Zurich Research Laboratory

Chips for Life: Microfluidics for Advanced Diagnostics

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IBM Zurich Research Laboratory

Built in 1962 in Rüschlikon (CH)
~ 350 people

IBM

Research Division
- └ Zurich Research Laboratory
  - └ Science & Technology Department
    - └ Nanoscale Devices & Structures
      - └ Experimental Biosciences

Engelberg

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Agenda

- Objectives of our work on experimental biosciences
- Immunoassays and challenges
- Benefits of miniaturization for bioassays
- Some of our approaches for miniaturizing bioassays
  - Microfluidics & the microfluidic probe
- Concluding remarks

Goal

To present some work derived from non-conventional lithographic techniques and nanotechnology, which might be helpful for healthcare

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Objectives of our work

- goal: to solve important challenges/problems in biology and medicine
- techniques used: surface chemistry, micro/nanotechnology, biochemistry, cell biology
- strategy: develop miniaturized, high-performances bioanalytical methods/devices
- current projects:
  1. development of fast and accurate point-of-care immunodiagnostics for the detection of cardiac markers
  2. screening ultra large libraries to develop vaccines against avian influenza virus (Project Checkmate)

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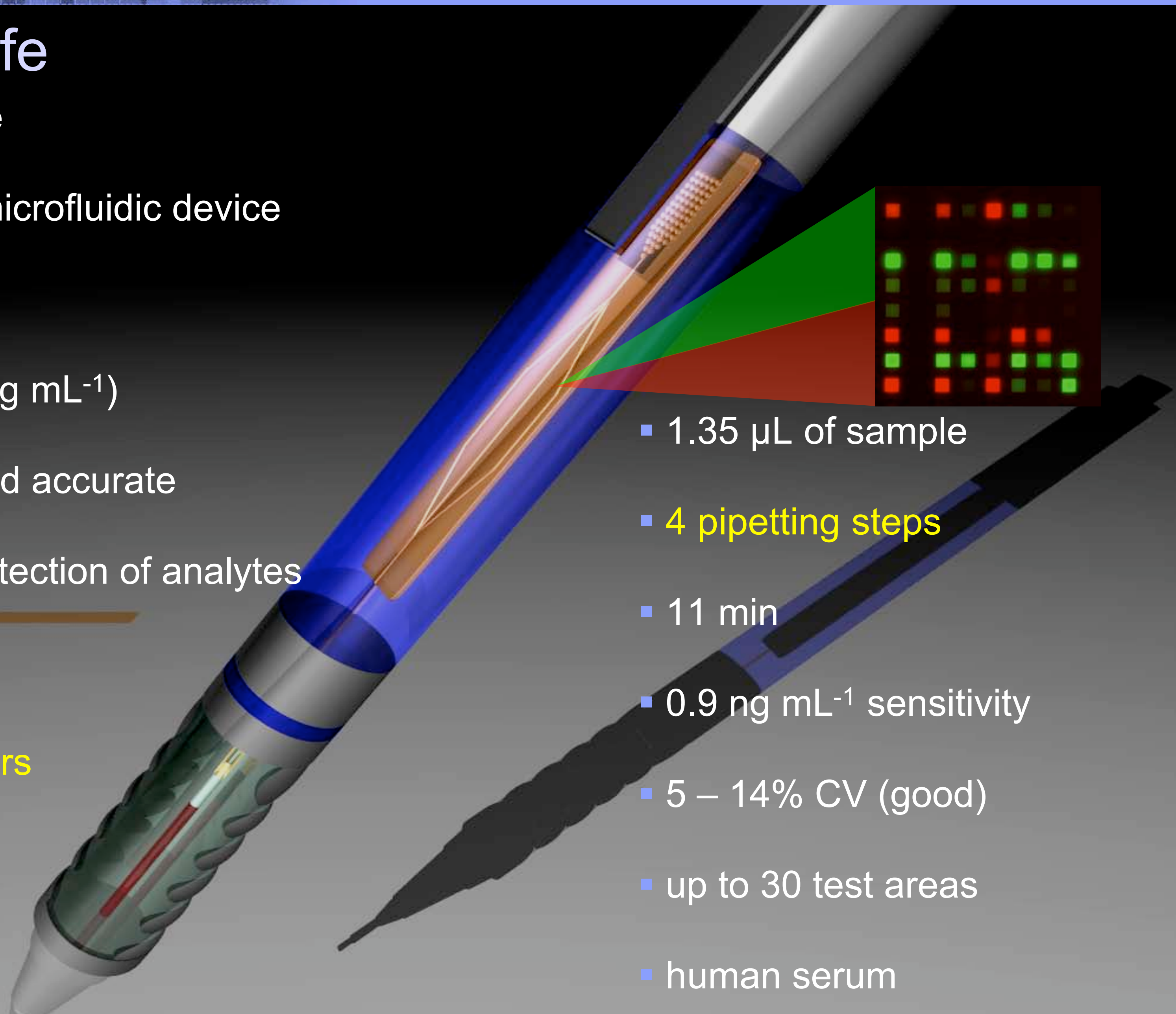
Chips for life

- 1 μL of sample
- autonomous microfluidic device
- fast (<10 min)
- sensitive (<1 ng mL^{-1})
- quantitative and accurate
- multiplexed detection of analytes

- blood sample
- non expert users



- 1.35 μL of sample
- 4 pipetting steps
- 11 min
- 0.9 ng mL^{-1} sensitivity
- 5 – 14% CV (good)
- up to 30 test areas
- human serum

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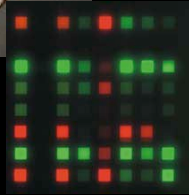
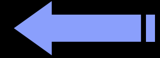
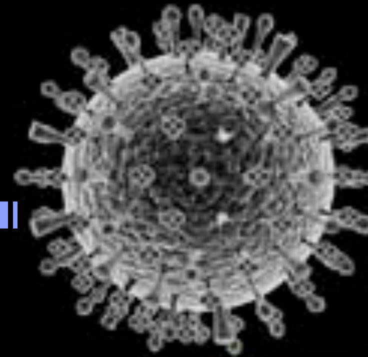
Checkmate – Microfluidics for identifying virus-neutralizing antibodies from ultra-large libraries

random library of 10^9 different antibodies (Scripps)



screening using microfluidic chips (Scripps/IBM ZRL)





random library of 10^4 bird flu receptors (Scripps)

prediction of virus mutations (Scripps/IBM WRC)

antibodies for vaccines (Scripps/Mt Sinai School of Medicine)

vaccines ready ahead of time pandemic prevented

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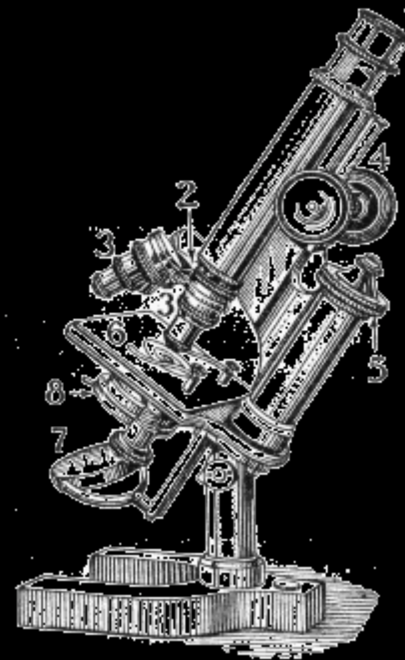
Example 1 - Breakthroughs

- improvement of optical microscopes and lenses
  - Anton van Leeuwenhoek, 1632 – 1723
  - discovered single cells, bacteria, blood flow in capillaries, muscle fibers, spermatozoa, etc.
  - microbiology was founded



http://en.wikipedia.org/wiki/Anton_van_Leeuwenhoek

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Example 2 – Stepwise Progress

- Assay for Prostate Specific Antigen (PSA)
  - PSA is a protein found in blood and used to help diagnosing prostate cancer in men
  - 2-4 ng/mL considered a critical threshold
  - ELISA

- "Sensitive" PSA immunoassays (1997)
  - used to detect very early recurrent cancer after prostatectomy
  - sensitivity < 0.1 ng/mL

- "Ultrasensitive" assays for PSA
  - pg/mL
  - PSA found in many non-prostatic sources
  - detection of PSA might be useful for the prognostic of some cancers (breast cancer, ovarian cancer) in women

http://www.cancer.gov/cancertopics/factsheet/Detection/PSA

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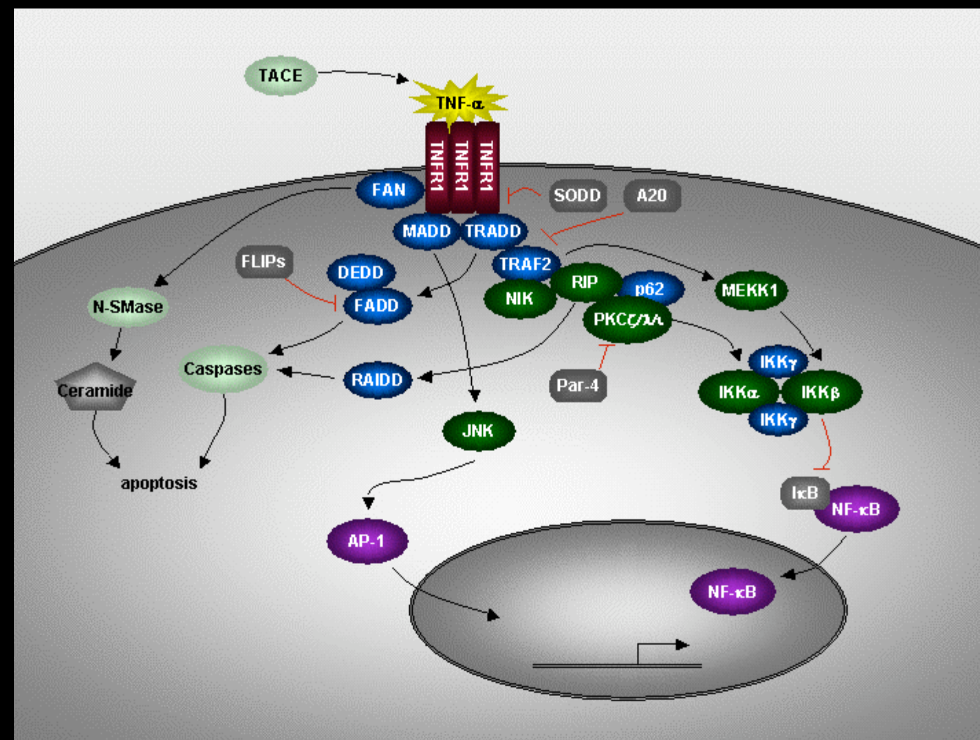
Example 3 – Bioanalysis needs a context

