

Femtosecond Laser Patterned Templates and Imprinted Polymer Structures

A Dissertation Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

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May 2013

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This dissertation is dedicated to my mother

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude towards my major adviser Dr. William H. Hofmeister for his guidance and support in successfully carrying out this work. I earnestly thank Drs. Lloyd M. Davis, Michael L. Simpson, George M. Murray, and Lino Costa who served on my doctoral committee. I owe a deep sense of gratefulness to Dr. Lino Costa, Ms. Kathleen Lansford, and Mr. Alexander Terekhov, who deserve a special recognition for their cooperation and unstinting support extended throughout my doctoral research at the University of Tennessee Space Institute.

I extend my gratitude towards Dr. Brian Canfield for carrying out some important experiments required for my dissertation. I sincerely acknowledge Mr. Doug Warnberg for his assistance in making certain experimental set-ups. I would like to place my sincere gratitude towards Drs. Jacqueline Johnson, Charles Johnson, Christian Parriger, and Zhongren Yue for their helpful suggestions and encouragement, and summer intern Ms. Julia Shi for her assistance in the lab.

I am greatly indebted to our collaborators Mr. Spencer Crowder, Mr. Lucas Hofmeister, Mr. Daniel Balikov, and Dr. Hak-Joon Sung at Sung Laboratory from the Department of Biomedical Engineering, Vanderbilt University for their invaluable support in conducting some of the most important experiments required for my dissertation. I am also thankful to our collaborators Drs. Christopher M. Rouleau, Alexander A. Puretzky, and David B. Geohegan from the Center for Nanophase Materials Sciences, Oak Ridge National Laboratory for their assistance with some laser machining experiments.

I am very grateful to Ms. Joyce Miller of the MTSU Interdisciplinary Microanalysis and Imaging Center (MIMIC) for her assistance with SEM imaging on their FE-SEM and critical point drying of some biological samples. I also thank Mr. Jamil Clarke of Hitachi High Technologies America, Inc., Jason D Fowlkes of Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, and Mr. Naoki Kikuchi of JEOL USA for their assistance with FIB sectioning / imaging and chemical analysis of some samples.

I am highly thankful to the staff of UTSI, Ms. Carole Thomas, Ms. Rebecca Layman, Ms. Brenda Brown, Ms. Kathy Hice, Ms. Brenda Brooks, Ms. Penny Oliver, and Ms. Jennifer Boyles for their assistance with office supplies, reading material, and other administrative works. I am also very thankful to Mr. Wesley McMinn and Ms. Susan Murphy for their assistance with my travel arrangements.

I express my heartfelt thanks to my friends, Dr. George Owens, Dr. Abhilasha Verma, Dr. Subhashini Chitta, Samuel Williams, Jaya Prakash Nanna, Ajay Katta, Lee Leonard, Chris Foerster, Sharon Gray, and James Rogers for their suggestions, support, and encouragement.

Finally, a very special recognition goes to the Tennessee Higher Education Commission for financially supporting my research work through a grant to the Center for Laser Applications, The University of Tennessee Space Institute, Tullahoma.

Tullahoma, Tennessee

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May 2013

ABSTRACT

Femtosecond laser machining is a direct-write lithography technique by which user-defined patterns are efficiently and rapidly generated at the surface or within the bulk of transparent materials. When femtosecond laser machining is performed with tightly focused amplified pulses in single-pulse mode, transparent substrates like fused silica can be surface patterned with high aspect ratio ($>10:1$) and deep ($>10\text{ }\mu\text{m}$) nanoholes. The main objective behind this dissertation is to develop single-pulse amplified femtosecond laser machining into a novel technique for the production of fused silica templates with user-defined patterns made of high aspect ratio nanoholes. The size of the nanoholes, both lateral and vertical, is controlled to a certain degree by controlling laser machining parameters or by chemical etching in a post-machining treatment.

Fused silica templates produced by this new technique, both as-machined and chemically etched, are shown to be useful for imprinting polymer structures by a simple replication procedure using polymer thin films or solutions. In particular, a solution-based replication procedure, termed *solution casting*, is developed to imprint polymer structures from fused silica templates. Polymer structures in the form of nanowires, nanocones, and micropillars are successfully imprinted from various polymer types. Imprinted polymer structures are easily functionalized by subsequent surface treatment processes like cryogenic sputter coating and vapor deposition. A novel low-temperature chemical vapor deposition process is developed to coat polymer nanowires with silica to

produce silica nanoneedles. Silica nanoneedles thus produced are shown to be useful as synthetic cell culture substrates to study the behavior of NIH 3T3 fibroblasts.

In the final part of this dissertation, a report is given on more in-depth collaborative experiments to study the role of optical aberrations as part of the mechanism for producing high aspect ratio nanoholes by single-pulse amplified femtosecond laser machining. The results indicate that (i) precise optical alignment of the focusing lens is needed to avoid coma, which significantly deteriorates the ability to produce nanoholes, and (ii) 10-micron deep nanoholes can be produced by focusing a beam without spherical aberration but even deeper nanoholes are formed when the beam is focused with undercorrected spherical aberration.

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LIST OF ABBREVIATIONS

AAO	Anodic Aluminum Oxide
ATR-FTIR	Attenuated Total Reflectance – Fourier Transform Infrared
CA	Cellulose Acetate
CVD	Chemical Vapor Deposition
EBL	Electron Beam Lithography
ECM	Extra Cellular Matrix
EDS	Energy Dispersive X-ray Spectroscopy
EMT	Epithelial-Mesenchymal Transition
FFT	Fast Fourier Transform
FIB	Focused Ion Beam
HA	Hard Anodization
HDPE	High Density Polyethylene
LDPE	Low Density Polyethylene
LSA	Longitudinal Spherical Aberration
MA	Mild Anodization
MET	Mesenchymal-Epithelial Transition
MIMIC	Micro Molding in Capillaries
NCG	Nanochannel Glass
NIL	Nanoimprint Lithography
PCL	Poly(ϵ -caprolactone)
PCL-PEG	Poly(ϵ -caprolactone) – Polyethylene Glycol

PDMS	Polydimethylsiloxane
PE	Polyethylene
PMMA	Polymethylmethacrylate
PSF	Point Spread Function
PVA	Polyvinyl Alcohol
PVAc	Polyvinyl Acetate
PVC	Polyvinyl Chloride
REM	Replica Molding
RMS	Root Mean Square
SAMIM	Solvent Assisted Micromolding
SEM	Scanning Electron Microscope
SP-AFL	Single-Pulse Amplified Femtosecond Laser
TGF	Transforming Growth Factor
UHMWPE	Ultra High Molecular Weight Polyethylene
α SMA	Alpha Smooth Muscle Actin
μ CP	Micro Contact Printing
μ TM	Micro Transfer Molding

Chapter 1

Background

Feynman's 1959 legendary talk entitled "There's plenty of room at the bottom" emphasized the technological merits of materials on a small length scale and laid the foundation for *nanotechnology* [1]. Instruments capable of observing and manipulating materials on the atomic scale led the race to the bottom as a desired goal across many scientific disciplines [2, 3]. In fact, the main competition today is to achieve the tiniest and the thinnest because of the very common belief: "*the smaller, the greater*" [4]. Nanotechnology, in general, encompasses the colossal field of preparation, manipulation, and applications of nanoscale materials and structures. Also, labeled as "*the technology of the future*," nanotechnology is prophesied to shape the future of technology and change virtually every aspect of our lives. The primary reason behind going down in length scale is because of the deviation in the material properties (physical, chemical, and mechanical) due to the manifestation of the statistical-mechanical and quantum-mechanical effects that are otherwise not observed in their bulk counterparts [5]. For example, the electronic properties significantly alter with the reduction in the size scale of the material because of pronounced quantum mechanical effects, which are more dominant when less than 100 nm size scale is achieved. Indeed, the terminology **nano**, from a materials perspective, is now strictly reserved for materials with at least one dimension less than 100 nm so that the quantum size effect can be observed and exploited for practical purposes [6]. Of the many known and envisioned applications of nanotechnology, the most prominent ones are in the field of micro/nanoelectronics, energy, and medicine.

Nanostructures are nanoscale materials with at least one dimension less than 100 nm per current scientific definition. Although this definition of nanostructures /

nanomaterials is now widely accepted, it is still very common to regard anything on sub-micron ($< 1 \mu\text{m}$) length scale as *nano* for simplicity on a case-to-case basis. Nanostructures or nanomaterials are considered to be the basis of nanotechnology; other aspects being nanofabrication, nanoscale characterization, and nanodevice systems. Nanofabrication is a collection of fabrication strategies used for the preparation of nanomaterials or nanostructures, and is broadly classified into *top-down* and *bottom-up* techniques [7]. Nanoscale characterization is the examination of properties of nanoscale materials with the use of highly sophisticated analytical instruments, whereas nanodevice systems are mission-oriented nanoscale machines or apparatus made from several nanoscale objects or just molecules. While a review of each of these topics is beyond the scope of this dissertation, only the most essential and the most relevant are briefly discussed.

One of the most common bottom-up nanofabrication techniques used for the preparation of nanostructures is **template synthesis** [8]. It is also regarded as one of the simplest and the most economical of all the nanofabrication techniques known to date, and has been successfully used to prepare one-dimensional (1D) nanostructures from a variety of materials [4]. Nanostructures of metals, elemental and compound semiconductors, superconductors, insulators, and conducting polymers have been created by template synthesis [9-14] and reported for applications in energy, electronics, photonics, biomedicine, and pharmaceuticals [15-20]. Based on their morphology, nanostructures are commonly classified as nanowires, nanoribbons, nanopyramids, nanopillars, and nanocones, and are synthesized either as topographical

features on films or substrates via nanofabrication techniques or by solution synthesis [4].

Template synthesis is essentially a casting process in which a mold is subjected to a deposition process to create structures within the holes/pores of the mold. The morphology and dimensions of the resulting structures depend on that of the holes in the mold from which they are cast. The four generic casting methods employed in template synthesis are:

- 1) soft lithography,
- 2) electrochemical or electroless deposition,
- 3) vapor deposition, and
- 4) injection molding.

Although there are several different types of molds used in the template synthesis of nanostructures, the four most commonly used are: i) anodized aluminum oxide (AAO); ii) nanochannel glass (NCG); iii) ion track-etch templates; and, iv) lithographically patterned templates (referred to as *master pattern* in soft lithography)

1.1) Anodized Aluminum Oxide (AAO)

AAO templates are one of the widely used nanoporous materials used extensively in the fabrication of 1D nanostructures. AAO templates are prepared by subjecting high purity aluminum films to electrochemical oxidation in an acidic electrolyte in a process called *anodization*. Although AAO prepared by anodization of aluminum has been in use for more than a century, the preparation and use of AAO with

self-ordered nanopores as a template for structure replication was first reported by Masuda and Fukuda in 1995 [21]. They reported the formation of self-ordered hexagonal arrays of high density nanoscale-diameter-holes, referred to as ***nanoholes*** from this point onwards, of cylindrical morphology in alumina with high regularity, and successfully created gold and platinum nanohole arrays by two-step replication. Since their report on AAO based template synthesis of nanostructures, there has been a continuous effort by scientists till this day from perfecting the anodization process to fabricating novel nanostructures by replication [22-25]. Besides applications in the template synthesis of nanostructures, self-ordered nanoporous AAO has also been reported for applications in the fields of sensing, storage, and separation processes [26-30].

There are two aluminum anodization practices known for AAO template fabrication: ***mild anodization*** (MA) and ***hard anodization*** (HA) [24]. MA, based on Masuda's original approach, is conventionally a slow process in which oxide growth rate is usually in the range 2-6 $\mu\text{m/h}$. MA has three major regimes for nanopore self-ordering that exist in a very narrow process frame, details of which are shown in Table 1.1. Such a process is not viable in an industrial set-up for volume production as the production rate is very low [24]. The better alternative to mild anodization in terms of production rate is the process of hard anodization in which oxide growth rate of 50-100 $\mu\text{m/h}$ can be easily achieved [31].

A generic MA process to obtain self-ordered nanoporous alumina is conducted in two steps for which an electropolished high purity aluminum film with nanoscale roughness is used as the starting material. The first step is usually performed in 0.3 M

oxalic acid ($\text{H}_2\text{C}_2\text{O}_4$) solution at 0°C and at a constant voltage of 40 V for 12-24 hours. The alumina layer formed in the first step contains highly ordered cylindrical cells arranged in a hexagonal close packed structure at the base that develops into nanopores with irregular arrangement as the electrochemical oxidation progresses (termed as protective oxide layer). The protective oxide layer on alumina with irregular nanopores is then removed by soaking in a mixture of phosphoric acid and chromic acid at room temperature, which leaves behind a texture of highly ordered hexagonal-packed cells at the surface of the aluminum. The second anodization step is performed on the textured aluminum thus obtained under the same conditions as the first anodization step. The duration of the second anodization step depends on the thickness of the final alumina film desired. The final structure of alumina (AAO) after the second anodization step consists of highly ordered and well-defined cylindrical nanopores arranged in a hexagonal closed packed structure. The unreacted aluminum is then removed by dissolving in a saturated HgCl_2 solution if required. The diameter of the nanopores obtained after the second anodization step is enlarged by immersing the AAO in phosphoric acid. A schematic of the generic MA procedure is shown in Figure 1.1.1.

Table 1.1 Process conditions used in the three known regimes in mild anodization (MA) of aluminum that produce self-ordered nanopores in alumina (AAO).

Electrolyte	Applied Potential (V)	Interpore Separation (nm)	Reference
Sulfuric acid	25	63	[32, 33]
Oxalic acid	40	100	[21, 32, 34]
Phosphoric acid	195	500	[35, 36]

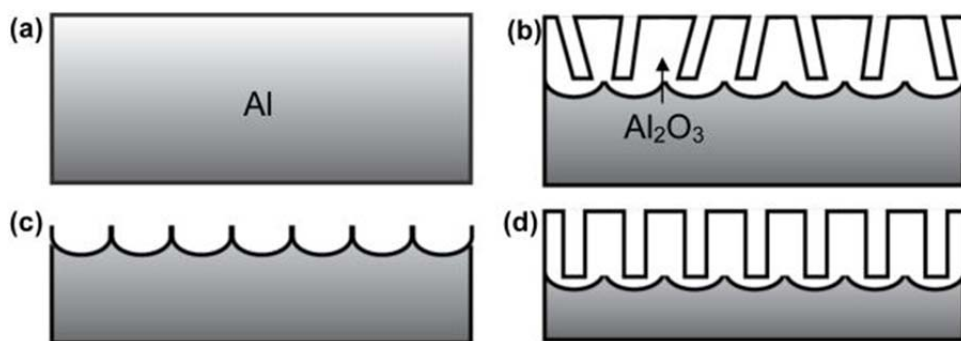


Figure 1.1.1. A schematic of a generic form of two-step anodization process. The first step involves electrochemical oxidation of an electropolished high purity aluminum (a), which forms a very thin highly ordered nanopores at the bottom and thick irregular nanopores for most part of the alumina film (b). The resulting alumina film is dissolved to leave behind a very thin layer of highly ordered hexagonal-packed nanopores on aluminum, which acts as a patterned substrate for the second anodization step (c). The second step is also performed under the same conditions as the first step, which forms a highly ordered nanoporous alumina desired for template synthesis (d). The second anodization step is usually followed a third step in which the pores are enlarged to the desired level by subjecting the nanoporous alumina to phosphoric acid treatment.

Invented and practiced since the 1960s, HA is an old process that has been widely used to produce hard surface protective coatings for numerous industrial applications. It was originally based on the electrochemical oxidation of aluminum in sulfuric acid at relatively low temperatures and high current densities. However, due to poor process control on pore size, interpore spacing, and aspect ratio of nanopores in anodized alumina, HA had never been encouraged for the fabrication of AAO (for template synthesis of nanostructures) until a 2006 report by Lee and coworkers [24]. A comparison of MA and HA from Lee's original work that revived interest in HA for template synthesis is shown in Figure 1.1.2. In their work, MA was done at 40 V and HA was explored in the range 110-150 V. Following their work on HA of aluminum, self-ordered nanoporous AAO has been a subject of growing interest across many scientific disciplines for template synthesis of 1D nanostructures for myriad applications. Indeed, recent reports indicate that this new generation of HA can be used to produce template synthesis grade AAO with unprecedented control on nanopore size, density, interpore spacing, and nanopore aspect ratio [37-41]. Nevertheless, it must be noticed that AAO based template synthesis is oftentimes a destructive method in which AAO is eventually dissolved to release the cast nanostructures.

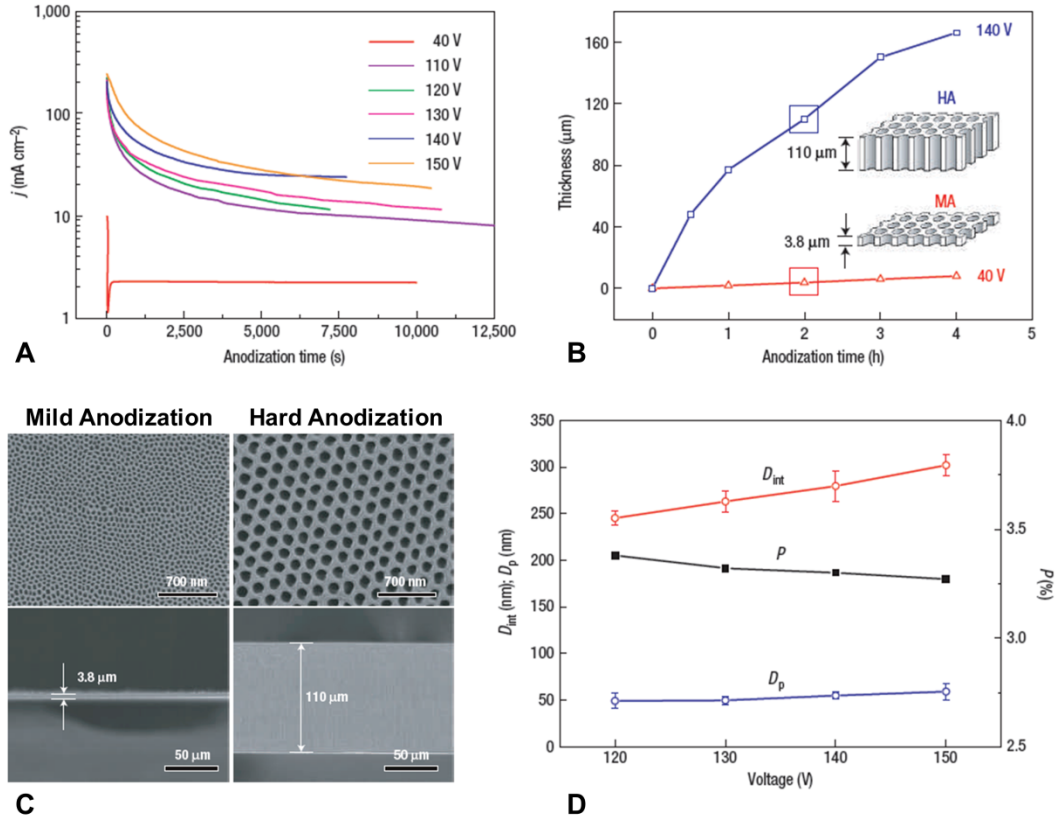


Figure 1.1.2 A pictorial summary of Lee's work on hard anodization of aluminum performed at 1°C and its comparison with mild anodization [24]. (A) current density vs anodization time: red bottom curve corresponds to mild anodization (40 V). (B) film thickness as a function of anodization time: red bottom curve corresponds to mild anodization where total thickness achieved after 4 hours is of the order of a few microns. (C) SEM images of nanopores in alumina obtained after 2 hours (left MA, right HA). Nanopore characteristics of AAO in HA (interpore separation D_{int} , pore diameter D_p , and porosity P (%)) as a function of the applied voltage.

1.2) Nanochannel Glass (NCG)

Nanochannel glass is essentially a dense bundle of parallel, hollow, and uniform nanochannels (or nanoholes) in glass. It was developed at the Naval Research Laboratory, Washington DC, in the early 1990s [42]. NCG preparation requires two different glass materials: a host glass and a channel glass. The channel glass is always an acid-etchable glass in the form of a cylindrical rod. The channel glass and the host glass are chosen in such a way that the etching rate of the channel glass is at least a thousand times more than that of the host glass for that particular acid that dissolves the channel glass. The channel glass rod is inserted into the host glass after ensuring that the dimensions of the two match such that a glass clad is formed. The glass clad is then drawn at high temperature under vacuum to reduce its overall cross-section so that it takes the shape of a fine filament. Several of these filaments are then stacked and fused to form a bundle. The resulting bundle is then redrawn under same conditions as were used for drawing the initial glass clad. The process of drawing-bundling-drawing continues until the desired nanochannel diameter is obtained. The nanochannel diameter and the inter-channel spacing in the NCG can be reasonably regulated by varying the cross-section of the host glass and that of the channel glass in the initial glass clad. Once the drawing operation is over and the desired nanochannel size is obtained, the NCG material is cut into pieces perpendicular to the nanochannel direction and polished to optical flatness. The polished side of the NCG pieces is then subjected to acid etching to dissolve the nanochannel glass to leave behind a large array of nanoholes in the host glass, also termed as *nanochannel array glass*. NCG materials with nanohole diameter of up to 17 nm and nanohole density greater than 10^{11} per cm^2

have been reported [43]. Schematics of the NCG preparation and some SEM images of the NCG cross-section with different nanochannel diameters are shown in Figure 1.2.1. NCG materials thus prepared have been used to produce replica membranes that can be used as deposition masks e.g., platinum [43, 44]. The replica membranes are produced from the NCG material by first coating a thin buffer layer, followed by the membrane material. The intermediate buffer layer is then dissolved in a chemical solution to release the replica membrane. The replica membrane is then placed on an appropriate substrate depending on the final application. The replica membrane thus prepared has been successfully used as a deposition mask to produce patterned substrates from different materials by replication e.g., platinum nanodot arrays on a silicon (110) wafer. Schematic of the preparation of NCG replica membrane and SEM images of NCG replica membrane and platinum nanodots array replicated from it are shown in Figure 1.2.2.

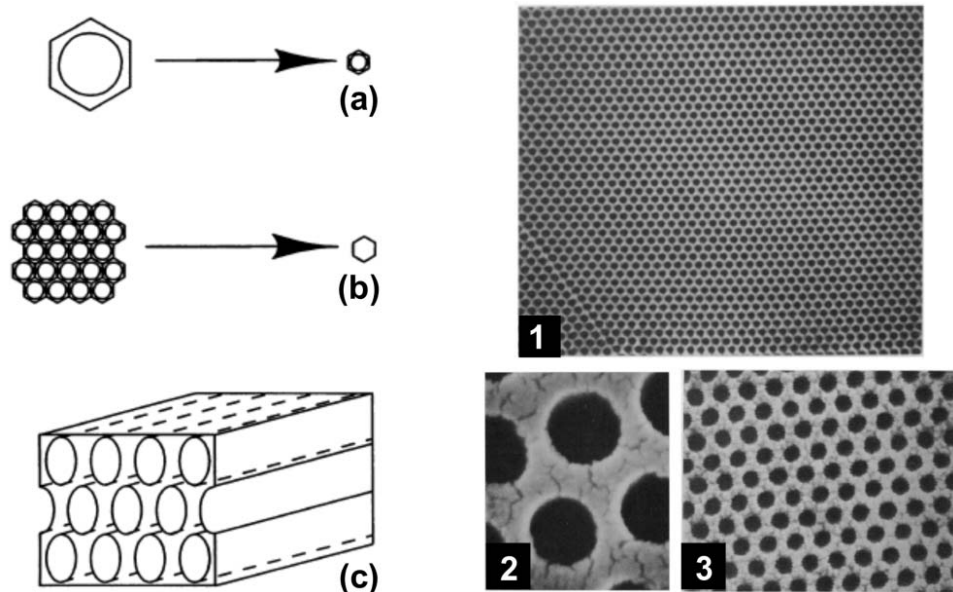


Figure 1.2.1. Schematic of the NCG template fabrication. A glass clad (a) is subjected to drawing at elevated temperature under vacuum, followed by bundling (b) and then redrawing until the desired nanochannel size is obtained (c). SEM images of nanochannels with different diameters produced by this procedure: (1) 450 nm; (2) 95 nm; and, (3) 33 nm. [reference: [42]]

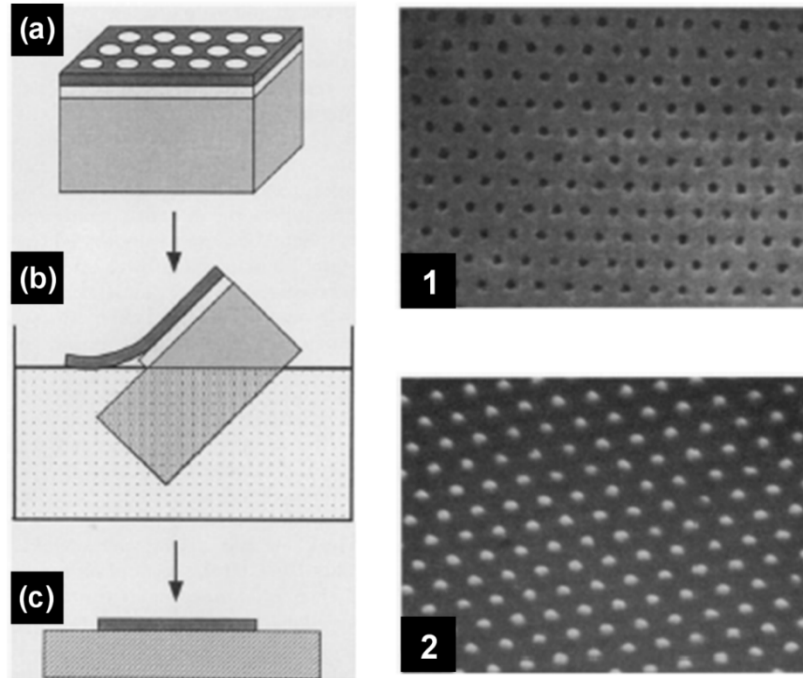


Figure 1.2.2. Schematic of the preparation of NCG replica membrane (left). A bilayer consisting of an intermediate buffer layer and the membrane material layer is first deposited on the NCG template (a). The NCG template is then soaked in a chemical solution to dissolve the buffer layer and release the replica membrane layer (b). The replica membrane layer is carefully removed from the buffer solution and placed on a substrate for further use (c). An SEM image of the replica membrane made on platinum (1) and an SEM image of the platinum nanodots array produced using this replica membrane (2). The width of the SEM image 1 is 2.3 μm , whereas that for the SEM image 2 is 4.6 μm . [reference: [43]]

1.3) Track-etch Templates

Track-etch templates are membrane structures with randomly arranged pits or voids produced on substrates such as mica and polycarbonate films by a two-step treatment [45, 46]. The first step involves irradiating the substrate film with charged or neutral particles (e.g., alpha particles and neutrons), which produces latent nuclear tracks of voids at the surface. The latent tracks are then subjected to chemical etching in the second step to expose or enlarge the voids, and the size of the voids is controlled by varying the etching time. The history of track-etching dates back to the 1950s when the effect of nuclear irradiation on the physical and chemical properties of solid materials was studied. The first material reported to have shown this effect was a lithium fluoride single crystal (100) by Young [47]. The work on track-etch membranes was carried out by several other research groups immediately following the work of Young, and the first review on this subject was published as early as 1968 by Becker [48]. However, the use of track-etched substrates to produce structures by replication was first reported in the year 1970 by Fleischer and coworkers in which they used a silicone rubber to replicate nuclear tracks formed in the helmets of Apollo astronauts [49]. The replication procedure was found to be helpful in identifying and quantifying the heavy ions that astronauts are exposed to during their space missions. As a consequence, track-etch templates were soon realized to be useful for the production of multitude of structures by replication or double-replication for applications such as electron emitters, ion emitters, and solid-state electronic devices [50, 51]. A brief graphical summary of nuclear track-etch substrates as templates for structure imprinting is presented in Figure 1.3.

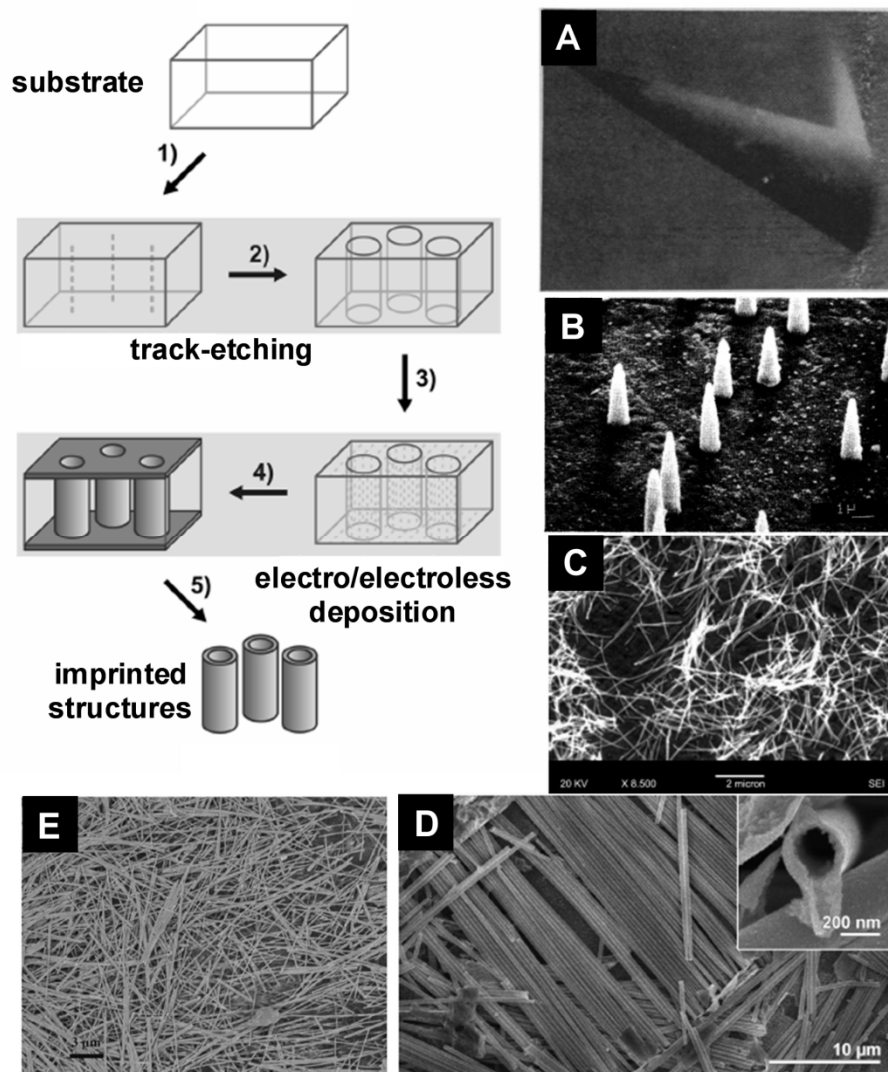


Figure 1.3. Schematic in the top left describes the preparation of a nuclear track-etch template (steps 1-2), which is subsequently subjected to electro/electroless deposition to imprint structures by replication (steps 3-5). A-E show the SEM images of some of the imprinted structures. (A) replica of a nickel track produced on a silicone rubber [49], (B) random arrays of copper pins produced by electrodeposition [50], (C) gold nanofibers produced by electroless deposition [52], (D) rhodium nanotubes produced by electroless deposition [53], and (E) polypyrrole nanostructures produced by electrochemical polymerization [54].

Most materials (metal, semiconductor, insulator, superconductor, and polymer) have been imprinted by template synthesis using AAO, NCG, and track-etch substrates over the years. Equal attention has been paid to both the template preparation and imprinting strategies, so that high quality nano/micro structures can be fabricated and used. Although these templates have been useful for numerous applications, there still are many applications where they cannot fulfill the requirements. The biggest disadvantage of these templates is the inability to control the physical arrangement of the nanoholes. Their size (diameter and depth) can be reasonably modulated. Furthermore, in case of AAO and track-etch based template synthesis, the template is eventually dissolved in a chemical solution to harvest the cast structure. In summary, reusable templates with user-defined arrangement of nanoholes cannot be fabricated by any of these three techniques. By comparison, nanolithography offers ever improving control on the physical arrangement of the nanoholes that a user can pattern on rigid substrates.

1.4) Nanolithography

Nanolithography is essentially a patterning technique in which structures (e.g., arrays of nanoholes) of user-defined designs are produced at the surface of substrates such as silicon, fused silica, and acrylic glass. The lateral and vertical dimensions of the nanoholes are usually controlled post-patterning by an etching treatment (to increase the hole size) [55] or a deposition treatment (to decrease the hole size by coating its sidewalls) [56]. Nanolithography constitutes a set of lithography techniques of which

most routinely used are photolithography, electron beam lithography, and soft lithography [57]. A brief description of these techniques is presented below.

1.4.1) Photolithography

Photolithography is a multi-step lithography process in which user-defined patterns are transferred to substrates with the help of ultraviolet light and a pattern-carrying mask [57]. In this process, the substrate is first thoroughly cleaned with wet chemical treatments to render the surface free of organic and inorganic impurities. The substrate is then coated with a layer of photoresist by spin coating after exposing the substrate to a chemical (e.g., hexamethyldisilazane) that improves adhesion between the photoresist and the substrate. The photoresist coated substrate is then heated at 80°C - 100°C on a hot plate for up to a minute to expel excess photoresist solvent from the photoresist layer in a process called *pre-baking*, so that the substrate is ready for UV exposure. The photoresist is then exposed to UV light through a photomask carrying the pattern, and then subjected to another baking process called *post-exposure baking*. A photomask is essentially a fused silica plate with user-defined patterns made on chromium. The UV light exposed photoresist gets chemically modified, which develops patterns onto the photoresist. Two types of photoresists exist: positive photoresist and negative photoresist. The UV exposed region on a positive photoresist is washed out upon developing, whereas the opposite results in case of a negative photoresist, i.e., the unexposed region is washed out. The penultimate step in photolithography is the pattern transfer process in which the patterned photoresist is subjected to an etching treatment (dry or wet) for a time period corresponding to the desired feature size, which removes parts of the substrate not covered with the photoresist. Dry etching is an

anisotropic etching process, which is primarily employed when high aspect ratio and deep features are desired. The most common of all the dry etching processes is deep reactive-ion etching (DRIE) based on the Bosch process in which several hundred microns deep features can be produced on the substrate [58]. In comparison, wet chemical etching is an isotropic etching process in which the maximum attainable depth for a feature equals the thickness of the photoresist used. The final step is the removal of the left-over photoresist. Schematics of a generic photolithography process are shown in Figure 1.4.1. The resolution of the feature that can be produced on the substrate depends on the wavelength of the UV light used to expose the photoresist; in principle, the shorter the wavelength, the smaller the feature that can be produced. Thus, high resolution features can be produced by reducing the wavelength. Over the years, many different types of UV sources have been used starting from gas-discharge lamps with up to 365 nm wavelength (till mid-1980s) to excimer lasers with 193 nm (current technology), which is expected to be superseded by the extreme ultra violet (EUV) source with up to 13.5 nm (also termed as *next generation lithography*; currently in the research stage) [57]. However, the depth of focus is negatively affected by the wavelength of the UV light, which restricts the thickness of the photoresist and eventually the depth of the final pattern.

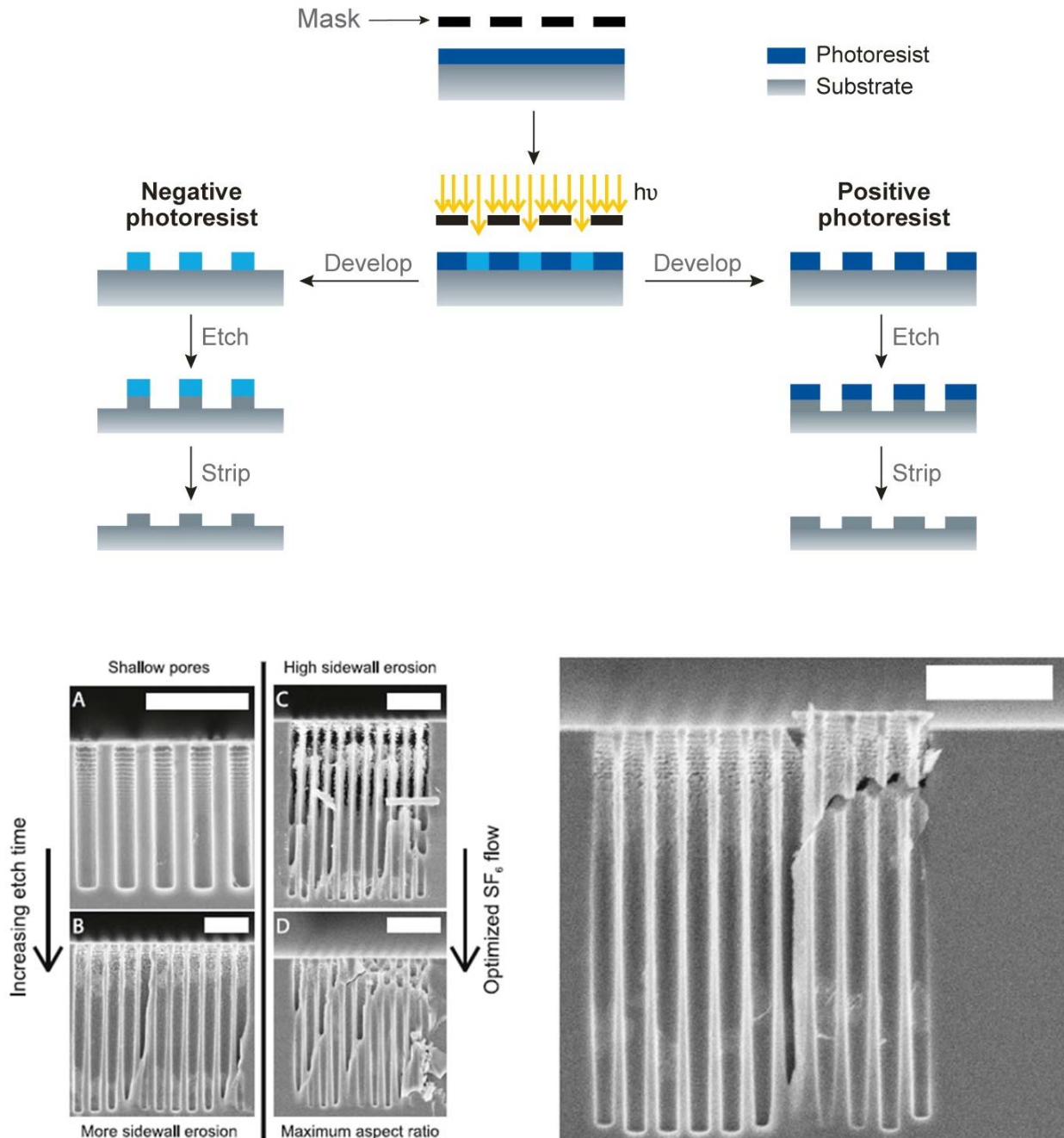


Figure 1.4.1. (Top) A general schematic of the photolithography process with negative (left) and positive (right) photoresists. (Bottom) SEM images of nanoholes made by a combination of deep UV lithography and reactive ion etching (in SF_6). The left image shows various nanoholes made by varying the etching time and flow rate of SF_6 . Shown in the right are some deeply etched nanoholes with aspect ratio of ~ 15.2 . The nanoholes are $6.4 \pm 0.14 \mu m$ deep and their diameter is $423 \pm 22 nm$. (the white scale bar corresponds to $2 \mu m$ in all the images) [references: [55, 59]]

1.4.2) Electron Beam Lithography (EBL)

EBL is a maskless lithography technique by which complex features are produced on a substrate with very high resolution [57]. The operational principle of EBL is similar to that of photolithography with the exception that EBL is a direct-write process where patterns are directly engineered onto the substrate without the need of a mask. The substrate is coated with a thin layer of resist (e.g., polymethylmethacrylate) by spin coating, pre-baked, subjected to pattern writing in an electron beam lithography system (*e-beam system*), followed by resist development and pattern transfer. A schematic of a generic EBL process and SEM images of some of the structures thus produced are shown in Figure 1.4.2. The major advantage of EBL is that it is not diffraction-limited like photolithography, and features with resolutions of up to 20 nm can be easily produced; indeed, sub-10 nm features can also be produced by EBL. However, the resolution of EBL is limited by the scattering of electrons in the resist. Furthermore, the throughput of EBL is very low as the processing time is directly proportional to the pattern area for a certain dose given by the equation $T \cdot I = D \cdot A$, where T is the exposure time, I is the beam current, D is the dose in Coulombs/cm², and A is the exposed area. In other words, it would take approximately 12 days to pattern a 1 cm² area with a 1 nA beam current and 1 mC/cm² dose. Of the many applications of EBL known to date, the most common are micromachining, fabrication of photomasks for photolithography, and fabrication of master patterns for soft lithography [60].

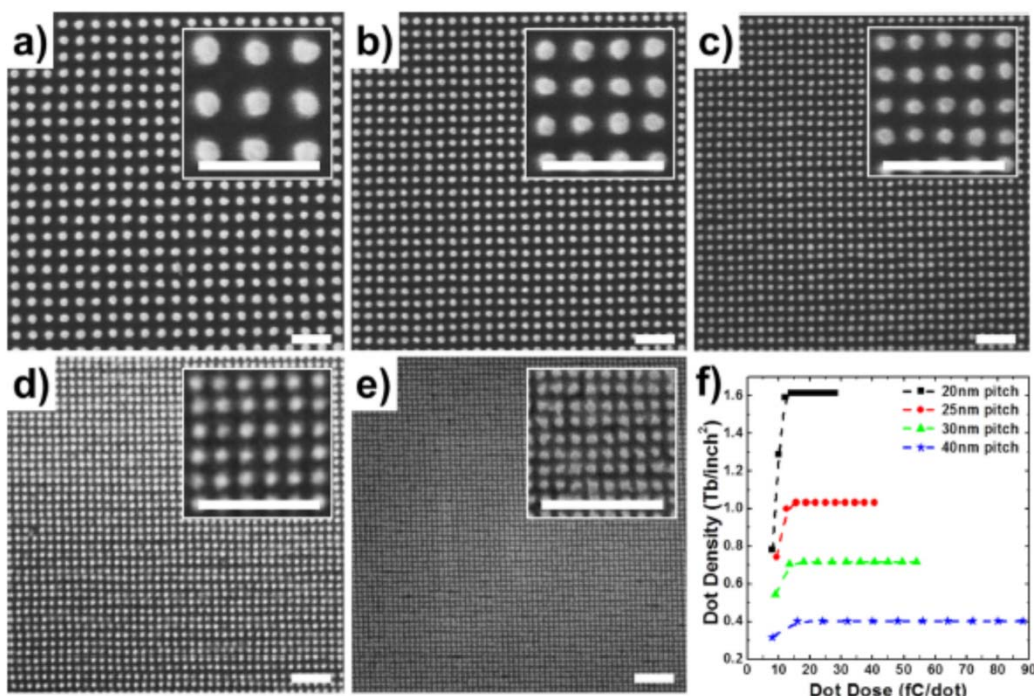
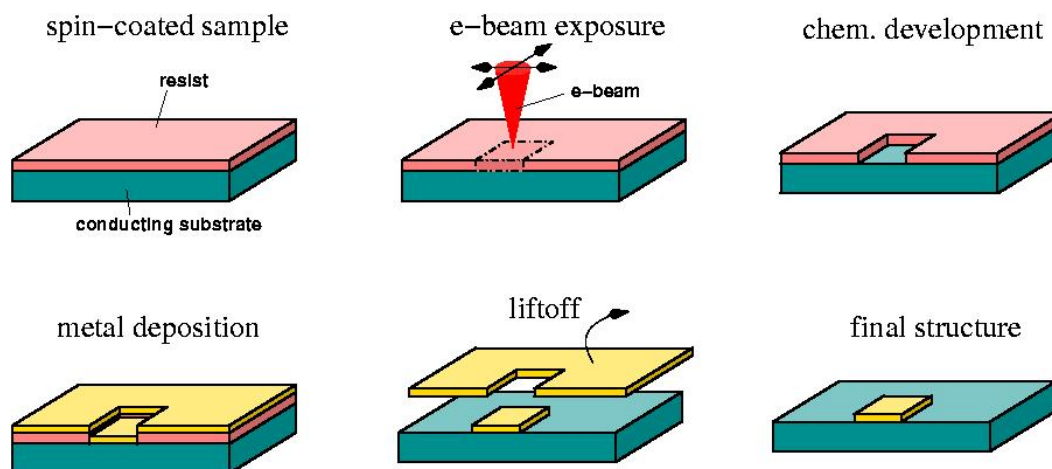


Figure 1.4.2. (Top) Schematic of an EBL process showing the formation of a metal structure on a substrate via electron beam patterning of a positive tone e-beam resist. (Bottom) SEM images of silicon nanodot arrays with varying pitch obtained by regulating the e-beam dose: (a) 40 nm, (b) 30 nm, (c) 25 nm, (d) 20 nm, (e) 15 nm, and (f) density of nanodots as a function e-beam dot dose (all scale bars correspond to 100 nm). These 60 nm deep silicon nanodot arrays were prepared by reactive ion etching ($\text{SF}_6 + \text{O}_2$) of a 20 nm thick negative tone e-beam resist, hydrogen silsesquioxane (HSQ), patterned by a 100 kV e-beam system. [reference: [61]]

1.4.3) Soft Lithography

Soft lithography is a form of nanolithography, which constitutes a set of various non-photolithographic techniques centered on the principle of self-assembly and replication (or *imprinting*) [62]. The term “soft” in soft lithography is derived from the physical nature of the mold used (i.e., soft) for fabricating structures, where the soft mold itself is prepared from rigid templates. Rigid templates, also termed as master patterns, are reusable patterned substrates that are usually prepared by photolithography, EBL, or micromachining on materials like silicon and silica. The master pattern is first subjected to cast molding to form its replica on an elastomeric material like polydimethylsiloxane (PDMS) to prepare the soft mold or stamp, which is then used to generate structures or patterns by various techniques that are collectively termed as soft lithography. Over the years, soft lithography has evolved into one of the most economical and most convenient nanolithography techniques where features as small as sub-10 nm size scale have been successfully fabricated by many research groups. Several review articles have also been communicated from time to time focusing on key developments in soft lithography techniques all over the world [7, 57, 62-64]. Some commonly used soft lithography techniques are: replica molding (REM), microtransfer molding (μ TM), micromolding in capillaries (MIMIC), solvent-assisted micromolding (SAMIM), and microcontact printing (μ CP).

1.4.3(a) *Replica molding (REM)*: REM, Figure 1.4.3(A), is a simple and single-step replication technique used to create micro/nanoscale features by curing prepolymers cast into PDMS molds. UV or thermally curable prepolymers without any solvent and low shrinkage (typically less than 3% on curing) are used for REM. Polyurethane (PU) is

an example of a UV curable prepolymer used extensively for replica molding. REM has also been employed against rigid molds for mass production of a variety of materials including compact discs (CDs), diffraction gratings, holograms, and micro-tools.

1.4.3(b) *Microtransfer molding (μ TM)*: In a μ TM process, Figure 1.4.3(B), a liquid prepolymer is first applied to the surface of a PDMS mold and the excess is removed by scraping, or blowing by passing a stream of gas, and then the liquid prepolymer filled mold is placed on the surface of a substrate. The prepolymer is thermally or UV cured to become solid and attached to the substrate after which the PDMS mold is delicately removed to leave behind the patterned structure on the substrate. Thermally curable epoxy resins and UV curable polyurethanes have been used as prepolymers for μ TM.

1.4.3(c) *Micromolding in capillaries (MIMIC)*: MIMIC is a very versatile soft lithography method used to create micro/nanoscale features from a variety of materials, such as UV or thermally curable polymers with no solvents, functional polymer solutions, glassy carbon or ceramic forming precursor polymers, inorganic salts, sol-gel materials, colloidal solutions, polymer beads, and biologically functional macromolecules. In this method, as shown in Figure 1.4.3(C), a PDMS mold with channels is first placed on a substrate and then a low-viscosity prepolymer is placed at the open ends of the channels. The prepolymer fills up the channels by capillary action, which is then solidified by curing. The PDMS mold is then slowly removed from the substrate to create patterned features of the polymer.

1.4.3(d) *Solvent-assisted micromolding (SAMIM)*: SAMIM is the only soft lithography method which utilizes polymer films to create nanostructures on their surface from

PDMS molds. However, only those polymer films can be used whose solvents do not affect the PDMS mold during micromolding. In this method, the surface of the PDMS mold is first wetted with a solvent that can dissolve (or soften) the polymer whose nanostructures are to be created. The PDMS mold with the solvent is then brought in contact with the polymer film held on a substrate. The solvent dissolves (or softens) a thin layer of the polymer and forms a gel that fills the patterns in the mold. When the solvent evaporates the gel inside the patterns freezes into solid polymer and forms polymer nanostructures on the substrate. A schematic of the SAMIM process is shown in Figure 1.4.3(D).

1.4.3(e) *Microcontact printing (μ CP)*: μ CP was originally developed by George M. Whitesides at the Harvard University. It is essentially a printing process in which a soft mold like PDMS is covered with a chemical solution called *ink* and then brought into physical contact with a substrate after the ink has dried. A commonly used ink is a thiol solution, such as hexadecanethiol. Upon physical contact with the substrate, the thiol is transferred to the surface of the substrate resulting in the reproduction of the mold pattern. The three known configurations to transfer the ink, based on the geometry of the mold and the substrate, are: i) planar mold onto a planar substrate; ii) nonplanar mold onto a planar substrate; and, iii) planar mold onto a nonplanar substrate. Figure 1.4.3(E) shows the schematics of these three configurations. A derivative of μ CP called nanocontact printing (NCP) is reported to pattern sub-50 nm features. μ CP (and NCP) have been investigated for applications in micromachining (where the ink acts as a resist for an etching process) and biological engineering (by patterning cells, DNA, and proteins) [65].

