Micro PIV in two-fluid flow

B.J. Kim, Y.Z. Liu and H.J. Sung

Abstract Micro PIV was applied to two-fluid flow. Glycerol solutions were chosen as working fluids. Equal volume streams of two fluids were injected into a Y-shape microchannel with cross-sectional size of $300 \times 50 \ \mu m$. Experiments of three mixing cases were carried out, e.g., $\phi = 0$ and $\phi = 0.2$, $\phi = 0.1$ and $\phi = 0.5$, and $\phi = 0$ and $\phi = 0.6$. The experimental results showed a good agreement with the numerical simulations at the same conditions. To see whether the difference of the refractive indices of two fluids influences two-fluid measurement, the position difference in the focused planes of two fluids was derived, and then compared with the out-of-plane solution of micro PIV. As a result, it was found that the measurement of two-fluid flow was seriously affected by the numerical aperture of an objective lens even at small refractive index difference of two fluids.

1 Introduction Two-fluid mixing at microscale is an essential process in many microfluidic devices (Beebe et al. 2002). For example, various biomedical and biochemical processes involve the mixing of two fluids, including DNA purification, polymerase chain reaction, and enzyme reaction. The performance of such processes relies on effective and rapid mixing of samples and reagents. Fluid properties such as density, viscosity, and diffusivity vary with changes in variables such as temperature and mass fraction of species; hence, these variations should be taken into account when evaluating the extent to which two fluids mix.

Most previous experimental studies on fluid mixing in microfluidic devices have used flow visualization to probe the mixing performance. Koch et al. (1999) used red and green ink dissolved in ethanol to see the mixing degree. Liu et al. (2000) and Beebe et al. (2001) used phenolphthalein and sodium hydroxide solutions dissolved in ethyl alcohol to test the mixing performance of a three-dimensional serpentine mixer. Lee et al. (2000) and Stroock et al. (2002) used distinct streams of a fluorescent and a clear solution to evaluate the mixing performance of an active mixer with a pressure perturbation and of a staggered herringbone mixer. However, few studies have been performed up to now to elucidate the variation in mixing behavior with changes in the difference of two fluids (Liu et al. 2003).

Micro PIV (Particle Image Velocimetry) is widely used to measure velocity profiles, which uses a microscope to magnify hundreds nanometer particles (Meinhart et al. 1999). Most of micro PIV measurements were made to one-fluid flow such as water. However, when the working fluid are two fluids with different properties, several factors should be taken into account, e.g., the two-fluid difference in refractive index and density. Moreover, advanced PIV algorithms should be applied since velocity gradient might be very strong where the properties of two fluids are different.

In the present study, the refractive indices and densities of two fluids were examined that may influence micro PIV measurements of two-fluid flow. Two miscible and not-chemically-reactive fluids (glycerol solutions) then were chosen as working fluids. The mixing behaviors were obtained by measuring the velocity profiles with micro PIV. In the system studied, the difference in the properties of the two mixing fluids was adjusted by varying the amount of glycerol in the glycerol-water mixture (mass fraction $\phi = 0 \sim 0.6$). Two fluids were injected into a simple channel which was fabricated by bonding a PDMS replica and slide glass. For all experiments, the mean velocities at two inlets were 2 mm/s. Numerical simulation results were provided for comparison.

2 Experimental set-up

2.1 Focused planes in two fluids

Standard PIV uses a camera with large depth-of-focus, and its measurement plane is illuminated by laser sheet.

B. J. Kim, Department of Mechanical Engineering, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Korea

Y. Z. Liu, Department of Mechanical and Power Engineering, Shanghai Jiaotong University, Shanghai 200030, P.R.China

Correspondence to:
Prof. H. J. Sung, Department of Mechanical Engineering, Korea Advanced Institute of Science and Technology, 373-1 Kusong-dong, Yusong-ku, Daejeon 305-701, Korea, E-mail: hjsung@kaist.ac.kr
Accordingly, the refractive index of working fluid is not usually taken into consideration. However, measurement plane and out-of-plane resolution of micro PIV with volume illumination are determined by depth-of-field by a microscope objective lens, so that it is necessary to consider influence of the refractive index of working fluid. Moreover, for measurement of two-fluid flow, the refractive index difference and the density difference of two fluids should be small enough to accommodate one measurement plane and to make particles follow the flow faithfully. Studies on the density problem have been performed and established well (Raffel et al. 1997). The refractive index problem will be discussed more hereafter.

