

Electronic Supplementary Information (ESI)

"Drill and fill" lithography: Fabrication of platinum electrodes and their use in label-free immunosensing

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1. Experimental

1.1 Fabrication of the Electrode

The initial fabrication process (Scheme 1) involved the patterning of Cu electrodes (200nm thick; 10 μm width) with connecting pads (2 mm \times 2 mm) on silicon wafers pre-coated with 200nm SiNx on both sides (University wafer, Boston) using standard photolithography and lift off processes. The photomask was designed using layout editor (L-Edit V15, Tanner Research Inc.) and printed onto 7" \times 7" high precision emulsion plate (Konica-Minolta, Japan) using flatbed photoplotter (MIVA 1624E T3, Miva Technologies, GmbH). Briefly, the silicon wafer coated with SiNx was further coated with hexamethyldisilazane in a vapour prime oven. A positive photoresist (AZ6612, Microchemicals) was then spin coated to obtain a 1.2 μm thick resist layer, followed by a pre- exposure bake step at 110°C for 50s. The positive photoresist coated wafer was exposed under UV light using a mask aligner (Quintel Ultraline 7000 series machine) and developed in the AZ726 MIF developer solution. Thereafter, a 200 nm Cu layer was deposited on these wafers using a sputter coater. Cu-electrodes and their connection pads were developed *via* lift-off process. In the second step of the fabrication, a thick SiNx layer (500 nm) was deposited on the Cu electrodes using Plasmalab 100 dual frequency PECVD system (Oxford instruments). Each wafer was then diced into individual devices for FIB milling. The FIB lithography (dual beam FEI Helios 600 Nanolab FIB system with software xT Microscope control) was employed for direct milling of SiNx passivation layer to drill pores of desired diameter. Gallium assisted Pt deposition was carried out to fill these pores in succession with optimized Z size and ion beam currents to establish desired Pt-Cu contact. The ion beam current measured was recorded as a function of milling time (as a function of Z size as well) with a sharp increase in current indicating the time required to expose the underlying Cu electrodes. The ion beam currents of 0.92 nA, 0.46 nA, 9.7pA and 1.5 pA with the Z size of 0.65 μm were used for

fabricating 10, 5, 1 μm and 500 nm electrodes, respectively. The fabricated Pt electrodes were characterized using SEM at an operating voltage of 15 KV.

1.2 Electrochemical Experiments

Electrochemical experiments were conducted at room temperature ($22 \pm 1^\circ\text{C}$) by using an electrochemical analyzer CHI 650D (CH Instruments, Austin, TX), where fabricated Pt electrodes were used as a working electrode, a Pt wire counter electrode and a Ag wire as quasi reference electrode. CVs were conducted in 1.0 M KCl containing 10 mM $[\text{Fe}(\text{CN})_6]^{4-}$. The EIS spectra were recorded in 1.0 M KCl electrolyte solution containing a 10 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ using an alternating current voltage of 5 mV, under the frequency range of 0.1 Hz to 100KHz.

1.3 Electrochemical Immunoassay

Pt electrodes were first cleaned with acetone/isopropyl alcohol and dried under a stream of nitrogen. Surface functionalization of the electrodes was done with a mixture of 1 mM 6 mercaptohexanoic acid and 6-Mercapto-1-hexanol (Sigma Aldrich) (1:20) for 24 hr at room temperature. The carboxylic groups of the monolayer were activated using 400 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and 100 mM N-Hydroxysuccinimide (Sigma Aldrich) in 100 mM MES buffer (pH 5.2) with gentle shaking for 2 hr. Subsequently, electrodes were incubated in 100 $\mu\text{g}/\text{ml}$ mouse monoclonal anti- HER-2 (Invitrogen) for 2 hr. The electrode surface was blocked with 3% BSA/10 mM PBS (Phosphate buffered saline) for 1 hr to avoid non-specific adsorption, followed by 2 hr incubation of 1 ng/mL HER-2 (R&D systems) spiked in 10 mM PBS. Target specific immunocapture was assessed by incubating the non-specific antigen, human CA-125 (1 ng/mL; R&D systems), spiked in 10mM PBS for 2 h. To assess false positive response, the sensor was incubated in a serum like buffer, fetal

bovine serum (without any specific target antigen), for 2 h. The formation of SAM and the attachment of the antibody/antigen complex were followed by CV and Faradaic EIS measurements.

