Embedded Passivated-Electrode Insulator-Based Dielectrophoretic Chromatography

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ABSTRACT

The detection and identification of particles within fluid samples is key in the prevention of the spreading of disease. This has created a growing need for devices able to successfully separate and identify multiple particles for this purpose while operating at a high enough throughput to be applicable in the field. A well investigated method of manipulating particles in this way is Dielectrophoresis (DEP), which is the use of varied electric fields gradients to generate a force on small particles. The strength of DEP depends of the properties of the particle medium, the signal generating the electric field, and the properties of the particles themselves. This method and its interaction with all small particles, including biological particles such as blood and cancer cells, has allowed devices utilizing this idea to be investigated for various biological purposes. This thesis investigates methods to increase the throughput of these types of devices in order to increase their ability to process large amounts of samples in reasonable amounts of time. This is done in primarily two methods. One approach uses the application of chromatographic methods to DEP devices to separate particles by altering their individual transit time through a device, allowing identification during constant flow. Another method is through mass parallel channels which each individually operate as a standard DEP particle trapping device. This allows for the summation of the maximum flow through the device due to its design layout.

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GENERAL AUDIENCE ABSTRACT

Micrometer scale devices are popular for the identification, separation, and characterization of micron scale particles. This includes uses in biological fields for the manipulation of particles such as blood cells, cancer cells, and bacteria. A common method of manipulating these particles is Dielectrophoresis, a force that causes particles to be repelled or attracted to geometric designs within the device generated by an applied electric field. The strength and direction of this force on the particles is dependent on the properties of the electrical signal applied to the device, the physical properties of the particles, such as size and shape, and the properties of the medium the particles are suspended in within the device. Biological devices utilizing this force have been tested before, allowing for particles to be separated out of mixed particle solutions. Most of these devices operate by moving through very little material at one time, somewhere in the microliter per hour range. This thesis explores attempts to increase the rate at which samples can be processed by these devices in multiple ways. Chapter 2 explores methods of DEP by applying Chromatography principles, which is to constantly move samples through the device at a high rate and slow the target particles, so they exit the device at a different time than other particles. Chapter 3 investigates increasing device throughput by replicating a standard DEP channel multiple times on one device so that several may operate all at once.

To Sheyenne E. Ervin

Acknowledgements

While at Virginia Tech, I have been able to work with some wonderful people that have done great things for my educational journey. First, I would have to recognize those in the VT MEMS Lab Group and my advisor, Masoud Agah. The ability to work in this facility has been an experience I could not have had anywhere else. The fascinating research done here by people that are a constant help has been crucial to my success in this program. Specific recognition should be given to my lab colleague Shanshan Zhao, who has been a crucial help in the final months of my work here.

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1. Introduction

1.1 Background

Devices for the manipulation and separation of biological particles have seen rapid development and investigation in recent years [1-3]. This is due to the ability of these devices to target a wide range of synthetic and biological particles and manipulate their position within a sample solution [1-8]. These devices have been shown to effectively manipulate particles in several ways in previous studies, yet operate at a slow pace, processing samples at a rate in the range of microliters per hour [9,10]. This is due to the applied methods of particle manipulation being dependent on a summation of forces required to move the particle, which must be able to overcome any flow force in the sample. This creates a hard limit to the rate at which the sample can be effectively processed by these methods using conventional means. Development of methods to increase this processing rate of devices without reducing their effectiveness therefore has high relevance to these devices further development towards application within the biomedical field.

Micro scale devices stemming from the Microelectromechanical systems (MEMS) and Micro Total Analysis Systems (µTAS) fields have been the prominent types of devices used for particle manipulation in bio-medical applications [9-11]. Utilizing these styles of devices and their related particle manipulation methods presents several advantages. These advantages include low device cost and ease of producibility. The methods used to fabricate these devices are highly developed over years of processing techniques. By fabricating device structures on silicon wafers to function as reusable molds, multiple devices can be made in short amounts of time using cheap silicone polymer with the same wafer mold, needing only the electrodes for each new device. Additionally, these devices are very small, with features within the channel ranging in size at times smaller than 5 micrometers [12]. This small scale and feature size allows micro-scale forces to dominate the activity within the channel [14]. These advantages allow MEMS devices to operate as a sensitive particle manipulation device in terms of both time and position within the device.

The phenomena these devices use to alter the forces within the device and manipulate particles is Dielectrophoresis (DEP) [13-18]. DEP describes the movement of particles in a solution when subjected to an electric field gradient due to an induced dipole moment. There are multiple forms of DEP devices, each with its own benefits and drawbacks. eDEP devices are a common version, which contain the electrodes necessary to impart the electric field gradient directly within the device in contact with the solution. However, these devices are less robust, have comparatively high costs as the whole device is integrated within the electrodes, and can have issues with sample electrolysis at the fluid-electrode boundary [19]. Additionally, iDEP devices are common for particle manipulation, which utilize insulator structures within the device to alter the electric field. This version of the device overcomes several of the issues common with DEP, however the electrodes still contact the sample leading to contamination problems, and the method of applying the electric field through the insulating material requires higher voltages for efficient operation [20]. Examples of these device types can be seen in Figure 1.1.

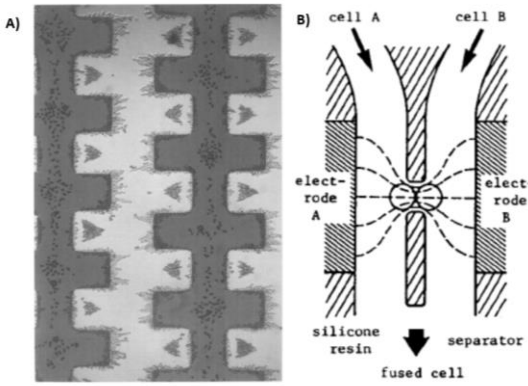

Figure 1.1: Examples of DEP Device types A) an eDEP device used to separate yeast cells [21] B) an illustration of the iDEP method used to fuse cells [22]

An additional method of DEP device developed by the VT MEMS laboratory and P. Zellner prevents the issues common with eDEP and iDEP devices by utilizing the benefits of each method while eliminating direct contact of solution with electrodes. This is done by adding a thin passivation layer over the electrodes, then embedding the passivated electrodes within an insulator device (EπDEP) [23,24]. The insulator structures generate strong electric field gradient areas, while the thin passivation layer of insulator over the electrodes prevents contamination and electrolysis while alleviating the need for as high of voltages as with iDEP. This thesis presents various methods of use for EπDEP devices in an attempt to increase the throughput of the devices compared to other DEP devices. These results demonstrate such devices ability to function at rates high enough for practical application.

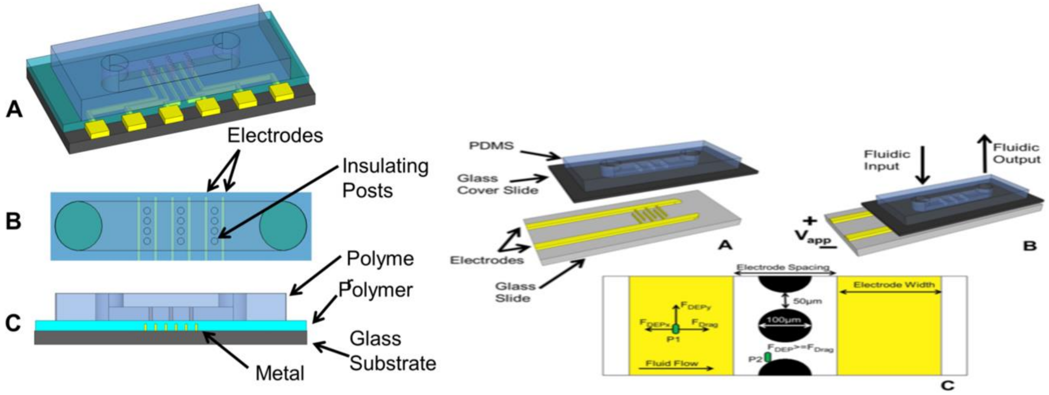

Figure 1.2: Examples of the πDEP device methods introduced by P. Zellner [25]

In addition to the development of new device types, new applications for DEP have been developed within recent years. A number of these new methods were introduced by the VT MEMS lab and K. Kikkeri, including DEP systems such as DEP Fiber and DEP Chromatography [26].

These systems presented new methods for the application of DEP that had not been previously investigated, each of which creates unique opportunities for the advancement of DEP as a whole.

