

Low-cost Electrochemical Microfluidic Immunoarrays for Cancer Diagnostics



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Abstract

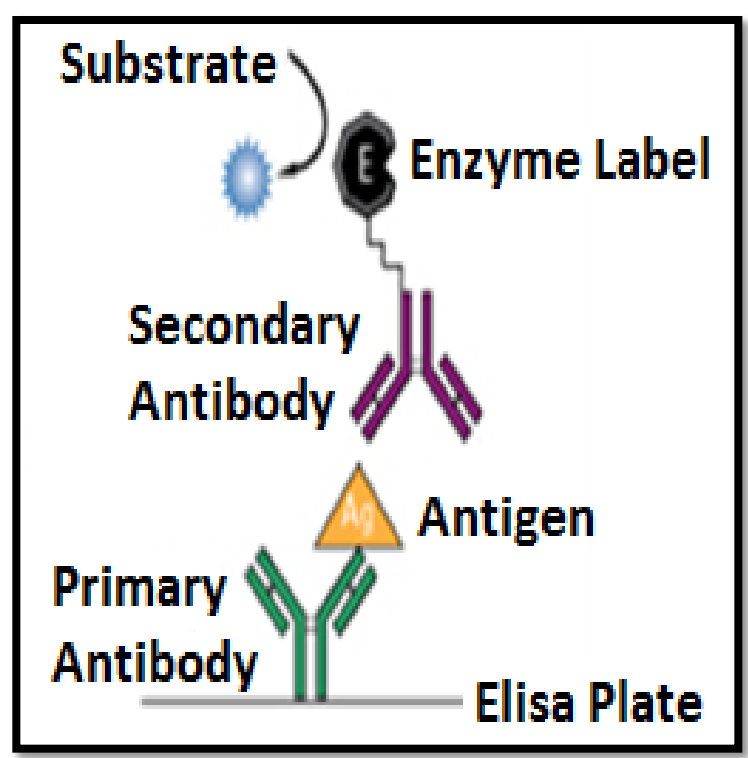
Rapid, accurate and sensitive detection of multiple biomarker proteins holds significant promise for early diagnosis of cancer and personalized therapy guidance. Here we describe a simple, low-cost, modular microfluidic system for on-line capture and detection of cancer protein biomarkers. The system features a small chamber for on-line protein capture from serum by magnetic beads labeled with many copies of analyte-specific antibodies and signal-transducing enzyme labels, positioned upstream of a detection chamber housing a nanostructured 8-electrode sensor array. Microfluidic chambers are made by templating polydimethylsiloxane (PDMS) channels on machined aluminum molds and mounting on hard flat poly(methylmethacrylate) (PMMA) plates equipped with inlet and outlet panels. The chambers are interfaced with a sample injector, syringe pump and switching valves to deliver sample and reagents. Gold immunoarrays fabricated by ink-jet printing (\$0.2) or commercial screen-printed carbon arrays (\$6) are fitted into the microfluidic detection chamber to achieve high sensitivity. Ultralow detection limit in the low fM range was achieved for multiplexed detection of four oral cancer biomarker proteins from as little as 5 μ L sample within 30 minutes. The incorporation of electrochemical immunoassays for protein biomarkers in a microfluidic platform thus provides a rapid, sensitive and effective tool for cancer diagnostics.

Cancer

- Cancer is the second leading cause of death in the US accounting for nearly 1 out of every 4 deaths.
- In 2014, there will be an estimated 1.67 million new cancer cases diagnosed and 585,000 cancer deaths in the US.

CURRENT CANCER DIAGNOSTICS

- Imaging technologies
 - CT scans
 - MRI
 - Mammograms
- Invasive techniques based on taking a biopsy
- ELISA



Cancer Biomarkers

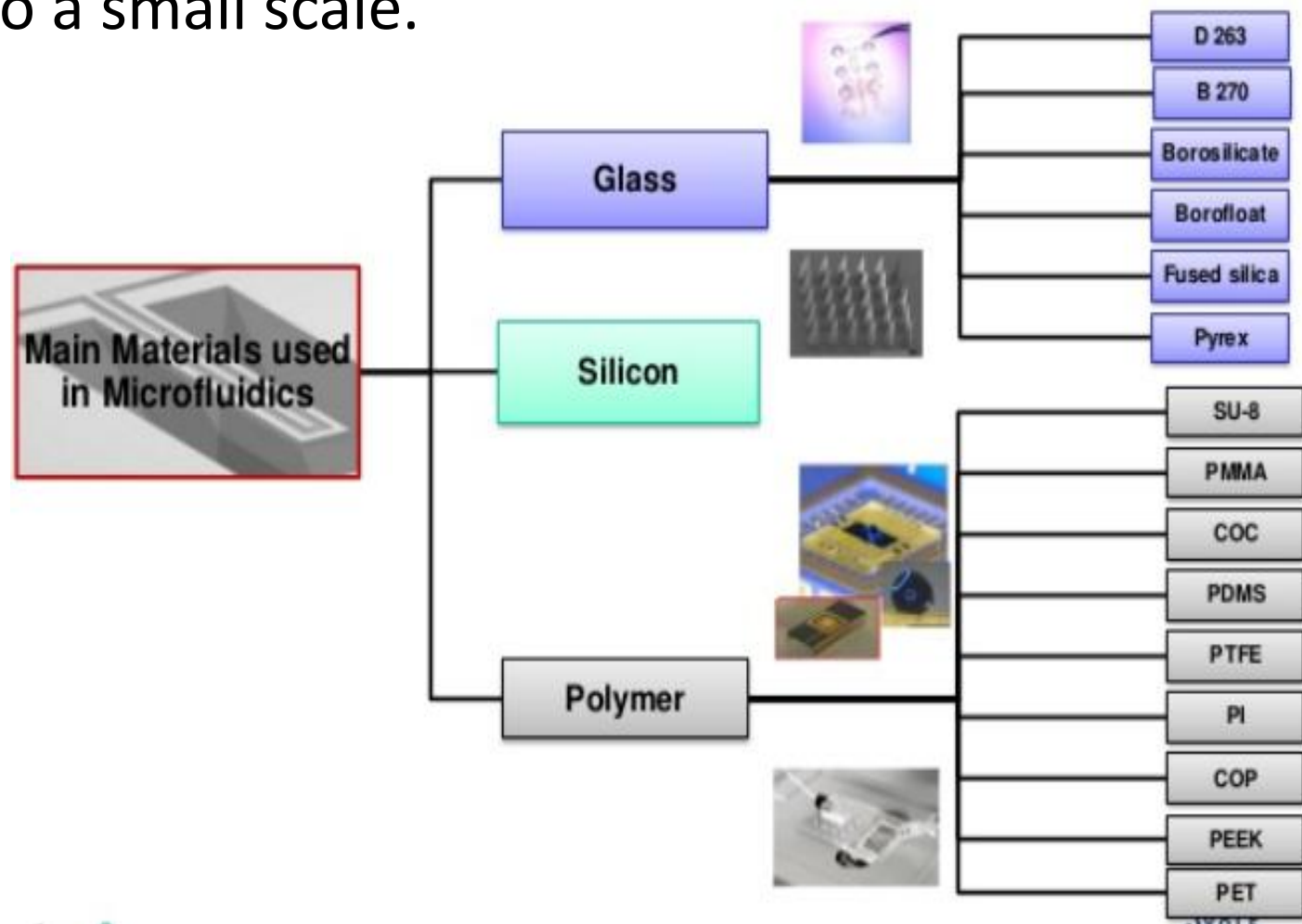
- Any measurable or observable factors in a patient that indicate normal or disease-related biological processes or responses to therapy.
- Genomic biomarkers - DNA
- Transcriptomic biomarkers - RNA
- Proteomic biomarkers - Proteins
- Metabolomic biomarkers – Metabolites
- Cancer biomarkers can enable early cancer detection, accurate pre-treatment staging, monitoring of the disease progression and response to cancer therapy.

