Experimental characterization of a metal-oxide-semiconductor field-effect transistor-based Coulter counter

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(Received 10 September 2007; accepted 24 March 2008; published online 20 May 2008)

We report the detailed characterization of an ultrasensitive microfluidic device used to detect the translocation of small particles through a sensing microchannel. The device connects a fluidic circuit to the gate of a metal-oxide-semiconductor field-effect transistor (MOSFET) and detects particles by monitoring the MOSFET drain current modulation instead of the modulation in the ionic current through the sensing channel. The minimum volume ratio of the particle to the sensing channel detected is 0.006%, which is about ten times smaller than the lowest detected volume ratio previously reported in the literature. This volume ratio is detected at a noise level of about 0.6% of the baseline MOSFET drain current, clearly showing the amplification effects from the fluidic circuits and the MOSFETs. We characterize the device sensitivity as a function of the MOSFET gate potential and show that its sensitivity is higher when the MOSFET is operating below its threshold gate voltage than when it is operating above the threshold voltage. In addition, we demonstrate that the device sensitivity linearly increases with the applied electrical bias across the fluidic circuit. Finally, we show that polystyrene beads and glass beads with similar sizes can be distinguished from each other based on their different translocation times, and the size distribution of microbeads can be obtained with accuracy comparable to that of direct scanning electron microscopy measurements. © 2008 American Institute of Physics. [DOI: 10.1063/1.2931026]

I. INTRODUCTION

The Coulter counter was first developed and patented in 1953 by Coulter¹ and is based on the principle that the electrical resistance of a small, electrolyte-filled channel increases as particles flow through the channel. This is because when a nonconducting particle flows through a small channel, it displaces a volume of electrolyte equivalent to its own volume, and hence increases the Ohmic resistance of the channel. This resistance modulation will lead to a corresponding decrease in the ionic current or an increase in the voltage drop across the sensing channel, which can be experimentally measured and used to detect particles in the channel. This technique is widely known as resistive-pulse sensing or the Coulter principle.

Coulter² first demonstrated that a device based on this principle could be used to count micron-sized particles such as red blood cells at a high count rate of ~ 6000 particles/s. DeBlois and Bean³ developed a theory to predict the change in resistance when a particle flows through the sensing channel and showed that the relative resistance modulation, $\Delta R/R$, is approximately equal to the volume ratio of the particle to the sensing channel if the particle diameter is small compared to the diameter of the channel. They also showed that they were able to detect a minimum volume ratio of 0.06% by using a tapered pore with end diameters of 490 and 450 nm as polystyrene beads that are 91 nm in diameter flowed through the pore. DeBlois and Wesley⁴ then showed that the resistive-pulse sensing could be used to de-

tect the size and concentration of viruses using submicron pores. As further optimization of the Coulter counter device continued, Sikdar and Webster⁵ showed that they were able to count particles at a high rate and accurately measure the mean and variation in the particle size distribution. Berge *et al.*⁶ developed a technique to reverse the flow of particles through the sensing channel to study the dissolution of air bubbles and radial migration of particles as a function of time.

The development of Coulter counters since the 1990s has been focused on using nanoscale pores for single molecule/nanoparticle sensing and making on-chip Coulter counters.⁷ Bezrukov *et al.*⁸ demonstrated the counting of polymer molecules as they passed through a single alamethicin pore that is ~ 5 nm long and ~ 2 nm in diameter. Kasianowicz *et al.*⁹ showed that they were able to sense individual strands of DNA and RNA as they translocated through a protein nanopore (α -hemolysin). These promising results sparked many studies of DNA translocation and dynamics in biological nanopores.^{10–13} However, the biological nanopores are not very robust and size tunable. Therefore, efforts have been made to fabricate artificial, solid-state nanopores to overcome the above-mentioned limitations.

Kobayashi and Martin¹⁴ fabricated gold nanotubules with a diameter of < 2 nm and used them to measure the concentration of divalent cations $[\text{Ru}(\text{bpy})_3]^{2+}$ and methylviologen (MV^{2+}). Sun and Crooks¹⁵ used a multiwall carbon nanotube (150 nm in diameter)-based device to sense 60–100 nm in diameter polystyrene particles. This method was then improved by Crooks and co-workers^{16,17} to simultaneously

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detect the size and surface charge of polystyrene particles. In 2005, Fan *et al.*¹⁸ reported an on-chip device based on silicon dioxide nanotubes and the detection of DNA translocation through the nanotube.

Li *et al.*^{19,20} were the first to fabricate solid-state nanopores by using ion and electron beam technologies and used these devices to study DNA translocation and dynamics. Similar devices based on SiN_x or SiO_2 nanopores were then developed^{21–27} to detect DNA translocation. Chang *et al.*²¹ studied DNA translocation through SiO_2 nanopores that are 50–60 nm long, 4–5 nm in diameter and demonstrated that the ionic current in the nanopore could increase due to the mobile counter ions adsorbed on the DNA itself. Chen *et al.*^{22,23} fabricated ~ 15 nm in diameter SiN_x nanopores and showed that coating the nanopore surface with alumina could reduce the electrical noise. Heng *et al.*²⁴ made ~ 1 nm in diameter SiN_x nanopores and reported that they were able to distinguish single-stranded DNA from double-stranded DNA and resolve the length of the molecule. Fologea *et al.*²⁵ reported the possibility of slowing down DNA translocation by a factor of 10 by fine-tuning the experimental conditions. Storm *et al.*²⁶ observed the translocation time and the length of the DNA segments and found a power-law relationship between them. Smeets *et al.*²⁷ studied DNA translocation through ~ 10 nm in diameter SiO_2 nanopores for various concentrations of electrolyte. Both Smeets *et al.*²⁷ and Fan *et al.*¹⁸ found that the ionic current could either decrease or increase depending on the concentration of the electrolyte used. The decrease in the ionic current is expected from the Coulter principle. The increase in the ionic current observed at low salt concentrations was attributed to the contribution to channel conductance from the mobile counter ions adsorbed on the DNA molecule.

