

## Electronic Supplementary Information (ESI)

# Fabrication of immunosensor microwell arrays from gold compact discs for detection of cancer biomarker proteins

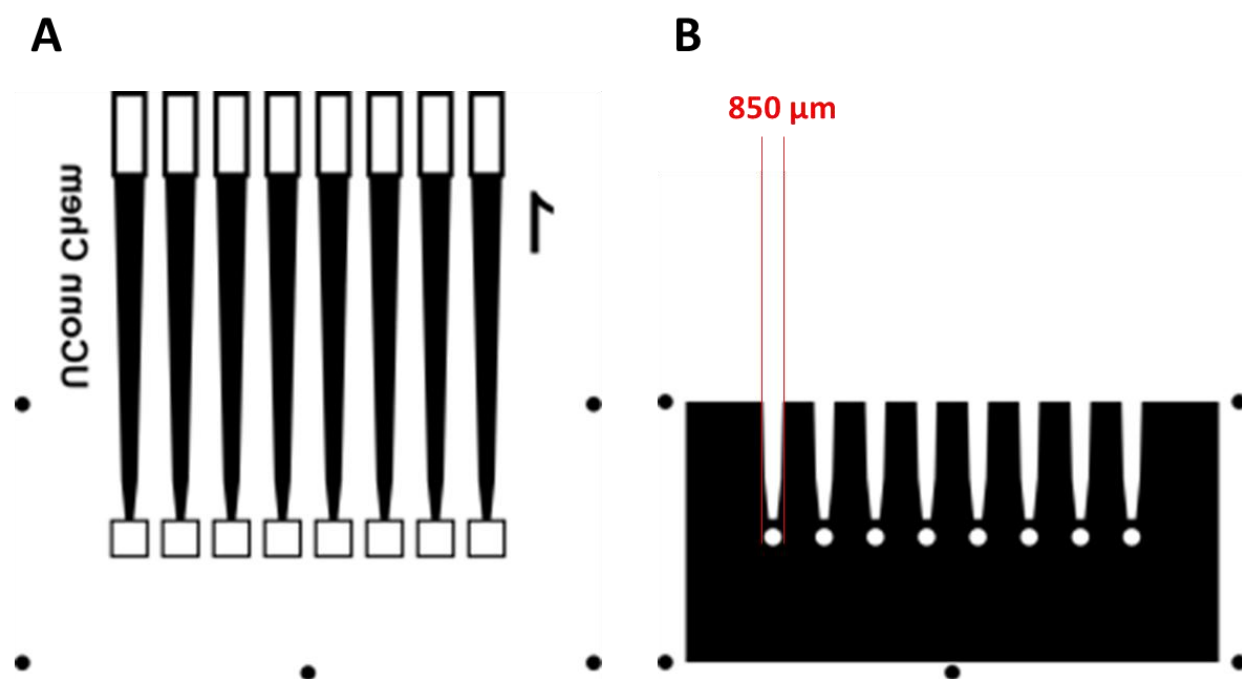
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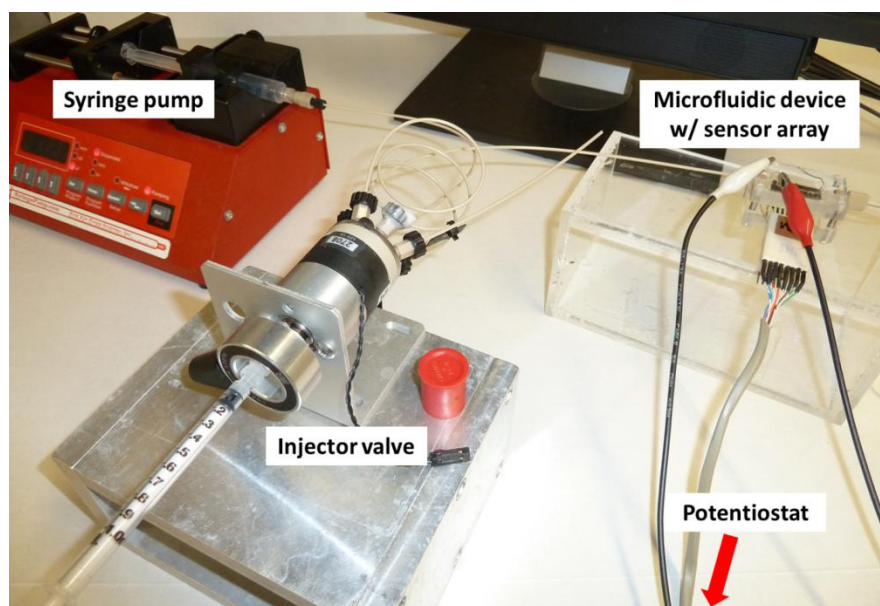
## Supplementary Figures



