Direct Electrical Detection of Target Cells on a Microfluidic Biochip

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Target Cell Detection

- Pathogenic bacteria detection is extremely important in:
  - Medical diagnosis
  - Food industry
  - Bioterrorism
- Ecoli O157 has been major cause of haemorrhagic colitis and haemolytic syndrome
  - Steady increase in number of outbreaks worldwide
  - High mortality rate

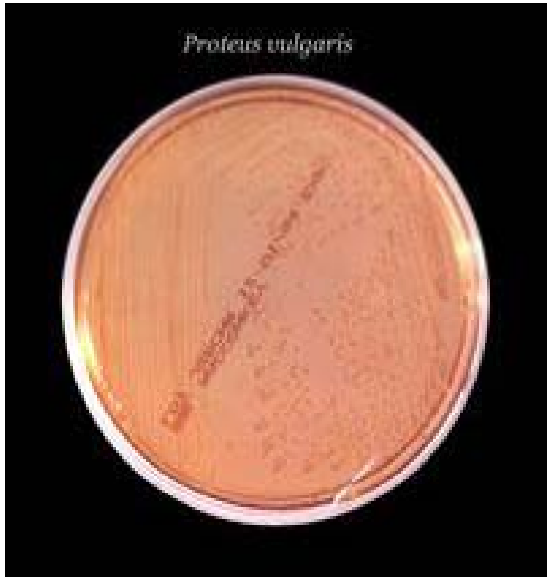
Bacteria sample grown on plate

Traditional method of Ecoli O157 Detection

- EcoliO157 does not ferment sorbitol (a sugar alcohol) rapidly as opposed to other strains of Ecoli
- Selective media developed based on this quality
- If EcoliO157 not present, pH will be lowered

Drawbacks

- Must test individually on each colony in sample
- Each test takes 24-48hours due to incubation time

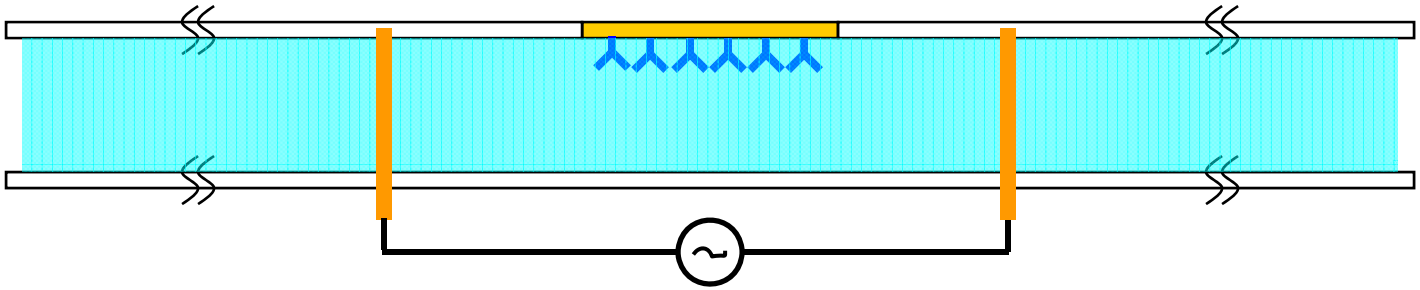


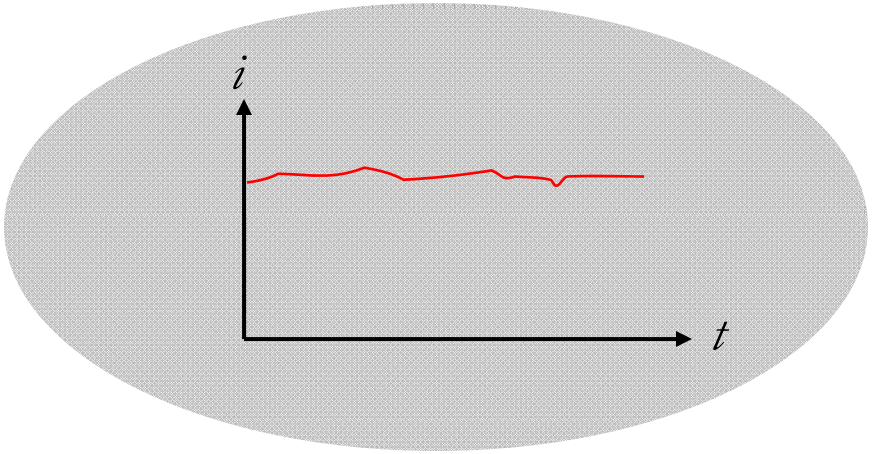
MacConkey Agar Plate with an active Bacteria Culture

Constraints for Sensors

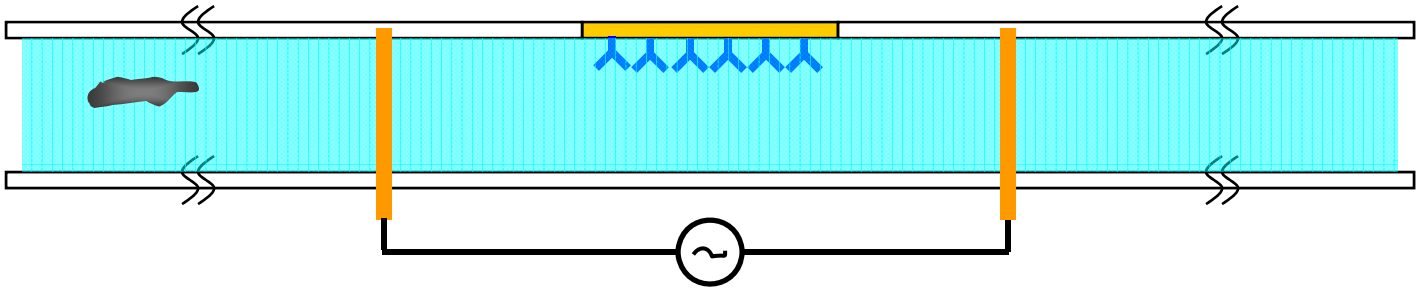
- Ultra-sensitivity
- Rapid Analysis Time
- Inexpensive

