**A SILICON MICROFABRICATED APERTURE FOR
COUNTING CELLS USING
THE APERTURE IMPEDANCE TECHNIQUE**

by

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Abstract

Blood cell counting is a very important test required in medicine for the diagnosis of many illnesses. However, one common feature of all of the existing blood cell counters is the fact that they are large and therefore not portable. A hand-held, battery operated, blood cell counter becomes very useful when field tests are required to be performed in remote areas. Such a micro blood cell counter could also be used for home-care or point-of-care analysis in doctors' offices, clinics and hospitals.

In this research we fabricated an aperture that could be used in the counting of blood cells. The device is based on the Aperture Impedance Technique which is described below. The resistance of the aperture in the absence of any cells or particles is determined by its geometry and the resistance of the electrolyte occupying it. However, when a cell or other non-conducting particle crosses the aperture, the aperture impedance increases sharply, giving rise to a voltage pulse, if a constant current is maintained across the aperture between two electrodes. By counting these pulses one can obtain the cell count. The amplitude of the signal is proportional to the volume of the cell, and therefore this information can be used to distinguish the three different blood cell types, i.e. red blood cells, white blood cells and platelets.

Two designs were fabricated for the micro-aperture using bulk silicon micromachining and tested by using latex beads to simulate blood cells. The micro fabricated aperture of the first design was pyramidal in shape while the second was rectangular. Preliminary testing of the pyramidal aperture produced signals that were comparable to those obtained by commercially available desk-top blood cell counters. The shape of the

signals were also roughly gaussian as it is for the commercial cell counters. Another interesting result obtained was that it was not necessary to use a cylindrical aperture as was thought previously by the scientific community, and that even truncated pyramidal shaped apertures such as ours, could produce signals of comparable quality. The rectangular aperture design, although more compact and convenient for packaging, did not produce signals that were clearly detectable.

To explain the functionality of the micro-aperture we modeled it using resistor networks and simulated it using H-Spice. This thesis describes in detail, the fabrication technology, testing scheme, experimental results obtained and the modeling and simulation, for the microfabricated apertures.

Dedications. . . .

I would like to dedicate this thesis to

- *my late father Robel Robert Pathacharige*

- my mother Malani Robert

- my sister Chandrika Waduge

and to

- my brother Bandula Pathacharige

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Chapter 1

Introduction

Most people have had to take a blood test at some point in their lives. This shows the significance of a blood test. Blood is the vital fluid of our body and its biochemistry is an indication of the condition of one's health. Blood is composed of two parts - the blood cells and the liquid medium in which the cells are suspended. Besides the chemical composition of the liquid portion of the blood, the number of cells per unit volume (cell count) is a characteristic indication of one's health. For many clinical diagnoses, the cell count is used as a measure to determine the condition of ailment.

Most of the blood cell counting today is done by sending the blood sample to a centralized laboratory. This means that the turn-around time for the results is quite long. This prolongs the diagnosis and as a result the patient not only has to pay for an extra doctors' visit but also has to suffer for a longer period of time. However, if it were possible to perform the blood cell count at the doctor's office in a short time, then the physician could make a diagnosis sooner and the patient would be treated sooner as well.

1.1 The Centralized Approach for Analyzing Blood

The hospital laboratory which is at the core of the centralized approach to blood analysis has seen a significant growth over the last few decades. In spite of this, it is becoming clear that such a centralized approach for blood analysis is not adequate[1]. Physicians treating critical care patients especially, have found this approach unsatisfactory. If a critical care patient is brought to the emergency room a doctor needs to

assess the situation without delay, or else it may cost the patient his/her life. The physician usually uses several analytical tools to provide qualitative and quantitative physiological information. The physician may use a stethoscope, an electrocardiograph machine and a blood pressure meter. However, in order to obtain a complete picture of the patient's condition one will also require a blood cell count and a blood chemistry report. In the absence of quick access to such information the physician is not be able to make an accurate diagnosis.

Generally, to obtain a blood cell count, a sample of blood is drawn from the patient and sent to a clinical chemistry laboratory for testing. This could take several hours even if the sample is required to be processed urgently (STAT¹ order). However, for critical care patients such delays in obtaining critical information, is a major factor affecting timely commencement of treatment.

The general procedure for obtaining a blood cell count is very time consuming as there are many steps to be done in the appropriate sequence before the results are sent to the physician. First, a blood sample is drawn from the patient. It is then labelled and sent to the central laboratory where a technician has to log it and only then is the blood cell count performed. After this, it has to be logged again before it is sent back to the physician. The reason why the blood cell counter has to be kept at a remote location is because it is a highly complex system and requires skilled personnel for its operation and maintenance.

In order to solve part of the problems associated with the centralized approach, several schemes have been implemented [1]. One such solution is for the

1. A clinical terminology for prioritizing.

physicians to be able to request a STAT order. One important time-consuming factor not taken into account with this solution is transportation of the sample. To deal with this issue several hospitals have devised a pneumatic tube system for transporting STAT samples from various departments to the central laboratory [1]. Another solution for the transportation problem was to create satellite laboratories close to critical care centers. However, both solutions are very costly to implement and are not economically viable in remote areas and in many third world countries that cannot afford such expenses.

This gives rise to the question, “but then how does one improve the turn-around time in an economical manner”? One way to do that is to bring the blood cell counter (or the automated analyzer) to the physicians’ office or to the bedside of the patient. This certainly does not imply that one has to equip every physicians’ office with blood cell counters that exist in the laboratories, because this would not be very economical. However, if one could miniaturize the blood cell counter so that it would be economically viable to have one in every physicians office or at the bedside of a patient then that would certainly reduce the turn-around time.

This method is generally termed “point-of-care” analysis [2]. Clearly the development of an analytical system that can be used quickly and reliably by a physician or nurse at the point-of-care, provides a significant opportunity for improved patient care. However, needless to say, this task has also presented itself as a major technological challenge.

1.2 Point-Of-Care Analysis (POCA)

As mentioned above the POCA system is the means by which a faster turn around time can be acquired. However, the challenge lies not only in the miniaturizing of

the testing equipment but in reducing the cost of such a device while maintaining or improving accuracy. This is conceivable with the aid of microfabrication technology which is instrumental in fabricating POCA systems.

1.2.1 Issues Relating to Microfabrication

When considering using microfabrication manufacturing processes for the development of a new product it is imperative to assess technical and economical issues. Since the overhead costs associated with a microfabrication facility are relatively high, it is essential to make a product that is required in high volume.

1.2.2 Future Prospects of Point-of-Care Testing

It is worth assessing the short and long term future for point-of-care diagnostic systems and the application of microfabrication in the area of medical diagnosis. The current health care trend is toward reducing the cost while maintaining the quality of health care. One vision of the future is that all high-acuity testing will be contracted out to a high volume regional laboratory [1], the argument for this being to achieve the lowest cost per test. The arguments for point-of -care testing are as follows.

- (1) The costs associated with the reagents in the point-of-care technology are more than offset by the savings of fixed and variable costs associated with a laboratory;
- (2) Given the rapid turn-around time required for high acuity patients some fraction of the tests cannot be sent out;
- (3) Putting the analytical technology in the hands of the care givers makes more sense, as it puts them in control;

There is significant commercial interest in non-invasive sensor based measurements, some involving microfabricated devices as shown in the following examples.

Blood glucose testing and blood gas measurements have attracted much of the commercial research and development in the recent past. For instance, bio-sensors that use electroosmosis or a micro-syringe to extract interstitial fluid through the skin seem promising [3] in the area of glucose testing. One group is presently working on developing electrochemical glucose bio-sensors for control of an insulin pump [4]. Non-invasive measurement using near-infrared light adsorption through the skin to determine glucose is another technology that has attracted interest [5][6]. In the area of blood gas measurement, a system where fiber optic sensors introduced through an arterial catheter has also been commercialized [7]. All these examples clearly show the commercial interest in microfabricated sensors for bio-medical analysis.

Another interesting issue is the application of microfabrication processes in the future for general diagnoses. A recent interest from the bio-medical community has been in relation to the human genome project [1]. Microfabrication techniques such as photolithography were used to form arrays of DNA at specific locations on a silicon chip. The same approach has also been used for assembling arrays of polypeptides for screening for antibody binding in drug development [8]. Other areas of interest include microfabrication and micromachining to form filtration devices, pumps and reaction chambers within a silicon chip. This is with a view to creating devices that integrate pre-analytical sample manipulations with sensor detection.

From a medical perspective, point-of-care testing is one of the most rapidly growing areas of clinical diagnostics. From the point of view of a scientist or engineer, it appears that there is sufficient interest and need for the fusion of bio-technology, analytical chemistry and microfabrication for the development of future POCA systems.

1.3 A model POCA system: the i-STAT clinical analyzer

The i-STAT point-of-care blood analysis system comprises of a single-use cartridge containing an array of electrochemical sensors capable of measuring the electrolytes such as Cl^- , K^+ , Na^+ , Ca^{++} , glucose and also partial pressure of blood gases such as pO_2 , pH and pCO_2 , in only a few minutes and with only a few drops of whole blood [9]. The cartridge contains a means for automatic calibration and runs in conjunction with a battery powered hand held analyzer as shown in Figure 1.1.

1.3.1 Selecting the Manufacturing Technology

Given that the concept of a disposable cartridge containing sensors has been adopted for blood analysis and given that the number of such sensors that would be required annually would be large, a manufacturing approach that produced sensors in high volume and at low cost is clearly required. Therefore, a preferable candidate for an economical manufacturing technology is the microfabrication technology.

1.3.2 Microfabrication as the Manufacturing Technology

The objective of any mass manufacturing operation is to develop economic processes that assure continuous output of a high quality product. Nowhere is this better exemplified in modern manufacturing than in the application of microfabrication processes. The precise dimensional control attainable over small structures, the high quality of products and the comparatively low unit cost at high volume has a clear appeal for the manufacture of microsensors. These manufacturing techniques, could clearly be used to expand the horizons of microfabrication beyond passive information-processing devices: in this case, to form sensors that acquire bio-chemical information from a complex biological fluid such as whole blood.