![Objective-imaging schematic diagram](image)

When the image of a particle in fluid is captured by a CCD camera, the light rays pass through the working fluid, the glass and the fluid between glass and the objective lens, as shown in Fig. 1. \( n_0, n_{\text{glass}} \) and \( n \) are the refractive indices of the fluid between the objective lens and the glass, the glass and the working fluid, respectively. \( t_{\text{glass}} \) denotes glass thickness. Let \( z_0 \) and \( z_i \) be the distance from the glass bottom to the focused point \( P_0 \) when \( n_i = n_{\text{glass}} = n_0 \), and the distance from the glass bottom to the focused point \( P \) with the glass. Then, in the general case that \( n_0 \leq n_{\text{glass}} \) and \(-t_{\text{glass}} \leq z_0 \) by large \( t_{\text{glass}} \), \( z_i \) can be derived from ray optics as follows:

\[
\begin{align*}
z_i &= \alpha z_0 + \beta t_{\text{glass}}, \\
\alpha &= \frac{n_i^2 - (n_0 \sin \theta_0)^2}{n_0^2 - (n_0 \sin \theta_0)^2}, \\
\beta &= \frac{1}{n_0^2 - (n_0 \sin \theta_0)^2} \left( \frac{n_i^2 - (n_0 \sin \theta_0)^2}{n_{\text{glass}}^2 - (n_0 \sin \theta_0)^2} \right) - \frac{n_i^2 - (n_0 \sin \theta_0)^2}{n_{\text{glass}}^2 - (n_0 \sin \theta_0)^2}. 
\end{align*}
\] (1)

Of various lenses, in the cases of air-immersed lens (\( n_0 = 1.000 \)) and of oil-immersed lens (\( n_0 = 1.520 \)), \( \alpha \) and \( \beta \) are plotted as a function of \( n_0 \sin \theta_0 \) in Figs. 2 and 3 for \( n_i = 1.333 \) and \( n_{\text{glass}} = 1.520 \). Because \( n_0 \sin \theta_0 \) can be increased up to the numerical aperture (\( NA \)) of the lens, the objective lens with high \( NA \) may make the rays more unfocused, which brings in a blurred particle image. Applying \( n_0 \sin \theta_0 \ll 1 \) to Eq. (1) gives the following approximated equation:

- Air-immersed lens (\( n_0 = 1.000 \leq n_i \))
The focused plane is changed with the refractive index of working fluid, by which discontinuous velocity profiles can be measured in two-fluid flow. However, if the position difference between two focused planes is much smaller than the out-of-plane resolution, one measurement plane can be achieved despite using single objective lens.

$|z_i - z_j| < \delta z$ \hspace{1cm} (4)

where $\delta z$ is the out-of-plane resolution of micro PIV system.

$\delta z = \frac{3n_i\lambda_0}{NA^2} + \frac{2.16d_p}{\tan(\theta_{0})_{\text{max}}} + d_p$ \hspace{1cm} (5)

Here, $\lambda_0$ is the wavelength of light imaged by the optical system and $d_p$ is the particle diameter (Meinhart et al. 2000). It is revealed that $NA$ should be high enough to reduce the out-of-plane resolution and be small enough for micro PIV to measure two-fluid flow, satisfying Eq. (4).

### 2.2 Experimental methods

As mentioned earlier, glycerol solutions were adopted as the working fluids. The refractive index of glycerol solution was changed by adjusting $\phi$. The mass fraction of glycerol in water is defined as

$\phi = \frac{m_g}{(m_g + m_w)}$ \hspace{1cm} (6)

where $m_g$ and $m_w$ are the masses of glycerol and water in the mixture, respectively. A simple Y-shape microchannel was employed in the present study. A schematic diagram of the channel is shown in Fig. 4. The cross-sectional size of the channel was 300×50 μm. Equal volume streams of two fluids were injected into the channel. The velocity profile is strongly dependent on the ratio of fluid properties at two inlets, such as the mean velocity
ratio, the viscosity ratio, the density ratio and the diffusivity ratio, etc. The properties of water-glycerol mixture with increasing $\phi$ are listed in Table 1. The solution with 0 % concentration is defined as the infinitely diluted glycerol solution. Both the diffusivity and viscosity of the mixture are very sensitive to $\phi$, with the diffusivity decreasing and the viscosity increasing with increasing $\phi$. The diffusivity varies approximately inversely with viscosity, showing a large drop on going from dilute ($\phi = 0$) to the concentrated solution.

