# SEQUENTIAL PRECONCENTRATION BY NANOFLUIDIC ELECTROSTATIC SIEVING FOR HIGH SENSITIVE ANALYSIS OF NEUROTRANSMITTERS RELEASED BY SINGLE CELLS

**[1]Ren-Guei Wu, [1]Ying-Ting Chen, [1]Pei-Ju Wang, [2]Chung-Shi Yang 1,[3]Fan-Gang Tseng**

*[1]Engineering and System Science Dept., National Tsing Hua University, Hsinchu, Taiwan*

*[2] National Health Research Institutes, Zhunan, Miaoli, Taiwan*

*[3]Applied Science Center, Academia Sinica, Taipei, Taiwan*

## ABSTRACT

This paper introduces a nanofluidic electrostatic sieving (NES) microdevices incorporating electrochemical sensor with sequential applied electric fields to pre-concentrate scarce neurotransmitters released from single PC-12 cells for continuous detection. The NES device consists of insulating layer coated silicon nanowires (SINWs) array for not only the electrostatic gating of charged molecules but also the sieving of different size molecules. For sequential preconcentration, in every 30 s successively, and the voltage of 10 V was applied at the concentrator after 240s to collect charged biomolecules by the electrostatic repulsion. A detection limit of 30~75 zeptomoles was achieved, which is close to the levels released by a single neuron in vitro. As a result, the device has potential to improve the detection limit of that requires time-dependent analysis of single-cells.

## INTRODUCTION

Various methods for high sensitive detection of neurotransmmiter have been reported. Carbon-fiber-microelectrode arrays were utilized to electrochemically image neurochemical secreted from individual PC-12 [1] in 2D manner. However, without pretreatment and pre-concentration, high sensitive detection of biomolecules can still encounter many non-specific binding and interference issues. Therefore, the integration of online sampling to gather almost all of the molecules released from single cells and perform sample pre-concentration immediately is necessary before high sensitive detection. In this paper, we introduce a novel platform for single cell–cell interaction studies by Electropreconcentration with Charge-Selective Nanochannels [2, 3].

## EXPERIMENTAL

The chip integrates a serial of NES electrodes operated in different time to perform sample preconcentration; a dual-asymmetry electrokinetic flow (DAEKF) for sample re-stacking [4] and an electrochemical detector for signal detection in nanochannel (Figure 1). Both of NES electrodes are compose of silicon nanowires etched in $AgNO_3$(0.03 M)/HF(50%) solution (1:1 v/v), and then passivated by $SiO_2$ layer. The wafers we used are p-type, with a thickness of 525 μm and a low resistance of less than 10 Ωcm. When electric fields applied onto the nanowires, the polarization appears in the insulate layer and pre-concentration of biomolecules occurs in front of the filter. When electric fields applied onto the nanowires, the polarization appears in the insulate layer and pre-concentration of biomolecules occurs in front of the filter. Figure. 2 shows the fabrication process of NES devices and nanofluidic channels on silicon substrate in detail.

## RESULTS AND DISCUSSION

In order to see the serial control of pre-concentration, a continuous injection of FITC-BSA solution (10 $\mu$M diluted to 10 nM with DI water) was load into the buffer loading channels using hydrodynamic flow controlled by a syringe pump for 10 min with the flow rate 1 $\mu$L/min. The results of sequential preconcentration by NES were shown in Figure. 3a. As a result of NES preconcentration, the sample concentration of FITC-BSA in sequentially is increasing at different position of NES electrode with time by voltage controlled switching power supply. To examine the quality of NES devices, the SEM image of SINWs array was used for the calculation of the density and pore-size of nanochannels made of SINWs array (Figure. 3b). After the injection of sample containing catecholamine, electric fields are applied to the NES electrodes for after 10-minute-preconcentation and then the signal changes can be recorded at every 5 min, the result was shown in Fig. 4. The simulated solutions (50

zeptomoles dopamine with 1X cell culture medium) were introduced into the channel in every 50 s successively, and the voltage of 10 V was applied at the concentrator after 200s. We can see the electrochemical signal of dopamine under the NES effect is about 3 times larger than background signal (Figure 5).

## CONCLUSION

A SINWs-NES device is proposed in this paper for protein (FITC-BSA) preconcentration and neurotransmitter separation. The advantages of this device include a simple fabrication process, high efficient concentration in shorter time, good ion selection property, and flexible size sieving capability for biomolecules, thanks to the controllable and highly-dense charges induced on the SiO2-SINWs-NES surface. This preconcentrator serving as an ion-selective nanogate have achieved a $10^{3\text{-}4}$ fold concentrations of BSA in sequential 30 min, respectively. This device also demonstrates the successful separation of a neurotransmitter mixture with size and electrostatic sieving capability for the concentration (100 nM in 10 $\mu$L) positive and negative charge molecules in 400 sec. This on-chip protein preconcentration and neurotransmitter separation device would be a useful component in a fully integrated microanalytical system for high sensitive biosensing.

