RESISTIVE PULSE SENSORS FOR POLLEN PARTICLE MEASUREMENTS

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ABSTRACT

Micro-Coulter counter is a device that allows measurements number and size of particles in the electrolyte. The sensor measures the electrical resistance of a micro-channel that connects two solution-filled reservoirs. When a particle passes through the micro-channel, it displaces conducting electrolyte, thus a transient resistive pulse of the pore resistance is generated. The magnitude and the duration of the pulse is related to the size, surface charge and mobility of the particle that caused it.

We have developed a prototype resistive pulse sensor, and measurement system platform for performing pollen and micro-particle analysis. We used the device with a single micro-channel with 120µm to measure 20µm and 40µm polymethyl methacrylate (PM) particles and then to detect pollen particles in various KCL concentrations. We experimentally demonstrated the theoretical relation between the particle geometry and the pulse height with 20 µm and 40 µm polymethyl methacrylate particles. For the first time we found that the resistance of the electrolyte-filled micro-channel decreases rather than increases when a pollen particle passes through the micro-channel in low ionic concentration solutions. The phenomena might be attributed to the differences between the surface properties of the pollens and polymethyl methacrylate (PM) particles, and is potentially useful for differentiating and counting different pollen particles.
In order to address the long standing problem of long-standing disadvantages of traditional single-channel resistive-pulse sensors, namely low throughput, we demonstrated a multi-channel resistive sensor that is able to detect the micro-particles through its multiple micro scale sensing channels. The usage of multiple channels allows a large volume of sample solutions to be analyzed at one time. Thus the sensing efficiency was improved multiple times. The multi-channel sensing mode was achieved by miniaturization and integration of micro-fluidic channels into one chip, without sacrificing the sensitivity. The resistive pulses due to particles passing each micro-channel were recorded simultaneously and continuously. Our preliminary experimental results on multi-channel sensor indicated that it is feasible for the future development of high throughput micro-machined device that integrates micro-fluidic channels, multiple sensing channels and multiplexed detection for pollen and other bio-particle analysis.
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LIST OF SYMBOLS

ρ: resistivity of electrolyte medium

f: volume fraction factor, the volume ratio of a particle with the channel

d: the diameter of a particle

D: the diameter of micro-channel

L: the length of micro-channel

R: resistance of channel

ΔR: resistance changes due to particle passing through the channel

V(r,θ): electrical potential distribution inside micro-channel

E_x: electrical field strength distribution along with x-axis

Ψ: electrical potential distribution near the charged particles

σ±: net ion density inside the channel

ε: permittivity of electrolyte inside the channel

n_0: ion concentration in bulk solution

ξ: electrical potential in the charged particle surface, also called “zeta potential”

J: current density

µ±: ionic mobility

Re: Reynolds constant

η: fluid density
v: fluid viscosity

τ_{flow}: characteristic time

ΔP: the hydrostatic pressure difference
LIST OF ABBREVIATIONS

KCL: Potassium Chloride

PDMS: Polydimethylsiloxane

NI DAQ system: National Instrument Data Acquisition system

PM particles: Polymethyl Methacrylate particles

DI water: Deionized water

A/D: Analogy signal converting to digital signal

S/N: Signal to noise ratio
CHAPTER I
INTRODUCTION

The need to count and size individual micro/nano scale biological particles exists in a wide variety of applications. The size and shape of the particles are amongst their most important characteristics because they determine many other features of particles suspended in an electrolyte. In addition, a number of the important theoretical concepts for colloid science are introduced from the practical problem of determining the particle size. Particularly, particle sizing is a critical area for the applications, such as environmental monitoring, if it can be shown to be effective.

1.1 General techniques for particle sizing

There are many methods for particles sizing, which include traditional methods utilizing force balance for particles distributions, such as gravitation and centrifugation; directly optical methods measuring particles size by optical images or shape features, such as microscopic and flow cytometry; and indirectly electrical method sensing the electrical resistance changes, as the Coulter Counter (Resistive pulse sensor). The information of a particle size and shape can be obtained by one or some methods depending on the applications and the range of particle size. Figure 1.1 shows the diameter range of particles each method could measure [1].
Gravitation and centrifugation are two of the most widely used methods for indirectly determining particles size by forces balance. The gravitation method utilizes the force balance with gravity force, up-thrust force and friction force when particles suspending in liquid. The particle size distribution could be built up by the concentration as a function of time. The Centrifugation method employs the force balance with centrifuge force and friction force when a particle immerses in water. By computing the friction force coefficient, a particle radius can be estimated [1]. Those methods are mainly used for separating particles, where only group particles with similar size could be determined. But, the time required, even for a large particle, to settle through a reasonable distance under the influence of gravity alone makes that procedure rather limited in some applications, for instance, environmental monitoring and poison particles detecting.
While, the centrifugation method only offers the information of particle’s size distribution, an individual particle size, which is critical in biological research areas, cannot be obtained.

Direct microscopic observation, a widely used method to directly determine particle size, however, has the following drawbacks. First, the sample for optical observation must be dried out, in which it is not the situation for many biological particles. Second, only a tiny fraction of the material can be examined, which limits the inspection for whole samples in many biological researches and thus causes the waste of precious biological materials. Furthermore, the sampling error, for example in microscopy, can be very large if the sample is polydisperse. Therefore, the direct microscopic observation cannot be applied to certain situations in which the individual particle size needs to be examined continuously, such as in environmental monitoring and pollen particles counting.

Another optical method, flow cytometery are widely used for the particle sorting and counting purpose by researchers [2, 3]. The basic principle of a flow cytometery is to line-up particles utilizing the hydrodynamic focusing effect and then the particle samples can pass through the detection area one-by-one [4]. In general, flow cytometery can detect particles within a thin core stream optically or electrically as they flow individually pass a stationary optical/electrical detection system. Fluorescence measurement of dye-labeled particles is the most commonly adopted method for flow cytometry [5, 6]. Figure 1.2 shows the typical structure of flow cytometery. Practically, it is essential that the particle pass precisely through the central region of a focused laser beam in order to optimize the detection signal, hence improving the reliability of the captured particle
information [7]. However, delicate optical components including focused laser beams, various optical detecting devices and complicated control circuits make the system relatively expensive.

![Figure 1.2: A typical structure of a flow cytometry with optical spectrum sensing paths.](image)

Coulter counters or so-called resistive pulse sensors are well-established method for particle counting by measuring the electrical resistance changes, which are caused by non-conductive particles passing through a pore. It was first invented by W. H. Coulter in 1953 [8]. Since then the coulter counter has been used widely in the medical and industrial fields to determine the size distribution of particles [9, 10]. Figure 1.3 shows the work principle of Coulter counter. Typically, Coulter counters consist of two reservoirs of particle-laden solution separated by a membrane and connected by a single...
pore through that membrane. When a particle passes the pore, the resistive change is measured in the electrolyte from one side of the pore to the other side, due to the volume displacement of the electrolyte caused by the particle. The recorded signals from the resistive measurement will be shown as current or voltage pulses for each passing particle. The height of pulse is proportional to the size of a particle and the number of the pluses is represented the number of particles. Thus, the device could be used to determine the size of the individual particles from the height, area of the pulses and to count the particles in a unit volume [11, 12].

Because of the Coulter counter’s simple structure, the Coulter Counter type sensors could detect the sizes of particles with good reproducibility and resolutions. However, for existing Coulter counter instrument, the expensive fragile sensing tube that made by the

![Figure 1.3: Left: Schematic sketch of Coulter counter work principle; Right: shows the resistive pulse when a particle passes the pore.](image)

Because of the Coulter counter’s simple structure, the Coulter Counter type sensors could detect the sizes of particles with good reproducibility and resolutions. However, for existing Coulter counter instrument, the expensive fragile sensing tube that made by the
glass with a pore and the complicated computer software make the Coulter counter instrument difficult to apply in the daily routine testing fields for environmental monitoring. In addition, frequent maintenance, such as cleaning and replacing the sensing tube, and low throughput even increase the cost. Those drawbacks limit the applications of Coulter counter in variety fields. Thus, to develop a cheaper, robust and minimized Coulter counter that satisfies criteria of outdoor environmental monitoring is in demand. Benefited by modern micro fabrication technologies, a single channel resistive pulse sensor could be developed in MEMS lab. Thus, a practical resistive sensor has been able to be utilized in environmental monitoring, such as pollen detection. In the next section, we will briefly review the existing single channel Coulter counters for various applications.

1.2 Existing Coulter counter

Coulter counter has been used to count and analyze different types of particles, ranging from biological cells to colloidal particles [9, 13]. In the following, we will review the state-of-art research in existing single channel Coulter counter. In brief, the Coulter counter research can be divided into two major topic areas: decreasing the diameter of the pore and integrating the instrument on a single chip.

Since the sensitivity of a single channel resistive pulse sensor is related to the diameter of the pore, in which the height of the resistive pulse is cube of the ratio with the diameter of particles and the pore, it becomes obvious that the smaller pore must be obtained to detect smaller particles. Many research groups have developed the methods on fabricating the tiny pore in past decades. DeBlois and Bean [14] described a Coulter-
type device in which a pore with diameter of 500nm was created in a track-etched polycarbonate membrane for detecting the virus of 122 nm in diameter. The track-etch process entails bombarding a solid material (10µm thick polycarbonate film) with a collimated beam of high-energy nuclear fission fragments to create parallel damage tracks in the film. The diameter of the pores is determined by the etch time and the etch-solution temperature. The damaged membrane was etched by NaOH to form pores in a density of 5X10^3/cm². By controlling the etching time, the 450 nm pores can be obtained. The device was calibrated by 90nm polystyrene latex spherical particles. Another experimental Coulter type device, Li and Crooks [15], utilized a glass fiber to imbed in a gold film, which was used as the template to form the sensing pore. The preliminary data show that this device can be used to count 440 nm diameter polystyrene spheres.

The pore size decreased to several nanometers in recent years, and the more smaller particles such as DNA molecular can be detected. Some studies have involved in using nano-techniques to form the small pore. For example, Takash Ito et al. [16] proved that the multi-wall nanotube (MWNT) with diameter 132 nm could be used as a pore and their devices can detect polystyrene nanoparticles with sizes and surface charger simultaneously. Chang et al. [17, 18] used 5nm micro-channel to directly measure DNA molecular. The device was fabricated on silicon wafer; and the channel was form by growing silicon dioxide layer. The micro-channel is 4 to 5 nm in diameter and 50 to 60 nm in thickness.

Another research area is to integrate the Coulter counter in a single chip to form a lab-on-chip. Larsen et al. reported the first micro coulter counter device in 1997 [19]. The device is made on silicon and adopted principles used in conventional coulter counters. It
proved the standard micro fabrication techniques including RIE, wet silicon etching, metallization and anodic bonding for the Coulter counter type particle-sizing sensor. However, no experiments were conducted.

