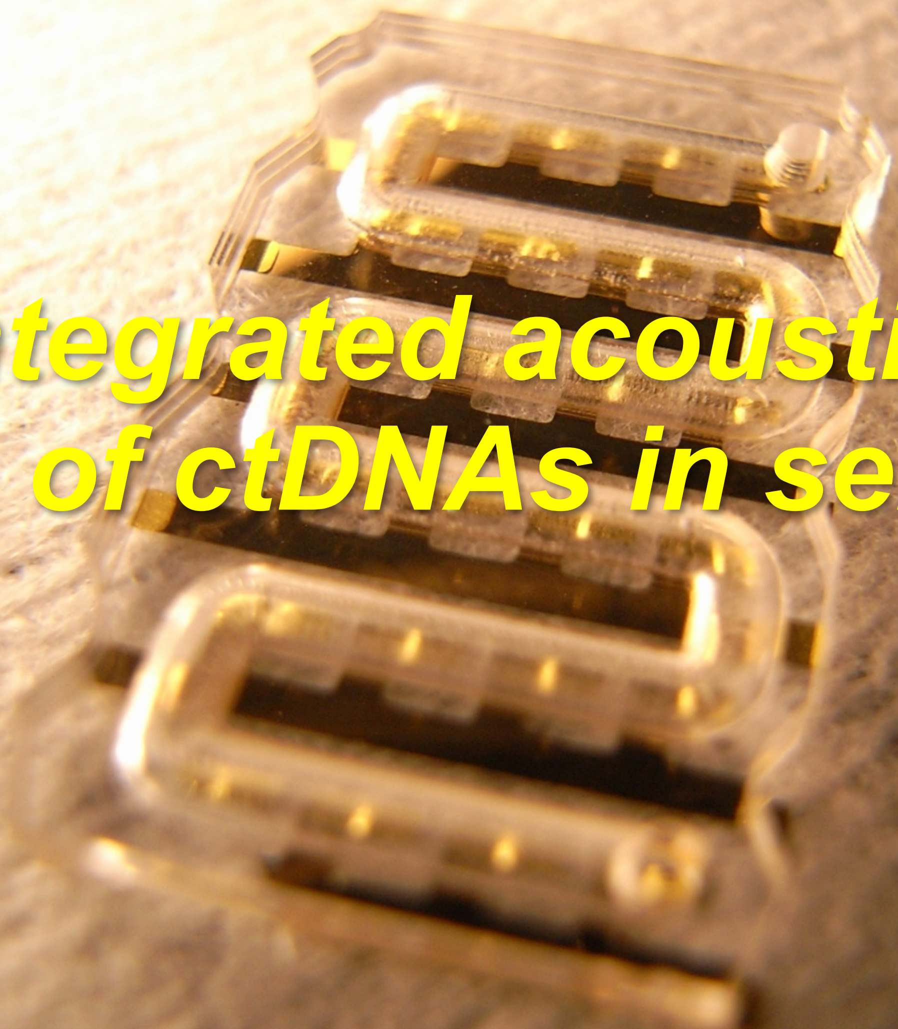
Catch-U-DNA

1st REVIEW MEETING



WP6 integrated acoustic detection of ctDNAs in serum

Jobst Technologies GmbH

Capturing non-Amplified Tumor Circulating DNA with Ultrasound Hydrodynamics

WP6 (Jobst, Forth, AWS, UoC)

Integrated acoustic detection of ctDNAs in serum

- **Objectives**

1. Develop/optimize/characterize sensor array compatible with passive/active microfluidics maximizing sensitivity

2. Integrate the measurement electronics, fluidics control, and assembled chips interface into one PC connective instrument.

Task 6.1 Microfluidics and assembly (JOBST, FORTH, M1-M15)

Task 6.2 Fluidics control (JOBST, M10-M24)

Task 6.3 Instrument (AWS, JOBST, FORTH, M16-M30)

Achievements - Task 6.1 Microfluidics and assembly

Benchmarks elaboration

1. To enable high shear rate to allow for
   - Maximizing binding rate
   - Detaching of loosely bound materials
   - Regeneration of chip surface (detaching Avidin)

   Applying **up to 100pN drag force** to a 200nm Liposome

2. To enable a fluid resting period to allow for
   - "threshold contact times for bond formation"

   At around **10ms no-flow condition** for bond formation in a consecutive process

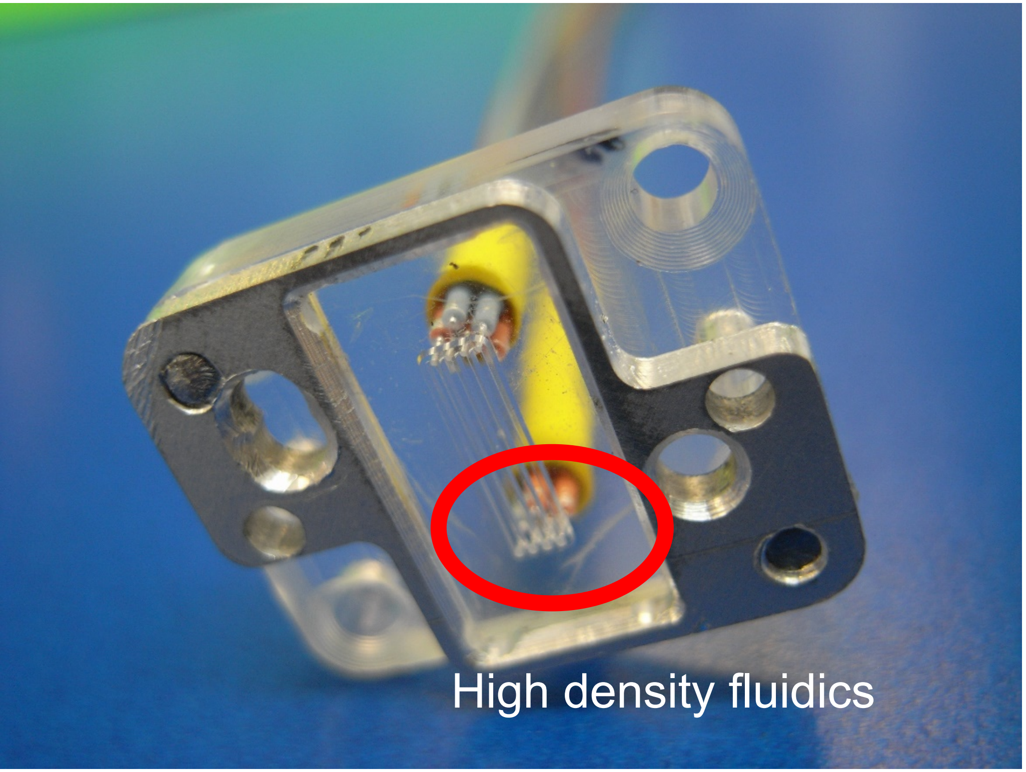
3. Not to break the 10microns thin sensing windows of the Quartz chip
   - Burst pressure calculations yield

