



The Abdus Salam
International Centre for Theoretical Physics


United Nations
Educational, Scientific
and Cultural Organization


International Atomic
Energy Agency



SMR.1670 - 28

INTRODUCTION TO MICROFLUIDICS

8 - 26 August 2005

Microarrays and Biochip Assays

R. Luttge
University of Twente, Enschede, The Netherlands

8. Microarrays and biochip assays

Topics in this lecture

Genomic impact

Why are arrays and biochip developments so successful....

Conflicting technologies?

Microelectronics versus classical biological experiments...

Assay applications

Microfabrication is linked to high through-put, however, here also examples are presented that make use of unique properties found on the microscale.

- Introduction
- Strategic developments of surface- and bead-based assays
- Added value by integration
- Examples of integrated microfluidic assays
- Outlook: Future developments
- Summary

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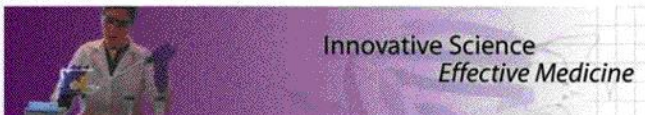


Topics in this section

Basic research and techniques



Microtechnology-assisted



Introduction

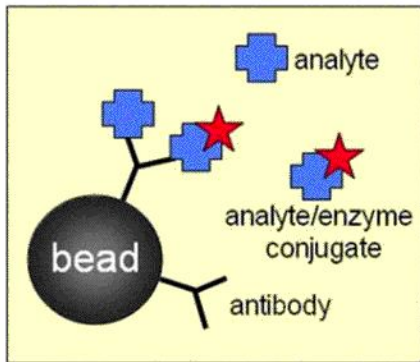
- Impact of Genomics
 - The beginning of understanding life (video)
- High-throughput screening
 - Bead chemistry
 - Electrical arrays
 - Optical arrays

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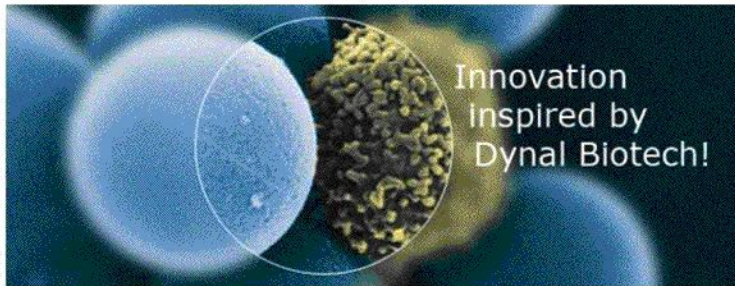
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Bead chemistry



- Biodetection methodology based on multiple use of instrumentation exploiting bead attachment chemistry.
- Enable rapid parallel analysis of the many thousands of proteins



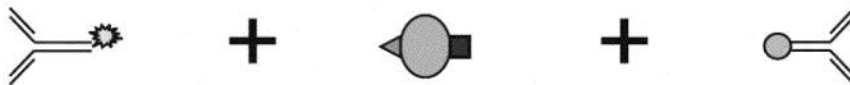
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Functionalizing chemistry

Sequential immunoassay



Detection antibody conjugated to e.g. fluorophore or enzyme

Analyte present in sample

Biotin-conjugated capture antibody



Combine antibodies and sample, mix and incubate

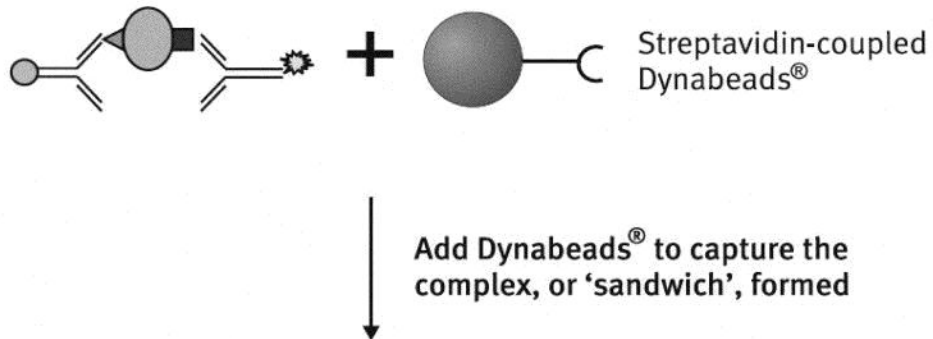
www.dynalbiotech.com

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8.1. Introduction



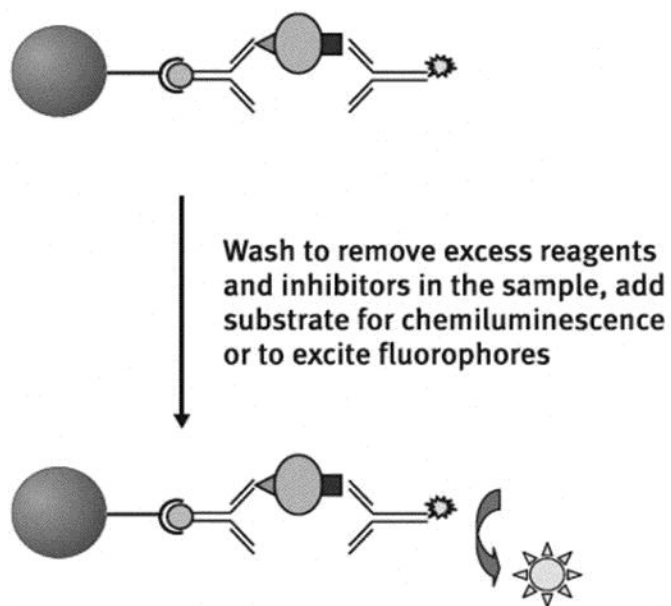
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8.1. Introduction



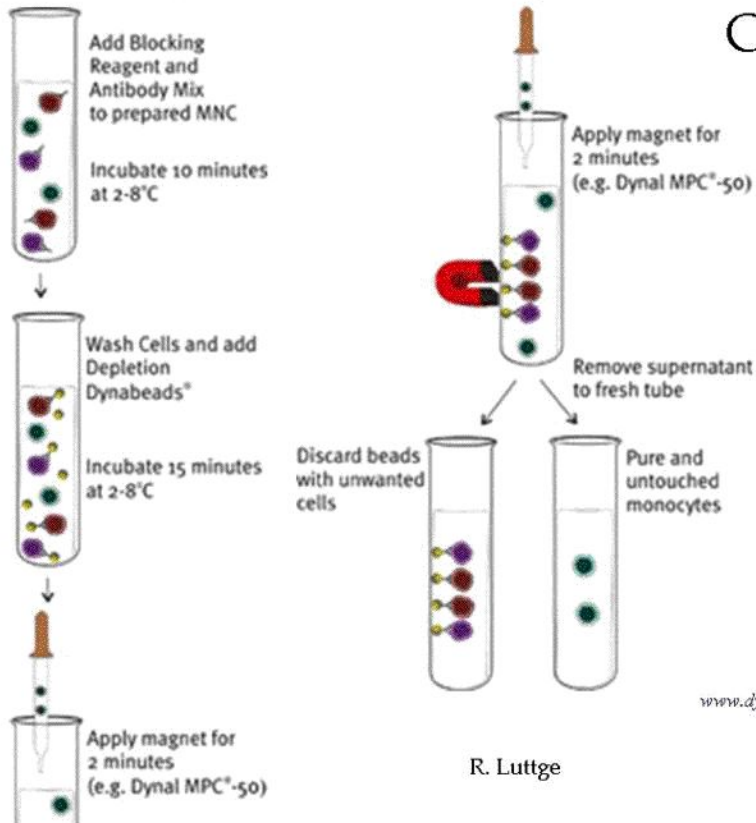
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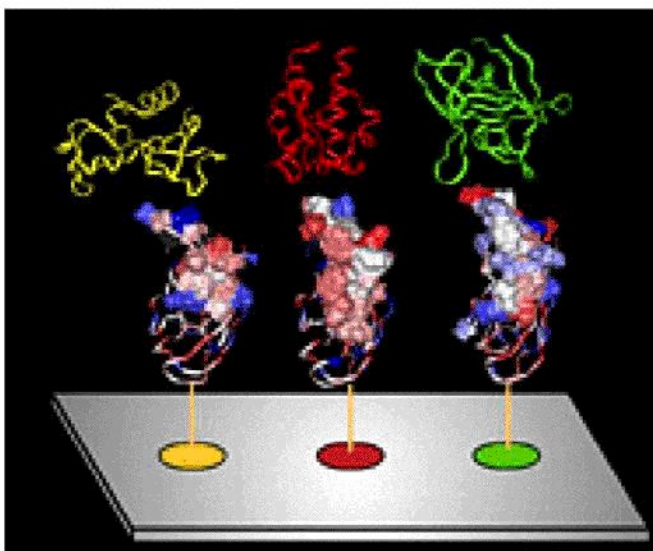


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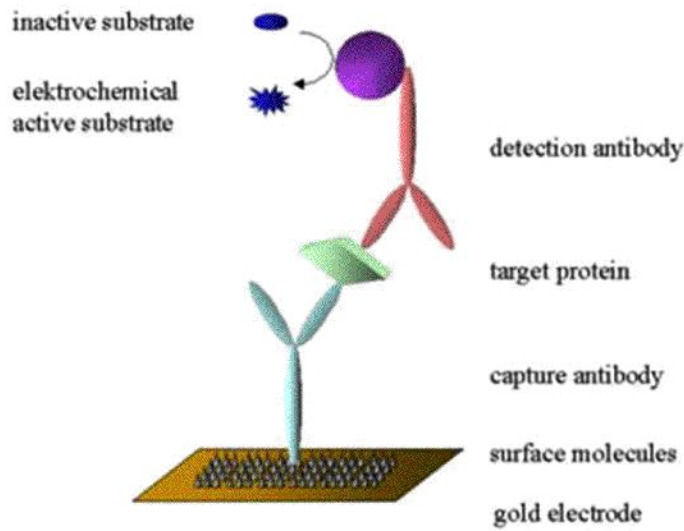
Functionalized surface arrays



- Protein targets approaching immobilized antibody array
- Hydrogel binding or thiol-based self assembled monolayer (SAM)

8.1. Introduction

Functionalized surface detection



www.chemie.uni-hamburg.de

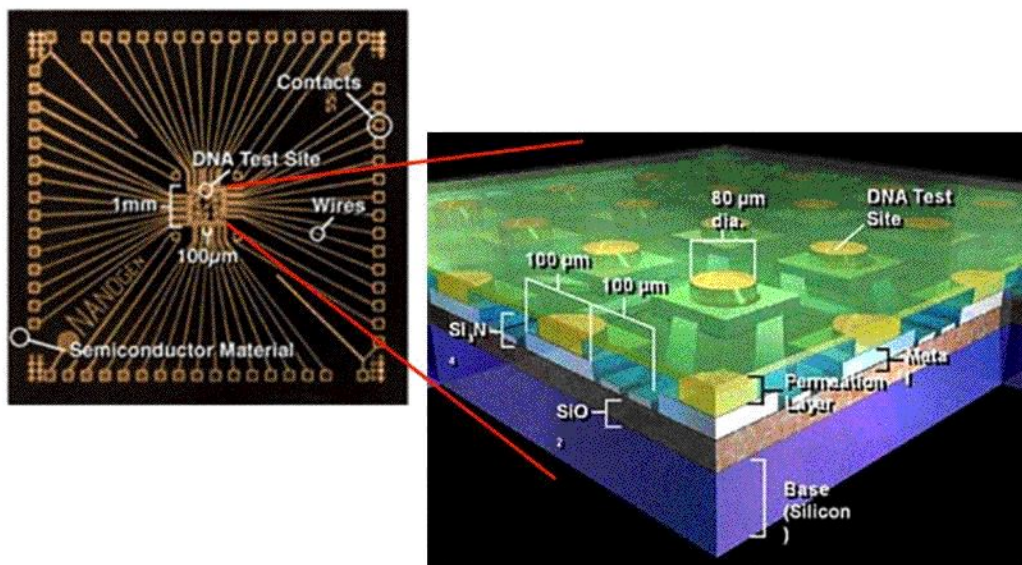
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8.1. Introduction

