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Miniaturization of analytical systems

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Miniaturization has been a long-term trend in clinical diagnostics instrumentation. Now a range of new technologies, including micromachining and molecular self-assembly, are providing the means for further size reduction of analyzers to devices with micro- to nanometer dimensions and submicroliter volumes. Many analytical techniques (e.g., mass spectrometry and electrophoresis) have been successfully implemented on microchips made from silicon, glass, or plastic. The new impetus for miniaturization stems from the perceived benefits of faster, easier, less costly, and more convenient analyses and by the needs of the pharmaceutical industry for microscale, massively parallel drug discovery assays. Perfecting a user-friendly interface between a human and a microchip and determining the realistic lower limit for sample volume are key issues in the future implementation of these devices. Resolution of these issues will be important for the long-term success of microminiature analyzers; in the meantime, the scope, diversity, and rate of progress in the development of these devices promises products in the near future.

Miniaturization of analytical and bioanalytical processes has become an important area of research and development during the past 10 years *(1–3)*, as a continuation of the general trend in size reduction of clinical laboratory analyzers. The original type of floor-standing analyzer (e.g., AGA Autochemist, Technicon SMAC) *(4, 5)* has been successively reduced in size, first to bench top and then to portable and hand-held devices. Micrometer-sized microchip devices, and ultimately nanometer-sized nanochip devices, represent the endpoint of this progression. A number of benefits are identifiable with miniaturization, notably reduction in manufacturing costs, ease of transport and shipping, and minimal space requirements in a laboratory. In addition, a microminiature device is easier to hold and manipulate, reduces requirements for power and consumable reagents, and offers the possibility of high-density testing and integration of individual steps in a multistep analytical process.

Several factors are fueling current interests in miniaturization. These include point-of-care testing *(6)*, high throughput drug discovery *(7)*, detection of biological warfare agents *(8)*, and astrobiology *(9)*. Analyzers for point-of-care testing need to be small, lightweight, and portable with low power requirements; all of these design goals can be achieved via miniaturization. In high throughput drug discovery, the sheer scale of testing (thousands of candidate drugs to be tested against thousands of biological targets) and the need to conserve the limited quantities of archival compounds or substances produced by combinatorial synthesis procedures *(10)* require high-density arrays of microvolume reaction vessels. The emerging demands to monitor and detect the release of biological warfare agents (e.g., *Clostridium botulinum* toxin and anthrax) by aggressors in a battlefield or by terrorists in a domestic situation may best be met by microminiature detection devices. Likewise, the limitations on space and weight for rocket payloads strongly supports miniaturization of analyzers designed for astrobiological tasks.

The new generation of microminiature analyzers and the proposed nano-sized devices will be built on a scale that would have been difficult to comprehend when the first automatic analyzers were introduced into the clinical laboratory >30 years ago *(11)*. Dimensions of the smallest structures in microchips are typically 10–100 μm (the diameter of a human red cell is 7 μm), and the proposed nanochips will be several orders of magnitude smaller.

In the current miniaturization trend, four main types of microminiature analytical devices are emerging: (*a*) high-density arrays of microreaction wells, (*b*) surface microarrays of reagents, (*c*) microchips, and (*d*) nanochips.

High-density Arrays of Microreaction Wells

The new emphasis on high throughput drug discovery methods in which vast numbers of candidate drugs are screened against equally large numbers of biological targets has generated new demands for microminiature analysis. Essential characteristics for an analytical drug discovery method include rapid, automated, and simultaneous testing of microvolumes of candidate drug com-

