BACHELOR ASSIGNMENT

THE RESISTIVE-PULSE TECHNIQUE AS A METHOD FOR DETERMINING SPERMATOZOA MORPHOLOGY

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ABSTRACT

To improve spermatozoa analysis for the livestock industry a novel method using microfluidic systems is developed. The resistive-pulse technique is a microfluidic system that has been used for detecting and sizing particles passing through a micropore but not yet for determining the exact shape of a particle. To determine the morphology of spermatozoa using the resistive-pulse technique, this study evaluates three methods for making a suitable micropore design that differentiates the resistive pulses of different shaped spermatozoa. The first two methods are based on mathematical models that approximate the behaviour of the resistive-pulse system and they show that information about a particle morphology can be found in the slope of its current pulse. The third method uses current pulses from simulations to show that short constrictions in a micropore can differentiate particles with the same volume but a different shape better than constrictions of greater length. The results from this study could help in developing a technique that can compete with computer aided sperm analysis currently in use.

CONTENTS

1 INTRODUCTION

Artificial insemination (AI) is used in the livestock industry worldwide. With 90% of the pigs and dairy cattle bred with artificial reproductive technologies, it is the most used form of reproduction in the European Union. AI was first introduced to decrease the spread of disease, the need of transporting animals for breeding was removed and sperm could be treated with antibiotics. Now AI is also used to select the specimen with the best semen and pre-process the semen in such a way that the highest chance of fertility can be guaranteed [1]. Spermatozoa are selected on morphological criteria that, if abnormal, are an indication of a decrease in fertility. Common defects include spermatozoa without a head, short, coiled or kinked tails, cytoplasmic droplets, corkscrewed mid pieces and/or acrosomal deformation. The first selection process was based on using samples of an ejaculate that a technician could observe using light microscopy [2]. The drawback of this method is the cost due to the need of a trained lab professional and it is prone to subjective observations. With the rise of CASA, computer-aided sperm analysis, computer technology has become the standard for objectively gathering information about sperm motion and morphology. These systems have reduced the time to analyse sperm significantly and can more accurately and objectively describe the quality of a sperm sample. While this technique looks at spermatozoa individually, it can only give an average quality of a sample. If this quality is below a certain threshold that whole sample is discarded even if viable spermatozoa are still present [3]. Therefore it is explored to use microfluidic systems to analyse and sort spermatozoa individually as seen in the work of Segerink et al. [4] and De Wagenaar [5]. This technique promises to improve the quality and yield of semen and due to the ease of parallelisation of microfluidic systems high throughput can be guaranteed.

The Coulter counter, or resistive-pulse technique, is a microfluidic system that is commonly used to count and size the volume of particles. The setup consists of two reservoirs filled with particles suspended in an electrolyte, like PBS, connected by a micropore. A voltage is applied over the micropore by two electrodes suspended in the reservoirs as seen in figure 1.1. The resistance of the micropore can be determined by measuring the current flowing between the electrodes. When a particle moves through the micropore it dipslaces its volume of electrolyte, therefore the electrical resistance of the micropore changes. DeBlois and Bean [7] made a simplified analysis of the change in resistance when a spherical particle transverses trough a

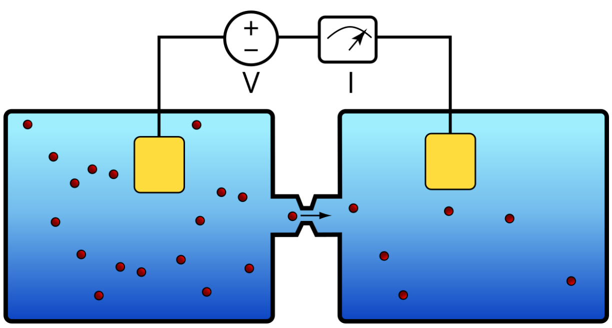


Figure 1.1: Schematic diagram of resistive-pulse sensing, in which particles flow through a micropore and are measured by electrodes placed on either side of the pore. [6]

micropore of constant width.

$$\Delta R = \frac{4\rho_m d^3}{\pi D^4} \tag{1.1}$$

ρ_m is the resistivity of the medium and the change in resistance is proportional to the cube of the particle diameter (d) and inversely proportional to the fourth power of the pore diameter (D). This only holds for particles that have a much smaller diameter than the pore diameter. For pores with particles in the same size order the expression for the change in resistance is as follows:

$$\Delta R = \frac{D}{L}(\frac{sin^{-1}(\frac{d}{D})}{\sqrt{1-\frac{d}{D}^2}} - \frac{d}{D}) \tag{1.2}$$

Pevarnik et al. [8] looked at the resistive pulses from micropores with non-uniform width. They saw that from the resistive pulses the structure of the pore could be determined when spherical particles move through it. They and Qiu et al. [9] proposed to use pores with longitudinal irregularities in the resistive-pulse technique to study morphology of particles because it is impossible to determine particle morphoplogy by using micropores of constant width.

The goal of this work is to answer the question: What is a suitable pore structure for determining the morphology of spermatozoa with the resistive-pulse technique? The micropore must be shaped in such a way that the morphology of different spermatozoa can be identified from their unique resistive pulse. To achieve this goal the system is first described with two mathematical models. The idea is to calculate the micropore structure with a healthy spermatozoa morphology and a wanted resistive pulse. Then, this could be applied to calculate back from a measured resistive pulse and the known micropore wall structure to the morphology of different spermatozoa passing through the pore. The first model uses the convolution theory based on Papadimitriou's work [10] and is evaluated in chapter 2. The second model is explored in chapter 3 and models the system as a series of electrical resistances. In chapter 4, two wall structures are experimentally evaluated using COMSOL simulations. Every chapter is individually discussed. A global conclusion is given in chapter 5. This work closes with recommendations for future research in chapter 6.

2 CONVOLUTION

Papadimitriou [10] introduced convolution as a method to model the resistive pulse of a micropore with a non-constant width. This model could be the key for determining spermatozoa morphology by deconvoluting a measured current response with the known micropore wall structure to get a function of the particle shape. In this chapter I will compare results from simulations with convolution calculations to determine if the convolution method is suitable for modelling a resistive-pulse sensing system.

2.1 Background

Convolution is a mathematical operation between two functions and for functions with respect to x is defined by equation 2.1.

$$y(x) = (f \otimes h)(x) = \int f(\chi)h(x - \chi)d\chi \tag{2.1}$$

A concise definition of convolution is given by Smith [11]: "The convolution formula can be described as a weighted average of the function $f(\chi)$ at position x where the weighting is given by $h(-\chi)$ simply shifted by amount x. As x changes, the weighting function emphasizes different parts of the input function." So, convolution describes how the shape of a function is altered by another function.

