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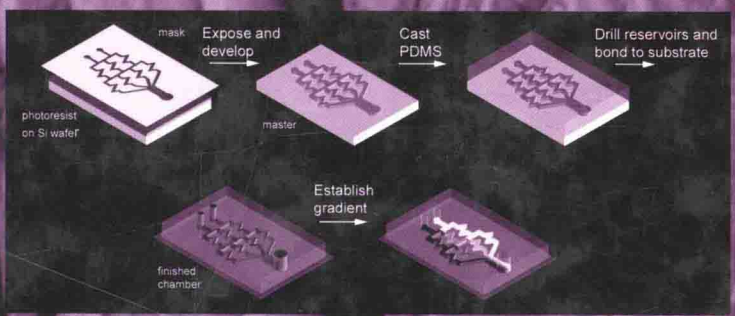
Microfluidic Techniques

Reviews and Protocols

Edited by

Shelley D. Minteer



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Preface

Microfluidic Techniques highlights recent advances in microfluidic techniques for biological applications. The first section of the book contains chapters that focus on the most popular techniques for fabrication of microchips (photolithography, laser ablation, and soft lithography), while the remaining chapters will focus on microfluidic techniques for bioanalytical assays and bioprocesses, such as DNA analysis, PCR, immunoassays, and cell reactors.

The chapters found here should provide molecular biologists and biochemists with the state-of-the-art technical information required to perform readily reproducible microscale bioassays and bioprocessing in the laboratory.

Shelley D. Minteer

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Contents

1

Overview of Advances in Microfluidics and Microfabrication

Shelley D. Minteer and Christine M. Moore

Miniaturized instrumentation and reactors have attracted great interest in the last decade. The first reported use of a microchip was in 1979, when a gas chromatograph air analyzer was fabricated on a silicon wafer *(1)*. It was not until several years later, when flow injection analysis was performed on a chip, that microchips gained attention *(2)*. Over the last decade, research in integrated microfluidic devices (which are typically referred to as lab-on-a-chip devices or micro total analysis systems [μTAS]) has expanded to include sample preparation, fluid handling, microreactors, separation systems, cell handling, and cell culturing. The incorporation of these techniques has led to microfluidic devices that have been used to perform capillary electrophoresis-based separations, magnetic microparticle-based separations, immunoassays, DNA analysis, and clinical diagnostics, along with the design of highly efficient microreactors *(3,4)*. They have been applied in medical analysis, environmental monitoring, biochemical analysis, and microchemistry *(4)*.

Advantages of such systems include high performance, design flexibility, reagent economy, miniaturization, and automation *(3)*. The use of small flow channels, typically between 1 and 100 μ*M* is important when considering the networks of microscopic channels in substrates in which analytes are transported, mixed, and separated *(5)*. Miniaturization allows high-throughput screening, portability, and high-density arrays on a small scale. By decreasing the dimensions of devices, space, time, and the amount of analyte decrease *(5)*. Smaller apparatuses are lower in cost, consume less energy and material, and are minimally invasive when referring to a biological application. The use of less material is protecting our limited resources and the disposability of the microdevices helps to avoid contamination. More sophisticated systems can be

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built using small parts, and further development of science can occur because the macro laws do not always apply to microsystems *(6)*.

Although this book focuses on biological applications of microchip technology, it is divided into two sections. The first section describes fabrication techniques. It contains detailed background and methods for photolithography, soft lithography, and laser ablation. The second section provides details of the different applications of microchip technology in molecular biology. Methods for DNA amplication through PCR-on-a-chip, DNA sequencing and separation, magnetic separations, immunoassays, cell culturing, and cell analysis are described.

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I

Microfabrication Methods

2

Introduction to Microfabrication Techniques

Rabih Zaouk, Benjamin Y. Park, and Marc J. Madou

Summary

The advent of photolithography literally brought about the integrated circuit (IC) revolution of the latter part of the twentieth century. Almost all electronic devices that we use today have one or more ICs inside. Improving lithography techniques led to smaller and smaller transistors, which translated into faster and more efficient computing machines. Photolithography also powered the advent of MicroElectroMechanical Systems (MEMS), which are now starting to become more and more diverse in commercial products from mechanical to biomedical devices, helping to change the way people perceive the applicability of IC technology. In this chapter, we examine basic photolithography techniques and their uses in soft lithography and MEMS.

Key Words: Photolithography; microlithography; lithography; soft lithography; microfabrication; MicroElectroMechanical Systems; BioMEMS, microfluidics.

1. Photolithography: An Overview

The word *lithography* (Greek for the words *stone* [*lithos*] and *to write* [*gráphein*]) refers to a process invented in 1796 by Aloys Senefelder. Senefelder found that stone (he used Bavarian limestone) when properly inked and treated with chemicals could transfer a carved image onto paper ***(1)***.

The most widely used form of lithography is photolithography. In this process, a pattern is transferred to a photosensitive polymer (a photoresist) by exposure to a light source through an optical mask. An optical mask usually consists of opaque patterns (usually chrome or iron oxide) on a transparent support (usually quartz) used to define features on a wafer. The pattern in the photoresist is then further transferred to the underlying substrate by subtractive (etching) or additive (deposition) techniques. The combination of accurate alignment of a successive set of photomasks and exposure of these successive

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patterns leads to complex multilayered structures. Photolithography has matured rapidly by continuous improvements in the ability to resolve ever-smaller features. Research in high-aspect-ratio resist features, driven by the field of MicroElectroMechanical Systems (MEMS), is also being actively pursued, as opposed to the essentially two-dimensional processes used traditionally. This is especially important in the fabrication of microfluidic molds.

