

Food Safety and Quality with Microsystems

AmI for Food Quality and Safety – Firenze 15-16 November

S Setford, Cranfield University, UK

Microsystems technology solutions for the rapid detection of pesticides

Objectives

- Design & development of innovative immuno-diagnostic microarrays/multi-analysis systems for pesticides
- Primarily aimed at the wine processing industry, but also applicability to fruits (apples, fruit juices)
- Development of compatible instrumentation & automated liquid handling systems
- Address pesticides *traceability* issue within the wine production process
- Assess role of device within the AmI scenario

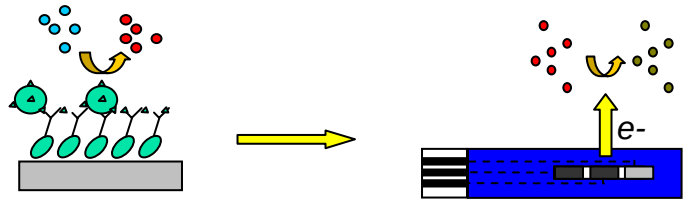
Amperometric and Impedimetric

- Amperometric
  - Very low-cost disposable sensing devices
  - Indirect method, measures assay label (specificity)
  - Build-up of product
  - More specialist reagents required
  - Modular unit with down-stream electrochemical detection

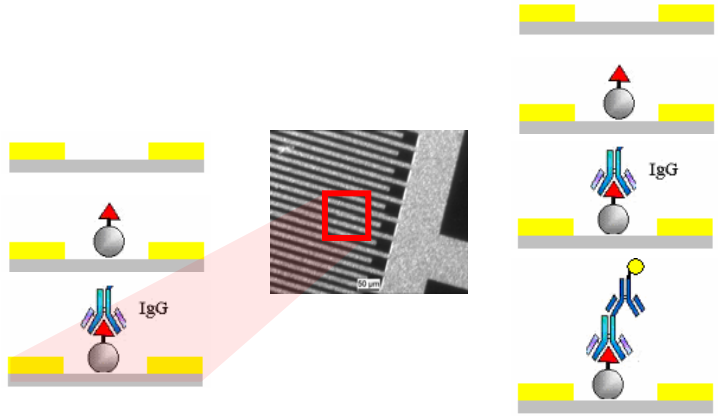
- Impedimetric
  - Simple (measure changes in resistance/capacitance due to antigen binding to antibody immobilised on inter-digitated micro-electrode structure)
  - Minimal need for specialist reagents
  - Potentially low cost (glass/plastic substrates)

- **Each method has a niche application within food analysis scenario – can both operate under a single device platform**

'Indirect' (label + amperometric detection)



