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Fabrication Development Of Micron Scale Retroreflector Assays Using Wet Etching

A Thesis

Presented to

the Faculty of the Department of Electrical and Computer Engineering

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in Materials Engineering

by

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August 2013

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Abstract

We are developing a new ultra-sensitive pathogen detection platform for rapid, point-of-care diagnostics. The platform uses micron-scale retroreflecting surfaces to allow for accurate and automated detection of virus and/or bacteria using an autonomous machine vision system. For this work, we have optimized the manufacturing processes for forming the retroreflecting patterns by switching to a negative tone resist and incorporating a wet etch in place of a liftoff step. We have eliminated low yield steps and have replaced them with a more repeatable fabrication sequence. The total number of processing steps have been reduced and repeatability has been improved, resulting in an overall 20 times increase in throughput. In parallel, we have increased the reflectivity of the structures by optimizing retroreflector pattern geometry as well as the reflective coating layer. Finally, we have also optimized a suspended retroreflector cube manufacturing approach that now integrates colored dyes for multiplexed readout.

Table of Contents

Abstract	vi
Table of Contents	vii
List of Figures	x
List of Tables	xiii
1 Introduction and background	1
1.1 Background	3
1.1.1 Planarized linear retroreflector platform	5
1.1.2 Corner cube retroreflectors as ultra-bright optical labels	7
1.2 Scope of the project	10
1.3 Literature review	12
2 Fabrication Tools	17
2.1 Evaporators	17
2.1.1 Thermal evaporator	17
2.1.2 Electron beam evaporation	21
2.2 Helium ion beam system	23
2.3 Reactive ion etching system	28
3 Membrane mask fabrication	31
3.1 Membrane formation	31
3.2 Membrane patterning-palladium	33
3.3 Membrane patterning-copper	36

3.4	Summary	38
4	Linear Retroreflector Liftoff Developement	41
4.1	Introduction	41
4.2	Patterning	43
4.3	Reflective layer coating and planarization	46
4.4	Summary	50
5	Wet etch technique development	52
5.1	Introduction	52
5.2	Polystyrene as a negative tone resist	53
5.3	Wet etching analysis	57
5.3.1	High purity copper deposition	60
5.4	Summary	62
6	Optimized linear retroreflector process	64
6.1	Introduction	64
6.2	Linear retroreflector wet etch process flow	64
6.3	Results and discussion	66
6.3.1	Retroreflector edge analysis	66
6.3.2	Retroreflector pattern geometry optimization	72
6.3.3	Chip layout optimization	73
6.4	Summary	74
7	Cube fabrication	76
7.1	Introduction	76
7.2	Cube manufacturing process	78
7.3	Results and discussion	79
7.3.1	Colored cubes	85

7.4	Chapter Summary	88
8	Summary and Future Work	90
8.1	Summary	90
8.2	Future Work	92
	Bibliography	94

List of Figures

1.1	Typical immunoassay steps used to detect the concentration of a target using labels (Diagram from ref. [4]).	2
1.2	Schematic for assay using linear retroreflectors embedded in transparent polymer underneath a microfluidic channel (Diagram from ref. [4]).	5
1.3	Linear retroreflector assay setup	6
1.4	Drag assay schematic, a platform for using retroreflecting cubes as a label (Diagram from ref. [4]).	9
1.5	Sample micro-bubble flotation assay readout comparing a positive sample to a control [8].	9
2.1	Thermal evaporation system schematic (Diagram from ref. [4]).	19
2.2	Plot of Cu thickness thermally evaporated on a 4 inch wafer and measured with ellipsometry (Plot from ref. [4]).	20
2.3	Custom chamber addition to e-beam evaporation system.	22
2.4	Basic proximity lithography schematic (Schematic from ref. [14]).	24
2.5	Normalized current density of the atom beam as a function of detector position, source voltage is 10 kV and source current is 1 mA (plot from ref. [14]).	25
2.6	Diagram of helium on beam system with image of the actual tool (Diagram from ref. [14]).	27
2.7	Reactive ion etcher schematic (Schematic from ref. [4]).	29
3.1	Membrane process flow using palladium (Diagram from ref. [14]).	34
3.2	Membrane process flow using copper (Diagram from ref.[15]).	36

3.3	(a) Image after PMMA developement (b) Reactive ion etching (c) Gold deposition (d) Mounted membrane on proximity lithography stage. . . .	38
3.4	200 nm lines and squares on membrane following reactive ion etching. .	39
4.1	The process flow for linear retroreflection fabrication using liftoff technique.	42
4.2	Two different ways PMGI etch rate variaiblity causes liftoff failure. . . .	44
4.3	(a) 140 nm copper hard mask (b) 180 nm angstroms copper hard mask .	45
4.4	(a) Retroreflector brightness post aluminum deposition. (b) Retroreflector brightness after planarization and gold deposition	47
4.5	(a) Projected light paths assuming air medium and (b) projected light paths accounting for SU-8 planarization layer.	47
4.6	(a) PMMA void formation (b) Profilometry over chip area.	49
4.7	(a) Linear rerotreflector wafer planarized with PMMA (b) Retroreflector viewed with camera.	50
5.1	Fabrication using polystyrene as photoresist.	56
5.2	Printed linear retroreflector pattern with polystyrene on silicon. Dose is $0.4 \mu\text{C} \cdot \text{cm}^{-2}$	57
5.3	Retroreflector pattern results following wet etch of copper.	58
5.4	Higher purity copper wet etch results	61
5.5	Optical microscope images of retroreflectors following wet etch of electron beam evaporated copper. (a) $50\times$ magnification and (b) $20\times$ magnification.	62
6.1	Fabrication using polystyrene as photoresist.	65
6.2	Linear retroreflectors fabricated with wet etch process.	67
6.3	Optical images of different copper hard mask thicknesses following reactive ion etching with a 30% over-etch time.	68

6.4	Retroreflector images of different copper thicknesses following aluminum deposition.	69
6.5	SEM images of side wall roughness of (a) 300 nm copper patterns and (b) 10 nm copper patterns.	70
6.6	Retroreflector patterns printed with test mask following (a) aluminum reflective coating and (b) PMMA planarization.	73
6.7	Fabrication using polystyrene as photoresist.	74
7.1	The process flow for linear retroreflection fabrication using liftoff technique.	80
7.2	Optical images of cubes following citric acid undercut.	81
7.3	SEM images of corner cube retroreflectors [4].	82
7.4	Optical images of cubes after (a) reflective layer coating and (b) lift off. .	83
7.5	Optical images of cubes after (a) silver reflective layer coating and (b) lift off.	84
7.6	Camera images of retroreflecting cubes suspended in solution.	85
7.7	Absorbance data for dyed SU-8.	86
7.8	Optical images of colored cubes following the first citric acid etch (a) red and (b) blue.	87
7.9	(a) Retroreflecting blue cubes in water. (b) Non dyed retroreflecting cubes with blue filter in water.	87

List of Tables

3.1	Etch rates in argon plasma.	34
3.2	Etch rates in tetrafluro methane and oxygen plasma.	34
3.3	Etch rates in argon plasma.	37
3.4	Etch rates in sulfur hexafluoride and oxygen plasma.	37

Chapter 1

Introduction and background

Using microfabrication processes to create high throughput and low-cost bioassays has been widely recognized as a new emerging field of research for the development of bio-analytical systems [1]. Processes originally intended for the microelectronic device industry have allowed for the creation of several new bioassay platforms such as microfluidic devices and shape-engineered particles. The goal of this work lies in the fabrication process development of a new ultra-sensitive integrated platform for the detection of biological agents, based on the technology of microfabricated retroreflectors.

Diagnostic tools are used to identify diseases at their onset when the chances of overcoming the disease are greatest. Immunoassays are an important diagnostic tool that utilize highly specific biochemical binding to quantify the concentration of an analyte. Common to many immunoassays are the use of a labeling technique, such as fluorescent molecules, enzyme-linked reactions, nanoparticles, and colored particles [2]. Each of these labeling techniques have been incorporated into biosensors and distinguish themselves with varying emphases on portability, sensitivity, selectivity, operational complexity, sample to answer time and cost [3]. A generic immunoassay utilizing anti-body binding is shown in Figure 1.1 [4]:

(a) antibodies that are specific to an antigen of interest are attached to the substrate.

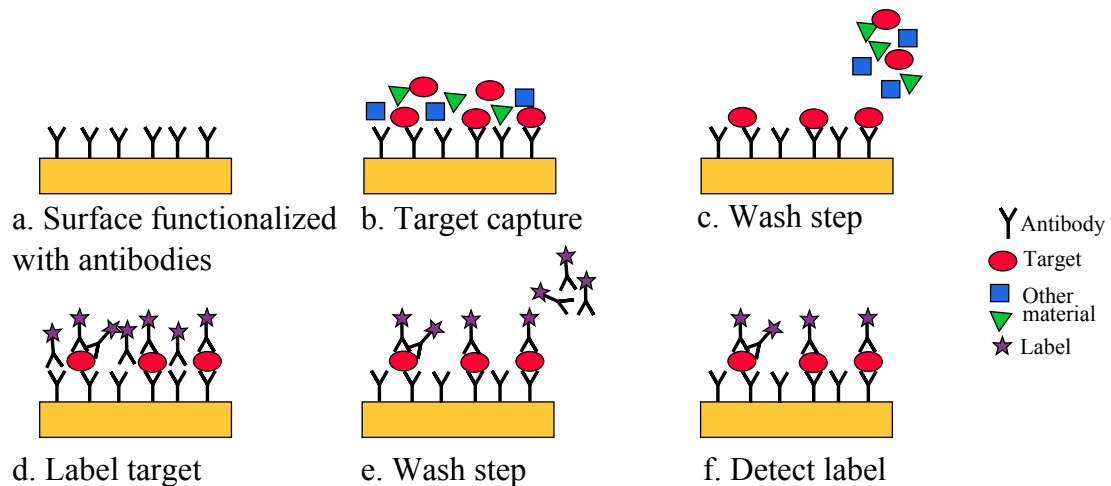


Figure 1.1: Typical immunoassay steps used to detect the concentration of a target using labels (Diagram from ref. [4]).

- (b) A sample containing the target pathogen is added to the surface. The target interacts with the surface through antibody-antigen binding and becomes specifically bound, while all other entities will remain free.
- (c) A rinsing step removes unbound material, and
- (d) antibody-coated labels are introduced to the surface, which specifically bind to the target pathogen and produce a signal.
- (e) Additional washing is performed to remove any labels that may not bound to the surface.
- (f) The signal is analyzed and concentration of the target is quantified.

One of the most important roles of an immunoassay is to detect the presence of a disease before it spreads throughout the body, making early detection an important factor for a favorable prognosis. However, during the early stages of a disease the concentration of the pathogen in the body is typically sparse, requiring assays to have ultra-high sensitivity for early detection. One of the many hurdles to achieve ultra high sensitivity in immunoassays is being able to detect an analyte

with a very small surface area relative to sample volume ratio. Pathogens, such as viruses, have traditionally been hard to detect due to their relatively small size (on the order of 100 nm in size) [5]. An immunoassay with the ability to pre concentrate the labeled target in a small volume is thus well suited for high sensitivity detection. The Naval Research Laboratory has developed a biosensing system for which multiplexed, un-amplified, femtomolar detection of both DNA and proteins in complex matrices (including whole blood, serum, plasma, and milk) have been achieved in minutes using as few as two reagents in a sandwich assay approach [3].

In this setup, analytes are captured onto a microarray surface coated with antibodies and are labeled with magnetic microbeads, a controlled laminar flow is then used to apply microfluidic forces sufficient to remove only nonspecifically bound beads. The density of beads that remain bound is proportional to the analyte concentration and can be determined with either optical counting or magnetoelectronic detection of the magnetic labels using giant magnetoresistive sensors. This assay combines easy to use force fluid discrimination techniques with the high sensitivity of microfluidic-chip based binding assays allowing for the possibility of a simple, potentially handheld, platform capable of both nucleic acid hybridization assays and immunoassays, including orthogonal detection and identification of bacterial and viral pathogens, and therefore suitable for a wide range of biosensing applications [3]. With the rising demand for onsite portable, easy to use high sensitivity diagnostics, a new platform for point-of-care diagnostics is explored.

1.1 Background

A retroreflector is an object that returns incident light back to its source over a broad range of incoming angles, making them extremely detectable using simple,

low-cost optics [4]. Retroreflectors have most commonly been used for signaling the presence of an object or person in the form of reflective sheets for use in vehicle reflectors, road signs, and safety outfits [6]. Recent work has also shown that retroreflectors can be used for providing a bright optical signal that can be used to indicate the presence of a pathogen. When compared to other immunoassay labels, retroreflecting objects can be fabricated to be similar in size and much brighter than dyed particles, can be readily imaged using lower cost optics than fluorescent particles, do not suffer from photobleaching, and can easily incorporate magnetic films and other materials in the particles themselves for various applications. Although techniques for direct monitoring of antibody and antigen binding events have become available in recent years (e.g., surface plasmon resonance, SPR, or related techniques), most quantitative assays rely on the indirect approaches based on labels for generating a clear signal [2].

A new class of labels has been prototyped that utilize retroreflecting surfaces for the direct detection of a target analyte based on the changes in light propagation properties of the sample. When compared to other labels, retroreflecting objects can be fabricated to be similar in size and much brighter than dyed particles, and can be readily imaged using low-cost optics. Using a low numerical aperture lense restricts the range of light acceptance angles of the camera to only light reflected back from the retroreflectors themselves, providing for a low noise high contrast image readout. Using microfabrication technology, the size of retroreflectors can also be made to be on the order of the diffraction limited resolution of the optics used to image them. Recently, Tim Sherlock at the University of Houston has prototyped two novel biosensing platforms for pathogen detection utilizing retroreflectors as a labeling technique [4].

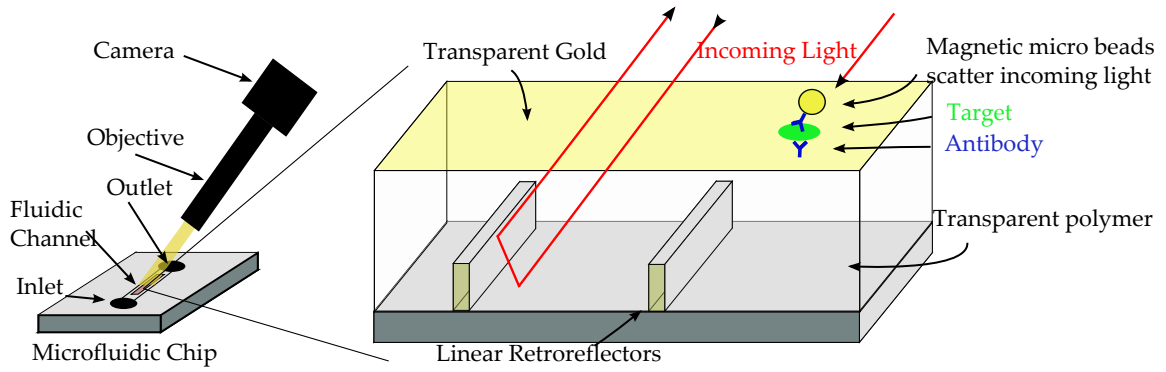


Figure 1.2: Schematic for assay using linear retroreflectors embedded in transparent polymer underneath a microfluidic channel (Diagram from ref. [4]).

1.1.1 Planarized linear retroreflector platform

In the first approach, a microfluidic chip is used for large area imaging with a simple CCD camera. Shown in Figure 1.2, linear retroreflecting surfaces are fabricated in an array of long lines and embedded in a layer of transparent polymer with a thin layer of gold on top. The microchip, when viewed at an angle, results in large bright areas at the location of the retroreflecting surfaces that are surrounded by a dark background where the light is reflecting away from the camera. The silicon chip from which they are fabricated is then incorporated into a microfluidic setup, in which the chip will lie just below a fluidic channel. The gold coated surface above the retroreflectors can then be used for biosensing using a sandwich assay approach with biotinylated antibodies and avidin conjugates. Figure 1.3 gives an outline of how the proposed assay works.

First, magnetic beads coated with a specific antibody is added to a sample containing the pathogen of interest. The sample is then incubated and a magnet is used to concentrate the beads in a small volume of buffer. The microfluidic chip containing retroreflecting surfaces is spotted with Abs and then sealed in a microfluidic cartridge containing micro channels. The sample containing target pathogen is then deposited into the micro channels and are allowed to settle. A controlled

laminar flow is then used for force fluid discrimination to rinse the surface of non-bound beads. Using a CCD camera to image the assay, the pathogen attached beads bounded to the retroreflecting surfaces will scatter incoming light and result in a signal change. If the target pathogen is not present in the sample, no beads will bind to the surface and the retroreflecting signal will be unaffected, resulting in a negative test. This type of labeling technique also allows for the possibility of multiplexing. The use of different colored beads enables the simultaneous detection of many other analytes in the same sample.

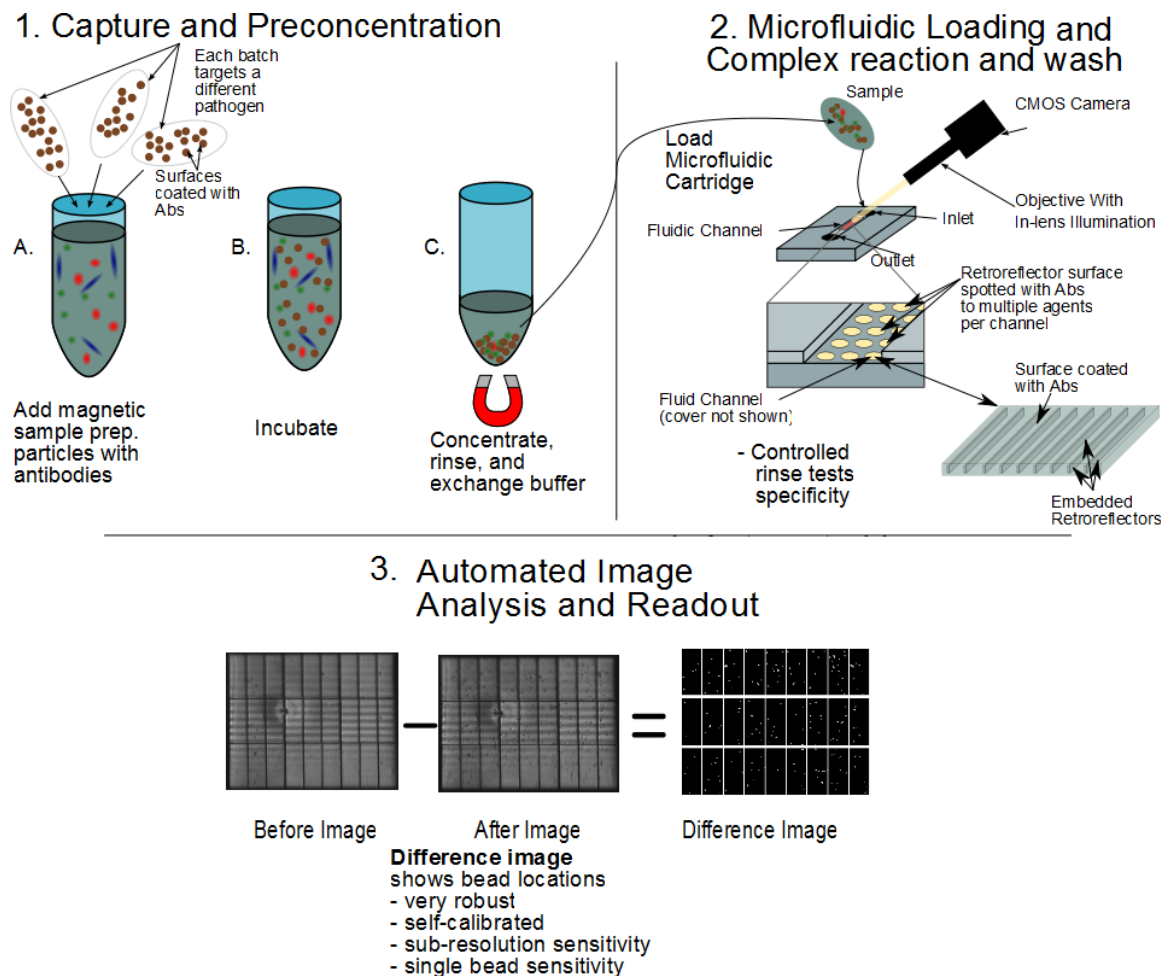


Figure 1.3: Linear retroreflector assay setup

The purpose of this assay is to be able to compare the differences in the image of the assay before and after exposure to the specimen of interest. One of its main advantages is the ability to automate the image read out. Most computer programs for shape recognition are based on locating and measuring selected patterns which are then compared with corresponding measurements of known shape features [7]. A similar approach can be used for automated alignment and focus of the retroreflecting areas for image capture. Since the shape of the retroreflector pattern is already known and are printed at predetermined locations, a computer program is able to locate retroreflector patterns on the chip and automatically align the image, as well as find a best focus. Following alignment and focus, a complex difference imaging algorithm has been developed to analyze the change in retroreflecting signal before and after exposure. The difference image can then be used to determine the number of beads present on the surface of the assay. To count the number of beads, the difference image is thresholded to a black and white image. Automated scripts can then be used to identify continuous regions of white pixels and calculate their pixel area. By then dividing the pixel area by the number of pixels a single bead occupies, the number of beads in each retroreflecting region can be determined. Figure 1.3 is an example of a difference image output. Dark regions of the after image that were previously not shown in the before image reveals the presence of beads scattering light and results in the lack of reflected light detection by the camera in those regions.

1.1.2 Corner cube retroreflectors as ultra-bright optical labels

A second platform has been prototyped using suspended micron-scale retroreflecting corner cubes. These transparent cubes have three reflective, mutually perpendicular surfaces, and much like linear retroreflectors, return light directly to its source over a broad range of angles. Functionalized magnetic beads are used to

bind to the cubes and are then differentiated from other non-bounded cubes with a magnet. Figure 1.4 shows an example platform for using retroreflecting cubes as a method of detection:

- (a) Cube retroreflectors and magnetic sample preparation particles, both coated with antibodies to a target analyte, are added to a sample for analyte detection.
- (b) The target analyte, if present, is captured by antibodies on the cubes surfaces and binds the cubes to the magnetic particles. A magnetic field is then applied that draws only the cubes bounded by magnetic particles to the surface of the sample container.
- (c) If the target analyte is present, the magnetically bound cubes are readily detected using inexpensive optics to image the surface of the container. Cubes that are not bound to magnetic particles settle to the bottom of the sample and are not able to be imaged by the camera.

Currently this size of cubes for this assay are 5 μm in size, this size has been chosen as a compromise between binding efficiencies between both the 2.8 μm sized beads and the target pathogen. Currently this assay has only been used to detect large bacteria and proteins. Previous work would suggest that this method for detection is transferrable to a smaller scale by using smaller particles for binding. Jwa-Min Nam at the Institute for Nanotechnology of Northwestern University has reported successfully bridging gold nanoparticles to 1 μm magnetic particles with DNA and using magnetic separation for bar-code DNA readout [5].

An alternative assay setup for cube discrimination has been prototyped using glass micro-bubbles. Glass micro-bubbles have been used in a variety of applications, including isolation of biomolecules, cell separation, and immuno- and DNA-based assays. In this assay, 3M Glass Bubbles iM30K are used as floatation devices for dragging bounded cubes to the sample surface for direct pathogen detection.

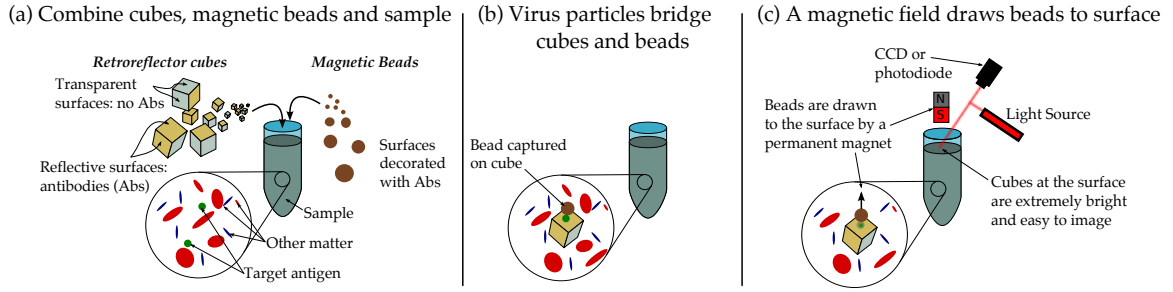


Figure 1.4: Drag assay schematic, a platform for using retroreflecting cubes as a label (Diagram from ref. [4]).