Another salient trend of the Coulter counter development since the 1990s is the development of on-chip devices. Larsen *et al.*²⁸ and Koch *et al.*²⁹ were the first to report the design and fabrication of microchannel-based Coulter counters by using silicon micromachining techniques. Saleh and Sohn³⁰ fabricated a microchip Coulter counter on a quartz substrate and showed that they were able to sense individual nanoparticles that are 87 nm in diameter with a sensing channel that is $8.3\ \mu\text{m}$ long and a cross-sectional area of $0.16\ \mu\text{m}^2$. Later, they developed a polydimethylsiloxane (PDMS)-based device with a sensing channel that is 200 nm in diameter and $3\ \mu\text{m}$ long and showed that they were able to detect $16\ \mu\text{m}$ long λ -DNA segments.³¹ Carbonaro and Sohn³² then fabricated a PDMS-based device to detect the size change of 490 nm diameter latex colloids upon antigen-antibody binding on the antibody-coated colloid surface. More recently, novel designs^{33–37} of on-chip Coulter counters were also developed by other researchers to realize a high throughput counting and sizing, hydrodynamic focusing, etc.

Despite all the success in the fabrication of nanoscale features leading to a dramatic reduction in the volume of the sensing channel, the lowest detectable volume ratio by using the resistive-pulse sensing technique has been 0.06%, as reported by DeBlois and Bean³ in 1970, which is approximately the same as the relative modulation in the ionic cur-

FIG. 1. The schematic of the experimental setup (not to scale) (Ref. 38). The fluidic and MOSFET circuits are commonly grounded. The resistances of three segments of the horizontal microchannel are denoted as R_2 , R , and R_1 , respectively.

rent. We have recently reported a design of fluidic sensors based on the resistive-pulse sensing principle, which integrates the fluidic circuit with a metal-oxide-semiconductor field-effect transistor (MOSFET) and detects the MOSFET drain current to sense the resistance modulation in the fluidic sensing channel as particles flow through it.³⁸ We showed that amplification can be achieved through both the fluidic circuit and the MOSFET, and a minimum volume ratio of 0.006% could be detected. In this paper, we report the detailed experimental characterization of the performance and capability of this MOSFET-based Coulter counter. We first demonstrate the amplification effect and perform noise analysis to show why enhanced sensitivity can be achieved. Detailed analysis of the device sensitivity as a function of the MOSFET gate voltage and the applied electrokinetic bias through the fluidic circuit are also presented. We then show some results of the capability of the device to distinguish different kinds of particles with similar sizes and to characterize the size distribution of particles.

II. DEVICE DESIGN AND MEASUREMENT SETUP

The performance of the MOSFET-based Coulter counter was demonstrated with a microfluidic device fabricated with PDMS microchannels bonded to a glass substrate in conjunction with a commercial MOSFET (2N7000 *N*-channel FET, Fairchild Semiconductor Co.), as shown in Fig. 1. The microfluidic chip consists of four microchannels, which are all $30\ \mu\text{m}$ deep and fabricated by soft lithography using patterned SU-8 as a master. The left channel (denoted by its resistance R_2) is $7.5\ \text{mm}$ long and $800\ \mu\text{m}$ wide, the sensing channel (denoted by its resistance R) is $150\ \mu\text{m}$ long and $16\ \mu\text{m}$ wide, and the right channel (denoted by its resistance R_1) is $15\ \text{mm}$ long and $160\ \mu\text{m}$ wide. The vertical microchannel connecting the downstream end of the sensing channel to the gate of the MOSFET is $6\ \text{mm}$ long and $300\ \mu\text{m}$ wide. Three reservoirs, each of $3\ \text{mm}$ in diameter, were punched through the PDMS to facilitate fluid injection and removal. Platinum wires were used as the source, drain, and gate electrodes to connect the fluidic and electronic circuits. An electrokinetic bias ($V_+ - V_-$) was applied by using two dc power supplies (Agilent E3612A and Agilent E3617A) to drive microbeads through the microchannels and to facilitate the adjustment of the MOSFET gate voltage.

FIG. 2. $\sqrt{I_D}$ as a function of V_G for a typical MOSFET we used when $V_{DS} = 0.15$ V and 0.5 V, respectively. The threshold voltage for each case is determined by the value of the x intercept.

