BIO-PARTICLE COUNTING AND SIZING USING MICRO-MACHINED MULTICHLANNE L COULTER COUNTER WITH WAVELET BASED DE-NOISING

A Thesis
Presented to
The Graduate Faculty of The University of Akron

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

Rupesh Sawant
December 2007
BIO-PARTICLE COUNTING AND SIZING USING MICRO-MACHINED MULTICHANNEL COULTER COUNTER WITH WAVELET BASED DE-NOISING

Rupesh Sawant

Thesis

Approved:            Accepted:

____________________  ____________________
Advisor               Department Chair
Dr. Jiang Zhe        Dr. Daniel B. Sheffer

____________________  ____________________
Co-Advisor            Dean of the College of Engineering
Dr. Narender Reddy   Dr. George K. Haritos

____________________  ____________________
Committee Member      Dean of the Graduate School
Dr. Dale Mugler       Dr. George R. Newcome

____________________
Date
ABSTRACT

The micro-machining and testing of a multi-channel micro-fluidic sensor for simultaneous counting of micro-particles is presented. The device is fabricated using micro-machining techniques including photolithography, wet etching, and chip bonding. The sensor consists of multiple sensing micro-channels, sensing electrodes, and sample reservoirs fabricated on a single chip. The testing is performed by loading various micro-scale particles (including Chinese Hamster Ovary Cells) into the central reservoir and is driven to the peripheral reservoirs through the micro-channels that connect the central reservoir and peripheral reservoirs. The detection is based on the Coulter counting principle. According to this principle, a particle causes a change in electrolyte-filled micro-channel resistance (or a resistive-pulse) as it passes through the micro-channel. The particle size, surface charge and concentration can be correlated to the magnitude and the duration of the pulse. Testing results show that the multi-channel sensor is able to rapidly count pollen, polystyrene particles and Chinese hamster ovary cells with a 300% increased throughput.

One challenge for the multi-channel micro Coulter Counter measurement is the high noise level which reduces its sensitivity. A novel discrete-wavelet-transform signal denoising method is applied on the measured signals obtained from multi-channel Coulter counters. Single channel data obtained from multi-channel Coulter counters processing
polystyrene, Juniper pollen particles and Chinese hamster ovary cells are used to demonstrate the application of wavelet transforms. Parameters for the signal de-noising algorithm are chosen using a cross-validation procedure; these parameters include the choice of the particular wavelet filter, the number of levels of decomposition, and the threshold value. Additionally, removal of 60 Hz power line interference is achieved by implementing a band reject filter (notch filter) in the wavelet domain. Baseline estimation (or correction) algorithm is also demonstrated on the Coulter counter signals; this processing allows accurate detection of particles and aids in computation of particle size and concentration. Overall, wavelets are presented as a tool to aid in the processing and analysis of data obtained from multi-channel Coulter counters. The results indicates that the de-noising procedure increases the signal to noise ratio (SNR) thereby improving the sensitivity of the sensor.
ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Dr Jiang Zhe for his constant support and co-operation all through my Master’s Thesis. This thesis would not have been possible without his guidance and persistent help.

I would also like to thank my co-advisor, Dr Narender Reddy for giving me valuable suggestions in this research project and his invaluable inputs in the entire course of the project.

A special thanks to my committee- Dr Dale Mugler and for his time and effort and especially for their invaluable suggestions in wavelet de-noising.

I would like to take a chance to thank my Dr. Richard Steiner for his valuable suggestions in Biostatistics part.

Thanks to my friends, who encouraged me, and helped made the journey more bearable. My special thanks to Ashish, for his careful readings and valuable suggestions during my experiments. I would also like to thank my other fellow lab-mates, especially Zheng, Yiou, Abhay, Nidhi and Sheela, for the opportunity to learn and progress together throughout my graduate school years.

I would also like to convey my special gratitude to Dr. Paul Lam and Dr. Julie Zhao for their advice, support and kindness during the “STEM” workshop.
Lastly, I would like to express my gratitude towards my parents for their faith and who were always there for me all through the progress of my thesis and eventually my degree.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>LIST OF TABLES</th>
<th>xi</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td>xiii</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>I. INTRODUCTION</td>
<td></td>
</tr>
<tr>
<td>1.1 MEMS and Micro-fluidics</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Need of a high speed bio-particle detector</td>
<td>2</td>
</tr>
<tr>
<td>1.3 Coulter Counter for bio-particle detection</td>
<td>4</td>
</tr>
<tr>
<td>1.4 Advancement in the coulter counter device</td>
<td>6</td>
</tr>
<tr>
<td>1.5 Signal de-noising using wavelets</td>
<td>8</td>
</tr>
<tr>
<td>1.6 Research objectives</td>
<td>10</td>
</tr>
<tr>
<td>1.7 Research hypothesizes</td>
<td>11</td>
</tr>
<tr>
<td>II. SENSOR DESIGN</td>
<td>3</td>
</tr>
<tr>
<td>III. SENSOR FABRICATION</td>
<td>20</td>
</tr>
<tr>
<td>3.1 Micro-machining processes</td>
<td>20</td>
</tr>
<tr>
<td>3.1.1 Substrate cleaning</td>
<td>21</td>
</tr>
<tr>
<td>3.1.2 Lithography</td>
<td>21</td>
</tr>
<tr>
<td>3.1.3 Fabrication of micro-channels</td>
<td>22</td>
</tr>
<tr>
<td>3.1.4 Fabrication of electrodes</td>
<td>22</td>
</tr>
</tbody>
</table>
3.1.5 Drilling of channel reservoirs .....................................................23
3.1.6 PDMS glue bonding .................................................................23

3.2 Final device and leakage testing ...................................................25

IV. EXPERIMENTAL PROTOCOLS .........................................................27

4.1 Solution preparation ......................................................................27
4.2 Experimental setup .......................................................................28
4.3 Measurement procedure and data acquisition ...............................29
  4.3.1 Inducing particle flow ............................................................29
  4.3.2 Device interface .................................................................29
  4.3.3 Data acquisition .................................................................30

V. SINGLE PARTICLE DETECTION .......................................................32

5.1 Detection of 30 µm polystyrene particles .....................................32
  5.1.1 Determination of particle size ...............................................34
  5.1.2 Determination of particle concentration ..................................36
5.2 Analysis of 20 µm juniper pollen .................................................37
  5.2.1 Determination of particle size ...............................................43
  5.2.2 Determination of particle concentration ..................................44
5.3 Analysis of chinese hamster ovary cells (CHO cells) .....................45
  5.3.1 Determination of particle size ...............................................48
  5.3.2 Determination of particle concentration ..................................49
5.4 Discussions .................................................................................50
  5.4.1 Variation in the measurement of particle size ..........................50
  5.4.2 Statistical analysis for particle height ......................................51
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 Expected height and measured height for polystyrene particles</td>
<td>36</td>
</tr>
<tr>
<td>5.2 Expected concentration and measured concentration for polystyrene particles</td>
<td>36-37</td>
</tr>
<tr>
<td>5.3 Expected height and measured height for juniper pollen</td>
<td>44</td>
</tr>
<tr>
<td>5.4 Expected concentration and measured concentration for juniper pollen</td>
<td>44</td>
</tr>
<tr>
<td>5.5 Expected height and measured height for CHO cells</td>
<td>49</td>
</tr>
<tr>
<td>5.6 Expected concentration and measured concentration for CHO cells</td>
<td>49</td>
</tr>
<tr>
<td>5.7 Results of the paired t-test for expected size vs. measured size for polystyrene particles</td>
<td>52</td>
</tr>
<tr>
<td>5.8 Results of the paired t-test for expected size vs. measured size for CHO cells</td>
<td>53</td>
</tr>
<tr>
<td>5.9 Results of the paired t-test for expected size vs. measured size for juniper pollen</td>
<td>54</td>
</tr>
<tr>
<td>5.10 Results of the paired t-test for expected concentration vs. measured concentration</td>
<td>56</td>
</tr>
<tr>
<td>6.1 Relation between r-value and status of cross-talk between the channels</td>
<td>60</td>
</tr>
<tr>
<td>6.2 Results of the correlation test for crosstalk</td>
<td>64-65</td>
</tr>
<tr>
<td>8.1 List of wavelet filters used in the de-noising process for optimization of the choice of the wavelet filter</td>
<td>79-80</td>
</tr>
<tr>
<td>8.2 Threshold value using Universal Threshold ($U_T$) method for the entire length of the signals</td>
<td>86</td>
</tr>
</tbody>
</table>
8.3 Optimized threshold values for each signal using the CV Method .......................87
8.4 Summary of optimized parameters for de-noising all the three signals..............91
9.1 Table of measured particle size and concentration for 30 µm polystyrene particles, 20 µm juniper pollen and 12.5 µm CHO cells........ 101-102
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Principle of Coulter Counter</td>
<td>4</td>
</tr>
<tr>
<td>2.1</td>
<td>Top view of the “Micro-Machined Multi-channel Resistive Pulse Sensor”</td>
<td>13</td>
</tr>
<tr>
<td>2.2</td>
<td>Magnified view of a setup of a single channel</td>
<td>15</td>
</tr>
<tr>
<td>2.3</td>
<td>Equivalent measurement circuit for one sensing channel</td>
<td>15</td>
</tr>
<tr>
<td>2.4</td>
<td>Measurement circuit for four channel sensor with the inter-channel resistances</td>
<td>17</td>
</tr>
<tr>
<td>2.5</td>
<td>Electrical equivalent of the four channels showing the bypass of the four inter-channel resistances. Dotted line represents the bypass</td>
<td>18</td>
</tr>
<tr>
<td>2.6</td>
<td>Overall measurement circuit for four channel sensor</td>
<td>19</td>
</tr>
<tr>
<td>3.1</td>
<td>Fabrication process for micro-channels and gold electrodes</td>
<td>20</td>
</tr>
<tr>
<td>3.2</td>
<td>Drilled reservoirs in the patterned glass slide</td>
<td>23</td>
</tr>
<tr>
<td>3.3</td>
<td>“Stamp and Stick” bonding procedure</td>
<td>24</td>
</tr>
<tr>
<td>3.4</td>
<td>(a) Photograph of the Micro-Machined Multi-channel sensor (b) Photograph of a single sensing micro-channel/electrode configuration</td>
<td>25</td>
</tr>
<tr>
<td>3.5</td>
<td>Picture of leakage testing done with diluted black ink</td>
<td>26</td>
</tr>
<tr>
<td>4.1</td>
<td>Block Diagram of the experimental setup</td>
<td>30</td>
</tr>
<tr>
<td>5.1</td>
<td>Microscopic picture of 30 µm polystyrene particle</td>
<td>32</td>
</tr>
<tr>
<td>5.2</td>
<td>Multi-channel voltage signal for 30µm polystyrene particles</td>
<td>33-34</td>
</tr>
<tr>
<td>5.3</td>
<td>Multi-channel relative resistance change signal for 30µm polystyrene</td>
<td></td>
</tr>
</tbody>
</table>
5.4 Microscopic picture of 20 µm juniper pollen particle ...........................................37
5.5 Multi-channel voltage signal for 20µm juniper pollen particles.......................... 38-39
5.6 (a) Current response for non-conductive particle
(b) Current response for particle with surface charge .............................................40
5.7 Multi-channel relative resistance change signal for 30µm polystyrene particles ................................................................. 42-43
5.8 Microscopic picture of Chinese hamster ovary cells ............................................45
5.9 Multi-channel voltage signals for CHO cells...................................................... 45-46
5.10 Multi-channel relative resistance change signal for CHO cells....................... 47-48
5.11 Signal 3 showing two of rapid pulses ...............................................................55
6.1 Cross-Correlation analysis for CHO cells between channels
   a) channel 2 –channel 3, b) channel 2 –channel 4
   c) channel 2 –channel 1 d) channel 4 –channel 4
   e) channel 3 –channel 1 f) channel 4 –channel 1 .............................................59
6.2 Cross-Correlation analysis for polystyrene particles between channels
   a) channel 2 –channel 3, b) channel 2 –channel 4
   c) channel 2 –channel 1 d) channel 4 –channel 4
   e) channel 3 –channel 1 f) channel 4 –channel 1 ...........................................61-62
6.3 Cross-Correlation analysis for Juniper pollen between channels
   a) channel 2 –channel 3, b) channel 2 –channel 4
   c) channel 2 –channel 1 d) channel 4 –channel 4
   e) channel 3 –channel 1 f) channel 4 –channel 1 .............................................62-63
7.1 Discrete wavelet transform showing decomposition up to three levels..........67
7.2 Discrete Wavelet Transform showing reconstruction for three levels.........68
7.3 Flowchart for cross-validation procedure to obtain Integrated
   Square Error (ISE) ...............................................................................................73
7.4 Decomposition tree for a signal using wavelet packets ...............................75
8.1 Voltage response across single channel of the multi-channel sensor for
(a) signal 1: Channel 2-30µm PM particle,
(b) frequency content of signal 1,
(c) signal 2: Channel 1-20µm Juniper pollen,
(d) frequency content of signal 2,
(e) Signal 3: Channel 1-10µm CHO cells,
(f) frequency response of signal 3,
(g) Signal 4: Channel 2-20µm Juniper pollen and
(h) frequency response of signal 4 ................................................................. 77-78

8.2 ISE values for thirty-two different wavelets, given nine levels of decomposition and hard thresholding according to the universal threshold rule ................................................................. 81

8.3 Cross-validation results for (a) signal 1 using wavelet bior 3.9, (b) signal 2 using bior 3.7, (c) signal 3 using bior 2.8 and (d) signal 4 using bior 2.4 ........................................................................... 82-83

8.4 Comparison of ISE obtained using Universal Threshold value when applying hard and soft threshold strategies for (a) signal 1, (b) signal 2, (c) signal 3 and (d) signal 4 ................................................................. 84-85

8.5 Reconstructed signals obtained using optimized filter parameters for (a) signal 1 using hard threshold strategy, level 3 and threshold = $17 U_T/20$, (b) signal 1 using soft threshold strategy, level 2 and threshold = $9 U_T/20$ (c) signal 2 using hard threshold strategy, level 3, threshold = $20 U_T/20$, (d) signal 2 using soft threshold strategy, level 3, and threshold = $12 U_T/20$ (e) signal 3 using hard threshold strategy, level 3 and threshold = $19 U_T/20$ (f) signal 3 using soft threshold strategy, level 3 and threshold = $20 U_T/20$ (g) signal 4 using hard threshold strategy, level 2 and threshold = $12 U_T/20$ (h) signal 4 soft threshold strategy, level 2 and threshold = $9 U_T/20$ ................................................................. 88-89

8.6 Hard threshold optimization for varied decomposition level for (a) signal 1 using bior 3.9, (b) signal 2 using bior 3.7, (c) signal 3 using bior 2.8 and (d) signal 4 using bior 2.4 ................................................................. 90

8.7 (a) De-noised signal 1 (b) Frequency content of de-noised signal 1 (c) De-noised signal 2 (d) Frequency content of signal 2 (e) De-noised signal 3 (f) Frequency content of de-noised signal 3 (g) De-noised signal 4 and (h) Frequency content of de-noised signal 4 ............. 92-93

8.8 Wavelet Packet decomposition tree showing bandwidth in nodes at each decomposition ........................................................................................................... 94
8.9 De-noised signal 3 using parameters optimized using cross-validation method (a) with 60 Hz noise and (b) with 60Hz noise removed using wavelet packets .................................................................95

8.10 (a) Signal 3 voltage converted to equivalent channel resistance (b) Estimated relative resistance change in the channel due to 12.5 µm CHO cells with baseline drift eliminated (c) Signal 4 voltage converted to equivalent channel resistance (d) Estimated relative resistance change in the channel due to 20 µm Juniper Pollen particles with baseline drift eliminated ......................... 96-97

8.11 (a) Peak detection: De-noised signal and (b) Peak detection: Noisy signal .................................................................98

10.1 Resistive pulses for particles with different mobility ..........................................................106

10.2 Resistive pulses due to anti-body binded bio-particle and simple bio-particle .................................................................107
CHAPTER I

INTRODUCTION

1.1 MEMS and Micro-fluidics

Micro Electro Mechanical Systems (MEMS) have evoked great interest in the scientific and engineering communities. This is primarily due to several advantages that MEMS offer; improved performance (application specific), reduced weight, and lower cost than their bulk device counterparts. Batch fabrication reduces manufacturing and assembly costs; reduced size and weight typically results in increased system design flexibility. Consequently, the MEMS have applications in various areas like RF MEMS, MOEMS, Micro-fluidic MEMS, Bio-MEMS etc. Micro-fluidics is one of its most potential examples dealing with transport phenomena at microscopic length scales. The concept of using planar fluidic devices for performing analysis of a small volume of chemical or biological solution has enabled many new micro devices [1,2,3,4] in analytical chemistry and biology. Micro-fluidic based detection of micro-particles including bioactive aerosol and pathogenic bacteria is one of the most important and upcoming research in biological and chemical systems [5,6].
1.2 Need of a high speed bio-particle detector

Bioactive aerosol particles and pathogenic bacteria represent an important class of environmental threat to public health and homeland security. For instance, more than 35 million Americans have allergic rhinitis, also known as “hay fever” caused by the excessive amount of pollen in air [7]. In addition, infectious diseases caused by pathogenic bacteria account for nearly 40% of the total 50 million annual estimated deaths worldwide, and these diseases constitute the major cause of death in many developing countries [8]. Terrorists commonly formulate biological agents, such as anthrax, into particles for air releases [9]. Rapid detection and counting of an aerosol release and pathogenic bacteria will enable timely implementation of protective measures to protect civilians and minimize the extent of contamination.

