SELECTIVE MICROORGANISM CONCENTRATION USING A DIELECTROPHORESIS-BASED MICROFABRICATED DEVICE.

by

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S.B. in Electrical Engineering and Computer Science, 2005

Massachusetts Institute of Technology

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

at the Massachusetts Institute of Technology

February 2007

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ABSTRACT
Detection of pathogenic microorganisms is a significant challenge in medicine, environmental protection
and biological threat safety because samples are often contaminated. This work presents a method of
separating bacterial spores from typical air contaminants such as sand or soot by means of
dielectrophoresis. Spores of *B. subtilis* as well as model airborne interferents are characterized electrically;
based on their properties, schemes of their electrical separation are proposed. The separation based on
the frequency of applied electric field is implemented using microfabricated chips. To relate the data
obtained from these microtools to particle concentration, a computer program is designed, implemented
and validated. Experiments show that separation of bacterial spores from soot and sand suspended in
water based on electrical properties of particles is possible and may serve as a way of enhancing spore
concentration in mixtures.

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To Mom and Dad
ACKNOWLEDGMENTS

Many individuals have supported and helped me along the way of working on this thesis.

First, I would like to extend my thanks to my parents, Anna and Kazimierz Puchała, without whom I would never be able to come close to starting the work I have a privilege of summing up in this thesis. They nurtured my passion to learn and they have always been supportive of my plans, even if these were as implausible as going to the other end of the world to study.

I am grateful I was able to work for Professor Joel Voldman. His patience, trust and understanding in teaching me the rigorous ways of research made my three years with the group fun and stimulating.

Not less inspiring were the amazing individuals that I worked with in the group. Ever since Lily taught me “PMDS” processing, as I called it at the time, she has remained a resourceful mentor and a great colleague. Adam’s effortless enthusiasm and his caring support have brightened up many of my days. Brian’s sharp wit and great knowledge continue to motivate me to learn. Mike’s humor and passion for research led to many inspiring discussions. I am fortunate to have collaborated with Hsu-Yi, whose rigor and patience I admire. I am grateful for being able to interact with such smart and enthusiastic colleagues as Nick, Joseph, Nina, Asiri, Salil and Pat. Thank you all for your help.

My work would not go as smoothly if not for the staff of Research Laboratory of Electronics, Microfabrication Technology Laboratory and Electrical Engineering and Computer Science Department. I appreciate help and enthusiasm of Debb Hodges-Pabon, Maxine Samuels, Mary Young and Susan Davco. I would not have been able to fabricate my chips without the help of Dave Terry, Paul Tierney and Kurt Broderick. Thanks to Anne Hunter, Vera Sayzew, Linda Sullivan and Lisa Bella for their quick assistance with administrative issues.

My friends have supported me immensely during my work. I appreciate caring encouragement of Osama Nayfeh, Emad Zand and Hugh Robinson. I am thankful for Ola Hosa, who has been a patient listener and an eager helper. I am grateful to Daniel Dumitran for always inspiring me to try new things. Thanks to Kathryn Hankin for her continuous support and understanding.

I am glad to have had Professor Dina Katabi as a mentor, professionally and personally. Her insight, optimism and kindness continue to be an inspiration for me.

I would not have the honor of working on this project if not for funding coming from Charles Stark Draper Laboratories and MIT Department of Electrical Engineering and Computer Science. Thank you.
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GLOSSARY

**Conductivity.** A measure of material's ability to conduct electric current.

**Crossover, crossover frequency.** The frequency, at which the transition from negative to positive DEP occurs.

**Dielectrophoresis.** Force exerted on a dielectric particle subject to non-uniform electric field.

**Dipole, electric dipole.** Separation of positive and negative charge.

**Drag.** A force that impedes the movement of a solid through a liquid.

**Permittivity.** A measure of material’s ability to transmit (permit) electric field.

**Polar.** Displaying polarity or separation of charges.

**Polarization.** The process of separating opposite charges within an object.
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>$\vec{F}_{\text{DEP}}$</td>
<td>dielectrophoresis force</td>
</tr>
<tr>
<td>$r$</td>
<td>radius</td>
</tr>
<tr>
<td>$\varepsilon_m$</td>
<td>permittivity of medium</td>
</tr>
<tr>
<td>$E_{\text{rms}}$</td>
<td>root-mean-square electric field</td>
</tr>
<tr>
<td>$K(\omega)$</td>
<td>Clausius-Mosotti factor</td>
</tr>
<tr>
<td>$\varepsilon_\rho$</td>
<td>complex permittivity of a particle</td>
</tr>
<tr>
<td>$\varepsilon_m$</td>
<td>complex permittivity of medium</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>permittivity</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>conductivity</td>
</tr>
<tr>
<td>$\omega$</td>
<td>angular frequency</td>
</tr>
<tr>
<td>$f_K$</td>
<td>crossover frequency</td>
</tr>
<tr>
<td>$C_p$</td>
<td>liquid capacitance</td>
</tr>
<tr>
<td>$C_o$</td>
<td>air capacitance</td>
</tr>
<tr>
<td>$R_p$</td>
<td>equivalent parallel resistance</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>liquid fixture correction coefficient</td>
</tr>
<tr>
<td>$C_{\text{mem}}$</td>
<td>membrane capacitance</td>
</tr>
<tr>
<td>$\varepsilon_s$</td>
<td>permittivity of free space; constant, $\varepsilon_s = 8.85 \times 10^{-12} \text{ Fm}^{-1}$</td>
</tr>
<tr>
<td>$\varepsilon_{\text{mem}}$</td>
<td>membrane permittivity</td>
</tr>
<tr>
<td>$d$</td>
<td>membrane thickness</td>
</tr>
<tr>
<td>$G_{\text{mem}}$</td>
<td>membrane conductance</td>
</tr>
<tr>
<td>$\sigma_{\text{mem}}$</td>
<td>membrane conductivity</td>
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Symbols are identified in the order, in which they appear in the text.
CHAPTER 1: INTRODUCTION

1.1 Overview

This work focuses on improving a microorganism concentrator previously described by Gadish and Voldman (1) to allow for selective concentration of bacterial spores in real-world complex liquids contaminated with substances such as dust or sand. The device is an intermediary between an air sampler that suspends air particles in a liquid (deionized water) and a micro-scale detector, which detects the presence of toxic cells, such as Bacillus anthracis spores, in the solution. The detector is limited in its sensitivity and can reliably detect concentrations of spores not lower than 10⁹ cells/mL (2); however, B. anthracis dispersed in the air remains toxic for humans even at concentrations as low as 2.5 · 10³ cells/mL (3). We have previously developed a microfabricated concentrator that can enhance the concentration of a pristine sample of Bacillus subtilis. Current work examines whether remediation of B. subtilis spores from their mixture with air contaminants is possible and how the microconcentrator should be operated in order to achieve it. In this work, I study the properties of typical air particles such as sand, dust, and soot. Given their electrical properties, I propose, test and evaluate schemes of device operation to achieve the highest sample purity.

1.2 Challenge overview and existing methods

Detection and identification of pathogenic microorganisms are significant challenges in medicine, environmental protection and biological threat safety. Infectious diseases, caused by microorganisms such as bacteria, spread in air, water and solid materials, are a major cause of deaths worldwide (4). Changing environmental factors as well as microorganism evolution are causing increased number of reported losses caused by microbes, for example damage to buildings, crops, and stored foodstuff (5, 6). Additionally, with advances in biology and biological engineering, bioterrorism is becoming a serious concern (7). In this situation, new tools are being deployed to help first responders and clinicians detect the pathogens in air or water so that proper measures with regards to microbe surveillance, treatment and control measures can be implemented.

Microorganism detection is usually performed based on nucleic acids or protein analysis of the sample (8), whereby the presence of a certain genes or proteins specifically characteristic to bacteria is detected. These analytical methods are hindered by extreme dilution of microorganism in air or water. In the case of nucleic acid analysis, polymerase chain reaction (PCR) that amplifies the amount of available genetic material is used to improve sensitivity. Immunoassays that rely on protein detection are usually limited by the sample concentration. Therefore, typically the first step in pathogen detection is sample concentration. An additional burden in sample preparation is also its contamination, dependent on the method of harvesting the sample. Air-borne pathogen samples typically contain air contaminants such as dust, sand and other biological particles in addition to the microorganism of interest. Therefore, for proper microorganism identification, the cells not only need to be concentrated; they have to be concentrated selectively.

Air samples contain a variety of interferants. These particles come from natural sources such as wind-borne pollen, salt particles resulting from evaporation of organic vapors and sea spray, fungi, mold, algae, yeast, rusts, debris from live and decaying plant and animal life, particles eroded by wind from beaches, desert, soil, rock; particles from volcanic and geothermal eruptions; the particulate matter polluting the air is also the result of human activities such as fuel combustion, automobile exhausts, cooking, house maintenance and recreation (9). In order to model this very heterogeneous mixture of air particles, I have chosen to represent different classes of particles present in the air by means of model
particles such as chitin (organic molecule), pollen (wind-borne grains), dust (natural and human exhausts), sand (natural erosion) and soot (natural and human combustion processes).

Many existing approaches can potentially be useful for selective bacterial concentration. Some methods might be prohibitively difficult for microscale format. Conventional bacterial sample preparation methods include lengthy culture steps for amplification (10) and require extensive gas exchange accomplished easily in bioreactors but not necessarily in microfluidic format. Concentration methods more amenable to microfluidic work include mechanical filters, which provide size-selectivity but not organism or particle specificity. Magnetic cell separation (11) allows one to separate cells after they have been selectively conjugated with magnetic beads. This method can provide the high throughput and selectivity needed in the endeavor of purifying a mixture of bacteria and air-borne interferents and is amenable to microscale separation; however, the mixture has to be chemically conjugated with magnetic particles first. This procedure requires a special linking molecule that needs to be designed for each microorganism. Another method allowing for selective sample enrichment is fluorescence-assisted cell sorting (12), which relies on optical analysis of the sample. Still, the cells need to be pre-treated with a fluorescent particle, specific to each cell type of interest. Although it achieves high degrees of purity, it is not portable since a laser is necessary to analyze the cells. Moreover, this method acts relatively slowly as each particle in the sample (millions of cells) needs to be analyzed individually with a laser.

The challenge of selective bacterial cell concentration discussed here has specific requirements. First, a continuous or semi-continuous operation of the concentrator is necessary for sampling the input during uninterrupted detection. This constraint stems from analyzing large volume samples of air to achieve efficient concentration enhancement. Second, the concentrator acts as an intermediary between a microscale pathogen detector and a regular air sampler suspending air particles in a liquid. Thus, the device needs to interface with both and it needs to be itself, microfabricated.

In order to fulfill both requirements, we harness the phenomenon of dielectrophoresis in the challenge of selective bacterial concentration. Dielectrophoresis (DEP) provides an attractive alternative to methods mentioned above because it can collect microparticles rapidly, selectively and reversibly (13, 14, 15). Given the application of the right operating conditions based on established particle properties, DEP accomplishes both concentration and selectivity. It operates at the microscale and is easily amenable to microfabrication as well as allowing for long periods of particle collection at a high flow rate. Dielectrophoresis, however, relies on a particle's electric properties and can only separate particles if there are electrical differences between them. These electrical properties of the particle of interest need to be determined first in order to establish the operating conditions. Yet, this calibration is a one-time commitment performed with a sample of cells in a laboratory setting; the result is exploited in tuning the parameters for device operation without the need to repeat the calibration measurement for recurring field operation. The material sampled in the field needs not to be treated before subjecting it to dielectrophoresis since DEP relies on internal particle properties and not specific labeling. It allows for high-throughput separation and has been demonstrated to not adversely affect the cells. Dielectrophoresis works at the microscale, matching the size of bacteria and fits well in the microfabricated framework of pathogen detectors. Thus, in comparison with other methods, dielectrophoresis is a good choice for selective concentration of bacterial cells.

1.3 Proposed solution

Our group’s research focuses on engineering a microfabricated dielectrophoretic device to perform the following functions: (i) concentration of bacterial cells to maximize sensitivity of down-stream detection; (ii) active filtering of bacteria out of their mixture with typical air contaminants such as sand, soot, dust. Both functions are achieved using positive dielectrophoresis while the mixture is being passed over electrodes setting up the field (Figure 1-1). Selectivity of the dielectrophoresis can be achieved through modulating different factors such as frequency of the field, conductivity of the solution and polarizability of the particles under test (Figure 1-1).
We have previously developed a concentrator and shown that we can fulfill aim (i) (1). This work focuses on aim (ii), specifically determining if and how electrical differences can be used for selective bacterial cell concentration, finding the right operating conditions in terms of frequency of the applied field and media conductivity, so that the concentrator can collect the \textit{B. subtilis} cells most efficiently and separate them from other particles in mixtures.

