A Combined Microfluidic/Dielectrophoretic Microorganism Concentrator

by

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Bachelor of Science, Electrical Science and Engineering

Submitted to the Department of Electrical Engineering and Computer Science in Partial Fulfillment of the Requirements for the Degree of

Master of Engineering in Electrical Engineering and Computer Science

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ABSTRACT

This thesis presents the development of a high-throughput microfluidic microorganism concentrator for pathogen detection applications. Interdigitated electrodes lining the bottom of the channel use positive dielectrophoretic forces to trap particles. A passive mixer is used to circulate the liquid in the channel, transporting particles to within the trapping region of the electrodes. Samples are extracted and their concentrations measured using a spectrophotometer. Concentration enhancements up to 40× at 500 µl/min flowrate using polystyrene microspheres and up to 10× at 100 µl/min flowrate using B. subtilis spores are achieved.

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Chapter 1: Introduction

This thesis presents the development of a microfabricated electric-field device for microorganism concentration to aid in the detection of biological warfare agents. The intended objective of this concentrator is to preconcentrate a sample of bacterial endospores in deionized water for downstream detection.

1.1 Background

Pathogen sensing is essential in many applications, including medical systems, water purification, and biological warfare agent detection. Among the approaches that exist for pathogen detection, microfluidic devices are especially appropriate as they operate optimally with particles on the micron scale. One such microfluidic pathogen detector is the μFAIMS, introduced by R. A. Miller et al. [1]. A limitation of this and other microfluidic pathogen detectors is the dependence of their sensitivity and detection threshold on the concentration of the sample they process. A concentrated sample of a given particle makes that particle easier to detect.

Another limitation, which is generally common in microfluidics, is the mismatch between the volume of samples obtained by standard collection methods and the volume a microfluidic device can process in a reasonable amount of time. Collected sample volumes are often on the order of milliliters, whereas microfluidic devices process volumes on the order of microliters. This mismatch should be corrected not by discarding the majority of the sample but by discarding the majority of the liquid while keeping all of the particles of interest.

A concentrator can be used to overcome both the sample preparation and the volume mismatch limitations by accumulating the particles of interest from a sample and resuspending them in a smaller volume of solution. The result is a highly concentrated sample of a volume that can easily be processed by a pathogen detector or other microfluidic device.
Concentration requires selectively applying a force to the particles of interest, but not to the liquid in which they are suspended. Common methods of concentration include membrane filtering and centrifugation.

Centrifugation accelerates particle settling in a liquid medium by applying a centrifugal force that acts on the difference between the particle and the medium densities. Centrifugation is fast, processing volumes on the order of 10 ml in a few minutes. It is often performed using a bench-top centrifuge, however, and requires human intervention steps (pipetting, aspirating, loading samples). In general, it does not readily lend itself to miniaturization and is impractical for integration with microfluidics.

Membrane filtering utilizes a porous membrane to selectively trap particles larger than the pores while the medium flows by unaffected. Membrane filtering is efficient in trapping particles above a certain size. However, as particles accumulate on the membrane, they obstruct the flow path of the medium; membranes are therefore prone to clogging. Additionally, it is difficult to remove a sample from the membrane. Analysis must be performed at the membrane, so in this case the concentration method shapes the design of the rest of the system, as in [2]. Following analysis, a backflow has to be generated to remove particles from the membrane, requiring a more complicated flow setup. Finally, once a membrane filter has been installed in a fluidic system, it cannot easily be disabled or removed.

Both centrifugation and membrane filtering are generally macro-scale techniques. It is desirable, however, to achieve concentration on the micro-scale. A micro-scale concentrator could be integrated with a microfabricated detection element, creating a complete microfabricated system. Direct integration of the two components eliminates the need for human intervention in delivering the concentrated sample to the detector.

Several methods exist for concentrating particles at the microscale. Several acoustic microconcentrators have been developed [3-5] that concentrate particles by focusing them to the nodes or antinodes of standing acoustic waves. Membrane filtering has also been performed on the microscale [2].

An alternative approach to concentrating particles on the microscale is to use electric fields to either apply forces to a charged particle (termed electrophoresis), or to
induce a dipole in a particle and act on the dipole. The latter method is known as
dielectrophoresis (DEP).

DEP refers to the force on polarizable particles in a spatially non-uniform electric
field, and provides a suitable method for manipulating microparticles in liquid suspension. It applies forces on the microscale and therefore works optimally with microscale particles and devices, integrating seamlessly with microfluidics. Two types of forces can be exerted: particles can be pulled toward points of maximum electric field (termed positive DEP, or pDEP) or pushed toward locations of minimum electric field (termed negative DEP, or nDEP). Dielectrophoretic forces can be produced using simple planar electrodes, making devices simple to fabricate. Moreover, electric fields can be quantitatively modeled, enabling optimization of device design. Finally, DEP is an active mechanism that can easily be turned on and off.

Several DEP concentrators have been presented in the literature. Particle accumulation has been demonstrated using both pDEP [6-8] and nDEP [9]. However, sample concentration is often evaluated optically on-chip. Furthermore, devices are often operated at low (~1 μl/min) flowrates and tested using large (~5-μm-diameter) particles.

The concentrator presented here is designed to prepare a sample for pathogen detection; specifically, it is designed to interface between an air sampler, which suspends particles from air into a known liquid, and a pathogen detector. The concentrator is therefore targeted to concentrate B. subtilis endospores (~1 μm diameter) suspended in deionized water at 500 μl/min throughput. It is implemented as a microfluidic device so that it is integrable into a microfluidic system. Concentrator performance is assessed quantitatively, by extracting a sample and directly measuring its concentration.

1.2 Device Overview

The initial device design consists mainly of a simple microfluidic channel, with electrodes lining the bottom and grooves lining the top (Figure 1-la). The concentrator utilizes pDEP, pulling particles to points of maximum electric field, to collect particles. A passive mixer circulates the liquid in the channel, bringing particles to within the trapping region of the electrodes, as illustrated in Figure 1-1b. As a sample of low particle
concentration flows through the channel, the electrodes selectively apply a downward force to the particles. The surrounding medium is not affected and is sent to waste. When enough particles have been accumulated on the electrodes, the force is deactivated and the particles are resuspended into a smaller volume, thereby producing a concentrated sample (Figure 1-1c).

Interdigitated electrodes are used to apply a pDEP force to collect particles. This force is limited by the applied electric field and decreases exponentially with distance from the electrodes. Moreover, for a given voltage, the field exhibits a characteristic tradeoff between its strength at the bottom of the channel and its reach into the channel. The device must be designed such that the DEP force is strong enough to both trap and hold particles in high flowrates.

Figure 1-1: Device overview

(A) Illustration of the main concentrator components, showing an enclosed microfluidic channel. Grooves in the top of the channel create a passive mixer that circulates the fluid and particles as illustrated in (B). Interdigitated electrodes lining the bottom of the channel trap and accumulate particles, as illustrated in (C). Accumulated particles are released into a concentrated suspension.
1.3 Thesis Overview

In this thesis, I present the development of the microorganism concentrator. I begin with an overview of dielectrophoresis and a description of the modeling and its outcomes in Chapter 2. I then outline the fabrication and packaging processes in Chapter 3. In Chapter 4 I describe the materials and methods I employed when performing experiments. In Chapter 5, I present the results I obtained, followed by challenges I encountered and possible improvements in Chapter 6. I conclude the thesis in Chapter 7.
Chapter 2: Design and modeling

Developing a system that produces a concentration enhancement at sufficiently high throughput requires a quantitative characterization of the system. Fortunately, the expertise of our research group lies in modeling systems that combine dielectrophoresis with simple microfluidics. We have developed modeling software [10] that calculates the trajectory of a particle subjected to electric fields and flow. Systems designed in our lab [9-13] show that the predictions made by this tool are close to experimental results.

This concentrator consists of two main components: the electrodes, which trap particles, and the mixer, which circulates the liquid to bring particles to within reach of the electrodes. To fully characterize the device, it is necessary to model both of these components, as well as the particle suspension to be concentrated. I modeled the system to predict its behavior and to determine optimal dimensions and operating parameters. In this chapter I describe the modeling process and how I used the results to design the concentrator.

2.1 Dielectrophoresis

The concentrator utilizes the properties of dielectrophoresis (DEP) to trap and concentrate B. subtilis spores. Dielectrophoresis refers to the force exerted on a polarizable particle by a spatially varying electric field. By acting on differences between the electrical properties of the particle and those of its surrounding medium, the electric field polarizes the particle and then acts on the induced polarization. If the particle is small compared to the length scale typical of the field nonuniformity, the induced polarization can be approximated as a dipole similar to that induced by a uniform electric field. The spatially varying field exerts a force of different magnitude on either side of this dipole, resulting in a nonzero net force and therefore in particle motion.

Based on differences in electrical properties between the particle and the medium, the particle will exhibit either negative dielectrophoresis (nDEP), in which it is pushed toward electric-field minima, or pDEP, in which it is pulled toward electric-field maxima.
If the particle is more polarizable than the surrounding medium, the path of least resistance for the electric field is through the particle. Negative charges will aggregate at the end facing the positive electrode directing the electric field into the particle; positive charges aggregate at the end of the particle facing the negative electrode, directing the electric field back out of the particle. The result is a dipole oriented opposite the electric field, as illustrated in Figure 2-1a. Opposite charges attract and the particle is pulled toward the point of maximum electric field, thus undergoing pDEP.

**Figure 2-1: Dielectrophoresis**

(A) Positive dielectrophoresis. A dipole is induced opposite the direction of the electric field, and is attracted toward the point of maximum electric field. (B) Negative dielectrophoresis. The dipole is oriented in the same direction as the electric field, and is repelled toward electric field minima.

The opposite is the case if the particle is less polarizable than the medium. In this case, positive charges aggregate at the end of the particle nearest to the positive electrode, diverting the electric field out of the particle and toward the more polarizable medium. The dipole is oriented in the direction of the electric field, as illustrated in Figure 2-1b, and gets repelled away from the point of maximum electric field, thus undergoing nDEP.
The polarizability of the particle is a lumped measure of its electrical conductivity and permittivity. For a uniform particle, the polarizability is defined as

\[ \epsilon_{m,p} = \epsilon_{m,p} + \frac{\sigma_{m,p}}{j\omega} \]  

(2-1)

where \( \epsilon \) is permittivity, \( \sigma \) is electrical conductivity, \( j = \sqrt{-1} \), and \( \omega \) is the frequency of the applied electric field. Subscripts \( m \) and \( p \) indicate properties of the medium and particle, respectively. This equation shows that polarizability is dominated by conductivity at sufficiently low, and permittivity at sufficiently high operating frequencies.

