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Fabrication of nanostep for total internal reflection fluorescence microscopy to calibrate in water

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ABSTRACT

Total internal reflection fluorescence microscopy (TIRFM) is a powerful tool for analyzing *x*–*y*–*z* (3D) fluid flow in nano- or 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 an attempt to fabricate a calibration plate to be used in water ambient we employed a polymer with a refractive index close to that of water. In this study, we have developed a lift-off technique for making polymer resin pattern on a glass substrate by employing the use of polyvinyl alcohol film and metal mask previously fabricated by nano transfer printing (nTP). In summary, we have succeeded in the fabrication of nanosteps made of a polymer resin and used it for the calibration of the fluorescence intensity in water.

1. Introduction

Micro- and nano-scale fluid flow has received lots of attention because of its well sought out application in lab on a chip, such as in high-precision sensor, high-efficiency chemical reactor, and fast drug discovery, etc. [1–3]. 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) [4] and particle tracking velocimetry (PTV) [5] 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 [6] and total internal reflection fluorescence microscopy (TIRFM) with multilayer technique and index matching [7] 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. Therefore, a combination of magnesium fluoride (MgF_2) nano-step on a glass substrate and 1-propanol, is generally used because the thickness of MgF_2 layer is easy to control to a very precise degree via vacuum deposition method. However, water, although highly desirable for the task cannot be used for the measurement with MgF_2 layer because of the difference of their refractive indices. As a result, the only available fluid for the multilayer TIRF method was 1-propanol.

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 [8,9]. The conventional calibration plates use nano-steps made of MgF_2, which is an inorganic material, thus, the normal lift-off process with an organic solvent is possible. In contrast, the key point of our technique is using the nano-steps with a polymer, MEXFLON, whose refractive index is close to water. However, the durability of MEXFLON for an organic solvent is not cleared because MEXFLON is expected for using in only water. Although the required pattern width for the calibration plate is micro-scale, the edge shape of the pattern is very important. For instance, we examined vacuum deposition method with a stencil mask. Fig. 1 shows the cross-section of the obtained MEXFLON pattern measured by a profilometer (Alpha-Step IQ, KLA-Tencor Co.). However, this sample was impossible to be used as the calibration plate because the width of the slope was over 30 μm, which is wider than a field of view of our TIRFM system of 30 μm × 30 μm, and we could not distinguish

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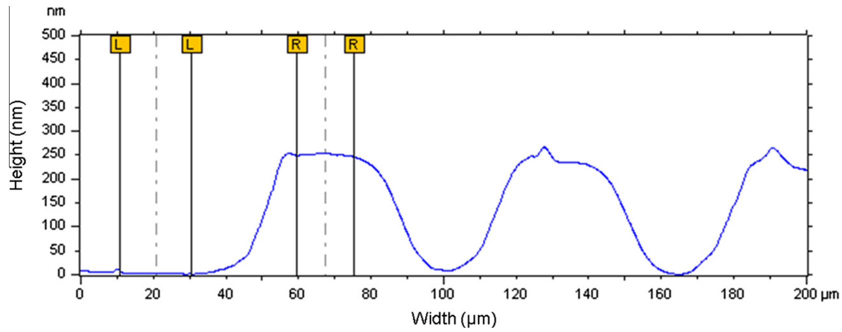

Fig. 1. Cross-section of the fabricated MEXFLON pattern using a stencil mask.

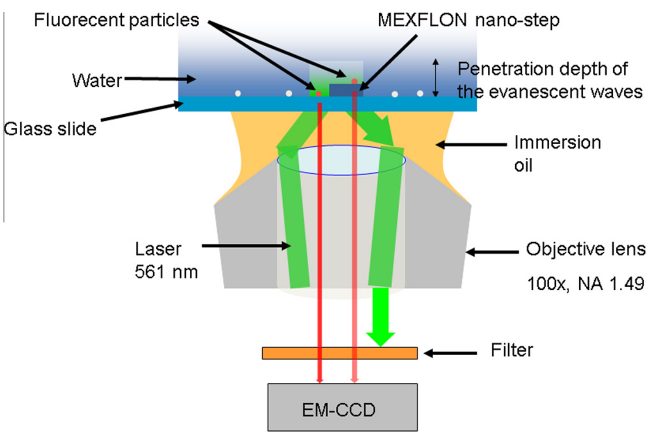

Fig. 2. Schematic view of TIRF measurement system.

