http://nanobio.kaist.ac.kr

Miniaturized Bioanalytical Systems for Biotech Industry

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- Technology Overview of Bioanalytical Systems
  - Biofluidic Devices and Systems
  - High Throughput Screening Systems
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"Two technologies will dominate the 21st century scientifically and industrially : *biotechnology and information technology*"

Bill Gates
http://www.microsoft.com/billgates/default.asp

"There is Plenty of Room at the Bottom"

Prof. Richard P. Feynman
December, 1959
California Institute of Technology
http://www.zyvex.com/nanotech/feynman.html

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

"Biology is not only for biologists"

Harold E. Varmus
The 1989 Nobel Prize Winner in Physiology or medicine

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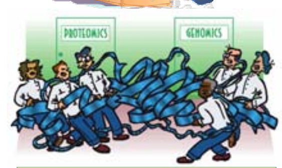
New Paradigm for Bio-Related Industry



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

- Microfluidic Technology
- High Throughput Screening

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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.

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Technology Overview of Bioanalytical Systems

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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 PCO_2
by ion-selective electrode potentiometry.

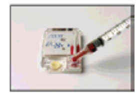
Urea is first hydrolyzed to ammonium ions in a reaction catalyzed by the enzyme urease.
The ammonium ions are measured by an ion-selective electrode.

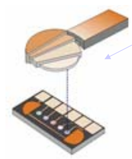
Glucose is measured amperometrically.

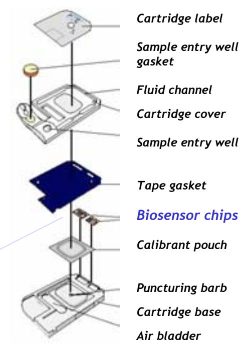
PO_2 is measured amperometrically.

Hematocrit is determined conductometrically.

HCO_3, TCO_2, BE, sO_2,
Anion Gap and Hemoglobin.







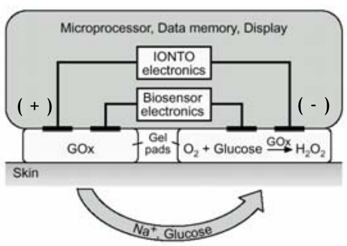
i-STAT Co. (Princeton, NJ)

Painless Blood Glucose Monitor

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

GlucoWatch Biographer

AutoSensor



Cygnus, Inc. (Redwood City, CA)

Triage® Cardiac Panel & BNP Test

TRIAGE® CARDIAC SYSTEM

- 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

TRIAGE® BNP TEST

- A quantitative test for measurement of B-type Natriuretic Peptide (BNP), a protein marker for Congestive Heart Failure (CHF)

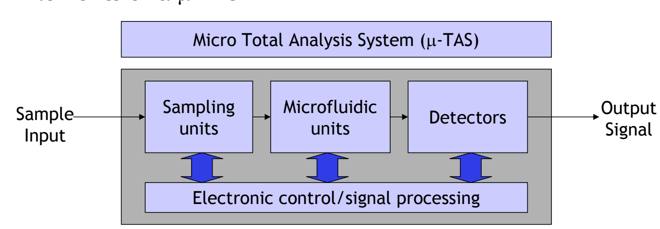
Over 500,000 Cardiac & BNP protein chips on an annualized basis

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

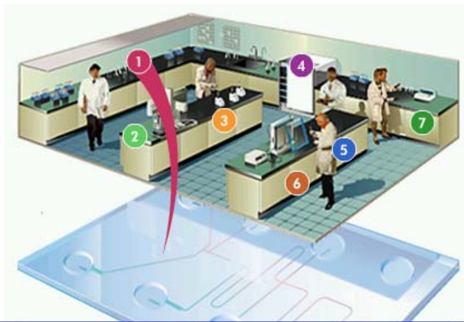
Microfluidic Systems

- **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



www.calipertech.com

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
Detection
Lead Optimization

