

# Chapter 1

## Introduction to BioMEMS

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### 1.1 What is BioMEMS?

BioMEMS, or *biomedical microelectromechanical systems*, has emerged as a subset of MEMS devices for applications in biomedical research and medical

microdevices.\* Merging traditional MEMS devices with discussion of bioMEMS is essential, because some readers will not have engineering backgrounds or familiarity with MEMS technology. For the purpose of teaching bioMEMS, the following subjects will be covered:

- (1) Microfabrication of silicon, glass, and polymer devices
- (2) Microfluidics and electrokinetics
- (3) Sensors, actuators, and drug-delivery systems
- (4) Micro-total-analysis systems ( $\mu$ TAS) and lab-on-a-chip devices (LOC)
- (5) Clinical laboratory medicine
- (6) Detection and measuring systems
- (7) Genomics, proteomics, DNA, and protein microarrays
- (8) Emerging applications in medicine, research, and homeland security
- (9) Packaging, power systems, data communication, and RF safety
- (10) Biocompatibility, FDA, and ISO 10993 biological evaluations

The bioMEMS industry is increasing rapidly with a growth rate of 11.4%, with projected revenues of \$850 million in 2003 to over \$1 billion in 2006 [Rebello, 2004].

### 1.1.1 Fabrication

Traditional fabrication techniques previously imported from integrated circuit manufacturing to MEMS devices have undergone yet another transition for bioMEMS devices, with an increased awareness of microfluidic physics and the surface science of silicon, glass, polymers, and ceramics. Modification of surfaces for biomedical assays and biocompatibility has emerged as a complex science, with abundant opportunities for creating novel techniques and applications that can be patented and ultimately brought to market. New techniques are being developed for molding, replication, casting, and bonding that are essential for mass production with reproducibility and functional reliability at low cost, both of which are vital to the medical disposables market. In addition, the 3D construction of bioMEMS devices, not just in shape but in the embodiment of electromechanical, chemical, and biological materials, reaches beyond the basic concepts of cantilevers, inertial masses, and diaphragms of typical MEMS devices.

Fabrication process steps are becoming more complex as integrated electronics, once thought better left to side-by-side devices in the MEMS realm, are now becoming essential as microreactor chambers, and as detection schemes become incorporated into the device itself. In addition, packaging for safety and biocompatibility poses a significant challenge for the bioMEMS engineer.

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\*In Europe the term *microsystem or microstructure technology* (MST) is used instead of MEMS.

### 1.1.2 Structure

BioMEMS devices can typically be considered as having at least one feature's dimension in the submicron to micron range (100 nm–200  $\mu\text{m}$ ), and other dimensions of up to several millimeters. On one end of the application scale they may be the platform for nanotechnologies, while on the other end they may be the key component to a much larger device such as a medical imaging machine. They may operate *in vivo* or *in vitro* (inside or outside a living system), and have self-contained or external power sources. They may be *smart systems* with integrated microprocessors, and operate as either an open-ended (sensor or actuator) system or a closed-loop system (autoregulation). They may be all encompassing devices, but more typically they are integrated with other components and perform one or more functions in a chain of operations connected by tubing or other conduits. Implanted devices may be part of a distributed system such as fiber optic sensors that provide continuous information via light from various parts of the body to a central medical device.

Among the advantages of biochip miniaturization are lower manufacturing costs, reproducibility, small sample size, and reagent use. Improved signal-to-noise ratio, improved response time, precise control of mixing, reacting, and discarding of waste products, in-line or embedded detection methods, and high throughput are also advantages of miniaturized biochips.

### 1.1.3 Goal of this book

Upon completing this book you will have acquired an understanding of the many necessary skills for conceiving, and designing bioMEMS and medical microdevices, and for applying them to research and medicine. This includes fabrication with silicon, glass, and polymers substrates; covalent and noncovalent surface modifications; self-assembled monolayers (SAMs); transport processes such as laminar flow and electrokinetic phenomena (electro-osmosis, electrophoresis, dielectrophoresis, and electrowetting); and sensor and actuator concepts.

