

**REAL TIME ANALYSIS OF CYTOSKELETAL FILAMENTS USING
MEMS TRACTION SENSOR AND FLUORESCENT MICROSCOPY**

by

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ABSTRACT

The ability of cells to resist and exert mechanical forces allows them to perform many essential tasks such as cell growth, orientation, migration, and division. Cytoskeleton is the protein network of the cells that provides this ability as well as mechanical strength. In the absence of cytoskeletal components, cells experience significant embrittlement and this causes various human diseases. Understanding the mechanisms that determine how cells withstand and exert the mechanical stimuli is therefore significant to the diagnosis and treatment of cytoskeleton-related diseases. For this purpose, two different technologies with the major objective of observing the behavior of cytoskeletal filaments under local loads are developed.

The first technology is based on the idea of building a smart surface comprising of MEMS sensors that can measure in-plane forces acting on the cytoskeletal filaments. The design of force sensors is geared towards optimizing both the mechanical sensitivity and piezoresistive response. Fabrication work is carried out through submicron patterning and doping by ion implantation. The initial fabrication results on silicon test and SOI wafers are presented.

The second technology developed within the scope of this study is to construct a miniaturized stretcher integrated with a fluorescence microscope, which can load very thin diaphragms and measure associated minute forces. The integration is also enhanced with a synchronization enabling us to keep a certain area in the field of view during the entire duration of stretching. By applying sub-pixel particle tracking algorithm to the deformed micrographs, deformation and in-plane strains in the cells or in the substrates can be determined with a high spatiotemporal resolution. This work addresses two current shortcomings of non-contact strain measurement of soft matter: i) real-time measurement, and ii) high spatial resolution. The proposed technique can overcome this challenge and provides a displacement measurement resolution of 116 nm and a strain resolution of 0.04% over a gage length of 300 μm .

ÖZET

Hücrenin üzerine etkiyen kuvvetleri algılama ve yönlendirmedeki kabiliyeti hücrenin büyüme, yönelme, göç etme ve bölünme gibi birçok olayın oluşmasında önemli rol oynar. Protein yapılardan oluşan hücre sitoskeleti hücreye bu kabiliyeti kazandırmasının yanında hücreye mekanik dayanıklılık da sağlamaktadır. Sitoskelet bileşenlerinin oluşmaması durumunda hücrenin dış kuvvetlere olan direnci azalır ve bu da birçok hastalığın meydana gelmesine sebep olur. Hücrenin mekanik uyartılara nasıl dayandığı veya nasıl yönlendirdiğinin açığa kavuşturulması, sitoskelet ile ilişkili olan hastalıkların teşhisi ve tedavisi için büyük önem taşır. Bu amaç doğrultusunda, sitoskelet iplikçilerin davranışlarını gözlemlemeye dayalı iki teknoloji geliştirilmiştir.

Geliştirilen birinci teknoloji, sitoskelet iplikçilere etkiyen düzlemsel kuvvetlerin algılanmasına olanak sağlayan ve MEMS algılayıcılardan oluşan bir akıllı yüzey geliştirmeye dayanmaktadır. Algılayıcıların mekanik duyarlılığı ve piezoresistif tepkisi optimum seviyede olacak şekilde tasarlanmıştır. Üretim aşamasında mikron-altı şekillendirme ve iyon yoluyla katkılama imkanlarından yararlanılmıştır. Silisyum test ve SOI pullar üzerinde yapılan üretim çalışmaları başarıyla gerçekleştirilmiştir.

Bu proje kapsamında geliştirilen ikinci teknoloji ise ince diyaframlar üzerinde ekilmiş hücrelere kontrollü bir şekilde kuvvet uygulanabilmesini sağlayan ve flüoresans mikroskopisi ile uyumlu olan bir minyatür çekme cihazı geliştirilmiştir. Çekme cihazı, mikroskop ile senkronize edilerek istenilen alanın veya hücrenin tüm bir deney esnasında görüntüde kalmasını mümkün kılmıştır. Çekme sırasında elde edilen görüntüler için piksel-altı parçacık izleme algoritması geliştirilerek hücre veya hücre alttaşılarında oluşan deformasyon ve düzlemsel gerinimleri yüksek çözünürlükte mekân-zamansal analizi yapılmıştır. Bu çalışma literatürde polimer malzemelerde üzerinde yapılan çalışmalardaki iki önemli kusuru giderebilecektir. i) gerçek zamanlı ve ii) yüksek uzamsal çözünürlükte ölçüm yapabilme. Önerilen teknik 116 nm yer değiştirme ve 300 mikrometre gibi kısa bir uzunluk üzerinden 0.04% gerinime kadar ölçebilme kabiliyetine sahiptir.

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NOMENCLATURE

AFM	Atomic force microscopy
BOX	Buried oxide
C	Right Cauchy-Green deformation tensor
CMOS	Complementary metal-oxide-semiconductor
CNC	Computer numeric control
DAPI	4',6-diamidino-2-phenylindole
DAQ	Data acquisition
DC	Direct current
DIC	Digital image correlation
DM	Dichroic mirror
DTI	Deep Trench Isolation
E	Green-Saint-Venant strain tensor
$\vec{E}$	Electric field
EMCCD	Electron Multiplying Charge Coupled Device
EmF	Emission filter
ExF	Excitation filter
E_{eff}	Effective elastic modulus
F	Deformation gradient tensor
FCC	Face-centered cubic
FEM	Finite Element Modeling
G	Piezoresistance gauge factor
HF	Hydrofluoric acid
I	Identity tensor
IF	Intermediate filaments
I/O	Input/Output
$\vec{J}$	Current density
M	Mirror
MEMS	Micro-electromechanical systems
MERIE	Magnetically enhanced reactive ion etching
MSD	Mean square displacement
N	Dopant concentration
NW	Nanowire
PC	Personal Computer

PDMS	Polydimethylsiloxane
PMMA	Poly(methylmethacrylate)
P(N,T)	Piezoresistance factor as a function of dopant concentration and temperature
RC	Resistor-Capacitor
RIE	Reactive ion etching
rpm/s	Revolution per minute /second
RMS	Root mean square
R^2	Coefficient of correlation
SEM	Scanning Electron Microscope
SMU	Source-measure unit
SOI	Silicon on insulator
SU-8	Epoxy-based negative photoresist
t	Time
T	Temperature
$\mathbf{u}$	Displacement vector
UV	Ultraviolet
v	Stretching speed
ν	Poisson's ratio
WLI	White light interferometer
$\mathbf{x}$	Positions of beads at any time
$\mathbf{X}$	Initial positions of beads
x_1, x_2, x_0	Coordinates of the edges and center point of sample in x direction
y_1, y_2, y_0	Coordinates of the edges and center point of sample in y direction
$\Delta I/I_0$	Relative change in current
$\Delta R/R_0$	Relative change in resistance
$\Delta \rho/\rho_0$	Relative change in resistivity
$\Delta \rho_{ij}$	Change in resistivity tensor
ϕ	Position function
γ	Shear components of strain
ε	Axial strain applied on the nanowires
ε_{xx}	Longitudinal component of strain
ε_{yy}	Lateral component of strain
$\varepsilon_{xy}, \varepsilon_{yx}$	Shear components of strain
ε_1	Axial strain on the horizontal nanowires
ε_2	Axial strain on the vertical nanowires
θ	Angle of the applied force vector with the positive x-axis
λ	Stretch ratio
π_{ijkl}	Piezoresistance tensor

π_L, π_T	Effective longitudinal and transverse piezoresistance coefficients
$\boldsymbol{\rho}_{ij}$	Resistivity tensor
ρ_0	Resistivity in the unstressed state
ω_c	Cut-off frequency of the passive low pass filter
δ_{ij}	Kronecker delta
σ_a	Axial stress on the nanowires
$\boldsymbol{\sigma}_{kl}$	Stress tensor
σ'	von-Mises stress
$\nabla \mathbf{u}_{iK}$	Displacement gradient tensor



Chapter 1

INTRODUCTION

Studies have revealed that cells regulate their functions in response to mechanical forces generated either externally or internally [1]. Cells within tissues routinely experience a variety of non-uniform mechanical loads [2] and the ability of these cells to resist and exert forces allows them to perform many essential tasks. During physiological processes such as cell growth, orientation, migration, and division, these stimuli play essential roles for maintaining cell structure and function [3]. Many cells maintain their internal components organized by a combination of internal pulling and pushing forces. Embryos, for example, develop into organisms with well-defined anatomy through the combinations of cell growth, divisions, and shape changes [4]. Understanding the mechanisms that determine how cells withstand and exert the mechanical forces is therefore crucial.

One popular approach to study how mechanical stimuli regulate cell functions is to reconstitute a biomimetic system in which the state of cells *in vivo* environments can be imitated. Biomimetic systems are simplified and purified environments and their base is mostly polymer. The reduced complexity compared to tissues makes it easier to conduct quantitative experiments that can be linked to quantitative physical theories [4].

In this project, an experimental study is carried out regarding the observing the behavior of cytoskeletal filaments under external loads. The available methods suffer either from real-time analysis or spatiotemporal distribution. Some methods also lack high resolution in addition to these factors mostly due to size scale of cells and small magnitude of forces. Despite these challenges, it is of utmost important to observe cellular functions under controllable external forces. For this reason, a technique based on micro-electromechanical system utilizing piezoresistive silicon nanowires is developed. This technique, requires sub-micron patterning

and doping through ion implantation. Despite the complexity of fabrication processes and the challenging nature of the technical infrastructure requirements, considerable improvements have been made.

Another technique is developed in this project for simultaneous measurement of biomechanics events in cells while they are being stretched. This technique mainly focuses on the evaluation of strain and deformation that cells experience with a high spatiotemporal resolution. For this purpose, a uniaxial tensile stretched is designed, manufactured, and ultimately integrated with a fluorescent microscope. Using the cleanroom facility, we prepared thin elastomeric films with micropatterns and they are loaded under the fluorescent microscope. The current setup provides a displacement measurement resolution of 116 nm and a strain resolution of 0.04% over a gage length of 300 μm . The technique is also adapted to biological studies. First, cells are stained with fluorescent dyes and cultured on PDMS substrates. They are then stretched under a fluorescent microscope where the fluorescent images of the cell and microspheres are simultaneously captured. This way, we can extract the deformation and strain in the substrate by evaluating micrograph of microspheres and at the same time we can analyze the biochemical events in the cells from fluorescent images of cells.

In the remain part of the thesis, a review of mechanical forces on cell morphology and the theories used in describing the effects of mechanical loads on cells' behaviors will be discussed. The shortcomings of the methods developed so far to measure the forces acting on cells and strain that cells experience will be highlighted in Chapter 2, literature review.

Chapter 3 describes the details of experimental setup and operation of tensile stretcher integrated with fluorescent microscopy. It also includes the preparation and surface characterization of PDMS samples. The robustness of the proposed technique and some applications are summarized.

Chapter 4 provides the details regarding the electromechanical design, fabrication process flow and some initial fabrication results of MEMS traction sensor. As the sensor utilizes the piezoresistive silicon nanowires as transducers, backgrounds on piezoresistance effect in single crystal silicon is given in this chapter.

Chapter 2

LITERATURE REVIEW

2.1. The Response of Cells to Mechanical Forces

Cells are composed of different mechanisms each of which evolves in a different way to sense the physical properties of the location and the mechanical forces generated within them. Sensing and assessing the external loads during the interaction of cells with neighboring cells or tissues play an important role in forming the cellular functions and organizing the cellular components [5-8]. Cell migration, moving of cells from one place to another place, is affected not only by biochemical composition but also the mechanical properties of the extracellular environment [9]. Cells can sense the physical properties of attached surfaces by pulling or pushing them [10-12]. During organ formation, for example, the stiffness of the extracellular environment changes dramatically. This causes some changes in cytoskeletal organization and cell differentiation [13, 14]. Recent studies have shown that both external and internal forces act through the cytoskeleton to affect cellular behavior and local mechanical properties [15]. Now scholars wonder how cytoskeletal generate, transmit, and respond to mechanical signals.

As studies on the cytoskeleton are extended, more examples of cells responding to mechanical cues through cytoskeleton have been revealed. This also brings new questions of how, what, and where physical forces are sensed. Evidence implicating stress-induced changes in focal adhesions and adherent junctions has been found and several molecules in cells have

been identified as mediators of mechanical inputs. Depending on substrate stiffness, for example, mesenchymal stem cells differentiate into cells of various lineages. Differentiation of cells based on elasticity sensing is blocked by inhibiting the protein non-muscle myosin indicating that changes in gene expression may be the end of mechanosensing [15]. Although this transformation can be observed in cells in culture, the mechanism of storing information about physical interaction with mechanical microenvironment is unclear.

Differences in external conditions and random events *in vitro* environment can lead to different cytoskeletal structures for genetically identical cells. Endothelial cells in culture, for example, contains randomly oriented stress fibers under similar conditions. When these cells are exposed to shear stress, the fibers will orient in the direction of shear stress. After shear stress is removed, stress-oriented fiber distribution will disappear slowly. If the cells are detached from the surfaces immediately after stress is removed, then the elongated shape persists [16]. In this way, the cell's past mechanical interactions from the orientation of fibers in that cell can be extracted. The ability of the cytoskeleton in the transduction of mechanical signal from the external microenvironment in recording the cell's past, as well as its role as a scaffold for many reactions past may significantly affect the cell's future [17].

We know that the ability of animal cells to sense and response the external mechanical stimuli depends on the cytoskeletal structures [18, 19]. Three significant functions carried out by the cell's cytoskeleton are:

1. spatial organization of the cells' contents
2. physical and biochemical connections of cells to the external environments
3. force generation that enables cells to move and change their shapes

Although cytoskeleton carries out broad functions and forms a large number of structures, it comprises only three types of polymers [4]. Figure 2.1.1 shows these three types of cytoskeletal polymers: actin filaments, microtubules, and intermediate filaments. Among these three cytoskeletal polymers, intermediate filaments (IFs) are relatively strong and ropelike, but they are less dynamic than actin and microtubules. This property of IF maintains mechanical integrity for the living cells.

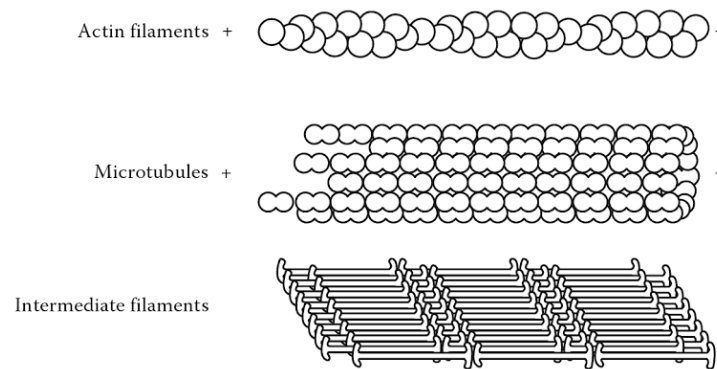


Figure 2.1.1 Cytoskeletal polymer structures: actin filaments, microtubules, and intermediate filaments [4].

The protein subunits of intermediate filaments may vary causing different type of IFs. Some cells may have specific types of IFs. For example, neurons have neurofilaments, muscle cells have desmin filaments, and keratins are found in epithelial cells. Some intermediate filaments are more commonly in cells. For example, lamins and vimentin filaments are found in all cell types.

The studies on cytoskeletal IF networks show that cells experience significant embrittlement in the absence of IFs which causes various human diseases [20-23]. The studies of Coulombe *et al.* [24] Chipev *et al.* [25] and Lane *et al.* [26] show that some skin diseases are caused by point mutation in keratins which have been related to structural alteration in IFs [27]. Janmey *et al.* [28] proved the role of IF in providing cell resistance to mechanical stresses by applying high strain to vimentin IF in vitro.

Attention is now focused on how external force distribution acting on the cells governs the formation of intermediate filaments, what the maximum stress that IF can withstand is, and whether cells can still survive under overloads on intermediate filaments. To answer these questions, a simultaneous measurement of force distribution and morphological changes in IFs should be conducted while cells undergo a deformation caused by chemical or physical methods. This way, information about the correlation between mechanical stimuli and dynamic changes in IF synthesis, assembly, and motility can be extracted.

Force measurement with simultaneous fluorescent imaging in those studies is the most challenging part as it requires active force sensing elements. To achieve active force measurement, it should be decoupled from the imaging system and should provide real-time

data. The next section of this chapter will discuss the methods that have been developed for force measurement in cell mechanics. The available techniques will be summarized and their shortcomings will be highlighted.

2.2. Force Measurement in Cell Mechanics

At the macro-scale, tissues can withstand compressive and tensile stresses up to hundreds of MPa. Bone, for example, can withstand a tensile stress of 100 MPa and a compressive stress of 170 MPa [29]. At the cellular level, forces range from pico-Newton to nano-Newton. Thus, the system used for force measurement should resolve forces in the order these magnitudes. One assumption at the micro-scale is to elucidate the mechanical properties of a cell as a singular unit [29]. At the micro-scale, however, this assumption is no longer valid as the cells are spatially and temporally heterogeneous.

Many techniques have been developed to measure the non-uniform force distribution caused by inhomogeneous mechanical properties of the cells. The most common way to measure the force is to characterize Young's modulus (E), shear modulus (G), stiffness (k), and viscosity (η) of the substrates and use these properties to interpret the force values. Figure 2.2.1 summarizes three common methods and mechanical parameters used in cell studies. In panel *i*, the flexible substrate with adherent cells is exposed to a uniaxial tension where both substrate and cells experience strain. The cells under tension increase their traction forces to balance the pulling force of the substrate. The relative Young's moduli of the substrate and the cells define the amount shared by cells and substrate. Panel *ii* shows shear force applied to an adherent cell by flowing fluid through a microfluidic chamber. Applied pressure on the fluid causes a flow in the microfluidic channel result in shear stress to act on the cell. The magnitude of the shear stress is a function of both velocity gradient and the viscosity of the fluid. The formulae used for this type of load are shown in the bottom right of Figure 2.2.1. Panel *iii* demonstrates the use of atomic force microscope (AFM) as a force measurement technique. This method is mostly preferred in cases where determining the Young's modulus of a cell is concerned. This technique requires the use of the Hertz model shown in the panel *iii* of Figure 2.2.1. The parameters for AFM cantilever, *e.g.*, stiffness constant (k), Young's modulus (E), and dimensions, must be known.

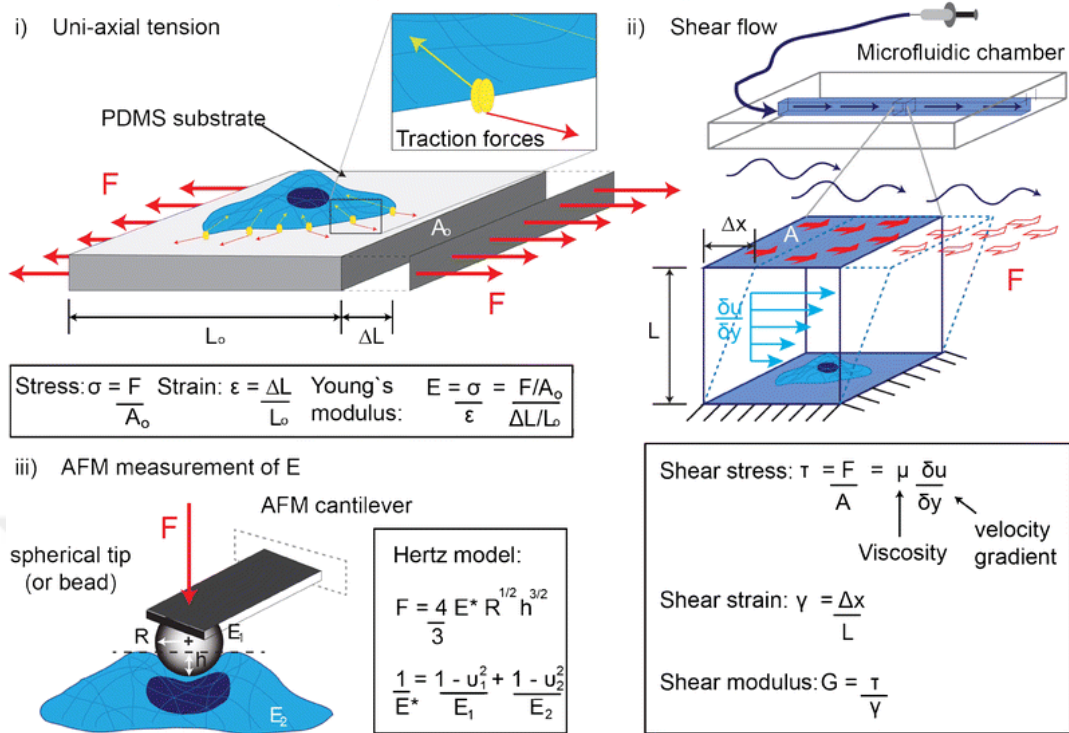


Figure 2.2.1 Methods and mechanical parameters relevant to cell and tissue studies [29].

Another method to measure the traction forces exerted by the cells on an elastomeric substrate is to microfabricate an array of polydimethylsiloxane (PDMS) pillars with known physical and chemical surface properties and convert the deformations of these pillars into force values [30]. First, Silicon wafers are patterned with an array of cylindrical holes using conventional photolithography. Prepolymer PDMS with desirable curing agent is mixed and poured over the patterned silicon wafers. The mixture in the mold is then cured at the optimum temperature. After PDMS is cured, it is peeled off from the mold (see Figure 2.2.2A). Since PDMS is hydrophobic, an additional process of plasma oxidation is conducted to make PDMS surface hydrophilic before cell culturing. Hydrophilic surfaces facilitate fibronectin adsorption. Cells are then cultured on these micropillars as shown in Figure 2.2.2B and local deformation of the pillars are measured by using a tracking algorithm.

Once the deflection of the micropillars are obtained, the force values corresponding to these deflections can be extracted using proper force-deflection formulae. Roure *et al.* [30] used the following force formula for a pillar having a length of L and radius of r :

$$F = \left(\frac{3}{4}\pi \frac{r^4}{L^3}\right) \Delta x$$

where E and Δx are the Young's modulus and the deflection of the pillar.

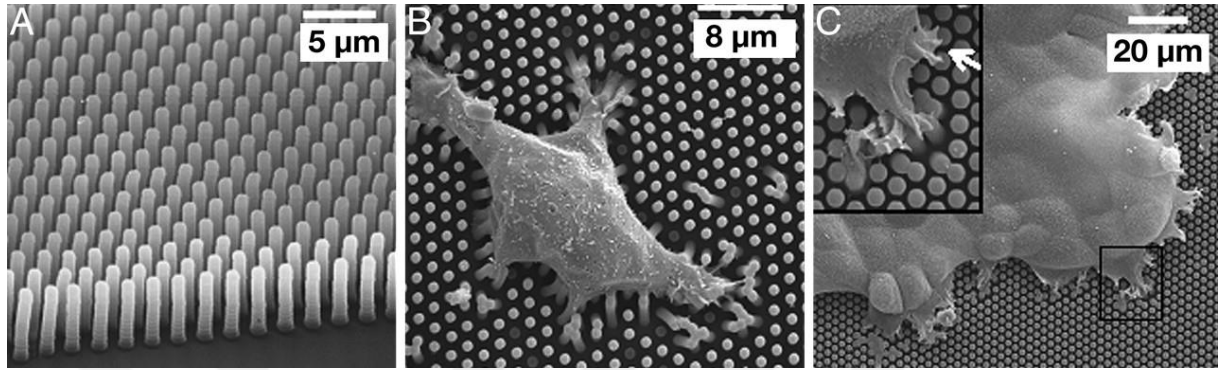


Figure 2.2.2 Micrograph of fabricated PDMS pillars (A), an individual cell lying on the microdimensional force sensing array (B), A cell monolayer with close-up view in the inset (C) [30].

2.3 Non-Contact Strain Measurement Techniques in Cell Mechanics

Microfabrication technology is utilized frequently for preparing microscopic structures on various biocompatible polymers with a particular focus on biomedical applications such as scaffolds in tissue engineering, drug delivery systems, microfluidic devices, and biosensors [31, 32]. Two commonly used techniques among various others are soft lithography and photolithography. Both methods are the combinations of simple and cost-effective patterning processes [33]. Elastomers, including gels, are convenient materials for microfabrication processes and are widely found in microvalves, microsensors, and interfaces with biological materials [34-36]. They also serve as isolation layers in electromechanical systems protecting the components against environmental factors. Polydimethylsiloxane (PDMS), in particular, is optically transparent and nontoxic, exhibits a low surface tension and tunable elasticity, and therefore it is well suited for biological samples in aqueous environment. Due to its ease of fabrication and adjustable surface properties, it is highly favored for stretchable electronics, microfluidic systems and bio-micro electromechanical systems (MEMS) within the same size scale as biological samples [35, 37-39]. Therefore, its mechanical properties have been subject to various experimental and modeling studies [40-43].

When micropatterns or fabrication imperfections are generated on a polymeric substrate, the strain profile under loading exhibits a point-to-point variation. Such strain gradients result due a heterogeneity in material properties or discontinuity in the cross-section. Nonuniform strain distribution may vary the properties of small domains on polymers used to study the mechanical response of cells that may be affected adversely by such small strain gradients in their microenvironment [2, 44]. The microscopic response is then no longer connected to the bulk response by simple scaling laws [45, 46]. Hence, a direct local measurement of the mechanical response of the microenvironment is required in the case of inhomogeneous soft materials under loading. Such measurements should exhibit a high spatial resolution and sensitivity to the variations in the local response and the presence of steric constraints [46]. To obtain local strain distribution precisely in a time-resolved manner, a spatiotemporal analysis throughout the whole loading is required [47]. For soft materials, such as elastomers, non-contact measurements are preferred as the weight and clamping force of the strain

measurements device and mechanical impedance at the point of contact can lead to unreliable results.

An earlier method proposed for such a contactless measurement is the digital image correlation (DIC). It is a robust, fast, and affordable tool for the measurement of heterogeneous deformation and strain on the sample surface, and so far it has been successfully employed for soft materials under deformation, *i.e.*, polymers, foams, textiles, and biological tissues [48-50]. In previous studies, this technique is employed to capture deformation and strain of the entire test specimen using a fixed camera aligned to be in-plane with the specimen. It is an optical method of strain measurement based on the principle of comparing speckle patterns on the sample surface [48, 51, 52]. Images of speckle patterns before and after deformation are captured by the camera, the patterned sample surface is then divided into small identifiable sub-images and displacements of these sub-images are tracked. Finally, in-plane strains are extracted from the displacement data by applying the appropriate strain formulation.

An alternative non-contact method is single-particle tracking (SPT), a recently demonstrated tool to obtain the paths of individual particles from consecutive images during sample stretching, which are then used to compute in-plane strains [53]. It is preferred in biophysics and fluid mechanics studies such as tracking of proteins during cell proliferation and measuring diffusion coefficients of membrane lipids obtained by the mean-square displacement of single-particle trajectories [54-56]. SPT provides coordinates of the individual particles. As this requires additional steps to define strain components in terms of displacements, it makes SPT less appealing than DIC [57, 58]. However, the accuracy of the strain in DIC depends on the ambient conditions and set-up parameters. It suffers from temperature variations, vibrations, changes in light intensity, and similarities of the speckle patterns [51]. Histogram equalization and filtering are often applied on microscopy images in SPT to eliminate the effects of ambient conditions and to enhance particles' contrast. Contrary to SPT, image enhancement techniques are not suggested in DIC since smoothing and equalization may remove the characteristics of speckle patterns created deliberately to enable the strain measurement in the first place.

The DIC technique is further extended to the study of Scanning Tunneling Microscope (STM) [59, 60] and Scanning Electron Microscope (SEM) [61] images further enhancing the spatial resolution. The mechanical properties of metallic materials at the microscale are effectively determined using a combination of high-resolution scanning microscopes (STM or

SEM) and DIC. These combinations are carried out mostly for metallic materials. For polymers, however, the non-contact strain measurement techniques have so far not been fully integrated with optical microscopes as an *in-situ* strain measurement tool. The main hurdle of performing such measurements is the difficulty of carrying out imaging during the application of load [62]. Two conditions, namely, the compatibility of the non-contact measurement set-up with the microscope and the synchronization of the loading device with the microscope stage, must be met to adapt these techniques at the microscale. Herein, we propose a non-contact technique for the heterogeneous strain measurement at the microscale with a high spatial resolution. Using a tensile stretcher integrated with inverted microscopy, sequential images of deforming surfaces are taken. SPT algorithm is then applied on images to extract the positions of the embedded microspheres. SPT is selected over DIC due to the physical nature of the experimental set-up and the ease of computation [53]. Furthermore, it proves to be more suitable to track the positions of these microspheres compared to tracking the subimages of speckle patterns, when it comes to the validation of the experimental technique via the Finite Element Method (FEM) as frequently demonstrated for measuring elastomer deformations [63]. Once the geometry is constructed in the FEM software, the initial positions of the microspheres can directly be implemented, where each microsphere is represented by a node in the FEM mesh. This modeling ability leads to an equivalent point trajectory corresponding to each experimental bead trajectory. Thus, displacement, deformation or strain of any point in the experiment and FEM model are accurately compared.

The proposed method can easily be adapted to cell mechanics studies to replace the commonly employed measure of strain. Quantification of sample deformation in cell stretching experiments is mostly based on the cross-head displacement [64-66] or FEM analysis [67, 68]. Using cross-head displacement, strain is calculated as the ratio of the total elongation to the original length of the sample. Some studies utilize FEM simulations of the substrate to calibrate the local strain in terms of cross-head displacements [67, 68]. To do this, the sample geometry is constructed in FEM software and a cross-head displacement is applied incrementally. For each cross-head displacement, a corresponding strain value of the area of interest is obtained. Instead of measuring the instantaneous strain in the cell stretching experiment, these strain values obtained from FEM simulations are utilized as references. Strain measurement based on

cross-head displacement or FEM calibration of the substrate assumes that strain in the sample under test is uniform and matches well with the simulation results.

The strain distribution in actual physiological tissues or living cells, however, is inevitably inhomogeneous [2]. Similarly, geometric discontinuities in the substrate such as patterned holes [1] or pillars [69, 70] lead to a similar strain nonuniformity. Therefore, the following two aspects of non-contact measurement are of utmost importance to develop a technique where nonuniform strain in stimulated *in-vitro* environment can be measured successfully: i) real-time measurement and ii) high spatial resolution:

- i) As far as real-time measurements are concerned, full-field DIC method does not match the speed of strain gauges [71, 72]. When DIC is utilized in the estimation of plastic zone size under cyclic loadings [73] or the detection of fatigue damage [74, 75], a postprocessing step is required to retrieve strain or deformation from data saves as images. To reduce processing time and apply DIC in a real-time manner, the calculation for correlation is carried out only for two mark points instead of the whole field of view. This way, strain data can be extracted at a frequency of 10 Hz and fed back to the control system of the testing equipment [71, 76]. Using current setup, microscopy images throughout the entire stretching can be captured at a frequency of 17 Hz for full field strain analysis.
- ii) In terms of resolution, a few studies report the minimum detectable strain or displacement. Lu *et al.* [77] pioneered the use of DIC to determine the surface deformation of thin-walled cylindrical specimens subjected to combined axial and torsional loadings. They managed to resolve a displacement of 3.5 μm over a gage length of 5 mm, which corresponds to a strain value of 0.07%. Kanchanomia *et al.* [76] used DIC to conduct strain-controlled fatigue tests. Aiming at measuring the strain at a high speed, they reduced the number of areas of the interest to two pieces of black tapes with a gage length of 8 mm. The smallest displacement they could control was 8 μm corresponding to $\pm 0.08\%$ strain. Tao *et al.* [71] improved the minimum detectable strain value to 0.01% using two markers on the specimen with a gage length of 25 mm. In their work, however, the minimum measurable displacement associated with this length was 2.5 μm . Using two high-speed cameras of the DIC system mounted on the movable frame, Jerabek *et al.* [51] succeeded to

measure 0.006% strain (3 μm displacement) for a gage length of 50 mm. Although, the precision in strain measurement is improved using different subpixel algorithms and DIC test setups, the minimum displacement reported in these studies is still in the order of a few micrometers. This challenge is addressed in the present work by integrating optical microscopy with a uniaxial stretcher and developing subpixel single-particle tracking algorithm. The area of interest is deliberately reduced to $820 \times 820 \mu\text{m}^2$ to increase the spatial resolution. Another issue frequently encountered in this field is the minimum resolvable displacement for small gage lengths. The smaller gage length provides a better spatial resolution but it requires a higher precision in displacement measurement. Our approach provides submicron displacement measurement together with strains on the order of 10^{-4} . Furthermore, the measurements are carried out not only for an average strain between two marked points, but for the all particles that exist in the field of view. This enables us to determine strain spatially and makes it possible to capture non-uniform deformation around micropatterned structures.

Overall, the goal of this study is to develop a non-contact strain mapping technique to capture the complex strain distribution in microscale patterns with a high spatial resolution. A direct local measurement for microenvironments is carried out to obtain microscopic response of the sample with embedded microscopic rhombus-shaped thru-holes. This microscopic response is expected to be significantly different from the bulk response.

In the remainder of this work the experimental setup is introduced first along with the sample preparation technique. This is followed by a series of discussions on the tracking algorithm, the discretization technique for FEM modeling and the computation of strain components. Strain distribution around rhombus-shaped micropatterns with a side length of 200 μm is provided. The work is concluded with a validation of the proposed technique by comparing measurements against FEM predictions.