For systems in which higher orders of polarization (e.g. quadrupoles, octopoles) can be neglected, the dielectrophoretic force is derived by calculating the force exerted by a spatially varying electric field on a dipole induced by a uniform field [14]. For a homogeneous spherical particle of radius \( R \), the dipole moment is induced by a uniform electric field \( \vec{E}_0(r) \) is

\[ \vec{p} = 4\pi \epsilon_m R^3 \cdot \vec{E}_0(r) \cdot \left( \frac{\epsilon_p - \epsilon_m}{\epsilon_p + 2\epsilon_m} \right) \]  

(2-2)

Based on the assumption of negligible higher-order polarization, this moment is approximately equal to that induced by a spatially varying electric field \( \vec{E}(r) \) that is similar to \( \vec{E}_0(r) \) near the particle. The force exerted on a dipole by an electric field \( \vec{E} \) is defined as \( \vec{F} = \vec{p} \cdot \nabla \vec{E}. \) Applying this to the dipole moment above yields the DEP force:

\[ \vec{F}_{\text{DEP}} = 2\pi \epsilon_m R^3 \cdot \text{Re} \left\{ C(\omega) \right\} \cdot \nabla |\vec{E}(r)|^2 \]  

(2-3)

15
\( \vec{E}(r) \) is the complex applied electric field and \( \omega \) is the frequency of the field.  

\[ CM(\omega) \] is the complex Clausius-Mosotti (CM) factor, defined as:

\[
CM \triangleq \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m}
\]  \( \text{(2-4)} \)

The CM factor is a lumped measure of the polarizabilities of the particle and the medium relative to each other. For a homogeneous spherical particle, the real part of the CM factor can vary between -0.5 and 1. The sign of the CM factor dictates the polarity of the dielectrophoretic force. If the CM factor is negative, the particle will undergo nDEP and will be pushed toward electric-field minima; if the CM factor is positive, the particle will undergo pDEP and will be pulled toward electric-field maxima. Moreover, the magnitude of the DEP force is proportional to the magnitude of the CM factor. These observations imply that, for exactly similar field gradients, a stronger force can be produced by pDEP than nDEP, motivating the use of pDEP in the device.

In this device, both the medium conductivity and the operating frequency are design parameters. To achieve a pDEP force of high magnitude, I chose deionized water as the medium because of its low conductivity. I also chose to operate the device at low (\(-500 \text{ kHz} \leq 1 \text{ MHz}\)) frequencies so that conductivity dominates in the region of operation. Bacterial spores have higher conductivity than deionized water, so these choices enable the use of pDEP to accumulate, and thereby concentrate, the spores.

### 2.2 Spores

The target organism for concentration in this device are endospores of the bacteria *Bacillus subtilis (B. subtilis)*. An endospore is a resting stage in the life cycle of some bacteria that enables a bacterial colony to survive harsh conditions [15]. Spores are therefore resistant to a variety of environmental stresses such as lack of nutrients, chemical disinfectants, and even radiation. *B. subtilis* spores are non-pathogenic and are often used as a safe substitute for *B. anthracis*, commonly known as Anthrax. Anthrax is
used as a biological warfare agent, and is distributed in spore form due to its resistant nature as such.

![Microscope image of B. subtilis spores](image)

*Figure 2-2: Microscope image of B. subtilis spores*

Taken using a 63× objective on the microscope described in Section 0. Using image processing techniques, I have estimated the average spore radius to be approximately 500 nm.

Most of the information that exists about *Bacillus* endospores describes their composition and size [16, 17], as well as methods for killing them, *e.g.* [18-20]. To my knowledge, no published information exists about their dielectric properties. I have used microscope photographs of the spores, such as the one shown in Figure 2-2, to estimate their size at about 1 μm in diameter, which agrees well with published values. However, without information on their dielectric properties, it is impossible to calculate a CM factor for the spores and thus predict their behavior when subjected to electric fields.

To overcome this limitation, I have modeled the spore as a homogeneous spherical particle 1 μm in diameter and assumed a CM factor of 0.3 for all simulations. Section 2.1 showed that the CM factor for positive dielectrophoresis has a maximum value of 1. A CM factor of 0.3 is therefore a reasonably conservative estimate.
2.3 Electric Fields

The electric fields in this concentrator are produced by a set of interdigitated electrodes lining the bottom of a microfluidic channel. Interdigitated electrodes produce a wide region dense with electric field maxima at which particles can be trapped, resulting in near-uniform trapping along the length of the channel. Due to their periodicity and symmetry, interdigitated electrodes are relatively simple to microfabricate and to analyze.

A closed-form analytic solution exists for the electric field produced by interdigitated electrodes, published by Chang et al. in [21]. Streamlines of the electric field produced by this solution are shown in Figure 2-3. I coupled this solution with our modeling software to predict the dielectrophoretic force on a particle of known CM factor or dielectric properties.

Our modeling software, ForceSim, is a suite of Matlab software that was developed by our research group [10]. This software calculates the trajectory of a particle suspended in a medium, as determined by the flow rate of the medium and by an electric field. The suite takes as its inputs the system parameters: an electric-field geometry, dielectric properties of the particle and its surrounding medium, and channel dimensions, among others. It uses these inputs to compute the drag, dielectrophoretic, gravitational, and lift forces exerted on the particle. For a particle to be trapped, the dielectrophoretic force on the particle has to be stronger than the drag force imposed by the flow. The program balances these forces, and uses the resulting net force to compute and draw the trajectory of a particle as it flows through the channel, and thus determine whether a set of operating conditions enables the electric field to trap the particle.
I used this tool to predict the trajectory of a spore in a channel, while varying parameters such as flow rate, electrode width and spacing, particle start height, and CM factor. Based on these simulations, I have come up with relationships that have enabled me to design the concentrator.

Interdigitated electrodes exhibit a tradeoff between the strength of the electric field and how far it reaches above the surface of the electrodes. For a given applied voltage, this tradeoff is controlled by the dimensions of the electrodes ([electrode width] = [spacing between electrodes] = \( d \)). Narrow electrodes spaced close to each other result in stronger, more localized electric fields. Wider electrodes spaced farther apart increase the reach of the field but decrease its strength.

In the context of this concentrator, the reach of the electric field controls how well the device can trap particles from within the channel. The strength controls the ability to hold particles against flow once they have been trapped. To determine the optimal electrode dimensions, I found it useful to decouple the reach and strength from each other and investigate each separately. I used simulations of the electric field reach to define the electrode dimensions, and simulations of the strength to set the operating conditions, as described in the next few paragraphs.

The reach of the electric field controls the height from which particles can be trapped, as well as the distance they travel in flow before they are trapped. Every set of operating conditions has a maximum flowrate associated with it beyond which the electric field is unable to hold particles against flow. Reach can be considered separately from strength if the flow is slower than the maximum flowrate associated with the simulation operating point. I investigated electric-field reach by starting a particle at varying heights \( h \) above the channel bottom and measuring the resulting capture distance. I have defined the capture distance to be the distance in the direction of flow that a particle travels before it is trapped (see Figure 2-4a). One instance of an electric-field reach simulation is shown in Figure 2-4a. The particle starts at 15 \( \mu \)m above the channel bottom and is gradually pulled toward the bottom by the electric field.
In the results of the reach simulations, I observed that for every height at which the particle starts, there exists an electrode width $d$ that minimizes the capture distance (Figure 2-4b). This result is independent of voltage, flowrate, and CM factor. Varying these parameters changes the value of the capture distance, but the electrode width that minimizes the capture distance remains the same. The relationship between electrode width and start height is a one-to-one linear relationship and is shown in Figure 2-5.

I used this relationship, in combination with an expected particle starting height, to determine the characteristic electrode dimension $d$. I chose the starting height based on the flow profile in the Staggered Herringbone Mixer, discussed further in Section 0. Published mixer results confirm mixing only in the central 50% of the channel cross-section [22]. This implies that the lower 15% of the 100-μm-deep channel is less well-mixed than the central region. Within these 15 μm, the mixer no longer circulates particles as efficiently, so this is where trapping needs to be optimized.

---

**Figure 2-4: Electric-field reach simulation.**

(A) ForceSim simulation output showing a particle with starting height $h = 15 \, \mu m$ being trapped by 10-μm-wide electrodes, resulting in a capture distance of 100 μm. (B) Plot of capture distance vs. electrode width for 15 μm start height, with same conditions as in (A). For $h = 15 \, \mu m$, the electrode width that minimizes capture distance is approximately 15 μm.
Figure 2-5: Picking an optimal electrode width.

(a) Plot of capture distance vs. electrode width for particles starting at 5, 10, and 15 μm above the channel bottom. For every start height, there is a corresponding electrode width that minimizes capture distance. (b) There is a linear relationship between start height $h$ and the corresponding electrode width that minimizes capture distance. This relationship is independent of flowrate, voltage, and CM factor, though varying these parameters does change the capture distance.

Ideally, to optimize trapping within 15 μm, the start height should be 7.5 μm. However, based on the relationship in Figure 2-5b, this would require about 6-μm-wide electrodes, which pushes the processing capabilities available to me. For a geometry that is relatively simple to fabricate, I chose 10 μm as the nominal electrode width and spacing. 10 μm is still relatively narrow, increasing the electrodes’ vulnerability to shorting. I therefore fabricated two wider sizes as well. First, I chose a 50-μm width, which targets a start height that is half the channel depth and is therefore optimal for a channel without a mixer. Second, I chose a 25-μm width as it combines the ease of fabrication of wide electrodes with the electric field strength of narrow electrodes.

