# Biochip Technology

Edited by

Jing Cheng

and

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# PREFACE

Microminiaturization is one of the fastest growing fields in the analytical sciences. Over the past ten years a diverse range of micrometer-scale devices has been fabricated in silicon and in glass, and more recently in different types of plastic. The scope of applications for the new microminiature analytical devices (biochips, microchips, "lab-on-a-chip") spans analytical chemistry and the biomedical sciences. Devices for genetic testing have attracted particular attention from the microminiaturization community, and a fully integrated genetic analyzer that would accept a minute sample of whole blood and produce a result without further human intervention will soon be a reality. Lab-on-a-chip devices would act as personal laboratories that could be used for a broad range of home testing and directly contribute to health maintenance and quality of life. Today, the microchip devices are making major contributions to the drug discovery process. In this application, a capability for rapid high-throughput multiplexed analysis using low volumes of sample and reagent is paramount, and the microchip devices offer a convenient and cost-effective approach to this type of analytical process. Microarray devices (DNA chips, gene chips, microspot chips) comprising surface arrays of micrometer-sized patches of antibodies, cDNA, or oligonucleotides are also having a major impact in biomedical research; particularly in gene expression studies, mutation detection, and protein analysis. The ability of microanalyzers to accomplish complicated analytical tasks, particularly with samples containing cells, is increasing. These new devices (biochips) contain microelectrodes, microfluidic elements, and other microfabricated features that orchestrate a variety of sample manipulation and analytical steps.

Against this background, the objective of this book is to provide up-to-date coverage of some of the emerging avenues of research and development in the field of microchip devices. The book contains descriptions of chip fabrication (micromachining, hot-embossing, patterning), system development, microarrays (polypyrrole-based, nylon, glass), assays, cell isolation, and manipulation using microfilters and bioelectronic devices, and applications ranging from clinical testing (PCR chips, portable laboratories) to plant genome analysis to biohybrid organs. This book is intended to be a starting point for anyone interested in the possibilities and potential of the diverse opportunities afforded by microminiaturized analysis in a chip format.

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Cheng developed the world's first laboratory-on-a-chip system in 1998, and the work was featured in the front cover story of the June 1998 issue of *Nature Biotechnology*, and also cited as the breakthrough of the year by *Science* that same year. He was awarded Nanogen's most prestigious award, the NanoAward, and China's Outstanding Young Scientist Award, both in 1999. Cheng has published over fifty peer-reviewed papers, of which over twenty are related to biochips. In addition, he holds more than twenty European and US patents and disclosures. He has presented at many international conferences. His current research interest is the development of microchip-based laboratory systems and ultra-high-throughput drug screening systems.

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# CONTENTS

# Microchips, Bioelectronic Chips, and Gene Chips

## Microanalyzers for the Next Century

Larry J. Kricka

### INTRODUCTION

An important direction in the development of analytical techniques is toward microminiaturized analyzers. Generic names for these new micrometer-featured devices include "micro-total analytical system" (μ-TAS) (Manz *et al.*, 1990a), lab-on-a-chip (Colyer *et al.*, 1997; Moser *et al.*, 1995), biochip, or, simply "chip." In some cases devices have been named based on their particular application, for example, PCR chips, gene chips, while for others the device is named for a characteristic structural feature, for example, microspot or microarray (Table 1). The common theme for all of these devices is the microminiaturization of an analytical process or part of an analytical process into a device built on a small piece of glass, plastic, or silicon (Beattie *et al.*, 1995a; Becker and Manz, 1996, Berg and Bergveld, 1995; Berg and Lammerink, 1998; Collins and Jacobson, 1998; Hacia *et al.*, 1998a; Kopp *et al.*, 1997; Kricka, 1998a,b; Manz, 1998; Ramsay, 1998).