Koch et al. [20] tried to fabricate the whole Coulter counter in a silicon wafer in 1999. Their work proved the concept that the Coulter counter could be a system on chip. They pointed out that integration of elements to form a complete system is one of the major advantages of microelectronics. This can be extended further to particle sorters and flow sensors to generate a library of micro-fluidic devices where integrated systems are possible because of the common fabrication process. Like Larsen’s work, this research focuses on the micro-fabrication of Coulter counter. No experiment work was conducted to demonstrate the sensing mechanism.

Later on, Saleh et al. [21] first presented an integrated micro-scale Coulter counter fabricated in PDMS or glass wafer. They reported quantitative sensing of nano-scale particles using micro-machined coulter counter. Different from prior research, this device is made in quartz or PDMS using IC compatible micro-machining process. The micro-channel was 1µm in diameter and 10µm in length. The electrolyte was KCL solution, which passes through the channel by pressure difference or electrophoresis. The device can quantitatively measure 60 nm particle and DNA molecules since the exactly geometry sizes of micro-channel were known.

1.3 Overview of Current Microscopic Technologies about Pollen Counting

The determination of pollen particles distribution is an important topic in the environmental monitoring. Pollens are tiny, egg-shaped male cells of flowering plants,
which are small and cannot be seen by naked eyes. They may cause big problems for allergic suffers, such as sneezing, rhino head and nasal congestion allergic symptoms, along with headache, poor concentration and irritability. Those symptoms affect people’s social, physical and emotional functioning.

In America, allergic rhinitis is becoming a common chronic illness and a significant burden for people’s social life in terms of effecting work performance and health care cost. Statistic data show that it affects as many as 35 million people annually; of these, an estimated 19 million are employed adults. Overall, up to 40 percent of children and 10 to 30 percent of adults are affected. The annual medical care costs of allergic rhinitis are estimated to $7.7 billion including indirectly costs [22].

To lessen allergic rhinitis, people usually avoid in exposing to the pollens. The index of pollen existence is announced with weather forecast. However, such information is not reflecting the condition of individual habitation space for a very wide range field. People with allergies need more information about pollen distribution to schedule their outdoor activities during the pollen peak season.

For the pollen monitoring networks, the traditional methods based on microscopic inspections are widely used. There are two widely – used methods: the Rotorod sampler and the Burkhard spore trap. Both methods include three typical stages, collecting pollen particles by sampler, staining particles in laboratory and inspecting particles under microscope.

As for the Rotorod sampler, it contains two silicones covered, greasy rods that are attached to a motor and timer. For collecting pollen, this device spin rapidly, at 2,500 revolutions per minute; pollen become projectiles as they drift by and become deeply
embedded in the silicone surface of the rods. After the sampling is complete, the next step is to stain the rod with Calberla’s stain. The acrylic rods are placed into a rod holder and the stain drops are placed on both side of the rod. Once stained, pollen particles are counted under a microscope and the final counts are adjusted to reflect a total count per cubic meter of air.

Compared to the Rotorod sampler, there is a difference in first stage of collecting pollen particles by utilizing Burkard spore trap. Burkard spore trap uses suction to pull air through a slit-type opening. Inside the slit is a greased, flat surface (a collection type) that advances in increments over time. The capture of the pollen grains occurs by impacting a sticky surface, which is then adequately prepared for examination under the optical microscope.

Both methods appear a big challenge in the third stage of counting pollen. Counting pollen can be complex and requires knowledge of their size, shape and texture. On the other hand, it is labor – intensive. For instance, a skilled lab technician takes about 45 minutes to an hour to differentiate the grains among the various pollens [23]. Nobody has developed a satisfactory automatic method for counting pollen [24].

Recently, a few researchers are utilizing Coulter counters for pollen detection. Pollen represents an important class of bio-particles that are critical to public health and homeland security. Size selective aerosol sampling for pollen allergen analysis has been a concern for the researchers and different samplers and filters had been proposed. Reidiker et al. [25] proposed the use of high volume cascade impactors with silicone grease for collection of pollen as the sampling technique for optical microscopy and other microscopic techniques. Nevertheless, microscopic detection is time consuming and
required skilled personnel. A method of pyrolysis-gas chromatography-mass spectrometry (GC/MS) was utilized to identify biological micro particles. [26,27,28]. In this method, bio-micro-particles are thermally decomposed to produce gas mixtures which are analyzed by MS of using shape index and pattern recognition methods to distinguish pollen from other particles. Chemo-metric comparisons of the sample with those of stored standard samples allow identification of the biological samples as shown by Kalmen et al. [29]. Other researchers utilize Coulter counter instrument to determine the biological properties of pollen particles. Kelly et al. [30] discussed a method to estimate pollen viability from pollen size variation by the Coulter counter Model Z1 dual (Coulter, Miami, Florida, USA). Because the variation in the size of pollen grains related to their viability, by analyzing the histograms of particle size distribution, the pollen viability can be directly estimated. The statistic results from the size distribution of pollen grains suggest an alternative to more labor-intensive methods for estimating pollen viability. However, the finding reported in their study has an obvious limitation so that relationship between pollen sizes and pollen variability has to be evaluated on a case-to-case basis.

The sizes of pollen particles are from 10µm to 60µm. From technical respective, the pollen particles could be counted by their size and surface properties. With the proper diameter of the pore, the Coulter counter can measure pollen particle’s size and count the number. In this thesis, our researches focus on development of Coulter-type device to detect pollen particles. The research involves two steps: First, we will utilize a single channel Coulter counter setup to detect and differentiate pollen. Second, the multi-
channel resistive pulse sensor will be designed to improve the sensing efficiency and thus to
reach the criteria of the application.

1.4 Objectives

While these current pollen detection methods require expensive equipment and complicated algorithms, an automatic, portable and mass-producible device for detecting and differentiating biological micro particles is still unavailable for field deployment to fulfill the strong needs in environmental monitoring and homeland security. With the long-term aim for developing a sensing method suitable for micro particles of biological activities, we will examine the potential of a Coulter counting device for pollen analysis. In this thesis we will report the preliminary results in detecting and counting pollen particles and distinguishing them from other particles. To the best of our knowledge, the use of a Coulter counting device for pollen or bacteria spore detections has not been reported in the literature. Our results indicated the method is feasible for the future development of portable and automated devices for sampling and differentiating bio-micro-particles such as pollens and bacterial spores.

On the other hand, traditional Coulter counters, which use a membrane with a single micro-channel, however, have the limitation of being low in counting efficiency. Coulter counting measurement relies on particles passing through a single micro-channel one by one. Because of the low travel velocity of the particles in a micro or nano scale channel, it could be prohibitively long to analyze a small amount of particle solutions. One could increase the particle velocity by applying large pressure gradient. However, this will lead to the loss of detailed information (particle shape, surface charge, mobility)
about the particles because of the shape of the measurement pulses. Thus it is not a
practical tool for detection and counting a large number of micro/nano scale particles in
the solution. So far, a portable, electronic device that can fast analyze one electrolyte
solution is not available yet. As the second part of this thesis, we designed and fabricated
a high throughput multi-channel sensor for micro-scale particle detection. The
preliminary experiments demonstrated that the sensor indicates the potentials of
improvement in counting efficiency.

The content of this thesis includes five chapters. Chapter II presents some
theoretical considerations about the Coulter-type devices. Chapter III describes the
experimental setup and discusses the experimental data for a single channel Coulter-type
sensor for pollen detection. In Chapter IV, the design and testing of the resistance pulse
sensor with multi-channels is discussed. Chapter V contains the summary and
conclusions of this thesis work. The future research to be conducted by our group to
improve the sensor performance is also included in Chapter VI.
CHAPTER II
THEORIES OF RESISTIVE PULSE SENSOR

The resistive pulse resulting from the passage of particles through a micro-channel can be well described by the pulse height, which relies upon the relative sizes of the pore and the particle to be measured. In this chapter, we will introduce some theoretical considerations, which are later used in interpreting experimental results.

2.1 The increase in electrical resistance

Figure 2.1: Schematic of micro-channel with coordination. X is the direction of fluid flow; r is the vector from the center, θ is the angle. A spherical particle with diameter d is located in the center of cylindrical channel of diameter D, length L.

Figure 2.1: Schematic of micro-channel with coordination. X is the direction of fluid flow; r is the vector from the center, θ is the angle. A spherical particle with diameter d is located in the center of cylindrical channel of diameter D, length L.
A micro-channel in a typical Coulter counter is shown in Figure 2.1. If a non-conducting particle suspended in a conducting medium moves into the channel, it increase the resistance of the channel relative to that of the pore filled with conducting medium alone. The central theoretical problem appears that of determining the increase in resistance of the channel caused by the insertion of a non-conducting sphere far form the ends. The magnitude of this increase in resistance is related to the particle size. In the following, we will discuss several theories that quantitatively describe the resistance change as a particle passes through the micro channel.

**A. Maxwell approximation**

Using an exceptionally ingenious argument, an expression of the micro-channel resistance can be obtained from the effective resistivity, $\rho_{\text{eff}}$, of a dilute suspension with non-conductive spheres in a solution of resistivity $\rho$[31]. Considering that the micro-channel is a cylindrical shape with diameter $D$, length $L$, and a spherical particle with diameter $d$ is located in the center of the channel, an expression of the effective resistivity $\rho_{\text{eff}}$, in terms of the volume fraction $f$ of the spheres in suspension, was given by Maxwell as following.

$$\rho_{\text{eff}} = \rho (1 + \frac{3f}{2} + \cdots) \tag{2.1}$$

Where $\rho_{\text{eff}}$ is effective resistivity of the solution, $f$ is the volume fraction of solution occupied by the particle, $\rho$ is the resistivity of the electrolyte. If we consider the micro-channel without a spherical particle, the micro-channel resistance approaches
\[ R_1 = \frac{4 \rho L}{\pi D^2} \]  
(2.2)

For \( L \) comparable to \( D \), one should substitute \((L + 0.8D)\) for \( L \) to make an approximate correction for end effects [32]. If a particle with diameter \( d \) is located in the center of the micro-channel, the volume fraction of the particle to the total channel volume is

\[ f = \frac{2d^3}{3D^3L} \]  
(2.3)

Substitute \( f, \rho_{\text{eff}} \) to the equation 2.2, the resistance of the micro-channel with a particle is

\[ R_2 = \frac{4 \rho_{\text{eff}} L}{\pi D^2} = \frac{4 \rho L}{\pi D^2} \left(1 + \frac{d^3}{D^2L} + \cdots \right) \]  
(2.4)

Thus, the increase in resistance \( \Delta R \) for \( d \ll D \), in first order, is as

\[ \Delta R = R2 - R1 \approx \frac{4 \rho d^3}{\pi D^4} \]  
(2.5)

