



Two-dimensional electrostatic actuation of droplets using a single electrode panel and development of disposable plastic film card

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Abstract

In order to form large micro-droplet-arrays, we have developed a device that can merge and transport microdroplets in two directions. Transportation of droplets is performed by electrostatic actuation technique. The speciality of this method is that it uses only one electrode panel, based on standard printed circuit board (PCB), and in it, only 18 independent controls are needed. We have also developed disposable plastic films cards, which can substantially reduce the development cost because the actuation chip has been separated into two parts: a fixed electrode panel and a less costly removable card. The droplets array is formed and analyzed in the card.

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1. Introduction

Electrostatic droplet manipulation is a powerful technique that has received a large interest in the recent years, because it has potentially great applications in lab-on-a-chip systems. Basically, every experiment involving manipulation, mixing, cutting and sorting of microvolumes of samples and reagents is in the scope of this technique. Some advantages, compared to microchannel-based methods, are low dead-volumes, no limitations related to interdependence of channels and no need of micropumps or microvalves [1].

The electrostatic-based actuation methods vary in detail and have therefore been given various names (electrowetting [2,3], dielectrophoresis [4], electrostatic [1], etc.), but they all have the same origin, i.e. the electrostatic force resulting from the interaction between an electrostatic field and charges (induced, like dipoles, or not) in the solution.

Depending on the application, simple designs can be used that only require a limited number of independent electrodes. For instance, Srinivasan et al. [5] have presented an integrated device for glucose assay that uses only few independent electrodes. Similarly, Fouillet et al. [6] have performed polymerase chain reaction (PCR) with a fluidic microprocessor which operates 16 reservoirs and 100 electrodes with 16 relays. However, the design is very specific to their small-scale application. On the other hand, large arrays of electrodes, which could enable complex and reconfigurable device, are in development. These are based on CMOS technology [4]. The relative complexity of the device, with high voltage switching circuitry buried beneath the electrode surface and the communication and addressing control logic included on-chip, make it costly, although necessary if one really needs reconfigurable system.

In this paper, the principle of the actuation technique will be explained, and the force acting on the droplet will be discussed. Then some finite element analysis (FEA) results that are helpful to interpret some unexpected experimental observation related to the insulator thickness will be presented. A description of the special design and voltage sequence that enables the merging of different solutions and the preparation of microarrays

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with a single electrode panel will be given followed by experimental results. Such technique is ideal to perform experiments that need to combine different solutions in various quantities. A typical application is high-throughput protein crystallization, where protein solutions have to be mixed with various reagents in order to get many droplets with different conditions of concentration, pH, etc. [7,8] Finally, an account of novel cards using a plastic film for both the bottom frame and the insulator will be described.

2. Materials and methods

2.1. Actuation principle

Fig. 1 shows a side view of the electrode panel used for droplet manipulation. An array of electrodes is patterned on a non-conductive substrate. The electrodes are covered with an insulator layer to avoid electrolysis that would occur if the droplet solution could directly come in contact with the electrodes. Finally, a hydrophobic layer (Teflon or Cytop, thickness of the order of few hundreds of nm) is spin-coated on the top of the insulator to increase the contact angle and thus to facilitate droplet actuation and sliding. Among the recent works that are using the so-called “electrowetting” configuration most of them need two electrode panels, a bottom one and a top one, especially when actuation in two directions is necessary. Due to a special design that will be described later, we can achieve actuation in two directions using a single bottom panel.

A voltage pattern is applied on the electrode array. Typically, a sequence of three positive and three grounded electrodes, which is repeated along the electrode array, is applied and regularly shifted in a given direction [9,10]. Due to electrostatic force, the droplets are shifted together with the voltage pattern. The number of voltage sources needed is fixed by the length of the pattern. Usually a six-phase pattern is used. Every six electrodes are connected together. Thus, only six voltage sources are needed irrespective of the transportation distance.

The droplets are transported in oil for the following reasons: first, the use of oil limits evaporation of droplets which is critical in this range of volume (nanoliter); second, we observed that the droplet contact angle is larger inside the oil. Moreover, the

contact angle hysteresis, which is believed to be at the origin of the voltage threshold, is reduced in the oil [11,12]. As a consequence, the actuation voltage is smaller in the oil. Finally, the presence of a thin layer of oil between droplet and hydrophobic surface limits the surface contamination [2]. Different type of oils can be used, the most important characteristic being the viscosity. The larger is the viscosity, the higher the actuation voltage. In our experiments, we are usually using silicon oil with a viscosity of 2cSt (Dow Corning Toray Silicone Co. Limited (DCTS), Tokyo).

