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THE CONSTRUCTION OF A MICRO-COULTER COUNTER DEVICE

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ABSTRACT

This report explains the general applications of the Coulter counter, discusses its benefits, and reviews literature in which researchers have used the device as a research tool and in biosensor applications. Coulter counters are electrofluidic devices commonly used to measure the size and the number of particles in a sample. The paper also reports the design and fabrication of a Coulter counter device constructed using Polydimethylsiloxane (PDMSX) and also one of a carbon nanotube based Coulter counter. These devices were desired to sense particle size through a change in resistance displayed through a current spike. Microfabrication was required to construct the device and prepare it for electrical and optical sensing. Potential concerns such as fabrication optimizing and mishaps, troubles with trapping, and as pore filling issues were evaluated and tested. Not all issues were resolved, and a functional Coulter counter device was not completed. However, with detailed microfabrication steps, revised trapping procedures, and improved troubleshooting, the groundwork for a functional device was laid out for implementation in the near future.

Table of Contents

1. Introduction
2. Background
    - 2.1 Coulter Principle
    - 2.2 Resistance Change and Sensor Performance
3. Literature Review
    - 3.1 Characterizing Yeast Cells
    - 3.2 Efficiency of Aerosol Samplers
    - 3.3 Implementation as a Biosensor
4. EXPERIMENTAL METHODS
    - 4.1 PDMS Device Design
    - 4.1.1 Testing
    - 4.1.2 Results
    - 4.2 Silicon/Glass Wafer Device
    - 4.2.1 Device Design
    - 4.2.2 Testing
    - 4.2.3 Silicon/Glass Wafer
5. DISCUSSIONS AND CONCLUSIONS
6. ACKNOWLEDGMENTS

1. INTRODUCTION

Science and engineering are being driven by the emerging field of nanotechnology. Many researchers believe that nanoscience may serve as a building block in the development of a sustainable society. One potential nanoscale device is the Coulter counter.

Coulter counters are electrofluidic devices commonly used for measurement of microscopic particles [1] such as in blood sample analysis. The Coulter counter helped revolutionize the complete blood count (CBC), the standard initial blood test for a majority of medical practices. Blood quality can be analyzed rapidly, objectively, as well as quantitatively [2].

The Coulter counter can also be used as a chemical sensor or biosensor. Counters have been readily found capable in the detection of target analytes binding to functionalized nanoparticles. As the analytes bind to the nanoparticles, the visible mass of the particles seems to change and can be readily detected by observing the change in the electric current's magnitude passing through the nanotube or nanopore. Because of the favorable scaling laws in microfluidics, specifically surface-to-volume ratios, it is desirable to scale down the Coulter counter for use as a chemical or biosensor. Minimizing the counter can vastly improve these applications, as well as making procedures more chemically safe, cheaper, and more efficient than ever before [1].

This paper provides an overview of the basic Coulter principle as well as covering some specific device designs and experiments done in an attempt to implement a carbon nanotube based Coulter counter. Coulter counters' use as a research tool and in biosensor applications is also briefly reviewed.

2. BACKGROUND

2.1 Coulter Principle

The Coulter principle is illustrated in Figures 1 and 2.