It is useful to work with the relative increase in resistance, as the following:

\[ \frac{\Delta R}{R_1} \approx \frac{4 \rho d^3}{\pi D^3} \approx \frac{d^3}{LD^3} \]  
(2.6)

This expression shows simple relation between the diameter of particles and micro-channel geometry, in which the magnitude of a resistive pulse is proportional to the cube of the diameter and inversely proportional to the channel diameter and length. Thus, this effect can be used to size particles if the pore diameter is known. The Eq.2.6 is frequently used later in interpretation of experimental date. The limitation of Eq.2.6 is that the diameter of the particle is much smaller than the diameter of the pore so that the high order terms are ignored in Eq.2.5.
In order to obtain an expression that is valid over a broader ranger rather than small particles $d \ll D$, one must use approximations. One of the most useful approximations for the resistance of a micro-channel of varying cross section is

$$R = \rho \int \frac{dx}{A(x)}$$

(2.7)

where $A(x)$ is the cross sectional area perpendicular to a length coordinate $x$. The electrical field strength is assumed uniform across a cross section while the total current is conserved. While this expression gives a lower bound to resistance of micro-channel, a larger resistance will be given for any non-uniformity of current density. The elements of area in Eq. 6 are the annular rings of solution between the surfaces of the spherical particle and the micro-channel.

$$A(x) = \frac{\pi}{4} \left( D^2 - d^2 + 4x^2 \right)$$

(2.8)

The complete solution, through expansion in powers of the particle’s diameter, is as following:

$$\Delta R = \frac{8 \rho \pi d^3}{3 \pi D^4} \left[ 1 + \frac{4}{5} \left( \frac{d}{D} \right)^2 + \cdots \right]$$

(2.9)

This expression gives a resistance less than the Maxwellian value by a factor of two thirds. Considering the case that when the diameter of the particle is very nearly equal to that of the micro-channel, the extra resistance is localized in the small annular region between the particle surface and the channel wall [33].

Off-axis particles
In reality, the particle can pass through the micro-channel at any position radically displaced from the central axis up until the particle surface contacts the channel wall. This eccentric motion affects the change in electrical resistance of the channel, and thus complicates attempts to set up the relation between the resistance changes to the particle diameter. As mentioned before, any non-uniformity in current density on micro-channel cross-sections will increase the channel resistance while a particle pinches the channel off on one side and opens it up on the other. The calculations of increase resistance are given by W.R. Smythe[34] with the effective length increase of the channel due to an off-axis particle. He found a significant off-axis effect on the electrical resistance, and his calculation results show that a 16% increase in resistance for a particle of size d/D=0.4 that travels off the channel axis by fractional radial distance x=b/R=0.5, where b is the radial distance between the channel axis and the particle center. Later, Berge et al. [33] corrected the Smythe’s numerical predictions by performing the experimental measurement. Their results show that the channel resistance changes are smaller than the prediction, but the pore resistance still increases 8-10% due to the off-axis effect [33]. Thus it is very important to take these effects into account when trying to measure precisely particle sizes using the resistive pulse technique.

**B. DeBlois Approximation**

Maxwell’s expression is based on the channel geometry, and assumes that the resistivity $\rho$ is the function of the volume fraction. While Eq. 2.5 is correct for a spherical particle small in comparison to the micro-channel diameter, DeBlois et al. described an expression in a broader range.
If a particle in a uniform field, the Laplace’s equation $\nabla^2 V = 0$ can be solved with the spherical boundary condition. The analytical solution is

$$V(r, \theta) = A(r + \frac{d^3}{16r^2}) \cos \theta$$  \hspace{1cm} (2.10)

The coordinates are shown in Figure 2.1, where $\theta$ is measured from X axis of the pore. The boundary condition of a non-conducting spherical particle is that the field normal to the sphere vanishes at its surface. $-\frac{\partial V}{\partial r} \bigg|_{r=\frac{d}{2}} = 0$. $A$ is a constant determined by the strength of $\bar{E}$ far from the particle.

The equation 2.6 is an exact solution of the electrical potential inside channel. From the equation 2.6, the axial component of the electric field is derived as:

$$E_x = -\frac{\partial V}{\partial r} \cos \theta + \frac{1}{r} \frac{\partial V}{\partial r} \sin \theta$$  \hspace{1cm} (2.11)

Furthermore, the total current $I$ through any cross section can be calculated by Ohm’s law:

$$I = \frac{2\pi}{\rho} \int_{\frac{d}{2}}^{D} E_x y dy$$  \hspace{1cm} (2.12)

The resistance of the micro-channel containing the particle is obtained by combine the Eq.2.6 and 2.8:

$$R_2 = \frac{\left[ V\left(\frac{L}{2}\right) - V\left(\frac{L}{2}\right) \right]}{I}$$  \hspace{1cm} (2.13)

By using the approximation

$$R_1 = 2\rho\int_{\frac{d}{2}}^{L} \frac{dx}{A(x)}$$  \hspace{1cm} (2.14)

Thus, DeBlois gave the expression of increase resistance as
\[ \Delta R_{(d/D) < 1, (d/L) < 1} \to \frac{4 \rho d^3}{\pi D^4} \left[ 1 + \frac{3}{8} \left( \frac{D}{L} \right)^4 + \cdots \right] \]  

(2.15)

For finite values of \( d/D \), the numerical factor \( F \) is introduced to correct the calculation

\[ \Delta R_{(D/L) < 1} = \frac{4 \rho d^3}{\pi D^4} \cdot F \left( \frac{d^3}{D^3} \right) \]  

(2.16)

\( F \) values are shown in table 2.1 [14].

<table>
<thead>
<tr>
<th>((d/D)^3)</th>
<th>(F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>0.1</td>
<td>1.14</td>
</tr>
<tr>
<td>0.2</td>
<td>1.32</td>
</tr>
<tr>
<td>0.3</td>
<td>1.55</td>
</tr>
<tr>
<td>0.4</td>
<td>1.87</td>
</tr>
<tr>
<td>0.5</td>
<td>2.31</td>
</tr>
<tr>
<td>0.6</td>
<td>2.99</td>
</tr>
<tr>
<td>0.7</td>
<td>4.15</td>
</tr>
<tr>
<td>0.8</td>
<td>6.50</td>
</tr>
<tr>
<td>0.9</td>
<td>13.7</td>
</tr>
</tbody>
</table>

The DeBlois expression gives broader relation of resistance change with particle size, ranging to diameter of particles near the channel. As shown in table I, if a larger size particle passes through the channel, such as \( d/D = 0.5 \) \([(d/D) = 0.125]\), so the numerical coefficient becomes 1.14 to 1.32, which introduce more inaccuracy for the calculation,
the theoretical prediction must be calibrated with experiments. The DeBlois’s expression is derived from the distribution of electrical potential inside the channel, in which more useful electrical parameters, such as current density distribution, and channel resistance can be obtained to further understand the behaviors of particles passing through the channel.

Both Maxwell and DeBlois expressions predict that the micro-channel resistance increases when a non-conducting particle passes through the micro-channel. If the voltage applied between the micro-channel keeps constant, the current flowing through the micro-channel will decrease due to increasing channel resistance. The relative changes in the current $I$ are equal to the relative changes in the resistance $R_I$ when $R_I$ is the dominant resistance in measurement circuit. In practice, either channel resistance or current is measured in order to obtain the size information of a particle.

An important assumption in DeBlois’s expression is that the resistivity $\rho$ of electrolyte keeps constant during the measurement. It is true if the electrolyte filled in channel is homogeneous. But when a particle with surface charge suspending in low ionic concentration solution passes through the micro-channel, the ionic distribution of electrolyte near the particle will distorted by the particle’s surface electrostatic potential; therefore, the electrolyte becomes ingenious and the resistivity $\rho$ inside micro-channel will not be a constant, but a variable.

2.2 The increase in ionic current

When pollen particles are immersed in a KCL solution, there is a general tendency for positive ions to absorb onto pollen particles, and for the negative ions to remain in the
neighboring fluid. The Figure 2.2 shows schematically what the electrostatic potential in the neighborhood of a negative charged pollen particle is expected to look like. Positive ions are attracted towards the particle by the electric field generated by the negatively charged surface but they are also subject to thermal motion, which tends to spread them uniformly through the surrounding medium. The result is a compromise in which a few positive ions remain very close to the surface and their concentration gradually falls off away from the surface, until it reaches that of the bulk solution. The distance over which this occurs depends upon the KCL concentration [1].

![Electrostatic potential distribution near a negative charged particle](image)

Figure 2.2: Electrostatic potential $\Psi$ distribution near a negative charged particle of diameter $d$. $\Psi_0$ is the zeta potential of the particle.

If a micro-channel is filled of the electrolyte with the KCL solution concentration $n_0$, the electrostatic potential $\psi$ at any point near the particle surface is related to charge
density $\sigma$, which is defined as the net charge presented in a small region of space divided by the volume of that region. For the spherical particles, the Poisson-Boltzmann equation is deduced to one dimension form

$$ \frac{d^2 \Psi}{dx^2} = -\frac{\sigma}{\varepsilon} \tag{2.17} $$

$\varepsilon$ is the permittivity of KCL solution. The equation explains how the potential $\Psi(x)$ is related to charge density. In an ionic solution the net charges arises from an imbalance in the numbers of positive and negative ions. For KCL solution, charge density $\sigma$ is given by Boltzmann equation

$$ \sigma = -en_0(e^{\frac{e\Psi}{K_bT}} - e^{\frac{e\Psi}{K_bT}}) = -2en_0\sinh\left(\frac{e\Psi}{K_bT}\right) \tag{2.18} $$

where $e$ is the charge of a proton, $K_b$ is the Boltzmann constant, $T$ is the absolute temperature. So the Eq.2.17 can be written as

$$ \frac{d^2 \Psi}{dx^2} = \frac{2en_0}{\varepsilon}\sinh\left(\frac{e\Psi}{K_bT}\right) \tag{2.19} $$

With boundary condition $x = d / 2$, $\Psi = \xi$, where $\xi$ is the electrical potential in the particle surface, also called “zeta potential” of the particle.