Several factors can be identified as underpinning the renewed interest in microminiaturization of analyzers. First, a range of analytical problems has emerged for which microminiaturization has obvious benefits, and these include high-throughput massively parallel testing for drug discovery (Devlin, 1997), small hand-held portable analyzers for point-of-care testing (e.g., clinical testing or biowarfare monitoring) (Kost, 1995), and lightweight analyzers for use on space exploration missions where payload is limited. Second, miniaturization offers a route to cost reduction in analytical processes because the amount of reagent used per assay can be drastically reduced compared to conventional analysis. Similarly, in drug discovery, where there is often only a limited amount of candidate drug compound, a reduction in the volume of sample tested translates into a larger number of tests with that particular compound. Finally, an important advantage of microminiaturization is the ability to integrate all of the steps in a complex multistep analytical process onto a single device. This finds a natural parallel with integrated electronic circuits produced on silicon wafers for the electronics industry. In these devices, thousands to millions of individual components are integrated into a single chip (e.g., an Intel Pentium III is produced using a 0.25-μm manufac-

**Table 1** Nomenclature of Analytical Microchip Devices

| | | |
|---|---|---|
| Biochip[a] | IVF chip | Oligonucleotide chip |
| Biologic chip | Lab-chip | PCR chip |
| DNA chip | Laboratory on a chip[b] | ProteinChip® |
| DNA MassArray™ | Lab-on-a-chip | SpectroChip™ |
| Expression chip | LifeGEM™ | Sperm chip |
| FeverChip® | LivingChip™ | UniGEM™ |
| Gene chip | Mesoscale device[c] | μ-TAS |
| Gene chip™ | Microarray | |
| Genosensor | Microspot® | |

[a]This term was originally used to refer to biological versions of electronic microchips (Tucker, 1984).
[b]This term was originally used in the context of computer-based experiments (Steber, 1987).
[c]Mesoscale refers to an intermediate scale, between that of large and small dimensions.

turing process and the CPU includes over 9.5 million transistors) (http://developer.intel.com/design/PentiumIII/prodbref/).

This chapter provides an introduction to microchip analyzers, their fabrication and applications, and discusses future trends in this emerging analytical science.

## HISTORICAL PERSPECTIVE

Current microanalyzers owe much to the early work of the micromachinists who were intrigued with the possibility of using silicon as a material for constructing different types of mechanical and microelectromechanical (MEMS) devices. They showed that it was possible to construct complex micrometer-sized devices such as cogs, movable mirrors, spanners, and more complex devices including an electric motor from micromachined silicon components (Amoto, 1989; Angell *et al.*, 1983; Mallon, 1992; Petersen, 1982; Stix, 1992). Practical devices based on micromachined components have emerged including sensors for measuring blood pressure and fuel flow in automobile engines, and a device that triggers airbags in automobiles. The latter has enjoyed considerable success and is based on a microfabricated silicon beam that bends under acceleration forces. Deflection of the beam is detected, and this triggers release of the airbag (Bryzek *et al.*, 1994).

One of the earliest microanalyzers was fabricated by Terry and colleagues. They constructed a gas chromatograph (GC) on the surface of a 2-inch silicon wafer that was then bonded to a glass plate (Terry *et al.*, 1979). There was then a hiatus of several years before interest was renewed in microanalytical devices. The next important landmarks in microanalyzer technology were in the early 1980s, with the development of the microphysiometer and the i-STAT analyzer. The microphysiometer is based on micromachined silicon, 50 μm square × 50 μm deep wells that incorporate a light-addressable pH sensor (McConnell *et al.*, 1992; Owicki and Parce, 1990; Parce *et al.*, 1989). This device was designed to assess cell metabolism for toxicity studies of new drug compounds. The i-STAT analyzer utilizes a dispos-

able cartridge that contains an array of microelectrodes and immobilized enzyme electrodes on a silicon microchip for whole blood analysis (e.g., blood gases, electrolytes, glucose, hematocrit) (Lauks *et al.*, 1992). By the end of the 1980s, research-and-development efforts directed toward microanalytical devices experienced a growth spurt. Some of the diverse range of analyzers, devices, tests, and procedures are listed in Table 2.