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pounds. Many of the compounds included in drug screening assays are archival and only available in a very limited quantity or are the products of combinatorial synthesis procedures and thus are only produced in microgram to milligram quantities. Conservation of valuable compounds is imperative, and miniaturization of assays is an immediate and viable route to this objective. The microplate has become the most popular format for drug discovery assays because it is readily integrated into an automated process and provides multiple simultaneous testing on a simple disposable device. The traditional 96-well format has proved inadequate and is being replaced by microplates with larger numbers of smaller wells *(12–18)*. These include plates with 192, 384, 864, 1536, 2025, 2288, 2304, 2400, 3456, 6144, 6500, 9600, and 20 000 wells with volumes that range from 125 μL to 50 nL. Microplates with 384 and 1536 wells (Fig. 1) are vying for acceptance as the new standard in high throughput screening; however, to date there has been no consensus on optimal well density or volume. These microminiaturized reaction devices have placed new demands on ancillary equipment. In response, a range of new micropipetting systems based on ink-jet principles (thermal-, solenoid-, or piezoelectric-actuated) have been developed for delivery of microliter to nanoliter volumes of sample or reagents *(19, 20)*. Injection molding is used for manufacture of most of the high-density microvolume microplate devices; however, other techniques such as polymer casting *(16)* and drilling *(13)* are also effective manufacturing techniques.

A diverse range of analytical methods have been adapted to the new high-density, low-volume microwell format. Most are simple mix-and-measure type homogeneous assays, such as scintillation proximity assays, fluorescence polarization assays, time-resolved fluorescence assays, reporter genes, and enzyme assays. To date, there has been relatively less progress in formatting multistep separation assays, such as ELISA, to a high-density microwell format.

Surface Microarrays of Reagents

Fabrication of surface microarrays of nucleic acids *(21–26)* and proteins *(27–30)* at discrete locations on small chips is another important direction in miniaturization methods. Chips are typically in the size range of 1–2 cm^2; the elements in the arrays vary from 1 to 200 μm but are usually 10–100 μm (diameter or length on side). Chips containing all 65 536 possible oligonucleotides in 8-nucleotide chains (8-mers) *(22)* and chips with 48 300 possible oligonucleotides in 20-nucleotide chains (20-mers) *(25)* are representative of the scale of array possible with this type of microminiature technology.

Microarrays of polynucleic acids or proteins are produced by a number of methods. In situ combinatorial synthesis uses photolithographic masks to define discrete array locations for photodeprotection-type synthetic reactions *(22, 24, 25)*. In this way, an oligonucleotide or a polypeptide molecule is synthesized one base or one residue at a time on the surface of a glass chip. Synthesizing an *n*-mer requires $4 \times n$ steps; thus 32 steps are required to synthesize the 65 536 possible 8-mers and 80 steps to produce the $\sim 10^{12}$ possible 20-mers. Alternatively, rubber spacers can be used to define orthogonal reaction channels on a glass surface *(31)*, or a 64-channel fluidic chemistry delivery system can be used to perform synthetic reactions at defined locations on a polypro-

