Active Mixing Strategy with Electromechanical Platform for Lab-on-a-chip Applications

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***Abstract*— The main purpose of this study is to present a new active mixing strategy that can be used for lab-on-a-chip applications to shorten analysis time. An electromechanical platform composed of stepper and DC motors is designed and manufactured. This platform allows rapid mixing in microwells of a polydimethylsiloxane chip for analysis. Mixing in microwells is performed with a stirring bar spun automatically using the electromechanical platform. Mixing experiments performed at different spinning speeds and different time intervals on the platform. It was observed that mixing was achieved only in 300 ms inside 100 µL microwell using 4300 revolutions per minute (rpm) spinning speeds. Hence, the proposed mixing strategy showed 200-fold faster mixing than pure diffusion-based mixing.**

Keywords— active mixing; lab-on-a-chip; electromechanical platform; polydimethylsiloxane.

I. INTRODUCTION

Mixing is significantly important for homogenization of solutions and reagents in lab-on-a-chip devices [1]. However, due to the laminar fluidic characteristics in these devices, mixing is generally governed by a slow diffusion process. Rapid analysis can be performed in lab-on-a-chip systems with different mixing strategies that allow operating at reaction-limited regime [2, 3, 4]. Passive mixing only relies on the flow itself without any external forces. On the other hand, active mixing provides better control with using extraneous forces [5]. Many different strategies are available for passive and active mixing strategies in the literature. Passive mixers have two main groups composed of chaotic advection and molecular diffusion-based micromixers. Chaotic advection is more preferred for large molecules that have little diffusion coefficient or for rapid mixing [6]. Passive mixing strategies can be applied by different types of techniques such as; t-type micromixer, altering flow direction laterally, anisotropic grooves-based chaotic mixers, and geometrically splitting and recombining sub-streams [7]. Active mixing can be classified as pressure driven, acoustic based, electrokinetic-based, temperature-induced and dielectrophoretic-based [6]. For instance, a fixed volume chaotic mixing is one of the active mixing concepts in this domain [5]. This mixing concept is based on multiple source-sink flows in a microfluidic chip using microplumbing technology. With this technology, various biological analyses such as DNA and protein purification can be conducted rapidly [8, 9].

Here, we present a novel electromechanical platform that allows rapid mixing in lab-on-a-chip devices with an active mixing strategy. To perform mixing, a stirring bar is spun automatically inside microwells of a polydimethylsiloxane (PDMS) chip. Mixing performance is analyzed and compared with pure diffusion based mixing strategy.

II. EXPERIMENTAL

A. Electromechanical Platform

Electromechanical platform was proposed with 2 bipolar stepper motors for 2-dimensional movement and DC motor for rotating and mixing fluids in the microwells of PDMS chip (Fig. 1). We designed the platform based on the size and shape of stepper motors and DC motor. Height and width were calculated, so that the stirring bar, at the end of the DC motor, is fully inserted into the microwells on the PDMS chip and does not touch the microwell walls. The platform base was designed to keep the PDMS chip stable and easy to replace since the PDMS chip and stirring bar could change for each analysis. The width and thickness of the platform were optimally designed to support the frame of the platform. The platform was fabricated using Ultimaker 2+ 3D printer from polylactide. 2 high quality bipolar stepper motors with screw shaft and nut were used to move the system in x and y directions (right-left and up-down movements). A high-performance micro DC motor with rotating speed of 3000 rpm at 6V operating voltage was used for mixing. DC motor has carbon brushes to achieve high performance and long life. In addition, it is possible to increase operating voltage up to 15V for this motor. It allows for mixing of compounds in the microwells at high speeds. Controlling of these motors was operated by Arduino Mega 2560 R3 microprocessor and L298N voltage regulator dual motor driver board. In addition, the Pololu 3081 encoder, which can operate at 2.7-18V voltage values, was added to the shaft extension at the rear of the DC motor. Thanks to the encoder, the rotational speed of the DC motor at different voltages was measured.