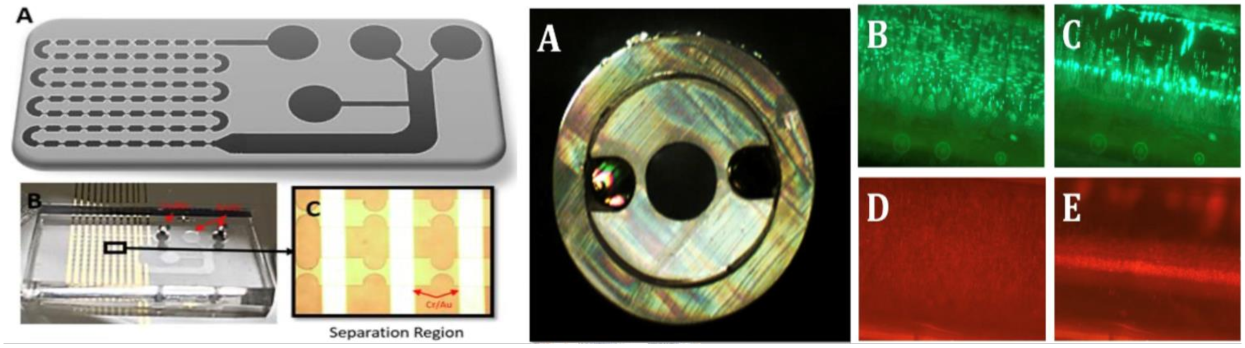

Figure 1.3: K. Kikkeri's novel DEP systems, DEP Chromatography (Left) and DEP Fiber (Right) [26]

1.2 Theory

DEP is the method in which particles are manipulated within a device via an applied electric field. This is done by the presence of an electric field gradient within the device in the region where particles are targeted. The movement of particles in this region is due to an induced dipole moment (**p**) within the particle due to its polarization while in the electric field. This dipole moment forces positive and negative charges (+/- Q) to opposite sides of the particle and can then be defined as a function of those charges and the distance vector between them (**d**) as shown in equation 1.1 [16].

$$\mathbf{p} = Q\mathbf{d} \tag{1.1}$$

This orientation of the particle diploe is dependent on the relative polarizability of the particle compared to that of the medium it is suspended in. If the particle is more polarizable than the medium, the dipole will align itself with the orientation of the electric field. In all other cases the particle will align inverse to the electric field orientation. This rotational alignment is to reduce the electric potential on the particle. In a non-uniform electric field, the two poles of the particle will experience different electrical forces, as each end of the particle will experience a differing strength of the field. This electric force is what induces movement of the particles in the field and is referred to as DEP force. The magnitude of the DEP force is dependent on the difference between the two charged sides of the dipole; thus DEP force may be expressed in terms of the dipole moment as:

$$F_{DEP} = (\mathbf{p} \cdot \vee \nabla)\mathbf{E} \tag{1.2}$$

This is, however, only the first order expression for DEP force. The remainder of the Taylor expansion can be found in [16]. While higher order moments are solvable, all orders beyond the first can be considered negligible to the total result. Additionally, when multiple particles are affected at once while in close proximity to each other, a dipole-dipole phenomenon known as "pearl chaining" may occur, resulting in the particles moving through the electric field region together along equipotential lines.

The magnitude of the induced dipole moment is directly proportional to the volume of the particle, the particle's polarizability, and the magnitude of the electric field used to induce DEP force. The dipole moment for a spherical particle of radius R can be defined as:

$$\mathbf{p} = 4\pi R^3 \varepsilon_0 \varepsilon_{r_{medium}} Re[CM]\mathbf{E} \tag{1.3}$$

where ε_0 is the permittivity of free space, $\varepsilon_{r_{medium}}$ is the relative permittivity of the medium, and Re[CM] is the Clausius-Mossotti factor [16]. Replacing **p** in equation 1.2 with equation 1.3 gives the following equation for DEP force.

$$F_{DEP} = 4\pi R^3 \varepsilon_0 \varepsilon_{r_{medium}} Re[CM] \vee \nabla \boldsymbol{E}^{\mathbf{2}} \tag{1.4}$$

DEP force is more commonly expressed in this form, as it can inherently be applied to both AC and DC generated electric fields without further changes.

The Clausius-Mossotti factor is often manipulated for DEP systems by fixing the particle radius, medium permittivity, and electric field gradient, leaving it as the key variable for DEP force. The CM factor for spherical particles is:

$$CM = \frac{\varepsilon^*{}_p - \varepsilon^*{}_m}{\varepsilon^*{}_p + 2\varepsilon^*{}_m} \tag{1.5}$$

where $\varepsilon^*{}_p$ is the complex permittivity of the particle, and $\varepsilon^*{}_m$ is the complex permittivity of the surrounding medium. The complex permittivity is defined as:

$$\varepsilon^* = \varepsilon + \frac{\sigma}{j\omega} \tag{1.6}$$

where σ is conductivity, j is $\sqrt{-1}$, and ω is the radial frequency of the applied electric field [16]. This relationship reveals the dominant factors in the CM factor depending on the frequency of the applied field. When the field is at a high frequency, the permittivity values dominate the CM factor equation, while the conductivity will dominate the CM factor at low frequencies. Additionally, it can be seen that depending on the comparative complex permittivity of the particles and the medium, the CM factor can be positive, negative, or zero. When the complex permittivity of the particle is greater than that of the medium, the particle will orient itself with the electric field as described above and the CM factor will be positive. This generates a positive DEP force value, attracting the particle towards the high field gradient areas in the region. This is known as positive DEP, or pDEP. The inverse case, where the complex permittivity of the medium is greater than that of the particle will have the opposite effect, generating a negative value for DEP force which acts as a repelling force to move the particle away from high field gradient areas. This is known as negative DEP, or nDEP. Lastly, in cases when the complex permittivity's of the particle and medium are equivalent, the CM factor becomes 0 and thus no DEP force is generated. This point is referred to as the crossover frequency [16]. Figure 1.4 illustrates the DEP forces on a particle under these various conditions.

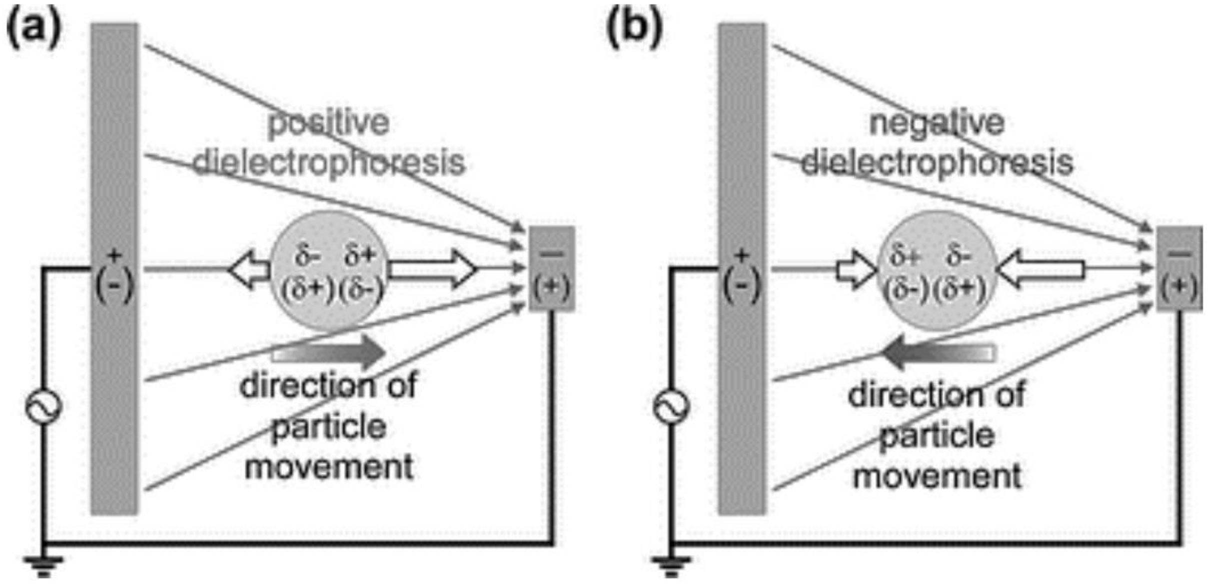

Figure 1.4: DEP force under nDEP and pDEP conditions [27]

Due to the complex nature of biological particles, several methods have been put forward to determine the effective complex permittivity for these particles. One such method is the shell, or multiple shell method, which models the particles as spherical shells. The shell method as illustrated below in Figure 1.5 demonstrates the characterization of the outer surface of a particle or the membrane of a cell as a consistent shell, while any interior material is combined into a secondary inner shell. Higher order representations may include continued levels of shells to make a detailed representation of complex systems, but the single shell method will suffice for most cases [13].

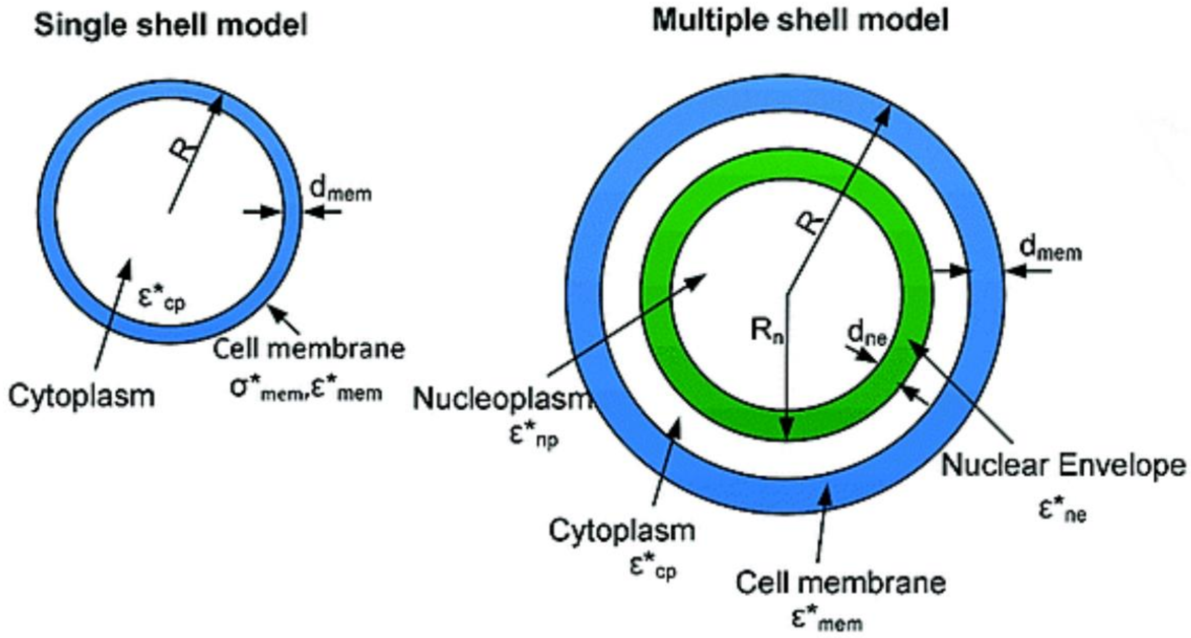

Figure 1.5: Examples of the shell method [13]