Fill cartridge with fresh whole blood and seal

Insert cartridge into hand-held analyzer.

View test results in 2 minutes.

Figure 1.1 i-STAT system, comprising a hand-held blood analyzer and single-use cartridge containing microfabricated sensors. [2]

1.4 Research Motivation

An extensive literature search conducted, revealed that there was no major effort done in the area of miniaturizing cell counters. We also consulted industry and confirmed that there was a need for a miniaturized cell counter. The aperture which is the main component of modern cell counters is presently made out of expensive materials such as ruby and sapphire and is not batch fabricated. Therefore, we were motivated to use silicon as an alternative material and use the batch fabrication nature of the Integrated Circuit (IC) technology to fabricate these apertures.

The lack of any miniaturized blood cell counting device and the need for a point-of-care cell counter also provided us motivation for this research. In addition, the I-STAT clinical analysis unit as well as other blood chemistry units do not have cell counters. Therefore, we were interested in building a cell counter that can be miniaturized and possibly integrated to a system such as the I-STAT blood chemistry analyzer.

1.5 Research Objective

The main objective of the research was to develop a design and fabricate a microfabricated chamber or microfabricated aperture that will aid in the eventual development of a hand-held blood cell counter. A secondary objective was to explore non-cylindrical aperture shapes and establish the use of silicon for fabricating apertures.

1.6 Research Methodology

The design was based on the Aperture Impedance Method which is an electrical means of counting cells. This principle is used in the commercially available blood cell counters. This technique will be discussed in detail in Chapter 3. The technology used for the fabrication is the bulk silicon micromachining technology.

1.7 Thesis Overview

Chapter 1 discussed the need for a miniaturized blood cell counter and introduced the concept of point-of-care-analysis systems. Chapter 2 is a study of hematology which in other words is the science of blood. In Chapter 3 we discuss the technology fundamentals for cell counting. The theory of the Aperture Impedance technique is presented and the challenges associated with a microfabricated aperture are discussed.

Chapter 4 presents the design and fabrication of design-1, which is a pyramidal shaped microfabricated aperture. In Chapter 5 we present the design, fabrication and test results of design-2 which was an attempt for an alternative design which could be undertaken for future development.

Chapter 6 presents the testing and results of design-1, which was the primary focus of this research. Modeling and simulation results of this aperture are also presented. Finally Chapter 7 presents the conclusions of this research and discusses the future work that could be carried out.

Chapter 2

A Study of Hematology

In order to fully appreciate what a blood cell counter does, one must understand why a blood cell count is necessary and appreciate all the illnesses it is needed for. Therefore, in this chapter we digress into the biological aspect of this research to study Hematology.

Hematology is the branch of medicine concerned with the study of the elements of blood and the blood forming tissues. Hematology laboratories perform tests to detect and monitor treatments for anemia, leukemias and inherited blood disorders such as hemophilia. The effects of certain treatments such as cancer chemotherapy, can be determined from hematological tests. Hematological tests also give information about a patient's general well being.

2.1 Composition of Blood

Blood is composed of cellular elements suspended in a fluid called plasma [10]. The plasma constitutes about 45-60% of the blood volume. The rest of the blood is composed of cellular elements, the majority of which are erythrocytes (red blood cells). Blood is 6-8% percent of the total body weight and the normal blood volume of an adult is approximately 5 litres.

2.1.1 Plasma

Plasma is a complex solution in which the blood cells are suspended. Plasma is more than 90% water. The remainder is dissolved solids such as proteins

(including antibodies, albumin and coagulation factors), lipids, carbohydrates, amino acids, hormones and electrolytes. These substances are measured in the clinical chemistry department.

2.1.2 Cellular Elements of Blood

The cellular elements of blood are commonly called blood cells. These include the erythrocytes (red blood cells), the leukocytes (white blood cells) and the thrombocytes which are commonly known as platelets. These are the elements in the blood that a blood cell counter would quantify.

2.1.2.1 Red Blood Cells: Erythrocytes

The red blood cells are the most numerous of the cellular elements in the blood. There are approximately 4-6 million red blood cells/mm³ of blood[11]. Erythrocytes transport oxygen from the lungs to the tissues and carbon dioxide from the tissues back to the lungs. This action is accomplished by the hemoglobin, the major component in the red blood cell.

If the red blood cell count falls below or rises above the normal range, an individual may experience a variety of symptoms. Information from the red blood cell count is helpful in the diagnosis and treatment of many diseases, which will be discussed in section 2.2.

2.1.2.2 White Blood Cells: Leukocytes

The leukocytes are the least numerous of the blood cells. The normal white blood cell count varies according to the age of the individual. New born infants usually have a white blood cell count of 9,000-30,000 /mm³ (or μ L). Within a few weeks after birth, the count drops rapidly and approaches the childrens' normal of 6,000-14,000/mm³. By adulthood the normal count is in the range of 4,500-11,000 /mm³ [11].

There are five types of leukocytes present in normal blood: neutrophils, basophils, eosinophils, lymphocytes and monocytes [11]. All of the white blood cells play important roles in immunity - the resistance to disease. Lymphocytes have important functions in immunity, one of which is production of antibodies. The number of lymphocytes usually increase in the event of a viral infection. Neutrophils provide defence against infections by directly attacking the invading organisms. The neutrophils usually increase in response to bacterial infections. The leukocytes have varied life spans some living a few days, others living several months. Each type of leukocyte has a different function but they are all associated with immunity or defence. Leukocytes spend most of their life in the tissues unlike red blood cells that perform their function from within the circulatory system. White blood cells only use blood as a means of transport.

2.1.2.3 Platelets: Thrombocytes

Platelets are not actually whole cells but are fragments of cytoplasm that have been released into the circulating blood from large cells in the bone marrow. Platelets are important in several stages of blood clotting also known as hemostasis. They act on vessel walls to form a plug to stop bleeding when a vessel has been injured and help initiate a series of enzymatic reactions that result in the formation of the blood clot.

Table 2.1 Comparison of blood cell sizes

Blood cell type	Size [μ m]
white blood cell	10-20
red blood cell	6-10
platelets	2-4

The platelet count is an important test used to investigate bleeding disorders, to assess clotting ability or to monitor drug treatments. Platelets are difficult to

count accurately because of their small size and because of their tendency to clump [11]. The normal platelet count is 150,000 to 400,000 platelets/ mm³[11]. Table 2.1 compares the sizes of the different blood cell types and as can be seen platelets are much smaller than red and white blood cells.

2.2 Diseases of the Blood

There are many diseases that involve primarily the blood cells. Some of these diseases are caused by improper or insufficient production of a cell type. For instance many factors may cause an increase or decrease in the white blood cell count. Factors such as stress, exercise, and anesthetics may cause temporary increases in the white blood cell count; these are called physiological increases. Changes in the white blood cell count due to disease continue until the illness is under control; these are called pathological changes. Usually the increase or decrease in the total white blood cell count is due to a change in only one type of cell.

An increase above normal in the total number of white blood cells is called leukocytosis [11]. Conditions that may cause leukocytosis include infections and certain diseases such as leukemias. In the leukemias although the patient has many leukocytes, the leukocytes are not matured properly and therefore cannot provide proper immunity. The patient may then be highly susceptible to infections even though the leukocyte count is high.

A decrease below normal in the total number of white blood cells is called leukocytopenia [11]. This condition may be caused by viral infections, exposure to ionizing radiation, certain chemicals and chemotherapy drugs. Infection by the Human Immunodeficiency Virus (HIV) is an example of a disease that causes a decrease in the

white blood cell count. Table 2.2 summarizes these changes in the white blood cell count.

Since the red blood cells carry oxygen, a reduction in their number results in decreased oxygen carrying capacity to body tissues. This condition is called anemia [11]. The symptoms of anemia include fatigue, weakness and headache. Extreme anemia causes increased heart rate. Examples of conditions in which red blood cell counts are decreased include iron deficiency anemia and sickle cell anemia.

Table 2.2 Causes of abnormalities in white blood cell count

Causes of increased White Blood Cell count	Causes of decreased White Blood Cell count
Pathological	Pathological
Infection	Some viral infections
Leukemias	Ionizing radiation
Polycythemia	Certain chemicals
Physiological	Chemotherapy drugs
Exercise	HIV infection
Exposure to sunlight	
Obstetric labor	
Stress	

Some anemias may be due to deficiencies of vitamins such as B12 or folic acid. Anemia can also result when blood loss occurs at a rate faster than the rate at which the bone marrow can produce cells. An example of this is the presence of a bleeding ulcer. An increased red blood cell count is called erythrocytosis [11]. People who live in high altitudes have erythrocytosis because the lower oxygen content of the air stimulates red blood cell production. Polycythemia vera is a disease in which the number of red blood cells as well as other blood cells is greatly increased [11]. Table 2.3 illustrates the different

conditions that affect the red blood cell count.

2.2.1 Inherited Hematological Diseases

Some diseases of the blood may be inherited, such as hemophilia, a condition in which one of the factors required for blood to clot is missing or is defective. Other inherited hematological diseases include the conditions in which the patients have abnormal hemoglobin function. An example of this is sickle cell anemia, a condition in which the abnormal structure of hemoglobin causes abnormal function of the hemoglobin.

Table 2.3 Conditions affecting red blood cell counts

Condition	Effect on Red Blood cell count
Anemia	
Iron deficiency	Decreased
Sickle cell	Decreased
B12 deficiency	Decreased
Folic Acid deficiency	Decreased
Erythrocytosis	Increased
Polycythemia vera	Increased

2.2.2 Secondary or Acquired Blood Diseases

Abnormalities in blood cells may occur due to conditions or diseases in other organ systems. These are called secondary or acquired conditions. For example, red blood cells that appear abnormal may be present in patients with severe hypertension because the cells may become damaged as they circulate through small blood vessels. Diabetics may have “lazy leukocytes”. This causes slow healing of wounds or infections because some of the white blood cells function inadequately. Lymphocytes - a type of white blood cell - develop an atypical appearance in infectious mononucleosis, a viral disease [11]. These will be noticed when a blood cell smear is observed microscopically.