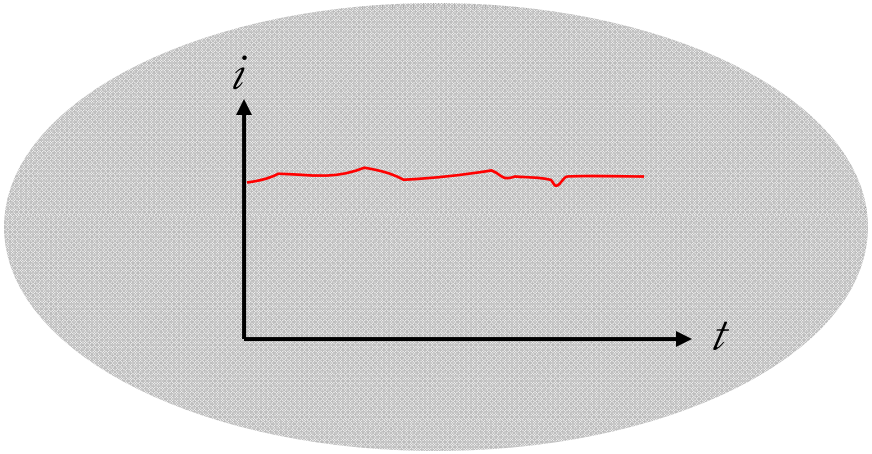
Micro-Channel Gating





Micro-Channel Gating (Cont.)





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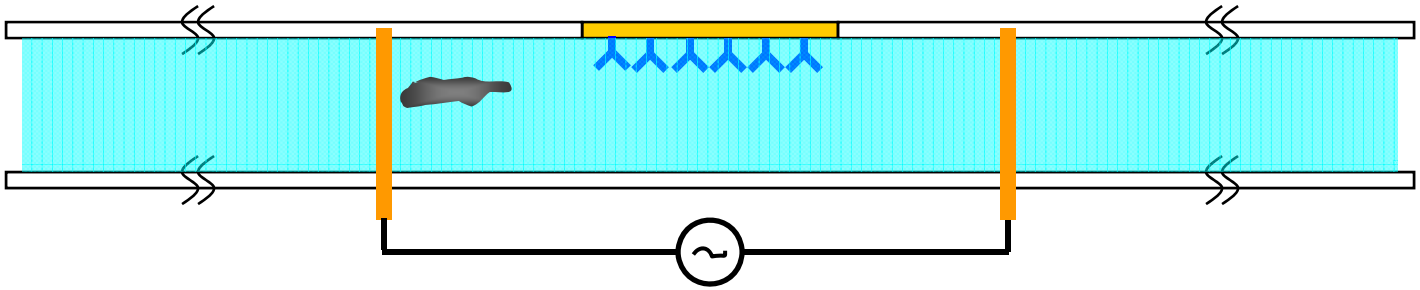

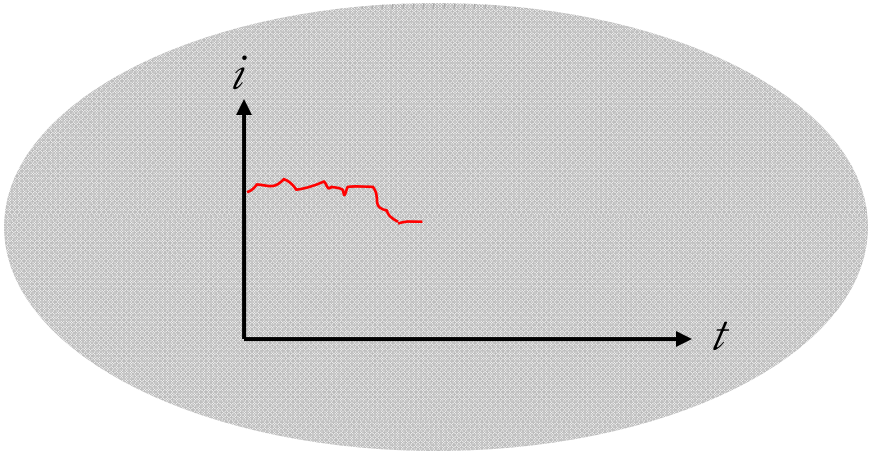

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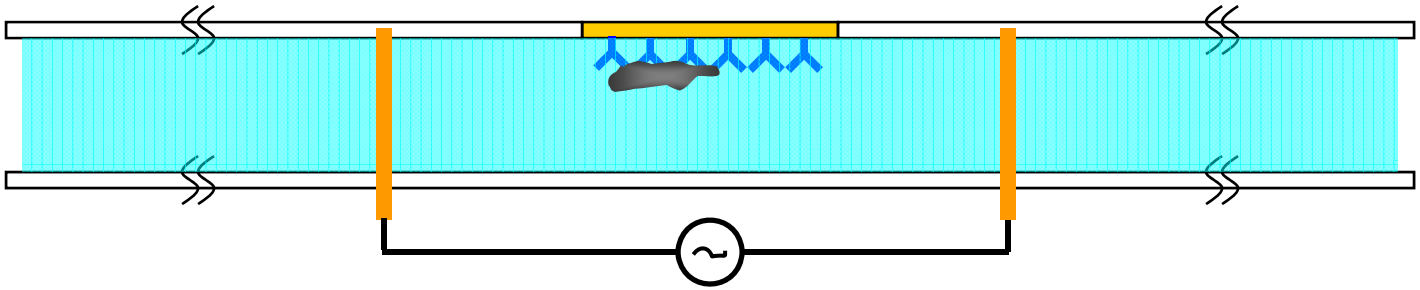