In addition to frequency, it has been found that the morphology of cells and particles can affect the CM factor. Figure 1.6 shows that membrane roughness, cytoplasm properties (conductivity, permittivity, and ration to nucleus), and particle size may modulate the CM factor. In a similar manner, the conductivity of the suspending medium may vary the maximum possible values of the CM factor. These factors may all be important for analyses when utilizing DEP systems for cell and particle manipulation.

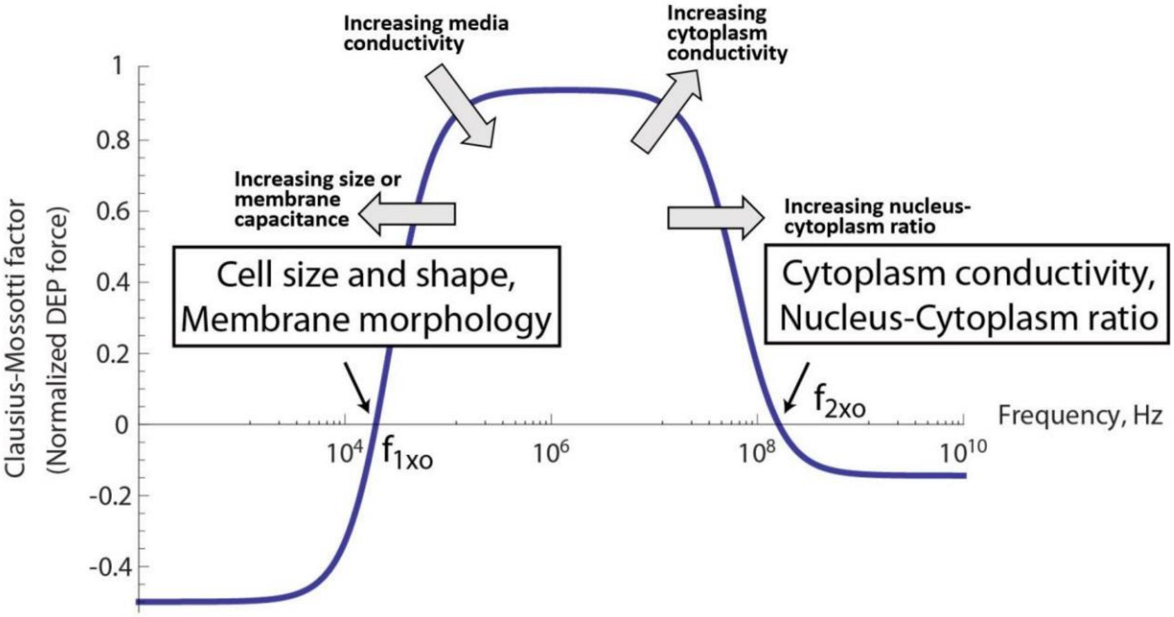

Figure 1.6: CM factor as a function of frequency [28]

Additional forces come into effect and must be accounted for in DEP devices. Microfluidic systems, such as those presented in this thesis, must account for the net force on a particle between the applied DEP force and Stoke’s Drag from the device’s fluid flow. When the DEP force dominates other present forces and is able to control the movement of the particle, this is typically called DEP trapping, as the particle is usually either held in place or moved and trapped within a particular portion of the device. When the DEP force is not high enough to overcome forces like Stoke’s drag, then the particles continue moving through the device with minimal to no disruption. Manipulation and balancing of the flow through the device and the strength of the DEP force allows for DEP devices to detect or separate different particles within a solution.

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2. DEP Chromatography via Input Signal Optimization

2.1 Introduction

A DEP Chromatography system is designed, fabricated, and tested. The DEP Chromatography system focuses on the separation of particles in continuous flow, achieving the desired result by reducing the speed of the targeted particle within the device rather than fully trapping it. Non-symmetrical triangular channel features create a DEP force that changes based on a particles position in relation to the geometry, allowing for a net change in particle speed across each feature. Under optimal signal and device conditions, separation of 80% may be achieved. Laboratory colleague Shanshan Zhao contributed to these findings in assisting with gathering data through experimental trials and generating figures.

2.2 Materials and Methods

2.2.1 Device and Microparticles

In this study, we utilized our groups previously developed passivated-electrode insulator-based dielectrophoresis device (EπDEP), which alleviates the shortcomings of the current eDEP and iDEP mechanisms. This is done by utilizing 3D insulator structures for generating strong electric field gradients and using passivated electrodes to apply the required AC voltage for selective isolation/trapping of bioparticles through capacitive coupling [1]. These structures within the device generate the field gradient necessary to generate a DEP force and manipulate nearby particles. Here we developed a novel DEP-chromatography system for particle separation based on microfluidic and chromatographic principles. The novel embedded passivated-electrode dielectrophoresis chromatography (EπDEP-C) chip shown in Figure 2.1 contains a preconcentration section, separation section and an impedance detection section. Although each region has its own unique results, this thesis will focus on the separation section and the DEP separation experiments performed to investigate the devices functionality and performance. Preliminary results show that the innovative design is able to both create separated bands of closely sized polystyrene beads and characterize 2um and 10um particles by their device retention time at the outlet.

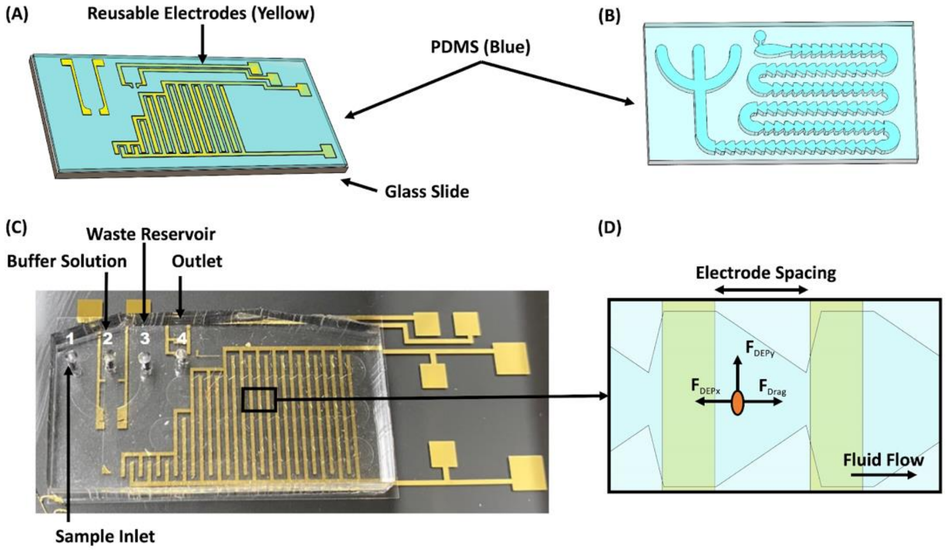

Figure 2.1: Layout of the EπDEP device (A) passivated electrodes with PDMS coating (B) PDMS Channel, the curve shape makes sure the particles in the sample solution start at the same time and the serrated channel can enhance the DEP force by utilizing the conception of iDEP (C) Actual Device with sample inlet, buffer solution inlet, waste reservoir, outlet marked (D) relevant forces inside the channel

Su-8 structures are fabricated on Silicon Wafers using photolithography techniques previously reported by our group (reference Zellner). PDMS is poured on the structures, degaussed for 10 minutes, and cured for one hour at 95°C. The microfluidic channel structures are 10cm long, 1.5mm wide, and 35um deep. Two variations of the channel are fabricated, one without alterations to the wall geometry of the channel and one with scalene triangle structures added in 1.5mm increments along the channel wall. Interdigitated chrome/gold planar electrodes are deposited onto a glass wafer and patterned using a standard liftoff technique. Electrodes are then spun with a coating of PDMS at 6000RPM for 5 minutes to apply a 4um thick passivation layer across the surface. PDMS channels are cut to the size of the electrode pattern and plasma bonded to the electrodes to form an EπDEP channel. This fabrication process is shown in detail in Fig.2.2.