The performance of the MOSFET was separately calibrated independent of the fluidic circuit before it was used in the experiments. The basic operating principles of MOSFETs have been extensively studied,^{39,40} so we only present the characterization of our MOSFETs. The MOSFET gate voltage was controlled by an accurate voltage source (Keithley 6487). A source-measure unit (Keithley 236) was used to apply a constant drain-source bias, V_{DS} , of either 0.15 or 0.5 V and to measure the MOSFET drain current. The MOSFET source terminal was commonly grounded with the voltage source and the source-measure unit. Figure 2 shows the measured $I_D^{1/2}$ - V_G curves for the MOSFET when $V_{DS}=0.15$ and 0.5 V, respectively. The threshold voltage V_T of the MOSFET was determined from the x intercepts of the $I_D^{1/2}$ - V_G curves in Fig. 2 to be ~ 2.1 V when $V_{DS}=0.15$ V and ~ 2.2 V when $V_{DS}=0.5$ V. The slight shift in the threshold voltage observed for different drain-source biases can be attributed to the substrate bias effect.³⁹ The typical commercial MOSFET used has only three terminals for the source, drain, and gate electrodes and no terminal to control the substrate bias. Therefore, the substrate bias was floating and caused the threshold voltage to shift when the drain-source bias was changed. However, this does not affect our measurements as long as we keep the drain-source bias fixed for each experiment.

In the experiment, a 7.5 mM sodium borate buffered solution (pH value of 9.45) was first filled into the microfluidic circuits and the liquid level in each reservoir was allowed to equilibrate. Then, the same borate buffer with dilute suspended polystyrene microbeads was added to the reservoir that would be positively biased. After a short period for the liquid level to reach equilibrium again, the electrokinetic bias ($V_+ - V_-$) was applied to induce electro-osmotic flow through the horizontal fluidic channel. When a microbead translocates through the sensing microchannel, the conductance in the sensing channel decreases because the sensing channel is partially blocked by the microbead, causing a drop in the ionic current through the channel, which is the most common sensing mechanism of modern Coulter counters. However, in the MOSFET-based Coulter counter, the downstream end of the sensing channel is connected to the gate of a MOSFET and the electrical potential modulation at the end of the sensing channel is monitored by measuring the MOSFET drain current. In our experimental setup, the MOSFET

drain current was measured by a current preamplifier (DL Instruments 1211) and the signal passed through a low-pass filter (Stanford Research Systems SR560) before it was fed into the digital data acquisition system. This sensing scheme presents an amplified percentage of modulation to the baseline MOSFET drain current as compared to the percentage of modulation to the baseline ionic current, which will be discussed in Sec. III.

III. THEORETICAL ANALYSIS OF THE AMPLIFICATION EFFECTS

A concise version of the amplification effect has been discussed in a recent paper about the MOSFET-based Coulter counter.³⁸ In this section, we present a more detailed analysis of the amplification effect.

Since the microbeads are in diluted suspension, their effects on the electrical resistance of the large fluidic channels on each side of the sensing channel are negligible. Denoting the increase in resistance of the sensing channel as ΔR when a bead is in the sensing channel and referring to the resistance in the three segments of the horizontal channels in Fig. 1, the ionic current without (I) and with (I^*) a bead in the sensing channel can be written as

$$I = \frac{V_+ - V_-}{R_2 + R + R_1} \quad \text{and} \quad I^* = \frac{V_+ - V_-}{R_2 + R + \Delta R + R_1}. \quad (1)$$

The gate potentials (V_G) of the MOSFET are then

$$V_G = V_- + IR_1 \quad \text{and} \quad V_G^* = V_- + I^*R_1. \quad (2)$$

The modulation of the gate potential can be derived as

$$\frac{\Delta V_G}{V_G} = \frac{\Delta IR_1}{V_G} = \frac{IR_1 \Delta I}{V_G I} = \frac{IR_1 \Delta R}{V_G R_t} = \left(\frac{IR_1 R}{V_G R_t} \right) \frac{\Delta R}{R}, \quad (3)$$

where $\Delta I = I - I^*$ and $R_t = R_2 + R + \Delta R + R_1$. Equation (3) indicates that the percentage modulation in the gate potential to the baseline gate potential is amplified by a factor of $A_{\text{fluidic}} = (IR_1/V_G)(R/R_t)$ compared to the percentage modulation in the channel resistance. This factor is defined as the amplification factor from the fluidic circuit.

A MOSFET can be operated in different regimes, in which the drain current differently behaves as a function of the gate potential. Amplification effects can be achieved when the MOSFET is working in either the saturation region under the conditions of $V_G > V_T$ and $V_{DS} > V_G - V_T$ or in the subthreshold region under the condition of $V_G < V_T$.

When the MOSFET works in the saturation region, the MOSFET drain current modulation as a function of the gate voltage^{39,40} can be written as $I_D = k_{\text{sat}}(V_G - V_T)^2$, where k_{sat} is a constant. For a small modulation, ΔV_G , the drain current modulation is then

$$\frac{\Delta I_D}{I_D} = \frac{2V_G}{V_G - V_T} \frac{\Delta V_G}{V_G}. \quad (4)$$

By combining Eqs. (3) and (4), we have

$$\frac{\Delta I_D}{I_D} = \left(\frac{2V_G}{V_G - V_T} \right) \left(\frac{IR_1}{V_G} \right) \left(\frac{R}{R_t} \right) \frac{\Delta R}{R}. \quad (5)$$

So the amplification factor from the MOSFET can be expressed as $A_{\text{FET,sat}} = 2V_G / (V_G - V_T)$. To achieve a large total amplification factor, we designed the fluidic circuit as $R_1 = 10R$ and $R_t = 12R$, so as long as the voltage drop across the sensing channel, IR , is larger than $1.2V_G$, A_{fluidic} is larger than 1. For the MOSFET, a large amplification factor can be achieved if $V_G - V_T \ll V_G$, so the gate voltage selected should be only slightly higher than the threshold voltage.

