IBM Research - Zurich

Stamps and chips for tackling hard problems in biology and medicine

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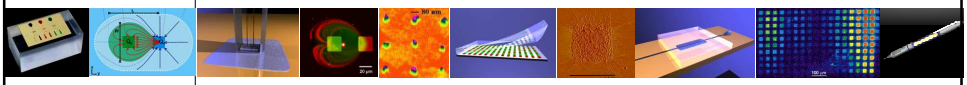
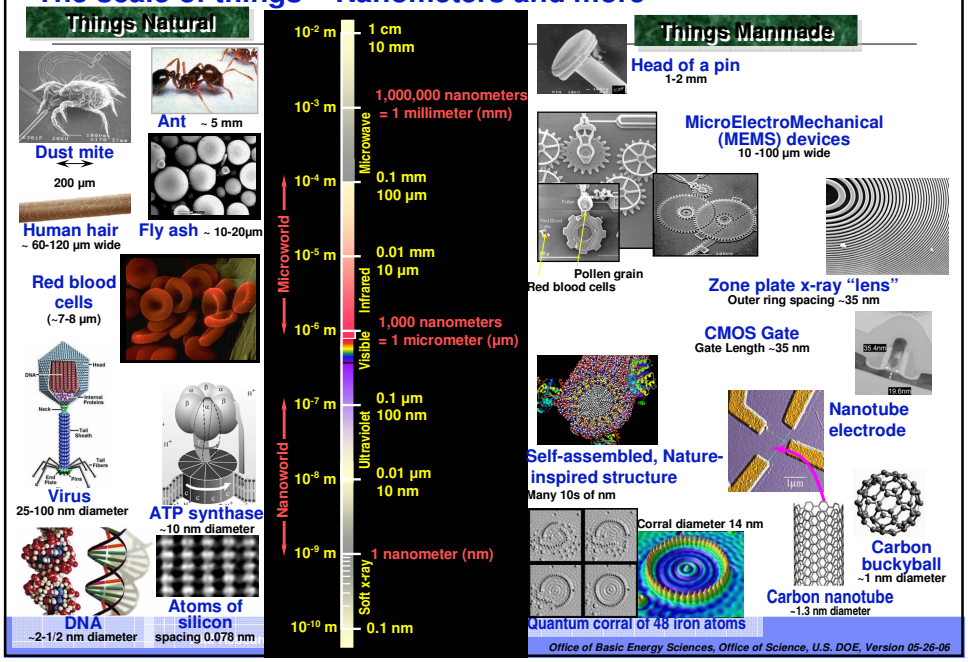
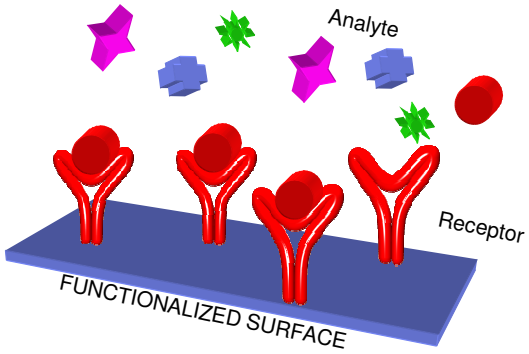


The scale of things – Nanometers and more



Discoveries using bioanalytical platforms

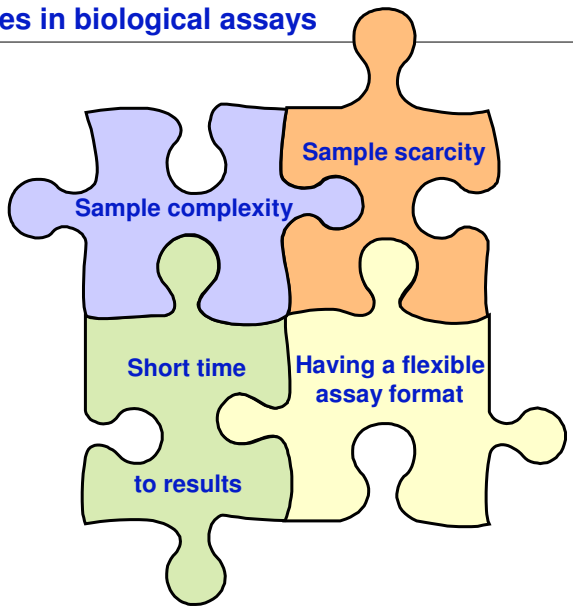


Transduction Principles

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

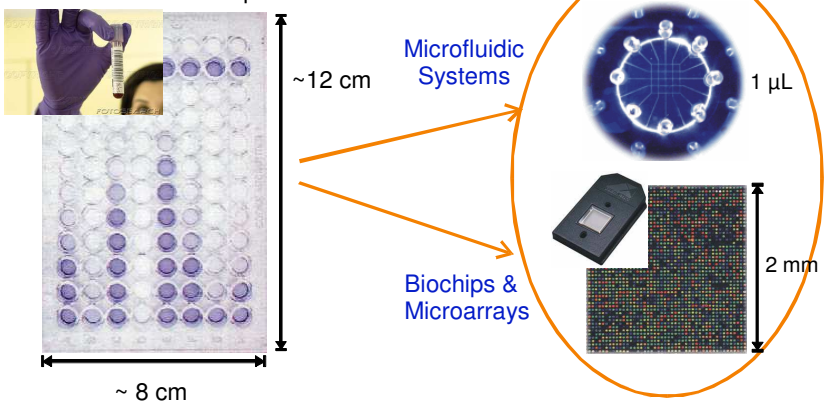
biological assays are broadly used for research in the life sciences, diagnostics, therapy monitoring, environment monitoring, and food safety.