- BCDF (B-cell differentiation factors)
- BCSF (B-cell stimulating factor)
- BSF-2 (B-cell stimulating factor-2)
- BSF-p2 (B-cell stimulating factor p2)
- CDF (CAT development factor , choline acetyltransferase development factor)
- CDF (Cytolytic differentiation factor for T-lymphocytes)
- CDF (Cytolytic T-lymphocyte differentiation factor)
- CDF (cytotoxic T-cell differentiation factor)
- CPA (colony promoting activity)
- CSF-309 (Hematopoietic colony stimulating factor-309)
- DIF (differentiation inducing factor)
- Differentiation inducing factor for human monoblastic leukemia cells
- FDGI (fibroblast-derived growth inhibitor)
- Fibroblast derived differentiation inducing factor for human monoblastic leukemia cells
- HGF (hybridoma growth factor)
- HP1
- HPGF (hybridoma/plasmacytoma growth factor)
- HSF (hepatocyte stimulating factor)
- HSF-1 (hepatocyte stimulating factor-1)
- IFN-beta-2 (Interferon-beta-2)
- ILHP1 (Interleukin-hemopoietin-1)
- ILHP1 (interleukin-hybridoma/plasmacytoma-1)
- L-GI factor (murine lung-derived growth inhibitory factor)
- L-HGF (L929-derived hybridoma growth factor)
- MGI-2A (Macrophage-granulocyte inducer-2A)
- Mk potentiator
- Myeloma GF (myeloma growth factor)
- Natural killer cell activity-augmenting factor
- NKAF (natural killer cell activating factor)
- PCT-GF (plasmacytoma growth factor)
- TAF (T-cell activating factor)
- Thymocyte growth factor
- TPO (thrombopoietin)
- TSF (thymocyte stimulating factor)
- WI-26-VA4 factor



- TNF-α is a small protein (MW 17 500) and plays an important role in the generation and regulation of inflammation and immune responses and is present at very low concentration in body fluids

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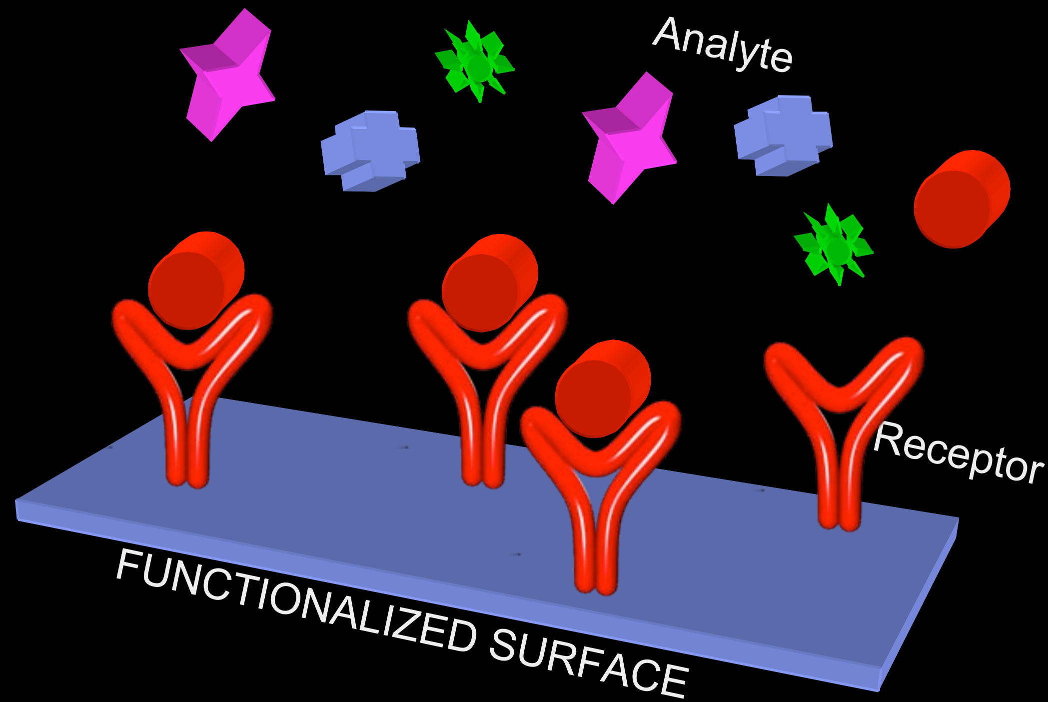
Ideal bioanalytical platforms



Transduction Principle

- current
- luminescence
- fluorescence
- radioactivity
- mass
- surface stress
- refractive index
- heat

- specific
- sensitive
- fast
- accurate
- reliable
- portable
- cost effective
- connected
- simple to use

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Real-life bottlenecks

- non-specific
- insensitive
- slow
- inaccurate
- fragile
- bulky
- expensive
- IT-poor
- complex to use

- scarce samples
- unstable samples
- heterogeneous samples
- competition
- IP
- business model

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Agenda

Objectives of our work on experimental biosciences

Immunoassays and challenges

Benefits of miniaturization for bioassays

Some of our approaches for miniaturizing bioassays
Microfluidics & the microfluidic probe

Concluding remarks

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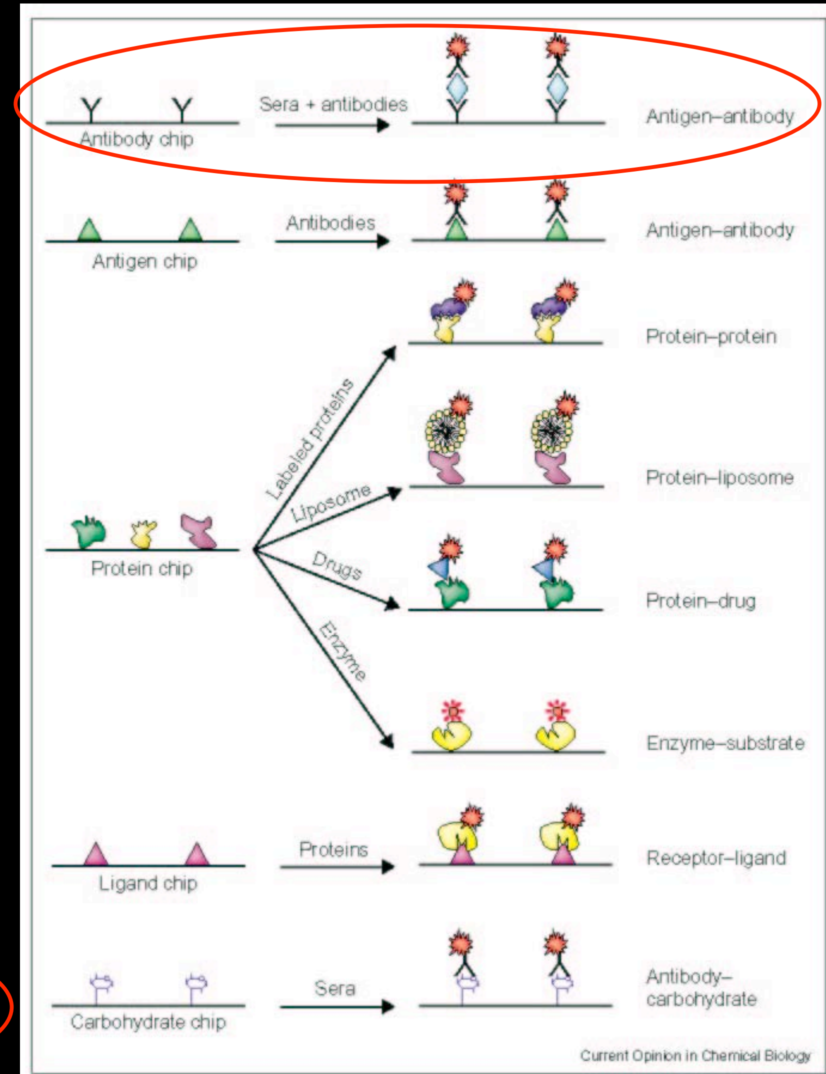
Introduction to Biological Assays & Immunoassays

- **principle**
  - Assay: a receptor is used to bind and detect a ligand (analyte)
  - Immunoassay: the binding deals with antibodies and antigens
  - Surface Immunoassay: an antibody immobilized on a surface is used to selectively capture analyte antigens from solution
  - Sandwich surface immunoassay: after capture to the surface, the antigen is bound by a detection antibody carrying a signal generating molecule
  - Fluorescence sandwich surface immunoassay: fluorescent centers are attached to the detection antibodies



Zhu, H., Snyder, M. *Curr. Opin. Chem. Biol.* **2003**, *7*, 55-63.

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Immunoassays are like cooking recipes

Assay procedures (ELISA)

1. Secure the desired number of coated wells in the holder.
2. Dispense **100µl** of standards, specimens, and controls into appropriate wells.
3. Dispense 100µl of **zero buffer** into each well.
4. **Thoroughly mix** for 10 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at room temperature (18-25°C) for **60 minutes**.
6. Remove the incubation mixture by emptying plate contents into a waste container.
7. Rinse and empty the microtiter wells **5 times** with running tap or distilled water.
8. **Strike the wells sharply** onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense **200µl** of Enzyme Conjugate Reagent into each well. **Gently mix** for 5 seconds.
10. Incubate at room temperature for **60 minutes**. ***Prepare TMB solution 15 minutes before use.***
11. Remove the incubation mixture by **flicking plate** content into a waste container.
12. Rinse and flick the microtiter wells **5 times** with running tap or distilled water.
13. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
14. Dispense 200µl of TMB solution into each well. Gently mix for 5 seconds.
15. Incubate at room temperature for 20 minutes **without shacking**.
16. Stop the reaction by adding 50µl of 2N HCl to each well.
17. **Gently mix for 30 seconds to make sure that the blue color changes to yellow color completely**.
18. **Within 30 minutes**, read the optical density at 450nm with a microtiter plate reader.

