SIMULTANEOUS MEASUREMENT TECHNIQUE OF DISSOLVED OXYGEN CONCENTRATION AND VELOCITY FIELD USING OXYGEN SENSITIVE PARTICLE IN MICROCHANNEL

Hyun Dong Kim[1], Seung Jae Yi[1], Kyung Chun Kim[1,c]

[1]School of Mechanical Engneering, Busan, 609-735, Republic of Korea

[c]Corresponding author: Tel.: +82515102324; Fax: +82515157866; Email: kckim@pusan.ac.kr

KEYWORDS:
Main subject(s): *simultaneous visualization of velocity and concentration field*
Fluid: *Microchannel flow, water,*
Visualization method(s): *Laser Induced Phosphorescence, Particle Image Velocimetry*
Other keywords: *oxygen sensitive particle, UV LED, dissolved oxygen concentration*

ABSTRACT : *This study introduces a simultaneous measurement technique of velocity and dissolved oxygen concentration (DOC) fields in micro-scale water flow using oxygen sensitive functional particles. Oxygen Sensitive Particle (OSParticle) used as oxygen sensor and tracer particle of Particle Image Velocimetry (PIV) were fabricated by dispersion polymerization method. Platinum (II) octaethyporphrin (PtOEP) known as typical oxygen indicator molecule and liquid polystyrene (PS) and Azoisobutyronitrile (AIBN) reagent were synthesized under the 80 ℃ of temperature. Diameter of OSParticle distributed in the range of 2.2 ~ 3.9㎛ and could be controlled by adjusting the amount of AIBN reagent. Water and OSParticle solutions having DOC values of 0% and 100% were injected to Y-shaped microchannel by using double loading syringe pump to measure velocity and dissolved oxygen concentration fields simultaneously. A ultra violet light emitting diode (UV-LED) with wavelength of 385 nm was used as a light source and phosphorescence OSParticle images were stored by using a CMOS high speed camera with an olympus BX51 microscope system. 20x objective lens and a 590nm long-pass optical filter were adopted to obtain clean images of OSParticles. With the Stern-Volmer equation of the luminescence of OSParticles with respect to the DOC plotted by in-situ calibration, the DOC field over the whole channel cross section area can be quantified. The velocity vector field inside of Y-shaped microchannel was extracted from the phosphorescence particle images using conventional two frame cross correlation algorithm.*

1 Introduction

The dissolved oxygen is one of the essential substrate in aerobic microbial processes such as water treatment, fermentation, cultivation of microorganisms and production of industrial chemicals. Especially, in the micro-bioreactor technology, it is crucial to supply sufficient oxygen during bio-reaction processes due to the low solubility of oxygen in culture broths (aqueous solutions), which is only worsened by laminar flow and difficult mixing circumstances. Scarcity of dissolved oxygen can affect the performance of micro-bioreactor system. Therefore, efficient supply of dissolved oxygen into the micro-bioreactor by providing sufficient mixing or increasing oxygen content in the supply gas is important. Moreover, measurement of DOC and accurate estimation of oxygen transfer rate (OTR) under the different conditions has a relevant role for the design, selection and scale-up of micro-bioreactor. [1].

Conventionally, the dissolved oxygen is measured by using electro-chemical sensors such as polarographic and galvanic one measuring the change of current generated by oxidation and de-oxidation. In recent years, to measure DOC in micro scale ultra-microelectrode array (UMEA) and optode sensor technique measuring the fluorescence intensity which varies with the DOC is also developed. [2]

However, electro-chemical sensor has low accuracy at low concentration of dissolved oxygen and optode sensor can measure the concentration of a particular point, so they are not suitable for measuring the dissolved oxygen field and the diffusion coefficient. To overcome these drawbacks, the laser induced fluorescence (LIF) method using oxygen indicator molecule has been studied. Philip et al. mapped distribution of DOC across air-water wavy interface using the LIF technique. Dani and Francois visualized oxygen transfer phenomena near the single bubble rising in water by measuring the time variation of fluorescent intensity of ruthenium complex. Song et al. measured the DOC field on the bottom wall of Y-shaped microchannel with oxygen sensitive film sensor. [3, 4, 5]