2.2. Materials

The materials of the substrate and the electrodes are not critical as regard to the actuation performance. We have successfully used various materials; such as, ITO (Indium Tin Oxide) on glass, printed silver paste on polyester, or copper on polyimide and glass epoxy (standard PCB); without significant variations in transportation characteristics. However, in order to connect every six electrodes together, we need either to perform a multi-layer process, or to make connections through the substrate. The first technique was used for the printed silver paste on polyester samples, and the second technique was used with the PCB samples. The choice of insulator and hydrophobic material layers might be more important. An insulator with a large dielectric constant is preferable [3] but not crucial.

2.3. Theoretical considerations about the force

There are two main tendencies to describe the force acting on the droplet in this type of configuration. The first theory, often referred as EWOD that stands for electrowetting on dielectric, is based on the electrowetting phenomena [3,11], i.e. the increase of the surface wettability by applying a voltage. The principle is that under the applied potential there is a redistribution of charges and dipoles inside the liquid that lowers the solid–liquid surface tension. As a consequence, the contact angle at the interface between the droplet and the energized electrode is reduced. If a portion of the droplet also overlaps a grounded electrode, the droplet meniscus is deformed asymmetrically and a pressure gradient is established between the ends of the droplet which result in bulk flow toward the energized electrode.

The second theory is referred as dielectrophoresis (DEP) [4]. DEP is the translational motion of neutral matter caused by polarization effects in a non-uniform electric field [13]. As it depends only on the dielectric properties of the droplet, non-conductive solutions can be actuated. AC fields are usually used in order to avoid charge-trapping phenomena to occur. Moreover, the direction of the displacement can be controlled by the frequency of the field, due to the frequency dependence of the dielectric constant of materials.

Recently, efforts have been made to unify these two theories, showing that EWOD and DEP effects are the low- and high-frequency limits, respectively, of a more general electrical force arising when a liquid is placed in an electric field [14,15]. Moreover, low- and high-frequency limits correspond to the cases of

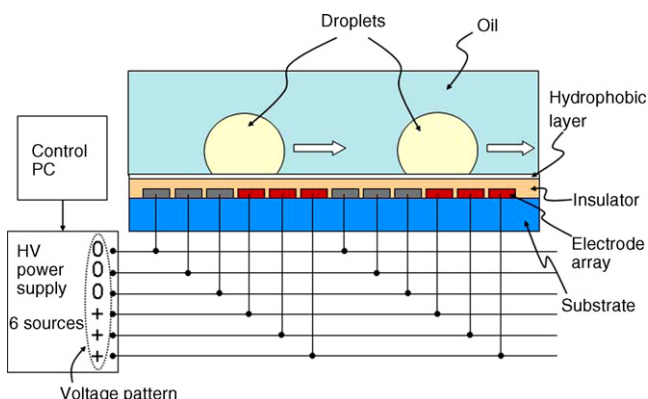


Fig. 1. A side view of the electrode panel used for droplet manipulation.

conducting and insulating liquids, respectively. The calculation of the force is based on the integration of the Maxwell stress tensor. This formulation can lead to an analytical solution only in simple geometries. In the case of droplets actuated on a single electrode panel, the electric field is not uniform, and numerical calculations are necessary if one wants to evaluate the forces and understand in details the behavior of the liquid droplets. That's what was done by Zeng and Korsmeyer [16] The electrohydrodynamics of droplets was studied by the use of simulations, and one of the conclusions is that the deformation of the droplet is a consequence of the occurrence of electrowetting on dielectric, not a condition for the droplet displacement. In other words, if the droplet was not deformable, the force would be the same. As a consequence, forces can be calculated without having to take into account the deformation of the droplet, which reduce largely the complexity of the calculation.

In our experiments, we used a conducting solution (water containing various ions) and we applied a DC voltage. Thus, if we had to define the force in one of the two most used terms, we should call it electrowetting rather than dielectrophoretic; but as discussed above, given that electrowetting is merely a consequence, not the origin, of the force we usually prefer the term 'electrostatic force' which is more general.

On the basis of these remarks, we have performed finite element analysis (FEA) with a commercial software (ANSYS) to evaluate the forces acting on the droplets in our configuration (no upper plate). The droplet is represented by a conducting non-deformable truncated sphere. The model is shown in Fig. 2. We have included a substrate, 12 electrodes, the insulator, the droplet and the surrounding inert medium. The basic set of parameters and their value are shown in Table 1. The signification of the parameters is explained in Fig. 3. F_x and F_z are the horizontal and vertical components, respectively, of the calculated electrostatic force [17].

