Autonomous sensor platform development for organic environmental contaminants

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DCU | Water Institute

Trends and advancements in the sampling and preservation of samples for the identification of contaminants of emerging concern

March 2nd and 3rd 2016

CIENS Forum, Gaustadalléen 21, 0349 Oslo, Norway

Outline

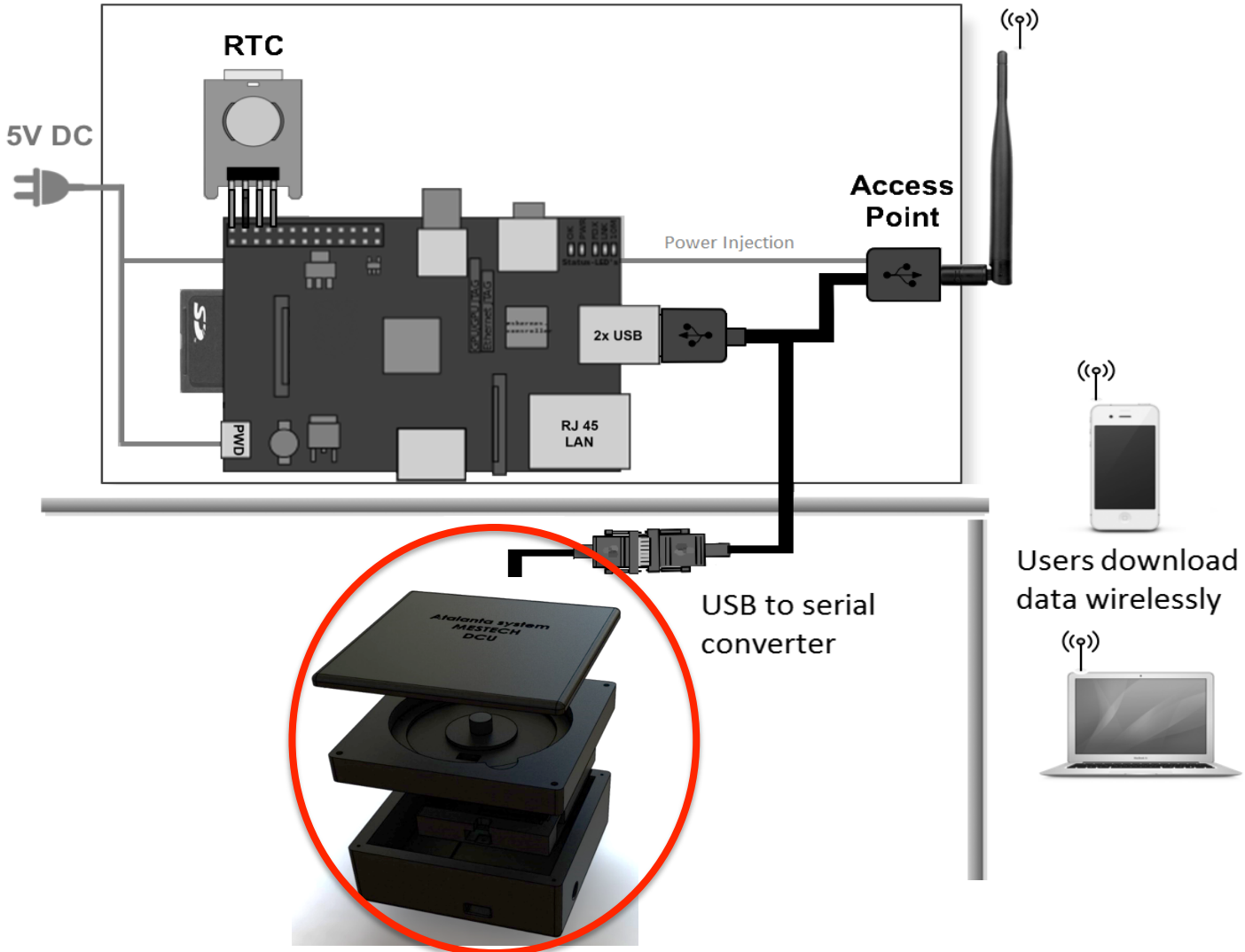
- Analytes – chemical toxins
- Sensing platform concept
- Antibody generation
- Centrifugal microfluidics
- Platform design
- Results
  - Test results for microcystin
  - Algal toxins
  - New chemicals

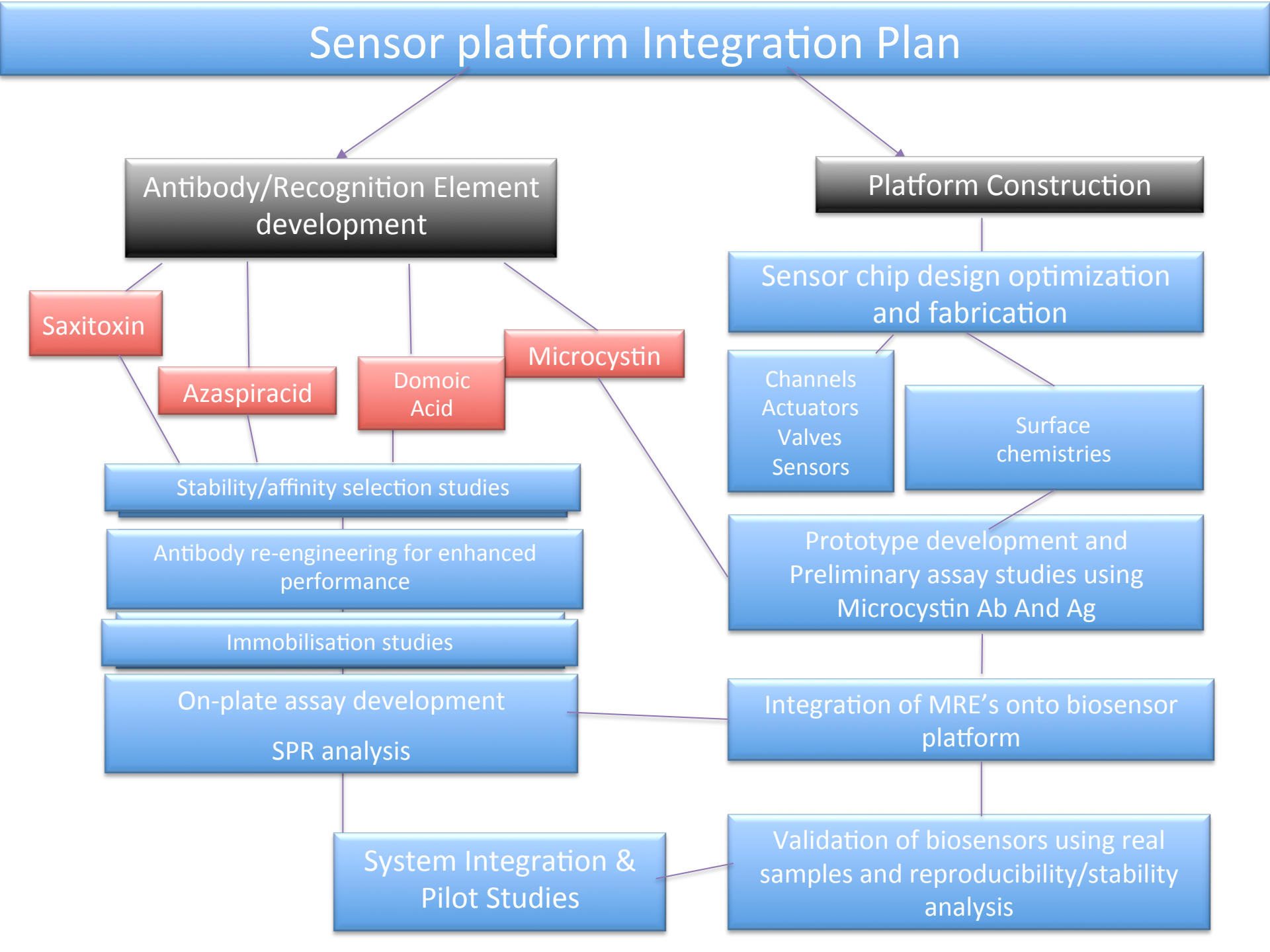
Algae's toxic threat

Agricultural and **urban run-off** causes increased pollution of lakes, rivers, streams and coastal areas causing **eutrophication** leading to Harmful Algal Blooms (HABs)

- 2% of algae produce harmful toxins.
- Microcystin is the most ubiquitously occuring cyanobacterial toxin and is present in fresh and brackish waters.
- Development of recombinant antibody sensors towards algal-toxins ➔ rapid, 'easy-to-use', portable toxin detection devices.