Papadimitriou showed that the convolution of a wall structure with a particle shape reasonable resembles the current response from simulations. He also showed that differences in particle shape made different pulse shapes when moved through a micropore with a complex structured wall [10]. To see how a micropore can be modelled with convolution first the current pulse needs to be defined. Looking at a micropore with constant width filled with medium the current response of it can be written with Ohms law.

$$I = G\Delta U \tag{2.2}$$

Here $G[S]$ is the conductance and $\Delta U[V]$ is the applied voltage difference over the micropore. The conductance described here is proportional to the cross-sectional area, $A[m^2]$, of the micropore.

$$G = \sigma \frac{A}{L} \tag{2.3}$$

The conductivity of the medium inside the micropore is given as $\sigma[Sm^-1]$ and $L[m]$ is the length of the micropore. In the case of a micropore which does not have a uniform width, the cross-sectional area varies along the x-axis. Thus, becomes the function $A(x)$. If the particle moving through the micropore is fully insulating it blocks parts of the local area. This can be expressed in the blocking factor γ where the particle area is subtracted from the local micropore area and divided by the local area.

$$\gamma = \frac{A_m - A_p(x)}{A_m} \tag{2.4}$$

The subscripts m and p describe the micropore and particle respectively. This blocking factor has a value between 0 and 1, where 0 is completely blocking the channel and 1 is no obstruction in the micropore at all. The particle is moving through the micropore thus is also a function of x. With this definition of a blocking factor the conductance of the micropore can be described analogous to the definition of convolution.

$$G(x) = \frac{\sigma}{L} \int_0^L A(\chi)\gamma(x-\chi)d\chi \tag{2.5}$$

The current response from equation 2.2 can now be rewritten as:

$$I = \frac{\Delta U \sigma}{L} A \otimes \gamma \tag{2.6}$$

A property of convolution is that it is reversible [11]. This means theoretically a measured current response can be deconvoluted with the shape of the micropore to get the blocking factor of the particle. With this blocking factor and the shape of the micropore the function of the particle shape could be determined.

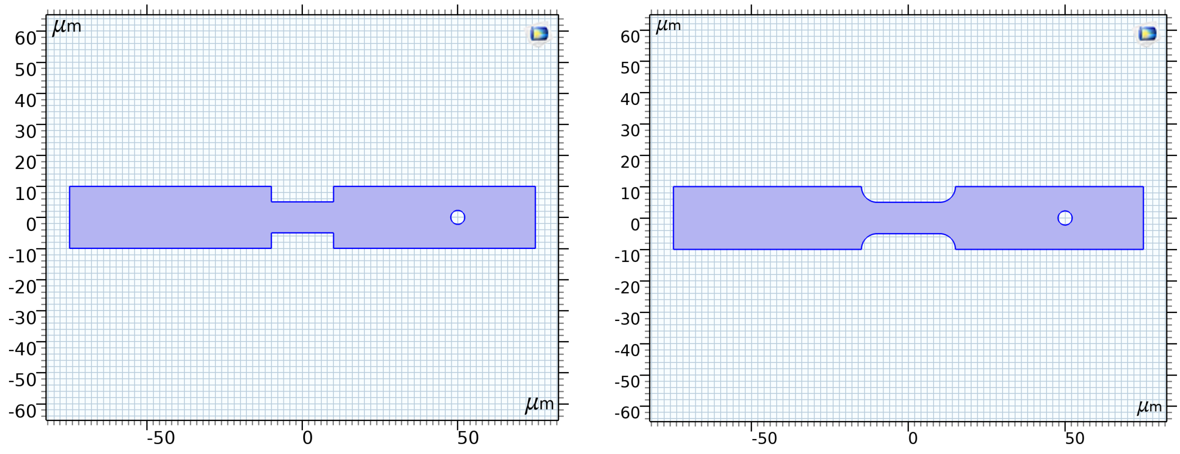
2.2 Method

Two different micropore structures are used to test the convolution theory. Both micropores are $150\mu m$ long and $20\mu m$ wide with one narrow section of $10\mu m$. Micropore 1 has indents on both sides which are rectangular and $20\mu m$ long. Micropore 2 has the ends of the indents extended with two round edges with a radius of $5\mu m$ which gives the narrow section a length of $30\mu m$. To be in the same range as spermatozoa a spherical particle of $4.5\mu m$ in diameter is send through the pore. The micropore is filled with a medium that resembles Phosphate-buffered Saline (PBS) with a conductivity of $1.4[Sm^-1]$. These two setups are made into a 2D model in COMSOL as seen in figure 2.1 and they are simulated with the electric currents physics module. For ease of calculation and because joule heating is not taken into account in this model, a potential of 1 Volt is applied with the positive terminal at the right end of the micropore. With the centre of the channel at $x = 0$, the particle starts at $x = -50\mu m$ and moves for $100\mu m$ to $x = 50\mu m$. The particle movement is divided in 500 data-points, which means $0.2\mu m$ per step. It is chosen to start the particle already in the pore to keep distance between the particle and the terminals at the end of the pore to prevent edge effects. The assumption is made that the particle moves along the middle of the channel and is fully insulating.

MATLAB R2020a is used to make the convolution calculations. The top half of the 2D models and half of the particle are converted to discrete functions with a stepsize of $1nm$. This can be done because the micropores and particle are symmetric in their x-axis. The results of half the setup can be doubled to get the results of the whole channel. The channel and particle are also symmetric around their y-axis and therefore, the reversal with convolution will not be a point of error. The convolution is implemented with the 'input first' method described by Smith [11]. The MATLAB code for making the functions is found in section A.1 and the code for convoluting the function can be found in section A.2.

2.3 Results

Figure 2.2 shows the results of the simulation with micropore 1 (figure 2.1a) in COMSOL. Decrease in current can be seen when the particle begins to approach and enters the narrow part of the pore. The lowest current measured with the simulation is $0.1535A$. The maximum current measured through the micropore is $0.1589A$. This corresponds to a current reduction of 3.4% and a resistance increase of 3.5%.