2.4. Plastic film cards

Usually the insulator layer is directly coated on the electrode panel, thus any digital microfluidic card must include the

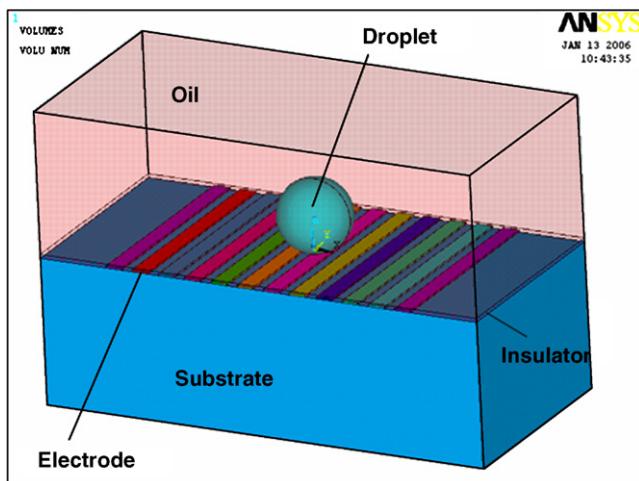


Fig. 2. Model used for the finite element analysis (FEA).

Table 1
Basic set of parameters used in the simulations

Parameter	Value
Dielectric constant of the substrate ϵ_s	3.4
Dielectric constant of the insulator ϵ_i	2.8
Dielectric constant of the surrounding medium ϵ_m	2.5
Electrode pitch p (μm)	300
Electrode width w (μm)	180
Insulator thickness t (μm)	10
Droplet diameter D (μm)	800
Droplet volume V (nl)	200
Contact angle θ ($^\circ$)	110
Voltage (V)	100

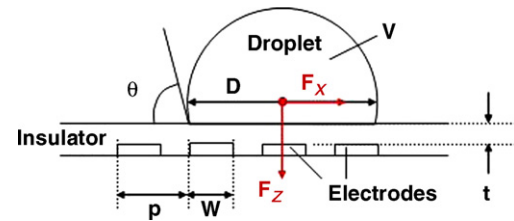


Fig. 3. Parameters used in the simulation.

electrode array. We have developed a separable card that uses a thin plastic film as an insulator (Fig. 4). The card containing the droplet samples is a simple plastic film fixed to a holder and coated with a hydrophobic layer. The film thickness can range from few micrometers to few tens of micrometers. The obvious advantage is that the separable card cost is considerably reduced

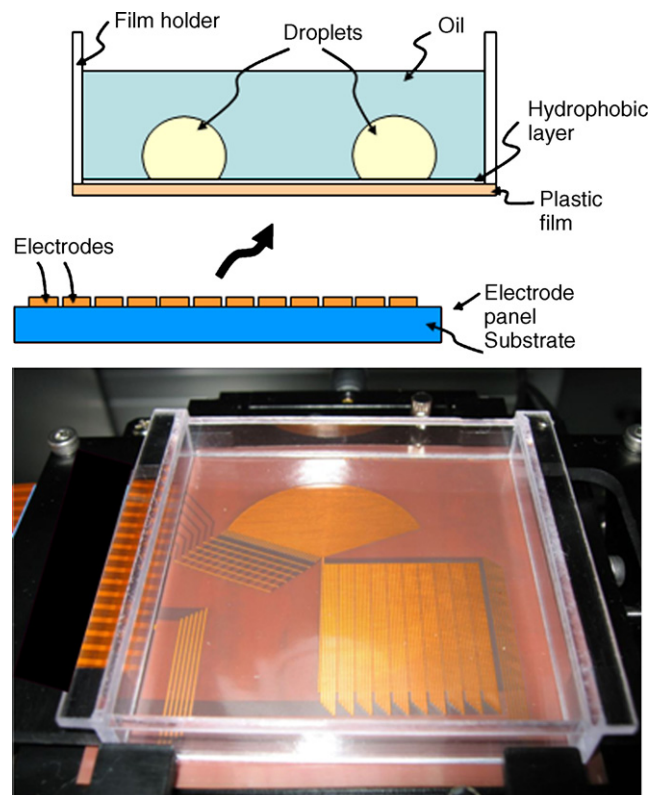


Fig. 4. Separable card: principle (top sketch) and picture (bottom).

as it does not contain the electrodes. Only one electrode panel is needed and reused with each card. This results in another benefit. Although it is barely mentioned in literature, we think, from our own experience, that it is quite difficult to keep a reliable actuation for a long period of time using the same chip. Charging of the insulator layer, degradation of the hydrophobic layer due to long period of immersion in the oil, contamination of the surface beneath droplets standing still for some time, or simply dust deposition, are some of the difficulties that must be faced in those kinds of devices. The use of inexpensive disposable cards can circumvent that issue. One card is used for one experiment and disposed off. For example, in the protein crystallization device, one card is used for the preparation of an array of droplets. Once the array is prepared, the card is stocked and the crystals growth is monitored periodically. When this experiment is completed the card is disposed off.