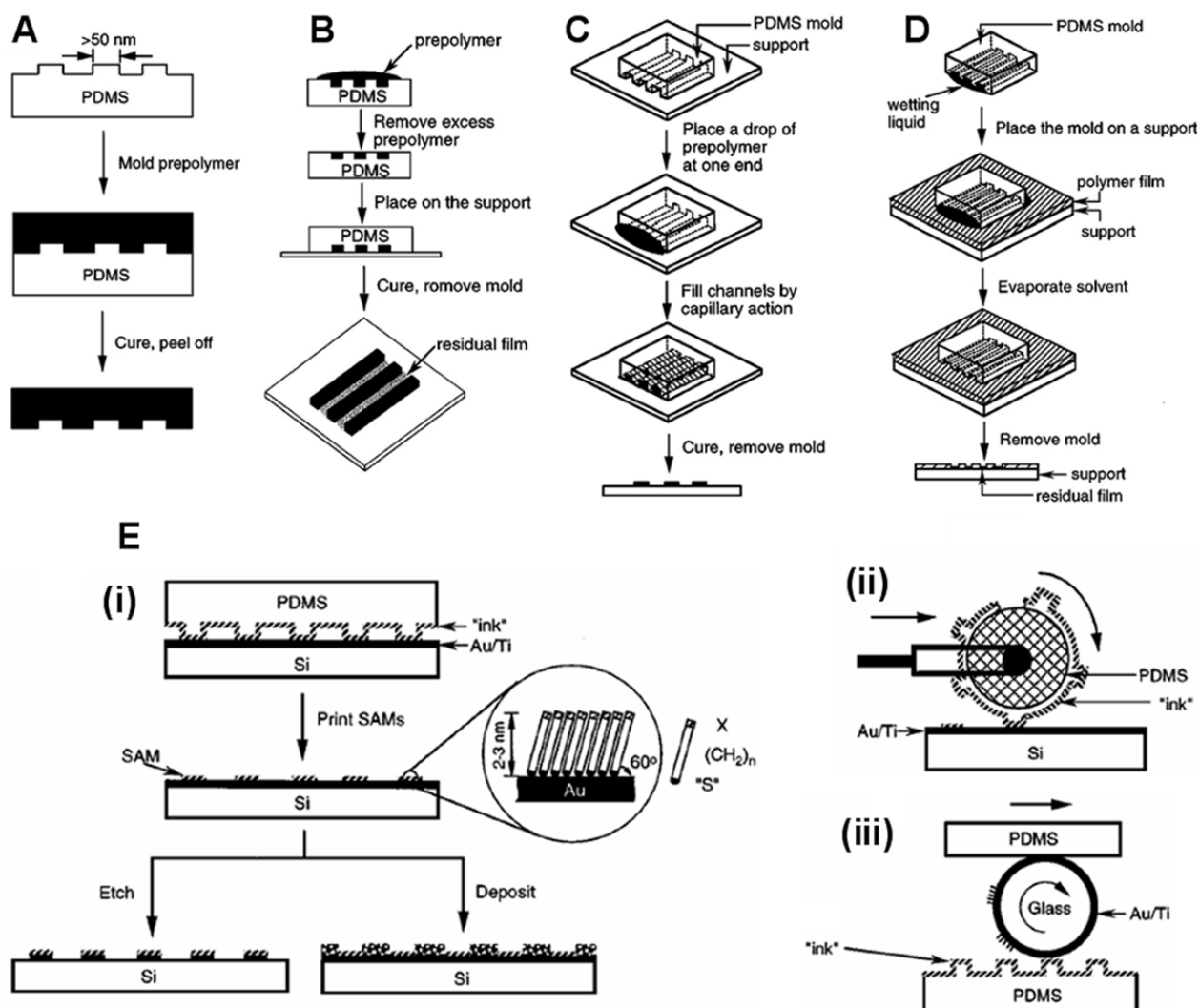


Figure 1.4.3. Schematics of some commonly used soft lithography techniques: (A) replica molding (REM); (B) microtransfer molding (μ TM); (C) micromolding in capillaries (MIMIC); (D) solvent-assisted micromolding (SAMIM); and, (E) microcontact printing (μ CP) in three different configurations:- i) planar mold – planar substrate, ii) rolling mold – planar substrate, and iii) rolling substrate – planar mold. [reference: [62]]

1.4.4) Nanoimprint lithography (NIL)

In NIL, an imprint resist is first spin coated on the surface of a substrate and then pressed against a rigid mold whose features are to be reproduced [66, 67]. Once the right pressure is achieved between the imprint resist and the template, the imprint resist is either subjected to a thermal treatment or a UV light treatment for curing depending on the type of resist. If a thermoplastic polymer is chosen as the imprint resist, it is heated above its glass transition temperature to soften and acquire the impression of the template. The template is then slowly separated from the imprint upon cooling. The acquired impression in the imprint is the negative replica of the template, which is further subjected to a pattern transfer process (usually by reactive ion etching just like photolithography) to transmit the imprinted pattern in the resist to the substrate. This nanoimprinting technique is termed as thermoplastic nanoimprint lithography (T-NIL) or hot embossing lithography (HEL), which was developed at the University of Minnesota by Dr. Stephen Chou's research group in the mid 1990's. In fact, the word nanoimprint lithography was coined by Dr. Chou's group [67], which performed and reported the first studies on nanoimprint lithography, and later developed it into a manufacturing technology.

Very similar to this technique is the photo nanoimprint lithography (P-NIL), also called cold embossing or UV nanoimprint lithography (UV-NIL), in which a photoresist is used as an imprint material. The photoresist is either spin coated or dispensed with a scanning inkjet head (step and flash imprint lithography [68]) on a transparent substrate, brought in contact with the template to maintain a certain pressure in between, and then cured by exposing to UV light. The photoresist undergoes crosslinking and hardens,

and acquires the impression of the template after which they are slowly detached from each other. This step is also followed by a pattern transfer process to transmit the features from the imprint into the substrate. This technique was developed by Dr. Grant Willson's research group at the University of Texas at Austin [69].

Schematics of T-NIL and P-NIL are shown in Figure 1.4.4. Developed as a low manufacturing cost and high-throughput method to fabricate nanoscale features, the NIL technique was demonstrated to create features from 25-nm resolution [67] up to sub-10 nm resolution [70], and was successfully applied to produce magnetic nanostructures [66], quantized magnetic disks [66, 71], silicon field effect transistors [72], semiconductor nanowires [73], and nano-compact disks [74]. Indeed, it was shown that NIL can be scaled up to fabricate large area patterns as well [75-77], and with further developments came several different versions of NIL, including hybrid techniques, commercial systems, and timely review articles [57, 69, 78, 79].

1.4.5) Other nanolithography techniques

Many more techniques have been investigated and reported for patterning substrates, such as laser based nanolithography techniques including femtosecond laser micromachining [80], dip pen lithography (DPN) [81], extreme UV lithography (EUV) [82], proximal-probe lithography [83], x-ray lithography [84], ion beam lithography including focused ion beam milling [85], proton beam writing [86], and more recently neutral particle lithography [87].

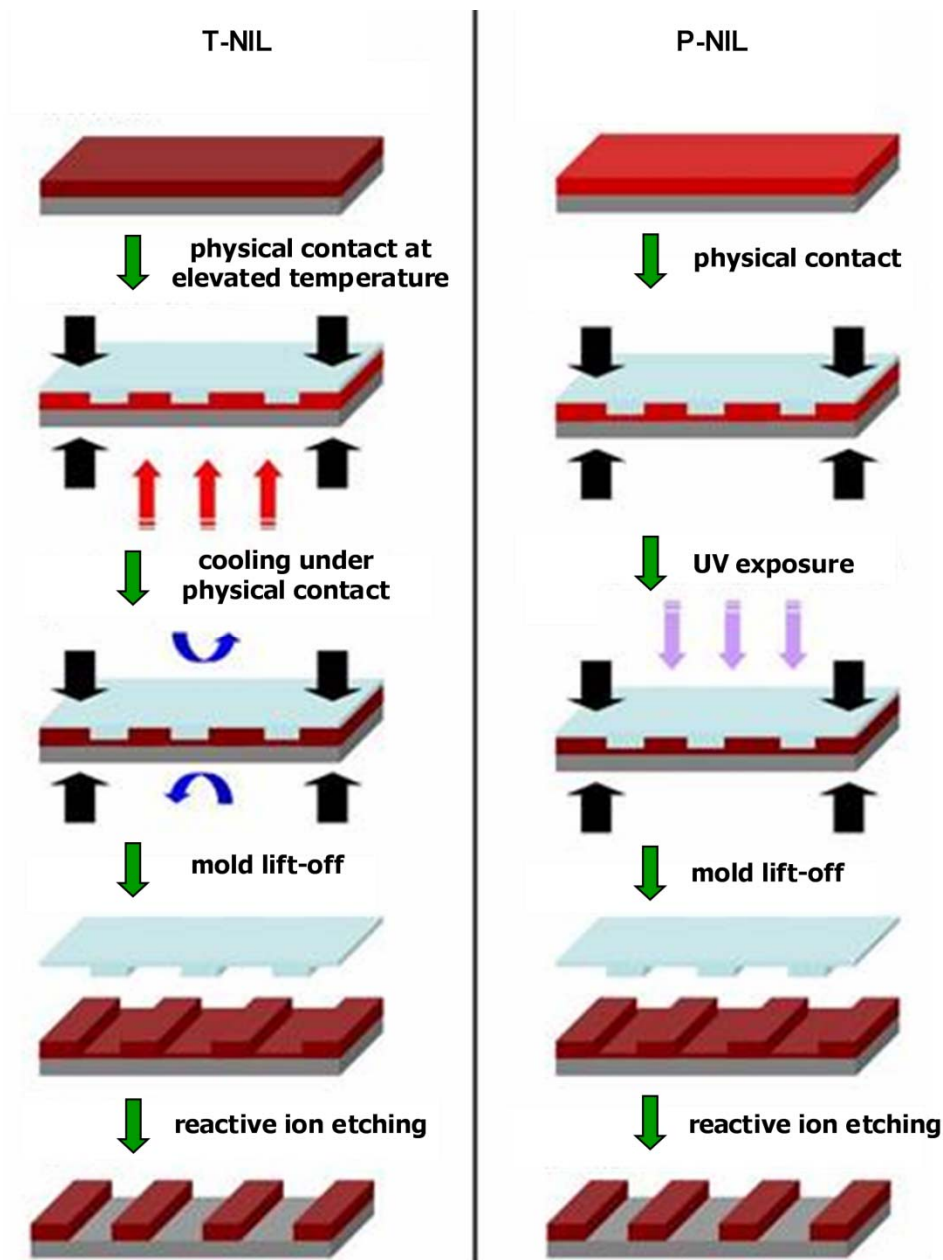


Figure 1.4.4. Schematics of thermoplastic nanoimprint lithography (left) and photo nanoimprint lithography (right). Both the techniques use a polymer resist preplaced on a substrate that is pressed against a mold to get the impression of the pattern (either by heat or by UV exposure), and subsequently subjected to reactive ion etching to transmit the pattern into the underlying substrate. All the other known nanoimprint lithography techniques, such as roller nanoimprint lithography (RNIL) and jet and flashTM imprint lithography (J-FIL), are derivatives of these two techniques.

Summary

Nanotechnology is undoubtedly one of the greatest scientific achievements of mankind, which holds the key to our future from many perspectives. Originally coined by Richard P. Feynman and later advocated by many eminent scientists, the field of nanotechnology has built a phenomenal reputation over the years for being a *solution* seeking *problems*. Nanofabrication is the heart of nanotechnology by which nanomaterials and nanostructures are fashioned. This chapter covered some of the nanofabrication techniques known hitherto with a particular emphasis on those based on template assisted synthesis. A short summary of conventional templates made on anodic alumina, nanochannel glass, and nuclear track-etch structures and how they are used to create nanostructures was presented. Nanolithography is the current state-of-the-art nanofabrication technique, which is used to craft nanostructures on solid materials with any desired design and with very high resolutions. Four of the most important nanolithography techniques (photolithography, electron beam lithography, soft lithography, and nanoimprint lithography) were briefly described.

However, none of the nanofabrication techniques reviewed here has so far been reported to produce large area patterns of high aspect ratio (at least 10:1) nanoholes of depths more than 10 μm at high production rates other than single-pulse amplified femtosecond laser machining. Substrates with user-defined patterns of high aspect ratio nanoholes of depths more than 10 μm can be used in template synthesis of micro/nano structures by replication. The next chapter, Introduction, covers single-pulse amplified femtosecond laser machining by which >25:1 aspect ratio and >10 μm deep nanoholes have been produced. Templates produced by this technique can be used to fabricate

structures by imprinting methods. Also discussed in brief are some applications where these imprinted structures can be used.

Chapter 2

Introduction

Materials modification with lasers has been studied extensively since the invention of the first working laser and remains a key research area in the development of materials with extraordinary properties for advanced technologies [88]. Many different types of materials like metals, alloys, glass, semiconductors, refractories, composites, and polymers have been subjected to laser treatment and explored for myriad applications. Some examples of laser materials modification techniques are laser cutting, drilling, welding and brazing, alloying and cladding, surface coating, surface hardening, surface texturing, milling, machining, semiconductor doping, interference lithography, and solid freeform fabrication [89, 90]. Both continuous and pulsed modes of laser operation are used to accomplish these materials modification processes. In particular, pulsed mode is employed where high peak intensities are required for material modification [90]. The work presented in this dissertation concerns the ablation of optically transparent materials that require high peak intensities for permanent structural modification.

This chapter highlights the fundamental principles and some early work done on laser ablation of optically transparent materials using femtosecond pulses, and how it led to the development of single-pulse amplified femtosecond laser machining as a nanofabrication strategy for patterning transparent materials. The later part of this chapter underscores the importance of this technology as a direct-write nanolithography tool to fabricate templates for creating high aspect ratio polymer structures via imprinting techniques, and how the imprinted structures can be used in various applications. Towards the end of the chapter, the motivation and the aims and objectives behind this dissertation are presented.

2.1) Laser-induced Breakdown in Optically Transparent Materials

Laser ablation of transparent materials is based on the process of laser induced optical breakdown caused by high intensity ultra-short laser pulses [91]. In this process, optical breakdown is realized when sufficient energy is supplied to the electrons for excitation from the valence band to the conduction band. **Photoionization** and **avalanche ionization** are two excitation mechanisms that contribute towards the nonlinear absorption of photons [80]. Photoionization occurs when a bound electron of an atom gets excited by the strong electric field of the incident laser beam. It is further divided into two categories depending on the laser intensity and frequency: **tunneling ionization** and **multiphoton ionization**. Although questioned for its suitability in certain conditions [92], Keldysh parameter (γ), also known as adiabaticity parameter, is often used to determine which of the two photoionization processes will be more dominant for the given laser intensity and frequency. It is defined as [93]

$$\gamma = \frac{\omega}{\omega_t} = \frac{\omega}{e} \sqrt{\frac{m_e c n \epsilon_0 E_g}{I}} = \sqrt{\frac{E_B}{2U_p}}$$

where I and ω are the laser intensity at focus and the frequency of the incident laser beam, respectively, ω_t is the tunneling frequency, e is the charge of the electron, c is the speed of light, m_e is the electron rest mass, n and E_g are the refractive index and bandgap of the material, respectively, E_B is the ionization potential of the atom, and U_p is the ponderomotive potential. For fused silica at 800 nm, according to Schaffer et al. [91], tunneling ionization occurs when $\gamma < 1.5$, whereas multiphoton ionization occurs when $\gamma > 1.5$.

2.1.1) Tunneling ionization

When the oscillating electric field of the incident laser beam is sufficiently strong, it lowers the Coulomb potential barrier of the bound electron in the atom. The lowering of the potential barrier is due to vector addition of the electric fields of the laser beam and the atom. The electron can then escape or tunnel through this reduced potential well and ionize the atom. This photoionization mechanism is termed *tunneling ionization* or *optical field ionization*, which dominates for lasers with strong fields and low frequencies [91], and is schematically described in Figure 2.1.1.

2.1.2) Multiphoton ionization

When several quanta are simultaneously absorbed by the bound electron in the valence band, excitation to the conduction band can occur if the total energy gain of the excited electron is equal to or greater than the bandgap. This mechanism is termed *multiphoton ionization* and it dominates for lasers with high frequencies [91]. The rate (Φ) of a multiphoton absorption process is given by the equation $\Phi(n) = \sigma_n I^n$, where n is the number of photons required to excite an electron and σ_n is the multiphoton absorption cross-section of n photons. The minimum number of photons (n) required for multiphoton ionization of an atom can be calculated by the energy balance equation: $n = E_g / \hbar\omega$, where E_g is the ionization energy and ω is the laser frequency. A pictorial depiction of multiphoton ionization is shown in Figure 2.1.2.



Figure 2.1.1. Ionization by tunneling of the bound electron due to suppression of the potential barrier caused by the strong electric field of the laser beam. This is called tunneling ionization or optical field ionization. This graph represents the potential (electrostatic) energy of the electron in the electric field of the laser. (empty circle = hole; solid circle = electron; the solid line is a graph of the Coulomb potential energy of an electron (y-axis) as a function of the radial position (x-axis) of the electron.)

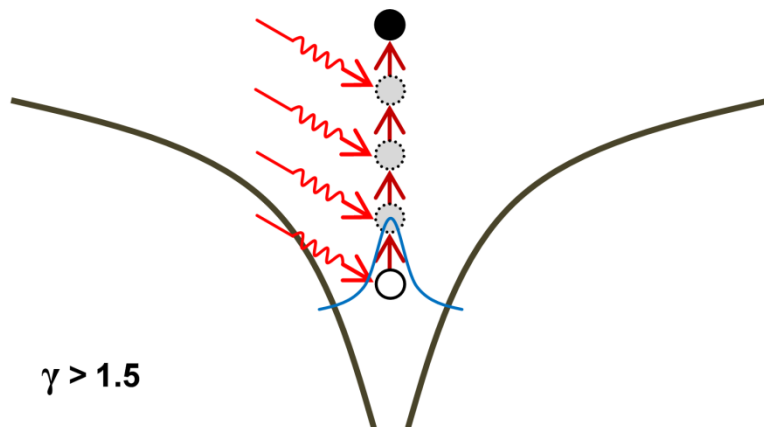


Figure 2.1.2. Ionization by excitation of the bound electron due to multiphoton absorption. This is called multiphoton ionization. In this example, a bound electron absorbs 4 photons and ionizes the atom. (empty circle = hole; solid circle = electron)

2.1.3) Avalanche ionization

Free electrons created by photoionization, thermal excitation or arising from crystal defects, are driven into oscillation by the strong alternating electric field of the incident laser beam and accelerate to higher energy states in the conduction band. A high energy state electron in the conduction band further excites a bound electron in the valence band by impact ionization provided the following energy criterion is met: $m\hbar\omega \geq E_g$, where m is an integer, $\hbar\omega$ is the photon energy, and E_g is the bandgap of the material. This higher energy state electron in the conduction band transfers part of its energy (equal to or more than the bandgap of the material) to a bound electron in the valence band forming an additional excited electron. One high energy state electron can impact ionize hundreds of bound electrons. This cascade of excitations of valence band electrons by impact ionization populates the conduction band, and continues as long as the free electrons have excess kinetic energy. This excitation mechanism is referred to as *avalanche ionization* [91] and is schematically shown in Figure 2.1.3.

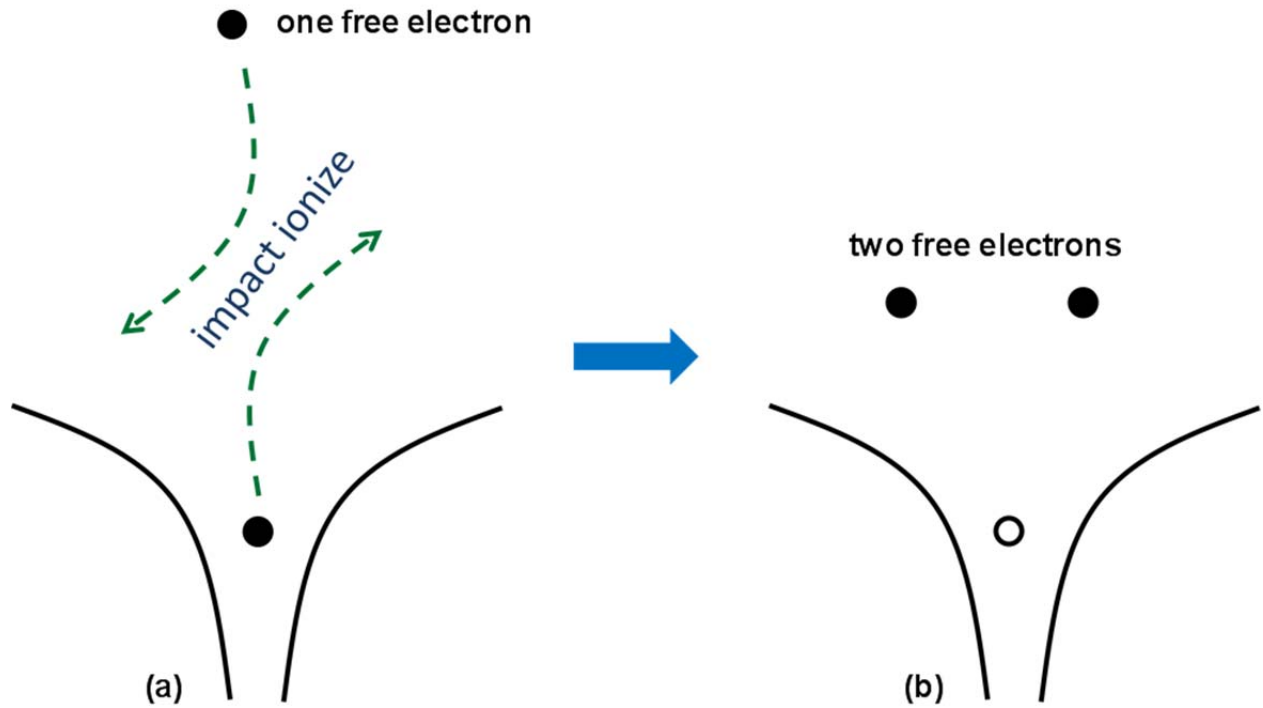


Figure 2.1.3. A simplified schematic of avalanche ionization in a wide bandgap material. A free electron in the conduction band excites to higher energy state upon interaction with an intense laser beam and impact ionizes a bound electron (a) to populate the conduction band (b). Practically, one high energy state electron can impact ionize many bound electrons.

2.2) Femtosecond Laser Machining of Transparent Materials

Femtosecond lasers have been widely used for micromachining of a variety of transparent materials due to their versatility in producing geometrically complex structures in three-dimensions with very high precision [80]. Nonlinear absorption of laser energy by means of multiphoton ionization or tunneling ionization followed by avalanche ionization causes *optical breakdown* in transparent materials [94, 95]. Micromachining of wide band gap transparent materials with femtosecond laser pulses has been reported for applications like binary storage data, optical waveguides, waveguide splitters, waveguide optical amplifiers, nanogratings, microfluidics, and flexures [96-99]. Transparent materials like fused silica, borosilicate glass, soda lime glass, BK7 optical glass, plastic, single-crystal quartz, diamond and sapphire have been successfully micromachined with femtosecond laser pulses [94, 95, 98, 100, 101].

Fused silica is one of the key transparent substrates used extensively for lab-on-a-chip device applications. Fused silica offers a combination of properties like wide range of spectral transparency, low autofluorescence, good biocompatibility, chemical inertness, near zero thermal expansion, excellent thermal shock resistance, and low dielectric constant and losses [94]. Laser induced breakdown in fused silica with femtosecond pulses was first reported by Du et al. in 1994 [102]. Micromachining of fused silica for lab-on-a-chip applications has been accomplished by numerous investigators [103-106].

2.3) Single-Pulse Amplified Femtosecond Laser (SP-AFL) Machining

A highly intense and tightly focused laser pulse from an amplified femtosecond (fs) laser system can induce a permanent structural change in the bulk of a transparent material due to nonlinear absorption. Making use of this nonlinear absorption phenomenon, White et al. [94] demonstrated the formation of high aspect-ratio nanoscale diameter holes, or *nanoholes*, of depths greater than 10 μm at the front surface of fused silica by focusing single pulses of temporal length 160 fs using a high numerical aperture ($NA = 0.85$) microscope objective lens. They determined the hole depths by measuring the lengths of the nanowires produced by cellulose acetate (CA) polymer replication (also termed as *nanoimprinting*) of the patterned fused silica templates, and then validated those measurements by focused ion beam (DualBeamTM SEM/FIB) sectioning of the nanoholes. It was found that the nanoholes' depth measurement by CA polymer nanoimprinting slightly underestimates their actual depth, which makes cellulose acetate polymer nanoimprinting a valid method for nanohole depth estimation. Figures 2.3(a) and 2.3(b) show nanoholes and an array of CA nanowires obtained by nanoimprinting, respectively. Schematic of FIB sectioning of nanoholes and an SEM image of the sectioned nanoholes are shown in Figure 2.3(c).

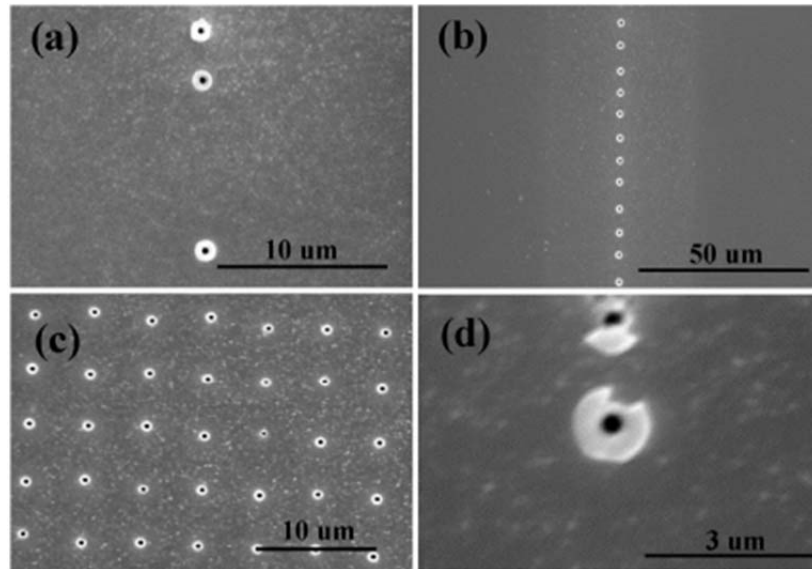


Figure 2.3(a). Nanoholes produced at the surface of fused silica at different energy values by single-pulse femtosecond laser machining using a 60x microscope objective lens of numerical aperture $NA = 0.85$. (a) $E = 1.2 \mu\text{J}$; (b) $1.6 \mu\text{J}$; (c) $2.4 \mu\text{J}$; (d) $1.2 \mu\text{J}$. [reference: [94]]

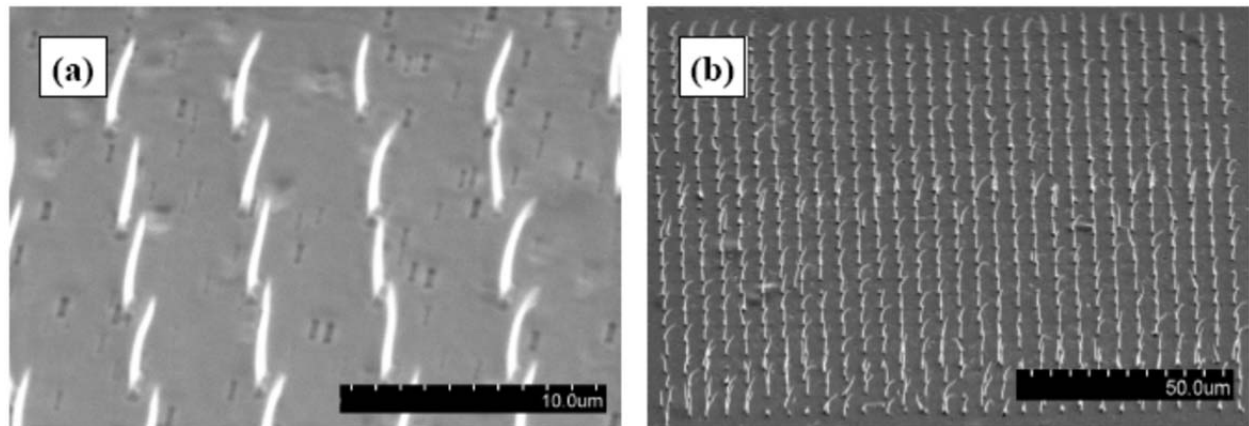


Figure 2.3(b). SEM images of the replicas of femtosecond laser machined fused silica shown in Figure 2.3(a). The replicas are of nanoholes machined at pulse energy of (a) $E = 1.6 \mu\text{J}$; and, (b) $E = 2 \mu\text{J}$. [reference: [94]]

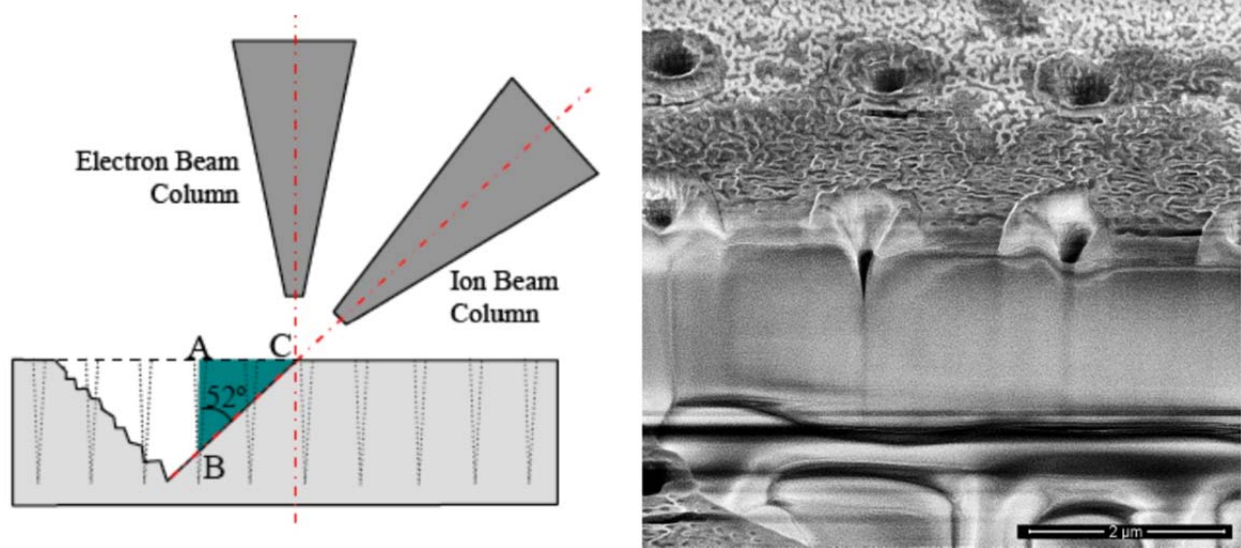


Figure 2.3(c). Schematic of the FIB milling and simultaneous SEM imaging of the nanoholes made at the surface of fused silica (left) and an SEM image of the cross-section of nanohole entrance taken at 52° stage tilt (right). The DualBeamTM SEM/FIB experiments were conducted at the Center for Nanophase Materials Sciences (CNMS), Oak Ridge National Laboratory, Tennessee. The nanoholes were shown to be at least $10\text{ }\mu\text{m}$ in depth. [reference: [94]]

Several other authors have also reported the formation of nanoholes in transparent materials by single-pulse femtosecond laser machining. Herbstman et al. [107] produced 8 μm deep holes by focusing single shots of temporal length 600 fs at the rear surface of a Corning 0211 glass cover slip using a microscope objective with numerical aperture $NA = 0.65$. Courvoisier et al. [108] reported the formation of 20-30 μm deep nanoholes at the front surface of a Corning 0211 glass cover slip patterned using single-shot femtosecond diffraction-free Bessel beams. The mechanism(s) responsible for the formation of such deep holes by tightly focused single-pulses from an amplified femtosecond laser are still unclear and open to discussion. White et al. proposed the longitudinal spherical aberration in the focal region of the laser beam caused by the microscope objective lens combined with self-focusing to be responsible for the formation of nanoholes, whereas Herbstman et al. proposed the role of self-focusing. Furthermore, studies by Delobelle et al. [109] have hypothesized that the laser beam undergoes reshaping upon interaction with the glass surface, which leads to the formation of deep nanoholes. As yet, none of the reported mechanisms have fully expounded the formation of nanoholes. Part of this dissertation is to address the reason behind the formation of deep nanoholes with a particular emphasis on the role of spherical aberration.

2.4) SP-AFL machined fused silica as templates

As mentioned in Chapter 1, there is no nanolithography technique known yet that can produce high aspect ratio ($>10:1$) and deep ($>10\ \mu\text{m}$) nanoholes economically, conveniently, and at high production rate. Although in its infancy, single-pulse amplified

femtosecond laser machining by White et al. appears to have filled this gap by which >25:1 aspect ratio and >10 μm deep nanoholes have been successfully produced at the surface of fused silica. Moreover, cellulose acetate replication of these nanoholes unveils a novel-yet-simple imprinting strategy for creating high aspect ratio and tall polymer structures. In addition, it is possible to modify the physical and chemical properties of these imprinted structures by functionalizing them to create novel structures. For example, silica nanowires can be created by coating a thin layer of silica on top of CA nanowires. Similarly, alumina and titania nanowires can also be created. As these polymer structures have high surface area, are physically arranged in a controlled manner, and can be subsequently functionalized, they are useful for a number of applications, such as photovoltaics, high surface area electrodes, and synthetic cell culture substrates.

2.5) Imprinting strategies

Nanostructures are routinely fashioned via replication (also termed *imprinting*) of patterned substrates. Some of the well-established replication strategies that use nanofabricated templates as molds are discussed in Chapter 1. Replication strategies used in soft lithography [62] and nanoimprint lithography [66] are of particular interest as they make use of templates with customizable nanoholes, just like SP-AFL machined templates. CA film replication of SP-AFL machined fused silica substrate (White et al.) resembles solvent-assisted micromolding (SAMIM) of soft lithography. The only difference being the type of mold used; SAMIM uses soft templates (PDMS molds), whereas White et al. used rigid templates (fused silica molds). Soft molds like PDMS

cannot be used with many solvents because of their affinity towards chemical reactions, such as swelling, which severely limits their application as templates. Nanoimprint lithography uses rigid templates like silicon and fused silica, but the replication material reported so far has always been in liquid phase, such as photoresist and PMMA, which is subjected to either UV curing or thermal treatment to form the replica [67, 110]. Moreover, the ultimate objective of the polymer replica in nanoimprint lithography is to act as a mask to transfer the pattern into the substrate by reactive ion etching. Since nanoimprint lithography is dependent on polymer type, its application as a replication strategy for imprinting polymer structures is also limited.

One of the objectives of this dissertation is to explore the capabilities of SP-AFL machined fused silica substrates as templates for imprinting polymer structures by film based and, in particular, solution based replication strategies. Indeed, solution based replication offers several advantages over film based replication (explained later), which makes it more versatile in its applicability. One of the major applications of polymer structures imprinted from SP-AFL machined templates is in cell biology, where they can be used as synthetic cell culture substrates with tunable topography. A brief discussion on the effect of substratum topography on cell behavior and its importance is presented in section 2.5.1.

2.5.1) Cellular response to substratum topography

The regulation of cytoskeletal configuration of a cell in response to variations in extracellular neighborhood is a long established phenomenon, and has remained a key research area in developmental biology, biomedical engineering, and pharmacology [111, 112]. This regulation is governed by sensory mechanisms of the cell that respond

to physical and chemical signals arising from the micro/nano environment. The cell can assay these signals and undergo a change in its morphology, conduct, and/or motility; collectively termed *morphogenesis*. The cellular response to externally applied mechanical forces is currently being explored by many researchers to understand the physical behavior of different cell types. Topography, compliance, texture, and anisotropy of the extracellular matrix (ECM) or the cell culture substratum affect these physical forces and influence cell phenotype and activity responsible for embryogenesis, cell regeneration, and wound healing [113-115]. Hence, it is essential to understand the characteristics of the physical interaction between the cells and their interaction with substrate features to further advance cell biology and regenerative medicine.

The effect of substratum topography on cell physiology was predicted as early as 1890's, and experimental observations of these effects coincide with the genesis of cell culture in the 1910's by Harrison [116, 117]. Since then studies on cellular response to solid substrata, also referred to as *stereotropism* or *thigmotaxis*, have been actively pursued. Indeed, topographical control of cell behavior has been the subject of numerous review articles over the years, focusing on the physical characteristics of the interaction between different cell types and different topographical features [112-115, 118-122]. Early investigations on stereotropism were done on fibrous, mechanically roughened, and mechanically patterned (grooves and ridges) substrata with unrefined definitions [116, 117, 123-126]. More precisely patterned substrata with good control on topography and geometric profiles were made available with the advent of photolithography and electron beam lithography [122, 127-129]. Present studies on

topographical control of cell physiology are mostly done by culturing cells on flat substrata with 2D topography in which feature size is normally in the range 10 nm to 3 μm . 2D topography is obtained by creating arrays of features like nanogratings, nanopillars, and nanoholes on cell culture substrata *via* various micro/nanolithography and nanofabrication techniques [121, 130]. The size scale of these topographical features is typically smaller than the cell in both lateral and vertical dimensions, and has been found to affect the morphology, survival, adhesion, proliferation, and locomotion of certain cell types [113]. When features are considerably larger in vertical dimension and are spaced at least one cell dimension apart, they tend to behave as physical barriers and provide 3D environments that specifically influence cell morphology, spreading, and alignment by providing more cell-matrix interaction sites, particularly observed in case of fibroblast attachment and motility [131-134].

Cell motility is governed by the polarization of the protrusions (extension and contraction) of its plasma membrane caused by polymerization and depolymerization of actin filaments [135]. Surface receptors like integrins and membrane proteoglycans in the protrusions facilitate physical interaction of the cell with its ECM, and provide adhesion to the neighboring stroma [136]. However, presence of any feature or obstacle in the vicinity of the cell is sensed by these receptors, which influences its protrusions and eventually affects cell attachment, orientation, viability, and motility. The phenomenon of *contact guidance* is an example of such a cellular response to topographical features [113, 124]. From materials standpoint, a variety of metals, ceramics, and polymers exhibiting suitable biocompatible properties are used to make substrata for *in vitro* cell culture studies [113, 114, 118]. Currently, polymers are being

explored as cell culture substrata as they can be easily and efficiently patterned via soft lithography with nano/microscale features, for example polydimethylsiloxane (PDMS) [118]. Micropillar arrays of PDMS and silicon prepared via nanolithography are commonly used to study behavior of different cell types in 3D microenvironments [131, 134, 137].

Polymer structures imprinted from SP-AFL machined fused silica are a great candidate as synthetic cell culture substrates to study cell behavior as they are created by a simple replication technique with very high throughput and reproducibility. Also, fused silica is chemically inert to organic solvents, so templates made on fused silica last for a very long time. With solution based replication strategy, structures from virtually any polymer or polymer blend (that makes continuous films) can be easily imprinted for cell study e.g., polycaprolactone (PCL). Imprinted polymer structures can be subsequently functionalized to create bioactive structures, such as silica nanocones, and used in biological applications as explained in section 2.5.2.

2.5.2) Functionalization of imprinted structures: Importance of silica

Silica and silica-coated materials constitute a special class of materials, which is used in a variety of biological and biomedical applications [138-141]. Silica is chemically inert to most organic substances, which makes it a suitable host material for many biological environments. Silica is a well-known catalytic support. Also, silica can be easily functionalized as the hydroxyl groups (-OH) present at its surface can covalently bond with other biochemical molecules, such as amino (-NH₂) and carboxyl (-COOH) groups. Functionalized silica allows further conjugation with other functional groups and biological agents, thus making silica a material of choice for many bioapplications.

Therefore, materials intended for use in biological applications (e.g., nanoparticles and quantum dots) are often coated with silica to improve their biocompatibility. Biosensing, bioimaging, and drug targeting are some examples where functionalized silica is consistently used [142-145].

Nanoscale surface coatings or nanoencapsulation based on silica has also been a subject of great interest [146-149]. Nanoscale silica has been successfully produced by methods like chemical vapor deposition (CVD) [150], catalyst-controlled room temperature chemical vapor deposition [151], microemulsion [152], atomic layer deposition [153], sol-gel synthesis (Stober's method) [154], and liquid phase deposition [155]. Silica coatings for bioapplications are most commonly produced by solution-based methods of sol-gel and microemulsion. However, these methods require prolonged soaking in chemical solutions, which is unsuitable to imprinted polymer structures (such as the ones replicated from SP-AFL machined templates) as they can swell or disintegrate. Furthermore, imprinted polymer structures cannot be exposed to high temperatures as well because they can oxidize or may even burn. Thus, the only way to coat polymer structures with silica is by low temperature CVD process. Indeed, coating these polymer structures with any material should be done at low temperature to maintain their structural integrity. Besides low temperature CVD, imprinted polymer structures can also be functionalized by sputtering and thermal evaporation methods. Coating processes such as sputtering emit fast-moving ions and thermal evaporation emit radiation that can distort the shape of thin polymer structures, thus cooling the polymer below its glass transition becomes imperative to sustain the impact of ions and heat.

Summary

Single-pulse amplified femtosecond laser machining has emerged as a new direct-write nanolithography technique for patterning transparent materials with user-defined arrangement of nanoholes. It is based on the principle of nonlinear absorption of photons leading to optical breakdown in the bulk of the transparent material. Nanoholes are produced by tightly focusing amplified femtosecond laser single pulses through a high numerical aperture microscope objective lens at the surface of the transparent substrate such as fused silica. Cellulose acetate replication and DualBeamTM SEM/FIB cross-sectioning of the nanoholes made on fused silica reveal that the holes are >10 μm deep with aspect ratio > 25:1 (White et al.). The reason behind the formation of deep nanoholes by single femtosecond laser pulse ablation is not yet clear, but longitudinal spherical aberration, self-focusing, and laser pulse reshaping are some of the proposed mechanisms.

CA film replication of the nanoholes produced by this technique by White et al. illustrates a facile method of creating high aspect ratio polymer structures that are suitable for many applications. Besides polymer films, polymer solutions can also be used for replication, which will offer complete freedom on the final chemistry of the polymer structures and will also allow replication from thermosets. Replicated polymer structures can be subsequently functionalized by coating with various materials such as oxide dielectrics and metals. Silica coated polymer structures can be used in biological applications.

Goals

Direct-writing of user-defined nanohole patterns by an amplified femtosecond laser system combined with the formation of high aspect ratio polymer structures by a simple replication approach together form the conception of a novel yet facile, potential nanofabrication process, which has yet to be explored from a technology standpoint. The goal of this dissertation is to explore this concept and develop it into a technology that is simple, economically feasible, and appropriate for high volume production of nano/micro- structures for a variety of applications.

Aims & Objectives

This dissertation is based on the following aims and objectives:

- 1) The reason behind the formation of high aspect ratio nanoholes by tightly focused single femtosecond laser pulses is still not clear and open to discussion. A clear understanding of nanohole formation is important to the fabrication of templates as it is to the current debate, as it could lead to improved hole parameters, such as diameter, depth, and conical profile. Of all the proposed mechanisms, the role of spherical aberration on nanohole formation can be investigated by optical modeling and ablation experiments using an aspheric lens. An aspheric lens is an optical element with non-spherical surface geometry designed to eliminate spherical aberration in the focal region of a perfectly collimated laser beam fully filling the entrance pupil of the lens. In this dissertation, an aspheric lens is used for laser ablation experiments in collimated (no spherical aberration) and non-collimated (with spherical aberration) conditions to study the role of spherical aberration on nanohole formation.
- 2) Fused silica templates prepared by single-pulse amplified femtosecond laser machining can be used as molds to create polymer structures by replication. A new replication procedure based on polymer solutions, termed *solution casting*, is investigated alongside the already known film based replication. Different types of polymer structures are imprinted from these templates to demonstrate the versatility of the replication procedures.

3) Modifying the properties of these polymer structures by functionalizing or coating with other materials will further advance their practical applications. A low-temperature chemical vapor deposition process is developed to coat the polymer structures with silica to create silica structures. As silica is used extensively in bioapplications, silica structures produced by this technique are investigated for applications in tissue engineering as cell culture substrates to study cell behavior.

Chapter 3

Experimental Procedure

Details of experimental techniques and testing methodologies are covered in this chapter. Experimental studies related to cell culturing were performed at the Department of Biomedical Engineering, Vanderbilt University. Experiments pertaining to femtosecond laser machining with an aspheric lens were conducted at the Center for Nanophase Materials Sciences (CNMS), Oak Ridge National Laboratory. Fused silica templates for imprinting polymer structures were prepared at the Nanofabrication Laboratory, Center for Laser Applications, University of Tennessee Space Institute, using an amplified femtosecond laser machining system described below.

3.1) Amplified Femtosecond Laser Machining System

The femtosecond laser machining experiments were performed at controlled ambient temperature (21°C) and atmospheric pressure in a class 1000 cleanroom facility using an amplified femtosecond laser machining system. Mounted on a vibration isolated optical table the amplified femtosecond laser machining system consists of two parts: 1) amplified femtosecond laser set-up; and, 2) machining workstation, details of which are described in sections 3.1.1 and 3.1.2, respectively.

3.1.1) Amplified Femtosecond Laser Set-up

The set-up consists of a diode pumped frequency doubled neodymium-doped yttrium orthovanadate (Nd: YVO₄) laser operating at 532 nm (Coherent, Inc., Verdi V18), a titanium-sapphire laser oscillator (Spectra Physics Inc., Tsunami Model-3941) operating at 76 MHz, and a 250 kHz titanium-sapphire regenerative amplifier (Coherent, Inc., RegA 9000). The titanium-sapphire laser oscillator seeds the titanium-sapphire regenerative amplifier to generate amplified femtosecond laser pulses at the rate of 250 kHz, while both the oscillator and the regenerative amplifier are pumped by the 532 nm

green diode laser. Figures 3.1.1(a) and 3.1.1(b) show the schematic and the photograph of the femtosecond laser set-up, respectively.

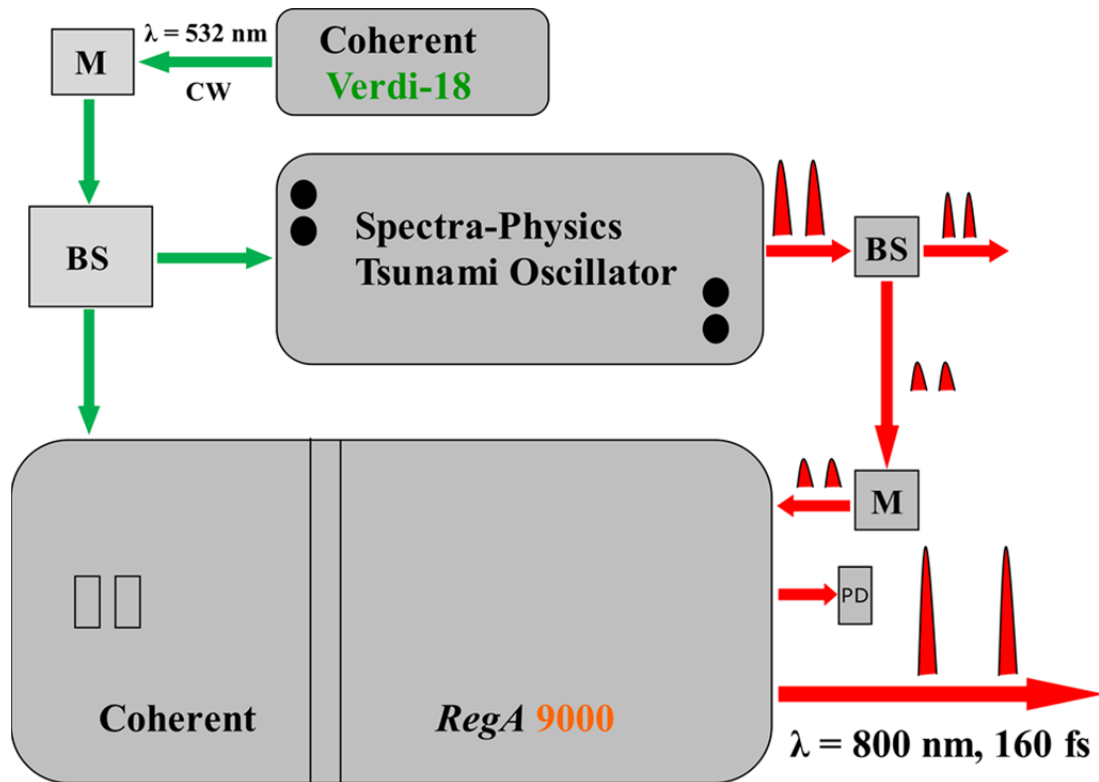


Figure 3.1.1(a) Schematic of the amplified femtosecond laser pulses generation in the amplified femtosecond laser set-up. A continuous wave green laser (Coherent Verdi-V18) of wavelength 532 nm pumps a titanium:sapphire laser oscillator (Spectra-Physics Tsunami) and a titanium:sapphire regenerative amplifier (Coherent RegA 9000), whereas the laser oscillator seeds the regenerative amplifier. The output from the regenerative amplifier is a train of 800 nm wavelength amplified femtosecond laser pulses at the rate of 250 kHz. The temporal length of each pulse is 160 fs. The maximum power of these pulses at 250 kHz is ~1.3 W, which gives the pulse energy of ~5.2 μ J. The Pockels cell within the regenerative amplifier enables the emission of single pulses for machining.

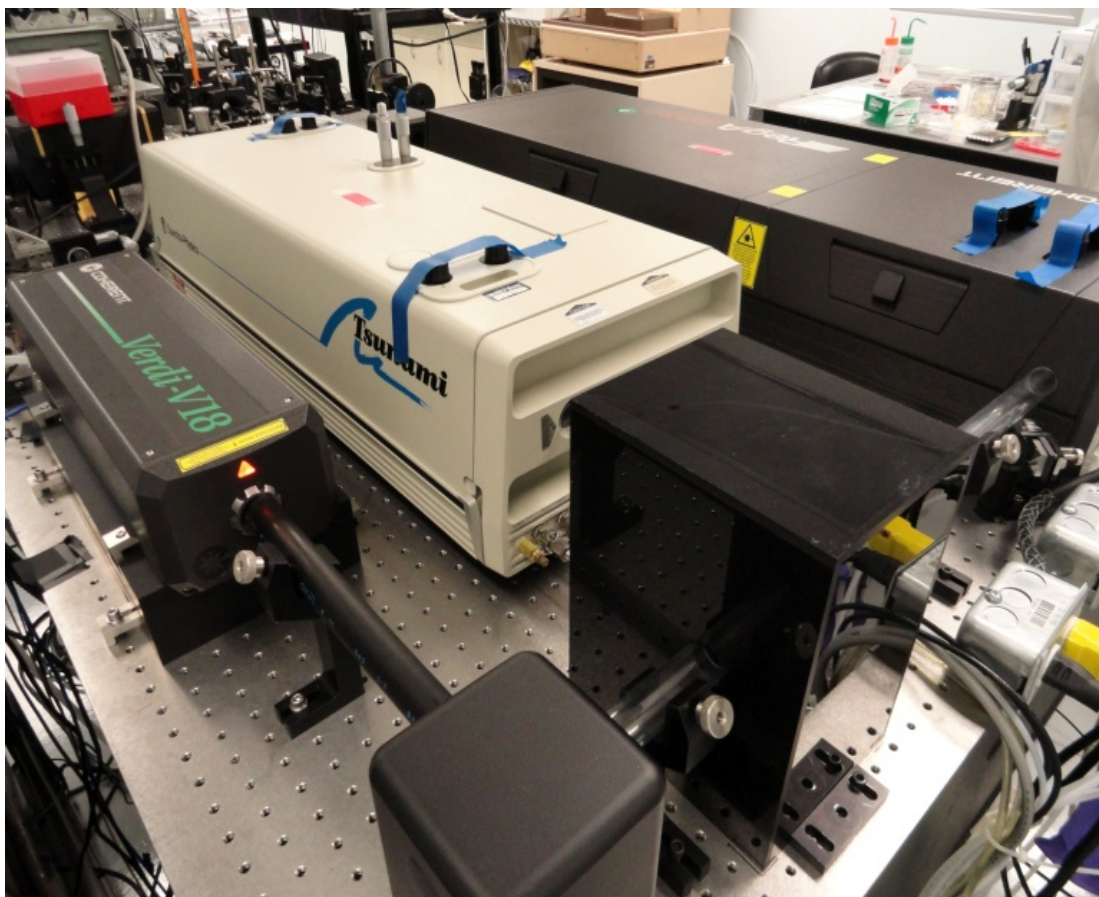


Figure 3.1.1(b) A photograph of the amplified femtosecond laser set-up. The output is a train of 250 kHz amplified femtosecond laser pulses of wavelength ~ 780 nm, temporal length 160 fs, and maximum pulse energy ~ 5.2 μ J.

The technical specifications of the 532 nm diode-pumped solid state laser, the titanium-sapphire laser oscillator, and the titanium-sapphire regenerative amplifier are shown in Tables 3.1.1(a), 3.1.1(b), and 3.1.1(c), respectively. The central wavelength of the amplified laser pulse is ~780 nm with a temporal length of 160 femtoseconds. The average power (P_{avg}) of the amplified laser pulses at 250 KHz was measured to be ~1.3 W, which gives the maximum pulse energy (E_p) to be ~5.2 μ J ($E_p = P_{avg}/250 \times 10^3$). The femtosecond laser pulses generated by the amplified femtosecond laser set-up travel through a neutral density filter and a beam expander consisting of two lenses, and delivered to the machining workstation via a periscope assembly for the machining experiments as shown in Figures 3.1.1(c) and 3.1.1(d). The periscope assembly consists of two mirrors at 45°, which elevates and guides the laser beam to enter the machining workstation at the right orientation. The laser pulse energy required for machining is regulated by adjusting the neutral density filter, whereas the distance between the two lenses in the beam expander is adjusted to collimate the beam. The electro-optic switch within the Titanium-Sapphire regenerative amplifier, also called *Pockels cell*, is externally triggered during machining to enable the emission of single laser pulses for the experiments. A detailed description of the amplified femtosecond laser set-up used in the present work can be found in references [95, 156].