Once a particle is trapped, the strength of the electric field determines the ability to hold it against flow. I quantified strength using the maximum flow rate against which the electric field can hold a spore. To decouple the strength from the reach of the electric field, I simulated particles starting on the channel bottom ($h = 0$). This demonstrates how a particle behaves after it has been trapped, and eliminates the need to simulate the
trapping, which shortens the length (in the direction of flow) that needs to be simulated: due to the periodicity of the electric field, all electrodes exhibit equal strength and I needed to simulate only one electrode. Figure 2-6a shows an output of a simulation in which the particle is held by the electric fields. The trajectory of the center of the particle is plotted. The particle starts and remains at the bottom of the channel (center 0.5 μm, which is the particle radius, above channel bottom) and travels (in the direction of flow) only until it reaches the point of maximum electric field.

\[ Q_{\text{max}} \propto \frac{1}{d} \]
\[ Q_{\text{max}} \propto V^2 \]
\[ Q_{\text{max}} \propto \text{Re}\{CM\} \]

\( Q_{\text{max}} \) is the maximum flowrate against which a particle can be held; \( d \) is the electrode width, also equal to the spacing between electrodes; \( V_{pp} \) is the peak voltage.
difference between the electrodes; and $\text{Re}[CM]$ is the real part of the particle CM factor. Of these four parameters, only $d$ is set in the device design. The remaining three, while coupled together, do not need to be set in the device design. These parameters can be adjusted during testing to tune the performance of the device to account for the unknown spore properties and meet the design specification.

For example, if the actual CM factor of the spores is 0.3, which is the value I used in simulation, then the modeling predicts that 18 V$_{pp}$ are required to hold the spores against a flowrate of 500µl/min. If the CM factor of spores is lower than 0.3, the applied voltage can be increased to compensate. If the CM factor is higher than the value used in the simulations, the voltage can be lowered, or a high voltage can still be used to achieve an increased throughput. Thus, the device is robust to a range of CM factors and the dielectric properties of the spores do not need to be extracted. Moreover, for a conservative estimate of the CM factor, simulations predict that the device meets the design specification.

2.4 Mixer

The passive mixer used in this concentrator is the Staggered Herringbone mixer, introduced by A. Stroock et al. in [22]. The mixer consists of grooves in the channel top or bottom that are oriented diagonally with respect to the direction of flow (Figure 2-7b). The grooves create helical flows in opposing directions (Figure 2-7a), and the mixer mixes by alternating the direction of the helix every half-cycle (Figure 2-7c).

The resulting fluid behavior is chaotic and is therefore difficult to model and integrate with the electric field models. However, while the flow profile throughout the channel is different in a simple rectangular channel than in a channel with the mixer, the average fluid velocity close to the channel walls is similar. Because spores are trapped by electrodes on the channel bottom, it is reasonable to use a rectangular channel to predict the strength of the electrodes and thus the maximum flow rate against which they can hold a particle.
Results published in [22, 23] define relative channel dimensions that produce optimal mixing. The channel and mixer are fabricated using a two-layer SU-8 process, where the first layer defines the channel and the second defines the mixer. To simplify the fabrication process, I chose a channel depth of 100 μm, as uniformity becomes difficult to control beyond this thickness. The remaining channel dimensions are defined by relationships in the publications ([22, 23]). The dimensions are illustrated in Figure 2-7b and summarized in Table 1.
Table 1: Channel dimensions based on mixer publications.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Governing relationship</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td>Average channel depth</td>
<td><em>Chosen to simplify fabrication</em></td>
<td>125 μm</td>
</tr>
<tr>
<td>w</td>
<td>Channel width</td>
<td>$w/h = 2$</td>
<td>250 μm</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Half groove depth</td>
<td>$\alpha = 0.2$</td>
<td>25 μm</td>
</tr>
<tr>
<td>l</td>
<td>Length of one mixing cycle</td>
<td>$l/h = 12$</td>
<td>1.5 mm</td>
</tr>
<tr>
<td></td>
<td>Channel length</td>
<td>$\exists 15$ mixing cycles</td>
<td>25 mm</td>
</tr>
<tr>
<td>r</td>
<td>Groove asymmetry</td>
<td>$r = \frac{2}{3}$</td>
<td>166 μm</td>
</tr>
</tbody>
</table>
Chapter 3: Microfabrication

The two main components of the concentrator – the base containing the electrodes, and the channel with patterned mixing grooves – are fabricated separately and then aligned to each other manually on the die level. Both are microfabricated in a cleanroom by a process that uses mainly photolithography and common MEMS fabrication techniques. The base of the channel is made of Pyrex and contains electrodes patterned in gold. This piece not only serves as the bottom of the channel but also produces the electric fields and provides fluidic access to the device. The channel and chaotic mixer are molded in poly(dimethylsiloxane) (PDMS) using an SU-8 structure. In this chapter, I describe the process I used to fabricate both parts of the device, as well as the packaging that facilitates interfacing between this micro-scale device and its macro-scale inputs and outputs.

3.1 Masks

The two parts – electrodes and channel – of the microconcentrator are fabricated separately and each requires its own set of masks for photolithography. I used one mask to pattern the electrodes and two to pattern the channel: one each for the channel and mixer. All three were 7” chrome masks (Fineline Imaging, Colorado Springs, CO). I chose dimensions for the masks based on the results of the modeling presented in Chapter 2. In this section, I describe some of the key features of these masks in detail.

3.1.1 Electrode Mask

The first mask, used for patterning the electrodes, is shown in Figure 3-1. This is a bright-field mask so that it can be used in a liftoff process. The mask patterns 13 functional dice and contains features designed to simplify fabricating and using the device.
An important design feature of this mask is that the interdigitated electrodes on each die are patterned in four isolated sections, rather than one long section. One such section is shown in Figure 3-1a, for 25-μm electrodes. I used this approach to increase my device yield. Because the resources and facilities I used are intended for research and prototyping, they cannot reliably produce large sets of 10-μm lines spaced 10 μm from each other, as required for interdigitated electrodes. Thus, it is likely that at least one pair of adjacent electrodes will be shorted to each other. I defined four isolated sets of electrodes so that if a pair of electrodes in one section is shorted, the remaining three still behave as desired.

Each of the 13 dice on this wafer has electrodes of 10-, 25-, or 50-μm width and spacing. Building electrodes with a variety of dimensions enabled me to test the characteristic tradeoff between strength and reach in the electric field produced by these electrodes. Each die also contains a resistor whose width is the same as that of the

Figure 3-1: Electrode mask

Background shows an AutoCAD drawing of the mask used for patterning electrodes. Magnifications show: (A) one of four sections of 25-μm interdigitated electrodes; (B) alignment mark; (C) device label; and (D) marker for fluidic access hole. Images were acquired by splicing together several micrographs, giving rise to the variations in shading across the image.
electrodes on the same die and whose resistance is nominally 100 Ω. The purpose of the resistor is to evaluate process parameters, namely the gold thickness or width. Resistances on the 50-µm devices were consistently within 5% of 100 Ω. Resistances on the 10-µm devices were observed to be less accurate and ranged from approximately 120 Ω to 250 Ω.

There are also visual markers on the die- and wafer-level that are not electrically functional. On the die-level, there are crosshairs (Figure 3-1d) to indicate the desired location of the fluidic access holes, as well as labels that identify each device (Figure 3-1c). On the wafer-level, there are alignment marks (Figure 3-1b) to enable the addition of a second mask if necessary. I did not use a second mask, but one could be used, for example, to cover only certain areas of the die with a layer of passivation. Also on the wafer-level are dicing streets and resolution features to help assess the quality of the photolithography (shown on SU-8 mask, Figure 3-2e).

### 3.1.2 SU-8 Masks

I built the microfluidic channels by means of a molding technique in which microfabricated, raised SU-8 structures are used as a mold to define grooves in PDMS. The SU-8 master mold required two layers of SU-8 patterning: one to define the channel, the other to pattern the mixing grooves. Each layer required one photolithography mask. The two SU-8 masks are shown in Figure 3-2. The first mask (shown in blue) patterns 28 channels all having the same dimensions. The second mask (shown in purple) adds the mixing grooves. Figure 3-2b shows a portion of the two masks in more detail. Both masks are dark-field masks because SU-8 is a negative photoresist.

I included mixing variability in the mask design to facilitate evaluating the effect of the mixer on the device. The mixer mask adds mixing grooves to only some of the channels, while others remain completely smooth (simple channel without mixer). Some of the mixing channels have grooves along the full length of the channel, while some have smooth and mixing sections. In theory, if chaotic flow is detrimental to the holding characteristics of the device, this alternating design should provide sections that circulate
particles and section that trap them. In every channel containing the mixer, the width of the mixing grooves is 5 \mu m less than that of the channels to provide alignment tolerance.

Similarly to the electrode mask, the SU-8 masks also contain visual markings essential to fabrication and packaging. I used cross-hair alignment marks (Figure 3-2c) on both masks to align the masks to each other. A window around the alignment mark on the second mask provides visual access to the cross-hair produced by the first mask. I have also included identifying labels (Figure 3-2d), dicing borders (Figure 3-2d), and resolution features (Figure 3-2e), all similar to those on the electrode mask.

**Figure 3-2: SU-8 mask**

Background shows an AutoCAD drawing of the masks used for patterning channels (purple) and mixer grooves (blue). Magnifications show, patterned in SU-8: (A) fluid reservoir and one end of a mixing channel; (B) detail of mask drawing; (C) alignment mark; (D) die label and dicing streets; and (E) features for assessing photolithography. Images were acquired by splicing together several micrographs, giving rise to the variations in shading across the image.
3.2 Microfabrication Process

I fabricated the concentrator in MIT’s Technology Research Laboratory class 100 cleanroom. The fabrication consists of standard MEMS processing and photolithography steps. I have provided a complete outline of the fabrication process in the Appendix, and describe it in detail in the following sections.

3.2.1 Gold Processing

I patterned the electrodes on 6" Pyrex wafers (Bullen Ultrasonics, Eaton, OH) using a gold liftoff process. I chose Pyrex as the substrate because it provides electrical isolation. Due to its low thermal conductivity, Pyrex is sometimes avoided in integrated microfluidic/electronic systems, especially those with biological applications, to prevent overheating bioparticles. At low medium conductivity, however, little power dissipates through the device, thus temperature rises are much less significant. This device is intended for operation with low-conductivity fluid (deionized water), and Pyrex is therefore an appropriate choice.

The liftoff process is a straightforward method of patterning metals that utilizes standard photopatterning and metal deposition techniques and eliminates the requirement of a specific metal (i.e., gold) etchant. In the liftoff process, a uniform layer of the metal is evaporated onto patterned photoresist, which is subsequently dissolved in acetone. The metal in direct contact with the substrate remains, while the metal that was deposited onto photoresist is lifted off when the resist is dissolved.