(a)

(b)

Figure 2.1: The 2D models made in COMSOL: (a) Micropore 1 with the square indents (b) Micropore 2 with the indents with rounded sides

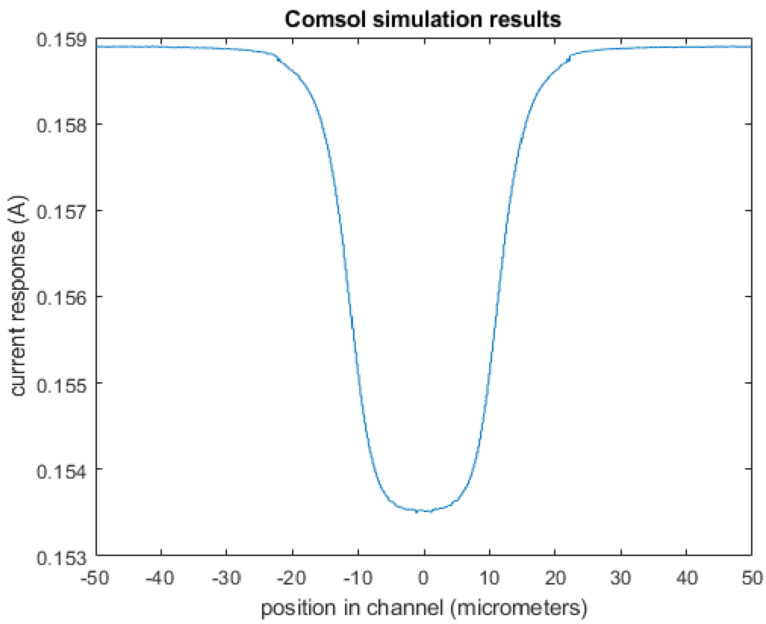


Figure 2.2: The current response of the particle moving through micropore 1 in the COMSOL

With the convolution done in MATLAB the results are depicted in figure 2.3. The maximum current calculated is $691.75A$ and the minimum is $271.7A$. This means a current reduction of 61%. These high current values are due to the fact that the convolution is multiplied by $\frac{1}{L}$ with a micropore length of $L = 150\mu m$. If this factor is neglected the maximum current would be $104mA$ and the minimum current would be $41mA$. What is also notable about these results is that the slope is exactly the length of the particle, $4.5\mu m$.

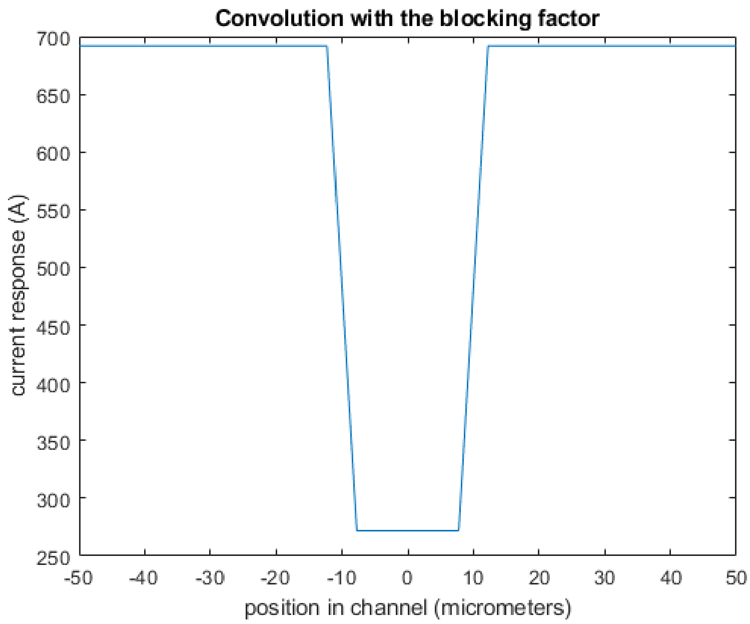


Figure 2.3: The current response of the convolution with micropore 1 and the round particle calculated as described in equation 2.6 using MATLAB

Because the absolute values are not in the same range the above mentioned results are normalized. With their maximum value on 1 and their minimum value on 0, the current change with respect to the position of the particle can be compared (figure 2.4). The convolution calculation certainly has much sharper changes in comparison to the simulation results. Also the simulation current begins to drop a lot earlier than the calculated current response. This happens because COMSOL accounts for the curving of the electric field around the particle and the indents in the pore wall. When the particle approaches the pore, the area of the pore in front of the particle, looking in the x-direction, does not change. But the area that the electric field can pass through between the indent and the front of the particle gets smaller. This higher electric field density signifies a local increase in resistance and therefore a decrease in current flowing through the micropore before the particle is even in the constriction.

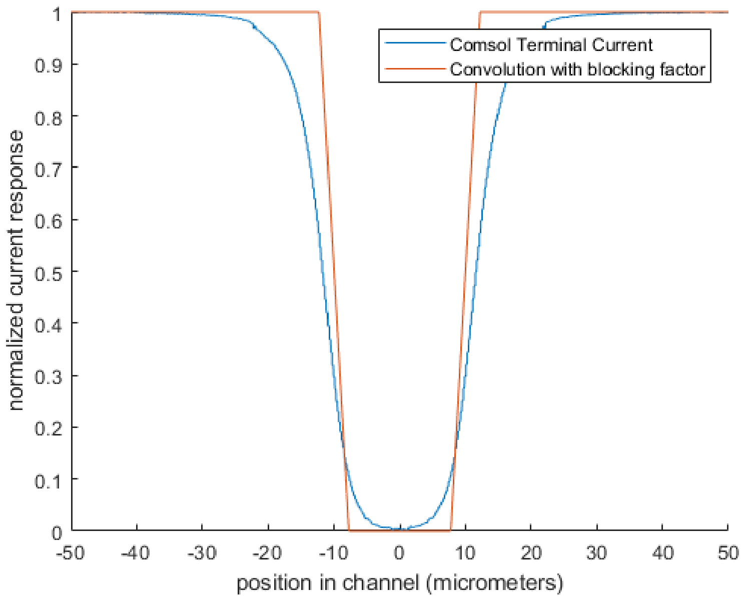


Figure 2.4: The normalized current response of both results shown in figures 2.2 and 2.3

To account more for the curving of the electric field, micopore setup 2 is used. In figure 2.5 it can be observed that the calculation now also follows a little more rounded path. But as seen in the previous results the simulations still has a much earlier drop in current than the calculated current response.