Table 3.1.1(a). Technical specifications of the continuous wave pump laser (Verdi-V18, Coherent, Inc.).

Output Power	> 18 W
Wavelength	532 nm
Linewidth	< 5 MHz
Beam Diameter	2.25 mm $\pm$ 10%
Beam Divergence	< 0.5 mrad
M ²	< 1.1
Pointing Stability	< 2 μ rad/ $^{\circ}$ C
Power Stability	$\pm$ 1%
Noise	< 0.03% rms
Polarization	vertical, > 100:1

Table 3.1.1(b). General specifications of the laser oscillator (Tsunami Model 3941, Spectra Physics).

Repetition Rate (nominal)	80 MHz
Noise	< 0.2
Stability	< 5%
Spatial Mode	TEM ₀₀
Beam Diameter (1/e ²)	< 2 mm
Beam Divergence, full angle	< 1 mrad
Polarization	> 500:1 vertical

Table 3.1.1(c). System specifications of the regenerative amplifier (RegA 9000, Coherent, Inc.).

Repetition Rate (nominal)	250 kHz
Pulse Width (FWHM)	160 fs
Polarization	linear, horizontal, 500:1
Energy Stability (% rms)	< 1
Average Power Drift (% rms)	< 1

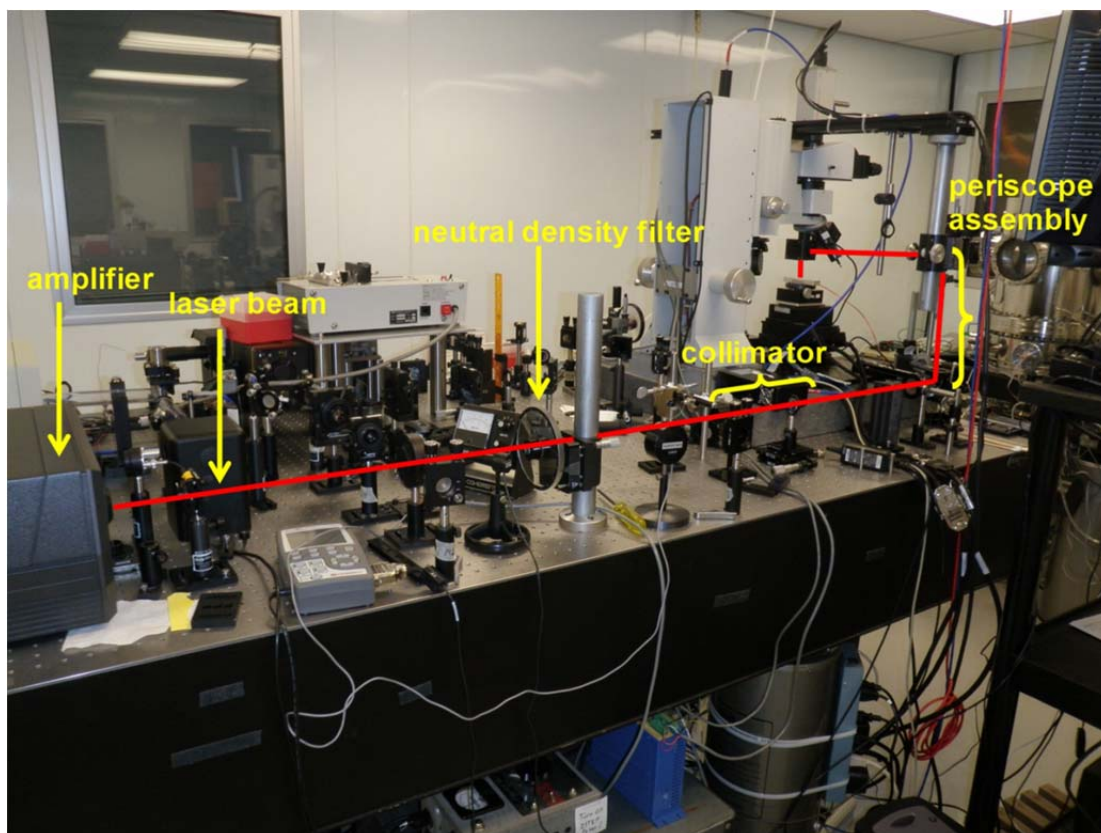


Figure 3.1.1(c). A photograph showing the femtosecond laser beam propagation path (shown as red line) after it comes out of the regenerative amplifier. The beam first goes through a neutral density filter and then a beam expander consisting of two lenses (shown as collimator in the picture) after which it is delivered to the machining workstation via a periscope assembly made up of two mirrors at 45° .

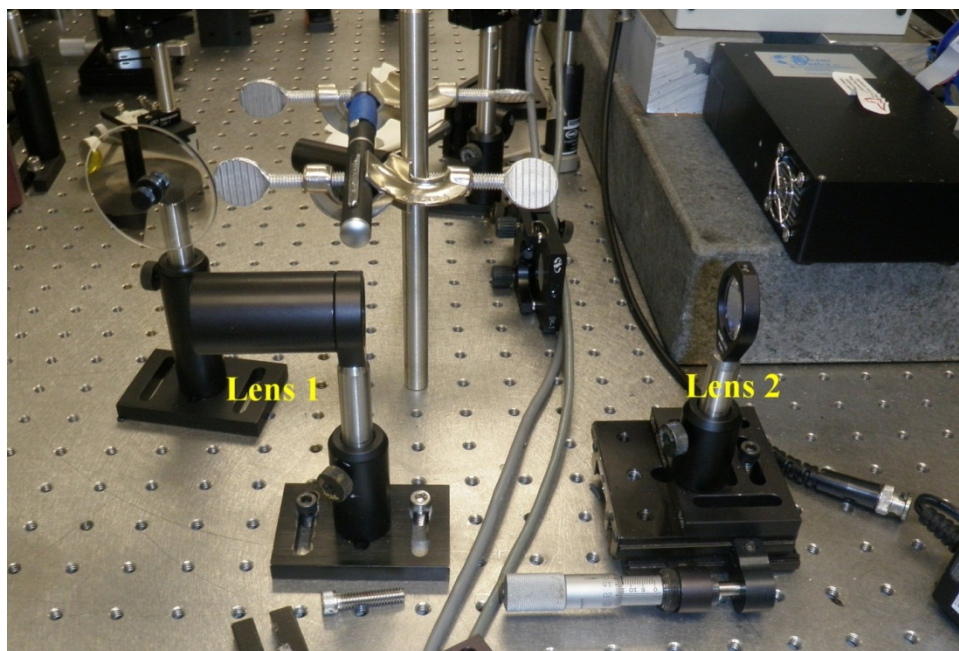


Figure 3.1.1(d). A close-up photograph of the beam expander. The beam enters Lens 1 from the left and diverges before entering Lens 2. Lens 2 focuses the laser beam, which then travels to the microscope objective lens via a periscope assembly. The spacing between Lens 1 and Lens 2 is adjusted to vary the diameter of the laser beam exiting Lens 2.

3.1.2) Machining Workstation

The laser pulses delivered by the amplified femtosecond laser to the machining workstation via the periscope assembly are reflected by an 800 nm dichroic filter mirror into the microscope objective lens. The microscope objective lens focuses the laser pulses onto the surface of a fused silica substrate mounted on a stage assembly for machining. The stage assembly consists of two independent stages capable of moving the substrate in XYZ directions with nanoscale precision controlled with a LabVIEW™ program and Aerotech basic software. The stage assembly consists of two stages mounted on top of each other: 1) ANT95-3-V for vertical translation (z-stage), and 2) ANT95-50-XY nMT for lateral translation (xy-stage). Both the stages are by Aerotech, Inc. and have a resolution of 1 nm. Figure 3.1.2(a) shows a photograph of the stage assembly with the z-stage mounted on top of the xy-stage, whereas a photograph and schematic of the machining workstation are shown in Figures 3.1.2(b) and 3.1.2 (c), respectively. Large vertical motions (Z direction) are controlled manually and by an additional stepper motor.

3.2) Fused Silica Substrates

500 μm thick, double side polished, UV grade fused silica wafers (Mark Optics, Inc.) were used to prepare templates by femtosecond laser machining. Technical specifications of the fused silica wafers used in the experiments are shown in Table 3.2. Unless otherwise specified, fused silica substrates of size 1 cm x 1 cm were diced from large wafers (100 mm diameter) to be used for machining experiments. All substrates were cleaned in acetone and isopropyl alcohol in order to remove any organic matter and dust from the surface prior to machining.

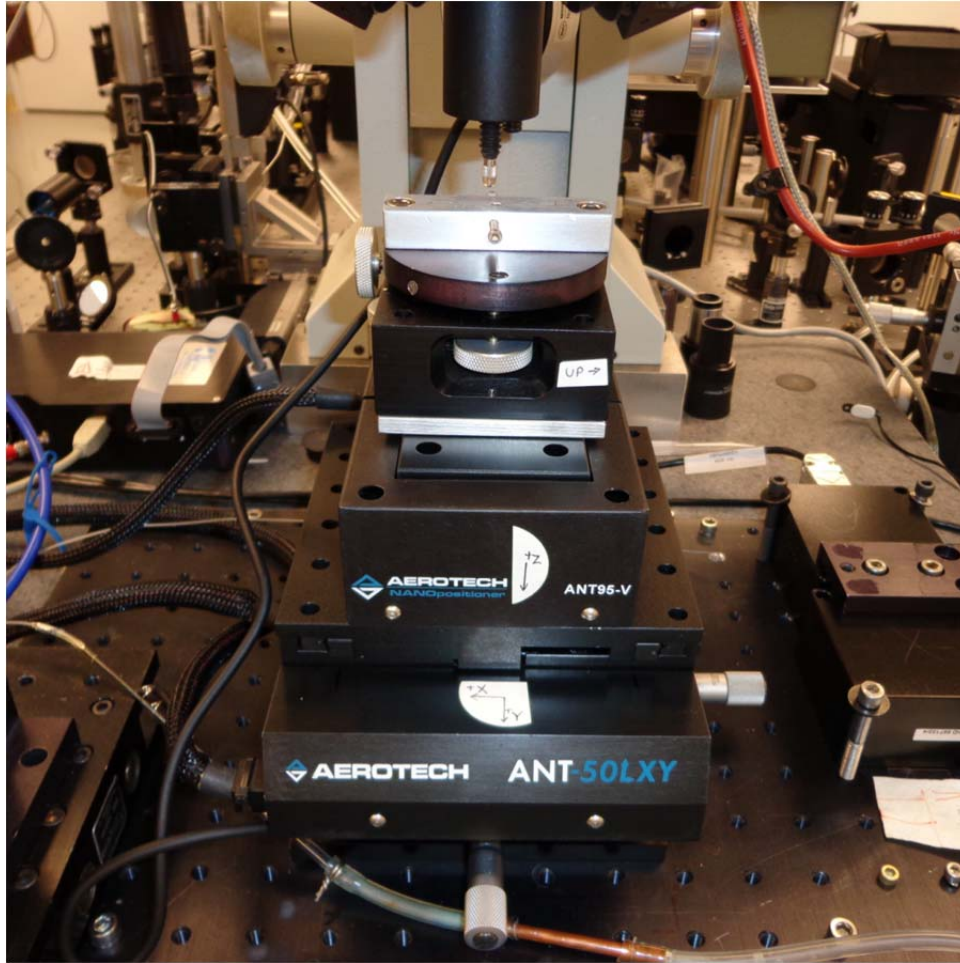


Figure 3.1.2(a). A photograph of the stage assembly on which the substrate is mounted for femtosecond laser machining. The z-stage is mounted on top of the xy-stage, both of them have 1 nm resolution and are controlled with a LabVIEW™ program during machining of fused silica.

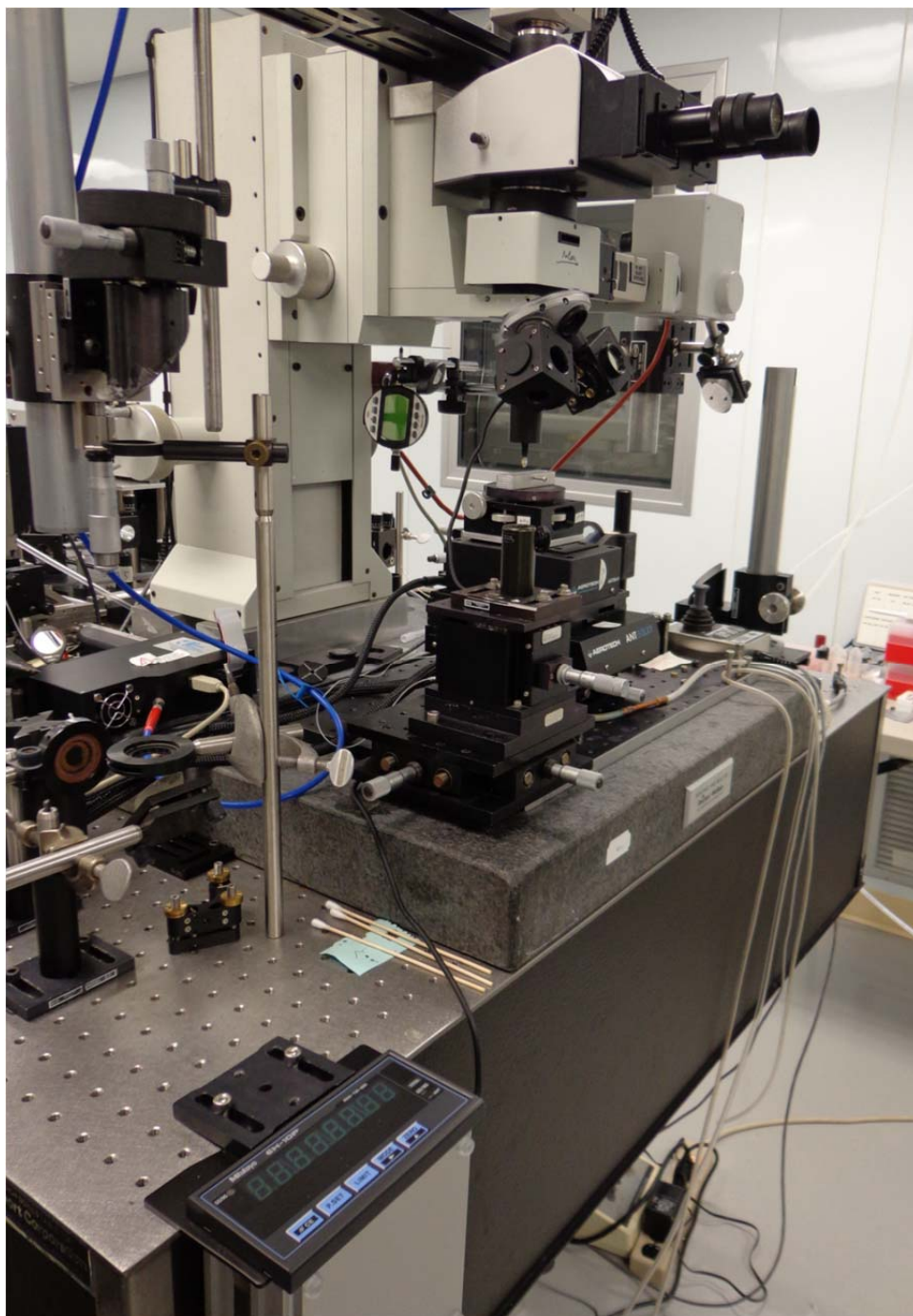


Figure 3.1.2(b). A photograph of the workstation used for amplified femtosecond laser machining of fused silica substrates. Schematic of this workstation is shown in Figure 3.1.2(c).

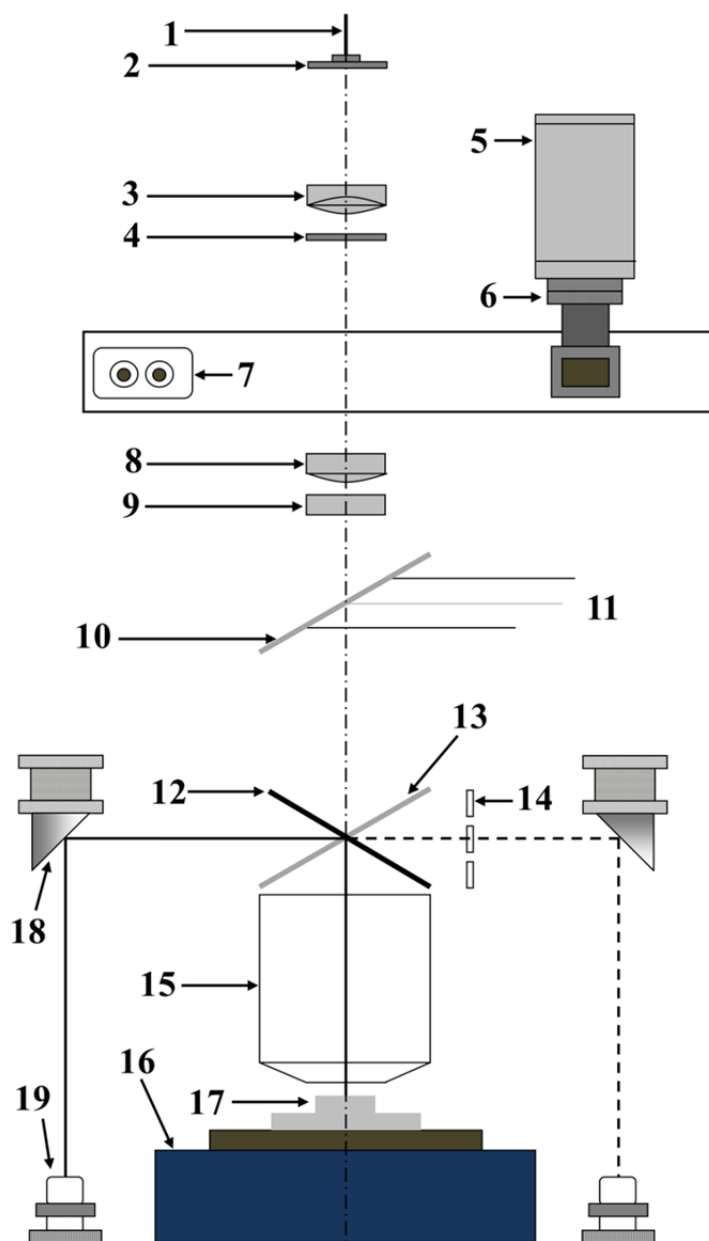


Figure 3.1.2(c). The optical assembly of the workstation used for laser machining of fused silica. (1) multimode fiber, (2) fiber optic endplate, (3) focusing lens, (4) long wave pass Raman edge filter, (5) 12 bit digital camera, (6) 9 mm computer TV lens, (7) eye piece, (8 and 9) tube lens, (10) half-mirror, (11) white light illumination produced from a reflected light vertical illuminator, (12) 800 nm dichroic filter mirror, (13) 532 nm dichroic filter mirror, (14) 532 nm narrow bandpass filter, (15) infinity corrected microscope objective, (16) xy-stage, (17) z- stage, (18 and 19) periscope assembly for redirecting and elevating the laser beam.

Table 3.2 Technical specifications of the UV grade fused silica used for experiments.

Outside Diameter (O.D.)	100.00 mm $\pm$ 0.20 mm
Thickness	500 μ m $\pm$ 25 μ m
Polished Surface	Both sides polished
Surface Quality (S.Q.)	60/40
Surface Roughness	20 Å
Total Thickness Variation (T.T.V.)	< 25 μ m

3.3) Single-Pulse Femtosecond Laser Machining of Fused Silica

Prior to laser machining, a stylus-based profilometer scans the surface of the substrate to obtain a level surface and more importantly map its topography. It is done in two steps in series:

- The first step involves the leveling of the substrate so that it is nearly normal to the laser beam during laser machining. Leveling is a process of reducing the substrate inclination as a function of stylus extension and compression. It is done by manually adjusting the tilt of the substrate in x and y directions while the stylus scans the substrate surface. The tilt adjustment is continued until a reasonable leveling or flatness is obtained.
- The second step involves fine scanning of the substrate surface to obtain detailed topographical information. The topography arises due to the presence of hills and valleys (waviness of $\sim 2 \mu$ m) native to the substrate surface, which alters its relative position with respect to the optical focal point, and eventually affects the machined nanohole size. Thus, it is important that all the points in the

patterning area of interest maintain the same relative position in order to produce identical nanoholes. The topography data obtained by fine scanning are then fed to the nanostage during laser machining, which helps maintain the desired relative position for the entire processing area by adjusting the stage movement. A photograph of the stylus and a fine scan of the surface topography are shown in Figure 3.3(a).

Early work by White et al. was done with a 60× microscope objective lens with a numerical aperture of $NA = 0.85$ and an adjustable correction collar [94]. The collar was set to compensate for focusing through a 0.17 mm glass coverslip, but the laser was actually focused on the top side of the substrate, so that the focus exhibited a significant amount of spherical aberration. In the present work, three different microscope objective lenses were used for machining in air: (1) 60× ($NA = 0.85$; Nikon CF Plan Achromat 79173), (2) 160× ($NA = 1.25$ in water; Leitz Wetzlar Model: $\infty/0$ PL APO), and (3) aspheric singlet lens ($NA = 0.68$; Thorlabs C330TME-B). Note that the 60× objective lens was used to focus and machine at the top surface, as in Ref. [94], but in a slightly differently configuration from that used in [94] in that the laser beam entering the objective was collimated, whereas the 160× objective lens was used dry with an input laser beam that was collimated. The 60× and 160× microscope objective lenses were used to make fused silica templates for polymer imprinting purposes, whereas the aspheric lens was used to study the role of spherical aberration on nanohole formation. A photograph of these three lenses is shown in Figure 3.3(b).

Patterns are made on the substrate by moving the xy-stage pre-programmed in a LabVIEW™ program. A graphical user interface of the LabVIEW™ program is shown in Figure 3.3(c). The machining platform and the LabVIEW™ program for the stage control were not developed as part of this dissertation research but were already available. Machining experiments are done by varying two parameters: a) the relative position of the optical focal point with respect to the substrate surface (Z); and, b) the pulse energy (E_p) of the laser beam. Optical (visible eye) focus is defined as the focus through the eyepiece on the surface of the fused silica substrate in air with visible light, and it is different from the focus of the 780 nm wavelength laser. The distance Z is adjusted by moving the z-stage up or down. The pulse energy (E_p) is determined by the formula $E_p = P/250 \times 10^3$, where P is the laser power at 250 kHz measured using a power meter, and is varied by adjusting the variable neutral density filter placed in the path of the laser beam, see Figure 3.1.1(c).

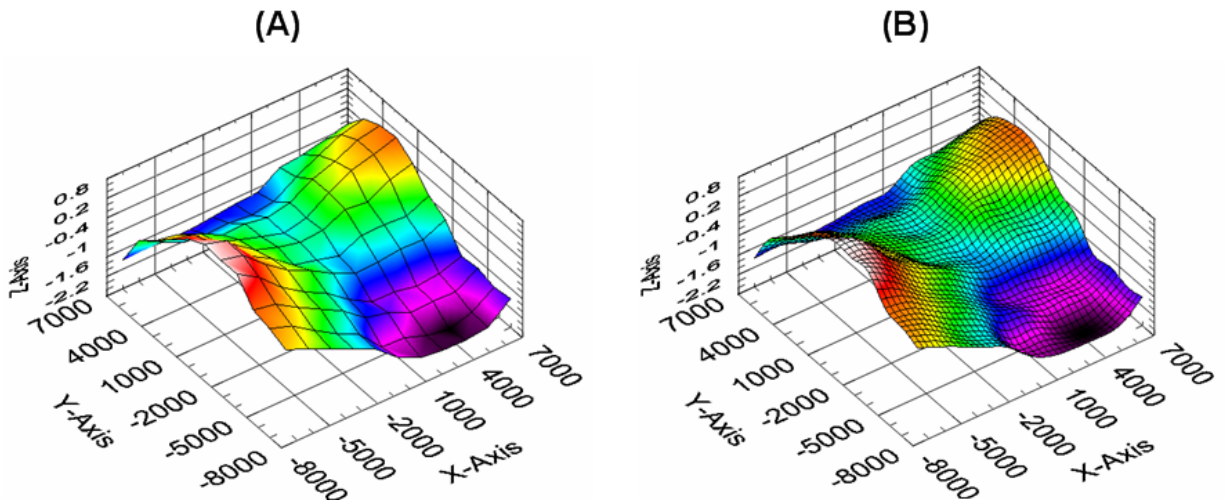


Figure 3.3(a). (Top) A photograph of the stylus based surface profiler used for optical leveling of the substrate. (Bottom) Surface topography of the substrate mapped by the stylus shown on top: fine scan (A) and interpolation of the fine scan (B). The scale of the z-axis in both A and B is from -2.2 to +0.8 microns.



Figure 3.3(b). A photograph of the lenses used for laser machining: left: 60 $\times$; middle: 160 $\times$; right: 60 $\times$ aspheric lens.

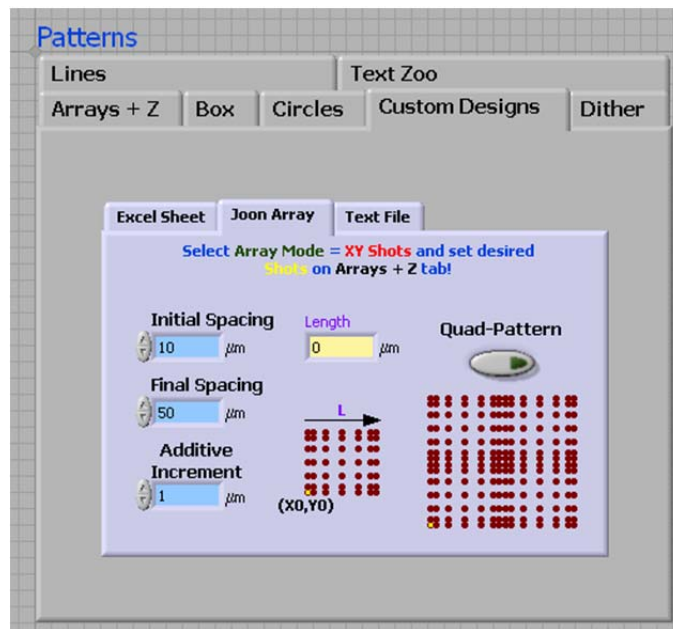
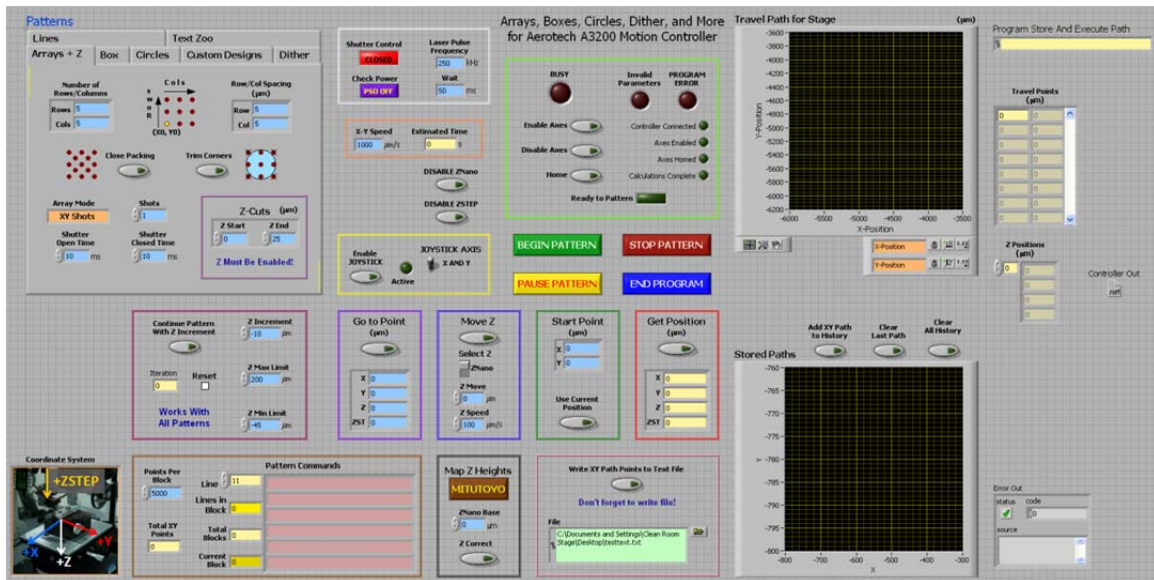


Figure 3.3(c). A screenshot of the graphical user interface (GUI) of the LabVIEW™ program used for the machining experiments (top) and an enlarged view of the patterns section of the GUI showing different types of patterns that can be machined (bottom).

3.4) Polymer structures by imprinting

Various different types of polymers are used to imprint polymer structures from single-pulse femtosecond laser machined fused silica templates by solution based replication called *solution casting*. The only exception is cellulose acetate (CA) structures, which are usually made by CA films. CA film replication involves wetting the surface of the template with solvent (acetone) followed by placing a piece of 35 μm thick CA film on the pattern. The solvent softens (or may even dissolve) the CA film, and fills the nanoholes by the action of capillary force. The softened polymer within the nanoholes molds against the sidewall of the nanoholes resulting in the formation of a replica of the pattern. Once the solvent dissipates, the imprinted polymer film is gently peeled off from the template surface to get the replica, which consists of polymer structures. Schematic of the CA film imprinting is shown in figure 3.4(a).

For solution casting, a polymer solution prepared by mixing the polymer in a suitable solvent (e.g. polycaprolactone in chloroform) is used. The pattern on the template is covered with a few drops of the polymer solution and then allowed to dry in normal atmospheric conditions or placed in a vacuum desiccator until the solvent has dissipated and a film has formed on the surface. The resulting film is the imprint or the replica of the pattern, which is carefully peeled off from the template similar to CA film imprinting. Sometimes, surface treatment of the templates becomes necessary for the removal (also called *lift off*) of the polymer imprint, which is described in section 3.4.1. Figure 3.4(b) shows a schematic of the solution casting process. Some of the polymer solutions used to make polymer structures by imprinting are shown in Table 3.4.

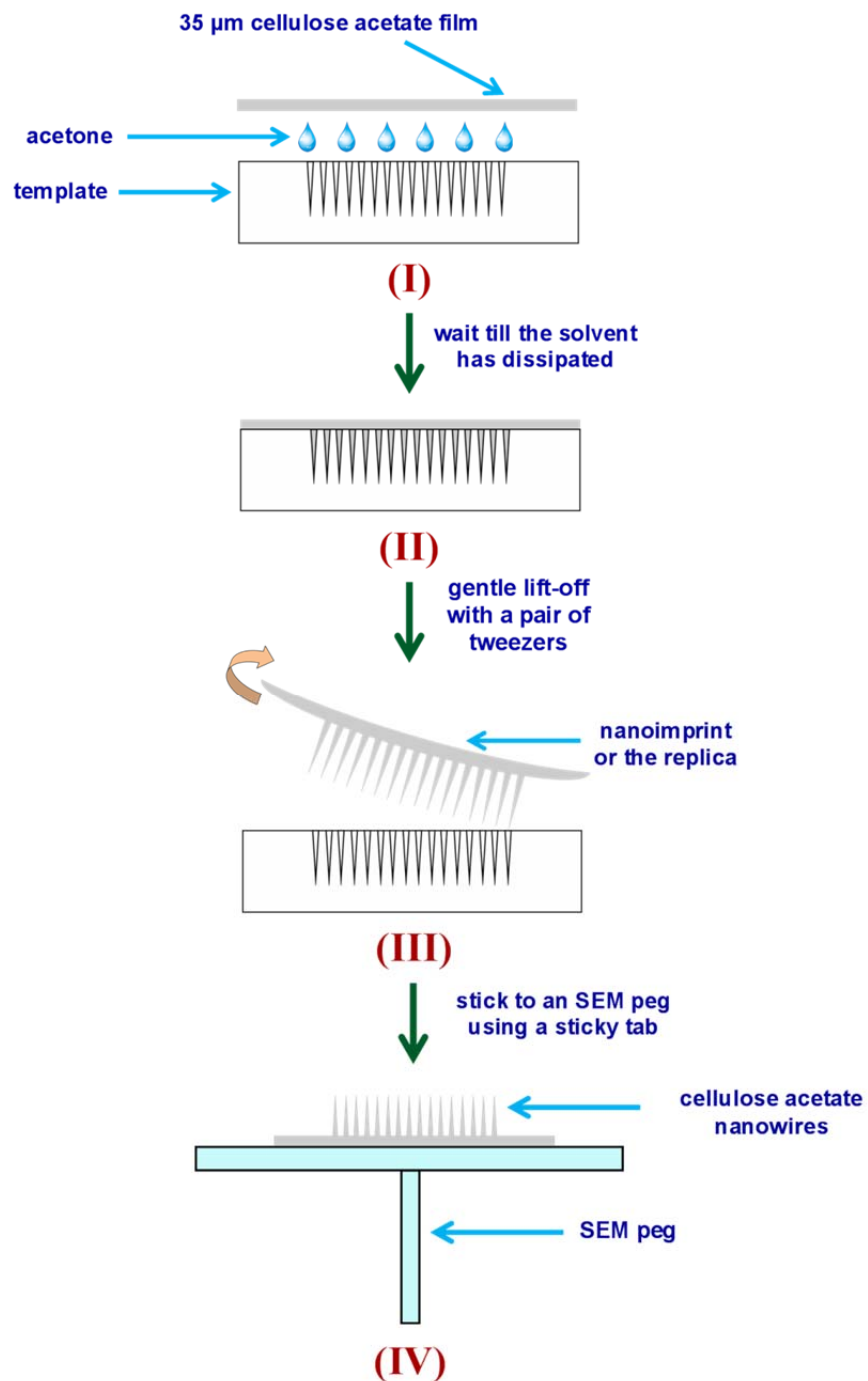


Figure 3.4(a). Cellulose acetate nanowires prepared by nanoimprinting of a single-pulse femtosecond laser nanomachined fused silica template using a 35 µm cellulose acetate film. The nanoimprint is the negative replica of the nanoholes in the pattern.

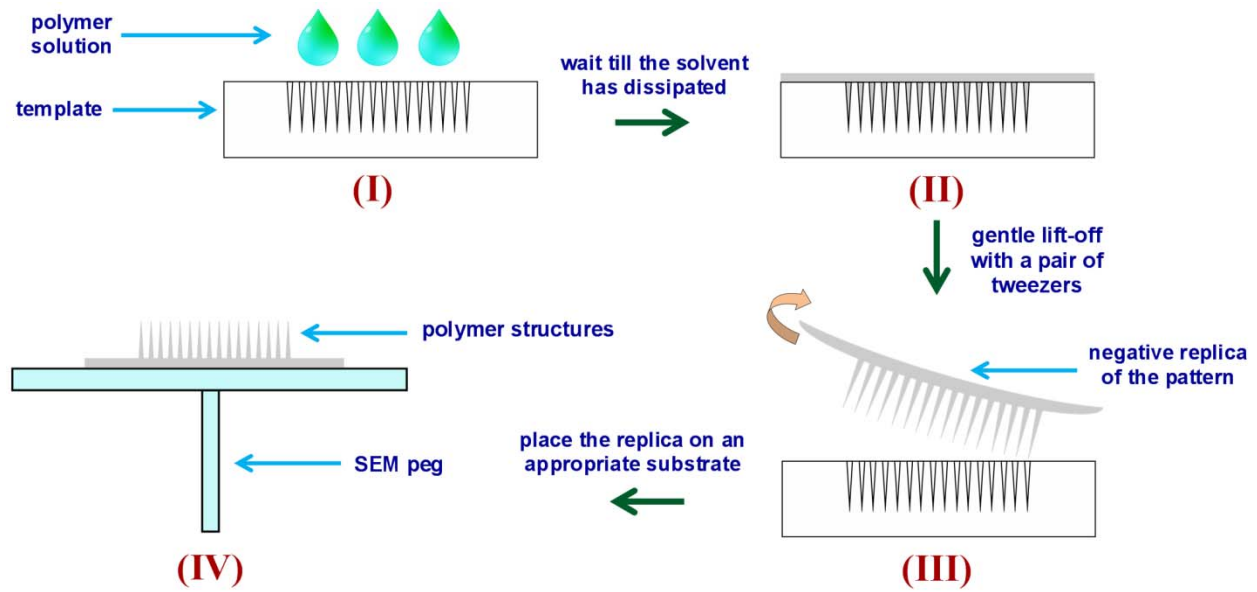


Figure 3.4(b). Schematic of the nanoimprinting procedure to make polymer nanowires by solution casting. The procedure of solution casting is similar to nanoimprinting with cellulose acetate film, see figure 3.4(a), with the only exception that the polymer in this case is in solution.

Table 3.4. Polymers used to make polymer structures by solution casting, their respective solvents, concentrations, and sources.

Polymer	Solvent	Wt. %	Origin
Cellulose acetate (CA)	Acetone	2 - 10	Sigma Aldrich
Polycaprolactone (PCL)	Chloroform	2	Sigma Aldrich
PCL-Polyethylene Glycol (PEG)	Chloroform	2	BME, Vanderbilt
Polyethylene (PE)	Toluene	10	SPI Supplies
Polydimethylsiloxane (PDMS)	Heptane	25	Dow Corning
Polymethylmethacrylate (PMMA)	Toluene	5-10	Sigma Aldrich
Polyvinyl alcohol (PVA)	De-ionized water	5	Alfa Aesar
Collodion	Amyl acetate	2	EMS, PA

Polymer structures from all the polymers mentioned in Table 3.4 except polyethylene (PE) are made by the solution casting process shown in Figure 3.4(b). Polyethylene solution after solvent evaporation does not form a film, but a loosely connected network of polyethylene powder, which is white in color. The template with the white powder network is then heated to about 155°C and thermally soaked at this temperature for 2 minutes to melt and coalesce to form a transparent and continuous polyethylene film. The white powder network starts melting around 110°C and becomes almost clear at a slightly higher temperature. The template is then cooled slowly after 2 minutes of thermal soaking at 155°C by turning the hot plate off, which takes around 2 hours cooling time to room temperature before the film is peeled off from the template.

3.4.1) Lift off of polymer replica

A monolayer of antistick coating based on fluorocarbons is prepared on the surface of the template prior to imprinting for those polymers that have a tendency to adhere to the template and affect lift-off of the imprint e.g., PMMA, PDMS, and PVA. In the present study, antistick coatings are prepared from 1H,1H,2H,2H-perfluorodecyltrichlorosilane. The template with the hole-side facing upwards is placed in a desiccator right next to a coverslip glass, and then a drop of fluorosilane is placed on top of the coverslip glass. As the vacuum is pulled in the desiccator, the fluorosilane starts to evaporate and coats the template with a layer of fluorosilane. A coating similar to Teflon in chemical structure, $(-\text{CF}_2-\text{CF}_2-)_n$, is formed. A photograph of the antistick coating process is shown in Figure 3.4(c).

3.4.2) Size control of polymer structures

The dimensions of the imprinted structures depend on the dimensions of the nanoholes (lateral and vertical) whereas the dimensions of the nanoholes depend on the machining parameters used during template fabrication. The depth and the cross-section of the nanohole can be reasonably decreased by reducing the pulse energy. However, in order to increase the dimensions of the nanoholes, the glass template needs to be chemically etched. Hydrofluoric acid (HF) is known to etch fused silica isotropically and concentrated aqueous solution of potassium hydroxide (KOH) is known to preferentially attack femtosecond laser affected region in fused silica [157]. A combination of isotropic etching in HF and anisotropic etching in KOH can thus be used to enlarge the nanoholes. In the present case, the nanoholes were enlarged by sequential etching, first in HF (LodyneTM, BOE, 6:1) and then in 10M KOH at 80°C. The etching duration depends on the desired dimensions of the etched holes. Etched holes with up to 2.5 μm diameter can be easily produced. Both as-machined and chemically etched templates are used to imprint polymer structures in the present study. To distinguish between the two, polymer structures imprinted from as-machined templates are termed nanostructures (e.g., nanowires and nanocones), whereas those imprinted from etched templates are termed microstructures (e.g., micropillars). The term nanoimprinting used frequently in this dissertation refers to imprinting of nanostructures.

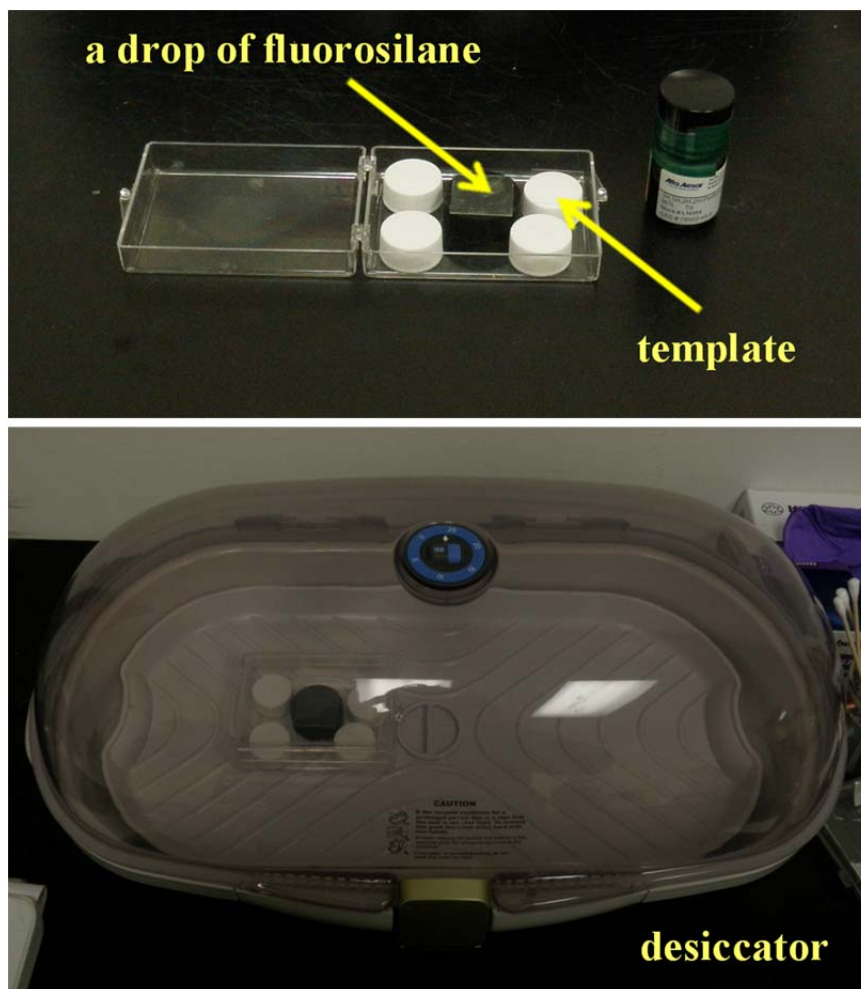


Figure 3.4(c). A photograph showing the antistick coating deposition procedure on a template. First, a drop of fluorosilane is placed on a glass piece and kept close to the template in a small box (top picture), and then the box is closed and placed in a desiccator (bottom picture). The antistick coating begins to form on the template after pulling the vacuum inside the desiccator, and the template is taken out after 1 hour of operation.

3.5) Functionalization of polymer structures

Imprinted polymer structures such as nanowires are functionalized by coating with different materials, mainly noble metals like gold, palladium, and platinum, and dielectric materials like silica, titania, and alumina.

3.5.1) Gold, platinum, and palladium Coating

Gold, platinum, and palladium are deposited by sputtering at cryogenic temperatures (normally when the probe temperature near the sample reaches $< -165^{\circ}\text{C}$) in a conventional sputtering machine modified with a cryo-stage connected to a liquid nitrogen container. A photograph of this modified sputtering machine for cryogenic deposition is shown in Figure 3.5.1. The thickness of the metal coating is a function of sputtering time and current applied for the given metal. Usually, 15-20 mA current is applied for these metals.

3.5.2) Silica deposition

Silica is deposited on CA nanowires in a low temperature chemical vapor deposition process at 65°C . Figure 3.5.2(a) shows a pictorial description of the silica deposition process which takes place in two separate glass beakers. First, a silicon tetrachloride solution (~ 5 mole%) is prepared by mixing 0.6 ml SiCl_4 and 9.4 ml CCl_4 in a 25 ml scintillation vial with a magnetic stir bar. The scintillation vial is immediately capped after putting SiCl_4 , CCl_4 , and the magnetic stir bar to avoid any escape of SiCl_4 by vaporization. Two glass beakers (Wide Mouth, Ball[®] Glass Preserving Jars, Wal-Mart Stores, Inc.), one with the scintillation vial (beaker 1) and the other with a small quantity of de-ionized water (beaker 2), are then placed on a digital stirring hot plate (VWR International, LLC) maintained at 65°C . The beakers are maintained at that temperature

for a minimum of 30 minutes before the start of the experiments, which ensures the formation of water vapor in beaker 2. A fresh silicon tetrachloride solution is prepared for each deposition experiment. (Caution: Silicon tetrachloride is highly corrosive and can cause chemical burns, hence extreme care must be exercised.)

A CA nanoimprint with erect nanowires prepared from CA film, as described in Figure 3.4(a), is first affixed to an SEM peg using a Lift-n-PressTM sticky tab with the nanowires facing upwards, and then the SEM peg is mounted on a brass holder. The cap of the scintillation vial in beaker 1 is opened and immediately the brass holder is placed on the neck of the beaker. The beaker is positioned such that the CA nanowires on the SEM peg face downwards just above the scintillation vial and are exposed to silicon tetrachloride vapor for adsorption. The exposure time in beaker 1 (t_i) is very critical and is recorded right after the brass holder is placed on the beaker. It is varied from 1 minute to 6 minutes to study the effect of SiCl_4 exposure time on silica coating thickness, whereas the exposure time in beaker 2 with deionized water is kept constant at 5 minutes for all the experiments. It was found that 5 minutes exposure to water vapor in beaker 2 is sufficient to hydrolyze all the silicon tetrachloride molecules adsorbed on the surface of cellulose acetate in beaker 1; hence, longer exposure times are not required. Figure 3.5.2(b) shows a photograph of the brass holder with an SEM peg mounted (top) and the hot plate with two glass beakers (down) used for the experiments. Silica coated cellulose acetate nanowires are termed silica nanowires or silica nanoneedles in this dissertation.



Figure 3.5.1. A photograph of the sputtering machine modified with a liquid nitrogen cold trap to coat gold, platinum, and palladium on polymer nanowires at cryogenic temperatures.

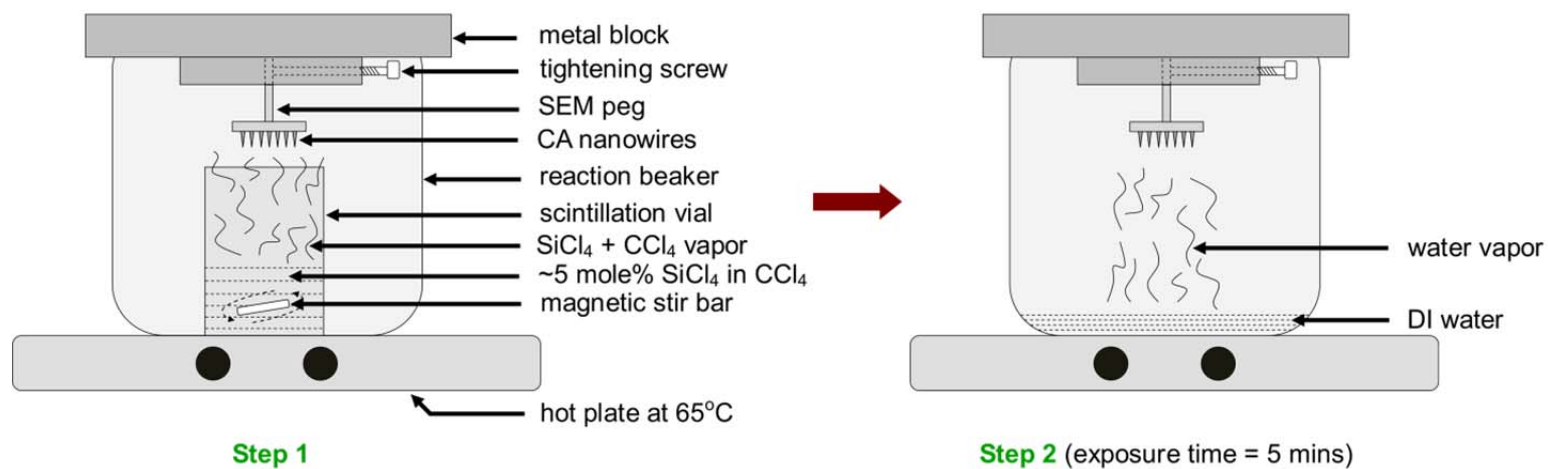


Figure 3.5.2(a). Schematic of the silica deposition process. Silica deposition on CA nanowires takes place in two steps: adsorption of silicon tetrachloride on the surface of CA nanowires in step 1; and, hydrolysis of the adsorbed silicon tetrachloride in step 2.



Figure 3.5.2(b). A brass holder used for mounting an SEM peg with CA nanoimprint (top) and two beakers sitting on a hot plate (bottom) used for silica deposition.

3.6) Characterization

Nanoholes, etched holes, imprinted polymer structures (nano and micro), and functionalized polymer structures are individually characterized.

3.6.1) Scanning electron microscope imaging

Holes produced at the surface of fused silica (as-machined nanoholes and chemically etched microholes), imprinted polymer structures (from as-machined and chemically etched fused silica templates), and functionalized polymer structures were imaged with various field emission scanning electron microscopes, most commonly FE-SEM (JSM 6320, JEOL) and FIB-SEM (Lyra 3, TESCAN). As the length of the imprinted polymer structure corresponds to the depth of the hole, the length of the polymer structure is measured in the SEM image to determine the hole-depth. Unless otherwise stated, all imprints are imaged at 45° stage tilt of the SEM. The length of the polymer structure measured in the SEM image is that of its projection at 45°, so the true length is determined by multiplying the projected length by $\sqrt{2}$. However, it is true only for those structures that are perfectly standing without bending or falling over, and only those structures that were perfectly standing were used for calculations.

Silica coating deposited on cellulose acetate nanowires described in section 3.5.2 was characterized to understand the physical behavior of the deposition process and the chemical composition of the coating. SEM images of silica nanoneedles produced at different values of exposure time (t_i) to silicon tetrachloride (beaker 1) were taken to determine the deposition rate of silica. The deposition rate was determined by measuring the width of the silica nanowires at a projected length of 6 μm . A total of 30

measurements on separate nanowires were made to obtain sufficient data for statistical analysis. The coating thickness (d_{t_i}) was determined using the formula:

$$d_{t_i} = \frac{D_{\text{SiO}_2}^{t_i} - D_{\text{CA}}}{2}$$

where, $D_{\text{SiO}_2}^{t_i}$ is the width of the silica nanowire for the corresponding soaking time t_i and D_{CA} is the mean width of the uncoated cellulose acetate nanowires measured in the same manner on the control sample. The standard deviation in the coating thickness ($\sigma_d^{t_i}$) for each t_i was expressed in terms of the standard deviation in the width of the silica-coated nanowire ($\sigma_{\text{SiO}_2}^{t_i}$) and the standard error in the mean width of the uncoated cellulose acetate nanowire (σ_{CA}^m) using the equation:

$$\sigma_d^{t_i} = \frac{\sqrt{(\sigma_{\text{SiO}_2}^{t_i})^2 + (\sigma_{\text{CA}}^m)^2}}{2}$$

3.6.2) Chemical analysis

An energy dispersive spectrometer (EDS) spectrum of the tip of a silica nanowire was taken with a Thermo EDS System 7 on a JSM-7600F thermal FE-SEM. Although EDS information is not quantitative, it does reveal the elemental composition of the coating.