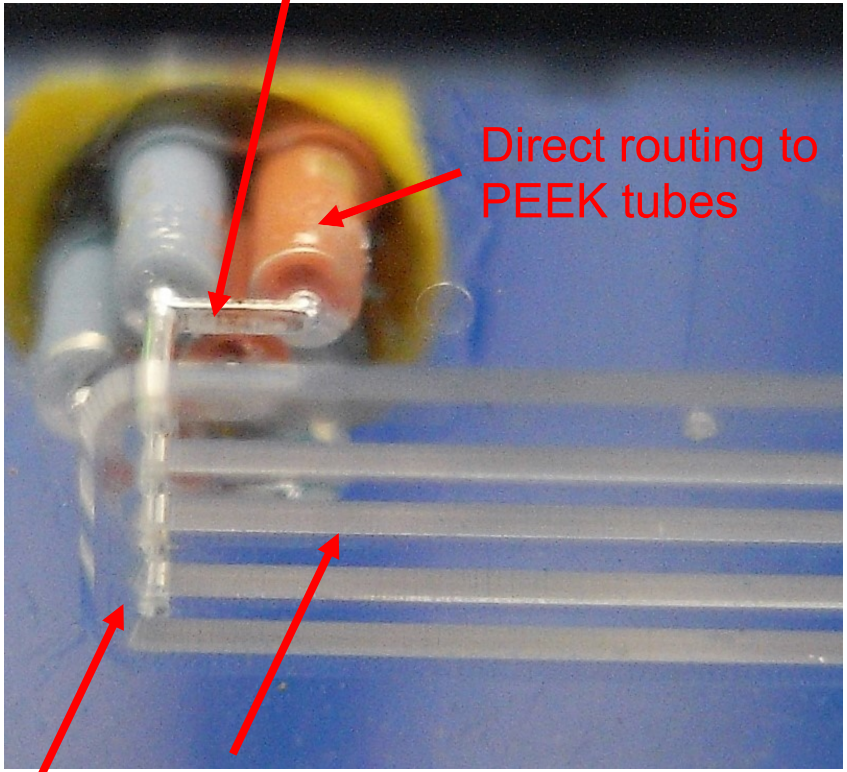
   A **pressure limit of 1.0bar**

$$Th = \sqrt{\frac{1.1 * (P) * (R)^2 * SF}{MR}}$$

Material	Modulus ot rupture [psi]	K	Pressure [bar]	Diameter [mm]	Safety Factor SF	Thickness [mm]
Fused silica (SiO2)	7.000	1,1	1	0,4	1	0,010

Achievements - Task 6.1 Microfluidics and assembly
Capabilities for reusable flow cell – to be used for evaluation work





