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Parametric Studies for the Fabrication of DNA Chip

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Abstract

Biochip technology, an innovation for genomic science, is an interdisciplinary high technology involving physical sciences, biomedical science, electronic engineering, and optoelectronic technology. We have developed a protocol that can routinely be used in our laboratories. Optimized conditions and parameters for a successful fabrication of DNA chip are described in this report.

Keywords: DNA Chip, Gene Expression, Nucleic Acids Probe Array

1. Introduction

In order to increase chance of success in DNA chip fabrication, many parameters in chemical reactions need to be optimized. (Ting *et al.*, 2000) Here, we describe the problems encountered during a cycle of fabrication and also propose solutions, done or yet to be done, to each problem. A protocol we adapted to fabricate a DNA chip includes several stages:

- Coating of underlayer (polyimide)
- Coating of photo resist (SU-8) layer
- Exposure of photo resist
- Developing of photo resist
- Etching of underlayer (polyimide)
- Synthesis of probe nucleotide
- Stripping off photo resist layer

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Each stage exists some problems. Some are of little importance; yet, some can be fatal.

2. Materials and Methods

2.1 Coating of underlayer (polyimide):

Underlayer is to separate photo resist layer and chip surface. Without this layer, photo resist SU-8 will stick on chip surface and cannot be completely stripped off easily. A good choice of this underlayer is polyimide. We use polyimide from Toray because we are not able to purchase this chemical from other sources at the moment. Although polyimide from CIBA-Geigy was used in the literature (McGall *et al.*, 1996), polyimide from Toray seems to work properly as well. Nevertheless, other polyimide sources are worth trying in future R&D.

Coating process relies on the quality of a spin-coater. Performance of a spin-coater does affect uniformity and thickness of coating layer. Moreover, a dust-free space is necessary to have a clean and uniform coating. At present, we are lack of these conditions. Nevertheless, the central region of substrate seems to be uniform and this is the region we want to synthesize nucleic acid probes on. In order to uniformly coat polyimide layer and later photo resist layer to almost whole 3" wafer or standard slide, it is strongly recommended to use a sophisticate spin-coater such as a Karl Suss RC8 Gyrset for better performance.

Polyimide from Toray is insoluble in anisole, a solvent used by Affymetrix group. A suitable solvent for Toray's polyimide (SP341) is pyrrolidinone. We also use this solvent to dilute polyimide. When 30%(v/v) of polyimide in pyrrolidinone is used, we can produce a 1-micron thick coating layer. (Spin rate is 1,200 rpm, 15sec and then 4,000 rpm, 30 sec. Baked on a hot plate of 100℃ for 2 min.) Accordingly, 50%(v/v) polyimide under same conditions can produce a layer around 5 microns in thickness. For convenience, low consumption, and good performance, we use 25%(v/v) polyimide for underlayer coating.

When a fine pattern (*e.g.* 10 μm ×10 μm cell size) is required by design, only thin underlayer can meet the goal. Since we are using chemical etching, *i.e.* wet etching, in our process, lateral etching is just as fast as vertical etching. Polyimide layer needs thorough etching in vertical direction. Unavoidably, this also produces unwanted lateral etching that causes troubles in making fine patterns. Besides, it cannot help but extend the space between two cells.

Therefore, a polyimide layer of less than 1 micron is a better choice when fine pattern is needed. One should be more skillful in running reactions when thin underlayer is used. In order to obtain a reliable and consistent biochip, fine pattern design is not recommended.

2.2 Coating of photo resist (SU-8) layer:

After trying some positive and negative photo resists, we choose the negative resist SU-8 (MicroChem Corp.). This is the only resist we know that can remain stable during DNA synthesis cycles. We follow a protocol developed by the Affymetrix group. (McGall *et al*., 1996) To coat resist layer, spin-coating was set to 1,200 rpm for 15 sec and then 2,500 rpm for 20 sec. Soft bake was done at 80℃, 1 min. This gave a nominal resist layer of less than 1 micron, which is good enough to protect chip surface during probe synthesis. Again, resist layer is not uniform with our facility. Yet, results are acceptable in our pilot research.