An absorption spectrum of the silica coating was recorded on an attenuated total reflectance – Fourier transform infrared spectrometer (ATR-FTIR model ALPHA-P by Bruker Optics) in the frequency range 4000 - 375 cm^{-1} . The FTIR discerns the presence of different chemical bonds present in the coating. A small piece of silica coating deposited on gold coated glass coverslip (5 minutes in beaker 1) was scratched to

obtain a sample for recording the absorption spectrum. Several samples were tested from different silica coating samples (all made at 5 minutes) to ensure reproducibility of the results.

3.6.3) Focused ion beam sectioning

A silica nanowire (made at $t_i = 5$ minutes) was subjected to focused ion beam sectioning to see the coating cross-section using a Hitachi NB5000 NanoDUE'T FIB-SEM with a gallium current of 20 μA . The cuts were made at different positions of the nanowire starting near the tip of the nanowire to up to 9 μm projected length from the substrate base. SEM images were continually captured while the cuts were made.

3.7) Silica nanoneedles as cell culture substrates

Silica coated cellulose acetate nanowires, or silica nanoneedles, were used as cell culture substrates to study cell behavior in 3D microenvironment created by silica nanoneedles. Cell culture experiments were performed using NIH 3T3 mouse embryo fibroblasts at the Department of Biomedical Engineering, Vanderbilt University. Fibroblasts from 3T3 cell lines were cultured on silica nanoneedles and silica flat substrate (as control for comparison), both of which were prepared by depositing silica on cellulose acetate under similar conditions.

3.7.1) Template fabrication

For cell culture experiments, the pattern made on the template was a 2D gradient in nanohole spacing consisting of four identical quadrants, where each quadrant is made up of one gradient pattern of nanoholes. The pattern was made by focusing single

laser pulses ($E_p = \sim 5.2 \mu\text{J}$) through a $160\times$ ($NA = 1.25$ in water) microscope objective lens in air. In each gradient pattern, the spacing between the successive nanoholes was increased by $1 \mu\text{m}$ in both x and y directions starting from $10 \mu\text{m}$ at the densest location (edge) up to $50 \mu\text{m}$ at the sparsest location. The spacing was then reduced by $1 \mu\text{m}$ per hole from $50 \mu\text{m}$ to $10 \mu\text{m}$ to complete the 2D gradient quadrant. Figure 3.7.1 shows a demonstrative picture (not to scale) of the gradient pattern made on the template. The template thus prepared was subjected to chemical etching in buffered oxide etch (LodyneTM, BOE, 6:1) for 30 seconds followed by rinsing in DI water, and then neutralized in 10M KOH for 3 minutes followed by rinsing in DI water (all at room temperature) prior to further use. The depth of the nanoholes was determined to be at least $14 \mu\text{m}$ by cellulose acetate replication. The average entrance diameter was 750 nm post-etching. This template with 2D gradient nanoholes is termed *wide gradient template* in this dissertation.

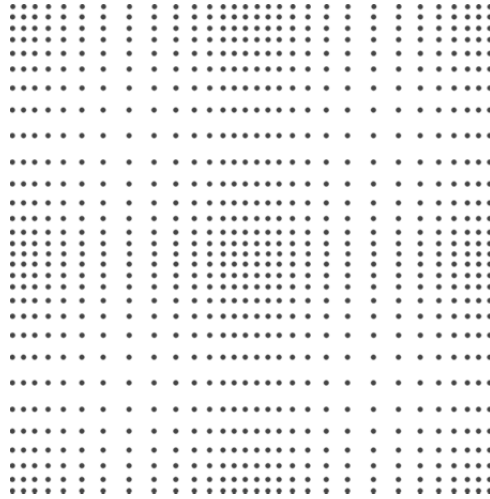


Figure 3.7.1. A representative picture of the 2D gradient pattern (reduced density for visual clarity and not to scale) fabricated on a 500 μm thick fused silica substrate by single-pulse femtosecond laser machining. Here, each black dot represents a nanohole made by focusing a single laser pulse through a 160 $\times$ microscope objective lens. The pattern is a 2x2 matrix of four quadrants. Each quadrant is formed by increasing the spacing between successive nanoholes by 1 μm starting from 10 μm in the densest location to 50 μm in the sparsest location and then decreasing from 50 μm to 10 μm at 1 μm decrements. A quadrant is a matrix of 84x84 nanoholes, and a 2x2 matrix of these quadrants forms the 2D gradient pattern with 168x168 nanoholes i.e., a total of 28,224 nanoholes in the pattern.

3.7.2) Creation of silica nanoneedles and flat silica substrates

A CA replica imprinted from the wide gradient template and a flat CA film (both 35 μm thick) were glued to two separate 170 μm glass coverslips using a thin layer of uncured PDMS (10:1 mass ratio, Sylgard® 184, Dow Corning Corporation) as an adhesive. Flat-CA-on-uncured-PDMS-on-glass-coverslip and CA-replica-on-uncured-PDMS-on-glass-coverslip were then left at room temperature for 24 hours for the uncured PDMS to crosslink, which makes their further handling easier during silica deposition. After PDMS curing, both the glass coverslips were placed on aluminum SEM pegs using sticky tabs (Lift-N-Press™, Structure Probe Inc., West Chester, PA) and subjected to silica deposition as described in section 3.5.2 and shown schematically in Figure 3.7.2. In both the cases, the exposure time in beaker 1 (i.e., to silicon tetrachloride solution) was kept 5 minutes followed by 5 minutes in beaker 2 for hydrolysis to form silica. It results in the formation of 240 ± 40 nm thick layer of silica. Both silica nanoneedles and flat silica substrates were used as cell culture substrates to study NIH 3T3 fibroblasts as described in section 3.7.3.

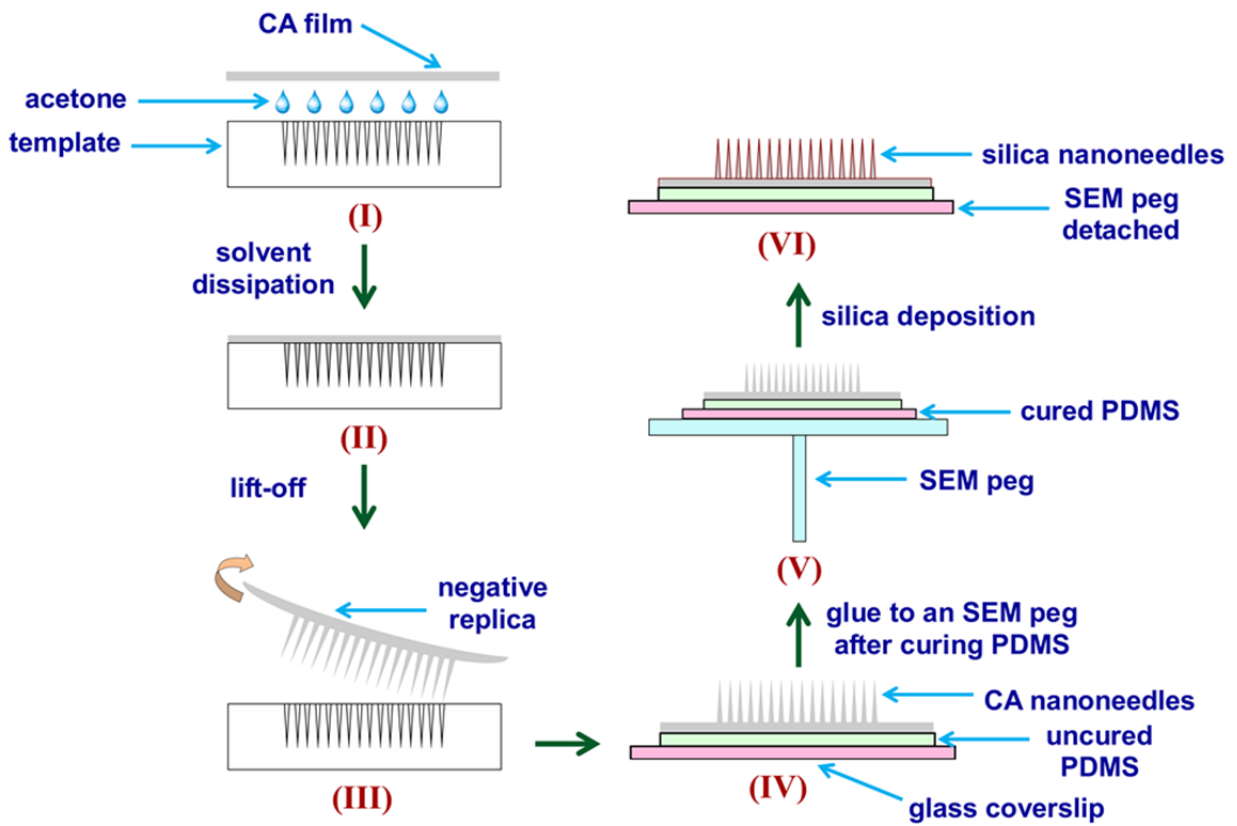


Figure 3.7.2. Schematic of silica nanoneedles preparation for cell studies using NIH 3T3 mouse embryo fibroblasts.

3.7.3) Cell culturing on silica

Cell culture experiments were performed at the Department of Biomedical Engineering, Vanderbilt University, by Sung Laboratory personnel. NIH 3T3 mouse embryo fibroblasts were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Gibco Cell Culture, Invitrogen, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco) with 1% penicillin-streptomycin (P/S, Gibco) on tissue culture polystyrene in a water jacketed incubator at 37°C and 5% CO₂. For experiments, cells were seeded at a density of 80,000 cells / cm² and cultured either overnight or for three days for attachment/viability or immunocytochemistry experiments, respectively, on both silica nanoneedles and flat silica control substrate. For attachment and viability experiments, cells were treated with 4 μM Calcein AM (Molecular Probes, Invitrogen) in phosphate buffered saline (PBS, Gibco) for 15 minutes at 37°C. For immunocytochemistry, cells were fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.25% Triton X in PBS for 10 minutes, and blocked with 10% goat serum in PBS. Unless otherwise stated, all steps were performed at room temperature. Cells were then incubated with phalloidin (Molecular Probes) for 20 minutes or 1:100 rabbit anti-mouse α smooth muscle actin primary antibody (αSMA, Abcam, Cambridge, MA) overnight at 4°C, followed by treatment of 1:50 FITC-conjugated goat anti-rabbit secondary antibody (Abcam) for 2 hours. Cells were imaged using a Nikon Eclipse Ti inverted fluorescence microscope (Nikon Instruments, Inc, Melville, NY). Images were analyzed with Image J (National Institutes of Health, Bethesda, MD) and $n \geq 50$ cells from $n \geq 5$ images were used for quantification.

Individual cells, not those in aggregates, were quantified for shape descriptors (i.e., average cell area and average cell perimeter).

Cell morphology was further investigated by SEM analysis after cell fixation at the University of Tennessee Space Institute. For SEM analysis, cells were fixed on silica nanoneedles and flat silica control with 2.5% glutaraldehyde in PBS for 2 hours at room temperature followed by rinsing in PBS (×2) and then ultrapure water (×3). Fixed cells were treated with 1% osmium tetroxide in PBS for 1 hour at room temperature followed by rinsing in ultrapure water (×3). Fixed cells were then subjected to dehydration, first in graded ethanol in water (30%, 50%, 70%, 95%, and 3x 100%) and then in graded ethanol in HMDS (30%, 50%, 70%, 95%, and 3x 100%) followed by supercritical drying (Polaron E3000) before SEM analysis.

Chapter 4

Results & Discussion

This chapter presents results and discussion on the preparation and applications of single-pulse amplified femtosecond laser patterned fused silica templates. In the present work, unless otherwise specified, all fused silica templates were patterned at the maximum pulse energy of 5.2 μJ using the laser system explained in section 3.1.

4.1) Nanohole size dependence on the relative position of optical focus

It is known that the size of the nanoholes patterned by femtosecond laser machining is affected by the position of the focal spot within the substrate as it alters the location of the optical breakdown. In the present study, the effect of the relative position of the optical focus with respect to the surface of fused silica substrate on the nanohole size was investigated. The patterning experiment was performed in air using a 160 $\times$ microscope objective lens ($NA = 1.25$) at the maximum pulse energy of 5.2 μJ after leveling the substrate as described in section 3.3. The sharpest image of the substrate seen through the microscope objective lens with the visible light was marked as the optical focus, and its position was recorded as $Z = 0$, where Z is the position of the vertical axis. It must be noticed that the focus of the 780 nm wavelength is different from the visible light focus, and is comparatively more difficult to determine. Also, the position of the optical focus recorded as $Z = 0$ may vary from user to user as it is determined with the naked eye. The relative position of the optical focus was varied by adjusting the vertical movement of the substrate mounted on a nanostage (z-stage) with respect to the optical focus at $Z = 0$. Nanoholes were patterned on a 500 μm thick fused silica substrate covering a range of vertical motions above and below the optical focus. The z-stage was moved at an increment of 1 μm from $Z = -6 \mu\text{m}$ to $Z = +2 \mu\text{m}$ passing through the optical focus at $Z = 0$. Here, positive values of Z mean the optical focus is above the

substrate surface by that amount and is obtained by moving the z-stage down. Schematic of the z-stage movement is shown in Figure 4.1(a). SEM images of nanoholes thus produced and their cellulose acetate replicas are shown in Figures 4.1(b) and (c), respectively.

It can be seen from the SEM images that the relative position of the optical focus affects the size of the nanohole. Also, it can be seen that the entrance of some nanoholes is not fully circular, which can be attributed to the spatial profile of the laser pulse used for machining. The spatial profile of the laser beam in the present case was elliptical with near-Gaussian intensity distribution. At $Z = -6 \mu\text{m}$, the position of the optical focus is so deep into the substrate that the nanoholes do not fully open and remain embedded in the substrate. This is possibly due to the inability of the plasma to escape from such a depth.

The nanohole depth was determined by measuring the length of the corresponding cellulose acetate imprint in the SEM image. Since the length of the imprint in the SEM image is that of its projection at 45° , the actual length was determined by multiplying it with $\sqrt{2}$ as shown in Figure 4.1(d). The dependence of nanohole size (diameter and depth) on the relative position of optical focus with respect to the substrate surface is shown as graphs in Figures 4.1(e) and (f).

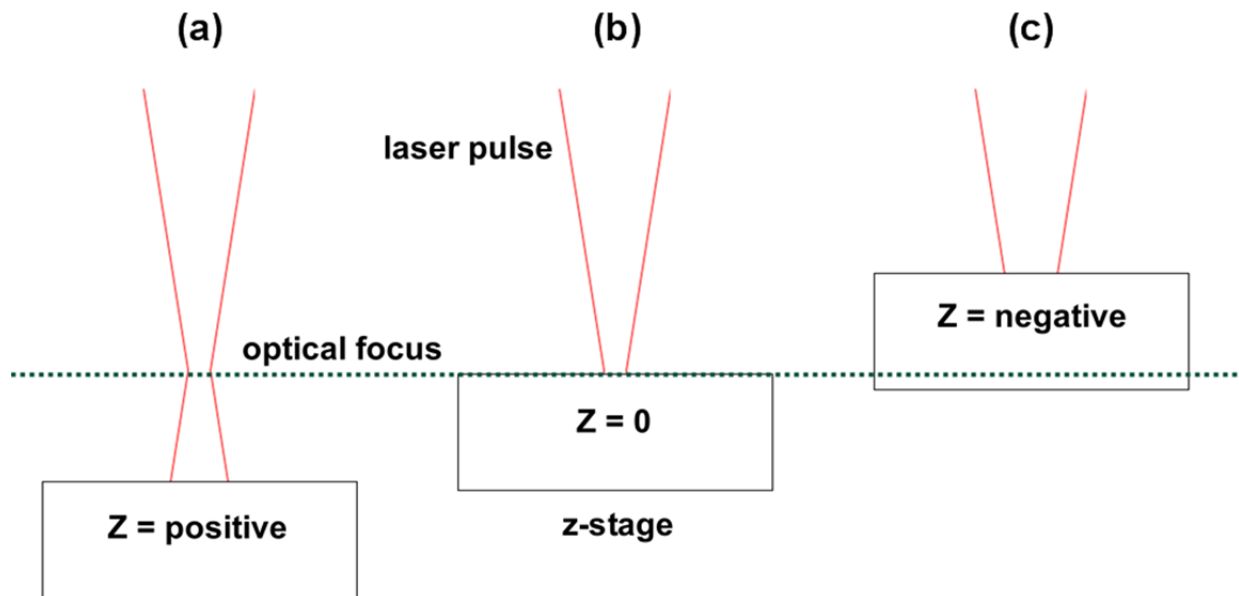


Figure 4.1(a). Schematic of the z-stage movement during femtosecond laser machining of fused silica. The laser pulse (red) is focused through a microscope objective lens onto the substrate (rectangular box) mounted on the z-stage. The stage is moved up and down to change the position of the optical focus with respect to the substrate surface. $Z = 0$ corresponds to optical focus on the substrate surface (b); $Z = \text{positive}$ corresponds to optical focus above the substrate obtained by moving the stage down (a), and $Z = \text{negative}$ corresponds to optical focus within the bulk of fused silica obtained by moving the stage up (c). The blue dotted line corresponds to the optical focus $Z = 0$.

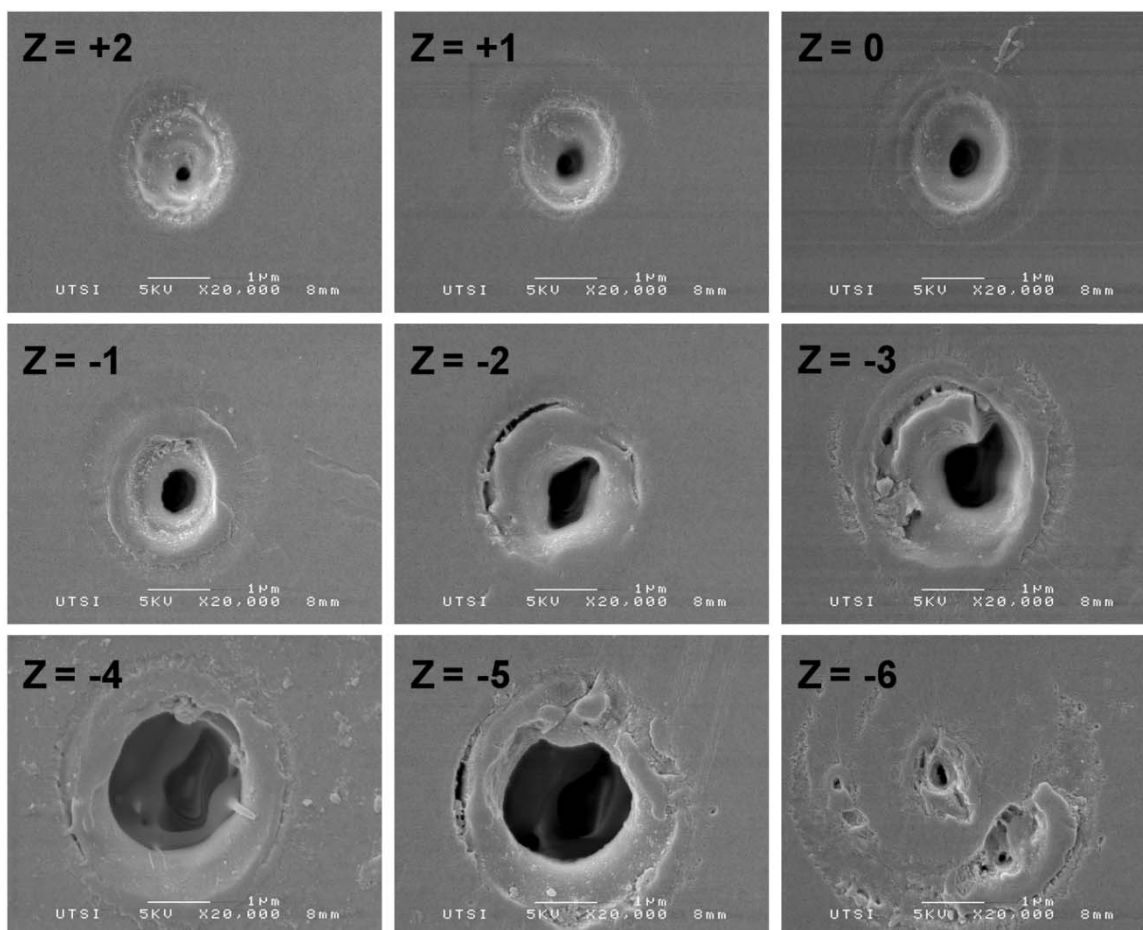


Figure 4.1(b). SEM images of nanoholes made at the surface of fused silica using a 160 $\times$ microscope objective lens in air at different values of Z and pulse energy $\sim 5.2 \mu\text{J}$. The template was coated with platinum for 45 seconds before SEM analysis. All scale bars correspond to 1 μm .

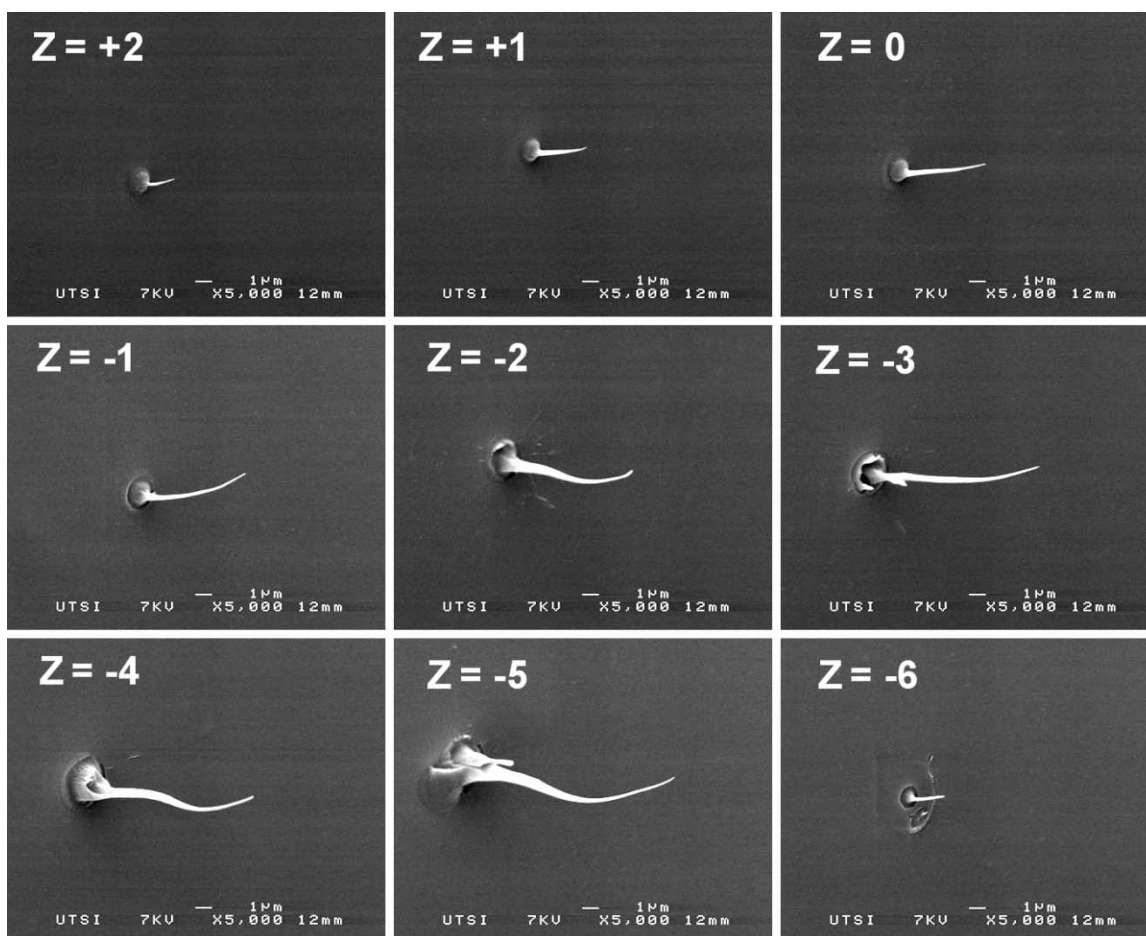


Figure 4.1(c). SEM image of the cellulose acetate replica imprinted from nanoholes (taken at 45° tilt) shown in figure 4.1(b). The replica was coated with gold for 45 seconds at -165°C before SEM analysis. All scale bars correspond to 1 µm.

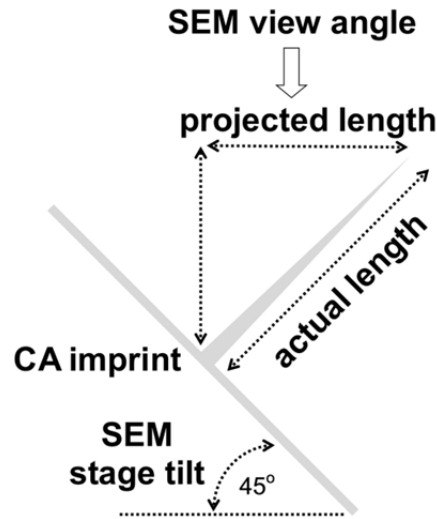


Figure 4.1(d). Schematic of SEM imaging of a cellulose acetate imprint of a nanohole showing its projected length and the actual length. Actual length = $\sqrt{2}$ Projected length.

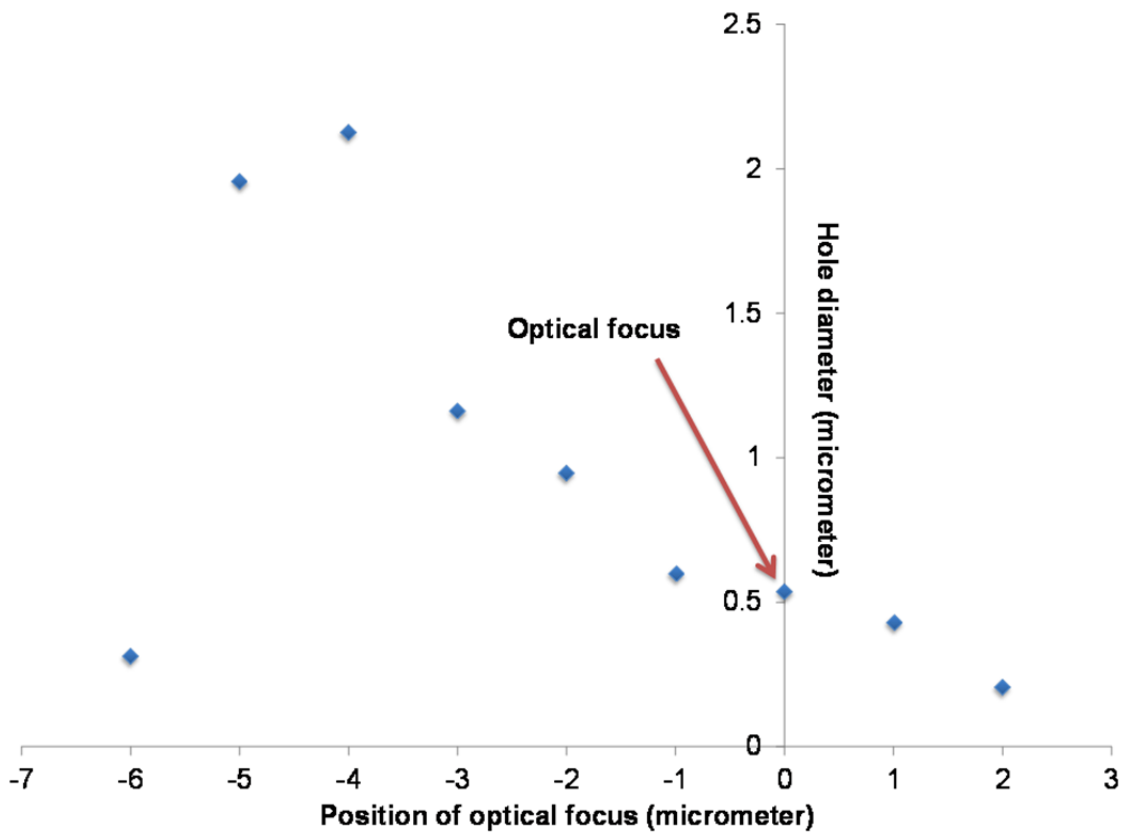


Figure 4.1(e). The dependence of hole diameter on the position of optical focus with respect to the substrate surface. The hole diameter was determined from the SEM images shown in Figure 4.1 (b).

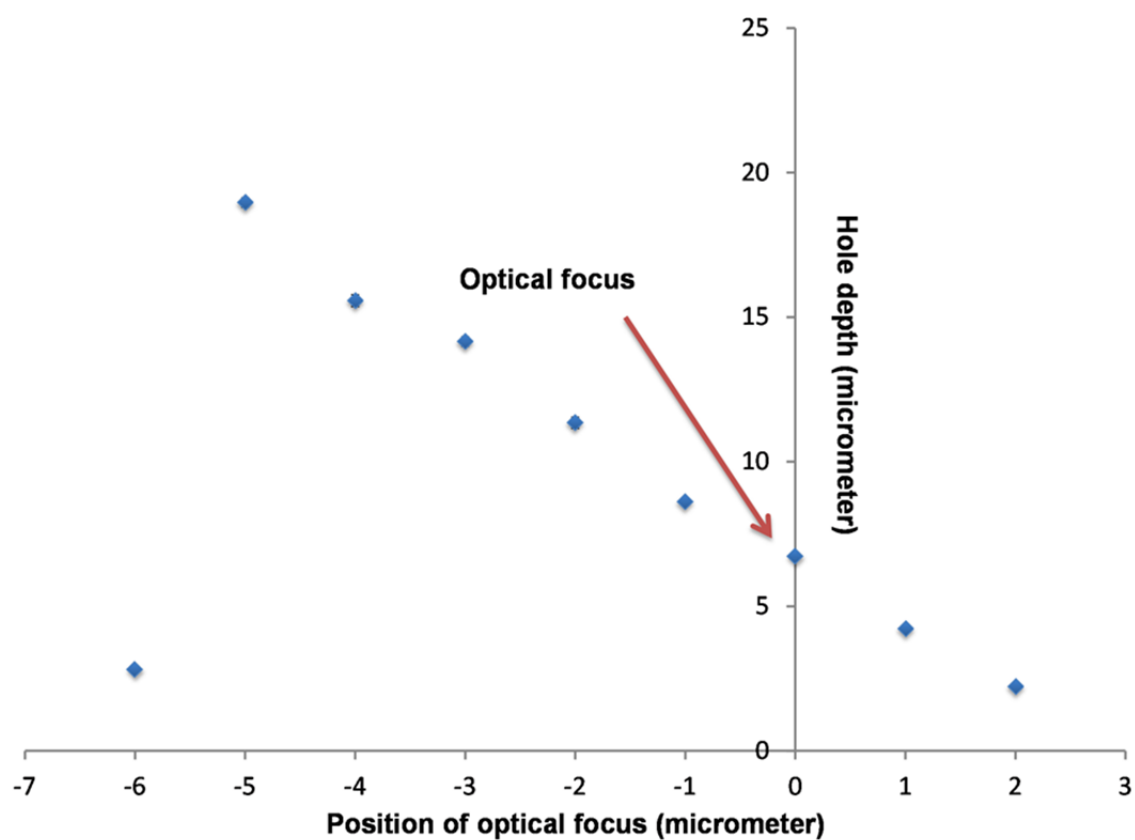


Figure 4.1(f). The dependence of hole depth on the position of optical focus with respect to the substrate surface. The hole depth was determined from the length of the cellulose acetate wires imprinted from the nanoholes shown in Figure 4.1(c).

4.2) Polymer structures imprinting by solution casting

Both as-machined and chemically etched fused silica templates, described in section 3.4, are used for solution casting to create polymer structures. Polymer structures imprinted from as-machined templates are termed *nano*, whereas those from etched templates are termed *micro* to differentiate between the two. Also, depending on the morphology, polymer structures imprinted from as-machined templates are referred to as *nanowires* or *nanoco*nes, whereas those from etched templates as *micropillars* in the course of discussion in this dissertation. The process of solution casting is so versatile that any polymer that forms continuous film, with or without the assistance of external energy like heat or UV light, can be used to imprint structures from these templates. Polymer solutions not only refer to polymers dissolved in solvent, but also polymers that can be obtained in liquid form e.g., resins. In the present study, select polymers with different physicochemical properties, but of current research interest, are subjected to solution casting to create nano- and micro- structures. Table 3.4 shows some of the polymers that have been successfully imprinted. A commonly used synthetic fiber – cellulose acetate (CA); a ubiquitously used linear chain hydrophobic polymer - *polyethylene* (PE); two biosorbable polymers - *polycaprolactone* (PCL) and PCL-polyethylene glycol (PEG); a water soluble polymer - *polyvinyl alcohol* (PVA); a widely used multi-purpose hard plastic - *polymethylmethacrylate* (PMMA); a 3D cross-linking polymer - *polydimethylsiloxane* (PDMS); a commercially available nitrocellulose – Collodion; a commonly used structural plastic – polyvinyl chloride (PVC); and a proton conducting ionomer used in fuel cells - *Nafion 117®*. All these polymers structures were imprinted from the same as-machined and etched templates.

4.2.1) Polycaprolactone (PCL) and PCL-polyethylene glycol (PEG) structures

Polycaprolactone (PCL) is a low melting point biodegradable polymer. Chemically, it is a polyester of molecular formula $(C_6H_{10}O_2)_n$ and is synthesized by ring opening polymerization of ϵ -caprolactone in the presence of stannous octoate [158]. PCL finds applications in biomedical engineering such as long term implants and (targeted) drug delivery vehicles because of its slow degradation in physiological conditions by the hydrolysis of its ester linkages [159, 160]. PCL is hydrophobic in nature and is often copolymerized with hydrophilic polymers (e.g., polyethylene glycol and hyperbranched polyglycidol) to synthesize amphiphilic block copolymers [161-163]. In the present study, nanowires and micropillars of PCL and PCL-8 wt%PEG are made by solution casting of their respective solutions made in chloroform. Figures 4.2.1(a) and 4.2.1(b) show the SEM images of PCL nanowires and micropillars, respectively. PCL-PEG nanowires and micropillars are shown in Figures 4.2.1(c) and 4.2.1(d), respectively.

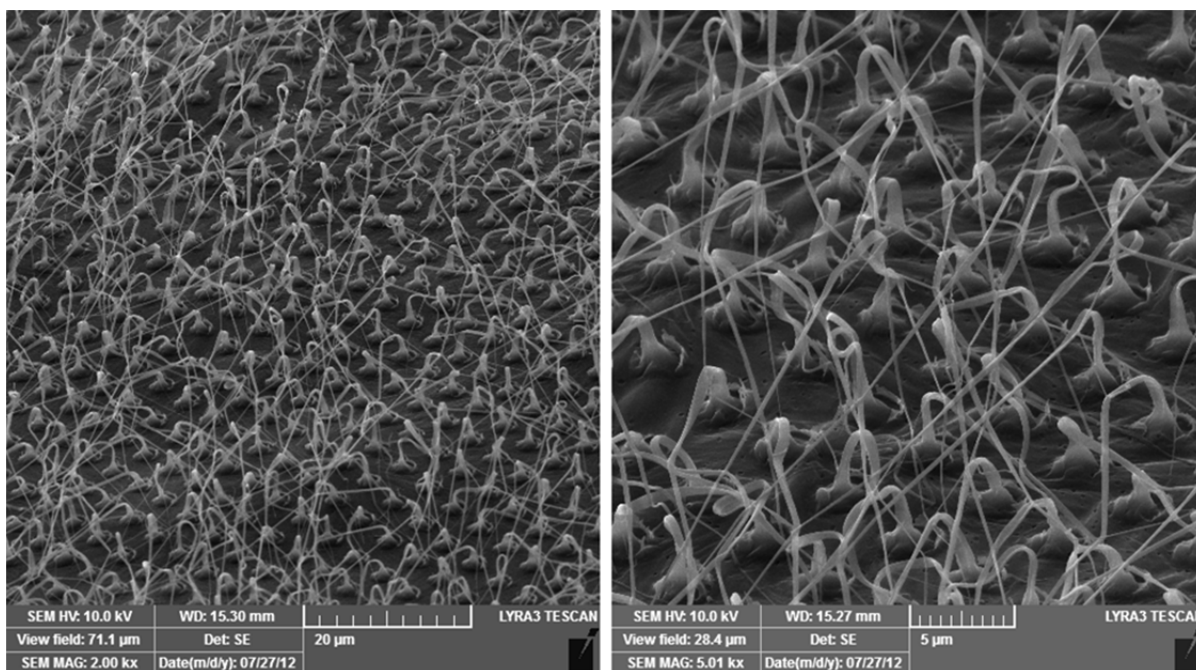


Figure 4.2.1(a). PCL nanowires imprinted from an as-machined fused silica template using 2 wt% PCL solution in chloroform.

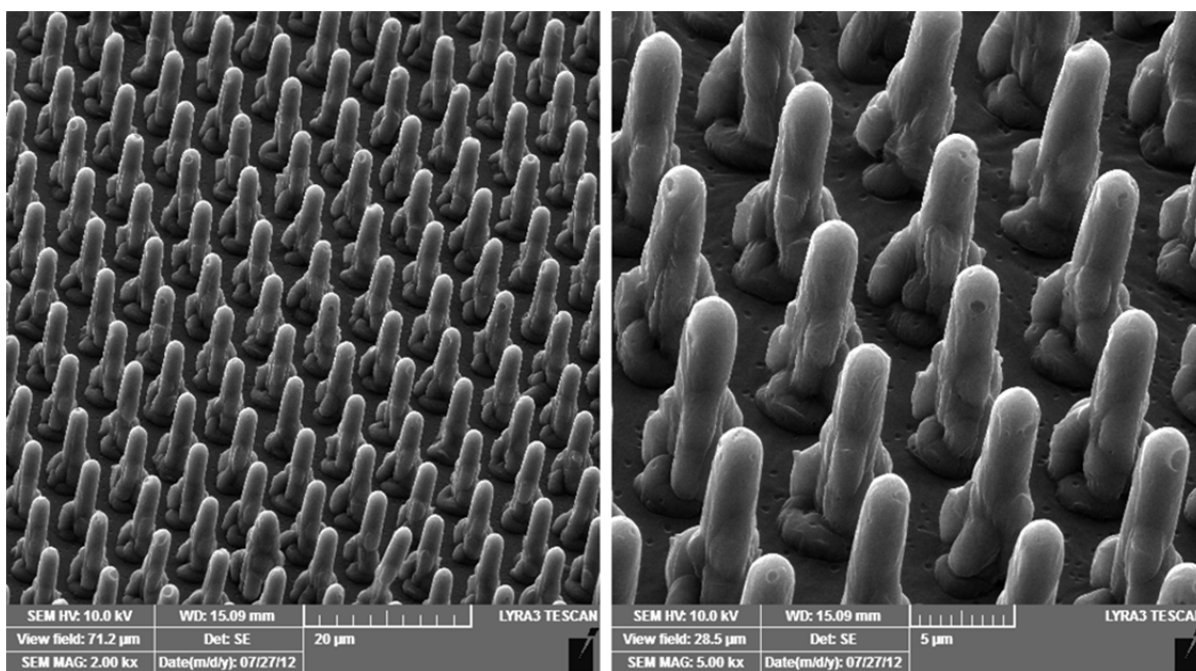


Figure 4.2.1(b). Polycaprolactone micropillars imprinted from an etched fused silica template using 2 wt% polycaprolactone solution in chloroform. The right image is taken at high magnification in both the figures for clarity.

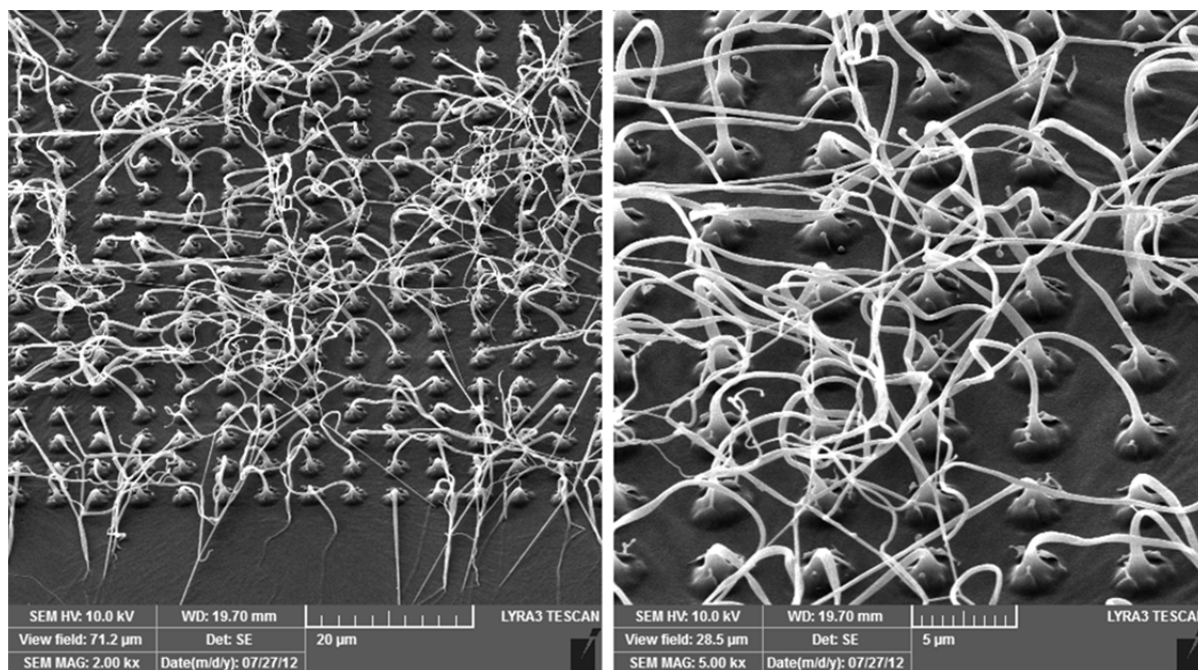


Figure 4.2.1(c). PCL-PEG nanowires imprinted from an as-machined fused silica template using 2 wt% polycaprolactone solution in chloroform.

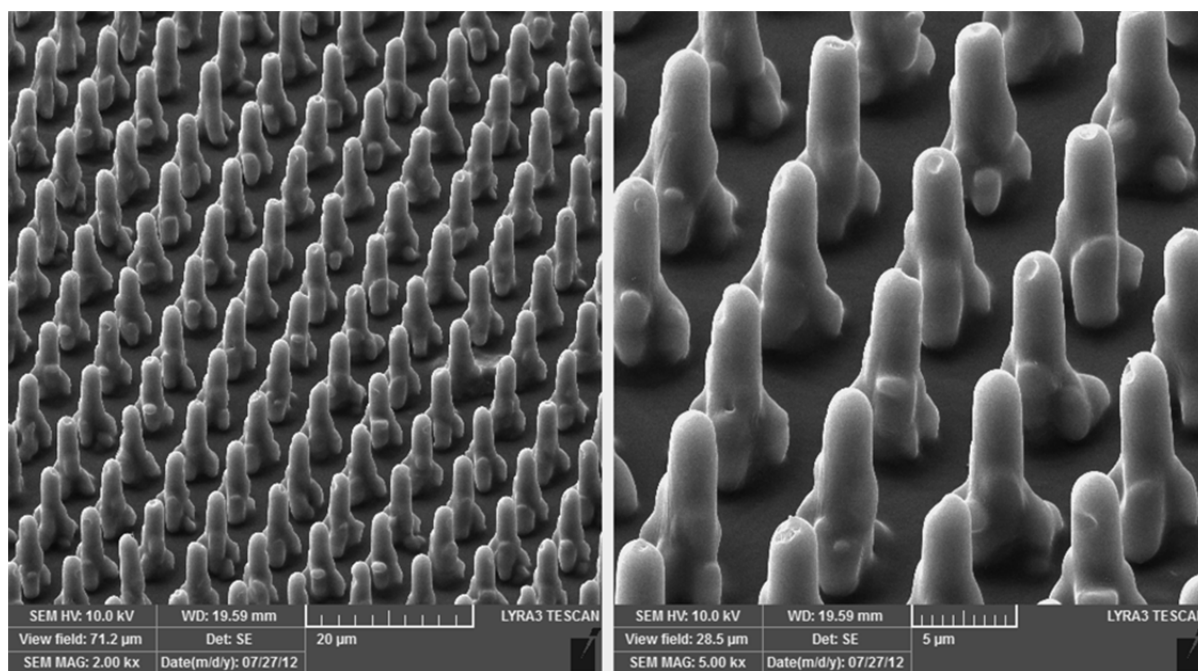


Figure 4.2.1(d). PCL-PEG micropillars imprinted from an etched fused silica template using 2 wt% PCL-PEG solution in chloroform. The right image is taken at high magnification in both the figures for clarity.

4.2.2) Cellulose acetate structures

Chemically, cellulose acetate is the acetate ester of cellulose, a naturally occurring polymer. It is mostly used in the form of films and fibers. Cellulose acetate films were first introduced in photography as a replacement for the highly flammable nitrocellulose films as base materials for photographic emulsions. Later, cellulose acetate films were shown to be very useful as a replication material in microscopy for metallographic studies of metallic and biological samples. Indeed, White et al. used microscopy grade cellulose acetate films to determine the depth of femtosecond laser machined high aspect ratio nanoholes by replication, and showed that cellulose acetate films can replicate these deep features without any observable stretching [94]. Cellulose acetate is also used as a semi-permeable membrane in separation processes and films/filters in biomedical applications [164-168]. One of the most common methods for the preparation of cellulose acetate fibers is *electrospinning*, and the cellulose acetate fibers thus produced in the form of mats have been examined as antimicrobial surfaces and drug delivery agents [166, 168]. The size of electrospun cellulose acetate fiber is usually controlled by making adjustments to the solvent system, and fibers with diameters in the range 100 nm to 5 μm have been successfully fabricated. In the present study, ACS grade acetone is used as the solvent to prepare cellulose acetate solution for solution casting. It was found that 5 wt% cellulose acetate (average molecular weight $\sim 30,000$) solution forms films that can be easily peeled off from the template. Dilute solutions of cellulose acetate (e.g., 2 wt%) can also be used to form peelable films by casting multiple layers. Figures 4.2.2(a) and 4.2.2(b) show cellulose

acetate structures imprinted from as-machined templates (CA nanowires) and etched templates (CA micropillars), respectively.

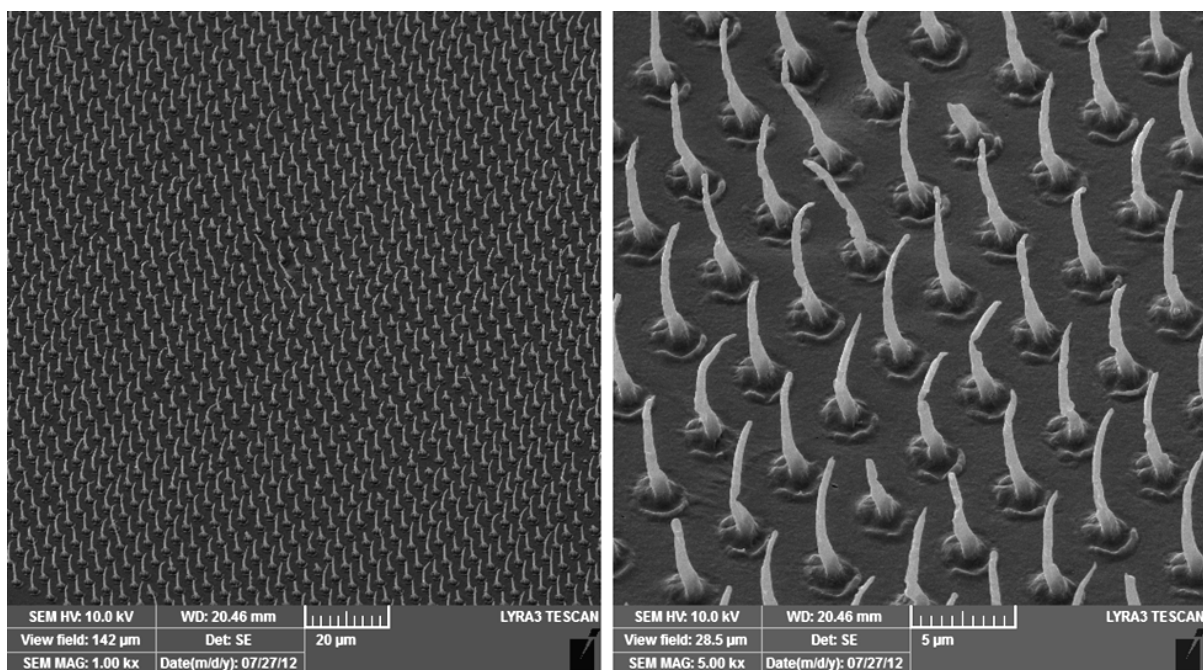


Figure 4.2.2(a). Cellulose acetate nanowires (nanocones) imprinted from an as-machined fused silica template using 5 wt% cellulose acetate solution in acetone.

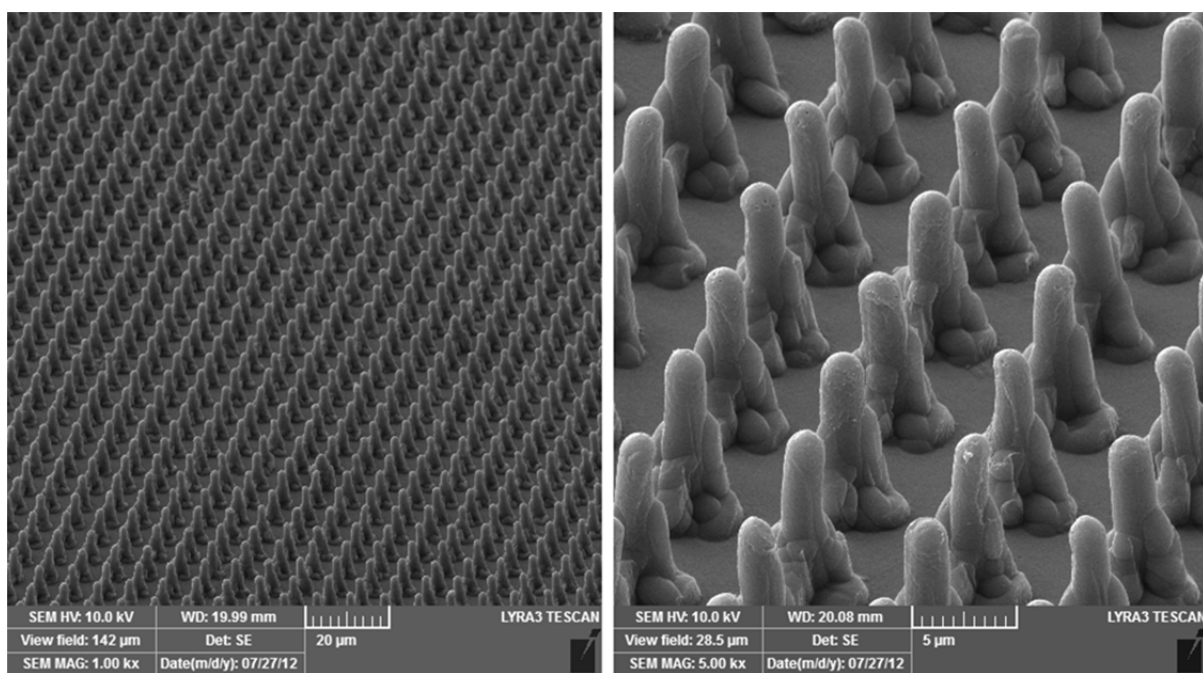


Figure 4.2.2(b). Cellulose acetate micropillars imprinted from an etched fused silica template using 5 wt% cellulose acetate solution in acetone. The right image is taken at high magnification in both the figures for clarity.

