Fabrication of versatile calibration samples for 3D super-resolution optical imaging with

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Fabrication of versatile calibration samples for 3D super-resolution optical imaging with <5nm Z resolution using greyscale e-beam lithography and Nano-Imprint replication

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Topic: Etching – Lithography – Nano-patterning

Abstract **— We present a 3D mold fabrication process to produce high precision calibration samples with few nanometers steps in the vertical direction. After imprinting into a resist layer of adequate refractive index using nano-imprint lithography, these molds can produce calibration samples valuable for super-resolution Direct Optical Nanoscopy with Axially Localized Detection (DONALD). This novel method is dedicated to the 3D mapping of labeled biomolecules into biological samples by fluorescence and requires calibration samples with well-characterized steps ranging from 5 nm to 125 nm in height.**

I. Introduction

To study the architecture of biological structures with a very high precision [1], numerous super-resolution optical fluorescence imaging techniques have been recently developed such as: STED (Stimulated Emission Depletion microscopy), SMLM (Single Molecule Localization Microscopy), SAF (Supercritical Angle Fluorescence microscopy), among others.

SMLM combined with SAF technique lead to an original configuration called DONALD microscopy [2]. This technique can achieve typically an isotropic localization precision of less than 20 nm within an axial range of ∼150 nm above the coverslip. Unfortunately, this precision decreases as a function of the distance between the emitting fluorescent biomolecule and the surface of the coverslip. It is thus necessary to use some calibration samples to maintain the Z resolution over distances of interest when investigating biological structures such as those found at the membrane of living cells or into their cytoskeleton.

In order to produce this calibration sample easily and at low-cost, we propose to use UV-Nanoimprint Lithography (UV-NIL) technique which consist in replicating a template (also called a mold) in a fluid solvent-free UV-sensitive material using pressure and UV-light [3]. In our case, as the calibration sample needs to be observed by transparency through a coverslip, the template for NIL can be made in a bulk silicon substrate because reticulation of the UV-sensitive material during NIL can be performed by UV illumination through the cover slip.

In this abstract we present a robust mold fabrication process combining: grey-scale e-beam lithography and Reactive Ion Etching (RIE).

II. Experiments and Results

A. Greyscale e-beam lithography

The needed Z calibration sample consists in a 5x7 array of 5x5µm^2 squares, each square corresponding to a precise topographical level. Greyscale e-beam lithography is a very well-known technique that has been employed in this work for creating resist features of different thickness through a precise adjustment of the deposited dose and resist development. Using a RAITH150 e-beam writer we exposed a spin-coated 135nm thick PMMA layer (molecular weight x960.000) on bulk silicon substrate using a beam current of 15pA and an acceleration voltage of 30kV. Different doses were applied ranging from 10 to 85µC/cm^2. PMMA development was made in pure MIBK for 30s.

B. Reactive Ion Etching

After grey-scale e-beam lithography, the samples were etched using an Inductively Coupled Plasma - Reactive Ion Etching machine (ICP-RIE ALCATEL AMS4200). Process parameters are as follows: pressure 8.10^{-3}mbar, power 450W (coil) / 30W (platen), gas mixture SF_6:C_4F_8:O_2 with respectively 30:45:3sccm of flow rate and an etching time of 96s.

C. Replication using Nano Imprint Lithography (NIL)

Replication of the mold was made using a dedicated NIL machine (Nanonex – NX2500) in a UV sensitive resist (NOA138 – Norland) which exhibits a low viscosity (20-40cps) and a low refractive index (1.38). Process is composed of 2 steps: a first replication in a soft transparent polymer itself used for UV-replication in NOA138. This transparent soft-mold allow us to replicate on transparent or non-transparent substrates.

D. Characterization

We use a calibrated AFM BRUCKER DIMENSION ICON to precisely reveal the various topographical levels in PMMA, on silicon substrate after etching (using the previous grey-scaled PMMA as a mask) and in NOA138. Measurements represent the mean value of each square depth and error bars represent the Root Mean Squared Surface Roughness (Sq).

We obtained a grey scale thickness of resist ranging from ~0 to ~90nm (Figure 1). Roughness increases as depth increases ranging from 1nm to 3.5nm. This roughness is inherent to any under exposed resist. As the local PMMA thickness left after grey-scale exposure does not depend linearly on the exposure dose (Figure 1), we have screened a large number of grey-scale levels in order to determine the best doses for PMMA exposure to get the adequate topographical steps after etching.