4 challenges in biological assays



Miniaturization brings benefits to biological assays

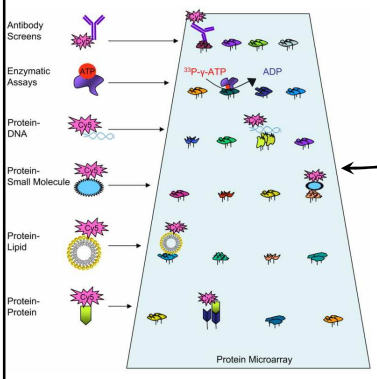
Test tubes / Microtiter plates



- (i) reduced sample consumption; (ii) faster reactions; (iii) parallelization
- high-throughput screening

Patterning surfaces by means of *microcontact printing*

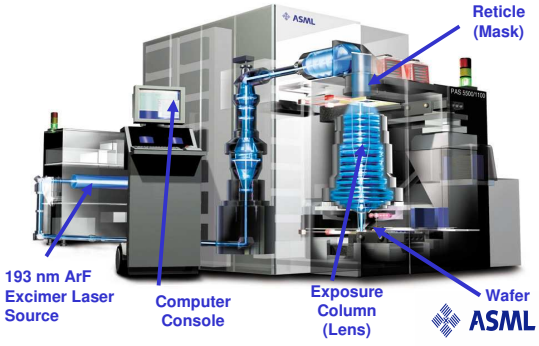
Why patterning proteins?

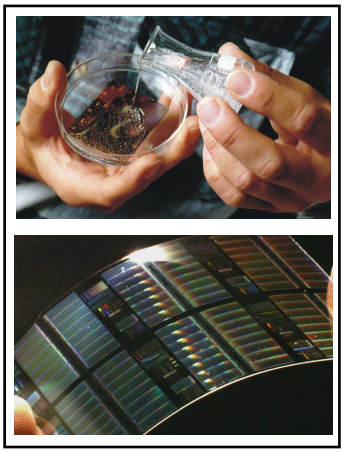


Snyder et al, *Mech. Ageing Dev.* **2007**, *128*, 161-167.

"CD"	"pitch"
10s nm / 100s nm	▪ to study single proteins on surfaces
10s nm / 1 µm	▪ to array elements of large libraries on surfaces for screening
10s nm / 1 µm	▪ to study protein-ligand interactions
µms	▪ to screen libraries for preclinical research
10s nm / 10s µm	▪ to study mechanisms of cellular adhesion
10s µm	▪ to pattern cells
10s µm	▪ to functionalize specific areas of sensors
10s µm	▪ for enabling miniaturized (immuno)assays
mm	▪ for cellular assays on cells and cell populations
cm	▪ to engineer tissues

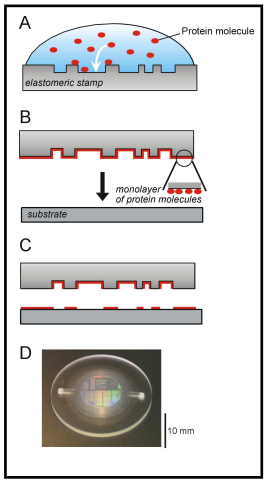
Lithography and microcontact printing



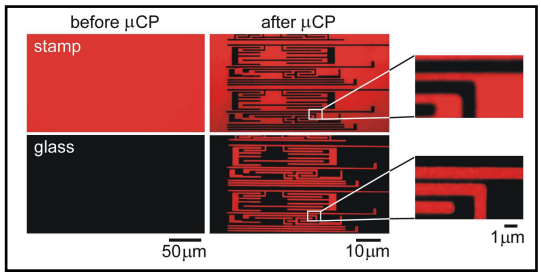


Kumar, A., Whitesides, G. M. *Appl. Phys. Lett.* **1993**, *63*, 2002-2004.

Microcontact printing proteins – Principles



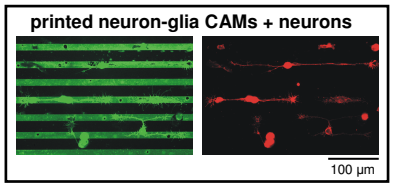
- an elastomeric stamp is covered with a solution of protein
- proteins spontaneously adsorb to the hydrophobic stamp
- the stamp is rinsed, dried and used to print the proteins
- proteins transfer from stamp to substrate due to adhesion forces
- simple and accurate method to pattern proteins

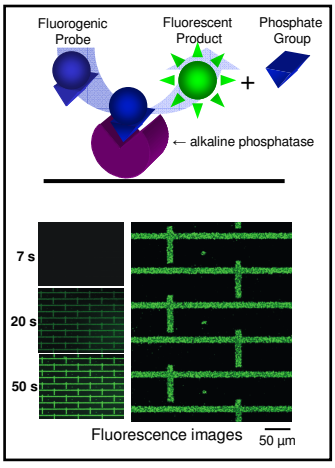


Bernard, A., Delamarche, E., Schmid, H., Michel, B., Bosshard, H. R., Biebuyck, H. *Langmuir* **1998**, *14*, 2225-2229.