2.3 Exposure of photo resist:

Since we did not have a mask aligner yet, a xenon lamp and a custom-designed stainless steel chamber were substituted in our current experiment. Unfortunately, there are double beams in the light source. We must reduce the exposure time to only 5 sec for reflective silicon wafer substrate and about 8 sec for transparent glass slide substrate. This can greatly eliminate the undesired double images with a cost on the stability of cross-linked resist. Images in *Fig. 1* show the differences between 5-sec exposure (left row) and 10-sec exposure (right row) when silicon wafer pieces were used as substrate.

2.4 Developing of photo resist:

We use the Developer provided by the MicroChem Corp. to develop the photo resist. Since our resist layer is very thin compared to other MEMS works, it is quite straightforward to develop the resist.

Only completely remove the resist inside a cell can we etch polyimide underneath. Incomplete developing or rinsing of resist will mess up chip surface and no synthesis can be successful afterwards. Indeed, it is somehow tricky to completely remove the dissolved SU-8. Special attention was paid in our protocol. Whole slide was immersed in a dish of Developer for 2 min and rinsed (5 to 10 sec) with fresh Developer from a washing bottle. Whole slide was

transferred to and agitated very mildly in another petri dish with fresh Developer for 1 min. Again, it was rinsed (5 to 10 sec) with fresh Developer from a washing bottle.

Since improper rinsing liquids will leave white stains on chip surface, we have been carefully using acetonitrile to rinse the surface after developing. Whole slide was rinsed with acetonitrile and vacuum-dried. Again, it was baked at 100℃ for 1 min. It should be noted that flushing the surface strongly may lift-off cross-linked resist and whole cycle has to be restarted.

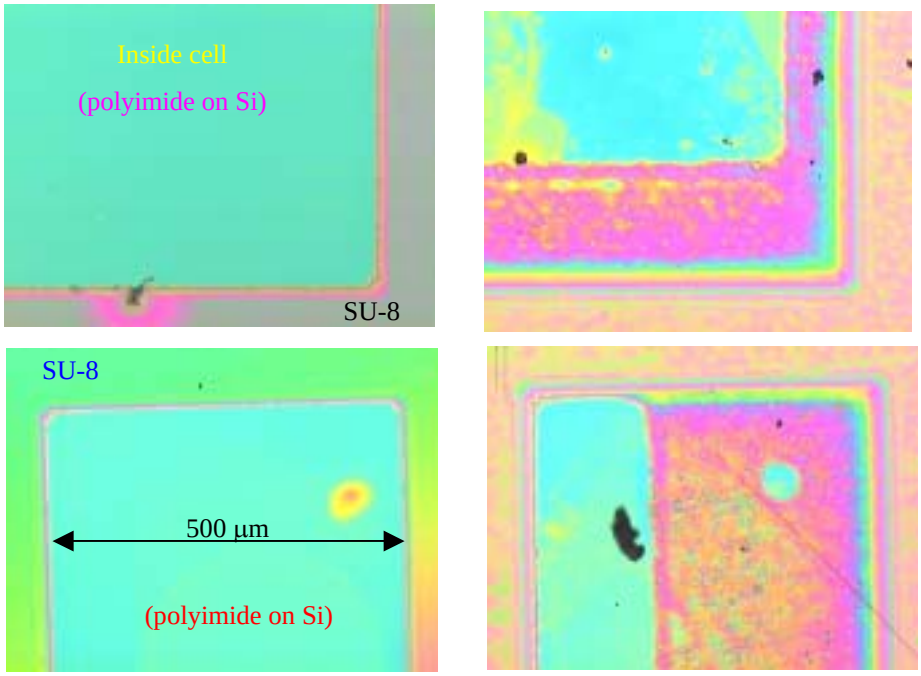

Figure 1. Differences are seen between 5-sec (left row) and 10-sec (right row) exposures. [Images, with same magnification, were taken after developing.]