The analytical or numerical solution of electrical potential $\Psi$ can be solved from Eq.2.19 according to the different boundary conditions; once the potential distribution is obtained, the charge density distribution could be derived from Eq.2.18. If electrical field and charge density distribution are known, the total current flowing through any cross-section of the micro-channel can be calculated as [35]:

$$ I_A = \int A J_A \cdot dA_A \tag{2.20} $$
Where $A$ is the area element, and the current density $J_A$ equals:

$$J_A = \sigma_+ U_+ + \sigma_- U_- \quad (2.21)$$

$U_+$ and $U_-$ are the average velocities of positive and negative ions, respectively; they are equal to:

$$U = \mu_i \overline{E} \quad (2.22)$$

$\mu_i$ is the mobility of ions; usually, they are treated as the constant, for $K^+ 76.2 \times 10^{-9}$, $Cl^- 79.1 \times 10^{-9}$ m$^2$s$^{-1}$V$^{-1}$. So the current density can be expressed as

$$J_A = \sigma_+ \mu_i \overline{E_x} + \sigma_- \mu_i \overline{E_x} \quad (2.23)$$

where $\sigma_+$ and $\sigma_-$ are positive and negative ion density; they are the function of bulk KCL concentration and net charge distribution near a charged particle. If we take the coordinates in Fig2.1 and the micro-channel is cylindrical geometry, the total current can be written as:

$$I_A = 2\pi \int_0^D \frac{D}{2} (\sigma_+ \mu_+ \overline{E_x} + \sigma_- \mu_- \overline{E_x}) dy \quad (2.24)$$

where $D$ is the diameter of micro-channel.

Eq.2.24 describes that the total current is the function of the cross-section area $A$, electrical field $E_x$ and charge density distribution $\sigma_+$ and $\sigma_-$. In other words, resistance change of the micro-channel is affected by the particle size, and charge density distribution inside the channel.

The total current flowing through the micro-channel is dominated by the effective cross-section of the channel if the charge density inside the micro-channel keeps constant. When a non-conducting particle inhabits the micro-channel, the area of cross-section is decreased, and then the total current is decreased; therefore, the channel
resistance increase, and a positive resistive pulse is generated due to the passage of the particles, as shown in Fig 2.3 which is the experimental result of a PM particle passing through the channel.

Figure 2.3: The positive pulse of PM particle passing through the micro-channel, where Y-axis is the relative resistance change and X-axis is the time, the height of pulse is near 0.014 and the width of pulse is near 0.5 ms, which represents the fluid velocity of 0.2 m/s.

On the other hand, when a charged particle introduced to the micro-channel, the charge density distribution will be affected by the particle surface charge. In case of electrolyte is low ionic solution; the total current may be dominated by the charged density distribution. Although the cross-section area is deduced by a presenting particle, the charge density may be increased because of the zeta potential of the presenting particle. Thus, the total current could increase due to the increasing of charge density. Since the increase of current $I$, the channel resistance will be decreased; and one can
observe the negative resistive pulse as shown in Fig 2.4, which are experimental result of tree pollen particles passing through the micro-channel in dilute KCL concentrations. Chang, et al. [20] reported the increasing current during the translocation of dsDNA through the nanopore. In our experiments, the negative pulses were also observed when pollen particles passed through the pore.

![Graph showing the negative pulse](image)

Figure 2.4: The negative pulse when a tree pollen particles passing through micro-channel as KCL concentration is 0.01M, where the height of pulse is near –0.009 and the width of pulse is near 0.5ms. The height of pulse is the function of particle size and surface charge properties.

2.3 Noise sources and measurement uncertainties

The effects of inhomogeneous solution, non-uniform electrical strength, off-axis motions and large particles generally cause uncertainties in evaluating particles’ size. In previous parts for deriving the formula of particle size with channel resistance changes, the medium –electrolyte, is assumed in static state and homogenous, where the influences of fluid flow are ignored. Since the complicate physical mechanism of inhomogeneous
effects, one could tread them as a noise source in sensor testing and data interpreting. Generally, this noise source includes two parts, low frequency drifts and high frequency ripples. The drifts are caused by the fluid velocity difference when particles are carried by fluid flow with pressure driven, and those drifts are represented as base current/voltage changes which can be removed by data processing. High frequency ripples are probably because of the medium inhomogeneous or non-uniform electrical current density, which are difficult to predict from the theoretical formula, and have to be estimated from experiments.

Another noise source is the position of a particle passing through the channel. As mentioned before, particles can pass through in any positions from centerline up to channel wall. Those various positions cause the measurement data distributions when uniform particles are selected to pass the channel, and therefore there are uncertainties in resulting the particle’s size. This effect usually generates 6-8% difference according the fore-researchers’ studies [33].

The uncertainty of measurement instruments is one of the main error sources during the testing. The errors in measuring the geometry of micro-channel and the ADC resolutions will be represented in final measurement data. Those errors are random, and could be estimated with standard error derivation. Later in data processing of our experiments, the errors are estimated by repeating measurements.

2.4 Pulse width

The width of resistance pulse is an important measurable parameter, which indicates the transit time of a particle through the pore. By the knowledge of Reynolds number $Re =$
\( \eta U S/\mu \), where \( \eta \) is the fluid density, \( U \) is a typical fluid velocity, \( S \) is a typical length scale, and \( \mu \) is the fluid viscosity, for the length scale \( S \sim 100\mu m \) and fluid velocity \( \sim 0.1m/s \), Re can be calculated as \( Re \ll 1 \). So the laminar flow is built inside channel and the Poisuelle parabolic velocity distribution is immediately set upon entering the micro-channel [36]. The particle suspension is made to flow by a hydrostatic pressure difference maintained across the pore, so that the flow velocity defined the duration of the resistance increase. Then, the width of a pulse can be calculated from the system hydrodynamics. It can be estimated by the characteristic time that takes the fluid to pass the pore along its axis under the laminar flow condition.

\[
\tau_{\text{flow}} = \frac{4\eta L^2}{\Delta PD}
\]  

(2.18)

where \( \Delta P \) is the hydrostatic pressure difference, \( D \) is the radii of the pore, \( \eta \) is the fluid density and \( L \) is the thickness of the pore. In practice, the longer transit time means the wider width of pulses; the more information about the particle’s behavior could be obtained from the pulse. Meanwhile the sensing efficiency is low when fluid slowly passes through the pore. This problem could be solved by the multi-channel structure, in which the fluid flows through each micro-channel in low velocity; but there are \( N \) channels in the sensor where the flow volume rate keeps high and maintains the sensor’s efficiency.

In the next chapters, based on the above theoretical considerations we describe the sensor designs and experimental results including a single channel and a multi-channel Coulter-type sensor.
CHAPTER III

SINGLE CHANNEL SENSOR FOR POLLEN DETECTION

In this chapter, we will show the setup and experimental results of a single channel resistive sensor for pollen detecting and counting. First the accountability of a single channel resistive pulse sensor is demonstrated by various sizes of polymethyl methacrylate (PM) particles. The experimental results are in good agreement with the Coulter counting theory. Next, we also indicated that the pollen particles could be differentiated from PM particles because of their surface properties.

![Figure 3.1: Schematic of experimental set up for the single channel resistive pulse sensor.](image)

29
Figure 3.1 shows the experimental setup for the single channel resistive pulse sensor device. The sensor consists of two plastic blocks and one plastic membrane sandwiched between the blocks. There is a reservoir (1.5 cm length and 0.5 cm in diameter) in each block. A mini channel (diameter of 0.4 cm and length of 1 cm) is fabricated by drilling. By carefully piercing a heated sharp micro-needle through the plastic membrane (thickness of 100 µm), a pore is created. A set of membranes with pores is produced. The pores are inspected under a 400X high precision microscope. Membranes with pores’ diameter 120 µm (D) are chosen for following experiments.

The two plastics blocks are clamped together tightly with the membrane in between to form a sensing zone so that the liquid can flow from the main reservoir to the sink reservoir passing through the pore. Two Ag/AgCl electrodes (1 mm diameter) are placed 1 cm away on each side of the membrane in the mini channel for measuring resistance of the pore.

In order to establish the accountability of the sensor we have chosen standard PM particles of diameter 20, and 40 µm (Fluka Chemie, Sigma-Aldrich Inc.) to check the response. After that, tree pollen particles from Juniperus Scopulorum, and grass pollen particles from Secale Ceral (Sigma-Aldrich Inc.) are tested by the sensor.

In the experiments, the KCL solution with fixed concentration is slowly injected to both reservoirs by a micro syringes. The concentrations of the neutral KCL solutions are ranged from 0.001 to 0.1 M. The device is kept at horizontal position for the solution to reach an equilibrium level in both reservoirs. Then 0.1 ml particle solution is added to the KCL solution in the main reservoir. The solution is then forced to flow through the pore by applying pressure difference along the channel, where the main reservoir was elevated.
1cm higher than sink reservoir sets pressure difference. The solution is agitated before each experiment so that the particles are well dispersed. When particles pass one by one through the pore, current or voltage pulses are detected and recorded. The data obtained is then converted to the resistance change ratio and the diameter and concentration of particles are calculated.

3.1 Measurement by Gamry Potentiostat

3.1.1 40um particle detection

The measurement is performed by Gamry Analyzer (Gamry®PCI4/300™ Potentiostat/Galvanostat/ZRA), which can apply DC voltage from 0 to 5v and measure the ionic current simultaneously. Real time data can be monitored through a computer interface with the Gamry software. In the experiments presented below, a 200Hz-sampling rate is selected. The device is placed inside a Faraday cage to reduce the measurement noise.

40 µm PM particles, with solid content of 10% at the time of purchase, are diluted with deionized water (DI) to form fresh particle solution. Then 0.1 ml particle solution was added to 0.01 M KCL solution in the main reservoir. The particle concentration in the KCL solution is approximately 1.3X10^3 ml⁻¹. An electrical potential of 1.5v is applied across the electrodes and the current flowing through the pore is monitored. Figure 3.2 shows the normalized current (ΔI/I) trace when the particles pass the pore (120µm in diameter). Each pulse represents a particle passing through. The normalized current is useful because it does not depend on the total current passing through the pore.
According to the Ohm’s law, ∆R/R = -∆I/I, the normalized current is equal to the resistance change, from which the particles diameter (d) can be calculated. The average ∆I/I pulse height is 0.012 to 0.02 in Figure 3.2. The particle diameters calculated from the pulses’ heights (Eq.2.5) are in the range of 31.6 -36.8µm. Two typical pulses ‘a’ and ‘b’ are marked in Figure 3.2, in which the diameters are estimated as 35.5µm and 33.1µm. Those calculation results are in good agreement with the actual diameter of the particles.

By measuring the level changes of the solution in sink reservoir at time t = 0 and 2 seconds, the average volume flow rate during the interval can be estimated. From number of pulses appeared in the period of 2 seconds and the estimated average volume flow rate, the concentration of particles in the solution is calculated to be 2X10³, in the same order
of magnitude of the estimated concentration. The results indicate that the device is capable to measure a particle’s size and count the number.

3.1.2 Detection of 40µm and 20µm particles in the mixture

An experiment with mixing 20 and 40µm particles solution was performed to test the ability of the device to distinguishing particles by size. The 0.1ml mixing solution was added to 0.01MKCL solution in the main reservoir, while the pore size is 120µm and applied voltage is 1.5v. The experimental results are shown in Figure 3.3. There are two distinguished regions of the current pulses heights. The calculated particle diameters are 36.3-39.0 µm for 40µm particles and 17.8-22.5µm for 20µm particles.