Autonomous System: Integrated sensor with comms/telemetry

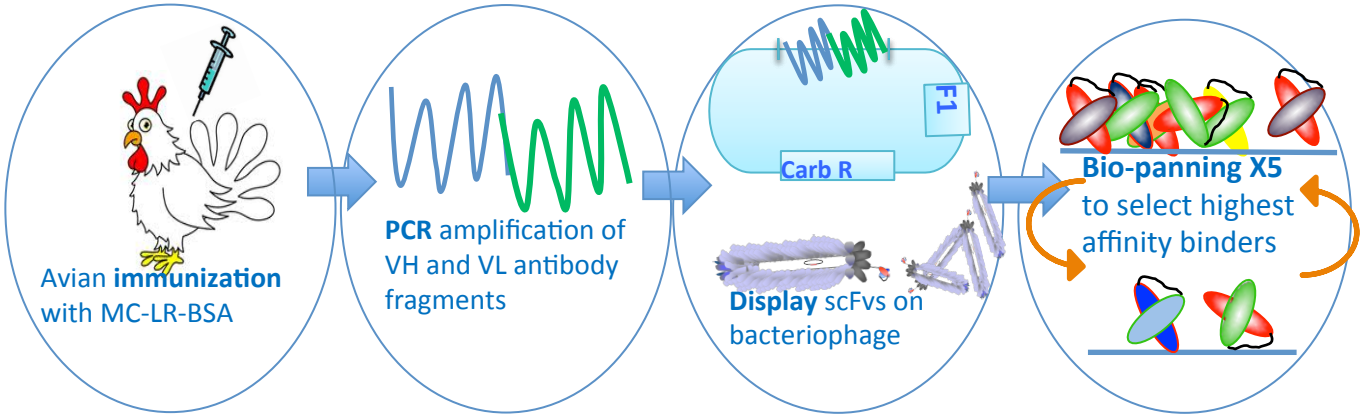


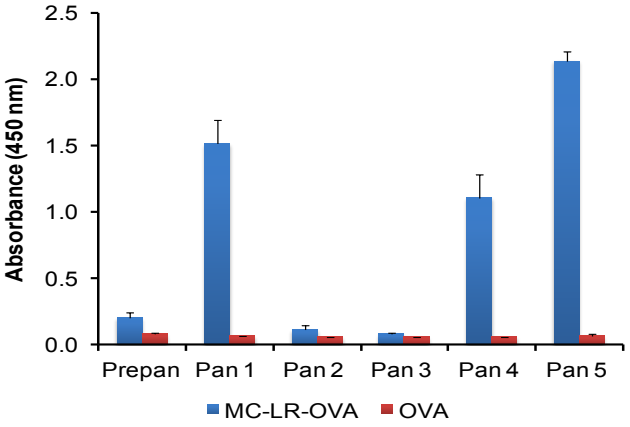

Sensor platform Integration Plan
Antibody/Recognition Element development
Platform Construction
Saxitoxin
Azaspiracid
Domoic Acid
Microcystin
Sensor chip design optimization and fabrication
Channels
Actuators
Valves
Sensors
Surface chemistries
Stability/affinity selection studies
Antibody re-engineering for enhanced performance
Immobilisation studies
On-plate assay development
SPR analysis
Prototype development and Preliminary assay studies using Microcystin Ab And Ag
Integration of MRE's onto biosensor platform
System Integration & Pilot Studies
Validation of biosensors using real samples and reproducibility/stability analysis

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

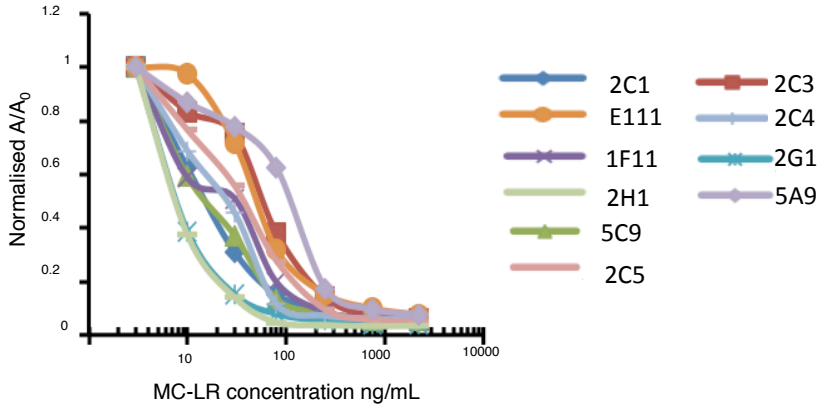
Methodologies

- Development of anti-microcystin recombinant antibodies



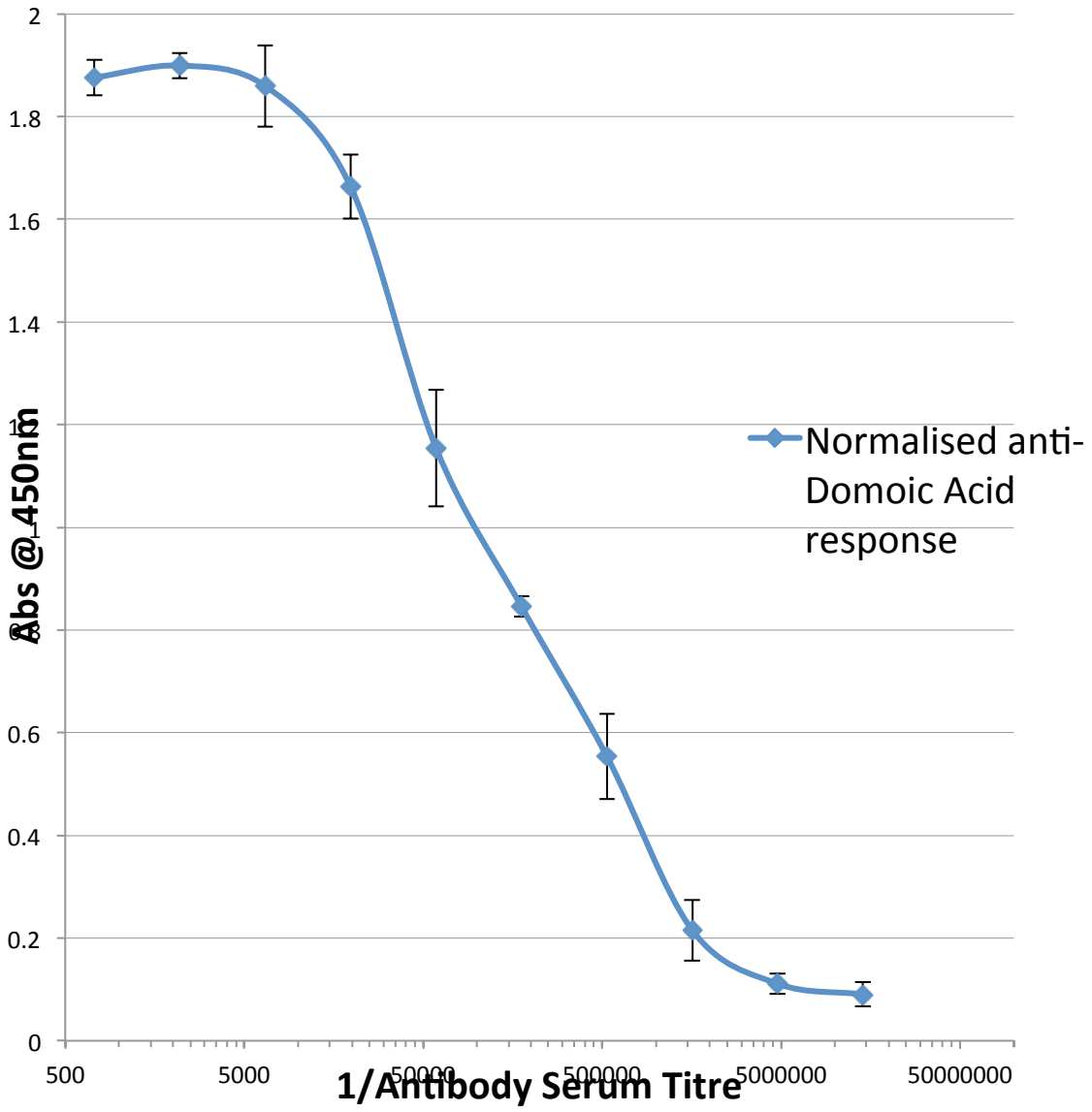


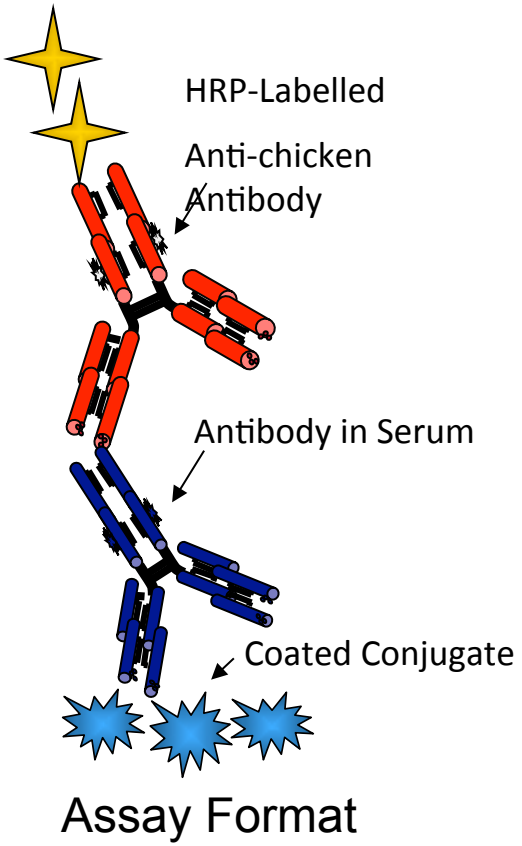
Antibodies produced from each round of biopanning