2.5. Electrode design

Various electrode designs were developed. An array of straight electrodes enables actuation in one dimension. An array of arc-shaped electrodes is a simple solution to perform mixing of droplets containing different solutions. If this arc-shaped electrode array is followed by a straight electrode array of equal or larger pitch, mixed droplets can be transported away.

Two-dimensional (2D) transportation can be achieved by using a second electrode panel that is flipped and placed over the first panel, separated by a given gap. The electrode array of the top panel is perpendicular to the bottom one. Fig. 5 shows such a set-up that has successfully transported droplets in two directions [18]. However, with this set-up the benefits of low cost and simple packaging of the single panel configuration are lost. Moreover, it may be preferable in some cases to have direct access to the droplets. For instance, in the previously mentioned application of protein crystallization, once crystals have been grown in some droplets, they need to be harvested. This task is greatly facilitated if no top electrode is present.

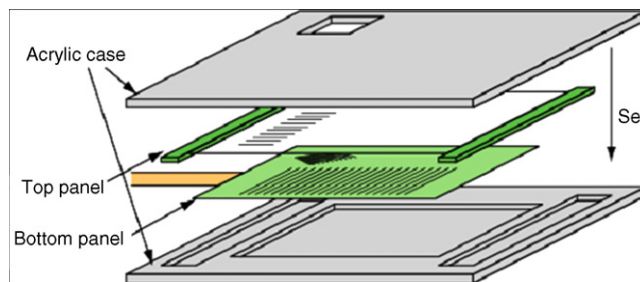


Fig. 5. Two-electrode panel configuration.

For this reason, the design in Fig. 6 was created. It includes three electrode arrays. Each array needs six voltage sources as every six electrodes are connected together via through holes. This design enables merging followed by 2D actuation of droplets using a single panel and only 18 voltage sources, whatever the size of the panel, are required. No relays are needed for switching electrodes connections and the control is relatively simple.

Droplets of different solutions are dispensed on the arc-shaped electrode array A (Fig. 6a). Then, they are transported toward the center of the electrode where they merge together. The resulting larger droplets are transported downward using the electrode array B. At this time, the voltage of the “horizontal” electrode array C is fixed depending on the charging properties of the droplets. Indeed, even neutral solutions, if dispensed using dispenser with tips made of plastics, will become charged, depending on the plastic material. For the manual dispensing of droplets we were using commercially available dispensers with tips made of polypropylene. It appeared that after dispensing, pure water droplets became positively charged, as they were repelled by the positive electrodes. Thus, during transportation of these positively charged droplets with B, C must be maintained to a positive voltage, otherwise the droplets will drift from B to C. On the other hand, if the dispensed solution is connected to the ground or to a slightly negative voltage, the generated

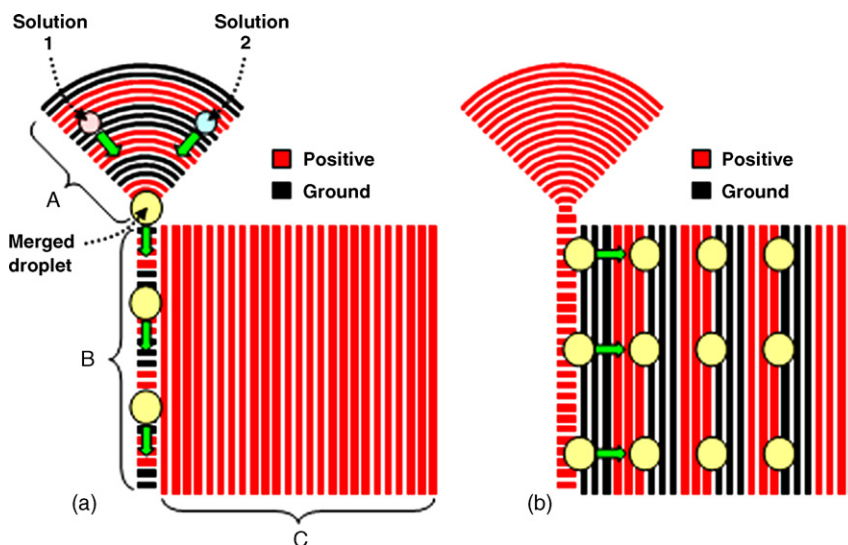


Fig. 6. The design for merging and two-dimensional actuation.