1.4 Dielectrophoresis

A particle placed in an electric field experiences a force. If the particle is polarizable, spatial inhomogeneities in the field induce dielectric polarization in the particle. The force exerted on the induced dipole moment of a polarizable particle in a non-uniform electric field is called dielectrophoresis (DEP).

The time-averaged DEP force acting on a particle due to an electrical field using the dipole approximation is (16)

\[ \overline{F}_{\text{DEP}} = 2\pi r^3 \varepsilon_m \text{Re}\{K(\omega)\} \nabla |E_{\text{rms}}|^2 \]

where \( r \) is the radius of the particle, \( \varepsilon_m \) is permittivity of the surrounding medium, \( E_{\text{rms}} \) is the applied electric field, \( \omega \) is the angular frequency, and Re refers to the real part of a variable. The Clausius-Mossotti factor \( K(\omega) \) is a measure of the effective polarizability of the particle with respect to the suspending medium, and for a uniform sphere is defined as

\[ K(\omega) = \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m} \]

where \( \varepsilon_p \) and \( \varepsilon_m \) are the complex permittivities of the particle and the medium defined as
\[ \varepsilon = \varepsilon - i \frac{\sigma}{\omega} \]  

(1.3)

where \( i \) is the complex unit equal to \( \sqrt{-1} \), \( \varepsilon \) is permittivity, \( \sigma \) is conductivity, and \( \omega \) is the angular frequency. Thus, the dielectrophoretic force is strongly dependent upon the frequency of the field (16). The response of a non-uniform particle to the non-uniform electric field is approximated by single-shell or smeared-out models and will be discussed in section 2.1.2.

For a sphere under the dipole approximation, its Clausius-Mossotti factor is bounded between 1.0 and -0.5 causing the DEP force to be either attractive or repulsive depending on the polarizability of the particle in relation to the medium (Figure 1-2). Positive DEP (pDEP), where the particle is attracted towards electric-field maxima, occurs when \( \text{Re}\{K(\omega)\} > 0 \) (pictured in red curve and a fragment of green curve in Figure 1-2). When \( \text{Re}\{K(\omega)\} < 0 \), the particle is pulled towards the minima of the electric field, a phenomenon termed negative DEP (nDEP) (shown in blue and rightmost portion of the green curve in Figure 1-2). For a sphere, the real part of the CM factor varies between -0.5 and 1, suggesting that the maximum magnitude of positive DEP can be made greater than the magnitude of negative DEP if all other parameters (geometry, excitation voltage, etc.) are kept constant.

Variations in the structure of materials result in variations of their conductivities and permittivities and thus the polarization factor. For example, soot made of very conductive carbon (conductivity \( 2.9 \times 10^4 \text{ Sm}^{-1} \)) is likely to act as a metal (for comparison, conductivity of germanium, a metalloid, is \( 2.2 \text{ Sm}^{-1} \) and that of copper \( 5.8 \times 10^7 \text{ Sm}^{-1} \)) and be strongly attracted to electric field maxima for a range of frequencies (17). It will experience pDEP when placed in water (Figure 1-2 red curve) for all practical frequencies. Sand made of insulating silica (conductivity about \( 1 \times 10^{-12} \text{ Sm}^{-1} \)) exhibits negative DEP when subjected to AC field while in water (Figure 1-2 blue curve) (17). From this example, where two particles made of different materials experience different DEP force when placed in the same electric field and the same medium, we see that DEP force may be a powerful separating factor for electrically heterogeneous materials. Bacterial spores, highly heterogeneous particles, will experience a complex behavior in the AC field, with pDEP and nDEP depending on frequency (Figure 1-2 green curve).

Not only does DEP vary across different types of materials; the same particle will experience DEP force different in sign and magnitude for different frequencies of the AC field. The complex permittivity of the media or the particle, for sufficiently low frequencies, is roughly equal to their respective conductivities. If the particle is more polarizable (higher conductivity) than the surrounding medium, it will experience a greater force and it will be attracted towards electric field maxima. If the medium is more conductive than the particle, then the medium will be attracted towards the electric field maxima thus pushing the particle out towards electric field minima. Accumulation of charge at the interface between the particle and the media causes a change in the electric field. Polarization of spherical particles by AC fields at low frequencies when placed in media of is presented in Figure 1-3.
For a nonuniform AC field, the magnitude and direction of the DEP force depends on the frequency, free charges surrounding the particle and changes in the surface charge density. The frequency at which the DEP changes its sign is termed the crossover frequency ($f_K$). Most particles have only a single crossover frequency; these particles are repelled from the electric field maxima above $f_K$ (nDEP) and attracted towards electric field maxima below $f_K$ (pDEP).

Dependence of the force on the frequency and conductivity enables the use of dielectrophoresis as a means of separating particles. Since the magnitude and direction of the dielectrophoretic force depends on the properties of the particles and the medium in which they are present, by appropriately matching the operating parameters (frequency of electric field, conductivity of the medium), the DEP force can be optimized to selectively capture one type of particle (e.g., a bacterial spore) or filter out another (interferent particle, such as dust). Using dielectrophoresis at frequencies where particles of interest experience positive DEP and other particles are repelled, we may be able to collect the former selectively.

The curves presented in Figure 1-2 picturing the real part of the polarization factor are not experimental observations results but rather model simulations for the behavior of the polarization factor for different types of particles. Obtaining the actual curves requires particle studies. This thesis presents methods to obtain the information about the crossover frequency of particles, that is the frequency at which the polarization factor changes its sign, as well as its usage in performing binary separations of B. subtilis spores from interferents.

Our model interferants used for testing the device include the following particles: chitin, dust, pollen, sand and soot. We used these paired with B. subtilis spores to test binary separation of an interferant from the bacterial cell. These substances correspond to classes of pollutants found in the air. Chitin is an organic material shed by animals; dust is a mixture of splinters, diesel exhaust and inorganic materials produced by households, animals and industries; pollen represents cells produced by plants, sand is an example of an inorganic, inert material; and, soot is an inorganic produced by human industrial activity, heating, etc. In Chapter 2, I will present a more in-depth discussion of relative properties of these materials.
1.5 Approaches to selective concentration using DEP

The selectivity of dielectrophoresis to the electrical properties of particles provides for a number of different ways of purifying mixtures of bacterial cells and interferants. Selective capture refers to purification during the concentration step by means of collecting only the particle of interest but rejecting interferants. This method could be effective in the case of sand because it undergoes negative DEP for all regions of conductivity and frequency (Figure 1-2). Alternatively, selective release refers to capturing multiple types of particles but releasing only the one of interest. Additionally, one might imagine releasing all but the particle of interest. This method could be effective if soot and spores were held differently or responded differently to some chemical agent. Then, upon capturing both of them, releasing one of them specifically would result in a selectively concentrated sample.

Alternatively, capturing particles with temporal or spatial resolution on the chip might allow for selective concentration. For example, soot and spores are collected in similar regions of conductivity and frequency, but this region is larger for soot. Therefore, there exist frequencies and conductivities for which soot will be collected while spores will not. Driving a preliminary region at these parameters would collect soot selectively and thus would clear the solution of soot. This situation offers two advantages; if effective enough to leave all the soot behind, it would produce purified solution of spores or it opens up stage for spores to be nonselective captured in a later part of the chip, purified of the interfering soot by the initial soot-clearing section of the device.

1.6 Device overview

The device used for spore concentration is essentially composed of two layers (Figure 1-4). A layer of gold electrodes for setting up the electric field, patterned on glass lines up the bottom of a micro channel that is molded in a clear polymer called poly(dimethylsiloxane). As the analyzed liquid with bacteria is being passed through the channel, the electrodes set up a non-uniform electric field, which attracts the particles of interest using dielectrophoresis, thus capturing them from the flowing mixture. Both the electrodes and the channel are micro scale components so as to ensure their compatibility with the micron scale particles that they deal with.

The electrodes have the form of two interdigitated combs, excited with voltage of opposite signs. This design ensures that a particle passing along the length of the channel in the direction orthogonal to the alignment of electrodes, is subjected to the electric field generating the DEP contour plot given in Figure 1-5. We previously found that this geometry will allow for effective particle collection in a continuous manner while particles are being flown over electrodes.

The comb of the interdigitated electrodes is divided into four equal sections along the channel length. This design allows for excitation of different sections of electrodes with different signals. Various signals may be necessary to access various particles present in the mixture, i.e., some particles may be...
trapped with one field frequency while others may require a different field. Moreover, sections may serve as different functional units of the device, such as filter and concentrator.

![Electric field streamlines](image)

**Figure 1-5:** Electric field streamlines (blue) set up by the interdigitated electrodes (black and red) excited with varying sign voltage.

1.7 Study overview

The approach that I take in this study included electrical characterization of the model particles, *B. subtilis* spores, as well as interferant particles used to model the air contaminants: chitin, dust, pollen, soot, sand. I summarize the results of characterization are in Chapter 2. These findings led me to designing the operating conditions for the concentrator and testing the selectivity performance on binary mixtures of spores and one of the interferents. In Chapter 3, I describe the process of fabricating the device; in Chapter 4, I outline the experimental protocols, methods and data analysis. I present the results of the experiments in Chapter 5 and in Chapter 6 I present the discussion of the findings and possible improvements to the selective concentration procedure.
2.1 Methods of characterization

In order to harness the electrical selectivity offered by dielectrophoresis, I need to determine whether the airborne interferents and the bacteria of interest are electrically distinct enough to be differentiated by the electric field. Then, I need to choose the right operating conditions for the device. There are many variables to choose from; for example, I could choose operating field frequency, liquid conductivity, excitation voltage, flow rate among others. I focus on determining the field frequency appropriate for separating bacterial spores from air interferants. To select this condition most effectively, I first characterize the particles’ electric properties. I explored two methods of determining electrical properties of particles. First, I used impedance measurements of the suspension of particles in liquid; second, I used the crossover frequency method. Given the available equipment and time constraints, only the latter method gave data usable in further studies. Here I describe both, giving the principles, advantages and disadvantages of each of them and results.

Since separation of particles and effectiveness of the separation may be aided by particles’ characteristics different from their electrical properties, I characterize their size distribution and absorbance as well.

2.1.1 Impedance measurement of particle suspension

The impedance measurement of a suspension of particles serves to establish particle’s dielectric constant and its variation with frequency. This information may lead to establishing its CM factor necessary for effective dielectrophoretic separation. By measuring the impedance of a certain volume of liquid as well as the impedance of the same liquid with some of its volume replaced with a volume of particles of interest and comparing the two, it is possible to establish the dielectric properties of the materials displacing the medium.

In order to obtain reliable repeatable results, I use a specially designed liquid test fixture (16452A, Agilent Technologies) to perform dielectric measurements of materials used in the study of the

![Figure 2-1: Liquid test fixture used for establishing dielectric properties of liquids (18)](image)
concentrator. The fixture is composed of two flat electrodes separated by a spacer to enclose a liquid chamber (Figure 2-1 A). The structure can be taken apart (Figure 2-1 B) by unscrewing four screws holding the electrodes together. When set up, the chamber is accessible through three channels (Figure 2-1 A): liquid inlet on a side, liquid outlet on the bottom and air inlet at the top. Surrounding the air inlet are four electrode connectors. The fixture acts as a round capacitor filled with liquid medium in-between the electrodes, whose dielectric properties it is designed to measure. Two high and two low voltage electrodes allow for cancellation of stray capacitance. The electrode diameter is 38 mm. There are four available spacers of widths: 1.3 mm, 1.5 mm, 2 mm and 3 mm providing electrode separations of 0.3 mm, 0.5 mm, 1.0 mm and 2.0 mm respectively. They allow for better accuracy in measurements.

To obtain dielectric properties of particle solutions, I prepare the liquid test fixture by cleaning the electrodes with deionized water, ethanol and drying them with nitrogen. I assemble the fixture with electrode distance 0.3 mm (1.3 mm spacer) and a shorting plate in-between electrodes as required for short compensation of the fixture. I close the fixture’s drain at the bottom and connect the electrodes to the impedance analyzer (4294A, Agilent Technologies). I use standard 1-m-long cable provided with the fixture and set the cable compensation parameters in the impedance analyzer so that the instrument automatically carries out the cable correction. I measure the capacitance and resistance of the shorted system. After confirming that the electrical performance of the system is within bounds required by the manufacturer, I perform the actual liquid measurement.