Chapter 3

SPATIOTEMPORAL ANALYSIS OF STRAIN DISTRIBUTION

Micropatterns are generated on a vast selection of polymeric substrates for various applications ranging from stretchable electronics to cellular mechanobiological systems. When these patterned substrates are exposed to external loading, strain field is primarily affected by the presence of microfabricated structures and similarly by fabrication-related defects. Capturing of such nonhomogeneous strain fields is of utmost importance in cases where study of the mechanical behavior with a high spatial resolution is necessary.

Image-based non-contact strain measurement techniques are favorable and have recently been extended to Scanning Tunneling Microscope (STM) and Scanning Electron Microscope (SEM) images for the characterization of mechanical properties of metallic materials, e.g. steel and aluminum, at the microscale. A similar real-time analysis of strain heterogeneity in elastomers is yet to be achieved during the entire loading sequence. The available measurement methods for polymeric materials mostly depend on cross-head displacement or precalibrated strain values. Thus, they suffer either from the lack of any real-time analysis, spatiotemporal distribution, or high resolution in addition to a combination of these factors.

In this chapter, these challenges are addressed by integrating a tensile stretcher with an inverted optical microscope and developing subpixel particle tracking algorithm. As a proof of concept, the patterns with a critical dimension of 200 μm are generated on polydimethylsiloxane (PDMS) substrates and strain distribution in the vicinity of the patterns

is captured with a high spatiotemporal resolution. Current noncontact techniques on elastomers can deliver a strain resolution of 0.001% over a minimum length of 5 cm. More importantly, inhomogeneities with this quite large region cannot be captured. In the field of strain measurement, there is always a tradeoff between minimum measurable strain value and spatial resolution. The proposed technique can overcome this challenge and provides a displacement measurement resolution of 116 nm and a strain resolution of 0.04% over a gage length of 300 μm . Similarly, the ability to capture inhomogeneities is demonstrated by mapping strain around a thru-hole.

The robustness of the technique is evaluated by reducing number of triangles or the area of interest, where no appreciable change in strain measurement is observed despite the significant variations imposed on the mesh. The proposed approach introduces critical improvements for the determination of displacement and strain gradients in elastomers regarding the real-time nature of strain mapping with a microscale spatial resolution.

As an application of the technique, we stained cells with different fluorescent dye and cultured them on the PDMS substrate. Then, we indirectly stretched the cells by stretching the substrate and imaged the cell while they are stretched.

3.1. Experimental Setup and Operation

The overall mechanical design of the uniaxial tensile stretcher is laid out with focus on flexibility, easy customization, and most importantly, compatibility with inverted microscope setups. The stretcher (Fig. 3.1.1) utilizes a linear actuator (Part 1 in Fig. 3.1.1) that applies tensile load, and a load cell (Part 10) to measure the tension in the specimen. The linear actuator is a stepper motor with a lead screw (Part 2) as the motor shaft. The sample (Part 9) is gripped tightly at two ends by custom-made aluminum grips (Parts 8). One of the grips is movable while the other is fixed. The motion of the movable grip is actuated by the stepper motor and a linear guide rail system (Parts 4) and carts (Parts 5) are used to achieve a stable linear motion of the grip. The movable grip is attached to the motor shaft via a mounting piece (Part 6) and the fixed grip is attached to the load cell via another mounting piece (Part 7). The grip mounting pieces are designed for permanent use while the grips themselves can easily be replaced. Hence, a variety of different grips can be used with samples of different sizes and shapes. The linear

actuator, load cell, and the grips are assembled onto two pieces of T-slotted aluminum extrusion pieces (Parts 12) that make up the backbone of the stretcher and enable easy mounting and customization. The entire assembly can be mounted onto most inverted microscope stages using simple L-shaped brackets (Parts 13) that can be custom-made for the microscope stage and attached to the T-slotted extrusion pieces on the stretcher assembly.

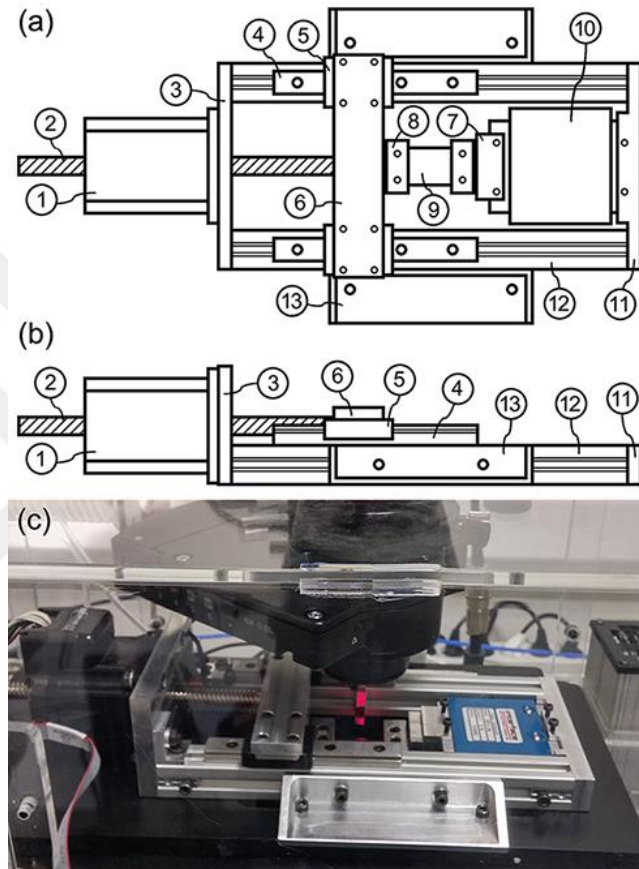


Figure 3.1.1 Schematic (a) top view and (b) front view of the stretcher illustrating the basic components: The stepper motor (1) and lead screw (2), motor mounting plate (3), linear guide rails (4) and carts (5), movable grip mounting piece (6), fixed grip mounting piece (7), grips (8), specimen (9), load cell (10), load cell mounting plate (11), T-slotted aluminum extrusion pieces (12), and brackets for mounting the stretcher onto a microscope stage (13). (c) View of the tensile stretcher mechanism mounted onto the stage of an inverted microscope.

This custom-made stretcher is preferred due to its compatibility with inverted microscopy setups and synchronization with motorized stages of the microscopes. The entire stretcher is mounted onto an inverted microscope stage using simple, custom-built L-shaped brackets. For mechanically driven stretchers, the synchronization is required to implement motion compensation which makes it possible to keep a certain area in the field of view during the entire duration of stretching [78, 79], thereby enabling the application of large strains.

To implement motion compensation between the stretcher and the motorized stage, first, the coordinates of the observed area and sample boundaries are recorded by scanning the specimen surface. The area of interest is thus selected. Then, the velocity of the stage is adjusted in accordance with the stretcher velocity based on the ratio of the distance of the observed area from the fix end and the initial length of the sample. For example, the stage speed is adjusted to be half of the stretching speed in order to keep the central region of the sample in the field of view. For any other region, the ratio is recalculated and the speed is readjusted before each experiment. This way, the beads that are initially captured remain in the field of view up to high stretching limits, whereas for the case of no motion compensation, the beads gradually move out of the field of view.

System characterization

The uniaxial tensile stretcher is operated and controlled using a PC and the LabVIEW development environment. Both the stepper motor and the load cell are powered by a DC power supply and the appropriate electrical connections are made between the motor, load cell, and the PC. A block diagram of the stretcher system is shown in Fig. 3.1.2(a), detailing the electrical connections between the power supply, stepper motor, load cell, and the PC.

The linear stepper actuator has an integrated driver and a programmable controller with two communication interfaces; a general purpose digital I/O port and an RS-485 communication port. The RS-485 communication port is connected to the PC via a communication converter and is used to assign functions to the general-purpose I/O channels for driving the motor. The general-purpose I/O channels are then connected to the PC via a shielded connector block and a DAQ card and the motor is controlled through this interface using a LabVIEW subroutine.

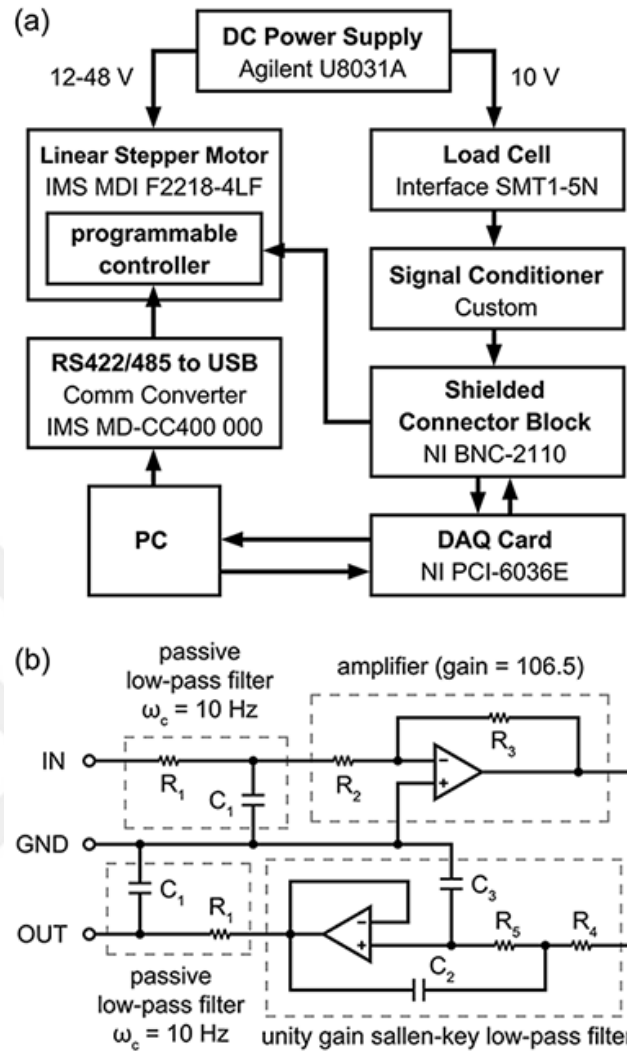


Figure 3.1.2 (a) Block diagram of the stretcher system, (b) schematic of the custom-built signal conditioner circuit for filtering and amplifying load cell readout signal.

The tension in the specimen is measured by a load cell consisting of strain gauges configured as a Wheatstone bridge. A DC power supply provides 10V excitation to the load cell and the output signal is filtered and amplified using a custom signal conditioner circuit as depicted in Fig. 3.1.2(b). The conditioned output is then connected to the PC through the shielded connector block and DAQ card, and the same LabVIEW subroutine that controls the linear stepper motor is used to record the load cell output.

Due to the lack of an encoder or a displacement sensor to provide displacement feedback for actuation, the linear actuator is controlled in an open loop configuration. This requires meticulous characterization of the actuator mechanism to ensure the accuracy of the applied displacement. The linear stepper actuator uses a lead screw to convert the rotary motion of the

rotor to linear motion. As specified on the motor datasheet, the stepper motor has a step angle of 1.8° and the lead screw travels $50.8 \mu\text{m}$ for one full step of the stepper motor. The integrated driver is capable of dividing the full 1.8° step angle into 256 bits or “microsteps” by modulating the current input to the electromagnets in the stator, resulting in a gain value of $0.198 \mu\text{m}/\text{microstep}$. The applied displacement is characterized by attaching a scale bar onto the movable grip and measuring the actual displacement generated by the motor by taking images of the scale bar under a microscope and implementing image processing. A linear relationship is observed between the measured displacement (in μm) and the motor command (in number of microsteps) with a coefficient of determination of $R^2 = 0.9999$, and a gain value of $0.196 \mu\text{m}/\text{microstep}$ which is in close proximity of the value reported on the datasheet.

The load cell is also calibrated before experimentation. It has a load capacity of 5 N and the rated output value specified in the calibration certification is 2.1095 mV/V resulting in a sensitivity of 4.2189 mV/N . To enhance the measurement resolution, the raw output signal of the load cell is amplified and filtered using a custom-built signal conditioning circuit (Fig. 2(b)). The signal conditioning circuit consists of two passive RC low-pass filters, an operational amplifier with a gain value of 106.50, and a unity gain Sallen-Key low-pass filter. The load cell is calibrated using precise weights ranging from 1 gram to 20 grams. The output voltage of the signal conditioner circuit is measured and recorded for each different load. There is a highly linear relationship between the measured output voltage and applied load with a coefficient of determination value of $R^2 = 1.0000$, and a sensitivity of 0.4339 V/N . The sensitivity value obtained by multiplying the raw output sensitivity (calculated from the calibration certification) with the amplifier gain of the signal conditioner circuit is in agreement with the calibration results.

Imaging Mechanism

After the stretcher is assembled and calibrations are carried out, the stretcher is integrated with Olympus IX81 inverted microscope (Fig. 3(a)). The images are recorded with an Andor Ixon3-897 reinforced by a quantitative digital camera technology called Electron Multiplying Charge Coupled Device (EMCCD). A 10x air objective with 0.30 numerical aperture is used for magnification. The integrated system enables recording both white light and fluorescence images during stretching.

The maximum tracking distance of any bead embedded in the sample across the frame is $819.2\text{ }\mu\text{m}$ due to the small field of view of the 10x objective and pixel size of the camera. Moreover, the sample is fixed at one end and stretched from the other end, as a result of which, the group of fluorescent beads that are initially captured gradually move out of the field of view as the sample is stretched. This makes it challenging to track the same set of beads (and cells) at high strains as they move along the pulling direction and eventually leave the field of view. This is an issue commonly encountered in stretching systems and it can be resolved by implementing motion compensation, *i.e.* moving the entire setup to keep the objects of interest in the field of view [80]. In the present work, this issue was addressed by using the motorized stage of the microscope to move the entire stretcher assembly in a direction opposite to the stretching direction to compensate for the motion of the sample. First, the center point of the sample is located by scanning the sample and recording the coordinates of its boundaries. Then, the stage is synchronized with the stretcher and the stage speed is adjusted to be half of the stretching speed. This way, the beads around the center point of the sample can be kept in the field of view while the sample is stretched up to high strains. A comparison of stretching without stage control and stretching with stage control to compensate for the motion of the beads is shown in Fig. 3.1.3(b) and Fig. 3.1.3(c), respectively. The same cross-head strain is applied to the samples in both cases and the trajectories of the beads are recorded. At 8% cross-head strain, for the case of no stage control, 70 of the original 107 beads remain in the field of view (Fig. 3.1.3(b)), *i.e.*, 37 beads move out of the field of view, whereas for the case of stretching with stage control, all 107 beads remain in the field of view (Fig. 3.1.3(c)).

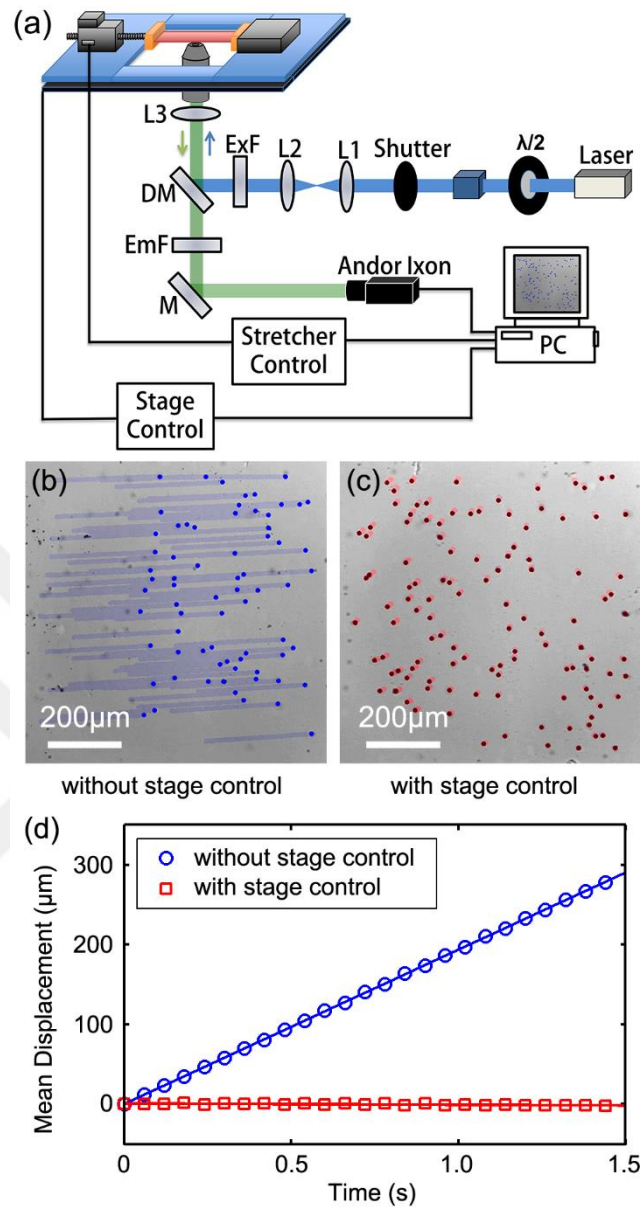


Figure 3.1.3 (a) Schematic representation of optical setup. (b) (multimedia view) Trajectories of beads recorded under white light illumination without stage control and (c) (multimedia view) with stage control. (d) Comparison of the mean displacement of beads with and without stage control.

3.2. Sample Preparation and Surface Characterization

PDMS is broadly used in drug delivery systems, microfluidic devices, biomedical systems and biosensors due to its straightforward fabrication, optical transparency, flexibility, resistance to high temperatures, and low cost [40, 81-83]. The sample used in the present work is prepared

by mixing PDMS precursor and curing agent (Dow Corning Sylgard 184) in a 10:1 weight ratio. Polystyrene microspheres (Phosphorex Inc.) with a 5- μm diameter are then mixed with precursor and curing agent at a concentration of 7.6 $\mu\text{L/g}$. The bead concentration in the mixture should provide an adequate number of beads in the field of view to capture strain heterogeneity. Similarly, a measurable distance between the beads should be attained. The prepolymer mixture is spun with a stir bar on a magnetic stirrer for 6 minutes to prevent conglomeration and ensure a homogeneous mixture.

The sandwich molding procedure illustrated by Jo *et al.* [84] is utilized for the patterning of the PDMS sample. First, micropatterns for the definition of rhombus-shaped holes are fabricated on a 4-inch-diameter, <100> silicon wafer. For this purpose, SU-8 photoresist (MicroChem Corp.) is spin coated on the cleaned silicon wafer at 1000 rpm. The resist-coated wafer is first baked at 65°C for 7 minutes and then at 95°C for 25 minutes on a hotplate. After this soft bake, the exposure is carried out for 30 seconds in contact mode in a UV mask aligner with an incident flux of 22 mW/cm^2 , followed by post baking at 65°C for 5 minutes and 95°C for 15 minutes. Finally, the pattern is developed by immersing the wafer in SU-8 developer by MicroChem. This process summarized in Table 3.2.1 results in 135- μm high rhombus-shape SU-8 structures on the silicon wafer in the form of a master mold (Fig. 3.2.1(a)).

Process Name	Process Parameters	Duration
Wafer Priming	Acetone and Isopropyl Alcohol	-
Spin Coating	$v=100$ rpm, $a=100$ rpm/s	1 min
Soft Bake	65 °C	7 min
	95 °C	25 min
Expose	Incident Flux = 22 mW/cm^2	30 sec
Post Bake	65 °C	5 min
	95 °C	15 min
Develop	Immersion	-
Rinse and Dry	Water and N_2	-

Table 3.2.1 Process parameters for microfabrication of SU-8 mold.

The uncured PDMS compound is poured over the master mold and the bubbles generated during pouring are removed by degassing (Fig. 3.2.1(b)). The prepolymer mixture in the SU-8 mold is then carefully covered with a flexible film, allowing the surface tension to pull down the film and hence, a continuous contact with the mixture is formed to prevent possible bubbles that may be generated at the interface. The stack of mold-mixture-flexible film is covered by a Plexiglas plate and then a mild pressure is applied using a clamping device. (Fig. 1(c)). The final stack with clamps is left to polymerize at room temperature for 48 hours. After curing, the PDMS sample is taken out of the SU-8 mold (Fig. 3.2.1(d)).

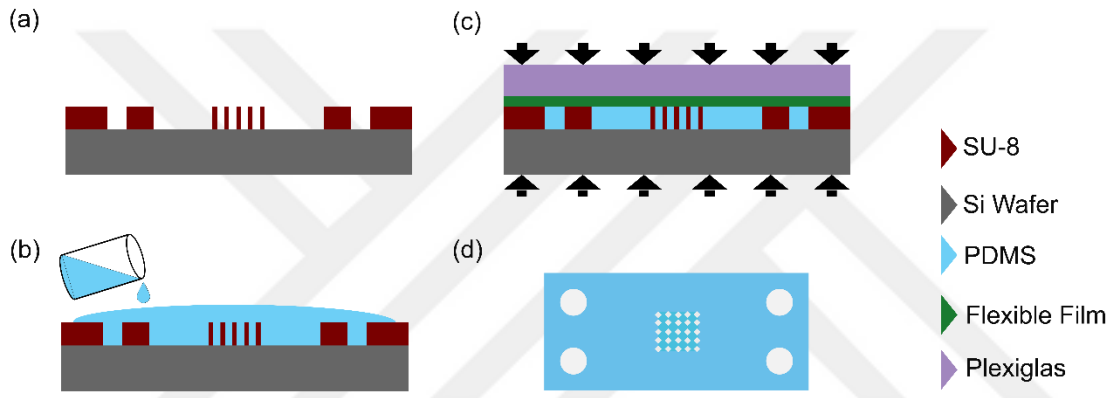


Figure 3.2.1 Schematic description of the sandwich molding process. (a) Silicon wafer with SU-8 pattern (b) Casting of uncured PDMS compound over the master mold. (c) Covering of PDMS with a flexible film and a Plexiglas plate followed by clamping. (d) Top view of the cured PDMS sample.

In order to ensure that all patterns on the mold are transferred to the test sample and a smooth sample surface is obtained after peel off, surface profile and roughness measurements are carried out. Quantitative topographical data, compositional images, and depth profile of the sample are gathered from atomic force microscopy (AFM), SEM, and white light interferometry (WLI), respectively. Figure 3.2.2(a) depicts the overall geometry of the specimen. The sample has a rectangular geometry with a length of 55 mm and a width of 24 mm with gripping regions at both ends for mounting to the stretcher. An array of 13×13 rhombus-shaped holes is visible in the central region of the PDMS sample. These holes have a side length of $200 \mu\text{m}$, a depth of $135 \mu\text{m}$ and make an angle of 45 degrees with the stretch direction. There is an $800\text{-}\mu\text{m}$ distance between the centers of two consecutive holes in both directions. Figure 3.2.2(b) shows the AFM image including the surface profile of the sample with the scan area of $90 \times 90 \mu\text{m}^2$.

PDMS surface roughness in the close vicinity of the hole (designated by the black line) is found to be only 1.82 nm RMS. The red curve shows the depth profile along the red line passing through the hole, where the AFM tip cannot reach the bottom surface of the hole. To verify the symmetric distribution of the rhombus structures on the sample and confirming the distance between consecutive rhombi, SEM images are taken as well (Fig. 3.2.2(c)). Figure 3.2.2(d) shows a close-up SEM image of an individual structure on the sample. Height uniformity is accurately determined by WLI. Top and oblique views of the sample are shown in Fig. 3.2.2(e) and Fig. 3.2.2(f), respectively, where a uniform depth of $130 \pm 5 \mu\text{m}$ is evident. Achieving a narrow distribution of depth is important to prevent thickness-dependent variations in strain. Hence, the sample profile attained in this study allows a 2D analysis of strain. Overall, both the depth distribution and surface smoothness of the sample verify that the sandwich molding is applied successfully to PDMS patterning.



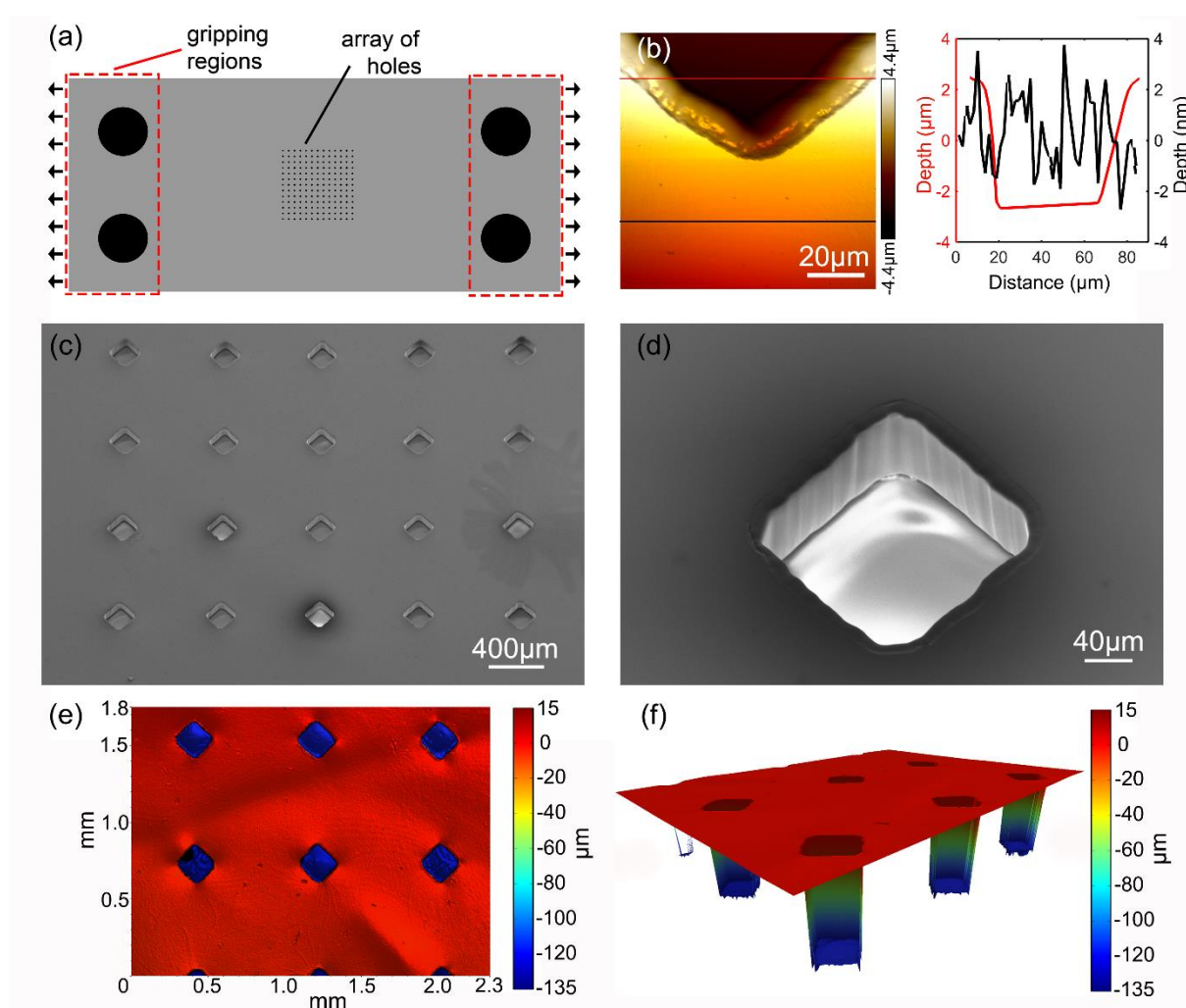


Figure 3.2.2 Surface characterization of microfabricated rhombus-shaped structures on the PDMS sample. (a) A schematic drawing of the PDMS sample, where dots in the center indicate a 13 by 13 array of rhombus-shaped thru-holes. (b) AFM image showing the topography around the holes (left), where black and red line scan data are provided in the attached graph (right). (c) Scanning electron micrograph of a part of the array of holes. (d) A close-up of a single hole with a side length of 200 μm. (e) White light interferometer image showing top view with depth information. (f) An oblique view of the sample.

3.3. Tracking of Microspheres

To track the positions of the embedded microspheres, sequential frames of the PDMS sample are captured at 17 frames/s with Andor Ixon EMCCD camera as the sample is stretched. Then, the trajectories of the embedded beads in the field of view are determined from these sequential frames using a single-particle tracking method. The method is based on the computer-aided

analysis of video images to track the particles such as microspheres, particles, and small cellular components such as organelles and proteins.

The particle tracking method used in this work consists of four steps: image filtering, identification of beads, estimation of particle coordinates, and linking particle coordinates between frames to compose trajectories (Figure 3.3.1). Despite the transparency of PDMS and the high-contrast ratio of small embedded beads, a Gaussian filter is applied to convolve spatially all images for reducing the discretization random noise [85]. Since the focal plane of particles remains unchanged during stretching, a low pass filter with a small sigma of 1.0 pixel is used for all processed images. Moreover, images are subjected to background subtraction and averaging boxcar filtering for image averaging and effective suppression of remaining variation [86]. Borders surrounding each image are simply discarded due to the distortion that is usually introduced by boxcar filtering. Once all images are smoothened, retained particles in each image are identified by the presence of either a local minimum or a local maximum. Positions of these extrema are assigned to particles as their coordinates. The coordinates of particles in the first frame are used as the initial estimate for their positions in the second frame, and this method of linking the particle's trajectory is repeated for the consecutive frames. Trajectories are composed by comparing each registered location in a pair of consecutive frames and minimizing the Euclidean distance of centroids. Due to the elongation in the stretching direction, some beads unavoidably leave the field of view and they are not considered. All trajectories are recorded for the subsequent strain computation.

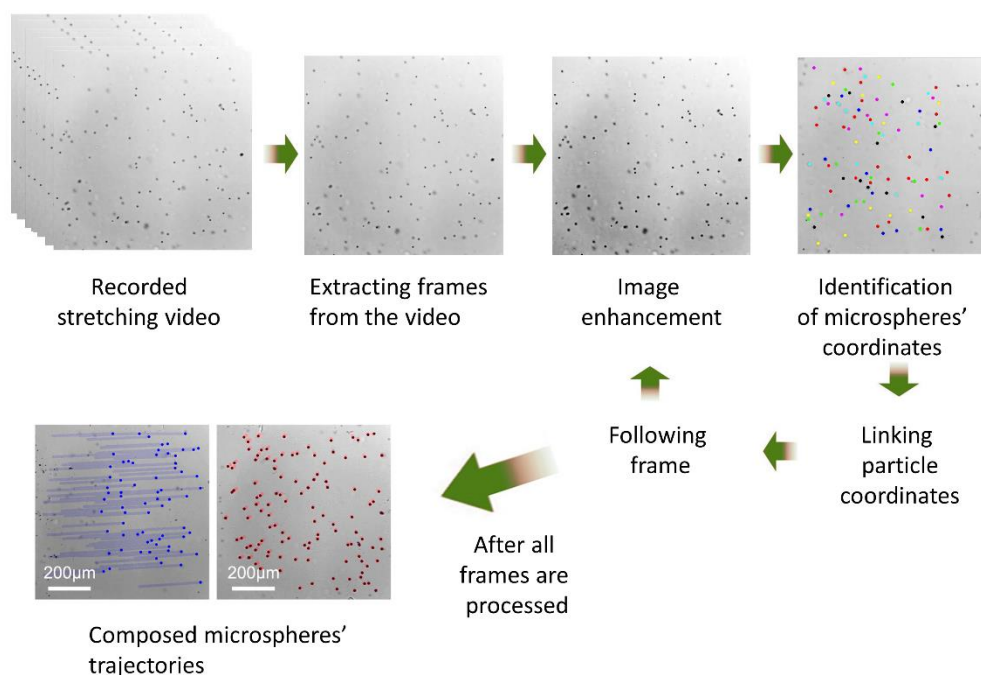


Figure 3.3.1 Figure 3.3.1. Extracting beads trajectories using image process.

After beads trajectories are composed, the motility parameters can also be extracted from those trajectories, i.e. the mean square displacement (MSD) that is an indicator of the spread of trajectory (Fig. 3.3.2(c)). Since bead motion is driven by active directional stretching, MSD follows a parabolic trend that results from the migration pattern of the beads. Small errors on MSD even at longer lag times result from the uniform stretching of the beads and indicate that the beads do not move randomly. Lastly, the angular displacements of the beads are measured and shown in Fig. 3.3.2(d). This is the angle that the trajectory curve makes with the horizontal axis. Probability close to 0° has the highest magnitude indicating that the majority of the beads move horizontally during stretching. The smaller probability between 15° - 30° is due to the small lateral motion of the beads which is also apparent in Fig. 3.3.2(a). The highly directional and uniform motion of the beads indicates a good adhesion of the beads to the PDMS substrate and validates the assumption that the motion of the beads is a result of the applied uniaxial deformation. The results obtained from tracking the positions of fluorescent beads are then used for the evaluation of displacement and strain fields.

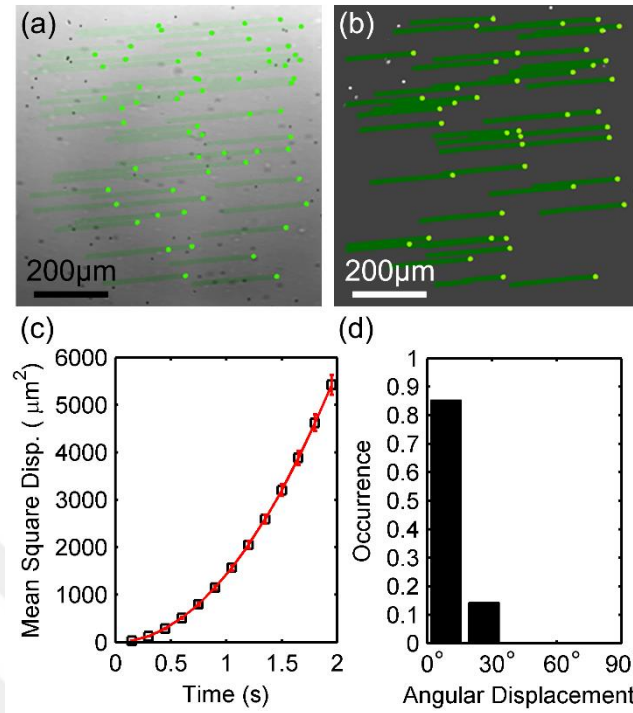


Figure 3.3.2 Tracking the movement of single beads during the stretching and analysis of the motion. (a) Trajectory of beads recorded under white light and (b) fluorescence illumination captured at 16 frames per second for 2 seconds. After trajectories of beads from both illuminations were separately determined, they were overlaid as faded lines to the images. (c) The measured mean square displacement of the beads as a function of time at short time intervals was computed from trajectory of beads as well as (d) the angular displacement of beads along the direction of stretching.