Multi-level fluidics in PMMA

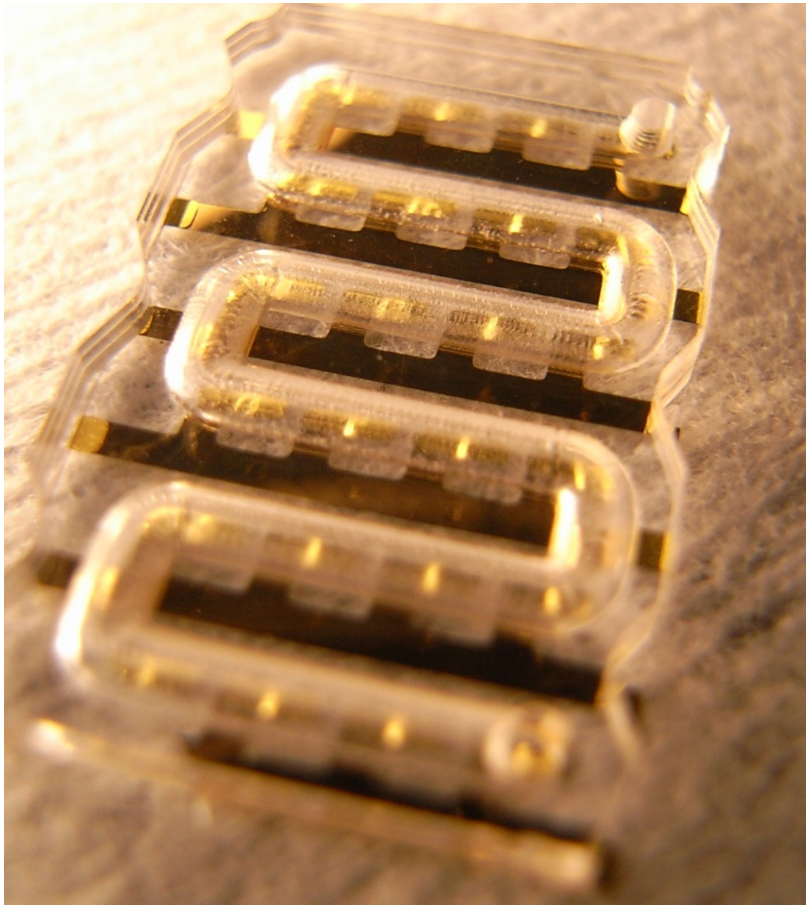
Seal lip width from 60 microns

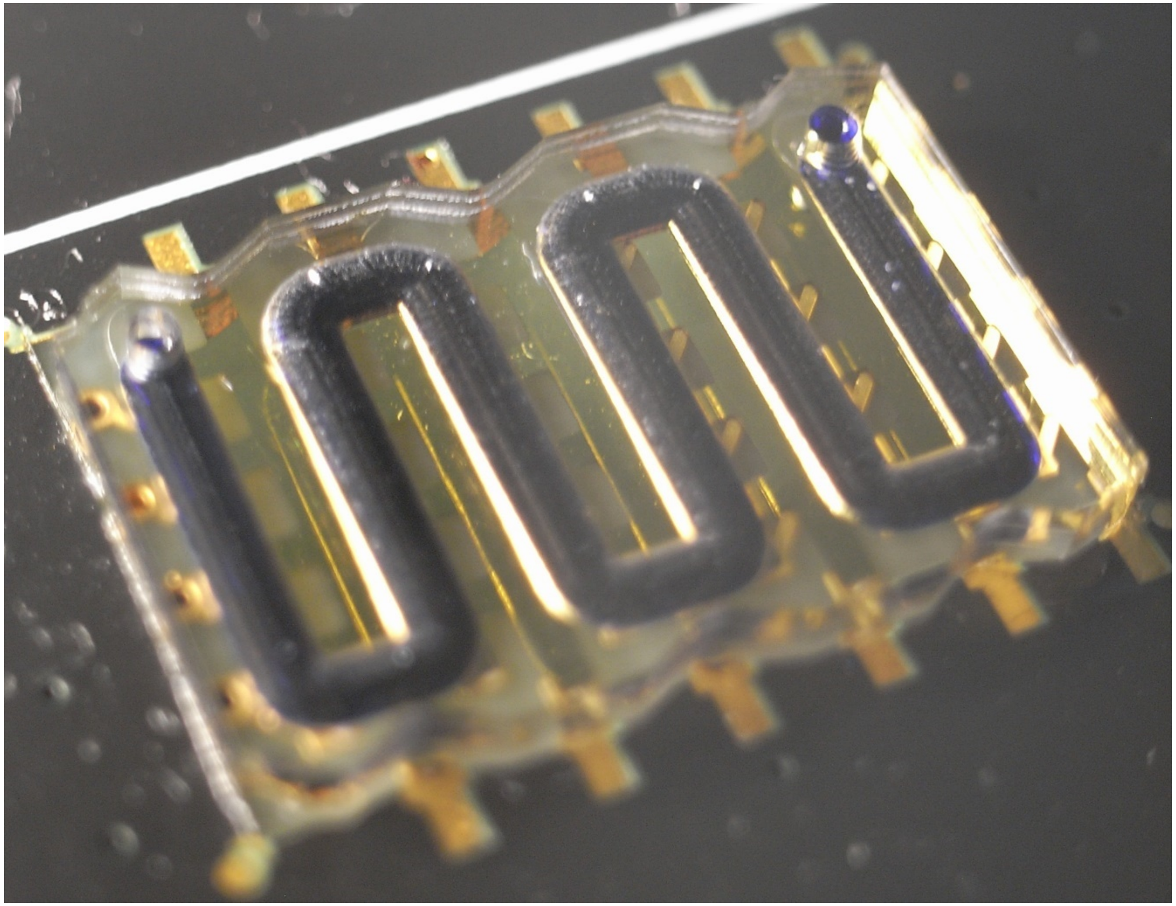
PDMS in-situ grafts to PMMA

Achievements - Task 6.1 Microfluidics and assembly

A flow cell permanently bonded to a 24x chip of AWS

(flow cells machined in PMMA and bonded with UV curing underfiller)

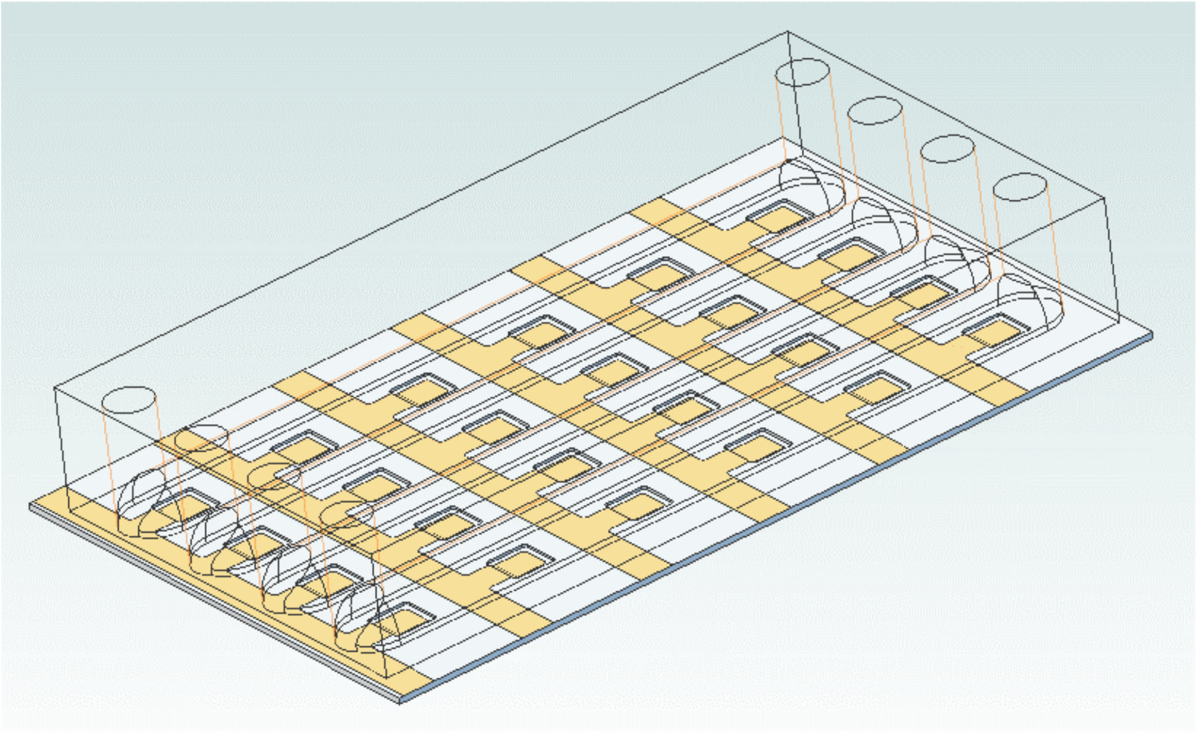




Achievements - Task 6.1 Microfluidics and assembly

But pressure drop adds up for each sensing site covered.