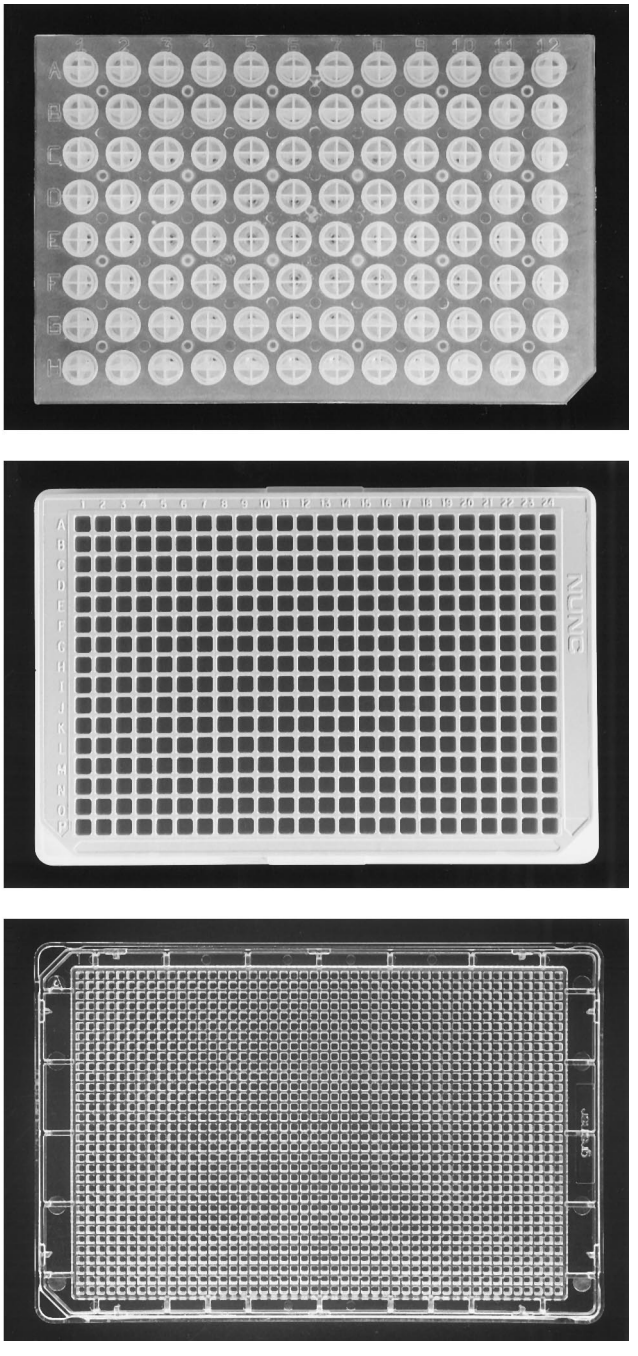

Fig. 1. High-density microwell plates.

(*A*) 384-split well microplate (Robbins Scientific); (*B*) 384-well microplate (Nalge Nunc International Corp); (*C*) 1536-well microplate (Greiner Labortechnik).

pylene chip *(21)*. Other options include direct attachment of preformed oligonucleotides to an activated chip surface (e.g., aminated polypropylene or polyacrylamide gel pads) *(23)* or attachment of pyrrole-substituted oligonucleotides to arrays of 50 μm × 50 μm polypyrrole-coated gold electrodes via electrosynthetic reactions *(32)*.

Direct arraying of the reagent onto the chip can be by a deposition process using a syringe microdispenser *(33)*, an array of ink-jet print nozzles *(25)*, micropins (e.g., 100 μm thick, 1-nL transfer volume) *(23)*, or open-capillary tips *(34)* that are simply dipped into the substances to be arrayed. Table 1 *(35–44)* lists examples of the applications of the array devices in immunological and genetic testing assays. Most devices are oligonucleotide, cDNA, or polypeptide arrays; however, arrays of unnatural polymers based on aminocarbamate monomers linked via a carbamate backbone have also been prepared *(45)*.

Microchips

A typical microchip is 1.5 cm × 1.5 cm in size and a few millimeters thick. Materials for microchip fabrication include silicon, glass, quartz, and plastics such as Teflon, polymethylmethacrylate, and polycarbonate *(1–3)*. In the case of silicon microchips, a range of fabrication techniques have been adopted from the microelectronics industry, including wet etching using KOH and reactive ion etching processes *(46)*. Other techniques include laser ablation/drilling, electrodischarge, injection molding, polymer casting, printing, and Lithographie Galvanoformung Abformung (LIGA) *(46–48)*. Ablation or drilling with a Nd:YAG laser offers a simple one-step process for fabrication of features <30 μm on a variety of materials. It is particularly useful for cutting curved and irregular shapes that are more problematic for conventional etching methods *(47)*.

Hot embossing is emerging as a highly promising method of making plastic microchips; this is important because it would lead to high-volume low-cost continuous production methods that may be easier to implement than batch etching of silicon wafers for silicon-based microchips (Fig. 2). The range and scope of microchip assays and analyzers continue to grow; some recent examples of devices for different types of assays or analytical procedures are listed in Table 2 *(49–74)*.

Table 1. Applications of microarray devices.

Arrayed reagent	Application
cDNA	Inflammatory disease *(35)*, human cancer *(36)*, gene expression–heat shock-regulated genes *(37)*, *Arabidopsis* genes *(38)*
Oligonucleotide	Oligonucleotide-olignucleotide interactions *(39)*, human mitochondrial genome analysis *(40)*, expression monitoring *(41)*, HIV-1 strain identification *(22)*, *BRCA1* mutations *(42)*, cystic fibrosis mutation detection *(21)*, β-thalassemia mutations *(43)*, hepatitis C genotyping *(32)*, HLA typing *(44)*
Antibody	Simultaneous immunoassay *(27,28)*, antimouse IgG assay *(29)*