CMOS integrated array device for advanced electrochemical assays



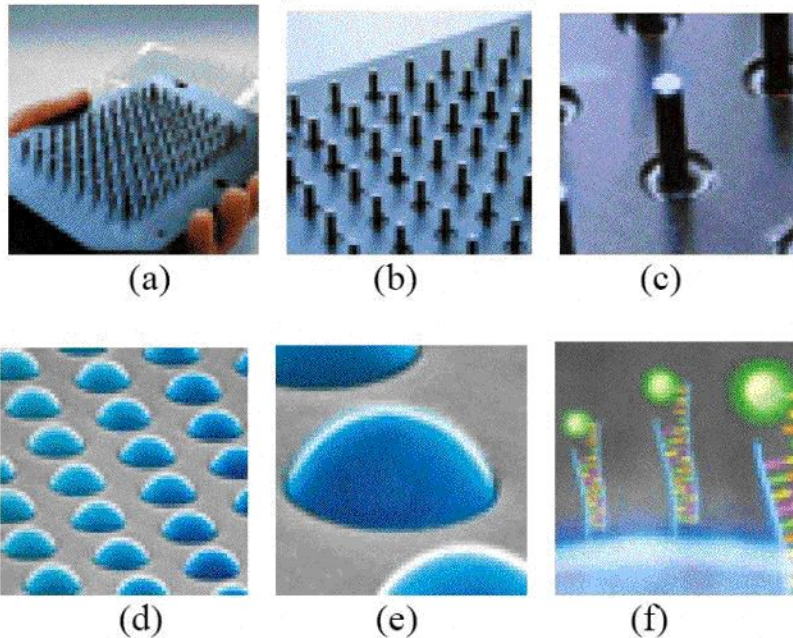
www.nanogen.com

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Optical integrated bead array



www.illumina.com

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Strategic developments of surface- and bead-based assays



- Spotted surface arrays
 - Spotting, antibody capture and binding relation
 - Examples of optical and electrochemical arrays
 - Microelectromechanical arrays
- Bead-based microchips
 - Bioanalysis using stationary trapped beads

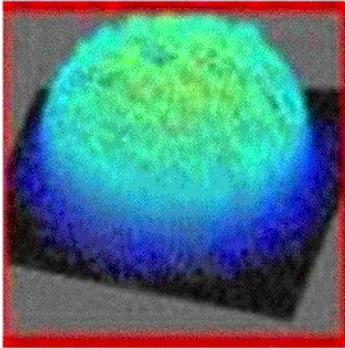
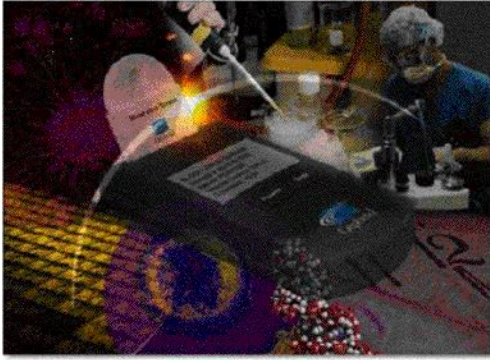
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8.2. Strategic developments of surface- and bead-based assays

Successful biochips?



- Reliable, accurate
- Sensitive
- Low non specific binding (NSB)
- High spot density (the more the better/cm²)
- Comparable
- High quality spot
- Repeatable

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8.2. Strategic developments of surface- and bead-based assays

Spotting technologies

- Split pen, contact printer
- Pin and ring spotter
- Piezo, contactless spotters
- Ink-jet spotting (Agilent)
- Top Spot (Biofluidix)
- Photolithography (Affymetrix)
-New developments....

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TopSpot Technology

Spot different ligands on a glass slide



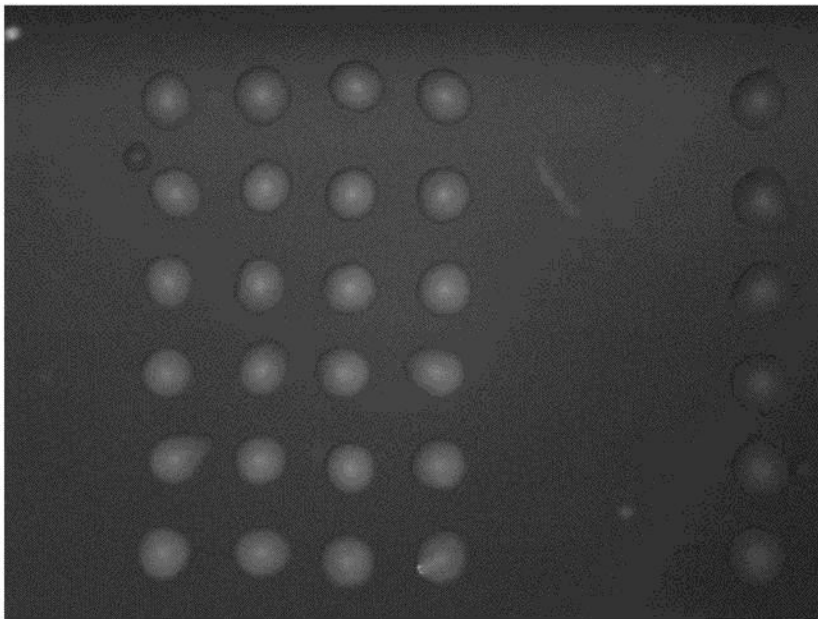
- **BioFluidiX** (www.biofluidix.com)
 - Liquid handling for the Life Sciences enabled by Microsystems technology.
 - Print head with 24, 96, or 384 different media.
- **Company spin-out from IMTEK, Freiburg, Germany** (www.imtek.de)

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Top-spot protein array



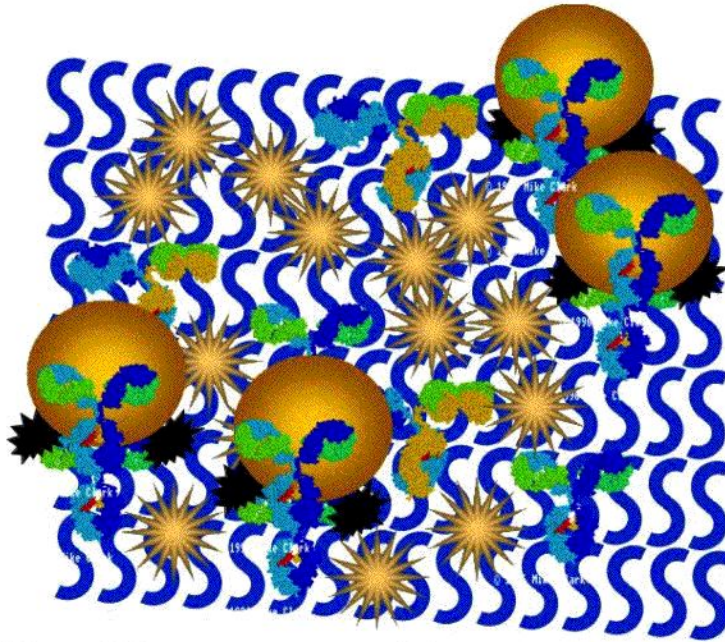
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8.2. Strategic developments of surface- and bead-based assays

Biomolecular interaction



From: R. Schasfoort, Biochip group, University of Twente

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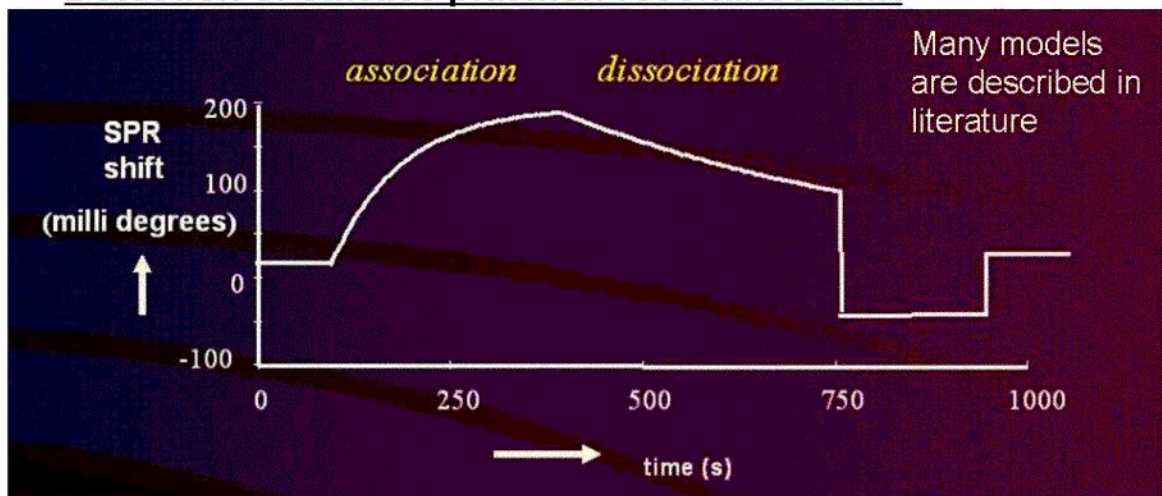
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8.2. Strategic developments of surface- and bead-based assays

Interaction kinetics

Detection of surface plasmon resonance shift

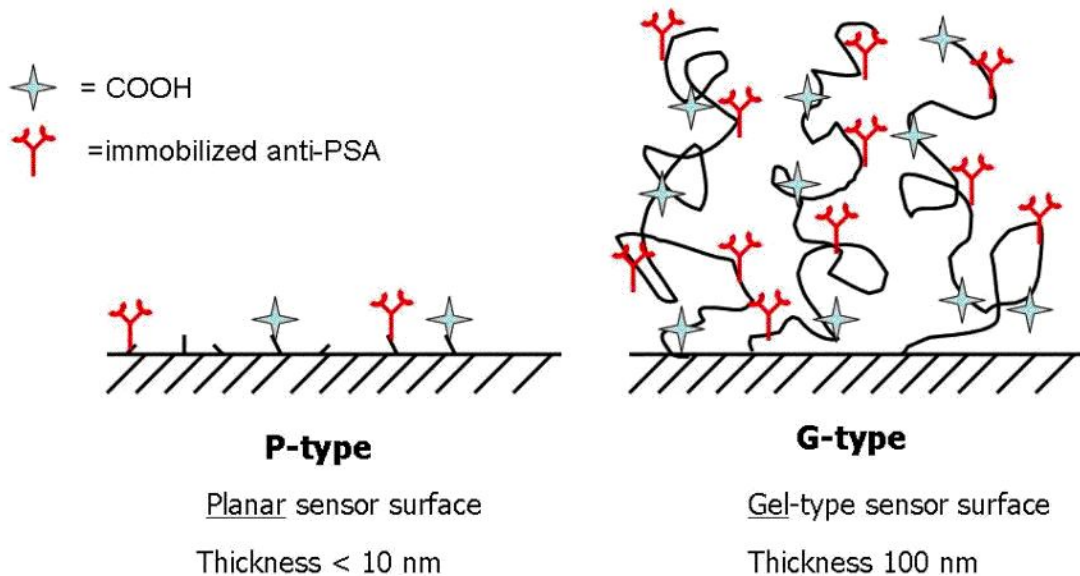


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Sensoric surface immobilization



