Fabrication Methods, Operation and Measurement of Electrokinetic Fluid Manipulation Devices

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ABSTRACT

Lab-on-a-chip BioMEMS devices have developed in recent years as a way to rapidly perform total biological analysis. With the complexity of fabrication of mechanical parts in microfluidic devices comes the desire to create devices which use alternative methods of pumping, mixing and manipulating fluids and suspended microparticles for reactions – such as electrokinetics. This paper presents novel ways of fabricating electrokinetic devices, which use the phenomenon of dielectrophoresis (DEP) for manipulation of suspended microparticles. Affects of driving waveforms are investigated. Visual results from microparticles are used to show the effect of operation of electrokinetic devices. Improved device designs and device operation are discussed.

Keywords: Dielectrophoresis, BioMEMS, Lab-on-a-chip, Microfabrication, Electrokinetics

1. INTRODUCTION

Much research has been done in the past 10-15 years in using dielectrophoresis (DEP) (Allsopp *et al* [14]) and travelling-wave dielectrophoresis (TWD) (Goater *et al* [13]), for the selective manipulation of suspended microparticles, such as animal and plant cells and nucleic acids, in lab-on-a-chip micro-total-analysis systems (μ TAS). A number of different electrode geometries have been conceived and used ([5], [10]-[12]) to selectively concentrate, fractionate or transport microparticles in a μ TAS.

In this paper, after covering theory in electrokinetics, methods of fabricating electrokinetics devices are introduced, as well as studying the effects of waveforms for driving TWD devices. Finally, the operation of simple TWD devices is illustrated.

2. THEORY

Electrokinetics covers electrophoresis, dielectrophoresis, electrorotation, electroosmosis, and electrowetting, and anything else that uses electric fields to induce particle motion.

2.1. Electrophoresis

Electrophoresis is simply the migration of charged colloidal particles suspended in a liquid, under the influence of an electric field [15].

Electrophoresis has been used for decades in biotechnology for separating cellular components of biological specimens [15].

The simple equation for the electric force acting on a charged particle in an electric field, is given by:

$$F_E = QE \quad (1)$$

Where Q is the charge of the particle, in Coulombs,
 E is the electric field strength, in N/C.

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Obviously, electrophoresis requires that the particles to be moved have a nonzero net charge.

2.2. Dielectrophoresis

The term Dielectrophoresis was first coined by Pohl in 1978 [7].

DEP occurs when an electric field interacts with an induced dipole in a particle. Dielectrophoresis differs from electrophoresis in that the force is the result of an interaction between an electric field and an induced dipole moment. Therefore dielectrophoresis can apply to charged or uncharged particles.

The dipole moment induced by an electric field E , is given by (X. B. Wang *et al* 1994 [6]):

$$m(\omega) = 4\pi\epsilon_m r^3 f_{CM}(\omega)E \quad (2)$$

where ϵ_m is the complex permittivity of the suspending medium
 r is the particle radius
 E is the electric field strength
 f_{CM} is the Clausius-Mossotti factor defined as:

$$f_{CM}(\epsilon_p^*, \epsilon_m^*) = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \quad (3)$$

Where ϵ_m is the complex permittivity of the suspending medium.
 ϵ_p is the complex permittivity of the particle.

The complex permittivity is given by:

$$\epsilon^*(\omega) = \epsilon - \frac{j\sigma}{\omega} \quad (4)$$

Where ϵ is the normal permittivity
 σ is the conductivity
 ω is the angular frequency (rad/s)
 j is $\sqrt{-1}$

The force acting on a charge-neutral suspended particle, as a result of the particle's dipole moment can be given by (M. P. Hughes, 1999 [7]):

$$F(\omega) = 2\pi\epsilon_m r^3 \text{Re}[f_{CM}(\omega)]\nabla E(rms)^2 \quad (5)$$

Equation 5 shows that the force acting upon the suspended particle is related to the real part of the Clausius-Mossotti factor, $\text{Re}[f_{CM}(\omega)]$, as well as the gradient of the electric field strength.

It is easily possible to create electrode geometries which produce a non-uniform electric field (i.e. Fields where the electric field strength has a gradient). If a fluid with suspended particles is placed in such a structure, the fluid will experience dielectrophoresis.

Note that the direction of the force depends on whether the particle has a higher or lower permittivity than the medium, as governed by the equation for the Clausius-Mossotti factor (Equation 3).

Figure 1 - Non-uniform electric fields, as required for dielectrophoresis.

If the particles are more polarisable than the suspending medium, they will be attracted to the places of higher electric field strength. This is called positive dielectrophoresis.

If the suspending medium is more polarisable than the particles, the particles will be attracted to places of lower electric field strength. This is called negative dielectrophoresis.