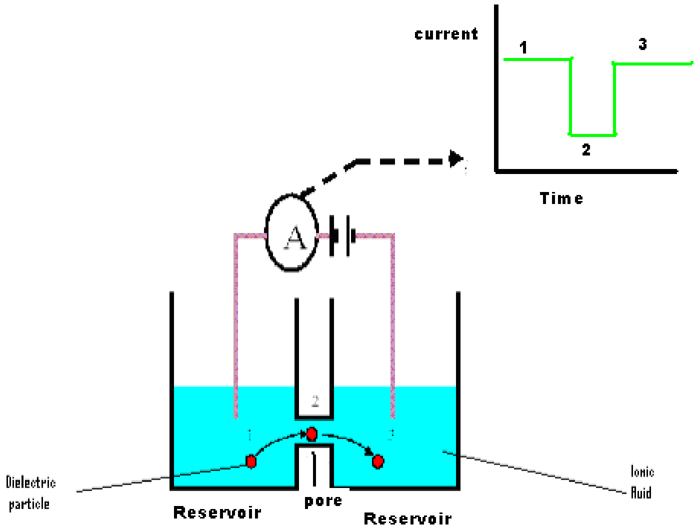
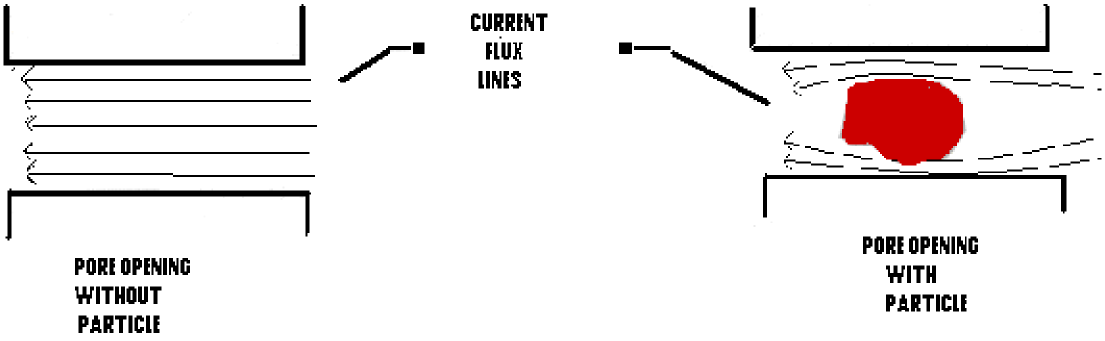


Figure 1. Basic Coulter principle – resistive pulse technique.



a) b)

Figure 2. Particles in the pore bending the electrical flux lines.

As Figure #1 shows, a dielectric particle immersed in ionic fluid [2] is transferred through a pore by electrophoresis. As the particle enters the pore the ionic current fluctuates (See Figure #2). If the dominant resistance in the system is the pore, this can be modeled as two resistors in parallel; otherwise the resistance with the particle in the pore will be greater. The resistance in Figure 2a will be significantly less than the resistance in Figure 2b. The change in resistance will appear as a current or resistance spike. The magnitude can be used to determine the volume of the particle [3].

The relationship between Δresistance and volume can be expressed mathematically using equation 1:

$$\Delta R = \frac{-2\rho_f \pi^2}{A} \int_0^b \frac{(b^2 - l^2)}{\left[1 - \frac{\pi(b^2 - l^2)}{A}\right]} dl = -\frac{4\rho_f}{\pi D}\left[\frac{\sin^{-1}(d/D)}{\sqrt{1-(d/D)^2}} - \frac{d}{D}\right]$$

Where ρ_f = electrical resistance of the fluid, D = diameter of pore, and d = diameter of particles.

2.2 Resistance change and sensor performance

With other conditions such as noise, a device is assumed able to detect only approximately five percent of change in resistance. Figure #3 displays the resistance change in the pore with the addition of about 20 nm in diameter to the particle. For this test, it is assumed that the ratio of the pore diameter and particle diameter will be constant, 2 to 1.

