Diffusion in Microchannel Analyzed by Chemiluminescence*

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Abstract
We investigate diffusion and mixing in a microchannel by using luminol chemiluminescence (CL) to estimate the local chemical reaction rate. The degree of mixing in micromixers is generally evaluated from the deviation of concentration profiles measured by fluorescence from a uniform concentration profile. The degree of mixing measured by this method is a macroscopic estimate, which is inappropriate for investigating diffusion and mixing in microchannels. In this study, the luminol CL reaction is used to visualize and quantitatively measure local diffusion and mixing at an interface between two liquids in a microchannel. Blue CL is observed where luminol reacts with hydrogen peroxide at the mixing layer. Diffusion and mixing in a microchannel are investigated by sequentially measuring the CL and fluorescence. The experimental results are compared with the results of a numerical simulation that involves solving transport equations including the chemical reaction term. By calibrating the CL intensity with the chemical reaction rate estimated by the numerical simulation, the local chemical reaction can be quantitatively estimated from the CL intensity profile.

Key words: Microchannel, Chemiluminescence, Fluorescence, Luminol Reaction, Diffusion

1. Introduction
Mixing and diffusion processes along the liquid–liquid interface are important in micromixers. Mixing in a microchannel occurs mainly by molecular diffusion at the interface between two liquids due to laminar flow at a low Reynolds number. When the channel width is about 100 μm, molecular diffusion can mix two liquid streams in several seconds. However, a long mixing channel is required for relatively high velocities. In biological and chemical analyses, solutions contain large molecules such as globular proteins whose diffusion coefficients are one or two orders of magnitude lower than those of most liquids (1). Micromixers that employ various mixing techniques have been developed. Examples include micromixers that have geometries that induce secondary flow (2), that have multiple laminations that reduce the diffusion length (3), that generate alternating flow from inlets (4), that utilize ultrasonic oscillations (5), and that generate alternating flow in an expansion chamber (6). We have developed an actively controlled micromixer that generates oscillating flow by electroosmosis (7).

The degree of mixing is generally evaluated by fluorescence. One of the solutions is marked by a fluorescent dye and its concentration profile is visualized by the fluorescence intensity. The degree of mixing is characterized by the deviation of the obtained concentration profiles from a uniform concentration profile. Thus, the degree of mixing is a
macroscopic estimate for the whole microchannel, which is inappropriate for investigating diffusion and mixing in the mixing layer. Estimation of local diffusion and mixing processes in a microchannel will enable the local mass transfer to be controlled and contribute to the development of microfluidic systems. Ichiyanagi investigated the mixing and chemical reaction fields in a microchannel. The local mass flux distribution was evaluated from the concentration gradient measured by LIF and μ-PIV.

Chemiluminescence (CL) is light emitted by chemical reactions. Unlike fluorescence measurements, CL measurements do not require a light source. CL has been widely used in analytical chemistry for sensitive and selective analysis, especially over the last decade. Tsukagoshi et al. proposed using CL in chemical analysis of the liquid–liquid interface of laminar flow in a microchannel; this technique is known as microchannel CL analysis (MCCLA).

In this study, the CL reaction of luminol is used to visualize local diffusion and mixing at an interface between two liquids in a microchannel. Luminol emits blue CL when it reacts with hydrogen peroxide at the mixing layer. The CL intensity profile is used to visualize the mixing of the two liquids. Diffusion and mixing in a microchannel are investigated by sequentially measuring the CL and fluorescence. The experimental results are compared with the results of a numerical simulation that involves solving transport equations including the chemical reaction term. By calibrating the CL intensity with the chemical reaction rate calculated by the numerical simulation, the local chemical reaction, which occurs due to diffusion and mixing, can be quantitatively estimated from the CL intensity profile.

2. Chemiluminescence

Chemiluminescence reaction exhibits a light emission $h\nu$ when the chemical reaction between the chemical species A and B produces the product C, as shown in Eq.(1).

$$A + B \rightarrow C + h\nu$$  \hspace{1cm} (1)

The luminescence intensity is proportional to the concentrations of A and B. Since the catalyst concentration also affects the CL reaction, CL has been used for quantitative analysis of metallic ions, enzymes, and other catalysts. The present study uses the CL reaction between luminol and hydrogen peroxide. The luminol reaction was first reported in 1928. It is the most widely used CL reaction. The luminol reaction can be expressed by

$$\text{luminol} + \text{H}_2\text{O}_2 \xrightarrow{\text{Cu}^{2+}} \text{3-aminophthalate} + \text{N}_2 + \text{H}_2\text{O} + h\nu$$  \hspace{1cm} (2)

Luminol oxidizes in an alkaline solution of hydrogen peroxide to give 3-aminophthalate, nitrogen, and blue emission ($\lambda = 420–460$ nm). In this study, Cu(II) acetate was used to catalyze the luminol reaction. Figure 1 shows a photograph of CL of luminol in a beaker.