We also note that the force is independent of the electric field direction, and is only dependent upon the electric field gradient. Therefore an AC or DC field can be used in dielectrophoresis.

2.3. Travelling-Wave Dielectrophoresis

In travelling-wave dielectrophoresis, interdigitated electrodes create a longitudinal electric field wave, which induces an electric dipole in a particle, which lags behind the field in magnitude. The interaction between the lagging dipole moment and the field causes a linear force.

The force on a particle caused by TWD is given by (Huang *et al*, 1992):

$$F_{TWD} = \frac{-4\pi\epsilon_m r^3 \text{Im}[f_{CM}(\omega)] E^2}{\lambda} \quad (6)$$

Where ϵ_m is the permittivity of the suspending medium

r is the particle radius

f_{CM} is the Clausius-Mossotti factor

E is the electric field strength

λ is the wavelength of the travelling wave (e.g. 4 times the electrode spacing)

Figure 2 - Travelling Wave Dielectrophoresis. Linear motion of the particle is induced by the travelling-wave.

Note that this is similar to equation 5 for dielectrophoresis, except that it depends on the electric field strength, not the gradient of the electric field strength, and the imaginary part of the Clausius-Mossotti factor, not the real part.

It has been theoretically shown that the maximum value of the magnitude of the imaginary part of the Clausius-Mossotti factor occurs at an applied frequency of the order of 1MHz [7] (but can be as low as 100kHz or as high as 10MHz), for many biological microparticle and fluid combinations.

3. TWD DEVICE DESIGNS

TWD devices consist of electrode structures, which aim to create a longitudinal travelling electric field wave. Most TWD devices appearing in the literature have one thing in common – they all use 4 sets of interdigitated electrodes which are energised by sine waves which are 90° phase-shifted.

The two main electrode configurations are linear (flow) or radial (concentrator) types. Linear TWD devices aim to simply accelerate suspended particles in one direction, or the opposite direction, perpendicular to the electrodes. An example of a linear TWD design is shown in Figure 4.

Radial TWD devices aim to concentrate suspended particles to a point on the structure. An example of a radial TWD concentrator is shown in Figure 3. Again, particles are accelerated perpendicular to the electrodes, but this time inwards to the centre of the device, or outwards, to the circumference of the device.

3.1. Mask Design

In this paper, the TWD device fabricated was a spiral (concentrator) design, similar to what has been previously published [9]. This type of design is easier to fabricate than a linear design, as no vias are required. Vias are interconnections between layers on a multi-layer structure. The geometry of the spiral, with four electrodes intertwined means that electrodes never overlap. The geometry of the linear array however, is such that vias are required to overlap the electrodes without making electrical contact, unlike a more common interdigitated electrode array, which has 2 sets of interdigitating electrodes (commonly used in capacitance, conductivity sensing, etc) instead of 4, which is required for TWD.

Figure 3 - Design of mask for TWD spiral concentrator. Close-up of spiral on the right. Spiral tracks have width of $50\mu\text{m}$. Notice that only 1 lithography layer is required.

Figure 4 - Design of linear TWD device. Close up of the device on the right. Notice the requirement for 3 layers of lithography because vias are required.

4. ELECTROKINETIC DEVICE FABRICATION

Electrokinetic Devices are commonly fabricated in a planar manner using similar techniques to the ones the microelectronics industry uses. One of the main advantages of electrokinetics over other techniques of microparticle manipulation (such as those which use fluid flows and channel structures to manipulate particles) is that fabrication of electrokinetic devices is very inexpensive and relatively simple. The most common way is to pattern a thin metal layer on an insulating substrate.

4.1. Fabrication method used

The glass substrates (half-microscope-slides) were cleaned using detergent. After cleaning thoroughly using detergent-soaked (lint-free) cloth, they were blown-dry with nitrogen, then left to sit in sealed petri dishes to dry for 24 hours.

An Aluminium film was evaporated onto the top side of the glass substrate. This was done at a vacuum of 10^{-5} Torr for about 120s and left an aluminium film of thickness 100 ± 20 nm with a resistivity of about $5 \Omega/\text{square}$.

After a quick rinse with isopropanol and blow-dry with Nitrogen, Microposit S1813 photoresist was spun-onto the sample at 1500rpm for 10s. The samples were then soft-baked in a temperature-controlled oven at 90°C for 15 minutes.

The sample was exposed to ultraviolet (UV) light using a halogen polychromatic lamp for 75s and the resist was developed using a 25% V/V solution of AZ-400K developer, and then rinsed in deionised (DI) water and blown-dry with nitrogen.