Activity of microcontact printed proteins

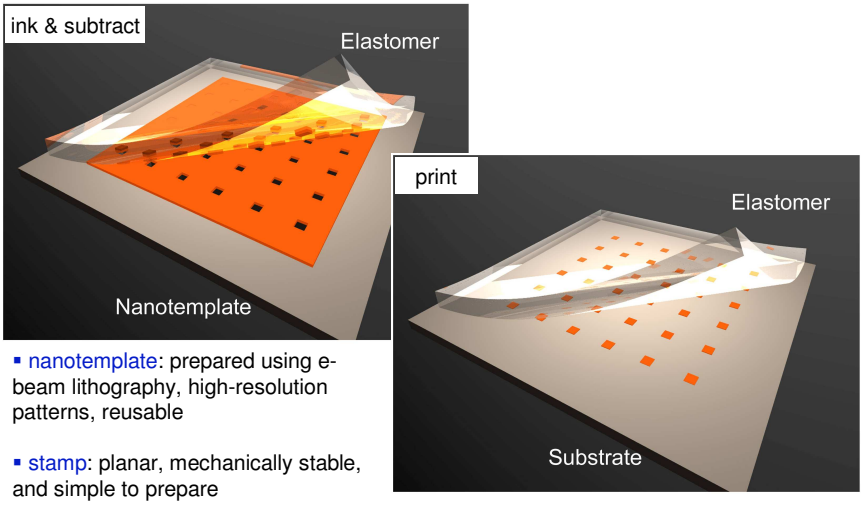
protein	measure/assay	rel. performance
polyclonal chicken IgG	immunostaining	100%
polyclonal rabbit IgG-TRITC	direct fluorescence	100%
monoclonal mouse IgG	immunostaining	90%
phosphatase (calf intestine)	p-nitrophenylphosphate (pNPP)	100%
cytochrome c (horse heart)	ellipsometry	100%
bovine serum albumin (BSA)	ellipsometry, ELISA-block	100%
streptavidin, avidin	biotin-phosphatase/ pNPP	80%
protein A (staphylococcus areus)	binding of TRITC-labelled polyclonal IgG	100%
proteinase K (tritirachium album)	ellipsom., protein as substrate / ninhydrin	~50%
peroxidase (horse radish)	H_2O_2, ABTS (chromogenic substrate)	60%
chymotrypsin (bovine pancreas)	N-benzoyl-L-tyrosinethylester/phenol red	70%
cell adhesion molecule (NgCAM)	cell attachment / cell growth	~70%





Bernard, A., Delamarche, E., Schmid, H., Michel, B., Bosshard, H. R., Biebuyck, H. *Langmuir* **1998**, *14*, 2225-2229.
Bernard, A., Renault, J.-P., Michel, B., Bosshard, H. R., Delamarche, E. *Adv. Mater.* **2000**, *12*, 1067-1070.
Bernard, A., Fitzli, D., Sonderegger, P., Delamarche, E., Michel, B., Bosshard, H. R., Biebuyck, H. A. *Nature Biotechnol.* **2001**, *19*, 866-869.

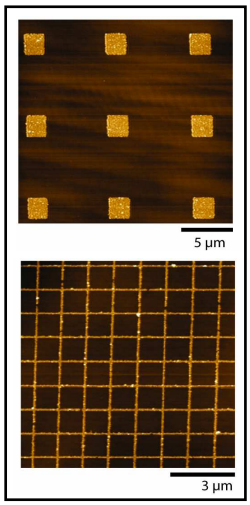
Inking, Subtraction, and Printing – ISP Method

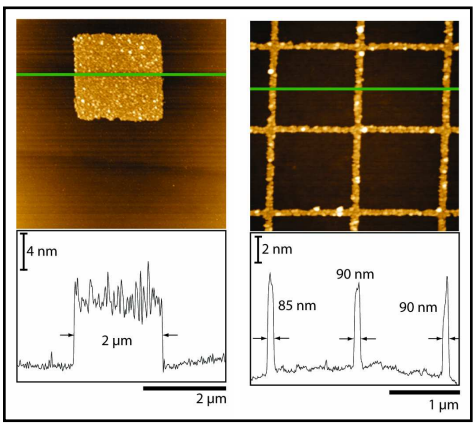


- nanotemplate: prepared using e-beam lithography, high-resolution patterns, reusable
- stamp: planar, mechanically stable, and simple to prepare

S. Coyer, A. Garcia, E. Delamarche, *Angew. Chem.*, **2007**, *46*, 6837.

AFM images of antibodies patterned onto silicon

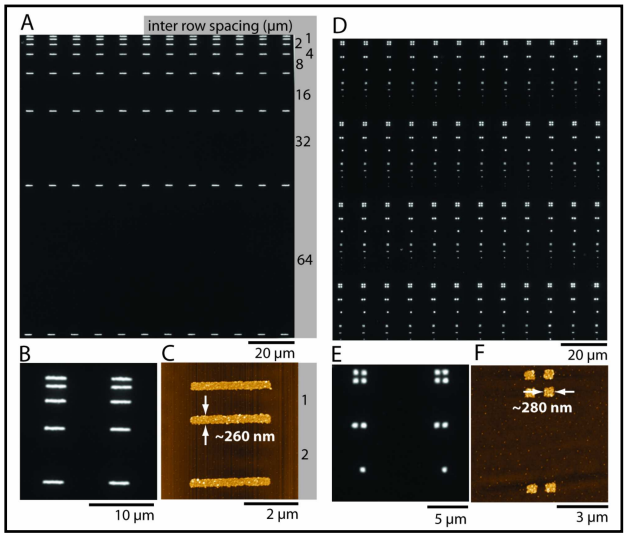




- Patterns repeated over large areas
- High contrast – limited background proteins
- High resolution – less than 100 nm

