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Citation: **72**, 4449 (2001); doi: 10.1063/1.1419224

View online: <http://dx.doi.org/10.1063/1.1419224>

View Table of Contents: <http://aip.scitation.org/toc/rsi/72/12>

Published by the [American Institute of Physics](#)

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# Quantitative sensing of nanoscale colloids using a microchip Coulter counter

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(Received 27 June 2001; accepted for publication 20 September 2001)

We have fabricated a microchip Coulter counter on a quartz substrate, and have used it to detect individual nanoscale colloidal particles with a sensitivity proportional to each particle's size. We demonstrate the ability of this device to sense colloids as small as 87 nm diameter, and to distinguish between colloids whose diameters differ by less than 10%. Further reductions in the pore size, easily done with current nanofabrication techniques, make our device applicable to measuring biological macromolecules, such as DNA and proteins. © 2001 American Institute of Physics. [DOI: 10.1063/1.1419224]

## I. INTRODUCTION

Quantitative measurements of the size and concentration of nanoscale particles are critical for studies of colloidal and macromolecular solutions. Traditionally, this is accomplished through ultracentrifugation, chromatography, gel electrophoresis,<sup>1</sup> or dynamic light scattering.<sup>2</sup> Here we discuss an alternative method based on the Coulter technique of particle sensing.<sup>3</sup> Coulter counters typically consist of two reservoirs of particle-laden solution separated by a membrane and connected by a single pore through that membrane. By monitoring changes in the electrical current through the pore as individual particles pass from one reservoir to another, a Coulter counter can measure the size of particles whose dimensions are on the order of the pore dimensions. While this method has long been used to characterize cells several microns in diameter,<sup>4,5</sup> its relative simplicity has led to many efforts to employ it to detect nanoscale particles,<sup>6-9</sup> including viruses.<sup>10</sup>

In this article, we present the first working realization of a Coulter counter on a microchip. Our device, fabricated on top of a quartz substrate using standard microfabrication techniques, utilizes a four-point measurement of the current through the pore. We are able to control precisely the pore dimensions, which we can easily measure using optical and atomic force microscopies. Knowing the exact pore dimensions allows us to predict quantitatively the response of the device to various sized particles. We have fabricated pores with lateral dimensions between 400 nm and 1  $\mu\text{m}$ , and used them to detect latex colloidal particles as small as 87 nm in diameter. Furthermore, we demonstrate the ability of the device to detect  $\sim 500$  nm diam colloids with a resolution of  $\pm 10$  nm. The device has numerous applications in sizing and separating nanoscale particles in solution, and is easily integrated with other on-chip analysis systems.

## II. EXPERIMENT

Our device, shown in Fig. 1(a), is fabricated in multiple stages. Each stage consists of lithographic pattern generation, followed by pattern transfer onto a quartz substrate using either reactive ion etching (RIE) or metal deposition and liftoff. The first stage is the fabrication of the pore. A line is patterned on the substrate using either photolithography (PL) for linewidths  $\geq 1 \mu\text{m}$ , or electron-beam lithography (EBL) for linewidths between 100 and 500 nm, and then etched into the quartz using a  $\text{CHF}_3$  RIE. The substrate subsequently undergoes a second stage of PL and RIE to define two reservoirs that are 3.5  $\mu\text{m}$  deep, separated by 10  $\mu\text{m}$ , and connected to each other by the previously defined channel [see Fig. 1(a)]. The length of the pore is defined in this second stage by the separation between the two reservoirs. The final stage consists of patterning four electrodes across the reservoirs, followed by two depositions of 50/250  $\text{\AA}$  Ti/Pt in an