The most sensitive binder was determined by inhibition ELISA

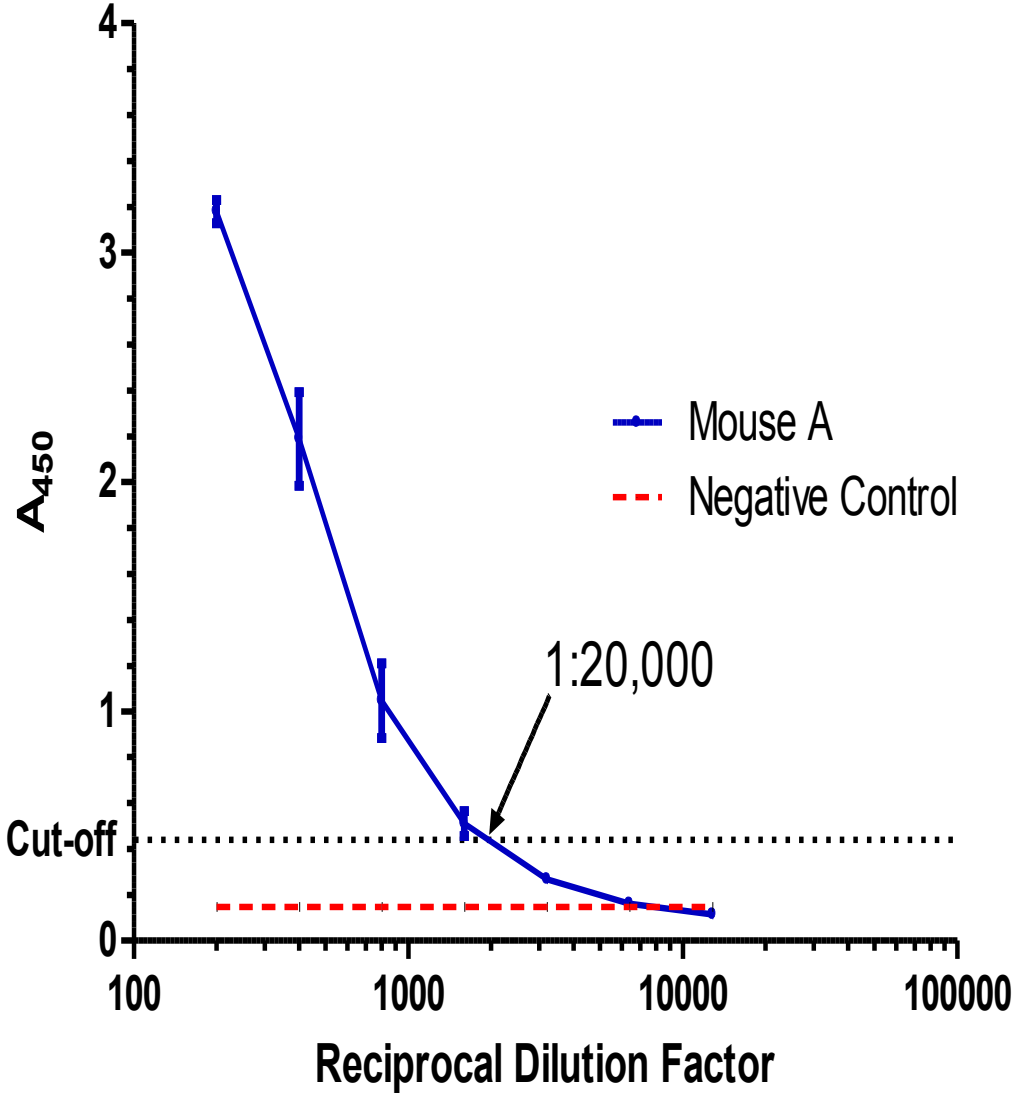
Domoic Acid Chicken Serum Titre





Assay Format

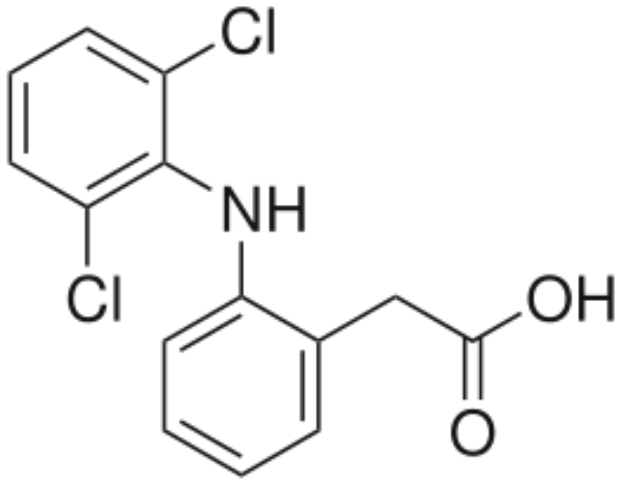
Azaspiracid



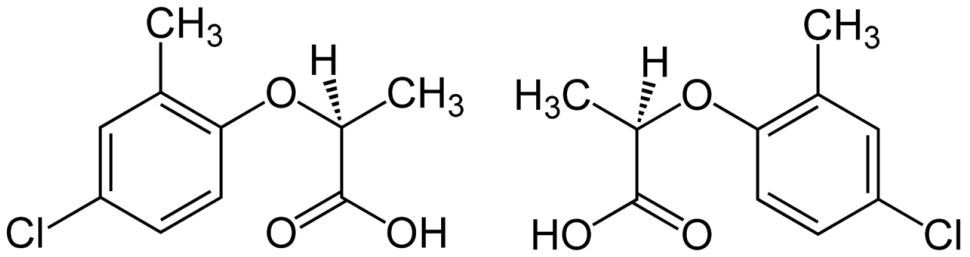
- ScFv library successfully constructed
- Preparation of conjugates underway for biopanning
- Polyclonal antibodies successfully isolated