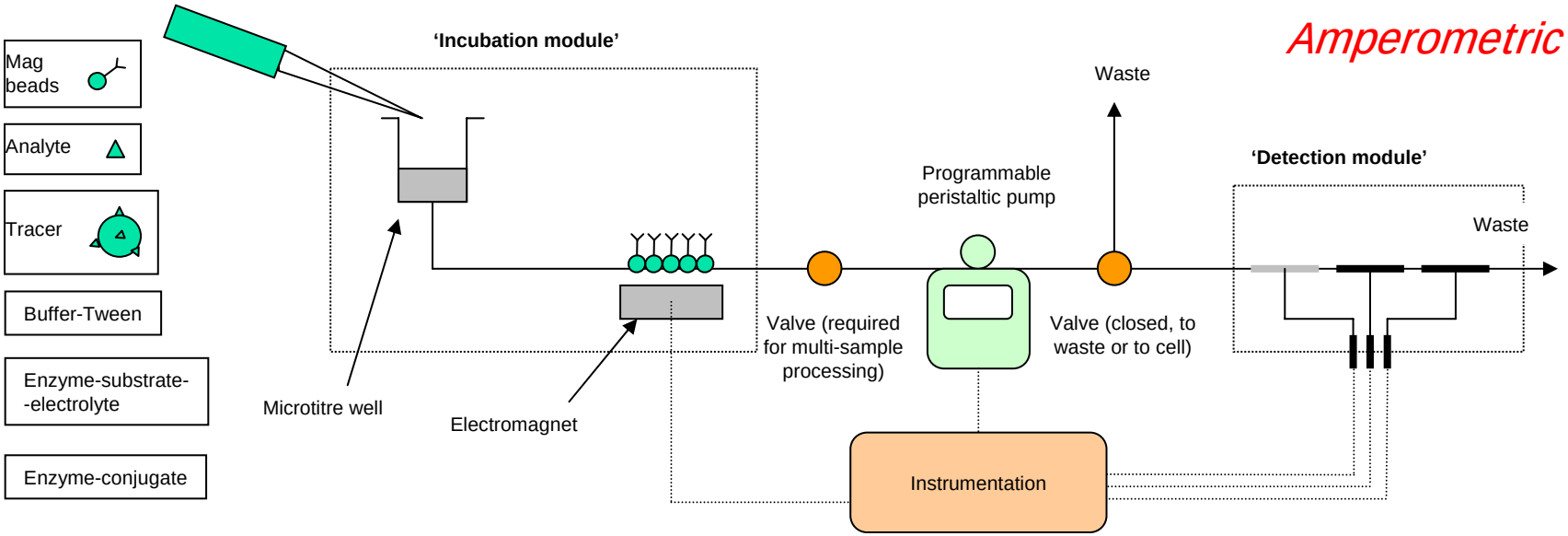
Immunoassay **Amperometric detection**

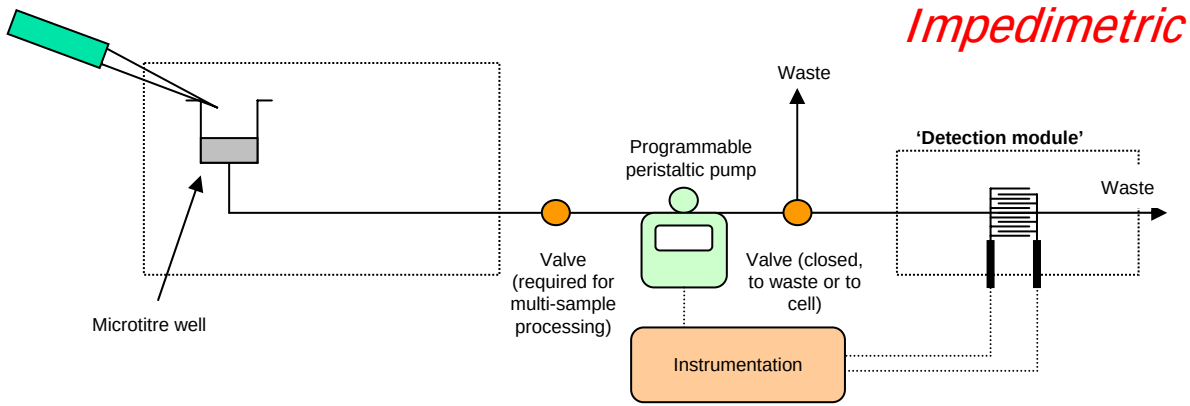


Capacitive: Changes in dielectric properties of spaces between IDE fingers

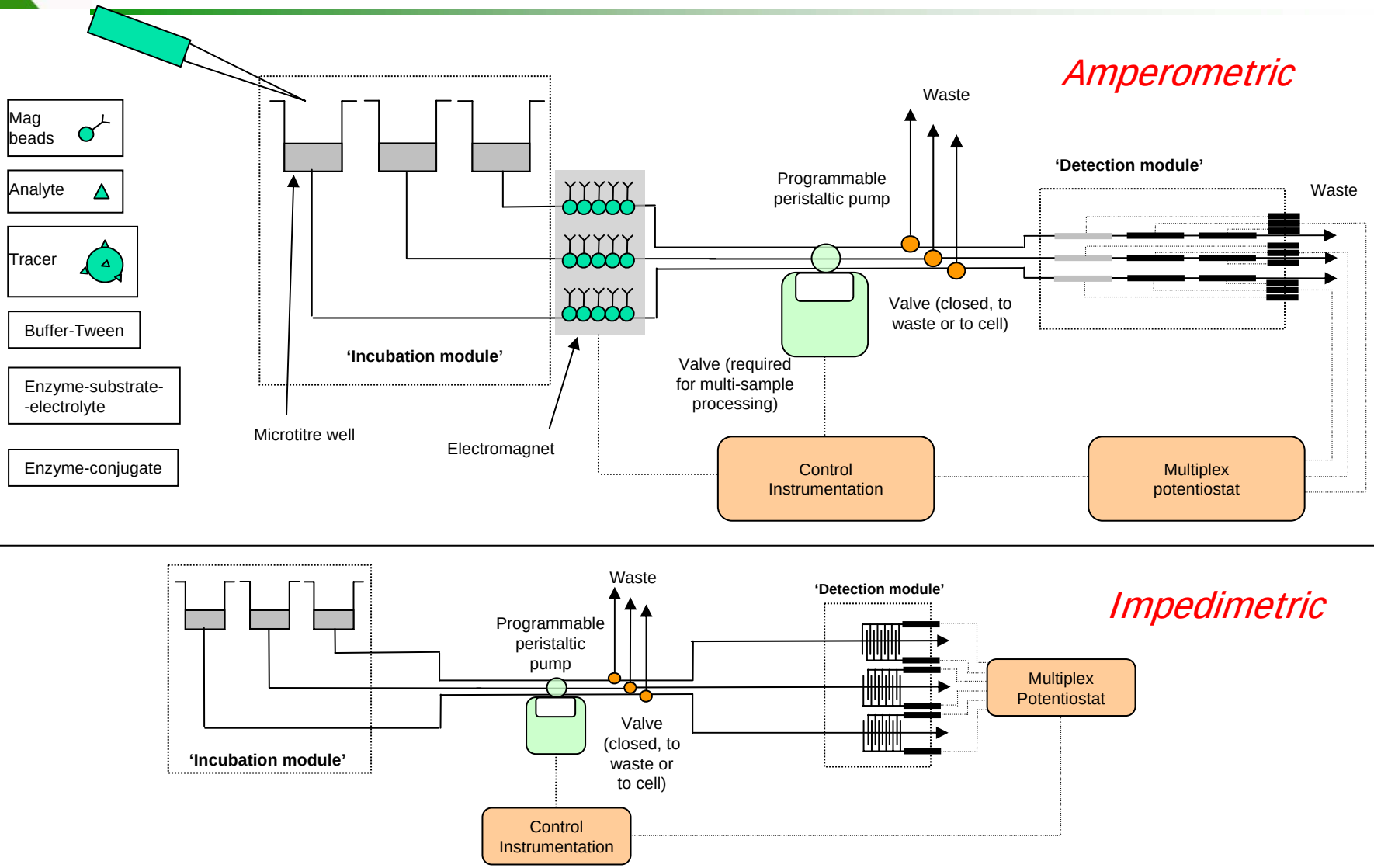
Conductive: Film conductance depends on the concentration of gold labeled antibodies.

Proposed solution (single channel or single detection cell)



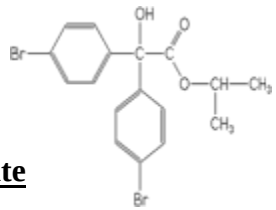


Alternative solution (multi-channel system)

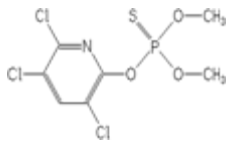


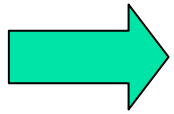
Selection of Targets

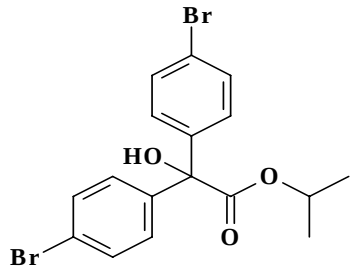
Simazine
Methiocarb
Dichlofluanid
Metalaxyl
Dinocap
Bromopropylate
Fenarimol



Organothiophosphate
Chlorpyrifos
Chlorpyrifos-methyl
Fenitrothion
Parathion-methyl
Malathion

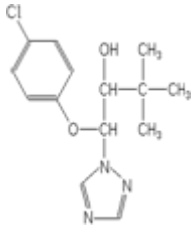




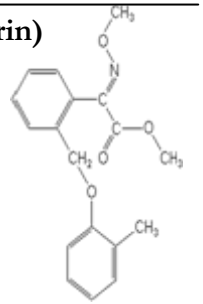


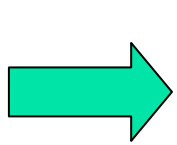
BROMOPROPYLATE
(MRL: 2 mg kg^{-1})

