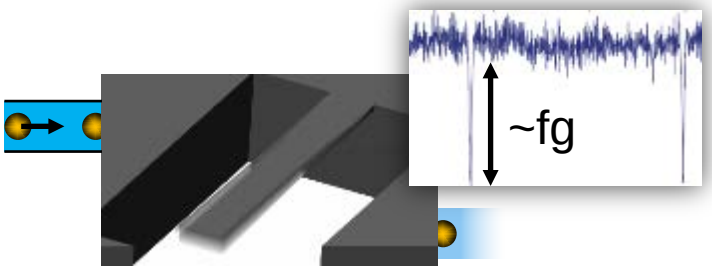
# Max Planck Institute for Biophysical Chemistry

Thomas P. Burg

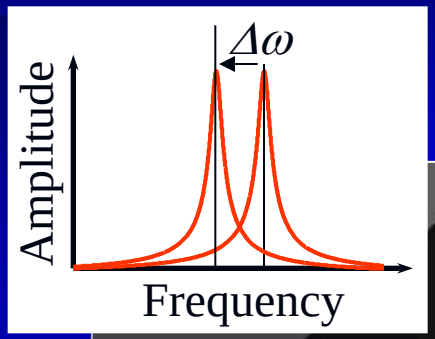
***Microfluidics for nanoparticle characterization***