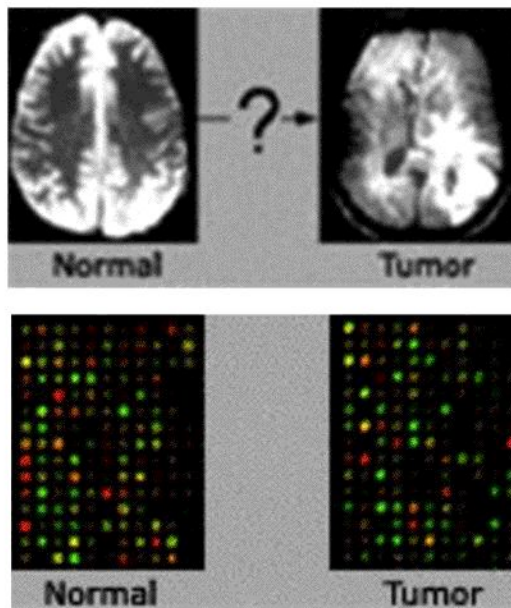
Slide prepared by R. Schasfoort, Biochip group, University of Twente

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The brain tumor project



- Using "Biochips" carrying 1000's of spotted DNA samples for detecting cancer-causing DNA in tumor tissue samples. After being exposed to an extract of the tissue, the brain tumor biochip is analyzed to provide digitized data on the tumor's genetic structure.

<http://www.vcu.edu/lifesci/phd/research/biochip.html>

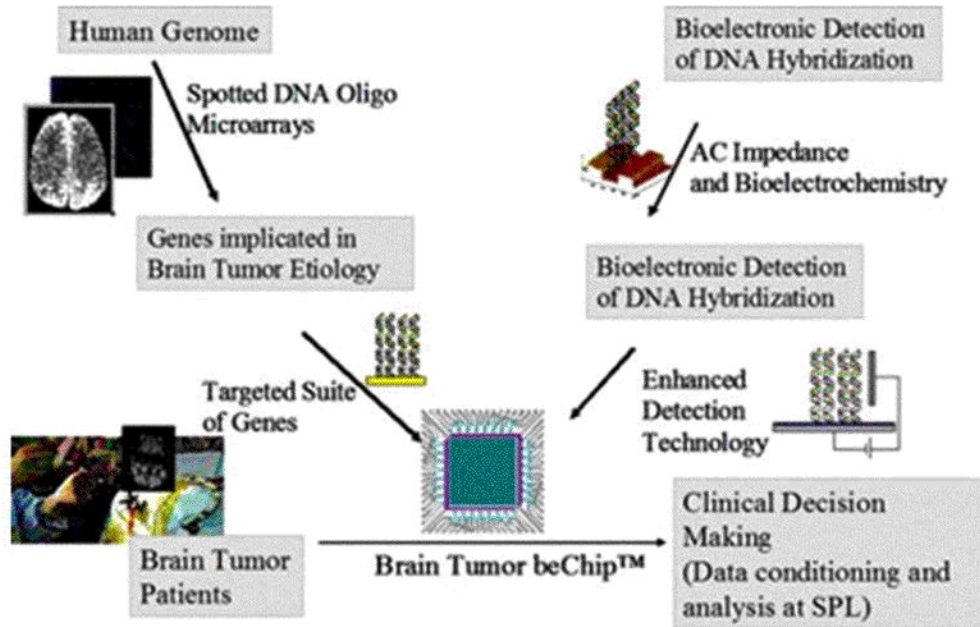
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8.2. Strategic developments of surface- and bead-based assays

Optimization by impedimetric DNA arrays



<http://www.vcu.edu/lifesci/phd/research/biochip.html>

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8.2. Strategic developments of surface- and bead-based assays

Bioelectronic array

The screenshot shows the Motorola website page for Biochips & Cartridges. The Motorola logo and tagline "Intelligence everywhere" are at the top. The page title is "Biochips & Cartridges". The main content area includes a navigation menu on the left with links for "Life Sciences", "Overview", "CodeLink™ Bioarray System", "eSensor™ DNA Detection System", "Products", "Technology", "Biochips & Cartridges", and "How the System Works". The main text describes the eSensor™ DNA Biochip, which consists of a small circuit board with gold electrodes and single-stranded DNA molecules. It detects different DNA sequences or SNPs by recognizing their complementary partners. A diagram on the left shows a cross-section of the chip with a "TARGET" strand, a "SIGNALLING PROBE", and a "CAPTURE PROBE". On the right, there is a photograph of two biochips, one in a clear plastic holder and one on a circuit board. The Motorola tagline "Making things smarter and life better" is at the bottom of the page.

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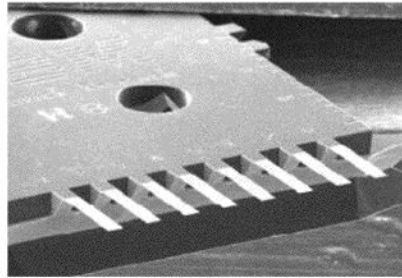
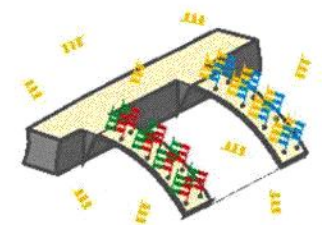
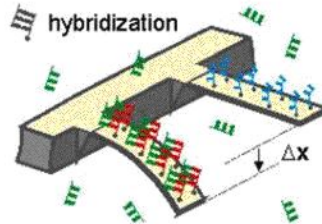
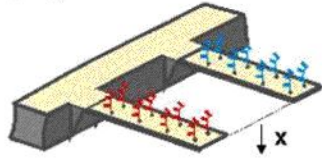
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8.2. Strategic developments of surface- and bead-based assays

oligonucleotide

Bio-MEMS array



- Microcantilever with bio-coating.
- Molecule interaction of test sample with bio-coating will lead to bending.
- Specific binding identification by resonance shift.



<http://www.zurich.ibm.com/st/nanoscience/cantilever.html>

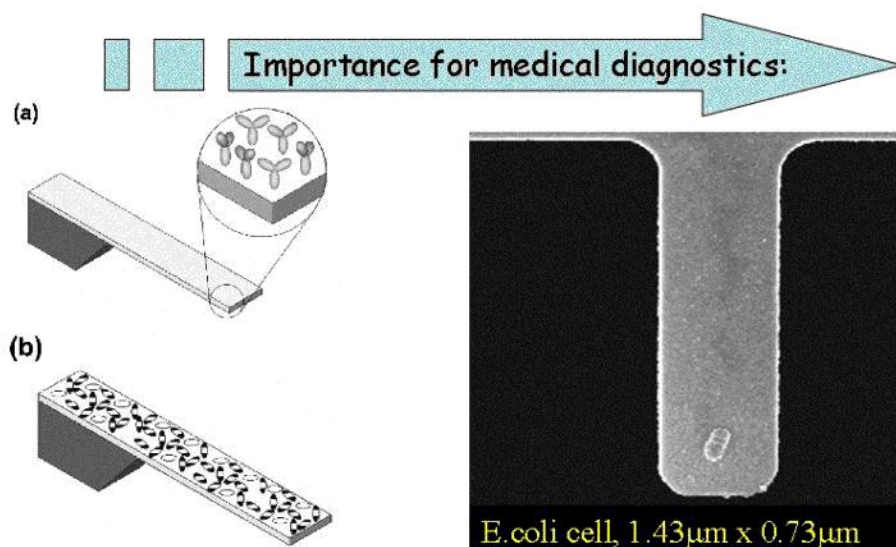
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Single cell detection



Ilic et al., JVSTB, vol. 19, iss. 6, pp. 2825-2828, 2001

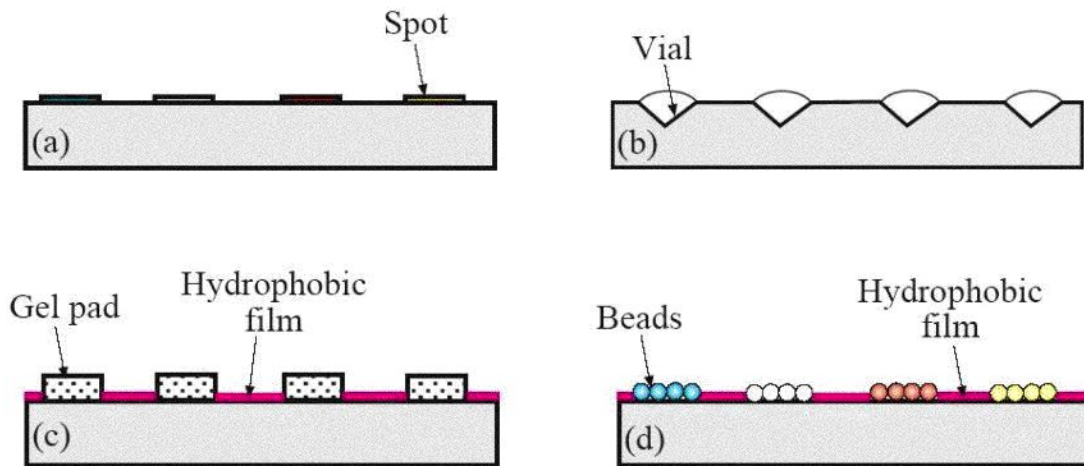
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8.2. Strategic developments of surface- and bead-based assays

Bead arrays



H. Andersson, PhD-Thesis

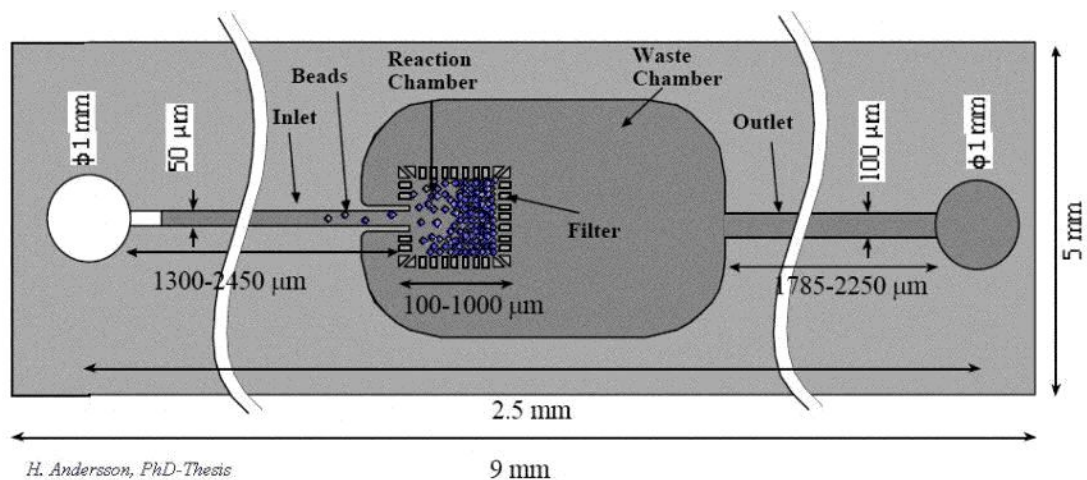
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8.2. Strategic developments of surface- and bead-based assays