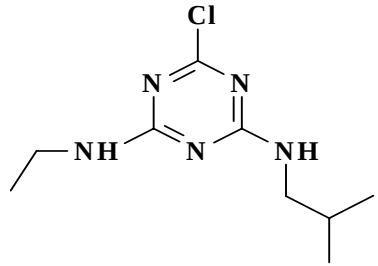
Fungicide (conazole)
Myclobutanil
Penconazole
Hexaconazole
Triadimenol



Fungicide (strobilurin)
Azoxystrobin
kresoxim-methyl

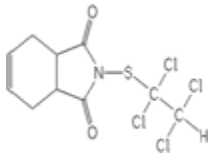




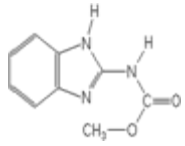


ATRAZINE
(MRL: 2 mg kg^{-1})

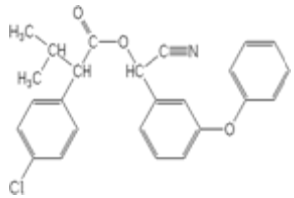
Fungicide (phthalimide)
Captafol
Captan
Folpet

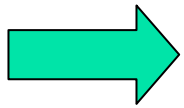


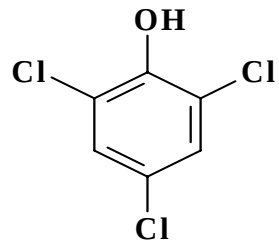
Fungicide benzimidazole
Benomyl
Carbendazim



Insecticide (pyrethoid ester)
Fenvalerate
Cyhalothrin

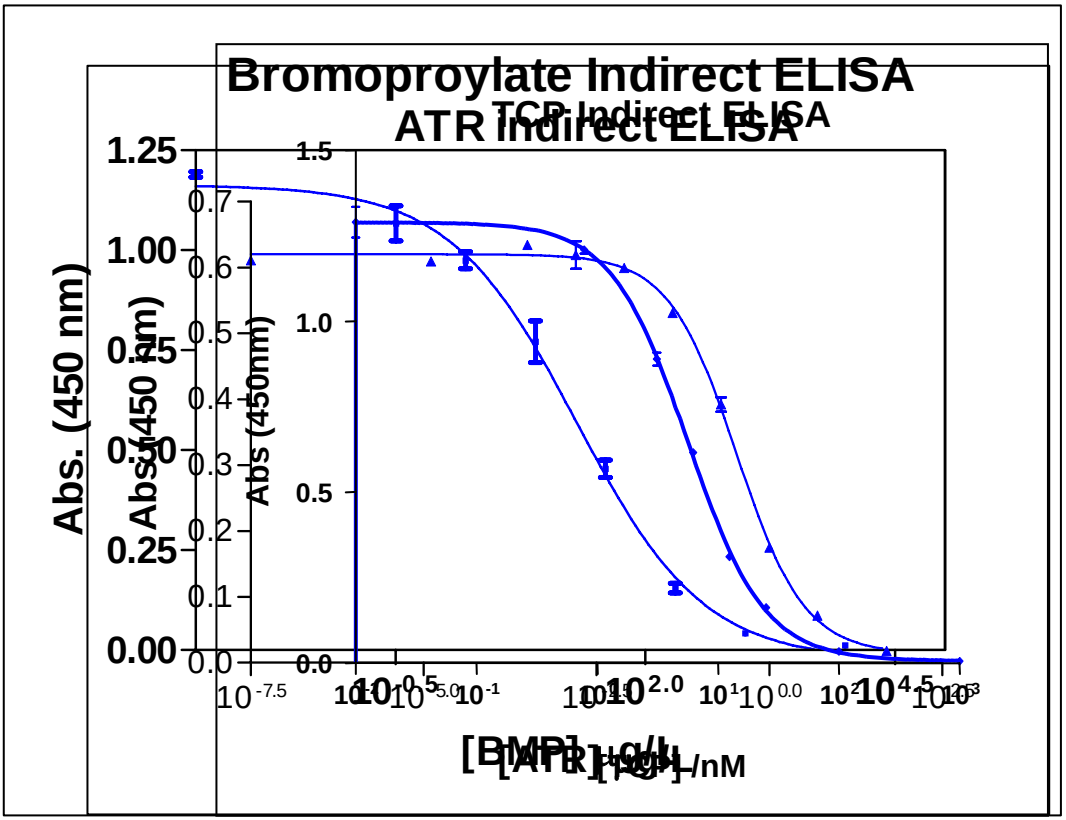






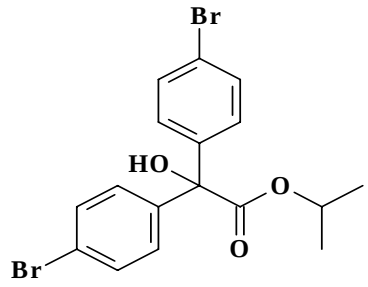
TRICHLOROPHENOL
(off flavours)

Assessment of Pesticides ELISAs

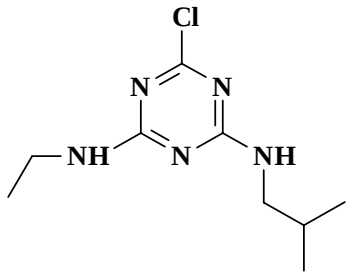


Amax	1.16	0.99±0.027
Amin	0.02	0.007±0.003
IC_{50}, µg L^{-1}	20.7	9.22±0.204
LOD µg L^{-1}	0.6	0.2

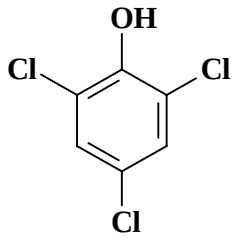
(non optimized assay)