Blood cells may also be affected by treatments or medications. High doses of aspirin cause abnormal platelet function. Cancer chemotherapy is designed to prevent the growth of cancer cells. However, these drugs are not selective and may also prevent the production of blood cells. Patients receiving chemotherapy must have regular blood cell counts to be sure that their blood cell concentrations do not fall to dangerous levels.

2.3 Summary

In this chapter we introduced the subject of hematology. We discussed the components of blood and the various diseases that can be diagnosed from a blood cell count. In the next chapter we will discuss the history and the development of modern blood cell counters and the technology fundamentals associated with blood cell counting.

Chapter 3

Cell Counting

Before beginning the discussion of modern cell counting systems it is interesting to review the history of the field of cell counting and the development of the technology which was ultimately applied to quantification of the elements of blood. Then we will discuss the technology fundamentals of modern blood cell counters. We will also discuss the theory of aperture impedance and the challenges associated with a microfabricated aperture.

3.1 History of Cell Counting

The first attempt to count blood cells was done by Leeuwenhook who used a microscope and a glass capillary tube with graduation marks of measured dimension to count red blood cells of chicken [12]. Later on, techniques for diluting the blood were introduced which resulted in easier and more accurate counting by using a shallow rectangular chamber with a thin cover glass into which the diluted blood was injected [12]. In the early 20th century Moldovan et. al [13] invented a technique using a photoelectric device to count cells. However, due to the unreliability of the photoelectric device at that time this attempt for cell counting did not develop.

In the mid 1950's Walter H. Coulter [14] invented a technique for counting cells and introduced an automated blood-cell counter. This technique was based on the fact that the resistivity of blood cells is much higher than that of the diluting fluid the cells are suspended in. We will discuss this technique in detail later in section 3.3.1. This

principle of cell counting based on the impedance of the cells, namely the Aperture Impedance technique or the “Coulter Principle”, is the principle this research is based on. Since the 1950’s this technique has been extensively developed and serves as the basis for most modern cell counters.

3.2 Challenges for Modern Cell Counters

The objective of a cell counting instrument is to count the cells one at a time. However, this problem is more technically challenging than it may appear. The challenge for the modern cell counter arises due to a few reasons. Firstly, the nature of the blood cells, secondly the difficulty in accounting for the volume of liquid due to dilutions and finally the need to obtain a cell count separately for each cell type. In the following few sections we will briefly describe the impact of these general problems.

3.2.1 The Nature of Blood Cells

The main technical challenge comes from the fact that blood cells are very small in size and, in addition, very concentrated. Therefore the counting device must be able to separate the signals from the cells and the spurious signals arising from tiny particles or other sources of background noise. Secondly, the device must have a sensing area that must be small enough to let the particles cross one at a time as well as be large enough to allow the cells to pass. Since the cells are so concentrated there is a high probability that more than one cell can cross the sensing area at any given moment. This is called coincidence count [15] and the resulting counting error is called a coincidence error. This coincidence error can be minimized by diluting the sample appropriately and by having proper control over the parameters associated with the sensing area, and thereby reducing the probability of coincidence. After the cells are diluted they must be made to

pass one at a time through a sensing area (microfabricated aperture) which is sensitive enough to detect the traversal of the cells.

3.2.2 Sample Dilution

After the cells are counted, the volume referred to in the concentration is the original whole blood volume. Therefore, one must keep track of the volumes in all the dilutions in order to be able to refer back to the initial volume. One problem associated with accounting for the volume is the fact that blood is a fluid that contains particles at different densities. Therefore constant attention must be given to the homogeneity of the solutions since the cells can settle or stick to the surfaces. Another problem is that the red blood cells are flexible and so this can create inhomogeneities in cell concentration under different flow conditions. Goldsmith et. al [16] showed that as a result of the deformability of the red blood cells they tend to crowd toward the centre of a cylindrical tube and cause inhomogeneity in the solution. Therefore, traditional laboratory techniques such as pipetting can distort the original cell concentration even if it was a homogeneous solution.

In a commercial cell counter the required dilution must be done without losing track of the relationship between the cells and the original sample volume. This problem is usually addressed by carefully controlling the sample and fluid delivery in absolute and in relative terms. However, since the intent of this thesis is mainly to prove that the principle behind modern cell counters can be realized using silicon, and not to produce commercial micro cell counters, steps such as dilution of the whole blood will not be automated in this study.

3.2.3 Classification of Different Cell Types

Another challenging issue is that the total count of all cells in the blood has no medical significance. Medical value is obtained by obtaining a separate cell count for each cell type. The concentrations of the different cell types differ by orders of magnitude, as was pointed out in section 2.1.2. In a healthy patient there are 100 red blood cells for every white blood cell and 20 red blood cells for every platelet [16]. In simpler terms this corresponds to a ratio of 100:1:5 for red blood cells, white blood cells and platelets respectively.

In general, the greater the ability to discriminate between the different cell types, the greater the medical utility of the cell count. In order to sub-classify the cell count, the treatment of the cells prior to cell counting is made more complex. Steps are added, such as hemolysis (lysing)² [11] of the red blood cells to enhance the cell counters' ability to discriminate between cell classes.

3.3 Technology Related Issues

There are a few important technology issues that need to be addressed, that are basically common for all cell counters. The inputs (blood samples) and the outputs (hematology parameters) are the same for all cell counters, and therefore the technical challenges are similar. In particular the following concepts are important technological issues.

1. The detection technology
2. The fluid transport technology

2. A chemical process that destroys the cell membrane so that it no longer behaves as an insulating object.

3. Signal processing

3.3.1 Detection Technology

A cell or particle is usually detected by the signal generated from the disturbance it causes to an electric or electromagnetic field. The Coulter technique [14], which is also commonly known as the Aperture Impedance Technique, makes use of a static electric field and the disturbance of this electric field is measured in order to detect the cells. Another technique which is based on the scattering of light is also commonly exploited in modern cell counters. The details of these two techniques will be discussed in the following sections.

3.3.1.1 Aperture Impedance Method

The Coulter method or the Aperture Impedance method of measuring cells is illustrated in Figure 3.1. This is the method that was employed in this research.

Figure 3.1 Illustration of the Coulter Principle [14].

The container shown above is filled with an electrolyte medium, typically saline. In this medium the particles or cells that are to be counted are suspended. The container is separated into chambers A and B by a nonconducting wall. The only connection between the two chambers is a small aperture that is typically about 50-100 μm in diameter. Chamber A is connected to a vacuum which when applied sucks the fluid from chamber B to chamber A. The reason a vacuum is used here is because when the fluid is sucked through the aperture it streamlines the cells due to a process called hydrodynamic focusing and makes them flow one after the other. The main reason we get a focusing effect is because the volume of the fluid in chamber A is much larger than the volume of fluid in the aperture and this makes the fluid flowing near the walls of the aperture to act as a sheath fluid³. Most commercial cell counters presently use vacuum for the fluid transportation as opposed to using positive pressure. [12][14][21][25]

Two platinum (Pt) electrodes are placed on either sides of the aperture and are connected to a constant current circuit as shown. In the absence of any particle in the aperture the resistance seen by these electrodes is a function of the resistivity of the electrolyte and the geometry of the aperture. i.e. the smaller the aperture diameter the higher the resistance and vice a versa.

Blood cells act as biological resistors if the membrane surrounding them is intact. In other words the cells act as very high impedance particles as compared to the resistivity of the electrolyte. In the presence of a particle in the aperture, the resistance observed by the Pt electrodes increases because the non-conducting cell displaces the conducting electrolyte and increases the total resistance. Since we have a constant current

3. A term used in fluid dynamics to describe a fluid that streamlines particles in flow.

across the electrodes this change in resistance will result in a voltage pulse as expected from Ohm's Law. Applying Ohm's Law for the case where there are no particles in the aperture, one obtains for the resistance

$$R = \frac{V}{I} = \rho \frac{l}{a} \quad (3.1)$$

where

a is the cross sectional area of the aperture

l is the length of the aperture

ρ is the resistivity of the electrolyte

V is the voltage between the electrodes

I is the current flow through the aperture and

R is the resistance of the aperture and the surrounding electrolyte⁴.

The size resolution of the aperture impedance detection system is ultimately limited by the ratio of the signal voltage to the electrical noise associated with the aperture resistance. This signal to noise ratio (SNR) increases as the square-root of the ratio between the particle size and the aperture size [12]. Therefore, extending the range over which the signal amplitude is proportional to the particle size by increasing the aperture diameter will compromise the SNR. In other words, if the aperture is too large, detection of small particles is compromised due to the reduction of signal and if the aperture is too small, the linear range of the particle size measurement is reduced. In commercial cell counters apertures of approximately 50 μm are used for counting red blood cells and platelets and an aperture of about 100 μm is used for counting white blood cells [17].

4. The resistance of the electrolyte surrounding the aperture amounts to less than 2% of the total resistance.

3.3.1.2 Optical Method (light scattering)

The light scattering method for measuring cells is illustrated in Figure 3.2 [18]. Light from an optical source propagating in a medium with an index of refraction n_o strikes a cell with a certain volume, shape and index of refraction n_p . This results in a scattered wave whose intensity varies with the angle of scattering. A dark stop is used to send a portion of this scattered wave to a photo detector. The angular distribution and intensity of the scattered radiation is a function of the properties of the scattering particle; i.e. its size, shape and refractive index.

Figure 3.2 Example of the light scattering method of measuring cells [18].

Kerker et.al [19] has developed the theory (Mie scattering) of scattering of electromagnetic radiation for homogeneous spheres. Unlike the Aperture Impedance technique, the optical signals from blood cells are strictly dependent upon the content of the cells and not on the integrity of the membrane [12]. Therefore, when red blood cells are re-sealed (after lysing) the signals are much smaller than those obtained from the original blood cells.