4.2.3) Polyethylene structures

Polyethylene is one of the most widely used plastic materials in the world with majority of its applications in packaging industry. It is produced by polymerization of ethylene and its chemical formula is usually $(C_2H_4)_n$. It is further classified into several categories depending on the density and degree of branching. The two most important classes of polyethylene used predominantly are high density polyethylene (HDPE) and low density polyethylene (LDPE). HDPE ($\rho \geq 0.941 \text{ g/cm}^3$) has strong intermolecular forces and high tensile strength due to a low degree of branching, whereas LDPE ($\rho = 0.91 - 0.94 \text{ g/cm}^3$) has weak intermolecular forces and low tensile strength due to a high degree of short and long chain branching. HDPE is generally used to make structures and components for storage applications, whereas LDPE is commonly used to make films and rigid containers. Besides HDPE and LDPE, ultra high molecular weight polyethylene (UHMWPE) is also used in a variety of applications because of its high toughness in addition to excellent chemical resistance and low wear rate. UHMWPE also finds applications in biomedical engineering to make parts for hip and knee implants [169]. Although it is always coated with hydroxyapatite or calcium phosphate to improve its cytocompatibility, it was recently shown that nanostructured polyethylene shows increased osteoblast and endothelial cell adhesion and can be explored for its applications in orthopedic and vascular applications [170]. In the present study, nanowires and micropillars of polyethylene are imprinted from the templates by solution casting in which an additional heating step is required. The process is described in section 3.4. SEM images of polyethylene nanowires and micropillars are shown in Figures 4.2.3(a) and 4.2.3(b), respectively.

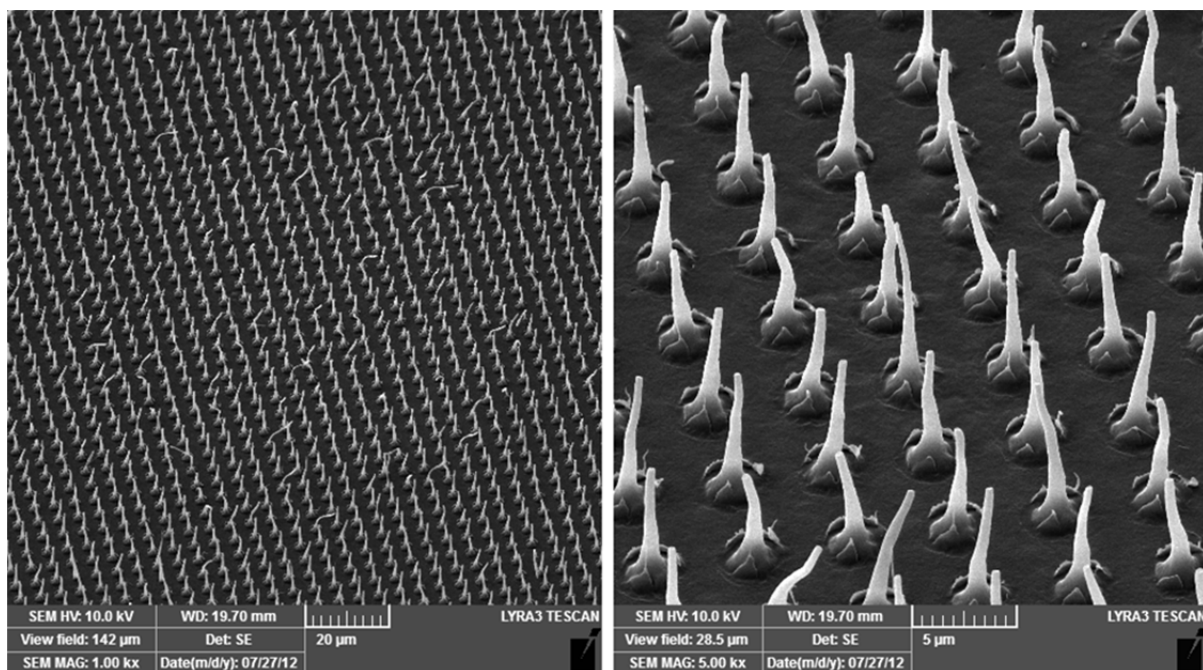


Figure 4.2.3(a). Polyethylene nanowires (nanocones) imprinted from an as-machined fused silica template using 10 wt% polyethylene solution in toluene.

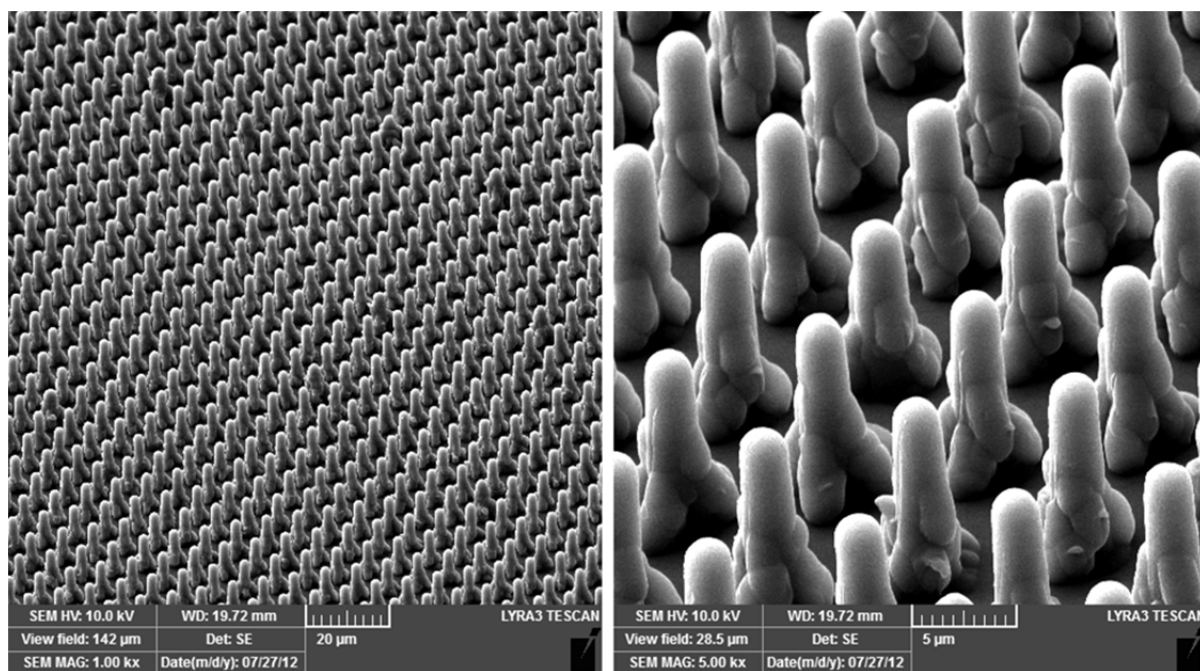


Figure 4.2.3(b). Polyethylene micropillars imprinted from an etched fused silica template using 10 wt% polyethylene solution in toluene. The right image is taken at high magnification in both the figures for clarity.

4.2.4) Polyvinyl alcohol (PVA) structures

PVA is a non-toxic and water-soluble synthetic polymer with molecular formula $(C_2H_4O)_n$. The structural monomer of PVA is vinyl alcohol, which is unstable. It only exists in its tautomer form acetaldehyde, which is a more stable compound. Thus, PVA is not prepared by polymerization of its monomer like other polymers. Instead, it is prepared from polyvinyl acetate (PVAc) by hydrolysis where the ester linkages in PVAc are substituted with hydroxyl groups. Some common uses of PVA are: preparation of contact lens and polarizer, water transfer printing, and as a packaging material in food industry. PVA is also a potential biomaterial and is currently being explored [171-173]. In this study, PVA (mol. wt. $\sim 30,000$) structures are made from its solution prepared in DI water, and are shown in Figures 4.2.4(a) and 4.2.4(b).

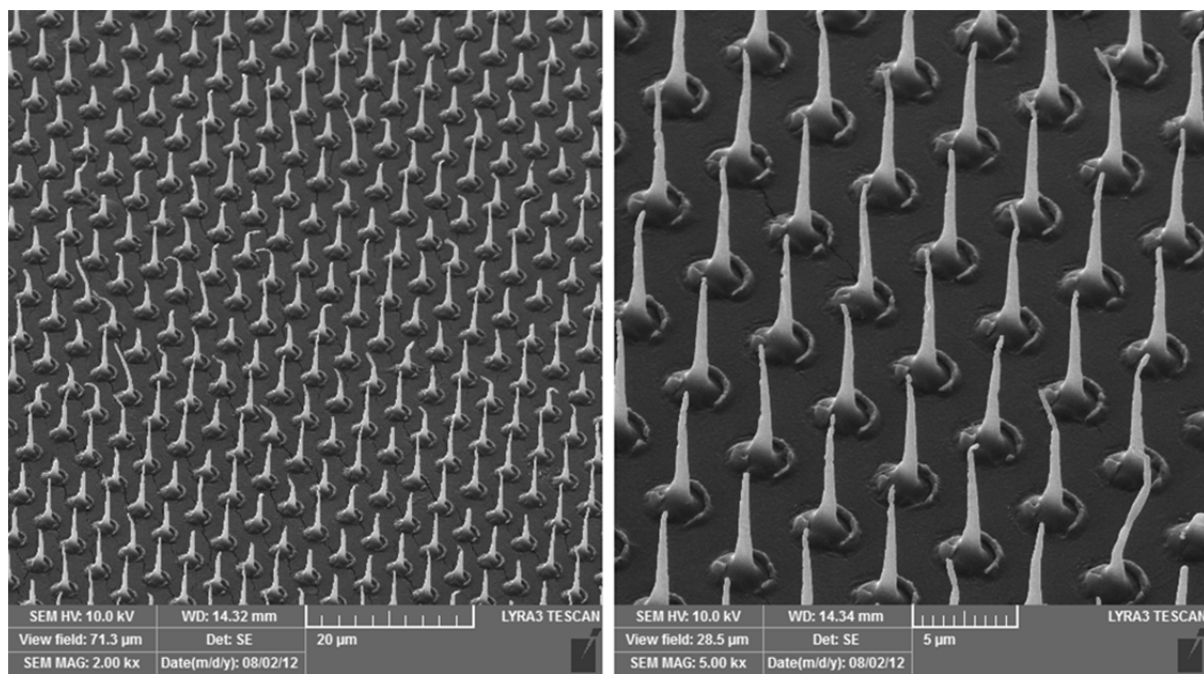


Figure 4.2.4(a). PVA nanowires imprinted from an as-machined fused silica template using 5 wt% PVA solution in DI water.

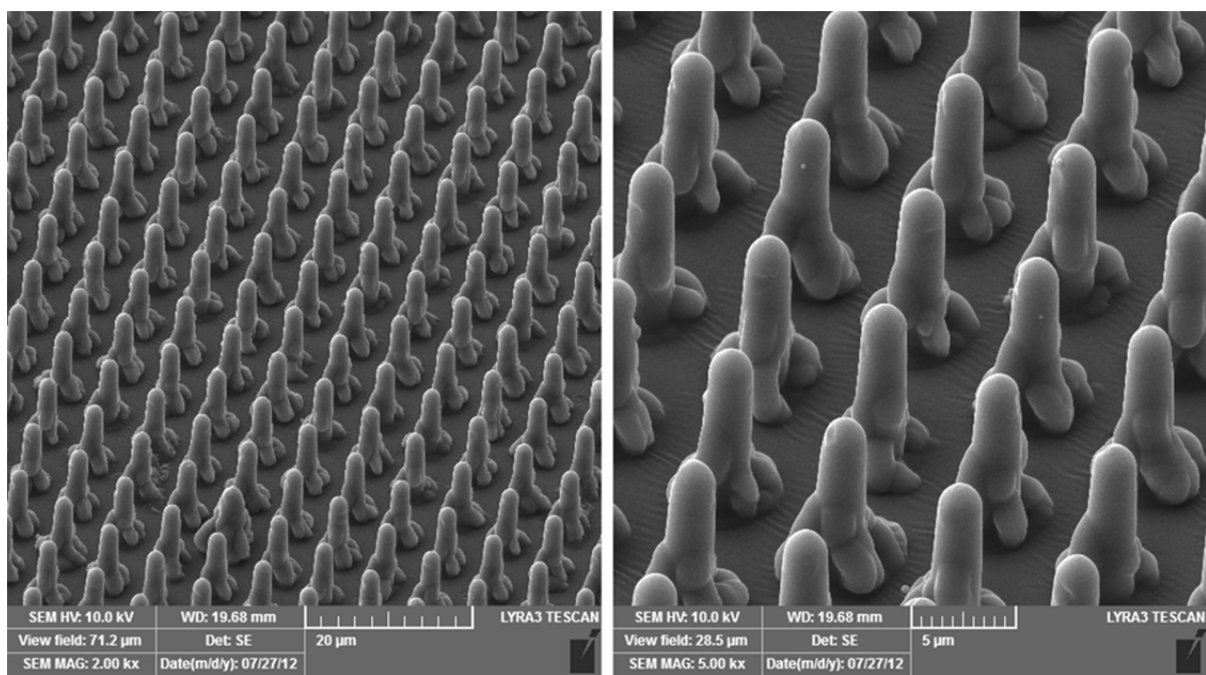


Figure 4.2.4(b). PVA micropillars imprinted from an etched fused silica template using 5 wt% PVA solution in DI water. The right image is taken at high magnification in both the figures for clarity.

4.2.5) Polymethylmethacrylate (PMMA) structures

PMMA is a transparent polymer with molecular formula $(C_5O_2H_8)_n$. It is a lightweight thermoplastic with good resistance to shattering, and is often used as an alternative to inorganic glass and polycarbonate. It is thus also called acrylic glass. PMMA is usually chemically modified for practical purposes. Due to its low cost and transparent nature, PMMA is used in a variety of applications, such as lenses and windows for automobiles and aircrafts, viewing ports in underwater vehicles, aquariums, daylight redirection systems, and aesthetic structures. Because of its good compatibility human tissue, PMMA is also used in medical technologies and preparation of biological implants e.g., cosmetic and orthopedic surgery [174-176]. PMMA is also found to be an attractive material for microfluidic lab-on-a-chip devices [177, 178]. In the present study, PMMA structures are imprinted using its solution in toluene. SEM images of PMMA nanowires and micropillars are shown in Figures 4.2.5(a) and 4.2.5(b), respectively.

4.2.6) Collodion structures

Collodion is essentially a solution of nitrocellulose. It was first used in photography in the 1850s. It finds applications as a glue to attach electrodes to the head for electroencephalography, cleaning agent for telescope optics, and more importantly as a replication material in microscopy [179-181]. In the present study, Collodion structures are imprinted using its commercially available 2% solution in amyl acetate (Parlodion). Figures 4.2.6(a) and 4.2.6(b) show the SEM images of Collodion nanowires and micropillars, respectively.

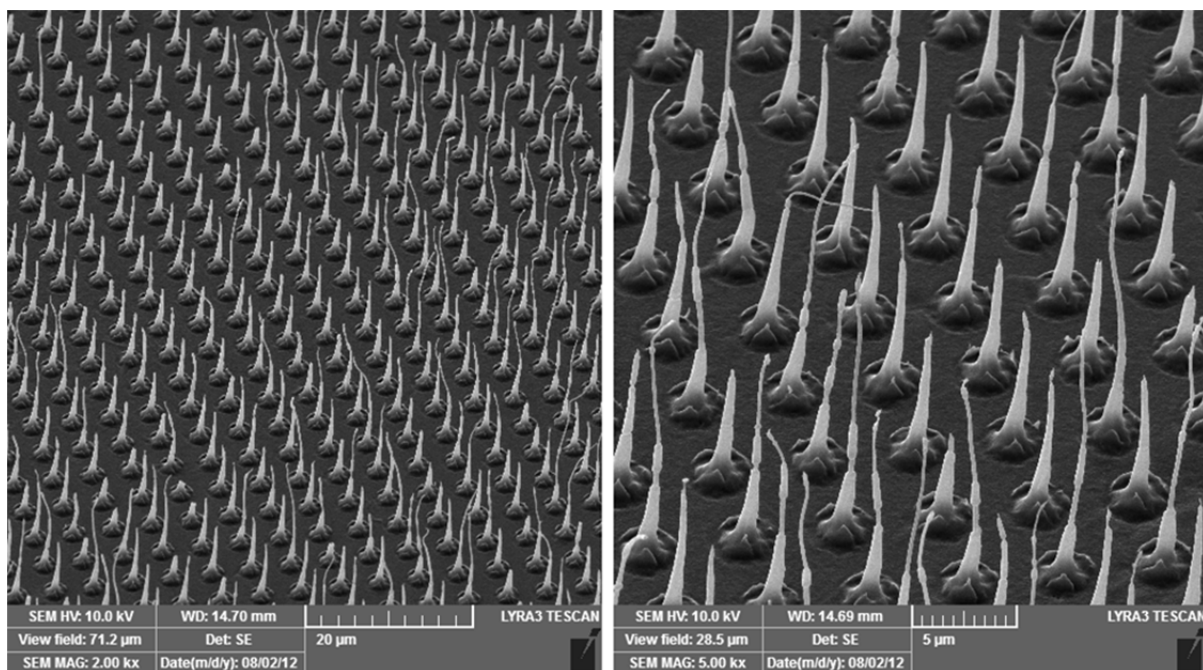


Figure 4.2.5(a). PMMA nanowires imprinted from an as-machined fused silica template using 10 wt% PMMA solution in toluene.

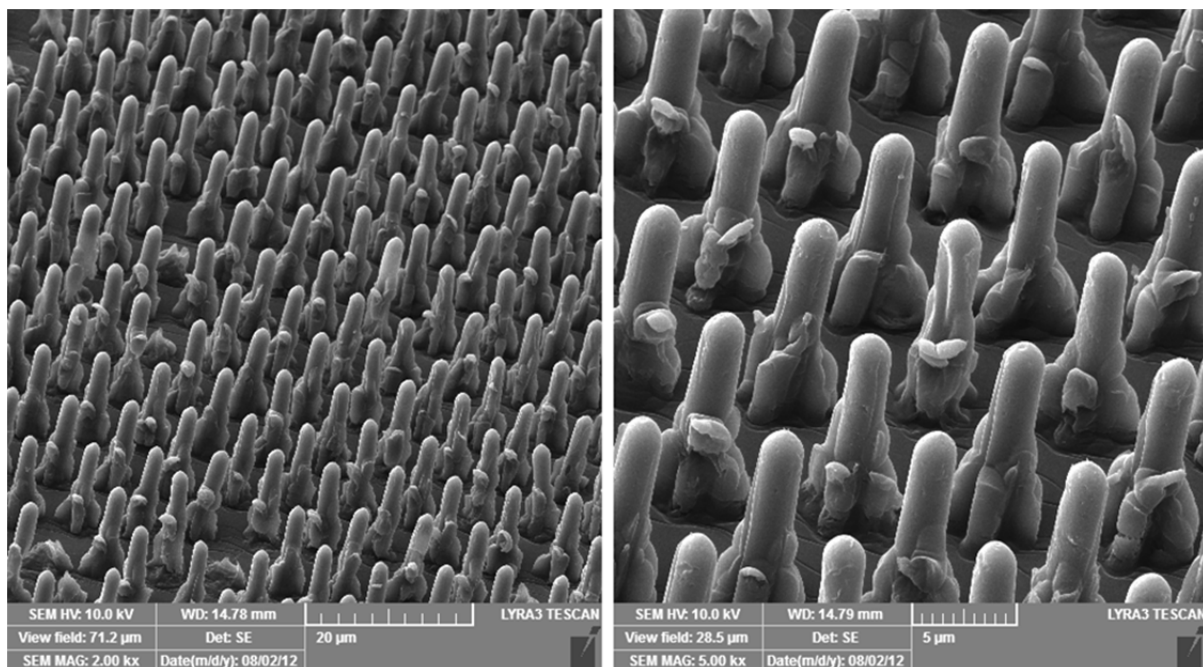


Figure 4.2.5(b). PMMA micropillars imprinted from an etched fused silica template using 10 wt% PMMA solution in toluene. The right image is taken at high magnification in both the figures for clarity.

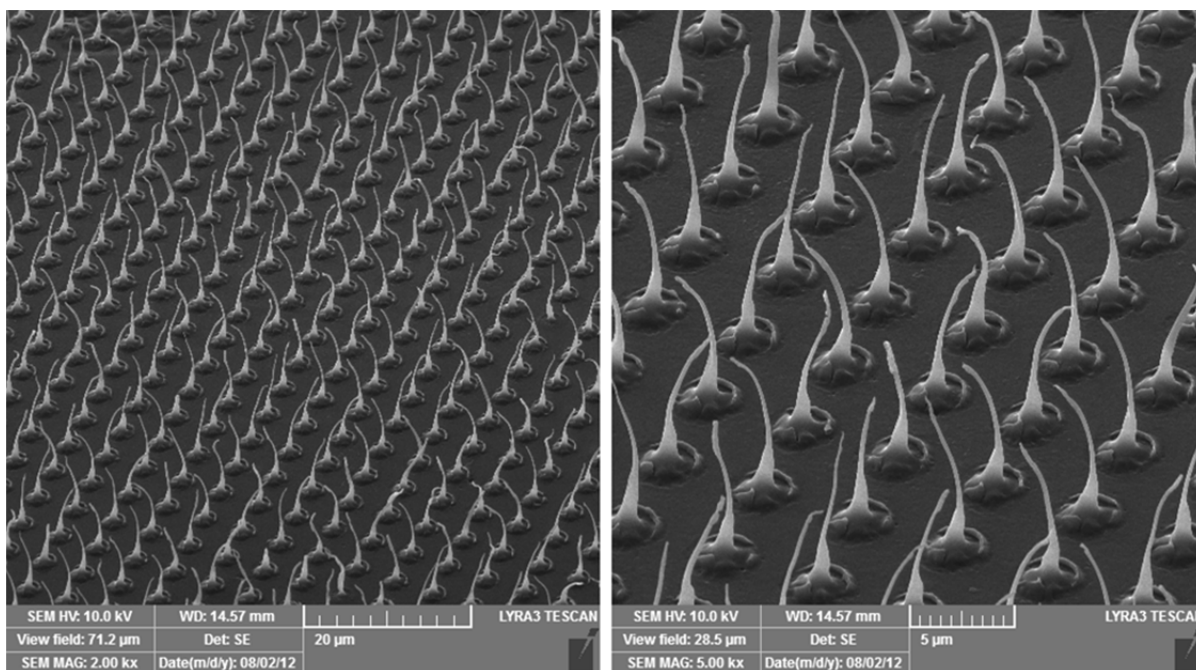


Figure 4.2.6(a). Collodion nanowires imprinted from an as-machined fused silica template using a 2% Collodion solution in amyl acetate.

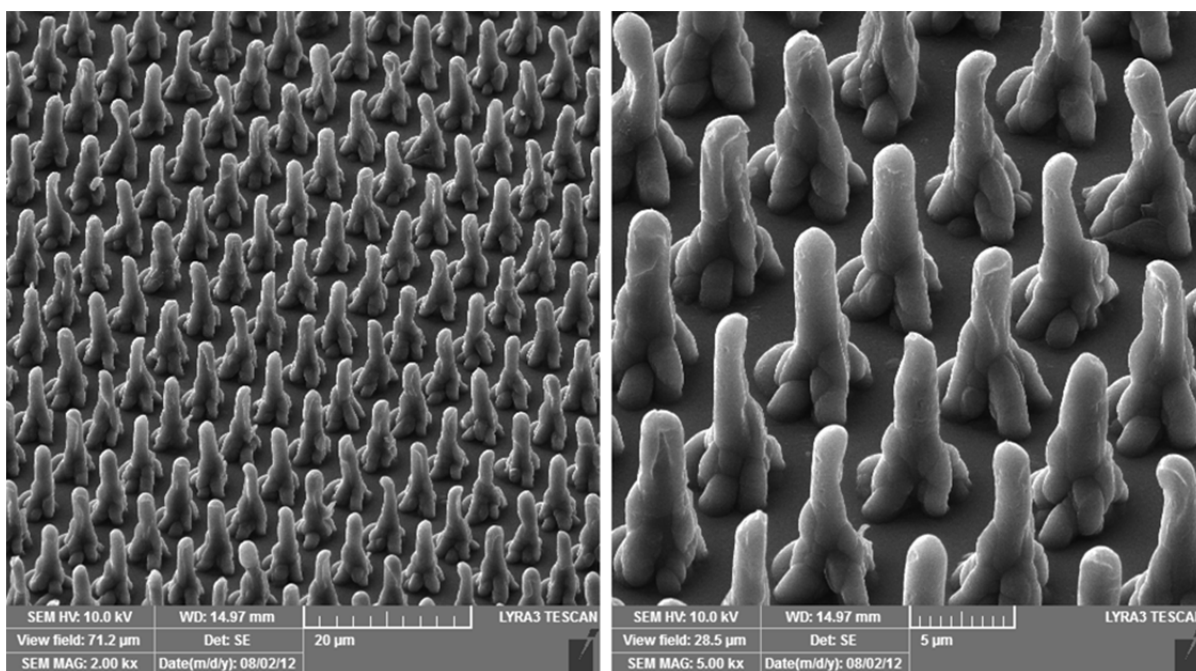


Figure 4.2.6(b). Collodion micropillars imprinted from an etched fused silica template using 2% Collodion solution in amyl acetate. The right image is taken at high magnification in both the figures for clarity.

4.2.7) Polydimethylsiloxane (PDMS) structures

PDMS is also a transparent polymer and it belongs to the family of organosilicon compounds called silicones. Its molecular formula is $(\text{C}_2\text{H}_6\text{OSi})_n$. It is hydrophobic, non-toxic, not flammable, heat resistant, and inert to most chemical substances. It finds applications as contact lenses and medical devices. PDMS is currently a material of choice for microfluidic lab-on-a-chip devices and elastomer stamps for soft lithography [62, 182]. Commercially available two part silicone elastomer kit Sylgard 184 is the most common material used to prepare PDMS [183, 184]. The two parts, base and curer, are usually mixed in 10:1 mass ratio and cured either in room temperature or at elevated temperature (usually 80°C for 2 hours) for cross-linking. The mechanical properties of PDMS can be altered by varying the base to curer ratio [184]. Uncured PDMS mixture is too viscous to fill the nanoholes of the templates, so its viscosity was reduced by mixing in n-heptane. In the present study, PDMS structures are imprinted using a 33 wt% PDMS (10:1 base to curer ratio) solution in n-heptane. SEM images of PDMS nanowires (nanococones) and micropillars are shown in Figures 4.2.7(a) and 4.2.7(b), respectively.

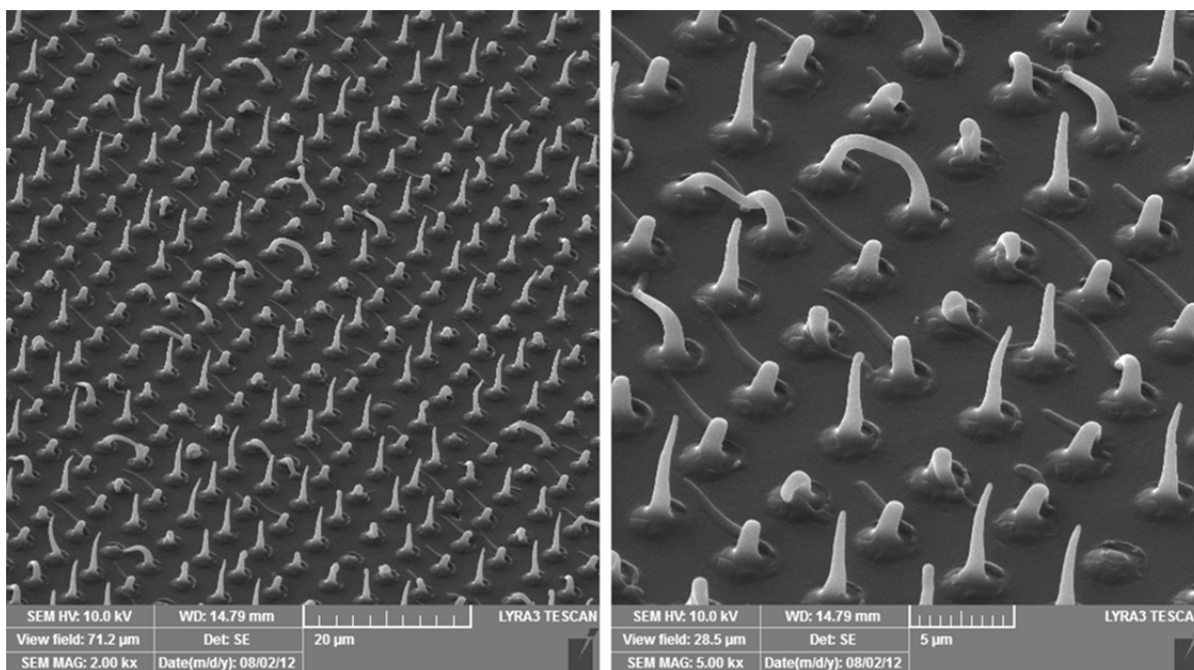


Figure 4.2.7(a). PDMS nanocones imprinted from an as-machined fused silica template using 33 wt% PDMS solution in n-heptane.

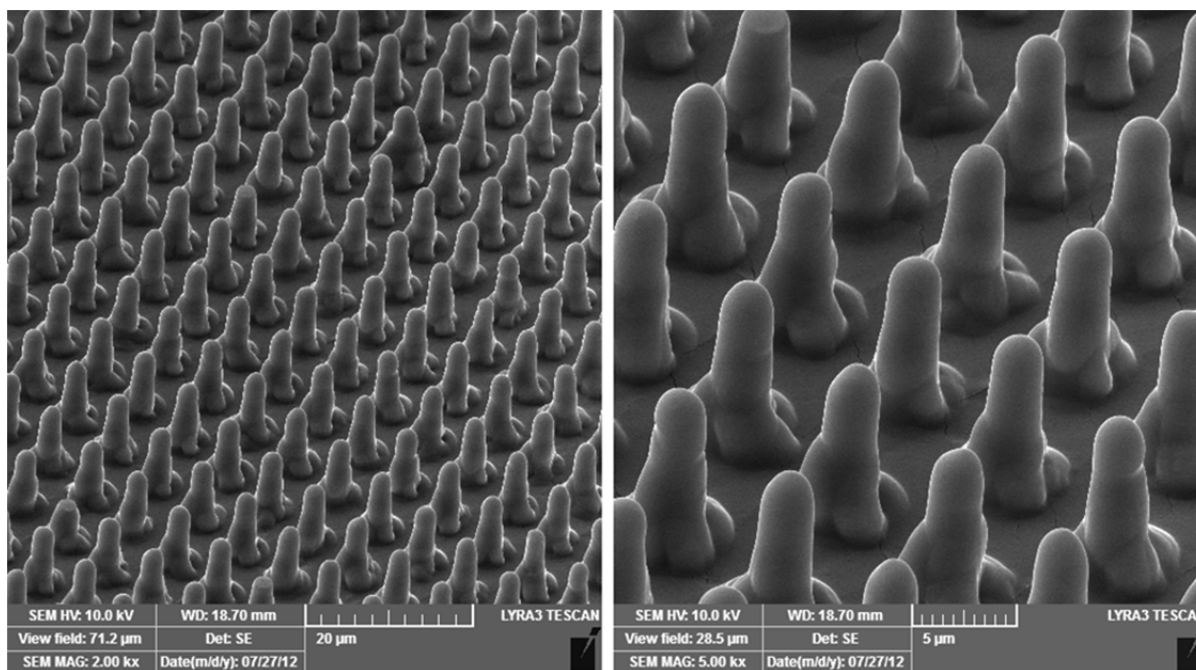


Figure 4.2.7(b). PDMS micropillars imprinted from an etched fused silica template using 33 wt% PDMS solution in n-heptane. The right image is taken at high magnification in both the figures for clarity.

PCL and PCL-PEG structures imprinted from as-machined fused silica templates are shown in Figures 4.2.1(a) and 4.2.1(c), respectively. The imprinted structures appear to be 30-40 μm long nanowires compared to ~ 15 μm deep nanoholes they are imprinted from. Apparently, these nanowires undergo a lot of stretching during the imprint lift-off process. It can be reasonably explained with the help of various forces acting on the polymer within the nanoholes during their lift-off. The adhesive strength between the glass nanohole and the polymer exceeds the tensile strength of the respective polymers (PCL and PCL-PEG). Consequently, the polymers yield during their lift-off, which results in their stretching. In fact, it was noticed that PCL and PCL-PEG nanowires stretch even when the templates are coated with antistick to help release the imprint. Furthermore, freezing the PCL and PCL-PEG imprints below their glass transition temperature ($< -60^{\circ}\text{C}$) before lift-off also did not avoid their stretching. These nanowires were measured to be around 100 nm wide near their tip. Since the wires come out very long (> 30 μm) due to stretching and are not stiff enough to carry their weight, they fall over acquiring the appearance of nanocarpets or nanomats. The stretched and fallen PCL and PCL-PEG nanowires have very high surface area and aspect-ratios greater than 200:1.

SEM images of CA, PE, PVA, PMMA, and Collodion structures imprinted from as-machined templates are shown in Figures 4.2.2(a), 4.2.3(a), 4.2.4(a), 4.2.5(a), and 4.2.6(a), respectively. The dimensions of these structures are fairly close to that of the nanoholes and morphologically they appear as nanowires or nanocones with their base diameter around 750 nm and tip diameter around 150 nm. It can also be seen that most of the polymer nanocones/nanowires slightly bend in one common direction whereas

some tend to bend in random orientations. The yield of the imprinted structures (i.e., the total number of imprinted structures with respect to the total number of nanoholes in the pattern) was found to be very high as most of the nanoholes are successfully replicated. However, some polymer nanocones tend to break (CA and PE) or stretch (PMMA) during lift-off. The yield, to a certain degree, improves if adequate time is given for the solvent to dissipate before the imprint lift-off. Most PDMS nanocones, shown in Figure 2.7.1(a), tend to fall over unlike any other polymer nanocones, but without breaking. It can be attributed to the low Young's modulus of PDMS, which is <1 MPa for any given base to curer ratio (Sylgard 184) [185]. Moreover, the chemical structure and mechanical properties of PDMS are very different from other polymers used in the present study. Its 3D cross-linked structure together with a combination of very long Si-O skeletal bond, unobstructed skeletal oxygen atoms, and large Si-O-Si bond angle of 143° provide it with extraordinary dynamic flexibility and deformability [186]. All the polymer structures imprinted from the etched template adopt the shape and dimensions very close to that of the cylindrical holes they are cast from. Morphologically, they appear as micropillars though their textures vary because of their physicochemical properties. The yield was found to be 100% in all the polymers, and none of them was observed to stretch, break, or bend.

4.2.8) Solution casting *versus* thin film imprinting

Thin film imprinting is suitable only for those polymers that can be obtained or prepared in the form of thin films (e.g., cellulose acetate and polyvinyl chloride) and at the same can be softened by a readily available solvent, thus making it a highly selective process. Thus, polymers that do not soften or dissolve in any solvent cannot

be used to make polymer structures by thin film imprinting e.g., thermosets like polydimethylsiloxane (PDMS). In comparison, solution casting can be used to imprint polymer structures from virtually any polymer that forms continuous films. Blending different polymers or reinforcing polymers with other polymers or non-polymeric materials leads to the formation of multifunctional polymers with novel properties for a variety of applications in particular biomedical engineering [187-189]. Such multifunctional polymer structures with varying chemical composition and tunable physical properties can be easily and more efficiently prepared by solution casting than thin film imprinting. Furthermore, heterogeneous structures composed of multiple layers of different polymers can be easily prepared by solution casting, which are otherwise not possible with thin film imprinting. Thus, solution casting offers several advantages over thin film imprinting, and can be employed for fabrication of novel polymer structures for advanced technologies.

4.3) Functionalization of imprinted polymer structures

Polymer structures imprinted from these templates can be subsequently functionalized by coating with different materials in order to modify their physical, chemical, and biological properties. Functionalized polymer structures are useful for a number of applications e.g., photovoltaics, high surface area electrodes, and synthetic cell culture substrates. In the present study, polymer structures are coated with noble metals (gold, platinum, and palladium) by sputtering and silica by chemical vapor deposition. Noble metals are coated at cryogenic temperatures in a modified sputtering machine described in section 3.5. The thickness of the metal coating is varied by

changing the sputtering time and applied current/voltage. However, sputtering is a line-of-sight deposition process, which affects the metal coating coverage on the surface of the imprinted polymer structures. The sidewalls of the polymer structure receive considerably less amount of metal coverage when compared to the tips of the polymer structures. Nevertheless, the coating coverage can be improved by tilting and spinning the polymer imprints for which the sputtering instrument will need a stage for sample manipulation. All the SEM images of the polymer structures shown in this dissertation are taken after coating with noble metals, mostly platinum, below -165°C . Besides noble metals, the imprinted polymer structures can be easily coated with other materials as well, such as insulators and metals, provided the deposition temperature is low enough to avoid polymer softening or disintegrating.

In the present study, a low temperature chemical vapor deposition process was developed to coat cellulose acetate nanowires with silica to prepare silica nanowires (or nanoneedles). It is a two-step chemical vapor deposition which deposits a thin layer of silica atop thin film imprinted CA nanowires at 65°C by hydrolysis of silicon tetrachloride (SiCl_4). The process is described in detail in section 3.5.2. Two separate beakers are used for silica deposition: one with silicon tetrachloride solution and the other with water. The surface of CA nanowires is covered with SiCl_4 in the first beaker, which is hydrolyzed to form SiO_2 in the second beaker. The silica coating thickness was studied as a function of exposure time in beaker 1. The exposure time in beaker 2 was kept constant at 5 minutes, which is sufficient for the complete hydrolysis of SiCl_4 molecules adsorbed at the surface of CA nanowires in beaker 1. The coating thickness was determined for the first 6 minutes with the help of SEM images of the corresponding

silica nanowires. 30 individual measurements were made and a detailed statistical analysis was done, as explained in section 3.6.1.

The rate of silica deposition as a function of exposure time in beaker 1 (SiCl_4 solution) thus determined is shown in Figure 4.3(a). Figure 4.3(b) shows an SEM image of an array of silica nanowires obtained after 5 minutes exposure in beaker 1. In order to confirm the formation of silica, a silica nanowire was subjected to elemental analysis using EDS. The EDS analysis, spectrum shown in Figure 4.3(c), confirms the presence of silicon and oxygen. Although it is a qualitative measurement, the EDS spectrum does reveal the presence of silicon and oxygen in the coating, thus confirming the formation of silica. To further characterize the silica coating, an absorption spectrum was recorded on an ATR-FTIR and is shown in Figure 4.3(d).

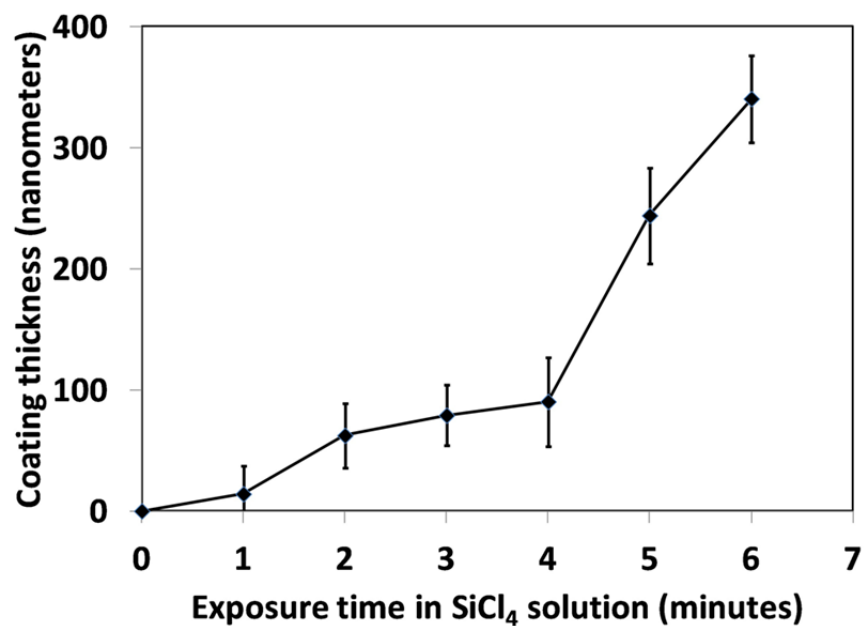


Figure 4.3(a). Rate of deposition of silica as a function of exposure time in beaker 1.

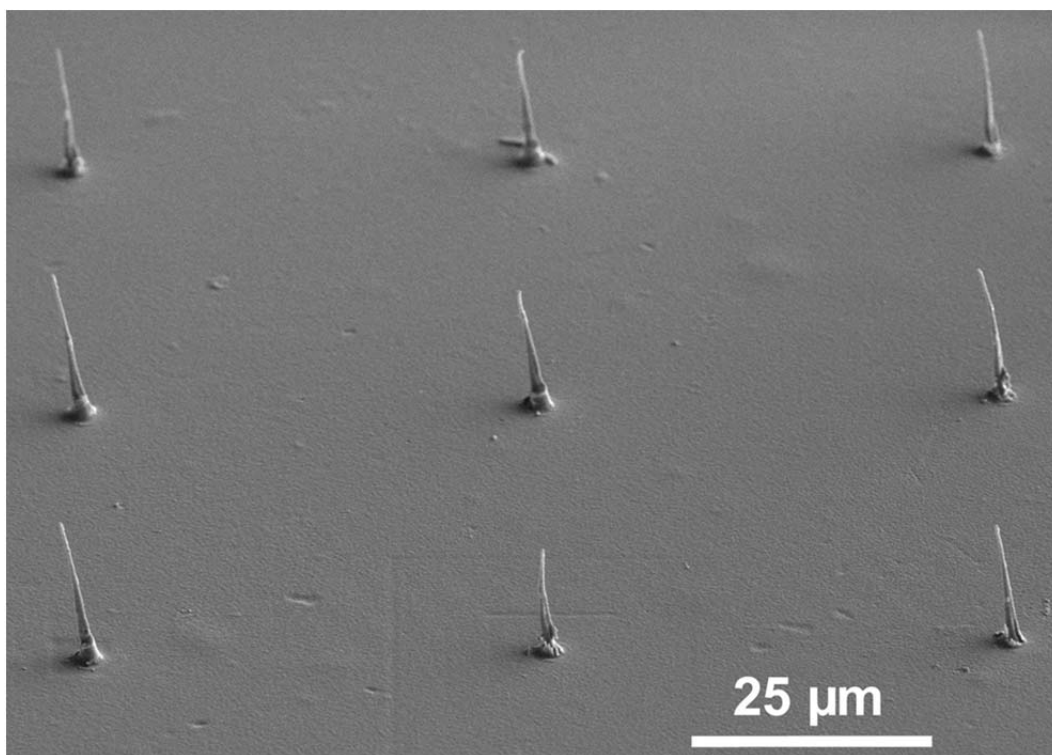


Figure 4.3(b). An array of silica nanowires obtained by coating cellulose acetate nanowires with silica for 5 minutes in beaker 1.

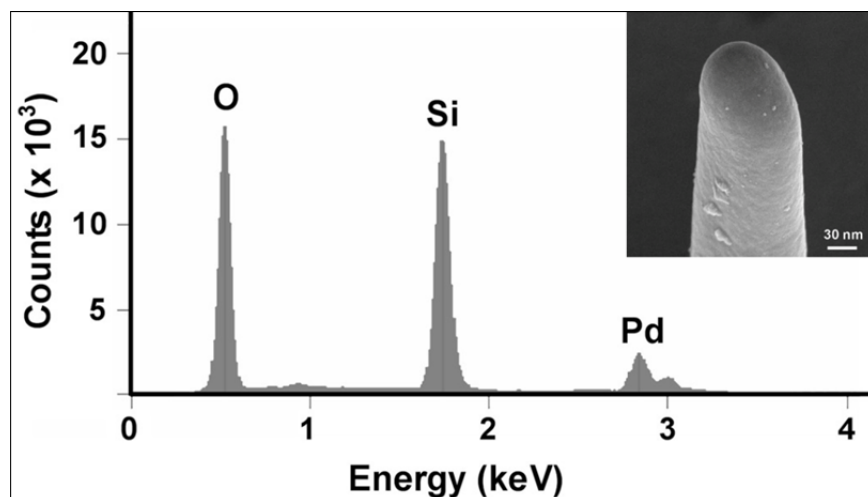


Figure 4.3(c). An EDS spectrum of the tip of a silica nanowire shown on top right. It reveals the presence of silicon and oxygen in the coating. The sample was coated with palladium prior to SEM/EDS analysis, thus shows up in the EDS spectrum.

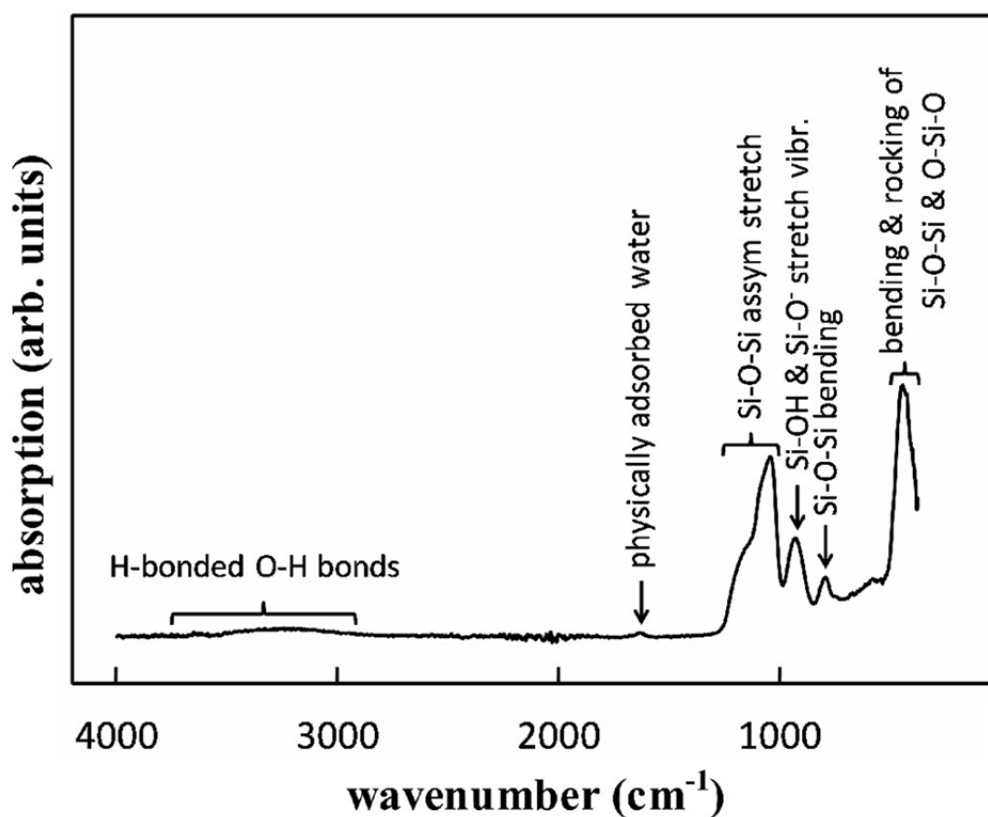


Figure 4.3(d). Absorption spectrum of the silica coating recorded on an ATR-FTIR. It shows the presence of various Si-O-Si and O-H vibration modes.

The mid-infrared absorption spectrum of the coating shown in Figure 4.3(d) reveals the presence of various vibrational modes in silica, and gives more information about its chemical structure. The broad band in the interval 3700–3000 cm^{-1} corresponds to the intensive overlapping of the stretching modes in hydrogen-bonded hydroxyl bands produced by O–H bonds in water and Si–OH [190, 191]. A small peak at $\sim 1635 \text{ cm}^{-1}$ is usually attributed to the deformation vibrations of the O–H groups in physically adsorbed molecular water at the surface of silica coating [192] and its weak intensity confirms the presence of very low amount of molecular water in the coating. A sharp peak at $\sim 927 \text{ cm}^{-1}$ appears due to the stretching vibrations of Si–OH or Si–O⁻ groups in silica [193], which indicates the presence of a large number of silanol groups on the coating surface. The band at 1000–1300 cm^{-1} appears due to the anti-symmetric stretching of Si–O–Si bonds in silica [194]. This band can be resolved into a peak at 1041 cm^{-1} (TO₁) and a shoulder around 1156 cm^{-1} (TO₂). The TO₁ peak in silica is usually found at $\sim 1082 \text{ cm}^{-1}$ and its shifting towards lower frequencies, 1041 cm^{-1} in the present case, has been reported to be associated with oxygen deficiency [195, 196] and lower three-dimensionality [197] in the structure of silicon oxide. It indicates that the stoichiometry of the silica coating in the present case is SiO_x with $x < 2$. Furthermore, the peak appearing at 799 cm^{-1} is due to the bending vibration of Si–O–Si bonds [194, 198]. A peak due to the bending modes in Si–OH bonds also appeared around $\sim 790 \text{ cm}^{-1}$ [199]; however, it is very close to the Si–O–Si bending mode and cannot be seen clearly in the spectrum. A series of peaks appears in the lower frequency region from 448 to 424 cm^{-1} , which could have arisen due to the combination of bending and rocking

modes of Si–O–Si or O–Si–O bonds in the coating [199-201]. However, it is very difficult to resolve and identify all these peaks in detail.

A silica nanowire produced by coating for 5 minutes was sectioned using a focused ion beam instrument to view its cross-section. A series of SEM images were captured while sectioning the silica nanowire, and are shown as a composite picture in Figure 4.3(e). The tip of the silica nanowire was cut with a gallium current of 20 μ A at the projected length of ~ 9 μ m from the base (i.e., ~ 13 μ m actual length). The inset (A) clearly shows the CA nanowire at the center of the structure. A sequence of cuts was made further down the silica nanowire; two of them are shown in (B) and (C), where the silica coating and CA can be easily distinguished by contrast. Also, the silica coating thickness and CA nanowire diameter can also be clearly seen as well. The FIB milling angle was slightly changed to drill into the CA nanowire and reveal the interior of the silica nanoneedle (D). It indicates that the silica coating is around 125 nm thick and the CA nanowire is 175 nm in diameter at that particular location on the silica nanowire. It must be noticed that the FIB-sectioned hole appears elliptical because it was imaged at 58° from the horizon in the FIB/SEM.

