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RESEARCH PAPER

Micro-reagent Dispensing Method Based on Pulse Driving & Controlling of Micro-fluids Technology and Application Research

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Abstract: With the development of bioanalytical technique and high throughput screening techniques, the amount of reagent was decreased to nanoliter and even picoliter level. To achieve high precision of micro-reagent dispensing, a two-channel micro-reagent dispensing system based on pulse driving & controlling technique of micro-fluids was developed. Then, taking glycerine solution as dispensing reagent, influences of viscosities, inner diameter of the micro-nozzle, driving frequency, and voltage amplitude on the dispensing volume were investigated. With the voltage amplitude of 70 V, driving frequency of 4 Hz, and 100 μm inner diameter of the micro-nozzle, different ratios of Na_2HPO_4 to KH_2PO_4 solution were prepared to study the mixing-reaction. In the experiment, a 3 × 3 phosphate buffer arrays solution with a pH gradient was first prepared. pH indicator was then added to detect the acidity and alkalinity of the mixed solution. The results showed that the relative standard deviation (n = 9) of spots diameter of the prepared pH gradient arrays was 0.8%; the spots were fully reacted, and uniform colors and significantly changed gradients were achieved. By the micro-reagent dispensing method based on pulse driving & controlling of micro-fluids technique, parallel, automated and micro volume (picoliter) dispensing with higher dispensing precision and higher repeated accuracy could be realized, and especially, micro reaction with different ratios could be achieved without separate “reaction pools”.

Key Words: Micro-reagent dispensing; Two-channel; Pulse driving and controlling of micro-fluids technology

1 Introduction

High-speed and accurate micro-reagent dispensing which is an indispensable technology has been widely used in the fields of life science such as protein crystallization, drug screening, gene sequencing and so on[1–5]. To realize micro reaction and analysis, different kinds of reagents are usually needed to be transferred into the same “reaction pools”. With the development of bio-analysis technique and high-throughput screening technique, the sample volumes should be reduced to increase the screening chance with a given amount of sample. Therefore, high dispensing speed and accuracy are required in a single experiment. It is of great significance to develop novel method of automatic liquid dispensing for transferring micro-reagent, which can improve the dispensing accuracy, decrease the time involved and reagent consuming, thus enhance experimental techniques in biomedical areas[6–9].

Currently, automatic liquid dispensing mainly includes contact and non-contact methods. In the contact dispensing method, the nozzle contacts with the substrate, and reagent is transferred to the substrate due to interface external force. Although this method is simple and flexible, it may bring pollution while the nozzle contacts the substrate and it is difficult to achieve accurate dispensing when reagent volume reduces to nanoliter scale[10].

In non-contact dispensing method, the nozzle needs not to

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contact with the substrate, an external force is used to provide enough energy to overcome liquid surface tension and viscosity, and then the droplet could be ejected out of the nozzle. Compared with the contact dispensing method, non-contact dispensing method is a mainstream way for micro-reagent dispensing due to its high-speed, non-pollution and micro volume. The current non-contact micro-dispensing technology varies on the basis of the driving types such as piezoelectric, thermal and pneumatical driving. Piezoelectric dispensing is realized by the displacement of piezoelectric elements applied with a pulse voltage signal, with which the volume of the chamber can be changed and a droplet is jetted out of the orifice. It has several advantages such as fast response, wide frequency range and picoliter dispensing volume. However, the piezoelectric head is structural complexity, high cost and difficult to disassemble and repair[11]. The thermal driving type is simple and can be used in high-density integration of nozzle arrays. However, it may affect the activity of the biological sample as it needs to be heated while the droplet is ejected from the nozzle[12].The pneumatic driving type, with a simple structure, is widely used and can work at higher temperature environment. However, unsteady ejection may occur with the gradual decline of liquid level in the chamber, which will result in poor droplets controllability[13].