3.4. Finite Element Modeling

FEM is employed to analyze the spatial distribution of strain and verify experimental results. The dimensions of the test sample with the array of holes are used to construct the FEM mesh. The orientation of the sample under the microscope is matched with that of the FEM such that x , y , and z axes correspond to stretch, transverse, and out-of-plane directions, respectively. In addition to the overall geometric construction, the initial positions of the embedded microspheres as obtained from the microscopy of the undeformed sample are implemented in FEM such that each embedded microsphere corresponds to a node in the simulations (see Fig. 3.4.1). The positions of the microspheres are extracted in terms of pixels from the micrographs. However, the implementation requires the positions in physical dimensions. Thus, they are converted into μm and the conversion coefficient is calculated by counting the number of pixels

across a known length. Tracking nodes corresponding to these microspheres in the PDMS sample provides an accurate pointwise comparison of the FEM and experimental results.

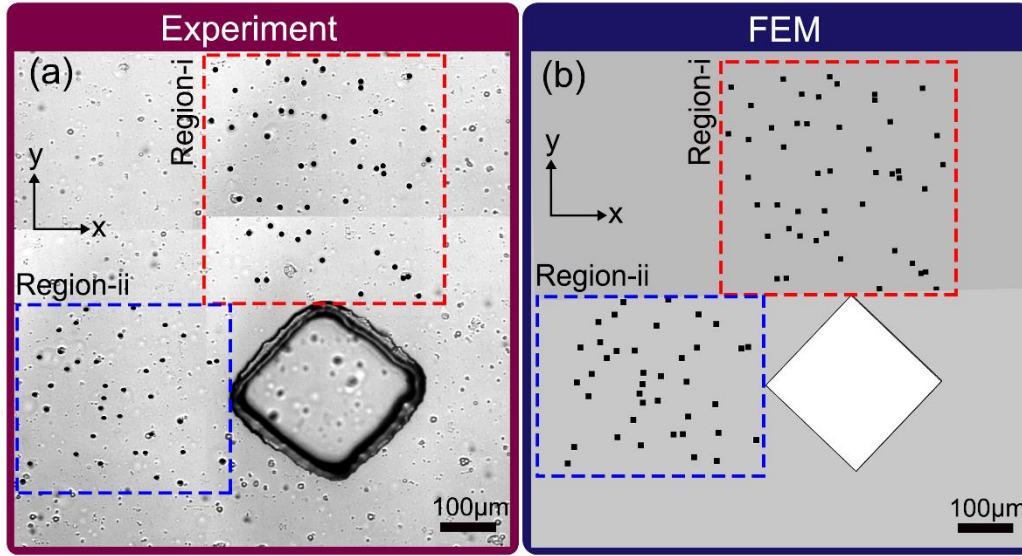


Figure 3.4.1 Initial positions of the microspheres (a) captured in experiment and (b) implemented in the FEM geometry.

After the geometry is constructed and the nodal positions are defined, a tetrahedral mesh is applied to the entire geometry using a commercial software package. As far as the boundary conditions are concerned, one end of the sample is fixed and a prescribed displacement is applied on the opposite end such that the sample is stretched up to 1.2 times of its original length replicating the experimental condition. Since recorded videos have 500 frames, this stretch is applied in 500 steps in order to have corresponding nodal positions for each frame of the video. The material model used for PDMS sample is the first-order Mooney-Rivlin model with the material constants of $c_{10}=90.35$ kPa and $c_{01}=12.82$ kPa. This model is preferred due to its validity for the hyperelastic behavior of rubber-like materials under large strains up to 50% [68, 87]. Once the simulations are carried out, the nodal positions corresponding to the trajectories of embedded beads in the experiment are extracted and recorded for further use in strain computation.

3.5. In-plane (2D) Strain Calculation

The positions of the embedded beads that are obtained from both micrographs and FEM simulations are used in strain computation. To maximize the spatial resolution, the set of positions is connected by Delaunay triangulation (Fig. 3.5.1). This computational technique tends to avoid sliver triangles, which have one or two extremely acute angles, by maximizing the minimum angle of all triangles. It also yields a set of triangles for which no point is enclosed within another triangle [88]. After triangulation is applied to the set of positions, the corresponding strain tensor for each triangular element is computed, which results in a high degree of spatial detail in the strain field [62].

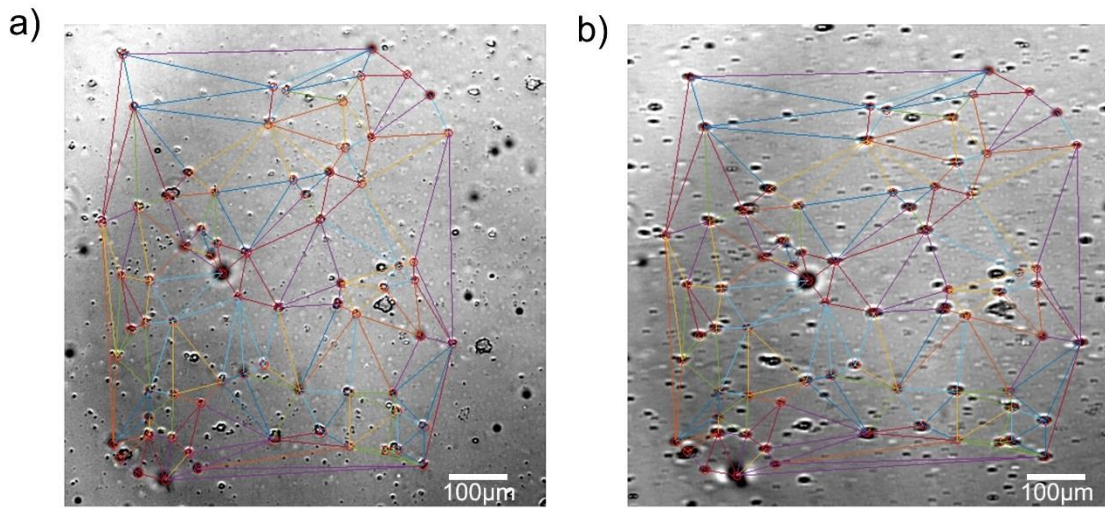


Figure 3.5.1 Delaunay Triangulation of microspheres (a) in undeformed and (a) deformed sample.

The position of a certain particle during deformation can be defined as a function of its initial position and time. In Lagrangian coordinates, the instantaneous positions, x_i , in the deformed configuration can be defined as

$$x_i = \phi(X_i, t) \quad (1)$$

where X_i denotes the initial position of particle i in the field of view and t is time. Since the undeformed and deformed positions are measured in the same coordinate system, the *displacement vector* is simply defined as

$$u_i(X_i, t) = \phi(X_i, t) - X_i \quad (2)$$

Positions and displacements are measurable physical quantities that can be directly obtained from microscopy images. However, strain is a derived quantity, which can be defined in terms of the displacement in various ways such as Cauchy, Green, Almansi, and Hencky strains [89]. The selection of the appropriate strain definition is of critical importance to avoid the misrepresentation of the mechanical response of the material [90]. In this study, *Green strain* is used throughout the entire study, since it is applicable to large elastic deformations [91].

To calculate the strain tensor, first the *displacement gradient tensor* is defined as

$$\nabla u_{ij} = \frac{\partial u_i}{\partial X_j} \quad (3)$$

The *deformation gradient tensor* is then defined in terms of the displacement gradient tensor as

$$F_{ij} = \nabla u_{ij} + \delta_{ij} \quad (4)$$

where δ_{ij} is the Kronecker delta. Then, the *right Cauchy-Green deformation tensor* is defined as

$$C_{ij} = F_{ki} F_{kj} \quad (5)$$

The right Cauchy-Green deformation tensor is preferred over the deformation gradient tensor since it eliminates the effect of rigid body rotation in strain computation. Lastly, the *Green–Saint-Venant strain tensor*, in short the *Green strain*, is defined as

$$E_{ij} = \frac{1}{2}(C_{ij} - \delta_{ij}) \quad (6)$$

In 2D Euclidian space, the strain tensor in Eq. (6) can be expressed in the component form as

$$E = \begin{bmatrix} \varepsilon_{xx} & \varepsilon_{xy} \\ \varepsilon_{yx} & \varepsilon_{yy} \end{bmatrix} = \begin{bmatrix} \varepsilon_{xx} & \gamma_{xy}/2 \\ \gamma_{yx}/2 & \varepsilon_{yy} \end{bmatrix} \quad (7)$$

where the x and y axes correspond to the longitudinal (stretching direction) and transverse axes of the sample. The scalar components ε_{xx} , ε_{yy} , and γ_{xy} in Eq. (7) are the longitudinal normal, transverse normal, and the shear components of strain, respectively. To ensure consistency in comparing local strains computed from bead displacements with applied cross-head strain, ε_{CH} , the cross-head strain is also computed using the Green strain definition as

$$\varepsilon_{CH} = \frac{1}{2} \left[\left(\frac{l}{l_0} \right)^2 - 1 \right] = \frac{1}{2} (\lambda^2 - 1) \quad (8)$$

where l_0 is the original length, l is the instantaneous length of the sample, and λ is the stretch ratio [91]. The elongation ratio is taken as the cross-head stretch with the magnitude of $\lambda - 1$.

Appendix A shows the formulae for Cauchy, Green, Almansi, Swainger, and Hencky strain definitions.

3.6. Results and Discussion

Results Using Cross-head Values

Elastomers are known to exhibit stress relaxation under constant stretch [92], and it has also been shown that substrate stress relaxation affects cell spreading and proliferation [93]. The stress relaxation response of the PDMS samples in this study was tested by keeping the samples under constant 40% stretch and recording the tension measured by the load cell. The nominal stress was then calculated as the ratio of tension to original cross-sectional area, and all stress values were normalized by the initial stress. 6% stress relaxation was observed over a period of 24 hours and the first 4% of this relaxation takes place during the first hour (Fig. 3.6.1). The capability to quantify this behavior through the integration of a load cell enables the present system to be used in future studies to investigate the effects of stress relaxation on adherent cells.

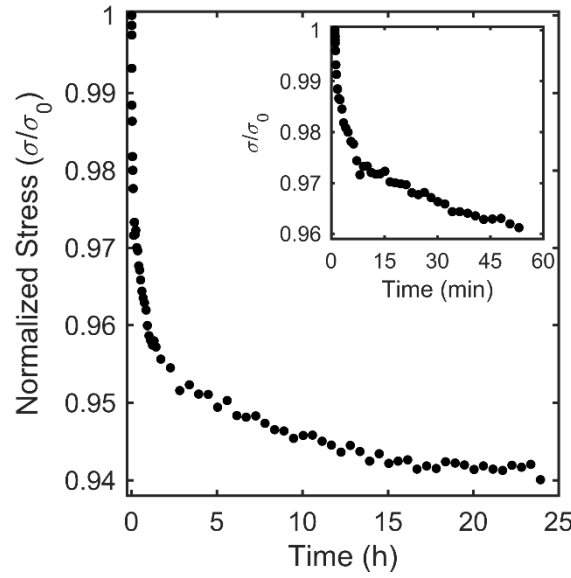


Figure 3.6.1 Stress relaxation curve of PDMS at room temperature for 24 h. The inset shows stress relaxation during the first hour [53].

Local Strain Mapping

Using bead displacements obtained from experiments and FEM simulations, in-plane strain components are computed and color-plots of the longitudinal strain are obtained at different locations on the sample. To demonstrate the inhomogeneity of the strain field, two critical regions around a rhombus-shaped thru-hole are selected: Region-i and Region-ii correspond to the immediate vicinity of the holes along the lateral and longitudinal axes, respectively. Figures 3.6.2(a) and (b) summarize experimental results. Figure 3.6.2(a) shows the locations of the critical regions encircled by dashed lines and the beads' initial positions. After microscope images are post-processed, 48 and 39 beads are tracked in Region-i and Region-ii, respectively. Delaunay triangulation is applied to the set of beads' positions and the area of each triad is colored according to its longitudinal strain value, ϵ_{xx} . Color-maps for two critical regions before the experiment are identical (Fig. 3.6.2(a)) indicating zero longitudinal strain. As the sample is stretched, the color of each triad changes in accordance with its strain value computed via Eqns. 1-7. An average cross-head Green strain of 0.220 corresponding to the 20 % cross-head stretch is applied to the sample in 30 seconds and the color-plots of longitudinal strain in Region-i and Region-ii are depicted in Fig. 3.6.2(b). This observation

reveals that the region along the lateral axis, Region-i, undergoes a higher deformation than the region along the longitudinal axis, Region-ii. A quantitative comparison will be provided next.

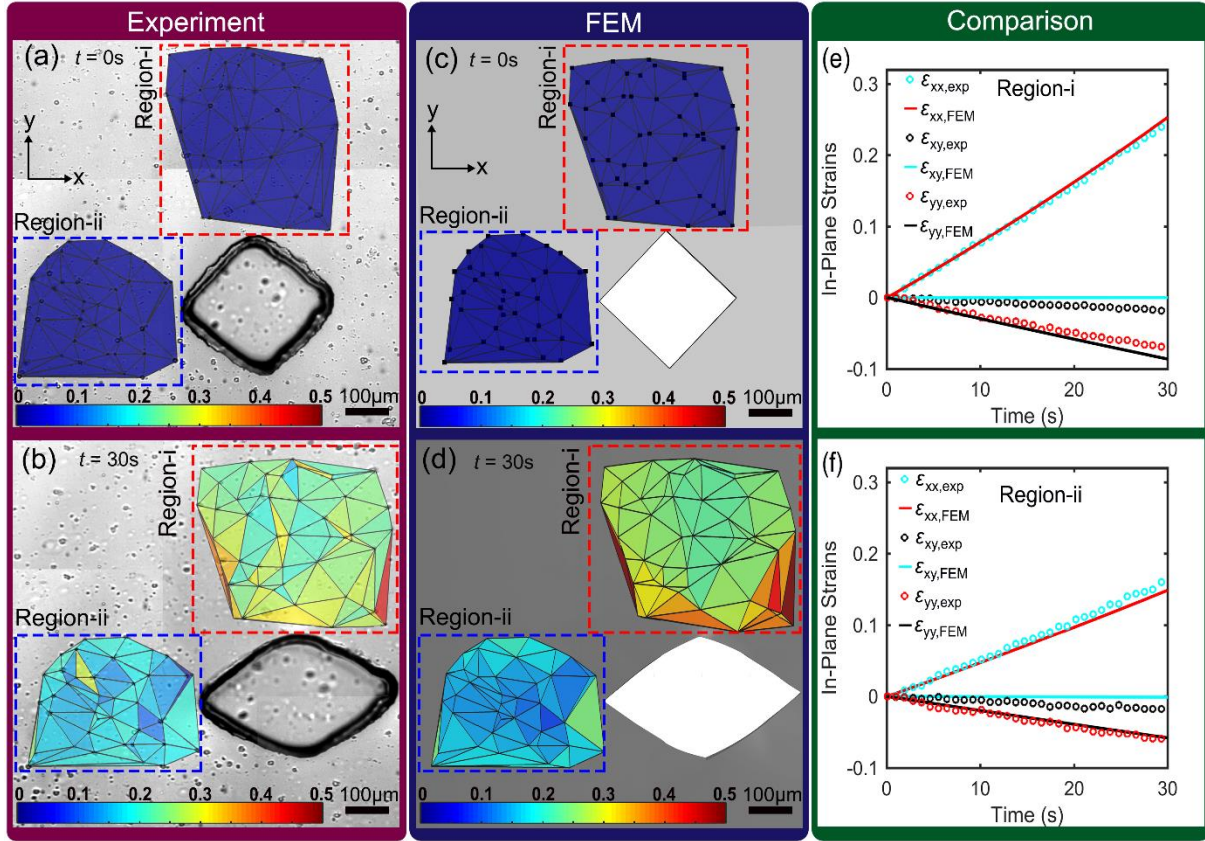


Figure 3.6.2 (a) Experimental longitudinal strain field for the sample at rest and (b) under 20% stretch. (c) Simulated longitudinal strain field for the sample at rest and (d) under 20% stretch. Comparison between time evolution of experimental in-plane strain components and FEM results for (e) Region-i and (f) Region-ii.

After the experiment, initial positions of tracked beads are implemented as nodal positions on the constructed geometry and they are connected by Delaunay triangulation. This procedure ensures the same number of triads being utilized in FEM as in the experiment. FEM mesh in Fig. 3(c) depicts this initial configuration. Then, the FEM simulation is run for the same crosshead stretch of 20% in 500 steps. Instead of obtaining an average strain value for the entire geometry, the *Green strain* formulation is applied to each triad to find in-plane strains. Figure 3.6.2(d) shows the longitudinal strain distribution for the applied stretch of 20% and it correlates well with the experimental results of Fig. 3.6.2(b).

For a quantitative comparison of all in-plane strain components between experiment and simulation one can consult Figs. 3.6.2(e) and 3.6.2(f) for Region-i and Region-ii, respectively.

Both figures show the temporal evolution of average in-plane strain components. The average values are calculated as the sum of strain values of the triads divided by the number of triads. As depicted in these figures, Region-i always exhibits a much higher longitudinal strain value compared to Region-ii. For example, after the sample is stretched for 30 seconds corresponding to a 20% cross-head stretch, the average longitudinal strains for Region-i and Region-ii are 0.25 and 0.17, respectively. As expected in uniaxial loading, there is a negative transverse strain and its magnitude in Region-i is higher than the one in Region-ii. Shear strain component does not depend on the location and it remains approximately zero in both regions (Figs. 3.6.2(e) and 3.6.2(f)).

In addition to comparing average values, one can further carry out a comparison between experiment and simulations based on the strain values of each triad. The histogram in Fig. 3.6.3(a) depicts experimental values of longitudinal strain in both Region i and Region ii for a cross-head stretch of 20%. The longitudinal strain in Region-ii is constrained mostly between 0.10 and 0.21 whereas Region-i exhibits a broad range of strain distribution from 0.20 up to 0.40. The corresponding histogram of FEM results is shown in Fig. 3.6.3(b) and it has a strain distribution similar to that of Fig. 3.6.3(a). Region-i and Region-ii exhibit longitudinal strain values of 0.10-0.20 and 0.21-0.38, respectively. This good correspondence achieved in Fig. 4 indicates that the positions of the beads are tracked accurately from the stretching videos and implemented successfully to FEM models.

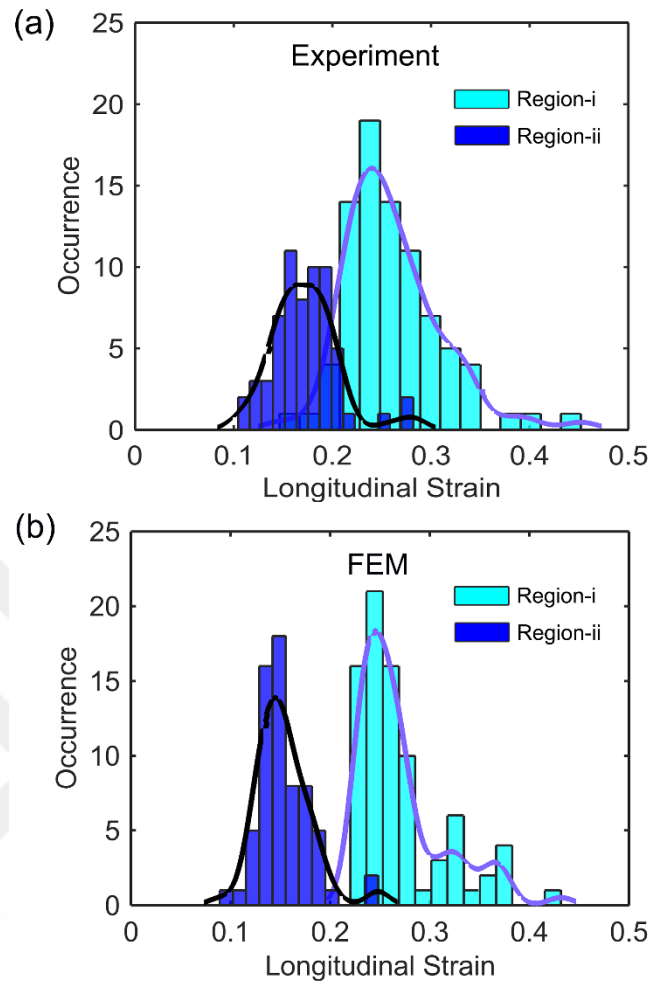


Figure 3.6.3 A histogram showing experimental longitudinal strain for Region-i and Region-ii. (b) Longitudinal strain obtained from FEM for Region-i and Region-ii. Both histograms are generated for a cross-head stretch of 20%.

So far we have focused on inhomogeneous strain distribution due to non-uniformities in the specimen geometry. If one is closer to a notch, longitudinal strains can surpass the far-field strain (Region i) or strain relaxation takes place and longitudinal strains remain lower than the far-field strain (Region ii). On the contrary, a region that is free from defects and sufficiently far away from any cross-sectional discontinuities will undergo a uniform deformation. Hence, one can demonstrate the spatial resolution of the strain measurement technique by showing the dependence of strain on the choice of location over the sample. For this purpose, five different regions are selected as shown in Fig. 3.6.4(a). Two regions, Region 1 and Region 2, are placed near the notch with varying distances along the lateral axis, whereas Region 4 and Region 5 are placed along the longitudinal axis in a similar fashion. Region 3 is sufficiently away from any

stress concentration. The regions in the vicinity of the hole are separated by dashed lines whereas the far-field region is not drawn to scale and placed as an inset in the Fig. 3.6.4(a). After a 20% stretch is applied to the sample, longitudinal strains in Regions 1-5 are obtained and plotted in Fig. 3.6.4(a). Average strains in each region are also computed and plotted in Fig. 3.6.4(b) as a function of time. Several arguments can be made based on these color-plots in Fig. 3.6.4(a) and line graphs of average longitudinal strains in Fig. 3.6.4(b):

1. The magnitude of the longitudinal strain decreases as the area of interest moves away from the hole in the lateral direction. Region 1 which is relatively close to the hole has higher strain values compared to those exhibited by Region 2. In fact, average longitudinal strain values of Region 1 and Region 2 under 20% stretch are found to be 0.276 and 0.239, respectively. The opposite is true for the longitudinally placed regions. Longitudinal strains of a region far away from the hole are higher than those in an area nearer to the hole. For example, Region 4, which is relatively far, has an average longitudinal strain of 0.170, whereas the average longitudinal strain value of Region 5 is found to be 0.166 for an average cross-head Green strain of 0.220. These results show that significant errors arise when cross-head displacement data is used to estimate local strain value, and the model proposed in this work can capture such strain variations at the microscale. The corresponding percentage error between FEM and experimental results for Region 1 is 6.7%.
2. According to the Saint Venant's principle, the strain in any region that is sufficiently removed from the area of the application of load is expected to be distributed uniformly. Region 3 of Fig. 5(a) satisfies the Saint Venant's criterion and it is also sufficiently away from any hole. Therefore, this region has a uniform longitudinal strain distribution with an average value of 0.214. This measured value is in close correspondence to 0.220. The corresponding percentage error for these results is 2.7%.

All claims listed above are valid through the entire stretching and Fig. 3.6.4(b) provides a comparison of the temporal evolution of the average longitudinal strain in Regions 1-5. Results in Fig. 3.6.4(c) further show, how the longitudinal strain component changes with distance from the hole. A set of points is implemented in the FEM geometry as shown in the inset in Fig. 3.6.4(c). The red path indicates the lateral (y) axis, while the black is aligned with the longitudinal (x) axes (stretching direction). The plot in Fig. 3.6.4(c) provides corresponding

experimental (circles) and FEM (solid lines) results, where the maximum strain is captured at the edge of the hole located along the y axis. The minimum strain is found along the x axis at the apex of the hole.



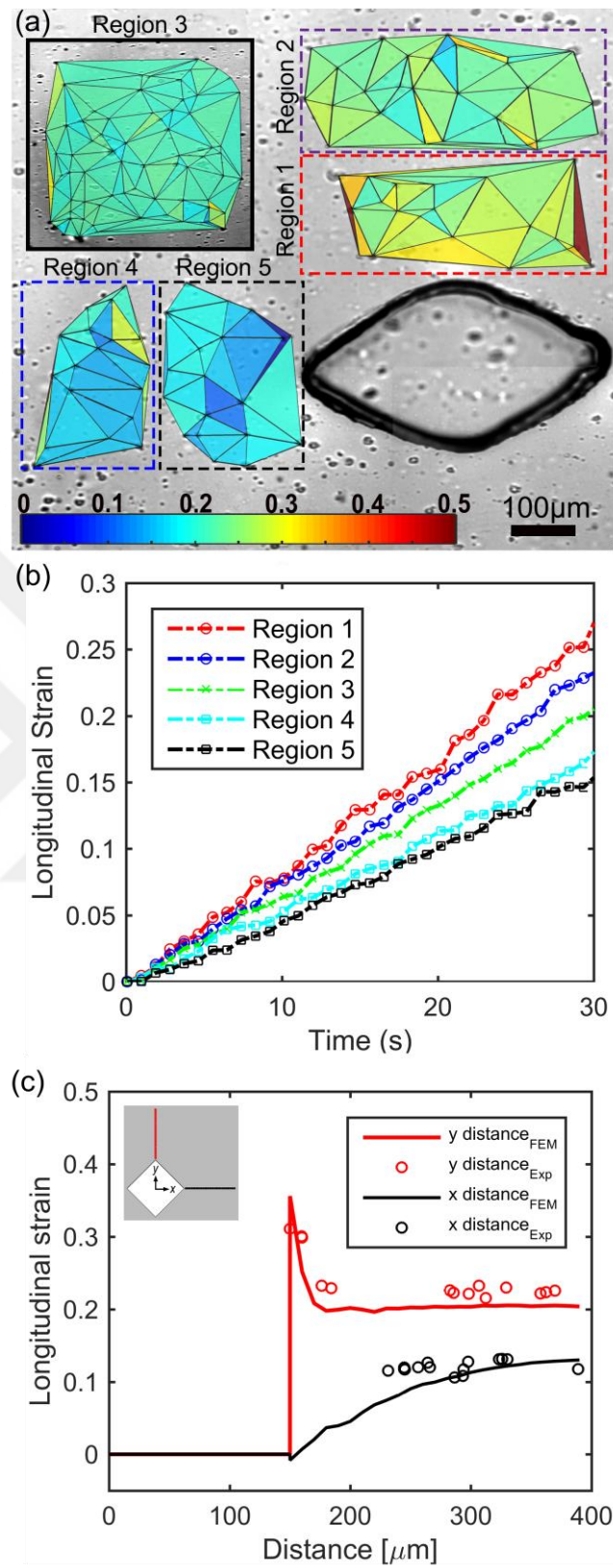


Figure 3.6.4 (a) Color-maps for the longitudinal strain for selected far-field and near-field regions. (b) A quantitative comparison of the average longitudinal strain for the specific five

regions. (c) Local longitudinal strain measurement along longitudinal (x) and lateral (y) axes with the origin placed at the center of the rhombus.

3.7. Accuracy and Robustness of The Technique

A further study is carried out to evaluate accuracy and robustness of the presented method in measuring submicron displacements. Figure 3.7.1 depicts the displacement of two beads having a gage length of 300 μm and corresponding strain values for the first 11 steps of stretching. Blue markers indicate experimental results corresponding to 11 individual measurements. The black solid line is the linear regression for these experimental data. The minimum measurable displacement, *i.e.* the step size between two consecutive frames, is 116 nm. This is consistent with previous work that measures the displacements of biomolecules as small as 35 nm [94]. This displacement forms the x-axis interval between experimental data points. It also corresponds to the minimum measurable strain of 4×10^{-4} . To the best of our knowledge this is the best reported value in similar studies. The measurement of such a small step size can be achieved thanks to the subpixel accuracy of the tacking algorithm and the magnification of the 10X objective. The velocity of the stepper motor and camera speed, however, limit the minimum measurable displacement. During stretching, the microscope sequentially takes an image and moves the stage for motion compensation. The duration of each cycle is 58ms, which is minimum duration for the current setup, and it repeats throughout the stretching. Using a high-speed camera shortens the cycle time, and consequently, the displacement between two consecutive frames will be smaller. Similarly, by lowering the speed of the stepper motor the displacement between two consecutive frames can be further decreased. Furthermore, the objective of the microscope is also a limit factor for the minimum detectable displacement. Theoretically, a 11.6-nm displacement can be resolved preserving the same experimental setup and switching the microscope objective to 100X. In this work, 10X objective is preferred due its field of view and the relative size of the micropatterns. The displacement resolution of 116 nm is reported here using current setup parameters and the 10X objective.

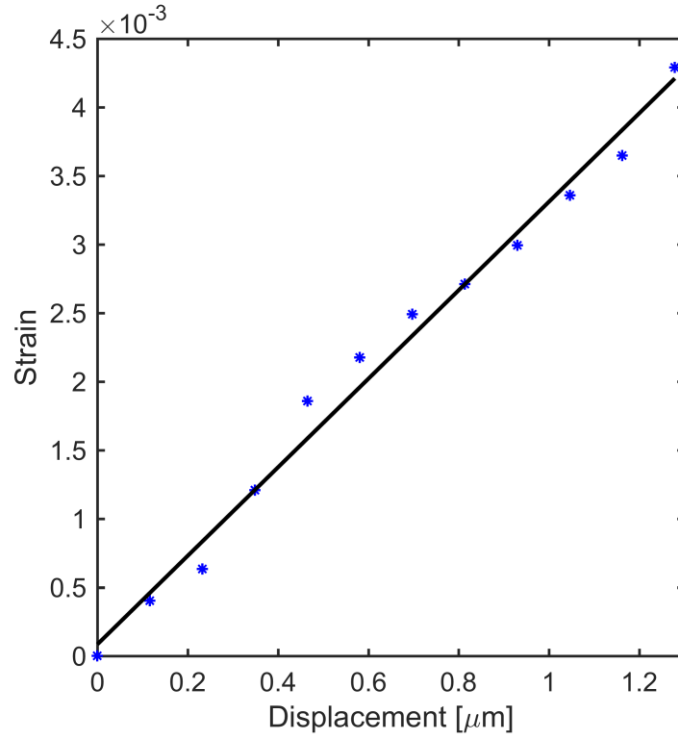


Figure 3.7.1 Minimum measurable displacement and strain values over an area of $820 \times 820 \mu\text{m}^2$.

A final note on the capabilities of the proposed strain measurement technique can be made regarding the number of necessary particles for the measurement reliability. This aspect will be demonstrated using the strain uniformity over Region 3. The bead concentration utilized in this work provides 72 particles in the field of view spanning an area of $820 \times 820 \mu\text{m}^2$. However, intrinsic heterogeneity, low transparency of the elastomer, low occurrence of beads per unit area at the desired location may limit the number of points for tracking. This might ultimately hinder the computation of strain. Therefore, it is highly advantageous to be able to compute strain accurately for any region having a low number of beads or similarly a region of small area. For this purpose, different numbers of beads and different areas are considered for Region 3. The resultant longitudinal strain fields for each condition are mapped and the average local longitudinal strains are compared with the cross-head strain. First, keeping the area of Region 3, the number of beads is randomly reduced from 72 (as depicted in Fig. 5(a)) to 24 as shown in Fig. 7(a). As the next operation, the area of Region 3 is reduced from 0.379 mm^2 to 0.135 mm^2 while keeping the number of beads as 24 (Fig. 7(b)). In the last operation, the number of beads is reduced to 6 while keeping the area as large as that of Fig. 7(a) (Fig. 7(c)). The areas

of interest for the first and third operation are almost identical. They are 0.379 mm^2 and 0.380 mm^2 , respectively. Color-maps of the longitudinal strain for 20% stretch are shown in Fig. 7(a)-(c). A quantitative comparison of the average longitudinal strains for three scenarios is given in Fig. 7(d) as a function of applied stretch. In this figure, average longitudinal strains obtained through the aforementioned three operations and the associated cross-head *Green* strain overlap, following a slightly nonlinear curve due to the quadratic term in the Green strain definition.

Figures 3.7.2(e) and 3.7.2(f) show the average longitudinal strain for 20% stretch using different numbers of Delaunay triangles and different average areas of triangles, respectively. The number of Delaunay triangles is varied from a single triangle to 140 triangles (Fig. 3.7.2(e)). Similarly, the average area of these triangles is changed from $1200 \text{ }\mu\text{m}^2$ to $200 \text{ }\mu\text{m}^2$ by removing relevant beads (Fig. 3.7.2(f)). In both plots, strain values for different cases remain constant despite the significant variation of mesh properties. Hence, in a uniform state of strain the proposed strain measurement technique is shown to be insensitive to the number of triangles or the average area of triangles. All results summarized in Figs. 3.7.2(d)- 3.7.2(f) demonstrate that the particle tracking algorithm used in this work is adaptable to cases where a few particles can be tracked.