1.4 xT Microscope Control Software

Figure S1 shows a snapshot of the xT Microscope control software with the ion beam Panel on the top right; SEM Panel on the top left and CCD image of the sample at bottom right. The bottom left Panel can be used for either ion beam or SEM. The ion beam Panel shows two circles (yellow and green) merged together on the SiN_x substrate. The yellow circle was used for the ion beam milling and green circle used for subsequent filling the pores with Ga-assisted Pt deposition. After fabrication, the electrodes were imaged in the SEM Panel at 52° and 0°.

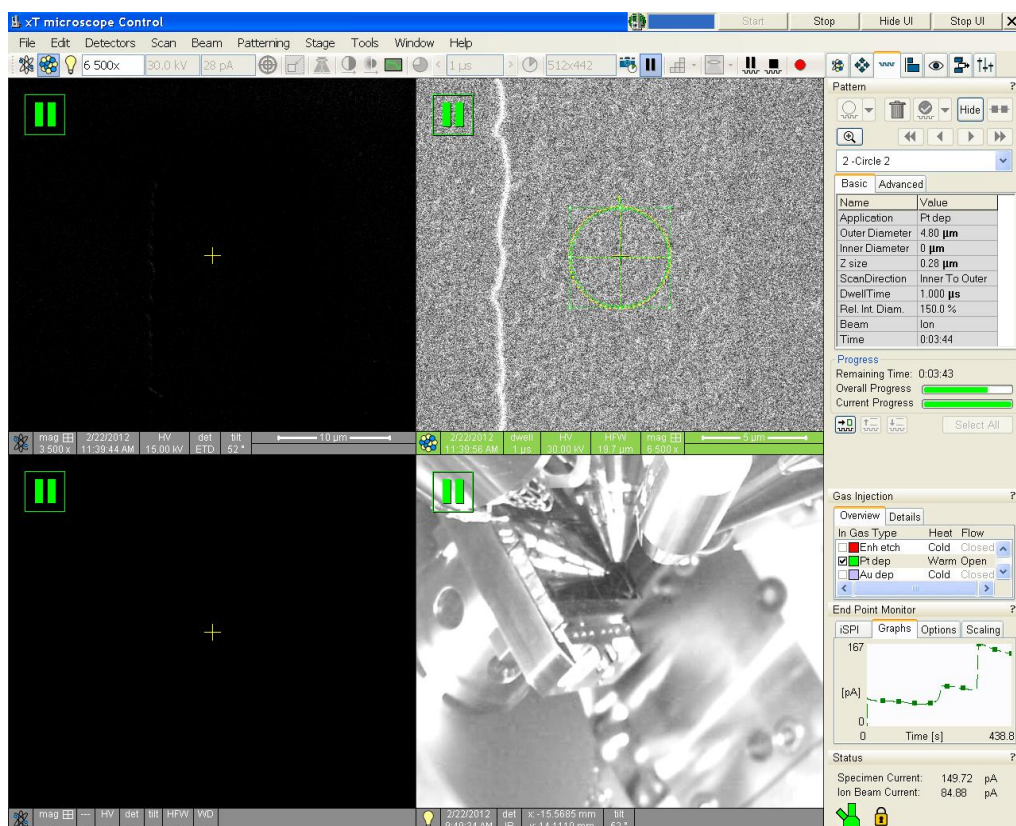


Fig. S1 A snap shot of xT microscope control software.

1.5 Energy Dispersive X-ray Spectroscopic Study

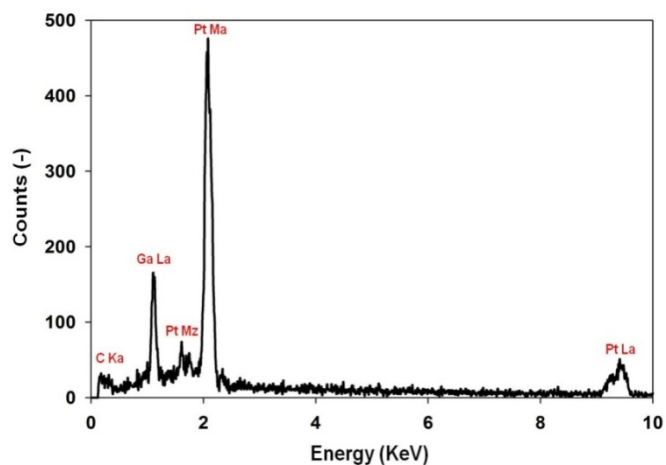


Fig. S2 EDS spectrum for the deposited Pt. The spectrum shows the presence of 72.44% platinum, 17.29% gallium, 8.74% carbon and 1.53% silicon.

