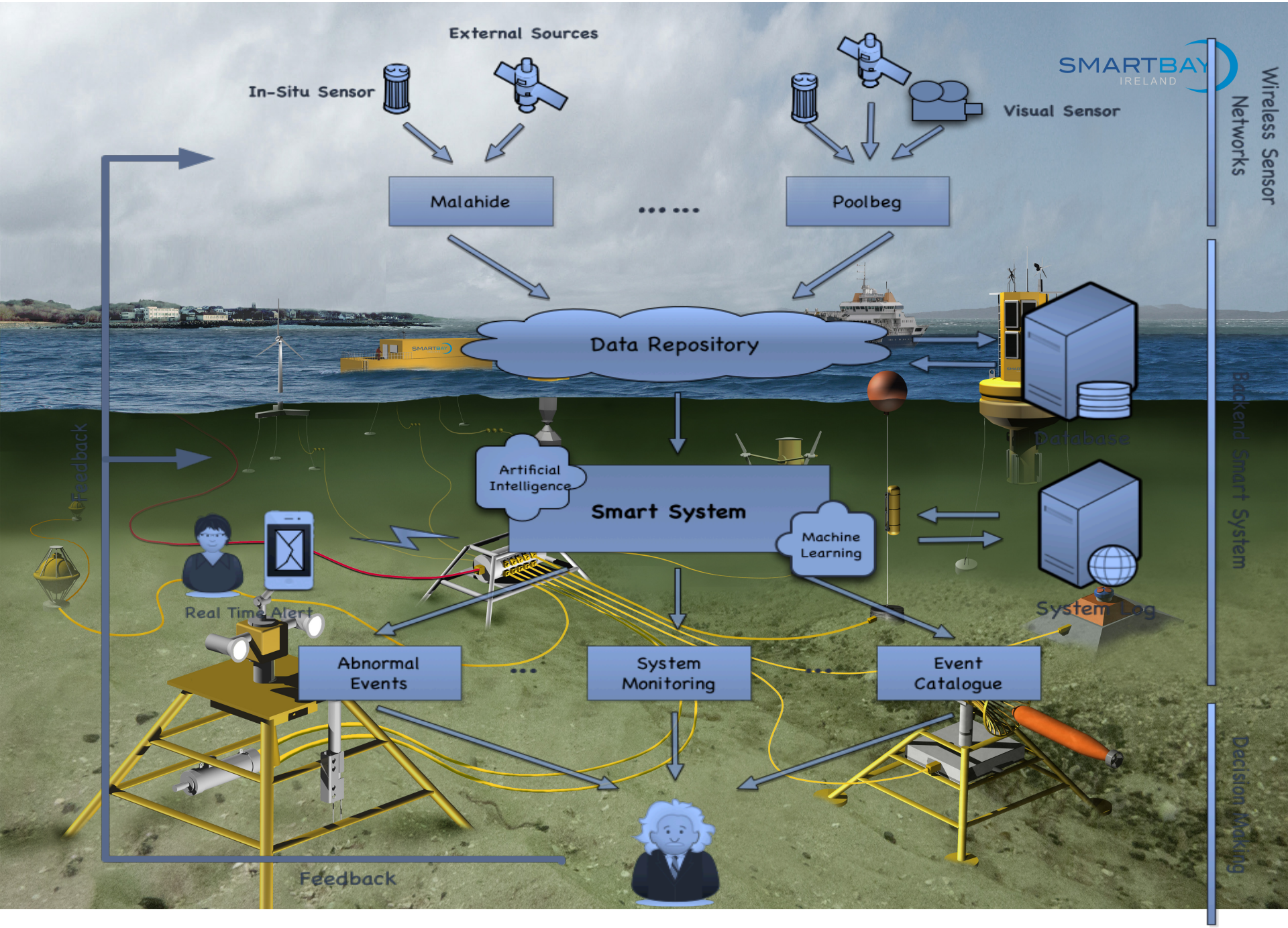
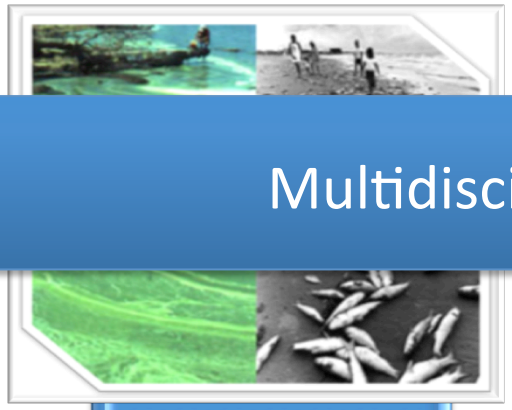
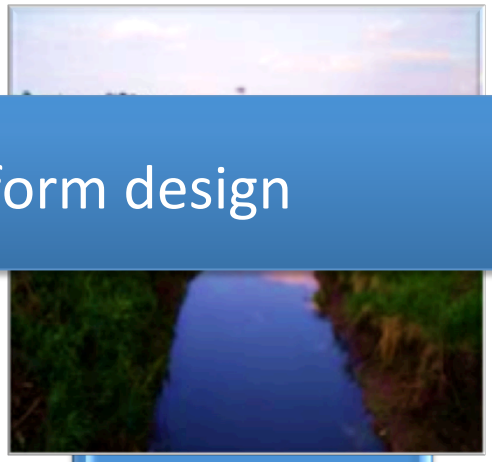
DCU | Water Institute

Europt[r]ode March 20-23 2016, Graz


External Sources
In-Situ Sensor
Visual Sensor
SMARTBAY
IRELAND
Wireless Sensor Networks
Malahide
Poolbeg
Data Repository
Backend Smart System
Database
Artificial Intelligence
Smart System
Machine Learning
Feedback
Real Time Alert
System Log
Abnormal Events
System Monitoring
Event Catalogue
Decision Making
Feedback

Outline





Multidisciplinary approach to platform design

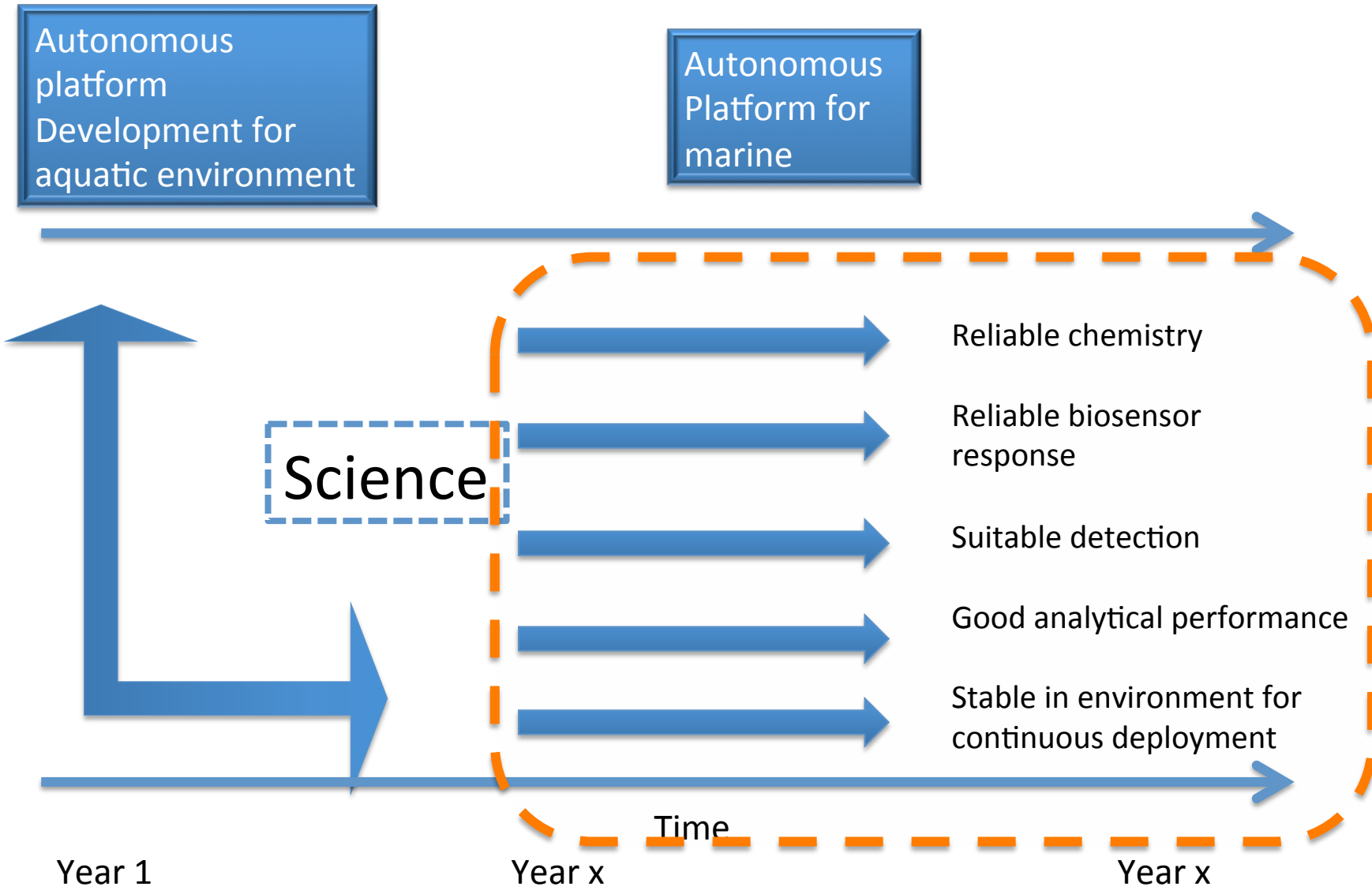
Antibody-based assay Fluorescence detection

Enzyme-based assay Fluorescence detection

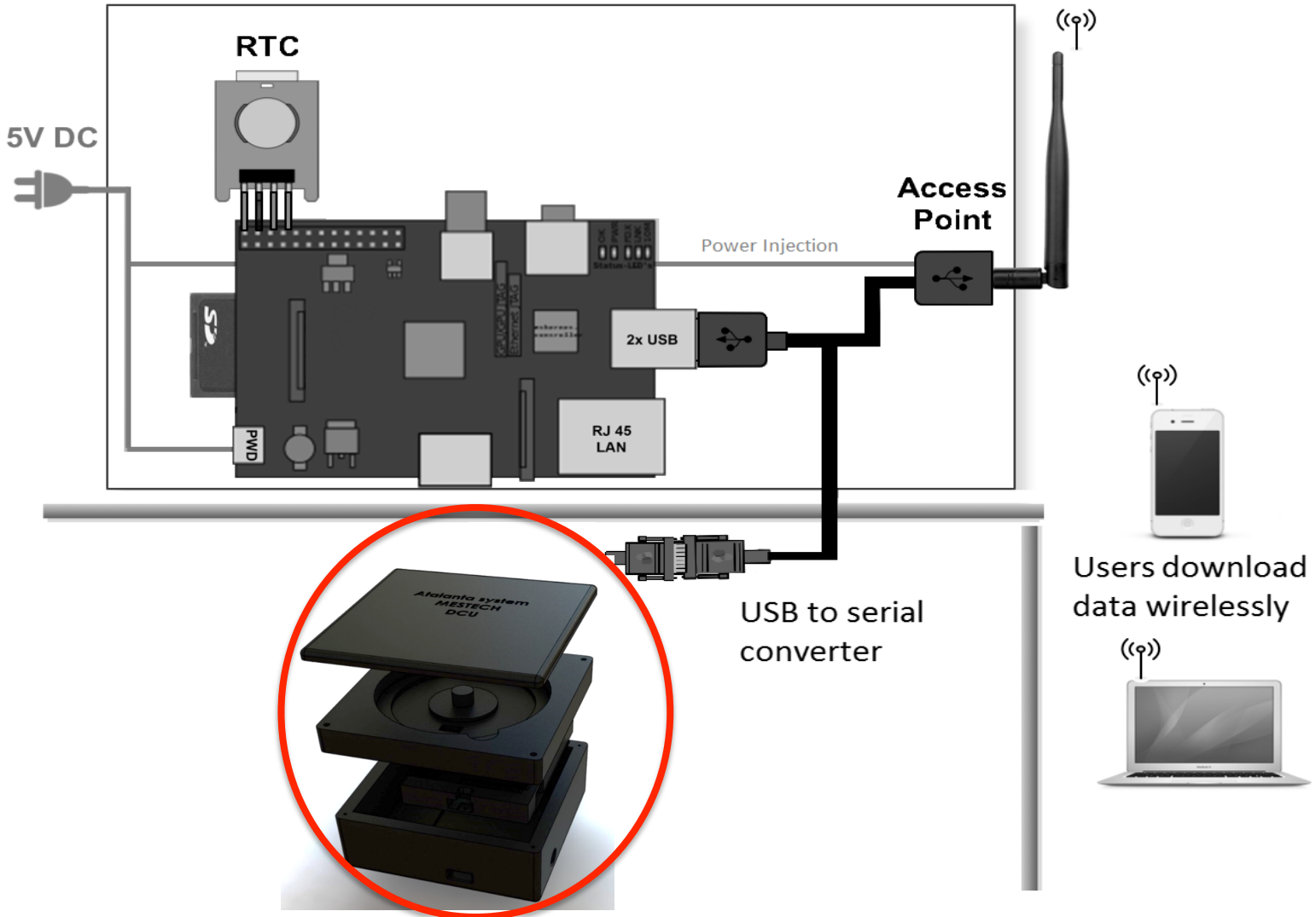
Colorimetric detection

Simple design, reliable chemistry, application to real challenge, generic platform where possible

System development



Autonomous System: Integrated sensor with comms/telemetry



- Algal toxins & emerging contaminants
  - Microcystin
  - Antibody development for new toxins
  - Device design & development

Sensor platform Integration Plan

Antibody/Recognition Element development

Saxitoxin

Azaspiracid

Domoic Acid

Microcystin

Stability/affinity selection studies

Antibody re-engineering for enhanced performance

Immobilisation studies

On-plate assay development

SPR analysis

System Integration & Pilot Studies

Platform Construction

Sensor chip design optimization and fabrication

Channels
Actuators
Valves
Sensors

Surface chemistries

Prototype development and Preliminary assay studies using Microcystin Ab And Ag

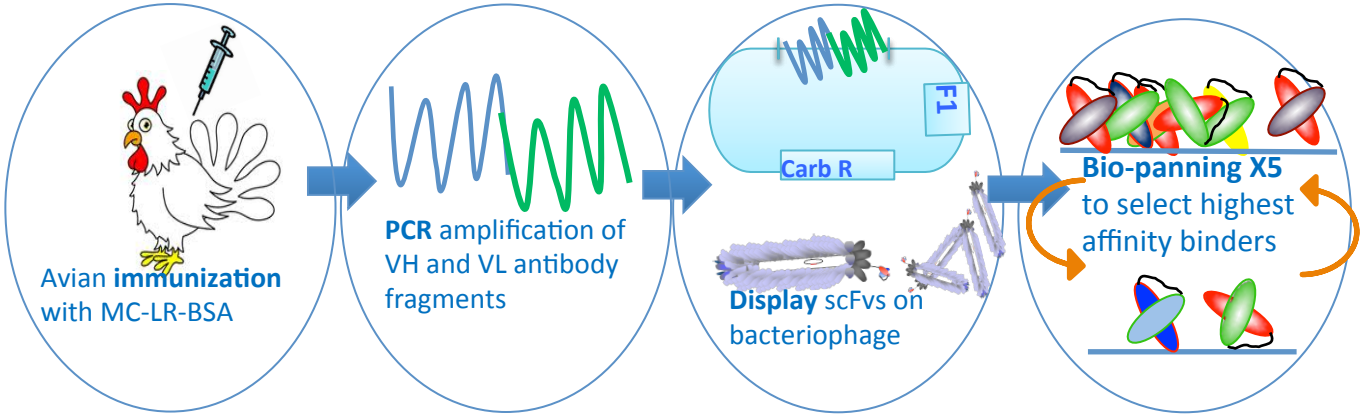
Integration of MRE's onto biosensor platform

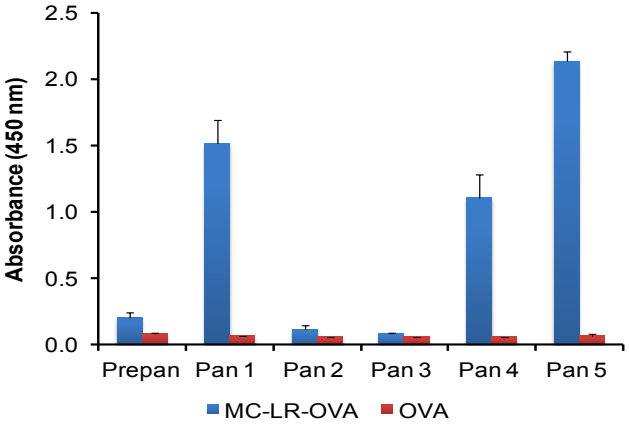
Validation of biosensors using real samples and reproducibility/stability analysis

Generation of **antibody-based biosensors** using **recombinant antibody technology**