$\Rightarrow$ Make parallel channels with separate in- and outlets



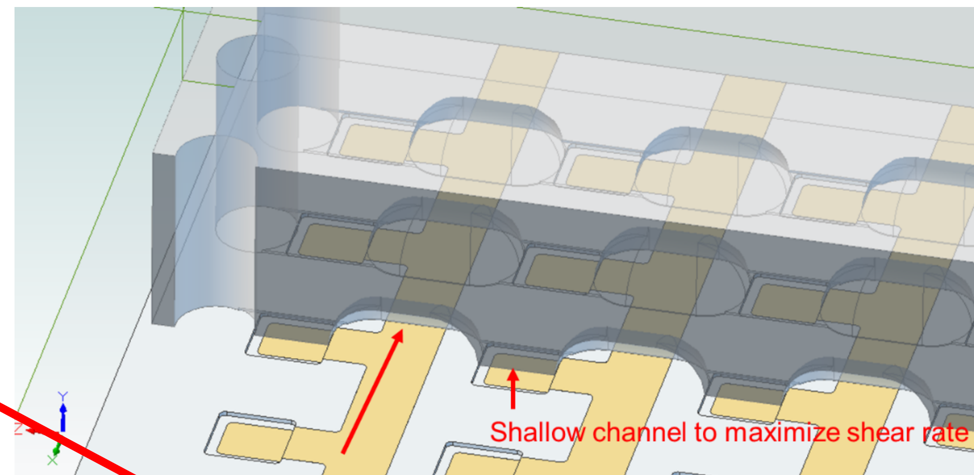
Achievements - Task 6.1 Microfluidics and assembly

$\Rightarrow$ And localize pressure drop to sensing site only

Just yields 50pN

At the burst limit

Just 25µm flow cell



Wide channel as bubble trap PLUS low pressure drop

Scenario channel per 6 sensors

Pressure drop calc for laminar flow only

			dp [mbar]	dp [bar]
η [mPa.s]	1			
v. [µl/min]	1.650	SUM	**1.019,9**	**1,0**

If value set: width of rectangle – otherwise tube

Geometry

round $\tau = \frac{4\eta Q}{R^3}$/pi.

rect. $\tau = \frac{6\eta Q}{h^2 W}$

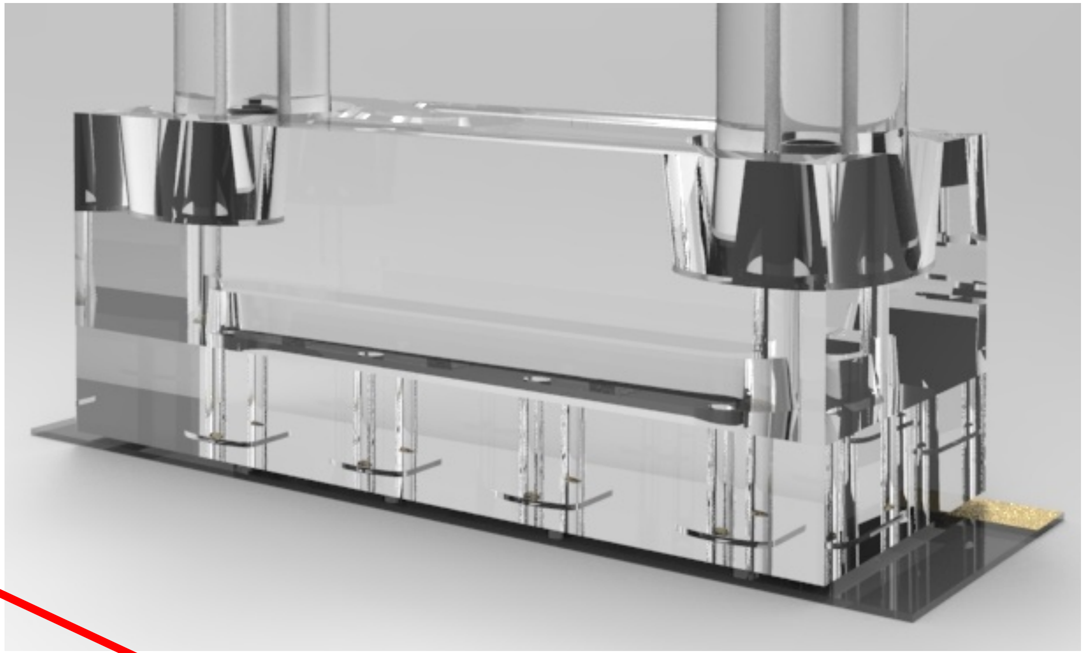
Sphere diameter [um]: 0,2

$F_d = 6\pi\,\eta\,R\,v$

	l [mm]	d/a [µm]	b [µm]	dp [mbar]	dp [bar]	% pD	v [mm/s]	Re	Shear rate [s-1]	Stokes drag [pN]
conn. Tube	50	500		8,964	0,009	0,9%	140,1	70,0	2,24	
in via	0,4	225		1,749	0,002	0,2%	691,6	155,6	24,59	
Flow cell S1 6x	**6**	**25**	**1000**	998,514	0,999	97,9%	1.100,0	53,7	264,00	50
out via	0,4	225		1,749	0,002	0,2%	691,6	155,6	24,59	
conn. Tube	50	500		8,964	0,009	0,9%	140,1	70,0	2,24	

Achievements - Task 6.1 Microfluidics and assembly

$\Rightarrow$ Flow cell for each single sensing site



Yields 100pN

Savely < burst limit

Scenario channel per 1 sensors

Pressure drop calc for laminar flow only!

			dp [mbar]	dp [bar]
n [mPa.s]	1			
v. [µl/min]	3.300	SUM	375,7	0,4
		If value set: width of rectangle – otherwise tube		

Geometry

round $\tau = \frac{4\eta Q}{R^3}$/pi.