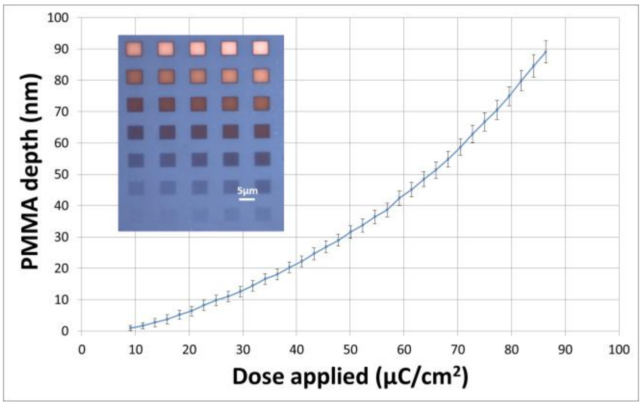

Figure 1. PMMA depth after e-beam exposure and pure MIBK development as a function of the exposure dose. Measurements were made on the sample shown in the embeded image exhibiting different colors corresponding to different topographical levels of the resist. Matrix is composed of 5x5μm^2 squares and dose is increasing from left to right and from bottom to top.

Figure 2 represents the etching depth into the silicon wafer as a function of the e-beam dose over the same dose variation. Transferred roughness ranges from 1.2nm to 4.6nm meaning that RIE etching process does not add significant roughness to the initial roughness of the PMMA mask.

This kind of calibration curve enables to adjust on-demand the exposure dose for generating any targeted topographical level in the silicon mold.

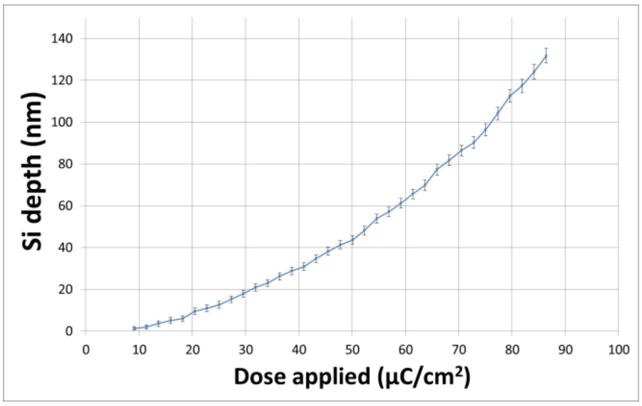

Figure 2. Si mold depth after transfert by ICP-RIE using previous grey-scaled PMMA as a mask

After molding [4] in NOA128 on a bulk Si substrate, we replicate the topographical levels of the silicon mold with a very good accuracy as it can be seen in Figure 3.

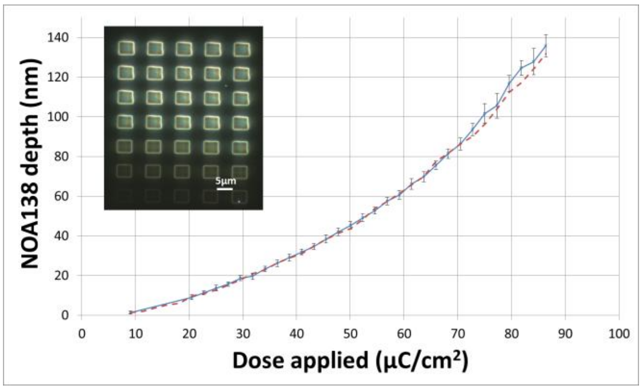

Figure 3. NOA138 depth after UV-NIL on bulk Si as a function of the exposure dose. Measurements were made on the sample shown in the embeded image made in darkfield optical microscopy due to the poor contrast between NOA138 and the substrate in brightfield illumination. Border brightness of each square increases with the resist height in each square corresponding to different topographical levels. Dashed line represent the silicon mold depth as it can be seen in Figure 2 for comparison.

III. Conclusion

We produce a template for UV-NIL replication technique that allows us to produce precise topographical steps with a resolution below 5nm. By UV-NIL replication we are able to replicate these topographical levels with a very high precision in a polymer whose refractive index is 1.38, on a bulk Si substrate, at "low cost". This versatile nanofabrication process can be applied to different refractive index resist materials for fitting with the constraints imposed by the 3D optical imaging technique to be calibrated. It can be extended to produce different calibration samples covering different ranges of topographical steps by tuning the initial PMMA resist thickness. Using an intermediate transparent mold for UV-NIL allows us to work with transparent and non-transparent substrates.

Acknowledgment

The project was financed by the French CNRS program “Instrumentation aux limites”.

This work was supported by LAAS-CNRS micro and nanotechnologies platform member of the French RENATECH network.

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