<table>
<thead>
<tr>
<th>Mass fraction $\phi$</th>
<th>Density g/cm$^3$</th>
<th>Viscosity $\times 10^{-3}$ Pa·s</th>
<th>Diffusivity $\times 10^{-10}$ m$^2$/s</th>
<th>Refractive index</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.000</td>
<td>0.888</td>
<td>10.6</td>
<td>1.333</td>
</tr>
<tr>
<td>0.1</td>
<td>1.021</td>
<td>1.147</td>
<td>8.98</td>
<td>1.345</td>
</tr>
<tr>
<td>0.2</td>
<td>1.044</td>
<td>1.527</td>
<td>7.52</td>
<td>1.357</td>
</tr>
<tr>
<td>0.3</td>
<td>1.067</td>
<td>2.137</td>
<td>5.81</td>
<td>1.370</td>
</tr>
<tr>
<td>0.4</td>
<td>1.091</td>
<td>3.130</td>
<td>4.18</td>
<td>1.384</td>
</tr>
<tr>
<td>0.5</td>
<td>1.117</td>
<td>4.991</td>
<td>3.24</td>
<td>1.398</td>
</tr>
<tr>
<td>0.6</td>
<td>1.143</td>
<td>8.640</td>
<td>2.30</td>
<td>1.413</td>
</tr>
<tr>
<td>1.0</td>
<td>1.264</td>
<td>887</td>
<td>-</td>
<td>1.476</td>
</tr>
</tbody>
</table>

Table 1. Properties of glycerol solutions at 25 ℃ (Dorsey 1940 and Ternstrom et al. 1996)

The microchannel was fabricated by bonding a PDMS (polydimethylsiloxane) replica and slide glass. To fabricate the PDMS replica, SU-8 was spin-coated onto a silicone wafer to create a mold master with a 50 $\mu$m thick structure. The pattern on the mask was photolithographically transferred to the SU-8 coated silicon wafer. After development, the master was treated with fluorocarbon (CHF$_3$) plasma for easy removal of the PDMS replica from the master after curing. The prepolymer of PDMS and a curing agent were mixed in the ratio of 20:1, stirred thoroughly, and then degassed in a vacuum pump. The prepolymer mixture was poured onto the master, and then cured at 80 ℃ for 1 hour. After cooling down, PDMS replica was peeled off from the master. The ports for inlet and
outlet were punched. PDMS replica was oxidized in a plasma cleaner for the strong bonding, and then attached to a glass substrate.

Velocity profiles were measured by micro PIV. A 12-bit CCD camera (1280×1024), two 20x/40x objective lenses (NA = 0.45 / 0.6) and a two-head Nd-Yag laser (λ = 532 nm) were used. The fluorescent particles (ρ_p = 1.05 g/cm³, d_p = 0.7 μm, λ_abs = 540 nm, λ_em = 612 nm) were chosen as seeding particles. Pulses were generated and delayed by a pulse/delay generator. Figure 5 shows a schematic diagram of the micro PIV. Velocity vectors were acquired using iterative multi-grid algorithm with window offset and multiplication of two adjacent 2D correlation planes to increase signal-to-noise where velocity gradient is strong (Scrano and Riethmuller 1999, Hart 2000). When the window size was decreased from 64 to 16 pixels during image processing and the 40x objective lens was used, the spatial resolution was 2.3×2.3×5.7 μm.

Commercial fluorescent particles are usually diluted in deionized water so that particles can be used to measure water velocity profiles. However, since the glycerol-containing solutions were used in this study, the solutions with particles for micro PIV should be carefully prepared. It is known that the proper ratio of particle volume to the working fluid is 0.05 ~ 0.1 %. For example, a 60 % glycerol solution with particles was prepared by mixing the commercial particle-containing water (1 % particle volume ratio) with a 65.2 % glycerol solution at the volume rate of 1:10.

3 Results and discussion
In the present study, experiments of three mixing cases were carried out, e.g., φ = 0 and φ = 0.2, φ = 0.1 and φ = 0.5, and φ = 0 and φ = 0.6. All experiments were performed at room temperature 25°C. The glass thickness was 1 mm, and NA was 0.60 except for the mixing of φ = 0.1 and φ = 0.5 (NA = 0.45).