3. Experimental Apparatus and Procedure

Figure 2 shows a schematic diagram of the microchannel chip used in this study. The microchannel was formed in a 3-mm-thick PDMS substrate. The microchannel is T-shaped and connects two inlets and one outlet. Each of the three branches of the microchannel is 15 mm long. The inlets and the outlet have microchannel widths $d$ of 200 μm. The microchannel depth $t$ is 50 μm. The PDMS chip was bonded with a 170-μm-thick cover glass by bringing their surfaces into contact. An acrylic base plate was attached to the rear
surface of the PDMS chip, as shown in Fig. 3. Solutions of the two reagents were delivered to the microchannels by the syringes (Hamilton, 1002TLL 2.5ml) using the syringe pump systems (KD, Scientific KDS100). The syringe was pushed by the linear actuator using a stepping motor equipped in the syringe pump system. The volume flow rate $Q$ was controlled by the stroke of the linear actuator.

Fig. 1 Photograph of luminescence emitted by luminol reaction ($5 \times 10^{-3} \mu \text{mol/mm}^3$ luminol, $500 \times 10^{-3} \mu \text{mol/mm}^3$ hydrogen peroxide, and $0.5 \times 10^{-3} \mu \text{mol/mm}^3$ Cu(II)) obtained using a Nikon D3x camera with a 30 s exposure (ISO 1600).

In this study, CL and fluorescence images were obtained sequentially by changing the optical unit of an inverted microscope (Olympus, IX71). Therefore, the CL reagent and a fluorescent dye were added to the same solution. Figures 4(a) and (b) schematically depict the configurations of the experimental system for CL and fluorescence measurements, respectively. Images were obtained using an EM-CCD camera (Hamamatsu Photonics, ImagEM C9100-13) mounted on the inverted microscope. This camera produced images with $512 \times 512$ pixels. A $20 \times$ magnification objective lens (N.A.: 0.45, Olympus, LUCPLFLN 20×) was attached to the camera.
CL images were observed directly through the microscope, as shown in Fig. 4(a). The EM-CCD sensitivity was set to an electron multiplication factor of about 150 times for a 10 s exposure due to the extremely weak CL intensity. The fluorescent dye (uranine) absorbs excitation light from a mercury lamp. Uranine was excited by blue light (485 nm) and emitted green light (515 nm). Fluorescence images were selected through a mirror-filter unit and recorded by the EM-CCD camera, as shown in Fig. 4(b).

Table 1 lists the compositions of the CL and fluorescent compounds used. Reagent 1 was a mixture of $5\times10^{-3}\ \mu\text{mol/mm}^3$ luminol and $0.5\times10^{-3}\ \mu\text{mol/mm}^3$ Cu(II) acetate in $1\times10^{-2}\ \mu\text{mol/mm}^3$ sodium hydroxide. Reagent 2 was $500\times10^{-3}\ \mu\text{mol/mm}^3$ hydrogen peroxide. $0.5\times10^{-6}\ \mu\text{mol/mm}^3$ of the fluorescent dye, uranine, was mixed with reagent 1.

![Experimental configurations of inverted microscope.](image)

Table 1 Reagent compositions.

<table>
<thead>
<tr>
<th>Reagent 1 (Inlet 1)</th>
<th>Reagent 2 (Inlet 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>luminol $5.0\times10^{-3}\ \mu\text{mol/mm}^3$</td>
<td>$\text{H}_2\text{O}_2\ 500\times10^{-3}\ \mu\text{mol/mm}^3$</td>
</tr>
<tr>
<td>Cu(II) acetate $0.5\times10^{-3}\ \mu\text{mol/mm}^3$</td>
<td></td>
</tr>
<tr>
<td>uranine $0.5\times10^{-6}\ \mu\text{mol/mm}^3$</td>
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</table>
4. Results and Discussion

4.1 Chemiluminescence and Fluorescence Images

Figure 5 shows CL and fluorescence images obtained at four different average velocities $u_{ave}$.