S. Coyer, A. Garcia, E. Delamarche, *Angew. Chem.*, **2007**, *46*, 6837.

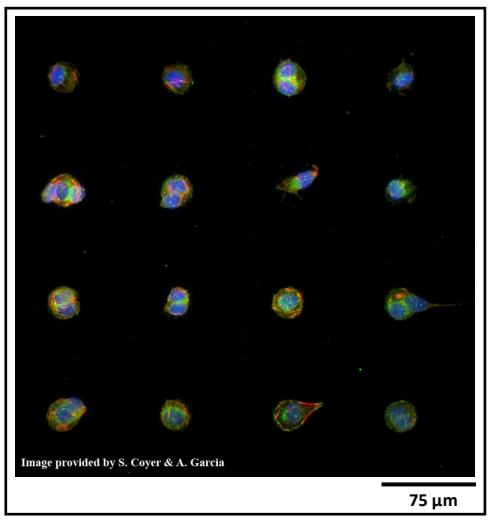
Protein patterns with large spacing and arbitrary patterns



- Spacing between features virtually unlimited
- Resolution from 500 nm to 50 nm?
- Various geometries
- A patterned stamp for microcontact printing would not be stable

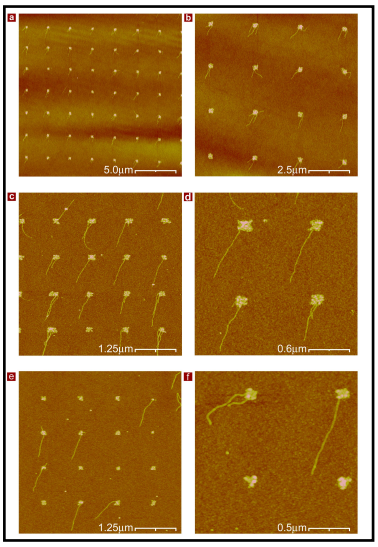
S. Coyer, A. Garcia, E. Delamarche, *Angew. Chem.*, **2007**, *46*, 6837.

Cell adhesion arrays on 10 µm islands

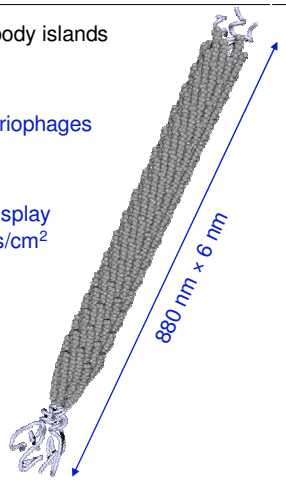


- Cell adhesion limited to printed protein pattern
- Uniform arrays of adherent cells achieved over entire sample
- Successful arrays produced with various patterns

Arrays of individual bacteriophages



- 90 × 90 nm^2 antibody islands on Si/SiO_2
- Single M13 bacteriophages bound to islands
- Arraying phage display library at 10^8 virus/cm^2



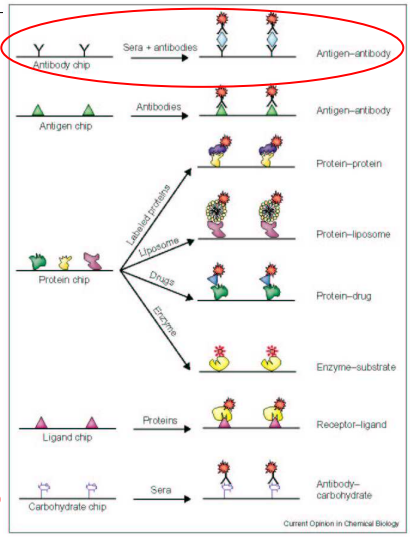
M13 Bacteriophage Virus

D. Solis, S. Coyer, A. Garcia, E. Delamarche, *Adv. Mater.*, **2009**, *22*, 111.

Microfluidics for immunoassays

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 molecules are attached to the detection antibodies



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

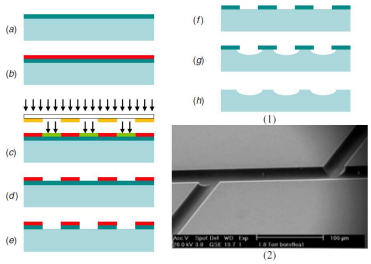
Microfluidics

- "*Microfluidics*" stands as a summation of the extraordinary properties that liquids reveal when confined within microscale boundaries, and of the use of these properties in miniaturized, microfluidic systems.
  - laminar flow. no turbulences, mixing is diffusion-limited
    when Re < 1, the flow is laminar (nearly always the case in microfluidics)
    $$\frac{f_i}{f_v} = \frac{\rho U L}{\eta} \equiv \mathrm{Re}$$
  - small volumes. 1 nL = 100 × 100 × 100 μm^3 but surface/volume ↑
    1 pL = 10 × 10 × 10 μm^3
  - small diffusion times / fast processes. TNF-α diffuses along 10 μm in ~1 s
    urea diffuses along 10 μm in ~72 ms

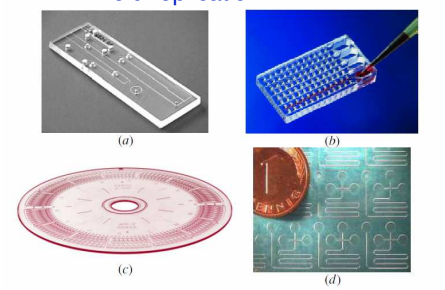
T. M. Squires, S. R. Quake, *Rev. Modern. Physics,* **2005**, *77*, 977-1026.

