國 立 交 通 大 學

工學院精密與自動化工程學程

碩 士 論 文

以深紫外光微影技術製造之次微米蛋白質微圖形
應用於細胞生物學之研究

DUV photolithography generation of protein micropatterns with submicron accuracy for cell biology applications

研 究 生：吳元薰
指導教授：羅一中、徐文祥

中華民國 九十三年 七 月

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國 立 交 通 大 學

工學院精密與自動化工程學程

碩 士 論 文

A Thesis
Submitted to Degree Program of Automation and Precision Engineering
College of Engineering
National Chiao Tung University
in Partial Fulfillment of the Requirements
for the Degree of
Master of Science
in
Automation and Precision Engineering
July 2003
Hsinchu, Taiwan, Republic of China

中華民國 九十三 年　七 月

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以深紫外光微影技術製造之次微米蛋白質微圖形應用於細胞生物學之研究

DUV photolithography generation of protein micropatterns with submicron accuracy for cell biology applications

合於碩士資格水準、業經本委員會評審認可。

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班主任：

中 華 民 國 九十三 年 七 月 二十八 日

以深紫外光微影技術製造之次微米蛋白質微圖形應用於細胞生物學之研究

學生：吳元薰　　指導教授：羅一中
徐文祥

國立交通大學工學院精密與自動化工程學程

摘　　要

在基礎的細胞生物學研究中，細胞必須被培養在特定的位置或形成某種順序以構成有組織的結構。而蛋白質微圖形是目前於微米級尺寸中，最被細胞生物學所廣泛運用的結構。然而，在某些特定的研究中，實驗結果與距離或圖形尺寸的精準卻有著極大的關聯性，因此，在更進一步的研究中，次微米蛋白質微圖形的製造是確實必要的。

在本篇論文中，我們成功的製造出次微米蛋白質微圖形，為了製造次微米蛋白質微圖形，某些新的步驟被導入深紫外光微影之微接觸印刷法，如 TEOS 膜、RIE 蝕刻與 AR3 塗層。為了彈性的深寬比需求，我們採用硬幕技術，於光阻層與矽基材間加入 TEOS 膜，此技術可增加其蝕刻選擇比。根據實驗的結果，藉由蝕刻參數的控制，深寬比可被控制於 1.5 至 4.5 之間。當圖形尺寸進入次微米時，矽基材與 PDMS 層間之黏著問題會更為嚴重，此外亦與微圖形之尺寸、間距

與形狀有強烈的關係。因此，我們分析其間之化學機制並選擇適當的材料做為緩衝層來避免矽基材與 PDMS 層間產生鍵結，我們選用了 AR3 層為緩衝層並成功減輕了矽基材與 PDMS 層間之黏著問題。最後，我們觀察神經與細胞生長於此特殊設計之次微米蛋白質微圖形的情況。這些新研發的製程可成功導入深紫外光微影之微接觸印刷法，並可藉此製造出有組織的表面結構，可利於細胞生物學之廣泛應用。

DUV photolithography generation of protein micropatterns with submicron accuracy for cell biology applications

Student：Yuan-Hsun Wu

Advisors：Prof. Yi-Chung Lo,
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Degree Program of Automation and Precision Engineering
College of Engineering
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ABSTRACT

In fundamental cell biology studies, cultured cells need to be positioned at specific location and in certain orders to create organized structures. Protein patterns are the most widely used as the structures in cell biology with micro size. In some specific investigations, the experimental results are strong dependence with the accuracy of distance or pattern size. Thus, protein micropatterns with accuracy of sub-micro are indeed required for further research.

In this thesis, we successfully demonstrated the realization of fabricating protein sub-micro patterns for cell biology applications. To successfully generate the protein sub-micro patterns, some new designed procedures were involved into the DUV photolithography with micro contact printing process, such as TEOS film, RIE etching and AR3 coating. For the requirements of flexible aspect ratio , TEOS film was added as a hard mask between photoresist and substrate for increasing the etching selectivity. According to our experimental results, the aspect ratio could be offered from 1.5 to 4.5 by RIE recipe tuning. When the pattern size is into sub-micro level, the sticking issue between silicon and PDMS will be getting worse. Besides, it also has

very strong dependence with pattern sizes, pitches and shapes. We analyze the chemical mechanisms and select suitable material to be the buffer layer to avoid the crosslinking from these two materials. The AR3 layer was employed to be a buffer layer and successfully reduce the issue of pattern sticking in sub-micro size. After that, we observed the neuron and cell outgrowth in these special designed protein sub-micro patterns. The investigated patterning process that combining with DUV photolithography and micro contact printing could be used to generate functional surfaces for cell biology applications

誌　　謝

感謝我的指導教授羅一中老師與徐文祥老師，謝謝他們的教導與寬容，不僅於實驗的嚴謹度，更多在於人生態度與待人處世上的啟發。還有謝謝洪景華班主任，由於他的鼓勵，使我有信心重新拾起書本，回到母校完成碩士學位。

另外要感謝我的內人劉秀慧小姐，全心全力照顧我的家庭，讓我完全沒有後顧之憂，此外，也要感謝我的家人，特別是我的父母與岳父母，於求學期間對我的家庭充分支援與關懷，使我能專心完成學業。亦要感謝我的兩個寶貝女兒庭萱與亭蓉，每當我快要放棄時，她們天真無邪的笑容是我能堅持下去的原動力。

當然也要感謝南亞科技的林正平處長、施江林部經理與呂燦課經理，在資源與人力的全力支援，於困難時給予技術上的建議與諮詢，並在工作時間上給予我最大的彈性，使我能順利於最短時間內完成學業。還有我的師門同學家聖、同事文彬、玉宗、永隆、嘉聰、漢明、譽耀、逸騮與垂福於專業領域上的協助，好友奕盛、鑫宏、文菁、主巨的鼓勵，在此致上由衷的感謝。

最後特別感謝我的好友釋龍，要不是他陰錯陽差的邀約，使我報考了專班，也幸運的考上專班，更在事業、家庭兩頭忙時，有驚無險中順利的完成了學業。最後僅以一段詩句來形容目前的心境，「兩岸猿聲啼不住，輕舟已過萬重山」，謝謝大家。

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Chapter 1 Introduction

1.1 Motivation

During nerve regeneration, injured neurons often exhibit a significant capacity for re-growth, but the repair usually fails because the newly developed neurites can not be directed to appropriate target locations where they can form functional synapses with other cells. The long-distance targeting that is characteristic of nerve cells suggests that neurite growth and regeneration might be steered by adhesive pathways and topographic channeling. In developing embryo, neurons *in vivo* are presented with a complex array of guidance cues that direct neurons extend their processes in a highly oriented fashion [1].These guidance cues that are functional bio-molecule of **extracellular matrix (ECM)** protein such as laminin is one of the most effective substrate to control neuronal cell growth in vitro. Thus, the accuracy of micropatterns would be very important to precisely control aspects of neurite growth *in vitro*.

In the last two decades, several techniques have been developed to generate patterns of functional bio-molecules on artificial surfaces to be used for biosensors, for cell biology studies and tissue engineering applications. The methods include local deposition of molecules using ink-jet techniques and other microfluidic systems, micro contact printing (**μCP, soft lithography**) technique, photochemical patterning technique and photolithography technique (**Ex. lift-off and plasma etching techniques**). [2]

Currently, the most popular technique is micro contact printing (**μCP, soft lithography**). Here a polymer printing stamp, usually PDMS, PolyDiMethySiloxane is cast using a master that is produced with photolithography and silicon etching techniques. The stamp is then used to imprint bio-molecules from an aqueous solution onto a culture substrate. The printing process can be carried out rapidly without the need

for expensive equipment and allows the transfer of molecules to surfaces in a wide concentration range with high efficiency, and also on curved surfaces. [2, 3]

But some further investigations, sub-micro patterns are indeed required such as artificial neuron networks combining with thin-film microstructure, where distances between outgrowing neurons and electrode surfaces may significantly influence the signal transfer. [2]

In some cell growth and migration studies, cells were shifted from growth to apoptosis by using substrates that contained ECM coating adhesive islands of decreasing size. The results illustrate that the growth index depends on the size of islands [4, 5]. Sub-micro patterns could not only offer new opportunities to push the growth rate but also provide other new applications of cell biology.

Recently, investigators have employed high-resolution patterns techniques, such as photolithography techniques adapted from IC or MEMS industries [6, 7]. The resolution has been pushed into sub-micro by KrF scanner, etc (**DUV process**) and globally be used in fabricating [6, 8]. It makes possible to fabricate sub-micro protein patterns by integrating micro contact printing and DUV photolithography.

In this thesis, several sizes, pitches and shapes of patterns were designed to verify the accuracy of the process. Some new designed procedures will be involved to increase the control on aspect ratio of patterns and the reliability of process. Then, cells outgrowth will be observed and recorded in each size and shape of protein patterns. Finally, we successfully demonstrated the realization of fabricating protein sub-micro patterns for cell biology applications.

1.2 Related Researches

Breakthroughs in scientific research often rely on the development of new technologies. There are several techniques to fabricate protein micropatterns, such as Micro Contact Printing (μCP) method, lift-off method, plasma–etching method and lift-off combining plasma etching method *etc*. Thus, we will begin to introduce the applications of these techniques as below.

1.2.1 Micro Contact Printing (μCP) method [4,9]

The Micro Contact Printing (μCP) technique is a basic and most popular method for generating protein micropatterns.