Microfluidics

- Microfluidics deals with the behavior, precise control and manipulation of fluids that are geometrically constrained to a small scale.

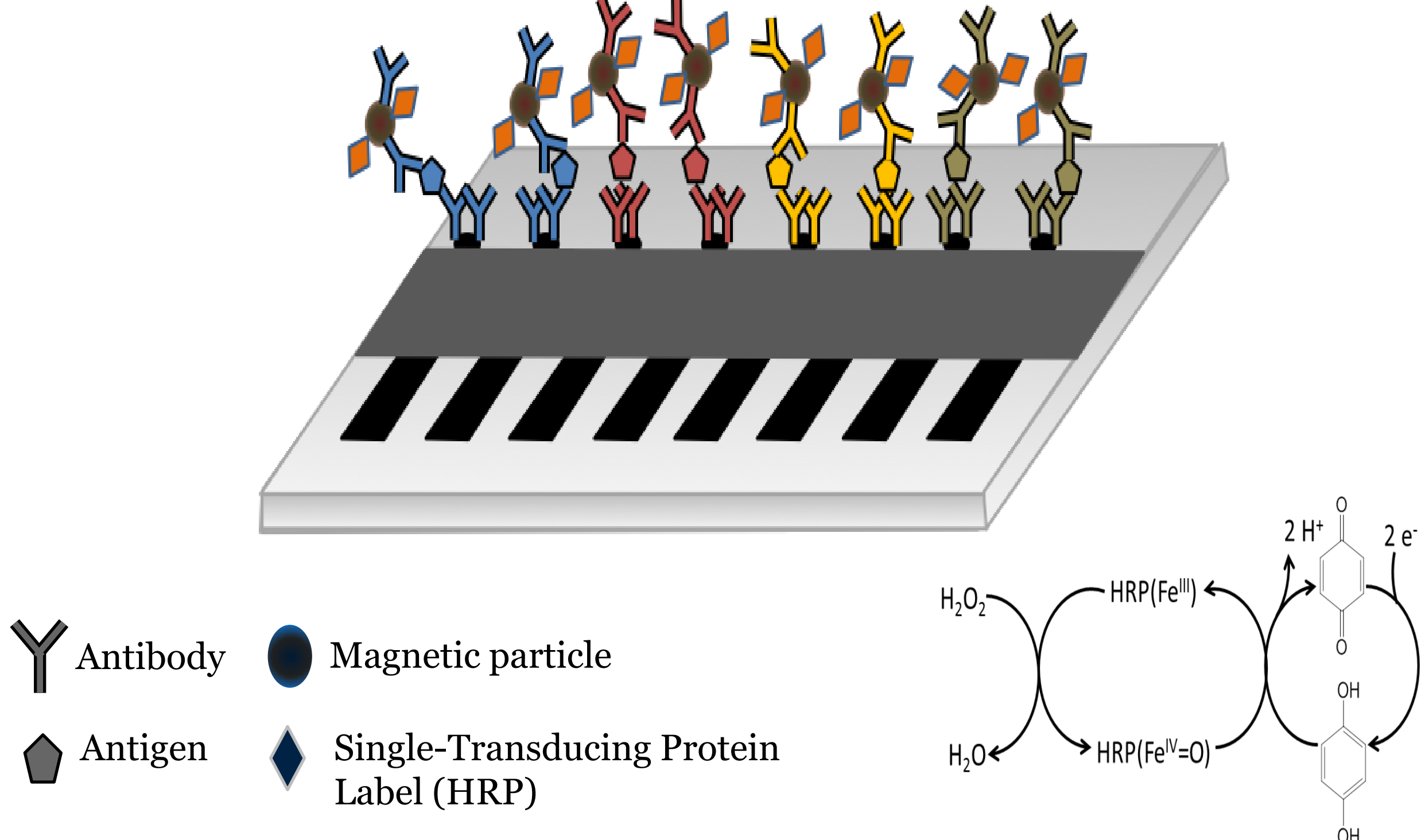
Why Microfluidics?