Fabrication of microfluidic devices

microfabrication



mold replication



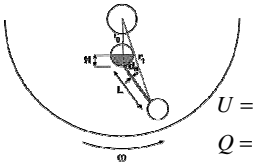
- materials: mostly silicon/glass/metals
- techniques: photolithography, etching, metal deposition
- fast and accurate (<1 µm)
- expensive, cleanroom

- materials: plastics/elastomers
- techniques: mold injection, hot embossing, soft lithography
- fast and relatively accurate (~2 µm)
- mass production, cheap

see e.g. P. Abgrall, A. M. Gué, *J. Micromech. Microeng.*, **2007**, *17*, R15-R49.

Microfluidic elements – moving liquids

centrifugation-driven microfluidics

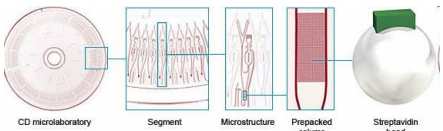


$$U = d_H^2 \rho \omega \bar{r} \Delta r / 32 \eta L$$

$$Q = AU = A d_H^2 \rho \omega \bar{r} \Delta r / 32 \eta L$$

$$(d_H = 4A / P)$$

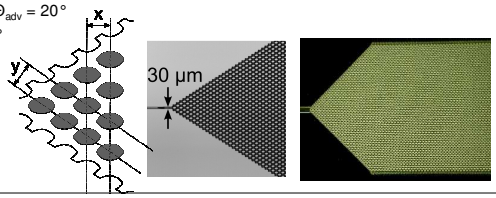
Duffy, Kellogg et al. *Anal. Chem.*, **1999**, *71*, 4669-4678.



(Gyros)

capillary-driven microfluidics

- contact angles of microfluidic chip with human serum:
  3 walls (chemically-treated Si surface) Θ_{adv} = 20°
  1 wall (sealing PDMS layer) Θ_{adv} = 116°
  smallest flow rates: ~1.8 nL/s

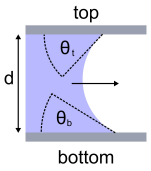


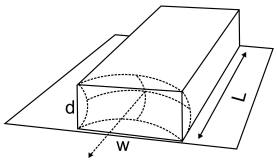
Capillary pressure in microstructures

- The capillary pressure P_c of a liquid-air meniscus in a rectangular microchannel is:

$$P_c = -\gamma\left(\frac{\cos\alpha_b + \cos\alpha_t}{d} + \frac{\cos\alpha_l + \cos\alpha_r}{w}\right)$$

  - Each wettable wall ($\alpha < 90°$) contributes to generating a negative pressure and to filling
  - Small structures generate larger pressures (in absolute values)





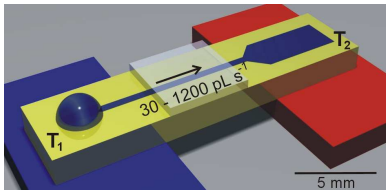
Flow rate resistance

$$R_{FR} = \left[\frac{1}{12}\left(1 + \frac{5a}{6b}\right)\frac{AR_H^2}{L}\right]^{-1} \qquad R_H = \frac{2A}{P}$$

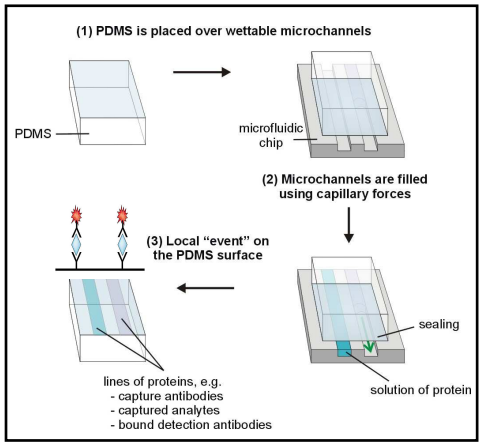
a and b: width or depth, $b > a$

- The mass flow rate in the microchannels is $D = \frac{1}{\eta}\frac{\Delta P}{R_{FR}}$
  - Flow is proportional to the capillary pressure but decreases with the viscosity of the liquid and is affected by the friction of small channels

Capillary-driven microfluidics for surface immunoassays

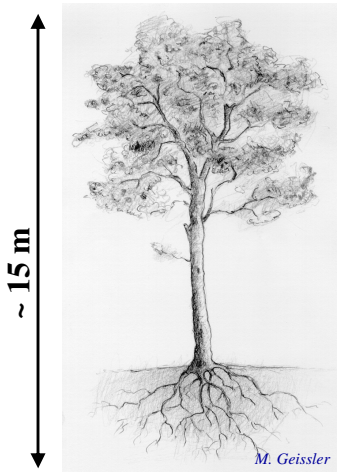


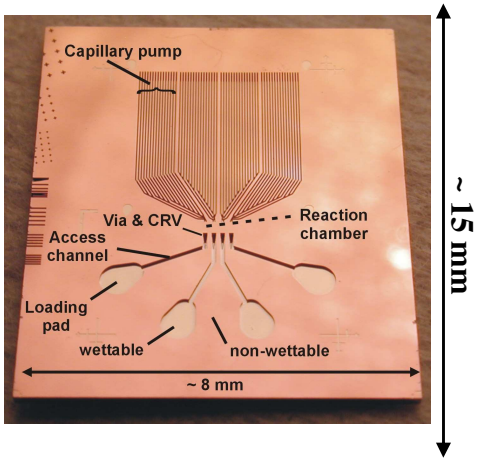
- solid support for assay: PDMS block
- PDMS ensures efficient, reversible sealing of independent flow paths
- solutions needed for an assay are sequentially flushed through microchannels



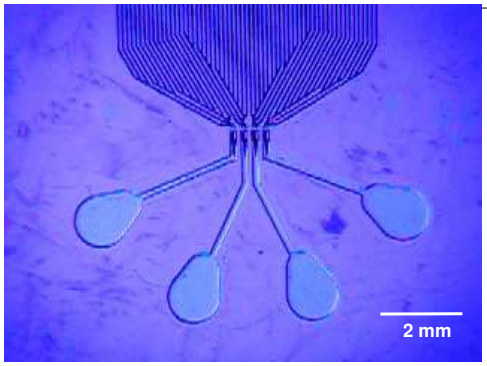
Delamarche et al. *Science* **1997**, *276*, 779-781
Delamarche et al. *J. Am. Chem. Soc.* **1998** *120*, 500-508.

