Microfluidic technologies and their applications in cell biology

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VISION

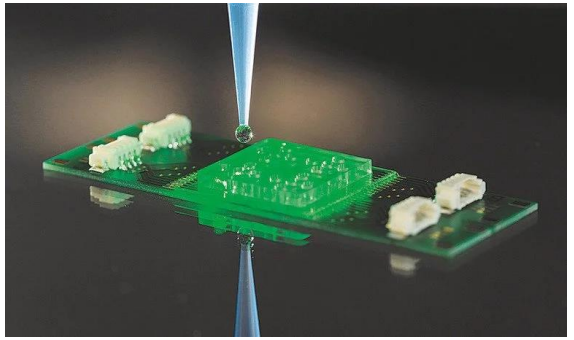
STRATEGIES TO STRENGHTEN SCIENTIFIC EXCELLENCE AND INNOVATION CAPACITY FOR EARLY DIAGNOSIS OF GASTROINTESTINAL CANCERS

Outline

- Basics of microfluidics
- Design and fabrication of microfluidic modules
  - Materials
  - Fabrication technologies
- System integration
- Examples of biological application areas and technological solutions @ IBMT

Microfluidics

- Since the 1980's
- Multidisciplinary field – physics, engineering, chemistry, nanotechnology,
- Behaviour, precise control, and manipulation of fluids that are geometrically constrained to a small scale (typically sub-millimeter)
- Transport, mixing, separation, …
- Applications: inkjet printheads, DNA chips, lab-on-chip, cellular biophysics, …
- *"In the same way that integrated circuits allowed for the miniaturization of computers from the size of a room to the size of a notebook, miniaturization has the potential to shrink a room full of instruments into a compact lab-on-a-chip." (Figeys and Pinto, 2000)*
- "Micro" features:
  - Small volumes (µL, nL, pL, fL)
  - Small size
  - Low energy consumption
  - Microdomain effects



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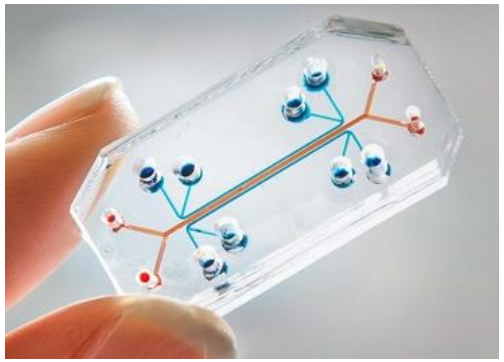
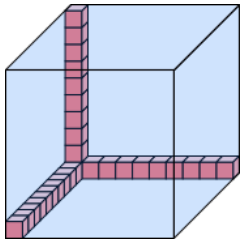
https://www.powerelectronicsnews.com/driving-piezoelectric-actuators-in-industrial-inkjet-printers/
https://medizin-und-technik.industrie.de/technik/forschung/lab-on-a-chip-diagnostik/

Basics: macrofluidics vs. microfluidics



Influencing factors and effects	Macro	Micro
Intertia force	X	
Friction forces (viscosity)		X
Weight force	X	
Capillary force		X
Turbulent flow	X	
Laminar flow		X
Diffusion		X
Boundary surface effects		X
Surface-to-volume ratio		X



Edge length	Surface / volume
1 km	0.006 m^{-1}
1 m	6 m^{-1}
1 mm	6,000 m^{-1}
1 µm	$6*10^6$ m^{-1}

→ 1 µl = 1 mm^3 → Droplet: ~ 50 µl

https://lillagreen.at/wp-content/uploads/2017/12/Danube-Bratislava.jpg
http://www.elveflow.com/wp-content/uploads/2014/08/organs-on-a-chip-microfluidics.jpg
http://blog.timeforslovakia.com/wp-content/uploads/2015/09/11082542_1610982419134738_4653258437494370439_n.jpg
http://www.wisegeek.org/what-is-a-capillary-tube.htm#capillary-tube-to-collect-blood-sample
https://experiencelife.lifetime.life/article/a-drop-of-blood-can-reveal-every-virus-youve-ever-had/

Basics: turbulent flow vs. laminar flow

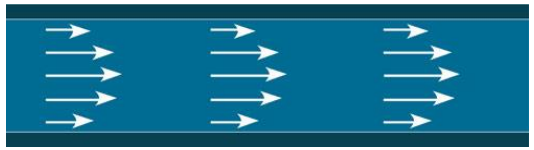
Turbulent flow

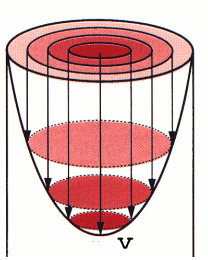
- Inertia >> inner friction
- Flow profile depends on wall surface
- Random, non-stationary movement, turbulences
- Flow resistance:

$$F = \frac{1}{2}\rho A v^2$$

F: flow resistance
ρ: density
A: cross section
v: velocity

Laminar flow





- Inner friction >> Inertia
- Parabolic flow profile
- No time dependency, stationary movement
- Flow resistance (Stoke's law)

$$F = 6\pi\eta v r$$

F: flow resistance
η: dynamic viscosity
v: velocity
r: particle radius

http://www.mobilehydraulictips.com/wp-content/uploads/2015/07/2110_laminar-and-turbulent.jpg
http://arstechnica.com/science/2008/11/is-turbulence-a-chaotic-repeller-or-attracter/
http://web.physik.rwth-aachen.de/~hebbeker/lectures/ph1_0102/tubevisc.gif

Basics: viscosity

Viscosity: measure of a fluid's resistance to deformation at a given rate.

Newton's equation: $$\eta = \frac{F/A_0}{dv_x/dy}$$

$$[\eta] = \frac{N * s}{m^2} = Pa * s$$

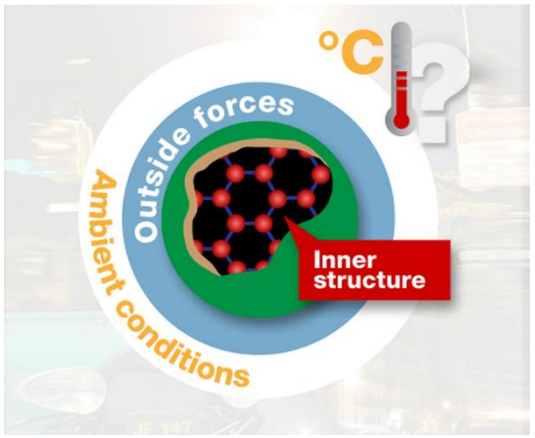
$$\frac{F}{A_0} = \eta \frac{dv_x}{dy}$$

shear rate

shear stress

viscosity

- Newtonian fluids: viscosity is independent of the external force (e.g. water, solvents, salad oils)
- Non-Newtonian fluids: viscosity depends on external force (e.g. ketchup, blood)



https://wiki.anton-paar.com/ch-de/grundlagen-der-viskositaetsmessung/#viscosity-and-viscometry

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Basics: Hagen-Poiseuille's law

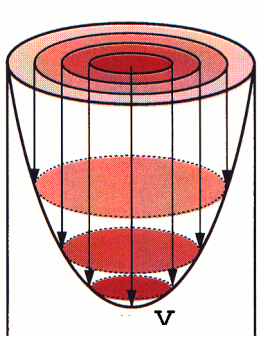
- Pressure gradient in capillary (microchannel)→ laminar flow
- Laminar flow of a homogeneous Newtonian fluid through a tube
- *No-slip condition*: flow velocity at the wall-fluid-interface is zero → adhesion of fluid molecules at the edge layers

$$Q = \frac{\Delta V}{t} = \frac{\pi R^4}{8\eta L}\Delta p$$

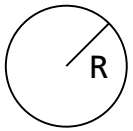
Fluidic resistance

Q: volumetric flow rate
V: volume
t: time

R: radius of capillary
L: length of capillary
η: viscosity
Δp: pressure difference

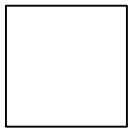


- Volumetric flow rate strongly depends on the diameter of the capillary: $V \sim \Delta p R^4$
- Example: blood vessels



$$\frac{8\eta L}{\pi R^4}$$

$$\frac{12\eta L}{bh^3}$$



$$\frac{28{,}454\eta L}{a^4}$$

Q: http://web.physik.rwth-aachen.de/~hebbeker/lectures/ph1_0102/tubevisc.gif

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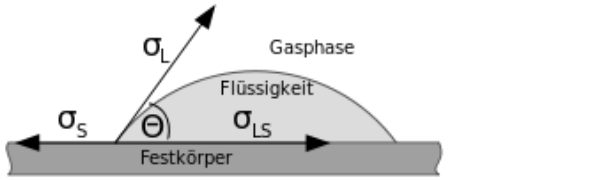
Basics: interface effects

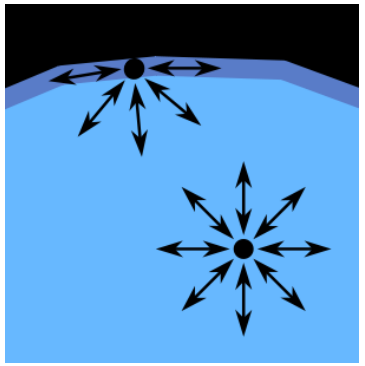
Surface tension and capillary effect

- Interaction of molecules with each other (cohesion)
- Striving for minimum surface area
- Liquid on solid surface: adhesion force
- Adhesion > cohesion → wetting
- Contact angle: measure of the relative strength of cohesion and adhesion force

- Young's equation: $$cos\theta = \frac{\sigma_S - \sigma_{LS}}{\sigma_L}$$



σ_L = Oberflächenspannung der Flüssigkeit
σ_S = Oberflächenenergie des Festkörpers
σ_{LS} = Grenzflächenenergie zwischen Flüssigkeit und Festkörper
Θ = Kontaktwinkel



- $\theta < 90°$ → hydrophilic surface
- $\theta > 90°$ → hydrophobic surface

http://www.wissenslogs.de/wblogs/gallery/51/previews-med/oberflaechenspannung.png
http://www.rheinfaktor.de/uploads/tx_mmdamfilelist/altprev_Essilor_CrizalA2_02.jpg
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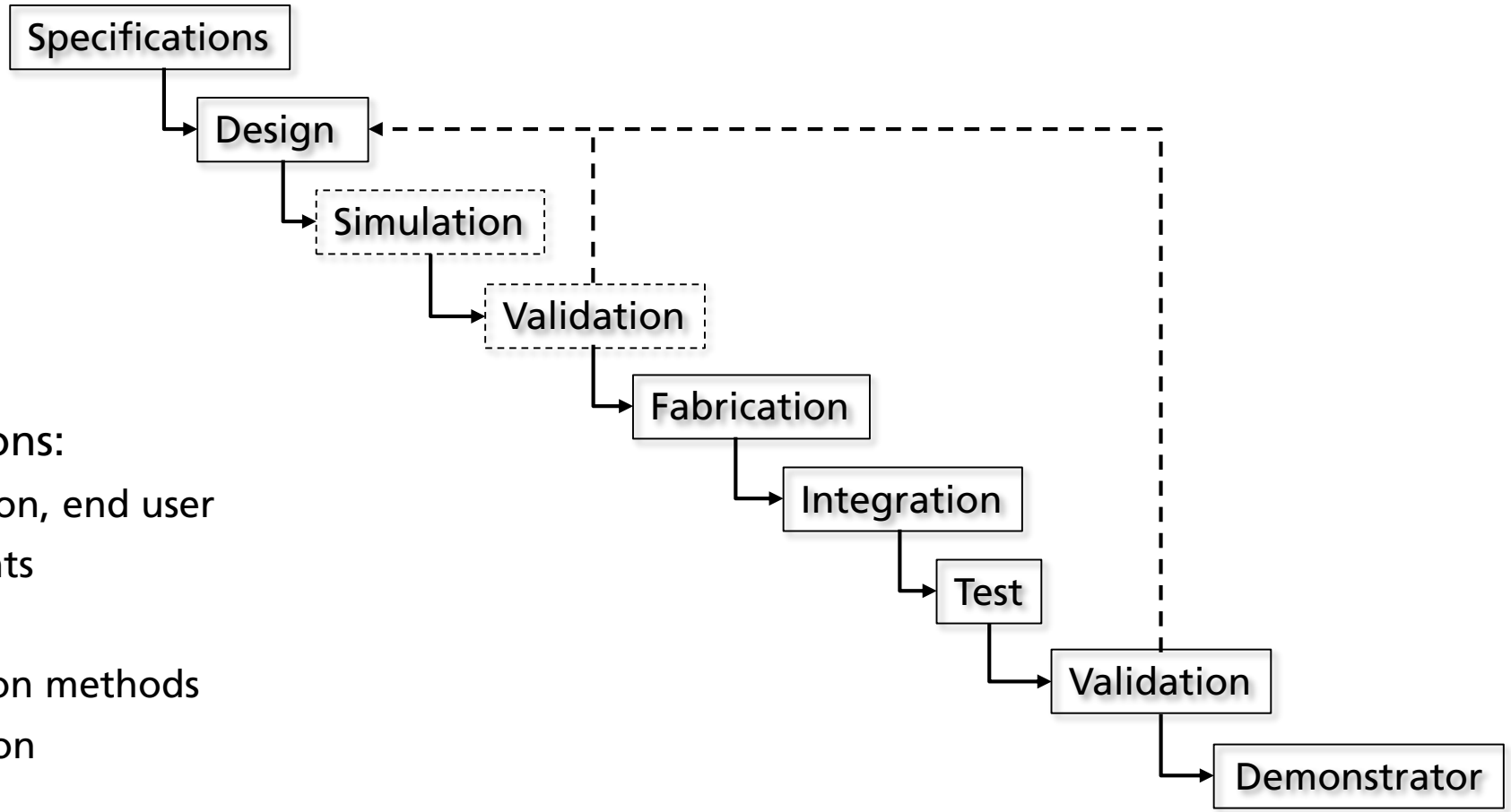
http://www.tamar.co.il/wp-tamar_content/uploads/2014/02/Capillary-Blood-Collection1.jpg