Filter chamber design



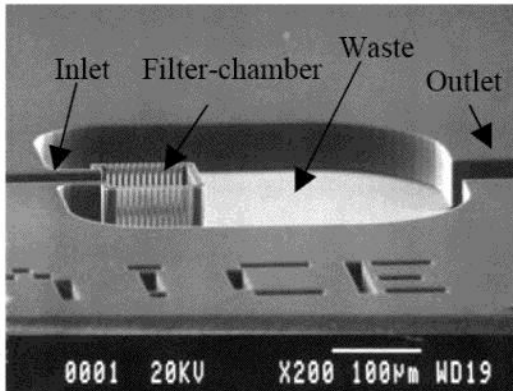
H. Andersson, PhD-Thesis

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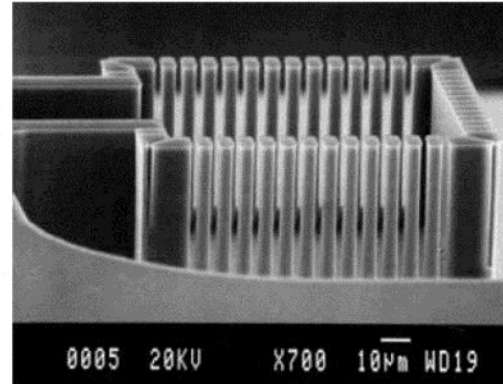
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Fabrication result



H. Andersson, PhD-Thesis



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Filter chamber bioassay

- Adding liquids and bead solutions manually or by microdispensing.
- 3kPas constant overpressure applied at the inlet of the flow-through filter chamber.
- Streptavidin-coated polystyrene beads with a diameter of 5.5µm were used.
- Samples collected at the outlet (microscope) were controlled to confirm that beads did not pass through the filter.

*H. Andersson, PhD-Thesis and
Andersson et al., Electrophoresis, 2001, 22, 249-257*

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Luciferase reaction

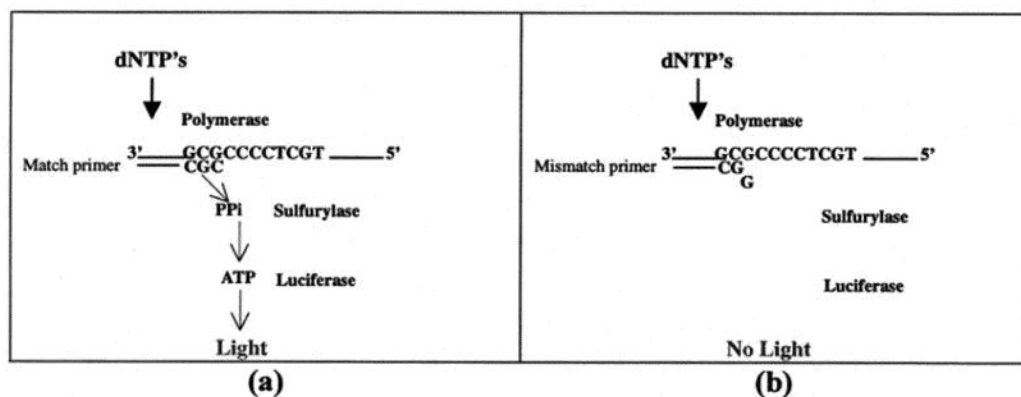


Figure 6. Principle of allele-specific pyro-extension. (a) Match primer annealed to the single-stranded DNA will result in detectable light. (b) 3'-End mismatch primer will not be extended by DNA polymerase, resulting in no light.

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Snapshot results

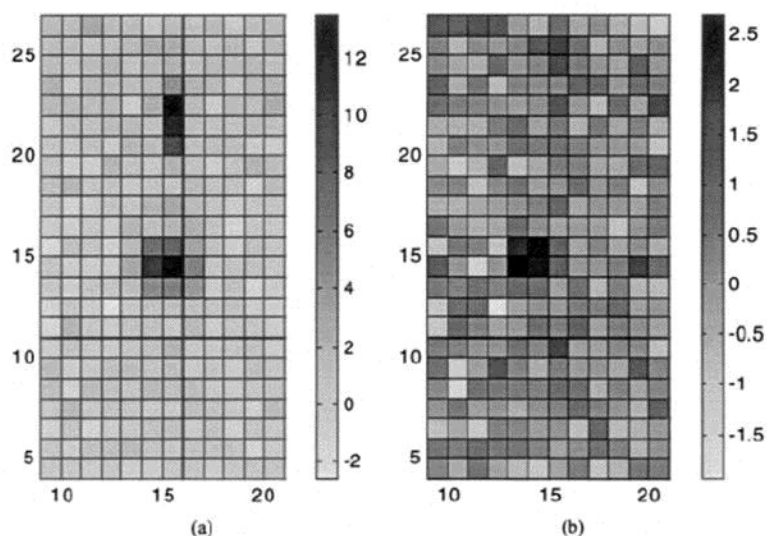


Figure 8. (a) A snapshot of the match pyro-extension. Light is detected in the filter-chamber and outlet. (b) A snapshot of the mismatch pyro-extension. A weak signal (background level) is detected in the filter-chamber.

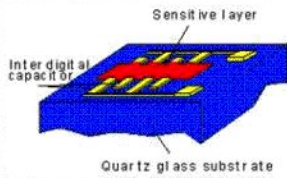
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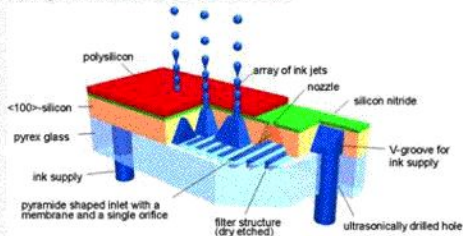
Topics in this section

Basic research and techniques



Microtechnology-assisted arrays

Ink-jet head



<http://www-mat.ee.tu-berlin.de>

- Facilitating array feedstock
- 3-D capacity (flow-through) arrays

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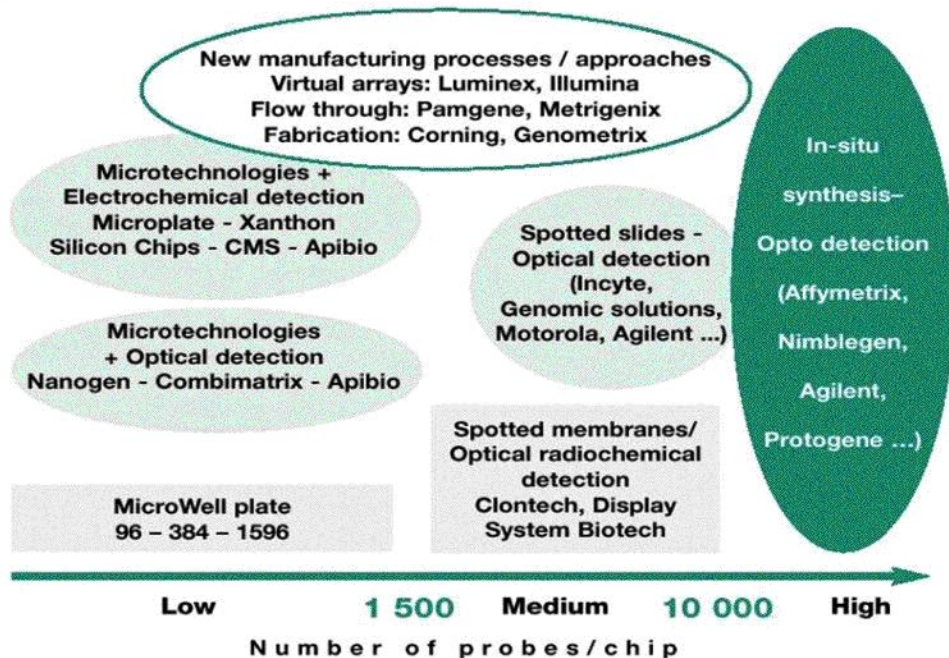
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8.3. Added value by integration

The BioFab report gives market and technologies analysis and more than 130 worldwide companies profiles in the field of Biochips, Microfluidic components and Biosensors.

Why small?



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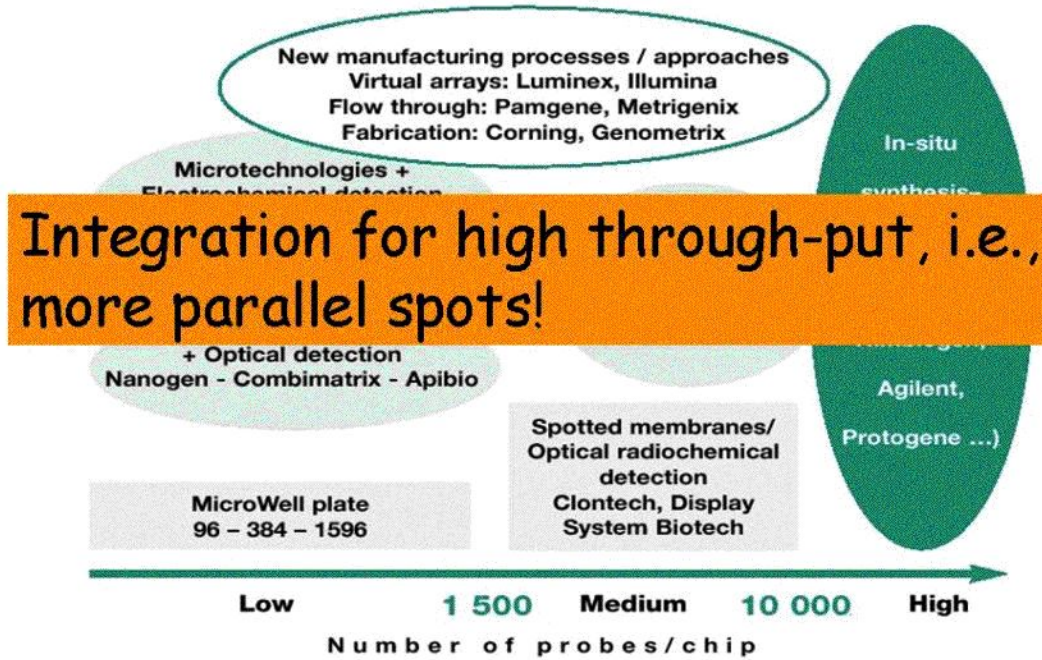
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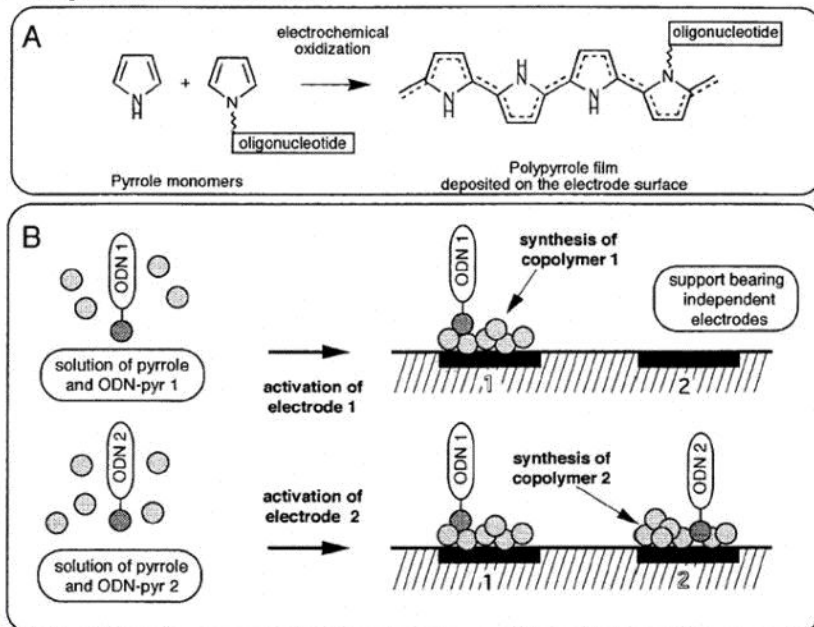
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8.3. Added value by integration