It uses an elastomeric stamp or mold, prepared by casting the liquid prepolymer of an elastomer against a master that has a patterned relief structure. Masters are produced with photolithography. Most of the research based on Micro Contact Printing (μCP) has used PDMS (PolyDiMethySiloxane) as the elastomer. The stamp is then used to imprint bio-molecules from an aqueous solution onto a culture substrate. (Figure 1.1)

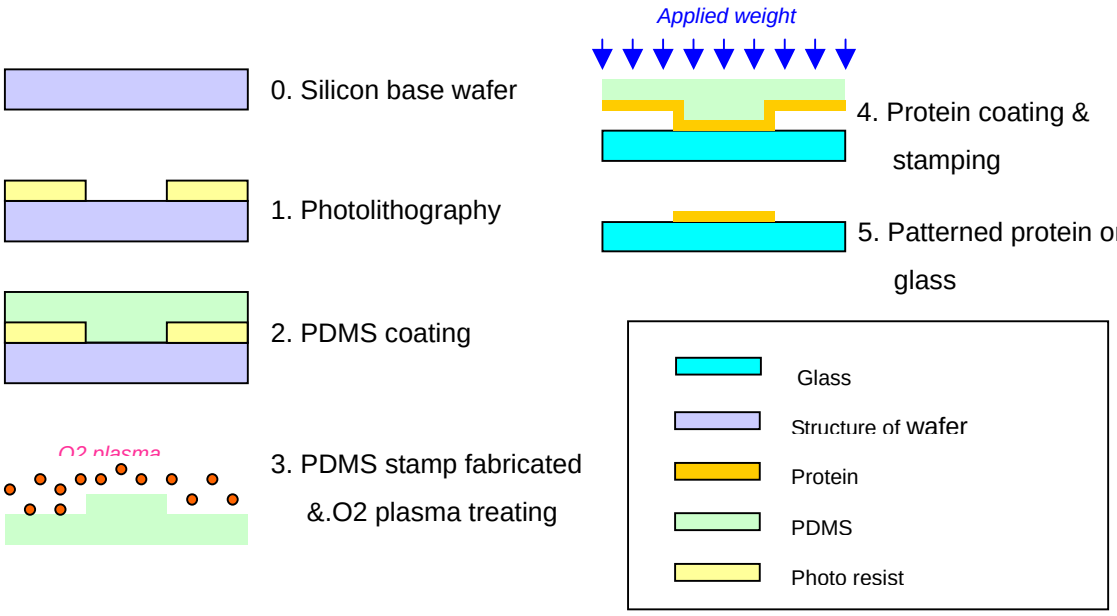


Figure 1.1 Schematic illustration of the procedure of Micro Contact Printing [4]

Figure 1.2 shows that protein patterns were generated by μCP method as below. (The size of dw=6.7 um, dl=43 um). Two geometric parameters that described each pattern, the width (dw) and length (dl) of the linear segments, are indicated in this image. The scale bar=50 um.

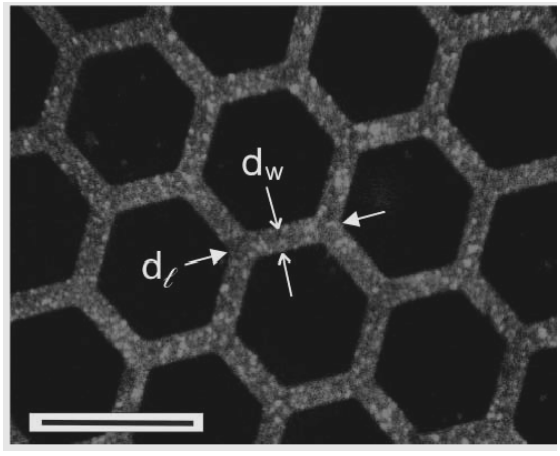


Figure 1.2 Hexagonal lattice patterns of protein were generated by μCP method [9] .

The geometry of this pattern was consisted of a series of linear segments arranged in an interconnected network (figure 1.2). Each pattern contained identically sized

segments measuring. (A) dw=7.5 um, dl=13 um (B) dw=6.7 um, dl=43 um (C) dw=2.6 um, dl=43 um. When we directed neuronal attachment and axonal outgrowth on the protein patterns, neurons were identified by a neuron-specific subunit of tubulin with red, and are shown in green-yellow. (Figure 1.3)

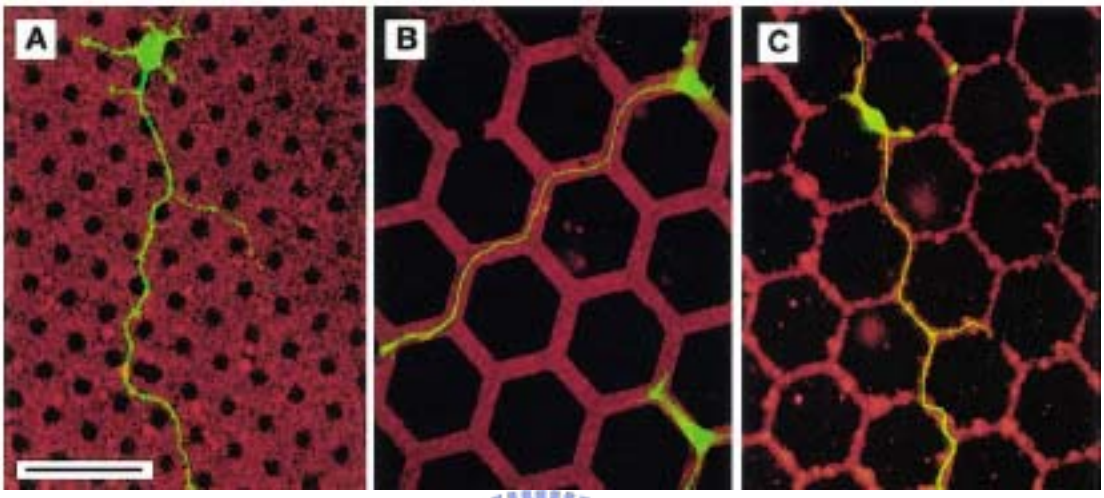


Figure 1.3 the images of directed neuronal attachment and axonal outgrowth on the protein patterns [9].

As Figure 1.4 shows the image of bioactivity of axonal outgrowth.(A) Neurons selectively extended axons along patterns of protein .(B) No extended axons outgrowth if neurons are not attach on protein patterns

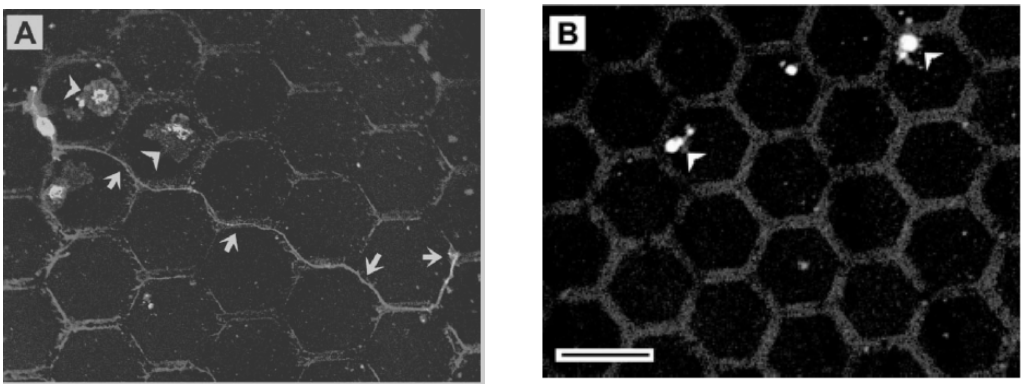


Figure 1.4 Image of bioactivity of axonal outgrowth [9].

1.2.2 Lift-off method [2]

The photoresist is first spun onto the surface and is photolithographically

structured. The proteins then immobilized on the sample and the resist structure is removed together with the overlying parts of the protein layer using a resist remover. Then the protein micropatterns could be generated on the substrate (Figure 1.5). The pattern definition is mainly given by the primary photoresist structure.

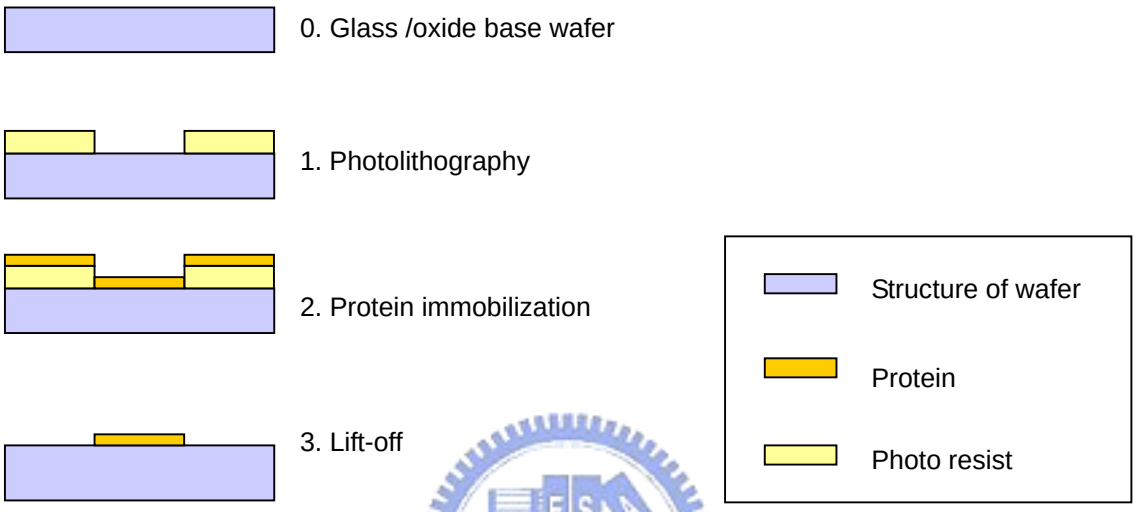


Figure 1.5 Schematic illustration of the procedure of Lift-off method [4].