rect. $\tau = \frac{6\eta Q}{h^2 W}$

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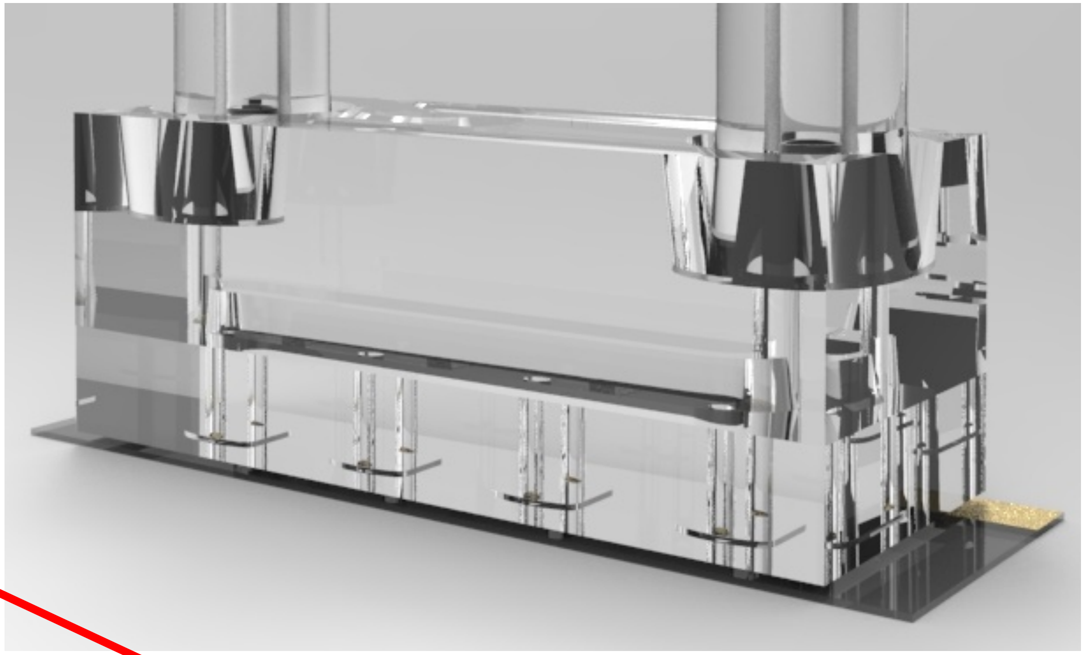
conn. Tube	50	500		17,927	0,018	4,8%	280,1	140,1	4,48	
in via	0,4	225		3,497	0,003	0,9%	1.383,3	311,2	49,18	
Flow cell S1	**1**	**25**	**1000**	332,838	0,333	88,6%	2.200,0	107,3	528,00	99
out via	0,4	225		3,497	0,003	0,9%	1.383,3	311,2	49,18	
conn. Tube	50	500		17,927	0,018	4,8%	280,1	140,1	4,48	

$F_d = 6\pi\eta R v$

Achievements - Task 6.1 Microfluidics and assembly

⇒ Flow cell for each single sensing site



Yields 100pN

Savely < burst limit

Scenario channel per 1 sensors

Pressure d[illegible] calc for laminar flow only!

n [mPa.s]	1		dp [mbar]	dp [bar]
v. [µl/min]	3.300	SUM	375,7	0,4
		If value set: width of rectangle – otherwise tube		

Geometry
round $\tau = \frac{4\eta Q}{R^3}$/pi.
rect. $\tau = \frac{6\eta Q}{h^2 W}$

Sphere diameter [um]: 0,2

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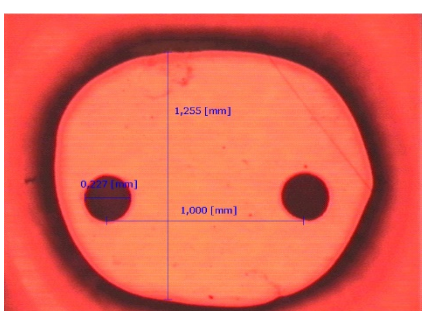
$F_d = 6\pi\eta R v$

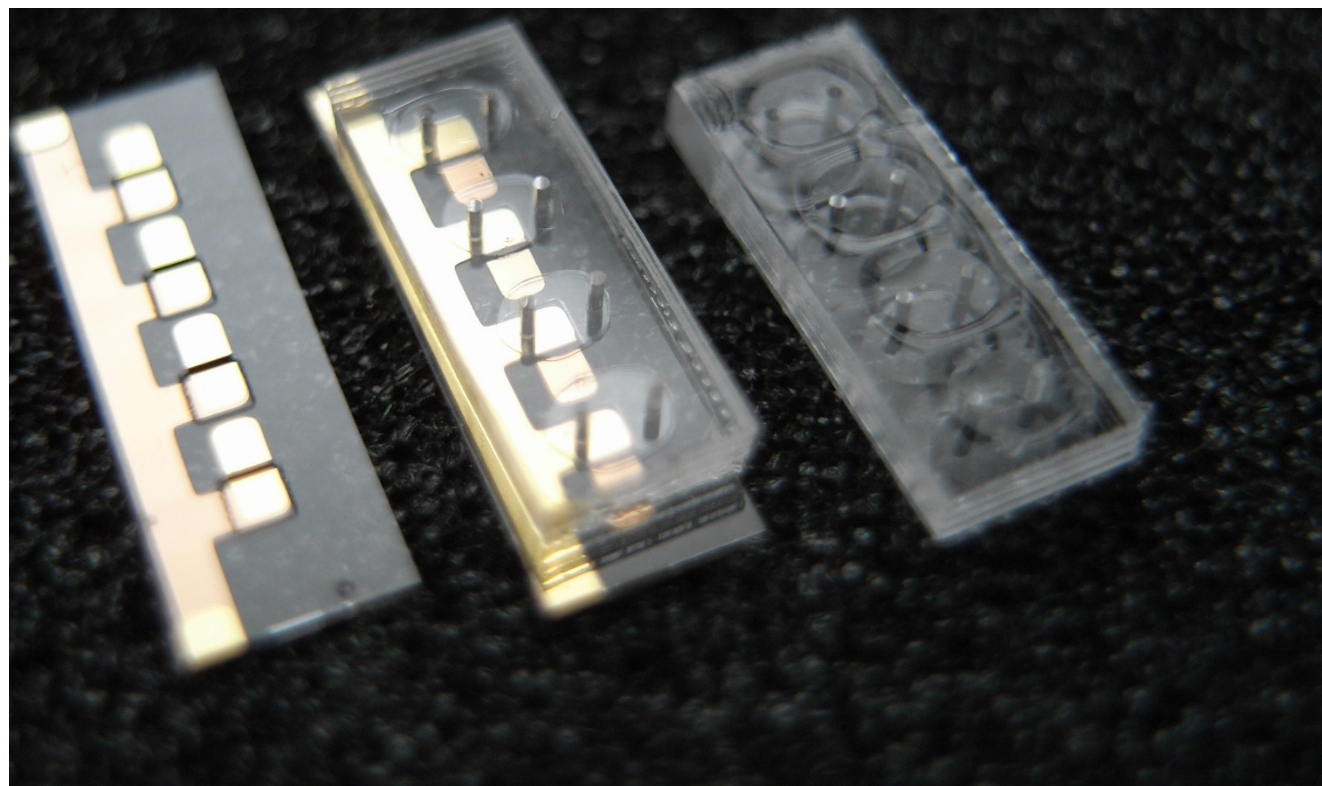
Achievements - Task 6.1 Microfluidics and assembly

