Abstract

BHATT, KETAN HARENDRAKUMAR. On-Chip Manipulation and Controlled Assembly of Colloidal Particles using Alternating Electric Fields. (Under the direction of Dr. Orlin D. Velev.)

Alternating (AC) electric fields have been investigated as a versatile tool for rapid particle and fluid manipulation in micro-Total Analysis Systems (µTAS). Different onchip electrode geometries and different particle suspensions were explored in this study with an aim to acquire a fundamental understanding of particle behavior under applied fields. Aqueous suspensions of particles of sizes ranging from nanoparticles to microspheres and having varied electrical properties (dielectric or conductive) were studied. For each system, detailed electrostatic simulations were carried out to identify the forces acting on the particles and fluid. Control of the particle-field, fluid-field and particle-particle interactions, by fine tuning the applied field, lead to the desired assembly of particles. Dielectrophoresis (DEP), the interaction between induced particle dipoles and the spatially non-uniform electric field, was used to assemble gold nanoparticles into microwires and for manipulating fluid droplets containing suspended particles in a novel liquid-liquid microfluidic system. AC Electrohydrodynamics (EHD) driven liquid flows were used for the transportation, redistribution and collection of suspended particles inside experimental cells.

Suspensions of metallic nanoparticles in water were assembled via DEP into wires of micrometer thickness between planar electrodes. Two modes of microwire assembly, one through the bulk of the suspension, and one as half-cylinders on the glass surface between the electrodes were identified. The operating conditions responsible for the two assembly modes were recognized. Control of the process parameters allowed making, for example, straight single connectors, or massively parallel arrays of microwires on the surface of the chip. The direction of microwire growth was guided by introducing conductive islands or particles in the suspension. The microwire assembly process was modeled using finite element electrostatic calculations. The experiments, supported by electrostatic calculations, showed that the wires grew in the direction of highest field intensity, "automatically" making electrical connections to the objects between the electrodes. The results point the way to
controlled dielectrophoretic assembly of nanoparticles into on-chip electrical connectors, switches and networks.

A new EHD effect arising from the application of alternating electric fields to patterned electrode surfaces was observed. AC fields were applied to dilute suspensions of latex microspheres enclosed between a patterned silicon wafer and an ITO-coated glass slide in a small chamber. The latex particles became collected in the center of the conductive "corrals" on the silicon wafer acting as bottom electrode. The particle collection efficiency and speed depended only on the frequency and strength of the field and were independent of the material properties of the particles or the electrodes. EHD flow emerging from the spatially non-uniform field was described and interpreted by means of electrostatic and hydrodynamic simulations. The technique allowed three dimensional microfluidic pumping and transportation using two-dimensional patterns. On-chip collection of latex particles, yeast cells and microbes using EHD flows was demonstrated.

DEP based manipulation of freely suspended droplets was used for designing a novel liquid-liquid microfluidic chip. Water or hydrocarbon droplets containing suspended particles were floated on a dense fluorinated oil layer and manipulated by applying spatially varying electric fields using an electrode array present beneath the oil. Two distinct equilibrium positions dependent upon the pattern of electrode energization were identified. Full scale simulation of the electric field distribution showed that if a single electrode within the array was energized the droplet levitated on top of the energized electrode in order to balance the high intensity DEP regions on either side of the electrode. Alternatively, if pairs of electrode were energized, the floating droplet got entrapped in the gap between an energized and grounded electrode where there was a high field intensity region. The on-chip system was capable of transporting multiple droplets in parallel, for rapid mixing of the contents of droplets and for carrying out chemical reactions and precipitations. Evaporation of water droplets was used to create binary and ternary "supraparticles". This novel liquid-liquid microfluidic system could find uses for compartmentalizing single living cell or genetic material into individual droplets and performing subsequent biochemical reactions, for precipitation assays, for high throughput drug and toxin screening and for material syntheses.
On-Chip Manipulation and Controlled Assembly of Colloidal Particles using Alternating Electric Fields

by

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Chair of the Advisory Committe
this dissertation is dedicated to
my family
without whose love and support
I would not be where I am
Biography

Ketan Bhatt, was born on New Year’s Day 1980 in Jaipur in the state of Rajasthan in India. The city of Jaipur, also known as the Pink City because of the pink facades of its old palatial buildings, is the state capitol and the largest city in Rajasthan. He was raised along with his sister in the city of Vadodara, in the Gujarat state of India. Vadodara is named after the Banyan trees (Vad in Gujarati) that grow in abundance on the banks of the Vishwamitri River and is home to major pharmaceutical, petrochemicals and plastics industries that include heavyweights such as the Gujarat State Fertilisers & Chemicals, Indian Petrochemicals Corporation Limited, Gujarat Alkalies and Chemicals Limited, Gujarat Refinery, ONGC and Alembic Industries. The vast industrial expanse around the city is one of the main reasons that Ketan decided to pursue the field of engineering. The high demand for chemical engineers in the state of Gujarat lead to the subsequent choice of Chemical Engineering as a major during his college career.

At an early age, Ketan was interested in Mathematics and Science and participated enthusiastically in school level quiz contests and showcases on those subjects. He carried out his high school education at Bright School in Vadodara, culminating his schooling days by placing 9th statewide (1st in Vadodara school district) in Gujarat State Higher Education Board Exam (Grade 12 equivalent). These high marks made it possible for Ketan to pursue a degree program in Chemical Engineering at the Birla Institute of Technology and Science (BITS) in Pilani, Rajasthan, one of the premier undergraduate institutions in the country. While at BITS, he undertook summer internship at National Physical Laboratory located in New Delhi, India during his second summer and followed it by another semester of internship at Birla Research Institute, Grasim Industries, Nagda, India. His experiences at both these institutes instilled in him a strong desire to continue his studies and drove him to pursue a graduate research career. After getting his B.E. (Honours) Chemical degree from BITS, Pilani in Spring 2001, he joined the PhD program at North Carolina State University the ensuing Fall. Since then he has been carrying out his graduate research under the guidance of Dr. Orlin Velev.
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Chapter 1

Alternating Electric Field Applications in Micro-Total Analysis Systems

* Partially based on Velev and Bhatt, Review article submitted to *Soft Matter*. 
1.1. Introduction
Micro-Total Analysis Systems (µTAS) or Lab-on-a-Chip systems that carry out sample pretreatment, analysis and detection are revolutionizing the fields of drug delivery, chemical and materials syntheses and analyses, point-of-care clinical devices, biowarfare and biochemical agent detection, high throughput screening, and water quality control among others [1]. A recent survey among nanobiotechnology (NBT) experts conducted by the Nano2Life Consortium found that the highest overall impact and prospect among emerging nanobiotechnology applications is of Lab-on-a-Chip systems [2]. This survey, conducted by the Interdisciplinary Center for Technology Analysis at the Tel Aviv University was an effort to understand the view of worldwide NBT experts on the anticipated developments in NBT and the impacts and prospects of these developments. The other leading areas deemed to have highest overall impact were self-assembly, biomimetic materials, single molecule biosensors and biodetection with tailor made nanosurfaces. The aim of my graduate research has been to develop tools and techniques for manipulation and assembly of colloids using alternating electric fields that are suitable for Lab-on-a-Chip applications. To this aim, this chapter overviews the field of µTAS, elucidates the reasons why electric fields are very well suited for µTAS and explains the various forces available for manipulating and assembling colloidal particles using electric fields.

1.2. Micro-Total Analysis Systems
Microfabrication & lithography technologies have led to a revolution in the electronic industry, scaling down devices from rooms full of power-hungry bulky vacuum tubes to chips packing as much as 700 million transistors with 65 nm features (Itanium 2 processor released in 2002 by Intel Corporation) on a single chip 15 cm² in size [3]. The advances in these technologies have naturally led to the development of miniaturized devices where chemical and materials processes are scaled down. The first miniaturized chemical analytical device, a gas chromatographic analyzer designed on a silicon wafer with intent on making portable air quality detectors was developed in 1979 by Terry et al. [4]. It included a spiral capillary column 1.5 m long fabricated on a 5 cm diameter silicon wafer and was able to separate simple mixtures in matter of seconds leading to a final package dimensions similar to a pocket sized calculator. In the early 90s, the term micro-Total Analysis Systems (µTAS)
was introduced by Manz et al. [5]. It was envisioned that a small on-chip device would be capable of analyzing the input streams, performing the necessary pretreatment and concentration stages, and carrying out analysis and detection steps automatically without human intervention. The qualitative flow chart for such a device is shown in Fig. 1.1.

![Figure 1.1. Flow chart of the steps necessary for a μTAS – The on-chip device envisioned should be able to do sampling, sample pretreatment and detection automatically. From Manz et al. [5].](image)

Early efforts in μTAS were focused on developing microfluidic devices for chemical analysis. These efforts lead to development of micro-pumps and micro-valves fabricated on-chips, for controlling and manipulating small volumes of fluid [6-9]. A further boost to μTAS concept was received when Manz et al. developed a device to achieve fast and efficient separation of amino acids using electroosmotic flows and electrophoretic separations in 100 μm capillaries [10, 11]. The use of electrophoresis and electrokinetic fluid handling has been a major cornerstone since then for development of μTAS devices.

In recent years, the field of μTAS has gained tremendous popularity. It is partially due to benefits offered by miniaturization such as low cost, reduced sample and reagent
consumption, very fast analysis, high throughput, extensively parallel architectures, compact design, reliability and ease-of-use. A large part of the popularity is also due to a belief that entirely new techniques will become available, opening up possibilities of experiments and innovations that would have been impossible by traditional methods. Excellent reviews on µTAS devices, their applications to separation systems, proteomic and genomic analysis, clinical and forensic analysis, point-of-care clinical diagnostic systems have been published in recent years [12-21].

From the early days, electric fields have been found to be of considerable significance in µTAS devices. A major advantage of using electric fields on a chip is the relative simplicity and availability of the experimental chips and equipment needed. The microlithography facilities used in electronic circuit fabrication allow facile fabrication of any kind of "chips" with microelectrodes for this type of application. The interfacing of the chips to the control circuitry is done by direct electrical contact and there are no mechanical or optical components involved (except for observation purposes). In contrast, for example, using optical “tweezers” for particle manipulation requires the construction of laser traps that include complex mechanical, optical and electronic components.

The use of electric fields also allows precisely tuning the forces exerted on the particles and fluid by the field and the field-induced particle-particle interaction. The parameters characterizing the electric signal applied to electrodes include magnitude, frequency, wave shape, wave symmetry, and phase (when multiple electrodes are involved). All of these parameters can be controlled individually by “turning knobs”, and all can influence in different ways the behaviour of particles and fluid between the electrodes. Harvesting the convenience of electrical fields, however, requires knowledge and prediction of the response of the particles and liquid inside the experimental cells to the fields applied to the electrodes. Their response is often quite complex, as generally, electric fields drive motion of both particles and liquid as explained in more detail below. Rich varieties of field-driven effects have been revealed and are the subject of active investigation. An overview of the different field generated forces on particles and the fluid is presented in the next section.
1.3. Electric Field Induced Forces on Colloids

Colloidal particles suspended in water readily respond to alternating (AC) or direct current (DC) electric fields. Charged particles in DC fields are attracted to oppositely charged electrodes by electrophoresis. Dielectrophoresis, particle mobility in AC fields, allows precise manipulation of particles through a range of parameters including field strength and frequency and electrode geometry. Simultaneously, DC or AC electrokinetics drive liquid flows inside the experimental cells, which also leads to transport and redistribution of the suspended particles. To understand the particle and fluid behavior in electric fields it is vital to examine the formation of electrical double layer around particles and at substrate–solution interfaces within the experimental cell which is discussed below.

1.3.1. Electrical Double Layer Formation at Interfaces

Electrical double layers are formed at all particle-solution interfaces irrespective of the presence of an applied field. For any material or phase, even for a metal in vacuum, there is a separation between the positive and negative components (electrons, atoms, molecules, ions), such as it generates potentials of the order of one volt across distances of the order of few molecular diameters at the surface [22]. Charge can also be generated by dissociation of surface groups or by adsorption of specific ions or molecules from the solution onto the surface. Thus, when a surface is brought into contact with an electrolyte solution, counterions bearing charge opposite to the charge on the surface are preferentially attracted towards the surface and form the electrical double layer as depicted in Fig. 1.2.

The electrical double layer, as the name suggests, consists of two distinct layers of ions – the Stern layer and the diffuse layer. The ions in the layer closest to the surface usually are physically adsorbed onto the surface and are thus immobile. This layer of bound ions is known as the Stern layer and is usually 1 – 2 nm thick. The Stern layer is further subdivided into the inner and outer Helmholtz layers. The inner layer consists of non-hydrated ions that are specifically adsorbed on to the surface and consists of both co-ions and counterions whereas the outer Helmholtz layer consists of electrostatically bound hydrated counterions. The plane between the inner and outer Helmholtz layers is known as the inner Helmholtz plane (IHP).
The ions in the diffuse layer are farther away from the surface and have the ability to move transverse to the particle surface or in and out of the double layer to the bulk solution phase. The plane between the Stern layer and the diffuse double layer is called the outer Helmholtz plane (OHP). The thickness of the diffuse layer is characterized by the Debye length of the suspension, $1/\kappa$, and is usually of the order of $3/\kappa$ to $4/\kappa$. The Debye length of the suspension is dependent solely on the liquid properties and is given by.

**Figure 1.2.** Electrical double layer formation at substrate-electrolyte interface and the plot of electric potential generated at the interface. The electric potential decays from $\psi_s$ at the surface to zero in the bulk solution leading to an electroneutral substrate-double layer complex.

The ions in the diffuse layer are farther away from the surface and have the ability to move transverse to the particle surface or in and out of the double layer to the bulk solution phase. The plane between the Stern layer and the diffuse double layer is called the outer Helmholtz plane (OHP). The thickness of the diffuse layer is characterized by the Debye length of the suspension, $1/\kappa$, and is usually of the order of $3/\kappa$ to $4/\kappa$. The Debye length of the suspension is dependent solely on the liquid properties and is given by.
\[
\frac{1}{\kappa} = \left( \sum_i \rho_{\infty, i} e^2 z_i^2 \epsilon \epsilon_0 k T \right)^{-1/2} \text{[meters] (1.1)}
\]

where, \( \rho_{\infty} \) is the bulk concentration of ions, \( e \) is the electron charge, \( z \) is the ion valency, \( \epsilon \) and \( \epsilon_0 \) are the dielectric permittivities of the medium and vacuum respectively, \( k \) is the Boltzmann’s constant and \( T \) is the temperature of the system. The summation is over all species \( i \) of the ions. Small amounts of added electrolyte can drastically change the behavior of particles in aqueous suspensions. For ultra pure water at 25 °C, the Debye length is 960 nm, whereas it is 30.4 nm and 9.6 nm for 0.1 and 1 mM NaCl solutions respectively. The Debye length also plays an important role in suspension stability by maintaining electrostatic repulsion between particles and prevents them from aggregating due to van der Waals forces. However, addition of about 50 mM NaCl (or similar monovalent salt) can decrease the Debye length and sequentially the electrostatic repulsion and leads to particle aggregation.

The potentials generated at the surface-solution interface and at the outer Helmholtz plane are denoted by \( \psi_s \) and \( \psi_d \) respectively. The electric potential decays as one goes away from the particle surface and through the diffuse layer and reaches zero in the bulk of the solution phase as seen in Fig. 1.2. For aqueous suspensions, it has been found that the OHP is also the plane of no-shear observed for electrokinetic fluid flow. Thus the OHP potential, \( \psi_d \) is the equivalent to the so-called zeta potential, \( \zeta \). The zeta potential is usually of the order of 100 mV and based on the surface charge could be positive or negative. The zeta-potential can be independently measured by a Zetasizer, which measures the dynamic light scattering spectra of particles drifting due to an applied electric field.

The zeta potential and the electrical double layer play important roles in electrokinetic phenomena. The amount of counterionic charge in the diffuse layer and hence the surface conductivity of the particle can be easily calculated from the zeta potential. The conductivity of the particle, necessary for determining dielectrophoretic force, is the sum of this surface counterionic conductivity and the bulk particle conductivity. The charge density for the diffuse layer is given by

\[
\sigma_d = -\frac{\epsilon_0 e \zeta}{1/\kappa} \text{ (1.2)}
\]

The amount of counterionic charge in the diffuse layer cancels out the charge on the particle resulting in an electroneutral particle-double layer complex. However, when a
constant (DC) electric field is applied to the suspension, the field “strips” away the counterions present in the diffuse layer, resulting in a particle with apparent potential, $\zeta$. The particles of effective potential $\zeta$ are then attracted to the opposing electrode. This motion of particles is termed electrophoresis. If instead alternating (AC) electric fields are applied, the ions in the counterionic layer diffuse around the particle and concentrate at the point nearest to the opposing electrode at the particle surface, thus, creating an electric dipole. The particle behavior in the AC field is strongly dependent on the strength of this “induced” dipole, and the force is termed dielectrophoresis. Furthermore, the counterionic double layer plays an important role in all electrokinetic flow phenomena, as the flow velocity is directly proportional to the zeta-potential. The different forces acting in a simple system where particles are suspended between two planar electrodes can now be understood in context of the interaction of the electric field with the ions in the electrical double layer.

### 1.3.2. Electrophoresis and Electroosmosis

The motion of a charged particle in a constant DC field is known as electrophoresis. The qualitative schematic of the electrophoretic force on a particle suspended in a system with planar electrodes is shown in Fig. 1.3a. As has been discussed above, application of a DC field will strip away some of the counterionic surface charge around a particle, revealing a surface of effective potential $\zeta$. The parameter that characterizes the response of a charged particle to applied electric field, is the electrophoretic mobility, $\mu$ defined as the ratio of the particle velocity, $u$ to the applied electric field, $E$ and is given by

$$\mu = \frac{\bar{u}}{E}$$

The electrophoretic mobility is dependent on the particle zeta-potential and the viscosity of the surrounding media, $\eta$. It increases with an increase in the zeta-potential and decreases on increasing the viscosity. The electrophoretic mobility is also dependent on the Debye length of the suspending media. The mobility of a “small” particle, defined as having radius $r << 1/\kappa$, is given by the Hückel equation

$$\mu = \frac{2 \varepsilon \varepsilon_0 \zeta}{3 \eta}$$

(1.4)
The electrophoretic mobility of particles much larger than the Debye length, \( r >> 1/\kappa \) is given by the Helmholtz-Smoluchowski equation

\[
\mu = \frac{\varepsilon \varepsilon_0 \zeta}{\eta}
\]  

(1.5)

Tabulated values for the numerical coefficient in the right hand side of these equations for the case of particles of size comparable to the Debye length are available in the literature [23]. The electrophoretic mobility determined by measuring the particle velocity as a function of the applied external field, as done by a Zetasizer allows measuring the particle \( \zeta \)-potential. The actual velocity of a charged particle in a real experimental situation of two electrodes inside a cell, however, is going to be equal to the theoretical one only in a very limited volume, where the liquid in the cell is immobile and there is no contribution of electroosmotic force.