A main constraint for this process sequence results from the protein immobilization procedure (figure 1.6). Organic solvents such as toluene often used for silanisations dissolve the photoresist, thus destroying the pre-formed structure. It is found that the photoresist structures were stable in solutions of the silanes APTES and MPTMS in dry iso-octane or hexane. The covalent binding of protein to the silane-treated surfaces is carried out in aqueous solutions at neutral pH. Under these conditions, the photoresist tolerate incubations over long periods of time.

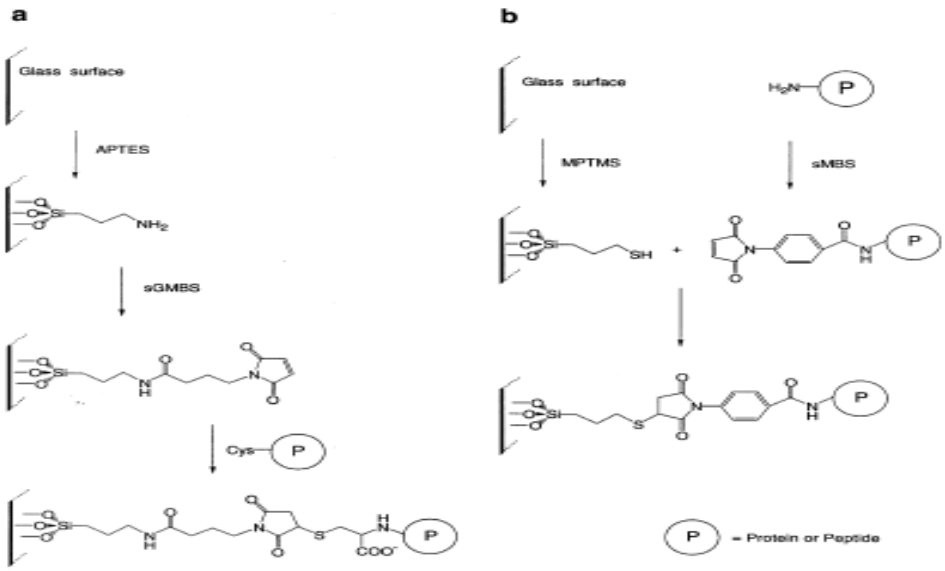


Figure 1.6 Covalent protein immobilization on glass or oxide surfaces using (a amino- and (b) mercapto-silanes and heterobifunctional crosslinkers [4].

For the lift-off step either organic solvents or water based resist removers may be used which usually contain amines or alkali-hydroxides at a pH of 9-11.**This restricts the applicability of the lift-off process relatively robust molecules such as small peptides and robust proteins such as avidin or antibodies.**

Figure 1.7 shows images of a photoresist test structure and a fluorescence image of the corresponding protein pattern. **Down to 2 um, no loss of resolution was found for the transfer of the pattern into the protein layer.**

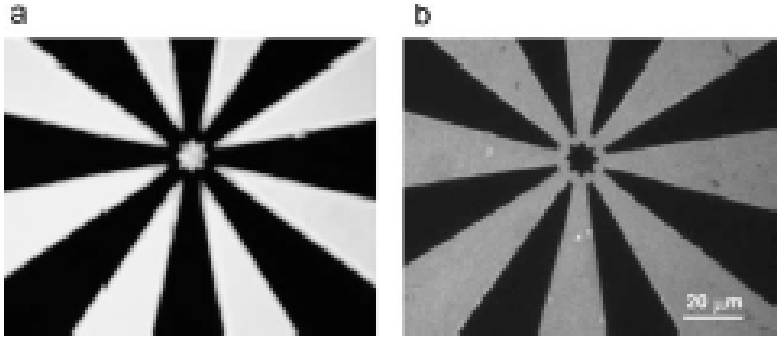


Figure 1.7 Photoresist test structure (a) and the corresponding protein pattern (b) generated in a lift-off process [4].

1.2.3 Plasma–etching method [2]

In this process sequence (see Figure 1.8), the structured photoresist is used as a mask during a dry etch process to transfer the pattern into underlying thin films. First, the protein is immobilized onto the sample and then is protected by embedding into a sucrose layer. The photoresist is applied on the later and photolithography structured. Subsequent etching in oxygen plasma removes those parts of the sucrose-protein layer which are not overlaid by photoresist. Finally the remaining photoresist is dissolved and the protein is reconstituted in the buffer solution.

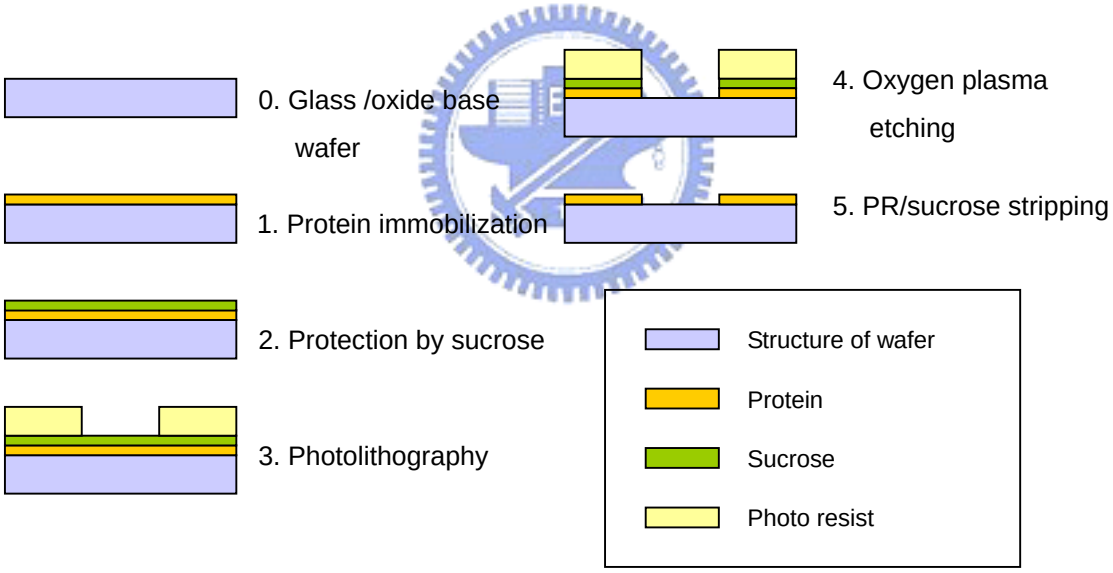


Figure 1.8 Schematic illustration of the procedure of Plasma-etching method [2]

In general, the etching method is more complicated than the lift-off process and its resolution was found to be slightly lower. Nonetheless, it is favored in cases, for example, **when layers of sensitive protein are patterned. Here the sucrose maintains a suitable environment for the proteins throughout the process and it efficiently**

protects the protein from direct contact with organic solvents.

Figure 1.9(a) shows images of protein patterns were generated using sucrose-protection neurite and plasma-etching process Figure 1.9(b) shows neurite outgrowth along the protein of the grid patterns.

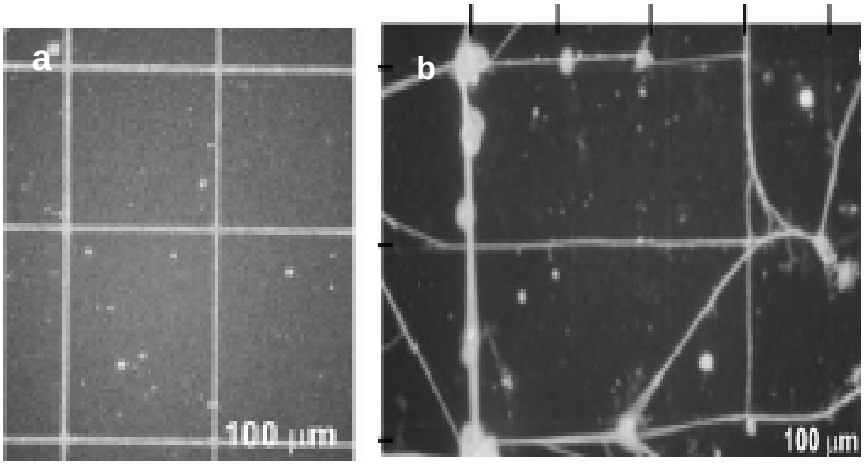


Figure 1.9 Neuron cultures on rectangular grid patterns of protein (axonin-I) on glass surfaces [2].