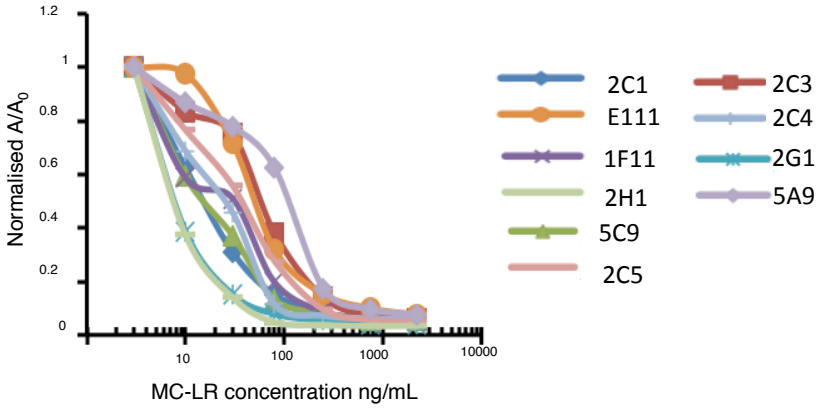
Methodologies

Development of anti-microcystin recombinant antibodies



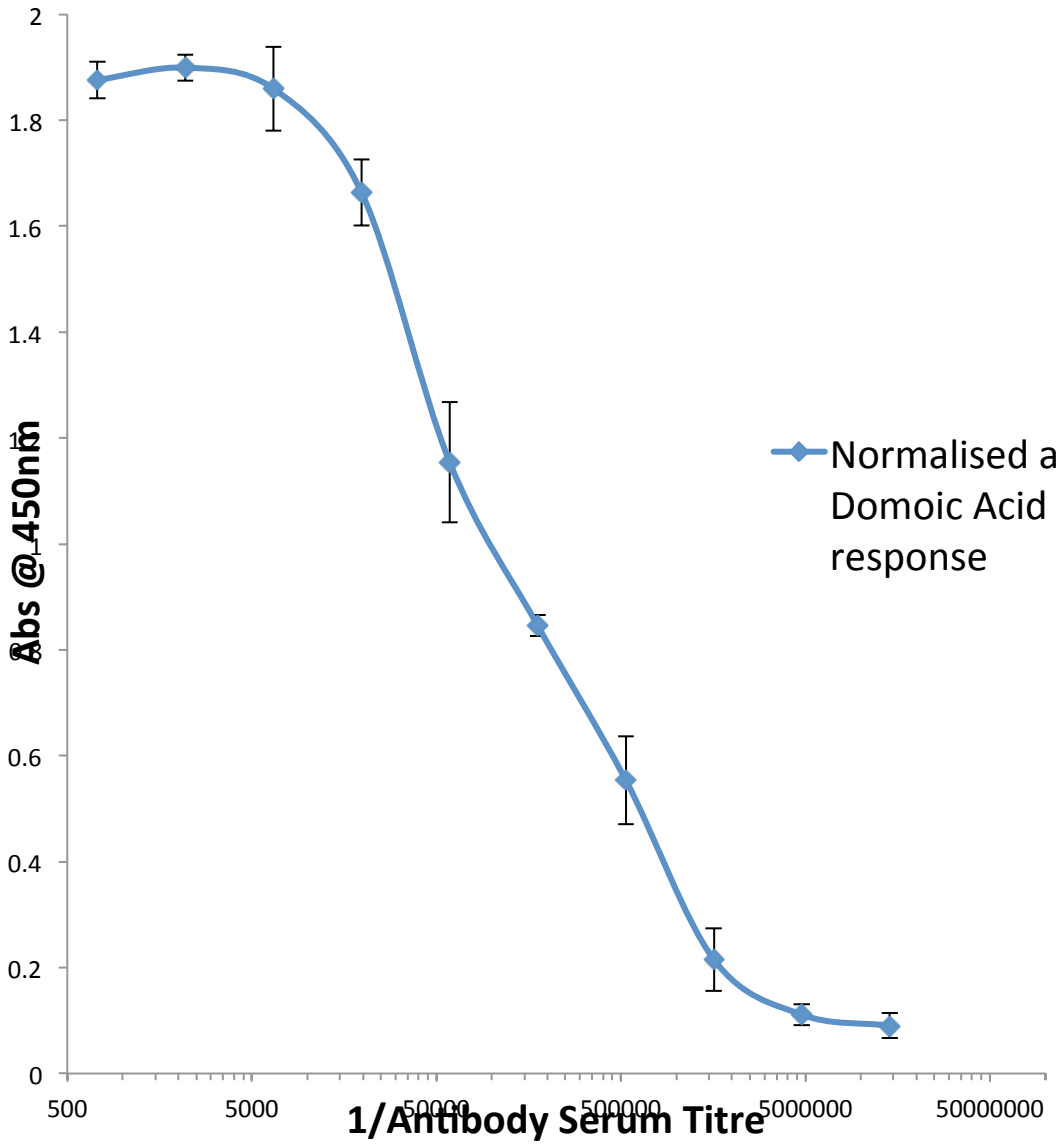


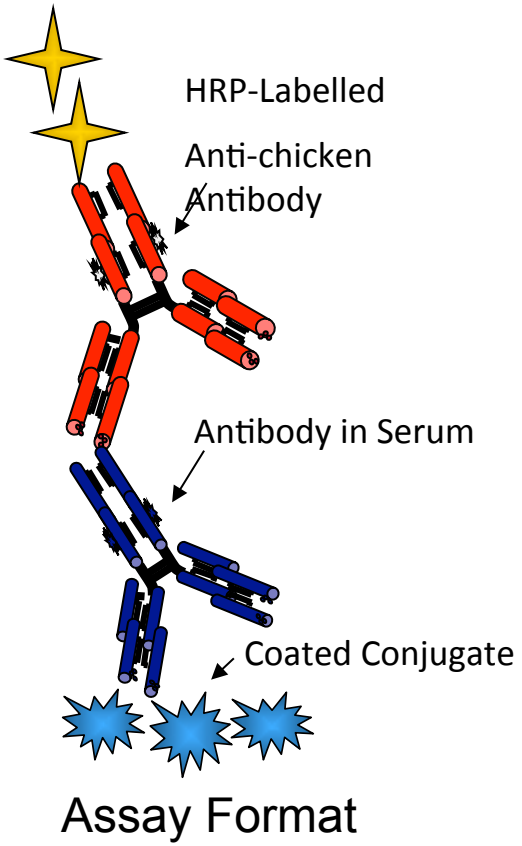
Antibodies produced from each round of biopanning



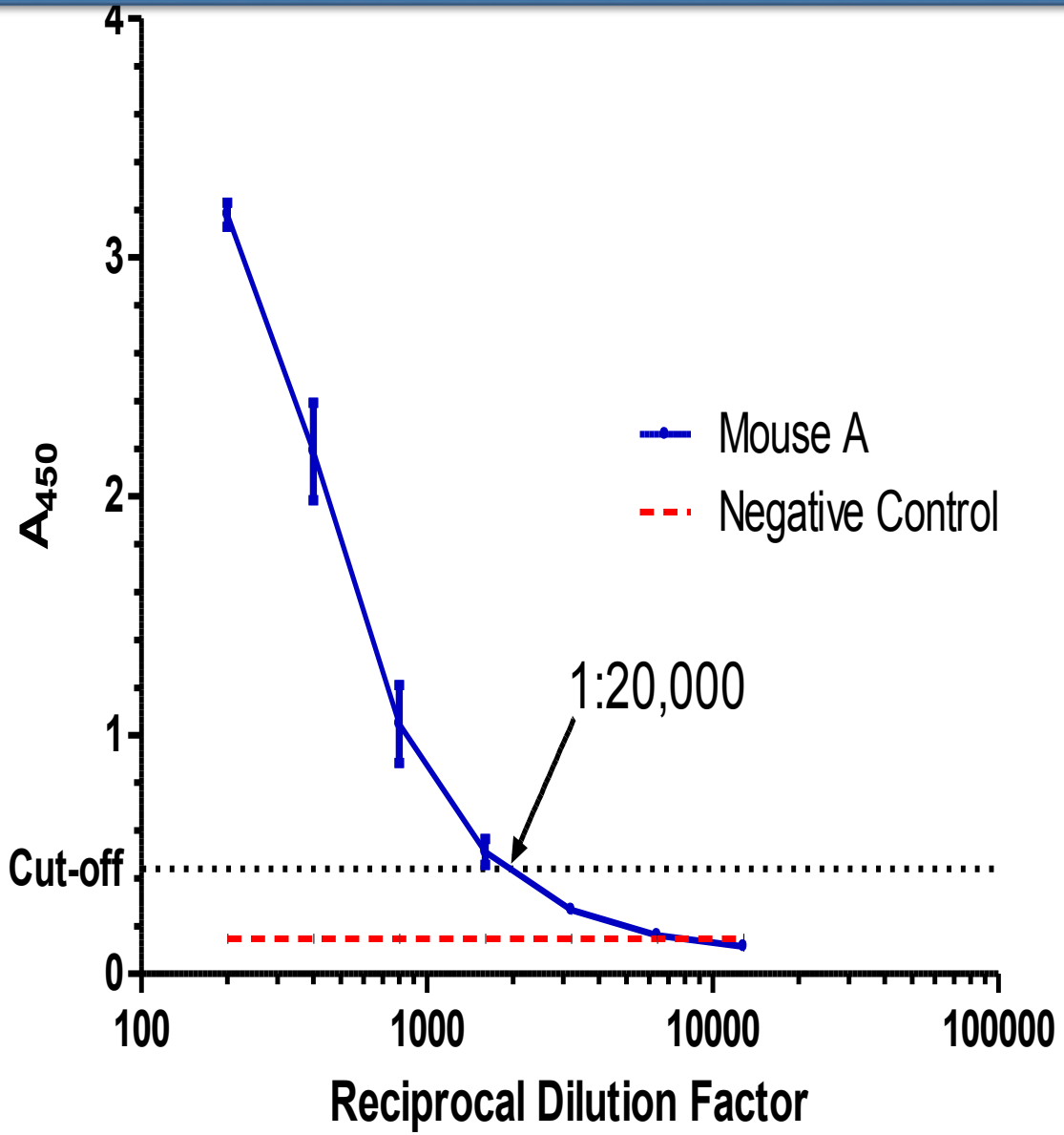
The most sensitive binder was determined by inhibition ELISA

Domoic Acid Chicken Serum Titre





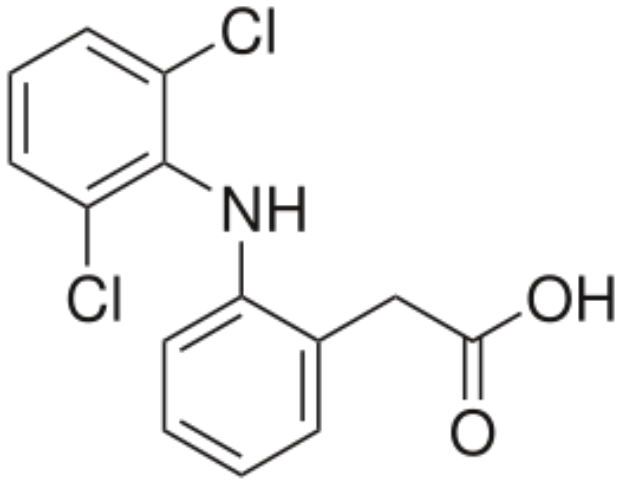
Azaspiracid



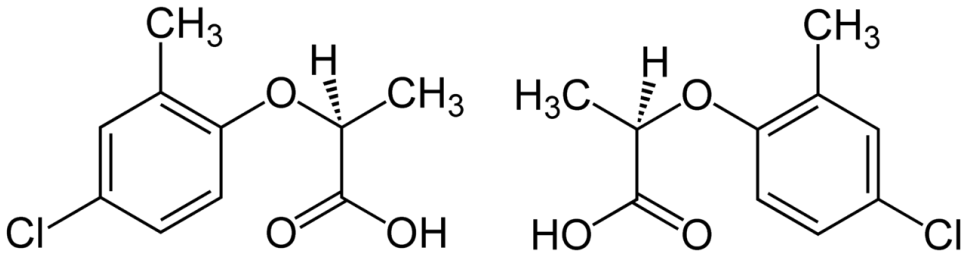
- ScFv library successfully constructed
- Preparation of conjugates underway for biopanning
- Polyclonal antibodies successfully isolated

New emerging chemicals – Recombinant antibody generation

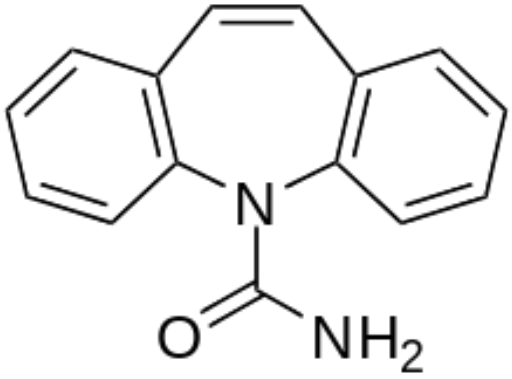
Diclofenac



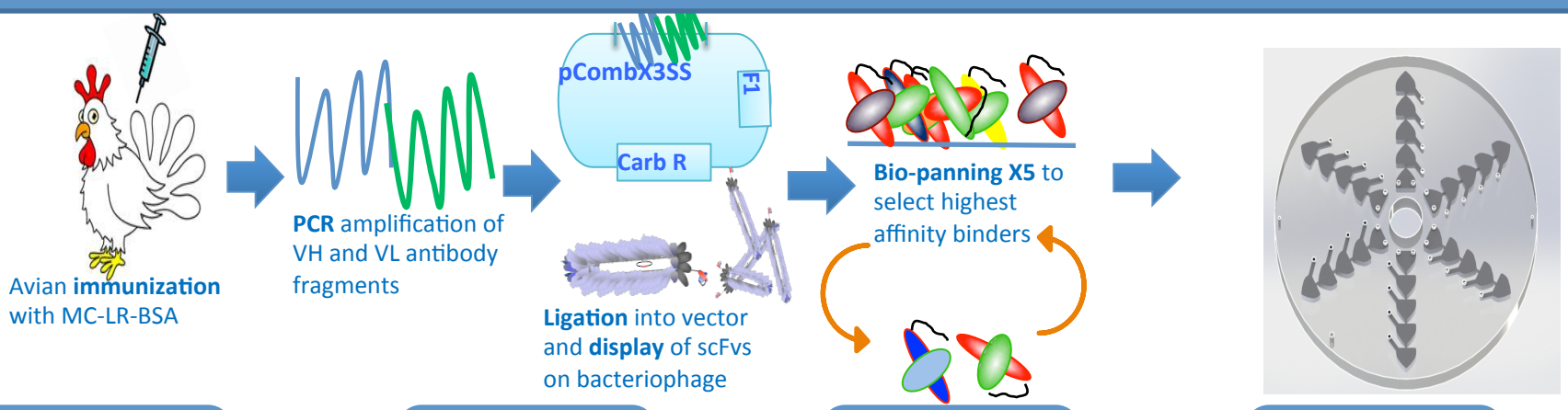
Mecoprop



Carbamezapine



Recombinant antibody-based microfluidic sensor



Immunisation

- Diclofenac, mecoprop and carbamapazine were conjugated to proteins and were immunised into an avian host.
- Immune response was identified using a polyclonal ELISA.

