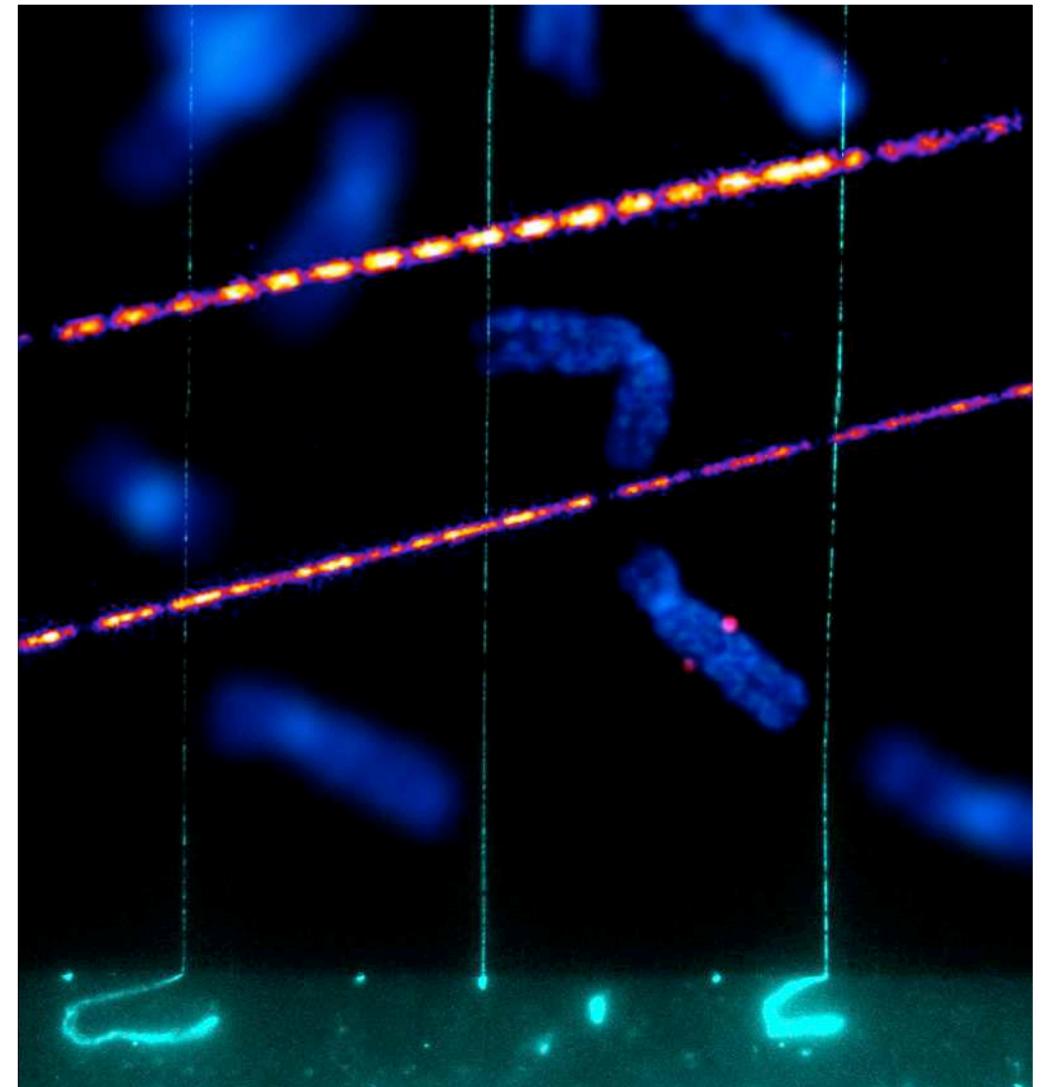


# Nano-2 F1 DNA in Nanofluidics

Rodolphe Marie



# Today's Programme

Time	Topic
8:00	lecture: "Nano-confined DNA"
	Exercise
11:30	Lecture on application (melting mapping)

# Outline

- DNA: a semiflexible polymer
- Motivation for optical mapping of DNA
- Statistical models
- DNA free in solution
- DNA in 1D (nanochannel) confinement
- ... in 2D confinement
- ... in other type of confinements
- Applications overview.

## Keywords:

- Free energy of DNA.
- Entropy barrier.
- Entropic spring and recoil.
- Statistical models: identify contributions.
- Scaling laws and units.
- Persistence length.
- Entropic confinement.
- De Gennes and Odijk regimes.

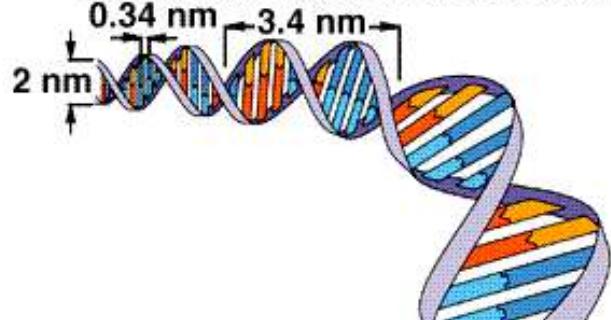


# 'Crash course' in cell biology

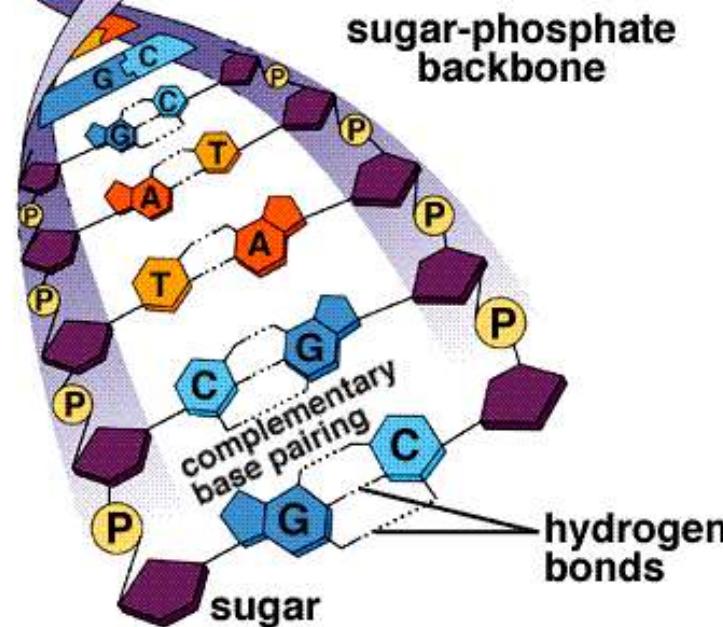


...ATGCGCCTAAT





# Watson and Crick Model of DNA



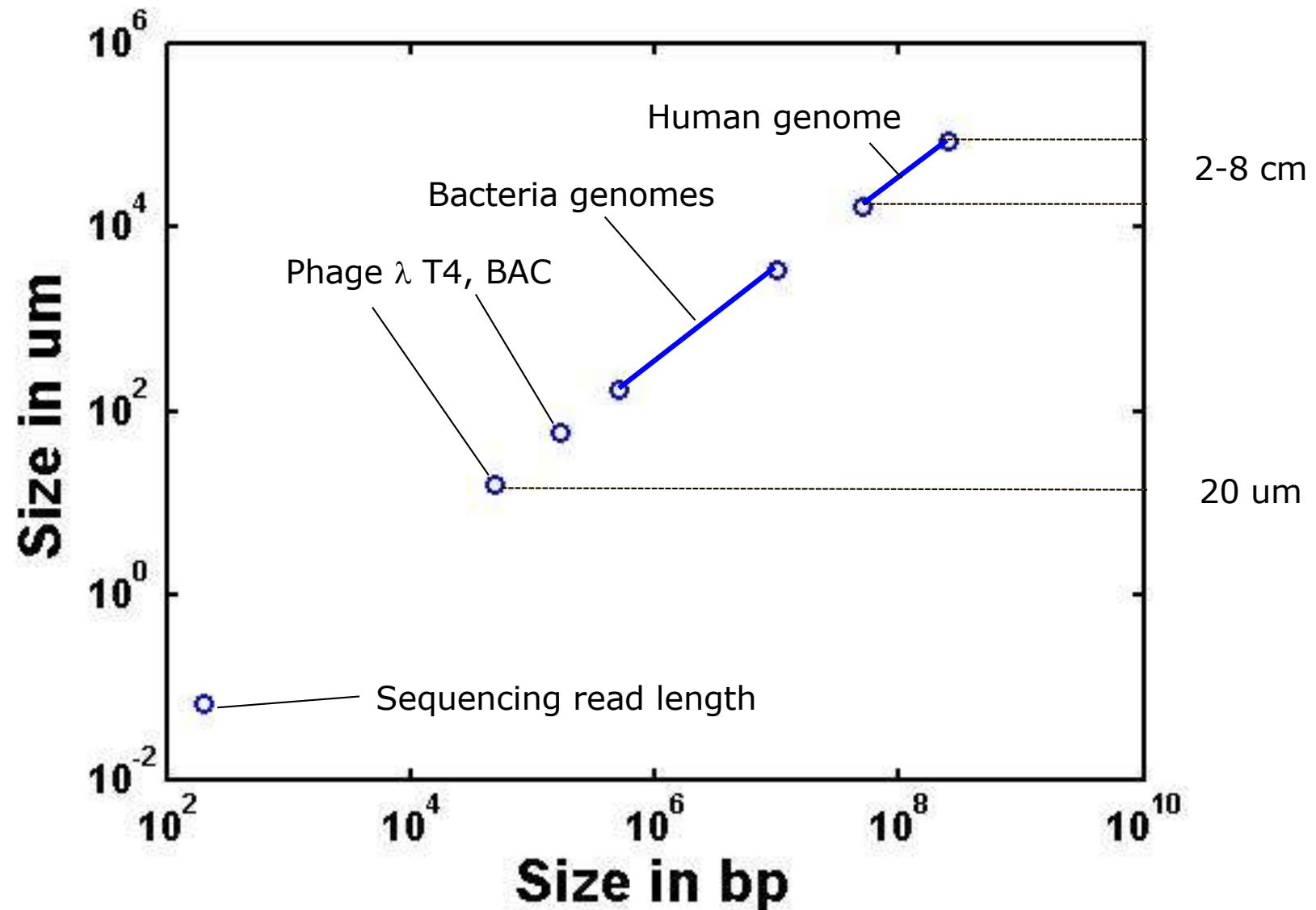
Supercoiling



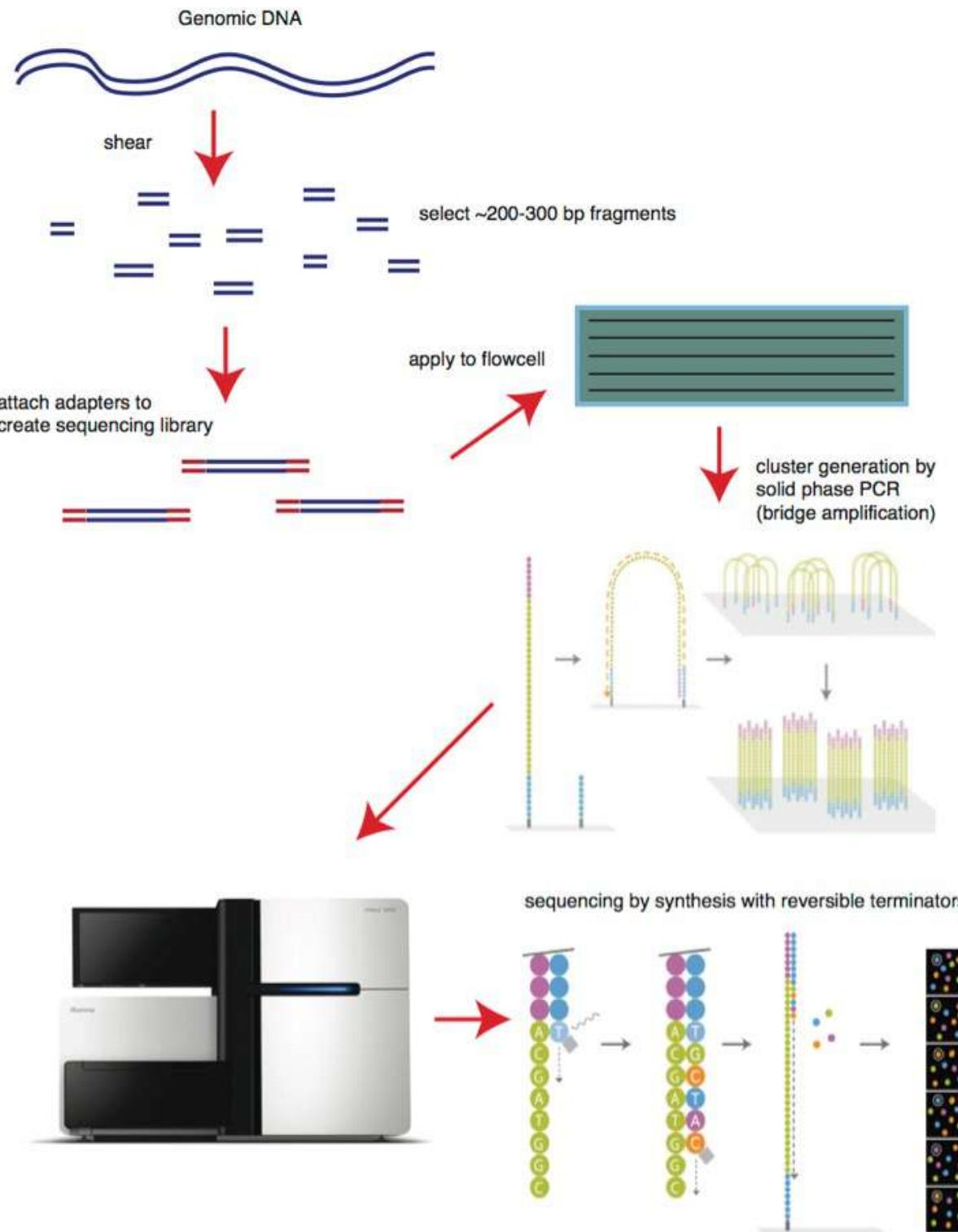
Electrostatic repulsion

Hydrogen bonding of base pairs  
+  
base stacking (hydrophobic/Van der Waals)

# How Long DNA Molecules?

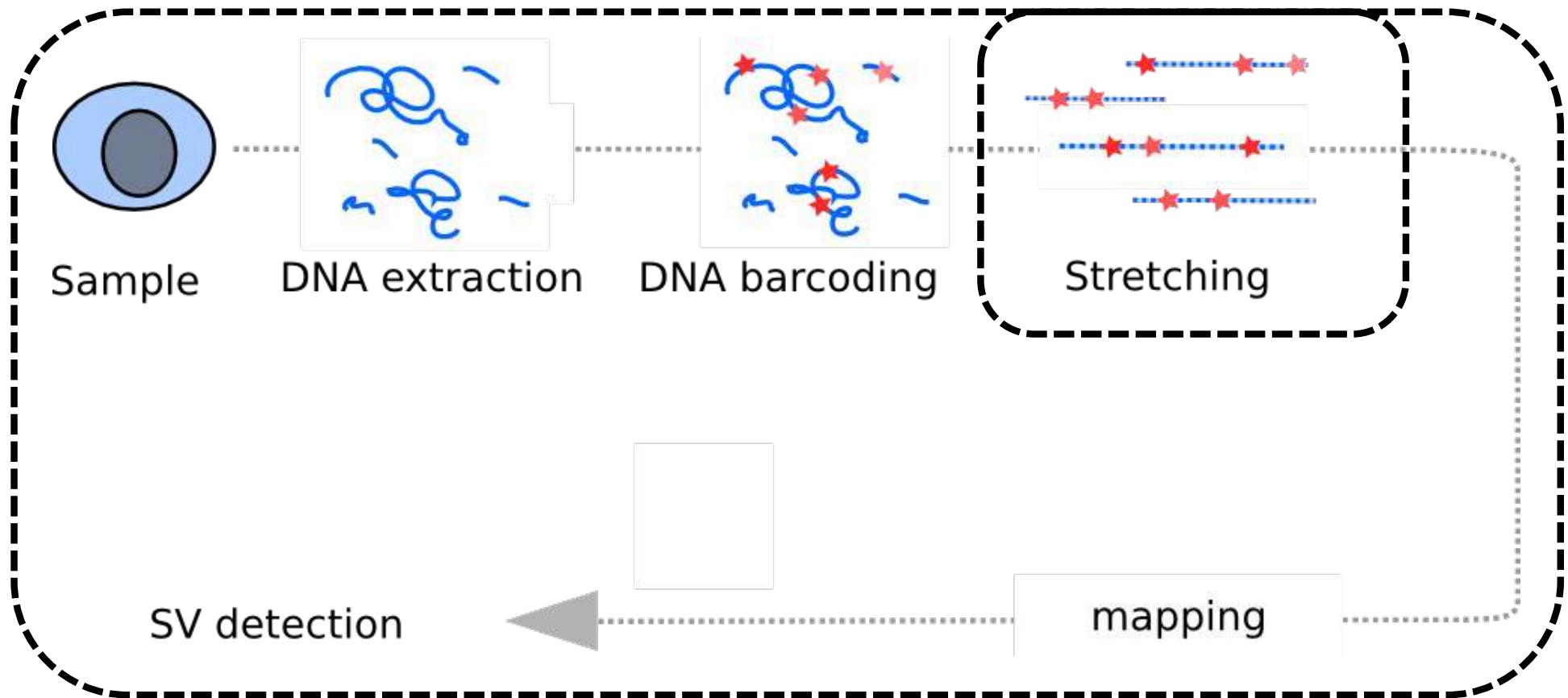


# DNA Sequencing



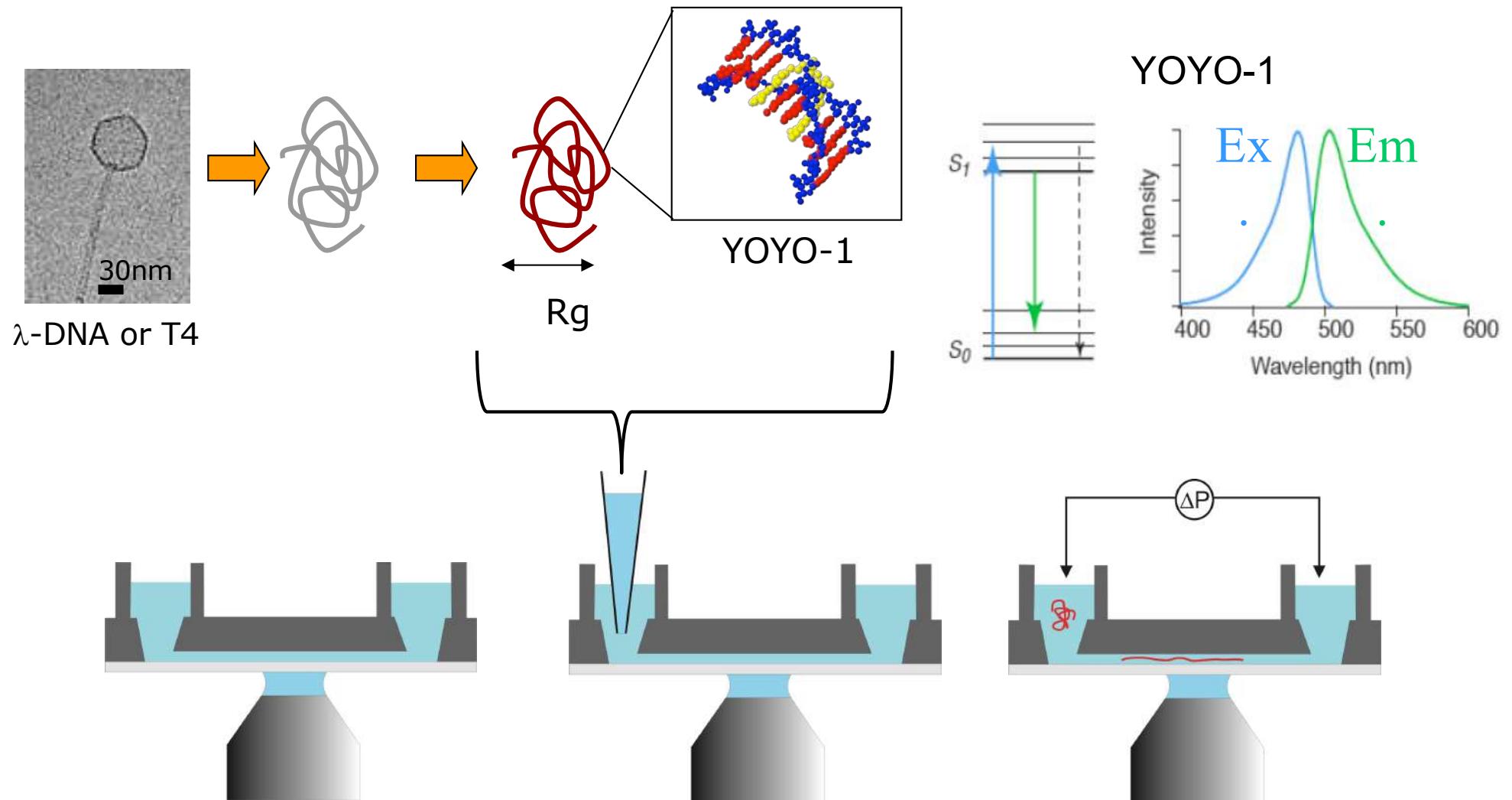
Illumina.com

# What are we aiming at with this technology?

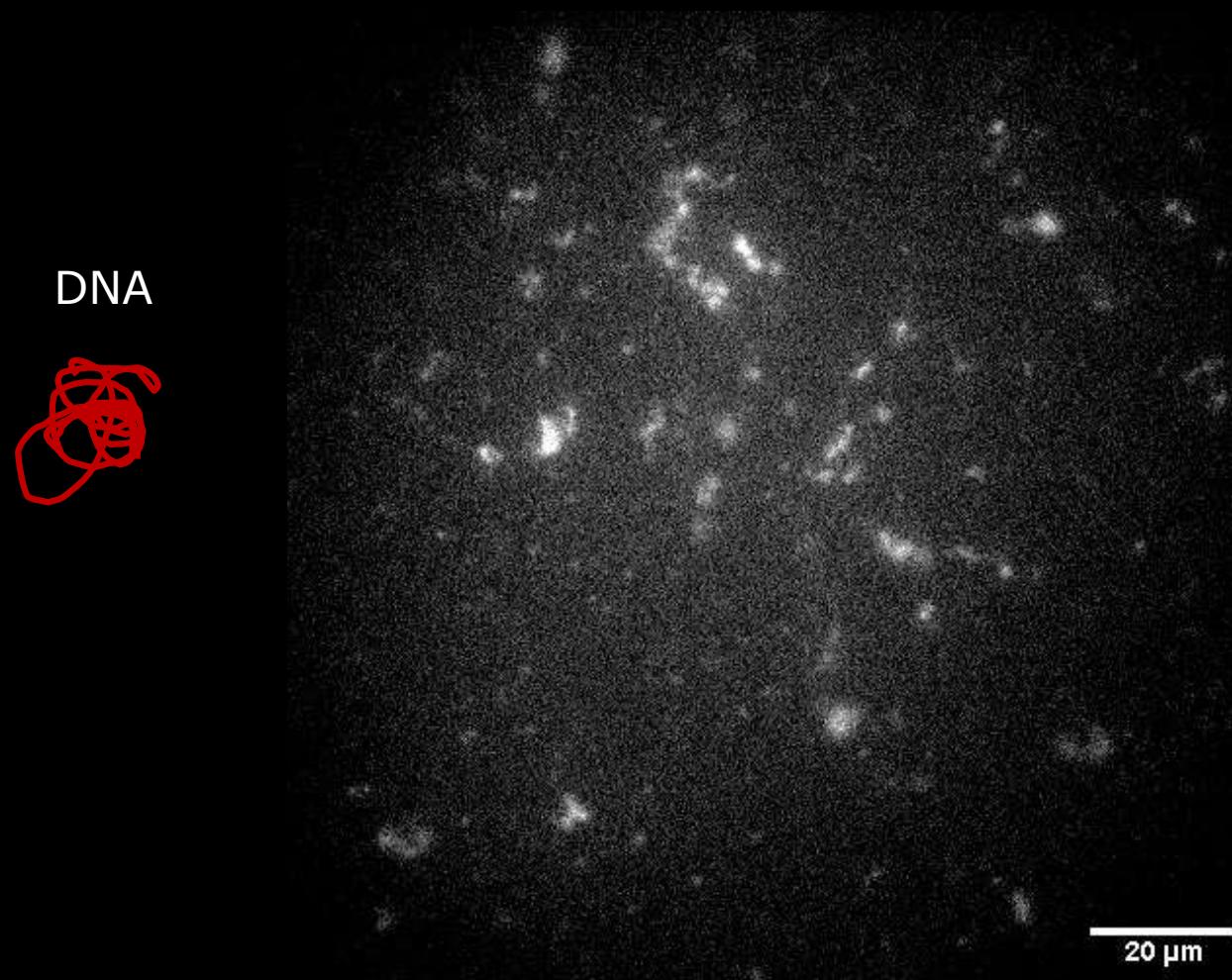


→ To access genome information such as structural variations (SV) on large, continuous and linearized DNA molecules.

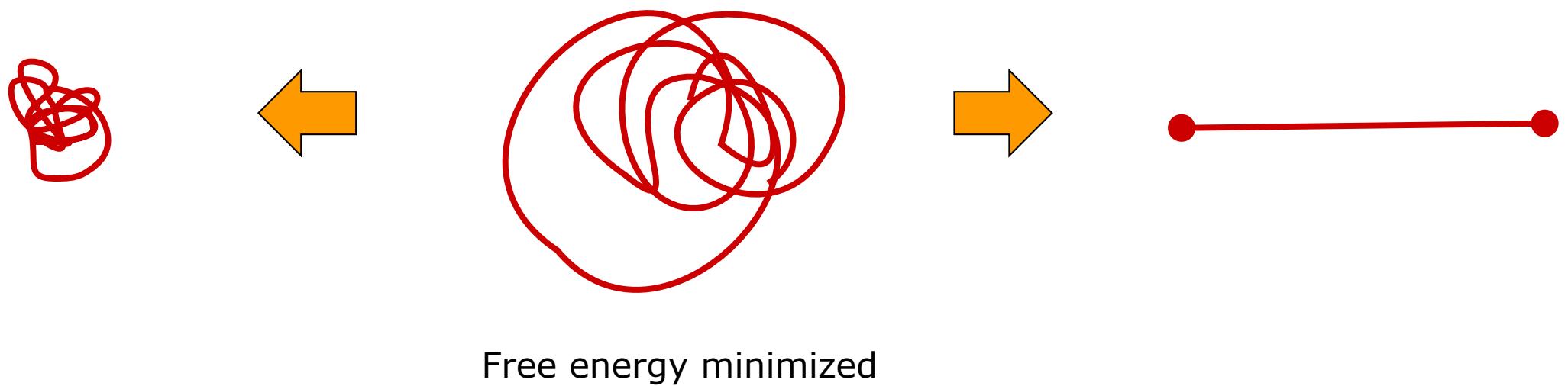
# single DNA molecules in confinement: Experimental set-up



# Result: genomic DNA in solution

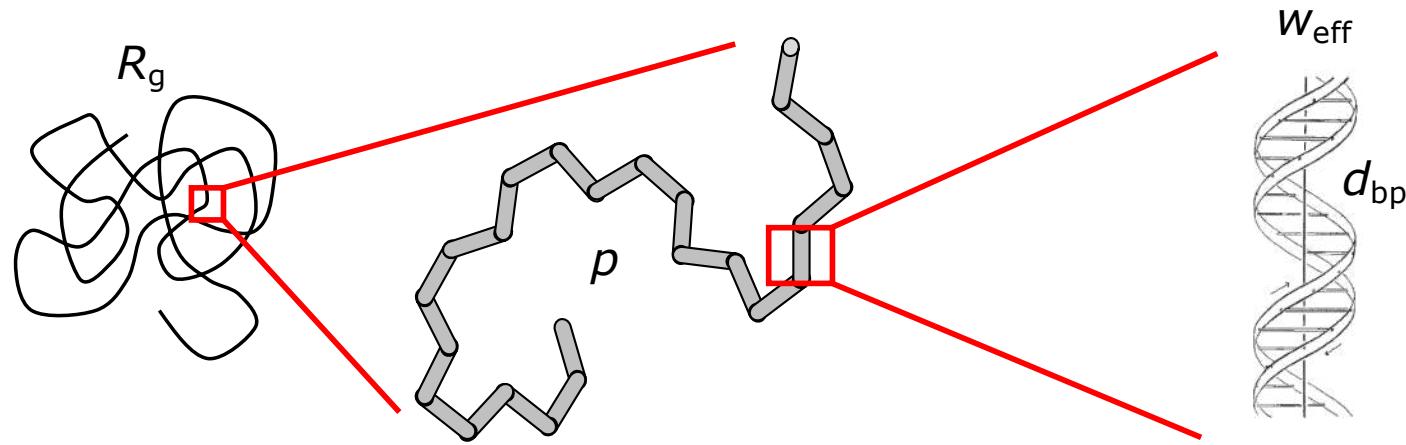


# What makes DNA coil, but not too much?



→ There is an **entropic barrier** to move the system away from it's equilibrium. DNA is an **entropic spring**.

# Important DNA length scales



Bulk solution radius of gyration,  $R_g$  depends on contour length

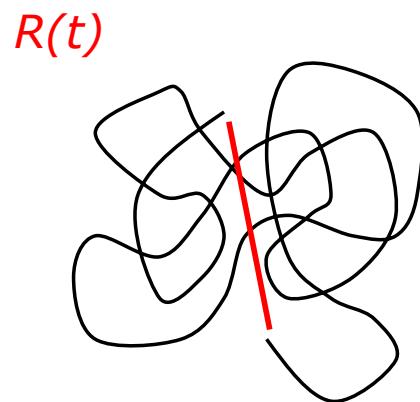
Contour length (length along the molecule),  $I_c = \text{bp} \cdot 0.34 \text{ nm}$

Persistence length,  $p \sim 50 \text{ nm}$

Effective width,  $w_{\text{eff}} \sim 5 \text{ nm}$

Base pair spacing,  $d_{\text{bp}} \sim 0.34 \text{ nm}$

# The end-to-end distance $R$ and the Radius of gyration $R_G$



They both describe the size of the 'coil' formed by the polymer chain:

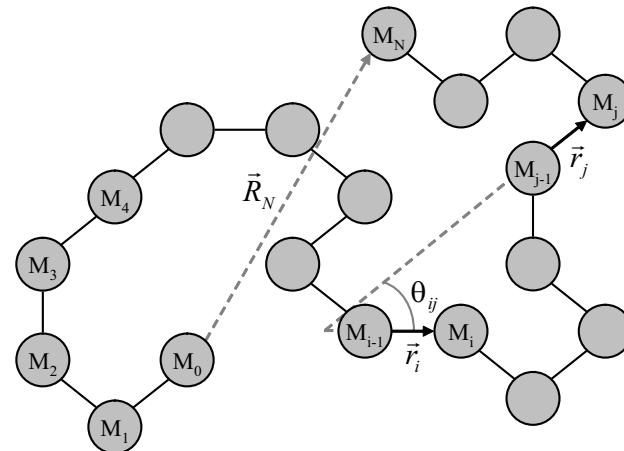
- $\langle R^2 \rangle$  is the time average of the end-to-end distance squared.
- $R_G = \sqrt{\frac{\langle R^2 \rangle}{6}}$  represents how far are the chain segments from the center of mass.

# DNA as a physical object: FJC

**The freely joined chain (FJC) model:**

Freely rotating segments of length  $l$

The contour length is  $lc = N l$



End-to-end distance can be calculated to be in the ideal case:

$$\langle R^2 \rangle = l^2 \sum_{i=1}^N \sum_{j=1}^N \delta_{ij} = Nl^2.$$

In practice this is a scaling law.

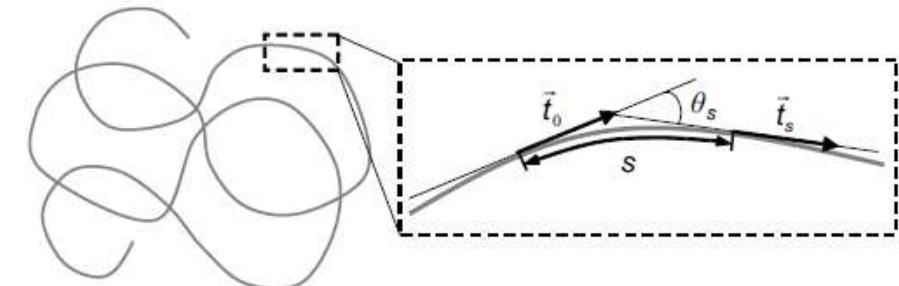
$$\langle R^2 \rangle \approx C_\infty N l^2.$$

# DNA as a physical object: WLC

## The worm-like chain (WLC) model

Persistence length

$$\langle \cos \theta_s \rangle = \exp\left(-\frac{s}{p}\right)$$



Bending free energy

$$\Delta F_b = \frac{k_B T s p}{2 R_c^2}$$

End-to-end distance:

$$\langle R^2 \rangle = 2l_p l_c - 2l_p^2 \left[ 1 - \exp\left(-\frac{l_c}{l_p}\right) \right]$$

### Limits:

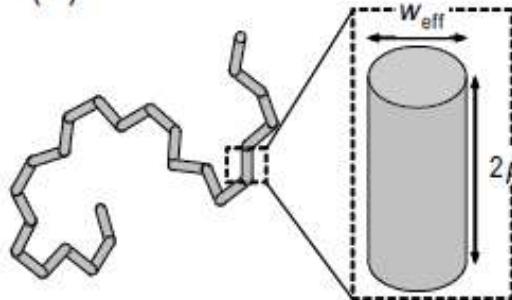
Kuhn length

$lc \gg lp : \langle R^2 \rangle = 2lp lc$  This is the FJC model with  $l_k = 2lp$  and  $lc/l_k = N$

$lc < lp : \langle R^2 \rangle = lc^2$

# DNA as a physical object: exclusion volume

(a)



## Self avoidance:

- The chain exclude itself
- The chain cannot cross itself

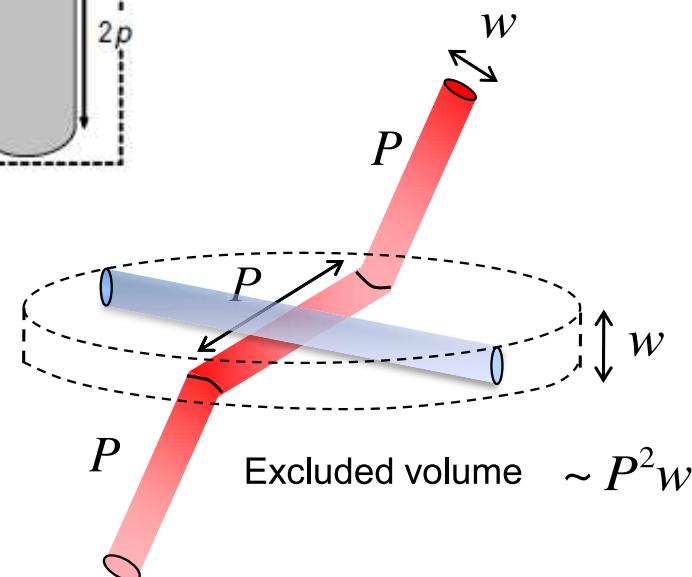
I [mM]	500	100	50	10	2
$\xi_{\text{eff}} [e^-/\text{nm}]$	25.90	6.08	4.15	2.43	1.78
$w_{\text{eff}} [\text{nm}]$	3.41	5.60	7.40	15.70	36.30
$p [\text{nm}]$	50.06	50.32	50.65	53.24	66.20

Effective width  $> 2\text{nm}$

The end-to-end distance is called the Flory radius:

$$R_F \approx (w_{\text{eff}} p)^{\frac{1}{5}} l_{\text{con}}^{\frac{3}{5}}$$

Reisner et al. Rep. Prog. Phys. **75** (2012)



**Figure 3.** A statistical segment of length  $\sim P$  excludes a volume of size  $\sim P^2 w$  to neighboring segments of the same length.

# Force vs. extension model

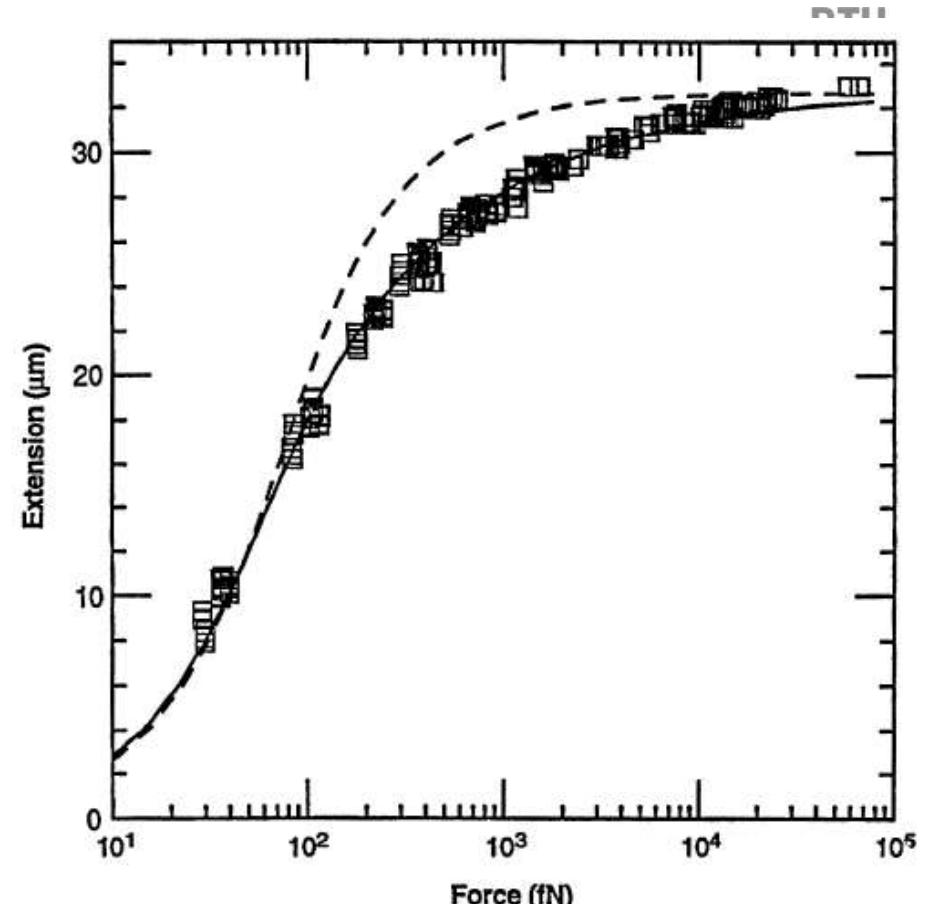
Persistence length  $p$

$$FA/kT = \frac{1}{4}(1 - x/L)^{-2} - \frac{1}{4} + \frac{x}{L}$$

where  $k$  is Boltzmann's constant,  $T$  is temperature, and  $L$  is the molecular contour length.

