Demonstration of Coulter counting through a cylindrical solid state nanopore

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Abstract. The technique known as a Coulter counting, or resistive-pulse sensing, can be used to measure the size of a nanoscale analyte as it passes through a fluidic constriction separating two reservoirs. We have developed a fabrication procedure capable of reproducibly manufacturing cylindrical nanopores with diameters as small as 20 nm using a silicon-on-insulator substrate and electron beam lithography. The ionic conductance of these nanopores was measured across six orders of magnitude in electrolyte concentration. Polystyrene nanoparticles were then passed through the cylindrical pores while monitoring the current that flowed due to a constant bias voltage. Current pulses due to the passage of individual nanoparticles of various dimensions through a nanopore were observed and compared to theory.

1. Introduction
There are various applications for nanoscale apertures that provide a fluidic path between two reservoirs. These applications include patch-clamp type measurements [2], Coulter counting or resistive-pulse sensing [3] and molecular studies [4]. The reduction in aperture dimensions results in operational improvements in many of these applications [5].

In the application of Coulter counting, the ionic current through a fluidic impedance is monitored while applying a constant bias voltage [3]. The presence of a constant bias generates a continuous ionic flux. Through various transport mechanisms, analytes with dimensions similar to the diameters of the cylindrical aperture may pass through the channel. These transport mechanisms include diffusive flux due to a concentration gradient, pressure driven flow, electrophoretic transport and electroosmotic transport.

The passage of analytes through the aperture reduces the volume of the channel, resulting in an increased impedance, Figure 1. The momentary increase in impedance, \( \Delta R \), is observed as a reduction in the ionic current. The amplitude of the resistance increase due to a spherical analyte is described by:

\[
\Delta R = \frac{2}{3} \frac{4 \rho d^3}{\pi D^2} \left[ 1 - 0.8 \left( \frac{d}{D} \right)^3 \right]^{-1}
\]  

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where \( \rho \) is the solution resistivity, \( d \) is the particle diameter, and \( D \) is the aperture diameter [6]. Equation (1) includes a number of end corrections as described in [6]. The magnitude of the current spike, \( \Delta I \), is described by:

\[
\Delta I = I_{\text{init}} \left( \frac{\Delta R}{R_{\text{init}} + \Delta R} \right)
\]  

(2)

Therefore, for an aperture of known geometry, the size of a passing analyte may be measured by observing the induced current spike.

2. Experimental Platform
We have developed a process capable of reproducibly fabricating cylindrical apertures in a silicon-on-insulator substrate with diameters less than 30 nm that can be used for the Coulter counting of nanoscale analytes. The fabrication process utilizes electron beam lithography for the lithographic definition of the apertures enabling accurate control of final device dimensions while maintaining an anisotropic etch profile resulting in a cylindrical aperture. A cross-sectional scanning electron micrograph of a single nanopore is shown in Figure 2. The nanopores are fabricated in the 340 nm thick device layer of a silicon-on-insulator wafer with micron scale cavities etched through the buried silicon dioxide layer and the handle wafer. After a final oxidation step the length of the pore is increased to 360 nm. The details of the fabrication procedure have been published elsewhere [7, 8].

The inset to Figure 2 shows some narrowing of the pores (~ 20%) at either end before they flare out at the entrance/exit to the pore. Numerical simulations of the final oxidation step indicate that for thin oxides (<20 nm) the narrowing of the pores is negligible with the oxide planes parallel to the etched silicon surfaces. For longer oxidations (25 nm < \( t_{\text{ox}} < 100 \) nm), the corners became rounded resulted in a cylindrical aperture with a flared entrance and exit as shown in Figure 2. For very long oxidations (>200 nm), apertures initially patterned as circles resulted in squares or rectangles.

Figure 2. Cross-sectional scanning electron micrograph of a single 40 nm cylindrical nanopore of length 360 nm in a silicon-on-insulator substrate. The top layer containing the nanopore is the device silicon layer while the cavity below it is a void fabricated in the buried oxide layer. The handle wafer is still present in this image to allow for manual scribing of the device.
3. Experimental Results

The ionic conductance of various apertures as a function of KCl concentration has been measured for pores of different diameter as shown in Figure 3. It was found that at high electrolyte concentrations the ionic conductance through the aperture is consistent with a cylindrical impedance having a constant resistivity equal to that of the bulk electrolyte. However, at low electrolyte concentrations the conductance is enhanced due to the presence of a surface conductance mechanism.

Polystyrene nanoparticles of known dimensions were suspended in 0.1 M KCl electrolyte solution with a concentration of $5 \times 10^{11}$ spheres/ml. Triton X-100 surfactant (0.1% w/v) was added to the suspension to prevent the aggregation of the nanoparticles.

Post characterization of a 240 nm aperture, the fluid reservoirs were exchanged with the solution containing the nanoparticles. Voltage sweeps from 0 mV to 200 mV were conducted in 25 mV increments while monitoring the steady-state current. Nanoparticle solutions were prepared with nanoparticles having diameters of 60 nm, 100 nm and 130 nm. Shown in Figure 4 and Figure 5 are example current traces at 100 mV showing nanoparticle transition events.

![Figure 3](image_url)  
**Figure 3.** Ionic conductance measurements of four cylindrical nanopores of varying diameters, from 78 nm to 240 nm, measured with KCl buffer concentration over six orders of magnitude.

![Figure 4](image_url)  
**Figure 4.** Ionic current passing through a 240 nm cylindrical aperture while applying +100 mV bias in the presence of 100 nm nanoparticles. The downward spikes originating at the baseline represent individual transitional events.

![Figure 5](image_url)  
**Figure 5.** Magnified individual transitional event from Fig. 4 of a particle passage through the nanopore. The magnitude of the current pulse is approximately 5% of the baseline current.
The transition of the different size nanoparticles was monitored at various applied biases and the average depth of the current spikes, $\Delta I$, was measured and compared to the theoretical values determined from equations (1) and (2), as shown in Fig. 6.

![Figure 6. Comparison of the theoretical $\Delta I$ (lines) as compared with the experimentally observed $\Delta I$ (points) for 130 nm, 100 nm and 60 nm diameter nanoparticles (from top to bottom) passing through a 240 nm diameter nanopore.](image)

4. Conclusion
Cylindrical nanopores of length 360 nm with diameters in the range 78-240 nm have been fabricated using standard silicon processing techniques. The ionic current flowing through a pore of diameter 240 nm showed very clear Coulter counting events in the presence of 60nm, 100nm and 130nm nanoparticles. By optimizing the length and diameter of the nanopore it will be possible to characterize the size distribution of nanoparticles down to the sub-10 nm regime.

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