After baking, the sample was etched in an aluminium etchant solution³ at 60°C for about 20s, or the time required for etching to visibly be completed. Hard-baking of the photoresist wasn't required before the etching step, the soft-baked resist did not appear to deteriorate in the etching solution. The sample was then rinsed in DI water and then blown-dry with nitrogen. The sample was immediately rinsed in acetone to remove the remaining photoresist. This is because the remaining photoresist seems to trap etchant, even after the sample has been rinsed in DI water. If the sample was left for hours after etching, without firstly removing the remaining photoresist, the Aluminium continued to slowly etch over a period of hours, under the photoresist. After rinsing in acetone, the sample was rinsed in DI water and blown-dry with nitrogen.

The sample was then electrically-tested for continuity between soldering pads.

An attempt was made to use an insulating layer over the electrodes, to protect the electrodes and prevent electrolysis during operation. However, as explained later in this paper, this was later found to prevent any observable electrokinetic phenomena. PDMS (Polydimethyl siloxane) (Dow Corning - Sylgard 184) was spun-onto the sample at 6000rpm for 10s to obtain a thin ($\sim 30\mu\text{m}$) PDMS layer. PDMS was rubbed-off of the sample above the soldering pad areas using a dry cotton tip. Any residue on the soldering pads was then removed using an OS-20-soaked cotton tip⁴. The sample with the PDMS layer was baked in an oven at 120°C for 60 minutes to cure.

Figure 5 - Photographs of fabricated TWD spiral concentrator.

³ The etching solution consisted of 80 parts Phosphoric Acid 85%, 16 parts Acetic Acid 100%, 4 parts Nitric Acid 70%.

⁴ OS-20 is the commercial name for a silicon rubber solvent produced by Dow Corning.

After the device itself was fabricated, wires were soldered to the bonding pads using a standard manual soldering process.

4.2. Other Fabrication methods commonly used

In 2001, Jones *et al* [1] used a vapour-deposited Aluminium-on-Glass structure for dielectrophoretic actuation of liquid, which is very similar to the fabrication explained in this paper. In 2003, Ahmed *et al* [2] did the same, but with a dielectric layer on top for protection, that was Teflon AF (amorphous fluoropolymer) or SU-8 to avoid boiling and electrolysis. In 1989, Burt *et al* [3], fabricated a device that consisted of 'paxolin' substrates with copper patterned using standard photolithography techniques. In 1993, Huang *et al* [4] used gold as the electrode material, 100nm thick, vacuum-evaporated onto a seed layer of chromium on glass. In 1996, Muller *et al* [5] used 100nm titanium covered by 50nm gold as the electrodes, on a quartz substrate.

Glass was chosen as the substrate material in this paper because of its very low cost, the fact that it is an electrical insulator, and the fact that it is optically transparent, to aid in visual inspection of the device operation. Aluminium was chosen as the electrode material because of its low cost, high conductivity, high resistance to corrosion, and the simple evaporation procedure. PDMS was originally chosen as the insulating layer because it is simple to apply and cure, and is very cheap. However, as explained later in this paper, the PDMS was discarded as it prevented observable electrokinetic effects because it was too thick, and the bare electrodes were used instead. Teflon AF such as that used by Ahmed *et al* [2] may be more suitable as an insulator.

5. ELECTROKINETIC DRIVING SIGNALS

5.1. Driving Signals Used

The driving signals used were generated using a HP 33120A signal generator, an RC phase-shift filter, and 3 BJT inverting amplifier circuits (see Figure 6). This allowed adjustable signals of up to 10Vp-p and frequencies from 100kHz to 5MHz. The signals were four identical sine waves separated in phase by 90° (as in Figure 7)

Figure 6 - Block diagram representation of signal source for TWD driving signals, and schematic diagram of BJT inverting amplifier circuit.

Figure 7 - Signals produced by the system of Figure 6.

6. OPERATION OF ELECTROKINETIC DEVICES

Saccharomyces Cerevisiae yeast cells were used as the suspended microparticles to test the operation of the device. These were of strain Y514 (commonly used in ale brewing). These have a measured cell diameter of approximately $2.5\mu\text{m}$.

An aqueous sodium chloride solution was used as the suspending medium. A concentration of sodium chloride was used to give a conductivity of 1.8mS/m , as measured by a TPS LC81 conductivity meter and TPS conductivity cell.

An uninsulated device was connected to the signal source by soldering wires directly to the soldering pads of the substrate (see Figure 5a). The device was then secured under a microscope.

A droplet of the fluid was placed on the spiral (electrode) area of the device. For the first observation, the electric signal source of 1.3MHz , 8Vp-p was turned on.

The fluid was then wiped off of the device, and another droplet of fluid was placed onto the spiral area of the device. For this second observation, the electric signal source was turned off.

