CMOS-compatible silicon-nanowire-based Coulter counter for cell enumeration

Yu Chen,∗ Jinhong Guo, Hamidullah Muhammad, Yuejun Kang, Sunil K. Ary

Abstract—A silicon-nanowire-based Coulter counter has been designed and fabricated for particle/cell enumeration. The silicon nanowire was fabricated in a fully complementary metal–oxide–semiconductor (CMOS)-compatible process and used as a field effect transistor (FET) device. The Coulter counter device worked on the principle of potential change detection introduced by the passing of micro-particles/cells through a sensing channel. Device uniformity was confirmed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Current–voltage measurement showed the high sensitivity of the nanowire FET device to the surface potential change. The results revealed that the silicon-nanowire-based Coulter counter can differentiate polystyrene beads with diameters of 8 and 15 µm. Michigan Cancer Foundation-7 (MCF-7) cells have been successfully counted to validate the device. A fully CMOS-compatible fabrication process can help the device integration and facilitate the development of sensor arrays for high throughput application. With appropriate sample preparation steps, it is also possible to expand the work to applications such as rare-cells detection.

Index Terms—cell counting, Coulter counter, field effect transistor, microfluidics, silicon nanowire

I. INTRODUCTION

S
ince the nineteenth century, blood counts of various types (white blood cells, red blood cells, platelets, etc.) have been used for clinical purposes [1]. Abnormally high or low counts of each type of cell/platelet may indicate the presence of certain diseases. The result of such a count provides the patient’s general health status and can be used for better treatment of patient illness [2]. For example, the circulating tumor cells (CTCs) may represent the tumor population that is most likely to develop overt metastases. The number of CTCs gives a surrogate through which the tumor’s genetic and phenotypic status can be evaluated through a relatively non-invasive procedure [3]. Conventionally, a hemocytometer, which uses counting chambers that hold diluted blood and divide it with grid lines to calculate red blood cells and white blood cells, is most commonly used for cell counting. Counting cells using a hemocytometer is fast and the cost is low. However, manual counting is labor-intensive, time consuming, and also subject to human errors due to the limited number of cells counted under a microscope [4]. The flow cytometer has become the gold standard for clinical hematologic assays after half a century of development [5]. In flow cytometry, cells flow in a narrow stream in front of a laser beam and light scattered from the cells is detected for cell counting and analysis. The flow cytometer has been used to analyze the shape of the cells and their internal and external structures. However, the flow cytometer is sophisticated and expensive. Another common method of cell counting is based on electrical resistance measurement [6, 7], which is known as the Coulter counter concept. This is based on the fact that the blood cells show greater electrical resistance compared with the plasma where the blood cells have settled.

Recently, silicon-nanowire-based sensing devices have been reported for highly sensitive detection of ions and biomolecules [8–11]. Silicon-nanowire-based sensors utilize the field effect of the silicon for sensitive measurement. When the charged particles or molecules are attached to the surface of the silicon nanowire, a potential change of the nanowire surface is induced, which can accumulate or deplete the charge carrier of the nanowire device and thus change the conductance of the nanowire. Because of the high surface-to-volume ratio and other unique characteristics of the silicon nanowire, the device shows great sensitivity and fast response time as a biosensor.

Using the Coulter counter concept, we are reporting on a system using silicon nanowire for the particle/cell counting. Silicon nanowire has been used as a highly sensitive FET device for the detection of the potential change produced by the presence of micro-particles/cells. The device was calibrated by micro-beads of different sizes, and circulating tumor cells (MCF-7) were used as a model to show the capability of the device for cell counting. Because of the compatibility with the CMOS process, a silicon nanowire array can be easily fabricated for high throughput application. The potential-based Coulter counter can solve the interference problem of the conventional Coulter counter when multiple channels are used [12, 13].

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∗Yu Chen, Muhammad Hamidullah, and Sunil K. Ary are with Institute of Microelectronics, A∗STAR (Agency for Science, Technology and Research), 11 Science Park Road, Singapore 117685, (e-mail: chenyl@e.ntu.edu.sg, hmdoel@gmail.com, aryask@ime.a-star.edu.sg).