![Figure 3.3: The experimental results of mixture 20 and 40µm particles.](image-url)
The above experiments indicate that the sensor is capable of detecting the size of particles. Next we will utilize this measurement for pollen detection.

3.1.3 Juniperus Pollen Detection

Tree pollen particles from Juniperus Scopulorum are mixed with DI water to form the particles solution. The microscopic picture of tree pollen particles is shown in Figure 3.4, where the diameter of tree pollen particles is estimated as 20µm. 0.1 ml particles solution was added to the main reservoir and the current pulses are monitored when particles pass the pore. In experiments, we found that there are positive current pulses when diluted KCL solutions are used, which means that the ionic current flowing through the pore increases when a tree pollen particle passes through and the resistance of the pore decrease. A series controlled experiments are performed to understand the signal changes utilizing pollen particles at different concentrations of KCL solution.

![Microscopic image of Juniperus Scopulorum tree pollen particles with 20µm PM particles; the diameter of tree pollen particles is estimated around 20 µm.](image-url)
A set of KCL solution with concentrations of 0.1M, 0.01M and 0.001M are prepared. The membrane with a pore (120µm) is the same as the previous experiment. Figure 3.5 shows the normalized current pulses of the sensor at three different KCL concentrations when tree pollen particles pass through the pore. In Figure 3.5(a), where the concentration of KCL solution is 0.1M, all pulses are negative. The magnitudes of those pulses are in the range of 0.006-0.012, which represent the calculated particle diameters as 24-32 µm, agree with the size measured by microscope.

![Figure 3.5](image)

(a)

Figure 3.5: Normalized current pulses verse time for tree pollen particles measured at 1.5v with 120µm pore (a) in 0.1 M KCL solution.
Figure 3.5, continued: Normalized current pulses verse time for tree pollen particles measured at 1.5v with 120µm pore (b) in 0.01 M KCL solution, (c) in 0.001 M solution.
As the KCL concentration was decreased to 0.01M, it is found that both positive and negative pulses are presented, shown in Figure 3.5(b). The average magnitudes of positive pulses are in the range of less than 0.005 whereas negative pulses are in the range of less than 0.005. When we decreased the KCL concentration further to 0.001M, more positive pulses are presented and the magnitudes increase to 0.01 (Figure 3.5(c)). It shows a tendency that as the KCL concentration decreases, the height of positive pulse increases.

Those results are different comparing to the experimental observations obtained from PM particles, which generate negative pulses regardless of KCL concentration. A possible explanation is presented in our publication [37]. The occurrence of the positive current pulses can be attributed to the surface charges of the tree pollen particles. Consider that the ionic current flow through the pore is affected by two factors: (i) the particle size, (ii) the particle surface charge. Let $N_0$ is the number of ions passing through the pore where no particle is presented. If the volume of pore in $V$ and the ion density is $\sigma$ then

$$N_0 = V\sigma \quad (3.1)$$

If a particle (volume $v$) whose surface charge is negligible passes the pore, it displaces the same volume $v$ of ionic solution. So the number of ions $N_i$ passing through the pore is decreased appropriately

$$N_i = (V-v)\sigma \quad (3.2)$$

$N_i$ is smaller than $N_0$ and leads to a negative current pulse. But, if a particle with surface charge that cannot be neglected, Eq.3.3 applies

$$N_2 = (V-v)\sigma + Z_+ \quad (3.3)$$
where $Z_+$ is the number of ions on the particle surface.

$Z_+$ will dominate in Eq 3.3 if the surface charge is large and the ion density is low, as a result that produces the increase in number of ions and generates a positive pulse. Same conclusion can be derived from previous Chapter II (Eq.2.17). Predicted by Poisson-Balzmann equation, the ion density distribution is contributed by zeta potential of a charged particle and the concentration of bulk solution. The total current is the compromise of the ion density re-distribution and cross-section area. At low KCL concentration (0.001M), the particle’s surface charge dominated the current; hence when a charged particle passes the pore, the current increases and a positive pulse presents. From experimental results, it appears that pollen particles are high surface charged, while PM particles are slightly charged.

3.1.4 Grass Pollen Detection

![Image of grass pollen particles and PM particles](image)

Figure 3.6: The microscopic image of grass pollen particles and 20µm PM particles; the diameter of grass pollen particles is estimated as 40µm.
Figure 3.7: Normalize current pulses verse time for grass pollen particles measured at 1.5v in (a) 0.1M KCL solution; (b) 0.001M solution.
Grass pollen particles from Secale Cereale have been tested using the same experimental set up and procedure. The microscopic image is shown in Figure 3.6, in which the grass pollen particle size is estimated as 40µm. Grass pollen is first tested with 0.1M KCL concentrations. Figure 3.7 shows the negative pulses when particles pass through the pore, which indicate the decrease of the ionic current. The magnitudes of the normalize pulses are in the range of 0.018-0.035. The calculated diameters are 33.5-44.3µm, which is in good agreement with the microscopic measurement.

As the 0.001M KCL concentration was chosen, there are both negative and positive pulses presented when grass pollen particles pass through the pore. The heights of positive pulses are in the range of 0.012 to 0.006 and the negative pulses are less than 0.003 in Figure 3.7 (b). Comparing with the tree pollen particles, the grass pollen particles generate lower positive pulses in dilute KCL solution. It might be because of the different surface properties of grass and tree pollen particles.

3.1.5 The differentiation of PM and Grass Pollen

The experimental results indicate that it is possible to detect and count pollen particles using a resistive pulse sensor, in which the size of pollen particles can be determined with high KCL concentration and the concentration of particles can be counted from the number of pulses. At same time, the surface properties could be utilized to differentiate the pollen particles in low KCL concentration, where the height of the positive pulses are different with tree and grass pollen particles. Figure 3.8 shows the results of mixture 40 PM particles with grass pollen particles, in which the grass pollen
particles generated the large positive pulses and PM particles generated the larger negative pulse in contrast.

![Graph showing experimental results](image)

Figure 3.8: The experimental results of mixture 40 PM particles and grass pollen in KCL concentration of 0.001M.

3.2. Measurement by Labview based DAQ

Gamry PCI4/300TM potentiostat can precisely measure current changes (full scale range +/- 3 nA, 100fA). However, the big disadvantages are the high cost ($30,000) and low sampling rate (200Hz). A low sampling rate will cause the loss of useful information from current pulses. For example, if a particle passes through the channel fast, it might not be detected because of the low sampling rate. Targeting at our long-term goal of developing a lab-on-a-chip multi-channel resistive pulse sensor, another measurement setup is built using National Instrument Data Acquisition board (NI DAQ). Instead of
monitoring current, the DAQ setup monitors the voltage on an external sampling resistor, as shown in Figure 3.9(a). NIDAQ-6220 is a low cost, high precision data acquisition system that has 8 differential analogue input channels, 250KS/s sampling rate per channel, and wide analogue input ranges ($\pm 10v$ to $\pm 0.2v$). The absolute accuracy is only $360\mu v (0.02\%)$ when analogue input is in $\pm 1v$ [38]. The system needs 15 minutes to warm up and runs under LABVIEW.

![Resistance pulse sensor cell](a)

![NI DAQ](b)

Figure 3.9: The sketch of NI DAQ set up; (a) the resistance pulse sensor with NIDAQ measurement system, $V_c$ is the voltage of a battery; $R_c$ is the resistance of the sensor; $R_s$ is the external sensing resistor; (b) the equivalent resistance series circuit; $V_s$ is the measured voltage.

The resistance change of the micro-channel due to the passage of a particle will cause the measured voltage pulse across the external sampling resistor $R_s$. The voltage pulse are measured and recorded by NIDAQ, which is connected to a computer. Then, the resistive pulse is converted from a voltage pulse. The equivalent circuit is shown in
Figure 3.9(b), in which the micro-channel resistance $R_c$ is in series with the sampling resistance $R_s$ (100K or if modified to match the micro-channel resistance). A battery is used to apply a stable DC voltage (3v). The normalized micro-channel resistance change can be derived from the measurement voltage $V_s$ and battery voltage $V_c$.

$$\frac{\Delta R_c}{R_c} = \frac{\Delta V_s}{V_s} \cdot \frac{1}{(1 - \frac{V_s'}{V_c})}$$

(3.4)

where $\Delta R_c$ is the resistance change due to a particle passing through the pore, $\Delta V_s$ is the voltage change in sampling resistor, $\Delta V_s = V_s - V_s'$; $V_s'$ is the measured voltage when a particle pass through the pore.

The measured voltages were recorded with the NIDAQ and a LABVIEW program. A 50 KHz sampling frequency is chosen in following experiments. After the data was stored in computer, we use Eq.3.4 to process the raw data and obtain resistance change. The digital filtering and a custom-written MATLAB program are used to find pulses and count the pulse number.

3.2.1 PM particle Detection

The experimental procedure was the same as that described in previous experiments with Gamry Potentiostat. The membrane with a pore 120µm in diameter and 100 µm in thickness is used. 0.1 ml particle solution was added to main reservoir, while the fluid flow caused by pressure difference. By elevating the main reservoir higher than sink reservoir, the fluid carrying particles flows through the pore in low velocity. KCL solutions with concentration 0.1 M to 0.001 M are used during the experiments.
solution was well agitated by a syringe before each recording. Sometimes we observed air bubbles might block the pore, but those bubbles could be removed by agitating the solution with a syringe. In order to reduce the environmental Electrical-Magnetical-Interference noise, all components are place inside Farady cage on an isolation table.

Responses of the resistive pulses sensor and measurement system were first checked by uniform size PM particles with diameters 20 and 40µm in KCL concentration of 0.01M. Figure 3.10 shows the converted $\Delta R_c/R_c$ versus time trace when 40µm PM particles pass through the pore and Figure 3.11 shows 20µm PM particles where KCL solution was in same concentration (0.01M). Each upward pulse in Figure 3.10 and Figure 3.11 represents a single PM particle passing the pore. All pulses shown in those two figures are upward direction, which indicate that the resistance of the pore is increased when PM particles pass through.