7. RESULTS

Upon placement of the fluid, the yeast cells were randomly distributed throughout the droplet. Sedimentation forces caused the yeast cells to slowly fall towards the surface of the device and the microelectrodes. In the first observation (with driving signals on the electrodes), as the cells got close to the surface (around $10\text{-}15\mu\text{m}$ above, as estimated by varying the focus depth and comparison with the height of the cells on the device surface) they experienced TWD motion. This was observed as a steady straight motion directly towards the centre of the spiral. Unfortunately the properties of the fluid and the cells were such that they experienced positive DEP, which trapped a large portion of the particles on the edges of the microelectrodes, where the electric field strength was highest. This is clearly visible in Figure 8.

Figure 8 - Photograph of Dielectrophoresis causing yeast cells to adhere to microelectrode edges, when signal source turned on. Dark areas are electrodes, light areas are glass.

To ensure that the observed adherence of particles to the electrode edges was a result of the electric field, the same procedure was repeated with the signal source turned off. Figure 9 shows the resulting random distribution of particles, instead of the adherence to the electrode edges.

Figure 9 - Same set up as Figure 8, but with no electric signal applied. Yeast cells randomly distributed instead of adhering to microelectrode edges.

8. DISCUSSION

8.1. Negative Dielectrophoresis for cell levitation

Much of the literature about the operation of TWD devices discusses the use of negative dielectrophoresis to oppose the sedimentation forces and levitate the cells just above the electrodes to allow TWD to continue [13]. Looking at equation 5, this means ensuring that the properties of the suspending fluid, suspended particles, and the driving frequency are such that the real part of the Claussius-Mossotti factor (3) is negative.

To change the Claussius-Mossotti factor, the particle or medium permittivity, and/or particle or medium conductivity, and/or applied frequency must be changed. In the practical use of such a device, we may not have a choice about the changing the suspended particles, so we may have to adjust the conductivity of the suspending medium by adding sodium chloride (to increase the conductivity) or diluting the medium (to reduce the conductivity). Our other options are to use a different type of medium (e.g. liquid with different permittivity than water), or to use a more suitable (most likely higher $\sim 3\text{MHz}$ [7]) driving frequency, where negative dielectrophoresis will occur.

8.2. Problems with the Insulating Layer

The attempt at using a thin insulating layer of PDMS failed, and no electrokinetic effects were observable for these devices. This could perhaps be due to the reduced proximity of the yeast cells to the electrodes. The measured thickness of the PDMS layer was about $30\pm 5\mu\text{m}$.

When electrokinetic effects were observed, such as TWD (explained in section 7), using the uninsulated devices, the TWD-induced motion occurred only when the yeast cells were within $10\text{-}15\mu\text{m}$ of the electrode surface.

To alleviate this problem, a much thinner insulating layer may be required (e.g. $< 1\text{-}2\mu\text{m}$). This may be obtainable by spinning-on the PDMS with a much lower viscosity (probably through diluting with a thinner) to obtain a thinner layer, or through the use of a different insulating material with naturally better viscosity in the liquid phase.

8.3. Improvements to Fabrication Methods

There is increasing interest in microfluidics devices being process compatible with MEMS and microelectronics to allow integration of microfluidics, MEMS and microelectronic devices on the one substrate. As mentioned above, electrokinetic devices are commonly fabricated by patterning metal on glass. This is generally not compatible with microelectronics nor MEMS fabrication. MEMS and microelectronics are quite commonly silicon-based.

DEP devices require arrays of coplanar microelectrodes, which are conductive paths for current flow, and perhaps a very thin insulating layer over these electrodes, to protect the electrodes, and to prevent charge exchange between the electrodes and the suspending liquid. In IC manufacturing, conductive paths or tracks can be created either by the doping of silicon to increase the carrier concentration, or the deposition and patterning of metal tracks on top of the device.

Two proposed methods of fabricating electrokinetic devices in parallel with CMOS processes, are to use the metal layer as the electrodes, or to use the doping used in transistor fabrication to create a conductive electrode path in silicon. The latter may allow the growth of an ultra-thin insulating layer of silicon oxide.

Another possible way of fabricating electrokinetic devices with smaller dimensions is through vertical layer fabrication. This may also enable easier patterning of vias and other features.

9. CONCLUSION

The theory of electrokinetic phenomena such as electrophoresis, DEP, and TWD were explained. The fabrication of a TWD device was shown, as well as suggestions for improvements of fabricating electrokinetic devices. The driving signals used with the TWD device were discussed. Last of all, the operation of an electrokinetic device was covered, visual results were shown, the results were discussed, and suggestions were made for enhancing the procedure.

One of the main findings of the results was that the electrokinetic forces on the particles were only observable when the particles were within $10\text{-}15\mu\text{m}$ of the device surface. Another finding was that the positive DEP forces attracted the particles to the electrode edges and that changes would need to be made to the suspending medium, and/or the applied frequency to result in negative DEP instead of positive DEP, to levitate the suspended particles to a suitable height for TWD.

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