New emerging chemicals – Recombinant antibody generation

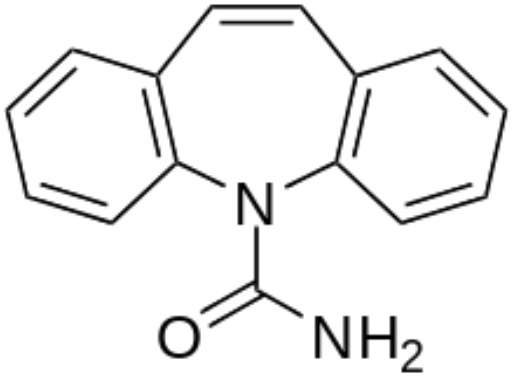
Diclofenac



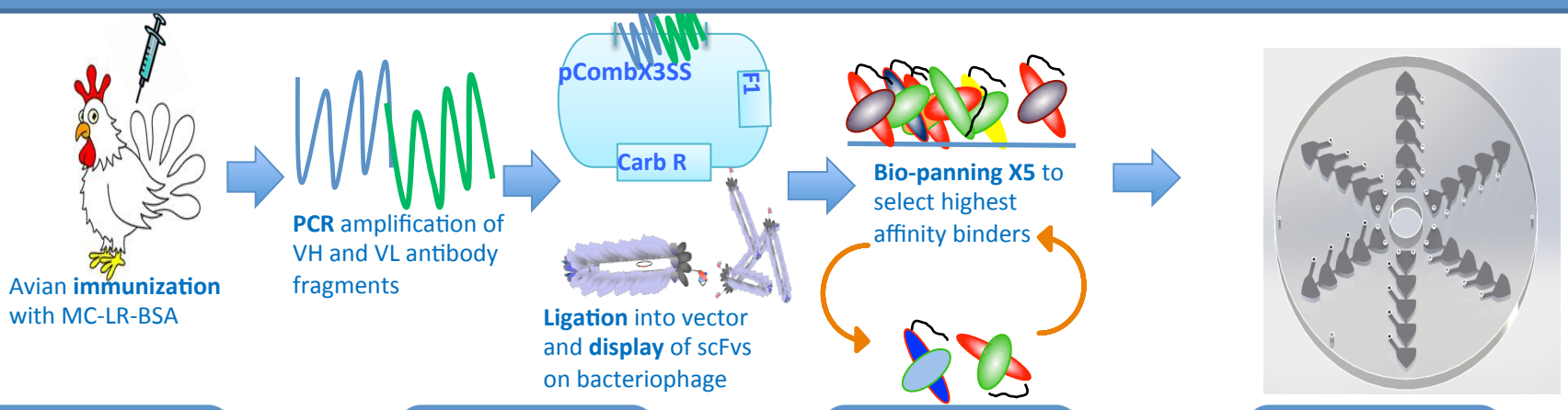
Mecoprop



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

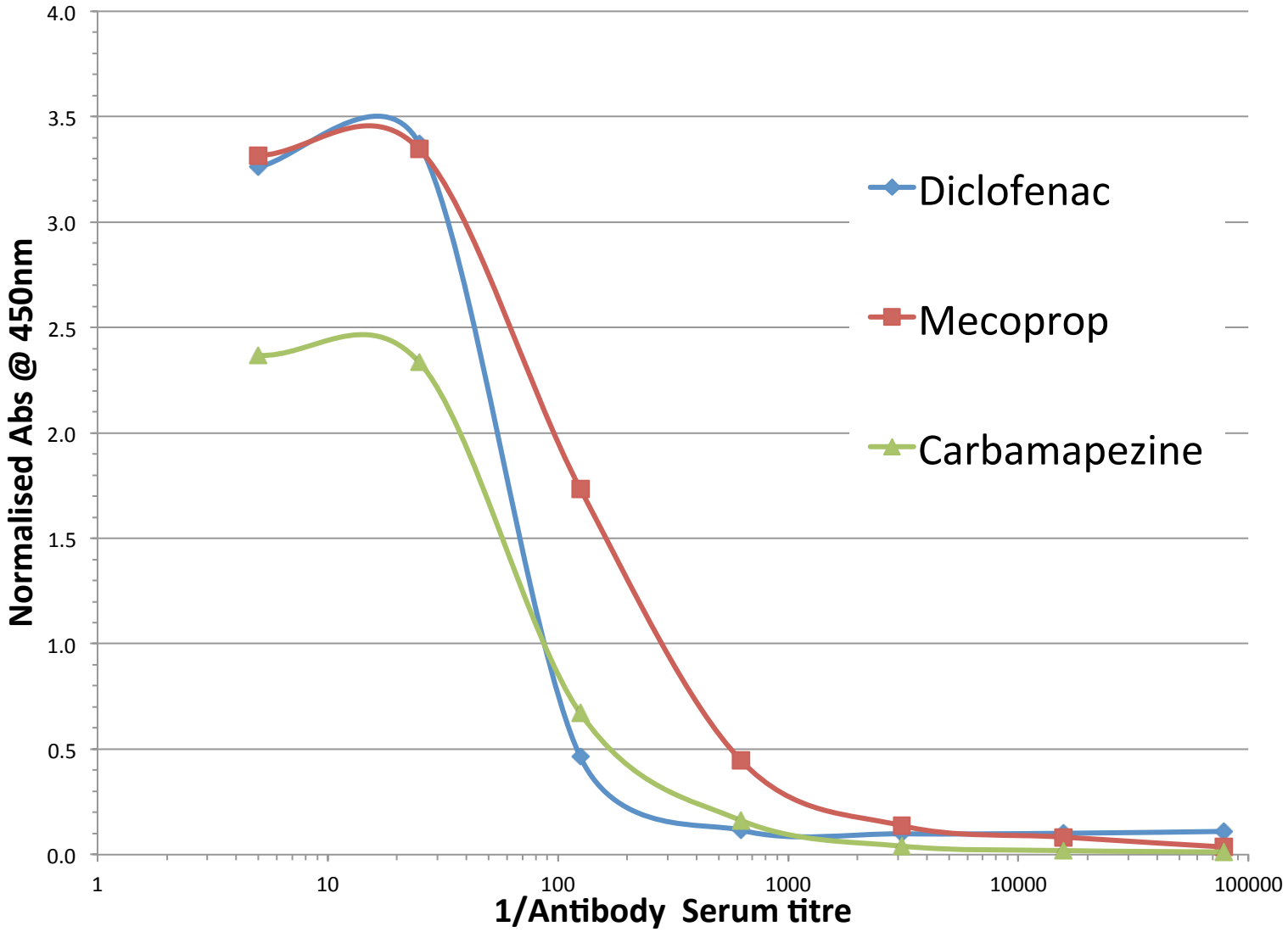
Antibody identification

- **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

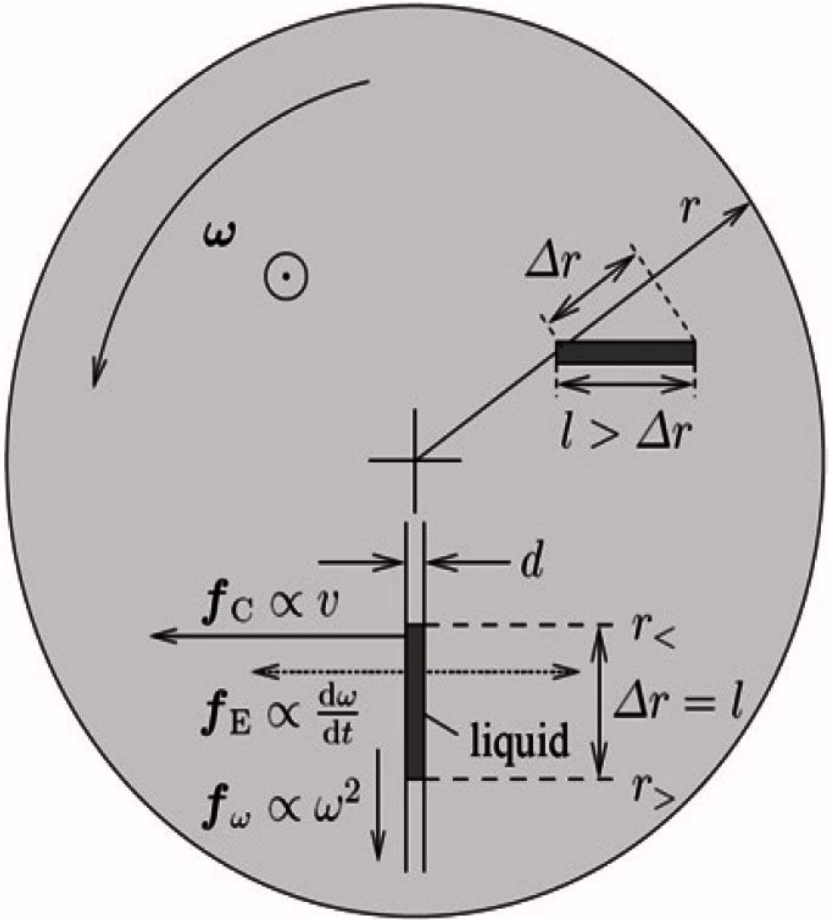
- The antibodies and chemical conjugates will be incorporated onto a microfluidic disc- based immunosensor.