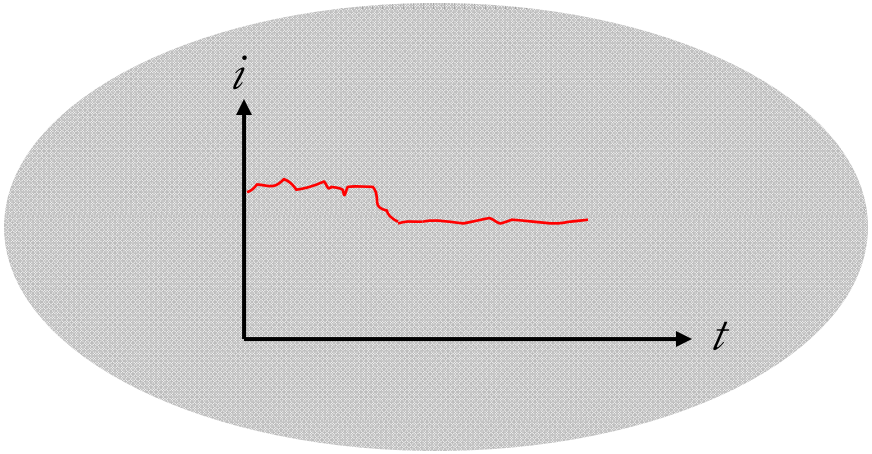

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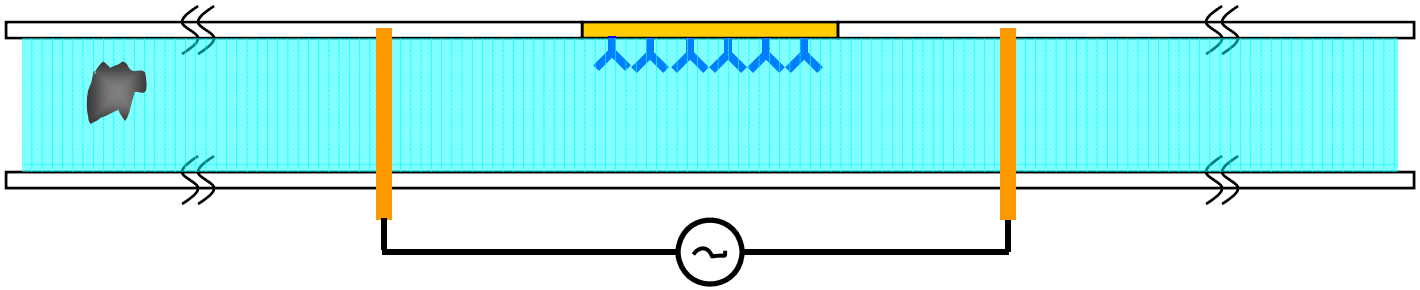

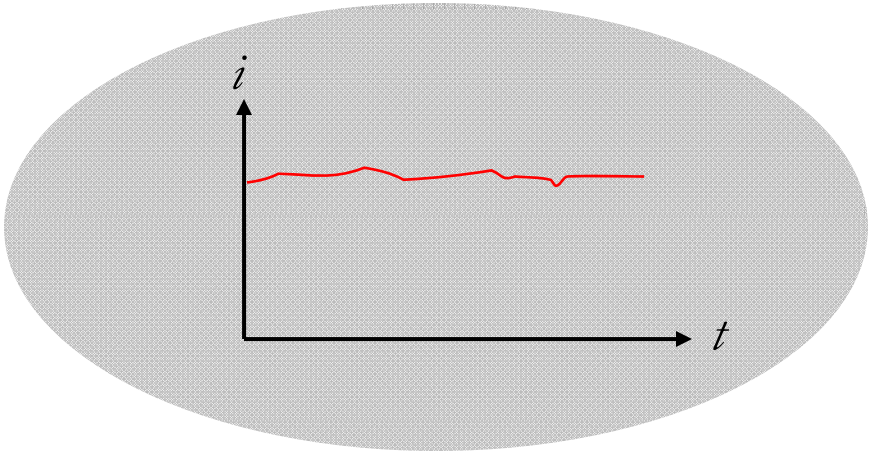

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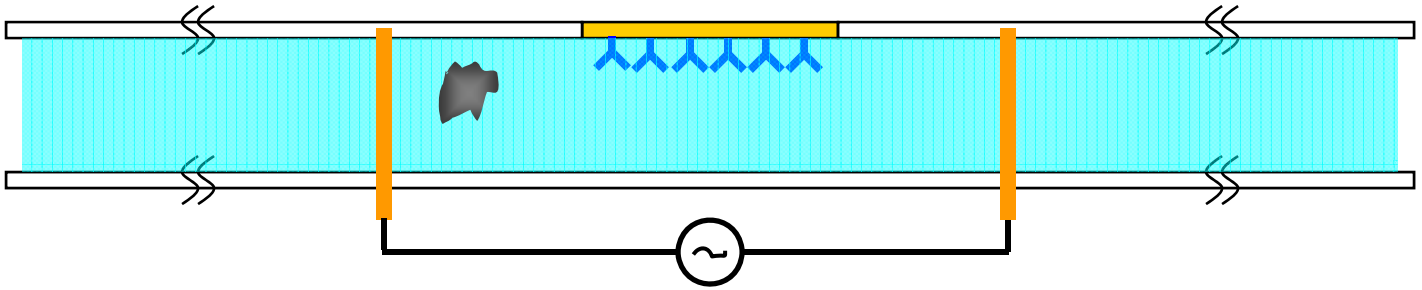

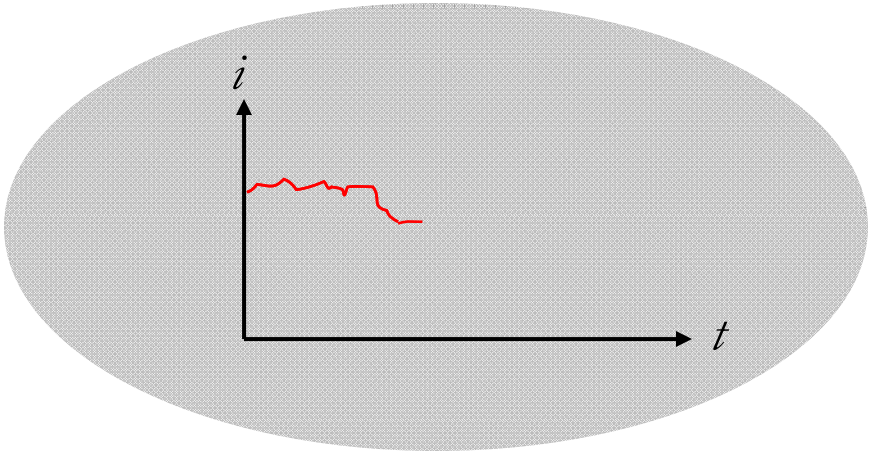

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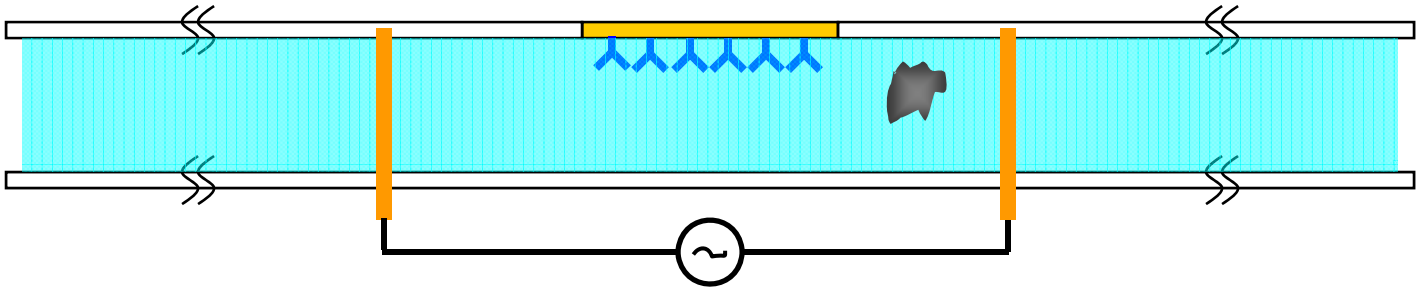