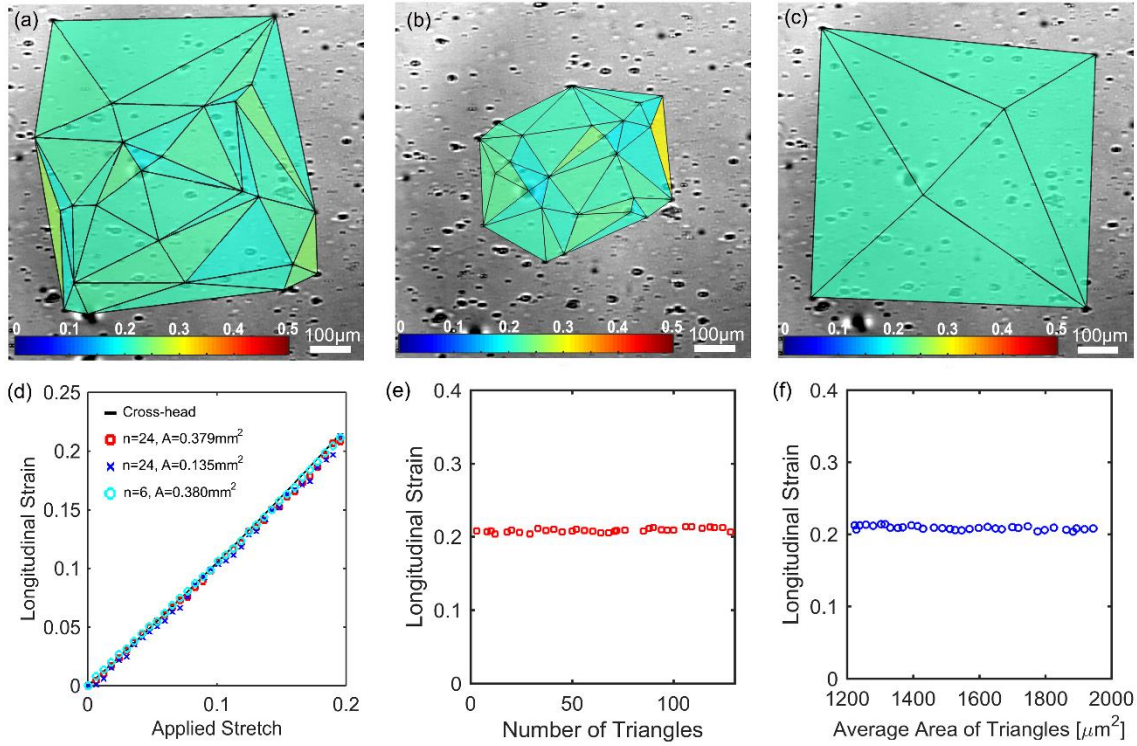


Figure 3.7.2 Color-maps of the longitudinal strain component at an applied stretch of 20% for (a) a random selection, (b) area (A) reduction, and (c) point (n) reduction. (d) Comparison of longitudinal strains obtained through three different operations and cross-head strain. Average longitudinal strain for 20% stretch using (e) different numbers of Delaunay triangles and (f) different average areas of triangles.

Although the ability of selecting a low number of points leads to a reduction in the computation time, it should be emphasized that it will also lead to missing the strain heterogeneities and hence, it should not be applied to regions undergoing nonuniform deformation. In this work, the point reduction is only used to express the stability of the particle tracking technique for yielding highly accurate measurements. Therefore, it is only utilized in the presence of uniform deformation. For achieving a high spatial resolution over areas with strain gradients, the choice regarding the number of points will depend strongly on the length scale of interest.

3.8. Application of The Technique: Strain Mapping in Cell Stretching

As an application of the presented technique, we managed to conduct cell stretching experiments without any further modification. First, we prepared PMMA mold and cured

PDMS in it, which creates a sample having gripping regions at both ends and a well in between (Fig. 3.8.1). The well contains culture media enabling long term cell experiments. We then prepared a recipe to culture cells on PDMS substrate and stretched the cells indirectly by stretching the substrate. Throughout the stretching, brightfield images of cells and fluorescent images of microspheres are simultaneously captured. Fluorescent imaging offers a suitable system for labeling and strain computation of the substrate.

The surface of PDMS is hydrophobic which requires a surface treatment to enhance the biocompatibility. Thus, plasma oxidation was applied to the PDMS substrate to make the surface hydrophilic (see Table 3.8.1).

Process Name	Process Parameters
Base Pressure	500 mT
Maximum Pump Time	240 sec
Stable Time	15 sec
MFC1(Ar)	0%
MFC2 (O ₂)	100%
Power	50 W
Plasma Time	60 sec
Temperature	70 °C

Table 3.8.1 Table 3.8.1 Recipe for plasma oxidation.

After plasma oxidation, Rat-tail collagen is applied to the surface for a further enhancement of cell adhesion. The sample with collagen is then incubated at 37 °C until a monolayer of fibers forms on the surface. HeLa S3 cells are cultured in a cell culture dish and transferred into the PDMS well when they reach 80% confluency. They are incubated overnight until cells reach 60 % confluency to ensure that the functionalized PDMS surface is a suitable substrate for cell attachment and growth [53].

The sample with adherent cells is then stretched and displacements of beads are computed by tracking their positions (Fig. 3.8.1(b)). A dual-color imaging, a simultaneous imaging of brightfield and fluorescent, of stretched cells and deformed substrate is carried out to localize the beads with respect to the cells. The overlay of brightfield and fluorescent signals (Figure

3.8.1(e)) shows us that beads are localized around the cells and cells are attached to the surface during the entire stretching. The fluorescence of microspheres enables dual-color imaging.

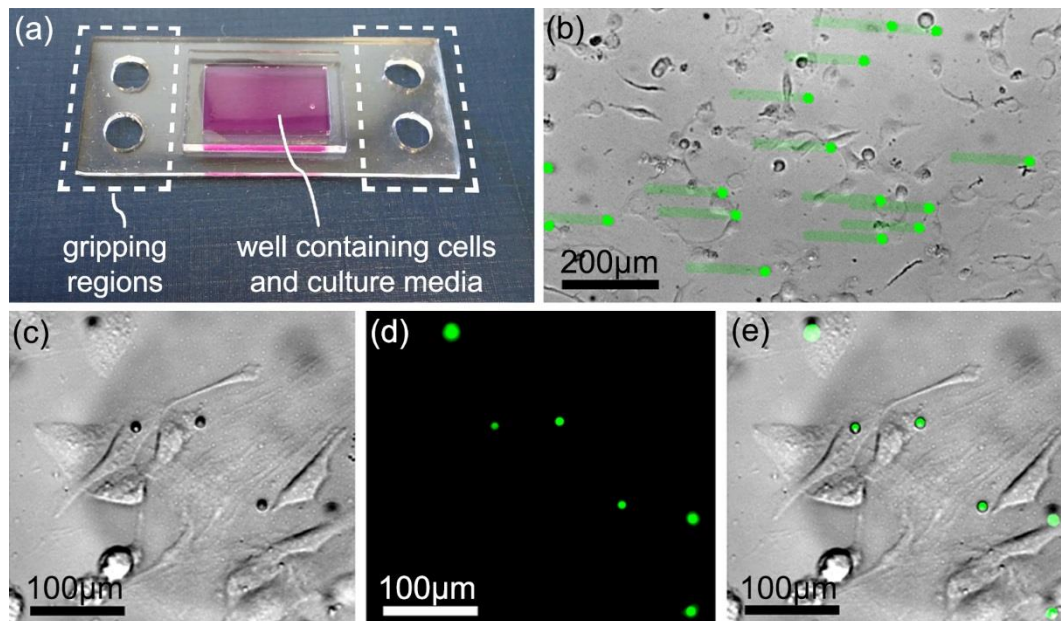


Figure 3.8.1 The application of the technique in cell stretching. (a) PDMS sample having a 12×19 mm well that contains culture media. (b) Trajectories of fluorescent beads are overlaid onto the brightfield image of adherent cells for a sample that is stretched without motion compensation to better illustrate the bead trajectories. (c, d, e) Close-up brightfield, fluorescence, and overlaid images showing beads embedded in the sample with adherent cells [53].

Cell stretching experiments continued with stained cells. In our studies, we stained nucleus and tubulin filaments of HeLa S3 cells and tubulins of retinal pigment cells. The size of an individual HeLa S3 cell is about 20 nm and the size of cytoskeletal filaments is approximately 2 nm. This scale of length requires higher magnification of imaging. Therefore, we switched the objective of the microscope from 10X to 60X.

The tubulin filaments of retinal pigment cells are stained with red dye and they were stretched under a fluorescence microscope. Figure 3.8.2 shows the undeformed and deformed (90% stretched) images of stained tubulin filaments of a retinal pigment cell.

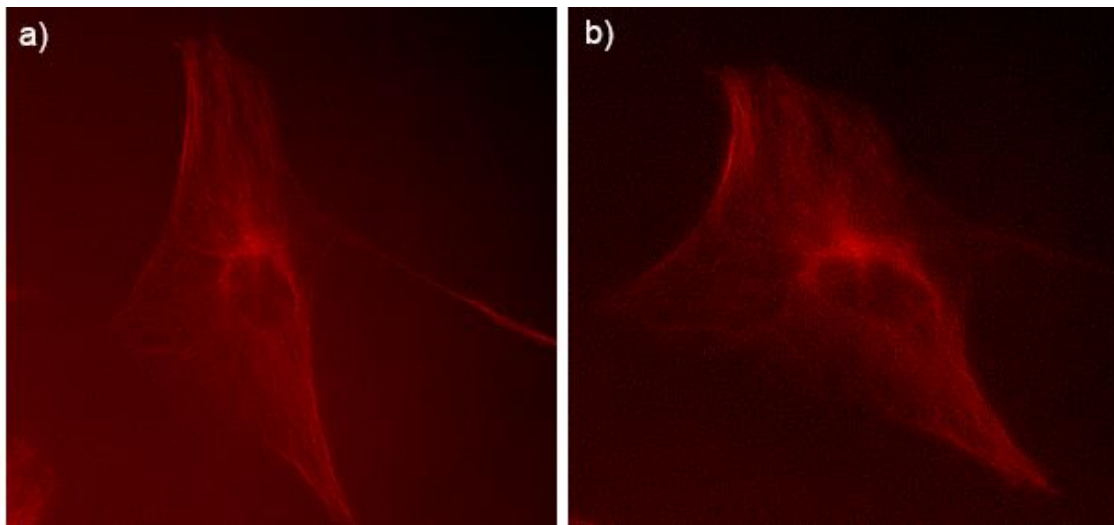


Figure 3.8.2 Fluorescent images of tubulin filaments of a retinal pigment cell (a) before and (b) after stretching.

The same staining protocol was applied to the tubulin filaments of HeLa S3 cells with green dye (Alex flour 488) and cells were stretched under the fluorescence microscope. Since their size is smaller than the size of retinal pigment cells, tens of cells were captured in the field of view. Figure 3.8.3 shows green labelled HeLa S3 cells at undeformed (24% stretch) and deformed states.

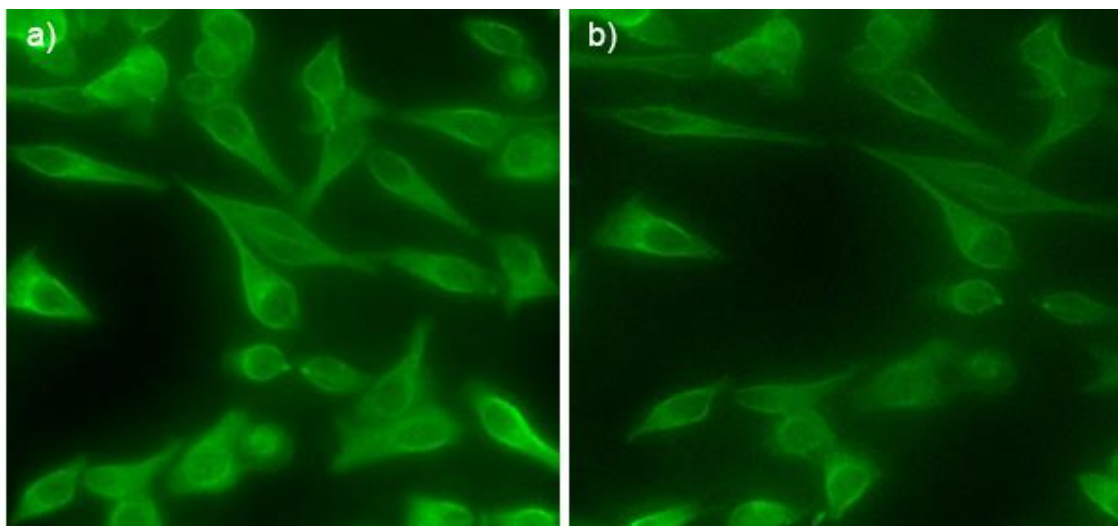


Figure 3.8.3 Images of Green labelled tubulin filaments in HeLa S3 cells that are captured (a) before and (b) after stretching.

The nuclei of HeLa S3 cells are stained with 4'-6-diamidino-2-phenylindole (DAPI) which is a fluorescence stain extensively used in fluorescence microscopy. DAPI has a maximum absorption at 358 nm (ultraviolet) and a maximum emission at 461 nm (blue). Thus, DAPI channel of fluorescence microscope excites DNA with ultraviolet light and detects through a blue filter. Figure 3.8.4 shows overlapped images of green labelled tubulin filaments and blue labeled nuclei in HeLa S3 cells.

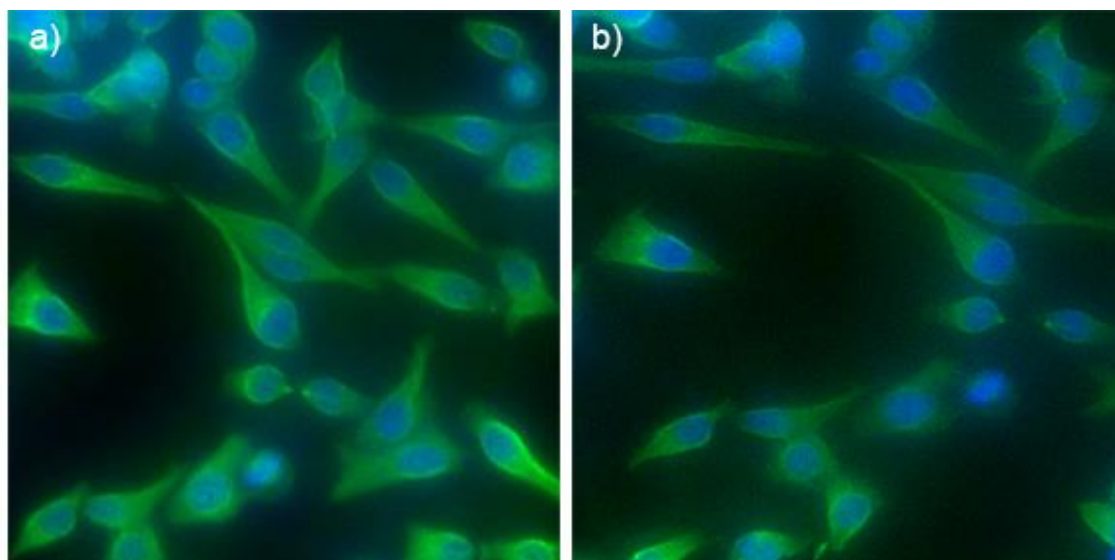


Figure 3.8.4 Overlapped images of HeLa S3 cells at (a) undeformed and (b) deformed states.

3.9. Discussion and Outlook

It is shown that contactless strain measurement by tracking of the bead displacements allows an accurate strain field determination in elastomeric samples with complex micropatterns. The presented method is particularly suitable for determining nonuniform strain distribution in microenvironments with strain inhomogeneity and it can be adapted for cell stretching experiments.

Local strain tracking microscope equipped with a uniaxial stretcher and high-magnification objectives is utilized to yield in-plane strains at a high spatiotemporal resolution. The integration of a custom-built stretcher with microscopy enables us to observe a small area of the sample during stretching. Using 10X objectives, an area of $820 \times 820 \mu\text{m}^2$ is observed and a minimum bead displacement of 116 nm can be resolved. Such small displacement measurement enables us to capture strains on the order of 10^{-4} . A PDMS sample with an array of rhombus-

shaped thru-holes and embedded microspheres is used to demonstrate particle tracking method and strain measurement at microscale. Additionally, the higher spatial details in strain field are obtained by applying Delaunay triangulation to tracked particles along with the Green strain formulation.

As expected in uniaxial loading, a uniform strain field is obtained over an area that is sufficiently far away from the cross-sectional inhomogeneities. It is proven that for this area, the number of the tracked particles does not affect the strain measurement. Similarly, the magnitude of the measurement area can be reduced without introducing any errors. All strain values obtained by point operations on this area match well with the applied cross-head strain.

Furthermore, the spatial distribution of strain components is captured by analyzing an area around a rhombus-shaped thru-hole in the PDMS sample. The variation in the strain is caused by the cross-sectional non-uniformity of the sample and strain value of a region depends on its position with respect to the hole and stretching direction. Using holes with a critical dimension of 200 μm , the method is shown to capture highest and lowest longitudinal strains in respective critical locations around each hole. With increasing distance from the hole, the longitudinal strain values for all regions approach the cross-head strain.

For the verification of experimental measurements, FEM is utilized. Bead locations obtained by image processing of the micrographs of the undeformed sample are directly implemented in an FEM mesh as nodes. The aforementioned triangulation with the same strain formulation is employed leading to a close correspondence with experimental observations.

This work addresses two current shortcomings of non-contact strain measurement of soft matter: i) real-time measurement, and ii) high spatial resolution. Capturing deformed images at 17 frames per second throughout the entire stretching enables one to map strain distribution with a high temporal resolution. Thanks to the subpixel tracking algorithm and integration with an optical microscope, a minimum strain value on the order of 10^{-4} is achieved without compromising the spatial resolution. To the best of our knowledge, this is the best reported value in studies of elastomers at microscale. Overall, this technique constitutes a robust method for the measurement of the spatially resolved strain distribution in micropatterned elastomers.

Chapter 4

REAL-TIME FORCE MEASUREMENT USING MEMS SENSORS

Design and fabrication of MEMS traction sensor is carried out within the scope of this project to create a smart surface that can measure in-plane forces acting on cytoskeletal filaments of cells. The smart surface comprises of a two-dimensional array of traction force sensors. MEMS based sensors enable us to measure the traction forces between adherent cells and the substrate without a help of external instrumentation such as fluorescence microscopy. As available methods depend on microscopy and require post-processing of microscopy images, the technique introduced here has a clear advantage over them.

The envisioned setup is capable of measuring cell-substrate interactions forces and simultaneously imaging the cells for biochemical events or cellular processes. The focus of this work is to establish correlations between mechanical response of the cytoskeletal protein network and external local loads. In other works, this work aims to characterize the response of cytoskeletal filaments of cells to external mechanical forces. Previous studies showed that such studies provide new insights into the various diseases.

This chapter provides a comprehensive overview of working principle of a piezoresistive transducer and piezoresistance effect in a single crystal silicon. It continues with electromechanical design and fabrication of MEMS traction sensors. Characterization and electrical readout of the sensor will be discussed at the end of this chapter.

4.1. Electromechanical Design of MEMS Sensor

The MEMS traction sensor is designed to map the forces acting on the cytoskeletal filaments of a single cell. Therefore, electromechanical design is restricted by many factors which are the size, force capability, and the fabrication technology. The upper limit and the lower limit of the sensor size is determined by the cell size and the fabrication technology, respectively. Due to fabrication restriction, the minimum dimensions of the MEMS sensor that we could fabricate are $20 \times 20 \times 2 \mu\text{m}$. The size of a single sensor is bigger than some cell types. We can still find many cells that are bigger in size and obtain force values at multiple points.

The developed MEMS sensor utilizes piezoresistive silicon nanowires (NWs) as electromechanical transducer. Piezoresistivity is the change in the electrical resistivity of a material induced by an applied mechanical stress. A comparison of capacitive displacement sensor and piezoresistive silicon nanowire carried out by Allain *et al* [95] revealed that piezoresistive NWs are superior to capacitive sensing mechanism in terms of noise and measurement resolution. Due to their these advantages, piezoresistive devices are widely preferred in various sensors such as strain gauges, accelerometers, force sensors, and pressure sensors.

With the discovery of the piezoresistance effect in silicon by C.S. Smith [96], piezoresistive sensing became one of the most desirable sensing schemes for MEMS technologies. In the following subsection, we will discuss the mathematical analysis of piezoresistive effect and piezoresistive properties of single crystal silicon.

4.1.1. Piezoresistance Effect in Single Crystal Silicon

For a single crystal material, resistivity and conductivity are generally anisotropic and relation between electric field ($\vec{E}$) and current density ($\vec{J}$) can be written in matrix form as,

$$\begin{pmatrix} E_X \\ E_Y \\ E_Z \end{pmatrix} = \begin{pmatrix} \rho_{xx} & \rho_{xy} & \rho_{xz} \\ \rho_{yx} & \rho_{yy} & \rho_{yz} \\ \rho_{zx} & \rho_{zy} & \rho_{zz} \end{pmatrix} \begin{pmatrix} J_X \\ J_Y \\ J_Z \end{pmatrix} \quad (4.1.1)$$

Where ρ is the resistivity tensor of second rank. The resistivity tensor is a symmetrical tensor, i.e., $\rho_{xy} = \rho_{yx}$. Therefore, it has only six independent components and can be shown as

$$\rho = \begin{pmatrix} \rho_1 & \rho_6 & \rho_5 \\ \rho_6 & \rho_2 & \rho_4 \\ \rho_5 & \rho_4 & \rho_3 \end{pmatrix} \quad (4.1.2)$$

The resistivity change in a crystalline material due to applied stress can be formulized in a general form as,

$$\begin{pmatrix} \Delta\rho_1 / \rho_0 \\ \Delta\rho_2 / \rho_0 \\ \Delta\rho_3 / \rho_0 \\ \Delta\rho_4 / \rho_0 \\ \Delta\rho_5 / \rho_0 \\ \Delta\rho_6 / \rho_0 \end{pmatrix} = \begin{pmatrix} \pi_{11} & \pi_{12} & \pi_{13} & \pi_{14} & \pi_{15} & \pi_{16} \\ \pi_{21} & \pi_{22} & \pi_{23} & \pi_{24} & \pi_{25} & \pi_{26} \\ \pi_{31} & \pi_{32} & \pi_{33} & \pi_{34} & \pi_{35} & \pi_{36} \\ \pi_{41} & \pi_{42} & \pi_{43} & \pi_{44} & \pi_{45} & \pi_{46} \\ \pi_{51} & \pi_{52} & \pi_{53} & \pi_{54} & \pi_{55} & \pi_{56} \\ \pi_{61} & \pi_{62} & \pi_{63} & \pi_{64} & \pi_{65} & \pi_{66} \end{pmatrix} \begin{pmatrix} \sigma_{11} \\ \sigma_{22} \\ \sigma_{33} \\ \sigma_{23} \\ \sigma_{31} \\ \sigma_{21} \end{pmatrix} \quad (4.1.3)$$

Where ρ_0 is the resistivity of unstrained state and π_{ij} is the piezoresistive coefficient tensor.

The silicon crystal has a diamond structure with face-centered cubic cell. Therefore, in the crystallographic coordinate system of the crystal, there are only three non-zero elements of the piezoresistive coefficient tensor which are $\pi_{11} = \pi_{22} = \pi_{33}$, $\pi_{44} = \pi_{55} = \pi_{66}$, and $\pi_{12} = \pi_{21} = \pi_{31} = \pi_{13} = \pi_{23} = \pi_{32}$. Thus, the relation between relative change in resistivity and stress for silicon can be written as,

$$\begin{pmatrix} \Delta\rho_1 / \rho_0 \\ \Delta\rho_2 / \rho_0 \\ \Delta\rho_3 / \rho_0 \\ \Delta\rho_4 / \rho_0 \\ \Delta\rho_5 / \rho_0 \\ \Delta\rho_6 / \rho_0 \end{pmatrix} = \begin{pmatrix} \pi_{11} & \pi_{12} & \pi_{12} & 0 & 0 & 0 \\ \pi_{12} & \pi_{11} & \pi_{12} & 0 & 0 & 0 \\ \pi_{12} & \pi_{12} & \pi_{11} & 0 & 0 & 0 \\ 0 & 0 & 0 & \pi_{44} & 0 & 0 \\ 0 & 0 & 0 & 0 & \pi_{44} & 0 \\ 0 & 0 & 0 & 0 & 0 & \pi_{44} \end{pmatrix} \begin{pmatrix} \sigma_{11} \\ \sigma_{22} \\ \sigma_{33} \\ \sigma_{23} \\ \sigma_{31} \\ \sigma_{21} \end{pmatrix} \quad (4.1.4)$$

The three non-zero elements of the piezoresistive coefficient tensor were found by Smith [96] for high resistivity silicon material. Test configurations used by Smith are illustrated in Figure 4.1.1. Using these test configurations Smith found the coefficients as shown in Table 4.1.1.

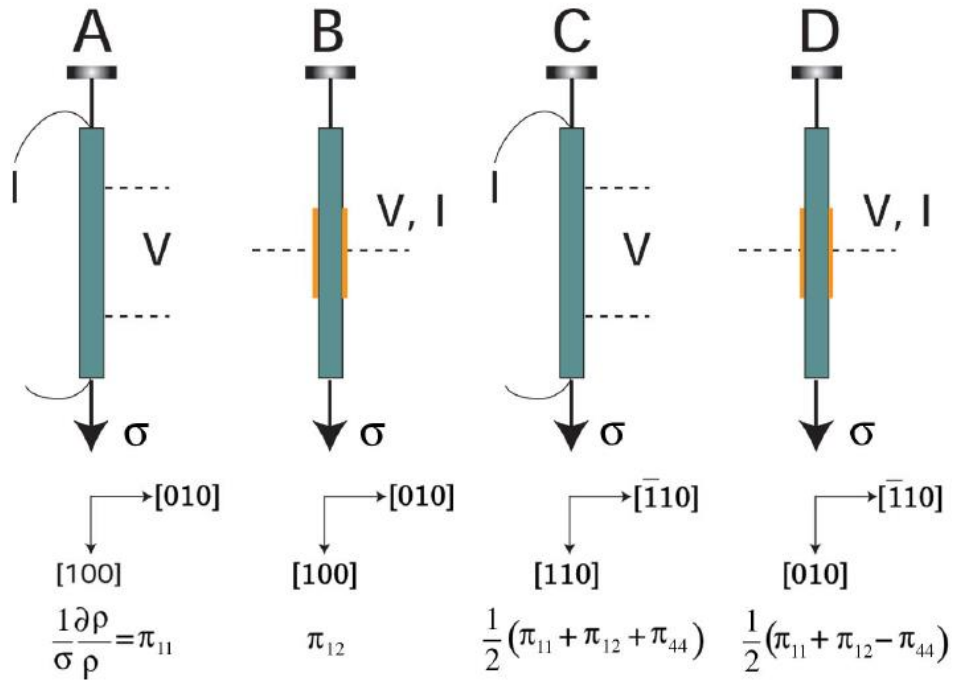


Figure 4.1.1 Test configurations used by Smith to measure the effective piezoresistive coefficients [97].

	π_{11}	π_{11}	π_{11}
p-Si (7.8 $\Omega \cdot \text{cm}$)	6.6e-11 [Pa^{-1}]	-1.1e-11 [Pa^{-1}]	138.1 e-11 [Pa^{-1}]
n-Si (11.7 $\Omega \cdot \text{cm}$)	-102.2e-11 [Pa^{-1}]	53.4e-11 [Pa^{-1}]	-13.6e-11 [Pa^{-1}]

Table 4.1.1 Components of the piezoresistive coefficient tensor for n-typed and p-typed doped silicon.

As seen from Table 4.1.1, doping type changes the piezoresistive coefficients. The other factors that piezoresistive coefficients depend on are device doping, crystal orientation, and temperature. These contributing factors are carefully taken into consideration while selecting parameters for sensor design.

Piezoresistive coefficients in a single crystal silicon are direction-dependent due to its anisotropic nature. This effect is extensively studied by Smith [96] and graphically represented by Kanda [98]. Figure 4.1.2 shows graphical representations of the piezoresistive coefficients in arbitrary directions demonstrating how these coefficients vary with respect to orientations.

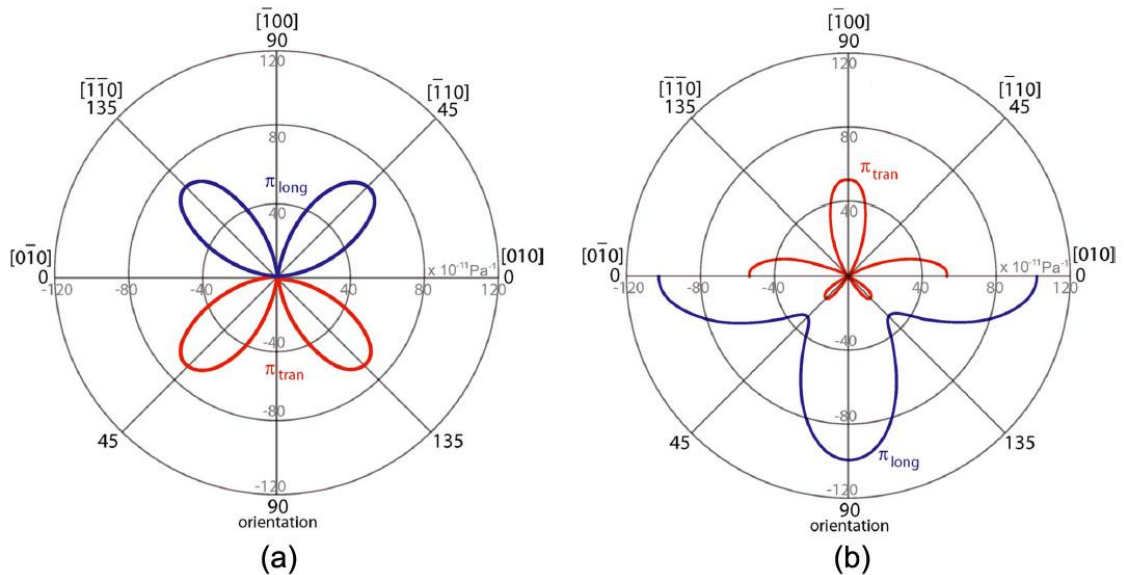


Figure 4.1.2 The longitudinal and transverse piezoresistive coefficients for (a) p-type and (b) n-type silicon on (100) plane [98].

For a comprehensive understanding of direction-dependency of the coefficients, we better discuss miller indices and the crystal structure of silicon. In miller indices, a direction

index $[hkl]$ denotes a vector normal to the plane (hkl) . Angle-bracket indices, $\langle hkl \rangle$ represent all direction equivalent to $[hkl]$ by symmetry (see Figure 4.1.3). Crystalline silicon has diamond-cubic structure which is equivalent to two interpenetrating face-centered cubic (FCC) lattices (see Figure 4.1.3a). Commonly used wafer surface orientations in microfabrication are (100), (111), and (110).

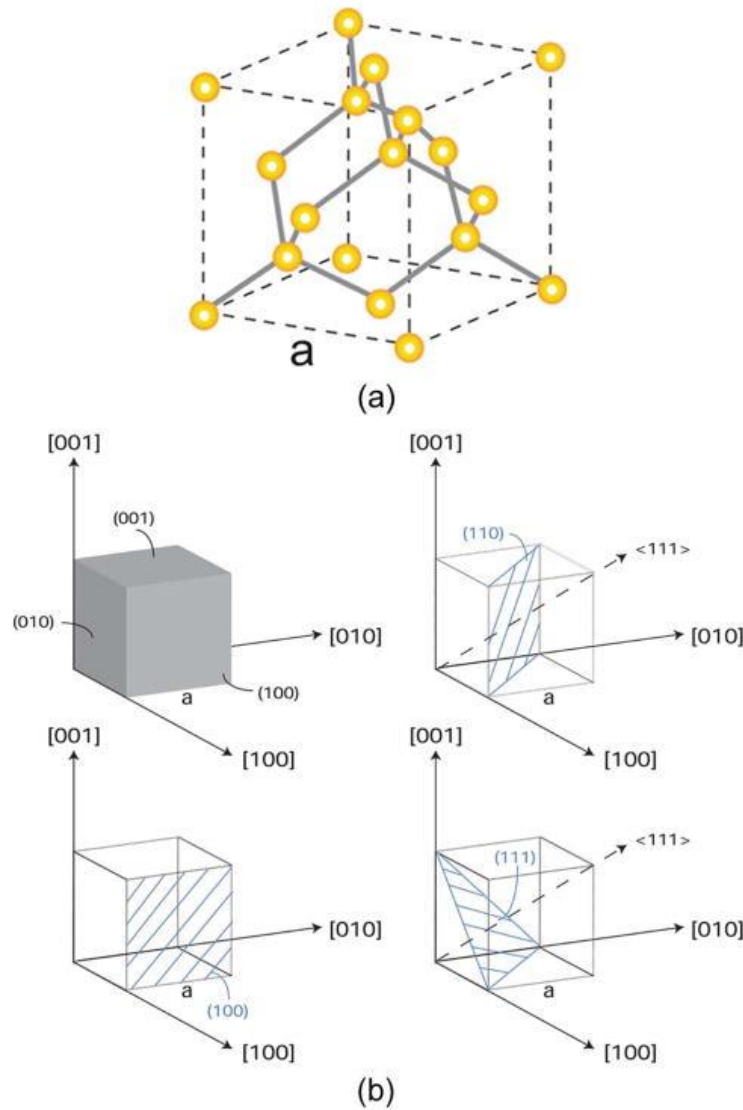


Figure 4.1.3 (a) cubic structure and crystal planes of silicon [97].