Design and fabrication of microfluidic components and systems



Specifications:

- Application, end user
- Constraints
- Materials
- Fabrication methods
- Integration

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Materials for microfluidic components and systems

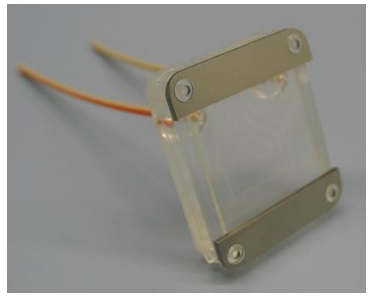
Tab. 9.2 Comparison of materials commonly used in microtechnology.

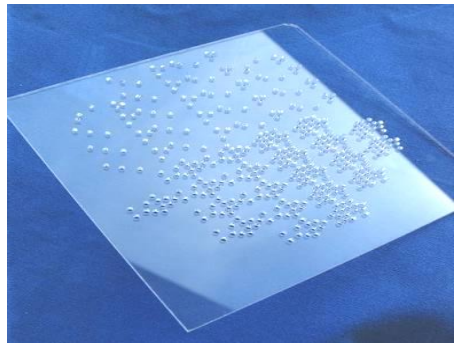
Material		*Cost*	*Frac-ture*	*Metal-lization*	*Machin-ability (common methods)*	*Dielectric constant*	*Young's Modulus E(GPa)*	*Thermal Conduc-tivity ($W\,mK^{-1}$)*
Single crystal	Si	$$$$	b,s	Good	Very good	11.8	165	150
	Quartz	$$$$	b,s	Good	Poor	4.4	87	7
	GaAs	$$$$$	b,f	Good	Poor	13.1	119	50
	Sapphire	$$$$$	b,s	Good	Poor	9.4	490	40
Amor-phous	Fused silica	$$$–$$$$$	b,f	Good	Poor	3.9	72	1.4
	Plastic	$$	T,s	Poor	Good	–	–	–
	Paper/ cardboard	$$	T,s	Poor	Fair	–	–	–
	Glass	$$–$$$$	b,f	Good	Poor	4.6	64	1.1
Polycrys-talline	Alumina	$–$$$$	b,s	Fair	Poor	9.4	400	~30
	Alumi-num	$$$	t,s	Good	Very good	–	77	~240

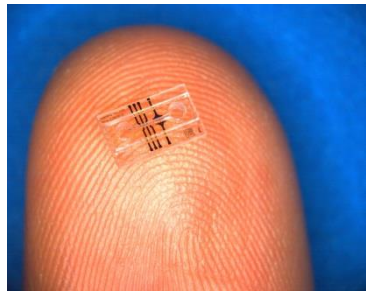
Note: b=brittle, t= tough, s=strong, f=fragile, $=very cheap, $$$$=very expensive.

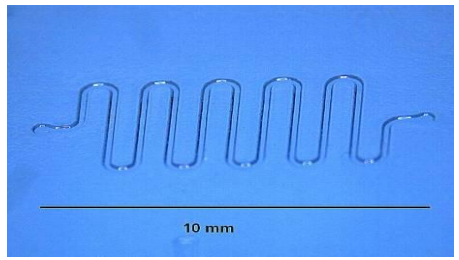
Materials used @ IBMT

- Silicon
- Glass
- PMMA
- Polymer films (PC, COC, PET)
- Photoresist
- PDMS elastomers









Geschke, Klank, Telleman, Microsystem Engineering of Lab-on-an-Chip Devices, S. 208

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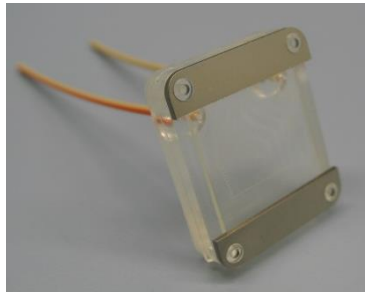
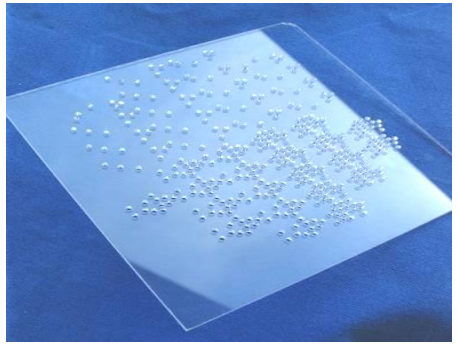
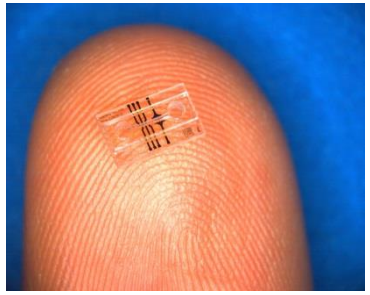
Fabrication methods for microfluidic components and systems

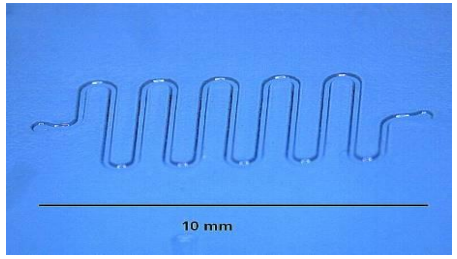
Criteria:

- Geometry (e.g. channels, reservoirs), feature size
- Materials (compatibility with different media, cytotoxicity)
- Handling, usability
- Single use or multiple use
- Numbers of pieces
- Fabrication costs



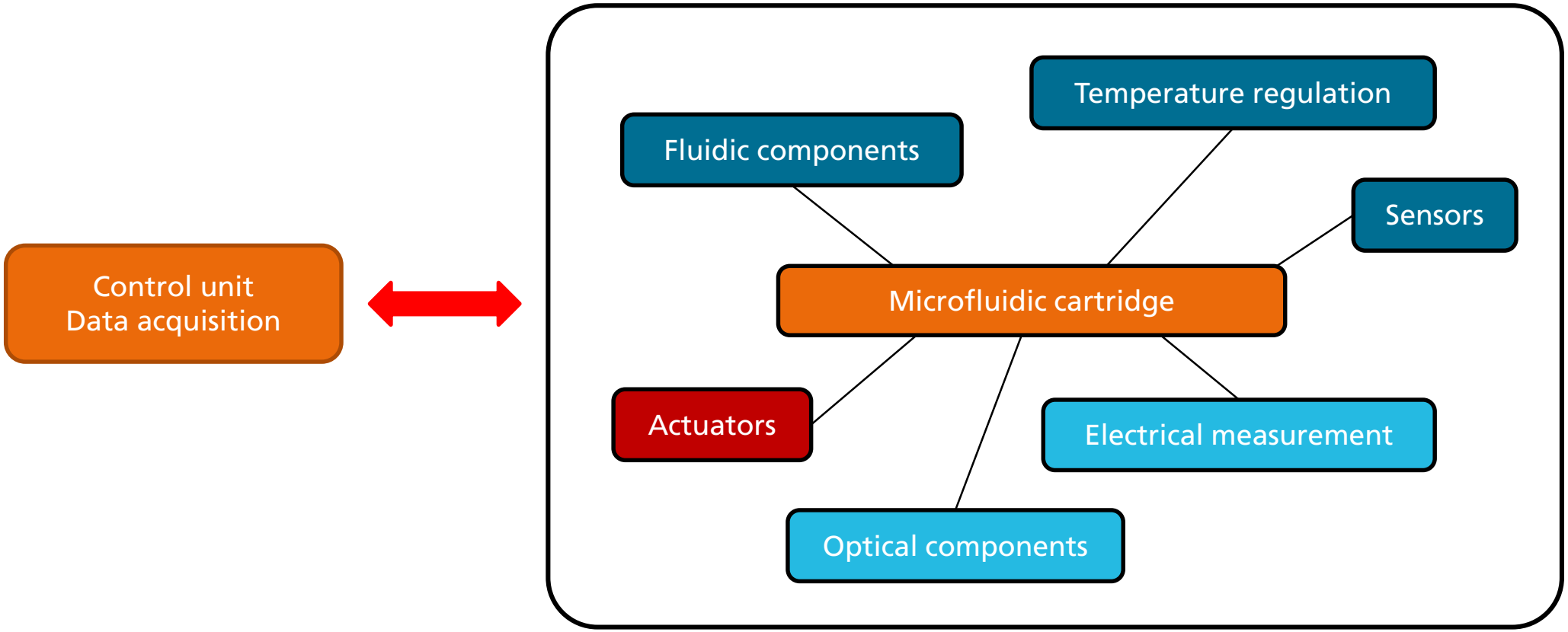






Material	Fabrication method
Silicon, glass	Lithography
	Metallisation (sputtering)
	Etching (wet and dry)
Polymers	Lithography (photoresists)
	Replication techniques (PDMS moulding, injection moulding)
	Printing, embossing
	Die cutting (e.g. adhesive tapes)
	CNC-milling
	Laser structuring
	3D printing

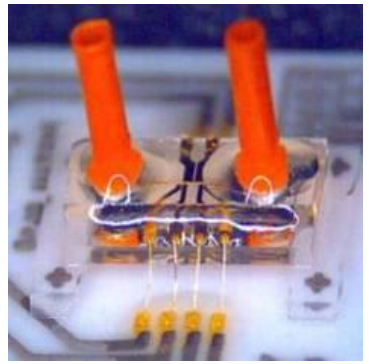
System integration issues

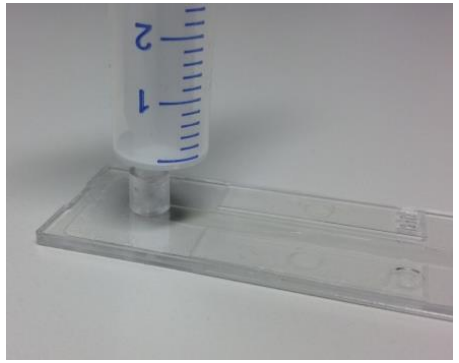


System integration issues

Peripheral fluidic components

- Tubing
  - Elastomer (silicone, Tygon)
  - PEEK
  - …
- Pumps
  - Hose pumps (peristaltic pumps)
  - Syringe pumps
  - Membrane pumps
- Others
  - Bubble traps
  - Filters
- Valves
  - Magnetic valves
  - Stop-cock valves
  - Clamps
  - …
- Adapters
  - Luer
  - Mini-Luer
  - Ferrules
  - Chip holders
  - …



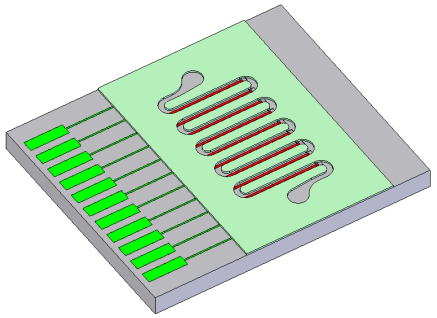


http://www.syringepump.com/images/NE-1000%20white.jpg
http://www.halmapr.com/bcf/pinch_valve.jpg

System integration issues

Optical, electrical, thermal etc.