Figure 3-3 outlines the main steps of the gold liftoff process. I begin the process with blank Pyrex wafers (Figure 3-3a) that I clean for 10 minutes in a Piranha (1:3 H₂O₂:H₂SO₄) solution to remove organic contaminants, then rinse with deionized water and spin dry. I dehydrate the wafers (20 mins, 150°C) and then deposit vapor-phase Hexamethyldisilizane (HMDS) onto them at 150°C. HMDS promotes adhesion of photoresist to the substrate. Next, I spin (final speed 3000 rpm) (Figure 3-3b) and pattern (Figure 3-3c) a layer of AZ5214 photoresist using an image-reversal process, such that
the areas to be covered with gold are bare. AZ5214 is a positive resist whose polarity can be reversed via a flood expose. The thickness of the layer of resist is not critical as it serves solely as a mask for gold patterning, but in my case was about 1.7 μm. To pattern the photoresist, I first pre-bake it (30 mins, 90°C), expose (2.3 sec, 10 mW/cm²/sec, 365-405 nm) and post-bake (30 mins, 95°C). I then flood expose the entire wafer (60 sec, 10 mW/cm²/sec, 365-405 nm) to reverse the polarity of the photoresist and finally develop the resist (70 seconds, AZ 422 developer).

Figure 3-3: Electrode fabrication process
Illustration of the gold liftoff process used to fabricate the electrodes. The process begins with a clean Pyrex wafer (A), onto which photoresist is spun (B). The photoresist is patterned (C) and a layer of gold is evaporated onto it (D). The photoresist is dissolved in acetone, lifting off the gold on top of it and leaving only the desired structures patterned in gold.
I have observed the photolithography process to be very sensitive to both the pre- and post-bake steps. More specifically, changes in temperature during these steps have often resulted in photoresist not developing uniformly across the wafer; that is, there are areas of the wafer in which the resist does not develop. There is a vent in the oven that blows hot air into the chamber to keep it at the desired temperature. I found that baking wafers so that the backsides face the vent prevents this problem.

Following photolithography, I evaporate 100 Å of titanium onto the wafer, followed by 2000 Å of gold (Figure 3-3d). Titanium promotes adhesion of the gold to the substrate. I soak the wafer in acetone for about 3 hours to remove the photoresist and lift off the unwanted gold. This results in the desired structures patterned in gold on a clean Pyrex substrate (Figure 3-3e).

The final step in this process is to dice the wafer. Prior to dicing, I spin (final speed 500 rpm) and bake (45 mins, 90°C) a thick, protective layer of photoresist onto the entire wafer so that die saw slurry does not contaminate the patterned structures. A photograph of a fully-processed die is shown in Figure 3-4.

3.2.2 SU-8 Processing

I built the microfluidic channels by molding them in PDMS using a microfabricated SU-8 master mold. SU-8 is a photodefinable, epoxy-based negative resist. Fully patterned SU-8 structures are rigid and durable, and molding is therefore a widely-used technique for patterning microchannels.
I patterned the SU-8 master mold using a two-layer process, illustrated in Figure 3-5, defining the channels in the first layer and the mixer in the second. I carried out the process on silicon test wafers (WaferNet, San Jose, CA) to minimize cost, as there are no electrical considerations for this process. Following a dehydration bake (30 mins, 200°C) to promote adhesion of the SU-8, I spin the first layer, a 100-µm layer of SU-8 2050 (MicroChem, Newton, MA) using a final spin speed of 1680 rpm (Figure 3-5b). To pattern, I soft-bake the wafer (15 mins, 95°C), expose it (30 sec, 10 mW/cm²/sec, 365-405 nm) and post-bake (9 mins, 95°C) to harden the SU-8 (Figure 3-5c). Without developing, I repeat the process for the second layer. This time I spin 50-µm thick SU-8 2025 (final speed 2250 rpm) (Figure 3-5d), pre-bake (40 mins, 95°C), expose (23 sec, 10 mW/cm²/sec, 365-405 nm), and post-bake (10 mins, 95°C) (Figure 3-5e). I then develop both layers simultaneously (Figure 3-5f) in PM Acetate for approximately 10 minutes (visual stop).

I obtained estimated spin speeds, bake and exposure times for this process from datasheets published by MicroChem {#67; #68}. I used these parameters as a starting point for experimentation to achieve the desired results. I used spin speeds lower than those published to achieve 50- and 100-µm-thick layers. Additionally, I baked the second SU-8 layer for significantly longer than the suggested bake times. For example, I increased the prebake time from the suggested 6 minutes to 40 minutes. I did this to adjust for the presence of the first layer, as the MicroChem datasheets are intended for single layer processes.

I cast the fabricated mold with PDMS to create the channels (Figure 3-5f). Prior to molding, I silanize the wafer to make it inert to PDMS. Silanization is a vapor-phase deposition of HMDS, performed by placing the wafer and a small volume of liquid-phase HMDS in a vacuum chamber. The HMDS satisfies dangling bonds on the wafer to which PDMS would otherwise bond. I pour a 3-mm layer of PDMS (10:1 base:curing agent by weight) onto the entire wafer and set it to cure at 65°C for about 3 hours. The cured PDMS cleanly peels off the wafer, the desired channel and mixer features having been molded into it by the SU-8. I use a razor blade to cut the PDMS into individual channels.
Figure 3-5: Channel fabrication process

Illustration of key steps in the process used to fabricate the SU-8 master structures used for molding PDMS channels. The process begins with a Pyrex wafer onto which two layers of SU-8 are spun (B, D) and patterned (C, E). Both layers are developed simultaneously and the resulting structure is used for molding PDMS (F).
3.3 Packaging

Packaging provides a way to interface between the micro-scale concentrator and the macro-scale world, and is therefore an essential part of the device design. The concentrator packaging provides external electrical and fluidic access to the device, supplying power to the electrodes and a flow path into and out of the channel. It also holds together the Pyrex base and the PDMS channel. I designed the device with packaging in mind: features such as large bond pads and ample alignment tolerance result in a simple packaging process that takes about two hours to complete.

3.3.1 Components

I employed a packaging scheme based on that developed in [25]. The package consists of a custom-designed printed circuit board (PCB) (ExpressPCB, Santa Barbara, CA) and an aluminum base, as illustrated in Figure 3-6. The PCB provides electrical access to the device, using a pin and jumper configuration that allows each of the four electrode sections to be turned on or off on the fly. The PCB also provides fluidic access via holes that align with holes in the Pyrex die. Standoffs between the aluminum base and the PCB provide clearance for tubes that route the fluidics into the bottom of the device, so that the tubing does not obstruct optical access to the channel. The aluminum base provides a stable platform for the device. Its magnetic underside, formed by an adhesive magnetic sheet, keeps the device in place on a steel microscope stage. This added steadiness is

![Illustration of a packaged device](image)

*Figure 3-6: Illustration of a packaged device*
valuable for microscope observation of the device during experiments.

I designed the PCB to enable on-the-fly reconfiguration of electrical connections to the device, so that if one section of electrodes is shorted, it can easily be disconnected. The electrical setup consists of three pins on the PCB corresponding to each bond pad on the device (refer to Figure 3-6). The middle pin is connected to the bond pad via a trace in the bottom of the PCB. Of the remaining two pins, one is connected to a voltage and the other to ground, both obtained from the function generator. The middle pin can be shorted to either the voltage or ground pin using a jumper. In this way, each section of electrodes can be turned on by connecting one of its bondpads to a voltage and the other to ground, or off by leaving the bondpads unconnected (or connected at the same voltage). There is also an option to connect the bondpads to two different, non-zero voltages.

### 3.3.2 Assembly

To assemble the device package, I first prepare the Pyrex die. I drill fluidic access holes according to the markers (Figure 3-1d) using a 0.75-mm-diameter diamond drill bit (C. R. Laurence Co., Inc., Los Angeles, CA). The Pyrex die has a protective coat of photoresist applied prior to dicing the wafer, which I leave as a protective coat for drilling. When the holes are drilled, I rinse the die in a sequence of acetone, methanol, and isopropanol to remove the photoresist.

To minimize nonspecific adhesion of particles to the electrodes during experiments, I coat the Pyrex die with a layer of Sylgard® Prime Coat (Dow Corning, Midland, MI). The purpose of this surface modification is further explained in Section 4.1.3. I apply the prime coat by dipping the Pyrex die in a diluted solution (10:1 Heptane:Sylgard® Prime Coat). I submerge the die in the solution for about 10 seconds and then remove it using tweezers, being careful not to let the die reenter the prime coat solution once it is removed. The prime coat dries almost instantaneously, so introducing the die back into the liquid solution would produce a second coating. Only a thin layer of the prime coat is required to minimize nonspecific adhesion, and any additional thickness has the unwanted effect of masking the electric field. After dipping the device in the
prime coat, I let it dry for about 5 minutes. I then pour a few drops of water onto it to verify that the surface is hydrophobic and therefore does not wet.

Next, I populate the PCB with header pins for electrical connections, and then prepare it for attaching the Pyrex die. I use NanoPort adhesive rings (Upchurch, Oak Harbor, WA) to simultaneously affix the die to the PCB and seal the fluidic access holes in the PCB to those in the die. I clean the surface of the PCB with isopropanol and apply the adhesive rings. I then darken the region of the PCB between the fluidic access holes using a black Sharpie marker. This decreases the background fluorescence emitted by the PCB during fluorescence microscopy so that beads, and especially dimmer spores, can be visualized more easily.

To affix the device, I place it on the PCB, aligning corresponding fluidic access holes, and clamp the two together using small binder clips. I use scrap pieces of PDMS to prevent direct contact between the binder clips and the device. I leave this configuration at 65°C for a few hours to let the adhesive set.

I make electrical connections by gluing wires to bond pads on the device using conductive Epoxy (ITW Chemtronics, Kennesaw, GA). I solder the other end of these wires to corresponding through-holes in the PCB. Finally, I pressure-fit PEEK tubing (Upchurch) into the fluidic access holes in the backside of the PCB, and use High Performance Epoxy (Loctite Inc., Rocky Hill, CT) to further seal this connection. I let the Epoxy set at 65°C for about 15 minutes.

Finally, I align a PDMS channel to the electrodes by matching the reservoirs at either end of the channel to the fluidic access holes in the Pyrex die. I set the PDMS on the Pyrex base with a glass slide on top and clamp using large binder clips. Binder clips provide enough pressure to prevent leaking at the desired flowrates and up to about 1000 μl/min. The glass slide aids in distributing the force exerted by the binder clips more evenly along the length of the device. If the force is still uneven and a leak is formed between the PDMS and the Pyrex die, I add another layer of PDMS and another glass slide to further distribute the force.