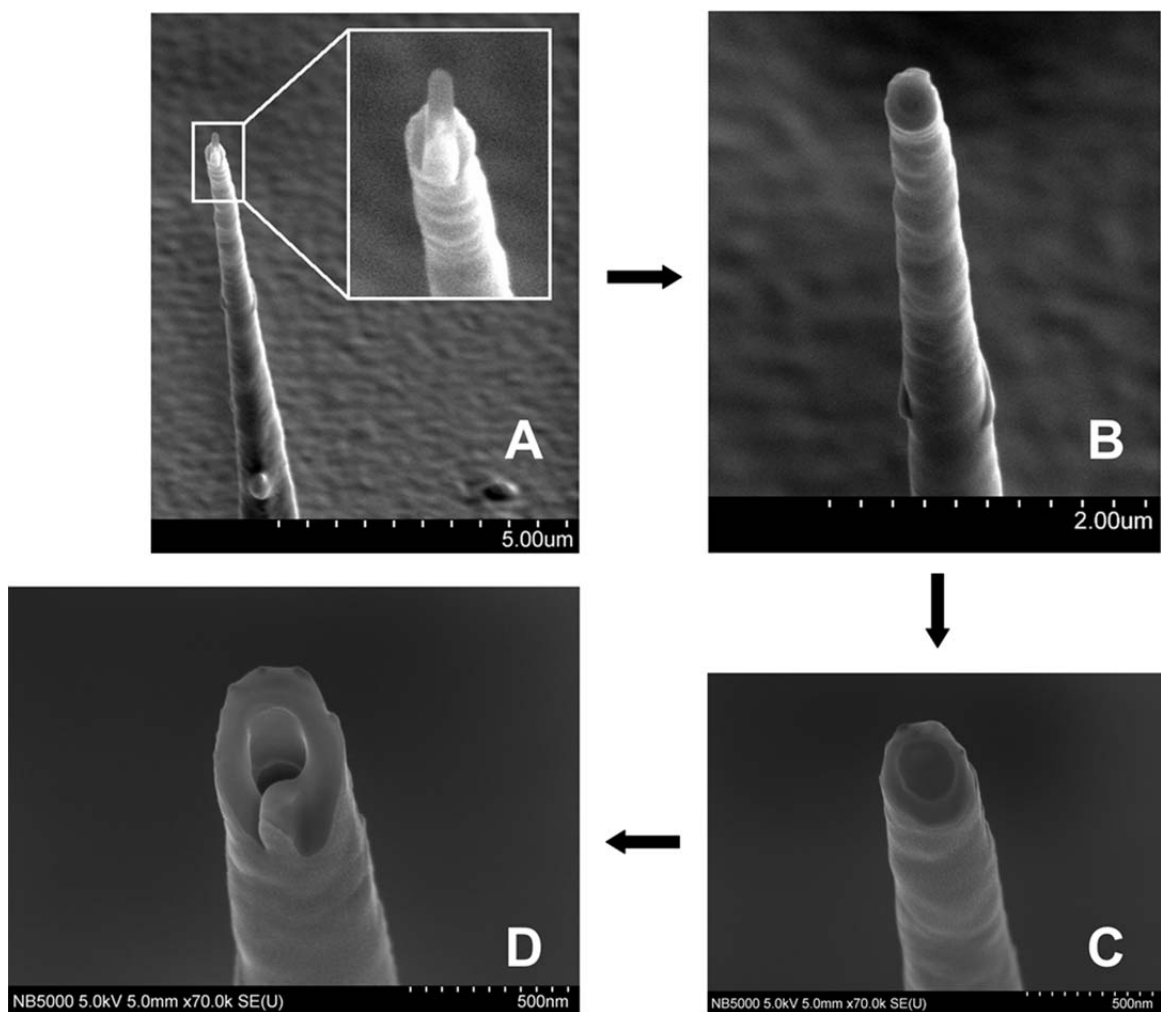


Figure 4.3(e). A silica nanowire subjected to gallium ion sectioning in an SEM/FIB. Each image represents a different section further down the silica nanocone from (A) to (D). The final image (D) shows an open-tip silica nanoneedle.

4.4) Silica nanoneedles as cell culture substrates

Figures 4.4(a) and (b) show the SEM images of the nanoholes from an edge of the 2D gradient pattern and silica nanoneedles, respectively. Flat silica coated cellulose acetate and silica nanoneedles were used as cell culture substrates to cultivate NIH 3T3 fibroblasts as described in section 3.7.3. The effect of substrate topography i.e., flat vs. presence of nanoneedles on the response of fibroblasts was studied.

The cellular response was found to alter in the presence of silica nanoneedles as compared to flat silica surfaces. It can be seen in Figure 4.4(c) that the fibroblasts “ball up” or agglomerate on flat surfaces (A), whereas they extend their foci to adhere to the nanoneedles (B). Most strikingly, cell adhesion to surfaces with silica nanoneedles was an order of magnitude higher than that on to flat surfaces ($p = 0.005$, Figure 4.4d(A)), while viability was maintained for cells adhered to either surface (Figure 4.4d(B)). Fibroblast spreading was also found to be influenced in the presence of silica nanoneedles. Fibroblasts were found to exhibit a larger area and perimeter on silica nanoneedles than those cultured on flat surfaces ($p < 0.005$ and $p = 0.007$, Figure 4.4d(C) and 4.4d(D), respectively).

The presence and spacing of silica nanoneedles strongly influenced competitive cell-cell versus cell-matrix interactions and can be seen in Figure 4.4(e). Cells either spread out individually or formed large, multicellular aggregates (A) on flat silica substrates that did not display any noticeable organization or regularity (B). In comparison, the presence of silica nanoneedles prevented cell aggregation, interrupted cell-cell contacts, and promoted interactions with the matrix (C-F).

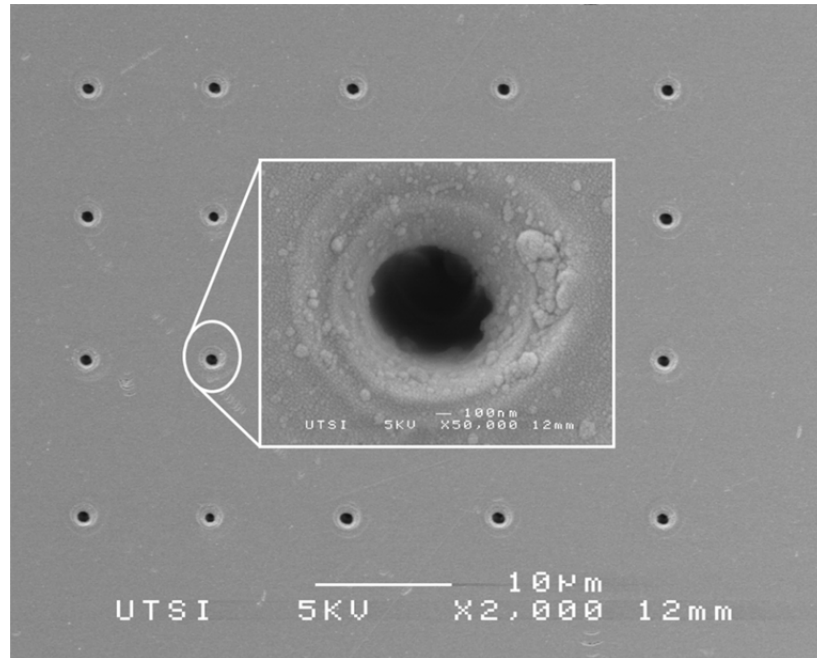


Figure 4.4(a). An SEM image of nanoholes from an edge of the 2D gradient pattern. The average diameter of the nanoholes is 750 nm and the depth $\sim 14 \mu\text{m}$.

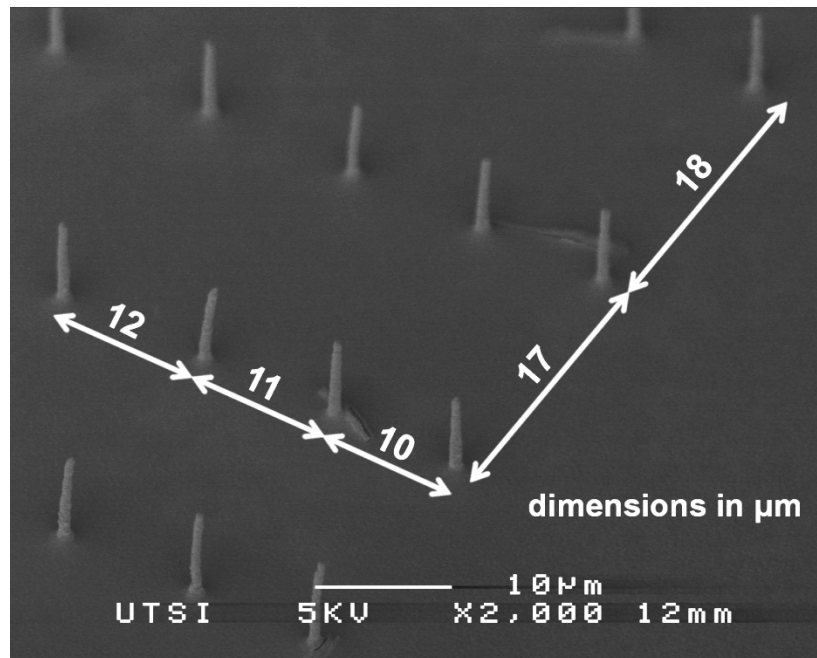


Figure 4.4(b). An SEM image of silica nanoneedles made by coating cellulose acetate nanowires with silica. The thickness of the silica coating is $240 \pm 40 \text{ nm}$.

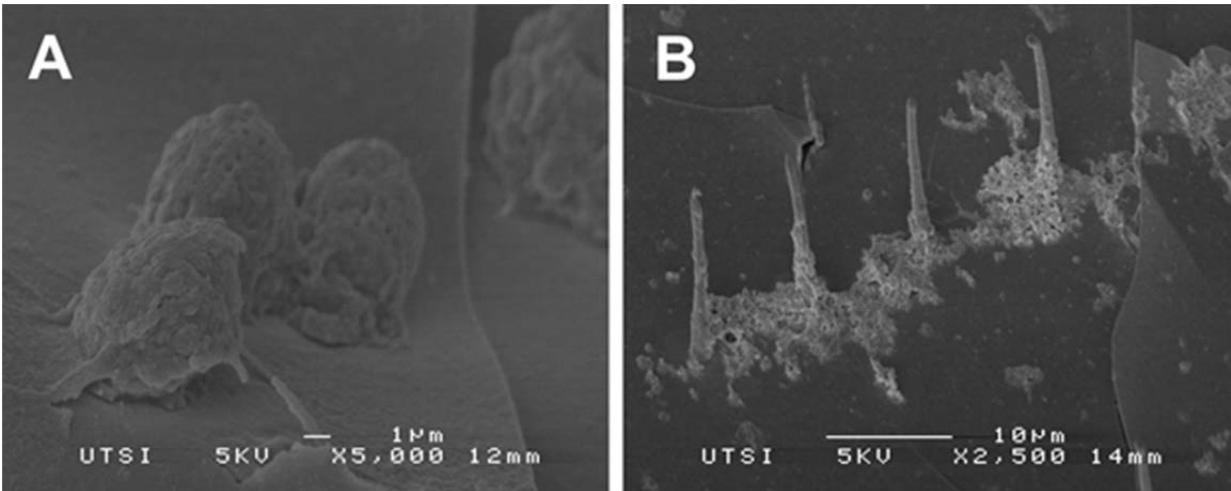


Figure 4.4(c). SEM images of cells fixed on flat silica (A) and silica nanoneedles (B) showing how the morphology of NIH 3T3 fibroblasts is affected by the surface topography of the substrate. Cell agglomeration on flat silica is an indication of poor adhesion, whereas cell spreading on silica nanoneedles is an indication of good adhesion. Silica coating cracks during cell fixation and can be seen in these SEM images.

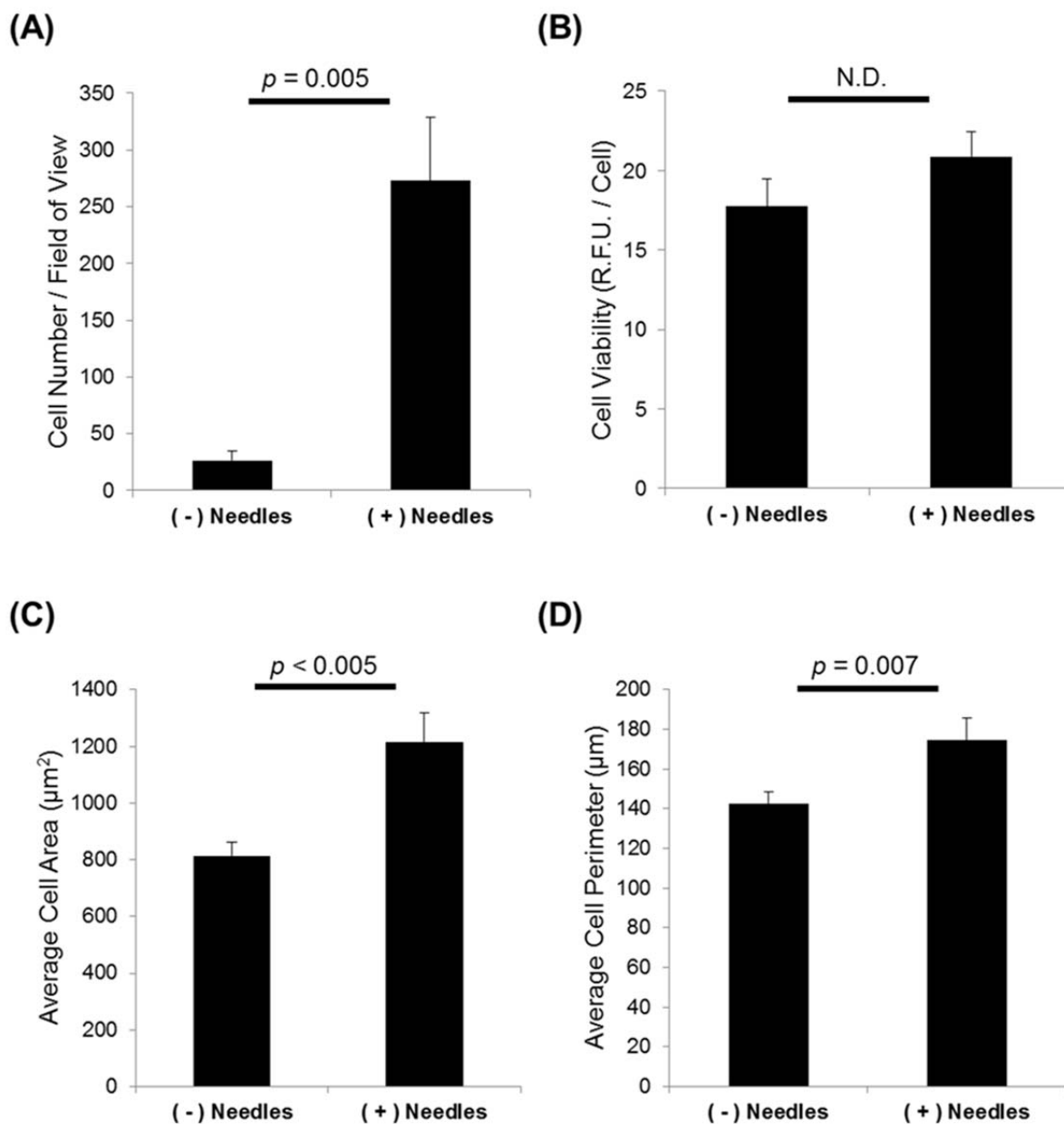


Figure 4.4(d). Comparison between cellular response to flat silica and silica nanoneedles. Silica nanoneedles were found to promote cell attachment and spreading. Significantly more cells attach to nanoneedle-containing surfaces (A) while cell viability is unaffected (B). Cell spreading (area, perimeter) are increased on nanoneedle-containing surfaces (C and D).

It was interesting to note that the fibroblasts preferentially aligned themselves along tightly spaced nanoneedles in gradient regions with sparse spacing in the orthogonal direction. In regions with sparse distributions in both directions, cells interacted both with adjacent needles as well as those located in the parallel rows (C-D). In general, parallel rows of silica nanoneedles with substantial spacing between them (i.e. $>30\text{ }\mu\text{m}$) sequestered cells and biased them towards cell-matrix interactions. This spacing also controlled the size and geometry of any small cell clusters that were present (E). A critical spacing between silica nanoneedles appeared to exist that regulated the ability of cells to adhere and spread in two dimensions (F).

Relative spacing ratios for local regions of needles regulated cell spreading. Sparse spacing in two dimensions allowed single cells to spread in two dimensions, whereas tight spacing in one dimension but wide spacing in the orthogonal direction influenced cells to spread only in one direction (see insets in Figure 4.4(e)C). It was found that the cells tend to spread in one dimension when the ratio between nanoneedles spacing in orthogonal directions was less than 0.5 (given that the nanoneedle spacing allowed for cells to attach between parallel rows). In contrast, the cells tend to spread in two dimensions when the nanoneedles spacing ratio was greater than 0.5. No significant difference in αSMA expression between the tested flat silica and silica nanoneedles substrates was observed. Furthermore, αSMA stress fibers were not observed on either substrate. Western blotting and PCR were not performed due to the presence of areas of un-patterned silica at the periphery of nanoneedles substrates, and the gradient spacing of silica patterns.

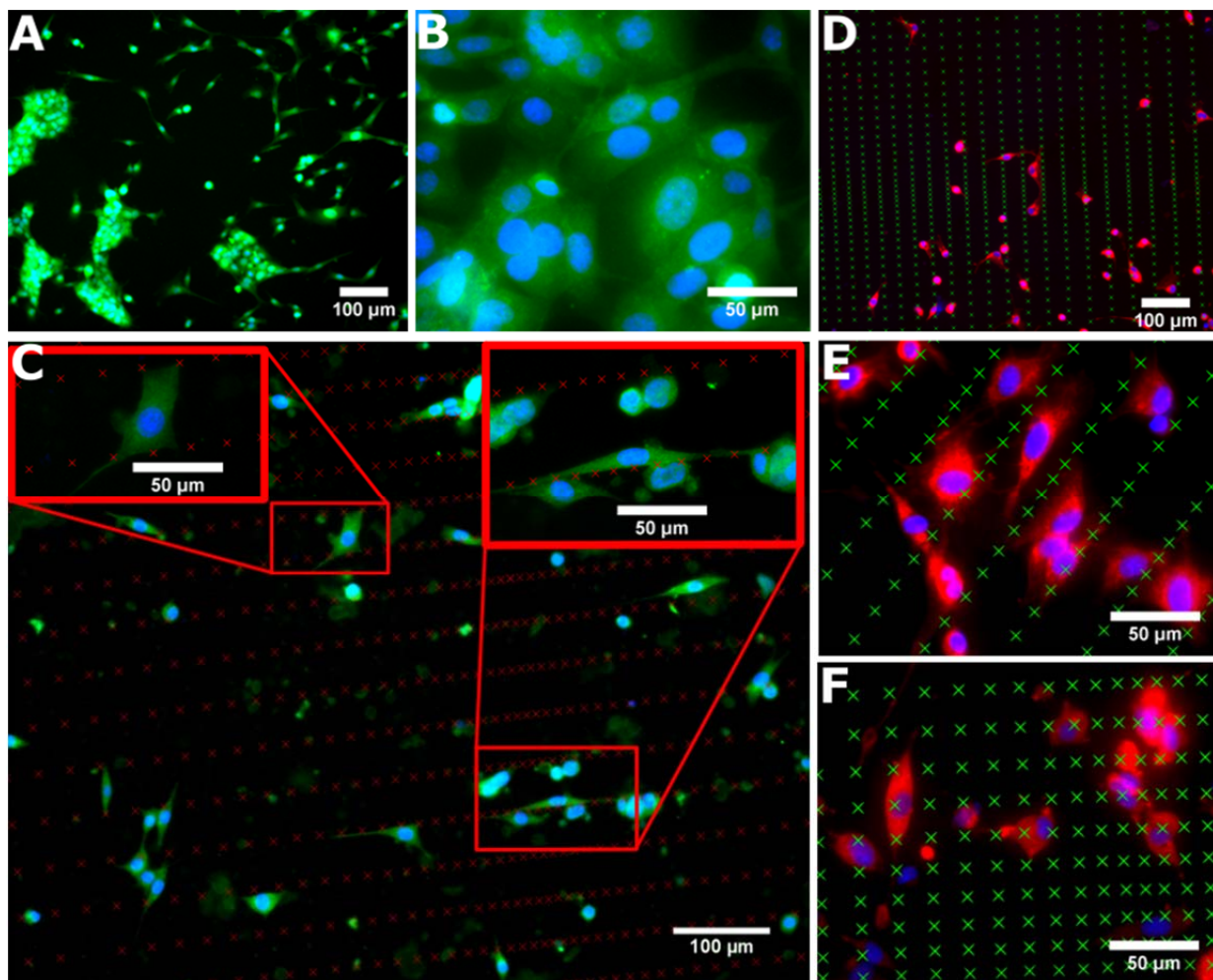


Figure 4.4(e). Cell behavior is influenced by the presence and spacing of silica nanoneedles. Flat surfaces allow for the formation of large, unorganized cell aggregates and independent, single-cell growth (A-B). Cells interact directly with the needles and only small cell aggregates, if any, are able to form (C-D). Parallel rows of tightly spaced needles sequester cells between them (E). Few, if any, cells are able to attach in areas of densely packed needles, but attachment improves as spacing becomes sparser (F).

In this investigation, the response of NIH 3T3 fibroblasts to silica nanoneedles was investigated as a means to modulate cell-cell versus cell-matrix interactions. Competitive cell-cell and cell-matrix interactions are a fundamental regulator of embryogenesis [120] and have been manipulated for complex tissue engineering strategies [202]. Cells of different lineages exhibit biased interactions depending upon their physiological location and function. For example, sheet-like epithelial and endothelial cells are dominated by cell-cell contacts through which various signals can be propagated, yet cells of a mesenchymal lineage are primarily cell-matrix interactive. Intricate lineage transition events, such as mesenchymal-to-epithelial transition (MET) and its reverse (EMT), can be driven solely by shifting the balance between these competitive events. In certain cancers, malignant cells in a primary tumor can undergo such a transition, allowing them to escape from their current location and metastasize [203]. By probing and modulating cell adhesion events, fundamental processes involved in embryogenesis, tissue engineering, and cancerous transformation can be intimately studied, controlled, and exploited for therapeutic benefits.

In the work undertaken in this study, the presence of silica nanoneedles was found to improve cell adhesion to the surface and stimulate cell spreading without affecting viability. Qualitative analysis comparing cellular response to flat silica and silica nanoneedles is shown in Figure 4.4(d). It can be seen that direct interactions between fibroblasts and the silica nanoneedles interrupted the formation of large cell aggregates that were seen on flat silica surfaces. Parallel rows of nanoneedles effectively constrained the cells and regulated the geometry of any small aggregates that formed (Figure 4.4(e)). Furthermore, the cellular response was dependent upon nanoneedles

spacing. The ratio of nanoneedles spacing in orthogonal rows was found to correlate with cell spreading in one versus two dimensions, suggesting that the cells actively sensed the 3D microenvironment and that nanoneedles spacing can be used as a critical parameter to intricately regulate cell behavior.

Chapter 5

Spherical Aberration & Optical Modeling

Hitherto, the mechanisms by which a deep nanohole with high aspect ratio is formed by tightly focusing a single femtosecond laser pulse are under discussion. There are two issues that must be explained by the mechanisms. First, the depth of the nanoholes produced is greater than the depth of focus of the laser beam, i.e., the depth over which the beam remains tightly focused (measured as the depth over which the area of the beam increases by less than a factor of two). Second, the plasma formed due to optical breakdown and avalanche ionization, under most conditions, very quickly becomes over-dense, opaque, reflective, and self-limiting, resulting in the formation of a shallow crater. Thus, additional mechanisms further to those introduced in chapter 2 are needed to explain the formation of high aspect ratio and deep nanoholes. Three mechanisms have been proposed by different research groups: spherical aberration, self-focusing, and beam reshaping [94, 107, 109, 204]. These proposed mechanisms are not exclusive and may all play a role to some degree in the formation of deep, high aspect ratio features.

Spherical aberration is an optical phenomenon in which light rays passing through a spherical lens focus at different locations along the optical axis so as to cause to a prolonged focal region. The effect of spherical aberration on the formation of high aspect ratio nanoholes can be reasonably investigated using an aspheric lens. An aspheric lens has a non-spherical surface geometry designed to greatly reduce spherical aberration in the focal region of a perfectly collimated laser beam fully filling its entrance pupil. Thus, an aspheric lens ($NA = 0.68$; Thorlabs, *C330TME-B*), shown in Figure 3.3(a), was used for machining experiments and optical modeling. This particular

aspheric lens was chosen because of its high numerical aperture and the availability of its detailed specifications and optical coefficients required for optical modeling.

5.1) Aspheric lens machining experiments

Initial experiments on aspheric lens machining of fused silica were performed with the machining platform presented earlier (section 3.1). The lenses of the beam expander, shown in 3.1.1(d), were adjusted so that the laser beam fully fills the entrance pupil of the aspheric lens. The entrance pupil of the aspheric lens used in this study is 5 mm in diameter. The machining experiments were performed at different relative positions of the optical focus with respect to the substrate surface (Z) as explained in section 4.1(a). The laser pulse energy was kept constant at $5.2 \mu\text{J}$. SEM images of the nanoholes made on a fused silica substrate by aspheric lens machining and their corresponding cellulose acetate (CA) replicas are shown in Figures 5.1(a) and (b), respectively. The deepest nanoholes were measured to be around $4 \mu\text{m}$ for $Z = +52$ to $Z = +60$ from their CA replicas. The cellulose acetate replicas show that the aspheric lens machined nanoholes are not as deep as those machined by the $60\times$ and $160\times$ microscope objectives. The aspheric lens minimizes the spherical aberration in the focal region of the laser beam even when its entrance pupil is fully filled. Thus, the formation of shallow nanoholes (maximum $4 \mu\text{m}$) by aspheric lens machining might be expected to be due to the reduced spherical aberration in the focal region of the laser pulse. However, it can be seen from the SEM images in Figure 5.1(a) that the single amplified femtosecond laser pulse undergoes multiple fragmentations upon focusing through the aspheric lens to form several nanoholes arranged in a crescent shape that resembles the structure of a cat's paw (from $Z = +52$ to $Z = +60$). The formation of crescent-shaped

laser damaged regions is due to the fact that the aspheric lens has a high numerical aperture and hence is very sensitive to minor beam misalignments, which may result in optical aberrations in the focal region. In fact, it is well known that misalignment of a high-NA lens results in comatic aberration or *coma*.

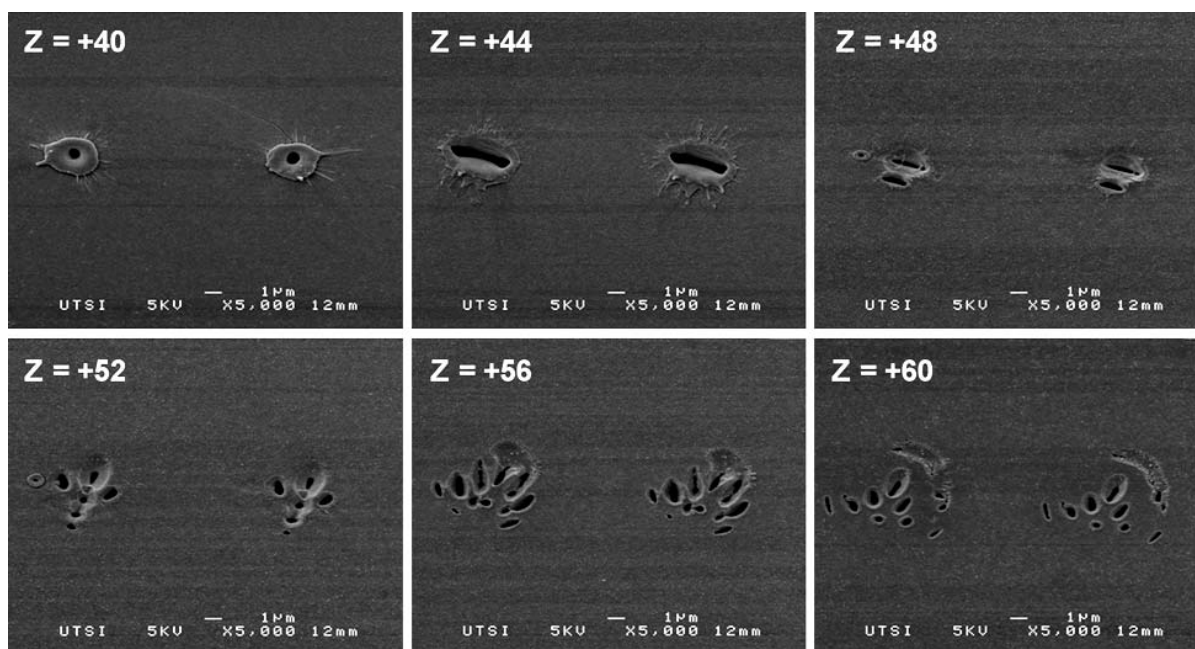


Figure 5.1(a). Nanoholes made at the surface of fused silica by aspheric lens machining at different positions of Z and laser pulse energy of $5.2 \mu\text{J}$ when the laser beam fully fills the entrance pupil of the aspheric lens.

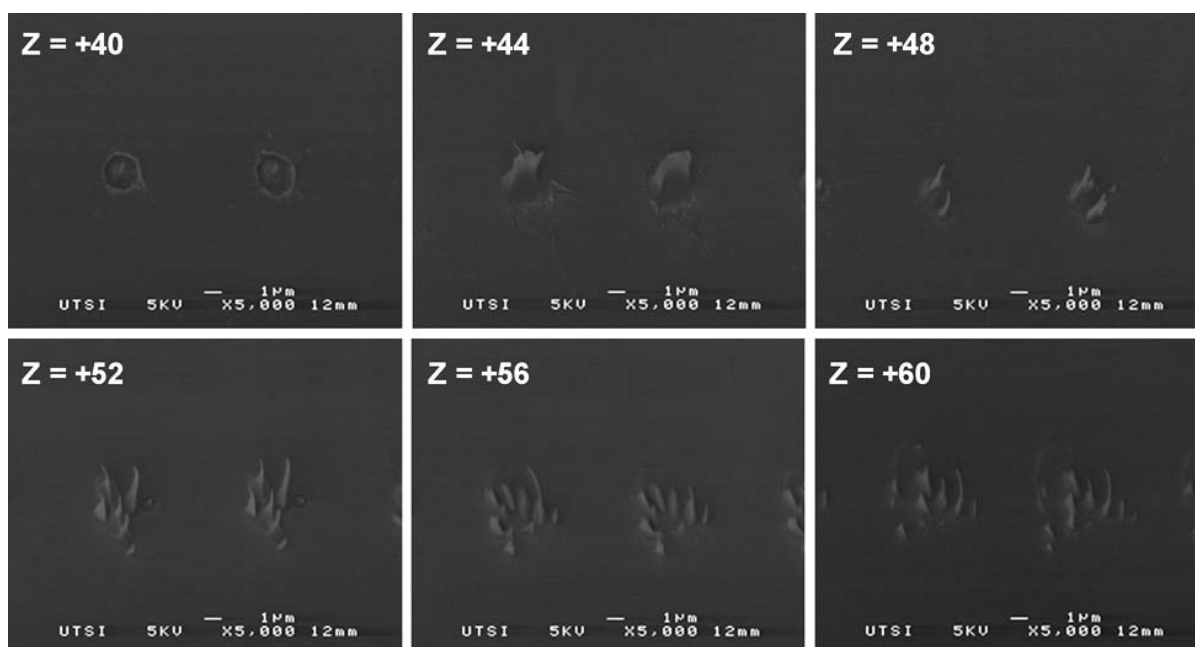


Figure 5.1(b). Cellulose acetate replica of nanoholes shown in Figure 5.1(a). It can be seen from these replicas that the depth of nanoholes decreases when the laser beam fully fills the entrance pupil of the aspheric lens.

5.2) Optical modeling

Three intuitively chosen configurations were modeled in an optical design software (Zemax by Radiant Zemax, LLC) to demonstrate that the cause of the structures was misalignment of the aspheric lens. These configurations are based on the propagation path of the laser beam (i.e., laser beam axis) with respect to the optical axis of the aspheric lens and are schematically shown in Figure 5.2.1. The configurations modeled were:

- A. ideal condition (i.e., the laser beam axis coincides with the optical axis),
- B. the laser beam decentered 0.5 mm in both X and Y axes, and
- C. the laser beam tilted at an angle of 0.5° to the optical axis.

The laser beam intensity profiles at and near the focal region for the aforementioned conditions were estimated by Zemax modeling. Two assumptions were made:

- a) the spatial profile of the laser beam is perfectly Gaussian; and,
- b) the entrance pupil of the aspheric lens is fully filled with the laser beam (i.e., the $1/e^2$ intensity radius of the Gaussian beam equals the 2.5 mm pupil radius of the lens).

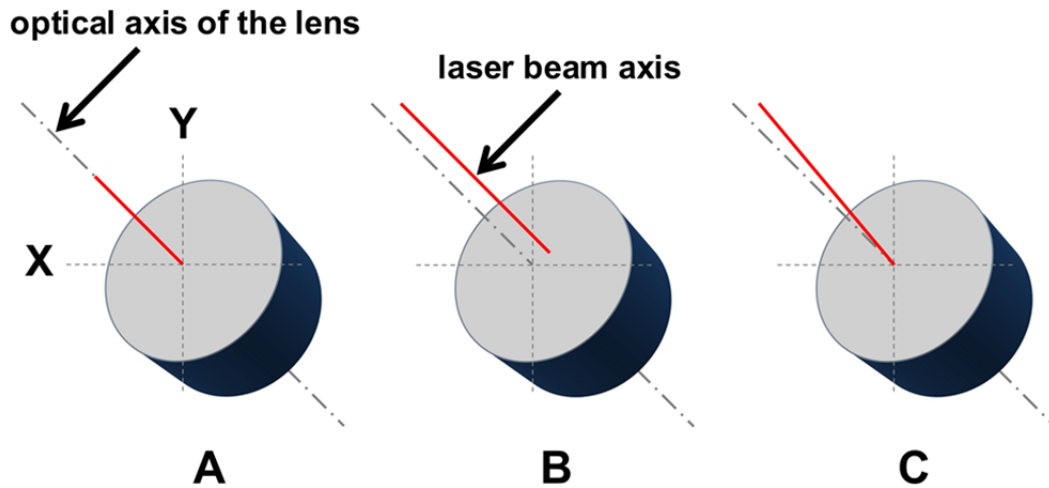


Figure 5.2.1. Schematic of the configurations modeled in Zemax: A) ideal condition: the laser beam axis is parallel to the optical axis of the aspheric lens and travels through the center of its entrance pupil, B) the laser beam axis is decentered 0.5 mm each in X and Y directions, and C) the laser beam axis is tilted by 0.5° to the optical axis of the lens. The red line shows the axis of the laser beam, where the actual laser pulse fully fills the entrance pupil of the aspheric lens.

Figures 5.2.2 and 5.2.3 show the focal spot diagrams and the corresponding Huygens point spread functions (PSF), respectively, for the three configurations modeled in Zemax. The PSF was obtained using a fast Fourier transform (FFT) function built-in in the Zemax. The spot radius is within the diffraction limit in the ideal condition, as indicated by the rays falling within the Airy disk (black circle) in Figure 5.2.2(A), and its root mean square value (RMS) of $\sim 0.21 \mu\text{m}$ being less than the radius of the Airy disk. Similarly, the spot radius in the second case (the laser beam is decentered 0.5 mm each in X and Y directions) also appears to be within the diffraction limit, but with a slight increase in its RMS value to $\sim 0.27 \mu\text{m}$. The shape of the spot, however, is no longer completely circular and shifts marginally in the XY directions; some rays form elliptical shapes but yet remain within the Airy Disk as seen in Figure 5.2.2(B). It indicates that decentering the laser beam up to 0.5 mm each in X and Y directions is still close to the ideal condition. Figure 5.2.2(C) shows the spot diagram when the laser beam arrives at an incidence angle of 0.5° with respect to the optical axis of the aspheric lens. It can be seen that this slight tilt can result in pronounced comatic aberration as the spot radius is larger than the diffraction limit, and the spot radius RMS value has drastically increased to $\sim 6.5 \mu\text{m}$ (nearly 30 times larger than that in the ideal condition). This comatic aberration (or *coma*) due to tilting is most likely the cause for the laser damage region to acquire the crescent shape, see Figure 5.1(a).

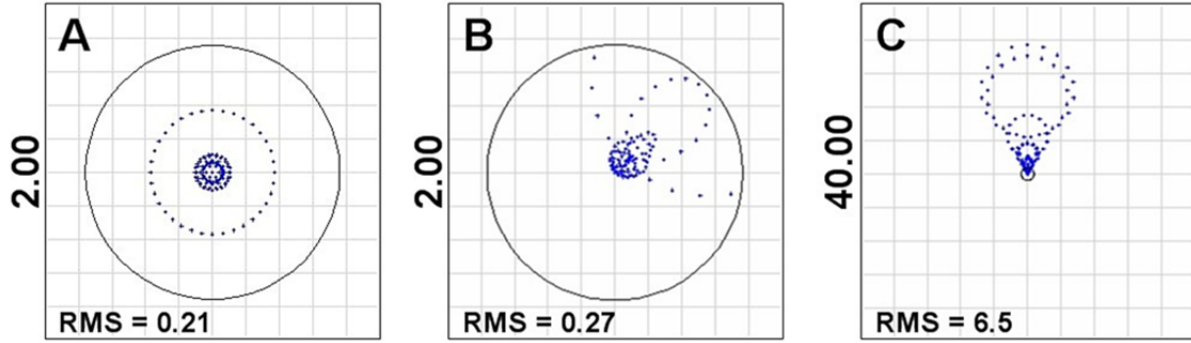


Figure 5.2.2. The spot radius at the image plane after the laser beam focused through the aspheric lens. A) ideal condition, B) 0.5 mm decenter each in X-Y, and C) 0.5° tilt with respect to the optical axis of the lens. The black circle represents the Airy Disk. The scale bar and RMS are in micrometers (μm).

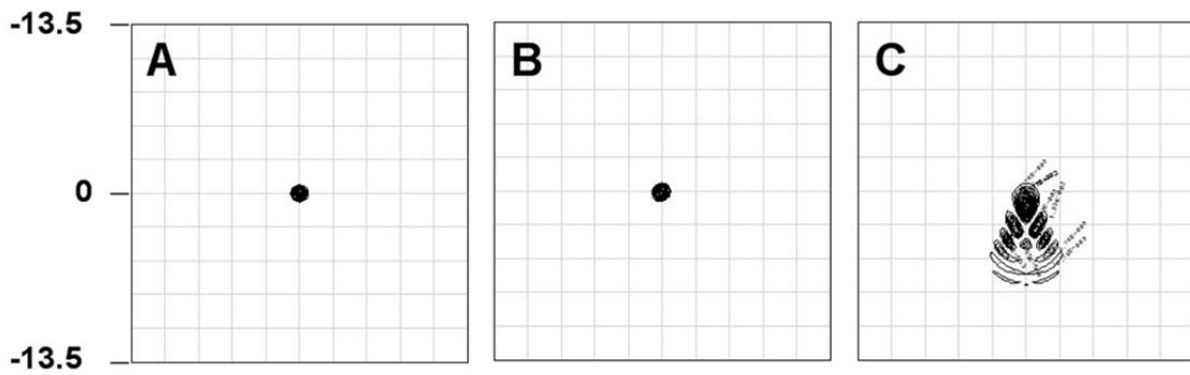


Figure 5.2.3. Surface contour of the fast Fourier transform (FFT) of the Huygens point spread function (PSF) at the image plane. A) ideal condition, B) 0.5 mm decenter each in X-Y directions, and C) 0.5° tilt with respect to the optical axis of the lens. The scale bar is in micrometers (μm).

Figure 5.2.3 shows the surface contours of the Huygens point spread functions (PSF) for the three configurations. It estimates the intensity of the focused laser beam when diffraction is accounted for. It can be concluded from Figures 5.2.3(A) and (B) that the 0.5 mm decentered laser beam and the ideal condition are very similar in terms of intensity distribution as the intensity of the focused beam predominantly lies in a tight spot in both the cases. However, in the case of the laser beam being tilted with respect to the optical axis of the lens, the surface contour shows a distribution of intensity forming the shape of a tear drop, Figure 5.2.3(C), which resembles the cat's paw structure formed due to multiple fragmentations observed in nanoholes in Figure 5.1(c). Based on these results, it can be concluded that an angle as small as 0.5° between the incident laser beam axis and the optical axis of the aspheric lens can lead to comatic aberration in the focal region of the laser beam. Furthermore, the comatic aberration in the focal region of the laser beam leads to multiple fragmentations of the intensity distribution resulting in the formation of cat's paw structure.

5.3) Aspheric lens machining with zoom lens and optical modeling

Optical modeling shows that aspheric lens machining of fused silica is very sensitive to any tilt between the optical axis of the lens and the laser beam propagation axis. Thus, it is imperative to remove any angular deviation between the laser beam axis and the optical axis of the aspheric lens to conclusively study the role of spherical aberration on nanohole formation. In order to obtain precise beam alignment to eliminate comatic aberration, a new platform was designed by Dr. Lloyd Davis for aspheric lens machining experiments, which also allowed the manipulation of spherical

aberration in the focal region of the laser beam. A set of three lenses, two identical plano-concave ($f = -100$ mm) and one biconvex ($f = 100$ mm), referred to as **zoom lens** from this point onwards, was placed before the aspheric lens in the machining platform. A photograph of the zoom lens and the substrate stage is shown in Figure 5.3(a). Zemax software was used to model the zoom lens to find different configurations, i.e., lens separations, for which spherical aberration was either minimum or significant in the focal region of the laser beam. Conditions for both positive and negative longitudinal spherical aberration were determined using Zemax. Configurations obtained by Zemax modeling, i.e., with and without spherical aberration, were subsequently used for laser machining experiments of fused silica substrates. Fused silica substrates thus machined were characterized by SEM analysis of the laser damaged regions and the respective cellulose acetate replicas to determine the size of the nanoholes. Results obtained were compared and analyzed to understand the role of spherical aberration on the formation of high aspect ratio deep nanoholes.

The aspheric lens machining experiments with the zoom lens were performed in collaboration as part of the ongoing research at the Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, under two user proposals [205, 206]. The machining experiments were carried out by the collaborators, whereas optical modeling to determine the machining conditions (i.e., the separations between the lenses in the zoom lens to alter the amount of spherical aberration in the focal region of the laser pulse) was done by the author at the University of Tennessee Space Institute. The amplified femtosecond laser system used for machining was different from the one discussed in section 3.1 and so were the laser beam characteristics and the LabVIEW™

program. This system consists of a 527 nm Q-switched Nd:YLF pump laser (Evolution, Coherent, Inc.), a titanium-sapphire laser oscillator (Micra, Coherent, Inc.), and a titanium-sapphire regenerative amplifier (Legend, Coherent, Inc.). The output from the amplifier is a train of up to 1 kHz laser pulses of wavelength 800 nm; but the laser is operated so as to give a single pulse on-demand. The pulse duration is 40 femtoseconds with maximum energy per pulse of 1.6 mJ. The laser system is shown in Figure 5.3(b) and a screenshot of the LabVIEWTM program used to manipulate the substrate is shown in Figure 5.3(c). The LabVIEWTM program also generates a trigger pulse (from a DAQ card) to command the laser to give a single pulse when needed.

In order to understand the role of spherical aberration on nanohole formation, it is necessary to machine and compare nanoholes made with a laser beam exhibiting no longitudinal spherical aberration (LSA) and one exhibiting significant LSA in its focal region, while at the same time taking care to maintain the same numerical aperture and to avoid overfilling of the focusing lens, which leads to energy deposited around the focal point due to diffraction. In the present study, a zoom lens consisting of three spherical lenses is used to obtain minimum LSA and significant LSA in the focal region of the laser beam focused through an aspheric lens. There are two signs of LSA: negative LSA and positive LSA. Negative LSA (also called overcorrected spherical aberration) results when the marginal rays refract *less* than the paraxial rays, whereas positive LSA (undercorrected) results when the marginal rays refract *more* than the paraxial rays. Changing the degree of collimation of the beam that enters the aspheric focusing lens is an effective way to introduce a significant amount of LSA in the focal region. A converging laser beam focused through the aspheric lens (but fully filling the

entrance pupil) results in positive LSA, whereas a diverging laser beam results in negative LSA. Thus, in the present study, negative and positive LSAs are intentionally introduced in the focal region of the laser beam by varying the amount of collimation.

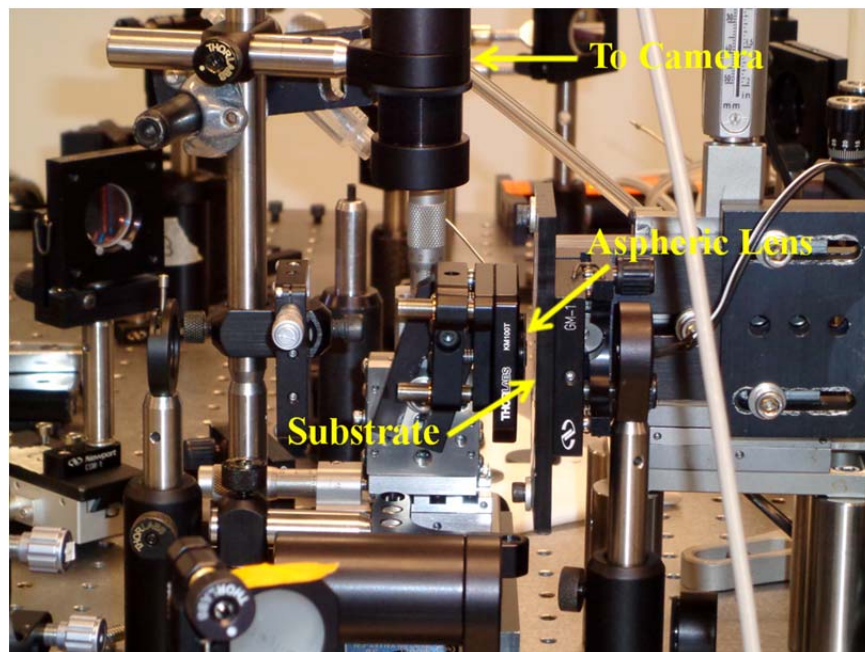
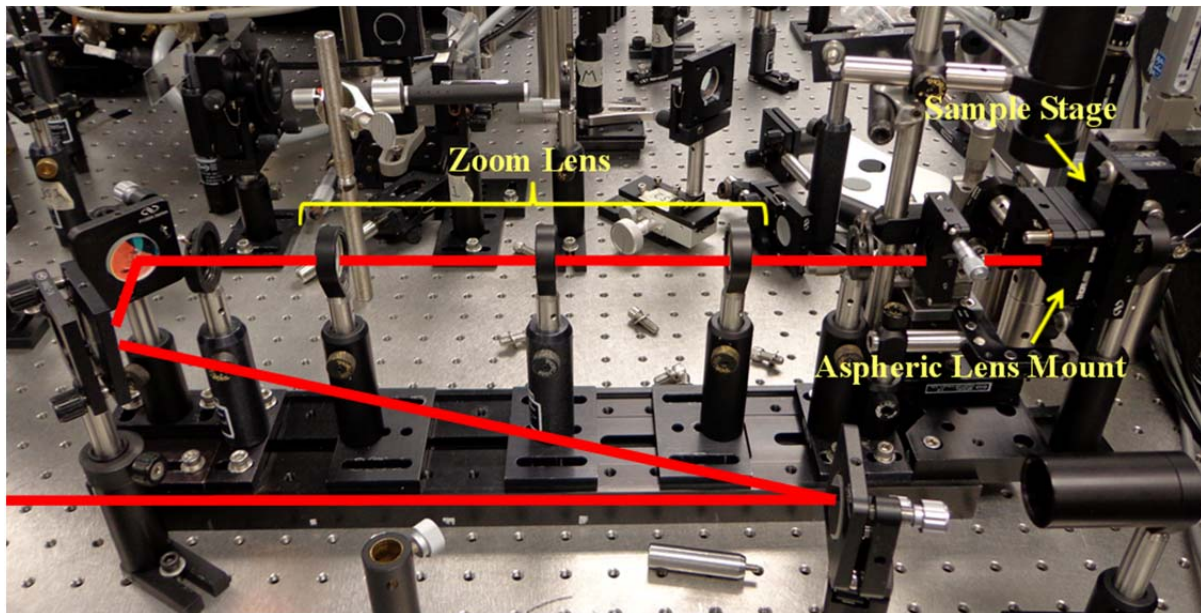


Figure 5.3(a). Photographs of the set-up used for machining with aspheric lens. Top photograph shows the beam path originating from the amplified femtosecond laser set-up (bottom left), passing through the zoom lens, and focusing on the substrate (mounted on a motorized stage) through the aspheric lens. A front view close-up of the aspheric lens and the substrate stage is shown in the bottom photograph.



Figure 5.3(b). A photograph of the amplified femtosecond laser set-up used for aspheric lens machining of fused silica. The output is a train of 1 kHz amplified femtosecond laser pulses of wavelength ~ 800 nm, temporal length 40 fs, and maximum pulse energy ~ 1.6 mJ.

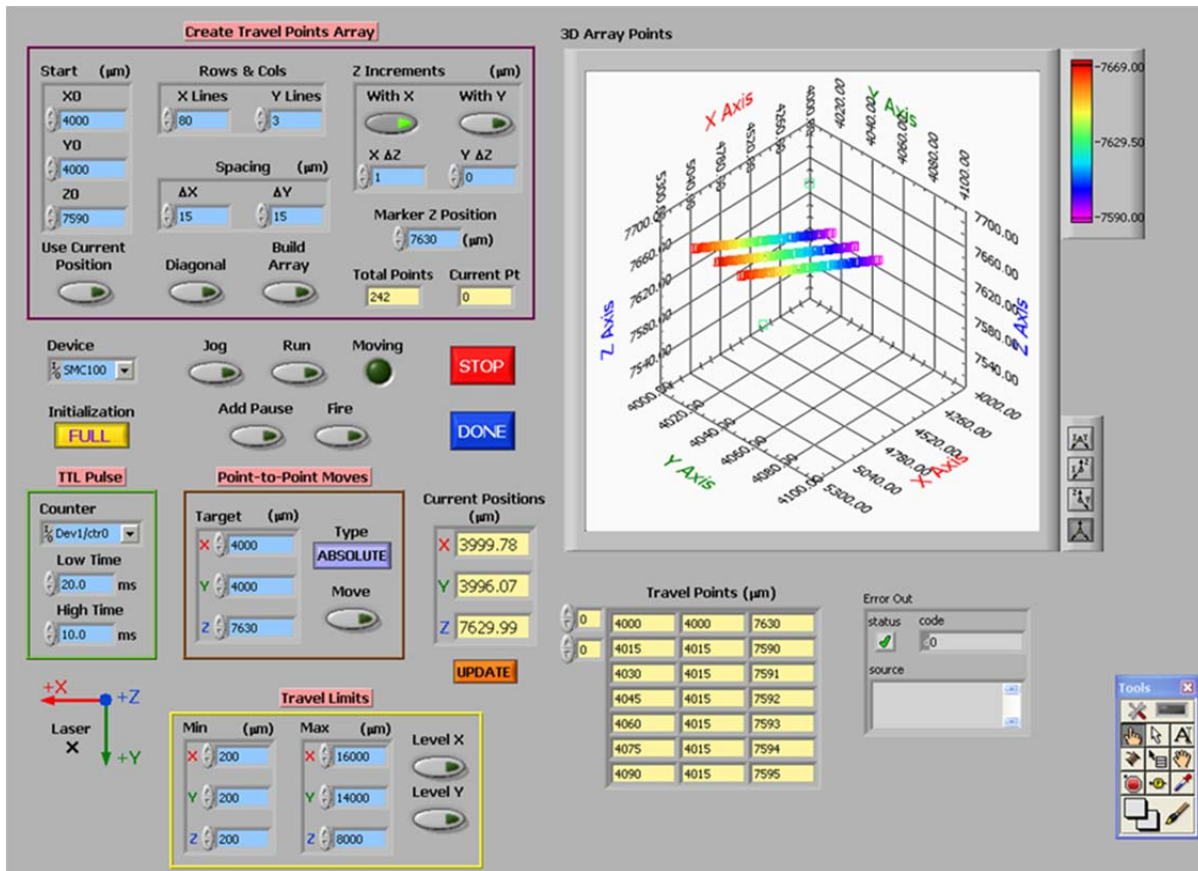


Figure 5.3(c). A screenshot of the graphical user interface (GUI) of the LabVIEW™ program used for aspheric lens machining of fused silica (developed by Dr. Brian Canfield).