Recombinant antibody generation

- Genetic material was then isolated for use in the production of recombinant antibodies (rAbs).
- Generation of three immune antibody libraries for each chemical

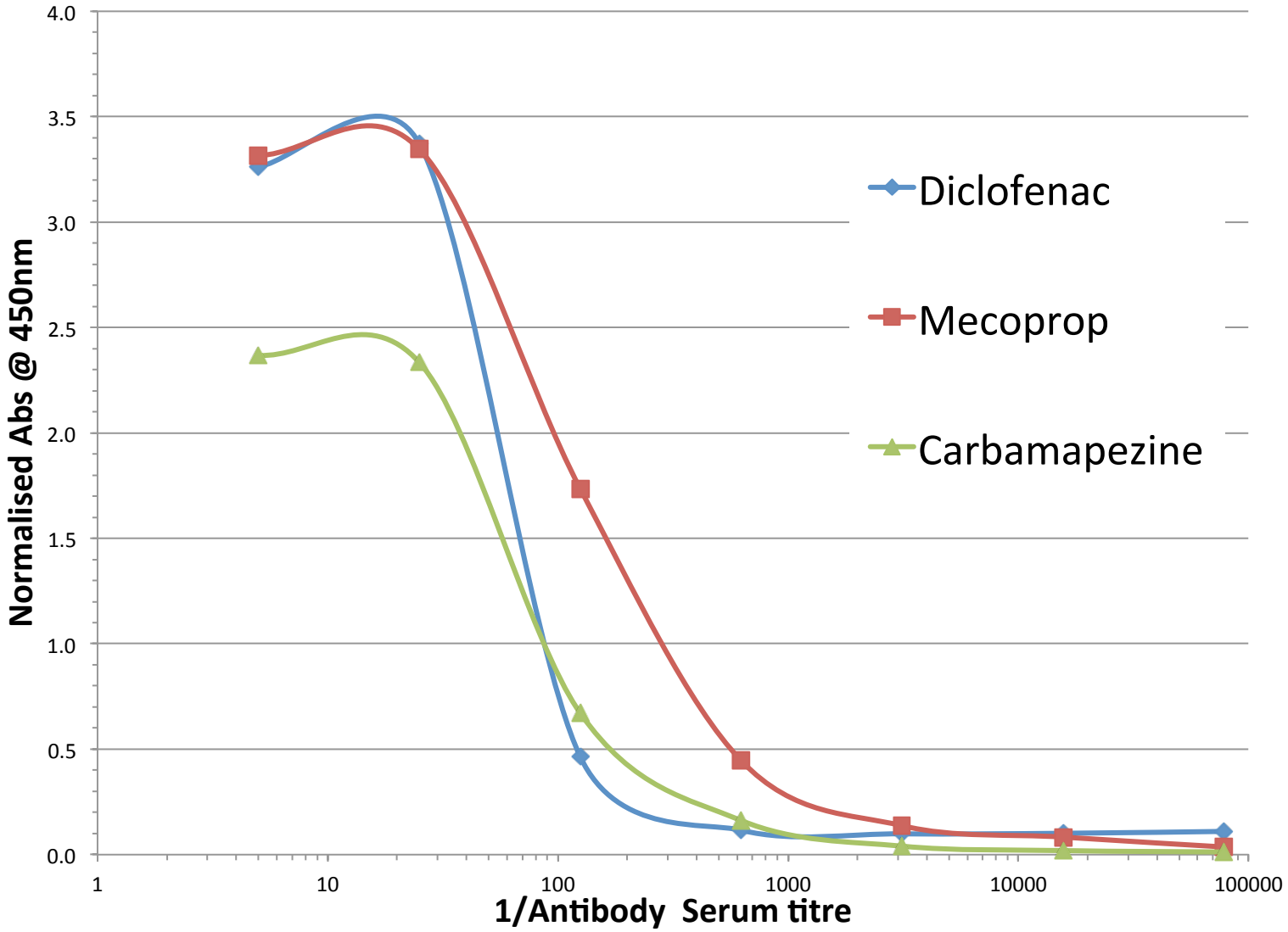
Antibody identification

- **Phage display** and **bio-panning** will be used to isolate most potent binders.
- They will be tested in matrix and cross-reactivity studies.
- Affinity tested using SPR.

Microfluidic disc

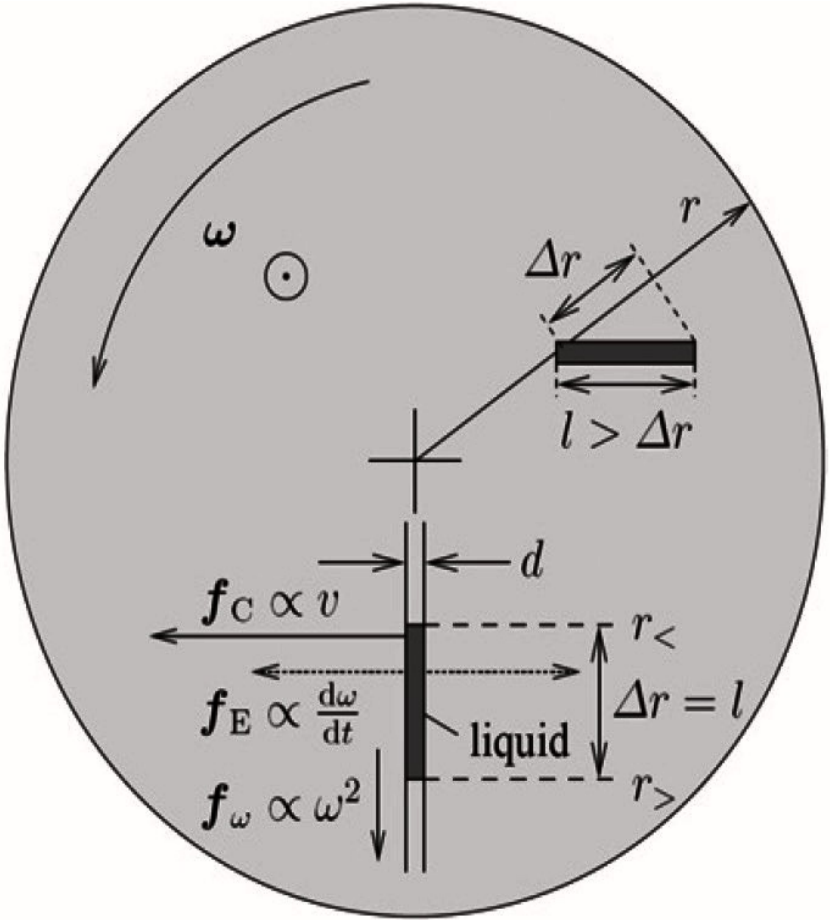
- The antibodies and chemical conjugates will be incorporated onto a microfluidic disc- based immunosensor.