Pulse driving and controlling of micro-fluids technique, also called micro fluidic digitalization technique, could realize micro-reagent (picoliter level) dispensing with simple, pyrogen-free and low-cost micro dispensing system[14]. In order to solve the problems mentioned above in the micro reagent dispensing technology, a novel precision dispensing system based on pulse driving and controlling technique of micro-fluids was developed, and automated two-channel dispensing of micro-reagent was realized in this study. The impacts of system parameters on the dispensing amount were experimentally investigated. Moreover, a pH gradient microarray of phosphate buffer solution was prepared by mixing Na_2HPO_4 and KH_2PO_4 solution in different ratios with the two-channel dispensing system.

2 Materials and methods

Pulse inertia force generated by various impact or vibration is the driving source to produce droplets in the pulse driving and controlling of micro-fluids system. In this study, piezoelectric actuator was used as the driving source to provide micro-fluidic pulse inertia force, since the pulse inertial force generated by piezoelectric devices had many merits, such as high response frequency and excellent dynamic response of voltage-displacement. The parameters of pulse driving and controlling of micro-fluids system were as follows: driving voltage waveform (driving waveform), driving frequency *f*, voltage amplitude *U* and micro-nozzle inner diameter *d*. As shown in Fig.1, by applying a driving waveform, piezoelectric actuators generated a pulse inertia force[15]. When the amplitude of the voltage rose instantly from 0 V to the maximum, the direction of the acceleration generated by piezoelectric actuator would be changed, which provided pulse inertia force to the micro-nozzle. The acceleration of the piezoelectric actuator could be adjusted by changing the voltage amplitude, which was used to control the pulse inertia force, and thus to accurately control the volume of a single injection of the liquid. The devices with neither micro movable parts nor embedded microcircuits have the advantages of high reliability, resisting solid particles jam and bubbles block, simple structure, low-cost, and appropriate working conditions which is of benefit to maintain the biological activity[16].

The schematic diagram of the two-channel micro-reagent dispensing system is shown in Fig.1.

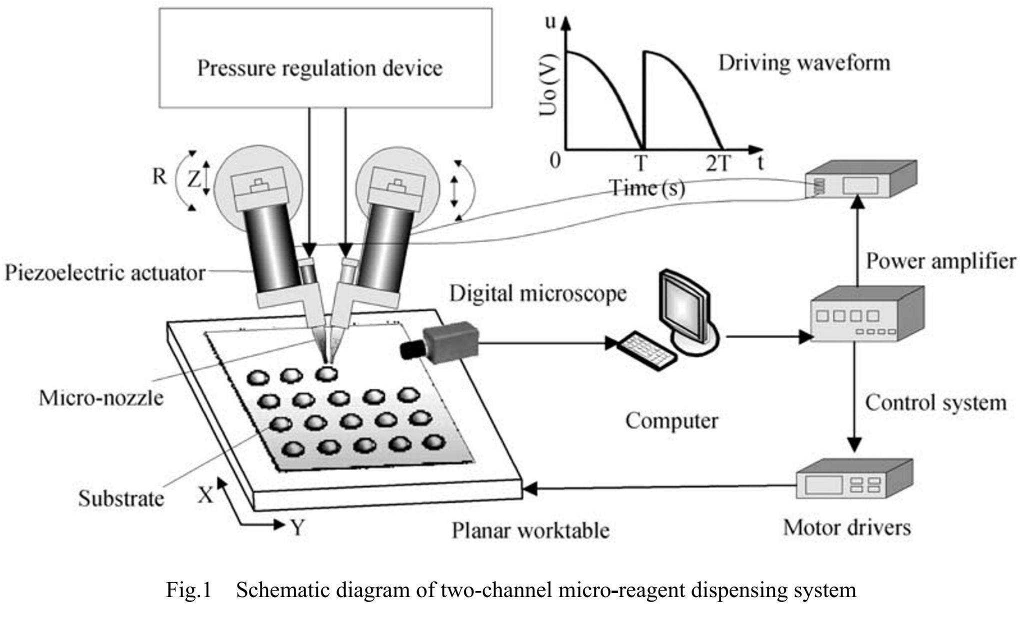

Fig.1 Schematic diagram of two-channel micro-reagent dispensing system