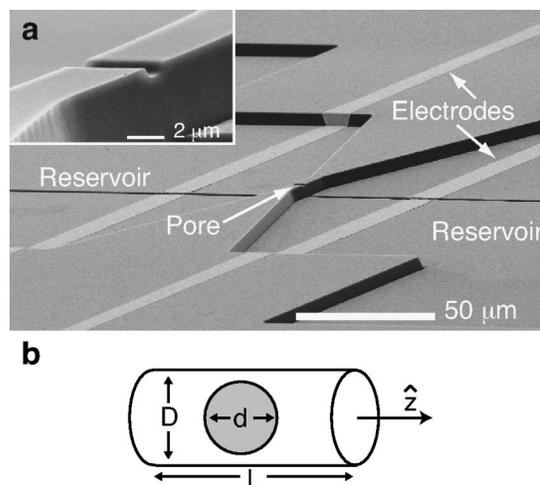


FIG. 1. (a) Scanning electron micrograph of our microchip Coulter counter. The 3.5  $\mu\text{m}$  deep reservoirs and the inner Ti/Pt electrodes, which control the voltage applied to the pore but pass no current, are only partially shown. The outer electrodes, which inject current into the solution, are not visible in this image. The inset shows a magnified view of this device's pore, which has dimensions  $5.1 \times 1.5 \times 1.0 \mu\text{m}^3$ . (b) A schematic diagram of a spherical particle of diameter  $d$  in a pore of diameter  $D$  and length  $L$ .

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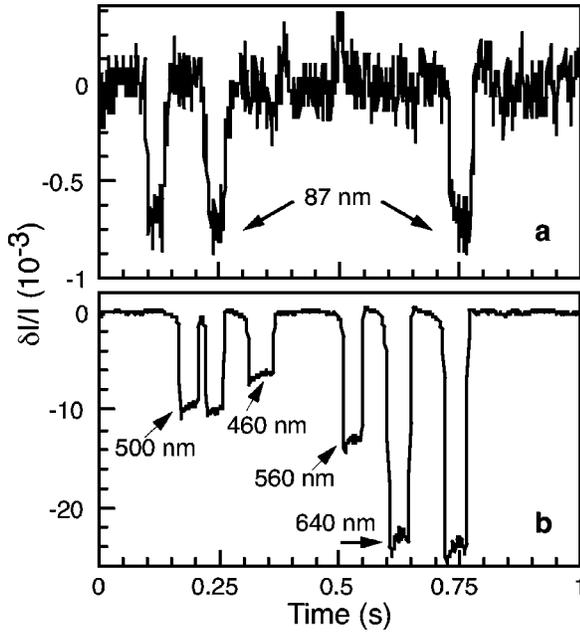


FIG. 2. Relative changes in baseline current  $\delta I/I$  vs time for (a) a mono-disperse solution of 87 nm diam latex colloids measured with an EBL-defined pore of length  $8.3 \mu\text{m}$  and cross section  $0.16 \mu\text{m}^2$ , and (b) a poly-disperse solution of latex colloids with diameters 460, 500, 560, and 640 nm measured with a PL-defined pore of length  $9.5 \mu\text{m}$  and cross section  $1.2 \mu\text{m}^2$ . Each downward current pulse represents an individual particle entering the pore (Ref. 14). The four distinct pulse heights in (b) correspond to the four different colloid diameters.

electron-beam evaporator with the sample positioned  $\pm 45^\circ$  from normal to the flux of metal to ensure that the electrodes are continuous down both walls of the reservoirs.

The device is sealed on top of with a silicone-coated (Sylgard 184, Dow Corning Corp.) glass coverslip before each measurement. Prior to sealing, both the silicone and the substrate are oxidized in a dc plasma to ensure the hydrophilicity<sup>11</sup> of the reservoir and pore and to strengthen the seal<sup>12</sup> to the quartz substrate. After each measurement, the coverslip is removed and discarded, and the substrate is cleaned by chemical and ultrasonic methods.<sup>13</sup> Thus, each device can be reused many times.

We have measured solutions of negatively charged (carboxyl-coated) latex colloids (Interfacial Dynamics, Inc.) whose diameters range from 87 to 640 nm. All colloids were suspended in a solution of  $5\times$  concentrated Tris–Borate–EDTA (TBE) buffer with a resistivity of  $390 \Omega \text{ cm}$  and pH 8.2. To reduce adhesion of the colloids to the reservoir and pore walls, we added 0.05% volume to volume (v/v) of the surfactant Tween 20 to every solution. The colloidal suspensions were diluted significantly from stock concentrations to avoid jamming of colloids in the pore; typical final concentrations were  $\sim 10^8$  particles/ml. The pore and reservoirs were filled with solution via capillary action.

