Three-dimensional Flow Measurement around Micro-obstacles in a Water by Total Internal Reflection Fluorescence Microscopy and Refractive Index-matching Method

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Abstract Total internal reflection fluorescence microscopy (TIRFM) is a powerful tool for analyzing 3D fluid flow in micro-channel. To visualize the 3D fluid flow via TIRFM, intensity calibration of a fluorescent particle is required by using calibration plates, carrying a set of nano-steps of different heights. In the present study, to discern the square obstacles in water, TIRFM visualization is carried out by a refractive index-matching method using a water employed as a working fluid. The water is chosen to be able to adjust its refractive index to match to that of the MEXFLON obstacles with an index of 1.33. We have demonstrated a fabrication technique for making the square obstacles on a glass substrate by employing thermal nanoimprint. In summary, we have succeeded in the fabrication of square obstacles made of a polymer resin and used it for the visualization of the fluorescence intensity in a water.
Keywords: TIRFM, Nanoimprint, Refractive index-matching method

1 Introduction

A detailed understanding of the fluid flow in the micro- and nano-scale channel is a key factor to improve the precision and efficiency of lab on a chip. Therefore, various analytical methods for the micro- and nano-scale fluid flow have been investigated in labs around the world. Particle image velocimetry (PIV) [1] and particle tracking velocimetry (PTV) [2] are commonly employed for this purpose. In the cases of PIV and PTV, the fluid flow is analyzed by observing spherical particles suspended within a fluid, at regular time intervals. Recently, the observation methods for x-y-z (3D) fluid flow are reported like the ones for the x-y (2D) fluid flow have been. For instance, stereoscopic PIV [3] and total internal reflection fluorescence microscopy (TIRFM) with multilayer technique and index matching [4] are powerful tools to analyze the micro- and nano-scale fluid flow. However, the resolution of stereoscopic PIV is approximately 10 μm that makes it difficult to measure the flow that is very close to the channel wall. On the other hand, multilayer PIV with TIRFM has sub-micron scale resolution. To visualize the 3D fluid flow via TIRFM, the intensity calibration of a fluorescent particle would be required by using a calibration plate bearing nano-steps with different heights. In a case like this, the refractive indices of the nano-steps and of the fluid must be close to each other. In an attempt to fabricate the calibration plates for use in water, and to measure the water flow, we employed a material MEXFLON, the refractive index of which is nearly equal to that of water [5]. As a result, the available fluid for the multilayer TIRF method was water. We have demonstrated a fabrication technique for making the square obstacles on a glass substrate by employing thermal nanoimprint. We have succeeded in the fabrication of square obstacles made of a polymer resin and used it for the visualization of the fluorescence intensity in a water.

2 Experimental apparatus and procedure

Figure 1 shows a schematic view of our TIRF measurement system. We used a continuous-wave green laser (λ=561 nm, 85-YCA-025-040, CVI Melles Griot Co.) as an evanescent light source. The TIRFM system, that carried the incident light, comprised NIKON Eclipse Ti-E with a 100x objective lens (NA=1.49, CFI Apo TIRF 100x, Nikon Instruments Inc.). In this system, the green laser source was filtered by a barrier filter so that only the fluorescence could be captured by the EM-CCD camera (Andor, iXon3 860). The resolution of EM-CCD pixel was 24 μm. For the fluorescent particles R200 (Thermo Scientific Co.) with diameters of 200 nm were used. This particle was diluted by 0.01 % with deionized water. For the fabrication of the nano-steps a material MEXFLON E-PDx (UNIMATEC Co. LTD.) was used [6], which has its refractive index about same as that of water. The refractive indices of water and MEXFLON are 1.333 and 1.330, respectively.