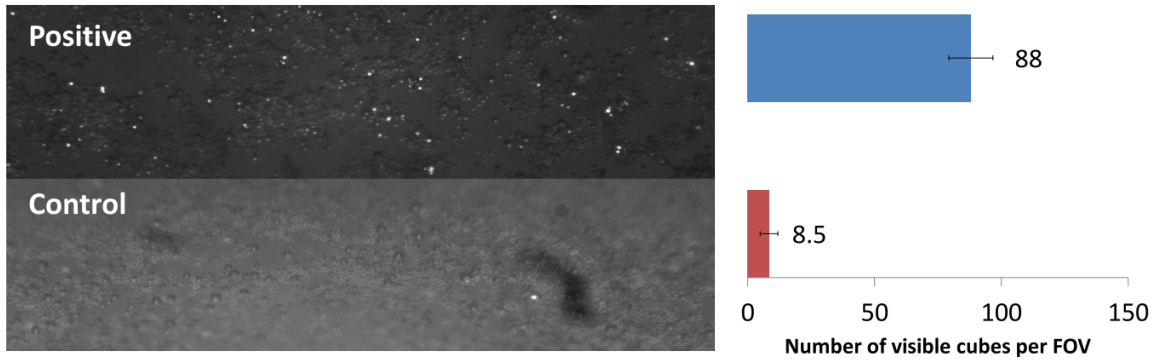


Figure 1.5: Sample micro-bubble flotation assay readout comparing a positive sample to a control [8].

Well established silanization chemistry is used for functionalizing the cubes and silica micro-bubbles [8]. The sample containing a pathogen is added to a tube containing functionalized cubes and micro-bubbles, the tube is then mixed and held stationary. Analytes sandwiched between the cubes and glass micro-bubbles are lifted to the top of the tube where signal generated by retroreflecting cubes are easily detected.

Figure 1.5 shows results of micro-bubbles and cubes functionalized with *Escherichia coli* antibodies in the presence of *E. coli*. Images are taken using a CMOS USB camera at $4\times$ magnification, each represents a $1.4 \times 0.4 \text{ mm}^2$ sample area. Error bars represent a ± 1 standard deviation from three different regions imaged at the top of the assay tube. The details of the antibody binding strength to cubes and micro-bubbles are not fully understood. These details are important for char-

acterizing an assay as particular attention needs to be placed on the bond strength. This is not easy to do as biomolecules come in a range of sizes and each require perhaps a different bonding interaction to efficiently bind to cubes. The requirements on antibody-antigen interaction will need to be studied further as the assay is developed.

1.2 Scope of the project

To move these assays further along in development, many more were needed to be manufactured for testing. In parallel, we attempted to improve assay performance with modifications to the manufacturing process based on feedback from assay results. The original process for manufacturing retroreflectors was sufficient for initial prototyping and testing. However, some steps in this process suffered from reliability issues while taken an absorbent amount of time to complete, resulting in a low overall yield of assay production. Thus development of a new process to mass fabricate these assays in a reasonable amount of time was needed. We aimed to create a new process that utilizes the same fabrication techniques used to mass produce microchips. The fabrication requirements for both linear retroreflectors and cubes are similar therefore only one general process was needed to be developed with the second only needing slight modifications but will remain very similar to the first.

Previously, a bi-layer resist liftoff technique was used for forming micron scale patterns across a large four inch silicon wafer. There are three key components to patterning phase of this process; the resist layer for lithographic patterning (poly(methyl methacrylate) (PMMA)), the undercut layer for greater contrast (poly(dimethyl glutarimide) (PMGI)), and the metal hard mask layer for reactive ion etching and pattern transfer (copper). First the PMMA layer is patterned using a

proximity helium ion beam system. The underlying PMGI layer is then isotropically wet etched for greater pattern contrast. Next a layer of copper is evaporated, followed by copper liftoff by dissolving PMMA with acetone. This is the step where the highest amount of chip failure occurs during the fabrication process. If the PMGI layer is wet etched too deep and the PMMA extends too far above the undercut, the PMMA can collapse and form a continuous copper bridge once copper is evaporated. This results in acetone being unable to dissolve the PMMA and the subsequent liftoff is unsuccessful. If the PMGI is under etched, the copper deposition step can deposit on the side walls of the features and leave no areas for the acetone to dissolve the PMMA.

The liftoff step is highly sensitive to substrate thicknesses and PMMA undercut, to achieve successful liftoff requires each layer to be consistently processed with little deviation from the norm. To do this in a university laboratory has proved challenging as there are no means for fine temperature and humidity control or industry standard equipment for reliable fabrication. It takes roughly 10 hours of fabrication time to create a wafer of 4 chips using Tim Sherlock's process and equipment available at the University of Houston. Approximately 1 in 6 chips were useable for testing. Reducing this production time and increasing the yield was imperative to the testing and optimization of this assay. In order to fabricate these assays with high reliability and throughput, a manufacturing process with a large room for deviations was created. Equipment previously used to fabricate retroreflectors was upgraded or replaced to improve further improve throughput as well.

Chapter 2 will describe the equipment used and any modifications or replacements done throughout the project. A proximity lithography tool was used for large scale patterning and requires the fabrication of thin silicon nitride membranes masks for exposing a pattern into the resist. Flexibility in changing pattern

parameters such as pitch, density, and feature size were critical to the development of retroreflectors. Fabricating a new membrane mask was required as the original linear retroreflector pattern geometry needed to be optimized to reduce blind regions and increase contrast in the assay. However, membrane mask fabrication has proved challenging, the original developed process had little room for error and many masks failed during processing. Chapter 3 will discuss a new process for reliably fabricating membrane masks. In Chapter 4 we attempted to improve the original fabrication method for producing retroreflector assays by identifying the lowest yield steps and modifying them for greater reliability. We also discuss how to improve retroreflector performance through changes in assay materials and geometry.

Chapter 5 presents findings on using polystyrene as a negative tone resist for lithographic patterning instead of PMMA. A new process utilizing a wet etch for manufacturing micron scale features on a silicon wafer is discussed. In chapter 6 we attempted to form linear retroreflector patterns using this new method and transitioned to full assay production. In Chapter 7 fabricating corner cube retroreflectors is discussed. The wet etch process was modified by adding a sacrificial copper layer to the substrate for releasing cubes into solution. Colored cubes are also explored for the possibility of multiplexing as well as different metal reflective layers. Finally, in chapter 8 we report our overall results and discuss future efforts for further developing retroreflector based assays into a complete point of care diagnostic platform.

1.3 Literature review

Microfabricated devices for biological application have existed for over 30 years, with several applications attaining commercial and/or scientific success. Although

there have been a few applications to biology or medicine during that time, only in recent years have a closer union emerged. Commercially, high-throughput, low-volume-consumption technologies such as whole-genome sequencing projects and drug discovery have created a need for these devices. Scientifically, the ability to design and control experiments at the micrometer scale has attracted the interest of the medical field, which have started devising fundamental studies using this technology. When applied in the right instances, microfabrication can either significantly enhance a device in relation to its conventional counterpart or enable entirely new devices. The purpose of this section is to describe immunoassay technology in detail and report on past findings of other microfabricated devices.

Rongsheng E. Wang and affiliates have reported femtomolar level detection of platelet-derived growth factor B-chain homodimer (PDGF-BB) using an Enzyme-linked immunosorbent assay (ELISA) with aptamers instead of antibodies [9]. In this assay two aptamers that bind to PDGF-BB were conjugated to the surface of a green fluorescent ferritin nanoparticle and the bottom of wells respectively for a sandwich ELISA. Higher sensitivity than antibody-based ELISA was achieved due to the multivalency effect of aptamer conjugated-nanoparticles. This technology has also reported high sensitivity towards other various small analytes such as metal ions, atp, adp and cocaine and is easily multiplexed with the use of different color dyes. However, only in-vitro testing has reported promising results and very few of these biosensors are commercially available due to operational complexity and portability issues. For commercial applications, it is essential to design easy-to-use, accurate, and cost-effective sensors or kits. For example, many extremely rapid (<5 min) and simple lateral flow immunoassay kits are available from a number of sources for quick application, but these kits perform poorly for pathogen detection and are not easily multiplexed [3]. There are also many different silver staining gels readily available for use and can detect as little as nano

grams of analyte but are generally restricted to larger analytes such as proteins and nucleic acids.

Because of the small dimension, most of the applications of microbiotechnology in molecular diagnostics fall under the broad category of biochips and micro arrays. The main goal of many of these microchip based assays are to bring otherwise labor-intensive tests that are restricted to professional laboratory environments, to less restricted settings for convenient access. Current methods for molecular-based diagnosis of disease rely heavily on modern molecular biology techniques for interrogating the genome for aberrant DNA sequences. However, to study DNA sequences in length often required the use of the time-consuming nature of slab gel electrophoresis. Recently, a multichannel microchip containing alternative electrophoretic formats has been developed in the form of capillary electrophoresis for obtaining diagnostic information in just under 3 minutes [10]. They have demonstrated separation of B- and T-cell gene rearrangement PCR products on microchips for providing critical diagnostic information in certain types of diseases with no loss of diagnostic capacity when compared with current methodologies for lab based tests. This type of technology has given rise to many "Lab-on-a-chip" assays for which portable low cost devices are now beginning to be used in remote settings, as a result of developments in integrating fluid actuation, sample pre-treatment, sample separation, signal amplification, and signal detection into a single device. As another example, an immunoassay developed by McDevitt and colleagues to measure levels of C-reactive protein in saliva has been incorporated into a micro chip with a 1000-fold improvement in sensitivity over ELISA in microtiter plates by using a microfluidic chip for more favorable transportation properties [11]. This property of microchips has also been transferred into other microfluidic chip setups for the detection of other pathogens.

The biomarker C-reactive protein (CRP) is an acute phase reactant and a well-accepted indicator of inflammation. Numerous clinical studies have established elevated levels of CRP as a strong indicator of cardiovascular disease diagnosis. A sandwich-type immunoassay on a microfluidic chip termed "electronic taste chip"(ETC) has been developed that can analyze the level of CRP in serum or saliva samples [12]. In this work, a polyclonal rabbit CRP-specific antibody coupled to an agarose microbead sequesters CRP within and around the bead. A detecting antibody is then used to visualize the bead-captured analyte. In this case, a fluorescent-based detection with an Alexafluor-488-conjugated antibody was used for imaging. Signals generated on the beads are visualized and captured by a CCD camera positioned above the array. CRP measurement using ETC is tested against other conventional methods for CRP detection using same serum and saliva samples. A clinically validated hsCRP ELISA kit from ALPCO (Windham, NH) was used for comparison. The CRP standards provided in the kit were also tested against standards used in the ETC method, and a correction factor derived from that comparison was applied in the validation studies of the ETC method. Results showed that the ETC performed better than conventional CRP ELISA tests. The standard ELISA method suffers from slow transport and ineffective rinsing of reagents and analytes occur due to the poor transport properties to the planar surface of the ELISA plate. However, one of the benefits of microfluidic chip based assays are that they are not diffusion limited in transport properties. Within the ETC lab-on-a-chip system, the integrated microfluidic structures are used to actively transport reagents to the microporous bead elements which also assist in creating a long effective path length for antibody interaction, allowing for greater capture efficiencies and thus CRP can be detected at extremely low concentrations. These assays have proven to be effective in detection of a single specific pathogen,

but lack the flexibility to be able to detect a range of pathogens. In this work, an assay platform with the ability to detect multiple pathogens is developed.

Chapter 2

Fabrication Tools

The following is a list of tools used to contribute to the development of retroreflectors. In the beginning of the project, fewer tools were available and it later became clear that new systems were needed to increase yield and reduce process times. Some of these tools are no longer used and have been completely replaced but still played a pivotal role in testing and developing retroreflectors.

2.1 Evaporators

When this project first entered the development phase, long after the first generations of retroreflectors showed promise, only a custom built thermal evaporation system was available as a means to deposit metals. The system was very useful in the prototyping stage of retroreflectors but the process time was great (>1.5 hours) and it became apparent that in order to increase production throughput of retroreflectors a system that can deposit metals more quickly was needed.

2.1.1 Thermal evaporator

This is a custom thermal evaporation system built by a previous graduate student and a more detailed description is given in ref. [4]. Thermal evaporation is a common choice in thin film deposition applications where parameters such as mechanical stress are important and can be well controlled. High deposition rates of metals can be achieved without the use of a plasma. In certain applications thermal evaporation is preferred because it has a poor ability for step coverage of materials

deposited on the substrate and can be used as an advantage in lift-off processes. This thermal evaporation system uses a single rotary vane mechanical pump to both rough the main vacuum chamber as well as back the high vacuum diffusion pump. Electrically controlled pneumatic valves are used to control the foreline, high vacuum, and rough vacuum valves. In thermal evaporation, current is sent through a filament carrying the target material until the filament becomes heated enough to evaporate the target. As such, two sets of electrical feed throughs are located in the middle of the chamber floor for placement of two evaporation sources. The substrate holder is connected to a rotary feed through to allow for angle evaporations on the substrate. A moveable shutter is positioned below the substrate holder to allow control for stop and start of deposition. A quartz crystal oscillator is positioned below the shutter so the deposition rate can be measured while the shutter is closed. Finally, to measure the pressure the system uses two thermocouple gauges and a single ion gauge to measure lower pressures. Figure 2.1 shows an outline of the system.

This setup allows for sequential evaporations of two different sources in one pump-down cycle. Being able to evaporate two different metals at once can be critical if the first metal does not act favorably in atmospheric conditions. For example, titanium has very different adhesion qualities than titanium oxide which forms when titanium is exposed to atmosphere. Another advantage to this system is the ability to measure deposition rate without depositing on the substrate. This is particularly useful in thermal evaporation systems where often times a filament is contaminated with other materials that evaporate as well during the heating process. Before evaporating a material, it is recommended to "outgas" the system. This is typically done by heating the filament to the point where the target material begins to evaporate. The system is then left in this state allowing time for unwanted impurities to outgas from the filament until the system returns to its base pres-

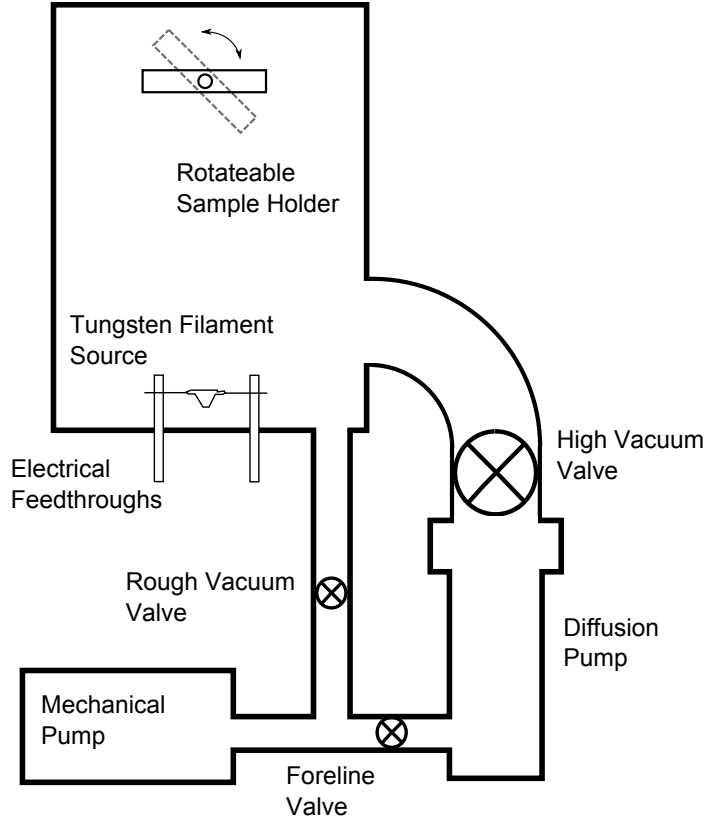


Figure 2.1: Thermal evaporation system schematic (Diagram from ref. [4]).

sure. The type of filament used is dependent on what metal is being evaporated. For this project we are interested in the deposition of Cu, Ti, Al, Au, Cr, Pd, and Ag. As such, two different types of filaments were used as recommended by a filament manufacturer (R.D. Mathis, Long Beach, CA). The first is a tungsten wire basket that is used for evaporating materials such as aluminum and titanium. The second is an alumina coated tungsten wire basket. The alumina coated tungsten basket is used for metals that do not sublime such as Cu and Au. Uniformity of film deposition is dependent on angle of incidence which is dependent on substrate size and distance from source. Modeling evaporation rate from a disc-shaped source gives a $\cos(\theta)^4$ distribution described by,

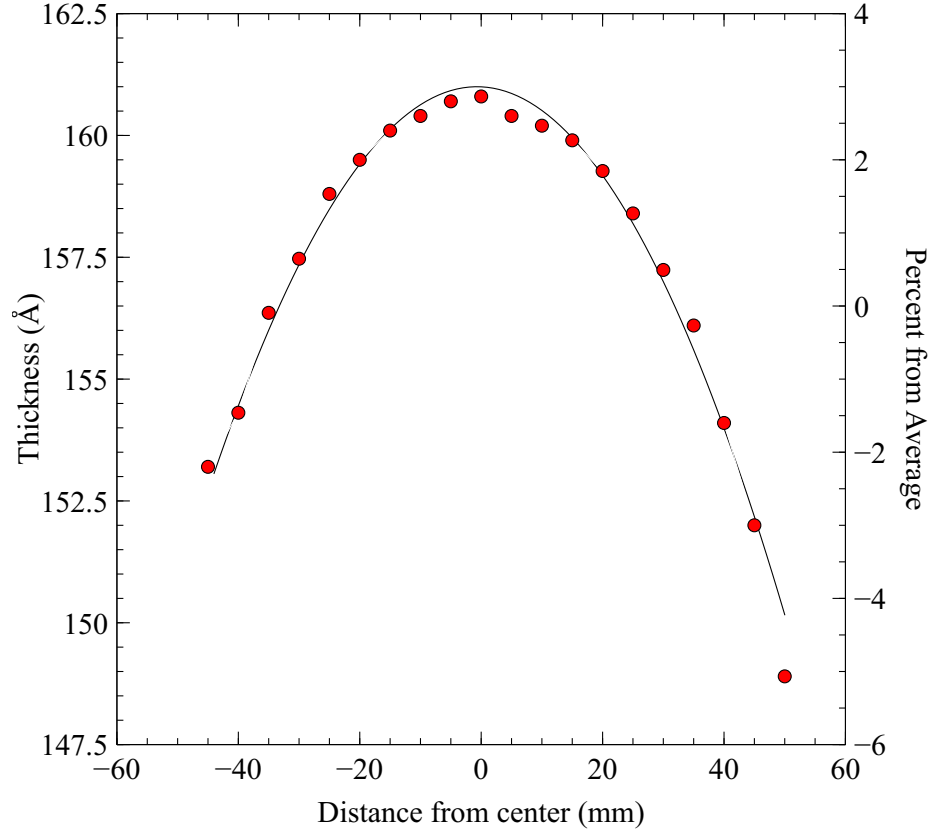


Figure 2.2: Plot of Cu thickness thermally evaporated on a 4 inch wafer and measured with ellipsometry (Plot from ref. [4]).

$$R_s = \frac{Q \cdot \cos^4 \theta}{\pi r^2}, \quad (2.1)$$

where R_s is the deposition rate determined by the flux perpendicular to the substrate at point s , Q is the total evaporation rate into the hemisphere from a disc-shaped source, r is the distance between the substrate and source, and θ is the angle of incidence from the source onto the substrate at any point s from the center of the substrate [13]. The substrate size used in this project is a 4 inch diameter silicon wafer, a film uniformity plot over 4 inch is shown in Figure 2.2 and indeed shows a $\cos^4 \theta$ distribution.

Figure 2.2 shows that the maximum difference in thickness from the edge of the wafer to the center is approximately 4% and is well within the requirements of this project. When the system was first built, a base pressure of 3×10^{-6} Torr was recorded, but the system has since then become significantly less clean due to material deposition on the walls of the chamber. Pump down time has increased significantly to well beyond an hour and only a base pressure of 2×10^{-5} Torr is achieved. Adhesion problems also started to arise from the decline in film purity so a new method for metal deposition is needed to increase throughput.

2.1.2 Electron beam evaporation

An electron beam evaporation system located at the University of Houston nanofabrication facility has been made available for use. Electron beam evaporation is a process similar to thermal evaporation, i.e., a source material is heated above its boiling/sublimation temperature and subsequently deposited onto a surface wherever the evaporated atoms strike. A noticeable advantage of e-beam evaporation over thermal evaporation is the possibility to add a larger amount of energy into the source material. This yields a higher density film with an increased adhesion to the substrate [13]. Because the electron beam only heats the source material and not the entire crucible, a lower degree of contamination from the crucible will be present than in the case of thermal evaporation.

Most of this system was purchased from a vacuum systems company (Thermionics Vacuum Products, Hayward, CA) with the main pump supplied by the University. There are many advantages to this system over the thermal evaporation system that allows for higher throughput and yield. Using a cryogenic pump, the base pressure of this system is approximately 1×10^{-7} Torr. Evaporation processes for this project were carried out at approximately 7×10^{-6} Torr using this system with a pump down time of approximately 10 minutes, which is a huge improve-

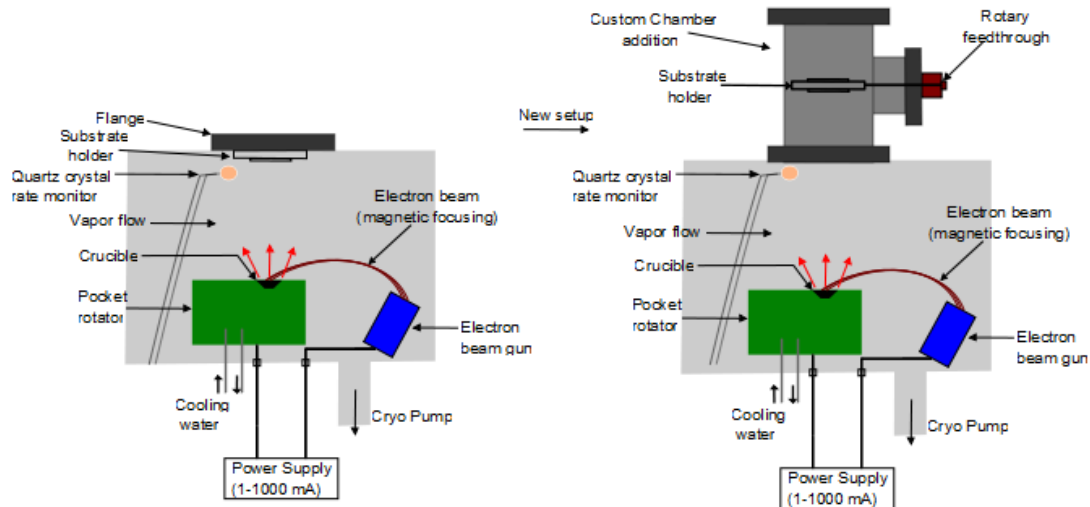


Figure 2.3: Custom chamber addition to e-beam evaporation system.

ment upon the hour or more pump down time of the thermal evaporation system to achieve 2×10^{-5} Torr. A rotary pocket controller is used to store up to 8 different metals for evaporation, allowing for multiple evaporations of different metals in one pump cycle. A glassy-coated graphite crucible is used for evaporation as recommended by the manufacturer. This system also has the advantage of being stored in a dust free clean room with humidity and temperature control, allowing for less variability of process parameters. Film uniformity of electron beam evaporation roughly follows the same relationship as thermal evaporation described in Section 2.1.1.

However, this system was unable to carry out angle evaporations so a modified chamber was custom made. A tee flange mated with a rotary feed through was placed on top of the current chamber, a sample holder with a front and back side was connected to the end of the rotary feed through. This setup allows for tilting of the sample for angle evaporations as well as the ability to deposit on two different samples in one pump cycle. By placing a second sample on the backside of the substrate holder and rotating the sample holder 180° , evaporation on to a second sample in the same run is made possible.

Changing the position of the substrate holder results in a change in deposition rate and uniformity. Equation 2.1 shows that an increased distance from source to substrate will result in decreased deposition rate as well as increased uniformity. Recalibration of the system is thus needed to compensate for this change, specifically the change to deposition rate must be characterized. To do this, a water color marker was used to draw a line across the center of a 4 inch wafer. Copper was then evaporated onto the wafer and then set in an acetone bath. Water color marked areas then “lifts off” the copper by being dissolved by acetone. A profilometer (Tencor, Alpha step-200) was then used to measure the change in step height across the wafer. The factory given resolution of the profilometer is + or - 10 nm. In this particular experiment 1 μm of copper was chosen for deposition as to give a nice thick copper layer for easy step height measurements and also to remain well within the resolution capabilities of the profilometer. An approximate value of 420 nm was measured for actual deposition on to the substrate, giving a deposition rate loss factor of 58% relative to the original sample holder position.