Important Notes

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 3 minutes. A full plate of 96 well may be used if automated pipetting is available.

Duplication of all standards and specimens, although not required is recommended.

obviously, platforms for immunoassays must be flexible…

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Immunoassays have proven importance

- detection of analytes for research in life sciences (Research, HTS)
- detection of disease markers (Diagnostics)
  - e.g. cardiac markers in blood samples
  - cancer markers
  - detection of infectious agents
  - allergen detection
- pregnancy testing (Point-of-care/over-the-counter diagnostics)
- monitoring of therapy (Healthcare)
- detection of pesticides and pollutants (Environmental testing)
- detection of doping substances
- cellular assays, animal testing, clinical trials (Drug development)
- quality monitoring of bio-engineered molecules (Biotechnology)

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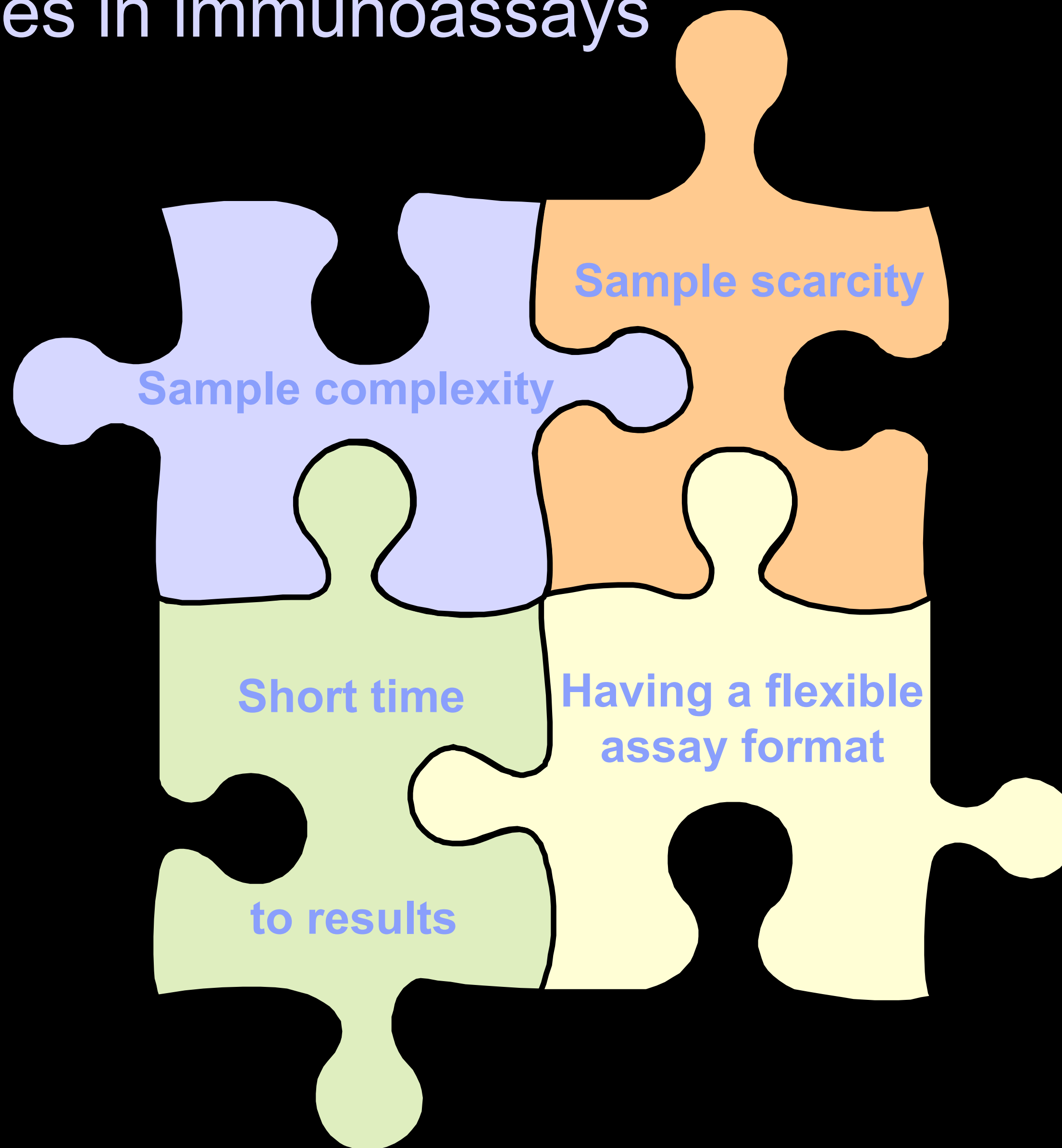
4 challenges in immunoassays



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Example of specific solutions

home testing

point-of-care testing

clinical analyzer

HTS for drug discovery

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Microfluidics & the microfluidic probe

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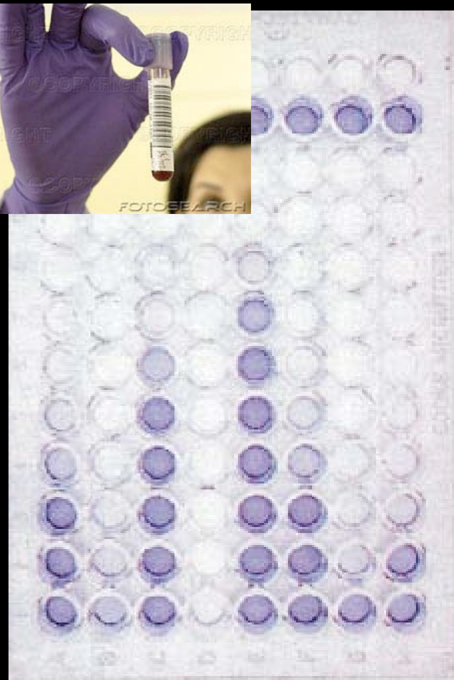
Miniaturization helps solving some challenges

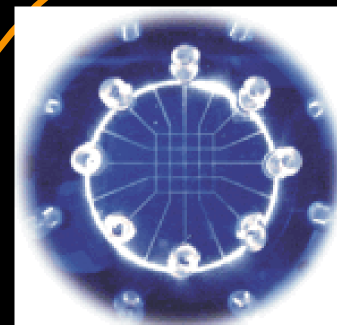
Microtiter Plates / Test Tubes



~12 cm

~ 8 cm

Microfluidic Systems



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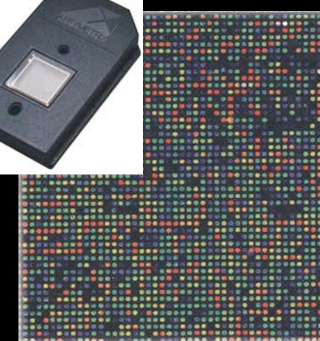

Biochips & Microarrays

2 mm

EACH DOT IS A DISTINCT CHEMICAL ENTITY!

- Nanotechnology enables totally new biosensing platforms useful for many healthcare and LS applications
- (i) reduced sample consumption; (ii) faster reactions; (iii) parallelization
- high-throughput screening

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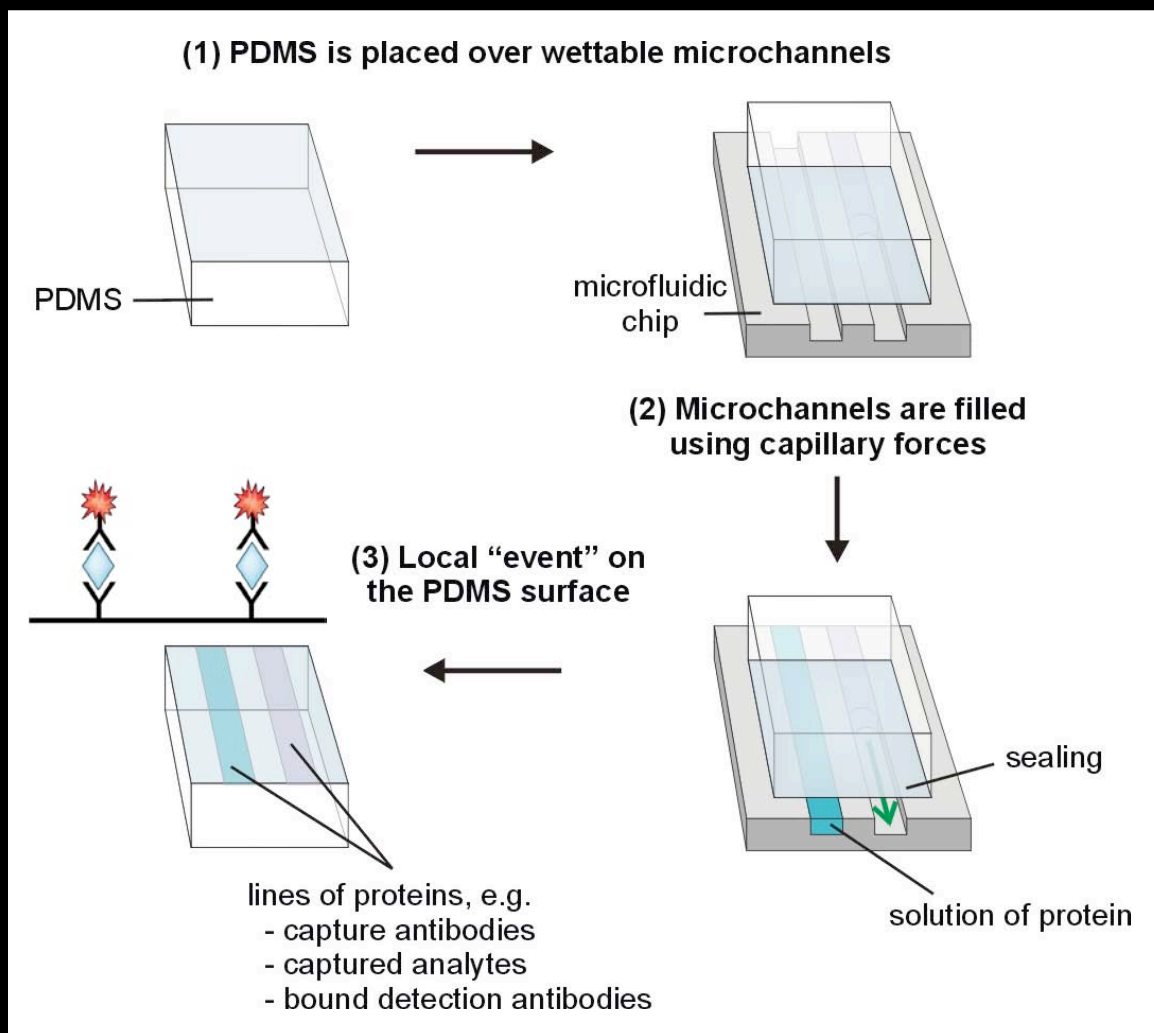
Capillary-driven microfluidics for surface immunoassays



- assay takes place on the surface of a PDMS block, which is placed over the chip
- PDMS block ensures efficient, reversible sealing of independent flow paths
- all the solutions needed for an assay can be sequentially flushed through one microchannel
- preferred format for the assay: fluorescence surface immunoassay

Delamarche et al. *Science* **1997**, *276*, 779-781
Delamarche et al. *J. Am. Chem. Soc.* **1998** *120*, 500-508.