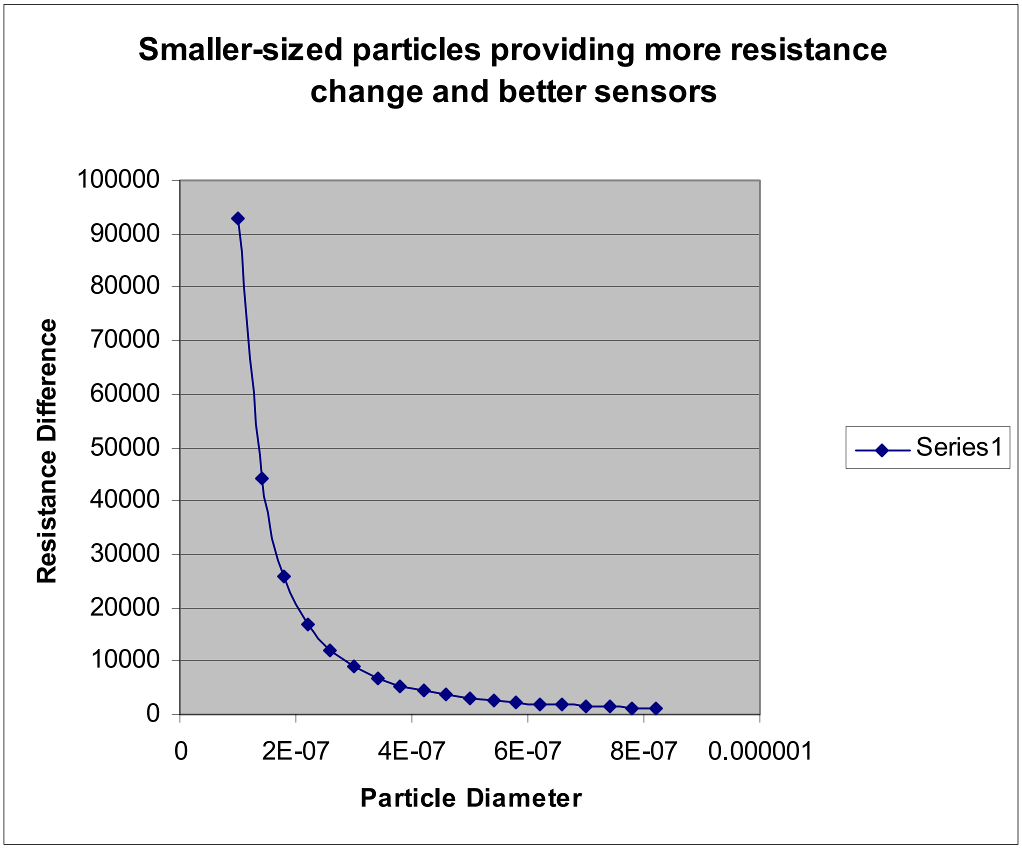


Figure 3: Shows how smaller-sized particles provide a greater resistance difference

The size of particles counted must be comparable to the size of the pore used in order for detection to occur [4]. Figure #4 shows how the surface to volume ratio exponentially increases as the particle size decreases. To obtain the surface area to volume ratio, the equations for surface area and volume were used:

$$\text{Surface Area} = 4 \times \pi \times Radius\verb|^|2$$

$$\text{Volume} = \frac{4}{3} \times \pi \times radius\verb|^|3$$

$$S/_{V} \text{ Ratio} = \frac{4 \times \pi \times Radius\verb|^|2}{\frac{4}{3} \times \pi \times Radius\verb|^|3} = \frac{3}{Radius}$$