BROMOPROPYLATE

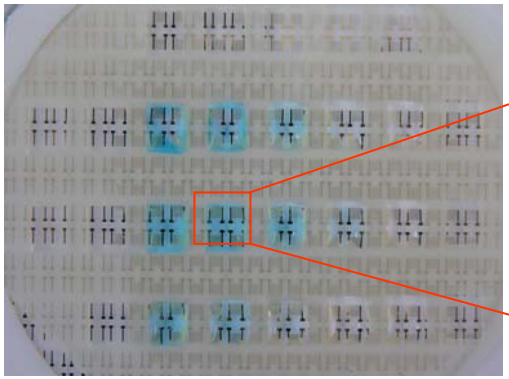


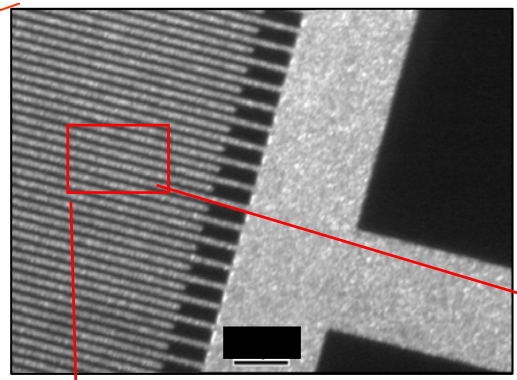
ATRAZINE

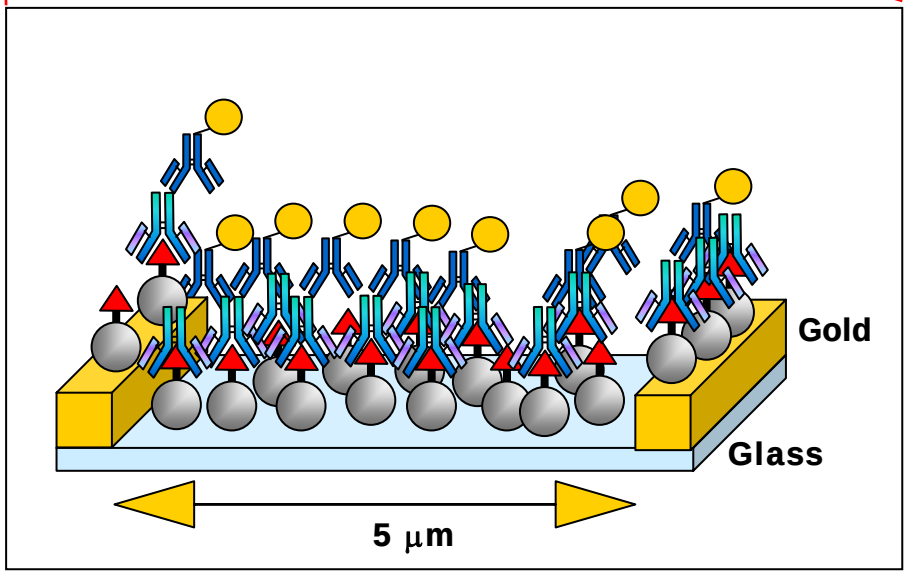


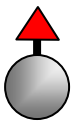
TRICHLOROPHENOL

Impedimetric Immunosensor:





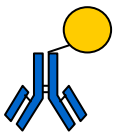




Atrazine-BSA

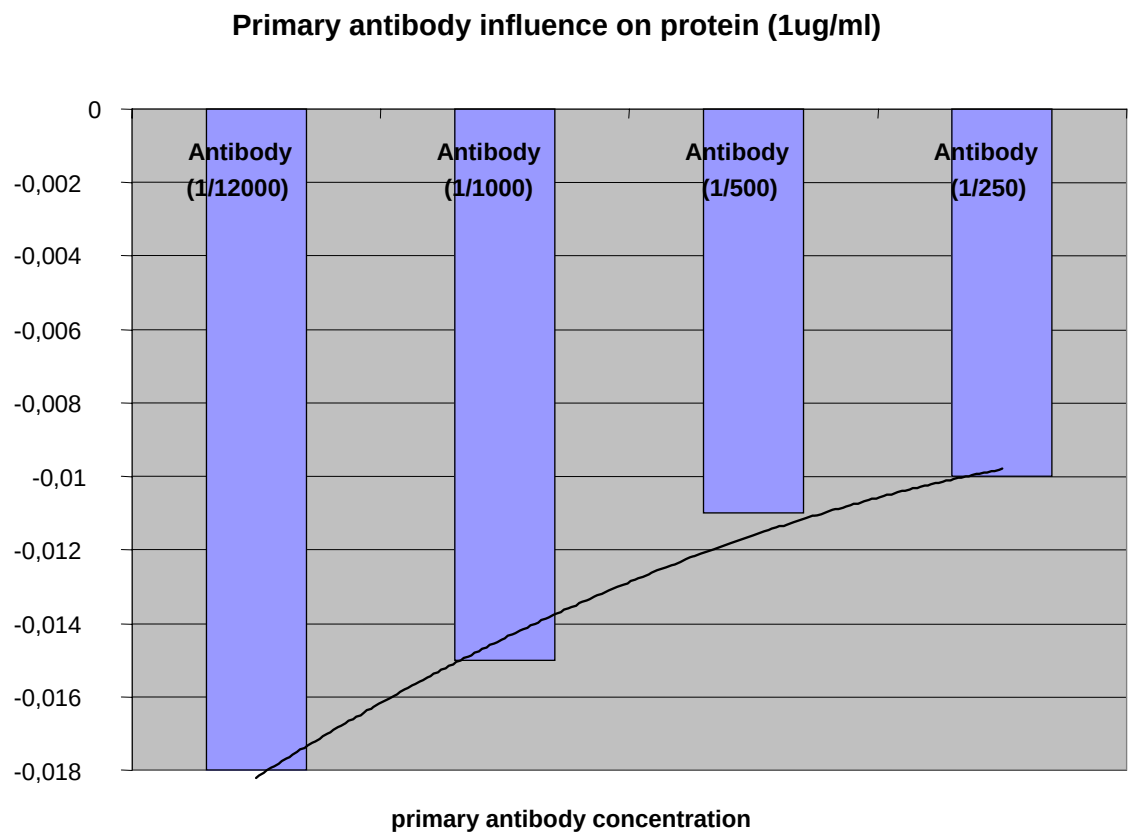


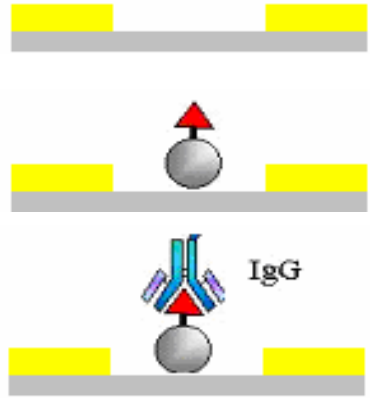
Specific antibody: IgG
(Conductance and capacitance)



Anti-IgG labelled with gold
(Conductance)