⇒ Flow cell for each single sensing site

First prototype made

25µm flow cell realized by dispensing of adhesive rubber to flow cell body





Achievements - Task 6.1 Microfluidics and assembly

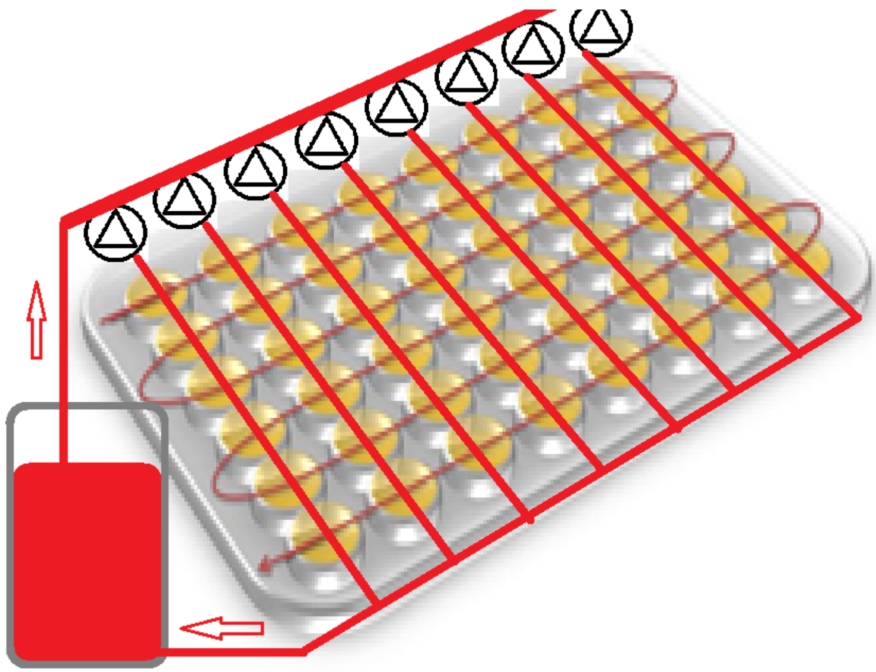
Conclusion:

- Required benchmarks elaborated
- Flow cells for AWS 24x and 4x chips designed
- Manufactured
- And assembled
- Successfully
- Performance follows expectations

Achievements - Task 6.2 Fluidics control

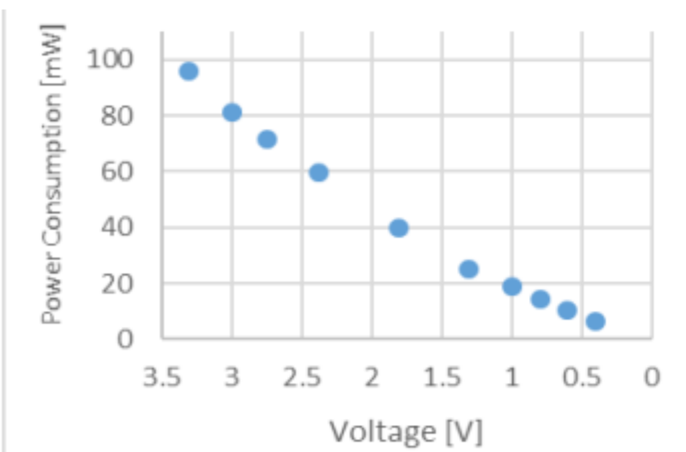
To achieve 100pN drag a total flow rate of 80ml/min is diverted into the 24 flow cells

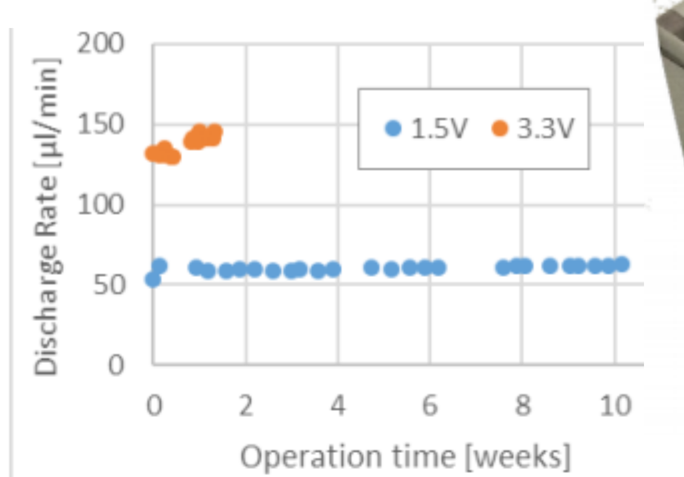
Split into 8 lines feeding 6 flow cells each => 10ml/min per pump