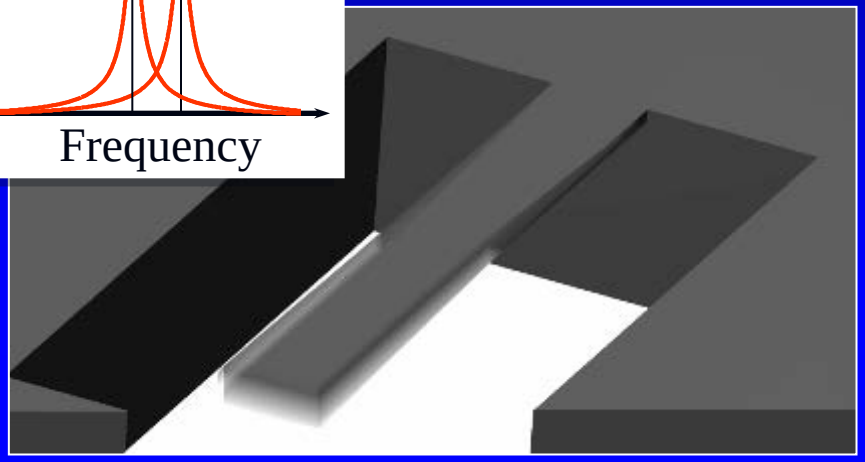

***Nanofluidic methods for membrane separation***

***Cryofixation with high time-resolution***

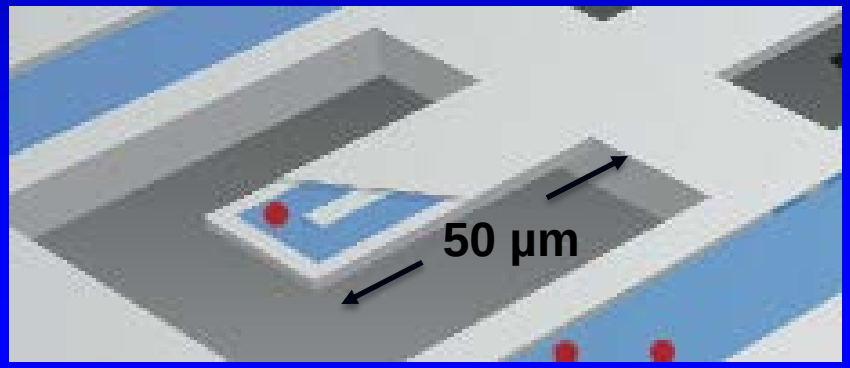
# Nanomechanical Mass Sensing




**In air or vacuum**

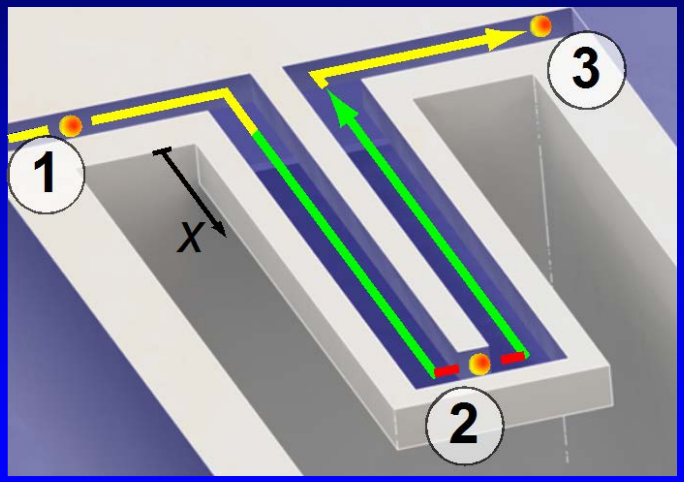


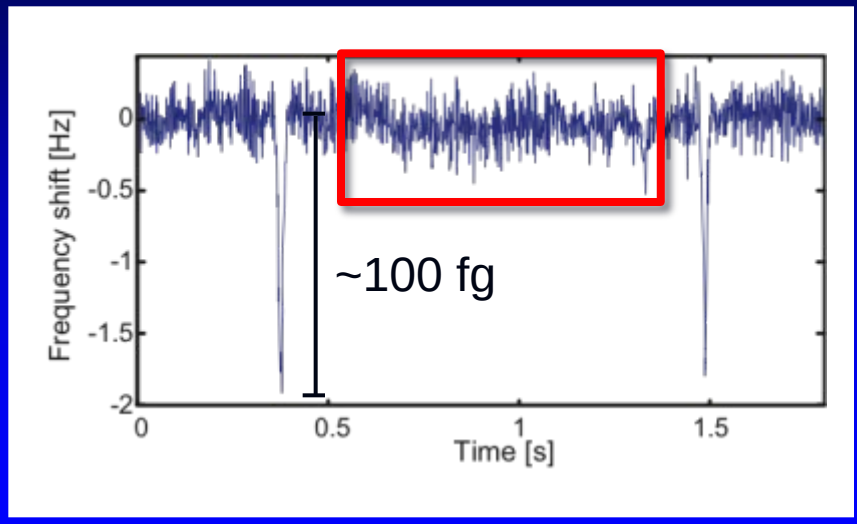
**In liquid**

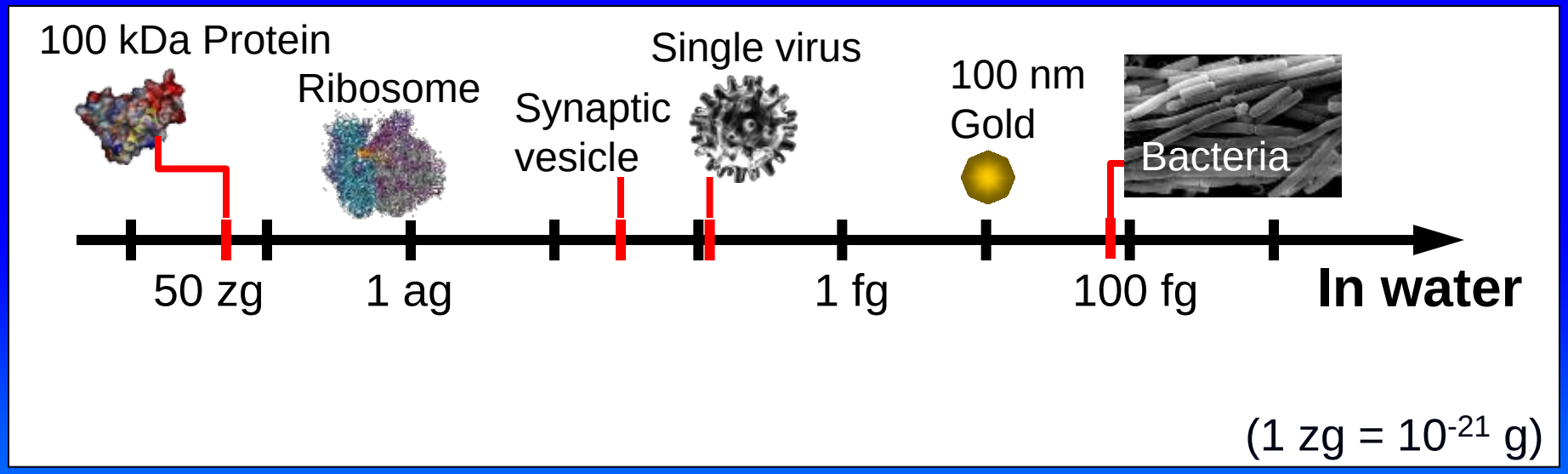



- Vibrating beam: Change in mass → change in resonance frequency
- Sensitivity improves with mass of the device (smaller is better)
- Damping limits resolution. Sensing in liquid is therefore challenging.

# Sensitivity of Single Particle Measurements



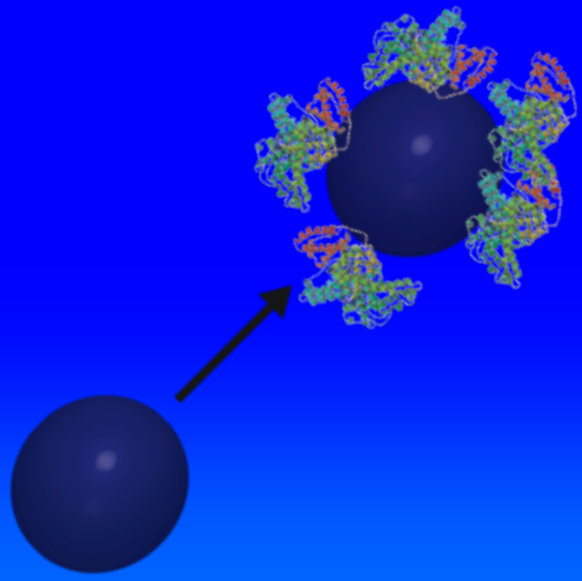



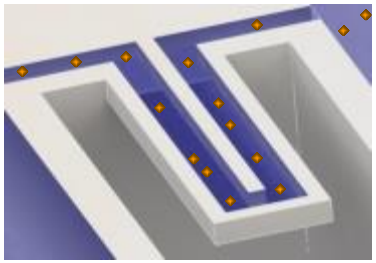
# *Not all applications require single-particle detection. For example:*

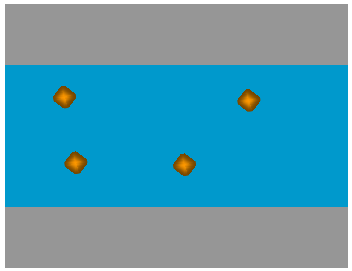
- *Label-free measurement of **polymerization** and **protein aggregation***
- ***Detection** of particulate contamination*
- *Characterizing **mass** and **size** of nanoparticles in solution*
- *Measuring **adsorption/dissolution** of coatings on particles*