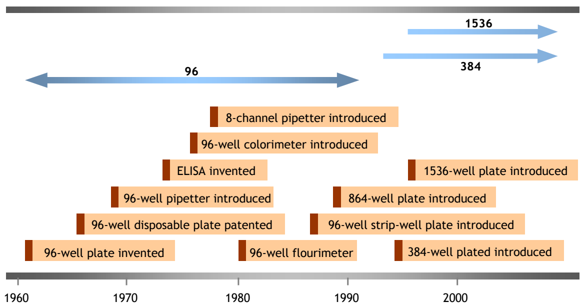
Genomic Drug Discovery
Microarrays and Gene Expression
SNPs & Pharmacogenomics
Identification of Gene Targets
Validation of Genomic Targets

Supporting Technologies
Rapid compound characterization
Miniaturization
LIMS
Lab automation

Chemoinformatics
Diversity & Library Design
Virtual Screening
Analysis of HTS Results
Integration

Milestones in Miniaturization

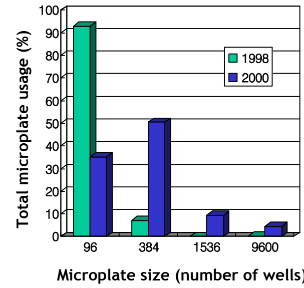
- From Micro-well to Nano-well -



(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



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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

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Issues on Microfluidic Array Applications

Needs for Microfluidic Components

High throughput screening
- •Microtiter plates
- •Sub-microliter dispensing technologies

Genomics
- •Amplification
- •Separation

Hybridization, Sequencing

Proteomics
- •Separation
- •Spraying
- •Crystallization

Mass spectrometer, X-ray diffraction, Protein-protein interaction devices

Cellular analysis
- •Cell sorting
- •Cell counting
- •Electrophysiology

Screening & diagnosis applications

Design & Fabrication
Sample Preparation Issue
Detection
Integration

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Microfluidic Array Applications

Design & Fabrication Issue

Sample Preparation Issue

Detection Issue

Integration Issue

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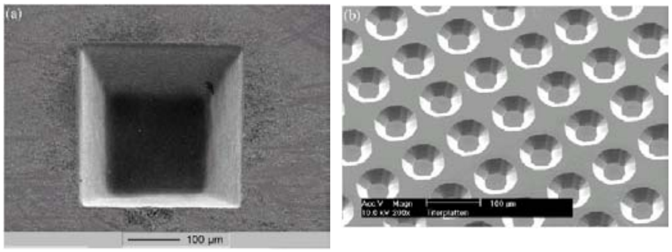
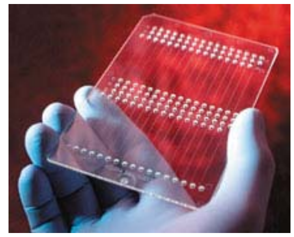
Nano-Well Plates



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.

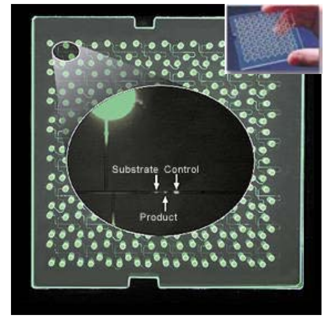
Becker and Klotzbucher (1999)

LabCard Microfluidic Devices



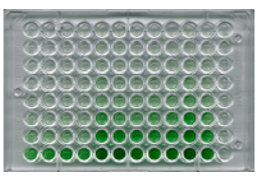
Genomic and proteomic applications, such as RNA and DNA analysis, as well as high-throughput pharmaceutical drug screening