On-site rapid detection of bacteria has become an increasingly important concern worldwide. One major difficulty in detecting pathogenic bacteria is that they are generally present only at very low concentrations; there are typically fewer than 100 colony-forming units (CFU)/ml. Current technology for real-time bacteria detection is not sufficient; it has a typical sensitivity in the range of $10^6$ to $10^7$ CFU/ml. Typical requirements for an effective aerosol detector include high sensitivity (100CFU/Liter air), small size, high reliability and low cost. While it is often difficult to detect aerosol directly in the air, researchers typically collect them in electrolyte for biochemical analysis. This collection method often creates a large volume with very dilute bio-particle solution.

For such diluted samples, the conventional standard is to concentrate the bio-particle to detectable levels via pre-enrichment. However, the pre-enrichment procedure
may take one or two days and is often not feasible for on-site testing [10]. Another challenge is that the most common detection instrumentations are based on antibody capture or real-time polymerase chain reaction (PCR). Antibody capture-based immunoassay typically requires the labeling of the antibody. PCR, a lab-based pre-enrichment method for enzymatic amplification of DNA, requires a complex multi-protocol. Both technologies add to the time and the cost of developing and using the instrumentations. Because of these limits, available instrumentations typically require labeling of bio-particles, have low detection sensitivity and require pre-enrichment. While well-equipped research centers or laboratories have achieved success in analysis of micro-scale bio-particles using pyrolysis-GC-IMS [11] and pyrolysis-GC-MS [12], these methods require sophisticated sample preparations and little biological information can be obtained from the pyrolytic fragments. Fluorescence-based spectroscopy [13] and optical flow cytometry [14] provide accurate and sensitive analysis of micro-scale particles. These techniques use a light beam perpendicular to the flow of the particles; the degree to which the light is scattered gives a measure of the size of the particles. However, these systems require complex optical setups, and require fluorescence labeling or native luminescence. Immunoassay is a prevalent method for accurately detecting biomolecules with antibodies [15], but it requires labeling of the antibodies with chemical, radioactive or fluorescence tags, which are usually difficult and time consuming to prepare. Hence, it is important for a new analytical device to be portable, require no complex set-up, and sample preparations. Further, the devices should be inexpensive, robust, and mass-producible. In particular, there is an urgent need for an on-site detection
device which is portable and which can detect aerosol bio-particles in a reasonably small volume with small assay time, and without labeling the bio-particles.

1.3 Coulter Counter for bio-particle detection

Coulter counters [16] are well-developed instruments that are used to measure the size and concentration of biological cells and colloidal particles. While this fluid based particle detection method has long been used to characterize cells several microns in diameter [17,18], its relative simplicity has led to many efforts to employ it to detect nano-scale particles [19]. With standard extraction techniques [20], the bio-particles can be collected in an electrolyte solution. A Coulter counter can be adapted to detect these bio-particles.

![Coulter Counter Diagram]

Figure 1.1: Principle of Coulter Counter
W.H Coulter invented the method of resistive pulse sensing in 1953 [16]. As shown in Figure 1.1, Coulter counters typically consist of two reservoirs of particle-laden solution separated by a single pore/channel. By monitoring changes in the electrical current through the pore/channel as individual particles pass from one reservoir to another, a coulter counter can measure the size of particles whose dimensions are of the order of the pore/channel dimensions. This change in the electrical current is recorded in a form of pulse whose height is proportional to the size of the particle and the number of pulses represents the number of particles traversed through the pore/channel. Thus, coulter counter can be used to determine the size of the individual particle from the height and the area of the pulse and to measure the particle concentration [21,22].

For traditional coulter counters with a single sensing channel, the throughput is proportional to the cross sectional area of the sensing channel. If such devices are used to detect a nano-scale particle then the diameter of the sensing channel should be reduced accordingly so that the presence of the particle in the channel causes a significant measurable resistance change inside the channel. Not doing so will deteriorate the sensitivity of the device. This results in detection of a small sample volume at a time which in turn causes low throughput of the device. This is a significant limitation for onsite analysis of bioactive particles that appear only in low concentrations. The smaller the channel the smaller the range of particle sizes that can be assayed since particles larger than the channel must be screened to prevent clogging. Thus, reducing channel size reduces the dynamic range of the device. In order to increase the throughput, multiple sensing channels can be used.
1.4 Advancement in the coulter counter device

A constraint that resistive pulse sensing requires the size of analyzed particle to be of the same order as the pore size, triggered DeBlois and C.P Bean to invent a “Nanopar” device [23]. This device had a plastic sheet joining two reservoirs and containing a single pore of 0.5-3 µm in diameter and several microns in length. DeBlois and Bean realized that the homogeneous electric filed in long, thin pore helped in achieving greater analytical precision in relating the pulse height with the particle size [24]. Later on this device was used by DeBlois to measure the diameter (< 100 nm) of the colloidal particles [24]. DeBlois and Uzgiris [25,26], Feuer et al [27] successfully characterized the viral particles with the aid of the Nanopar device. Schulthess et al investigated the agglutination of the antigen coated particles by antibody [28]. Analyte specific molecular coulter counting was successfully achieved by Bezrukov et al [29]. Current recordings indicated the passage of a single molecule through the nanometer scale ion channel. Resistive pulse sensing based on ion-channels was further explored to achieve specificity for the molecules of interest [30,31,32,33,34,35]. Li et al [36,37] used a focused ion beam technique to prepare a single channel within a Si$_3$N$_4$ membrane that was ~3-10 nm in diameter and ~ 5-10 nm in length. Li used this device to measure the length and folding characteristics of dsDNA [37]. Mara et al prepared conical shaped coulter cells (2-7 nm constriction at the tip) in track-etched polyimide membrane [38]. These cells were used to study the transport of ds DNA. Saleh and Sohn integrated the small micro-fluidic channel using common lithography techniques [39,40]. In one case, this group fabricated a single channel resistive pulse sensing device on a quartz substrate [39]. The channel of this device was 400 nm to 1 µm in depth and was used to detect 87 nm latex spheres. In
another case, micro-molding technique was used to prepare the channels within the PDMS (Polydimethylsiloxane) mold [40]. The fabricated channels were 200 nm in depth and were used to sense the presence of $\lambda$-phage DNA. Saleh and Sohn used the same device to measure the small difference in size of ~500 nm diameter, between the streptavidin-coated beads resulting from antibody binding to the surface [41]. Sun et al measured the transport of 440 nm polymer particles by using the channels as small as 1.5 $\mu$m in diameter [42]. The channels were prepared by using the template based technique [42]. Ito et al reported a carbon nano-tube coulter counters having an interior diameter of 132 nm and a length of 0.94-1.26 $\mu$m [43,44]. This device was used to simultaneously determine the size and surface charge of carboxylate-terminated polymeric nano-particles ranging from 28-90 nm in diameter. Since all the above mentioned devices used only single channel/pore to detect the micro-particles/nano-particles, the throughput was very low and took longer time to assay large volume of particle laden analyte. To overcome this problem Carbonaro and Sohn [45] used multiple Coulter counter cells on a single chip to detect different antigens rapidly and simultaneously. By doing so, the two researches were able to increase the throughput of the device. However, it was difficult to integrate large number of Coulter counter cells on a single chip as each channel had its own power supply, detection electronics and flow system in order to avoid crosstalk (electronic coupling) between the channels. With a long term goal of developing a multi-channel resistive pulse sensor, we demonstrate a micro-machined multi-channel coulter counter device which can detect large volume of micro-particle laden analyte simultaneously with its multiple sensing channels. In this sensor the channels are electrically connected via fluid which may cause a crosstalk between the channels. The
electronic coupling is reduced by shorting the negative electrode (cathode) [46]. The power source is shared by all the channels.

1.5 Signal de-noising using wavelets

As discussed in section 1.3, a Coulter counter’s sensitivity is limited by the size of the sensing channel. Particles whose diameter is less than about 10% of the channel’s width do not cause a large enough change in resistance to reliably detect; the small change is of the same order as measurement noise, so that the signal-to-noise ratio is small. The device response could further deteriorate for onsite measurements, where ideal laboratory conditions are not available. Also, reducing the size of detector channel causes a significant increase in the channel resistance, reducing the base current in the device from µA to nA or even pA level [44]. Such a small signal can be easily buried in noise, making measurement and the associated instrumentation a challenge. Although the signal-to-noise ratio in the measured current or voltage of a channel can be improved by embedding sophisticated detection electronics in the device, doing so increases device cost and makes integration of large number of channels in a single device difficult. Hence a low cost efficient method to de-noise the coulter counter signals should be implemented in order to improve the detection accuracy and particle sizing range of the device.

The most common method for eliminating noise is the use of digital filters based on Fourier transform. Techniques employing Fourier transform give information only in the frequency domain and are best suited for stationary signals [47]. Coulter counter signals are non-stationary with pulses of varied shape and width. Methods based on wavelet transforms, which contain information about both time and frequency domains
simultaneously, are more appropriate, and can easily adapt to dynamic changes in pulse width and shape [47]. The wavelet filter selected is based on the shape characteristics of the signal. Wavelet domain techniques based on wavelet transforms allow for qualitative improvement of the measured response by removing the noise components.

The wavelet transform has been established with the Fourier transform as a data-processing method in analytical chemistry which includes micro-fluidics and micro-fabricated devices. The main fields of application in analytical chemistry are related to de-noising, compression, variable reduction, and signal suppression [48]. Weidong et al [49] used wavelet transform to remove the noise from the capillary electro-phoresis-electrochemiluminescence electropherograms. In his study, the RMS (root mean square error) between the reconstructed signal and pure signal is used to evaluate the extent of de-noising. Signal de-noising and baseline correction using wavelet transform were described for microchip capillary electrophoresis by Liu et al [50]. In this study the researcher used visual inspection to optimize the wavelet filter for efficient de-noising. Perrin et al [51] applied wavelet transform to de-noise electropherograms in capillary electrophoresis. The wavelet de-noising was proved efficient by comparing the de-noised results with the simulated pure data.

The main idea of de-noising is to minimize the difference between the de-noised signal and the ideal (noiseless) signal. The studies mentioned above used ideal signals and calculated the error between the de-noised signal and the ideal signal to optimize the de-noising procedure. In our study, we did not have any ideal noiseless signal. So we followed the Cross validation algorithm by Pasti et al [52] which is based on wavelet transform. By using this algorithm an estimation of error between the ideal and the de-
noised signal is obtained without using the ideal signals. This method gives a quantitative base for the optimization of wavelet filter, thresholding method and decomposition level for improved wavelet de-noising.

1.6 Research objectives

There are three main objectives of this thesis aiming at developing a micro-machined multi-channel resistive pulse sensor for rapid, onsite detection and counting of bioactive particle.

First, we will utilize the micro-machining technology to fabricate a lab-on-a-chip multi-channel sensor. We will micro-fabricate sensing micro-channels, mini-channels, detection electrodes and reservoirs on one microchip.

Second, we will demonstrate the high throughput of this sensor using polymer particles, pollens and Chinese hamster ovary cells. In a single channel coulter counter, only a very small volume of particle solution can be assayed at a given time. Thus the single channel sensor has low throughput and low counting efficiency. In this thesis, we will demonstrate a coulter counter with multiple sensing channels, all operating simultaneously to improve the counting efficiency. The use of four sensing channels is expected to improve the throughput by approximately 300% over a single channel device without sacrificing the sensitivity, reliability and efficiency.

Finally, we will develop a signal de-noising techniques to improve the signal to noise ratio (SNR). The signals gathered from the multi-channel sensor are affected by environmental noise, which lowers the SNR and deteriorates the sensitivity of the sensor
thus making it unreliable for micro-particle detection. To overcome this problem, a wavelet based signal de-noising algorithm will be used for de-noising the signals.

1.7 Research hypothesizes

Based on the proposed method of multi-channel resistive pulse sensing of bio-particles, the following null hypothesis (\(H_{01}, H_{02}, H_{03}, H_{04}\) and \(H_{05}\)) and the respective alternative hypothesis (\(H_{11}, H_{22}, H_{33}, H_{44}\) and \(H_{55}\)) are tested:

\(H_{01}: \mu_d = 5 \mu m\), the mean of the absolute difference between actual and measured polystyrene particle size is \(5 \mu m\)

\(H_{11}: \mu_d < 5 \mu m\), the mean of the absolute difference between actual and measured polystyrene particle size is less than \(5 \mu m\)

\(H_{02}: \mu_d = 4 \mu m\), the mean of the absolute difference between actual and measured CHO cell size is \(4 \mu m\).

\(H_{22}: \mu_d < 4 \mu m\), the mean of the absolute difference between actual and measured particle size is less than \(4 \mu m\).

\(H_{03}: \mu_{actual} = \mu_{measured}\), there is no significant difference between the means of actual juniper pollen size and the measured juniper pollen size.

\(H_{33}: \mu_{actual} \neq \mu_{measured}\), there is significant difference between the means of actual juniper pollen size and the measured juniper pollen size.
$H_0^4$: $\mu_d = 0.5 \times 10^4 \text{ ml}^{-1}$ for polystyrene particles and $\mu_d = 0.5 \times 10^5 \text{ ml}^{-1}$ for juniper pollen and CHO cells.

