

## Research Article

### Simulation and Experiment Research of Non-contact Micro-liquid Reagent Dispensing

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**Abstract:** With the development of biological analytical techniques and high throughput screening techniques, a large number of automated biological agents dispensing systems are widely used in the field of life science research. Non-contact dispensing method characterized by its small dispensing volume, high dispensing precision and quick service speed which satisfies the requirements of biological agent's distribution, becomes the mainstream dispensing method used in reagent dispensing systems. However, the difficult control way, complex system of non-contact dispensing method and its vulnerable dispensing process, which can be easily affected by characteristics of the agent, have hindered the application and development of the method. In this study, simulation model of the separation process of micro scale biological reagent are constructed in order to solve the above problems. The prerequisites of the non-contact dispensing method, the relationship between dispensing volume and precision and characteristics of reagents, formation of system and control parameters are researched through theoretical analysis and numerical simulations. The experiment results are quite consistent with the simulation results; therefore it well verifies the reliability of the modeling and simulation method and also lays a firm theoretical foundation for the system design of non-contact micro-liquid reagent distribution and control optimization.

**Keywords:** Micro-liquid dispensing system, micro-liquid reagent, non-contact dispensing, VOF

## INTRODUCTION

In the field of life science, it is often needed to analyze all kinds of liquid reagents. At this point, the volume reduce has great significance to increase the heat exchange rate between different reagents, shorten the time to reach equilibrium of binding reaction and decrease the consumption of expensive biological reagents (Horrocks *et al.*, 2012). With the development of bio-analytical techniques, the volume of single reagent used in related life science has been reduced to microliters and even nanoliters (Minh *et al.*, 2010). Then the exact distribution of micro-liquid reagent becomes an important factor in the success of experimental study. With the development of automation technology, various types of automated liquid dispensing devices have been widely used in the life sciences. These devices which have the characteristics of small volume of distribution reagent, fast operating speed and high precision allocation have played an important role in life science (Tropmann *et al.*, 2012; Liu *et al.*, 2007a). However, in different areas of life science, types of reagents used are very different and the range of required dispensing volume is very wide (Minh *et al.*, 2010; Li *et al.*, 2009), all of

these differences have different effects on the dispensing process and make different requirements for structure and control methods for micro liquid dispensing systems. In order to ensure that different types of micro reagent can be distributed precisely and provide theoretical basis for the designing and optimization of control parameters of automatic micro-liquid dispensing system, it is necessary to study the process of micro-liquid distribution.

Currently, there are 2 distribute methods for automatic micro-biological reagents operation: contact and non-contact dispensing (Liu *et al.*, 2007b; Sun *et al.*, 2008; Yao *et al.*, 2011). And the contact dispensing method which rely on the contact between reagent nozzle and substrate to achieve liquid transfer has the problems of slow operation, difficult for micro volume's distribution and has the risk of contact pollution. In the existing liquid distribution systems, this method is used not frequently (Jia *et al.*, 2007). Differently the non-contract distribution method relies on the external energy to overcome the liquid's surface tension, viscosity and other effects to push out the liquid from the nozzle with a high-speed. This method overcomes the problems of low dispensing speed and easy polluted occurred in contract method and more

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suitable for the dispensing of micro volume reagents (Liu *et al.*, 2007a; Sun *et al.*, 2008). Therefore, this method has been widely used in micro-liquid handling systems. But this method has the characteristics of complex principle and control and sensitive to reagent specificity, volume, dispensing speed and nozzle.

In this study, simulation and experimental research methods are used in the study of non-contact distribute process of micro-liquid reagent to obtain the factors' impact on the non-contact dispensing process and provide theory support for the control of non-contact dispensing method and the design of liquid reagent dispensing system.

### THEORETICAL MODELING OF DISPENSING PROCESS

**Construction of theoretical model:** Considering that there are 2 immiscible fluids which are gas and liquid when the micro reagent jets out from the nozzle, VOF flow model (Abe and Koro, 2006) adopted to establish the solution equation. For the purpose of easy study, give the following assumptions: the spray reagent is always axisymmetric in the evolution, all the phases are incompressible Newtonian fluid, each phase is in laminar flow and the flow is one-dimensional.