The sizes of particles are easily differentiated from single particle events by their pulse heights. In Figure 3.10(a), most of pulses are in the range of 0.012-0.026, which represent the particle diameters are 30.1-40.2µm. Figure 3.10 (b) shows a magnified region from Figure 3.10 (a), where the calculation diameters are 39.8 and 34.7µm, agree with the particle’s true diameter, 40µm. In Figure 3.11(a), the heights of Pulses are 0.0014-0.0025, in which the particle diameters are estimated as 14.8-18.6µm. The magnified region is shown in Figure 3.11 (b), where the calculation diameters are 17.6 and 18.9µm, in good agreement with the actual diameter, 20µm. Those results prove that the resistive pulse sensor and measurement system have correct responses for regular PM spheres.
Figure 3.10: Normalized resistive pulses verse time traces; (a) 40μm PM particles in 0.01 M KCL solution; (b) magnified region where the calculation diameters are 39.8 and 34.7μm.
Figure 3.11: Normalized resistive pulses verse time traces; (a) 20µm PM particles in 0.01M KCL solution; (b) magnified red region, where the calculation diameters are 17.6 and 18.9µm.
Figure 3.12: Histogram of experimental data of 40 and 20 µm particles (a) 40 µm PM particles; (b) 20 µm PM particles.
The accountability of the sensor and measurement system can be confirmed from the histograms of experimental data in 40µm and 20µm PM particles. Figure 3.12(a) shows a histogram of events versus pulse height for 40µm PM particles, where mean value is 0.0162 in total 87 events. Figure 3.12(b) shows a histogram of number versus pulse height for 20µm PM particles, in which mean value is 0.00259 in total 266 events.

It can be seen that the pulse height distribution of 40µm PM particles is different with 20µm PM particles. This result confirms the ability of the sensor and measurement system to detect particles by size. In addition, the sensor’s response for 40µm PM particles is 34.4µm at mean value, and 18.7µm for 20µm PM particles, which agrees with particle’s true size.

3.2.2 Pollen Detection

Previous experiments with Gamry Potentiostat show the specific behaviors when pollen particles pass through the pore. Similar experiments were performed with NIDAQ measurement system. Figure 3.13 (a) shows normalized resistance change traces versus time when tree pollen particles pass through the pore with KCL concentration at 0.01M. There are both positive and negative resistive pulses in the Figure 3.13(a). The heights of negative pulses are less than 0.01. Figure 3.13 (b) shows the magnified region, in which it clearly shows that the height of negative pulse is larger than positive pulses.
Figure 3.13: The normalize resistance verse time traces. (a) tree pollen particles in 0.01M KCL solution; (b) magnified region, where the heights of positive pulse are less than 0.01, and the negative pulse are less than 0.005.
Figure 3.14: Normalized resistance verse time; (a) Tree pollen particles in 0.001M KCL solution; (b) magnified region, where the height of positive pulse are in 0.008 to 0.02.
Figure 3.15: Normalized resistance verse time; (a) grass pollen particles in 0.01M KCL solution; (b) magnified region, where the pulses represent the diameters of particles 41.9 and 39 µm.
Figure 3.16: Normalized resistance verse time; (a) Grass pollen particles in 0.001M; (b) in very dilute solution.
When KCL concentration decreases, the higher negative pulses appear, which is shown in Figure 3.14 (a). In magnified region of Figure 3.14 (b), the heights of negative pulse are 0.008 to 0.02, almost 2 times higher than in 0.01M. Those results confirm the previous experimental results obtained by Gamry Potentiostat for tree pollen particles that the pore resistance decreases by passage of tree pollen particles. When electrolyte of the sensor is high concentration KCL solution, the upward pulses are generated when tree pollen particles pass through the pore and the resistance of pore increase. But if the electrolyte is low ionic concentration, there are both positive and negative pulses generated with tree pollen particles passing through. Along with more dilute the KCL concentration; the height of negative pulse becomes higher comparing with high KCL concentration.

Then, the grass pollen particles are tested by the sensor. In Figure 3.15, the normalized resistance changes versus time curves shows only positive pulses when grass pollen particles pass through the pore with KCL concentration 0.01 M. The diameters of the grass pollen particles can be estimated as 41.9 and 39\(\mu\)m as shown in Figure 3.15 (b). When KCL concentration decreases to 0.001 M, both positive and negative pulses are appeared as grass pollen particles passing through the pore. The heights of negative pulses are less than 0.005 in Figure 3.16 (a) as KCL concentration of 0.001M. With very diluted electrolyte such as water, grass pollen particles generate higher negative resistive pulses which are near 0.01, as shown in Figure 3.16 (b).
Figure 3.17: Histogram of tree pollen particles; (a) in 0.01 M; (b) in 0.001 M.
Figure 3.18: Histogram of grass pollen in: (a) 0.001M; (b) in very dilute solution.
Furthermore, the histograms of experimental data for pollen particles are used to analyze the tendency of negative pulses with KCL concentrations. Figure 3.17 provides the histograms of events versus positive and negative pulse heights for tree pollen particles. Figure 3.17(a) presents a histogram versus normalized pulse height of tree pollen particles at KCL concentration 0.01M, in which the mean value of positive pulses is 0.0139 of total events 2340, and 0.00806 for negative pulses with total number 1600. In Figure 3.17(b), the mean values are 0.00472 of total events 2390 of positive pulse, and 0.0322 for negative pulses with total number 2460, respectively, where KCL is 0.001M. Those results show that when KCL concentration decrease from 0.01 M to 0.001M, the average positive pulse height decreases from 0.0139 to 0.00472; but the average negative pulse height increases from 0.0086 to 0.018. In addition, the ratio of positive pulses to positive pulses changes from 1.46: 1 to 1: 0.9. These statistical data confirms that as the electrolyte concentration decreases, the charged pollen particles bring in extra charges into the micro-channel and results in increasing channel resistance as negative resistive pulses.

The similar trend can be seen from histograms of grass pollen particles. Figure 3.18 shows the histogram of experimental data. Figure 3.18(a) shows a histogram of events versus pulse height for grass pollen particles in 0.001M KCL concentrations. It can be seen in Figure 3.18(a), the negative pulse height are less than 0.005 and positive pulse height are less than 0.004; but in Figure 3.18(b), the negative pulse height increase to 0.01 and the positive pulse height decrease to less than 0.002, where the electrolyte was diluted to near water. The statistic analysis for pollen particle experimental data confirms the previous experiment observations for pollen particles behaviors.
3.3. Discussion

There are variations for the sensor’s response when the uniform size particles pass through the pore. In common, spherical particles passing through the pore of a resistive pulse sensor can travel at any radial position relative to the pore axis up until the sphere contacts the pore wall. Off-axis particles will create a larger resistance change than the same size on-axis particles [33]. In addition, there are non-uniform edges in the pore, which is made from fabrication process. The non-regular geometry of a pore will affect the sensor’s responses.

The uncertainty method is utilized to estimate the particles size. There are three uncertainty sources in estimation of the size. The first source is due to uncertainty in measurement of micro-channel diameter and length. Due to the asymmetric shape and the fabrication variation of the micro-channel, these uncertainties are ±20% for diameter, and ±50% for channel length. These uncertainties contribute ±8.2% uncertainty in particle size evaluation. Note that this source of uncertainty would systematically alter the estimated the particle diameters. The second source of uncertainty is due to the fluctuations in the measured voltages, which are about ±0.5% at voltage levels of 0.2V. These fluctuations could be due to either flow unsteadiness or the measurement system and appear to have no systematic trend. This uncertainty contributes to an uncertainty of ±1.96% in particle diameter estimation. The third source of uncertainty is due to the off-axis position when one particle passes through micro-channel. This uncertainty source will create the uncertainty in resistive pulse of ±4.6% that corresponds to an uncertainty of ±1.1% in particle size. Combining the three uncertainty sources, the uncertainty of particle size estimation is ±9.6%.
3.4. Summary

The experimental data presented in this Chapter demonstrate that the measurement setup can be utilized to measure diameter PM particles and differentiate pollen form PM particles. We found that pollen particles generate negative resistive pulses in very diluted electrolyte solutions while PM particles still generate positive resistive pulses. An explanation is provided to explain the negative resistance pulses when pollen particle pass through the micro-channel.
CHAPTER IV
MULTI-CHANNEL RESISTIVE PULSE SENSOR MEASUREMENTS

One big limitation of single channel resistive pulse sensor is its low counting efficiency. The sensor measures the size and counts the number of particles when they pass through a single micro-channel one by one. Because of the low travel velocity of the particles in a micro or nano scale channel, it could be long time to analyze a small amount of particle solutions. For instance, if a 1ml pollen particles sample solution needs to be tested, the single channel resistive pulse sensor with a pore of 50µm in diameter will take 14 hours to finish testing, assuming the fluid velocity at the micro-channel is 0.01m/s. One could increase the particle velocity by applying large pressure gradient. However, this will lead to the loss of detailed information (particle shape, surface charge, mobility, etc) about the particles because of the sharp shape of the measurement pulses. Or one has to use expansive high speed, precious amplifiers and A/D converters to capture the high frequency peaks due to high particle travel velocity. Therefore, it is not a practical tool for detection and counting a large number of micro/nano scale particles in the solution. Furthermore, the large pressure difference required to keep a high fluid velocity will more easily damage the micro-channel and thin membrane; thus the sensor’s lifetime will be shortened. Finally, in pollen detection, the volume of sample particle solutions can easily reach more than 1 ml, due to collecting methods. In summary, the low counting efficiency of a single channel resistive pulse sensor has limited its
applications, especially in real time environmental monitoring of pollen and other aerosol. So far, a portable, electronic device that can fast analyze solution is not available yet. As the second part of this thesis, we designed and fabricated a high throughput multi-channel sensor for micro-scale particle detection.

To address this problem, one solution is to increase the number of sensing channels to form a multi-channel resistive pulse sensor. The flow volume rate is increased by the numbers of the micro-channel whereas the fluid flow velocity can be kept low. Hence the testing time is reduced, and the system efficiency is improved. With the low particle travel velocity, there is no need to construct the measurement system using expensive amplifiers and A/D converters. Thus, it is possible to develop a low cost, high efficiency sensing system for micro/nano scale particle detection including pollen detection.

In this chapter, we will describe the design concept of the unique multi-channel sensing structures and show the capability of a micro scale multichannel sensor for counting of the PM particles through its multiple sensing channels simultaneously. This experimental work demonstrates the multi-channel resistive pulse sensor could be used to size PM particles in terms of voltage pulses with a 300% improvement in counting efficiency. This part of work laid the groundwork for the use of this multi-channel resistive pulse sensor for actual environmental monitoring.

4.1. Design of a 4-channel resistance pulse sensor

The design objectives of the multi-channel resistive pulse sensor are: 1) multi-channel resistive pulse sensor should have same or similar sensitivities as a single channel resistive pulse sensor; 2) the sensor’s structure should be simple so that the
micro-fabrication process can be easily achieved; 3) measurement system should be easily connected to the sensor without introducing more noise, or in other words, the sensor should be well grounded to avoid testing noise; 4) The multi-channel measurement should not bring in significant cross talk among sensing channels.

Figure 4.1: A diagram of four-channel sensor.

Following these design goals, the concept diagram of multi-channel resistive pulse sensor is sketched in Figure 4.1. The sensor consists of two reservoirs to load the
electrolyte solutions, four micro-channels inside the sensor for detection and counting particles, two major electrodes for application of DC voltage, and 4 sensing electrodes for control or measurement purposes. The four identical rectangular micro-channels are separated by isolation islands. Each channel has the dimensions of 50µm in width, 100µm in height and 350µm in length. The four sensing electrodes (S1-S4) are located in the center of micro channels and the two power (E1, E2) electrodes are located in the two reservoirs.