2.2 Helium ion beam system

A custom in house proximity helium ion beam lithography system is used for substrate patterning. A brief description is provided in this section, a more extensive description of design and characteristics is published in [14]. Proximity lithography is chosen over more common types of lithography such as optical lithography due to diffraction resolution limits set by wavelengths typically used in optical lithography. Techniques, such as electron beam lithography, do not suffer from diffraction limits due to the short wavelength of electrons and can print with nanoscale precision. However electron beam lithography suffers from low throughput. To print a dense array on the nanoscale with electron beam lithog-

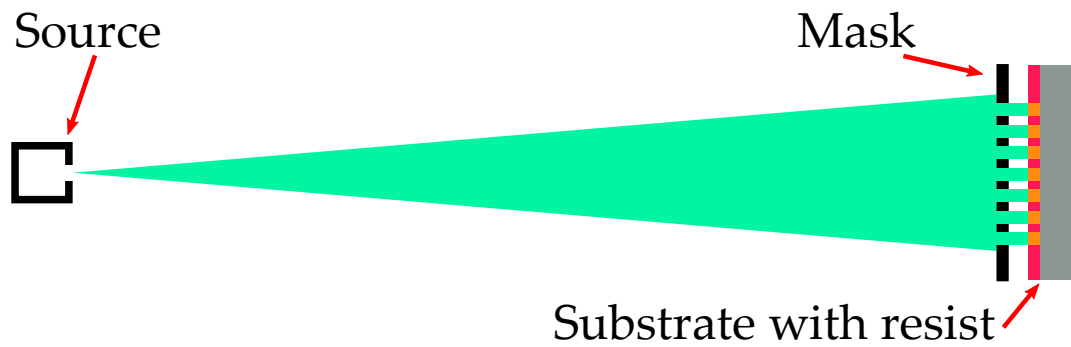


Figure 2.4: Basic proximity lithography schematic (Schematic from ref. [14]).

raphy can take several hours just to expose 1 mm^2 of pattern area making it not suitable for mass production. This system is capable of patterning 1 cm^2 areas in seconds. Thus proximity lithography is used as a compromise for high throughput while maintaining high resolution and Figure 2.4 illustrates how a typical proximity lithography tool works.

In this system, a collimated beam of energetic helium ions is used to irradiate a mask placed in close proximity to a substrate coated with ion sensitive resist. The ions that pass through the mask penetrate the resist producing secondary electrons along its path. The secondary electrons cause chemical reactions in the ion sensitive resist at the exposed areas. The resulting solubility change of exposed regions will classify the resist as positive or negative tone. For positive tone resists, the secondary electrons cause an increase in solubility of the exposed regions. The mechanism for this solubility change are chain scission events caused by the scattering secondary electrons that decrease the molecular weight of the resist polymer in exposed regions [14]. For negative tone resists, secondary electrons cause cross-linking events that increase the molecular weight of the resist resulting in decreased solubility of exposed regions. The changes in solubility of the exposed

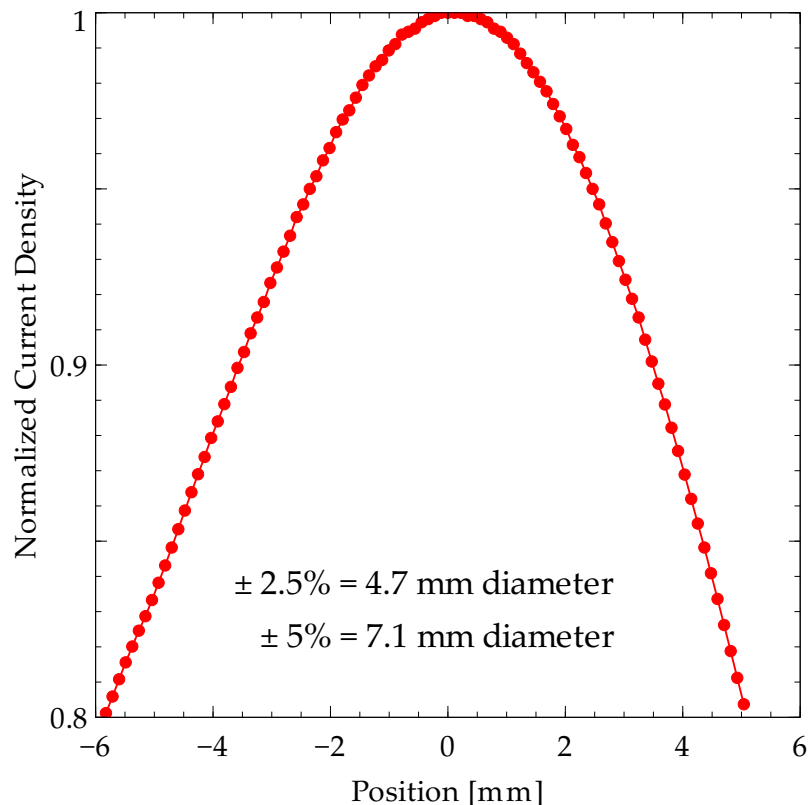


Figure 2.5: Normalized current density of the atom beam as a function of detector position, source voltage is 10 kV and source current is 1 mA (plot from ref. [14]).

regions allow for the use of a solvent to remove either the patterned or unpatterned areas depending on the tone of the resist.

This tool uses a saddle field ion source capable of patterning high resolution nanoscale features [14]. The source size ranges from 0.3-0.9 nm, depending on source voltage and current, with an average brightness value of $15 \text{ nA/cm}^2 \cdot \text{sr}$. A turbo pump backed by a rotary vein mechanical pump is used for creating a vacuum. Flushing the system with helium during pump down is recommended in order to keep the system as oxygen free as possible. The source is made of aluminum and is susceptible to oxidation. Oxidation of the anode causes the source to become unstable and prone to arcing. This instability requires polishing of the source to remove any thin oxide layers. For this reason, a valve has been installed

at the beamline in order to constantly keep the beamline under vacuum even while the chamber is vented. A piezo $x - y$ stage (SmarAct GmbH, Oldenburg, Germany) with 1 nm positioning resolution and $100 \times 120 \text{ mm}^2$ of travel is used for moving the substrate for high spatial fidelity patterning. An ion-secondary electron detector is mounted to the $x - y$ stage in order to measure the beam current. The detector reads current density as the inlet to the detector is 1 mm in diameter, a simple current divided by area calculation will give the beam current and current times area divided by time will give dose. The stage is controlled by interfacing with a custom LabView scripting program, this allows for automatic current measurements and sample movement for mass lithographic patterning. A razor blade attached to a pneumatic linear feedthrough in front of the source allows blanking of the beam during long stage moves. After the energetic helium ion beam is formed at the source, the emitted beam upon leaving the source contains a large fraction of neutralized atoms as ions leaving the source are neutralized by charge-exchange collisions. For this reason, the beam column contains a set of electrostatic deflector plates that are used to sweep the energetic ions out of the way leaving only a neutral atom beam. The deflector plates are necessary for high resolution patterning because the ions and atoms have slightly different trajectories due to the affects of the earth's magnetic field on ions.

Figure 2.5 shows the energy of the beam as a function of position. For many resist layers, such as PMMA and polystyrene, the exposure latitude for printing nanoscale features is high enough that the beam can pattern a 1 cm^2 area in a single exposure. Sources of error for high resolution printing with this tool are ions scattering at the mask, ion scattering in the resist, penumbral blur, and, to a lesser extent, diffraction. Diffraction is negligible for micron-scale features, as the wavelength of helium ions is much lower than 1 nm. Using thin stencil masks and thin resist layers, ion scattering events are minimized and blur becomes the greatest

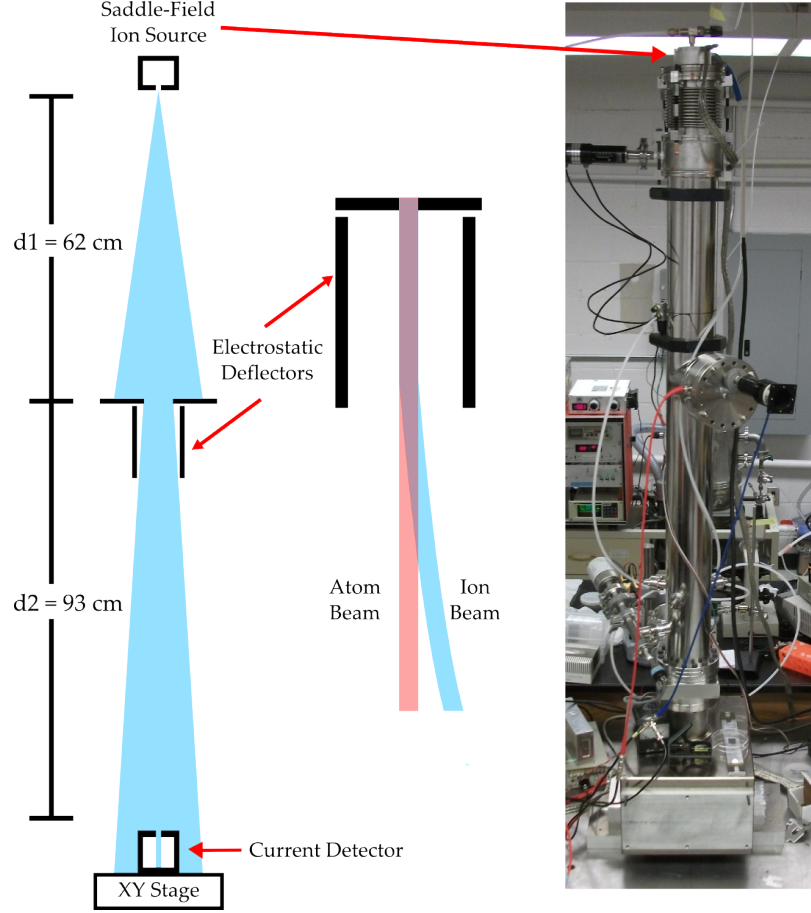


Figure 2.6: Diagram of helium on beam system with image of the actual tool (Diagram from ref. [14]).

resolution limiting factor. Penumbral blur refers to the shadow cast from the mask onto the resist layer, this is because the source is not infinitely small. Regions of the beam will transmit through the mask openings at an angle and cause nearby regions to become exposed as well. The magnitude of the region of partial exposure is a function of source size, distance from the source to the mask (beamline), and distance from the mask to the substrate (gap). The relationship is described by

$$R_{Blur} = \frac{G \cdot FWHM_S}{L}, \quad (2.2)$$

where G is the gap, $FWHM_S$ is the full width half maximum of the source, and L is the length of the beamline. The source size is a fixed parameter and is a charac-

teristic of the type of ion source being used. Greater resolution can be achieved by increasing the beamline length, however the current density of the ion beam drops with the square of distance, so throughput becomes less efficient with longer beamlines. The parameter easiest to modify for decreasing blur size is the gap, which is set by placing precision ground stainless steel shims under the mask holder to raise the membrane a precise distance above the substrate. A laser position sensor (Keyence, LK-081) with 3 μm detection resolution is used to verify the gap distance. The beamline is approximately 1.3 meters in length, the FWHM_S is 250 μm , and the gap typically used is approximately 180 μm , which gives a blur of 45 nm. The minimum features size that can be printed is typically twice the blur, which in this case is 90 nm. For manufacturing features on the submicron scale, this is an acceptable resolution limit, gaps less than 100 μm are difficult to set due to the risk of damaging the fragile membrane mask. This system facilitates high throughput with nanometer scale resolution by using masks fabricated with electron beam lithography as described in Chapter 3.

2.3 Reactive ion etching system

Lithographically patterned resist are normally too thin for applications that require features to be thicker than hundreds of nanometers, thus etching is commonly used in fabrication processes for transferring a thin lithographically patterned resist layer into a thicker more robust layer. In this project, reactive ion etching is used for transferring the ion beam patterned PMMA layer into SU-8. In general, reactive ion etching is a chemically enhanced etching technique that selectively removes a material deposited on a wafer through a chemical reaction. This technology uses a chemically reactive plasma to react with the substrate forming a gaseous product. One of the major advantages of this process is the ability to

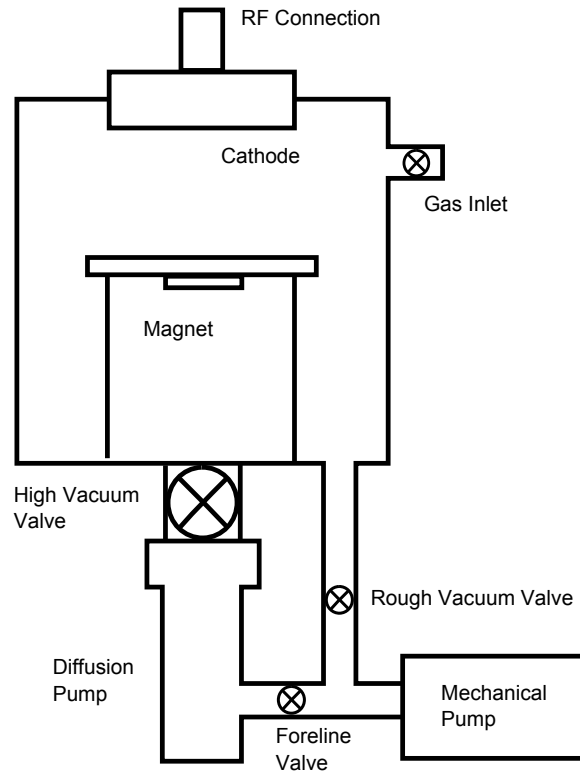


Figure 2.7: Reactive ion etcher schematic (Schematic from ref. [4]).

have high etch rates that are normal to the substrate. A magnetic field is created at the substrate by a magnet positioned below the anode. The magnetic field increases the pathing distance of electrons through the plasma, resulting in more energetic collisions and formation of ions. This modification increases ion density, enhancing the etch rates at lower pressures, and increasing the number of normal incident ions. Smooth sidewalls prove to be critical to the reflectivity of retro reflecting surfaces. Plasma parameters for forming smooth sidewalls as well as maintaining high throughput is described in Chapter 4.

A custom built reactive ion etching system is shown in Figure 2.7 is used in this project for the selective removal of the SU-8 resist layer. Similar to the vacuum system setup of the thermal evaporator, this system uses a diffusion pump backed by a basic rotary vein mechanical pump and electronically controlled pneumatic valves. However, unlike the thermal evaporation tool, this system requires a

sustainable plasma. For this, a gas inlet manifold capable of adding two different gasses is connected to the chamber. An ion gauge is used to measure the base pressure and a Baratron gauge for measuring the process pressure. A 5 inch electrode is driven with a 13.56 MHz RF signal 4 inches above the grounded anode plate. A magnet is positioned below the anode and creates a 50 Gauss magnetic field at the substrate. The base pressure of this system is measured to be 5×10^{-7} Torr.

One of the primary uses of the etching system for this project is in etching SU-8 along a 4 inch diameter silicon wafer. Thus, it is important to test the uniformity by measuring the etch rate at the center (where the highest density of ions strike) to the edge. For etching small area substrates (radius <1 inch) the difference in rates between the center and edge is assumed to be negligible. For a 4 inch wafer, a quick plasma etch and profilometry across the diameter of the wafer revealed the etch rate at the edge to be approximately 86% of the etch rate at the center. Etch times need to be adjusted to compensate for this etch non uniformity. This will require hard masks used to be thick enough to compensate for an approximate 16% over etch time the center of the wafer receives relative to the edge. Other tools used for fabrication were

- Elipsometer Model LSE (Gaetner Scientific Corporation, Skokie, Illinois),
- CL-1000 Ultraviolet Crosslinker (Ultra Violet Products, Upland, CA) 254nm shortwave UV,
- Spincoater Model 02551 (Laurell Technology Corp., North Wales, PA),
- Tencor Alpha 200,
- Electron Beam Writer Model JBX 5500FS (JEOL Ltd., Tokyo, Japan), and
- Laser Position sensor (Keyence LK-081).

Chapter 3

Membrane mask fabrication

Flexibility in changing pattern parameters, such as pitch, density, and feature size, are critical to the development of retroreflectors. Due to the limitations of electron beam lithography, proximity lithography is used alternatively for patterning large areas. The major disadvantage of this type of lithography is the necessity of a membrane mask for forming patterns, and testing new pattern parameters requires the fabrication of a new membrane mask each time. Membranes are delicate and difficult to fabricate and the current process for forming membranes has little room for error and suffers from low yield. Thus, efforts have been made for a more robust process for reliably forming patterned membranes. This work was a collaboration with Prithvi Basu and further details can be found in his thesis work [15].

3.1 Membrane formation

The mask fabrication process consists of two main stages. Formation of the membrane and deposition the necessary layers for patterning. The membranes are formed from a double-side polished four inch wafer coated with a 600 nm thick layer of non-stoichiometric, low stress silicon nitride. The first step is to etch multiple openings equal to the desired membrane size into the silicon nitride on one side of the wafer. To do this, a transparency film is cut and used as an etch mask during reactive ion etching. Since the helium ion beam is uniform over a 1 inch

diameter, membrane windows are fabricated to be 1 cm wide squares for both uniformity and mechanical stability.

The process parameters for the etch are 0.2 mTorr of O_2 and 0.8 mTorr of CF_4 with a power setting of 30 watts at 2×10^{-5} Torr. The exposed silicon surfaces are then wet etched using a solution of KOH. The solution is prepared by dissolving 1 kg of KOH pellets with 2 liters of water at 80°C. A magnetic stir bar at 400 rpm is used to evenly disperse the dissolved KOH to the solution, once the KOH is completely dissolved the wafer with the etch silicon nitride windows is placed in the solution. The KOH will etch away silicon while leaving the silicon nitride unaffected. The etch takes about 7 hours to remove the silicon, followed by another 1 hour of over etch time.

The wafer is then quickly transferred to an already heated 80°C deionized water bath with the aforementioned stir bar for 20 minutes. This is a critical step in order to form clean membranes. KOH at room temperature will become solid and result in salt deposits on the thin silicon nitride membranes. Membranes not properly washed will result in nonuniform spin coating and likely subsequent failure during patterning steps. The wafer is then placed in deionized water at room temperature to wash off any remaining residue and to transition the wafer back to room temperature. The wafer is then rinsed with isopropanol and dried by carefully blowing nitrogen parallel to the surface. The next step is to deposit the layers required for patterning. Two methods have been used for forming membrane patterns, the original method used palladium as a mask during reactive ion etching with a CF_4 and O_2 plasma. The second method improves on the first and uses copper as a mask with a SF_6 and O_2 plasma for etching.

3.2 Membrane patterning-palladium

After forming membranes, the patterning stage begins. For the first method a 20 nm layer of palladium is deposited onto the front side of the wafer using thermal evaporation. Next, a 100 nm thick layer of PMMA is spincoated into the front side of the wafer. The wafer is then baked at 180°C for one hour as before, for driving out the solvent from the PMMA. The membranes are then carefully cleaved into separate pieces using a diamond tipped cutter and then printed on using electron beam lithography. The PMMA is then developed in a 3:1 solution IPA and MIBK. An argon plasma at 1 mTorr and 13 watts is used to remove the palladium using the PMMA layer as an etch mask. This step is highly variable and can change depending on the contamination of the chamber. PMMA is sensitive to oxygen and will immediately etch away when exposed to oxygen plasma. Water vapor or contamination from previous processes can easily introduce oxygen into the argon plasma and result in a large portion of the PMMA layer being removed. Thus, the chamber needs to be conditioned to help minimize contamination. An argon plasma at 5 mTorr and 30 watts for 30 minutes will remove much of the residue in the chamber from previous runs as well as water vapor introduced from the presence of atmosphere.

Once the pattern has been driven into the palladium layer, the nitride layer is etched with the same conditions the windows were etched during membrane formation, but with a power of 10 watts instead of 30 watts to increase the selectivity. This is the step with the highest chance of failure. By the time the 500 nm thick silicon nitride membrane is etched all the palladium hard mask is etched away as well, leaving little room for error. Table 3.1 and 3.2 shows the relevant etch rates of each layer in the presence of both plasmas.

The obvious fix is to increase the palladium layer thickness in order to have a thicker palladium hard mask during the silicon nitride etch. However, the pal-

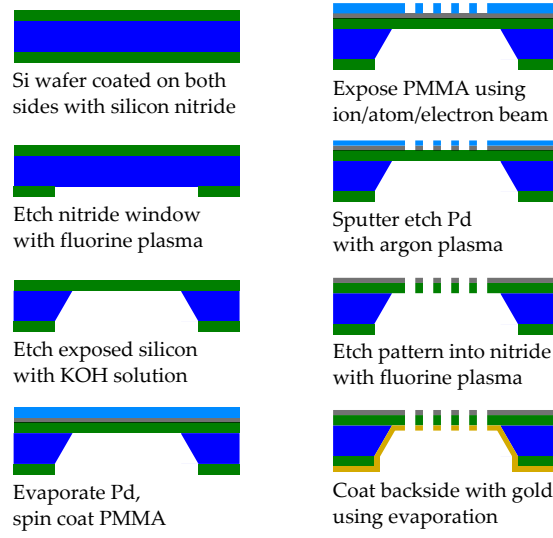


Figure 3.1: Membrane process flow using palladium (Diagram from ref. [14]).

Table 3.1: Etch rates in argon plasma.

Argon plasma (15 mminutes)	Initial thickness (nm)	Rate (nm/min)	Final thickness (nm)
Palladium	20	2	0
PMMA	100	6	10

Table 3.2: Etch rates in tetrafluro methane and oxygen plasma.

CF ₄ and O ₂ plasma (50 minutes)	Initial thickness (nm)	Rate (nm/min)	Final thickness (nm)
Silicon Nitride	500	11	0
Palladium	20	0.4	0

ladium layer is restricted to 20 nm as a maximum allowable thickness due to the limitations of lithography tools used. An ideal membrane mask for the proximity tool would be of 1 cm² area, however the electron beam lithography tool at hand was unable to pattern such a large area at once. In order to make a membrane with a large enough area suitable for mass production with proximity lithography, the electron beam tool is used to pattern a small 1 mm² area and is then copied several times onto another membrane using the proximity lithography tool, resulting in a copy that is a larger area membrane mask.

The restriction to the palladium layer of 20 nm is due to the fact that increasing the palladium layer would also require the PMMA layer to be thicker. While increasing the PMMA layer thickness would not be a problem for the electron beam lithography tool, the proximity lithography tool used for copying the pattern onto a large area has a maximum penetration depth of approximately 100 nm. Therefore, the PMMA is restricted to 100 nm, allowing only for 20 nm of palladium to be etched in the time frame that the PMMA can withstand the argon plasma without failure. If the silicon nitride etch is successful, then the backside is coated with 100 nm of Au.

The Au layer serves two purposes. the first is to prevent charging of the mask by serving as a conductive layer, and the second is to act as the actual hard mask for the patterned membrane during sample exposures. This process is highly unreliable and many masks fail during the silicon nitride etch. Due to the restriction of the PMMA layer to 100 nm the only alternative is to explore other metals for more favorable sensitivities during exposure to both plasmas or to explore different plasma parameters for etching. A new recipe for fabricating membrane masks have been developed in the next section.

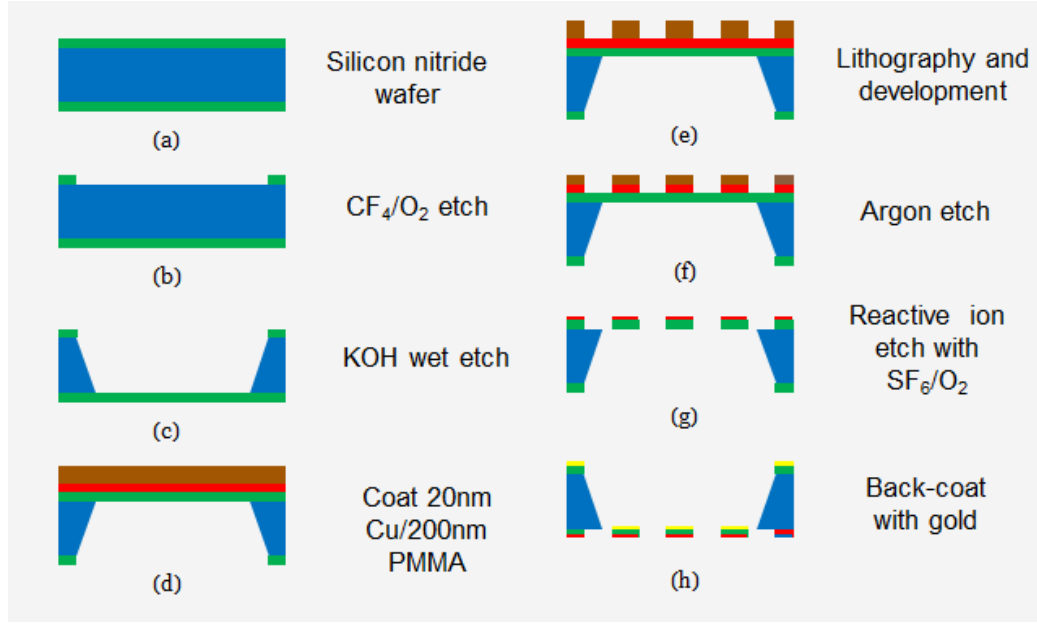


Figure 3.2: Membrane process flow using copper (Diagram from ref.[15]).

