The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific
and Cultural Organization

International Atomic
Energy Agency

World Year of PHYSICS 2005

SMR.1670 - 28

INTRODUCTION TO MICROFLUIDICS

8 - 26 August 2005

Microarrays and Biochip Assays

R. Luttge
University of Twente, Enschede, The Netherlands

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.

8. Microarrays and biochip assays

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

Topics in this section

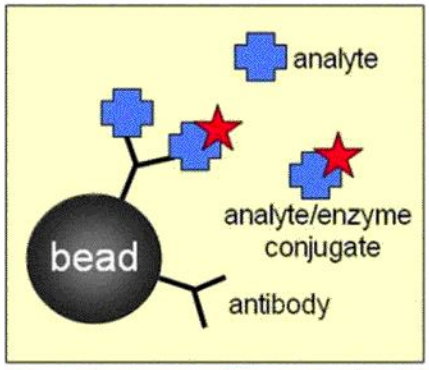
Basic research and techniques

Microtechnology-assisted

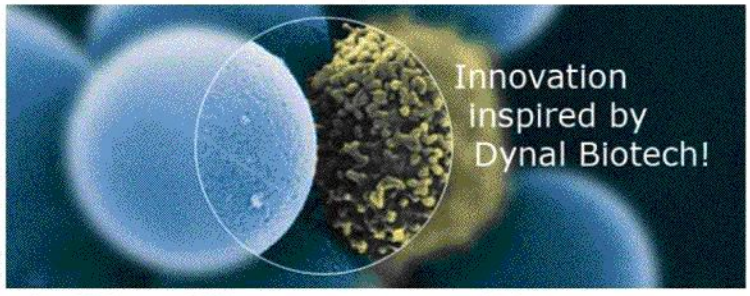
Introduction

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

Bead chemistry

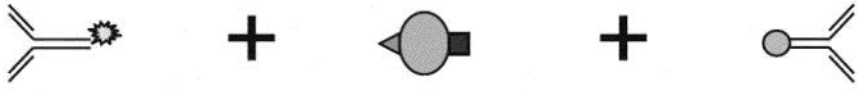


- Biodetection methodology based on multiple use of instrumentation exploiting bead attachment chemistry.
- Enable rapid parallel analysis of the many thousands of proteins



Functionalizing chemistry

Sequential immunoassay



Detection antibody conjugated to e.g. fluorophore or enzyme

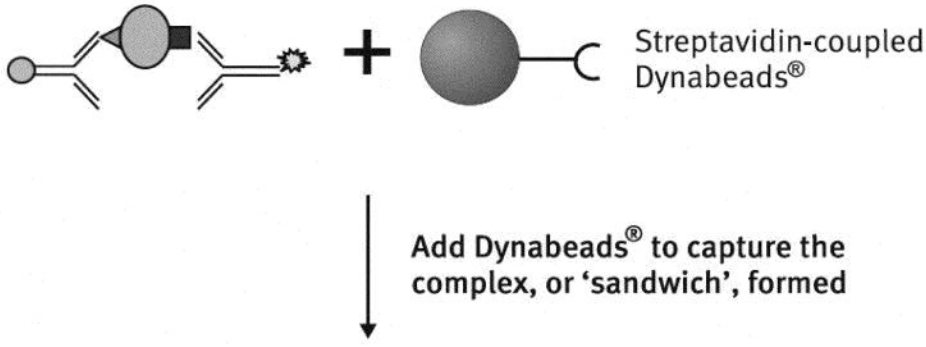
Analyte present in sample

Biotin-conjugated capture antibody

Combine antibodies and sample, mix and incubate

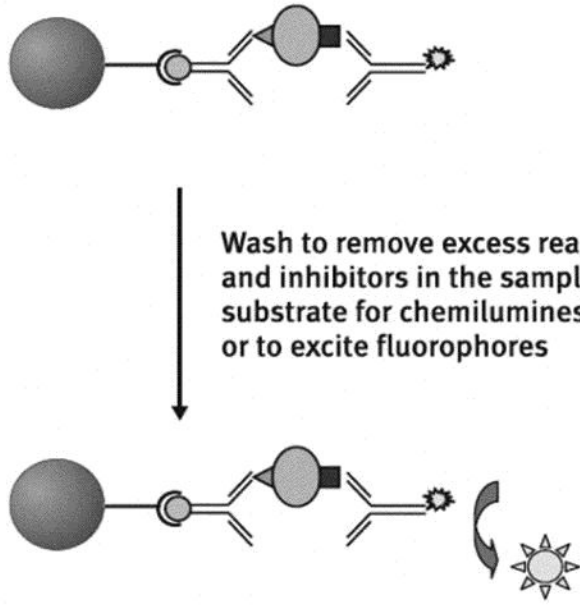
www.dynalbiotech.com

8.1. Introduction



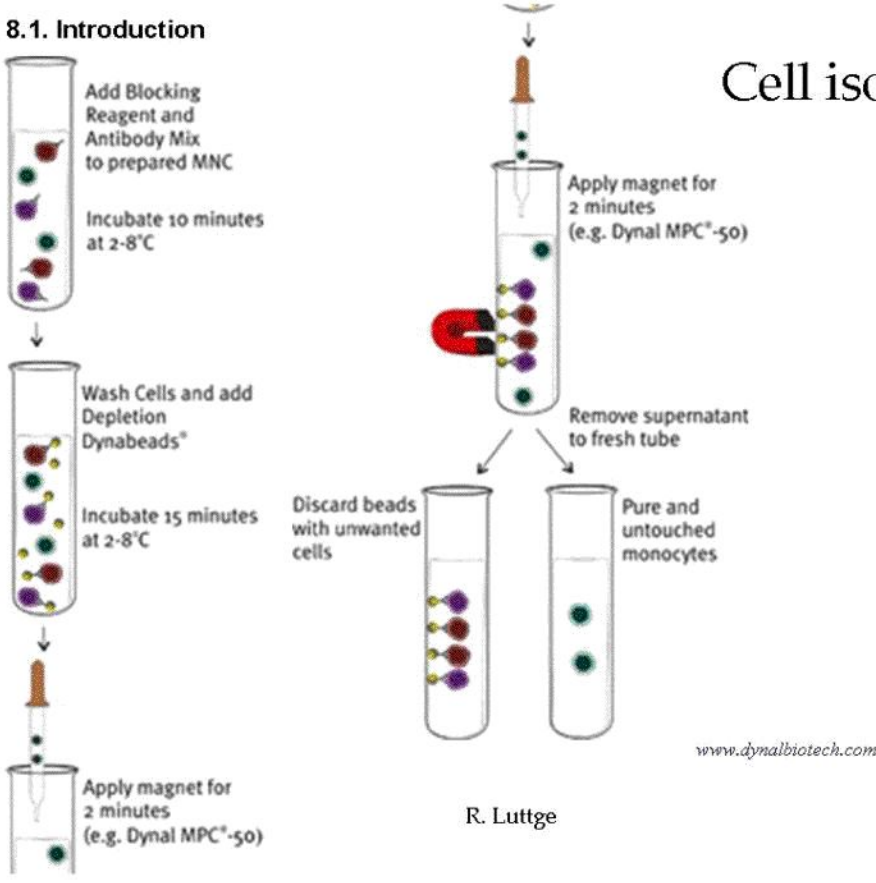
www.dynalbiotech.com

8.1. Introduction

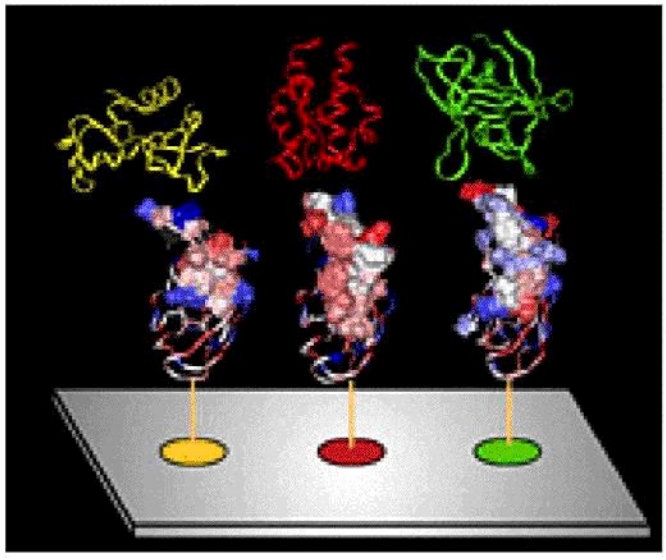


www.dynalbiotech.com

Cell isolation

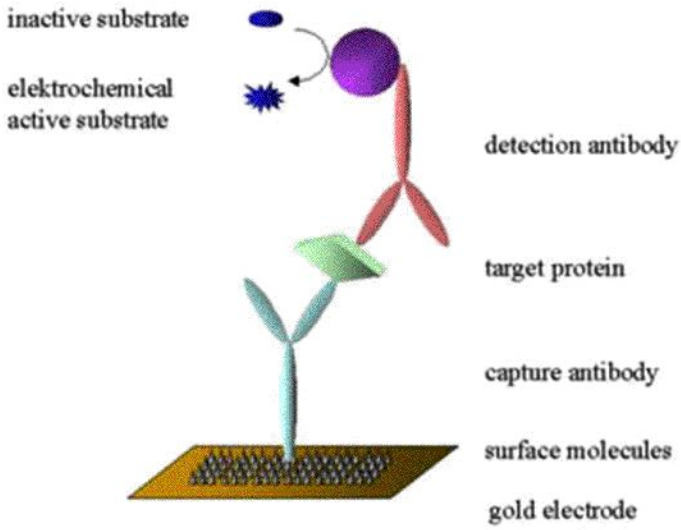


Functionalized surface arrays



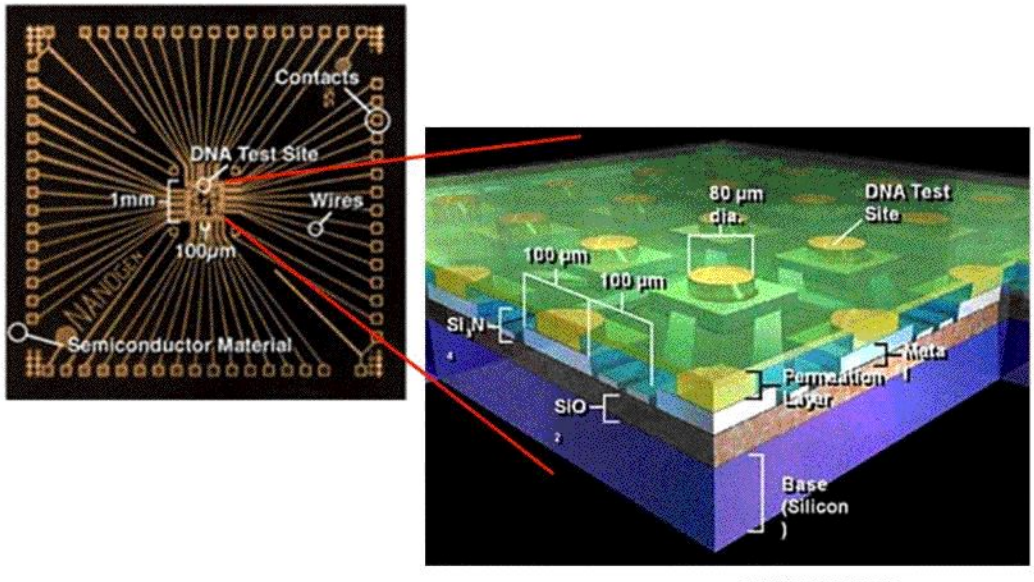
- Protein targets approaching immobilized antibody array
- Hydrogel binding or thiol-based self assembled monolayer (SAM)

