DESIGN AND TESTING OF AN INTERPOLATING MIXING ARCHITECTURE FOR ELECTROWETTING-BASED DROPLET-ON-CHIP CHEMICAL DILUTION

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ABSTRACT

An on-chip dilution scheme for an electrowetting based microfluidic system is presented in this paper. The dilution scheme uses the mixing and splitting of two droplets of different concentrations to result in an intermediate concentration, as the fundamental operation. A range of dilution factors can be obtained repeating the simple two-droplet mixing and splitting cycles, in various combinations. The accuracy of the dilution scheme primarily depends on two factors – variation in droplet volume during dispensing and splitting, and homogenous mixing. The proposed on-chip dilution architecture was tested for the dilution factors of 2, 4 and 8, using a red food dye as the sample solution and 0.1M KCl solution as the buffer. The concentrations were measured using an optical absorbance measurement system, consisting of an LED and photodiode. The error in the dilution factor was found to be ~15% for a dilution factor of 4 and ~25% for a dilution factor of 8. Droplet volume variations in dispensing and splitting contribute to ~80% of this error.

INTRODUCTION

Dilution of samples is an important step in almost all bio-analytical systems. The dilution step is done as part of the sample preparation process (pre-reaction) and/or during the reaction by controlling sample and reagent volumes. Sample dilution is done primarily for two reasons – to reduce the effect of interfering substances and to increase the linear range of operation of devices. A typical example is the enzymatic glucose assay (Trinder's reaction) where a dilution factor of 200 or more is used for the reaction to be linear up to glucose concentrations of 40mM. In the case of the glucose assay the dilution is typically done by mixing a small volume (50μL) of the sample with a large volume of reagent (1mL). Sample glucose concentrations greater than 40mM are typically pre-diluted with buffer.

In microfluidic chips based on continuous flow, the fluid is driven either by applying external pressure or by using electrokinetic methods. (electroosmosis or electrophoresis). Dilution is done in these systems by varying the relative pressures or voltages applied to sample and reagent/buffer ports, which affects the flow rates and correspondingly the volume ratios [1]. However, accurately performing large dilutions is difficult, partly due to the fact that the relative pressure/voltage required is dependant on the properties of the fluid and geometry of mixing chamber/channels.

An alternative approach towards microfluidic systems, which has received a lot of interest recently, is to manipulate the liquid as discrete "unit" droplets. This approach, which we refer to as "digital microfluidics", has several advantages over continuous-flow systems, the most important being the ease of fabrication, and reconfigurability and the scalability of the architecture. Electrowetting is one of several techniques that have been proposed to actuate microdroplets [2]. Electrowetting refers to the modulation of the interfacial tension between a conducting liquid phase and a solid electrode, by the application of an electric field. The use of electrowetting for droplet dispensing, transport, merging, mixing, and splitting, has been shown previously [2][3]. More recently an enzymatic glucose assay has also been demonstrated on an electrowetting-based microfluidic device [4].

The problem of obtaining on-chip dilution in a digital microfluidic system however, has not been addressed before. It is therefore necessary to develop a scheme for this much needed sample preparation step in a fully functional droplet-based microfluidic device. The ability to digitally manipulate droplets in an electrowetting device enables a new framework for on-chip dilution. The mixing and splitting of two unit droplets of different concentrations to result in an intermediate concentration, is used as the fundamental operation in the proposed scheme. As opposed to a continuous-flow system, our approach allows a range of dilution factors to be obtained by using multiple passes of the two-droplet mixing and splitting, in various combinations.

INTERPOLATING DILUTION ARCHITECHTURE

The mixing of a unit sample droplet of concentration C and a unit buffer droplet, results in a droplet with twice the unit volume, and a concentration of C/2. Splitting this larger drop, results in two unit droplets of concentration C/2. Continuing this step in a recursive manner using the diluted droplet as the sample, an exponential dilution of 2^N can be obtained in N steps. This two-fold dilution step can be extended to two droplets of different concentrations C1 and C2. This would result in two unit droplets with an interpolated concentration of (C1+C2)/2 each. By cascading the exponential and interpolating dilution steps in a serial fashion, arbitrary dilution factors can be obtained. For example by mixing and splitting two unit droplets of concentration C/8 and C/16 we can obtain a concentration C/10.67. By mixing the C/8 and C/16 in higher ratios such as 1:4, 1:8, 4:1 and 8:1, we can get droplets with concentrations of C/12.8, C/14.2, C/9.14, and C/8.53 respectively. This

