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Extremely thin planarized grating for sub-diffraction (<100 nm) far-field optical imaging of living cell membranes

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ABSTRACT

A planarization technique has been developed to embed in a very thin (<20 nm) transparent layer a sub-wavelength hole grating patterned in an amorphous silicon layer on a glass substrate. Such an embedded high-index grating is of interest for far-field imaging of biological cells through sub-diffraction optical probing. Obtaining a planar surface is mandatory to get observation conditions similar to those with usual microscopy bare glass coverslips. The bio-compatibility of the embedding material has been successfully assessed.

1. Introduction

Far-field optical imaging of living cells at the highest resolution is still challenging. Sub-diffraction limit (~100 nm) is required for imaging at the cell's membrane scale and for following its dynamics. A specific illumination mode can provide such a high resolution once the sample is deposited onto a periodically nanostructured glass substrate. Thanks to a resonant effect, a significant part of the incident beam is diffracted into a Bloch mode whose wavenumber is much higher than what can be reached in free space. The resulting evanescent wave is used to probe the finest features of a sample close to the grating surface, thus giving sub-diffraction limit information in the far-field [1,2]. In order to faithfully image the plasmic membrane of a living cell without altering its geometry, the substrate has to be flat. After being etched, the required grating thus has to be embedded so as to recover a planar surface. Planarization has to be performed with the thinnest possible layer (<20 nm) to maintain a large optical coupling of evanescent waves with the observed cell.

We have successfully applied the degassing assisted patterning (DAP) technique with UV curable resist for NanoImprint Lithography (NIL) which fills the holes of the grating and produces a flat surface located 10 nm above the holes. The material used for planarization has demonstrated full bio-compatibility under living cells culture condition.

2. Embedded grating design and fabrication

The silicon geometry produces a resonant grating filter that provides the desired structured illumination [3]. Specifically, the grating is patterned in a high-index 33-nm-thick amorphous Silicon (a-Si) layer deposited on a glass substrate. The grating consists of a triangular array of holes of period 200 nm and hole diameter 100 nm. The a-Si layer is only partially etched in order to keep a thin guiding layer below the grating to better support the resonant grating's tightly-guided mode.

The amorphous silicon layer a-Si is deposited by PECVD on a glass substrate. The process parameters have been optimized in order to produce a low deposition rate and smooth surface material. That last point requires a deposition regime far from the plasma powder regime as explained in [4], thus operating at a low gas flow and low chamber pressure.

The grating is patterned by e-beam lithography on a positive poly-methyl metacrylate (PMMA) resist using a 2.5 nm writing resolution. The e-beam resist is then used as the etch mask. A dedicated SF_6–CHF_3 process has been optimized in order to produce smooth and vertical side walls. The etching rate is deliberately low in order to accurately control the etched depth. Indeed, when operating with a high index material all the geometry inaccuracies translate into large changes in optical property.

Different techniques have been investigated to embed the grating and obtain a flat surface. For inspection of the holes filling and layer surface profile, a test grating has been patterned on top of a 100 μm thick Si substrate which can be easily cleaved.

As a first attempt, a thinned PMMA A2 resist (2% solid contents), calibrated to deposit a 20-nm-thick layer, has been spin-coated. After solvent evaporation, the PMMA recovers its original high viscosity and produces a stretched thin layer which does not fill the holes Fig. 1(a). A wavy surface is obtained which would translate in a variable coupling of the evanescent waves, modulated by the variable separation from the grating.