3.3 Membrane patterning-copper

A new e-beam writer tool has been made available with enough travel and throughput to pattern a whole 1 cm^2 membrane. Thus, the restrictions of the PMMA layer thickness in the proximity lithography tool is no longer a problem as no copies are required for full membrane fabrication. Membrane formation for this process is the same as described in Section 3.1. Although the ability to increase the PMMA thickness allows for more palladium deposition for the previous patterning process, the selectivity between palladium and silicon nitride remains unfavorable. Copper was explored as a replacement to palladium and sulfur hexafluoride with oxygen was used for etching. In this process, a 20 nm layer of Cu is electron-beam evaporated onto the front side of the membrane followed by a 200 nm layer of PMMA to create a nice robust layer during the argon etching step. The new electron beam writer is then used for patterning the whole membrane at once, followed by the same argon etch previously described.

Table 3.3: Etch rates in argon plasma.

Argon plasma (15 minutes)	Initial thickness (nm)	Rate (nm/min)	Final thickness (nm)
Copper	20	4	0
PMMA	200	6	110

Table 3.4: Etch rates in sulfur hexafluoride and oxygen plasma.

SF ₆ and O ₂ plasma (30 minutes)	Initial thickness (nm)	Rate (nm/min)	Final thickness (nm)
Silicon Nitride	500	27	0
Copper	20	0.13	16

A sulfur hexafluoride with oxygen plasma is then used to drive the pattern into the silicon nitride layer. The parameters used for this etch is 0.8 mTorr SF₆ and 0.2 mTorr O₂ at 15 watts for 60 minutes with a base pressure of 2×10^{-5} Torr. Table 3.3 shows the measured etch rates using copper and SF₆ plasma. The selectivity for copper in both plasmas have improved significantly. The etch rate of copper in argon is twice that of palladium. More importantly, the selectivity of copper in SF₆ plasma is much greater than that of palladium in CF₄. In the previous process the selectivity during the silicon nitride etch was 27.5. The copper and SF₆ plasma has improved the selectivity to approximately 207, nearly an 8 times increase in the selectivity.

The original cube mask was damaged and a replacement was needed, a 5 μ m square mask is made using the new process. Figure 3.3 shows optical images of the membrane patterning steps. (a) PMMA is exposed with electron beam lithography and developed in MIBK and IPA. (b) Pattern is driven into the silicon nitride layer by argon and SF₆ plasma. (c) Gold is deposited on the back side and inspected. (d) A mounted membrane on the proximity lithography stage that is ready for use.

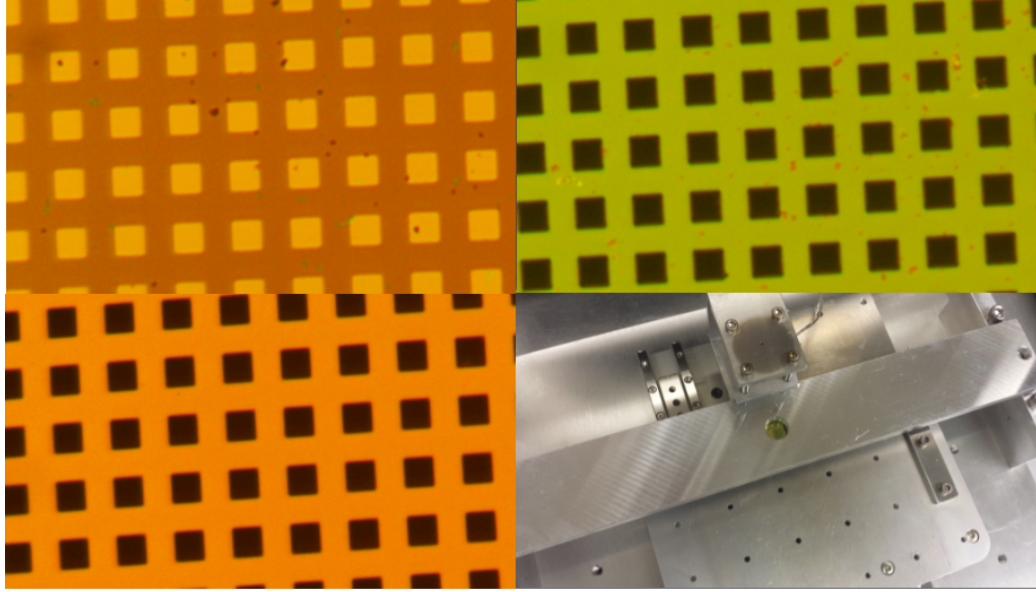


Figure 3.3: (a) Image after PMMA development (b) Reactive ion etching (c) Gold deposition (d) Mounted membrane on proximity lithography stage.

3.4 Summary

A SF_6 and O_2 plasma for pattern transferring into a thin silicon nitride membrane using copper as a hard mask has been developed for thin membrane mask fabrication. Mask fabrication using the previous CF_4 and O_2 plasma with palladium as a hard mask has shown reliability issues and substrate parameters are limited by the low selectivity etch between palladium and silicon nitride. The first argon plasma etching step has been improved by increasing the pmma layer to 200 nm and using copper as a hard mask instead of palladium. Over etch time has been increased during this step due to the fact that copper sputters twice as fast in argon than palladium. A thicker PMMA layer also ensures that the initial oxygen burst during argon plasma will leave a thick enough pmma layer behind to resist the full argon etch and over etch time.

Transferring the pattern into the silicon nitride membrane layer has been improved by using copper and SF_6 plasma instead of palladium and CF_4 plasma. SF_6

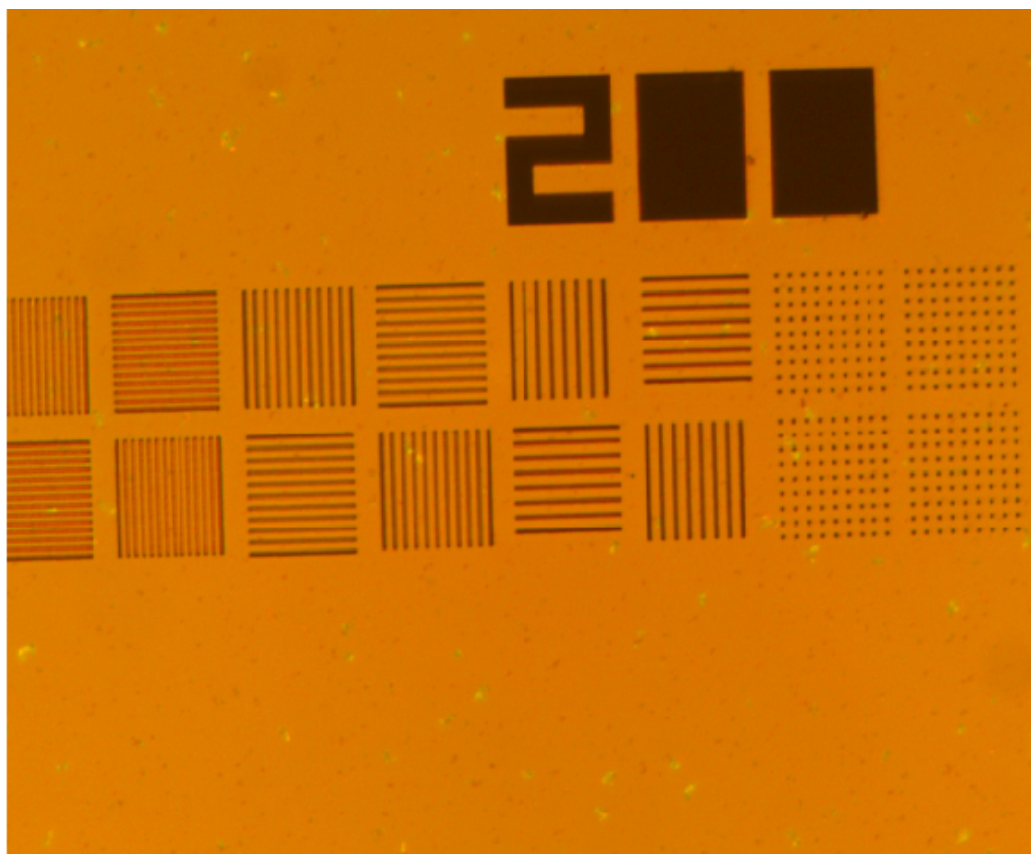


Figure 3.4: 200 nm lines and squares on membrane following reactive ion etching.

plasma etches silicon nitride approximately two and a half times faster than CF_4 while removing the copper hard mask at a third the rate the CF_4 plasma removes palladium. However, images have shown the silicon nitride layer etch rate to be variable across the membrane in the presence of SF_6 plasma. Silicon nitride etch rates on a silicon wafer would suggest that only 30 minutes of etching is needed to clear the membrane, however in practice this number is more closely to 70 minutes.

Future efforts should be made for printing small sub-micron features using this process. The inclusion of the new JBX 5500-FS electron beam writer for mask patterning has allowed for the possibility of printing features as small as 10 nm. However, etching 10 nm features into a 500 nm thick silicon nitride membrane will

prove difficult due to the large aspect ratio. Currently 200 nm mask features have been fabricated with the new copper and SF₆ plasma process^{3.4}. To print smaller features, a thinner membrane should be explored as an option as well as further refining the current SF₆ plasma process parameters. The more favorable etch rates of SF₆ plasma with copper has made a more flexible and robust process for readily manufacturing new membrane masks for proximity lithography.

Chapter 4

Linear Retroreflector Liftoff Developement

4.1 Introduction

The following is the initial process flow for manufacturing linear retroreflectors originally prototyped by Tim Sherlock. First, a 5 μm layer of SU-8 5 photoresist (Microchem, Inc., Newton, MA) is spin-coated at 2000 rpm for 1 minute onto a silicon wafer, followed by a 90°C bake for 3 minutes. The sample is then exposed to a 254 nm wavelength UV light source (UVP CL-1000, Upton, CA) for 3 minutes and then baked at 95°C for 3 minutes to cure the epoxy-based resist. A 300 nm layer PMGI (poly(methyl glutarimide)) is then deposited by spin coating at 2000 rpm for 2 minutes and then annealed at 180°C for 5 minutes. Next, a 70 nm layer of PMMA (poly(methyl methacrylate)) resist is spin coated and annealed at 180°C for 1 hour. The rotational speed for this step varies from batch to batch and is dependent on PMMA-to-solvent ratio. PMMA is mixed 1:4.5 by volume with chlorobenzene and an approximate speed of 3000 rpm is used to obtain 70 nm thickness.

The pattern is printed using an in-house helium ion beam lithography system described in Section 2.2. After patterning, the resist is developed in a 3:1 solution of isopropanol (IPA) and methylisobutylketone (MIBK) for 30 seconds, rinsed in IPA for an additional 30 and then dried with nitrogen. The PMGI is then isotropically etched in a 2.3% solution of tetramethyl ammonium hydroxide (TMAH) for 20 seconds as to undercut the PMMA layer. A 160 nm layer of copper is thermally evaporated for shielding patterns during reactive ion etching. Next, the PMMA is dissolved in an acetone bath and results in the lift off of copper in all areas

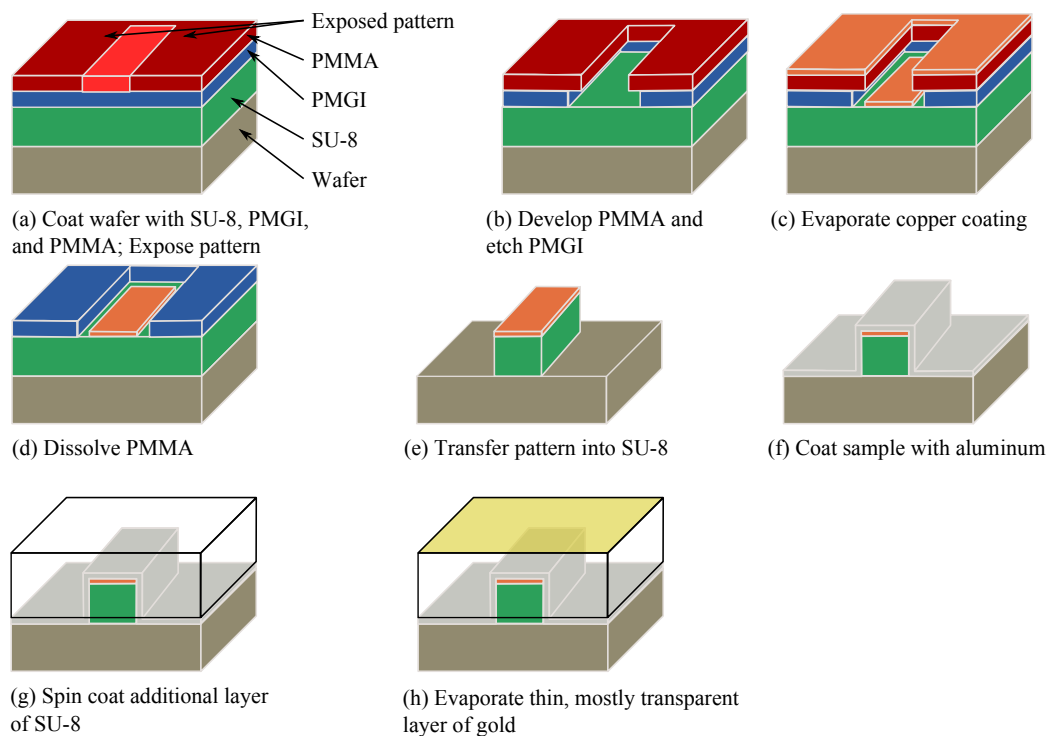


Figure 4.1: The process flow for linear retroreflection fabrication using liftoff technique.

that are not patterned. Afterwards, the sample is placed in the reactive ion etching system for transferring the pattern into the SU-8. The process parameters of the etch is 0.1 mTorr CF_4 and 0.9 mTorr O_2 at 50 watts for 1 hour. Next, a 150 nm layer of aluminum is deposited at a 45° angle in the thermal evaporator to form the reflecting surfaces. An additional layer of SU-8 is deposited using the same spin-coat settings mentioned before in order to planarize the surface. Finally, a 10 nm thin transparent layer of gold is thermally evaporated on the sample as the functionalization surface. The chips at this stage are now ready to be cleaved and assembled into the microfluidic device for use.

The aforementioned process flow has been sufficient for initial prototyping and testing. However, some steps in this process suffer from reliability issues resulting in a low overall yield of the assay production. Therefore, each stage of retroreflector fabrication will be analyzed for possible improvements of yield, throughput,

and performance. There are three main stages to retroreflector fabrication: the substrate preparation, patterning, and the reflective layer coating and planarization. The substrate preparation step is dependent the results of the patterning stage, thus patterning is looked at first for improvements.

4.2 Patterning

The first step of patterning is to expose the PMMA layer using the helium ion beam proximity tool. To ensure that the PMMA is given an optimal exposure, a dose series is printed on the side of each wafer for inspection throughout processing. The copper liftoff step in Figure 4.1(d) is the step in which the highest amount of chip failure occurs during the fabrication process. Resist side walls with a large undercut are required for lift-off applications. The liftoff technique used has three key components; the resist layer for lithographic patterning (PMMA), the undercut layer for greater contrast (PMGI), and the metal hard mask layer for reactive ion etching (copper). Previous research has shown that the greatest amount of failure occurs during this step, therefore each layer is analyzed for inconsistencies in fabrication. Experiments have shown that the PMGI etch rates vary greatly with the atmospheric weather conditions during processing. During hot/humid weather, etch rates as high as 100 nm/sec have been recorded, while cold, dry weather has given etch rates as low as 15 nm/sec. Understanding the PMGI etch rate is the most critical step of for ensuring successful liftoff. Figure 4.2 outlines how both PMGI over and under etch can result in liftoff failure.

In the first demonstration shown in Figure 4.2(a), the PMGI is too heavily wet etched and undercuts the PMMA too far. However, in the second demonstration (b), the PMMA collapses during copper deposition and forms a continuous layer between features. This results in acetone being unable to dissolve the PMMA and

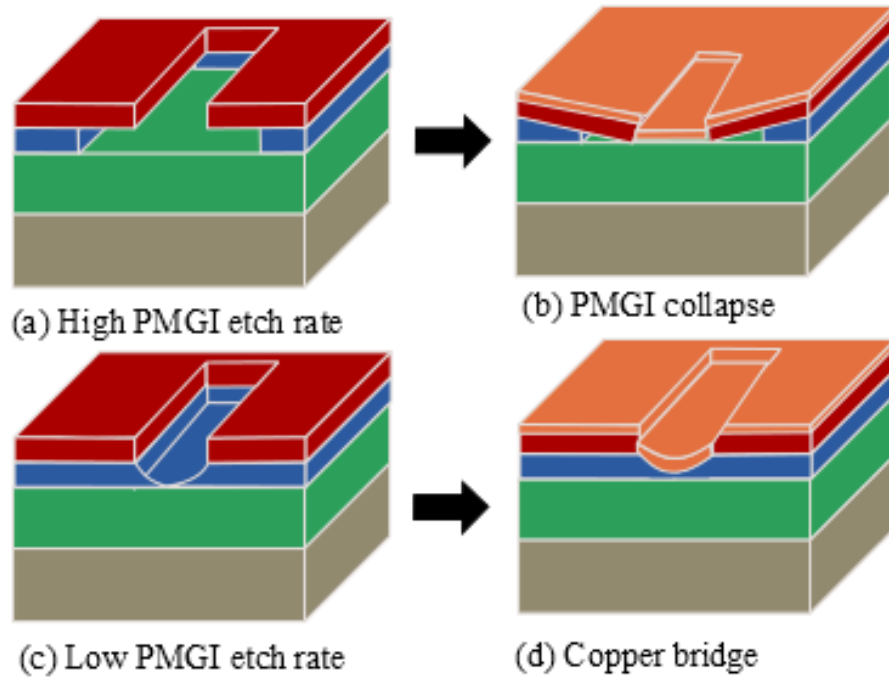


Figure 4.2: Two different ways PMGI etch rate variability causes liftoff failure.

the subsequent liftoff is unsuccessful. For the second case (c), the PMGI is under etched, (d) a continuous bridge is formed leaving no areas for the acetone to dissolve the PMMA. The liftoff step is also sensitive to the amount of copper that can be deposited without liftoff failure. Copper thickness is an integral part of the fabrication as the thickness of the copper plays an important role in how smooth the features are after withstanding the hour-long reactive ion etch.

The maximum copper thickness that can be deposited is determined by the PMGI layer thickness. The additional thickness of the PMGI layer is important for creating a greater separation between the PMMA undercut and the bulk SU-8. Initial experiments showed that a PMGI thickness of 200 nm often resulted in copper blockage of the acetone and a 450 nm PMGI thickness resulted in PMMA collapse and subsequent liftoff failure. With a measured etch rate of roughly 15 nm of the copper during reactive ion etching, at least 90 nm of copper is needed to survive 1 hour. However, faceting of the edges of the copper must be taken into considera-

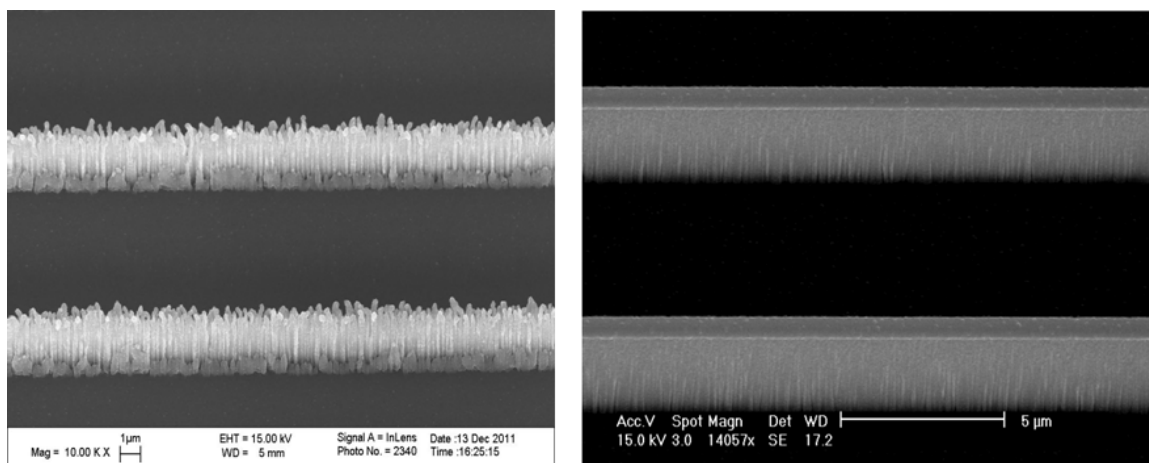


Figure 4.3: (a) 140 nm copper hard mask (b) 180 nm angstroms copper hard mask

tion. Faceting gives much higher etch rates on the edges of the features and results in roughness of the feature walls. It was experimentally determined that approximately 200 nm of copper is thick enough to compensate for faceting and result in straight edges. However, 200 nm of copper required a thicker PMGI layer which resulted in greater mechanical instability of the PMMA layer and subsequent collapse and liftoff failure. Figure 4.3 shows SEM images of two different retroreflectors taken with an SEM: (a) a retroreflector fabricated with 140 nm copper hard mask layer and (b) a retroreflector fabricated with 180 nm of copper.

In Figure 4.3, (a) Clearly shows that the 140 nm copper hard mask failed during the reactive ion etching step. While the 180 nm copper hard mask pattern seemed to remain mostly intact. Part (b) shows that at 180 nm the sidewalls are smooth and there is little to no mask failure of the copper during the etch. A compromise between PMGI and copper thickness is the only possible conclusion for solving the lift off yield problems without changing processes all together. Since 180 nm of copper yielded relatively smooth sides, increasing the PMGI thickness for this amount of copper to work is tested. Experiments are performed on three different wafers of different PMGI thicknesses. The three thicknesses used were 250 nm

, 300 nm , and 375 nm. Varying PMGI wet etch times for each wafer from 1-60 seconds showed 18 seconds at 375 nm to have had the highest liftoff yield following copper liftoff. Approximately 50% of the retroreflectors lifted off successfully. However, later runs with these parameters produced lower yield wafers once conditions changed due to PMGI etch variability with atmospheric conditions. Wafers are continued to be fabricated using these parameters in order to keep producing wafers for assay testing.

4.3 Reflective layer coating and planarization

Once the retroreflectors are fabricated past the lift off stage, a layer of aluminum is deposited to create the reflective layer. Other metals, such as gold, chromium, and nickel chromium, were tested as possible replacements. Experiments showed aluminum was the brightest option as it reflects greatest across the the visible spectrum. ImageJ program is used to measure the contrast between the retroreflectors and background at different steps to track the changes in contrast. The contrast following aluminum deposition was measured to be 25, the contrast following SU-8 planarization was measured to be approximately 12.4, and the contrast following a 10 nm gold functionalization layer was measured to be approximately 6.1.

A contrast of 6.1 is unacceptable as these retroreflectors have yet to be assembled into the microfluidic chip setup where contrast will once again be reduced. A high contrast is required for the image difference algorithm to pick up a positive result. Both SU-8 planarization and gold resulted in approximately 50% drop off in contrast. It was realized that the original geometry of the retroreflector pattern (1 μm wide, 5 μm tall, and with a 7 μm pitch) assumed an index of refraction of $n=1$ in it's design.