When $V_G < V_T$, the MOSFET works in the subthreshold region and the drain current exponentially changes with the gate voltage as⁴⁰

$$I_D = k_{\text{sub}} \left[1 - \exp\left(-\frac{qV_{DS}}{kT}\right) \right] \exp\left(\frac{q\psi_S}{kT}\right) \left(\frac{q\psi_S}{kT}\right)^{1/2}, \quad (6)$$

where k_{sub} is a constant and q , k , and T are the electronic charge, the Boltzmann constant, and the temperature, respectively. ψ_S is the surface potential of the MOSFET electronic channel and is given by

$$\psi_S = V_G - V_{\text{FB}} - \frac{a^2 kT}{2q} \left\{ \left[1 + \frac{4}{a^2} \left(\frac{qV_G}{kT} - \frac{qV_{\text{FB}}}{kT} - 1 \right) \right]^{1/2} - 1 \right\}, \quad (7)$$

where $a = 2(\epsilon_s / \epsilon_{\text{ox}})(d_{\text{ox}} / L_D)$. ϵ_s and ϵ_{ox} are the permittivity of the semiconductor and the oxide, respectively. d_{ox} is the thickness of the MOSFET gate oxide, L_D is the extrinsic Debye length, and V_{FB} is the flat-band voltage.

Based on Eqs. (3), (6), and (7), we can derive the theoretical drain current modulation when the MOSFET is working in the subthreshold region as

$$\frac{\Delta I_D}{I_D} = \left(\frac{qV_G}{kT} - \frac{V_G}{2\psi_S} \right) \left(\frac{d\psi_S}{dV_G} \right) \left(\frac{IR_1}{V_G} \right) \left(\frac{R}{R_t} \right) \frac{\Delta R}{R}. \quad (8)$$

This equation is cumbersome to use as we do not have the necessary information such as the dopant concentration, oxide thickness, and flat-band voltage to evaluate the variables a and V_{FB} , and hence ψ_S .

In ideal situations, i.e., if we assume that there is no fixed oxide charge, interface traps, or difference in work function between the semiconductor and the gate metal, and if the diffusion current can be neglected, then ψ_S is approximately equal to V_G . Equation (8) can then be simplified as

$$\frac{\Delta I_D}{I_D} \approx \left(\frac{qV_G}{kT} - \frac{1}{2} \right) \left(\frac{IR_1}{V_G} \right) \left(\frac{R}{R_t} \right) \frac{\Delta R}{R}. \quad (9)$$

Therefore, an amplification factor for MOSFET working in the subthreshold regime can be written as $A_{\text{FET,sub}} = qV_G / kT - 1/2$. The typical value of q/kT is around 40 at room temperature and for a threshold voltage of 2.1 V, a FET amplification factor of around 80 can be achieved.

FIG. 3. (a) The MOSFET drain current modulation as a function of time for a mixture of 4.1, 5.9, 7.26, and 9.86 μm in diameter polystyrene beads when the MOSFET works in the saturation region. (b) The amplitude in the drain current modulation as a function of the microbead volume. The points represent experimental values derived from (a) and the solid line represents the theoretical prediction derived from Eq. (5).

IV. RESULTS AND DISCUSSION

A. Experimental demonstration of the amplification effects

The demonstration of the amplification effect was performed with the 7.5 mM sodium borate buffer containing polystyrene beads. We first characterized the amplification effect for the MOSFET working in the saturation region by setting $V_- = -29$ V, $V_+ = 11.98$ V, and $V_{DS} = 0.5$ V. The MOSFET gate voltage was inferred from the $I_D^{1/2} - V_G$ curve in Fig. 2 to be ~ 2.31 V, so the MOSFET was working in the saturation region. Under these conditions, the calculated amplification factors are $A_{\text{fluidic}} = (IR_1 / V_G)(R / R_t) = 1.13$, and $A_{\text{FET,sat}} = 2V_G / (V_G - V_T) = 42$, yielding a total theoretical amplification factor of 47.46 for the MOSFET drain current modulation compared to the resistance modulation of the sensing channel.

The measured MOSFET drain current as a function of time is shown in Fig. 3(a) when a solution with polystyrene beads that are 4.1, 5.9, 7.26, and 9.86 μm in diameter was added. The different-size polystyrene beads can be easily distinguished by the different amplitudes of the drain current modulation observed as each microbead was translocated through the sensing channel, which was verified by concurrent observation by using an optical microscope (Nikon Eclipse TE-2000U). As pointed out in Refs. 3 and 30, if the diameter of the microbeads is much smaller than the diameter of the sensing channel, the ratio $\Delta R / R$ can be approximated by the ratio of the volume of the microbead to the volume of the sensing channel. Therefore, in Fig. 3(b), we

FIG. 4. The MOSFET drain current modulation as a function of time for a mixture of 2 and 9.86 μm in diameter microbeads when the MOSFET works in the subthreshold region. “Twins” in the figure indicates a group of two beads that translocate together through the sensing channel.