![Chemiluminescence and Fluorescence Images](image)

Fig. 5 Chemiluminescence and Fluorescence images.
The averaged velocity $u_{ave}$ is calculated as dividing the volume flow rate $Q$ by the crosssectional area of the microchannel, $u_{ave}=Q/(d \cdot t)$. In this figure, reagents 1 and 2 flow into the T junction from the top and bottom of the images, respectively. The images in Fig. 5 are composites of two images in the flow direction due to the limited field of view of the microscope; the joining line between these two images (at $x = 0.6$ mm) is smooth. For low fluorescent dye concentrations, the fluorescence intensity is proportional to the dye concentration; thus, the concentration field was estimated by shading compensation of image-processing using 100% and 0% concentrations of the fluorescent dye. In this experiment, the CL images were normalized by the maximum intensity obtained, which was at $x = 0.79$ mm for $u_{ave} = 50$ mm/s.

Figure 6 shows concentration and CL intensity profiles at $x = 0$, 0.4, 0.8, and 1.2 mm. The fluorescence images and the profiles of Figs. 5 and 6 show the development of a concentration boundary layer at the T junction. Its thickness increases slightly with decreasing velocity. The interface between the two liquids was located at the center of the microchannel.

The CL intensity profiles clearly correspond to the mixing layer observed in the fluorescence image. The mixing layer thickness increased with decreasing $u_{ave}$. However, the maximum CL intensity was shifted to the left of the microchannel center (i.e., to the luminol reagent side). This deviation from the microchannel center increased with decreasing velocity.

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![Fig. 6 Concentration and CL intensity profiles.](image-url)
4.2 Numerical Simulation of Transport Equation

The numerical simulation was performed on the two-dimensional straight channel by solving the transport equations with the boundary-layer approximation that includes the production term of the chemical reaction:

\[
\frac{u}{\partial x} = D_1 \frac{\partial^2 c_1}{\partial y^2} - kc_1^2 c_2 \tag{3}
\]

\[
\frac{u}{\partial x} = D_2 \frac{\partial^2 c_2}{\partial y^2} - kc_1^2 c_2 \tag{4}
\]

where \(c_1\) and \(c_2\) are the concentrations of the species in reagents 1 and 2, respectively; thus, \(c_1\) represents the concentrations of luminol and uranine and \(c_2\) is the hydrogen peroxide concentration. Since the mass diffusivity is species dependent, different diffusivities \(D_1\) and \(D_2\) were used for the two species.

The second term on the right-hand side of Eqs. (3) and (4) represents the amount of solute consumed by the chemical reaction \(-kc_1^2 c_2\). The power of \(c_1\) and \(c_2\) in the production term was principally one because the luminol reaction of Eq. (2) is a second-order chemical reaction. However, the luminol reaction depends on the concentration of the catalyst Cu(II) acetate; thus, the chemical rate constant \(k\) is a function of the concentration \(c_1\). In this calculation, the production term was taken to be that of a third-order reaction with a fixed chemical rate constant \(k\).

Figure 7 shows the grid system for the numerical simulation. The number of the grid points is 60 in the spanwise direction for 200μm in width, and 280 in the streamwise direction for 5.6mm in length. The velocity profile in \(y\) direction in this numerical simulation is applied the developed velocity profiles of Hagen–Poiseuille flow in the whole of the calculation domain. The inlet shape of the microchannel in the numerical simulation is different to that in the experiments. However, Reynolds number is \(Re=4.0\) for the maximum averaged velocity \(u_{ave}=50\text{mm/s}\), in which the entry length to be a fully developed velocity profile is estimated as less than the hydraulic characteristics length \(d_h\). In this study, therefore, the numerical calculation of the concentration field on the downstream channel of the T-junction is carried out on the straight channel. On the experiment, the mass diffusion starts at the stagnant point of the T-junction at \(x=–0.2\text{mm}\), thus, the entrance length \(x_0\) is applied to the numerical simulation, as shown in Fig. 7. The boundary conditions are shown in Fig. 7.

The diffusion term in Eqs. (3) and (4) is approximated by a second-order central finite difference. The convection term is used the first-order upwind scheme. The production term is non-linear function of \(c_1\), thus, the linear form is adopted according to the Ref.(16). The finite differential equations are solved by Gauss-Seidel scheme.