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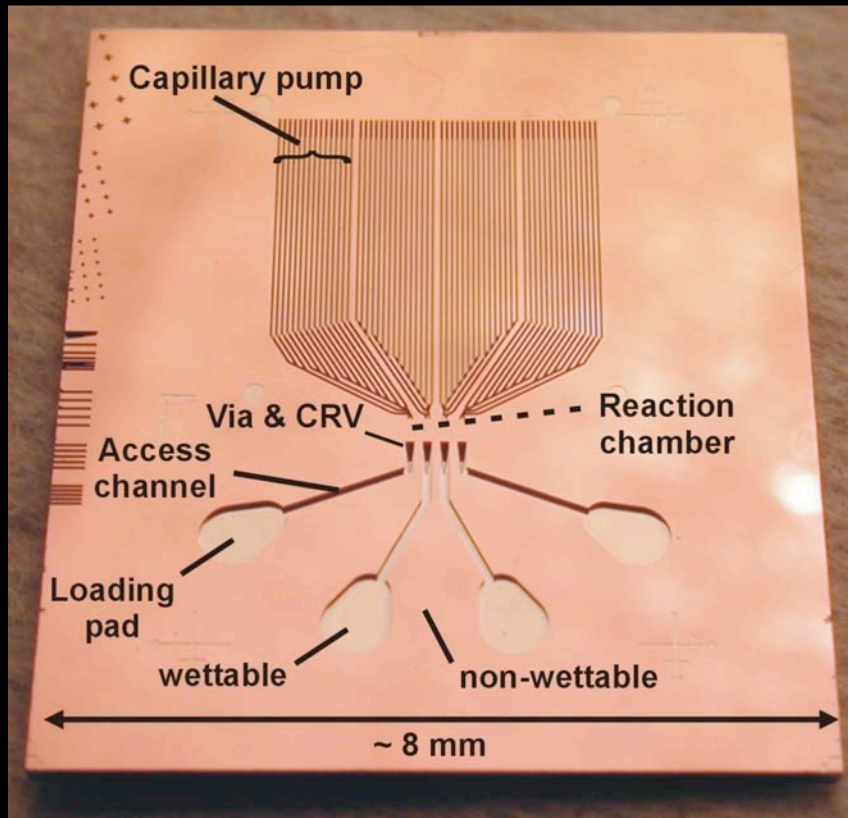
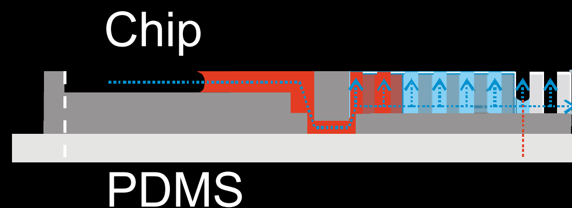
(3D) Capillary-driven Microfluidics





- Chips microfabricated in Si
- Chips are autonomous (e.g. no strict need for pumping or peripheral equipment)
- Solutions are directly pipetted by hand onto the chip
- Several independent flow paths can be used to make assays in parallel
- Assays take place on the surface of a PDMS block (not shown here), which is reversibly placed over the chip

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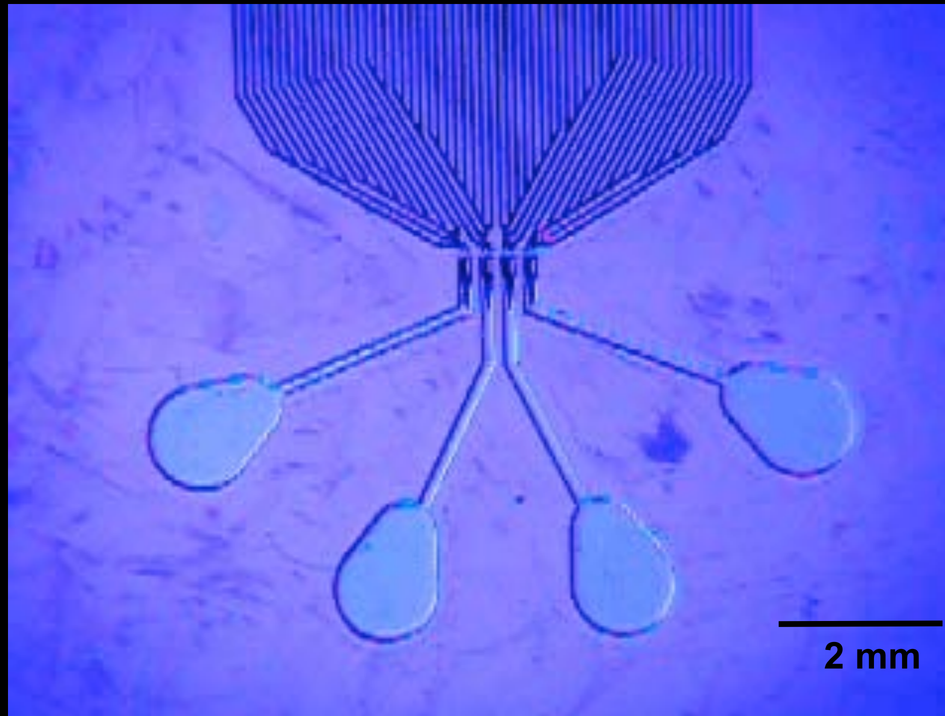
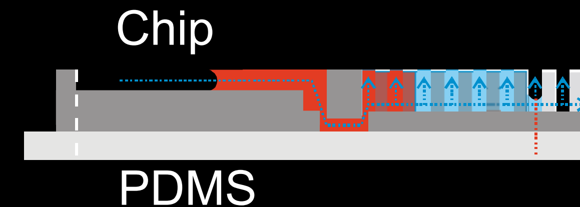
Filling a chip with 4 independent flow zones (topview)





- ~150-nL-aliquots are sequentially added to loading ports
- the aliquots spontaneously flow from the loading ports to the capillary pumps
- flow rate is here ~ 220 nL s^{-1}

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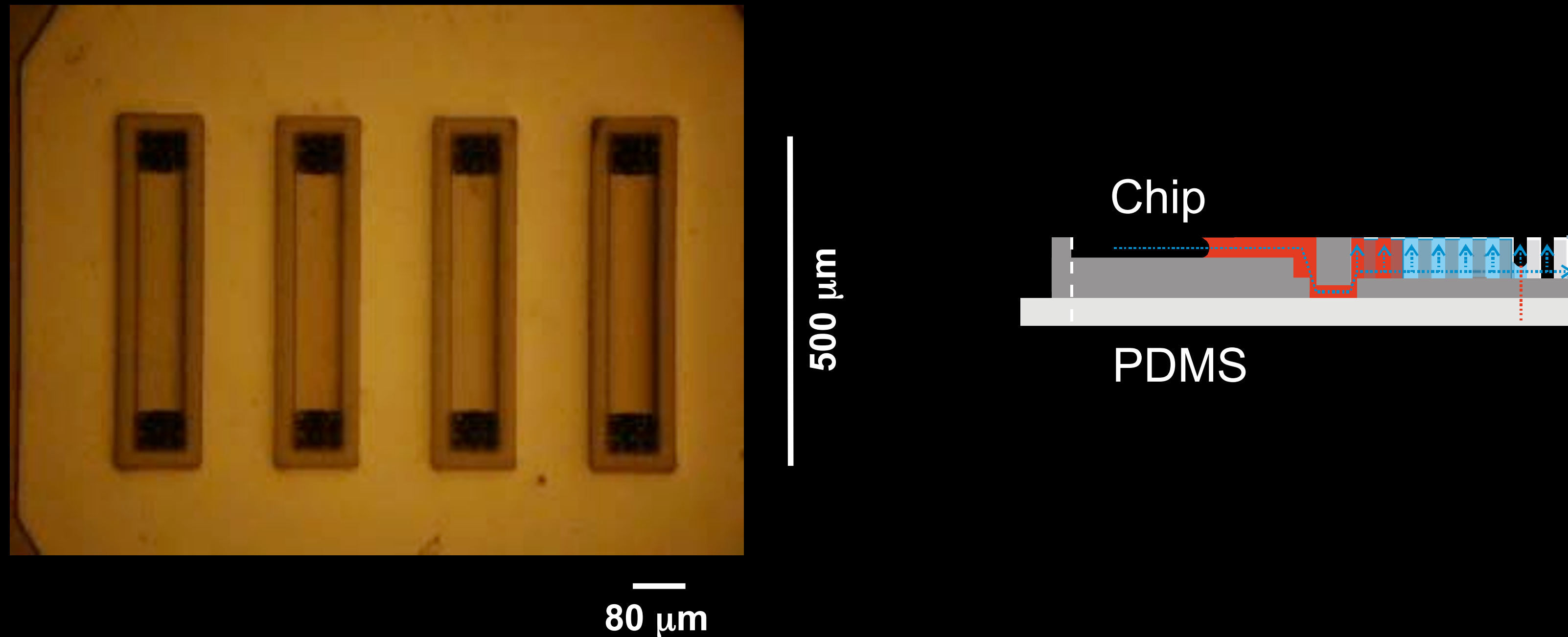
View through the PDMS substrate (reaction chamber)



