MIXING MEASUREMENTS USING LASER INDUCED FLUORESCENCE

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Abstract
In this article, we present a novel method of quantitatively measuring mixing using laser induced fluorescence. This method is independent of flow and light sheet characteristics. The premise of this work is that molecular level mixing can be quantified through a scalar pH field in an aqueous solution. A two dimensional pH field can be determined in the solution by imaging the laser induced fluorescence of a pH sensitive dye which has been calibrated for fluorescence versus pH. This work differs from previous work in that the dye we selected exhibits dual band emission characteristics. By forming a ratio of the intensity image of each band's fluorescence, variations in dye concentration, laser light absorption and laser intensity are normalized out and a relationship between the ratio value and pH can be determined experimentally to within 0.1 a pH unit.

Introduction
To date, an efficient technique to quantitatively measure instantaneous two dimensional mixing has not been developed. Qualitative mixing measurements using laser induced fluorescence (LIF) have been carried out by Dimotakis1-4 where fluorescein was used to indicate areas in a jet combustion field that have "reacted"- mixed with the ambient, versus areas in the jet that have "not reacted"-not mixed with the ambient. Bellerose5 advanced mixing measurements using LIF quantifying the amount of mixing instead the binary mixed/not mixed type of data. Since the intensity of fluorescein is proportional to pH, local pH measurements were made using the digital video image. Therefore, if solutions of differing pH are combined, the amount of mixing (via an acid base reaction) between the solutions at each point is quantitatively reflected in the local pH. The major limitation to this technique's accuracy, however, is accounting for laser light absorption from upstream fluorescence. That is, regions in the shadow of high pH see a lower laser power and fluoresce less causing spurious pH predictions. This method also required extensive calibration in order to account for the Gaussian distribution in the laser light intensity across the light sheet.

In the present work, we have selected a new fluorescent dye, carboxy-SNARF, which overcomes the calibration limitations imposed by fluorescein. Carboxy-SNARF is also a pH sensitive fluorescent dye which fluoresces in two separate wavelength bands. Figure 1 (taken from Molecular Probes) shows how each bands fluorescence intensity varies with pH when excited with 514 nm laser light6. The major advantage of a dual band emission dye is that the fluorescent intensity image of each band can be ratioed. This normalization process removes variations introduced by laser light absorption, laser power and changes in dye concentration. Therefore, we can obtain a two dimensional map of ratio values which reflects the mixing history at each point. It is important to ensure that the convective time scales are much faster than the diffusion time scales to prevent diffusion from the hydrogen ion gradient from influencing the results. This condition is equivalent to requiring high Schmidt numbers.

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**Experimental setup**

Figure 2 shows our experimental setup. A particular line of the argon ion laser (514 nm or 488 nm) is passed through a cylindrical lens (f=5 mm) and into a water tank containing a $10^{-4}$ molar solution of carboxy-SNARF dye. Carboxy-SNARF (seminaphthorhodafluor) is a man-made chemical purchased from Molecular Probes. The pH of the tank was changed using HCl and NaOH and measured on a PHH-70 model Omega pH meter which has a resolution of 0.01 pH units, an accuracy of 0.02 pH units and a range of 0-14 pH units. A Sony Video Hi8 video recorder was positioned perpendicular to the laser sheet in front of the tank. The camera is accurate to 7 bits due to tail effects at either end. Two filters were used to find the intensity of light in each band. The filters were centered at 580 nm and 640 nm respectively and had a bandwidth of 0.10 nm. The images were frame grabbed and processed on a Macintosh Quadra 950 computer.

**Results**

Figures 3-5 show our preliminary results on the response of the dye. We examined the effect of laser power on the ratio values to ensure laser power was normalized out as we expected. As a matter of convention all ratios were calculated by dividing the red image by the yellow image. Figure 3 shows the variation in the ratio values as a function of laser power at a pH of 6. The y axis represents the ratio image at a given laser power divided by the ratio image at 120 mW. The x axis is the ratio of the laser power. These average values show less than an 4% difference for laser power variations over a span of four times the starting laser power.

The next step was to determine the calibration curve of ratio values versus pH. Figures 4 and 5 show the results of ratio values versus pH at the excitation wavelengths 514 nm and 488 nm respectively. In figure 4, the square points represent data from a camera while the diamonds are data points from a United Detector Technology power meter. The normalized ratio reported was the mean of the ratioed image at a given pH. The standard error within each image varied with pH. Typical standard error is under 5% for a pH range of 6 to 8 and under 12% for a pH range of 8 to 9.
Finally, figures 8 a,b,c,d show an unsteady case of a droplet of base into an acidic environment using 514 nm excitation light. The images show a burette injecting the base droplet into the acidic solution. Figures 8a and 8b show the red and yellow images respectively. The high pH droplet appears bright in the red image and dark in the yellow image which is consistent with what is predicted by the response curve in figure 1. Figure 8c shows the ratio of figures 8a and 8b. Again the high pH droplet appears brighter which is consistent with the experimentally determined curve in figure 4. From the ratio value in figure 8c we were able to determine the pH at every point and figure 8d shows the pH contours from those calculations. To determine a pH from the ratio image we fit the points in figure 4 with a fifth order polynomial to relate the ratio values to pH. Finally, one point of interest is that the ratio image and the contour map indicate there is a high pH region at the top of the image. This is the result of buoyancy effects causing the base to float to the top and collect there. The same effect is causing the droplet to float to the top.

Discussion
Since the power variations within the interrogation region of the laser sheet are less than the factor of 4, from figure 3, we can expect under 4% error from any variations in the sheet. The pH range being considered for mixing measurements is between 6-8.5 pH units. This is because outside of this range one of the fluorescence bands intensity drops below the camera’s sensitivity.
By fitting a fifth order polynomial to our data points in figure 4 and taking into account the standard error in each data point, an approximate resolution of ratio versus pH can be obtained. In the 6-8 range, pH differences of approximately 0.1 unit can be resolved, while between 8 and 8.5 pH can be resolved to approximately 0.5 units. The droplet images show that small variations in pH do cause the fluorescence to change significantly. This is especially true in the red and yellow images where the intensity differences are very pronounced. However, a more accurate calibration curve is needed so that the intensity information can be used for greater resolution in pH measurements.

**Conclusion**

Our preliminary results show that ratio imaging using a dual emission dye is a valid technique for measuring pH and thus mixing in an aqueous solution. Ratio imaging to determine pH in cells is already an established technique in the biochemistry industry\(^7\), although not for measuring pH in bulk systems. Our system does present some unique limitations. First for the technique to measure mixing, a high Schmidt number flow is required. In addition, for cellular measurements two 1 nanometer width filters can be selected to optimize the calibration curve of the ratio values versus pH. We require much more light than is available in any one wavelength and therefore we cannot be as selective in the fluorescence light we use. Some of the same limitations we encounter are that our method is limited to acid base measurements of fluids in the range of 6-9 pH units. Below 6 and above 9 pH units one of the wavelengths intensity drops to zero. Another limitation that we have in common is photo bleaching. This is when the excitation light destroys the dye molecule. However, our system is much larger than biochemists and the same dye molecule will not be exposed to the laser light as much as it would be in cellular measurements. Therefore it is unclear how great of a problem photo bleaching will be.

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**References**