1.6 Additional Data

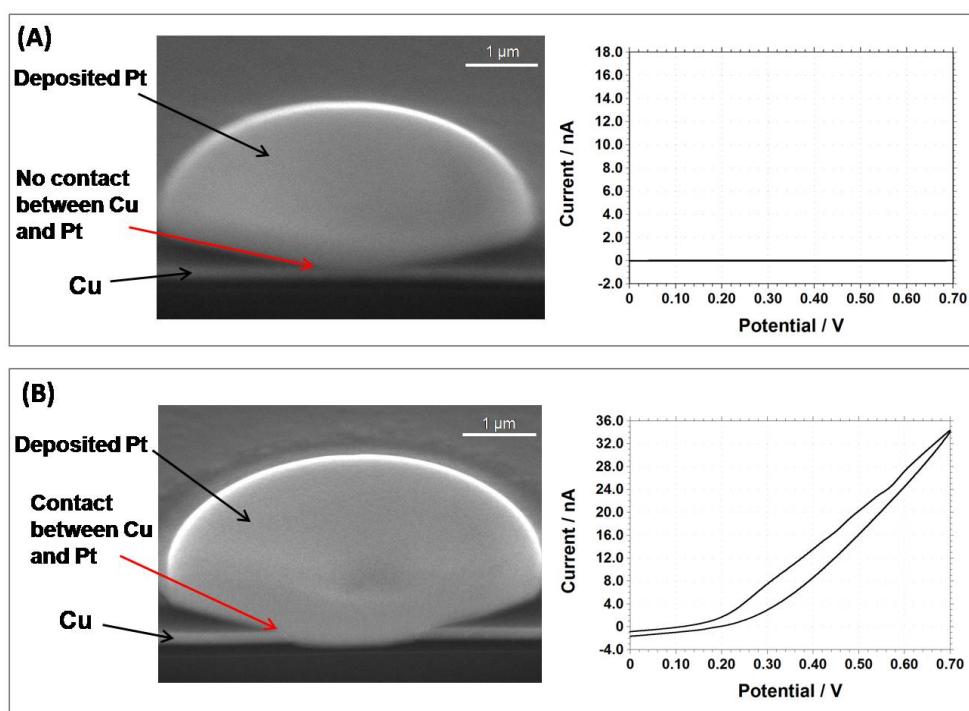
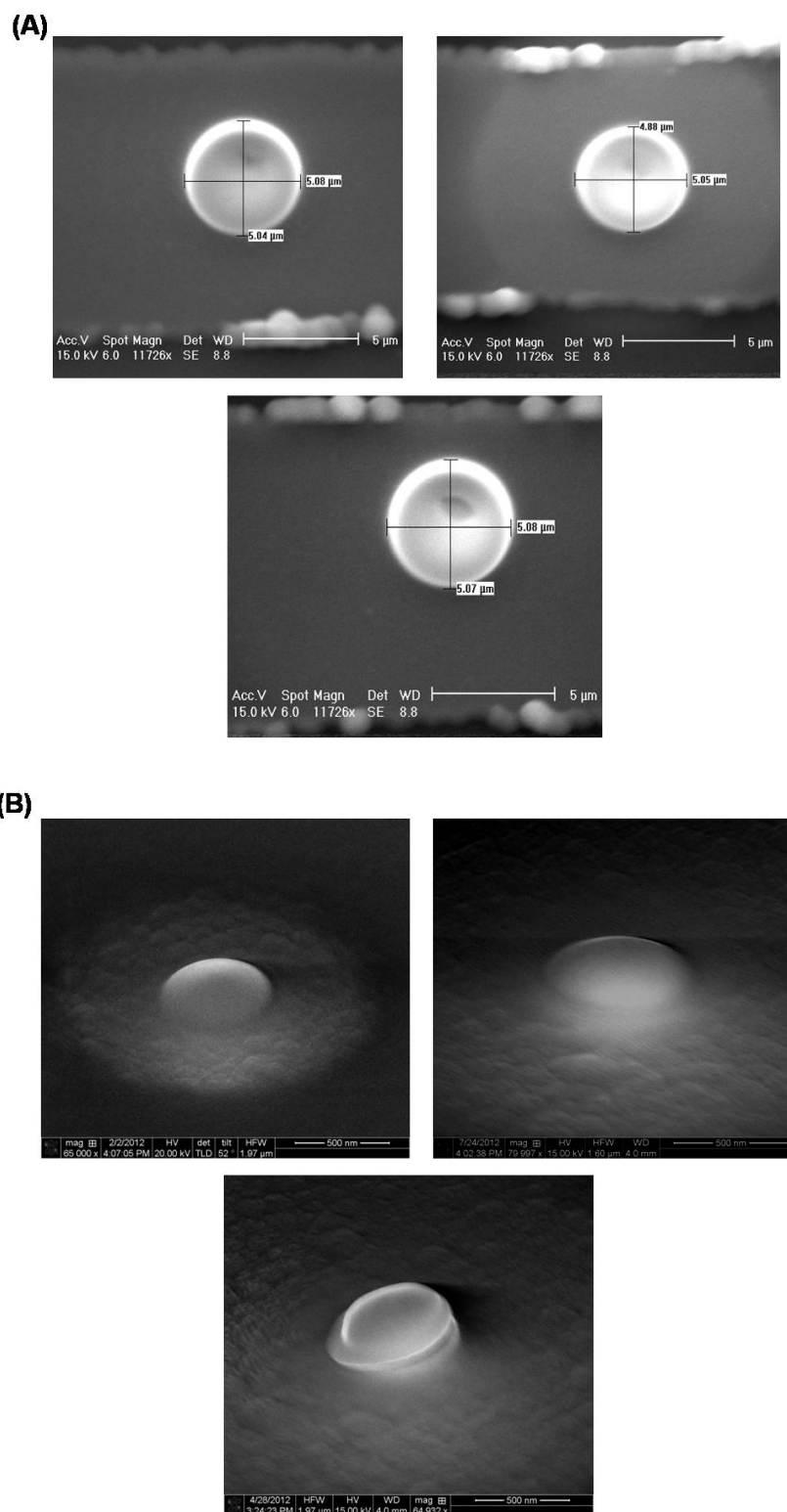