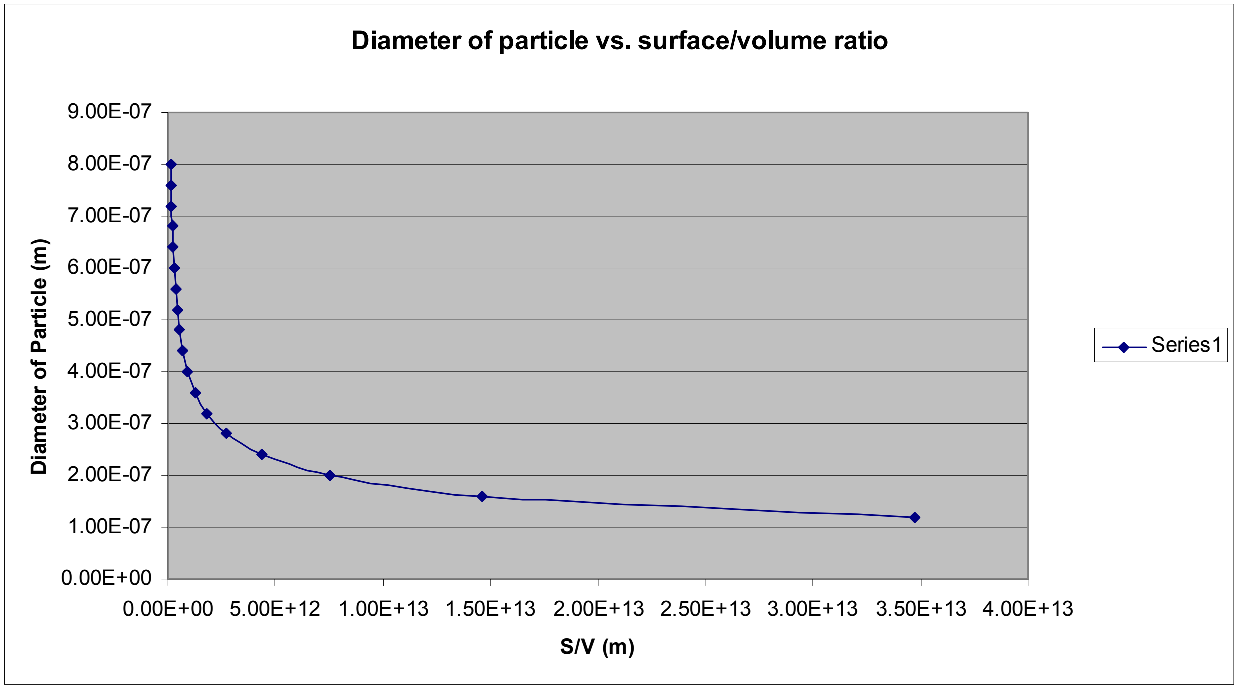


Figure 4: Smaller particles have greater surface to volume ratios

As the radius approaches infinity the surface-to-volume ratio approaches zero, while as the radius approaches zero, the surface-to-volume ratio approaches infinity.

As Figure #4 shows, the particles with the smaller diameter have greater slopes in their resistance, while the larger-sized particles have a change in resistance that tends to flatten out, thus showing that the smaller particles are the only ones whose resistance will change by more than five percent. The data from these graphs clearly show that the resistance is greatly affected by the volume of the particle that enters the pore. Figures # 3 and 4 show that the smaller particles have a greater surface-to-volume ratio, and thus a greater change in resistance. The charts are combined in figure #5 to display the connection more clearly. This supports the notion that particle volume plays an essential role in resistivity.