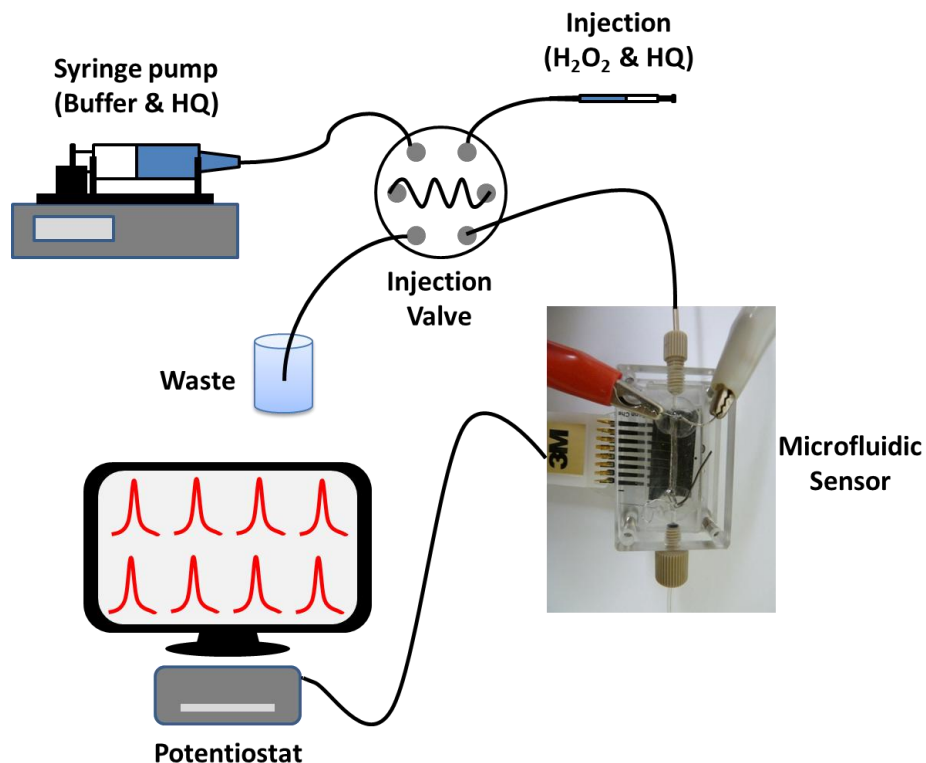
**Supplementary Figure S1.** (a) A computer generated pattern of the sensor array with 8 sensor spots using Canvas 11 graphical software. A mirror image is printed to get proper transference of the laserjet toner onto the gold CD-R surface. (b) A computer generated pattern of the second layer. Each circle represents on sensor spot with a diameter of 850  $\mu\text{m}$ . The second layer helps define the electrode area and also form hydrophobic wells around the sensors. The five dots around the patterns are used to align the arrays and the second layer manually.