Functionalized surface detection



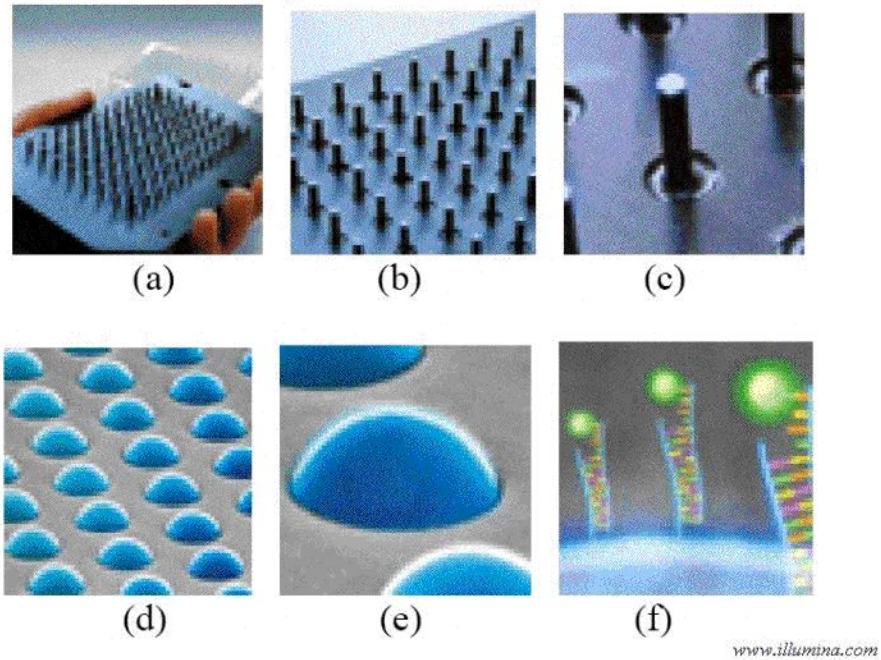
www.chemie.uni-hamburg.de

CMOS integrated array device for advanced electrochemical assays



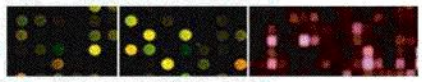
www.nanogen.com

Optical integrated bead array

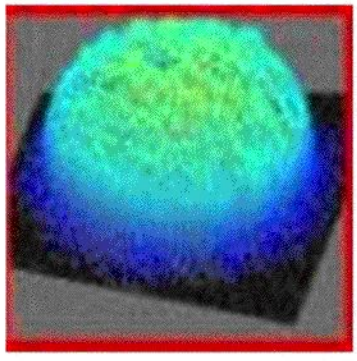


www.illumina.com

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



Successful biochips?

- Reliable, accurate
- Sensitive
- Low non specific binding (NSB)
- High spot density (the more the better/cm^2)
- Comparable
- High quality spot
- Repeatable

Spotting technologies

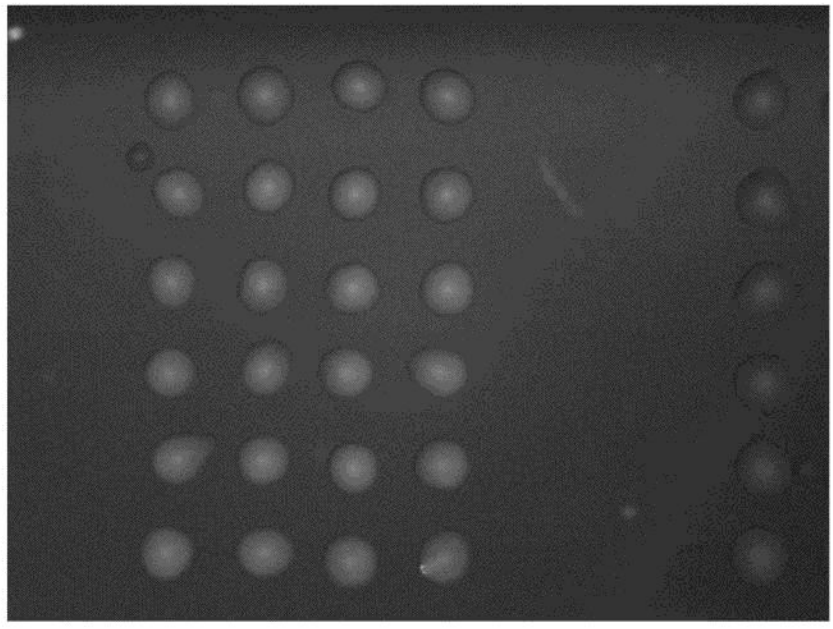
- Split pen, contact printer
- Pin and ring spotter
- Piezo, contactless spotters
- Ink-jet spotting (Agilent)
- Top Spot (Biofluidix)
- Photolithography (Affymetrix)
- .....New developments....

TopSpot Technology

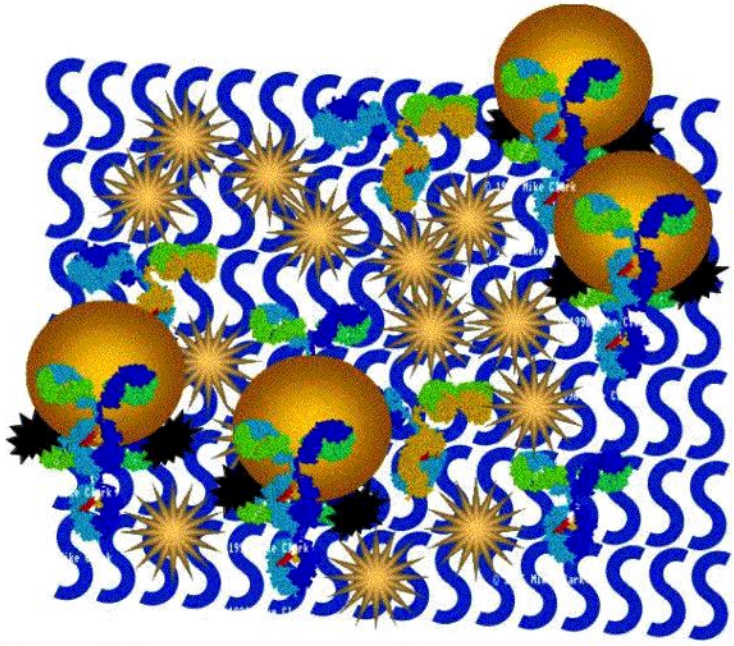
Spot different ligands on a glass slide

- 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)*

Top-spot protein array



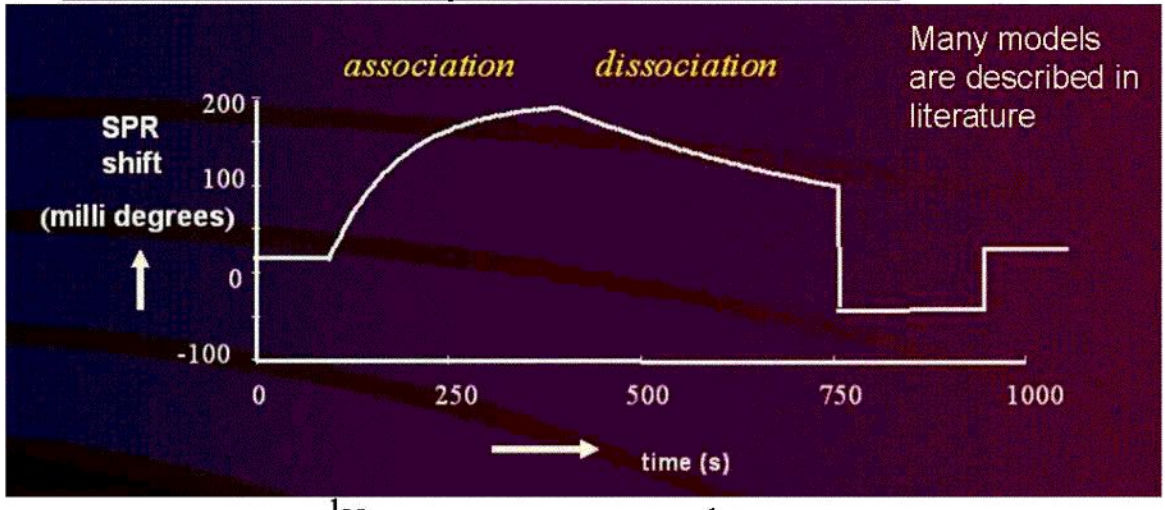
Biomolecular interaction



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

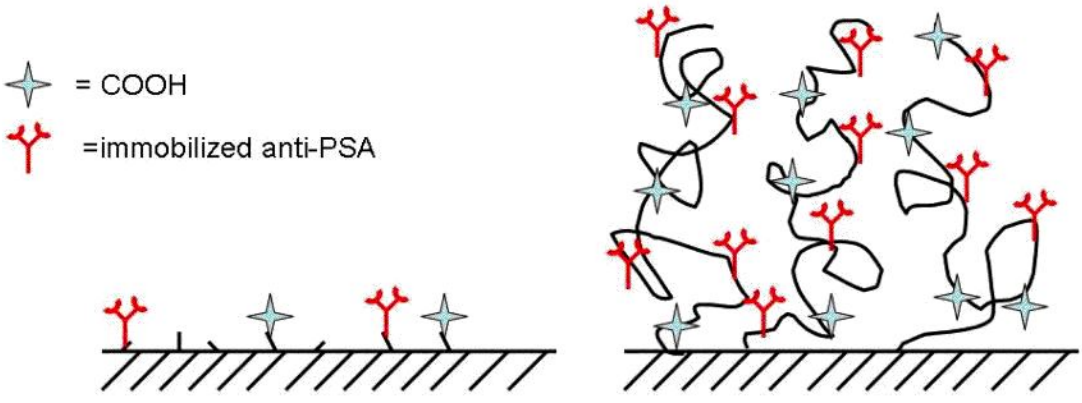
Interaction kinetics

Detection of surface plasmon resonance shift



$$Ab + Ag \underset{kd}{\overset{ka}{\rightleftharpoons}} AgAb \qquad \frac{ka}{kd} = K_A$$