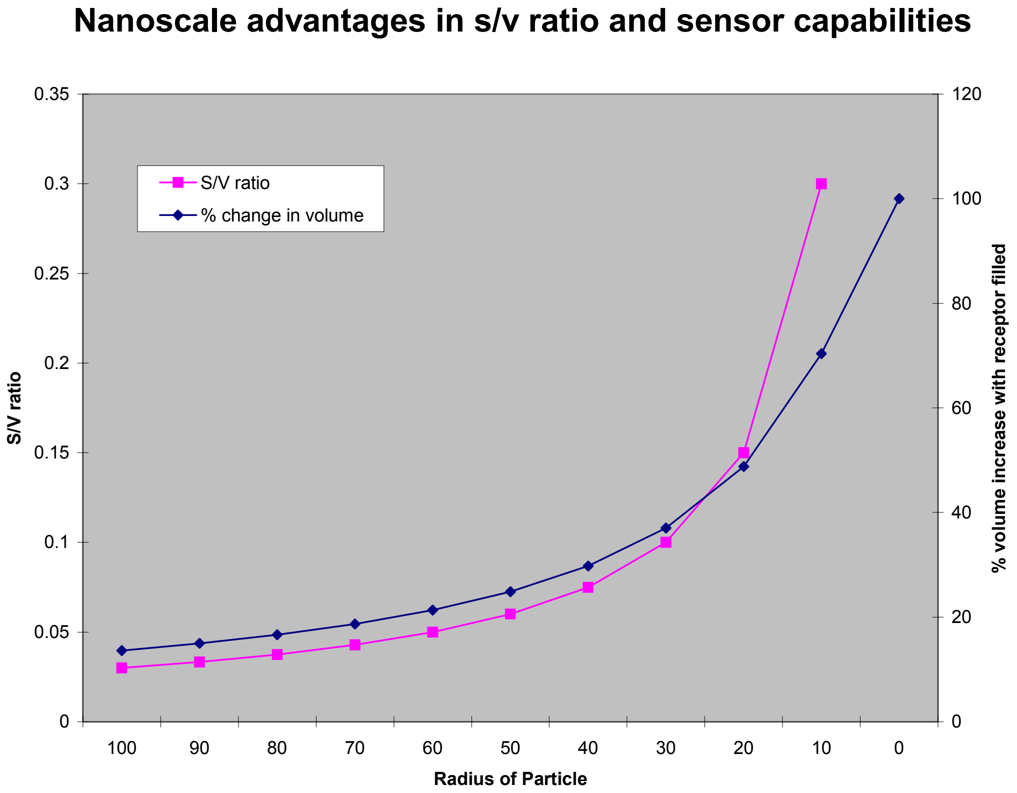


Figure 5: Particle volume playing a key role in s/v ratio and resistivity

3. Literature Review

The Coulter counter has been used in biosensor applications with particle sizes as small as 50 nm in diameter. These counters have been used for everything from blood analysis, to counting biological cells and colloidal particles, to recording debris number in cell tissue [5]; to even evaluating mass transport rates through nanoporous materials [6]. The smaller the tube's (pore) diameter the greater the device's sensitivity. Researchers predict that further reduction in pore size will allow them to build Coulter counters that can detect large polymeric molecules such as antibodies and viruses. Along with its other benefits, including simple construction, rapid production with good reproducibility, and miniaturization, counters will create many new opportunities because many proteins, polysaccharides, and DNA do not have the electrochemical properties or are too small to be detected by available analytic mechanisms.

Various groups used Coulter counters as research tools and attempted to implement Coulter counters as biosensors. The counter produced among the most effective results for their tests. A few of the more significant studies are described below.

3.1 Characterizing Yeast Cells

In a 1997 study, Teeraoatar Srinorakutara [7] at the Thailand Institute of Scientific Research used the Coulter counter to find the cell wall thickness and cell diameter of yeast cells. This experiment involved comparing the yeast cell size distributions using the counter to the size obtained using image analysis. The results showed that although the mean cell diameter could be effectively measured using either image analysis or Coulter counting. Coulter counters were more efficient, because of the simplicity of the device, the mass transport and number capabilities, and its ability to render fast, accurate results. Any direct effects of vacuolation on the cell diameter, such as changing the resistance of the cell, were thought to be small. This theory proved consistent with the notion that volume is proportional to the pulse height and response of the instrument, as there is extremely little effect of particle resistivity on the diameter of the cell.

3.2 Efficiency of Aerosol Samplers

P. Gorner, R. Wrobel, and J.-F. Fabries of the National Institute of Research and Security in France [8] used a Coulter counter to measure the efficiency of aerosol samplers. Aerosol samplers measure the concentration of health-related aerosol fractions in work areas. They must satisfy the international requirements of sampling performance, including the penetration efficiencies for inhalable dust laid down by such hygiene organizations as the International Standards Organization in Geneva. The efficiency is normally measured and determined by comparing the ratio of concentration of sampled and referenced aerosols, as a function of particle aerodynamic diameter which is usually measured by time of flight method. However, for their case of high speed winds, normally a few meters per second, high sampler flow rates, or coarser particles, the researchers used the Coulter counter method. First, the test aerosol was generated, and then neutralized and weighed. The particles were recovered in an electrolytic solution with some surface active agent added. The electrolytic particles were forced through a calibrated pore using platinum electrodes on each side. The penetration efficiency data for the sample were also assessed compared using the time of flight method. The experiment determined that the Coulter counter produced comparable results but was slightly more effective than the time of flight method in determining the efficiency of aerosol samplers [6].

3.3 Implementation as a Biosensor

O.A. Saleh and L.L Sohn of Princeton University [2] implemented the Coulter counter as a biosensor. They fabricated a microchip counter on a substrate made of quartz, and used it to try to detect individual nanoscale colloidal particles that had a sensitivity in proportion to the size of each individual particle. The device was able to sense colloids as small as 87 nm in diameter, and to also discriminate among colloids whose diameters differed by less than 10%. Consistent with the Coulter principle, the researchers performed a test in which four electrodes were patterned across the reservoirs, followed by small samples of platinum in an electron beam evaporator for conductive purposes. The tests were done using solutions of colloids of various diameters and varied applied voltage. It was found that the downward current pulses were inversely proportional as the applied voltage, as was expected. When the experimental data were compared to the predicted data, the measured and calculated values were found to be comparable. By further reducing pore sizes, the researchers concluded, their device would be applicable for measuring macromolecules, including DNA and proteins [2].

4. EXPERIMENTAL METHODS

This section reports on efforts to fabricate and implement a Coulter counter device. Discussed are two methods used: PDMS Coulter counter devices and a microfabricated device using both glass or silicon wafer chips and carbon nanotubes.