## ADVANTAGES AND LIMITATIONS OF MICROANALYZERS

There are a series of compelling reasons why microanalyzers will find widespread use for analysis (Table 3). Microminiature analyzers are small and compact and thus suitable for use in non-laboratory settings (e.g., point-of-care testing) where hand-held portable analyzers are required. Miniaturized arrays of different reagents on planar surfaces (e.g., plastic, glass, or silicon) permit simultaneous testing of a sample for specific components. The volume of sample required for analysis is reduced in microanalyzers (e.g., nL–pL volumes), and this is beneficial in a clinical setting as it reduces the amount of blood that must be drawn from a patient. There is also a reduction in the volume of reagent required per test, and this provides an economic benefit.

**Table 2** Micromachined Analyzers, Devices, and Assays

**Analyzers and devices**

Biocapusule (Desai *et al.*, 1998)
Capillary electrophoresis analyzer (Jacobson and Ramsey, 1995; Seiler *et al.*, 1993)
Controlled release system (Sheppard *et al.*, 1996)
Blood gas analyzer (Arquint *et al.*, 1994; Shoji and Esashi, 1995)
Electrochemiluminescence detector (Arora *et al.*, 1997)
Electrolyte analyzer (Moritz *et al.*, 1993)
Electroporation system (Murakami *et al.*, 1993)
Flow-injection analyzer (Manz *et al.*, 1991; Suda *et al.*, 1993)
Gas chromatograph (Terry and Hawker, 1983; Terry *et al.*, 1979)
Haemorheometer (Tracey *et al.*, 1995)
*In vitro* fertilization chamber (Kricka *et al.*, 1995)
Liquid chromatograph (Manz *et al.*, 1990b; Ross *et al.*, 1998; Xue *et al.*, 1997a,b)
Thermal cycler (Northrup *et al.*, 1996; Wilding *et al.*, 1994)

**Test or procedure**

Antibody analysis (Rodriguez *et al.*, 1997)
Cell movement and responses (Oakley and Brunette, 1995)
Cell traction force (Galbraith and Sheetz, 1997)
DNA analysis (Shalon *et al.*, 1996; Sheldon *et al.*, 1993)
DNA sequencing (Drobyshev *et al.*, 1997; Southern, 1996)
Expression monitoring (Schena *et al.*, 1995; Lockhart *et al.*, 1996; Wodicka *et al.*, 1997)
Immunoassay (Koutny *et al.*, 1996; von Heeren *et al.*, 1996; Song *et al.*, 1994)
Mutation testing (Gingeras *et al.*, 1996; Hacia, 1999; Hacia *et al.*, 1997)
Nerve regeneration (Zhao *et al.*, 1997)
Nucleic acid hybridization (Beattie *et al.*, 1995b; Fodor, 1993; Southern *et al.*, 1999)
PCR (Belgrader *et al.*, 1998; Cheng *et al.*, 1996a; Kopp *et al.*, 1998; Waters *et al.*, 1998a,b)
Semen testing (Kricka *et al.*, 1997)
Serum protein analysis (Colyer *et al.*, 1997)
Topographic guidance of cells (Oakley *et al.*, 1997)

**Table 3** Advantages and Disadvantages of Microanalyzers

| | |
|---|---|
| **Advantages** | Disposable |
| Portable | Fast response time |
| Low power consumption | |
| Low production costs | **Disadvantages** |
| Mass production | Human interface |
| Diverse range of applications | Obtaining a representative sample |
| Integration of steps in an analytical process | Exceeding the analytical detection limit |

Fabrication of microanalyzers derives benefit from the manufacturing processes used in the microelectronics industry that are geared to high-volume production. Many different designs can be simultaneously fabricated on the same wafer and then tested. This allows rapid design cycles and the potential for more design iterations than would be normally possible for a macroscale device.