1.2.4 Lift-off combining plasma-etching method. [2]

Two complementary structures can be generated by lift-off combining plasma etching method and only using the same photolithographic mask. By this method, complementary patterns of two proteins can be generated on a single substrate. The first protein layer is patterned according to the etching process, but the photoresist is not removed as it serves as the mask for pattern definition of the second protein. The second protein then immobilized on the sample and the resist structure is removed together with the overlying parts of the protein layer using a resist remover. (Figure 1.10)

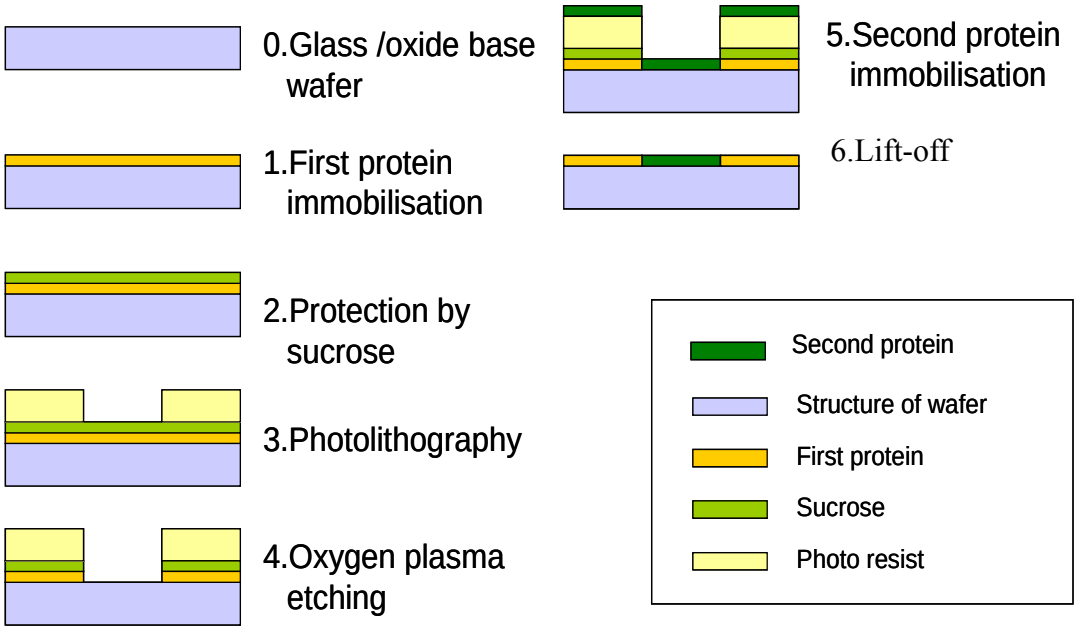


Figure 1.10 Schematic illustration of the procedure of Lift-off combining plasma etching method [2].

As Figure 1.11 shows the fluorescence images of two complementary patterns of proteins (2 and 5 um lines) were generated by lift-off combining plasma etching method. The top half of the image shows the first protein layer and structured by etching; the bottom half of the image shows the second protein layer and structured by lift-off. For the 5 um lines the patterns are well defined for both proteins; for the 2 um lines the pattern are poor for both. This demonstrates that the optimum resolution of the pattern transfer not only depends on the process parameters, but also on the shape of the transferred pattern. The limitation of resolution of the combined process is estimated to be 1~2 um.

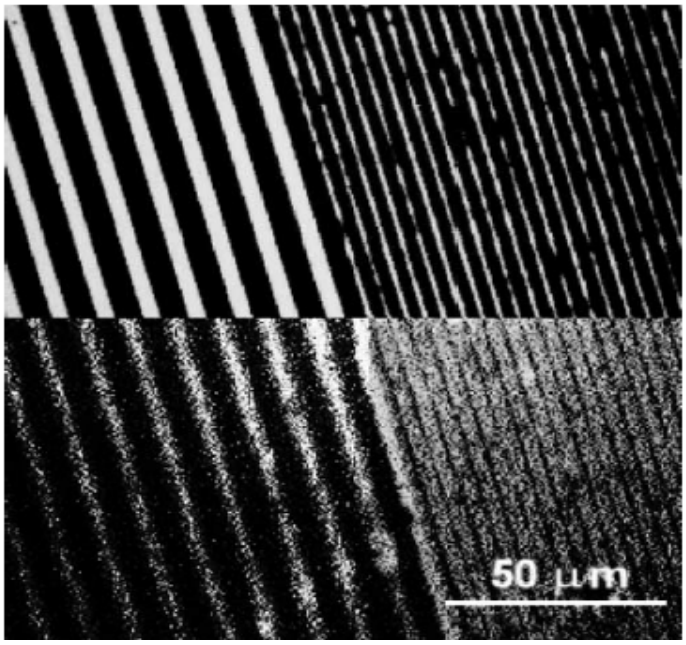


Figure 1.11 Complementary proteins (2 and 5 um lines) were generated by lift-off combining plasma-etching method [2].

1.3 Objectives

Our ultimate goal is to fabricate protein micropatterns with accuracy of sub-micro resolution. But, there are so many protein micropatterns fabricating techniques mentioned in the foregoing section, which one is good for us?

In our approach, we choose Micro Contact Printing (μCP) technique to fabricate protein micropatterns, because there are five **advantages** as follows:

1. It is biocompatible, permeable to gases, and can be used for cell culture.
2. It is optically transparent down to about 300 nm.
3. Because it is elastomeric, it can contact non-planar surfaces conformally.
4. Its interfacial properties can be readily modified by treating the surface with plasma. (figure 1.1 step3)
5. It is a durable elastomer. One PDMS stamp approximately 100 times over a period of several months without noticeable degradation in its performance. [4]

Table 1.1 Comparison of three major technologies to realize protein patterns

Item	μCP	Lift-off	Plasma-Etching
Accuracy	Middle	Highest	High
Popularity	High	Normal	Normal
Protein restriction	No	Yes	No
Complexity	Middle	Low	Middle
Cost	Middle	Low	Middle
Reserve	Good	Low	Low
Re-use	Good	Low	Low
Carry-out	Good	Bad	Bad

As shown in table 1.1, Lift-off method has the highest accuracy but in items of protein restriction, reserve, re-use and carryout, the results are poor. Although plasma-etching method has no protein restriction but still poor in items of reserve, re-use and carryout.

Micro Contact Printing (μCP) method not only has no protein restriction issue but also well for reserve, re-uses and carryout. [4, 9] If we could offer a high accuracy Micro Contact Printing (μCP) method with sub-micro resolution, it will be the best fabrication technology for protein micropatterns.

In short, our **main objectives** could be listed to six items as follows:

1. To integrate with MEMS/IC and biologics fields.
2. To offer a **high accuracy, stable, convenient and workable fabrication technology for protein micropatterns.**
3. To offer **new opportunities for further cell biology application into sub-micro**

level.

4. To fabricate protein micropatterns with accuracy of sub-micro resolution by DUV photography and Micro Contact Printing (μCP) method.
5. To Compare the pattern accuracy with variable size (0.3, 0.6 um), pitch (1:1~1:4), aspect ratio (1:0.5~1:2) and different shapes in each steps.
6. To observe the neuron and cell outgrowth in different sizes and shapes of sub-micro protein patterns.

Chapter 2 Principle and Applications

2.1 Sub-micro lithography [6, 8]

Lithography is a manufacturing process for producing highly accurate and microscopic patterns in photosensitive resist material. These patterns are replicas of a master pattern on a durable mask/reticle, typically made of a thin patterned layer of chromium on a transparent quartz plate. At the end of the lithographic process, the photoresist is used to create a useful structure in the device that is being built [6].

In this chapter, we will introduce some basic conceptions of sub-micro lithography including light source, excimer laser, mask, stepper and scanner, etc. We will choose the most suitable component of route to fabricate sub-micro patterns.

2.1.1 Light source

Resolution of the projection optics is determined by the wavelength and numerical aperture using Rayleigh`s formula,

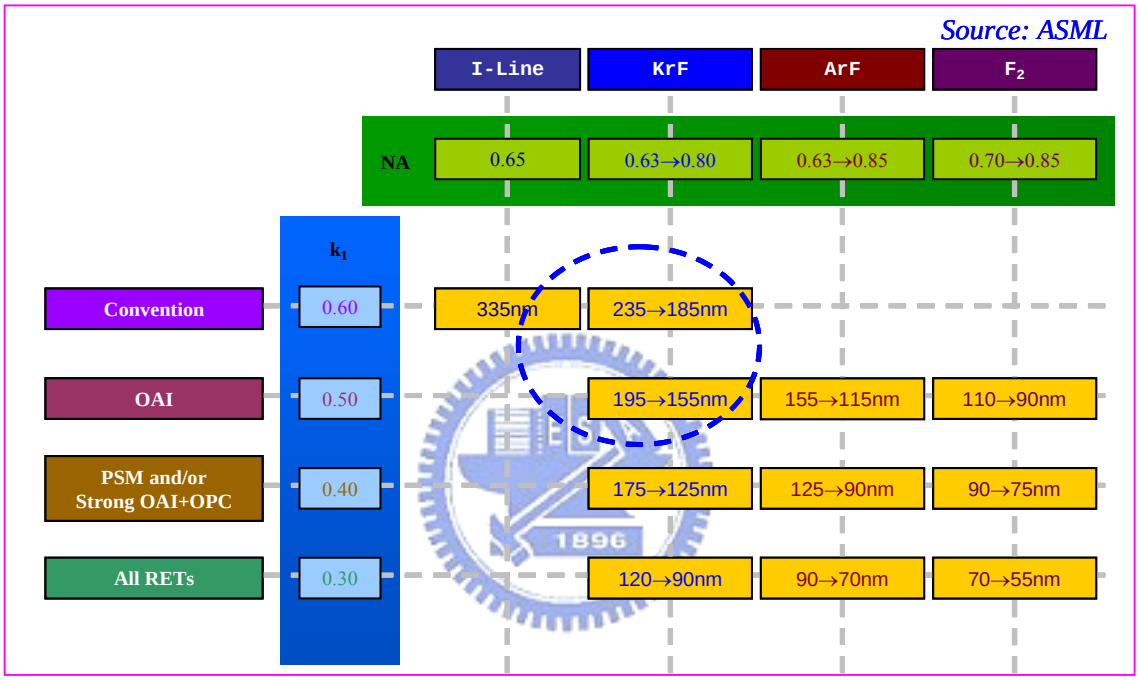
$$R = k_1 \frac{\lambda}{NA}$$

where R is the minimum dimension that can be printed, λ is the exposure wavelength, and NA is the numerical aperture of the projection lens [6].The proportionality constant *k1* is a dimensionless number in an approximate range from 0.6 to 0.8 when we use conventional illumination system. If we use OAI (Off Axis Illumination) system, the constant k1 could be down to 0.5. [12]

In this thesis, we will fabricate the pattern size from 0.6um to 0.3 um. As shown in table 2.1, we could not use the I-line(λ =365 nm) because the minimum resolution is

only 0.335 um. The KrF(λ =248 nm) could offer the resolution from 0.235 um to 0.185 um in conventional illumination system and 0.195 um to 0.155 um in OAI illumination system. According to table2.1, KrF(λ =248 nm) seems to be a suitable light source for our requirement.