Figure 6 also shows the numerical simulation results. The following simulation procedure was employed:

1. The uranine concentration profile was calculated using Eq. (3) with no production term and the diffusivity of uranine was determined by fitting with the experimental results. The calculation results (shown by the concentration profiles in Fig. 6) show excellent agreement with the experimental results though in the different inlet shape of the microchannel. The diffusivity of uranine was determined to be \(0.50 \times 10^{-3} \text{ mm}^2/\text{s}\), which is close to the value given in Ref. (17). The entrance length \(x_0\) of the concentration boundary layer formation were respectively set to –0.2, –1.2, –2.0, and –8.0 mm for average velocities \(u_{ave}\) of 1, 5, 10, and 50 mm/s.

2. Applying a diffusivity of \(0.50 \times 10^{-3} \text{ mm}^2/\text{s}\) to luminol and hydrogen peroxide, the species concentrations of the luminol reaction were calculated using Eqs. (3) and (4) with
the production term \(-kc_1^2c_2\). The boundary conditions at the inlet were set to \(5 \times 10^{-3} \mu\text{mol/mm}^3\) for luminol and \(500 \times 10^{-3} \mu\text{mol/mm}^2\) for hydrogen peroxide based on the experimental conditions. The calculation results for the production term \(kc_1^2c_2\) are indicated by the dashed line in the CL intensity profiles in Fig. 6. A chemical rate constant \(k\) of 800 \((\mu\text{mol/mm}^3)^2\text{s}^{-1}\) was obtained by fitting the experimental results for CL. However, the peak position differed from that of the experimental results.

3. The mass diffusivity depends on the species, especially the molecular mass. Uranine, luminol, and hydrogen peroxide have molecular masses of 376.28, 177.16, and 34.01, respectively. Hydrogen peroxide is estimated to have a higher mass diffusivity than luminol and uranine. The mass diffusivity \(D_2\) of hydrogen peroxide was set to \(2.0 \times 10^{-3} \text{mm}^2/\text{s}\), which is very similar to that of oxygen \((2.1 \times 10^{-3} \text{mm}^2/\text{s})\) \((18)\). The diffusivity of luminol was set to \(0.50 \times 10^{-3} \text{mm}^2/\text{s}\). The calculation results for these conditions are shown by the solid line in Fig. 6. They show excellent agreement with the experimentally observed CL intensity for \(u_{ave} = 50\) and 10 mm/s. The properties used in the numerical simulation were listed in Table 2.

![Fig.7 Grid system and boundary conditions for the numerical simulation.](image)

<table>
<thead>
<tr>
<th>Table 2 Properties for the numerical simulation.</th>
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<tbody>
<tr>
<td>Mass diffusivity of luminol (D_1)</td>
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<tr>
<td>Mass diffusivity of hydrogen peroxide (D_2)</td>
</tr>
<tr>
<td>Chemical rate constant (k)</td>
</tr>
</tbody>
</table>

### 4.3 Quantitative Estimation of Local Chemical Reaction

The CL profiles obtained agree with the numerical simulation. By calibrating the CL intensity with the chemical reaction rate \(kc_1^2c_2\) obtained from the numerical simulation results, the local chemical reaction rate can be quantitatively estimated from the CL intensity profile. Figure 8 shows the relationship for between the CL intensity and the chemical reaction rate calculated with the production term \(kc_1^2c_2\), which has units of \((\mu\text{mol/mm}^3)/\text{s}\). The dispersion in Fig. 8 is caused by the misalignment of the profiles of the numerical simulation and the experimental results, as shown in Figs. 6. Therefore, the dispersion in Fig. 8 becomes large by comparing the local distribution of the chemical reaction rate and the CL intensity. However, approximately-linear relationships between the CL intensity and \(kc_1^2c_2\) are observed at \(u_{ave} = 10\) and 50 mm/s, although there is some scatter in the data. The gradient is given by the ratio of \(2.5 \times 10^{-3} \ (\mu\text{mol/mm}^3)/\text{s}\) to the CL intensity. By comparing the peak values of the chemical reaction rate and CL intensity in Figs. 6(c) and (d), the uncertainty is estimated as 11%. This implies that the maximum CL intensity in Fig. 6 corresponds to the \(2.5 \times 10^{-3} \ (\mu\text{mol/mm}^3)/\text{s}\) of the chemical reaction rate. Therefore, the CL image in Fig. 5 can be quantitatively converted into the profile of the chemical reaction rate.
Fig. 8 Relationship between CL intensity and $k_{c1^2c2}$.