Next you will learn about micro-total-analysis systems ( $\mu\text{TAS}$ ) and lab-on-a-chip (LOC) devices, and basic genomics and proteomics as a prelude to DNA and protein microarrays. You will also develop an understanding of biocompatibility based on the ISO 10993 Standard, and the options for powering and packaging devices to obtain optimal performance and safety.

Although this book is suitable for a one-semester course at the graduate level, instructors may additionally incorporate a laboratory experience to provide hands-on design and fabrication of a bioMEMS device. Moreover, this book may serve as useful extension for a MEMS engineering class, technology management course, or elective study for anyone in related fields.

## Chapter 9

# Micro-Total-Analysis Systems ( $\mu$ TAS)

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## 9.1 Lab-on-a-Chip

### 9.1.1 Introduction

*Micro-total-analysis systems* ( $\mu$ TAS), and the subset of devices referred to as *lab-on-a-chip* (LOC), derive from application of “hard” and “soft” fabrication techniques for the manufacture of miniaturized devices that perform all or part of a biochemical analysis.  $\mu$ TAS may be hybrids of multiple chips, integrated electronics, and external supports; while LOC refers more specifically to a microfluidic chip or other device that performs a well-defined analytical task [Guber et al., 2004]. *Biochips* more generically include the LOC devices and *microarray* devices. The definition of these terms varies with different authors and may be used interchangeably. Commonly used materials, fabrication techniques, and the history of these devices have been discussed in previous chapters.

Microanalysis devices can be broadly classified as either microfluidic-based or microarray-based microdevices [Krishnan et al., 2001]. *DNA chips* (for the study of DNA and RNA) include both DNA LOC devices and *DNA microarrays*, while *protein chips* include both LOC devices and *protein microarrays*. These are discussed in Chapters 11 and 12.

The goal of  $\mu$ TAS and LOC devices is to achieve increased efficiency through smaller scales and to undertake analysis that cannot be done conveniently by other means. Advantages of smaller scales include the following:

- (1) improved transport through use of electrokinetic effects and miniaturized pumps;
- (2) efficient cells, molecular and particle separation, and immobilization;
- (3) smaller sample requirements and carrier volumes;
- (4) reduced reagent consumption; and
- (5) integration of channels, mixers, separators, reactions chambers, electrodes, and detectors into single devices.

Improved throughput of analytes occurs as a consequence of miniaturization and integration. LOC devices may incorporate microfluidic components, microsensors, microactuators, and customized surfaces created by chemical modification or coatings with inorganic and organic materials. Microspheres and beads are also integral to numerous LOC devices.

Most of the techniques employed for  $\mu$ TAS and LOC devices are investigational; hence, much of the discussion here derives from current research efforts. While certain representative works have been selected to introduce basic concepts, not all techniques are reported, and much can be gained by going directly to the literature and searching a topic of interest.

### 9.1.2 Chemical analysis

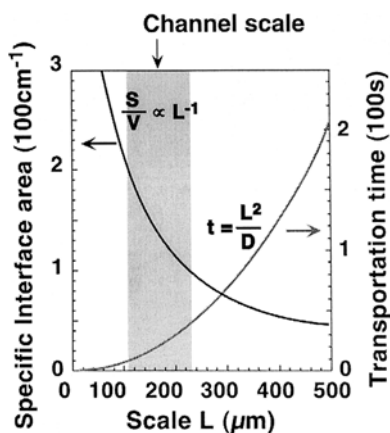
Although electrophoretic methods, especially for DNA analysis, have been the catalyst for LOC devices, new technologies have emerged for continuous and

sequential chemical processing. These devices are typically microfluidic in design, and incorporate either novel electrokinetic effects or pressure-driven systems for movement of fluids and solids.