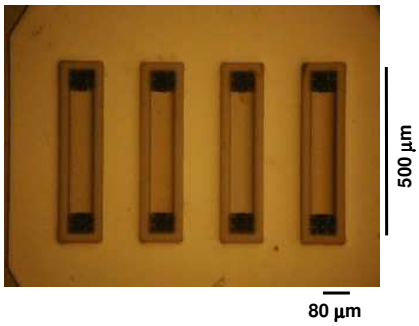
Capillary-driven Microfluidics





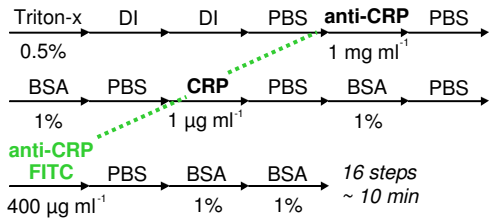
Filling a microfluidic chip with 4 independent flow zones

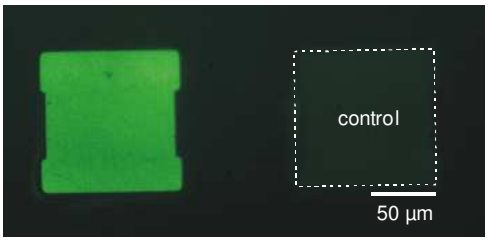




- ~150-nL-aliquots are sequentially added to loading ports
- the aliquots spontaneously flow from the loading ports to the capillary pumps
- Reaction chamber is 15 pL in volume, flow rate is ~ 220 nL s^{-1}

Fluorescence immunoassay for CRP





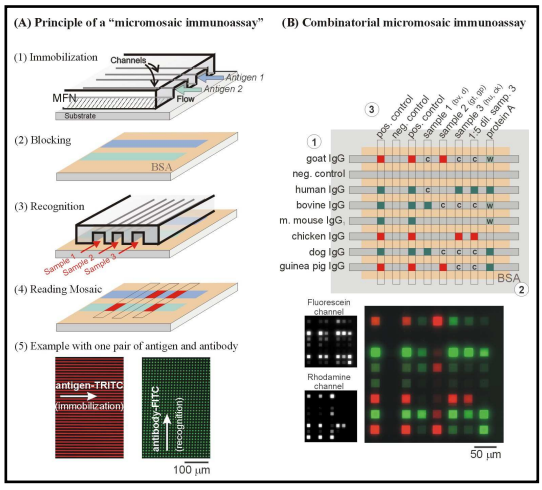
Fluorescence image, positive result

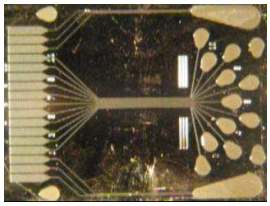
Negative control

- 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.

Micromosaic immunoassays





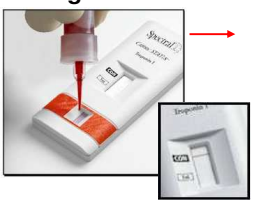
300-nL-aliquots are added to each of the 11 loading ports
(video: courtesy of G. Dernick and C. Fattinger, Hoffmann-La Roche)

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.

Toward portable microfluidic diagnostics

- There is a gap between accurate clinical analyzers and fast and portable
- diagnostics

non-quantitative point-of care diagnostics



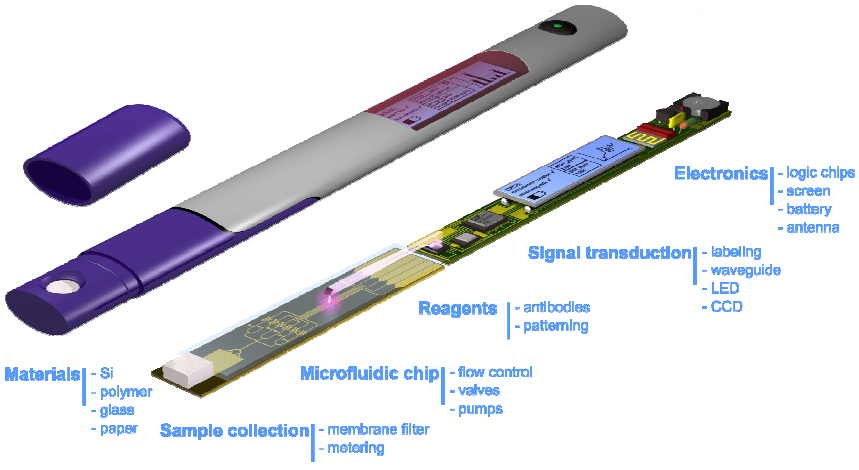
"ideal tech"

Quantitative clinical analysis

Cardiac STATus™ point-of-care test
- disposable
- yes/no answer within 15 min
- 150 µL minimum volume