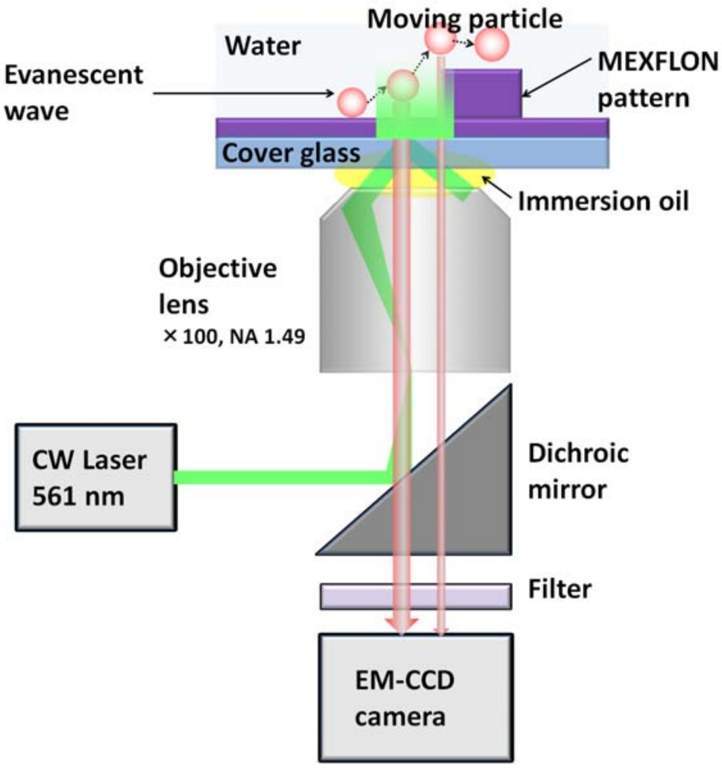

Fig. 1 Experimental apparatus

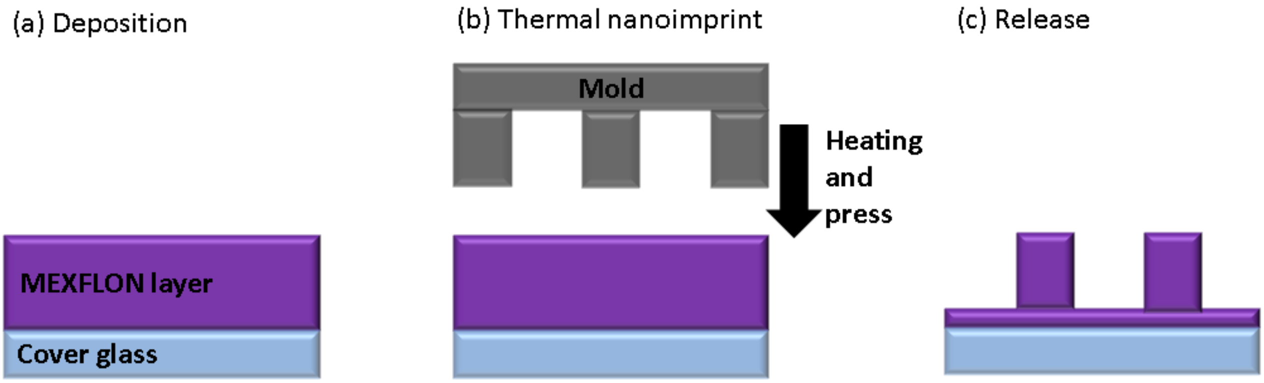

Fig 2. Nanoimprint using a deposited MEXFLON layer on the cover glass.

At first, MEXFLON layer was deposited onto a cover glass using vacuum evaporation method. After that, thermal nanoimprint [7] was carried out to obtain a nano-step pattern (Fig. 2). The fabricated pattern on the cover glass was shown in Fig. 3.

We measured the obtained MEXFLON pattern by AFM, as shown in figure 4(a). The width was 11 μm, and the height of the pattern was 362 nm. Figure 4(b) shows the optical images of MEXFLON pattern without water. In this case, the images were recorded by the standard optical microscope with a halogen lamp.