Marko-Siggia equation:

$$f = \frac{k_B T}{p} \left[ \frac{1}{4 \left(1 - \frac{r}{l_{\text{con}}} \right)^2} + \frac{r}{l_{\text{con}}} - \frac{1}{4} \right]. \xrightarrow{\text{low force limit}} f = \frac{3k_B T}{2pl_{\text{con}}} r.$$

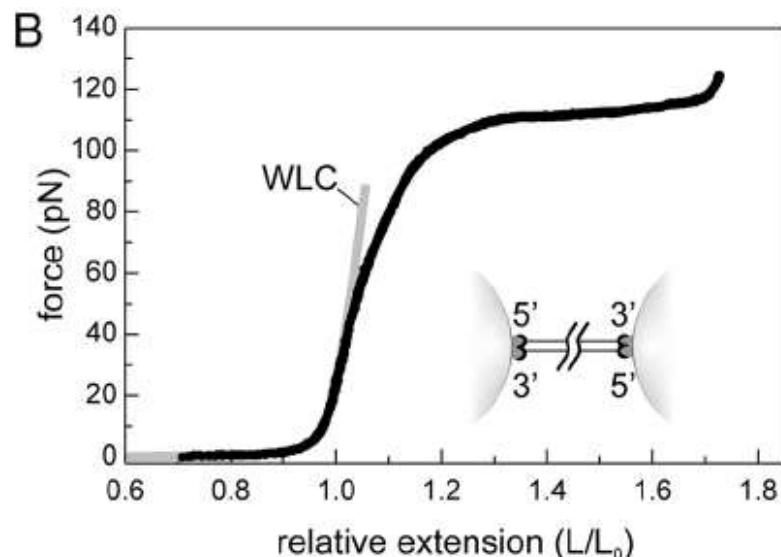


This is a spring!

Bustamante 1994 Science

# Experiments for stretching DNA

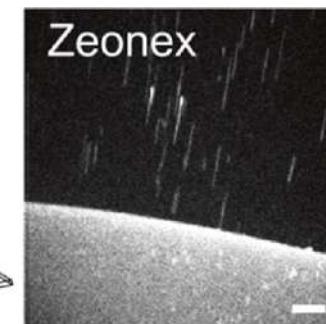
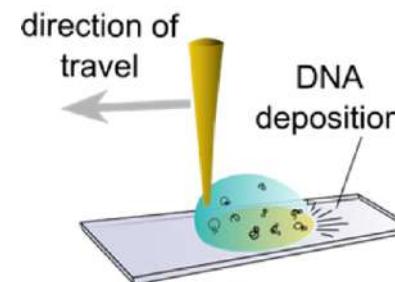
→ Optical tweezers



Van Mameren 2009 PNAS

→ Thermophoresis

→ Surface combing



Deen 2015 ACS Nano

→ Electrophoresis

→ Hydrodynamic drag

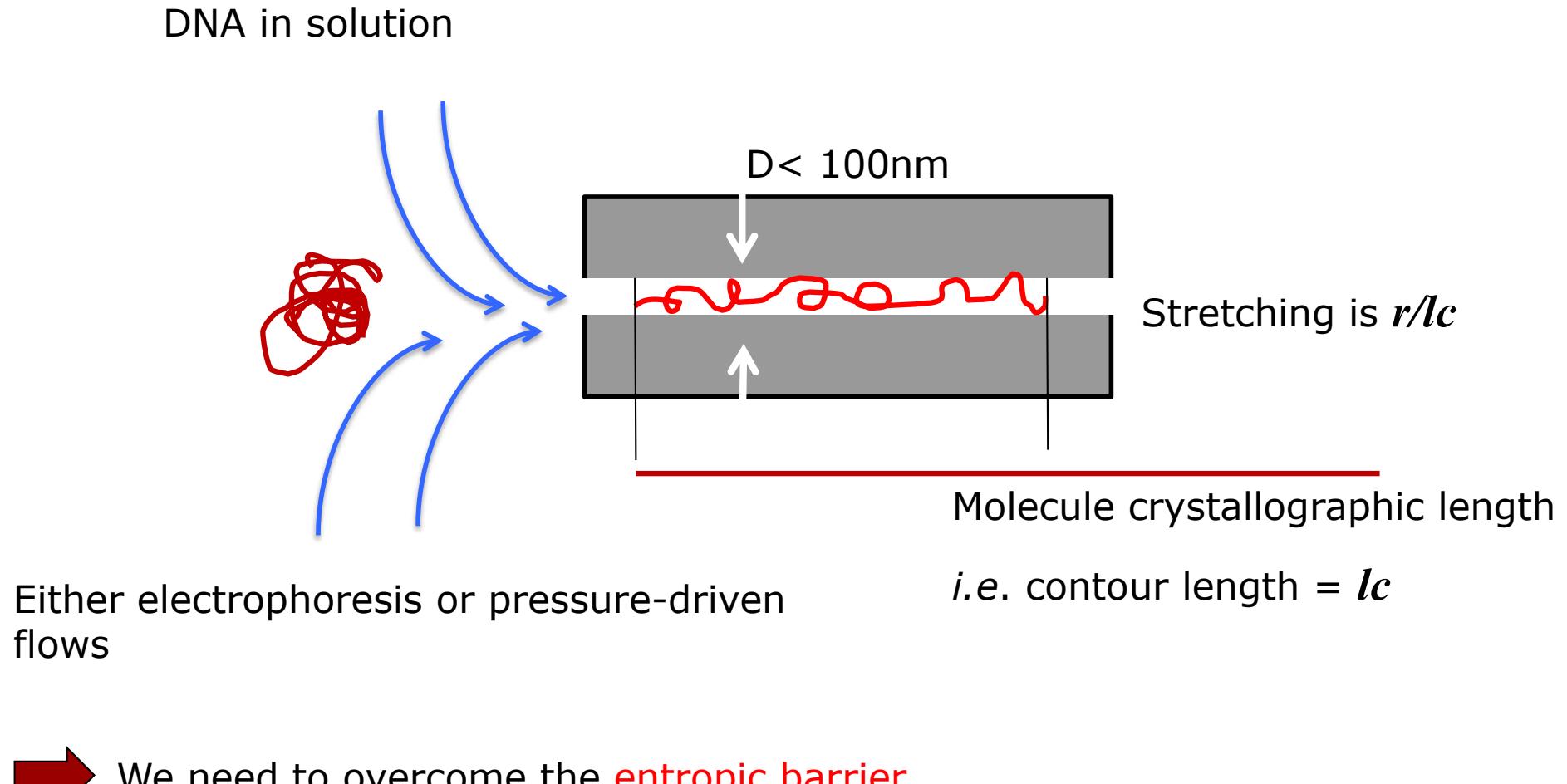
- Elongation flow

- Tethered/semi tethered DNA

- Double elongation flow

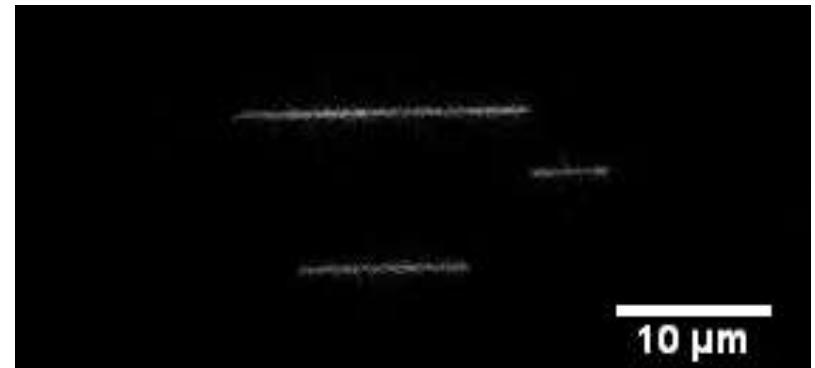
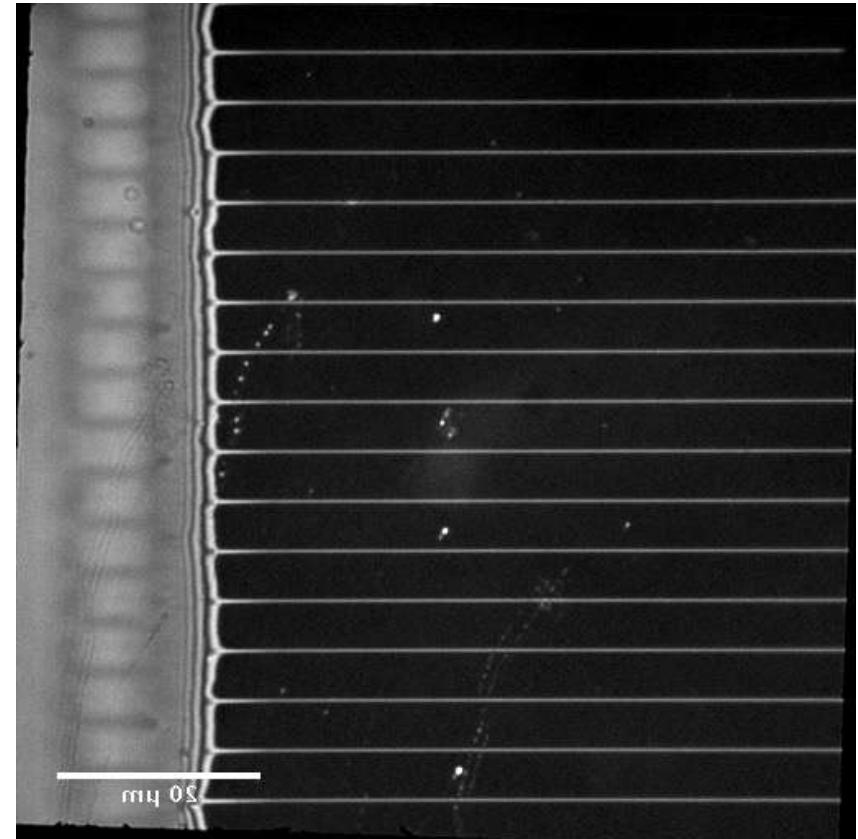
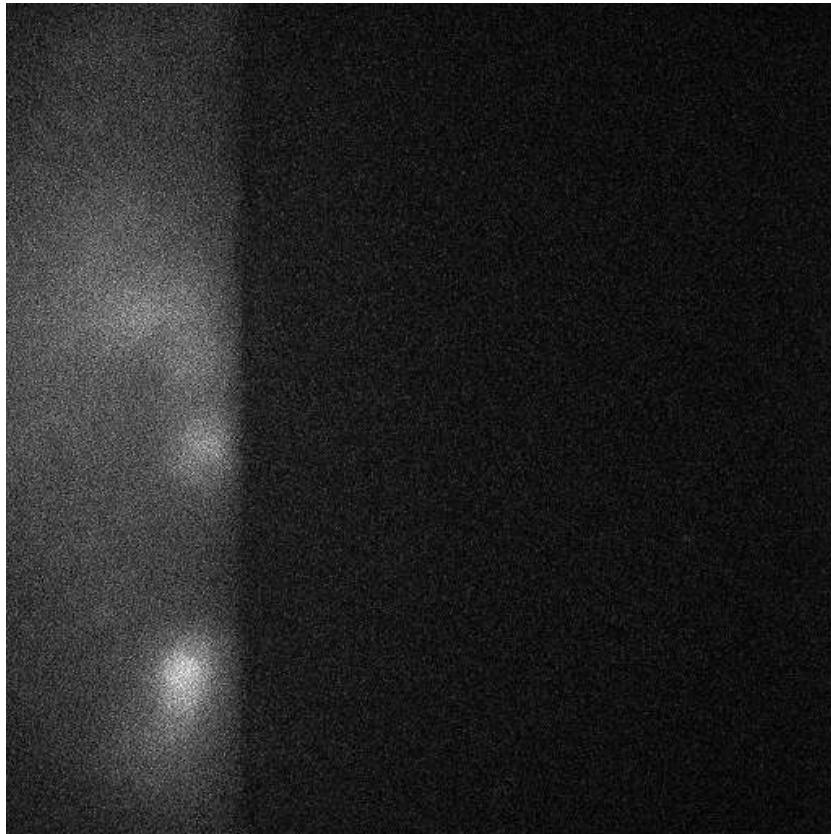
See also review article: F. Persson and J. O. Tegenfeldt, Chem. Soc. Rev. 39, 985 (2010)

# DNA stretching by confinement



Tegenfeldt (2004) PNAS

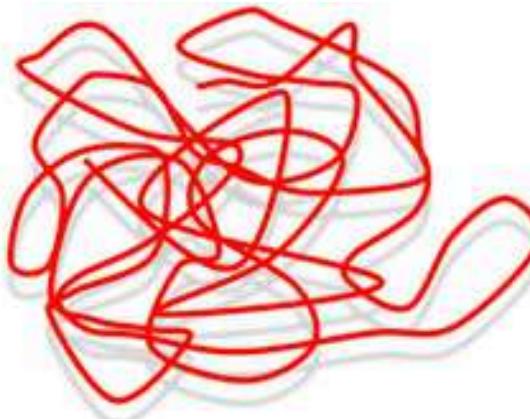
# DNA in nanochannels



Down to 140 nm nanochannels (gives 43% stretching)

# Confined DNA Overview

Free Solution (in 3D)



$$D_{av} > R_G$$

De Gennes



$$P < D_{av} < R_G$$

Odijk (1D confinement)



$$D_{av} < P$$

# The Odijk regime

Molecule Extension:

$$R_{\text{Odijk}} = L \cos(\theta) = L \left[ 1 - A \left( \frac{D}{P} \right)^{2/3} \right]$$

L is the contour length

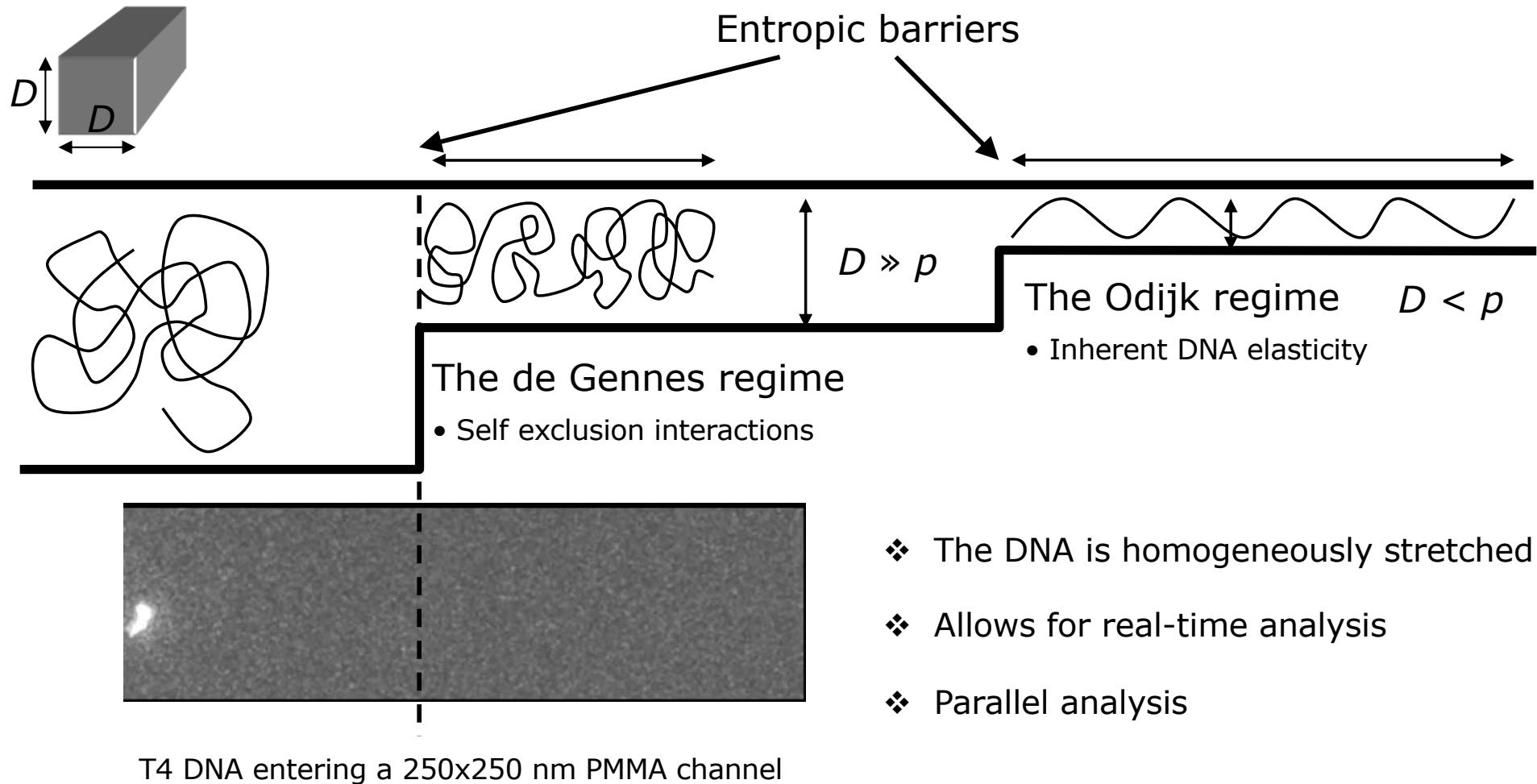
A is a geometrical factor

D is the channel diameter

P is the persistence length

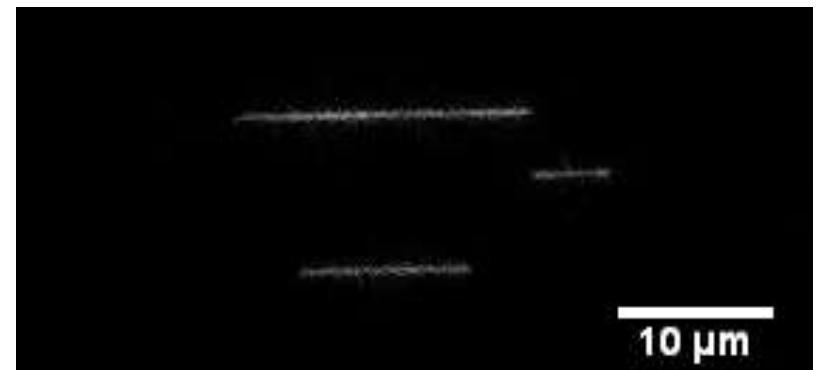
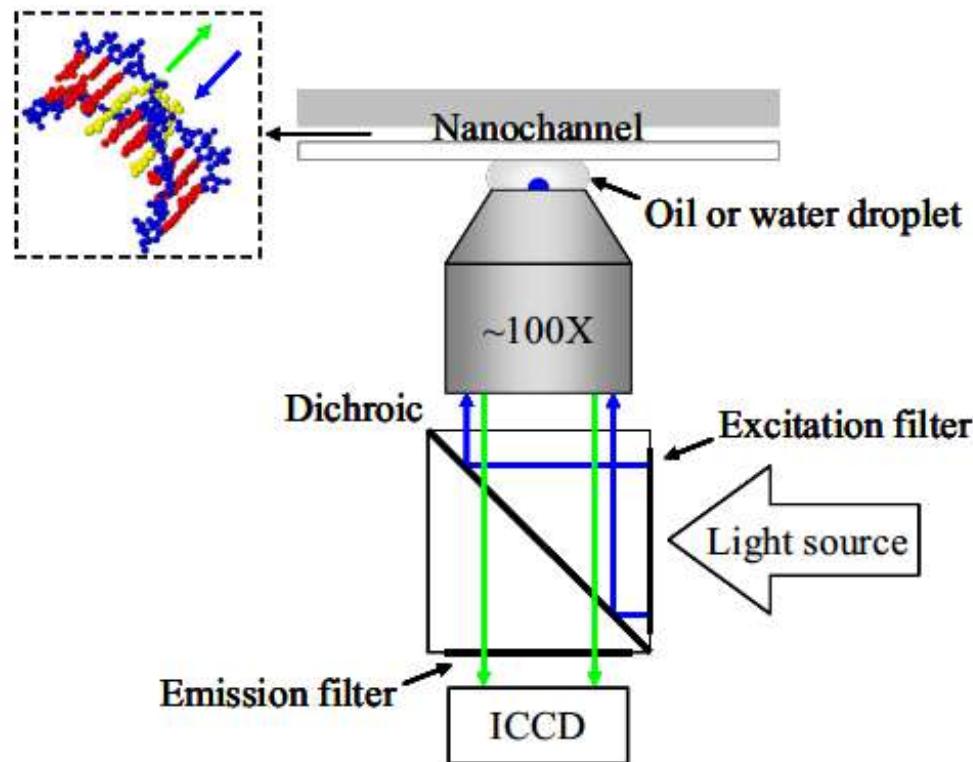
See further refinements and numerical values in Reisner et al 2012 Rep. Prog. Phy.

# De Gennes regime: experiment

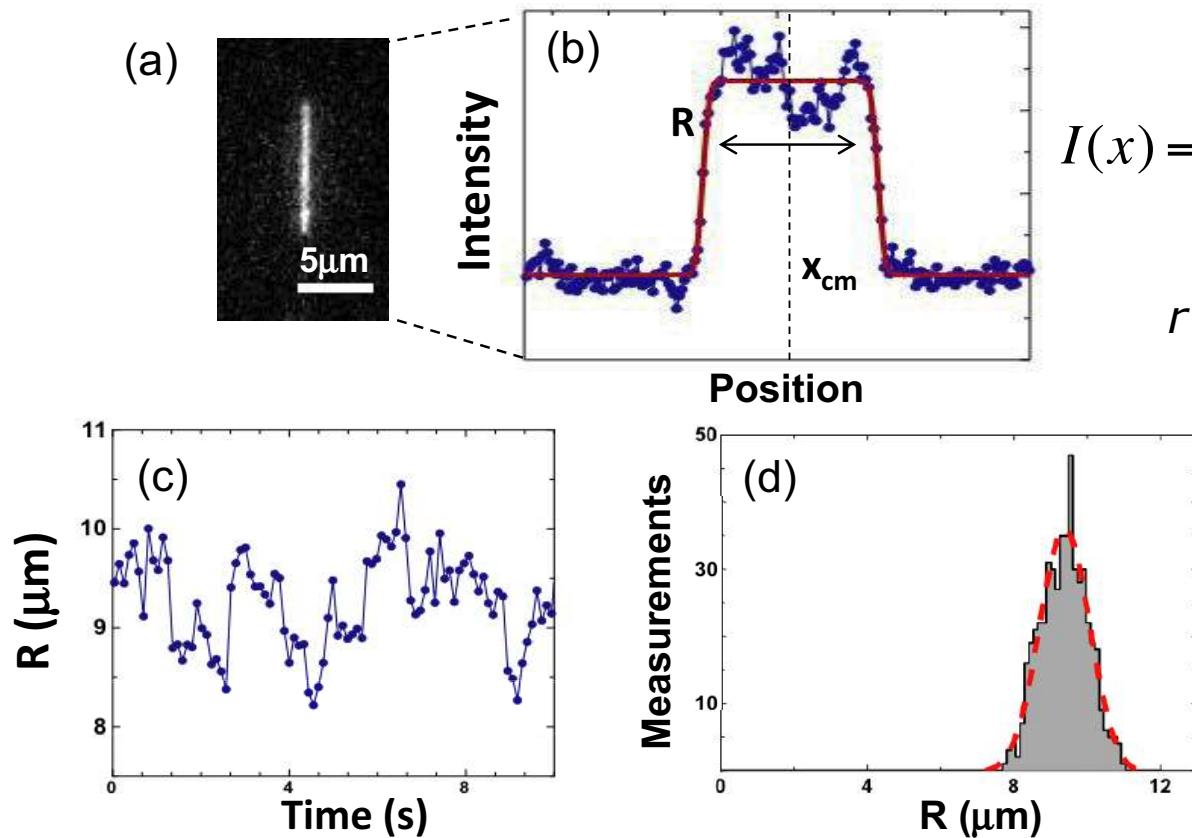


- ❖ The DNA is homogeneously stretched
- ❖ Allows for real-time analysis
- ❖ Parallel analysis

# Experimental measurement of elongation length, $r$



# Extract the elongation length, $r$

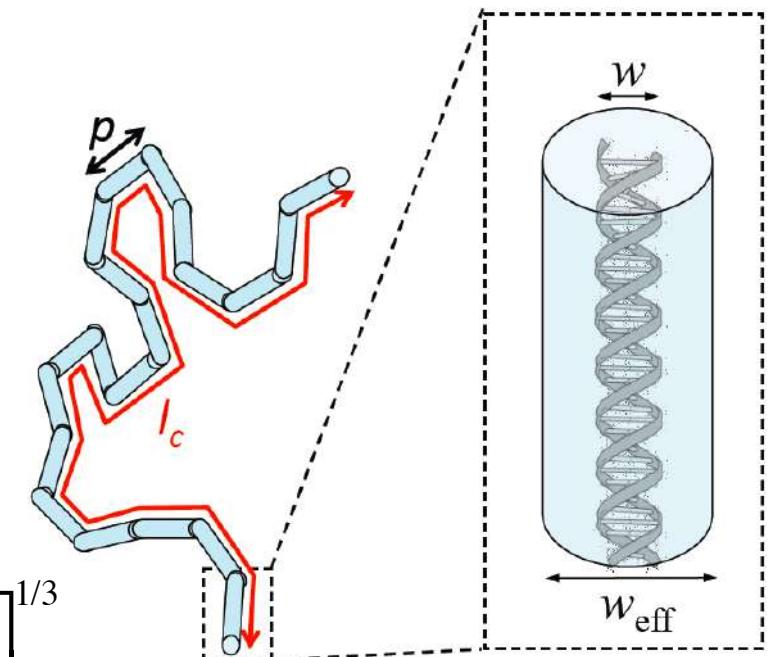
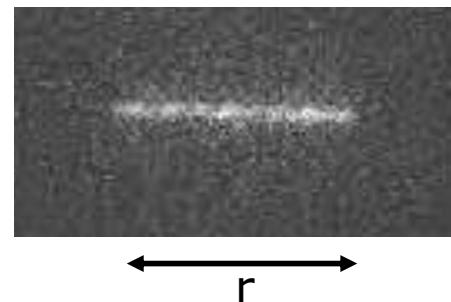
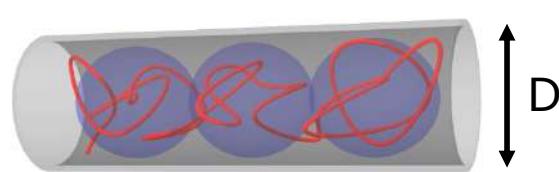


$$I(x) = \frac{I_0}{2} \left[ \operatorname{Erf}\left(\frac{x}{\sigma_0 \sqrt{2}}\right) - \operatorname{Erf}\left(\frac{x-r}{\sigma_0 \sqrt{2}}\right) \right]$$

$r$  and  $\sigma_0$  are fitting parameters

$$\operatorname{Erf}(x) = \frac{2}{\sqrt{\pi}} \int_0^x e^{-t^2} dt$$

# Confined DNA – De Gennes regime, $R_g > D > p$

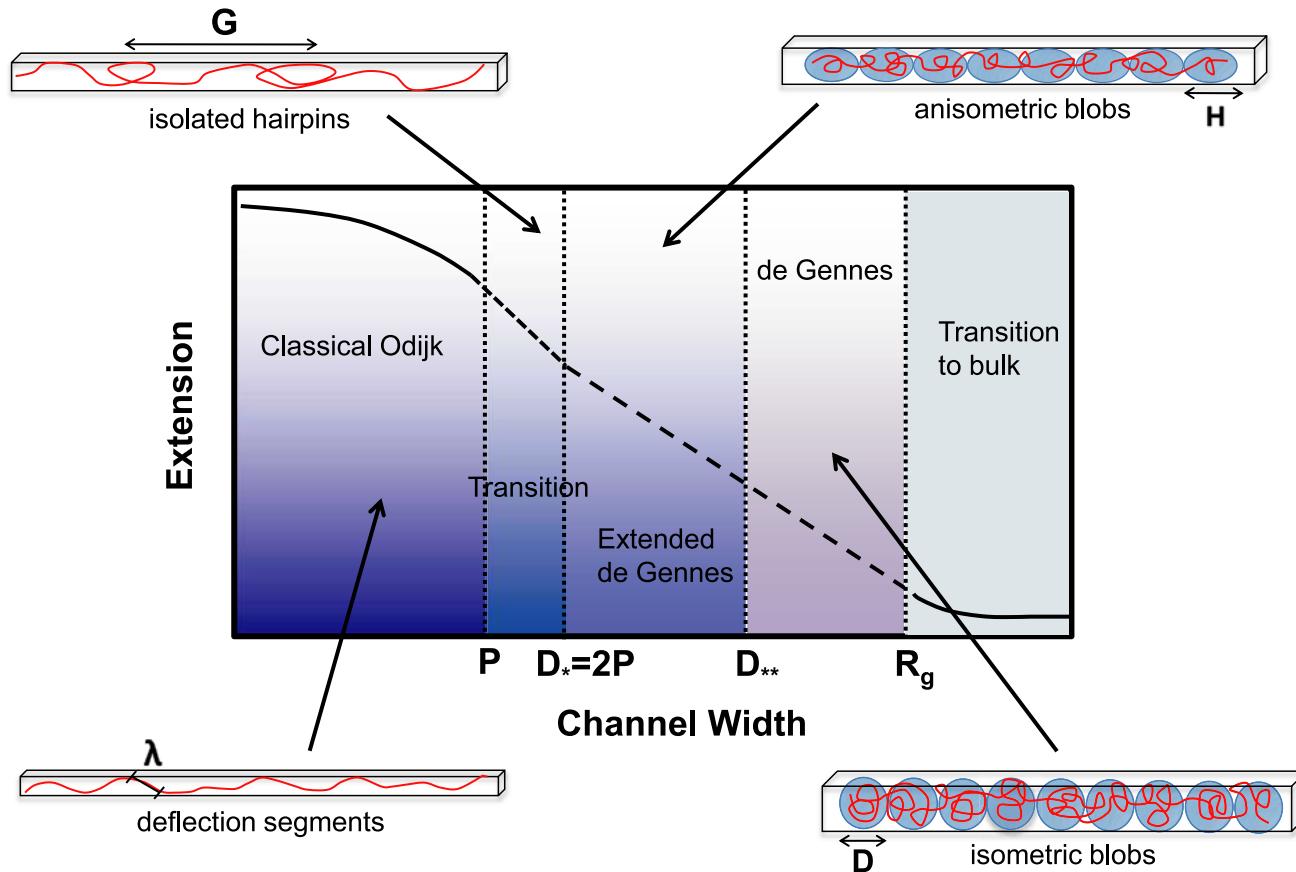


Molecule Extension:  $r \approx \frac{(w_{eff} \cdot p)^{1/3}}{D^{2/3}} \cdot l_c$

(Entropic) Spring Constant:  $k \approx \frac{15}{4} \frac{k_B T}{l_c} \left[ \frac{1}{p \cdot w_{eff} \cdot D} \right]^{1/3}$

RMS length variance:  $\langle \delta r^2 \rangle = \frac{k_B T}{k} = \frac{4 \cdot l_c}{15} [p \cdot w_{eff} \cdot D]^{1/3}$

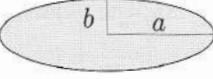
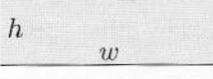
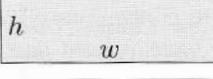
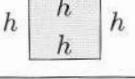
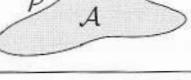
# A more complex picture



Reisner 2012 Rep. Prog. Phys.

# Design consideration: Hydraulic Resistance

**Table 4.1** A list over the hydraulic resistance for straight channels with different cross-sectional shapes. The numerical values are calculated using the following parameters:  $\eta = 1 \text{ mPa s}$  (water),  $L = 1 \text{ mm}$ ,  $a = 100 \mu\text{m}$ ,  $b = 33 \mu\text{m}$ ,  $h = 100 \mu\text{m}$ , and  $w = 300 \mu\text{m}$ .

shape		$R_{\text{hyd}}$ expression	$R_{\text{hyd}}$ [ $10^{11} \frac{\text{Pa s}}{\text{m}^3}$ ]	reference
circle		$\frac{8}{\pi} \eta L \frac{1}{a^4}$	0.25	Eq. (3.39b)
ellipse		$\frac{4}{\pi} \eta L \frac{1 + (b/a)^2}{(b/a)^3} \frac{1}{a^4}$	3.93	Eq. (3.38)
triangle		$\frac{320}{\sqrt{3}} \eta L \frac{1}{a^4}$	18.5	Eq. (3.46)
two plates		$12 \eta L \frac{1}{h^3 w}$	0.40	Eq. (3.30)
rectangle		$\frac{12 \eta L}{1 - 0.63(h/w)} \frac{1}{h^3 w}$	0.51	Eq. (3.58)
square		$28.4 \eta L \frac{1}{h^4}$	2.84	Exercise 4.4
parabola		$\frac{105}{4} \eta L \frac{1}{h^3 w}$	0.88	Eq. (3.80)
arbitrary		$\approx 2 \eta L \frac{P^2}{A^3}$	-	Eq. (3.27a)

From 'theoretical microfluidics' H. Bruus.

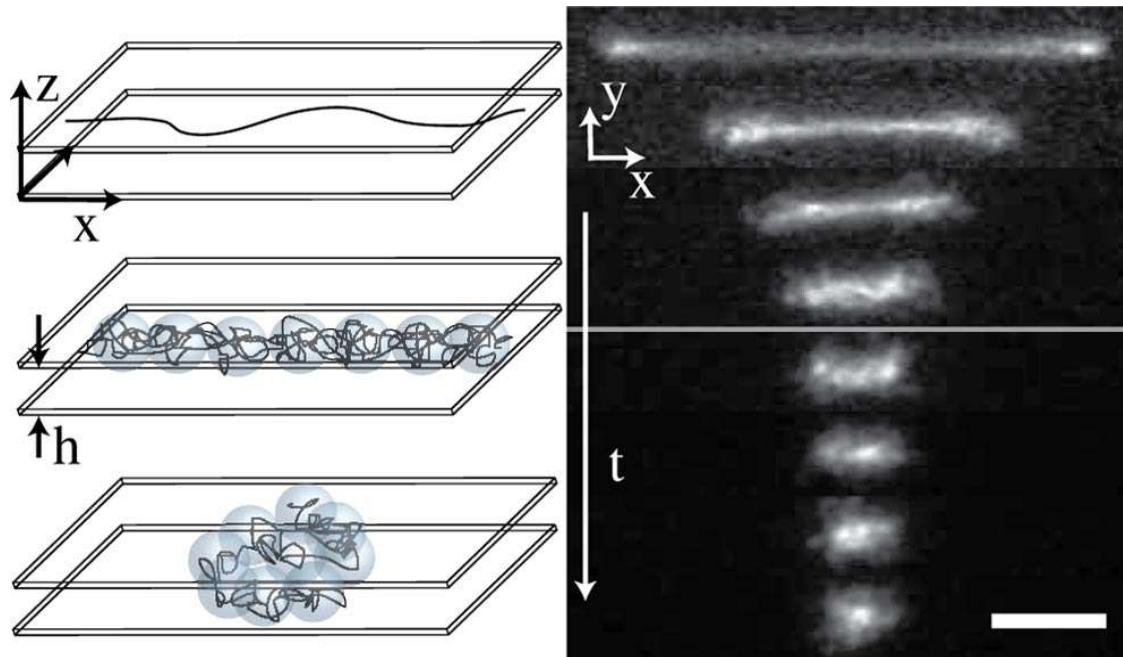
# Exercise F1

- See separate slides

## Exercise sum up

- Go through questions during the exercise
- Numerical results will be uploaded on campus net

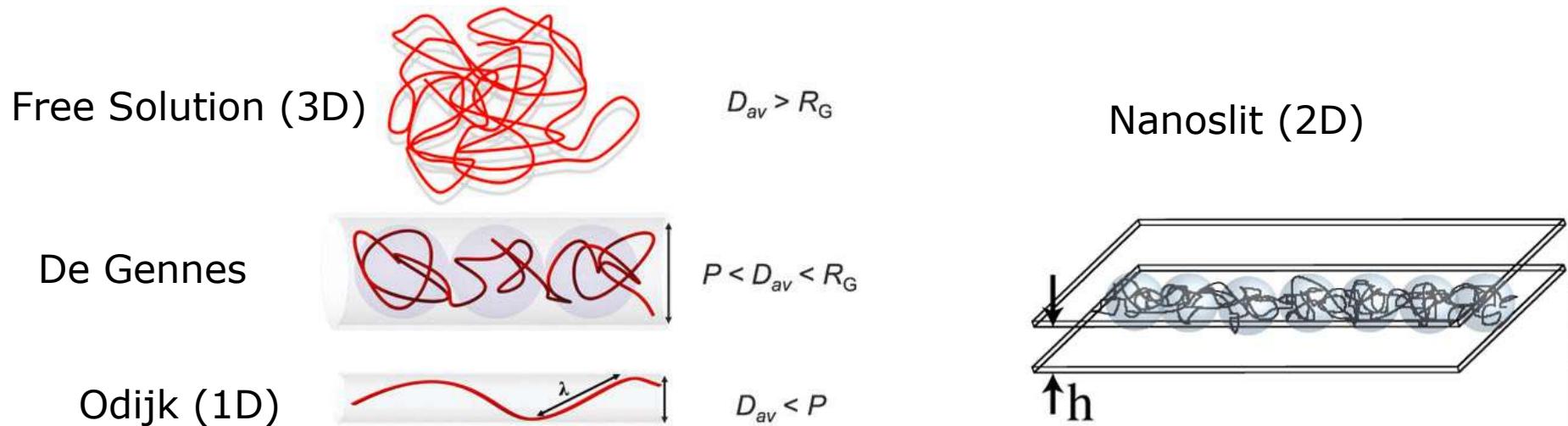
## DNA in slit-like confinement (2D)