- 150-nL-aliquots flowing through independent reaction chambers
- No mixing between aliquots due to laminar flow
- Reaction chamber is 15 pL in volume, flow rate is ~ 220 nL s^{-1}, linear rate 55 mm s^{-1}

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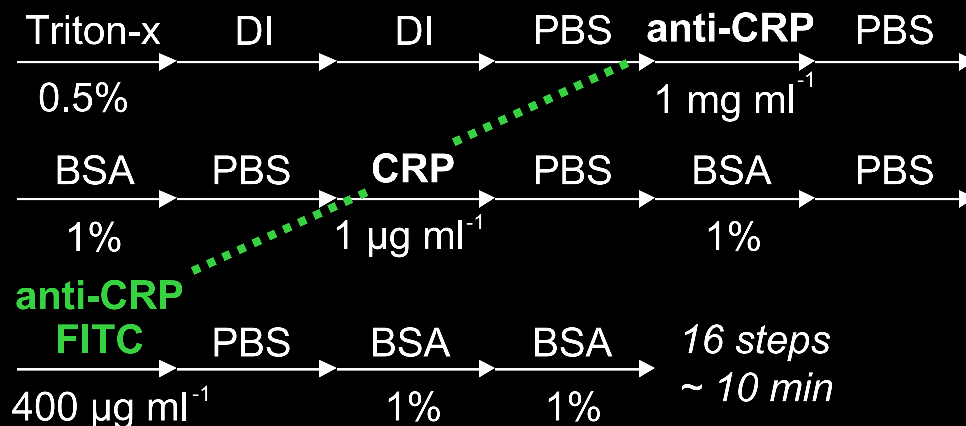
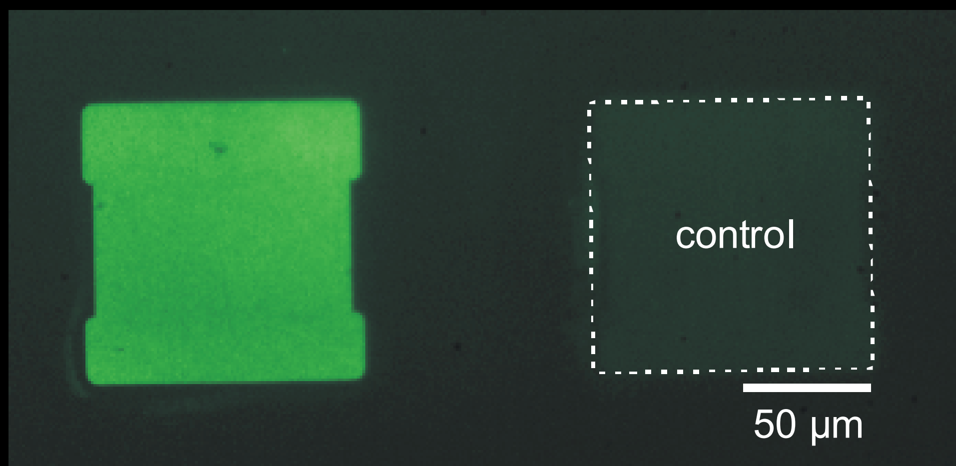
Fluorescence immunoassay for CRP





Fluorescence image, positive result

Negative control

- CRP: C-reactive protein. Protein found at high concentration in the blood of people having e.g. a bacterial infection or acute myocardial infarction
- ~10× faster than conventional immunoassays
- ~100× less volume (200 nL aliquots)
- High-quality signals, high-signal density, well-defined background signal
- Fluorescence is a very convenient type of signal to detect

Juncker, D. et al. *Anal. Chem.* **2002**, *74*, 6139-6144.

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Micromosaic immunoassays

- combinatorial screening of antigens and antibodies!
- even faster and more preservative of reagents than previous examples
- many pharma, diagnostic, and instrument companies have shown interest in using this technology
- was extended for drug toxicity testing with very high sensitivity (1 pM) with Hofmann-LaRoche

Bernard, A., Michel, B., Delamarche, E. *Anal. Chem.* **2000**, *73*, 8-12.

Cesaro-Tadic, S., Dernick, G. et al. *Lab-on-a-Chip* **2004**, *4*, 563-569.

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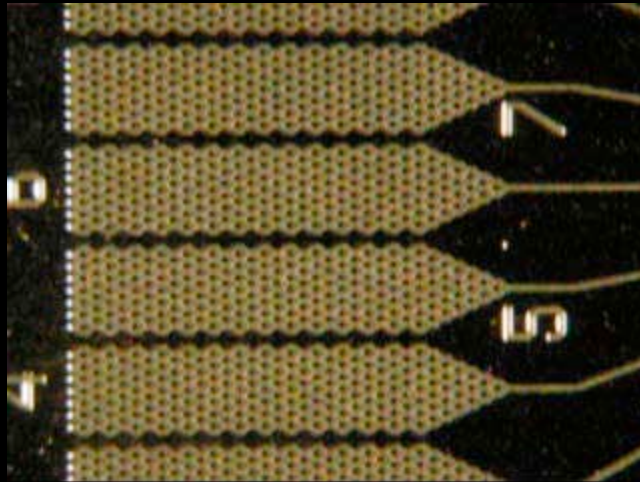
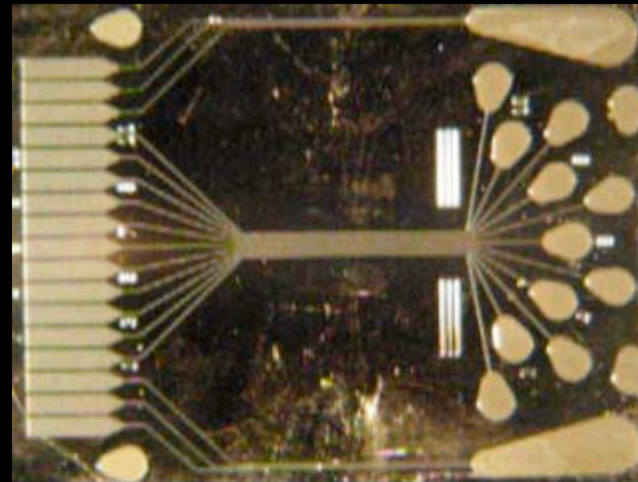
Filling a chip having 11 independent flow zones



detail of capillary pumps



autonomous microfluidic chip

- 300-nL-aliquots are added to each of the 11 loading ports
- the aliquots spontaneously flow from the loading ports to the capillary pumps

(courtesy of G. Dernick and Ch. Fattinger, *Hoffmann-La Roche*)

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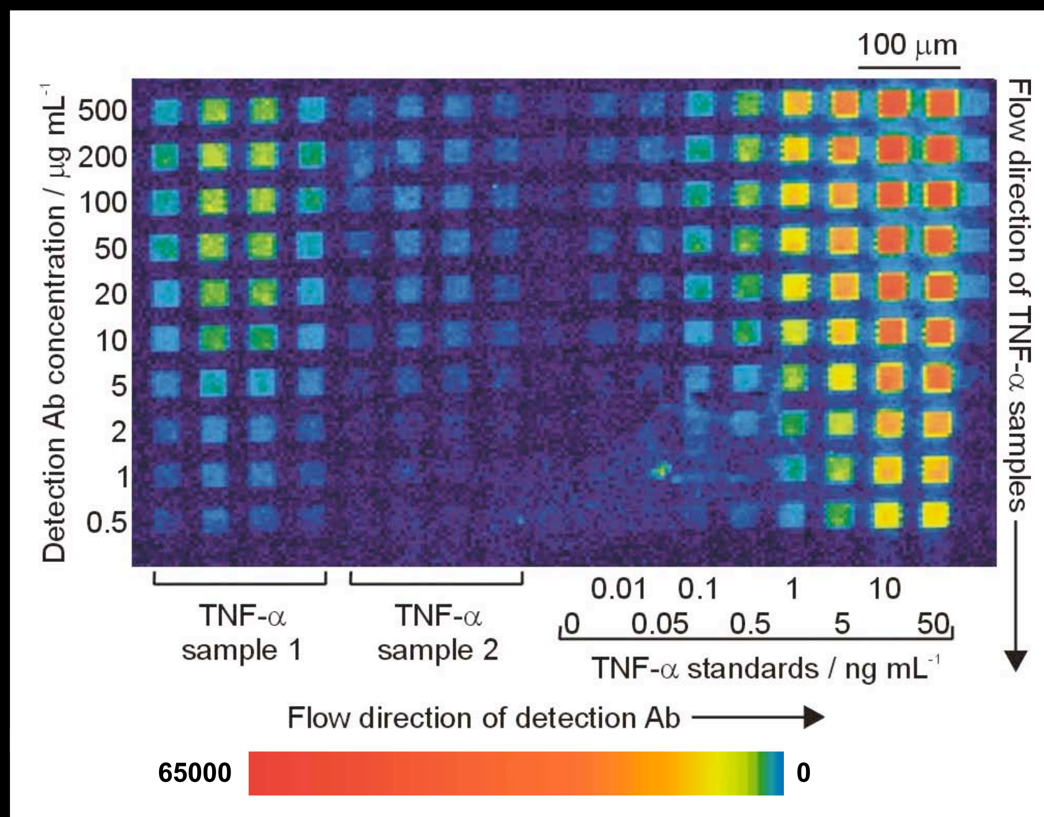
Micromosaic immunoassay for TNF-α



fluorescence micrograph obtained using a biochip scanner

- capture antibody (anti-TNF-α) was homogenously coated on PDMS block
- solutions containing TNF-α were provided (vertical direction) for the capture step using a first chip having 17 flow paths
- solutions with detection antibody were provided (horizontal direction) using a second chip having 10 flow paths
- assay developed with Hoffman-LaRoche
- 1 pM sensitivity

Cesaro-Tadic, S., Dernick, G. et al. *Lab Chip* **2004**, *4*, 563-569.

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Sample volume reduction for testing drugs?