Integration-assisted derivatization

GENOTYPING hepatitis C virus



T. Livache et al.,
Anal Biochem 1998;255:188-94.

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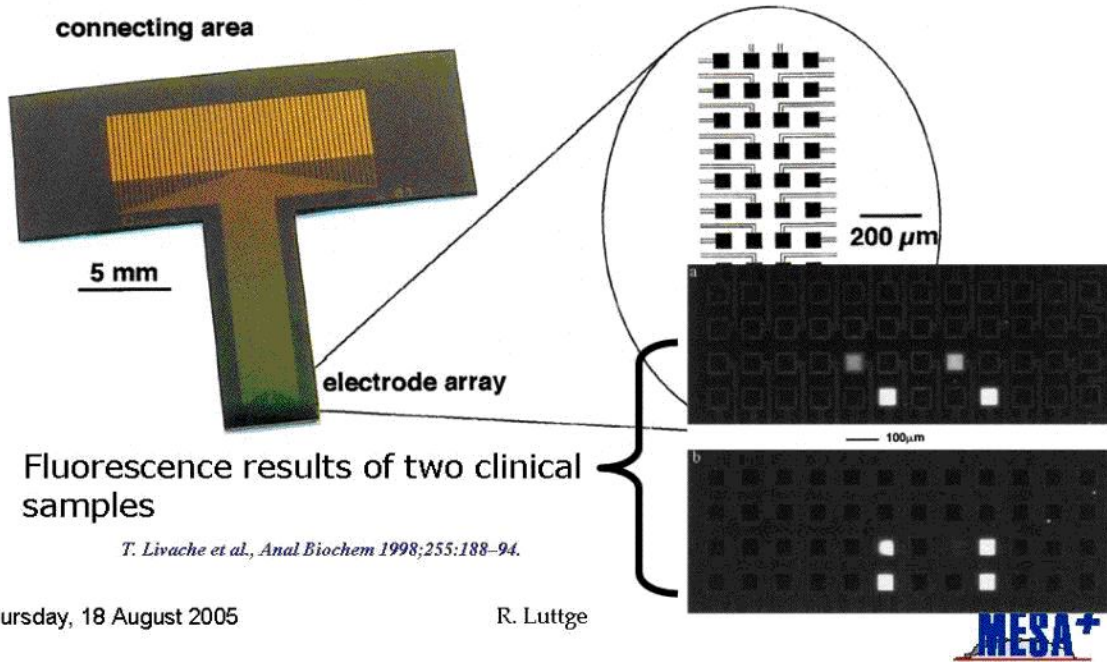
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8.3. Added value by integration

Array test result

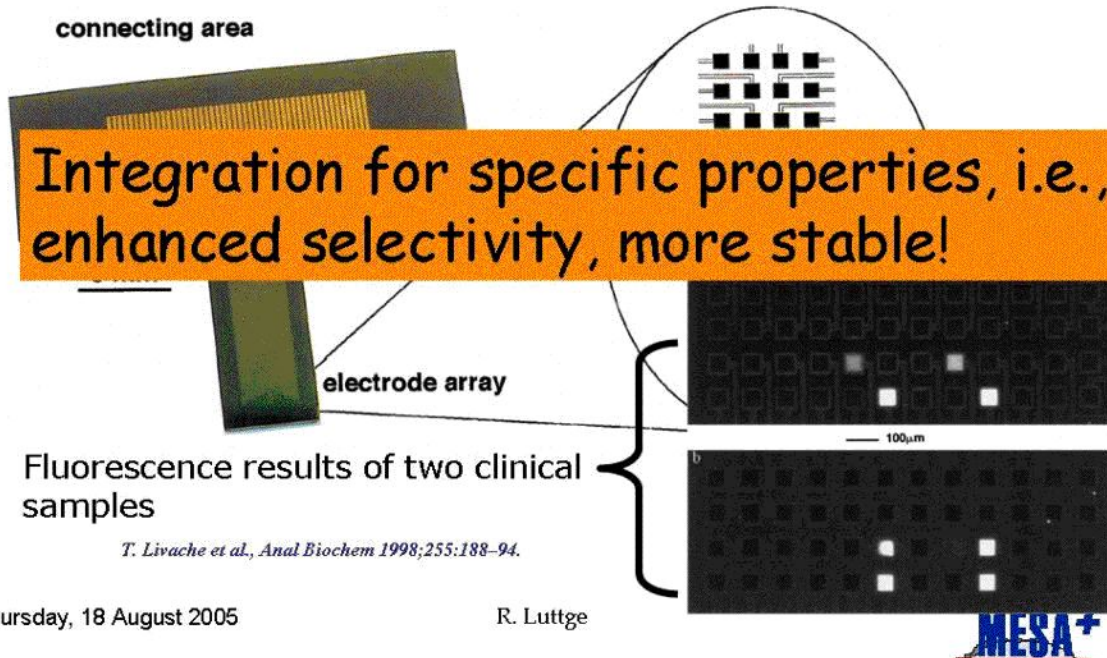
hepatitis C virus SILICON CHIP



8.3. Added value by integration

Array test result

hepatitis C virus SILICON CHIP

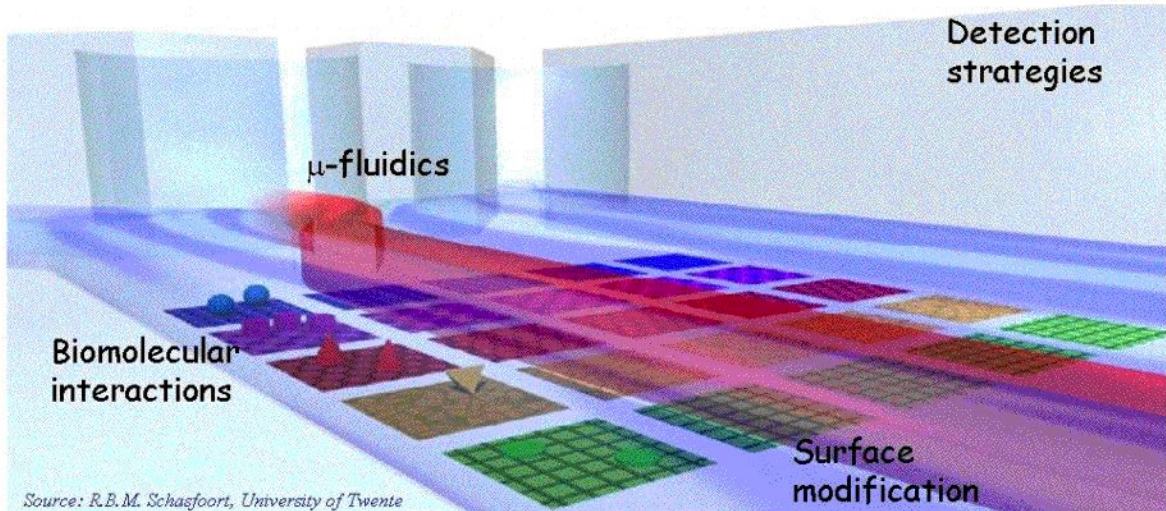


8.3. Added value by integration



Biochip Group
University of Twente

Advanced diagnostic systems



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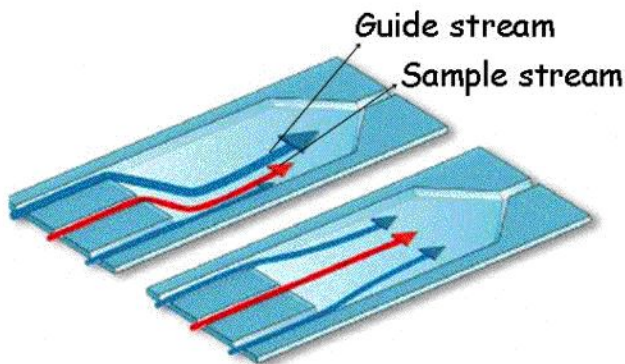
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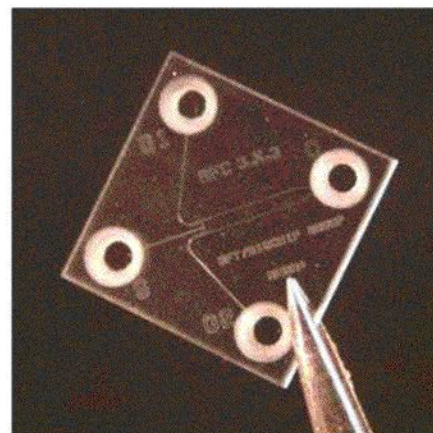
8.3. Added value by integration

Guiding streams

The Address flow technique



Source: R.B.M. Schasfoort, University of Twente



15mm x 15mm glass microchip with isotropically etched channels and powder blasted reservoirs

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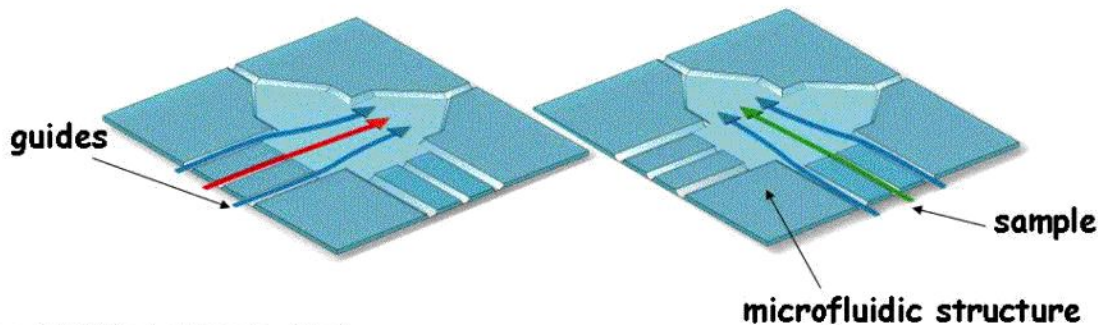
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8.3. Added value by integration

Perpendicular address-flow

- Control of two perpendicular fluid streams containing functional molecules to build up a protein array.
- Since it is a closed system, proteins are not exposed to air.
- Flow is induced electroosmotically allowing a simple chip design.