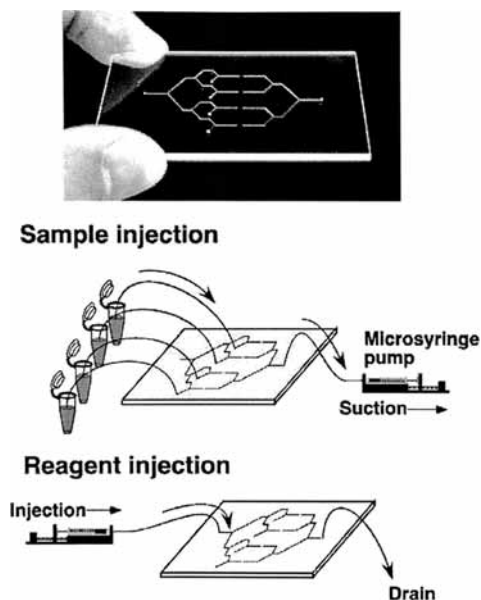
Recall from the discussion on diffusion in Chapter 5 that the mean-square displacement of a particle from its origin is proportional to time, and that surface area to volume is proportional to the scale of the microchannel. As the scale decreases, surface area to volume increases, while time for diffusion decreases. Figure 9.1 shows the scale dependence of the molecular transportation time and the specific interface area. In LOC devices, the channel size of 150–250  $\mu\text{m}$  means diffusion across the channel may occur in seconds.

Pressure-driven continuous-flow chemical processing chips with stabilized laminar flow, suitable for a variety of inorganic, organic, and physical chemistry reactions, are described by Sato et al. (2003). Applications include chemical analysis, multi-ion sensing, immunoassays, and cell analysis. An immunoassay chip, for example, is shown in Fig. 9.2. The following summarizes the process steps required to fabricate this chip:

Mechanically polished 0.7-mm thick Pyrex glass plates were used (top and bottom plates). Inlet and outlet holes were drilled by ultrasonic sandblasting on the top plate. For good contact between the substrates and the photoresist, and protection of the substrates during glass etching, 20-nm thick Cr and 100-nm thick Au layers were deposited on the substrates by physical vapor deposition. 2- $\mu\text{m}$  thick positive photoresist was spin-coated on the Au metal layer and baked at 90°C for 30 minutes. UV light was exposed through a photomask by using a mask aligner to transfer the microchannel pattern onto the photoresist. The photoresist was developed and a pattern with 10- $\mu\text{m}$  wide lines was obtained. The Au and Cr layers were etched with  $\text{I}_2/\text{NH}_4\text{I}$  and  $\text{Ce}(\text{NH}_4)_2(\text{NO}_3)_6$  solutions. The bare glass surface with the microchannel pattern was etched with a 50% HF solution at an etching rate of 13 mm/min. After glass etching, the remaining photoresist was removed in acetone and metals were removed in  $\text{I}_2/\text{NH}_4\text{I}$  and  $\text{Ce}(\text{NH}_4)_2(\text{NO}_3)_6$  solutions.

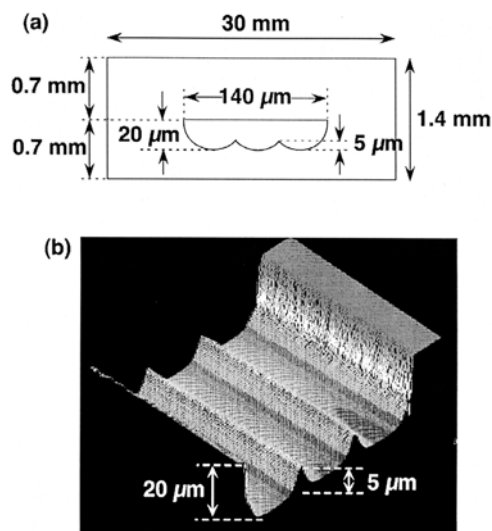


**Figure 9.1** Scale dependence of the molecular transportation time and the specific interface area. [Reprinted with permission from Sato et al. (2003), copyright Elsevier.]



**Figure 9.2** Immunoassay chip. [Reprinted with permission from Sato et al. (2003), copyright Elsevier.]

Microchannels were designed with guide structures (Fig. 9.3), forming a stable liquid-liquid interface. These required three photomasks and three independent etching cycles. Surface-chemical modification with octadecylsilane stabilized liquid flow in organic solvent carrying channels.