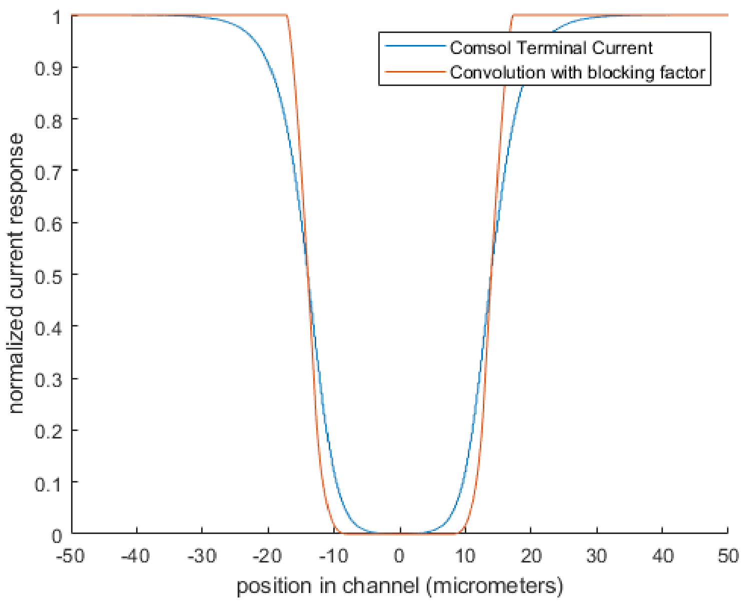


Figure 2.5: The normalized current response of the simulation and convolution of micropore 2

2.4 Discussion

The results show that there is a big difference between the absolute values of the calculated current response and the simulated response. This raises the question if the simulation is in the right direction. The simulation has been verified by changing the width of the constriction in micropore setup 1 from $10\mu m$ wide to $0.2\mu m$ wide, the steps taken were $0.1\mu m$. This setup is compared to a variable resistor in a circuit with 3 resistors in series, two represent the wider parts at the beginning and the end of the micropore and the variable resistor represents the constriction of the pore. With only a maximum absolute deviation of 1.8Ω the comparison proved that the simulation is in the right current range and not the convolution calculation.

While the absolute values are not comparable the changes in the pulse can be evaluated. The expected change in resistance can be determined using the analysis from DeBlois and Bean given in equation 1.2. Taking the constriction in the pore as an individual micropore with a diameter of 10μ and length of 20μ an estimation can be made for the change in resistance. This gives an increase in resistance of 3.6% which is very close to the increase in resistance seen with the simulations.

Also, when just the slopes of the convolution model are compared with the simulation in figure 2.4 and figure 2.5, the convolution model has not proven to be accurate. But it has showed that a morphology change shows up in the slope of the current response. This can be seen by the change in slope when comparing the calculated current response from micropore 1 and micropore 2. Where the result from micropore 2 has a more rounded slope when it also has indents with rounded edges. This difference in slope between the convolution model and the simulation can be explained by the fact that the convolution model assumes a uniform electric field moving only parallel to the x-axis. COMSOL accounts for the interactions that the electric field lines have with the particle and channel wall as explained in the results.

3 SERIES OF RESISTANCES

The series of resistances model is already briefly used in the discussion of the convolution model to see if the absolute values of that model and the simulations were in the right direction. This simplification is also used by DeBlois and Bean [7] to verify maximum deviations and sensitivity. In this chapter it is explored if this model can be used to determine particle morphology. This is done by comparing the shape of the current curve calculated with the earlier presented simulations.

3.1 Background

The current response of the micropore will for this model be described with the resistance instead of the conductance as previously done in equation 2.2.

$$I = \frac{\Delta U}{R} \tag{3.1}$$

The resistance here is proportional to the cross-section of the micropore and is given with:

$$R = \frac{L}{\sigma A} \tag{3.2}$$

When the area of the pore changes with respect to x it can be cut up in an infinite amount of resistances in series. If the current distribution through the micropore is taken to be uniform, equation 3.2 can be changed to an integral.

$$R = \frac{1}{\sigma} \int_0^L \frac{1}{A(x)} dx \tag{3.3}$$

If the particle that moves through the micropore is taken to be fully insulating the area of the particle can be subtracted from the local pore area to get the total area of the medium that the current can pass through in that part of the micropore. If the function is made discrete by cutting up the micropore in n amount of pieces, the resistance of the whole micropore with the particle becomes a summation of those pieces.

$$R(x) = \frac{L_n}{\sigma} \sum_0^n \frac{1}{A_m(n) - A_p(n-x)} \tag{3.4}$$

Here L_n is the length of one discrete part, $A_m(n)$ the local area of the pore in that part and $A_p(n-x)$ is the particle area in that part shifted by the amount x that it is in the channel. This equation does not account for any interactions of the electric field with the particle or pore wall.

3.2 Method

As described in section 2.2 the model will be evaluated with two micropore setups. Micropore 1 with square indents and micropore 2 with indents with rounded edges. Both setups are shown

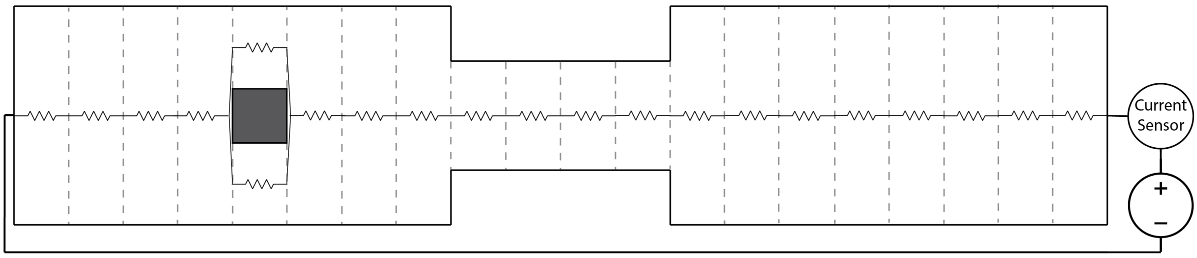


Figure 3.1: A visualisation of how a micropore with a constriction and square particle is divided in pieces that each have their on resistance in an equivalent electric circuit

in figure 2.1. The particle moving through the pore is again taken to be a sphere with diameter of $4.5\mu m$. The setups are modelled in 2D in COMSOL and simulated using the electric currents physics module. The models are evaluated with the particle moving from left to right starting at $-50\mu m$ and stopping at $50\mu m$ with the middle of the channel on $0\mu m$. The particle is moved in steps of $0.2\mu m$ which gives 500 data-points. This is consistent with the COMSOL simulation done in the previous chapter about the convolution method.

A simple visualisation of the calculation method can be observed in figure 3.1. The schematic outline of the method depicts a micropore with a square particle which is divided in 21 pieces. Each division is modelled as an individual resistor in a network of resistances. The calculations for this method are done using MATLAB R2020a. The discrete functions of the micropore profile and particle from previous chapter are used to calculate the area profiles. To only calculate in 2D like the simulation, the depth of the setups is taken to be one. The micropore is divided in $n = 150 \times 10^3$ pieces. Every section of the micropore that will be seen as a separate resistance is therefore $1nm$ long. The code used to calculate the resistance network can be found in section A.3.