Sensoric surface immobilization



P-type

Planar sensor surface

Thickness < 10 nm

G-type

Gel-type sensor surface

Thickness 100 nm

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

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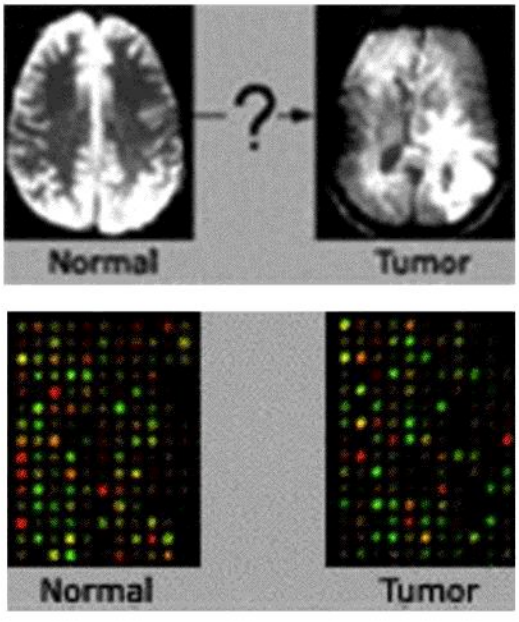
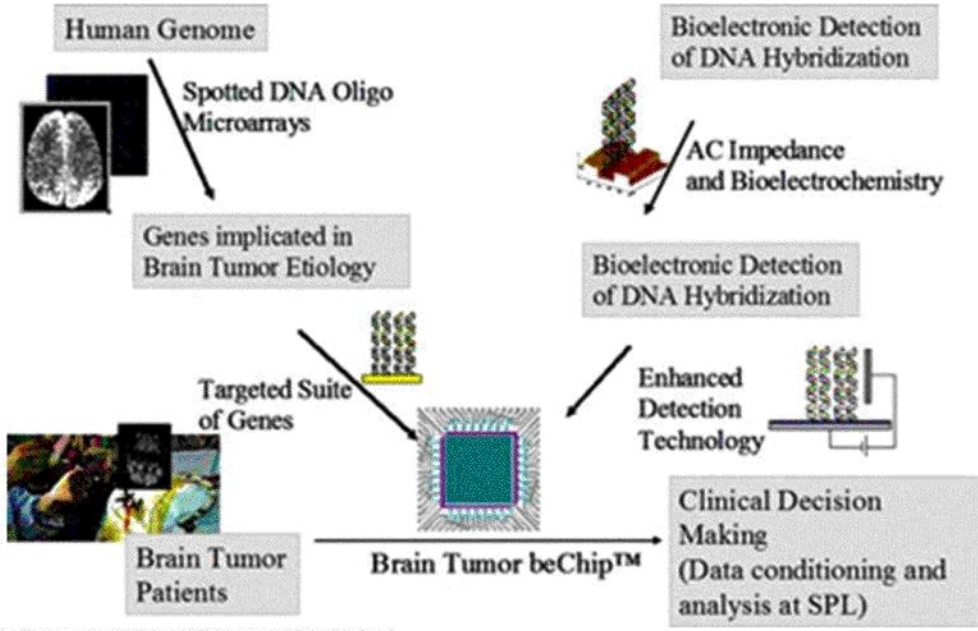
The brain tumor project



- 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

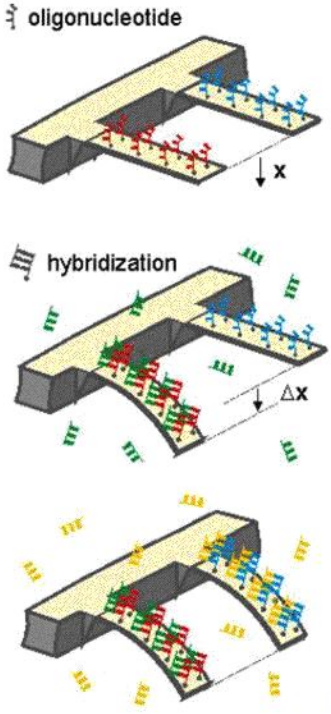
Optimization by impedimetric DNA arrays

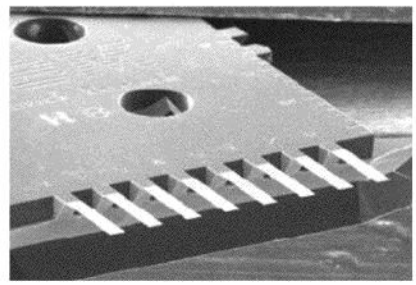


http://www.vcu.edu/lifesci/phd/research/biochip.html

Bioelectronic array

Bio-MEMS array



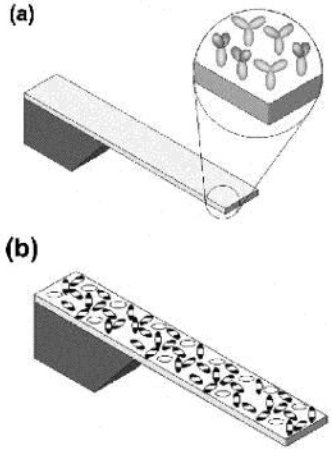


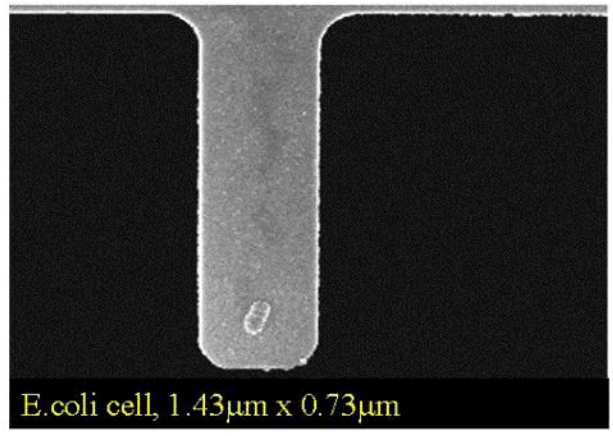
- Microcantilever with bio-coating.
- Molecule interaction of test sample with bio-coating will lead to bending.
- Specific binding identification by resonance shift.

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

Single cell detection

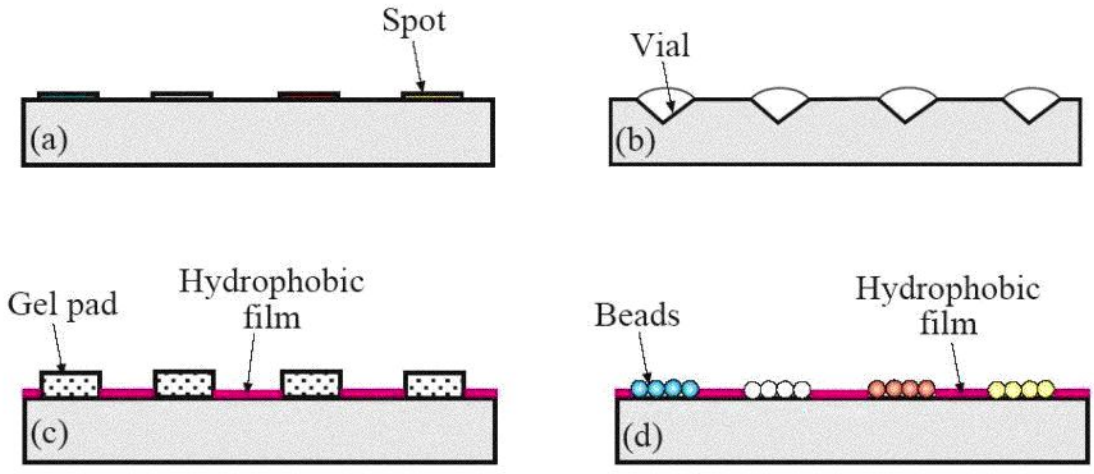
Importance for medical diagnostics:





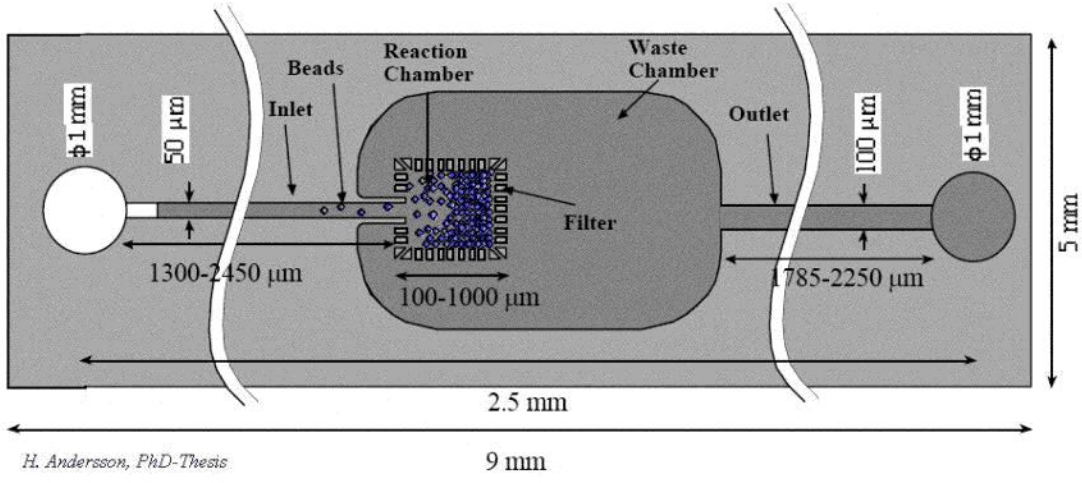
Ilic et al., JVST B, vol. 19, iss. 6, pp. 2825-2828, 2001

Bead arrays



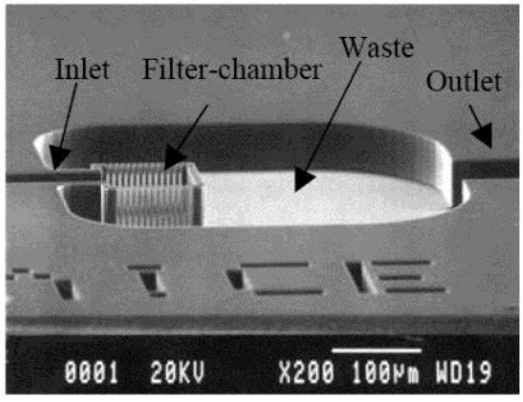
H. Andersson, PhD-Thesis

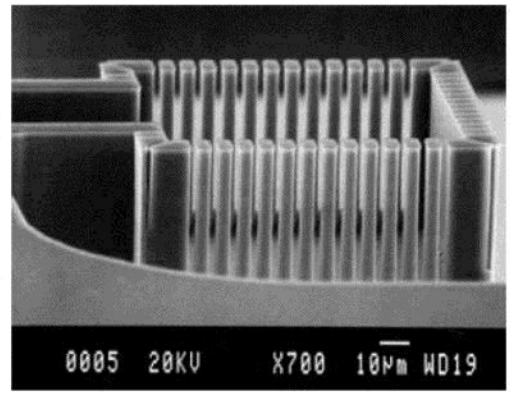
Filter chamber design



H. Andersson, PhD-Thesis

Fabrication result





H. Andersson, PhD-Thesis

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 polysterene 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

Luciferase reaction

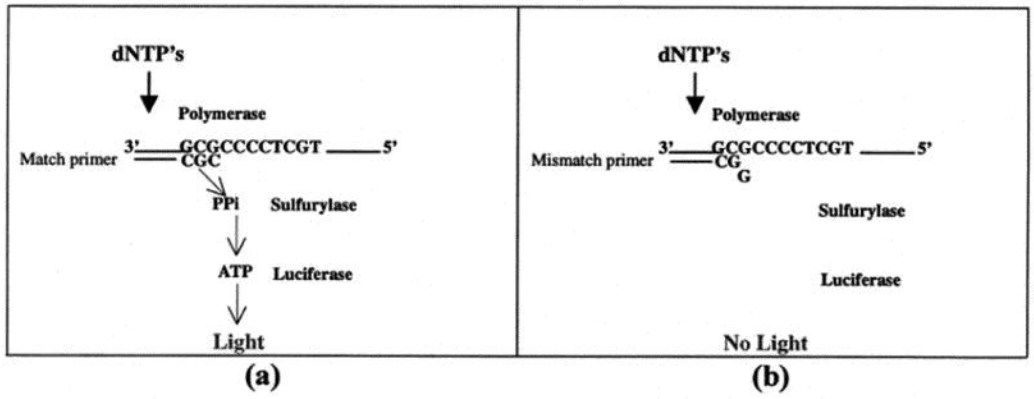


Figure 6. Principle of allele-specific pyro-extension. (a) Match primer annealed to the single-stranded DNA will result in detectable light. (b) 3'-End mismatch primer will not be extended by DNA polymerase, resulting in no light.