![Figure 4.2: (a) Microscopic image of multi-channel resistance pulse sensor setup; (b) The picture of the device.](image)

The reservoirs and micro-channels are formed in PDMS layer using soft lithography technologies. The electrodes are fabricated by deposition and patterning of Au thin film (1000Å) on a glass substrate. Once these two components are fabricated, they are bonded together to form the sensor. A picture of a micro-machined multi-channel sensor is shown in Fig 4.2 (b). Fig 4.2(a) shows the magnified area including the four sensing channels and the sensing electrodes.
4.2. Electrical equivalent circuits

Because the sensing electrode is located in the middle of the channel, each micro-channel can be modeled as two resistors, front half channel and end half resistance, which are connected serially (see Figure 4.3). Thus the multi channel resistive pulse sensor can be described as a resistance network with four series resistance branches in parallel. In resistance network, each branch is independent with others if the resistance between power electrodes to the micro-channel is ignored.

![Figure 4.3: The equivalent resistor circuit of four channels.](image)

Figure 4.3 shows equivalent electrical circuit of four branches. Assume a particle passes through the channel 1, it results in a change in the resistance of the channel 1. The resistance change ratio can be derived from the measured voltages $V_s$, and power supply voltage $V_c$. The measured voltages without particles are
\[ V_{s1} = \frac{R_{c12}}{R_{c11} + R_{c12}} V_c \]  

(4.1)

where \( R_{c11} \) is the resistance of front half channel; \( R_{c12} \) is the resistance of end half channel, \( V_{s1} \) is the measured voltage taken at the sensing electrode of channel 1. When a particle inhabits in the front half channel, \( R_{c11} \) increases, and \( V_{s1} \) decreases. So a negative resistive pulse is generated:

\[
\frac{\Delta R_{c11}}{R_{c11}} = \frac{\Delta V_{s1}}{V_{s1}} \frac{1}{1 - \frac{V_{s1}}{V_c}}
\]

(4.2)

When particles inhabit the end half channel, \( R_{c12} \) increases, and \( V_{s1} \) also increases. So a positive resistive pulse is generated:

\[
\frac{\Delta R_{c12}}{R_{c12}} = \frac{\Delta V_{s1}'}{V_{s2}} \frac{1}{\frac{V_{s1}'}{V_c} - 1}
\]

(4.3)

where \( \Delta V_s = V_s - V_s' \), \( V_s \) is measured voltage without particle, \( V_s' \) is voltage as particle passes through the micro-channel.

For channel 2, 3 and 4, similar equation can be obtained. Note that here we ignore the resistance between the major electrodes and each channel. Hence the voltage across each channel (voltage across each pair of resistance \( R_{c11} + R_{c12}, \ i=1, 2, 3, 4 \)) is kept constant. Thus any resistance change in one sensing channel due to the passage of particles will not cause the changes in other channels. Therefore the four micro-channels are expected to measure particles simultaneously and hence improve counting efficiency.
4.3. Experimental procedure

The multi-channel resistive pulse sensor is mounted on a thick plastic plate to prevent movement during the experiments. The whole setup is positioned in a damping-isolation optical table to avoid random vibration. A solution of 0.01M KCL is prepared. A few drops of Tween 100, a nonionic surfactant, is added in to prevent particle coagulation and micro-channel plugging. In order to test the sensor, PM particle solution is added in main reservoir by a syringe, which is also used for driving the fluid moving from Reservoir 1 to Reservoir 2 through the four sensing channels. The voltages at sensing electrodes are then measured by NIDAQ system. The data was taken after the agitation.

![Figure 4.4: Experiment set up of multi-channel measurement.](image_url)
Figure 4.4 shows the picture of the experimental setup. The voltages taken at the sensing electrodes are connected to the NI DAQ measurement system via the 4 probes. A constant 3V voltage is applied between the two major electrodes. 20KHz-sampling frequency is chosen for the following experiments. The whole setup is placed in a Farady cage on top of a damping isolation table.

4.4. Results and Discussion

Two measurement modes are utilized for testing of the micro-machined multi-channel sensing. The experimental setup is described below.

4.4.1 Measurement Mode 1

Measurement mode 1 is shown in Figure 4.3. A constant voltage 3V is applied across the two main electrodes. The voltage drop $V_{si}$ for each individual channel is taken between the central electrode and the main electrode than is grounded. From equation (4.2) and (4.3), we expect to see a downward $V_{si}$ pulse (when particle is in the front half channel) followed by an upward $V_{si}$ pulse (when particle is in the end half channel). Note that the sequence of upward and downward pulse is depended on the fluid direction, if the fluid flow through reverse direction, then the sequence will be first downward and upward. Figure 4.5 shows the typical the measured voltage changes traces ($\Delta V_{si}$) of micro-channel 1 (Figure 4.5(a)) and micro-channel 4 (Figure 4.5(b)) when 40µm PM particle solution is loaded. Note here that signals from channel 2 and channel 3 are not
Figure 4.5: Measured voltages changes using measurement Mode 1. (a) micro-channel 1; (b) micro-channel 4.
Figure 4.6: (a) Single pulse ‘a’ of measured voltages of channel 1; (b) single pulse ‘b’ of channel 4.
shown here because they are blocked by particles. The voltages of each channel are recorded by NIDAQ system. We observed a pair of voltage pulses consisting of an upward voltage pulse and a downward voltage pulse as one 40µm particle passes through one micro-channel. The pair of pulses appeared in different time point; for instance, the pair pulse “a” in Figure 4.5(a) was appeared at time 800 ms, but at same time, there is no such large pair pulse in Figure 4.5(b); similarly, the pair pulse “b” in Figure 4.5(a) is in 930 ms, and there are no pulses in same time point founded in Figure 4.5(a). The results confirm that the pulses in channel 1 does not create distorted signal in micro-channel 4.

From the heights of a pair of pulses, the diameter of PM particle can be estimated by Eq.4.2 and 4.3. In Figure 4.6(a), the first half pulse peak of signal “a” is upward (ΔV = 15mv), the calculated particle diameter is 36µm, where the hydrodynamic diameter is 134µm, and the baseline voltage Vs is 1.7v. The second half pulse is downward (ΔV=30mv), the particle’s diameter is estimated as 46µm. Similarly, the particle diameter can be calculated from a pair pulse “b” in Figure 4.6(b), for the voltage changes are 8mv and 16mv, the estimated particle diameters are 30 and 36µm, respectively. Although there exist the variations, those calculated diameters from the pulse heights are close to the actual particle diameter, 40µm.

The experimental data presented here indicates that it is possible that every single micro-channel can be monitored simultaneously. Since the sensor can sense the particles through all its multiple sensing channels, the counting efficiency is increased.

If a high electrolyte concentration is used, the resistance formed between main electrodes to micro channels can be neglected. But when very diluted electrolyte is used, the resistance cannot be neglected. Thus, a voltage pulse of one channel will cause
voltage variations (cross talk) of other channels. The cross talk among channels introduces fake voltage pulses for other micro-channel and therefore affects the sensor’s performance. In our test (Figure 4.5), 0.01 M KCL solutions is used and we did not find obvious cross talk. One possible way is to reduce the cross talk is to keep the voltage across each channel resistance constant value. In the next chapter, Measurement Mode 2 will be used for this purpose.

4.4.2 Measurement mode 2

The power electrode E1 connects to battery positive polarity whereas the external sensing resistors (100K) connect the sensing pad S and battery negative polarity; the NIDAQ system measured the voltages at sensing pads. The equivalent circuits are as single channel measurement set up (Figure 3.9(b)).

Figure 4.7 shows typical voltage traces of channel 1, 4. The external sampling resistance for each channel is 100 K. In experimental mode 2, there are only negative pulses observed because of the external resistors. Each downward pulse shown in Figure 4.7 represent the passage of a 40µm PM particle through one micro-channel. From Figure 4.7, the downward pulses are presented in different time instant between two channels and also in the certain observation period, the number of particles is great than a single channel. Those results illustrate the improvement of counting efficiency. Because no obvious cross talk among channels is found, each channel measurement is independent.
Figure 4.7: Voltage drop between sensing resistors (100k) of channel 1 and 4; KCL concentration is 0.01 M, sampling particles are 40µm PM particles. (a) channel 1; (b) channel 4.
Figure 4.8: the normalized resistive change; (a) channel 1; (b) channel 4.
Figure 4.8 shows the converted resistive changes, which can be used to calculate the particles diameter. In Figure 4.8 (a), the heights of positive pulses are 0.12 to 0.2, and in Figure 4.8 (b), the heights of pulse are 0.05 to 0.1. If we directly calculate the particles diameters from those values using the Eq 3.4, the calculated diameters of particles are larger than their true value. After repeat the experiments, the normalized pulse heights are in the same range of 0.1 when 40µm PM particles pass through the micro-channel. Probably, those larger normalize pulse heights might be attributed by electrode polarization.

Since there is the current flowing through the sensing electrodes in measurement mode 2, the phenomenon called polarization occurred at gold electrodes [39]. The electrode polarization describes the interrelation of potential drop between electrode to electrolyte interfacial layer and the passage of current. Because of the large potential drop at electrodes shown in Figure 4.7; the voltage drop at external sampling resistance (100KΩ) are less than 0.1 V while the battery voltage is 3v. Because of polarization at electrodes, we need to include this effect into Ohm’s law to derive the relation between voltage changes on sensing resistor and channel resistance changes.

The electrodes polarization is illustrated as diagrammed in Figure 4.9. A significant voltage drop exists in the interface of power electrode and electrolyte. One can consider the voltage equation as follows:

\[
V_c = E_n + V_{act} + V_{con} + V_{ch} + V_s
\]  

(4.4)

Where \(V_c\) is the battery voltage, \(E_n\) is the electrode contact potential, \(V_{ch}\) is the voltage drop of channel resistance, \(V_{ch} = IR_{ch}\); \(V_{act}\) is the activation overvoltage; \(V_{con}\) is the concentration overvoltage; \(V_s\) is the measured voltage on external resistance, \(V_s = IR_s\).
Figure 4.9: Schematic of the potential drop caused by polarization on electrodes in solution. $V_c$ is the power supply; $E_n$ is the electrode contact potential; $V_{act}$ is the activation overvoltage; $V_{con}$ is the concentration overvoltage; $V_{ch}$ is the voltage drop on channel resistance; $V_s$ is the voltage drop on external sensing resistance.