plotted the amplitude of the observed drain current modulation as a function of microbead volume together with the theoretical drain current modulation calculated from Eq. (5) by using the theoretical amplification factor of 47.46, as derived above. The resistance modulation in the calculation is taken as the volume ratio of the microbead to the sensing channel. From Fig. 3(b), it can be seen that the agreement between the theoretical prediction and experimental results is excellent for microbeads that are 4.1 and 5.9 μm in diameter and quite good for microbeads that are 7.26 μm in diameter. However, for the 9.86 μm in diameter beads, there is a difference of about 17.8% between the theory and the experiment. We believe that this is related to the fact the MOSFET is working close to the threshold voltage, and its I - V relationship is highly nonlinear. Therefore, Eq. (4) may not be very accurate for the relatively large modulation in the 9.86 μm in diameter beads. The volume ratio of the 9.86 μm beads to the sensing channel is only 0.7%, so one might think that the voltage modulation is still small. However, with the 47.46 times amplification, Eq. (5) will lead to a 100% MOSFET drain current modulation for a 2.1% resistance modulation (or volume ratio if the resistance modulation is regarded to be equal to the volume modulation).

In addition to characterizing the device for the MOSFET working in the saturation regime, we also characterized the device performance for the MOSFET working in the subthreshold regime by setting $V_- = -58$ V, $V_+ = 19.56$ V, and $V_{DS} = 0.15$ V. In this case, the gate voltage is derived to be ~ 1.7 V in the same manner as above, which is smaller than the threshold voltage for $V_{DS} = 0.15$ V. Thus, the MOSFET was working in the subthreshold regime. The amplification factors from the fluidic circuit and the MOSFET are $A_{\text{fluidic}} = 1.5$ and $A_{\text{FET,sub}} = 67.5$, respectively, giving a total theoretical amplification factor of 101.25 with respect to the resistance modulation in the sensing channel. The total amplification factor is more than twice the total amplification factor for the MOSFET working in the saturation regime (47.46). To demonstrate the enhanced amplification effect, we added the sodium borate buffer with a mixture of 2 and 9.86 μm in diameter polystyrene beads to the fluidic circuits and the measured MOSFET drain current as a function of time is shown in Fig. 4.

FIG. 5. The MOSFET drain current modulation as a function of time for 5.9 μm diameter microbeads when the rise time of the current preamplifier is set to 0.3 ms. The experimental setup was shielded in a Faraday cage, and $V_{DS} = 0.15$ V, $V_+ = 9.03$ V, and $V_- = -26.3$ V.

Figure 4 shows that with the higher amplification factor in the subthreshold regime, we were able to detect particles as small as 2 μm in diameter, which has a volume ratio to the sensing channel of about 0.006%, or ten times smaller than the smallest volume ratio previously reported in the literature (0.06%).³

The modulations shown in Figs. 3(a) and 4 are triangular in shape because the rise time of the current preamplifier was set to 100 ms, which was comparable to the time taken for the beads to translocate through the sensing channel (~ 300 ms). Hence, due to signal integration, we do not observe an ideal rectangular-shaped signal. However, the long rise time was required only because we tried to do concurrent electrical and optical measurements of particle translocation through the sensing channel and did not have a Faraday cage that is large enough to contain the whole setup. In real applications wherein only the electrical signal is desired, the setup can be shielded with a Faraday cage and a much shorter rise time can be used. This has been verified, and Fig. 5 shows the MOSFET drain current as a function of time when we added the sodium borate buffer with microbeads that are 5.9 μm in diameter to our fluidic circuits shielded in a Faraday cage. For this experiment, $V_{DS} = 0.15$ V, $V_+ = 9.03$ V, and $V_- = -26.3$ V, and the rise time of the current preamplifier is 0.3 ms with a cut-off frequency of the low-pass filter set as 100 Hz. As can be seen from Fig. 5, the microbead translocation signals observed have a shape close to the ideal rectangular shape, wherein the flat region at the bottom corresponds to the microbead traversing the sensing channel. In addition, in Fig. 5, we also observed overshooting of the MOSFET drain current signal when the microbead enters the sensing channel, which may be due to the sudden resistance and capacitance changes caused by the microbead.

It is worth noting that direct measurement of the ionic current through the fluidic circuit with the same instrument shows no discernible dips when microbeads pass through the channel, as evidenced by concurrent optical observation. Figure 6 shows the measured ionic current through the fluidic circuit when the solution with 9.86 μm in diameter polystyrene beads was driven through the sensing channel. No discernible dips can be observed from the ionic current measurement.

FIG. 6. Measured ionic current in the fluidic circuit for the microbead suspension containing $9.86 \mu\text{m}$ polystyrene beads.

B. Noise analysis

The sensitivity and limitation of any experimental measurement are ultimately dominated by noise and a more sensitive sensing scheme has to provide a higher signal to noise ratio. The MOSFET-based Coulter counter provides a higher percentage modulation compared to the percentage modulation to the baseline ionic current for traditional Coulter-type sensors. However, the concept is useful only if the noise level does not increase to the same extent. If we define the noise level as the ratio of the peak to peak value of the noise to the average baseline value of the signal, then in our measurement, the noise level of the ionic current through the fluidic circuit was about 0.3%, and the noise level of the MOSFET drain current was between 0.5% and 0.9%, which varied from one experiment to another. Note that the noise level of the ionic current in the literature ranges from $\sim 0.02\%$ (Ref. 3) to more than 10%,²⁴ so the noise level we observed is comparable to those reported in the literature. As was discussed in Sec. IV A, the percentage in the modulation can be magnified from 47.46 to 101.25 times depending on the working regime of the MOSFET and applied electrokinetic bias. In comparison, the noise level of the MOSFET drain current is only two to three times higher than that of the ionic current through the fluidic channel. So compared to the traditional Coulter counters, the reported MOSFET-based Coulter counter demonstrates an enhanced sensitivity of up to 50 times. (The sensitivity here is defined as the ratio of the percentage modulation from the particles to the noise level.)

