Miniaturized Bioanalytical Systems for Biotech Industry

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“Two technologies will dominate the 21st century scientifically and industrially: biotechnology and information technology”

“There is Plenty of Room at the Bottom”

Nanoparticles, Proteins, and Nucleic Acids: Biotechnology Meets Materials Science

“The Challenges for Biotech Business

- Scientific Creativity
  - Discovery: You have discovered something wonderful.
  - Platform Technology: You can do something wonderful.
  - Research has “fashions”: The biotech industry follows the fashion.

- Market Need
  - Product company
  - Tools company
  - Solution providers

- Competitive Advantage
  - You hold the patent on doing it.
  - You have the tools necessary to do it.
  - You have the skills necessary to do it.
  - You have a lot of resources or money to do it.
  - You are the first to do it.

New Paradigm for Bio-Related Industry

Miniaturization of chemical instrumentation has become an increasingly important component for genomic and proteomic applications, as well as drug discovery and development research

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Bioanalytical Systems

- **Biosensor**, Point-of-care (POC) hand-held devices
- **Biochips** (DNA chip, Protein chip, Cell chip, Neuron chip)
- **micro-TAS**, Biofluidic devices, Lab-on-a-chip
- Conventional clinical analyzer
- High-Throughput Screening System
- Diagnostic and therapeutic Biomedical instruments

Microfluidic and Biosensor Chip Technology

Sodium, Potassium, Chloride, Ionized Calcium, pH and PCO₂ by ion-selective electrode potentiometry.

**Urea** is first hydrolyzed to ammonium ions in a reaction catalyzed by the enzyme urease. The ammonium ions are measured by an ion-selective electrode.

**Glucose** is measured amperometrically.

**PO₂** is measured amperometrically.

**Hematoctrit** is determined conductometrically.

**HCO₃, TCO₂, BE, sO₂, Anion Gap and Hemoglobin.**

**i-STAT Co.** (Princeton, NJ)

Painless Blood Glucose Monitor

Cygnus’s GlucoWatch uses electroosmosis to draw glucose molecules from the skin into a dermal patch for analysis.

**Cygnus, Inc.** (Redwood City, CA)

Triage® Cardiac Panel & BNP Test

- A rapid, one-step whole blood test to aid in the diagnosis of AMI (Acute Myocardial Infarction)
- Quantitative results of three Cardiac markers
  - Myoglobin
  - CK-MB
  - Troponin I
- Simultaneous measurement of three AMI markers may improve sensitivity and specificity
- Results in approximately 15 minutes

**Biosite’s Protein chip**

www.biosite.com

Over 500,000 Cardiac & BNP protein chips on an annualized basis

Trends in Biosensor & Biochip

- Improvements in health care, therapeutics
- Integrated point-of-care diagnostic systems (Lab-on-a-chip)
- Synergetic integration of nano/micro system technology to biotech
- Standardization of platform technology
- Emerging e-Health business areas
- Need for personalized biological information services

μ-TAS (Total Analysis System)

- Classifying the μ-TAS field
  - The analyte phase (liquid/gas)
  - Mode of operation: flow-type & separation type
  - Application-oriented classification
- Elements of a μ-TAS
What is Microfluidics?

Technologies/devices capable of controlling and transferring tiny quantities of liquids to allow biological assays to be integrated and accomplished on a small scale

- Microfluidics is the science of designing and manufacturing devices and processes for manipulation of extremely small volumes of liquid
- Microfluidics promises to minimize the time and cost associated with routine biological analysis while improving reproducibility
- The first wave of micro-laboratories (referred to as µ-TAS, micro total analysis systems)
- The next generation of drug discovery tools

Microfluidic Systems

- Aqueous flow is generally laminar, not turbulent
- Diffusion is an efficient process for mixing the dissolved contents of two or more fluids
- Particles can also be separated by diffusion according to their size

Advantages of Microfluidics over Macro-Scale Lab Operations

- **Miniaturization**
  - Need only a few tens or hundreds of nanoliters of sample and reagent for each process
  - Reaction in miniature is more accurate and faster than that on a macro scale
  - There is a very high surface-to-volume ratio in channels in microfluidic devices
- **Automation**
  - Can be done on standardized chips with little human intervention
- **Integration**
  - Can be designed with multiple “on-board” functions with the sample being automatically guided from one place to another on the chip

Putting The Lab on a Chip

Test tubes, beakers and other glassware are replaced by microscopic channels in the chip

The Growth of Microfluidics and Lab-on-a-Chip Market

- To increase throughput and reproducibility
- To decrease cost and time to do experiments in order to streamline and accelerate laboratory assays
- To supply analytical tools and solutions dedicated primarily to the drug development research.
- To speed up and simplify the sample preparation steps in genomic and proteomic experiments
- To offer high-throughput, low-cost versions of traditional research methods

High-Throughput Technologies

High Throughput Screening
- Assay development
- Cell-based screens
- Lead Optimization

Genomic Drug Discovery
- Microarrays and Gene Expression
- SNPs & Pharmacogenomics
- Identification of Gene Targets
- Validation of Genomic Targets

Supporting Technologies
- Rapid compound characterization
- Miniaturization
- Lab automation

Chemoinformatics
- Diversity & Library Design
- Virtual Screening
- Analysis of HTS Results
- Integration
Milestones in Miniaturization

- From Micro-well to Nano-well -

- 96-channel pipetter introduced
- 96-well colorimeter introduced
- ELISA invented
- 96-well pipetter introduced
- 384-well plate introduced
- 96-well disposable plate patented
- 96-well plate invented
- 1536-well plate introduced
- 864-well plate introduced
- 96-well strip-well plate introduced
- 96-well fluorimeter invented
- 384-well plated introduced


(Source) Dr. Jonathan Burbaum, Pharmacopeia, Inc.