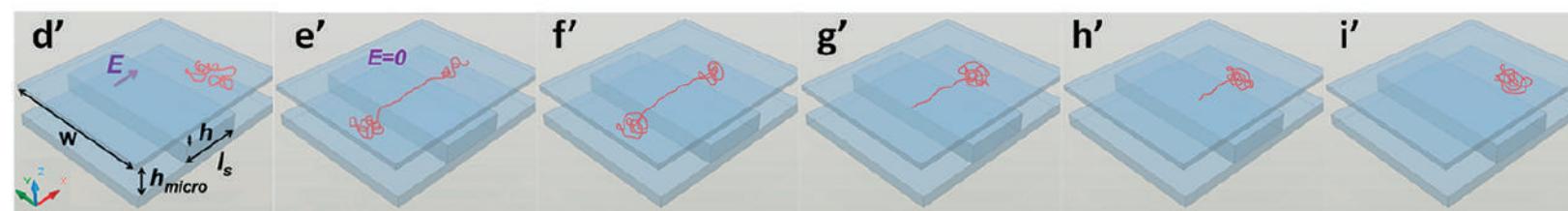
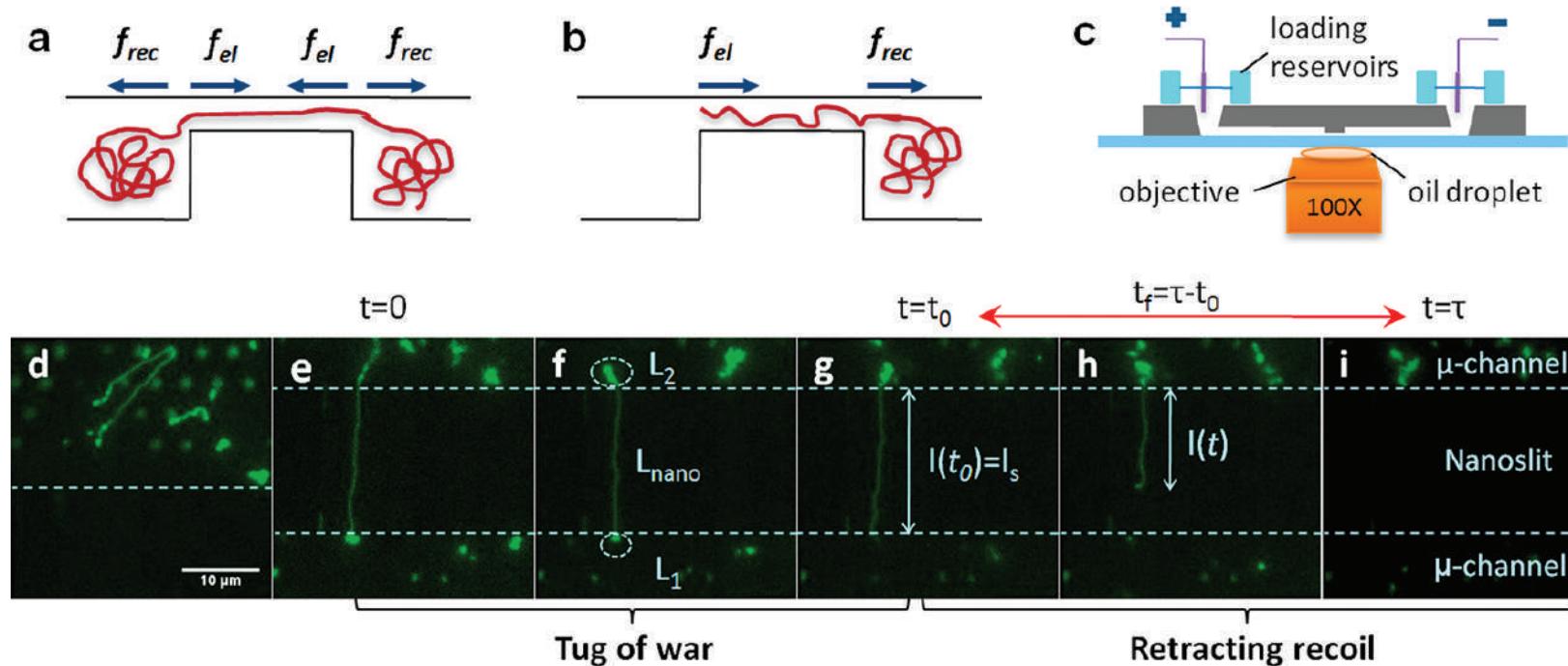
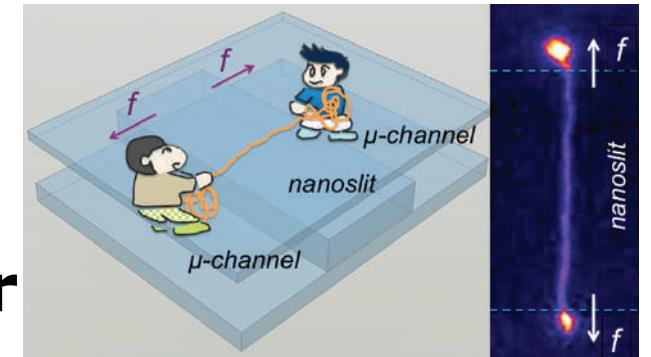
Like a DeGennes regime where blobs are free to move in 2D

Balducci 2007 PRL (*relaxation in a nano slit*)

# Overview

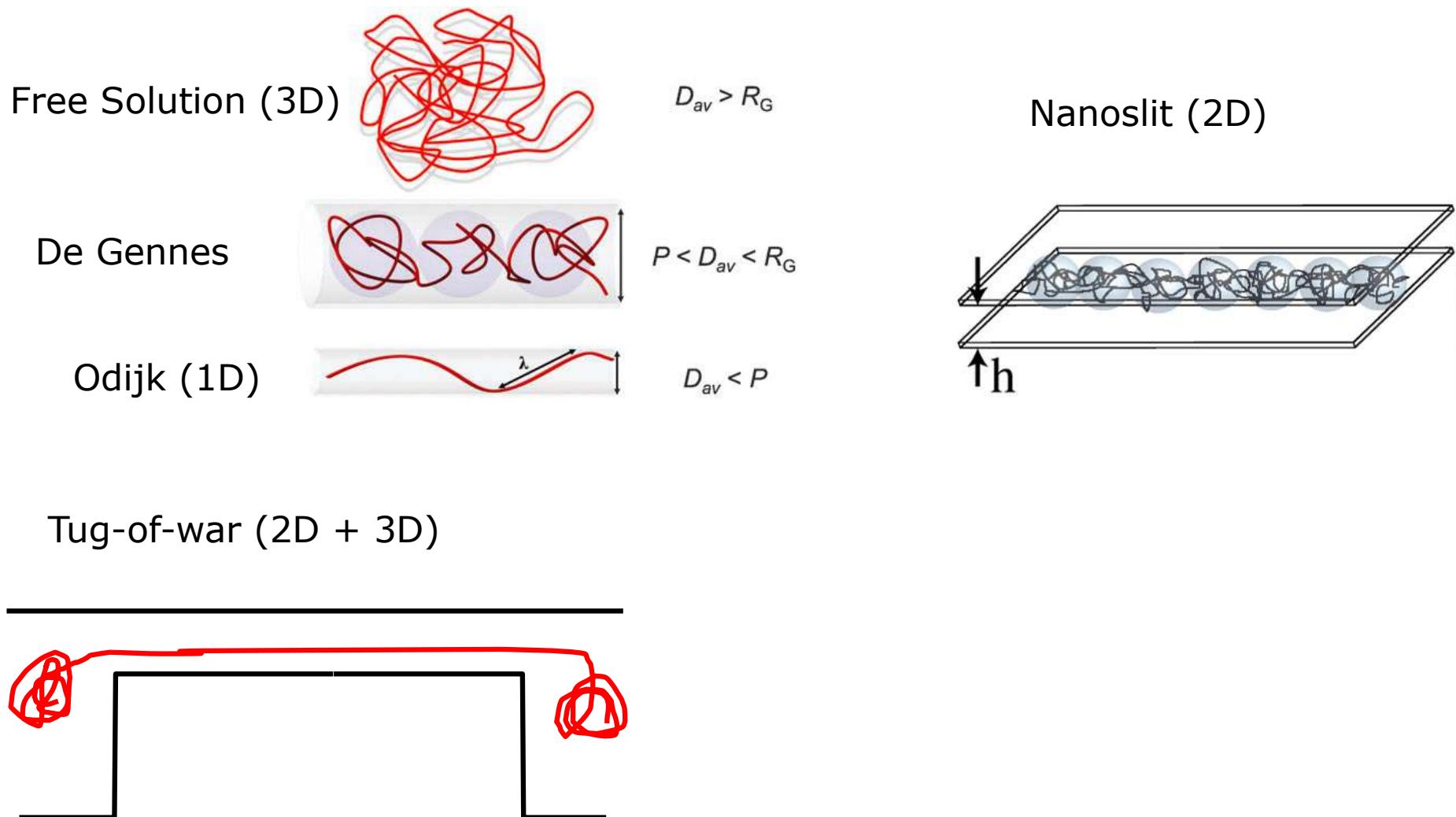


# Additional stretching: Tug-of-war

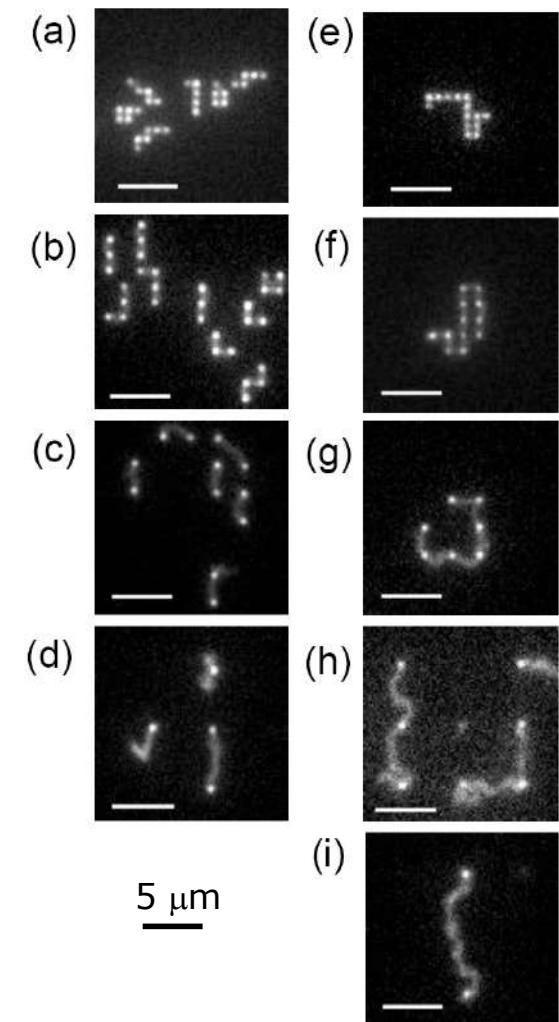
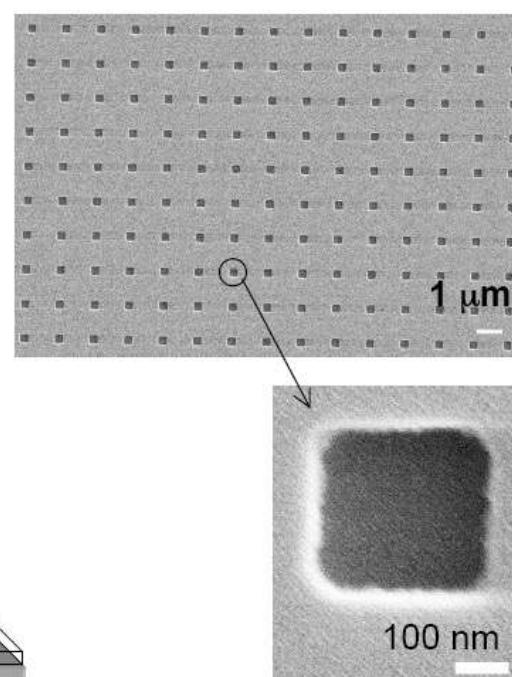
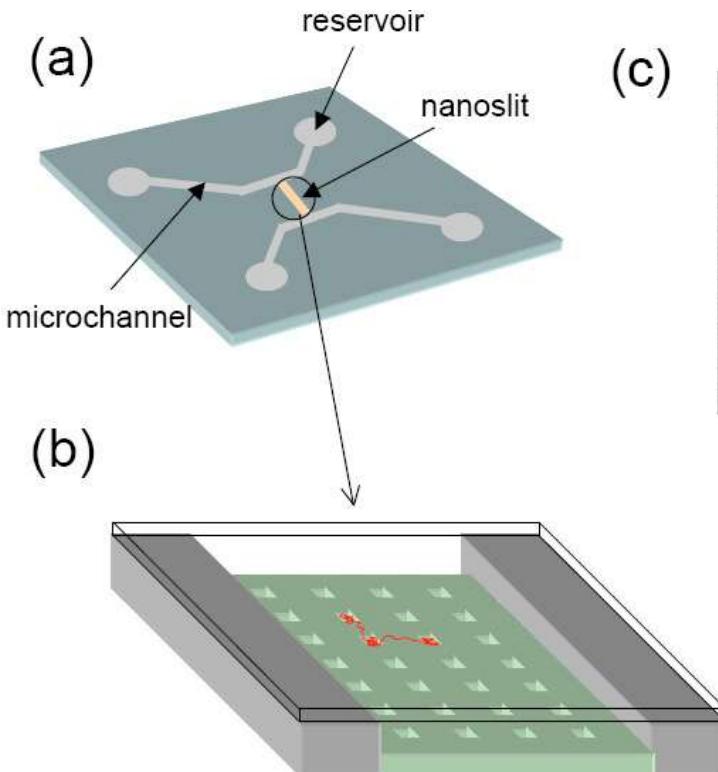


Yeh, Nano Lett 2012

# Overview slide

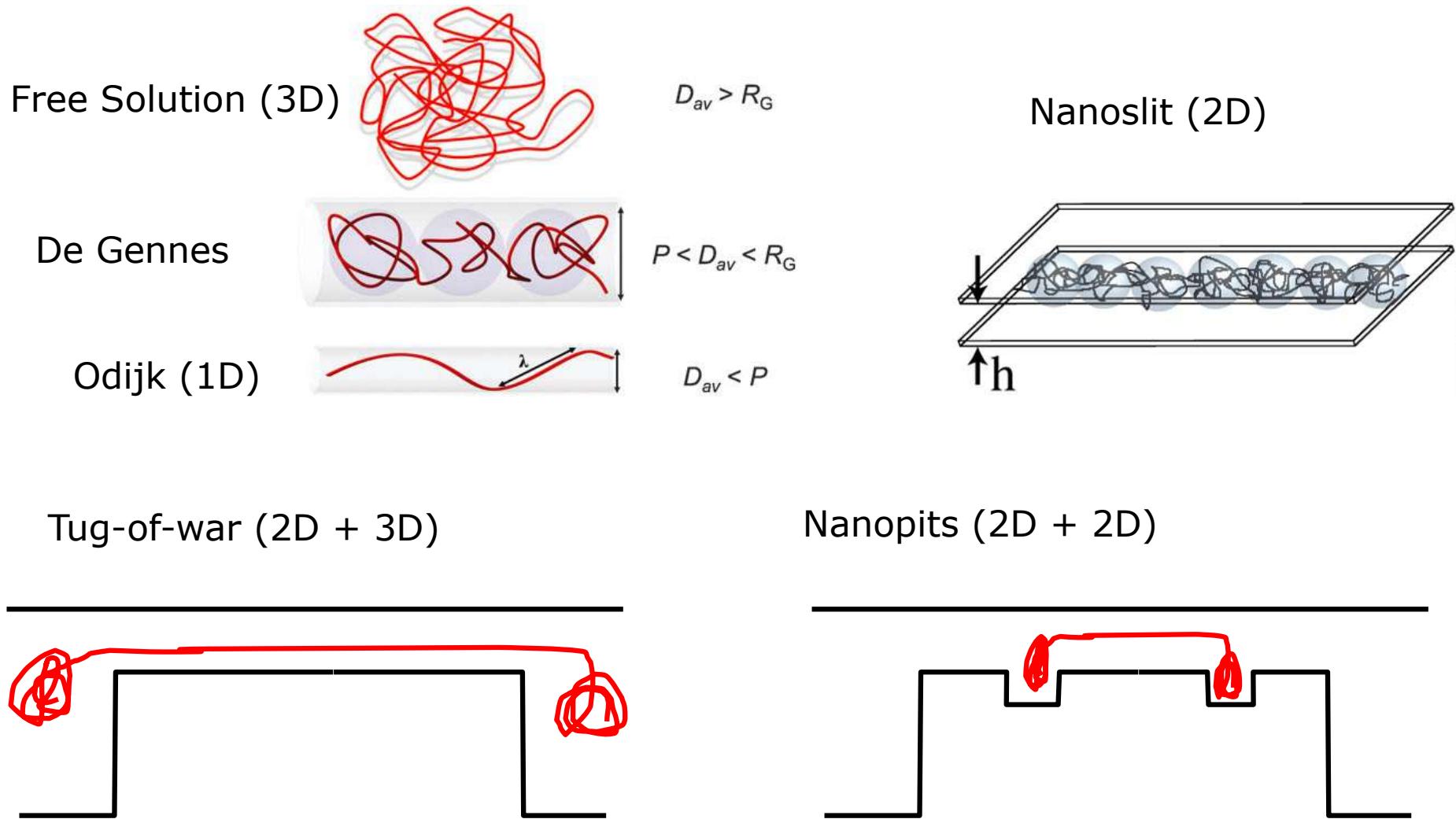


# DNA immobilization in nanopits

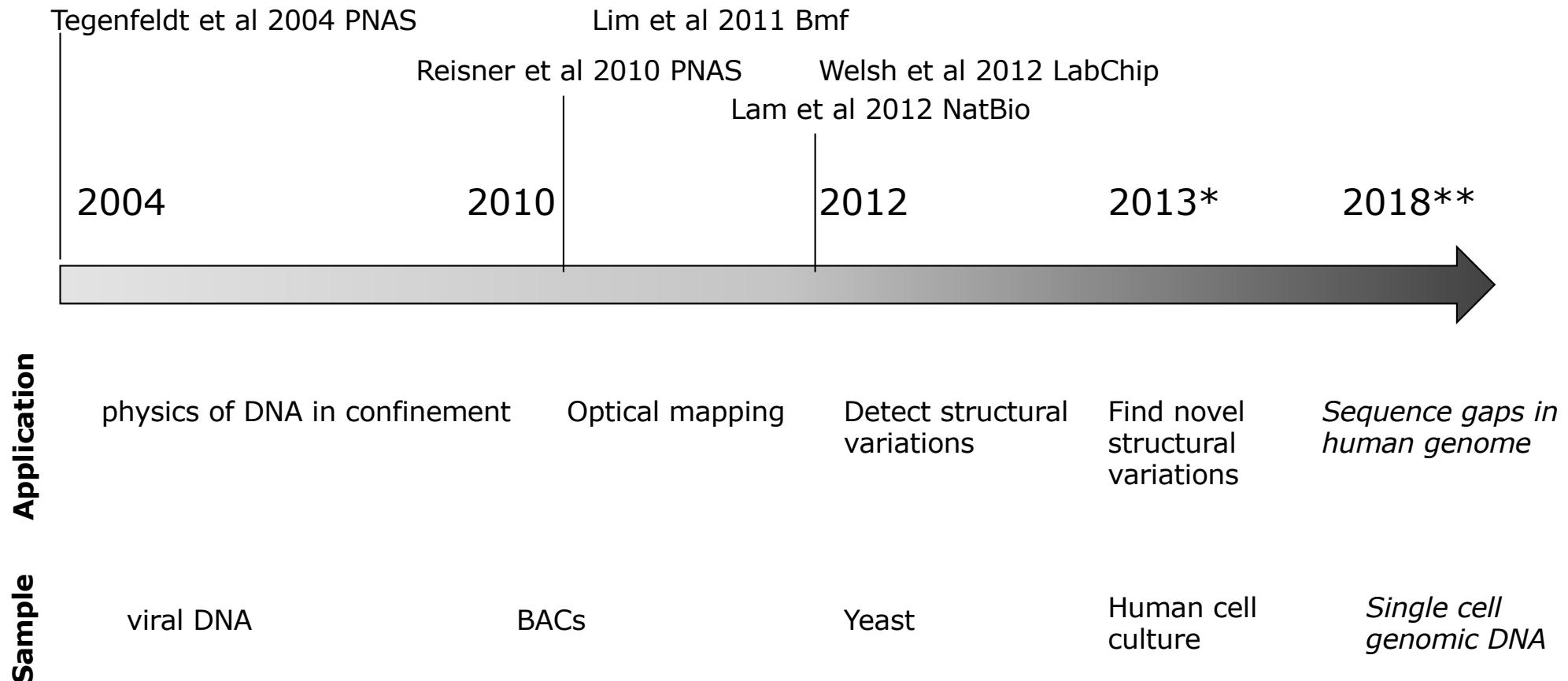


**W. Reisner, N. B. Larsen, H. Flyvbjerg, J. O. Tegenfeldt and A. Kristensen**  
**Proc. Natl. Acad. Sci. USA, Vol. 106, pp. 79-84 (2009)**

# Overview slide again



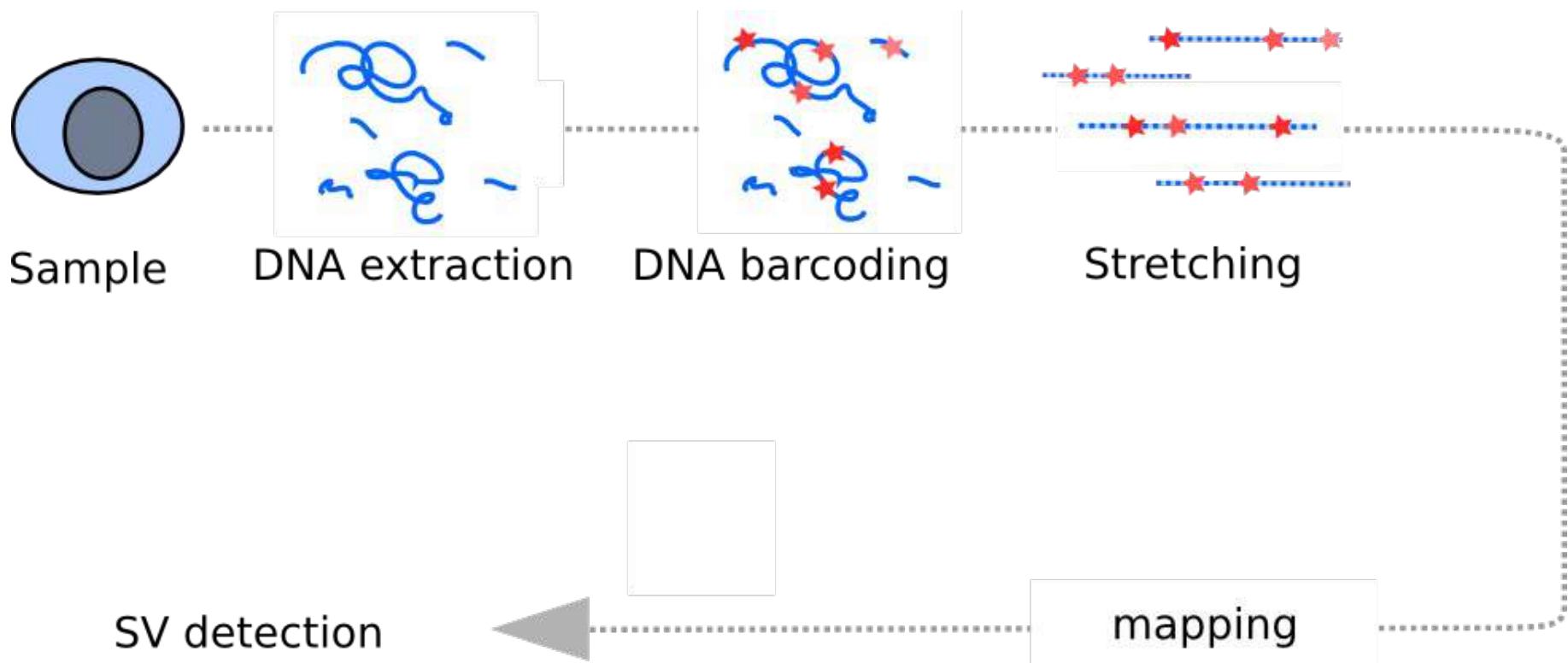
# Samples and applications



\* Marie R. et al, '**Integrated view of genome structure and sequence of a single DNA molecule in a nanofluidic device**', *PNAS* 2013 110(13) Open Access

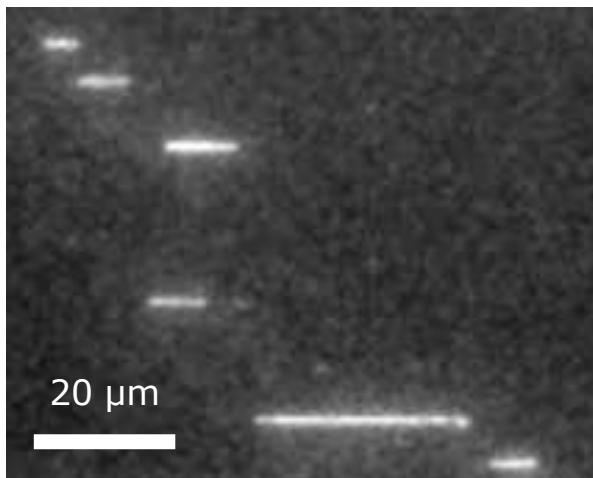
\*\* Marie et al, '**Single-molecule DNA-mapping and whole-genome sequencing of individual cells**', *PNAS* 2018

# What are we aiming at with this technology?



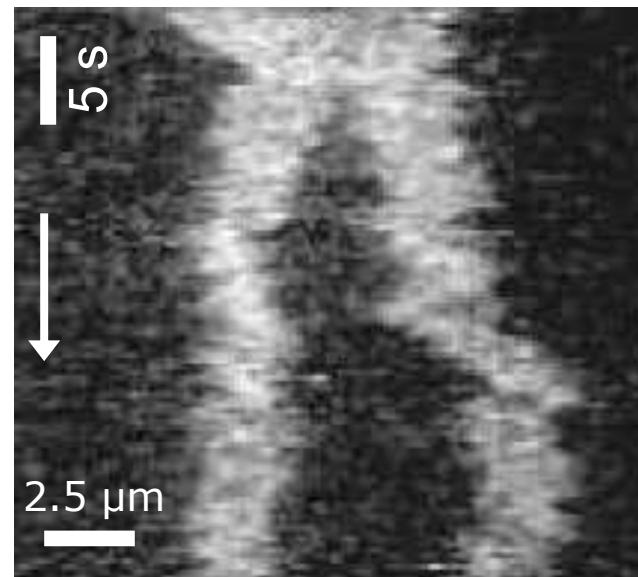
# Toward Next generation DNA sequencing: genome mapping

Determine size of DNA

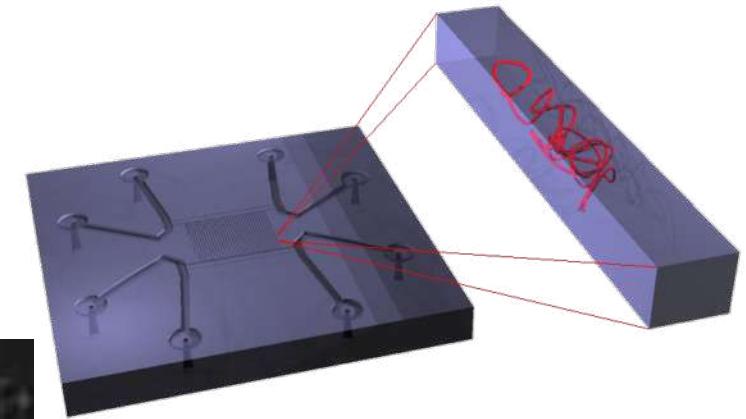


Tegenfeldt, PNAS, 2004

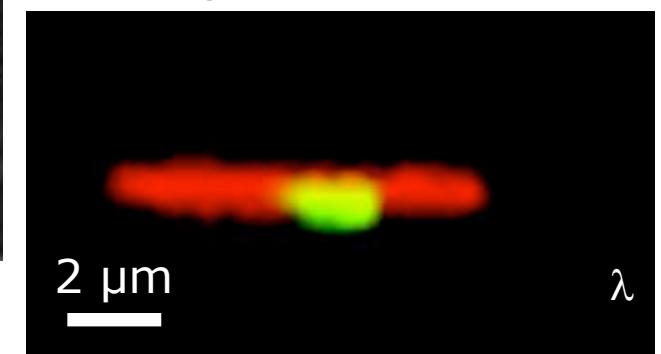
Restriction Mapping



Riehn, PNAS, 2005

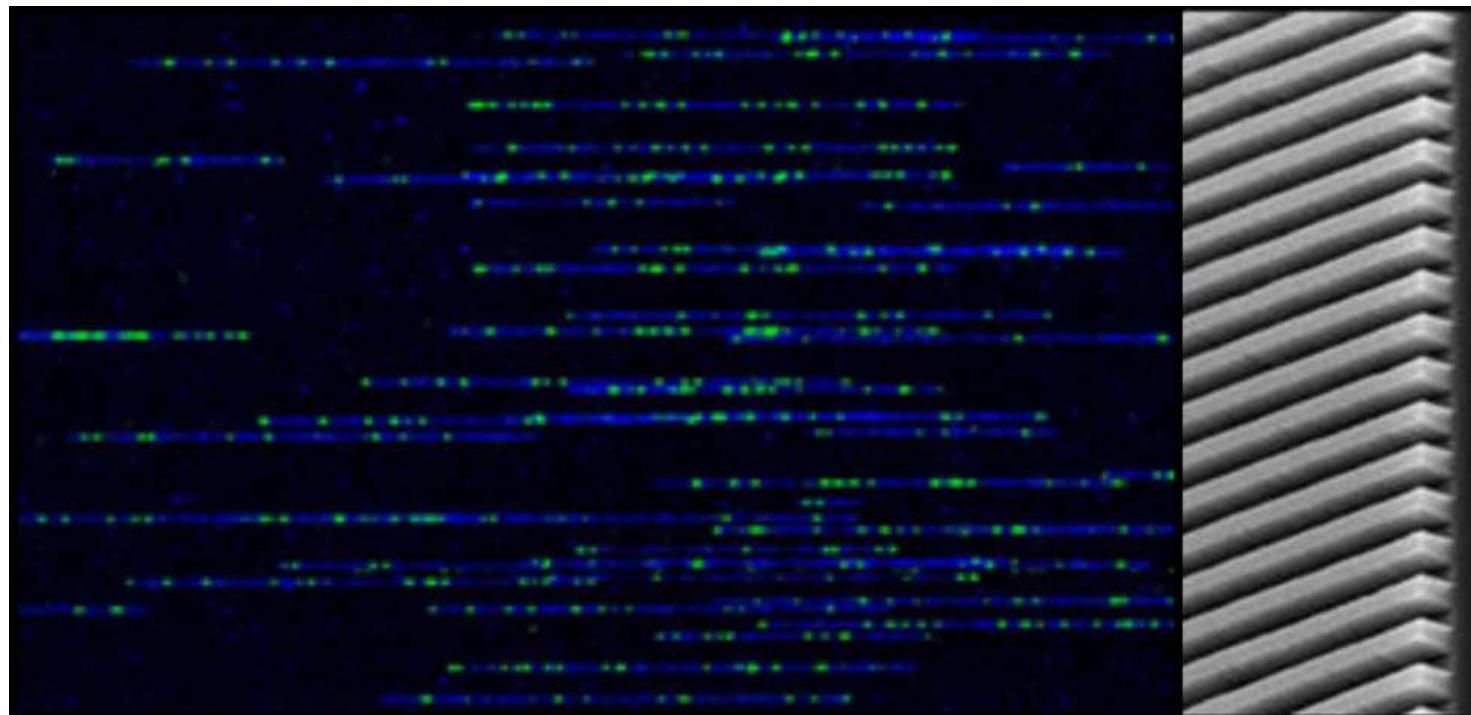


Biological interactions



Wang, PNAS, 2005

# Current genome mapping in nanochannels



Nick labelling on DNA stretched in 45x45nm nanochannels  
(BioNanoGenomics)

Rodolphe Marie

10321

**Theme F2: Solvent and solute  
transport in gradients.**

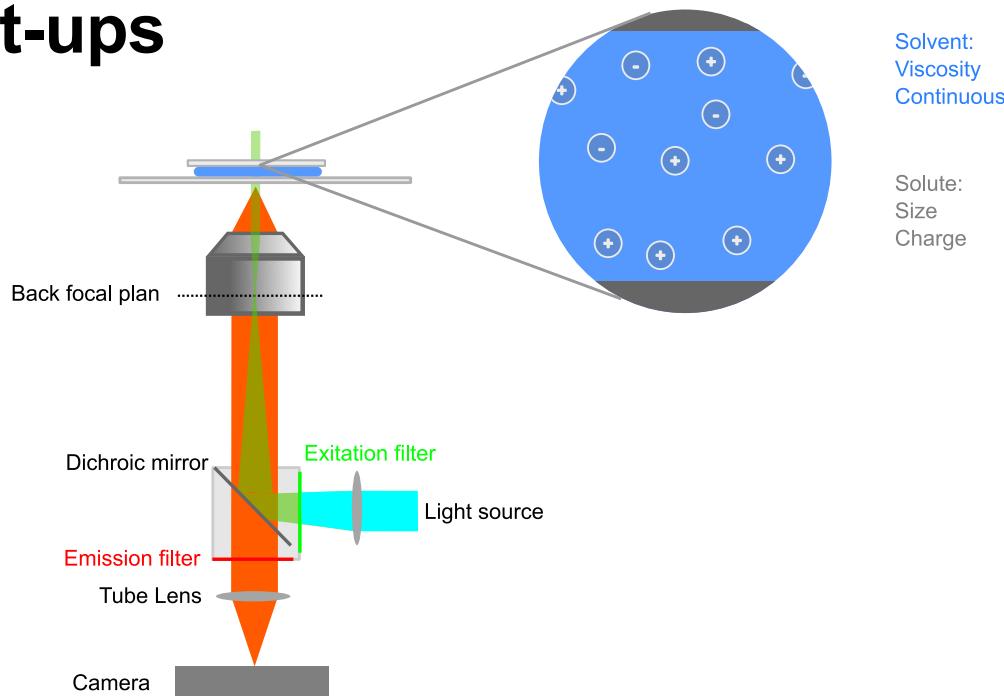
# Programme for today

Time	Topic
13:00	Lecture part 1 + 2
14:30	Exercise (paper Lee PRL 2014)
16:30	Lecture on application (paper Rasmussen Nat Comm 2020)

Learning objective 10321:

- Analyse the behaviour of DNA molecules confined in nanofluidic channels (F1)
- **Describe optothermal solute and solvent transport in nanofluidic devices (F2)**

# Definitions and set-ups



- In the following lecture we will consider: the solvent and the solute.  
The solute can be ions, molecules, particles.  
In some cases the ions are seen as a part of the solvent (if something else is the solute)  
To visualize the solute (molecules and particles) we use fluorescence microscopy.

# Part 1: Transport due to thermal gradients

# Experiment 1: DNA and a hot spot

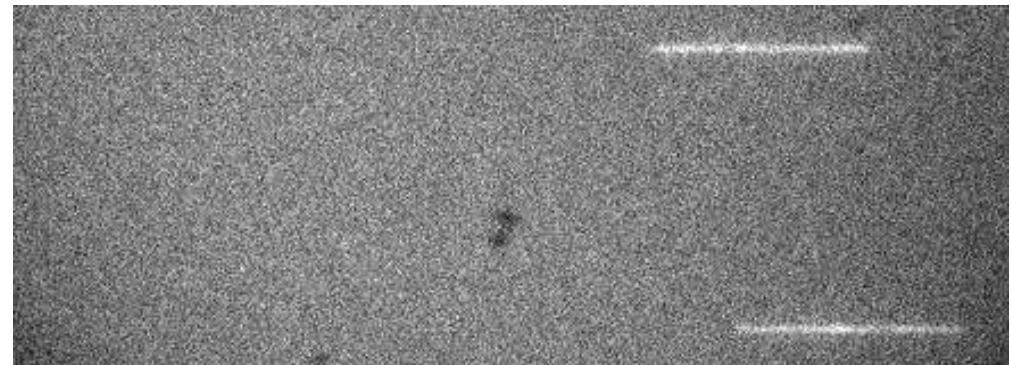
Genomic DNA in a microfluidic channel,  
observed using fluorescence microscopy.



On the solute, thermophoresis works like a ‘finger’  
pushing the object.

Solvent: buffer solution  
Solute: Fluorescent DNA with yoyo-1

Stretched genomic DNA confined in a nanochannel.

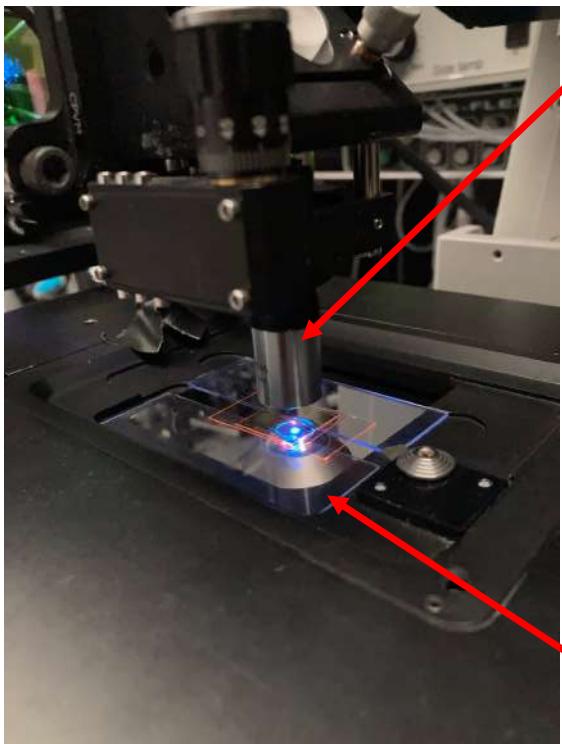


When pushed, the DNA compresses.

Data by Lasse H. Thamdrup

# How to create a hot spot?

Solvent: buffer solution  
Solute: Fluorescent Ruthenium complex



infrared laser objective.

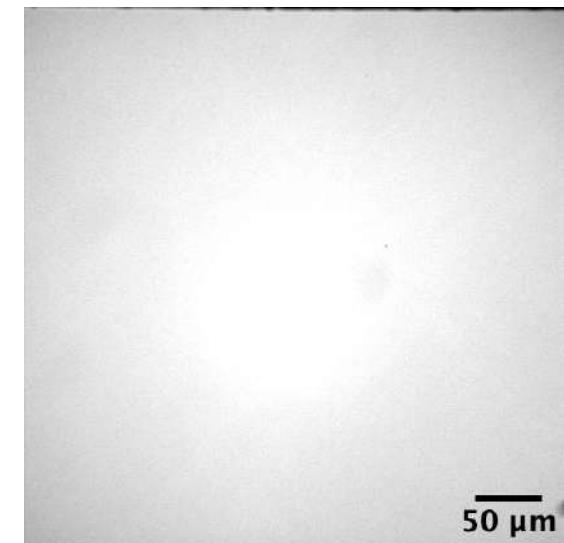
We excite with blue light.

We image the fluorescence of the Ruthenium complex (orange)

The concentration of the Dye is homogeneous.