$H_{44}$: $\mu_d < 0.5 \times 10^4 \text{ ml}^{-1}$ for polystyrene particles and $\mu_d < 0.5 \times 10^5 \text{ ml}^{-1}$ for juniper pollen and CHO cells.

$H_{05}$: $r = 0.1$, there is a cross talk between the channels.

$H_{55}$: $r < 0.1$, there is no crosstalk between the channels.

This thesis is organized by chapters in the following way: Chapter II presents a sensor design for multi-channel resistive pulse sensor with all theoretical considerations. Chapter III describes the micro-fabrication processes for the multi-channel resistive pulse sensor. Chapter IV describes the experimental setup and testing protocols. Chapter V discusses the results for the measurement with the multi-channel coulter counter for a variety of testing particles. In Chapter VI, the cross-correlation study is performed on the single particle measurement data. Chapter VII introduces to wavelet transform technique and briefly explains the CV algorithm used for de-noising the obtained coulter counter signals. Chapter VIII discusses the de-noising results obtained using wavelet transform. Chapter IX contains the summary and conclusion of this thesis work. The future research to be conducted by our group to improve the sensor’s performance in detecting and differentiating the multi-particle analyte solution is also included in Chapter X.
CHAPTER II
SENSOR DESIGN

The micro-machined multi-channel resistive pulse sensor design concept is illustrated in Figure 2.1. The multi-channel sensor consists of four peripheral reservoirs and a central reservoir. Every reservoir has a diameter of 2000 µm. Each peripheral reservoir is connected to the central reservoirs through a mini channel. A micro-channel of dimensions 40 µm (depth) x 100 µm (width) x 180 µm (length) is positioned in the middle of each mini channel and is used for particle sensing. When the bio-particle solution is forced through the channels, the particles are detected simultaneously by all the four channels. This increases the throughput of the device by 300% as compared to the single channel coulter counter sensor.

Figure 2.1: Top view of the “Micro-Machined Multi-channel Resistive Pulse Sensor”
As shown in Figure 2.1, the gold electrodes of dimensions 0.1µm (thickness), 80µm (width) are placed on opposite sides of the micro-channel with their position perpendicular to the direction of the fluid flow. These electrodes are used for applying a constant dc voltage \( V_{cc} \). For the four channel sensor, the four negative electrodes were electrically shorted near the central reservoir to ensure that each channel has the same constant dc voltage \( V_{cc} \). In this way, variation in the resistance in one micro-channel does not cause any voltage variation (cross talk) in any other channel.

Figure 2.2 shows the sectioned schematic diagram of a single sensing channel along with the measurement setup. \( R_s \) is known external sampling resistor. It consists of a constant dc supply \( (V_{cc}) \) connected to a common negative electrode at one end and a sampling resistor \( (R_s) \) at the other end. \( V_s \) is the voltage to be measured across \( R_s \).

The measurement circuit for one sensing channel is equivalent to the circuit in Figure 2.3, where \( R_c \) is the resistance of the electrolyte filled micro-channel. The electrolyte containing micro-particles is forced to move from the central reservoir to the peripheral reservoirs through the multiple sensing micro-channels. When a particle passes through a channel, it displaces some of the electrolyte solution in the micro-channel thus causing a change in the resistance \( R_c \) of the electrolyte filled micro-channel. This change in resistance is represented by \( \delta R_c \) which in turn leads to a change in the measured voltage \( V_s \) across the sampling resistor \( R_s \). From the circuit model in Figure 2.2, the relative change in the resistance of the micro-channel in terms of measured voltage is given by
Figure 2.2: Magnified view of a setup of a single channel

\[
\frac{\Delta R_c}{R_c} = \frac{(V_s - V_0)V_{cc}}{(V_{cc} - V_s)V_s}
\]  \hspace{1cm} (2.1)

Where \( \Delta R_c \) is the change in the channel resistance when a particle passes through the micro-channel, \( V_s \) is the voltage measured across the sampling resistor when the channel is filled only with electrolyte solution and \( V_s' \) is the peak voltage measured across the sampling resistor as a particle passes through the micro-channel.

Figure 2.3: Equivalent measurement circuit for one sensing channel
For a micro-channel of length $L$ and diameter $D$ as shown in Figure 2.2, the change in resistance as particle passes through it is given by [23].

\[
\frac{\delta R_c}{R_c} = \frac{d^3}{L D^2} \left[ \frac{D^2}{2L^2} + \frac{1}{\sqrt{1 + \left(\frac{D}{L}\right)^2}} \right] \tag{2.2}
\]

where $d$ is the particle diameter, $L = L + 0.8D$ is the corrected aperture length that accounts for end effects, and $F\left(\frac{d^3}{D^3}\right)$ is a correction factor that accounts for the bulging of the electric field lines into the micro-channel wall. For the rectangular micro-channel, we use the characteristic diameter $D = \sqrt{4A/\pi}$ in equation 2.2, where $A$ is the cross sectional area of the micro-channel. For the micro-channel present in the sensor, the characteristic diameter $D$ is calculated as 76.61 micron. The equivalent holds true when $(d / D)^3 < 0.1$ [53]. Thus, the particle diameter can be calculated from the relative change in resistance as

\[
d = \left\{ \frac{\delta R_c L D^2}{F\left(\frac{d^3}{D^3}\right)} \right\}^{\frac{1}{3}} \left[ \frac{D^2}{2L^2} + \frac{1}{\sqrt{1 + \left(\frac{D}{L}\right)^2}} \right] \tag{2.3}
\]
For the four channel sensor, there are four sampling resistor $R_{s1}, R_{s2}, R_{s3}$ and $R_{s4}$, across which four voltage measurements $V_{s1}, V_{s2}, V_{s3}$ and $V_{s4}$ are measured. The electrical equivalent of the four channel sensor is shown in Figure 2.4, where $R_{c1}, R_{c2}, R_{c3}$ and $R_{c4}$ are the resistances of the four micro-channels. $R_{14}, R_{12}, R_{23}$ and $R_{34}$ are the inter-channel resistances between channel 1-channel 4, channel 1-channel 2, channel 2-channel 3 and channel 3-channel 4 respectively.

One challenge for using multiple sensing micro-channels is the electronic coupling or crosstalk among the channels because the electrolyte electrically connects all the channels. When a particle passes through a channel, it causes a resistance change in this channel. Since all the channels are electrically connected, a resistance change in one channel can possibly cause a current change in other channels, and in turn induce a
voltage change across the sampling resistors of other micro-channels. This voltage change will be translated into a resistance change signal for other channels where there is no passage of particles, resulting in false detection. As shown in Figure 2.5, by electrically shorting the four negative electrodes the inter-channel resistances are bypassed thus eliminating the crosstalk between the sensing channels.

![Figure 2.5: Electrical equivalent of the four channels showing the bypass of the four inter-channel resistances. Dotted line represents the bypass](image)

The overall electrical equivalent of the four channel sensor with modification crosstalk elimination is shown in Figure 2.6.
Figure 2.6: Overall measurement circuit for four channel sensor

The voltage pulses across each sampling resistor are recorded and analyzed separately. Unlike single channel Coulter Counter, the sensor can detect particles through its four sensing micro-channels simultaneously; thus enabling higher throughput.
3.1 Micro-machining Processes

The fabrication of the micro-sensor includes three major steps: 1) micro-machining of micro-channels on a glass substrate; 2) micro-machining of detection electrodes on another glass substrate with drilled reservoirs; 3) bonding of the two glass slides. The micro-machining process for micro-channels and detection electrodes is illustrated in Figure 3.1.

Figure 3.1: Fabrication process for micro-channels and gold electrodes
3.1.1 Substrate Cleaning

Both glass substrates are cleaned in a boiling Piranha Solution \( \text{H}_2\text{SO}_4 \text{ (%)}:\text{H}_2\text{O}_2 \text{ (%)} = 3:1 \) for 20 min, then rinsed with DI water and blown dry with dry air. The dehydration process is carried out by baking the glasses on hot plate (100ºC) for 3 min to remove residual water molecules.

3.1.2 Lithography

The glasses are then spin coated with Hexamethyldisilazane (HMDS) solution (AZ Electronics) and baked on a hotplate (100ºC) for 3 min in order to improve the adhesion of the photoresist. The primer-treated substrates are coated with AZ P4620 positive photoresist (AZ Electronics), and a two step baking process is used in this study. First, a soft-baking process is carried out at 100ºC for 1 min, 120ºC for 1.5 min, and then maintained at 100ºC for another minute. This two step process can reduce the thermal stress between the glass substrate and the photoresist, resulting in a longer survival time of the positive photoresist in Buffered oxide etchant \( \text{BOE (H}_2\text{O:HF} = 6:1) \). The thickness of the positive photoresist is approximately 2.5 µm after soft baking. The UV photolithography is processed using a mask aligner (OAI-200) and the exposure dose is 288 mJ cm\(^{-2}\). The developing of the photoresist is accomplished in 3 min by immersing the exposed substrate into the AZ P4620 developer (one part AZ A400K developer to four parts DI water). After rinsing in DI water and drying by dry air, the hard baking of the photoresist is carried out at 140ºC for 30 min.
3.1.3 Fabrication of micro-channels

After baking the photoresist, the glass substrates were immersed in the BOE. The etching is carried out in polypropylene container with continuous manual agitation. In order to remove the precipitated particles, we interrupted the etching process every 5 min and dipped the substrates in a 1 M HCl solution for 10 s during the etching process. After the HCl dipping, the substrates are cleaned by dipping in DI water and immersed into the BOE again. The etching and de-precipitation process are iterated until the etching process was finished.

3.1.4 Fabrication of Electrodes

Industrially deposited, (titanium [thickness, \( t_t = 5 \text{ nm} \)] followed by gold [\( t_g = 100 \text{ nm} \)]) slides are used for the fabrication of electrodes. The electrode containing slide is termed as bottom substrate in the entire fabrication process. Ti sputtered on the glass substrate acts as an adhesion layer for the subsequently deposited Au layer [54]. The electrode pattern on the mask and the glass slide is aligned under a professional mask aligner and then exposed with UV light. The developing of the photoresist is accomplished in 3 min by immersing the exposed substrate into the AZ P4620 developer. After rinsing in DI water and drying by dry air, hard baking of the photoresist is carried out at 100°C for 60 min. The etchant used for etching away the Au film layer is KI: I\(_2\) complex from Transene Chemicals. The etching period in the solution is typically 15-20 sec for 200 nm Au film. Once the upper layer of the Ti/Au film is removed, the underlying Ti adhesion layer is etched for approximately 10 sec with H\(_2\)O: HF: H\(_2\)O\(_2\) mixed in a ratio of 20:1:1 at room temperature.
3.1.5 Drilling of channel reservoirs

Diamond drills from A & M Instruments are used to drill holes of 2 mm diameter in the top glass substrate with patterned micro-channels. Figure 3.2 shows the side and top view of the patterned glass slide with drilled reservoir.

![Figure 3.2: Drilled reservoirs in the patterned glass slide](image)

3.1.6 PDMS glue bonding

Figure 3.3 shows the stamp and stick procedure followed in this study for device bonding. A constraint for our bonding procedure is the necessity of low viscosity glue, ensuring a thin layer after spin-coating. Polydimethylsiloxane (PDMS) and Toluene are obtained from Dow Corning Corporation and EMD Chemicals respectively and used as received. An adhesive solution is made by dissolving PDMS in Toluene in a ratio of 1:4 by weight [55]. The mixture has low viscosity suitable for our application. The vial containing the adhesive solution is stored at 4°C until use to avoid curing of PDMS in it. To selectively apply this glue layer to the bonding surface and prevent contact with the channel structures, a stamp and stick method [56] is used. During the adhesive bonding step, the adhesive solution is applied with a dropper on a bare glass slide and spin-coated at 1000rpm for 60 sec. The top wafer containing the fluidic channels and the ports is
brought in contact with the glue coated glass slide with the help of a mask aligner. The speed for the spin coating is optimized to obtain a thin glue layer that does not enter the channels when in contact with the micro-fluidic wafer. The filling of a wafer pair interface can be observed under the aligner microscope. Immediately after the full glue coverage, the wafers are separated.

Any shearing of both the wafers has to be avoided during this action to assure that no glue is smeared into the channel structures. This is achieved by using a blade to separate the wafer pair. The glue covered top wafer is aligned and then brought into contact with the electrode wafer with the help of a mask aligner. According to our experiments, the time between separation of the glue transfer wafer from the fluidic wafer and the contact of this wafer with the fluidic wafer is approximately 3 min. This time has

Figure 3.3: “Stamp and Stick” bonding procedure

24
to be minimized to avoid evaporation of the glue. After alignment and contact of both the wafers, the temporarily bonded device is transferred in a vacuum oven which is set at 70°C. The device is kept in the oven for 4 hours for the PDMS glue to cure properly.

3.2 Final Device and leakage testing

A photograph of our intact micro-machined multi-channel resistive pulse sensor device is shown in Figure 3.4. The chip shown in Figure 3.4(a) has sensing micro-channels of size 40 µm x 100 µm x 180 µm and 2 mm drilled holes for sample reservoirs. Figure 3.4(b) shows a magnified view of a single sensing channel with basic two electrode detection configuration. The electrodes are fabricated at the either ends of a sensing micro-channel.

![Figure 3.4: (a) Photograph of the Micro-Machined Multi-channel sensor (b) Photograph of a single sensing micro-channel/electrode configuration](image)

After the device is micro-fabricated, it is tested for leakage by using a black ink. The black ink is mixed with DI water in a ratio of 2:1 (DI water: Black Ink). The diluted ink is passed through the channels and the channels are visually observed under a high
precision optical microscope. It can be seen in the Figure 3.5 that the channel is filled
with an ink and shows minimal leakage (3-4 µm sideways).

Figure 3.5: Picture of leakage testing done with diluted black ink
CHAPTER IV
EXPERIMENTAL PROTOCOLS

The experimental protocol for multi-channel resistive pulse measurement includes three steps: preparing the solution to be analyzed, experimental setup and acquiring the data. In this chapter, we describe in general terms the procedures we have used to accomplish each step.

4.1 Solution Preparation

In order to establish the accountability of our micro-machined device in detecting and differentiating biological and non-biological micro particles we have chosen polystyrene particles (Sigma Aldrich Inc.), juniper pollen particles (Sigma Aldrich Inc) and Chinese hamster ovary cells (CHO cells) for the study.

Polystyrene particles with well characterized diameters of 30 µm (30 µm ± 0.5 µm) (Sigma Aldrich Inc.); are chosen for testing. 30µm Polystyrene particle solution is prepared by diluting 0.1ml original particle solution, which has 10% solid content, in 7 ml of de-ionized water. Thus the yield particle concentration of 30µm polystyrene particle solution is approximately 9.534 x 10^4 ml⁻¹.

Juniper Pollen particles of size 20 µm ± 2.5 µm as specified by the vendor are measured using a multi-channel device. The Juniper Pollen particle solution is prepared
by diluting 10 mg of Juniper tree pollen in 10 ml of water. Taking an estimated density of 1.1 gm cm$^{-3}$ and an average diameter of 20 µm, the concentration of the prepared sample solution is estimated as $2.39 \times 10^5$ ml$^{-1}$.

We detected the passage of cells through multiple micro-channels of the micromachined resistive pulse sensor. The cells selected in this case are the Chinese hamster ovary cells which are having varying sizes between 10-15 µm. CHO cells are obtained from Dr. Yun’s laboratory (Department of Biomedical Engineering, The University of Akron). Standard experiments are performed in 0.1M KCl, 0.1M KH$_2$PO$_4$, 4M NaCl, 0.575M of anhydrous Na$_2$HPO$_4$, pH = 7.4. A CHO cell concentration of $2.7 \times 10^5$ ml$^{-1}$ is used for the experiments.