In Eq.4.4, three overvoltages - $V_{ch}$, $V_{act}$ and $V_{con}$ are the functions of current. Depending on the circumstances, these three voltages may have similar magnitudes. When a particle inhabits the micro-channel, the current $I$ changes to $I_f$. Thus, the measured voltage $V_s$ becomes $V_s'$, the voltage on micro-channel resistance changes to $V_{ch}'$; because of $V_{act}$ and $V_{con}$ are the function of the current; they change to $V_{act}'$ and $V_{con}'$, respectively. Let $\Delta V$ represent the voltage change and give the definitions of changed voltage as following:

$$V_s' = V_s + \Delta V_s$$

$$V_{ch}' = V_{ch} + \Delta V_{ch}$$

$$V_{act}' = V_{act} + \Delta V_{act}$$
\[ V'_{\text{con}} = V_{\text{con}} + \Delta V_{\text{con}} \]

Substitute the changed voltages to Eq.4.4, we have

\[ V_c = E_n + V_{\text{act}} + \Delta V_{\text{act}} + V_{\text{con}} + \Delta V_{\text{con}} + V_{\text{ch}} + \Delta V_{\text{ch}} + V_s + \Delta V_s \]  

(4.5)

Combining Eq.4.4 and 4.5, we obtain

\[ \Delta V_s = - (\Delta V_{\text{act}} + \Delta V_{\text{con}} + \Delta V_{\text{ch}}) \]  

(4.6)

Here we make an assumption that the sum of two overvoltage changes, activation and concentration overvoltage, \((V_{\text{act}} + V_{\text{con}})\), is \(\alpha\) time of voltage change on channel resistance.

Thus, the voltage change on channel resistance can be modeled as

\[ \Delta V_s = -(\alpha + 1) \Delta V_{\text{ch}} \]  

(4.7)

Meanwhile, let \(\beta\) be the ratio of channel resistance with external sensing resistance.

\[ \beta = \frac{R_{\text{ch}}}{R_s} \]  

(4.8)

So we have

\[ V_{\text{ch}} = \beta V_s \]

Now the relation of voltage change on channel resistance with the measured voltages can be written as:

\[ \frac{\Delta V_{\text{ch}}}{V_{\text{ch}}} = \frac{1}{\beta(\alpha + 1)} \frac{\Delta V_s}{V_s} \]  

(4.9)

The Eq. 4.9 is used to estimate the particle diameters from experimental data. First, \(\beta\) could be estimated from channel resistance. The micro-channel has length \(L = 150\mu\text{m}\) and the cross-section area \(A = 50\times100\mu\text{m}\). If the electrolyte is the 0.01M KCL solution, then the channel resistance could be estimated as 290K \((R=\rho L/A)\) while the external
sensing resistance is 100KΩ. So we take $\beta$ as 3. Second, the value of $\alpha$ is estimated as 1 when non-Ohm’s overvoltages have same contribution to channel resistance change. Based on the above assumptions, the particle diameters are estimated from experimental data. The average measured voltage change ratio in Figure 4.8 (a) is 0.18 when a 40µm PM particle passing through the micro-channel. Corrected by $\alpha$ and $\beta$ factors, the particle diameter is estimated as 41.6µm. The average measured voltage change ratio in Figure 4.8 (b) is 0.08, where the particle diameter is estimated as 31.5µm.

![Figure 4.10: A normalized resistance pulse of 20µm PM particles in channel 1.](image)

The similar procedure is applied to 20µm pulse, where the measured voltage change ratio is 0.031 as shown in Figure 4.10; with correctional factors, the estimated diameter is 23.1µm. The calculated diameters are closed to the particle’s actual diameters. Those results confirm the multi-channel resistive pulse sensor can be used to measure the
size of particles including the polarization effects in electrodes. However, with the polarization effects, the evaluation procedure becomes more complex. More experiments are needed for future investigation.

4.5. Uncertainty Analysis

As discussed previously in chapter 3, the uncertainty of the resistive pulse sensor measurement includes 1) uncertainty in measurement of micro-channel diameter and length, 2) uncertainty due to the fluctuations in the measure, 3) the off-axis position when one particle passes through micro-channel. Because of the regular shape and precise dimension of micro-channel, the measurement uncertainly is reduced in contrast to the single channel resistive pulse sensor reported in Chapter 3. Considering the noise sources, the ultimate sensitivity of sensor is the results of Johnson noise of every single micro-channel resistance adding with measurement system noise. In order to reduce the uncertainty source 2, good shielding and low noise measurement system are desired. Also note when the fluid flows in high velocity carrying the particles, the capacitive effects at the micro-channels and electrodes may limit the bandwidth of the sensor.

4.6. Summary

The preliminary experimental data presented here shows the potentials of counting the particles through its four sensing channels simultaneously. The multi-channel resistance pulse sensor is tested by two measurement setups. Each sensing micro channel’s resistive changes can be individually monitored by the measurement system. Because the four –channel sensor allows more particle solutions to pass through than a
single channel sensor, the sensing efficiency is expected to improve multiple times. Future work will be done to improve the sensor reliability. It has potentials to be used to improve the throughput of pollen detection, which normally has a large volume of sample solutions (more than 1 ml), due to particle collecting methods.
CHAPTER V

CONCLUSIONS

In this thesis, we have presented several important experimental results for microparticles and pollen particles detection using resistive pulse sensor. First, we set up a single channel resistive-pulse sensor and demonstrated its capability of measuring the diameters of particles; second, for the first time, we demonstrated an all-electronic approach to detect and count pollen particles based on the single channel resistive pulse sensor. Third, we designed and tested a micro-machined multiple channel sensor that has significantly improved the counting efficiency.

First, the response of single channel resistive pulse sensor is demonstrated. PM particles with different sizes are used to evaluate the sensor performance. The experimental results indicate the sensor’s capability of measuring the particles’ size. The resistive pulse sensor was tested with two different measurement systems, where either ionic current flowing through the micro-channel (using Gamry Potentiostat) or voltages drops between the pore (using DAQ) are measured. The low cost, high precision data acquisition system (DAQ) offers the potential to integrate the resistive pulse sensor with measurement system on a chip, which is the long-term goal for MEMS based sensor. Digital filter techniques and statistical methods are employed for data processing, from which the sensor’s capabilities can be improved by digital signal processing (DSP) techniques.
Second, we are the first group to show that the pollen particles present the different behaviors when they pass through the sensing channel from other particles. The resistance of the liquid-filled micro-channel decreases with passage of pollen particles in very diluted electrolyte solution. In addition, the resistance pulse of micro-channel is varied with different electrolyte concentration when pollen particles pass through it. We observed this phenomenon using both Gamry Potentiostat and NIDAQ measurement system. The phenomena may be explained in terms of the number of free ions in electrolyte solution and surface charge of pollen particles. In low concentration the surface charge of pollen might be the dominant factor for the resistance changes. Statistical data analysis shows that different pollen particles present the different negative pulses tendencies at low ionic concentration, indicating the possibility to separate the pollen particles with their surface charge. Furthermore, the pollen particles could be sized with high ionic solution, in which the particle volume dominates the channel resistance change.

Considering the low throughput of the single channel resistive-pulse sensors, we designed and tested a micro-machined multichannel sensor. The concept of using multiple sensing channels to count the particles simultaneously is confirmed by preliminary experiments. Two measurement modes are utilized in the sensor testing. Because of the unique but simple multi-channel sensing structure, flexible measurement setups can be utilized. The multiple micro-channels could be easily fabricated by micro-machining process compatible with microelectronic fabrications. Therefore, the sensing structure can be easily integrated to lab-on-chip applications. Preliminary experimental tests show the micro-machined multichannel sensor has the following capabilities: 1)
each sensing channel can be monitored individually and the sensor’s sensitivity and response are similar to the signal channel sensor; 2) The sample flow volume rate is increased and the sensing efficiency is improved significantly because of the multiple sensing channels. This preliminary work lays a solid background for developing a low cost, portable, lab-on-a-chip system for pollen detection and environmental monitoring.

In general, we demonstrated an all-electronic approach based on single channel and multichannel resistive-pulse sensors for detection and counting micro scale particles. Experimental results indicated the presented approach is feasible for the future development of portable, high throughput and automated devices for sampling and differentiating bio-micro-particles such as pollens and bacterial spores.
CHAPTER VI
FUTURE RESEARCHES

There are several foreseeable extensions of this thesis. More experiments needed for deeply understanding the physical principles of the specific features of pollen particles, in which the negative pulses have more important information waiting to investigate. The mechanical structure of multi-channel sensor could be redesigned to improve the performance, where the measurement system could be easily recorded the data with better shielding. And more experiments on electrodes polarization are needed to set up more accurate relation between the pulse height and particle diameters.

The accurate measurement is very important for resistive pulse sensor. Here we will describe an idea to increase signal to noise ratio.

The sensitivity of the multi-channel resistive pulse sensor is directly related to the noise level of the measured voltage pulses. The small signals from the resistive pulse sensor bring a challenge on detection sensitivity. To design a low noise, high precision front-end amplifier following the resistance pulse sensor is a method to increase the signal to noise (S/N) ratio of the sensing system.

Figure 6.1 shows a typical measured voltage pulse traces when 20 µm PM particles passing through 120 µm pore, where Y-axis is measured voltage and X-axis is time. The NIDAQ system is operated at 50 KHz sampling rate with an analog input voltage range
of ± 1V. NIDAQ system has absolute error 0.36mv in this input range. As shown in the figure, the DC offset is around 0.927v; the magnitude of peak ‘a’ is around 1 mv and the peak ‘b’ is around 2mv. The measurement system S/N ratio is 6 and A/D converter does not be used efficiency because most bits of the DAQ are for DC offset.

![Figure 6.1: Raw data of the measured voltage on a 50KΩ sensing resistor.](image)

The concept of using the front-end amplifier is to separate a small pulse signal from large DC offset (Figure 6.2). MOSFET transistor is chosen because of its high input impedance and the property that there is no current flowing through its gate electrode. When it connects to resistive pulse sensor, the amplifier will not distort the sensor. Q1 and Q2 form two identical branches to pick voltage signal form the sensor. Capacitor “C” forms a low pass filter, so any measured pulses and high frequency signals will be filtered. The voltage at point B is the DC offset line. The voltage at point A follows the DC
voltage of the sensor. The operation amplifier A1 compares the voltage of A and B, thus the DC offset will be removed at the output. As a result, only pulse signals will be obtained at O1. The output of A2 follows the input, same voltage of B. Therefore, the pulses signals without base are obtained at O1, while the DC offset will be obtained at O2. The A/D converter will convert two signals separately with different full-scale range.

Figure 6.2: Schematic of front-end amplifier.

The pulse signal and DC offset also can be amplified by A1 and A2. In this design the noise can be significantly reduced, while the DAQ system keeps same measurement accuracy and the magnitudes of signals are amplified. With a careful circuit design, the front-end amplifier will not introduce more noise, thus S/N ratio will be improved.
REFERENCES


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