An assumption of the scattering theory is that the cells are homogeneous particles. This is a reasonable assumption for red blood cells but is a very poor assumption

for white blood cells that vary among themselves not only in size but also in their internal structure and composition. Therefore the optical method has its own limitations.

3.3.2 Preparation and Transportation of the Sample

3.3.2.1 Sample Preparation

For cell counting studies, researchers use latex beads to simulate blood cells [20][21][22]. Latex beads come in different sizes ranging from about 0.1 μm to several mm in diameter. In our experiments latex beads of 5, 10 and 20 μm were used to simulate blood cells of different sizes. The latex beads were diluted in 0.9% NaCl which is ordinarily known as saline. Saline is the standard electrolyte medium used in commercial cell counters. For preliminary testing of our device the exact particle count was not an issue and so the concentration of the beads varied in our experiments.

3.3.2.2 Transport to the Detector

After preparation of the sample and electrolyte, it was required to guide this solution to flow through the microfabricated aperture. The flow can be controlled by a good vacuum source or by an advancing column of mercury moving in a cylinder of known dimensions. In our experiments we used a laboratory aspirator as the vacuum source.

Figure-3.3 shows two typical flow geometries commonly in use. In Fig 3.3a the particle and electrolyte medium fills the aperture. However, in Fig 3.3b the diluted blood sample is constrained to the centre of the aperture by the surrounding particle-free sheath fluid.

The technique used in Fig 3.3b is generally called hydrodynamic focusing [23] or “sheath flow”. The problem of non-axial particles and re-circulation are resolved by constraining the sample flow to the centre of the sensing zone. This advantage is

Figure 3.3 Two typical flow cell geometries [12]. (a) without sheath flow (b) with sheath flow
gained, however, at the expense of mechanical complexity and additional fluid flow.

Two designs were implemented for the microfabricated aperture in this research. One of the designs used the flow pattern shown in Figure 3.3a while the other took advantage of the hydrodynamic focusing shown in Figure 3.3b (the shaded area represents the channel walls). The details of the designs will be discussed in Chapters 4 and 5.

3.3.3 Signal Processing

In fully automated cell counters the output seen on the display screen (i.e. oscilloscope) is a train of electrical signals each corresponding to the passage of a cell or particle. This must be processed in order to get the final count. Processing includes separating and classifying signals, minimizing coincidence error and organizing the final results. However, since a fully automated cell counter was not part of the scope of this

research, the signals obtained were not fully processed as is done in the commercial counters.

3.3.3.1 Coincidence Correction

In spite of the fact that cell counting detectors have relatively small sensing volumes, it is necessary to apply a correction for the possibility that more than one cell is in the aperture at the same time, especially when counting red blood cells, since they are more numerous. When the sensing volume is well known, coincidence correction can generally be applied effectively using relatively straightforward approaches.

The probability of coincidence is given by the cell rate times the pulse width or the total time that the aperture is occupied. This is called the “dead time” and it can be used to correct the count as follows:

$$\text{Corrected count rate} = \frac{r}{1 - (DT)} \quad (3.2)$$

where r is the observed rate and DT is the dead time [12].

The technique we use to minimize coincidence is simple dilution. By diluting the sample to a low particle concentration one is able to reduce the coincidence dramatically and so the error introduced due to coincidence can be minimized. If the error is thus minimized then one can tolerate the error since the value obtained for the concentration will be a very good approximation to the true value, for all intensive purposes.

3.4 Theory of Aperture Impedance Technique

The principle of electrical impedance was discussed briefly in section 3.3. However, the complete theory of the electrical impedance technique was not dealt with. Many problems arise due to the nature of the small aperture involved in this technique.

The theory and the problems due to the small aperture will be discussed in this section.

3.4.1 Electrical and Physical Relationships

The determination of the particle or cell volume is based on the Aperture Impedance method or Coulter method described in 3.3.1.1. Let us assume that the particles to be counted/sized are spherical in shape and that a uniform electrical field exists in the aperture. In this case, a mathematical relation derived by Maxwell can be applied for calculation of the pulse height-particle volume relation [24]:

$$\rho = \frac{2\rho_1 + \rho_2 + p(\rho_1 - \rho_2)}{2\rho_1 + \rho_2 - p(\rho_1 - \rho_2)} \times \rho_2 \quad (3.3)$$

Eq.3.3 gives the total resistivity of a medium with resistivity ρ_2 which has spheres of resistivity ρ_1 embedded in it. The ratio of the volume of the embedded spheres to the volume of the aperture is, $p = \Delta V/V$. The distance between the spheres are considered long enough so that the influence of one sphere on others can be ignored. Let us consider a particle in a cylindrical aperture of a cross section a and length l , where the diameter of the aperture, is large with respect to the particle diameter. The volume of the aperture can be stated as,

$$V = a \times l \quad (3.4)$$

The resistance of the aperture in the absence of any particle is R_2 . In the presence of a particle the resistance increases and can be stated as,

$$R = R_2 + \Delta R_2 \quad (3.5)$$

or in terms of resistivity,

$$\rho = \rho_2 + \Delta R_2 \left(\frac{a}{l} \right) \quad (3.6)$$

Since,

$$R = (\rho \cdot l)/(a) \quad (3.6^*)$$

The voltage change detectable at the electrodes from Ohm's Law can be written as,

$$\Delta U = i \times \Delta R_2 \quad (3.7)$$

By equating (3.3) and (3.6) and solving for ΔR_2 and then substituting in (3.7), we obtain for the height of the voltage pulse at the electrodes,

$$\Delta U = \frac{\Delta V(\rho_2 \cdot i)(\rho_2 - \rho_1) \cdot l \cdot (-2)}{(v(\rho_2 + \rho_1 \cdot 2) + v(\rho_2 - \rho_1))a} \quad (3.8)$$

However, the particle volume is much smaller than the aperture volume, i.e, $V \gg \Delta v$ and the particle resistivity is much higher than the electrolyte resistivity, i.e, $\rho_1 \gg \rho_2$.

Therefore, Eq. (3.8) may be simplified to get,

$$\Delta U = \frac{\Delta V(i \cdot \rho_2)}{a^2} \quad (3.9)$$

Equation (3.8) identifies the factors that influence the pulse height in an aperture if the field is homogeneous and the particles are spheres. The pulse height is proportional to the current i and the particle volume V and inversely proportional to the square of the cross section of the aperture. The pulse height depends on the resistivity of the particles ρ_1 and the electrolytes ρ_2 .

3.4.2 Flow Conditions in the Aperture

When considering fluid flow in channels or pipes there are mainly two types of flow as categorized in fluid dynamics. They are laminar flow and turbulent flow [24]. In laminar flow the flow lines do not mix and the layers of fluid slide side by side without generating eddies or swirls. In turbulent flow the flow lines and layers get mixed and distorted due to generation of eddies and swirls in the system. Turbulent flow usually occurs at high flow rates. A dimensionless number called the Reynolds number defines

whether the flow is laminar or turbulent. The Reynolds number is calculated from the following formula [24],

$$R = \frac{d \cdot v}{\nu} \quad (3.10)$$

where:

v = average velocity of the flow

D = inside diameter of the tube

ν = is the dynamic viscosity of the fluid.

The critical Reynolds number $Re_{kr} = 2300$ describes the transition between laminar and turbulent flow. Below Re_{kr} the flow is laminar. In general, the Reynolds number in small apertures is far below the critical number [24].

3.4.2.1 Coincidence Theory

Coincidence is not something that one can get rid of completely. Therefore the next best choice is to minimize the coincidence probability. Wales et.al [26] has described 2 types of coincidence, namely the horizontal and vertical coincidence. Horizontal coincidence is when two particles enter the aperture in such a way that two pulses are generated but only the larger one is counted by the peak detector and vertical coincidence is when both particles are in such proximity that only one pulse is generated with double the pulse amplitude.

If one assumes a pulse rate of N particles per second with a flow velocity of v and an effective aperture length of x then from [25],

$$\gamma = x \cdot \frac{N}{v} \quad (3.13)$$

particles are present on average in the aperture. The probability $(p(n))$ of having n particles in the aperture can be expressed by Poisson's Law [25], where

$$p(n) = \gamma^n \cdot \frac{e^{-\gamma}}{n!} \quad (3.14)$$

Therefore, as can be seen, the coincidence probability is a function of the flow velocity, the average number of particles passing through the orifice and the effective aperture length.

The ratio of the probability of the presence of 2 particles to the probability of one particle in the aperture $p(2)/p(1)$, indicates how many double particles are expected to cross the aperture if the probability of single particle crossing is 100%. For instance if we have a typical flow rate of $v=5\text{m/s}$, and $N=1000$ particles/sec and if we assume an effective aperture length of $50\text{ }\mu\text{m}$ the coincidence probability is about 0.5%. This indicates that by proper control of parameters associated with the aperture one can reduce the probability of coincidence to an insignificant level.

3.5 Summary

In this chapter we discussed the history and the development of the modern blood cell counter and the technology related to it. The theoretical description of the aperture impedance technique was presented and the problems that arise in a small aperture were analyzed.

The next chapter will deal with the design and fabrication sequence of the main design, design-1, and the packaging of it.

Chapter 4

Design and Fabrication: Design-1

This chapter will discuss the design and fabrication of our first design (design-1). It also details the equipment and the experimental set-ups that were used in the process and the packaging of the functional device. The experimental results and the modeling and simulation results will be presented later in Chapter 6.

4.1 Design

4.1.1 Design Requirements

Human blood cells are in the order of $10\text{ }\mu\text{m}$ in size as was shown in Table 2.1 in Chapter 2. As mentioned in section 3.3.1 and illustrated in Figure 3.1, the detection technology for the Aperture Impedance Technique involves the passing of cells or particles through an aperture that is a few times the size of the cell/particle. Literature suggests [24] an aperture size of about $50\text{-}100\text{ }\mu\text{m}$ in diameter and a length of at least the size of the diameter. Therefore, the apertures we designed were made with consideration to these requirements.