**Figure 1.3.** Schematics of forces in DC fields. (a) Electrophoresis – the negatively charged particle is attracted towards the anode. (b) Electroosmosis – the cations in the double layer are attracted towards the cathode resulting in fluid flow towards the cathode. The particle velocity in both cases is indicated by the blue arrows. The net particle velocity under DC fields is the sum of the electrophoretic and the electroosmotic velocities.
The interaction of the counterions in the double layer around an “immovable object” (typically, the experimental cell walls) with a tangential DC field leads to a net bulk fluid flow. This phenomenon has been termed electroosmosis and is illustrated schematically in Fig. 1.3b. The ions in the diffuse layer are attracted towards the oppositely charged electrode. These ions in turn drag the liquid or solvent molecules around them resulting in a bulk fluid flow. The fluid velocity is zero at the substrate surface and the electric potential at this “no-slip” plane is same as the \( \zeta \) potential of the substrate. Since the substrate can be considered a particle with an effective radius \( r \) much greater than \( 1/\kappa \), the electroosmotic velocity is well estimated by the Helmholtz-Smoulchowski equation (Eqn. 1.5). However, to account for the “inverse” direction of the fluid velocity, it needs to be multiplied by -1. Thus, the electroosmotic velocity is given by

\[
\bar{u} = -\frac {\varepsilon \varepsilon_0 \zeta}{\eta} \hat{E}
\]  

(1.6)

The electroosmotic liquid flow can drag the particles and the net particle velocity will be equal to the one caused by the electrophoretic effect, plus the electroosmotic velocity of the surrounding liquid (which changes with the position inside the cell). This is a common problem arising in particle characterization or assembly experiments with DC fields. For example, while using a Zetasizer to determine particle \( \zeta \) potential it is necessary to identify regions of the experimental cell where there is no electroosmotic liquid flow. The moving liquid can also drag the particles in arbitrary direction and hence distort the assembled structures. Thus, care must be taken while using DC fields for particle manipulation in \( \mu \)TAS devices.

The ability to drive the particles towards an oppositely charged electrode can be used for their concentration, deposition, or colloidal crystallization. If the field is strong enough, the particles can also be attached irreversibly to the surface. One of the first processes for electrophoretic deposition of ordered arrays of gold and latex particles onto surfaces of conductive carbon-coated copper grids under the action of electric field of 1-5 V/cm has been reported by Giersig and Mulvaney [24]. Adsorption, nucleation and growth of polycrystalline arrays have been observed with increasing periods of time. Multilayer latex arrays have been formed at higher field strengths. Such processes can be used to crystallize the particles by concentrating them near electrodes. The technique can be viewed as speeding up the
deposition of dense particle crystals that would otherwise be formed under gravity. Wide cells are usually used in order to decrease the magnitude of electroosmotic flows.

Direct electrophoretic control of the deposition speed of silica spheres for the fabrication of high quality crystals was first reported by Holgado et al. [25]. DC field applied in the vertical direction in cylinders with sedimenting spheres was used to control the speed at which the spheres deposit on the bottom. The vertical electrophoretic mobility of the spheres can add up to the Stokes sedimentation velocity so crystals from small spheres can be assembled rapidly. When the field is applied in the opposite direction, it can slow the downward mobility of large spheres whose Stokes sedimentation speed is too high in order to slowly grow well-ordered crystals. Electrophoretic deposition of colloidal particles has been used to prepare various crystals and multilayered deposits [26-35]. Electrophoretic redistribution of encapsulated particles has been used in "electronic ink" and flexible displays [36, 37].

The electroosmotic effect is conveniently utilized in microfluidic pumps [38, 39]. By placing electrodes at the ends of a capillary or a channel, electroosmotic fluid flow can be produced along the entire length of the capillary. One main advantage of electroosmotic pumps is that they do not contain any mechanically moving parts and hence lead to uniform flow in the channel. Moreover, the flows in different branches can be controlled independently by switching on/off different electrodes without needing valves. Harrison et al. used electroosmotic pumping to drive fluid flow and electrophoresis to separate amino acids in 1 to 10 cm long capillaries with 300 \( \mu \text{m}^2 \) cross-section etched in glass chips within seconds [9]. Jacobson et al. demonstrated parallel and serial mixing of fluid samples in microfluidic devices by applying a single voltage across the ends of the device [40]. By scaling the channel length to a desired voltage, mixed samples of different dilutions can be prepared without needing multiple electrodes. Morf et al. predicted the flow rates and back-flow characteristics of electroosmotic flows in complex capillary systems based on the electric and hydraulic resistances of the microfluidic channels [41]. The theory developed allowed to design an optimized microfluidic device that uses electroosmotic pumping to carry out volumetric nano-titrations [42]. Moosavi et al. extended the application of electroosmosis to organic solvents for pumping reagents in microreactors leading to controlled mixing and subsequent chemical reaction [43]. Furthermore, the combination of
Electroosmosis and electrophoretic separation in capillaries, also known as Capillary Electrophoresis, has been a major tool for biological separation of proteins and DNA molecules based on their electrophoretic mobility [44-48].

1.3.3. Dielectrophoresis and Particle Chaining Force

The application of a non-uniform AC field across particle suspensions leads to emergence of dielectrophoretic (DEP) force. As the sign of the electrode polarization changes constantly, the particles are not attracted by direct charge-electrode electrostatic interactions (apart from oscillations at low frequencies of the order of tens of Hertz). Instead, the DEP force arises via interaction of the induced dipoles with the gradient of the (inhomogeneous) field as shown in Fig. 1.4. The resultant force, \( F_{\text{DEP}} \), is dependent on the gradient of the field squared, \( \nabla E^2 \) and the particle radius, \( r \) [49-53]

\[
F_{\text{DEP}} = 2\pi\varepsilon_1 \text{Re}[K(\omega)] r^3 \nabla E^2 \tag{1.7}
\]

Its sign and magnitude are dependent on the effective polarizability of the particle, which is described by the real part of the Clausius – Mossotti function, \( K \)

\[
\text{Re}[K(\omega)] = \frac{\varepsilon_2 - \varepsilon_1}{\varepsilon_2 + 2\varepsilon_1} + \frac{3(\varepsilon_1\sigma_2 - \varepsilon_2\sigma_1)}{\tau_{MW}(\sigma_2 + 2\sigma_1)^2 \left(1 + \omega^2\tau_{MW}^2\right)} \tag{1.8}
\]

In the above formulae, \( \varepsilon_1 \) and \( \sigma_1 \) are the dielectric permittivity and conductivity of the media and \( \varepsilon_2 \) and \( \sigma_2 \) that of the particles. If \( \text{Re}(K) > 0 \), the particles are attracted towards the areas of high electric field intensity and the DEP force is said to be positive DEP. Metallic and other highly polarizable particles are always attracted along the gradient to the regions of high field intensity and undergo positive DEP. However, if the medium is more polarizable than the particle, i.e., \( \text{Re}(K) < 0 \), the particles are repelled from the areas of high electric field intensity and undergo negative DEP. Negative dielectrophoresis is often used for levitating particles above electrodes.

It is important to note, that the major contribution to the low frequency polarizability of almost any type of particle dispersed in water comes from the surface conductance of the counterionic layer near the surface. Even though dielectric particles have lower bulk polarizability, they almost always have charge in water, so at low frequencies \( \text{Re}(K) > 0 \) and the particles are attracted to the high field intensity areas. \( K \) changes sign, i.e., the force changes from attractive to repulsive, at a crossover frequency.
\[ \omega_c = \frac{1}{\tau_{MW}} \]  

(1.9)

where \( \tau_{MW} \) is the Maxwell-Wagner charge relaxation time given by

\[ \tau_{MW} = \frac{\varepsilon_2 + \varepsilon_1}{\sigma_2 + 2\sigma_1} \]  

(1.10)

This frequency-dependent change of sign of the force is commonly observed with polymer microspheres in water [49-52] and allows a high degree of particle control via the field frequency.

![Figure 1.4. Dielectrophoretic force on suspended particles between planar electrodes. The electric field intensity gradient is directed towards the plane of electrodes and towards the electrode edges. Positive DEP (right) – particle is more polarizable than the medium and is attracted towards regions of higher field intensity. Negative DEP (particle on the left) – particle less polarizable than the medium is repelled from the high electric field regions.](image)

The dielectrophoretic force is not limited to AC fields. The largest possible magnitude of the induced dipoles will be realized in DC fields and DEP effects will be present in inhomogeneous fields in electrophoretic cells. The use of DC dielectrophoresis, however, is impractical, due to the much smaller magnitudes of the fields that could be applied in aqueous media. The use of AC voltage, on the other hand, allows manipulation of virtually any type of particle in any type of media and has the advantage of permitting high field strengths without water electrolysis and strong electroosmotic flows.

The DEP effects become much more complex (and interesting) when large numbers of particles are present between the electrodes. The processes of interaction and assembly of the particles can be explained in intuitively clear, albeit simplified, way by assuming that the
field induces a dipole within each particle. These induced dipoles interact not only with the external field, but also with each other if the particles are close enough. The biggest energy gain is realized when the particles align in chains along the direction of the field lines (see Fig. 1.5). This “chaining” force, $F_{\text{chain}}$, is dependent on the field strength, $E$. A generalized expression for the force between adjacent particles is

$$F_{\text{chain}} = -C \pi \varepsilon_0 r^2 K^2 E^2$$

(1.11)

where the coefficient $C$ ranges from $3$ to $>10^3$ depending on the distance between the particles and the length of the particle chain [50]. Particles of the same type always align along the field lines, regardless of whether their polarizability is higher or lower than the media, while mixtures of particles of lower and higher polarizabilities than the media could form alternating chains in the perpendicular direction [54]. Dipolar chaining and 3D structuring were first observed and studied in relation to electrorheological fluids, and are presently a major tool in the assembly of organized particle materials and various structures and devices.

![Figure 1.5. Schematics of particle chaining force arising from dipole-dipole interaction. The electric field induced polarization is indicated by the arrows inside the particle. The yellow particles have higher polarizability compared to the medium and the green particles have lower polarizability. Particles of the same type (with polarizability either higher or lower) always align in the direction of the electric field, whereas mixtures of particles form chains perpendicular to the field direction.](image)

The polarized particles not only interact with each other but also modify the field intensity in the region of the experimental cell. Dielectric and conductive particles have different effects on the electric field intensity within the cell. An illustration of these two
distinctive cases of particle behavior under AC fields is presented in Fig. 1.6. The color-coded plot of the simulated intensity of the field allows easy visualization of the direction and range of the forces that arise within the system. Dielectric particles with effective permittivity higher than the medium will be attracted in the direction of the higher intensity (warmer colors in Fig. 1.6a). The simulation shows how the field intensity between two polarizable particles increases and the particles moves towards each other (similarly to the predictions based on dipole-dipole interactions, Eqn. 1.11). The disturbances of the field around the particles, however, are relatively minor, regardless of the position of the chain within the chamber.

Figure 1.6. Simulation illustrating the two general cases of electric field intensity distribution in chambers with colloidal particles. (a) Dielectric particles ($\varepsilon = 5$) and (b) conductive particles ($\varepsilon \rightarrow \infty$) suspended in a medium with lower relative dielectric permittivity $\varepsilon = 2$. The orange bars on either side represent the electrodes. The simulation demonstrates how the particles attract each other in chains, due to the high field intensity area in-between them (analogous to chaining force). However, conductive particles near electrodes also modify the electric field distribution in the whole chamber by extending the electrode into the solution (left pair in b); consequently, the pattern of assembly of conductive particles is different.
The application of electric field across suspension of conductive (typically metallic) particles creates a different electrostatic pattern in the cell. The metallic particles are nearly infinitely polarizable, and the Clausius – Mossotti factor takes its limiting value of $K = 1$. The DEP and chaining forces are maximized. In addition, particles at the electrode edges will connect to the electrode, effectively extending it to the outer end of the particle chain (see the simulated example in Fig. 1.6b). The tip of the conductive chain formed will distort the field intensity across the whole cell, and establish a gradient towards the end particle. Other particles will then be attracted to the tip, extending it further out into the suspension in the direction of the other electrode. The conductive chain of metallic particles will keep on extending until the electrodes become short-circuited. An example of such a process is the aggregation of gold nanoparticles into microwires discussed later in Chapter 2 and also one recently demonstrated by a study where gold nanoparticles form a pearl chain between electrodes separated by micron sized gaps [55].

1.3.3.1. Dielectrophoretic Manipulation and Assembly of Nonconductive Particles

This research area is by far the largest and best developed, due to the convenience and precision of the dielectrophoretic techniques. The potential of devices with various electrode configurations to move, rotate and separate dielectric particles has been recognized for more than 50 years. Many of the pioneering works and much of the research today are focused on the manipulation of DNA [56-62] and live cells [63-75]. The frequency-dependent cell membrane polarizability depends on a variety of factors and can be used to separate streams of live and dead cells and cells of different genotype in flow-through devices [64, 67, 76-83]. Precise adjustment of the field frequency allows separating various types of cells and polymer spheres in the areas of lowest and highest field intensity between electrodes [51, 84, 85]. Bennet et al. [86] combined negative DEP with fluid flow to achieve phase separation in particle suspensions. The suspension containing particles flows in a microfluidic channel on one side of which are situated multiple electrodes perpendicular to the channel. The particles undergoing negative DEP are barred from crossing the area of high field intensity near the electrodes, which thus serve as “dielectrophoretic gates” in the channel. Suehiro et al. [87] have demonstrated a DEP filter where yeast cells from a flowing suspension are collected and concentrated on glass beads placed in between electrodes. Local areas of high electric
field intensity are created where the glass beads touch each other laterally. The yeast cells are preferentially concentrated by positive DEP in these regions. In both of these methods, switching off the field allows for the release and collection of the trapped particles and cells.

The combined action of DEP and chaining forces can be used as a tool for AC field-driven assembly of particle structures. Our group has used alternating electric fields applied to the gap between planar electrodes for rapid and switchable assembly of colloidal crystals from polymer and silica microspheres [88-90]. These two-dimensional crystals are specifically oriented by the field without the need for prior templating by microlithography or micromolds. Schematics of the DEP cell and images of the stages of particle assembly observed in these experiments are presented in Fig. 1.7. The first, rapid, stage of the crystallization is the assembly of particles in chains along the direction of the field by dipolar attraction. The particle chains are then attracted to the surface of the glass plate between the

Figure 1.7. Dielectrophoretic assembly of latex spheres into 2D colloidal crystals. (a) Schematics of the experimental cell designed for assembly of particles under DEP. (b) Optical micrograph taken during the initial rapid particle chaining stage. The latex spheres form chains due to dipole-dipole interaction. The chains are attracted towards the plane of electrodes by DEP slowly merging into 2D crystals. (c) Micrograph taken after the 2D colloidal crystal is formed. The particles in the micrographs are 1.4 µm in size. [88-90]
electrodes by dielectrophoresis. The chains confined to the surface assemble and crystallize into hexagonal particle crystals arrays, one axis of which is always aligned in the field direction (transverse to the electrode gap). This second slower crystallization stage is also driven by lateral attraction between the particle chains. The model for crystallization driven by a combination of induced dipole chaining and dielectrophoresis is supported both by direct microscopy observation and by reconstructing the structure from the laser diffraction pattern. The threshold field intensity for crystallization $E_{\text{th}}$ of particles of different radii $r$ has been measured at varying frequencies and shown to form a constant group $E_{\text{th}}^2 r^2 = \text{const}$ as expected on the basis of Eqn. 1.11 [90].

The size of these switchable 2D crystals could be larger than 25 mm$^2$. The laser diffraction patterns of all consecutively formed crystals are identical, which points out to the formation of a single crystal domain, unlike the multicrystalline materials assembled by convective deposition [89, 90]. If the field is turned off, the arrays disassemble within seconds as the particles diffuse out of the crystal plane. Thus, the electrically tunable crystallization can be used to make rudimentary optical switches. The transitions between ordered and disordered states can be repeated tens of times, although the quality of the crystals in the vicinity of the electrode edges slowly degrades due to the action of the AC electrokinetic flows. The precision of determining the lattice constant from the laser diffraction pattern is $\approx 10$ nm, which allows quantifying how the electrolyte concentration can be used to tune the electrostatic repulsion between the spheres. Various combinations of DEP, EP and chaining forces are likely to find applications in more elaborate techniques for precise assembly of ordered structures [91].

The use of AC electric fields further allows electrical measurements of the properties of captured and assembled particles, which can be used to detect target analytes present in the system. This application has been first demonstrated by Velev and Kaler, who used dielectrophoretic assembly of functionalized latex particles as a tool to make microscopic on-chip biosensors [92]. Small patches of latex particles captured and aggregated in the micrometer sized gaps between on-chip electrodes selectively bind complementary immunoglobulin molecules. The presence of captured immunoglobulin is detected by tagging with gold nanoparticles, and silver enhancement, which leads to direct electrical "short circuiting" of the electrodes. The result is detected by measuring the conductance of the
circuit including the particle patch. More recently, silver enhancement of DNA strands functionalized with gold nanoparticles and complimentarily captured onto substrates have been used for detecting the presence of target DNA molecules [93, 94]. Zheng et al. describe the use of a lock-in-amplifier to measure the impedance of DNA and proteins trapped under positive DEP [95]. Electric measurements and detection is a natural combination to electric field assembly that reveals the full potential of on-chip devices.

1.3.3.2. Dielectrophoretic Assembly of Conducting Particles
The assembly of conducting colloidal particles provides means to making electrical microcircuits and other functional structures such as biosensors, DNA detecting probes, etc. The use of dielectrophoresis offers the combination of speed, easy control and precision that might not be readily available through the more traditional colloidal assembly techniques. Quantum dots (CdSe semiconductor nanoparticles), carbon nanotubes (CNT), gold nanoparticles, DNA and protein molecules and oligonucleotides functionalized with gold nanoparticles, metal nanowires and nanorods can all be assembled using dielectrophoresis between suitably spaced electrodes. The state of the art in the preparation and use of nanoparticles in different biological, electrical and optical applications is given, e.g., in recent reviews by Alivisatos [96] and Tang and Kotov [97]. The assembly of CNTs [98-106] and gold nanoparticle conjugated to DNA [94, 107], in particular, has been extensively studied due to their potential in biosensors.

1.3.4. AC Electrohydrodynamics
Uniform AC fields applied normally or tangentially to a charged wall do not engender fluid flows as the DC fields, since the fluid velocity in consecutive half-cycles will cancel each other out. Fluid flows, however, are generated in areas near the electrodes where a strong electric field gradient, gives rise to a strong tangential field at the solid-liquid interface. The interaction of the ions in the double layer atop the electrodes with this tangential field gives rise to liquid drag near the dielectric wall adjacent to the electrodes. These flows are referred to as AC electrohydrodynamics (EHD). Schematics of the double layer formation and the electric field distribution in a system with two planar electrodes are presented in Fig. 1.8.
The external applied voltage at electrode surfaces modifies the native charge on the surface thereby leading to an "induced" zeta potential different from the intrinsic zeta potential. Furthermore, for AC fields the induced double layer charge changes sign synchronously with the electric field frequency. For example, in the next half-cycle for Fig. 1.8, the left electrode will have positive applied voltage which leads to accumulation of negative counterions in the double layer. Thus, the ions in the double layer have to move in and out of the layer during subsequent half-cycles of the electric field. This leads to induced zeta potentials that may be different for the positive and negative half-cycles of the AC field, but are always of sign same as that of the field applied to the electrodes [108]. The electric field is normal to electrode surface; however, there is a tangential component of the electric field at the electrode edges. The ions in the double layer then react to these tangential electric fields and lead to a bulk electrohydrodynamic flow directed towards the electrodes. Notably even though an AC field is applied, the bulk flow in different half cycles points in the same direction along the field gradient resulting in a net fluid flow.