4.1 PDMS Device Design

The first device was basic in design. It contained a 2 micron inner diameter tube, which served as the pore. The outer coating of the tube was melted away by a Bunsen burner, enabling the pore to be transparent. Two small tops were to be taken, preferably saucer-style plastic tops. The tops, equal in size, served as the reservoirs in which the particles were submerged. A drill containing a bit designated for plastic materials was used. One tiny hole penetrating each top was drilled. After the tube was connected to the tops, the device was sealed with a silicone coated material called polydimethylsiloxane (Dow Corning Corp.), PDMS, made by mixing a 10 to 1 ratio of the PDMS material with its curing agent. The PDMS was placed in a vacuum in order to remove the air bubbles and was then cured. It generally takes 16-24 hours for the PDMS to cure in air, but with heat applied it cures quicker. After about two hours the still liquid PDMS was poured onto the device and allowed to cure on it. When the PDMS had finally cured, the micron tube was removed, thus leaving a small channel imbedded in the PDMS layer. Next the caps were removed as well, leaving two reservoirs overtop a thin PDMS coat. What remained were two reservoirs connected to a channel, similar to the one displayed in figure #6.

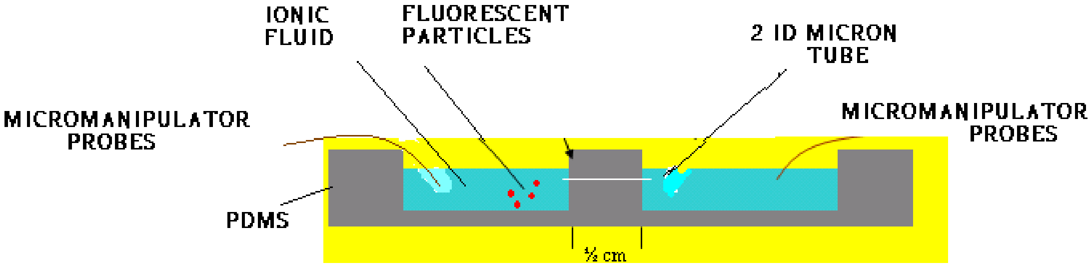


Figure 6. Model of Coulter counter device, similar to the one designed

4.1.1 Testing

For the PDMS device, fluorescent particles were placed into one of the reservoirs, which contained an electrolytic solution, so that the particles could be viewed optically in the pore. Attached at opposite ends of the pore were electrodes attached to micromanipulator probes. The probes were connected to an AC field power source; thus, when the power was turned on, an electric field within the pore was to be created. The voltage between these two fluidic reservoirs was to drive electroosmotic flow, and the fluorescent particles were to be transported by electrophoresis. The voltage needed to drive the particles through was then applied. An arbitrary potential of 10 volts was selected. The fluorescent particles were thus supposed to be viewed traveling through and out of the pore. If the particles were too small to be observed by the naked eye, a microscope would then be used and the device would be constructed under the microscopic lens and viewed at a necessary magnification.

4.1.2 Results

The PDMS device encountered a significant number of problems and therefore was disregarded over time. The first device was faulty in that the PDMS did not cure properly. There were many bubbles within the device, especially near the opening of the pore. The PDMS was also not solid in some areas. Several attempts were made to properly cure the PDMS as well as vacuum out any bubbles that arose during the curing stages. These attempts finally provided successful results. However, after the device was made, problems developed when filling the pore. As the particles were poured into the reservoir containing water and a hydrolytic solution, a 10 volt potential was applied. Neither the particles nor any liquid traveled through the pore, nor did any liquid. Tinkering with the voltage appeared to have no real effects on the device. Something within the pore was not allowing for transport, perhaps an air bubble. After weeks testing out possible solutions, the use of a PDMS device was halted and a silicon/glass device was introduced.

4.2 Silicon/Glass Wafer Device

Other Coulter counter devices used in the experiment employed glass or silicon wafer chips in the microfabrication of a functional device. The fabrication of these devices meshed customary microfabrication techniques with the dielectrophoretic trapping of single carbon multi-walled nanotubes.