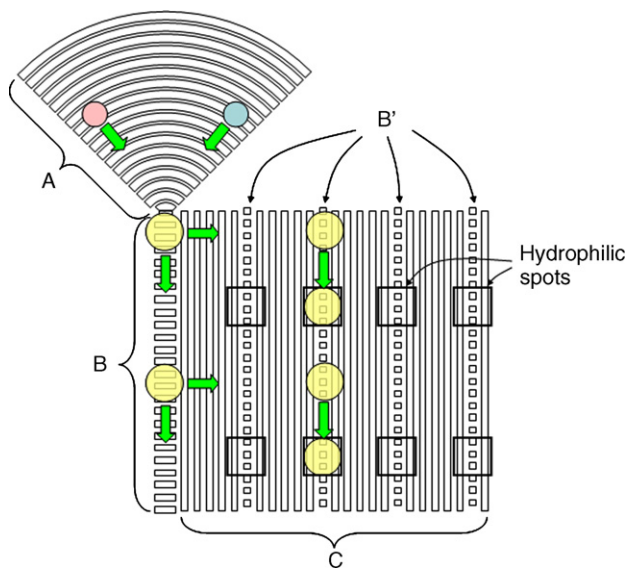


Fig. 7. Additional vertical electrode array for the transportation of droplets to hydrophilic spots.

droplet is neutral and C must be grounded during downward transportation with B.

After actuation in the first direction, droplets can be transported in the second direction by applying the traveling voltage pattern to C (Fig. 6b). Simultaneously, B is switched to a positive voltage or to the ground for charged or neutral droplets respectively. By this way, a 2D array of droplets can be prepared. The volume of the droplets depends on the dimension of the electrodes, and on dispensing methods and conditions.

In the next development, we added additional electrodes to introduce more flexibility in the droplet displacement (Fig. 7). Every six lines in the array C were replaced by an array of electrodes B' that can transport the droplets downward. The arrays B' are connected to the array B from the backside of the substrate. Consequently, they cannot be used separately, but the advantage is that no additional controls are needed.

The benefit can be readily understood if we once again take the example the protein crystallization application. In that application, an array of droplets has to be prepared, and the card containing this array will have to be transported from a place to another, for instance from the array formation stage to the storage unit or to the protein crystallization monitoring unit. That will cause vibration and shocks, and droplets, if not firmly fixed on the surface of the card, will move and merge together. This can be avoided by introducing hydrophilic spots on the hydrophobic surface. If droplets are transported to the hydrophilic spots, they will attach to this point, and not move during card transportation. The additional electrodes B' can be used to transport the droplets to the hydrophilic spots, as shown in the Fig. 7.

3. Results and discussion

3.1. Electrostatic force as a function of the droplet position

In order to understand in more detail the behavior of the droplet when a voltage pattern is applied to the electrode array,

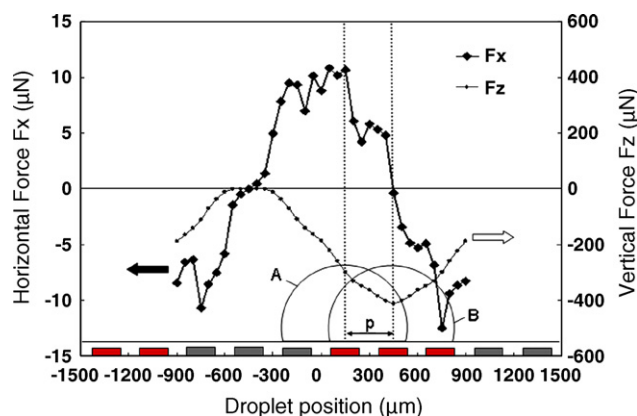


Fig. 8. Magnitude of the horizontal (F_x) and vertical (F_z) forces exerted on the droplet as a function of the droplet position.

we have performed force calculations as a function of the droplet position. The force was separated in two components, a horizontal force F_x responsible for the droplet displacement, and a vertical force F_z acting downward. Simulations results are shown in Fig. 8. The x -axis represents the position of the center of the droplet. The gray and red rectangles represent the position of the grounded and energized electrodes, respectively. If the initial position of the droplet is $x = 0$, right between grounded and energized electrodes, it will experience a positive horizontal force that will transports it to the equilibrium position represented by the circle B in Fig. 8. The equilibrium position corresponds to a null horizontal force. If the voltage pattern is switched by one step to the right (which is the same as shifting the droplet by one pitch to the left regarding the force calculation), the new droplet position relative to the energized electrode will correspond to the circle A in Fig. 8. At this position, the horizontal force is positive, therefore, the droplet will move to the right until it reaches its equilibrium position B again, and so forth.

This mechanism is applicable for a given range of droplet diameter that was estimated to be one electrode pitch to five electrode pitches. Thus, for an electrode pitch of $300 \mu\text{m}$, the volumes of droplets that can be transported ranged from about 11 nl to 1.3 ml.