For this study, a wafer with (100) orientation is selected and nanowires are fabricated along $\langle 110 \rangle$ direction. This way of designing enables high equal and opposite longitudinal and transverse piezoresistive coefficients and easy fabrication.

Dopant concentration has a profound effect on piezoresistance performance. Kanda carried out studies on how piezoresistive performance varies with dopant concentration. Barlian et al compared Kanda's suggestion and previous experimental data in Figure 4.1.4. According to Kanda's notation, the piezoresistive coefficient is multiplied by a piezoresistive factor, $P(N,T)$ where N and T are impurity concentration and temperature, respectively (see Figure 3.1.5). Theoretical calculation done by Kanda agrees well with the experimental data for low dopant concentration but it deviates at the higher level of dopant concentration such as $3 \times 10^{19} \text{ cm}^{-3}$.

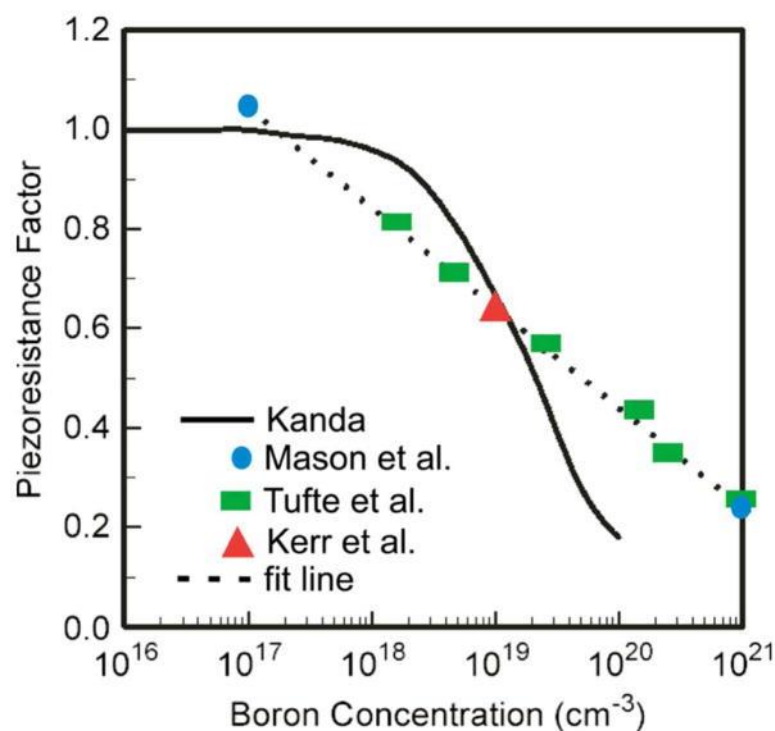


Figure 4.1.4 Piezoresistance factor as a function of Boron Concentration [97].

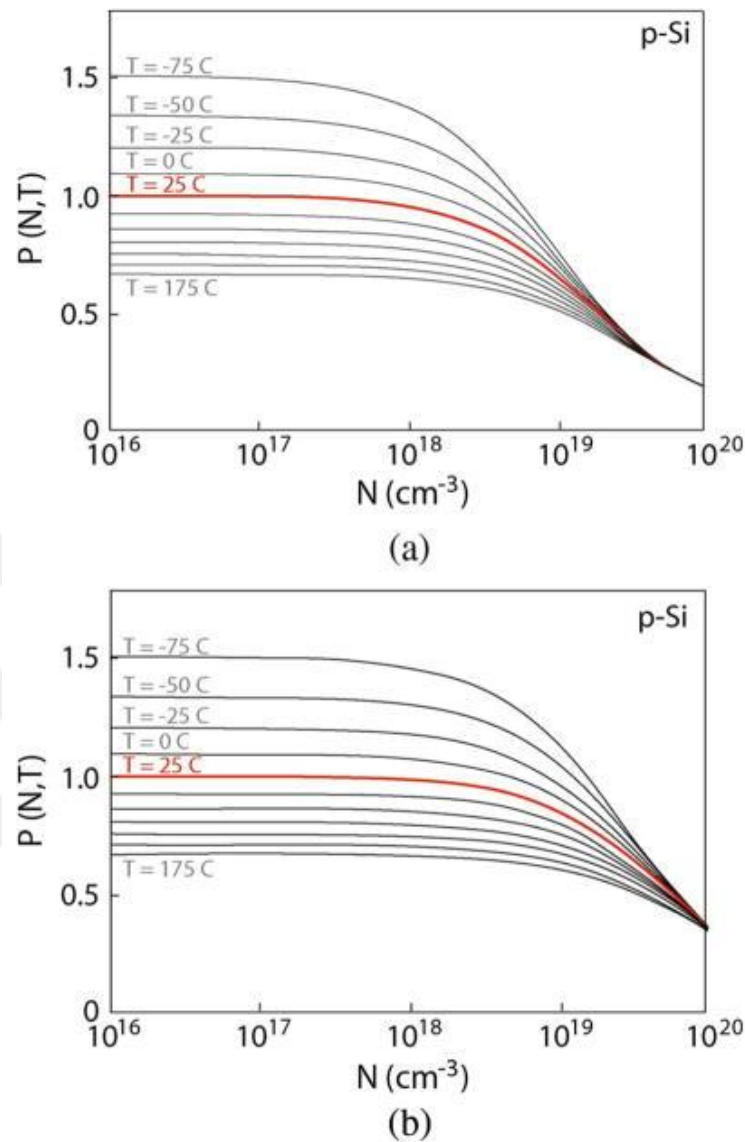


Figure 4.1.5 Piezoresistance factor as a function of impurity concentration and temperature for (a) p-type and (b) n-type silicon [97].

Although temperature affects the piezoresistive coefficients, the fabricated piezoresistive force sensor will operate at a temperature of 37°C with $\pm 0.5^\circ\text{C}$ variation. This narrow range of temperature significantly reduces the temperature effects. Considering all factors affecting piezoresistance performance listed above, the material type and process parameters are listed in Table 4.2

Dopant type	p-type (Boron doping)
Dopant concentration	10^{17} cm^{-3}
Orientation	<110> directions on the (100) plane

Table 4.1.2 Material type and process parameters for the sensor design.

An advantage of our sensor is that piezoresistive nanowires are design such that they undergo either uniaxial tension or compression which is called uniaxial stress-state. This particular state reduces Equation 4.1.4 to a single scalar equation as,

$$\Delta\rho / \rho = \pi_{eff} \sigma \quad (4.1.5)$$

where $\Delta\rho / \rho$, π_{eff} , and σ are the total relative change in resistivity, the effective longitudinal piezoresistance coefficient along the uniaxial stress direction, and the uniaxial stress, respectively. When a nanowire is subjected to a uniaxial stress, the dimensions of it changes as well, which is an additional contributing factor of resistance change. In other words, the nanowire elongates in the direction of the applied stress and shrinks in the lateral directions, which consequently changes the geometry of it. The geometry change induces electrical resistance change. We now should define the resistance change as a function of geometric change $(1 + 2\nu)$ and fractional resistivity change $(\Delta\rho / \rho)$.

$$\Delta R / R = (1 + 2\nu) + \frac{\Delta\rho}{\rho} \quad (4.1.6)$$

For silicon and germanium resistance change due to resistivity change is 50-100 times larger than the resistance change due to geometric change [97]. Thus, the geometric term $(1 + 2\nu)$ can be neglected and we can rewrite Eq. 4.1.6 as,

$$\Delta R / R = \frac{\Delta\rho}{\rho} \quad (4.1.7)$$

Substituting Eq. 4.1.5 into Eq. 4.1.7 we get,

$$\Delta R / R = \pi_{eff} \sigma \quad (4.1.8)$$

This simplification is very useful as resistance measurement is more convenient than measuring resistivity. Assuming that the piezoresistive nanowires operates in the linear elastic regime, we can further modified Eq. 4.1.8 by implementing Hook's law,

$$\Delta R / R = \pi_{eff} E_{eff} \varepsilon \quad (4.1.9)$$

where E_{eff} is the effective Young's modulus along the axial direction of the nanowire, and ε is the applied strain. The effective elastic modulus in single crystal silicon depends on crystal orientation. Figure 4.1.6 shows the elastic modulus of silicon in different orientations. The elastic modulus for silicon in $\langle 110 \rangle$ crystallographic direction, for example, is 169 GPa. The term $\pi_{eff} E_{eff}$ in Eq. 4.1.9 is called piezoresistive gauge factor, G .

$$G = (\pi E)_{eff} = \frac{\Delta R / R}{\varepsilon} \quad (4.1.10)$$

The gauge factor of a nanowire defines its piezoresistive sensitivity which is the total relative change in resistance per unit applied strain. Using the piezoresistance coefficients listed in Table 4.1.1, one can find the gauge factor for the desired nanowires. The value of the gauge factor for a $\langle 110 \rangle$ oriented p-type silicon nanowire with a dopant concentration of 10^{17} cm^{-3} is found to be 121.34.

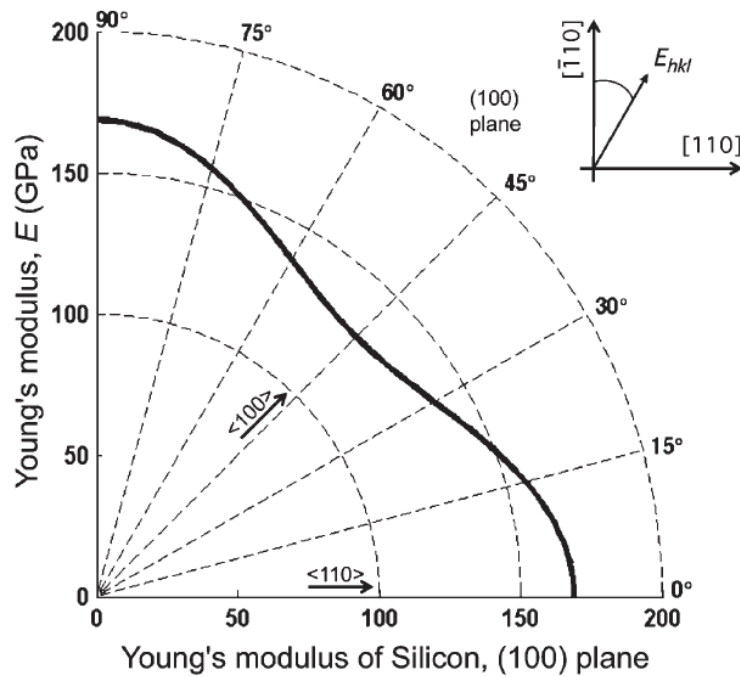


Figure 4.1.6 The Young's modulus of silicon for different orientations [99].

4.1.2. Mechanical Design of The Sensor

The factors affecting electromechanical design are force scale, function, and fabrication technology. To measure force at multiple points in the cell, the size of the sensor must be smaller than the size of the cell. Thus, the lower limit of the sensor size is determined by fabrication technology that was available to us and it covers an area of $20 \times 20 \mu\text{m}$. Although, this size is larger than the sizes of some cells, we are limited by fabrication facility in Turkey.

The traction sensor is designed to use piezoresistive nanowires to transduce the mechanical forces into electrical signals. Sensitivity and efficiency of the piezoresistive nanowires is optimized in the design of the sensor. Figure 4.1.7 shows the three main components of the traction sensor which are interaction platform, piezoresistive nanowires, and springs.

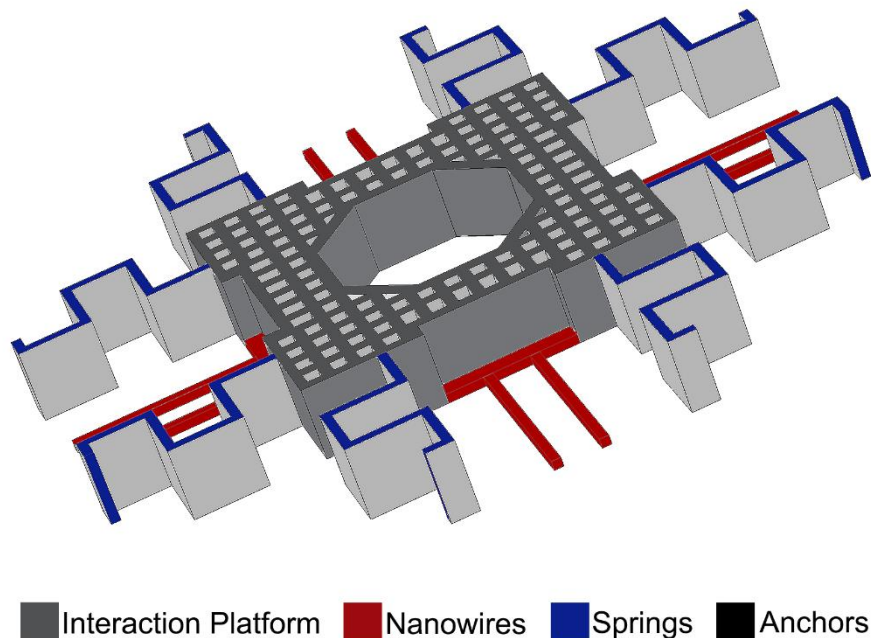


Figure 4.1.7 Main components of the traction sensor

Interaction platform is a movable plate where cells going to adhere and apply forces on it. It is anchored by nanowires and springs. The rigidity of the platform is very high compared to the other components of the sensor. The duty of the platform is to transmit the forces acting on it to the nanowires without undergoing any deformation. The nanowires are the electromechanical transducers of the sensor. They enable us to convert forces into resistance change. As the focus in this study is to map the in-plane traction forces, out-of-plane motions should be restricted. The springs are used to keep the platform's motion in-plane.

The out-of-plane motion of the platform causes bending stress in addition to uniaxial stresses. This tilting motion combines the stresses and negatively affects the sensitivity of the sensor. The springs in the design constrain out-of-plane motion and enable the platform to move in-plane freely. In this way, the force acting on platform can be transmitted to the nanowires and the springs, which induces tensile and compressive stresses in the components. Due to double-folded shape of the springs, all forces are obtained by the nanowires.

Dimensions for the top view of the traction sensor and cross-section views of the nanowires and springs are shown in the Figure 3.1.8. All components of the sensor are fitted to an area of $20 \times 20 \mu\text{m}$. In this work, in-plane dimensions are referred as width and length whereas the dimensions perpendicular to the in-plane dimensions are referred as height. Nanowires and springs have the same width of $0.25 \mu\text{m}$ but different heights. The height of the nanowires is kept as $0.25 \mu\text{m}$ where springs have a height of $2 \mu\text{m}$, the thickness of the device layer of SOI wafer (Figure 3.1.8b). Decreasing the heights of nanowires reduces the cross-sections of them. The smaller cross-section area enables the higher strain values per unit tensile force. The reason for not reducing the heights of the spring is to keep stiffness constant the spring in out-of-plane direction as much high as possible.



The direction of a force applied in an arbitrary direction can be determined (Figure 4.1.9b) by taking relative compressive strains in the neighboring nanowire pairs. Taking the scenario in figure 4.1.9b as an example for this case where force makes an angle θ with the x-axis, the magnitude of the strains on the horizontally oriented nanowires (ε_2) and the vertically oriented nanowires (ε_1) should be considered. The direction of the force (θ) can simple be found as,

$$\theta = \tan^{-1}(\varepsilon_2 / \varepsilon_1)$$

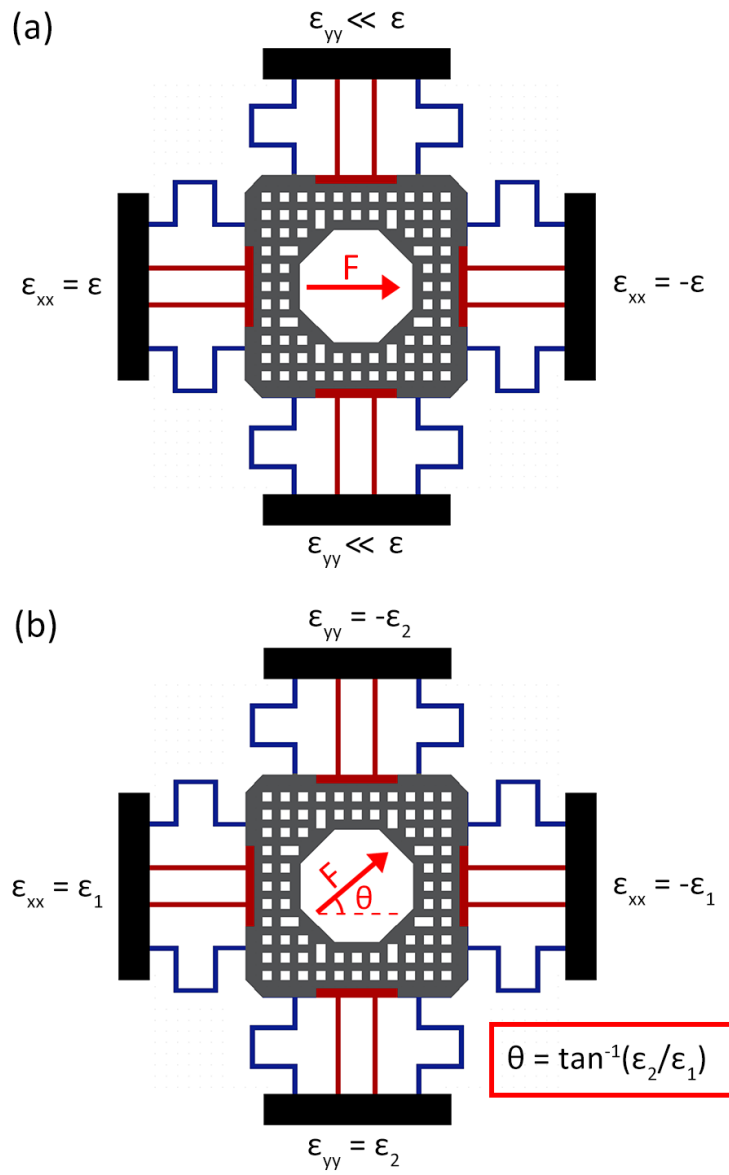


Figure 4.1.9 The method for detection of the direction of the in-plane forces.

4.2 Sensor Fabrication

Fabrication Flow Process

MEMS traction sensor is designed to be fabricated on a silicon-on-insulator (SOI) wafer. SOI wafers have three different layers that are device layer, buried oxide (BOX) layer, and handle layer. The buried oxide layer is sandwiched between device and handle layers. The device layer is the top layer of the SOI wafer where devices are fabricated. The buried oxide layer is an oxygen rich layer and it acts as a natural stop for silicon etching processes. The specifications for the SOI wafers used in this study are given in Table 4.3.1.

Diameter	100 mm
Orientation	(100)
Doping type	p-type (Boron)
Resistivity	20 – 30 Ωcm
Device layer thickness	$2 \pm 0.5 \mu\text{m}$
Buried oxide thickness	$2 \mu\text{m} \pm 5\%$
Handle layer thickness	$500 \pm 10 \mu\text{m}$

Table 4.2. 1 Specifications of the SOI wafers.

The fabrication process flow of the MEMS traction sensor is shown in Figure 4.2.1. The figure depicts the main fabrication steps for two different cross-sections that are shown in Figure 4.1.8. These steps are silicon etchings, doping with different Boron concentrations, metallization, passivation, BOX etching with HF vapor, and passivation removal.

Sensor fabrication starts with a silicon etching process by magnetically enhanced reactive ion etching (MERIE). This process (Figure 4.3.1a) etches the device layer at a depth of $2\mu\text{m}$ to define spring and platform geometries and it also outlines the nanowires geometry. The mask of the silicon etchings is thermal growth oxide mask. First, an oxide layer is growth and photoresist is coated on it. The photoresist is then patterned using photolithography. After the photolithograph process, photoresist is removed and the patterned oxide mask is used for the first silicon etch process. The same oxide layer will be used for the second silicon etch as well.

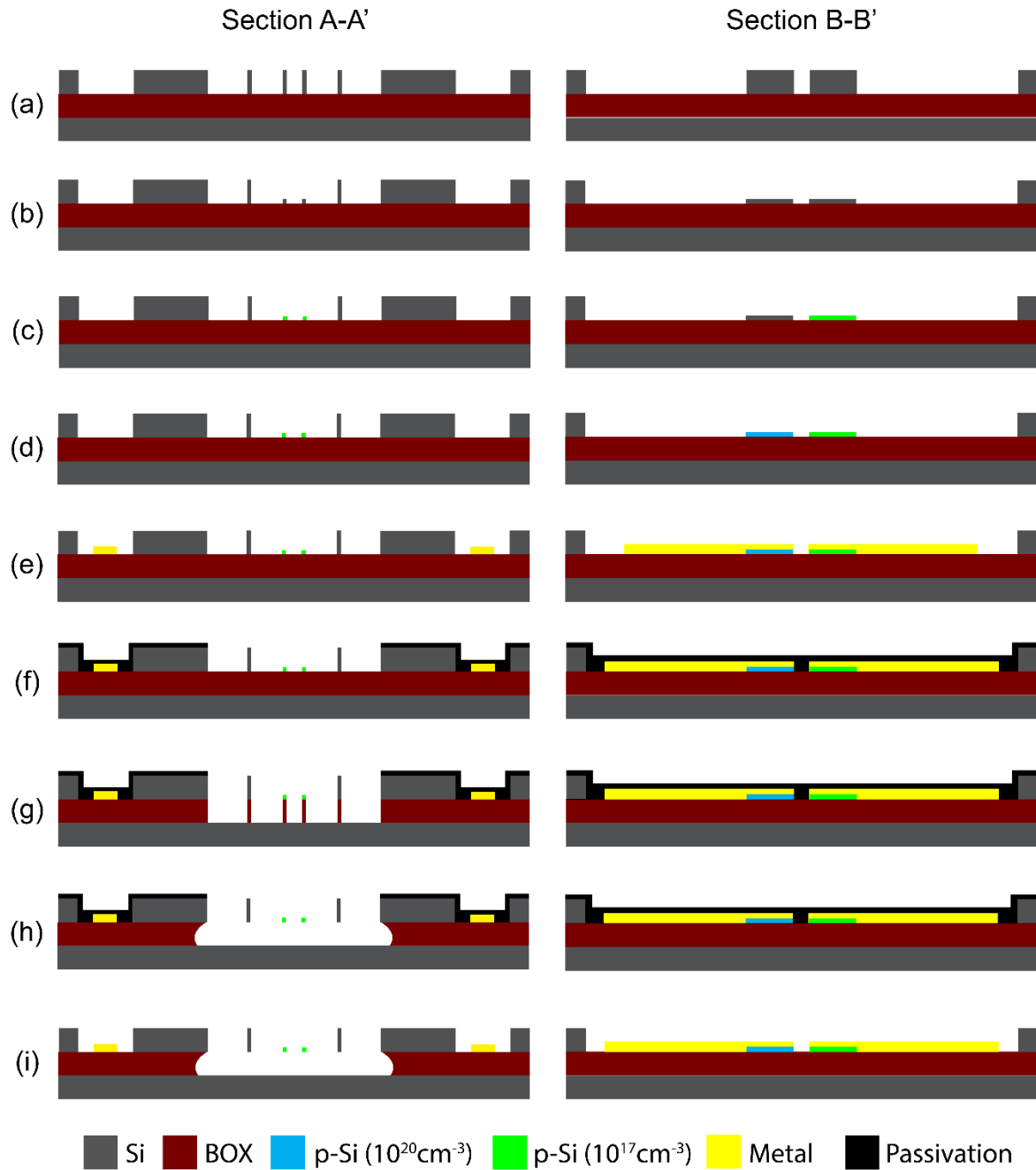


Figure 4.2.1 Overview of the process flow of the traction sensor for AA' and BB' cross-sections.

The next step in the fabrication flow is the second silicon etch step where the heights of the nanowires is reduced from $2\mu\text{m}$ to $0.25\mu\text{m}$ (Figure 4.3.1b). The etch mask for this step is the remained oxide mask from the first etch step. The only oxide layer on the top of the nanowires is removed and the rest of the mask remains. After this step, the height of nanowires is reduced

to desired values. The oxide layer used as masks for the silicon etch processes is removed after this step.

The third step is the ion implantation where nanowires and metal contact pads are doped with Boron (Figure 4.3.1c). The desired dopant concentration for this step is 10^{17} cm^{-3} . The second ion implantation is required to achieve a good ohmic contact between doped contact pads and metal (Figure 4.3.1d). In the second ion implantation, the desired regions are doped with a dopant concentration of 10^{20} cm^{-3} . Due to sensor array design, only half the pads are doped at high concentration whereas the remains are kept at low concentration. Schottky diodes are used with the nanowire bridges. In order to have a good Schottky contact between p-type doped silicon and metal, the dopant concentration of silicon needs to be low [100].

The following step is metallization (Figure 4.3.1e). For metallization process, first the entire surface of the wafer is coated with metal using sputtering. The commonly used metal in YITAL is aluminum with a titanium seed layer. Then the deposited metal layers are patterned using photolithography and finally the resist is removed. After metallization is completed, the metal surface is coated with polymer for passivation (Figure 4.3.1f). The first choice of polymer materials was polyimide. We had a hard time finding commercially available polyimide for a short time period, we then decided to use TI-35E image reversal resist from MicroChemicals Inc for passivation. Passivation layer protects the metal layer against hydrofluoric acid (HF) which will be used to etch BOX layer.

Before suspending the sensor's components using HF vapor, we suggest the fabrication group to use Freon RIE (Figure 4.3.1g) for an anisotropic etch. This reduces HF release time. Note that the nanowires could be also etched by Freon RIE. Therefore, the selectivity, etch rate of Si/SiO₂, of the recipe that will be used for Freon RIE should have very high selectivity.

The next step in fabrication process flow is HF vapor release (Figure 4.3.1h). This process removes the buried oxide layer under the sensor components and suspends the platform, nanowires, and springs. The final step in the fabrication process flow is the removal of the passivation layer by oxygen plasma ashing (Figure 4.2.1i). This process enables us to access the metal connections.

Fabrication Results

The fabrication of the MEMS sensor was carried out in collaboration with YITAL as they have equipment to reach submicron patterning and desired doping concentration. Collaboration with YITAL is particularly advantageous to us to do all fabrication processes at one place.

For the first Silicon etch step, we started with a modification of a recipe called Deep Trench Isolation (DTI) recipe. It is extensively used in CMOS technology and can etch a depth of 7.8 μm . In the first version of the recipe (DTI-1A), a photoresist mask was used and an inclined sidewall profile was obtained. To increase the sidewall angle and achieved the desired etch depth several recipes are developed. All recipes and fabrication results using these recipes are listed in Appendix C.

Using the optimized fabrication recipe, we could achieve an etch depth of 2.47 μm and a sidewall angle of 89.5°. After process parameters are optimized on prime silicon wafers, we decided to apply this recipe to the SOI wafers. The initial results on SOI wafer is shown in Figure 4.3.2. The etch depth in this micrograph is 1.9 μm and the sidewall angle is 89.7°.

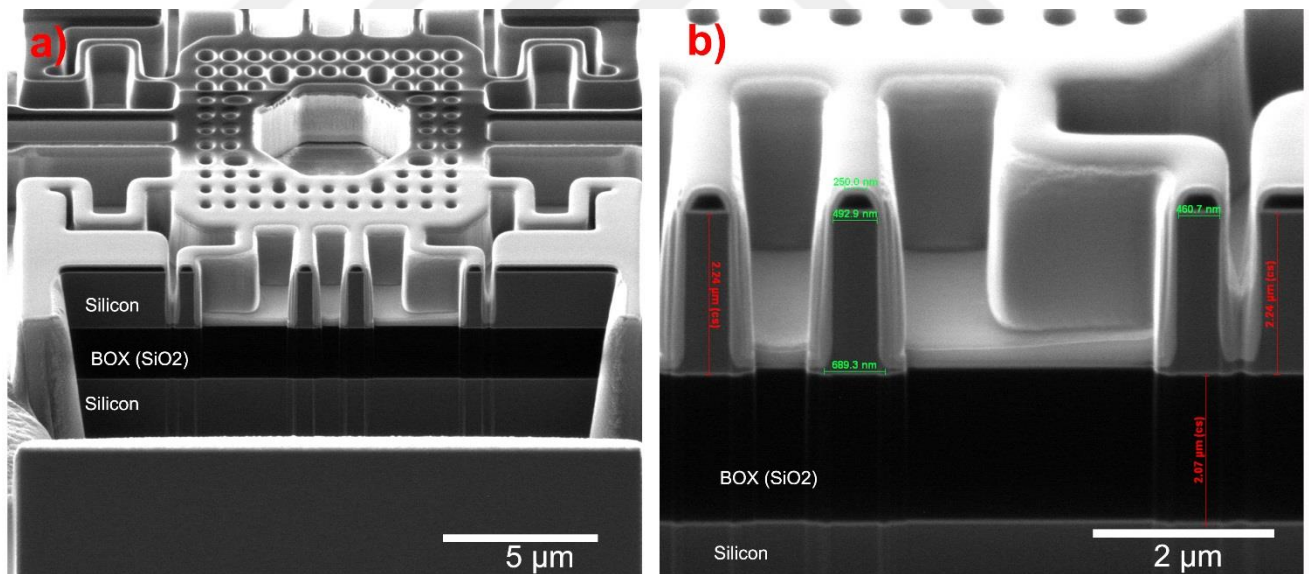


Figure 4.2.2 Initial fabrication results on SOI wafer.

The second silicon etch by MERIE was carried out on a single-layer silicon wafer. The initial results for this step is shown in Figure 4.2.3. The thickness difference between silicon nanowires and springs can be observed.

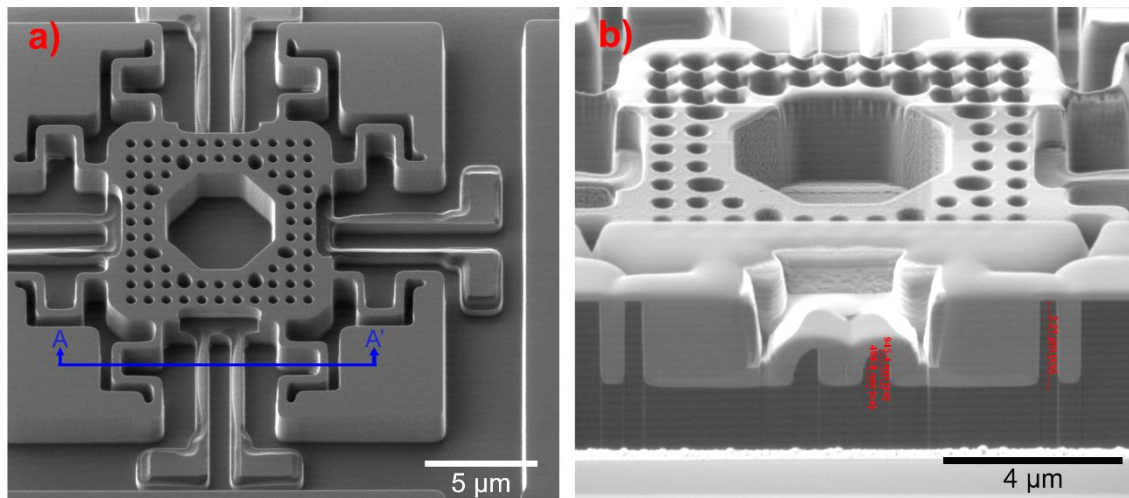


Figure 4.2.3 Top and cross-section views of the fabricated structures after second Silicon etch.

4.3. Electrical Readout and Characterization

There different electrical readout connections were developed. These connections are shown in Figure 4.4.1. Each piezoresistor in the first electrical readout design is addressed independently. This design makes ohmic contact with the metal lines and it requires 8 connections. Each piezoresistor in the second design complements as the fourth resistor in Wheatstone bridge circuit. This design requires 16 connections. In the third design, each piezoresistor has two different contact types, ohmic and Schottky contacts. This configuration is equivalent to a resistor connected in series with a Schottky diode. Such an electrical connection scheme enables multiplexing the piezoresistors which reduces the number of connections to 4 while each piezoresistor can still be addressed individually. The multiplexing scheme is a convenient approach for the electrical connection of an array of sensors.

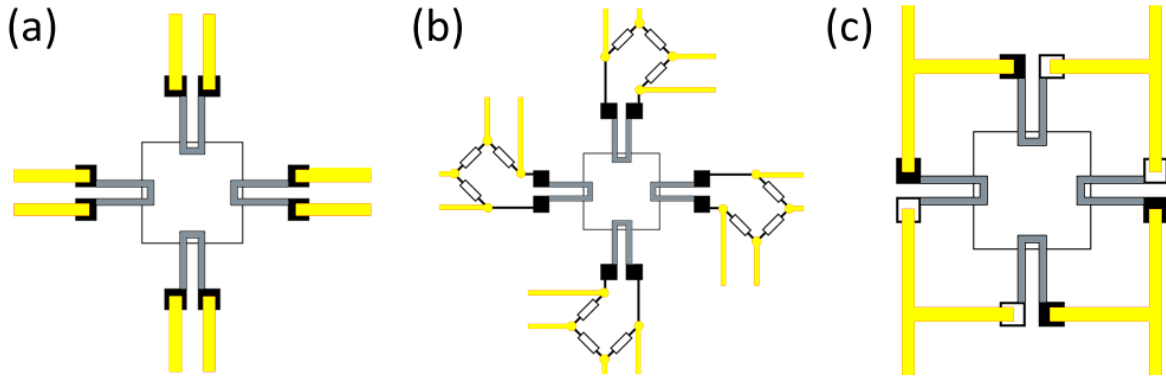


Figure 4.3.1 Electrical readout connection schemes.