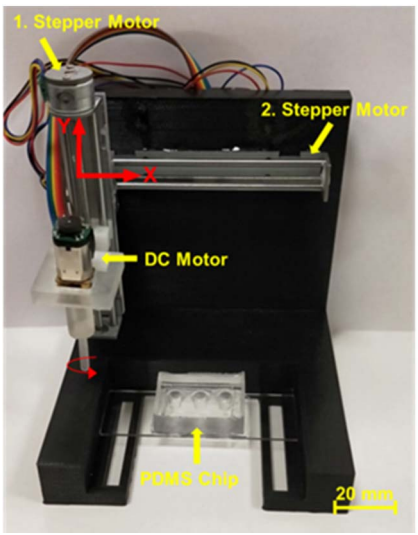

Fig. 1. Components of the electromechanical platform designed and fabricated for the study.

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B. PDMS Chip

PDMS chips were fabricated using soft lithography technique (Fig. 2). To do so, molds were fabricated using Formlabs Form2 3D printer. After a glass slide was treated with air plasma, it was placed between the molds. Then, molds were assembled together with screws. 3D printed pillars were introduced inside the molds to define microwells in the PDMS chip. After that, PDMS mixture prepared 10:1 weight ratio was poured inside the assembled molds and it was incubated overnight at 60°C. Then, molds were disassembled and PDMS chip was pulled out from the molds. The resulted PDMS chip had 3 equal dimension microwells with 5 mm diameter and 10 mm height.

C. Stirring Bar

Stirring bars having is 23 mm length, 3 mm width and 1.5 mm thickness (Fig. 3) were fabricated using Formlabs Form2 3D printer using Clear v2 resin. These bars are mounted on electromechanical platform and they are changeable in order to eliminate cross-contamination between the tests. The bars can easily go inside and go outsides of PDMS microwells with the electromechanical system.

D. Mixing Performance

To analyse mixing performance, 1 µL of red food color solution was pipetted in a microwell filled with 99 µL of water. After that, mixing was performed immediately.

The mixing index was used to measure the mixing performance inside a PDMS chip. To do so, PDMS microwell images were obtained using Zeiss Axio Vert A1 inverted microscope equipped with 1.25 × objective and color camera. The captured images were analyzed to get time dependent mixing index values (MI) as follows [10]:

$$MI(t)=\sqrt{\frac{1}{N}\sum_{k=1}^{N}\left(\frac{I(t,k)-I_{avg}(t)}{I_{avg}(t)}\right)^2} \quad (1)$$

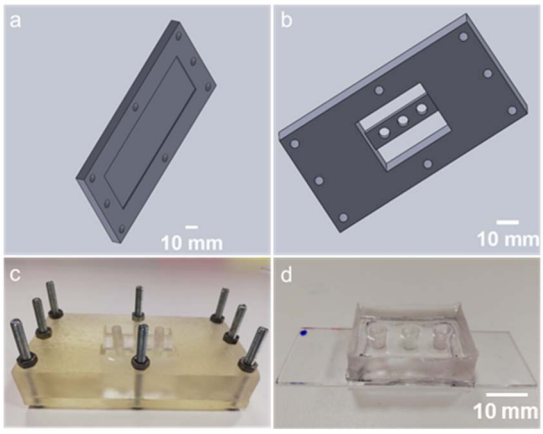

Fig. 2. Design and fabrication of PDMS chip. a) Bottom and b) top molds, which are assembled together with screws. c) Photographs of assembled mold pieces. d) Fabricated PDMS chip having three microwells

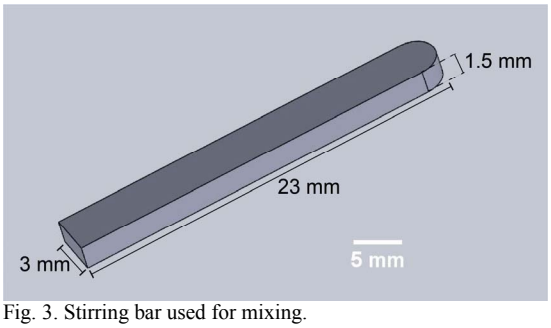

Fig. 3. Stirring bar used for mixing.

In this equation, t is the time, N represents the total number of pixels, $I(t,k)$, the intensity of the pixel k at time t, and $I_{avg}(t)$ represents the average density value of all pixels at the time t. As the mixing index value approaches zero, the mixing homogeneity is obtained. Good mixing profile can be achieved when mixing index value reaches to 0.1 [5].