Source: R.B.M. Schasfoort, University of Twente

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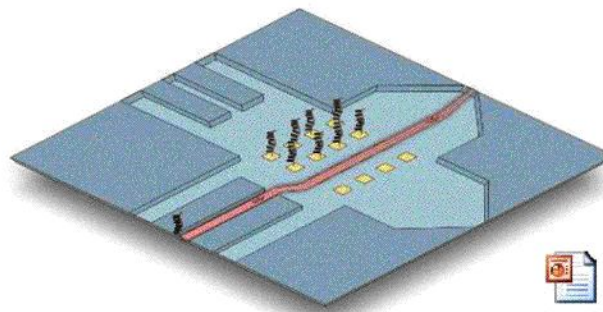
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8.3. Added value by integration

Perpendicular address-flow: application

Animation: principles of building up the microarray



Microsoft
PowerPoint Slide Show

Source: R.B.M. Schasfoort, University of Twente

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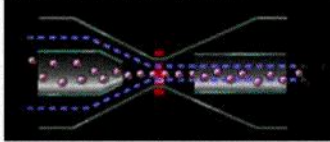
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Topics in this section

New array and assay techniques

Liquid and particle sheathing defined by microfluidic laminar flow.



Examples of integrated microfluidic assays

- Hybrid-integrated microfluidic assays
 - Addressed flow PDMS array
 - Infineon *Flow-Thru Chip*
 - Bead-based microfluidic assay

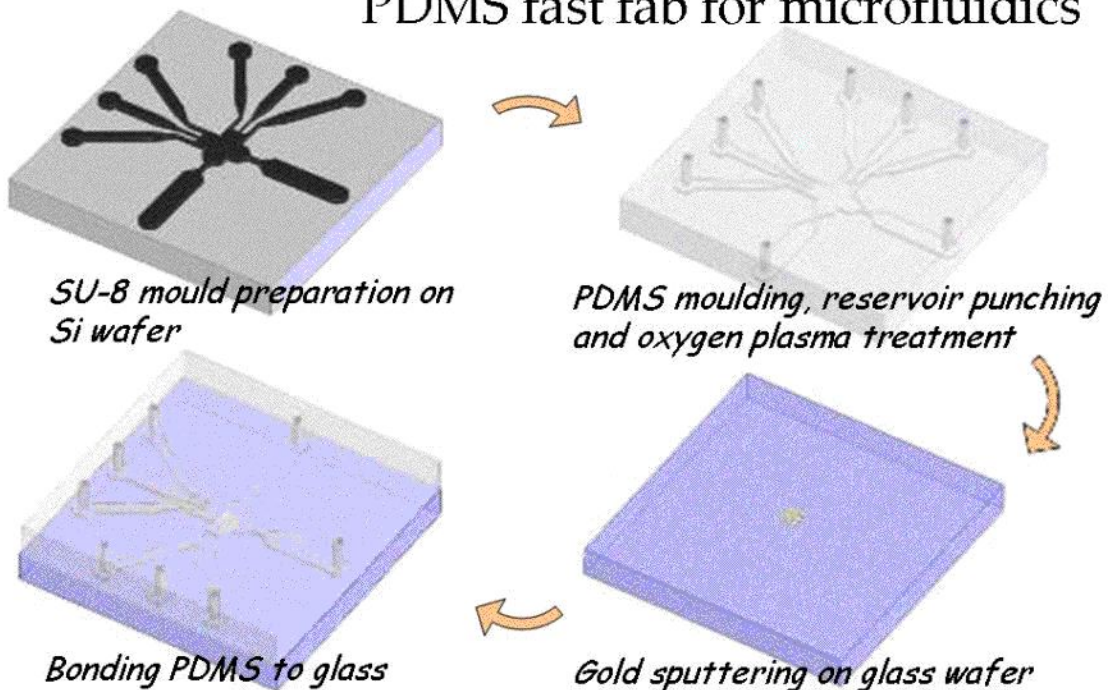
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8.4. Examples of integrated microfluidic assays

PDMS fast fab for microfluidics



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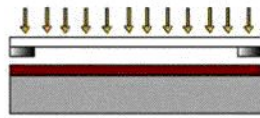
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Slide prepared by: R.B.M. Schasfoort, University of Twente



8.4. Examples of integrated microfluidic assays

Overview: technical process



Photolithography on Si wafer with 35 μ m SU-8



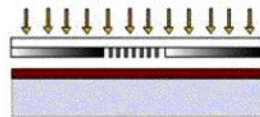
SU-8 development



PDMS molding, Curing time: 90 mins at 70°C



Reservoir punching and oxygen plasma treatment



Photolithography on Pyrex glass



Resist development and 50nm gold sputtering, lift-off



Pressure bonding of PDMS to glass

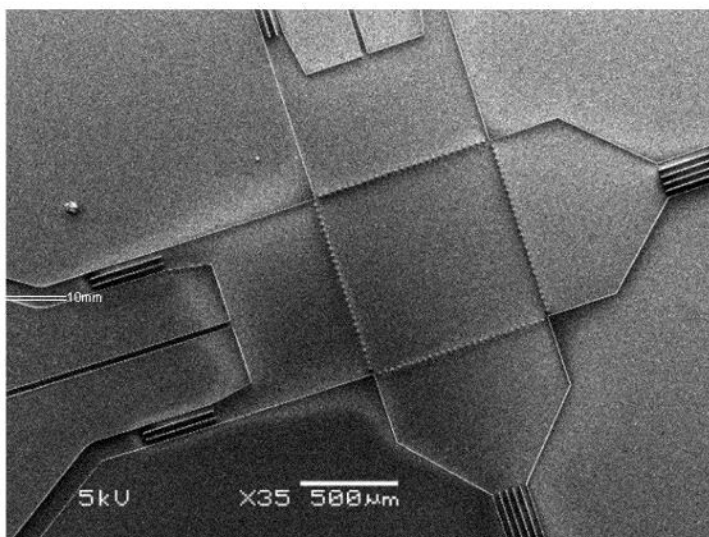
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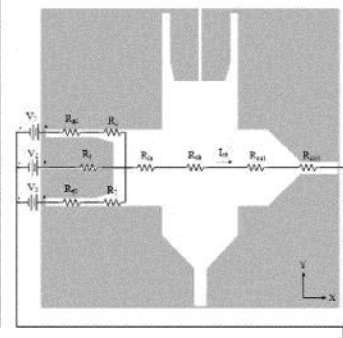


8.4. Examples of integrated microfluidic assays

PDMS microchip



Electrical analogy of the fluid structure



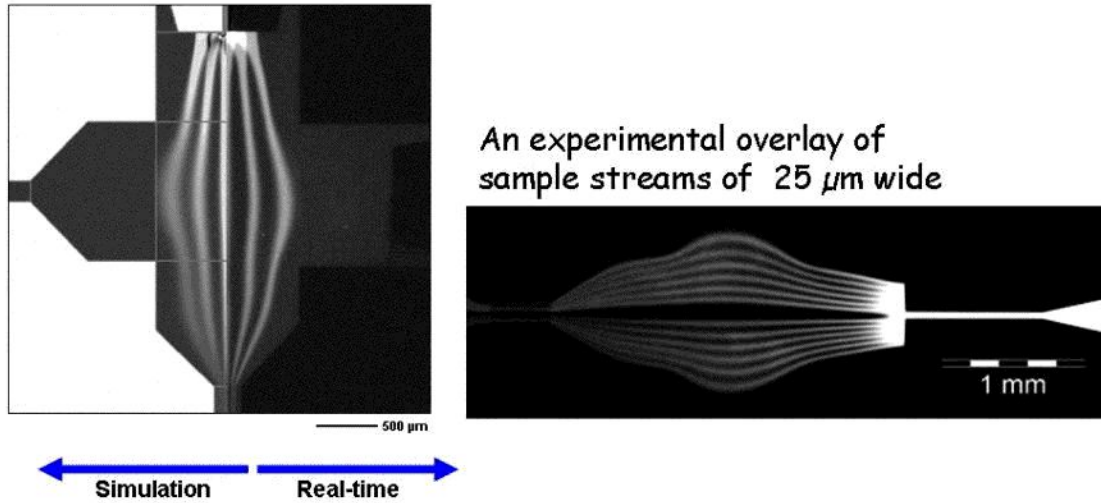
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Source: R.B.M. Schasfoort, University of Twente
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8.4. Examples of integrated microfluidic assays

Experimental results



Source: R.B.M. Schasfoort, University of Twente

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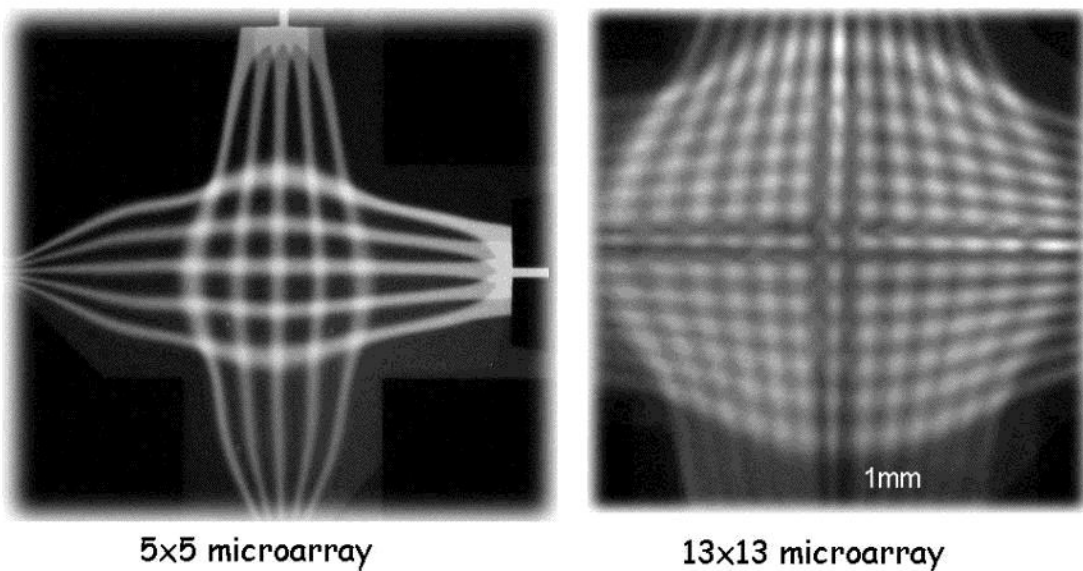
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8.4. Examples of integrated microfluidic assays

Perpendicular addressed flow results

Image overlay assembly



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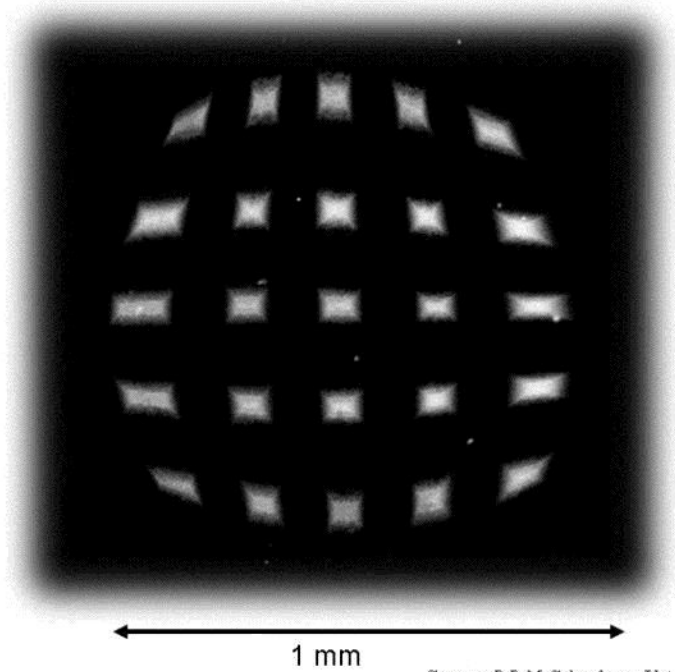
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Source: R.B.M. Schasfoort, University of Twente