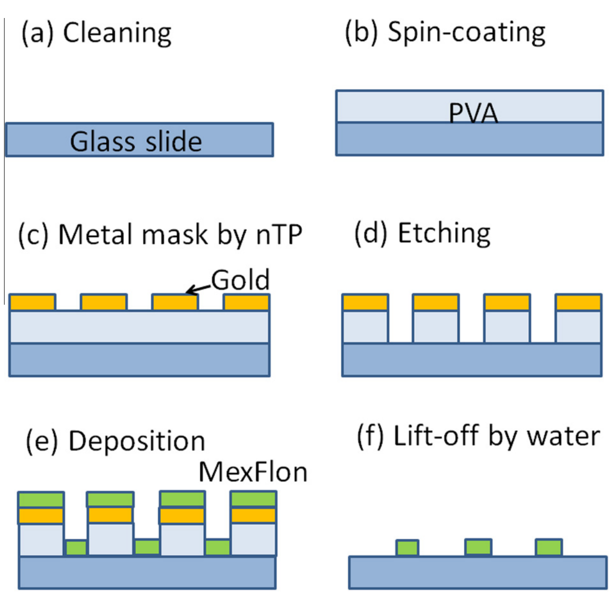

Fig. 3. Line and space patterning process by using a metal mask and lift-off with water.

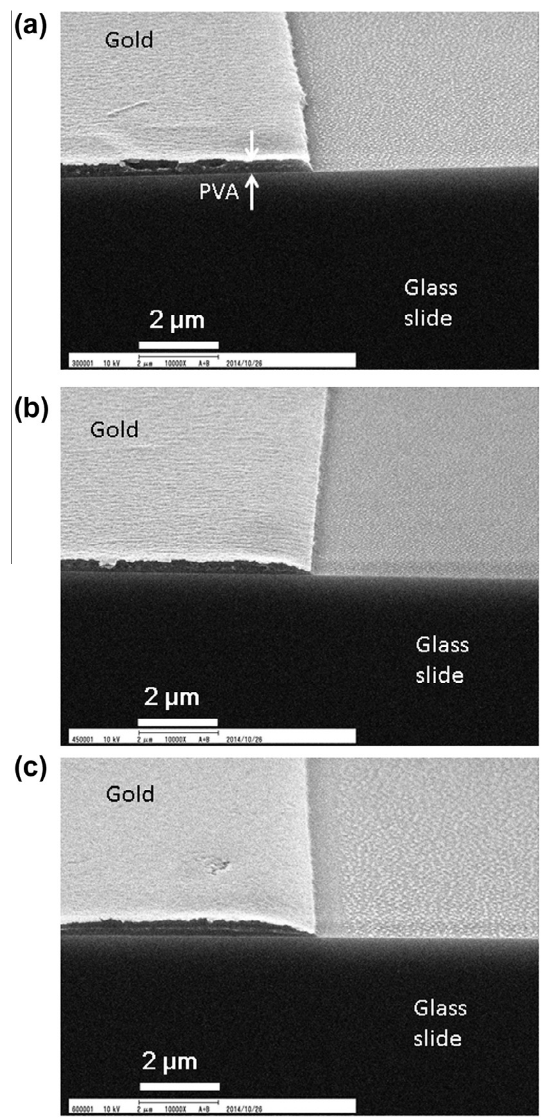

Fig. 4. The cross-sectional SEM images after (a) 30 s, (b) 45 s, (c) 60 s etching with the power of 100 W.

the pattern edge from the glass surface. Consequently, the lift-off process with water is needed. In this study, therefore, we used polyvinyl alcohol (PVA) for the lift-off process [10,11] because PVA is soluble in water whereas other organic solvents can cause damage to a polymer resin, and definitely not recommendable. As shown in the references, some PVA lift-off techniques have already been reported. However, the technique of Ref. [10] requires an additional etching process using CHF_3 to obtain a reverse tapered shape, which is helpful to carry out the lift-off process easily. On the other hand, an UV curable resin is used for patterning in Ref. [11]. In this case, the PVA layer was removed by water. If an oxygen plasma was used for etching the PVA layer, the UV curable resin