As the applied electric field induces double layer formation and then leads to bulk fluid flow by acting on its own induced charge, these flows are also referred to as “Induced Charge Electro Osmosis (ICEO)” [109, 110]. AC EHD and electroosmosis are types of ICEO flows. The AC EHD flow velocity is given by

Figure 1.8. Origins of AC Electrohydrodynamics effects. The tangential electric field present atop the electrodes close to the edges leads to EHD flow. The sign of the counterions in the double layers on the electrodes and the electric field change concurrently in subsequent half-cycles to give net fluid flow directed towards the electrodes.
\[ u = -\frac{\varepsilon \varepsilon_0 \zeta_{\text{ind}} E_t}{\eta} \]  

(1.12)

where, \( \zeta_{\text{ind}} \) is the induced zeta potential due to the applied external field and \( E_t \) is the tangential component of the electric field [111, 112].

As AC EHD is not dependent on the particle properties, it is suitable for application in \( \mu \)TAS devices where the liquid, rather than the particles is manipulated. Ramos et al. [113] were first to identify AC EHD flow generated at low frequencies (< 500 kHz) on microelectrode structures. During their experiments with latex particles undergoing positive DEP in a system with parallel finger electrodes, they observed that instead of concentrating at the electrode edges, where the field intensity is highest, the particles become collected on top of the electrodes. They attributed this flow to the interaction of the electric field with the ions in the double layer on top of the electrode. In their subsequent papers they made a detailed investigation of the EHD flow velocity as a function of field frequency and the position on the electrode and found that the particles are moving from the interelectrode gap towards the electrode [114] as demonstrated in Fig. 1.8. The particle velocity was found to be highest at the electrode edge and decreased as it moved across the electrode. The velocity was also found to depend on the frequency with a maximum occurring at a characteristic frequency and tending to zero at low and high frequencies [115].

A detailed theoretical model based on the assumption of a thin double layer formation on the electrodes was developed, and scaling laws for the various forces acting on particles suspended in a microelectrode system with applied AC field have been established. It was concluded that EHD is the dominant force at low frequencies and for small system sizes [116]. Electrothermal forces, which occur due to the temperature-dependent changes in the permittivity and conductivity of the suspension, dominate at high frequency and high voltages. In most \( \mu \)TAS EHD systems, thus, it is advisable to work at low to moderate frequencies and at low voltages to prevent electrothermal effects. A novel technique to independently measure the particle and liquid velocity in electrokinetic systems using two color micro- Particle Image Velocimetry (\( \mu \)PIV) has been recently developed by Wang et al. [117] It allows decoupling the DEP contribution to particle velocity from the EHD and electrothermal contributions.
Various particle-trapping techniques that use AC EHD have been developed. Chang et al. [118-120] designed impedance spectroscopy detectors that take advantage of AC EHD to rapidly concentrate bioparticles, leading to enhanced sensitivity due to reduction of the transport time to the detector. Chip designs that combine DEP with EHD flow for particle concentration have been extended by Hoettges et al. [121] and Wong et al. [122] Particle separation can also be achieved by utilizing the ability of negative DEP to levitate particles and thereby allowing EHD to form distinct patterns of collected particles [84].

Another important application of EHD flow in µTAS is for pumping fluids [123, 124]. The use of EHD instead of electroosmosis requires lower voltages, and also prevents electrolysis and bubble formation in the suspension. However, as AC fields create rolls on top of electrodes, it is essential to design electrode configurations that generate a net fluid flow in the device. Several such designs have been proposed and implemented. Ajdari [125] detailed the formation of fluid rolls on a charge-modulated surface in presence of an applied external field. He proposed the use of an undulated surface close to the charge-modulated surface to create biased rolls, leading to a net flow in the channel formed by the two surfaces. Asymmetric electrode arrays can be used to break the symmetry of the rolls created in EHD [126, 127]. A practical demonstration of using asymmetric electrode for pumping water in microfluidic channel was presented by Brown et al. [128] Interdigitated electrodes with a large electrode followed by small gap and a small electrode followed by a large gap lead to net flow of fluid from the large electrode towards the small electrode across the large gap. A further improvement of the performance of the system was achieved by placing two interdigitated electrode arrays, one on the top and one on the bottom of the system [129]. Fluid velocities in excess of 450 µm/s were obtained in channels of 280 µm heights. Debesset et al. [130] developed an AC electroosmotic micropump that works by placing interdigitated electrodes in a circular channel. This makes it useful for circular chromatography applications, which require a closed loop system. Furthermore, micro-vortexes for mixing applications can be generated electroosmotically in channels with patterned walls or at microchannel junctions [131-134].
1.4. Layout of this Thesis

The case for the use of electric fields for µTAS applications has been laid out in the preceding sections. My graduate research has focused on the manipulation and assembly of colloidal particles of any size scale using alternating electric fields as a way to develop new tools and techniques for µTAS. Chapter 2 describes the use of dielectrophoresis for assembling on-chip microwires from metallic nanoparticle suspensions. These wires can be used for in situ wet electronic circuits, for bioelectronic interfacing of live cells, as detectors for biosensors, and for anisotropic thermal and electrical conductors. Chapter 3 details the use of device where dielectrophoresis and AC electrophorodynamics were combined to make a microfluidic device that collects and concentrates colloids from dilute suspensions. Latex microspheres, yeast cells, microbes and DNA strands were collected in patterned “corrals” in this device. A novel liquid-liquid microfluidic system that operates by manipulating floating liquid droplets using dielectrophoresis was developed. This novel system is presented in Chapter 4. This new chip allows performing chemical reactions, immuno-agglutination assays and forming “supraparticles”. Chapter 5 summarizes my graduate work and lays down areas conducive for extending this research, which can lead to new developments in the field of µTAS.

1.5. References


Chapter 2

Assembly of Colloidal Particles into Conductive Microwires by Dielectrophoresis*

2.1. Introduction

Traditional areas of colloid assembly deal with the crystallization of microparticles into colloidal crystals, which can be conducive, for example, to the fabrication of photonic materials [1-4]. More recently, the assembly of particles of nanometer size has attracted significant interest for applications such as sensors [5-7] and nanocoatings [8, 9]. The assembly of conductive nanoparticles can be used to make \textit{in situ} electrical contacts. This in turn could be used for bioelectronic interfacing of live cells to electrical circuits or for doing impedance measurements for biosensor applications. Both of these processes could be used as detection stages in \mu{TAS} devices.

Various techniques have been developed for making \textit{in situ} electrical connections. Bradley \textit{et al.} used electrochemical growth of copper wires to connect silicon oxide microelements and form a diode [10]. Mirkin’s group have employed silver enhancement of DNA strands attached with gold nanoparticles to facilitate formation of silver bridges between electrodes. Conductivity measurements on the silver wires formed were used for detecting the presence of target DNA molecules in the system [6]. Magnetic fields and fluidic alignment of nanowires and nanotubes has also been investigated. Tanase \textit{et al.} used magnetic fields to align nickel nanowires forming chains that can be hundreds of microns long [11]. Huang \textit{et al.} assembled electrical circuits out of GaP, InP and Si nanowires by flowing the nanowires perpendicular to the electrode gap [12]. The self assembly of nanoparticles, including monolayer protected clusters, polymers and polyelectrolytes can be used for making electronic devices such as junctions, heterojunctions and single electron transfer devices [13]. Not all assembly processes, however, offer the combination of speed, simplicity, low-cost and precision desired for technologically feasible applications.

One tool for rapid assembly of micro- and nanoparticles is dielectrophoresis (DEP), the particle mobility and interactions imparted by an alternating electric field [14-16]. Electric fields have been used in the assembly of complex particles [17, 18], colloidal crystals [19-27], biosensors [5, 6] and linear aggregates of particles [28-31]. The use of alternating (AC) fields in dielectrophoresis, in particular, allows avoiding electroosmotic and electrolysis effects, which significantly limit the precision and usability of the direct current (DC) fields.

One process of specific interest to applications in electrical circuits and sensors, that has been reported previously by our group is the assembly of metallic nanoparticles into long
wires of micrometer thickness [31]. The metal particles of diameter 10-20 nm assembled in millimeter to centimeter-sized gaps between two planar electrodes on a surface under applied AC fields. The long, porous fibrillar assemblies grow when the nanoparticles are attracted and aggregated by the DEP force at the end of the microwires. The speed of assembly of the microwires can be as high as 50 \( \mu \text{m/s} \). The microwires formed have good Ohmic conductance for both AC and DC currents. They can form rudimentary self-repairing "wet" circuits and hold promise for applications in sensors, bioelectronic circuits, or other devices where electrical connectors are assembled in situ inside a liquid instead of being made by microfabrication [31]. A similar process has been recently used by others to assemble gold nanoparticles between nanometer spaced electrodes using AC fields and then creating a small electrode gap by breaking the wire with DC currents [30].

For gold particles in aqueous suspensions, the Clausius–Mossotti factor attains its limiting value of \( K = 1 \) and leads to positive dielectrophoresis at all field frequencies, The gold nanoparticles are, thus, attracted towards the areas of high field intensity usually situated on and near the electrodes. Microwires begin assembling at the electrode surface due to the collective aggregation of the nanoparticles and continue to grow until they bridge the gap between the electrodes, resulting into a short circuit. The use of such microwires in nanotechnology applications requires detailed understanding of the factors involved and control over the assembly process, which were the first goals of my dissertation research. This chapter describes the effect of different operating parameters on the assembly of nanoparticle wires and identifies the methods for controlling the dielectrophoretic assembly process. Iterative 2D electrostatic models for the microwire growth process are presented and the microwire structures and the growth direction predicted by these models are compared to experimental observations. On that basis, methods to engineer specific microwire structures are demonstrated.

2.2. Materials and Methods

2.2.1. Substrate Fabrication
Glass microscope slides were cleaned by immersing in Nochromix solution overnight, and then washed thoroughly with deionized water and dried in an oven at 60 °C. The dried slides were covered with TEFOLON thread seal tapes in areas where no electrodes were desired. The
Tapes acted as masks when metal vapor deposition was carried out. A 10 nm layer of chromium was deposited on the glass first, to provide better gold adhesion to the substrate. A 100 nm gold layer was then deposited on top of the thin chromium layer. The distance between the two planar electrodes formed by this procedure was controlled by varying the width of the masking tape. The gap for different electrodes was varied from 4 to 8 mm. For some experiments, conductive islands were created in the gap between the gold electrodes by depositing spots of silver paint, traditionally used for electron microscopy applications. The diameter of these conductive islands was varied from 1 to 3 mm.

2.2.2. Gold Nanoparticle Suspensions

Gold nanoparticle suspensions were prepared by the method of Slot and Gueze [32]. Auric acid (10 mL-1 wt% in 800 mL solution) was reduced with sodium citrate (40 mL-1 wt% in 200 mL solution) in the presence of tannic acid (100 \( \mu \)L, 0.1 wt%). This process yielded gold nanoparticle suspensions with average particle diameter of 16 ± 3 nm.

The concentration of gold nanoparticles as synthesized, \( \approx 10^{12} \) particles/mL, was not sufficient for the microwire assembly experiments. The nanoparticle suspension was further concentrated by centrifuging at 1500-g through Millipore BioMax membrane filters. Before collecting the nanoparticles from the membrane, they were washed with distilled water to remove any remaining salts left from the reduction process. This process concentrated the suspension \( \approx 100\)-fold to a nanoparticle concentration of ca. \( 10^{14} \) mL\(^{-1}\). Depending upon experimental conditions the suspension was used as is or after further concentration. The gold nanoparticle concentration in the suspensions was determined by measuring the absorbance peaks at 520-525 nm with a JASCO V-550 spectrophotometer (JASCO Corporation, Japan). The absorbance peak for suspensions with gold concentration \( 10^{12} \) mL\(^{-1}\) (as synthesized by the Slot and Gueze method without further concentration) was around 1.1.

2.2.3. Experimental Setup

The nanoparticle suspension above the electrodes was contained in a 0.1 mm thick and 9 mm in diameter chamber created on the microscope slides by using SecureSeal Imaging Spacers obtained from Grace Bio Labs, OR. For some experiments the spacers were cut into stripes and the area of the chamber was kept about 25-50 mm\(^2\). 3-8 \( \mu \)L of concentrated gold
nanoparticle suspension was loaded in the chamber and sealed with a microscope glass cover slip.

The glass slide with the electrodes was then connected to the electrical circuit outlined in the bottom part of Fig. 2.1. An EZ-Digital (Korea), FG-7002C Sweep/Function generator was used as a source of square waves in the frequency range 100-3000 Hz. A Burleigh (NY), RG-71 Ramp generator amplified the signal to desired working voltage of 40-150 V. A 1 µF capacitor was included in the circuit to filter any direct current component of the signal. Digital multimeters connected in the circuit read the voltage applied and the current through the colloidal suspension. A master switch allowed starting and stopping the process whenever needed. The assembly process was continuously observed using a Meiji (Japan), EMZ series zoom stereomicroscope and was recorded on a Toshiba digital camera.

**Figure 2.1.** Outline of the experimental setup and schematics of the two different modes of microwire growth – bulk microwire assembly and surface microwire assembly. The chamber and nanoparticle sizes are not to scale.

### 2.3. Modes of Microwire Growth

Two qualitatively different modes of microwire assembly were identified and characterized. These two assembly modes are referred to hereafter as bulk microwire assembly and surface microwire assembly. In the bulk microwire assembly, wires grew through the bulk of the suspension as described in the earlier work [31]. The second mode of microwire assembly, where the wires grew directly on the glass surface, has not been previously reported. The
nanoparticles in these surface-supported wires aggregated in half-cylinders, compared to the cylindrical aggregation in the bulk. The two types of structures could be easily distinguished by high-magnification optical microscopy, as the surface microwires were always in the focal plane at the plate surface, while parts of the bulk ones might be out of focus, and the surface of the plate was not seen concurrently. Experimental images of bulk and surface microwires are shown in Fig. 2.2.

![Typical experimental images of the two types of microwire structures obtained.](image)

**Figure 2.2.** Typical experimental images of the two types of microwire structures obtained. (a) Bulk microwire growth. Note that some of the branches are not in the focal plane. (b) Surface microwires. (c) Scanning electron micrograph of the surface microwire structure. Scale bars: (a) 100 µm; (b) 20 µm.

The first major challenge of this research was to understand and control the mode of growth. In many of the initial experiments, surface or bulk wires began assembling without a clearly distinguishable trigger, and sometimes both types grew simultaneously. If the wire assembly began as bulk, it continued in the bulk mode until there was a short circuit in the chamber. If the wire assembly began on the surface, it usually continued on the surface for most of the distance, however at small separations between the tips of the microwires growing from opposite electrodes, the assembly mode often switched from surface to bulk.

The parameters used to characterize the microwire growth in the two modes were the assembly speed and the surface density. The assembly speed was determined by following the growth process in real time with a microscope. The microscope eyepiece scale marks were aligned with the electrodes and the microscope zoom was adjusted so that the scale length was equal to the electrode gap. The distances covered by microwires growing from the
two electrodes were recorded. The data for microwire growth were plotted in coordinates of percentage electrode gap covered versus time. The assembly speed was then calculated from the slope of the data in these plots.

Surface density was used as a qualitative parameter based on an estimate of the fraction of the total area covered by the microwires when viewed from the top after the assembly process had completed and the electrodes have short-circuited. It effectively combined the degree of branching, thickness and the number of microwires formed into one parameter. Surface density increased with increase in any one of the above individual parameters. The surface density of the bulk microwire structures was found to be always smaller than that of the surface microwires (See Fig. 2.2a and b).

![Figure 2.3. Speed of microwire assembly in bulk and surface modes plotted as percentage of electrode gap covered versus time. Two microwires were grown in each assembly mode to evaluate the reproducibility of the process. Lines are guides to the eye.](image)

Typical plots of the assembly process for bulk and surface microwire assembly are shown in Fig. 2.3. The data are for four different experiments carried out on the same glass slide with a gap between the electrodes ≈ 5 mm. The concentration of gold nanoparticles was ≈ 2.23×10^{13} \text{ mL}^{-1}. The microwires grew through the bulk suspension when the initial voltages applied were 95 and 96 V/cm at a frequency of 1200 Hz. When the initial voltage was decreased to 90 and 88 V/cm, the microwires grew in the surface assembly mode. The data from Fig. 2.3 also illustrate that the microwire assembly speed is well reproducible as
long as the wires grow in the same mode, surface or bulk. The assembly speed for bulk microwires in this and other similar experiments was always higher than that for surface microwires (cf. the slope of the lines in Fig. 2.3). The highest recorded assembly speeds of bulk microwires were 50 µm/s and the highest speeds of surface microwire growth were 20 µm/s.

The most intriguing feature of the data displayed in Fig. 2.3 was the linear relationship between the time elapsed and the gap covered. Thus, the microwire growth speed did not depend upon the electric field intensity, which increased monotonously during the process as the distance between the tips of the wires from opposing electrodes decreased. This observation implied that the assembly process was controlled by a diffusion-limited or a diffusion-like process where the wire grew after enough nanoparticles were transported to its ends. The macroscopic structures obtained were similar to those observed for electric field induced diffusion-limited aggregation of microspheres [33, 34]. The only evidence of "reaction-controlled" regime was found at low field intensities, where, as has been reported earlier [31], the microwire assembly started abruptly only above a certain "threshold" intensity, when the field was strong enough to initiate aggregation. Thus, the formation of microwires can be interpreted as collective aggregation of gold nanoparticles that have diffused from the bulk suspension to the areas of high electric field intensity (electrodes and the tips of the growing microwires).

As the external AC field drives the mass transfer process, any parameter that changes the forces acting on the particles will modulate the speed and specifics of the growth process. The effect of a number of such parameters, including frequency, electrolyte concentration, viscosity and dielectric constant of the media is presented in the next sections.

The microwire assembly process was also influenced by the electrohydrodynamic fluid motion in the cell, which increased substantially at high field intensities. The observed transition of microwires from surface to bulk growth mode as the wires from the opposing electrodes approached each other, noted earlier, was usually accompanied by intensive circulation of the fluid near the tips of the microwires indicating substantial electrohydrodynamic flow. This surface to bulk mode transition can be prevented by decreasing the applied voltage as the growing ends approach each other, which, in principle,
could be done by running the experiment at constant currents. This suppressed the electrohydrodynamic motion and uniform surface wires were obtained in the electrode gap.

2.4. Effect of Operating Parameters on Assembly Speed of Microwires

The next thesis objective was to identify the parameters that affected the microwire assembly process and characterization of their effect on microwire growth. Conditions under which only bulk or surface microwires can be grown were also sought. These data allowed achieving control of the microwire assembly process and obtaining microwire structures of specific type.

The parameters that were recognized to control the growth of microwires were frequency, the concentration of electrolyte in the suspension, the viscosity and dielectric constant of the media, and the concentration of particles in the suspension. The effect of some of these parameters on the growth process is shown in Fig. 2.4 by typical plots of microwire assembly evolution under different conditions. The experimental details and conclusions for all parameters studied are outlined below.