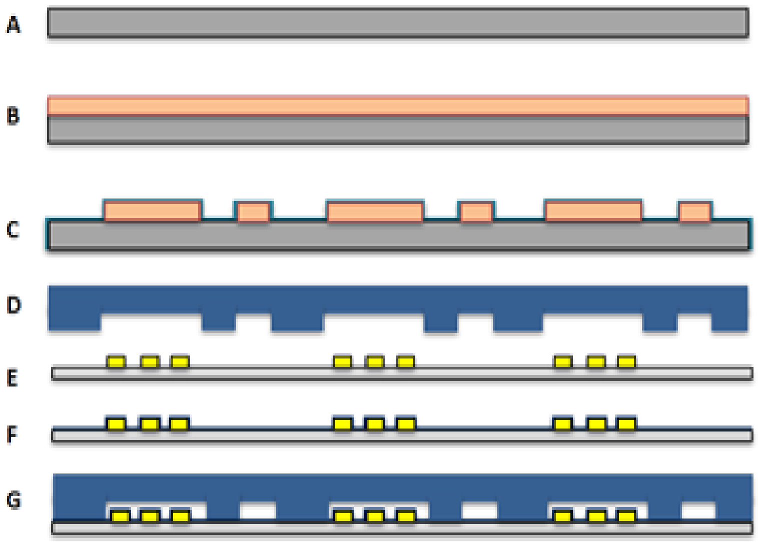

Figure 2.2: Fabrication process flow of the DEP Chromatography device a) Silicon wafer is cleaned and dehydrated b) the wafer is spin coated in SU-8 photoresist c) The photoresist is patterned via photolithography techniques and developed. Remaining SU-8 structures are coated to protect the mold during PDMS application and removal. d) PDMS is poured onto the wafer, cured, and then removed to make the form of the device channel e) Cr/Au electrodes are deposited on a glass wafer and patterned with liftoff techniques f) the electrode wafer is spin coated with a thin layer of PDMS for protection g) the PDMS form from (d) is aligned and plasma bonded to the electrode wafer, completing the device

Two sizes of fluorescently tagged microparticles purchased from Fischer Scientific were used to make three different suspension concentrations. All particles were suspended in deionized water. 10um particles were suspended in concentrations of 1:100 while 2um particles were suspended in concentrations of 1:1000. 2um and 10um particles were combined in one suspension in DIW at a concentration of 1:10:1000. Medium and particle concentrations are carefully selected as the conductivity will modify the strength of the DEP effect via the Claussius-Mossotti factor.

2.2.2 Equipment and Experimental Procedure

In order for chromatography to take place, particles must enter the channel at the same time. To do this, the cross-channel inlet is used to create a packet of particles within the channel that enter at the same time and are separated by the effects of the channel. (See Fig.2.3) The channel is prepared for testing by continuously injecting DIW in the main channel through opening 2 at the target flow rate (.1mL/min, .05mL/min, or .01mL/min). To create a packet of particles for testing, the constant DIW injection is halted, and the clamps removed from openings 1 and 3. Particle solution is then injected to opening 1 at a constant .05mL/min for 5 seconds. This injection is then stopped, and clamps are reapplied to openings 1 and 3. Constant injection of DIW to opening 2 is resumed allowing the portion of particle concentration placed within the center channel intersection to be pushed through the device. Microscope imaging observing the end of the channel length will observe the packet after it has passed through the device and DEP force has been applied.

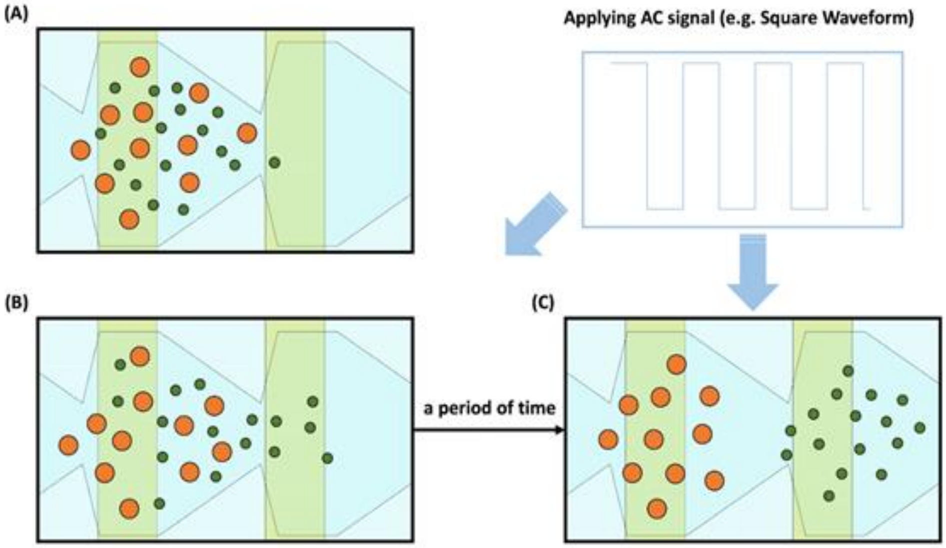

Figure 2.3: (A) Shows two different sized particles at the beginning of the channel (B) Shows these two particles gradually separated after applying an AC signal (C) After a period of time, two particles are fully separated at the end of the channel.

Microparticles in the channel were recorded using a Zeiss Axio Observer Z.1 inverted microscope and processed using ZenPro. Applied signals for the DEP Chromatography process are created using a BK Precision 4079 function generator and amplified by a FLC Electronics AD800I amplifier. The crossflow inlet of the channel is attached to a Chemyx syringe pump, connecting openings 1 and 2 to syringe channels 1 and 2, respectively. Openings 3 and 4 are connected to tubing leading to a waste vial. Clamps are applied to the tubing connected to openings 1 and 3 to prevent undesired sample flow during testing. Particles in the sample packet are marked to enter the channel when the syringe pump begins injection at time = 0s. The travel time of the packet for each particle is recorded by observing the end of the channel and noting the start and end times of the packet relative to the initial t=0s. The packet is marked as present when more than 50% of the channel at the recording point is occupied by particles. A schematic of the experimental setup can be seen in Figure 2.4.

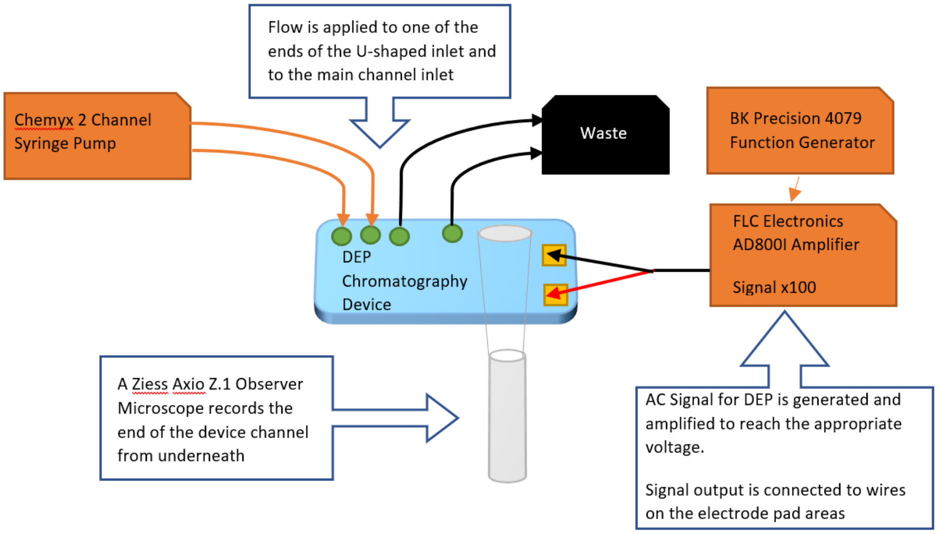

Figure 2.4: Connections and system layout for the DEP Chromatography system

2.3 Results and Discussion

First, an appropriate electrical signal and sample injection speed must be selected to perform the DEP Chromatography process. 2u and 10u particles were tested on both blank and triangular channel structures under varying frequency and flow rate conditions. The time required to traverse the channel and the length of the packet were recorded. Voltage is held constant at

400Vp-p while flow rate is swept from 0.01mL/min to 0.1mL/min and frequency is varied from 10-500kHz for each flow rate. Comparing the travel times and length of packets between the three tested flow rates, each flow rate tested can be seen to have distinctly different transit time responses. Additionally, comparing the transit times between blank and triangular geometries shows that due to geometric changes in the flow of the device, the blank channel slows the travel of the packet compared to the triangular channel.

For each flow rate, frequency effects are visible on both channel geometries, particularly around the middle of the frequency band from 50-250 kHz. As the input signal approaches these middle frequencies the two particles are affected differently, and the two particles separated from each other begin forming their own individual packets. Figure 2.5 indicates that under various conditions, one particle will be slowed more than the other, allowing particles to be seen individually at the channel end. As these particle separations focus and vary based on the input signal frequency, it can be confirmed that this is a result in the alteration of the CM factor.