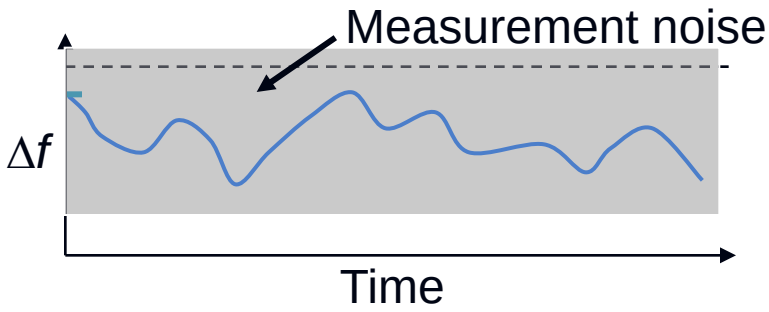
Protein aggregation



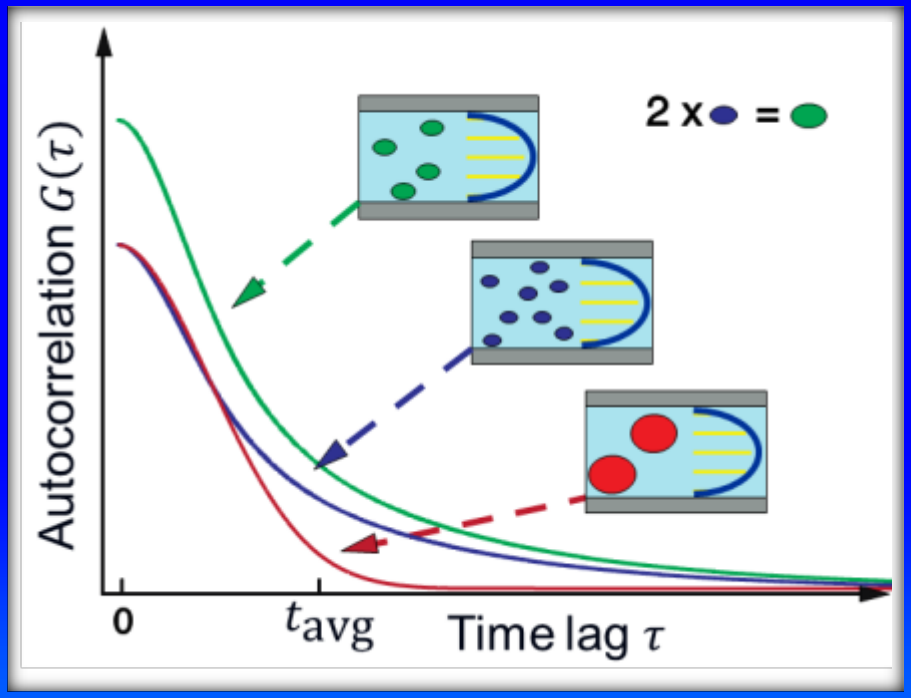
# Beyond the single-particle limit








***High concentration, light particles (below noise floor)***

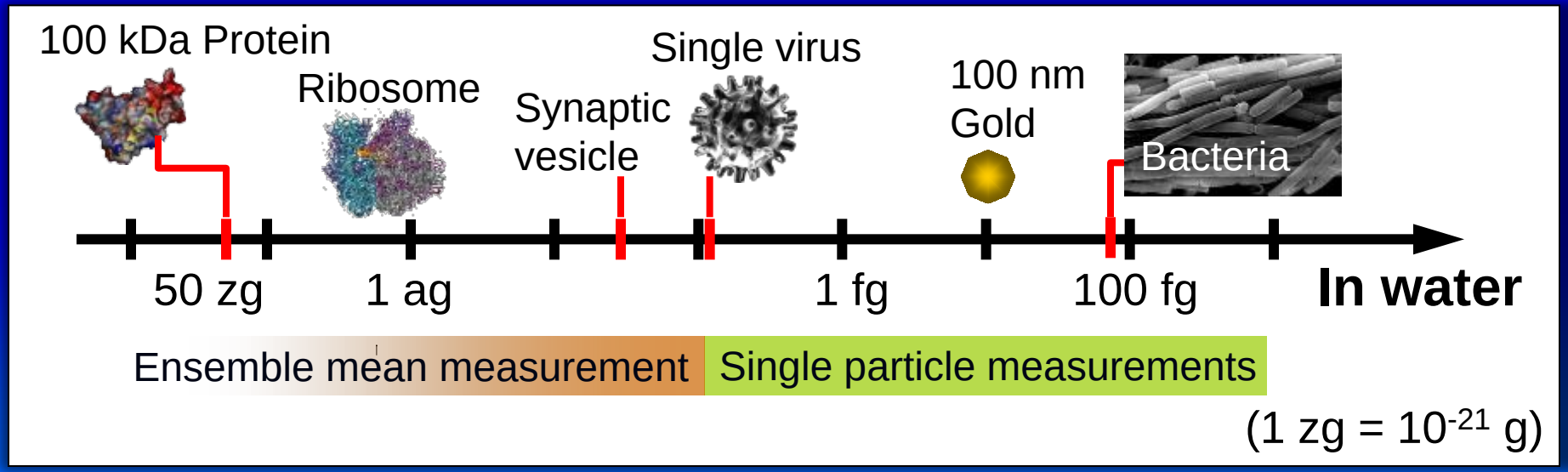



***Correlation analysis allows detection of particles far below the single-particle limit.***

Amplitude → Mass

Width: $t_{avg}$ depends on

- flow rate
- Particle size
- diffusivity


100 kDa Protein
Ribosome
Synaptic vesicle
Single virus
100 nm Gold
Bacteria
50 zg
1 ag
1 fg
100 fg
In water
Ensemble mean measurement
Single particle measurements
(1 zg = 10-21 g)

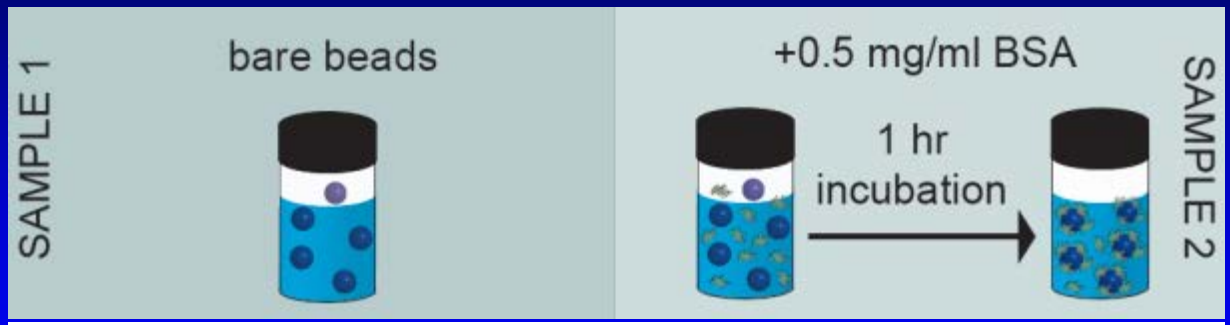
# Measuring protein adsorption

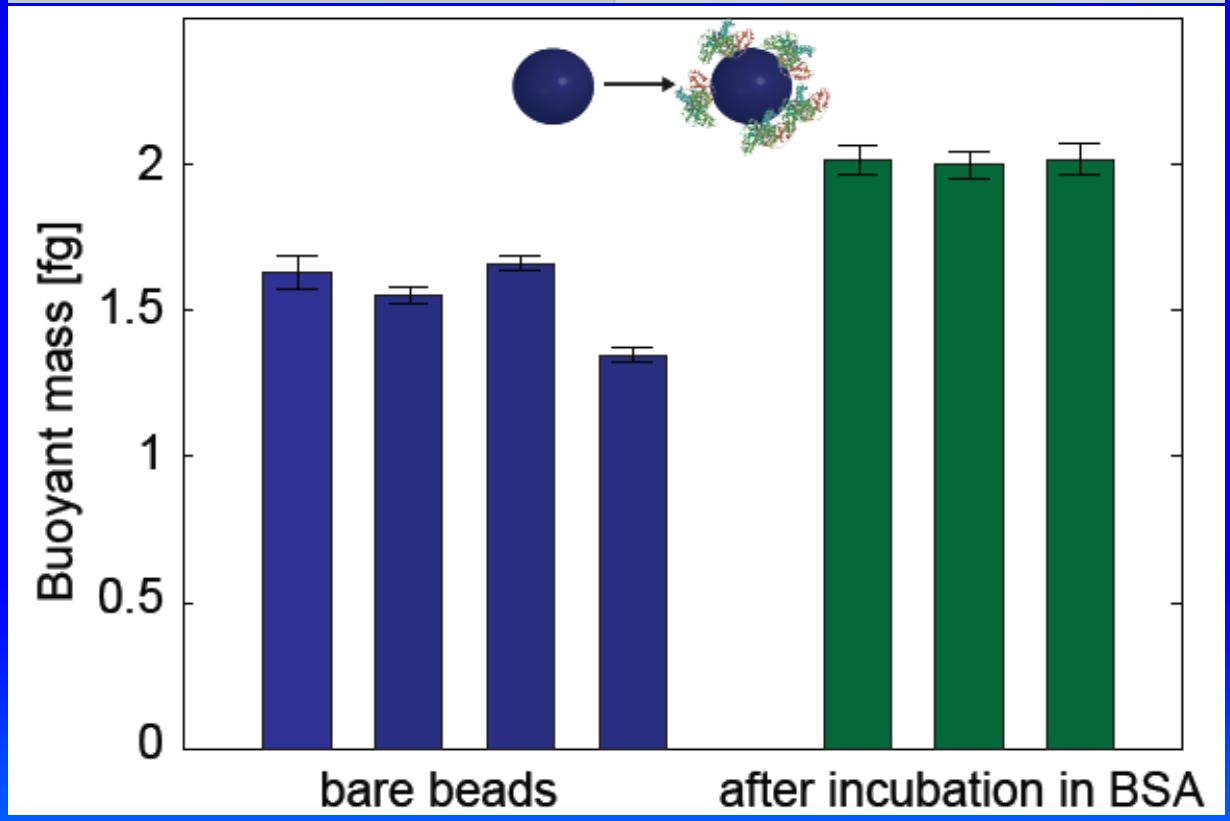
**Polystyrene beads**
Diameter = 390 nm
Concentration = $3 \cdot 10^9$ $mL^{-1}$