Jinhong Guo is with School of Electronic Engineering, University of Electronic Science and Technology of China, 610054, P. R. China (Email: guojinhong@uestc.edu.cn)

Yuejun Kang is with the School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore 637459 (Email: yuejun.kang@ntu.edu.sg).
II. MATERIALS AND METHODS

A. Silicon nanowire fabrication

A silicon-nanowire FET device can be fabricated by either the “top down” or the “bottom up” method. In the “bottom up” method, nanowires are first grown in a furnace and then poured onto the substrate. The metal line electrodes are then defined using the lithography process. As the nanowire device needs to operate in a buffer solution, the electrodes need to be passivated to ensure the stability of the device. It is difficult to realize mass production using such “bottom up” method. Here we describe a “top down” method where a fully CMOS compatible process is used for the fabrication of a silicon nanowire FET device (Fig. 1). A silicon-on-insulator (SOI) wafer (Soitec, p-type, resistivity = 14–19 Ω⋅cm) with a device layer of 70 nm was oxidized and etched by HF (1:100). The device layer was thinned down to be around 30 nm (Fig. 1b).

Low Pressure Chemical Vapor Deposition (LPCVD) was used to coat silicon nitride as a hard mask for later fabrication, and photolithography (Nikon Stepper S203B) was used to define the nanowire structure. The width of the photoresist mask was around 180 nm and then it was trimmed to around 100 nm by an AMAT P5000 etching system (He–O2:40 sccm + N2: 20 sccm, pressure: 30 mTorr, power: 40 W) (Fig. 1c). Silicon nitride was etched to form the hard mask for TMAH (Tetra-Methyl Ammonium Hydroxide) etching (25% at 50 °C) of silicon and the photo resist (PR) was stripped away (Fig. 1d). After TMAH etching and removal of the nitride hard mask, the device was coated with 10 nm of Al2O3 by atomic layer deposition (ALD) (Fig. 1e). Then the wafer was coated with 200 nm of SiO2 by plasma-enhanced chemical vapor deposition (PECVD), and the two sides of the nanowire (source and drain) were exposed for the doping and metal contact (Fig. 1f). Aluminum was deposited on the wafer and lithography and wet etching processes were used to pattern the metal line (Figs. 1g). Finally the silicon oxide on the sensing area of the nanowire was etched and exposed by reactive ion etching (RIE) (Figs. 1h). Because of the high etching selectivity of SiO2/Al2O3 when low etching power was used, the etching process was stopped at Al2O3 with minimal over-etching (less than 2 nm). The remaining Al2O3 served as the gate oxide layer for the FET sensor device.

Silicon-nanowire fabrication was characterized using SEM (scanning electron microscopy) and TEM (transmission electron microscopy). Fig. 1i shows the SEM image of the fabricated nanowires. The total length of the nanowires is 18 μm, whereas only 10 μm is exposed as the sensing area. There are five nanowires in each bundle and they are at a distance of 500 nm from each other. Only the nanowire in the center is activated (connected to the source drain electrodes), whereas the other four are dummy wires to ensure device uniformity. The cross-section of the nanowire is shown in Fig. 1j. It is clear from the figure that nanowires in a trapezoidal shape are consistent and uniform with a bottom width of around 90 nm.

B. Microfluidic channel fabrication

The microfluidic channel is fabricated following the typical soft lithography fabrication method. First, photolithography is used to make the SU8 mold (SU-8, 25 Microchem, MA, USA) on silicon wafer. After soft and hard baking, polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning, MI, USA) and curing agent in a volume ratio of 10:1 were mixed well, cast onto the SU8 mold, and baked at 80 °C for two hours. Finally, the PDMS replica was peeled off the mold and bonded with the silicon-nanowire chip after plasma O2 activation for 1 min at a power of 70 W using a plasma asher. The micro-fluidic channel was aligned so that nanowire FET sensor was located right on the left of the micro-sensing channel. The chip was then baked at 70 °C for 24 hours to improve the bonding strength between the PDMS and silicon-nanowire chip. The sensing channel (smaller central micro-channel) of the PDMS chamber is designed to be 30 μm wide and 100 μm long. The bigger micro-channels on two sides of the small micro-channel are designed to be 800 μm wide and 5 mm long, whereas the PDMS channel is 30 μm in height.
C. Theoretical calculation