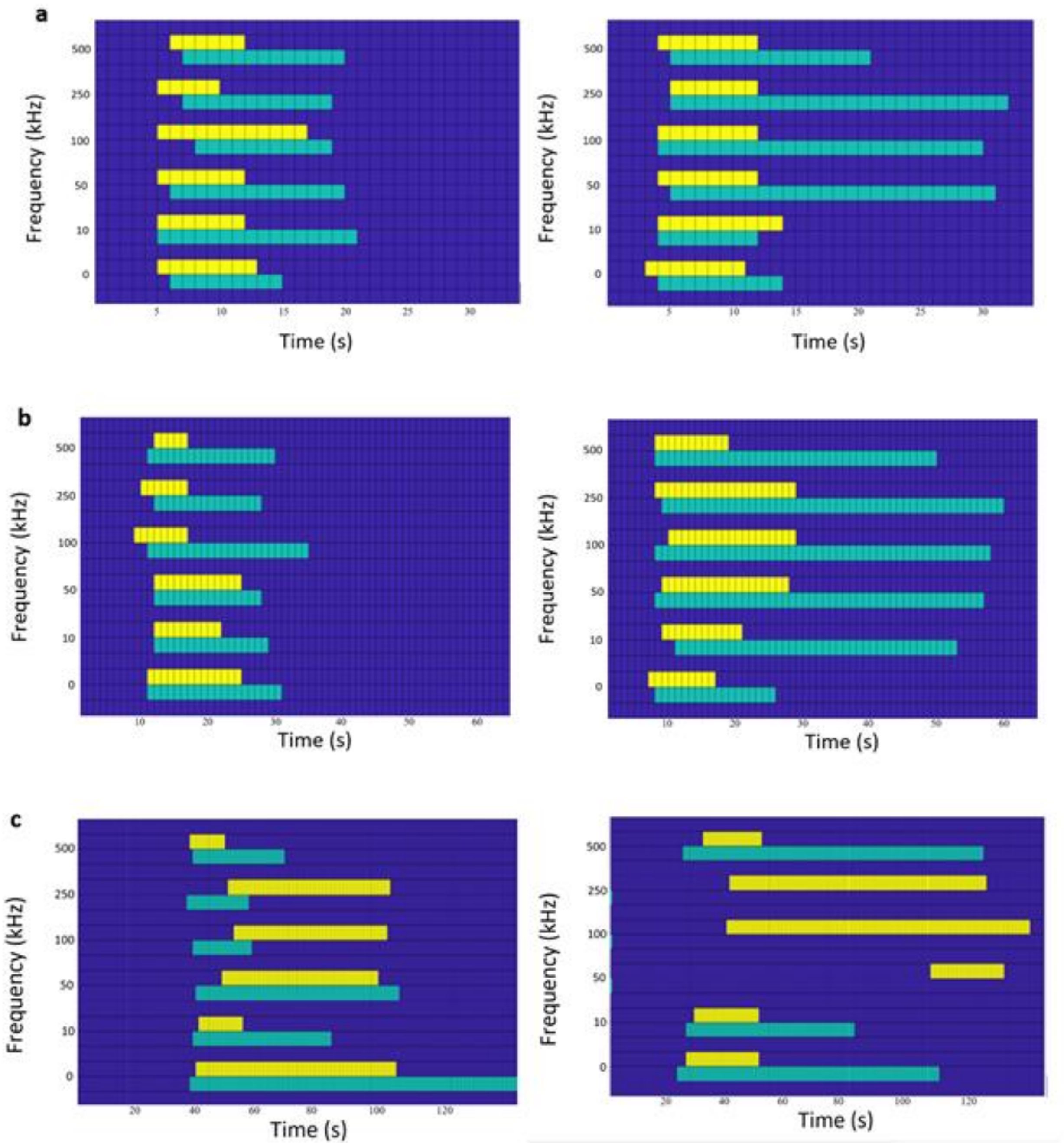

Figure 2.5: A) .1 mL/min flow rate for Blank and Triangular channel form B) 0.05 mL/min flow rate for Blank and Triangular channel form C) .01 mL/min flow rate for Blank and Triangular channel form

For all plots, 10um beads are yellow, 2um beads are teal

Triangular geometry on the right, Blank geometry on the left

Different transit times in each sized particle between geometries may also be observed. The triangular channel form gives noticeably longer sample lengths, particularly of 2um beads.

Under low flow rate conditions of .01 mL/min, effective particle separation can be achieved by the blank channel form. These geometric effects result from increased and altered field gradients within the device from the interior insulator geometries, thus changing the DEP force strength and direction at different points in respect to the flow of the sample. At higher flow rates of .05 and .1 mL/min, some particle separation can be achieved to a lesser degree due to the increasing drag force of the sample flow. Additionally, these higher flow rates invert the order of detection for the two particles. Of the two channel geometries, the triangular form has greater overall effects on the particles and is selected to be used for further testing.

In addition to variations in the channel geometry and sine wave frequency, the input waveform can be changed to further separate the two particles within the channel. A square wave is selected for testing, with the same 400Vp-p as the sinusoidal wave. The same frequency and flow rate sweep is performed as on the triangular geometry. Figure 2.6 shows the comparison of the square wave applied signal to that of the sinusoidal signal. For the square wave, separation of the two particles is increased and the travel times and packet sizes decreased.

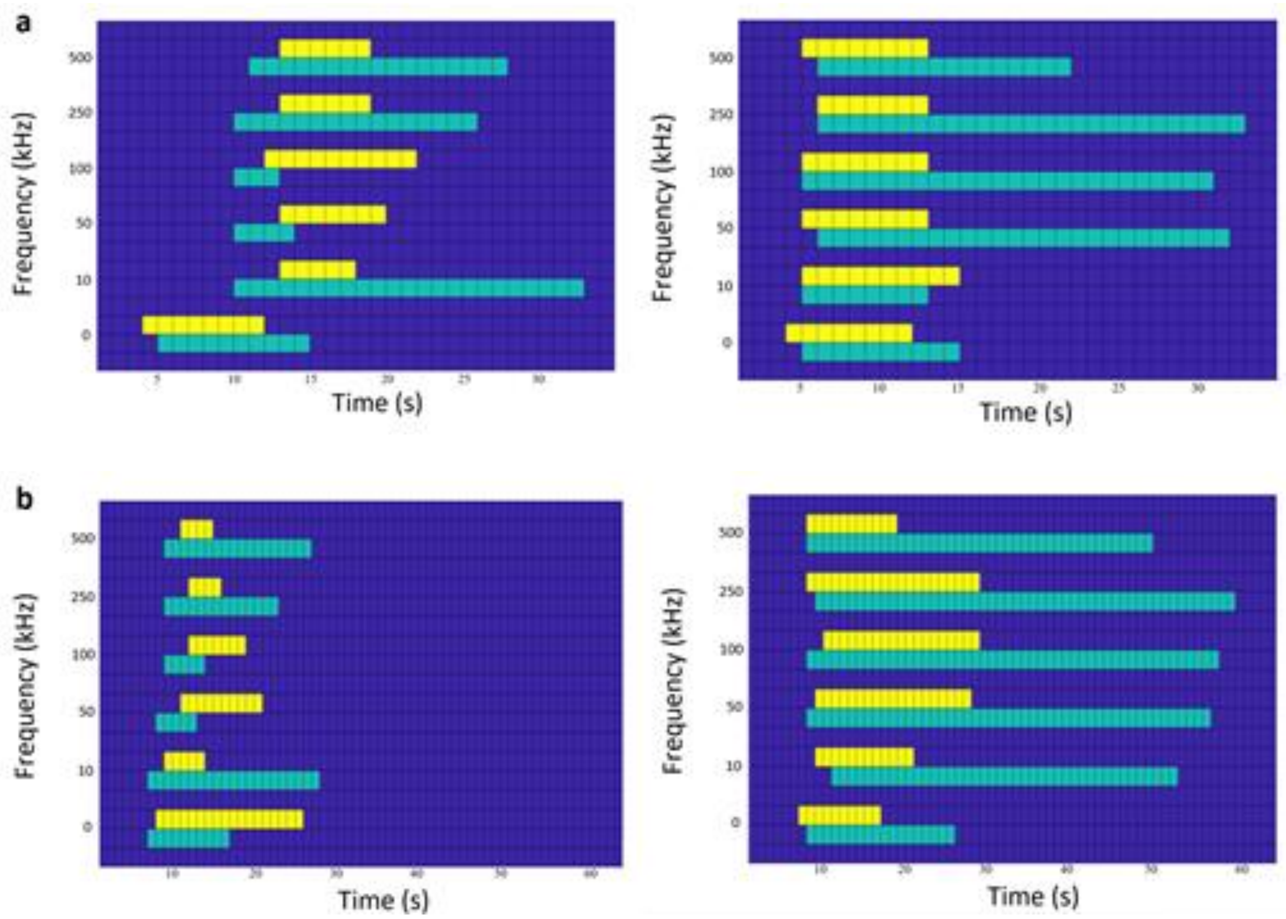

Figure 2.6: A).1 mL/min flow rate for the triangular channel with applied square and sine wave B) 0.05 mL/min flow rate for the triangular channel with applied square and sine wave
For all plots, 10um beads are yellow, 2um beads are teal
square wave left, sinusoidal right

When comparing the input waveforms, the square wave gives higher degrees of separation compared to similar signals using sinusoidal waveforms. At .1mL/min and 100 kHz, the highest degree of separation is achieved, with 80% separation of the two particles. This particle separation can be seen in Figure 2.7, where it is also compared to the separation achieved by the same parameters on the sinusoidal waveform.