In the entire flow field define a function  $C$ , which is the ratio of the fluid volume to computing grid volume, suppose the computational domain is  $W$ , the domains are respectively  $W_1$  and  $W_2$  occupied by 2 fluids. Define the function:

$$\alpha(x_r, t) = \begin{cases} 1 & x_r \in W_1 \\ 0 & x_r \in W_2 \end{cases} \quad (1)$$

Then the VOF equation can be expressed as:

$$\frac{\partial C}{\partial t} + v_r \cdot \nabla C = 0 \quad (2)$$

where,  $v_r$  represent the fluid velocity field. And in every grid  $I_{ij}$ , define  $C_{ij}$  as  $\alpha(x_r, t)$  integral on the grid:

$$C_{ij} = \frac{1}{\Delta V_{ij}} \int_{I_{ij}} \alpha(x_r, t) dV \quad (3)$$

Define air as the main phase and liquid reagent as the second phase, then the volume fraction of liquid reagent meets the following:

$$\frac{\partial C_2}{\partial t} + v_r \cdot \nabla C_2 = 0 \quad (4)$$

The volume fraction of air is:

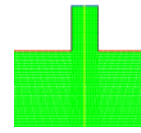


Fig. 1: Grid figure of simulation model

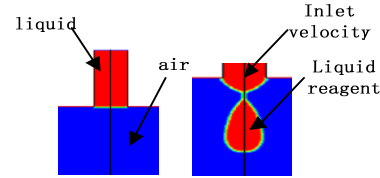


Fig. 2: CFD simulation model of dispensing process

$$C_1 = 1 - C_2 \quad (5)$$

where,  $C_1$  and  $C_2$  respectively represent air and liquid reagent volume fraction.

In the control equation, the calculation of volume fraction average parameters (density, viscosity, temperature, etc.) can be obtained by averaging the volume fraction of each fluid on each grid cell. Such as the volume fraction average density is:

$$\rho = C_2 \rho_2 + (1 - C_2) \rho_1 \quad (6)$$

**Finite element mode:** In the course of reagent injection, the nozzle axial direction is parallel to the gravitational field and the fluid in nozzle can be treated with axisymmetric and non-circumferential speed, thus axis boundary condition is adopted in simulation geometry area, inlet velocity boundary condition is adopted on the surface of the liquid flow in nozzle, that is the speed direction along the axial, in the injection time, the fluid flows into the needle with the speed of  $U$ , when the injection stop the speed is 0, the pressure outlet boundary condition is adopted in the gas interface, the relative pressure is 0, Fig. 1 shows the 2-dimensional axisymmetric model and mesh effects based on GAMBIT. The initial state of simulation is set that the nozzle is filled with liquid and the reagent jets out from the nozzle with a certain speed under the external pressure, the model analysis can be simplified as shown in Fig. 2, where the red part is liquid reagent and the blue part is air.

In micro reagent dispensing, there are four forces influent the dispensing process: fluid inertia, viscous drag forces, surface tension and gravity. The relationship among these forces can be described as following dimensionless parameters: Reynolds number  $Re$ , represent inertia force and viscous force ratio, capillary number  $C_a$ , represent viscosity and surface tension force ratio, Weber number  $We$ , represent inertia

Table 1: Parameters used in simulation

Characteristics	Value ranges
Density $\rho$ (kg/m <sup>3</sup> )	500~5000
Surface tension $\sigma$ (N/m)	0.01~0.3
Viscous $\mu$ (Pa.s)	0.0001~0.05
Distribution volume V ( $\mu$ L)	0.01~20
Distribution needle radius R (mm)	0.076~0.15
Flow speed u (m/s)	0~2.0

and surface tension rate, bond number G, represent gravity and surface tension ratio. And each parameter is show in Eq. (7) to (10):

$$G = \frac{\rho R^2 g}{\sigma} \quad (7)$$

$$Re = \frac{\rho u R}{\mu} \quad (8)$$

$$We = \frac{\rho u^2 R}{\sigma} \quad (9)$$

$$Ca = \frac{We}{Re} = \frac{\mu u}{\sigma} \quad (10)$$

where,

$\rho$  = Density

$\sigma$  = Surface tension

$\mu$  = Viscosity

u = Speed

R = Distribution needle radius

Obviously the dimensionless parameters mentioned above is related to reagents viscosity, surface tension, density, nozzle size, flow rate, etc., the ranges of these parameters are shown in Table 1.