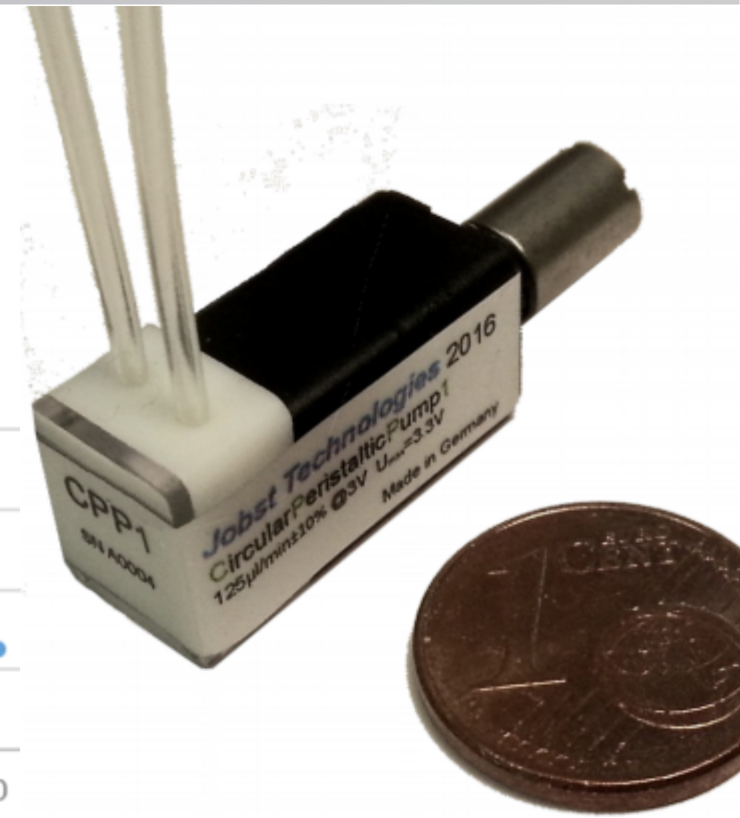


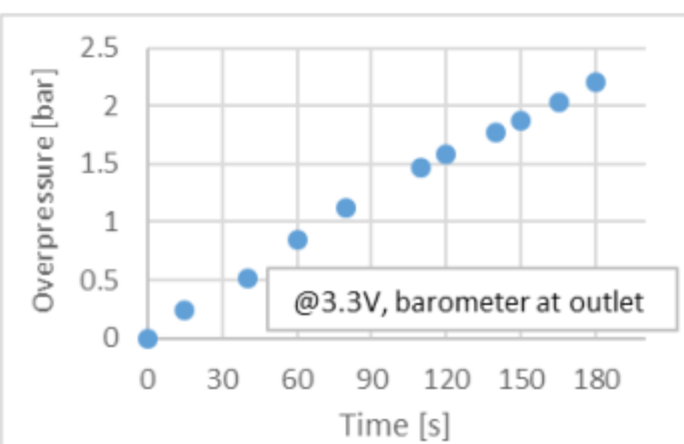
Achievements - Task 6.2 Fluidics control - pumps

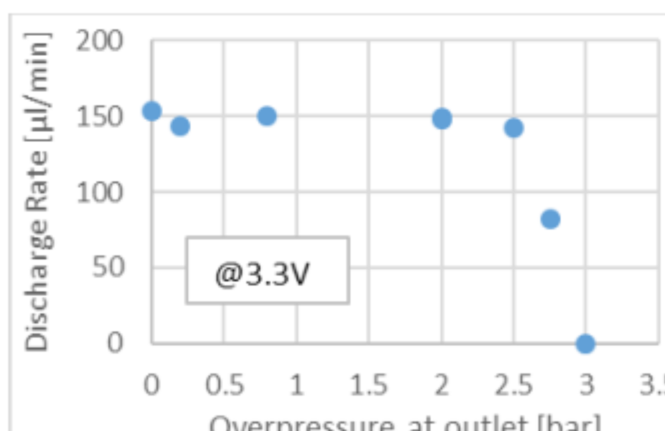
JOBST Micro-pumps performance

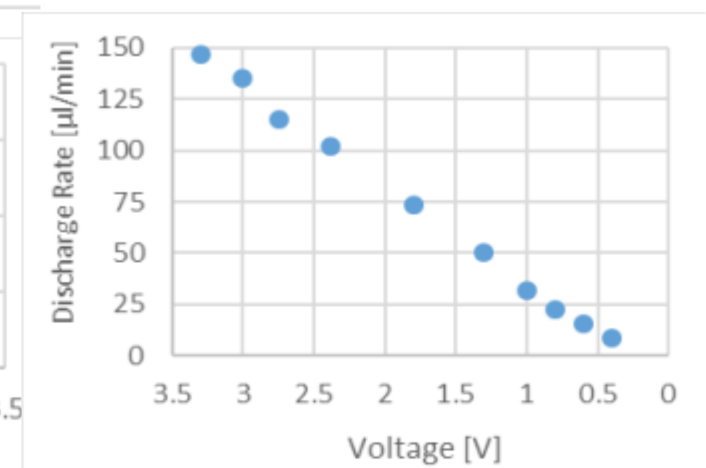






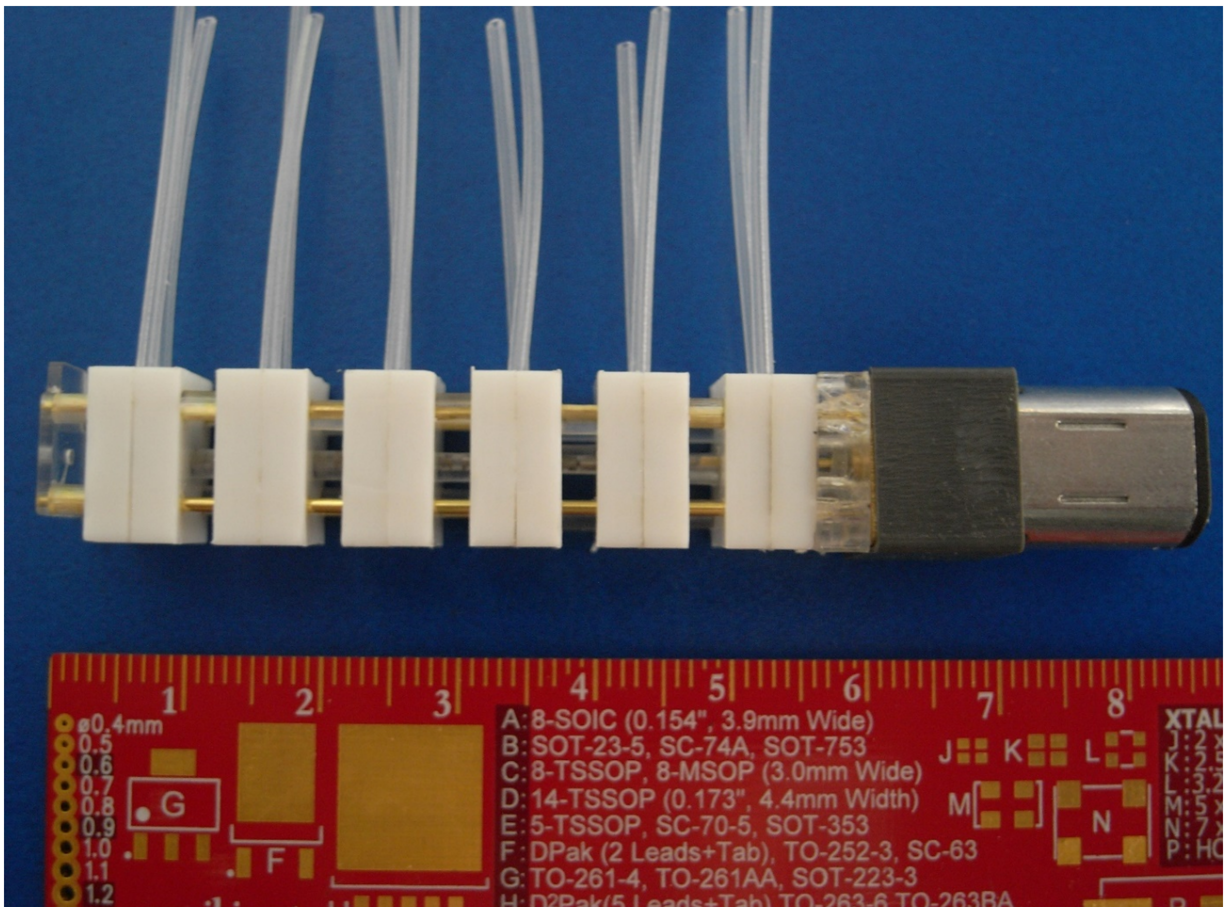






Achievements - Task 6.2 Fluidics control - pumps

The first version of a compact 6 channel multi-head pump



Deviations

- WRT to DOW:
- **Deliverable D6.1** Report on electrical and fluidics assembly providing high signal quality and compatibility with bio-functionalization **(**M15)