The lens separations in the zoom lens required to obtain these three conditions (positive LSA, minimum LSA, and negative LSA) were found with the help of Zemax modeling. In the Zemax model, the lens separations were optimized so that the aspheric lens pupil is fully filled and so as to give the greatest divergence, maximum collimation, and greatest convergence of the beam entering the aspheric lens, which in turn results in most positive LSA, close to zero LSA, and most negative LSA in the focal region. The diameter of the laser pulse ($1/e^2$ value) and the beam divergence required for Zemax modeling were measured to be 6.44 mm and -0.122 milliradians, respectively, by using a power ratio method, which is explained in Appendix IV together with detailed calculations. The zoom lens decreases the beam size to exactly fill the pupil of the asphere, without overfilling it, and it also adjusts the collimation of the beam before the asphere and hence the longitudinal spherical aberration in the focal region of the laser pulse. Since the zoom lens is mounted on a rail of fixed length, the maximum achievable positive and negative LSAs are limited. The three conditions found by Zemax modeling used for aspheric lens machining experiments are: negative LSA of -49 μm , close to zero LSA (termed *best collimation*) of +2 μm , and positive LSA of +27 μm . Figure 5.3(d) shows the Zemax ray tracing of these three conditions and the corresponding Huygens point spread function.

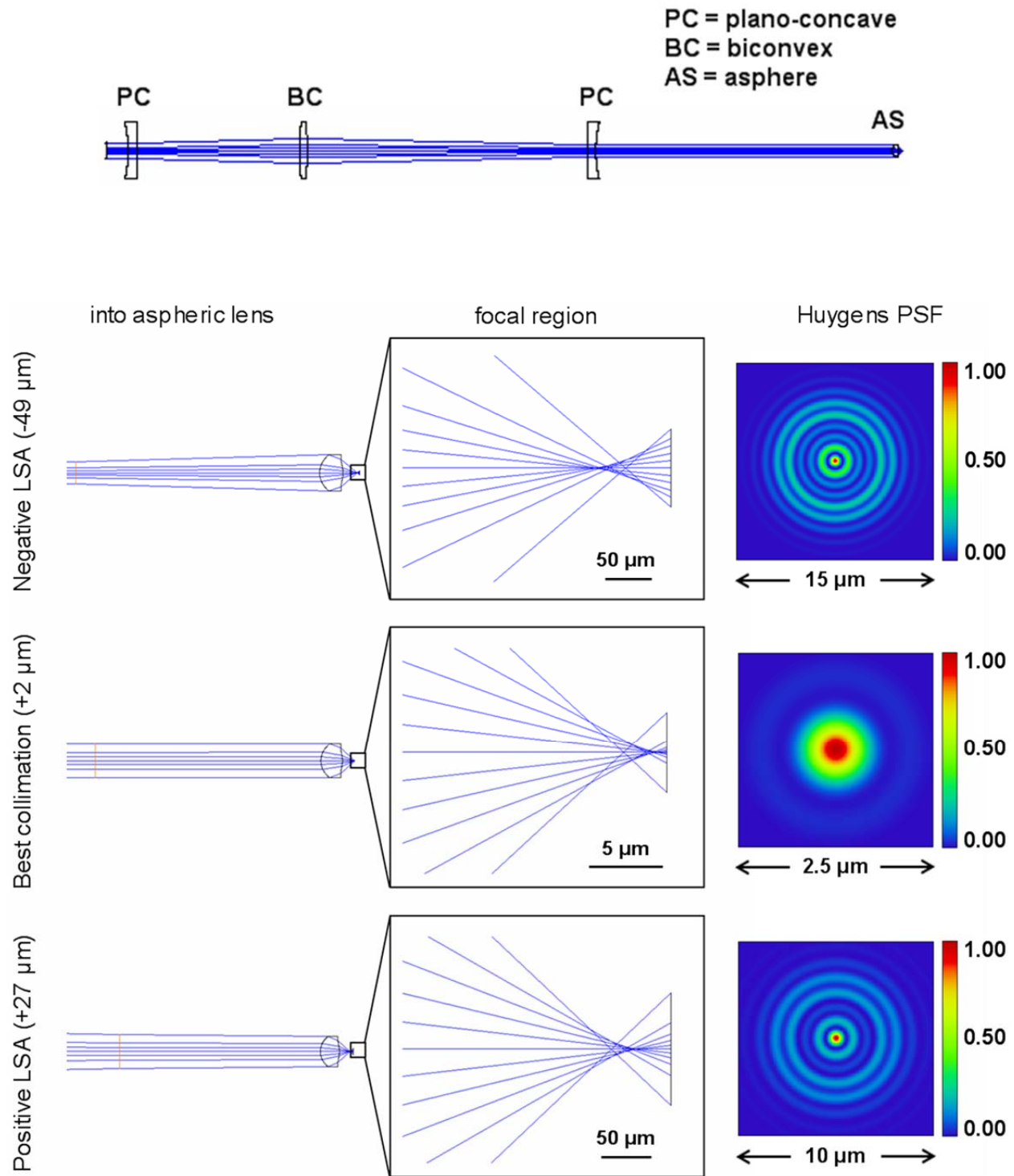


Figure 5.3(d). Zemax layout showing the zoom lens and the aspheric lens (top). Zemax ray tracing of 800 nm wavelength laser beam entering the aspheric lens after going through the zoom lens (bottom). The Huygens PSF is plotted at the focal point from the front of the lens on the optical axis for the three machining conditions. The distance from the front of the lens to the focal position for each of these conditions are: 1.9 mm for negative LSA, 1.76 mm for best collimation, and 1.711 mm for positive LSA.

The aspheric lens machining experiments under these three conditions (negative LSA, best collimation, and positive LSA) were done at three different values of laser pulse energy (1.2 μJ , 3.5 μJ , and 38 μJ) after aligning the optical elements and the substrate. In order to remove any comatic aberration arising due to angular deviation between the laser beam path and the aspheric lens optical axis, the three lenses of the zoom lens and the aspheric lens were aligned with the help of a green laser pointer (532 nm wavelength). The green laser was chosen because it is in the visible range and is reflected by the aspheric lens coated with an anti-reflective coating for the 600 nm – 1050 nm wavelength range. Furthermore, the optical leveling (described in section 3.3) of the fused silica substrate was also performed with the green laser beam in lieu of the stylus, so that identical holes are machined for analysis. Note that the calibration of the surface profile with the stylus prior to femtosecond machining provides the advantage that the focal position of the sample can be adjusted to compensate for surface irregularities in the substrate. SEM images of the cellulose acetate replicas of the aspheric lens machined nanoholes are shown in Figure 5.3(e).

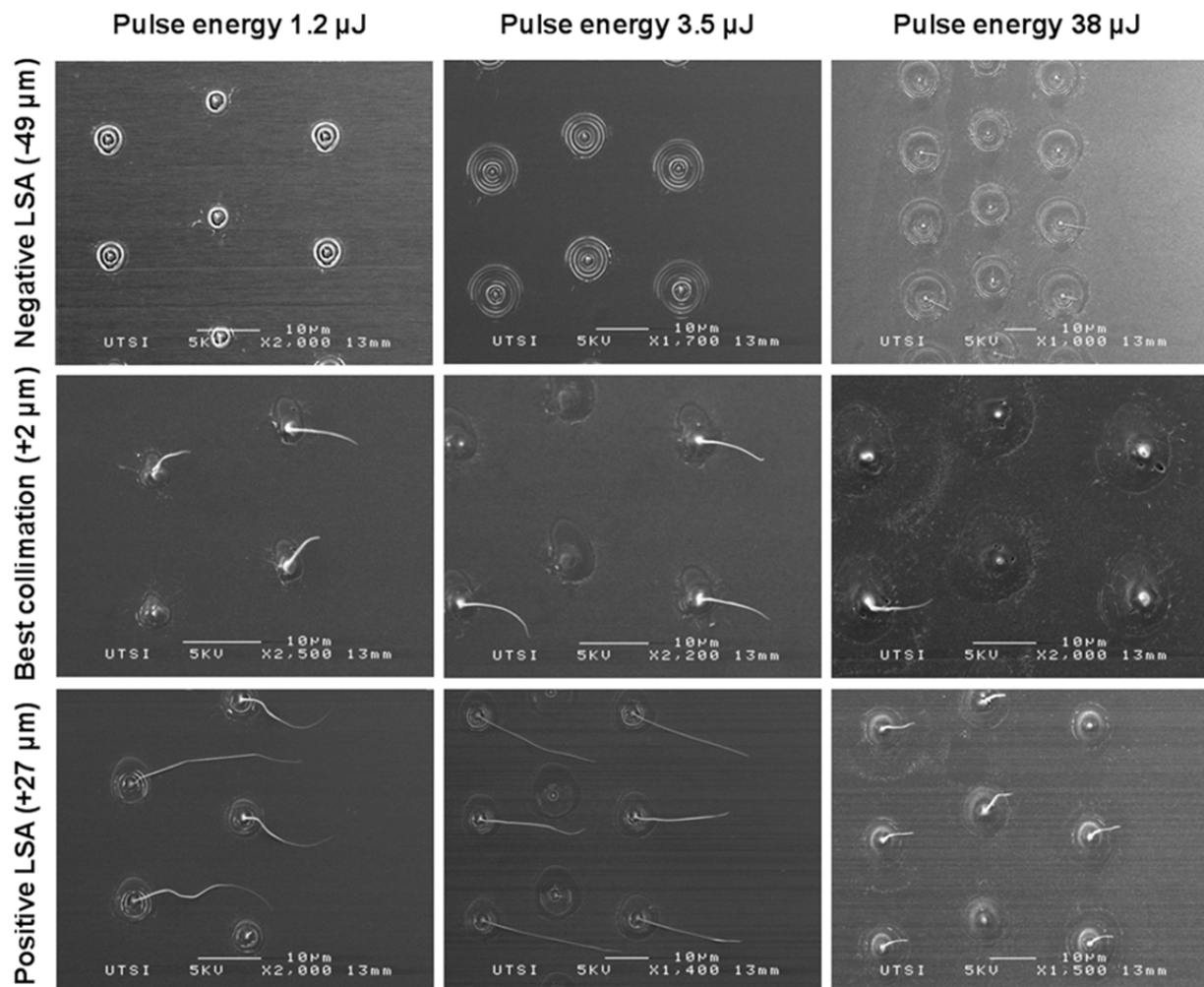


Figure 5.3(e). SEM images of cellulose acetate replicas of nanoholes made at the surface of fused silica by aspheric lens machining. These images were taken at zero degrees stage tilt.

5.3.1) Negative LSA (-49 μm)

SEM images of the CA replicas show that the aspheric lens machining of fused silica with negative LSA did not usually produce deep nanoholes for the three pulse energies tested. However, some of the nanoholes made with 38 μJ appear to be $\sim 8\ \mu\text{m}$ deep from their CA replicas. The most striking feature of the nanoholes made with negative LSA is the appearance of concentric rings in the laser damaged region (or the *laser footprint*) as seen from their CA replicas. The concentric rings appear to be due to diffraction of the laser pulse and can be seen in the Huygens point spread function (PSF) in Figure 5.3(d) for negative LSA.

5.3.2) Best collimation (+2 μm)

It can be deduced from the SEM images, shown in Figure 5.3(e), that the nanoholes produced in best collimation condition (+2 μm LSA) are considerably deeper than the expected depth of focus of the tightly focused laser beam and are also deeper than the shallow crater that is usually observed from a self-limiting over-dense laser-induced plasma. The average length of the CA nanowires imprinted from these nanoholes is 10 μm for all the values of laser pulse energy, thus the nanoholes are at least up to 10 μm in depth.

5.3.3) Positive LSA (+27 μm)

It can be seen from the SEM images that even deeper nanoholes are produced with positive LSA. For LSA of +27 μm and pulse energy of 3.5 μJ , the length of the CA nanowires was measured to be $\sim 32\ \mu\text{m}$, which indicates that the nanoholes are at least as deep as 32 μm . Furthermore, the nanoholes made at 1.2 μJ pulse energy with the positive LSA of +27 μm also appear to be around 30 μm deep as indicated by their

cellulose acetate replicas. However, the nanoholes made by 38 μJ pulse energy are only $\sim 8\ \mu\text{m}$ in depth. The size of the laser footprint, as depicted by the CA replica, was found to increase with laser pulse energy irrespective of the sign and the magnitude of LSA. The depth of the nanohole, however, was not found to increase with the laser pulse energy. In fact, the deepest nanoholes were machined with 1.2 μJ (30 μm deep) and 3.5 μJ (32 μm deep) laser pulse energy values compared to 38 μJ (8 μm deep) in positive LSA conditions.

5.4) Discussion

The formation of 10 μm deep nanoholes in fused silica with essentially no spherical aberration in the focal region (best collimation condition, LSA +2 μm) indicates that spherical aberration is not required to create holes deeper than the depth of focus of the beam. For a diffraction-limited focused Gaussian beam, in the paraxial approximation ($NA = \sin \theta \approx \theta$ (radians)), the depth of focus is two times the Rayleigh range, $2z_R = 2\lambda/(n\pi NA^2)$, where z_R is the Rayleigh range, $\lambda = 0.8\ \mu\text{m}$ is the laser wavelength, $n = 1.47$ is the refractive index of fused silica, and NA is the numerical aperture of the focusing lens. For the aspheric lens with $NA = 0.68$, this gives $2z_R = 0.75\ \mu\text{m}$. However, the plasma may be created over a greater depth provided the laser irradiance is above the damage threshold, which is about $10^{13}\ \text{W}/\text{cm}^2$ for fused silica. Figure 5.4(a) shows the irradiance of the femtosecond laser pulse as a function of axial position around the focal region for the best collimation condition (focus at 1.76 mm) in air computed using Zemax for a pulse energy of 1.2 μJ . It can be seen that the depth over which the irradiance is above the damage threshold is more than 19 μm . This

depth of damage threshold (ΔZ) is adequate to account for the observed nanohole depths (10 μm) made by focusing laser pulses with essentially no spherical aberration provided that the laser pulse energy can propagate through the plasma. While only a shallow feature might be expected because laser-induced plasmas are reflective if overdense (when the electron density is high enough such that the plasma frequency is above the optical frequency), in the case of breakdown induced by a femtosecond laser pulse, deeper features could be produced if a significant amount of the pulse energy propagates before the plasma is created, as has been predicted in a previous report [207].

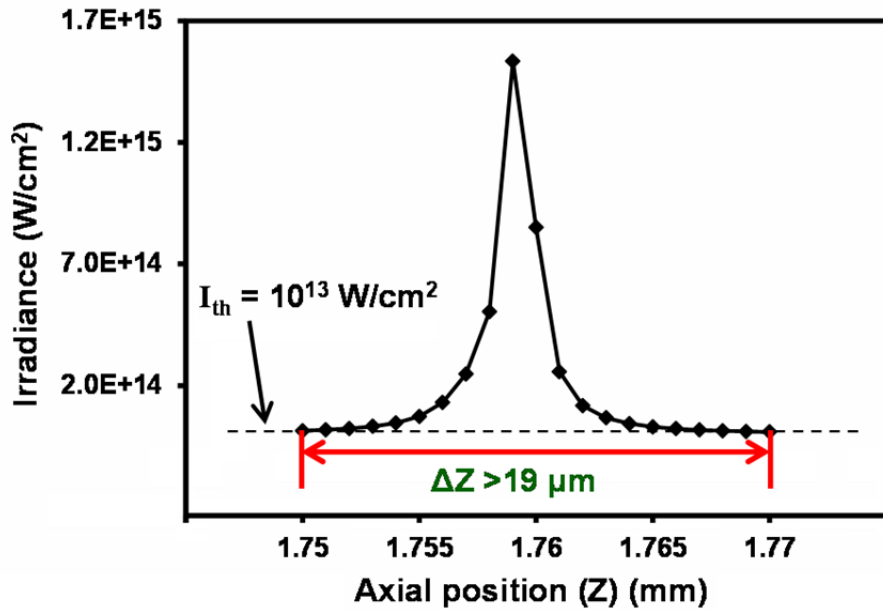


Figure 5.4(a). Irradiance profile of the femtosecond laser pulse with +2 μm LSA (best collimation) as a function of axial position near the focal region (focal position = 1.76 mm). The depth of damage threshold (ΔZ) was found to be $>19 \mu\text{m}$. The damage threshold for fused silica is $I_{\text{th}} = 10^{13} \text{ W/cm}^2$.

When significant positive spherical aberration is introduced in the focal region of the laser pulse (LSA of +27 μm), the depth of the nanoholes was found to be up to 32 μm , which is much deeper than those made with no or negative spherical aberration. The increase in the nanohole depth occurs even though the calculated depth of damage threshold is less than that for no spherical aberration. When significant negative spherical aberration is introduced (LSA of -49 μm), the nanoholes were found to be very shallow at pulse energies of 1.2 and 3.5 μJ and up to about 8 μm for 38 μJ . As seen in Figures 5.4(b) and (c), the depth of damage threshold in air along the axis was calculated to be ~ 14 μm for LSA of +27 μm and ~ 15 μm for LSA of -49 μm . However, it should be noted that in all cases, refraction of the beam as it enters the surface induces spherical aberration near the focus that is not accounted for in the above calculations. It is possible that this surface-induced aberration may further extend the depth of damage threshold along the axis to enable >30 μm deep nanoholes to be created in fused silica for the case of initial positive spherical aberration. Also, the above calculations do not account for non-linear effects, such as Kerr-induced self-focusing and/or possible beam front reshaping into a diffraction-free Bessel beam at the surface of fused silica. Such effects may contribute as mechanisms for deep-hole formation.

In summary, spherical aberration has been found to significantly alter the depths of the created holes, with positive LSA creating deeper holes than no or negative LSA. However, more investigation is required to understand the mechanisms of these findings.

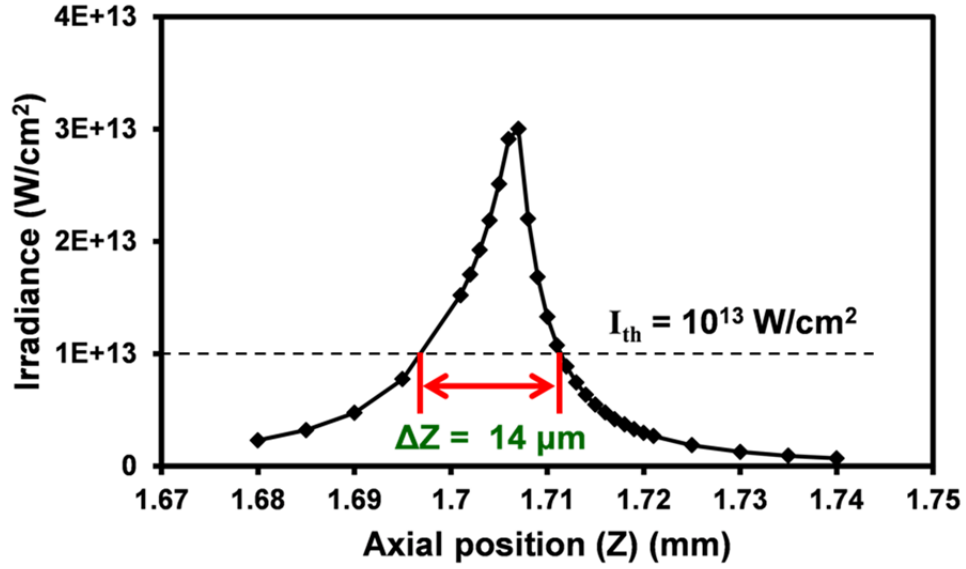


Figure 5.4(b). Irradiance profile of the femtosecond laser pulse with +27 μm LSA as a function of axial position near the focal region (focal position = 1.711 mm). The depth of damage threshold (ΔZ) was found to be $\sim 14 \mu\text{m}$.

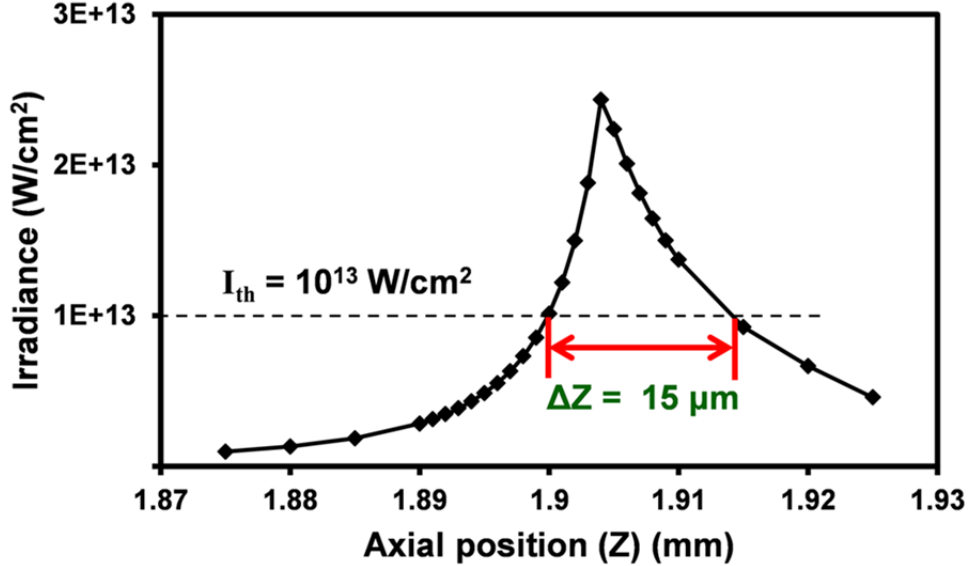


Figure 5.4(c). Irradiance profile of the femtosecond laser pulse with -49 μm LSA as a function of axial position near the focal region (focal position = 1.9 mm). The depth of damage threshold (ΔZ) was found to be $\sim 15 \mu\text{m}$.

Conclusions

Single-pulse amplified femtosecond laser machining has been developed into a new nanofabrication technology for production of templates on transparent substrates. It is essentially a direct-write nanolithography technique, which can pattern the surface of wide band gap materials like fused silica with user-defined arrangements of nanoholes with aspect ratios $> 10:1$ and depths $> 10\text{ }\mu\text{m}$. The lateral and vertical dimensions of the nanoholes are shown to be reasonably controllable during laser machining by regulating processing conditions and by chemical etching post-machining. The process is shown to be simple, economical, and less time consuming, thus making it ideal for large scale production of templates. Some of the salient features and applications of the single-pulse amplified femtosecond laser patterned templates investigated in this dissertation are summarized below.

1) The nanoholes made by amplified femtosecond laser single pulses of energy $5.2\text{ }\mu\text{J}$, pulse duration 160 femtosecond , and wavelength 780 nm focused through a microscope objective lens of numerical aperture $NA = 1.25$ in air are shown to be at least as deep as $14\text{ }\mu\text{m}$ by cellulose acetate replication of the patterned fused silica surface. It has been shown that the diameter and the depth of the nanoholes are affected by the relative position of the optical focus with respect to the substrate surface during laser machining. The deepest nanoholes are shown to form when the optical focus is within the bulk of the substrate at an optimized location from the substrate surface. Furthermore, the size and the morphology of the nanoholes made on fused silica substrates have been shown to modify by a combination of sequential etching in hydrofluoric acid and potassium hydroxide.

2) The templates made by single-pulse amplified femtosecond laser machining have been shown to be useful as molds for imprinting high aspect ratio polymer structures by replication procedures. Two replication methods for imprinting polymer structures have been demonstrated: film based replication and solution casting. The film based replication procedure uses commercially available polymer films, whereas solution casting is based on polymer solutions made in common organic solvents. Solution casting presents several advantages over film based replication, and is thus explored and presented in detail. Solution casting offers full control over the final chemistry of the imprinted polymer structures and also allows imprinting of thermoset polymers, which are otherwise not possible with film based replication. Nanocones and micropillars of polycaprolactone (PCL), PCL – polyethylene glycol (PEG), cellulose acetate (CA), polyethylene (PE), polyvinyl alcohol (PVA), polymethylmethacrylate (PMMA), Collodion, and polydimethylsiloxane (PDMS) have been successfully imprinted from as-machined and etched fused silica templates, respectively, by solution casting. PCL and PCL-PEG structures imprinted from as-machined templates have been found to stretch during imprint lift-off and take the form of nanocarpets or nanomats with very high surface area and aspect ratios greater than 200:1. All the aforementioned polymers except PCL and PCL-PEG adopt the shape and dimensions close to that of the nanoholes they are imprinted from though some stretching of the nanocones is also observed. Furthermore, most of the PDMS nanocones have been found to fall over, but without breaking or stretching, because of the low Young's modulus of PDMS. None of the polymer micropillars have been found to stretch, break, or fall over due to stiffness.

3) Polymer structures imprinted from amplified femtosecond laser machined fused silica substrates have been functionalized by coating with thin layers of gold, platinum, and palladium by sputter coating and silica by chemical vapor deposition. Sputter coating of noble metals was performed at cryogenic temperatures to avoid bending or collapsing of polymer nanowires by impact of fast moving ions. For this purpose, the sputtering system was modified with a liquid nitrogen cold finger so that the polymer nanowires become stiff below their glass transition temperatures and withstand the impact of ions during coating deposition. A low-temperature chemical vapor deposition process was designed for deposition of silica on cellulose acetate nanowires to fabricate silica nanowires. It is based on the hydrolysis of silicon tetrachloride molecules adsorbed at the surface of CA nanowires, which completes in two separate beakers and results in the formation of a thin layer of silica. The thickness of the silica coating was found to increase with the increase in the exposure time to silicon tetrachloride.

Silica nanowires produced by this technique have been demonstrated to be useful as synthetic cell culture substrates with tunable 3D microenvironment for cell behavioral studies. Studies done on NIH 3T3 mouse embryo fibroblasts show that the cells adhere and align along silica nanowires, thus making silica nanowires a promising material for tissue engineering. Furthermore, focused ion beam sectioning of a silica nanowire tip demonstrated the possibility of fabricating open-tip silica nanoneedles that can find applications as cellular patch clamps for intravital imaging and electrophysiological studies.

4) The role of spherical aberration on the formation of high aspect ratio and deep nanoholes was investigated with the help of aspheric lens machining and optical modeling using Zemax software. An aspheric lens of $NA = 0.68$ was used to machine nanoholes at the surface of fused silica by varying the amount of longitudinal spherical aberration (LSA) in the focal region of the laser pulse determined by optical modeling. Negative LSA, minimum possible LSA, and positive LSA were intentionally introduced in the focal region of a 40 femtosecond laser pulse of wavelength 800 nm by varying the collimation at the entrance pupil of the aspheric lens. The depths of the nanoholes thus produced were determined by cellulose acetate replication, and it was observed that the deepest nanoholes were made by positive LSA, the next deepest by minimum LSA and the shallowest by negative LSA. The nanoholes made by minimum LSA of $+2\ \mu\text{m}$ were found to be $10\ \mu\text{m}$ deep for pulse energy in the range $1.2 - 38\ \mu\text{J}$, which indicates that spherical aberration is not required to create holes deeper than the depth of focus of the beam. However, provided that sufficient pulse energy can pass through or around the plasma, the calculated depth of damage threshold along the axis is adequate to account for the observed hole depths. When positive longitudinal spherical aberration of $27\ \mu\text{m}$ is introduced in the focal region of the femtosecond laser pulse, the depth of the nanoholes increases to $30 - 32\ \mu\text{m}$ for pulse energies of $1.2 - 3.5\ \mu\text{J}$, even though the calculated depth of damage threshold decreases to $14\ \mu\text{m}$.

It is concluded from this study that the formation of deep and high aspect ratio nanoholes cannot be exclusively attributed to the presence of spherical aberration in the focal region of the laser pulse though hole-depth was found to increase with the increase in positive longitudinal spherical aberration up to a certain pulse energy.

Transmission of a significant part of the pulse energy through or around the first part of the laser-induced plasma as well as self-focusing due to Kerr nonlinearity and/or nonlinear laser pulse reshaping into a non-diffractive Bessel beam could also be important in the formation of deep and high aspect ratio nanoholes at the surface of fused silica by single-pulse femtosecond laser ablation.

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Appendices

Appendix I

Silica Coating of Polymer Nanowires Produced via Nanoimprint Lithography from Femtosecond Laser Machined Templates

(This paper was published in the journal **Nanotechnology**, IOP Publishing)

Silica coating of polymer nanowires produced via nanoimprint lithography from femtosecond laser machined templates

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Received 27 November 2011, in final form 18 January 2012

Published 24 February 2012

Online at stacks.iop.org/Nano/23/105304

Abstract

In this paper we report on the fabrication of regular arrays of silica nanoneedles by deposition of a thin layer of silica on patterned arrays of polymer nanowires (or polymer nanohair). An array of high-aspect-ratio nanoscale diameter holes of depths greater than $10\ \mu\text{m}$ was produced at the surface of a fused silica wafer by an amplified femtosecond laser system operated in single-pulse mode. Cellulose acetate (CA) film was imprinted into the nanoholes and peeled off to form a patterned array of standing CA nanowires, a negative replica of the laser machined nanoholes. The cellulose acetate replica was then coated with silica in a chemical vapor deposition process using silicon tetrachloride vapor at 65°C . Field emission scanning electron microscopy, focused ion beam sectioning, energy dispersive x-ray analysis and Fourier-transform infrared spectroscopy were used to characterize the silica nanoneedles. Precisely patterned, functionalized arrays of standing silica nanoneedles are useful for a number of applications.

1. Introduction

The ability to produce a patterned array of high-aspect-ratio, nanoscale diameter holes at the surface of fused silica by single-pulse femtosecond laser machining was first demonstrated by White *et al* [1]. Nanoholes more than $10\ \mu\text{m}$ deep were produced by using a high numerical aperture ($\text{NA} = 0.85$) microscope objective to focus 160 fs laser pulses onto the surface of fused silica. Cellulose acetate (CA) replication and DualBeamTM scanning electron microscopy/focused ion beam (SEM/FIB) sectioning confirmed the formation of high-aspect-ratio nanoholes. Herbstman *et al* [2] performed similar experiments by focusing single-shot 600 fs laser pulses using a microscope objective with $\text{NA} = 0.65$ on a Corning 0211 glass cover slip. CA replication showed the formation of $8\ \mu\text{m}$ deep holes when the laser beam was focused on the back side of

the glass cover slip. Furthermore, single-shot femtosecond laser machining using diffraction-free Bessel beams has been reported to produce nanoholes up to $20\text{--}30\ \mu\text{m}$ in a borosilicate glass [3, 4]. Surface patterning of glass with high-aspect-ratio nanoscale diameter holes has been envisioned for applications in nanofluidics, nanophotonics, and nanoimprinting. Patterned arrays of standing nanowires produced by nanoimprinting have a large number of potential applications as an engineered surface, e.g. for membranes. Arrays of nanowires patterned and functionalized for cell growth and differentiation purposes can further open the design and innovation space in cellular biology.

Silica and silica-coated materials constitute a special class of materials which are used in a variety of biological and biomedical applications [5–8]. Silica is chemically inert and can be easily functionalized by covalently bonding the $-\text{OH}$ groups on the surface to a variety of active biomolecules.

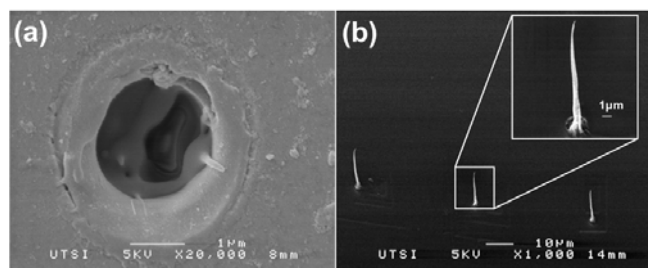


Figure 1. SEM images of (a) a nanohole produced on the surface of fused silica by single-pulse femtosecond laser machining and (b) cellulose acetate nanowires produced by nanoimprinting (Pd coated, 45° stage tilt-JEOL 6320 FE-SEM).

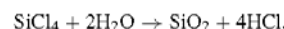
Functionalized silica allows further conjugation with other functional groups and biological species, thus making silica a material of choice for many bioapplications, for example biosensing, bioimaging, and drug targeting [9–12]. Surfaces of nanoparticles and quantum dots are often coated with silica to modify or enhance surface properties [13–16]. Nanoscale silica has been successfully produced by chemical vapor deposition (CVD) [17], catalyst-controlled room-temperature CVD [18], microemulsion [19], atomic layer deposition [20], sol–gel synthesis (Stober’s method) [21], and deposition from aqueous solution [22]. Silica coatings for bioapplications are most commonly produced by solution-based methods of sol–gel and microemulsion. Amorphous silica nanowires have been successfully produced by laser ablation [23], catalyst-controlled thermal evaporation [24, 25], high-temperature catalytic chemical vapor deposition [26], and more recently, room-temperature catalyst-free CVD [27]. Precisely placed, periodic arrays of silica nanowires or nanoneedles have not been reported.

2. Experimental procedure

The femtosecond laser nanomachining of the fused silica templates was carried out in a class-1000 clean room, details of which can be found elsewhere [28, 29], with an exception that the motion system was a linear motor ANT95-50-XY nMT (Aerotech, Inc.). The amplified laser beam central wavelength was 780 nm and the single-shot, temporal pulse length was 160 fs. The average power (P_{avg}) of the laser beam at 250 kHz repetition rate (R) was measured to be 1.3 W, which gives an energy per pulse (E_p) of 5.2 μJ (P_{avg}/R). Double side polished fused silica of thickness 500 μm was used for all templates (Mark Optics, Inc.). The fused silica substrate was cleaned in acetone and 2-propanol and then affixed to an aluminum SEM stub and mounted on the computer controlled XY stage. Arrays of holes were patterned at the surface of the fused silica substrate at laser energy of 5.2 μJ per pulse using an infinity corrected, water immersion 160× microscope objective lens in air (NA = 1.25 in water, Leitz Wetzlar, Germany). The nanohole patterned fused silica template was used as a mold to extract a negative replica of CA nanowires by polymer casting. A few drops of acetone were placed on the fused silica template and then a

small piece of 35 μm thick cellulose acetate film (Electron Microscopy Sciences, Hatfield, PA, USA) was placed on the pattern. The acetone softens the CA polymer so that it fills the nanoholes. After 1 h the CA film was slowly peeled from the template surface and transferred to an aluminum SEM stub with the nanowires facing upwards. Figure 1 shows the scanning electron micrographs of the entrance of a nanohole and a CA replica obtained by nanoimprinting. The entrance of the nanohole shown in figure 1(a) is around 2 μm and this feature appears as a pedestal on the replica. The nanohole is less than 500 nm in diameter 2 μm into the fused silica and further tapers to around 100 nm at its tip in figure 1(b). The hole entrance feature is common in single-pulse femtosecond ablation and is speculated to arise from reshaping of the beam as the pulse interacts with the surface [30].

Seven replicas were extracted from a single fused silica template, six for coating and one as a control. The cellulose acetate nanowires were coated with silica to make high-aspect ratio silica nanowires in a simple two-step deposition process. Two beakers were placed on a hot plate maintained at 65 °C. Beaker 1 contained 10 ml of ~5 mol% SiCl₄ solution (in CCl₄) in a magnetically stirred scintillation vial, and beaker 2 contained a small quantity of de-ionized water. The CA nanowires were mounted on a metal block and placed above the solution in the first beaker so that the nanowires faced down as shown in figure 2. The sample was first placed inside beaker 1 and exposed to the SiCl₄ vapors for the exposure time (t_i) and then transferred to beaker 2 for a 5-min treatment in water vapor. The CA nanowires adsorb SiCl₄ on the surface in beaker 1 and then the adsorbed SiCl₄ is hydrolyzed in water vapor to form a silica coating in beaker 2 according to the following reaction:



3. Analysis of silica coating

The exposure time (t_i) in beaker 1 was varied from 1 to 6 min at regular intervals of 1 min to create six separate samples. The time in beaker 2 was kept constant at 5 min, sufficient to hydrolyze all the adsorbed SiCl₄ molecules in this series of experiments. Figure 3 shows the silica thickness on CA nanowires for each of the six samples. In order to

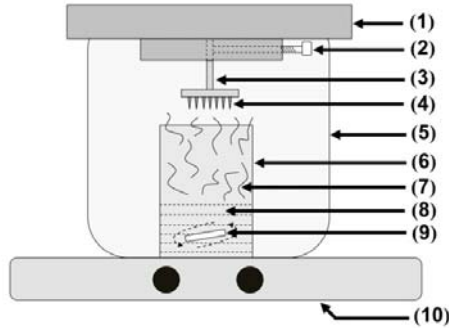


Figure 2. A schematic of the apparatus used for the evaporation of SiCl_4 on CA nanowires in beaker 1: (1) metal block, (2) screw, (3) SEM mounting stub, (4) CA nanowires, (5) beaker, (6) scintillation vial, (7) $\text{SiCl}_4 + \text{CCl}_4$ vapor, (8) ~ 5 mol% SiCl_4 in CCl_4 solution, (9) magnetic stir bar, (10) hot plate maintained at 65°C .

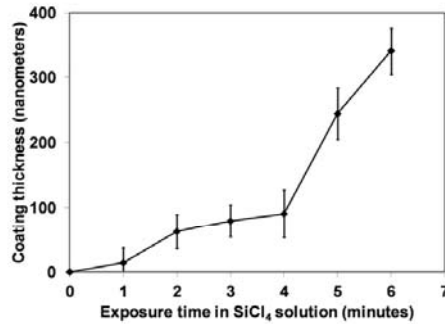


Figure 3. Silica deposition rate determined by statistical analysis of the coating thickness of silica-coated cellulose acetate nanowires as a function of soaking time in SiCl_4 solution. The measurements were made at projected length of $6\ \mu\text{m}$ above the base of the nanowires.

calculate the coating thickness, the diameters of silica-coated nanowires and the uncoated control samples were measured in the scanning electron microscope (SEM) after sputtering with a 20-nm layer of palladium. The SEM stage was tilted at 45° and the nanowire width was measured at $6\ \mu\text{m}$ projected length above the substrate. The actual length is the projected length divided by $\sin 45^\circ$, as shown in figure 4. A total of 30 measurements on separate nanowires were made at each soaking time (t_i) to determine the coating thickness (d_i) using the formula

$$d_i = \frac{D_{\text{SiO}_2}^{t_i} - D_{\text{CA}}}{2}$$

where $D_{\text{SiO}_2}^{t_i}$ is the width of the silica nanowire for the corresponding soaking time t_i and D_{CA} is the mean width of the uncoated cellulose acetate nanowires measured in the same manner on the control sample. The standard deviation in the coating thickness ($\sigma_d^{t_i}$) for each t_i was expressed in terms

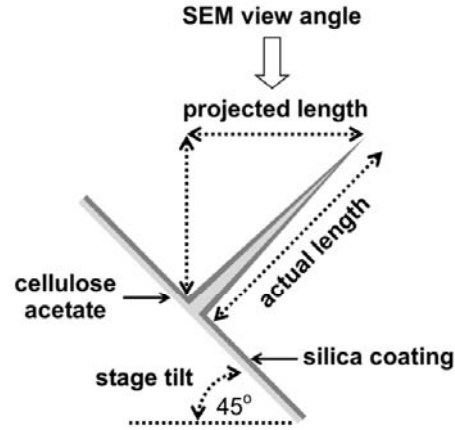


Figure 4. A pictorial description of a silica nanowire during SEM imaging. The SEM stage is tilted at 45° in order to image the silica nanowires. The length of the nanowire measured using the SEM image gives the projected length, which is then multiplied by $\sqrt{2}$ to get the actual length of the nanowire.

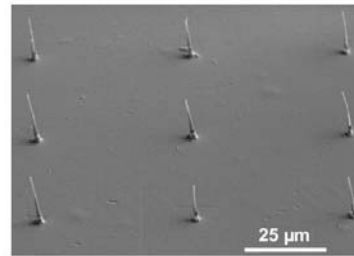


Figure 5. An array of silica-coated nanowires ($t_i = 5$ min) imaged at 45° stage tilt (palladium coated, JEOL JSM-7600F, Naoki Kikuchi, JEOL USA, Inc.).

of the standard deviation in the width of the silica-coated nanowire ($\sigma_{\text{SiO}_2}^{t_i}$) and the standard error in the mean width of the uncoated CA nanowire (σ_{CA}^m) using the equation

$$\sigma_d^{t_i} = \frac{\sqrt{(\sigma_{\text{SiO}_2}^{t_i})^2 + (\sigma_{\text{CA}}^m)^2}}{2}.$$

The standard deviation is shown in the error bars in figure 3. The graph shows that the deposition rate is slow for the first 3–4 min, which we believe is the incubation period for complete coverage of the nanowire, and then the film grows linearly at the rate of about $125\ \text{nm min}^{-1}$.

An array of silica-coated nanowires (coated for 5 min) is shown in figure 5. A scanning electron micrograph of the tip region sputter coated with palladium is shown in figure 6 along with an energy dispersive spectrometer (EDS) scan of the tip region. While not quantitative, the EDS spectrum does reveal the presence of silicon, oxygen, and the palladium coating.

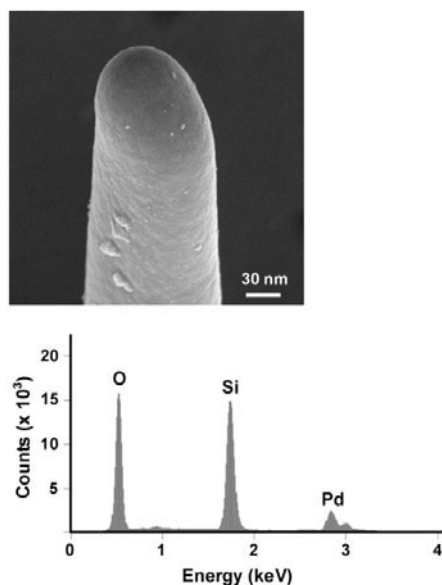


Figure 6. A SEM image and the EDS spectrum of the tip of a silica-coated nanowire (sputter coated with palladium for SEM analysis) confirmed the presence of silicon and oxygen, and the formation of silica. (Micrograph and EDS data by Naoki Kikuchi, JEOL USA, Inc., JSM-7600F with Thermo EDS System 7.)

A silica-coated nanowire was sectioned using a Hitachi NB5000 NanoDUE'T FIB-SEM. In figure 7(a) the silica tip of the cone was cut with a gallium current of 20 μ A, showing the CA nanowire at the center of the structure (see inset). Cuts were made further down the silica-coated nanowire in figures 7(b) and (c) where the silica and CA can be distinguished by contrast and the silica coating thickness and CA nanowire diameter are clearly seen. In figure 7(d) the milling angle was changed slightly to cut into the CA nanowire and reveal the interior of the silica nanoneedle.

The FIB-sectioned part shown in figure 7(d) reveals that the silica coating is around 125 nm thick and the CA nanowire is 175 nm in diameter. The FIB-sectioned hole appears elliptical because it was imaged at 58° from the

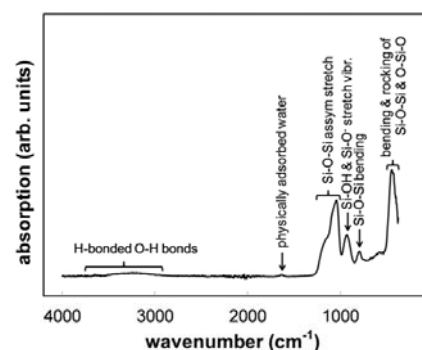


Figure 8. ATR-FTIR absorption spectrum of the silica coating showing the presence of various Si–O–Si and O–H vibration modes.

horizon in the FIB/SEM. This cut was made at the projected length of about 9 μ m from the substrate base.

Figure 8 shows the absorption spectrum of the silica coating in the frequency range 4000–375 cm^{-1} recorded on an attenuated total reflectance-Fourier transform infrared spectrometer (ATR-FTIR, model ALPHA-P, Bruker Optics). The broad band in the interval 3700–3000 cm^{-1} corresponds to the intensive overlapping of the stretching modes in hydrogen-bonded hydroxyl bands produced by O–H bonds in water and Si–OH [31, 32]. A small peak at $\sim 1635 \text{ cm}^{-1}$ can be attributed to the deformation vibrations of the O–H groups in physically adsorbed molecular water at the surface of silica coating [33] and its weak intensity confirms the very low amount of molecular water in the coating. A sharp peak at $\sim 927 \text{ cm}^{-1}$ appears due to the stretching vibrations of Si–OH or Si–O[−] groups [34], which indicates the presence of a large number of silanol groups on the coating surface. The band at 1000–1300 cm^{-1} has been reported to appear due to the anti-symmetric stretching of Si–O–Si bonds [35] and it can be resolved into a peak at 1041 cm^{-1} (TO₁) and a shoulder around 1156 cm^{-1} (TO₂). The TO₁ peak in SiO₂ is normally found at $\sim 1082 \text{ cm}^{-1}$ and its shifting towards lower frequencies (in the present case it shifted to 1041 cm^{-1}) has been associated with oxygen deficiency [36, 37] and lower three-dimensionality [38] in the structure of silicon oxide. This indicates that the silica coating is SiO_x with $x < 2$. The

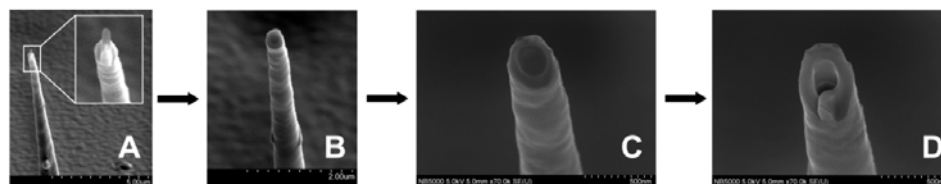


Figure 7. A silica-coated nanowire subjected to gallium ion sectioning in an SEM/FIB. Each photo shows a different section further down the nanocone from (a) to (d). (Micrographs and FIB sectioning by Jamil Clarke, Hitachi High Technologies America, Inc., Hitachi NB5000 NanoDUE'T FIB-SEM.)

peak at 799 cm^{-1} is due to the bending vibration of Si–O–Si bonds [35, 39]. A peak due to the bending modes in Si–OH bonds also appeared around $\sim 790\text{ cm}^{-1}$ [40]; however, it is very close to the Si–O–Si bending mode and cannot be seen clearly in the spectrum in figure 8. A series of peaks appears in the lower frequency region from 448 to 424 cm^{-1} , which could have arisen due to the combination of bending and rocking modes of Si–O–Si or O–Si–O bonds in the coating [40–42].

4. Discussion

In metallic films nanoholes of aspect ratio up to 10:1 can be created using focused ion beams [43, 44] and an aspect ratio of 25:1 was achieved by Patterson *et al* [45]. The Bosch process for deep reactive ion etching can create deep structures in silicon with straight sidewalls but the masking techniques cannot maintain submicron entrance hole diameters [46]. In transparent materials holes of submicron size with aspect ratios greater than 10:1 cannot be made by any method other than femtosecond laser machining where 100:1 aspect ratios have been demonstrated [3]. In addition, the process is carried out at room temperature in the open laboratory; it does not require vacuum or reactive gases. Holes can be created at the pulse repetition rate of the laser (in this case 250 kHz for the Coherent RegA 9000) but the speed limitation of the practical process is the motion control system for sample manipulation. We can pattern a 1 cm^2 template with a $3\text{ }\mu\text{m}$ pitch of over 11 million holes in under half an hour. Replication of these nanohole templates follows normal imprinting and molding techniques; however, the ability to reproduce the high-aspect-ratio structures is totally unexpected [47]. The nanowire yields from nanoimprinting are close to 100% for lengths of $15\text{ }\mu\text{m}$ on an area of 1 cm^2 with very few wires broken or missing. While we have not done studies of mold life, tens of nanoimprints have been made from single templates. The fused silica molds are very robust and can be easily cleaned. All in all, the process of machining templates, nanoimprinting, and silica coating is amenable to low cost, volume production.

5. Conclusion

High numerical aperture femtosecond laser machining of fused silica is a fast and flexible direct-write method of fabricating nanoimprint templates for nanowire fabrication with virtually any pattern and spacing. We have developed a deposition methodology to coat cellulose acetate nanowires with silica. The silica coating is dense and exhibits a large number of surface silanol groups. Arrays of silica-coated nanowires produced by this technique can be functionalized and used as substrates for cell motility, differentiation and interaction studies. Topological gradient arrays of silica-coated nanowires are being explored for cell interaction studies in mouse embryo fibroblasts (NIH 3T3). Opening the silica nancone tips by FIB (as shown in figure 7) enables the fabrication of silica nanoneedles. The array of open-tip silica nanoneedles with cellulose acetate removed

can be transferred and attached to a microfluidic platform where individually addressable silica nanoneedles could be used to inject bioactive molecules into living cells. Given the high dielectric strength of silica, addressable hollow silica nanoneedles may prove suitable as cellular patch clamps in single-cell electrophysiology studies.

Acknowledgments

This work was supported by the Tennessee Higher Education Commission through a grant to the Center for Laser Applications, University of Tennessee Space Institute (UTSI). The authors acknowledge Dr George Owens (UTSI) for assistance with FTIR analysis and Dr George Murray (UTSI) for helpful discussions. The authors extend their gratitude to Naoki Kikuchi (JEOL USA, Inc.) for SEM/EDS analysis and Jamil Clarke (Hitachi High Technologies America, Inc.) for performing FIB sectioning and imaging.

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Appendix II

Cell Interaction Study Method Using Novel 3D Silica Nanoneedle Gradient Arrays

(This paper was published in the journal **Colloids and Surfaces B: Biointerfaces**,
Elsevier B.V.)



Short communication

Cell interaction study method using novel 3D silica nanoneedle gradient arrays

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ARTICLE INFO

Article history:

Received 12 April 2012

Received in revised form 19 July 2012

Accepted 25 July 2012

Available online xxx

Keywords:

Cell–cell interactions

Silica

Femtosecond laser

Nanoimprinting

Nanofabrication

Fibroblasts

ABSTRACT

Understanding cellular interactions with culture substrate features is important to advance cell biology and regenerative medicine. When surface topographical features are considerably larger in vertical dimension and are spaced at least one cell dimension apart, the features act as 3D physical barriers that can guide cell adhesion, thereby altering cell behavior. In the present study, we investigated competitive interactions of cells with neighboring cells and matrix using a novel nanoneedle gradient array. A gradient array of nanoholes was patterned at the surface of fused silica by single-pulse femtosecond laser machining. A negative replica of the pattern was extracted by nanoimprinting with a thin film of polymer. Silica was deposited on top of the polymer replica to form silica nanoneedles. NIH 3T3 fibroblasts were cultured on silica nanoneedles and their behavior was studied and compared with those cultured on a flat silica surface. The presence of silica nanoneedles was found to enhance the adhesion of fibroblasts while maintaining cell viability. The anisotropy in the arrangement of silica nanoneedles was found to affect the morphology and spreading of fibroblasts. Additionally, variations in nanoneedle spacing regulated cell–matrix and cell–cell interactions, effectively preventing cell aggregation in areas of tightly-packed nanoneedles. This proof-of-concept study provides a reproducible means for controlling competitive cell adhesion events and offers a novel system whose properties can be manipulated to intimately control cell behavior.

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1. Introduction

Studying a regulatory role of extracellular matrix (ECM) in cell behavior remains a key research area in developmental biology, biomedical engineering, and pharmacology [1,2]. Cells sense physical and chemical signals arising from surface features (e.g., topography, compliance, texture, and anisotropy) of ECM and synthetic culture substrates, resulting in changes of their morphology, transcriptional behavior, and/or motility that are responsible for developmental processes and wound healing [3–6]. Hence, it is essential to understand the characteristic interaction between cells and specific surface features to further advance cell biology and regenerative medicine.