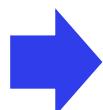
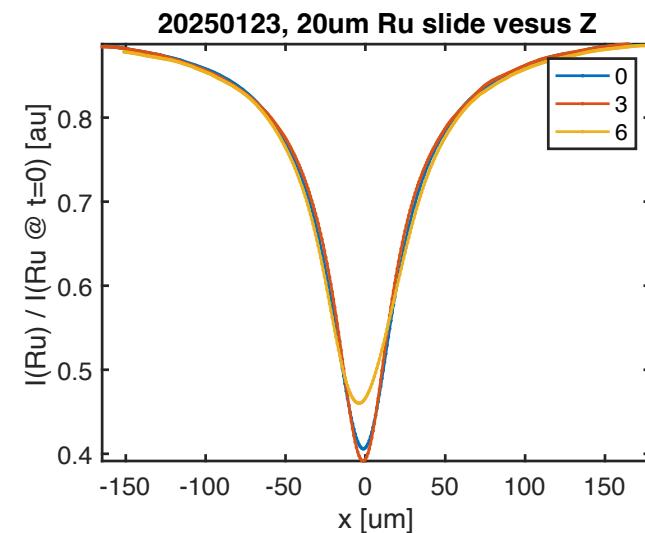
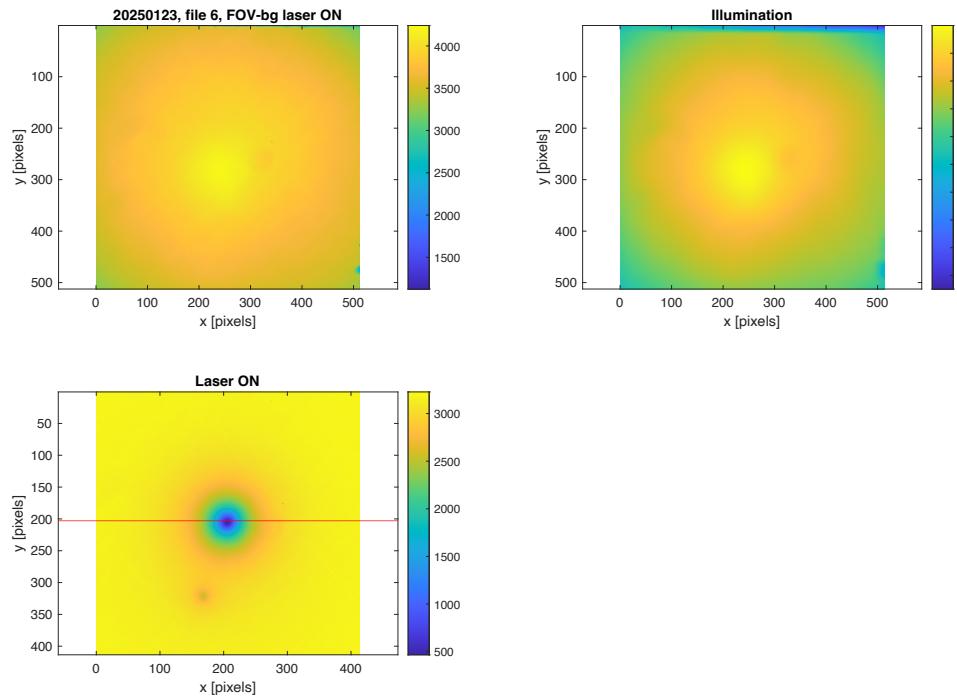
We focus an infrared laser in the middle of the field of view.

Imaging objective.



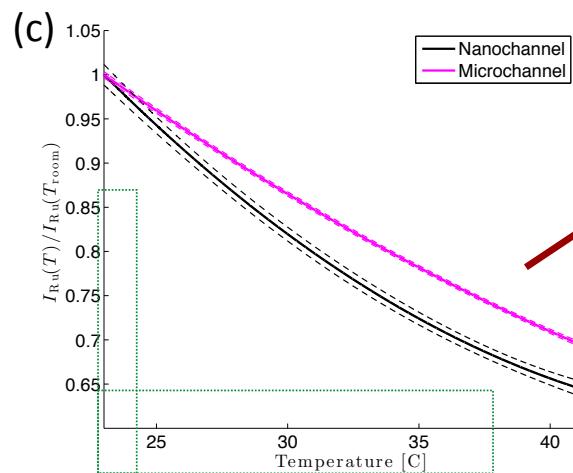
- The fluorescent dye has a quantum efficiency that depends on the temperature.
- The hot spot is fast but not instantaneous as seen for the cooling.

# How to measure a local temperature gradient



- The infra red laser spot is only a few micrometers.
- The hot spot is about  $50\text{ }\mu m$ -wide ie much larger than the laser spot.
- Temperature is actually raised over the whole sample slide due to dissipation.

# Ruthenium calibration



A change of  $I(T) / I(T_{room})$  of 0.25 means  $T = T_{room} + 15 \text{ K}$

or

0.01 units change means  $T_{room} + 0.6 \text{ K}$

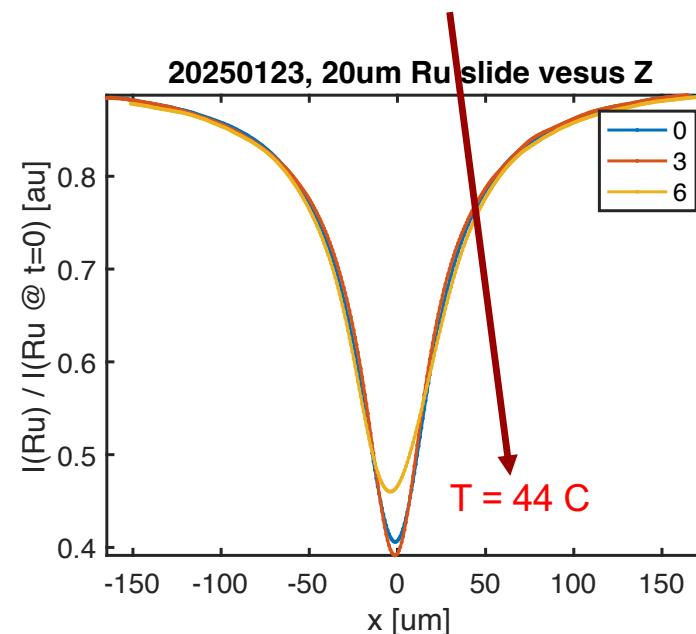


FIG. S1. Calibration of temperature vs. light intensity.  
 (a) Average intensity per pixel measured over the nanochannels for an integration time of 50 ms for the sweeps with increasing (blue) and decreasing (red) temperature, respectively. Dashed lines mark standard errors on the fitted values.  
 (b) Averaged calibration curve  $\mathcal{I}_{\text{calib}}(T)$ , including standard errors for the two sweeps shown in Panel (a).  
 (c) Averaged calibration curve for the two sweeps in Panel (b) for the measurements over the nanochannels (black). A similar calibration curve over the microchannels is shown for comparison (magenta).

- The fluorescence intensity change is converted into a local temperature change.

Calibration from Pedersen et al, PRL 2014.

## Description of thermophoresis

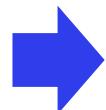
The drift velocity due to the gradient is:  $\vec{v} = -D_T \nabla T.$

The balance between thermophoresis and diffusion is represented by the **Soret coefficient**:

$$S_T = D_T/D.$$

This is assuming:

- Dilute solution = no particle-particle interactions.
- Small Temperature gradients.



In experiment 1, the genomic DNA moves **away from the hot spot** due to thermophoresis.  
Molecules are large and has a **small diffusion coefficient D**.  
DNA is a **negatively charged** solute.

Duhr and Braun PNAS (2006) 103 52

# Physical origin of the thermophoresis

The particle moves to minimize the free energy  $F=U-TS$ .

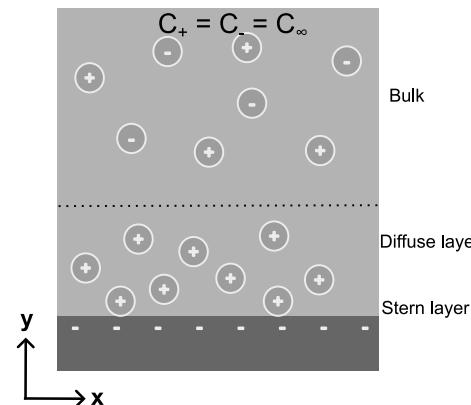
The Soret coefficient can be modelled as:

$$S_T = -S/k_B T$$

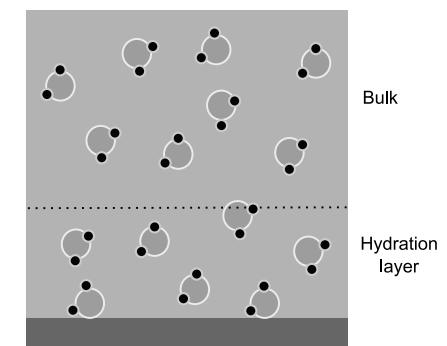
There are three contributions to the **entropy  $S$**  of the solute/solution system:

- The **ionic shielding** at the surface
- The **hydration** of the surface
- The Brownian motion (which can be neglected for large DNA molecules and large particles)

Ionic shielding (ie. the EDL formation)



hydration entropy



The hydration layer extends a few molecules in the solution.

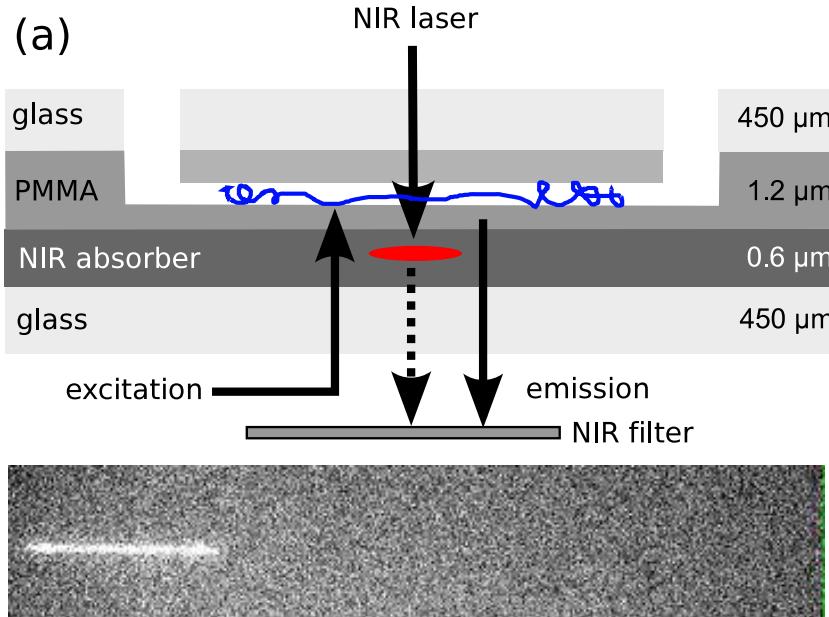
Usually  $S_{hyd} < 0$ : water is ordered at the surface.

The ‘order’ results from the interaction between water and the surface: electrostatic, hydrophilic/phobic, steric.

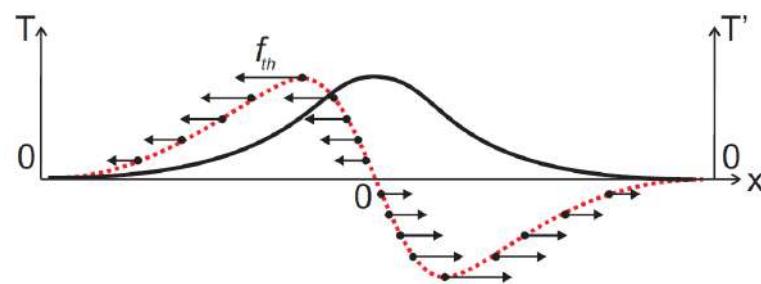
Duhr and Braun PNAS (2006) 103 52

## Experiment 2: Confined DNA over a hot spot

Solvent: buffer solution  
Solute: Fluorescent DNA

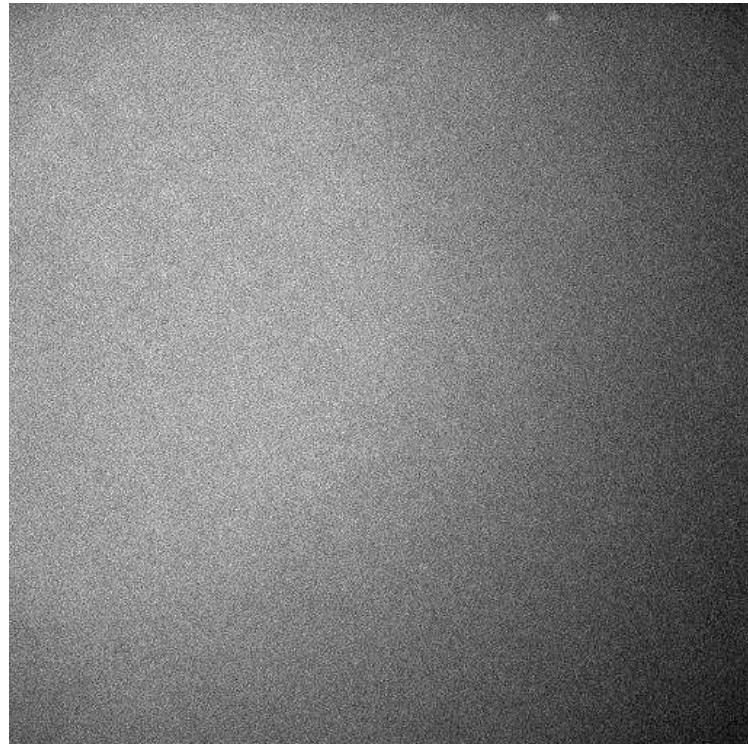


In experiment 1, the genomic DNA is arranged in a tug-of-war.  
The DNA is more stretched over the hot-spot than on the sides.  
The stretched part act as a spring on the two ends.



Pedersen et al, PRL 2014.

# Thermophoresis yes, but what about the solvent?



Solvent: buffer solution  
Solute: Fluorescent particles

Due to thermophoresis, the concentration of particles, at steady state is:

$$c/c_0 = \exp[-S_T(T - T_0)]$$

Discuss in groups:

What happens with the solute (nanoparticles)?

What does the movement of the particles tell about the solvent?

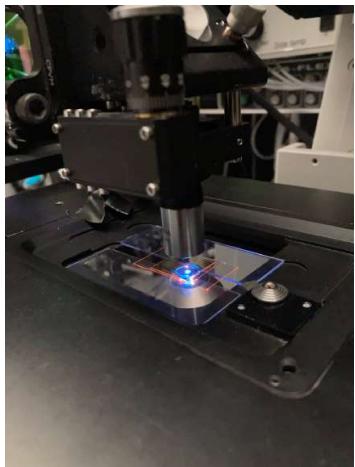


The video shows an accumulation, so there must be a competing transport of the particles: the thermal gradient induces a **convection** flow.

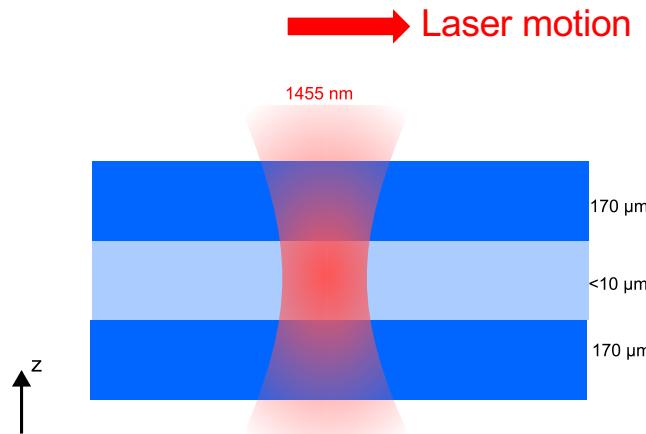
# Moving thermal gradients

Transport induced by a moving hot spot.

# Experiment 3.

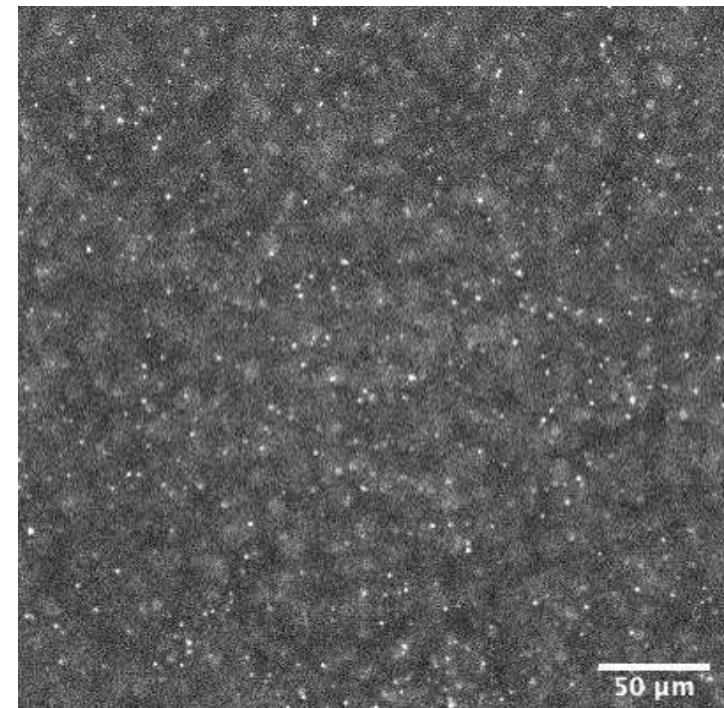


Solution contains 140 nm fluorescent particles  
Laser spots moves in a circle motion.



Solvent: buffer solution with increased viscosity  
Solute: Fluorescent particles (diluted)

10 x speed up video.



Discuss in groups what phenomena can or cannot explain the solute motion.

# Experiment 3:

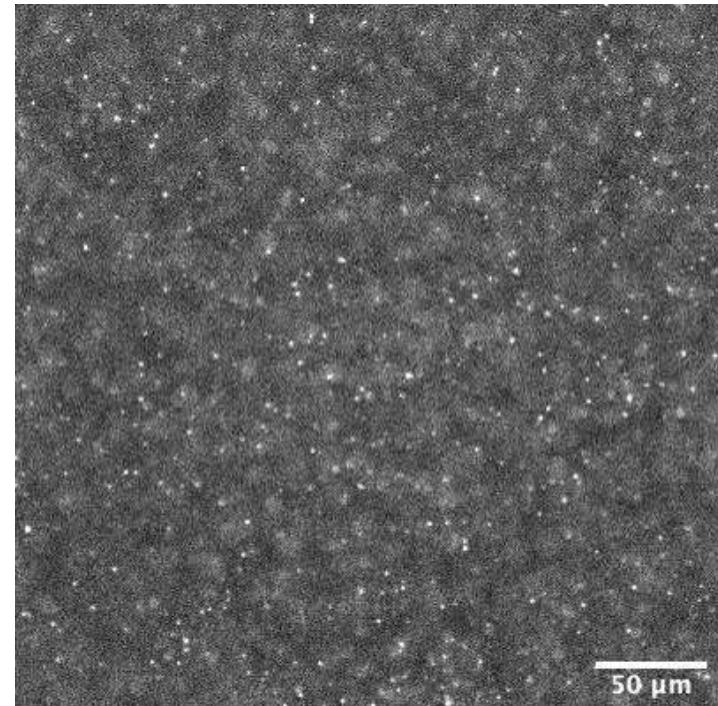
## Experimental details:

- Fluid thickness >10  $\mu\text{m}$  (about 20  $\mu\text{m}$ )
- Data shown is at IR laser  $I_{LD} = 400 \text{ mA}$ .
- The laser passes at each spot 50 times per second (ie Repetition rate is 50 Hz).
- We add glycerol to the solution.

→ The particles are **not** moving much: diffusion is suppressed.  
The particles' motion is actually revealing the solvent flow.

Solvent: buffer solution with **increased viscosity**  
Solute: Fluorescent particles (diluted)

10 x speed up video.



# Experiment 3: particle tracking

## Result from tracking:

The particles move in the ‘opposite’ direction than the laser beam motion.

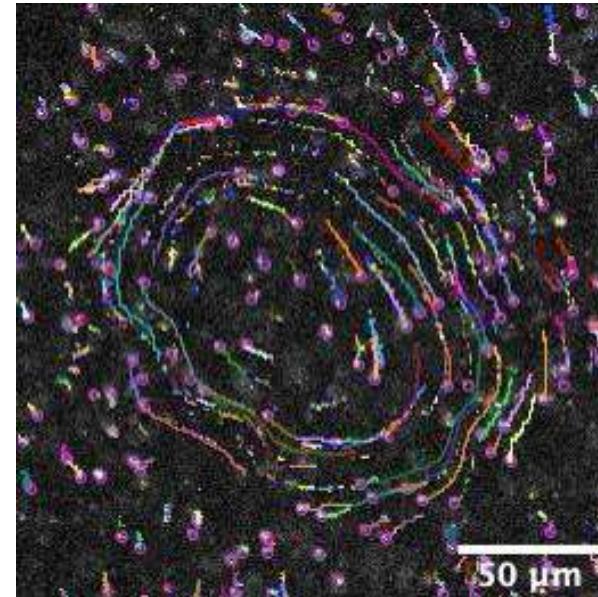
Can thermophoresis and convection explain the motion?



The particles are **not** moving because of thermophoresis

The motion is **not** due to a **convection** flow.

Real time video with particle tracking result.



# Thermoviscous flows shown by Weinert & Braun

JOURNAL OF APPLIED PHYSICS **104**, 104701 (2008)

## Optically driven fluid flow along arbitrary microscale patterns using thermoviscous expansion

Franz M. Weinert and Dieter Braun<sup>a</sup>

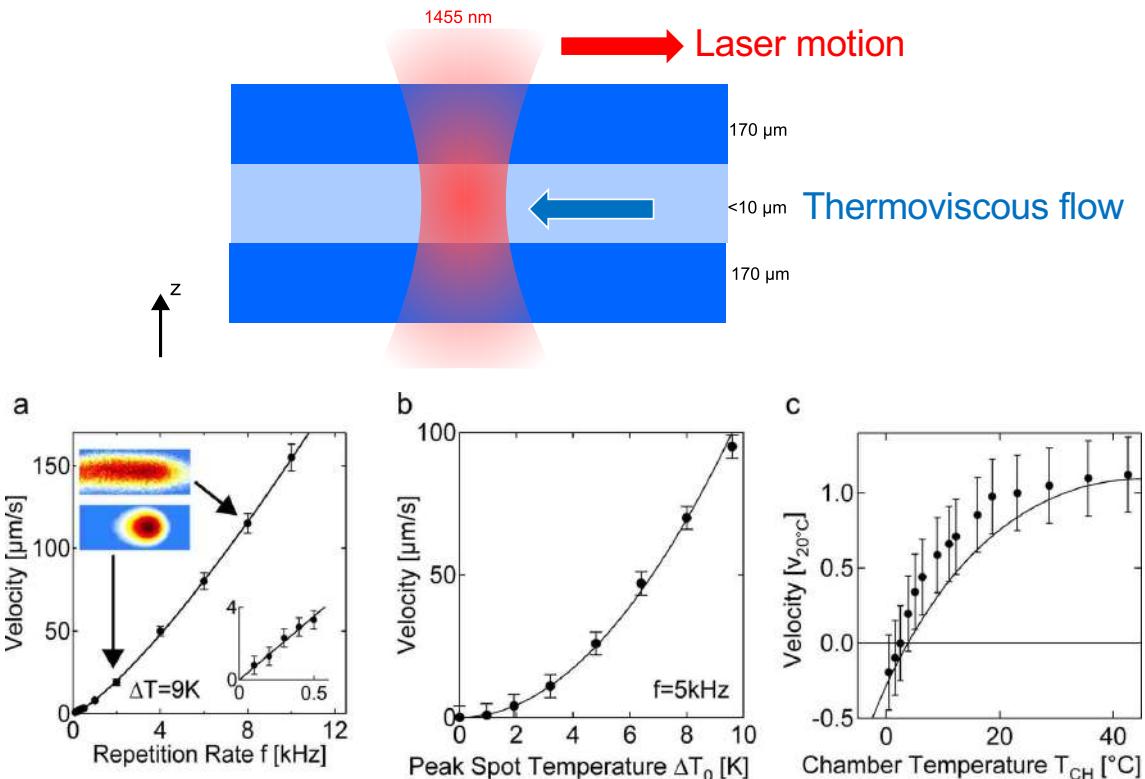
<sup>a</sup>Systems Biophysics, Functional NanoSystems, Center for Nanoscience (CENS), Department of Physics, Ludwig-Maximilians University Munich, Amalienstr. 54, 80799 Munich, Germany

(Received 25 June 2008; accepted 8 October 2008; published online 25 November 2008)

We show how fluid can be moved by a laser scanning microscope. Selected parts of a fluid film are pumped along the path of a moving warm spot which is generated by the repetitive motion of an infrared laser focus. With this technique, we remotely drive arbitrary two-dimensional fluid flow patterns with a resolution of 2  $\mu\text{m}$ . Pump speeds of 150  $\mu\text{m/s}$  are reached in water with a maximal temperature increase in the local spot of 10 K. Various experiments confirm that the fluid motion results from the dynamic thermal expansion in a gradient of viscosity. The viscosity in the spot is reduced by its enhanced temperature. This leads to a broken symmetry between thermal expansion and thermal contraction in the front and the wake of the spot. As result the fluid moves opposite to the spot direction due to both the asymmetric thermal expansion in the spot front and the asymmetric thermal contraction in its wake. We derive an analytical expression for the fluid speed from the Navier-Stokes equations. Its predictions are experimentally confirmed without fitting parameters under a number of different conditions. In water, this nonlinearity leads to a fluid step of  $<100$  nm for each passage of the spot. Since the spot movement can be repeated in the kilohertz regime, fluid speeds can exceed 100  $\mu\text{m/s}$ . Using this technique, we pump nanoparticles over millimeters through a gel. An all-optical creation of a dilution series of DNA and biomolecules by aliquotting and mixing is demonstrated for fluids sandwiched between untreated and unstructured, disposable microscope cover slips. The shown optical remote control of fluid flow expands the microfluidic paradigm into previously inaccessible regimes of tiny volumes, closed flow paths, fast switching between flow patterns, and remote fluid control under extreme fluid conditions. © 2008 American Institute of Physics. [DOI: [10.1063/1.3026526](https://doi.org/10.1063/1.3026526)]

### Weinert experimental:

- fluid thickness  $< 10 \mu\text{m}$ .
- 1  $\mu\text{m}$  PS beads in 75% glycerol.
- repetition rates  $>$  kHz .



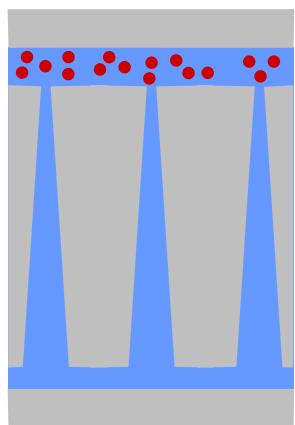
Weinert 2008 Journal of Applied Phys 104 104701

# Part 2: A failed experiment with salt

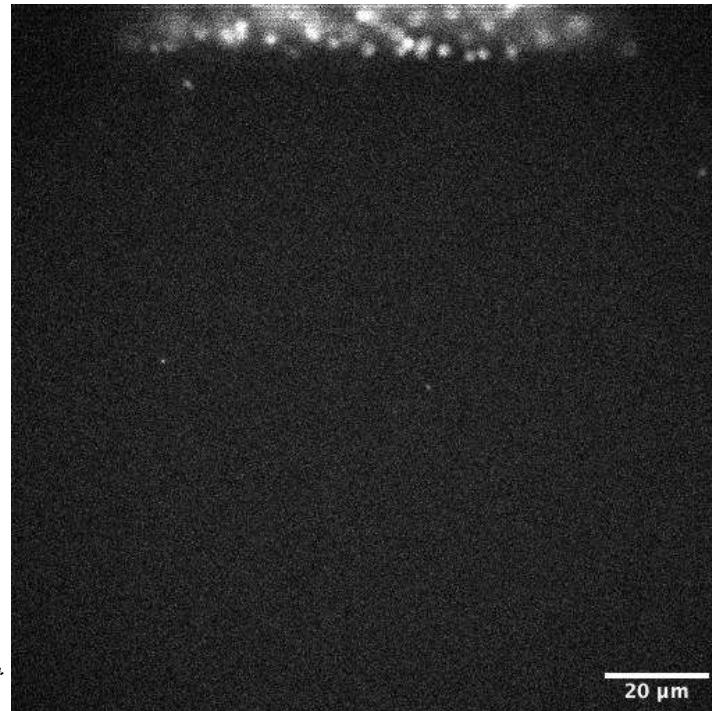
# Experiment in the lab

A little while later...

particles in low salt

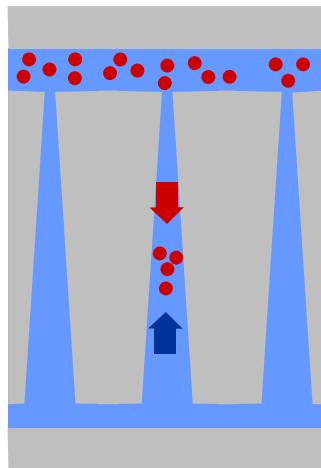


high salt



# Hypothesis

particles in low salt

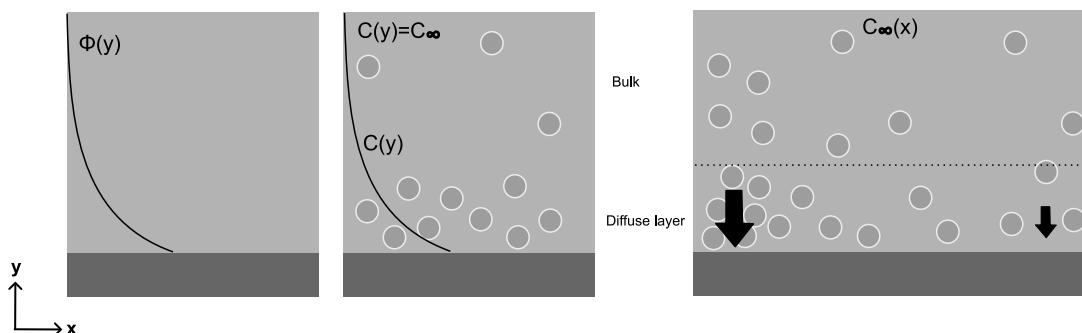


Discuss what happens in this experiment?

- There is diffusion
- But also two competing transports.
- Both due to the salt gradient.

# Transport of solvent in a neutral solute gradient

- The neutral solute experiences a **surface potential**.
- There is a concentration profile along  $y$
- The concentration gradient in the bulk results in a pressure gradient in the diffuse layer



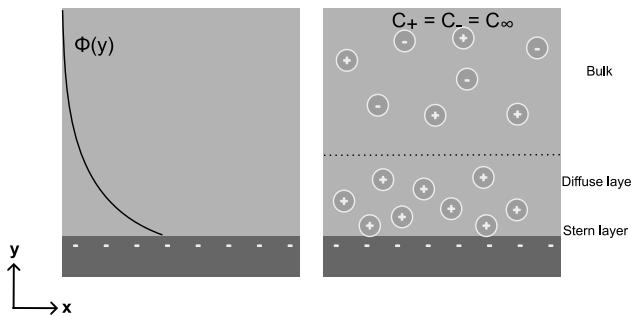
$$\begin{aligned} v^s &= -\frac{k_B T}{\eta} \frac{dC}{dx} \int_0^\infty y [e^{-\Phi(y)/k_B T} - 1] dy \\ &= -\frac{k_B T}{\eta} \frac{dC}{dx} K L^* \end{aligned}$$

And so in this example, the liquid flow to the right.

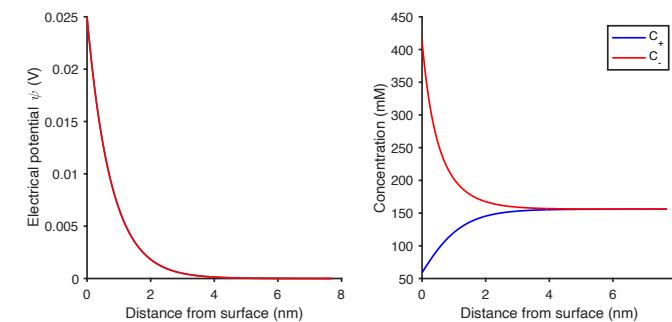
OBS: This is the topic of part 1 & 2 of the exercise.

# Charged solutes: the formation of the EDL

- Electrolytes experience an electrical potential at the surface
- Positive and negative ions form the **electrical double layer** (Stern layer and diffuse layer).



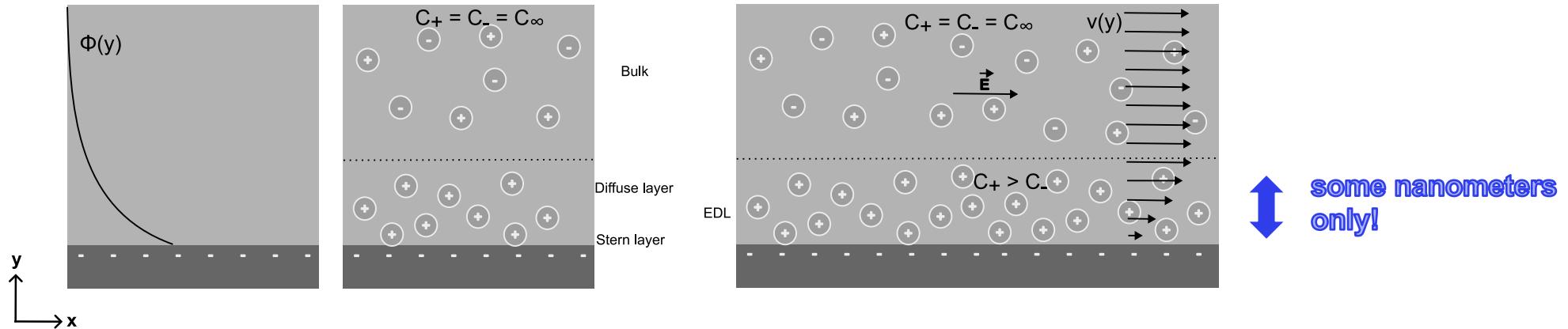
We can calculate the ions concentrations.



The Debye length is **1 nm at 100 mM** monovalent electrolyte (salt).

# Detour: the EDL can be used for transport by electroosmotic (EO) flow

- The EDL formation induces a net volume charge of the liquid.
- The liquid moves when an electric field is applied along x.



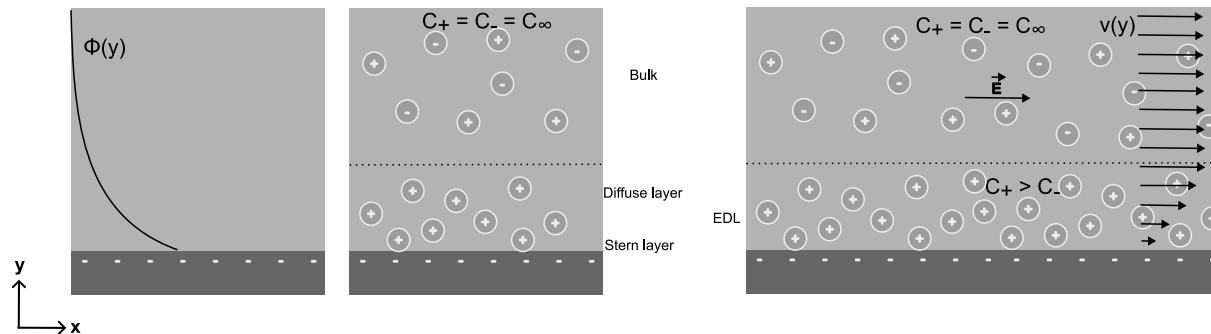
The shape of the fluid velocity along  $y$  is that of a **plug flow**.

# Plug flow versus Poiseuille flow

Discuss what the difference is between a pressure driven flow and an electroosmotic flow

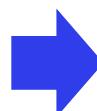
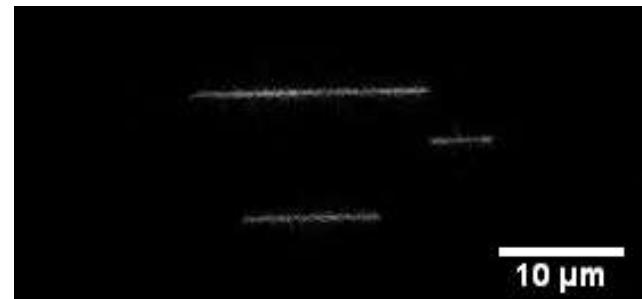
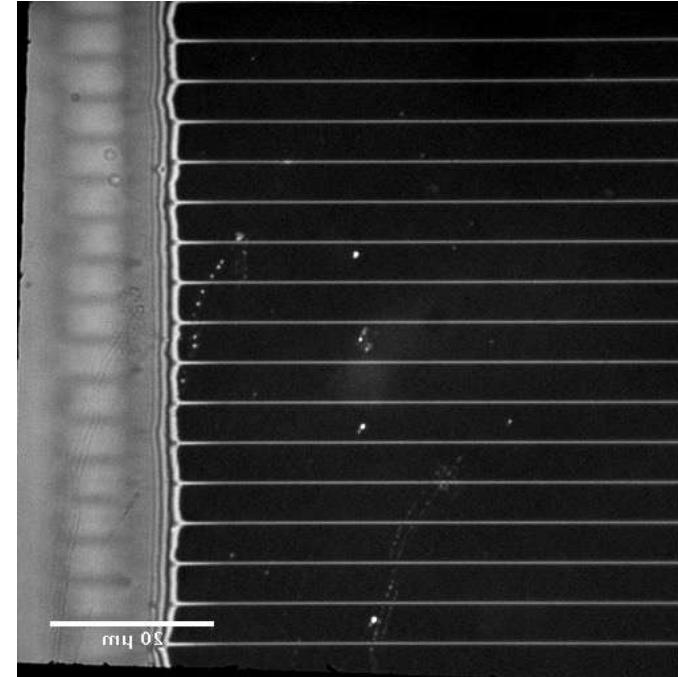
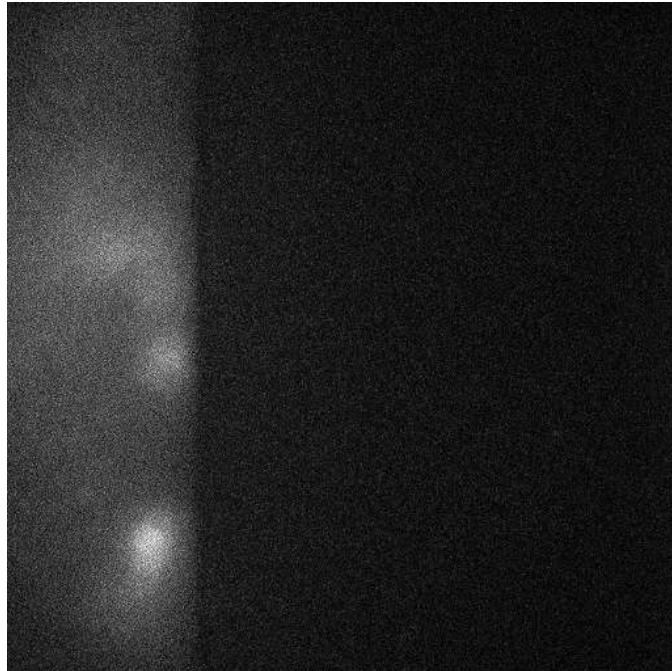
# Transport of solute using Electrophoresis.

- When applying an electric field, a charged solute would also move: **electrophoresis**



- 
- The electrophoretic force is a **body force**.
  - The solute is still subjected to the viscous drag (Stokes drag)...
  - ... and so to fully use the electrophoretic transport of a solute, we often suppress the EO flow.

## Example from last lecture: DNA in nanochannels



- The **electrodes** are placed in the microchannels.
- A **surfactant** is added to suppress the EO flow.
- The **electric field**  $E$  is strongest in the 'narrow' nanochannels.

## Back to our failed experiment

- There is no electric field imposed with electrodes.
- The solvent and the particle motion are both induced by the salt gradient.



This leads us to consider diffusioosmosis induced by a charged solute

# Transport of solvent in an electrolyte gradient

The diffusioosmotic flow:

$$\beta = \frac{D_+ - D_-}{D_+ + D_-} \quad (\simeq -0.20 \text{ for NaCl}),$$

$$D_{DO} = \frac{\epsilon k_B T}{\eta Ze} \left[ \beta \zeta - 2 \frac{k_B T}{Ze} \ln(1 - \gamma^2) \right]$$

$$v_x(\infty) = -D_{DO} \frac{d \ln C_\infty(x)}{dx}$$

The electrolyte has ions with different diffusion coefficients

The DO mobility depends on the charge of the channel wall.