Recombinant antibody assessment

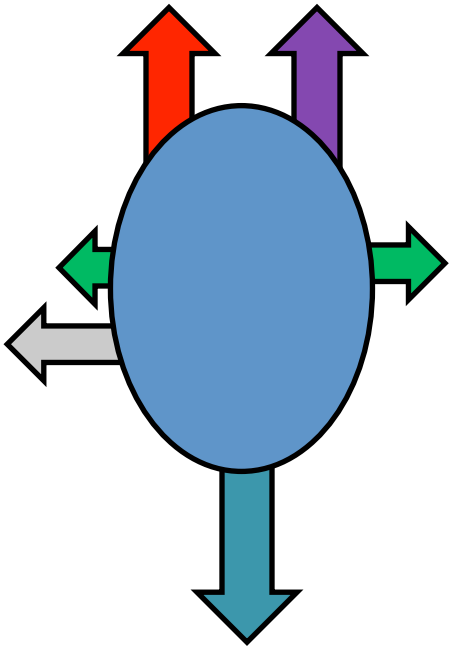


Centrifugal sensing platforms

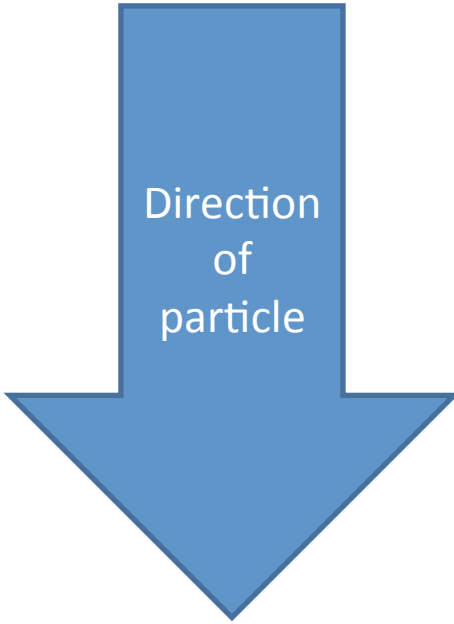
Fluidic movement on rotating platform



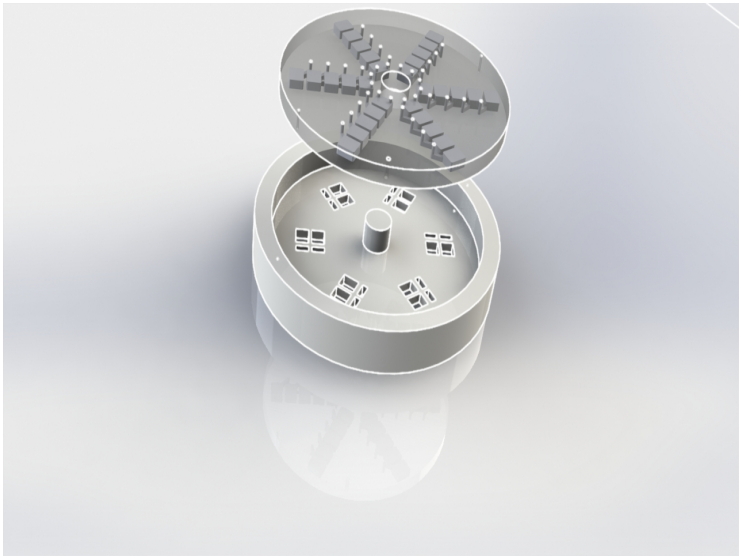
Forces on acting on a rotating disc

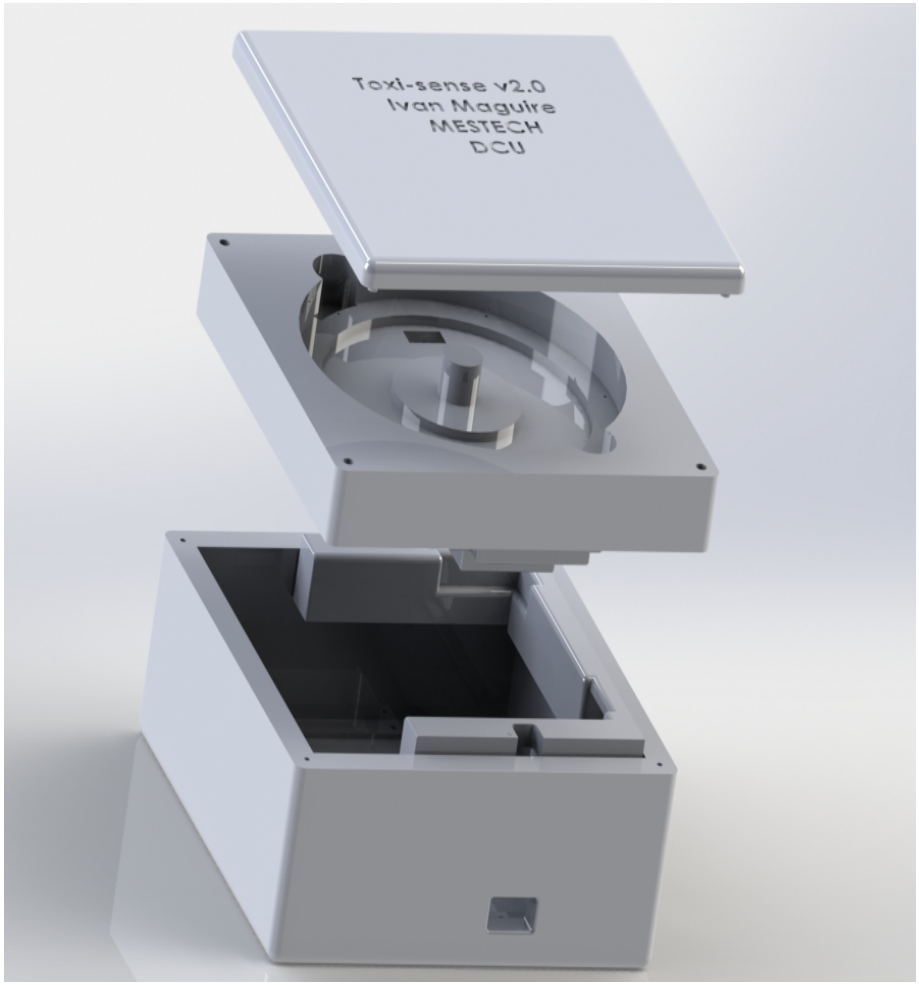


Particle sedimentation through a fluid on anti-clockwise rotating disc

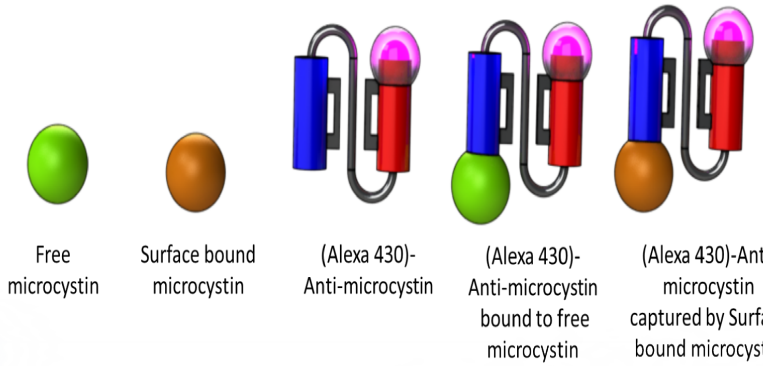


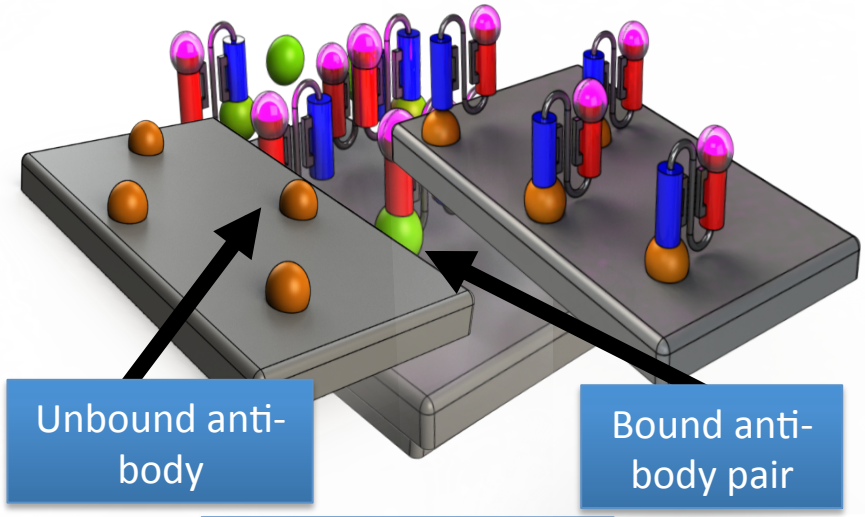
ToxiSense microfluidic System



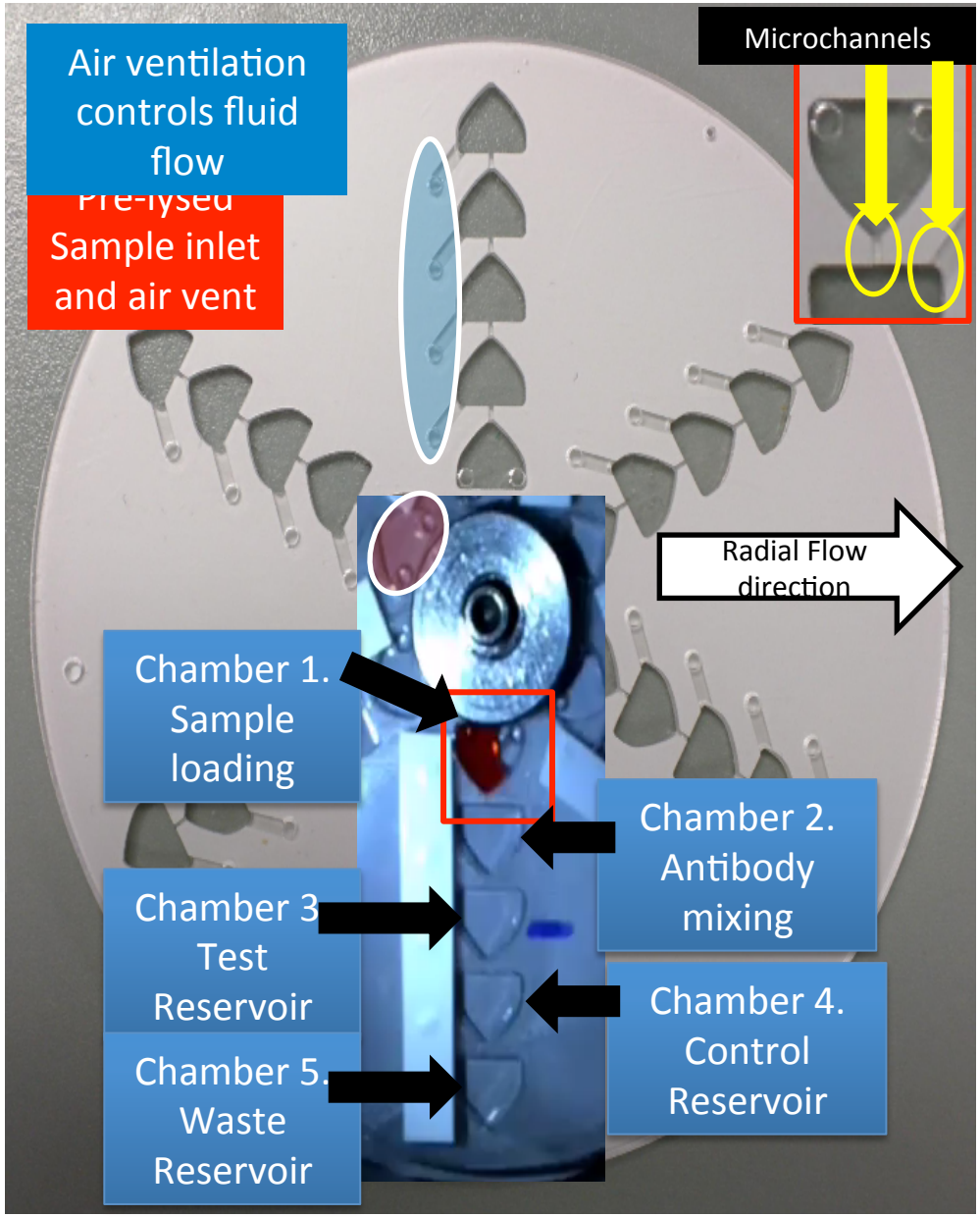


Single assay proof of concept using pneumatic pressure valving

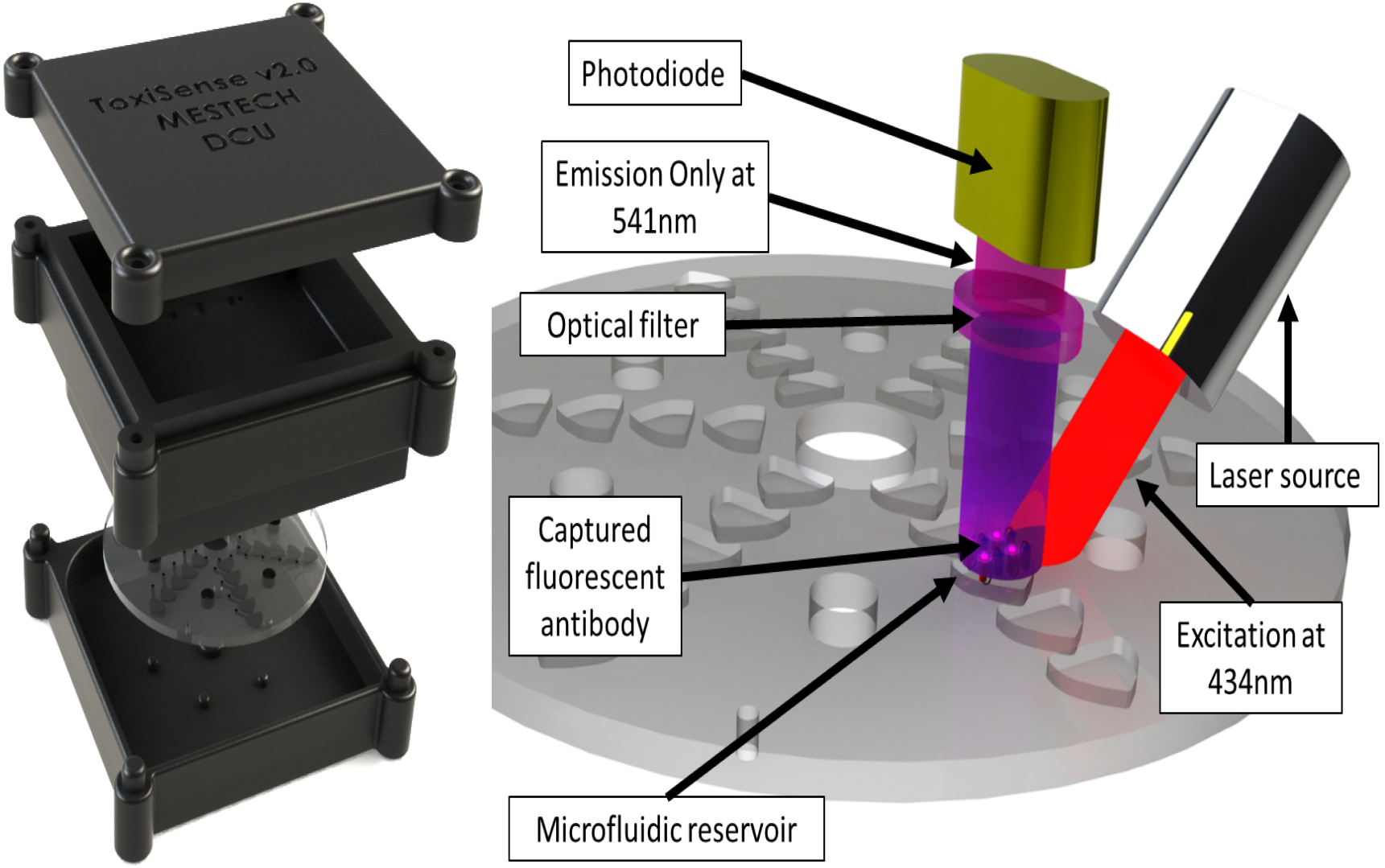




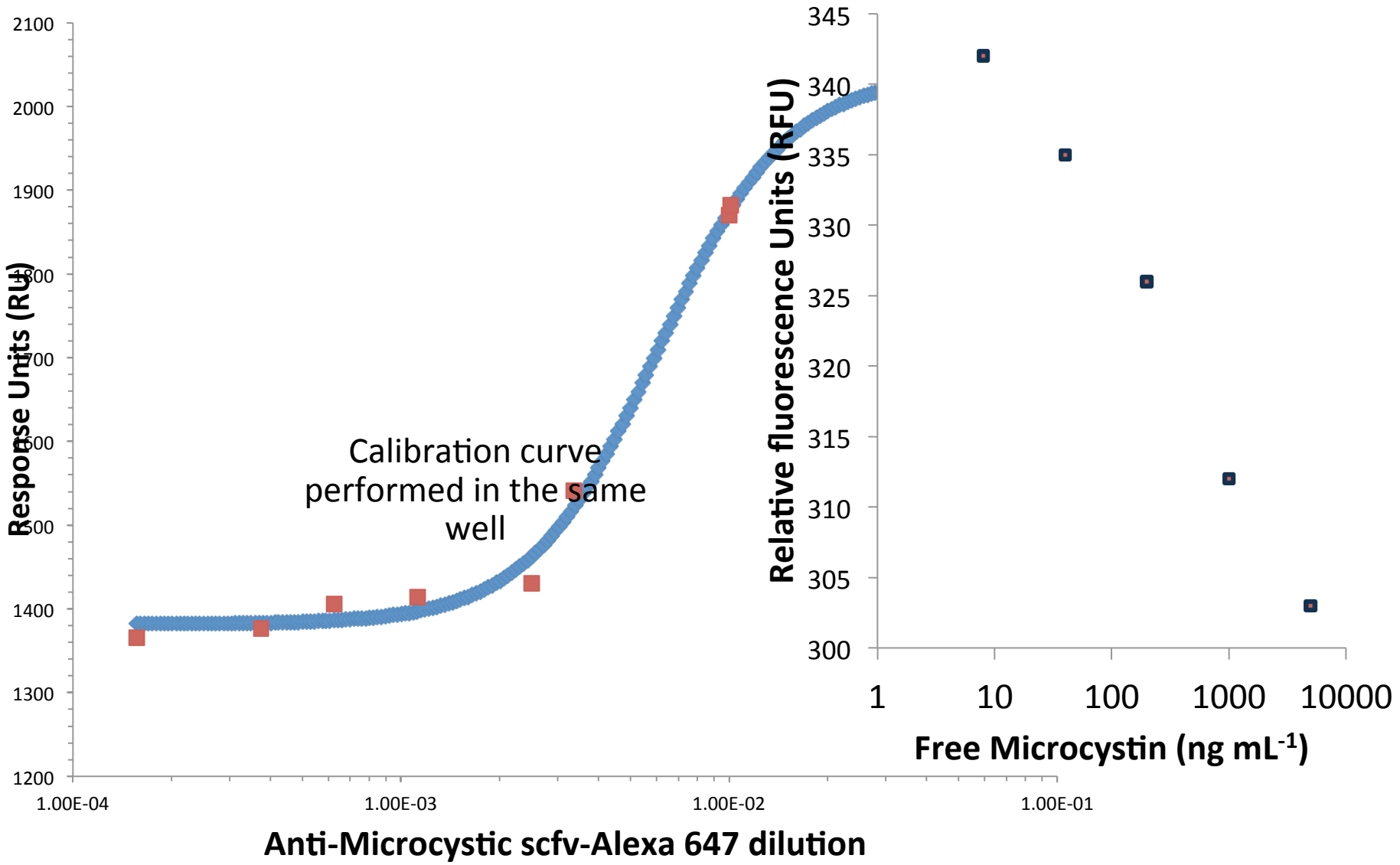
Chamber 4. Control Reservoir Before (left) and after (right)



ToxiSense – optical arrangement

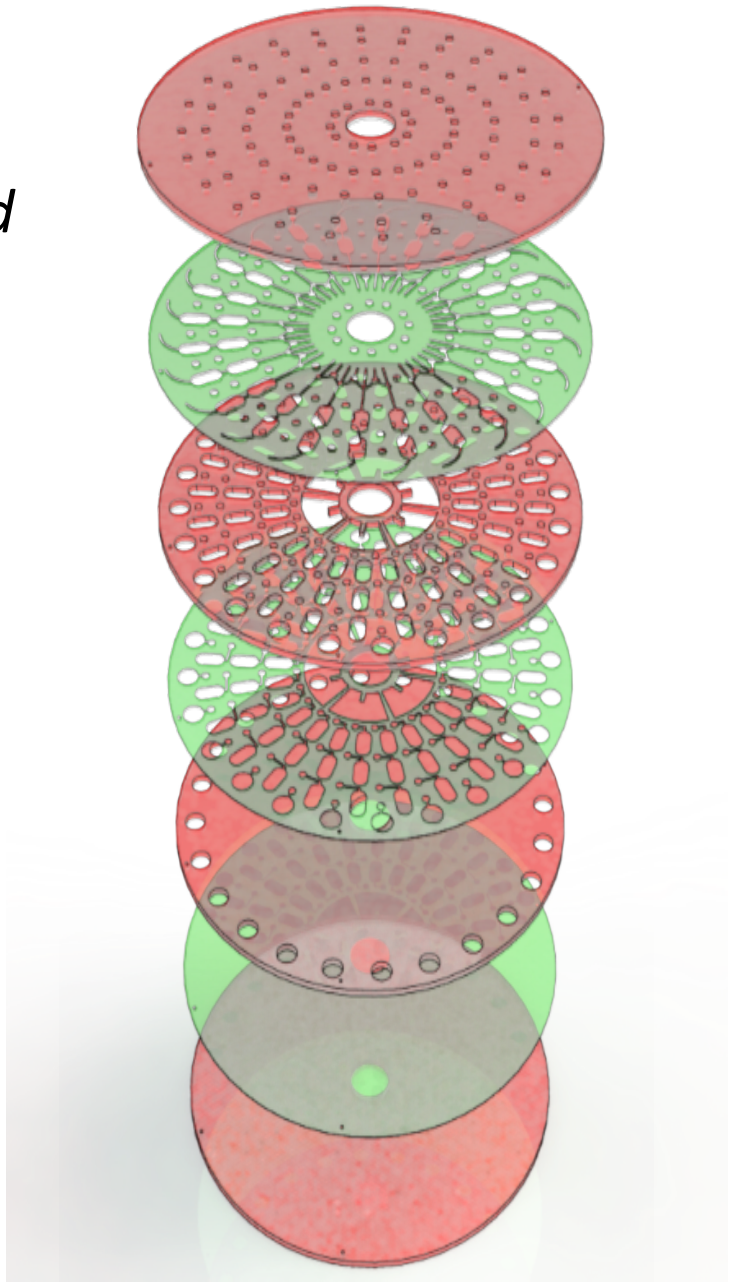


Analysis of capture raw optical data generated



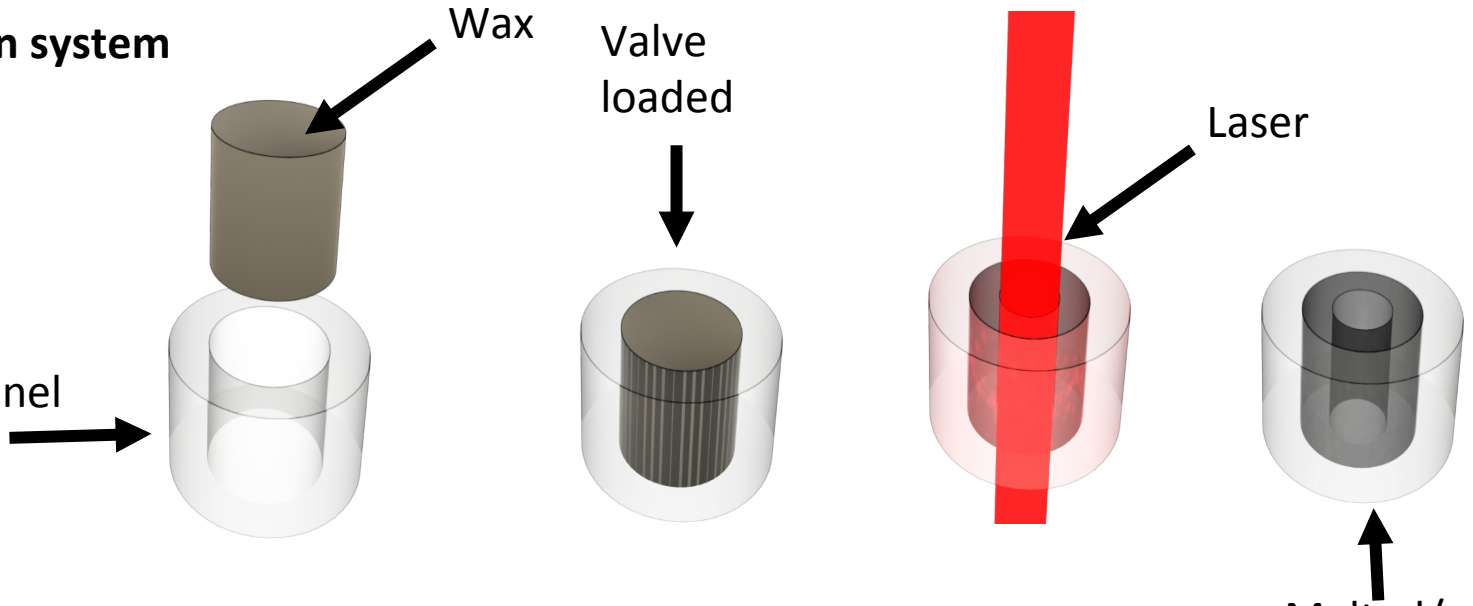
Lab-On-A-Disc platform

- *Poly(methyl methacrylate) (PMMA) (Red) (Radionics™) and pressure sensitive adhesive (PSA) (Green)(Adhesives Research Inc. ™)*
- Easily modifiable
- Microcystin-LR detection: Proof of concept
- Low sample size, cheap to manufacture