Table 2.1 Resolution tables (Light source vs. K1) **[12]**



2.1.2 Excimer laser [6]

The term “excimer” comes from “”excited dimmer,” a class of molecules that exist only in upper excited state but not in the ground state. The excimer molecule has short upper state lifetime, and it decays to the ground state through disassociation while emitting a photon. There are two types of excimers: rare gas excited dimmers such as Xe2 and Kr2*, and the rare gas halogens such as XeF*, XeCl*, KrF*, and ArF*.The latter classes of excimers are of greater interest because they emit deep-UV photons (351,308, 248 and 193 nm). [6, 8]

There are three advantages of excimer laser: [12]

1. High power output
2. Make the task of developing suitable photoresist materials simpler
3. Narrow spectral linewidth (~pm).

Figure 2.1 shows the energy diagram for a KrF* excimer laser .KrF* is formed via two reaction channels. It decays to the ground state via disassociation into Kr and F while emitting a photon at 248 nm. [6]

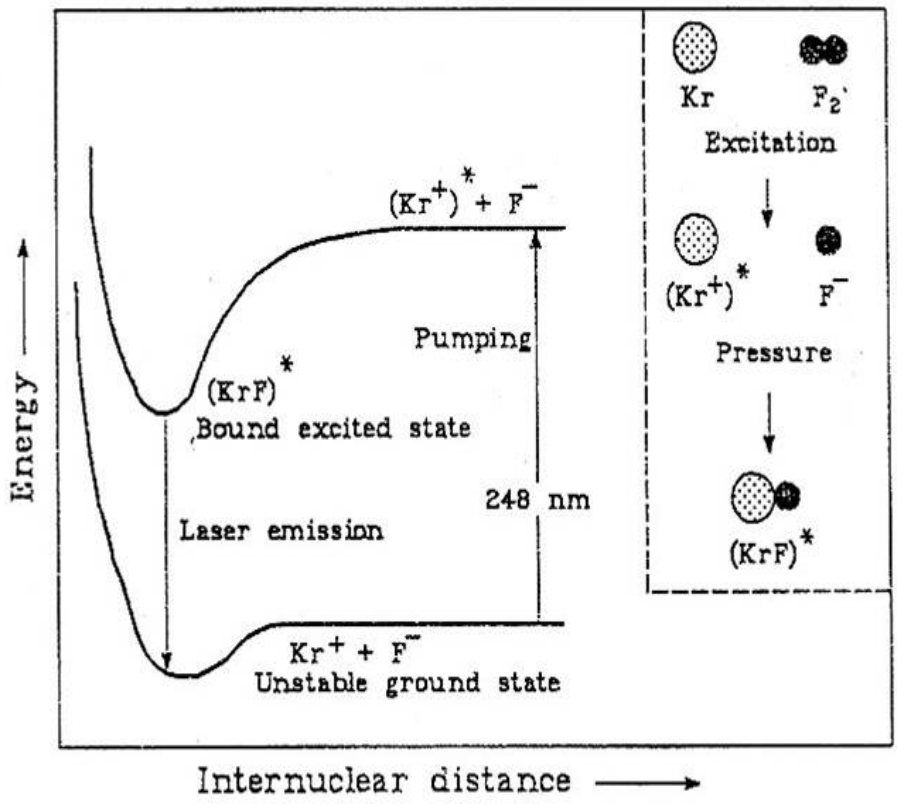


Figure 2.1 shows the energy diagram for a KrF* excimer laser **[6]**

2.1.3 Mask [6, 7]

Optical masks are made on a substrate of glass or quartz. Typical masks for a 4X reduction stepper /scanner are 6 X 6 inches square and 0.025 inch thick.

Quartz is usually used in the deep-UV portion of the spectrum from 248 to 193

nm, because other types of glass are not sufficiently transparent at these wavelengths. Chromium has for many years been the material of choice for the patterned layer on the mask's surface. A layer of chromium less than 0.1 um thick will block 99.9% of the incident light. Above the layer of chromium, material of ARC (Anti reflective coating) is chrome oxide to suppress interferences at wafer surface.

Masks must be generated from an electronically stored original pattern. Some sort of direct-writing lithographic technique is required to create the pattern on a mask blank coated with photoresist. Both electron beam and laser beam mask writers are in common use. After the resist is developed, the pattern is transferred to the film of chromium absorber, usually with a wet etch process. But in some smaller feature size, dry etching is needed to control the critical dimension. [6]

2.1.4 Stepper

The optics printing of stepper is designed to use step-and-repeat (S/R) projection systems [12]. It employs reduction optics and exposes only one chip at a time. The 4Xor 5X mask remains stationary with respect to the lens (figure 2.2). After each chip is exposed, a high –precision stage moves the position where the next exposure will occur. If the chip pattern is small enough, two or more chips may be printed in each exposure. [6]

There are some properties of stepper: [12]

1. The most common printing technique in modern technologies
2. Image size is projected 1:1 or reduced in size 2x-5x (10x)
3. Advantages: easier to fabricate mask, mask defects and imperfections are reduced in size
4. Reductions by factors larger than 5x become difficult when the chip

size increases

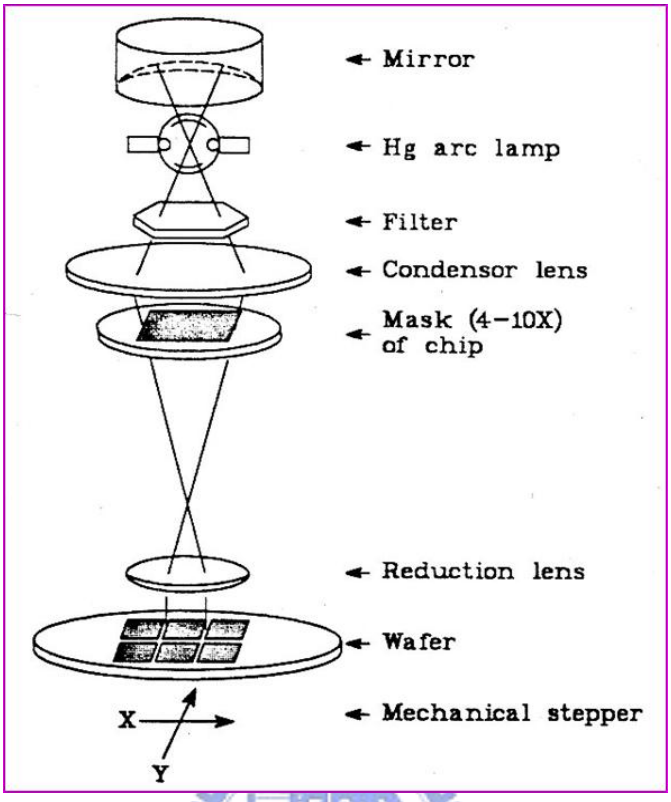


Figure 2.2 Stepper (Step-and Repeat) Projection systems. **[6]**

2.1.5 Scanner [6]

The optics printing of scanner is designed to use step-and-scan (S/S) projection systems [12].In which a reduction lens is used to scan the image of a large exposure field onto a portion of a wafer. The wafer and mask are simultaneously scanned across the field aperture. At the end of the scan, the wafer is stepped to a new position, where the scanning process is repeated. (Figure 2.3)

There are some properties of scanner: [12]

1. Reticle stage and wafer stage are both in motion
2. Larger field sizes (20mm x 32.5mm or above)
3. Increasing dimensional control