**Incubation in**
0.5 mg/mL BSA

**Added mass:**
0.46 ± 0.15 fg

**Surface coverage:**
380 ± 120 ng/$cm^2$



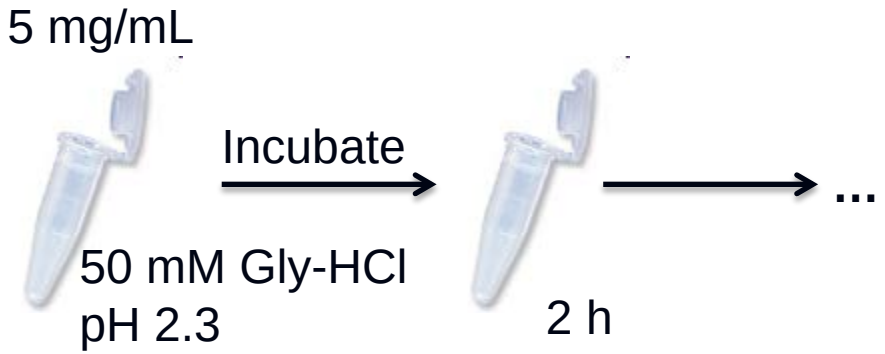
# *Monitoring Insulin aggregation by Mass Correlation Spectroscopy*

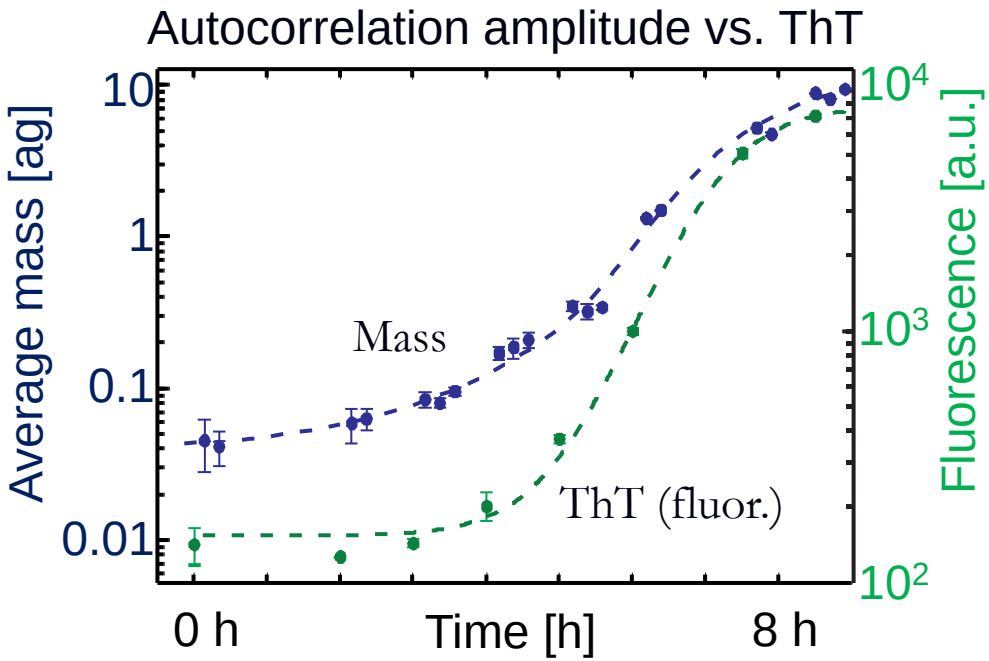
Monomer

Dimer

Oligomer

Fibril

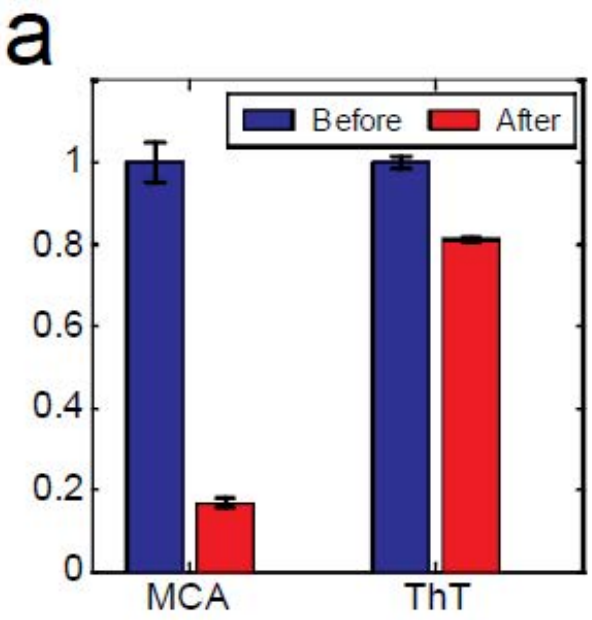


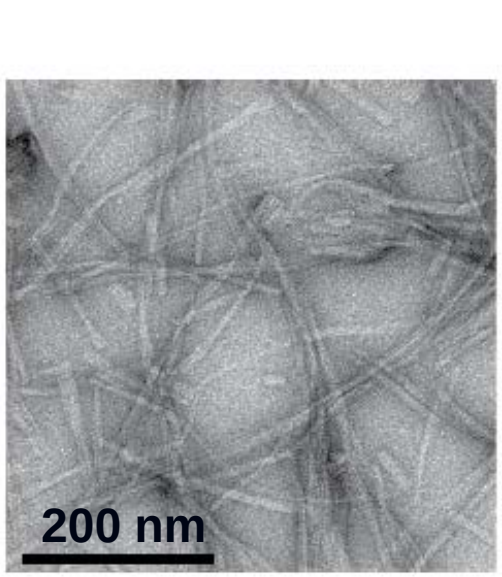

Mario M. Modena et al., *Lab on a Chip*, **14** (2014)