8.4. Examples of integrated microfluidic assays

Resulting microarray



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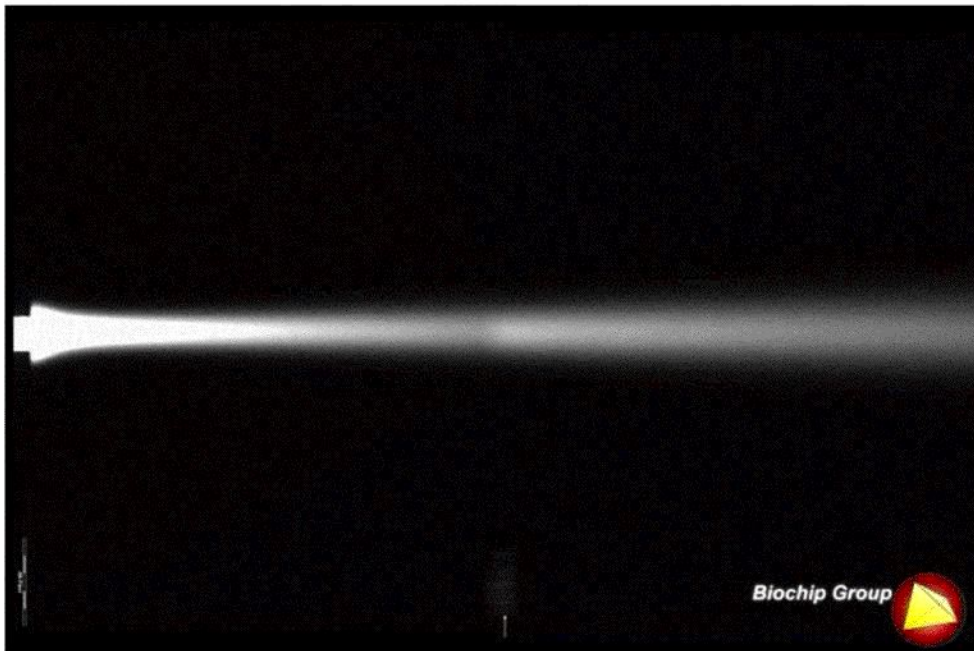
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Source: R.B.M. Schasfoort, University of Twente



8.4. Examples of integrated microfluidic assays

Addressed flow experiment



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Source: R.B.M. Schasfoort, University of Twente



8.4. Examples of integrated microfluidic assays

Microfluidic chip array

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In the biotechnology sector, it is Infineon's aim

- to develop and manufacture low-cost semiconductor-based solutions through further development of existing semiconductor technologies, and
- to simplify, improve, reduce costs and speed up drug development and medical diagnosis through the use of innovative technologies.

Currently, the focus is on four bioscience themes: production of porous silicon, Flow-thru Chip® (FTC) co-developed with MetriGenix, DNA chip with fully electronic readout and electronic neurochip.

Whereas the fully-electronic DNA chip and the neurochip are still in the research phase, the flow-thru chip is available to solve questions in the area of gene expression profiling and genotyping.

Together with our partner MetriGenix we offer:

- Medium and low density Flow-Thru Chips that enable speed and convenience for data collection.

4D Array

The Flow-Thru Chip platform utilizes a porous substrate constructed of a network of micro-channels. Specific probes are spotted and bound inside a number of channels within a defined area using a proprietary process.

more...

Fully Electronic DNA Chip

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8.4. Examples of integrated microfluidic assays

Bead-based integrated microfluidics

- **Re-circulating bead flow**
 - Experimental design study
 - Bioanalysis using dynamically trapped beads
- **Magnetic bead assays**

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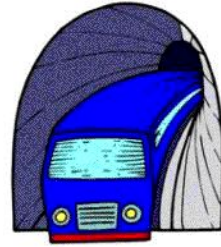


8.4. Examples of integrated microfluidic assays

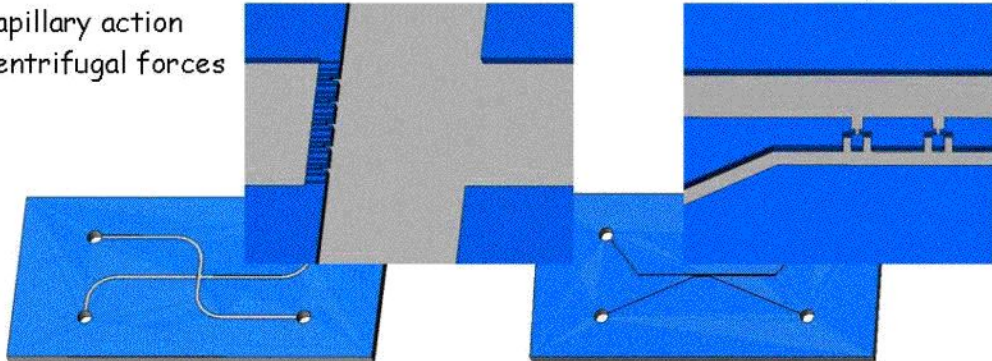
Microfluidic bead transport

Microfluidic performance

- Evaluation of microfluidic design
- Chip fabrication
- Transport test protocols
 - Pressure-driven flow
 - Electroosmotic flow
 - Capillary action
 - Centrifugal forces



A. Valero et al., BIOS, University of Twente



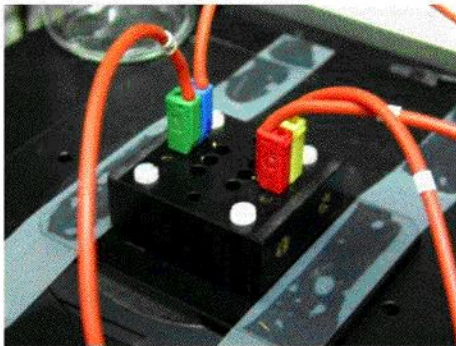
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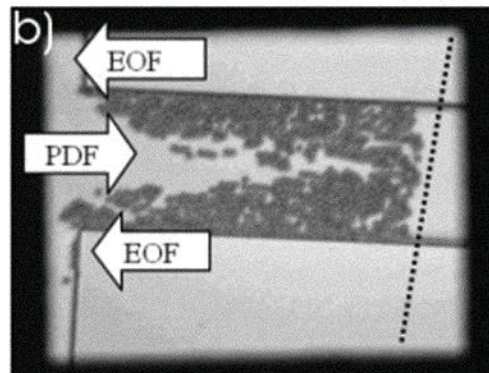
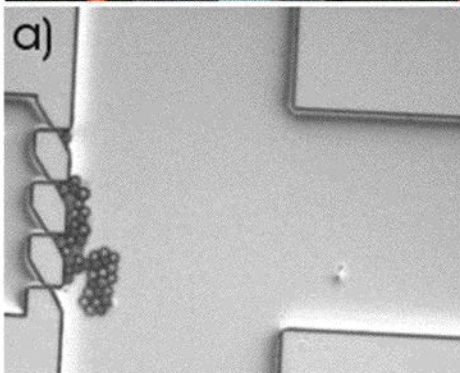
8.4. Examples of integrated microfluidic assays

Re-circulating beads



- Polystyrene beads of 10 μm used as a model for HL60 cells.
- Four point voltages scheme at all reservoirs.

A. Valero et al., University of Twente

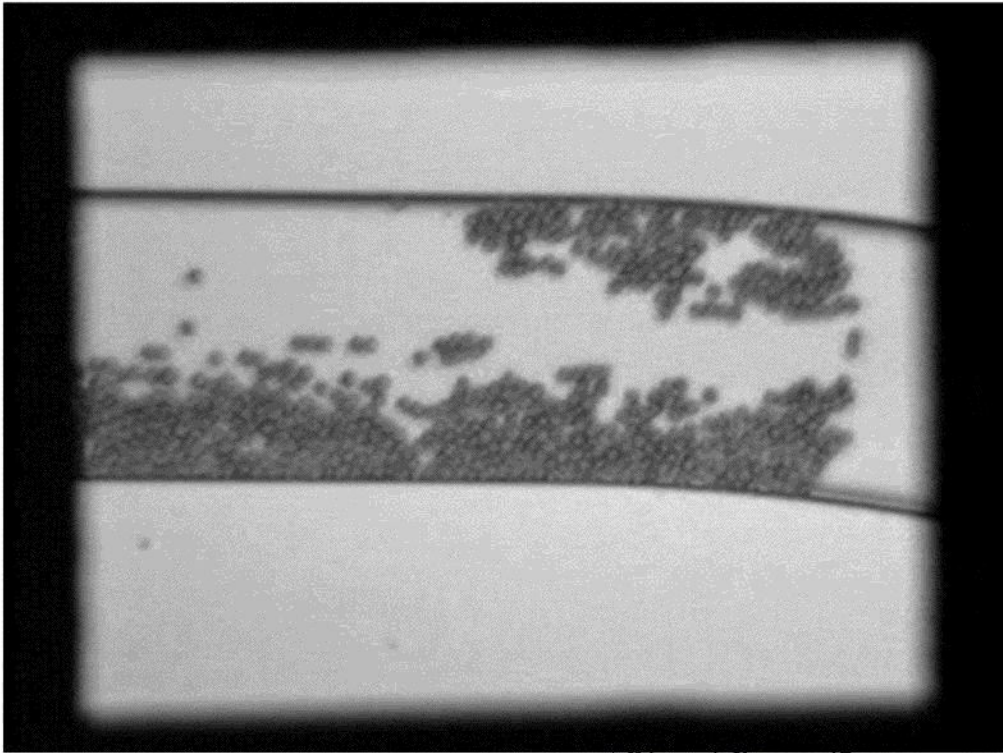


Thursday, 18 August 2005

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Thursday, 18 August 2005

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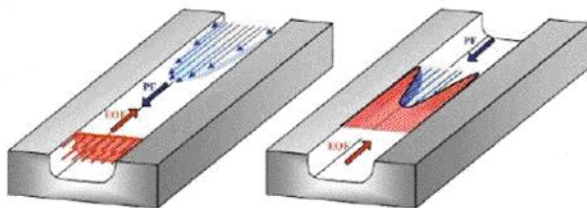
A. Valero et al., University of Twente



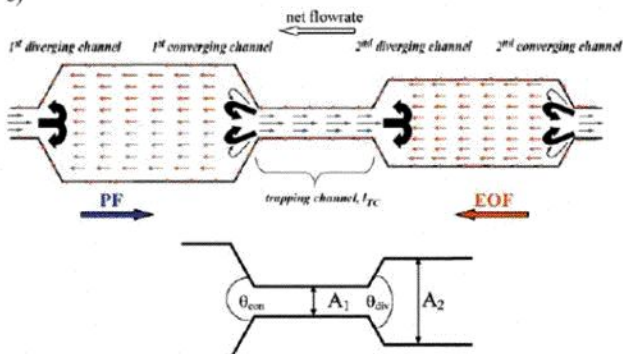
8.4. Examples of integrated microfluidic assays