4. Increasing throughput
5. Reduction ratio: e.g., 4:1
6. Adapted choice for next generation of optical lithography

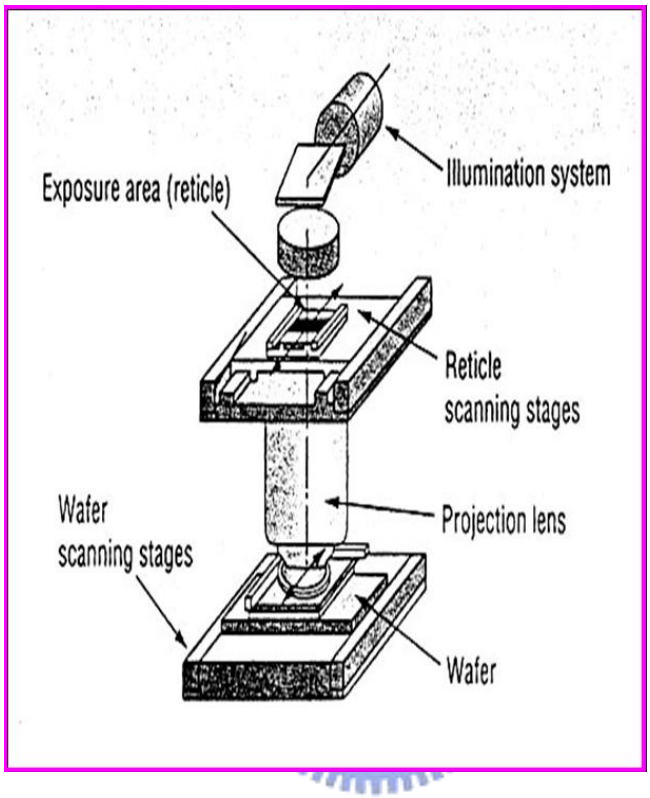


Figure 2.3 Scanner (Step-and scan) Projection systems. **[6]**

Comparing with stepper and scanner, there are some benefits of scanner. Scanner could provide a large exposure field that is good for increasing the chip size. Besides, a slit-shaped exposure field aperture is used .It means smaller projection lens required than stepper and lens productivity will be improved (figure 2.4).In the same standard of lens manufacturing, scanner will get the larger N.A and that is also better for resolution. [12]

There are also a few more subtle advantages of step-and-scan system. Because the exposure field is scanned, a single feature on the mask is imaged through a number of different parts of the lens. Any localized aberrations or distortions in the lens will be

reduced by averaging along the scan direction. Also, any local nonuniformity of illumination intensity is unimportant, as long as the intensity integrated along the scan direction is constant. (Figure 2.5)

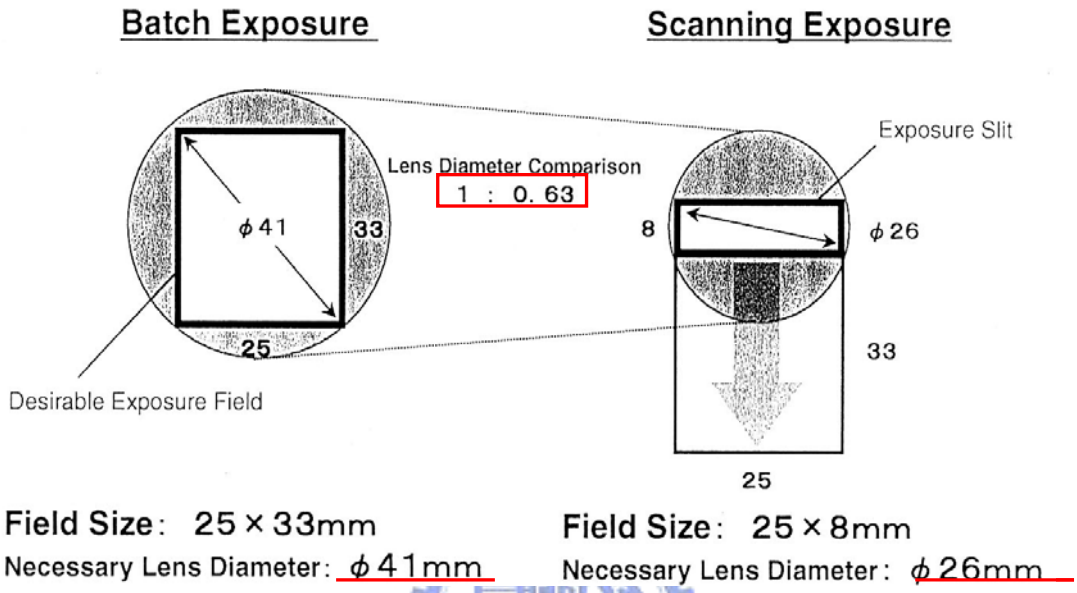


Figure 2.4 Lens required comparisons with scanner and stepper **[6]**

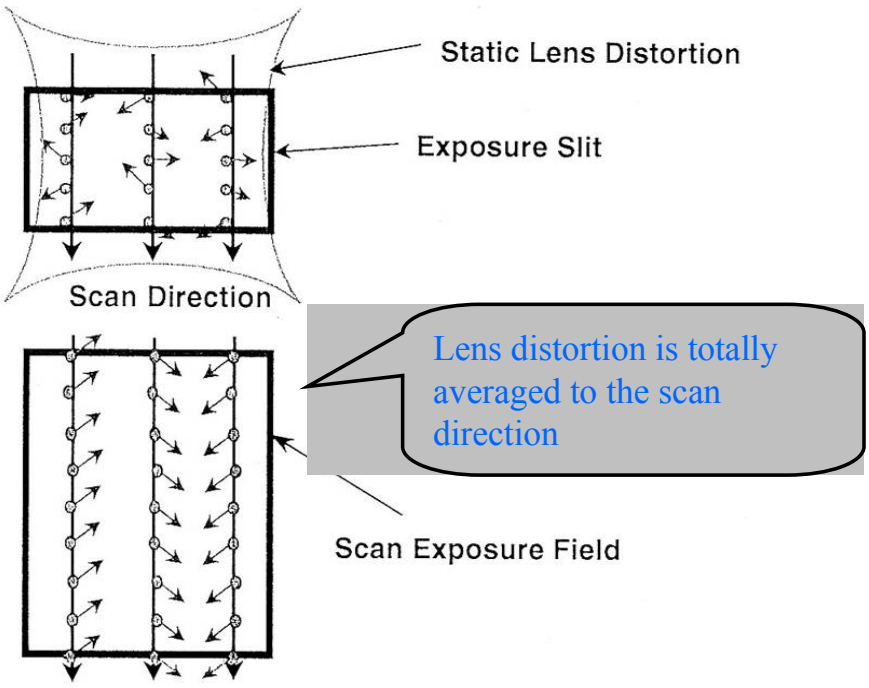


Figure 2.5 Averaging effect due to scanning **[6]**

2.1.6 RIE Etching

A standard, parallel-plate reactor design is shown in Figure 2.6, where RF power is applied to the electrode housing the wafer. In this mode, termed reactive ion etching (RIE), the DC self-bias builds up on the powered electrode where the wafer rests, yielding higher energy (50-500eV) ion bombardment. If high magnetic fields are present, the electron trajectories can be modified to increase of these types of reactors are the magnetron and the transformer coupled plasma (TCP).[6]

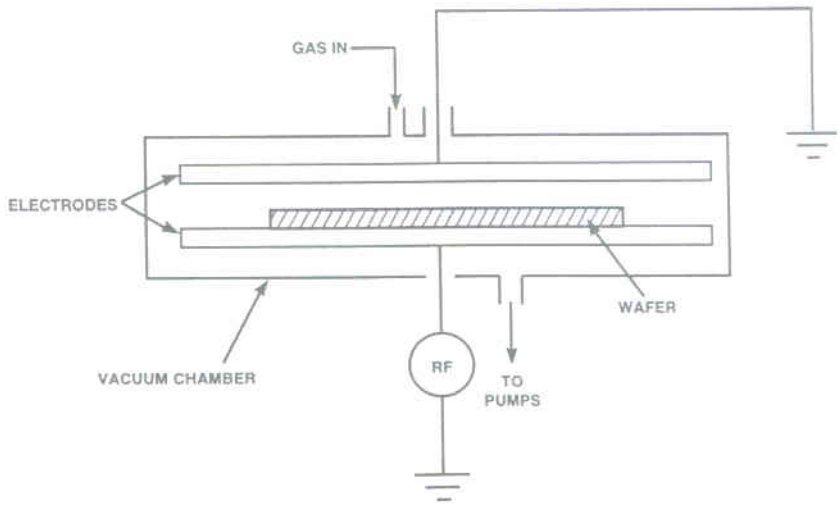


Figure 2.6 A schematic diagram of standard RIE etcher **[6]**

As shown in figure 2.7, an RF-powered wafer chuck is located in close proximity to a high–ion-density plasma source, such as an electron cyclotron resonance (ECR) or helicon RF plasma. The high-ion-density source provide high ion fluxes at relatively low pressures (<5 mtorr) and the RF-powered wafer chuck can be used to control the ion kinetic energy independently. In this arrangement, the wafer can be either immersed

in or remote from the plasma source. [6]