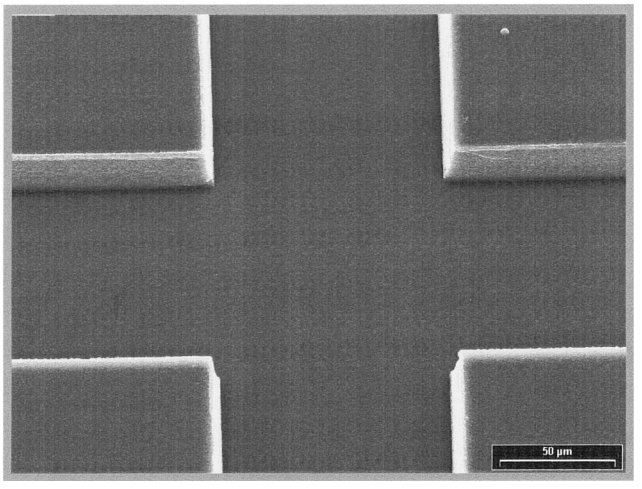

Fig. 2. Polymethylmethacrylate microstructure produced by hot embossing.
Channel width is 100 μm, depth is 25 μm. (JENOPTIK Mikrotechnik Gmbh).

Nanochips

One vision of the future direction of miniaturization is nanochip devices that are built at the nanometer scale from individual atoms and molecules *(75–77)*. The nanotechnologist's view is that the counterparts of machine components can be found among natural molecules and biological assemblies of molecules: for example, collagen is a cable, an antibody is a clamp, DNA is a memory device, and membrane proteins are pumps. Currently there are no examples of nanochips; however, progress in

Table 2. Microchip assays and analyzers.

Application	Reference
Blood gas analyzer	*49*
Capillary electrophoresis	*50–52*
Cell analysis	
Isolation	*12, 53, 54*
Deformability	*55*
Motility	*56*
Flow cytometry	*57*
Enzymatic assays	*58*
Gas chromatography	*59*
Glucose analyzer	*60*
Immunoassay	*61–64*
Mass spectrometry	*65–67*
Nucleic acid amplification	
PCR	*68–71*
Multiplex PCR	*72*
DOP-PCR[a]	*72*
Probe ligation	
LCR	*73*
Restriction fragment analysis	*74*

[a] DOP, degenerate oligonucleotide primed; LCR, ligase chain reaction.

self-assembling molecular structures, e.g., 0.5-μm diameter, 30-μm long lipid tubules *(78)*, 0.7- to 0.8-nm diameter cyclic peptide nanotubes *(79)*, and the design and synthesis of molecules that mimic mechanical devices provide the grounds for some optimism for this avenue of development (e.g., recently, a metallocene molecular gear was successfully synthesized) *(80)*.

Scaling Issues for Microanalytical Devices

Implementation of microanalytical devices presents a series of issues related to the physical size of the device and the scale of the reaction volumes.

If a human interface is anticipated, then a microanalytical device must be mounted or packaged into some type of holder or cartridge that provides a convenient means of introducing a sample. This approach has been adopted for the Affymetrix microarray chips *(22)*, microParts microspectrometers (microParts), and the i-STAT chip (i-STAT Corp.) *(81)*.

Successive reduction in the volume of the sample analyzed in a microanalytical device may compromise analysis either because the measurement limit of the analytical method is exceeded or because the sample is no longer representative of the bulk specimen. For example, a 1-μL sample of a specimen containing an analyte at a concentration of 1 fmol/L contains 6020 molecules. Further reduction in sample size to 1 nL leads to a sample containing only 6 molecules of analyte, which may be substantially less than the detection limit of the analytical method formatted into the microchip.

Analysis of rare cells poses yet another challenge for microminiaturized analysis. Fetal nucleated red cells are present in very low abundance in the maternal circulation. For example, 18 mL of maternal blood may only contain 20 fetal cells among a total cell population of nearly 10^8 nucleated cells *(82, 83)*. Sampling a microliter volume is unlikely to provide a sample containing even a single fetal cell; thus, other strategies are required, such as on-chip flow-through capture/concentration techniques.