In order to fill the grating holes and reach a flat surface, a low viscosity material with optimal surface wetting properties has

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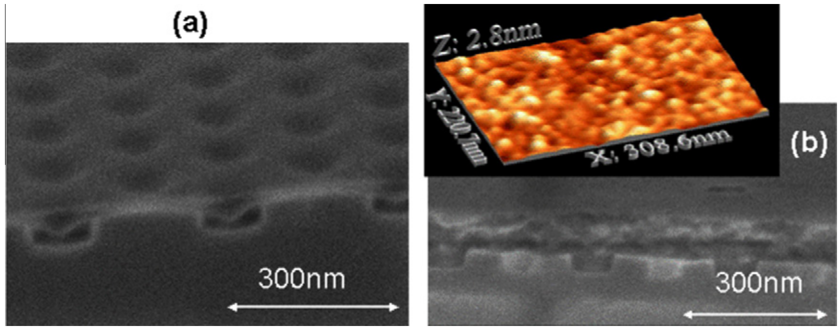

Fig. 1. (a) Cross view of A2-PMMA resist film stretched above the holes. (b) diluted Amonil spin-coated and UV-cured after ICP etching (inset) AFM scan of the resist surface.

been chosen. A good candidate is Amonil resist from Amo GmbH, a UV curable resist for Soft UV Nanoimprinting Lithography (Soft UV NIL). Amonil resist (MMS10 - Amo GmbH) has been spin-coated on the grating and then cured by UV light. While the deposited resist correctly fills the holes and provides a flat surface, its thickness remains too large (∼70–100 nm). An attempt to reduce the resist layer thickness has been investigated through ICP-RIE HBr-based etching process. However, due to the dual carbon-based and silicon-based chemistry of the Amonil resist, it was not possible to find an optimal etching process that could produce a perfectly flat surface. Fig. 1(b) does show the SEM cross view image of correctly filled holes, but the resist surface after etching measured by AFM evidences a large roughness (Fig. 1b inset). Moreover, attaining a very thin residual layer proved difficult because controlling the resist layer thickness during etching by reflectometry was impossible due to the transparent glass substrate.

3. Embedded grating by degassing assisted patterning

A further attempt has been tested by using Amonil resist diluted in 2-Isopropoxyethanol in combination with Soft UV NIL and a flat hard-PDMS/PDMS stamp [5]. Because of the uniform pressure applied to the stamp, the air trapped in the nanoholes prevents their desired filling by the resist as shown in Fig. 2(a). To finally overcome this issue, we have successfully applied the concept of degassing assisted patterning (DAP). DAP is a new lithographic technique that has first been proposed for nanopatterning biological species at the micrometer scale [6]. Due to its high porosity, PDMS has a high gas solubility which obeys Henry's law, which states that the equilibrium concentration of gas dissolved in PDMS is proportional to the partial pressure of the gas around the PDMS [7]. Therefore it can be degassed in a desiccator and once brought back to the atmosphere, the PDMS piece behaves like a sponge by