Aclara Biosciences

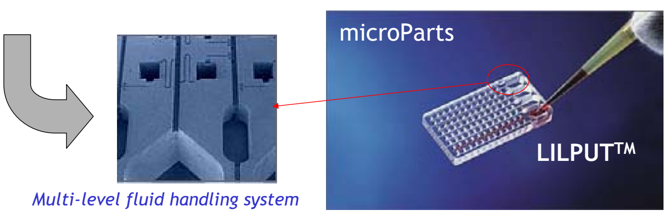


Microfluidic arrays for high-throughput submicroliter assays using CE

Microtiter Plates Based on the Capillary Action



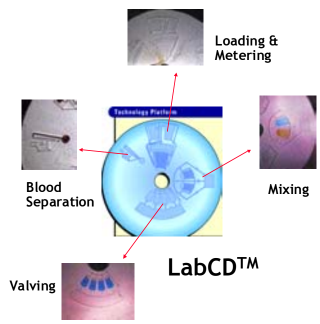
96 Well

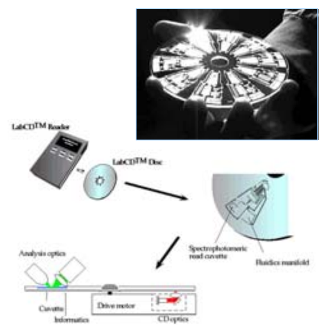


Multi-level fluid handling system

Centrifugal Microfluidic System: LabCD™

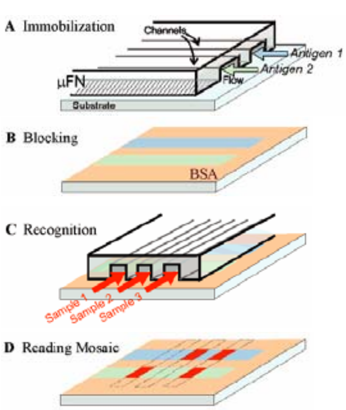
A plastic disc (CD) with a network of chambers, channels and vents (fluidics) and information (informatics) about the specific function of the analytical Lab incorporated on the disc

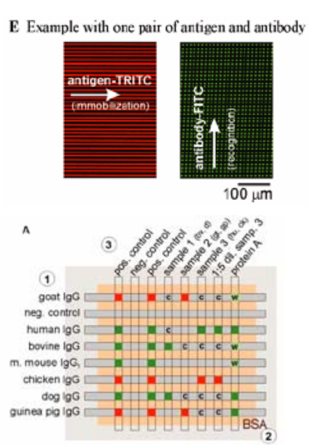




Tecan

Micromosaic Immunoassays





Anal. Chem. (2001). 73: 8-12

Microfluidic Array Applications

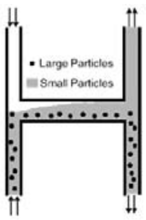
Design & Fabrication Issue

Sample Preparation Issue

Detection Issue

Integration Issue

Microfluidic Disposables

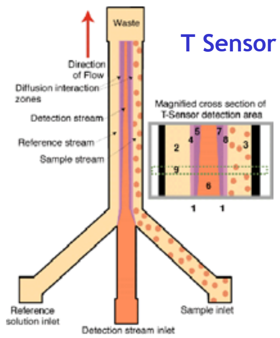


H-Filter™

Membrane-free inline separation and extraction of compounds in solution

→ Drug discovery, chemical synthesis, environmental

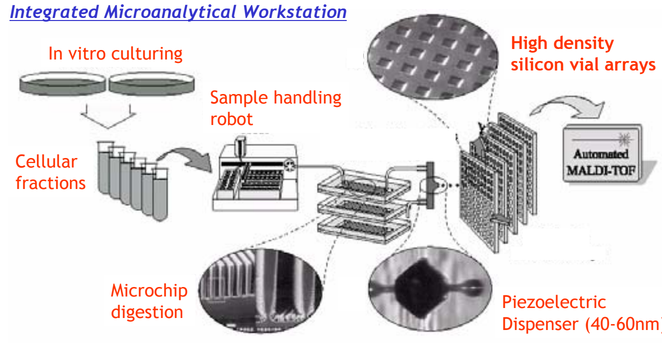
MICRONICS



Science 283: 346-347 (1999)

On-line Protein Digestion

Integrated Microanalytical Workstation



AstraZeneca, Sweden

Microfluidic Strategies

Pumping Techniques

Centrifugal
Tecan, Gyros

Capillary forces
Steag MicroParts
Biosite Diagnostics

External actuation
bioMerieux, Micronics

Magnetism/ Acoustic
Aviva BioSciences

Electrophoresis/ Capillary/ Osmosis
Caliper Technologies, Nanogen, Philips Research Center Agilent Technologies

Piezoelectric
IMM, IEMN, Tronic's Microsystem,

Electrowetting on Dielectric
UCLA

Sorting by Diffusion

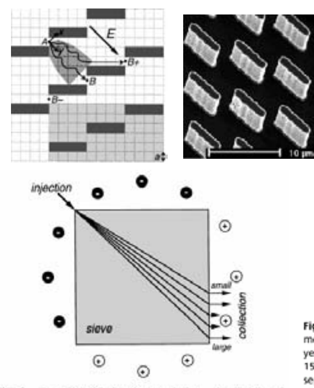


FIG. 2. Operation of the sorting device. A mixture of molecules is injected into the microchamber at the top corner. The fractionated components of the mixture are collected at intervals along the opposite edge.