- Optics
  - Microscopy
  - Fluorescence microscopy
  - Standard microscopy systems
  - Customized solutions
- Electrical measurements
  - Impedance (TEER)
  - Cyclovoltammetry
- **Operation**
  - Ease-of-use
  - Exchange / cleaning
- Temperature regulation
  - Peltier elements
  - Heating cartridges
  - …
- Sensors
  - Oxygen
  - pH
  - …
- External actuators
  - Ultrasound
  - RF
  - …



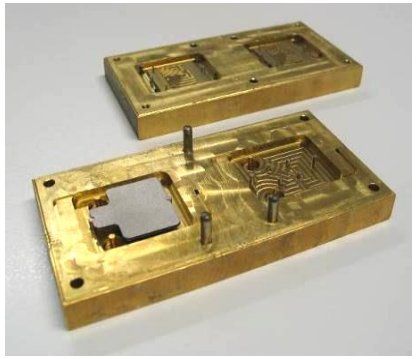
https://www.laborgeraete-muenchen.de/CO2-Brutschrank+BBD+6220.htm

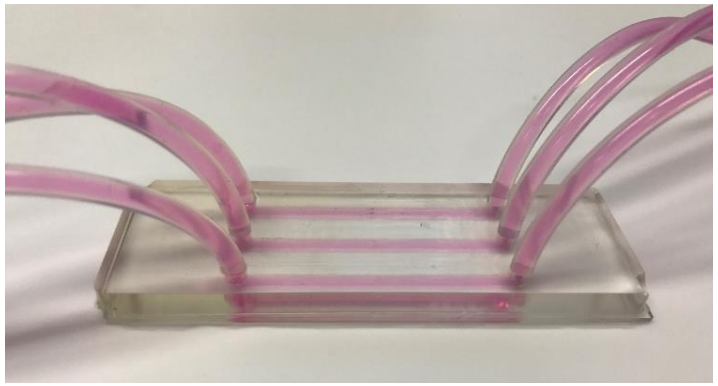
From basics to practice – biological application areas and technological solutions @ IBMT

- Biological applications
  - Single-cell analysis
  - Cell sorting / cell separation
  - Barrier models, transport studies
  - Tumor models (spheroids)
- Technological solutions
  - PDMS flow modules
  - Polymer microchannels
  - Microhole array chip
  - Modular microfluidic cartridges

PDMS flow modules

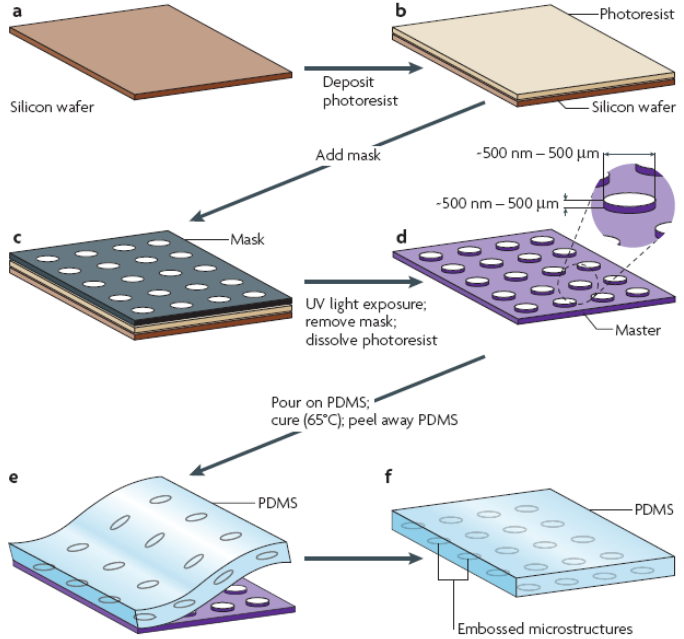
- Applications
  - SPR imaging
  - Tumor spheroids
- Characteristics
  - Variable channel geometries
  - Robust
  - Re-usable
  - 50 – 100 pcs.
  - Channel height: 0.1 – 2 mm
  - Channel width: 0.1 – 15 mm
  - Simple fluidic interface
- Fabrication
  - Mould fabrication (CNC milling, 3D printing, microfabrication)
  - PDMS moulding (Sylgard 184)
  - Plasma-activated bonding (PDMS + glass)

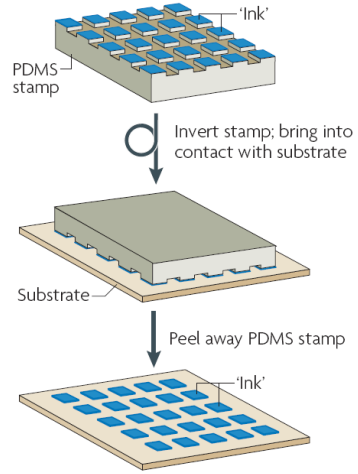




Cast moulding with PDMS elastomers

- „Soft Lithography“ (George Whitesides, 1998)
- Feature size down to < 1 µm
- Fast fabrication of small numbers up to several hundred units
  - Fluidic components
  - Silicone mould for further replication steps
  - Stamp for „micro contact printing“
- Silicone elastomer: Polydimethylsiloxane (PDMS), e.g. Sylgard 184 (Corning)
- PDMS: high gas permeability
- Casting mould
  - Metal (aluminium or brass)
  - Silicon with structured functional layer (photoresist)
  - Polymer (PMMA, PC,…)



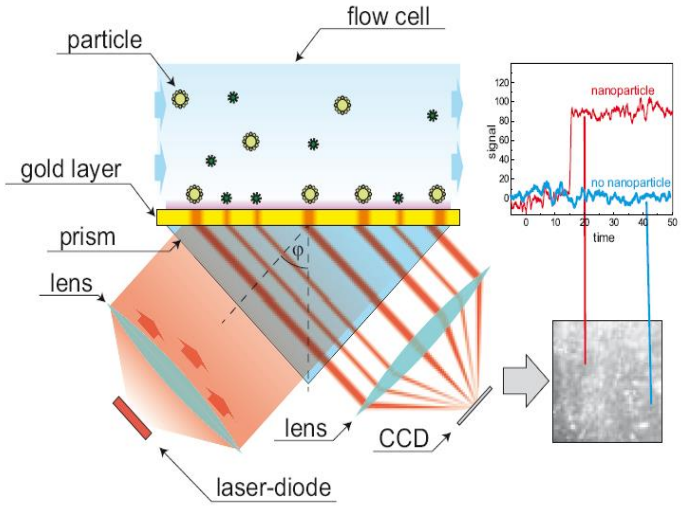


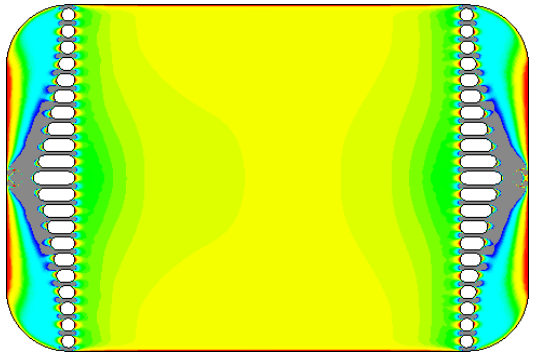
http://www.elveflow.com/wp-content/uploads/2013/05/Soft-lithography-process-scheme.png
http://www.elveflow.com/wp-content/uploads/2013/05/Microcontact-printing.png

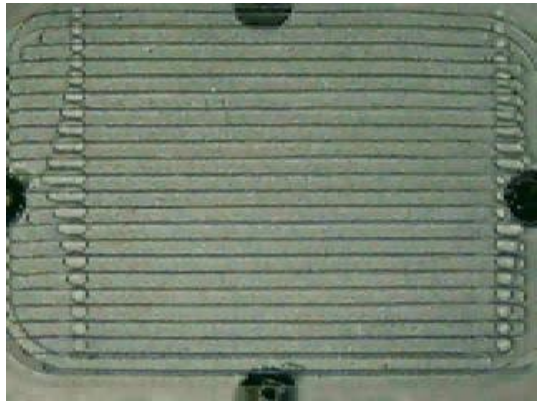
PDMS flow modules

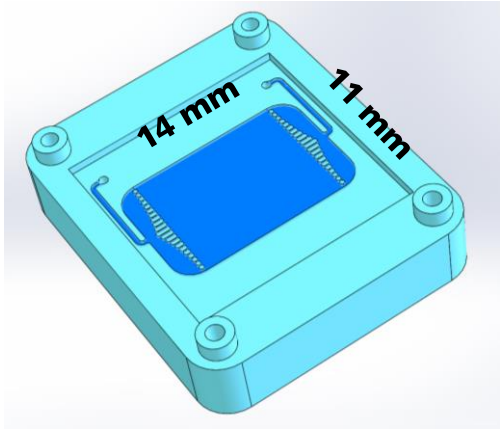
SPR imaging, virus detection

- Immunosensing diagnostic assay without labels and amplification
- Single-virus detection
- Homogeneous flow distribution





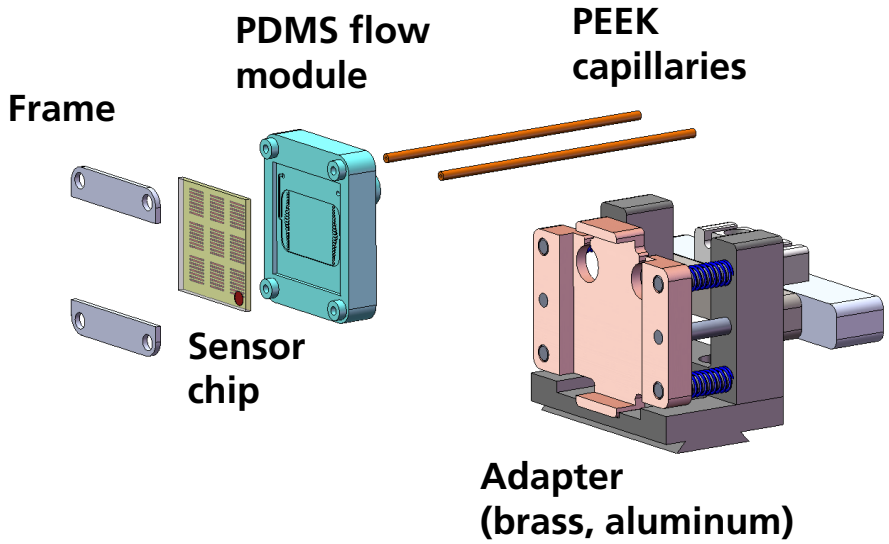


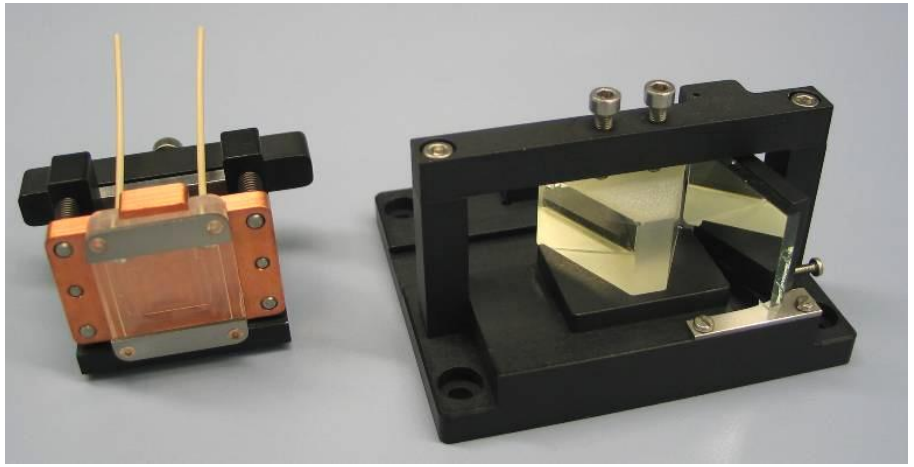


- Active area: ~ 1.4 cm²
- Chamber height: 0.5 mm

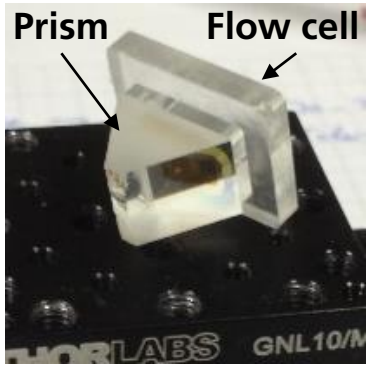
PDMS flow modules

SPR imaging, virus detection





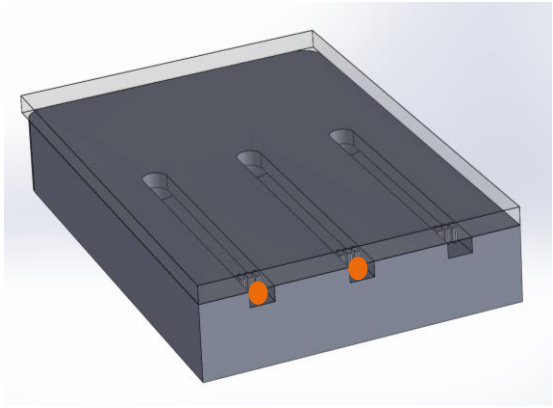
- Combine flow cell with prism
- Plasma-activated bonding of PDMS with glass