2.4.1. Effect of Electrolyte Concentration

0.1 mM NaCl were added to gold nanoparticle suspensions and microwire formation was studied simultaneously for suspensions with and without added electrolyte (Fig. 2.4a). In order to exactly duplicate the experimental conditions for the two systems being compared, the two suspensions were simultaneously loaded in two adjacent chambers on the same glass slide and thus followed parallel growth under identical conditions of electric field of 80 V/cm at 1200 Hz applied across an ≈ 5 mm electrode gap. In both chambers, the microwire formation began in the surface assembly mode and then transited to bulk as the wires approached each other. As demonstrated by Fig. 2.4a, the addition of electrolyte increases the speed of growth for surface microwires. The assembly speed of the surface microwires decreases in the "depletion" region in Fig. 2.4a because by this point of the process most of the particles in front of the growing wires have been depleted. The decreased concentration of gold nanoparticles in the suspension in front of the growing tips is easily observed optically, since the suspension color becomes less intense.
The increase of the speed of surface assembly in the presence of electrolyte correlates to the increase of the speed of bulk assembly with electrolyte reported earlier [31]. The effect of electrolyte can be explained in terms of the suppression of the repulsive electrostatic interactions between the gold nanoparticles. The nanoparticles in our suspension are stabilized by the inherent negative surface changes only, as no surfactants or thiols were added. In the presence of electrolyte, the electrostatic repulsions between the gold nanoparticles are suppressed; however, the attractive van der Waals interactions remain the same. The aggregation of gold nanoparticles into microwires is thus facilitated and the assembly speed increases.

Figure 2.4. Effect of operating parameters on the assembly speed of microwires, (a) electrolyte; (b) frequency; (c) viscosity and dielectric constant of the medium; and (d) particle concentration. In all experiments, the other parameters were kept constant. Note that in all plots the growth speed for bulk assembly is higher than that for the surface assembly. Solid lines are guide to the eye.
2.4.2. Effect of Frequency

Frequency was found to be an important switching parameter in controlling the mode of microwire growth. At frequencies close to 100 Hz, the microwires grew only in bulk assembly mode. At frequencies $\geq 1000$ Hz, the wires grew predominantly in the surface assembly mode.

The data from experiments performed at three different frequencies while keeping all other parameters the same are plotted in Fig. 2.4b. The field intensity was 110 V/cm and the electrode gaps were approximately 5 mm. The microwire grew in the bulk assembly mode at 200 Hz. At 1200 and 3000 Hz, the wire growth started on the surface and transited to bulk as the two tips approached each other and the intensity increased. The assembly speed of the bulk microwires remained approximately constant at different frequencies. Frequency, however, had an effect on the assembly speed of the surface microwires, which decreased as frequency was increased.

It is hypothesized that the decrease in the speed of growth with the increase in the frequency is related to the suppression of the displacement of the counter-ionic atmosphere around the particles and the resulting decrease of the dielectrophoretic force. The gold nanoparticles in the suspension are negatively charged and surrounded by counterions. This counter-ionic atmosphere is a source of additional dipolar polarization under the action of electric field. When an external field is applied, the atmosphere moves in the direction opposite to the one of the charged particle, effectively resulting in the formation of a dipole. The reorientation of the dipoles to match the phase of the alternating electric fields is limited by the relatively slow diffusion of the counter ions surrounding the particles. At higher frequencies the counterions can not follow the rapidly oscillating field and the dipole polarization and interaction with the electric field decreases [35]. This is the likely reason for the decrease of the microwire growth speed at higher frequencies. These data show that while the conductive metal cores of the gold nanoparticles are easily polarizable, the polarization of the counter-ionic atmosphere possibly also contributes to the dielectrophoretic attraction and microwire assembly. The counter-ionic polarization can also be suppressed by increasing the viscosity, as discussed below.
2.4.3. Effect of Media Viscosity and Dielectric Constant

The effect of the frequency on the microwire assembly shows the importance of the counter-ionic mobility as a source of field-induced dipoles. The ionic mobility can also be suppressed by changing the viscosity, and thus viscosity was expected to be a major controlling parameter for this process. It is difficult to distinguish the effect of the viscosity of the medium and the effect of the dielectric constant of the medium from each other. Addition of almost any liquid to the water-based suspension will decrease its dielectric constant. On the other hand, the viscosity may increase or decrease depending upon the properties of the liquid added. To characterize the effect of medium properties, samples containing 33% ethanol by volume and 50% glycerol by volume were prepared. The dielectric constants for both of these mixtures are the same and $\approx 63.4$ [36]. The ethanol based suspension has a viscosity of about 2.08 cP whereas the glycerol based suspension has a viscosity $\approx 6.5$ cP [36]. The concentration of gold nanoparticles was kept constant in all suspensions.

2.4.3.1. Suspensions containing Ethanol

The speed of growth in bulk assembly mode in ethanol-water suspensions was lower than that in normal aqueous suspensions (Fig. 2.4c). This could be readily explained by the lower dielectrophoretic force at lower media permittivity (see the formula for $F_{DEP}$ in chapter 1, Eqn. 1.7) and the higher viscosity of the medium.

2.4.3.2. Suspensions containing Glycerol

This media has the same dielectric permittivity as ethanol, but three times higher viscosity. The more viscous media had a drastic effect on the microwire assembly, to the point where the mechanism of growth changed significantly. First, three to four times higher voltage was required for initiation of the microwire growth. Even at these higher fields, the microwires began to grow only after an initial induction period of about 1 min. However, once the process began the wires grew with assembly speeds as high as 2 mm/s and short-circuited in a matter of seconds. The formation of these wires was always in the bulk and surface growth was never observed. The most intriguing and practically important characteristic of these glycerol-based microwires was that they were single, without branching and very straight.
Experimental observations suggested that the mechanism of the assembly process for glycerol-based suspensions was different from that in aqueous suspensions. The higher viscosity of glycerol did not readily allow the nanoparticles to quickly diffuse and aggregate in the areas of high intensity. Instead, it appeared that the nanoparticles slowly accumulated on the surface of the slide or near the periphery of the chamber (optically observed by intensification of the suspension color in these areas). The growth then proceeded at a high rate inside the highly concentrated nanoparticle phase. This could be seen as a transition to "reaction-controlled" process, as the particles are pre-concentrated at the electrodes and the mass-transfer to the growing wire is very fast.

Table 2.1. Effect of different operating parameters on the assembly speed and the surface density of microwires growth in bulk and surface assembly modes (↑ = increases, ↓ = decreases, ↔ = no change).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Type</th>
<th>Assembly speed</th>
<th>Surface density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>100-3000 Hz</td>
<td>Bulk</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td></td>
<td>1000-3000 Hz</td>
<td>Surface</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Media viscosity</td>
<td>0.9-6.5 cP</td>
<td>Bulk</td>
<td>very rapid formation of a single wire</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface</td>
<td>no surface growth seen at high viscosities</td>
<td></td>
</tr>
<tr>
<td>Media dielectric constant</td>
<td>60-80</td>
<td>Bulk and surface</td>
<td>↑</td>
<td>↔</td>
</tr>
<tr>
<td>Particle concentration</td>
<td>$10^{13}-10^{15}$ mL$^{-1}$</td>
<td>Bulk and surface</td>
<td>↑</td>
<td>↔</td>
</tr>
<tr>
<td>Electrolyte concentration</td>
<td>0-0.1 mM</td>
<td>Bulk and surface</td>
<td>↑</td>
<td>↔</td>
</tr>
</tbody>
</table>

2.4.4. Effect of Nanoparticle Concentration

The effect of gold particle concentration on the microwire assembly speed was straightforward. The assembly speed of the microwires directly increased with an increase of the concentration of gold nanoparticles in the suspension (Fig. 2.4d). The same was true for the effect of field strength, which always increased the speed of microwire growth and was not investigated in detail here. A summary of the effect of the different parameters on the
assembly speed and the surface density of microwire growth is presented in Table 2.1 and the key switching parameters between bulk and surface growth have been identified in Table 2.2.

Table 2.2. “Switching parameters” identified that allow controlling the mode of microwire assembly, bulk or surface.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bulk</th>
<th>Surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>100 Hz</td>
<td>1000 Hz</td>
</tr>
<tr>
<td>Viscosity of media</td>
<td>1:1 Glycerol-Water mixture (~6.5 cP)</td>
<td>Water-like (1 cP)</td>
</tr>
<tr>
<td>Field strength</td>
<td>Surface to bulk transition at increased field strengths</td>
<td></td>
</tr>
</tbody>
</table>

2.5. Modeling of Microwire Growth Process by Electrostatics Calculations

Microwire assembly is driven by the electric field acting between the electrodes. Controlling of the process requires understanding the role of electric field in microwire formation, branching, assembly mode, and direction of growth. In order to achieve this, two types of electrostatic calculations and modeling were performed. The first type focused on the local assembly process by calculating the field distribution at the growing tip of the microwire. These calculations provided information on the structure and branching of microwires and the mode of microwire assembly. To capture the effect of the chamber walls on the wire structure and growth mode, these calculations were performed in the vertical plane perpendicular to the chamber. Second, to predict the general direction of growth in the thin chamber, electrostatic calculations were performed in the horizontal plane of the chamber. These calculations were used to determine the direction of microwire growth based on the electrode structure and in the presence of conductive elements in the gap.

All calculations were performed on symmetrical systems with constant applied field using TRICOMP – 2D electrostatics software based on finite element method available freely from Field Precision [37]. Details about the software and the finite element method are provided below.

2.5.1. Electrostatics Calculations using TRICOMP

The TRICOMP software includes a mesh generator MESH, an electrostatic calculator EStat, and an interactive results display module VESStat. In electrostatic calculations using finite element method, the solution space is divided into number of small elements. It is assumed
that the properties within each element remain constant; hence, there are no elements shared between different objects or across boundary lines. The electrostatic calculations for microwire assembly were performed on a mesh of variable isosceles triangles.

As the electric field has discontinuities at the surface of different dielectrics (which is the case in the microwire process, where water, glass and gold electrodes have different dielectric constants) field calculations are carried out using Poisson equation for the electric potential for each element

\[ \nabla^2 \phi = -\frac{\rho}{\varepsilon_0} \]  

(2.1)

where \( \phi \) is the potential, \( \rho \) is the charge density \((\text{C/m}^3)\) and \( \varepsilon_0 \) is the permittivity of free space \((8.854 \times 10^{-12} \text{ C}^2/\text{Jm})\). The division of the solution space into small elements with constant properties allows linearizing the Poisson equation. The potential in the mesh element is calculated by weighing out the potential of its neighbors. The weighing factors are dependent upon the material properties (dielectric constants, conductivity) of the elements. The potential distribution for the solution space is obtained by iterative calculations. The field intensities are then calculated from the potential distribution. More details about the TRICOMP software and the finite element method are given in Reference [38].

For all electrostatic calculations, detailed specifications of the electrode geometry and the surrounding regions (nanoparticle suspension, glass slide, cover slip) were provided as regions in a 2D plane to MESH to generate a variable triangular mesh. The physical properties of the materials, viz. dielectric constants, conductivity, and potential values were specified into EStat and numerical calculations were performed based on the mesh generated. The solution matrix generated by EStat was viewed with VEStat in the form of plots of equipotentials and electric field lines for the whole system and for each element.

2.5.2. Mechanism of Assembly

The flowchart of the iterative algorithm used for modeling the dynamics of microwire growth in both bulk and surface assembly modes are shown in Fig. 2.5. This algorithm was based on the fact that as the dielectrophoretic force pulls the gold nanoparticles along the direction of the electric field gradient, they end up in the areas of highest electric field intensity. The
growth occurs in these areas of high intensity. In general, this is the reason why the particles accumulate at the tips of the microwires, where the field intensity is the highest.

It was assumed that at any stage of microwire growth, the nanoparticles fill up the areas with highest field intensity. The initial electric potential solution was generated by EStat and the field intensity inside each element of the triangular mesh was calculated by VEStat. At the next stage of the iterative procedure, all elements with field intensity empirically chosen to be higher than 80% of the maximum were identified (the change in the threshold intensity changes the scale, but not the pattern of growth). In the third step, these elements were added to the microwires as newly assembled elements. At this step, occasional empty mesh triangles in between the new elements added and the metal structure were also filled up to maintain consistent microwire shape. The file with the coordinates of the new geometry of the system was then passed on to MESH to generate a variable triangular mesh, and then back to EStat to perform the next electrostatic calculation for the new geometry, and so on. The iterations were repeated until the wires short-circuit.

The results of the iteration procedure were in remarkable agreement with the experimental data. The simulations demonstrate the formation of two opposing wires with multiple periodic large and small branches, which grow towards each other and short-circuit at the end of the process. Snapshots of the electrode assembly at various intervals during

Figure 2.5. Algorithm for electrostatic simulation of the mechanism of microwire growth.
modeling process for bulk (Fig. 2.6a-g) and surface (Fig. 2.6i-k) microwire assembly are compared with actual experimental images in Fig. 2.6.

To initiate the computational procedure for bulk microwire assembly, only a small slant away from the glass surface in the initial electrode geometry was required (Fig. 2.6a). The electric field distribution at the end of first iteration for bulk microwire assembly displayed in VEStat is shown in Fig. 2.6a. The highest intensity of the electric field is near

**Figure 2.6.** Snapshots of the simulation process at various growth stages for both bulk and surface microwire assembly compared with actual experimental images. Bulk microwire assembly – (a) initial geometry, (b) branching of wire, (c) and (d) propagation of branches, (e) field intensity at the tip of the first branch drops below 80% of the maximum leading to termination, (f) the first branch stops growing, (g) short circuit of the wires growing from opposing electrodes. Surface microwire assembly – (i) initial geometry, (j) the wire stays on the surface and does not form long branches, (k) wires are still on surface even though nearing short circuit. The electrode and the microwires formed are shown in white, and the solid line next to electrode delineates the suspension and the glass surface. Actual experimental images: (h) branching pattern for bulk microwires, (l) scanning electron micrograph of surface microwires. Compare (g) and (h), (k) and (l). Note that a slight change in the initial geometry, (a) and (i), changes the assembly mode. Scale bar: 10 μm.

To initiate the computational procedure for bulk microwire assembly, only a small slant away from the glass surface in the initial electrode geometry was required (Fig. 2.6a). The electric field distribution at the end of first iteration for bulk microwire assembly displayed in VEStat is shown in Fig. 2.6a. The highest intensity of the electric field is near
the edge of the electrode as indicated by the bright red triangle element of the mesh used in the electrostatic calculations. The elements with electric field intensity equal to or greater than 80% of the maximum for this iteration are the red triangle and the two surrounding yellow triangles. These elements are added to the electrode geometry for the next iteration. The elements in between the electrode and the red and yellow triangles are added as well to maintain the microwire structure. As the bulk wire grows, it widens and the electric field intensity distribution splits the wire into two different branches (Fig. 2.6b and c).

Branches continue to grow as long as the electric field intensity of the elements near the tips is high enough. Once the branches are left behind the growing wire and the field intensity on their tips decreases, they stop growing (Fig. 2.6d-f). The pattern of branches for the simulated structure of bulk microwire indicates large branches with some smaller branches in between. The results of this simulation match very well the branching patterns observed experimentally in typical bulk microwires (Fig. 2.6h).

In order to initiate the computational procedure for the surface microwires, the edge of the electrode was slanted towards the surface of the glass plate (Fig. 2.6i). In this specific geometry, the highest intensity element is located in the glass slide (red triangle in Fig. 2.6i). This reflects the physical law that the electric field intensity is higher in media with lower dielectric permittivity, which in this case is the glass slide (dielectric permittivities of glass and water are 4.0 and 80 respectively). The local gradient of the field points towards the glass surface and the particles will be accumulated there. The wire proceeds to propagate on the surface (Fig. 2.6j and k). The branching structure observed in the scanning electron micrograph of surface microwire at high magnification (Fig. 2.6l) is similar to the one predicted by the modeling. Thus, simulations explain the periodic branching and the termination of growth once the branches are left behind the areas of high field intensity. More importantly, they prove that the mode of growth, bulk or surface, depends on minute differences in original conditions, which may be imperceptible in the actual experiment. This immediately explains, why in the majority of experiments, random assembly of both types of wires was observed without a clear trigger for the formation of either type.

The switching parameters favoring the growth of microwires in the bulk or on the surface, which were identified, are likely to operate via more complex mechanisms, not taken into account here. For example, low frequency is likely to favor bulk growth by disruption of
the surface growth by the electrohydrodynamic motion of the liquid in the vicinity of the glass wall. The existence of fluid movement near the electrode for DEP has been shown to displace particles away from the electrode at low frequencies [39].

2.5.3. Predicting Microwire Growth around Conductive Objects

It has been demonstrated by earlier research in our group that microwires will "automatically" connect to any conductive objects present in between the electrodes [31]. This is a potentially useful property of this process, as the microwires can be used as a tool to make in situ connections to objects in liquid-filled chambers and thus make electric circuits
by field-driven assembly. To understand why the microwires grow towards these objects in the gap and how to direct the growth, simultaneous experiments and calculations of the electric field in the chamber were carried out.

In the experiments, conductive “islands” of silver paint were deposited on the glass slide between the electrodes. Microwires were then grown inside the chamber to investigate the effect of the islands on the wire formation and growth direction. In the supporting electrostatic calculations, the field intensity in the chamber with a conductive object in the middle was calculated. The results of electrostatic calculations compared with actual experimental images are shown in Fig. 2.7.

The calculated intensity of electric field is highest around the object. Based on the model for growth in the areas of highest intensity, this result suggests that the wire formation will start from the island. Such a theoretical prediction is somewhat counterintuitive, as it expects that the wires would begin assembling at the island that is not electrically connected, instead at the electrodes where the field originates. However, it was directly confirmed by experiments such as the one shown in Fig. 2.7b, where images of the initial stages of wire formation from a conductive island are captured. Thus, the DEP-based model of microwire assembly was proved and its predictive power was established. For example, when the microwires growing from the islands, and the ones from the electrodes, are displaced the calculations show that they will grow towards each other and short circuit (Fig. 2.7c). Similar situation is evident from the actual experimental image shown in Fig. 2.7d. Thus, whenever there is a conducting object in between the electrodes, the wires are very likely to spontaneously connect to it and include it in the circuit. This provides a useful tool for the in situ assembly of circuits, which is discussed in more details in the following section.

2.6. Microwire Assembly for Nanotechnology Applications
The assembly of electrically functional structures from suspensions of gold nanoparticles under the action of AC fields can be useful in various applications such as chemical sensors, "wet" electronic circuits, interfacing biological cells or tissues with electric systems, etc. The results of this work allowed taking the first steps towards the control of the microwire growth process and the engineering of structures with specific functionality.
2.6.1. Engineered Microwire Assembly

After understanding the effect of various parameters on microwire formation and with the help of electrostatic calculations, desired microwires of specific structure can be grown by controlling the assembly process. Fig. 2.8a shows how a single, straight and unbranched microwire could be grown through the bulk of the suspension. This wire was obtained at a frequency of 1000 Hz in suspensions containing 50% glycerol by volume. Alternatively, microwires on the surface can be made to assemble in the form of multiple parallel arrays. Fig. 2.8b shows surface microwires grown parallel to each other. These wires were obtained at a frequency of 1200 Hz, electrode gap of 5 mm, area of the chamber $\approx 20 \text{ mm}^2$, and 75 V/cm initial applied electric field, which was gradually decreased as the growing wires from the opposing electrodes approached each other, to avoid transition to bulk mode and thus achieve complete surface assembly.

**Figure 2.8.** Controlled assembly of microwires with a specific morphology: (a) single unbranched microwire growth through the bulk nanoparticle suspension containing 50% glycerol by volume; (b) array of parallel wires assembled on the surface of a glass slide. Scale bars: 50 $\mu$m.
The bulk microwires are stable as long as they remain in the liquid phase. However, they do not survive the drying process: the large capillary forces exerted on the long thin fibers by the withdrawing meniscus of the liquid typically lead to breakage and fragmentation. Thus, the bulk wires are limited in application to *in situ* assembly and function in liquid environment. This still makes them useful in devices such as biochips and sensors. The surface microwires reported here, however, are mechanically supported by the glass surface. These microwires remain intact even after removal and drying of the liquid phase. This important advantage opens a range of applications of these structures in novel materials. For example, arrays of parallel microwires similar to the ones shown in Fig. 2.8 can be used in materials that conduct electricity and heat in one specific direction or transmit light of specific polarization. Such arrays could also find use for heat management in MEMS devices and in various microchips.