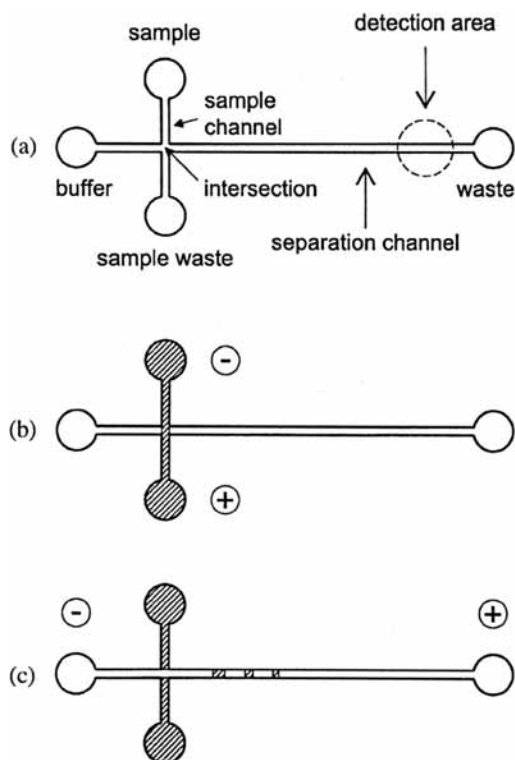
**Figure 9.3** Microchannels with guide structures forming a stable liquid-liquid interface. [Reprinted with permission from Sato et al. (2003), copyright Elsevier.]

As an alternative to traditional ELISA, this immunoassay chip is able to measure ultra-trace amounts of human carcinoembryonic antigen (CEA) (a serum colon cancer marker) and interferon. The methodology employs antibody-coated polystyrene beads packed in the microchannels and thermal-lens microscopy for detection.

## 9.2 Capillary Electrophoresis Arrays (CEA)

### 9.2.1 Capillary electrophoresis (CE)

A microfluidic system for capillary electrophoresis (CE) is shown in Fig. 9.4, where sample introduction and electrophoretic separation are accomplished in each of two crossing channels. The sample is driven through the short sample channel across the separation channel by application of a potential, and the



**Figure 9.4** A microfluidic system for capillary electrophoresis. (a) Sample introduction and electrophoretic separation are accomplished in each of two crossing channels. (b) The sample is driven through the short sample channel across the separation channel by application of a potential, and (c) the “plug” is electro-phoretically separated by application of another potential. [Reprinted with permission from Guber et al. (2004), copyright Elsevier.]



## Chapter 11

# Genomics and DNA Microarrays

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## 11.1 Introduction to Genomics

### 11.1.1 Definition

The science of genomics includes (1) understanding the mechanism of DNA replication, protein synthesis, gene expression, and the exchange and recombination of genetic material; (2) the understanding of restriction endonucleases and DNA ligases capable of cutting and rejoining DNA at sequence specific sites; (3) technical advances such as PCR and automatic DNA sequencing; and (4) bioinformatics, the storing, analyzing, and interpreting of data [Baldi and Hatfield, 2002]. Functional genomics is the overall effort of deconstructing the genome to assign

biological function to genes, groups of genes, and particular gene interactions.

*Bioinformatics* is a science that addresses the challenge of *knowledge acquisition* from *genomic data*. It includes (1) the establishment of informational databases for the storage and retrieval of molecular sequence and structure information, and gene expression data; and (2) facilitation of modeling of molecular interactions (*phemonics*), including cellular and subcellular messaging. This has involved computer scientists with special skills in string processing, computational geometry, and computer graphics. In addition, the large amount of expression data has involved data mining specialists, machine learners, and statisticians. With genome data from humans and other organisms, it is now possible to systematically deconstruct how the genetically programmed behavior of an organism's physiology is related to the constituent genes that makes its individual version of its species' genome. Available biological information includes DNA sequence, physical maps, gene maps, gene polymorphisms, protein structure, gene expression, and protein interaction effects. As a cautionary note, the comprehensive genomic measurement technologies, such as microarray-based expression profiling, have evolved and disseminated faster than the computation skills and analytical techniques required to properly interpret the data. The consequence is that many reports will not be substantiated in follow-up studies. Moreover, *raw genomic data*, as measured by the number of entries into databases such as GenBank, is increasing far more rapidly than our *knowledge* about these genes as measured by the number of articles appearing in the biomedical literature [Kohane et al., 2003].