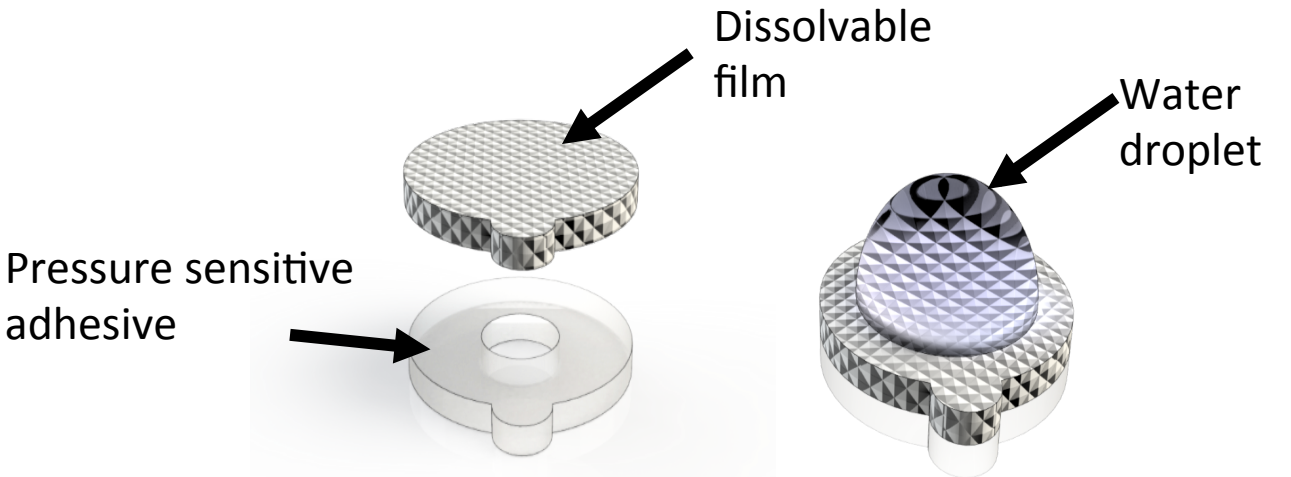


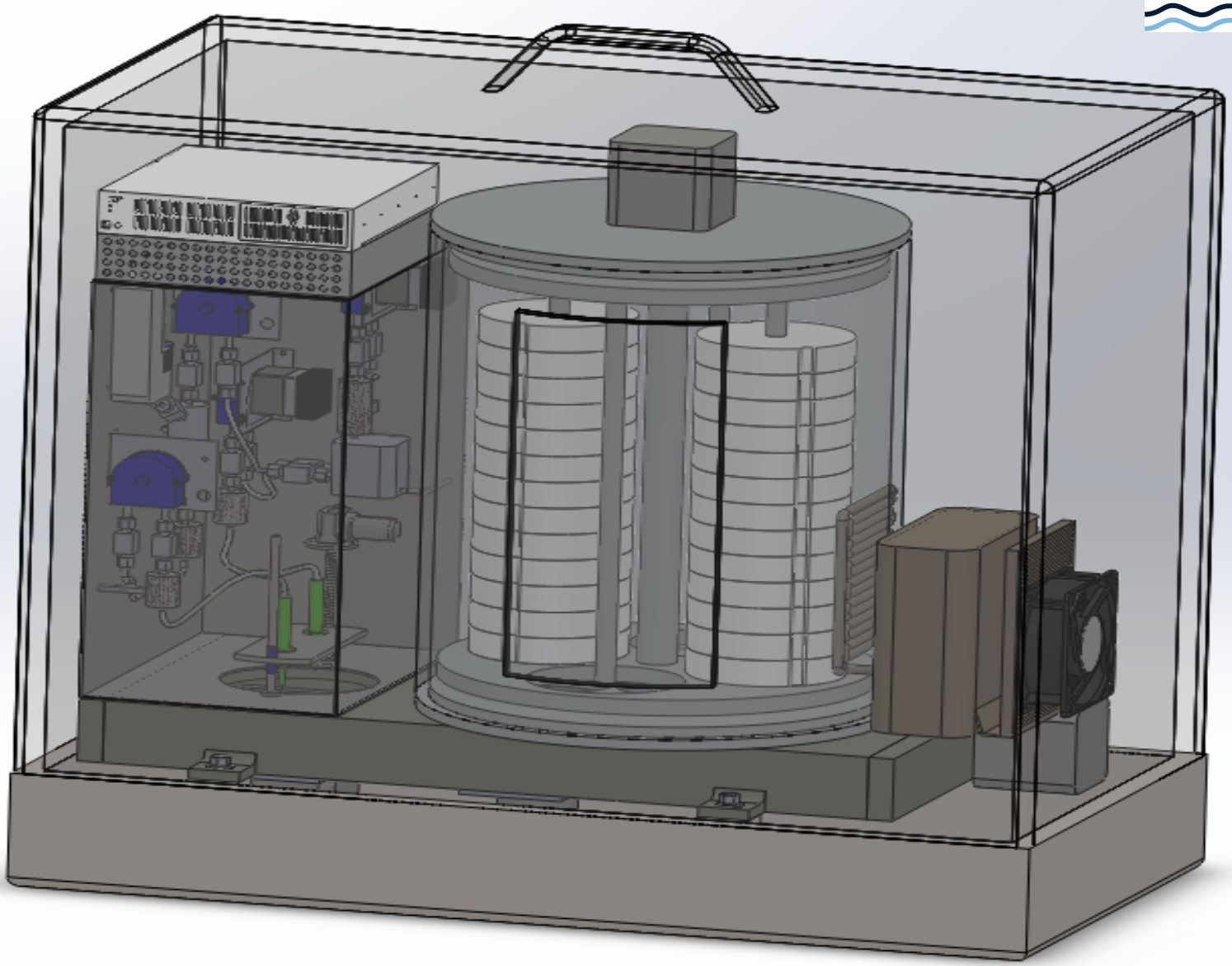
Some microfluidic valve solutions

Heat actuation system



Liquid actuation system





- EU Water Framework Directive (WFD)
- Phosphate limit: 0.1 mg L^{-1}

- Nutrients in Agriculture
  - Phosphate
  - Selection of wet chemical method
  - Design of sensor

Phosphate sensor I

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Molybdenum yellow method:

Sample + reagent ⟶ yellow product

PO_4^{3-} + Ammonium molybdate + Vanadium

⟶ Vanadomolybdophosphoric acid

Phosphate sensor II

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Molybdenum blue method:

Sample + reagent1 ⟶ intermediate

Intermediate + reagent2 ⟶ blue product

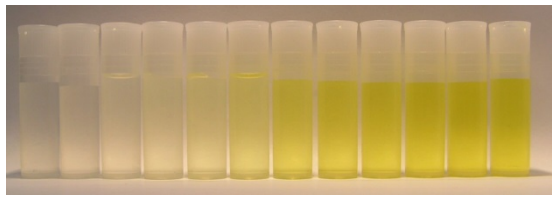
PO_4^{3-} + Ammonium molybdate ⟶ molybdophosphoric acid

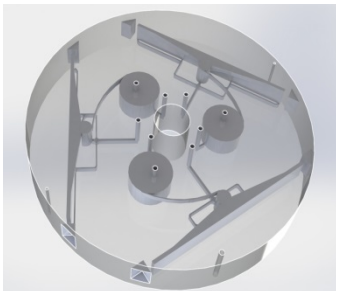
Molybdophosphoric acid —Reduced→ Molybdenum blue

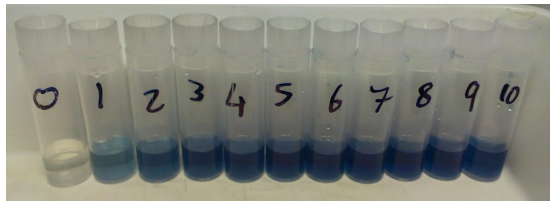
Selection of method

- High stability (>1 year)
- Widest linear range of all methods (0.1 – 20 µg L^{-1} P)
- Easiest to automate (Single step reaction)
- Improve LOD by increasing path length

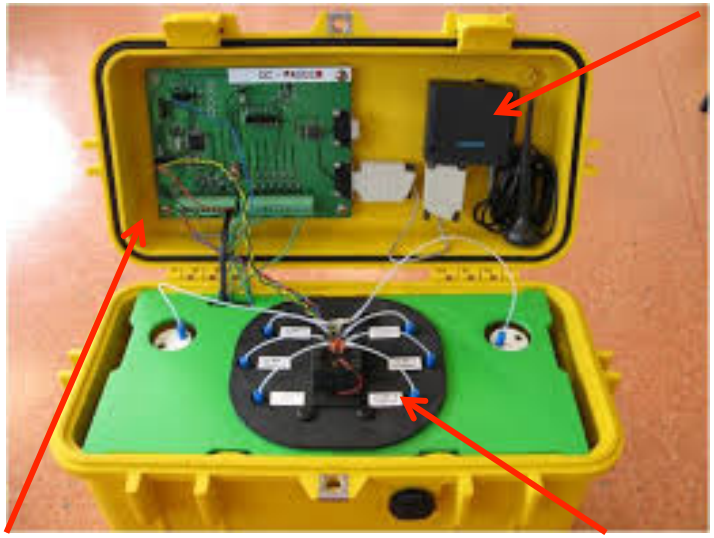
- Poor stability (1 week)
- Smaller linear range (0.01 – 2.0 µg L^{-1} P)
- More difficult to automate (Two step reaction)
- Lower LOD