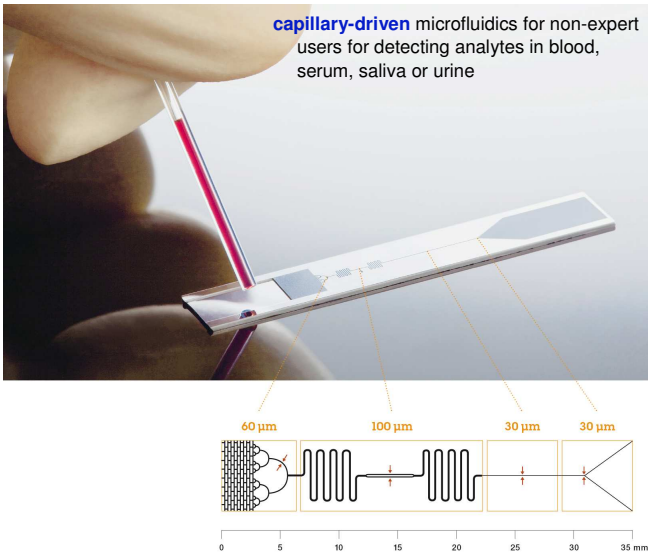
Roche Cobas e 601 module
- 425 to 1000 kg, up to 4 m long
- accurate concentration measured within 30 min?
- 50 µL minimal volume
- 155 assays for anemia, bone, cancer, hormones and infectious diseases

"Ideal" microfluidic diagnostic chip

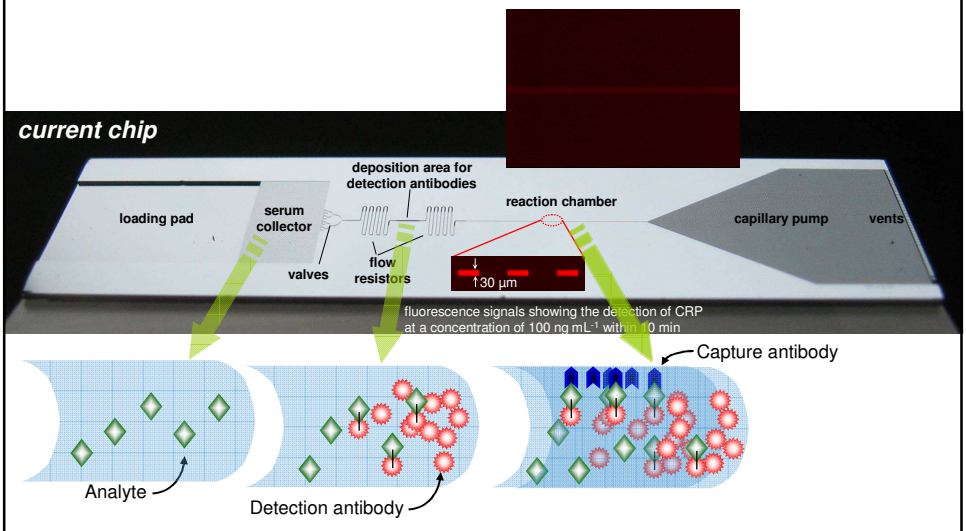


Gervais, L. and Delamarche, E. *Adv. Mater.*, manuscript under preparation.

Concept and example of diagnostic chip



"Chips for Life" – Microfluidics for point-of-care diagnostics

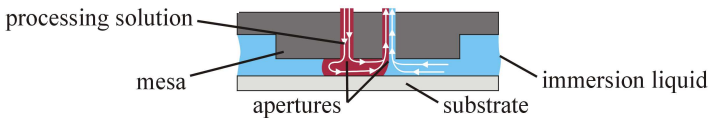


Gervais, L. and Delamarche, E. *Lab Chip*, **2009**, *9*, 3330.

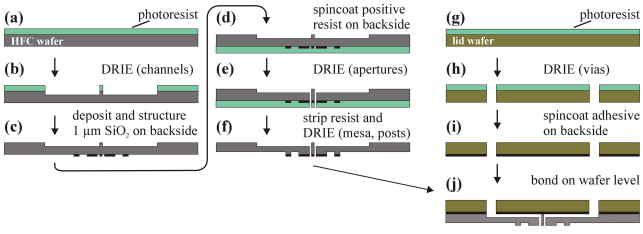
The Microfluidic Probe (MFP)

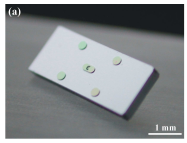
- *from closed to open microfluidics*
- *from contact to non-contact*

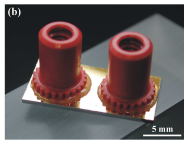
Principle of the MFP



Fabrication and packaging

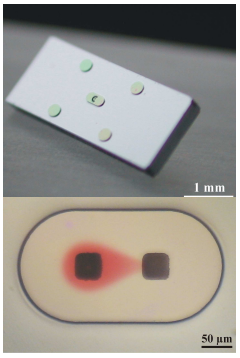




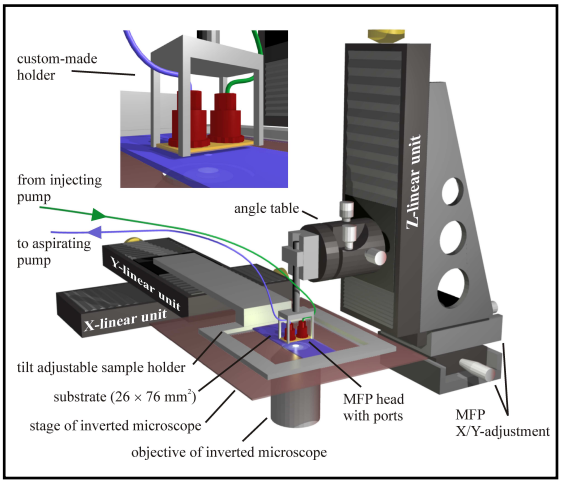


Juncker, D., Schmid, H., Delamarche, E. *Nature Materials* **2005**, *4*, 622-628.