4.2 Experimental Setup

After the device is fabricated, the N 124-S coned Nanoport assemblies from Upchurch Scientific are placed on the central reservoir for fluidic connections and sample injection. Micro-manipulators and wire holding arms are used to make contact with the bonding pads thus interfacing the device with National Instrument’s NI-6220 data acquisition board. The detector data acquisition is done in real time using Labview software. The entire assembly is kept in a home made aluminum faradic cage to minimize the interference from surrounding electromagnetic noise [57].
4.3 Measurement Procedure and Data Acquisition

Once the device micro-channels are wet filled with particle solutions, the particles are made to flow through the micro-channel and the electrical current through the channel are measured.

4.3.1 Inducing Particle Flow

The particles in the solution are made to pass through all four micro-channels by applying pressure to the central reservoir. The pressure is applied by injecting the fluid with the help of a syringe. Clogs in the pore occur occasionally due to colloidal aggregates. These clogs are removed by application of higher flow rates thus inducing high pressures or by sonicating the device in an Ultrasound Bath (Branson).

4.3.2 Device Interface

In our experiment, we used a constant voltage set-up. This setup maintains a constant voltage across the micro-channel while measuring the current. The equivalent measurement circuit for all the four channels is shown in Figure 2.6. Figure 4.1 shows the block diagram of the device interface with the data collection and recording system. As described earlier, the electrically shorted central electrode is connected to the negative terminal of a 4V constant DC voltage supply. Each channel of the DAQ system has two terminals. One terminal of each channel of DAQ is connected to the positive electrode of an individual micro-channel. The remaining four terminal of the DAQ are connected to the positive terminal of the voltage supply. Further the DAQ is interfaced with a computer where it is controlled by a Labview program.
In the electrolyte solution and DI water, electrode polarization causes the dc voltage applied on electrodes to be dropped across the double layers of the two electrodes. Thus, the voltage drop across the bulk solution is less than the actual applied voltage [58]. Due to the polarization effect of gold electrodes [59], a high voltage source is necessary to ensure that there is a high strength electric field within the electrolyte to record a noticeable voltage change across the sampling resistor. Thus an input voltage of $V_{cc} = 4V$ is applied across the micro-channels.

4.3.3 Data Acquisition

When the particle inhabits the micro-channel, it displaces the conducting fluid and there is a decrease in the background ionic current. The current through a micro-channel is converted into a voltage signal by NI-6220 data acquisition board. The signals are
digitized at 50 kHz using the DAQ. A Labview program is used to remotely control the DAQ. The program is used as a real time graphical user interface to display the voltage signals collected simultaneously from the four micro-channels of the device. A maximum of 3 second data is recorded. The data is stored within the computer by a Labview program. After the measurement is completed the raw data files are analyzed with a Matlab program that is designed to measure the height and width of every voltage pulse. These values are saved to a new file for further analysis.
CHAPTER V
SINGLE PARTICLE DETECTION

5.1 Detection of 30 µm polystyrene particles

Figure 5.1 shows the picture of polystyrene particle taken under a high precision optical microscope.

Figure 5.1: Microscopic picture of 30 µm polystyrene particle

Figure 5.2 shows the measurement results of voltage traces across the four sampling resistor during a specific period of time. It can be clearly seen that the voltage pulses appear in random sequence. Each downward pulse represents a single polystyrene particle transiting the micro-channel.
Figure 5.2: Multi-channel voltage signal for 30µm polystyrene particles
5.1.1 Determination of Particle Size

The voltage pulses of each micro-channel are converted to the ratio of the resistance change using equation 2.1. The results are plotted in Figure 5.3 as a function of time.
Figure 5.3: Multi-channel relative resistance change signal for 30µm polystyrene particles (continued)
The relative change in resistance of a micro-channel is used to calculate the polystyrene particle diameters using equation 2.3. The estimated particle diameters are shown in table 5.1.

Table 5.1: Actual size and measured size for polystyrene particles

<table>
<thead>
<tr>
<th>Channel</th>
<th>Actual size</th>
<th>Measured size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$(30 \pm 0.5) \mu m$</td>
<td>$(26.39 \pm 1.43) \mu m$</td>
</tr>
<tr>
<td>2</td>
<td>$(30 \pm 0.5) \mu m$</td>
<td>$(27.22 \pm 1.26) \mu m$</td>
</tr>
<tr>
<td>3</td>
<td>$(30 \pm 0.5) \mu m$</td>
<td>$(26.52 \pm 1.04) \mu m$</td>
</tr>
<tr>
<td>4</td>
<td>$(30 \pm 0.5) \mu m$</td>
<td>$(27.28 \pm 1.31) \mu m$</td>
</tr>
</tbody>
</table>

5.1.2 Determination of Particle Concentration

The particle travel velocity in the micro-channel is estimated by measuring the pulse width (i.e. the time one particle took to pass through the micro-channel) and the length of the micro-channel, which is used to estimate the particle concentration later. Because each resistive pulse represents a particle passing through a micro-channel, the concentration of the particles in the four channels is calculated from the number of pulses during a period of 1s. The measured concentrations are given in table 5.2.

Table 5.2: Actual concentration and measured concentration for polystyrene particles

<table>
<thead>
<tr>
<th>Channel</th>
<th>Actual concentration</th>
<th>Measured concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$9.534 \times 10^4 \text{ ml}^{-1}$</td>
<td>$(9.11 \pm 1.82) \times 10^4 \text{ ml}^{-1}$</td>
</tr>
</tbody>
</table>
Table 5.2: Actual concentration and measured concentration for polystyrene particles (continued)

<table>
<thead>
<tr>
<th>Channel</th>
<th>Actual concentration</th>
<th>Measured concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>$9.534 \times 10^4$ ml$^{-1}$</td>
<td>$(9.26 \pm 1.41) \times 10^4$ ml$^{-1}$</td>
</tr>
<tr>
<td>3</td>
<td>$9.534 \times 10^4$ ml$^{-1}$</td>
<td>$(9.08 \pm 2.08) \times 10^4$ ml$^{-1}$</td>
</tr>
<tr>
<td>4</td>
<td>$9.534 \times 10^4$ ml$^{-1}$</td>
<td>$(9.02 \pm 1.54) \times 10^4$ ml$^{-1}$</td>
</tr>
</tbody>
</table>

5.2 Analysis of Juniper Pollen

Figure 5.4: Microscopic picture of 20 µm juniper pollen particle

Figure 5.4 shows the picture of Juniper Scopulorum pollen taken under a microscope
Figure 5.5: Multi-channel voltage signal for 20μm juniper pollen particles
Figure 5.5: Multi-channel voltage signal for 20µm juniper pollen particles (continued)

As shown in Figure 5.5, in channels 1-4, the voltage pulses caused by juniper pollen are all upward. This implies an increase in micro-channel ionic current when a pollen particle passes through the channel. A similar phenomenon is reported by Chang et al [59] that a negatively charged DNA molecule generated an increase in current (decrease in resistance- downward resistive pulse) when it is passed through a nanopore channel. A similar phenomenon of increase in ionic current due to the presence of surface charge on pollen particles is explained by Jagtiani et al [46].
The ionic current across the micro-channel can be written as

Figure 5.6. (a) Current response for non-conductive particle (b) Current response for particle with surface charge
\[ I = \int_{A} \sigma_{E} \mu \, dA \quad (5.1) \]

Where the mobility of the free ions is “\( \mu \)”, \( E \) is the electric field applied and \( A \) is the cross section area. As seen in Figure 5.6(a), when a nonconductive particle traverses through a micro-channel, it displaces an electrolyte solution equivalent to its volume which reduces the amount of free ions in the channel and hence the ion density “\( \sigma \)”. As expected the presence of particle inside the micro-channel decreases the ionic current (\( \Delta I_{\text{volume}} \)). According to the phenomenon reported by Chang et al [59] and Jagtiani et al [46], when a particle bearing a surface charge passes through a micro-channel the ionic current decreases due to the physical displacement of the electrolyte. However, since the particle is having a high surface charge, it induces additional ions within the channel, and therefore the ionic current actually increases (\( \Delta I_{\text{charge}} \)) thereby increasing the channel ion density. So, once the charged particle (particle with surface charge) enters the micro-channel, the \( \Delta I_{\text{volume}} \) current decreases while the \( \Delta I_{\text{charge}} \) increases. When the particle (with surface charge) is about to leave the micro-channel, the \( \Delta I_{\text{charge}} \) is at its maximum value. In this case the increase in the \( \Delta I_{\text{charge}} \) current will overlap the \( \Delta I_{\text{volume}} \) current as the particle surface charge is high and the concentration of ions in the electrolyte solution is low. As soon as the particle (with surface charge) leaves the channel, the \( \Delta I_{\text{volume}} \) current recovers and the \( \Delta I_{\text{charge}} \) current decreases resulting in a net ionic current as shown in Figure 5.6(b).
Figure 5.7: Multi-channel relative resistance change signal for 20 µm juniper pollen particles
The overall effect of a particle with high surface charge passing through a micro-
channel is an increase ionic current and is noticed as a downward resistive pulse as 
shown in Figure 5.7. From the experimental results presented, it appears that pollen 
particles are highly charged while the polystyrene particles are only slightly charged. The 
height of downward resistive pulses might be indicative of the surface charge of pollen 
particles.

5.2.1 Determination of Particle Size

In our experiments, the pulses of the Juniper pollen vary in height; the variation 
might be attributed to the surface charge variation and the irregular shape of juniper 
pollen. The estimated particle diameters for channels 1-4 are given in table 5.3
Table 5.3: Actual size and measured size for juniper pollen

<table>
<thead>
<tr>
<th>Channel</th>
<th>Actual size</th>
<th>Measured size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$(20 \pm 2.5) \mu m$</td>
<td>$(36.03 \pm 2.7) \mu m$</td>
</tr>
<tr>
<td>2</td>
<td>$(20 \pm 2.5) \mu m$</td>
<td>$(32.81 \pm 3.2) \mu m$</td>
</tr>
<tr>
<td>3</td>
<td>$(20 \pm 2.5) \mu m$</td>
<td>$(33.46 \pm 1.5) \mu m$</td>
</tr>
<tr>
<td>4</td>
<td>$(20 \pm 2.5) \mu m$</td>
<td>$(32.78 \pm 3.3) \mu m$</td>
</tr>
</tbody>
</table>

5.2.2 Determination of Particle Concentration

The concentrations of particles are calculated from the number of downward peaks appearing in channel 1 to 4 during the period of 1 second. Table 5.4 shows the measured concentrations of the particles along with the actual concentration.

Table 5.4: Actual concentration and measured concentration for juniper pollen

<table>
<thead>
<tr>
<th>Channel</th>
<th>Actual concentration</th>
<th>Measured concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$2.39 \times 10^5 \text{ ml}^{-1}$</td>
<td>$(2.14 \pm 0.81) \times 10^5 \text{ ml}^{-1}$</td>
</tr>
<tr>
<td>2</td>
<td>$2.39 \times 10^5 \text{ ml}^{-1}$</td>
<td>$(2.06 \pm 1.26) \times 10^5 \text{ ml}^{-1}$</td>
</tr>
<tr>
<td>3</td>
<td>$2.39 \times 10^5 \text{ ml}^{-1}$</td>
<td>$(2.26 \pm 1.32) \times 10^5 \text{ ml}^{-1}$</td>
</tr>
<tr>
<td>4</td>
<td>$2.39 \times 10^5 \text{ ml}^{-1}$</td>
<td>$(2.10 \pm 1.71) \times 10^5 \text{ ml}^{-1}$</td>
</tr>
</tbody>
</table>

It can be seen from the table that the calculated concentrations from measured resistance pulses are in good agreement with the estimated actual concentrations.
5.3 Analysis of Chinese Hamster Ovary Cells (CHO cells)

In this work, we detect the passage of cells through multiple micro-channels of the micro-machined resistive pulse sensor. The cells selected in this case are the Chinese hamster ovary cells which had varying sizes between 10-15 µm.

![Microscopic picture of Chinese hamster ovary cells with scale](a)

Figure 5.8: (a) Microscopic picture of Chinese hamster ovary cells with scale and (b) Magnified image of CHO cells

Figure 5.8 shows the picture of CHO cells taken under a high precision optical microscope.

![Multi-channel voltage signals for CHO cells](a)

Figure 5.9: Multi-channel voltage signals for CHO cells
Figure 5.9: Multi-channel voltage signals for CHO cells (continued)
As observed in Figure 5.9, there are instances where the ionic current decreases (and thus the voltage) due to the passage of the cells through the micro-channel. After the cell passes through the micro-channel, the voltage level returns to its original value. Figure 5.10 shows the resistance change results for CHO cells. The resistance pulses are in upward direction indicating the increase of resistance in the micro-channel due to the presence of a CHO cell.

Figure 5.10: Multi-channel relative resistance change signal for CHO cells
5.3.1 Determination of Particle Size

In our experiments, the pulses generated by the CHO cells varied in height; the variation might be attributed to the irregularity in the size of the cells (10-15 µm) in the same solution. The estimated particle diameters calculated from the relative change in the resistance of a micro-channel for channels 1-4 are given in table 5.5.
Table 5.5: Actual size and measured size for CHO cells

<table>
<thead>
<tr>
<th>Channel</th>
<th>Actual size</th>
<th>Measured size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(12.5 ± 2.5) µm</td>
<td>(11.34 ± 2.74) µm</td>
</tr>
<tr>
<td>2</td>
<td>(12.5 ± 2.5) µm</td>
<td>(11.67 ± 3.26) µm</td>
</tr>
<tr>
<td>3</td>
<td>(12.5 ± 2.5) µm</td>
<td>(10.29 ± 1.52) µm</td>
</tr>
<tr>
<td>4</td>
<td>(12.5 ± 2.5) µm</td>
<td>(11.01 ± 3.37) µm</td>
</tr>
</tbody>
</table>

5.3.2 Determination of Particle Concentration

The concentrations of particles are calculated from the number of downward peaks appearing in channel 1 to 4 during the period of 1 second. The time duration of each voltage/resistance pulse has a direct correspondence to the number of cells having passed during that time interval.

Table 5.6: Actual concentration and measured concentration for CHO cells

<table>
<thead>
<tr>
<th>Channel</th>
<th>Actual concentration</th>
<th>Measured concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.7 x 10⁵ ml⁻¹</td>
<td>(2.23 ± 0.49) x 10⁵ ml⁻¹</td>
</tr>
<tr>
<td>2</td>
<td>2.7 x 10⁵ ml⁻¹</td>
<td>(2.56 ± 0.26) x 10⁵ ml⁻¹</td>
</tr>
<tr>
<td>3</td>
<td>2.7 x 10⁵ ml⁻¹</td>
<td>(2.26 ± 1.02) x 10⁵ ml⁻¹</td>
</tr>
<tr>
<td>4</td>
<td>2.7 x 10⁵ ml⁻¹</td>
<td>(2.44 ± 0.83) x 10⁵ ml⁻¹</td>
</tr>
</tbody>
</table>

Table 5.6 shows the calculated concentrations of the particles along with the expected concentration. It can be seen from the table that the calculated concentrations...
from measured resistance pulses are in good agreement with the estimated actual concentrations.