- **D6.2** Report on sensitivity increase scaling by local and temporal shear rate engineering (M24)
- **D6.3** Final instrument prototype (M30)

Deviations

- WRT to DOW:
- **Milestone MS11** First generation chip assembly operational (M15)
- **MS12** Sensitivity by shear rate engineering >100 fold (M24)
- **MS13** Demonstration of electronics control unit (M30)

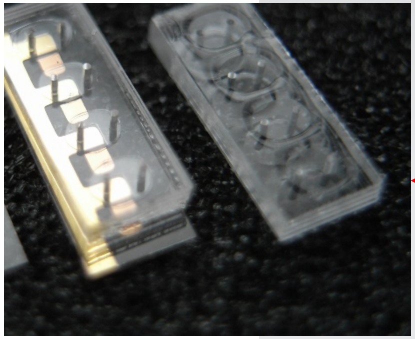
Future plans - Task 6.1 Microfluidics and assembly

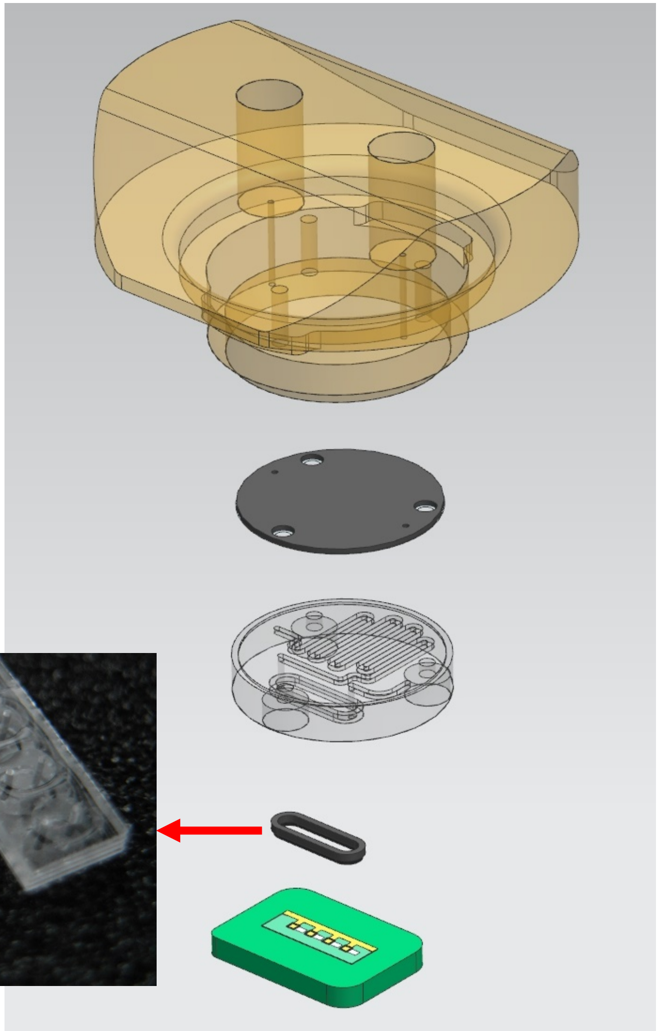
Realize integrated

Flow channel + seal manifold
For:

- Optimized shear @ low pressure drop
- Air bubble tolerant design
- Sealing
- Internal volume
- Thermal equilibration

Consider DNA electro-sorption





Future plans - Task 6.2 Fluidics control

- Get full process flow from sampling to readout elaborated

- Benchmark multi-channel pump

- Design pump heads delivering flow only for <50% of rotation
    - To enable >10ms resting period

Backup content…

T 6.1 Microfluidics and assembly
Shear force assisted performance improvement

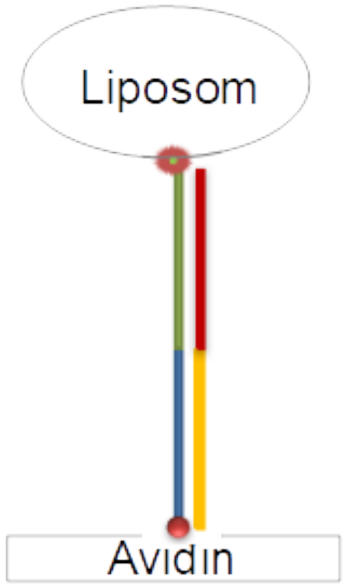
Kinds of binding: **unspecific binding** of DNA, micro-particles:

- Minimizing the surface to volume ratio of the fluidics minimizes analyte loss due to unspecific binding to walls.

- Realize drag forces in the range of 1-100 pN to detach unspecific bound microspheres[a]

a) M. Javanmard, F. Babrzadeh, R.W. Davis, "Microfluidic force spectroscopy for characterization of biomolecular interactions with piconewton resolution" Appl. Phys. Lett. 97, 173704

T 6.1 Microfluidics and assembly
Shear force assisted performance improvement



Method b: Direct immobilization to a Avidin-modified surface

Threshold contact times for bond formation:

10 milliseconds for antibody coated 4.5 microns micro-spheres[b)]

Shear stress resistance:

sensor surface- avidin (adsorption on Au)+

avidin/ biotin@5' DNA +++

cholesterol@3' DNA / liposom ?

b)..A. Pierres, A. Benoliel, C. Zhu, P. Bongrand, "Diffusion of Microspheres in Shear Flow Near a Wall: Use to Measure Binding Rates between Attached Molecules" Biophysical Journal 2001, 81, 25– 42