**Figure 2.9.** Various types of microwire electrical connections to conductive islands between the electrodes. Islands connected through (a) bulk microwire growth only, (b) a combination of bulk and surface growth, or (c) surface assembly only. Scale bars: 500 µm.

Another important property of the microwire assembly process is the wiring of conductive islands, which may be e.g. electronic or biological objects. As described in the electrostatic calculations the microwire growth in such systems will start at the island. Fig. 2.9 illustrates connections to conductive islands in the suspension achieved in various ways – bulk only, bulk-surface and surface only. This method could be used to connect
electrically objects in the suspension and to "grow" an electrical circuit out of them. More complex applications using parallel surface arrays may include multiple connections on electrical chips and microcircuits.

As an alternative to having the conductive objects present in the system and allowing the microwires to self-assemble on them, the conductive objects can be sequentially introduced after microwires have began to grow at the electrodes. The conductive objects will then be attracted towards the area of high field intensity and will “automatically” arrive at the microwire tips. This method can thus be used to make electrical circuits, in which the components are introduced into the suspension like an “assembly line” – alternatively growing microwire and connecting different objects.

2.6.2. Microwires Made from Other Materials

The insight gained from understanding the forces involved in microwire assembly from gold nanoparticles, can now be applied for assembling materials from other colloidal particles.

![Figure 2.10. Microwires assembled from different materials. (a) DMAP-stabilized 6 nm gold nanoparticles. (b) 640 nm Core-shell microspheres with polystyrene core and a thin gold shell. (c) Single walled carbon nanotubes. Scale bars: (a) and (b) 50 µm, (c) 500 µm.](image)

2.6.2.1. DMAP-stabilized Gold Nanoparticles

Samples of 6 nm diameter gold nanoparticles stabilized by 4-dimethyl aminopyridine (DMAP) were obtained from Caruso’s group at the University of Melbourne, Australia. These particles were positively charged as opposed to the negative charge of the gold nanoparticles produced by the Slot and Gueze method [40, 41]. The DMAP-stabilized positively charged gold nanoparticles assemble into similar microwire structures under an
applied alternating electric field. (See Fig. 2.10a). These experiments demonstrate that functionalized gold nanoparticles, irrespective of the charge on the surface or of the presence of a thin dielectric shell, assemble into microwires. The ability to assemble “functionalized microwires”, by using functionalized gold nanoparticles would be of tremendous advantage for biosensors. In particular, impedance measurements of microwires from immunoglobulin-tagged gold nanoparticles can be used as a way to detect complimentary antigens present in the suspensions.

2.6.2.2. Metallo-Dielectric Microspheres
Metallo-dielectric core-shell microspheres were also obtained from Caruso’s group. These particles were prepared by covering 640 nm polystyrene microspheres with eight layers of alternate positive and negative polyelectrolytes. The polyelectrolyte matrix was then infiltrated with 6 nm DMAP-gold nanoparticles. The result is particles with dielectric latex cores and thin metallic gold shell [40, 41]. These core-shell particles also assemble into microwires as shown in Fig. 2.10b. These wires can be very useful as sensors as they allow functionalizing the polymer shell relatively easily [42]. The core-shell particles, due to their gold nanoparticles infiltrated shell, are conductive in nature. Thus, an important observation that can be drawn from these experiments is that conductive particles assemble into microwires, whereas dielectric particles of the same size (latex microspheres, 0.5-1 µm) assemble into colloidal crystal (See Figs. 1.6 and 1.7).

2.6.2.3. Carbon Nanotubes
These experiments were performed in collaboration with the Zhou group at the University of North Carolina at Chapel Hill. Single walled carbon nanotubes were assembled under applied field of 100 V and at 400 Hz (Fig. 2.10c). CNTs in the suspension aggregated and formed bundles, which were then dielectrophoretically assembled into microwires. There is evidence in literature that the CNTs that assembled into microwires are metallic in origin [43], and this provides means for separating the metallic and semiconducting nanotubes. The assembly of CNTs has immense potential in the field of biosensors [44-51].
2.7. Conclusions

Assembly of gold nanoparticles from suspensions into microwires under the influence of alternating electric field has been investigated. Bulk and surface modes of microwire assembly were observed and the "switching parameters" allowing forming only one type of structure were identified. The assembly speeds are constant throughout the process and are independent of the electric field intensity. The operating parameters that influence the assembly speed and the surface density of the microwires were identified and the effect of these parameters on the microwire growth process was studied. Electrostatic modeling allowed us to simulate the mechanism of assembly and to predict the general direction of microwire growth.

Based on these results, we can now control the process through a range of operating parameters and engineer the microwire structure as needed. Two "extreme" cases of microwire assembly – single unbranched bulk microwire and arrays of massively parallel surface wires were demonstrated. We can also make in situ electrical connections to objects in the gap between the electrodes. These tools can be useful for nanotechnology applications, which require formation of electrical wires between miniature devices in liquid or biological environments, or in interfacing of biological cells with electronic devices. The process can also be useful in the formation of materials with anisotropic thermal or electrical conductivity.

2.8. Acknowledgments

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2.9. References


Chapter 3

AC Electrokinetic Collection of Particles and Cells

3.1. Introduction

The development of integrated microfluidic chips that can perform sample pre-treatment and analysis on a single chip, also known as micro-Total Analysis Systems (μTAS), is still in its infancy and is an area of extensive research [1-6]. μTAS devices are adaptable for extensive parallelization and automation and have the potential to drastically reduce both the time and cost for analysis. Electric fields are well suited for use in such devices as they have the ability to transport, manipulate and analyze most sample types including particles and cells. Forces of electrical origin that can be used in μTAS include electro-osmosis, dielectrophoresis (DEP) and AC electrohydrodynamics (EHD). These forces can be applied separately or simultaneously and their magnitudes can be easily adjusted by changing the amplitude or the frequency of the applied external field.

Dielectrophoresis is the interaction of an uncharged dielectric particle with a non-uniform electric field [7-9]. Particles are either attracted towards or repelled from the electric field maxima depending on their effective polarizability in the medium. DEP has been used by various researchers, including us, for forming linear aggregates of nanoparticles [10-13], colloidal crystals [14-18], and cell arrays [19]. In μTAS devices, DEP can be used as a tool for particle, cell and DNA manipulation and separation [20-24]. Large amount of research has been devoted to Field Flow Fractionation on microfluidic chips, where DEP along with pressure driven flow is used to separate particles based on their dielectric properties [25-27].

Electro-osmosis and AC electrohydrodynamics are electrokinetic effects arising from the interaction of ions in the electrical double layer formed on a surface and the tangential component of the electric field inside the double layer. Both these forces result in liquid flow and are suitable for pumping of liquids in μTAS devices [28-35]. AC electrohydrodynamics has been found to be a contributing force in colloidal crystal formation during electrophoretic deposition [36-45]. The term “induced-charge electro-osmosis” (ICEO) has recently been introduced to describe electrokinetic phenomena in which the applied electric field induces a charge on a conducting or a dielectric surface and the same field interacts with its own induced charge to produce fluid motion. Electro-osmosis and AC electrohydrodynamics can be classified as ICEO phenomena [46, 47].

Most microfluidic chips at present require that the samples containing the particles, cells or molecules of interest are pre-concentrated and pre-cleaned. Sample pre-treatment is
labor intensive and generally requires bench-top equipments. Filtration or centrifugation can be a major source of artifacts, in particular, for cell analysis due to the interactions of the cells, particles and contaminants in the concentrated cake [48, 49]. Collection and pre-concentration of cells thus remains a major problem in μTAS devices. Chips performing particle collection in a chamber using alternating electric field are being developed by various groups [50-54]. Bennet et al. used negative DEP and prevented particles in the chamber from crossing “dielectrophoretic gates”, the region of high electric field intensity [50]. Suehiro et al. created a DEP filter by filling up the space between electrodes in the chamber with glass beads [51]. Yeast cells were attracted to the areas of high electric field intensity on the surface of the glass beads. In both these chips, the collected particles were flushed out from the chamber by switching off the applied field. A drawback of these DEP-based chips is that they rely on the dielectric properties of the particles and cells and thus are not applicable to all sample types. Electrokinetic flows on the other hand are not dependent on the dielectric properties of the particles and thus they can be applied to all sample types. Hoettges et al. and Wong et al. have developed chips that combine EHD flow with DEP for particle collection on planar electrodes in small chambers [52-54].

This chapter describes a new electrokinetic technique that uses AC electric fields for collecting particles using patterned electrodes in a small chamber. The effect of various process parameters on the collection speed and efficiency are investigated. These parameters include the applied field strength and frequency, the electrolyte and particle concentration, the particle size and charge and the pattern geometry. An EHD flow model to describe the particle collection process is extended. The experimental results are interpreted by means of numerical simulations of the electric field and fluid velocity distribution in the chamber. The technique and the results of the model could be used to design chips that collect and concentrate particles, yeast cells and microbes from dilute suspensions.

3.2. Materials and Methods

3.2.1. Experimental Apparatus

Electric field was applied to latex particles suspended in a chamber by means of a patterned silicon electrode and an indium-tin oxide (ITO) electrode separated vertically by a spacer. The schematics of the patterned silicon wafer and the experimental setup are shown in
Fig. 3.1. A 1 µm thick layer of Shipley positive photoresist was deposited on a p-doped silicon wafer. The silicon wafer had sufficient conductivity (nominally $\rho = 1-20 \, \Omega \, \text{cm}$) to act as an electrode and the photoresist layer served as an insulator. The photoresist layer was patterned using standard photolithography techniques to expose parts of the underlying silicon wafer as shown in Fig. 3.1a. The pattern consisted of eight squares approximately 0.81 mm$^2$ in area and separated by 600 µm of photoresist-covered surface. These patterned squares acted as conductive corrals surrounded by the insulating photoresist layer. This patterned silicon wafer served as the bottom electrode in the experimental chamber (Fig. 3.1b). A glass slide coated with transparent conductive ITO layer was used as the top electrode for the chamber. The two electrodes were separated by a 0.75 mm silicone rubber spacer (Grace Bio-Labs, OR) with openings for adding and removing particle or cell suspensions from the chamber. The volume of the chamber formed was approximately 120 µL.

Figure 3.1. Schematics of (a) the patterned silicon wafer and (b) the experimental setup of the chamber used for particle collection.

The silicon wafer and the top ITO electrode were connected to electrical leads using Circuit Works CW2400 conductive epoxy (Chemtronics, GA). The leads were connected to an Agilent 33120A 15 MHz function generator (Agilent Technologies, CO) providing AC signal 2-10 V in peak to peak magnitude. The strength of the applied field and the current passing through the chamber were measured using multimeters connected in the circuit. The maximum currents measured in the experiments (~ 0.2 mA) were an order of magnitude
lower than the maximal permissible current load of the Agilent function generator and the voltage was very stable. An oscilloscope was connected to the circuit and no capacitive distortion of the square wave signal was observed when the load (experimental chamber) was connected into the circuit. The particles in the chamber were continuously monitored using Olympus BX-61 microscope equipped with reflection-mode and fluorescent-mode microscopy and images were recorded using Olympus DP-70 digital CCD camera.

3.2.2. Materials
Fluorescent latex microspheres of different diameters were purchased from Molecular Probes (Eugene, OR). The particles were centrifuged and washed twice with deionized water from Millipore RIOs system to remove any preservatives, surfactants, or electrolytes present in the media. The washed samples were diluted using deionized water to obtain latex suspensions with final particle concentration of 0.01% w/v particles. The conductivities of latex suspensions thus prepared were within the 0.05 – 0.07 µS/cm range. Sodium chloride was purchased from Fisher Scientific, PA and phosphate buffered saline (PBS) tablets were purchased from Sigma-Aldrich, MO. A PBS solution containing 1.37 mM NaCl, 0.027 mM KCl and 0.1 mM phosphate buffer was prepared by dissolving one PBS tablet in 20 L deionized water. Baker’s Yeast (dried, active) was purchased from MP Biomedicals, Inc., OH. The yeast cells were dispersed in the PBS solution to obtain a final concentration of 0.014% w/v yeast. The conductivity of latex suspensions with 0.5 mM NaCl added was 6.15 µS/cm and that of yeast suspensions in PBS was 17.5 µS/cm.

3.3. Results
When an alternating electric field was applied to the chamber the suspended latex particles began accumulating inside the conductive corrals on the bottom electrode. The particles near the photoresist-corral edges began moving into the corrals, whereas, the particles farther away from the corral edges on the photoresist surface remained stationary. Observing the particles with the 50× objective and following their three dimensional motion, evidence for fluid flow was found. Close to the photoresist surface, the particles clearly moved towards the conductive corrals; whereas, when the microscope stage was adjusted such that the plane of focus was elevated above the photoresist surface, most particles exhibited Brownian
motion but a few were observed to be moving away from the corrals. The velocity of particles moving into (or towards) the corrals exceeded the velocity of particles moving away from the corrals. Many more particles were moving towards the corrals than moving away, so that, over time the originally suspended latex particles collected at the centers of all conductive corrals inside the chamber. Typical results for collection of 2 µm latex particles in one of the conductive corrals when an alternating field was applied are shown in Fig. 3.2.

![Figure 3.2](image)

**Figure 3.2.** Time-lapse images of 2 µm latex particles collected from a 0.01% w/v suspension without added electrolyte in one conductive corral at a frequency of 100 Hz, and a field strength of 2.67 V/mm. (a) t = 1 min, the arrows show areas where particles began accumulating, (b) t = 15 min, (c) t = 30 min, (d) t = 45 min, (e) the equilibrium point, t = 60 min. (f) Zoom in micrograph at the center of the corral shows that the particles are loosely packed.

Inward fluid flow was generated around the corral edge immediately upon the application of the electric field. Within seconds the particles driven by fluid flow started moving into the conductive corral. The first results of particle accumulation were visible near the corral corners one minute after the start of the experiment (these regions are marked by arrows in Fig. 3.2a). As time went by, more latex particles from the sides were pushed
inwards into the corral. The particles accumulated near the corral edges were pushed further towards the center (Fig. 3.2b-d). One hour after the start of the experiment the particle accumulation was mostly completed and no further collection of particles was observed (Fig. 3.2e). Upon closer inspection it was found that the amount of particles moving into the corral close to the photoresist surface was balanced by the amount of particles moving away from the corral in horizontal planes some distance away from the photoresist surface. The collected particles formed an “X-shaped” accumulation pattern with four spikes corresponding to the four corners of the conductive corral. A small fifth spike (to the top left) in the observed particle accumulation pattern corresponds to a small notch in the corral edge illustrating the importance of the boundary area. At the center of the conductive corral, the particles were loosely packed (Fig. 3.2f) and did not form a closed packed crystal structure as in the previously reported case of electrophoretic deposition on patterned electrodes [18, 55].

3.4. Effect of Operating Parameters

In order to understand the particle accumulation process the effects of different operating parameters on the process were characterized in detail. The collection process was found to be affected by the frequency and strength of the applied field and concentration of electrolyte in the suspension. The effects of these three controlling parameters are described in detail below. A number of other operating parameters, including particle size (varied from 200 nm to 5 µm), particle charge (negative sulfate latex and positive amidine latex), concentration of particles in the suspension (0.001-0.1% w/v), corral size (1-115 mm²), and substrate material (silicon and gold) were also investigated. No remarkable difference was observed in the process of particle accumulation or in the collection pattern in the corral when these operating parameters were varied in the ranges listed above.

3.4.1. Effect of Applied Field Strength

The strength of the applied field was varied from 0.5 to 8 V/mm. The particle velocity increased on increasing the external voltage, which resulted in increased particle accumulation. Physical breakdown of the photoresist layer and deterioration of the conductive corrals took place when the applied voltage was increased beyond 8 V/mm. The
collection pattern inside the corral remained unchanged on increasing the strength of the applied field.

The particle velocity near the photoresist-corral edge was measured for different voltages using video microscopy (Fig. 3.3). Series of micrographs were taken using a 50× objective and the digital camera attached to the microscope. Time-lapse images were acquired for a total time of one minute, with one second interval between the frames. The image subtraction function in Adobe Photoshop was used to calculate the particle coordinates and velocity. Two frames in grayscale modes were superimposed and the second frame was subtracted from the first one resulting in one single image. Particles which did not move during the time between the two frames (typically stuck to the surface) were thus eliminated from the resulting image. The moving particles were visible as white or black circles that stood for their beginning and ending positions respectively. The distances traveled by each particle were then calculated from their coordinates in the image and the velocities were obtained by dividing them by the time difference between the two frames.

![Figure 3.3](image)

**Figure 3.3.** Particle velocities measured by video microscopy near photoresist-corral edges at 100 Hz. The lines are linear fits for (top) maximal particle velocity and (bottom) average particle velocity.

For each applied voltage between 0.67 – 4.67 V/mm, the velocity of a minimum of hundred particles was measured using this procedure. The maximum particle velocity and the average particle velocity were found to be proportional to the square of the applied field.
strength. The plots of the maximal particle velocity and the average particle velocity as a function of the squared applied voltage are shown in Fig. 3.3. The least squares fit for the coefficient of linear dependence were $1.57 \times 10^6 \mu m^3/(V^2 s)$ and $0.81 \times 10^6 \mu m^3/(V^2 s)$ respectively for the maximum and average particle velocities. Particle velocity as high as 50 $\mu m/s$ can be obtained with 5.33 V/mm applied field. For applied voltages higher than 5 V/mm, the particles move so fast that there are not enough particles remaining within the field of view of the digital camera in consecutive frames and hence the above outlined procedure for measuring particle velocity could not be used. The data will be discussed in the model section below.

![Image](image.png)

**Figure 3.4.** Effect of the frequency of the applied field (a) 2000 Hz, 4 V/mm, (b) 5000 Hz, 6.67 V/mm. Both images show collection of 2 $\mu m$ latex from 0.01% w/v suspensions and were taken 1 hour after the start of the experiment. Compare also with Fig. 3.2e.

3.4.2. Effect of the Field Frequency

Particle collection took place when the frequency of the applied field was between 10 and 5000 Hz. At low frequencies (< 10 Hz) and at high frequencies (> 5000 Hz) no fluid flow was observed and no collection of particles occurred. For the range of frequency where particle collection was observed (10 – 5000 Hz), the rate of particle accumulation diminished with increasing frequency. Experimental images for the collection of particles on a single conductive corral for two different frequencies – 2000 Hz and 5000 Hz are compared in Fig. 3.4. The voltage of the applied field was 4 V/mm at 2000 Hz and 6.67 V/mm at 5000 Hz. Also compare these images with Fig. 3.2e, where particles are collected at 2.67 V/mm, 100 Hz. Although the higher field should have increased the rate of collection of particles as described above, the amount of particles collected at 5000 Hz and 2000 Hz were significantly
lower than that at 100 Hz. At higher frequencies the particles also stayed close to the periphery of the conductive corrals rather than collecting at their centers.

3.4.3. Effect of Electrolyte Concentration

The effect of different concentrations of sodium chloride on the collection of latex particles inside the corrals was also studied. At electrolyte concentrations larger than 2 mM NaCl there was no particle collection and no observable fluid flow. For suspensions with less than 2 mM NaCl, the rate of particle collection remained approximately the same as suspensions without added electrolyte. However, the accumulation pattern formed was significantly different in the presence of electrolyte. The particles collected in the centers packed much more closely when electrolyte was added.