In practice, the surface tension of a variety of reagents are usually smaller than distilled water's (0.073 N/m) and the density is about 1000 kg/m<sup>3</sup>, combined with Table 1 and Eq. (7), the Bond number G value is approximately  $3.0 \times 10^{-3}$ , so the impact from surface tension to the micro reagent distribution process is much greater than the gravity. Therefore, in the micro-liquid reagent dispensing process the impact from the gravity can be ignored and the inertial force is the main factor to overcome the surface tension to push out the liquid reagent from the nozzle.

## SIMULATION RESULTS AND ANALYSIS

**Analyzing the effect of reagent properties on dispensing process:** First, the dispensing process of the reagents of density of 1000 kg/m<sup>3</sup> at a flow rate 1.0 m/s within the nozzle of a radius of 0.127 mm is simulated with dispensing volume 0.1  $\mu$ L. Under the situation of modifying surface tension and viscosity to change We

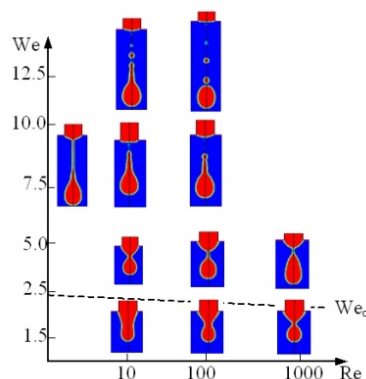


Fig. 3: Simulation results under different reagent We and Re number

and Re values, (Fig. 3) shows instant distribution simulation image of corresponding liquid reagents with different We, Re states. We can see, by the chart, the reagents spray or adhere to the nozzle in the form of drops.

Figure 3 shows that in the dispensing process of micro reagent, there exists a critical Weber number (referred to dotted line in the figure, which is shown as  $We_c$ ). When We actually is lower than the critical value, the reagent droplets still adheres to the nozzle end; only when We is larger than the critical value, can the reagent droplets be able to jet out from the nozzle tip to achieve non-contact dispensing, but at this time, part of the liquid is still sucked back to the nozzle tip; when  $We_c$  is located between 7.5 to 10.0, 3 to 4 times of the critical Weber number, all spray reagents can be separated from the nozzle in the form of droplets; later, with the continued increase of We, reagent droplet is stretched longer and the advents of satellite droplets and spray phenomena are appeared. The phenomenon can be understood like this: We stands for the ration of inertial force and the ratio of surface tension, when surface tension is larger, the pull of nozzle on the droplets is larger, inertial force is not sufficient to overcome the binding role of the tension in order to separate the droplet from the nozzle end; with the decreases of surface tension, the role of droplet inertia is enhanced. When it can balance the binding effect of surface tension, the droplets have the energy of being separated from the nozzle, but at this time, there are still some reagents being sucked back and the actual dispensing volume is lower than expected volume; with further strength of the role of inertia, spray droplets have a greater kinetic energy so that all reagents can spray from the nozzle in the form of droplets and the reagent accuracy is highest; when the force of inertia is too large, the energy of spray reagent is surplus so that the phenomenon of spray droplets and the satellite is

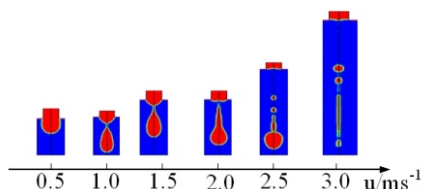


Fig. 4: Simulation results under different jet speed

appeared, which will seriously reduce the accuracy of distribution and should be avoided in the application.