To characterize the fabricated sensor a force with known magnitude and direction will be applied to the platform of the sensor and the sensor response will be measured. Due to small dimension of the sensor we cannot use commercial force sensors to apply force on the platform. We, finally decided to use AFM cantilevers to calibrate the sensor since AFM probe's dimensions can meet our size scale criteria. AFM also enables us to apply lateral forces of known magnitude. During the application of load by AFM tip, the resistance change of the nanowire bridges will be measured using a semiconductor parameter analyzer SMU.

4.5. Discussion and Outlook

In this part of the project, we developed a technology to measure the in-plane forces action on cytoskeletal filaments of cells using an array of MEMS sensors. Electromechanical design of the sensor is geared towards optimizing the piezoresistive response and mechanical sensitivity. Fabrication work was carried out in collaboration with Tübitak BİLGEM, through which access to submicron patterning and doping through ion implantation was secured. Innovative use of the IC manufacturing infrastructure toward the realization of extreme topographies associated with microsystems were brought up.

Although fabrication process flow given in Figure 4.2.1 can be perceived to be in its final version, we continuously improve the fabrication steps in accordance with new available materials and opportunities. For example, we decided to use aluminum with titanium seed layer for metallization part as fabrication group at YITAL has hands-on experience with these

materials. However, HF attacks aluminum-titanium and an additional step of passivation with polymer coating is required to protect the metals. We finally came up with an alternative combination of materials, gold with chromium seed layer. This gold-chromium combination has a self-protection against HF. This material selection takes away unnecessary steps that are passivation, Freon RIE, and passivation removal processes.



Chapter 5

CONCLUSION

Two technologies were developed regarding real-time analysis of cytoskeletal filaments under local loads. The first technology utilizes a miniaturized cell stretcher integrated with a fluorescent microscope enabling us to load thin substrates with adherent cells and to determine in-plane strains and deformation that cells undergo. This setup also provides a simultaneous fluorescent imaging, which can be used to interpret the biochemical events in the cells.

The integration and synchronization of the stretcher with the fluorescent microscope enables motion compensation and overcomes the challenges commonly encountered in stretching systems. Without motion compensation, for example, the area of interest that is initially captured by microscope gradually moves out of the field of view as the sample is stretched. In this way, we can capture sequential micrographs of the area of interest throughout the entire stretching.

We also developed an algorithm that can track the particles with a sub-pixel accuracy. Using the positions of the particles, we can determine deformation or in-plane strain components for critical regions on the samples. Micropatterns with a critical dimension of 200 μm are fabricated on a PDMS substrate and the substrate is then stretched up to 20% cross-head stretch. Two critical regions in the vicinity of the patterns is analyzed and strain distribution in those regions is captured with a high spatiotemporal resolution.

This technology facilitated imaging of a single cell and observation of its mechanical behavior throughout the whole experiment. Finally, the same technology was applied to observing the behavior of cytoskeleton in single living cells in a real-time fashion. Hence, the main scientific objective of cell mechanical observations in a quantitative manner was realized.

The second technology is based on a MEMS force sensor. This sensor is designed using piezoresistive nanowires as electromechanical transducers. Thus, extensive literature review on piezoresistance effect in single crystal silicon was carried out, and the effects of doping type and dopant concentration, crystal orientation, and temperature on the sensitivity of silicon piezoresistors were carefully examined. Final decision on the design is made towards maximizing the sensor sensitivity and piezoresistance response.

The fabrication process flow is developed in collaboration with the fabrication group at YITAL (Semiconductor Technologies Research Laboratory). The last version of the fabrication flow comprises reactive ion etching processes, doping by ion implantations, metallization, and HF vapor release. Fabrication stage of the MEMS sensor is currently in progress, and the fabrication results from the silicon etching steps are presented in Appendix C: Fabrication Results.

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Appendix A: Strain Definitions

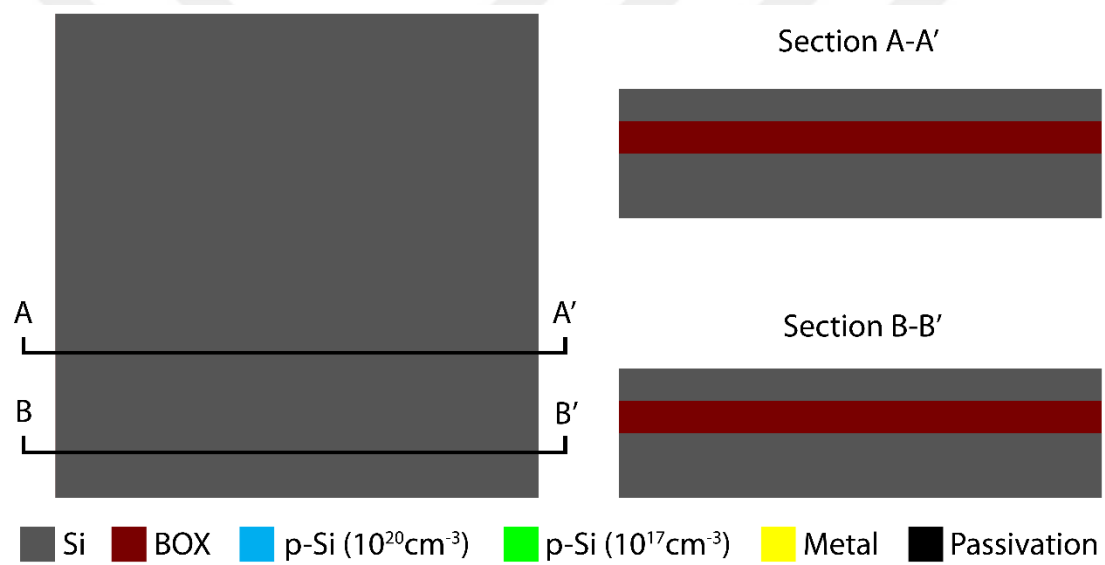
Strain Formulae for Different Strain Definitions

Cauchy	Green	Almansi	Swainger	Hencky
Beads' position $x_i = \phi(X_i, t)$	Beads' position $x_i = \phi(X_i, t)$	Beads' position $x_i = \phi(X_i, t)$	Beads' position $x_i = \phi(X_i, t)$	Beads' position $x_i = \phi(X_i, t)$
Displacement Vector $u_i(X_i, t) = \phi(X_i, t) - X_i$	Displacement Vector $u_i(X_i, t) = \phi(X_i, t) - X_i$	Displacement Vector $u_i(X_i, t) = \phi(X_i, t) - X_i$	Displacement Vector $u_i(X_i, t) = \phi(X_i, t) - X_i$	Displacement Vector $u_i(X_i, t) = \phi(X_i, t) - X_i$
Displacement Gradient Tensor $\nabla u_{ij} = \frac{\partial u_i}{\partial x_j}$	Displacement Gradient Tensor $\nabla u_{ij} = \frac{\partial u_i}{\partial x_j}$	Displacement Gradient Tensor $\nabla u_{ij} = \frac{\partial u_i}{\partial x_j}$	Displacement Gradient Tensor $\nabla u_{ij} = \frac{\partial u_i}{\partial x_j}$	Displacement Gradient Tensor $\nabla u_{ij} = \frac{\partial u_i}{\partial x_j}$
-	Deformation Gradient $F_{ij} = \nabla u_{ij} + \delta_{ij}$	Deformation Gradient $F_{ij} = \nabla u_{ij} + \delta_{ij}$	Deformation Gradient $F_{ij} = \nabla u_{ij} + \delta_{ij}$	Deformation Gradient $F_{ij} = \nabla u_{ij} + \delta_{ij}$
-	right Cauchy-Green deformation tensor $C_{ij} = F_{ki} F_{kj}$	right Cauchy-Green deformation tensor $C_{ij} = F_{ki} F_{kj}$	right Cauchy-Green deformation tensor $C_{ij} = F_{ki} F_{kj}$	right Cauchy-Green deformation tensor $C_{ij} = F_{ki} F_{kj}$
Cauchy Strain $E_{ij}^C = \frac{(\nabla u_{ij} + \nabla u_{ij}^T)}{2}$	Green–Saint-Venant Strain Tensor $E_{ij}^G = \frac{(C_{ij} - \delta_{ij})}{2}$	Almansi Strain Tensor $E_{ij}^A = \frac{(\delta_{ij} - C_{ij}^{-1})}{2}$	Swainger Strain Tensor $E_{ij}^S = \frac{(\delta_{ij} - C_{ij}^{-1/2})}{2}$	Hencky strain Tensor $E_{ij}^H = \frac{\log(C_{ij})}{2}$
Strain Components $E^C = \begin{bmatrix} \epsilon_{xx} & \epsilon_{xy} \\ \epsilon_{yx} & \epsilon_{yy} \end{bmatrix}$	Strain Components $E^G = \begin{bmatrix} \epsilon_{xx} & \epsilon_{xy} \\ \epsilon_{yx} & \epsilon_{yy} \end{bmatrix}$	Strain Components $E^A = \begin{bmatrix} \epsilon_{xx} & \epsilon_{xy} \\ \epsilon_{yx} & \epsilon_{yy} \end{bmatrix}$	Strain Components $E^S = \begin{bmatrix} \epsilon_{xx} & \epsilon_{xy} \\ \epsilon_{yx} & \epsilon_{yy} \end{bmatrix}$	Strain Components $E^H = \begin{bmatrix} \epsilon_{xx} & \epsilon_{xy} \\ \epsilon_{yx} & \epsilon_{yy} \end{bmatrix}$

Appendix B: Fabrication Process Flow

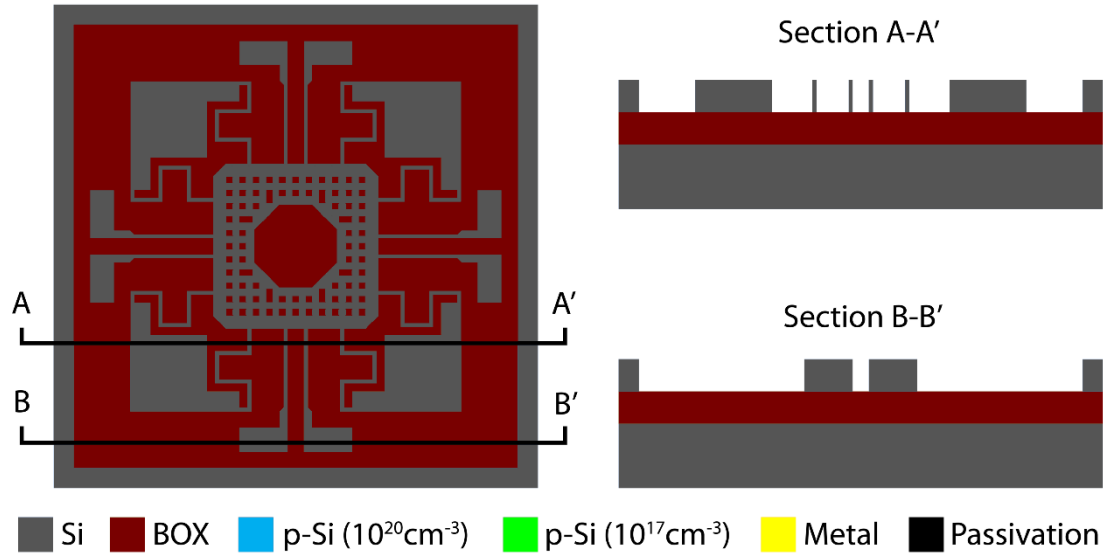
Appendix B includes the schematic representation of fabrication processes.

SOI wafer



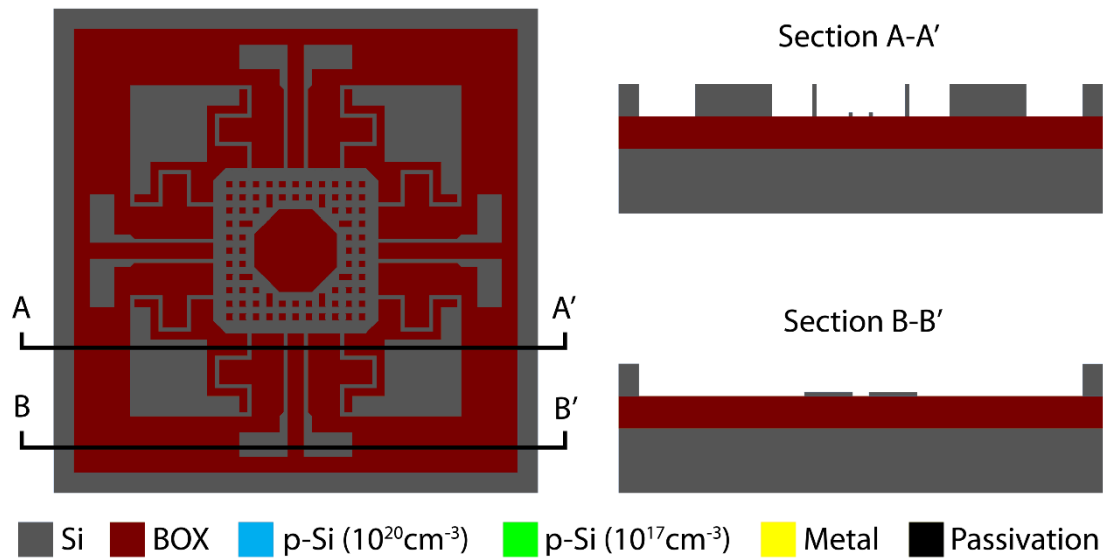
The figure shows two different cross-sections of an SOI wafer

First Silicon Etch with MERIE



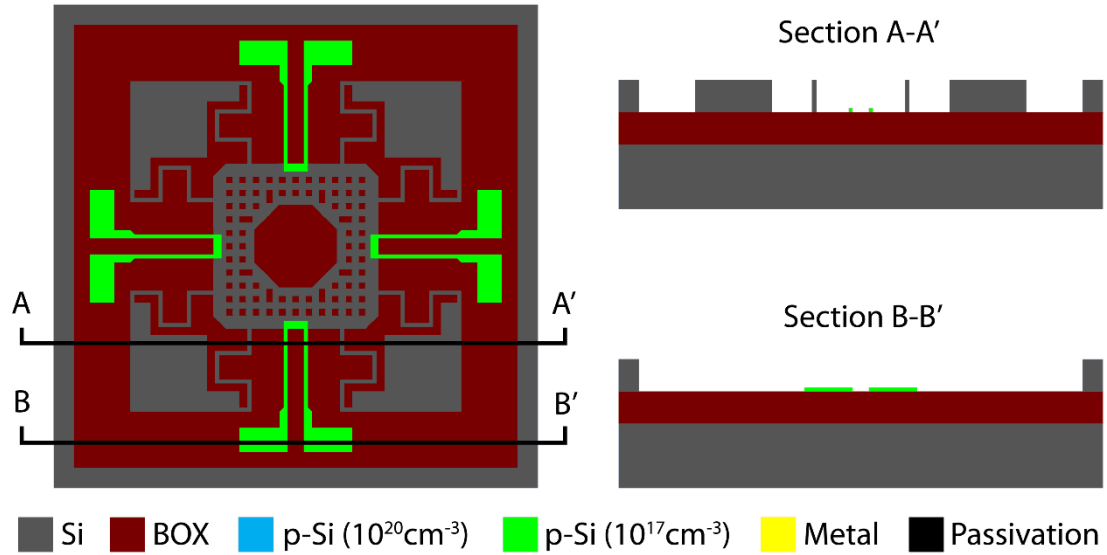
The anchors, springs, and the platform are defined and the nanowire bridge is outlined by a 2 μm MERIE etch.

Second Silicon Etch with MERIE



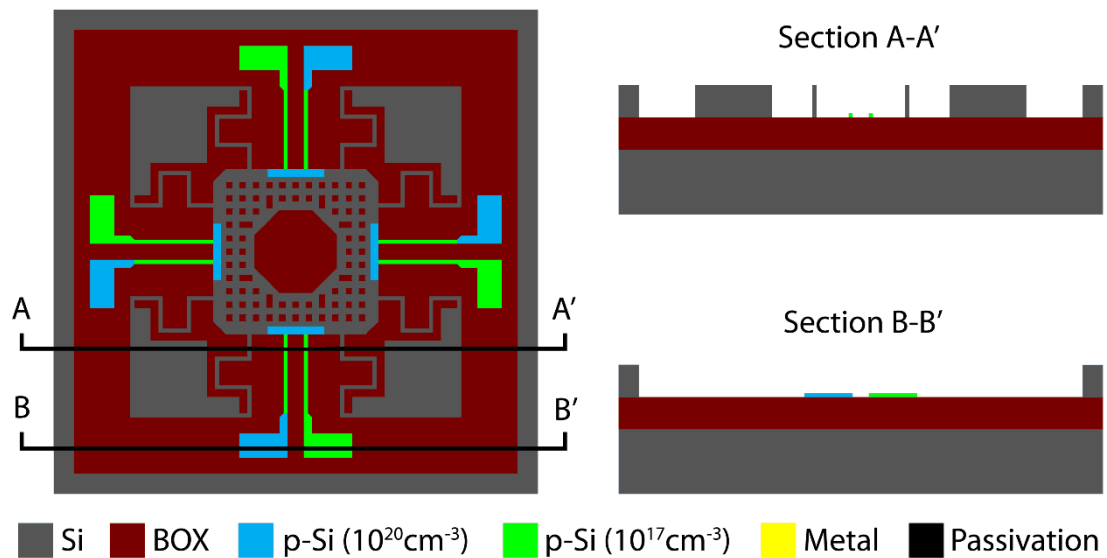
The thickness of nanowires is reduced to 0.25 μm .

Ion implantation with 10^{17} cm^{-3} dopant concentration



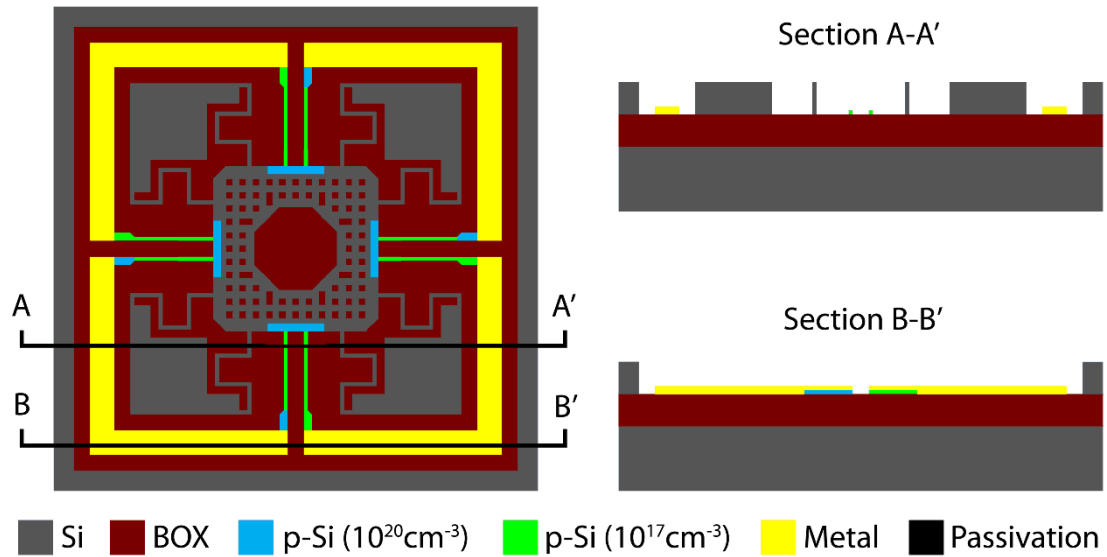
Green regions (nanowires and metal contact pads) are lightly doped.

Ion implantation with 10^{20} cm^{-3} dopant concentration



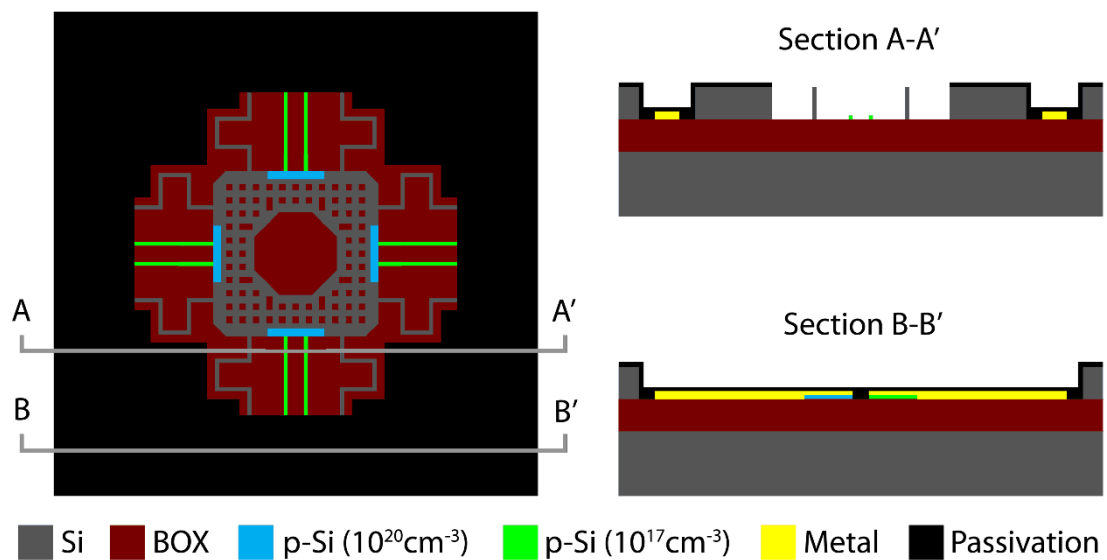
Doping of ohmic contact pads and unstrained piezoresistors with boron by ion implantation.

Metallization



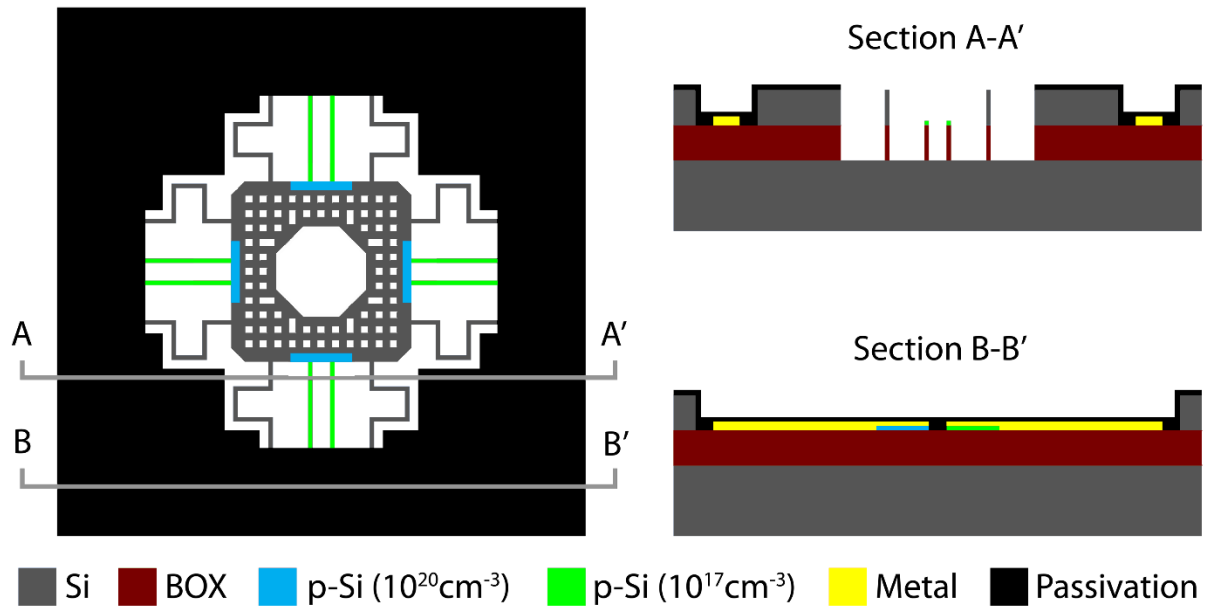
Metallization by sputtering.

Passivation



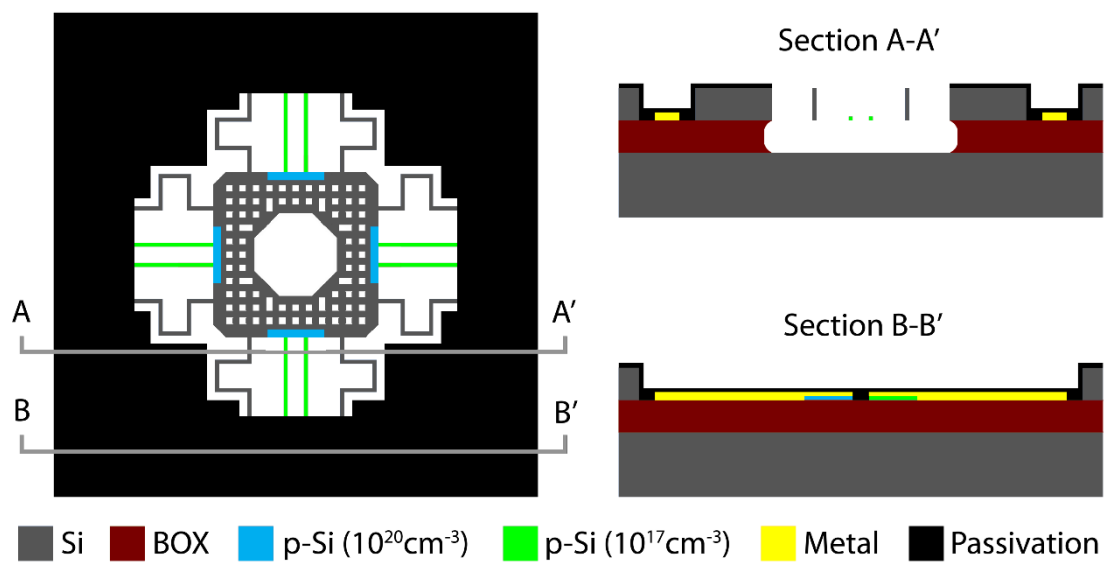
Passivation by polymer coating.

Freon RIE



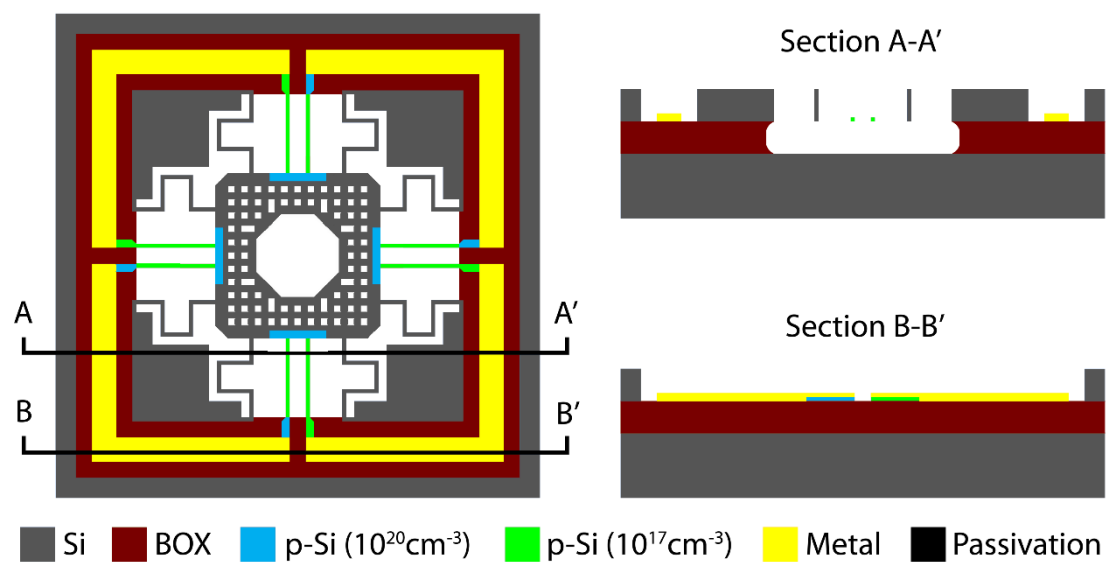
Box etching by Freon RIE.

HF vapor release



Metallization by sputtering.

Removal of passivation



Removal of the polymer passivation layer by oxygen plasma ashing.

Appendix C: Fabrication Results

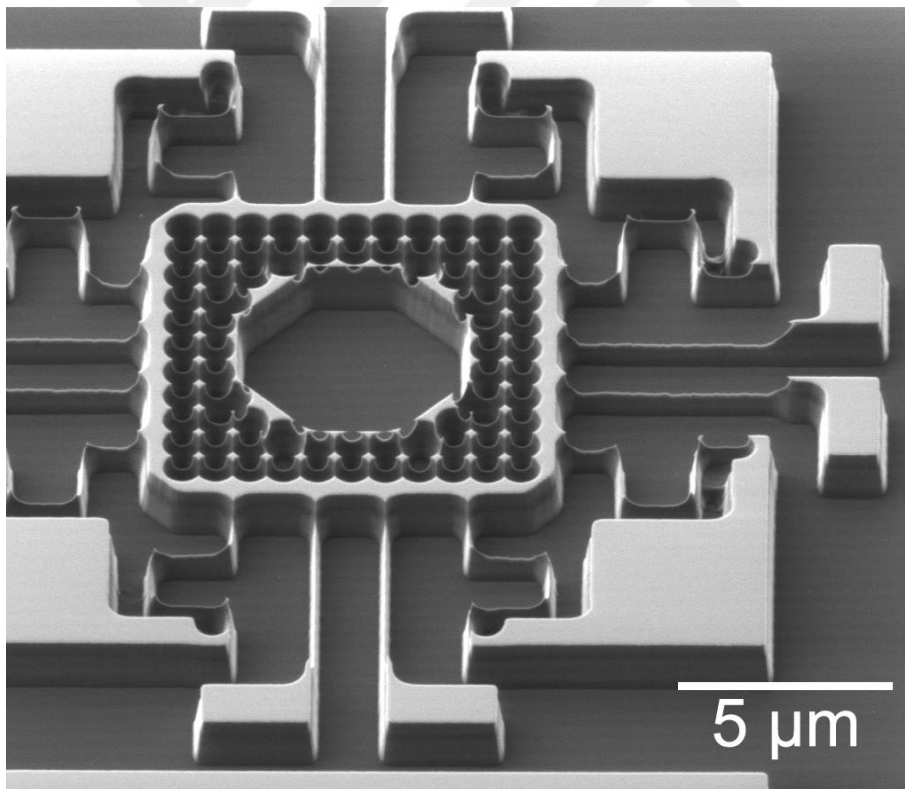
Fabrication Recipes for Silicon Etch Processes and Micrographs

Since we are not allowed to share process parameters, we only provide mask materials, captured micrographs, and shortcomings of each fabrication recipe.

Deep Trench Isolation 1A Recipe (DTI-1A)

Mask material: photoresist (6250A°)

Etch Depth: $\sim 1.2\mu\text{m}$



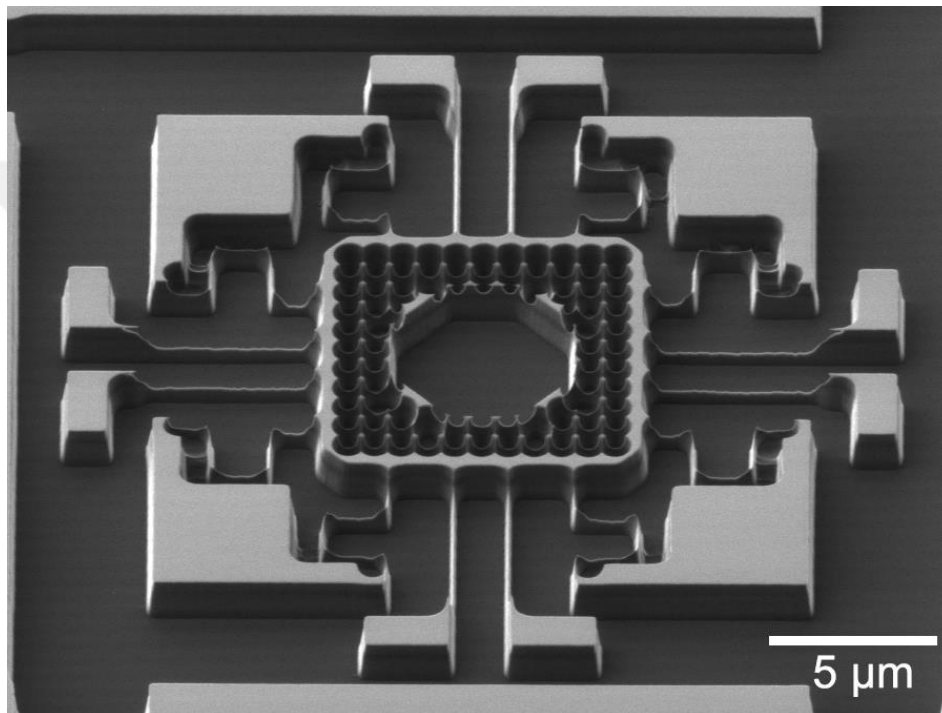
Fabricated structure using DTI-1A recipe.

Shortcomings: Inclined sidewall profile. Photoresist mask did not survive. Relatively, nanowires have good etch profiles than the springs.

Deep Trench Isolation 1B Recipe (DTI-1B)

Mask material: photoresist (6250A°)

Etch Depth: 1.2 μ m



Fabricated structure using DTI-1B recipe.