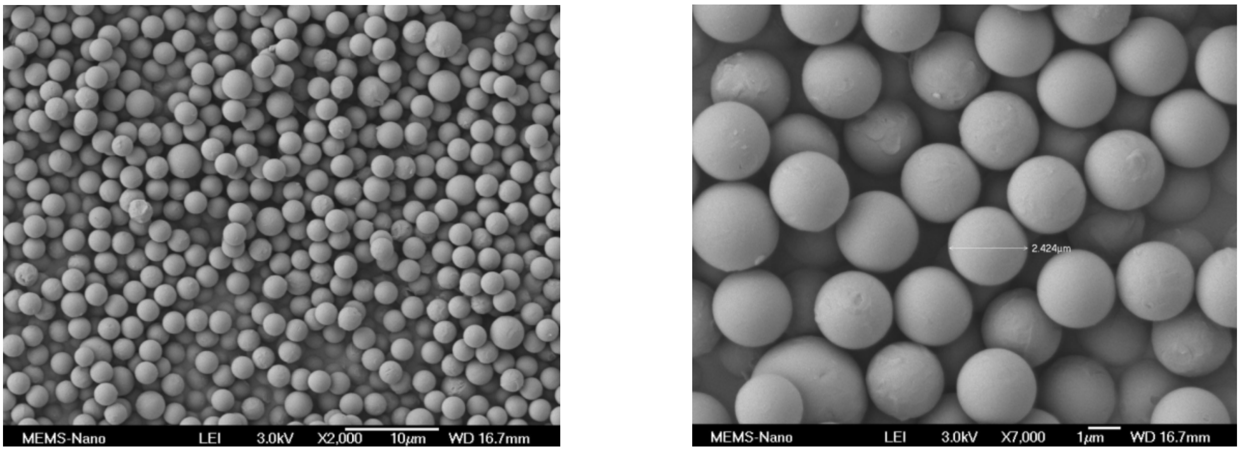
A particle based oxygen concentration monitoring or pressure measurement techniques have been studied by some research groups. Koo et al. measured change in oxygen concentration near the C6 glioma cells using PEBBLE (Photonic Explorer for Biomedical use with Biologically Localized Embedding) sensor fabricated with ruthenium complex and silica nano particles. PIV-PSP hybrid system using pressure sensitive particles (PSParticles), micro-balloons made of silicon dioxide coated with ruthenium complex, to get oxygen concentration field and velocity field around nitrogen gas jet was demonstrated by Abe and co-workers. While this experiment demonstrates a proof of concept, the researchers could only make oxygen concentration measurements over a limited dynamic range (0 ~ 1 %). Kimura et al. developed dual luminophore polystyrene microspheres (OSBeads) containing both an oxygen-sensitive platinum porphyrin luminescence and a pressure-insensitive silicon porphyrin luminescence and measured velocity and pressure field. [6, 7, 8]

However, DOC field measurement in the liquid phase flow with oxygen quenchable particles has never been tried. Considering that oxygen transfer phenomenon is highly related to internal flow characteristics in a few micro-bioreactor systems, it is important to obtain information about DOC and velocity field simultaneously for the efficient design and analysis of micro-systems. This paper will therefore discuss about simultaneous measurement technique of velocity and DOC fields in micro-scale water flow using oxygen sensitive functional particles.

2 Fabrication of Oxygen Sensitive Functional Particles

In this research, the oxygen functional particle made by dispersion polymerization method is adopted for the simultaneous measurement of oxygen concentration and velocity in the micro channel, so functional particle is synthesized by a dispersion polymerization process. 45 mL of ethanol and 5 mL of de-ionized water are put inside of a three-neck flask, then heated at 80℃ for 30 minutes. 0.04g of PtOEP, 5mL of styrene, and 0.2g of AIBN are added to the solution and the polymerization is progressed for 24 hours. Then those polymerized beads are dried and washed three times using ethanol. As the diameter of functional particle depends on how much AIBN is added, it is easy to change the