Figure 3 also shows that as  $Re$  increases,  $We_c$  is slightly reduced. By the formula (8) we can see,  $Re$  stands for the ratio of the inertia and viscous force, when the viscosity is reduced and leads to  $Re$  increases, the inertia force of droplets is relatively increased, it has a higher energy to spray from the nozzle. Therefore, the viscosity will increase  $Re$  number and decrease  $We_c$ . But when  $We$  is located between 3 ~ 4 times of critical value, changes of  $Re$  number doesn't change the separated state of reagent droplets. From (10) shows that the capillary number  $Ca$  stands for the ratio of the viscous forces and surface tension, with the increases of viscous force, the capillary number is also increasing. Figure 3 shows, when the capillary number increases, the droplet will be stretched longer.

**Analyzing the effect of jet speed on dispensing process:**

By the formula (8) and (9), we know that  $We$  and  $Re$  are related to the jet speed. For specific reagent, the reagent characteristics are determined. At this time,  $We$  and  $Re$  can only be adjusted by adjusting the jet speed. For specific reagents (surface tension of 0.109 N/m, viscosity 0.005 Pa.s), this study carries out simulation of 0.1  $\mu$ L distribution under different jet speed and the transient simulation image is shown in Fig. 4. In Fig. 4, when the flow rate is 0.5 m/s,  $Re$  is 25 and  $We$  is 0.5734, lower than  $We_c$ . At this time, the surface tension and viscous force are significant and the inertia force of spray reagents is small. Reagent droplet cannot be divorced from the nozzle; when flow rate is 1.0 m/s,  $We$  is 2.2935 and  $Re$  is 50, the droplet can be divorced from the nozzle, but there is still part of the reagent sucked back to the nozzle, resulting that the reagent distribution is lower than expected volume; when the jet speed increase to 2.0 m/s,  $Re$  is 100 and  $We$  is 9.174, located between 3 to 4 times of  $We_c$ , the spray reagent breaking point flushes with the basic end of the nozzle, then no residual liquid adhere to the nozzle, all of reagents blend into droplets; later, with the increase of velocity, spray reagent is stretched very long and a number of satellite droplets forms after break, which would seriously reduce the accuracy of dispensing and should be avoided in applications.

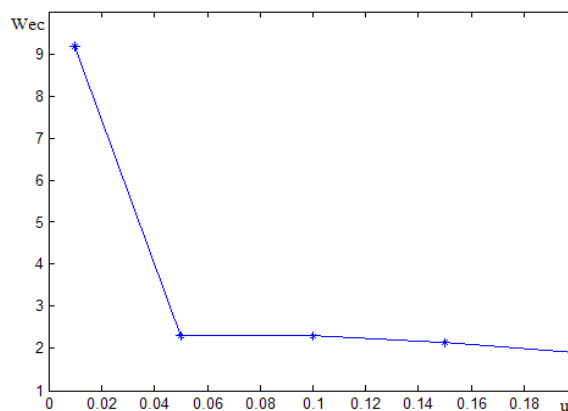


Fig. 5: Relations between  $We_c$  and dispensing volume

It can be seen, in order to make the droplets divorce from the nozzle to achieve a successful non-contact dispensing, the key is to make  $We$  located between 3 to 4 times of  $We_c$  by adjusting the jet speed.

**Analyzing the effect of reagent volume on dispensing process:**

This section researches the critical Weber number corresponding to the different dispensing volume by using the nozzle with a 0.127 mm radius. The simulation results are shown in Fig. 5.

By the figure shows, when the dispensing volume increases,  $We_c$  will be reduced. This is mainly because, as the dispensing volume increases, the gravity of spray reagent is also increasing. To some extent, it overcomes the binding effect of the surface tension and viscous force, leading to  $We_c$  reduced, when allocating volume reaches a certain level, the gravity force of reagent itself is sufficient to completely overcome the shackles of the force mentioned above, so that non-contact dispensing can be achieved at very low injection speed. Figure 5 also shows that when the dispensing volume is very small,  $We_c$  surges seriously, allocation of 0.02 reagents corresponding to  $We_c$  greater than 9 and a much higher jet speed is needed.