Solutions in the microwells on the platform must be distributed homogeneously for efficient mixing. To show that homogeneity is achieved, the time-dependent mixing index value was calculated [5]. In order to find the mixing index of the system, the DC motor was rotated at different speeds and the water and food dye in the microwell were mixed.

III. Results and Discussion

To locate microwells, the stepper motors moved the stirring bar in the horizontal and vertical directions with a speed of ~ 0.9 cm/s in the platform. DC motor is used to spin stirring bar inside PDMS microwells. Rotational speeds of the DC motor can be tuned with applied voltages (Fig. 4). When applied voltage is increased, motor rotational speed of the DC motor increase linearly ($R^2 = 0.997$).

Mixing homogeneity is expected to be reached rapidly while using high DC motor speeds. On the other hand, high DC motor speeds can spill liquid out of microwells. As shown in Figure 5, when the DC motor speed was larger than 4800 rpm (> 9V), liquid spills were observed during mixing process. Hence, DC motor speed was set to 4300 rpm by using 8V supply for mixing experiments.

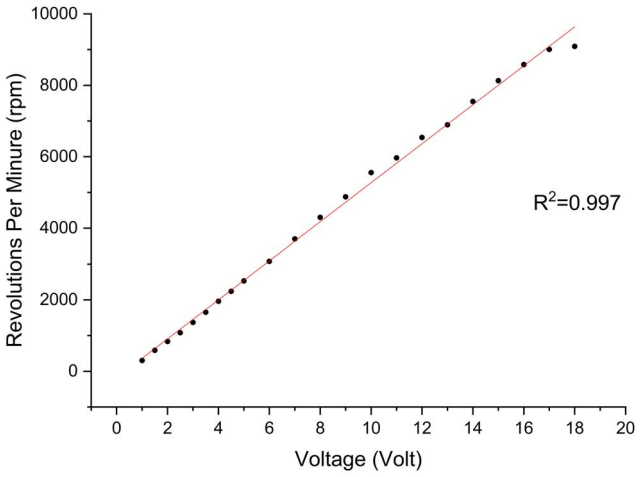

Fig. 4. Rotational speed of the DC motor with applied voltage.

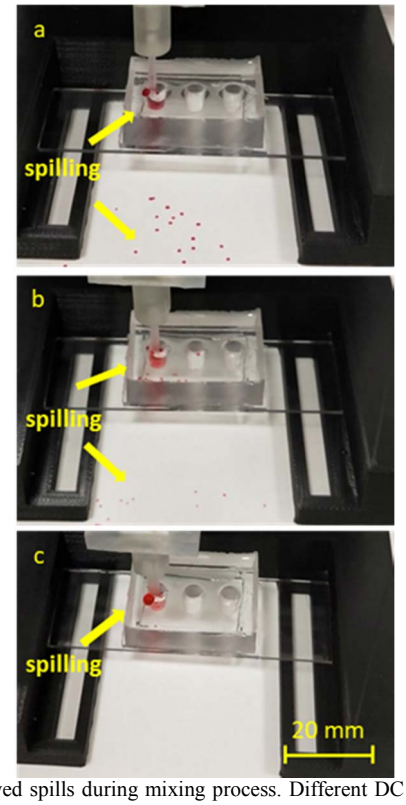

Fig. 5. Observed spills during mixing process. Different DC motor speeds were utilized by applying a) 10V, b) 12V and c) 15V.

We performed active mixing for 1s. Microwell photographs were captured under an inverted microscope at 100 millisecond intervals (Fig. 6). As a result of the mixing experiments, it was observed that the mixing index value reached below 0.1 after 0.3 s (Fig. 7).

Pure diffusion-based mixing was also characterized in PDMS microwells. For this purpose, stirring bar was removed from the platform and color homogeneity was inspected for 1 hr. Color homogeneity was obtained slowly in microwells (Fig. 8) and 0.1 mixing index value is reached nearly 60 s (Fig. 9).