The concept of the Coulter counter is demonstrated in Fig. 2. The fluidic channel includes three sections: the sensing channel (small central micro-channel with resistance \( R_0 \)) and two bigger micro-channels (with resistance \( R_L \) and \( R_R \)) on two sides of the sensing channel. Electrical voltages are applied from the inlet and the outlet (\( V_L \) and \( V_R \)). The nanowire FET device is located right next to the sensing channel (Fig. 2a) to measure the potential change. When non-conductive particles are occupying the big micro-channel (\( R_L \) and \( R_R \)), as the volume ratio of the particle and fluidics channel is small, the total resistance (\( R_{total} = R_L + R_0 + R_R \)) change is negligible (Fig. 2b). However, when the particles are occupying the sensing channel (\( R_0 \)), the total resistance (\( R_{total} \)) is not negligible because the volume ratio of the particle and the sensing channel is large. The current change is proportional to the ratio of the volume of the cell and sensing channel, which is in proportion to the conductance change introduced by the non-conductive cell in conductive buffer [14]:

\[
\Delta I = \frac{(V_L - V_R)}{R_{total}} \cdot \frac{(V_L - V_R)}{R_{total} + \Delta R} = \frac{(V_L - V_R) \Delta R}{R_{total}}.
\]

Here we assume that the resistance change \( \Delta R \) is much smaller than the resistance of the whole channel \( R_{total} \).

For the potential-based Coulter counter that we proposed, instead of measuring the current passing from left to the right, we can also measure the potential right next to the central micro-channel (\( R_0 \)) using a silicon nanowire (Fig. 2b), and the potential \( V_g \) can be expressed as

\[
V_g = V_R + (V_L - V_R) \cdot \frac{R_{eff}}{R_L + R_{eff}},
\]

where \( R_{eff} = R_0 + R_R \). When the non-conductive particle/cell is passing through the sensing channel, the resistance of the sensing channel changes to \( R_0 + \Delta R \), and thus \( R_{eff} \) is changed to \( R_0 + \Delta R \). The potential \( V_g \) can be expressed as

\[
V_g' = V_R + (V_L - V_R) \cdot \frac{R_{eff} + \Delta R}{R_L + R_{eff} + \Delta R}.
\]

So the potential change introduced by the passing of the cell can be calculated as:

\[
\Delta V_g = (V_L - V_R) \frac{\Delta R}{R_{eff}} \cdot \delta,
\]

where

\[
\delta = \frac{R_L}{R_{eff}} \cdot \frac{1}{(1 + \frac{R_L}{R_{eff}})^2}.
\]

In our fluidic channel design,

\[
\frac{R_L}{R_{eff}} \cdot \frac{R_0}{R_L} = \frac{5000}{800 \times 30} = \frac{5}{24} \approx 0.208,
\]

and \( \delta = 0.24 \). When \( V_L - V_R = 1 \) V, the change in potential introduced by one 10 µm particle is around 3.9 mV. This potential change can be captured by the nanowire resistance measurement. Also, the sensitivity limit can be further tuned by the potential applied to the left and right sides of the micro-channel.