Bioanalysis using freely moving beads

a)



b)



Lettieri et al., LabChip, 2003, 3, 34-39

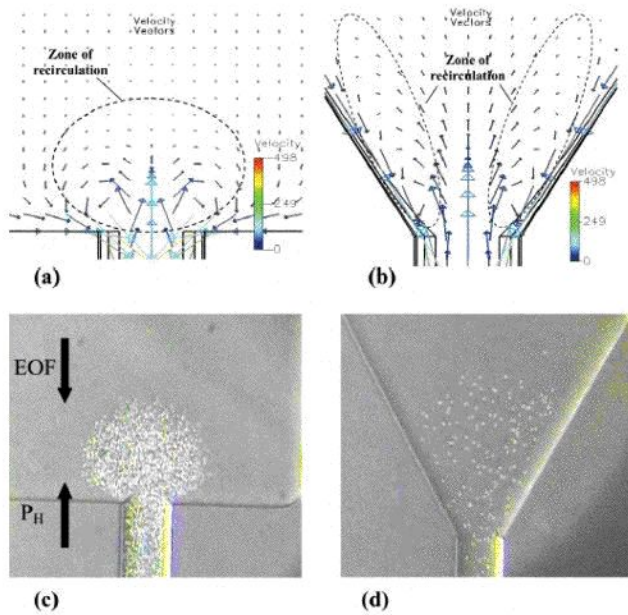
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8.4. Examples of integrated microfluidic assays

Particle re-circulation



- 1 μm diameter protein A-coated fluorescent beads

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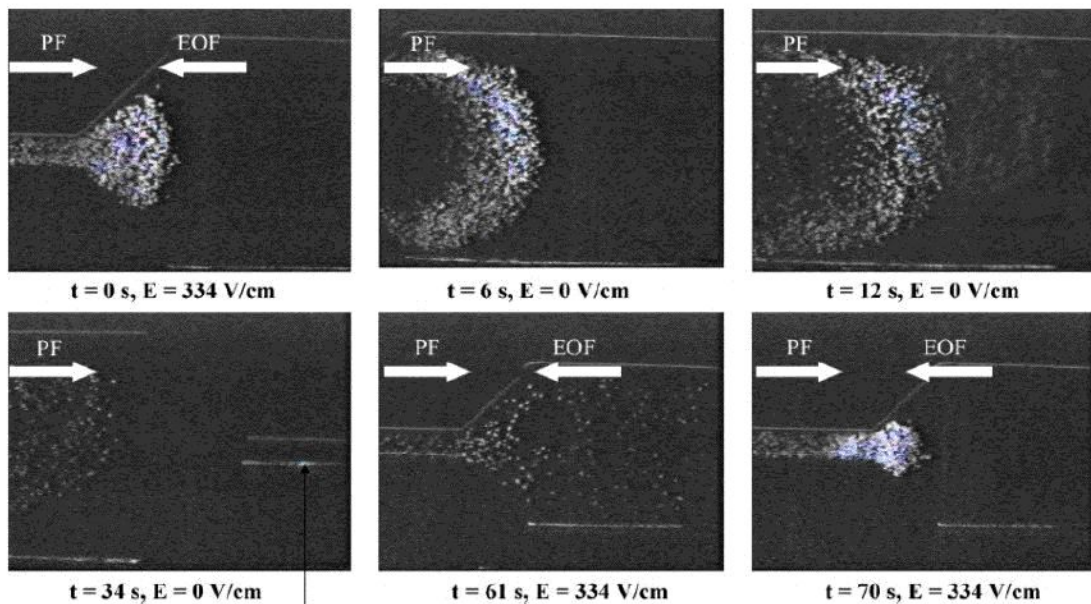
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Lettieri et al., LabChip, 2003, 3, 34-39



8.4. Examples of integrated microfluidic assays

Video capture of particle behaviour



Width of trapping channel: 50 μm

Lettieri et al., LabChip, 2003, 3, 34-39

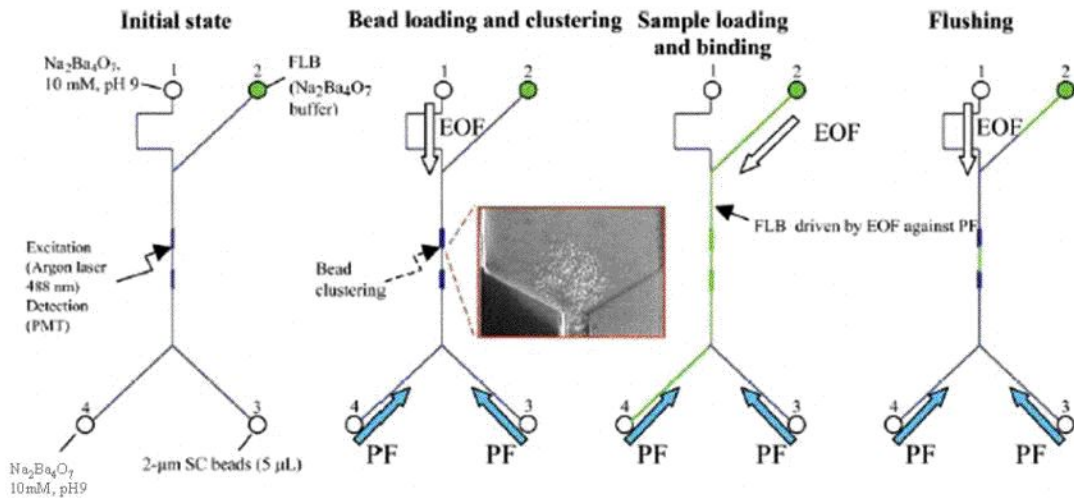
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8.4. Examples of integrated microfluidic assays

Bead-flow bioassay



Lettieri et al., LabChip, 2003, 3, 34-39

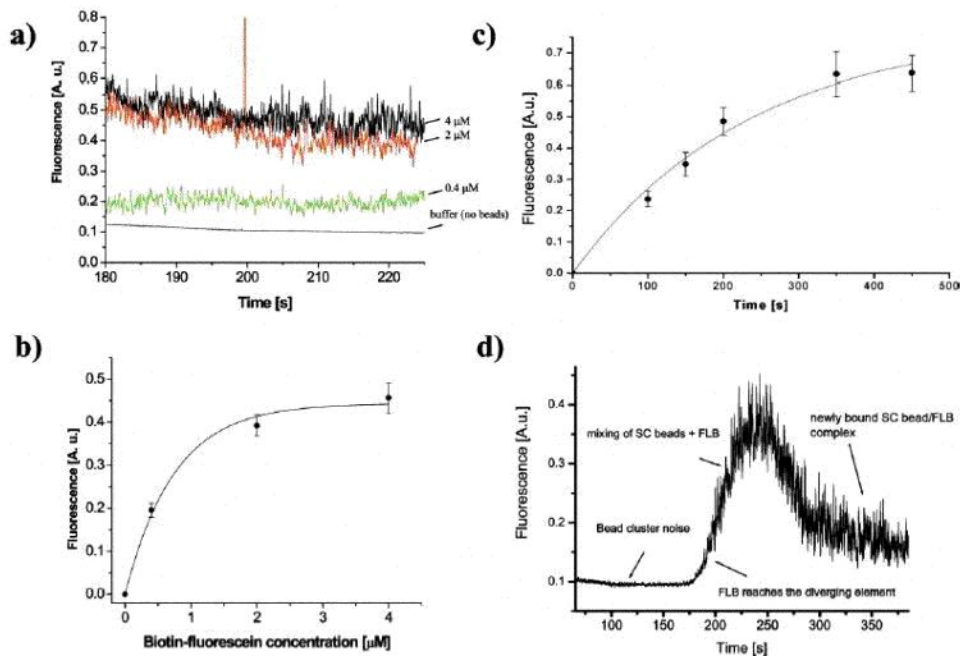
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8.4. Examples of integrated microfluidic assays

Biotin binding results



Lettieri et al., LabChip, 2003, 3, 34-39

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8.4. Examples of integrated microfluidic assays

Innovative developments

PHILIPS

Molecular World

New bio-electronic technologies extract genetic and protein information for timely, personalized treatment and prevention



<http://www.medical.philips.com>

- Philips has borrowed advanced materials and signal processing capabilities from its magnetic sensor activities to increase signals and reduce noise. Philips is currently prototyping a one-time usable biosensor, designed for low-cost manufacture, that could fit into a handheld reader.

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8.4. Examples of integrated microfluidic assays

Magnetic bead assay on chip

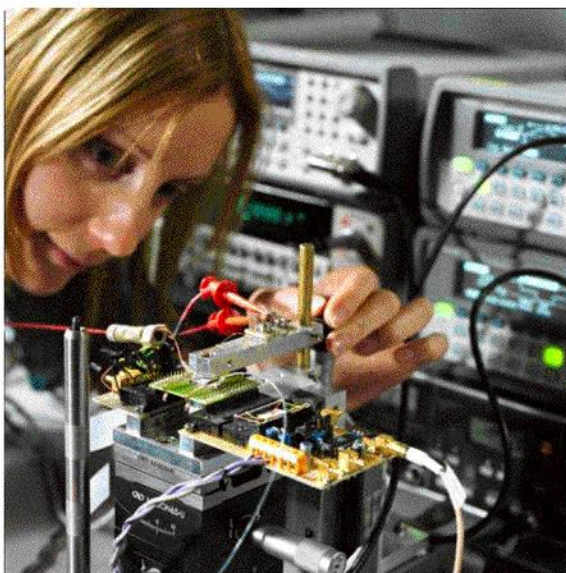


Photo: Philips

<http://www.philips.com>

- Magnetic biochip measurement setup.
- See also other groups working on magnetic assays: Pekas et al., Appl. Phys. Lett., Vol. 85, No. 20, 15 November 2004

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Outlook: Future developments



<http://www.seahawkbio.com/>

- Portable
 - E.g., using multi-use magnetic biochip technology to be used for animal diagnostics, food processing, and environmental testing.
- Living cells on chip
 - Cells as a transducer

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8.5. Outlook: Future developments

Living cell arrays

Diagram illustrating a living cell array. The array is shown as a grid of cells, with dimensions of 4.5 mm by 2 mm. The array is divided into two regions: 'Cell type 1' and 'Cell type 2'. The array is shown at 1.25x magnification, and a detailed view of the cells is shown at 20x magnification.

Single or multiple engineered cell types microarrayed in predetermined positions

Arrayed cell-specific ligands are used to direct the cells

The arrayed cells function as interrogators and reporters of for example drugs

Kapur, R., et al., (1999) Biomedical Microdevices, 2, 99-109.

Slide prepared by H. Andersson, University of Twente

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Summary

- Microarrays and microfluidics biochemical assays, the workhorses of Genomics and Proteomics.
- Arrays may give new insight into life-science processes, e.g.,:
 - “..endocytosis is essential for the efficient uptake of nutrients and other macromolecules into cells and for the regulation of signaling by cell-surface receptors.”
 - “Taking a biochemical approach, we (*Schmid et al.*) developed and use cell-free assays that faithfully reconstitute discrete events in clathrin-mediated endocytosis to discover new components of the endocytic machinery and to probe the hierarchy of interactions leading to coat assembly, cargo selection, vesicle budding, membrane fission, and CCV uncoating.”



*S.L. Schmid et al.,
New Insights Into the Regulation of
Clathrin-Mediated Endocytosis,
TSRI Scientific Report 2003*

<http://www.scripps.edu/>

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