I disconnect the fixture from the analyzer and take it apart to remove the shorting plate. I select the proper width spacer from among the available widths and assemble the fixture with the spacer. With the empty fixture, I measure the value of the capacitance of air \( C_o \). Next, I pour 10 mL of deionized water into the fixture and repeat the measurement. Note: the actual liquid volume between electrodes ranged from about 4 mL to 7 mL for various electrode separations; however, the liquid fills in the fixture drain and the liquid inlet as well. Subsequently, I open the drain of the fixture, drain the water out, dry the fixture with nitrogen, close the drain and pour 10 mL of particle solution in deionized water into the fixture. I tried various concentrations of solutions of particles under test (chitin, dust, soot, sand, pollen), the highest concentrations were 10 mg/mL of sand and pollen, and the substances were seen to sediment within a couple of seconds. This measurement is challenging as there exists a tradeoff between the particle concentration used (the higher the concentration, the more accurate the measurement) and the particle sedimentation (the bigger the mass of dense particles in the solution, the faster they fall to the bottom of the chamber interfering with the result). I settled on using solutions of concentrations around 4 mg/mL, which were seen to settle down within about 1 minute in 10 mL of deionized water. It was necessary for the mixture to remain well mixed with particles randomly distributed throughout for the period of about 30 s that the impedance measurement takes (probing 250 data points in frequencies varying from 100 kHz to 20 MHz).

The electrical model of the liquid test fixture is shown in Figure 2-2. Besides measuring capacitance and resistance of the liquid, the impedance output contains stray capacitance, which might alter the dielectric constant. The measurement procedure accounts for it and corrects it by measuring the air capacitance. Moreover, the manufacturer provides a correction coefficient based on fixture geometry and construction that is also used in calculations. Using the capacitive method for measuring dielectric properties models the complex dielectric parameter according to the following equation (18),
\[ \varepsilon = \eta \left( \frac{C_p}{C_o} - j \frac{1}{\omega C_o R_p} \right) \]  

(2.1)

where \( C_p \) and \( C_o \) correspond to liquid and air capacitance and \( R_p \) is the equivalent parallel resistance, \( \omega \) is the angular frequency. \( \eta \) is the correction coefficient given by the manufacturer to cancel stray capacitance of the electrodes.

I follow manufacturer’s suggestions to establish the dielectric coefficient of the medium as well as the medium containing the particles of interest. Next, given the percentage content of the particles, I establish the dielectric properties of the material displacing the original liquid. Using this method, however, small concentrations of particles under test (4 mg/mL) constitute a prohibitively small fraction of the solid material in the liquid medium to allow for a meaningful measurement. For example, the smallest electrode separation allows for around 4 mL of liquid in between the electrodes, which contains 16 mg of pollen at 4 mg/mL, accounting for less than 1% of the mixture by volume and by mass. Such a small fraction of particles does not affect the measurement results sufficiently to give a reliable value for the particle’s dielectric constant across multiple measurements if at all. To remedy the quick sedimentation, I add various concentrations of sucrose to deionized water thus matching the density of particles under study with that of the medium without changing the medium’s electric properties (sucrose is non-polar). Again, the highest practical concentrations that I am able to obtain this way are around 20-30 mg/mL, which produce similarly limited results.

The capacitance measurement method turned out very challenging for a number of reasons. It is not well suited for measuring dielectric properties of solids dissolved in liquids but rather designed for analyzing various liquids separately. Small fraction of solids that I was able to subject to tests was not sufficient to produce meaningful data. Unmatched density of solid/liquid mixture led to sedimentation of the solid material in the fixture, further limiting accuracy of the method. Accounting for the density difference by adding sucrose remedies the issue only partially while adding problems with fixture cleaning, since the sugar adheres to the surface of the electrodes. This issue forces me to reassemble the fixture for each measurement, further adding variability in fixture gap width across measurements.

### 2.1.2 Crossover frequency measurement

The crossover frequency measurement allows to measure crossover frequency of the material and subsequently uses particle models to establish dielectric properties. I used the measurement to model particle’s CM factor curve as applied the information about the DEP sign directly.

First, I prepare a liquid of certain known conductivity prepared by mixing phosphate-buffered saline (Dulbecos, Gibco, Carlsbad, CA) with deionized water. Since stock solutions are stored over a course of a couple of days in room atmosphere, the conductivity of solutions changes. Final conductivity varies but it is not essential for it to be exactly the same in each experiment and I always verify it prior to each experiment using a conductivity meter (Orion 555A pHuture, Thermo Fisher Scientific, Waltham, MA). The conductivity of the prepared solutions stayed within the range \( 6 \cdot 10^{-4} \) S/m up to \( 9 \cdot 10^{-1} \) S/m across different measurements. Each of the particles from among: chitin (C7170, Sigma Aldrich, St. Louis, MO), dust, pollen (P6770, Sigma Aldrich, St. Louis, MO), sand (274739, Sigma Aldrich, St. Louis, MO), spores (6633, SUS-1A-7, Raven Biological Laboratories, Omaha, NE), soot (572497, Sigma Aldrich, St. Louis, MO) was suspended in the prepared conductive medium. The exact concentration of the particle is not significant in this procedure; the particles were concentrated enough to be easily seen under microscope but dilute enough to distinguish separate particles in the field of view. In order to fulfill this requirement, the concentration was established by trial-and-error and stayed within \( 4 \cdot 10^{-4} \) g/mL and \( 9 \cdot 10^{-4} \) g/mL.

For visualizing small, otherwise hardly visible bacteria in fluorescent light, I stain the spores with concanavalin A conjugated with manufacturer’s proprietary stain Alexa Fluor 488 (C11252, Invitrogen, Carlsbad, CA) prior to each experiment. Concanavalin A is a carbohydrate-binding protein that selectively attaches itself to the mannose found on the surface of the spore (19), while Alexa 488 is a stain visible in
fluorescent light (20). All other particles are well visible in brightfield and therefore, I use this mode to image them.

I pipette 50 µL of each suspension onto the interdigitated electrodes (IDE) chip (described in detail in Chapter 3) and cover with it with a 25 mm by 25 mm coverslip (48366-249, VWR, West Chester, PA). Next, I apply time varying electric field of amplitude of 10 V \text{pp} to the chip. I vary the frequency of the field while observing the behavior of any given particle type under test on the slide. I sweep the frequency range from low frequencies of 10 kHz to high frequencies of 80 MHz and back in roughly logarithmic increments. I record the frequency regions of positive and negative dielectrophoresis exhibited by each particle for different media conductivities, ranging in conductivity from $6 \cdot 10^{-3}$ S/m up to $9 \cdot 10^{-1}$ S/m. I repeat the set of measurements for each particle on at least three different days with differed IDE chips and fresh medium each time.

An example of abbreviated results for spores of \textit{B. subtilis} stained with conanavalin A bound with Alexa Fluor 488 are given in Table 1. For a given field frequency applied to the solution of spores at a given conductivity, the kind of resulting force is noted (attractive, repulsive, no force). Table 1 shows a measurement for a certain particle type at a single conductivity. For each particle, many different conductivities were tested, each one of them at least three times on separate days. After performing this measurement for various liquid conductivities over a course of at least three different days, the result for a given particle are represented as bar graphs in frequency versus conductivity, and example of which is given in Figure 2-3. Each bar represents a measurement for a single conductivity. Bars are composed of areas marked in red, blue and orange. The orange square represents the frequency at which there was no force acting on particles, causing them to become stationary; this frequency is known as crossover frequency. The frequencies below the crossover are marked red and represent regions of attractive or positive DEP force experienced by the particle. The blue bar above crossover reflects the negative DEP or force repelling the particles from electrode edges.

Following the crossover frequency measurements, the obtained data was used in two ways. First, the bars of regions of DEP force were extrapolated to present continuous regions in frequency and conductivity in order to find the parameters for particle separation. Second, the crossover frequency values were used along with the single shell model to obtain possible CM factor curves for the particles as explained in Chapter 1. The DEP regions turned more conclusive and are presented here for each particle. The CM factor curves have an approximate value and some examples of these results are shows here as well.
While assuming no claim to the magnitude of the DEP force, the regions generated using the crossover measurements allow us to establish operating conditions for particle separation based on sign of the DEP force. Using the bar graphs generated earlier (Figure 2-3), the DEP force regions for each particle are established as follows. I connect the crossover frequencies for each particle, establishing a boundary between force regions. Since the boundary depends on the particle size and the populations of particles are heterogeneous in size, the boundary is not definitive. Surrounding the boundary is a region of weak and unclear DEP action. Using the smallest and the biggest crossover frequency values for a given conductivity, I establish the boundary of the weak/unknown region of the DEP force, which I present as a

Figure 2-4: DEP regions obtained for particles under study: chitin (A), dust (B), pollen (C), sand (D), spores (E), soot (F).
yellow region separating the red region of positive DEP and blue region of negative DEP in Figure 2-4, showing collected data for all studied particles. The width of the unknown region depends on the repeatability of crossover frequency measurements.

The DEP sign diagrams reflect differences in the internal structure of the particles. Highly polarizable materials such as soot or dust, whose main component is soot, undergo pDEP (showed in red) for all or almost all of the tested field frequencies and media conductivities. Insulating materials such as sand experience nDEP (blue) for all tested parameters. Biological particles such as spores, pollen, chitin have a more complex internal structure and experience pDEP and nDEP depending on the field frequency for a given medium. These differences in dielectric reaction to the AC field are the basis for the separation of particles.

For the purpose of electrical modeling, a biological multi-shelled sphere can be represented as a single-shelled sphere (Figure 2-5), where the internal layer represents the cell, whereas external layer represents membrane, cell wall and spore shell if applicable. Using the single shell model, which allows us to model a complex multi-shell cells as two-layer objects, the crossover frequency as a function of cell parameters can be given as

\[
 f_K = \frac{\sqrt{2}}{8\pi r C_{\text{mem}}} \sqrt{(4\sigma_m - rG_{\text{mem}})^2 - 9r^2 G_{\text{mem}}^2} 
\]

(2.2)

Where \( C_{\text{mem}} \) is membrane capacitance \( (C_{\text{mem}} = \varepsilon_0 \varepsilon_{\text{mem}}/d) \) and \( G_{\text{mem}} \) membrane conductance \( (G_{\text{mem}} = \sigma_m/d) \).

Simplifying and lumping the cell parameters together into constants \( \alpha \) and \( \kappa \), the formula can be written as

\[
 f_K = \kappa \sqrt{\alpha^2 \sigma_m + \alpha \sigma_n^2 - 2 \sigma_n^2} 
\]

(2.3)

Using this equation, I fit the crossover frequency and media conductivity values to obtain the parameters and use these to generate the CM curves. Given many measurements of the crossover frequency and media conductivity, I perform a search to minimize the error between the theoretically determined value and the experimentally measured one across all measurements. Example curves obtained for spores of \( B. \ subtilis \) in this way is given in Figure 2-6.

Overall, because of limited information regarding the cell parameters available in literature, the only reliable information is the sign of the DEP force rather than its relative magnitude presented in the
CM curves. Therefore, useful as an approximate tool, the exactness of the curves cannot be quantitatively determined with available means.

2.1.3 Size distribution measurement

In order to examine size-exclusion as a means of separating bacterial spores from their mixture with air contaminants, such as dust, pollen or sand, I measure the sizes of particles. These values might be useful in determining optimal filter mesh size. Particle size is also an important factor in particles’ interaction with the AC field and might be useful in modeling electrical behavior of the materials.

To measure their size, I make a suspension of each of the investigated particles from among: chitin (C7170, Sigma Aldrich, St. Louis, MO), dust (RM 8631, Reade Advanced Materials, East Providence, RI), pollen (P6770, Sigma Aldrich, St. Louis, MO), sand (274739, Sigma Aldrich, St. Louis, MO), spores (6633, SUS-1A-7, Raven Biological Laboratories, Omaha, NE), soot (572497, Sigma Aldrich, St. Louis, MO) in deionized water. The exact concentration is not important; I keep the suspensions dilute enough so as to distinguish individual particles in the field of view. I pipette 50 µL of each of the mixtures onto a 25 mm by 75 mm glass slide (48300-025, VWR, West Chester, PA), cover it with a 25 mm by 25 mm coverslip (48366-249, VWR, West Chester, PA), visualize it in its appropriate mode at the magnification of 20x using an inverted microscope (Axiotech 200, Zeiss, Thornwood, NY). Next, I take four photographs of randomly chosen regions on the slide using a microscope camera (Spot RT Color, Diagnostic Instruments, Sterling Heights, MI). Finally, I process the pictures with engineering software package Matlab (The Mathworks Inc., Natick, MA).