- Small volume, μ L/nL
- Fast response
- Portable and user-friendly
- Easy automation
- Less energy consumption
- Point-of-care diagnostics



Objective

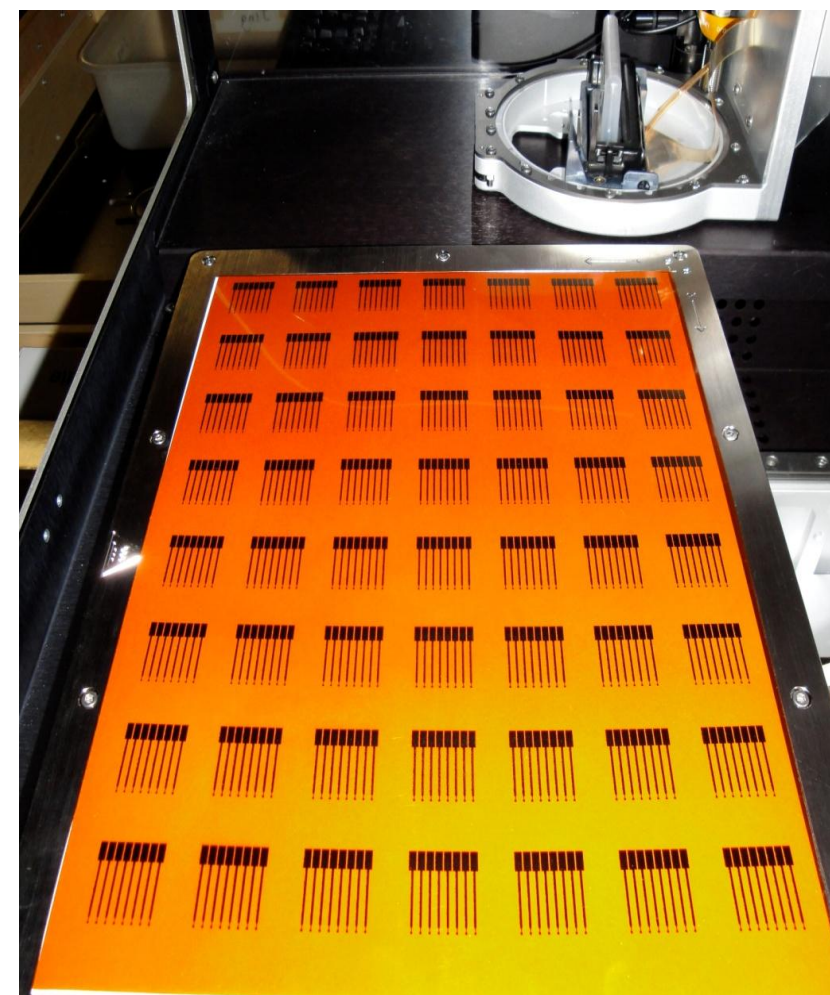
To develop an immunoassay capable of simultaneously measuring the levels of a panel of cancer biomarker proteins in a single sample.