![Fig. 6. Comparison of velocity profiles between φ = 0 and φ = 0.6 for V̇ = 2 mm/s](image)

One-fluid velocity profiles of φ = 0 and φ = 0.6 are compared in Fig. 6, which were measured independently in the middle plane of the channel when the mean velocities were 2 mm/s. Because the objective lens was placed to focus the middle plane of the channel in each case, the velocity profiles of φ = 0 is close to those of φ = 0.6, showing that the density difference could not affect the velocity profiles in this study.

The mixing of φ = 0.1 and φ = 0.5 is shown in Fig. 7. For comparison, numerical simulations were made. In the numerical simulations, variations of density, viscosity and diffusivity with change in mass fraction were taken into account. After joining of two fluids, the high viscosity fluid occupies larger region and pushes the low viscosity fluid to move at a higher speed. The measured velocity profiles 0.3 mm downstream from the inlet of the joined channel (300×50 μm) are compared with those of numerical simulations in Figs. 8 ~ 10. In the experiments, the objective lens was positioned to make particles in the high viscosity fluid be focused in the middle plane of the channel. As the viscosity ratio is high, the high viscosity fluid occupies larger portion of the cross-sectional area. The experimental data agree well with the numerical simulation curve for the mixing of φ = 0 and φ = 0.2.
Fig. 7. Mixing of $\phi = 0.1$ and $\phi = 0.5$: a) velocity profiles (experiment), b) velocity profiles (simulation), c) mass fraction distribution (simulation)

Fig. 8. Velocity profile 0.3 mm downstream for the mixing flow of $\phi = 0$ and $\phi = 0.2$

Fig. 9. Velocity profile 0.3 mm downstream for the mixing flow of $\phi = 0.1$ and $\phi = 0.5$
However, in the other cases, the velocity profile of the low viscosity fluid is shifted right compared with the numerical simulation curve. Specifically, this difference is large for the mixing of $\phi = 0$ and $\phi = 0.6$. Several factors are attributed to the deviation between experimental and numerical results.

The first reason is the inherent problem of the PIV algorithm. In the algorithm, since rectangular interrogation windows of finite size were used during image processing, it was not easy to detect an exact velocity where velocity gradient was strong. For example, the maximum velocity is about six times larger than the mean velocity of the low viscosity fluid in the mixing of $\phi = 0$ and $\phi = 0.6$, as shown Fig. 10. Two advanced algorithms were used to improve the ability to extract a velocity in a strong velocity region, as mentioned earlier. The time interval between two laser pulses was set in order that the displacement of a particle might be $9 \sim 10$ pixels in $\phi = 0$, and $1 \sim 2$ pixels in $\phi = 0.6$. Interrogation window size was decreased from 64 to 16 pixels, shifting the interrogation window based on a previous vector to compensate particle-pair loss.

The second reason may be the difference between flow rates through two inlets. The reliability of the syringe pump was examined by comparing experimental velocity profiles and analytical velocity profiles of one fluid (Poiseuille flow) in the middle plane of rectangular channels. The maximum velocities in the middle plane are analytically $3.46 \text{ mm/s}$ and $3.85 \text{ mm/s}$ in a rectangular channel with $300 \times 50 \mu \text{m}$ and a rectangular channel with $150 \times 50 \mu \text{m}$, respectively when the mean velocities are $2 \text{ mm/s}$, while the measured maximum velocities were $3.74 \text{ mm/s}$ and $3.88 \text{ mm/s}$, respectively. The syringe pump pushed a syringe at a higher speed than the desired speed. The systematical error of about 9 % was found. This error probably originates from the channel dimension error and the syringe pump using a step motor to push a syringe at a low speed. It is found in Fig. 9 that the area by integrating the measured velocity profile along the normalized width is close to that by integrating the simulated velocity profile. This means that the mean velocity of $\phi = 0.1$ was larger than $2 \text{ mm/s}$, and the mean velocity of $\phi = 0.5$ was smaller than $2 \text{ mm/s}$. In Fig. 10, the area by the integrating measured profile is higher than the simulated one, specially in $\phi = 0$. It is thought that the mean velocity of $\phi = 0$ was larger than $2 \text{ mm/s}$, but the mean velocity of $\phi = 0.6$ was nearly $2 \text{ mm/s}$.

The third reason may be the differences of the properties between experiments and simulations. Although Table 1 was obtained from literatures, the properties there are generally based on averaged values from measured data which are variable. For example, about 10 % variation was found in the measured diffusivity in the literature. Moreover, the experiments were performed at $26^\circ \text{C}$ actually due to fluctuating room temperature, while the simulations used the properties at $25^\circ \text{C}$. But these differences seem not to have a large contribution the deviation much.