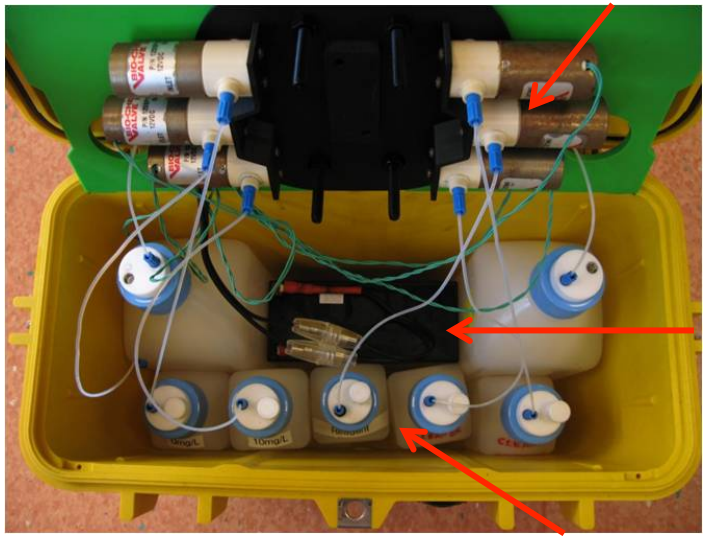


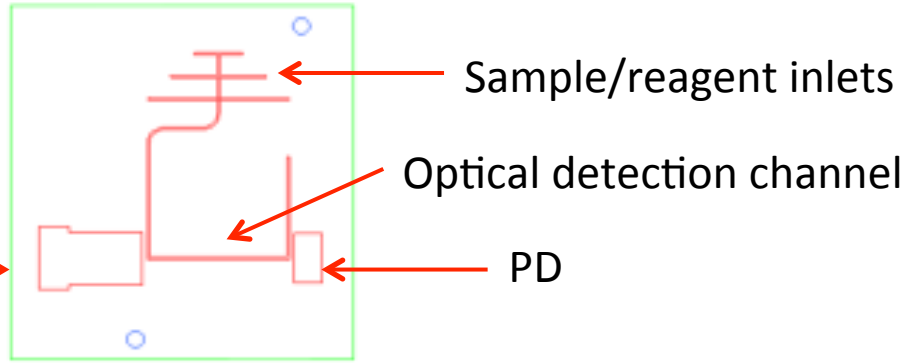




Autonomous phosphate sensor

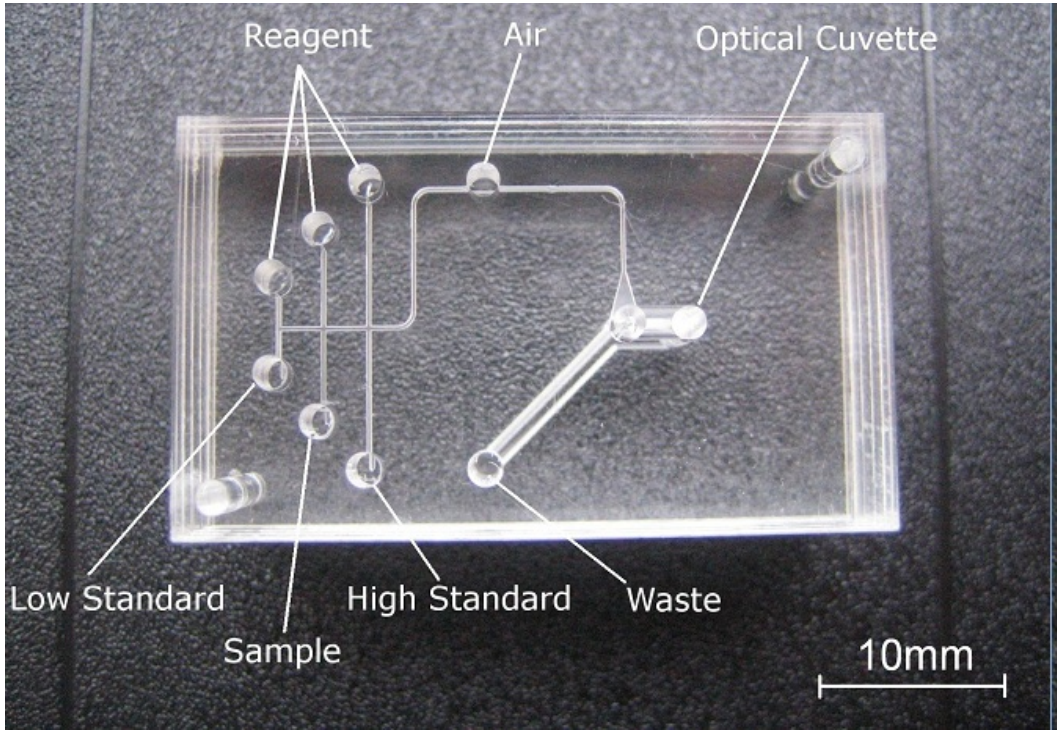


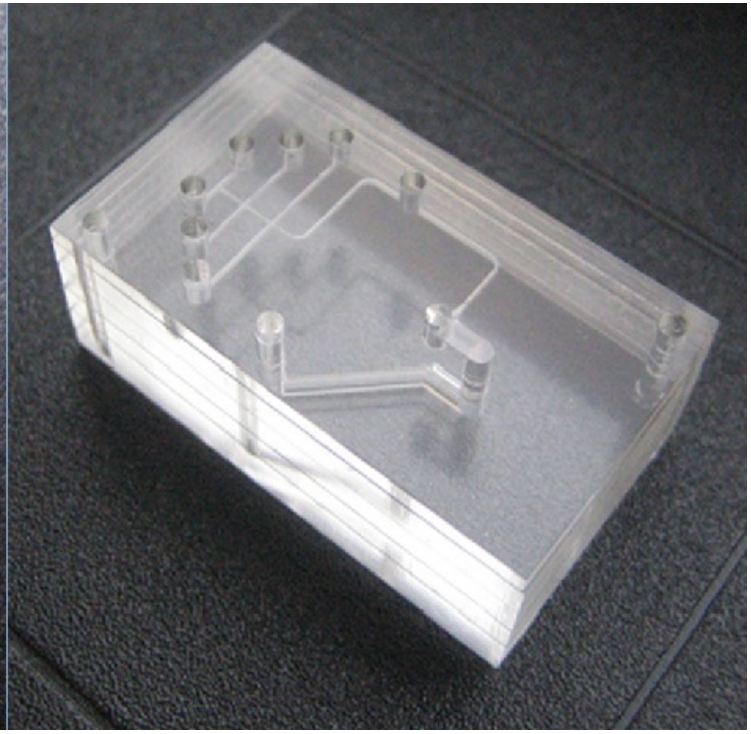




Microfluidic Chip

Reaction occurs on a microfluidic chip;
µL volumes





Sensor Data

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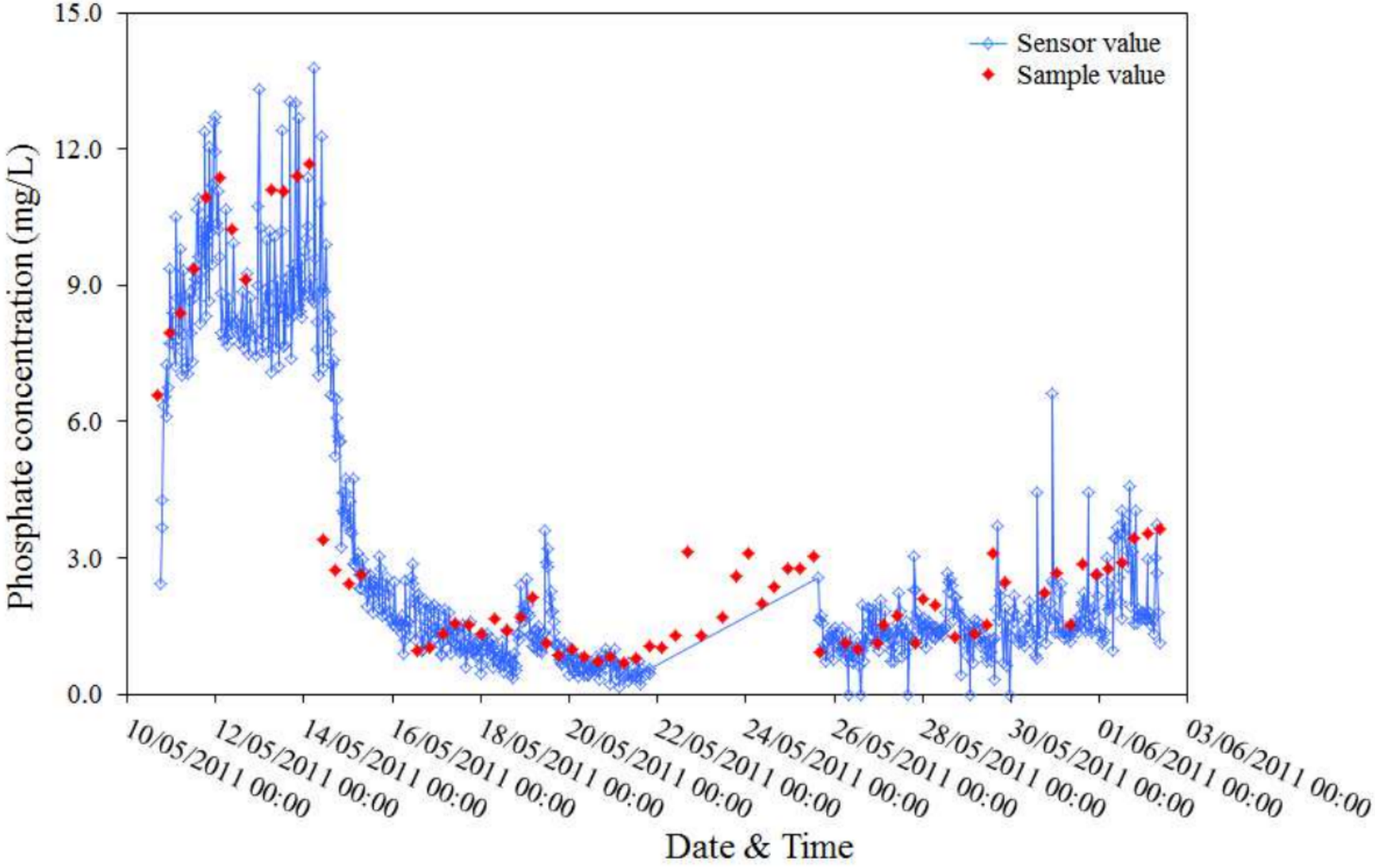
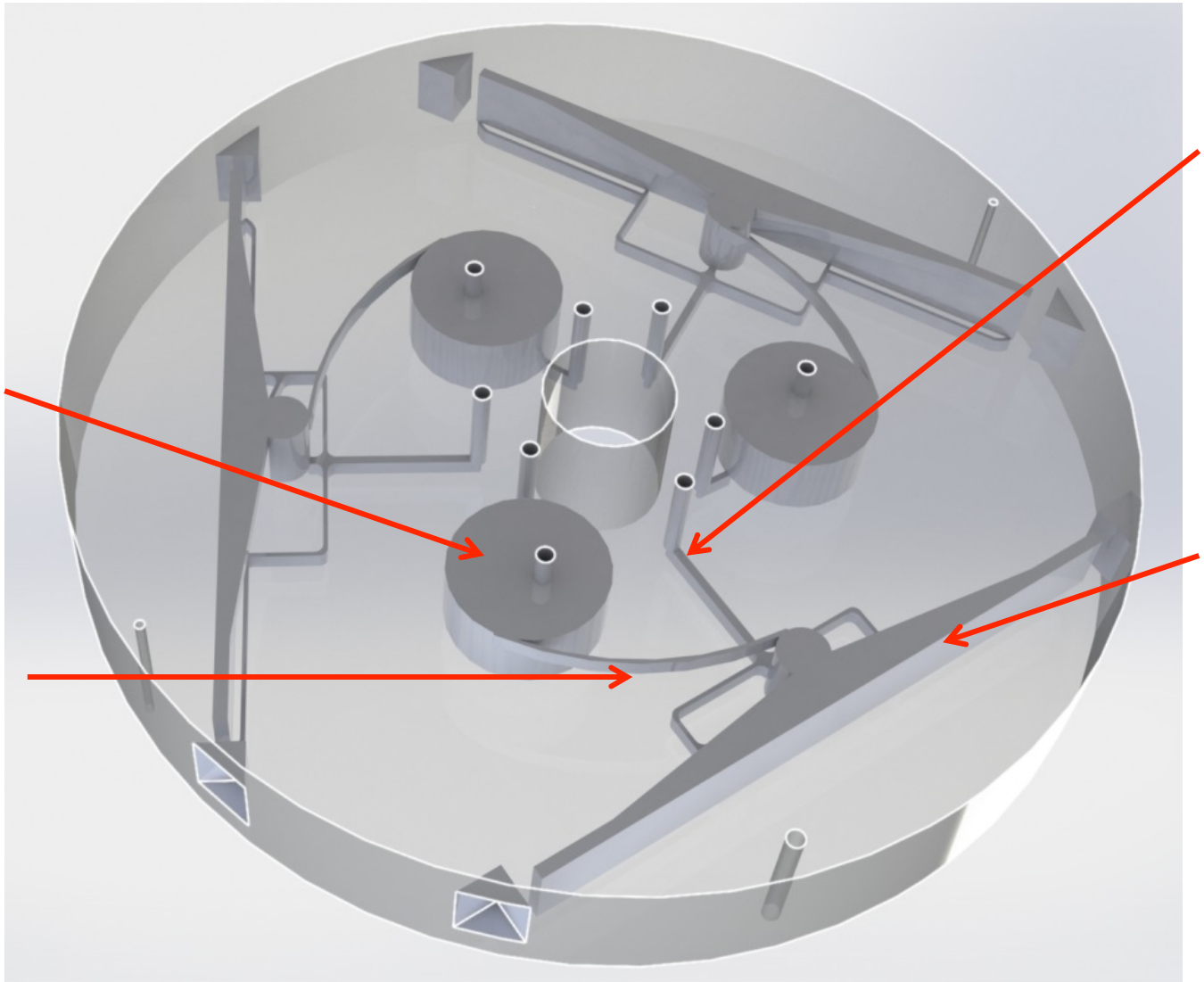
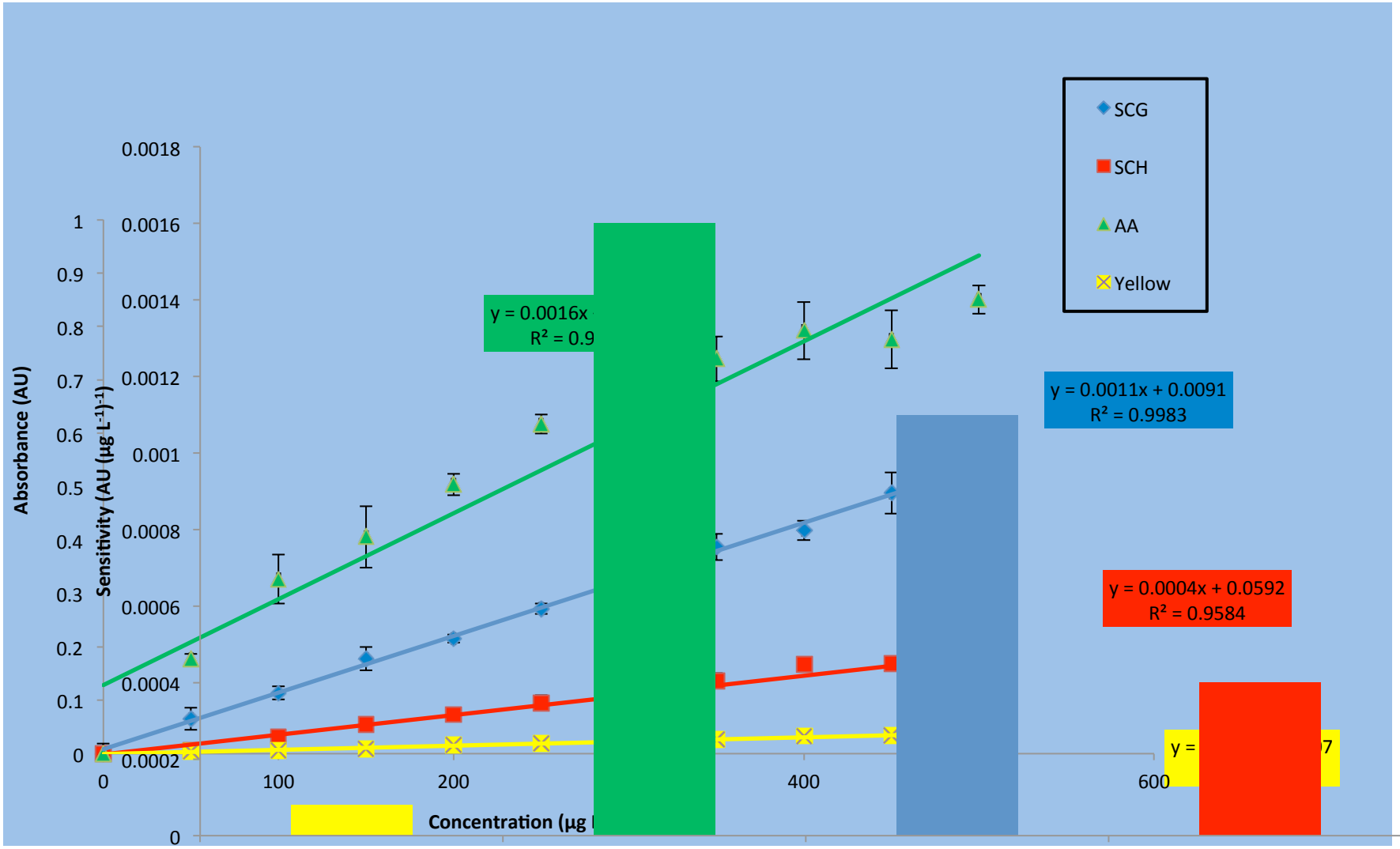


NCSR National Centre for Sensor Research
Marine Institute Foras na Mara
SeaChange Casadh na Taoide
SSTI Strategy for Science, Technology and Innovation
NDP National Development Plan
DCU

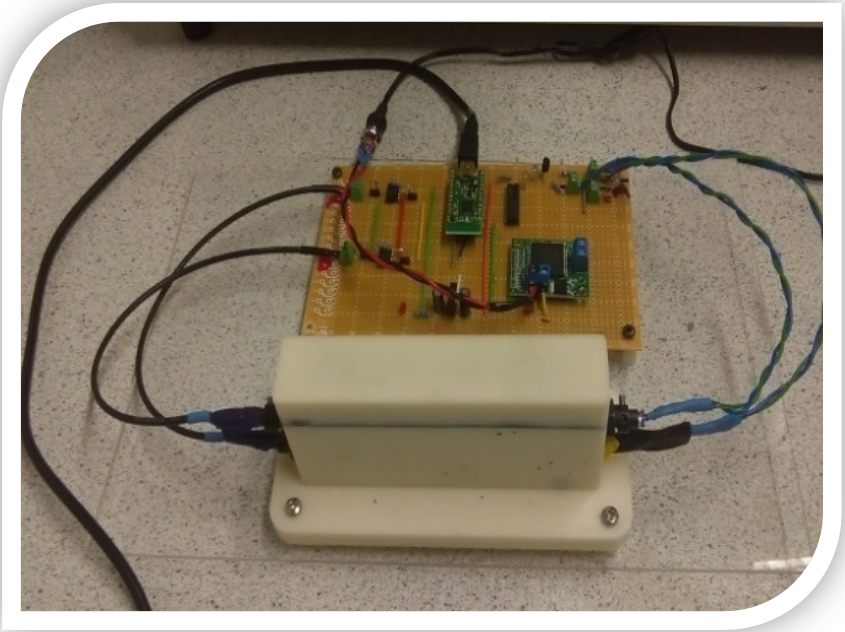
Centrifugal microfluidic device

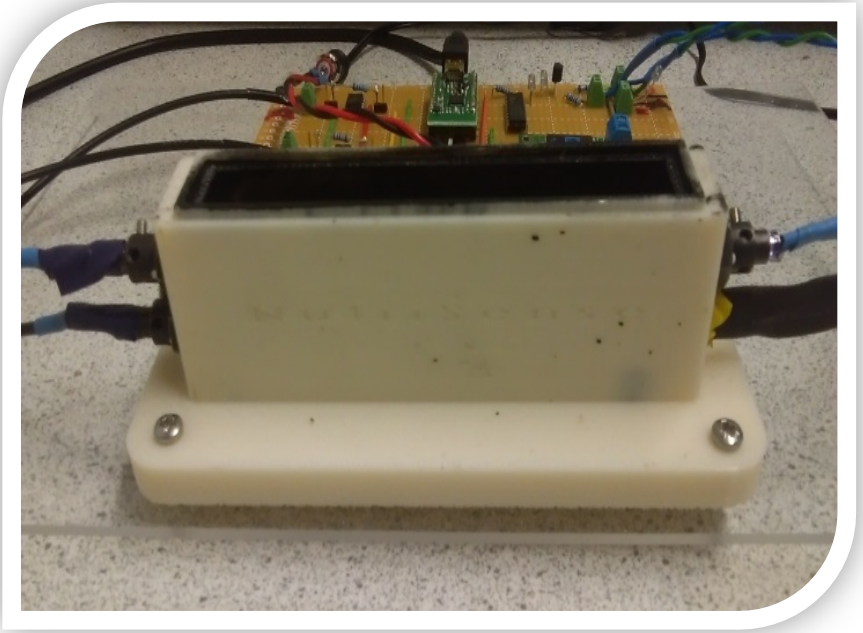


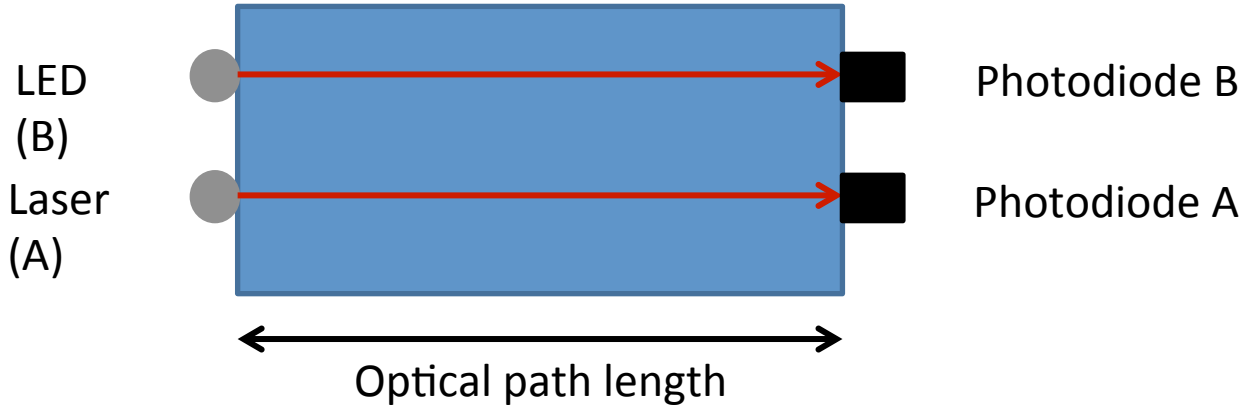
Selection of method for centrifugal system