Snapshot results

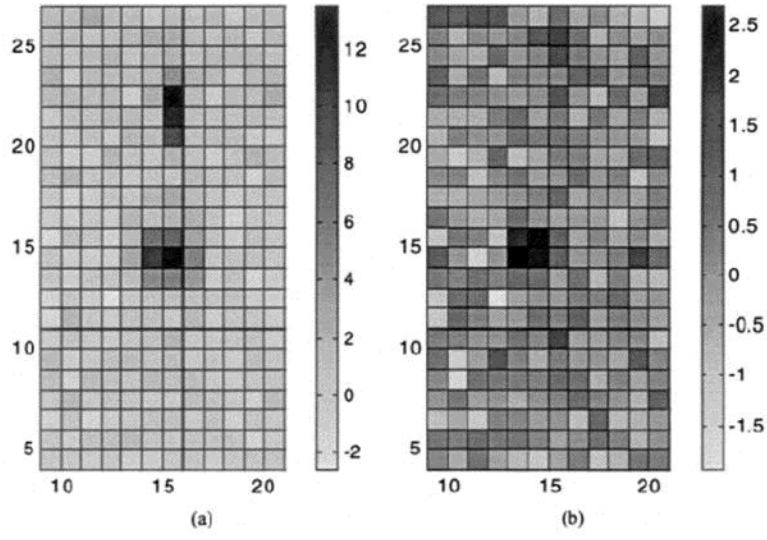
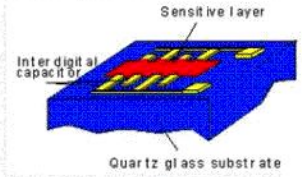


Figure 8. (a) A snapshot of the match pyro-extension. Light is detected in the filter-chamber and outlet. (b) A snapshot of the mismatch pyro-extension. A weak signal (background level) is detected in the filter-chamber.

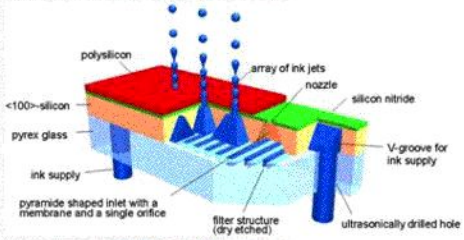
Topics in this section

Basic research and techniques



Microtechnology-assisted arrays

Ink-jet head



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

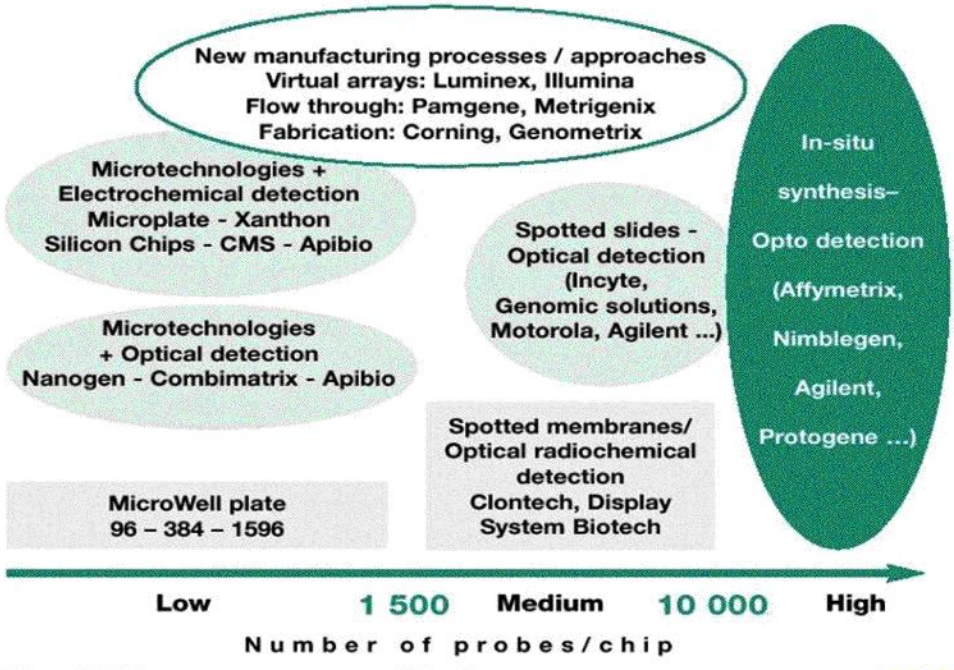
Added value by integration

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

8.3. Added value by integration

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

Why small?



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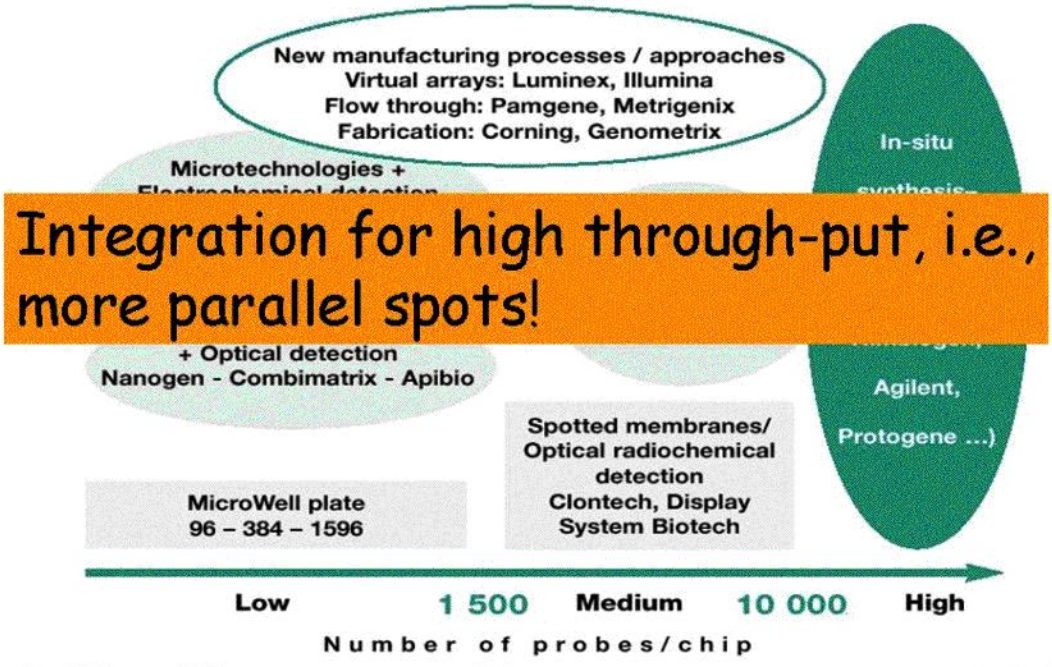
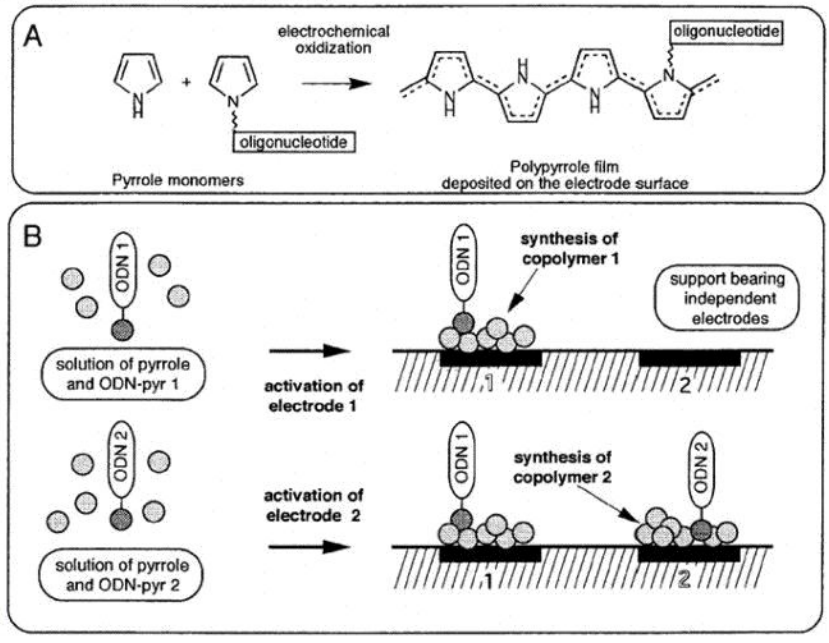


Integration for high through-put, i.e., more parallel spots!