Microanalyzers can improve analytical reliability through multiple test sites for simultaneous parallel assays. This degree of redundancy provides an analytical safeguard that cannot be easily achieved in macroscale analyzers, where duplicate assays represent the normal extent of repetitive assay of a specimen. Encapsulation of microscale devices provides extended operation over a wider range of environmental conditions of humidity and temperature than can be achieved with a conventional analyzer.

One of key advantage of microanalyzers is the opportunity to integrate all of the steps in a complex multistep analytical process into a single device. The scale of a microchip is such that it is feasible to design structures to perform individual tasks, including sample addition, processing, analysis, and read-out of the results, all on a microchip that is 2 × 2 cm or smaller. An even greater degree of integration is achieved by further combination of analytical steps into individual microstructures on the microchip (e.g., cell separation and nucleic acid amplification). Added to this is the availability of a large number of microminiaturized components (e.g., lasers, pumps, valves) that enhance the capabilities of the device. Table 4 lists some miniaturized components that are available for incorporation into microchip analyzers. This, of course, also includes the electronic circuitry to operate and control the analytical process, which would easily fit onto the surface of the type of devices currently being developed.

Microminiaturization does have some disadvantages. As the size of a sample is successively decreased, an immediate concern relates to how representative the sample is of the specimen from which it was derived. This is a problem for inhomogeneous biological specimens that contain a diversity of constituents (e.g., cells, proteins, lipids). For example, a submicroliter blood sample is unlikely to contain rare cells such as trophoblasts in maternal circulation, which may only be present at one per million or one per ten million cells. This problem can be overcome by developing flow-through sampling in which larger volumes of sample are flowed through a low volume-microminiature device. Another issue that arises as the volume of the sample is reduced is that of detectability. If an analyte is present at only 1 femtomole/L in the original specimen, then a 1 μL sample contains 600 molecules ($1 \times 10^{-15} \times 10^{-6} \times 6\ 10^{23}$). Further reduction of the sample

**Table 4** Microminiaturized Components

| | | |
|---|---|---|
| Accelerometer | Microbeam | Pump |
| Air turbine | Microbearing | Refrigerator |
| Anemometer | Microbridge | Relay |
| Cables | Microflexible arm | Resonator |
| Cantilever | Micromotor | Robot |
| Diaphragm | Microphone | Screw |
| Flow sensor | Micropipette | SFM and STM tips |
| Fuse | Microturbine | Sieve |
| Gears | Mirror | Solenoid |
| Hinge | Peltier heater/cooler | Tweezer |
| Laser | Pirani pressure gauge | Vacuum tube |
| Membrane | Pressure sensor | Valve |

size to 1 nL produces a sample that contains one thousand times fewer molecules, that is, less than one molecule, and this would not be detectable (Petersen *et al.*, 1998).

## FABRICATING MICROCHIPS

Microfabrication methods used to make the different types of microanalyzers are summarized in Table 5 (Qin *et al.*, 1998). In many cases the basic technology has been adapted from the microelectronics industry (e.g., photolithography for glass and silicon devices) or from the printing industry (e.g., ink jet printing). The size of the features that can be fabricated are in the micrometer range for photolithographic, molding, and printing methods and in the nanometer range for patterning. An important current direction in microfabrication is the manufacture of plastic microchips (Becker and Dietz, 1998; Ford *et al.*, 1999; Friedrich and Vasile, 1996; Friedrich *et al.*, 1997; McCormick *et al.*, 1997). These may be easier to manufacture and at lower cost than glass or silicon-glass chips and, additionally, may provide greater flexibility in design.

Fabrication of microanalyzers also requires ancillary processes for assembling the microcomponents (e.g., anodic and thermal bonding), and methods to introduce direct-access ports into structures formed by bonding microparts together (e.g., mechanical, ultrasonic, and laser drilling) (Shoji and Esashi, 1995). Handling and manipulating very small microchips is difficult, but this can be overcome by packaging the microchip into a substantially larger holder or by mounting one or more microchips onto a platform.