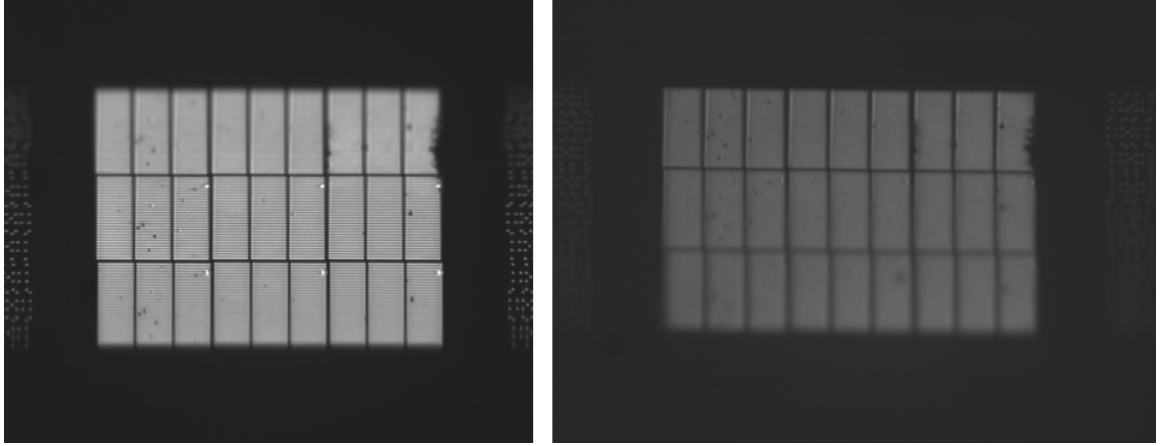


Figure 4.4: (a) Retroreflector brightness post aluminum deposition. (b) Retroreflector brightness after planarization and gold deposition

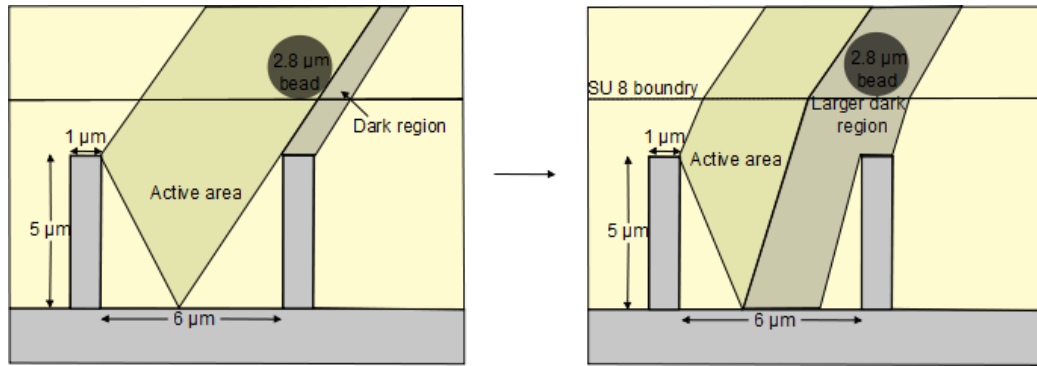


Figure 4.5: (a) Projected light paths assuming air medium and (b) projected light paths accounting for SU-8 planarization layer.

Since light moves from air to SU-8 which is a change from $n=1$ to $n=1.66$, the current spacing of retroreflectors do not allow for optimal reflectance of light. In addition, when mapping out the trajectory of light in the current assay setup it is shown that a large blind spot has formed as a result of the planarization layer. Using basic geometry and Snell's law, the current setup allows for only 50% of the area of the retroreflector pattern to reflect light back at the camera. Figure 4.5 (b) shows a microbead that will be completely undetected due to the enlarged dark region from refraction of light rays. For optimal light reflectance and reduced dark

region area, a new pattern pitch (P) is calculated using

$$P = 2h \tan \theta + w, \quad (4.1)$$

where h is the height of the retroreflector walls, w is the width, and θ is the angle of incidence from the camera lens to the substrate. Using $h = 5 \mu\text{m}$, $w = 1 \mu\text{m}$, and $\theta = 21^\circ$ for the adjusted incident angle, a new pitch of $4.68 \mu\text{m}$ is calculated. Approximately 90% of the area will be able to reflect light back to the camera using this new pitch. Using bigger bead sizes for detection can also reduce the blind spot area since the light travels to the camera from both directions. However larger bead sizes may make anti-body binding more difficult.

Printing structures with a different pitch requires a new membrane to be fabricated. Rather than fabricate a membrane with a pitch adjusted for SU-8 index of refraction, it was proposed that a thick layer of PMMA can be used to imbed the retro reflectors as an alternative to using SU-8. A new chemistry has been developed for which PMMA can be used as the functionalization layer. A quick modification procedure for PMMA surfaces yielding primary amino groups, has been found to be suitable for immobilizing different types of biomolecules, like DNA and antibodies. In this technique, PMMA is aminated using hexamethylene-diamine, following which antibodies oxidized using sodium periodate are covalently immobilized onto the substrate [16].

Using PMMA as both the planarization and functionalization layer solves multiple problems with the current setup. One of the main advantages being that the gold layer is no longer required. Thus, a 50% brightness loss following planarization can be avoided by skipping the thin gold deposition step. This will not only increase contrast between the retroreflectors and background, but also reduces assay cost and production time. It should also be noted that the index of refraction of PMMA is also closer to that of air. With an index of refraction $n=1.49$ as opposed to $n=1.66$, the light gathering capabilities of the retroreflector pattern are increased

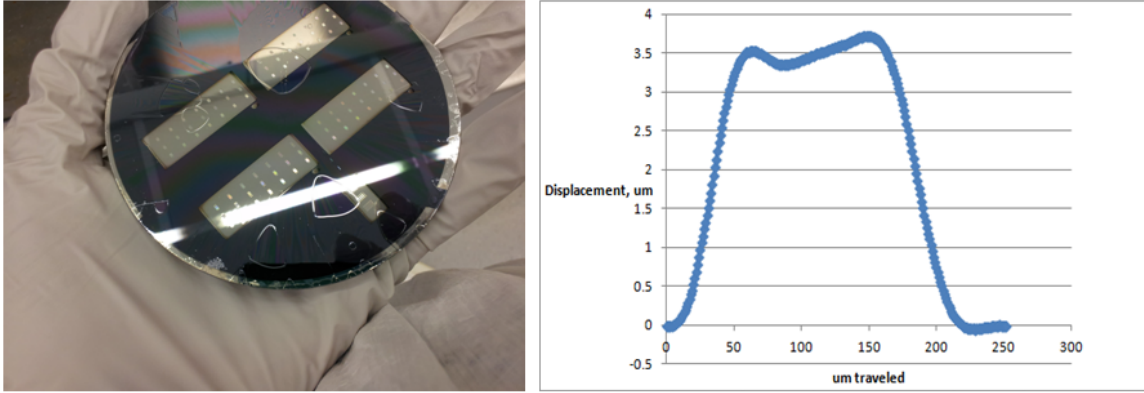


Figure 4.6: (a) PMMA void formation (b) Profilometry over chip area.

and blind spot area is reduced. To obtain thick enough PMMA to planarize the retroreflecting structures PMMA is diluted to 8% by volume in anisole solvent. A spin speed of 1500 rpm gives a 10 μm thick layer of PMMA on a bare silicon wafer. Figure 4.6 shows initial results when spincoating on an actual wafer.

When spincoating on an actual wafer adhesion of the PMMA onto the silicon wafer became a problem. Figure 4.6 (a) shows void formations across the wafer and (b) shows a non planar profilometry measurement across the retroreflecting surfaces. To fix this, following aluminum deposition, the wafer was exposed to an oxygen plasma in the reactive ion etcher for cleaning. A low voltage, high pressure plasma is used for sputtering away debris that maybe have deposited during thermal evaporation or previous reactive ion etching. The parameters for this etch are 10 mTorr oxygen with 10 watt power for 5 minutes. This etch seemed to fix the problem as no further void formations occurred and a profilometry verified that the PMMA properly imbedded the retrorreflectors.

Figure 4.7 (a) Clearly shows that void formation has disappeared and spincoat-ing is uniform. (b) Shows the increased brightness of the retroreflector pattern as viewed by the microfluidic camera setup. The background is measured to have a pixel value of 20 and the retroreflectors a pixel value of approximately 220. This

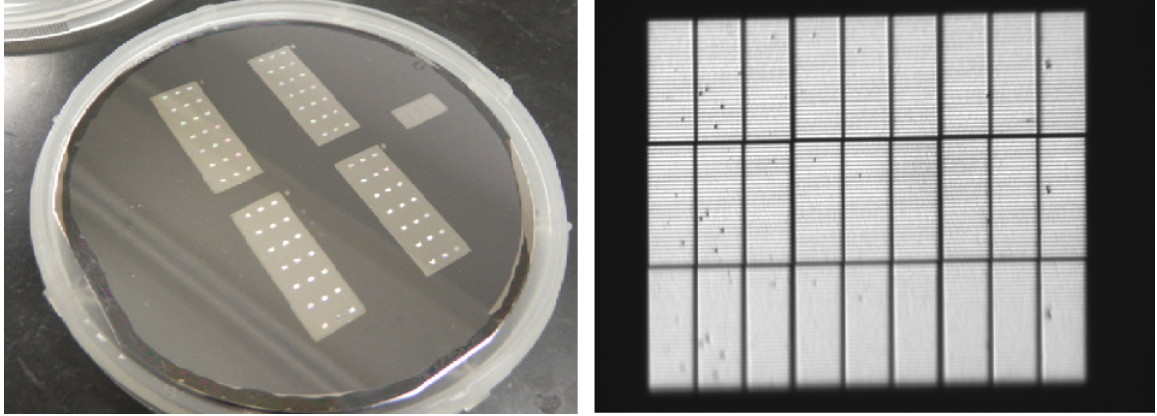


Figure 4.7: (a) Linear rerotreflector wafer planarized with PMMA (b) Retroreflector viewed with camera.

gives a contrast of 11, which is nearly a two fold increase in contrast relative to the retroreflectors planarized with SU-8.

4.4 Summary

An optimized process for manufacturing linear retroreflectors using liftoff has been explored. Testing showed the primary cause for fabrication yield issues is largely the result of unreliable PMGI etch rates. PMGI etch rates are important for controlling the amount of PMMA undercut, this undercut is critical for ensuring a successful copper layer liftoff. Etch rates for PMGI are observed to vary considerably between cold and hot weather. During summer months, the PMGI etch rate was too high to have reliable control over the undercutting of the PMMA layer resulting in nearly no product yield. Reducing PMGI etch rates for greater control has been attempted by further diluting the TMAH solution in water, but rates still showed high variability. The current facility used for processing does not have fine temperature and humidity control, thus to continue using PMGI for liftoff would require constant characterization of etch rates on a daily basis.

Retroreflector brightness was increased by thickening the copper hard mask layer. Previously, 140 nm of copper resulted in dim low contrast retroreflectors. Retroreflectors fabricated with 180 nm of copper resulted in much smoother sidewalls allowing for greater reflectance. Increasing the PMGI layer thickness to 375 nm from 200 nm was necessary to compensate for the increased copper thickness. The planarization and functionalization layer has also been modified by replacing the final SU-8 and gold layer with one thick coating of PMMA. PMMA serves well as both the planarization and functionalization layer and has more favorable optical properties than SU-8. In addition, using PMMA as the functionalization layer has negated the need for a final layer of gold which was also found to have reduced the brightness considerably. This change has resulted in nearly a two times increase in retroreflector brightness and background contrast while lowering fabrication time and cost.

While optimization of the PMGI and copper layer parameters has resulted in an increase of the overall yield of the process, throughput with this process is still too low. Initially, it took roughly 10 hours of fabrication time to create a wafer of 4 chips using Tim Sherlock's process. One in six chips on average were found to be useable. With the current developed process, it now takes approximately 8 hours of fabrication time and approximately one in four chips are useable. This represents an almost two times increase in throughput. However, due to the variability of the PMGI wet etch rates, PMMA collapse, and copper thickness limitations replacing the lift off step completely would be ideal to increase throughput. Other options should be explored to increase throughput such as UV contact printing and negative tone resists for pattern formation.

Chapter 5

Wet etch technique development

5.1 Introduction

The focus of this work is to increase throughput of retroreflector based assays by increasing product yield while decreasing process manufacturing time. The process described in Chapter 4 suffers major yield issues due to the sensitive nature of liftoff while taking an exorbitant amount of time to manufacture. In this work, a new process is explored for efficiently producing retroreflectors. Of the numerous known processes used in the micro-electronic fabrication industry, the wet etching process has had perhaps the most extensive acceptance [17]. The advantages of wet etching is that it is cost effective, stable, and can have ultra high selectivity relative to the underlying film or substrate. The main disadvantage of wet etching is that it etches in an isotropic manner and so is limited to low aspect ratio etching. A negative tone resist and wet etch process is explored for manufacturing 5 μm tall patterned features on a silicon wafer.

By using a negative tone resist for direct patterning, the need for liftoff to transfer a lithographic pattern on the substrate is eliminated. Negative tone resists are typically organic polymers and are highly reactive to oxygen and are thus not suitable for exposure to oxygen plasma. The strategy for preserving the lithographically exposed regions during pattern transfer into the SU-8 layer is to use a wet etch for transferring the pattern into an underlying metal hard mask layer sandwiched between the resist and the bulk SU-8 layer. The cross-linked negative tone resist regions will itself serve as a mask during the wet etch of the underlying metal

hard mask layer by blocking the chemical etchant from attacking the metal below each region. The patterning process is essentially reduced to three simple substrate layers with a pattern transfer step into each layer. The reduced amount of steps for pattern transfer into SU-8 represents a potentially large increase in throughput just by reducing manufacturing time alone.

Improving yield is also critical to increasing throughput and may become an issue if the wet etching step is not consistent. Copper is readily available for deposition and its selectivity relative to SU-8 has already been characterized in reactive ion etching, and it is also easily etched in the presence of a benign citric acid solvent. This makes it a good candidate for the wet etchable metal layer. Polystyrene is chosen as the negative tone resist and is discussed in detail in this chapter. By using a negative tone resist and wet etch instead of a liftoff technique for forming patterns, it could result in much more flexibility and consistency to fabrication while reducing manufacturing time.

5.2 Polystyrene as a negative tone resist

Positive tone resists are typically used for lithographic patterning in industry, largely because of the availability of the benchmark resist PMMA for electron beam lithography that offers high resolution with low cost and ease of process. ZEP520 (Zeon Corp) is arguably the second most popular positive tone resist for electron beam lithography due to its higher sensitivity and etching resistance than PMMA. Unfortunately, there is no negative resist that gains similar popularity as PMMA and ZEP520. Bilenberg et al. has selected four negative tone resists and analyzed their performance: Calixarene (Tokuyama Corp.), MA-N 2401 (Microresist Technology), SU-8 (Microchem Corp.), and MR-L 6000 (Microresist Technology) [18]. However, each of these resists suffered from either low contrast, low sensitivity, or

low resolution. In addition, these resists are commercially formulated with typically high cost and short shelf life.

It is preferable to have a negative resist like PMMA, a polymer with low cost and relatively unlimited shelf life, that can be diluted and dissolved easily with various solvents for desired film thickness. Polystyrene as a negative tone resist exemplifies many similar qualities as PMMA. It is simple and low-cost with easy process control and a long shelf life. The resolution limits are similar to that of PMMA and ZEP520 resists: Siqi Ma at Waterloo Institute for Nanotechnology has demonstrated fairly well-defined patterns of 20 nm period line arrays and 15 nm period dot arrays using electron beam lithography with polystyrene [18]. They also reported the contrast for polystyrene to be comparable to that of ZEP520 and PMMA. Polystyrene is also much more sensitive than PMMA and readily cross-links at lower doses, thus throughput can be even more increased with reduced patterning exposure times. It should be also noted that polystyrene is also considerably more resistant to dry etching than PMMA and can contribute to the hard mask during reactive ion etching.

As mentioned before, electron beam lithography lacks the throughput needed to quickly pattern large area substrates. Proximity lithography must be used for large area patterning, therefore the characterization of polystyrene using the helium ion beam system as described in section 2.2 is required. The choice of molecular weight is dependent on application. Swelling in negative tone resists during development is typically the main factor that limits resolution. Utilizing low molecular weight polystyrene reduces the amount of swelling in the resist which results in higher resolution capabilities than the high molecular weight polystyrene. Higher resolution comes at a cost however, as low molecular weight polystyrene is considerably less sensitive to cross-linking than higher molecular weight polystyrene. The threshold dose, where the contrast curve starts to rise is inversely propor-

tional to the molecular weight for simple negative polymer resists according to the Charlesby theory [19]. This is because the number of cross-links necessary to make the resist insoluble in the developers decreases with higher molecular weight.

Two different molecular weight polystyrene powders, 78.5 kg/mol and 10.3 kg/mol, were purchased (PolymerSource, Dorval, Quebec) and are tested for dose response to atom beam exposure. The solutions were prepared by dissolving 2.5% by volume powdered polystyrene in toluene solvent over night with a magnetic stir bar. The solutions are contained in UV resistant bottles since polystyrene is highly sensitive and easily cross-links under UV energies. Each solution is then filtered through a syringe equipped with a 0.45 μm polytetrafluoroethylene (PTFE) filter multiple times to remove any contaminants or cross-linked polystyrene residue. An approximate 70 nm polystyrene film was spin coated onto a silicon wafer at 2500 rpm for 1 minute. A 1 cm^2 aperture is used in the helium ion beam tool to extract a dose series response for each resist. Figure 5.1 shows a normalized HD curve of polystyrene thickness for both molecular weights versus dose using helium ion beam proximity exposure with a neutral atom beam.

Contrast for the 10,300 molecular weight polystyrene and 78,500 molecular weight polystyrene are approximated to be 2.58 and 2.09 respectively. The higher molecular weight polystyrene fully cross-linked around $3 \mu\text{C} \cdot \text{cm}^{-2}$ while the lower molecular weight fully cross-linked at approximately $0.5 \mu\text{C} \cdot \text{cm}^{-2}$. These results are in agreement with the general trend of lower molecular weight polymers having less sensitivity and higher contrast relative to higher molecular weight polymers. Since the goal of this work is to increase throughput, the higher molecular weight polystyrene is preferred due to the fact that it is roughly six times more sensitive than the lower molecular weight resist. The size of features being printed are on the micron scale, thus resolution limitations of higher molecular weight polystyrene are assumed negligible for this application. Figure 5.2 shows

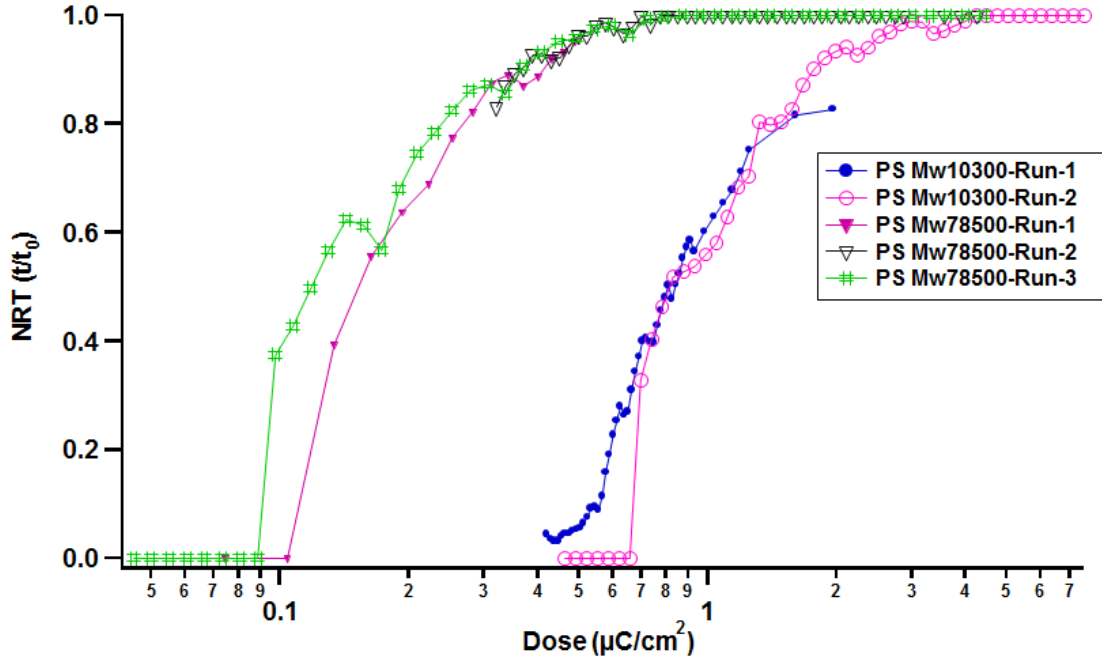


Figure 5.1: Fabrication using polystyrene as photoresist.

a polystyrene retroreflector pattern printed with the proximity ion beam tool on silicon. Image is taken with an optical microscope at $50\times$ magnification, lines are $1\text{ }\mu\text{m}$ wide with a pitch of $7\text{ }\mu\text{m}$ and an approximate field of view of $130\text{ }\mu\text{m}$.

A dose series ranging from $0.1\text{ }\mu\text{C} \cdot \text{cm}^{-2}$ to $2\text{ }\mu\text{C} \cdot \text{cm}^{-2}$ was printed with the linear retroreflector mask for inspection. The resist was developed in toluene for 1 minute followed by a nitrogen drying step. This method for resist development was determined experimentally, other developer solvents can be used such as xylene and cyclohexane for developing polystyrene. It was observed that there was very little visual difference between doses beyond $0.3\text{ }\mu\text{C} \cdot \text{cm}^{-2}$ with the exception of the higher doses being slightly wider than the lower doses. As mentioned before, negative tone resists tend to swell during development, so a $1\text{ }\mu\text{m}$ mask opening will likely result in a slightly larger crosslinked area on the substrate. In addition, it is not yet understood how well polystyrene protects the bottom layer



Figure 5.2: Printed linear retroreflector pattern with polystyrene on silicon. Dose is $0.4 \mu\text{C} \cdot \text{cm}^{-2}$.

during wet etching. Hence, to determine the correct dose for forming $1.1 \mu\text{m}$ features, the wet etching step must be analyzed as well.

5.3 Wet etching analysis

The low cost and high selectivity of wet etching previously made it a preferred method for fabrication in industry. However wet etching is no longer used in the current state of industry as it is limited to low aspect ratio etching due to the nature of the isotropic etch, requirements for fabricating submicron and nanoscale devices have increased, therefore the amount of use of the wet etch has decreased. However, linear retroreflectors are well suited for such a process as the feature size for the copper etch is $0.2 \mu\text{m}$ by $1 \mu\text{m}$, giving a low aspect ratio of 5:1. Wet etching is used for forming a metal hard mask for the SU-8 resist layer during reactive ion

Copper wet etch results

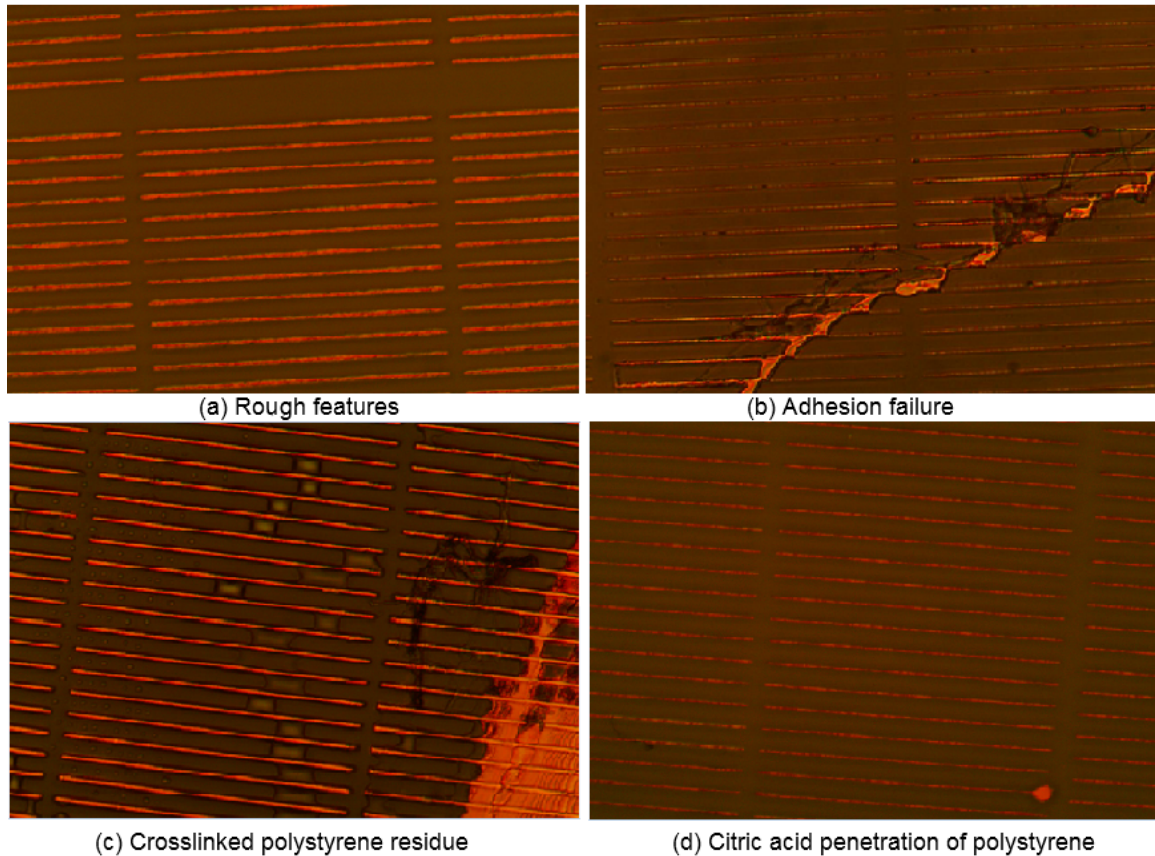


Figure 5.3: Retroreflector pattern results following wet etch of copper.

etching, a lithographically patterned crosslinked polystyrene is used to act as the mask to the bulk copper layer during the wet etch. The thickness of the copper layer is determined by how thick the copper hard mask needs to be to withstand the reactive ion etch process.