Capacitive Sensor: Results

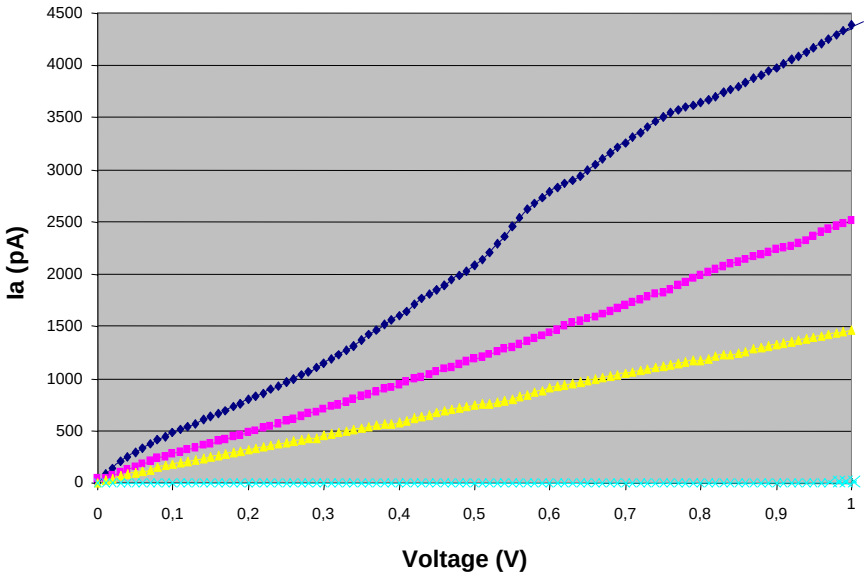




Change in capacitance produced by immobilization of antibody at different concentrations.

Conductive Sensors: Results

Secondary labelled with gold antibody influence

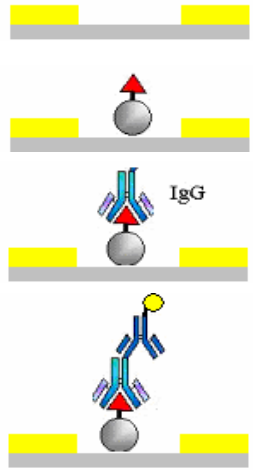


Primary antibody: 1:4000

Primary antibody: 1:8000

Primary antibody: 1:1600

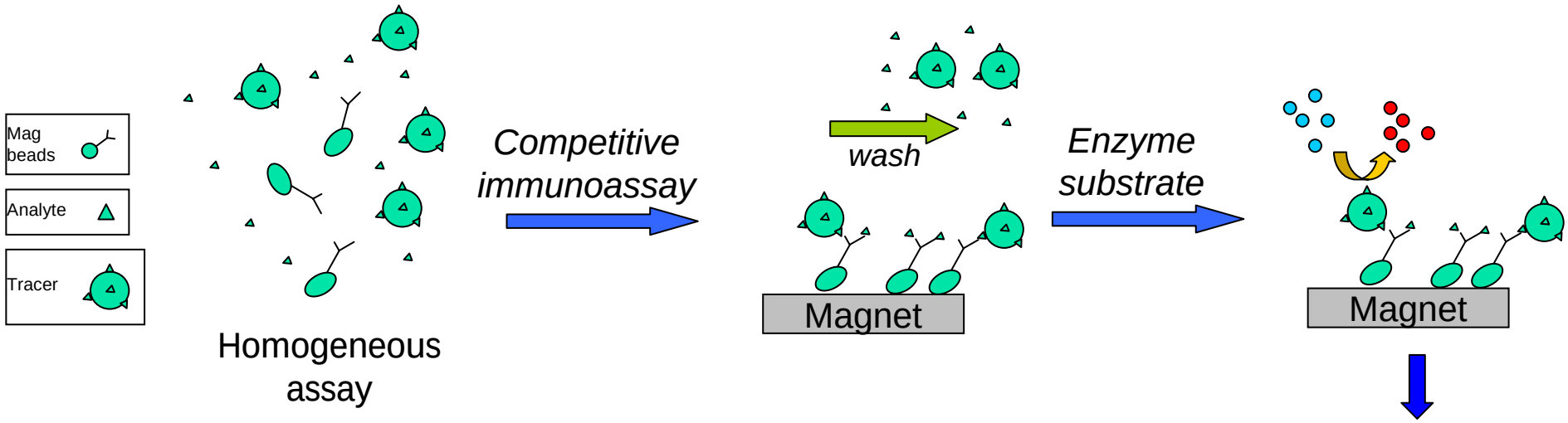
No primary antibody

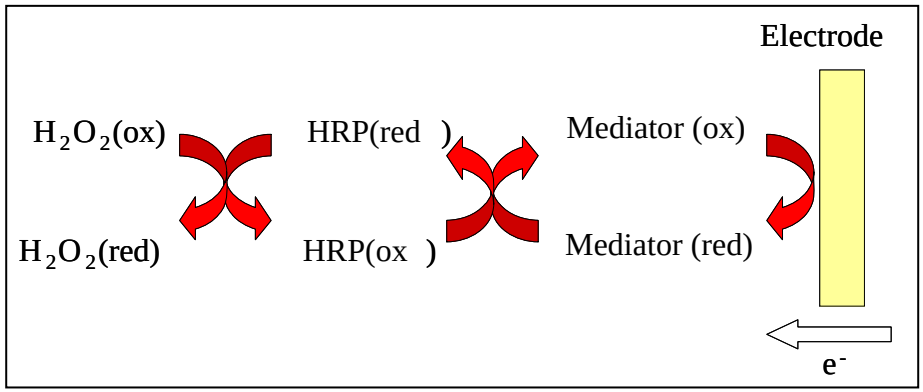


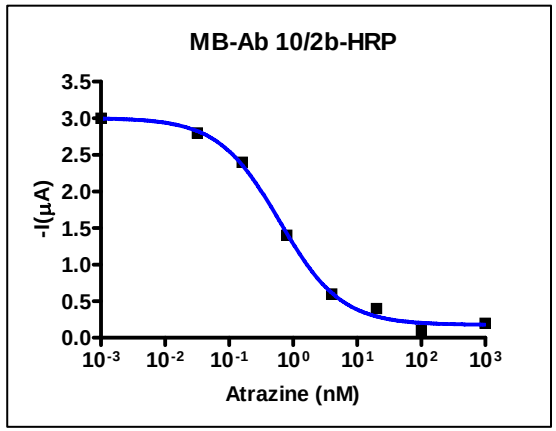
- Device response to increasing primary antibody concentrations. In this example, the distance between fingers is 5um, and the gold labels were 50nm beads.
- High linearity in this margin of concentration.
- Antibody signal is 4 orders of magnitude superior to background. Easy detection.