The forth reason may be the refractive index difference between two fluids. To estimate this contribution, the difference between the focused positions in $\phi = 0$ and $\phi = 0.6$ is calculated using Eq. (2).

$$
\frac{z_{60\%} - z_{0\%}}{z_{60\%}} = 0.0573 \frac{z_{60\%}}{z_{60\%}},
$$

Fig. 10. Velocity profile 0.3 mm downstream for the mixing flow of $\phi = 0$ and $\phi = 0.6$
where \( z_{\phi=0.6} \) and \( z_{\phi=0.1} \) are the distances from the glass bottom to the focused planes of \( \phi = 0.6 \) and \( \phi = 0.1 \), respectively. For \( z_{\phi=0.6} = 25 \ \mu m \) (channel depth = 50 \( \mu m \)), \( z_{\phi=0.6} - z_{\phi=0.1} = 1.4 \ \mu m \) is smaller than the out-of-plane resolution \( \delta_z = 5.7 \ \mu m \). Since \( z_{\phi=0.6} - z_{\phi=0.1} < \delta_z \), it is thought that the refractive index difference might not contribute to the deviation of experimental data from simulation results. Figure 11 shows a captured image of particles in the mixing of \( \phi = 0 \) and \( \phi = 0.6 \), with the interface of two fluids. Enlarging the interface neighborhood reveals that the intensity distribution is continuous, which supports \( z_{\phi=0.6} - z_{\phi=0.1} < \delta_z \).

The position difference of the focused planes is \( 3.2 \ \mu m \) in the mixing of \( \phi = 1 \) (pure glycerol) and \( \phi = 0 \) when the focused plain of \( \phi = 1 \) is placed at \( 25 \ \mu m \) below the glass bottom, using oil-immersed lens (\( n_o = 1.52 \)). And the out-of-plane resolution is \( 2.8 \ \mu m \) for \( NA=1.4 \). In this case, since \( z_{\phi=1} - z_{\phi=0} \) is larger than \( \delta_z \), an experimental result will be thrown out. This experiment was not performed because velocity gradient is too strong to measure due to about 1000 viscosity ratio and simulation is subject to the divergence.

In liquids, the Eykmann’s equation appears to be the most accurate empirical equation to estimate the refractive index of liquid (Lyman et al., 1982).

\[
n = \frac{R_d \rho + \sqrt{(R_d \rho)^2 + 1.6(R_d \rho) + 4}}{2},
\]

where \( \rho \) is the density, and \( R_d \) is the molar refraction which remains nearly constant with change of other properties (3 ~ 6\times10^{-5} \ m^3 \) in liquids). \( R_d \rho \) is so small that \( n \) can be rewritten as

\[
n \approx \frac{R_d \rho}{2} + \left( 1 + \frac{1.6}{4} R_d \rho \right) = 1 + 0.9 R_d \rho. \tag{9}
\]

From Eq. (9), the range of refractive indices of general pour liquids are not so broad that the position difference of the focused planes of two liquids may not be affected much by the refractive index difference of two fluids. Instead, since the out-of-plane resolution is greatly influenced by \( NA \), it is important to select an objective lens with proper \( NA \) because the smaller out-of-plane resolution makes two-fluid measurement more difficult.

4 Conclusions
Micro PIV was applied to two-fluid flow in a microchannel (300\times50 \( \mu m \)) to see the effect of the refractive indices of working fluids, with consideration of the refractive index and the thickness of glass. The properties of two fluids
were adjusted by varying the amount of glycerol in water. Three experiments were made of the mixing of $\phi = 0$ and $\phi = 0.2$, $\phi = 0.1$ and $\phi = 0.5$, and $\phi = 0$ and $\phi = 0.6$. The experimental results showed a good agreement with the numerical simulation results at the same conditions. Measurement of two fluids using single objective lens is possible only when the out-of-plane resolution of micro PIV system is larger than the difference of the focused positions in two fluids. When applying micro PIV to two different liquids, the refractive index difference of two fluids does not affect the measurement much. Instead, to make the out-of-plane resolution be larger than the position difference of the focused planes of two fluids, an objective lens with low $\text{NA}$ should be selected, at that time, $\text{NA}$ should be sufficiently large enough for small out-of-plane resolution.

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