4.2.1 Device Design

The first step in designing the device used a p-type silicon wafer. The wafer was cleaned with acetone and isoproponal. A small 1 micron layer of silicon dioxide (SiO_2) was applied to the top of the silicon. The SiO_2 layer serves as a thermal insulator for the chip, preventing heat or electrons from reflecting off the surface. With the use of an electron-beam machine, gold was then evaporated on the chip. The wafer is placed in a sealed, vacuumed chamber where a beam of electrons strikes a mound of gold and then evaporates gold onto the wafer. A layer of nickel chromium was also applied on top to serve as an electrical insulator. The chip is then ready for the photolithography process.

Photolithography is a process of transferring geometric shapes on a mask to the surface of a substrate [9], in this case glass. The first step was to chemically clean the chip and remove any remnants of organic, ionic, and metal impurities from the chip as well as any other unwanted residue. Once again cleaning was done by applying acetone, water, and isoproponal. Positive photoresist was then added to the surface of the chip. High-speed spin coating was performed to apply the photoresist. The chips were spun at 5000 rpms for approximately 25 seconds. The chip is then soft baked on a heater for approximately 4 minutes to remove almost all of the solvents from the photoresist coating. The chip is then ready for exposing.

The positive resist was exposed with ultraviolet light wherever underlying matter was removed. The chip was then aligned with a mask, a square glass plate with geometric-shaped designs on the surface. The mask was put in contact with the gold plate and exposed to UV light for 21 seconds, in this case. The photoresist has properties that allow the contents of the mask to be transferred onto the gold plate. After exposure the plate was developed for approximately 45 seconds in photoresist developer. The chip was immersed in water and dried. It was then hard baked in order to harden the photoresist and improve adhesion. Next a wet-etching technique was used to remove the nickel chromium layer from the chip. What remained was a chip with gold-patterned electrodes. Finally the chip was dipped into a positive resist stripper for a minute and then into water.

After the gold electrodes were patterned onto the chip, multi-walled carbon nanotubes were prepared. These commercially available carbon nanotubes were treated with heat and suspended in a mixture of Isoproponal and H_2O. The tubes were then placed in an ultrasonic machine for approximately a half hour, in order to break apart the tube bundles. The tubes were then once again suspended in the mixture and put in the ultrasonic machine for another half hour. The tubes were then trapped onto the chips using dielectrophoresis. The dielectrophoretic process involved connecting an oscilloscope, frequency generator, resistor, and voltmeter to a probe station. Although many trials and experiments were done using various voltages and frequencies, for one trial a peak-to-peak voltage of 15 VAC was applied as well as a 2 MHz frequency for approximately one minute. Examples of other trials included 3 VAC, 2MHz for 1 minute, and 18 VAC, 2MHz for 5 minutes. Micromanipulator probes were attached to each of the isolated electrodes. The gap spacing differed with the varying experiments, anywhere from 4 microns to 15 microns in length. After the power source was turned on the CNT solution was poured in between the two remote trapping electrodes. The tubes were then trapped in between those electrodes. With the 4 micron gap spacing an efficient single tube was produced.

Next, SU-8 reservoirs were placed around the tube ends with a mask that was superimposed with the previous mask. SU-8, a negative toned, thick, chemically amplified photoresist, had to be fabricated onto the chip. The substrate was cleaned and dried, and then a coat of SU-8 was spun onto the chip at 5000 rpms for 25 seconds. The chip was then soft baked at 65 ºC on a hot plate for one minute and then 95 ºC for five minutes in order to evaporate the solvent. After heating, the chip was exposed, with the new mask superimposed on the old mask, for approximately 42 seconds. Alignment marks on the masks made it easy to line up the two masks appropriately. After exposing, the chip was placed on the hot plate for one minute at 65 º and five minutes at 95 ºC once again for post baking. The chip was then submerged in MicroChem SU-8 Developer (five parts water, one part developer) for one minute. After developing the chip should be rinsed with water and air-dried. An SU-8 reservoir sidewall reservoir was patterned around the carbon nanotube endings and the gold electrodes. The device was then ready for testing.