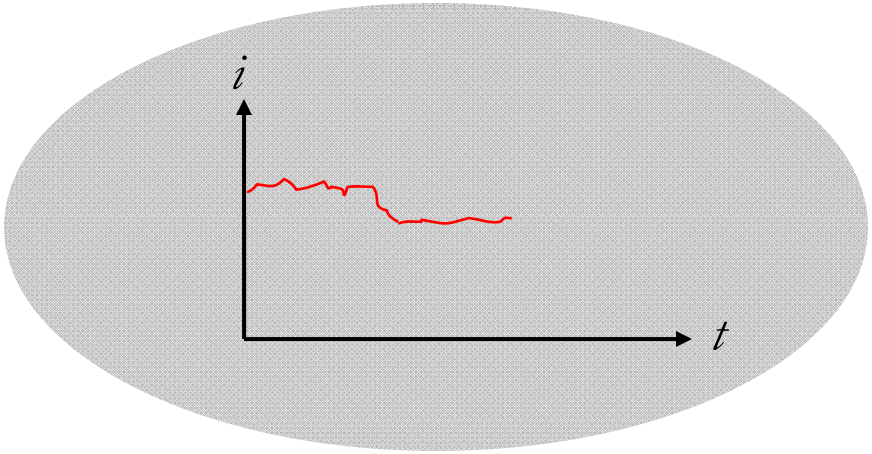

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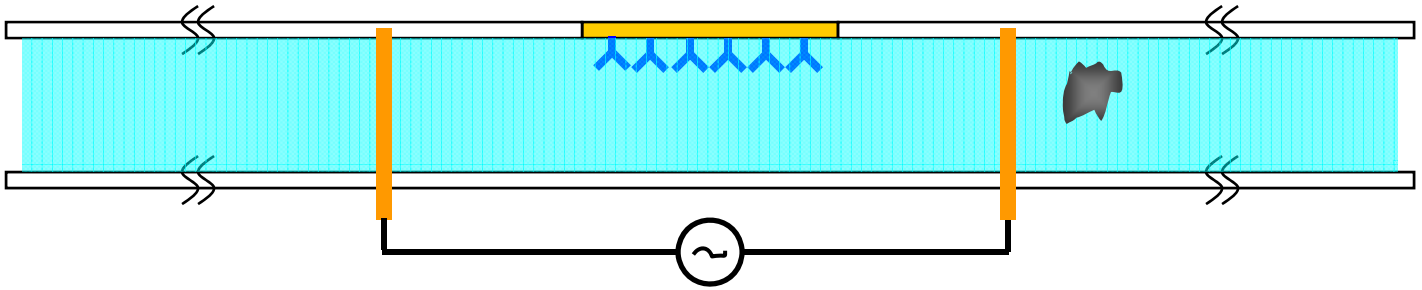
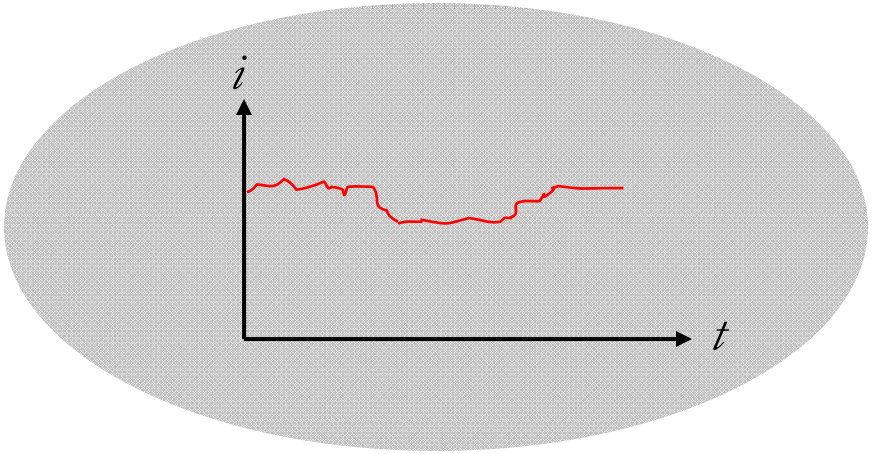




Performance Limits

- Time
  - time for solution analysis (limited by flow rate)
- Cost
  - Fabrication of devices using PDMS very inexpensive, high reproducibility
  - Label-free and direct measurement, low reagent costs
- Sensitivity
  - False Negatives: limited by rate of particles binding to electrodes
  - False Positives: limited by nonspecific binding

Monte Carlo Simulation Assumptions

- Diffusion 10^{-6} cm^2/s,
- characteristic diameter of 1.2 μm.
- Random displacement applied to each cell due to brownian motion
- Laminar flow causes cells to follow parabolic velocity profile