Recombinant antibody assessment

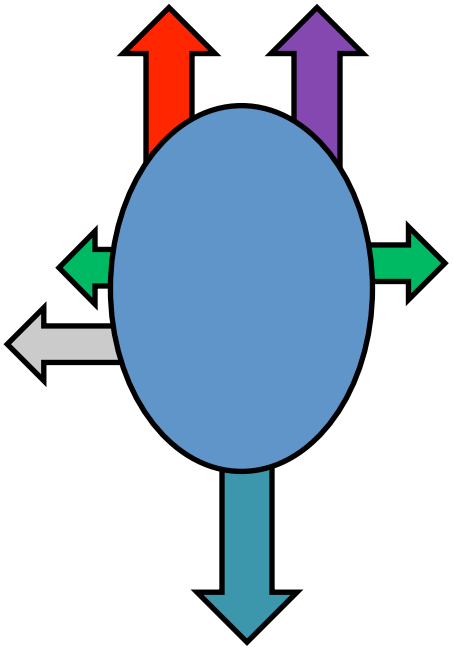


Centrifugal sensing platforms

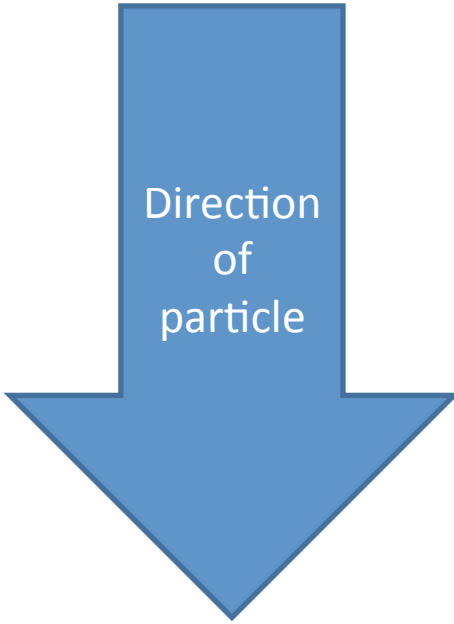
Fluidic movement on rotating platform



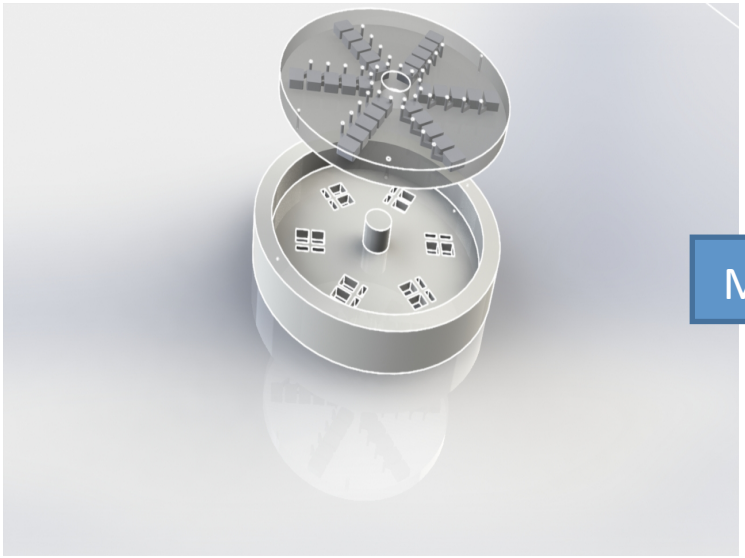
Forces on acting on a rotating disc



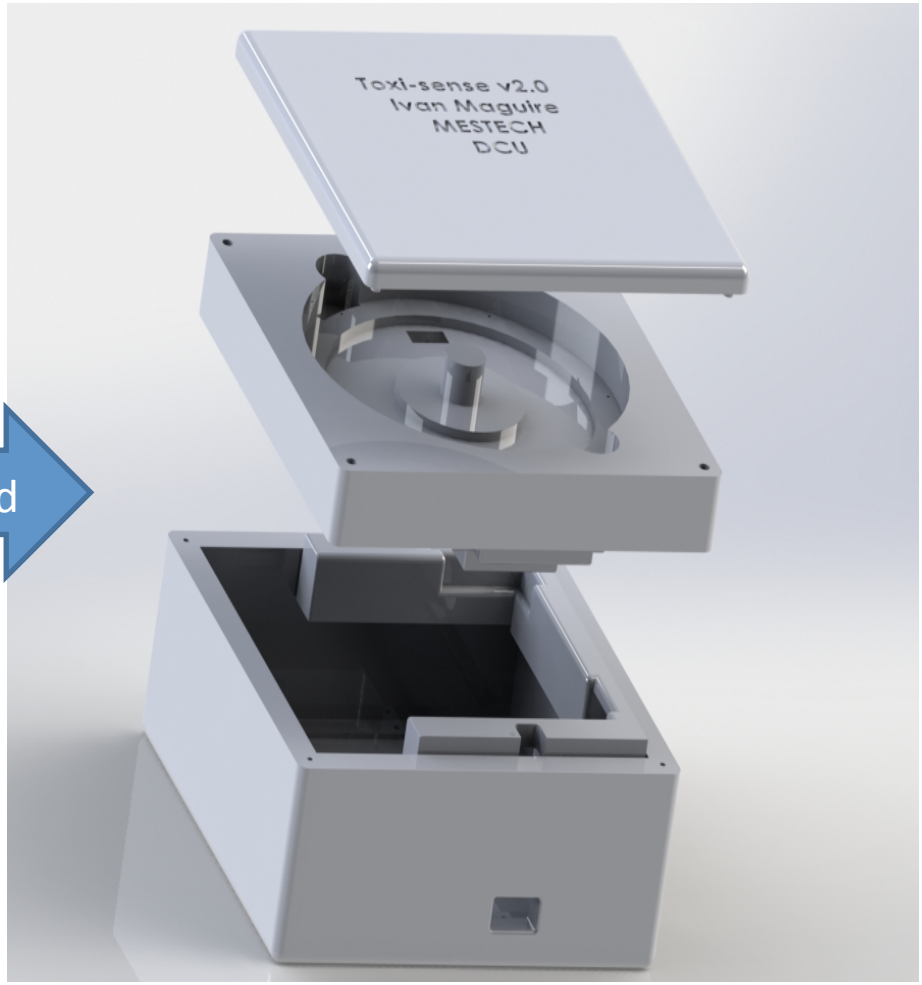
Particle sedimentation through a fluid on anti-clockwise rotating disc



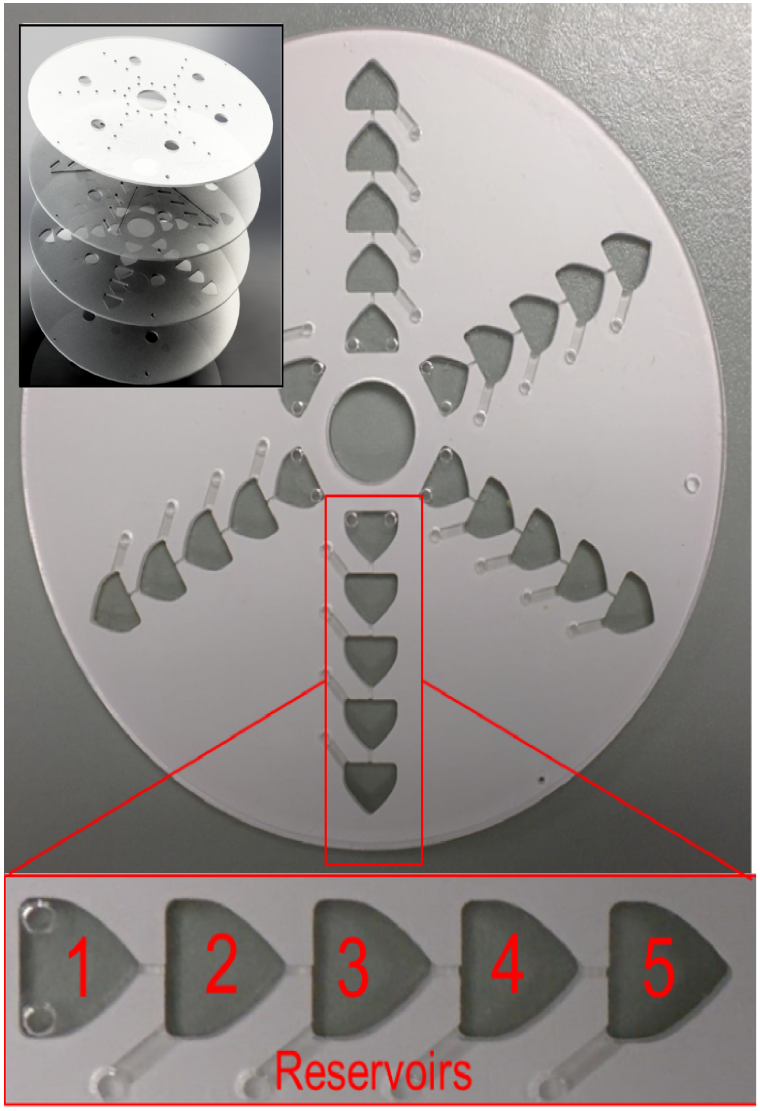
ToxiSense microfluidic System MK. 2

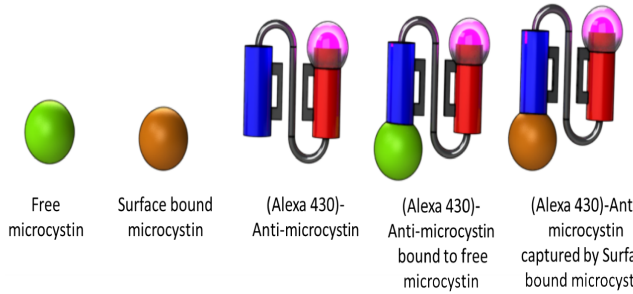


Modified



ToxiSense microfluidic system





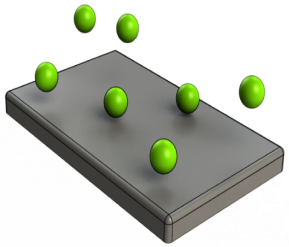
Free microcystin

Surface bound microcystin

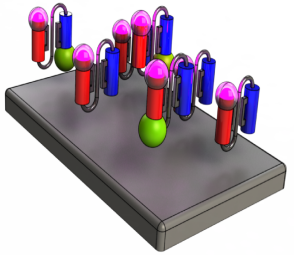
(Alexa 430)-Anti-microcystin

(Alexa 430)-Anti-microcystin bound to free microcystin

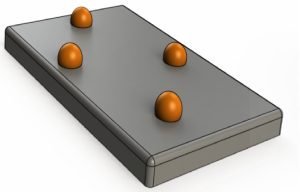
(Alexa 430)-Anti-microcystin captured by Surface bound microcystin