It is noticeable that the magnitude of the vertical force, which tends to squash the droplet against the surface of the insulator (as shown by the negative sign), is much higher, by about forty times than the magnitude of the horizontal force. Since the droplet is considered as a grounded conductor in the calculations, the vertical force is null when the droplet is positioned directly on the grounded electrodes. The vertical force only appears when the droplet is, at least partially, positioned over the energized electrodes. The experiments results concerning the effect of the insulator thickness presented in the next paragraph lead us to think that this high vertical force may have a detrimental effect on actuation performance.

3.2. Effect of the insulator thickness

It has been reported that the smaller is the thickness the better is the actuation performance, because the larger is the actuation

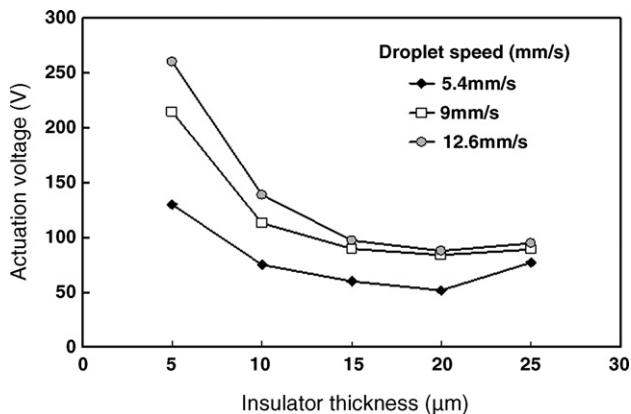


Fig. 9. The minimum actuation voltage as a function of the insulator thickness for three different droplet speeds. (Actuation on PCB panel, electrode pitch 300 μm, droplet volume 1 μl).

force [3]. We noticed the same behavior – reduction of the actuation voltage with the insulator thickness – until an “optimum thickness” is reached. When the insulator thickness is further decreased, the actuation voltage increases again. Fig. 9 shows the results of actuation experiments that were performed with different insulator thickness. In those experiments the insulator was not a plastic film, but a photo-imageable solder resist ink (SSR-6500-153, San Ei Kagaku Co. Ltd.) that was directly coated on the electrode. The optimum thickness, i.e. the one for which the actuation voltage is minimum, was 20 μm. The actuation voltage, at a given speed, is defined as the minimum voltage necessary for the droplet to be actuated.

The electrostatic force exerted on the droplet as a function of the insulator thickness were calculated by FEA. Both horizontal (F_x) and vertical (F_z) components increase when the insulator thickness decreases, but the vertical component increases much faster than the horizontal one. This becomes evident when we plot the ratio of the vertical force to the horizontal force F_z/F_x (Fig. 10). This ratio increases only by a factor 2 when the thickness changes from 50 to 20 μm, but it increases further by a factor 14 from 20 to 1 μm. We think that the fast increase of the vertical force is responsible for the deterioration of the actuation performance when insulator thickness decreases. This might be

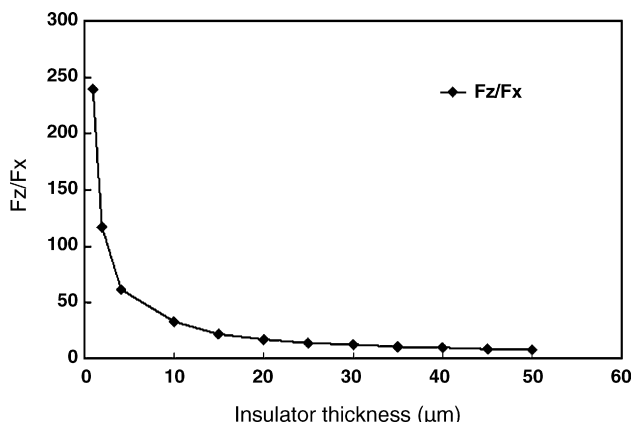


Fig. 10. The ratio of the vertical force (F_z) to the horizontal force (F_x).

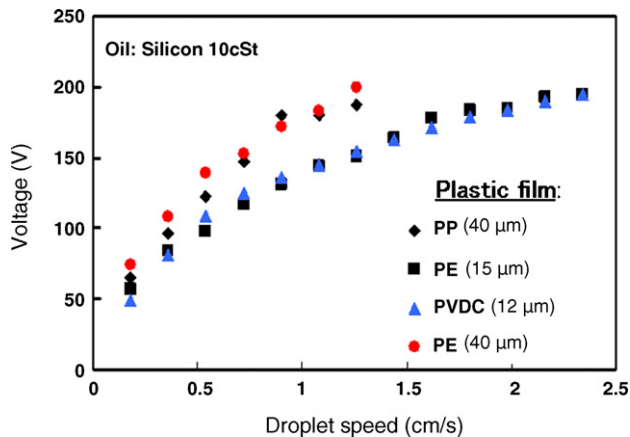


Fig. 11. Actuation characteristics of different film materials. The film thickness is indicated inside brackets. (PP: polypropylene, PE: polyethylene, PVDC: polyvinylidene chloride).