Figure 3.5. Particle collection for 2 µm, 0.01% w/v latex suspensions with 0.5 mM NaCl under electric field of 4 V/mm, frequency of 100 Hz. (a) t = 1 hr, particles pack more closely compared to suspensions without electrolyte. (Compare with Fig. 3.2e) (b) Zoom in image at the center of the corral shows close packed colloidal quasi-crystals. (Compare with Fig. 3.2f).

Typical experimental images for the process in suspensions containing 0.5 mM NaCl are shown in Fig. 3.5. The micrographs were taken from the particles collected in the center of a conductive corral one hour after the start of the experiment. The close ("quasi-crystal") packing is obvious when these images are compared with Fig. 3.2e and f (showing structure of the particles collected without added electrolyte). The addition of the electrolyte also increased the adhesion of the particles to the surface of the silicon wafer. For experiments without electrolyte, the collected particles were easily removed when the chamber was
flushed with water. However, when electrolyte was added to the suspensions almost half of the particles collected in the corral remained on the surface when the chamber was flushed with water. These particles remain adherent to the silicon wafer even after drying the chamber.

3.5. Interpretation of Results and Discussion
The forces that are exerted on the particles when an alternating electric field is applied to the chamber could include dielectrophoresis (acting directly on them) and AC electrohydrodynamics (moving them together with the surrounding liquid). The transport and collection of particles in the conductive corrals can be attributed to either or both of these forces. In order to understand how DEP and AC EHD might be involved in the particle collection process, it is necessary to determine the direction and magnitude of these forces in the chamber. Both forces are related to the magnitude and direction of the electric field, and hence to determine the DEP and EHD forces the electric field distribution inside the experimental system was modeled using FEMLAB.

3.5.1. Simulation of Electric Field Distribution
As the corral geometry was symmetric and all corrals within the chamber were identical, it was sufficient to simulate the field distribution on only one conductive corral and the surrounding photoresist. The electric field inside the chamber during the positive half-cycle of the applied voltage was simulated with 2D electrostatic calculations using the FEMLAB multiphysics modeling package (COMSOL, Burlington, MA).

The calculation of the electric field in FEMLAB required several steps. The geometry of the system to scale was specified as a side view of the chamber as shown in Fig. 3.6. The dielectric sub-domains were water \( (\varepsilon = 80) \) and the photoresist layer \( (\varepsilon = 2) \). The boundaries were the bottom silicon electrode (applied voltage = 1.5 V, positive half-cycle of the AC field) and the top ITO electrode (grounded). The two boundaries to the left and right were taken to be electrically symmetrical. Next the solution space was triangulated into a conformal mesh. The physical properties inside the elements remain constant and hence there are no mesh elements across sub-domains or boundaries. The solver was then initialized to
solve the Poisson equation for all elements to obtain the electric field distribution. The electrical field vectors inside the chamber, computed with FEMLAB, are shown in Fig. 3.6b.

![Figure 3.6](image)

**Figure 3.6.** Simulation of the electric field distribution inside the chamber using FEMLAB. (a) Boundary conditions and geometry specified, (b) Electric field vectors calculated for one conductive corral, (c) Zoom-in near the left photoresist-corral edge shows non-uniform field distribution. Black boxes illustrate the photoresist layer and are not to scale except in (c).

The electric field inside the chamber is mostly uniformly vertical as seen in Fig. 3.6b. However, there are small areas of non-uniformity near the photoresist-corral edges. Zoom-in of the area of non-uniformity in the electric field distribution close to the left photoresist-corral edge is shown in Fig 3.6c. The magnitude of the electric field, as determined by the length of the vectors, is highest near the photoresist-corral edge and decreases as one moves away from the edge. The first implication from these calculations is that there will be a local field gradient, which could attract the particles dielectrophoretically towards the corral edge. However, if DEP were the controlling force the particles would collect at the photoresist-corral edge and not inside the corral as seen in the experiments. The non-uniform field also does not exert any force on the bulk fluid medium but acts only on the suspended particles.
This could not explain the fluid circulation observed inside the chamber under the application of the electric field. Thus, the possibility of dielectrophoresis as the major controlling force was ruled out, due to the lack of evidence for particle attraction to the areas of highest field gradient.

An alternate hypothesis that AC electrohydrodynamics is responsible for particle collection inside the chamber was then considered. To ascertain this, the surfaces where field-induced electrical double layers are formed (in addition to intrinsic surface charging) need to be identified and the tangential electric field on these surfaces needs to be calculated. Double layer formation takes place both on the silicon wafer surface and on the photoresist surface. At the silicon wafer surface (of the conductive corral), for, e.g., the positive half cycle of the alternating electric field, negative counter-ions from solution are added to the electrical double layer. The photoresist layer behaves as a capacitor with the bottom electrode and the photoresist-solution interface acting as opposing plates. Thus, a positive half-cycle voltage applied at the bottom electrode also creates a negative charge build up in electrical double layer at the top photoresist-solution interface.

During the positive half-cycle of the electric field, the induced additional charge in the double layer will be negative. At the photoresist surface the tangential field is directed away from the corral and is stronger at its edges (see Fig. 3.6c). Thus the induced negative ionic charges in the double layer will be dragged towards the corral, generating an EHD flow in the same direction. During the negative half cycle of the AC wave the field on the electrode will be negative, the induced double layer charge will be positive and its interaction with the tangential field will again lead to EHD flow towards the corral. Thus there will be constant EHD "pumping" along the gradient of the field, which will occur only at the corral edges. No EHD flow will be generated in the middle of the corral, in the middle of the photoresist, or on the top ITO electrodes as the fields there are uniform.

The magnitude of the EHD flow can be estimated from the magnitude of the tangential electric field. The values of the tangential electric field at the patterned electrode surface were computed from the FEMLAB simulation of the electric field distribution. The plot of the tangential component of the electric field on the bottom electrode surface versus the distance from the photoresist-corral edge is shown in Fig. 3.7a. The tangential component of the field is highest at the photoresist edge and decreases rapidly as one goes away from the
The velocity of the generated EHD flow, thus, should increase from zero some distance away from the corral edge to reach its maximum at the edge and then decrease in the corral due to friction and lack of driving force. The particle velocity will be the same as that of the EHD flow. The particles may also face dielectrophoretic attraction towards the edge, which further decreases the particle velocity.

The above model-predicted relation between particle velocity and the distance from the photoresist-coral edge was verified experimentally by video microscopy measurements. The procedure followed was similar to that described in the Results section. In addition to calculating particle velocities, the distances from the photoresist-coral edge were measured along the direction of particle motion. The experimentally measured particle velocity as a function of the distance from the photoresist-coral edge for applied field of 2 V/mm, 100 Hz is plotted in Fig. 3.7b. The particles entrained by the EHD flow were accelerated as they arrive closer to the edge of the corral and their velocity reached a maximum at the corral edge in exact correspondence with the EHD model and simulations. After they crossed the corral edge, the particle velocity decreased as there is no tangential field gradient and no EHD driving force.

Figure 3.7. (a) Computed tangential electric field from FEMLAB simulation and (b) Experimentally measured particle velocities. The comparison proves that the particles (and liquid) get accelerated in area of high tangential field outside the conductive coral edge.
3.5.2. Simulation of Fluid Velocity Distribution

AC electrohydrodynamics explains well the fluid motion and particle dynamics in the proximity of the conductive corral. In order to model how this EHD "pump" generates macroscopic fluid flow in the cell, FEMLAB was used to simulate the fluid velocity distribution in the chamber.

The geometry used for this simulation was similar to the one for the electric field distribution (Fig. 3.8a). In this case there was only one sub-domain – water, with hydrodynamic properties – density = 1000 kg/m$^3$ and viscosity = 10$^{-3}$ kg m$^{-1}$ s$^{-1}$. To simulate the EHD flow generated on the photoresist surface, the surface velocity spanning 100 µm inside the photoresist edge was set equal to 4 µm/s (approximate highest particle velocity at the edge obtained experimentally in Fig. 3.7b, shown by red arrows in Fig. 3.8a). The fluid velocity distribution inside the chamber was then obtained by solving the Navier-Stokes equations. The resulting velocity distribution is shown in Fig. 3.8b. The EHD flow generated on the photoresist surface, results in bulk circulation of the fluid inside the chamber, which corresponds to the experimental observations. The simulation also shows that there is a stagnant region at the center of the conductive corral, where the particles were experimentally observed to collect.

Figure 3.8. Simulation of the fluid velocity distribution inside the chamber using FEMLAB (a) Boundary conditions and the geometry specified, (b) Computed velocity distribution inside the chamber. The liquid is accelerated at the corral edges and drags along the particles which are deposited in the stagnant region in the middle.
The results obtained from the FEMLAB simulations of the electric field distribution and the fluid flow point out that the major driving factor of the effects observed is the EHD flow. It drags and accelerates the particles as they approach the corral edge and pushes them towards the center of the corral. The particles settle in the center of the conductive corral where there is a stagnant flow region. DEP is not a leading force, but it may contribute by attracting the particles towards the photoresist-conductive corral edge, where they are entrained in the flow.

3.5.3. Comparing Experimental Results with Simulation Predicted Behavior

The experimentally observed effect of different operating parameters on the particle collection process can now be readily interpreted in terms of the above described model. The expected electrohydrodynamic behavior and the experimental observation for different operating parameters are compared in Table 3.1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range studied</th>
<th>Expected EHD behavior</th>
<th>Experimental observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC voltage</td>
<td>0.5 – 8 V/mm</td>
<td>( u \alpha E^2 )</td>
<td>( u \alpha E^2 )</td>
</tr>
<tr>
<td>AC frequency</td>
<td>0.5 – 8 V/mm</td>
<td>( E_t = 0, u = 0 )</td>
<td>( u = 0 )</td>
</tr>
<tr>
<td></td>
<td>&lt; 10 Hz</td>
<td>( \zeta_{ind} ) ( \downarrow )</td>
<td>( u \downarrow )</td>
</tr>
<tr>
<td></td>
<td>10 – 5000 Hz</td>
<td>( \zeta_{ind} = 0, u = 0 )</td>
<td>( u = 0 )</td>
</tr>
<tr>
<td></td>
<td>&gt; 5000 Hz</td>
<td>( \zeta_{ind} = 0, u = 0 )</td>
<td>( u = 0 )</td>
</tr>
<tr>
<td>Electrolyte concentration</td>
<td>0.5 – 2 mM NaCl</td>
<td>Debye length ( \downarrow )</td>
<td>Particles pack more closely</td>
</tr>
<tr>
<td></td>
<td>&gt; 2 mM NaCl</td>
<td>( E_t = 0, u = 0 )</td>
<td>( u = 0 )</td>
</tr>
<tr>
<td>Particle charge</td>
<td>Positive or Negative</td>
<td>( \leftrightarrow )</td>
<td>( \leftrightarrow )</td>
</tr>
<tr>
<td>Particle size</td>
<td>200 nm – 5 ( \mu )m</td>
<td>( \leftrightarrow ), but larger particles may experience more friction</td>
<td>Higher voltage required for collection of larger particles</td>
</tr>
<tr>
<td>Corral area</td>
<td>1 – 115 mm(^2)</td>
<td>( \leftrightarrow )</td>
<td>( \leftrightarrow )</td>
</tr>
<tr>
<td>Substrate material</td>
<td>Silicon or gold</td>
<td>( \leftrightarrow )</td>
<td>( \leftrightarrow )</td>
</tr>
</tbody>
</table>

The EHD flow is dependent on the induced charge in the double layer and on the tangential electric field (Eqn. 1.12). Any operating parameter which affects either of these
parameters will affect the EHD flow and consequently the particle collection inside the chamber. If the applied external field, $E$, is increased, the induced zeta potential will increase, $\zeta \propto V \propto E$. The tangential component will also increase linearly with the total electric field, $E_t \propto E$ [9]. The fluid velocity, and hence the particle velocity, which are proportional to the induced zeta potential multiplied by the tangential electric field (Eqn. 1.12), thus should increase as the applied voltage squared, $u \propto E^2$, as was experimentally observed (Fig. 3.3). This is an established relation for many polarization-dependent electric fields driven effects [33].

The effect of the applied field frequency on the particle collection process can be explained on the basis of the dynamics of the double layer formation process. For an alternating electric field the induced component of the double layer is formed by negative counter-ions in the positive half-cycle and by positive counter-ions in the negative half-cycle. The ions in the solution have to diffuse in and out of the double layer to the bulk solution with the changing sign of the alternating electric field. When the applied electric field frequency is high (of the order of few kHz) the ions do not have enough time to redistribute as the field goes from one half-cycle to the next [9, 56]. The induced charge in the double layer at high frequencies hence is zero and so there should be no EHD flow and no particle collection. On the other hand, when the applied field frequency is very low (of the order of a few Hz) the ions have enough time to form the double layer and suppress the field. The entire potential drop at low-frequency (or static) fields takes place in the double layer [9, 56]. The electric field inside the chamber will be zero and again there should be no EHD flow. The effect of frequency is similar to the one previously observed for aggregation of particles due to EHD flow [57, 58].

In the experiments it was observed that there is no particle collection for applied field frequency $< 10$ Hz or $> 5$ kHz, which is in accordance with the above concepts. Within the 10 Hz – 5 kHz range it was found that the rate of particle accumulation decreased as the frequency of the applied field was increased (See Fig. 3.4). As described above the ions in the double layer need a finite time to redistribute with the changing sign of the field. As the applied field frequency is increased, the time available for redistribution decreases which results into a decrease in the amount of the induced charge in the double layer [9, 56]. The
EHD flow at the surface hence will decrease as the frequency is increased and the particle collection will diminish.

For suspensions with high electrolyte concentration the large amount of ions present in the bulk solution will lead to a dense double layer that suppresses the applied field similarly to the case of low frequency. The entire potential will then drop within the thin double layer, the tangential electric field will not be able to drag excess ions outside the shear plane and no EHD flow will be generated. Correspondingly, it was observed that there is no collection of particles for suspensions with NaCl concentrations greater than 2 mM. For suspensions with NaCl concentrations less than 2 mM, it was found that the particles pack closely compared to the suspensions without the electrolyte. The closer packing of the particles (and stronger adhesion to the substrate) can be attributed to the decreased electrostatic repulsive forces between the particles.

The model based on EHD flow is conceptually similar to the earlier research on flows around and interactions between particles on electrodes. The new element in this work is the use of patterned surfaces leading to macroscopic circulation flows within the experimental chamber, rather than local flows around particles. The model presented here depends on only two parameters – the induced charge in the double layer and the presence of tangential field inside the double layer. The EHD-based collection does not depend on particle charge or size, the corral geometry or the electrode material and it was indeed observed experimentally that these parameters do not affect the particle collection. The good correlation between the expected electrohydrodynamic behavior and the experimental result (Table 3.1) confirms the model.

3.6. Potential Applications of the EHD Flow Technique

This new electrokinetic technique could have many potential applications in µTAS devices. One important application area is particle and cell collection and concentration from dilute suspensions. The EHD flow can also be used for pumping of fluids in microfluidic devices. The excellent correlation obtained between the EHD flow model and the experimental observation of particle collection can be utilized in predicting particle behavior on electrodes patterned with photoresist layers of various sizes and shapes. Two such applications of this new electrokinetic technique are demonstrated below.
3.6.1. EHD Focusing of Particles

The detection and analysis of particles or cells in μTAS devices generally require that the analytes of interest be concentrated or "focused" in a small area that is coincident with the foci of fluorescence illuminators and detectors. Such "particle focusing" using EHD flow was demonstrated by modifying the conductive patterns in the chamber. The eight square conductive corrals on the bottom electrode were replaced by one large 12 mm diameter circular corral. The chamber spacer dimensions were still maintained at $20 \times 13 \times 0.75$ mm$^3$.

Figure 3.9. EHD focusing of particles – 2 μm latex particles collected using 4 V/mm, 100 Hz alternating electric field for 75 minutes at the foci of the 12 mm diameter circular corral.
(L×W×H), cropping the pattern to the shape shown in Fig. 3.9. The particle collection process was similar to that for the smaller square conductive corrals; however, the pattern formed by collected particles was quite different. Initially the particles pushed into the corral from all sides and collected close to the periphery of the corral forming a concentric ring of concentrated particles. As time went by, the ring was pushed inward and the asymmetric distribution of particles along the two axis of the chamber resulted in a pattern of two concentrated regions – Fig. 3.9. Effectively, the result is that the two concentrated particle regions are formed at the foci of the curved arcs seen in the image. By bringing the two arcs closer to each other and decreasing the corral size, one can focus particles in one single concentrated region. On the other hand, if the arcs are so designed that there is an unbalanced fluid flow from different sides of the corral, chips that translate particles in one direction and result in a net pumping of liquid may be fabricated. EHD focusing and pumping has large advantages in µTAS devices as it has the possibility of generating fluid circulation within the chamber by applying electric fields of low strength.

Figure 3.10. Micrograph of yeast cells collected in a conductive corral from a suspension containing 0.0141% solids under an alternating electric field.

3.6.2. Collection of Live Cells and Microbes

Microfluidic chips for cell collection and manipulation are of large interest for bio-analytical applications [4, 5, 23]. For example, in water monitoring systems there is a need for chips that can concentrate microorganisms or cells of interest present in very dilute concentrations
The EHD flow generated in these experiments is not dependent on the physical properties of the particles and hence the technique can be easily adapted to pre-concentration and focusing of live cells from suspension. This was demonstrated by collecting yeast cells from a diluted phosphate buffer saline (PBS) solution using EHD flow. Optical image of yeast cell collection from dilute suspensions in one conductive corral under the application of 8 V/mm, 100 Hz electric field is shown in Fig. 3.10. The larger size of the yeast cells (5 µm compared to 2 µm for the particles) required a higher applied voltage. The surrounding solution contains approximately 1.37 mM NaCl, 0.027 mM KCl and 0.1 mM phosphate buffer and the presence of electrolyte led to the formation of quasi-packed array of yeast cells similar to the quasi-colloidal crystals obtained with latex particles. The advantages of collecting cells in a close packed area, where they can be easily examined and eventually discriminated by type, are obvious.

Preliminary experiments on Enterococcus faecalis and Escherichia coli, common microbes from river water samples were also successfully performed. These samples were obtained from Professor Wondwassen’s lab in the College of Veterinary Medicine at NC State. The microbes of initial concentration 10000 cells/mL were labeled with SyBr Green dye and successfully collected at the center of the square conductive corrals (Fig. 3.11). Furthermore, optical fluorescence reading from the center of the corral can be used to quantify the concentration of cells in the suspension.

Figure 3.11. Collection and concentration of microbes from river water samples. (a) A 1:1 mixture of Enterococcus faecalis and Escherichia coli stained by SyBr Green dye and collected at the center of the corral. (b) Same mixture, but only E. coli cells are dyed. The fluorescence can be used to quantify the concentration of cells in the suspension.
quantify the amount of cells or microbes present in the system. In Fig. 3.11a, both the *E. faecalis* and *E. coli* cells were labeled, whereas for the experiment in Fig. 3.11b only the *E. coli* cells are tagged with the fluorescence dye. The difference in the quantities is easily distinguished visually and can be quantified using a fluorescence meter.

Thus, it was proven that this electrokinetic technique is suitable for collection of cells and microbes from dilute suspensions and can be used in microfluidic chips for biosensors and bio-analytical purposes, among other possible applications.