The true advantage of the MOSFET-based Coulter counter is that the design locally amplifies the percentage in the resistance modulation. The noise of a Coulter-type sensor can come from three sources, i.e., the electrical noise from the measurement instruments external to the fluidic circuit, the noise from the electrochemical interactions at the electrode-electrolyte interface, and the noise from the fluidic circuit itself. Recently, there have been several discussions about the noise of nanopore-based Coulter-type sensors.^{23,41–43} All of these reports indicate that low-frequency ($1/f$) flicker noise is the dominant source of noise in nanopore-based Coulter-type sensors. The origin of this noise remains unknown but surface effects such as the inhomogeneity of the surface charge on the pore wall,²³ nanobubble formation,⁴¹ and underlying motions of biological membrane channels⁴³ have been proposed as possible sources for the flicker noise. To better understand the noise source in our measurement system, we performed a spectral analysis of our data.

Figure 7 shows the power spectra of the measured baseline MOSFET drain current for two cases: no Faraday cage

FIG. 7. PSD of the baseline MOSFET drain current for the cases of with and without Faraday cages.

with 100 ms rise time setting for the current preamplifier and with Faraday cage with 0.3 ms rise time setting for the current preamplifier. The power spectra are expressed in units of decibels with a reference of 1 A^2 . The solid line shows the power spectrum density (PSD) of the baseline MOSFET drain current shown in Fig. 5 when the experimental setup was shielded with a Faraday cage, and the dotted line shows the PSD of the baseline MOSFET drain current shown in Fig. 4 when a Faraday cage was not used. Figure 7 indicates that in addition to the $1/f$ flicker noise at very low frequencies ($<10 \text{ Hz}$), noise at 60 Hz and its harmonic frequencies are present in our measurement. Figure 7 indicates that the 60 Hz noise can be reduced to an acceptable level either by using a long rise time for the current preamplifier or by using a short rise time for the current preamplifier and shielding the experimental setup with a Faraday cage.

It is worth noting that we did not shield our experimental setup by using a Faraday cage because at the experimental characterization stage, we wanted to perform concurrent optical observations to validate the electrical signals. The noise suppression in the above reported results is mainly achieved by a relatively large rise time of the current preamplifier (100 ms) and a low cut-off frequency (30 Hz) of the low-pass filter, which may not be desirable for some applications. However, this is because the measurements were performed in open space without a Faraday cage, which is used in nearly all other Coulter-type sensors. Further noise characterization showed that if a shorter rise time was used, the observed noise was mainly 60 Hz, which can be eliminated by using a Faraday cage, as shown in Fig. 5. Using a Faraday cage to shield the measurement setup can help achieve a noise level of 0.4% with a much shorter rise time of the current preamplifier (0.3 ms) and a much higher cut-off frequency of the low-pass filter (1 kHz).

C. Device sensitivity as a function of V_G

As demonstrated above, the amplification of the sensing channel resistance modulation is a function of the MOSFET gate voltage and depends on the MOSFET operating region, as shown in Fig. 8. The characterization was performed with the 7.5 mM sodium borate buffered solution with $9.86 \mu\text{m}$ in diameter polystyrene microbeads and the settings of the power supplies were $V_{DS}=0.15 \text{ V}$ and $V_{-}=-29 \text{ V}$. In the experiment, the MOSFET gate voltage was varied by adjust-

FIG. 8. Drain current modulation for 9.86 μm diameter polystyrene microbeads as a function of gate voltage with $V_- = -29$ V and $V_{DS} = 0.15$ V. V_+ was adjusted to obtain different gate voltages on the MOSFET. The points represent experimental data and the solid curves represent theoretical prediction based on Eqs. (3) and (8). The dashed line represents the approximate theoretical prediction in the subthreshold region based on Eq. (9).

ing V_+ . It can be clearly seen from Fig. 8 that the amplification factor continuously varies as the gate voltage changes and the amplification effect is more significant when the MOSFET works in the subthreshold region. In the subthreshold region, the MOSFET drain current modulation only marginally changes with the MOSFET gate voltage, while in the region above threshold, the drain current modulation decreases as the MOSFET gate voltage increases.

The calculated values of the MOSFET drain current modulation as a function of the gate voltage are also calculated and presented in Fig. 8. In the saturation region, the theoretical prediction based on Eq. (3) fits the experimental results very well except in the transition region close to the threshold voltage. However, a significant difference was observed between the experimental results and the theoretical prediction [Eq. (9)] in the subthreshold region. This discrepancy indicates that the assumption that $\psi_s \sim V_G$ is not a good approximation in this case. Therefore, we fitted the experimental data in the subthreshold region to Eq. (8), which reflects the more realistic performance of the MOSFET. A set of fit parameters of $a = 6.6$ and $V_{FB} = -0.3$ V yielded an acceptable theoretical curve that fits the experimental data. These values are reasonable as the typical value of a ranges from 0.3 to 30, and V_{FB} depends on the material used for the gate metal and typically ranges from -0.5 to 0.5 V, as stated in Ref. 40.