The flow velocity depends on the log of the gradient (not just the gradient as for the neutral solute).



The dependency on the log of the gradient comes from the Electric field developed in the dilute layer of the EDL.

# Exercise (1h30)

- Calculations and plotting exercise based on the paper *Lee et al. PRL 2014*
- *Download the exercise and the paper from the LEARN content page.*

PRL 112, 244501 (2014)

PHYSICAL REVIEW LETTERS

week ending  
20 JUNE 2014

## Osmotic Flow through Fully Permeable Nanochannels

C. Lee,<sup>1,2</sup> C. Cottin-Bizonne,<sup>1</sup> A.-L. Biance,<sup>1</sup> P. Joseph,<sup>3,4</sup> L. Bocquet,<sup>1,\*</sup> and C. Ybert<sup>1,†</sup>

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(Received 28 February 2014; published 19 June 2014)

Osmosis across membranes is intrinsically associated with the concept of semipermeability. Here, however, we demonstrate that osmotic flow can be generated by solute gradients across nonselective, fully permeable nanochannels. Using a fluorescence imaging technique, we are able to measure the water flow rate inside single nanochannels to an unprecedented sensitivity of femtoliters per minute flow rates. Our results indicate the onset of a convective liquid motion under salinity gradients, from the higher to lower electrolyte concentration, which is attributed to diffusio-osmotic transport. To our knowledge, this is the first experimental evidence and quantitative investigation of this subtle interfacially driven transport, which need to be accounted for in nanoscale dynamics. Finally, diffusio-osmotic transport under a neutral polymer gradient is also demonstrated. The experiments highlight the entropic depletion of polymers that occurs at the nanochannel surface, resulting in convective flow in the opposite direction to that seen for electrolytes.

DOI: 10.1103/PhysRevLett.112.244501

PACS numbers: 47.61.Fg, 82.39.Wj, 47.57.jd

## Nano-2 Course

### Exercise on Diffusioosmosis and -phoresis

Rodolphe Marie and Jonas Nyvold Pedersen\*  
DTU Health Tech

#### Introduction

In this exercise we look at diffusiophoresis. Before starting, you should have read the lecture notes. During the exercises you will read the following paper: Lee et al 'Osmotic flow through fully permeable nanochannels' PRL 2014 [?].

#### Diffusioosmotic flow for a neutral solute.

We look at the case where a neutral solute experiences a 'Hard wall potential' at the surface. For spherical particles with radius  $a$  and a hard-wall potential, we can define the potential  $\Phi$  as:  $\Phi(y) = \infty$  for  $y < a$ , and  $\Phi(y) = 0$  for  $y > a$ .

**Question 1:** Calculate the expression of the integral  $K^*L$  in Equation 22 of the lecture notes (Section 3.4).

**Question 2:** Write the expression for the slip velocity ie. the velocity far from the wall  $v(y \rightarrow \infty)$ .

# Exercise (1h30)

- Calculations and plotting exercise based on the paper *Lee et al. PRL 2014*
- Download the exercise and the paper from the LEARN content page.

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The neutral solute is a Semi-flexible Polymer (Lee 2014 PRL paper)

We now consider a situation where the solute is not a hard sphere but instead, a semiflexible polymer. The entropic surface potential  $\Phi(y)$  experienced by a semiflexible polymer near a hard wall can be approximated by a scaling relation involving the radius of gyration  $R_g$ :

$$\Phi(z) \approx k_B T \left( \frac{R_g}{y} \right)^n \quad (0.1)$$

In the paper they write that for PEG, the mobility is:

$$\mu_{DO} \approx -\frac{k_B T}{\eta} R_g^2 \quad (0.2)$$

OBS: please note there is a typo in equation 4, Temperature is missing!

**Question 5:** Write the expression of the radius of gyration for double stranded

DNA. Hint you can either calculate the Flory radius first then use  $R_g = R_F/\sqrt{6}$  or calculate  $R_g$  using the worm like chain model. Do you expect the mobility to change as function of the salt concentration and why?

**Question 6:** Calculate the radius of gyration of  $\lambda$ -DNA which is 48 kbp long.

**Question 7:** Calculate the DO mobility and the flow rate in fL/min expected from a gradient  $(n_L - n_R) = 1 \mu\text{M}$  of  $\lambda$ -DNA. OBS: the gradient value needs to be converted from M to  $\text{m}^3$  units.

# Exercise (1h30)

PRL 112, 244501 (2014)

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DOI: 10.1103/PhysRevLett.112.244501

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## Diffusioosmosis for a salt gradient (Lee 2014 PRL paper)

We now consider the first part of the paper dealing with salt gradients. The diffusioosmotic flow is induced in a channel of length  $L = 150 \mu\text{m}$  a square cross section of width  $w = 5 \mu\text{m}$  and a zeta potential of -55 mV. The solute gradient is made by imposing a gradient of sodium chloride. You can find the necessary values for parameters in the lecture note (or the paper).

We first look at the experiment where the flow rate in the nanochannel is measured using a fluorescent 'tracer' molecule fluorescein gradient with  $C(x = 0) = C_L$  and  $C(x = L) = 0$ . The profile of concentration of the tracer molecule is the solution of the diffusion-advection equation:

**Question 8:** The solution is the concentration  $C(x)$  as function of  $x$ . This is

$$C(x) = C_L \left(1 - \frac{1 - e^{vx/D}}{1 - e^{vL/D}}\right). \text{ Plot the concentration profile for } v = 0, 1 \text{ and } 8 \mu\text{m/s}.$$

**Question 9:** Show that the tracer concentration  $C(x)$  reduces to the linear expression  $C(x) = C_L(1 - x/L)$  when  $v \ll D/L$ .

**Question 10:** Using the same equation but applied to the salt instead of the tracer, check that the assumption that the salt gradient is linear is verified in the paper. For this you need to use the concentration of salt in the left channel  $C^s(x = 0) = C_L^s$  and in the right channel  $C_s(x = L) = C_R^s$ .

**Question 11:** Assuming that the gradient of salt is linear, write the expression of the salt concentration  $C^s(x)$  as function of  $x$  with  $C^s(x = 0) = C_L$  and  $C^s(x = L) = C_R$ .

**Question 12:** Calculate the diffusioosmotic mobility  $D_{DO}$  using Eq. (4) in the Lee PRL paper.

**Question 13:** Now use your favorite program to plot the slip velocity  $v_s(x)$  as function of  $x$  between  $x = 0$  and  $x = L$ . Use the Eq. (2) of the paper or Eq. (78) of the lecture note. Do you see any problem here? If not plot the flow rate  $Q_{slip}$  as function of  $x$ . What fundamental law does this break?

# Part 3: Diffusiophoretic trapping application

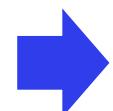
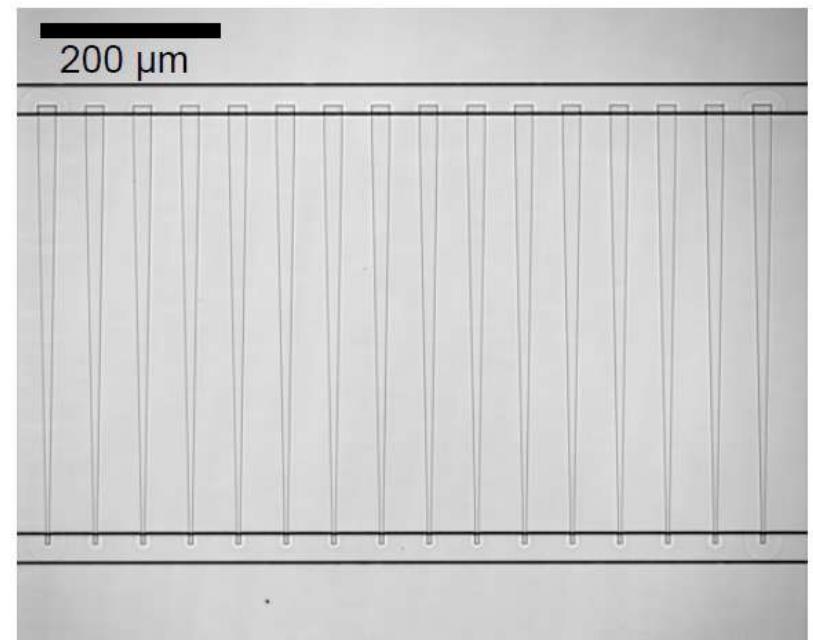
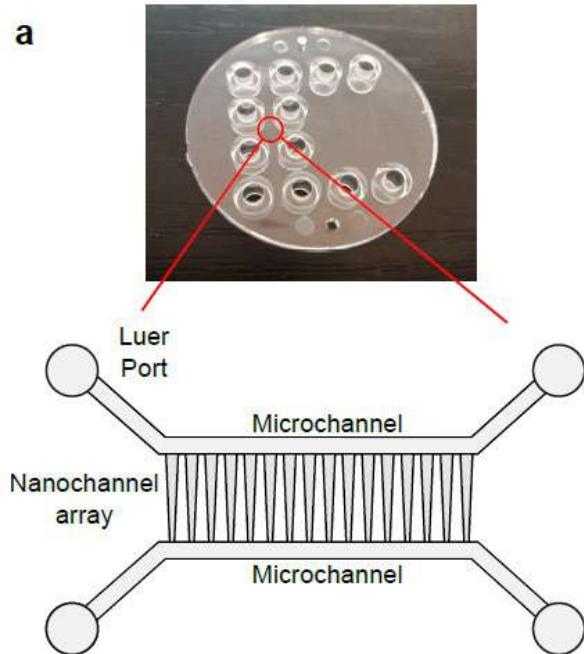
Based on the following papers:

M.K. Rasmussen, J.N. Pedersen & R. Marie  
"Size and surface charge characterization of nanoparticles with a salt gradient"  
Nature communications (2020)

M.K. Rasmussen, J.N. Pedersen & R. Marie  
"Label-Free Sensing of Biorecognition on Liposomes"  
ACS sensors (2020)

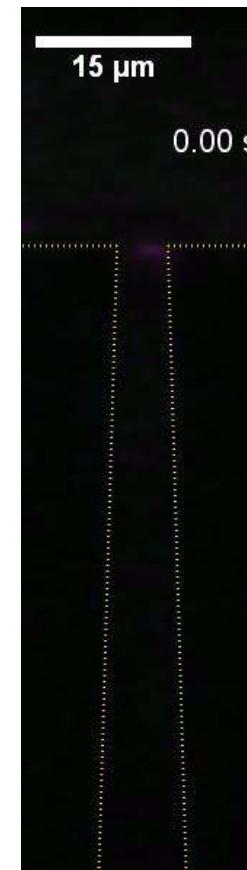
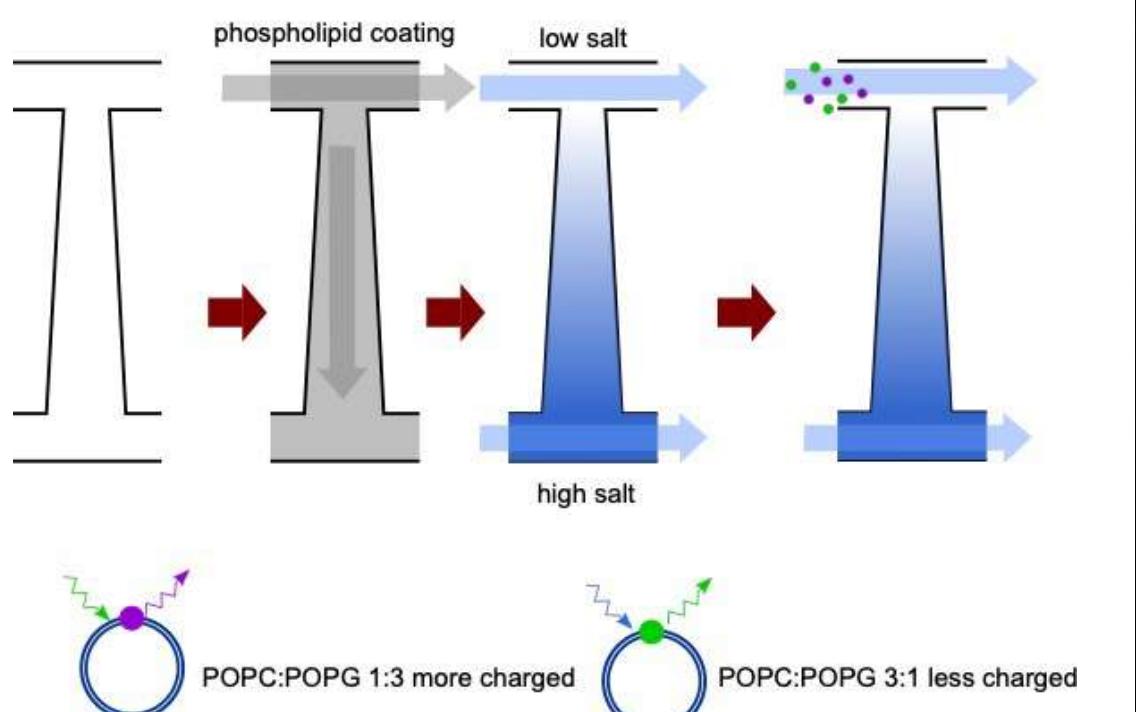
M.K. Rasmussen, R. Marie, J.N. Pedersen  
, Quantifying DNA-Mediated Liposome Fusion Kinetics with a Nanofluidic Trap, in preparation.

# Nanofluidic funnels fabricated by injection molding



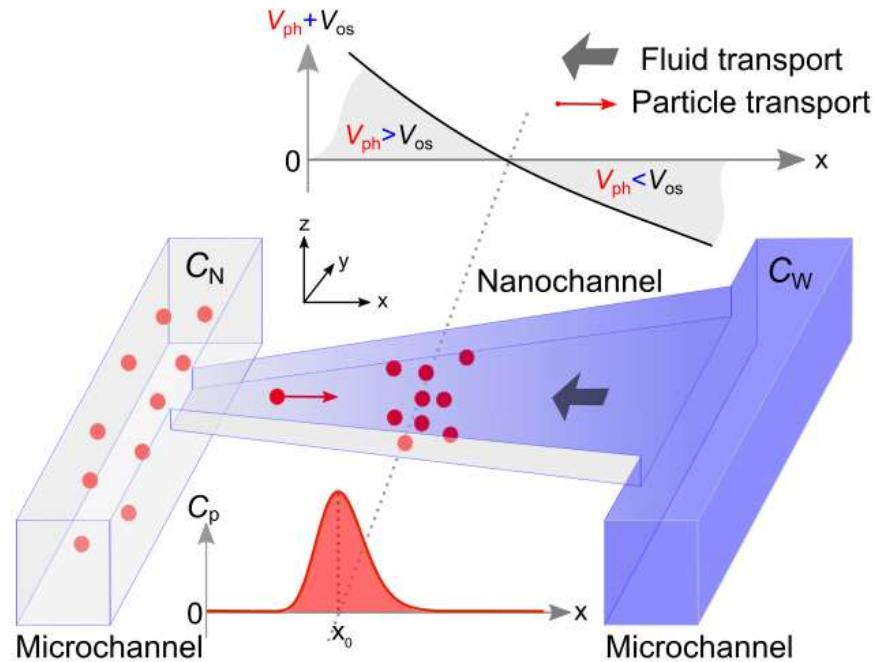
- Nanochannel depth is 295 nm.
- Each funnel is 5 to 20  $\mu\text{m}$  wide over 440  $\mu\text{m}$  length.
- Lid is a 150  $\mu\text{m}$  -thick TOPAS 5013 foil.

# Separating liposomes in a salt gradient

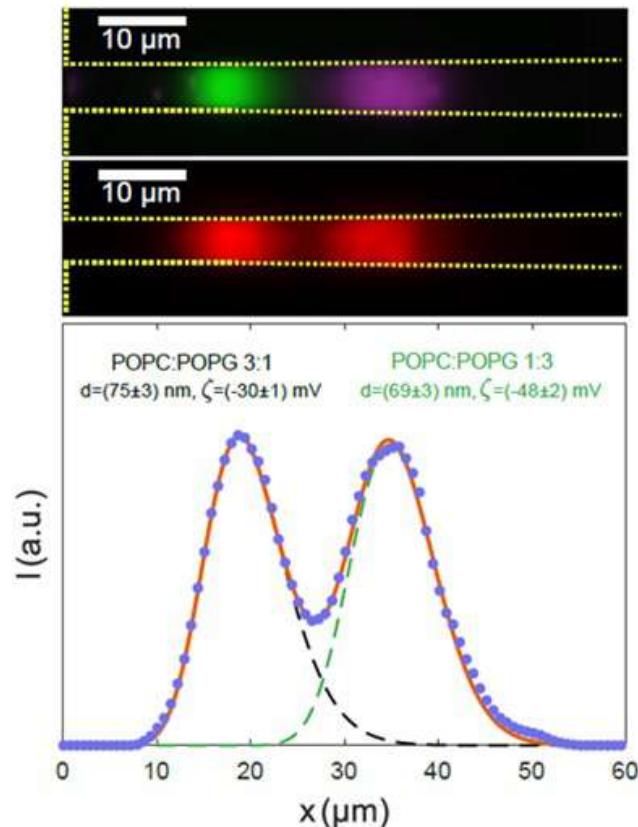


# Principle of the diffusiophoretic trap

- Nanofluidic device with static salt gradient.
- Diffusiophoresis = **particle** motion.
- Diffusioosmosis = fluid flow leading to Stoke's drag.



# Separating liposomes subpopulations



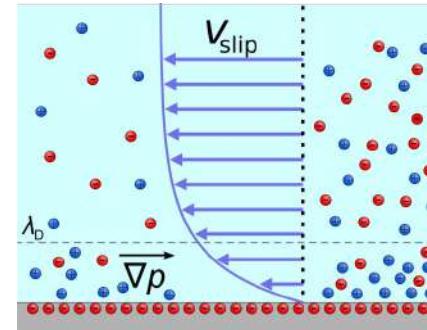
- Labeled separately.
- Labeled with the same fluorophore.

Particles are separated by size and/or zeta potential.

# Diffusioosmosis and diffusiophoresis

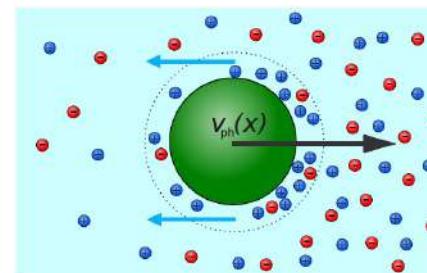
- Salt gradient induce diffusioosmotic flow

$$v_{\text{slip}}(x) = \Gamma_{\text{os}}(\zeta_{\text{surf.}}) \frac{\nabla C(x)}{C(x)}$$



- Diffusiophoresis for particles

$$v_{\text{ph}}(x) = \Gamma_{\text{ph}}(\zeta, d) \frac{\nabla C(x)}{C(x)}$$

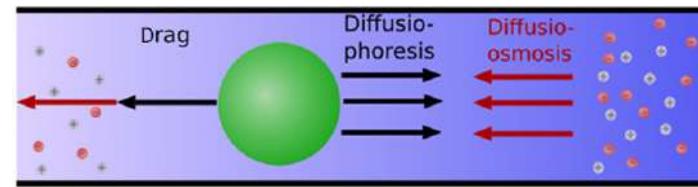


# Modelling the diffusiophoretic trap at steady state

- Condition for trapping is

$$v_{\text{ph}}(x) + v_{\text{os}}(x) = 0$$

- Model from Fick's first law with drift



$$C_p(x) = C_p(x_0) e^{\int_{x_0}^x dx' [v_{\text{os}}(x', \zeta_{\text{surf}}) + v_{\text{ph}}(x', d, \zeta)] / D_p(d)}$$

-Diffusioosmosis, calibration.

-Diffusiophoresis ( $d$  and  $\zeta$ ).

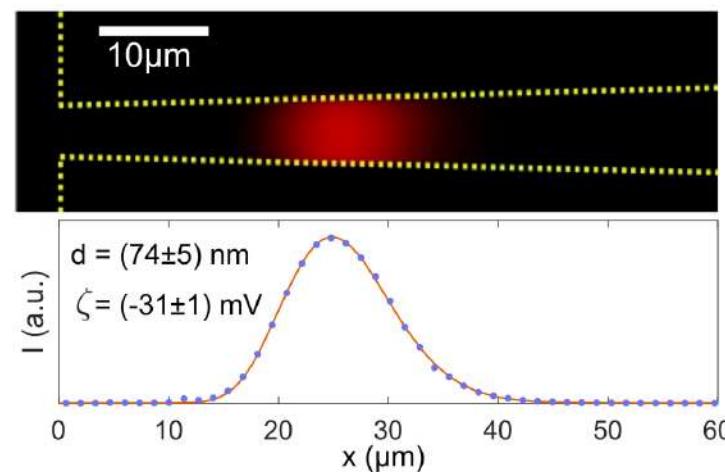
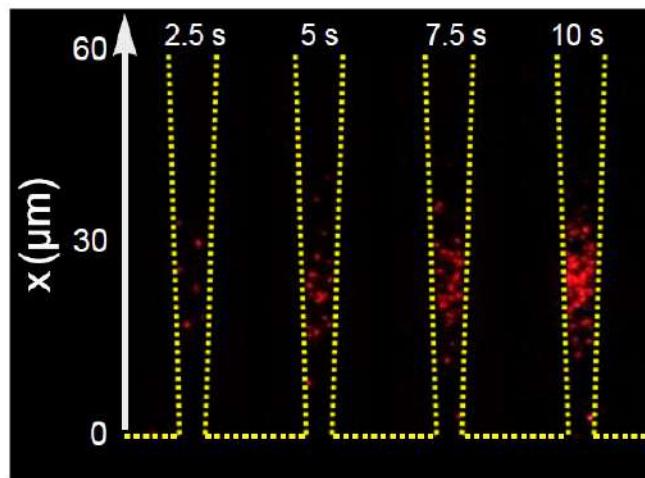
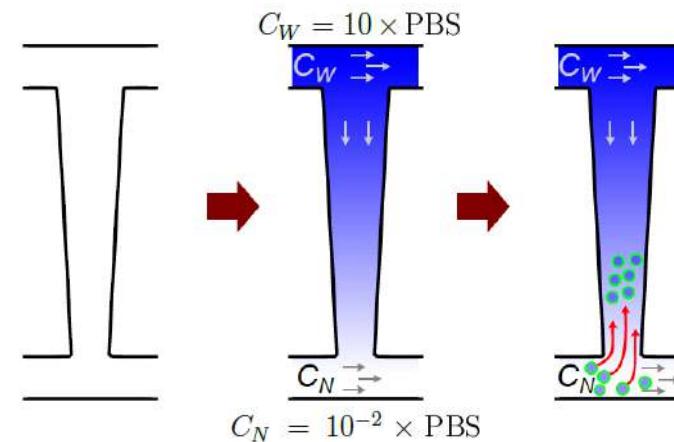
-Diffusion coefficient ( $d$ ).

→ we can infer  $d$  and  $\zeta$  from a fit to the concentration of particles  $C_p$ .

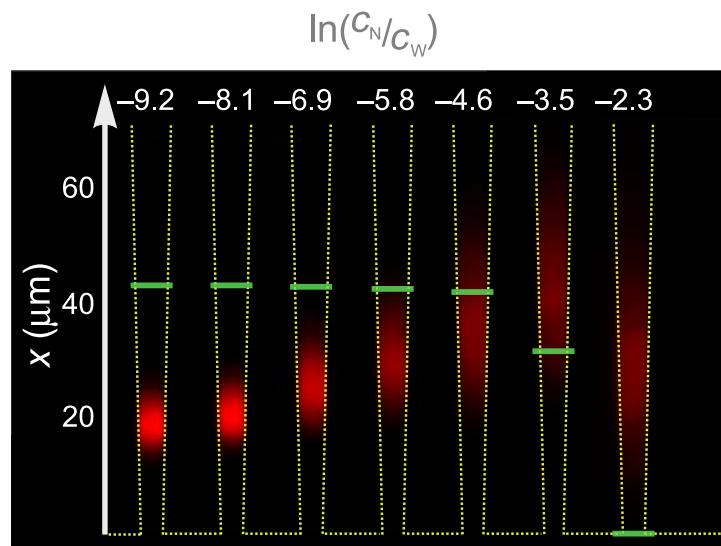
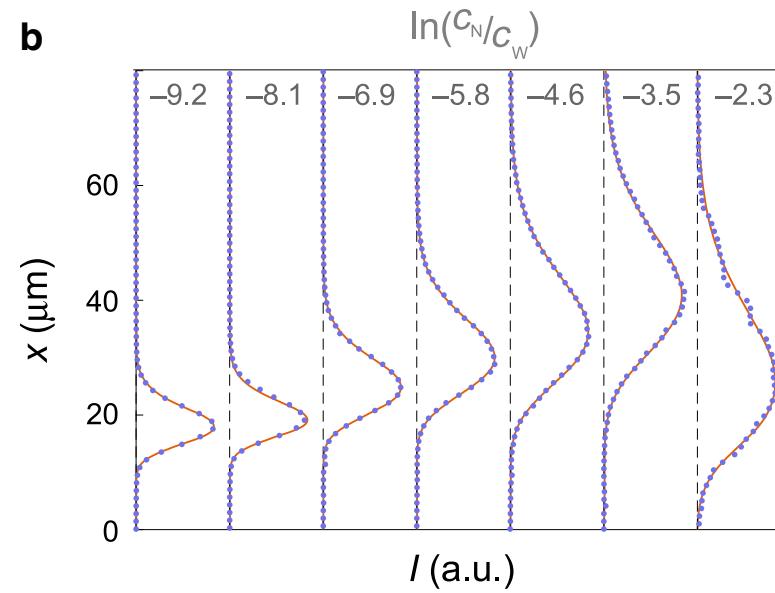
# Nanoparticle characterization

- POPC:POPG 3:1 liposomes.
- Concentrated ~400x times after 90 s.
- Capture efficiency ~6% for each trap.
- $d_{DLS} = (76 \pm 3) \text{ nm}$   
 $\zeta_{LDE} = (-28 \pm 1) \text{ mV}$

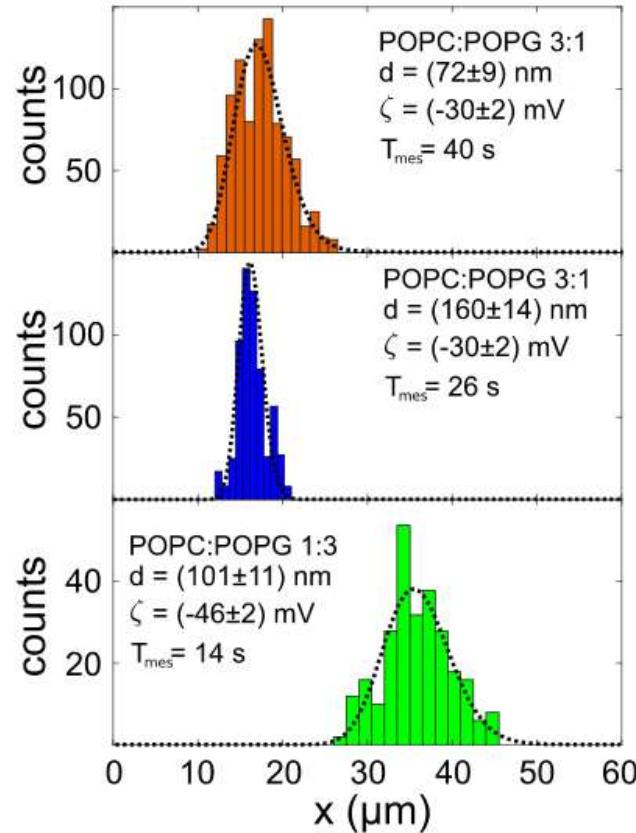
→ Size and  $\zeta$  from a single measurement.



# Trapping at different gradient strength

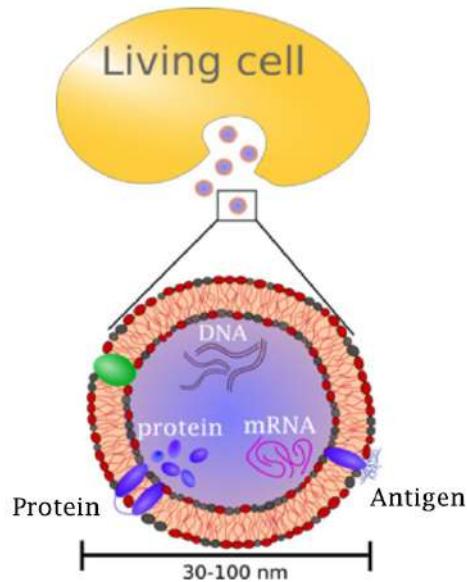
**a****b**

# Trapping and characterizing single particles



From single particle measurements, we could analyse heterogeneous samples.

# Motivation for analysing exosomes (extracellular vesicles ie. EVs)



## Purification of exosomes:

- Ultracentrifugation.
- Differential solubility.
- Membrane filtration.

## Characterizing size and $\zeta$ -potential:

- Dynamic light scattering (DLS).
- Nanoparticle tracking analysis (NTA)
- Cryo-electron microscopy.
- Tunable resistive pulse sensing (TRPS).
- Laser doppler electrophoresis (LDE).

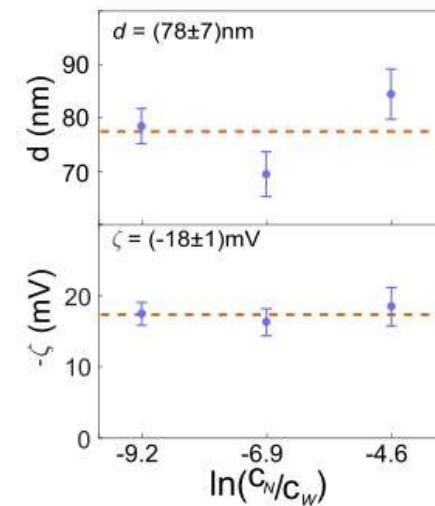
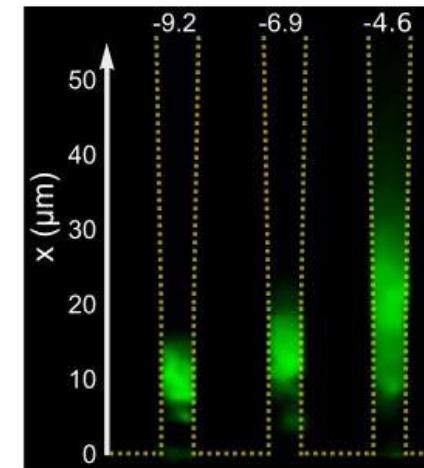
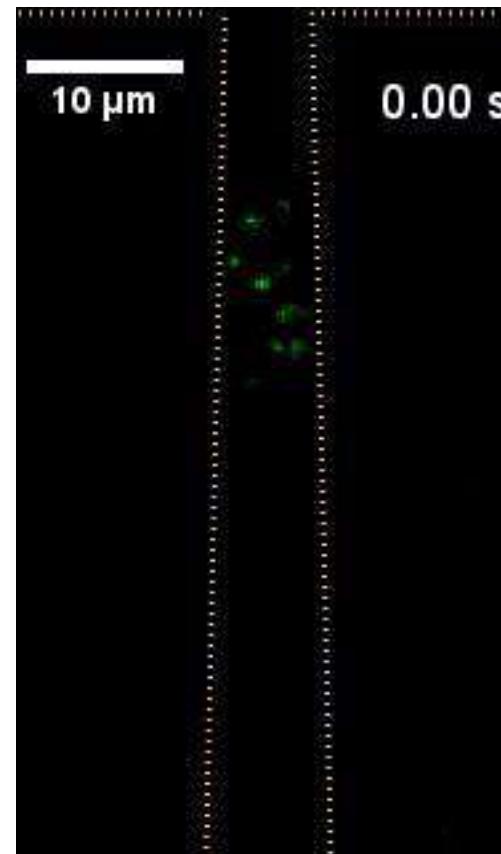
## Characterizing surface molecules:

- Fluorescence assays (Binding ligands)
- Quartz crystal microbalance with dissipation (QCM-D)
- Localized-surface plasmon resonance (L-SPR)
- Second harmonic generation (SHG)
- Tunable resistive pulse sensing (TRPS).
- Laser doppler electrophoresis (LDE)

→Bottleneck for improved sensitivity is the purification and characterization.

# Application to exosomes

- Exosomes from human blood serum, already purified.
  - Concentrated ~400x in 90s.
  - Measured at different salt gradients.
  - Consistent with NTA (provider's spec. sheet).
- Analysis of heterogeneous population.



## Conclusion and Outlook

- ✓ d and  $\zeta$  from a single measurement.
- ✓ Concentrating nanoparticles 400x in a few minutes.
- ✓ Application to exosomes isolated from blood plasma.
  - ❖ Monitoring DNA hybridization on liposomes.
  - ❖ Monitoring reactions between liposomes.
  - ❖ Further work with exosomes towards diagnostics applications.

## Acknowledgements

Assistant Professor Jannik Larsen, Technical University of Denmark.

Associate Professor Karen Martinez, University of Copenhagen.

PhD student Fatima Zahra Abd Issa, University of Copenhagen.

DTU Health Tech for funding of Martin K Rasmussen PhD.

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# End of the lecture

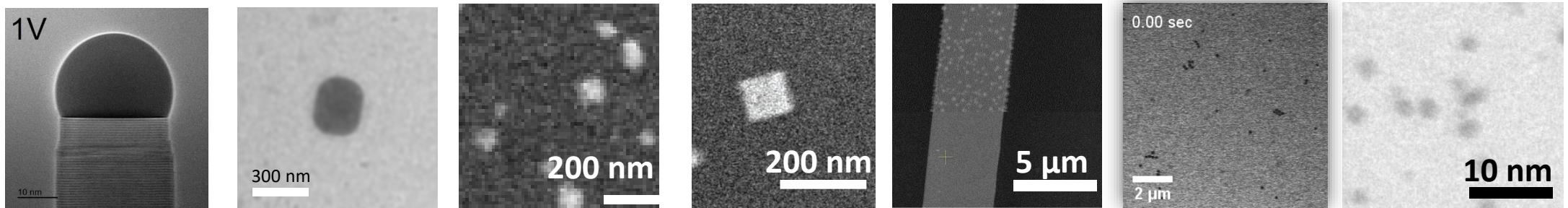
# Nano2 Theme F

## Analyzing Nanosystems using Liquid phase transmission electron microscopy

Murat Nulati Yesibolati, Hongyu Sun & Kristian Mølhave

DTU Nanolab

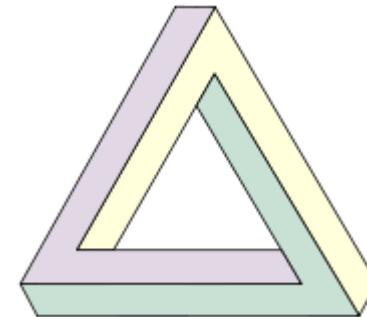
Molecular  
Windows



# Case F3+4 Program

## Today's lecture

13:00 - 13:35	<b>Session 1.</b> Course introduction
13:45 -14:30	<b>Session 2.</b> Transmission electron microscopy
14:40 -15:30	<b>Session 3.</b> Liquid phase electron microscopy
15:45-17:00	<b>Group work</b> on 'designing an experiment'

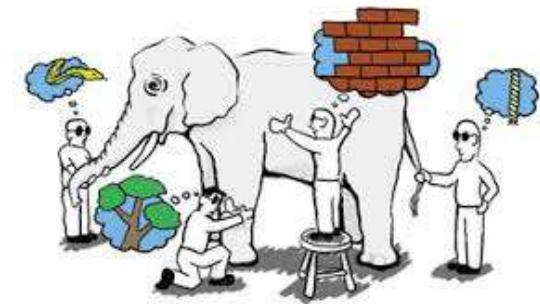


## Next lecture:

1. **Session 4:** Theory of Nucleation processes
2. **Session 5:** Nucleation processes viewed by LPEM
3. **Group Work Prepare presentations**
4. **Group presentations** (10 minutes) and discussion(5 minutes)

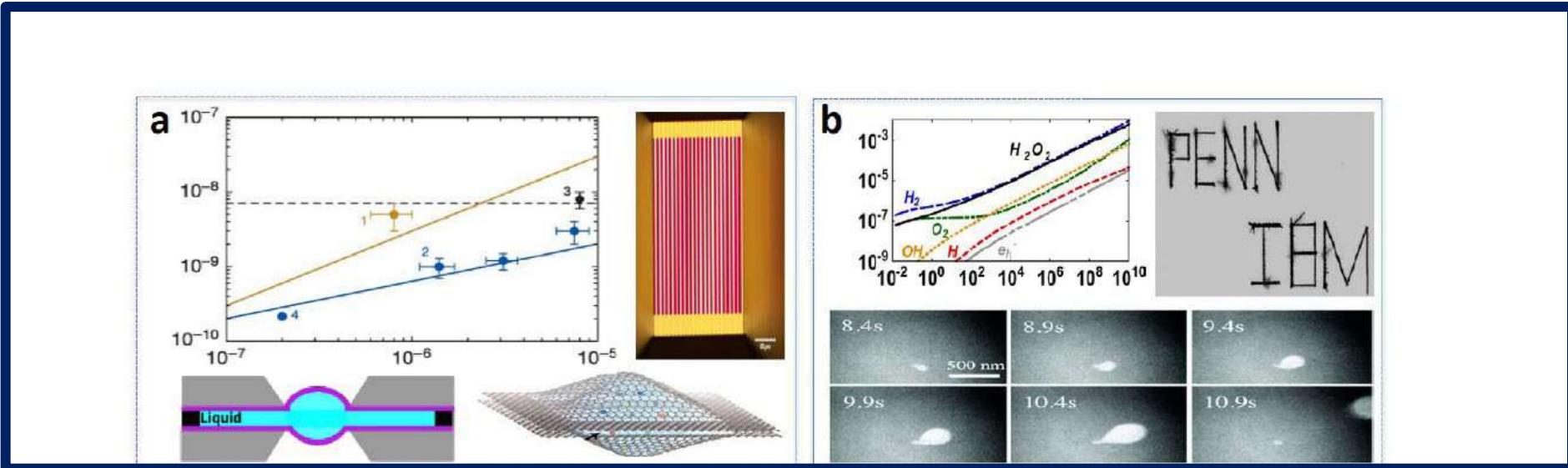
# Learning objectives for case F3,4

1. To explain liquid cell TEM methods based on the operating principles of a TEM
2. To interpret nanostructure growth measurements
3. To design an experiment for liquid cell studies
  - Group work and very short presentation in next class
4. To be able to choose an appropriate method for a given experiment (by knowing the above limitations)