## ON-CHIP DETECTION METHODS

Fluorescent detection methods currently dominate microchip analyses. Laser-induced fluorescence (LIF) is widely used with capillary electrophoresis chips to detect separated components (Cheng *et al.*, 1996b; Effenhauser *et al.*, 1993; Harrison *et al.*, 1993). Confocal fluorescence microscopy is the most common detection

**Table 5** Materials and Fabrication Processes

| | |
|---|---|
| **Materials** | |
| | Acrylic copolymer (McCormick *et al.*, 1997) |
| | Glass (Effenhauser *et al.*, 1993) |
| | Photoresist (Gorowitz and Saia, 1984) |
| | Polyacrylamide (Proudnikov *et al.*, 1998) |
| | Polycarbonate (Jenoptik Mikrotechnik, Jena, Germany) |
| | Poly(dimethylsiloxane) (Qin *et al.*, 1998) |
| | *Polymethyl methacrylate* (Jenoptik Mikrotechnik, Jena, Germany) |
| | Polypropylene (Matson *et al.*, 1995) |
| | Quartz (Danel and Delapierre, 1991) |
| | Silicon (Petersen, 1982) |
| **Processes** | |
| | Anodic bonding (Spangler and Wise, 1990) |
| | Contact printing (Jackman *et al.*, 1995) |
| | Covalent bonding (Drobyshev *et al.*, 1997) |
| | Deposition (Beattie *et al.*, 1995a; Shalon *et al.*, 1996) |
| | Electrochemical micromachining (Datta, 1995) |
| | Embossing (Becker and Dietz, 1998) |
| | Injection molding (McCormick *et al.*, 1997) |
| | Ink jet printing (De Saizieu *et al.*, 1998) |
| | *In-situ* synthesis (Fodor *et al.*, 1994; Southern, 1996) |
| | Laser ablation (Hennink, 1997; Zimmer *et al.*, 1996) |
| | LIGA (Lithographie, Galvanoformung, Abformung) (White *et al.*, 1995) |
| | Microcontact printing (Kane *et al.*, 1999) |
| | Micromilling (Friedrich *et al.*, 1997; Friedrich and Vasile, 1996) |
| | Pattering . (Sleytr *et al.*, 1992) |
| | Pattern transfer (Xia *et al.*, 1996) |
| | Reactive ion etching (Gorowitz and Saia, 1984) |
| | Silicon fusion bonding (Petersen *et al.*, 1991) |
| | Thermal bonding (Lasky, 1986) |
| | Ultrasonic impact grinding (Qin *et al.*, 1998) |
| | Wet-etching (Petersen, 1982) |

method for assessing antibody–antigen binding and hybridization on microarrays. This technique is highly sensitive and can detect 5–10 fluorescein labels per $\mu m^2$ (Chu *et al.*, 1996; Sheldon *et al.*, 1993). Fluorescence has also been used for TaqMan-type assays in arrays of glass microwells in combination with a charged-coupled device (CCD) (Taylor *et al.*, 1998). Both one- and two-color fluorescence procedures have been devised for use with microarrays. For example, in the microspot assay, the capture antibody is labeled with Texas Red and the detection antibody is labeled with fluorescein (Chu *et al.*, 1996; Ekins, 1998), whereas in the gene expression assays the test and control are labeled with lissamine and fluorescein or with Cy 3 and fluorescein, respectively (DeRisi *et al.*, 1996; Hacia *et al.*, 1998b). An alternative two-color detection strategy employs a β-galactosidase label and an alkaline phosphatase label detected using X-Gal and Fast Red TR/naphthol AS-MX substrates (Chen *et al.*, 1998).

Chemiluminescence methods have also been used to study reactions in microchips (Kricka *et al.*, 1994), for example, genetic (Rajeevan *et al.*, 1999) and immu-