In the actual distribution, not only require the reagent be divorced from the nozzle tip, but also ask for a higher dispensing accuracy (a small amount of residue) and dispensing quality, this study simulates the distribution of different reagent volumes with different  $We$ , the results are showed in Fig. 6. Figure 6a shows that, when  $We$  is 1.6667 and dispensing volume is 0.5  $\mu$ L, the droplet is still not able to spray from the nozzle end, but when the volume increased to 1.0, the droplets can be ejected from the nozzle, indicating that the dispensing volume increase can decrease  $We_c$ , which is consistent to Fig. 5. At this time, a number of large

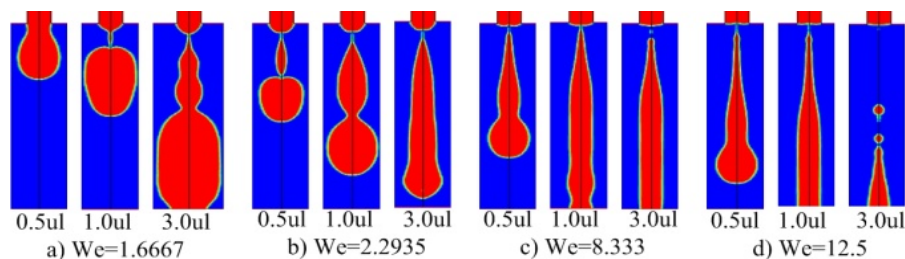


Fig. 6: Simulation results under different  $We_c$  and dispensing volume

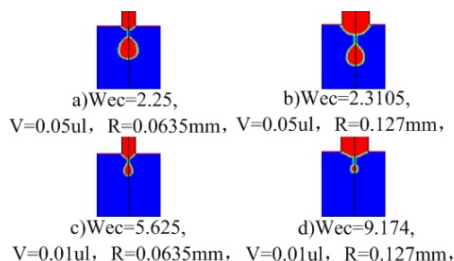


Fig. 7: Simulation results under different interior radius and dispensing volume

droplets, which is formed because of reagents' fracture, falls into the micro plate and can easily result in the dramatic impact of the liquid which has been in the micro plate and liquid splashing. At this time, part of the reagent has been sucked back to the nozzle. As  $We$  increases, the spray droplets have a greater kinetic energy. After ejection, it can be stretched longer and the residual liquid in the nozzle tip has also been reduced. As shown in Fig. 6c, when  $We$  is 8.333, the spray reagent ejected from the nozzle in the form of a long liquid column and almost no residual liquid exists in the nozzle tip, indicating that the distribution has a better effect. When  $We$  continue to increase, with the increases of reagent jet speed and dispensing volume, satellite droplets began to appear at the end of each distribution. It can be seen, when the large volume of reagent can spray in the form of liquid column into the micro plate format after injection without residual liquid in the nozzle end and formation of satellite droplets, the effect of distribution is best. Comparing Fig. 6 and 3, it can be showed that when  $We$  is located between 3 to 4 times of  $We_c$ , the reagent can still ensure a good dispensing results after the increases of volumes. Therefore, in the specific operation, the modulated system parameters can be applied to the reagent distribution of the large volume.

**Analyzing the effect of nozzle diameter on dispensing process:** Figure 5 shows that, when the volume becomes very small,  $We_c$  will dramatically increase. At this time, it requires a large speed to ensure

the reagent droplets can be divorced. But the high speed of reagent flow will bring some difficulties to the control system. Therefore, the study of how to achieve dispense small volume reagent (smaller than 0.1  $\mu\text{L}$ ) with smaller flow rate is very necessary. At the same jet speed, when the nozzle radius is reduced, the volume distributed during the same periods is reduced. Figure 7 shows the simulation results of divorced process when the nozzle radius is 0.0635 and 0.127 mm. This figure shows that when the nozzle radius is 0.127 mm and distribution volume is 0.05  $\mu\text{L}$ , the residue of droplet is large and when the radius is reduced to 0.15 mm, the spray reagent has little residue, indicating that the accuracy of distribution is high; in the distribution of 0.01  $\mu\text{L}$ , the nozzle with a radius 0.127 mm needs  $We_c$  number 9.174, but when 0.0635 mm is applied,  $We_c$  significantly decreased and the amount of reagent residues is small, indicating a high degree of accuracy.

It can be seen, when the dispensing volume is small, a smaller interior radius of the nozzle can not only reduce the critical Weber number, but also improve dispensing accuracy.