# *Mass Correlation Analysis reveals differences in fibril length not visible with ThT*

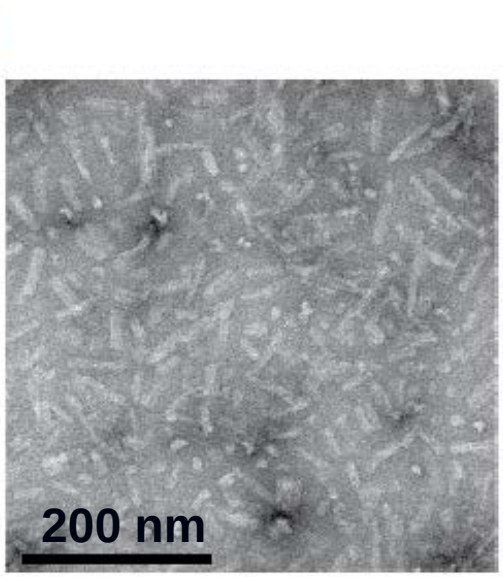
**Comparison Mass / ThT**




**Before Ultrasonication**



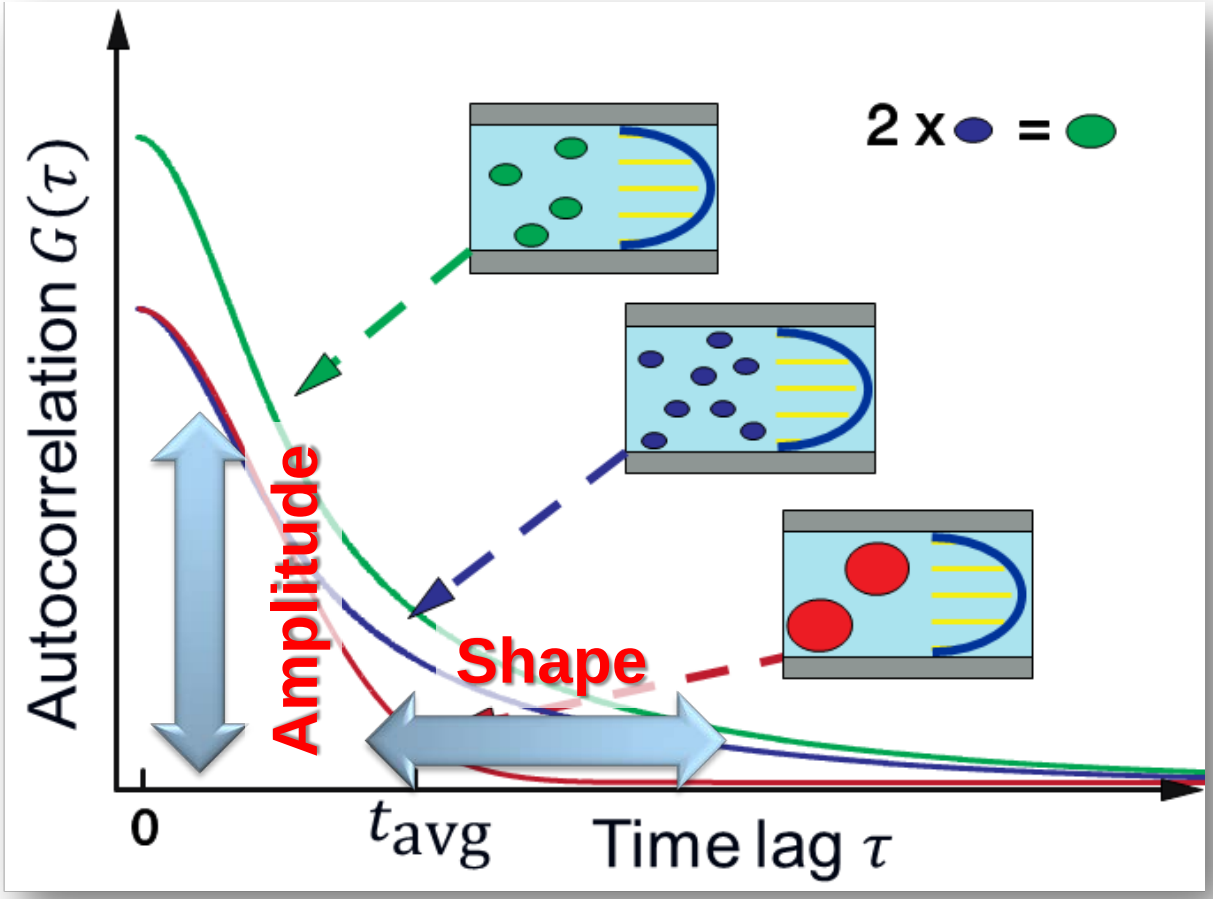

**After Ultrasonication**




***Mass correlation analysis is sensitive to fibril fragmentation.***

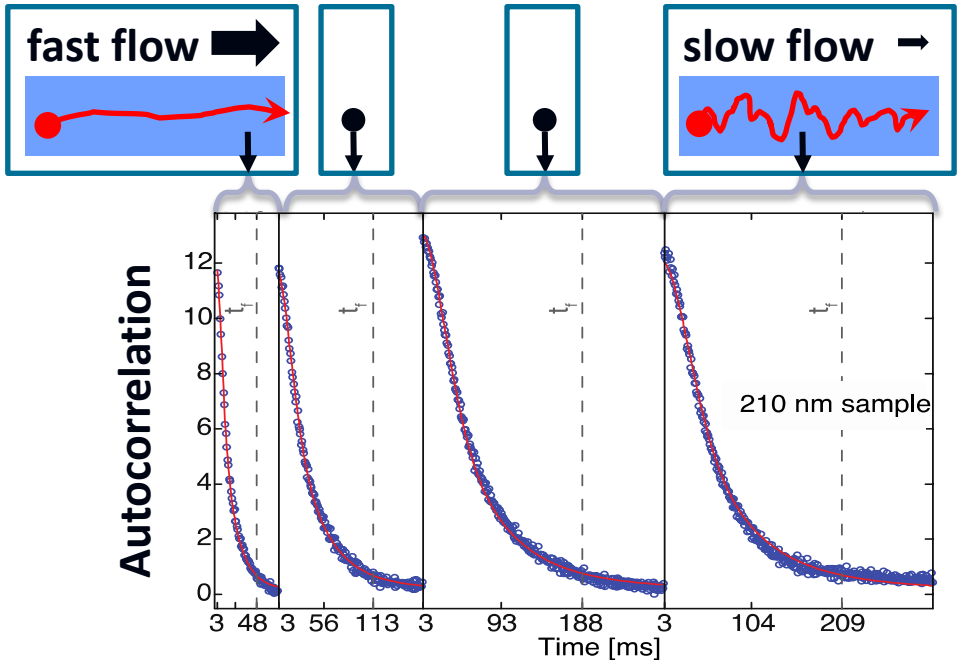
***Mass correlation measurements are more robust to presence of large aggregates than light scattering.***