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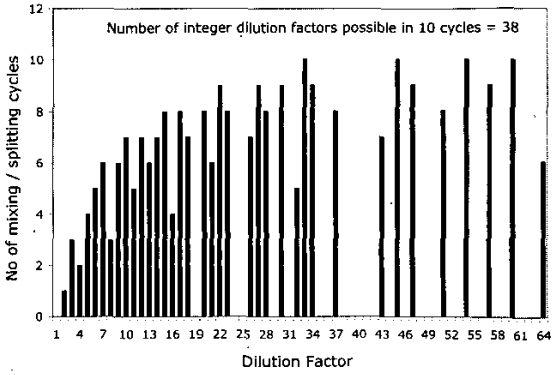

Figure 1 - The different dilution factors possible and the number of cycles required for each

scheme of obtaining the desired dilution ratio is referred to as interpolating serial dilution in this study.

Using the interpolating serial dilution method, a large number of dilution factors can be obtained with a relatively small number of dilution (mixing/splitting) cycles. Using less than 10 such cycles one can obtain 38 different dilution factors in the range of 2 to 64, given the constraint that only 64-fold exponential dilution and 16-fold interpolating dilution is possible. The 16-fold dilution constraint is to accommodate the limited volume of intermediate droplet mixtures generated in the 64-fold exponential dilution step. These achievable dilution factors and the respective dilution cycles required are shown in Figure 1.

To realize the dilution factors shown in Figure 1, it is essential that the electrowetting chip can handle parallel mixing and splitting operations, storage of the intermediate mixtures, and the routing of the sample and buffer droplets to the mixing area. A waste area is also required to discard unused droplets. This can be accomplished by using a simple physical design shown in Figure 2.

EXPERIMENTAL SETUP

Electrowetting set-up – The electrowetting setup is shown in Figure 4 and the fabrication is described elsewhere [2]. In the experiments reported in this paper we have used chips with an electrode pitch of L=750μm and a gap of H=90μm or H=200μm, unless otherwise specified. 1cSt

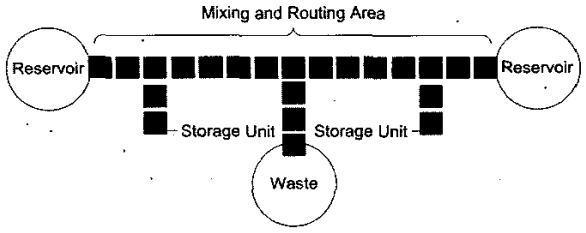

Figure 2 - Layout of an electrowetting chip capable of performing on-chip dilution

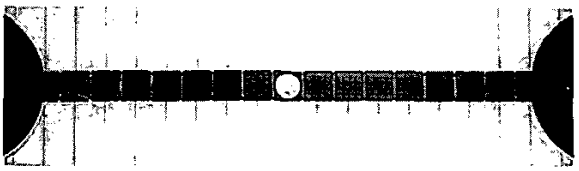

Figure 3 - Picture of the electrowetting chip used in the experiments

silicone oil is used as the filler fluid in all experiments. The electrowetting chip used in the experiments in this paper is shown in Figure 3.

Chemicals – A 0.1M KCl solution with 0.01% Triton-X and colored with a red food dye is used as the sample liquid. A 0.1M KCl solution with 0.01% Triton-X is used as the dilution buffer. Triton-X is added to lower the interfacial tension between the liquid and the oil, which assists both droplet formation and splitting.

Concentration measurement – An optical absorbance measurement setup is used to measure the concentration of the droplet. The setup consists of a cyan LED (λ=505nm) and a photodiode (TSL257, Texas Advanced Optoelectronic Solutions), as shown in figure 3. The voltage output of the photodiode (V) is proportional to the intensity of the light incident on it, and is related to the absorbance by the following equation.

$$A = \log(V/V_{BUFFER}) \propto 1/dilution \quad factor$$

V_{BUFFER} corresponds to the absorbance of the buffer solution. The absorbance A is directly proportional to the concentration of dyed droplet and therefore inversely proportional to the dilution factor.