3.3 Results

The current response calculated with the series of resistances method for the micropore with a square indent can be observed in figure 3.2. The maximum current calculated is $0.1638A$ and the minimum current is $0.1598A$. This gives a difference of 2.5% between the minimum and maximum current values which is smaller than the difference of 3.5% seen with the simulation in figure 2.2.

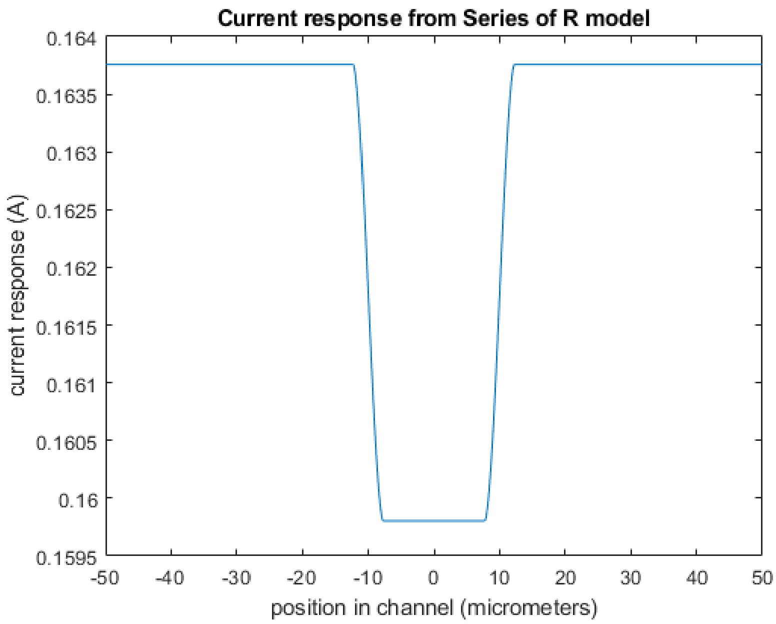


Figure 3.2: The current response of the series of resistances calculation for the setup with micropore 1

The simulated current response and the response calculated with the series of resistance model are plotted normalized in figure 3.3. It is observed that the transitions in the calculated response are far sharper than the response from the simulation. It can be noted that the slopes of the calculated current response are $4.5\mu m$ wide, which is as wide as the particle. The slope also has a small curve which reflects the round shape of the particle.

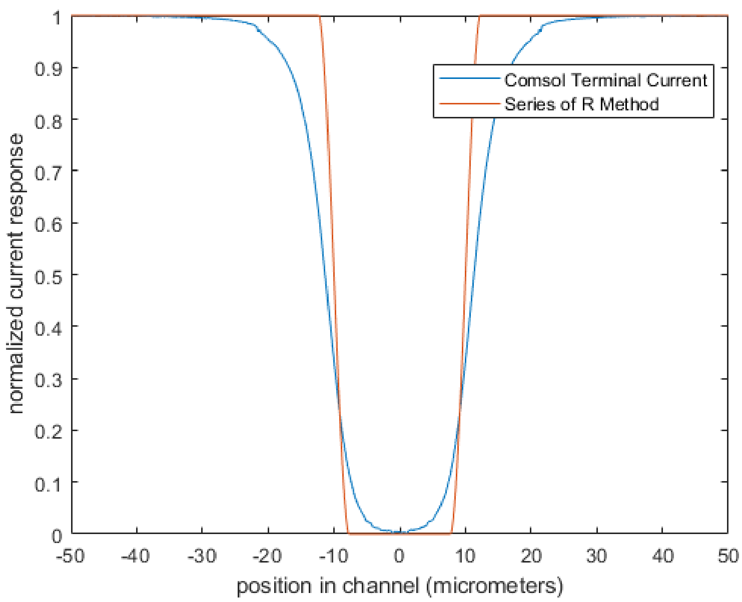


Figure 3.3: The normalized current response of the COMSOL simulation shown in figure 2.2 and the calculation in MATLAB from figure 3.2

For the setup with micropore 2 the results are shown normalized in figure 3.4. It is observed that the calculated response does have even more curved slopes when the pore also has indents with round edges compared with the results in figure 3.3. The slope is now also $5\mu m$

wider, which is exactly the radius of the added round edge to the indent. The simulation results still show a much earlier drop in current in comparison with the calculation.

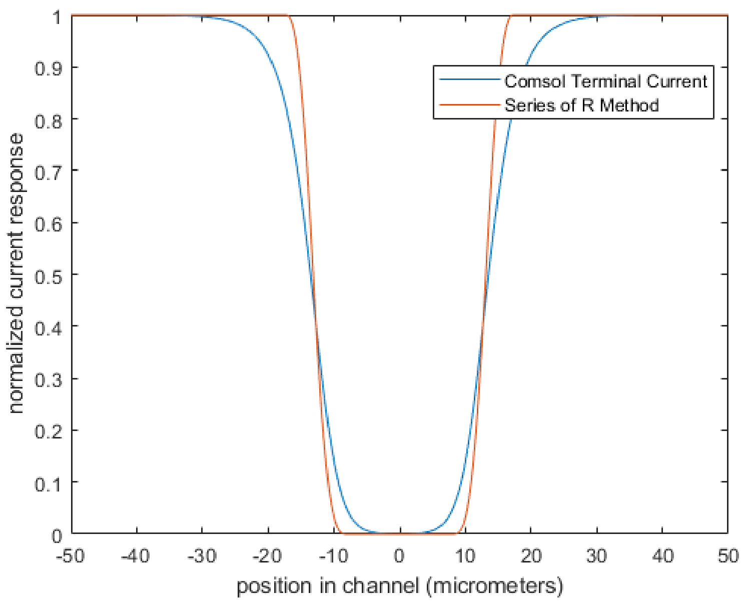


Figure 3.4: The normalized current response of the simulation and series calculation for setup with micropore 2

3.4 Discussion

While the absolute values calculated are in the same order of magnitude, the difference between the minimum and maximum values is observed to be smaller in the calculations. This is an implication of formulating the series of resistance model for a uniform electric field in the pore. In reality the electric field is denser in the constriction which makes the conductivity of the constriction lower than the surrounding micropore. Due to the limitation of using a uniform electric field for this method also the shape of the curve does not correspond to the simulation.