Micromosaic immunoassays:

- 45 min for the whole assay
- 600 nL sample volume for 10 test sites

DELFIA

- 150 µL of sample, 6–7 h

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There is a need for “open” microfluidics

- many samples cannot be loaded inside microchannels
  - tissue sections
  - microarrays/biochips
  - adherent cells
  - gels and other viscous substances
  - cells, when shear stress on microchannel walls disrupt the cells
  - samples from biopsies
  - samples with particulates
- many samples can be loaded inside microchannels but then some information might be lost
  - living cells outside of their environment

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The Microfluidic Probe (MFP)

- *from closed to open microfluidics*
- *from contact to non-contact*

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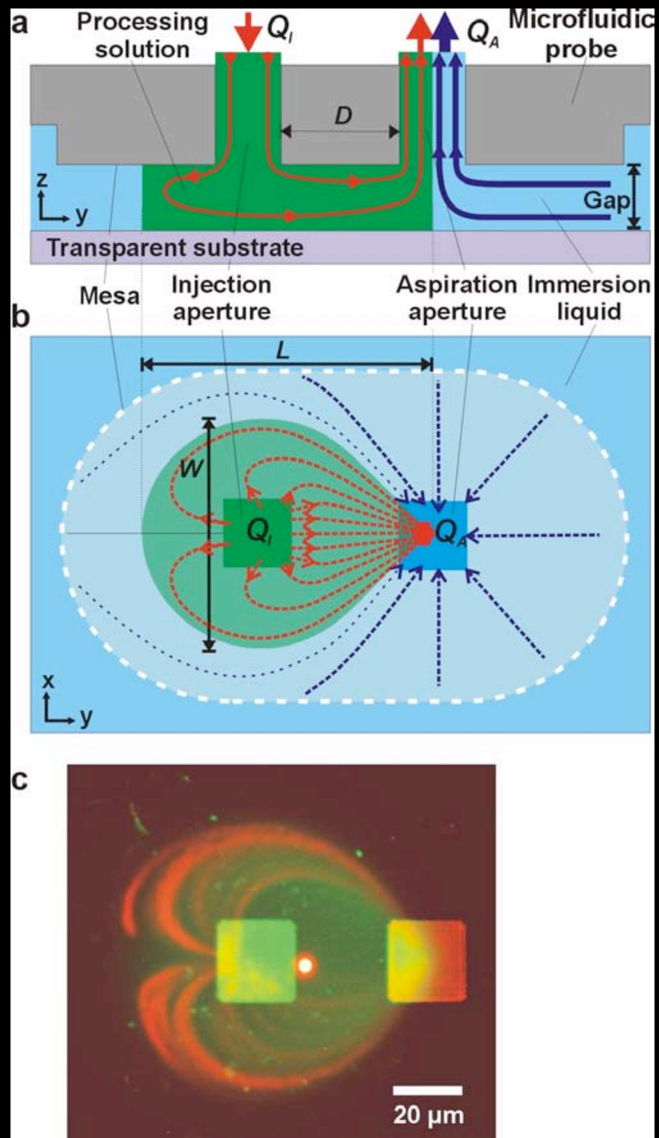
Principle of the MFP



a. Crossview of a MFP head over a substrate

- the MFP has 2 apertures
- one ejects a processing liquid at a rate Q_I
- the second one aspirates both the processing liquid and an immersion liquid at a rate Q_A

b. Topview showing the flow of liquids in the gap between the MFP head and the substrate

- the processing liquid is well confined by the immersion liquid
- hydrodynamic flow confinement

c. Fluorescence microscope image showing the confinement of a processing liquid over a glass substrate.

- the processing liquid contains green dyes and large red beads

Juncker, D., Schmid, H., Delamarche, E. *Nature Materials* **2005**, *4*, 622-628.

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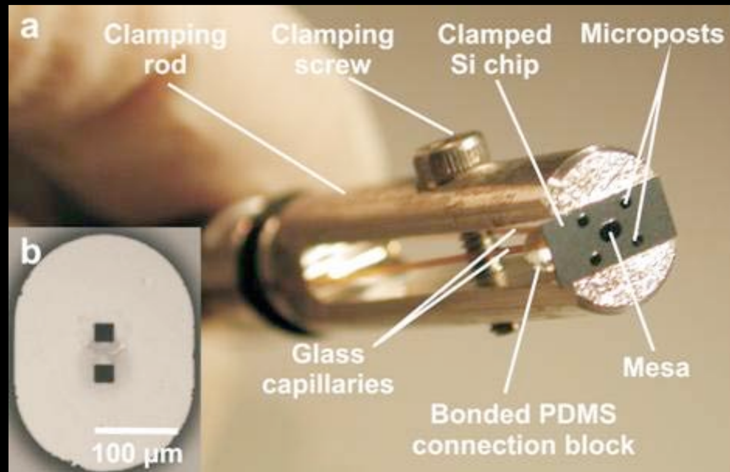
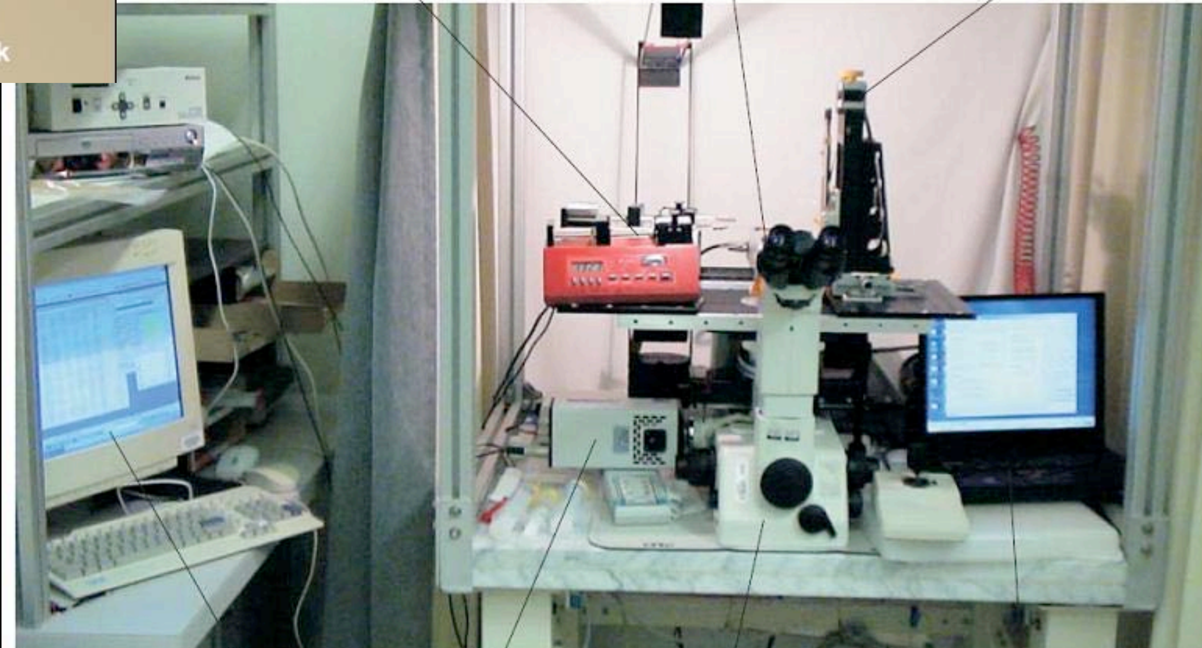
Implementation





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Parameters determining the hydrodynamic flow confinement

- variable parameters:
  - Q_a and Q_i (nL/min)
  - gap between the MFP and substrate (μm)
  - writing speed (mm/s)

Q_i-Q_a: 2-200 nL min^{-1}

Q_i-Q_a: 2-50 nL min^{-1}

Q_i-Q_a: 8-200 nL min^{-1}

Q_i-Q_a: 8-50 nL min^{-1}

apertures: 20 μm
distance between the apertures: 120 μm
gap: 2 μm

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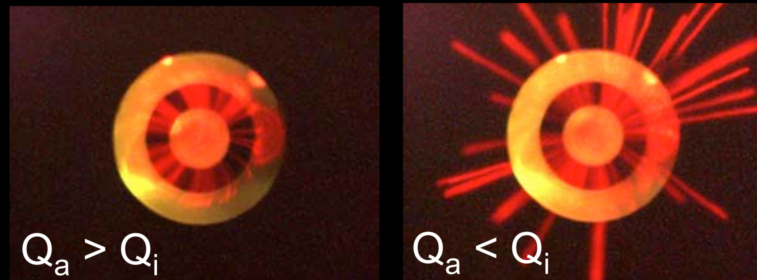
Parameters determining the hydrodynamic flow confinement

- fixed parameters
  - size and shape of the apertures
  - number of apertures
  - relative position of the apertures

MFP with 2 concentric apertures



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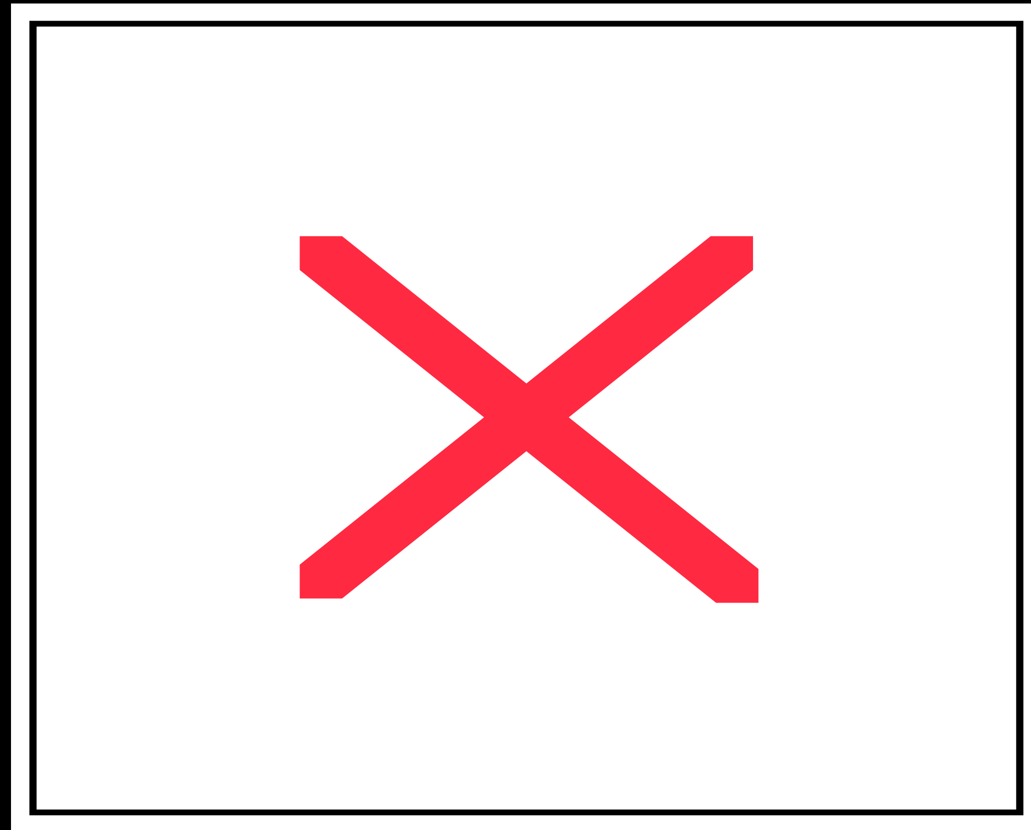
Video showing the confinement as a function of Qa/Qi