4.1.2 The Design-1

For simplicity we decided to produce a truncated pyramidal shaped aperture compared to the cylindrically shaped aperture found in commercial cell counters. Since the anisotropic wet etching process produces features bounded by the slanted $\langle 111 \rangle$ planes of silicon, a pyramidal shaped aperture was easy to produce.

Since the angle of slope of the $\langle 111 \rangle$ planes are known with respect to the $\langle 100 \rangle$ planes, based on simple geometry, the larger opening of the truncated pyramidal

aperture can be patterned appropriately to give the required opening at the base of the etch profile.

Figure 4.1 shows the design of the truncated pyramidal aperture. Part (a) shows the layout view of the aperture. The boundary of the large white square was the first pattern etched on silicon. This was etched to a depth of 450 microns leaving a 50 μm membrane illustrated by the dark square at the center. The second etching was done from the back side of the wafer. The small white square at the centre of Figure 4.1a is the opening of the aperture which was designed to be about 50 μm per side.

Figure 4.1b shows the cross sectional view of the design. As can be seen clearly in this drawing the second etch from the backside of the wafer intersects the first etch resulting in a truncated pyramidal aperture.

4.2 Fabrication of Design-1

The entire Fabrication Sequence of Design-1 is shown in Figure 4.2. Each process used in each of the steps will be discussed in detail below, in chronological order.

4.2.1 Layout Design and Mask

Prior to any fabrication, the masks must be designed and prepared to be used in the subsequent photolithographic step discussed in section 4.2.5. The designs for the masks were made with the aid of Cadence mitel 1.5 technology package. The designs from Cadence were then converted to cif (caltech interchange format) and fitted to the standard mask frame available in Kic. The cif files were then converted to Post Script format and submitted to a local desktop publishing company to be transferred onto mylar film. The image from the mylar film was then transferred onto a photographic glass emulsion plate to create a working mask plate for the lithographic steps.

Figure 4.1 Layout and cross-section of Design-1 (a) Schematic Illustration of Design-1 that utilized wet chemical etching (b) Cross-sectional profile at AA'.

4.2.2 Wafer Selection

Since the windows on the mask were designed to obtain a membrane of a certain size and since the membrane size is dependent on the depth to which silicon is etched, it is important that the thickness of the wafer be taken into account. The calculations for the designs were based on a wafer thickness of $500 \mu\text{m}$. However, since all wafers are not the same size, it was important to measure the wafer thickness before processing it. A digital Vernier Caliper was used to measure the wafer thickness and

Figure 4.2 Fabrication Sequence for design-1.

wafers with a thickness of $500 \pm 10 \text{ } \mu\text{m}$ were chosen.

4.2.3 Wafer Cleaning

Before the wafer was processed it was important to first clean the wafer. For this purpose a standard wafer cleaning process called RCA cleaning was used. This process removes any unwanted metals and organic materials existing on the wafer surface. Since the wafer was going into the oxidation furnace in the subsequent step, it was imperative that the wafer should not pollute the environment in the furnace. As a standard practice, the wafers always had to go through an RCA cleaning process⁵ before it enters the oxidation or diffusion furnace.

4.2.4 Oxidation

Figure 4.2a illustrates a silicon wafer that was oxidized on both sides. There are 2 types of oxidation. One is wet-oxidation and the other is dry-oxidation. Wet oxidation is done in the presence of water vapor and dry-oxidation in the absence of it. Wet-oxidation is a faster process but results in a low quality porous silicon dioxide. In comparison, dry-oxidation is a slower process but produces a more dense and better quality oxide.

The wafers were carefully inserted into the oxidation furnace at 750°C . Then the furnace was programmed to reach a temperature of 1100°C . By choosing an appropriate time, an oxide thickness of about $1.2 \text{ } \mu\text{m}$ was obtained⁶. Standard charts that display thickness versus time for different temperatures were used in choosing the above mentioned parameters.

5. Table 1 in Appendix A.3 gives the steps for the RCA cleaning process.

6. Table 2 in Appendix A.4 gives the details of the parameters.

4.2.5 Photolithography

After the oxide was grown, it was necessary to transfer the pattern from the mask onto the oxide. Patterning is the process that removes specific portions of the top layer(s) of the wafer surface. This process uses light and hence the name photolithography.

Photolithography is one of the most critical operations in Semiconductor Processing. It is the process that sets the surface dimensions of various parts of the device. The goal of this operation is two fold. First it has to create in and on the wafer surface a pattern the dimensions of which are as close to the design requirements as possible. This is called the resolution. The second goal is the correct placement of the pattern on the wafer, with respect to other patterns.

The transfer of the pattern happens in two steps. First the pattern on the mask-1⁷ is transferred onto a layer of *photoresist* (PR), which is a chemical with which the wafer is coated. This photoresist is a light sensitive material similar to the coating on regular photographic film. The silicon wafer was coated with a thin layer (~1 μm) of shipley SPR 2-10L. The spinner that was used to spin the PR on the wafer had a vacuum chuck that rotated at 4000 rpm's for 30s. After the wafer was coated with PR it was kept in the furnace for soft baking at 100° C for about 20 minutes. This process hardens the PR and prepares it for the subsequent step.

When exposed to ultra violet (UV) light, the solubility of the PR is either increased or decreased. The PR whose solubility increases is called positive photoresist and the PR whose solubility decreases is called negative photoresist. The PR we used was

7. The layout of mask-1 is shown in Appendix A.1.

a positive PR. A Quintel Mask Aligner was used to expose the wafer to UV light for 40 seconds.

Removing the portions where the solubility increased was done by using a chemical solvent (developer). AZ 351 developer was used for 45 seconds to develop the PR. After rinsing and drying the wafer it was cured in a hard bake oven at 120° C for 30 minutes. This process transferred the pattern from the mask onto the PR layer, which in turn acted as a mask for the subsequent step of oxide etching. Figure 4.3 illustrates the pattern transfer from the mask to the photoresist.

Figure 4.3 Pattern transfer from Mask to PR.

4.2.6 Oxide Etching

In the next step this pattern needed to be transferred to the oxide layer which in turn would act as a mask for the silicon etch. This was achieved by etching the oxide that was now exposed. Figure 4.2b shows the step where the oxide was etched. The oxide etchant used was called buffered oxide etchant (BOE). BOE is 6-7 parts of NH_4F added to 1 part of HF. The chemical reaction for the process was,

BOE is a reasonably selective etchant for oxide and etches silicon at a very slow rate. However, HF attacks PR to some extent but the presence of NH_4F reduces this effect.

The wetting properties of silicon and silicon dioxide is used for the end point detection of the oxide etch. Silicon is hydrophobic and so repels water but on the other hand silicon dioxide is hydrophilic and so attracts water. Therefore, water will be repelled from areas where the oxide pattern was etched and water will cling on to areas where the oxide was not attacked. Usually, a 1.0 μm thick oxide would take between 12-15 minutes to etch completely.

After the oxide was etched it was rinsed in de-ionized (DI) water. Then the PR was stripped off by dipping the wafer in acetone for about 5 minutes. The wafer was then rinsed in DI water thoroughly and dried. The wafer was now ready for the subsequent silicon etching step which creates the pyramidal pits. Due to the sensitivity of PR to white light all steps performed with the PR had to be performed in a yellow light environment.

4.2.7 Anisotropic Silicon Etching

The etchants used for the anisotropic etching of silicon were Ethylenediamine-Pyrocatechol (EDP) and Tetra Methyl Ammonium Hydroxide (TMAH). EDP is very toxic as opposed to TMAH. In addition EDP had a tendency of depositing a white residue on the silicon after several hours of etching. Etching with EDP also had a higher tendency of creating surface defects such as *hillocks* and *micro-pits* on the silicon membrane. Although 25 wt% TMAH had a higher etch rate for the masking oxide, it was not toxic, did not deposit any residue on the silicon surface and was less likely to create hillocks and micro-pits. As a result of the higher oxide etch rate, when using 25 wt% TMAH, it was necessary to use a thicker oxide layer and to take the lateral oxide etch into account when designing the mask. One other major difference between the two etchants were the etch rates for silicon. At 95° C EDP etched at about 85 μm per minute as opposed

to about 60 μm per minute for 25 wt% TMAH.

In order to take advantage of the faster etch rate of EDP and the better surface quality that results from etching with TMAH, a double etching process was used. This involved first using EDP for about 75% of the silicon etch, to speed up the etching process, and then using TMAH for the rest of the etch, in order to obtain a cleaner and defect free membrane surface.

Since we required a 50 μm thick silicon membrane and since we did not have any etch-stop layer in the wafer, it was imperative that the etch was carefully timed. Based on the etch rates and the fact that the wafer was about 500 μm thick, it was necessary to etch for a total of about 6 hours in EDP and TMAH combined, to obtain a 50 μm thick membrane. Figure 4.4 below shows the set-up used for TMAH and EDP etching.

The reflux system was used by circulating cold water through it so that the vapor formed inside the beaker condensed and returned into the solution, maintaining the concentration of the solution constant. A magnetic stirrer was placed inside the beaker as can be seen in Figure 4.5 in order to maintain a homogeneous solution throughout, thereby maintaining a fairly constant etch rate over the surface of the wafer. Once the temperature reached 95 °C and was stabilized the oxide masked wafers were immersed into the etchant after placing them in a wafer holder, as shown. After the timed etch, the wafer was carefully rinsed so as to not damage the membrane. Figure 4.2c shows the cross section of the etch profile after the anisotropic silicon etching process.

After the first silicon etch, the wafers were once again RCA cleaned since they had to be put into the oxidation furnace for another oxidation. The purpose of the

Figure 4.4 Apparatus used for EDP and TMAH etching.

second oxidation was to provide another mask for the second silicon etch that was to be followed. An oxide of about $0.5\ \mu\text{m}$ was grown in the second oxidation. This step is shown in Figure 4.2d.