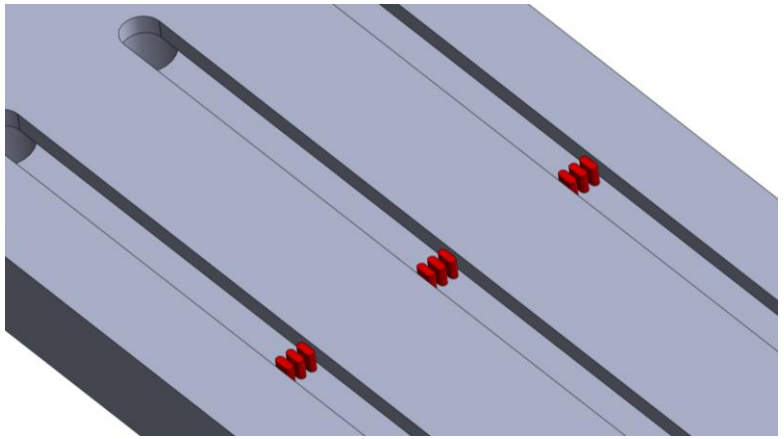


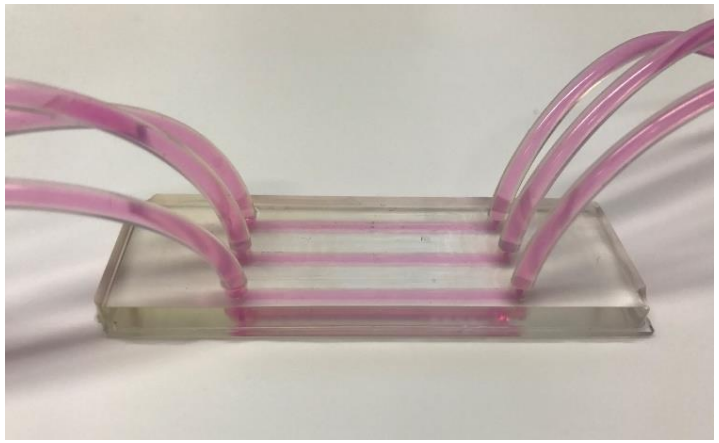
PDMS flow modules

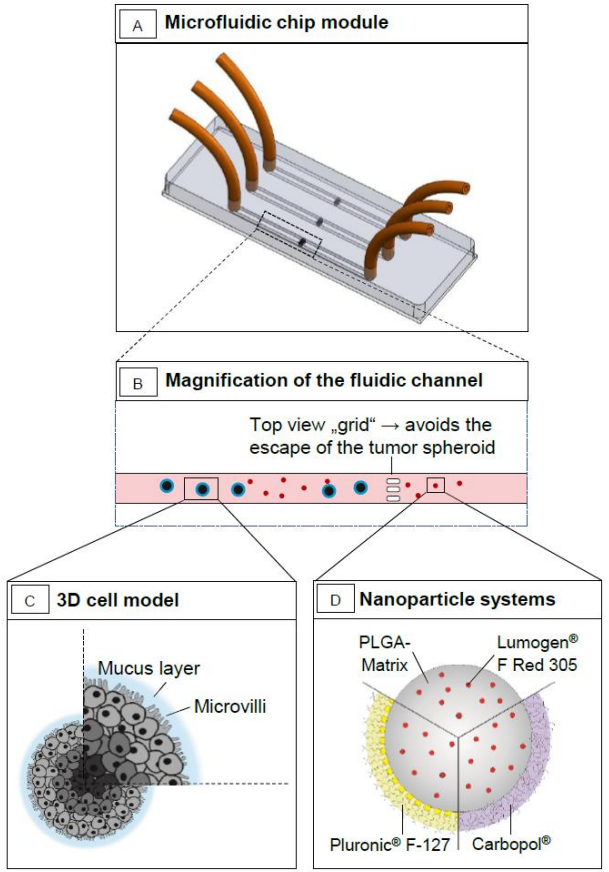
Spheroids under flow conditions

- Tumor spheroids
- Flow module with 3 channels
- Cross section: 2 mm x 1 mm (w x h)
- Grating to hold spheroids at defined position









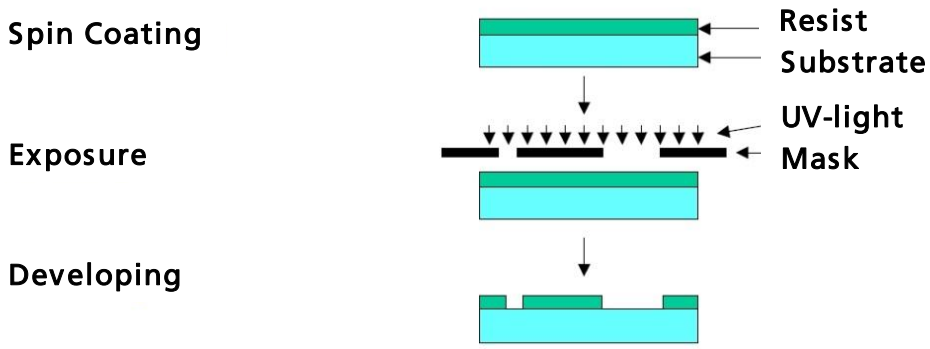
L. Elberskirch et al., Journal of Drug Delivery Science and Technologies (2021)

Microfabricated polymer microchannels

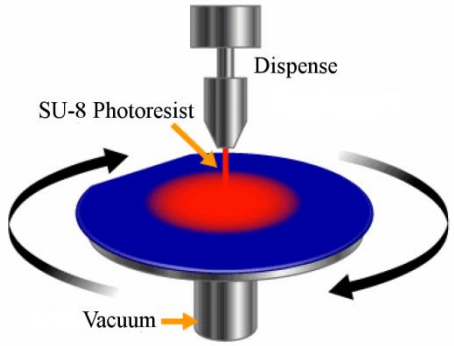
- Applications
  - Cell sorting
  - Micromixer
- Characteristics
  - Polymer layers on glass or polymer substrates
  - Multi-layer fluidic structure
  - Narrow flow channels (<< 0.1 mm)
  - Integrated electrodes
  - 10 – 100 pcs.
- Fabrication
  - Lithography (SU-8, dry film resist)
  - Sputtering and etching
  - Laminating

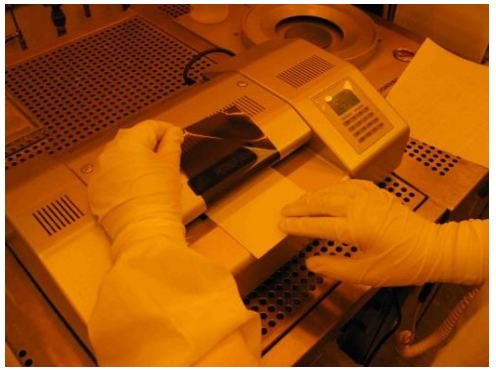
Microfabrication of microfluidic structures

- Microfabrication technologies
  - Lithography
  - Layer deposition (photoresist, metals, insulators)
  - Etching: wet chemical etching (acids and alkaline solutions), dry etching (plasma)



- Lithographic patterning
  - Negative photoresist, liquid
    - SU-8, AZ 125nXT
    - Thickness: between 1 and > 100 µm
    - High lateral resolution, depending on thickness
  - Negative photoresist, solid (DFR – dry film resist)
    - Lamination on silicon, glass or polymer substrates
    - Variable thickness
    - Lower lateral resolution



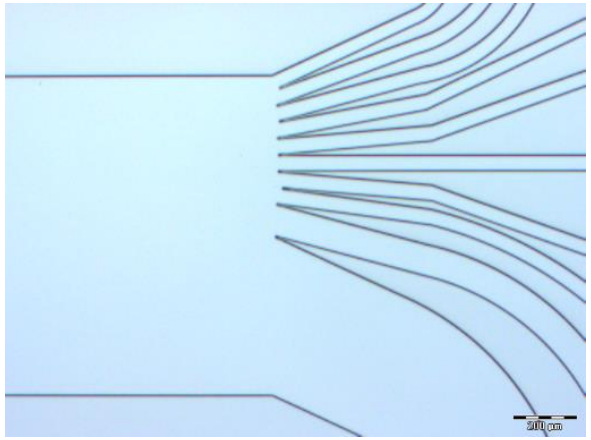


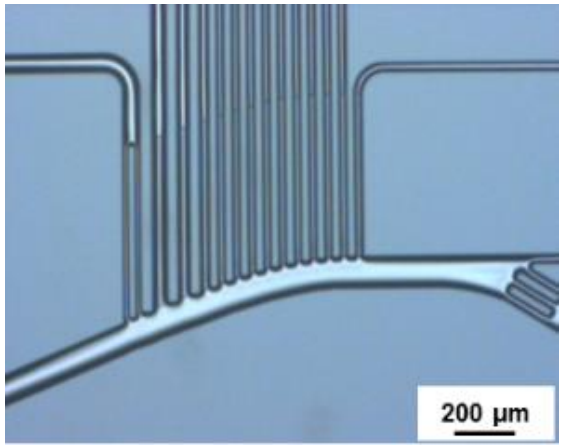
www.elveflow.com/wp-content/uploads/2013/04/Spin-coating-scheme.png

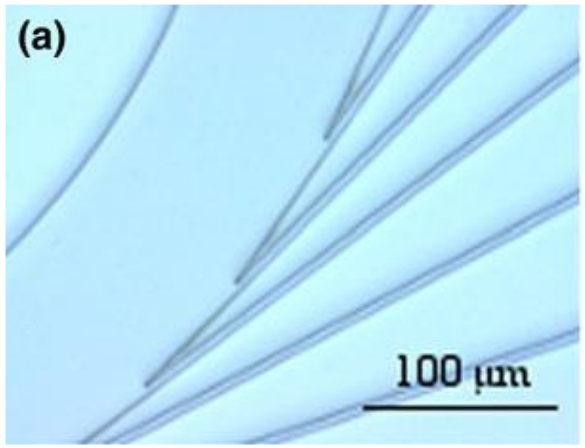
Microfabricated polymer microchannels

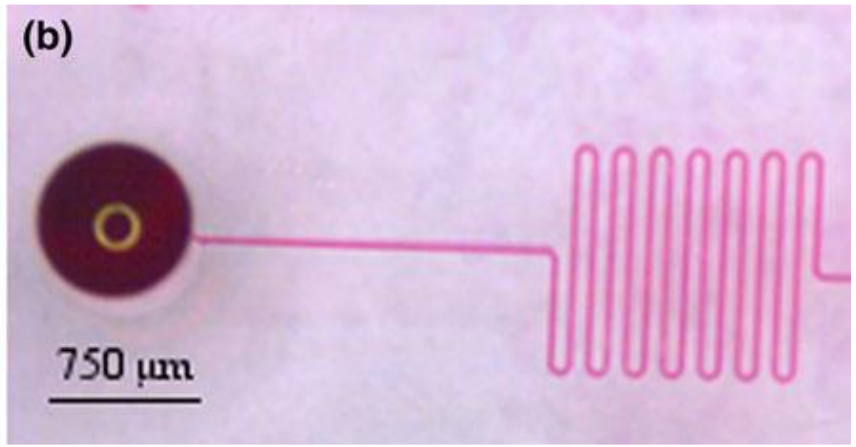
Cell sorting chip

- PMMA bottom and lid
- Epoxy channel (SU-8)
- Minimum cross section: 4 µm x 20 µm (w x h)
- Polymer-polymer bonding