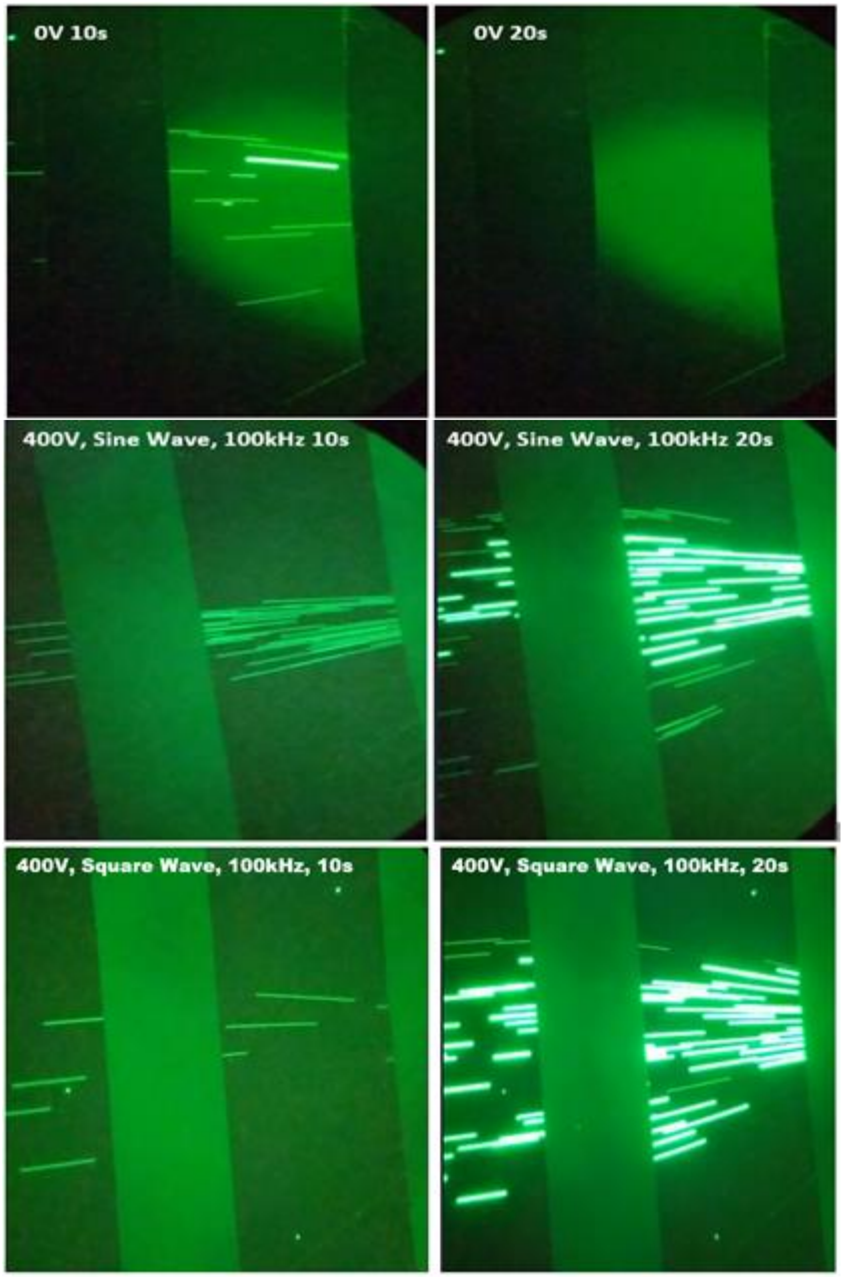

Figure 2.7: Example of the particle separation within the device for no signal, Sinusoidal waveform, and Square waveform

2.4 Conclusion

In this study we have examined the optimization of a dielectrophoretic device for chromatographic separation of particles. The EπDEP form of device allows for the manipulation of particles under various AC signals and device parameters, enabling effective separation of particles to be achieved. Previous studies have investigated microfluidic chromatography using DEP forces as a possibility. However, successful separation by particle size via channel transit time is a new application of these ideas. Electric field gradients to generate DEP force are focused through the PDMS geometry of the channel, and are easily transferred by capacitance coupling through the insulating layer into the channel to affect the particles due to the direct presence of the electrodes within the channel. This novel design allows a greater range of conditions to affect the particles such as a wider frequency range and higher flow rates including 50 - 250 kHz and 0.1 - 0.01 mL/min flow rates. Particle separation of over 80% can be achieved under the optimal combination of these conditions.

By isolating each DEP condition that affects DEP force on a particle, including flow rate, frequency, input waveform and PDMS geometry, and varying it within the possible range of effect, the device and signal properties can achieve a high degree of separation. This separation may also occur at frequencies and flow rates that would otherwise be unachievable with other device and signal methods. The reported results indicated that the separation of differently sized particles is achievable to some degree at several different system settings. Particle separation under optimal conditions can be achieved at 80% separation up to flow rates of .1 mL/min. The given transit time of each particle when the system settings are known allows for identification by size. This allows for several conditions to achieve distinguishable separation and identification of particles within the device. While the unmodified blank channel form does not give a high degree of particle separation on its own, when put in parallel with another channel with the triangular geometry, distinct particle signals can be observed. Under 0.1 mL/min flow and 100 kHz, these two geometries are able to individually identify opposite particle sizes due to the transit time including a portion of one particle without the second particle. These two signals operating in parallel would then allow for identification of the present particles by analyzing the output time data of both devices. In a similar manner, two triangular geometry devices in parallel but with alternate input waveforms could produce the same single particle identification system, such as under 0.1 mL/min flow and 100 kHz conditions. Here, each waveform provides overlapping transit times, but contain portions of opposite particles individually, and thus may each identify one particle. This device allows for the testing of particles under a greater range of flow rates and frequencies than typical DEP devices. Due to its wide channel design and sharp triangular features, the triangular geometry channel allows for high flow rates to pass through while maintaining strong and varied electric fields via the geometric features. These features allow for high resolution separation under a variety of conditions. Higher resolution separation may be achievable through continued investigation of input waveforms and their properties.

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3. High-Throughput Parallel Channels

3.1 Introduction

As discussed previously, DEP Microfluidic devices have shown capabilities in manipulating biological particles for use in chemical treatment testing. Processing whole blood and other live particle samples, while promising in result effectiveness, lacks the ability to be used in practical applications due to the long time to process large sample sizes. Typical microfluidic devices process samples at a rate of microliters per hour, requiring over 5 weeks to process a 10mL sample on a device operating at 10 µL/hr. This limit stems from the physical factors involved with the device design. First, devices such as these are designed on the micrometer scale such that macroscale forces are negligible in order to successfully manipulate small particles. Additionally, the small features within the channel generates variance in the electric field when using insulator-based MEMS DEP devices. The small channel sizes only allow a small amount of volume to occupy the channel at a given time without damaging the device. Additionally, DEP devices operate at low flow rates to allow the DEP force to dominate other forces such as drag.

These present hard physical limits to the processing capabilities of microfluidic DEP devices. Increasing device size or increasing flow rate will subsequently reduce particle trapping efficiency of the device. In order to increase throughput and device processing rates, a method for increased sample testing must be developed that does not modify the individual DEP channel. While the dimensions of the testing channel must remain within the rage of previously tested dimensions, the size of the total device is irrelevant when considering sample testing when compared to tabletop sized testing equipment otherwise used. If a singular DEP channel is capable of processing 10 µl/hr of sample, two identical devices operating at the same time should process exactly double that quantity. This method of simultaneously operating channels is theoretically scalable to any degree as long as the total size of all channels may fit within a predefined space, and all channels are able to receive the same amount of fluid flow. To create a higher throughput device without changing fabrication methods, all channels must fit on one wafer, giving a maximum space of a 4-inch circle. In order to ensure all channels on the device receive equal fluid flow, an interwoven, branching inlet system must be designed that connects all operating channels to one inlet and one outlet, with a volume capacity equal to the volume of all DEP channels on the device. This then requires the inlet and outlet both be equivalent in size to the total size of all channels on the device. With these parameters, a DEP microfluidic device that operates several channels in a parallel manner can be designed to increase the throughput of the total system by a factor of the number of channels included on the device. The final fabricated DEP device can be seen in Figure 3.1.

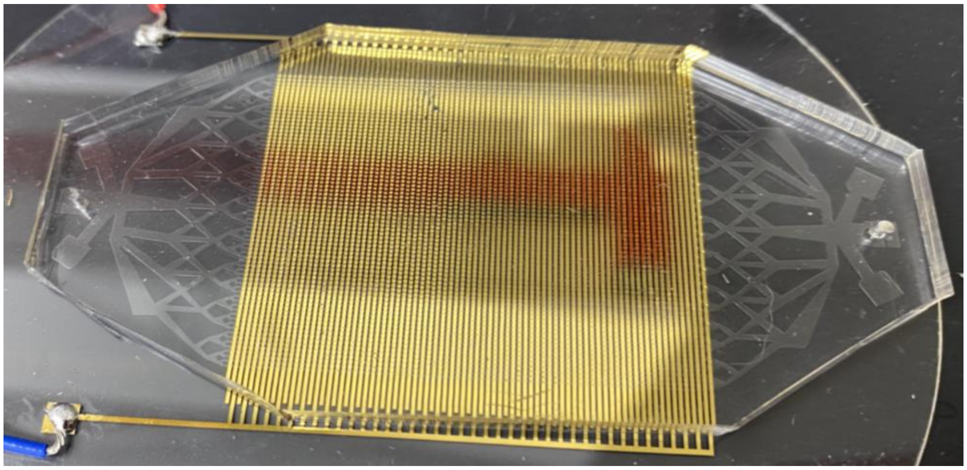

Figure 3.1: The final fabricated High Throughput DEP device, featuring a branching inlet and planar electrodes that cross each channel to ensure equal inputs

3.2 Device Fabrication

A DEP device consisting of 72 identical, parallel particle trapping devices is designed. The ends of each channel are connected in tiers of pairs to evenly disperse flow between all the channels at the same time. Utilizing the same EπDEP fabrication method discussed in Chapter 2, a silicon wafer is patterned with the designs for the DEP channels. Photolithography is performed to create SU-8 structures on the wafer matching the designed pattern, then developed to remove excess photoresist. PDMS mixture is poured over the finished wafer, then left to cure on a hotplate for 24 hours at 65 ºC. Once cured the PDMS is peeled off the wafer mold and is ready to be bonded to create the device channels. The completed PDMS channel form can be seen in Figure 3.2. Interdigitated planar electrodes are deposited on a glass wafer via e-beam evaporation. This electrode wafer is then coated in a thin layer of PDMS via spinning to protect the electrodes within the device during operation. The PDMS channel form and electrode wafer are then plasma bonded together to form the total device.