size of particle. However, as the functional particle become larger, their size distribution was also broadened. Fig. 1 shows the SEM images of fabricated functional particle. Diameter of OSParticle distributed in the range of 2.2 ~ 3.9μm and mean diameter estimated from the images was 2.56μm (STD ~ 0.2218). We can confirm that the particle size is quite uniform and the shape is totally sphere.
After fabricating functional particle, the oxygen sensitivity of fabricated particle was measured by a spectrometer. The spectrum of luminescence from the particle was obtained with de-ionized water sample having different dissolved oxygen concentration. Fig. 2 shows the luminescence spectra of functional particle when they are excited with 385 nm UV LED (M385L2, Thorlabs). The wavelength of peak for each spectrum is same at 646 nm and the value of peak is decreased with the increase of dissolved oxygen concentration. By the Stern-Volmer equation, the luminescent intensity of a luminophore in a polymer matrix decays naturally by the first order process and quenched bimolecularly by oxygen.



(a) (b)

Fig. 1 SEM images of OSParticles fabricated by dispersion polymerization method (a) 2000X (b) 7000X.

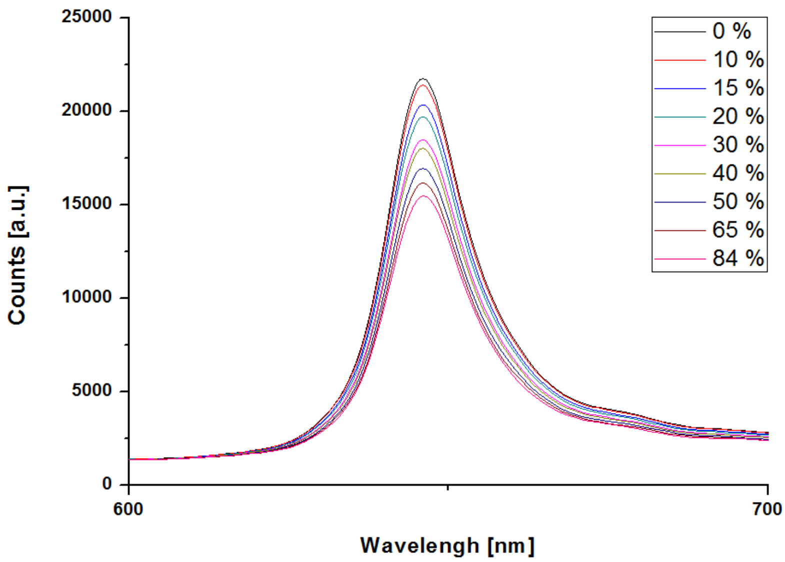


Fig. 2 The Variation of luminescent intensity by increasing DO concentration

3 Experimental Setup

The oxygen sensitive functional particle made by dispersion polymerization method is used for simultaneous measurement of oxygen concentration and velocity field in the microchannel. Figure 3 shows the experimental setup for simultaneous measurement. The Y-shaped microchannel was used to flow the different dissolved oxygen concentration water mixed with functional particle. To protect from the oxygen permeation, the micro channel was made by glass and silicon wafer. The micro channel consists with two inlet branches (200 μm width) width and one outlet channel (400 μm width) and depth of channel is 75 μm. The micro channel was fabricated by dry etching, sand blasting, and anodic bonding processes. A double loading syringe pump (KD Scientific) is used to inject water samples with two different DO concentrations. The 0 % and 100% of water sample were prepared by using DO meter (YSI 550A, YSI, USA) and sodium sulfite. The channel was loaded on the microscope (Olympus BX51) and 385 nm UV LED was adopted in the microscope to exciting the particle in the water sample. The image data was captured using a CMOS high speed camera (Photron, Fastcam SA1.1) with 60 frames per second and an Olympus 20x objective lens was adopted. The luminescence from the particle was filtered with a 590 nm long-pass optical filter**.**