### 3.7. Conclusions

The new electrokinetic technique reported here allows collection and concentration of colloidal particles suspended in a chamber using a combination of AC electrohydrodynamics and dielectrophoresis. This technique was found to be applicable to particles, cells and bacteria irrespective of their size, shape or charge as it is only dependent on the formation of the double layer at the electrode surface and not on the physical properties of the suspended particles. The EHD model and the FEMLAB electrostatic and hydrodynamic simulations were consistent with the experimental observations. The effect is strong, easy to control, and may be of importance in many microfluidic and µTAS systems involving AC fields. The EHD simulations can be used to understand and predict flows generated in corrals of various sizes and shapes and may be exploited in designs of patterned electrodes for microfluidic pumping. This technique has potential in various µTAS devices as it can easily be combined with various detection and analysis methods, especially if they are based on electric fields. Microfluidic chips that are capable of focusing latex particles and collecting yeast cells from buffer solutions were demonstrated.

### 3.8. Acknowledgements

This research was performed in collaboration with Dr. Sonia Grego (RTI International, Research Triangle Park, NC). Prof. Rachel Noble of UNC-Chapel Hill and Prof. Gebreyes Wondwossen of the College of Veterinary Medicine at NC State were kind enough to provide the river water samples containing *Enterococcus faecalis* and *Escherichia coli*. This study was supported by NER and CAREER grants from the National Science Foundation and by the Camille and Henry Dreyfus Foundation.
3.9. References


Chapter 4

Dielectrophoretic Manipulation of Freely Suspended Droplets*

4.1. Introduction

Micro-Total Analysis Systems (µTAS) systems often require handling and manipulation of small volume of colloidal suspensions containing solid particles, live cells, viruses and proteins [1]. Traditional microfluidic systems with prefabricated channels are poorly suited for flexible and reconfigurable handling of such samples and can be severely hampered by problems such as channel clogging due to particle and cell adhesion [2]. Proteins and lipids adsorb strongly onto many surfaces, while bulky DNA molecules may be delayed for entropic reasons [3]. Living cells may adhere to channel walls and can be disrupted by valves or pressure build-ups.

One alternative route to “channel-free” µTAS is transportaion of the liquid as droplets. Various techniques for manipulation of small droplets on solid surfaces have been proposed and demonstrated [2, 4-8]. Popular among these are the device prototypes operating on electrowetting. The applied electric field in these chips changes the contact angle of the liquid adjacent to a dielectric layer and moves the droplets by a combination of capillary effects and dielectrophoresis [9-21]. However, any device where the droplets are in contact with solid walls may encounter problems due to adsorption and fouling similar to those in conventional chips with microchannels. The surface fouling may increase the contact angle hysteresis of droplets on solid surfaces, which either increases the power dissipation and leads to heating or causes immobilization of the droplets.

These problems can be avoided by manipulating liquid droplets suspended in or floating on another immiscible liquid. A number of studies have focussed on droplet transport within microchannel devices. Droplets with diameters much smaller than the channel orifice can be formed by “flow-focusing” in microchannels [22]. Specially designed channels can further break up the droplets into smaller sizes [23]. Droplets flowing within channels can be diverted along different tracks by application of DEP [24]. Moreover, multiple liquid streams can be combined into single droplet whose contents are mixed rapidly without dispersion into the surrounding liquid [25]. The EHD forces acting on droplets formed using these techniques have been characterized by Zheng et al. [26]. Such systems, however, still operate within channels and thus loose some of the flexibility required for µTAS applications.
This chapter presents the development of a novel liquid-liquid microfluidic system for manipulation of freely suspended micro- and nanoliter droplets using dielectrophoresis. This allows for flexible microfluidic transport without contact with any solid surface, avoiding many of the problems with present lab-on-a-chip devices.

4.2. Experimental Setup

The schematic of the liquid-liquid microfluidic system developed is presented in Fig. 4.1a. Droplets, which may be from water or hydrocarbons, were suspended on the surface of a dense fluorinated oil (F-oil). The droplets were captured and manipulated by spatially inhomogeneous electric fields applied by means of an electrode array present underneath the F-oil.

![Schematics of the liquid-liquid microfluidic chip. (a) Microdroplets suspended at the surface of fluorinated oil are manipulated by timed switching of the electrodes underneath the F-oil. (b) Example of the printed circuit board design. The red and green leads are on the top and bottom respectively and are connected through the holes.](image)

The electrodes and the electrical leads for the chips were fabricated on two-sided printed circuit boards with electrode patches on one side and the connecting leads on the other. Most of the chips designed had a 1.5 mm pitch between electrodes. The electrodes were either circular or square in shape. An example of a circuit board design used for mixing two droplets is shown in Fig. 4.1b. The electrode boards were immersed inside small Petri dishes with FC-70 (F-oil), an inert, dense benign liquid with low dielectric permittivity. Droplets of volume 500-1000 nL were then dropped onto the F-oil surface with an ultra micropipette. The distance between the bottom of the droplets and the electrodes was sustained between 0.5 and 1.5 mm by adjusting the depth of the F-oil.
The water droplets typically contained suspensions of micro- and nanoparticles. The droplets were driven with AC or DC voltages in the range of 200-600 V. The AC frequencies were in the 50-5000 Hz range. The transition between AC to DC signal could be made gradually by varying the symmetry ratio of the AC waves, from full negative, to symmetric AC, to full positive voltage. All electrodes that were not switched to the high voltage source were grounded.

For making “supraparticles” the droplets were composed of mixtures of micro- and nanoparticles (usually, polystyrene and silica microspheres and gold nanoparticles), along with polypyrrole, 1,6-hexanediol diacrylate (HDDA) monomer and/or SU-8 25 photoresist (Microchem, MA). Supraparticles were formed due to on-chip droplet evaporation in the presence of Drierite desiccant. The polymer supraparticles formed from HDDA and SU-8 25 were photopolymerized on the chip by exposure to 365-nm UV radiation for 30-40 min with a B-100A UV lamp (Blak-Ray, CA). HDDA was polymerized in the presence of an initiator comprised of 3% Igracure 184, 0.3% Igracure 819 and 1% divinylbenzene.

4.3. Results and Discussion

When aqueous droplets were suspended on the denser F-oil, they nearly submerged in the F-oil leaving only a small cap exposed to air. When an AC signal was then applied to a single electrode within the array, floating droplets positioned themselves on top of the energized electrodes. After the droplets were captured on the energized electrodes, sequentially switching on/off single electrodes in the track, lead to the droplets following the switching direction, always staying on top of an energized electrode as illustrated by the cartoon in Fig. 4.1a.

The application of the spatially inhomogeneous AC field to the floating droplet suspended atop the electrode array leads to a dielectrophoretic force on the microdroplets. The microdroplets behave as large particles suspended in a media of low dielectric permittivity and the sign and magnitude of the DEP force depends on the effective polarizability of the droplet in this media. As the dielectric permittivity of water droplets ($\varepsilon = 80$) was higher than that of the F-oil ($\varepsilon = 2$), they were always attracted along the field gradient to regions of high field intensities by positive DEP. To determine the droplet response to applied AC fields, a full-scale simulation of the electric field distribution for the
floating droplet geometry was performed in FEMLAB. Electric field intensity distribution for two configurations of the energized electrode on the chip is shown in Fig. 4.2a. Optical microscopy images of floating 750 nL droplets containing suspended latex microspheres (top, pink) and gold nanoparticles (bottom, red) are shown in Fig. 4.2b.

![Figure 4.2. On-chip manipulation of floating microdroplets using dielectrophoresis. (a) Simulation of the electric field intensity distribution in the vertical plane at two distinct droplet equilibrium positions. For electrodes energized at the end of the array, the droplet is attracted towards the middle of the gap between energized and grounded electrodes; otherwise the droplet positions itself on top of the electrode. The color scale to the right is in the order of increasing electric field intensity. (b) Optical image of 750 nL droplets containing polymer microspheres (top) and gold nanoparticles (bottom) suspended above two tracks of electrodes. The experimental observed positions correspond to the theoretical predicted ones.](image)

Different equilibrium positions of the floating droplets were identified depending on the pattern of energized electrodes below them. If the first electrode at the end of the array was energized, while the second and third electrodes are grounded, or alternatively, if the electrodes were energized and grounded in pairs, the floating droplet positioned itself above the centre of the gap between the energized and grounded electrode(s). This placed the droplet above the area of highest field intensity where the DEP force was strongest. This situation is illustrated by the left droplet in the simulation and on the real chip shown in Fig. 4.2. However, if only a single electrode was energized in an electrode array where two
neighboring electrodes were grounded, the droplet positioned itself directly above the energized electrode (see the droplet to the right in Fig. 4.2b). The reasons for this positioning are illustrated by the electrostatic simulation for the droplet to the right in Fig. 4.2a. Two areas of high field intensity were created in this electrode configuration in the gaps to the left and to the right of the energized electrode. The droplet minimized the DEP energy by symmetrically balancing the attractive forces towards the two gaps by hovering above the electrode between them. As long as the field was present, the droplets were firmly held in place and resisted attempts to displace them by air or liquid currents. When the electrodes in the array were turned on/off sequentially, the captured droplet moved along the path of the energized electrodes in either configuration.

The mobility of microdroplets on a variety of fluidic chips, including the linear tracks shown in Fig. 4.1, circular "racetracks", and matrixes, was studied in order to understand and optimize the electrostatic forces operating in the chips. The effect of the basic system parameters on the droplet mobility and the results are summarized in Table 4.1.

*Table 4.1.* Effect of experimental parameters on the responsiveness and mobility of suspended microdroplets. Increases (↑), decreases (↓) or no effect (↔)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Range studied</th>
<th>Effect on droplet responsiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC amplitude</td>
<td>0 – 700 V</td>
<td>↑; proportional to $E^2$</td>
</tr>
<tr>
<td>AC frequency</td>
<td>20 – 5000 Hz</td>
<td>↔</td>
</tr>
<tr>
<td>DC bias</td>
<td>0 – 500 V</td>
<td>Attraction or repulsion; moves both water and oil droplets; very strong but erratic</td>
</tr>
<tr>
<td>Droplet volume</td>
<td>500 – 1500 nL</td>
<td>↑</td>
</tr>
<tr>
<td>Distance between droplet bottom and chip surface</td>
<td>0.5 – 1.5 mm</td>
<td>↓</td>
</tr>
<tr>
<td>Electrolyte in water droplets</td>
<td>None – 100 mM</td>
<td>↑ (small)</td>
</tr>
<tr>
<td>Fluorinated or non-fluorinated surfactant added to the droplet</td>
<td>0 – 0.01 wt%</td>
<td>↔</td>
</tr>
<tr>
<td>Electrode patch geometry</td>
<td>Square or Circular</td>
<td>Square at shorter droplet-electrode distance; circular at larger</td>
</tr>
</tbody>
</table>

The most reliable manipulation of water droplets was achieved by symmetric AC fields. The maximal speed at which the droplets moved in the AC mode was approximately
proportional to $E^2$ as depicted by the plot in Fig. 4.3. To record the maximal speed of the droplets, the switching on/off of electrodes was automated by using computer controlled relays. The velocities in Fig. 4.3 were observed for 750 nL water droplets suspended over a 1.15 mm F-oil layer and manipulated at a constant frequency of 200 Hz. Changing the frequency did not affect the droplet behavior as the polarizability of the droplets was weakly dependent on the frequency in the range studied.

**Figure 4.3.** Droplet speed plotted as a function of the field intensity squared. The data are for 750 nL aqueous droplets submersed in a 1.15 mm deep F-oil layer. The speed was measured by the smallest time required for the droplet to traverse an automated 8-electrode sequence forwards and backwards. Frequency was 200 Hz.

The droplets could also be moved by applying constant electrical voltages. Water droplets in constant fields were expected to behave similarly to those in AC fields of the same amplitude, as the polarization effects with static fields were similar to the ones at low AC frequencies. The droplet response to DC fields, however, was found to be very different, pointing out a variety of unexpected charge and polarization effects. Typically the droplets responded very strongly to DC fields by either moving rapidly away from the energized electrode, or by being strongly attracted towards it. The velocity of droplet motion and the range of the interactions were about two times larger than the AC-driven effects at the same voltage range (speeds as high as 2.0 mm/s were measured for 750 nL droplets driven with a
negative voltage of 500 V). This pointed out that the droplets possessed significant charge and/or dipole moments and thus responded by Columbic repulsion or attraction. The sign of this charge for droplets made from different suspensions varied from positive to negative; though we were not able to correlate the contents of the droplets to their apparent charge. Furthermore, charging and/or re-charging effects were observed at combined AC+DC voltages. Usually droplets that were initially repelled from the energized electrodes, drifted back within a few seconds, which was likely connected with the decrease of the droplet charge or dipole moment. Because of these re-charging effects, the use of DC fields was found to be more difficult to control.

The use of DC field also allowed manipulation of hydrocarbon oil droplets. These droplets did not respond to symmetric AC fields in accordance with the expected lack of polarizability (as their dielectric permittivity was close to that of the F-oil). Oil droplets however readily responded to constant fields in a manner similar to the water droplets. Charging and discharging effects for oil droplets were routinely observed. Thus, the mobility of both water and oil droplet in DC fields, depended on strong and complex polarization and charging effects. The droplets may become charged by collecting static charges from the F-oil interface or from charge transfer through the oil phase.

The power dissipation in the experiments was extremely low as the currents through the cell were smaller than the capacitance leaks in the circuit. The energy required to transport freely suspended droplets was estimated to be more than two orders of magnitude lower than that required to move the same volume of liquid as drops on solid surfaces or in microfluidic channels (Table 4.2.).

**Table 4.2.** Estimated energy required for moving a 500 nL water droplet, 1 cm at 2 mm/s by different methods.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Droplet moved in F-oil</th>
<th>Hemispherical droplet dragged on solid surface</th>
<th>Viscous flow in microfluidic channel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assumptions and approximations</td>
<td>✓ Stokes sphere in bulk liquid</td>
<td>✓ ( \theta_{\text{Advancing}} = 90 \text{ deg} ) ✓ ( \theta_{\text{Receding}} = 80 \text{ deg} ) ✓ No viscous dissipation</td>
<td>✓ Circular channel of diameter 20 µm ✓ Poiseuille flow</td>
</tr>
<tr>
<td>Type of estimation</td>
<td>Overestimate</td>
<td>Underestimate</td>
<td>Underestimate</td>
</tr>
<tr>
<td>Energy required (J)</td>
<td>( \leq 9.4 \times 10^{-10} )</td>
<td>( \geq 1.6 \times 10^{-7} )</td>
<td>( \geq 1.4 \times 10^{-4} )</td>
</tr>
<tr>
<td>Energy ratio</td>
<td>1</td>
<td>170</td>
<td>150000</td>
</tr>
</tbody>
</table>
4.4. Applications of the Liquid-Liquid Microfluidic System

The liquid-liquid chips described here could become a versatile experimental tool and an easy-to-implement technological platform for microdroplet transport, mixing, and chemical and materials synthesis. This potential was demonstrated in a variety of experiments that are described below.

4.4.1. Controlled Parallel Transport of Many Droplets

Multiple droplets can readily be transported and manipulated on chips with a large number of addressable electrodes. Snapshots of four droplets moved simultaneously by the electric field are shown in Fig. 4.4.

![Figure 4.4](image)

**Figure 4.4.** Parallel transport of multiple droplets. Four 750 nL water droplets containing, from top to bottom, gold nanoparticles, 2% white polystyrene, 2% pink polystyrene and 0.2% white polystyrene microspheres, were moved in parallel by switching on single column of electrodes within the array to an applied AC signal of 300 V/300 Hz. The droplets covered approximately 15 mm distance in 14 seconds.

Four 750 nL aqueous droplets containing gold nanoparticles, 2% white polystyrene latex, 2% pink polystyrene latex, and 0.2% white polystyrene latex were suspended on four separate tracks on the electrode array. Initially only one column of the electrode array was energized by applying an AC signal of 300 V at 300 Hz. The droplets positioned themselves on this energized electrode as shown by the image for time $t = 0$. For transporting the
droplets to the next column, the electrodes in the right column where switched on and at the same time the original column of electrodes was grounded. With the DEP force pulling the droplets now towards the right column, they reposition themselves on the right column in matter of seconds. This process of switching on and off columns of electrodes can be continued and the droplets follow the direction of field switching. The number of manipulated droplets is limited only by the size and complexity of the chip, thus, providing a technique to manipulate multiple droplets in parallel.

The droplets could also be directed along a desired path when the track branches by adding extra electrodes as switches near the path. Fig. 4.5 shows snapshots of this experiment. A water droplet containing latex microspheres is transported along the track to the point where the track branches. If now the extra electrode present near the desired branch is switched on (the top electrode at t = 6 seconds in Fig. 4.5), the droplet can be directed towards the top branch where it will continue following the electrode path. This ability to switch tracks could be used for separation of droplets based on their contents.

Finally, chips with two-dimensional matrixes of individually addressable electrodes permitted independent holding of droplets, with motion in arbitrary direction and mixing of droplets of various compositions (images not shown here).

Figure 4.5. Controlled transport of droplets. Water droplet containing latex microspheres is diverted along the top branch at the fork using an extra electrode close to both the fork and the top branch.
4.4.2. Mixing of Droplets and Encapsulation in Oil

Droplets from two separate transporters were coalesced in a 1:1 ratio at the track junctions and then the combined droplet was moved further along the tracks (Fig. 4.6, \( t = 0-10 \) seconds). This lead to a complete mixing within the droplets and the contents in the combined droplet homogenized within seconds. At many instances, as the droplets neared each other at the track junctions, they were also pulled by capillary forces and coalesced before reaching the junction aiding the mixing process.

Furthermore, the versatility of the method allowed developing encapsulation techniques. Water and hydrocarbon droplets on the chips can also be combined at track junctions. When a hydrocarbon-based surfactant such as sodium dodecyl sulfate was added to the water droplets, the balance of the interfacial tensions favors the complete engulfment of the water droplet inside the hydrocarbon one. Thus the combined droplet formed has the water phase symmetrically encapsulated inside a liquid hydrocarbon shell (Fig. 4.6, \( t = 17 \) and 21 seconds). These liquid-liquid capsules could be moved by both DC and AC fields; the whole capsule responds to DC fields, while only the internal water droplet responds to AC
voltages. This encapsulation process can be used for protection of the water droplets from evaporation, for long-term storage on the chip, and for the fabrication of capsules, core-shell composite particles and complex assemblies.

4.4.3. Chemical Reactions and Precipitations
The DEP droplet chips allow carrying out chemical reactions and precipitation on the microscale. A variety of mixing and precipitation experiments on track and matrix chips were performed by bringing pairs of droplets in contact. Precipitation reactions included the formation of Ca$_3$(PO$_4$)$_2$, CaCO$_3$ and Fe(OH)$_2$. Fig. 4.7 shows results of two such reactions being performed.

![Chemical reactions and precipitation](image)

**Figure 4.7.** Chemical reactions and precipitation. Two separate precipitation reactions are performed by synchronous movement of two pairs of droplets. On the top track solutions of CaCl$_2$ and K$_2$HPO$_4$ are combined to form the white precipitate, Ca$_3$(PO$_4$)$_2$. On the lower track drops of FeSO$_4$ and NaOH are mixed to form the green precipitate, Fe(OH)$_2$. The images at t = 600 seconds and t = 1200 seconds show the growth of the crystalline solids with time. All droplets are 750 nL in volume and driven by voltages of 400 V/200 Hz.