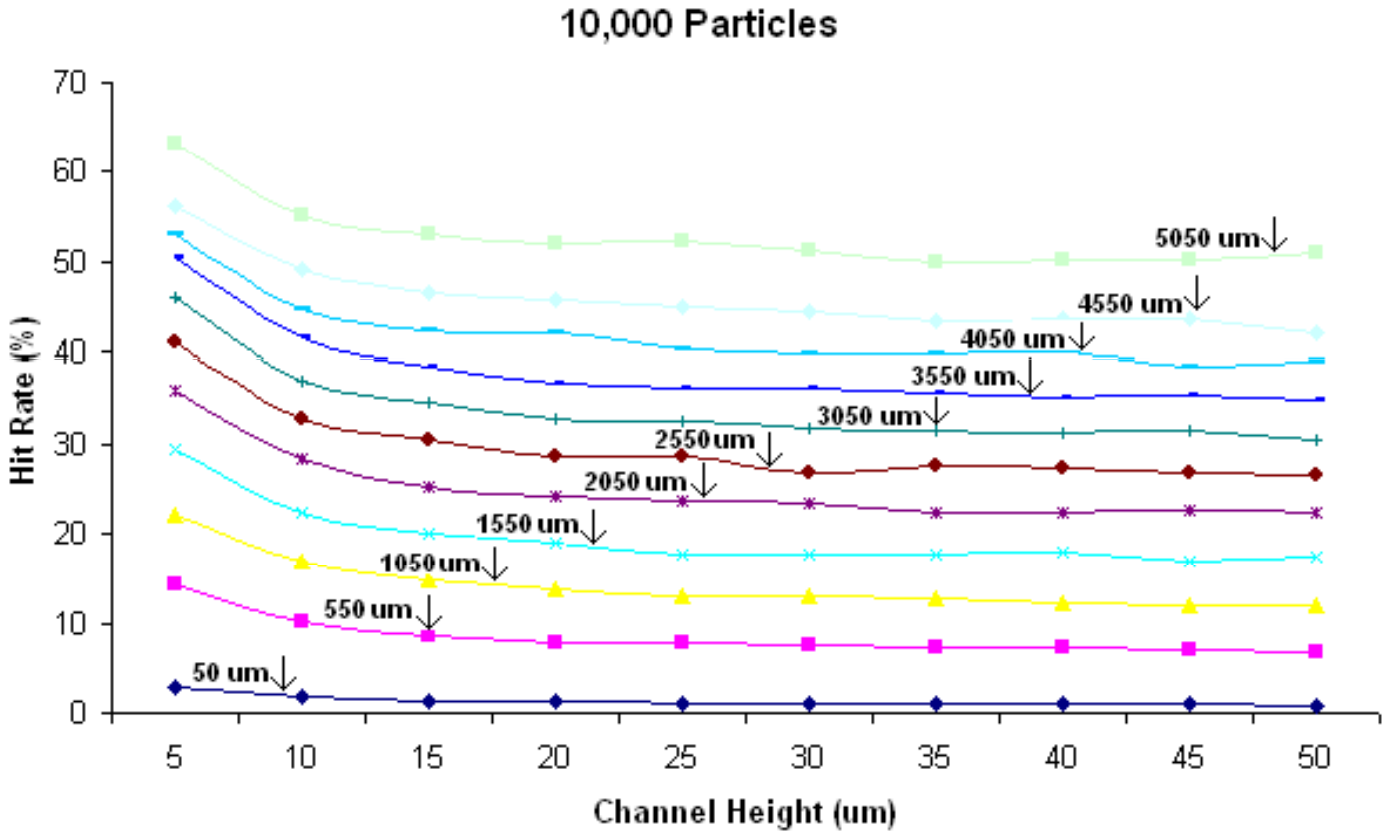
$$U_x(y) = \frac{6Q}{wh^3}[y(h-y)]$$

Q : volumetric flow rate w :width of the channel

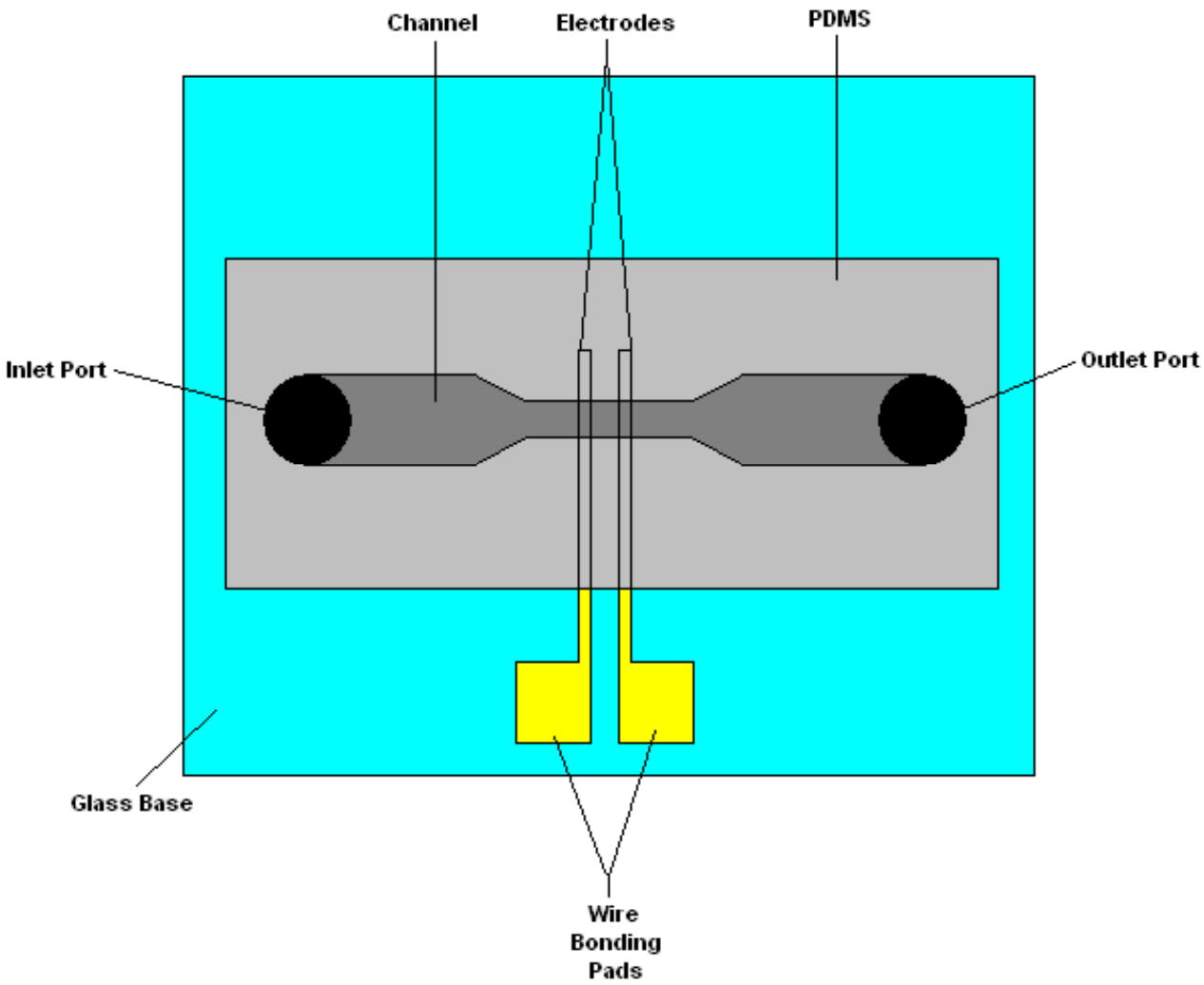
h : channel height y : vertical distance

- Cells exceeding walls of channel reflected back into the channel.