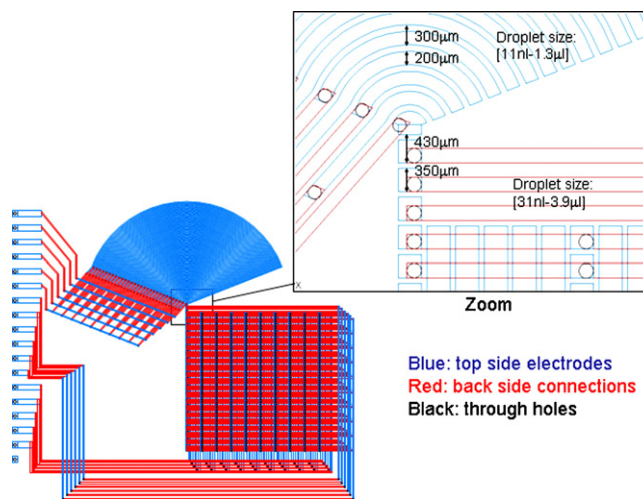


Fig. 12. Layout and dimensions of the electrodes for the preparation of droplets arrays.

due to the increased friction between the droplet and the surface beneath, or by the increase of the insulator charging, as it is known that the charging efficiency is related to the electrostatic force magnitude [19].

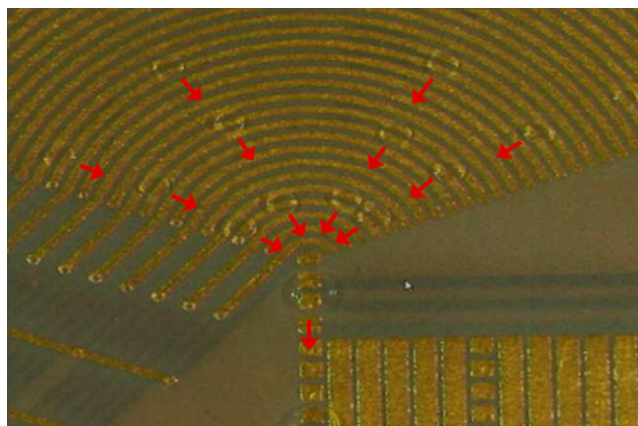


Fig. 13. The merging of four droplets and transportation of the merged droplet.

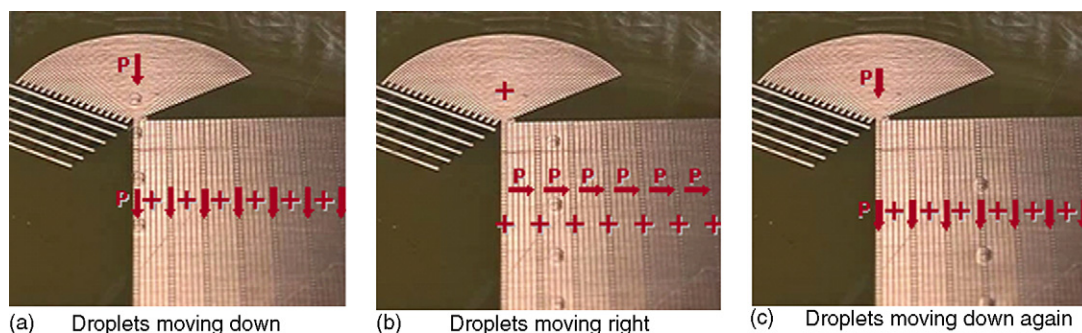


Fig. 14. Transportation in two dimensions.

3.3. Actuation in plastic film cards

We have tested several plastic materials. For each material, the actuation voltage as a function of the droplet speed was measured (cf. Fig. 11). A thin layer of Teflon was coated on the plastic films by spin coating. These tests were not exhaustive, but these results tend to show that the material has less effect on the actuation characteristics than the thickness of the plastic film. The polyethylene (PE) and the polypropylene (PP) films, both having thickness of 40 μm , had very close characteristics concerning the droplet actuation. Similarly the curves for 15 μm polyethylene film and 12 μm polyvinylidene chloride film (PVDC) almost superimposed. The actuation voltage for $\sim 15 \mu\text{m}$ films was lower than the actuation voltage for 40 μm films. For later experiments, a PVDC film was used because it is permeable to oil. The card frame was made of polycarbonate. The size of the card was 6 cm \times 6 cm. We fixed the PVDC film by heat-sealing at 140 $^{\circ}\text{C}$ for 2 min 30 s. A special aluminum holder was designed to heat only the bottom and lateral part of the frame, without heating the entire plastic surface.