Electrochemical Transduction

Magnetic bead immunoassay for Atrazine

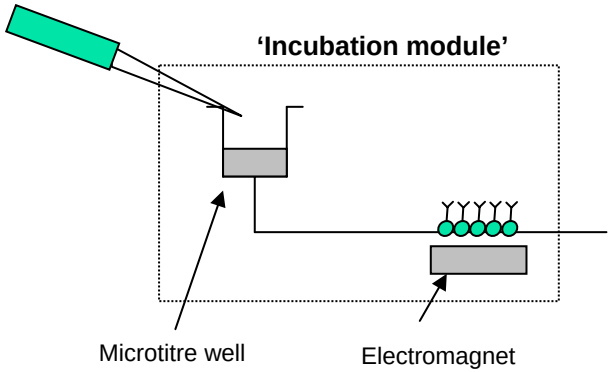






Mediator-based reaction scheme with electrochemical detection

Amperometric measurement process

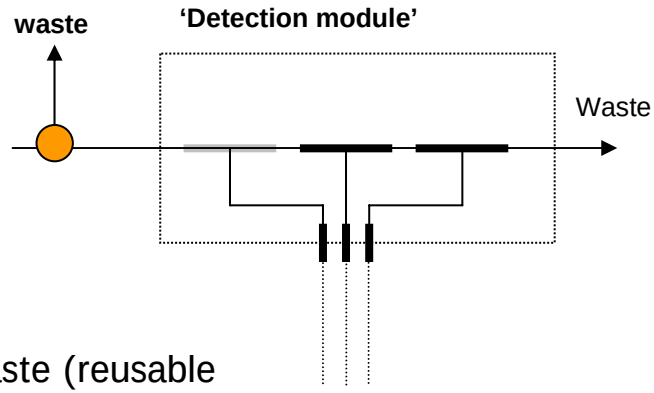


Immunoreaction (Typical example)

- Magnetic beads (immobilised antibody) introduced
- Trapped by electromagnet
- Addition of sample (pre-concentration? Interferent removal?)
- Addition of HRP-analyte tracer
- Incubate/wash
- Stop flow/active product generation/signal amplification

Measurement

- Only enzyme product contacts electrode (minimises fouling issues)
- Continuous flow gives superior data to 'stop' flow



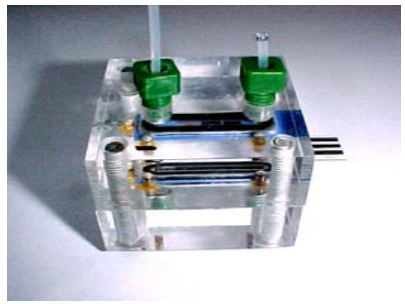
Note:

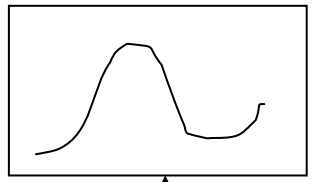
- Magnetic beads can be flushed from incubation module to waste (reusable incubation module)
- Multiple channels and multiplex potentiostat - or -sequential multi-analyte detection on single electrode element (valving system)
- Screen-printed electrodes ~replacement

Initial characterisation studies

Electrochemical device

Flowing downstream detection system
Currently tested with HRP reaction product (oxidised ABTS)





Electrochemical analyser

Sample in (e.g. from Immunoassay)

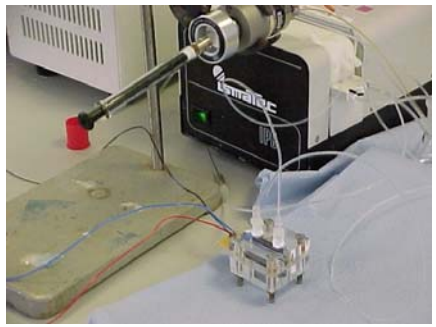
Buffer/KCl reservoir

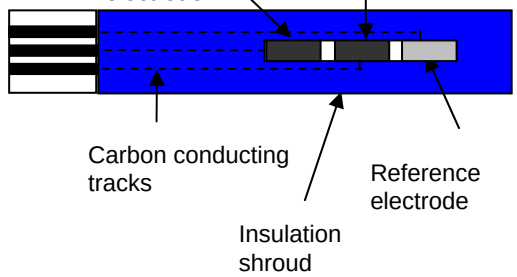
Peristaltic pump

6-port Injection valve

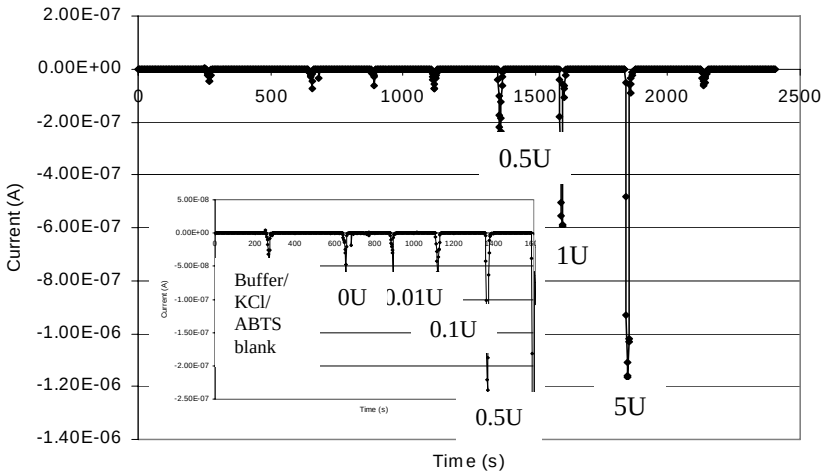
Waste

flowcell



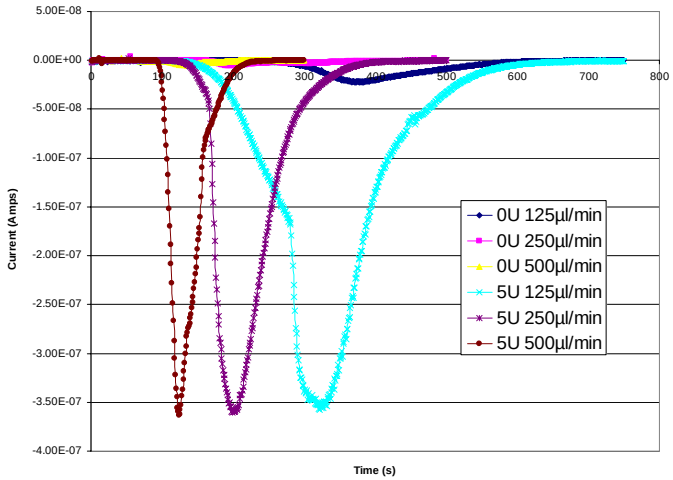


2. Technical Progress Overview of the first year



- Electrochemical response of varying activities of HRP.
- Immunoassay substrate: 48 µl 1% v/v H_2O_2, 192 µl 0.6%v/v ABTS in 40 mM buffer; +150mV Vs. Ag/AgCl;
- 1 ml/min flow rate; 3 min. reaction time (no acidification).