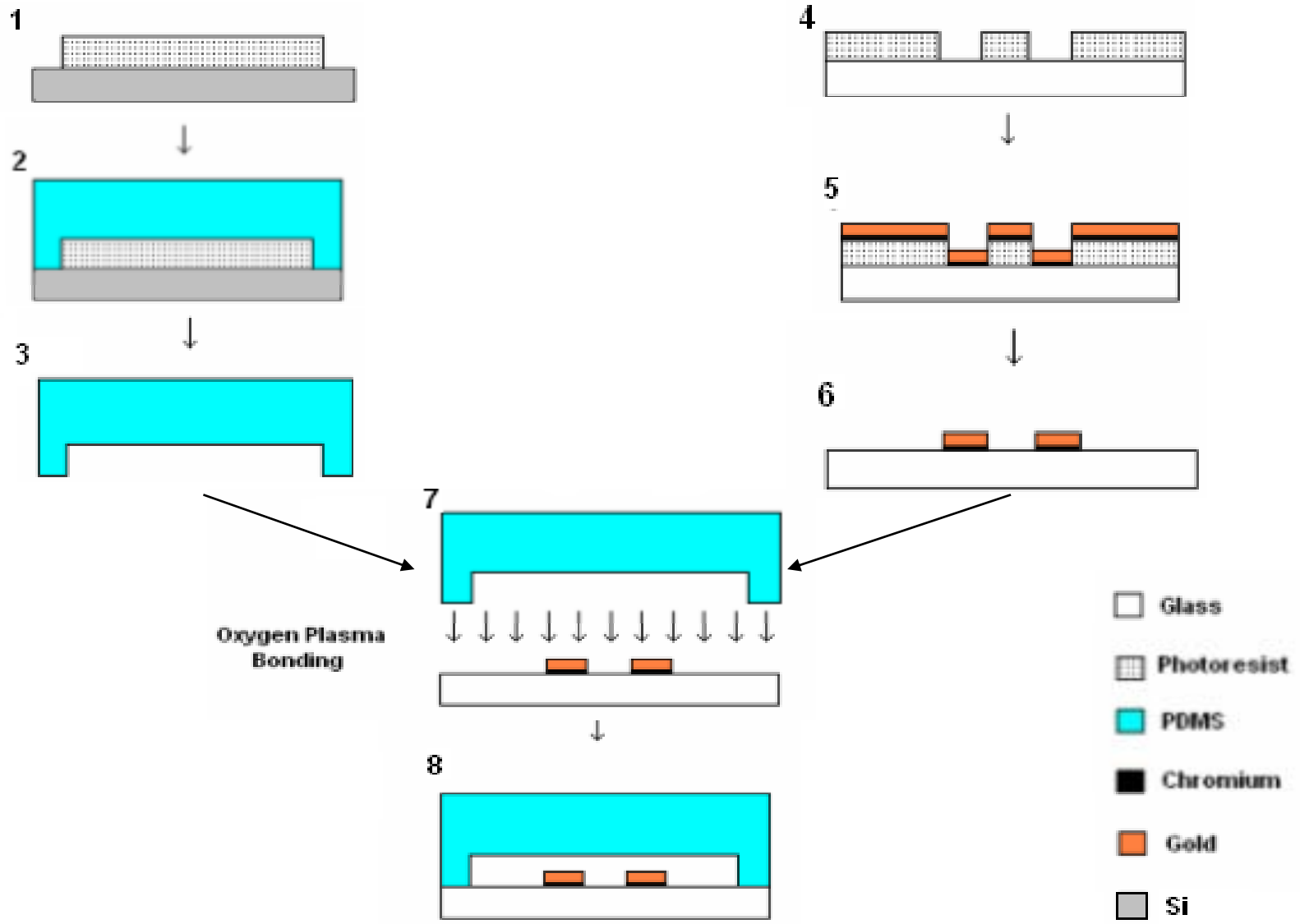
Hit Rate for Varying Sensor Active Area



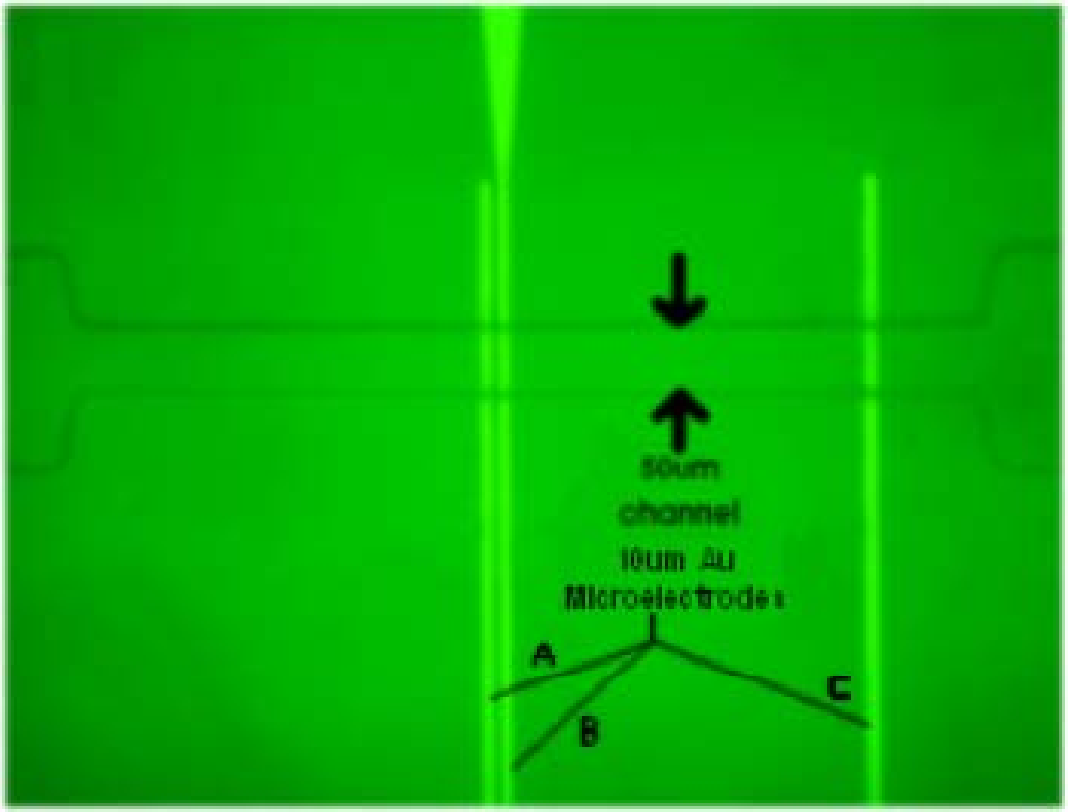
Biochip Schematic



Fabrication Process



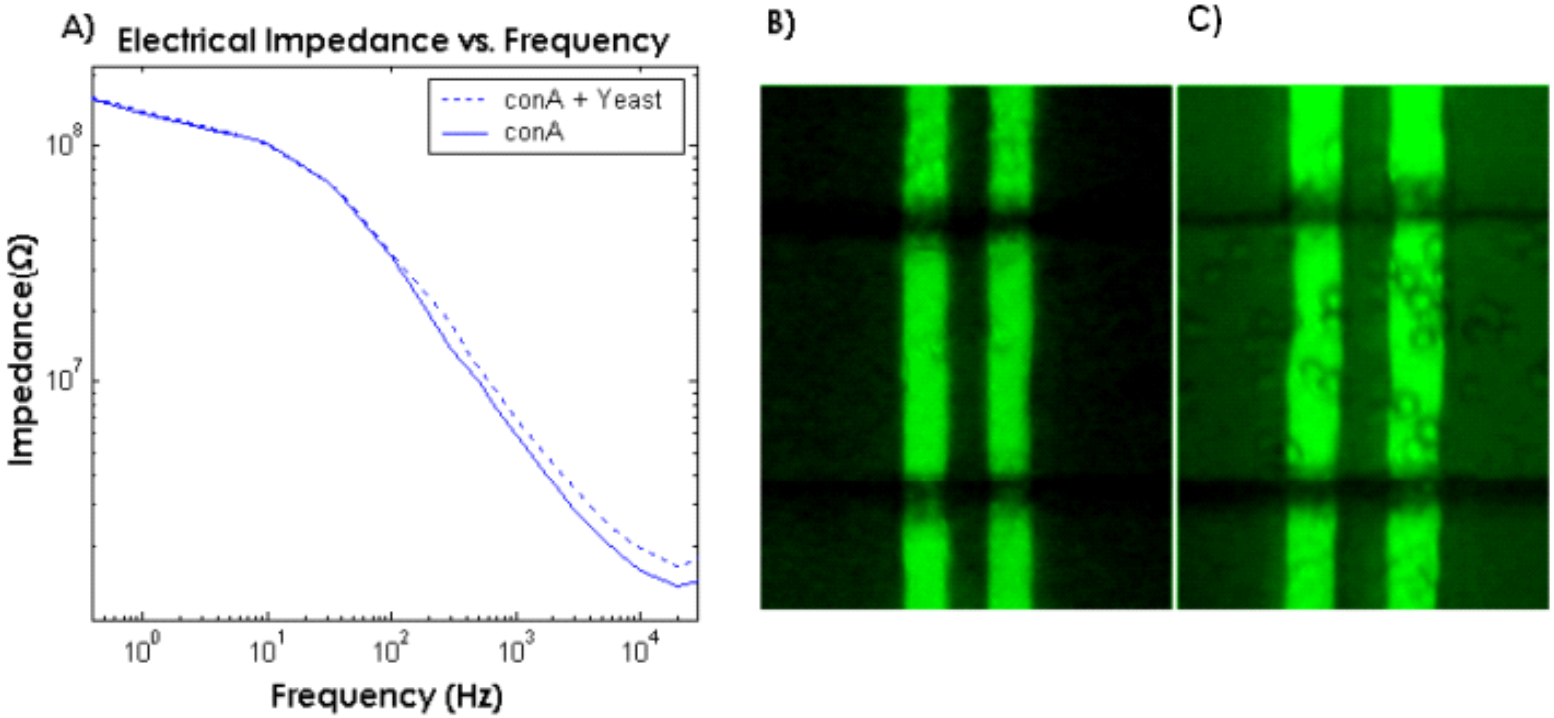
Fabricated Micro-channel



Detection Assay

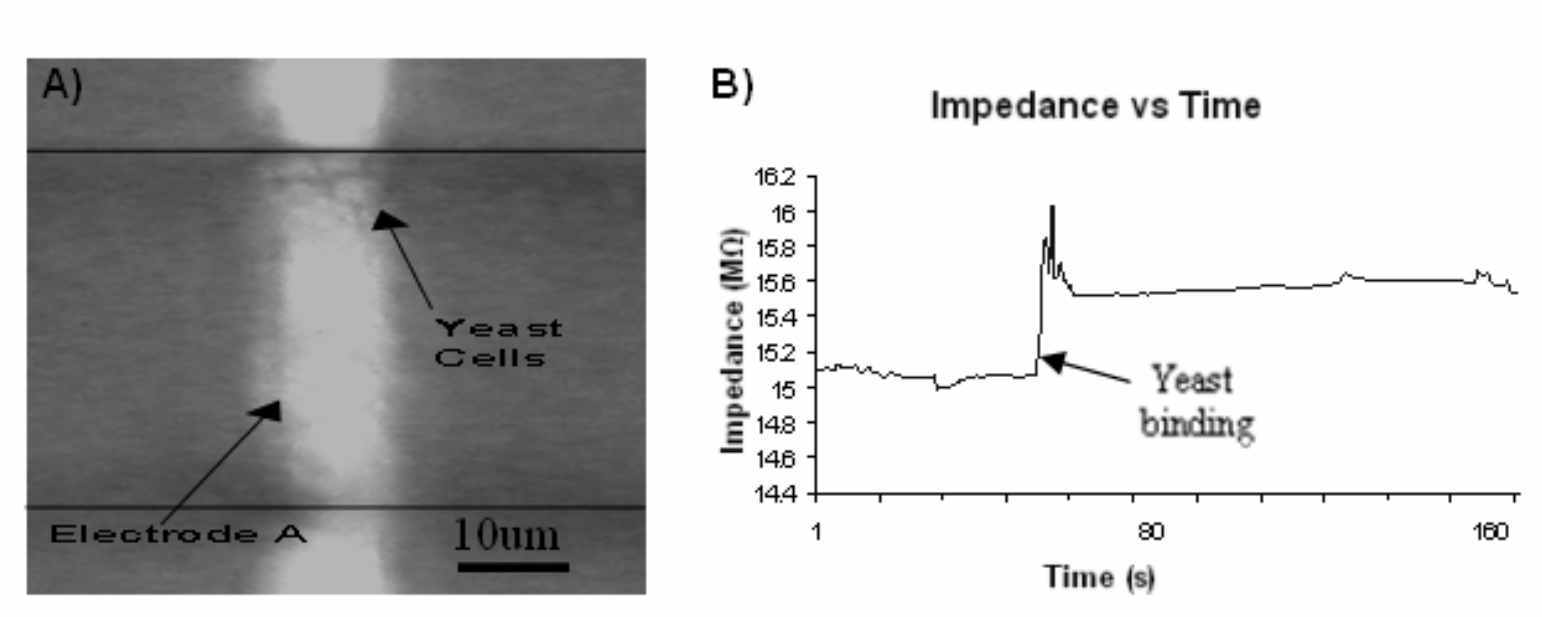
- Yeast Cells used as target cells
- Concanavalin A used in place of antibodies
- 10^7 cells/ml suspended in Hepes buffer with 200 mM KCL

Electrical Impedance Spectrum