5.4 Discussions

The variations in the particle size and concentration measurement are discussed below. Appropriate statistical tests are performed to show that the measured particle size and concentration are in good agreement with each other.

5.4.1 Variation in the measurement of particle size

As can be seen from the table 5.1 (polystyrene particle) and table 5.3 (CHO cells), the estimated particle size is having a divergence when compared with the actual particle size. This divergence can be attributed to four uncertainty sources in the estimation of the size. The first one is due to the uncertainty in the off axis position [60] when particle passes through the micro-channel. This uncertainty source will create uncertainty in the resistive pulses that corresponds to the uncertainty in the particle size. The second source of uncertainty is due to the fluctuations in the measured voltages. These fluctuations have no systematic trend. They can either due to the flow unsteadiness or the measurement system. Third source of uncertainty is due to the gold electrode polarization effect as discussed earlier in section 4.3.2 of Chapter 4. The electrode polarization effect can be minimized by using Ag/AgCl electrodes with large surface area [58]. Since the micro-fabricated Ag/AgCl electrodes have limited life time [61], we use gold electrodes in this study. The fourth source of uncertainty is the measurement of the micro-channel breadth.
Due to the wet etching of the channel, the channel sidewalls are concave. The surface profilometer needle treats these concave walls as vertical and measures the breadth.

For the juniper pollen particles (Table 5.3), the huge difference in estimated particle size as compared to the Juniper pollen size of 20 µm ± 2.5 µm (specified by the vendor) is due to the high surface charge on pollen and low concentrations of ions in the electrolyte solution. In the case of pollen, the second factor is dominant. Hence the overall effect of a pollen particle passing through a micro-channel is a downward resistive pulse. This phenomenon can be used to differentiate pollen from other slightly charged particles. The actual pollen size can be measured if we use an electrolyte solution of high ion concentration. In this case the particle size instead of the particle surface charge will play a dominant role in the size of the resistive pulse [62,63].

### 5.4.2 Statistical analysis for particle height

For polystyrene particles the following null hypothesis and alternative hypothesis are tested for all four channels to determine if the mean ($\mu_d$) of the absolute difference between actual and measured polystyrene particle size is less than 5 µm. As reported by Jagtiani et al [62], the uncertainty in measuring the 40 µm particle was ±12.5 %. In our study, we have selected the maximum value of $\mu_d$ as 5 µm to statistically show that the measured polystyrene size is almost close to the actual size,

$H_{01}$: $\mu_d = 5$ µm, the mean of the absolute difference between actual and measured polystyrene particle size is 5 µm

$H_{11}$: $\mu_d < 5$ µm, the mean of the absolute difference between actual and measured polystyrene particle size is less than 5 µm
A paired t-test is used for testing the hypothesis at an $\alpha$-level (confidence interval) of 0.05. If the probability (p) value obtained from the paired t-test is less than 0.05 than the mean of the absolute difference between actual and measured particle size is less than 5 $\mu$m. The test is performed on a sample size (number of experiment) of 5 for all four channels. The results from the paired t-test are reported in table 5.7.

Table 5.7: Results of the paired t-test for actual size vs. measured sized for polystyrene particles

<table>
<thead>
<tr>
<th>Channel</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-6.74</td>
<td>0.0013</td>
</tr>
<tr>
<td>2</td>
<td>-5.84</td>
<td>0.0021</td>
</tr>
<tr>
<td>3</td>
<td>-6.27</td>
<td>0.0017</td>
</tr>
<tr>
<td>4</td>
<td>-5.37</td>
<td>0.0029</td>
</tr>
</tbody>
</table>

The mean ($\mu_d$) of the absolute difference between actual and measured polystyrene particle size is less than 5 $\mu$m. As a result, we reject the null hypothesis $H_{01}$.

For CHO cells the following null hypothesis and alternative hypothesis are tested for all four channels. As the uncertainties in measuring the particle size as mentioned by Jagtiani et al [62] are the same as mentioned in this study, we select $\mu_d$ as 4 $\mu$m to statistically show that the measured CHO cell size is almost close to the actual size, $H_{02}$: $\mu_d = 4 \mu$m, the mean of the absolute difference between actual and measured CHO cell size is 4 $\mu$m.
H_{22}: \mu_d < 4 \mu m, the mean of the absolute difference between actual and measured particle size is less than 4 \mu m.

A paired t-test is used for testing the hypothesis at an \(\alpha\)-level (confidence interval) of 0.05. The test is performed on a sample size (number of experiment) of 5 for all four channels. The results from the paired t-test are reported in table 5.8.

Table 5.8: Results of the paired t-test for actual size vs. measured size for CHO cells

<table>
<thead>
<tr>
<th>Channel</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-11.32</td>
<td>0.0002</td>
</tr>
<tr>
<td>2</td>
<td>-12.63</td>
<td>0.0001</td>
</tr>
<tr>
<td>3</td>
<td>-9.04</td>
<td>0.0004</td>
</tr>
<tr>
<td>4</td>
<td>-7.91</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

The mean of the absolute difference between actual and measured CHO cell size is less than 4 \mu m. As a result, we reject the null hypothesis \(H_{02}\).

For juniper pollen the following null hypothesis and alternative hypothesis are tested for each channel to determine if there is a significant difference between means of actual juniper pollen size (\(\mu_{\text{actual}}\)) and the measured juniper pollen size (\(\mu_{\text{measured}}\)).

\(H_{03}: \mu_{\text{actual}} = \mu_{\text{measured}},\) there is no significant difference between the means of actual juniper pollen size and the measured juniper pollen size.

\(H_{33}: \mu_{\text{actual}} \neq \mu_{\text{measured}},\) there is significant difference between the means of actual juniper pollen size and the measured juniper pollen size.
A paired t-test is used for testing the hypothesis at an \( \alpha \)-level of 0.05. If the probability (p) value obtained from the paired t-test is less than 0.05 then there is significant difference between the means of the actual juniper pollen and measured juniper pollen size. The test is performed on a sample size (number of experiment) of 5 for each channel. The results from the paired t-test are reported in table 5.9.

<table>
<thead>
<tr>
<th>Channel</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-22.44</td>
<td>1.16 x 10^-5</td>
</tr>
<tr>
<td>2</td>
<td>-21.92</td>
<td>1.28 x 10^-5</td>
</tr>
<tr>
<td>3</td>
<td>-16.81</td>
<td>3.66 x 10^-5</td>
</tr>
<tr>
<td>4</td>
<td>-18.34</td>
<td>2.59 x 10^-5</td>
</tr>
</tbody>
</table>

There is a significant difference between the means of actual juniper pollen size and measured juniper pollen size. As a result, the null hypothesis \( H_{03} \) is rejected. This result statistically confirms that the surface charge rather than the size of the juniper pollen (DI water solution) plays a dominant role in peak height.

5.4.3 Variation in the measurement of particle concentration.

As seen in tables 5.2, 5.4 and 5.6 for polystyrene, juniper and CHO cells respectively, the difference between the measured concentration and the actual concentration can be due to the following reasons: First, due to the manual flow rate control there exist an uncertainty in the estimation of the flow rate and thus the measured
concentration. Second, the particles tend to deposit on the substrate during the experiments. Third, the particles tend to aggregate accounting for occasional large peaks as shown in Figure 5.3 (a & d) for polystyrene particles, Figure 5.7 (a) for juniper pollen and Figure 5.9 (b) for CHO cells. This results in the miscalculation of number of peaks and thus slightly lowers the measured concentration. As shown in Figure 5.11, the flow of more than one particle through the channel causes a series of rapid pulses with increased pulse width thus causing an error in concentration calculation. Finally, the difference in measured and expected concentration may also be due to the dilution errors and non-uniform mixing of the solution.

![Figure 5.11: Signal 3 showing two of rapid pulses](image)

5.4.4 Statistical analysis for particle concentration

The following null hypothesis and alternative hypothesis are tested for each channel to determine if the mean ($\mu_d$) of the absolute difference between actual and measured particle concentration is less than $0.5 \times 10^4$ ml$^{-1}$ for polystyrene particles, $0.5 \times 10^5$ ml$^{-1}$ for juniper pollen and $0.5 \times 10^5$ ml$^{-1}$ for CHO cells.
\[ H_{04}: \mu_d = 0.5 \times 10^4 \text{ ml}^{-1} \text{ for polystyrene particles and } \mu_d = 0.5 \times 10^5 \text{ ml}^{-1} \text{ for juniper pollen and CHO cells.} \]

\[ H_{44}: \mu_d < 0.5 \times 10^4 \text{ ml}^{-1} \text{ for polystyrene particles and } \mu_d < 0.5 \times 10^5 \text{ ml}^{-1} \text{ for juniper pollen and CHO cells.} \]

A paired t-test is used for testing the hypothesis at an \( \alpha \)-level (confidence interval) of 0.05. The test is performed on a sample size (number of experiment) of 5 for each channel. The results from the paired t-test are reported in table 5.10.

<table>
<thead>
<tr>
<th>Test Particles</th>
<th>Channel</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polystyrene particle</td>
<td>1</td>
<td>-2.61</td>
<td>0.0297</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-3.46</td>
<td>0.0129</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-3.71</td>
<td>0.0103</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-2.97</td>
<td>0.0206</td>
</tr>
<tr>
<td>Juniper pollen</td>
<td>1</td>
<td>-6.65</td>
<td>0.0013</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-6.57</td>
<td>0.0014</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-7.56</td>
<td>0.0008</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-6.11</td>
<td>0.0018</td>
</tr>
<tr>
<td>CHO cells</td>
<td>1</td>
<td>-4.31</td>
<td>0.0063</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-3.99</td>
<td>0.0081</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-3.89</td>
<td>0.0088</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-4.63</td>
<td>0.0069</td>
</tr>
</tbody>
</table>
The mean ($\mu_d$) of the absolute difference between actual and measured particle concentration is less than $0.5 \times 10^4 \text{ ml}^{-1}$ for polystyrene particles, $0.5 \times 10^5 \text{ ml}^{-1}$ for juniper pollen and $0.5 \times 10^5 \text{ ml}^{-1}$ for CHO cells. As a result, we reject the null hypothesis $H_{04}$. 
6.1 Cross-correlation study for crosstalk between the channels

Cross-Correlation is a well established method of estimating the degree to which two data series are correlated. It is used in many fields including signal processing [64] and image processing. In the field of EMG, cross-correlation is used to assess myoelectric cross talk [65].

In the sensor design chapter, the explanation for eliminating the crosstalk is based on the basic knowledge of electrical networks. The cross talk is eliminated by shorting the negative electrode thereby shunting the inter channel resistances. In this section, a statistical method known as normalized cross-correlation analysis is performed between the signals from two sensing channels at a time to prove that the device is free from crosstalk.

The normalized cross-correlation between the signals from a pair of sensing channel is performed as follows.

\[
r(d) = \frac{\sum \left[ (a(i) - \bar{a}) \times (b(i - d) - \bar{b}) \right]}{\sqrt{\sum (a(i) - \bar{a})^2} \times \sqrt{\sum (b(i - d) - \bar{b})^2}}
\]  

(6.1)
where \( a_i \) and \( b_i \) are the two signals between whom the cross-correlation is performed, \( i = 1, 2, \ldots, N-1 \) where \( N \) (5000 data points for 0.1 second data) is the length of a signal \( a \) or \( b \), \( \bar{a} \) and \( \bar{b} \) are the means of the corresponding signals. The cross-correlation coefficient ‘\( r \)’ is computed for all time lags (\( d \)) from 0 to N-1 which results in a cross-correlation coefficient series of twice the length as the original signal. In our study, a cross-correlation is a measure of cross-talk between two signals as a scalar between -1 to 1 [66]. Table 6.1 shows the relation between the ‘\( r \)’ value and amount of crosstalk to be considered between the channels.

Table 6.1: Relation between r-value and status of cross-talk between the channels

<table>
<thead>
<tr>
<th>r-value</th>
<th>Status of Crosstalk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Close to “1”</td>
<td>Perfect crosstalk</td>
</tr>
<tr>
<td>Close to “0”</td>
<td>No cross talk</td>
</tr>
<tr>
<td>Close to “-1”</td>
<td>Equal but opposite crosstalk</td>
</tr>
</tbody>
</table>

6.2 Results of the crosstalk analysis

To measure the crosstalk between all four channels, a cross correlation analysis is performed in pairs between channel 2 –channel 3, channel 2 –channel 4, channel 2 –channel 1, channel 3 –channel 4, channel 3 –channel 1, and channel 4 –channel 1. Figure 6.1 shows the cross-correlation analysis results for CHO cells.
Figure 6.1: Cross-Correlation analysis for CHO cells between channels:

- a) channel 2 – channel 3
- b) channel 2 – channel 4
- c) channel 2 – channel 1
- d) channel 4 – channel 4
- e) channel 3 – channel 1
- f) channel 4 – channel 1
It is obvious from Figure 6.1 that the cross-correlation coefficients ‘r’ are less than 0.1, indicating that there is negligible correlation among the pulses of different channels. Similarly, the typical results for normalized cross-correlation analysis for Juniper pollen and CHO cells are plotted in Figure 6.2 and Figure 6.3 respectively.

Figure 6.2: Cross-Correlation analysis for polystyrene particles between channels a) channel 2 – channel 3, b) channel 2 – channel 4 c) channel 2 – channel 1 d) channel 4 – channel 4 e) channel 3 – channel 1 f) channel 4 – channel 1
Figure 6.2: Cross-Correlation analysis for polystyrene particles between channels a) channel 2 – channel 3, b) channel 2 – channel 4 c) channel 2 – channel 1 d) channel 4 – channel 4 e) channel 3 – channel 1 f) channel 4 – channel 1 (continued)

Figure 6.3: Cross-Correlation analysis for Juniper pollen between channels a) channel 2 – channel 3, b) channel 2 – channel 4 c) channel 2 – channel 1 d) channel 4 – channel 4 e) channel 3 – channel 1 f) channel 4 – channel 1
We found that the cross-correlation coefficients “r” is less than 0.1, indicating that there is negligible correlation among the signals of different channels. This implies that the four sensing channels are able to simultaneously detect and count particles with negligible crosstalk among channels.
Hence the signals obtained from four different micro-channels of the multi-channel resistive pulse sensor are not correlated. This validates our sensor design (Chapter 2) for elimination of crosstalk.

6.3 Discussions

We performed an additional statistical analysis between each pair of channel for the particles tested. The following null hypothesis and alternative hypothesis is tested for each channel to determine if there is a crosstalk between the channels.

$$H_{05}: r = 0.1$$, there is a crosstalk between the channels.

$$H_{55}: r < 0.1$$, there is no crosstalk between the channels.

A value of 0.1 is chosen as it indicates very less crosstalk [67]. A z-test [67] is used for testing the hypothesis at an $\alpha$-level (confidence interval) of 0.05. According to this test, if the probability (p) value is less than 0.05 then there is no crosstalk between the channels. The results from test are reported in table 6.2.