The practical limit of this model, if it were to be used to analyse the morphology of spermatozoa, is due to the summation of the resistances. Just a current response can not be broken down in its equivalent series of resistances circuit. Calculating a micropore structure from a particle morphology and a wanted resistive pulse, or calculating the particle morphology from a measured current pulse and the known pore structure is not possible. It would be possible to use the model to make a data-set of current pulses of different particle morphologies moving trough a chosen micropore to compare measured current pulses against. If the model were accurate enough a certainty could be assigned of what particle morphology produced a certain current response. But because this model in its current state is not accurate enough the use of simulations for making a data-set is evaluated in the next chapter.

4 SIMULATION

The mathematical models are explored to eventually calculate back from a measured current response to a particle morphology. This proved to be a bigger challenge than initially thought because of the uniformity of the electric field needed in these models and therefore introducing discontinuities in the mathematical methods. It is explored if simulations can give some information on how a micropore must be structured to distinguish different particle morphologies and if the possibility exists for building a data set out of simulated current responses with which measured responses can be compared.

4.1 Background

Pevarnik et al. [8] made the observation that the size of the particle had a significant influence in distinguishing the shape of a pore using the resistive pulse technique. For larger particles the differences in measured current are bigger because they move more medium in the channel. They found that smaller particles have a higher spatial resolution than the bigger particles. But due to the smaller current differences noise becomes much more prevalent. In their setup the pore structure was unknown and they changed the particle size. In this work the particle morphologies are unknown, therefore the claim can be observed if a shorter constriction gives more resolution than a longer constriction.

The models discussed in chapter 2 and 3 have given insight in the fact that the information about the particle morphology is present in the slopes of the current response. Also it could be seen that gradual transitions in the pore wall increased the slope length of the current pulse. In this section it will be evaluated if the slopes of the current response again changes with a different particle morphology and if this is significant enough to distinguish particle shapes.

4.2 Method

Two 2D micropores are made in COMSOL. Both micropores are $150\mu m$ in length and $20\mu m$ wide. The first micropore has an indent of $5\mu m$ on both sides. The indent begins with a 45 degree slope, has a flat piece of $20\mu m$ long and ends in another 45 degree slope widening the channel again to $20\mu m$. The length of the flat piece is chosen to be longer than the longest particle to separate the change in current when the particle moves into the constriction and when it moves out of the constriction. The second micropore also has an indent of $5\mu m$ on both sides but only consists of two 45 degree slopes such that they form a sharp point to the middle of the channel. The 45 degree slopes by the indents are used to increase the slope of the current response which will give a broader range of data-points to observe possible differences. Through the pores three different particles are transferred which are all fully insulating. Each particle begins at $-30\mu m$, moves to the right and stops at $30\mu m$ from the middle. This is evaluated for 500 datapoints which means a stepsize of $0.12\mu m$. The first particle is a circle with a diameter of $4.5\mu m$, the second particle is a square with the same area as the circle (sides of $3.99\mu m$) and the last particle is an ellipse with the same dimensions as a boar sperm head (length of $9\mu m$

and width of $4.5\mu m$ [12]). In figure 4.1 both micropores are shown and all three particles can be seen in each pore.

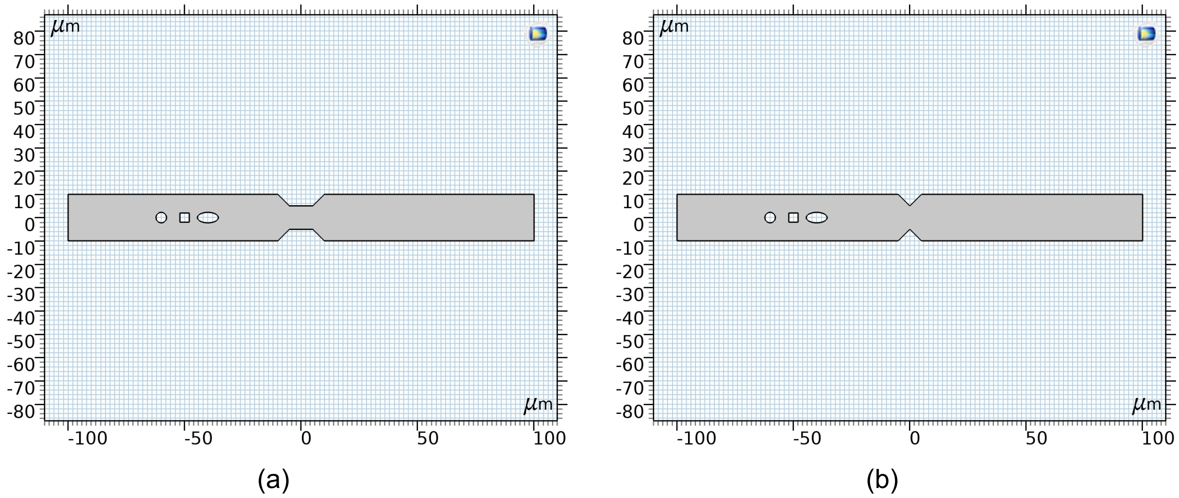


Figure 4.1: The 2D models made in COMSOL: (a) Micropore 1 with the long constriction (b) Micropore 2 with the sharp point as constriction

4.3 Results

The current response from the three particles through the first micropore is shown in figure 4.2. The ellipse with the bigger area, so more displacement of medium, is clearly distinguishable from the particles with the same area. It has a minimum difference of $0.51mA$ and a maximum difference of $2.3mA$ with the round particle. The square and round particle seem to have almost the same shape and absolute values with a maximum difference in current response of $0.11mA$. What is noticeable between the current response of the circular and square particle is that the square particle seems to have a lot more inconsistencies in the current values, especially when it is in the narrow part of the micropore.