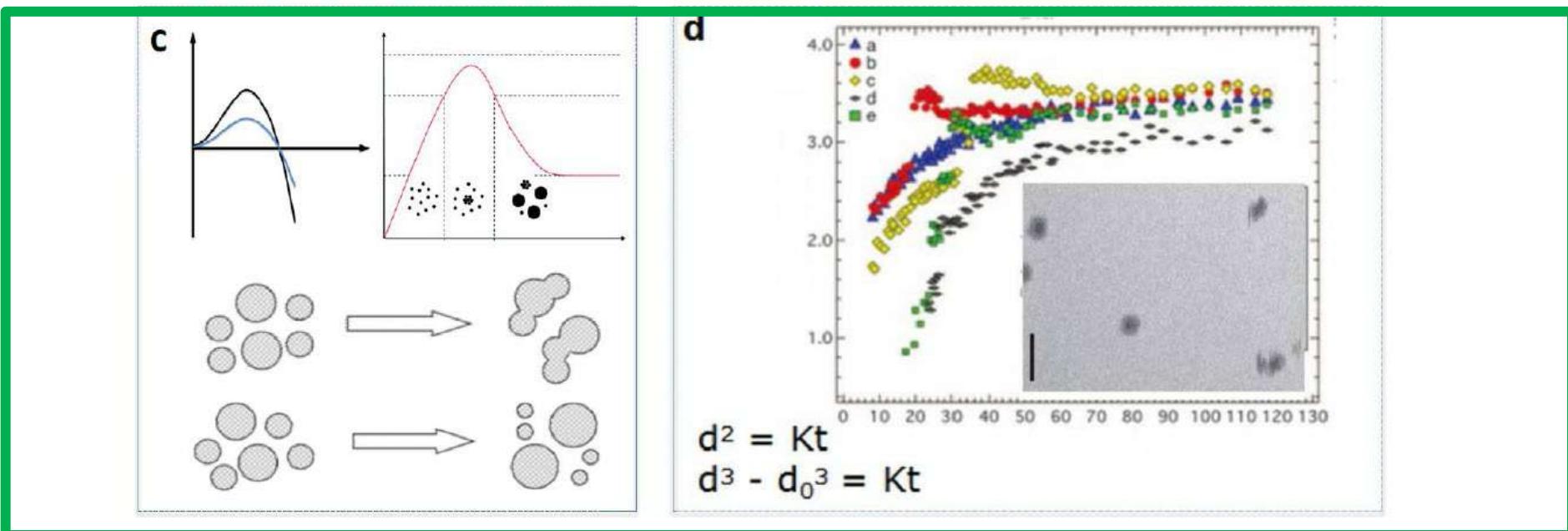


# What's in this case? (exam slide)

Today



Next lecture



Home assignment:

# Preparing for a short presentation

**Each group Design experiments :**

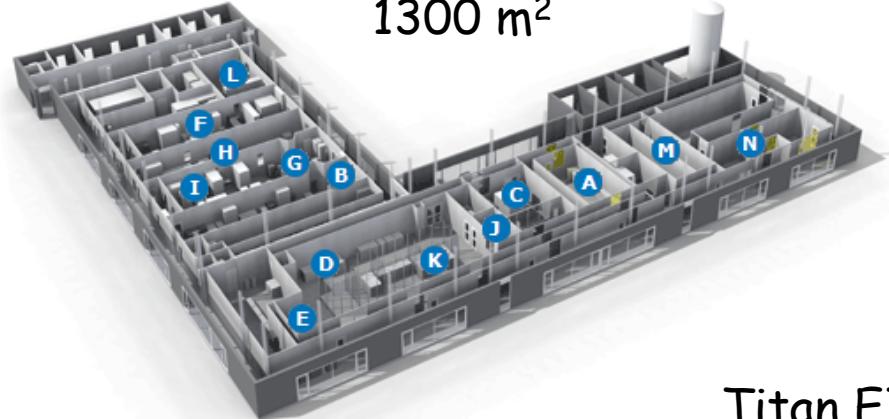
**You can choose to come up with your own suggestion or choose one below to further improve the previous studies**

1. We would like to observe the lithiation process of a silicon nanowire. They would be amazing battery electrode materials as they can store several Li ions pr silicon atom, but also swell to a far larger volume in the process making the battery quite difficult to make stable during use. How would make an experiment to observe the process? You can get inspiration from refs [1, 2] - how could you improve it?
  - a) Perspectives in in situ transmission electron microscopy studies on lithium battery electrodes. Current Opinion in Chemical Engineering, 2016, 12, 37-43.  
<http://www.sciencedirect.com/science/article/pii/S2211339816300089>
  - b) Demonstration of an Electrochemical Liquid Cell for Operando Transmission Electron Microscopy Observation of the Lithiation/Delithiation Behavior of Si Nanowire Battery Anodes. Nano Lett., 2013, 13, 6106–6112. <http://pubs.acs.org/doi/abs/10.1021/nl403402q>
2. Silicon is etched in KOH in the cleanroom, but the actual chemical process is not well characterized and details on how precipitates form near to the surface are making it difficult to use this etch to create a simple solution based etch process for the advanced new FIN-FET transistor designs in next generation microchips. Ref [3] would be useful for you to understand this etching process.
  - a) Transient Clustering of Reaction Intermediates during Wet Etching of Silicon Nanostructures. Nano Lett., 2017, 17, 2953–2958.  
<http://pubs.acs.org/doi/abs/10.1021/acs.nanolett.7b00196>
3. How to make graphene liquid cells?
  - a) "Strategies for Preparing Graphene Liquid Cells for Transmission Electron Microscopy" <https://pubs.acs.org/doi/10.1021/acs.nanolett.8b01366>
4. Energy research with liquid cell TEM
  - a) "Importance and Challenges of Electrochemical *In Situ* Liquid Cell Electron Microscopy for Energy Conversion Research"  
<https://pubs.acs.org/doi/full/10.1021/acs.accounts.6b00330>
5. Measure the electrode resistance during cycling or electrodeposition; possible factors that will affect the results. How to further improve the previous studies? You can refer to Refs [4, 5].
  - a) Electrochemical In-Situ Conductivity Measurements for Thin Film of  $\text{Li}_{1-x}\text{Mn}_2\text{O}_4$  Spinel. Chem. Mater. 2000, 12, 1367-1371. <http://pubs.acs.org/doi/pdf/10.1021/cm990696z>
  - b) . Electrochemical microsystem technologies: from fundamental research to technical systems. Electrochimica Acta 1999, 44, 3605-3627.  
<http://www.sciencedirect.com/science/article/pii/S0013468699000651>

Large facilities for creating and seeing the smallest structures:

Cleanroom w. student access

1300 m<sup>2</sup>



Cleanroom extension 700 m<sup>2</sup> in 2026



+ Softmatter & Polymer  
facilities ~200 m<sup>2</sup>

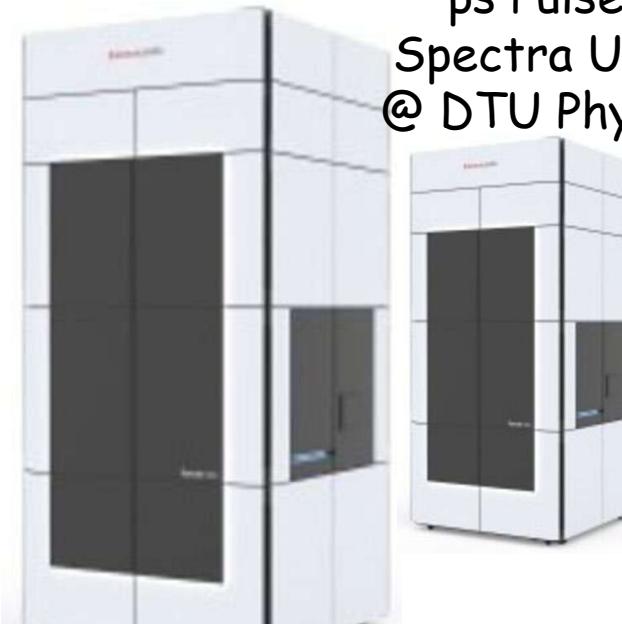
Wide range of  
TEM, FIB-SEM,  
SEM



Titan ETEM  
+ chip based in situ  
platforms



Spectra Ultra



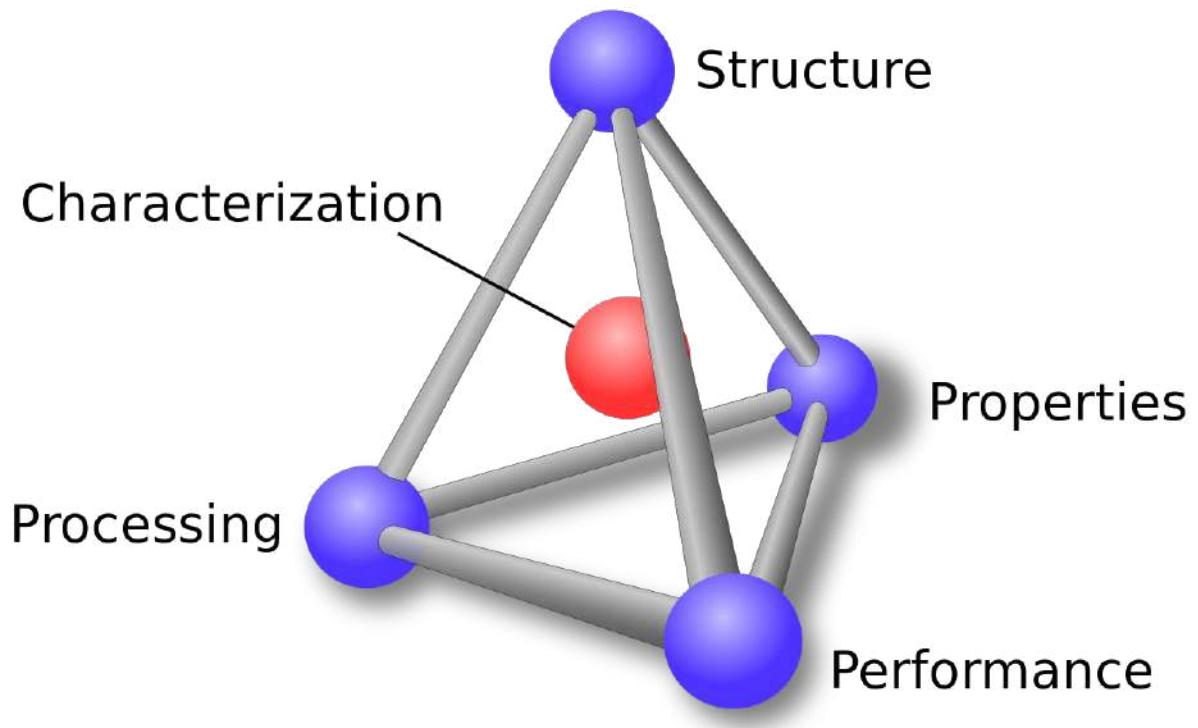
ps Pulsed  
Spectra Ultra  
@ DTU Physics

Molecular  
Windows

We make  
Microchips for  
Microscopy

# Why in situ microscopy

The materials science tetrahedron



<https://msestudent.com/what-is-materials-science-tetrahedron-paradigm/>

# Why in situ microscopy?

In the context of in situ and microscopy: manipulate or control the sample within microscope.

- Mechanically loading
- Heating
- External bias
- Changing the surrounding environment
- .....

Why is it valuable ?

- Real world dynamic interaction
- Fast reactions
- New insights
- Accelerate research and applications



imgflip.com

**House of tomorrow 1949**

# Why Liquid in situ microscopy?

- The majority of known chemical and biological processes occur in a liquid medium
- Knowing the liquid/particles structure and their evolution/dynamics at the molecular level will improve our understanding of the process, and engineer better solutions (ions, nanoparticle, living cells etc.)

## Energy Conversion and Storage



K. N. WOOD ET AL/ACS CENTRAL SCIENCE 2016

## Catalysis for chemical production and fuels

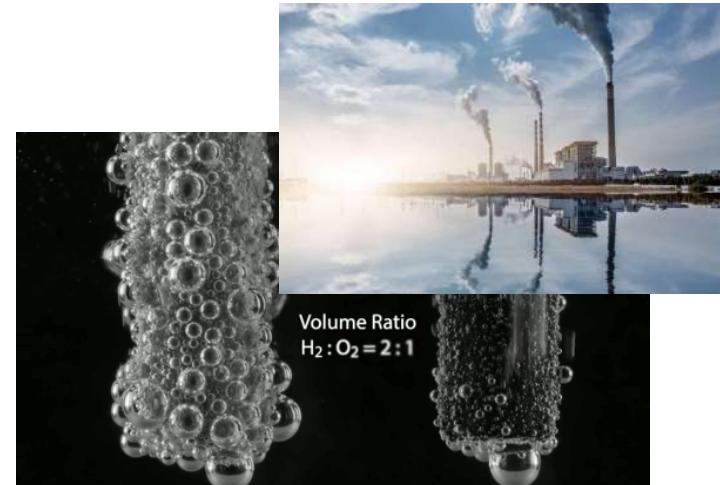


image credit: Beauty of Science)

## Softmatter and Nanomedicine

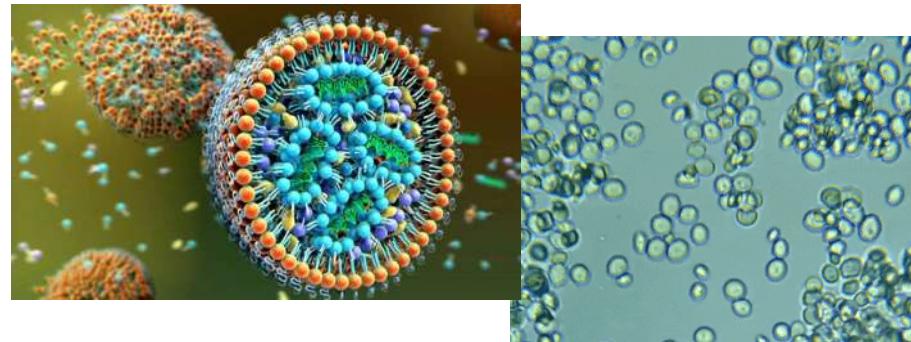
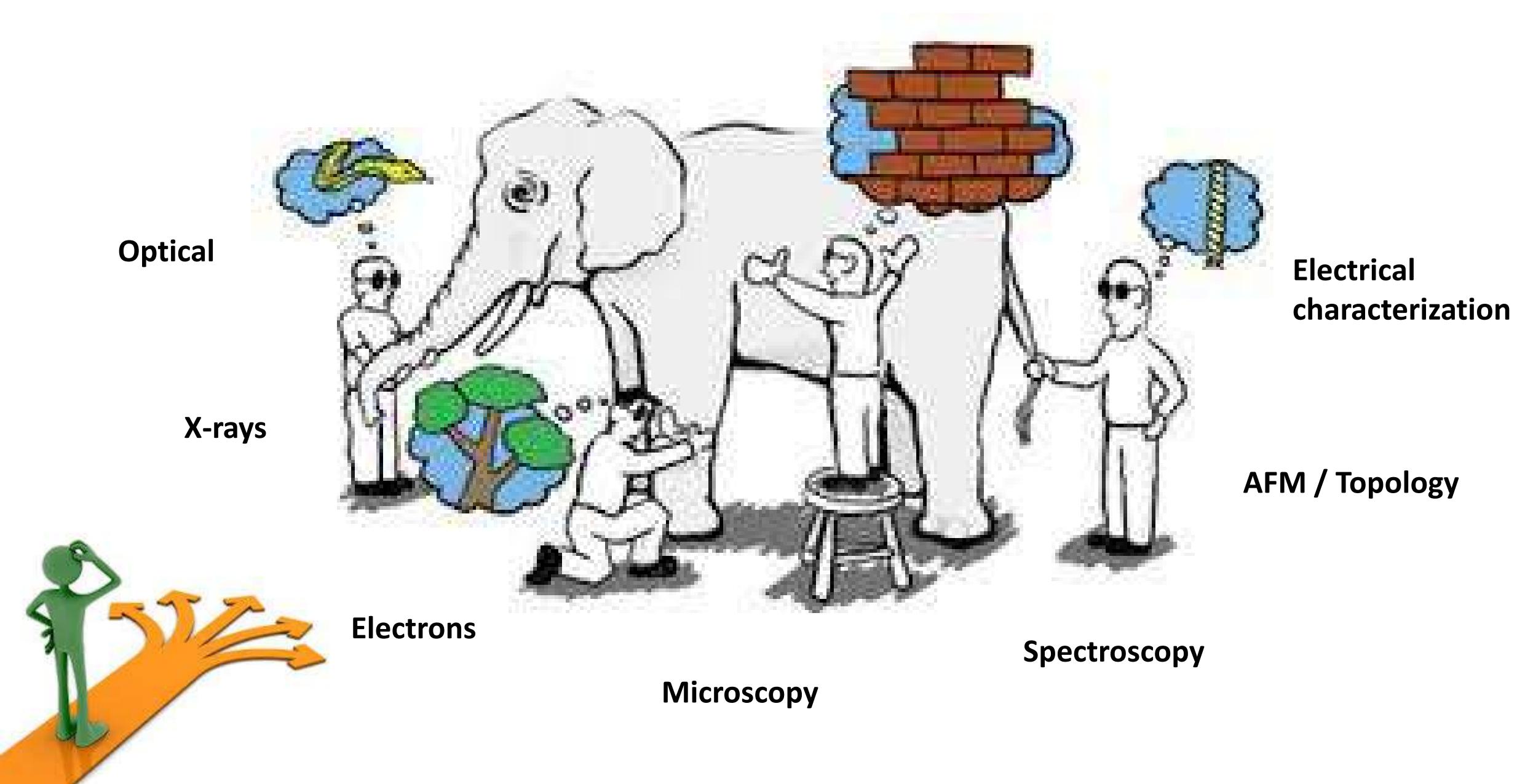


image source: YouTube: webbiocosm

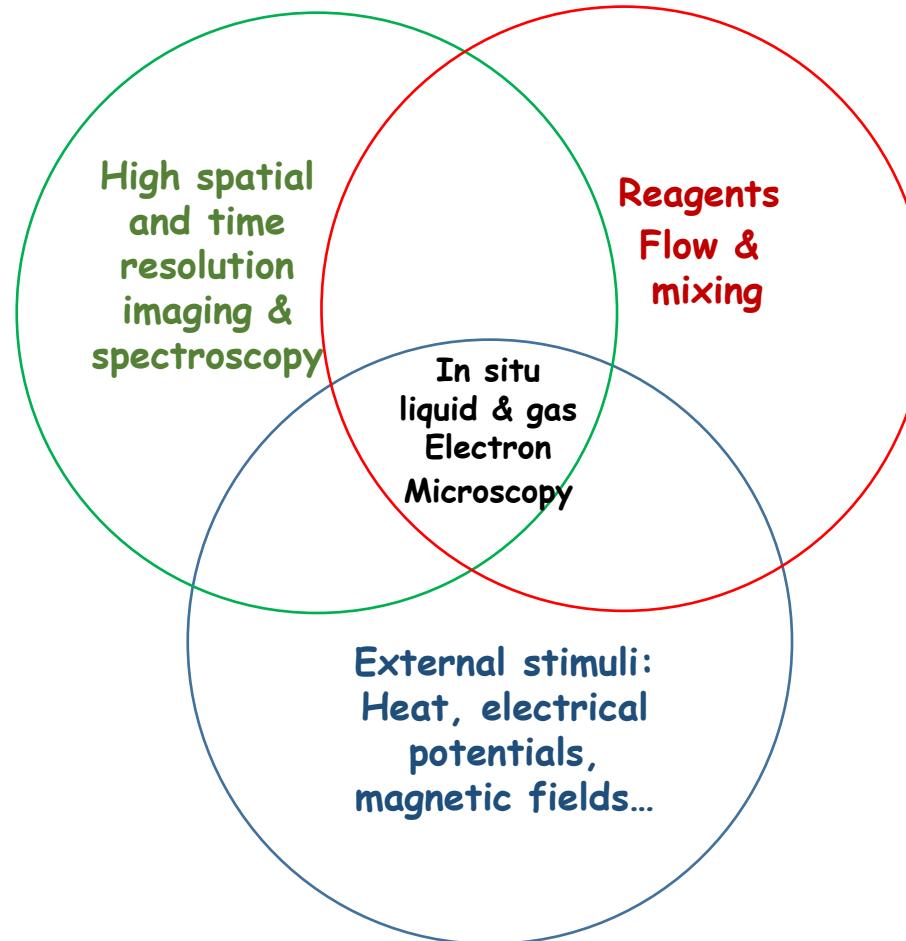
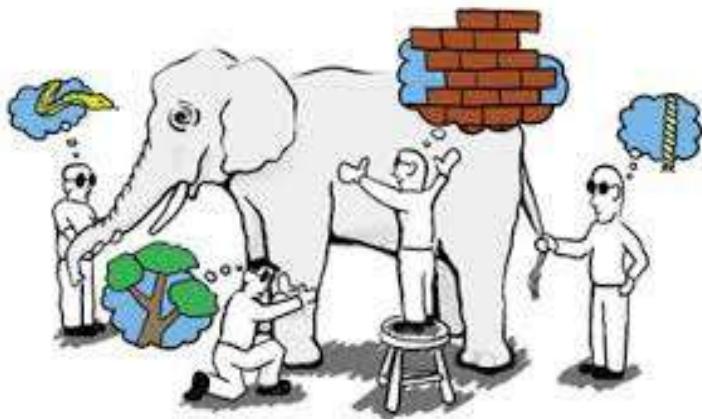
Progress slowed by nanoscale complexity...

# Studying Complex Nanoscale Processes...



# In situ Electron Microscopy

- Requires an ultrathin microchip nanoLaboratory
- Creating an All-in-one process analysis?



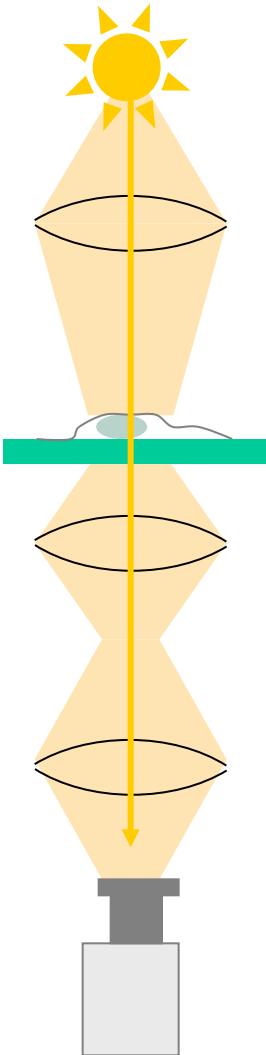
Electrons provide more information at a given beam damage level than x-rays



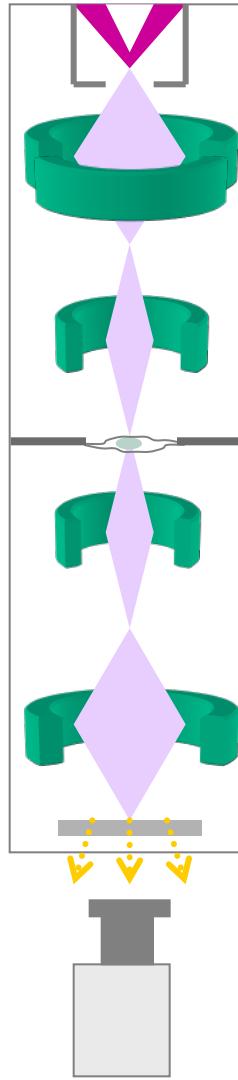
# Microscopes

Optical microscope      Transmission electron microscope      Scanning electron microscope

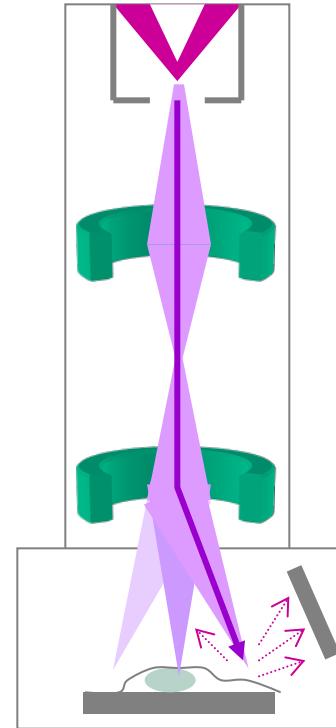
Optical



TEM



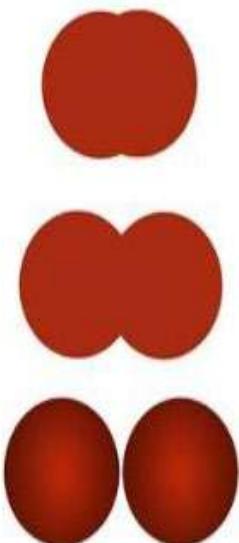
SEM



## Discuss with your neighbors

- What type of microscopes have you used ?
- What would be the pros and cons if you want to use it to image liquid process at the nanoscale ?  
E.g. Consider:

## Resolution ( spatial, temporal, spectroscopy )



Resolution allows us to see objects as separate from one to another → more details

Resolving power

- Unaided eye 0.1mm apart
- Optical microscopy – 0.2  $\mu\text{m}$  apart

Beam damage to the samples



# Next session 2: Todays Advanced TEMs

## The first TEM



Electron microscope constructed  
by [Ernst Ruska](#) in **1933**



The Titan Krios cryo-electron  
microscope looms over facility  
director Susan Hafenstein.  
*IMAGE: Nate Follmer / Penn State*

## Modern TEMs



*Osaka University's Ultra-High  
Voltage Electron Microscopy  
Research Centre on June 29, 2018*

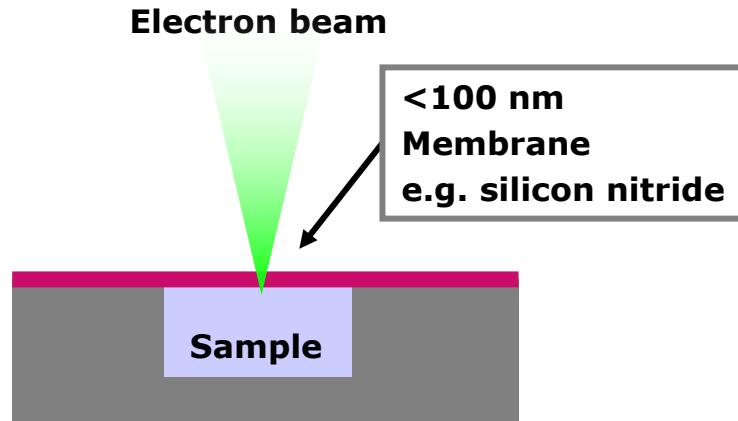
## Courses available at DTU

Course 41690, Electron microscopy  
analysis for materials research , Ph.D.

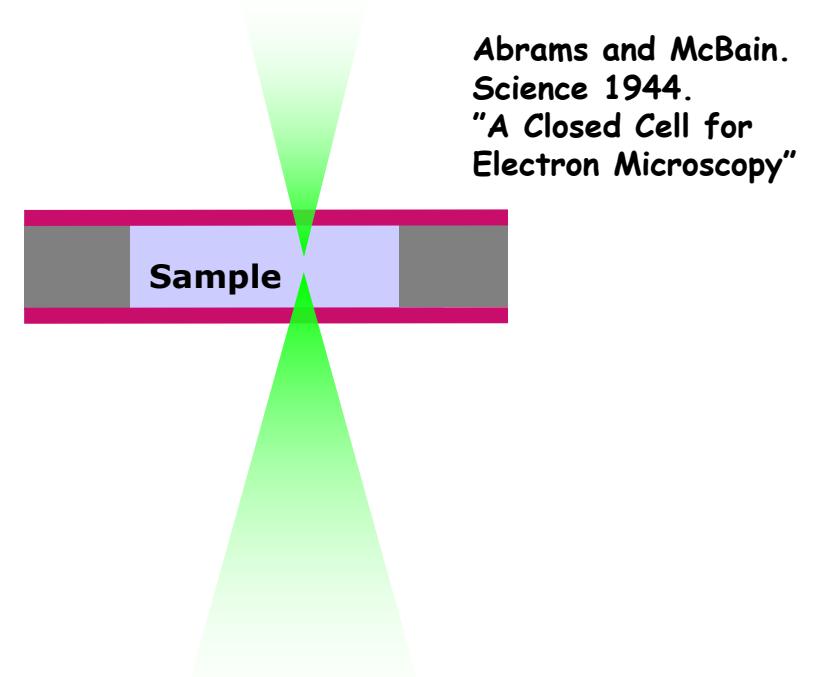
Course 47333, Electron microscopy  
for materials science, MSc.

# Session 3: Getting liquid water in a **vacuum** chamber...

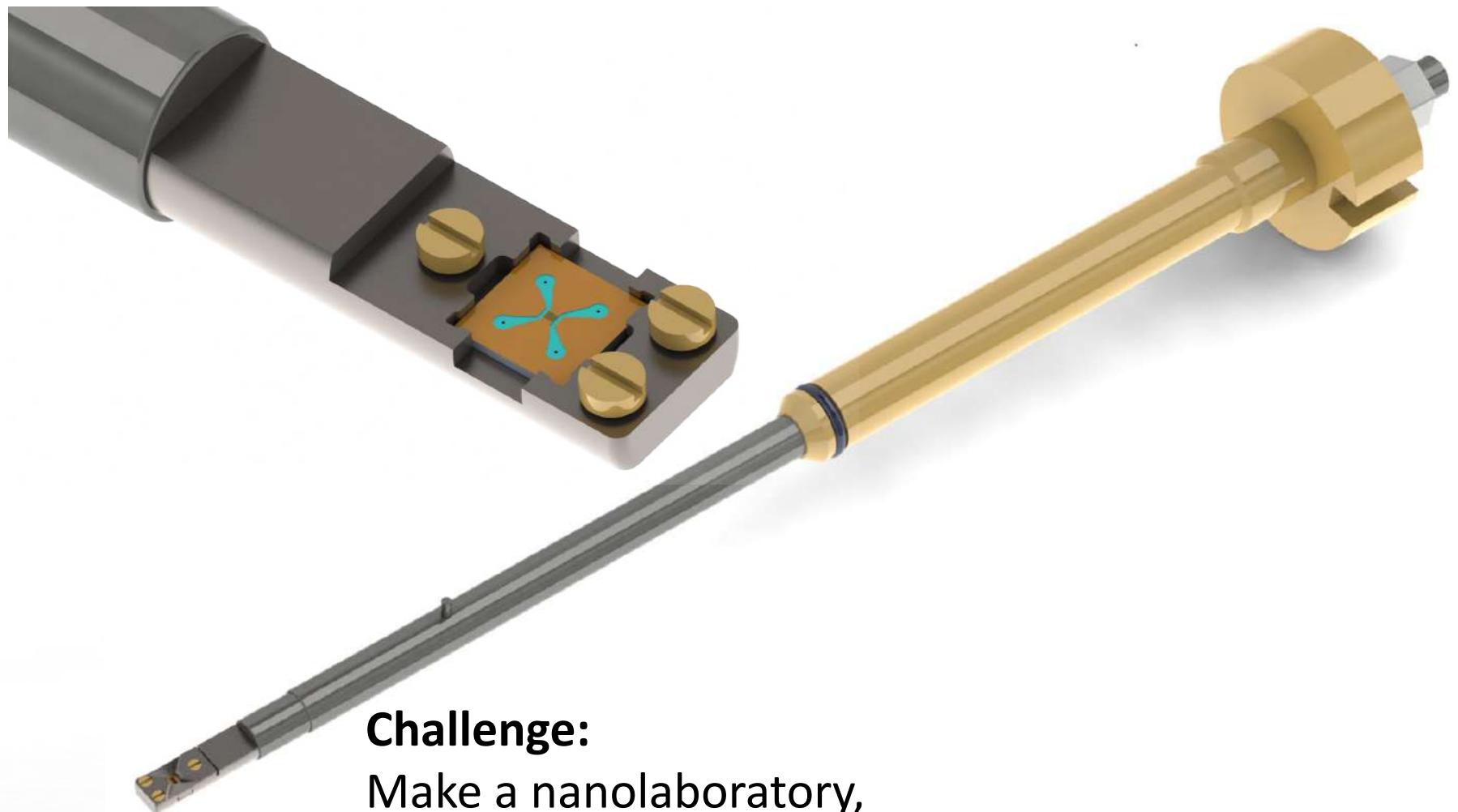
SEM



TEM



## Session 3: Liquid phase transmission electron microscopy



### Challenge:

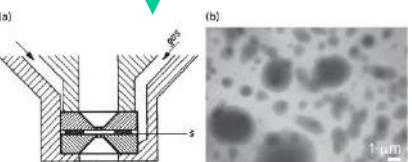
Make a nanolaboratory,  
that fit on to the tip of a pen,  
with the imaging area less than 2000 atoms thick

# Liquid in electron microscopy, a brief history

Here at DTU:

1931

First TEM



1944

First liquid sample in TEM

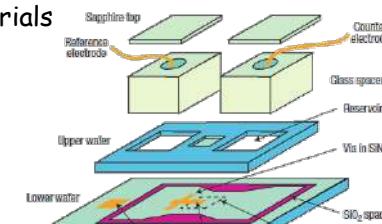
"A Closed Cell for Electron Microscopy"  
Abrams and McBain, Science

2003

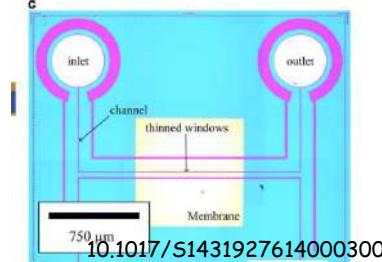
First Silicon nitride liquid cell

M. J. WILLIAMSON & ROSS

Nature Materials



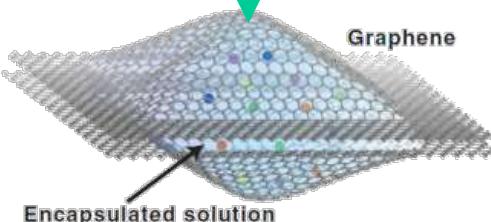
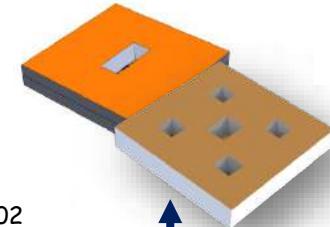
2014,  
Monolithic Chip  
By MoWin(DTU)



2017,  
Nanochannel V1  
By MoWin(DTU)



2020  
Nanochannel V2  
By MoWin(DTU)

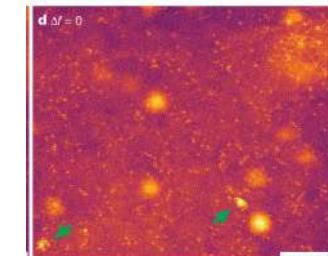
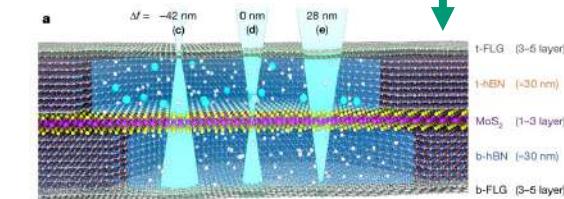


2012

Frist Graphene liquid cell

DOI: 10.1126/science.1217654

Atomic resolution

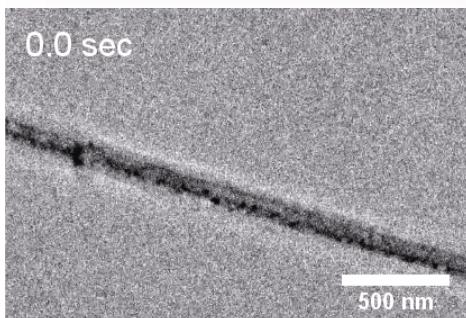


2022  
A double liquid  
cell structure

10.1038/s41586-022-05130-0

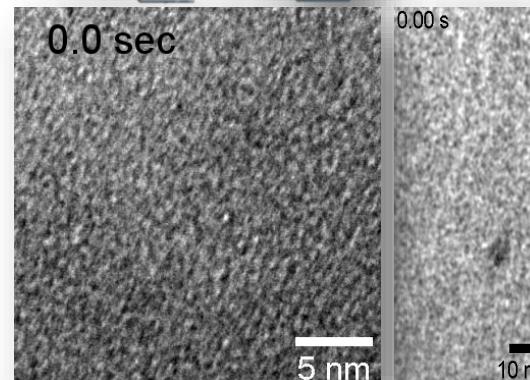
# Liquid phase electron microscopy applications

## Energy storage



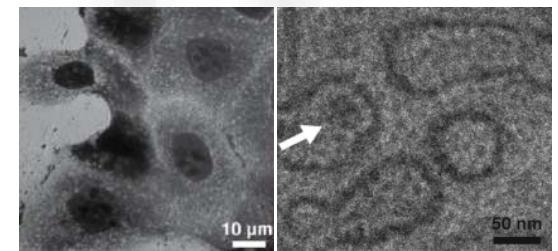
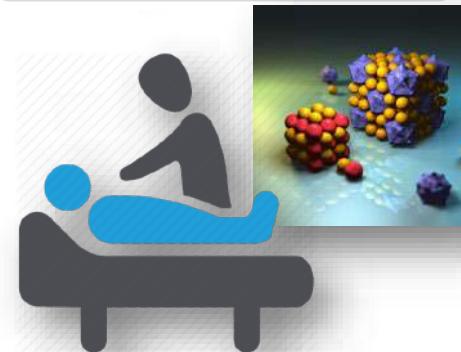
Lithiation of Si nanowire [1]

## Functional materials synthesis



Multistep nucleation of nanocrystals [2] Nanoparticle assembly [3]

## Bioscience

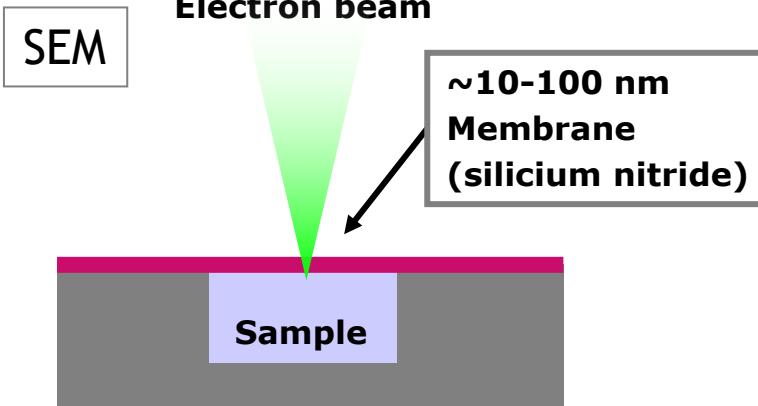


Cells and virus [4]

Liquid process dynamic information at the nanoscale is essential to improve our understanding the underlying chemistry and physics.