**Experiment results and analysis:** In order to verify the correctness of the simulation results, this study conducts non-contact micro-liquid reagent dispensing experiments on a self-built experiment platform. The experiment platform mainly composes of pressure regulating module, dispensing module and control module and it uses an external air source to drive the flow of liquid reagent in the pipeline to get a required flow speed and a high-frequency electromagnetic valve to control the pipeline to achieve the goal of quantitative distribution of the reagent. The composition and control principle of experiment platform is shown in Fig. 8.

In the experiment, distilled water, medical alcohol and 10% glycerol are used as test reagent; their relative properties are shown in Table 2.

**Experiment of micro-liquid departing:** Through simulation, we obtained the condition on how the micro-liquid will depart from the nozzle tip and effect

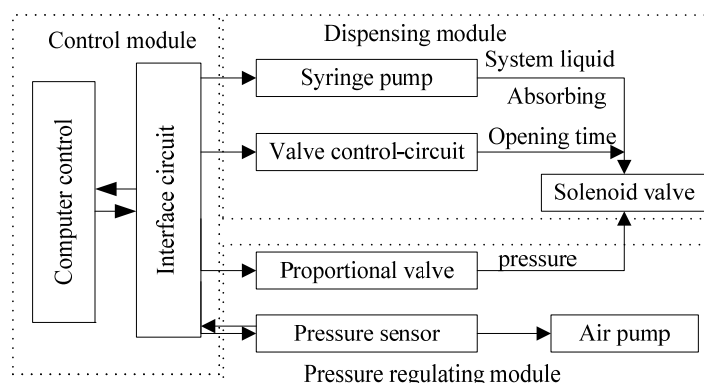


Fig. 8: Composition and control principle of experiment platform

Table 2: Liquid reagents used in experiments (standard atmospheric pressure, 25°C)

Reagents	Distilled water	Medical alcohol	10% glycerol
Surface tension (mN/m)	71.01	22.03	69.0379
Viscosity (mPa.s)	1	1.10	8
Density (kg/m <sup>3</sup> )	1000	800	1026

Table 3: Actual dispensing volume (radius: 0.0635 mm, length: 8.89 mm, expected volume: 5 μL)

Volume (μL)	Speed (μL/s)			
	30	40	50	60
Reagent				
Distilled water	4.845	4.758	4.758	4.750
10% alcohol	4.840	4.799	4.713	4.720
Medical alcohol	4.812	4.786	4.708	4.735

Table 4: Actual dispensing volume (radius: 0.0635 mm, length: 8.89 mm, expected volume: 10 μL)

Volume (μL)	Speed (μL/s)			
	30	40	50	60
Reagent				
Distilled water	9.925	9.811	9.778	9.772
10% alcohol	9.934	9.694	9.695	9.765
Medical alcohol	9.915	9.833	9.705	9.744

Table 5: Actual dispensing volume (radius: 0.038 mm, length: 8.89 mm, expected volume: 5 μL)

Volume (μL)	Speed (μL/s)			
	30	40	50	60
Reagent				
Distilled water	4.898	4.920	4.893	4.908
10% alcohol	-	-	-	-
Medical alcohol	4.914	4.690	4.670	4.718

of reagents' properties on departing process. Three reagents mentioned above are used on different conditions of system pressure, electromagnetic valve opening time and nozzle end size to verify the correctness of the simulation. Relative experiment phenomenon is concluded as follows:

- When other factors maintain the same, there is a critical value of the system pressure and only when the pressure is greater than the critical value, the popped reagent can depart from the nozzle tip.

- When other factors maintain the same, increasing the opening time of the electromagnetic valve and decreasing the interior radius of nozzle, the reagent departs more easily.
- When other factors maintain the same, the reagent departs more easily from a smaller nozzle end.
- When other factors maintain the same, the reagent departs more easily when the surface tension and viscosity decrease.

From the working principle of the experiment platform, we know, there is a critical jet speed accords to the critical system pressure, only when the reagent speed is greater than the critical jet speed can the reagent depart from the nozzle tip, this is supported by the simulation. Moreover, increasing the opening time of electromagnetic valve equals to increasing the volume of the jetted reagent, the increase of volume of jetted reagent and decrease of nozzle size will decrease the critical Weber Number, this will also make the reagent depart easily. When the surface tension and viscosity decrease, the jetted reagent meets a smaller binding force and thus also decrease  $We_c$ , the reagent drops easily. From the experiments, we see the experiment results are consistent with the simulation results.