Fig. S3 Cross sectional scanning electron microscopy images of Pt electrodes ($d = 5 \mu\text{m}$) measured at 52° and corresponding cyclic voltammograms for the oxidation of $10 \text{ mM } [\text{Fe}(\text{CN})_6]^{4-}$ at a scan rate of 25 mV/s (in 1 M KCl electrolyte). The red arrows pointed no connection (A) and a successful connection (B) between the underneath Cu and deposited Pt when Z values were $0.55 \mu\text{m}$ and $0.65 \mu\text{m}$, respectively.



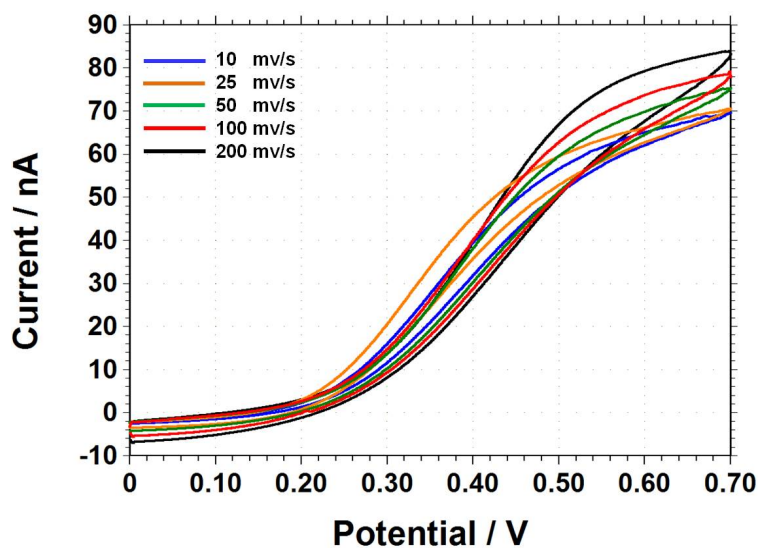


Fig. S5 Cyclic voltammograms for the oxidation of 10 mM $[\text{Fe}(\text{CN})_6]^{4-}$ (in 1 M KCl electrolyte) at 10.13 μm Pt electrode.

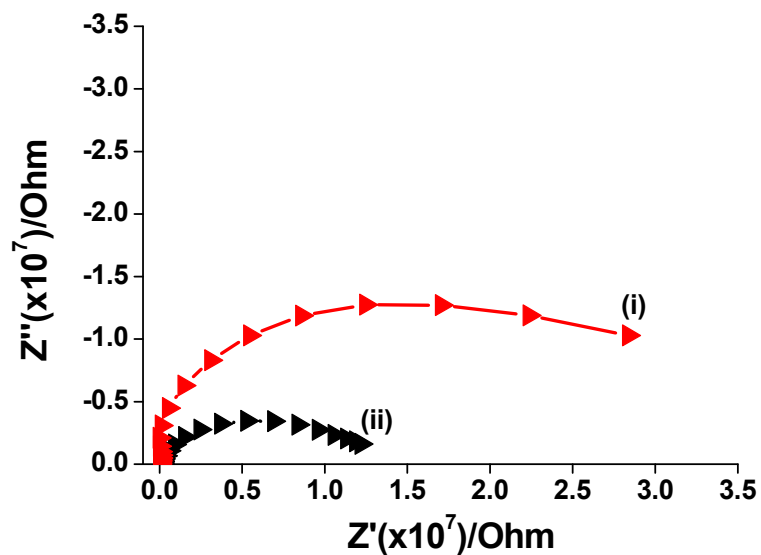


Fig. S6 Nyquist plots for the (i) 5.03 μm and (ii) 10.13 μm Pt electrodes in 1 M KCl electrolyte solution containing 10 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$.