In order to obtain the size distribution for each population, I write a program that detects and marks particles in the picture, calculates the number of pixels that comprise each particle and based on the area of a particle, modeling it as a sphere and using a calibrated pixel to µm factor, calculates its radius in µms. The data is averaged over the number of particles visible in each picture as well as over all the pictures. I give the size overview for each particle below.
2.1.4 Spectrophotometric absorbance measurement

To investigate optical identification and exclusion of particles as a means of mixture separation, I measure the absorbance spectrum of suspension of each particle. It might be helpful in identifying the presence of a certain type of interferant in a mixture. The fluorescence spectrum was not available at the time and therefore only interferents are investigated in the visible spectrum; fluorescently stained spores are easily distinguishable from the mixture.

I make a suspension of each of the interferants from among: chitin (C7170, Sigma Aldrich, St. Louis, MO), dust (RM 8631, Reade Advanced Materials, East Providence, RH), pollen (P6770, Sigma Aldrich, St. Louis, MO), sand (274739, Sigma Aldrich, St. Louis, MO), spores (6633, SUS-1A-7, Raven Biological Laboratories, Omaha, NE), soot (572497, Sigma Aldrich, St. Louis, MO) in deionized water. Although the value of absorbance depends on particle concentration, I normalize the measurement data to the highest value in any given measurement. Therefore, the exact concentration of each particle is not important. This way, I preserve the shape of the absorbance curve, while losing information on relative particle content. This action is justified when looking for specific optical benchmarks of given particle presence (e.g., a peak at 235 nm) rather than trying to deconvolve exact concentrations of particles in a mixture (e.g., overlay peaks at 235 nm and 248 nm).

Next, I pipette 2 µL of each suspension onto the detector of the microliter-volume spectrophotometer (ND 1000, NanoDrop Technologies, Wilmington, DE) and I apply the signal to read the spectrum. The absorbance spectrum for the range of wavelengths 220-750 nm is automatically saved by software provided with the instrument. I repeat the experiment three times on different days; results given here correspond to normalized averages of the instrument readings for each particle. Figure 2-7 shows the absorbance spectra for all particles used in the study.
Only pollen and soot show characteristic peaks at short wavelengths. Remaining spectra are non-characteristic, with high absorbance for short, close-to-ultra violet wavelengths falling off towards 800 nm. Overall, absorbance spectrum does not seem to be an effective tool in identifying any particular interferant.

### 2.2 Overview of particles used in the study

As mentioned earlier, the study focuses on separating bacterial spores out of a mixture containing other particles. I used five main classes of interferents modeling various contaminants found in the air as a result of human and natural activity: Model particles included chitin (organic molecule), pollen (wind-borne
grains), dust (natural and human exhausts), sand (natural erosion) and soot (natural and human combustion processes). The following section presents an overview of the particles.

2.2.1 Chitin

Chitin is a polysaccharide comprising cell walls of fungi and yeast, exoskeletons of anthropods and organs of animals, for example horse shoes (22). Chemically, this polysaccharide is composed of acetylglucosamine residues linked to one another with $\beta$-1,4 bonds (23). Acetylamine groups participate in hydrogen bonding between adjacent chains of polymers giving chitin its strength (22). Chitin forms translucent crystals. Since it is strong and biodegradable, it is used to make surgical threads, artificial blood vessels, contact lenses (22). Chitin is dispersed in the air by many sources. It is a by product of many human activites, such as cosmetics industry, agriculture, chemical, as well as coming as a waste material.

In this study, I determine the chitin particles sizes to range from 1 to 200 $\mu$m. The particles look like flakes of undefined shape, and are difficult to visualize (Figure 2-8). Based on their size, I suggest that most of chitin can be removed with a mechanical filter with the mesh size of about 1 $\mu$m out of a stream containing chitin and $B. subtilis$ spores, which are less than 1 $\mu$m in diameter.

Chitin absorbance is given in Figure 2-7 A. Its UV-Vis spectrum does not show any significant peaks or a specific marker. Chitin absorbs mostly in the UV range at about 200nm. Its absorbance drops rapidly. With its colorless appearance and unspecific spectrum, chitin cannot be easily detected based on absorbance.

Crossover frequency of chitin (Figure 2-4 A) shows the pDEP region for frequencies below about 30-40 MHz and conductivities below 0.2 S/m. Among the frequencies and conductivities considered, this is a large pDEP region, which may enable effective trapping of chitin possibly as a way of filtering it out of the solution.

2.2.2 Dust

Dust is a mixture of solid particles, whose diameters I determine to be less than 500 $\mu$m. Dust arises in the atmosphere from various sources, e.g., pollution, volcanic eruptions, animal and plant debris, soil lifted by wind, coagulated combustion residues (24). Therefore, what we call dust is a chemically heterogenous mixture of soot, textile fibers, plant splinters, sand and organic debris. Dust particles grow in size through coagulation and sticking as a result of turbulence and Brownian motion (25).

The absorbance spectrum of dust, as expected, shows no characteristic peaks. Dust is black in color; its absorbance spectrum supports this observation by confirming that it absorbs light wavelengths of all frequencies almost uniformly, except for the UV light, which it absorbs more readily. Again, the spectrum of dust is not a sufficient way to distinguish the substance out of its mixture with other particles as it is too generic.

The crossover frequency spectrum of dust also matches expectations. Dust is a mixture containing large and small particles, which are electrically heterogeneous. Small particles undergo pDEP for all frequencies and conductivities considered, which suggests that they are particles of soot. Larger particles, however, experience nDEP for conductivities greater than 0.1 S/m and frequencies in the range of tens of MHz, indicating a different source such as organic debris.

2.2.3 Pollen

Pollen is comprised by powder of microgametophytes, which serve to transport male gametophytes of seed plants (26). These grains are composed of two generative cells (genetic material) and
one vegetative cell (nutrition) that are enclosed in a protective coat of cellulose and a hard cuticular wall (26). This design allows for safe long-distance transport of the genetic material (26). The outer wall bears spikes and characteristic carvings specific to a given genus and species that uses that particular grain for reproduction (26). To model the pollen grains present in the air, in this work I use *Betula alba* pollen (Figure 2-9). Throughout the course of my work with pollen of *Betula alba*, I noticed that its grains have three carvings equidistantly spaced on the outer diameter of the granules.

The absorbance spectrum of *B. alba* pollen (Figure 2-7 B) shows distinct peaks in the wavelengths of about 250 and 350nm. The latter could be used to determine the content of this particle in the mixture with others because no other particle used in this study shows a peak at this wavelength. Pollen does not significantly absorb any other wavelengths than blue and UV light, which is manifested in its yellow color.

The crossover frequency spectrum of pollen (Figure 2-6 B) shows the typical complex spectrum of a multi-shelled biological particle (16). Pollen exhibits pDEP below frequencies of 10 MHz and conductivities of 0.02 S/m. Above these quantities, nDEP is displayed clearly.

### 2.2.4 Sand

Sand (Figure 2-10) refers predominantly to chemical silicon dioxide. It may also contain traces of other substances such as granite, chlorite, gypsum. As a particle, it is composed of fine grains of rock of sizes between several microns and 2 mm (27). Sand is transported by wind and water; naturally, it is deposited on beaches and dunes (27).

Sand shows an absorbance spectrum (Figure 2-7 B) very similar to that of chitin, with high absorbance in the UV range falling off towards higher wavelengths. This highly generic spectrum with no specific peaks or markers does not allow for differentiating sand in a mixture by means of absorbance.

Crossover frequency spectrum of sand (Figure 2-4 C) meets expectations for that of an insulating particle. For all frequencies and conductivities considered in this study, sand turns out not polarizable and thus exhibits nDEP. As such, it is easy to eliminate sand out of a mixture as it is repelled from electrodes and can be effectively filtered out while the particles of interest are being collected.

### 2.2.5 Spores

*Bacillus Subtilis* used in this study is a Gram-positive bacterium, non pathogenic to humans (28). It has an ability to tolerate harsh environmental conditions thanks to the ability to form tough protective exospore (28). It has become a model organism for scientific studies as it is easily manipulated genetically (28). This bacterium is often found in water and soil (28).

Spores are much smaller than the original bacterial cells themselves, on the order of 1 µm in length. They are extremely difficult to see under brightfield illumination and so need to be stained for visualization purposes. The crossover frequency spectrum of spores stained with fluorescent dye (Figure 2-4 D) shows a pDEP region that they exhibit when placed in conductivities lower than 0.1 S/m and frequencies lower than 30-40MHz.
2.2.6 Soot

Soot is the black or brown powder resulting from incomplete combustion of fuel with high carbon content such as coal, wood, and oil (29). Its main component is amorphous carbon although soot obtained from coal may also contain hydrogen, oxygen, sulfur and ammonia (29). Soot used in this study is composed of carbon in fullerene form.

Soot particles used in this study range in size between 1 and 10 microns. They are uniformly black in color. The absorbance spectrum of soot (Figure 2-7 E) shows a generic UV peak that appears in the case of many other aforementioned particles. Absorbance therefore is not a good way to distinguish soot from its mixture with other particles present in the air.

The dielectrophoresis diagram of soot (Figure 2-4 E) shows positive dielectrophoresis for all conductivities and frequencies considered. This result is expected because carbon used here to model soot is very conductive and polarizable. Therefore, soot can be easily trapped by dielectrophoresis at all frequencies.

2.3 Approach to selective concentration of spores using DEP

Given collected data on electrical properties of the particles considered, we decided to devote a part of our dielectrophoretic concentrator to filtering and trapping unwanted particles, such as soot and dust and operate it at relatively high frequencies (10 MHz) so that these particles would be collected while spores would not be efficiently trapped. The mixture depleted of these interferents would then be passed over a concentrator section of the interdigitated electrodes device, operated at 100 kHz, which would collect bacterial spores. The results of tests of this operating scheme are given in the following chapters.

The electrical characteristics of the model interferent particles and the B. subtilis spores indicate that there does not exist a combination of the field frequency and media conductivity values allowing for the exclusive collection of spores while all other particles undergo negative dielectrophoresis (Figure 2-4). Similarly, there are no such operating conditions that would make chitin, dust, soot, sand, pollen be attracted to the electrodes, while the spores are being repelled (Figure 2-4). Instead, the positive DEP (pDEP) region of spores overlaps with that of chitin, dust, pollen, and soot. Based on the characteristics of interferent particles, I expected the only substance that can be separated from spores based solely on the sign of the DEP force to be sand, which experiences nDEP for all tested operating conditions. Regardless of which frequency and media conductivity is used to capture the bacteria, in their mixture with sand particles B. subtilis should be captured exclusively while sand should be carried away with the stream of flowing media. As for the other interferents, I devised a scheme of device operation for selective spore capture based on the differences in magnitude of the DEP force that various particles experience in the same field.
CHAPTER 3 : DEVICE DESIGN AND FABRICATION

3.1 Overview

The device used for spore concentration is essentially composed of two layers (Figure 1-4). A layer of gold electrodes for setting up the electric field, patterned on glass lines up the bottom of a micro channel that is molded in a clear polymer called poly(dimethylsiloxane). As the analyzed liquid with bacteria is being passed through the channel, the electrodes set up a non-uniform electric field, which attracts the particles of interest using dielectrophoresis, thus capturing them from the flowing mixture. Both the electrodes and the channel are micro scale components so as to ensure their compatibility with the micron scale particles that they deal with. Both components of the device are fabricated in class 100 cleanrooms (MIT Microfabrication Technology Laboratory, Cambridge, MA). After fabrication, the components are assembled, packaged and interfaced to the macro scale technology in regular laboratory settings. Here, I describe the design of the device, fabrication steps of the electrodes and the channel, packaging and interface of the device.

3.2 Masks

Microfabrication requires the use of masks for transferring design patterns onto the base surfaces. The electrodes are patterned in gold deposited on Pyrex glass and require a mask outlining the electrode pattern. The channel covering the electrodes is molded in a polymer, polydimethylsiloxane, using a mold made out of photoresist called SU8 covering a silicon wafer. Shaping the photoresist is also accomplished by exposing it to UV radiation through a mask containing the channel pattern (in the process called photolithography). Both masks are custom-made 17.5 cm x 17.5 cm x 3 mm soda lime glass upright masks with the pattern printed in chrome with resolution 40,640 DPI (FineLine Imaging, Colorado Springs, CO).

3.2.1 Electrode Mask

The mask for the electrode photolithography allows for exposing photoresist deposited on Pyrex glass. Photoresist is a light-sensitive material. Negative photoresist is used here, which becomes relatively insoluble in the photoresist developer when exposed to light. Therefore, the dark fields on the mask will become the electrode profile (they allow for dissolving of the photoresist and subsequent attachment of gold onto the glass protected with resist), while bright fields of the mask will result in clear areas on the final wafer. This method is used so that the photoresist can be undercut and may be preferentially removed with a solvent during the lift-off (30,31,32).