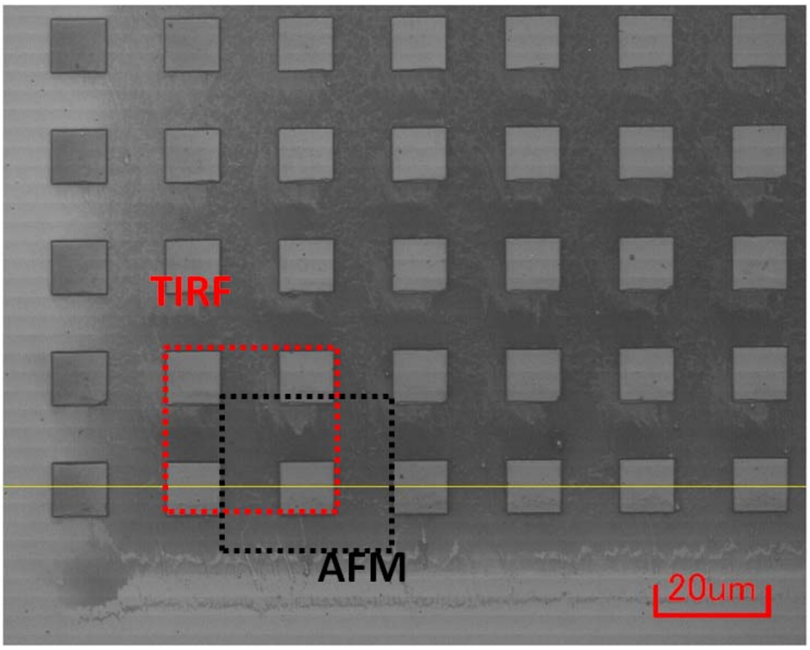

Fig. 3 Laser microscope image of the cover glass with MEXFLON pattern.

Figure 3 shows laser microscope image of the cover glass with MEXFLON pattern. The observation areas for TIRF and AFM are shown in two square-shaped segments red and black marked by chain lines

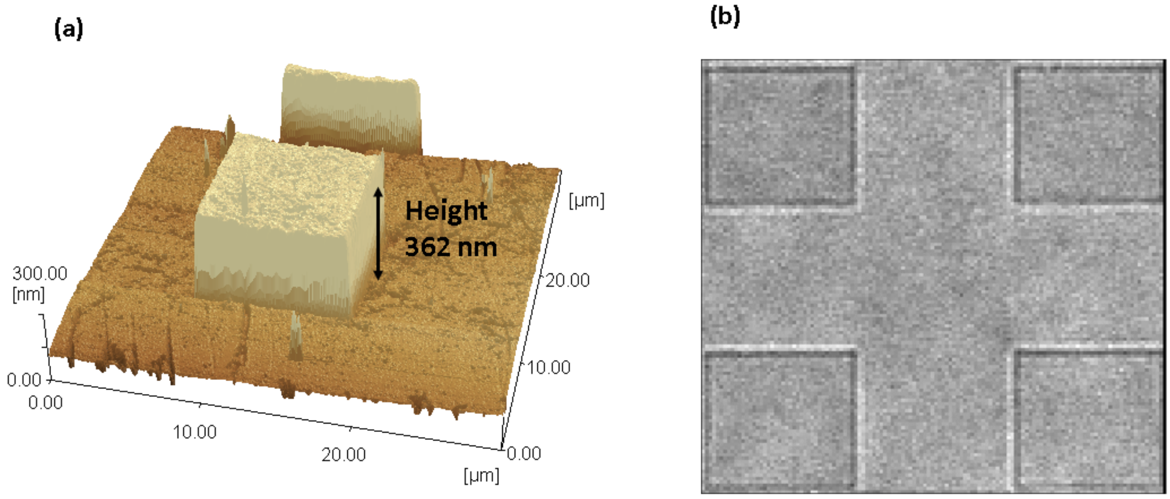

Fig. 4 (a) AFM image and (b) Microscope image of MEXFLON pattern using a halogen lamp without water.

3 Results and discussion

Figure 5 shows TIRF images of a moving fluorescent particle around the MEXFLON pattern: (a) 0 ms, (b)521 ms, (c) 2550 ms, and (d) 2654 ms. From each image, we can see the difference in the heights between the particles on the buttom and the particles on the MEXFLON obstacle. As the height of the particle position increases, we can notice a decrease in the brightness of a particle.