After the oxide was grown, the same steps of spinning PR, soft baking,

exposing to UV with the mask-2⁸, developing and hardbaking were performed as before. However, this time, the wafer had to be flipped upside down as the backside had to be patterned. The alignment of the mask-2 to the pattern of mask-1 was done with the aid of the infra-red alignment capability of the mask aligner and the alignment marks that were put in the masks as part of the design.

After hardbaking, the oxide was etched in BOE (Figure 4.2e), after which the PR was removed with acetone and then prepared for the second silicon etch. The second silicon etch was a shorter etch since only a 50 μm membrane was to be etched. Since the second etch was to etch all the way through the membrane, this time it was not crucial that the etch be timed. Also the oxide layer over the membrane acted as an etch stop layer. However, since the etchants have a non-zero etch rate for the $\langle 111 \rangle$ planes it was necessary to remove the wafers from the etchant in time, before the etchant widened the opening. The resultant etch profile after the second silicon etch is shown in Figure 4.2f. The next step was to etch all the oxide with BOE. Figure 4.2g shows the microfabricated aperture after all the oxide was removed.

4.3 Packaging

After all the oxide was etched the wafer was scribed with a diamond scribe and then diced to obtain the individual dies. Figure 4.5 is an illustration of the packaging of the microfabricated aperture for testing.

Holes were drilled in two pieces of plexi glass (Figure 4.5a,c) and glued on either side of the die shown in Figure 4.5b. Two tubes with platinum electrodes secured in them were then inserted into the holes of the plexi glass in a tight fit (Figure 4.5e), to com-

8. The layout of mask-2 is shown in Appendix A.2.

plete the packaging of the device.

4.4 Summary

In this chapter we discussed the design and entire fabrication sequence of Design-1, which was the main focus of this research. The experimental results as well as the modeling and simulation results will be presented in Chapter 6. Another design (Design-2) was designed, fabricated and tested. The next chapter describes the design, fabrication sequence and experimental results obtained for this design.

Figure 4.5 Packaging of the microfabricated aperture.

Chapter 5

Design, Fabrication and Test Results: Design-2

This chapter will outline the design, fabrication, assembly and test results of the design-2. Design-2 was an attempt for an alternative design. It is a more compact design in that it includes fluid flow chambers, as well as the microfabricated aperture. This is possibly a more elegant design that is convenient for packaging. Another difference between the two designs is that the aperture in design-2 is made on the substrate and not through the substrate, as in design-1.

5.1 Design-2

The requirements for this design were the same as those outlined in section 4.1.1. However, the etching of Silicon was done using a dry etching process, namely a deep reactive ion etching process (DRIE). Due to the highly anisotropic nature of the DRIE technology, vertical walls with very high aspect ratios were obtainable and hence it was convenient to design two micro chambers on either side of the aperture that served as the inlet and outlet ports for fluid flow.

A schematic of design-2 is shown below in Figure 5.1a. The shaded area in Figure 5.1a is the unetched silicon and the white area is the area to be etched. The mask was designed so that the feature shown in the centre is an aperture of width $70\text{ }\mu\text{m}$ and length $100\text{ }\mu\text{m}$. The depth to which it was etched was $70\text{ }\mu\text{m}$. The cross section across the aperture would appear as shown in Figure 5.1b.

Figure 5.1 Layout and cross-section of design-2. (a) Schematic illustration of the design-2 that utilized Deep Reactive Ion Etching. (b) Cross Section at AA'.

Although the cross section of the aperture shown in Figure 5.1a has perfectly vertical walls, that is not exactly the case, since there will always be some rounding at the edges. However, since the DRIE process has an aspect ratio of about 30:1, one may assume that the walls are nearly vertical. Hence the shape of the aperture obtained from this etching process is practically rectangular. Hereafter, this design will also be referred to as the rectangular aperture design.

The top chamber was divided into two compartments: A and B, as shown in Figure-5.1. The cell/particle containing electrolyte would enter into compartment A and pass through the aperture in the centre and then into the bottom chamber. This is achieved with the aid of a vacuum applied over the bottom chamber. The channels (compartment B) on the two sides of compartment A contain a “sheath fluid” for the purpose of focusing the cells so that the cells would tend to queue and flow, one after the other. A sheath fluid is simply an electrolyte solution that is void of any cells or particles. Such a mechanism used to focus the cells is called hydrodynamic focusing. This also has the advantage of preventing recirculation of the particles around the aperture.

5.2 Fabrication of the Design-2

The design for the microfabricated chamber was submitted to the Berkeley Micro-Lab at the University of California, Berkeley, where the mask was developed and the DRIE etch was performed.

5.2.1 DRIE Technology

The DRIE technology used in the Berkeley Micro-labs is the Advanced Silicon Etch (ASE) process offered by Surface Technology Systems (STS). The ASE process has the advantage of creating very high aspect ratio structures by implementing a

highly directional etch. The directional etch is based on a sequentially alternating etching and deposition of the protection layer in a timely fashion. In this cyclic way the etching and deposition can be balanced to provide accurate control of the anisotropy.

The DRIE process can be summarized as follows. First the deposition precursor gas is dissociated by the plasma to form ion and radical species. (Eq 5.1) [27]

These ion and radical species undergo polymerization reactions to result in the deposition of a polymeric layer or passivation layer. This layer $n\text{CF}_2\text{f}$ (f denotes film) is deposited on the surfaces of both the silicon and the mask during the first step as shown schematically in Figure 5.2(a).

The next step of the ASE process is the etch cycle. Here the gases are switched from CF_4 to SF_6 to allow for etching. During this etch step SF_6 first dissociates as following.

Next the fluorine radicals remove the surface passivation as shown in Eq. 5.4.

This is followed by the silicon etching that takes place according to Eq. 5.5 and 5.6.

Figure 5.2(b) shows the etching process schematically. After this the deposition step is repeated to begin the cycle again. Here the directionality of the etch is controlled by the ion bombardment in its role of aiding the removal of the surface

Figure 5.2 Illustration of the ASE process (a) Deposition of polymer layer (b) Etching of the passivation layer and silicon.

polymer. Fine control over the chemistry is required in order to maintain a good balance between the etching and deposition.

After the wafer was etched, the mask was stripped and the wafer was scribed and then diced to obtain individual dies that contained the fluid chambers.

5.3 Assembly

The assembly consisted of two parts. The first part was the etched silicon die and the second part was a glass part which served several purposes. The glass that was attached to the silicon provided extra support, it held the inlet and outlet tubes, and it also contained the sputtered metal electrodes for electrical connection. Figure 5.3 shows the assembly process schematically. The silicon part shown (Figure 5.3.a1) was etched using the ASE-etch (Advanced Silicon Etch) process (Figure-5.2) as discussed above. The pattern etched was that shown in Figure 5.1a and 5.1b.

The processing of the glass part is described below. First, two holes, roughly 1 mm in diameter, were drilled in the pyrex glass with a diamond drill (Figure 5.3b1). Then 50 nm of chromium (Cr) was sputtered followed by 20 nm of nickel (Ni), on both sides of the sample (Figure 5.3b2). The Cr/Ni layers served as the seed layer for the subsequent gold electroplating⁹. During the sputtering process the two holes served as vias allowing the Cr/Ni to pass through it. Next, photoresist was spun on both sides of the glass and then patterned. Two masks were used, one for patterning the bottom part and the other for the top. The pattern on the photoresist defined the areas for electroplating gold over the Cr/Ni layer (Figure 5.3b3). The gold on the bottom defined the coulter electrodes that were to be in contact with the fluid flowing underneath and the gold on the top served as the electrical connection from the electrodes to the outside circuitry. The undesired Cr/Ni was etched away after the photoresist was stripped. The Cr was etched with a Nitric Acid based etchant and the Ni with transene Aluminum etchant. Subsequently, two more holes were drilled in the glass to serve as the fluid inlet and outlet ports (Figure 5.3c1).

9. See Appendix A.5.

Figure 5.3 Fabrication sequence: Design-2. (a1) Silicon (a2) Advanced Silicon Etched (b1) Drilled pyrex glass (b2) Cr/Ni sputtered (b3) Gold electroplated (c1) Anodic bonding of glass to silicon (c2) Conductive epoxy contact.

Next, the Si and the glass components were anodically bonded¹⁰ (Figure 5.3c1) at 400 °C and 900 V, to provide a hermetic seal between the two interfaces. A silver-based conductive epoxy was then used to seal the two holes that had gold (Au) on both sides (Figure 5.3c2). This step assured a contact between the electrode in the bottom and the metal line connections on top. It also sealed the chamber for efficient fluid flow. A schematic of the complete device is shown below in Figure 5.4. Plexi glass-1 provided extra protection for the silicon chip and plexi glass-2 was glued over the inlet and outlet ports to provide support for the inlet and outlet tubes. These were secured with ordinary Krazy glue.

Figure 5.4 Complete assembly of design-2.

5.4 Testing

As was mentioned in section 3.3.2, in order to simulate blood cells, latex beads of the appropriate size were used. The inlet port was connected to a tube containing the particles suspended in electrolyte and a laboratory aspirator was connected to the

10. See Appendix A.6.

outlet port as the vacuum source. Two electrolytes were used, that were interchangeable. One was 0.9% NaCl, which is also known as the standard saline solution, and the other was a Phosphate Buffered Saline (PBS) solution (pH 7.4).

The gold (Au) electrodes were connected to the external circuitry¹¹ which consisted of a constant current source across the electrodes. The constant current was maintained at $500 \pm 2 \mu\text{A}$. The output from the circuit was connected to an oscilloscope for viewing the signal.

5.5 Results and Discussion

The signals obtained by the traversal of latex beads through the rectangular aperture of design-2 produced signals that were not clearly detectable. Two reasons were believed to cause this.