<table>
<thead>
<tr>
<th>Particles</th>
<th>Channel Pair</th>
<th>r-value</th>
<th>z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3</td>
<td>0.01603</td>
<td>-5.939101759</td>
<td>&lt; 2.87x10^{-7}</td>
<td></td>
</tr>
<tr>
<td>2-4</td>
<td>0.0178</td>
<td>-5.814259931</td>
<td>&lt; 2.87x10^{-7}</td>
<td></td>
</tr>
<tr>
<td>2-1</td>
<td>-0.0192</td>
<td>-8.431821138</td>
<td>&lt; 2.87x10^{-7}</td>
<td></td>
</tr>
<tr>
<td>3-4</td>
<td>0.0204</td>
<td>-5.630913339</td>
<td>&lt; 2.87x10^{-7}</td>
<td></td>
</tr>
<tr>
<td>3-1</td>
<td>0.0192</td>
<td>-5.715529765</td>
<td>&lt; 2.87x10^{-7}</td>
<td></td>
</tr>
</tbody>
</table>
Table 6.2: Results of the correlation test for crosstalk (continued)

<table>
<thead>
<tr>
<th>Particles</th>
<th>Channel Pair</th>
<th>r-value</th>
<th>z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polystyrene particle</td>
<td>4-1</td>
<td>0.0231</td>
<td>-5.440554281</td>
<td>&lt; 2.87x10^{-7}</td>
</tr>
<tr>
<td></td>
<td>2-3</td>
<td>0.0631</td>
<td>2.04184628</td>
<td>0.0045</td>
</tr>
<tr>
<td></td>
<td>2-4</td>
<td>0.0618</td>
<td>-2.711503788</td>
<td>0.0034</td>
</tr>
<tr>
<td></td>
<td>2-1</td>
<td>0.0011</td>
<td>-6.993294528</td>
<td>&lt; 2.87x10^{-7}</td>
</tr>
<tr>
<td></td>
<td>3-4</td>
<td>0.0536</td>
<td>-3.290428735</td>
<td>0.000233</td>
</tr>
<tr>
<td></td>
<td>3-1</td>
<td>0.0563</td>
<td>-3.0998823</td>
<td>0.00135</td>
</tr>
<tr>
<td></td>
<td>4-1</td>
<td>0.0596</td>
<td>-2.866895066</td>
<td>0.0021</td>
</tr>
<tr>
<td>Juniper pollen</td>
<td>2-3</td>
<td>0.0596</td>
<td>-2.866895066</td>
<td>0.0021</td>
</tr>
<tr>
<td></td>
<td>2-4</td>
<td>0.0141</td>
<td>-6.075255072</td>
<td>&lt; 2.87x10^{-7}</td>
</tr>
<tr>
<td></td>
<td>2-1</td>
<td>-0.0068</td>
<td>-7.552249639</td>
<td>&lt; 2.87x10^{-7}</td>
</tr>
<tr>
<td></td>
<td>3-4</td>
<td>0.027</td>
<td>-5.165645258</td>
<td>2.87x10^{-7}</td>
</tr>
<tr>
<td></td>
<td>3-1</td>
<td>-5.63E-04</td>
<td>-7.110881592</td>
<td>&lt; 2.87x10^{-7}</td>
</tr>
<tr>
<td></td>
<td>4-1</td>
<td>0.0356</td>
<td>-4.559546257</td>
<td>2.6x10^{-6}</td>
</tr>
<tr>
<td>CHO cells</td>
<td>2-3</td>
<td>0.0246</td>
<td>-5.334813546</td>
<td>&lt; 2.87x10^{-7}</td>
</tr>
<tr>
<td></td>
<td>2-4</td>
<td>0.0141</td>
<td>-6.075255072</td>
<td>&lt; 2.87x10^{-7}</td>
</tr>
<tr>
<td></td>
<td>2-1</td>
<td>-0.0068</td>
<td>-7.552249639</td>
<td>&lt; 2.87x10^{-7}</td>
</tr>
<tr>
<td></td>
<td>3-4</td>
<td>0.027</td>
<td>-5.165645258</td>
<td>2.87x10^{-7}</td>
</tr>
<tr>
<td></td>
<td>3-1</td>
<td>-5.63E-04</td>
<td>-7.110881592</td>
<td>&lt; 2.87x10^{-7}</td>
</tr>
<tr>
<td></td>
<td>4-1</td>
<td>0.0356</td>
<td>-4.559546257</td>
<td>2.6x10^{-6}</td>
</tr>
</tbody>
</table>

The statistical analysis proved that there is no crosstalk (r < 0.1) between the channels. As a result, the null hypothesis $H_{05}$ is rejected. By rejecting the null hypothesis we have proved that our sensor is capable of detecting the particles simultaneously through all the four micro-channels without having any crosstalk amongst them.
CHAPTER VII
WAVELET THEORY

Wavelets are becoming an increasingly important tool for image and signal processing [68]. Unlike the Fourier transform (FT), a Wavelet transform (WT) can utilize different types of wavelet functions each having different properties. These functions have one important property, i.e., their localization of both time and frequency domains simultaneously. The signal to be analyzed is multiplied with the wavelet function. The width of a wavelet function changes at each level, with lower levels giving good time resolution and poor frequency resolution, and higher levels giving good frequency resolution and poor time resolution.

7.1 Wavelet Transform

The wavelet transform decomposes the signal, \( x(t) \) in both time and frequency domains and can be mathematically represented as

\[
\psi_{k,j}(t) = \int_{-\infty}^{\infty} x(t) \cdot \psi_{k,j}(t) \, dt \quad (7.1)
\]
where \( j \) is the scaling factor and \( k \) is the translation factor, \( w_{k,j} \) are the wavelet coefficients. A set of dilations and translations \( \Psi_{k,j}(t) \) of a chosen mother wavelet \( \Psi(t) \) is used for the analysis of a signal \( x(t) \). This can be defined as

\[
\Psi_{k,j}(t) = \frac{1}{\sqrt{j}} \Psi\left(\frac{t-k}{j}\right) \tag{7.2}
\]

### 7.2 Discrete wavelet transforms (DWT)

The DWT is a sampled version of continuous wavelet transform. It is easy to implement and reduces the computation time and resources required as compared to the continuous wavelet transform. The DWT can be written into a vector or matrix form as,

\[
w = \Psi \cdot x \tag{7.3}
\]

where \( w \) is the wavelet transform of a discrete signal \( x \), with an orthogonal matrix of wavelet functions \( \Psi \). The signal to be analyzed is passed through analysis filters with different cutoff frequencies at different levels.

![Figure 7.1: Discrete wavelet transform showing decomposition up to three levels](image_url)
Figure 7.1 shows the computation of DWT by successive low and high pass filtering. The input signal is denoted by $x[n]$, where $n$ is an integer. The input signal is passed through filters with different cutoff frequencies at different levels. The low-pass filter is denoted by $G_0$ and the high-pass filter is denoted as $H_0$. At each level, the DWT decomposes the input signal into high-frequency components, called detail coefficients ($D[n]$), and the low-frequency components, called approximation coefficients ($A[n]$) [69]. The approximation coefficients obtained at the previous level are used as the input to the next level; i.e., the approximation coefficients are further decomposed to produce the next level’s approximation and detail coefficients.

At each decomposition level, the frequency resolution is doubled as the uncertainty in the frequency is reduced by half. Thus the decimation by 2 halves the time resolution as the entire signal is represented by only half the number of samples. With this approach, the time resolution becomes good at high frequencies and the frequency resolution becomes good at low frequencies. The filtering and decimation process is continued until the desired level is reached.

Figure 7.2: Discrete Wavelet Transform showing reconstruction for three levels
Figure 7.2 shows the reconstruction (reverse process of decomposition) of the original signal. It is basically a reverse process of decomposition in which the approximation and detail coefficients from the last level of decomposition are up-sampled by 2, passed through the low and high-pass filters respectively and then added. These filters are called synthesis filters and are represented as $G_1$ (low-pass) and $H_1$ (high-pass). The original signal is obtained by repeating the process for the same number of levels as in the case of decomposition.

7.3 Signal de-noising using DWT

The algorithm for signal de-noising using DWT involves three major steps: (a) application of the wavelet transform to convert the signal into the wavelet domain, (b) thresholding of the detail coefficients to remove or suppress undesired coefficients and (c) reconstruction of the signal by application of the inverse wavelet transform.

The first step (a) consists of application of the wavelet transform to decompose the signal and obtain the DWT coefficients $w$ (equation 7.3). For best results, the wavelet function $\Psi$ and number of levels of decomposition are chosen based on the signal to be de-noised.

In the second step (b), the undesired detail coefficients are removed or suppressed. The procedure makes use of a threshold value ($\delta$), which was estimated using the Universal Threshold ($U_r$) rule, defined as [70]:

$$\delta = \sigma \sqrt{2 \times \log N}$$  \hspace{1cm} (7.4)
where $N$ is the length of the signal and $\sigma$ is an estimate of the standard deviation of the noise which is computed from the detail coefficients of the first level of decomposition ($D^{(1)}$) [70].

$$\sigma = \frac{\text{median}(D^{(1)})}{0.6745}$$  \hspace{1cm} (7.5)

In DWT based signal de-noising, either soft thresholding or hard thresholding strategy [71] can be used. In the soft thresholding strategy, detail coefficients that are smaller than the threshold value ($\delta$) are set to zero, and those that are larger (or equal) are reduced by the threshold value [71]

$$w^* = \begin{cases} 0 & \text{if } |w| < \delta \\ \text{sign}(w) (|w| - \delta) & \text{if } |w| \geq \delta \end{cases}$$  \hspace{1cm} (7.6)

For hard thresholding, detail coefficients that are smaller than threshold value are set to zero, and those that are larger are kept unchanged:

$$w^* = \begin{cases} 0 & \text{if } |w| \leq \delta \\ w & \text{if } |w| > \delta \end{cases}$$  \hspace{1cm} (7.7)

Here, $w$ and $w^*$ are the wavelet or DWT coefficients before and after thresholding. Approximation coefficients are not thresholded; for them, $w^* = w$.  

70
In the final step, the original signal is reconstructed by applying an inverse wavelet transform using the thresholded DWT coefficients, \( w^* \).

\[
\bar{x}_d = \Psi^T w^*
\]  

(7.8)

The result \( \bar{x}_d \) is the de-noised version of the original signal \( x \) after the reconstruction by applying inverse wavelet transform.

7.4 Cross-Validation (CV) Algorithm

To achieve the best de-noising results for any given application, it is important to select the wavelet function, number of levels of decomposition, threshold value and thresholding strategy appropriately. The cross-validation algorithm described by Pasti et al [72, 52] is used here to optimize the wavelet filter, decomposition level, threshold value, and threshold strategy. The CV algorithm computes an estimate of the Root square error (RSE), also known as the Integrated square error (ISE). RSE is the difference between the de-noised signal and the ideal or noiseless signal. For experimental data, the ideal signal for calculation of RSE is not available; we have to use ISE, which is an estimate of RSE. The main goal of parameter optimization using CV is to obtain a minimum value of ISE. The procedure for the CV method is summarized below and is also shown in Figure 7.3.

1. The original signal, of length \( 2^p \), is divided into odd \( (x_o) \) and even \( (x_e) \) data points, which are then re-indexed \( (i=1 \text{ to } 2^{p-1}) \) into two different signals of half
length.

(2) An estimate $\hat{x}_e$ of the even signal is obtained by interpolating the odd signal $x_o$, and an estimate $\hat{x}_o$ of the odd signal is obtained by interpolating the even signal $x_e$.

(3) The odd and even estimates $\hat{x}_o$ and $\hat{x}_e$ are transformed into wavelet domain.

(4) A threshold value is applied to the odd and even signals, using either hard or soft thresholding with a threshold value determined from the Universal Threshold rule. In figure 3, $U_T$ denotes the thresholding operator.

(5) The reconstructed odd ($\overline{x}_o$) and even ($\overline{x}_e$) filtered signals are obtained by applying the inverse wavelet transform.

(6) The integrated square error (ISE) for the odd signal $ISE_o$ is obtained as the sum of the squared differences between the reconstructed signal $\overline{x}_o$ (step e) and its estimate $\hat{x}_o$ (step b). A similar calculation is done to find the ISE for the even function.

(7) The total ISE of the signal is calculated as the sum of the two ISE’s obtained from the odd and even functions.

The threshold value obtained using Universal Threshold rule in the above procedure is for half the data points ($N/2$ points) of the original signal length ($N$ points). The threshold value for the entire data length consisting of $N$ points is determined as,
\[ \delta_N = \left(1 - \frac{\log 2}{\log N}\right)^{\frac{1}{2}} \delta_{N/2} \]  \hspace{1cm} (7.9)

Once the parameters for DWT de-noising are optimized by cross-validation procedure they are used in DWT de-noising algorithm (section 7.3) to remove the noise from the measured data.

Figure 7.3: Flowchart for cross-validation procedure to obtain Integrated Square Error (ISE).

73
7.5 Wavelet Packets

As described in section 7.2, each level of wavelet decomposition splits the input signal into two sub-bands, the approximation coefficients (A) and detail coefficients (D). Multiple level decomposition is done by further splitting the approximation coefficients of one level to produce approximations and detail coefficients for the next level; N-level decomposition is comprised of N details sub-bands, one from each level, and the one approximation sub-band from the N\(^{th}\) level. A variation of the wavelet decomposition called a wavelet packet decomposes not only the approximation coefficients, but also the detail coefficients, so that N-level wavelet packet decomposition is comprised of \(2^{N-1}\) sub-bands. Figure 7.4 shows the structure of three-level wavelet packet decomposition. It shows the original signal decomposed into first level approximation (A\(^1\)) and detail (D\(^1\)) coefficients. At the second level, A\(^1\) is decomposed into its approximation (AA\(^2\)) and detail (DA\(^2\)), and at the same time D\(^1\) is decomposed into its own approximation (AD\(^2\)) and detail (DD\(^2\)) coefficients. AAA\(^3\), DAA\(^3\) and ADA\(^3\), DDA\(^3\) are the approximations and details for AA\(^2\) and DA\(^2\) at the third level, and similarly AAD\(^3\), DAD\(^3\) and ADD\(^3\), DDD\(^3\) are the approximations and details for AD\(^2\) and DD\(^2\).

In wavelet packets, decomposition at each level splits the frequency content of the signals into two distinct frequency bands; the resulting approximations and details each contain half of the frequency band as compared to the previous level. At higher levels of decomposition, the sub-bands further are split into smaller frequency bands. This allows separation of coefficients corresponding to a particular frequency band; this separation is taken advantage of to filter out or preserve frequency content of a signal. De-noising can
be done using wavelet packets; the steps are similar to those discussed in section 7.3 for standard DWT de-noising.