2.5 Etching of underlayer (polyimide):

We use pyrrolidinone to etch polyimide after pattern was developed. This reaction is critical. Polyimide etching in vertical direction should be thorough so that nucleotide can be added onto chip surface afterwards. However, lateral etching should be as less as possible. It is worth mention that polyimide layer can usually be dissolved within 5 sec. Yet, this reaction time is changed to more than 5 min after 25%(v/v) polyimide coating layer was covered by SU-8 photo resist. When 50%(v/v) polyimide coating layer is used instead, it still takes more than 2 min to

completely etch the underlayer.

When polyimide underlayer is completely etched, surface of substrate will emerge. On silicon wafer, it looks like mirror surface. On a slide, it is difficult to identify transparent surface through native eyes. Nevertheless, useful information still can be obtained through an optical microscope. Slide surface without polyimide appears as smooth domain. The pictures in *Fig. 2* show polyimide etching had reached surface of substrate.

Since the chemicals used in probe synthesis can further dissolve polyimide, spacing between two reaction cells should be adjusted to prevent cross contamination. Wrinkles, or even cracks, appeared on the resist layer after polyimide etching if SU-8 was underexposed. Chemistry of pyrrolidinone on SU-8 cross-linked film was not studied here. However, we are confident that SU-8 film remains stable during biochemical synthesis reactions. This SU-8 film seems intact after one cycle of nucleotide synthesis.

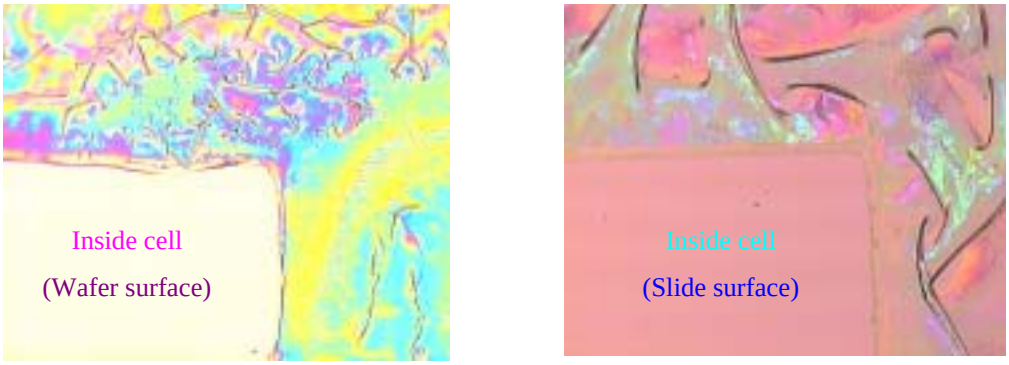

Figure 2. Polyimide layer has been completely etched. Left: A silicon wafer piece was used as substrate. Right: Glass slide was used as substrate. Magnifications are same as in Fig. 1.

2.6 Synthesis of probe nucleotide:

The method we used had been described in previous documents. Attentions should be paid to leaking or seeping in tubing fittings. Biochemical reaction steps have been optimized using the ABI 391 synthesizer. At the moment, we use homemade (by Academia Sinica) cassettes, to adapt glass slides only. We plan to make another cassette to adapt 3" silicon wafer. When the new cassette is used, we will calibrate the reaction volume again and then optimize new parameters of

the ABI 391 synthesizer.

2.7 Stripping off photo resist layer:

This is an important step in producing gene chip. Based on our experiences, SU-8 layer usually can be stripped off by agitation in the Remover, provided by the MicroChem Corp., at 65 ℃ for 30 min.

3. Conclusions

From the discussions stated above, we conclude that fabrication of biochip is straightforward. (Ting and Chiou, 2000) All procedures have been carried out in our laboratories. Necessary conditions to improve the efficiency and to increase success percentage include: (1) dust-free space for clean coating, (2) a high quality spin-coater for uniform coating, and (3) a good resolution mask-aligner with well-designed light sources for good patterning.

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基因晶片之製作參數研究

丁君毅*

摘　　要

基因晶片技術是針對基因體科學而發展的創新研究，連結物理科學，生物醫學，電子工程，與光電技術綜合領域的高等科技。基因晶片製程各步驟已在本實驗室中開發得出最適化參數。所得結果列述於本報告中。

關鍵詞：基因晶片、探針陣列、基因表現。

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