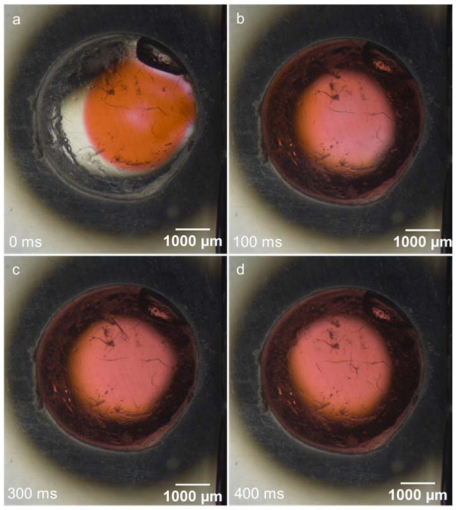

Fig. 6. Active mixing. Micrographs of microwells at a) 0 ms, b) 100 ms, c) 300 ms and d) 400 ms after starting experiment.

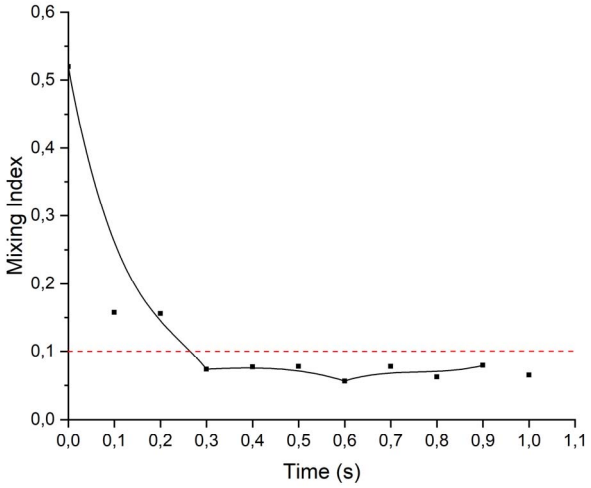

Fig. 7. Time-dependent mixing index values obtained for active mixing.

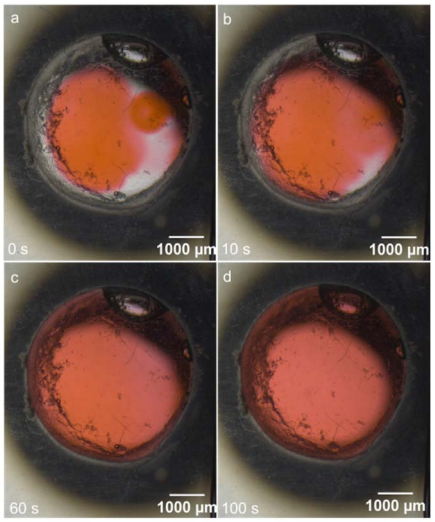

Fig. 8. Pure diffusion based mixing. Micrographs of microwells at a) 0 s, b) 10 s, c) 60 s and d) 100 s after starting experiment.

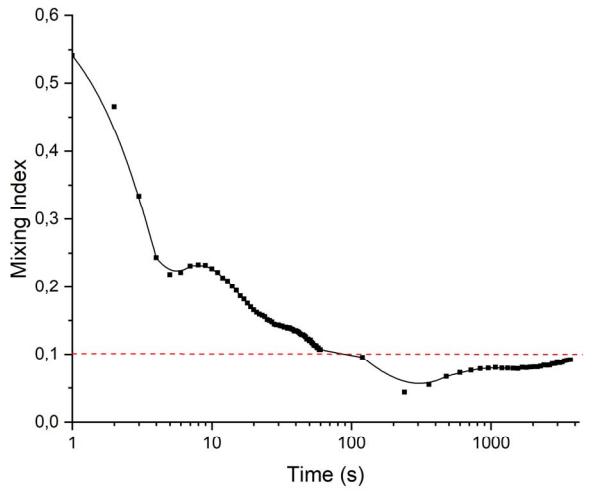

Fig. 9. Time-dependent mixing index values obtained for pure diffusion-based mixing.

These results show that the proposed mixing strategy can achieve a homogenous mixture 200-fold faster than pure diffusion-based mixing. With respect to these results, we can use this technique to mix any kind of solution in quantitative lab-on-a-chip analysis.

IV. Conclusion

Here, we presented a new mixing strategy for lab-on-a-chip applications using electromechanical actuations. Mixing index gives us information about the homogeneity of mixture. Therefore, we investigated the mixing index of this active mixing strategy. Then, this mixing strategy was compared with pure diffusion. It was seen that active mixing is 200 times faster than pure diffusion. The future aim of this study is to determine disease biomarkers automatically on this lab-on-a-chip platform for point-of-care analysis.

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