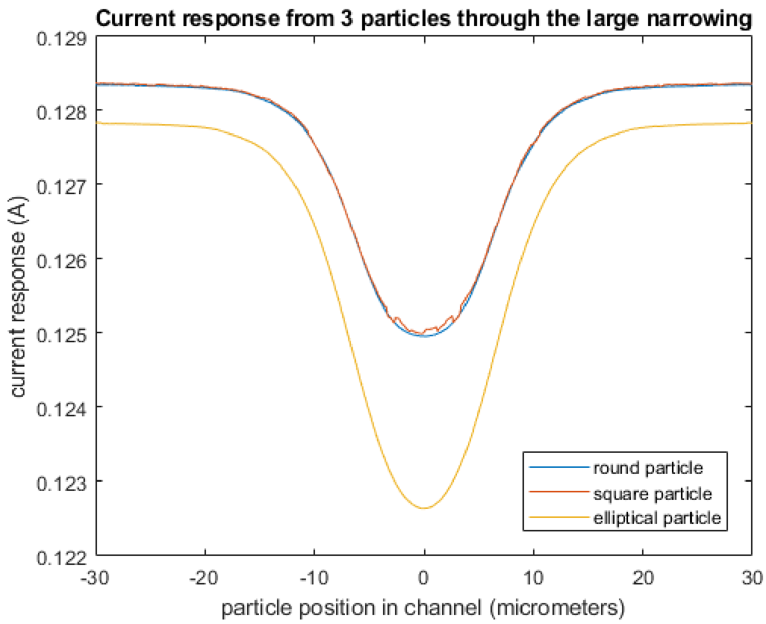


Figure 4.2: The current response of three different particles moving through the first micropore with the long constriction simulated in COMSOL

When the particles move through the second micropore the current responses are as shown in figure 4.3. The separation between the elliptical particle and the circular particle is still very significant with a minimum difference of $0.55mA$ and a maximum difference of $1.2mA$. The separation between the particles with the same area is now also more distinguishable with a maximum difference of $0.18mA$. The difference is also more noticeable across the whole curve instead only in the valley of the response. It can also be noted that for the square particle the current starts decreasing when it's later in the channel than the round particle. Overall the current responses wit this setup shows more sudden jumps compared with the pulses shown in figure 4.2.

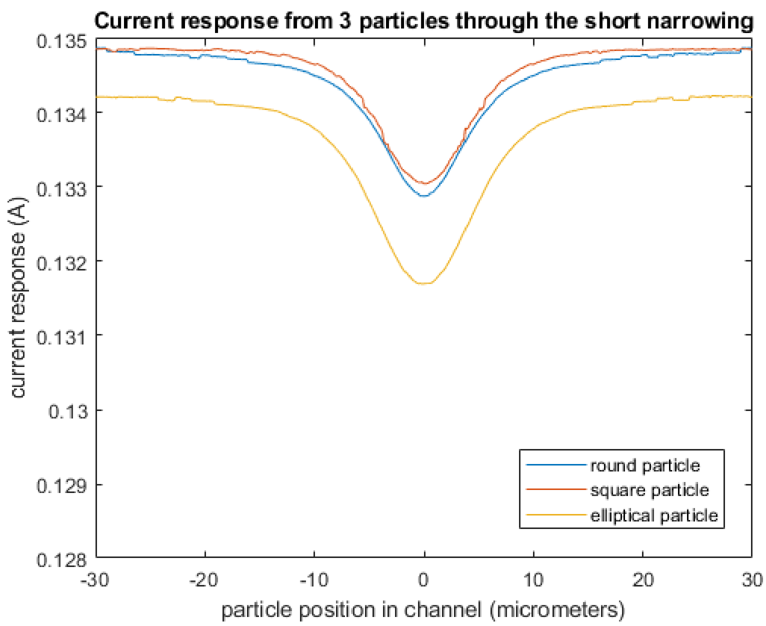


Figure 4.3: The current response of three different particle moving through the second micropore with the peak constriction simulated in COMSOL

4.4 Discussion

Looking back at the statement of Pavernik et al. that says there is a resolution difference between small and big particles, the same can be said about the constriction in the channel looking at the results shown. With a longer constriction the individual particles have bigger differences in their current response. The constriction that consists of a point differentiates the two particles with the same area but different morphology better.

The claim made in the background section that the slopes give the most information about the particle can not be evaluated. The begin of the current decrease can be an indication of the particle length if the current response from the elliptical and round particle are compared in the first and second setup. When the slopes of the square and round particle are compared only the steepness of the start of the curve differs in the second setup.

For the setup with the second micropore also the lowest current value differs between the square and the round particle. Since they are the same area, the particles displace the same volume of medium and do not differ in lowest current value for the setup with the first micropore. For the second micropore it can be explained that due to the use of a 2D simulation and an infinitely short constriction, only the height of the particle matters in blocking the constriction of the pore. The maximum height of the cube is always smaller than the maximum height of the circle and thus decreases the resistance of the micropore less than the circle.

The current pulses from the setup with the second micropore has shown to have more inconsistencies. It is thought that this happens because in theory the points of the indents are infinitely small and the simulation can not evaluate a consistent mesh around these points. The mesh in the simulation is used to divide the setup in sections for which all the physics equations can be evaluated. Because of this inconsistent mesh it shifts with some movements of the particle and therefore can have inconsistencies in the calculated current. These inconsistencies can be taken analogous with the increase in noise Pevarnik et al. observed when smaller particles were used to determine the structure of a micropore.

One limitation with a real application is these simulations do not take into account that in microfluidic systems spermatozoa are moved through the channel by means of laminar flow. This would cause the spermatozoa to speed up when the pore narrows and slow down when it widens again. These simulations are done with respect to the x position of the particle and not with respect to time as measured current responses would be.

5 CONCLUSION

To achieve the design of a suitable micropore for determining the morphology of spermatozoa, three different methods have been evaluated in this study. Two methods relied on a mathematical model. The idea was to first model the system and use that to calculate a micropore structure from a healthy spermatozoa morphology and desired resistive pulse. The first method for modelling the system used convolution and is based on the work of Papadimitriou [10]. It was shown that the pulses of this model did not approach simulations results due to not taking into account the curving of the electric field and therefore differences in electric field density. What could be seen from these results is that information about the particle or channel morphology is most present in the slopes of the current pulse. The second method modelled the system with an equivalent circuit composed of resistances in series. This method compared more to the simulations with its absolute values than the convolution method. The pulse shape did not approach the simulation because this mathematical model relied on a uniform electric field in the micropore. Also it was determined that it is impossible to reverse this calculation to determine a pore shape or particle morphology with the help of a wanted or measured resistive pulse. Because modelling the system accurately with mathematical models was harder than initially thought, COMSOL simulations were used to determine requirements for a micropore structure. The results showed that short constrictions in the channel differentiated particles with the same volume but different morphology more than longer channels.

The goal of this study was to answer the question: What is a suitable pore structure for determining the morphology of spermatozoa with the resistive-pulse technique? The answer to this question is not yet readily available. The method of convolution could be used to construct a structured micropore. Only, this method in its current state would not be accurate enough to use deconvolution again to calculate a particle morphology from a measured current response. It is determined that the method of series of resistances can not be used to answer this question due to the inability to reverse the calculation. One specification for a suitable micropore structure is determined, simulations showed that a shorter constriction in the channel can differentiate two particles better than a longer constriction. This last method, with using simulations to build a data-set of current responses, is very promising and could be further developed in future work.