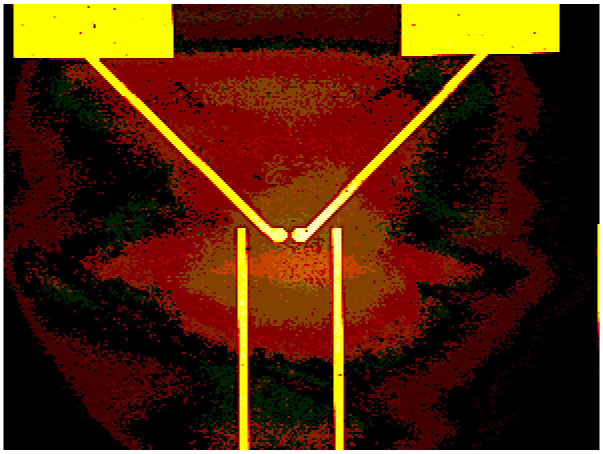


Figure 7. Gold electrodes in SiO_2 (MEAM laboratory University of Penn)

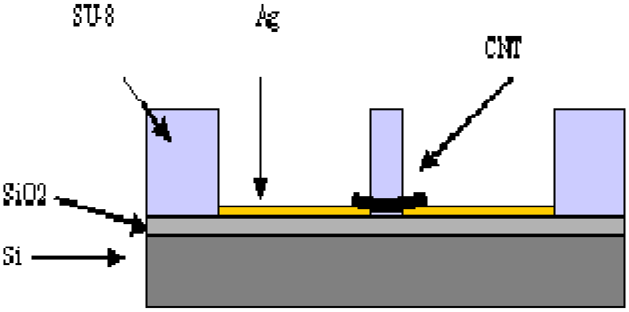


Figure 8. fabricated Coulter Counter device

4.2.2 Testing

The silicon wafers were to be tested electrically by measuring a change in resistance when the particles entered the pore. When the particle passed through the pore a current spike was intended to formulate. The carbon nanotubes that were trapped onto the chip were to then have spherical particles 49 nm in diameter driven through them. The particles are immersed in conductive fluid and then poured onto the substrate. They particles are suspended in the SU-8 reservoirs and once a potential was turned on they were to migrate through the pore. Attached to the device was a computer running a Matlab program that was to measure the current throughout the procedure.

4.2.3 Silicon/Glass Wafer

Trying to create the Coulter counter device using a silicon chip did not produce desired results. There was a problem with adhesion of the SU-8 onto the substrate as well as scum and other undesired particles forming on the chip. Methods were explored to remove such residue without damaging the chip altogether, and one seemed to work. The silicon wafer was dipped in a solution of hydrofluoric acid, which etched off a layer of oxide and therefore lifted off the scum with the oxide. This process was very effective and had very little effect on the chip. The next problem was the nanotubes trapping in between the electrodes. Clustered tubes and tubules not connecting to both electrodes continuously occurred, especially with an increased gap space and a higher potential. It is possible that the problem originated all the way from microfabrication. When wet etching was performed to remove the unwanted nickel chromium layer, the layer would be removed but some of the gold electrodes would be undercut and be etched away also, resulting in an increased and undesirable spacing of the electrodes.

The desired single tube was finally obtained with the specific 4 micron gap spacing of this chip. Once the tube was trapped, the test of driving particles and fluid through the pore commenced. The problem of tube filling also occurred with this device. The particles seemed to not be traveling through the pore. When trying to detect a current spike, the Matlab program did not perform enough readings per second. Also, when the current-voltage characteristics were measured there was relatively no change before and after the power was turned on. This in essence showed that the Coulter counter was not functioning. Flourescent particles were then poured onto the substrate and observed in a microscope. The particles looked to be trapped at the opening of the tube. Reducing the surface tension and contact angle of the pore and the particle by applying SDS detergent produced relatively no change in results.

5. DISCUSSIONS AND CONCLUSIONS

Although the Coulter counter devices did not work to the desired specification, some strides were made that suggest a functional device will be created in the near future. Extensive trial and error methods in fabrication as well as in testing have pinpointed prospective problems as well as changed and perfected specific techniques. For future tests, it will be necessary to measure the resistance of the pore before and after filling in order to determine if the Coulter counter is working properly. The numerous tests performed will make it easier to attack the operation from various angles in the future. Fabrication of the devices has been made simpler because of the different methods used in construction. Applications such as making the bake time 4 minutes instead of 2 minutes, spinning a specific layer of SU-8 on the chip, and curing the PDMS at specific temperatures, have all contributed to simplifying future experiments. When fluid and particles are properly filling the pore of the device, experiments characterizing and identifying particles will be possible.

6. ACKNOWLEDGMENTS

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