EXPERIMENTS

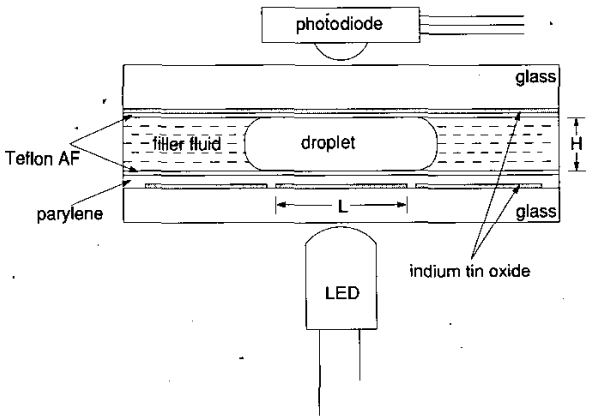

Figure 4 - Schematic of the electrowetting device (vertical cross-section)

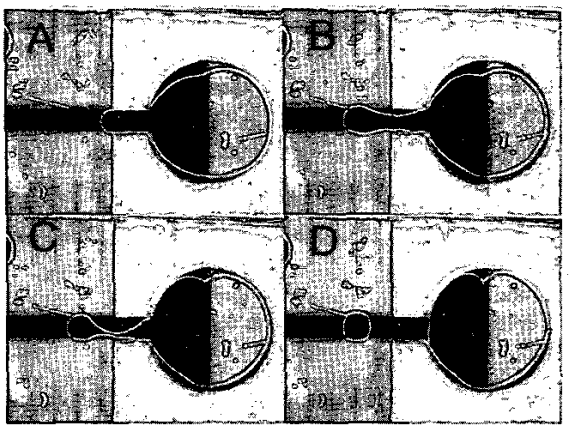

Figure 5 - Droplet dispensing from on-chip reservoir

Droplets of the sample and buffer solution are generated from an on-chip reservoir as shown in Figure 4. Mixing and splitting is done on the linear electrodes. The concentration of the mixed droplet is measured using the optical absorbance measurement setup described earlier. Images of the droplets are captured using a CCD video camera to obtain information about the volume variation, assuming the gap height does not vary across the chip. Due to the absence of a storage units on the chip, only exponential 2^N dilution factors were tested for N=1, 2 and 3.

RESULTS AND DISCUSSION

The primary sources of error that contribute to the inaccuracies in dilution are - droplet volume variation in dispensing and splitting operations, and inadequate mixing. The presence of insoluble chemicals in the droplet can also lead to concentration variations during dispensing and splitting.

Volume variation in droplet dispensing - Generating droplets of uniform volume is critical in controlling the accuracy of the dilution. Therefore the reproducibility of droplet volume is first evaluated for both on-chip dispensing and dispensing assisted by off-chip pressure source. The on-chip dispensing is accomplished by first extending a liquid column from an on-chip reservoir by activating a series of electrodes. The electrodes other than the one where the droplet is to be formed are then deactivated. The electrode in the reservoir is activated to retract the liquid and pinch-off a droplet. Figure 5 shows the various steps involving in forming KCl droplets from an on-chip reservoir. The accuracy and repeatability of this volume depends on several factors, including aspect ratio (L/H), droplet/oil interfacial tension, liquid volume in the reservoir, number of pinch-off electrodes, actuation voltage and control sequence. All these factors contribute to the variations of droplet volume. From data obtained in two different droplet generation experiments, the calculated variation of the volume is less than 3%. The variation in droplet formation assisted by off-chip pressure source and capacitance