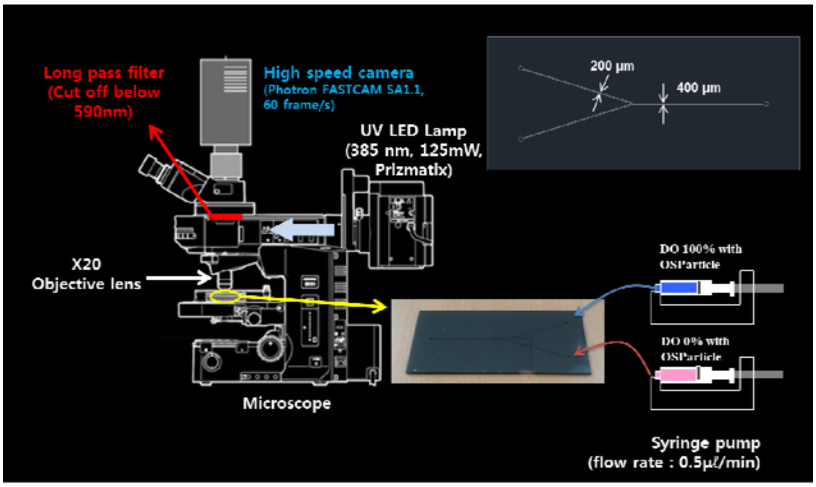


Fig. 3 Experimental setup for simultaneous measurement of DOC and velocity field in microchannel

4 Calibration of DOC and Luminescent Intensity

For the oxygen concentration calibration, an intensity based modified pixel by pixel calibration method was developed. The conventional pixel by pixel calibration method has been used for LIF in micro scale flows means each pixel of the camera should be calibrated with known concentration. This method is useful for continuous luminescence particle image to measure concentration field since non uniformity of excitation intensity is no longer problem compared to a single value calibration. However, in the present study, the concentration image data is not continuous in the field of view. Although we have thousand instantaneous images of OSParticles, but conventional ensemble averaging method with a single calibration curve cannot provide accurate information of the dissolved oxygen field in this micro scale experiment. Fig. 4 (a) demonstrates the flow chart of calibration procedure to obtain Stern-Volmer plot between phosphorescence intensity and DOC. To applying modified pixel by pixel calibration, we divided all of data images, including calibration images, to make a window with 8 x 8 pixels. The intensity value of OSParticles was filtered with 1 % noise filtering method. After that, intensity was ensemble averaged with particle number for each window and 50 % of window size was overlapped. Then we calculate calibration curve for each interrogation window from calibration images and the calibration curve was applied to the experiment data. Fig. 4 (b) shows Stern-Volmer plot of (61, 649) interrogation window.