Note that the vertical spread in the data can be attributed to the intrinsic size dispersion of the 9.86 μm microbeads used and this vertical spread is greater in the subthreshold region compared to that in the above-threshold region. This is because the device is more sensitive to the resistance change caused by the bead size dispersion when the MOSFET is operating in the subthreshold region since the total amplification factor is larger in this region, as shown in Sec. III.

D. Device sensitivity as a function of the electrokinetic bias

As mentioned in Sec. III the amplification factor is also a function of current through the fluidic circuit, and hence a function of the applied electrokinetic bias. The device sensi-

FIG. 9. Drain current modulation for 9.86 μm polystyrene microbeads as a function of V_- for a constant gate voltage of ~ 1.81 V and $V_{DS} = 0.15$ V. The points represent experimental data and the solid line represents the theoretical prediction based on Eq. (8) and the dashed line represents the theoretical prediction based on Eq. (10).

tivity is expected to linearly vary with V_- because ideally, the IR_1 term in Eqs. (3), (8), and (9) is equal to $V_G - V_-$. When the MOSFET is operating in the subthreshold region, the drain current modulation given by Eq. (9) is

$$\frac{\Delta I_D}{I_D} \approx \left(\frac{q}{kT} - \frac{1}{2V_G} \right) (V_G - V_-) \left(\frac{R}{R_t} \right) \frac{\Delta R}{R}. \quad (10)$$

The dependence of the drain current modulation on V_- is similar in the saturation region with the same IR_1 substitution made in Eq. (5).

The drain current modulation of 9.86 μm in diameter polystyrene microbeads was measured as a function of V_- while holding the MOSFET gate voltage constant at about 1.81 V. The MOSFET was operating in the subthreshold region. This was achieved by accordingly adjusting V_+ to keep the measured MOSFET drain current constant. Figure 9 shows the performance of our device as a function of V_- and a linear relationship between the drain current modulation and V_- is clearly observed, as expected.

The theoretical prediction based on Eq. (10) is also presented in Fig. 9 as the dashed line and again, some discrepancy is observed between the experimental results and the theoretical prediction, which confirms the observation in Sec. IV C that the approximation that $\psi_s \sim V_G$ is not a very good one. However, using Eq. (8) with the same fitting parameters ($a = 6.6$, $V_{FB} = -0.3$ V) as in Sec. IV C (Fig. 9, solid line) results in a much better match between the calculation and experiment. We were not able to measure drain current modulations for $|V_-| < 29$ V because the electro-osmotic flow was very slow and most of the beads stick to the channel walls after a short time of experiment.

E. Detection of particles with similar sizes but different surface charges

We tested the ability of our device to distinguish two similarly sized microbeads with different surface charges. Nominally 4.84 μm diameter polystyrene and 4.8 μm diameter glass microbeads were used to test this capability of the device. Glass microbeads are known to have relatively large negative surface charge densities⁴⁴ in aqueous electrolyte solutions and will experience an electrostatic force that opposes the electro-osmotic flow of the microbeads.⁴⁵ There-

FIG. 10. MOSFET drain current as a function of time for a mixture of $4.8 \mu\text{m}$ in diameter glass and $4.84 \mu\text{m}$ in diameter polystyrene microbeads with $V_- = -29.1 \text{ V}$, $V_+ = 11.52 \text{ V}$, and $V_{DS} = 0.15 \text{ V}$. The gate voltage of the MOSFET is determined to be about 1.84 V , so the MOSFET is operating in the subthreshold region.

fore, the net speed of the glass beads through the small microchannel will be reduced and the translocation time of the glass beads through the sensing channel should be longer than that of the polystyrene beads of much lower surface charges. Figure 10 shows the MOSFET drain current as a function of time when we added a mixture of the above-mentioned polystyrene and glass beads. The power supply settings are $V_- = -29.1 \text{ V}$, $V_+ = 11.52 \text{ V}$, and $V_{DS} = 0.15 \text{ V}$. The MOSFET gate voltage was $\sim 1.84 \text{ V}$, so the MOSFET was operating in the subthreshold region.

As expected, the magnitude in the drain current modulation observed for both types of microbeads is very similar because of their similar sizes. The glass microbeads took a longer time to translocate through the sensing channel compared to the polystyrene microbeads, as evidenced by the width of the drain current modulations. This has been confirmed by concurrent optical observations with the optical microscope. In fact, the time taken for the glass microbeads to translocate through the sensing channel was $\sim 600 \text{ ms}$, in comparison with $\sim 400 \text{ ms}$ for the polystyrene microbeads. This shows the ability of our device to distinguish particles of similar sizes but different surface charges.