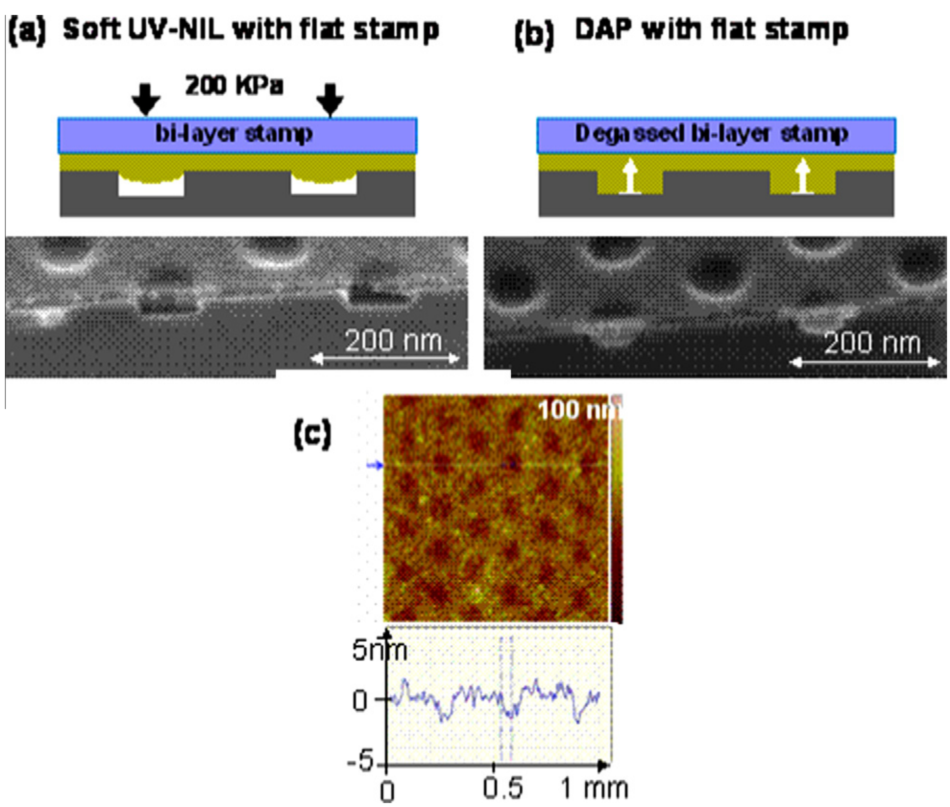

Fig. 2. (a) Diluted Amonil resist after Soft UV NIL with a flat bilayer stamp: in spite of the applied pressure, the air trapped in the nanoholes still prevents a complete filling. (b) diluted Amonil resist after DAP with the same flat bilayer stamp, (c) AFM characterization reveals a maximum fluctuation of 2 nm of the Amonil resist thickness over the holes and a rms roughness of Rq = 0.7 nm on 1 μm^2.

absorbing air toward the new equilibrium state. The time constant of a 3-mm-thick PDMS slab – the typical thickness of a bilayer hard-PDMS/PDMS stamp - is about 10 min. In this manner the degassed bilayer stamp can easily mold without trapped gas a substrate spin-coated with a resist. The difference of pressure then assists the capillary forces in sucking the resist in the nanoholes and allows the replication of structures as small as 15 nm [8]. In this work we used a flat stamp in order to planarize a nanostructured substrate: once the air trapped in the nanoholes is sucked in the degassed flat bilayer stamp, it is replaced by the NIL resist, and then cured by UV light before the release of the mold. Fig. 2(b) shows a perfectly filled grating with a residual layer of 10 nm on top. The lens shape could originate from capillary forces acting on the Amonil in liquid state before the UV curing. The shape is preserved after curing as is visible on Fig. 2(b). A maximum fluctuation of the Amonil thickness of 2 nm of the surface height over the holes along with a low roughness has been measured by AFM on an embedded grating patterned on a glass substrate Fig. 2(c).

The bio-compatibility of the Amonil low viscosity material has been assessed through seeding living cells and following their development. Cells correctly stick to the surface, and after 24 h a large multiplication of healthy living cells is observed. This confirms the bio-compatibility of the material used for planarization.

4. Discussion

In order to insure that their behavior conforms to our theoretical simulations, the embedded gratings have been optically characterized using near-field and far-field techniques. These characterizations will be reported elsewhere.

Recovering a planar surface is required to image cell membranes without altering their geometry, in order to avoid any unwanted bias in further biological studies. It is also important to prevent imaging artifact which could originate from a periodically patterned surface with holes. Indeed our super-resolution imaging technique, as well as the optical characterization of the gratings, relies on a good knowledge of the field at a defined height above the substrate.

The grating is patterned by e-beam lithography. A 2.5 nm writing resolution has been chosen in order to insure that disorder will not blur the image reconstruction process. Previous work related to the performances of two-dimensional resonant grating notch filters [9] has shown that field stitching has no impact on the filter linewidth, and has confirmed that a writing grid of 2.5 nm was sufficiently small to provide the filter expected linewidth in the case of a high index contrast grating. Thus, we took advantage of this former study to pattern the a-Si high index contrast grating with a 2.5 nm resolution, and we consider that the disorder encountered during the lithography of these a-Si fabricated gratings will not impact the image reconstruction process.

5. Conclusion

We have presented the degassing assisted patterning technique and demonstrated its relevance for the thin planarization of embedded grating. This technique can be extended to any geometry of nanopatterning (holes, lines, pillars) and different low viscosity sol–gels. The bio-compatibility of the material used has been validated. Such a planarized grating is of interest for sub-diffraction (<100 nm) living cells imaging below the classical resolution limit.

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