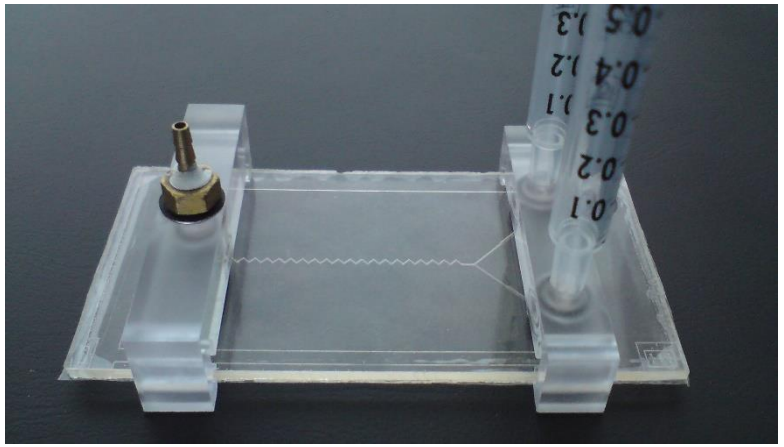


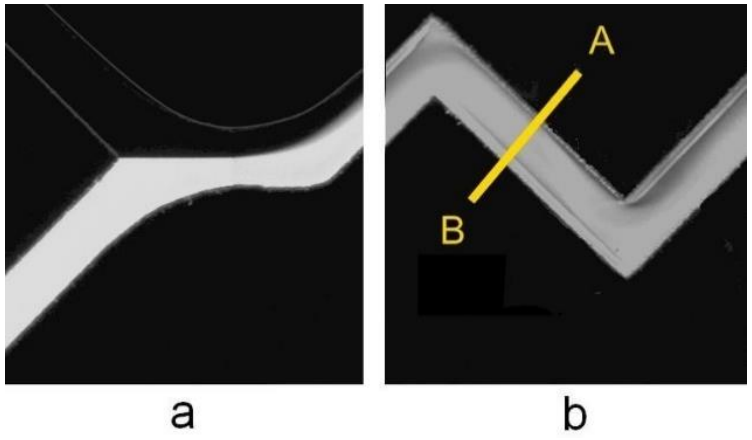
A. Krebs et al., Microsyst Technol (2012)

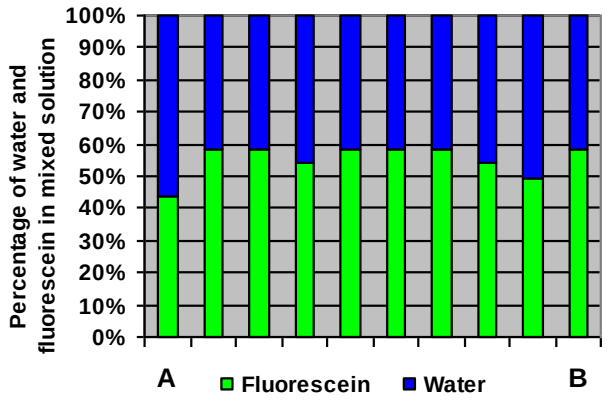
Microfabricated polymer microchannels

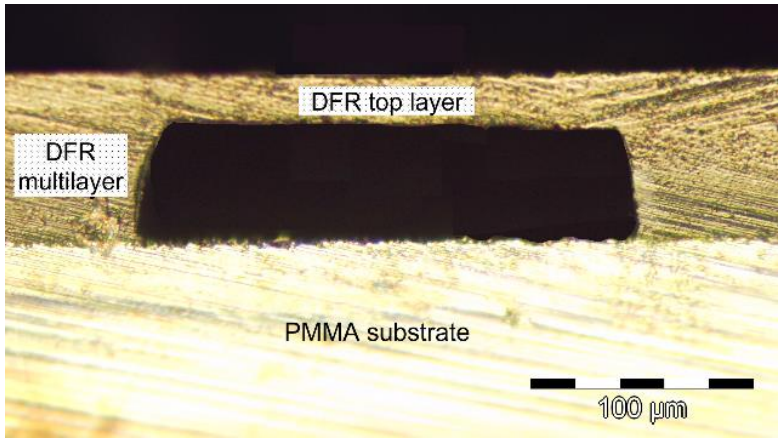
Micromixer

- PMMA bottom and lid
- Channel layer: Dry film resist (epoxy)
- Zig-zag channel
- Homogeneous mixing







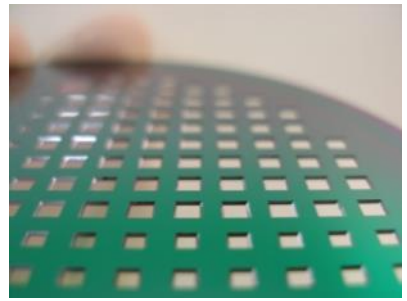


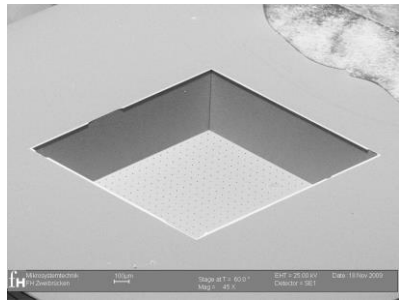
D. Nußbaum et al., Proc. 4M conference 2009

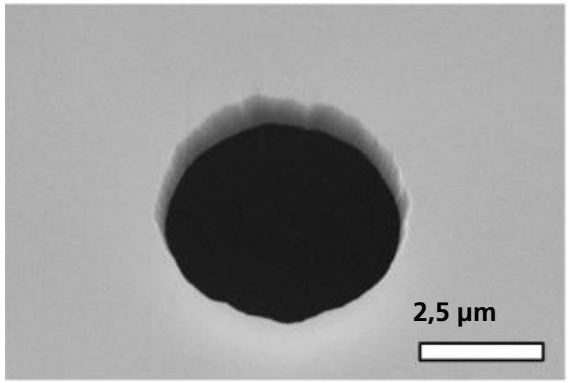
Microhole array chip

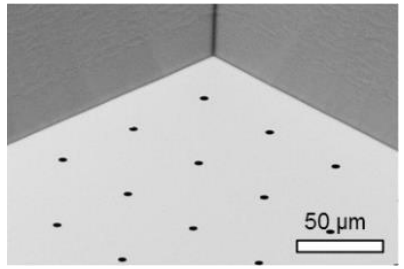
- Applications
  - Barrier models
    - Mucus
    - Lung, liver, intestine
  - Single-cell positioning and analysis
    - CTCs
    - Lung, liver, intestine
- Fabrication
  - Lithography
  - Deposition
  - Etching

- Characteristics
  - Hole diameter min. 3 µm
  - Regular array of microholes
  - Highly transparent membrane (Si_3N_4, 1.5 µm)





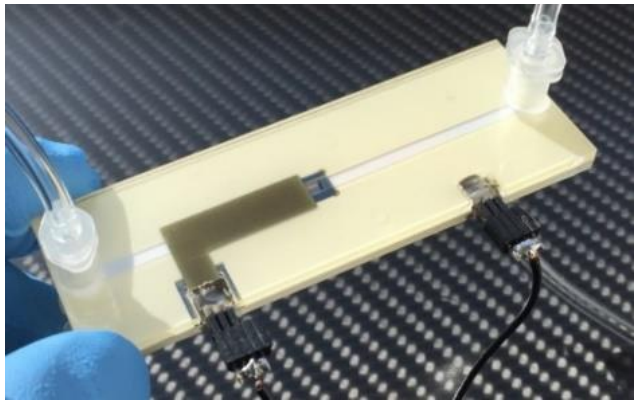


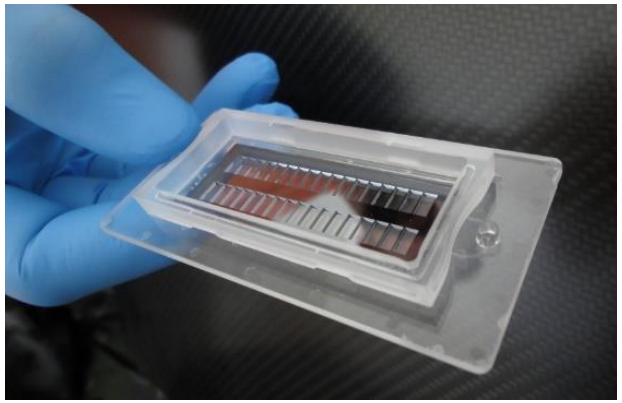


Fraunhofer IBMT

Microhole array chip

- Applications
  - Barrier models
    - Mucus
    - Lung, liver, intestine
  - Single-cell positioning and analysis
    - CTCs
    - Lung, liver, intestine
- Characteristics
  - Open and closed versions
  - Variable channel geometries
  - Electrodes
  - 10 – 200 pcs.
- Fabrication
  - Lithography, deposition, etching (cavity chip with microholes)
  - Injection-moulding
  - Die-cutting of PSA tape

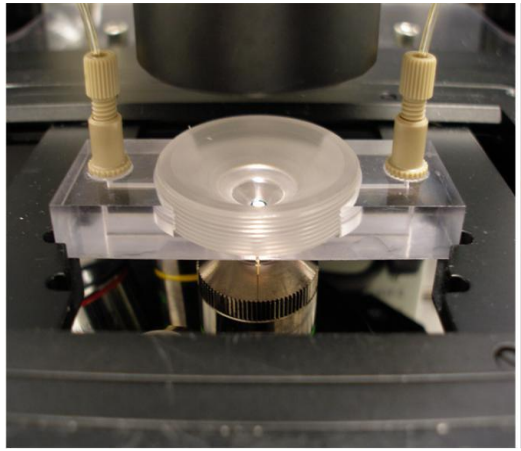


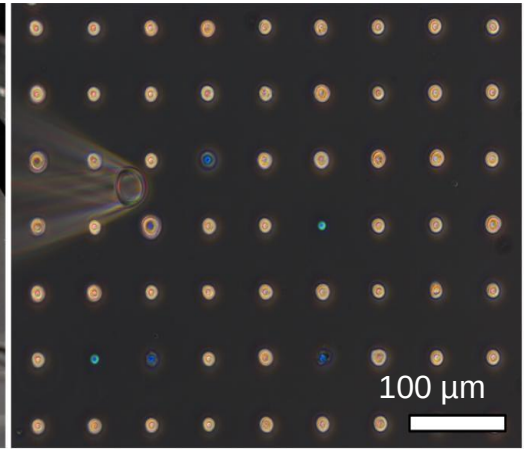


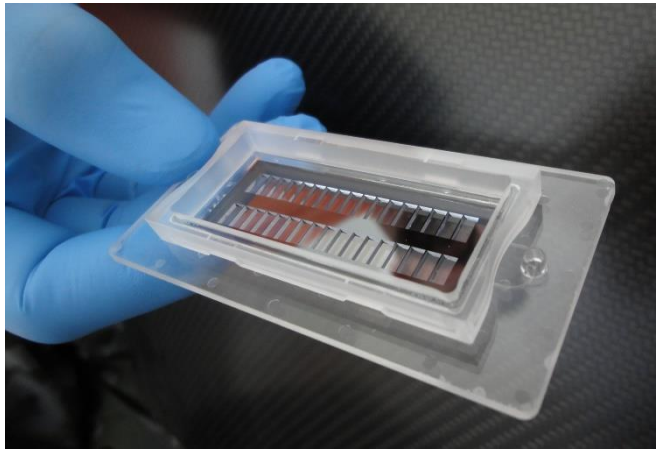
Microhole array chip

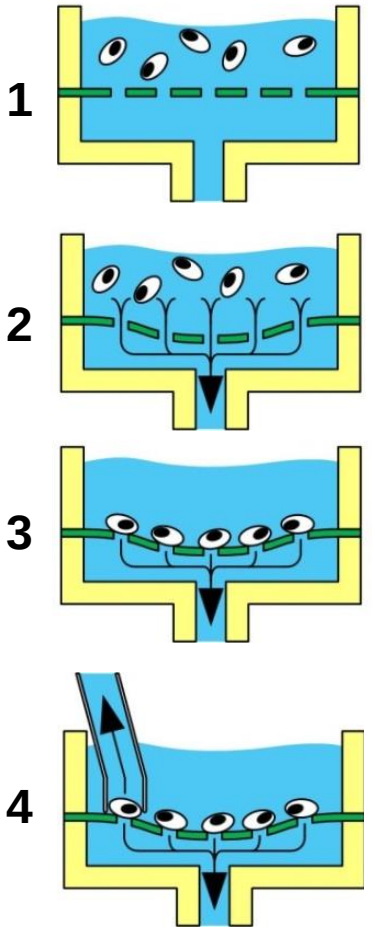
Positioning and analysis of single cells