It was previously determined that 200 μm of copper is enough to withstand the oxygen plasma during removal of the 5 μm thick SU-8 layer. Copper etchant 49-1 (Transene Company, Danvers MA) is chosen as the metal etchant and consists of dilute citric acid in water. The specified etch rate is 2.2 nm/sec at 30°C. Since wet etching is isotropic, an over etch step is needed to fully remove copper left near the edges. Once it is apparent through visual observation that the bulk copper is

removed, an additional 50% over etch time is applied to ensure a clean pattern transfer. Using a visual cue for copper removal gives a rough estimation of the relative etch rate during processing due to variation in process conditions. Citric acid dissolves copper through an oxidation reaction, the etched features are vulnerable to air bubbles from blocking further etching. Slight agitation during the etch is used to remove bubbles so that etching can continue. Figure 5.3 shows initial wet etching results, images are taken with an optical microscope at $50\times$ and an approximate field of view of $130\text{ }\mu\text{m}$.

Figure 5.3 shows the various defects encountered during testing. Figure 5.3(a) would suggest that the crosslinked polystyrene areas are not fully protecting the copper layer from citric acid etching. Initial hypothesis was that the isotropic nature of the citric acid wet etch is undercutting in a variable manner under each feature. This could possibly be due to variations in polystyrene size from improper lithography or as a result of poor adhesion between polystyrene and copper. However, analysis of an exposure dose series printed on the side of the same wafer would suggest that there is no discernable difference between each dose. A separate experiment was performed to test the adhesion of polystyrene to copper oxide. Poor adhesion between two substrates can often be the result of a large difference in wetting properties. If the copper layer is oxidized into copper oxide, it will become more hydrophilic and tendency for polystyrene to adhere to the surface can be significantly reduced.

Previously copper was deposited at a rate of 0.5 nm/sec at a base pressure of 2×10^{-5} Torr using the thermal evaporation system. For this experiment, copper is deposited at 0.05 nm/sec and at an elevated base pressure of 5×10^{-5} Torr. By lowering the evaporation rate and increasing the background pressure, copper is more thoroughly oxidized by the background gas during deposition and the resulting film will contain a higher percentage of copper oxide. Figure 5.3(b) shows

the results of the experiment and confirms that less pure copper suffers greater adhesion problems to polystyrene than a more pure copper film. It should also be noted that in both cases there seemed to be large areas of copper that remained unetched following citric acid exposure.

Figure 5.3(c) shows a large area of copper is blocked from citric acid due to residual polystyrene deposited on the surface. To fix this problem a separate fresh toluene bath is used during development. Following 40 seconds of toluene development, the wafer is transferred to a second fresh container of toluene for the remaining 20 seconds of development. The second toluene bath is less saturated with polystyrene and will result in a much cleaner development step. To further clean the wafer of polystyrene deposits, an isopropanol wash step is added following toluene development. Wafers cleaned in this manner showed essentially no polystyrene deposits following development. Figure 5.3(d) shows diffusion of citric acid through the cross-link polystyrene resist, resulting in “spotty” features. Tests indicate that this type of defect is a result of cross linked polystyrene unsaturated with toluene. Polystyrene is a porous polymer and when saturated with toluene, water is unable to diffuse through the cross-linked resist as toluene will prevent water from penetrating the resist through hydrophobic interaction. The immediate solution is to wet etch the copper immediately following resist development.

5.3.1 High purity copper deposition

In an effort to fix the adhesion problem between the copper layer and polystyrene, parameters for the thermal evaporation deposition step were modified to increase the copper purity. To do this, an outgassing step was added to the thermal evaporation process. It is possible that the humidity in the air is coating the crucibles with a thin layer of water that will eventually be evaporated along with

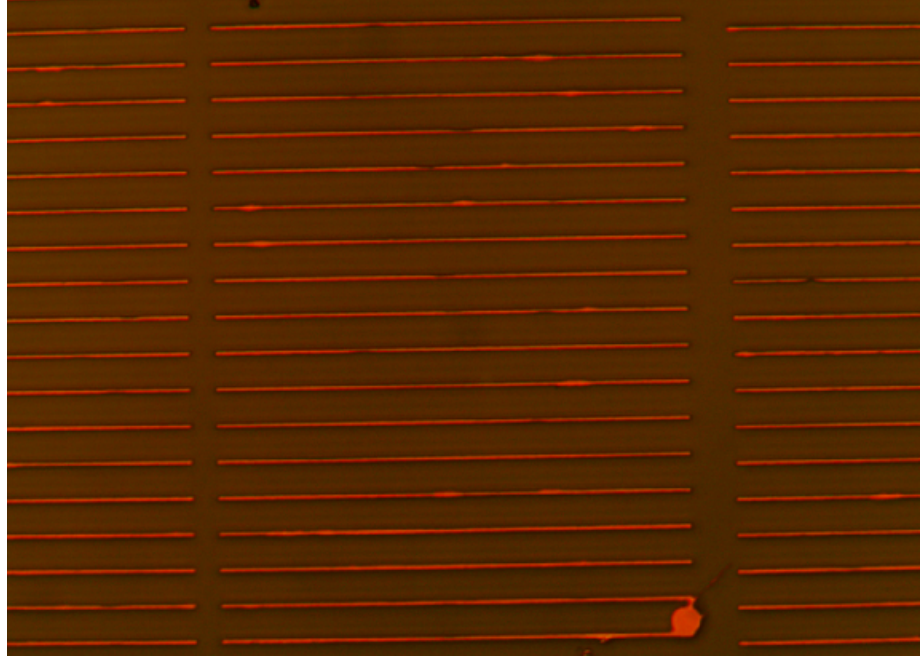


Figure 5.4: Higher purity copper wet etch results

the copper and contribute to the amount of oxygen in the chamber. Following outgassing of the crucible, the system was pumped down for an additional hour and a lower vacuum of 8×10^{-6} Torr is achieved. In addition, the substrate shutter is not opened until an evaporation rate of at least 1 nm/sec is reached. The wet etch results shown in Figure 5.4 represent a significant improvement with higher purity copper. The side walls are less rough and the polystyrene adhesion to copper is improved. However, the inconsistent line width of each feature is a concern because, once the reflective layer is deposited the reflectivity will vary across the pattern and result in nonuniform brightness.

The electron beam evaporation system was not yet operational at this time so thermal evaporation was the only choice for depositing copper. Retroreflector assays were fabricated using the thermally evaporated copper, but the quality of retroreflector images were low due to brightness gradients caused by variability in line width. Deposition using the available thermal evaporation system was also time consuming and was the largest portion of fabrication time. Eventually, re-

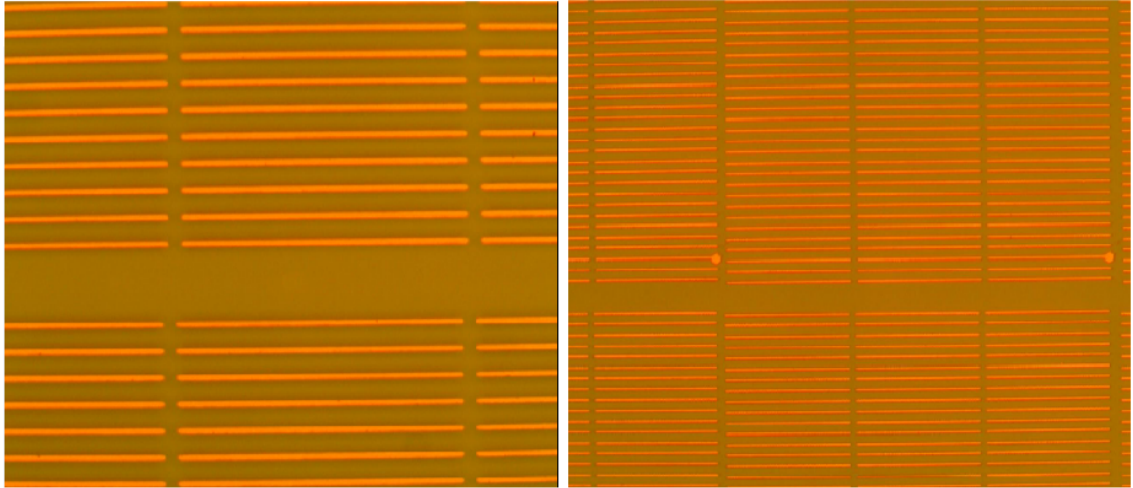


Figure 5.5: Optical microscope images of retroreflectors following wet etch of electron beam evaporated copper. (a) $50\times$ magnification and (b) $20\times$ magnification.

placing this step with electron beam evaporation proved to improve both quality and throughput of retroreflectors. As detailed in Section 2.1.2, the electron beam evaporation system pumps down at a much quicker rate and can achieve higher vacuum.

Figure 5.5 shows even greater improvement on pattern quality with increased copper purity. The line width is more uniform across the patterns and there is no visual evidence of poor adhesion between the copper and polystyrene. The large circular copper areas shown in (b) are a result of a defect in the membrane mask and will be subtracted during image analysis with the difference algorithm. Line width is approximately $2\mu\text{m}$ based on the $130\mu\text{m}$ field of view at $50\times$ magnification.

5.4 Summary

A robust process for forming micron scale patterned features on silicon wafers for retroreflector fabrication was developed. By using polystyrene as a negative

tone resist with a copper wet etch technique, the low yield liftoff step for retroreflector fabrication has been eliminated. Polystyrene is much more sensitive than PMMA and is easily cross-linked at low doses while maintaining high resolution. Cross-linked polystyrene proves to be an effective mask for wet etch pattern transfer into the copper layer. Citric acid was used as the wet etchant for dissolving copper. Etch rates of copper in citric acid are relatively independent of temperature and are easily controlled through visual observation alone. Throughput, as well as quality, have been increased significantly by using electron beam evaporation instead of thermal evaporation for copper deposition. Experiments indicate that polystyrene does not stick well to copper oxide. High purity copper deposition with the electron beam evaporation tool has resulted in near perfect retroreflector patterns. The full process for manufacturing retroreflectors with the developed wet etch technique is described in the next chapter.

Chapter 6

Optimized linear retroreflector process

6.1 Introduction

A complete retroreflector fabrication sequence has been designed utilizing the wet etch process described in Chapter 5. The main advantage of the wet etch process over liftoff is that it is much more stable and the amount of time and steps involved for the overall process are reduced. Retroreflectors are initially fabricated with the original linear retroreflector mask with 1 μm line width and 7 μm pitch for lithographic patterning. The geometry of the retroreflector pattern has yet to be optimized as the project required immediate linear retroreflector chips fabricated for functionalization chemistry tests.

6.2 Linear retroreflector wet etch process flow

Figure 6.1 shows the new process using polystyrene resist and copper wet etch. First, a 5 μm SU-8 5 photoresist (Microchem, Inc., Newton, MA) is deposited by spin-coating at 1700 rpm for 1 minute, and is baked on a hot plate at 95°C for 5 minutes. The sample is then exposed to a 254 nm wavelength UV light source (UVP CL-1000, Upton, CA) and baked at 95°C for 5 minutes to cure the resist. Next a 200 nm layer of copper is electron beam evaporated. The copper thickness in the process is less restricted than the liftoff process as the liftoff process is limited by the geometry of the PMMA undercut. However, for this process, no such limitation is in place and therefore a thick copper layer can be deposited for

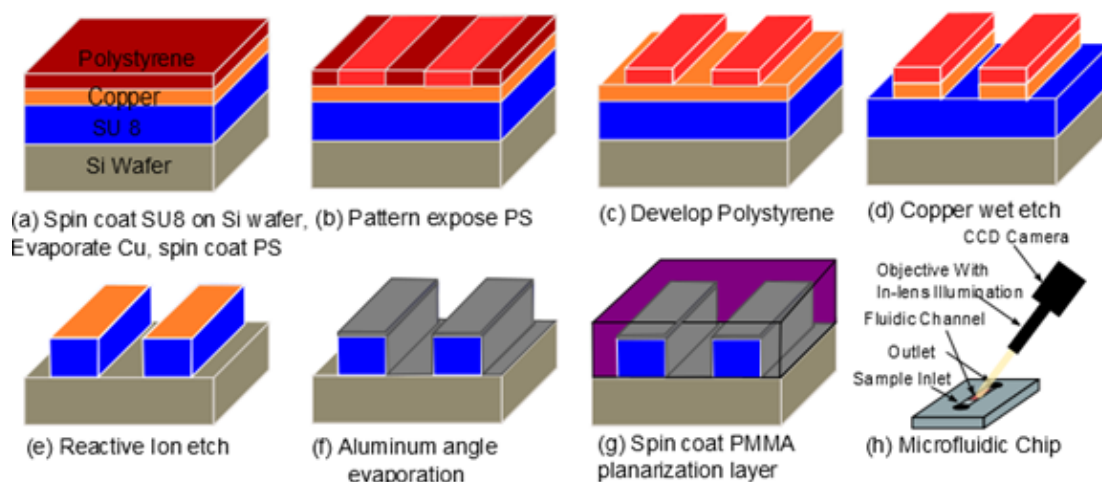


Figure 6.1: Fabrication using polystyrene as photoresist.

the patterned hard mask. Next, a 70 nm layer of polystyrene is spin coated on at 1700 rpm for 1 minute using a solution of 2.5% by volume 76.5 kg/mol molecular weight polystyrene in toluene solvent. The sample is then patterned using the same in-house helium ion beam system. Next, the wafer is developed in toluene for 40 seconds with slight agitation and rinsed in a separate toluene bath for an additional 20 seconds.

To finish the development step, the wafer is submerged in an isopropanol bath to rinse off any remaining polystyrene residue on the surface of the substrate. The wafer is then dried in nitrogen to remove the toluene from the surface. Next, the exposed copper is removed by citric acid etching for approximately 8 minutes with periodic agitation at 25°C and 50% humidity. The exact wet etch time is determined by observation, and varies slightly depending on parameters such as temperature and humidity. The wafer is then reactive ion etched with plasma parameters of 0.1 mTorr CF_4 and 0.9 mTorr O_2 at 50 watts for 1 hour. The sample is then coated with 100 nm of aluminum by thermal evaporation at a 45 degree angle to form the retroreflecting surfaces. For the planarization layer, a 20 μm layer of PMMA is spin coated on instead of SU-8.

PMMA works well as a functionalization layer and its optical properties are more favorable than the original SU-8 and thin gold layer setup. Tests have shown that PMMA attenuates the signal much less than the SU-8 and gold layers, resulting in brighter reflection and a greater contrast between the background and retroreflectors. PMMA also does not show any signs of delamination from aluminum, which allows us to remove the stencil mask during used evaporation and further reduce fabrication time. Following PMMA planarization the chips are ready to be cleaved for use in the microfluidic device setup.

6.3 Results and discussion

Figure 6.2(a) shows a sample linear retroreflector image fabricated with the wet etch process focused to the middle row. An approximate contrast of 12.5 is calculated with a maximum retroreflector pixel intensity of 250 and a dark background value of 20. Variations in brightness across the line arrays are shown as well, this was an expected result as during the citric acid wet etch of the copper the copper sidewalls immediately below the crosslinked polystyrene suffer some lateral wet etching and result in edge roughness. A close-up SEM image of a sidewall is shown in part (b). It can be seen that the side walls are indeed rough, while the top surface is essentially flat. Even with the roughness of the side walls, the retroreflector image quality is still high enough to use for assay testing and many retroreflectors are fabricated with the above conditions and handed in for testing.

6.3.1 Retroreflector edge analysis

One option for improving the side wall roughness without adding additional steps to the process is to reduce the copper thickness. Reducing the copper thickness lowers the citric acid etch times and exposes the sidewall to less lateral etch-

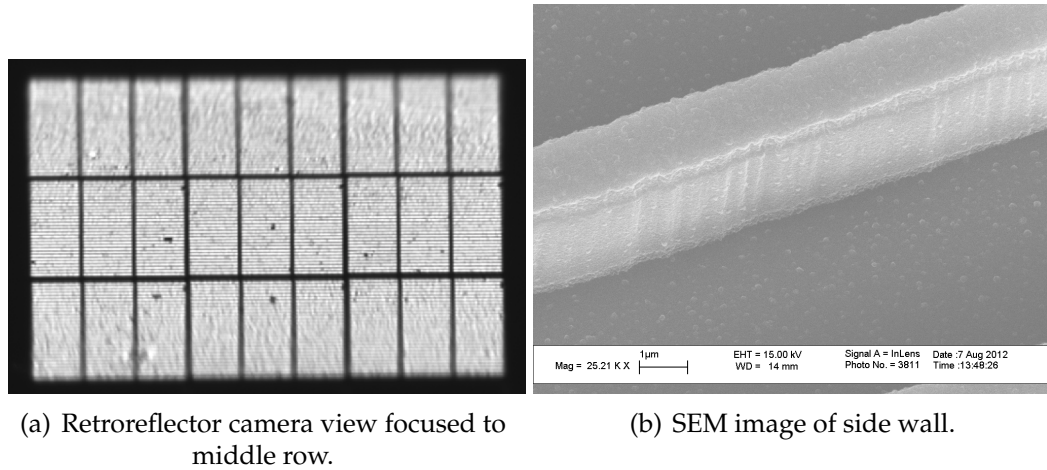


Figure 6.2: Linear retroreflectors fabricated with wet etch process.

ing. However, a thinner copper mask would be more susceptible to mask failure during the reactive ion etching step and would result in a rough surface as well. A compromise between the two extremes is explored for a minimum copper thickness while maintaining a robust hard mask during reactive ion etching. The minimum copper thickness required for the reactive ion etch mask can be estimated by using the relative etch rates of copper and SU-8 resist in the presence of the oxygen plasma. An SU-8 etch rate of approximately 140 nm/min and a copper etch rate of 1.4 nm/min is measured for these conditions.

It is expected that an amount of faceting of the side walls will occur as the copper layer is thinned, however the faceting effect isn't well characterized for this step so an iterative approach is taken to optimizing the process parameters. Without taking faceting into consideration, an absolute minimum copper thickness to clear $5 \mu\text{m}$ SU-8 in the presence of oxygen plasma with no over etch time is calculated to be approximately 53 nm . To minimize the amount of copper removed during reactive ion etching, the SU-8 over etch step is first tested for the minimum etch time for full SU-8 removal. The SU-8 resist is previously cleared with a total over etch time of more than 70%. In this experiment over etch times were varied starting with 10% and incrementally increased by 5% until the SU-8 is fully re-

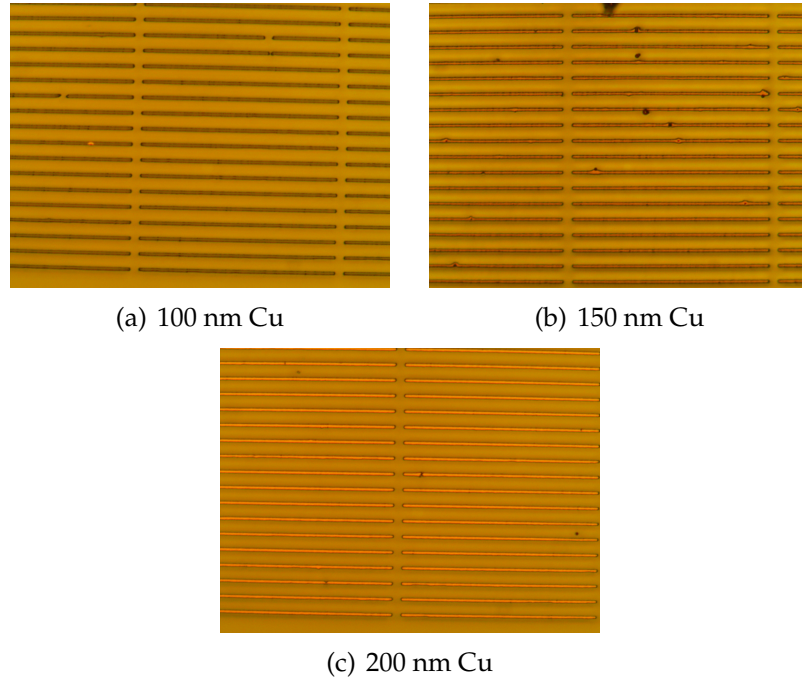


Figure 6.3: Optical images of different copper hard mask thicknesses following reactive ion etching with a 30% over-etch time.

moved. It was determined that the SU-8 layer is fully etched through at an over etch time of 40%, which comes to 49 minutes in our system.

Next, different copper thicknesses of 100 nm, 150 nm, and 200 nm are tested. Figure 6.3 shows the results:

- (a) Shows complete copper mask failure with 100 nm.
- (b) The 150 nm copper shows rough features with partial mask failure in the darker regions.
- (c) The 200 nm copper sample shows little to no mask failure.

Next, each wafer is deposited with an aluminum reflective layer for comparisons and is shown in Figure 6.4.

- (a) Shows almost no reflecting signal.

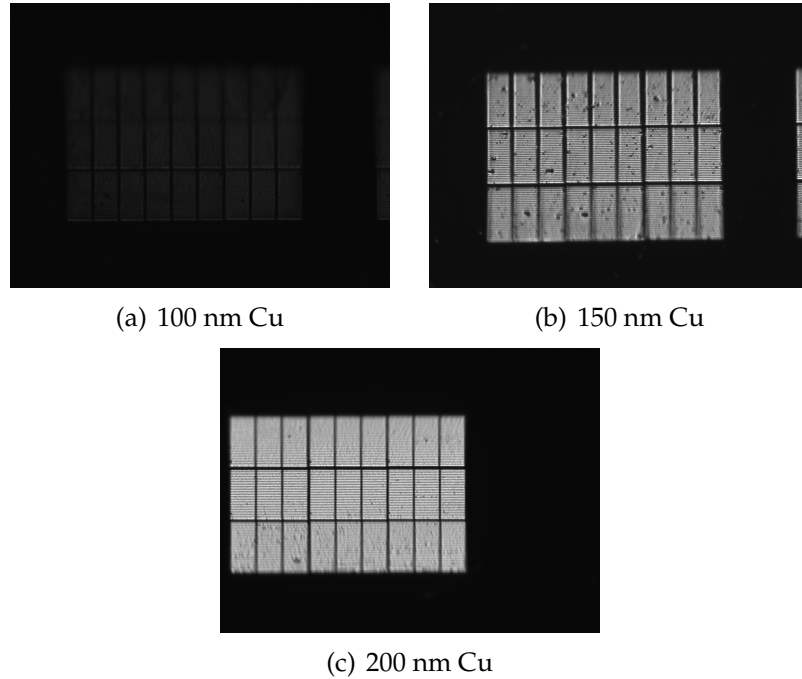


Figure 6.4: Retroreflector images of different copper thicknesses following aluminum deposition.

(b) Shows a large amount of defects, possibly caused by complete mask failure in those particular regions.

(c) The 200 nm copper sample shows a bright retroreflecting signal.