- Solution resistance dominant regime begins above 20 kHz

Large Channel Experiment

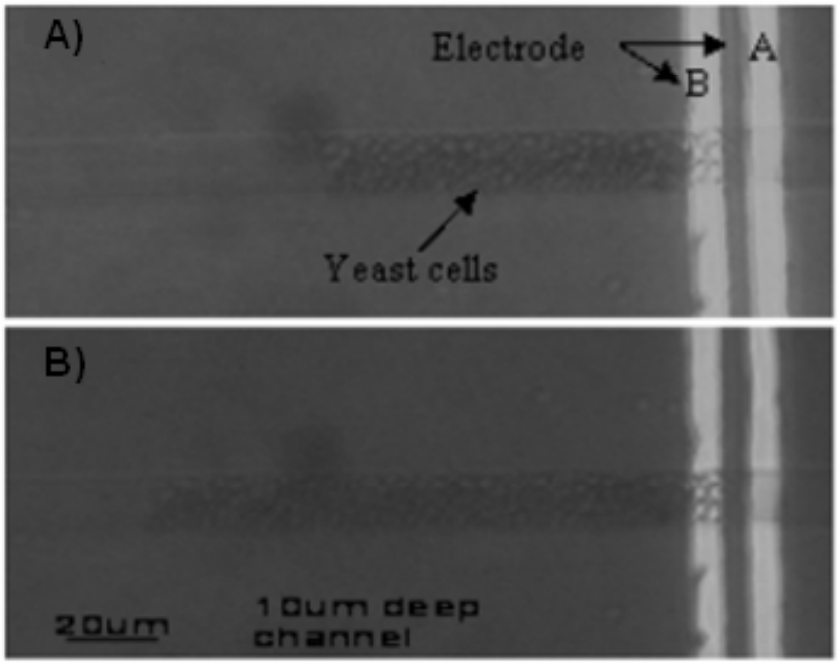


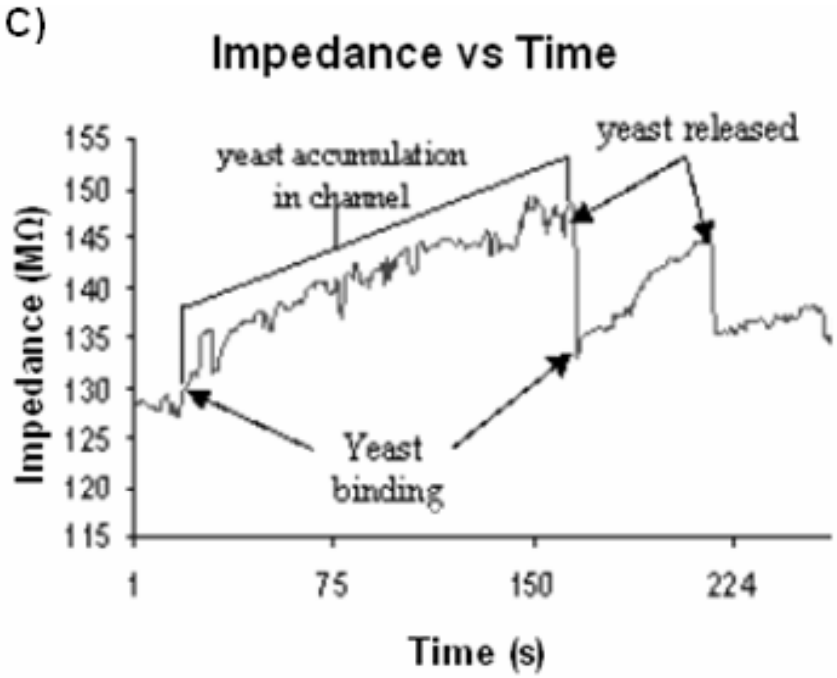
- Yeast binding occurs at t = 55 s

Binding Specificity

- Treated the surface of yeast with alpha-mannosidase and alpha-glucosidase for removing the sugars, mannose and glucose which have an affinity for ConA.
- Immobilized the channel with ConA and injected 50 µl of yeast solution with a flow rate of 100 nl/min.
- No binding of yeast occurred anywhere in the channel.

Small Channel Experiment





- Impedance increases steadily as cells accumulate in channel.
- Release of cells results in impedance drop @ t = 160 s. The same cycle is repeated until t = 220 s.
- No cells across electrodes after t = 220 s.

Conclusions

- Novel device for target cell detection has been modeled, fabricated, and tested.
- Proof of Concept achieved
- Can provide a low-cost solution for pathogenic bacterial cell determination.