There are a number of  $\mu$ TAS opportunities in genomics and proteomics. To appreciate what has already been accomplished and prepare for future devices, it is necessary to have some background in molecular genetics. While the following discussion offers some insight, a thorough understanding and link to the original literature can be obtained in the multipart series "Primer on Medical Genomics," published in the *Mayo Clinic Proceedings* between August 2002 and March 2004; and is cited several times below.

Genes that provide the code for all living things, both animal and plant, are located on *chromosomes*, and chromosomes are contained within the nucleus of the cell. A genome is composed of a series of nitrogenous *DNA bases* (adenine, guanine, thymine, and cytosine), and the specific order of base pairs provides the genetic code. There are about 3 billion such bases in the human genome, with about 20,000 to 25,000 genes. The function of only about 15,000 of these genes is known [Lorentz et al., 2002]. The function of a genome is to drive the generation of molecules (mostly proteins) for structural integrity and response to the environment.

There are 23 pairs of chromosomes in the human genome. *Diploid* refers to chromosomes in *homologous pairs*, with one chromosome from each parent. *Haploid* refers to a nucleus, cell, or organism possessing a single set of unpaired chromosomes, such as *gametes* (reproductive spermatozoa and oocytes).

Genomics permits us to understand the total characteristics displayed, or *phenotype*, by an organism under a particular set of environmental factors. It also allows the effects of *single nucleotide polymorphisms* (SNPs) and *mutations* (substitution, insertion, and deletion of base pairs) to be studied. Not all diseases

stem from single gene disorders with Mendelian inheritance patterns. There are many abnormalities that show a familial recurrence and have a clear genetic component, but do not show Mendelian segregation patterns. Genomics facilitates an understanding of these complex diseases as well [van Heyningen and Yeyati, 2004].

BioMEMS includes *cDNA chips* and *oligo chips* discussed below. These are rapidly evolving devices, and consulting the manufacturer's current product sheets is essential for realizing their full potential.

## 11.1.2 A brief history of genetics

### 11.1.2.1 Inheritance

The earliest understanding of inheritance began long ago when humans realized that specific traits were shared between parent and child. The first attempts to cultivate plants and domesticate animals can be traced back to ancient Babylonians and Assyrians from 5000 BC. Greek philosophers Hippocrates, Aristotle, and Plato wrote about the inheritance of human traits between 460 and 322 BC.

Cells were discovered by Hooke in 1665, and over the next two centuries it was recognized that cells with nuclei were the fundamental units of life and were formed by the division of other cells.

In 1859, Darwin published *On the Origin of Species* with his theory of natural selection. In 1865, Mendel published "Experiments in Plant Hybridization," introducing the principles of heredity and the concept of dominant and recessive genes. In the late nineteenth century it was recognized that chromosomes were located in the nucleus and were the basis of heredity.

### 11.1.2.2 Genetics

The term *genetics* was coined by Bateson (1900) at the beginning of the twentieth century in advocacy of Darwin's theory based on Mendel's principles of hereditary. An understanding of biochemical disorders based on genetic defects followed, as did an understanding of sex-linked traits and the linear arrangement of genes on chromosomes.

Chargaff (1951) discovered the ratio of purines to pyrimidines (adenine to thymine, guanine to cytosine) was 1:1. X-ray diffraction patterns by Franklin and Gosling (1953) and Wilkins et al. (1953) showed the helical structure of the DNA molecule. The DNA double helix was described by Watson and Crick in 1953, and soon after Crick introduced the concept that DNA makes RNA (ribonucleic acid), which in-turn makes proteins. In 1956, Tijo and Levan determined that humans had 46 chromosomes, and soon afterward chromosome syndromes were identified.

In 1960, Nowell and Hungerford discovered that an abnormality of chromosome 22 was associated with chronic myeloid leukemia, leading to the birth of *cancer cytogenetics*. Temin and Mizutani (1970), and Baltimore (1970)