Another complication for microchip devices is evaporation of microvolumes of sample or reagent from the microchip, thus compromising the volumes metered into the device. This problem has been addressed by designing pipetting systems that automatically replace fluid lost by evaporation or by enclosing the chip in a controlled environment *(84, 85)*.

Integration

A key benefit of miniaturization is the prospect of integration of all of the steps in an analytical process into a single device. For bench-top analyzers, entire fluidic modules have been machined into transparent plastic blocks to provide both integration and some degree of miniaturization (e.g., Unifluidics™ Technology, Bayer Corp.). For microchips, the range of components that have been miniaturized and that would be available as building blocks for fully-integrated analyzers is impressive and includes pumps, valves, lamps, filters, heaters, refrigeration, ion-selective electrodes, capillary electrophoresis, and electronic control circuitry *(1, 86, 87)*. Some of the analytical functions integrated into single-chip devices are listed in Table 3 *(88–95)*. Most devices integrate the different analytical structures by interconnection on the surface of the chip. Three-dimensional integration can be achieved by stacking or by fabricating chips into interconnected layers *(96, 97)*. Integrating the sample preparation step required in an analytical procedure is an important goal, and white cell isolation from whole blood followed by PCR analysis has been successfully combined on a single 15 mm × 17 mm silicon-glass PCR filter chip *(54)*.

Table 3. Combinations of components integrated into microanalytical systems.

Component	Combinations of components												
Microvalve							√	√	√				
Micropump			√	√			√		√	√			
Heater		√											
Electronic control circuitry									√				
Detector					√		√	√	√				√
Reaction chamber	√	√	√	√	√	√	√	√	√	√	√	√	√
DNA isolation											√		
Microdialysis													√
Cell isolation	√					√				√			
Cell lysis	√											√	
Fertilization						√							
PCR	√	√										√	
Capillary electrophoresis			√	√								√	
Enzymatic reaction			√										
Immunoassay				√									
Microarray					√								
Reference	*54*	*70*	*74*	*62, 88*	*89*	*84*	*90*	*49*	*91*	*53*	*92, 93*	*94*	*95*

Integration of an analytical procedure and detection is possible for a number of assays. For example, restriction fragment length polymorphism analysis can be performed on a glass microchip (~20 mm × 30 mm) that mixes a DNA sample with a restriction enzyme, incubates the mixture, and then delivers the 0.7-nL reaction mixture to an on-chip capillary electrophoresis system for analysis *(74)*.

Capillary electrophoresis in combination with laser-induced fluorescence is a popular on-chip detection option for integrated analysis because it is sensitive, versatile, and the sample volume required is low. It proved effective in the quantitation of bound and free fluorophore-labeled fractions in a competitive immunoassay for theophylline performed on a glass microchip. Steps involving mixing and incubating the sample and reagents and detecting the product (100-pL sample of reaction mixture) were performed on the chip. Quantitation of the bound and free fluorescein-labeled fractions took <1 min in a 58-μm wide, 7.5-cm-long, on-chip separation channel *(88)*.

Another integrated microchip device utilizes a series of electrodes coated with DNA capture probes. The 200-μm diameter electrodes facilitate electronic hybridization, washing, and dehybridization within the chip. Positively biased electrodes facilitate capture of negatively charged DNA, and by changing the bias to negative, captured target can be dehybridized and transported to a reaction site. This "complexity reduction" device has been used to capture DNA sequences from complex mixtures and to quantitate captured target by means of a charge coupled device and a fluorophore label (e.g., Bodipy Texas Red) *(92, 93)*.

Conclusions

Miniaturization continues to be an important consideration in the design and development of many assays and analyzers. The escalating interest in high-throughput screening for drug discovery and the large-scale analysis required for genetic studies will continue to be major factors influencing miniaturization. There are already numerous examples of assays and analytical processes that have been successfully adapted to a microchip format, and the goal of a "lab-on-a-chip" is realistic. The emergence of microchips fabricated from plastics *(98)* will help eliminate some of the materials issues encountered with silicon devices *(69)*. One of the next major challenges in miniaturization is the development of nanotechnology and fabrication of nanochips.

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