III. RESULTS AND DISCUSSION

The nanowire FET device was characterized by I-V measurement. The measurement setup was shown in Fig. 3c, where nanowire conductance was measured using Agilent semiconductor analyzer B1500A and two reference electrodes were injected at the inlet and outlet of the fluidics chamber. PBS buffer (10 mM, with 138 mM of NaCl, pH 7.4) was first introduced into the fluidic chamber and only one electrode (Ag/AgCl pellet, A-M system, 550008) from the left side was connected to the power source (\( V_g = V_L \)), while \( V_R \) was floated, and the potential of the gate of silicon nanowire was determined by \( V_L \). In Fig. 3a, the electrical current through the nanowire was recorded when the gate voltage was scanned from +1 to −0.2 V (black line for linear scale and green line for log scale). The field effect can be seen clearly with
negligible hysteresis. The silicon nanowire is slightly p-doped and the FET is operated at the depletion region. When a positive voltage is applied to the solution gate, the positive charge carriers of the p-type silicon are depleted and the source drain current decreases [15]. Further, when $V_L$ was floated and $V_R$ was connected to the power source ($V_s = V_R$), almost identical I-V characteristics (black dots and red lines) were observed (Fig. 3b) as the potential across the whole fluidic channel was the same. The real-time measurement of the current through the nanowire with constant gate voltage clearly reveals the nanowire current sensitivity to the applied gate voltage (Fig. 3c). The leakage current to the reference gate electrode is negligible, as a noise current at the level of +/-50 pA was observed instead of a potential-dependent current (data not shown). This confirmed a conformal coating of Al₂O₃ on the silicon nanowire. When $V_L$ and $V_R$ are connected to different potentials as described previously in Section II (C), there is a gradient of electrical potential across the fluidics channel and the surface potential of silicon nanowire can be determined by $V_L$ and $V_R$ as well as the resistance ratio of the microfluidics channels.

Polystyrene micro-beads of three different sizes (8, 15, and 20 µm) were used to characterize and validate the device-counting capability. The microfluidic channel was first incubated by PBS buffer with 5% BSA (bovine serum albumin) and then the solution with the micro-beads was driven through the channel (Fig. 4a) from the left side inlet at a flow rate of around 50 nL/min. Figure 4b show the real-time current measurement of the nanowire, where $V_L = 1$ V and $V_R = 0$ V. The passing of big micro-beads (15 µm) introduces a large change in current (around 800 pA), whereas small micro-beads (8 µm) introduce a small change in the current (around 130 pA). From Fig. 4b and 4c, the peak conductance change is approximately in linear with the particular size, and the change in peak conductance from 8 µm beads is small in comparison with the peak from 15 µm beads. As the conductance change is proportional to the particle volume, the size of unknown cells/particles can be estimated by comparison with the signal of the beads (Fig. 4c). When two beads were passing through the central micro-channel continuously with a short time interval, a potential drop with a double peak was observed (data not shown). This implies that a higher sampling rate is needed to identify particles at high flow rate or high concentration.

Recently, the importance of knowing the number of circulating tumor cells (CTCs) present in blood for cancer diagnosis and treatment has generated interest due to their clinical implication [16, 17]. CTCs shed into the vasculature from a primary tumor and circulate in the bloodstream. The presence of CTCs in the blood can trigger the subsequent growth of additional tumors in distant organs by metastasis and is the major reason for cancer-related deaths, and thus CTC detection and counting are critical for cancer management. Here we use MCF-7 as a model cell to validate our Coulter counter device. MCF-7 cells were spiked in the PBS at a concentration of around $6.6 \times 10^4$ cells/ml. The flow rate used is 10 µl/min. The change in conductance of silicon nanowire when MCF-7 cells were driven through the microfluidic channel was recorded. By comparing the results of Fig. 4c and Fig. 3c, the nanowire conductance change is approximately proportional to the gate voltage change when the voltage change is small (for a 20 µm bead, the gate voltage change is around 30 mV). The gate voltage change is proportional to the volume of the cell/bead passing through. Thus we can extract and normalize the MCF7 cell size as showed in Fig. 4d. The results clearly indicate that the device can be used for cell counting.

The current prototype is based on the size difference of the particles/cells and there is no specificity, which means it cannot differentiate particles/cells of the same sizes. For example, some big white blood cells have a similar size to the
CTCs, and thus the device cannot be used directly for clinical samples. In order to realize selective detection and counting of CTCs in blood, two possible approaches may be adopted during the sample preparation step: 1) depletion of all white blood cells before counting or 2) increasing the effective size of target cancer cells by selective conjugating microbeads [18, 19].

IV. CONCLUSIONS

In conclusion, a silicon-nanowire FET device was fabricated using a CMOS-compatible process. The nanowire is operated as a highly sensitive potential sensor for the particle counting using the potential-based Coulter counter concept. The counting capability was validated using micro-beads as well as the cancer cell MCF-7. The advantage of such a counting device is that it has the potential to improve on the low throughput of the conventional Coulter counter device with a multiple-sensor array.

REFERENCES