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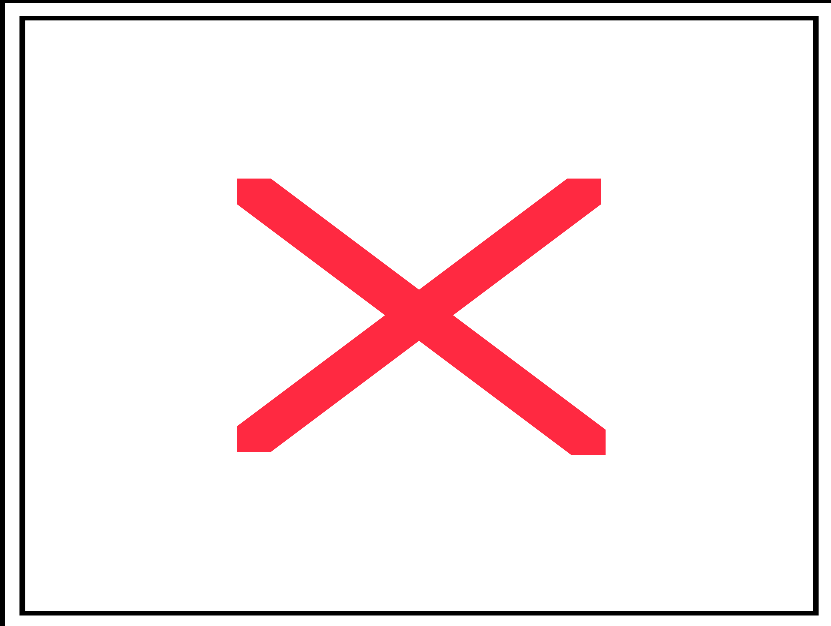
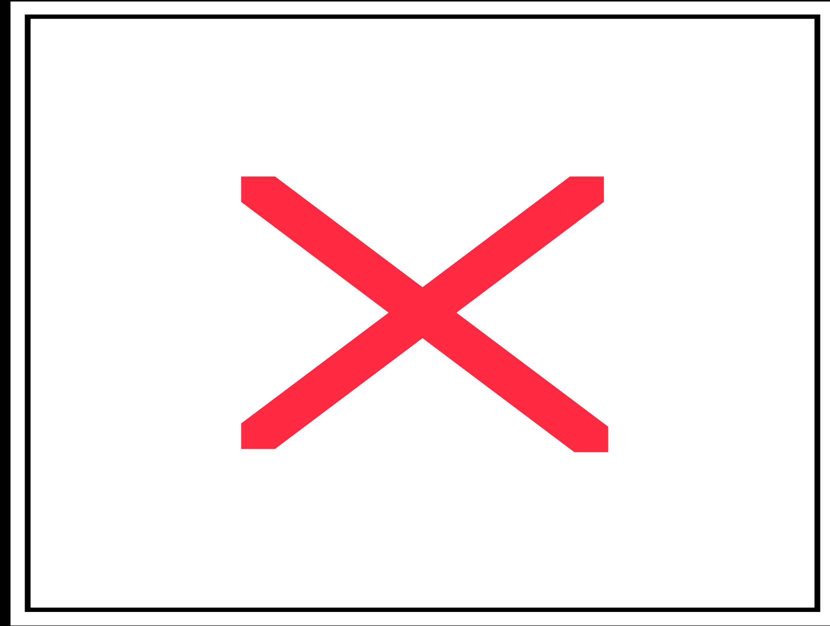
Writing using a MFP





- Proteins in the processing liquid deposit on the scanned surface

- no drying artifact due to the presence of the immersion liquid (biological buffer)

- moving quickly the MFP makes the immersion liquid inserting below the processing liquid → non-writing mode!

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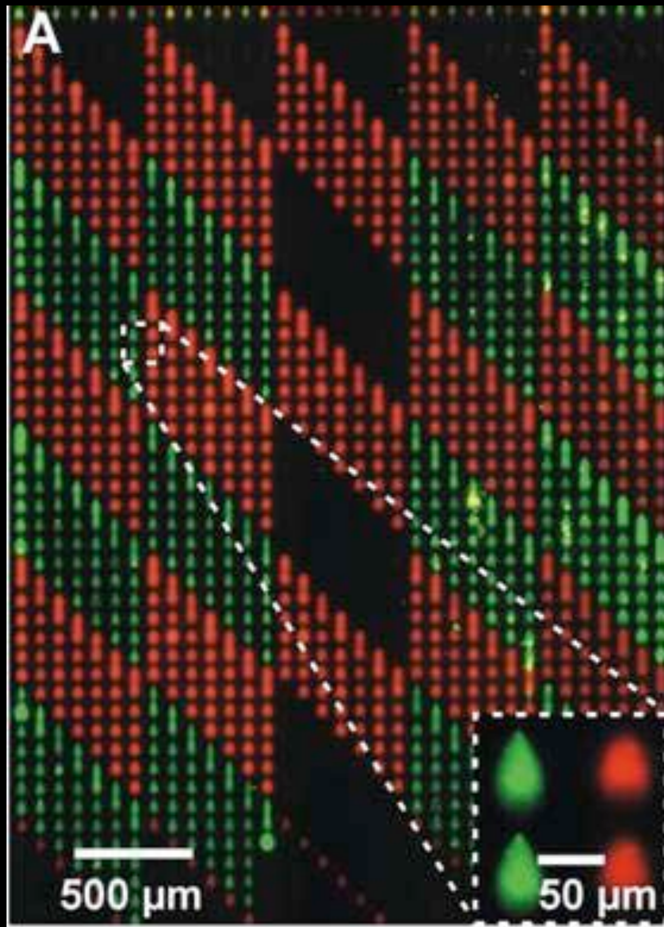
Patterning Proteins on a Surface using a MFP



Fluorescence microscope image

Juncker, D., Schmid, H., Delamarche, E.
Nature Materials **2005**, *4*, 622-628.

- 2 types of antibodies were subsequently patterned on an activated glass slide
- array has 1384 spots spaced 80 µm apart
- ~130 pL of antibody solution and 0.3 s writing time per spot
- array needed 300 nL of antibody solution and 15 min writing time

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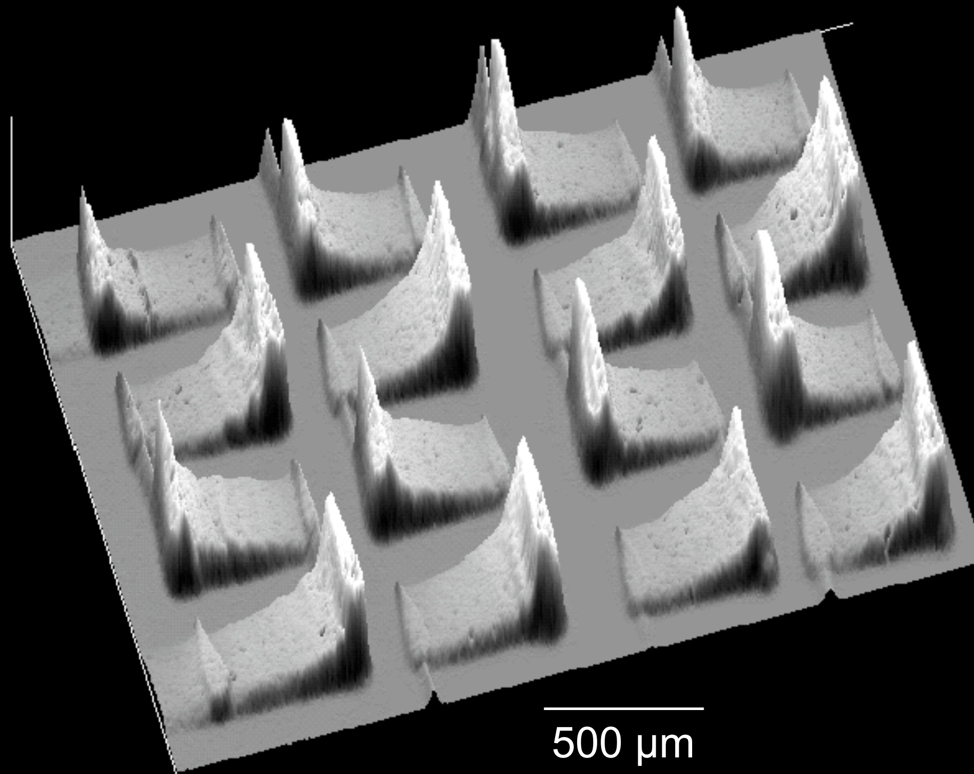
Making Surface-Density Gradients using a MFP



3D representation of a fluorescence microscope image

- surface-density gradients of proteins on a glass surface
- gradient was formed by varying the writing speed of the MFP