Since the effect of substrate topography on cell behavior was first studied with development of cell culture in 1890–1910s [7,8], topographical control of cell behavior has been the subject of numerous review articles over the years [2,3,5,6,9–13]. The topographical features that have been studied range from unrefined

patterns (e.g., grooves and ridges) [7,8,14–17] to precise topographical patterns and geometric profiles that are generated by photo or electron beam lithography [13,18–20]. Recent studies on topographical control of cell behavior have been mostly done on 2D substrates in which the size of topographical features usually ranges from 10 nm to 3 μ m [3] in an array form of nanogratings, nanopillars, or nanoholes [12,21]. When these features are considerably larger in vertical dimension and are spaced at least one cell dimension apart, the features act as 3D physical barriers that specifically influence cell morphology, spreading, and alignment by guiding cell–matrix interaction (“contact guidance”), particularly observed in the case of fibroblast attachment and motility [22–25]. The contact guidance is mediated by an integrin–ligand interaction [26], resulting in alternation of cell attachment, orientation, viability, and motility [3,15].

Three types of biomaterials (i.e., metals, ceramics, and polymers) are used for cell culture studies [3,5,9]. In particular, polymers are considered an efficient template to generate nano/microscale patterns via soft lithography. For example, micropillar arrays of polydimethylsiloxane (PDMS) and silicon prepared via lithographic techniques are used to study cellular responses to 3D microenvironments [9,22,25,27]. Thermal properties of polymers can also be

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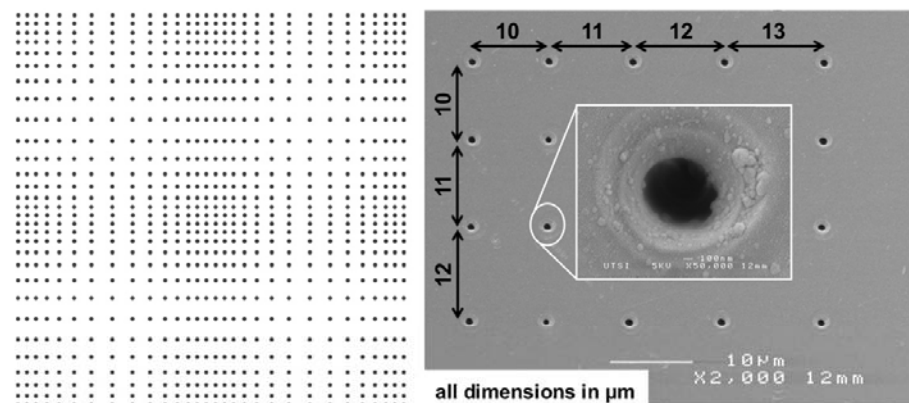


Fig. 1. Pattern prepared on fused silica substrate by single-pulse femtosecond laser machining. (Left) A schematic of the pattern displaying the 2D gradient in nanohole spacing (nanohole density reduced for visual clarity). The pattern is a 2×2 matrix of four quadrants, each formed by increasing the spacing between successive nanoholes by $1 \mu\text{m}$ starting from $10 \mu\text{m}$ in the densest location (edge) to $50 \mu\text{m}$ in the sparsest location (middle of the quadrant) and then decreasing from $50 \mu\text{m}$ to $10 \mu\text{m}$ at $1 \mu\text{m}$ decrements in both x and y directions. A quadrant is a matrix of 84×84 nanoholes, and a 2×2 matrix of these quadrants forms the pattern with 168×168 nanoholes. Each black dot in the schematic represents a nanohole made by focusing a single laser pulse. (Right) An SEM image of the nanoholes from one edge of the pattern.

used to produce micro- and nanoscale features, which influence cellular behavior [28].

In this study, we investigated the physical behavior of NIH 3T3 mouse embryonic fibroblasts on an array of patterned silica nanoneedles. We used a recently-developed nanomachining technique whose biological application has not been explored yet. The pattern was a spatial 2D gradient array wherein needle spacing was varied from $10 \mu\text{m}$ to $50 \mu\text{m}$ in one micron increments (spacing = $10, 11, 12, \dots, 50, 49, 48, \dots, 10 \mu\text{m}$) in both orthogonal directions. First, an array of nanoscale diameter holes (nanoholes) with 2D gradient spacing was prepared at the surface of fused silica by focusing single-pulses from an amplified femtosecond laser system using a high numerical aperture (NA) microscope objective lens (Fig. 1). The fused silica template was etched to remove damaged material and slightly enlarge the nanoholes. A negative replica of the template was extracted with cellulose acetate (CA) by molding a CA film into the pattern creating an array of standing CA nanoneedles (Fig. 2). A thin layer of silica was then deposited on CA nanoneedles by a low temperature chemical vapor deposition (CVD) process to form silica nanoneedles. This nanofabrication technique enables high resolution control of geometrical structures in a user-specified manner. NIH 3T3 fibroblasts were cultured on silica nanoneedles and their cell–cell and cell–matrix interactions were compared with those cultured on a flat silica surface prepared by the same CVD process. This proof-of-concept study provides a new gradient template method for controlling cell adhesion and function.

2. Materials and methods

2.1. Nanoneedle array preparation

An amplified femtosecond laser system was operated in single-pulse mode to pattern fused silica substrate of $500 \mu\text{m}$ thickness in air using a $160\times$ microscope objective lens ($NA = 1.25$ in water) with 780 nm laser pulse central wavelength, 160 fs temporal length, and $\sim 5.2 \mu\text{J}$ energy per pulse [29–31]. The pattern made on the template was a 2×2 matrix of quadrants, where each quadrant is a 2D gradient in nanohole spacing. In each quadrant, the spacing between the successive nanoholes, in both x and y directions, was first increased from $10 \mu\text{m}$ to $50 \mu\text{m}$ in $1 \mu\text{m}$ increments, and then reduced from $50 \mu\text{m}$ to $10 \mu\text{m}$ in $1 \mu\text{m}$ decrements, thus forming a 2D gradient in nanohole spacing. A schematic of the pattern (at

reduced scale) is shown in Fig. 1. The template was subjected to chemical etching in buffered oxide etch (Lodyne™, BOE, 6:1) for 30 s followed by rinsing in DI water, and then neutralized in 10M KOH for 3 min followed by rinsing in DI water (all at room temperature) prior to further use. Etching the template in BOE preferentially attacks laser affected regions in fused silica and widens the entrance of nanoholes. The depth of the nanoholes was determined to be at least $14 \mu\text{m}$ by cellulose acetate replication. The average entrance diameter was 750 nm post-etching.

A small piece of $35 \mu\text{m}$ thick cellulose acetate film was molded into the pattern using acetone solvent as shown in Fig. 2A. After a few hours the resulting film was peeled off from the template using a pair of tweezers to remove the negative replica, a patterned array of cellulose acetate nanoneedles. The replica film was then glued to a $170 \mu\text{m}$ glass coverslip using a thin layer of uncured base PDMS (Sylgard® 184, Dow Corning Corporation) as an adhesive. CA replica-on-PDMS-on-glass coverslip was then left at room temperature for 24 h for PDMS to crosslink for easy handling of the CA nanoneedles. The glass coverslip with CA replica was then placed on an aluminum SEM peg using a sticky tab (Lift-N-Press™, Structure Probe Inc., West Chester, PA) and subjected to a two-step chemical vapor deposition process at 65°C for silica deposition to form silica nanoneedles, details of which can be found in reference [29]. Briefly, CA replica was first exposed to silicon tetrachloride (SiCl_4) vapor for 5 min in a beaker and then to water vapor (H_2O) for 5 min in another beaker. Silicon tetrachloride molecules adsorbed at the surface of CA nanoneedles in the first beaker hydrolyze in the second beaker ($\text{SiCl}_4 + 2\text{H}_2\text{O} \rightarrow \text{SiO}_2 + 4\text{HCl}$) to form atop a thin silica coating of thickness $240 \pm 40 \text{ nm}$. Silica nanoneedles thus formed are shown in Fig. 2B and C, and were used as a cell culture substrate. Flat silica substrates were prepared with unpatterned glass coverslip as a template using the same method (CA cast attached to glass with PDMS, followed by chemical vapor deposition).

2.2. Cell experiments

NIH 3T3 fibroblasts were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Gibco Cell Culture, Invitrogen, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco) with 1% penicillin-streptomycin (P/S, Gibco) on tissue culture polystyrene in a water jacketed incubator at 37°C and 5% CO_2 . For experiments, cells were seeded at a density of

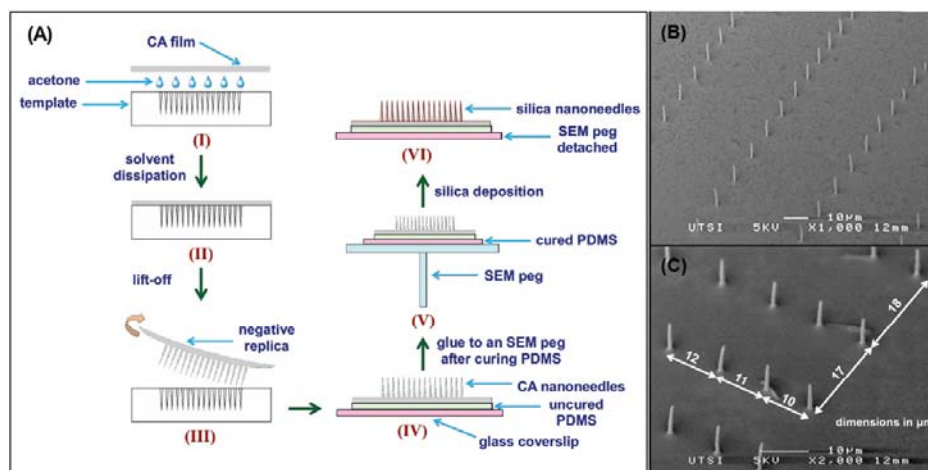


Fig. 2. Silica nanoneedle fabrication. (A) Schematic of the fabrication of silica nanoneedles starting from cellulose acetate (CA) replication of the laser patterned fused silica template (steps I and II). The replica with CA nanoneedles is then peeled off from the template and glued to a 170 μm glass coverslip with uncured PDMS, and cured at room temperature for 24 h (steps III and IV). The glass coverslip with CA nanoneedles is then affixed to an aluminum SEM peg (step V) and subjected to silica deposition to form silica nanoneedles. The glass coverslip with silica nanoneedles is then detached from the SEM peg by selectively dissolving the sticky tab adhesive in toluene. (B) SEM image of silica pattern in a specific location where nanoneedles are densely spaced in one direction and sparsely spaced in the orthogonal direction. (C) SEM images of silica nanoneedles (taken at 45° stage tilt) from a section of the 2D gradient pattern at one edge illustrating the x and y increment of 1 μm every successive nanohole.

80,000 cells/cm² and cultured either overnight or for three days for attachment/viability or immunocytochemistry experiments, respectively. For attachment and viability experiments, cells were treated with 4 μM Calcein AM (Molecular Probes, Invitrogen) in phosphate buffered saline (PBS, Gibco) for 15 min at 37 °C. For immunocytochemistry, cells were fixed with 4% paraformaldehyde for 15 min, permeabilized with 0.25% Triton X in PBS for 10 min, and blocked with 10% goat serum in PBS. Unless otherwise stated, all steps were performed at room temperature. Cells were then incubated with phalloidin (Molecular Probes) for 20 min or 1:100 rabbit anti-mouse α smooth muscle actin primary antibody (αSMA , Abcam, Cambridge, MA) overnight at 4 °C, followed by treatment of 1:50 FITC-conjugated goat anti-rabbit secondary antibody (Abcam) for 2 h. Cells were imaged using a Nikon Eclipse Ti inverted fluorescence microscope (Nikon Instruments, Inc, Melville, NY). Images were analyzed with Image J (National Institutes of Health, Bethesda, MD) and $n \geq 50$ cells from $n \geq 5$ images were used for quantification. Individual cells, not those in aggregates, were quantified for shape descriptors (i.e., average cell area and average cell perimeter).

3. Results and discussion

3.1. Cellular response to nanoneedles

The cellular response was altered in the presence of SiO₂ nanoneedles as compared to flat SiO₂ surfaces (Fig. 3). Most strikingly, cell adhesion to surfaces with nanoneedles was an order of magnitude higher than adhesion to flat surfaces ($p = 0.005$, Fig. 3A), while viability was maintained for cells adhered to either surface (Fig. 3B). Cell spreading was also influenced by the presence of nanoneedles. On nanoneedle-containing surfaces, cells exhibited a larger area and perimeter than those cultured on flat surfaces ($p < 0.005$ and $p = 0.007$, Fig. 3C and D, respectively), indicating increased cell spreading on the nanoneedle-containing surfaces. Therefore, the nanoneedle-containing surfaces were proven to promote cell–matrix interaction.

As seen in Fig. 4, the presence and spacing of nanoneedles strongly influenced competitive cell–cell versus cell–matrix

interactions. On flat substrates without nanoneedles, a significant number of cells formed large multicellular aggregates (Fig. 4A; calcein, green) that did not display any noticeable organization or regularity (Fig. 4B; αSMA , green; Hoechst, blue). In contrast, the presence of nanoneedles prevented cell aggregation, interrupted cell–cell contacts, and promoted interactions with the matrix (Fig. 4C–F; αSMA , green; Hoechst, blue; actin, red). Cells preferentially aligned themselves along tightly spaced nanoneedles in gradient regions with sparse spacing in the orthogonal direction. In regions with sparse distributions in both directions, cells interacted both with adjacent nanoneedles as well as those located in the parallel rows (Fig. 4C and D). In general, parallel rows of nanoneedles with substantial spacing between them (i.e., $>30 \mu\text{m}$) sequestered cells and biased them towards cell–matrix interactions. This spacing also controlled the size and geometry of any small cell clusters that were present (Fig. 4E). A critical spacing between nanoneedles appeared to exist that regulated the ability of cells to adhere and spread in two dimensions (Fig. 4F).

Relative spacing ratios for local regions of nanoneedles regulated cell spreading. Sparse spacing in two dimensions allowed single cells to spread in two dimensions (Fig. 4C, top left inset), whereas tight spacing in one dimension and wide spacing in the orthogonal direction allowed cells to spread only in one direction (Fig. 4C, top right inset). Given that nanoneedle spacing allowed for cells to attach between parallel rows, cells tended to spread in one dimension when the ratio between nanoneedles spacing in orthogonal directions was less than 0.5. In contrast, cells spread in two dimensions when nanoneedles spacing ratio was greater than 0.5. These data indicate that the cells actively sensed the microenvironment and that nanoneedle spacing can be used as a critical parameter to intricately regulate cell–cell and cell–matrix interactions.

In this study, we have employed a novel surface of patterned silica nanoneedles as a synthetic cell culture substrate and evaluated how needle presence and spacing affects cell attachment, morphology, and alignment. The impact of this work lies in the translation of a new material fabrication technique whose application to biology allows for the design of culture substrates that

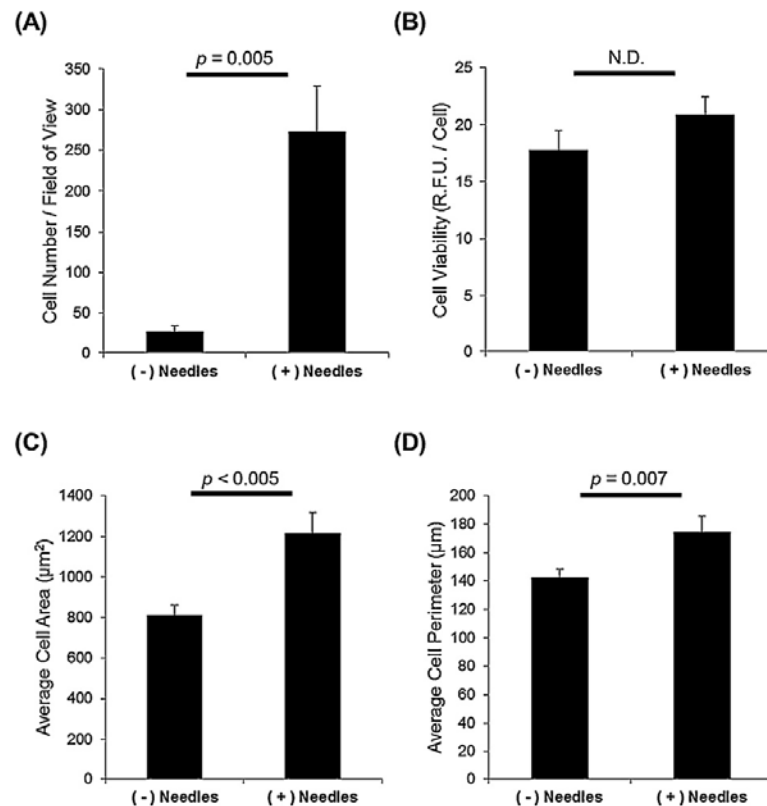


Fig. 3. SiO₂ nanoneedles promote cell attachment and spreading. (A) Significantly more cells attach to nanoneedle-containing surfaces and (B) cell viability is unaffected. (C and D) Cell spreading (area, perimeter) are increased on nanoneedle-containing substrates.

direct and regulate cell behavior. Specifically, the cellular response to silica nanoneedles provides a means to directly modulate competitive cell adhesion events. Competitive cell–cell and cell–matrix interactions are a fundamental regulator of embryogenesis [11] and have been manipulated for complex tissue engineering strategies [32]. Cells of different lineages exhibit biased interactions depending upon their physiological location and function. For example, sheet-like epithelial and endothelial cells are dominated by cell–cell contacts through which various signals can be propagated, yet cells of a mesenchymal lineage are primarily cell–matrix interactive. Intricate lineage transition events, such as mesenchymal-to-epithelial transition (MET) and its reverse (EMT), confer new characteristics to differentiated cells that can also involve pathological progression. In certain cancers, for example, malignant cells in a primary tumor can undergo such a lineage transition, allowing them to escape from their normal location and metastasize [33]. By probing and modulating cell adhesion events, fundamental processes involved in embryogenesis, tissue engineering, and cancerous transformation can be intimately controlled, studied, and exploited for therapeutic intervention.

Fibroblasts, a mesenchymal cell type, are involved in diverse body processes including normal wound healing and pathological fibrosis. In healthy wound healing, fibroblasts express a smooth muscle cell-like phenotype (myofibroblast) to aid in remodeling and contraction of wound sites [34,35]. Myofibroblasts are also associated with numerous pathological conditions, such as fibrous scarring, aortic valve calcification, and other fibrotic diseases,

usually when environmental variables result in an over-abundance of myofibroblasts [34,36]. Fibroblasts express basal levels of α SMA, however, increased α SMA expression and formation of α SMA stress fibers is indicative of myofibroblast phenotype [34,35,37]. There are several known modulators of myofibroblast phenotype, the most notable are stimulation with transforming growth factor β 1 (TGF- β 1), and mechanical forces arising from dynamically changing strain and substrate stiffness [35,36,38,39]. Furthermore, α SMA expression has been shown to correlate with fibroblast contractile ability, and is upregulated on stiffer substrates, and can be dynamically modulated by changing substrate stiffness [35,39,40]. In the present study, we investigated the cellular response to patterned SiO₂ substrates in the context of α SMA expression to determine if needles altered the contractility or myofibroblasts phenotype of 3T3 fibroblasts. Neo-expression of α SMA was not observed on any of the test substrates, α SMA expression was not significantly different, and α SMA stress fibers were not evident in fibroblasts cultured on either substrate (Fig. 4B and C). This indicates that culture of 3T3 fibroblasts on SiO₂ substrates does not induce a myofibroblast phenotype. However, fibroblasts which formed large clumps on flat substrates were not analyzed for α SMA expression. These clumps of cells demonstrate similar morphology to fibroblasts which form calcific nodules in aortic valve disease [40,41]. Because these test substrates have a high surface modulus, which has been shown to induce myofibroblast formation, we would expect to see the contraction of fibroblasts into nodules when seeded on SiO₂ substrates. Clump formation was significantly lower on patterned nanoneedles

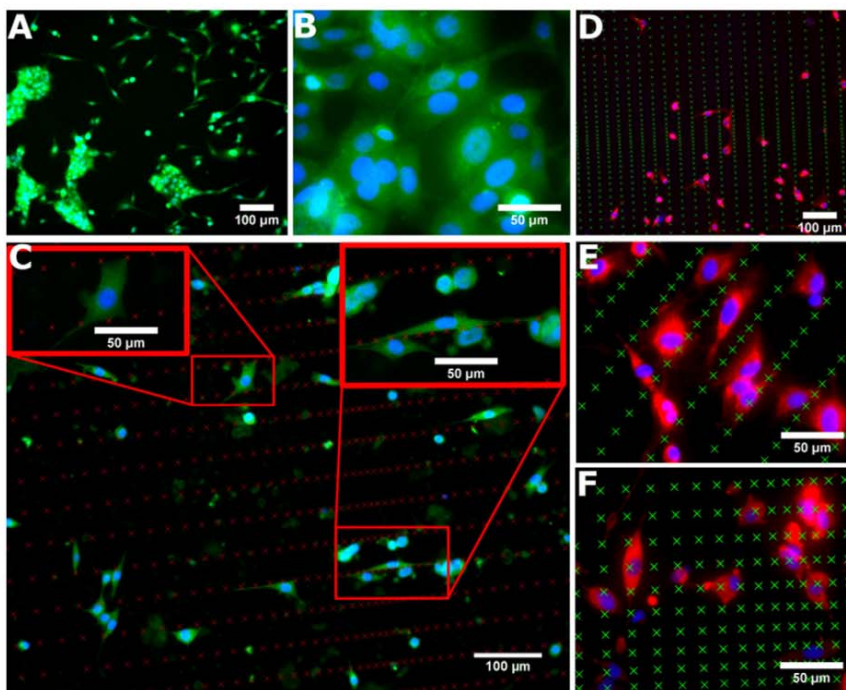


Fig. 4. Cell adhesion is influenced by the presence and spacing of SiO_2 nanoneedles. (A and B) Flat surfaces promote the formation of large, unorganized cell aggregates. (C and D) Cells interact directly with the nanoneedles and only small cell aggregates, if any, are able to form. (E) Parallel rows of tightly spaced nanoneedles sequester cells between them. (F) Few, if any, cells are able to attach in areas of densely packed nanoneedles, but attachment improves as spacing becomes sparser. Nanoneedle locations are indicated by X's in each image. A: calcein, green; B and C: α SMA, green; Hoechst, blue; D and F: actin, red; Hoechst, blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

substrates, which suggests that interrupting cell–cell interactions in favor of cell–matrix interactions decreases the contractile ability of fibroblasts, even when seeded on stiff substrates.

The utility of this surface format for basic and applied biological sciences and biomedical engineering lies in its ability to manipulate cell behavior in order to probe fundamental biological processes and pathological mechanisms. This technique is versatile and other materials, such as synthetic polymers, can be used in the place of CA through a simple solvent casting approach. Material properties (i.e., stiffness, hydrophobicity/philicity, charge) impact cell health and fate, and can be tuned through copolymerization [42,43]. For example, our laboratory has used copolymers with subunits of varying molar ratios to alter material properties and control cell health, proliferation, and differentiation [42,43]. By applying these polymers in the nanoneedle format, we plan to use synthetic surfaces to investigate tissue formation/regeneration and early mechanisms in cancerous transformation of human cells.

4. Conclusions

Single-pulse femtosecond laser machined templates were used to extract gradient patterns of CA nanoneedles by replication. After coating CA nanoneedles with silica, the resulting 3D silica nanoneedle patterns were used to study the effect of two dimensional gradient spacing on cell–cell and cell–matrix interactions that play a mechanistic role in tissue regeneration as well as pathogenesis. The technique is useful in understanding cell biology with possible application to the study of cell lineage transition, particularly the mesenchymal-to-epithelial transition and myofibroblast phenotype expression. The advantage of this process is the ease and

control of geometrical structuring by femtosecond laser machining. We foresee the use of this technology and advanced polymers to engineer synthetic surfaces for tissue regeneration.

Acknowledgements

The Tennessee Higher Education Commission supported this work through a grant to the Center for Laser Applications, University of Tennessee Space Institute. We also would like to acknowledge support from the National Institutes of Health (NIH HL091465) and the National Science Foundation (CAREER: CBET 1056046). This work was also supported by the Vanderbilt Institute of Nanoscale Science and Engineering (VINSE).

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Appendix III

Solution-Cast High-Aspect-Ratio Polymer Structures from Direct- Write Templates

(This paper was published in the journal **ACS Applied Materials & Interfaces**,

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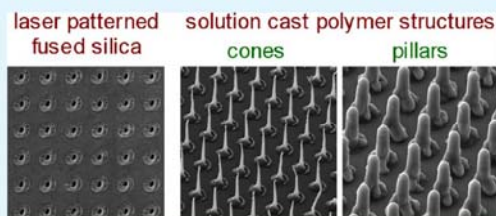
1 Solution-Cast High-Aspect-Ratio Polymer Structures from Direct- 2 Write Templates

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6 **ABSTRACT:** This letter presents a novel strategy for template
7 synthesis of polymer structures with laser machined substrates.
8 User-designed patterns of submicrometer holes with aspect ratios
9 >10:1 and depths >10 μm were produced by focusing 160 fs, 5.2
10 μJ laser pulses on the surface of fused silica with a high numerical
11 aperture microscope objective. Some holes were enlarged by
12 chemical etching. Polymer solutions were cast into the templates
13 to create high aspect ratio polymer structures using replication.
14 Engineered polymer structures prepared by this unique method
15 are useful for a number of applications such as high surface area
16 electrodes and biological substrates.

17 **KEYWORDS:** high-aspect-ratio holes, femtosecond laser machining, template synthesis, solution casting, replication, polymer structures



18 ■ INTRODUCTION

19 Template-synthesis is a conventional yet economical method of
20 creating topographical structures, where a patterned surface is
21 used as a mold.¹ In this method, structures are usually obtained
22 from templates by: (i) injecting molten material with the
23 application of heat and pressure; (ii) electrochemical or
24 electroless deposition; (iii) vapor deposition via physical,
25 chemical, or metallorganic vapor deposition techniques; or,
26 (iv) numerous soft lithography techniques.^{2–5} Anodized
27 aluminum oxide (AAO), nanochannel glass, track-etched
28 polymers, and lithographically patterned substrates are some
29 of the commonly used molds (also referred to as master pattern
30 in soft lithography) in template-synthesis. AAO templates are
31 prepared by electrochemical oxidation of high purity aluminum
32 films, and have high density of cylindrical and parallel holes,
33 also termed nanopores, arranged in hexagonal-packed arrays.⁶
34 Nanochannel glass (NCG) templates are fabricated by repeated
35 operations of drawing-followed-by-bundling the glass material
36 until the desired hole diameter is obtained.⁷ Track-etched
37 polymers are produced by chemical etching of the latent tracks
38 formed in polymer films by bombardment of energetic ions.⁸
39 However, none of these techniques allows complete control on
40 the physical arrangement of the holes produced on the
41 templates even though their diameter and depth can be
42 regulated.^{9,10} In comparison, lithography offers ever improving
43 control on the physical arrangement and size of the holes that
44 can be produced.¹¹

45 Photolithography and electron beam lithography are the two
46 most routinely used lithography techniques. Both these
47 template fabrication techniques are primarily based on
48 patterning polymer coated on the substrate followed by
49 reactive ion etching to transfer the pattern into the substrate.
50 Other lithography techniques that have been investigated for

51 patterning substrates are X-ray lithography,¹² ion beam
52 lithography including focused ion beam milling,¹³ proton
53 beam writing,¹⁴ and more recently neutral particle lithog-
54 raphy.¹⁵ However, these lithography techniques have so far not
55 been reported to produce patterns with high aspect ratio (at
56 least 10:1) nano/microscale diameter holes of depths more
57 than 10 μm on large areas at high production rates. Thus, there
58 exists an opportunity to look for alternative lithography
59 techniques that can pattern substrates with user-defined
60 arrangements of high aspect ratio (>10:1) holes.¹⁶

61 Femtosecond (fs) laser machining is a direct-write
62 lithography technique used to machine transparent substrates
63 like glass for applications such as photonics and microfluidics.¹⁷
64 White et al. demonstrated that tightly focused single amplified
65 femtosecond laser pulses can produce high aspect ratio holes at
66 the surface of fused silica.¹⁸ Holes of aspect ratios as high as
67 25:1 and depths more than 12 μm were produced by focusing
68 200 fs laser pulses of wavelength 800 nm through a microscope
69 objective lens of numerical aperture NA = 0.85 onto fused
70 silica. Hole dimensions (diameter and depth) were determined
71 first by cellulose acetate (CA) thin film replication, and then
72 the results of CA thin film replication were validated by imaging
73 the cross-section of the machined hole in a DualBeam FIB/
74 SEM. CA replication of holes results in formation of CA
75 topographical structures that have length-scales comparable to
76 that of the holes, proving that replication or imprinting of high-
77 aspect-ratio holes in fused silica templates is feasible and a novel
78 strategy for creating patterned polymer structures. Moreover, 78
79 these structures can be subsequently functionalized by coating

Received: September 20, 2012

Accepted: December 17, 2012



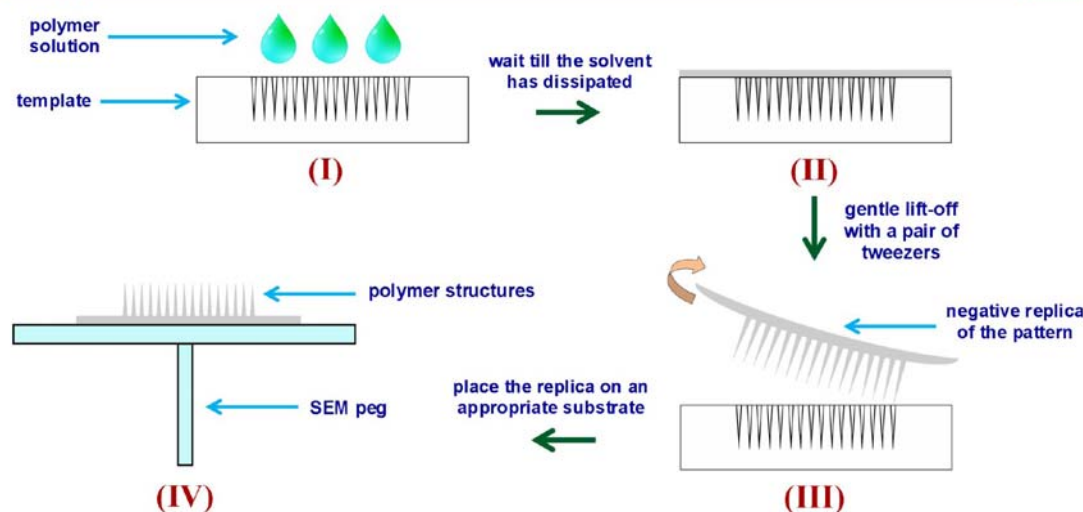


Figure 1. Schematic of the solution casting process used to extract polymer structures from as-machined and chemically etched fused silica templates using replication approach. PE requires an additional step between steps (II) and (III) in which the polymer network resulted from solution casting is heated to and thermally soaked for 2 min at 155 °C, and then slowly cooled in order to form a continuous film before lift-off.

Table 1. Details of the Polymers Used for Solution Casting and the Polymer Structures Created^a

polymer	source ^b	solvent	wt %	mol wt ($\times 10^3$)	as-machined template		etched template	
					yield	structure length	yield (%)	structure length
PCL	SA	chloroform	2	70–90	0	>30 μm (wires)	100	~13.5 μm micropillars
PCL-8 wt % PEG	VU	chloroform	2	60–70	0	>30 μm (wires)	100	~13.5 μm micropillars
PE	SPI	toluene	10	unknown ^d	100	avg. 8 μm (cones)	100	~13.5 μm micropillars
PMMA ^c	SA	toluene	10	120	100	avg. 9 μm (cones) ^f	100	~13.5 μm micropillars
PVA ^c	AA	DI water	5	57–66	100	avg. 9 μm (cones)	100	~13.5 μm micropillars
PDMS ^c	DCC	heptane	33	NA ^e	50	avg. 7 μm (cones) ^f	100	~12.5 μm micropillars

^aYield was determined as percentage of self-supporting polymer structures at 5000 $\times$ from Figures 3 and 4. The thickness of the final polymer imprint depends on the amount of the solution used initially for casting. Several layers are cast to build up the imprint thickness for convenient handling.

^bSA: Sigma Aldrich; VU: Vanderbilt University; SPI: SPI Supplies; AA: Alfa Aesar; DCC: Dow Corning Corporation. ^cTemplate coated with antistatic layer prior to solution casting. ^dPolyethylene homopolymer (SPI #01090C-AB Positive Replica Powder). ^eNot applicable (Sylgard 184 silicone elastomer kit). ^fStretched and fallen cones were ignored.

with different types of materials. For example, imprinted CA wires were converted to silica needles by depositing atop a thin layer of silica in a low-temperature chemical vapor deposition process,¹⁹ and silica needles thus produced were used as cell culture substrates for cell differentiation studies using NIH 3T3 mouse embryo fibroblasts.²⁰ As of yet, only CA thin film imprinting of single-pulse amplified femtosecond laser machined fused silica templates is reported and discussed for practical applications. In this letter, we propose the idea of creating patterned polymer structures from femtosecond laser machined fused silica templates by solution casting in which the diameter of the holes is in the range 750 nm to 2.5 μm and the depth ~15 μm .

EXPERIMENTAL PROCEDURE

An amplified femtosecond laser system consisting of a 532 nm continuous wave (CW) diode-pumped frequency doubled laser (Nd:YVO₄, Model Verdi-V18 by Coherent, Inc.), a 76 MHz laser oscillator (Model 3941 by Spectra Physics), and a 250 kHz laser amplifier (RegA 9000 by Coherent, Inc.) was used to machine fused silica substrates. The CW laser pumps the oscillator and the amplifier, while the oscillator seeds the amplifier to generate amplified

femtosecond laser pulses. The output from the amplifier is a train of pulses with central wavelength 780 nm, temporal length 160 fs, and repetition rate 250 kHz. The Pockels cell within the amplifier allows the emission of single amplified femtosecond laser pulses of energy 5.2 μJ . The laser beam delivered by the amplifier is guided through an assembly of periscopes to the microscope objective lens. The lens focuses the laser beam onto a fused silica substrate mounted on a high resolution nanostage (model ANT95–50-XY nMT by Aerotech, Inc.). The entire femtosecond laser machining system is controlled via a LabVIEW program where user-defined machining patterns are easily designed. Patterns can be machined at the repetition rate of the laser amplifier (i.e., 250 kHz). The motion control system limits the speed at which individual holes can be machined. Currently, with a nanostage velocity of 6 mm s⁻¹ and a laser repetition rate of 1.2 kHz, over 4 million holes can be machined at a 5 μm pitch on a 1 cm² wafer in less than one hour. Thus, user-defined large area patterns of high aspect ratio holes can be machined at high production rates by single-pulse femtosecond laser machining. Further details of the experimental setup can be found in the literature.^{21,22}

A 160 $\times$ microscope objective lens of numerical aperture NA = 1.25 (for water immersion; Leitz Wetzlar Model: $\infty/0$ PL APO) was used to pattern arrays of holes on a 500 μm thick double side polished fused silica substrate. Machining was done in air without water immersion, so the effective numerical aperture of the lens was less than 1. The as-

machined fused silica template was ultrasonically cleaned in acetone and then subjected to polymer solution casting to create polymer structures. A schematic of imprinting by solution casting is shown in Figure 1 and details of the polymer solutions used to make polymer structures are shown in Table 1. The pattern on the template was covered with a few drops of polymer solution (I) and then allowed to dry in normal atmospheric conditions or placed in a vacuum desiccator until the solvent had dissipated and a film was formed on the surface (II). The resulting film is the negative replica of the pattern, the imprint, which consists of structures that form within the holes. The imprint was then slowly peeled-off from the template surface with a pair of tweezers (III) and placed on an appropriate substrate for further analysis (IV) e.g., an aluminum SEM peg or a glass coverslip. In some cases the template was coated with an antistick layer (by vapor deposition of 1H,1H,2H,2H-perfluorodecyltrichlorosilane in a vacuum desiccator) to help release polymer films that stick to the template. Polyethylene (PE) solution does not form film upon solvent dissipation, but a loosely connected network of polymer powder. Thus, to form PE film, the template with the powder network was heated above the melting point of PE at 155 °C on a hot plate and thermally soaked at that temperature for 2 min before turning the hot plate off for a slow cooling. This process successfully forms PE imprints. Once the polymer imprint is peeled from the template, the template can be reused to extract more imprints. The authors have not found a limit to the number of polymer imprints that can be extracted from a single template.

RESULTS AND DISCUSSION

Virtually any polymer that forms a continuous film upon solution casting, with or without the assistance of external energy, can be used to produce high-aspect-ratio patterned polymer structures from these templates. In the current investigation, patterned areas as large as 1.5 cm × 1.5 cm have been successfully solution cast to create polymer structures with high reproducibility and without any observable shrinkage of the polymer film. The morphology of the cast structure depends on the hole morphology produced by single-pulse femtosecond laser ablation, which in turn depends on the spatial profile of the femtosecond laser pulse. Furthermore, the dimensions of the cast structures depend on the dimensions of the holes, i.e., cross-section (both entrance and tip) and depth, which in turn depend on the laser pulse energy used during template fabrication. For a near-Gaussian laser pulse of energy 5.2 μJ, conic submicrometer holes of base diameter 750 nm, tip diameter 150 nm, and depth up to ~12 μm are produced in fused silica templates. By reducing the pulse energy, the depth and the cross-section of the hole can be reduced.¹⁸ To increase the hole dimensions, we can etch the glass template. Hydrofluoric acid (HF) is known to etch fused silica isotropically. Concentrated aqueous solution of potassium hydroxide (KOH) preferentially attacks femtosecond laser affected region in fused silica.²³ The holes can thus be modified by a combination of isotropic etching in HF and a highly selective etching in KOH. Under optimized conditions in both the etchings, the morphology of the holes can be changed from conic to cylindrical. Figure 2 shows scanning electron micrographs of an array of as-machined submicrometer holes (A) and an array of microholes obtained postetching (B). The as-machined submicrometer holes were enlarged by sequential etching, first in HF (Lodyne, BOE, 6:1) for 2 min and then in 10 M KOH at 80 °C for 12 h. The resulting cylindrical holes are ~2.5 μm in diameter (measured at 5 μm depth into the substrate) and up to 15 μm in depth. However, the etched holes appear irregular in shape and larger in diameter at the hole-entrance due to etching of very fine nanocracks that

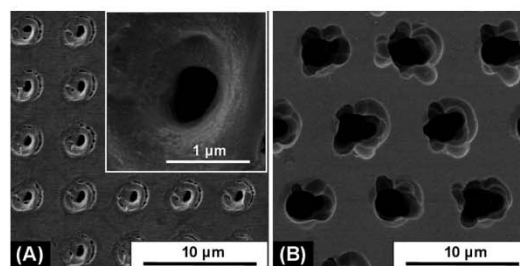


Figure 2. SEM images of two different single-pulse femtosecond laser machined fused silica templates. (A) As-machined femtosecond laser ablated submicrometer holes with average diameter of 750 nm (see inset). (B) Femtosecond laser ablated holes after sequential etching in HF (2 min, Lodyne, BOE, 6:1) followed by KOH (12 h, 10 M KOH solution at 80 °C) with average diameter of 2.5 μm.

develop near the hole-entrance during femtosecond laser machining.

SEM images of some of the polymer structures prepared by solution casting are shown in Figures 3 and 4, and only those polymers that are of current research interest are reported here: (A) polycaprolactone (PCL); (B) PCL, polyethylene glycol (PEG) block copolymer; (C) polyethylene (PE); (D) polymethylmethacrylate (PMMA); (E) polyvinyl alcohol (PVA); and (F) polydimethylsiloxane (PDMS). PCL and PCL-PEG structures, Figure 3A, B, appear to be 30–40 μm long wires when extracted from holes with ~12 μm depth. Presumably, these wires undergo stretching during the lift-off process. The adhesive strength between the glass hole and the polymer exceeds the tensile strength of the respective polymers, which results in their stretching during lift-off at room temperature. Because the wires come out very long (>30 μm) because of stretching and are not stiff enough to support their weight, they fall over as irregular-shaped wires. PE, PMMA, and PVA appear as cones with their base diameter around 750 nm and tip diameter in the range 150 to 450 nm as seen in Figure 3C–E. The height and tip diameter of the polymer cones depends on the filling of the hole which is dependent on the interfacial free energy balance between the solution, fused silica, and any trapped vapor. The fact that uniform structures can be formed with dimensions closely matching the hole morphology shows this energy balance is favorable for the wetting of the silica holes by the polymer solutions. PDMS prepared by Sylgard 184 (a silicone elastomer kit by Dow Corning Corporation) is routinely used in soft lithography and microfluidics, and its mechanical properties are tailored by varying the base-to-curer ratio. Although the glass transition temperature (T_g) of PDMS is very low (−125 °C), its 3D cross-linked structure assists the release of PDMS cones from the as-machined holes without breaking or stretching, as shown in Figure 3F. However, most PDMS cones tend to fall over, which may be attributed to low Young's modulus of PDMS (<1 MPa for any base-to-curer ratio). Furthermore, it was observed that most of the erect polymer cones slightly bend in the direction of lift-off whereas some tend to be randomly oriented. The polymer cone yield was found to be very high in all these polymers though some cones break during lift-off in case of PE (Figure 3C) and some tend to stretch near the tip in case of PMMA (Figure 3E). Figure 4 shows SEM images of polymer structures cast from microholes (obtained by etching the template) that appear as micropillars. All the

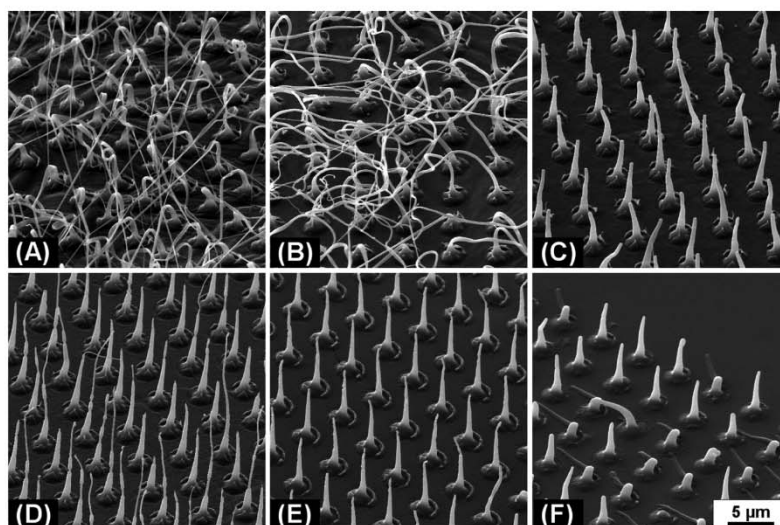


Figure 3. SEM images of polymer structures taken at 45° stage tilt. (A) PCL, (B) PCL-PEG, (C) PE, (D) PMMA, (E) PVA, and (F) PDMS. PCL, PCL-PEG, and PMMA were subjected to vacuum desiccation. PDMS was cured at 80 °C for 2 h.

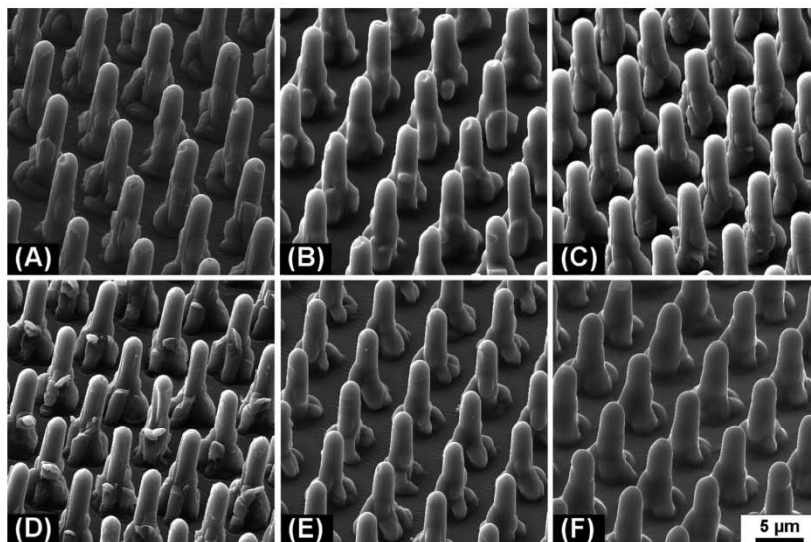


Figure 4. SEM images of polymer micropillars taken at 45° stage tilt. (A) PCL, (B) PCL-PEG, (C) PE, (D) PMMA, (E) PVA, and (F) PDMS. PCL, PCL-PEG, and PMMA were subjected to vacuum desiccation. PDMS was cured at 80 °C for 2 h. PDMS appears enlarged because it was cast from a solution made in heptane, and PDMS is known to swell in the presence of organic solvents.

polymers adopt the shape and dimensions very close to that of the cylindrical holes they are cast from; however, their textures vary because of their physicochemical properties. No stretching, breaking, or bending of pillars was observed and the yield was almost 100% in all these polymers. Besides these polymers, similar observations are recorded for Nafion117, cellulose acetate (CA), polyvinyl chloride (PVC), and Collodion. In thin film imprinting, the pattern on the template is first wetted by putting a few drops of the solvent (e.g., acetone for CA) and the polymer film is placed on top of the template. The

solvent softens the polymer so that it fills the holes. Once the solvent dissipates, the resulting polymer film forms the negative replica of the pattern; a process quite similar in operation to the aforementioned solution casting. Nevertheless, thin film imprinting is suitable only for those polymers that can be obtained or prepared in the form of thin films and at the same time can be softened by a readily available solvent, thus making it a highly selective process. As a consequence, polymers that do not soften in any solvent cannot be used to prepare topographical structures by thin film imprinting e.g., thermosets

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dx.doi.org/10.1021/am302009e | ACS Appl. Mater. Interfaces XXXX, XXX, XXX–XXX

like polydimethylsiloxane (PDMS). Blending different polymers or reinforcing polymers with other polymers or nonpolymeric materials leads to the formation of multifunctional polymers with novel properties. Such multifunctional self-supporting polymer structures with varying chemical composition and tunable physical properties can be easily and more efficiently prepared by solution casting. Furthermore, heterogeneous structures can also be prepared by casting multiple layers of different polymers using dilute solutions.

CONCLUSION

To conclude, we successfully created high-aspect-ratio (>10:1) patterned polymer structures by combining template-synthesis with solution casting. The templates were fabricated on fused silica by focusing single pulses from an amplified femtosecond laser using a high numerical aperture microscope objective lens, and patterns of high aspect-ratio (>10:1) submicrometer holes of depths more than 10 μm were produced at the surface. Submicrometer holes with conic morphology were converted to microholes with cylindrical morphology by subjecting the template to a chemical etching process. Six polymers of distinct physical properties (PCL, PCL-PEG, PE, PMMA, PVA, and PDMS) were solution cast into the fused silica templates to create polymer structures via replication approach. The solution casting process is highly reproducible and large area polymer structures with high yield and regularity are obtained. This novel-yet-simple strategy for creating high-aspect-ratio patterned polymer structures is currently being explored for applications in biomedical engineering, high-surface-area electrode structures, and photovoltaics.

AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work is supported by the Tennessee Higher Education Commission through a grant to the Center for Laser Applications, the University of Tennessee Space Institute, Tullahoma, TN. The authors gratefully acknowledge Dr. George Murray (UTSI) for kindly revising this manuscript during its preparation. We also acknowledge the MIMIC user facility at the Middle Tennessee State University, Murfreesboro for FESEM analysis. PCL-PEG polymer was kindly provided by Dr. Hak-Joon Sung, Vanderbilt University.

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APPENDIX IV

Beam size and beam divergence measurements for the CNMS laser

(Chapter 5: Spherical Aberration & Optical Modeling)

The beam diameter ($1/e^2$ value) and the beam divergence of the CNMS laser used for the aspheric lens machining experiments were determined by power ratio method. In this method, the values of the laser power are measured with and without the presence of an iris of a known diameter on the laser beam path using a power meter. The power ratio (R) is found as:

$$R = \frac{P(ir)}{P(o)}$$

where, $P(ir)$ is the power of the laser beam measured when the iris was placed on the beam path and $P(o)$ is the power of the laser beam measured without the iris on the beam path (i.e, the maximum power). The iris diameter was kept constant at 7.55 mm. The $1/e^2$ laser pulse diameter was determined using the power ratio (R) by the equation:

$$d = 2\omega_o = \sqrt{\frac{-2a^2}{\ln(1-R)}}$$

where, ω_o is the $1/e^2$ laser pulse radius and a is the diameter of the iris opening (i.e., 7.55 mm). The derivation of this equation is as follows:

$$P = \int_0^a I dA$$

where, P is laser power, a is the $1/e^2$ laser pulse radius, I is the laser intensity of a Gaussian beam, and dA is the area of the laser beam with the radius dr (i.e., $dA = 2\pi r dr$).

The intensity distribution of a Gaussian laser beam is given by:

$$I = I_o e^{\frac{-2r^2}{\omega_o^2}}$$

$$\Rightarrow P = \int_0^a I_o e^{\frac{-2r^2}{\omega_o^2}} 2\pi r dr$$

Integrating this equation gives the equation for the laser power as:

$$\Rightarrow P = I_o \left(\frac{\pi}{2} \omega_o^2 \right) \left(1 - e^{\frac{-2a^2}{\omega_o^2}} \right)$$

$$\Rightarrow P = P_o \left(1 - e^{\frac{-2a^2}{\omega_o^2}} \right)$$

$$\Rightarrow R = \frac{P}{P_o} = \left(1 - e^{\frac{-2a^2}{\omega_o^2}} \right)$$

Rearranging this equation gives the equation for the $1/e^2$ laser pulse diameter:

$$d = 2\omega_o = \sqrt{\frac{-2a^2}{\ln(1-R)}}$$

The beam divergence (half-angle) was determined by measuring the $1/e^2$ laser pulse radius (ω_o) at two different locations on the beam path using the equation:

$$\vartheta = \frac{\omega_{2_o} - \omega_{1_o}}{\text{separation between the two locations}}$$

where, ω_{2_o} and ω_{1_o} are the $1/e^2$ laser pulse radii measured at two different locations using the power ratio method. The value of the beam divergence (half-angle) was found to be -0.12185 milliradians (i.e., converging laser beam), and it was used to determine the laser beam diameter at the entrance of the first lens of the 3-lens zoom system. The value of the $1/e^2$ laser beam diameter at the entrance of the zoom lens was found to be ~6.44 mm. This value was used as the laser beam size for Zemax modeling to determine the lens separations for aspheric lens machining experiments.

Vita

Deepak Rajput was born January 03rd 1982 in Bhilai, Chhattisgarh, India. He received a B.Tech. (Bachelor of Technology) in Metallurgical Engineering from the National Institute of Technology (erstwhile Regional Engineering College) at Tiruchirappalli, Tamil Nadu, India, in May 2004 and an M.S. in Materials Science and Engineering from the University of Tennessee at Knoxville in December 2008. He worked in the Research & Development Center of Essar Steel Limited, Hazira, India, as a research engineer from June 2004 to August 2006. He worked on new products development in hot rolling, galvanizing, and cold rolled closed annealing sectors of flat steel products and was in charge of the Materials Characterization Laboratory at Essar Steel Limited. He joined the University of Tennessee Space Institute in Fall 2006 to pursue M.S. and Ph.D. in Materials Science & Engineering. He worked as a graduate research assistant to Dr. William Hofmeister at the Center for Laser Applications, UTSI.

Deepak Rajput received the UTSI Outstanding Graduate Research Assistant award in 2009 and the Lloyd W. Crawford fellowship for the academic year 2009-2010. He published eight journal articles and five conference papers till August 2012. He served as a reviewer for three international journals (Surface and Coatings Technology, Vacuum, and Lasers in Engineering). He is a student member of the Materials Research Society (MRS) and Materials Advantage (AIST, ACerS, TMS, ASM International). He is also a member of the Sigma Xi Honor Society affiliated to the Vanderbilt University chapter. More details about Deepak Rajput and his current activities can be found on his personal website <http://www.drajput.com>.