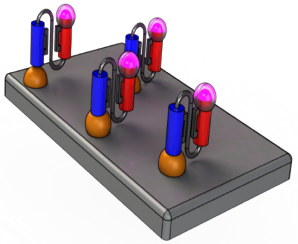
Reservoir one: Free microcystin present in sample



Reservoir two: All free microcystin binds to some (Alexa 430)-anti-microcystin

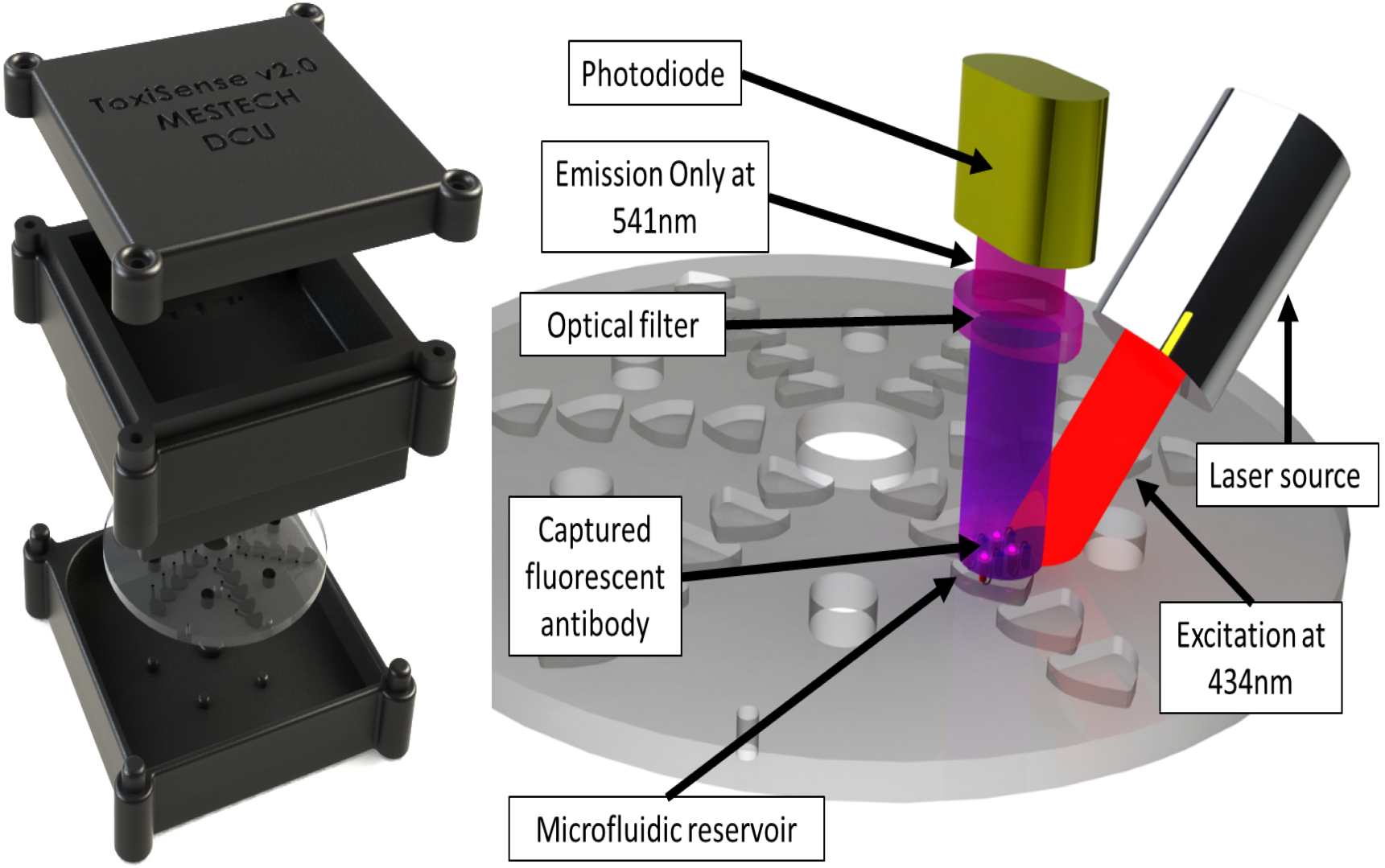


Reservoir three (before sample arrival): Reservoir surface is coated in surface bound microcystin.



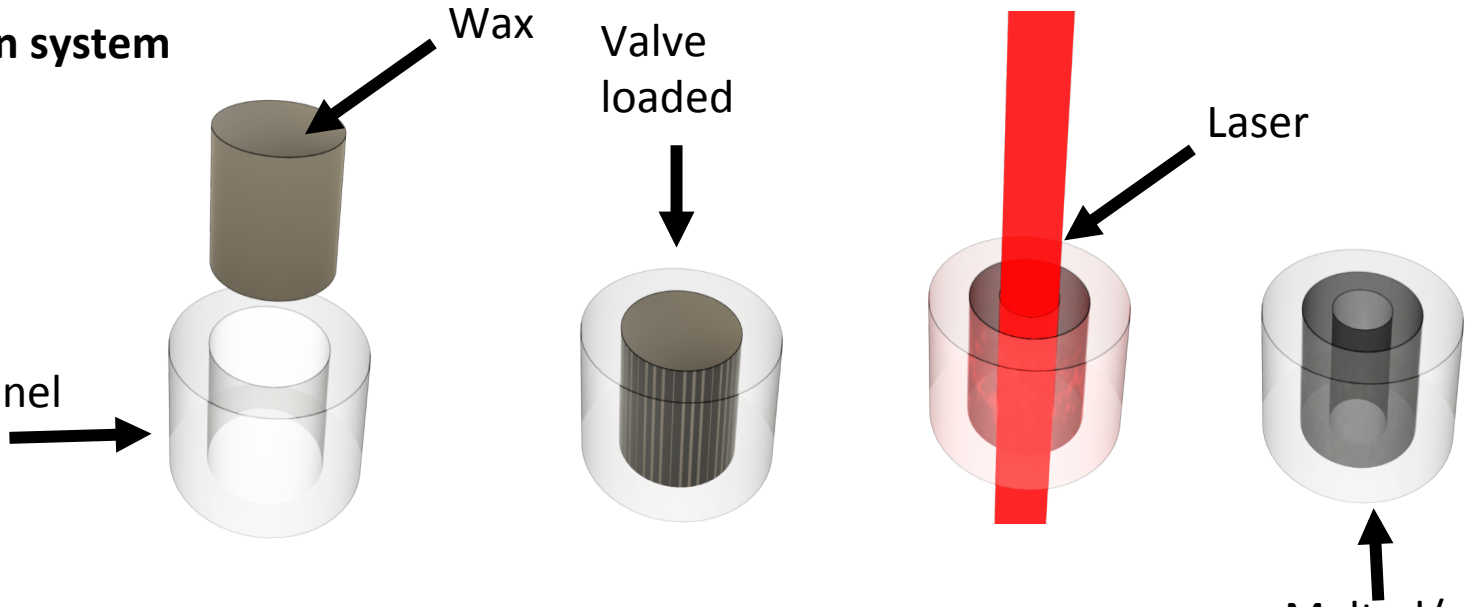
Reservoir three (after sample arrival): Remainder (Alexa 430)-anti-microcystin is captured by Surface bound microcystin and ready for detection.