Photolithography and pattern transfer involve a set of process steps as summarized in **Fig. 1**. As an example, we use an oxidized silicon (Si) wafer and a negative photoresist to transfer a pattern from a mask to a layer of silicon dioxide. An oxidized wafer (**Fig. 1A**) is coated with a 1-μm-thick negative photoresist layer (**Fig. 1B**). After exposure (**Fig. 1C**), the wafer is rinsed in a developing solution or sprayed with a spray developer, which removes the unexposed areas of photoresist and leaves a pattern of bare and photoresist-coated oxide on the wafer surface (**Fig. 1D**). The resulting photoresist pattern is the negative image of the pattern on the photomask. In a typical next step after development, the wafer is placed in a solution of HF or a mixture of HF and NH_4F that attacks the oxide at a much faster rate than the photoresist or the underlying Si (**Fig. 1E**). The photoresist prevents the oxide underneath from being attacked. Once the exposed oxide has been etched away, the remaining photoresist can be stripped off with a solution that only attacks the photoresist, such as a strong acid (e.g., H_2SO_4) or an acid-oxidant combination (e.g., piranha, H_2SO_4:H_2O_2) (**Fig. 1F**). Other liquid strippers include organic solvent strippers and alkaline strippers (with or without oxidants). The oxidized Si wafer with etched windows in the oxide (**Fig. 1F**) now awaits further processing. Recently, photoresists are increasingly being used in applications in which the resist is a permanent part of the final device rather than just a sacrificial layer for patterning the substrate.

2. Basic Photolithography Techniques

Each implementation of the photolithographic process has its own specific requirements, but there is a basic common flow of process that are common to most procedures. In this section, we introduce the basic lithography steps of preparation of wafer, resist application, exposure, development, and pattern transfer.

2.1. Preparation of Wafer

2.1.1. Cleaning of Wafer

Physical contaminants such as dust particles can hinder the lithography process by preventing light from exposing the photoresist or by disturbing the surface uniformity of a coated photoresist. Chemical contaminants may also react with various materials used in the lithography process, creating unwanted effects.

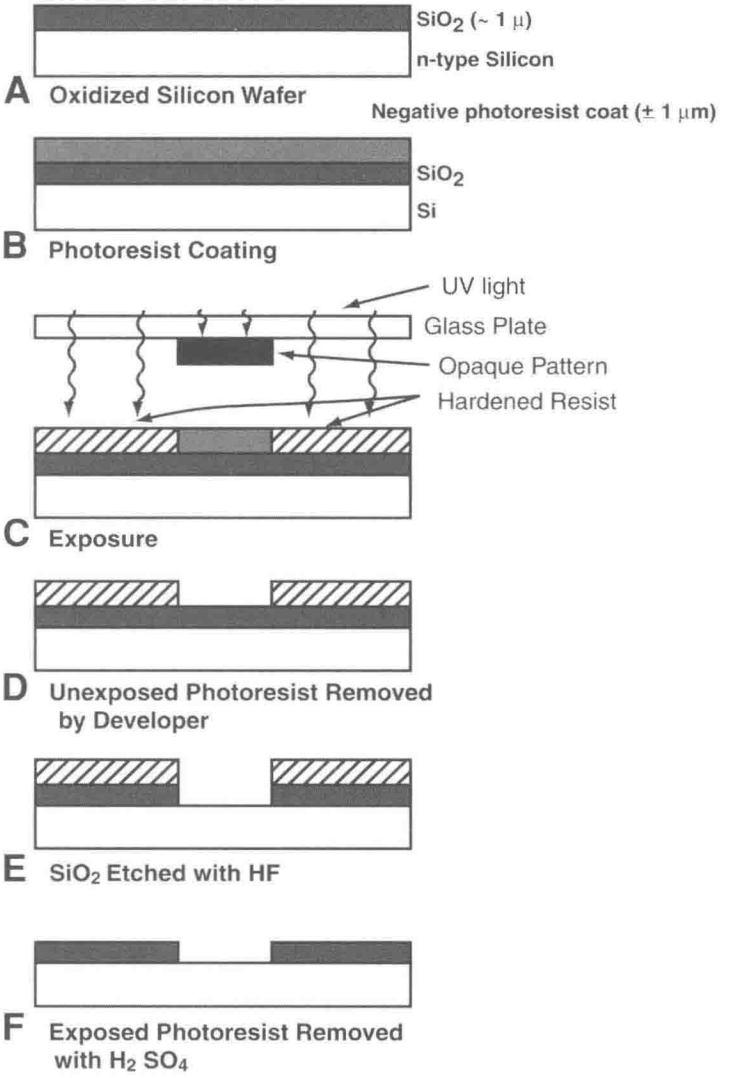

Fig.1. Process flow of basic photolithography followed by pattern transfer. The example uses (**A**) an oxidized Si wafer and a negative photoresist system. The process steps are (**B**) photoresist coating, (**C**) exposure, (**D**) development, (**E**) oxide etching, and (**F**) resist stripping and oxide etching. These steps are explained in detail in the text.