### III. THEORY

The sensitivity of a Coulter counter relies upon the relative sizes of the pore and the particle to be measured. The resistance of a pore  $R_p$  increases by  $\delta R_p$  when a particle

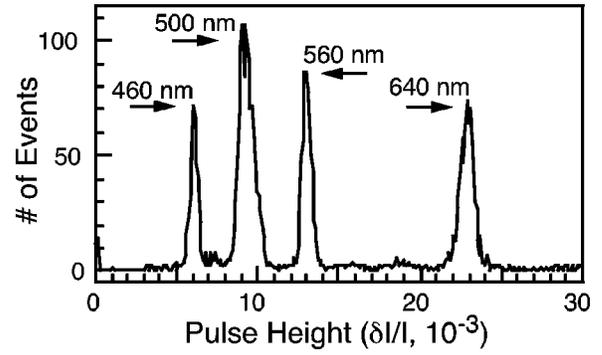


FIG. 3. A histogram of pulse heights resulting from measuring the poly-disperse solution shown in Fig. 2(b). The resolution for this particular device is  $\pm 10$  nm in diameter for the particles measured.

enters since the particle displaces conducting fluid.  $\delta R_p$  can be estimated<sup>5</sup> for a pore aligned along the  $z$  axis [see Fig. 1(b)] by

$$\delta R_p = \rho \int \frac{dz}{A(z)} - R_p, \quad (1)$$

where  $A(z)$  represents the successive cross sections of the pore containing a particle, and  $\rho$  is the resistivity of the solution. For a spherical particle of diameter  $d$  in a pore of diameter  $D$  and length  $L$ , the relative change in resistance is

$$\frac{\delta R_p}{R_p} = \frac{D}{L} \left[ \frac{\arcsin(d/D)}{(1-(d/D)^2)^{1/2}} - \frac{d}{D} \right]. \quad (2)$$

Equations (1) and (2) assume that the current density is uniform across the pore, and thus is not applicable for cases where the cross section  $A(z)$  varies quickly, i.e., when  $d \ll D$ . For that particular case, Deblois and Bean<sup>6</sup> formulated an equation for  $\delta R_p$  based on an approximate solution to the Laplace equation:

$$\frac{\delta R_p}{R_p} = \frac{d^3}{LD^2} \left[ \frac{D^2}{2L^2} + \frac{1}{\sqrt{1+(D/L)^2}} \right] F\left(\frac{d^3}{D^3}\right), \quad (3)$$

where  $F(d^3/D^3)$  is a numerical factor that accounts for the bulging of the electric field lines into the pore wall. When employing Eq. (3) to predict resistance changes, we find an effective value for  $D$  by equating the cross sectional area of our square pore with that of a circular pore.

If  $R_p$  is the dominant resistance of the measurement circuit, then relative changes in the current  $I$  are equal in magnitude to the relative changes in the resistance  $|\delta I/I| = |\delta R_p/R_p|$  and Eqs. (2) and (3) can both be directly compared to measured current changes.

This comparison is disallowed if  $R_p$  is similar in magnitude to other series resistances, such as the electrode/fluid interfacial resistance  $R_{e/f}$  or the resistance  $R_u$  of the reservoir fluid between the inner electrodes and the pore. We completely remove  $R_{e/f}$  from the electrical circuit by performing a four-point measurement of the current [see Fig. 1(a)]. We minimize  $R_u$  by placing the inner electrodes close to the pore ( $50 \mu\text{m}$  away on either side), and by designing the reservoir with a cross section much larger than that of the pore. For a pore of dimensions  $10.5 \mu\text{m}$  by  $1.04 \mu\text{m}^2$ , we measured  $R_p = 36 \text{ M}\Omega$ , in good agreement with the  $39 \text{ M}\Omega$  value pre-