Chemical reaction rate $\mu$mol/(mm$^3$/s)

- 0
- 2.5x10$^{-3}$

(a) $u_{ave} = 1$mm/s

(b) $u_{ave} = 5$mm/s

(c) $u_{ave} = 10$mm/s

(d) $u_{ave} = 50$mm/s

Fig. 9 Profiles of chemical reaction rate $k_{c1^2c2}$.
Figure 9 shows the local chemical reaction rate $k c_1 c_2$ estimated quantitatively from the CL intensity images of Fig. 5. In Fig. 9(a) for $u_{ave} = 1$ mm/s, luminol was consumed by the chemical reaction with a reaction rate of about $1.5 \times 10^{-3}$ (μmol/mm$^3$)/s near the T junction; however, the inlet concentration of luminol was only $5.0 \times 10^{-3}$ μmol/mm$^3$. The long residence time for the slow velocity of $u_{ave} = 1$ mm/s causes luminol to be consumed in the flow direction. Consequently, the luminol concentration decreases near the center of the microchannel. A further cause of the peak shift for the luminol reaction is luminol consumption near the center of the microchannel. In contrast, at $u_{ave} = 50$ mm/s, the residence time of a 1-mm-long section of the microchannel was about 0.02 s. Luminol was not consumed by the chemical reaction due to the short residence time. Therefore, the chemical reaction mostly occurred near the center of the microchannel.

By accounting for the local reaction rate in the mixing layer, the total reaction rate can be evaluated from CL images. Figure 10 shows the total reaction rate from $x = 0$ to 1.2 mm as a function of the Reynolds number. It shows that the total reaction rate is almost constant with the Reynolds number. The calculation results agree with the experimental results except at the low Reynolds number of $Re=0.08$ due to the different of the inlet shape of the channel between the calculation and the experiment. Figure 11 shows the ratio of the total reaction rate to the amount of luminol at the inlet. At $u_{ave} = 10$ mm/s, the total reaction rate is $5.04 \times 10^{-5}$ μmol/s. This is only 2.0% of the amount of luminol at the inlet. However, at $u_{ave} = 1$ mm/s, 22.9% of the luminol reacts in the microchannel over about 1.2 s and the CL profile is shifted to the left of the microchannel because luminol was consumed by the chemical reaction. The ratio of the total reaction rate to the amount of luminol at the inlet decreased rapidly with increasing $Re$.

![Fig. 10 Total reaction rate ($x = 0$ - 1.2mm)](image)

![Fig. 11 Total reaction rate to the inlet amount of luminol ($x = 0$ - 1.2mm).](image)
5. Conclusion

In this study, the luminol CL reaction was used to visualize and quantitatively measure local diffusion and mixing at an interface between two liquids in a microchannel. CL was clearly observed at the mixing layer in a T-junction microchannel. Numerical simulations performed by solving transport equations including the chemical reaction agreed with the experimental results. By calibrating the CL intensity with the chemical reaction rate estimated by the numerical simulation, the local chemical reaction could be quantitatively estimated from the CL intensity profile.

Nomenclature

\( c \) : concentration \[\mu\text{mol/mm}^3\]  
\( D \) : mass diffusivity \[\text{mm}^2/\text{s}\]  
\( d \) : channel width \[\text{mm}\]  
\( d_h \) : hydraulic diameter of microchannel, \( =4(d_t/2(d+t)) \)[mm]  
\( h \) : Planck's constant \[\text{J} \cdot \text{s}\]  
\( k \) : reaction rate constant \[(\mu\text{mol/mm}^3)^{-2}\text{s}^{-1}\]  
\( Q \) : volume flow rate \[\text{mm}^3/\text{s}\]  
\( \text{Re} \) : Reynolds number, \( =d_hu_{\text{ave}}/\nu_w \) \[\text{mm}\]  
\( t \) : channel depth \[\text{mm}\]  
\( u_{\text{ave}} \) : averaged velocity, \( Q/(d_t t) \)[mm/s]  
\( x \) : streamwise coordinate \[\text{mm}\]  
\( x_0 \) : entrance length \[\text{mm}\]  
\( y \) : spanwise coordinate \[\text{mm}\]  
\( \nu \) : frequency \[\text{Hz}\]  
\( \nu_w \) : viscosity \[\text{m}^2/\text{s}\]

References

(8) Ichiyanagi, M., “Combined Laser-Based Measurements on Micro-Scale Thermo-Fluid