(Right)
Effect of flow-rate on electrochemical response on carbon screen-printed electrodes. Measurement conditions as above.

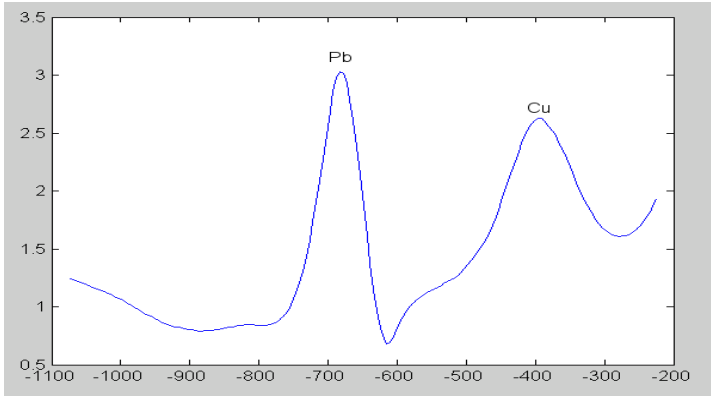


Other key parameters also studied: assay time, alternative mediator species etc.

Multiplex potentiostat

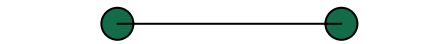
- 4 independent potentiostat channels
- Electrochemical drive voltages fully synthesised in software to 1mV, 5usec resolution
- PicoAmp resolution with full auto ranging.
- Onboard Microcontroller – fast, low current consumption
- Structured software design, (‘C’ language)
- On board data storage, or real time transmission over RS232 / Bluetooth link to PC / PDA
- Calibration data stored in on-board EEPROM, allows low cost hardware components to be used.
- Battery operated, robust, field-portable
- On board or PC based data analysis

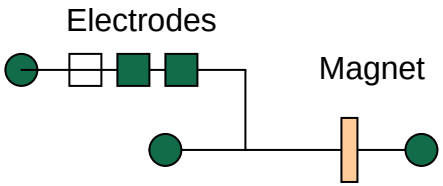


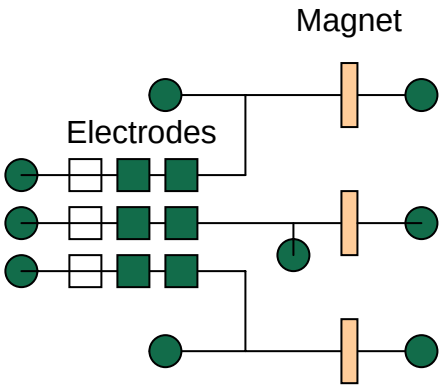


Heavy metals analysis using screen-printed electrode

Design and fabrication of prototype disposable microfabricated flow-cell







Simple channel to test electrode response

- Investigate response in static and flowing system using different channel sizes

Multiple channels

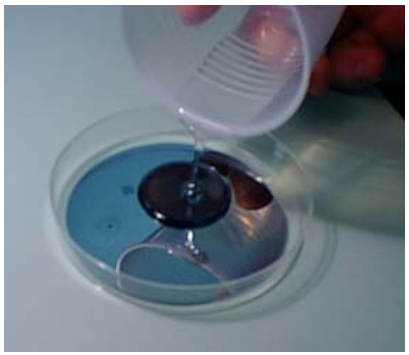
- to allow routing of wash solutions to waste not over electrodes
- incorporation of permanent/electro- magnet for immunomagnetic bead assay

Array approach for parallel high throughput sampling

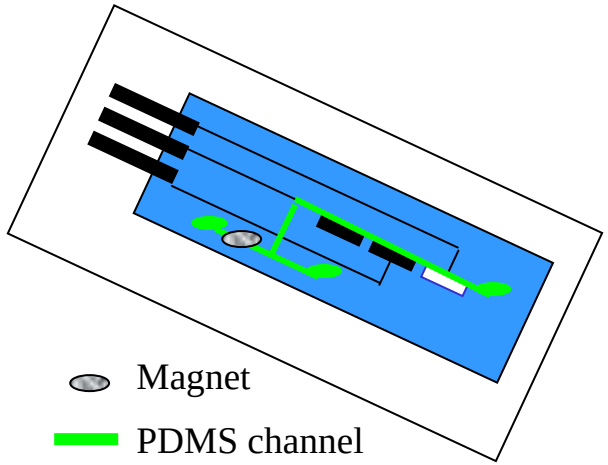
- under development

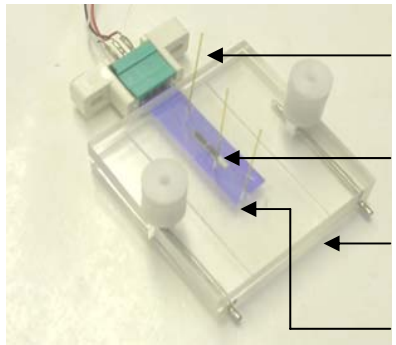
Microfluidic channel design

- Creation of desired microfluidic design in CAD package
- Creation of raised structures of SU-8 polymer on Si wafers using photolithography
- Casting of PDMS elastomer against master template and curing
- Removal and sealing of PDMS layer containing microfluidic channels to screen printed electrode
- Integration of fluidic inputs (clamp assembly) with syringe pump, magnet and system control software (in progress)