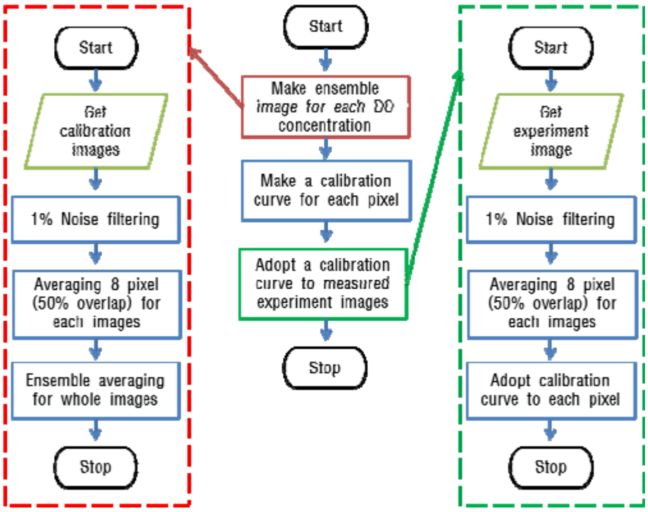


Fig. 4 The flow chart of modified pixel by pixel calibration procedure

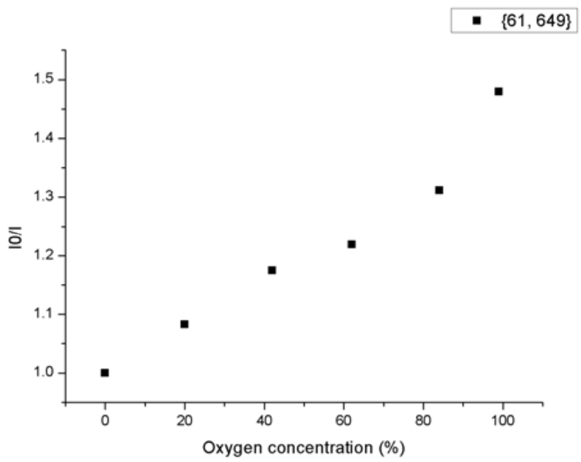
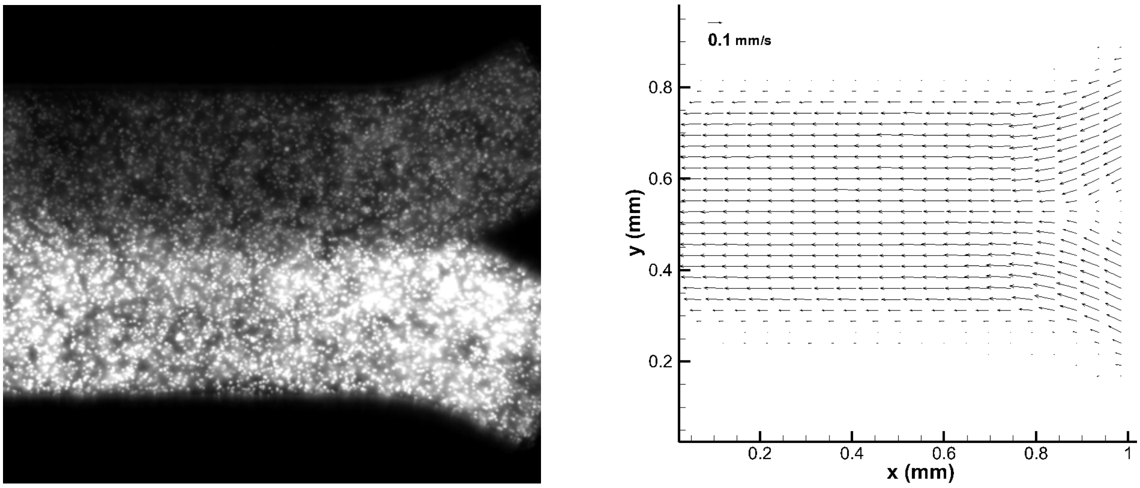


Fig. 5 Stern-Volmer plot at (61, 649) interrogation window

5 Results and Discussion

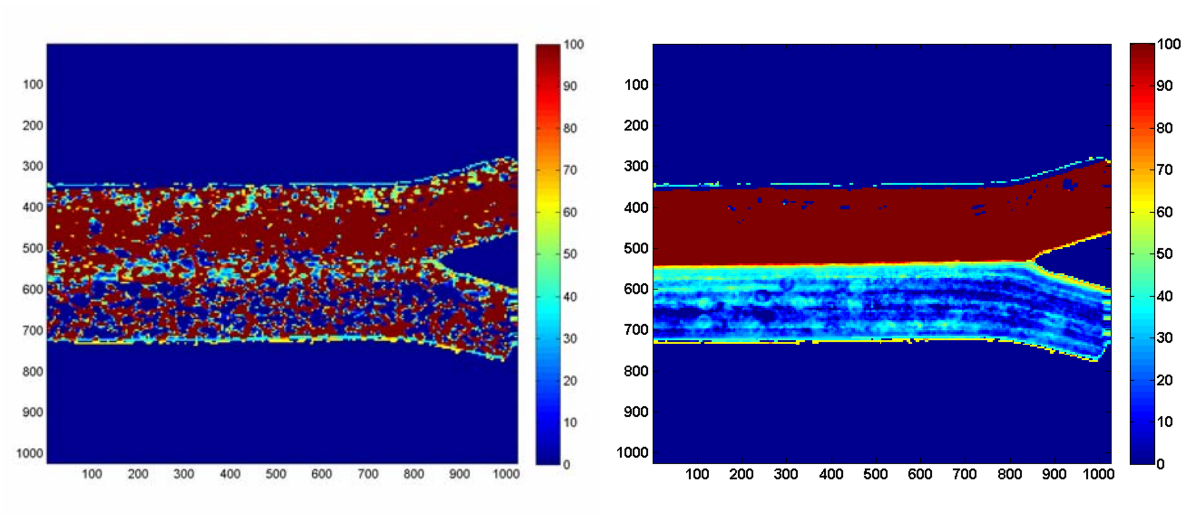
Fig. 6 (a) shows the instantaneous image of OSParticles for simultaneous measurement. The water having 0% of DOC was injected to upper branch and water of 0% DOC was injected to the other blanch. The intensity of OSParticle in the 0 % water sample is much higher than 100 % water sample. With these images, the velocity field was calculated by 48 X 48 pixel interrogation window using