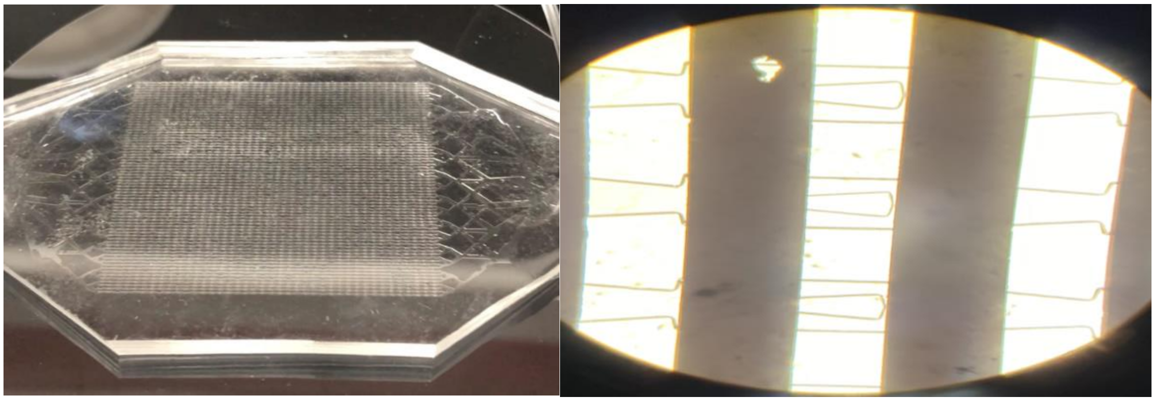

Figure 3.2: The PDMS multi-channel structure bonded to a glass substrate (Left) and the interior channel geometry (Right)

3.3 Experimental Setup

One end of the device is connected through microfluidic tubing to a 1mL syringe on a Harvard Apparatus syringe pump. The syringe is filled with a 2 micrometer diameter polystyrene beads solution, diluted to a concentration of 5:1000 in deionized water. Soldered wire connections to the planar electrodes are connected to an incoming electrical signal from a FLC Electronics AD800I amplifier increasing power from a BK Precision 4079 function generator.

Constant injection is provided via the syringe pump to the device and is maintained throughout testing at a selected flow rate. Visual recording of particles is taken to measure the trapping efficiency of the channels, comparing the total number of particles to pass through the device with no signal to the number able to pass through under applied DEP activity. Activity recording begins at time equals to 0s. At time equal to 5s, electrical signal from the function generator is turned on to generate a DEP force within the device from the embedded electrodes. This is continued for 10s before turning the signal off at time equal to 15s and allowing the system to return to base conditions.

3.4 Results

The mass channel DEP device is tested with a baseline 700 microliter per hour flow rate. Any lower flow will be less than the minimum to fill all channels repeatedly, leaving some without consistent flow through the device. A sine wave of 400Vp-p is applied across the electrodes with a varying frequency between 50kHz and 500kHz. At 100kHz, the applied signal is able to achieve over 95% trapping efficiency within the device at the base flow rate. Voltage is then reduced to examine the device trapping efficiency under lower strength signals. While at minimum flow rate, 200Vp-p signals produce noticeably less trapping, resulting in a total trapping efficiency of around

75%. With an ideal signal of 400Vp-p and 100kHz selected, flow rate is increased in stages to determine the maximum speed at which the device may continue to operate at high trapping efficiencies. Flow rate is nominally increased by multiples of 2, starting with the baseline flow rate of 700 ul/hr, then increasing to 1400 µl/hr, 2100 µl/hr, 4200 µl/hr, 8400 µl/hr, 10 ml/hr, and lastly 20 ml/hr. For all flow rates up to and including 10 ml/hr, the device is able to continue achieving over 95% trapping efficiency. Once 10 ml/hr is passed, trapping efficiency decreases proportionally with increasing flow rate.

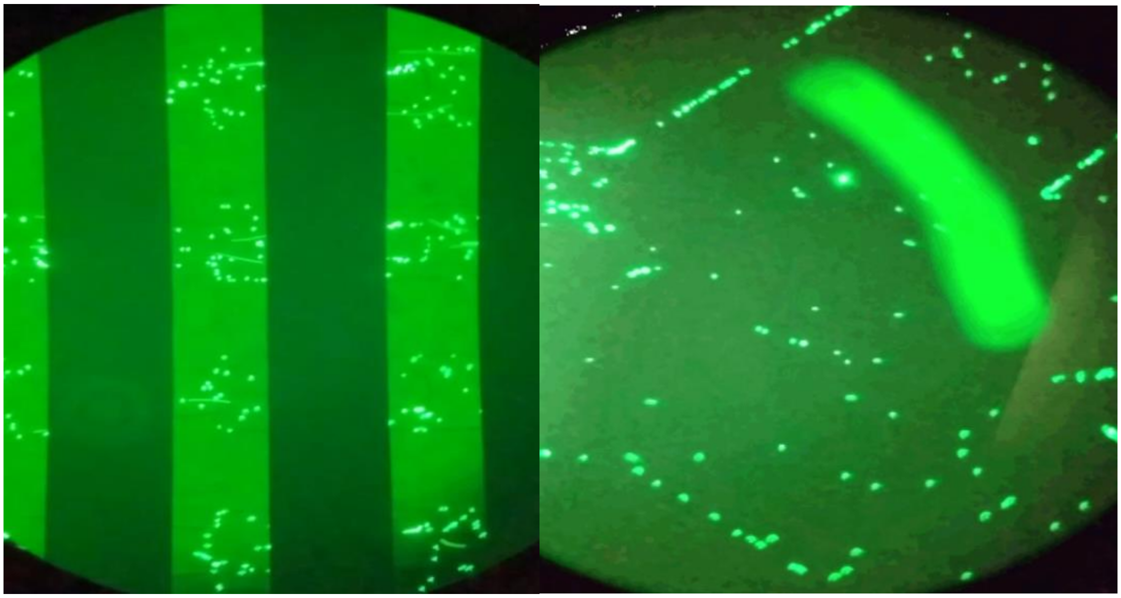

Figure 3.3: The High Throughput DEP Device in operation

3.5 Discussion

This device functions as a base proof of concept for the idea of mass parallel DEP channel devices. The ability to function at flow rates multiple times higher than an individual channel enables device systems operating in this method to process practical amounts of sample in short amounts of time. However, this initial iteration of the system presents a number of issues that must be accounted for when continuing to develop the method.

PDMS structures, while helpful in making DEP microfluidic channels due to their low cost and ease of producibility, are hydrophobic in nature. This can cause potential issues when operating on water-based solutions, as the formation of air pockets can be common. In addition to the material composition, the channel and inlet design of the device incorporates several sharp ends and corners, which continues to cause problems with air pockets within the device. When the outer edges of the channel resists water, sharp edges will force constant flow outward away from the edge, trapping pockets of air within the device that can prevent flow through entire areas of the device. Other DEP devices can have similar issues, requiring the device to be vacuum purged of air and cleansed with an ethanol and water mix to remove air pockets prior to operation. However, this device continuously generates air pockets within the device during operation, regardless of preparatory methods applied before testing. Future variants of this device could alleviate this issue by utilizing more rounded edges to avoid air pocket creation, or possibly utilize an alternative material to PDMS.

Due to the size of the device, visualization of all the interior channels at once using standard microscopic devices is impossible. The Zeiss microscope system used for fluorescent particle recording on other DEP devices can only observe four channels or the outlet connector for four channels at any given time. This prevents ongoing data acquisition from the entire device or being able to ensure that all channels are operating at full capacity during testing. For all previous tests completed on this device, results were taken on a randomly selected set of four channels on the device. These results are then generalized to be equivalent to every set of four channels on the device. Further testing on this system would benefit from investigating an observation method capable of recording either the entire channel or the larger outlet portion.

4. Future Work in the Area

4.1 Continuing DEP Chromatography through Signal Optimization

DEP Chromatography as discussed in Chapter 2 can be an effective method for particle separation and identification which has significant application in biological studies. This method not only separates particles on a time basis for effective identification but does so with continuous solution flow through the device which increases the sample processing capabilities of the system. Our findings on this subject so far have reported high throughput separation with an effective chromatography system, due to the optimization of the individual variables that contribute to DEP force, including channel geometry and electrical signal characteristics. The dependence on signal characteristics is inherent to DEP force as seen in the Clausius-Mossotti factor, however few signal characteristics apart from frequency and amplitude are modified in other studies. Our work has investigated the effects of alternate waveforms, which has shown notable changes to the functions of the device. In addition to input signal waveform, the effects of amplitude modulation signals and varied length duty cycles have possible results on DEP systems and DEP chromatography.