Firstly, the contact made with the conductive epoxy between the electrodes, as shown in Figure 5.3c2, was thought to be a source of error. It was not possible to carefully control the depth to which the conductive epoxy made its way since it was in a liquid/solid phase (similar to wet cement) when it first made contact with the electrodes. Although the epoxy was cured with heat immediately at 80°C , it was quite possible that it made its way all the way down, due to gravity, and made contact with the silicon substrate. If both electrodes on either side of the aperture made contact with the silicon substrate then the substrate would certainly be a means for electrical conduction which could result in electrical noise in the circuit.

The second reason was also related to the electrodes. The Cr/Ni and Au layers in Figure 5.3 were not drawn to scale since they were very thin. So, although the

11. See appendix A.7.

layers seemed thick, all layers together were less than 100 nm thick. Therefore, it was possible that the conductive epoxy was not making proper contact with the part of the electrodes under the glass. This too could result in poor quality signals.

Although this device did not produce good signals, its design was compact since it integrated the microfabricated aperture and the microfabricated chambers together. Future work can improve the electrode configuration which should result in better signals.

Chapter 7 discusses the testing, and the modeling that was done for design-1. Design-1 produced far better results than design-2.

5.6 Summary

This chapter discussed the design, fabrication, packaging, testing and test results of design-2. Although this design incorporates microfabricated chambers as well, apart from the microfabricated aperture, in a compact design, it requires further work to improve the signal quality.

The next chapter discusses the testing and test results of the truncated pyramidal aperture discussed in the previous chapter. It also discusses the modeling and simulation results.

Chapter 6

Testing, Results and Modeling: Design-1

This chapter will deal with the testing of the pyramidal aperture described in Chapter 4 and discuss the test results obtained. It will also discuss the modeling and simulations that were performed.

6.1 Testing

The inlet port in Figure 4.5e was connected to a tube containing the particles suspended in an electrolyte and a laboratory aspirator was used as the vacuum and connected to the outlet port. Again, 0.9% NaCl or PBS solution was used as the electrolyte medium. The platinum (Pt) electrodes were connected to an external circuit¹² that maintained a constant current of $500 \pm 2 \mu\text{A}$ across the aperture. As in the testing of design-2, the output of this circuit was connected to an oscilloscope. Pictures of the displayed signals were taken with the aid of a polaroid oscilloscope camera mounted on the oscilloscope screen.

6.2 Results - Signals from Particles

The size of beads used to simulate the cells were 5, 10 and 20 μm . These sizes roughly corresponded to the sizes of platelets, red blood cells and white blood cells respectively. Signals obtained due to the traversal of these particles across the aperture were very clearly observable. Figure 6.1 shows typical signals obtained with 10 μm beads. The signals in Figure 6.1a were obtained with a 45 μm aperture whereas the

12. See Appendix A.7.

Figure 6.1 Oscilloscope images. (a) Typical signals obtained for 10 μ m beads from a 45 micron aperture (b) Typical signals obtained for 10 μ m beads from a 50 micron aperture.

Figure 6.2 Oscilloscope images. (a) Signals obtained for 10 μ m beads from a 52 μ m aperture. (b) Signals obtained for 20 μ m beads from a 53 μ m aperture.

signals in Figure 6.1b were obtained from a 50 μm aperture. The amplitude of the signals were between 8-12 mV. This implies a resistance change of about 20 Ω for a 500 μA current. Figure 6.2(a) shows some more signals obtained with 10 μm beads. These signals are displayed on a larger time scale and so, many more pulses can be seen.

Figure 6.2 (b) shows typical signals obtained for 20 μm latex beads. As expected, the amplitude of these signals is higher, and is between 20-25 mV. This implies a maximum resistance change of about 45 Ω . Devices of aperture sizes ranging from 40-60 μm were also successfully tested for bead sizes of 10 and 20 μm . Devices were tested with 5 μm beads as well. However, this did not result in any appreciable signals. The resistance increase as a result of the traversal of 5 μm beads across the aperture was not sufficient in order to create clearly detectable signals. Therefore the particle resolution of our device is a particle of size between 5-10 μm . However, if the ratio of particle size to aperture size was larger for the 5 μm beads, then clearly we would obtain detectable signals. This could be achieved simply by using a smaller aperture.

6.3 Comparison of Signal Amplitude with Theory

From Equation 3.9 of chapter 3.4, we know that,

$$\Delta U = \frac{\Delta V(i \cdot \rho_2)}{a^2}$$

From Appendix A.11 $\rho_2 = 55.55 \Omega\text{-cm}$. Also, we get from Appendix A.12 for the average cross sectional area ($a=A_{av}$), $A_{av} = 84.5 \times 10^{-6} \text{ cm}^2$. $i = 500 \mu\text{A}$ and $\Delta V = 4.2 \times 10^{-9} \text{ cm}^3$ for 20 μm sized particles. By substituting these values we get a value of about 16 mV for the amplitude of the voltage pulse. Therefore, the theoretically predicted value is within 20% of the experimentally obtained value of 20 mV for 20 μm beads. The discrepancy is mainly due to the fact that the theory assumed a homogeneous electric field inside the

aperture. However, this is not true for the pyramidal aperture and so this introduces a small error in the calculations. Therefore, the difference between the theory and experiment is due to the inhomogeneous electric field in the pyramidal shaped aperture.

6.4 Signal Shape

Traditional Coulter counters with cylindrically shaped apertures produce signals that are roughly gaussian in shape as reported by Leif et. al [28]. Since we are using a non-cylindrically shaped aperture, we wanted to study the shape of the signals from our aperture and compare it to an ideal gaussian function. By plotting the points on our signals against a gaussian curve, we learned that the signals we obtained were nearly gaussian in shape as well as shown in Figure 6.3. This was a very important discovery, especially because not only did the truncated pyramidal aperture produce clearly detectable signals for micron sized particles, but the shape of the signals were also comparable to the signals obtained from conventional coulter apertures.

The correlation (R) of the fit in Figure 6.3 was calculated¹³ to be 93.8%. Many such graphs were plotted for different pulses. The average correlation was about 94%. This shows the near-gaussian shape of the signals.

6.5 Modeling

In order to verify the functionality of the Aperture Impedance technique we modeled our device using a resistor network. Figure 6.4 represents a general resistor model for a unit volume of electrolyte where the resistors in the x, y and z directions represent the resistances in each direction.

13. Calculation was performed by *KaleidaGraph* Graphical Analysis software using a least squares regression scheme.

Figure 6.3 A gaussian fit for the experimental data.

represent the resistances in each direction.

Figure 6.4 Resistor model for a unit volume of electrolyte.

For simplicity if we consider a symmetrical rectangular aperture across which a constant electrical field exists, it would appear as shown in Figure 6.5. We assume that the bulk current flow is in the direction of Y (Figure 6.4). The dotted vertical lines are the equipotential lines (electrical field lines are orthogonal to the equipotential lines). As a matter of fact, the ZX plane (Figure 6.4) is an equipotential plane by virtue of the nearly homogeneous field that exists in and around the aperture. Therefore, there is no current

Figure 6.5 Equipotential lines across aperture with constant current flow. [24]
 flow in the Z direction and X directions in the aperture, and in the vicinity of its openings.
 Hence, in and around the aperture the unit resistor model can be simplified to look like the
 model shown in Figure 6.6.

Figure 6.6 Simplified resistor model for unit volume of electrolyte.

Figure 6.7 approximates a volume that roughly represents the electrolyte in and around the aperture. A unit volume of the electrolyte is represented by the simplified unit lumped resistor model that was shown in the Figure 6.6. This unit volume can then be repeated (dotted lines of Figure 6.7) to fill the entire electrolyte volume inside and outside the aperture. By representing all the resistors in the X direction by the equivalent parallel

computational simplicity. The equivalent 2-D model thus obtained is shown in Figure 6.8.

Each dot in Figure 6.8 represents a resistor¹⁴. The size of the dots are not proportional to the resistance values. The value of each resistor is chosen such that the value is representative of the resistance of a unit volume of electrolyte. The volume of electrolyte in the aperture is much smaller than the volume of electrolyte outside it and therefore there are fewer resistors in parallel as opposed to outside the aperture. Therefore, the resistance value per unit volume inside the aperture will be higher than outside the aperture. This is consistent with the fact that the largest resistance appears inside the aperture, due to the constriction of electrolyte flow.

In Figure 6.8, only a few resistors are shown for the purpose of illustration. However, the density of these resistors can be chosen to be higher in the vertical and horizontal directions. As a matter of fact, the higher the density of resistors, the closer the simulation result will be to the actual situation. The largest dot shown, is the high resistive resistor network representing the non-conducting cell/particle moving across the axis of the aperture. The higher the density of resistors in the model, the larger the density will be for the resistors in the high resistive network representing the non-conducting particle.

A “C” program¹⁵ was written to generate the network of resistors shown above. The number of resistors used for the simulations were 1000. Simulations were done using the H-Spice circuit¹⁶ simulation software.

14. Resistor values are given in Appendix A.8.

15. The C program can be found in Appendix A.8.

16. The H-Spice circuit in the simulation is shown in Appendix A.9.

Figure 6.7 Illustration of the 3-D resistor network model

Figure 6.8 Illustration of the 2-D model

6.6 Resistor Values

In order to determine the resistance for a unit volume, we need to know the resistivity of the electrolyte. The calculations in Appendix A.11 find a value of $55.55 \text{ } \Omega \text{ cm}$ for the 0.9% NaCl electrolyte used. Using this value the calculation of the aperture resistance (Appendix A.12) gives a value of $4.63 \text{ K}\Omega$

6.7 Simulation Results

The simulations were performed using the values obtained above for the aperture resistance and the values obtained for the resistance of the electrolyte. The number of resistors in the network were 1000. This roughly corresponds to a matrix of 16×20 resistors in each of the three rectangles in Figure 6.8. Figure 6.9 below shows the variation of resistance change with increasing particle size. As expected from theory and suggested by [12], the resistance change increases roughly linearly with particle size. Figure 6.9a shows non-conducting particles that consisted of a high resistive resistor matrix of $n \times 1$ and Figure 6.9b consisted of a high resistive resistor matrix of $n \times 2$ resistors respectively, where $0 < n < 6$.