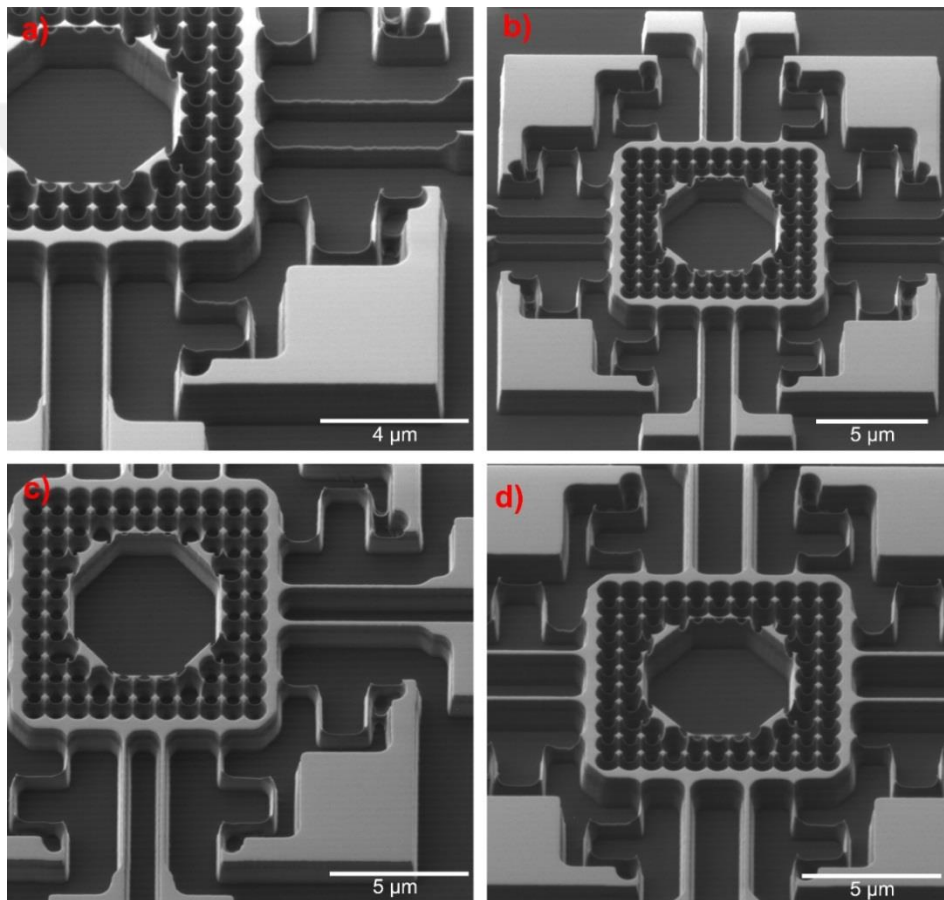
Shortcomings: Photoresist mask did not survive. Thus, it could not preserve the platform, nanowires, and springs during MERIE.

Deep Trench Isolation 1C Recipe (DTI-1C)

Mask material: Thermal growth oxide (500Å) and photoresist (~6000Å °)

Etch Depth: 1.4 – 1.6µm

Sidewall angle: 83°



Fabricated structures using DTI-1C recipe.

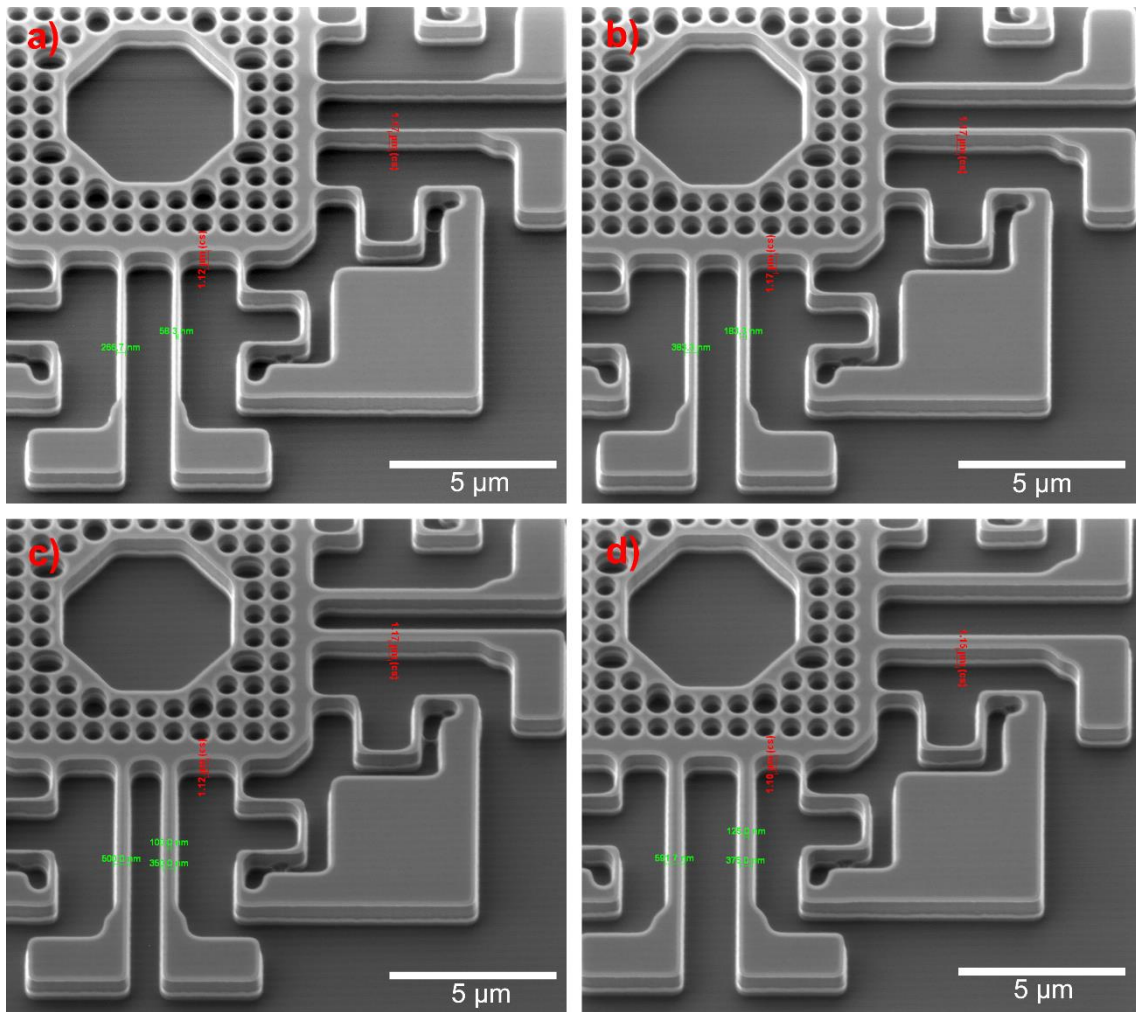
Shortcomings: Photoresist mask did not survive and thickness of oxide mask reduced to 191Å. Moreover, the oxide mask at the outer regions of the wafer, which was supposed to survive, completely etched during MERIE. This shows that Silicon etch rate was faster at the outer regions.

Deep Trench Isolation 1D Recipe (DTI-1D)

Mask material: Thermal growth oxide (500Å) and photoresist (~6000Å)

Etch Depth: 1.17μm

Sidewall angle: 85°



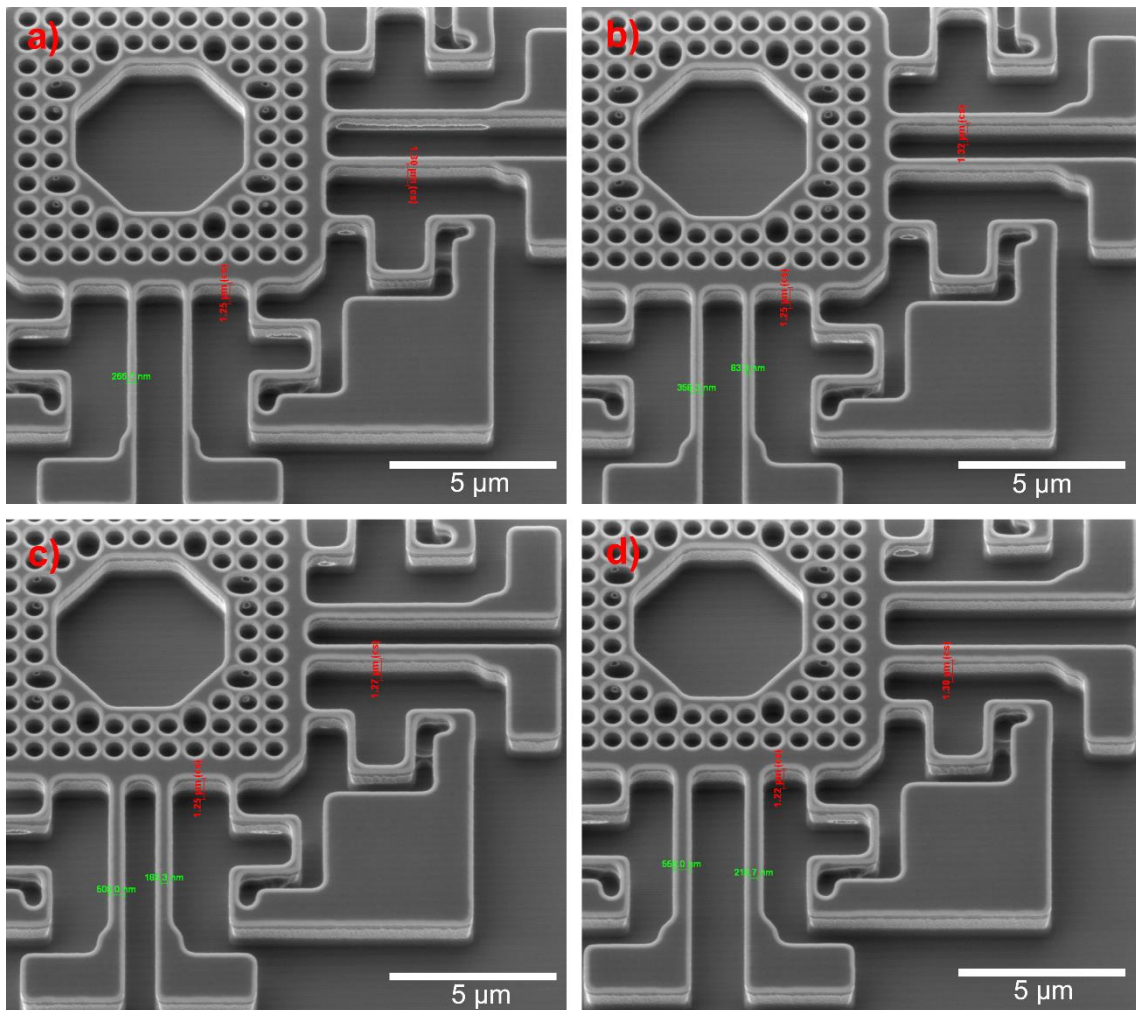
Fabricated structures using DTI-1D recipe. In-plane dimensions (in green) and depth values (in red) are shown on the micrographs.

Shortcomings: Etch mask survived and preserved the nanowires and the springs.

Deep Trench Isolation 1E Recipe (DTI-1E)

Mask material: Thermal growth oxide (500Å) and photoresist (~6000Å °)

Etch Depth: 1.22 -1.30 μm



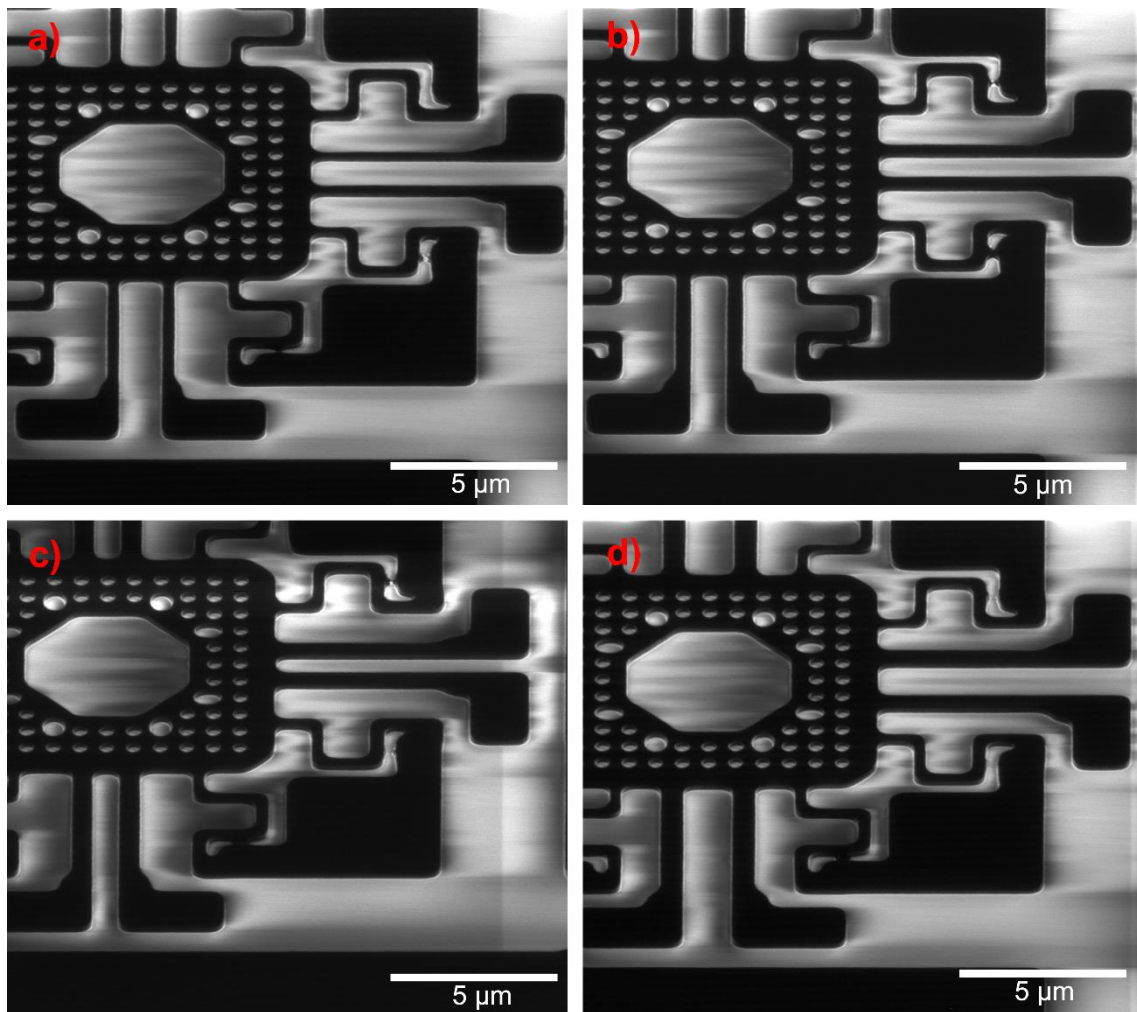
Fabricated structures using DTI-1E recipe. In-plane dimensions (in green) and depth values (in red) are shown on the micrographs.

Shortcomings: The rate of chemical etching was increased and this caused bowing profiles in etch holes of the platform.

Deep Trench Isolation 1F Recipe (DTI-1F)

Mask material: Thermal growth oxide (2000Å)

Etch Depth: not measured.



Fabricated structures using DTI-1F recipe

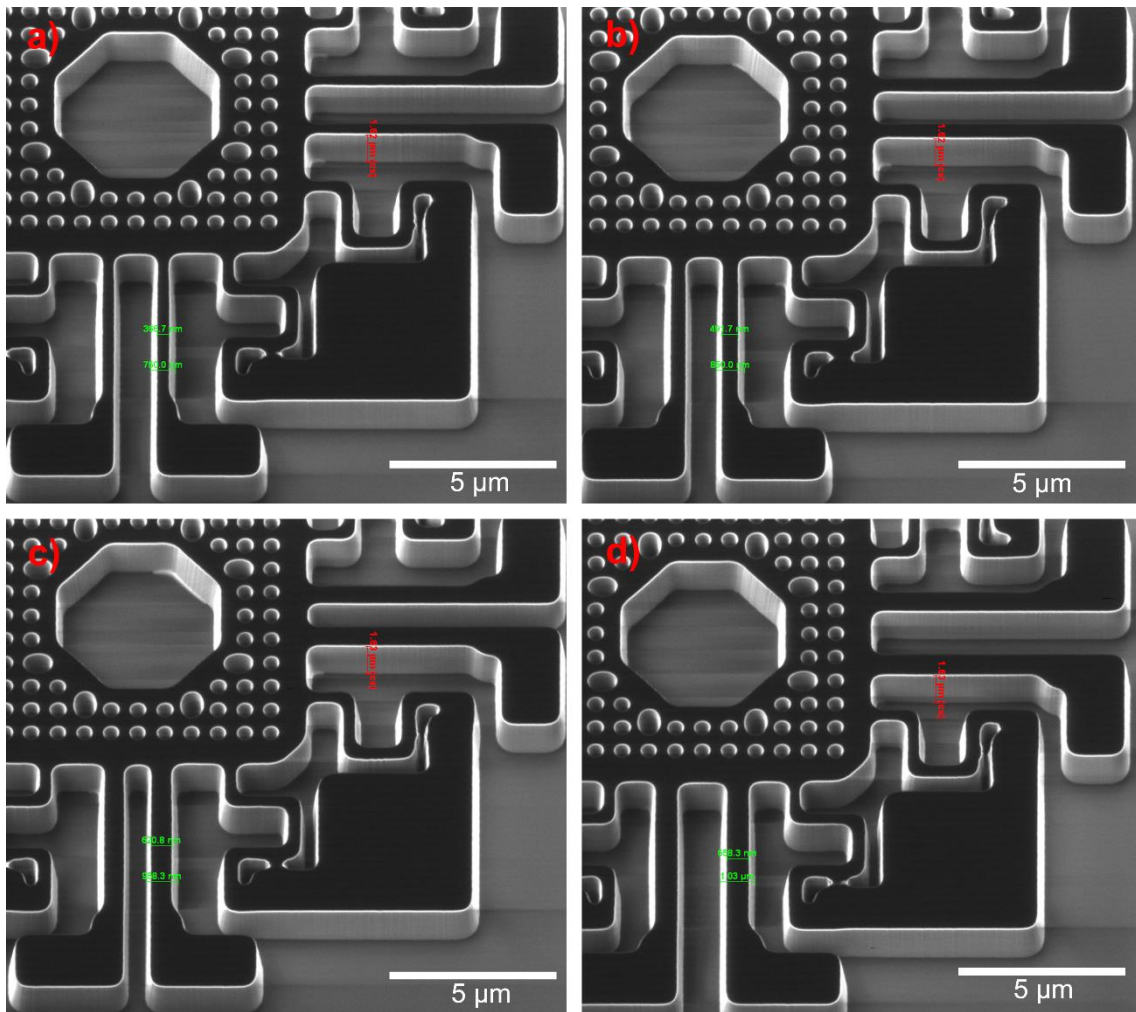
Shortcomings: Black Silicon was observed.

Deep Trench Isolation 1G Recipe (DTI-1G)

Mask material: Thermal growth oxide (2000Å)

Etch Depth: 1.62 μm

Sidewall angle: 83°



Fabricated structures using DTI-1G recipe (Oxide mask was not removed).

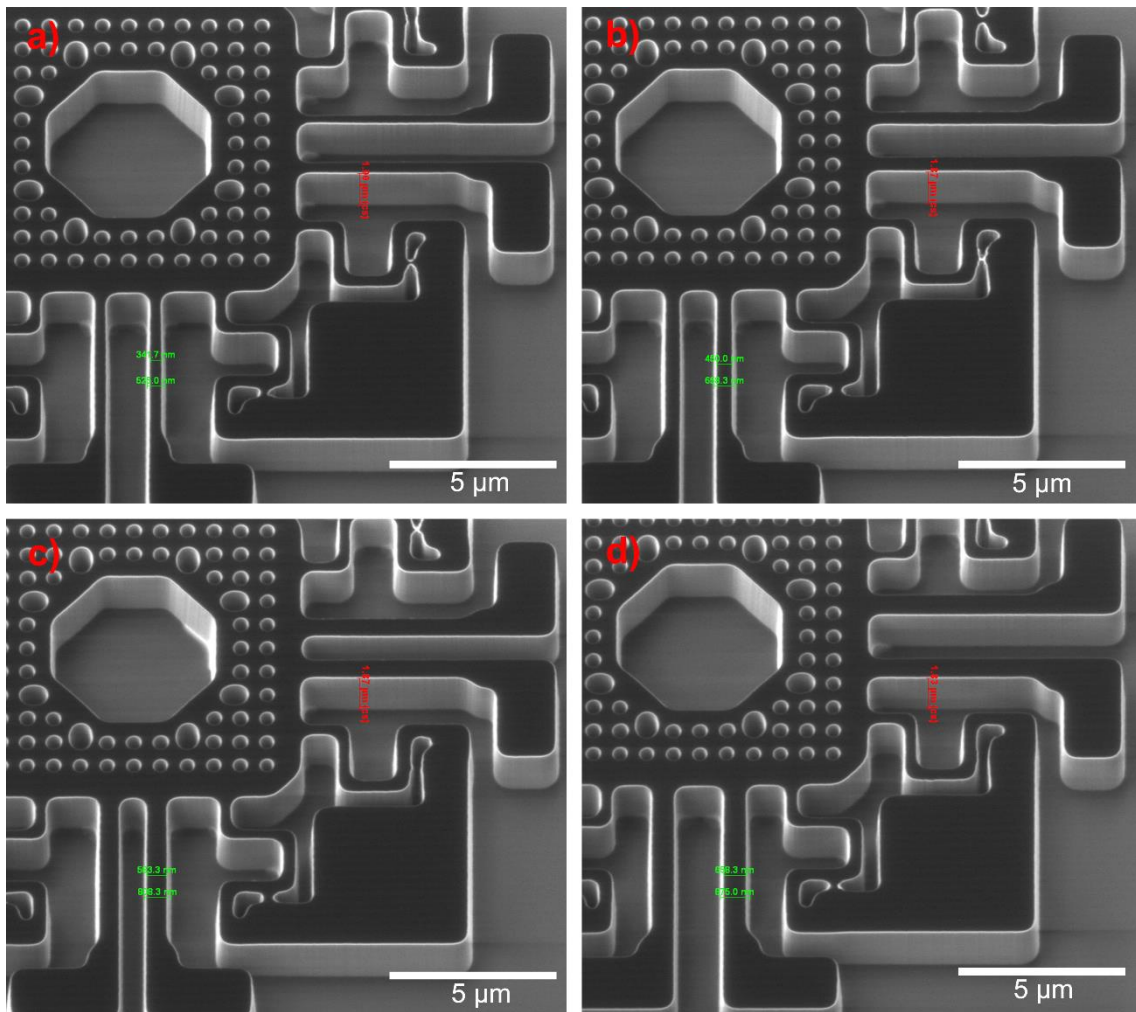
Shortcomings: The desired etch depth was not achieved.

Deep Trench Isolation 1H Recipe (DTI-1H)

Mask material: Thermal growth oxide (2000Å°)

Etch Depth: 1.90 μm

Sidewall angle: 87°



Fabricated structures using DTI-1H recipe (Oxide mask was not removed).

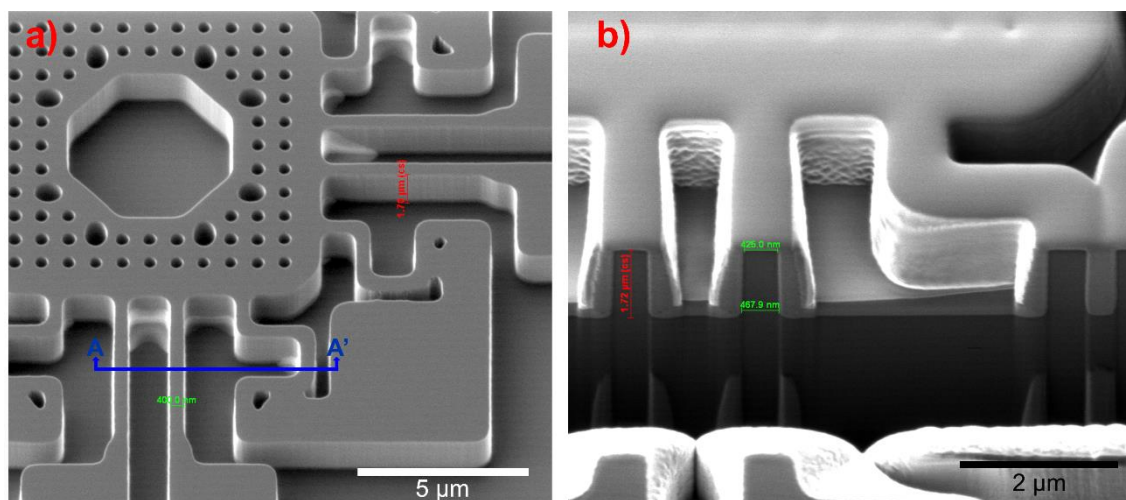
Shortcomings: The folds of springs could not completely remove from the anchors. For a fully functional spring, the shape of the spring should be double folded.

Deep Trench Isolation 1I Recipe (DTI-1I)

Mask material: Thermal growth oxide (2000A°)

Etch Depth: 1.72 μm

Sidewall angle: 89.3°



Oblique and cross-section view of a fabricated structures using DTI-1I recipe

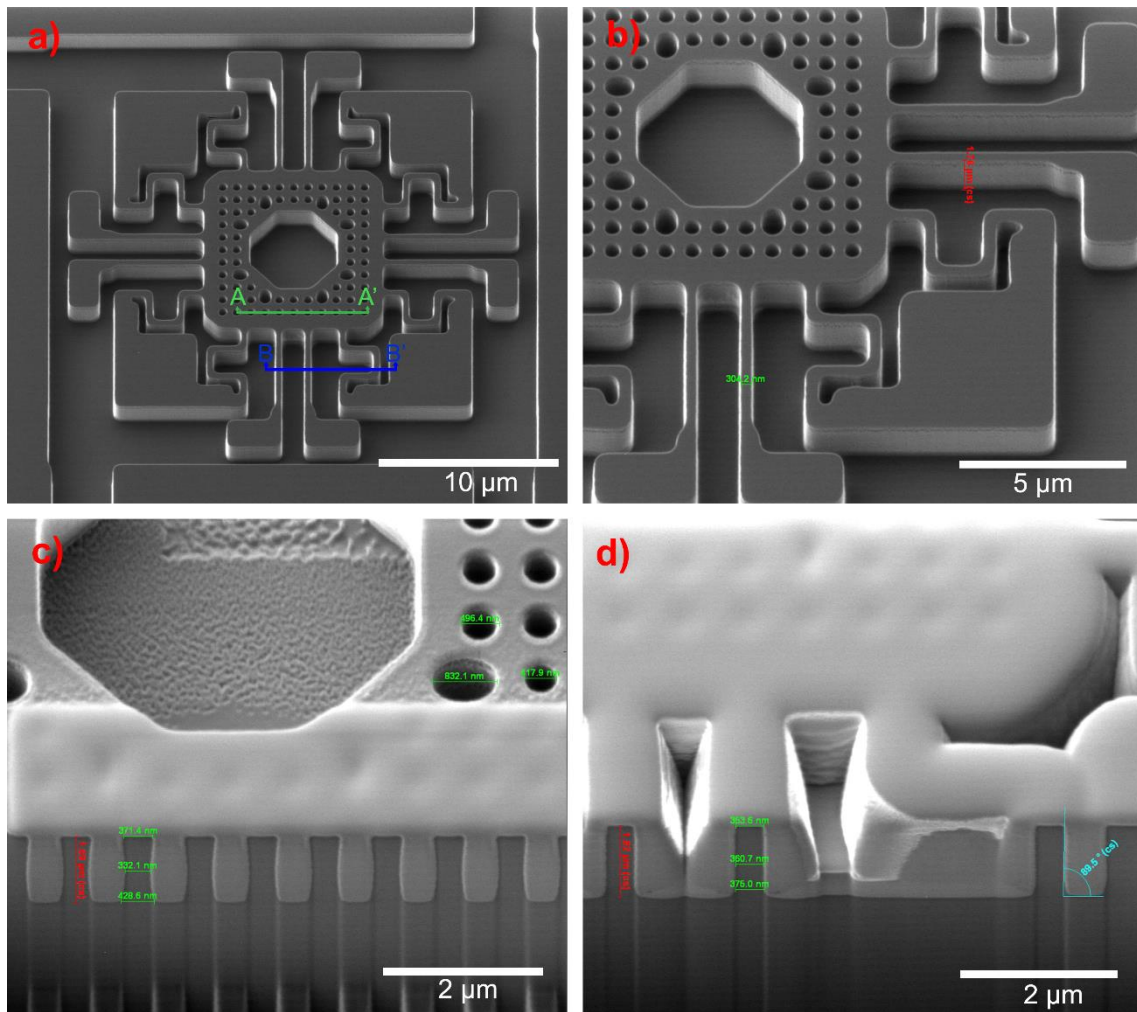
Shortcomings: Spring geometries were not well defined.

Deep Trench Isolation 1J Recipe (DTI-1J)

Mask material: Thermal growth oxide (2000Å°)

Etch Depth: 1.82 μm

Sidewall angle: 89.5°



Oblique, close-up, and cross-section views of a fabricated structures using DTI-1J recipe

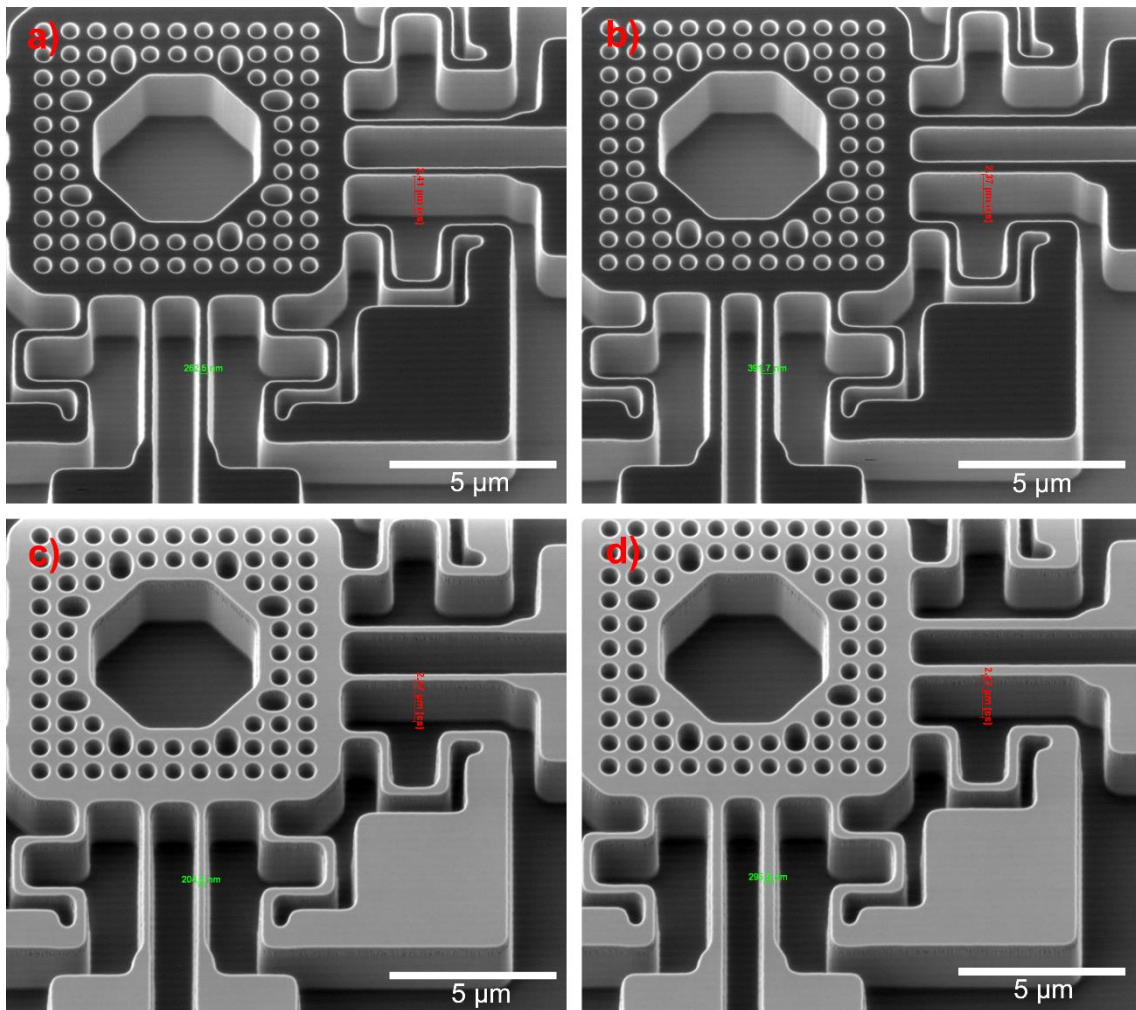
Shortcomings: Thickness variation between etch holes and nanowires depths due to aspect ratio dependent etching (ARDE).