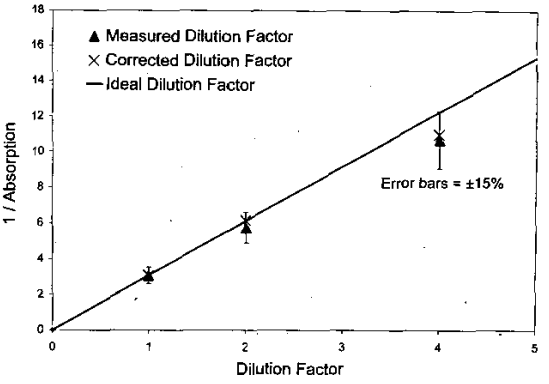

Figure 6 - Plot of expected vs. measured dilution factor for dilution factor of 2 and 4

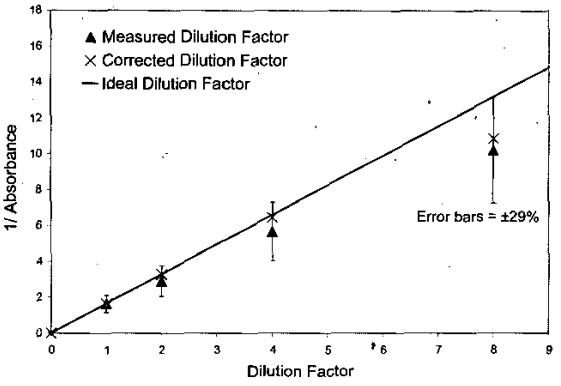

Figure 7 - Plot of expected vs measured dilution factor for dilution factors of 2, 4 and 8

feedback has been reported by us previously [5] and is less than 2%.

Volume variation in droplet splitting - Droplet splitting is another step that causes errors in the dilution factor. Splitting of a larger droplet into two smaller identical ones is highly sensitive to the volume of the larger droplet volume and timing of the switching sequence. The reproducibility of the volume in droplet splitting is tested over a range of droplet volumes (1.2μl-3.2μl), on electrowetting chips with an electrode pitch of L=1.5mm and gap of H=140um. The variation of volume (calculated as the volume after splitting divided by the total volume) is less than 7%.

Variation due to incomplete mixing – The dilution process can also be affected by incomplete mixing. In the dilution experiment described in this paper, mixing is assumed to be complete when the measured intensity (voltage) reaches a steady-state.

Error analysis for dilution –The total error in an N-step serial dilution is given by the following equation.

$$E_N = 1-(1-E_0)^N \qquad (1)$$

where E_N is the error in the N^{th} step, E_0 is the error in a single two-fold dilution step, and N is the number of dilution cycles. From this equation we can see that the error increases with the number of dilution cycles required.

The interpolating serial dilution architecture was tested for dilution factors of 2, 4 and 8. Figures 6 and 7 plot the inverse of the absorbance (1/concentration) as a function of the dilution factor. The straight lines in the plots correspond to the absorbance expected in the case of ideal dilution without any errors. 'Δ' (delta) represents the measured absorbance data. '×' (cross) corresponds to the expected absorbance, with a correction factor applied to account for volume variations. The volume variation is calculated by using the area of the droplet, obtained by processing the images captured before merging and after splitting. The deviation of the dilution factor from the ideal is ~15% for a dilution factor of 4 and ~25% for a dilution factor of 8. There is good agreement between the corrected dilution factor and the measured values, which shows that most of the error (~80%) in dilution is contributed by volume variations. The precision error for a single step 2-fold dilution is measured as ~8% (average from 5 independent results). From equation (1) we can calculate the error for the dilution factors of 4 and 8 to be 15% and 23%, which is consistent with the results observed.

CONCLUSIONS AND FUTURE WORK

An on-chip dilution architecture for an electrowetting based microfluidic chip has been demonstrated in this paper. Arbitrary dilution factors can be obtained using simple 2-droplet mixing and splitting operations. We have shown the on-chip dilution of a sample dye droplet by factors of 2, 4 and 8. The error in a single step dilution is ~8% and the total error increases with the number of dilution cycles. The total errors in the dilution factor are ~15% for dilution factors of 4 and ~25% for the dilution factor of 8, and is consistent with the expected values. Droplet volume variations in dispensing and splitting contribute to most of this error (~80%). The errors also increase with the number of mixing and splitting cycles as expected. Tighter tolerances are required on dispensing and splitting to reduce the dilution errors. Further experiments are also required to evaluate higher dilution factors.

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