Figure 7.4: Decomposition tree for a signal using wavelet packets
CHAPTER VIII

SIGNAL ANALYSIS USING WAVELETS

8.1 Signals gathered from coulter counter

In order to demonstrate the performance of the wavelet technique for both qualitative and quantitative processing, four different signals (shown in Figure 8.1) obtained from the multi-channel sensor are chosen. Signal 1 in Figure 8.1(a) is a multi-channel (channel 2) voltage signal for 30µm polystyrene particles. Signal 2 in Figure 8.1(c) and signal 4 in Figure 8.1(g) are the multi-channel (channel 1 and channel 2 respectively) voltage signals obtained for juniper pollen particles. Signal 3 in Figure 8.1(e) is a multi-channel (channel 1) voltage signal obtained for CHO cells. Fast Fourier Transforms (FFT coefficients “F”) of the four signals, shown in Figures 8.1(b), 8.1(d), 8.1(f) and 8.1(h), show that all signals contain frequency content distributed over the entire spectrum, but with concentrated content in two distinct regions. The first region, from DC to 5 KHz, contains the signal and 60 Hz noise; the second region, from 10 KHz onwards (high frequency noise) contains noise from an unknown source. Signal 3 shows significant amount of both 60 Hz and high frequency noise. Signal 3 and 4 are affected by a baseline drift. 60 Hz noise and baseline drift are not as prominent in signal 1 and 2. In all four signals, the effect of high frequency noise can be seen in the measured response.
Figure 8.1: Voltage response across single channel of the multi-channel sensor for (a) signal 1: Channel 2-30µm PM particle, (b) frequency content of signal 1, (c) signal 2: Channel 1-20µm Juniper pollen, (d) frequency content of signal 2, (e) Signal 3: Channel 1-10µm CHO cells, (f) frequency response of signal 3, (g) Signal 4: Channel 2-20µm Juniper pollen and (h) frequency response of signal 4
Figure 8.1: Voltage response across single channel of the multi-channel sensor for (a) signal 1: Channel 2-30µm PM particle, (b) frequency content of signal 1, (c) signal 2: Channel 1-20µm Juniper pollen, (d) frequency content of signal 2, (e) Signal 3: Channel 1-10µm CHO cells, (f) frequency response of signal 3, (g) Signal 4: Channel 2-20µm Juniper pollen and (h) frequency response of signal 4 (continued)

8.2  Optimization of de-noising parameters

Applying wavelets to de-noise a signal effectively requires appropriate selection of de-noising parameters such as the wavelet filter, the number of levels of decomposition, the thresholding method and the threshold value applied. The selection of the parameters is optimized using the cross-validation method already described in section 7.4 of Chapter 8, which allows evaluation of de-noising performance quantitatively in terms of the resulting integrated square error (ISE).

8.2.1  Wavelet Filter Optimization

The wavelet filter is chosen from the group of 32 wavelet filters listed in table 1. The cross-validation method is used to compute the ISE for each of the wavelets, and the wavelet filter that resulted in a minimum value of the ISE selected. For this selection, the other wavelet de-noising parameters are kept fixed; nine levels of decomposition and
hard thresholding with a threshold value chosen according to the universal threshold rule \((U_T)\) are used.

Table 8.1 List of wavelet filters used in the de-noising process for optimization of the choice of the wavelet filter

<table>
<thead>
<tr>
<th>Wavelet Number</th>
<th>Wavelet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Daubechies 2</td>
</tr>
<tr>
<td>2</td>
<td>Daubechies 4</td>
</tr>
<tr>
<td>3</td>
<td>Daubechies 6</td>
</tr>
<tr>
<td>4</td>
<td>Daubechies 8</td>
</tr>
<tr>
<td>5</td>
<td>Daubechies 10</td>
</tr>
<tr>
<td>6</td>
<td>Daubechies 12</td>
</tr>
<tr>
<td>7</td>
<td>Daubechies 14</td>
</tr>
<tr>
<td>8</td>
<td>Daubechies 16</td>
</tr>
<tr>
<td>9</td>
<td>Daubechies 18</td>
</tr>
<tr>
<td>10</td>
<td>Daubechies 20</td>
</tr>
<tr>
<td>11</td>
<td>Coiflet 1</td>
</tr>
<tr>
<td>12</td>
<td>Coiflet 2</td>
</tr>
<tr>
<td>13</td>
<td>Coiflet 3</td>
</tr>
<tr>
<td>14</td>
<td>Coiflet 4</td>
</tr>
<tr>
<td>15</td>
<td>Coiflet 5</td>
</tr>
<tr>
<td>16</td>
<td>Symmlet 4</td>
</tr>
</tbody>
</table>
Table 8.1 List of wavelet filters used in the de-noising process for optimization of the choice of the wavelet filter (continued)

<table>
<thead>
<tr>
<th>Wavelet Number</th>
<th>Wavelet</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Symmlet 5</td>
</tr>
<tr>
<td>18</td>
<td>Symmlet 6</td>
</tr>
<tr>
<td>19</td>
<td>Symmlet 7</td>
</tr>
<tr>
<td>20</td>
<td>Symmlet 8</td>
</tr>
<tr>
<td>21</td>
<td>Symmlet 9</td>
</tr>
<tr>
<td>22</td>
<td>Symmlet 10</td>
</tr>
<tr>
<td>23</td>
<td>Biorthogonal 2.4</td>
</tr>
<tr>
<td>24</td>
<td>Biorthogonal 2.6</td>
</tr>
<tr>
<td>25</td>
<td>Biorthogonal 2.8</td>
</tr>
<tr>
<td>26</td>
<td>Biorthogonal 3.3</td>
</tr>
<tr>
<td>27</td>
<td>Biorthogonal 3.5</td>
</tr>
<tr>
<td>28</td>
<td>Biorthogonal 3.7</td>
</tr>
<tr>
<td>29</td>
<td>Biorthogonal 3.9</td>
</tr>
<tr>
<td>30</td>
<td>Biorthogonal 4.4</td>
</tr>
<tr>
<td>31</td>
<td>Biorthogonal 5.5</td>
</tr>
<tr>
<td>32</td>
<td>Biorthogonal 6.8</td>
</tr>
</tbody>
</table>
Figure 8.2 shows the ISE values obtained for each of the thirty-two wavelets from the cross-validation procedure. The wavelets giving the best results for signal 1, signal 2, signal 3 and signal 4 are bior 3.9 (number 29 with ISE: $7.46 \times 10^{-5}$), bior 3.7 (number 28 with ISE: $1.757 \times 10^{-5}$), bior2.8 (number 25 with ISE: $5.13 \times 10^{-5}$) and bior2.4 (number 23 with ISE: $7.04 \times 10^{-5}$) respectively. Figure 8.2 shows that most of the biorthogonal filters considered in this study are good choices for the signals measured using this sensor. Although we tried to maintain the same experimental conditions for each experiment, variation in the type and level of the noise were inevitable. This variation led to different wavelet filters providing optimal de-noising in different experimental conditions; however further cross validation of an additional twelve signals confirm that the bior 2.8, bior 2.4 and bior 3.7 are good choices overall for signals measured from the sensor.
8.2.2 Decomposition Level optimization

The CV procedure is used to find the number of levels of decomposition that gives a minimum value of ISE. Each signal is processed using the best wavelet filter (from section 8.2.1), and hard thresholding with the universal threshold value is used. Figure 8.3 shows the variation of ISE with the number of levels of decomposition. For signals 1, 2, 3, and 4 the best number of levels is three, three, three and two, respectively. The best number of levels is always relatively low ($\leq 4$) and depends on the frequency content of the signal ($\leq 1$ KHz in this case). An additional twelve signals are analyzed to confirm that two to four levels of decomposition results in the lowest ISE and are suitable to be used for de-noising. Varying the flow rate at the input and/or varying the sampling rate of the DAQ would change the optimal number of levels.

![Figure 8.3: Cross-validation results for (a) signal 1 using wavelet bior 3.9, (b) signal 2 using bior 3.7, (c) signal 3 using bior 2.8 and (d) signal 4 using bior 2.4](image)

Figure 8.3: Cross-validation results for (a) signal 1 using wavelet bior 3.9, (b) signal 2 using bior 3.7, (c) signal 3 using bior 2.8 and (d) signal 4 using bior 2.4
8.2.3 Optimization of the Threshold Strategy

Soft and hard threshold strategies are applied to the measured signal to filter out the coefficients related to the noise in the signal. The threshold strategy giving the lowest ISE value is selected for de-noising the signal. ISE is computed for all the wavelet filters with the soft threshold strategy using 9th level of decomposition and the Universal threshold. These are compared with the ISE values calculated earlier using the hard threshold strategy in section 8.2.1. Figure 8.4 shows the comparison of the ISE values from both soft and hard threshold strategies for signal 1, signal 2, signal 3 and signal 4.
Figure 8.4: Comparison of ISE obtained using Universal Threshold value when applying hard and soft threshold strategies for (a) signal 1, (b) signal 2, (c) signal 3 and (d) signal 4.
Figure 8.4: Comparison of ISE obtained using Universal Threshold value when applying hard and soft threshold strategies for (a) signal 1, (b) signal 2, (c) signal 3 and (d) signal 4 (continued)
Figure 8.4 shows that for nearly all the signals and wavelet filters, hard thresholding results give lower ISE than soft thresholding. Hence, hard thresholding would prove to be a better thresholding strategy for signals from this sensor.

8.2.4 Optimization of the Threshold Value

A threshold value obtained using the Universal threshold rule for N data points (equation 7.9) is assumed as the initial threshold value. The initial threshold value \( U_T \) is then used to find the appropriate threshold value using an optimization process carried out by the CV method. Table 8.2 gives the threshold values obtained using the Universal threshold method for the four signals. Twenty threshold values are used for each signal, ranging from 0 to \( U_T \) in steps of \( 1/20 U_T \). The threshold value at which the least value of ISE is obtained is chosen as the optimized threshold value for each signal. The optimized values of the threshold are shown in table 8.3.

Table 8.2: Threshold value using Universal Threshold \( (U_T) \) method for the entire length of the signals

<table>
<thead>
<tr>
<th>Signal</th>
<th>Universal Threshold Value, ( U_T ) (x 10(^{-6}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal 1</td>
<td>671.81</td>
</tr>
<tr>
<td>Signal 2</td>
<td>138</td>
</tr>
<tr>
<td>Signal 3</td>
<td>142.31</td>
</tr>
<tr>
<td>Signal 4</td>
<td>698.61</td>
</tr>
</tbody>
</table>
Table 8.3: Optimized threshold values for each signal using the CV Method

<table>
<thead>
<tr>
<th>Signal</th>
<th>Filter</th>
<th>Optimized Threshold value</th>
<th>ISE value at optimized threshold value (x 10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bior</td>
<td>CV Threshold = 17/20 ( U_T )</td>
<td>7.3402</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>( U_T )</td>
<td>7.3480</td>
</tr>
<tr>
<td>2</td>
<td>Bior</td>
<td>CV Threshold = 20/20 ( U_T )</td>
<td>1.7066</td>
</tr>
<tr>
<td></td>
<td>3.7</td>
<td>( U_T )</td>
<td>1.7066</td>
</tr>
<tr>
<td>3</td>
<td>Bior</td>
<td>CV Threshold = 19/20 ( U_T )</td>
<td>3.8813</td>
</tr>
<tr>
<td></td>
<td>2.8</td>
<td>( U_T )</td>
<td>4.0249</td>
</tr>
<tr>
<td>4</td>
<td>Bior</td>
<td>CV Threshold = 12/20 ( U_T )</td>
<td>4.9811</td>
</tr>
<tr>
<td></td>
<td>2.4</td>
<td>( U_T )</td>
<td>5.4105</td>
</tr>
</tbody>
</table>

8.2.5 Verification of the Optimized parameters

As shown in section 8.2.3, hard threshold strategy gives better de-noising results than the soft threshold strategy. To verify these results the de-noising of the signals using both hard and soft threshold strategies are shown in Figure 8.5. It can be seen that there is peak attenuation along with false peaks and/or artifacts when the signals are processed using soft threshold strategy. In the case of hard threshold strategy, noise is reduced and peak magnitude is not as severely attenuated.
Figure 8.5: Reconstructed signals obtained using optimized filter parameters
Figure 8.5: Reconstructed signals obtained using optimized filter parameters for (a) signal 1 using hard threshold strategy, level 3 and threshold = 17 $U_T/20$, (b) signal 1 using soft threshold strategy, level 2 and threshold = 9 $U_T/20$, (c) signal 2 using hard threshold strategy, level 3, threshold = 20 $U_T/20$, (d) signal 2 using soft threshold strategy, level 3, and threshold = 12 $U_T/20$, (e) signal 3 using hard threshold strategy, level 3 and threshold = 19 $U_T/20$, (f) signal 3 using soft threshold strategy, level 3 and threshold= 20 $U_T/20$, (g) signal 4 using hard threshold strategy, level 2 and threshold = 12 $U_T/20$, (h) signal 4 using soft threshold strategy, level 2 and threshold = 9 $U_T/20$ (continued)

Figure 8.6 shows the variation of ISE as a function of different threshold values for different levels of decomposition. The threshold values and level of decomposition are optimized simultaneously to verify the results obtained for the optimized decomposition level and the threshold values discussed in the previous sections. A hard threshold strategy with the optimized wavelet filter from the previous sections is used. For each decomposition level, the ISE for all the 20 threshold values are obtained. The threshold value and the decomposition level corresponding to the same minimum ISE are chosen for de-noising. The results matched those obtained when decomposition level and threshold value are optimized individually.
Figure 8.6: Hard threshold optimization for varied decomposition level for (a) signal 1 using bior 3.9, (b) signal 2 using bior 3.7, (c) signal 3 using bior 2.8 and (d) signal 4 using bior 2.4

8.2.6 DWT de-noising using optimized parameters

Table 8.4 summarizes the results of the CV procedure. It lists the optimized parameters that are applied to de-noise each of the three signals. DWT de-noising with the optimized parameters is performed on the signals shown in figure 6. Figure 8.7 shows the de-noised signals, along with their frequency content obtained using FFT. From the frequency spectrum of signal 2 and 3, it can be seen that the large coefficients around
23.5 KHz are attenuated, with the response below 4 KHz mostly unchanged. From the frequency spectrum of signal 1 and 4, it can be seen that the high frequency noise beyond 5 KHz is attenuated. This indicates that the de-noising process using cross-validation method eliminates high frequency noise from the signal.

Table 8.4: Summary of optimized parameters for de-noising all the three signals

<table>
<thead>
<tr>
<th>Signal</th>
<th>Filter</th>
<th>Decomposition Level</th>
<th>Threshold Strategy</th>
<th>ISE ((x \times 10^5))</th>
<th>Threshold Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal 1</td>
<td>Bior 3.9</td>
<td>3</td>
<td>Hard</td>
<td>7.3402</td>
<td>CV Threshold = (17/20 U_T)</td>
</tr>
<tr>
<td>Signal 2</td>
<td>Bior 3.7</td>
<td>3</td>
<td>Hard</td>
<td>1.7066</td>
<td>CV Threshold = (20/20 U_T)</td>
</tr>
<tr>
<td>Signal 3</td>
<td>Bior 2.8</td>
<td>3</td>
<td>Hard</td>
<td>5.0436</td>
<td>CV Threshold = (19/20 U_T)</td>
</tr>
<tr>
<td>Signal 4</td>
<td>Bior 2.4</td>
<td>2</td>
<td>Hard</td>
<td>4.9811</td>
<td>CV Threshold = (12/20 U_T)</td>
</tr>
</tbody>
</table>
Figure 8.7: (a) De-noised signal 1 (b) Frequency content of de-noised signal 1 (c) De-noised signal 2 (d) Frequency content of signal 2 (e) De-noised: signal 3 (f) Frequency content of de-noised signal 3 (g) De-noised signal 4 and (h) Frequency content of de-noised signal 4
8.3 60 Hz noise removal

The decomposition of the signal using wavelet into approximation (A) and detail coefficients (D) results in division of the frequency content into low frequency and high frequency bands respectively. To filter the power line interference, the high frequency de-noised signal obtained from the CV method is processed by wavelet packets [73]. To remove 60 Hz noise, the coefficients corresponding to a band of frequency containing 60 Hz are set to zero. The wavelet packet decomposition tree for the signal used to filter out the 60 Hz noise is shown in Figure 8.8.
Figure 8.8: Wavelet Packet decomposition tree showing bandwidth in nodes at each decomposition level

As the sampling frequency of the experimental signals is 50 KHz, the original signal contains frequency components ranging from 0 Hz to 25000 Hz. Each level of wavelet packet decomposition divides the range in half; after one level, for example, the sub-band (1,0) has components from 0 Hz to 12500 Hz, and sub-band (1,1) has components from 12500 Hz to 25000 Hz. For adequate removal of 60 Hz noise, enough levels must be done to separate at a relatively small frequency band that contains 60 Hz.
In this case, nine levels are used; the resulting sub-band (9,1) has components from 48.83 Hz to 97.66 Hz. The 60 Hz noise is removed by setting the coefficients of the sub-band (9,1) to zero and reconstructing the signal. Figure 8.9 compares signal 3 after de-noising without and with 60 Hz noise removal.