As expected, the wafer with the thick 200 nm copper shows the best results. A direct comparison can be made as these samples were made from the same wafer and have received the same process treatment with the exception of the intentional different copper thicknesses and slightly different wet etch times. The background measurement is 10 for all three images, the contrast for (a) (b) and (c) are 3.5, 14, and 18 respectively. These results indicate that sidewall roughness is more so a factor of copper thickness during reactive ion etching than the amount of lateral etching during the citric acid wet etch. To confirm this another experiment is performed with two wafers of different copper thicknesses, one with 10 nm of copper and the other with 300 nm. Polystyrene is spin-coated on both and followed by

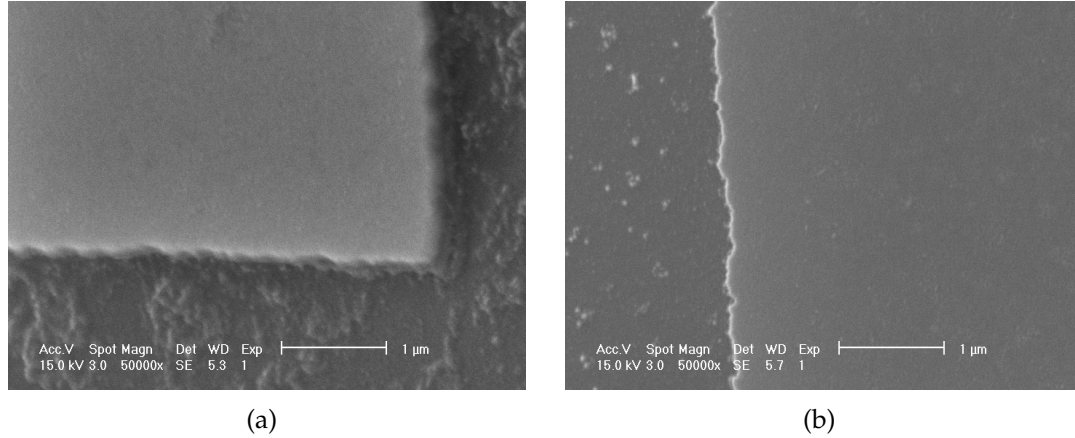


Figure 6.5: SEM images of side wall roughness of (a) 300 nm copper patterns and (b) 10 nm copper patterns.

crosslinking with the same dose as previously used to form the retroreflector patterns. The patterns are then developed in toluene and each sample is wet etched in citric acid. The citric acid wet etch time for the 10 nm copper sample will have to be calculated as 10 nm of copper is optically transparent and cannot be monitored by eye. Although the citric acid wet etch times are relatively constant, there are slight variations in etch rates between days and even greater variations between months. To get an approximation of how long it will take to wet etch the 10 nm copper sample, the 300 nm sample is etched first and a relative etch rate is extracted. Next, the samples are reactive ion etched for greater imaging contrast between the copper and silicon. This step is required as a 10 nm thick copper layer will be transparent to SEM imaging. The silicon in both wafers are etched in approximately 300 nm with a sulfur hexafluorid plasma for 1 minute. Figure 6.5 shows an image of the sidewall of both patterns taken with a scanning electron microscope.

No significant difference in sidewall roughness is observed between the thin and thick copper SEM images. To quantify the roughness of each sidewall, the ImageJ program is used to measure the total variation of each edge [20]. Using the SEM scale bar, an approximate pixels/ μm ratio is obtained and sidewall defect

distances are measured. Both the thin and copper sidewall roughness resulted in a measured sidewall roughness variation of 120 nm. With the same sidewall roughness for both the thin and thick copper, it is concluded that reduced citric acid etch times and thinner copper will not improve sidewall roughness of the features. The only remaining process that has yet to be analyzed for the source of the sidewall roughness is the lithographic patterning step.

It is possible that the sidewall roughness is a result of shot noise during lithography. Shot noise is normally observed in high sensitivity resists and results when a low dose of particles are used to crosslink a relatively large area. The low count of particles, in this case neutral atoms, cannot smoothly expose the resist due to the random nature of the particle bombardment coupled with the low number of particles used with low doses. One of the factors of influence on the line-width control is the statistics in the number of particles hitting the surface of the resist. This effect gives line edge roughness, or, in other words, a lack of control over the local position of the resist edge. This has long been recognized as a problem with high sensitivity resists and is well characterized in other lithographic systems such as electron beam lithography. Modeling has shown that the line edge position variation is inversely proportional to the dose used for the exposure of the resist [21]. This makes it impossible to increase the lithography throughput of a process using high sensitivity resists, like polystyrene.

Lower molecular weight polystyrene can be used to form smoother sidewall features but the sacrifice in throughput may not be worth the increase in performance. Ideally a low molecular weight polystyrene resist that crosslinks at energies similar to PMMA would be used in this process as earlier results showed that retroreflectors fabricated with PMMA have smooth sidewalls. The current high molecular weight polystyrene resist used cross links at 10% the energy required to expose PMMA, to use a low molecular weight polystyrene while maintaining high

throughput an improvement in source design for the lithography tool would need to be done to increase the beam current. Having not enough time to do this, retroreflectors are continued to be fabricated with the high molecular weight polystyrene. Although the quality of retroreflectors will continue to suffer from rough sidewalls the difference imaging algorithm is still able to account for these variations in pattern and the chips will still be useable for testing.

6.3.2 Retroreflector pattern geometry optimization

As discussed in 4.3 the geometry of the linear retroreflector pattern needs to be optimized to account for the index of refraction for the PMMA planarization layer. The geometry is critical to the light gathering capabilities of the assay as well as the assay's ability to detect pathogen presence. The original retroreflector pattern was fabricated assuming an index of refraction of that of air (1 μm wide 5 μm tall and 7 μm pitch). Taking into account the index of refraction of PMMA and with equation 4.1 an adjusted pitch of 4.68 μm is calculated. Printing new retroreflector patterns will require the fabrication of a new membrane mask. Using the mask fabrication process described in 3.3 a test mask has been fabricated, the mask contains retroreflector patterns with line width varying from 1-3 μm and pitch varying from 5-8 μm for a total of 12 different pattern dimensions.

Figure 6.6 shows results for retroreflector patterns fabricated with the test mask printed at the standard retroreflector exposure dose with atom beam proximity lithography. The brightest columns in (a) correspond to 1 μm line width and 7 μm pitch followed by 1 μm line width and 6 μm pitch. This is expected since the pattern is not yet planarized with PMMA. This observation agrees with our calculations as these are the optimal calculated parameters for retroreflectance in an air medium. We expect that the brightest retroreflector columns will shift once the PMMA layer is deposited. Shown in (b) the PMMA planarized retroreflector pattern

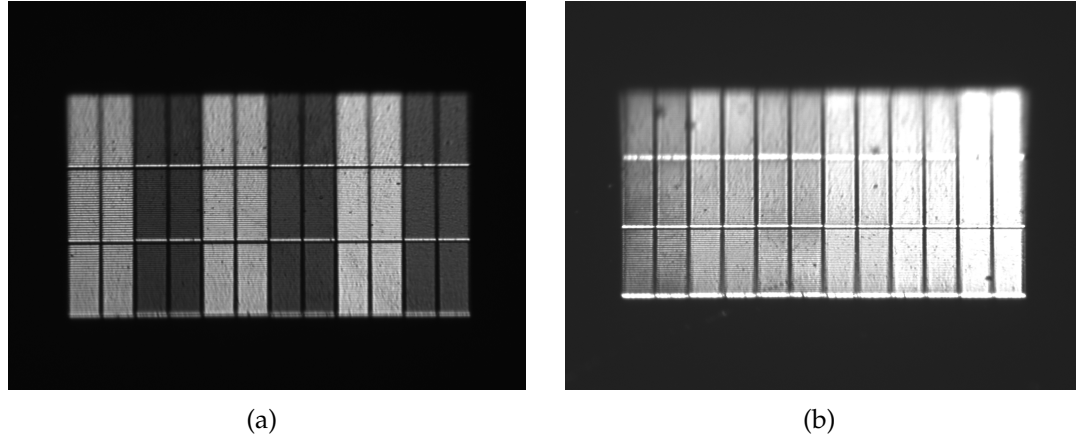


Figure 6.6: Retroreflector patterns printed with test mask following (a) aluminum reflective coating and (b) PMMA planarization.

has brightest columns corresponding to $1\text{ }\mu\text{m}$ line width and $5\text{ }\mu\text{m}$ pitch, which is close to our calculated $4.68\text{ }\mu\text{m}$ pitch for optimal retroreflectance. It should be noted that the pitch in these patterns decrease from left to right, and it can be seen that in (b) the brightness increases as the pattern pitch decreases. An exact contrast cannot be calculated from this image as the $1\text{ }\mu\text{m}$ line width and $5\text{ }\mu\text{m}$ pitch is saturated at 256 pixel value. Still, this test confirms that the retroreflector performance is increased with the adjust pattern dimensions.

6.3.3 Chip layout optimization

With a stable process for manufacturing retroreflector surfaces, chip and wafer layout is modified for further increased throughput. Previously, each wafer contained 4 chips each with 7 channels and 3 test areas per channel for a total of 84 test areas per wafer. The chip setup has been modified to now contain 7 channels with 6 test areas each. In addition, the chip placement has been changed to fit a fifth chip on each wafer. The dose series printed to the side of each wafer is replaced with a chip pattern and the dose series is moved to the middle of the wafer.

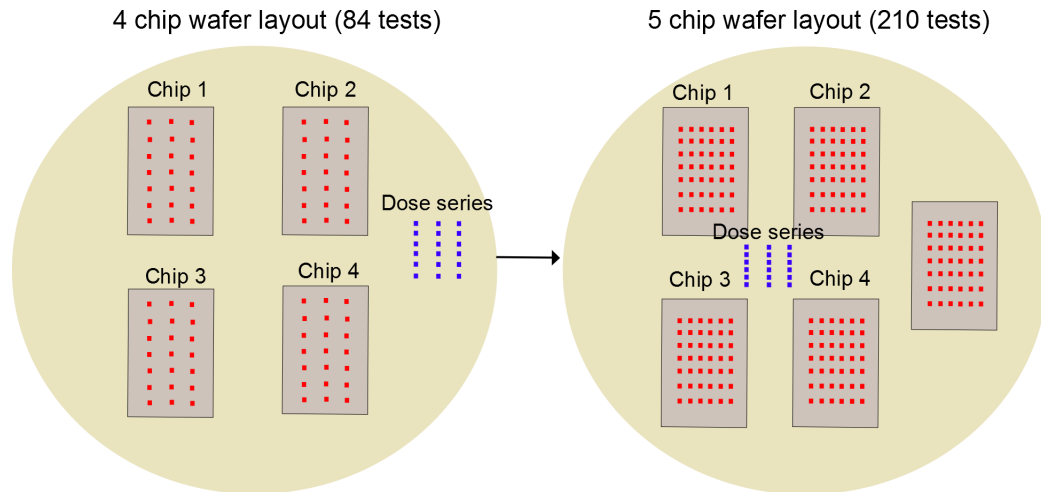


Figure 6.7: Fabrication using polystyrene as photoresist.

The resulting wafer will contain a total of 210 test areas, for a 150% increase in the number of tests per wafer.

6.4 Summary

A new robust process for forming retroreflecting surfaces has been developed. The original liftoff process suffers from low yield due to the unpredictable and inconsistent nature of the bilayer resist liftoff step. In this section, an alternative process for forming retroreflector surfaces without using liftoff is explored. Retroreflecting surfaces are formed by using a negative tone resist for patterning, followed by a metal wet etch step for driving the pattern into the underlying metal hard mask layer. The pattern is then driven into the SU-8 layer by reactive ion etching as before and followed by a reflective layer coating and PMMA planarization step. The main advantage of the wet etch process over liftoff is that it is much more reliable as the wet etch rates are not highly variable and the amount of time and steps involved have been reduced.

Polystyrene is chosen as the negative tone resist as it is cost efficient, stable, and easily crosslinked while maintaining high resolution. The low sensitivity of

high molecular weight polystyrene resist allows for quick patterning of the entire wafer in a short amount of time. This comes at a tradeoff as it is shown that high molecular weight polystyrene suffers from shot noise due to the low amount of energy used for crosslinking. This results in the retroreflector line width varying and causing the features to have rough sidewalls. Although the new process has rougher sidewalls, it is easily canceled out in the difference imaging algorithm. The old process suffers from brightness issues and background contrast is very low. This makes thresholding with the difference imaging unreliable and noisy when trying to pick up the presence of beads. To increase contrast the pattern geometry has been optimized to account for the index of refraction of PMMA and has resulted in much bright retroreflectance.

The high reliability and throughput of the current process proves to be much more effective than the liftoff technique previously used. Further efforts will be made to develop the current process and transition into a mass fabrication environment. Over fifty 4 inch wafers of linear retroreflector assays have been fabricated using this new process of which failure can be traced only to human error. The process prototyped by Mr. Sherlock required approximately ten hours of fabrication time per wafer and roughly one in four wafers had acceptable chips for testing, of which only two chips on average were useable. Using the new process and upgraded equipment the total fabrication time is down to three and a half hours per wafer with high yield. The wafer layout has also been improved for each wafer to contain 150% more test areas. Overall throughput has been increased nearly twenty times.

Chapter 7

Cube fabrication

7.1 Introduction

Bioassays are analytical techniques in which selectivity and sensitivity are generated by a biomolecular interaction. Many bioanalytical and diagnostic methods rely upon the use of labels, such as colored particles, fluorescent molecules, nanoparticles, and enzyme reaction product for generating a measurable signal. For example, immunoassays are based on the highly selective interaction and binding of antibodies to target antigens, which can be measured by use of a label attached either to the antigen or to the antibody. Although techniques for direct monitoring of antibody and antigen binding events have become available in recent years (e.g. surface plasmon resonance, SPR, or related techniques), most quantitative assays rely on the indirect approaches based on labels for generating a clear signal [2]. A new class of labels has been prototyped that utilize retroreflecting surfaces for the direct detection of a target analyte based on the changes in light propagation properties of the sample. When compared to other labels, retroreflecting objects can be fabricated to be similar in size and much brighter than dyed particles, and can be readily imaged using low cost optics.

The flexibility of retroreflectors also allow for incorporation of magnetic films and other elements in the particles themselves. Two different approaches have been designed for incorporating these labels into an assay platform, where the first assay uses predetermined, fixed locations of retroreflectors at the base of a channel. Functionalized opaque micro beads are then attached to the retroreflect-

ing surfaces. An automated image readout with difference imaging is then used and the change in retroreflectance of the sample is analyzed. The second approach uses free floating retroreflecting micron-scale cubes that are reflective from three sides of a single corner. Functionalized magnetic beads are used to bind to the cubes and are then differentiated from other nonbounded cubes with a magnet. Other forms for cube discrimination are being developed as well, including low density micro beads that can be used to cause bounded cubes to float to the top of a sample and can be easily imaged. Such retroreflecting particles are also well suited for multiplexing. By dyeing the SU-8 resist used to manufacture the cubes, a light color filter can be incorporated into the cubes and can be sensed by using a color camera.

Previously, we described the process development for fabricating linear retro-reflecting assays with high throughput. In this chapter we describe an optimized process for efficiently manufacturing cube retroreflecting particles. Much of the same process and techniques used for manufacturing linear retroreflectors are directly transferrable to manufacturing other assay platforms such as retroreflecting cubes. A wet etch method is used for driving a lithographic pattern into a copper metal layer by using crosslinked polystyrene resist as a barrier to a citric acid based etchant. Polystyrene has proven to be a robust negative tone resist with maximum resolution capabilities comparable to that of PMMA. High molecular weight polystyrene is used for increased sensitivity to facilitate high throughput of the lithography step while maintaining acceptable line width control. This wet etch technique is well suited for manufacturing particles with a release layer. By adding a sacrificial copper layer to the silicon wafer, the particles can easily be lifted off of the substrate and suspended into solution by submerging the sample in citric acid for complete copper layer removal.

7.2 Cube manufacturing process

The cube fabrication sequence is shown schematically in Figure 7.1. First, a 400 nm copper layer is deposited by electron beam evaporation onto a silicon wafer. Next a 5 μm SU-8 5 photoresist (Microchem, Inc., Newton, MA) is deposited by spin coating at 2000 rpm for 1 minute, and is baked on a hot plate at 95°C for 3 minutes. The sample is then exposed to a 254 nm wavelength UV light source (UVP CL-1000, Upton, CA) for 3 minutes and baked at 95°C for 5 minutes to cure the epoxy-based resist. A second copper layer is electron beam evaporated onto the substrate with a 200 nm thickness. Next a thin 70 nm polystyrene layer is deposited by spincoating a 2.5% by volume polystyrene powder in toluene solution at 2300 rpm for 1 minute. The cube pattern is then printed using the ion beam proximity lithography tool described in (lithosection). In this process, a gold coated stencil mask containing a $5 \times 5 \text{ mm}^2$ area of 5 μm square openings with a 10 μm pitch in a silicon nitride membrane, is exposed to a broad beam of energetic helium atoms. Particles that strike the surface of the membrane are stopped, while those that pass through the openings expose the polystyrene resist on the substrate.

After patterning, the resist is developed in a toluene bath for 40 seconds. Slight agitation of the wafer is recommended for this step as large polystyrene particulates are prone to sticking to the substrate. The wafer is then transferred to a second toluene bath for 20 seconds with slight agitation, the second bath helps ensure a clean development. The wafer is then briefly submerged in isopropanol and nitrogen dried. The underlying copper layer is etched with copper etchant TF-49 reagent (Transene company, Danvers MA). During this step the crosslinked polystyrene will act as a wet etch mask and transfer the lithographic pattern into the copper. The magnetically-enhanced reactive ion etcher tool described in 2.3 is used to transfer the pattern into the SU-8, leaving a cube structure. The plasma for the reactive ion etch consists of 0.9 mTorr of O_2 and 0.1 mTorr of CF_4 introduced to

a chamber with a base pressure of 2×10^{-5} Torr. A 5-inch electrode is driven with a 13.56 MHz RF signal at 50 watts in the presence of a 50 Gauss axial magnetic field for 1 hour. An SU-8 etch rate of approximately 140 nm/minute and a copper etch rate of 1.4 nm/minute is measured for these conditions.

With a copper selectivity of >150:1, a 200 nm copper layer thickness is sufficient for a 100% over etch step. Next, the bottom copper layer is dissolved and cube structures are undercut. A 100% over etch step following clearance of the bulk copper layer is generally enough to ensure a large enough undercut. The undercut is critical for providing an opening for citric acid to dissolve the remaining copper during the final liftoff step. Next, a 10 nm thick titanium layer is evaporated top down onto the substrate, immediately followed by a 100 nm layer of gold evaporated at an angle relative to the surface normal so that only three of the optically transparent SU-8 surfaces are coated. Gold has poor adhesion to silicon, and the addition of a titanium adhesion layer ensures that the gold deposited on the surrounding silicon will not delaminate during the final cube lift off and contaminate the final cube product in solution. Finally, the cubes are released in copper etchant solution.

7.3 Results and discussion

A new membrane mask was fabricated for printing cubes with proximity lithography. The membrane patterns consists of an array of 5 μm squares with a 10 μm pitch patterned over an area of 25 mm² on the membrane. Each print takes approximately 14 seconds with an atom beam current of 38.2 nA/cm² to crosslink the polystyrene resist. The membrane pattern is copied 184 times across a 4 inch wafer for a total of 4.6×10^7 cubes. Patterning takes a total of approximately 45 minutes, other approaches such as optical lithography, may have higher throughput. The

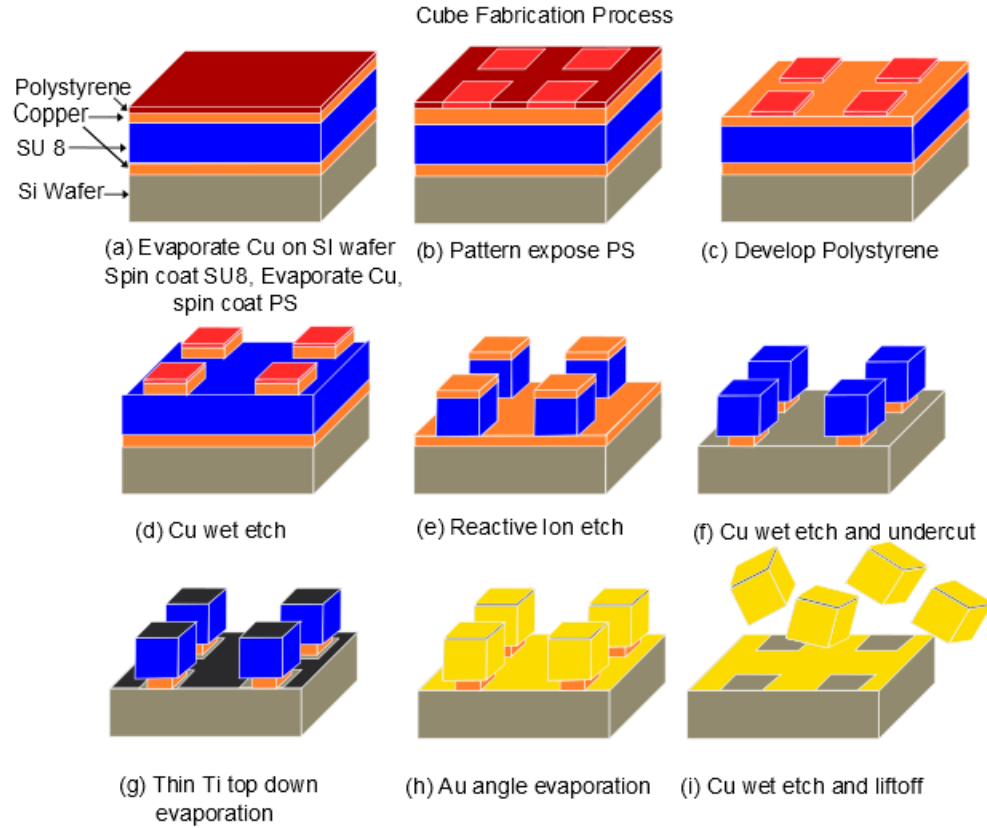


Figure 7.1: The process flow for linear retroreflection fabrication using liftoff technique.

throughput of our technique could be increased as well by increasing the source current, but existing throughput is sufficiently high for laboratory-scale manufacturing, as each test is expected to use less than 10^5 cubes per 1 mL assay. Cube manufacturing with wet etching, as opposed to liftoff, proves to be a quite reliable process as the results are very repeatable, with only failure occurring due to premature liftoff. Premature liftoff is a result of poor copper adhesion to SU-8 at high humidities. Copper adhesion has shown to be reduced by a factor of up to 30 times in the present of a wet environment, thus manufacturing in a humidity controlled dry environment is recommended to avoid premature cube liftoff [22].

This process is essentially the same as linear retroreflector manufacturing with the addition of a sacrificial layer and liftoff step and the absence of a planarization

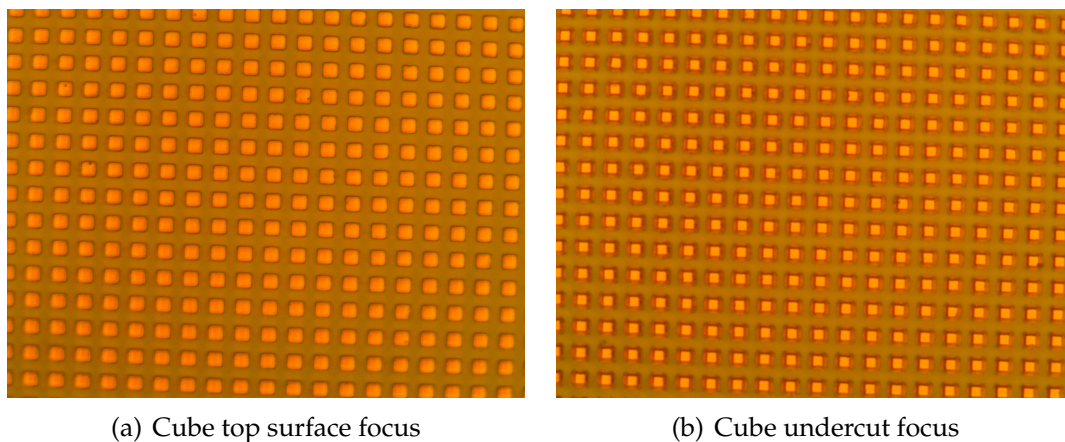


Figure 7.2: Optical images of cubes following citric acid undercut.

layer. There are three different citric acid etching steps. The first citric acid step drives the lithographic pattern into the metal hard mask layer. Following reactive ion etching, a second citric acid step undercuts the cubes. The third and final citric acid step dissolves the remaining copper and lifts off the cubes following reflective layer coatings.