6 FUTURE WORK

While the discussed implementation of the series of resistances method does not accurately represent a current pulse of a particle moving through a channel, it can be improved by adding expressions for the non-uniformity of the electric field. To know the movement of the electric field the structure of the micropore and particle morphology are needed. The improvement of this method would create a better understanding of the system but would not contribute to a method that determines spermatozoa morphology with the resistive-pulse technique.

The use of the simulation to build a data-set is very promising. By improving upon a channel design with multiple constrictions that are shorter than the particle, simulations can be done with a plethora of spermatozoa with different morphologies. The derivative of the current response can be compared with the derivative of measured current responses from a lab setup with the same micropore as used in the simulation. The measured current response can be correlated with a current response from a known particle which can indicate with a given certainty which spermatozoa morphology moved through the pore. Individually selecting spermatozoa would still be possible using this technique and due to the use of a microfluidic system could be integrated with other analysis and refinement methods, for example the ones discussed in the study of De Wagenaar [5]. With these improvements this approach for determining spermatozoa morphology could still be a competitor with the CASA systems currently in use.

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A MATLAB CODE

MATLAB version R2020a was used for running the code.

A.1 Micropore Construction

Micropore setup 1: Straight edges.

```
%% Construct particle
Hp = 4.5e-6;   % height of particle
Lp = 4.5e-6;    % length of particle
xp = 0:1e-9:Lp; % define the x-axis
yp = sqrt((Hp/2)^2-((Hp/2)/(Lp/2))^2.*(xp-(Lp/2)).^2);  % make half of
                                                        % circle as particle
%% Construct micropore with straight edges
Lm = 150e-6;    % length of micropore
Hm = 10e-6;     % height ot micropore
xm = 0:1e-9:Lm; % define the x-axis for plotting
ymbase = ones(size(xm))*Hm; % make the general straight micropore
ympulse = 5e-6.*rectpuls(xm-(Lm/2),20e-6);  % make a square in the middle
ym = ymbase - ympulse;  % subtract the square to give the micropore a
                        % structure
%% plot the setup
prepend = 10000;
append = length(ym)-prepend-length(yp);
yp_plot = [zeros(1,prepend) yp zeros(1,append)]; % particle array must be
                                             % the same lenght as micropore array
figure(1)
plot(xm, ym, xm, yp_plot)
title('Channel and particle as functions')
xlabel('Length (meters)')
ylabel('Height (meters)')
```

Micropore setup 2: Rounded edges.

```
%% Construct particle
Hp = 4.5e-6;   % height of particle
Lp = 4.5e-6;    % length of particle
xp = 0:1e-9:Lp; % define the x-axis
yp = sqrt((Hp/2)^2-((Hp/2)/(Lp/2))^2.*(xp-(Lp/2)).^2);  % make half of
                                                        % circle as particle
%% Construct micropore with rounded edges
Lm = 150e-6;    % length of micropore
Hm = 10e-6;     % height ot micropore
```

```
xm = 0:1e-9:Lm; % define the x-axis for plotting
ymbase = ones(size(xm))*Hm; % make the general straight micropore
ympulse = 5e-6.*rectpuls(xm-(Lm/2),20e-6);  % make a square in the middle
rad = 5e-6; % radius of rounded edges
xrnd = 0:1e-9:rad;
ymrnd1 = [zeros(1,59999) sqrt(rad^2-(xrnd-rad).^2) zeros(1,85001)];
ymrnd2 = [zeros(1,85e3) sqrt(rad^2-(xrnd).^2) zeros(1,60e3)];
ym = ymbase - ympulse - ymrnd1 - ymrnd2;  % subtract the square to give the
                                            % micropore a structure
%% plot the setup
prepend = 10000;
append = length(ym)-prepend-length(yp);
yp_plot = [zeros(1,prepend) yp zeros(1,append)]; % particle array must be
                                               % the same lenght as micropore array
figure(1)
plot(xm, ym, xm, yp_plot)
title('Channel and particle as functions')
xlabel('Length (meters)')
ylabel('Height (meters)')
```

A.2 Convolution

```
%% Convolution 'input first'
Conv = zeros([1 (length(xm)+length(xp)-1)]);    % set the array length
for i = 1:length(xm)
    for j = 1:length(xp)
        alpha = (ym(i)-yp(j))/ym(i);    % define the alpha variable for
                                        % every part of the particle in
                                        % that part of the micropore
        Conv(i+j-1) = Conv(i+j-1) + 2*1*ym(i)*alpha;  % add it to the
                                                      % array
    end
end
cResp = 1.4/(150e-6) .* Conv;

%% plot absolute convolution results
xc = -50:1e-3:50;                % define the x scale for plotting
figure(2)
plot(xc, cResp(27250:127250));
title('Convolution with the blocking factor')
xlabel('position in channel (micrometers)')
ylabel('current response (A)')

%% normalize results and plot
ManConNN = normalize(Conv(27250:127250),'range');
figure(3)
hold on
plot(xc, ManConNN)
xlabel('position in channel (micrometers)')
ylabel('normalized current response (A)')
```

A.3 Series of Resistances

```
%% Variables
Depth = 1; % depth of micropore and particle to calculate with areas
sig = 1.4; % conductivity of medium
V = 1; % voltage applied to the channel

%% calculate areas
Am = ym*2*Depth; % area of micropore
Ap = yp*2*Depth;     % area of particle

%% set up for loop
trvl = 100; % travel distance of particle in micrometres
strt = 25; % start location in channel in micrometres
R = zeros(1,(trvl*1000+1)); % set resistance array length
pst = strt*1000 - (length(xp)-1)*0.5;    % set particle starting point
Apl = [Ap zeros(1, (length(xm)-length(xp)))]; % lengthen particle array
Apl_loop = circshift(Apl,pst);  % shift particle to starting point

%% make array of channel resistance per x position of the particle
for i= 1:length(R)
    Diff = Am-Apl_loop;
    Rpieces = 1e-9./(sig.*Diff);
    R(i) = sum(Rpieces);
    Apl_loop = circshift(Apl_loop,1);
end
%% plot current
xplot = -50:1e-3:50;
I = V./R;
figure(2)
plot(xplot, I)
title('Current response from Series of R model')
xlabel('position in channel (micrometers)')
ylabel('current response (A)')

%% plot normalized
IN = normalize(I,'range');
figure(3)
plot(xplot, IN)
title('Current responses plot normalized')
xlabel('position in channel (micrometers)')
ylabel('normalized current response')
```