6.8 Summary

In this chapter we discussed the testing and test results obtained for the pyramidal aperture. A model consisting of a resistive network was presented for the modeling of the device. Simulation results of the modeling were presented and discussed.

Figure 6.9 Change in resistance versus particle size. (a) for nx1 resistors in the particle (b) for nx2 resistors in the particle. ($0 < n < 6$ particles)

Chapter 7

Conclusions and Recommendations for future research

Two designs for a silicon micromachined aperture that could be used in a miniature blood cell counter were fabricated using the bulk micromachining technology. The main design was a truncated pyramidal shaped aperture which was fabricated using wet chemical etching technology. The second design was a rectangular shaped aperture which was fabricated using a deep reactive ion etching technology (DRIE). The rectangular aperture design also consisted of microfabricated chambers that were capable of hydrodynamically focusing particles into the aperture. The principle of operation of both designs was the Aperture Impedance principal or Coulter principal.

The truncated pyramidal aperture proved to be a very promising design for cell counting purposes as it produced very clearly detectable signals. Experiments were conducted using latex beads to simulate blood cells. The shape of the signals were also roughly gaussian as observed with conventional apertures made of ruby or sapphire. This clearly proves the successful use of silicon as a suitable inexpensive material for the fabrication of microfabricated apertures that can be used in designing future hand-held point-of-care miniature blood cell counters.

The second design (rectangular aperture), although it offered more features, did not produce signals that were clearly detectable. Further improvement of this design seems promising for a rectangular shaped aperture.

Summary of contributions

- 1) Successfully used silicon microfabricated apertures to obtain clearly countable signals.**
- 2) Signals obtained from the pyramidal shaped silicon microfabricated apertures were as good as those conventionally obtained with ruby and sapphire aperture, proving that pyramidal shaped apertures are a good substitute to the cylindrical apertures.**
- 3) An inexpensive technology, namely the wet-chemical etching technology was used for the fabrication of the pyramidal aperture.**

APPENDIX A

A.1 Photolithography mask-1

A.2 Photolithography mask-2

**EMULSION
OTHER
SIDE**

 FILENAME APR-2-ETC2 POSITIVE POSTSCRIPT FILE

SIDE LIFE EXTENSION

**WE NEED POSITIVE EMULSION
EMULSION SHOULD LOOK LIKE THIS HANDCOPY
TRANSPARENT EVERYWHERE EXCEPT IMAGE FEATURES IT**

भारत

NO FINGER PRINTS IN IMAGE AREA PLEASE

A high-contrast, black and white image of a document page. The page is mostly white with a large black rectangular area in the center. The text "FINGERPRINTS IN IMAGE AREA PLEASE" is visible on the left side, and "FINGERPRINTS IN IMAGE AREA PLEASE" is visible on the right side. The page is framed by a thick black border.

NO FINGER PRINTS IN IMAGE AREA PLEASE

Photolithography Mask 2

A.3 RCA Cleaning Process

Table 1 The RCA cleaning process

Process Step	Chemicals	Process Conditions + comments
1	RCA SC-1 Clean (Organics) DI H ₂ O (1000 ml) NH ₄ OH, 30% (200ml) H ₂ O ₂ , 50% (200 ml)	Temperature: 80 +/- 5 C Time: 10 min
2	DI Water Rinse=	> 3min in running DI water
3	RCA SC-2 Clean (Metals) DI H ₂ O (1050 ml) HCl, 38% (175 ml) H ₂ O ₂ , 50% (175 ml)	Temperature: 80 +/- 5 C Time: 10 min
4	DI Water Dump Rinse	> 5 minutes in beaker of running DI water. Dump beaker. Repeat 2 more times

A.4 Oxidation parameters

Table 2 Oxidation parameters.

	dry	wet	dry	total thick-ness
1st Oxidation	time= 0:38 hr thickness = 0.10 μ m T=1100 deg. C	time= 2:00 hr thickness = 1.0 μ m T=1100 deg. C	time= 0:38 hr thickness = 0.1 μ m T=1100 deg. C	1.2 μ m
2nd Oxidation	time= 0:30 hr thickness = 0.09 μ m T=1100 deg. C	time= 0:20 hr thickness = 0.38 μ m T=1100 deg. C	time= 0:30 hr thickness = 0.09 μ m T=1100 deg. C	0.56 μ m

A.5 Gold electroplating set-up

A typical electroplating set-up.

The cathode was made by Ni/Cr. This was the seed layer on which Au was electroplated. The aqueous metal solution consisted of Au^{++} ions. When the power supply is turned on the positive Au^{++} are attracted to the negatively charged cathode where they accept electrons and deposit on the cathode creating a layer of Au. The Anode was made of Pt plated Ti.

A.6 Anodic bonding set-up

The Silicon and glass are heated to about 400 degrees C. At this temperature the ions in the glass become mobile. When the high voltage is applied between the two substrates with the glass substrate as the anode, the ions are depleted from the glass interface creating an ion dependent region about 1 micron thick with a high electric field. This high electric field pulls the silicon and glass surfaces close together so that oxygen forms new Si-O bonds at the interface. This technique forms a good hermetically sealed bond between silicon and glass.

A.7 External Circuitry

External circuitry connected to the micro-aperture.

A.8 Resistance calculations

Resistance of unit volume of electrolyte

In order to determine the resistance for a unit volume of electrolyte we need to first find out the resistivity of the electrolyte. From Appendix A.11 we get the resistivity of the 0.9% NaCl to be $55.55 \Omega\text{-cm}$. Therefore the resistance across a unit cm^3 is 55.5Ω from Eq. 4.41.

Resistance of micro-aperture

Consider the geometry of the truncated pyramidal aperture shown in Figure 6.5.

Geometry of the truncated pyramidal aperture.

Using Eq. 3.6* (from Chapter 3.4), we get a value¹ of $4.63 \text{ K}\Omega$ for the resistance across the two square faces. Each resistor inside the aperture, shown in Figure 6.8 has a value that is a fraction of $4.63 \text{ K}\Omega$. The exact value depends on the resistor density of the model, i.e., the number of resistors in each branch and the number of branches in the aperture. By appropriately dividing the values for the resistors in series and multiplying for the resistors in parallel the exact value can be obtained. For instance,

1. See Appendix A.12 for the calculation.

in one of the models simulated where there were 16 branches and 20 resistors in each branch, the exact value per resistor was $2.63 \text{ K}\Omega$. For the largest dot in Figure 6.8 that represented the moving non-conducting particle, a value of $2000 \text{ M}\Omega$ was used.

A.9 The C program

```
#include <stdio.h>

main(int argc, char**argv) {

    int i,j,k,l;

    char *r, *ra;

    r = (char *)malloc (128); ra = (char *)malloc (128);

    k = atoi (argv[1]);

    l = atoi (argv[2]);

    r = argv[3];

    ra = argv[4];

    for (i = 0; i < 2001; i+= 2000/k)

        for (j = 0; j < 4501; j+= 4500/l) {

            printf("R%d*%d A%d_%d B%d_%d %sK\n", i,j, i,j, (i+2000/k),j, r );

        }

    for (i = 2001; i < 3001; i+= 1000/k)

        for (j = 2000; j < 2501; j+= 500/l) {

            printf("R%d*%d A%d_%d B%d_%d %sK\n",i,j, i, j, (i+1000/k), j,ra );

        }

}
```

```

for (i = 3001; i < 5001; i+= 2000/k)

    for (j = 0; j < 4501; j+= 4500/l) {

        printf("R%d*%d A%d_%d B%d_%d %sK\n",i,j, i, j, (i+2000/k), j,r);

    }

}

```

A.10 Circuit used for H-Spice simulations

This is the simplified H-Spice external circuit. All resistors are represented with a dot. The resistors in the aperture have the highest unit resistance with a total aperture resistance of 3.3 K-Ohms. The total electrolyte resistance on each side is about 55 Ohms. The large dot represents the non-conducting particle with a resistance of several M-Ohms. Since it is impractical to show all resistor names and node names in the resistor network, only the external component names and the node names are shown.

A.11 Resistivity Calculations

Resistivity (ρ) is the reciprocal of conductivity or specific conductance.

$$\rho = \frac{1}{\kappa} \quad (6.1)$$

Since the conductance of an ionic solution depends on the number of ionic charges, it is convenient to define it in terms of the conductivity per unit concentration of ionic constituent,

$$\Lambda = \frac{\kappa}{C} \quad (6.2)$$

where Λ is the equivalent conductivity in [$\text{cm}^2/\Omega\text{-mol}$] and C is the concentration in [mol/cm^3] [29]. The concentration of 0.9% NaCl is 0.154 M. According to [30] the equivalent conductivity Λ for 0.154 M NaCl is $117 \times 10^{-4} \text{ m}^2/\Omega\text{-mol}$ at 25 °C. Therefore from Eq. (6.2) we get for the conductivity, $\kappa = 0.018 (\Omega\text{-cm})^{-1}$. Now from Eq. (6.1) we get for the resistivity $\rho = 55.5 \Omega\text{-cm}$.

A.12 Calculation of Resistance of Pyramidal Aperture

The average cross-sectional area a , of the pyramidal aperture, from Appendix A.8 is,

$$a = A(l) = (50 \times 10^{-4} \text{ cm} + 1.4 \times l)^2 =$$

$$\text{resistivity } \rho = 55.55 \Omega\text{-cm and } (50 \leq l \leq 120) \times 10^{-4} \text{ cm}$$

$$\text{Therefore, since } R = \rho \cdot l / A = \int_0^{50} \frac{55.55}{(50 + 1.4l)^2} dl, \text{ we get,}$$

$$R = 4.63 \text{ K}\Omega.$$

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