- Active positioning of single cells by applying negative pressure
- Isolation of single cells (~ 1,000 – 200,000)
- Analysis (fluorescence, impedance, Raman spectrum)
- Picking of designated cells by micro capillaries
- Cultivation, cloning





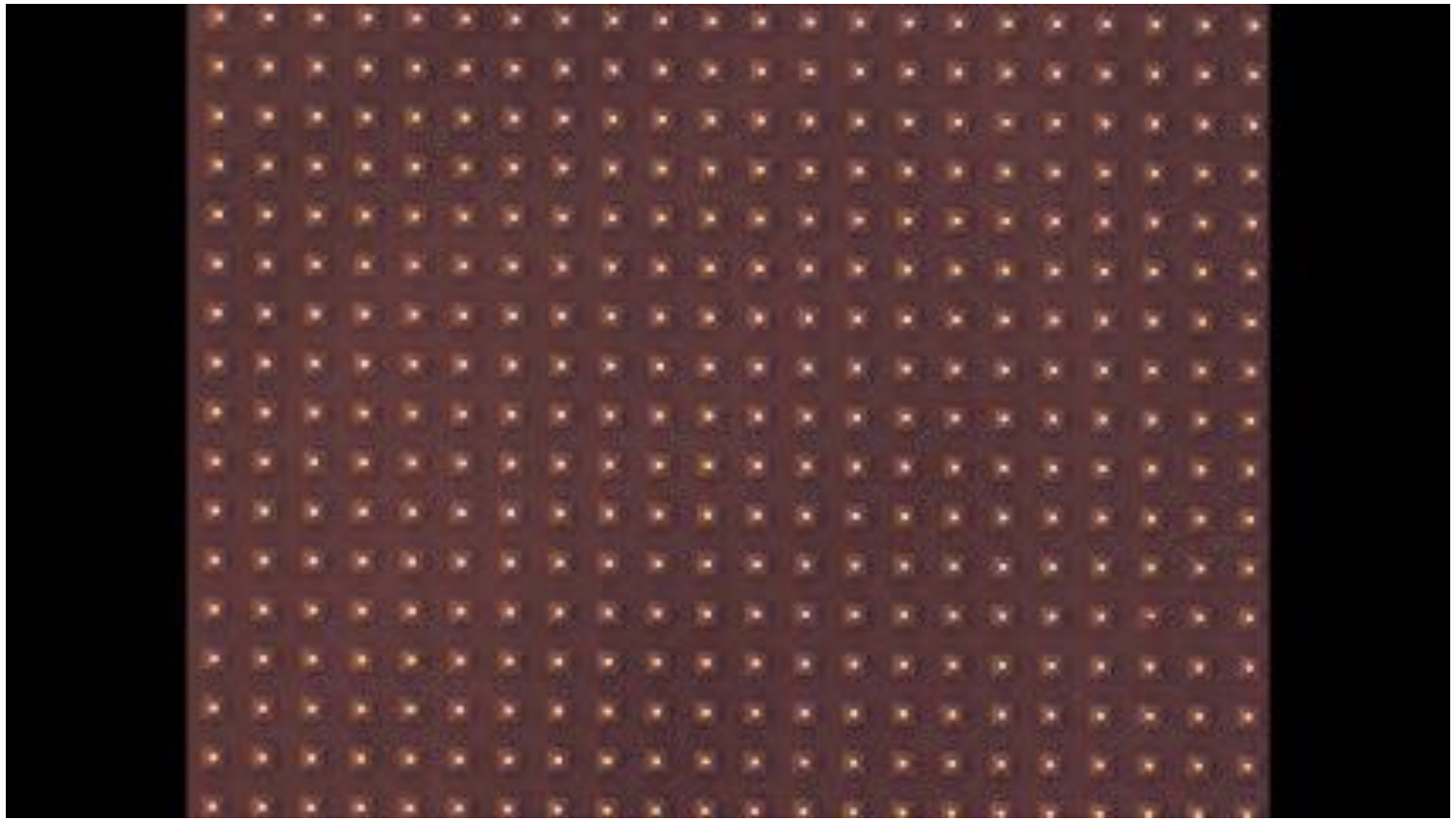


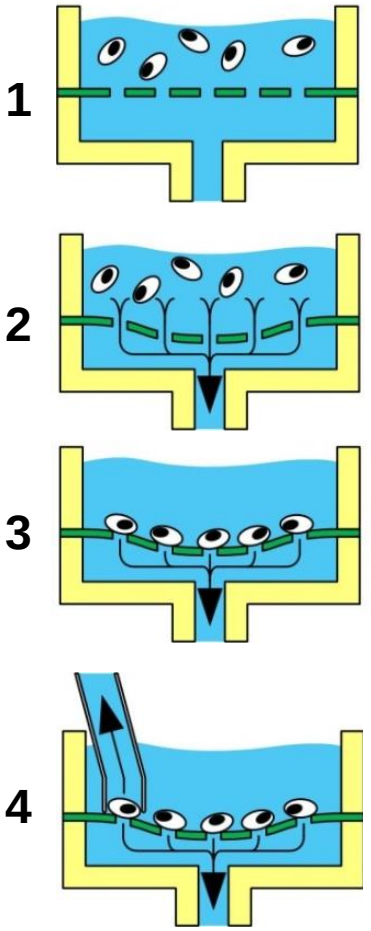


Microhole array chip

Positioning and analysis of single cells

- Positioning of hMSCs



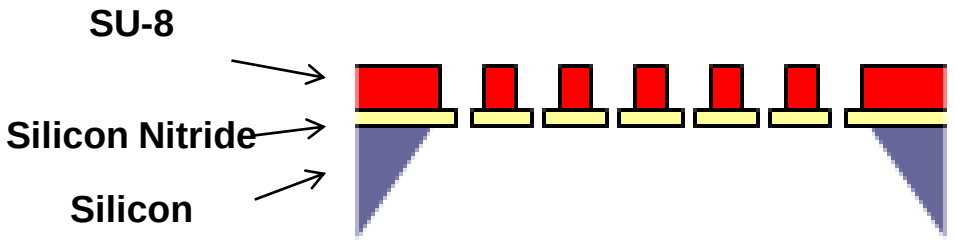


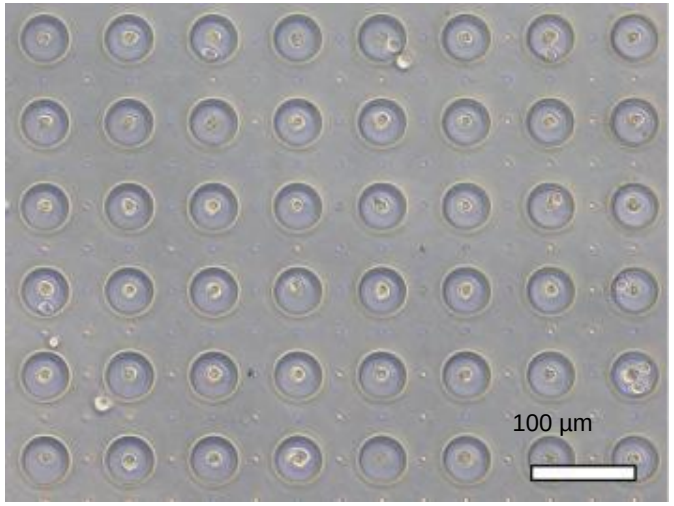
Fraunhofer IBMT

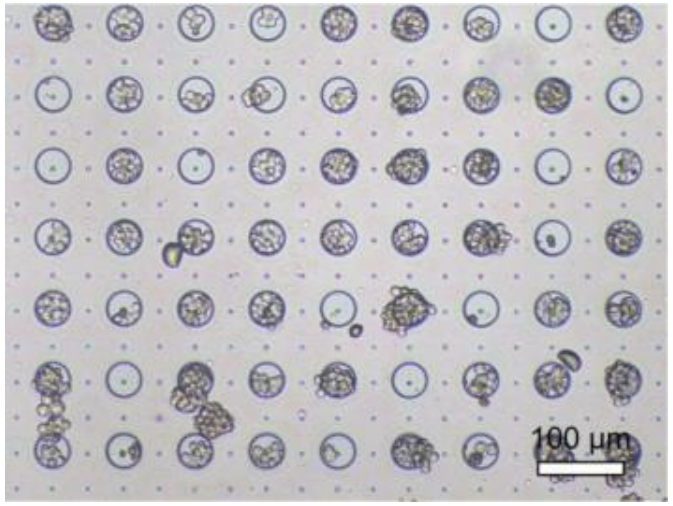
Microhole array chip

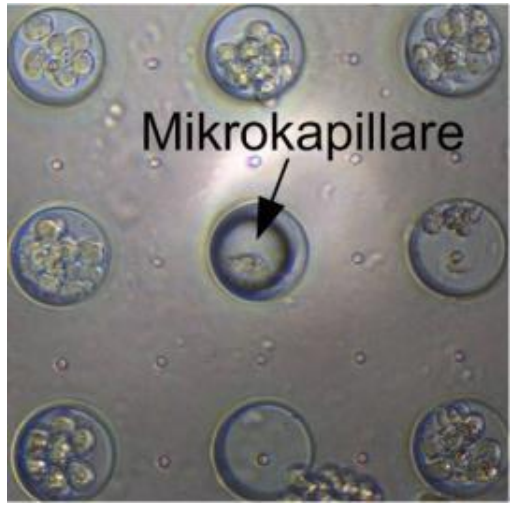
Cloning of single cells

- Polymer micro-wells on microhole array membrane
- ~1,000 cells (54K(1)-5 Hybridom)
- Medium: soft agar
- Cloning efficiency ~ 90 %





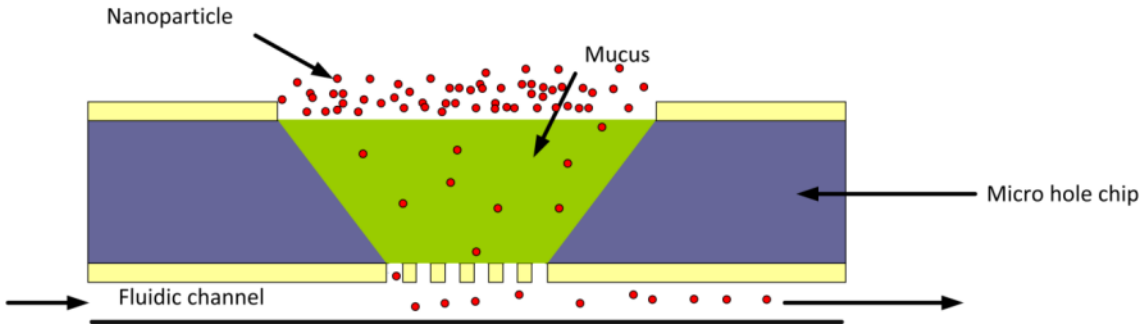


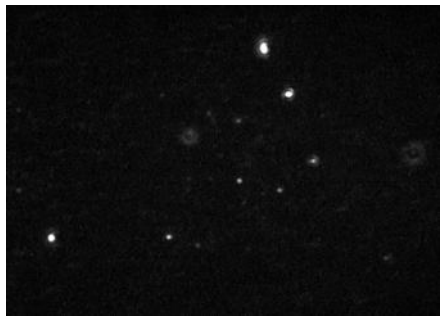


Microhole array chip

Permeation through mucus barrier

- Simulation of mucus barrier with micro-hole-chip
- Field: pharmacokinetic and drug delivery
- Investigation of oral drug administration
- Nanoparticle transport across the intestinal barrier
- Simulation of mucus barrier for first screening step
- Advantages of micro-hole array chip:
  small volumes and parallelization