ToxiSense – optical arrangement

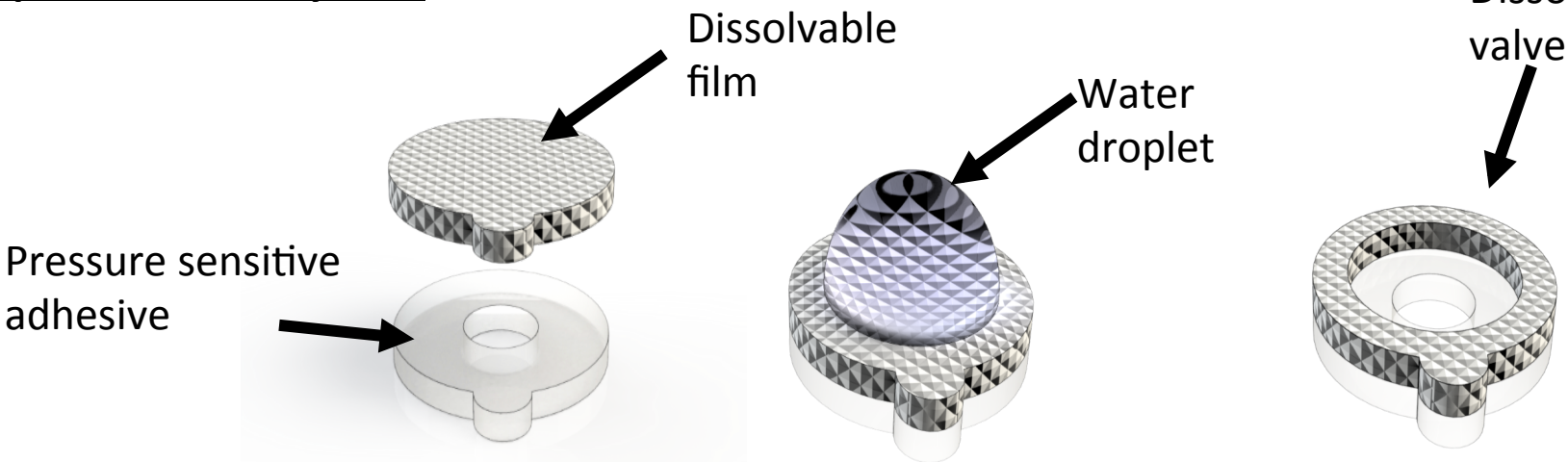


Some microfluidic valve solutions

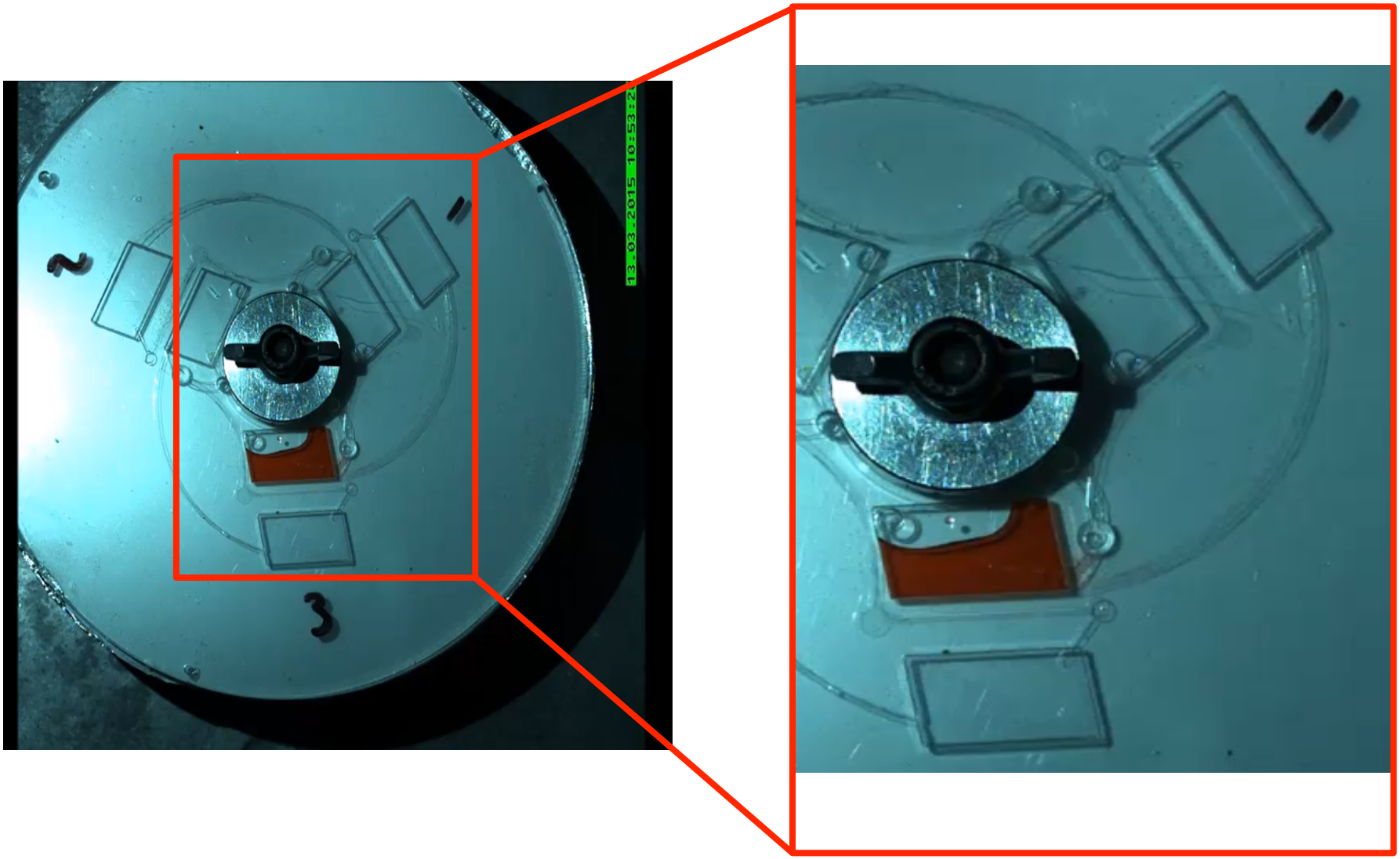
Heat actuation system



Liquid actuation system



Investigation into simple automatic ventilation mechanism

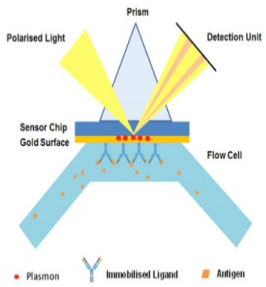


Video of automation by dissolvable film (DF) valve opening by pulsing motor speed

Detecting Microcystin

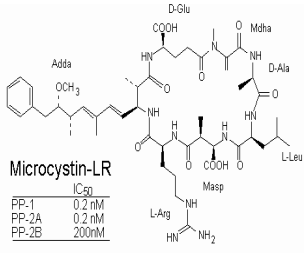
Surface plasma resonance (SPR)

LOD = 1.7ng



Protein phosphatase Inhibition (PPI)

LOD = 1.5ng mL^{-1}



Enzyme-linked immunosorbent assay (ELISA)

LOD = 1ng mL^{-1}

High-performance liquid chromatography (HPLC)