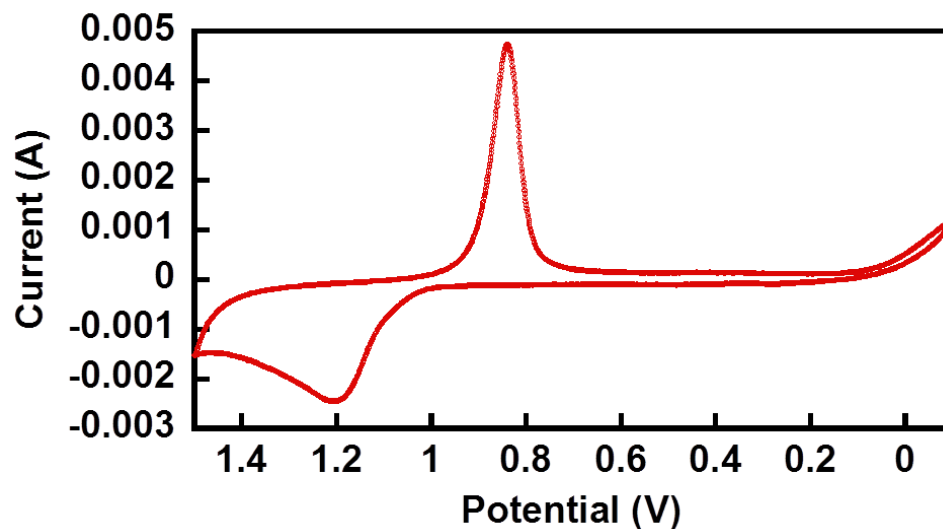
**Supplementary Figure S2.** Side view of the finished sensor array with 1  $\mu\text{L}$  droplets of PBS contained by the hydrophobic microwell on each sensor spot.



**Supplementary Figure S3.** Microfluidic setup<sup>1</sup>. A syringe pump was used to establish continuous flow of deoxygenated PBS into the microfluidic device at a flow rate of  $100 \mu\text{L min}^{-1}$ . Injections were performed by loading the  $100 \mu\text{L}$  sample loop of the injector valve with a mixture of deoxygenated  $100 \mu\text{M H}_2\text{O}_2$  and  $1 \text{ mM}$  hydroquinone and injecting into the microfluidic device at  $100 \mu\text{L min}^{-1}$ . A 3M test clip was used to connect the sensor array to an eight electrode CHI 1030 electrochemical workstation.



**Supplementary Figure S4.** Schematic of the experimental setup. A mixture of pH 7.4 PBS and 1 mM hydroquinone (HQ), purged with nitrogen for 30 min, was pumped through the channel using the syringe pump at  $100 \mu\text{L min}^{-1}$ . Then a deoxygenated mixture of  $100 \mu\text{M}$  hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and 1 mM hydroquinone (HQ) mediator in PBS is injected into the  $100 \mu\text{L}$  sample loop of the injection valve. A constant potential of  $-0.3 \text{ V}$  vs. Ag/AgCl was applied to the sensor array, and the change in current over the all eight sensors were monitored simultaneously. After reaching a steady baseline in the amperometric response (no change in current over a period of time), the injector was switched to injection mode. The flowing buffer and HQ from the syringe pump then pushed the peroxide and HQ over the sensor array, which generate amperometric responses that were proportional to the amount IL-6 antigen captured. Once finished, the sensor array was replaced with a fresh one and the above detection steps were repeated.



**Supplementary Figure S5.** A typical cyclic voltammetry (CV) of bare gold sensor array in 0.18 M sulfuric acid vs. Ag/AgCl reference electrode in microfluidic device between -0.1 V to +1.5 V at  $100 \text{ mV s}^{-1}$ . It shows similar peaks to those from bulk gold (data not shown) with the formation of gold oxide at +1.2 V and the reduction back to bulk gold at  $\sim +0.9 \text{ V}^2$ . This is also a cleaning method for the gold surface by removing the gold oxide layer. All immunosensor arrays undergo potential sweeping in sulfuric acid before immunoassay development.

## References

- 1 B. V. Chikkaveeraiah, V. Mani, V Patel, J. S. Gutkind, and J. F. Rusling, *Biosens. Bioelectron.*, **2011**, *26*, 4477–4483.
- 2 S. H. Cadle and S. Bruckenstein, *Anal. Chem.*, 1974, **46**, 16-20.