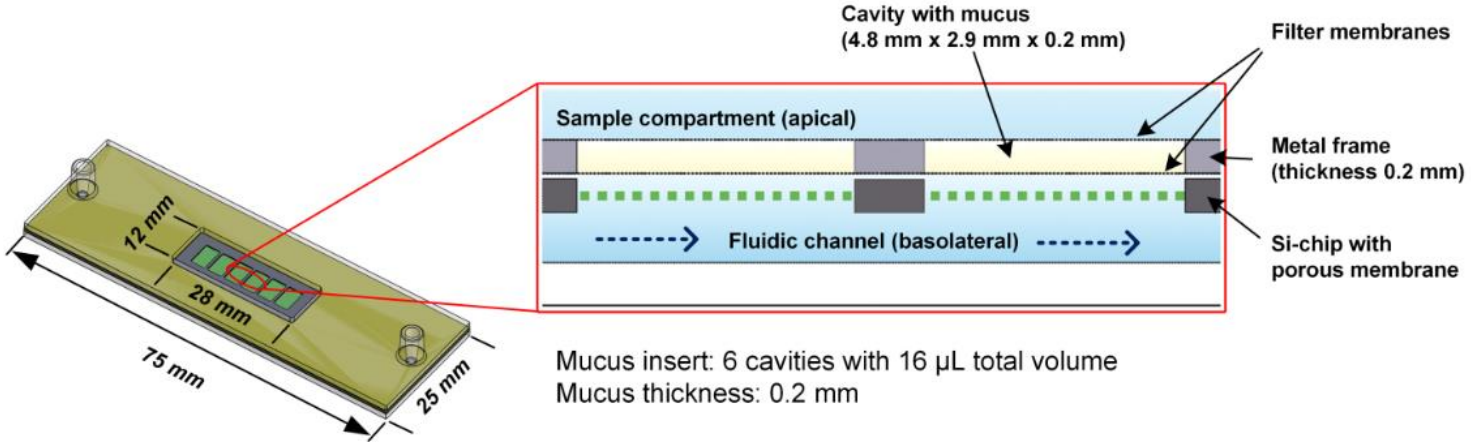


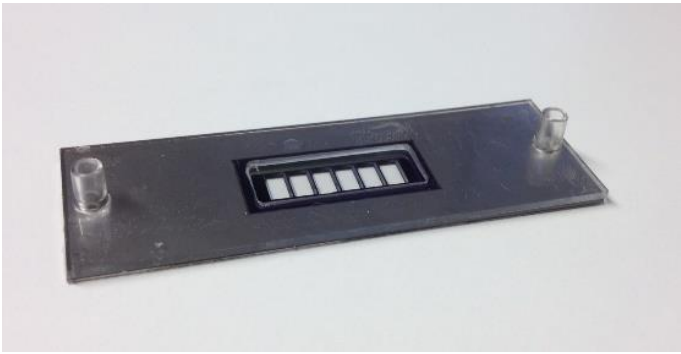
Nanoparticle tracking analysis

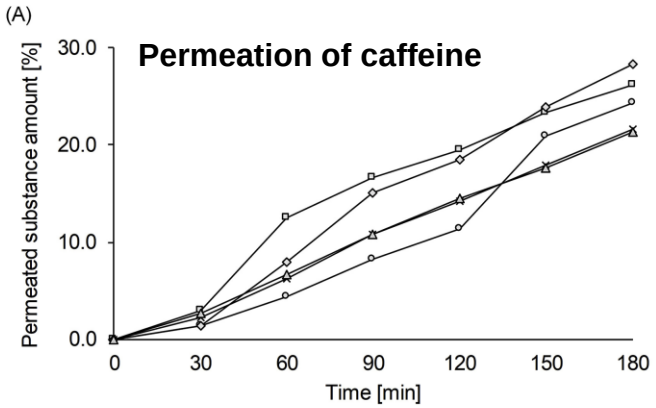
Fraunhofer IBMT

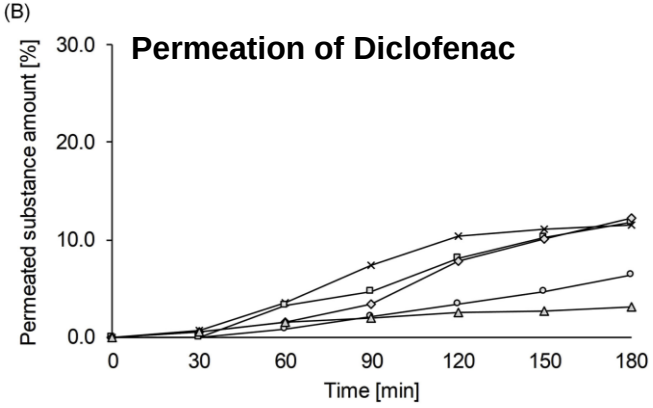
Microhole array chip

Permeation through mucus barrier





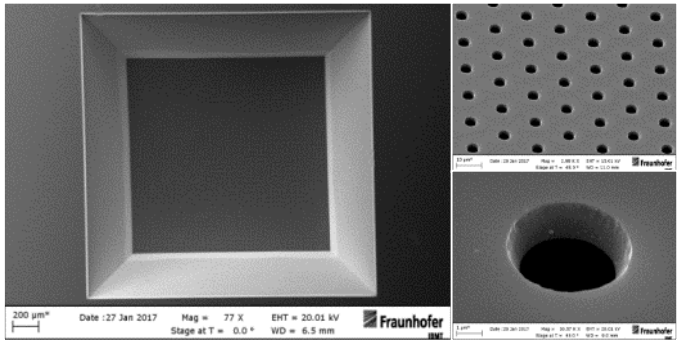




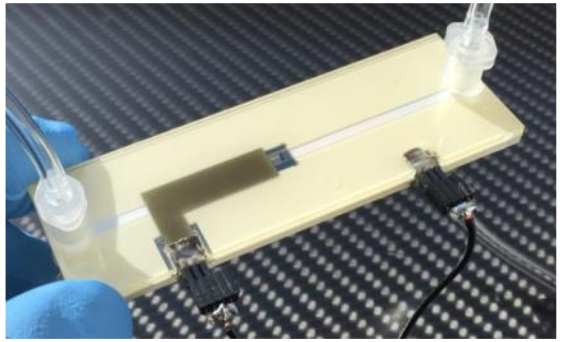
L. Elberskirch et al., Journal of Drug Delivery Science and Technology, 2019

Microhole array chip

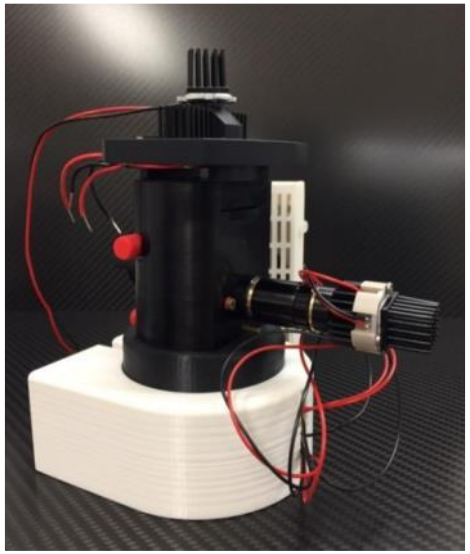
Platform for toxicity screening



1) Micro-hole array chip



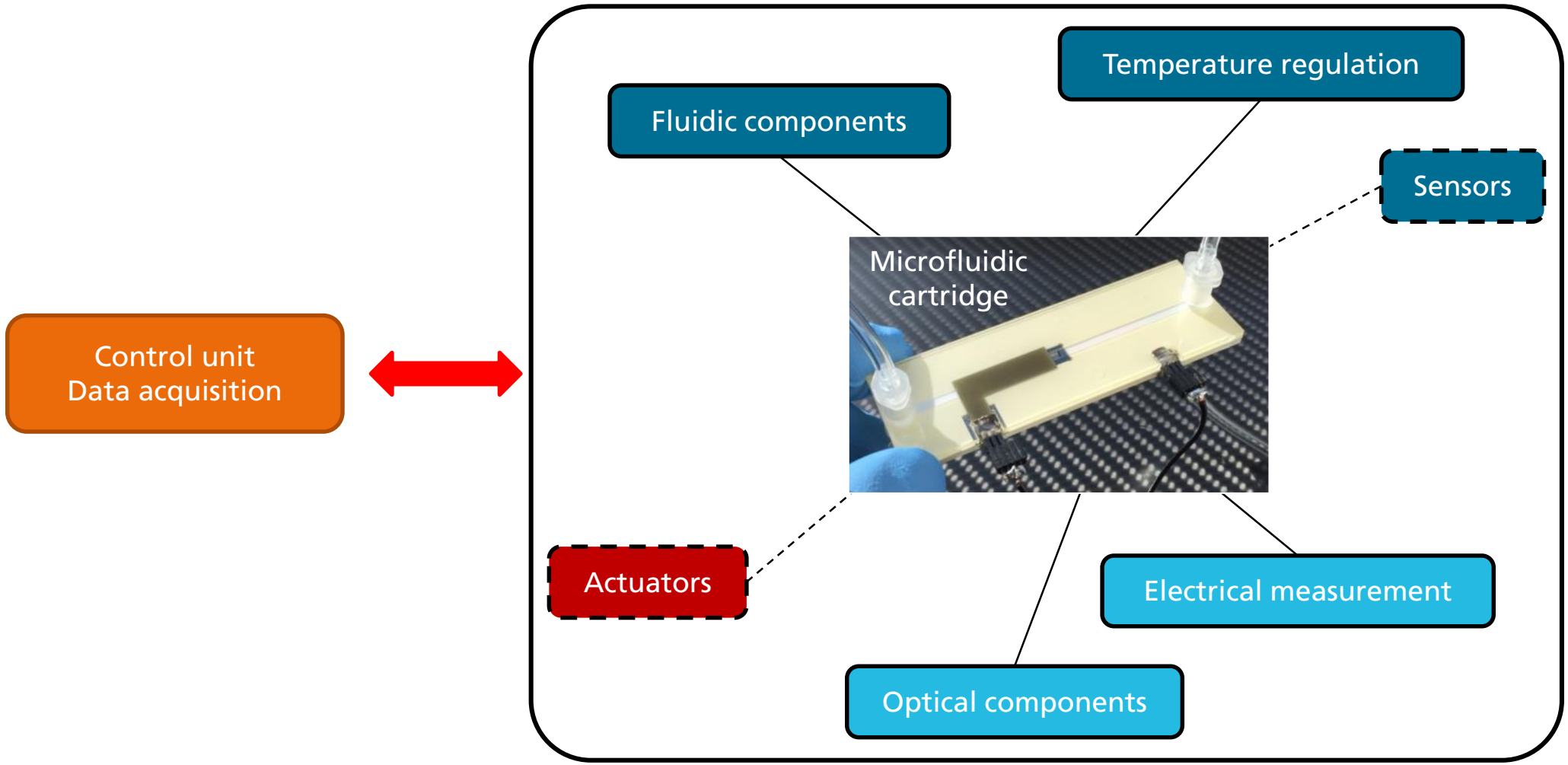
2) Microfluidic cartridge



3) Miniaturized incubator microscope

Y. Kohl et al., SMALL, 2021

System integration issues

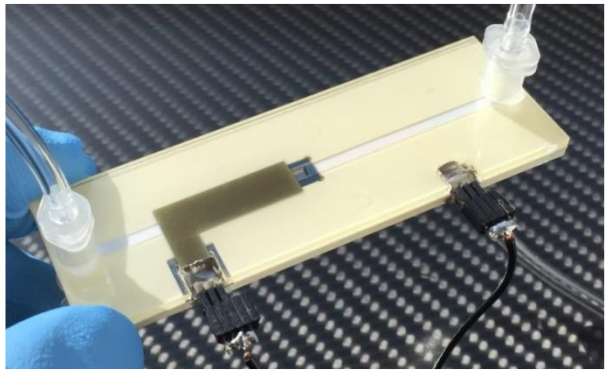


Microhole array chip

Platform for toxicity screening

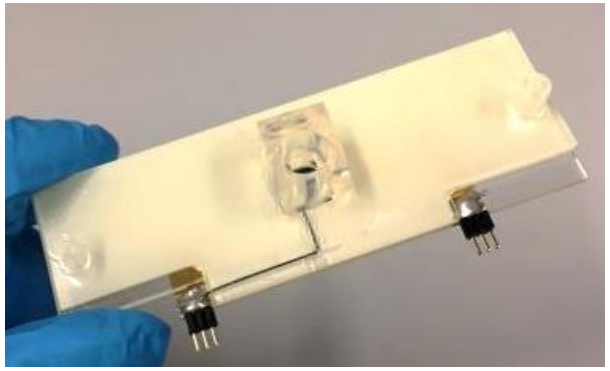
Closed system

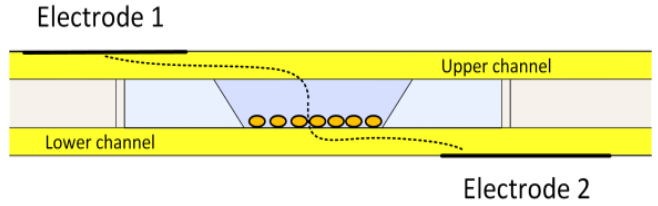
(barrier models)