I do not use a permanent bond to attach the PDMS to the Pyrex. Over the course of my experiments it has proven beneficial to have the ability to take the device apart, for purposes of thorough cleaning or replacing the channel. A more permanent bond would
necessitate a lengthy cleaning process to strip the PDMS off the Pyrex, and moreover, the PDMS would not be reusable. However, while a temporary seal is suitable for prototyping, a more permanent bond could be used in the final device design. The Pyrex die is coated with a layer of Sylgard® Prime Coat to which PDMS bonds when both surfaces are treated with oxygen plasma. This would eliminate the need to clamp the device components together.

The packaged device can be unpackaged and all the parts reused. The PDMS is not permanently bonded to the die, thus is easily removed from the package. I soak the remaining components of the package (PCB, Pyrex die, tubes) in acetone for about an to softens the Epoxies and NanoPort adhesives. This allows me to remove the Pyrex die and PEEK tubes from the PCB. I use a razor blade to remove the High Performance Epoxy from the back of the PCB. Acetone removes the conductive epoxy from the wires that connect to the bond pads. These wires are soldered in to the PCB and can be reused. To remove the Sylgard Prime Coat from the Pyrex die, I soak the die in KOH (100 mg/mL DI water; VWR, Westchester, PA) for at least 4 hours. I remove the die from the KOH and immediately rinse it in heptane (anhydrous 99%; Sigma-Aldrich, St. Louis, MO). The prime coat can then be reapplied and the die repackaged using the same PCB.
Chapter 4: Materials and Methods

An essential part of developing this concentrator was to design a method for evaluating its performance. In this chapter, I describe the materials I used and their role in my experiment setup. I then outline the test procedure I used in the laboratory to evaluate the concentrator.

4.1 Materials

I begin the chapter by describing the materials I used while conducting experiments. I first discuss the particles I concentrated in the device – spores as the target organism, and beads as test particles – and the advantages and disadvantages associated with each. I then introduce a method to overcome the challenge of nonspecific adhesion, in which I use Sylgard Prime Coat to modify the surface and Alconox to release particles.

4.1.1 Spores

This microconcentrator is designed to preconcentrate a sample of anthrax for delivery to an anthrax detector. The target organism to be concentrated in the device is bacterial endospores, which is the form of anthrax used in biological warfare. To avoid unnecessary risk, spores of the bacteria *Bacillus subtilis* were used as a simulant for *Bacillus anthracis*. I used *B. subtilis* ATCC #6633 (later renamed *B. atrophaeus*), purchased from Raven Biological Laboratories (Omaha, NE). Bacterial endospores are resistant by nature and can be stored in deionized water at 4°C, so no incubation or special care was required.

One challenge in optically evaluating the device performance was visualizing the spores under a microscope. The spores are small (~1 μm diameter [16, 17]), and blend into the background in bright field. Thus, they are difficult to see without fluorescence. However, the spores do not naturally fluoresce, so they must be stained. An ideal stain fluoresces brightly, stains all of the spores, and photobleaches as little as possible.
I explored three different fluorescent stains: a nucleic acid stain (Syto 9, Invitrogen, Carlsbad, CA) and two spore coat stains (Wheat germ agglutinin, Alexa Fluor 594 conjugate, Invitrogen; and Concanavalin A, Alexa Fluor 488 conjugate, Invitrogen). In each case, I added the recommended amount of stain to a sample of spores and let sit for about one hour at 4°C. I then removed the stain by centrifuging the sample (5 mins, 14000 rpm, Centrifuge 5417R, Eppendorf, Hamburg, Germany) and replacing the supernatant liquid with deionized water. I repeated this procedure three times to reduce the amount of stain in the solution and thus minimize the background fluorescence.
To evaluate the properties of each stain, I pipetted a sample of stained spores into a PDMS gasket on a glass slide. I covered the gasket with a cover slip to prevent evaporation. I observed the fluorescence intensity of the sample by taking an image every second for 10 minutes.

The results of this assessment, presented in Figure 4-1, show that spores stained with wheat germ agglutinin (WGA) photobleach very slowly, losing less than 10% of their intensity over 10 minutes. Spores stained with Syto 9 photobleach quickly, losing more than 50% of their fluorescence intensity over about 2 minutes. Syto 9 is a nucleic acid stain and has to not only penetrate the thick spore coat but stains a very small part of the spore, hence the photobleaching.

WGA-stained spores, though resistant to photobleaching, were also very dim and difficult to see under the microscope. The Concanavalin A (ConA) stain provided a good compromise of fluorescence intensity and stability.

4.1.2 Test Particles

Although the concentrator is intended for use with bacterial endospores, there are several challenges associated with using them for testing and characterization of the device. First, to my knowledge, there is no published information on the dielectric properties of spores. This makes it impossible to model the dielectrophoretic forces on them. Second, because they are biological particles, their size and properties likely vary from sample to sample, as well as within a sample. Finally, as mentioned in Section 4.1.1, spores are difficult to visualize and difficult to stain.

It is necessary to show that the device reliably works with spores. However, for testing and characterization of the device it is advantageous to use test particles, overcoming many of the challenges associated with using spores. Test particles often have well-defined, uniform properties. They are also brightly fluorescent and exhibit negligible photobleaching.
The test particles I used are 1-μm-diameter fluorescent pink Carboxyl magnetic microspheres (Spherotech, Libertyville, IL). These are spherical polystyrene particles coated with a layer of magnetite and stained with a fluorophore [26]. Their surface is modified with carboxyl groups.

Because polystyrene is less conductive than water, larger unmodified polystyrene beads do not exhibit pDEP at any frequency. Sufficiently small beads can exhibit pDEP due to surface conductivity, which can dominate the electrical properties of the particle due to its small size. The beads I used exhibit pDEP because of their surface modification. The carboxyl modification forms a negatively-charged layer around the particle that is highly conductive. Due to the small size of the particle, the surface charge is able to dominate the bulk properties and the particles undergo pDEP. I have qualitatively compared the behavior of these beads to that of carboxyl-modified beads not coated with magnetite. Beads without magnetite also undergo pDEP in the same range of operating frequencies, suggesting that the magnetite is not what enables the beads to undergo pDEP.

To determine the operating frequency for trapping beads in the concentrator, I fit experimental crossover frequency data to a CM factor model to predict the behavior of the beads. To do this, I pipette a suspension of beads into a gasket on the electrodes and cover the gasket with a glass coverslip. Using a microscope, I observe the behavior of the beads as I vary the operating frequency applied to the electrodes. I alternate between nDEP and pDEP, first at extreme frequencies, and then narrow in on a
crossover frequency. There is a range of frequencies (~1 MHz) over which no response is observed. I define the crossover frequency to be the middle of this range.

I use the data from this experiment in Equation (2-4) to solve for the effective bulk conductivity of the beads, assuming \( \varepsilon_r = 4\varepsilon_0 \). The bead conductivity changes with medium conductivity because the surface conductance, which dominates bead conductivity, is affected. Based on the resulting effective conductivity, I use Equation (2-4) again to plot the predicted CM factor.

This curve suggests that the optimal operating frequencies are frequencies lower than ~100 kHz. However, because there is a dielectric coating on the surface of the electrodes (see Section 4.1.3), it is advantageous to operate at higher frequencies to minimize the shielding effect. I chose to operate the device at 500 kHz, where the CM factor has not significantly decreased.

4.1.3 Sylgard Prime Coat

One of the major challenges I encountered during testing was nonspecific adhesion of beads and spores to the glass substrate and electrodes. The device uses pDEP to trap these particles, and when the voltage is released to turn off the force, many of the particles do not release, even at increased flowrates. To produce a high concentration enhancement, all particles must be released simultaneously, into as small a volume as possible. This result is unachievable in the presence of nonspecific adhesion.

To address this issue, I sought a chemical surface modification or release agent that would efficiently release particles but would not attenuate the electric field or otherwise adversely affect trapping.

My solution to this problem combined a surface modification with a release agent to effectively release both beads and spores. I modify the surface with Sylgard Prime Coat, and release particles using Alconox. Sylgard Prime Coat is a coat that, when applied to the Pyrex die, renders its surface hydrophobic. The prime coat does not independently prevent particles from adhering to the substrate. To release trapped particles, I flow Alconox, an anionic detergent, into the channel after particles have been...
trapped. The detergent forms a hydrophilic sheath around the particles, allowing them to be swept off the surface into a concentrated sample.

For an effective release, the influx of the agent in the channel must be synchronized with shutting off the DEP force. Otherwise, particles are released into a larger volume and therefore a less concentrated suspension. Using Alconox eliminates the need for precise timing. Alconox is anionic and is therefore highly conductive, but pDEP relies upon the presence of a low-conductivity medium. When Alconox is introduced into the channel, it releases particles by simultaneously turning off the force and relieving the sticking. An effective release requires the combination of these two things – simply turning off the force or using a non-ionic detergent is insufficient.

Alconox is a detergent that is known to lyse cells and other bioparticles. However, evidence in the literature suggests that spores are resistant to detergents [20]. Before using Alconox to release spores, I needed to verify that it does not harm the spores. I did

![Figure 4-3: Improving the release](image)

This figure shows micrographs of a section of a channel with beads accumulated on the electrodes (left) and after beads have been released (right). The top row shows these results for an unmodified surface, with the release performed simply by turning off the voltage to the electrodes. In the bottom row, the channel is coated with Sylgard Prime Coat and the beads are released into Alconox.
this by suspending spores in Alconox for 30 minutes, after which I dialyzed them back into DI water using dialysis tubing (Sigma Aldrich, St. Louis, MO). Following dialysis, I restained the spores and inspected them under both bright field and fluorescence using 63× magnification. I was able to restain the spores and visualize them in fluorescence. Additionally, I used the bright-field images (Figure 4-4a) to produce a histogram of the spore radii (Figure 4-4b). The most frequent radius of spores treated with Alconox is ~0.6 μm, similar to that of untreated spores, ~0.5 μm. Differences can be attributed to differences in the image processing used to measure the radii.

Figure 4-3 shows the effect of using the prime coat modification and Alconox. The top row shows an unmodified release, while the bottom row shows a release with Alconox and a prime coat-modified substrate. These pictures demonstrate the effectiveness of this method of particle release.

![Figure 4-3](image)

Figure 4-3: The effect of using the prime coat modification and Alconox. The top row shows an unmodified release, while the bottom row shows a release with Alconox and a prime coat-modified substrate. These pictures demonstrate the effectiveness of this method of particle release.