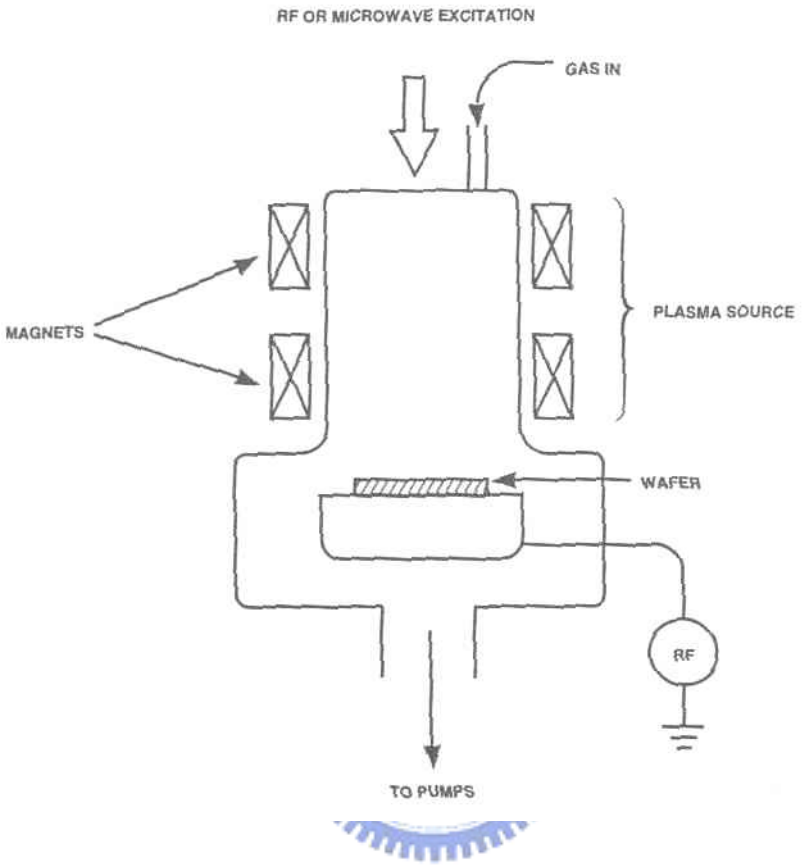


Figure 2.7 A schematic diagram of an advance RIE etcher (ECR) **[6]**

2.2Cell biology Applications

2.2.1 The Mechanism of Extracellular Matrix Pattern

[11]

In vertebrates and invertebrates many peripheral axons grow through connective tissue or along basal laminae. These patterns were initially thought to result from preferential extension through channels or along hard surfaces. Simple studies of outgrowth *in vitro* were instrumental in revising this view. For example, when neurons were grown on patterned substrates (i.e. stripes of one substance alternating with patches of a second), the axons extended preferentially along pathways of the more adhesive substrate, even when the less adhesive substance was quite capable of supporting neurite outgrowth on its own, as illustrate in Figure 2.8, growth cone extend *only* on the collagen-coated surface. We now know that axonal preferences correlate only imperfectly with adhesiveness, but the main point stands: growing axons recognize molecular difference among the substrates along which they grow, and these distinctions can regulate the direction and rate of their growth.

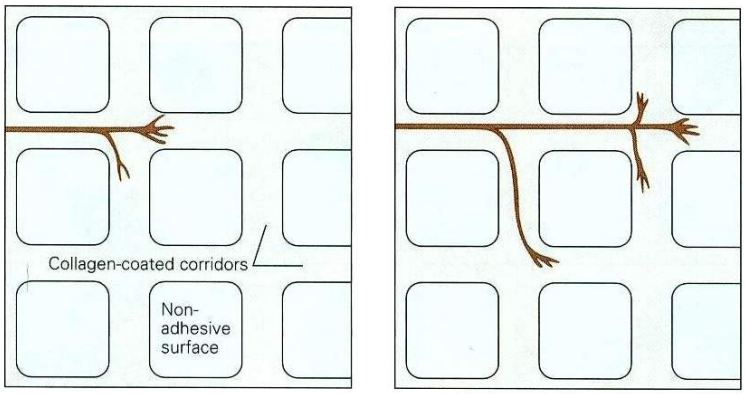


Figure 2.8 The growth cone extension on an extracellular matrix component. [11]

Numerous substances capable of promoting outgrowth *in vitro* have now been identified, including **laminin**, **collagens**, **fibronectin**, and some **proteoglycans**, and the laminin, collagens, fibronectin, and some proteoglycans are components of extracellular matrix (ECM).

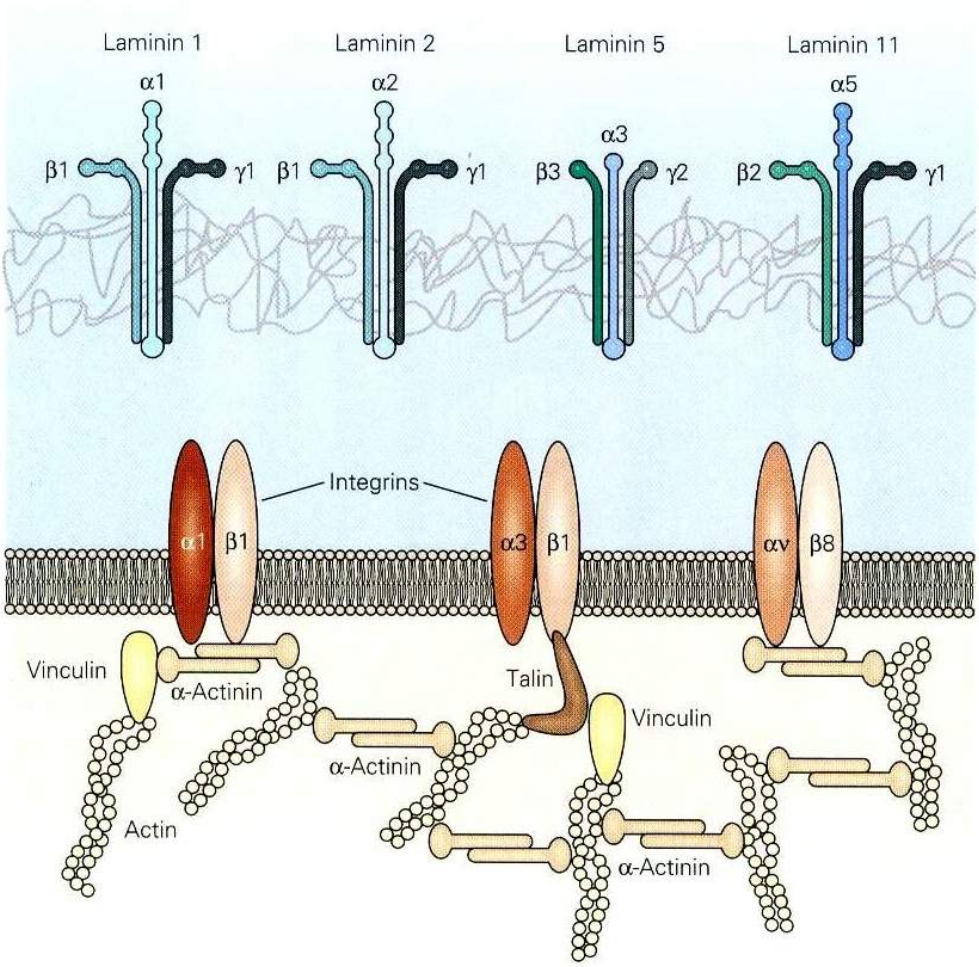


Figure 2.9 Laminins in basal laminae interact with integrins on growth cones. [11]

How do axons recognize growth-promoting molecules in the ECM? A variety of matrix-binding proteins have been isolate from neural cells, but the main signaling receptors appear to be the **integrins** and integrins are heterodimers of α and β subunits, drawn from a set of at least 16α and 8β chains. Essentially all cells in the body bear at least one integrin, and some express several. Each dimer recognizes a distinct set of ligands — α1β1, for example, binds to collagens and laminins, α4β1 binds to fibronectin, and so on. At least seven different integrin heterodimers bind to laminins, but they differ in the laminin isoforms they prefer and the domains on laminin that they

recognize (see Figure 2.9), laminins are major components of basal laminae and account for much of the axon outgrowth-promoting ability of the ECM. Laminins are cruciform heterotrimers of related α, β, and γ subunits, drawn from a family of at least 5α, 4β, and 3γ genes.) Together, the multiplicity of integrins and matrix components provides the potential for considerable subtlety and specificity in the interactions of growth cones with the ECM. So, extracellular matrix molecules promote neuron outgrowth and integrins are matrix-binding proteins in nerve cell.

Chapter 3 Design

3.1 Mask layout design

The layout of sub-micro protein patterns that we design is illustrated in Figure 3.1 and Table 3.1. 6 set of patterns to be plotted; including line patterns (0.3 and 0.6um), line combing dash line patterns, T shape (1.8um) and dash line patterns (0.6 um). Each of them has 4 different steps with variable pitch.

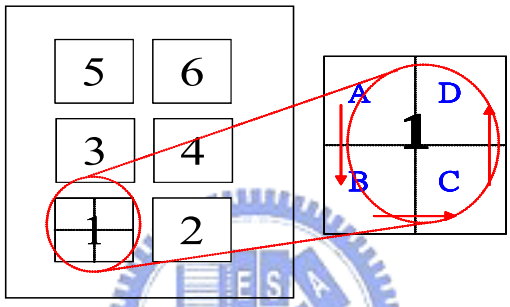


Figure 3.1 The design of mask layout.

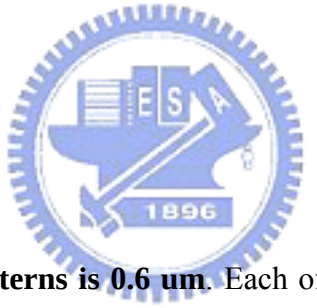
Table 3.1 The list of mask layout design.