Integration-assisted derivatization

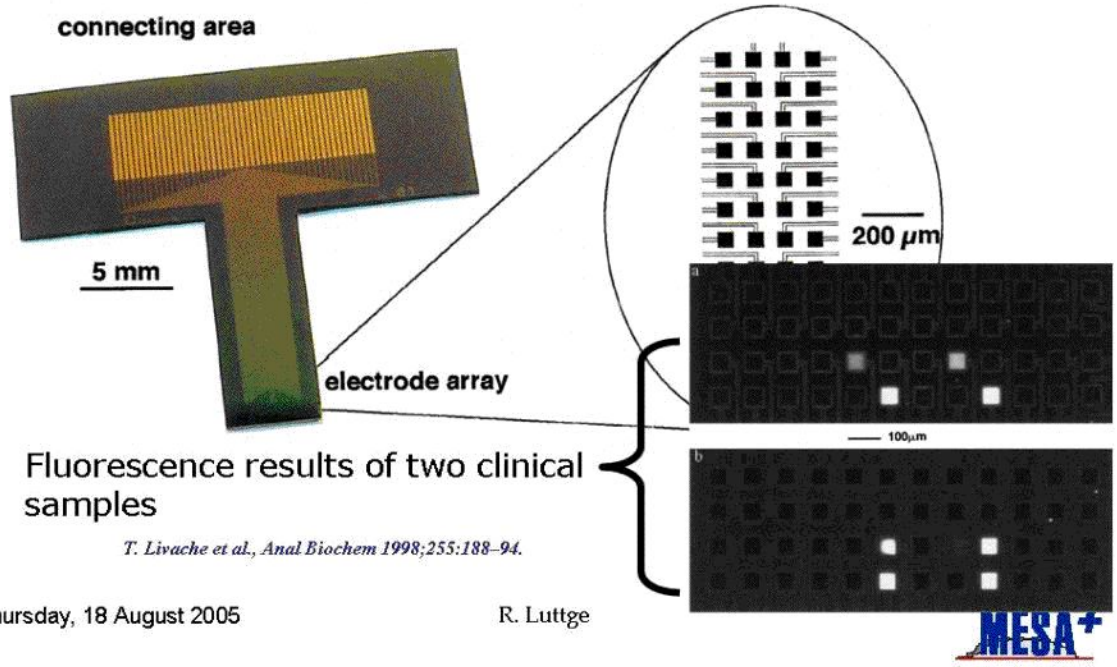
GENOTYPING *hepatitis C virus*



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

Array test result

hepatitis C virus SILICON CHIP



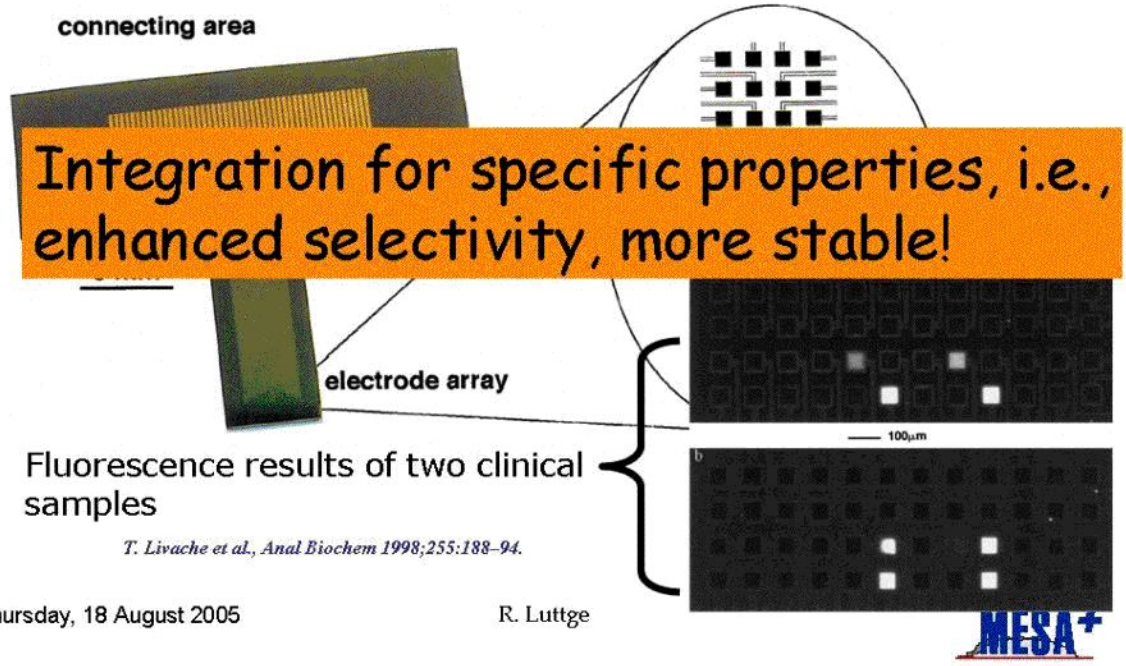
Fluorescence results of two clinical samples

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

8.3. Added value by integration

Array test result

hepatitis C virus SILICON CHIP



Integration for specific properties, i.e., enhanced selectivity, more stable!

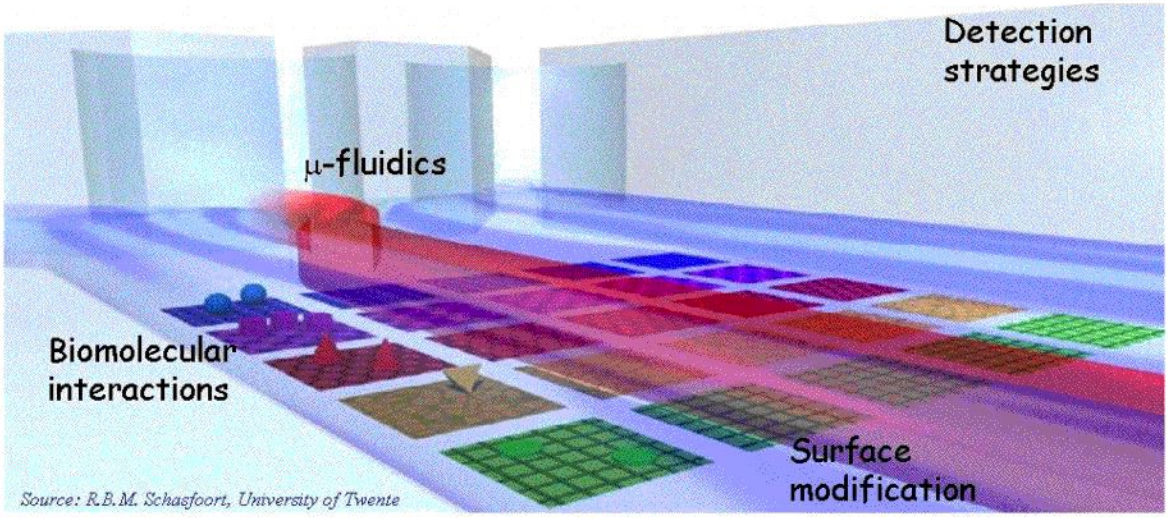
Fluorescence results of two clinical samples

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

Thursday, 18 August 2005 R. Luttge

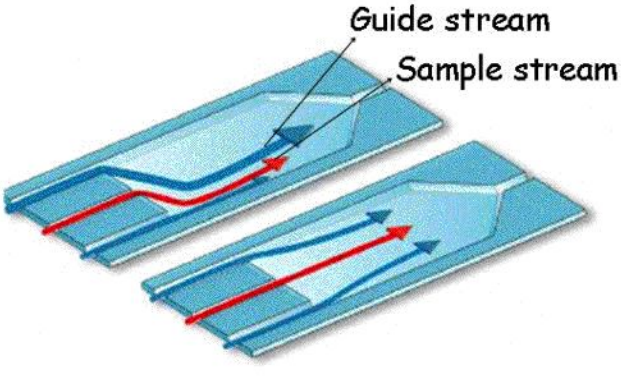
Biochip Group
University of Twente

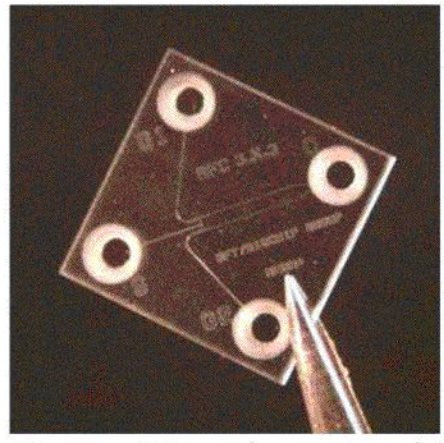
Advanced diagnostic systems



Guiding streams

The Address flow technique



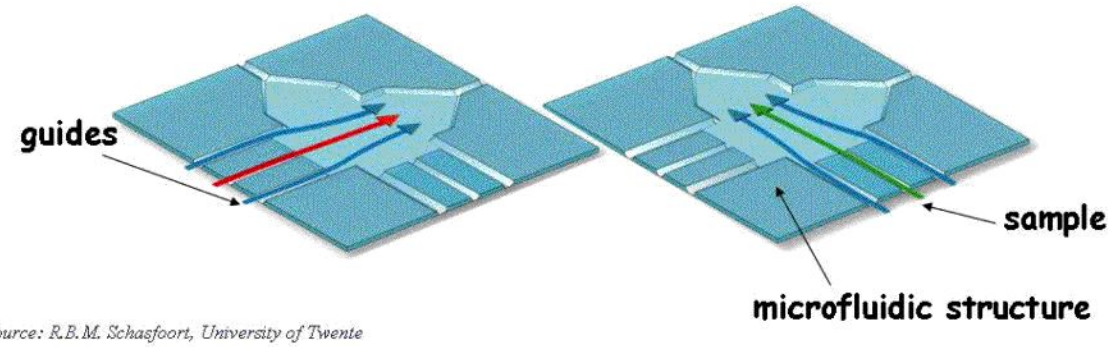


15mm x 15mm glass microchip with isotropically etched channels and powder blasted reservoirs

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

Perpendicular address-flow

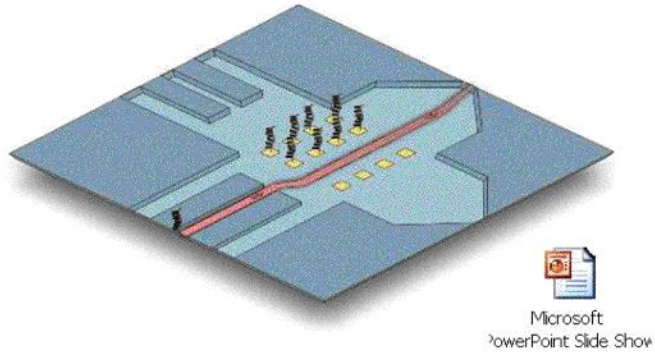
- Control of two perpendicular fluid streams containing functional molecules to build up a protein array.
- Since it is a closed system, proteins are not exposed to air.
- Flow is induced electroosmotically allowing a simple chip design.