![Figure 4-4](image)

Figure 4-4: Determining the effect of Alconox on spores
Bright-field images (A) and radius histograms (B) of untreated spores (top) and spores suspended in Alconox for 30 minutes (bottom).
4.2 Measurement Tools

During testing, I relied heavily upon two tools to visually inspect the operation and quantitatively assess the performance of my device. I used a microscope for visual observation and a spectrophotometer for quantitative characterization. In this section, I describe these two tools and how I utilized them.

4.2.1 Microscope

Optical characterization is useful for qualitative assessment of the device operation. I performed optical characterization of the device using a Zeiss Axioplan 2 upright microscope (Zeiss, Thornwood, NY). Images were acquired using a Sensicam QE cooled CCD digital camera (The Cooke Corporation, Romulus, MI).

The Axioplan microscope is equipped with several fluorescence filters. The two I used, Cy3 and FITC, are listed in Table 2 along with their excitation and emission spectra. Pink fluorescent beads and ConA AlexaFluor 488-stained spores fluoresce with different characteristics, also listed in Table 2. This table shows that Cy3 is appropriate for visualizing beads, and FITC is appropriate for visualizing spores.

Table 2: Fluorescence spectra

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<th>Excitation (nm)</th>
<th>Emission (nm)</th>
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<td>580-640</td>
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<tr>
<td>(41007a, Chroma, Rockingham, VT)</td>
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<td></td>
</tr>
<tr>
<td>FITC</td>
<td>460-500</td>
<td>510-550</td>
</tr>
<tr>
<td>(41001, Chroma, Rockingham, VT)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beads</td>
<td>560</td>
<td>590</td>
</tr>
<tr>
<td>(FCM-1058-2, Spherotech, Inc.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spores</td>
<td>495</td>
<td>519</td>
</tr>
<tr>
<td>(stained with ConcanavalinA, AlexaFluor 488 conjugate, C11252, Invitrogen)</td>
<td></td>
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</table>
4.2.2 Spectrophotometer

To fully characterize the device, quantitative assessments of the concentration enhancement are needed. Concentration enhancement is the figure of merit of this device, and is defined as [output concentration]/[input concentration].

It is not straightforward to measure the concentration of a suspension of 1-µm particles. Visual counting methods cannot be used reliably because the particles do not settle to the same focal plane. Conventional spectrophotometers require sample volumes on the order of 10’s-100’s of µl. For my application, sample volumes are on the order of single µl only. Diluting these small samples would reduce the concentration to levels below the measurement threshold of most spectrophotometers.

To overcome the measurement challenges, I used a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The NanoDrop is designed specifically for measuring 1-2-µl volume samples. It measures absorbance at wavelengths ranging from 220-750 nm.

![Figure 4-5: Calibration of NanoDrop measurements](attachment:figure_4-5.png)

Calibration plots correlating known bead (blue) or spore (green) concentrations to the resulting NanoDrop absorbance. Points show mean +/- standard deviation for three measurements.
I produced a set of calibration curves to correlate the NanoDrop absorbance measurements to sample concentration. I prepared a series of bead and spore suspensions of known concentrations and measured each three times with the NanoDrop. The results are plotted in Figure 4-5. This plot shows that the NanoDrop absorbance is linearly proportional to sample concentration.

4.3 Test Setup

I have developed a simple flow system and test setup around this package to assess the functionality of the concentrator. An illustration is shown in Figure 4-6. The flow into the channel is pressure-driven and is controlled by syringe pumps. The setup consists of two valves. The first is a 4-way valve that selects between water and Alconox. The second valve enables me to flow in the beads. Depending on the sample volume, this can be either a 6-port injection valve or a 4-way valve: for volumes up to 1 ml, I use a 6-port injection valve with a sample loop (shown in Figure 4-6). For larger volumes, I use a 4-way valve with an additional syringe.

4.4 Experiment
I have developed an experiment protocol for testing the device, which I used for all of my experiments. The protocol uses the packaged device, materials, and tools described in previous sections and allows me to assess the performance of the device. In this section, I describe the protocol I followed to obtain my results.

Prior to running an experiment, I set up the device as described in Section Test Setup and prepare the samples to be used. To obtain the appropriate input concentration of beads or spores, I begin with a known concentration and dilute with deionized (DI) water by the desired amount. I load the input sample, DI water, and Alconox into three separate glass syringes (Hamilton Company, Reno, NV), which I set up in syringe pumps (KD Scientific, Holliston, MA). I connect the syringes to the device using PEEK tubing and connectors (Upchurch).

To begin the experiment, I flush the channel with water at 500 µl/min to eliminate pockets of air. Bubbles are generally not a problem in this device due to the high flowrates and the simplicity of the channel, but the mixer grooves do tend to trap air initially. Next, I apply a voltage to the electrodes and turn Valve 2 (see Figure 4-6) to introduce particles into the channel, at 500 µl/min (beads) or 100 µl/min (spores). At this point, pDEP causes particles to be trapped on the electrodes while water and any untrapped particles go to waste. When the desired volume of beads has flown into the channel, I turn Valve 2 back. This allows water to flow through and clear the channel so that there are no particles in the flow. I then turn Valve 1 to flow Alconox into the channel at 500 µl/min and release the particles.

To assess device operation qualitatively, I can optically monitor the collection and release, and record images and videos. For more quantitative information, I can collect the output sample and compare its concentration to that of the input sample. I collect between 8 and 16 drops of the released sample in 200-µl PCR tubes, which I cap immediately after collection to prevent evaporation. During sample collection, Alconox is present in the channel, and its high conductivity leads to degradation of the electrodes. Thus, it is important to turn off the voltage to the electrodes immediately following sample collection. I then flush the channel with 3 ml of DI water to eliminate any Alconox residue.
I use NanoDrop absorbance values to assess the concentration of the collected drops. To reduce the noise threshold of the measurements, I average absorbances over a range of wavelengths from 400 nm to 600 nm. The presence of Alconox in the sample results in a peak in absorbance at low (~200-300 nm) wavelengths, but does not affect the rest of the spectrum, as indicated by Figure 4-7.

To translate an absorbance value to a concentration, I compare the measurement to a calibration curve. When performing experiments with beads, I use an existing calibration curve, shown in Figure 4-5. When performing spore experiments, I measure a calibration curve for every experiment due to variability in sample preparation procedures (staining, centrifugation, etc.).

Figure 4-8 shows the results of a typical experiment, plotting bead concentration vs. drop for 8 collected drops. In this figure, the mean and standard deviation of three identical experiments are plotted. The peak in this plot represents the collected sample of particles, in this case, beads. I define the output concentration of an experiment to be the peak concentration. In this case, the output sample produced a peak absorbance of ~0.03, corresponding to an output concentration ~3×10^7 beads/ml.

![Figure 4-7: Effect of Alconox on NanoDrop measurement](image)

Alconox causes a peak in absorbance at the lower wavelengths (~200-300 nm), leaving higher wavelengths unaffected.
Figure 4-8: Output data from a typical experiment

Plots mean +/- standard deviation of three identical trials in which beads at $6.7 \times 10^7$ are collected and released at 500 μl/min.
Chapter 5: Results

I have demonstrated device functionality, both optically via microscopy, and quantitatively by measuring and comparing input and output concentration. In this chapter, I present the quantitative data I have obtained to characterize and verify device performance.

5.1 Device Characterization

Although *B. subtilis* spores are the target organism for concentration in the device, polystyrene microspheres are more suitable to use for performing the device characterization because their properties are better defined and their fluorescence is brighter and more stable (see Section 4.1.2). Characterization with a controlled particle can later be used to predict the device operation with spores.

My objective for characterizing the device was twofold. First, I wanted to assess the trapping ability of the device at different operating conditions. Second, I wanted to validate my assumptions about the effect of the Staggered Herringbone Mixer. In this section, I describe the results I obtained without the mixer, and the effect of the addition of the mixer.

5.1.1 Trapping Characteristics

To assess the trapping characteristic of the device, I extracted a relationship between output concentration and input number of beads, shown in Figure 5-1. I vary the number of beads by changing the input volume while maintaining a fixed concentration to eliminate the need for mixing several bead suspensions. I used an input concentration of approximately $6 \times 10^7$ beads/ml. At this concentration, inputs of interest ranged between approximately 10-1000 µl. At a flowrate of 500 µl/min, this is a range of volumes that can be flowed through the device in reasonable amounts of time.

I first performed these experiments using a plain channel, without the Staggered Herringbone Mixer. For a very low range of inputs (Figure 5-1, [A]), below ~30 µl, the
resulting output concentration is below the measurement detection threshold and the output does not appear to change with variations in the input. The lower detection limit is about $5.5 \times 10^6$ beads/ml. For an intermediate range of inputs (Figure 5-1, [B]), approximately 30-500 μl, I assume that a constant fraction of the particles input into the device are trapped. Thus for this range the output concentration varies proportionally to the input number of beads. When the input exceeds this intermediate range (Figure 5-1, [C]), the finite trapping area (that is, the area of the bottom of the channel) sets a limit on the number of particles that can be trapped, and thus on the output concentration. This causes the output concentration to saturate at a value corresponding to the maximum number of particles that can be trapped. Saturation in this device is reached at input volumes around 500 μl, and produces an output concentration of approximately $6 \times 10^7$ beads/ml.

![Figure 5-1: Device characterization](image)

*Characterization of device performance.* Each data point corresponds to mean +/- standard deviation for three trials. All trials performed with the same device at 500 μl/min and with an input concentration of $6 \times 10^7$ beads/ml. (A) and (C) indicate lower and upper saturation regions of the curve, respectively, and (B) indicates the intermediate linear region.
5.1.2 Mixer

To assess the effect of the Staggered Herringbone Mixer on device performance, I repeated the experiment from Section 5.1.1 using a grooved channel. The results are presented in Figure 5-2, superimposed onto the results of the experiment without the mixer.

![Figure 5-2: Characterization of mixer effect on device performance](image)

Comparison of device characterization with (blue curve) and without the mixer (green curve). Both curves plot mean +/- standard deviation for three trials. All trials performed with the same device at 500 μl/min and with an input concentration of $6 \times 10^7$ beads/ml. (A) and (C) indicate lower and upper saturation regions of the curve, respectively, and (B) indicates the intermediate linear region.