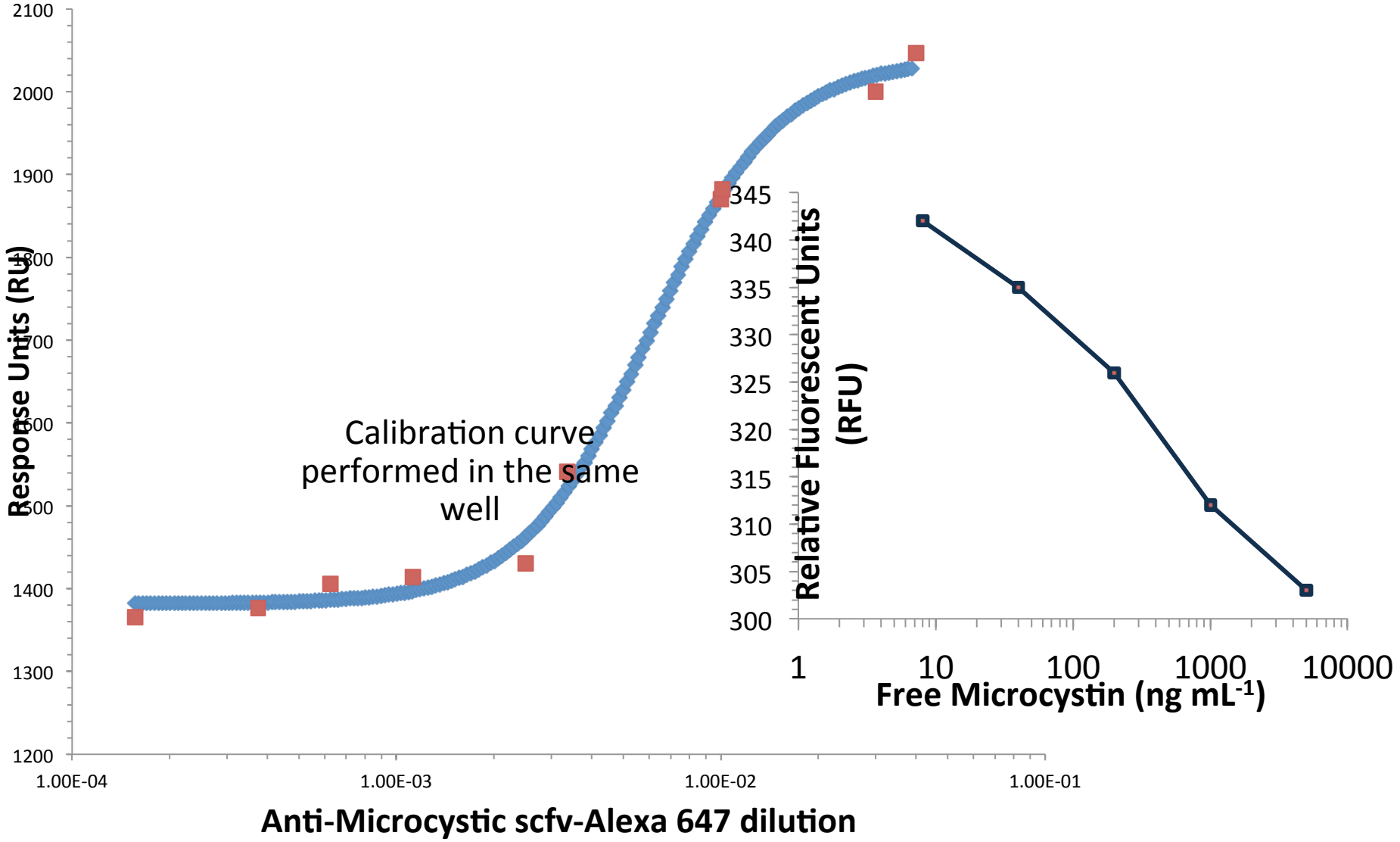
LOD = 1ng mL^{-1}

Microfluidic toxin-sensing system

LOD* = 8 ng mL^{-1}

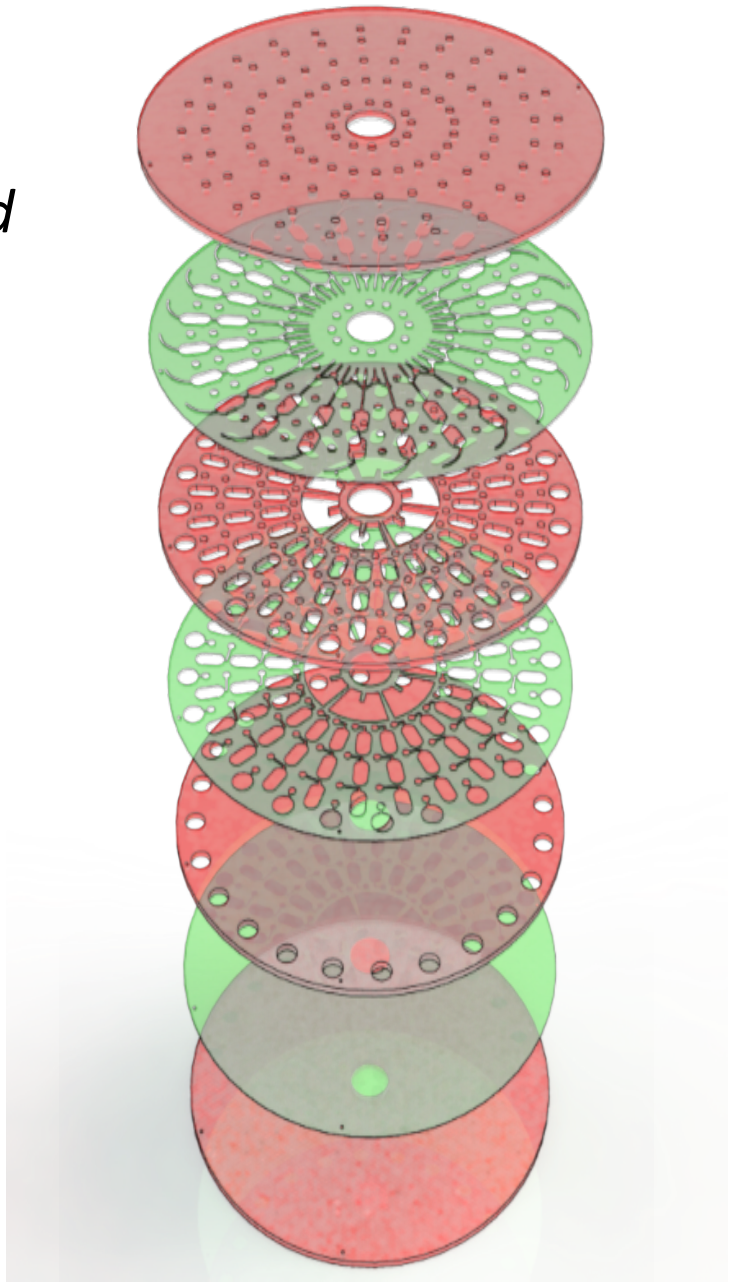
* This is the current Limit of detection (LOD) projection according to the latest sample recordings

Analysis of capture raw optical data generated

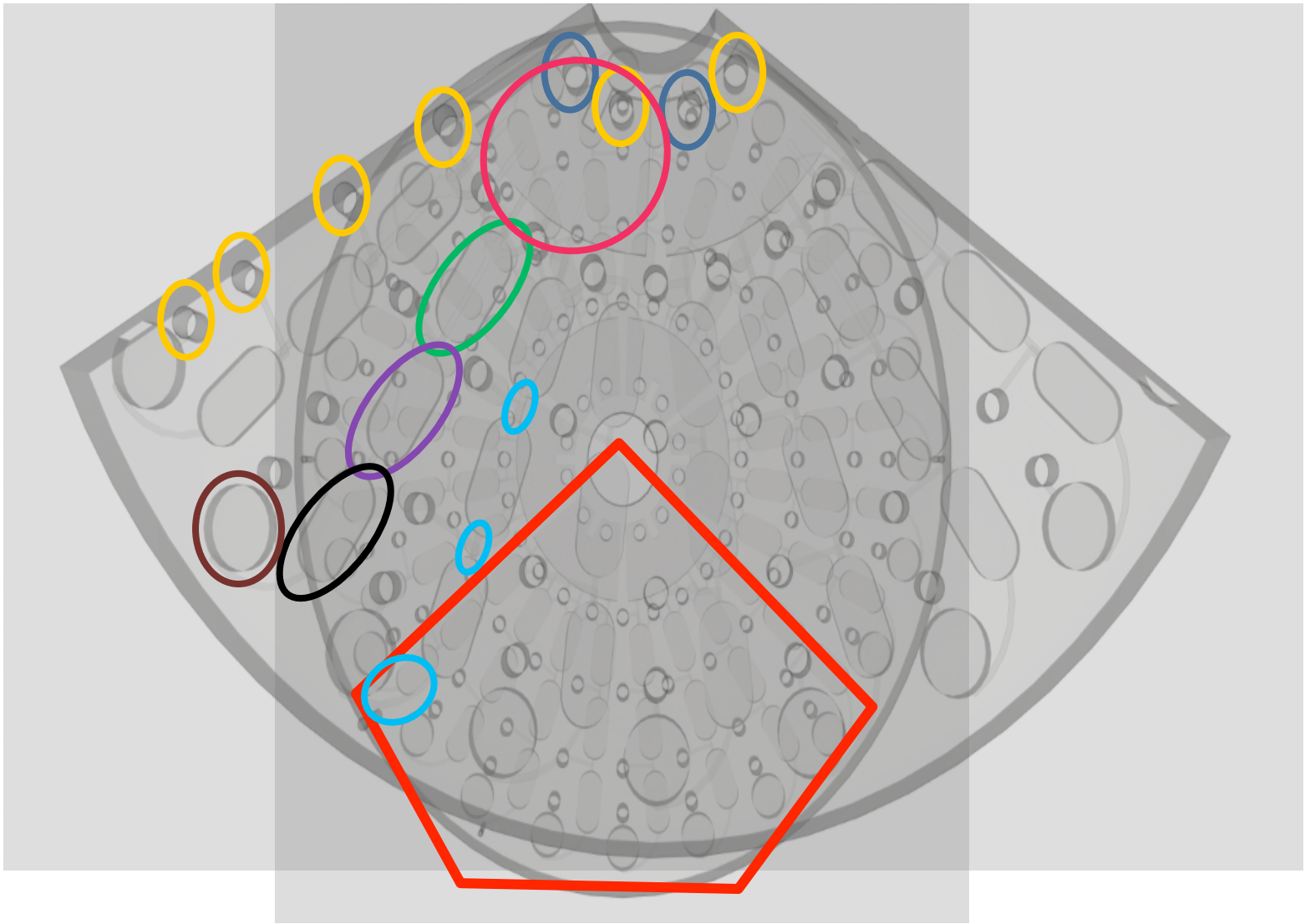


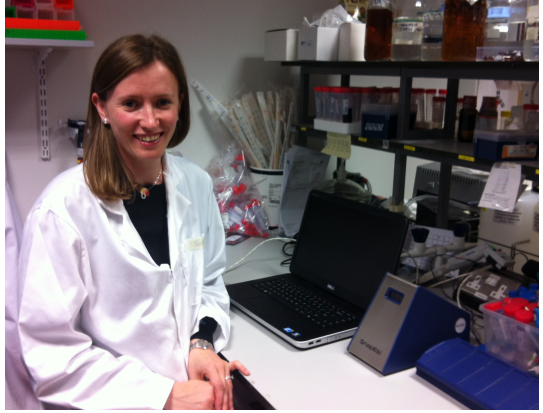
Lab-On-A-Disc platform

- *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



8 Test-assay disc per day





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Applied Biochemistry Group –
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Shortlisted - Irish Lab of the Year, 2013
DCU Award - Commercialisation, 2014

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