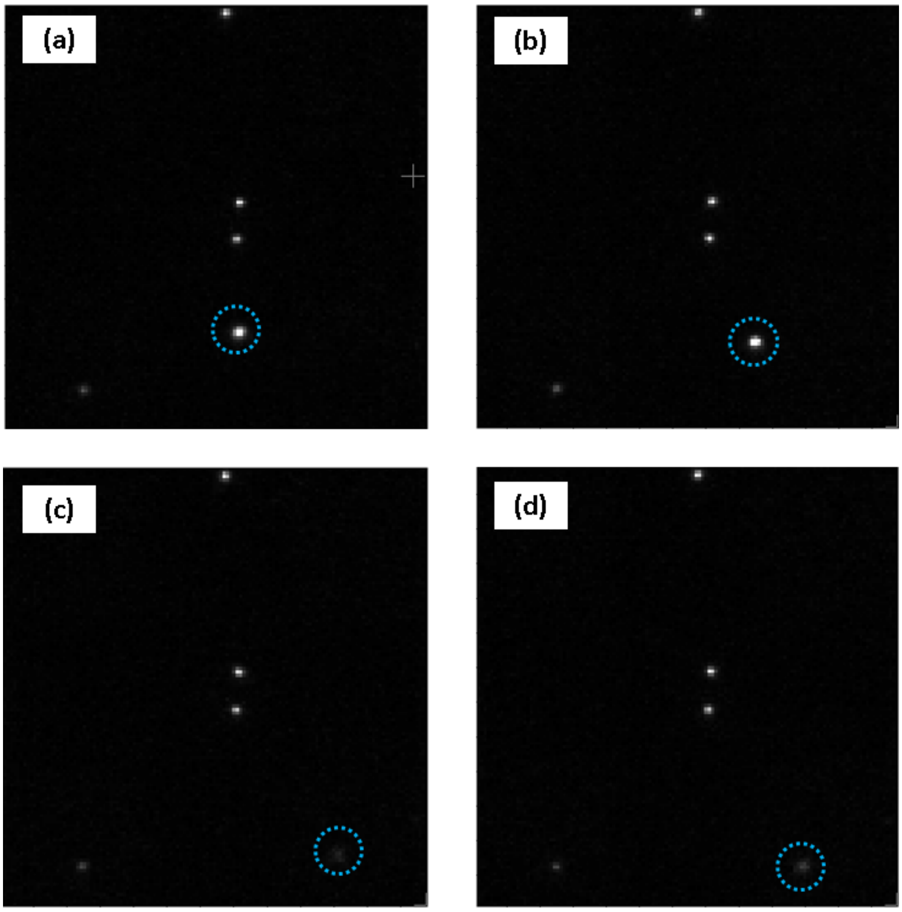

Fig. 5 TIRF images of a moving fluorescent particle around the MEXFLON pattern.
(a) 0 ms, (b) 521 ms, (c) 2550 ms, (d) 2654 ms

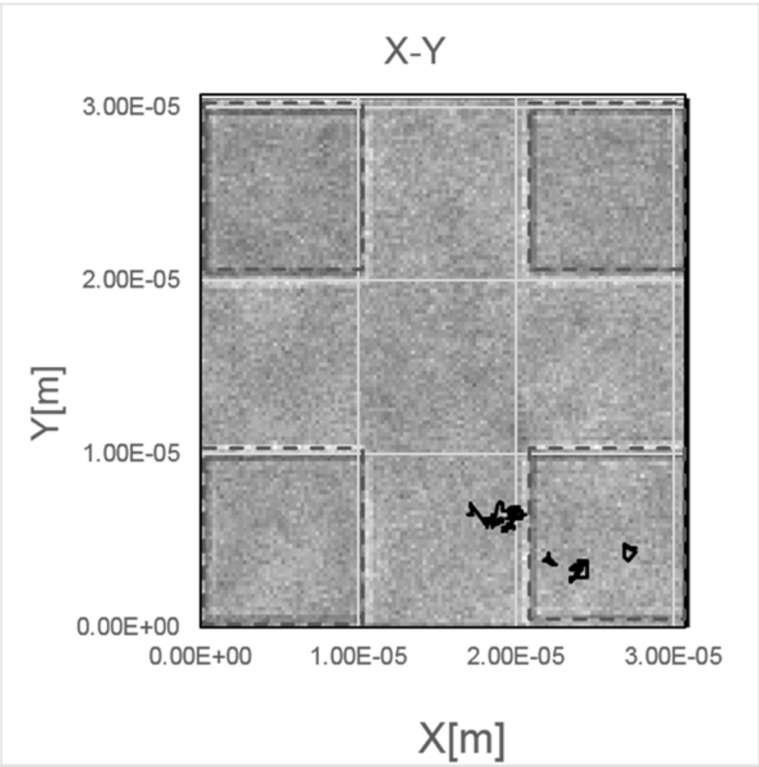

Fig. 6 Particle tracking result using layer-TIRFM method with the MEXFLON pattern.