Mario M. Modena et al., *Lab on a Chip*, **14** (2014)

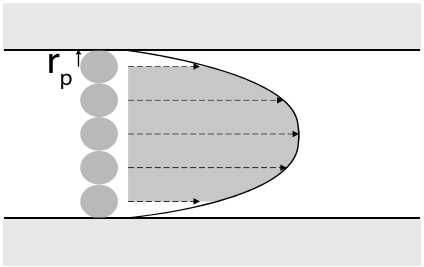

Autocorrelation $G(\tau)$
2 x
=
Amplitude
Shape
0
$t_{avg}$
Time lag $\tau$

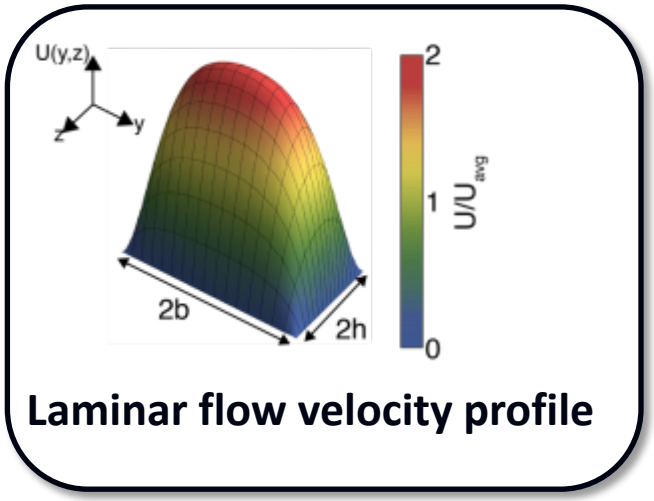
# Residence Time Distribution - An alternative way to measure size by correlation spectroscopy

## 1. Dispersion differences due to diffusion


## 2. Dispersion differences due to steric hindrance

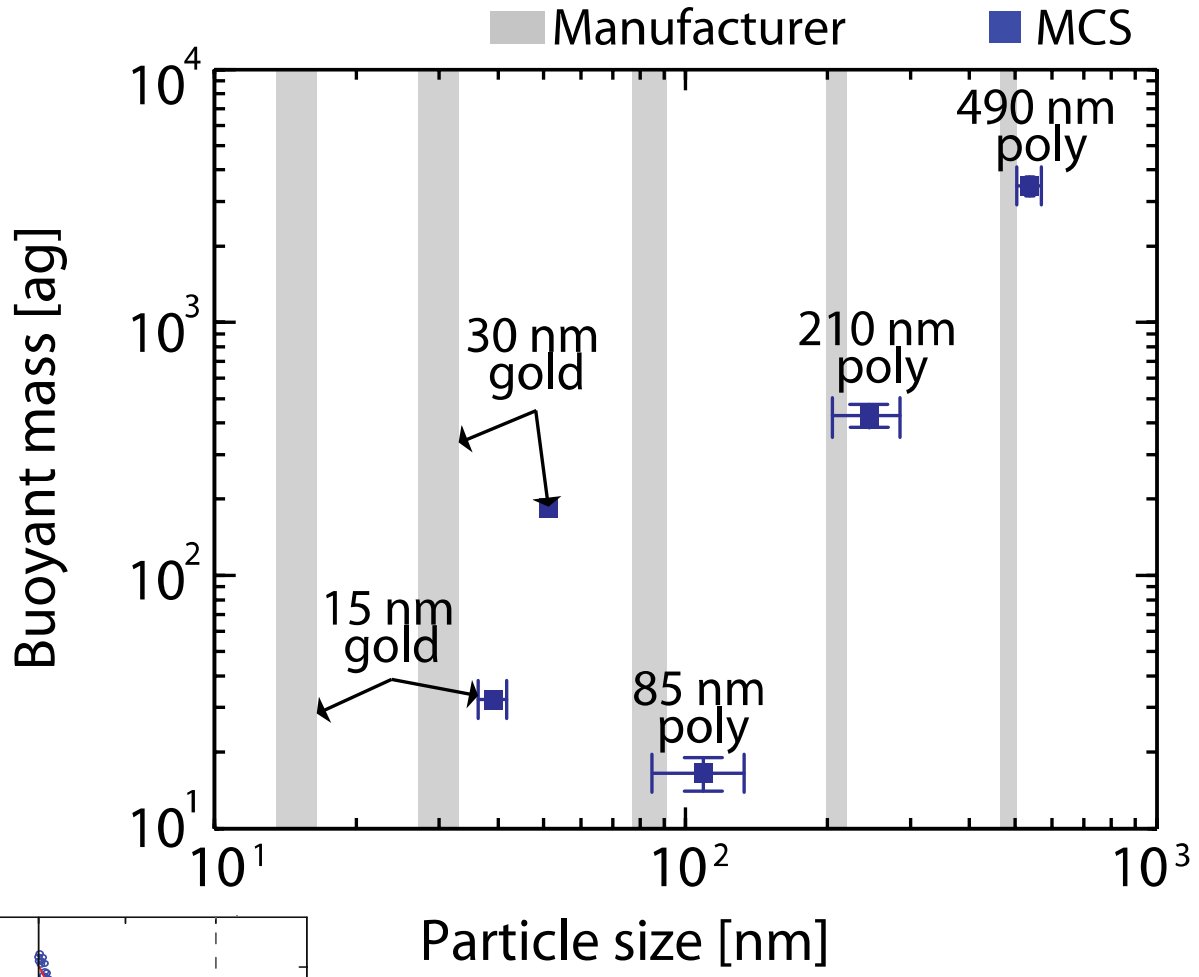


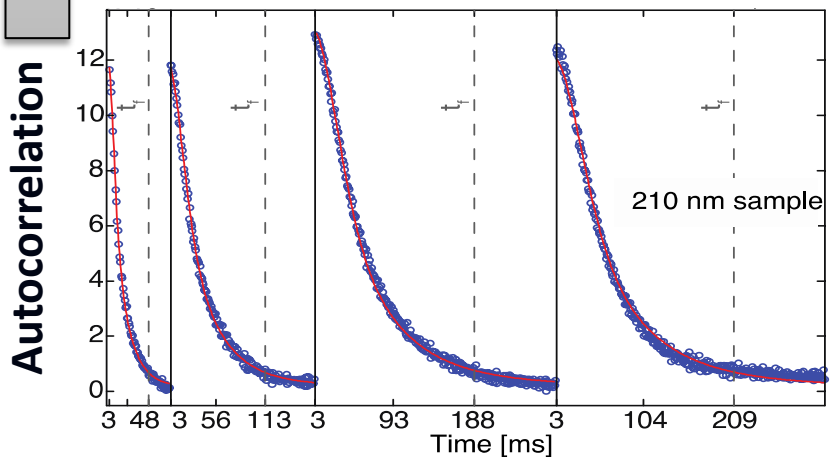




Mario M. Modena, *J. Appl. Phys.* 2015

# *Measuring density directly*

**Amplitude**




**Shape**

Mario M. Modena, *J. Appl. Phys.* 2015

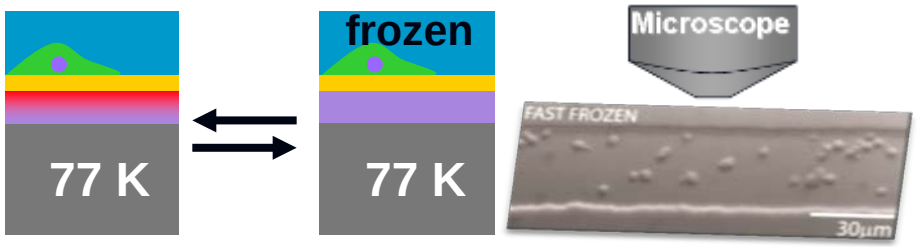
# Max Planck Institute for Biophysical Chemistry