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

Perpendicular address-flow: application

Animation: principles of building up the microarray

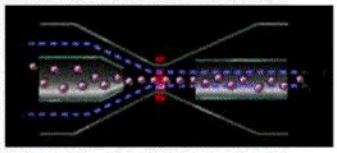


Source: R.B.M. Schasfoort, University of Twente

Topics in this section

New array and assay techniques

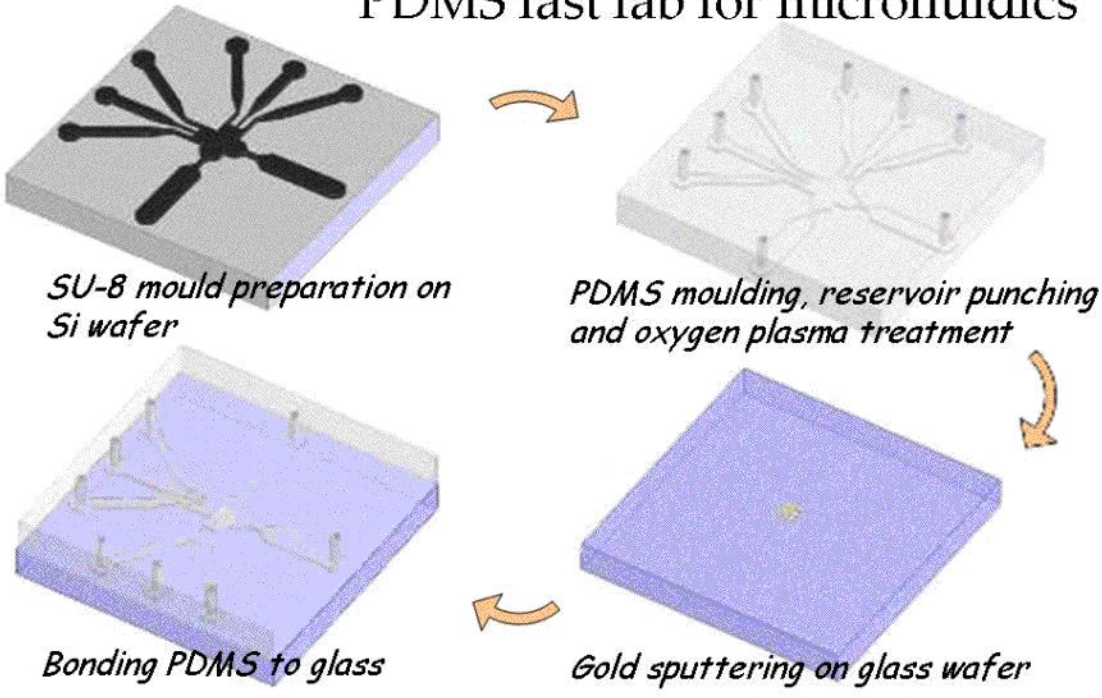
Liquid and particle sheathing defined by microfluidic laminar flow.



Examples of integrated microfluidic assays

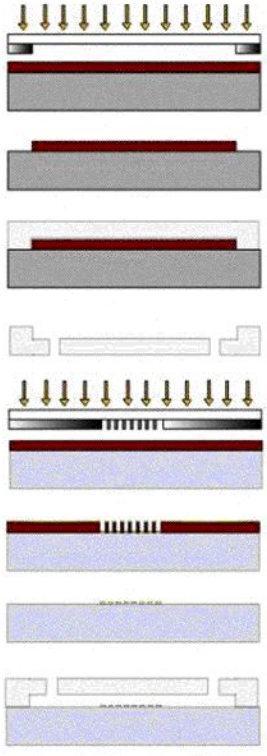
- Hybrid-integrated microfluidic assays
  - Addressed flow PDMS array
  - Infineon *Flow-Thru Chip*
  - Bead-based microfluidic assay

PDMS fast fab for microfluidics



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

Overview: technical process



Photolithography on Si wafer with 35μm SU-8

SU-8 development

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

Reservoir punching and oxygen plasma treatment

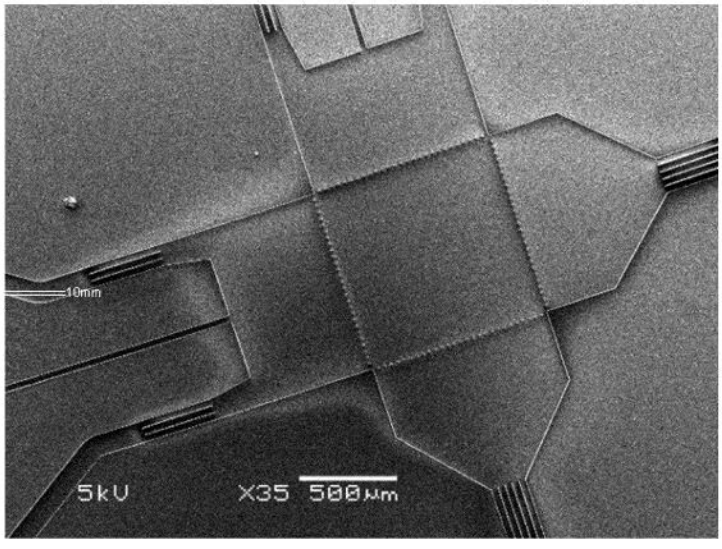
Photolithography on Pyrex glass

Resist development and
50nm gold sputtering, lift-off

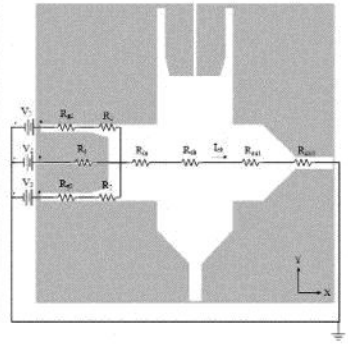
Pressure bonding of PDMS to glass

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

PDMS microchip

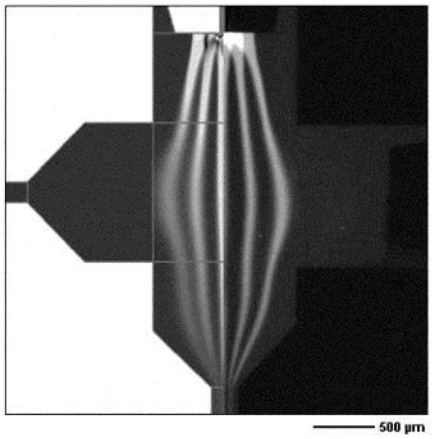


Electrical analogy
of the fluid structure



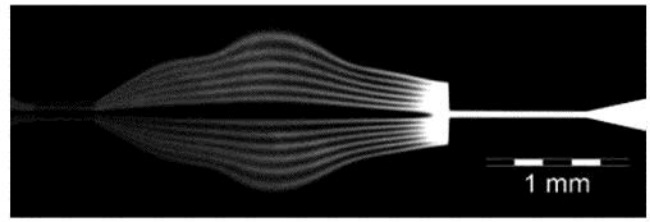
Source: R.B.M. Schasfoort, University of Twente

Experimental results



Simulation ⟷ Real-time

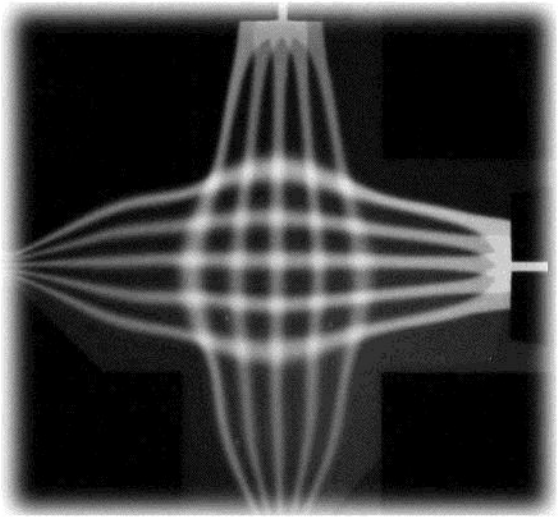
An experimental overlay of sample streams of 25 µm wide



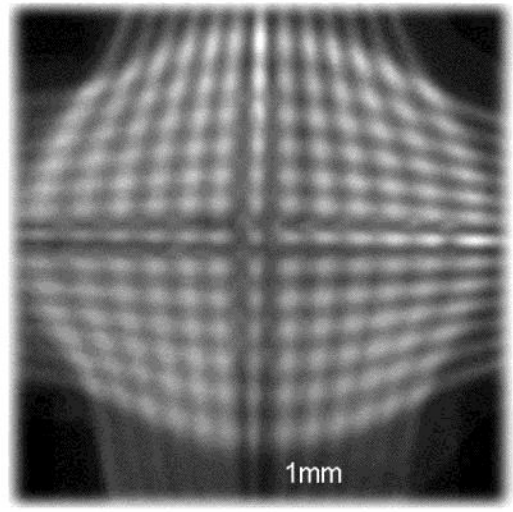
Source: R.B.M. Schasfoort, University of Twente

Perpendicular addressed flow results

Image overlay assembly



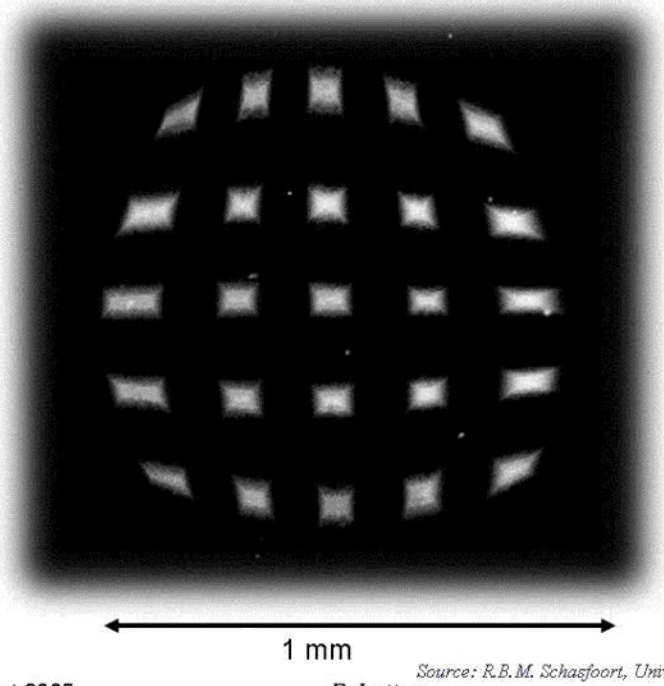
5x5 microarray



13x13 microarray

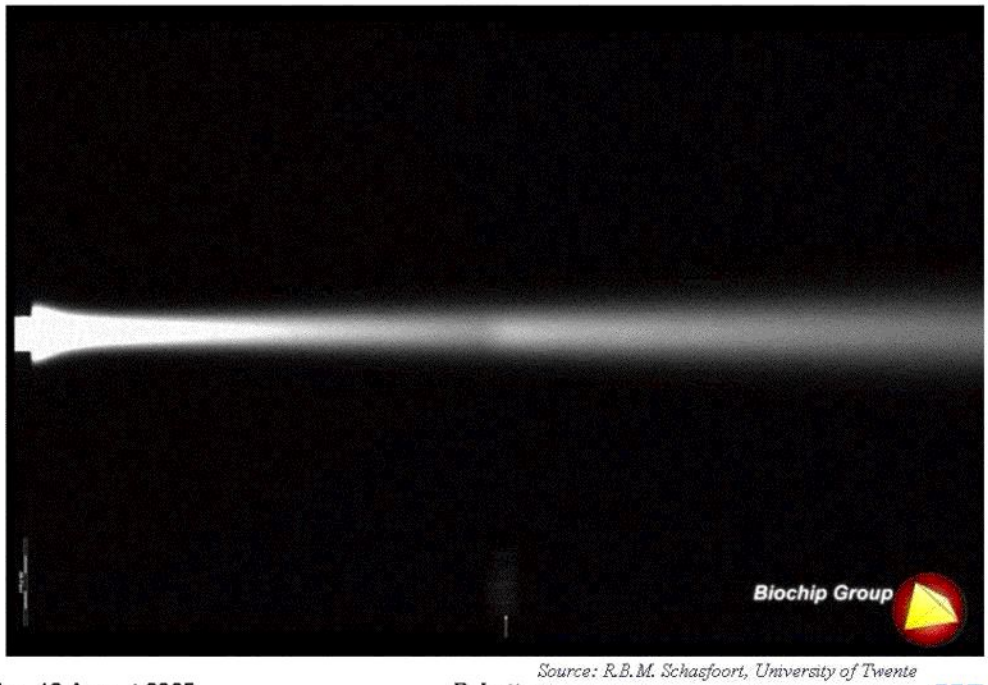
Source: R.B.M. Schasfoort, University of Twente