These results show that, qualitatively, the device operation remains the same with the addition of the mixer. The curve still exhibits an intermediate linear region (Figure 5-2, [B]) and flat regions at the high (Figure 5-2, [C]) and low (Figure 5-2, [A]) extremes. Quantitatively, the mixer improves some aspects of the device performance, but hurts others. Including the mixer in the device was expected to improve device performance by...
circulating the liquid in the channel, thus bringing more of the particles within reach of
the electrodes. This effect is seen in Figure 5-2. The curve produced by the device with
the mixer (shown in blue) lies to the left of the smooth channel curve (shown in green),
and reaches its linear region and upper limit at lower input volumes. This implies that
more of the beads are trapped in a device with a mixer, and thus the same input volume
produces a higher output concentration.

However, the two curves suggest that the mixer may slightly lower the device
saturation limit. If this is the case, it may be a consequence of the chaotic flow profiles in
the channel. While in some parts of the channel the flow is directed downward to bring
particles to the electrodes, in other parts it must be directed upward. Figure 5-3 is a top-
down view of a mixing channel in which beads have been collected, showing
qualitatively the effect of the mixer. Beads accumulate very densely at the sides of the
channel (bright regions) but do not trap in the center of the channel. These observations
agree well with published diagrams of the flow profiles created by the mixer [22], which
suggest that flow is directed upward in the center of the channel.

---

Figure 5-3: Microscope photograph of channel showing effect of mixer on trapping

Shows that beads do not trap as densely in the middle of the channel when the mixer is
present, due to chaotic flow profile. Without the mixer, beads accumulate uniformly
along the width of the channel (not shown).
5.2 Concentration Enhancement

It is important to note that the curves in Figures 5-1 and 5-2 do not show concentration enhancement. Both curves saturate around $5 \times 10^7$ beads/ml, which is lower than the input concentration of $6 \times 10^7$ beads/ml. I constructed these curves to verify the hypothesis that the output concentration would saturate.

Assuming that the output concentration saturates at the same value independent of input concentration and volume, achieving a 50× concentration enhancement should simply entail inputting a large enough volume to saturate the device at a concentration 50 times below the saturation.

By lowering the input concentration and increasing the input volume, I have been able to repeatably achieve concentration enhancements up to 40× using polystyrene microspheres. Figure 5-4 shows the outputs of three experiments, plotting sample concentration vs. drop number, in which concentration enhancements of 25×, 36×, and 40× were achieved.

![Figure 5-4: Three plots showing concentration enhancement with beads](image)

25× (left), 36× (middle), and 40× (right) concentration enhancements produced by the device. Blue lines show the output concentration measured for each of 8-11 collected drops. Dashed red line shows the starting concentration.

I have also achieved concentration enhancement using *B. subtilis* endospores, the target organism for this device. Because the electrical properties of spores are not
published, I have determined the operating point by experimentation. I operated the device at 100 kHz to concentrate spores. Moreover, rather than use 10-μm-wide electrodes at 20 V_{pp} as I did with polystyrene microspheres, I used 25-μm-wide electrode to increase the trapping reach, and increased the voltage to 40 V_{pp} to maintain the holding strength. Additionally, I decreased the flowrate to 100 μl/min to collect spores but maintained it at 500 μl/min for the release. At this operating point, I was able to achieve a 9x concentration enhancement of *B. subtilis* spores; the corresponding output concentration plot is shown in Figure 5-5.

![Figure 5-5: Concentration enhancement using spores](image)

9x concentration enhancement achieved using *B. subtilis* spores, the target organism for concentration in the device.

The results presented here are subject to non-idealities introduced by the experimental setup that do not reflect the performance of the device itself. A sample of beads is collected in a 0.5-μl channel and released in flow. Upon release, the sample disperses due to Taylor dispersion into several drops, each ~10 μl in volume. Even if the beads could all be released into a 0.5-μl volume, such a small volume is difficult to...
extract and measure. Resuspending the beads into any volume larger than the volume of the channel reduces the concentration enhancement.

It is possible to calculate an intrinsic concentration enhancement that describes the maximum achievable enhancement. Intrinsic output concentration is defined as \(\frac{\text{number of beads released}}{\text{channel volume}}\), where the number of beads released is calculated by integrating under the curves in Figure 5-4 and Figure 5-5. Intrinsic concentration enhancement, \(\frac{\text{intrinsic output concentration}}{\text{input concentration}}\), disregards the effects of the experimental setup and takes into account only the performance of the device itself.

The results presented in Figure 5-4 and Figure 5-5 correspond to intrinsic concentration enhancements of 750-1500× using beads, and 350-1000× using spores. It is important to note that the release is limiting in these experiments, especially in the case of spores. Because the spores are released at a higher flowrate than that at which they are collected, some of the collected spores are released due to the increased flowrate before the Alconox releases the rest of them. This results in a much more dispersed output sample and a decreased extrinsic concentration enhancement, but the intrinsic enhancement remains high.
Chapter 6: Challenges and Future Work

I have encountered several challenges in developing the experimental setup for the concentrator and comparing the results to modeled predictions. In this section, I describe these challenges and possible ways to overcome or work around them.

6.1 Modeling

After I had fabricated a device based on the results of a model, I would have liked to use experimental results to validate the model. Our modeling software has been validated using MEMS devices developed in our lab [9-13]. However, due to the nature of my device, I have not been able to compare simulated results to experiments.

6.1.1 pDEP

I have encountered a few challenges with modeling particle behavior under pDEP forces. In nDEP, particles are repelled from the electrodes, where the electric field is concentrated, and are usually subjected to a balance of well-defined forces: dielectrophoretic (some distance from the electrodes), drag (near the middle of the channel), and gravitational where applicable. Particles often reach equilibrium some distance away from the electrodes, where the electric and velocity fields vary over lengths much greater than the particle size, and where approximations of these fields are more accurate.

In the case of pDEP particles are attracted to the locations of electric field maxima, often at the electrodes or another physical barrier. In the case of the device presented here, particles reach equilibrium very near the bottom of the channel and at the electrode edges, where forces are less well defined. The dielectrophoretic force exhibits a singularity at the surface of the electrode Figure 6-1. One particle radius (0.5 µm) above the surface of the electrode, the singularity is smoothed out but the peak is still narrow. In simulation, the dielectrophoretic force has to be sampled at a very high resolution to capture this peak. Resolution is always a concern in discrete, numerical simulations such
as those performed by Matlab. I have observed that changing the resolution by about a factor of 3 can change the predicted maximum flowrate by about 20%.

![Graph showing dielectrophoretic force](image)

*Figure 6-1: Plot of z-directed dielectrophoretic force*

Z-directed (downward) DEP force plotted at $z = 0.05$ µm (dashed green) and $z = 0.5$ µm (solid blue), where $z$ is height above channel bottom. Electrode locations are shown in red and black.

The flow profile is also not well defined in this region. Flow is modeled for a simple rectangular channel, but the real channel has electrodes patterned on the bottom that are $\sim 0.2$ µm thick. While this dimension is very small compared to the channel depth, it is on the same order of magnitude as the particle radius and the particle might be subjected to the disturbance in the flow profile.

### 6.1.2 Missing Information

Some aspects of the device were difficult to model. Due to a shortage of published information on their properties, I did not model the spores and calculate their
CM factor. Without the CM factor, it is difficult to predict the force exerted on the spores by the electric field. It was also impractical to model the effects of the Staggered Herringbone Mixer. The flow profiles produced by the mixer are chaotic and would require complex simulations.

While I do not have information about the dielectric properties of spores, I could experimentally predict their CM factor \( \text{Re}[\text{CM}(\omega)] \) by measuring the crossover frequency at different medium conductivities and fitting these data to a model, as described in Section 4.1.2. However, spores are not necessary for validation of the model. I performed all of the device characterization using polystyrene microspheres, for which I was able to experimentally predict the CM factor.

Modeling the mixer flow profiles is much more complicated, but may not be necessary. If the goal is to validate the model using experimental results, then this can be done using a channel without a mixer.

6.1.3 Experiments

The final challenge that prevented me from using experimental results to validate my model was the fact that it is difficult to design an appropriate experiment. The ideal experiment would enable me to experimentally relate capture distance vs. starting height, and maximum flowrate vs. voltage.

To extract a relationship between capture distance and starting height, I would ideally set up an experiment to emulate the conditions of a simulation: that is, to input a single particle at a given height above the channel bottom, and measure the distance it travels before it is trapped. However, unlike in simulation, the experiment setup does not enable me to control the height at which a particle enters the channel. Moreover, I cannot perform an experiment with a single particle.

A possible alternative is to instead emulate the experimental conditions in simulation. This can be done using a Monte Carlo simulation, for example, as presented in [27]. Rather than predict the behavior of a single particle, this method simulates a bulk effect by simulating a collection of particles with randomly selected initial parameters. Theoretically, I would be able to compare these predictions to the results of an
experiment in which I flow an appropriate concentration of particles into the concentrator and observe where they trap.

One drawback is that it would take a long time to perform this simulation, as a trajectory would have to be computed for each particle, or at least for each starting height. Additionally, this simulation would not take into account interactions between particles, which may not be negligible in this system.

It is also difficult to validate the relationship between maximum flowrate and electrode voltage. In simulation, I acquire this relationship by starting a particle at the channel bottom and increasing the flowrate until the electric field no longer holds it in place. This should theoretically be possible to verify, by trapping particles on the channel bottom and increasing flowrate (or equivalently, decreasing voltage) until the particles release.

Practically, however, this is not straightforward to do, mainly due to nonspecific adhesion. Trapping particles at a low flowrate brings them to the bottom of the channel. However, when the voltage is turned off, many particles adhere to the substrate and do not release, even at high flowrates. Measurements can be done in which the maximum flowrate is considered to have been reached when a certain fraction of the particles release, as presented in [6].

6.2 Spores

Throughout this thesis, I have mentioned several challenges associated with the use of spores. In this section, I describe the difficulties with modeling and staining spores, and suggest possible solutions.

6.2.1 Modeling

One way to overcome the inability to model the spores is to build a device that is robust to variations in the CM factor. This is what I chose to do, compensating for changes in the CM factor by increasing the voltage. This device utilizes pDEP and therefore operates at reasonably low (<1 MHz) frequencies, so the voltage can be
amplified if needed. Another solution would be to extract the spore properties by measuring their crossover frequency in media of different conductivities and fitting these data to a model, as described in Section 4.1.2. Unlike the beads, the spores are not homogeneous and in fact consist of several layers of different materials. This means that more crossover frequency data would have to be measured, and the data would be fitted to a more complicated model. The modeling approach, however, is still valid.