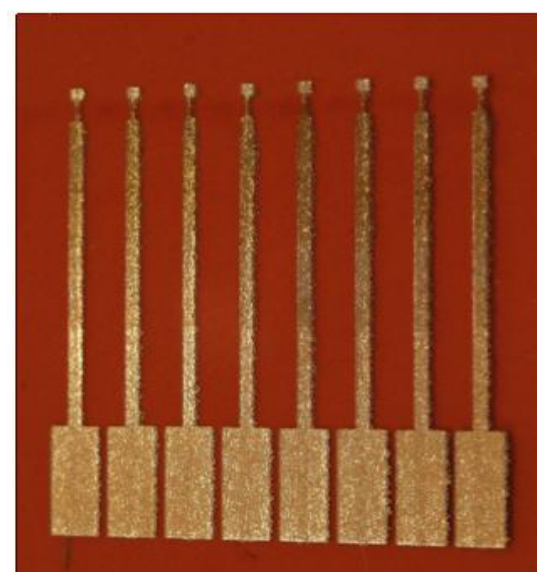
Array Fabrication



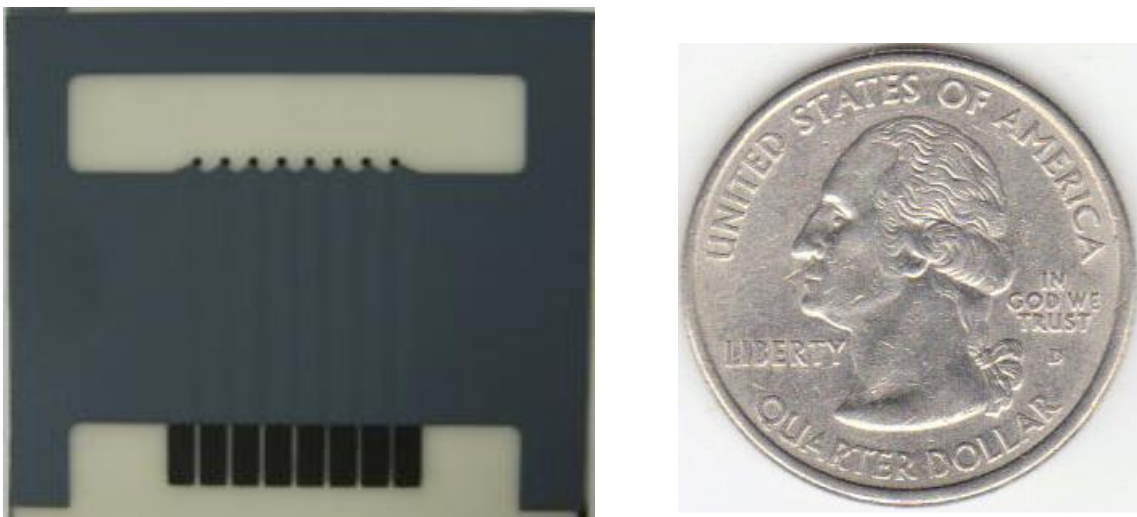
Dimatix Inkjet-Printer



Gold Arrays on the Surface of Kapton sheet

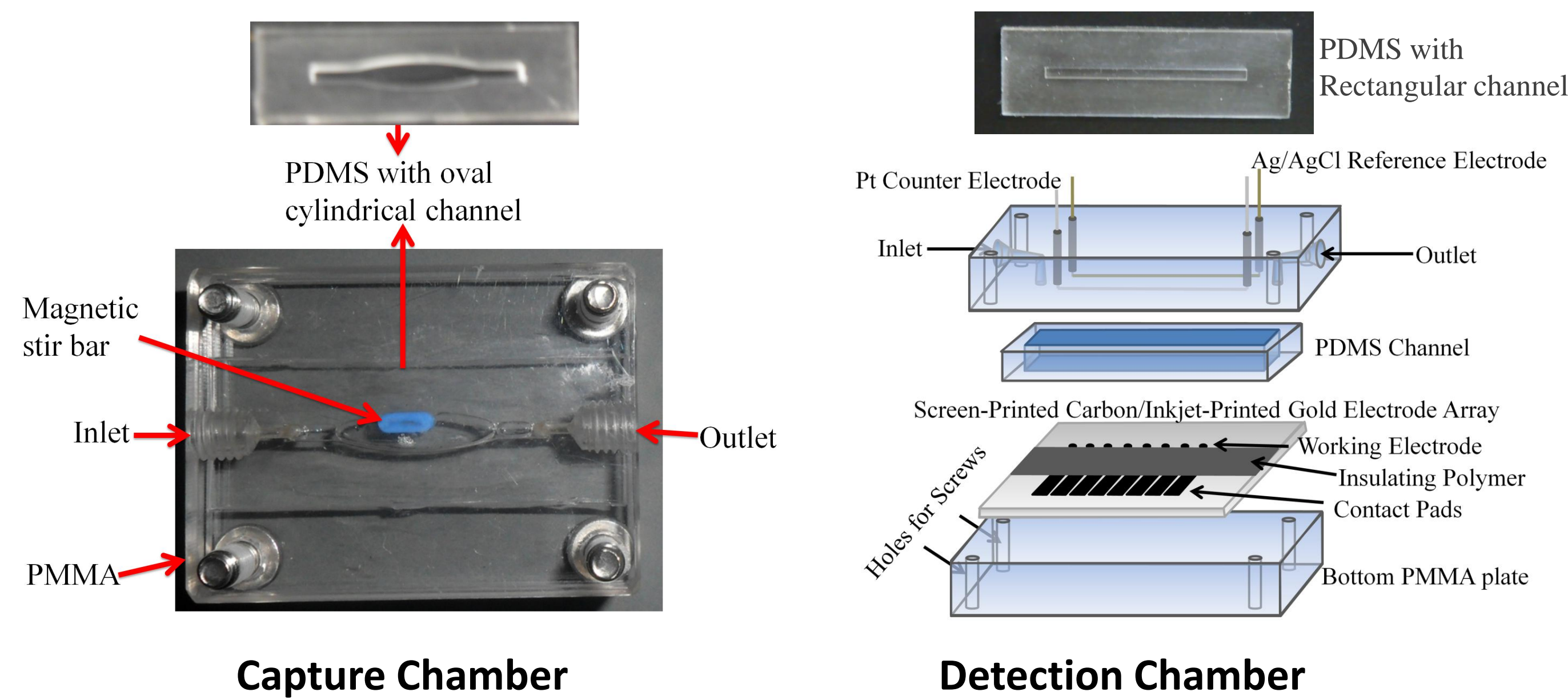


Inkjet-Printed Gold Arrays In the Laboratory