The card was just set down on the electrode without special adjustment. In that case, if the bottom of the frame is not perfectly flat, a small gap between the plastic film and the electrode may appear. This would of course have a large impact on actuation properties. However, when oil is poured in the card, the film gets in close contact to the electrode due to the weight of the oil.

3.4. Droplet merging and 2D actuation

The layout and dimensions of the electrodes for the preparation of droplets arrays is shown in the Fig. 12. Each of the three arrays of electrodes has six connections to voltage sources, because every six electrodes are connected together by the way of holes through the polyimide substrate.

The dimensions of the electrode and the range of droplets volumes that can be handled by those electrodes are indicated in Fig. 12. Considering the droplets volume ranges, a simple way to mix different solutions in various proportions is to change the number of droplets of each solution that form the merged droplet. For instance, a hundred of 20 nl droplets can be mixed using the arc-shaped electrodes to form a 2 μl droplet that will be transported by the straight electrode array. To change the ratio of the different solutions, another possibility is to directly produce droplets of various volumes on the arc-shaped elec-

trode. This last method could lead to smaller merged droplet, but would require a more extensive control of the dispensed droplets volume.

Fig. 13 shows a picture of the merging of four droplets and the transportation of the merged droplet. Fig. 14 shows the transportation in 2D. Pure water droplets were dispensed either manually using plastic tips (thus droplets were positively charged) or by using a home made syringe dispenser [20] connected to the ground (thus droplets were not charged). A dispenser was used for the droplet merging in Fig. 13. The droplets were dispensed manually in Fig. 14. The letter “P” associated with an arrow indicates that the voltage pattern is applied in the direction of the arrow. The plus sign indicate that a constant positive voltage is applied on the electrode array. The droplets are dispensed on the arc-shaped array A, and transported downward by the array B (Fig. 14a). When a positive voltage is applied to the electrodes of the array C, the droplets follow the vertical line without drifting away. Thereafter droplets are transported in the right direction by applying the voltage pattern on the array C, while the array B voltage is kept to a positive value (Fig. 14b). Finally, the line of droplets can be actuated downward again using one of the arrays B’ (Fig. 14c).

4. Conclusions and future work

The research and development on digital microfluidics systems based on electrostatic actuation of droplets show two trends: the first is the realization of small simple devices dedicated to specific and limited applications, and the second is the use of large reconfigurable arrays of dot electrodes, based on complex and expensive CMOS technology. The method presented in this paper stands somewhere between those two extremes. By using relatively low cost printed circuit board associated with a specific design of the electrodes layout, we can prepare droplet arrays as large as desired, with only 18 independent sources and no relays. Though it can’t offer as much versatility as CMOS devices, we think that our method is well suited for specific applications requiring preparation of large microarrays of samples with different conditions such as high-throughput protein crystallization assays and other proteomic related analyses. Moreover, because no top panel is necessary, the complexity of the packaging as well as the cost are reduced, and direct access to the samples, for characterization, or harvesting is made possible.

At present, samples volumes down to 11 nl are handled. However, by reducing the dimension of the electrodes array, especially the pitch between electrodes, smaller volumes, on the order of picoliter, are potentially achievable.

2D-actuation of droplets by using only one panel has been demonstrated. This is the first step for the formation of micro-droplet-arrays. The actual formation of a micro-droplet-array needs improvement of the control software. For large arrays, the question of the reliability would also become predominant because if only one droplet over a hundred or more get accidentally stuck temporarily or not to a critical position, for instance the merging area, it may prevent the use of a large part of the array.

Moreover, the droplets that will be arranged in an array needs to be fixed to their position by the use of hydrophilic spots. Such an array, containing hydrophilic spots, was fabricated on an electrode panel made of ITO-on-glass, by using a lift-off technique. This technique was not transposable to the plastic cards. A method based on patterning of different types of polymer on the plastic surface is now under development.

Eventually, pure water droplets were used in the 2D actuation experiments. The presence of proteins or other chemical elements in the droplets might change the characteristics of the actuation. We have previously demonstrated that the actuation of droplets containing proteins such as albumin, beta-amylase, lysozyme, thaumatin, and xalanase was possible [21]. However, for the formation of protein arrays, a more thorough investigation on that issue would probably be necessary.

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