Modified DTI-1J Recipe

Mask material: Thermal growth oxide (2000Å)

Etch Depth: 2.4 μm

Sidewall angle: 85.5°



Fabricated structures using modified DTI-1J recipe. The structure in panels a and b had oxide mask whereas oxide mask in panels c and d was removed

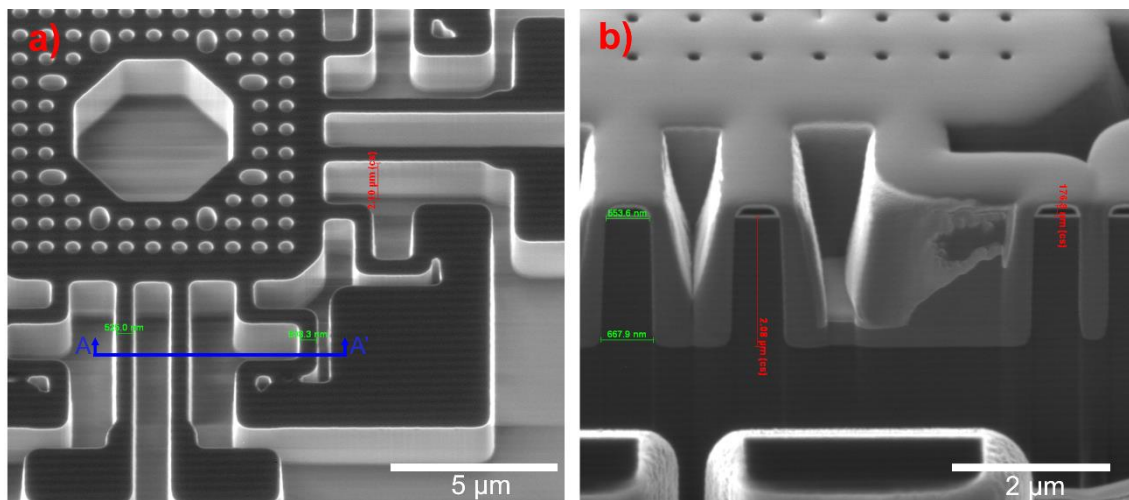
Shortcomings: This recipe provides acceptable results, but sidewall angle should approach to 90 °.

Modified DTI-1J Recipe

Mask material: Thermal growth oxide (3200Å°)

Etch Depth: 2.1 μm

Sidewall angle: 88.4°



Top and cross-section views of the fabricated structures using modified DTI-1J recipe.

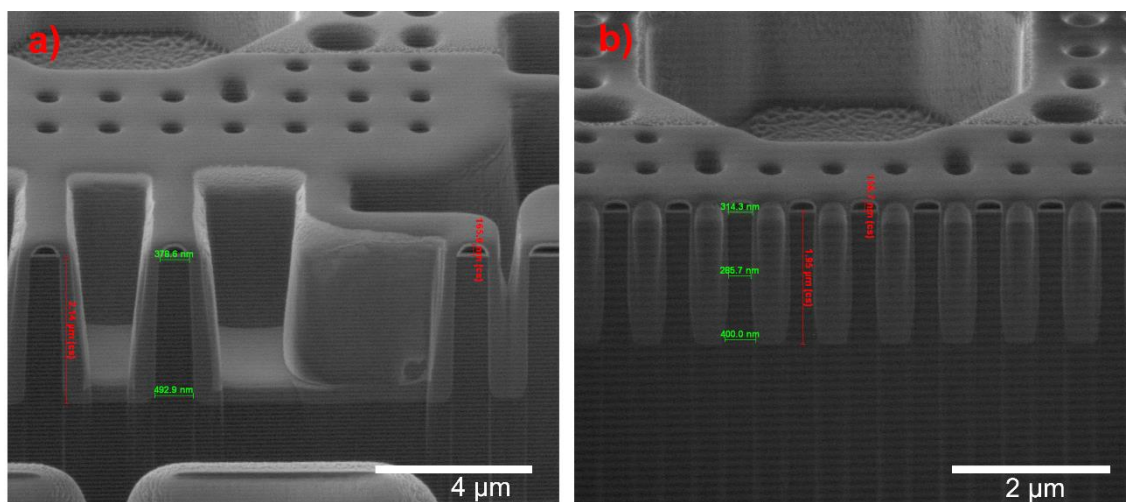
Shortcomings: This recipe provides acceptable results.

Modified DTI-1J Recipe

Mask material: Thermal growth oxide (3200A°)

Etch Depth: 2.15 μm

Sidewall angle: 88.5°



The cross-sections of (a) nanowires, spring, and (b) platform etch holes.

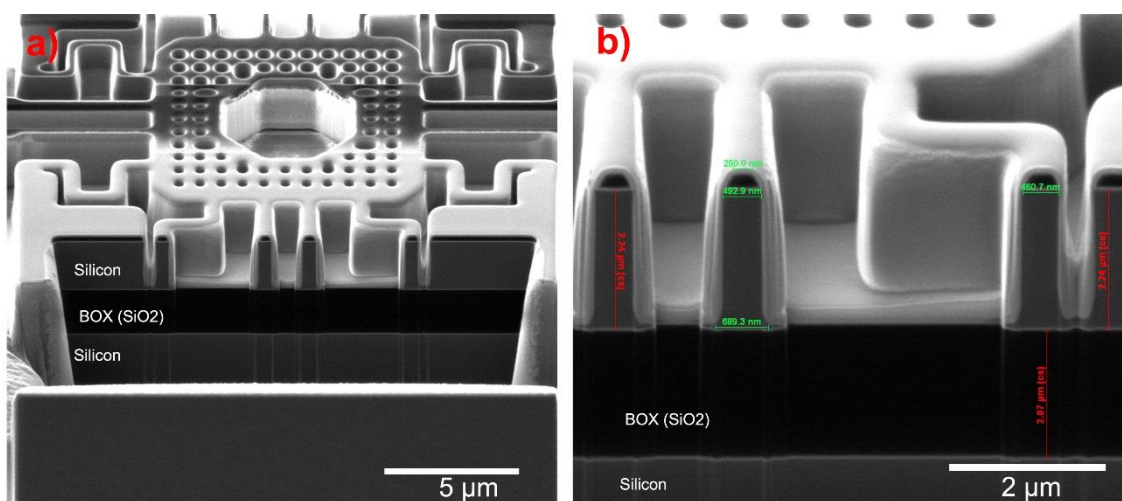
Results: This recipe provides an acceptable result.

Modified DTI-1J Recipe on SOI wafer

Mask material: Thermal growth oxide (3200Å°)

Etch Depth: 2.24 μm

Sidewall angle: 87.5°



The cross-sections of nanowires, spring on a SOI wafer.

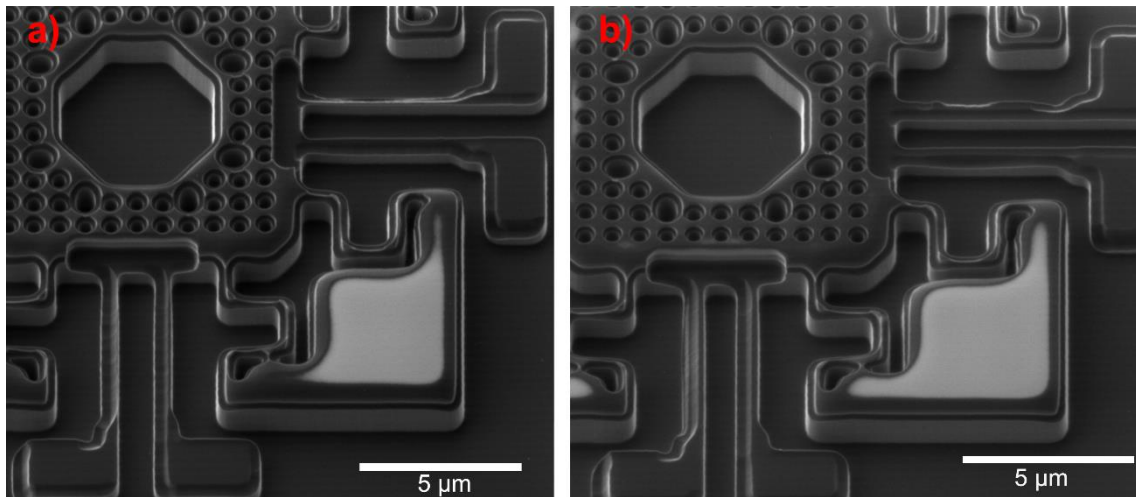
Results: This recipe provides acceptable results.

The following micrographs belong to second Silicon etch process.

Deep Trench Isolation 2A Recipe (DTI-2A)

Recipe used for the first Silicon etch: DTI-1I

Mask material: Photoresist. Photoresist has a thickness variation due to topography of the first Silicon etch process.



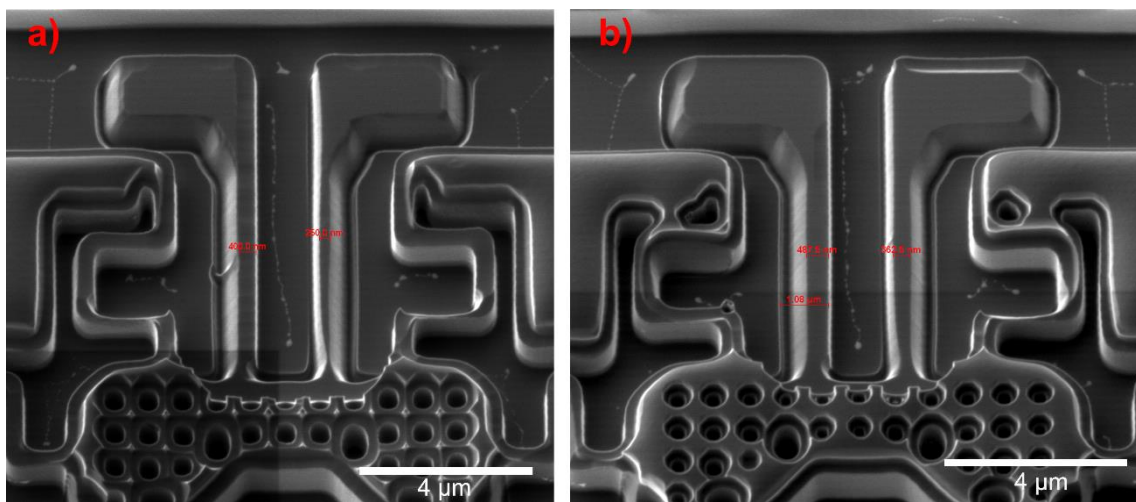
Fabricated structures using DTI-1I for the first Silicon etch and DTI-2A for the second Silicon etch.

Shortcomings: Photoresist did not survive. An anisotropic etching was observed.

Deep Trench Isolation 2A Recipe (DTI-2A)

Recipe used for the first Silicon etch: Modified DTI-1I

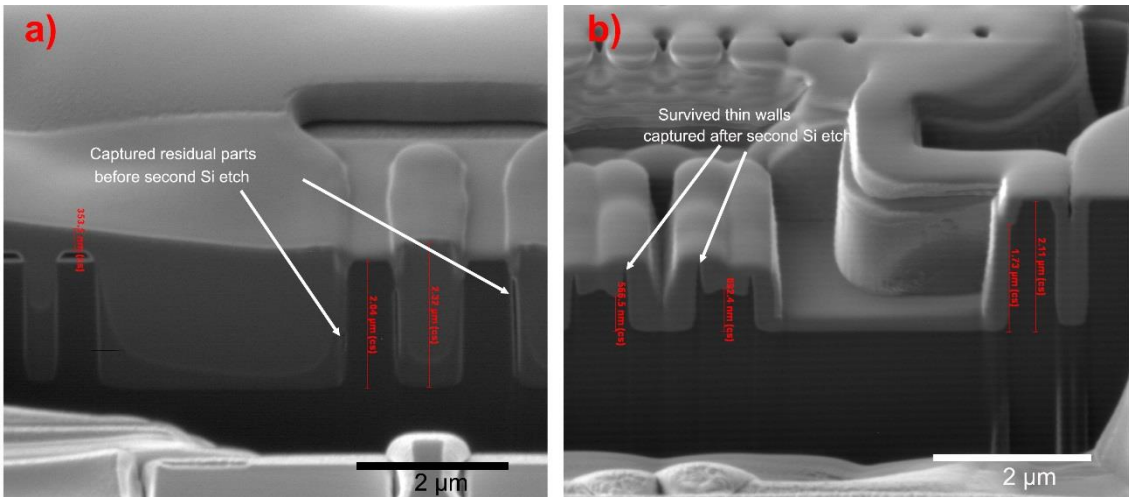
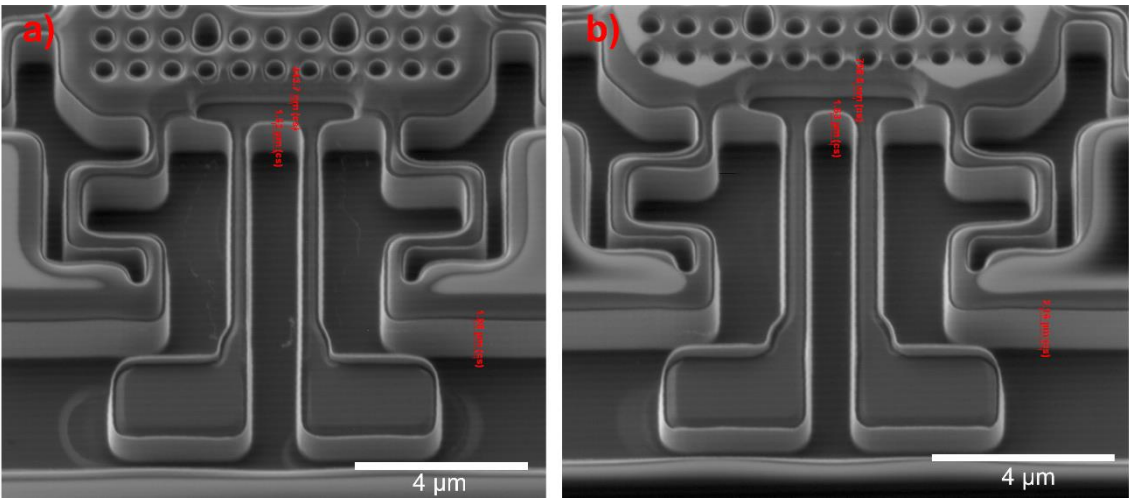
Mask material: Photoresist. Photoresist has a thickness variation due to topography of the first Silicon etch process.



Fabricated structures using modified DTI-1I for the first Silicon etch and DTI-2A for the second Silicon etch.

Shortcomings: Photoresist did not survive. An anisotropic etching was observed. Micro-trenched was observed.

Figure 1. The effect of the number of trials on the number of correct responses. The number of correct responses was plotted against the number of trials for each condition. The number of correct responses increased with the number of trials for all conditions. The number of correct responses was highest for the condition with the highest number of trials (10 trials) and lowest for the condition with the lowest number of trials (2 trials).

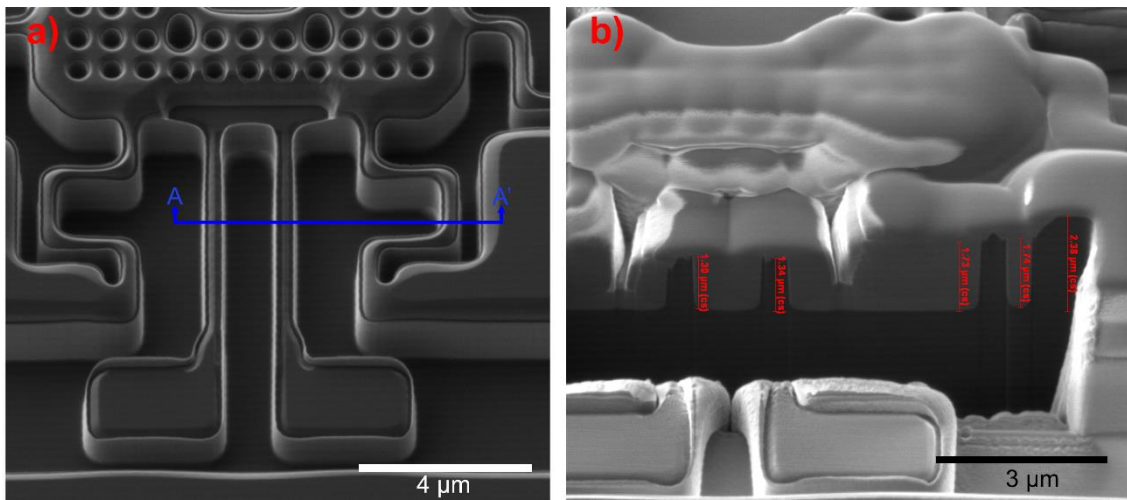


Deep Trench Isolation 2C Recipe (DTI-2C)

Recipe used for the first Silicon etch: DTI-1J

Mask material: Oxide

Etch Depth: 1.04 μm



Top and cross-section views of the fabricated structures using DTI-1J for the first Silicon etch and DTI-2C for the second Silicon etch.

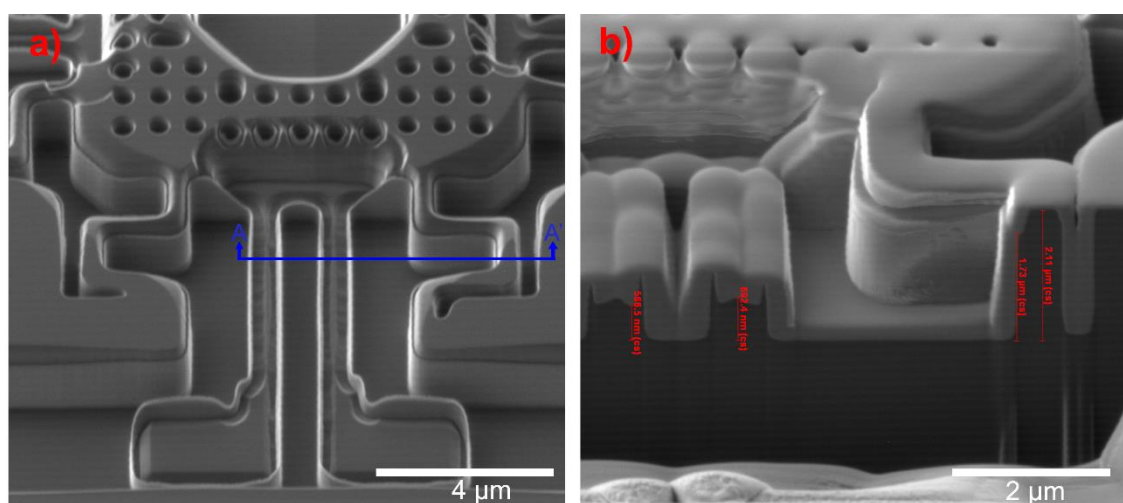
Shortcomings: The springs adversely affected, their depths were reduced by 0.64 μm .

Deep Trench Isolation 2C Recipe (DTI-2C)

Recipe used for the first Silicon etch: modified DTI-1J with 3200A oxide mask

Mask material: Oxide

Etch Depth: $\sim 1.44 \mu\text{m}$



Top and cross-section views of the fabricated structures using modified DTI-1J for the first Silicon etch and DTI-2C for the second Silicon etch.

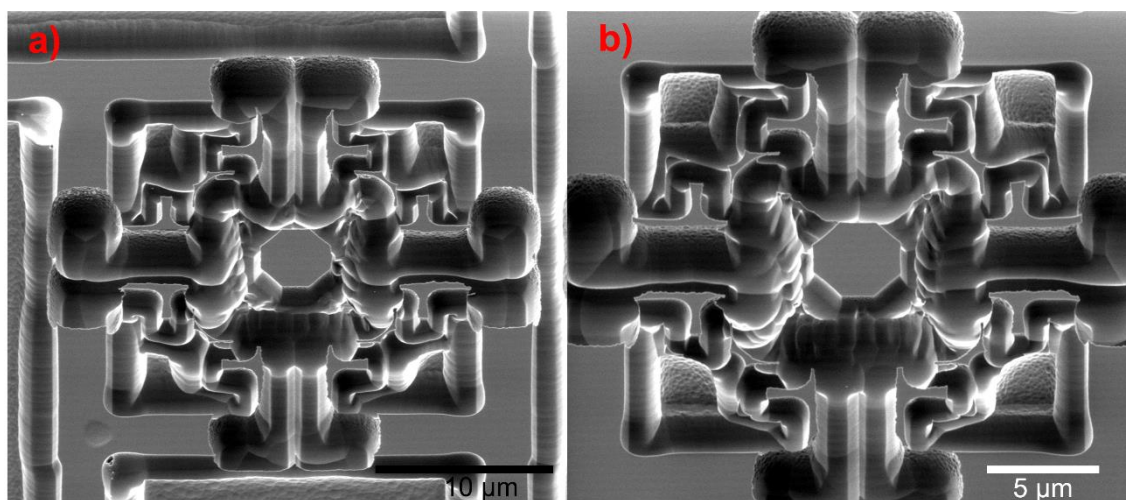
Shortcomings: An anisotropic etching was observed.

Deep Trench Isolation 2D and 2E Recipes (DTI-2D and DTI-2E)

Recipe used for the first Silicon etch: modified DTI-1J with 3200A oxide mask

Mask material: Oxide

Etch Depth: not measured



Micrographs of fabricated structures using (a) DTI-2D and (b) DTI-2E recipes.

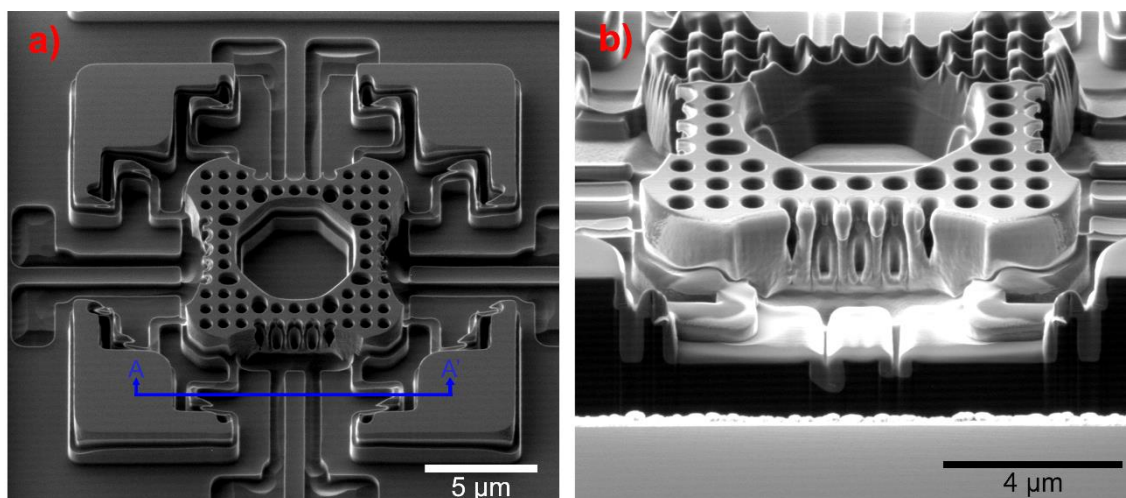
Shortcomings: These recipes caused very aggressive etch. All components are etched.

Deep Trench Isolation 2F Recipe (DTI-2F)

Recipe used for the first Silicon etch: modified DTI-1J with 3200Å oxide mask

Mask material: Oxide

Etch Depth: not measured



Top and cross-section views of the fabricated structures using modified DTI-1J for the first Silicon etch and DTI-2F for the second Silicon etch.

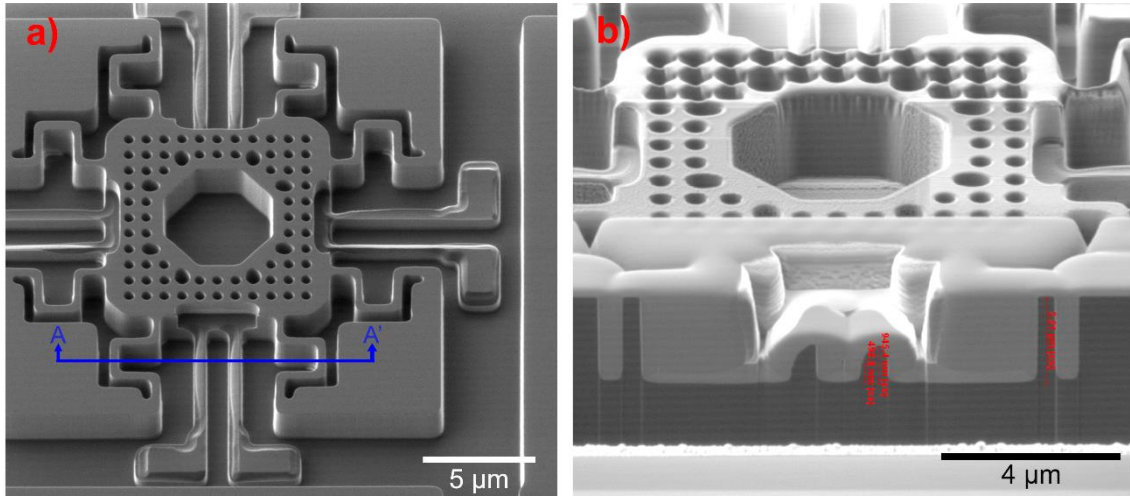
Shortcomings: These recipes caused an aggressive etch. Nanowires (completely) and springs (partially) were etched.

Deep Trench Isolation 2G Recipe (DTI-2G)

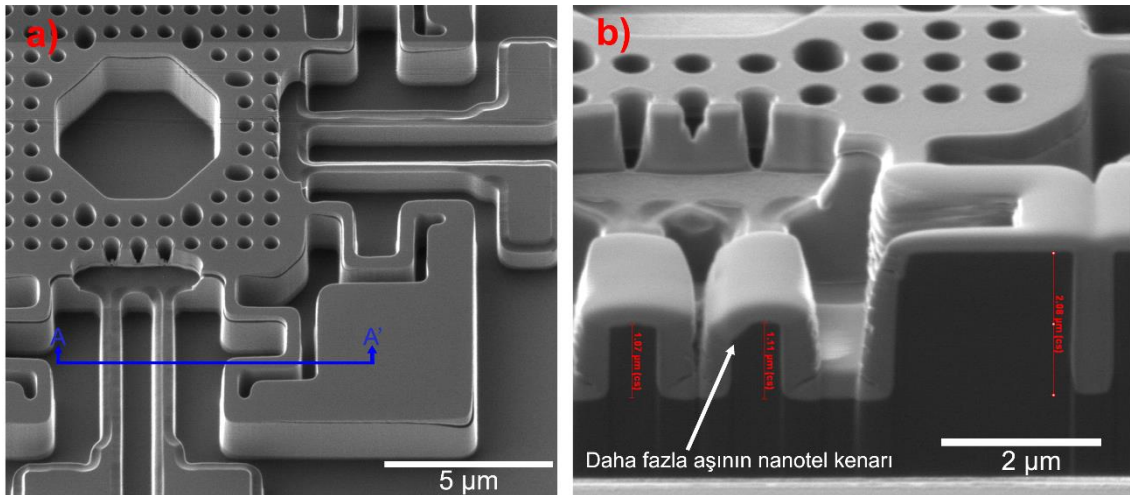
Recipe used for the first Silicon etch: modified DTI-1J with 3200A oxide mask

Mask material: Oxide

Etch Depth: 1.27 – 1.72 μm



Top and cross-section views of the fabricated structures using modified DTI-1J for the first Silicon etch and DTI-2G for the second Silicon etch.



Another structure on the same wafer

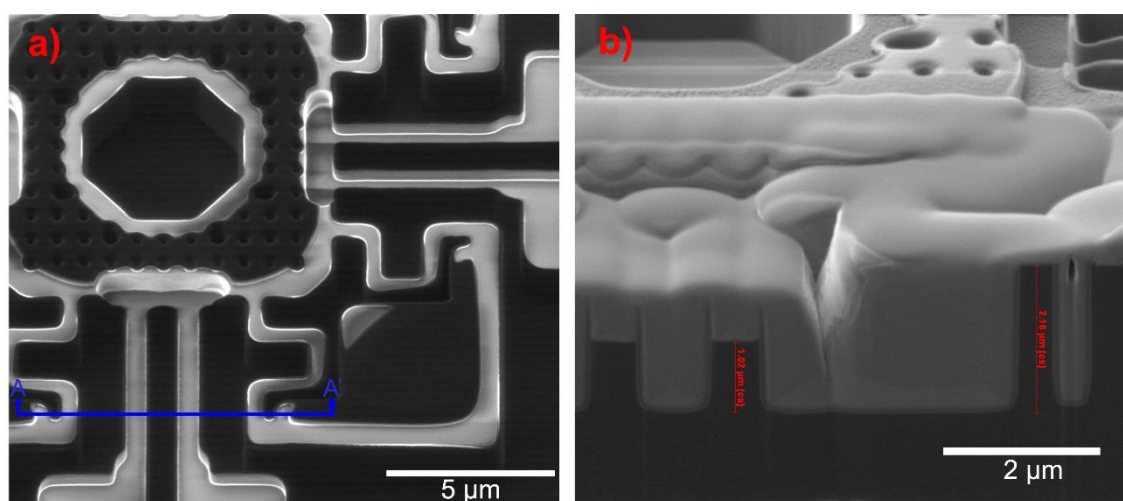
Results: Oxide mask survives throughout the etch processes and perfectly protects the platform and the springs. To achieve the desired depth, the duration should be increased.

Deep Trench Isolation 2H Recipe (DTI-2H)

Recipe used for the first Silicon etch: modified DTI-1J with 3200Å oxide mask

Mask material: Oxide

Etch Depth: 1.14µm

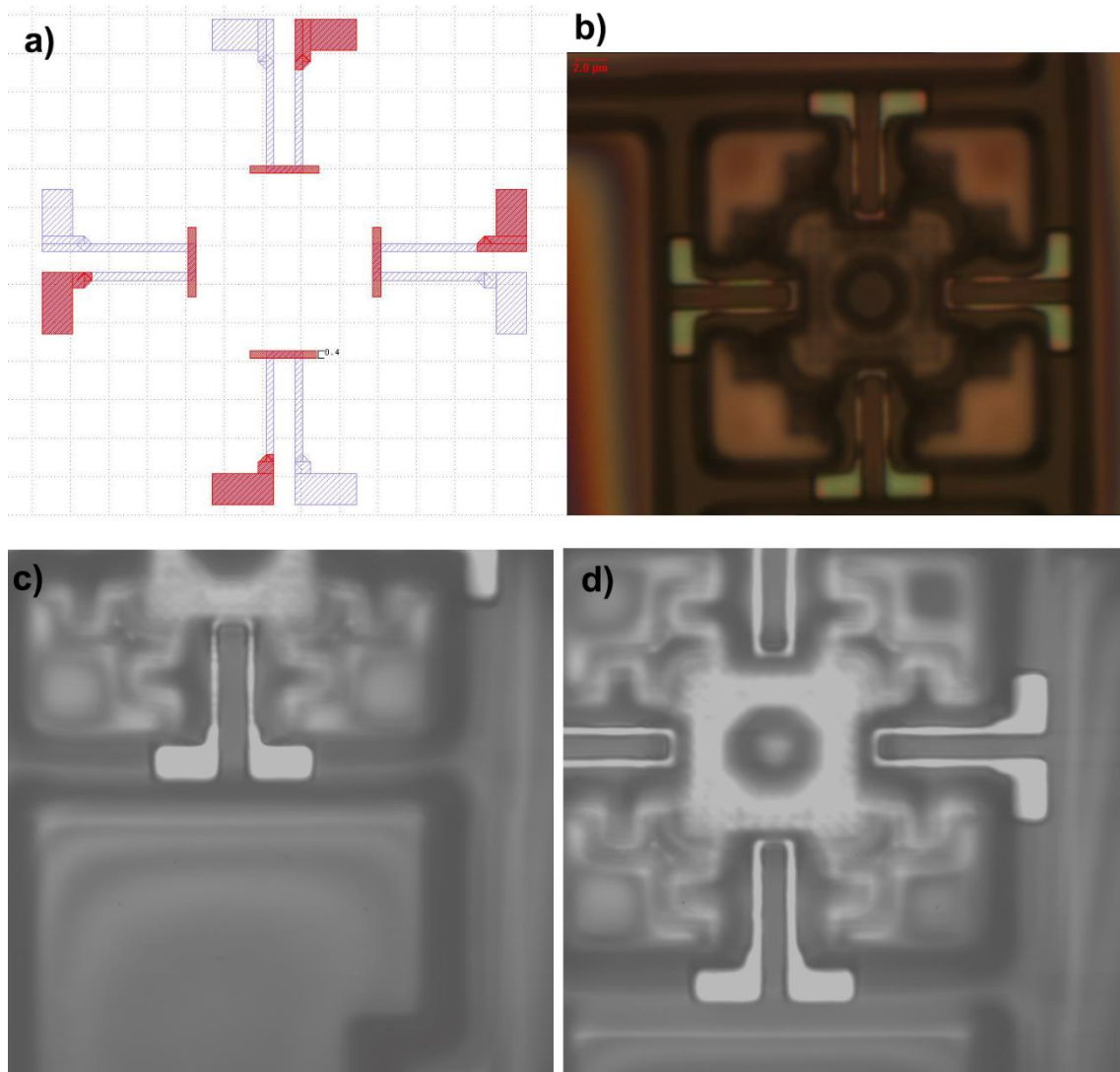


Top and cross-section views of the fabricated structures using modified DTI-1J for the first Silicon etch and DTI-2H for the second Silicon etch.

Results: We should increase process time to achieve an etch depth of 1.75 µm.

The following micrographs belong to ion implantation process.

Micrograph of Ion Implantation



Regions to be doped with Boron and micrographs of doped regions.

Blue regions are doped with Boron at a concentration of 10^{17}cm^{-3} and the dope concentration for red regions is 10^{20}cm^{-3} .