The mask contains 12 dies arranged in the same orientation and delineated with border lines for subsequent dicing. Each die has dimensions 25 mm by 64 mm and each one includes four sections of electrodes 25 µm in width and every 25 µm apart. These dimensions were chosen based on experiments with 10 µm, 25 µm and 50 µm electrodes performed previously (1). Sectioning of the device is essential to producing separate filter and concentrator segments, which are driven with different voltages. It also increases the yield, since a section or two may still be useable even if the entire device is not due to short-circuited electrodes in one of the divisions. Each device includes a pair of marks for fluidic access holes drilled later. Each die contains marking regarding the mask version, author and the electrode dimension.
3.2.2 Channel molding Mask

The channel that forms the top layer of the device is made out of a polymer called poly(dimethylsiloxane) (PDMS) (33), which is initially clear and soluble but becomes solid when exposed to a PDMS curing agent and heat. The soluble substance is molded using a microfabricated mold made of photosensitive called SU8 shaped on a silicon wafer. SU8 is a negative photosensitive and becomes insoluble in the developing material when exposed to optical radiation (34,35,36,37,38), therefore SU8 mask is dark field. This means that the features that are dark in the mask will be saved on the wafer, while bright spots on the mask will be removed on the final wafer.

The mask contains 18 dies, each with a channel, of dimensions 48 mm long and two widths 100 µm and 250 µm. Each die is delimited with a border line for precise slicing of the molded PDMS. Every channel terminates on both sides with a fluidic reservoir of diameter 3 mm used as inlet and outlet of the channel. Also included on a die mold is text describing the author and the device version.

3.3 Microfabrication

The fabrication process for the gold electrodes and the SU-8 mold is a standard photolithography and lift-off process described elsewhere. I explain the fabrication process below.

3.3.1 Gold Electrodes

The gold microelectrodes are deposited on borosilicate - Pyrex (Bullen Ultrasonics, Eaton, OH) glass because it is poorly conducting electrically (resistivity 8 x 10\(^{10}\) Ω/cm at room temperature) and thermally (thermal conductivity 1.1 W/mK at room temperature), transparent (transmission of 90% over the range 300-2200nm) and hard (Knoop hardness 418 kg/mm\(^2\)) material (39); therefore, providing electrical and thermal isolation, optical access and sound base for the device. I use Pyrex wafers of diameter 150 mm and thickness 762 µm because the microfabrication tools available in the cleanrooms are suited for this size of the substrate. According to the device design, low-conductivity media will be used for generation of positive DEP, so little power dissipation through the device is expected. Therefore, thermally-insulating Pyrex is a fine choice for the electrode substrate.

The electrodes themselves are made out of bio-compatible metal, such as gold, attached to glass via an adhesion layer of bio-compatible titanium. They are patterned using a lift-off process, relatively simpler than the available alternative - chemical etching, as it does not require experimental determination of the time and speed of the process. Additionally, heterogeneous films such as gold and its associated titanium adhesion layer can be patterned in one step. This is possible because the lift-off process following deposition removes the photosensitive (and as a by-product of its removal, gold and titanium) rather than targeting the metals themselves.

First, I bathe the Pyrex wafer in standard Piranha solution (1 part of 30% hydrogen peroxide to 3 parts of 98% sulphuric acid) for 30 minutes in order to remove organic contamination. Next, I wash the solution off of the wafer by bathing it in deionized water for 30 minutes and I dehydrate it in 120°C oven for 30 minutes. Next, I apply gaseous hexamethyldisilizane at pressure (1300 Pa) and temperature (125 °C) to the wafer in order to improve the adhesion of the photoresist to its surface later on.

Next, I spin a 1.4 µm layer of photoresist AZ5214-E (Hoechst Celanese, Sommerville, NJ) for 30s at the final speed of 3000 rpm using spin coater (5110, Manual Photoresist Coater, Solitec Inc., Milpitas, CA). AZ5214 is a positive photosensitive; however, its polarity can be reversed by a flood exposure. The process of producing negative images from a positive resist is referred to as image reversal. I use the resist AZ 5214-E for this lift-off process because it can produce a negative slope (features form an angle greater than 90° with the substrate) on the sidewall. The precise angle of the negative profile can be regulated using the exposure time; lower exposure time gives a more negative profile; this profile is necessary to prevent shielding of the resist walls with gold during the metal
evaporation process. Lift-off of gold is impossible when the sides are covered with the metal as the developing agent does not dissolve gold and undercutting is not possible.

Subsequently, I bake the photoresist at 90°C for 30 min in a convection oven (DDC – 146C, Blue M, White Deer, PA). Next, I align the wafer with the mask and expose it for 3 s at 8.7 mW/cm² (EV 620, Electronic Visions, Tempe, AZ) through the previously described chrome mask. Then, I bake the wafer again at 95°C for 30 min in the convection oven. Next, I flood expose the entire wafer for 60 s to reverse the polarity of the resist. Finally, I develop it in developer AZ422 (Hoechst Celanese, Sommerville, NJ) for 80 s. Subsequently, the wafer is rinsed with deionized water and spin-dried.

I deposit gold on the developed photoresist using an electron beam evaporator (Temescal Semiconductor Products, Livermore, CA). To ensure good adhesion between gold and Pyrex substrate, I deposit a 100 Å layer of titanium before depositing 2000 Å layer of gold. After deposition, I place the entire wafer in acetone for duration of a couple hours up to a couple of days, however long is necessary to dissolve the photoresist. During that time, I monitor the wafers to determine when the process is ready. As a result of dissolving the resist, the gold not attached directly to the wafer but rather to the resist is lifted off, leaving the patterned electrodes behind.

Finally, I coat the wafer with another layer of AZ5214E resist as before to protect it from contamination during dicing. I dice the wafer using a die saw (Model DAD-2H/6T, Disco Abrasive System, Shinagawa-ku, Tokyo, Japan) programmed with dimensions of my dice (rectangles of sizes 25 mm by 64 mm). I use a 2060 blade (cuts 220 µm wide). Dicing produces 14 devices from a 300-mm wafer.

3.3.2 SU-8 Mold and PDMS channel

The microchannel in which the liquid is passed over electrodes is molded in poly(dimethylsiloxane). PDMS is the most widely used silicon-based organic polymer for MEMS as it is clear, bio-compatible, inert, non-toxic and non-flammable (.40, 41). The mold used for producing the channel is made out of photodefinable, epoxy-based negative resist 2050 SU8 (MicroChem, Newton, MA) patterned on 150-mm-diameter 650-µm-thick silicon wafer (WaferNet, Inc., San Jose, CA). This resist can be used to fabricate high-aspect-ratio structures and can be used repeatedly for molding many PDMS channel duplicates; therefore, it is a good choice for microfluidic molds.

I start the fabrication of the mold by dehydrating the wafer. To accomplish this, I bake the wafer on a hotplate at 200°C for 30 min. Next, I spin 2050 SU8 using SU8 spinner. The exact time of the spin, final speed as well as the amount of dispensed SU8 is determined by the desired final thickness of the resist layer as well as the wafer diameter. For a 100-µm-thick SU-8 on a 150 mm wafer, the manufacturer (MicroChem, Newton, MA) suggests spinning 6 ml of the material and accelerating the speed of the spin at 100 rpm/s² up till the speed of 500 rpm/s and then holding that speed for 20 s to spread the resist throughout the wafer. Following this, I ramp up the speed until final speed of 1680 rpm/s with acceleration of 300 rpm/s² and hold it at this final speed for 30 s to achieve the desired thickness. The baking time and exposure time are also determined by the resist thickness; for a 100-µm tick layer, I prebake the wafer at 65 °C for 5 min, then at 95 °C for 10 min. Next, I align and expose the resist through the chrome channel mask mentioned earlier by delivering 10 mW/cm²/sec for 36 s at wavelengths 365-405 nm (EV 620, Electronic Visions, Tempe, AZ).

After exposing, I postbake the wafer at 65°C for 1 min and then at 95°C for 9 min. Next, I develop the resist by soaking the whole wafer in 1-methoxy-2-propanol acetate (PM acetate) and gently shaking the container until SU8 dissolves. To verify the end of the process, I squirt some isopropanol on the wafer; undeveloped SU8 will turn white on reaction to isopropyl alcohol. After the SU8 has been fully developed, I wash the wafer with PM acetate and dry it with a stream of nitrogen. Next, I silanize the SU8 surface by placing the wafer in a vacuum jar with several drops of hexa(dimethyl)siloxane (HDMS) for 1 hr. The process of silanization can neutralize the surface charges, thus eliminating non-specific binding preventing PDMS from bonding to SU8. With this step the wafer is ready to serve as a mold for PDMS channels.
In order to make the channel, I mix 10 parts of silicone (PDMS) elastomer base and 1 part of the curing agent (Sylgard 184/182, Dow Corning, Midland, MI) by weight. I blend the mixture very thoroughly since the materials are viscous and complete mixing is essential; next, I drive the air bubbles out of the mixture by degassing it in a vacuum jar for 40 min. I pour the PDMS mix on the wafer and bake it at 65°C for 2 hrs. The exact amount poured does not need to be precise since it only determines the thickness of the sheet, which is irrelevant in my studies; usually I dispense about 90 mL of the elastomer for a 150 mm diameter wafer. After PDMS sets, I gently peel it off the wafer and cut the sheet into individual devices following the borderlines between individual devices.

3.4 Packaging

Packaging provides the die with an electrical and fluidic interface to the external world and is an integral part of the device. Here, I describe the design of the printed circuit board and the assembly of all the components.

The printed circuit board (PCB) (ExpressPCB, Santa Barbara, CA) is the main component of the package providing the electrical and fluidic access (Figure 3-1). It contains a rectangle in the middle for easy alignment of the die. There are four connections on each side of the die for connecting each of the electrode sections to appropriate voltage. The two metal lines above and below the die with six 1-mm holes in them serve to provide the electrical signal. The outermost holes on each of the lines hold headers, onto which the cables connected to the signal generator may be clipped. Two of these access points on top and bottom of the die are provided for convenience; only two of them are used at any given time (one on top and one on bottom). Each line holds four headers in-between the access points. There are four corresponding jumpers on each side of the die placed in 1-mm holes as well but not linked in a line. Each one of these holes is instead connected to a 1.2 mm hole directly surrounding the die. The holes of 1.0 mm and 1.2 mm diameter are connected by traces on the bottom of the board and serve to connect the die electrically. There is a wire soldered into the 1.2 mm hole and this wire is glued with a conductive epoxy onto a single section of the die. The 1.0 mm holes aligned in pairs on each side of the die hold headers, which are selectively connected using shorting jumpers to the main voltage supply line coming from a pin of the voltage generator clipped onto the incoming clip. 1.5 mm diameter holes provide fluidic access connecting to the fluidic tubing and forming an inlet and an outlet of the micro channel.

To package the device after microfabricating the Pyrex die, I drill the fluidic access holes in the die following the gold alignment marks with a hand-drill (380-6, Dremel, Mount Prospect, IL) equipped with a .75-mm-diameter diamond drill bit (C.R. Laurence Co, Los Angeles, CA). After drilling the holes, I clean the dust and the protective resist by rinsing the die with acetone, methanol and isopropanol. I dry the die with a stream of nitrogen.

Next, I attach the die onto the PCB by placing two NanoPort adhesive rings (Upchurch, Oak Harbor, WA) around the fluidic access holes on the PCB and pressing the die with its access holes aligned with the rings. Not only do the NanoPort adhesive rings attach the die to the PCB, but they also seal the fluid access ports preventing liquid leaks. For a secure seal, I clamp the NanoPort rings to the die with binder clips and bake it in an oven at 65°C for 1 hour.

Next, I solder the wire into the PCB and glue onto the die on each section of the interdigitated electrodes using conductive epoxy (ITW Chemtronics, Kennesaw, GA). I also solder the headers into the 1.0-mm holes of the PCB. I insert 1.6-mm-outer-diameter PEEK tubing (Upchurch) into the fluidic
access holes from the back of the PCB. I glue the tubing into the holes with High Performance Epoxy (Loctite Inc., Pocky Hill, CT). I then cure the epoxy by baking the device at 65°C for 1~2 hours.

The electrode and the channel layer of the device are not bonded but rather clamped together. I clamp them with binder clips on each side of the die providing pressure to seal the microfluidic channel.

The device is then used in experiments as described in the following chapters.
4.1 Overview

This chapter describes the experiments conducted in order to see whether and how well the concentrator can selectively collect bacterial spores using dielectrophoresis. Here I propose the operating conditions based on the particle data presented in Chapter 2, the experiments used to test this operating scheme, and methods of analysis of the collected data leading to information on sample purity. The chapter finishes with a discussion of shortcomings of the experimental methods and suggested improvements.