## REFERENCES:


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Word Count: 491

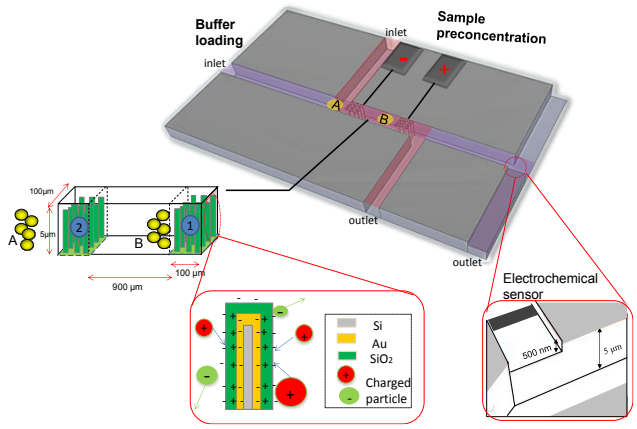



Figure 1. Schematic of a Nano-CEEC chip designed for sequential analysis of single cells. The polarization of silicon nanowire after we apply the electric field, and the nanowire will sieve the molecules with the charge opposite to the $SiO_2$ layer.

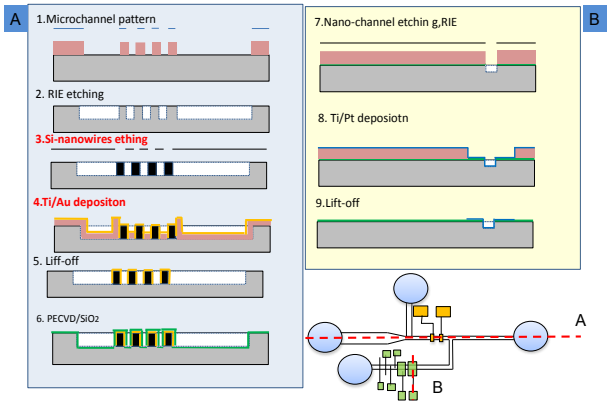
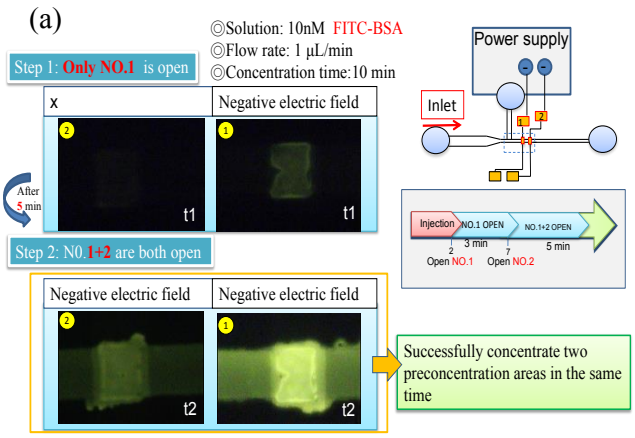



Figure 2. Microfabrication process of NES devices and nanofluidic CEEC channels on silicon substrate.



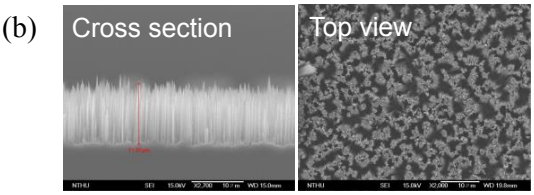




Figure 3. (a) The polarization of silicon nanowire after we apply the electric field, and the nanowire will sieve the molecules with the charge opposite to the SiO2 layer, (b) Cross-section of SINWs(Left), Top view of SINWs(Right).

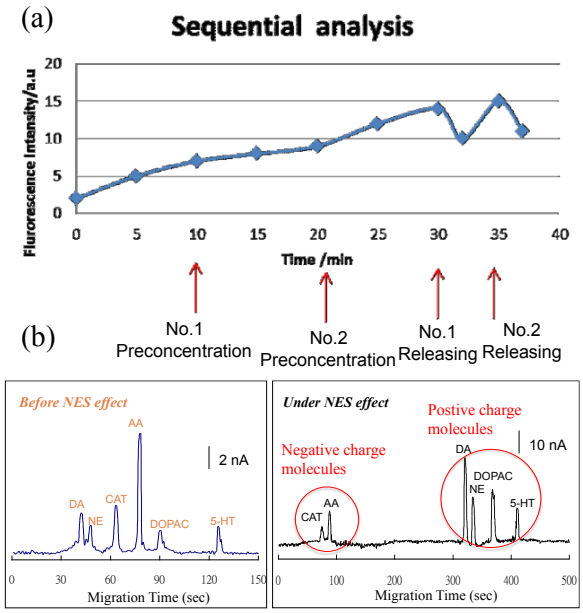
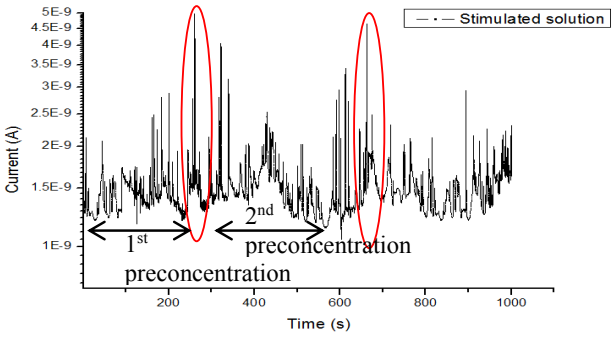



Figure 4. (a) The efficiency of sequential preconcentration by NES devices, (b) catecholamine separation before (left) and under (right) NES effect in nanochannel.




Figue 5. The Electropherogram of Sequential analysis of dopamine in culture medium under the NES effect.