Optical path length study

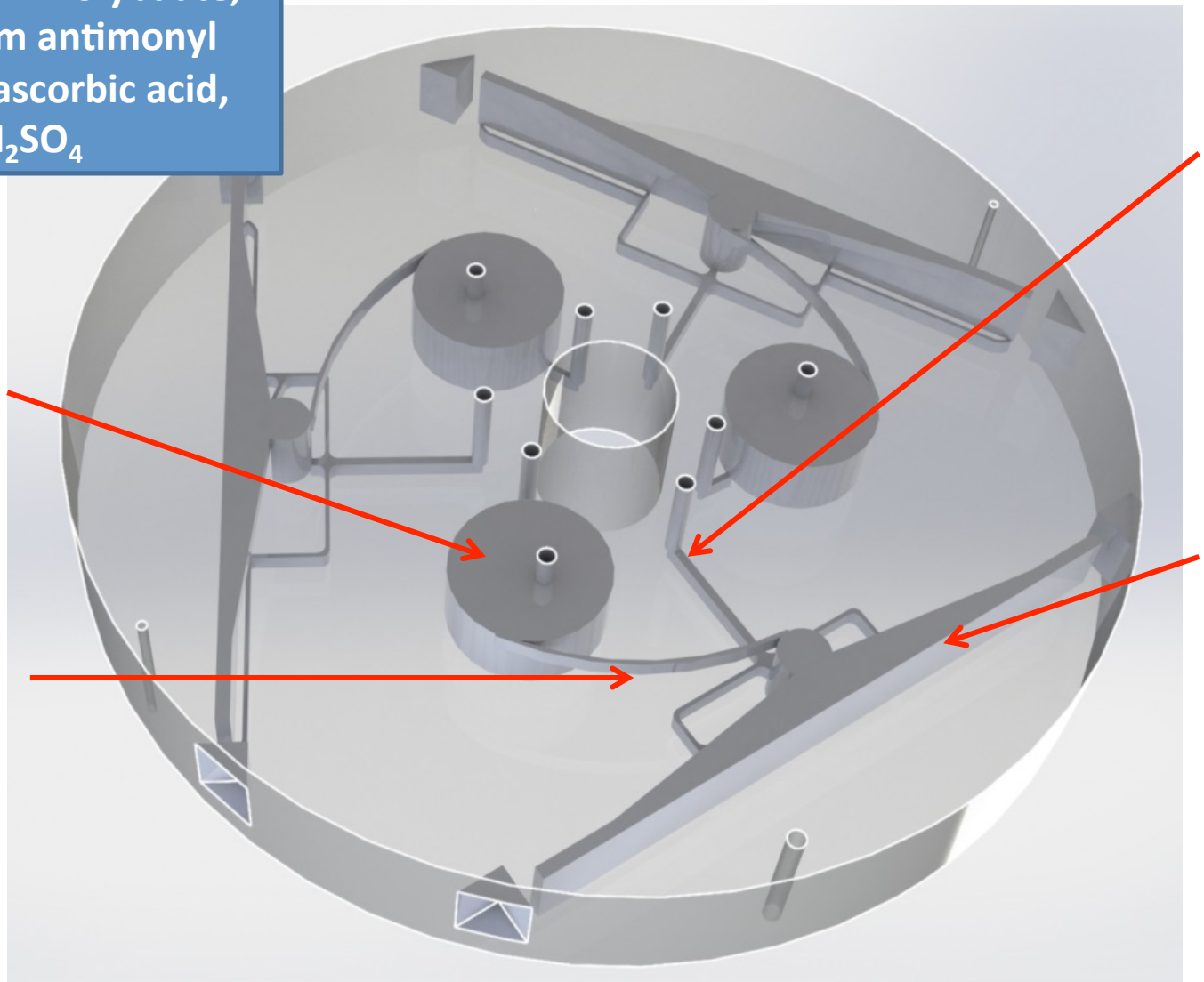




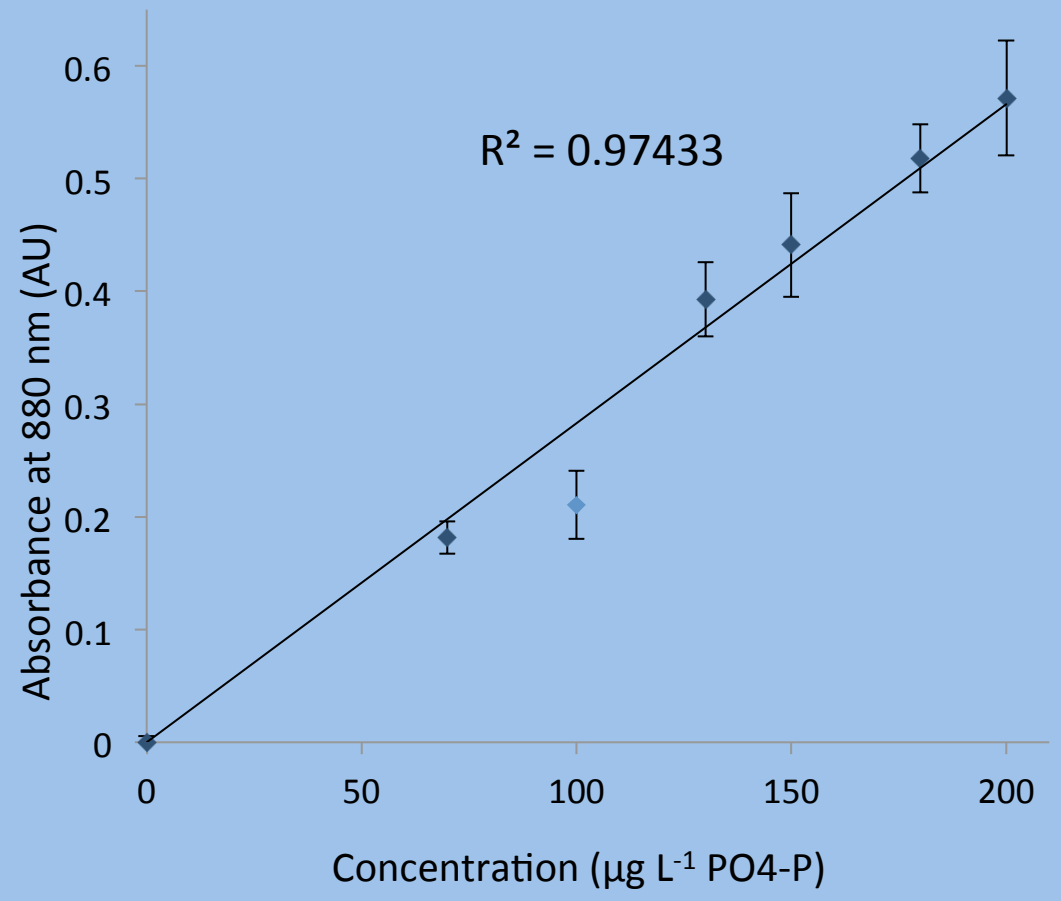


Centrifugal microfluidic device

Ammonium molybdate, potassium antimonyl tartrate, ascorbic acid, H_2SO_4



Portable NutriSense system



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- E-coli
  - Freshwater and bathing water
  - Chemical assay development
  - System & protocol design

Drivers

➢2006/7/EC BWD

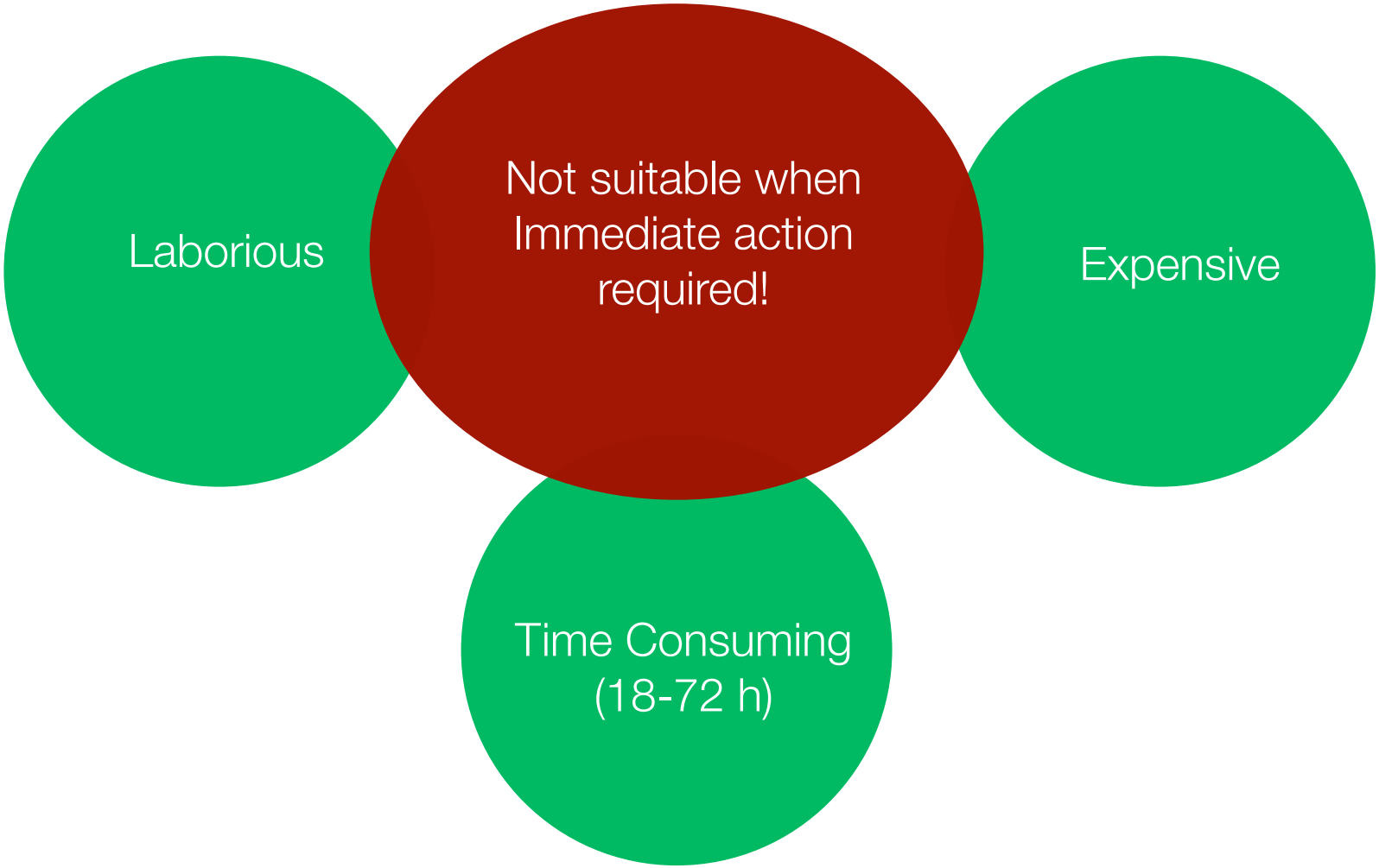
Inland waters					
	Parameter	Excellent quality	Good quality	Sufficient quality	Reference methods of analysis
1	Intestinal *Enterococci* (cfu/100mL)	200(*)	400(*)	330(*)	ISO 7899-1 or ISO 7899-2
	***Escherichia coli* (cfu/100mL)**	**500(*)**	**1,000(*)**	**900(**)**	ISO 9308-3 or ISO 9308-1

(*) Based upon a 95-percentile evaluation, (**) Based on a 90-percentile evaluation.

Coastal and transitional waters					
	Parameter	Excellent quality	Good quality	Sufficient quality	Reference methods of analysis
1	Intestinal *Enterococci* (cfu/100 mL)	100(*)	200(*)	185(*)	ISO 7899-1 or ISO 7899-2
	***Escherichia coli* (cfu/100mL)**	**250(*)**	**500(*)**	**500(**)**	ISO 9308-3 or ISO 9308-1

(*) Based upon a 95-percentile evaluation, (**) Based on a 90-percentile evaluation.

Drivers



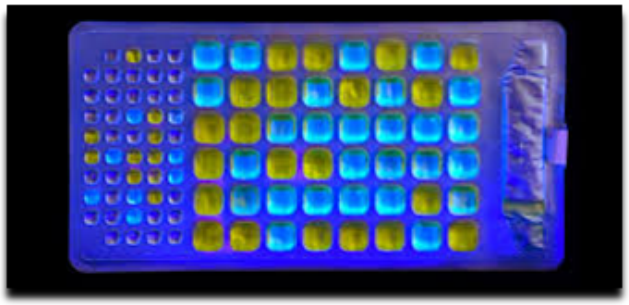
➢Need for early warning technologies

Background – Enzyme based methods

β-D-Glucuronidase (GUS)

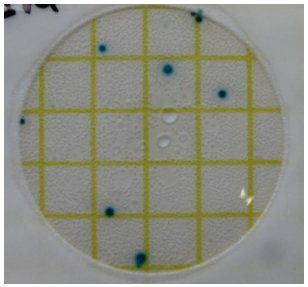
GUS extraction

GUS activity measurement



Colilert 18

Chromocult

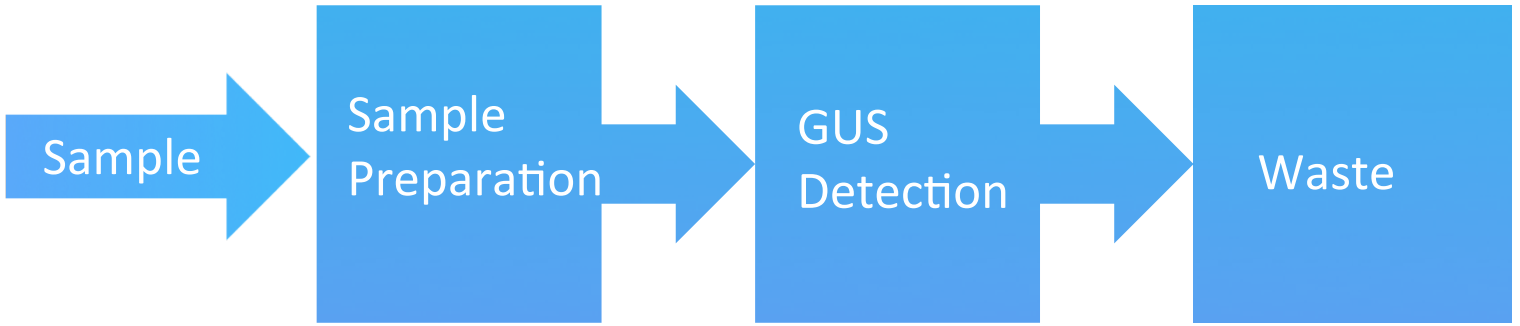


3M Petrifilms

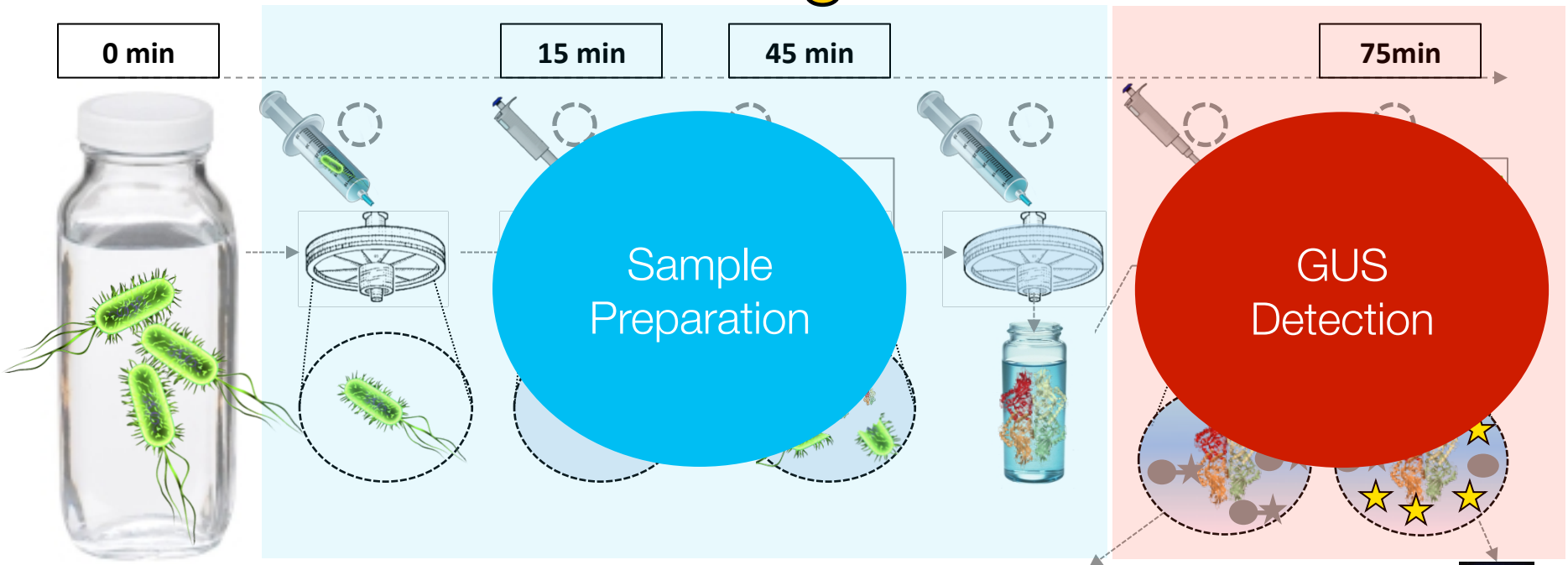
Substrate uptake

Outputs

- ❑ Continuous fluorometric method for the measurement of GUS activity.
- ❑ Sample preparation protocol to maximise GUS recovery from *E. coli* contaminated samples
- ❑ Sensing platform for *E. coli* detection in environmental waters.

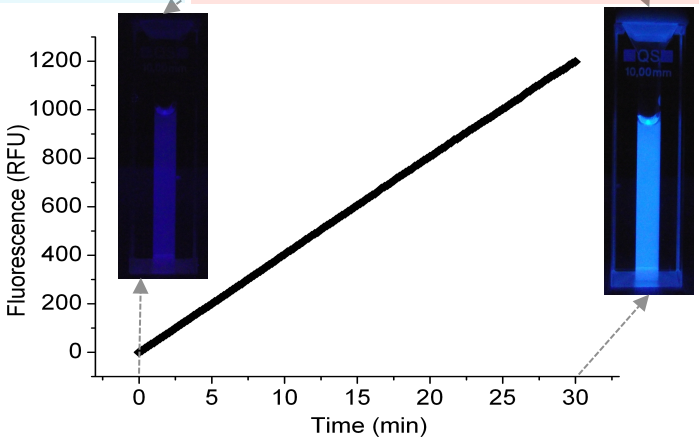


Protocol Description

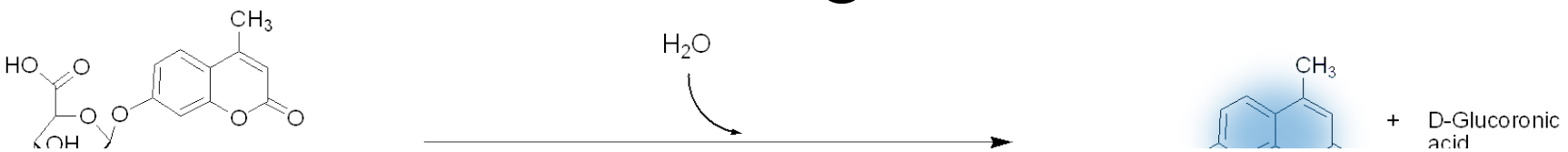


Legend

- *E. coli*
- *Incubation*
- *Substrate addition & detection*
- *Capture & pre-concentration*
- *GUS*
- *Fluorogenic substrate*
- *Lysing buffer addition*
- *GUS recovery*
- *Fluorophore*



Continuous Fluorometric Method for GUS



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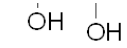
Continuous fluorometric method for measuring β-glucuronidase activity: comparative analysis of three fluorogenic substrates†