“The Microfluidic Probe” – Concept

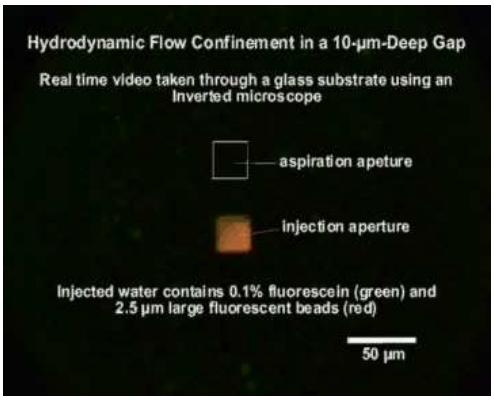


Microfluidic probe head (top) and liquid focused over a transparent surface (bottom)



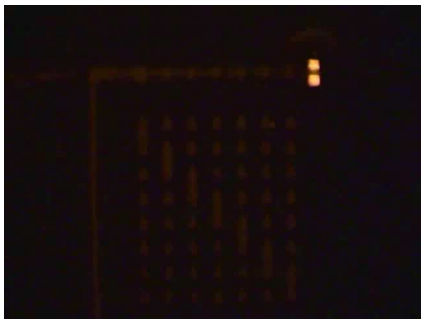
Juncker, D., Schmid, H., Delamarche, E. *Nature Materials* **2005**, *4*, 622-628.
Lovchik, R., Drechsler, U., Delamarche, E. *J. Micromech. Microeng.* **2009**, *19*, 115006.

Video showing the confinement as a function of Qa/Qi



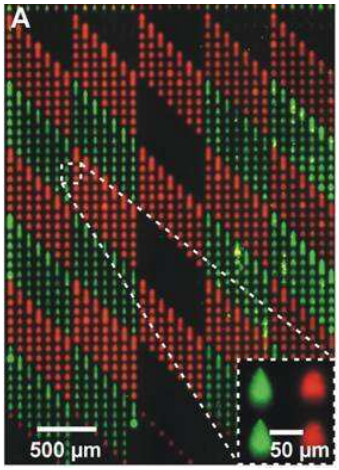
Writing using a MFP

- 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!

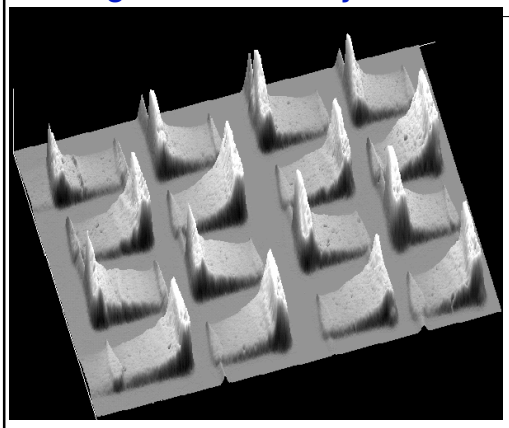
Patterning Proteins on a Surface using a MFP



Fluorescence microscope image

- 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

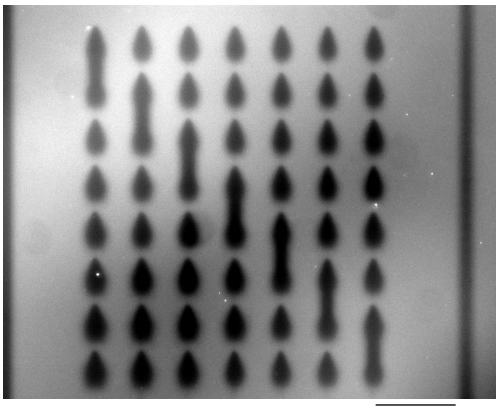
Making Surface-Density Gradients using a MFP



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

3D representation of a fluorescence microscope image

Erasing: Contact-Free Removal of Proteins

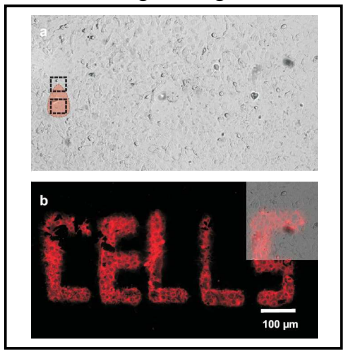


Fluorescence microscope image 100 µm

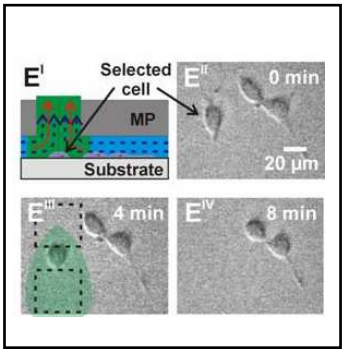
- the processing liquid contained a surfactant, a high pH and high ionic strength
- proteins adsorbed on a glass slide are removed by the processing liquid
- subtractive process

“The Microfluidic Probe” – Staining cells & picking cells

staining of living cells



selective removal of an adherent cell



IBM Research - Zurich

Thank you!