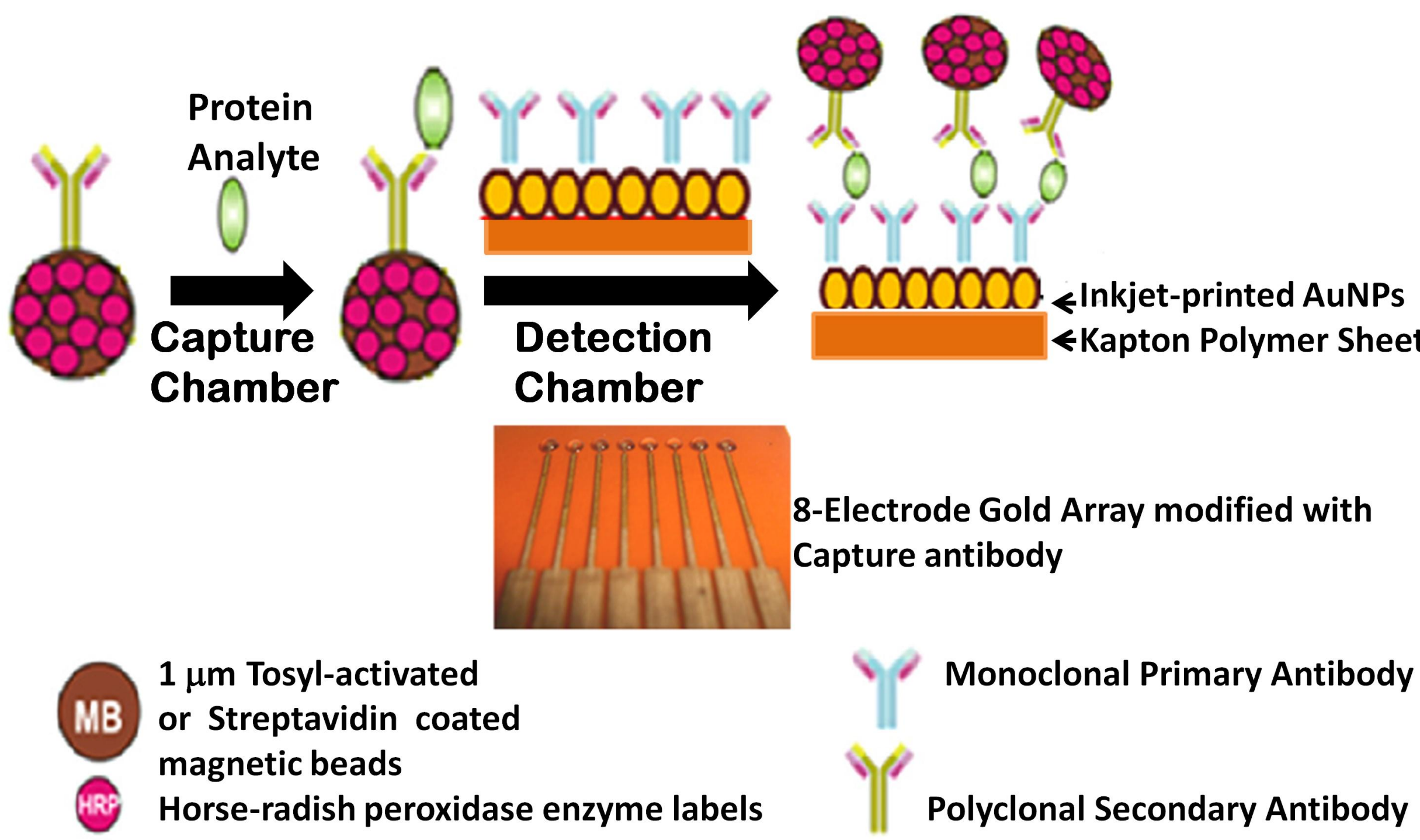


8-Electrode Screen Printed Carbon Array from Kanichi Research Limited, UK

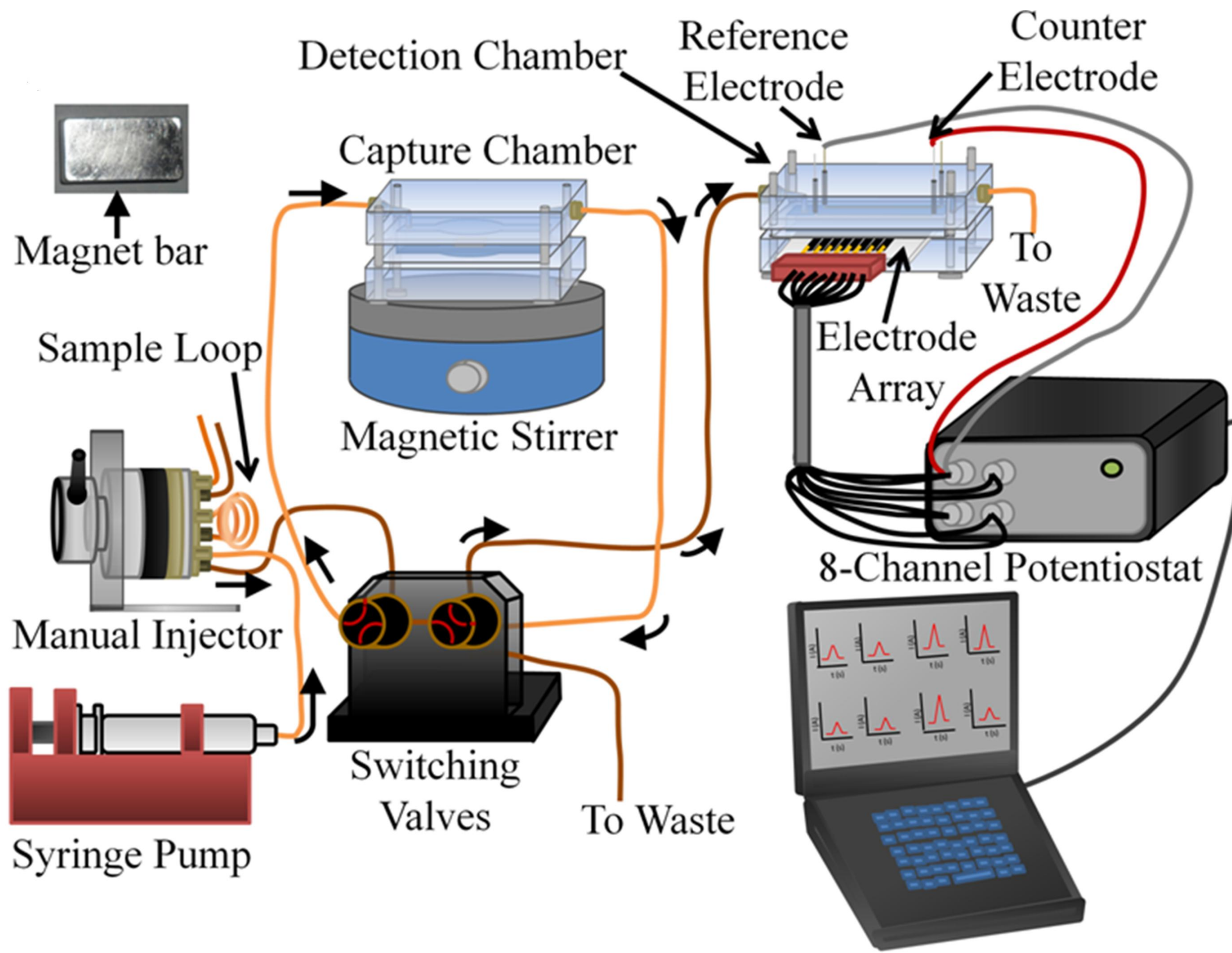
Microfluidic Devices



Immunoassay Protocol



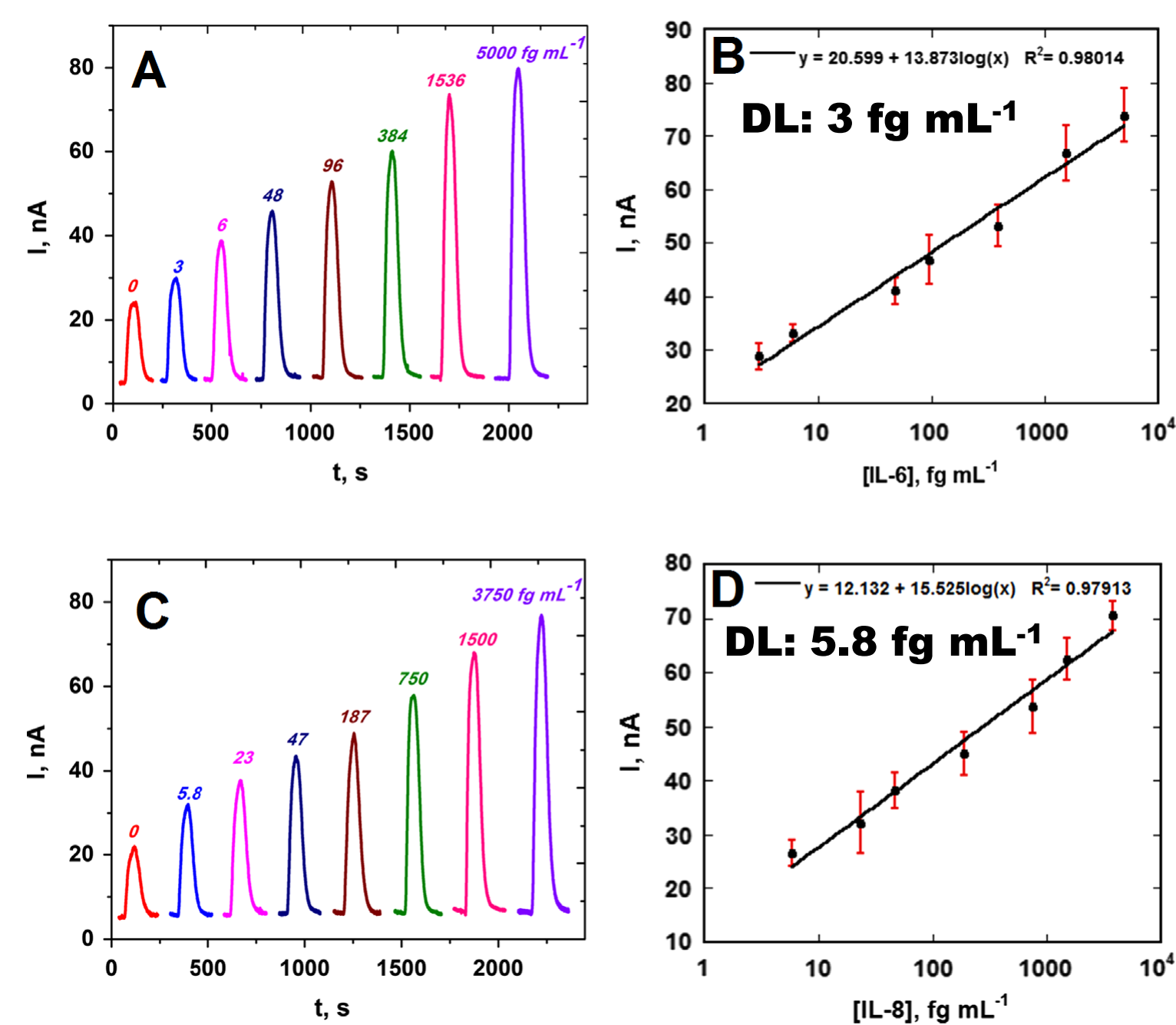
Experimental Set-up



Applications of Microfluidic Immunoarrays