**Experiments of micro-liquid dispensing accuracy:**

Non-contact reagent dispensing requires not only the reagent departing from the nozzle end, but also a high dispensing accuracy, this study also conducts experiments on the accuracy of distributed reagent. As the volume of the distributed reagent is so small to be measured directly, we firstly measure the weight of the reagent and then convert it to volume by the reagent density. The experiment results are shown in Table 3 to 5.

From the data in the tables, we can see, there is a relatively small difference between the actual distributed reagent volume and the desired volume. But as the jetted speed increasing, the dispensing accuracy is in a trend of decrease, the volume difference becoming bigger. From the results of simulation, when the Weber Number is controlled in the range of 3 to 4 times of the critical value, system obtained the highest dispensing precision, whereas, when  $We$  is smaller, there is too much reagent remain in the nozzle end after the main drop departed, this resulted of smaller dispensing volume and when  $We$  is too big, the jetted reagent is distributed in a large scale, this leads to atomization and air entrainment, all these end up with decrease in dispensing accuracy. When the kind of reagent and size of nozzle are confirmed, reagent jet speed is the only parameter which will affect  $We$ , so there is a range of flow rate at which the dispensing accuracy is the best. Comparing Table 3 with Table 4, at the same jet speed and dispensing volume, the accuracy is improved when the interior radius of nozzle is decreased, this is because a smaller nozzle size has decreased the critical Webber Number and in hence the jet speed plays a less important role in the dispensing accuracy. Table 3 and 4 also indicate that the dispensing accuracy increased when the reagent dispensing volume becomes larger, this is because, when the dispensing volume increases, the reagent jetted from the nozzle tip in the form of liquid column, then the remaining reagent in the nozzle end has a decreased proportion of the total volume, thereby increasing the dispensing accuracy.

From the analysis of the experiments data, we found that at the same jet speed and same dispensing volume but different kinds of reagent, the dispensing accuracy of medical alcohol, distilled water and 10% glycerin is in the trend of decrease. In the analysis of simulation, when the reagent surface tension and viscosity increase, the corresponding critical Webber Number will increase too, this also increase the best dispensing jet speed. In Table 2, the surface tension and viscosity of alcohol is the minimum, 10% glycerin's viscosity is 8 times of distilled water, so at the same dispensing jet speed, alcohol's dispensing accuracy is the highest, whereas 10% glycerin the lowest, this result is even more obvious when the jet speed is at low level. This phenomenon is basically consistent with the simulation results and it again verified the correctness of the simulation.

In Table 5, the dispensing experiment of 10% alcohol is not conducted to avoid the nozzle with a smaller radius being blocked by the high viscosity reagent. Moreover, the alcohol's evaporation property can affect the experiment results.

## CONCLUSION

This study builds a theoretical model of the non-contact micro-liquid reagent dispensing process, simulation on reagent properties, jet speed, dispensing volume, nozzle end size and some other parameters affecting the dispensing process is conducted based on the FLUENT software. The results of simulation indicates that there exists a corresponding critical Webber Number  $We_c$  of each certain reagent, only when the actual Webber is greater than the critical value can the droplet be ejected from the nozzle to achieve non-contact dispensing. Reagent surface tension and viscosity decrease will reduce  $We_c$  and then the reagent drops off more easily. In practice, through adjusting the jet speed to control the actual  $We$  in the range of 3 to 4 times of the critical value, the dispensing accuracy will be at its best level. Increase of the dispensing volume reduces  $We_c$ , this makes the reagent depart from the nozzle tip easily. A smaller size of nozzle end can realize the distribution of small reagent volume and achieve a higher dispensing precision. The experiment results conducted on the self-built platform are basically consistent with the simulation results, this verifies the reliability of the method of modeling and all these work lays a firm theoretical foundation for the system design and optimization of controlling method of non-contact micro-liquid reagent distribution.

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