F. Characterization of microbead size distribution

A useful function of a standard Coulter counter is to detect the size of particles. To characterize the device's ability of detecting the size distribution of microbeads, we measured the size distribution of the nominally monodisperse $9.86 \mu\text{m}$ diameter polystyrene beads with the MOSFET-based Coulter counter. We also measured the diameter distribution of the polystyrene beads with a scanning electron microscope (SEM) (Raith eLiNE SEM). The manufacturer (Bangs Laboratories, Inc.) quoted that nominal mean sphere diameter of the beads is $9.86 \mu\text{m}$ with a standard deviation of $0.65 \mu\text{m}$, as measured with a Coulter principle-based particle sizer. The measurement with the MOSFET-based Coulter counter was performed with the following parameters: $V_- = -29 \text{ V}$, $V_+ = 11 \text{ V}$, and $V_{DS} = 0.15 \text{ V}$, leading to $V_G \sim 1.79 \text{ V}$. Thus, the MOSFET was working in the subthreshold region.

Figure 11(a) shows the size distribution of the $9.86 \mu\text{m}$ diameter microbeads obtained with the SEM, in which the

FIG. 11. (a) Size distribution of $9.86 \mu\text{m}$ diameter polystyrene microbeads obtained from SEM measurements. (b) Distribution of drain current modulation observed for $9.86 \mu\text{m}$ diameter microbeads. The vertical bars represent a histogram of experimental data and the lines represent a Gaussian fit to the data.

vertical bars represent a histogram of experimental data and the curve represents a Gaussian fit to the data. The diameters of a total of 199 microbeads were directly measured by using the SEM, and a mean microbead diameter of $9.82 \mu\text{m}$ with a standard deviation of $\sigma = 0.29 \mu\text{m}$ was obtained from the Gaussian fit to the data. Thus, the direct SEM measurements showed that the size distribution of the $9.86 \mu\text{m}$ beads was about half of the quoted value from the bead manufacturer. Figure 11(b) shows the distribution in the drain current modulations observed by using the MOSFET-based Coulter counter when $9.86 \mu\text{m}$ in diameter polystyrene beads were translocated through the sensing channel. The total number of samples was kept the same as that for the SEM measurement, and from the Gaussian fit to the data, a mean drain current modulation of 54.4% , with a standard deviation of $\sigma = 1.95\%$, was obtained.

To enable a direct comparison between the size distributions obtained between the two measurements, the data were normalized by the mean of the respective Gaussian distributions: $9.82 \mu\text{m}$ for the SEM data and 54.4% for the MOSFET-based Coulter counter. Figure 12(a) shows the normalized data from the SEM measurement and Fig. 12(b) shows the normalized data from the MOSFET-based Coulter counter measurement. Because of the normalization, the mean in both Figs. 12(a) and 12(b) was 1.0. The standard deviation for the normalized SEM data shown in Fig. 12(a) was 0.028 and the corresponding value for the normalized MOSFET-based Coulter counter data shown in Fig. 12(b) was 0.035. Therefore, the size distribution of the $9.86 \mu\text{m}$ in diameter beads measured with the two techniques matches rather well.

FIG. 12. (a) Normalized size distribution of $9.86\ \mu\text{m}$ diameter microbeads obtained by direct SEM measurement. The normalized mean was 1.0 and the standard deviation was 0.028. (b) Normalized size distribution of $9.86\ \mu\text{m}$ diameter microbeads obtained using the new-concept Coulter counter. The normalized mean and standard deviation were 1.0 and 0.035, respectively.

It is worth noting that for the normalized distribution of spherical particles, we expected the standard deviation from the MOSFET-based Coulter counter to be three times of the standard deviation from the diameter measurement of the SEM because the signal from the Coulter counter is proportional to the volume of the beads. However, the observed standard deviation from the counter is only slightly higher than that from the SEM measurement. A full analysis of this difference will depend on understanding the intrinsic resolution of each probe and the manner in which the resolution function couples to the actual data.

V. SUMMARY

In summary, we report the detailed experimental characterization of a design of the Coulter-type fluidic sensor and demonstrate that this MOSFET-based Coulter counter can increase the sensitivity by amplifying the percentage of the modulation caused by the translocation of particles through the sensing channel. The detected minimum volume ratio between the particle and the sensing channel is about 0.006%, which is approximately ten times smaller than the lowest volume ratio previously reported in the literature. The sensitivity of the MOSFET-based Coulter counter has been characterized as a function of the MOSFET gate voltage, showing that the device is more sensitive when operated in the subthreshold region as compared to when it is operated in the saturation region. In addition, the sensitivity with respect to the applied electrokinetic bias V_{L} has been investigated and it is shown that the drain current modulation linearly

varies with V_{L} . The MOSFET-based Coulter counter can distinguish particles both with different sizes and with similar sizes but different surface charges based on the observed amplitude of the MOSFET drain current modulation and the translocation time. It is shown that the size dispersion of $9.86\ \mu\text{m}$ in diameter polystyrene beads measured with the MOSFET-based Coulter counter is comparable to that from direct SEM observation.

ACKNOWLEDGMENTS

The authors wish to acknowledge the financial support from NSF (Award Nos. CTS-0507903 and CBET-0643583), NIH (Grant No. 5R01HG002647), and DARPA (Grant No. W911NF-07-1-0046). The authors thank Dr. Ronald Schrimpf for helpful suggestions. M.S. thanks John Rozen and Eugene Donev for valuable discussions and D.X. thanks Xudong Wu for valuable discussions. A portion of this research was conducted at the Center for Nanophase Materials Sciences, which is sponsored at Oak Ridge National Laboratory by the Division of Scientific User Facilities, U.S. Department of Energy.

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