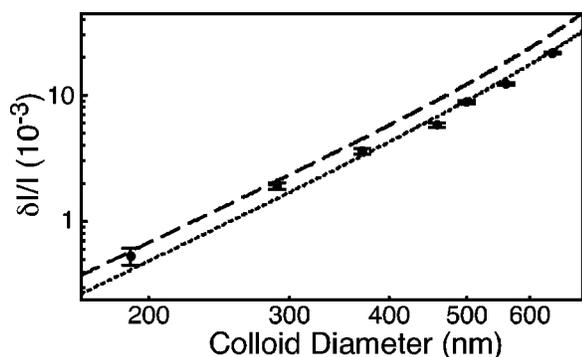


FIG. 4. Comparison of measured  $\delta I/I$  values (circles) to those predicted by Eq. (2) (dotted line) and Eq. (3) (dashed line). The measured data were taken over several runs on a single PL-defined pore of length  $10.6 \mu\text{m}$  and cross section  $1.04 \mu\text{m}^2$ . Error bars for the larger colloid sizes are obscured by the size of the plotted point. As the colloid diameter increases, there is a transition from agreement with Eq. (3) to Eq. (2). This reflects the fact that the derivation of Eq. (3) assumes the colloid diameter  $d$  is much less than the pore diameter  $D$ ; conversely Eq. (2) relies on an assumption that holds only as  $d$  approaches  $D$ , and breaks down for smaller colloids.

dicted by the pore geometry and the solution resistivity. This confirms that we have removed  $R_u$  and  $R_{eff}$  from the circuit.

#### IV. RESULTS AND DISCUSSION

Figure 2 shows representative data resulting from measuring a monodisperse solution of colloids 87 nm in diameter with an EBL-defined pore [Fig. 2(a)], and from measuring a polydisperse solution containing colloids of diameters 460, 500, 560, and 640 nm with a PL-defined pore [Fig. 2(b)]. Each downward current pulse in Fig. 2 corresponds to a single colloid passing through the pore.<sup>14</sup> For the data shown, 0.4 V was applied to the pore. In other runs, the applied voltage was varied between 0.1 and 1 V to test the electrophoretic response of the colloids. We found that the width of the downward current pulses varied approximately as the inverse of the applied voltage, as is expected for simple electrophoretic motion

Figure 3 shows a histogram of  $\sim 3000$  events measured for the polydisperse solution. The histogram shows a very clear separation between the pore's response to the differently sized colloids. The peak widths in Fig. 3 represent the resolution of this device, which we find to be  $\pm 10$  nm in diameter for the measured colloids. This precision approaches the intrinsic variations in colloid diameter of 2%–

4%, as given by the manufacturer. In this run, the maximum throughput was 3 colloids/s, a rate easily achievable for all of our samples. Event rates are limited by the low concentrations needed to avoid jamming.

We used a device whose pore size was  $10.5 \mu\text{m}$  by  $1.05 \mu\text{m}^2$  to measure colloids ranging from 190 to 640 nm in diameter. Figure 4 shows a comparison between the measured mean pulse heights and those predicted by Eqs. (2) and (3). As shown, there is excellent agreement between the measured and calculated values, with the measured error insignificant compared to the range of pulse heights. In addition, the measurements more closely follow Eq. (3) for small  $d$  and Eq. (2) for larger  $d$ , as was anticipated in the derivation of those equations.

#### ACKNOWLEDGMENTS

The authors would like to thank M. W. Wu for assistance in obtaining the electron micrographs. O.A.S. acknowledges support from the Fannie and John Hertz Foundation. This work was supported in part by DARPA and an NSF CAREER award.

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- <sup>13</sup>After removing the coverslip, the substrate is soaked in Amtex CCR (Amtex Chemical Co.) for several hours to remove silicone residue, then rinsed in de-ionized water. Remnant colloids are removed by briefly sonicating the substrate in toluene, then rinsing in methanol. Finally, all remaining particulate residue is removed using the RCA SC1 cleaning procedure, which consists of a heated bath of 5:1:1  $\text{H}_2\text{O}:\text{NH}_4\text{OH}:\text{H}_2\text{O}_2$ . The substrate is then ready to be resealed.
- <sup>14</sup>We have seen events that clearly correspond to two colloids simultaneously inhabiting the pore. Such two-particle events are easily differentiated from one-particle events by their anomalous pulse heights and widths.