Resulting microarray



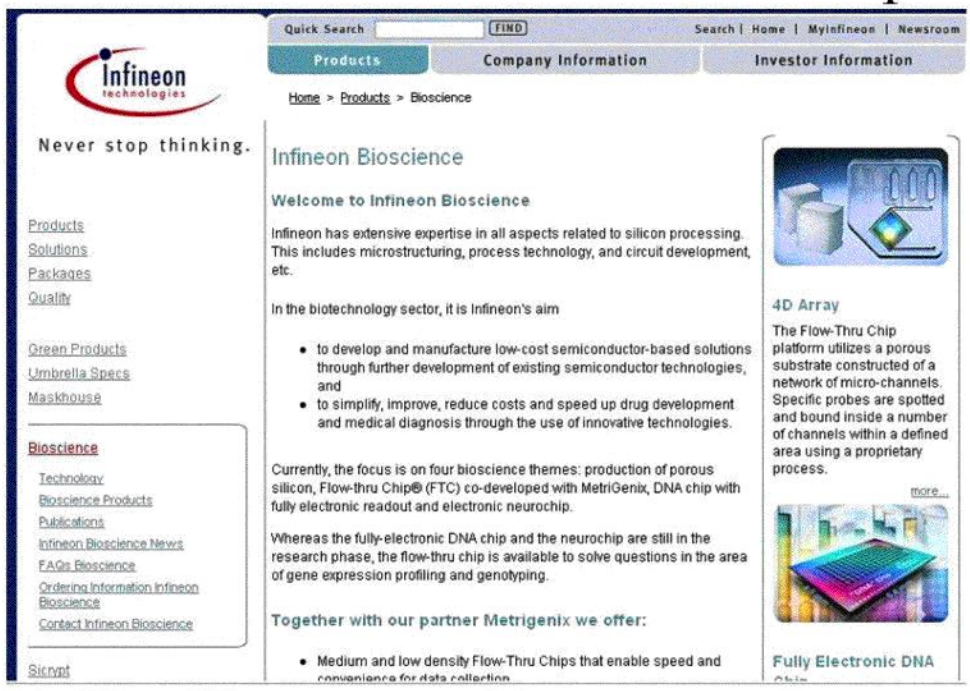
Source: R.B.M. Schasfoort, University of Twente

Addressed flow experiment



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

Microfluidic chip array



8.4. Examples of integrated microfluidic assays

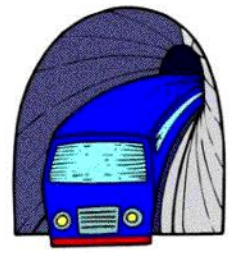
Bead-based integrated microfluidics

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

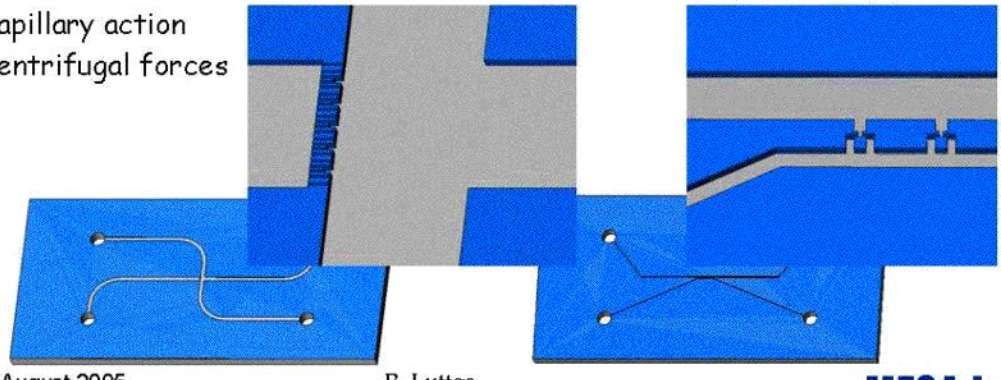
Microfluidic bead transport

Microfluidic performance

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

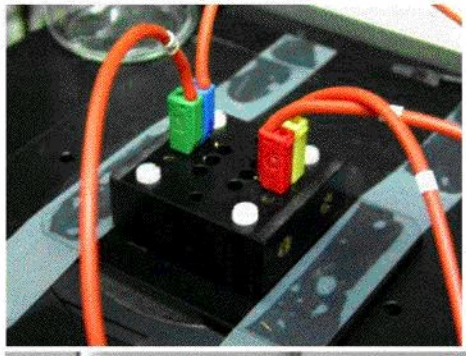


A. Valero et al., BIOS, University of Twente



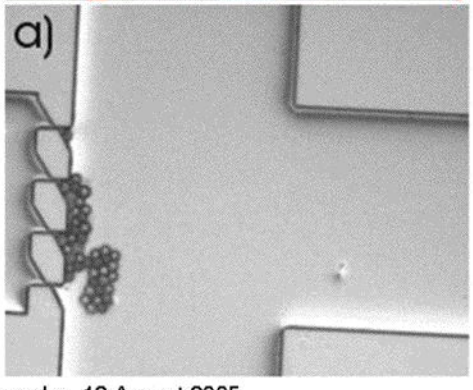
MESA+

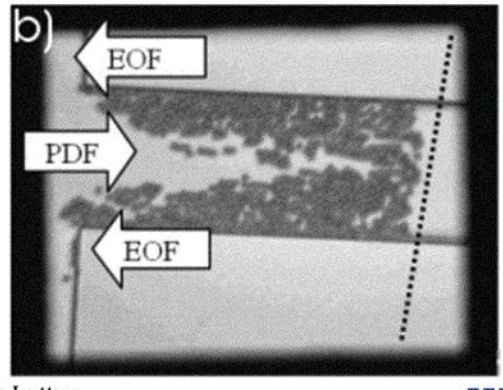
Re-circulating beads



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

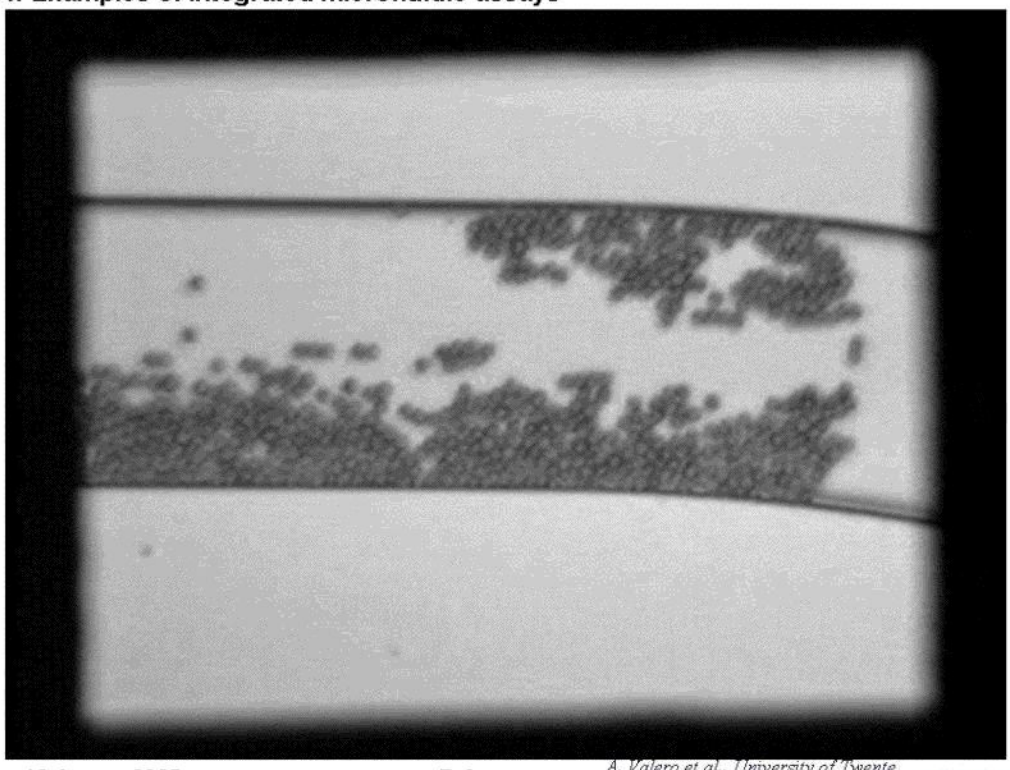
A. Valero et al., University of Twente





MESA+

8.4. Examples of integrated microfluidic assays

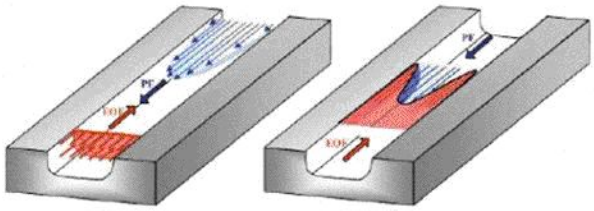


A. Valero et al., University of Twente

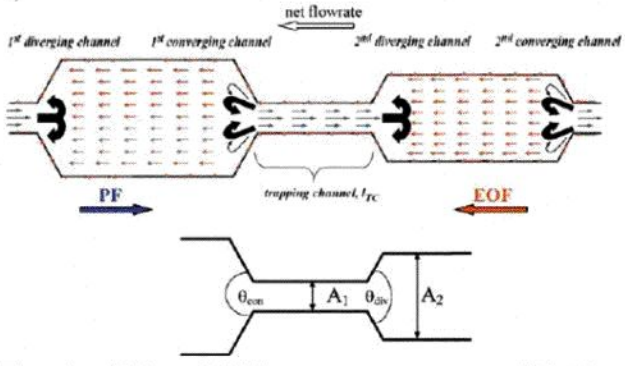
8.4. Examples of integrated microfluidic assays

Bioanalysis using freely moving beads

a)

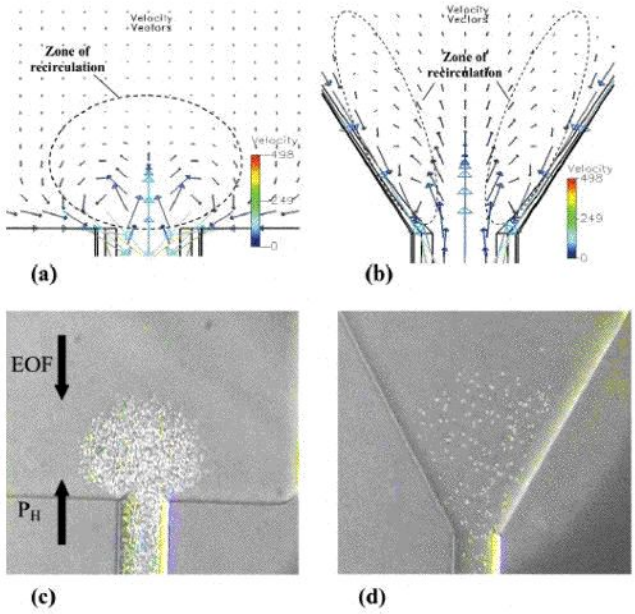


b)