Higher strength input signals have been shown to trap targeted particles within the DEP device without trapping others. This is beneficial for removing target particles from a solution but is not typically applicable to DEP chromatography as the targeted particles are held within the device rather than separated out in a constant flow. However, via the application of the chromatography principles of absorption and desorption, such high-power trapping signals can be applied to the DEP chromatography method. If such trapping signals can be turned on and off at a rapid rate, the targeted particles will be repeatedly partially trapped then released within the channel, while non-targeted particles will continue through the device with minimal interruption. If the input signal is amplitude modulated (AM) then this exact method can be replicated, both in terms of an on-off signal and in full strength to reduce strength signals for partial particle trapping. Additionally, along the same method and ideas, applying a square wave that ranges from off to a high voltage suitable for DEP trapping will operate in a similar on-off manner, allowing the trapping and releasing of the target particle within the device. Both of these systems are dependent on the rate at which the system turns the signal on and off, which is the AM frequency or duty cycle of the square wave.

Operating the same system outlined in Chapter 2, AM signals and square waves of varied duty cycles have been investigated in a preliminary manner. Both methods have shown altered transit times for the particles within the device, allowing for enhanced particle separation and identification compared to the findings from the investigation of DEP force optimization. These preliminary results can be seen in Figure 4.1. However, these results require further investigation. AM signals are both dependent on the characteristics of the base wave being modulated and the characteristics of the amplitude modulation itself, including the frequency of the modulation, percentage change in amplitude, and the form of the AM wave. Duty cycle on a square wave signal is less dependent on direct parameters but is affected by indirect effects of the signal generation. Such square waves tend to alternate between positive and negative values of the same max voltage to create a voltage peak-to-peak waveform rather than the target positive to zero voltage form desired for the absorption-desorption effect. Varying duty cycle also created voltage offset issues, where the further from a 50% duty cycle , the signal will shift the longer portion towards 0 proportionally, towards the effect of a 0% or 100% duty cycle being a constant voltage at the positive or negative value. These variables require further investigation to better understand the effects and capabilities of new and different electrical signals on DEP and DEP Chromatography devices.

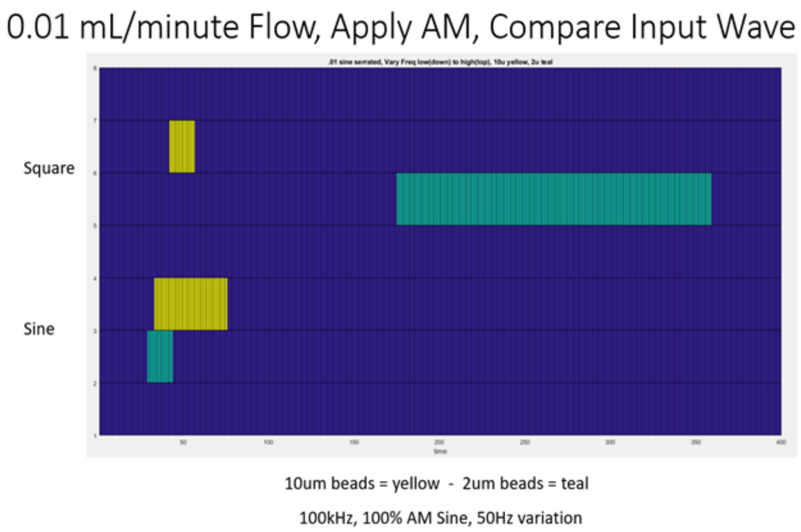

Figure 4.1: Preliminary results of the DEP Chromatography system using AM waves showing increased separation compared to standard AC Signals

4.2 Fiber Optic DEP Devices

The device types used in this thesis to investigate DEP effects have been MEMS devices, robust silicon and glass structures forming a channel to contain a fluidic sample while testing. These devices are beneficial for testing due to their low manufacturing costs; however, their applicable uses are limited due to their materials and occasional bulkiness. Other microscale devices have been tested for applications within the body. Of these alternative device types, optical fiber devices have shown promise in biological applications and particle research in areas such as neural study [1,2]. Applying DEP methods to these types of devices can then also allow for DEP particle manipulation in similar situations and conditions.

To utilize an optical fiber for DEP, the fiber is created with an internal channel similar to that of a MEMS device. This may be a circular or square channel, with or without geometric features inside. On opposing sides of this interior channel are two smaller channels, filled with copper wiring. These wires, when an electrical signal is applied across them, will create the electric field necessary to induce DEP force. The geometry of the interior channel and any geometric features such as grooves within the channel will generate high and low intensity areas of the electric field within the channel, allowing for nDEP or pDEP to take place on any particles within. Geometric features within the channel such as grooves or wells may function as trapping points to hold particles in place and separate them from a solution, or linear features may be constructed to attempt to recreate DEP chromatography effects within the fiber. Examples of these fiber devices can be seen in Figure 4.2.

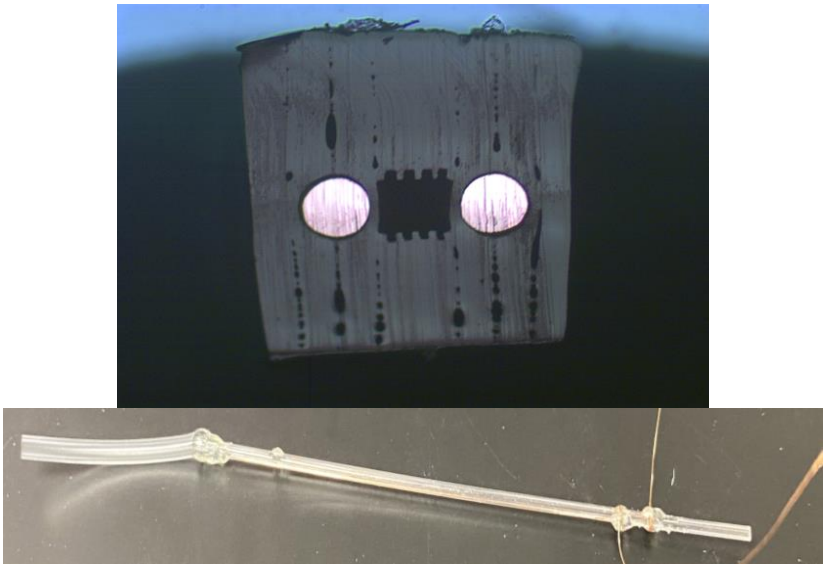

Figure 4.2: The DEP Fiber device viewable under microscope from one end to reveal channel structures and as a whole featuring inlet and electrode connectors

Testing with this form of devices was attempted in multiple manners. The common issue amongst DEP Fiber devices is shorting of the input signal between the copper wire electrodes in the device. This occurs in a manner of ways. The fiber device is open to all three channels on each end of the device. During fabrication, one end has the wires extended out of the device for connection points to the function generator and the opening of the main channel connected to tubing via epoxy, while the other end is not modified. This design allows for easy fabrication of each DEP device from a total length of fabricated fiber, however the open ends both in the epoxy coated inlet and at the device end create a connection between the two copper wire areas when fluid from the sample covers the open areas. This short connection burns through the fiber device and renders it useless. Multiple ways to address this issue have been attempted, including adding medical epoxy to seal the copper wire openings, altering the inlet and outlet connection methods, and changing the position and access points of the copper wires. These alterations made to the design process alleviated this signal shortage issue to a degree, yet the problem was still prevalent enough to prevent consistent testing of the devices.

References

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5. Conclusion

This thesis presents the culmination of my research on DEP microfluidic devices. Several devices and methods were tested, each developed and tested in order to investigate the ability to increase device throughput. Chapter 2 focuses on the last of these methods I developed, building off the work of previous students within our lab group on the concept of DEP chromatography. Utilizing an isometric triangle channel structure and EπDEP methods to perform a time-based separation of particles that are constantly moving through a device at high rates. A full investigation of the variables that affect DEP force and contribute to the chromatographic effect was completed to compile a full understanding of how to operate such a system and achieve usable results. Chapter 3 reviews the predecessor to the device in Chapter 2, a DEP device using the original version of the triangular channel design while operating 72 parallel DEP channels to achieve particle trapping at a much higher rate than possible with an individual system. Lastly, Chapter 4 reviews the remaining topics of investigation from my investigation of DEP microfluidics, including attempts to operate DEP in a fiber device and to continue the Chromatography project with higher separation results via the use of different input signals such as AM signals. These topics, while promising in initial results, require further investigation that could not be completed within the scope of this thesis.

In order to participate in the research presented in this thesis, I had to gain the skills necessary to fabricate and operate such systems. This included design software to plan and construct the designs for devices used, capable operation of cleanroom facilities and tools to fabricate said devices, and laboratory practice. This is not only to know how to accurately test these systems but to understand and be able to correct issues that arise. In addition to technical skills, my literacy on the subject has been enhanced via the reading of several recent and relevant papers on the topic, as well as in writing and compiling materials for grant proposals involving the topic.