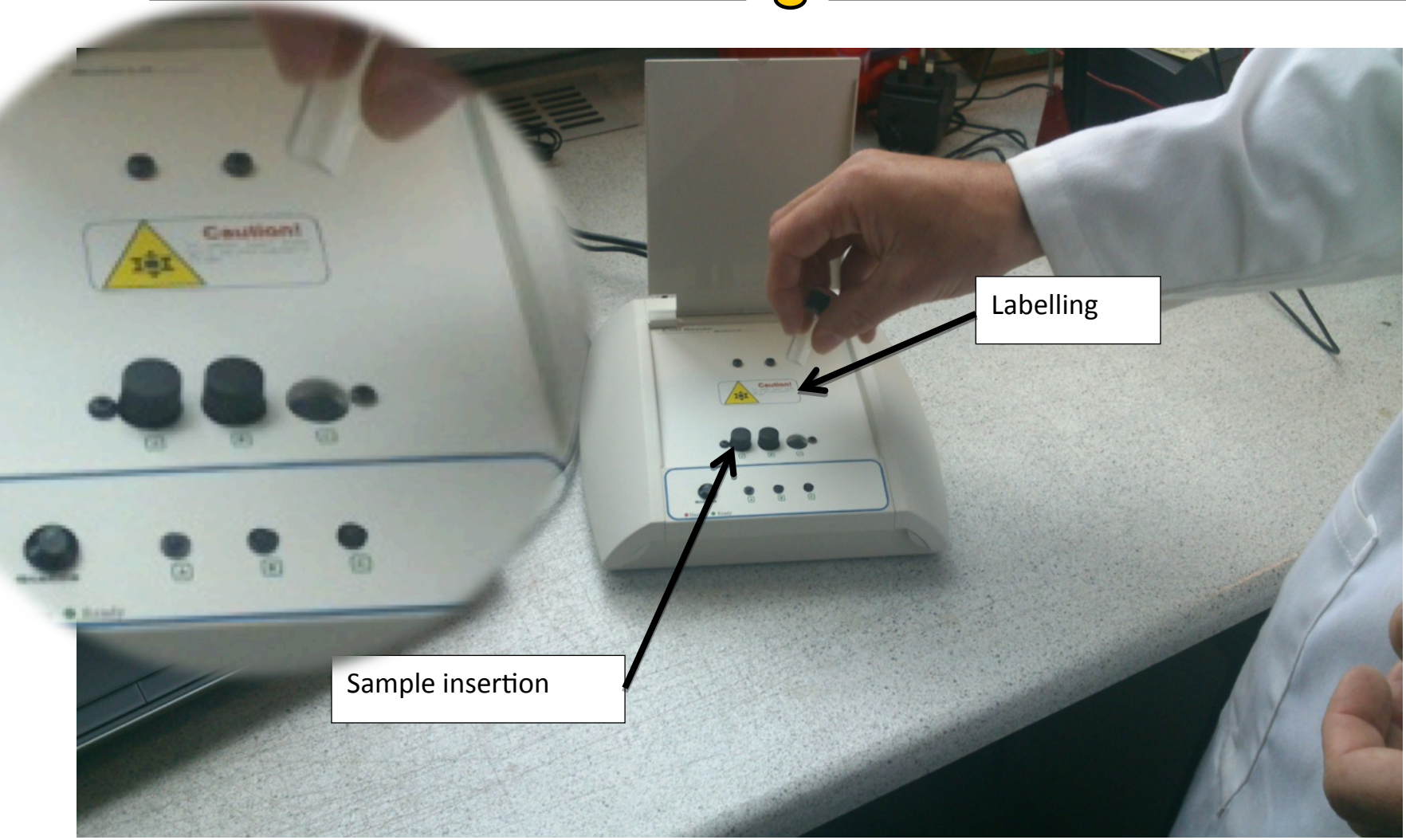
Ciprian Briciu-Burghina, Brendan Heery and Fiona Regan*



6-Chloro-4-Methylumbelliferyl-β-D-Glucuronide (6-CMUG)

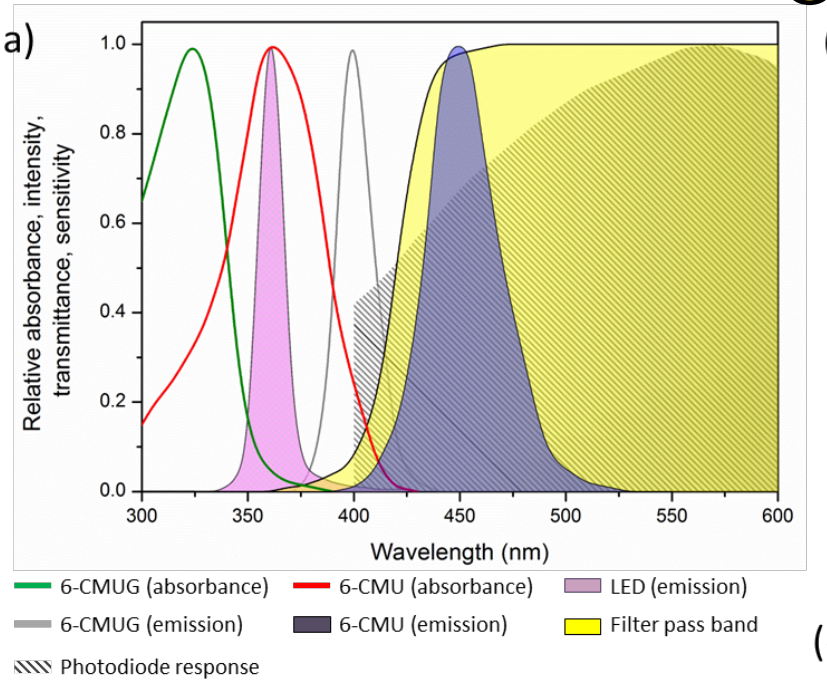
6-Chloro-4-Methyl-Umbelliferone (6-CMU)

ColiSense (GUS Detection)

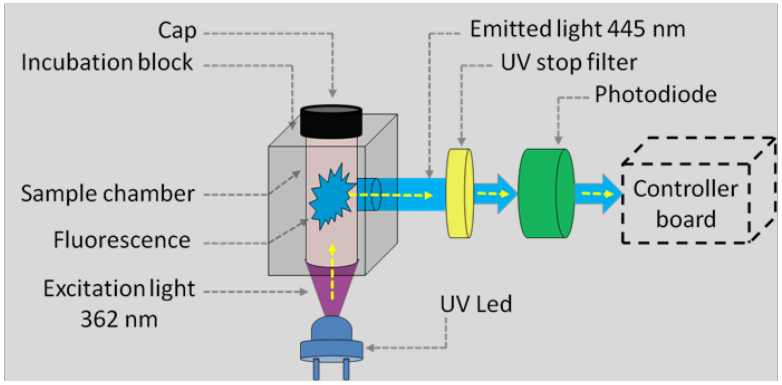


The Detection Platform

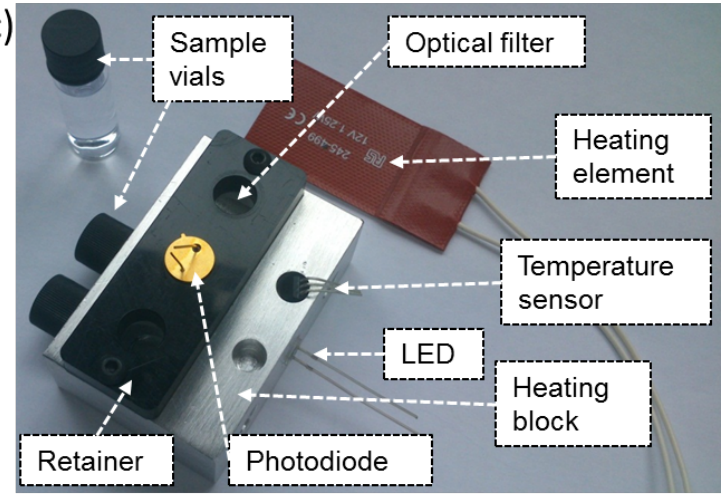
(a)



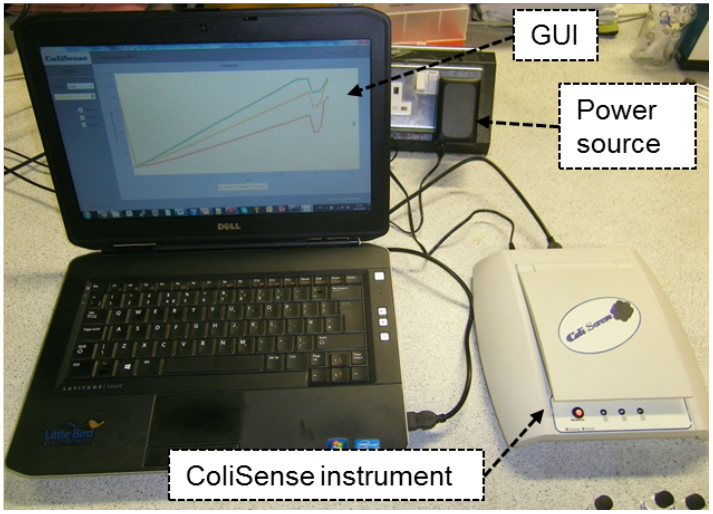
(b)



(c)



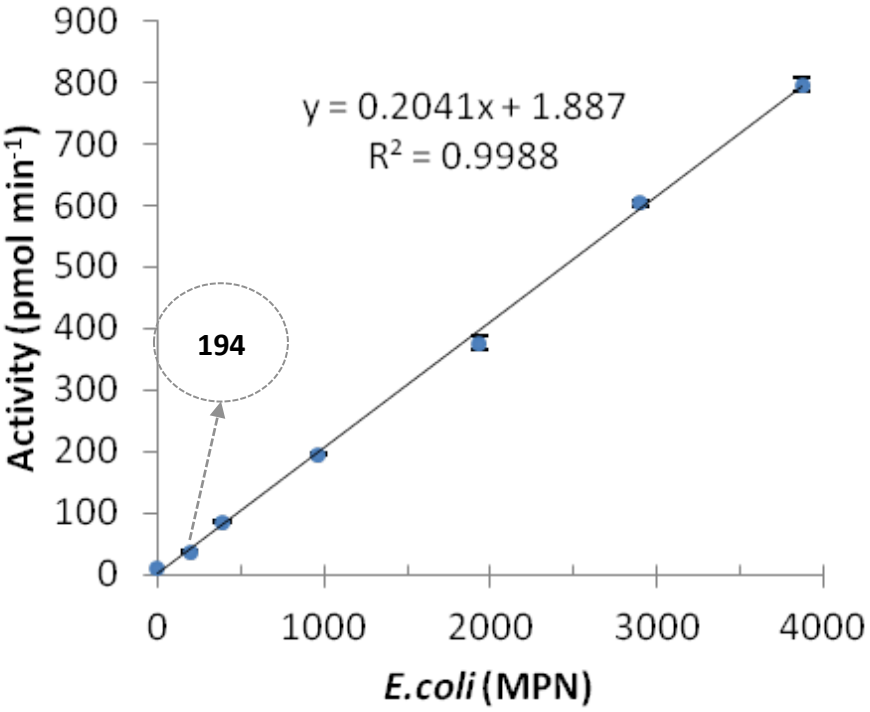
(d)



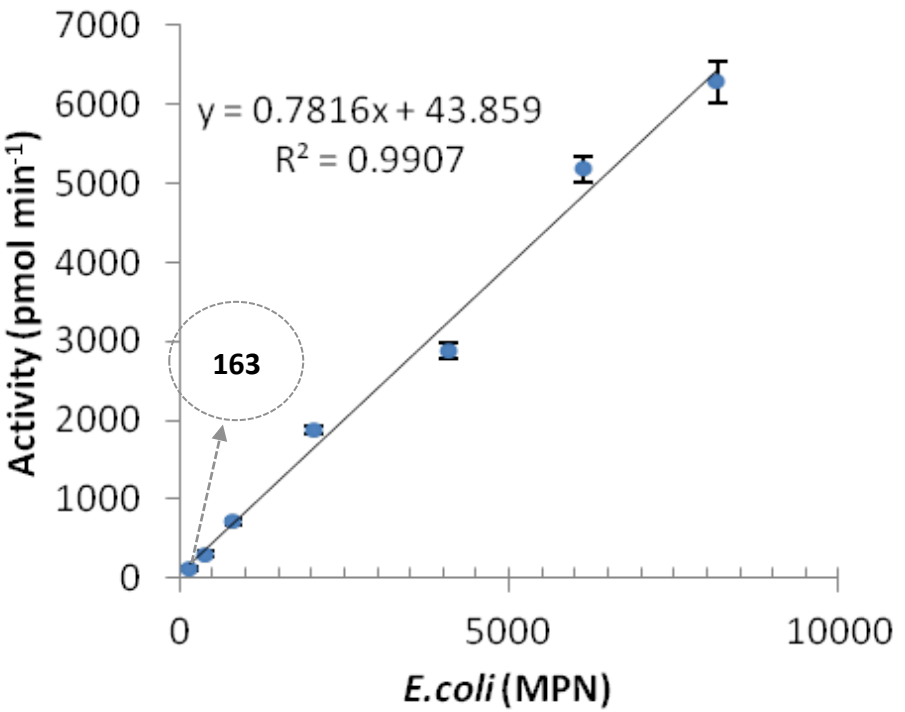
Sampling Sites

River and Seawater Samples

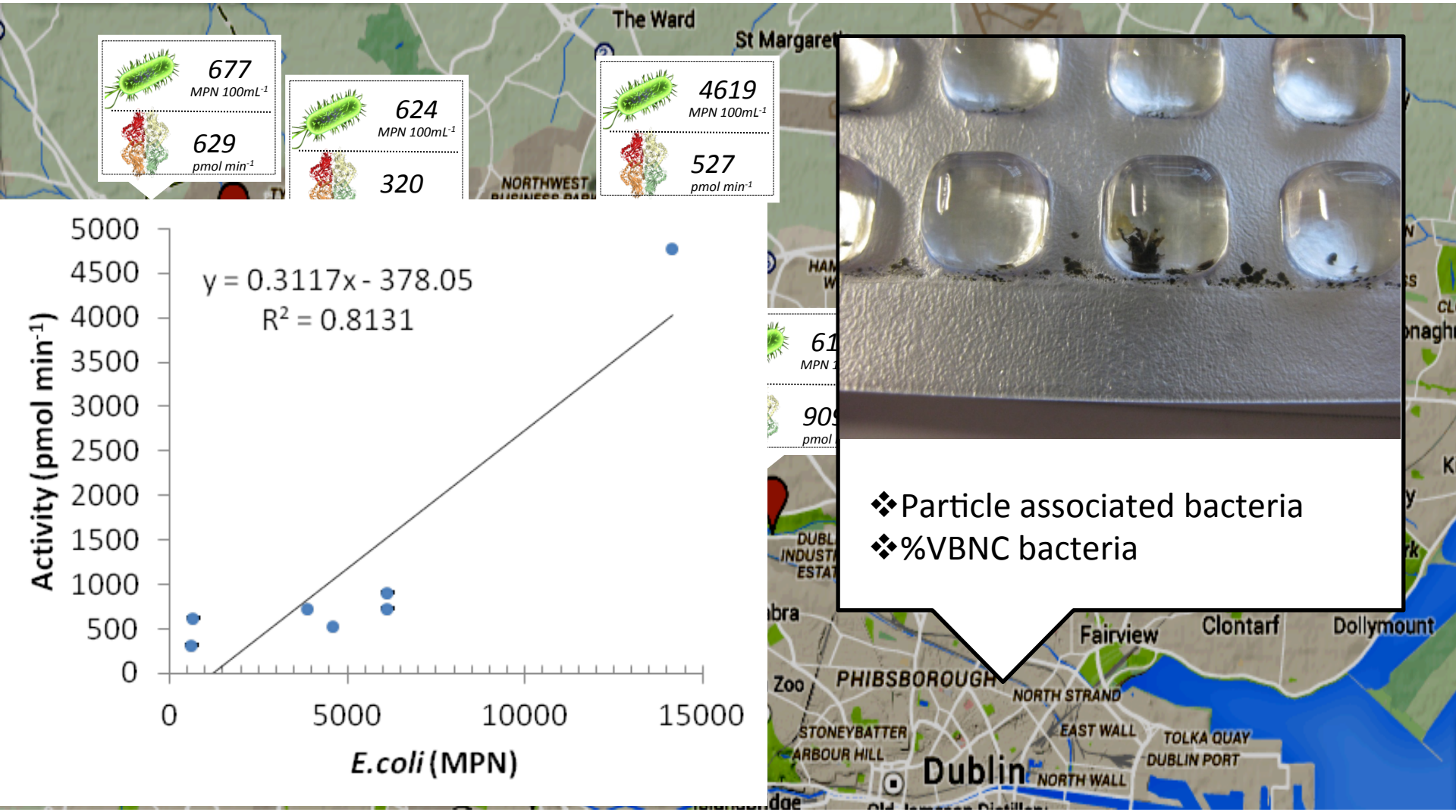
River water



Seawater



Field Testing



- Particle associated bacteria
- %VBNC bacteria

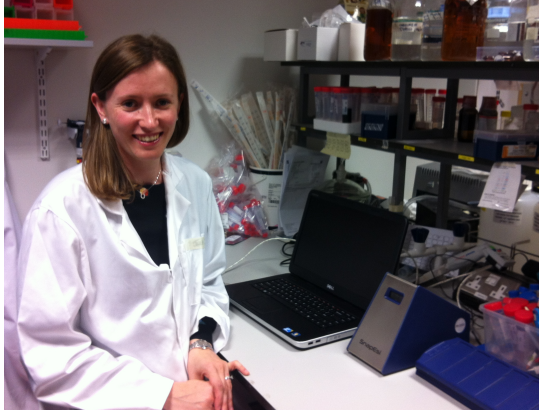
Observations

❖Method developed and applied for the detection of *E. coli* from environmental water samples and was successful in predicting *E. coli* concentrations below the EU threshold for "excellent quality" < 1.5 h.

❖Continuous fluorometric method for the determination of GUD activity has been developed (minimal sample manipulation, reagent consumption minimised)

❖Sample preparation protocol for recovery of GUS from *E. coli* contaminated environmental samples

❖Bathing water monitoring May-September 2016 Dublin Bay



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