Fabrication and Replication of Nanofluidic Devices for the Analytical Separation of Biological Nanoparticles

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The replication of complex nanostructures in soft materials not only enables their mass production, but also broadens their applicability in diverse fields involving biological materials. We previously reported the focused ion beam milling and replica molding of complex nanostructures with subnanometer resolution of vertical dimensions.[1,2,3] We demonstrated structure–property relationships of dielectric films for interferometry of device dimensions and integration of spectroscopic optics, and nanofluidic slits for analytical separation of fluorescent nanoparticles by size exclusion with subnanometer resolution down to particle diameters of approximately 20 nm.[2,3] Here, we replicate such devices in soft materials with nanometer resolution of vertical dimensions over a range of 200 nm, and we demonstrate their essential functionality of structural integrity. These advances will fulfill the potential of our devices for analytical separation of biological nanoparticles such as exosomes in this newly extended size range.

To form our device mold, we mill a parallel array of staircase structures into a thin film of silicon dioxide (Figure 1a). We characterize the structures by atomic force microscopy and report quantities as mean values ± two standard deviations. The 36 step depths vary from 40.2 nm ± 1.2 nm to 248.3 nm ± 4.4 nm below the zero plane, while the surface roughness remains constant at 0.26 nm ± 0.08 nm. We replicate the staircase structures in a bilayer of hard and soft silicone[4] by a double molding process (Figure 1b). The roughness of the replica remains nearly constant at 0.26 nm ± 0.24 nm. White light interference results in potential optical functions of both the mold and replica.[3] The staircase profiles of the mold (purple) and replica (gray) are generally consistent (Figure 1c). Artefactual discrepancies between the mold and replica are evident from combining multiple atomic force micrographs over distances of hundreds of micrometers. However, nanometer fidelity of pattern transfer is also evident in replication of local asperities (Figure 1c inset). We bond the zero plane of the replica to a fused silica substrate to enclose the staircase structure (Figure 1b). Nanofluidic slits in soft materials are prone to collapse due to lack of structural integrity.[4] However, our silicone staircases with width to depth aspect ratios exceeding 100 maintain their structural integrity with high yield, demonstrating nanofluidic functionality.

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[1] Total Nanofluidic Confinement Devices Nanofabricated by Focused Ion Beam Milling, K.-T. Liao, J. Schumacher, S. M. Stavis, EIPBN 2014

[2] FIB Milling and Replica Molding of Complex Surfaces with Atomic-Scale Precision, K.-T. Liao, J. Schumacher, H. J. Lezec, S. M. Stavis, EIPBN 2015

[3] Focused Ion Beam Milling of Complex Optical Films with Subnanometer Surface Topography, K.-T. Liao, J. Schumacher, H. J. Lezec, S. M. Stavis, EIPBN 2016

[4] Rapid Prototyping of Nanofluidic Slits in a Silicone Bilayer, T. P. Kole and K.-T. Liao *et al*., Journal of Research of the National Institute of Standards and Technology, 120, 252-269, 2015

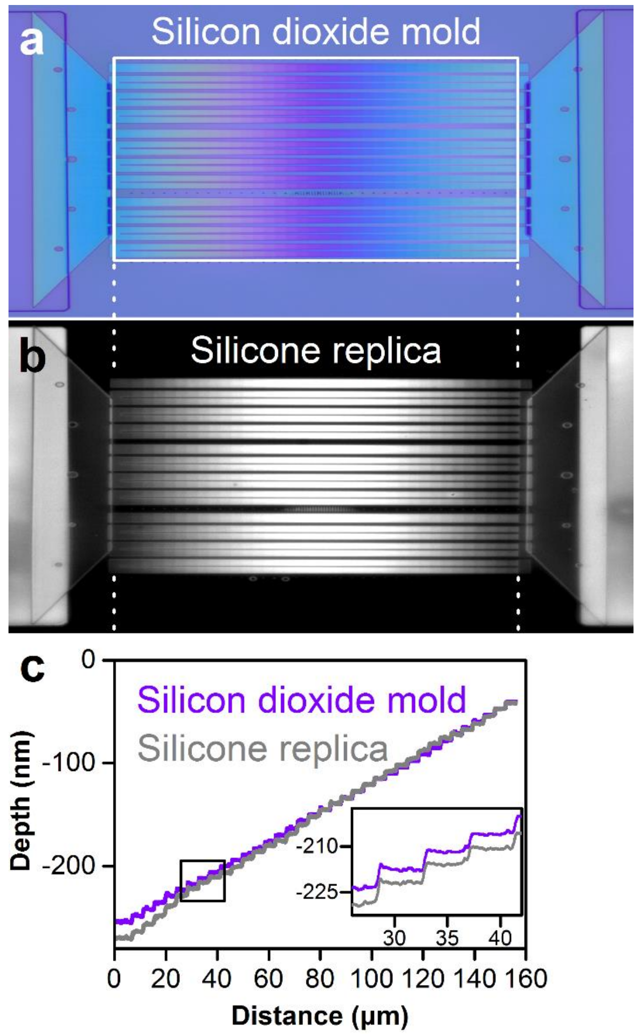

Figure 1: Silicone Replication of Nanofluidic Devices for Analytical Separation of Biological Nanoparticles. (**a**) Brightfield optical micrograph showing a parallel array of staircase structures milled into a silicon dioxide film with a thickness of approximately 600 nm. The staircase step depths vary from a minimum of 40.2 nm ± 1.2 nm to a maximum of 248.3 nm ± 4.4 nm below the zero plane, while the root-mean-square surface roughness of the steps remains constant at 0.26 nm ± 0.08 nm. (**b**) Brightfield optical micrograph showing a replica of the staircase structures molded in a bilayer of hard and soft silicone, after bonding to a fused silica substrate to form an enclosed nanofluidic device. Black regions are where the zero plane of the silicone replica has contacted the fused silica substrate, eliminating contrast from optical interference in a thin film. (**c**) Sections from atomic force micrographs comparing the profiles of the milled and molded staircase structures. Artefactual discrepancies between mold and replica structures are evident, particularly at the greatest depths below the zero plane, from leveling and stitching multiple atomic force micrographs over distances of hundreds of micrometers. However, the nanometer fidelity of pattern transfer is also evident by the precise replication of local asperities.