4.2 Device operation

The main goal I focus on is to operate the concentrator so as to capture the spores while the remaining interferents are either repelled, not captured or captured in a different section of the device. As shown above (Figure 2-4: DEP regions obtained for particles under study: chitin (A), dust (B), pollen (C), sand (D), spores (E), soot (F)), the spores undergo pDEP in media conductivities lower than 0.1 S/m and frequencies lower than 30-40 MHz. All other studied particles exhibit pDEP or weak pDEP within this range of conductivities (between 0.0006 S/m and 0.1 S/m); therefore, I chose deionized water as the medium (0.0006 S/m) to optimize the trapping of spores since increasing the conductivity would not exclude any additional particles. Spores, however, may potentially experience the strongest DEP attraction in the least conductive medium. Using preliminary trapping experiments, I noticed that spores are well trapped for frequencies in between 10 kHz and 1 MHz. Below the lower frequency, electrochemical reactions damage the electrodes, whereas above the upper cutoff of this range, DEP holding the spores to the electrodes is very weak. Thus, to optimize the device operation for spore trapping I chose deionized water as the medium and the frequency of 100 kHz. Since all other particles of interest (e.g., dust, soot) experience pDEP at this conductivity and frequency as well, optimizing the operating conditions for spore trapping does not necessarily mean optimizing them for mixture purification. In order to ensure improved selective trapping, I decided to deplete the mixture of the interferents (e.g., dust, soot) prior to trapping the particle of interest (spore). This way, I expected the contaminated solution to be initially and selectively cleared of interferent, while the spores can be carried further downstream to their trapping location.

Therefore, I divided the device into two parts: the first section - an active filter – that collects interfering particles such as soot and dust at 10 MHz but does not attract spores and the second section – the concentrator – that concentrates bacterial spores from a pre-purified solution at 100 kHz (Figure 4-1). The spores are not affected when passed through the filter but soot and dust experience pDEP since their pDEP region extends at least until 80 MHz at low conductivities. The goal of the filter is to deplete the mixture of these interferents so that the next section of the device, the concentrator, can capture predominantly spores despite operating in the pDEP region of both the bacteria and the interferents. For this purpose, I designated two functional parts of the device: an active filter driven with a sinusoidal voltage of frequency 10 MHz and amplitude 20 Vpp, and the concentrator section, driven at sinusoidal voltage of 100 kHz and 20 Vpp. Both parts are banks of interdigitated electrodes 25 μm in width and spaced uniformly at 25 μm distance from one another.
4.3 Experimental methods

Before every experiment, I clean the device thoroughly to remove any contamination first. I wash the die with acetone, isopropanol and methanol to remove organic residues, rinse it with deionized water and bathe it in Nanostrip 2x (Cyantek Corporation, Fremont, CA). After a thorough rinse in deionized water, I package the die on a custom-designed printed circuit board (PCBExpress, Mulino, OR) that provides electrical and fluidic contacts. I place the assembly on the microscope stage (AxioImager, Zeiss, Germany), attaching it with tape to prevent any movement during the experiment. I use PEEK tubing (Upchurch Scientific, Oak Harbor, WA) with an inner diameter of $1.59 \times 10^{-3}$ m and four-way valves (V-101D, Upchurch Scientific, Oak Harbor, WA) to attach the inlet of the device to a 10-mL syringe (VWR International, West Chester, PA) filled with a test mixture that is placed in a syringe pump (KDS200, KD Scientific Inc., Holliston, MA). The fluid is delivered to the device at the rate of 100 $\mu$L/min.

I use two signal generators (33250A, Agilent, Palo Alto, CA) to simultaneously apply two different sinusoids to the device; the electrodes of both are clipped to the pins on the PCB, which provides connection to the filter and the concentrator section of the device. I use two different frequencies of the signal during the experiment; the signal amplitude is kept at 20 Vpp. 20× magnification is obtained using a Spot RT Color camera (Diagnostic Instruments, Sterling Heights, MI) and an upright microscope (AxioImager, Zeiss, Thornwood, NY).
I prepare the test mixtures of particles as follows. First, I stain 1 mL of $10^7$ cells/100 μL of *B. subtilis* spores (SUS-1A-7, Raven Biological Labs, Omaha, NE) with 50 μL of 2 mg/mL solution of concanavalin A conjugated with Alexa Fluor 488 dye (C11252, Invitrogen, Carlsbad, CA) for 30 minutes at 37 °C. Next, I wash the spores four times with deionized water and resuspend them back into 1mL of water. Next, I add this 1mL of the stained spore solution spore to 9 mL of a solution of an interferent of interest, thus creating a binary mixture of spores and one other substance from among: chitin, dust, pollen, sand and soot. This ratio is used to form a standardized 10 mL of solution for each mixture under test.

I prepare the solution of the interferent as follows. I suspend soot (and other interferents, one by one) in deionized water up to a final concentration of 0.4 mg/mL (the concentration differs for different interferents as given below), forming a stock solution of soot, stored at room temperature for about a month. Each time I use soot as an interferent, I take 100 μL of this stock solution and dilute it with 8.9 mL of water, obtaining 9mL of interferent solution added to 1 mL of freshly stained spores solution each time. Stock solutions of other interferents have the following concentrations: dust – 0.4 mg/mL, pollen – 0.6 mg/mL, chitin – 0.4 mg/mL, sand – 0.6 mg/mL. I draw 10 mL of the obtained binary mixture into a syringe (309605, WVR International, West Chester, PA).

Next, I affix the device onto the microscope stage and place the syringe with the test mixture onto a syringe pump (210C, KD Scientific, Holiston, MA), which I program with desired parameters of the syringe (plastic, 10 mL). I connect the syringe to the input of the device with the 0.8-mm inner diameter, 1.6-mm outer diameter PEEK tubing (1533, Upchurch Scientific, Oak Harbor, WA.). I start flowing the mixture into the channel at 100 μL/min with the electrodes turned off.

After the channel has been filled in completely, I take an initial set of background pictures. Binary mixtures are visualized using the two available imaging modes: particles such as soot, sand, dust, etc. are visualized using bright field; spores are observed under fluorescence. Since I expect a difference in behavior from the filter and concentrator part of the device, I take pictures of both of these locations of the chip. Using the electrode sections’ boundaries, I always take pictures of the same mid region of the concentrator and filter section from each chip. Before the experiment, I take a brightfield and a fluorescent picture of each section (2 sections, 2 pictures each). Throughout this thesis, I refer to this set of pictures as either “before” or “background” images. An example set of brightfield pictures taken in a single experiment is given in Figure 4-2. Two of these images (Figure 4-2 A and C) are taken before the experiment and show clean electrodes.

Next, I turn on the preset voltages and with electrodes on flow 2 mL of the mixture through the device. After delivering the mixture (20 min), a set of pictures is taken again at the same locations as the background to evaluate the efficiency of particle trapping in the filter and concentrator sections respectively. I refer to this set of images as the “after” images. An example set of brightfield pictures taken in a single experiment is given in Figure 4-2. Two of these images (Figure 4-2 A and C) are taken before the experiment and show clean electrodes. After the experiment (Figure 4-2 B and D), particles have attached onto the electrodes. Similarly, Figure 4-3 shows analogous pictures from the filter (Figure 4-3 A, B) and concentrator (Figure 4-3 C, D) sections imaged using fluorescence. Subsequently, I switch the electrodes off, disconnect the fluidic access ports, take the setup apart and clean the device.
Figure 4-2: Brightfield images taken for each collection experiment. A. Background image of the filter section of the device. B. After image of the filter section with soot particles trapped on the electrodes. C. Background image of the concentrator section of the device. D. After image of the concentrator with soot on electrodes. Conditions: 100µL/min, filter – 10 MHz, concentrator – 100 kHz.

Figure 4-3: Fluorescent images taken for each collection experiment. A. Background image of the filter section of the device. B. After image of the filter section. C. Background image of the concentrator section of the device. D. After image of the concentrator with spores visible on the electrodes. Conditions: 100µL/min, filter – 10 MHz, concentrator – 100 kHz.
In order to extract information regarding the concentration and purity of collected mixtures of accumulated particles, I have developed an algorithm that quantitatively relates the resulting image intensity to the amount of captured material. It is implemented in MATLAB (Mathworks, Inc., Natick, MA) and includes the following steps. First, the program automatically aligns the respective corresponding images from the before and after set to restore the “before pixel to after pixel correspondence” since the relative locations of the stage, the device, or the camera might have shifted in the course of the experiment. As a result, the before (Figure 4-4 A) and after (Figure 4-4 B) pictures do not necessarily present the same exact area but are possibly slightly shifted with regards to one another in vertical and horizontal directions. If \( f(s,t) \) represents “before image” and \( g(s,t) \) represents “after image”, the after image is created from the before image by applying a shifting kernel \( k(s,t) \), expressed mathematically as \( f(s,t)=k(s,t)*g(s,t) \), where * denotes the process of convolution. The kernel, or the shifting function, can be found by considering the Fourier transforms of the images in frequency domain since \( F(u,v)=K(u,v)G(u,v) \), where \( F(u,v) \) corresponds to the Fourier transform of \( f(s,t) \). Thus, the kernel is obtained from \( K(u,v)=F(u,v)/G(u,v) \).

The algorithm finds the coordinates of the relative shift of the two pictures and crops them to constant area to account for the shift so that respective pixels are in the same locations in both images. An example of the processed pair of images is given in Figure 4-4 C, D.

![Figure 4-4](image.png)

Figure 4-4: A typical result of using the shift function. A. Background picture of an electrode section of the device. B. After picture of the same section with particles attached. Note: the image is shifted relative to the background. C. The corrected version of the background. D. The corrected version of the after image. Note: C and D have been shifted and cropped to make respective pixels correspond to one another.

Since aligning various sets of images requires shifts by different numbers of pixels, in order to obtain meaningful numbers across different experiments, all analyzed pictures need to be cropped to a constant size. The algorithm automatically crops the pictures to a preset size of 300 by 300 pixels. Based on multiple experiments, after accounting for shifts the pictures always have at least this area and therefore this size of the picture is appropriate for further analysis.
Each of the collected brightfield images contains some electrode-covered and electrode-less areas; to avoid variations due to different reflectance of the particles from varied backgrounds only the particles trapped and visible on electrodes are counted. To accomplish this differential counting, the electrode image is first extracted by creating a binary mask (Figure 4-5). White areas of the mask correspond to the gold-covered areas of the chip and they will be retained when the mask is applied to the collected images. The mask is created using the Otsu’s method that chooses the threshold between dark and bright pixels so as to minimize interclass variance between them (standard masking method used by MATLAB). The result of masking the background image is presented in Figure 4-5 B.

![Figure 4-5: Images illustrating the mask processing. A. Background image. B. Mask based on the background. C. Eroded mask.](image)

The mask is then applied to both before and after images in order to determine the differences between the two and thus count the particles that have accumulated over the course of the experiment. Before this happens however, the mask is eroded by one pixel in each direction to account for the fact that the focus plane of the microscope might have shifted in course of experiment, thus, changing slightly the locations of the electrode edges in the after images. The mask and the eroded mask are shown in Figure 4-5 B and C.

Finally, the algorithm integrates the trapped particle intensity. The after image is subtracted from the before image and the difference of the two gives the collected particle intensity (Figure 4-6). The greater the area covered by the particles, the more concentrated the solution and the greater the difference. Similarly, the greater the intensity of a given area, the more particles must have attached in that place and the greater the difference. The total particle intensity is related to the concentration of the particle for some initial particle concentrations as shown in the next section. At present the range over which this is true is

![Figure 4-6: Processing the images to obtain the final particle intensity count. A. Masked background image. B. Masked after image. C. Difference between the masked background and masked after image; shown are the particles in white.](image)
not known, therefore, only limited quantitative statements may be made about the results. The eroded mask is applied to the before, after and their difference images. Typical masked before, after and difference images are shown in Figure 4-6. Next, the algorithm integrates the intensity of the masked difference image producing the final number that relates the difference image intensity to the particle concentration. Since the same area of the picture is processed for each experiment and the electrodes have the same dimensions in each experiment, the effective area masked out is the same for all experiments. The same steps are followed for the fluorescent set of images; the shift coordinates and the masks applied to fluorescent images are taken from processing the brightfield channel.

Given the data obtained from the described algorithm, I compare the intensity value for the filter and the concentrator section of the device for each particle in the binary mixture. The results show brightfield and fluorescence result as bar graphs and are presented in the following Chapter 5.