On the top track droplets containing CaCl$_2$ and K$_2$HPO$_4$ were combined to precipitate Ca$_3$(PO$_4$)$_2$, whereas on the bottom track Fe(OH)$_2$ was precipitated by merging droplets containing FeSO$_4$ and NaOH (t = 0 to 23 seconds). The complex precipitation patterns inside the mixed droplets lead to the formation of crystal shell-like balls (t = 600 and 1200 seconds). The particles formed could be further moved intact along the electrodes, however,
their response to symmetric AC fields decreases as the water present within them dries out. Such shell-like crystalline particles might be used as biomimetic capsules and are of interest of their own in materials science. Combinatorial reactions can also be performed by mixing large numbers of nanoliter droplets of different chemical compositions in computer-controlled sequences and analyzing the results on a droplet-by-droplet basis.

4.4.4. Anisotropic Particle Synthesis using Suspended Droplets

Microdroplets suspended in organic and fluorocarbon oils have vast potential as compartments for the synthesis of new classes of structured particles. Shell-like ‘colloidosomes’ can be assembled by adsorption and binding of particles around droplets [27-32]. Alternatively, when droplets containing suspended microparticles and nanoparticles are dried, the particles confined inside them are compressed and assembled into ball-like ‘supraparticles’ [33-37]. The liquid-liquid chip system presented here can be used for synthesis of new types of supraparticles. Droplets of liquid monomers and the resultant solid supraparticles resulting from the synthesis were found to respond sluggishly in AC fields but could still be reliably trapped and manipulated. This was expected because of the lower difference in polarizabilities between the particles and F-oil, similar for the case with hydrocarbon droplets noted earlier. Better response was achieved by changing the symmetry of the applied signal to a maximum 95% positive or negative, wherein an additional electrostatic component of the force was present.

The ability to hold the individual droplets in place and observe them continuously with a microscope from above opens a region of possibilities, both in terms of designing new particles and in characterizing the assembly process. An example of the evolution of on-chip formation of a novel type of anisotropic “eyeball” supraparticle is presented in Fig. 4.8. The process of creating this type of particle began with depositing droplets on the F-oil, which contained a binary suspension of 0.21 wt% 12 nm gold nanoparticles and 20 wt% 0.65 µm polystyrene latex microspheres.

As the droplets began to dry by water evaporation from the top section exposed to air, a series of remarkable phenomena were observed. The internal hydrodynamic flux compensating for the evaporation brought the particles to the top surface of the droplets. The gold nanoparticles (red) were the first to become visibly concentrated on the top of the
droplet, where evaporation of water was taking place (Fig. 4.8a). As evaporation progressed, a phase of concentrated latex microspheres was also formed in the top section of the droplets. The latex particles organized in a colloidal crystal, which sparkled in a range of bright colors owing to the diffraction of light in the ordered array [33] (Fig. 4.8b).

Figure 4.8. Anisotropic “eyeball” supraparticle assembly by the evaporation of droplets from binary suspensions. Images show 1000 nL droplet containing suspended gold nanoparticles and polystyrene microspheres above the energized electrode, (a) 1 min after injection on F-oil, (b) 7 min of drying, (c) 18 min of drying, (d) 3 hours of drying. (e) Dried eyeball assembly. Inset shows the SEM of colloidal crystal formed by latex microspheres. (f) Array of eyeball particles entrapped on the chip. Scale bars: (a-d and f) 1 mm, (e) 500 µm, Inset 5 µm.

After several minutes of particle accumulation in the top droplet sections, a clear particle separation in two regions was observed: a dark cap of concentrated gold nanoparticles on the top of the droplets and a larger body of white iridescent microsphere crystal below it (Fig. 4.8c). The water in the droplet was completely dried after three hours, and the resulting products were solid eyeball particles made of iridescent latex crystals with a dark metallic spot on one side (Fig. 4.8d and e). Arrays of eyeball particles could be assembled on the chip (Fig. 4.8f), deposited on its surface by removing the F-oil, and extracted.

More complex ‘striped’ particles were created with this device from ternary mixtures of gold, fluorescent latex and silica particles (Fig. 4.9). First, droplets containing a mixture of 0.040 wt% 12 nm gold nanoparticles, 15 wt% 1 µm red fluorescent latex microspheres and
2.8 wt% 0.97 μm silica microparticles were added and the water was allowed to dry overnight. During the drying process the gold nanoparticles segregated on top of the balls owing to liquid evaporation (similarly to the eyeballs), and the (white) dense silica microspheres sedimented on the bottom, leaving a middle region of red fluorescent latex (Fig. 4.9a). When observed under ultraviolet (UV) illumination, the latex stripe in the middle of the particles fluoresced whereas the gold and silica portions were dark (Fig. 4.9b).

Another type of ternary particles was created by drying droplets of 0.013 wt% 12 nm gold nanoparticles, 1.67 wt% 190 nm yellow fluorescent latex microspheres and 0.92 wt% 0.97 μm silica particles floating on the surface of the fluorinated oil. Particle collection and phase separation within the droplets again occurred similarly to the red fluorescent latex assembly, except that in this case the gold nanoparticles did not separate on top but condensed in the middle of the droplets between the latex and the silica (Fig. 4.9c). The large silica spheres allowed unperturbed nanoparticle transport towards the top. However, the pores between the adjacent spheres in the crystal from 190 nm latex particles were too small.

Figure 4.9. Formation of “striped” multilayer particles by the evaporation of droplets from ternary particle mixtures. (a) Segregated multilayer assemblies of dried gold nanoparticles (top layer), fluorescent red latex beads (middle) and silica microspheres (bottom), (b) Complementary image in fluorescence illumination. (c) Segregated multilayer assembly of dried yellow latex beads (top), gold nanoparticles (middle), and silica microspheres, (d) Complementary image in fluorescence illumination.
to allow transport of the gold nanoparticles and the latter were accumulated in the middle brown gold stripe. Visualizing these particles in fluorescence mode showed the latex on top fluorescing green with a very dark band where the gold was concentrated and a less dark area for the silica phase (Fig. 4.9d). These striped multilayer particles illustrate the power of the method to make new particles with unique structure.

The chips also readily allowed the making of solid polymer spheres with special optical or electrical properties. Such particles were made by two different solidification mechanisms: photopolymerization of liquid monomer and evaporation of water from polymer solution. Droplets of the photopolymer SU-8 25 laced with 0.8 wt% 12 nm gold nanoparticles were polymerized with UV radiation while floating on top of the fluorinated oil layer. The resulting particles were solid droplets of insulating epoxy polymer with a uniform distribution of conductive gold particles through the interior (Fig. 4.10a). The larger epoxy particles reproduced the biconcave shape of the original floating droplet. To make conductive particles, droplets of 5 wt% polypyrrole in water were positioned on the electrode array and the evaporation of the water lead to the formation of black polymer spheres with metal-like appearance (Fig. 4.10b). These semiconductive particles had a resistance on the order of 100 kΩ when measured with a two-point probe.

**Figure 4.10.** Polymer-based and polymer-encapsulated particles: (a) Polymerized SU-8 droplets laced with uniformly dispersed gold nanoparticles, (b) Solid semiconductive polypyrrole spheres, (c) Dried “eyeball” supraparticle encapsulated in polymerized HDDA and (d) “Cups” from polymerized HDDA with polystyrene latex deposited inside by drying of the originally encapsulated water droplets. Scale bars: 1 mm.
Finally, core-shell particles were synthesized by encapsulation of dried supraparticles or droplets of aqueous suspension inside polymer shells (Fig. 4.10c and d). The droplets of liquid monomer were deposited on the chip near the target droplets or particles. The monomer droplets were attracted by the active electrode holding the target droplets. On contact, the monomer droplet encapsulated the core droplet or particle and formed one single larger particle. The liquid monomer shell was subsequently solidified by photopolymerization. The first type of particle created by this method was the anisotropic dried eyeballs encapsulated with a liquid monomer, 1,6-hexanediol diacrylate (HDDA). Dispersions for these particles were dried on the device for 2 hours and then encapsulated with a droplet of HDDA. After a wait of 30 min to allow for uniform encapsulation, the photopolymer layer of this composite microstructure was polymerized with UV radiation, resulting in eyeball particles with a solid polymer shell (Fig. 4.10c). The second type of encapsulated particle was made with droplets of 40 wt% 0.72 µm latex that were encapsulated with droplets of HDDA before being dried in a 1:1 ratio by volume. After 30 min of waiting, the HDDA shells were polymerized. The resulting particles were subsequently removed from the oil and dried overnight. Because the capsule had a small opening on the bottom of the original droplets, the water phase inside dried, depositing a layer of dried latex on the inside of the acrylic shell. The final capsules were solid polymer ‘cups’ partly filled with dried latex particles (Fig. 4.10d).

The technique can be scaled up as a continuous process taking place in chip ‘micro-factories’ for particle synthesis, where droplets are injected on one side of the chip and the particles formed are collected on the other side. There are no obstacles in principle to scaling down the electrode patterns by photolithography to allow the manipulation of nanoliter droplets and making micron sized particles. The size of the droplets can be reduced by automatic ejection techniques similar to ink-jet printing. On-chip synthesis processes are not likely to be advantageous for simple mass-produced particulates. They can, however, find niches of applications requiring particles with special structure and properties. Particles from conductive and semiconductive polymers could find application as self-assembling components for photonics and electronics. Eyeball and striped particles could find use in electronic paper and coatings with changeable properties. They could also be used as ‘bar-coded’ tags in biological and environmental research, and in advanced drug delivery and
targeted therapeutics (by combining particles that bind to specific tissues with particles providing local therapeutic effect). Creating particles with magnetic stripes of ‘eyes’ by this method is straightforward; such particles can be used in cell sorting. The closed and open capsules have potential in drug delivery, by using biocompatible and biodegradable polymers in the shells and by replacing the inner particle core with biologically active components.

4.5. Conclusions
Controlled transport and manipulation of free microdroplets containing particles and/or chemical reagents has been demonstrated in this chapter. The technique is simple, robust, easy to implement in a technological platform, and to scale down in droplet size. It allows the synthesis of single particles by aggregation or precipitation in the confines of droplet microreactors, fabrication of liquid-liquid encapsulated droplets, and syntheses of novel types of anisotropic supraparticles and their subsequent manipulation. The formation of eyeball and striped particles of a complexity that has not been achieved before in self-assembled structures, semiconductive and semitransparent polymer particles and closed and open capsules has been achieved. These chips can be particularly well-suited for confining single living cells or genetic material into individual droplet containers, and performing biochemical reactions, precipitation assays, high throughput drug or toxin screening, or other biotechnological processes on the microscale. However, the full potential of similar techniques for electric field controlled on-chip materials synthesis is yet to be realized.

4.6. Acknowledgements
This research was performed in collaboration with Brian Prevo and Jefferey Millman, fellow members of the Velev research group. David Woolard is thanked for assistance with some of the experiments performed. This study was supported by the National Science Foundation, USA grants CTS-0238636 and CTS-0403462.

4.7. References


Chapter 5

Summary & Outlook
5.1. Summary
The ability to manipulate and assemble particles, cells and liquids is of utmost importance in the field of microfluidics and micro-Total Analysis Systems (µTAS). The first goal of my dissertation was to explore the use of alternating (AC) electric fields as a possible tool for particle manipulation and assembly for µTAS applications. The second objective was to develop on-chip devices that use AC fields for particle assembly, collection and transport. Different on-chip electrode geometries and different particle suspensions were investigated. Emphasis at all stages was to understand the fundamentals about the particle interaction with the electric field and also the particle-particle field-induced interactions. At each stage detailed electrostatic calculations were carried out to identify the electric field induced forces and the resultant particle behavior. The resultant understanding developed helped in designing chips for the controlled assembly of gold nanoparticles into microwires, for particle collection and concentration from dilute suspensions and for droplet transport in microfluidic systems.

The first project that was carried out demonstrated assembly of metallic nanoparticles into microwires between planar electrodes using dielectrophoresis. Long, porous, cylindrical or half-cylindrical structures of micrometer diameter were grown from suspensions of gold nanoparticles with speeds as high as 2 mm/s. The microwires were assembled when the gold nanoparticles in the suspension were attracted to the high electric field intensity regions (at the electrodes and the growing wire tips) and aggregated. Using electrostatic calculations it was demonstrated, as described both in Chapters 1 and 2, how the field intensity distribution within the chamber gets modified due to the presence of conductive particle chains formed by dielectrophoresis and how the electrodes are extended into the chamber because of these chains. The result of this extension of electrodes into the solution by the accumulated gold nanoparticles was that the microwires grew until the electrodes were short circuited. The microwire formation was irreversible and the assembled wires remained in the chamber after removal of the applied electric field. The rate of microwire growth was found to be controlled by diffusion-limited aggregation.

Two distinct assembly modes for gold nanoparticles in aqueous suspensions were observed in this work. In the bulk assembly mode, the nanoparticles formed cylindrical microwires through the bulk of the suspension. In surface assembly mode the microwires
were assembled as half-cylinders on the surface of the glass slide. The assembly mode of the microwires and their branching and structure was controlled by the operating field parameters. Single bulk microwires were formed at low frequency in 1:1 glycerol:water suspensions, whereas parallel surface microwire arrays were formed at low field intensity and high frequencies from aqueous suspensions. The assembly process was simulated well by a model of electric-field driven dielectrophoretic assembly that was developed. The electrostatic model described realistically the dynamic process and the resulting pattern of microwire assembly, including the periodic branching and self-centering on conductive objects in the media.

These microwires had the ability to bridge any conductive objects present in between the electrodes and to form in situ electrical circuits. The ability of electric fields to assemble colloidal particles into microwires can be an important tool for nanotechnology applications such as bioelectronic interfacing of living cells to electrical circuits. The parallel surface microwires grown can be used in MEMS systems for one-directional heat conduction. Similar techniques have been recently developed for the assembly of gold nanoparticle and nanowires, carbon black particles and carbon nanotubes between micro electrode gaps.

The next stage involved modifying the simple planar electrode design to a system consisting of patterned vertical electrodes. This modification of the electrode design lead to the identification of a new electrohydrodynamics effect arising due to the interaction of the electric field with the ions in the electrical double layer at the pattern edges. Electric field was applied to latex particles suspended between a patterned silicon electrode and an Indium-Tin Oxide (ITO) coated glass slide separated vertically by a spacer. The bottom silicon electrode was fabricated by first depositing a thin photoresist layer and subsequently etching the photoresist to expose square corrals of the underlying silicon wafer. When an AC field was applied to the top and bottom electrodes, a fluid flow at the photoresist-silicon wafer edge emerged, resulting in the collection of the suspended particles in a neat "pile" in the center of the conductive corrals.

The mechanism behind this particle collection process was found to be field-induced EHD flow. The voltage applied to the silicon wafer beneath the dielectric layer created an opposite charged double layer on the photoresist-electrolyte interface by means of the photoresist layer acting as a capacitor. A tangential electric field was present on the
photoresist surface near the corrals and reached its maximum at the film-patch edge. This gradient generated the EHD flow. For different half-cycles of the electric field, the sign of the induced charge and the sign of the electric field changed concurrently, and thus the EHD flow was always in the same direction leading to particle transport towards the center of the corrals. This strong EHD flow, in some instances > 50 µm/s, lead to formation of electroosmotic flow roll cells throughout the chamber that dragged the particles in the chamber and deposited them at the stagnation regions in the center of the conductive corrals.

This technique is promising for various devices that collect and analyze particles on a chip. It was demonstrated that such devices could be used, for example, to collect yeast cells and microbes from dilute water suspensions. In more general plan, devices for particle collection could use a combination of DEP, AC electrokinetics and DC electroosmosis in new synergistic ways. Such chips are presently under development by many groups including ours.

Our research also showed how not only particles and fluid but also fluid droplets containing suspended particles could be manipulated by AC fields. A novel liquid-liquid microfluidic system for transport of droplets and particles was developed. Liquid droplets, which may be from water or hydrocarbons, were suspended on the surface of perfluorinated hydrocarbon oil (F-oil), an inert media with high density and low dielectric permittivity. The floating droplets were manipulated by AC fields originating at arrays of electrodes below the F-oil. The electrodes were either connected to the AC (“energized”) or grounded. The dielectrophoretically trapped droplets move along programmed paths when the voltages applied to the electrodes were switched on and off consecutively.

Full-scale simulation of the floating droplet geometry and electric field intensity distribution showed two possible equilibrium positions for the floating droplet. If the first electrode at the end of the array was energized, while the second and third electrodes were grounded, or alternatively, if the electrodes were energized and grounded in pairs, the floating droplet positioned itself above the centre of the gap between the energized and grounded electrode(s). This placed the droplet above the area of highest field intensity where the DEP force was strongest. However, if only a single electrode was energized in an electrode array where two neighboring electrodes were grounded, the droplet positioned itself directly above the energized electrode. Two areas of high field intensity were created in this
electrode configuration in the gaps to the left and to the right of the energized electrode. The droplet minimized the DEP energy by symmetrically balancing the attractive forces towards the two gaps by hovering above the electrode between them.

These droplets entrapped on chips are suitable for use as microreactors for chemical syntheses and can also be used for transport of the resultant precipitated solids. The droplets can be further encapsulated by dodecane oil caps to prevent evaporation. Alternatively, microparticle separation and liquid circulation can be effected within evaporating droplets by Marangoni effects at the liquid surfaces. Microseparation processes were used to form anisotropic supraparticles and to design immuno-agglutination microbioassays. This novel microfluidic platform may find applications in chips, where single living cells or genetic material are confined into individual droplet containers, and subsequent biochemical reactions, precipitation assays, high throughput drug or toxin screening, and other biotechnological processes on the microscale are carried out. The method also illustrates the potential of dielectrophoretic techniques to affect and manipulate not only particles, but droplets and potentially a variety of other heterogeneous systems on a chip.

5.2. Outlook

The investigation and development of techniques for particle manipulation and assembly based on electric fields was carried out during my graduate research. The use of dielectrophoresis and AC electrohydrodynamics for the assembly of gold nanoparticles into microwires, particle collection on patterned electrodes and droplet transport has been investigated. Other electric field induced forces and the possible on-chip electrode configurations are numerous and still largely unexplored. An interesting area of research could be the characterization of the dielectrophoretic response of particles of non-spherical shape, non-isotropic charge or surface composition, permanently embedded dipole moment or non-linear conductance. Experimental studies in this area are generally lacking, not in the least because few methods for the fabrication of such complex particles have been available. Recent advances in the area of making complex anisotropic particles, "supraparticles" and "colloidal molecules" are likely to make possible research on the dielectrophoretic and electrokinetic properties of such particles and reveal new effects and applications.
A large pool of potential applications of the results of my research is likely to emerge with the rapid progress in the area of microfluidic and bioassay devices. In addition to the established techniques for on-chip characterization and separation of cells, dielectrophoretic and electrokinetic methods are likely to find much larger use in the on-chip handling and separation of proteins and DNA. The electric field driven assembly of cells and nanoparticles can be used to create new types of biosensors, microbioassays and bioelectronic circuits. The AC electrokinetic effects are still incompletely understood on the microscopic (particle) scale and for the cases of more complex electrode and experimental cell designs. Many new lab-on-a-chip applications are likely to use AC electrokinetic techniques for liquid handling, as well as for particle collection and manipulation. Interesting new developments will probably come from the discovery of new AC dielectrophoretic and electrokinetic effects and from better theoretical interpretation of complex liquid mediated particle-particle interactions. The convergence of electrical microcircuits and soft colloidal materials is forthcoming and its future technology potential is high.