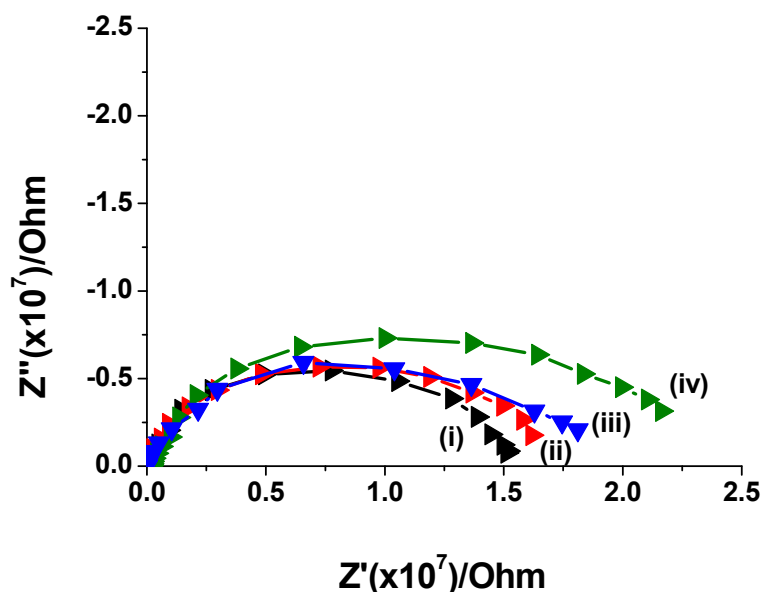


Fig. S7 Nyquist plots for the (i) Pt/SAM/anti- HER-2/BSA (ii) Pt/SAM/anti-HER-2/BSA/CA-125 antigen (1 ng/ml) (iii) Pt/SAM/anti-HER-2/BSA/FBS and (iv) Pt/SAM/anti-HER-2/BSA/HER-2 antigen (1 ng/ml) electrodes in 1 M KCl electrolyte solution containing 10 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$. Electrode diameter = 10.13 μm .

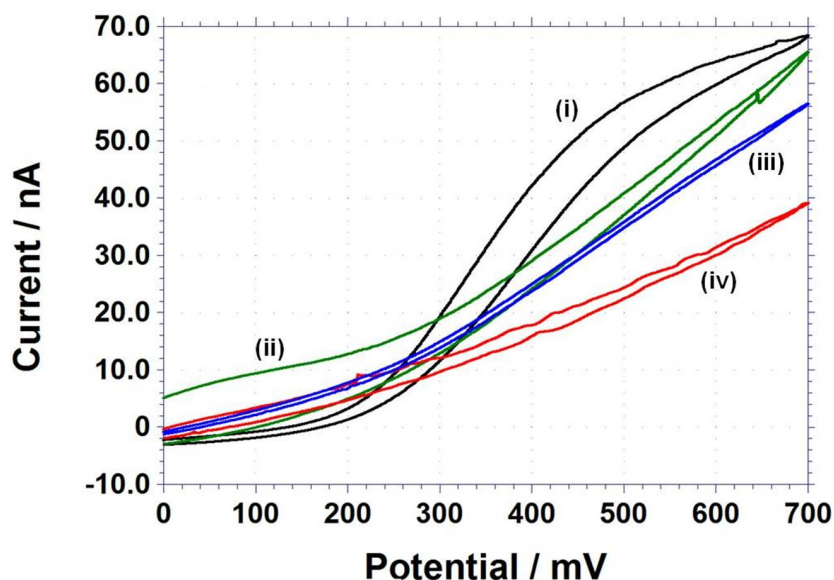


Fig. S8 Cyclic voltammetric profiles for the oxidation of 10 mM $[\text{Fe}(\text{CN})_6]^{4-}$ (in 1 M KCl electrolyte) at the (i) bare Pt, (ii) Pt/SAM (iii) Pt/SAM/anti- HER-2/BSA and (iv) Pt/SAM/anti- HER-2/BSA/HER-2 antigen electrodes. Electrode diameter = 10.13 μm .