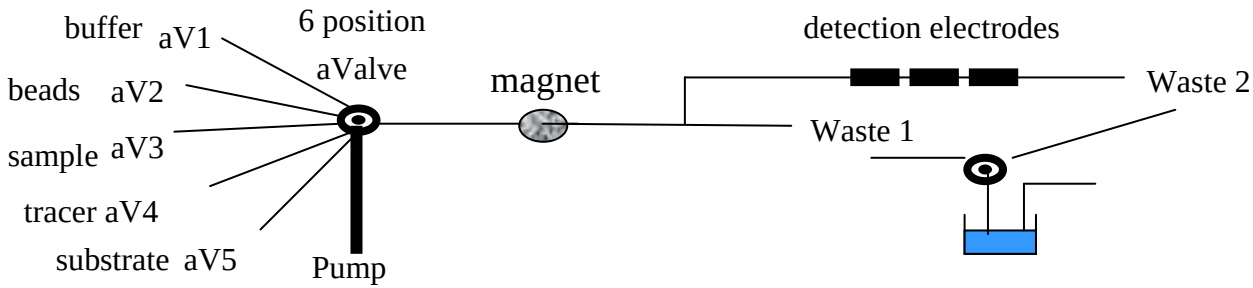
Casting PDMS against master





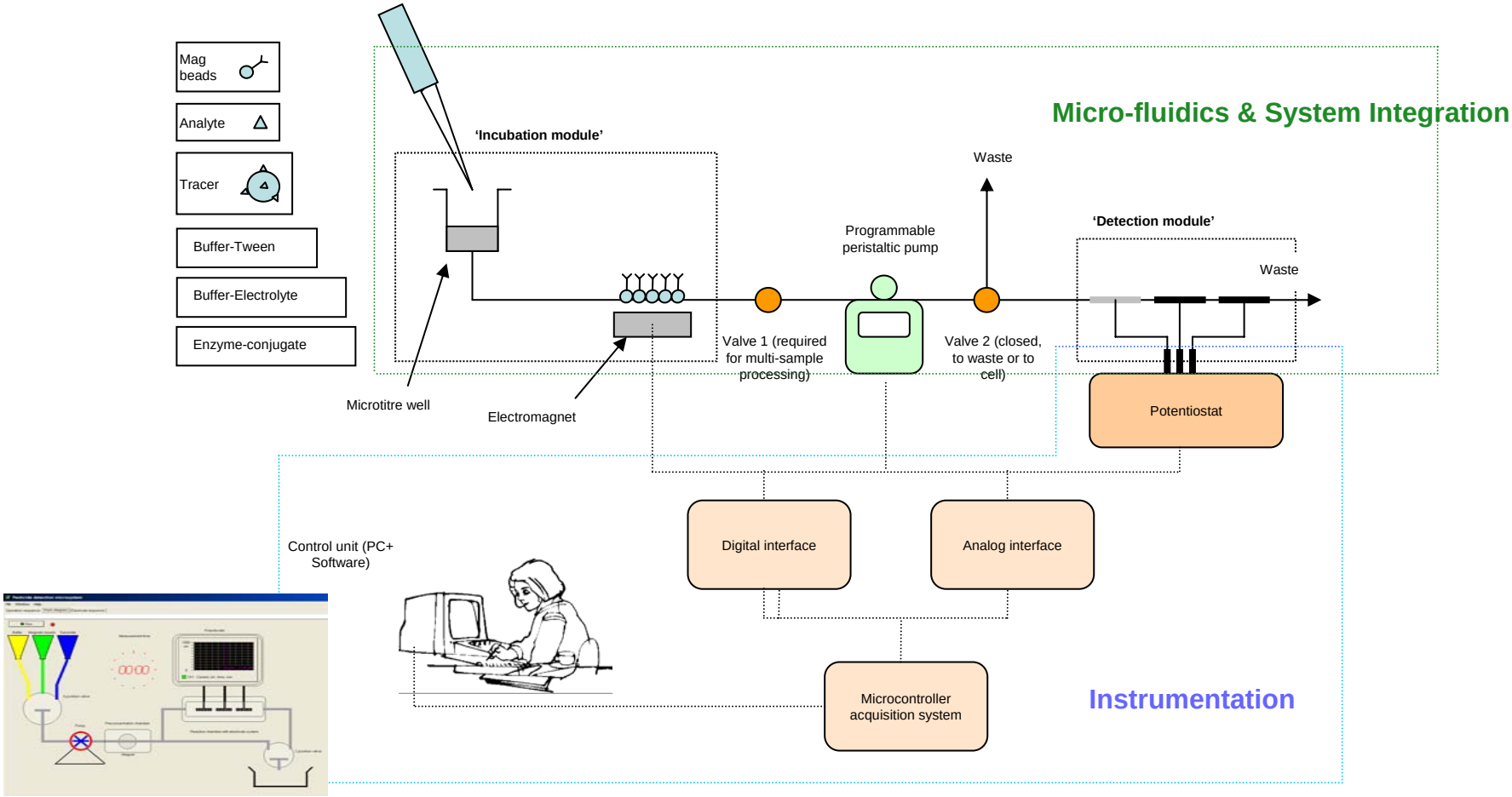
Microfluidic assembly

Proposed control sequence for immunoassay protocol



Sequence	Time	aV1	aV2	aV3	aV4	aV5	wV1	wV2	magnet	pump
1 buffer flush - turn on potentiostat after 2 mins	5 min	open	closed	closed	closed	closed	closed	open	off	on
2 bead flush&trap	3 min	closed	open	closed	closed	closed	open	closed	on	on
3 sample flush	3 min	closed	closed	open	closed	closed	open	closed	on	on
4 tracer flush	5 min	closed	closed	closed	open	closed	open	closed	on	on
5 wash step	3 min	open	closed	closed	closed	closed	open	closed	on	on
6 add enzy subst	5 min	closed	closed	closed	closed	open	open	closed	on	on
7 incubation	5 mins	closed	closed	closed	closed	closed	closed	closed	on	off
8 flush over elects	3 min	open	closed	closed	closed	closed	closed	open	on	on
9 cleaning flush	5 min	open	closed	closed	closed	closed	open	closed	off	on

Instrumentation Integration



Conclusion

- 2 main routes under investigation:
  - Impedance approach using interdigitated gold microelectrodes
  - Amperometric approach using screen-printed disposable electrodes
- Common measurement platform under development
- Initial impedance results promising
  - Binding of antibody to target antigen ‘recognised’
  - Competition assays to be developed
- Amperometric route
  - Magnetic bead immunoassay + enzyme label
- Prototype macro-scale system under development + flow-cell microfabrication
- **Control systems/instrumentation under development**

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- **Atrazine magnetic bead immunoassay**
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  - Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Argentina