4.4 Image Algorithm Validation

I verified that the algorithm reflects the difference in concentration of particles by collecting various known concentrations of different particles and monitoring the output of the algorithm for each of these experiments. The particle concentrations reflect concentrations of particles in mass per volume used for each experiment. The numbers computed based on the collected images are positively correlated with concentration. The results of validating the algorithm with soot, spores and sand are given in Figure 4-7, Figure 4-8 and Figure 4-9 respectively.

Figure 4-7 shows how the algorithm output varies with the input soot concentration (gray data markers). The aquamarine line shows a linear model for the data ($R^2 = 0.9909$). The integrated image intensity approaches linear dependence on concentration of particles. While the numbers may be fit to a different models as well (second degree polynomial - $R^2 = 0.9964$; third degree polynomial $R^2 = 0.9993$), the general relationship maintained by the numbers reveals that for increasing particle concentration, the output given by the algorithm increases and thus, the image intensity and the particle concentration are positively correlated. The error bars indicate a large variation between runs for very dense particle mixtures. This variation may be caused by saturating the image with collected particles, suggesting that there exist a dynamic range for possible concentrations that can be measured with this method, beyond which the measurement is prone to error. Similar effects occur for another brightfield particle, sand (Figure 4-8). Again, the data

![Figure 4-7: The image intensity output by the algorithm for different concentrations of soot.](image1)

![Figure 4-8: The image intensity output by the algorithm for different concentrations of sand.](image2)
may be fit to different models (linear fit shown by aquamarine line gives $R^2 = 0.8934$; second degree polynomial - $R^2 = 0.941$; third degree polynomial - $R^2 = 0.991$) but the variations for high sand concentrations are relatively large.

I verified that the algorithm is successful when imaging in fluorescence as well. It performs comparably well on the pictures of fluorescent particles such as stained spores. The results of validating the device with spore solutions of varying concentrations are given in Figure 4-9 below.

Fluorescent images produce the same positively sloped relationship of image intensity and particle concentration. This measurement also suffers from significant scatter for high input concentrations. During the experiments, brightfield as well as fluorescent particles are used at about 0.01 of the concentrations that give the relatively large errors (upper dynamic range limit) suggesting that the correlation of results thus obtained is less at a risk of suffering from high scatter that some of the calibration data. Fitting the data points to various models indicates a very good match even for the linear model (linear fit shown by aquamarine line gives $R^2 = 0.9609$; second degree polynomial - $R^2 = 0.9988$; third degree polynomial - $R^2 = 0.9995$).

Overall, the validation of the algorithm is successful in confirming a positive correlation between particle concentration and calculated image intensity for all particles considered. All tests included the particle concentration that was subsequently used in actual experiments. Given a good quality of the linear fit and the fact that the experimental concentrations are among the ones used for validation of the algorithm, I am convinced that the algorithm reveals a true positive correlation between the particle concentration and image intensity. Thus, regardless of the actual values of the pixel value-to-concentration correspondence, which would require more tests to be rigorously established, an increase in image intensity means an increase in concentration of the particles. The algorithm thus proves to be a helpful tool when evaluating relative effectiveness of different schemes of particle remediation.

All of the measurements show higher variations for high particle concentration suggesting saturation of the image and an end to the dynamic range of the evaluation method. To further confirm the existence of the dynamic range, the concentrations over which this data is evaluated could extend further.

### 4.5 Possible improvements to the experimental method

Next, the results are quantified using an on-chip measurement. In the process of releasing the particles off the chip (which is a challenge by itself), some of them are lost, some are not released and only a fraction of the particles quantified here is actually usable for further analysis. Therefore, the actual purity of the mixture achieved as an output of the concentrator will differ from the values presented here. Additionally, the release step in itself may be electrically specific further complicating the device operation.

The algorithm developed to count the particles reflects the concentration differences at its output. However, it shows significant scatter for high concentrations of particles. This effect possibly arises due to the saturation of image with the particles; dynamic range is extremely important not only in the case of light intensity, exposure time but also the number of particles visible in any given image. Using less concentrated solutions of particles is a possible solution to this shortcoming.
4.6 Imaging modality

Quantifying the operation of the device requires imaging the same area of the chip in two modalities: brightfield to quantify particles such as soot, dust, sand and fluorescence to quantify the concentration of spores. Imaging of one of the signals does not exclude the other and therefore, there is some amount of signal bleedover. Bleedover may obscure the results. For example, imaging fluorescent spores alone will result in a certain value but imaging them in a mixture with another particle, whose signal bleeds into the fluorescent channel (such as pollen) will produce another number.

In order to quantify and possibly correct for bleedover, I perform cross-channel algorithm validation, whereby I quantify the amount of bleedover coming every particle from their usual imaging modality to the other modality. To accomplish this, I image particles in their usual mode of visualization (brightfield for chitin, dust, pollen, sand, soot; fluorescence for spores) as well as I take pictures of the particles and their respective backgrounds in the opposite modality. I calculate the image intensity change that the presence of the particle produces in both modes.

The results of bleedover experiments are presented in Figure 4-10. The numbers thus obtained are hard to normalize across different particles. The normalization refers to individual particle normalization with respect to its background. Thus, a value of 1 or close to 1 means almost no change with respect to the background. The red square in the diagram outlines values of 1.00 ± 0.05 of intensity, which I chose to refer to as no different from the background value based on my experimental observation of particle visibility under various filters. According to this benchmark, spores produce an intensity change in the fluorescent modality without a noticeable effect on the brightfield picture. Similarly, chitin, sand and soot produce an intensity change in the brightfield modality without affecting the fluorescent image. Two particles, namely dust and pollen turned out problematic in this classification. Dust is well visible in brightfield; its fluorescent value of image intensity is however smaller than 1 as if dust did not reflect but instead absorbed radiation. This behavior may affect the values of the fluorescent intensity when dust is

![Figure 4-10: Image intensity of brightfield images (gray series) and corresponding fluorescence images (green). Red box exposes values within 0.05 value of the background. Particles imaged at their usual experimental concentrations: dust – 0.004 mg/mL, pollen – 0.006 mg/mL, chitin – 0.004 mg/mL, sand – 0.006 mg/mL, spores – 1 \(10^7\) cells/mL.](image-url)
placed in a mixture with spores. Similarly, pollen, which was meant to be visualized in brightfield turned out to be easily visible in the fluorescence channel. When mixed with spores, which only fluoresce in green, it may be distinguished however in that it also fluoresces in red.

Overall, bleedover measurement is a good way of validating the separation between two modalities. It is difficult however, to assign meaning to different values. For example, the fact that a certain concentration of chitin produces a change of 0.19 relative to its background is hard to relate to the corresponding change in its fluorescent intensity of 0.05. It does not mean that twice the concentration will produce 0.38 and 0.1 respectively nor does it mean that a concentration producing 0.38 in brightfield (whichever concentration of chitin it happens to be) will produce 0.1 in fluorescence. Depending on relative sensitivities of brightfield intensity with respect to fluorescent intensity and vice versa, the values may change linearly or not. Therefore, comparison across particles has limited quantitative value. The data only reflects what is seen with an eye, that brightfield particles except for pollen do not interfere with the fluorescent channel and that fluorescent spores do not affect the brightfield channel.
CHAPTER 5: RESULTS

5.1 Overview

After validating of the counting algorithm, I characterize the behavior of the device purifying the spore/soot and spore/sand mixtures using the proposed 10MHz-100kHz scheme (10-100, for short). In an attempt to learn more about the mechanisms of the device operation, I perform a set of control experiments as well. I also characterize the purification scheme for varying input concentration of the interferents and show that the effectiveness of the purification depends on these. I summarize the chapter with conclusions and evaluation of these experimental results.

5.2 Characterization of the 10 MHz-100 kHz scheme

First, I test the device with the proposed 100-10 scheme on a mixture of spores and soot. Soot contains a single substance with fairly simple electrical properties (highly conductive carbon; pDEP for all tested regions of operation), and is therefore a good preliminary interferent model. Intensity enhancement of the fluorescent signal for this mixture is presented in Figure 5-1. As shown, the difference in collecting frequencies leads to a difference in the amount of spores collected in both sections of the device. Little intensity increase (almost no spores collected) is visible in the 10 MHz section of the device (filter); the intensity of the fluorescent signal is about 7.0 ± 1.6 times that of what the original spore concentration amounts to. In the 100 kHz section of the device (concentrator) however, the signal rises to about 72.0 ± 11.0 times that of the initial value.

The collection of soot is reflected by the brightfield intensity enhancement shown in Figure 5-2. It is collected uniformly throughout the entire device despite the differences in the operating frequencies; its intensity is enhanced about 4.5 ± 0.8 times by the filter section and 4.2 ± 1.6 by the concentrator section. This result suggests that either the depletion of soot expected of the 10 MHz filter does not take place (there is too much soot to filter it out to begin with) or the decreased operating frequency in the concentrator (100 kHz) effectively offsets the difficulty of collecting the diluted particles by effecting a bigger pDEP force.
Since the calculated intensity value and the particle concentration are positively correlated, we can claim that this operating scheme leads to enhancement in concentration of both spores and soot. The image intensity value for spores is about 7 times larger and for soot 4.5 times as referenced to their initial intensity values for original mixture concentrations in the filter section. Similarly, in the concentrator section, the intensity is now about 72 times for fluorescent image and about 4.2 times for brightfield than before the experiment. As a result, if the mixture from the concentrator section of the device were to be released without any loss in material, this would mean that the ratio of the spore particles to the soot particles by their respective intensity scales would be increased with respect to their initial mixture concentrations. The exact increase in concentration cannot be identified from the increase in intensity values since these are not rigorously calibrated, however positive correlation between particle concentration and image intensity allows me to claim that the 10-100 operating scheme allows for a specific spore concentration increase in the concentrator section of the device.

To investigate the mechanism by which the device performs the purification, I perform the control experiments next. To see to what extent the device depletes the mixture of a given particle, I operate the entire device at a single frequency, either 10 MHz (Figure 5-3) or 100 kHz (Figure 5-4 and Figure 5-5).

Figure 5-3 illustrates the soot depletion by a 10 MHz signal. The entire device is driven with the same signal of frequency 10 MHz and yet about half as many particles are trapped by the concentrator (1.8 ± 0.7 enhancement) as by the filter (3.7 ± 1.4). Moreover, the intensity of the images in the concentrator section is close to that of the initial mixture composition (enhancement close to 1), which supports the view that the filter does in fact effectively deplete the soot particles but the change in frequency to 100 kHz in the concentrator region offsets this action. Given an initial section of 10 MHz, the second section collects less soot resulting in half
the intensity (cannot be well translated into concentration). In reality however, once the second section is driven with a sinusoid of frequency 100kHz, the magnitude of the DEP force that soot experiences in this region is much stronger and able to still pull more particles than 10 MHz signal as shown by Figure 5-1 (enhancement of 1.8 ± 0.7 by 10 MHz and a value of 4.2 ± 1.6 by 100 kHz in the concentrator section).

In the case of spores captured under 10 MHz-10 MHz scheme, there is no depletion effected by the filter, which agrees with the prediction that spores would not effectively react to this frequency. When collected at 10 MHz-10 MHz, their collection leads to intensity increase of 5.3 ± 2.0 and 4.5 ± 1.7 (graph not shown) in the filter and the concentrator section respectively. This uniform attachment throughout the device can be explained by the fact that the 10 MHz does not trap or hold spores very efficiently, therefore a significant fraction of the original content of cells remains in the solution after passing the filter section and is readily available to the concentrator.

Driving the entire device at 100 kHz serves as a control for the effectiveness of the concentrator section. The results of brightfield intensity increase by 100 kHz-100 kHz operation are shown in Figure 5-4. This result confirms the hypothesis that 100 kHz is able to overcompensate the depletion of soot effected by the filter section of the device. It also shows less soot collecting in the second section of the device, which may be a result of the depletion of the solution of soot. The concentrator section in this case collects a comparable amount of soot as the concentrator section in the 10 MHz-100 kHz case suggesting that the concentrator section driven at 100 kHz is strong enough to overcompensate the depletion caused by 10 MHz and 100 kHz filter. The experiments confirm that the concentrator section of the device driven at 100 kHz will collect about the same amount of soot regardless of which frequency is used in the filter section. In fact, the filter may even be turned off (data not shown) without a significant effect on the performance of the concentrator. Operating the device using the 100 kHz-100 kHz scheme reveals depletion of spores by the filter; the intensity enhancement of the brightfield images (soot) is 5.1 ± 1.4 and 3.8 ± 2.3 in the filter and the concentrator respectively. Spore ratios in this case are 93.9 ± 29.1 and 83.4 ± 25.0.

The main role of the filter was meant to be the depletion of the solution of the interferant particle such as soot. Control experiments presented so far did not allow concluding that effective depletion in fact occurs. This fact may be caused by too large concentration of soot. If there were fewer particles, the same sensitivity of the filter section would clear a bigger fraction of them from the solution and thus prevent their collection in the concentrator segment of the device.