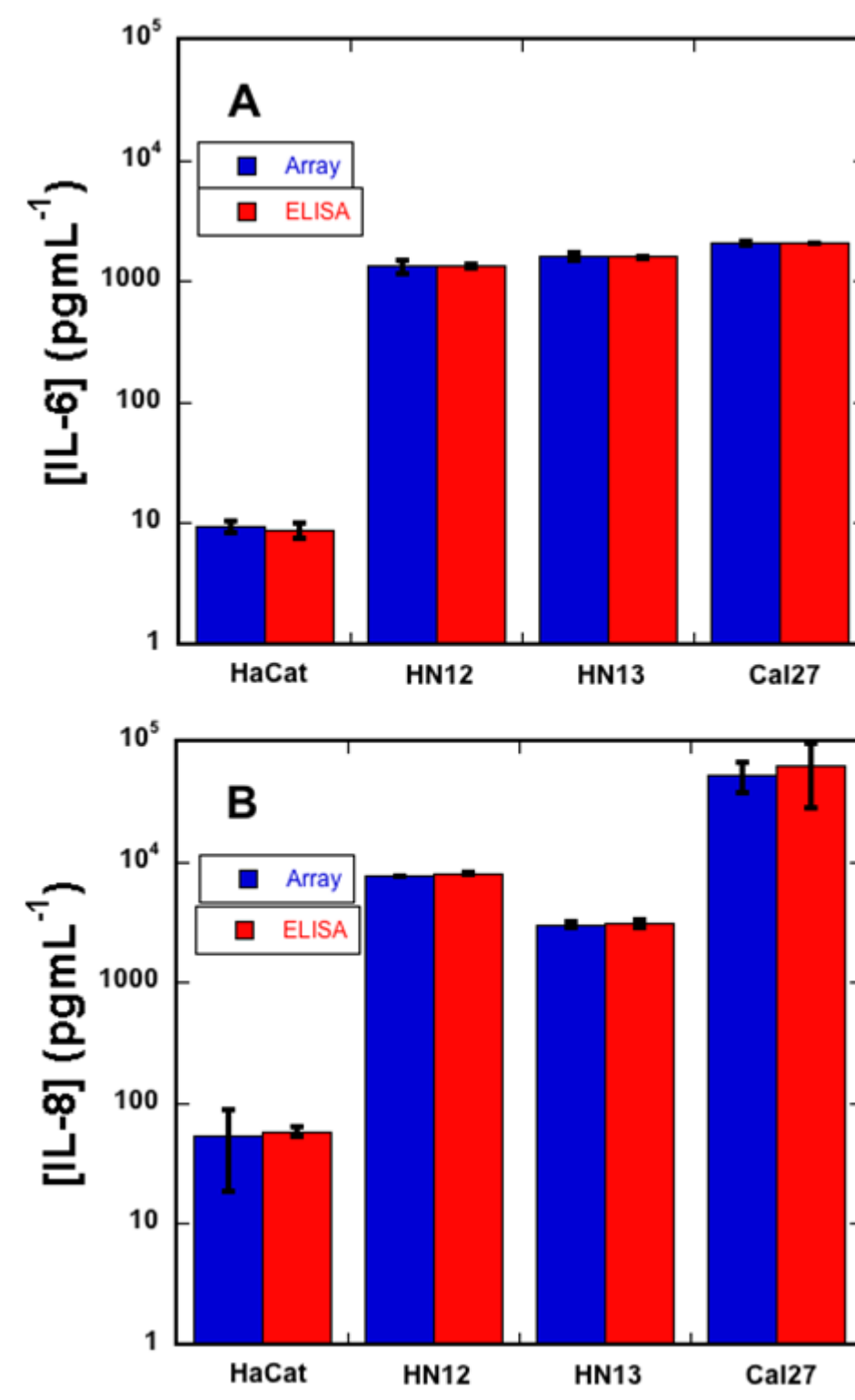
ORAL CANCER

- Biomarkers: IL-6, IL-8
- Healthy Individual: <6 pg/mL IL-6; <13 pg/mL IL-8
- Cancer Patient: 20-1000 pg/mL IL-6, IL-8



Amperometric responses for standard protein mixtures in diluted calf serum for (A) IL-6, (C) IL-8 and the corresponding calibration curve for (B) IL-6, (D) IL-8.

Assay Validation



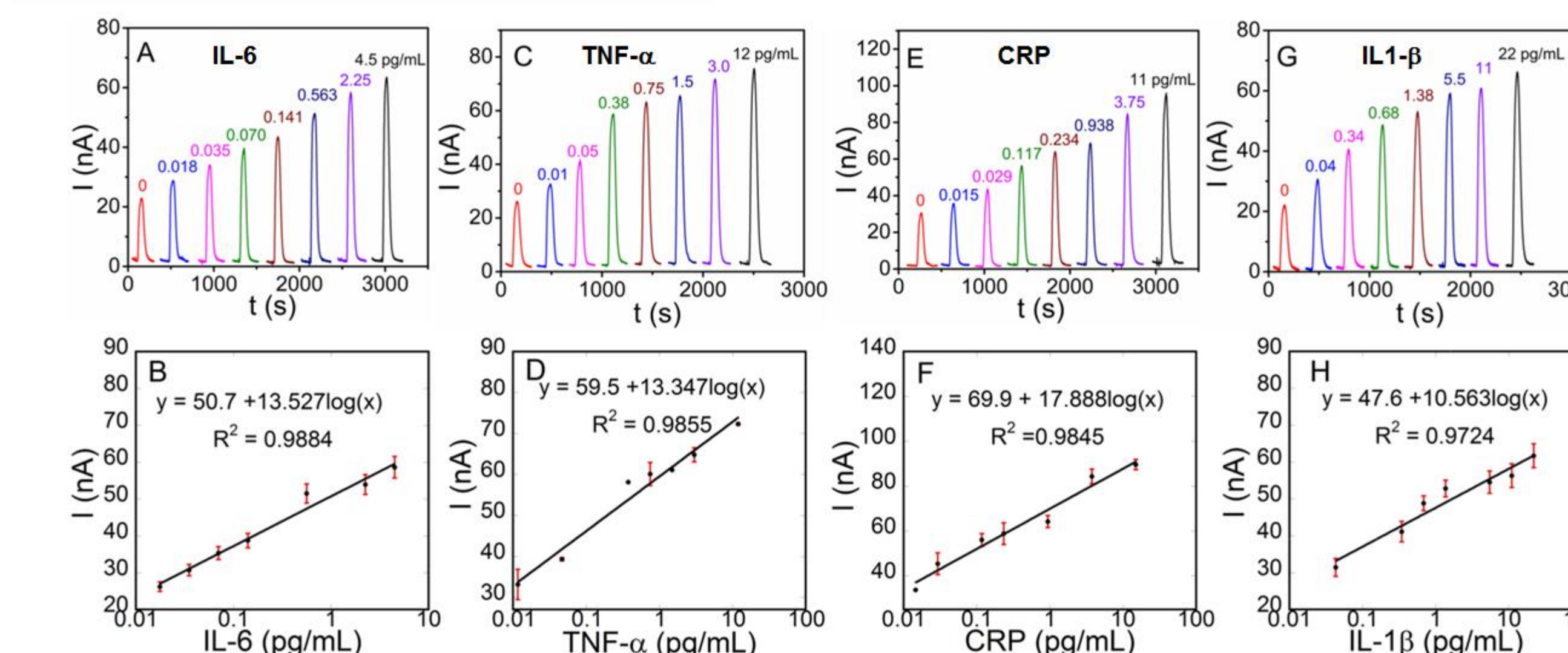
Immunoarray assay results for conditioned media for cells (HaCat, HN12, HN13 and Cal27) with standard ELISA assays for (A) IL-6 and (B) IL-8.