Initially cubes were not releasing into solution when attempting to dissolve the final copper layer for liftoff. It was thought that the cubes were not being undercut enough during the second citric acid etch step to allow for fluid flow underneath the cube. It was later observed that the undercut of the cubes can be monitored with an optical microscope by adjusting the focal plane distance. Following reactive ion etching, the sample is submerged in citric acid and monitored until the surrounding bulk copper layer is removed. The sample is then etched for an additional 50% over etch to undercut the cubes. At this point light can be transmitted through the cubes, allowing for the undercut layer to be imaged. Figure 7.2 (a) shows optical images of cubes following reactive ion etching and citric acid undercut focused to the top surface of the cubes and (b) shows the cube undercut focus. No definitive amount of undercut has been established for the successful liftoff of cubes, a 50% over etch step generally provides enough undercut to suc-

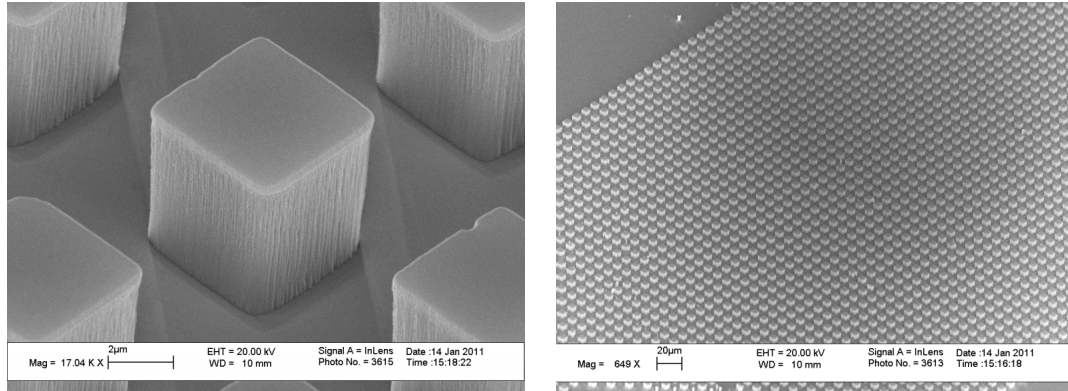


Figure 7.3: SEM images of corner cube retroreflectors [4].

cessfully dissolve the bottom copper layer. However, due to slight fluctuations in copper etch times even at 50% over etch some wafers have resulted in liftoff failure. Automating this process with as little variations between sample process parameters is preferred, the cubes are able to withstand much longer citric acid etch times before the bottom copper layer is fully removed. Changing to a 100% baseline over etch has yielded good results as cubes have consistently lifted off with high repeatability under these conditions. Figure 7.3 shows SEM images of cubes following the reflective layer coating.

The reflective layer coating step has been slightly modified from the original angle evaporation for better liftoff yield. Previously the angle evaporation step was done by depositing both titanium and gold at an angle that is incident to a single cube corner, resulting in three coated sides. The titanium layer is necessary as an adhesion promoting layer between gold and silicon as gold has weak adhesion to silicon and can contaminate the sample with large gold particulates during cube liftoff. In this work, titanium is instead evaporated top down with only gold deposited at an angle. Depositing titanium at an angle is unnecessary for depositing an adhesion layer onto the surrounding silicon substrate, the titanium can be more efficiently used to coat the silicon with a top down evaporation. Figure 7.4 (a) shows cubes after top down deposition of titanium followed by angle evaporation

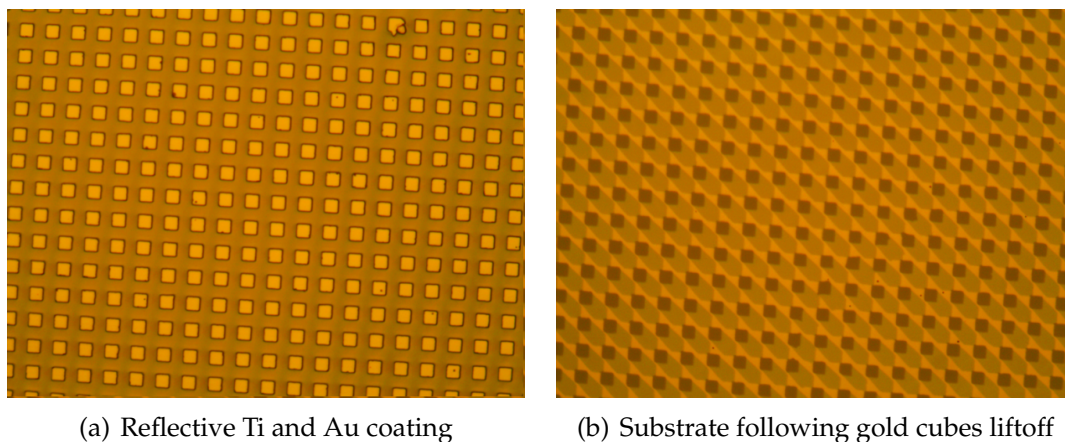


Figure 7.4: Optical images of cubes after (a) reflective layer coating and (b) lift off.

of Au and (b) shows the wafer substrate after cubes have been released in citric acid. The angled shadows casted during evaporation can be seen in 7.4 (b), the dark squares are released cubes and represent plain silicon, the lighter shadows angled in front of each square are titanium, and the surrounding brighter regions are both titanium and gold on silicon.

It is not entirely understood why cube liftoff has shown better results using a titanium top down evaporation as opposed to an angle evaporation. One possible explanation is that the cube undercut area has a larger opening for citric acid flow for smoother liftoff. Since titanium is deposited top down it is not present in the undercut to bind the additional 100 nm gold layer below the cube that may block the citric acid flow during liftoff. Other materials are available for the reflective coating layer as well, retroreflecting cubes have been fabricated with silver serving as the reflective layer. An adhesion layer is not required as silver sticks well to both silicon and SU-8. Silver is well suited as a possible reflective layer as it absorbs heavily in the 300-600 nm range [23]. Titanium reflectivity is relatively low and does not reflect nearly as well as gold and silver at these wavelengths. To allow for the current functionalization chemistries a small 10 nm layer of gold is deposited at

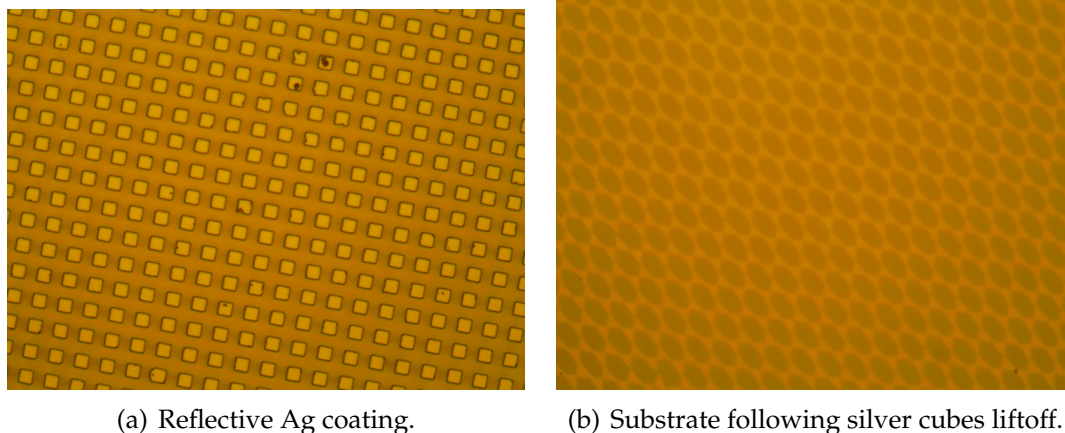
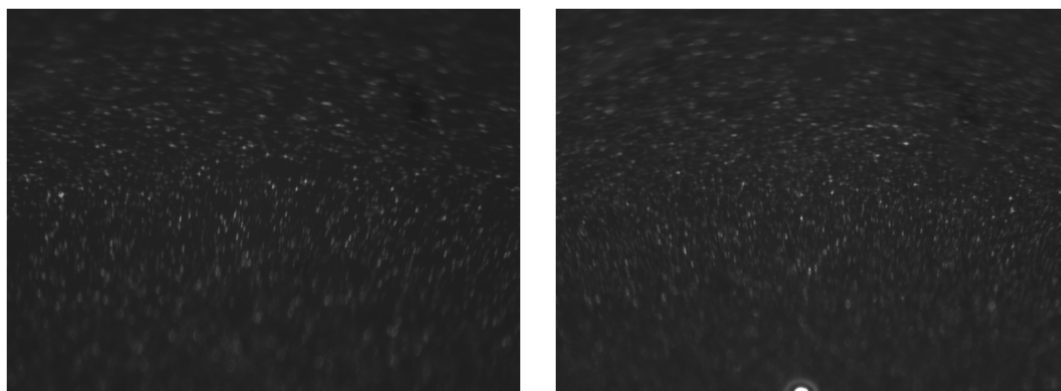


Figure 7.5: Optical images of cubes after (a) silver reflective layer coating and (b) lift off.

an angle following silver deposition. Figure 7.5 shows succesful liftoff using silver as a reflective coating, the darker regions are released cube areas and are plain silicon while the lighter regions consists of silver with a thin gold layer.

Following cube liftoff, the cubes are suspended in water and slowly settle to the bottom of the container. The cubes are then further concentrated by removing excess water from the container. A small sample is then spotted on a silicon wafer for viewing. Figure 7.6 (a) and (b) shows silver and gold retroreflecting cubes suspended in water with an approximate cube density of 10^5 ml^{-1} . A simple CCD camera is used for imaging. The background is kept constant between tests and is approximately 33-36 for both the gold and silver cube images. Comparing the two images show no substantial difference in the retroreflecting signal. More testing needs to be done to further characterize the differences if any between using gold versus silver as a reflective layer coating. It should be noted that the retroreflecting signal is optically filtered by the wavelength of the SU-8 resist and by how well the reflective layer behaves at such wavelengths. Spectroscopy of crosslinked SU-8 shows heavy absorbance in the 560-650 nm wave length range [24]. Gold and silver both reflect well across these ranges, which explains why the two images



(a) Silver cubes suspended in water

(b) Gold cubes suspended in water

Figure 7.6: Camera images of retroreflecting cubes suspended in solution.

are similar in value [23]. Further testing of gold versus silver cubes are needed to fully characterize this system, it is possible that the different reflectivity across the spectrum will provide another means for multiplexing.

7.3.1 Colored cubes

Multiplex assays are often used for high-throughput screening applications, where many specimens can be analyzed in a single process. It was shown earlier that it may be possible to multiplex cubes by using different reflective coatings as a method for differentiating between cubes. Another perhaps more robust method to achieve multiplexing with cube retroreflectors is done by dyeing the SU-8 layer different colors. The retroreflecting signal is generated by light transmitting through the cube before being reflecting back once it reaches the reflective coating sides, meaning the signal is filtered by the optical components of the SU-8 resist layer. The absorbance of red, blue, and yellow dyed SU-8 is shown in Figure 7.7.

The dyes are prepared by dissolving Orasol (BASF Chemical Company) dye in cyclopentanone, which is the SU-8 2010 solvent to a concentration of 40% by

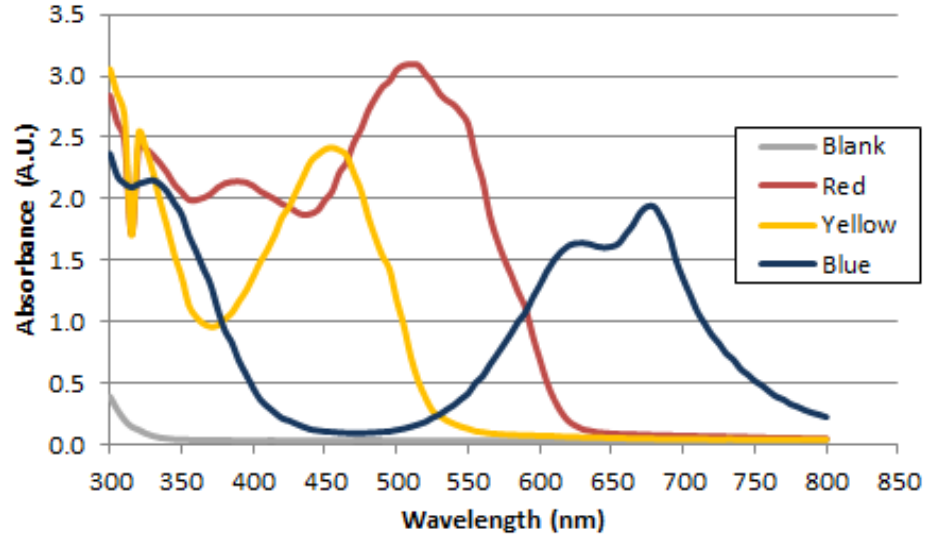


Figure 7.7: Absorbance data for dyed SU-8.

weight solution for each color. The solutions are then added to SU-8 2010 to a concentration of 8% weight by volume dye. SU-8 2010 series is used in place of SU-8 5 in order to achieve 5 μm thick films, due to the fact that the SU-8 is diluted by cyclopentanone, a series that spins on thicker is required. The dyed solutions are then spin coated onto clear glass slides at 3000 rpm for 1 minute, followed by a 95°C soft bake for 3 minutes. The sample is then exposed to a 254 nm wavelength UV light source for 3 minutes and baked at 95°C for 5 minutes to cure the epoxy-based resist. The glass slides are then exposed to reactive ion etching to verify that the dyed SU-8 can be etched in the same plasma as the normal SU-8. After verifying that the colored SU-8 does etch in oxygen plasma very similar to that of regular SU-8, colored cubes are immediately fabricated. Figure 7.8 shows red cubes and blue cubes following the first citric acid etch.

Figure 7.9 (a) shows blue cubes coated with gold spotted in 5 ml of water on a silicon wafer and imaged with a CCD camera (Edmonton optics). (b) Shows non dyed gold cubes viewed through a blue dyed SU-8 glass slide. The retroreflecting signal for non dyed cubes is noticeably less when viewed through the blue filter.

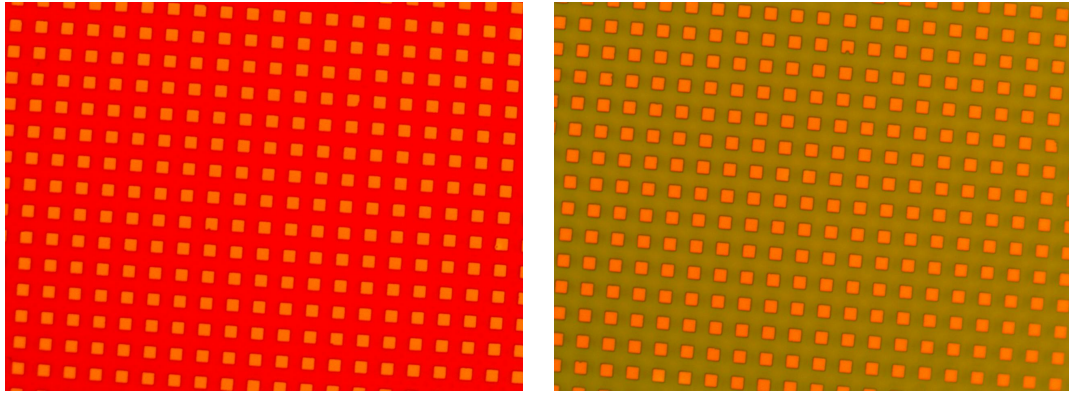


Figure 7.8: Optical images of colored cubes following the first citric acid etch (a) red and (b) blue.

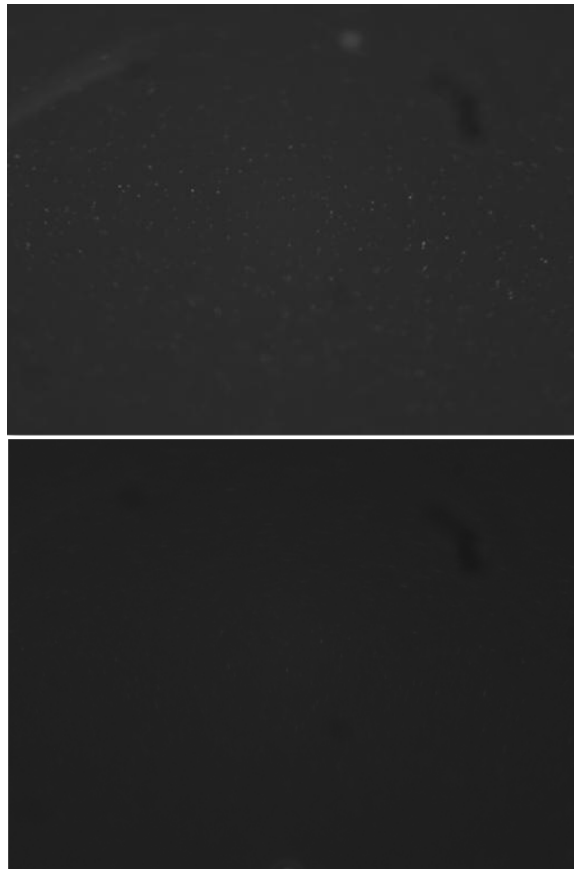


Figure 7.9: (a) Retroreflecting blue cubes in water. (b) Non dyed retroreflecting cubes with blue filter in water.

More work needs to be done to further characterize this multiplexing approach. It is not yet fully understood how the reflective coating interacts with the retroreflecting signal or how well colored cubes can be differentiated from cubes of different colors in the same sample. Addressing specificity issues between cubes with different anti bodies attached poses a whole new problem to the multiplexing assay that will require the fabrication of many more cubes.

7.4 Chapter Summary

Retroreflecting cubes have successfully been fabricated using the wet etching technique described in 5. A sacrificial copper layer has been added to the substrate for releasing cubes into solution. The technique originally used to fabricate cubes suffered greatly from liftoff problems and often resulted in very low to no yield cube samples. The unreliable bilayer resist liftoff method for forming a patterned hard mask has been replaced with a pattern transfer step using wet etching. Initially many cubes were lost due to premature liftoff of the cubes prior to a reflective coating layer deposition. It was realized that copper suffers from poor adhesion properties in the presence of wet environments, moving the process to a humidity controlled environment has resulted in approximately 100% yield with the only failure being attributed to human error or equipment failure. With the tools available in the lab, a full 4 inch wafer can be fabricated in under 5 hours, yielding approximately 4.6×10^7 cubes per wafer, which is equivalent to 460 tests assuming 10^5 cubes per test.

Throughput has been significantly increased with this process, with the prior cube process taking 10 hours for fabrication with low yield, overall throughput has been increased by more than 100%. The flexibility of this process also allows for easy manipulation of the cube properties. Both gold and silver metals have suc-

cessfully been used for creating the reflective layer coating of cubes. The different reflectivity properties of silver and gold at certain wavelengths may prove useful for multiplexing purposes in the future. Colored cubes have also successfully been fabricated for testing. Dyed SU-8 has been used for creating red, blue, and yellow cubes for imaging multiplexed samples. More work is needed to fully characterize the differences in retroreflecting signal between different colors for forming a robust multiplexing assay.

Chapter 8

Summary and Future Work

8.1 Summary

Retroreflectors are structures that return incident light directly back to the source. This principle makes them very detectable bio-sensing labels using low cost, low numerical aperture optics for readout. Previous efforts have been made towards integrating this new labeling technique as part of bio-sensing platforms to detect small quantities of target molecules. This work represents a continuation of that effort. Two methods for using retroreflectors as labels have been prototyped. For the first approach stationary retroreflector structures, termed “linear retroreflectors,” are fabricated on silicon wafers and are used in conjunction with microfluidic channels to detect attenuation of retroreflected light by labels that scatter and absorb it. The second approach uses suspended corner cube retroreflectors, five microns on each side, as ultra bright labels that can be bounded to magnetic sample preparation beads or buoyant micro bubbles in the presence of an analyte. The bounded cubes can then be differentiated from unbounded cubes by moving to an imaging site. For magnetic bead particles the bounded cubes can be moved by applying a magnetic field. Buoyant micro-bubbles are used for moving bounded cubes to the top surface of the sample for imaging. To further develop these assays as new platforms for point-of-care diagnostics, many more assays will need to be fabricated for functionalization and assay testing.

The fabrication of these retroreflector based assays have proved challenging and have suffered from low throughput. The original fabrication method for man-

ufacturing retroreflector assays involves a bi-layer resist liftoff technique using PMMA and PMGI and copper as a patterned hard mask during pattern transfer. In Chapter 4 we discuss this method and attempt to improve throughput by optimizing the lowest yield steps. Throughput and retroreflector quality was improved but ultimately the process suffered from inconsistent etch rates, the liftoff process itself is hard to control and requires much daily calibration in order to achieve pattern liftoff. The goal of this work is to develop a new robust process for manufacturing retroreflector based assays with high throughput to assist in the development of this labeling technique for integration into novel bio-sensing platforms.

Fabrication of micron scale retroreflectors requires the use of high resolution lithography, reactive ion etching, and deposition tools. Some tools have been upgraded while others have been replaced with newer models. These changes have resulted in increased process repeatability while reducing fabrication time. In Chapter 5 we discuss a completely new process for forming micron scale patterns on a silicon wafer using polystyrene as a negative tone resist and copper as a wet etchable underlying metal layer. It was demonstrated that this process is able to fabricate bright linear retroreflectors with high contrast for use in assay development. Chip and wafer layout have also been optimized to fit a greater number of tests per wafer. The overall throughput with the increased yield, reduced fabrication time, and improved wafer layout has resulted in nearly twenty times throughput increase relative to the original liftoff method. In addition, this process is directly transferrable to manufacturing corner cube retroreflectors. A sacrificial copper layer is added to the substrate for lifting off the cubes into solution with high consistency. Results have shown these cubes have been successfully bound to magnetic particles and buoyant micro bubbles. Colored cubes have also been successfully fabricated for creating a multiplexing assay. Different colored cubes have

shown to absorb different wave lengths of light and are able to be detected by using a color camera or light filters. Using different metal coatings for the reflective layer has also shown to impact the wavelength of light received by the camera. Both gold and silver cubes have been fabricated and have shown to reflect light differently under different color filters. This represents another possible avenue for multiplexing.

We have demonstrated the ability to efficiently fabricate retroreflecting assays with high throughput and repeatability. Retroreflector quality has been improved and many assays have been delivered for further development into a biosensing platform. This work allows for the development of retroreflector based assays as well as new possibilities for fabricating novel bio assays with micron scale features on a substrate at low-cost and with high reliability.

8.2 Future Work

This effort represents the manufacturing process development for two new retroreflector based assays first prototyped by Tim Sherlock at the University of Houston. In order to realize how truly powerful these novel biosensors are for pathogen detection, more work is needed. An improved process for manufacturing retroreflector based assays with high throughput has been developed in this work. Although the new wet etch method for retroreflector fabrication works, more fabrication work should be done to improve assay performance and even further improve throughput. In terms of small particle detection, efforts should be put towards fabricating sub-micron scale corner cubes and linear retroreflectors. This would allow for the use of smaller particles for pathogen capture that can more favorably bind with smaller pathogens such as viruses and DNA. To print sub-micron retroreflectors will require the fabrication of a new membrane

mask for proximity lithography. Since the ability to fabricate sub-micron features on membrane has already been demonstrated with this work, very little will need to be changed regarding membrane manufacturing. Until it is determined that sub-micron feature sizes will be required for small pathogen detection, other alternatives such as optical lithography for printing micron-scale retroreflectors should be explored for possibly an even higher throughput process. Recent work has shown that direct patterning of the SU-8 layer with micron-scale precision is possible with low cost optical lithography tools. While optical lithography's overall resolution is lower than than proximity lithography due to diffraction limitations, it is still a viable option for manufacturing retroreflectors in it's current form. Direct patterning of the SU-8 layer would remove the need for reactive ion etching and the only vacuum systems required would be evaporation systems for metal deposition.

Colored cubes have been fabricated by dying the resist layer. Many more colored cubes need to be delivered to develop a multiplexing platform as well as studying the affects of different reflective metal coatings on returning retroreflector light properties. While optical lithography represents a potential throughput increase, multiplexing corner cube assays will still need to be fabricated using proximity lithography. The ability of colored dye to absorb at the ultra violet wavelengths will render optical lithography unable to properly cross-link the full retroreflector depth. While the detection of labels, both light attenuating and suspended cubes, has been shown to work with repeatability by direct binding either to the surface or to particles, the experiments in which target molecules are bound have not been shown to be reproducible. Part of the reason for this is the low number of experiments performed due to the time it takes to fabricate retroreflectors and to prepare the experiment. With the new developed wet etch process, or by optical lithography for increased throughput, many more retroreflector assays should be fabricated to better demonstrate the reliability of the platform.

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