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

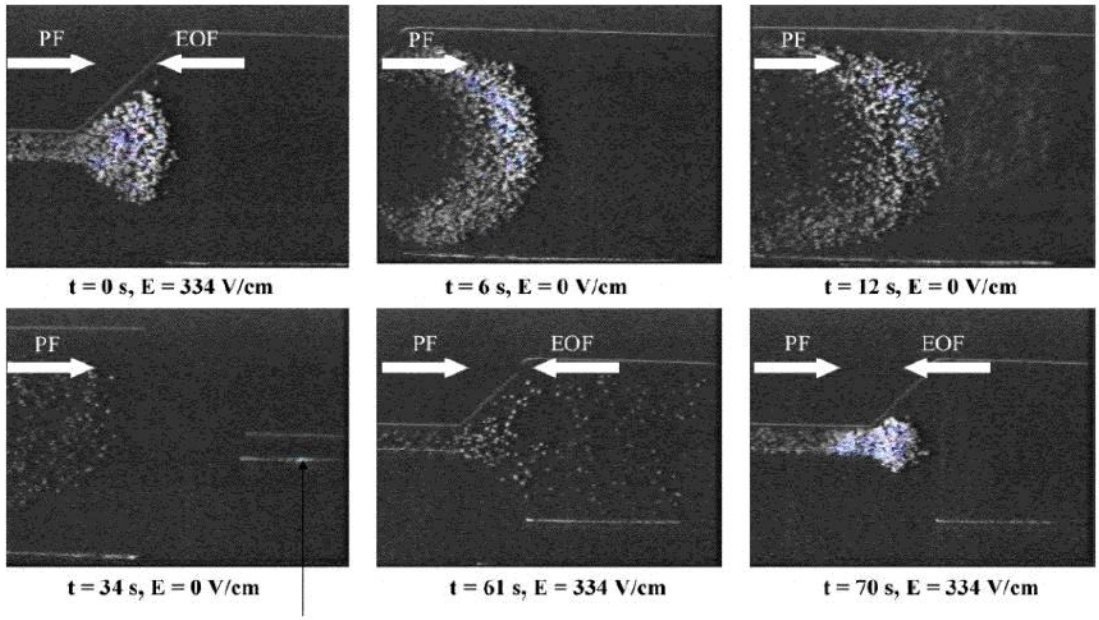
Particle re-circulation



- 1 μm diameter protein A-coated fluorescent beads

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

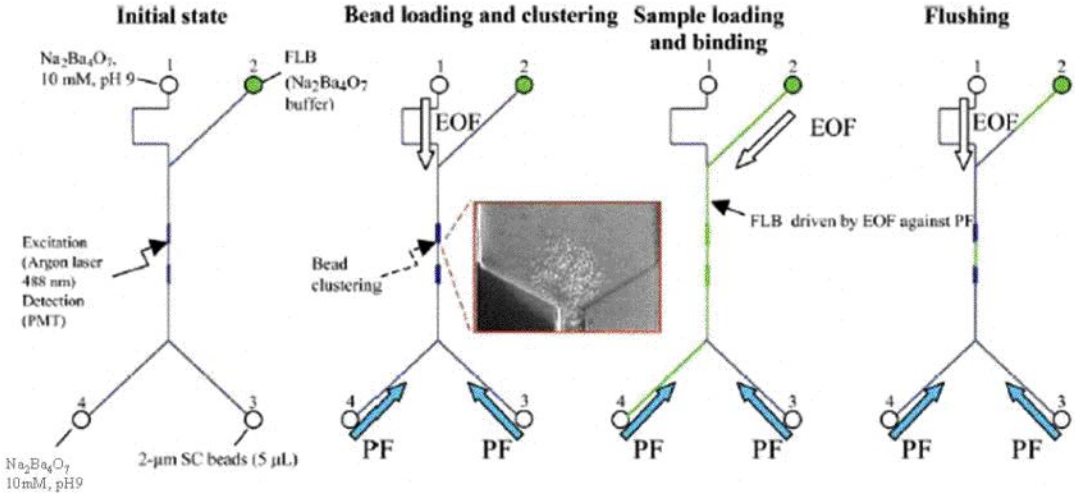
Video capture of particle behaviour



Width of trapping channel: 50μm

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

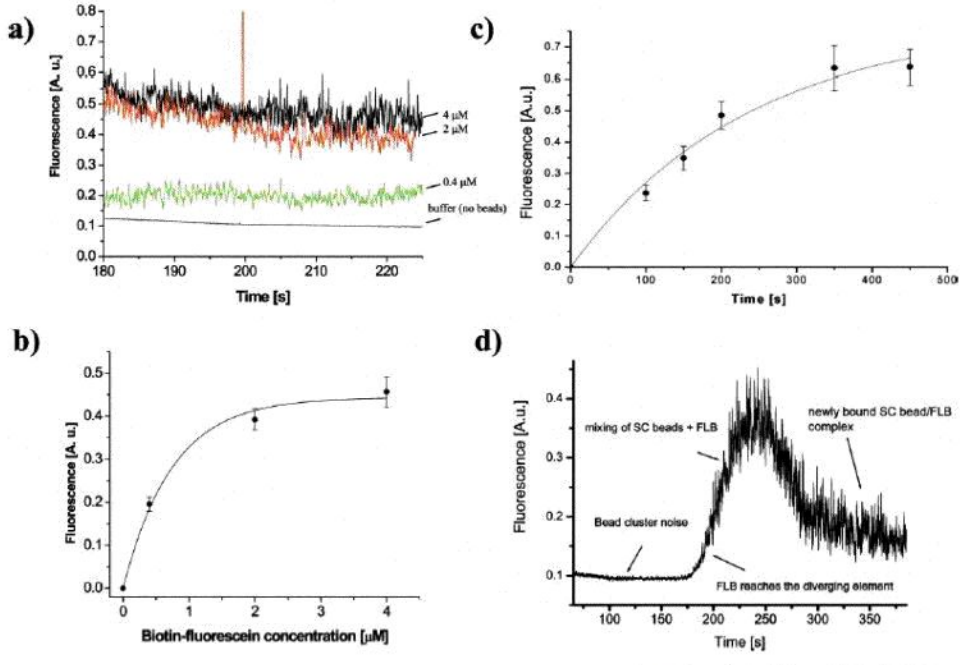
Bead-flow bioassay



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

8.4. Examples of integrated microfluidic assays

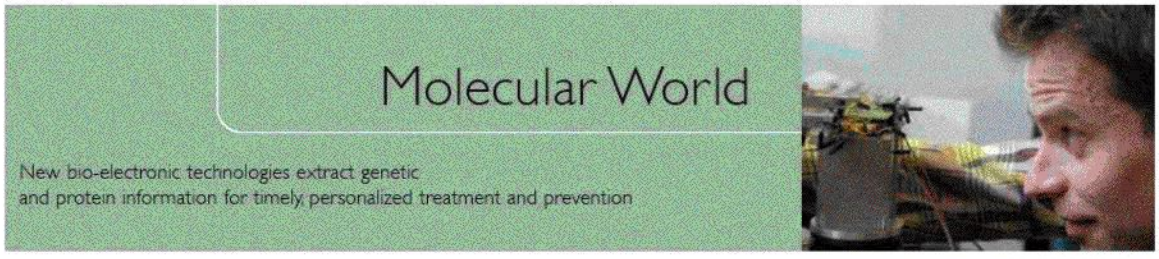
Biotin binding results



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

Innovative developments

PHILIPS



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.

Magnetic bead assay on chip

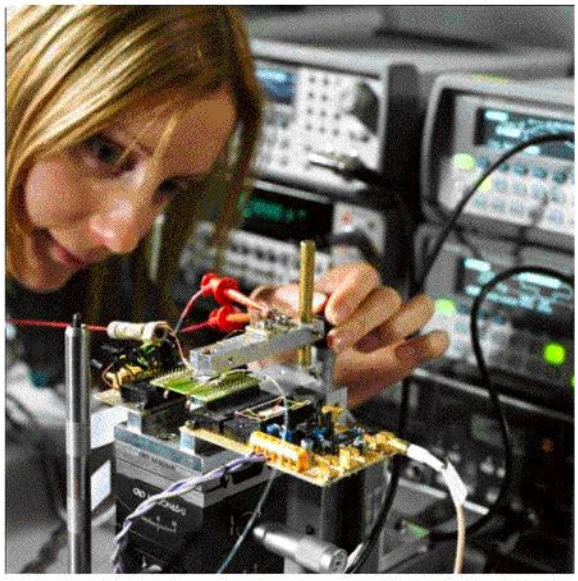


Photo: Philips

http://www.philips.com

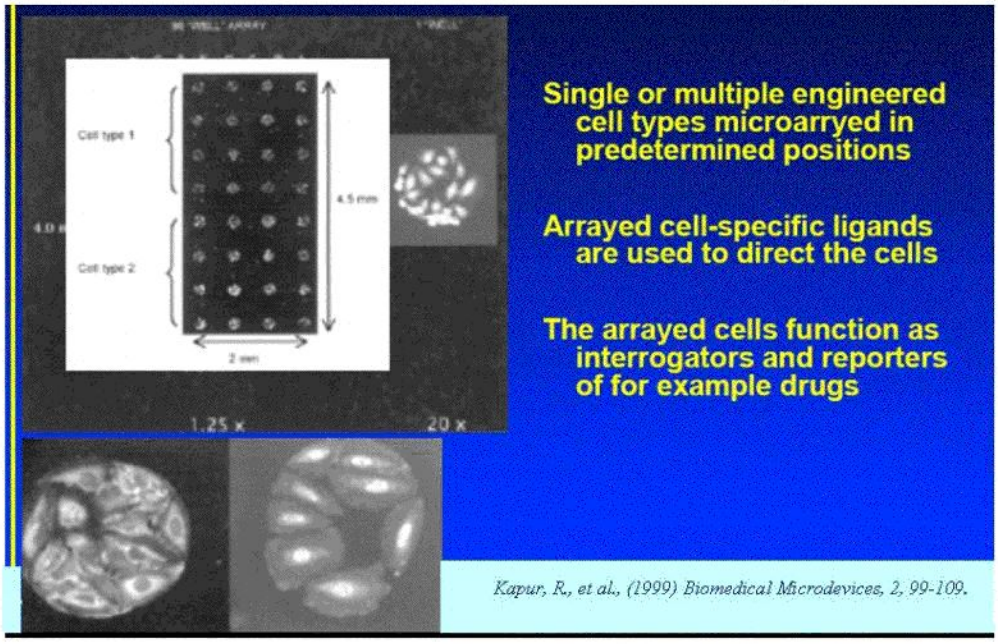
- Magnetic biochip measurement setup.
- See also other groups working on magnetic assays: Pekas et al., Appl. Phys. Lett., Vol. 85, No. 20, 15 November 2004

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

Living cell arrays



Slide prepared by H. Andersson, University of Twente

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/