conventional two frame cross correlation algorithm. It should be noted that DO concentration can be obtained where the OSParticles are placed in continuous media.
Fig. 6 (b) depicts the ensemble averaged velocity field extracted from the OSParticle images in the Y-shaped microchannel. The velocity field was obtained successfully in spite of intensity variation due to DOC difference between upper and lower branch. Since the injection flow rate of each branch is the same as 0.5μℓ/min, the velocity field in the micro channel looks a parabolic laminar 2D channel flow although DO concentrations are different.
Figure 7 (a) shows the instantaneous dissolved oxygen concentration field after applying the calibration procedure. Figure 7 (b) shows ensemble averaged DO concentration field. We could confirm that the oxygen concentration of upper branch is higher than that of the lower branch.



(a) (b)

Fig. 6 (a) The Luminescence OSParticle image in microchannel and (b) ensemble averaged velocity vector field extracted from the OSParticle images



(a) (b)

Fig. 7 (a) Instantaneous DO concentration field and (b) ensemble averaged DO concentration field

6 Conclusion

Oxygen sensitive functional particle was fabricated by using dispersion polymerization method and it was applied for simultaneous measurement of oxygen concentration and velocity field in the micro channel. The particle size is very uniform and oxygen tendency was confirmed. The velocity field was calculated successfully and we could confirm that the possibility of quantitative measurement of oxygen concentration in micro system.

Acknowledgments

This work was supported by the National Research Foundation (NRF) of Korea through a grant funded by the Korean Government (No. 2011-0000199, No.K20703001798-11E0100-00310). This work was supported by the second phase of Brain Korea21 Project in 2010.

References

1. Garcia-Ochoa F and Gomez E Bioreactor scale-up and oxygen transfer rate in microbial processes: An overview. *Biotechnol Adv*, Vol. 27, pp153-176, 2006.
2. Schäpper D, Alam Z, Szita N, Lantz A and Gernaey K. Application of Microbioreactors in Fermentation Process Development: A Review. *Anal. Bioanal. Chem*. Vol. 398, pp.679~695, 2009.
3. Philip T, and Steve R. Laser-Induced Fluorescence Studies of Oxygen Transfer Across Unsheared Flat and Wavy Air-Water Interfaces. *Ind. Eng. Chem. Res*. Vol. 40, pp.1985-1995, 2001.
4. Dani A., Guiraud P. and Cockx, A. Local Measurement of Oxygen Transfer around a Single Bubble by Planar Laser Induced Fluorescence. *Chem. Eng. Sci.,* Vol. 62, pp.7245~725, 2007.
5. Song D., Kim H. and Kim K. Dissolved Oxygen Concentration Field Measurement in Micro-scale Water Flows using PtOEP/PS Film Sensor. *Opt. Lasers Eng.,* Vol. 50 pp.74~81, 2012.
6. Koo Y., Cao Y., Kopelman R., Koo S., Brasuel M. and Philbert M, Real-time Measurements of Dissolved Oxygen Inside Live Cells by Ormosil (Organically Modified Silicate) Fluorescent PEBBLE Nanosensors. *Anal. Chem*., Vol. 76 pp.2498~2505, 2004
7. Abe S., Okamoto K. and Madarame H. The development of PIV-PSP hybrid system using pressure sensitive particles. *Meas. Sci. Technol*. Vol. 15, pp 1153-1157. 2004.
8. Kimura F., Khalil G., Zettsu N., Xia Y., Callis J., Gouterman M., Dalton L., Dabiri D. and Rodriguez M. Dual Luminophore Polystyrene Microspheres for Pressure-sensitive Luminescent Imaging. *Meas. Sci. Technol*. Vol. 17, pp 1254–1260, 2006.