Figure 6 shows particle tracking result using layer-TIRFM method with the MEXFLON pattern. The trajectory shows from the bottom of MEXFLON to the top of the obstacle.

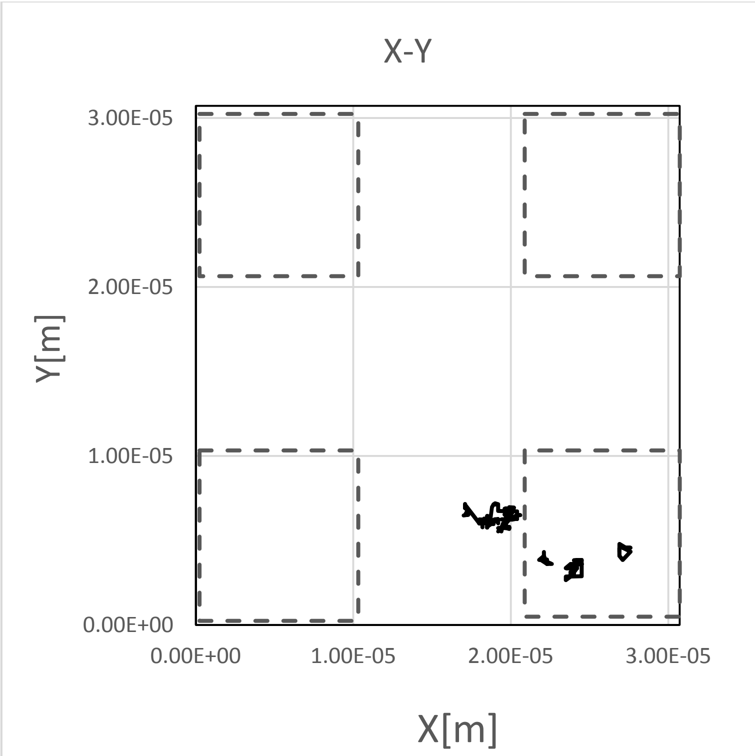

Fig. 7 Particle tracking result using layer-TIRFM method with the MEXFLON pattern in the x-y plane

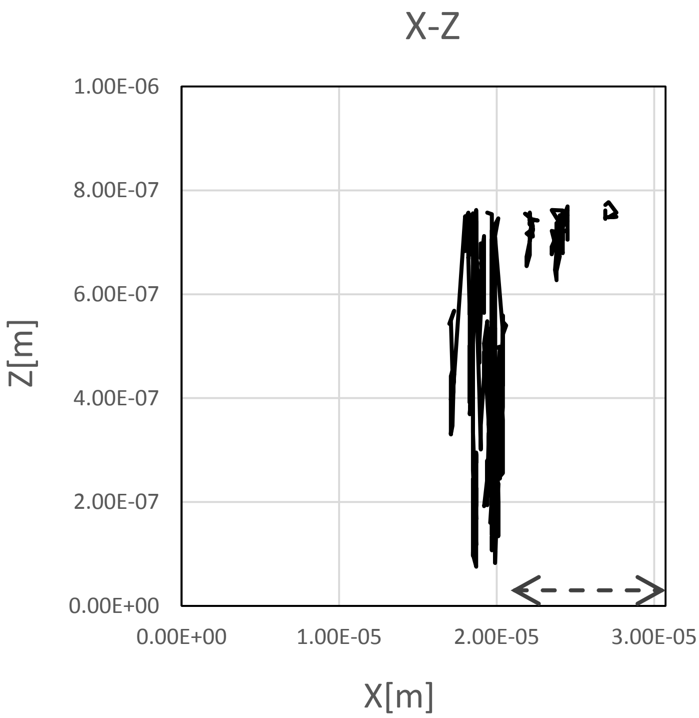

Fig. 8 Particle tracking result using layer-TIRFM method with the MEXFLON pattern in the x-z plane

We can find a particle moving across the obstacle on the x-y side in Fig. 7, but we can also confirm what the moving particle rises for the height of the obstacle in the x-z side in Fig. 8.

4 Conclusion

In this paper, three-dimensional particle position around Micro-obstacles in water was obtained by Total Internal Reflection Fluorescence Microscopy and Refractive Index-matching Method. The reconstructed particle position can be obtained from the fluorescence image of particle on micro-obstacles made of MEXFLON.

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