1. Gu, M., et al., Nano Letters, 2013.
2. N. Duane Loh et al. Nat. Chem. 2017
3. G Lin et al., ACS Nano, 2016
4. Jungwon Park et al. Nano Lett. 2015
5. Images from internet

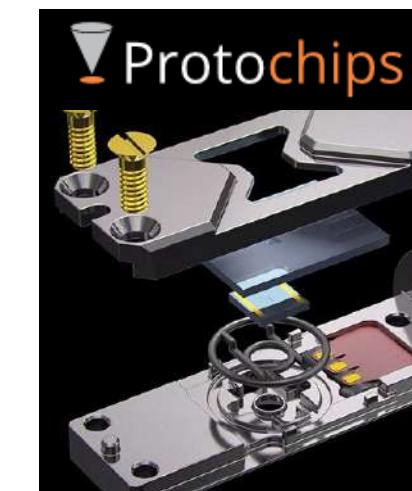
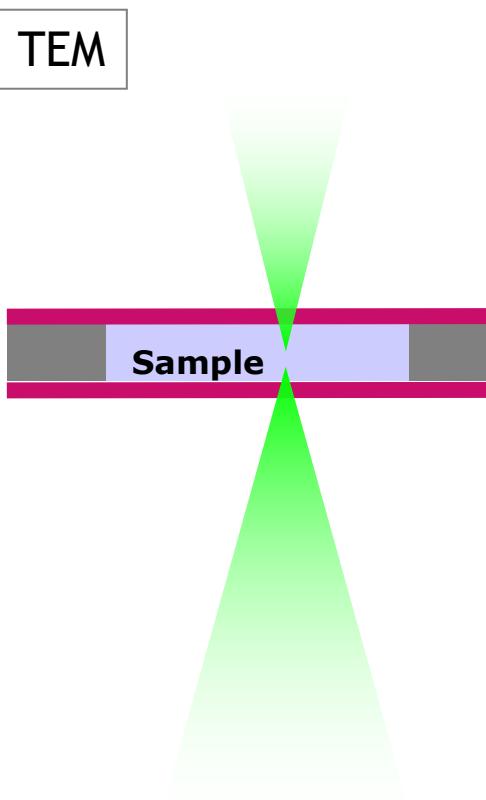
# Going Liquid – commercial systems



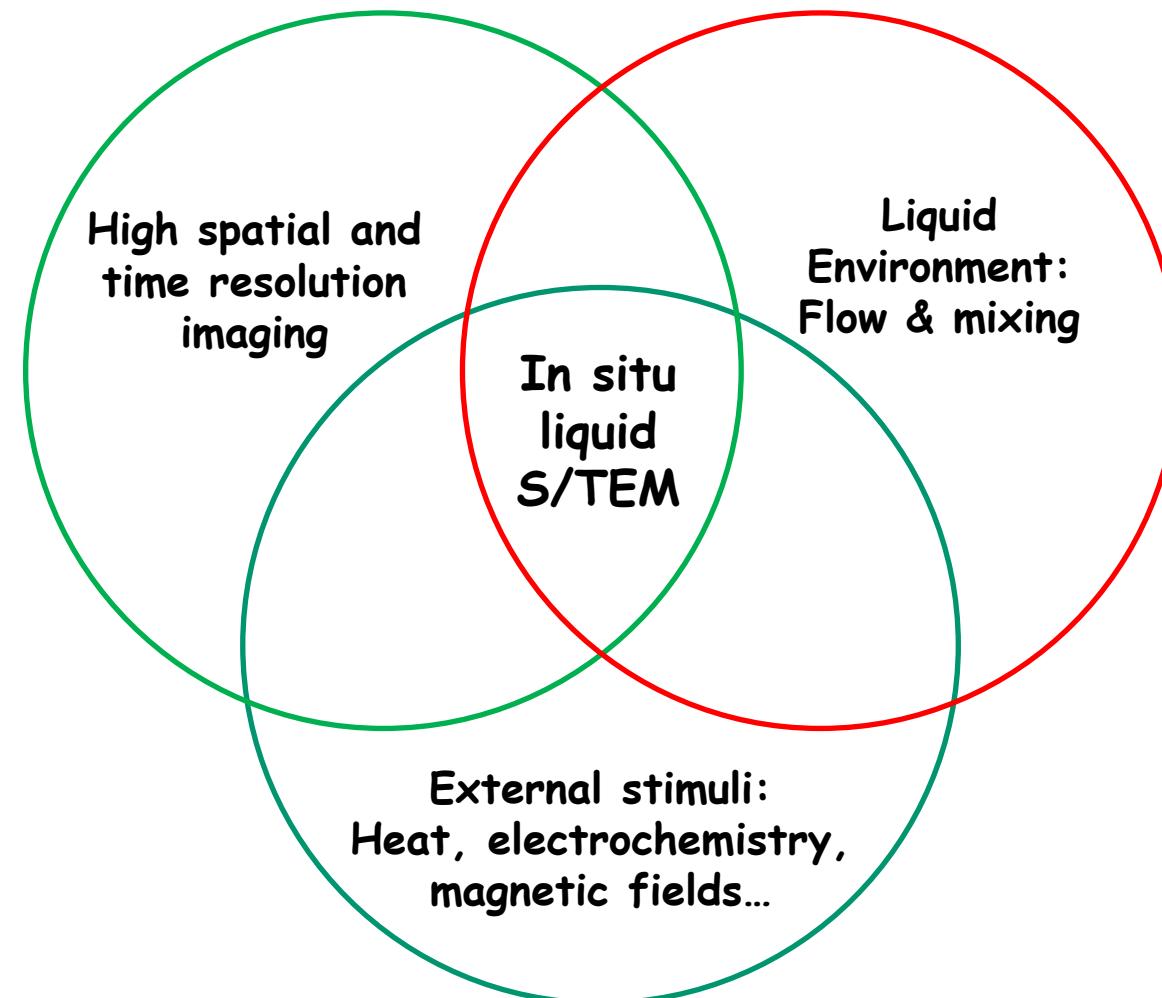
Quantomix WetSEM capsule



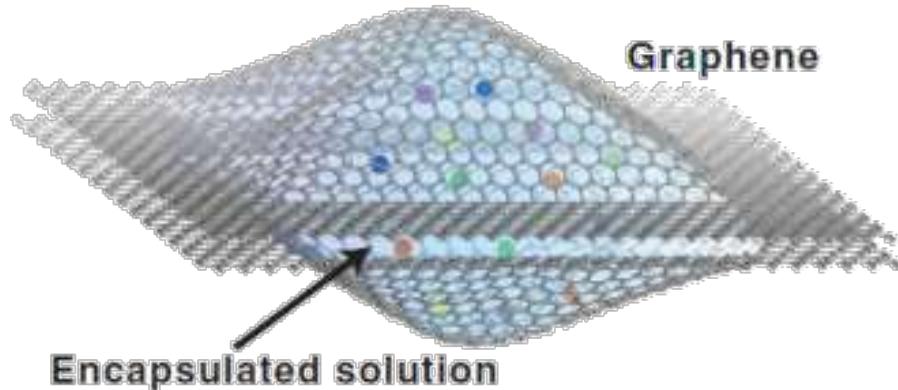
JEOL ClairScope™  
An 'inverted SEM' for  
special petridishes



# In situ Liquid Phase Electron Microscopy

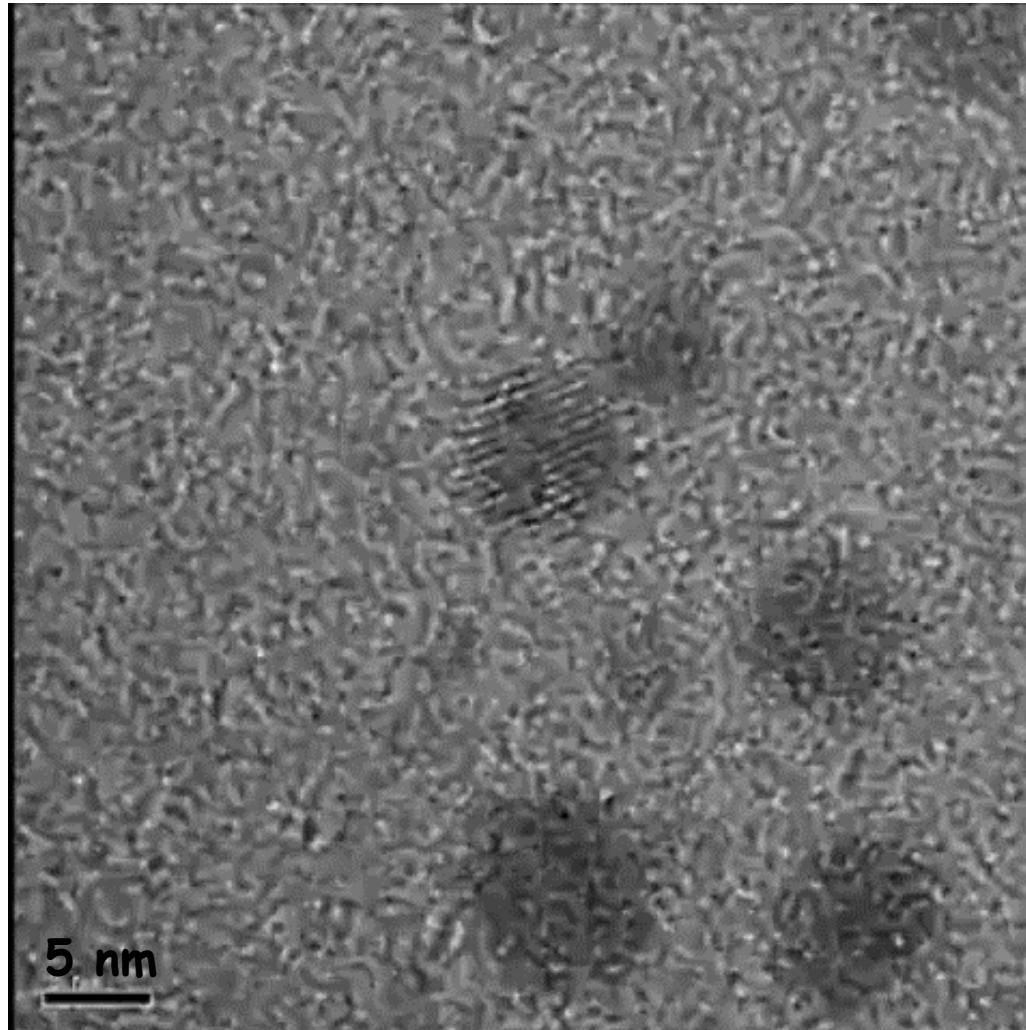


# Session 4 and 5: Nanoparticle nucleation and interaction



Nucleation phenomena observed  
in a graphene liquid cell

Decoding the movie:  
What do you see?

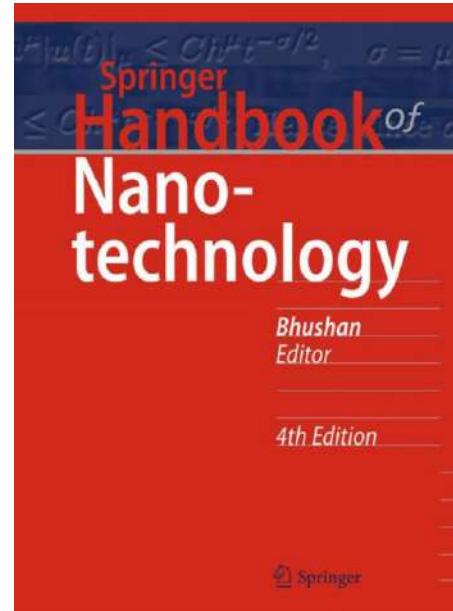
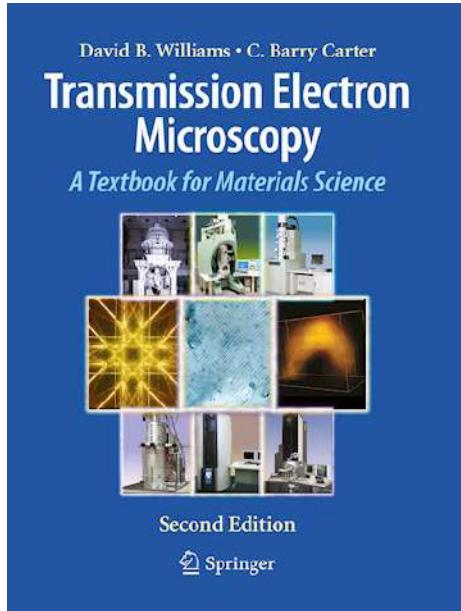
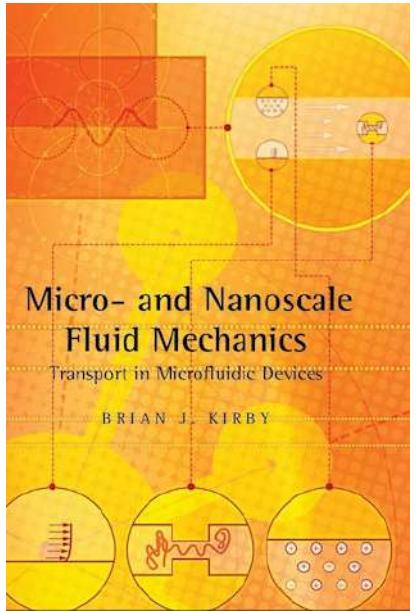


<https://science.sciencemag.org/content/336/6077/61>

# Nucleation and growth – a very fundamental process!

- What cases can you think of?
- How would you study the nanoscale initiation?

# The working space of Nanofluidic TEM

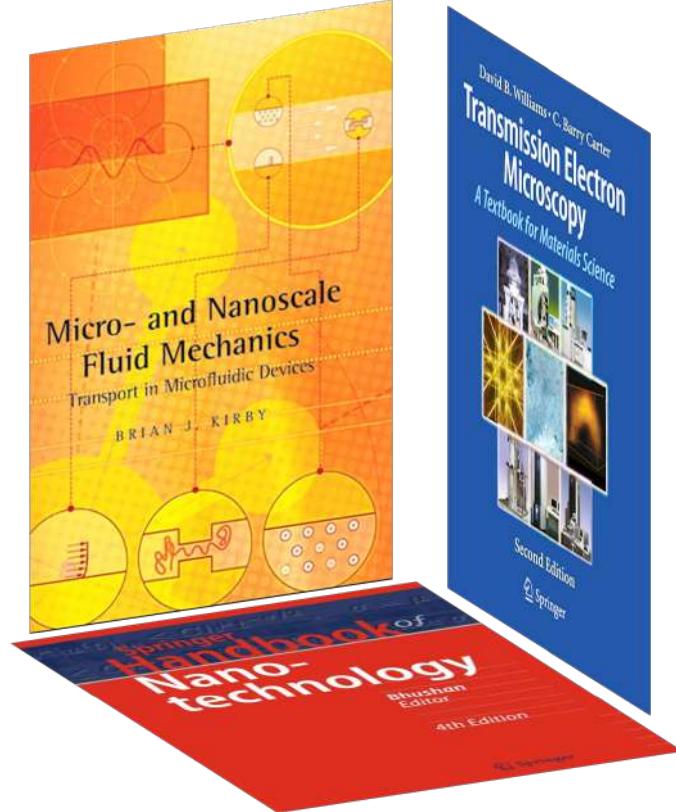


Fluidics  
Chemistry & Bio

TEM/SEM

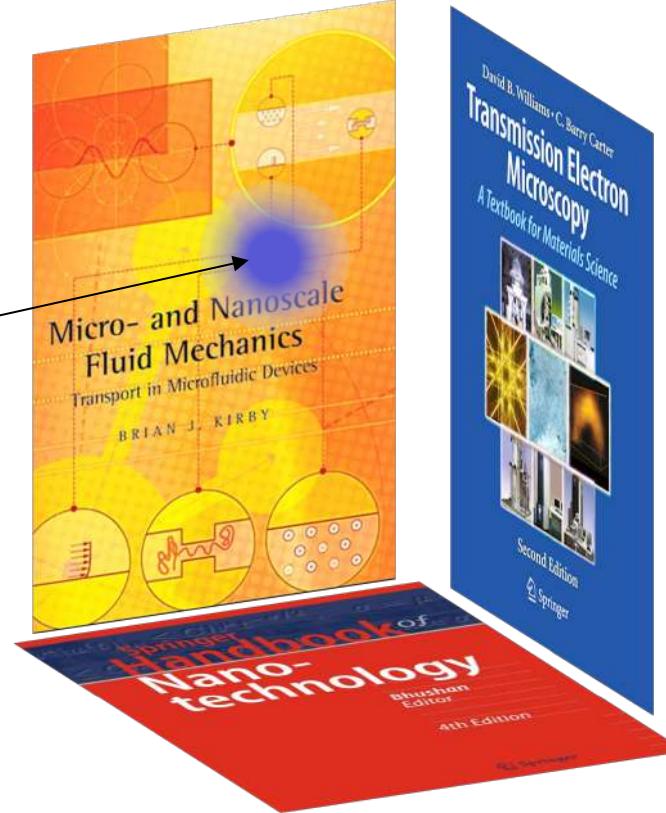
Physics,

# The working space of Nanofluidic TEM – in 3D



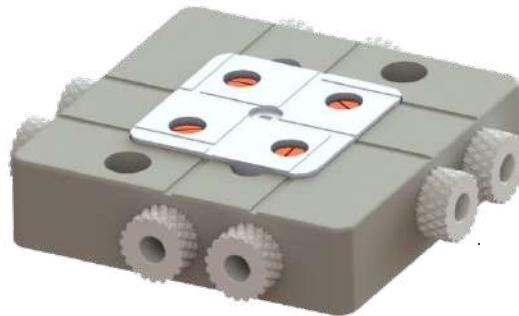
# The working space of Nanofluidic TEM – in 3D

One  
research  
group





A rapidly growing DTU Nanolab start up!



# Literature - If you want to know more:

Science Review paper: **Opportunities and challenges in liquid cell electron microscopy**

<http://science.sciencemag.org.proxy.findit.dtu.dk/content/350/6267/aaa9886>

Book: **Liquid cell electron microscopy**

<https://www.cambridge.org/core/books/liquid-cell-electron-microscopy/A668214DAFA539E0682ADF8672FE8C6C>

More broad in-situ TEM electrical measurement review book chapter

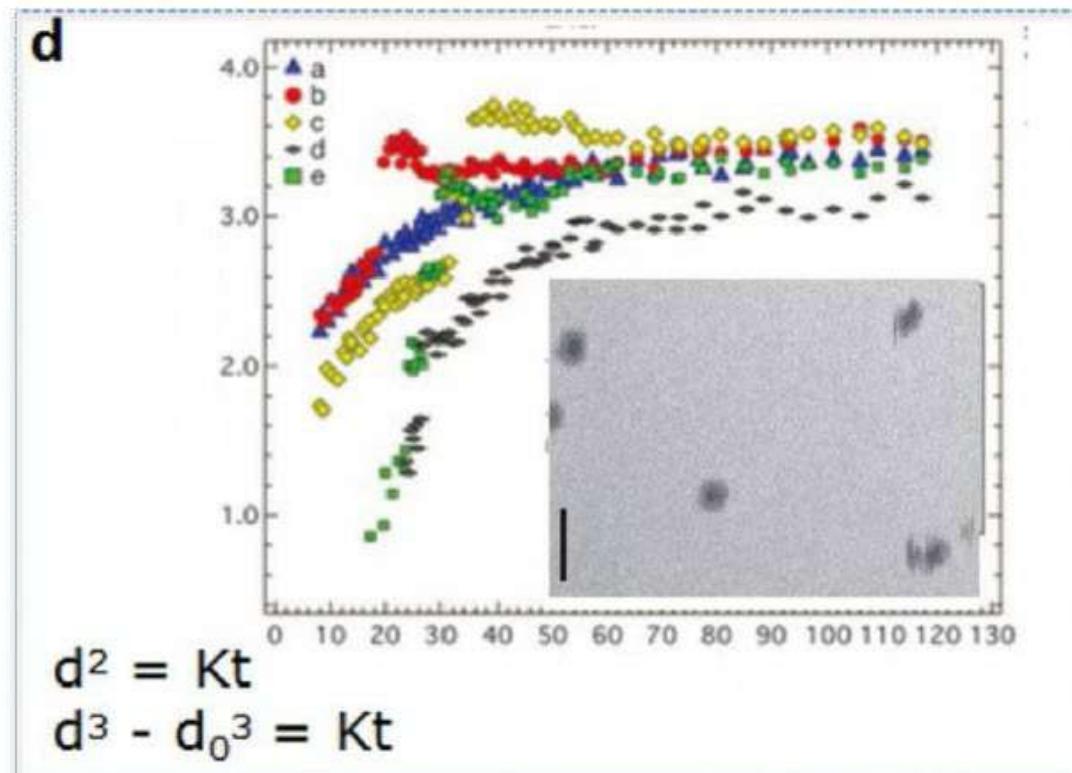
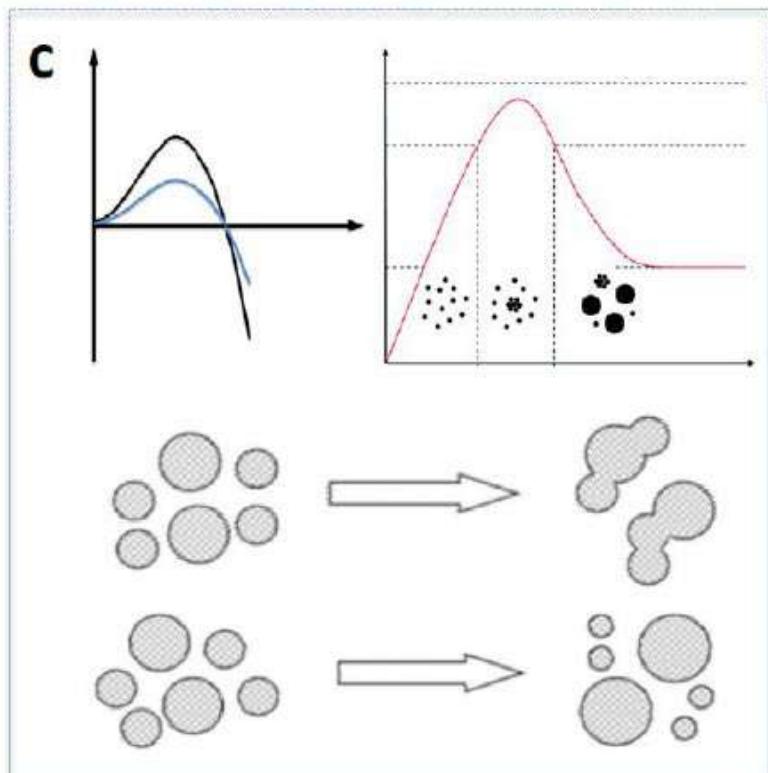
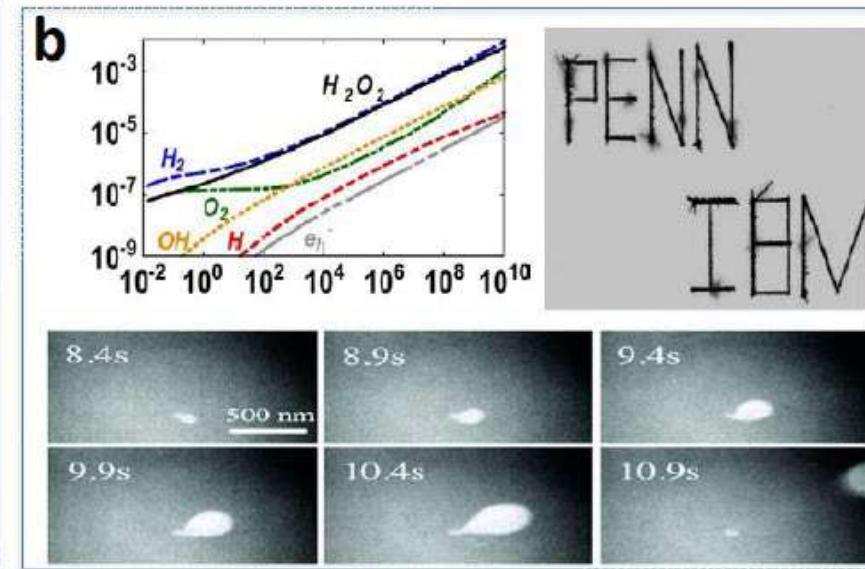
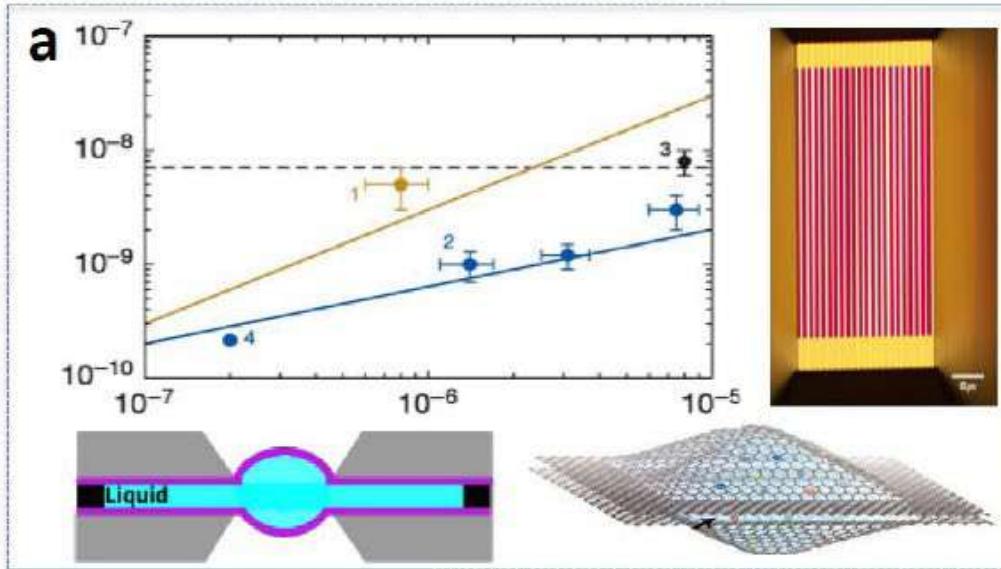
[https://link-springer-com.proxy.findit.dtu.dk/chapter/10.1007/978-3-319-22988-1\\_10](https://link-springer-com.proxy.findit.dtu.dk/chapter/10.1007/978-3-319-22988-1_10)

**Discuss with your neighbors:**

What could you think of using this method for?

Next session

## **2. Transmission electron microscopy**

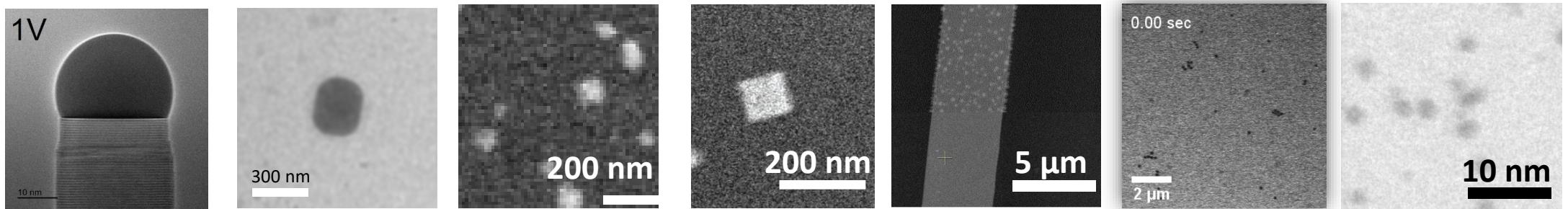


# 2. Transmission Electron microscopy

Murat Nulati Yesibolati, Hongyu Sun & Kristian Mølhave

DTU Nanolab

Molecular  
Windows



# Learning objectives

- What **electron gun** would you use? Why?
- What **imperfections** can you see in this glass **lens**
- At what **sample thickness** would you think that TEM becomes difficult to image and why
- Why is **biological TEM difficult**?
- And there are many other methods for characterization – what would you tell someone to do if they said:
  - I want to know if this TiO<sub>2</sub> powder is the anatase crystal phase?
  - What is the shape of these 100-1000 nm metal particles?
  - What is the structure of this 100 nm virus particle?
  - How can I follow the surface changes of my catalytic particle in gas at 700 C



# Advanced TEMs

## The first TEM



Electron microscope constructed  
by [Ernst Ruska](#) in **1933**

## Modern TEMs ( half angstrom)



e.g. The Titan Krios cryo-electron  
microscope. *IMAGE: Nate Follmer  
/ Penn State*



*Osaka University's Ultra-High  
Voltage Electron Microscopy  
Research Centre on June 29, 2018*

## Courses available at DTU

Course 41690, Electron microscopy  
analysis for materials research , Ph.D.

Course 47333, Electron microscopy  
for materials science, MSc.

# Why high energy TEM

Wave-particle duality

**1924**, Louis-Victor de Broglie

$$\lambda = \frac{h}{p}$$

First electron microscopy

**1931**, Ernst Ruska

## Electron wavelength

1200 pm @ 1 eV

12 pm @ 10 keV

1.9 pm @ 300 keV , 77% of  
the speed of the light

(relativistic electrons at  $\frac{1}{2}c$ )

# A Tour of The TEM

- An electron gun
- Electromagnetic lenses
- Image corrections
- Image
  - Resolution
  - Brightness and Contrast

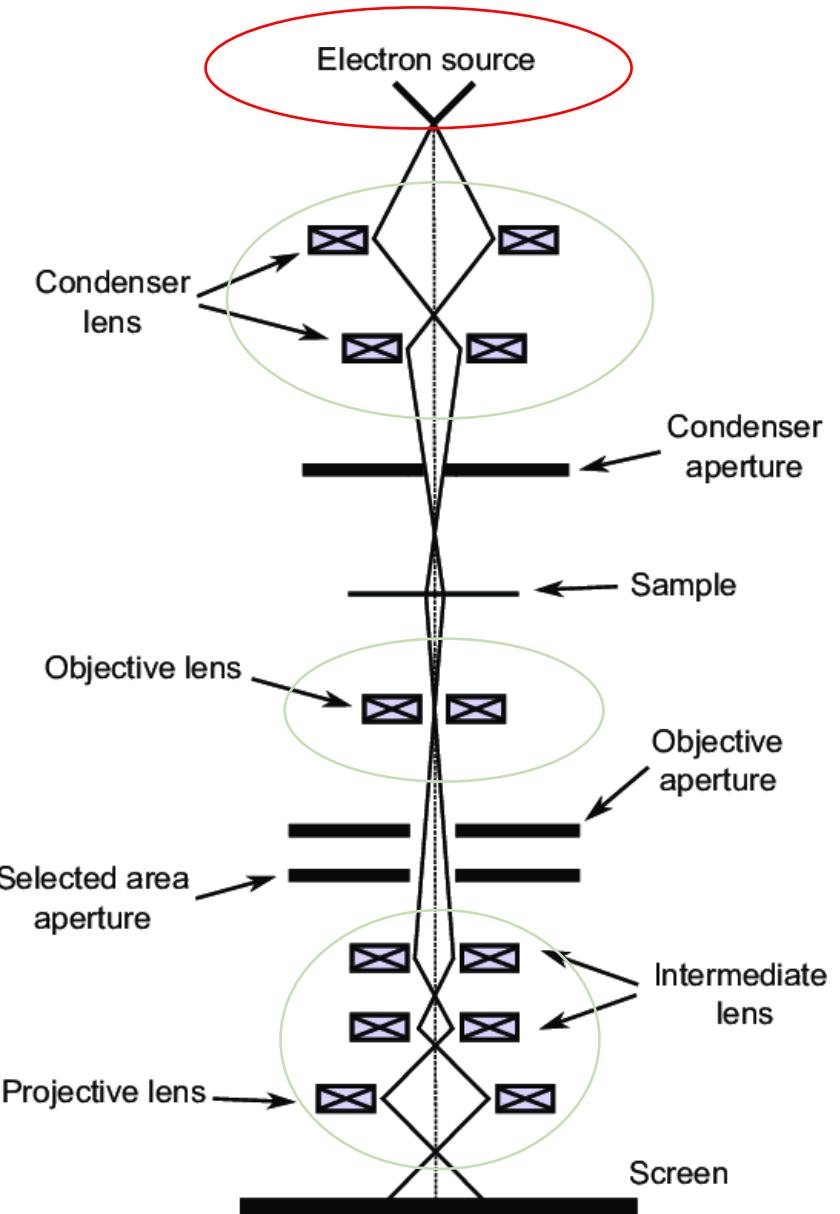
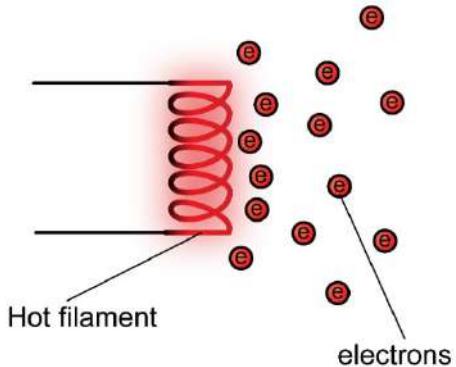


Image from Naresh Marturi, Vision and visual servoing for nanomanipulation and nanocharacterization in scanning electron microscope.

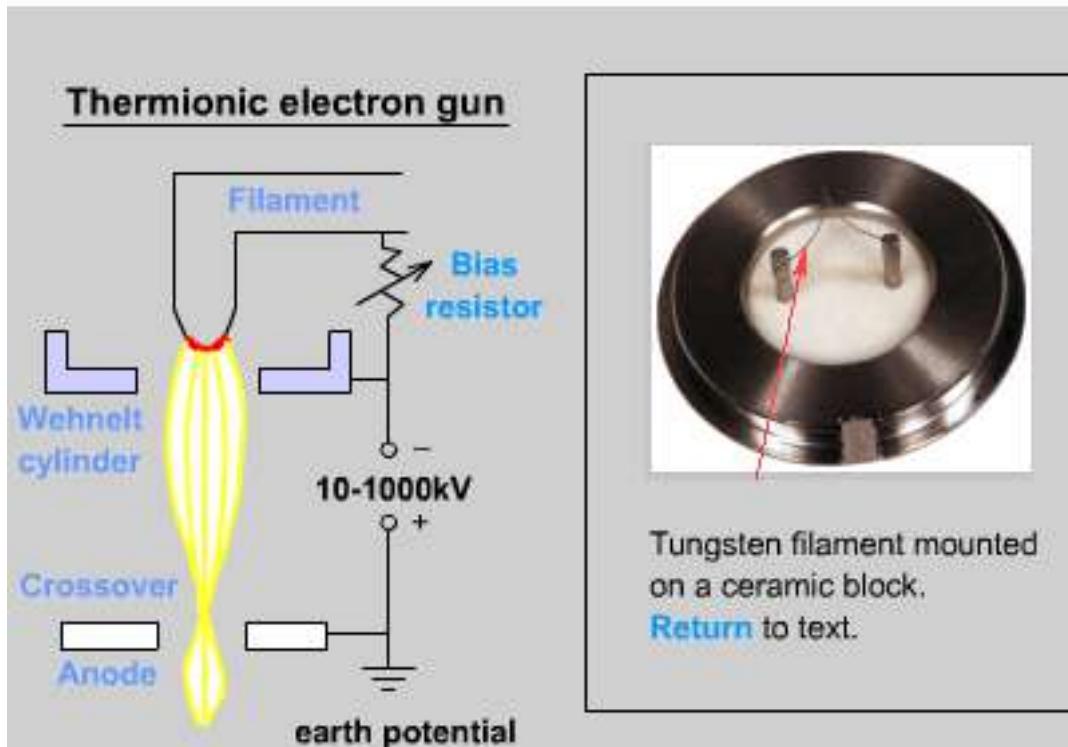
# Thermionic electron gun



Joule heating of the filament produces electron emission.

Electrons are accelerated and focused

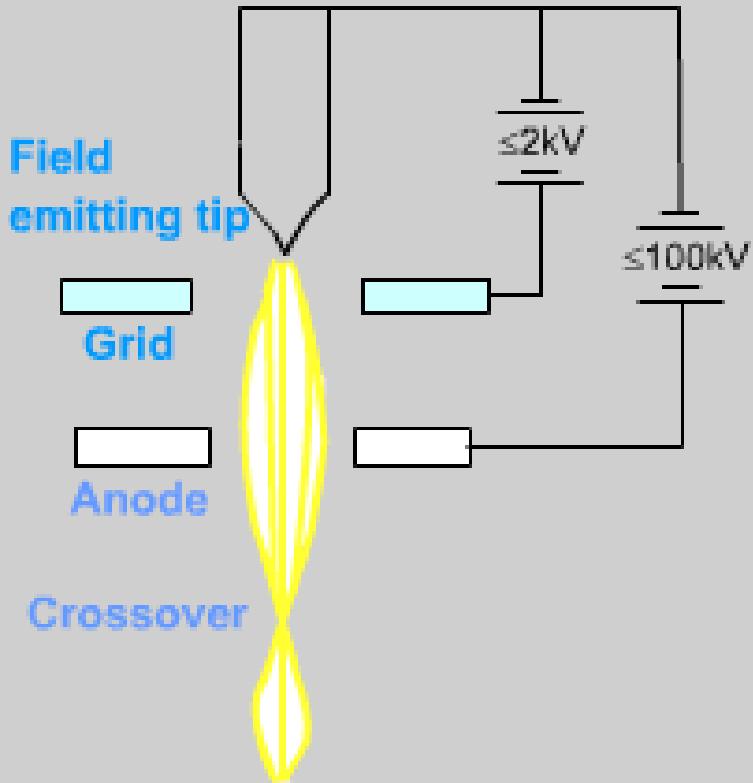
- Limited brightness
- Large energy spread (1-2eV)



Brightness = beam current density per unit solid angle ( $\text{Am}^{-2}\text{sr}^{-1}$ )

# Field emission electron gun

## Field emission electron gun

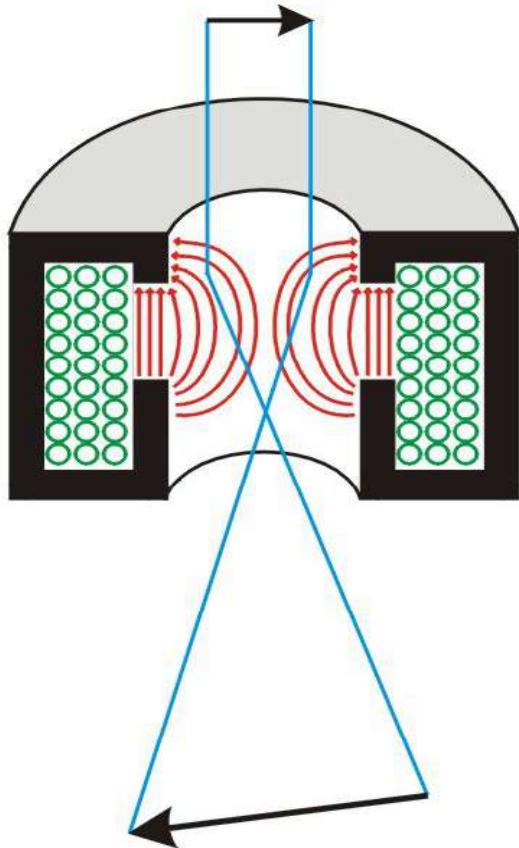


Field emitting tungsten tip.  
[Return to text.](#)

Electrons tunnel from sharp tip.