Therefore, subsequently I characterize the behavior of the device over a range of concentrations of both spores and soot. Figure 5-5 shows the enhancement in intensity achieved in the concentrator section for both soot (brightfield) and spores (fluorescence) when the soot concentration is varied. As the concentration of soot increases 10 times of its usual test value to 0.04 mg/mL, the intensity enhancement of the brightfield image (soot collection) increases about 6 times (from 3.6 ± 2.0 to 19.0 ± 2.9). At the same time, the collection of spores is impeded; the fluorescence intensity decreases from 71.9 ± 19.8 to 30.4 ± 6.9. While decreasing the soot concentration does not seem to decrease its rate of collection (the intensity stays at about the same level, increasing slightly), the collection of spores increases significantly (up to 214 ± 45.7). This result suggests that the final purification achieved for various solutions depends

![Figure 5-4: Intensity enhancement of the brightfield signal (soot) by the device driven with 100 kHz-100 kHz input signal.](image-url)
strongly on the input concentration of soot. Here I have shown that the spore concentration enhancement with respect to soot varies as 31, 20 and 0.6 with varying the soot input concentration (0.0004, 0.004, 0.04 mg/mL).

Similarly, the filter section (Figure 5-6) allows for different particle attachment depending on the initial soot concentration. However, the difference in spore attachment is not as striking and therefore, the achieved intensity enhancements are around 2, around 1.5 and 0.5. These two experiments suggest that the lower the input concentration of soot, the better sample purification is achieved by the concentrator without a significant loss of the spores in the filter. Even though 100 kHz section has been shown to collect at its maximum capacity (100 kHz-100 kHz experiment), preserving the un captured spores in the mixture may become important if several devices were cascaded back-to-back to improve the sample purity.

The variation in the input concentration of spores does show a similar dependence on the purity enhancement in the concentrator section. Decreasing the spore concentration 10 times its initial value (to $10^6$ cells/mL) causes the intensity enhancement of the concentrator to go down from about 20 to around 0.2 (graph not shown). In the filter section, the same action causes the intensity enhancement to fall from 1.5 to 0.3. These experiments establish the need to characterize the dynamic range better. I am convinced that the final purification of the spore samples by the device is dependent on the input concentration of the species. If the mixture is very contaminated, the spores are not easily pulled out of the solution and the purity enhancement is small.

Next, I test the 10 MHz-100 kHz operating scheme on an insulating particle, sand, as an interferent. Figure 5-7 shows that spore collection pattern is similar regardless of using sand or soot as an interferent. The fluorescent image intensity
increases from about 10.4 ± 8.3 in the filter section to about 75.1 ± 19.6 in the concentrator section. At the same time, the sand does not collect in either of the sections (Figure 5-8), remaining close in intensity its original values (enhancement about 1 for both sections), which enhances the spore concentration in the concentrator section in agreement with the design.

The values of the intensity of sand are slightly bigger than 1 for both sections perhaps because the initial mixture is being flushed into the device continuously over a period of time. Even though sand is not attracted to the electrodes with electrical specificity, it may nonspecifically attach to the inside of the channel. I observe this trend for all experiments with sand.

Control experiments with the sand as the interfering particle show that either 10 MHz or 100 kHz signal applied to either of the sections does not affect sand collection, or lack thereof. The collection of spores (Figure 5-9), however, undergoes an enhancement at 10 MHz applied to the filter as compared to the analogous filter of spore/soot mixture at 10 MHz – 10 MHz (5.3 ± 2.0 intensity enhancement as opposed to 16.8 ± 10.2). Although statistically prone to high standard deviation, this result suggests that in the absence of particles “competing for the field” spores are able to attach more effectively. In light of the previously mentioned saturation effect at 10 MHz, this experiment suggests that the upper range of electrode saturation depends on how many particles compete for the field and for the “real estate” of the electrode edges.

After gaining insight regarding the 10-100 operating scheme, I hypothesize that a different combination of frequencies may be more effective in selective collection of spores. Since the frequency of the filter does not seem to affect the soot or sand significantly, using a higher frequency such as 20 MHz may effectively decrease the amount of spores collected here without the detrimental effect on its filtering action. Decreasing the spore collection frequency to 20 kHz in the concentrator section of the device may lead to an increase in the sample purity (spore concentration versus the interferant concentration). These operating conditions were tested with spore/soot mixture.

The results of the experiments are shown in Figure 5-10 and Figure 5-11. In accordance with predictions, the spore intensity decreases in the filter section operated at 20 MHz to about 1.1 ± 0.1. Similarly, the spores are concentrated much better by the 20 kHz signal than before at 100 kHz; the
fluorescent intensity of this section has increased to 300.8 ± 34.3. Similarly, the intensity of soot has increased in the concentrator section at 20 kHz (7.5 ± 1.5), while staying about the same in the filter section (4.0 ± 0.6). However, the 20-20 scheme is consistent with overcompensation (suspected in 10-100 case as well) of the soot depletion by the decreased concentrator frequency. Nevertheless, it still achieves a much higher fluorescent intensity values in the concentrator section of the device, which allows to expect a significant increase in the resulting spore concentration as compared to analogous section under the 10-100 scheme.

Overall, the scheme of selective operation of the device is based on the difference in the magnitude of DEP force, when no real information regarding the DEP magnitude is available. The only established information about the particles is the sign of the DEP force they experience; based on particle properties, this characteristic allows to distinguish only sand from the other particles. All other particles present difficulties to electrical exclusion because of lack of information on their electrical properties.

Some particles are excluded from the mixture not based on electrical differences between them and the spores but based on other factors, such as density and size. Pollen particles are uniformly bigger than spores and are therefore excluded by filtration before even reaching the channel. Similarly, chitin is much denser than the medium and tends to stick to the tubing used to deliver the media and therefore does not reach the channel either. This methodology achieves the desired purpose of purifying the mixture but is not electrically specific.

Figure 5-9: Intensity enhancement of the fluorescent signal (spores) by the device driven with 10 MHz-10 MHz input signal.

Figure 5-10: Intensity enhancement of the fluorescent signal (spores) by the device driven with 20 MHz-20 kHz input signal.
5.3 Summary

Experiments I conducted confirm that the interdigitated device can be operated as a selective concentrator for purifying the mixtures of *B. subtilis* spores and enhancing their concentration. The device uses an active filter in the initial section of the channel, through which the mixture is passed, to stop the interfering particles. Next, the concentrator section collects and holds spores against the flow. While the filter effectively depletes the mixture of the interfering particles such as soot, the concentrator uses such a low frequency field to trap the spores that it effectively compensates for the dilution of the interferent and still traps it very effectively. It traps the spores however, much more efficiently, and therefore allows for electrically specific mixture purification.

This section does not present the results with other particles such as dust, chitin, pollen. Chitin and pollen are eliminated from the device by their size and density and dust needs more thorough evaluation since it is a heterogeneous mixture of electrically-different particles. Given more time, I would like to further my understanding of the behavior of the device with these particles and look into the schemes of effective release of spores without harming them.
Chapter 6: Conclusions and Future Work

6.1 Overview

In this chapter, I present the conclusions of my work, its contributions, suggested improvements to the experimental method and data analysis, as well as the challenges that lie ahead in the work of selective bacterial spore concentration using dielectrophoresis.

6.2 Conclusions

This thesis presents the first up-to-date attempts at electrically specific remediation of bacterial spores from their mixture contaminated with typical air interferents such as dust, sand or soot. The main conclusion of this work is that such a purification of the biological sample is possible using dielectrophoresis. In this work, I present possible schemes of operation of an interdigitated electrode device in order to increase the spore concentration with respect to that of the interferant (improve purity of the sample). Different sections of the device are driven with different sinusoids to achieve separate functions: active filtering of unwanted particles at the inlet of the channel and concentration of the desired particles close to the outlet. I have made an assumption that my method of quantifying the device operation allows for direct comparison of image intensity to particle concentration. Using this metric, I have shown that the purity of the sample may increase depending on the operating conditions and the input mixture concentrations.

Investigation into the mechanisms of action of the device let me conclude that it achieves the purity enhancement via the maximization of the spore collection by the concentrator section. I have shown that the interferents such as soot or sand are collected at about the same rate for all tested frequencies (20 kHz, 100 kHz, 5 MHz, 10 MHz, 20 MHz). This way, the purity enhancement, which is the ratio of the final spore concentration enhancement to the interferent’s concentration’s enhancement depends heavily on the spore concentration since the denominator stays close to constant. Spores are most readily collected at 20 MHz and this frequency is recommended for most efficient remediation of spores from their mixture with air interferents such as soot or sand.

Depletion of the interferent from the mixture effected by the active filter, which was designed to impede interferent’s collection in the concentrator section, is compensated for by lower frequency signal driving the concentrator section, which can trap more efficiently even the diluted particles. This action interferes with the designed scheme of depletion for high interferant concentrations. For lower concentrations, the scheme works as designed. High interferant concentrations require better filtering scheme; one possible improvement may be extending the filter area so that the mixture resides longer in the active area of the filter and so that it can trap more particles. On the other hand, this design could potentially reduce the throughput or increase the device area since more area would be devoted to particle filtering and less to concentration if the device size were to stay the same. Alternatively, increasing the signal amplitude in the filter section might lead to a better filtering action, “compensate back” and even better entrapment of undesired particles out of their diluted solutions.

6.3 Contributions

This work led to establishing electrical properties of air particles and bacterial spores. The electrical properties of substances such as dust, pollen, sand, soot or chitin have not been thoroughly investigated in the context of dielectrophoresis. In my work, I established their crossover frequencies for...
various media conductivities and suggested a plan to purify mixtures of these substances based on these electrical properties. This is the first step to investigate their permittivities and conductivities more thoroughly and establish the relative DEP strength spectrum for each particle, which would allow for an improved design of a separation scheme.

Before establishing the electrical properties of the particles under study, I had devised a method to establish their permittivities and conductivities based on the impedance of their solutions in water. Using the impedance measurement, their permittivity and conductivity and thus, relative DEP strength as a function of frequency can be shown. Even though this method turned out not sensitive enough for the limited amounts of particles that I had available, it can potentially work if an appropriate setup holding a small amount of liquid and allowing for repeatable measurements is built.

As a part of the impedance measurement experiment, I developed software for automated data collection by the impedance analyzer. I implemented the package in MATLAB and topped it with a graphical interface, making it potentially available to run an impedance measurement experiment from any place in the world provided the control computer is connected to the internet. In practice, the measurement usually requires an operator to change and clean the liquid test fixture and affix the electrodes. However, if a lengthy experiment is run with a single sample being tested and remaining in the fixture over a period of time, then the code can make the data collection and transfer much more efficient than using obsolete diskettes.

Another contribution is the design of the operating scheme for the device to achieve the maximum selectivity. The simple device consisting only of banks of interdigitated electrodes can be made into an efficient selective concentrator based solely on differential localization of input signals. The device achieves good intensity enhancement, which reflects well on its ability to concentrate particles. It can potentially be cascaded to further improve its selective performance and parallelized to better the throughput.

Finally, in the course of this work, I developed the imaging algorithm to relate the collected images to the input concentrations of particles. This software is very robust to deficiencies of the experimental procedure, which occur when developing an assay. It accounts for the shifts of the unfixed device; for uneven lighting in a room of variable light intensity and for individual differences in electrode morphology for different devices. It has been shown to convincingly relate the collected images to the input particle concentration making the on chip measurements feasible.

6.4 Challenges and further work

It is unclear how much better remediation scheme one could imagine if the exact properties of the particles of interest were known, that is if the exact DEP curves (rather than their respective signs) were established for each of the particles. A better characterization of the relative magnitude of the DEP force at all frequencies would make this question a reasonable starting point for the discussion. Therefore, the relative DEP magnitude characterization remains outstanding. It will most probably require a thorough observation of a small number of particles (e.g., electrorotation) or improved impedance measurements on more concentrated samples (decreasing the volume of the measurement vessel, increasing the particle fraction increasing the instrument sensitivity, eliminating experimental error).

Next, the spores should be released off the chip for further analysis. Releasing particles that are already stuck is a challenge by itself without having to deal with the issues of electrical specificity. Here the release scheme may additionally affect the collected mixture purity if it favors the release of one particle over another. Another issue in releasing spores is to preserve them in their usual state or if a change is necessary, it needs to be reversible so that they can be easily detected based down stream of the device.

Finally, given more time, I would like to conduct more experiments with the proposed 20-20 operating scheme as well as with the remaining particles in order to fully characterize the device and its selective concentration abilities.
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