Oral Mucositis Risk Assessment

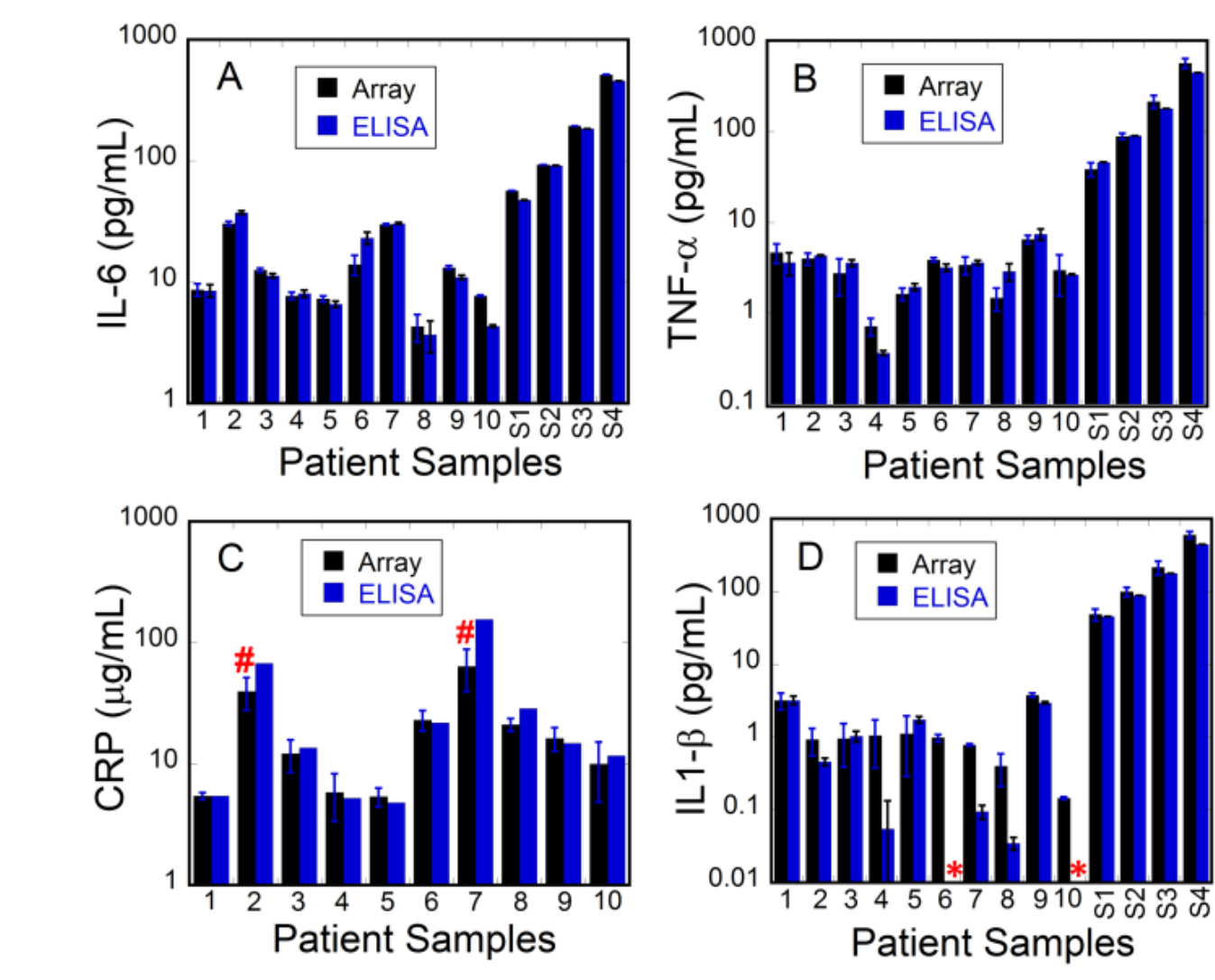
• Oral mucositis is an inflammatory lesion of oral mucosa caused by high dose chemo- and/or radiation therapy.

• These oral lesions adversely impact clinical management of cancer patients, and can result in hospitalization, infection, and delay of therapy.

• Proteins such as TNF- α , IL-6, IL-1 β and CRP were selected for their reported link to oral mucositis.



Amperometric responses for standard protein mixtures in 5-fold diluted calf serum and their corresponding calibration plots developed by injecting a mixture of 1 mM HQ and 0.1 mM H₂O₂ at -0.2 V vs. Ag/AgCl for IL-6, TNF- α , CRP and IL- β .



Detection limit
✓ 10-40 fg mL⁻¹
✓ 2.5-10 zeptomoles
✓ ~1500 molecules

□ Fast – 30 minutes (total assay time)
□ Multiplexed assay
□ Low-cost
□ Small sample volume

Immunoarray and ELISA assay results from serum samples of Head and Neck cancer patients serum for (A) IL-6, (B) TNF- α , (C) CRP, and (D) IL- β . S1-S4 correspond to patient samples spiked with 50, 100, 200, and 500 pg/mL respectively. (*) corresponds to values below the detection limits of ELISA and (#) corresponds to values above the dynamic range of the microfluidic immunoarray.

Conclusions/ Future Directions

- We have fabricated a low-cost modular microfluidic system from PDMS and standard accessories.
- Highly sensitive and promising immunosensors featuring on-line capture of analyte proteins on magnetic beads for multiplex detection of cancer biomarker proteins.
- Accuracy of the assay was confirmed by comparing the array results with that of the standard ELISA.
- Partial automation and short analysis time of the assay suggests promise for clinical diagnosis and therapeutic monitoring.
- This detection technique is easily adaptable to any panel of protein biomarkers.

References

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- (4) Krause, C.E.; Otieno, B.A.; Bishop, G.W.; Phadke, G.; Choquette, L.; Lalla, R.V.; Peterson, D.E.; Rusling, J.F. *Manuscript submitted to Anal. Chem.*

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