Thomas P. Burg

***Microfluidics for nanoparticle characterization***


***Nanofluidic methods for membrane separation***

***Cryofixation with high time-resolution***



***Existing methods of vitrification (freezing without ice crystals) require physical manipulation of the specimen and have a large dead time.***

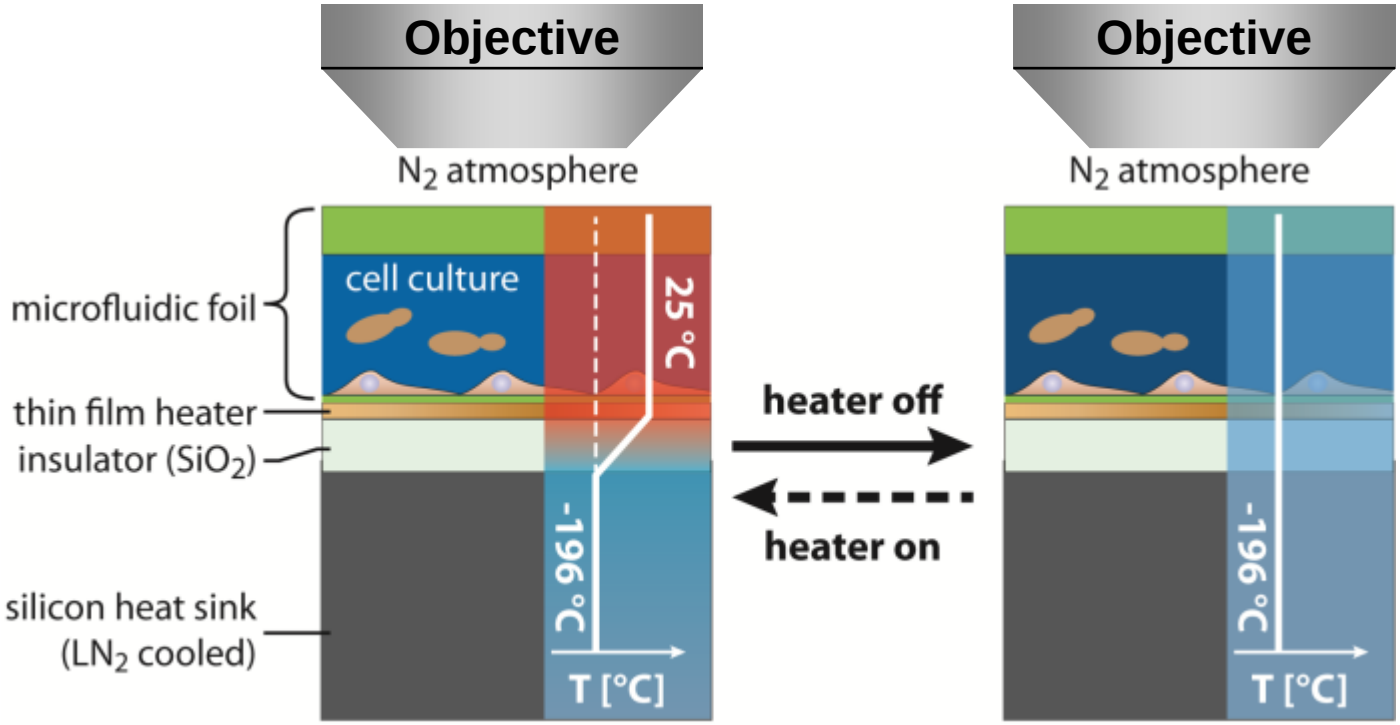
**Plunge freezing (since 1950‘s)**

**$>10^4$ K/s**

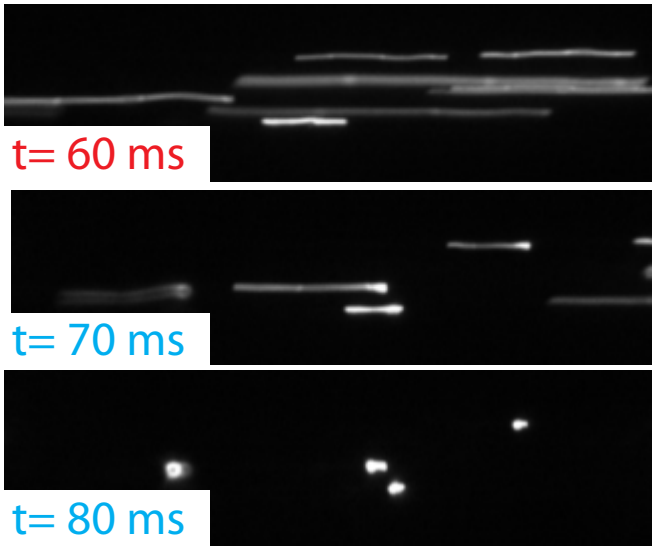
**High pressure freezing (Moor and Riehle 1968)**

**> 2000 bar**

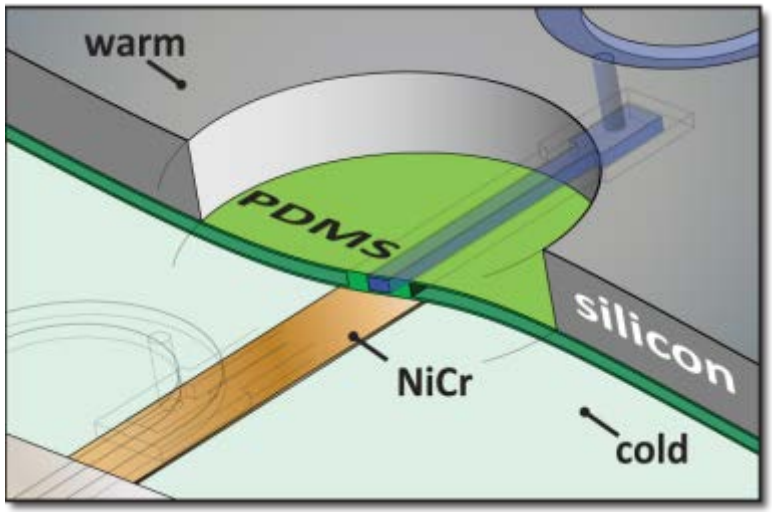
## Microfluidics enables a new concept:



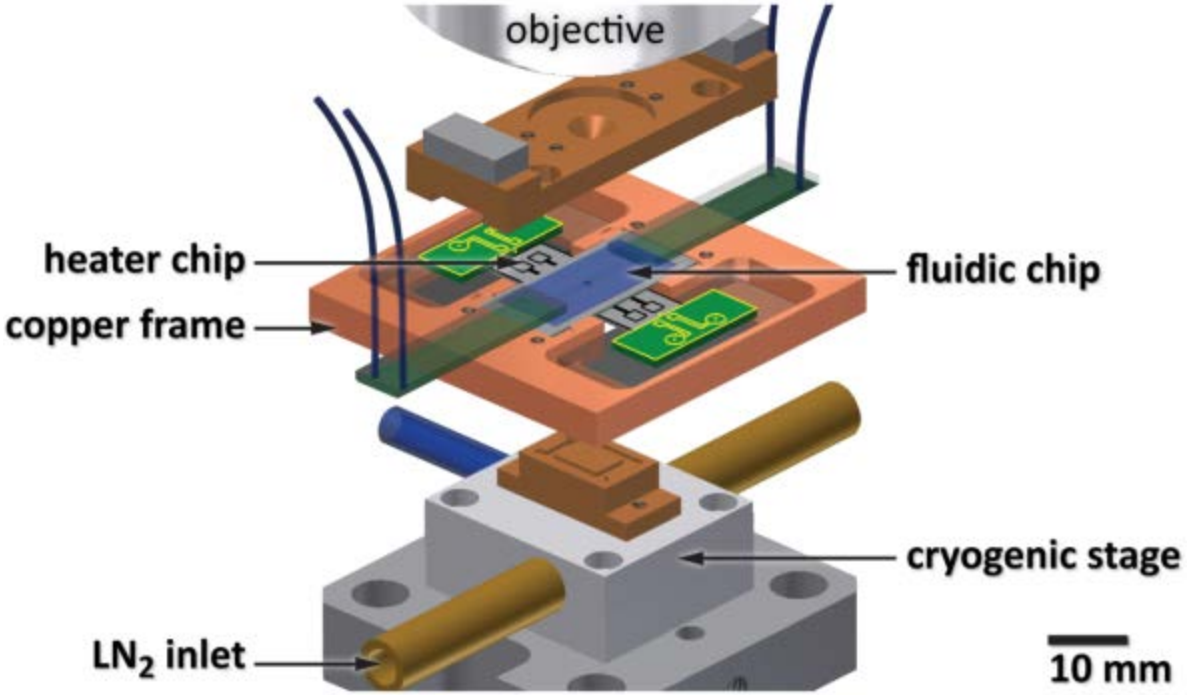

Mejia et al., *Lab on a Chip* 2014




Heater off



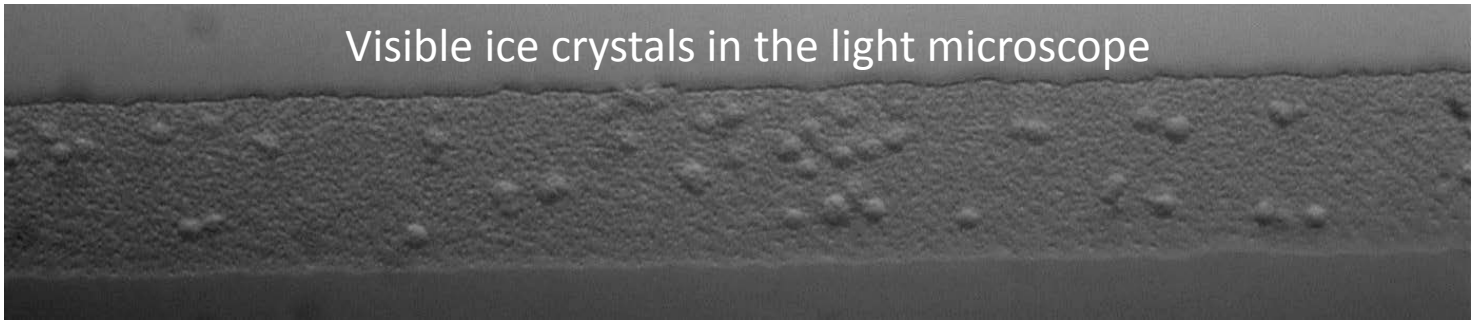

| | |
|---|---|
| **Heated area** | **0.2 x 2 mm** |
| **Power dissipation** | **50 – 100 W** |
| **Channel width** | **30 µm** |
| **Channel height** | **15 – 50 µm** |



# *Suppression of ice crystallization in the light microscope*

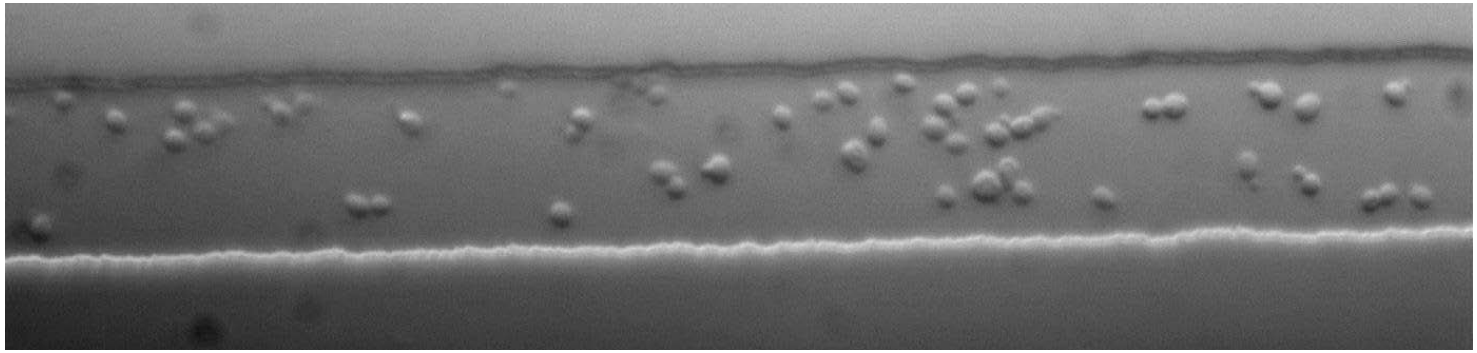
Frozen sample with crystalline ice

neg. control




Heater is turned on
→ sample becomes liquid

Heater is then turned off
→ sample becomes solid.
Sample remains free of visually detectable ice crystals (40x, NA=0.6, C-DIC)



frame rate = 2 Hz