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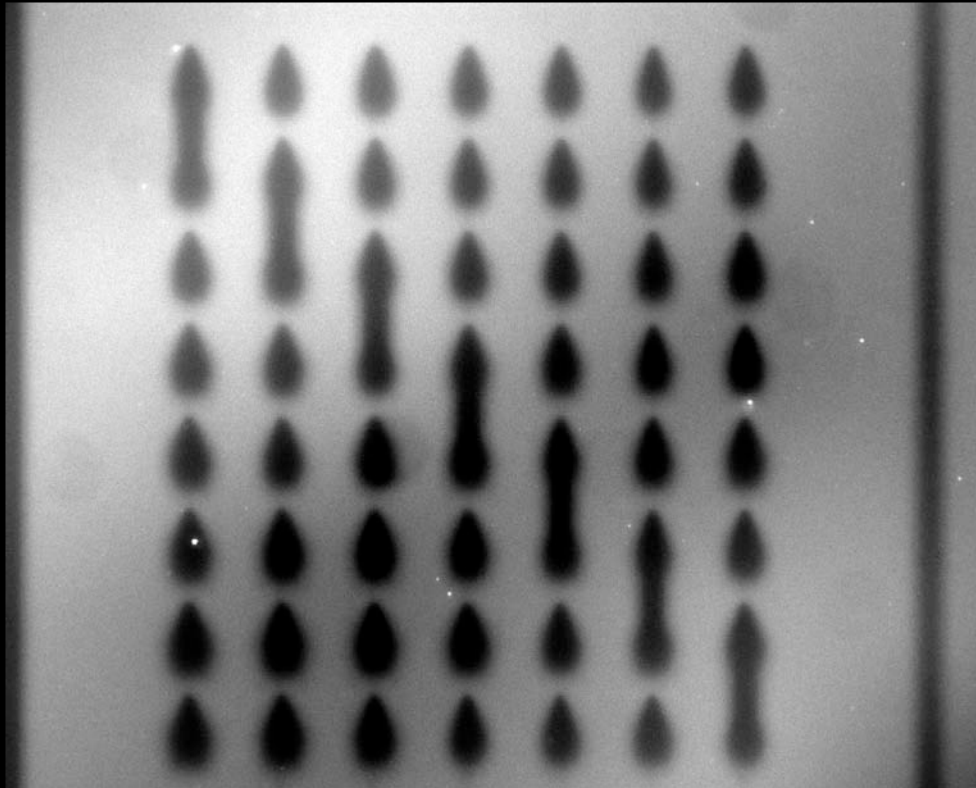
Erasing: Contact-Free Removal of Proteins



Fluorescence microscope image 100 µm

- the processing liquid contained a surfactant, a high pH and high ionic strength
- proteins adsorbed on a glass slide are removed by the processing liquid
- subtractive process

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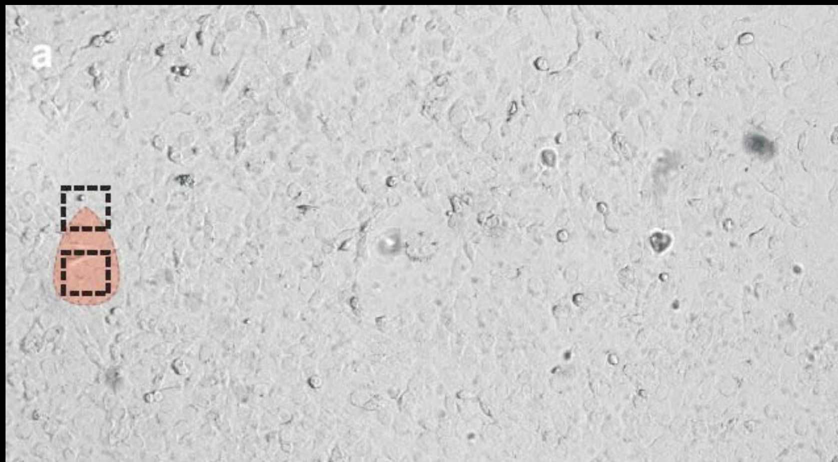
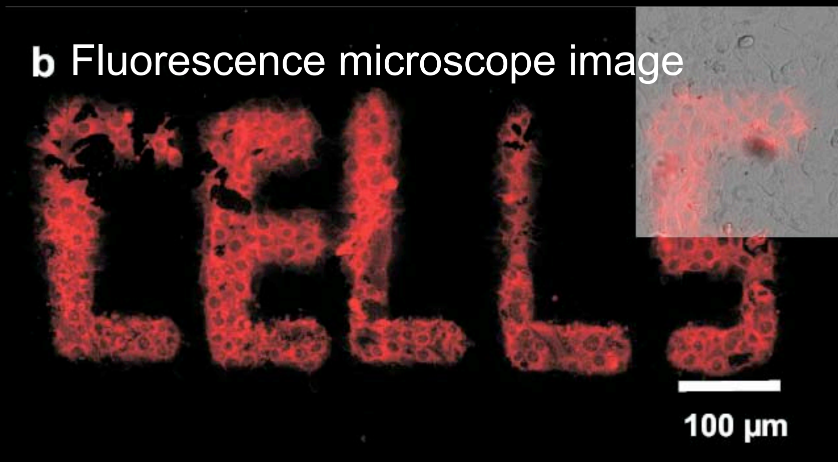
Local Staining of Cells

a Differential interference contrast image



b Fluorescence microscope image



- fibroblast cells fixed on a surface were selectively stained with carbocyanine dyes (red)
- staining done in 3 min
- could allow for local diagnostic and identification of cells

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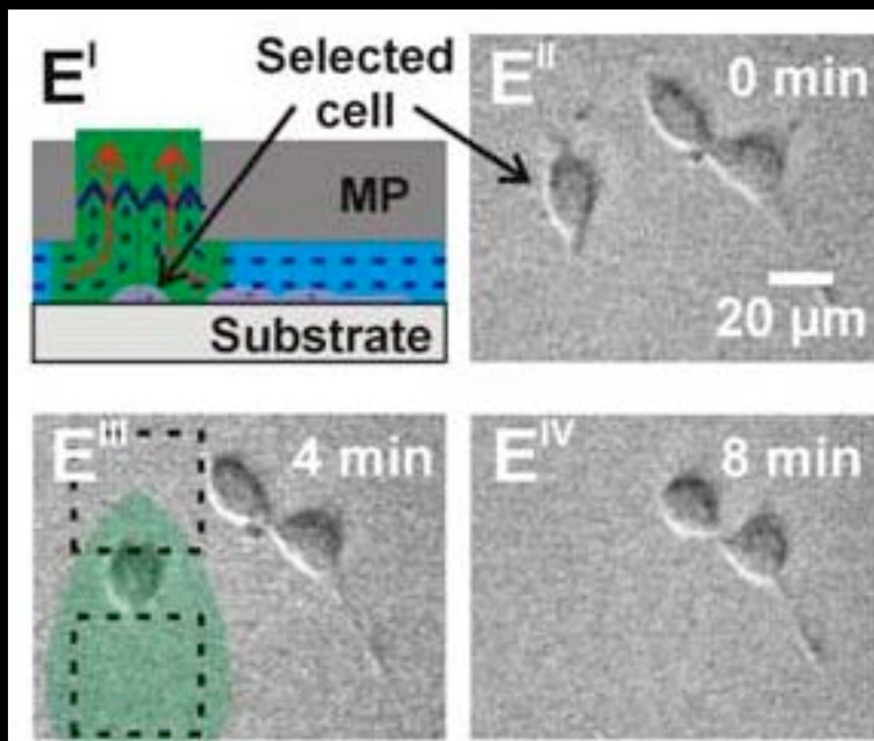
Contact-Free Selective Removal of a Living Cell



Differential interference contrast images

- MFP is placed over a selected fibroblast cell
- processing liquid contained trypsin, which degrades cell adhesion molecules from the fibroblast cell
- after 20 min, the fibroblast could not maintain its adhesion and was aspirated without affecting neighboring cells
- could allow for single cell handling and retrieval

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Agenda

Objectives of our work on experimental biosciences

Immunoassays and challenges

Benefits of miniaturization for bioassays

Some of our approaches for miniaturizing bioassays

Microfluidics & the microfluidic probe

Concluding remarks

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Concluding Remarks

- Micro/nanotechnologies are probably helpful for devising high-performance bio-analytical systems and discoveries in life sciences
- Miniaturization enhances the performances of bioanalytical devices
  - accurate patterning of biomolecular receptors for analytes
  - economy of samples and reagents
  - shorter time to results
  - portability
- Miniaturized bioanalytical platforms are often (too) complex
- A key challenge is to develop new, powerful bioanalytical that can easily be used by biologists/doctors

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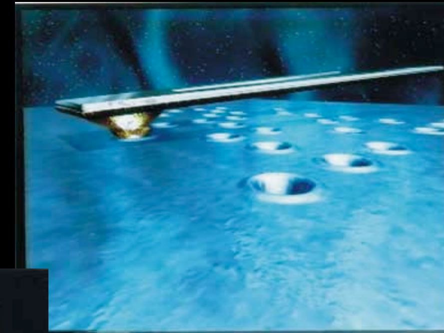

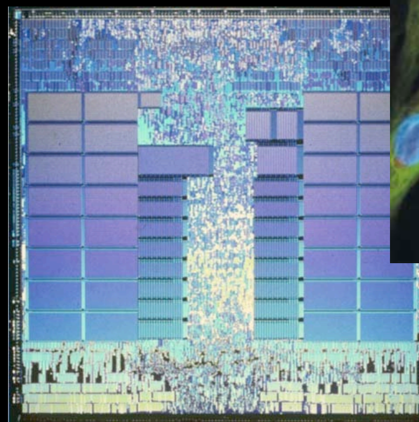

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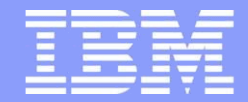

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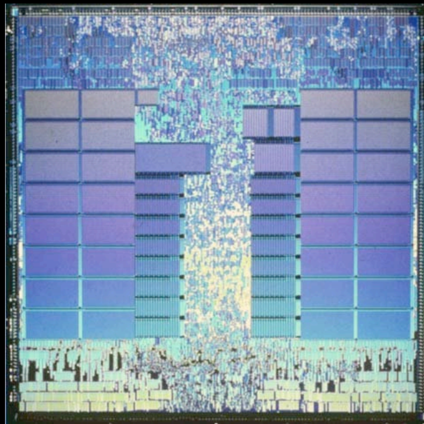

Thank you!