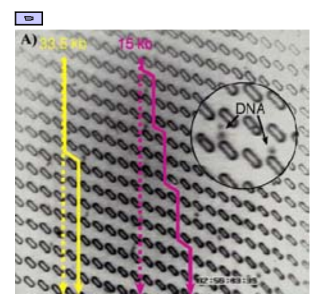


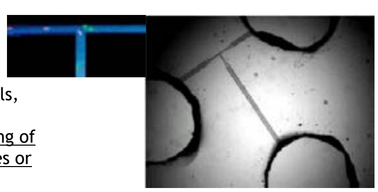
Fig. 3. (A) The trajectories of *Xho*I-digested bacteriophage λ DNA fragments. The straight lines show the average electric field direction. The solid yellow line traces the path of a 33.5-kbp fragment; the purple line traces a 15-kbp fragment. The frame size is 130 × 114 μm². Total dimension of the separation device is 3 × 4 cm, and electric field strength is 1.4 V/cm. (A video clip illustrating the process is available at http://suiling.princeton.edu.)

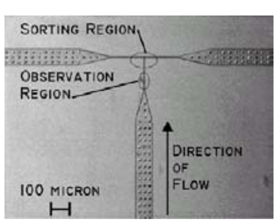
PNAS 96: 13762-13765 (1999)

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)





Nature Biotech. 17: 1109-1111 (1999).

Microfluidic Array Applications

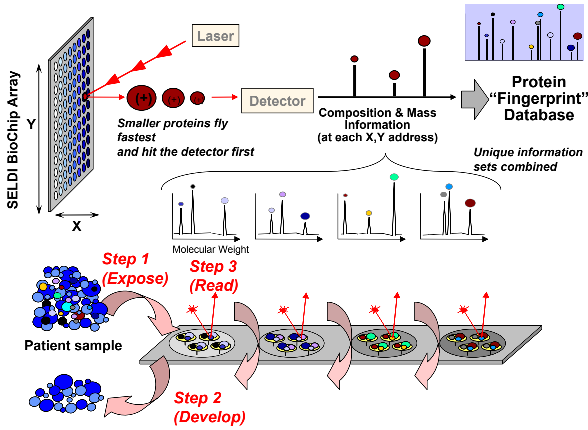
Design & Fabrication Issue

Sample Preparation Issue

Detection Issue

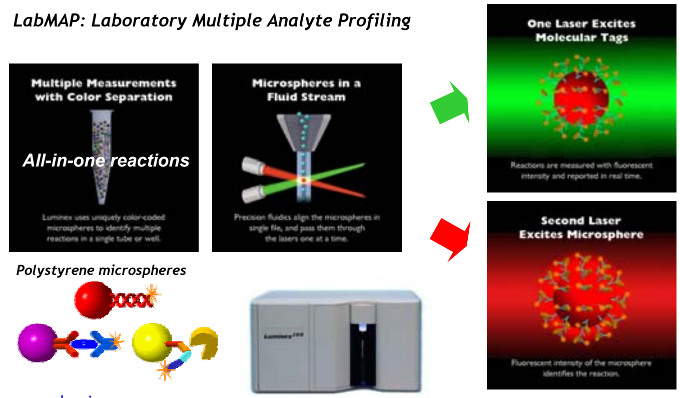
Integration Issue

Surface-Enhanced Laser Desorption/Ionization (SELDI) Process



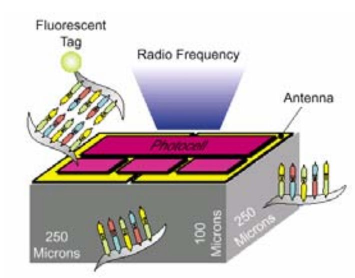
High Throughput Applications with Suspension Arrays