6.2.2 Staining

Due to the highly resistant nature of bacterial endospores, they are not easily penetrated by stains. ConA was the best of the three stains I explored, but by no means stained all the spores. For example, Figure 6-2 shows a comparison of a spore sample viewed in bright field (A) and in fluorescence (B). Noticeably more spores are visible in the bright field image, suggesting that many of the spores are not stained. Both images in this figure were taken under 63× magnification, so spores are clearly visible in the bright-field image. During an experiment, however, I view the channel using 10-20× magnification, making it more difficult to visualize the spores.

I use microscopy only as a way to qualitatively assess the operation of the device, and collect all my data using a spectrophotometer. Thus it is sufficient to view the behavior of the stained spores as an indicator of the behavior of all the spores. When I use the spectrophotometer to gather data, I am able to observe absorbances at a range of wavelengths that is not affected by the stain. Therefore, my ability to stain the spores does not adversely affect the data I collect.

However, it may still be desirable in the continuation of this project to improve the staining capabilities. One way to achieve this is to experiment with additional stains and staining parameters (such as concentration and staining times). Alternatively, published methods for staining spores can be attempted. One common spore staining method is the Schaeffer-Fulton method [28], which involves steaming the stain into the spores by holding the sample over a boiling water bath.
6.3 Achieving a concentration enhancement

As discussed in Section 5.2, I have been able to repeatably achieve concentration enhancements up to 40× using polystyrene microspheres. However, I also encountered non-idealities that limit the performance of the device.

6.3.1 With beads

Based on the shape of the curves in Figure 5-1, I hypothesized that the output concentration of the device saturates at a certain value, independent of input parameters. By decreasing the input concentration a factor of 50 from this value and flowing in a sufficiently large volume, I should be able to achieve the same output concentration, thereby having concentrated 50×.

In practice, this hypothesis was complicated by several non-idealities. First, the output saturation is not independent of input parameters, and in fact decreases with lower input concentration. This effect further increases the input volume required to achieve a 50× concentration.

Figure 6-2: Visual indication of spore staining

(A) Spores as viewed in bright-field illumination under 63× magnification. (B) Same sample of spores viewed using FITC fluorescent filter, 63× magnification.
Using a larger input volume adversely affects the device performance. One of the most difficult challenges I encountered was nonspecific adhesion of particles to the electrodes. As described in 4.1.3, I overcame this problem using a surface modification and a surfactant, which in combination released both beads and spores very efficiently. The surface modification, a layer of Sylgard Prime Coat, causes the surface to be hydrophobic. I have observed that the hydrophobicity of the surface decreases with prolonged exposure to electric fields. To obtain a concentration enhancement, I flow large volumes of a low concentration particle suspension into the device. Even at the 500-μl/min flowrate, this requires the electric fields to be on for about 50 minutes to obtain an output concentration measurable using the NanoDrop spectrophotometer. The resulting decrease in hydrophobicity causes a less efficient release and thus a lower output concentration.

6.3.2 With spores

I have done some initial work to achieve concentration enhancement using spores. Qualitatively, I have observed that spores collect on the electrodes and are released as desired. I have also been able to collect and measure the output of an experiment, achieving a concentration enhancement of 9x at 100 μl/min flowrate.

Due to time constraints, I have not explored the effects of different operating parameters on device operation using spores, nor have I measured their electrical properties. However, based on my observations, achieving a greater concentration enhancement of spores at a higher flowrate is possible with this concentrator and should only require some experimentation with input parameters.

6.3.3 Trapping Efficiency

Predictions from the modeling presented in Section 2.3 suggest that at 18 V_{pp} and under the simulated conditions, all particles should be trapped and held at the bottom of the channel. However, the “waste” output of the device (i.e. the output suspension while
the electrodes are on and collecting particles) contains a large fraction of the particles, implying that only a small fraction are collected. While a trapping efficiency specification was not defined for this project, an improved efficiency would greatly improve the throughput of the device, as a lower volume would be required to produce the same output concentration.

Further modeling of particle behavior and the mixer flow profile might indicate the cause of the low trapping efficiency. If electric field reach is limiting, wider electrodes could be used at a higher voltage to increase the reach while maintaining the holding capability. If efficiency is limited by holding capabilities, alternate electrode designs, such as castellated electrodes [8], could be explored. If the flow profile created by the mixer is known, electrodes could be tailored to the flow profile. A castellated or zig-zag electrode topology would also increase the trapping area of the device, thus increasing the maximum output concentration of the device.
Chapter 7: Conclusion

I have demonstrated the development and functionality of a microparticle concentrator for use with *Bacillus subtilis* endospores. I used modeling to size and operate the device optimally for the target organism. The device was designed to perform concentration enhancement at high throughput. I have achieved enhancements up to 40× at 500 µl/min flowrate using 1-µm-diameter polystyrene microspheres, and up to 9× at 100 µl/min flowrate using *B. subtilis* endospores.

The device consists of two main functional components: a set of interdigitated electrodes, and a passive mixer. The electrodes are designed to hold particles strongly against high flowrates. As a consequence, the region from which particles can be trapped is very localized to the bottom of the channel. The mixer is intended to circulate the fluid within the channel, bringing particles to the bottom of the channel, within the electrode trapping region.

Interdigitated electrodes were an appropriate choice for this device. They are simple to model and fabricate and use. A future iteration of this device might extend the electrode topology and use, for example, castellated or zig-zag electrodes to increase the trapping area and trapping strength. The chaotic mixer increased trapping efficiency but at the same time prevented particles from accumulating in certain areas of the channel. In a future iteration of this device, the mixer could be optimized to transport particles closer to the bottom of the channel. This would further decrease the need for trapping reach and enable the use of electrodes optimized for holding strength.

One of the most difficult challenges I encountered in testing this device was nonspecific adhesion of particles to the bottom of the channel. To overcome this challenge, I developed a protocol for releasing particles. I modify the surface of the channel to make it hydrophobic, and use a detergent to release particles. I have verified that this procedure does not harm *B. subtilis* spores.

I have demonstrated successful operation of the device using polystyrene microspheres as well as *B. subtilis* spores. A future iteration of the device could improve some of the design aspects, and could use experimental measurements of the electrical properties of spores to further enhance device performance.
7.1 Contributions

I have designed, modeled, built, and tested the first high-throughput DEP concentrator demonstrated to work with *B. subtilis* spores. I have achieved concentration enhancements up to 40× at 500 μl/min flowrate using polystyrene microspheres, and up to 9× at 100 μl/min flowrate using *B. subtilis* spores. I used a passive microfluidic mixer in combination with DEP trapping to enhance device performance. Most importantly, I have presented quantitative, experimental data on extracted concentrated samples of both polystyrene microspheres and *B. subtilis* spores.

I have developed a simple method for labeling *B. subtilis* spores. I have also developed a method for effectively releasing polystyrene microspheres and spores from the surface of the device, by modifying the channel surface and using a detergent to release particles. I have shown that this method does not harm *B. subtilis* spores.
Appendix: Fabrication Process Flow

Starting Materials:
- For electrodes: 150-mm-diameter, 762-μm-thick Pyrex wafers (Bullen Ultrasonics, Eaton, OH)
- For channel (PDMS) patterning: 150-mm-diameter, 650-μm-thick Silicon wafers (WaferNet, Inc., San Jose, CA)

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<th>Parameters</th>
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<td>UV expose</td>
<td>EV1</td>
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<td>AZ 422 developer</td>
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<td>post-bake oven</td>
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<tr>
<td>14</td>
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## Microfluidic Channel Pattern, on 6" Silicon wafer

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<td>SU-8 Spin</td>
<td>SU8-spinner</td>
<td>SU-8 2050 (100 um thickness):</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dispense ~6ml SU-8 (1ml per inch</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>diameter)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ramp to 500 rpm at 100 rpm/sec</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>accel and hold for 5-10 seconds</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ramp to 1680 rpm at 300 rpm/second</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>hold for total of 30 seconds</td>
</tr>
<tr>
<td>3</td>
<td>Prebake</td>
<td>hotplate</td>
<td>65°C for 4 mins; ramp to 95°C,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>keep at 95°C for 15 min; cool</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>gradually</td>
</tr>
<tr>
<td>4</td>
<td>UV expose</td>
<td>EV1</td>
<td>Mask 2, 30 sec, 30 μm separation</td>
</tr>
<tr>
<td>5</td>
<td>Post-expose bake</td>
<td>hotplate</td>
<td>65°C for 1 min; transfer to 95°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>for 9 min, ramp down to ~40°C to</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>cool</td>
</tr>
<tr>
<td>6</td>
<td>SU-8 Spin</td>
<td>SU8-spinner</td>
<td>SU-8 2025 (50 um thickness):</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dispense ~6ml SU-8 (1ml per inch</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>diameter)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ramp to 500 rpm at 100 rpm/sec</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>accel and hold for total of 30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>seconds</td>
</tr>
<tr>
<td>7</td>
<td>Prebake</td>
<td>hotplate</td>
<td>65°C for 2.5 mins; ramp to 95°C,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>keep at 95°C for ~40 min, cool</td>
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<td></td>
<td></td>
<td></td>
<td>gradually</td>
</tr>
<tr>
<td>8</td>
<td>UV expose</td>
<td>EV1</td>
<td>Mask 3, 23 sec, 30 μm separation</td>
</tr>
<tr>
<td>9</td>
<td>Post-expose bake</td>
<td>hotplate</td>
<td>65°C for 1 min; 95°C for 10 min,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ramp to 40°C to cool</td>
</tr>
<tr>
<td>10</td>
<td>Develop</td>
<td>Solvent-Au</td>
<td>~7-10 mins, visual stop</td>
</tr>
<tr>
<td>11</td>
<td>Silanize wafer</td>
<td>EML acid hood</td>
<td>Put 3-4 drops of HMDS into cup in</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>vacuum jar.</td>
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<td></td>
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<td></td>
<td>Place wafer(s) against wall of jar.</td>
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<td></td>
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<td></td>
<td>Close jar, turn on vacuum for 5-10</td>
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<td></td>
<td></td>
<td></td>
<td>minutes, then turn vacuum off, let</td>
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<td></td>
<td></td>
<td></td>
<td>sit for some time (~1 hour).</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>Vent.</td>
</tr>
</tbody>
</table>
References