Figure 8.9: De-noised signal 3 using parameters optimized using cross-validation method (a) with 60 Hz noise and (b) with 60 Hz noise removed using wavelet packets

8.4 Baseline estimation and correction

The measurement signals from Coulter counters often have baseline drift in the measured signal, which can be attributed to the flow variations through the detector channel. Base line drift affects the current through the channel and in turn affects the voltage measured across the sampling resistor. We use wavelet transform to estimate the base resistance $R_c$ and use this base resistance to compute the relative resistance change. The relative resistance change is
\[
\frac{\Delta R_c}{R_c} = \frac{R_c - R'_c}{R_c}
\]  

(8.1)

where \( R'_c \) is the measured channel resistance.

Figure 8.10 shows the resistance signal measured across the sampling resistor converted for one channel with juniper pollen particles. We use level 8 approximation coefficients generated by the bior 2.8 to estimate the baseline resistance \( R_c \). Although, the number of levels is selected by trial and error, it is closely related to the frequency of the drift signal. The resulting signal without a baseline drift is shown in Figure 8.10(b) and Figure 8.10(d).

Figure 8.10: (a) Signal 3 voltage converted to equivalent channel resistance (b) Estimated relative resistance change in the channel due to 12.5 µm CHO cells with baseline drift eliminated (c) Signal 4 voltage converted to equivalent channel resistance (d) Estimated relative resistance change in the channel due to 20 µm Juniper Pollen particles with baseline drift eliminated
8.5 Discussions

A main parameter in measuring the particle concentration is the calculation of number of peaks caused by the particles passing through the micro-channel in one second of time. The number of peaks is calculated by using a threshold peak detection algorithm. It is difficult calculate the number of peaks due to the presence on noise in the obtained signals. So to precisely calculate the peak number, the peak detection/counting algorithm is applied on a de-noised relative resistance change signal.

The threshold for peak detection is decided by calculating particle size from 4-5 individual peaks. These peaks are called as the reference peaks. If these calculated particle sizes are almost similar to the actual size then the threshold value is selected to neglect those peaks which are less than 70% of the size of the reference peaks.
Figure 8.11: (a) Peak detection: De-noised signal and (b) Peak detection: Noisy signal
As seen in Figure 8.11, the threshold value calculated for the de-noised signal is also applied on its noisy counterpart. The peak count for noisy signal is greater than the number of peaks counted for its de-noised version. Thus the counting of fake noisy peaks is almost avoided with the aid of wavelet signal de-noising.
CHAPTER IX

CONCLUSION

In this thesis, we demonstrate a micro-machined multi-channel resistive pulse sensor for sensing/counting of micro-particles. First, we micro-fabricate a multi-channel resistive pulse sensor and test it for leakage; second, by using the micro-machined multi-channel resistive pulse sensor we demonstrate a label free, high throughput, rapid, sensitive and reliable detection and counting of non-biological particle like polystyrene and biological particle like juniper pollen and CHO cells. Finally, we apply a wavelet de-noising method to de-noise the signals obtained from multi-channel coulter counter and thereby increase the sensor’s sensitivity.

The sensor micro-channels are bulk micro-machined on a glass substrate. The micro-channels are etched using a 6:1 (NH₄F:HF) BOE (Buffer oxide etchant) wet etching technique. The measured etch rate for BOE is 0.8 µm/min. The dimensions of the glass micro-channel are 40 µm (depth) x 100 µm (width) x 180 µm (length). The gold electrodes are having a thickness of 0.1 µm and width of 80 µm. The electrode slide and the patterned micro-channel slide is bonded using PDMS glue bonding technique. The device is tested for leakage using a diluted black ink. The leakage test shows a leakage of 3-4 µm sideways which is permissible in our study. The device is extremely compact, and uses sub-micro liter amounts of fluid to perform each measurement.
The use of a micro-machined multi-channel channel Coulter counter for rapid detection/counting of micro-scale particles is demonstrated. Non-biological particles like 30 µm polystyrene particle and biological particles like 20 µm juniper pollen and 12.5 µm CHO cells are tested to evaluate the performance of the sensor. The resistive pulses generated by the polystyrene particles and CHO cells are in positive direction whereas the juniper pollen generates negative resistance pulses. In case of juniper pollen, the ionic concentration of DI water is low and so the surface charge instead of size plays a dominant role (see section 5.2). The experimental results indicate that the sensor is capable of measuring the particle’s size and concentration. Table 9.1 shows the average measured particle diameters and concentration from a single experiment.

Table 9.1: Table of measured particle size and concentration for 30 µm polystyrene particles, 20 µm juniper pollen and 12.5 µm CHO cells

<table>
<thead>
<tr>
<th>Test particles</th>
<th>Channels</th>
<th>Average Measured Diameters</th>
<th>Average Measured Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 µm polystyrene</td>
<td>1</td>
<td>(26.39 ± 1.43) µm</td>
<td>(9.11 ± 1.82) x 10^4 ml⁻¹</td>
</tr>
<tr>
<td>particle</td>
<td>2</td>
<td>(27.22 ± 1.26) µm</td>
<td>(9.26 ± 1.41) x 10^4 ml⁻¹</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>(26.52 ± 1.04) µm</td>
<td>(9.08 ± 2.08) x 10^4 ml⁻¹</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>(27.28 ± 1.31) µm</td>
<td>(9.02 ± 1.54) x 10^4 ml⁻¹</td>
</tr>
<tr>
<td>20 µm juniper</td>
<td>1</td>
<td>(36.03 ± 2.7) µm</td>
<td>(2.14 ± 0.81) x 10^5 ml⁻¹</td>
</tr>
<tr>
<td>pollen</td>
<td>2</td>
<td>(32.81 ± 3.2) µm</td>
<td>(2.06 ± 1.26) x 10^5 ml⁻¹</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>(33.46 ± 1.5) µm</td>
<td>(2.26 ± 1.32) x 10^5 ml⁻¹</td>
</tr>
</tbody>
</table>
Table 9.1: Table of measured particle size and concentration for 30 µm polystyrene particles, 20 µm juniper pollen and 12.5 µm CHO cells (continued)

<table>
<thead>
<tr>
<th>Test particles</th>
<th>Channels</th>
<th>Average Measured Diameters</th>
<th>Average Measured Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 µm juniper pollen</td>
<td>4</td>
<td>(32.78 ± 3.3) µm</td>
<td>(2.10 ± 1.71) x 10^5 ml^-1</td>
</tr>
<tr>
<td>12.5 µm CHO cells</td>
<td>1</td>
<td>(11.34 ± 2.74) µm</td>
<td>(2.23 ± 0.49) x 10^5 ml^-1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>(11.67 ± 3.26) µm</td>
<td>(2.56 ± 0.26) x 10^5 ml^-1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>(10.29 ± 1.52) µm</td>
<td>(2.26 ± 1.02) x 10^5 ml^-1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>(11.01 ± 3.37) µm</td>
<td>(2.44 ± 0.83) x 10^5 ml^-1</td>
</tr>
</tbody>
</table>

The differences in the actual and measured particle size are likely due to the uncertainty of the micro-channel dimensions, electronic noise and the off-axis position when a particle passes through the micro-channel (see section 5.4 for details). A paired t-test is done for the polystyrene particles and CHO cells separately. The null hypotheses in both the cases are rejected which proves that the mean of the absolute difference between the actual and measured size is less than 5 µm for polystyrene particles and less than 4 µm for CHO cells. A paired t-test was performed for juniper pollen particles. The null hypothesis in this case is rejected which proves that there is a significant difference between the actual and measured particle size.

The average measured concentrations from a single experiment for all the tested particles are shown in table 9.1. The reasons for the divergence between the actual and measured particle concentrations are well explained in section 5.4. A paired t-test is
performed to prove that mean of the absolute difference between the actual and measured concentration is less than $0.5 \times 10^4 \text{ ml}^{-1}$ for polystyrene particles and $0.5 \times 10^5 \text{ ml}^{-1}$ for juniper pollen and CHO cells. Since the probability value obtained from the test is way less than 0.05, the null hypothesis is rejected. Thus the aim of the test is achieved.

Furthermore, a cross-correlation analysis is performed between the signals obtained from each channel of the multi-channel sensor. The cross-correlation coefficients ‘r’ is less than 0.1, indicating that there is negligible correlation between the signals of different channels. A correlation test is performed to statistically prove that the correlation coefficient between the different channels of the sensor is less than 0.1. The null hypothesis in this case is rejected implying that the four channels are able to simultaneously detect and count particles with negligible crosstalk among them. Thus we demonstrate that the micro-machined multi-channel sensor increases the throughput by approximately 300% as compared to the single channel sensor.

Considering the fact that the signals obtained from the multi-channel resistive pulse sensor are affected by noise, the signal de-noising using wavelet transform is demonstrated. The wavelet selection, decomposition level and the threshold value for DWT de-noising is optimized based on the cross-validation procedure to remove noise occurring in the high frequency range. Bior 2.8 (wavelet number 25) gives low values of ISE for all the four signals and can be considered as the best overall choice amongst the wavelet filter. The decomposition levels 2-4 are the best choice of levels to be considered for de-noising the coulter counter signals. Using the hard threshold policy, we not only filter the high frequency noise but also achieve negligible attenuation of the signal. Additionally, the 60 Hz noise is successfully removed from signal 3 using the wavelet
packet algorithm. The baseline estimation is used in the computation of the relative resistance change across the micro-channel. By using estimated baseline, the baseline drift is removed from the signals 3 and 4 successfully. The wavelet method presented here would aid in improved and faster analysis of data obtained from multi-channel Coulter counters.

In general, we demonstrate that a micro-machined multi-channel resistive pulse sensor based on coulter counter principle is capable of performing a label free detection of the bio-particle. It also enhances the overall throughput without sacrificing the detection sensitivity, reliability and accuracy. Further it is also described that the noise induced in the coulter counter signals is successfully removed by an algorithm based on wavelet transform technique which helps in improving the performance of the sensor.
Detection and differentiation of bioactive particles based on the shape characteristics of the pulse is a foreseeable extension of this thesis. In previous chapters, we showed the possibility of using multi-channel sensor to differentiate pollen particles and polymer particles with similar sizes in DI water. The differentiation is based upon the difference of polarity and magnitude of resistive pulses generated by particles with different surface properties.

However, many bioactive particle including cells can only survive in the culture solution. It is not always feasible to differentiate bioactive particles in much diluted electrolyte solution. For example, CHO cells can only survive in a CHO culture media. An attempt to differentiate CHO cell and 20 µm pollen is unsuccessful because both particles generate positive resistive pulses. This is because when the ionic concentration is high, the size of the particles instead of its surface charge plays a dominant role thereby both particles generate positive resistive pulses.
One possible way to differentiate is by applying a pattern recognition algorithm [74] which recognizes a particular peak shape generated by a particle as it passes through the micro-channel. The pattern reconciliation is feasible because the particles with different shapes, surface properties and mobility will certainly create different resistive pulses in terms of height, polarity, pulse width and shapes as shown in Figure 10.1. In the future work, our research group will work along this direction. To obtain a well defined peak shape for a particle, experiments needs to be performed at low flow rate and high sampling frequency (>200 kHz).
The sensitivity and the inherent specificity can be improved by using biorecognition antibodies [41]. As shown in Figure 10.2(a), to achieve the best specific sensing for bio-particles, it is necessary to specifically bind the antibody to a target bio-particle. The interaction between the bacteria and the probe antibody is specific; therefore the size, mobility and surface property will be changed. As shown in Figure 10.2(b), the changes will be reflected in the peak shape of the Coulter counting signal and be used as the identification for a particular bioactive particle such as cell and bacteria.
REFERENCES

1 Sridharamurthy S., Dong L., and Jiang H. 2007 “A micro-fluidic chemical/biological sensing system based on membrane dissolution and optical absorption”, Measurement Science and Technology 18, 201-207


16 Coulter W. 1953 Means for counting particles suspended in a fluid US Patent no 2656508


24 DeBlois R., and Wesley R. 1977 “Sizes and concentration of several type C oncornaviruses and bacteriophage T2 by the resistive-pulse technique”, *J. Virol.* 23 (2), 227-233

25 DeBlois R., Bean C., and Wesley K. 1977 “Electrokinetic measurements with submicron particles and pores by the resistive pulse technique”, *Journal of colloid and interface science* 61 (2), 323-335


30 Bezrukov S. 2000 “Ion channels as molecular Coulter counters to probe metabolite transport”, *Journal of Membrane Biology* 174 (1), 1-13

31 Bayley H., and Martin C. 2000 “Resistive-Pulse Sensing-From Microbes to Molecules”, *Chemical reviews* 100 (7), 2575-2594


34 Akeson M., Branton D., Kasianowicz J., Brandin E., and Deamer D. 1999 “Microsecond time-scale discrimination among polycytidylic acid, polyadenylic acid, and polyuridylic acid as homopolymers or as segments within single RNA molecules”, *Biophysical journal* 77 (6), 3227-3233


43 Ito T., Sun L.,and Crooks R. 2003 “Simultaneous Determination of the Size and Surface Charge of Individual Nanoparticles Using a Carbon Nanotube-Based Coulter Counter”, Analytical chemistry 75 (10), 2399-2406


46 Jagtiani A.; Sawant R.; and Zhe J. 2006 “A label-free high throughput resistive-pulse sensor for simultaneous differentiation and measurement of multiple particle-laden analytes”, Journal of Micromechanics and Microengineering 16 (8), 1530-1539


48 Ehrentreich F. 2002 “Wavelet transform applications in analytical chemistry”, Analytical and Bioanalytical Chemistry 372 (1), 115-121
49 Weidong C. 2003 “Discrete wavelets transform for signal de-noising in capillary electrophoresis with electrochemiluminescence detection”, *Electrophoresis* 24 (18), 3124-3130

50 Liu F. 2003 “Signal de-noising and baseline correction by discrete wavelet transform for microchip capillary electrophoresis”, *Electrophoresis* 24 (18), 3260-3265

51 Perrin C. 2001 “The Use of Wavelets for Signal De-noising in Capillary Electrophoresis”, *Analytical Chemistry* 73 (20), 4903-4917

52 Pasti L. 1999 “Optimization of signal de-noising in discrete wavelet transform”, *Chemometrics and Intelligent Laboratory Systems* 48 (1), 21-34


55 Wu H., Huang B., and Zare R. 2005 “Construction of micro-fluidic chips using polydimethylsiloxane for adhesive bonding”, *Lab on a Chip* 5, 1393-1398


58 Saleh O. 2003 “A novel resistive pulse sensor for biological measurements”, *PhD Thesis*


64 Sisbot S. 2005 “A cross-correlation technique as a system evaluation tool; application to blood flow measurement in extra-corporeal circuits”, *Flow Measurement and Instrumentation* 16 (1), 27-34

65 Morrenhof W., and Abbink J. 1985 “Cross-correlation and cross talk in surface electromyography”, *Electromyography and Clinical Neurophysiology* 25, 73–79