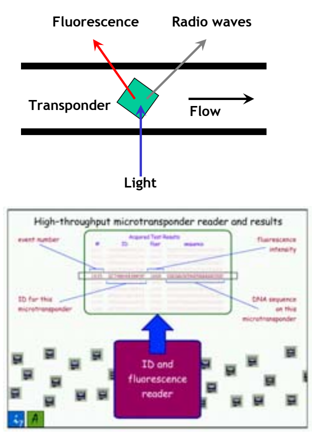
LabMAP: Laboratory Multiple Analyte Profiling



www.luminexcorp.com

Pharmaseq 's Microtransponders





For 8" wafers
100k ~ 1M/wafer
$100/wafer

www.pharmaseq.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

Microfluidic Array Applications

Design & Fabrication Issue

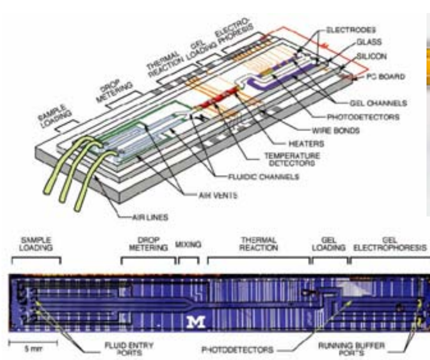
Sample Preparation Issue

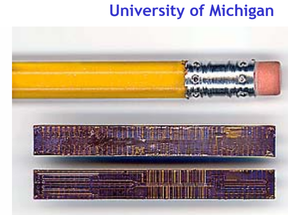
Detection Issue

Integration Issue

Micromechanical Integrated DNA Analysis

University of Michigan





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

Science 282: 484-487 (1998)

The μChemLab™ : Micro Total Analysis System

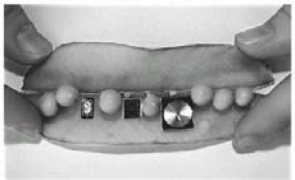


Fig. 2 Components of the μChemLab gas analysis subsystem—from left to right the SAW detector (an array of four), the sample collector/preconcentrator, and a 1 m gas chromatography column.

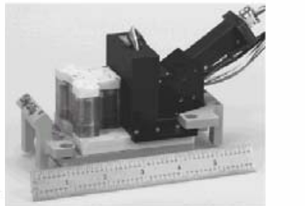


Fig. 4 Liquid analysis subsytem from the biotoxins analyzer. Injection ports to the left. Buffer and waste reservoir cartridge in the center. LIF detection subsystem to the right. The separations chip is underneath.

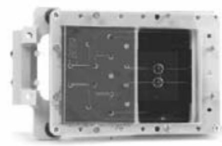
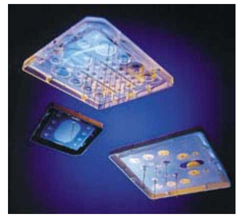
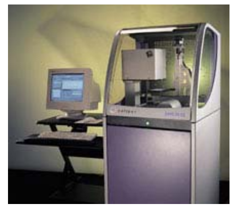


Fig. 5 Carrier mounted dual column (CZE and CGE) separations chip used in biotoxins analysis.

Sandia National Laboratories

Caliper's LabChip® High Throughput Screening System

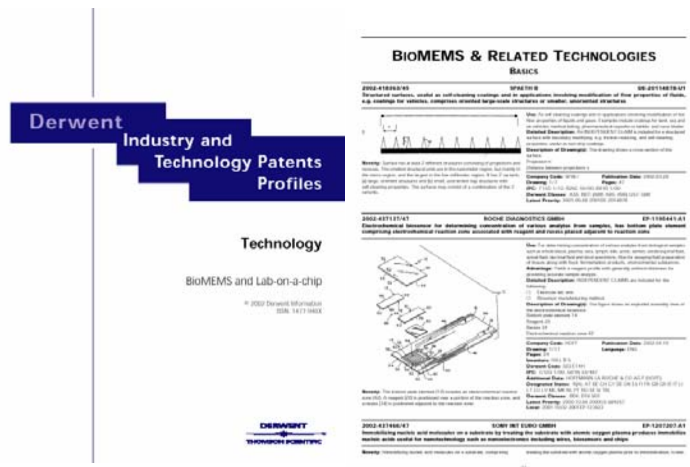




Caliper's high-throughput AMS 90 SE Electrophoresis system. This assay will analyze nanoliter sample volumes of protein mixtures from 96- and 384-well plates. The assay automatically determines constituent protein sizes and relative concentrations.

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.*



www.derwent.com