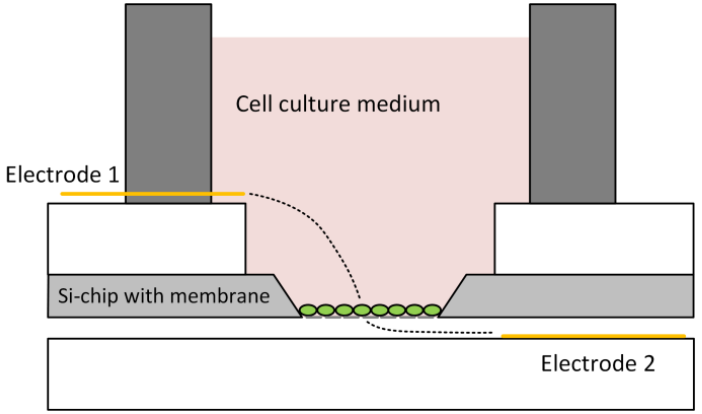


Open system

(single cells)



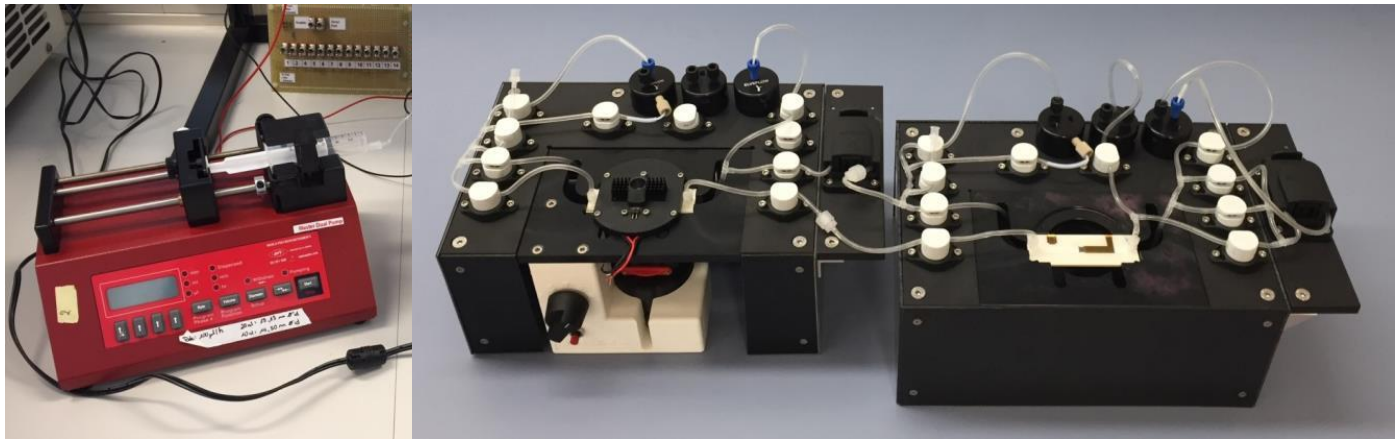


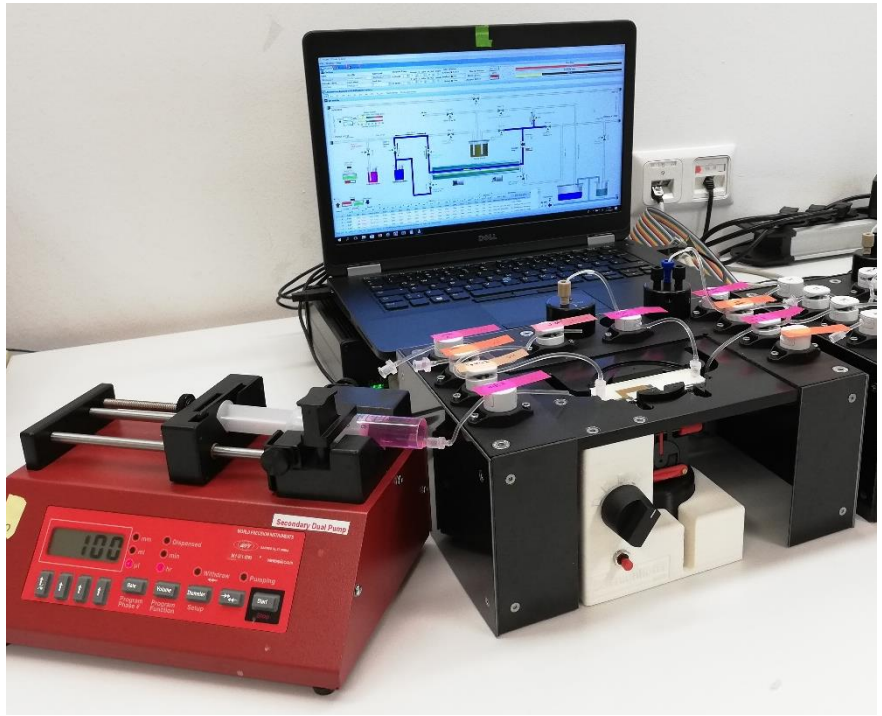


Y. Kohl et al., SMALL, 2021

Microhole array chip

Platform for toxicity screening





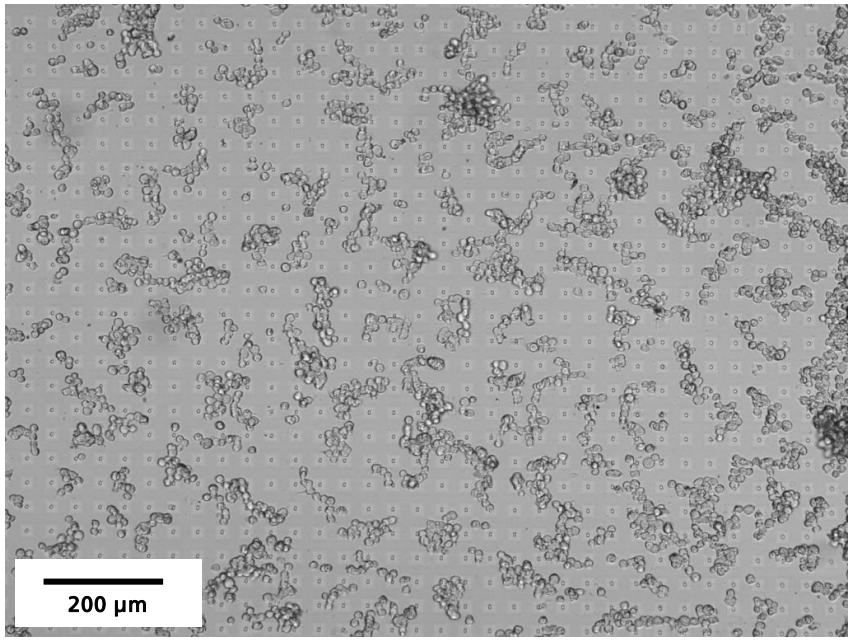
- Module footprint: 25 cm x 20 cm
- Miniaturized microscope for cell imaging, with integrated temperature unit
- Microfluidic module for cell positioning and cell cultivation
- Fluidic circuit for transport of cell suspension, medium and reagents
- Modules can be operated separately or connected with each other
- Electronic control of all platform components

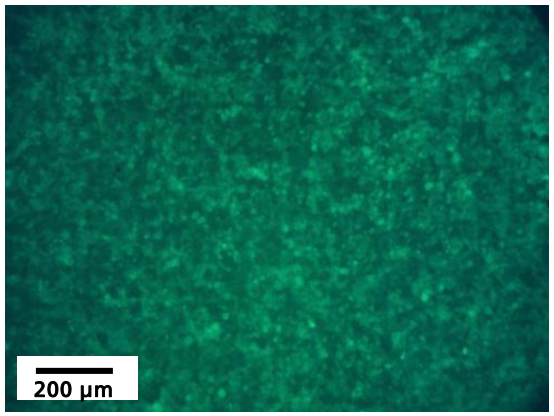
Y. Kohl et al., SMALL, 2021

Microhole array chip

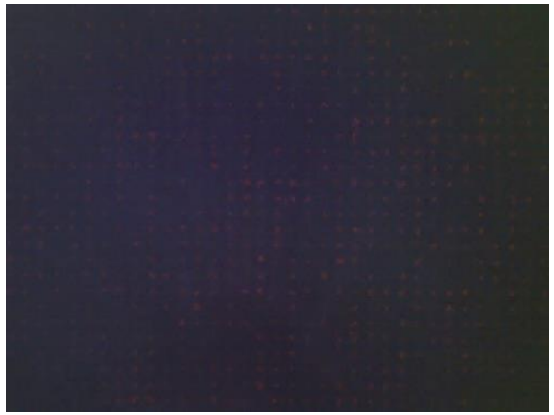
Platform for toxicity screening

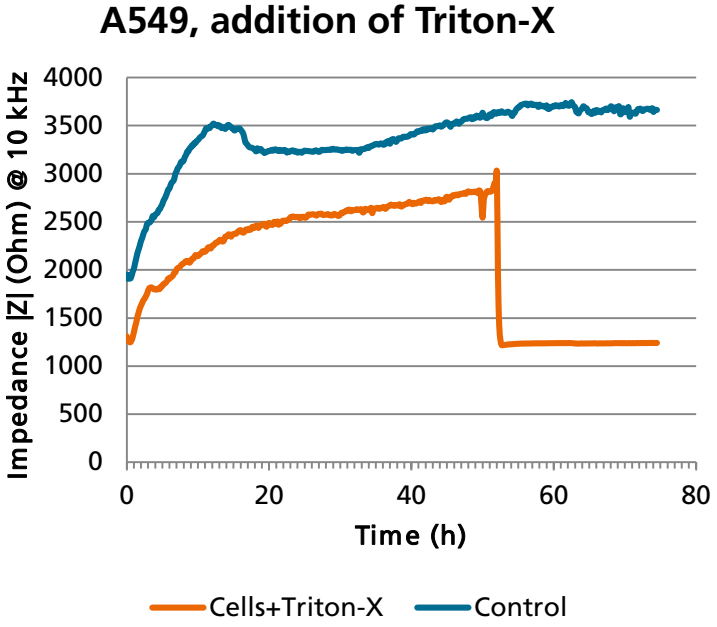
Cultivation of HepG2
time: 144 h, medium flow rate: 100 µl/h





Fluorescein diacetate (viable cells)
Propidium iodide (dead cells)

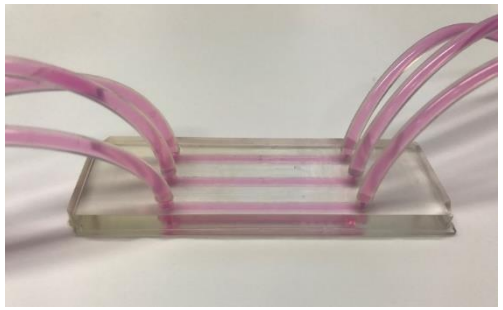


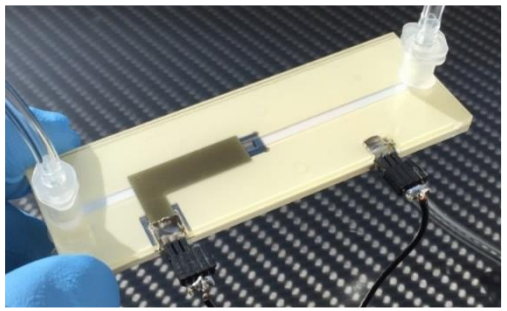


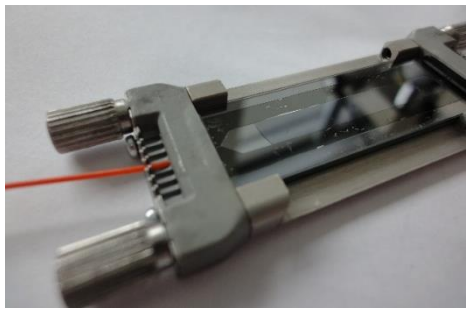
Summary

- Microfluidics technology: promising tools and systems for miniaturized solutions in biotechnology
- IBMT: technological solutions for a broad range of biological applications
- Individual customized combinations of system components
- Consideration of handling issues and cost-efficient fabrication

PDMS flow modules | Microhole array chip | Modular cartridge | Microfabricated channels







2D cell layers | Single cells | 3D aggregates | Mucus | Cell suspension

Nanoparticles | Nanoparticle systems | Chemicals | Drugs | Viruses

Thanks to all colleagues from Fraunhofer IBMT!

Thanks to all project partners!

Thank you very much for your attention!