編號	形狀（單位：um）		間距（單位：um）
1A	實線形矩陣（實線：0.3 × 900）	間距	實線間距：0.3
1B			實線間距：0.6
1C			實線間距：0.9
1D			實線間距：1.2
2A	實線形矩陣（實線：0.6 × 900）	間距	實線間距：0.6
2B			實線間距：1.2
2C			實線間距：1.8
2D			實線間距：2.4
3A	[實線形+虛線形]矩陣：（實線0.6 × 375；虛線0.6 × 1.2）	間距	虛線間距：0.6
3B			虛線間距：1.2
3C			虛線間距：1.8
3D			虛線間距：2.4
4A	T形矩陣：（T形由4個1.8 × 1.8的矩形組成）	間距	矩形間距：0.6
4B			矩形間距：1.2
4C			矩形間距：1.8
4D			矩形間距：2.4
5A	虛線組矩陣：（虛線：0.6× 12）	間距	虛線間距：0.6
5B			虛線間距：1.2
5C			虛線間距：1.8
5D			虛線間距：2.4
6A	虛線組矩陣：（虛線：0.6 × 60）	間距	虛線間距：0.6
6B			虛線間距：1.2
6C			虛線間距：1.8
6D			虛線間距：2.4

3.1.1 Narrow Line patterns

The design rule of **narrow line patterns is 0.3 um**. Each of them has 4 variations of steps with different pitch and named 1A, 1B, 1C, and 1D (Table 3.1). There are 4 purposes to design these patterns.

1. **Test the resolution limitation of μCP method**.
2. Compare the pattern accuracy with variable space/line ratio in each step of process.
3. Compare the pattern accuracy and neuron outgrowth between two different line widths (1A~1D groups, 2A~2D groups).
4. Observe the cell/neuron outgrowth with variable space/line ratio of protein patterns.



3.1.2 Line patterns

The design rule of **line patterns is 0.6 um**. Each of them has 4 variations of steps with different pitch and named 2A, 2B, 2C, and 2D (Table 3.1). There are 3 purposes to design these patterns.

1. Compare the pattern accuracy and neuron outgrowth between two different line widths (1A~1D groups, 2A~2D groups).
2. Compare the pattern accuracy with variable space/line ratio in each step of process.
3. Observe the cell/ neuron outgrowth with variable space/line ratio of protein patterns.

3.1.3 Small dash line with solid line patterns

The design rule of **small dash line with solid line patterns; line is 0.6 um and dash line is 0.6X1.2 um**. Each of them has 4 variations of steps variation with the distance between two dash lines and named 3A, 3B, 3C, and 3D (Table 3.1). There are 4 purposes to design these patterns.

1. Compare the pattern accuracy with variable space in each step of process.
2. Observe the cell / neuron outgrowth with variable space of protein patterns.
3. Test the maximum distance of dash line patterns that axonal outgrowth could across.
4. Count the number of dash line patterns that axonal outgrowth could across.

3.1.4 T shape patterns

The design rule of **T shape patterns is component of 4 squares (1.8X1.8 um)**.Each of them has 4 variations of steps with the distance between two squares and named 4A, 4B, 4C, and 4D (Table 3.1). There are 4 purposes to design these patterns.

1. Compare the pattern accuracy with variable space in each step of process.
2. Compare the pattern accuracy with different shape in each step of process.
3. Observe the cell/neuron outgrowth with different shape of protein patterns.
4. Observe the cell/neuron outgrowth with variable space of T shape protein patterns.

3.1.5 Middle dash line patterns

The design rule of **middle dash line patterns is 0.6 X 12 um**. Each of them has 4 variations of steps with the distance between two dash lines and named 5A, 5B, 5C, and

5D (Table 3.1). There are 4 purposes to design these patterns.

1. Compare the pattern accuracy with variable space in each step of process.
2. Observe the cell/ neuron outgrowth with variable space of protein patterns.
3. Test the maximum distance of dash line patterns that axonal outgrowth could across.
4. Observe variable line/width of dash line patterns (3A~3D, 5A~5D, and 6A ~ 6D) that axonal outgrowth could across.

3.1.6 Long dash line patterns

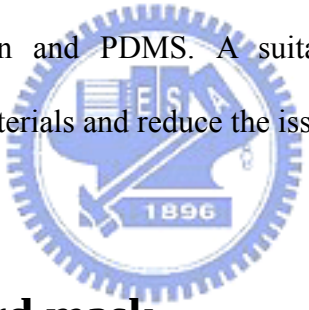
The design rule of **middle dash line patterns is 0.6 X 60 um**. Each of them has 4 variations of steps with the distance between two dash lines and named 6A, 6B, 6C, and 6D (Table 3.1). There are 4 purposes to design these patterns.

1. Compare the pattern accuracy with variable space in each step of process.
2. Observe the cell/ neuron outgrowth with variable space of protein patterns.
3. Test the maximum distance of dash line patterns that axonal outgrowth could across.
4. Observe variable line/width of dash line patterns (3A~3D, 5A~5D, and 6A ~ 6D) that axonal outgrowth could across.

3.2 New procedures of Micro contact printing (μCP) method

In figure 3.3, the rectangular frame marked by dashed outline represents the new procedures of Micro contact printing (μCP) method we re-designed. We deposited a TEOS film with 0.3 um thickness above the silicon structure of wafer. After photolithography, we use RIE etching and transfer of the patterns into the TEOS film, then using RIE etching again and the patterns have been successfully transfer into substrate of silicon.

When the pattern size is into sub-micro level, the sticking issue between silicon and PDMS will be getting worse. Buffer layers are indeed needed and used to avoid sticking issue between silicon and PDMS. A suitable material could avoid the crosslinking from these two materials and reduce the issue of pattern sticking .



3.2.1 TEOS film hard mask

In this thesis, we use the DUV lithography to fabricate sub-micro patterns. Through our related researches, photoresist base is used as the master [4]. But in DUV lithography, the thickness of photoresist is usually less than 1um.That means the aspect ratio will be limited.

Upon this, we re-design the procedure of Micro contact printing (μCP) method and add TEOS as a new film stacking above silicon structure of wafer. The TEOS is an oxide film and will be a hard mask to increase the etching selectivity between DUV photoresist and silicon. The range of aspect ratio will be tested by tuning RIE etching recipes, including the power, gas flow rate and components of gases.

We will use the new procedure of Micro contact printing (μCP) method to fabricate sub-micro protein patterns and compare the pattern accuracy with variable size (0.3, 0.6 um), pitch (1:1~1:4) and different shape in each step. In theory, we can get 5 times Photoresist/Silicon etching selectivity by TEOS hard mask.

3.2.2 Buffer layers

In step 5 of figure 3.2, some samples will be tested to be buffer layer. The types of materials are including organic, inorganic films and surfactants. We will choose the suitable one to avoid sticking issue between silicon and PDMS and gets a silicon base of master.

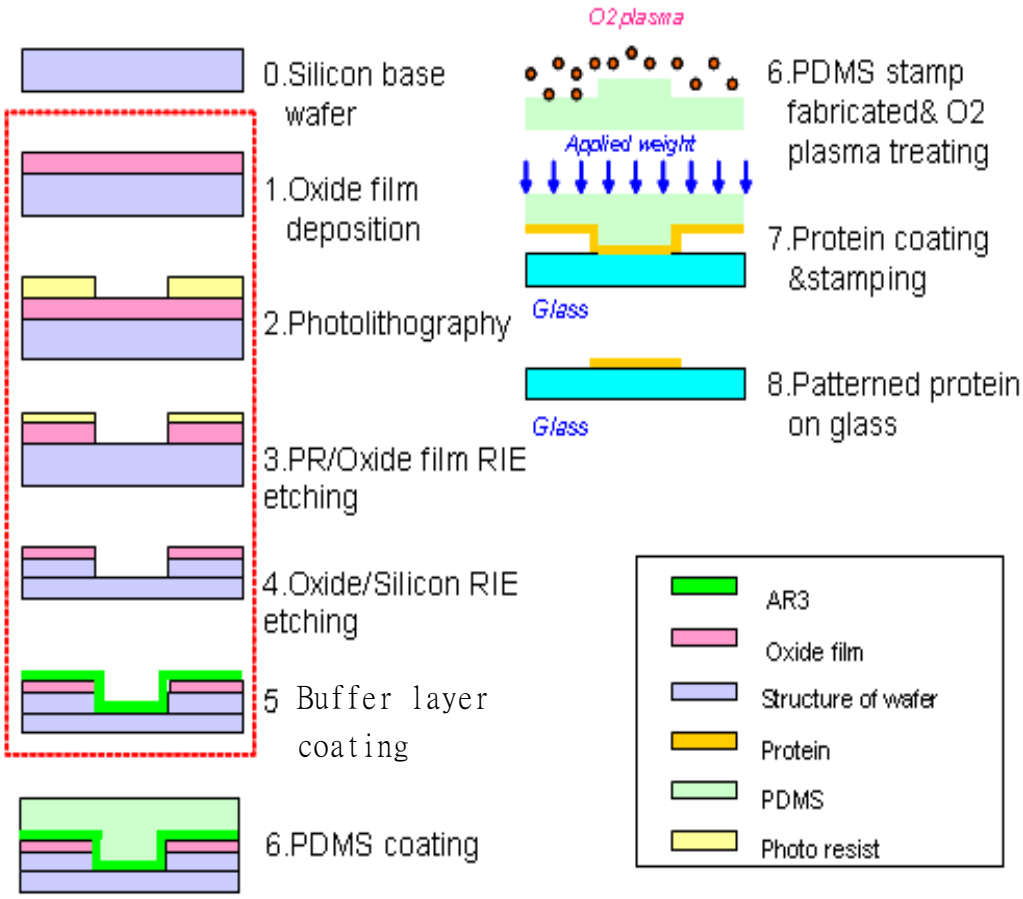


Figure 3.2 Schematic illustration of the procedure of silicon base of Micro Contact Printing