HTS Market Requirements

- Reduce overall cost of assay
- 100,000 assays per day
- Reduce use of target/compound library
- Compatible with existing robotics (96/384)
- Disposable
- Applicable to wide range of assay types
- Easy transfer of assay devices to HTS
- Scaleable to 500K assays per day

Miniaturization in High Throughput Screening

- To increase the productivity of drug discovery efforts
- To decrease time to market
- To reduce the cost of drug discovery
- To accommodate the enormous increase in compounds and targets over the past few years
- To increase throughput while decreasing costs

Issues on Microfluidic Array Applications

- Design & Fabrication Issue
- Sample Preparation Issue
- Detection Issue
- Integration Issue

Needs for Microfluidic Components

- High throughput screening
- Genomics
- Proteomics
- Cellular analysis

- Microtiter plates
- Sub-microliter dispensing technologies
- Amplification
- Separation
- Mass spectrometer, X-ray diffraction, Protein-protein interaction devices
- Cell sorting, Cell counting, Electrophysiology

Microfluidic Array Applications

- Design & Fabrication Issue
- Sample Preparation Issue
- Detection Issue
- Integration Issue
Nano-Well Plates

Reaction occurred in a static environment in miniaturized reaction vessels. The miniaturized version of the open micro-titer plate, the nanowell plate
(a) Laser ablated nanowell in PMMA with well volume of 11 nl.
(b) Hot embossed nanowell plate with a well volume of 700 pl.

Becker and Klotzbucher (1999)

LabCard Microfluidic Devices

Genomic and proteomic applications, such as RNA and DNA analysis, as well as high-throughput pharmaceutical drug screening

Aclara Biosciences

Microfluidic arrays for high-throughput submicroliter assays using CE

Microtiter Plates Based on the Capillary Action

96 Well

Multi-level fluid handling system

Centrifugal Microfluidic System: LabCD™

A plastic disc (CD) with a network of chambers, channels and vents (fluidics) and information (informatics) about the specific function of the analytical Lab incorporated on the disc

LabCD™

Microfluidic Array Applications

Design & Fabrication Issue

Sample Preparation Issue

Detection Issue

Integration Issue
Microfluidic Disposables

H-Filter™
Membrane-free inline separation and extraction of compounds in solution

Drug discovery, chemical synthesis, environmental


On-line Protein Digestion

Integrated Microanalytical Workstation

In vitro culturing
Sample handling
robot
Cellular
fractions
Microchip
digestion
Piezoelectric
Dispenser (40-60nm)

AstraZeneca, Sweden

Microfluidic Strategies

Pumping Techniques

Capillary forces
Steag MicroParts
Biosite Diagnostics

Piezoelectric
IMM, IEMN,
Tronic’s
Microsystem,

External actuation
bioMerieux, Micronics

Electrosporation/Capillarity/Osmosis
Caliper Technologies,
Philips Research Center
Agilent Technologies

Electrowetting
on Dielectric
UCLA

Sorting by Diffusion

FIG. 3. Operation of the sorting device. A mixture of molecules is injected into the microchannel at the top center. The separated components of the mixture are collected at locations along the opposite side.

PNAS 96: 13762-13765 (1999)

Fluorescence-Activated Cell Sorter

- Conventional FACSs ($250,000)
- µFACSs that rapidly sort live cells, particles, and single molecules would greatly facilitate screening of combinatorial chemistry libraries or cell populations during in vitro molecular evolution.

- Fluorescence based sorting
- PDMS molded from silicon master
- Feedback controlling fluid flow (electroosmotic flow)


Microfluidic Array Applications

Design & Fabrication Issue
Sample Preparation Issue
Detection Issue
Integration Issue
Surface-Enhanced Laser Desorption/Ionization (SELDI) Process

Step 1 (Expose)
Step 2 (Develop)
Step 3 (Read)

Patient sample

Smaller proteins fly faster and hit the detector first

Protein "Fingerprint" Database

Composition & Mass Information (at each X,Y address)

Unique information sets combined

High Throughput Applications with Suspension Arrays

LabMAP: Laboratory Multiple Analyte Profiling

Multiple Measurements with Color Separation

Microspheres in a Fluid Stream

Polystyrene microspheres

www.luminexcorp.com

Drivers for Seeking New Detection Modes

- Achieve simpler, one step, homogeneous assays
- Avoid radioactivity, safety, and disposal costs
- Increase sensitivity and go to higher density
- Lower cost and conserve reagents, targets and compounds
- Ensure compatibility with miniaturization
- Achieve uniform platform with fewer kinds of assays

Pharmaseq's Microtransponders

Fluorescence
Radio waves
Transponder
Flow
Light

www.pharmaseq.com

Microfluidic Array Applications

Design & Fabrication Issue
Sample Preparation Issue
Detection Issue
Integration Issue

Microfluidic Array Applications

University of Michigan

- Metering samples
- PCR reaction
- Gel electrophoresis
- Sensors and actuators

The μChemLab™: Micro Total Analysis System

Caliper's LabChip® High Throughput Screening System

Requirements for Microfluidics Product Development

- Patent Analysis
  - Based on selection of the appropriate patented portfolio to achieve the type of microfluidic processing needed for the individual application requirements

- Surface Chemistry/Materials Analysis
  - It is one thing to know the specific microfluidic approach ("How it’s done"). It is also critical to assess the environment ("Where it’s done") with respect to surface chemistries and possible interactions with materials used in the assay.

- Fluidic Modeling

- Detection
  - How the results of microfluidic interactions on a lab card will be detected, interpreted, and reported.

Thank You!