- High brightness
- Small energy spread (<0.5eV)
- Need high vacuum @ gun

# The electromagnetic lens



Lorentz Force:

$$F = e(v \times B)$$

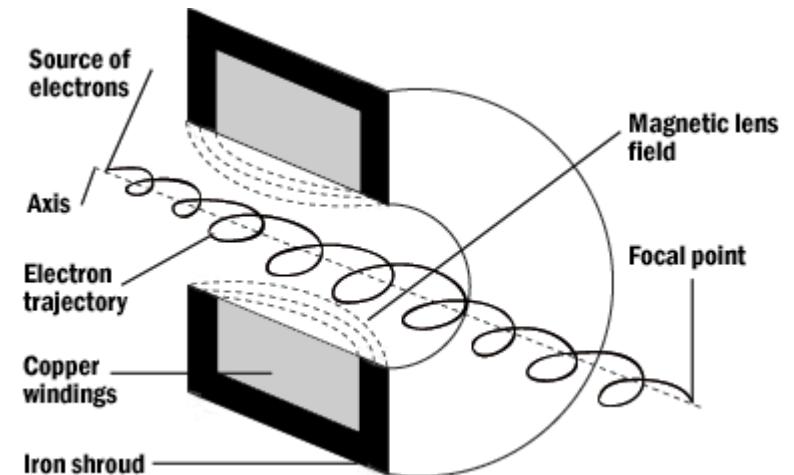
In two steps:

1: Downward beam  $\times$  Radial field

-> Tangential force -> spiralling motion

2: Spiralling velocity  $\times$  upward field

-> radial force focussing the spiralling beam



# Imperfect lenses... what do you see?



Kristian Mølhave krmo@dtu.dk

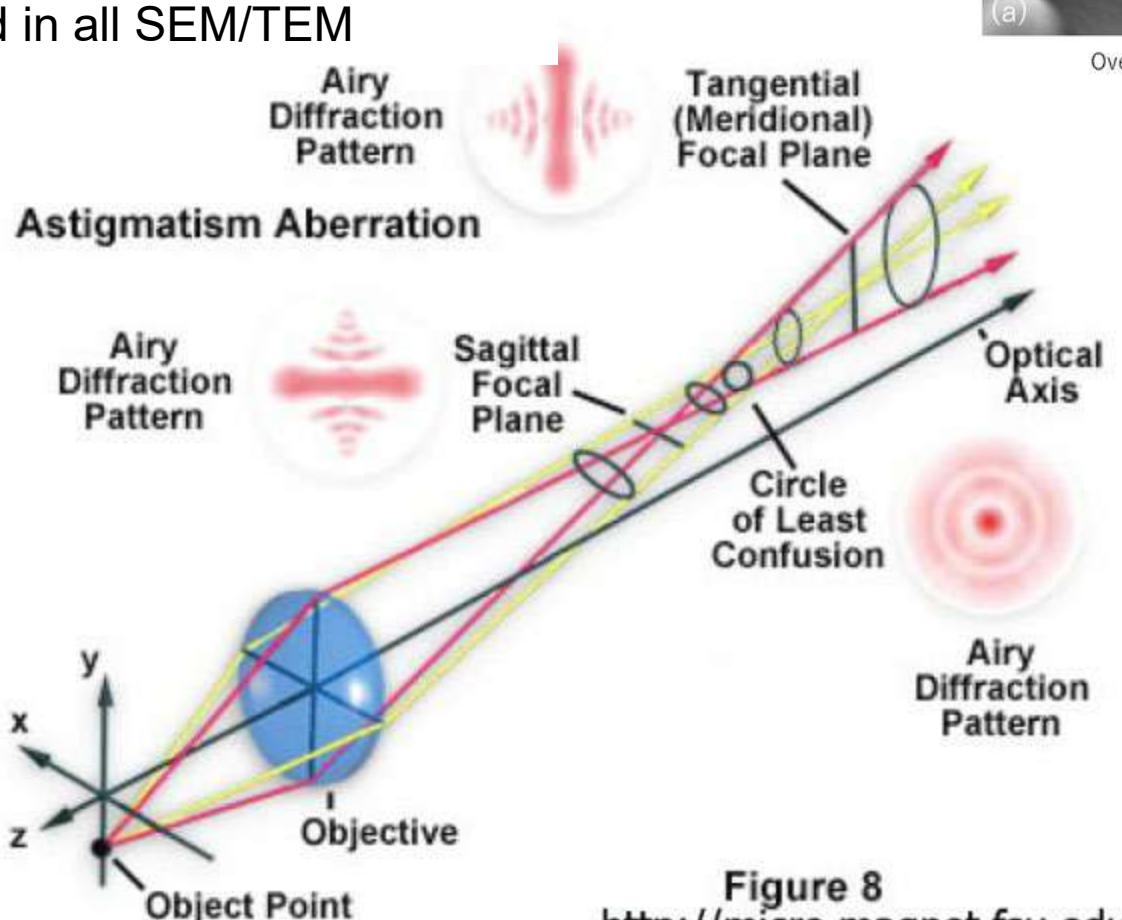
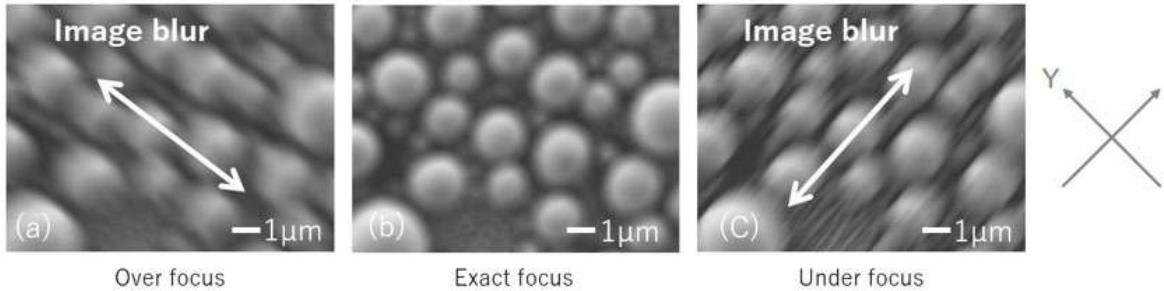
# Astigmatism correction

Astigmatism:

The effect of a cylindrical lens

-focal length varies in different planes.

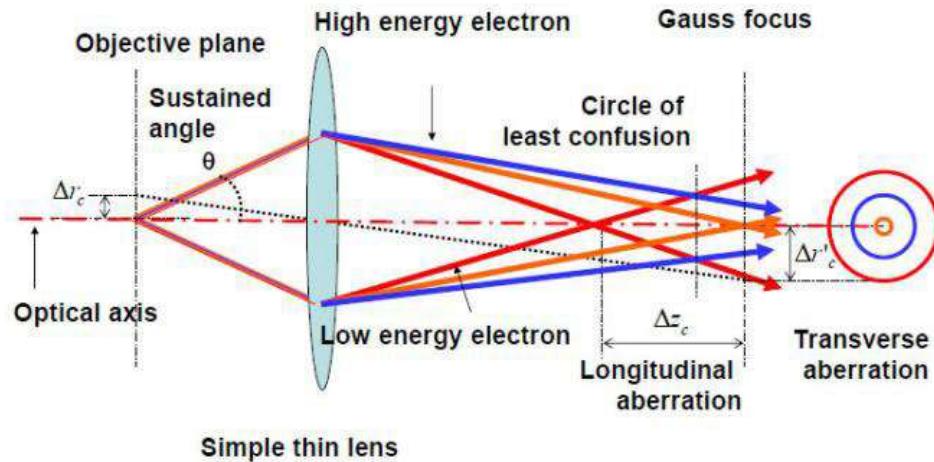
-Can be corrected in all SEM/TEM



Not a perfect lens:  
The lens are stigmatic

Figure 8  
<http://micro.magnet.fsu.edu/>

# Chromatic aberration

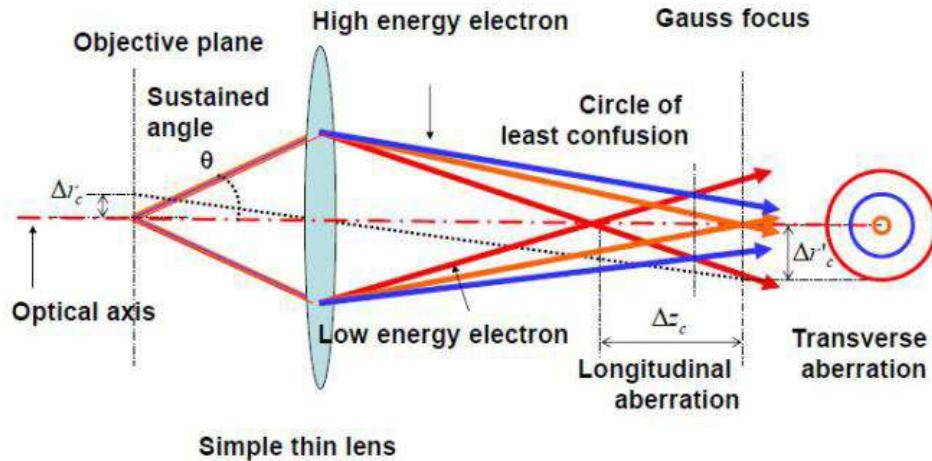


*Spread in focus  
due to Chromatic Aberration  
with energy spread  $\Delta E$*

$$d_c = \alpha C_c \Delta E / 2E$$

**How to solve this?**

# Chromatic aberration

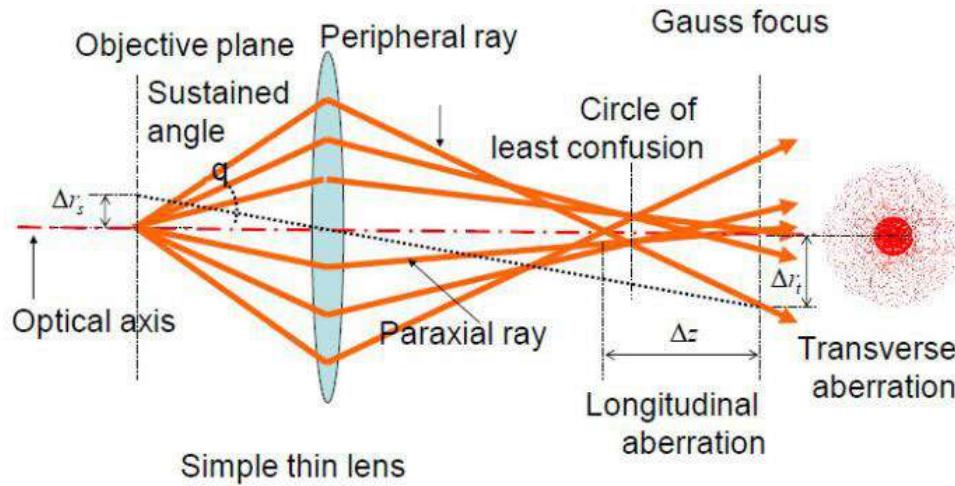


*Spread in focus  
due to Chromatic Aberration  
with energy spread  $\Delta E$  at energy  $E$*

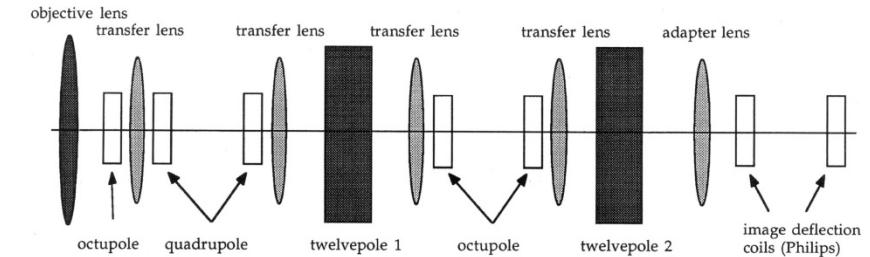
$$d_c = \alpha C_c \Delta E / 2E$$

**Solved by ‘monochromatic’ beam with low energy spread  $\Delta E$ , e.g. from FEG source and by high energy  $E$**

# Spherical aberration



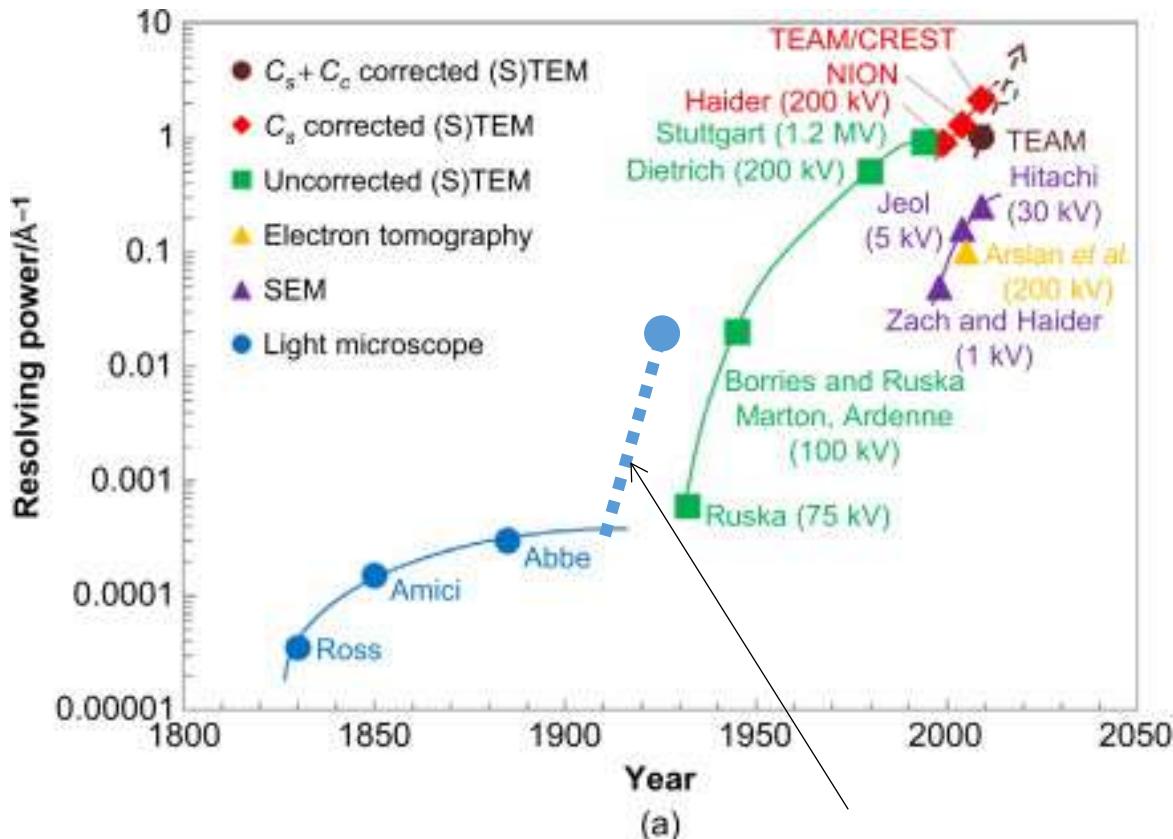
**Solved by very complex aberration correction coil system**



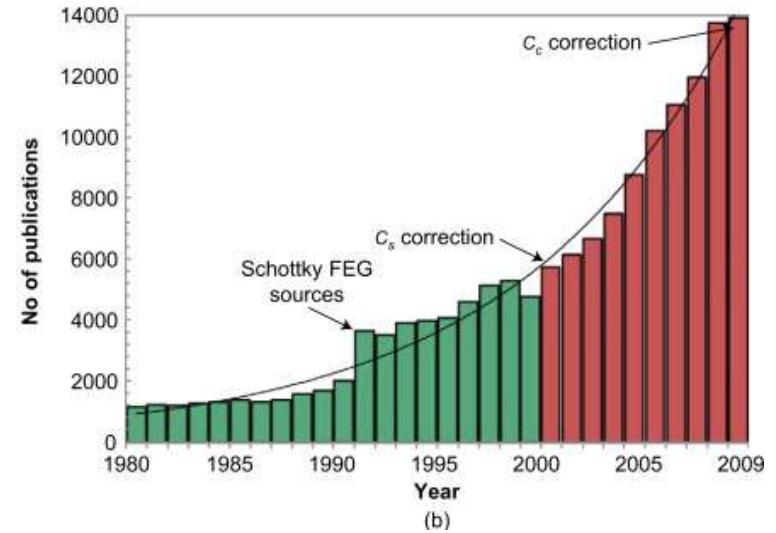
1947 → 1961 → 1967 → 1990s

<https://www.sciencedirect.com/science/article/pii/S0304399115000509#f0025>

# High resolution TEM

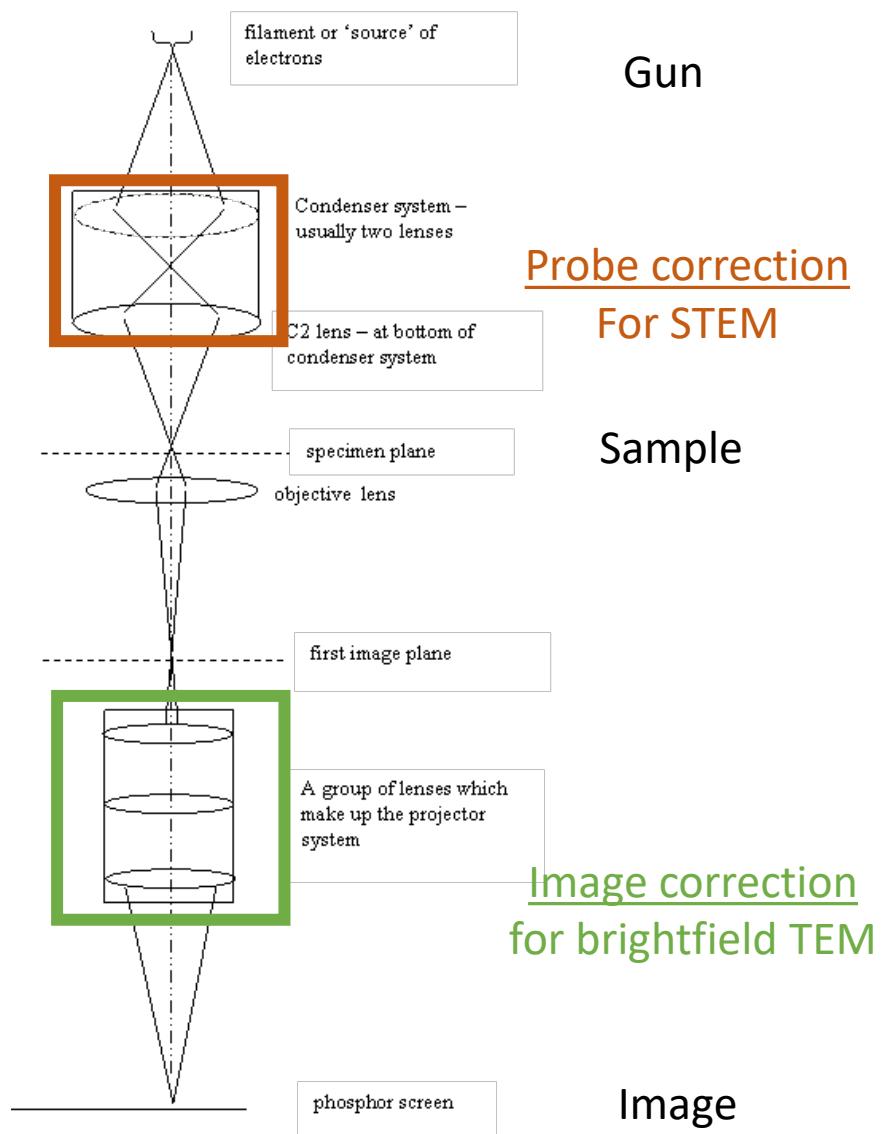


Advanced optical microscopy  
~10 nm resolution  
+ EUV microfab lithography at few nm



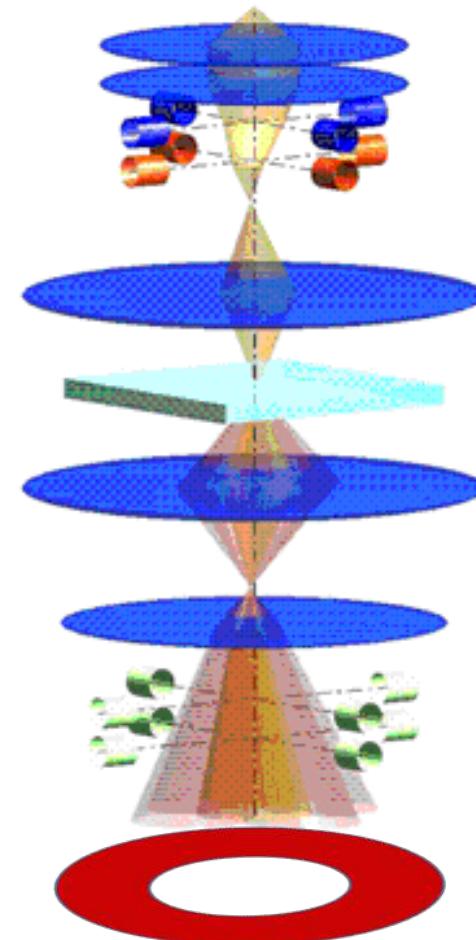
Rowan Leary, Rik Brydson, Advances in Imaging and Electron Physics Volume 165, 2011, Pages 73-130

# Abberation corrected TEM gives ultimate resolution



# Two TEM system TEM & STEM

- TEM - Transmission electron microscope
  - Resolution limited by overall thickness of sample scattering the beam
- STEM Scanning TEM
  - Resolution limited by probe focus
  - Contrast may be reduced with thick samples, but better resolution on top than bottom of sample



[https://www.physics.hu-berlin.de/en/sem/software/software\\_qstem](https://www.physics.hu-berlin.de/en/sem/software/software_qstem)

# Getting electrons through the sample

Beer-Lambert law

Beer-Lambert law of exponential intensity decay  
of transmitted beam with distance

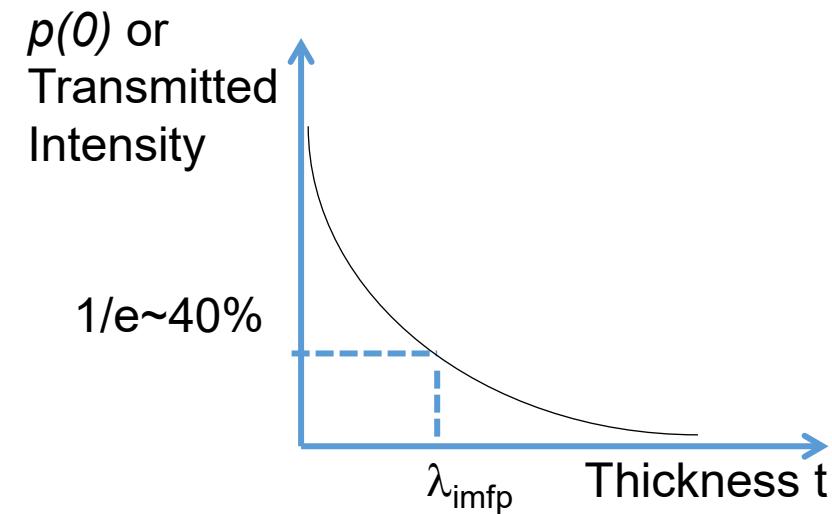
Probability of not scattering:

$$P(z, n) = \frac{1}{n!} \left( \frac{t}{\lambda_{mfp}} \right)^n e^{-\frac{t}{\lambda_{mfp}}}$$

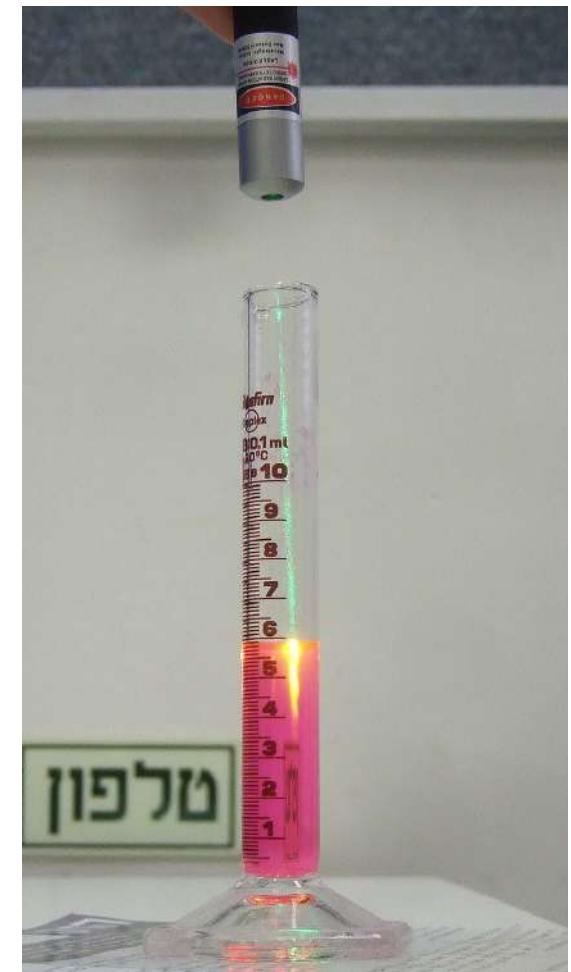


$$P(z, n = 0) = e^{-\frac{t}{\lambda_{IMFP}}}$$

IMFP: Inelastic Mean Free Path



Sample thinner than IMFP if we want mainly electrons that are not scattered.



A demonstration of the Beer–Lambert law:  
green laser light in a solution of Rhodamine 6B.  
[https://en.wikipedia.org/wiki/Beer%20-%20Lambert\\_law](https://en.wikipedia.org/wiki/Beer%20-%20Lambert_law)

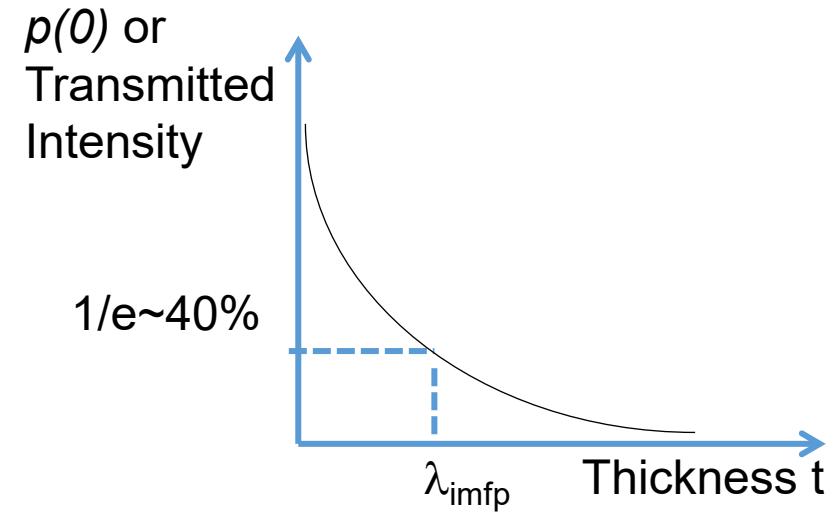
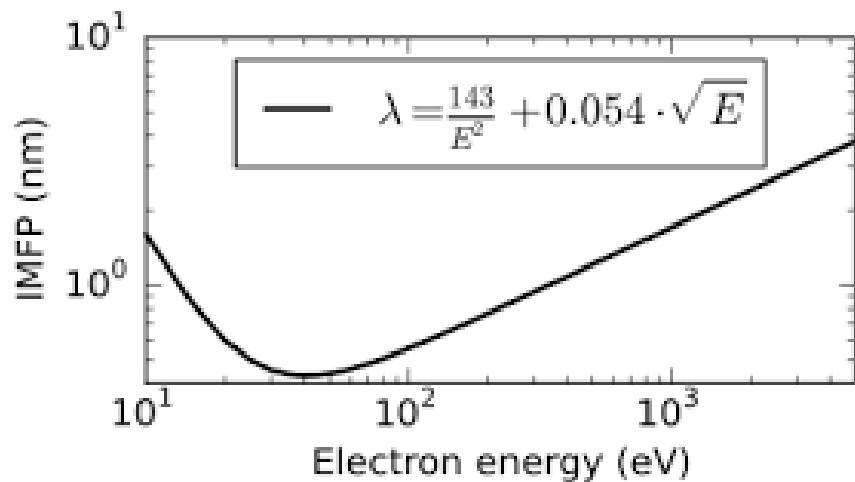
# Getting electrons through the sample

Beer-Lambert law of exponential intensity decay of transmitted beam with distance

- The Probability of not scattering:

$$P(z, n = 0) = e^{-\frac{t}{\lambda_{IMFP}}}$$

IMFP Universal curve approximation



Sample thinner than IMFP if we want electron not scattered.

# Getting electrons through the sample

Beer-Lambert law of exponential intensity decay of transmitted beam with distance

Probability of not scattering:

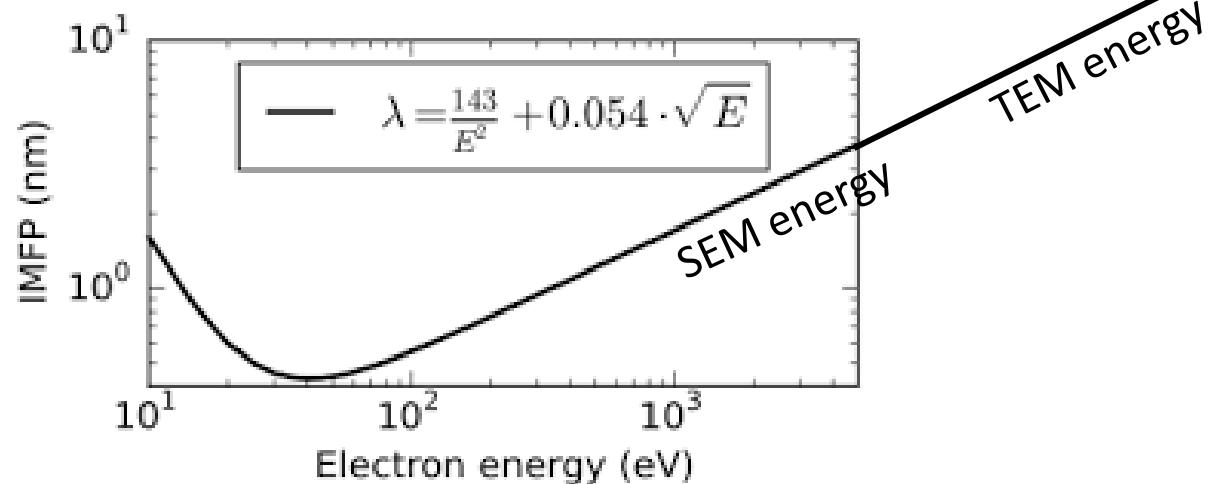
Higher E longer IMFP

At 300 kV ca 200 nm...

Only ~2000 atoms thick

$$P(z, n = 0) = e^{-\frac{x}{\lambda_{IMFP}}}$$

IMFP Universal curve approximation



# Getting electrons through the sample

Beer-Lambert law of exponential intensity decay of transmitted beam with distance

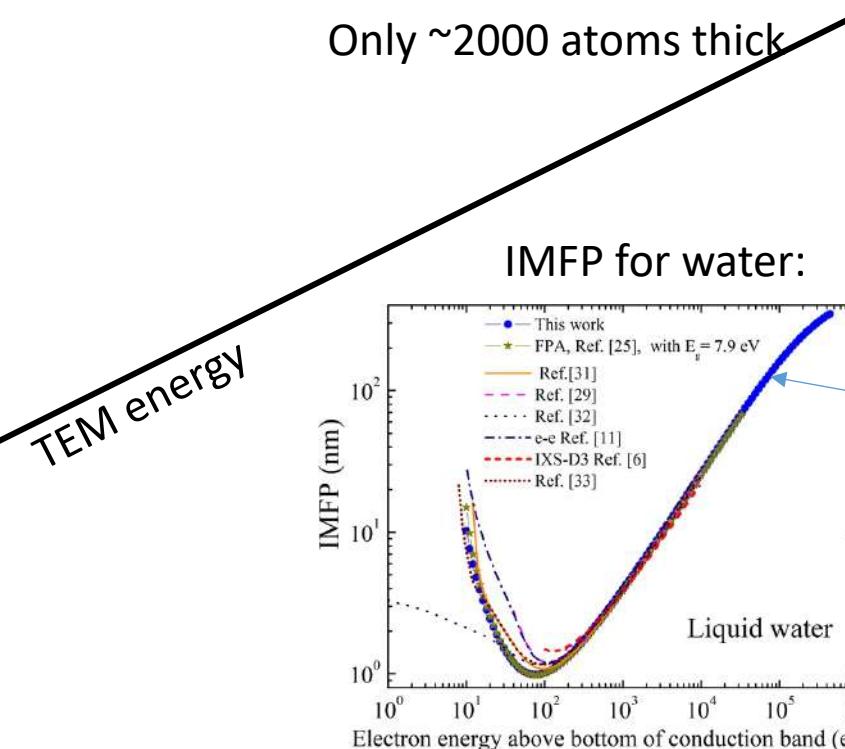
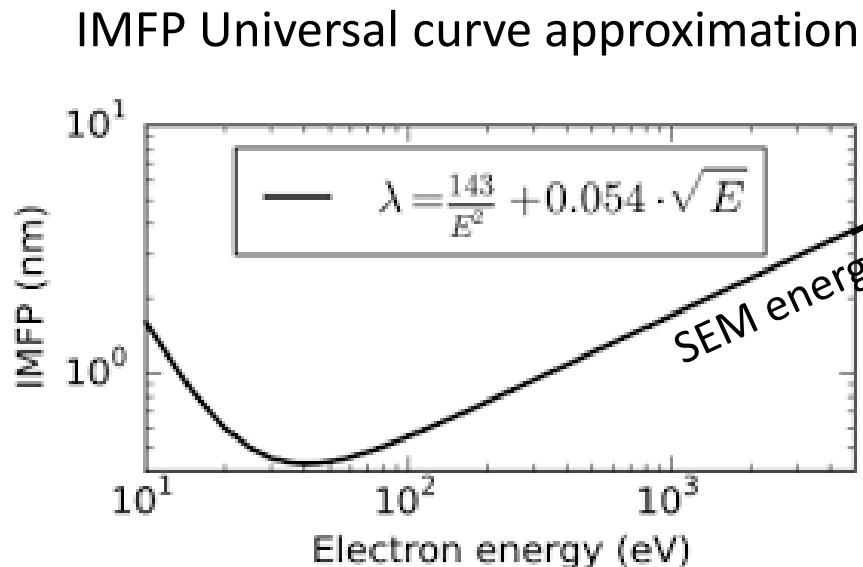
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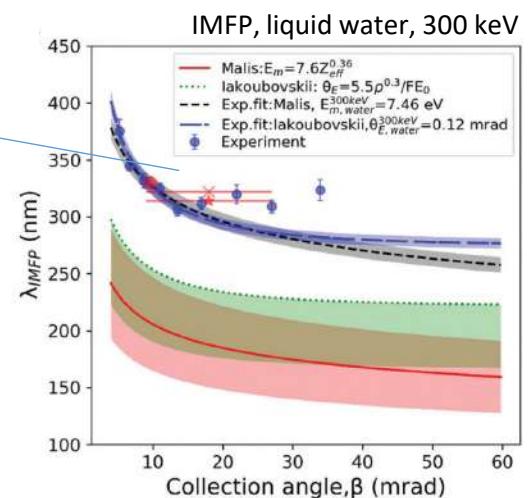
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Flores-Mancera MA, Villarrubia JS, Massillon-JL G, ACS Omega, 17 Feb 2020, 5(8):4139-4147  
DOI: 10.1021/acsomega.9b03872



Murat, Kristian Mølhav et al. Electron inelastic mean free path in water, Nanoscale, 2020, 12, 20649–20657

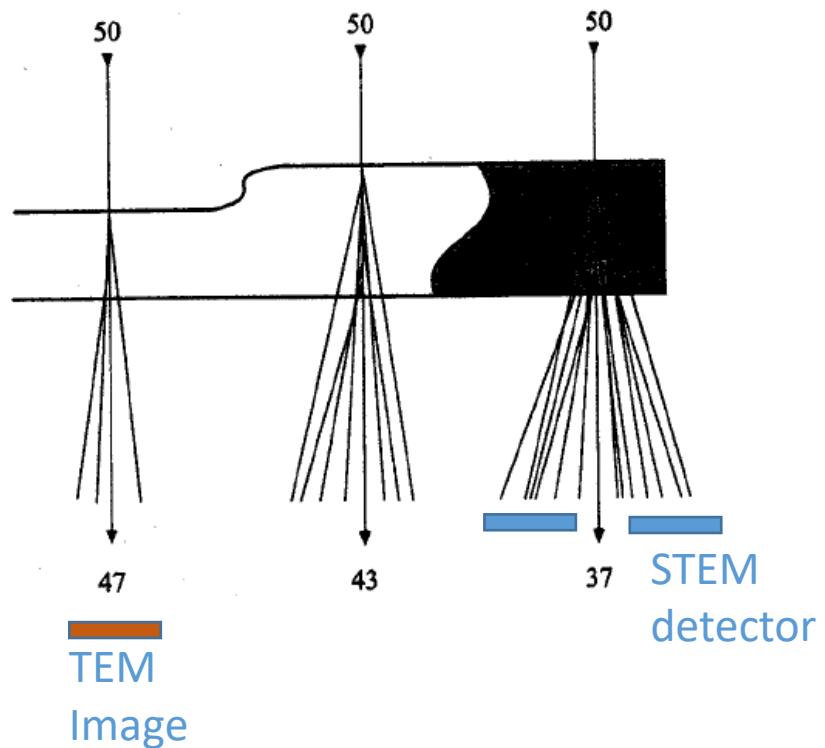
# What forms an image? Brightness and contrast!

Universal formula is only an approximation.

Gold scatters more than carbon or oxygen!

Nuclear charge scatters electrons:

$$\lambda_{\text{IMFP}} \sim 1 / Z^{0.36}$$



TEM measures transmitted beam with both no loss (coherent) and some energy loss (incoherent).

TEM form diffraction patterns, phase contrast images, absorption/scattering contrast images. Much like an optical microscope

STEM measures transmitted large-angle scattered incoherent electrons.

STEM cannot form an image due to large angle, so the beam is scanned to measure scattering in each pixel.

4D-STEM takes an image in each pixel and may give far more information (and a lot of data analysis)

# What forms an image? Brightness and contrast!

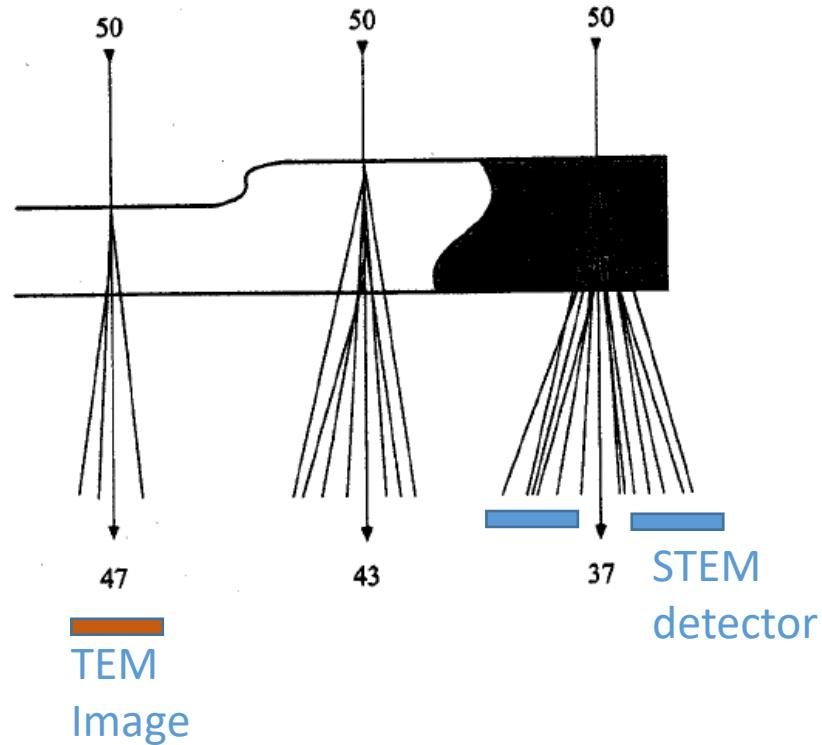
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TEM can form

- diffraction patterns,
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- absorption/scattering contrast images.

Much like an optical microscope

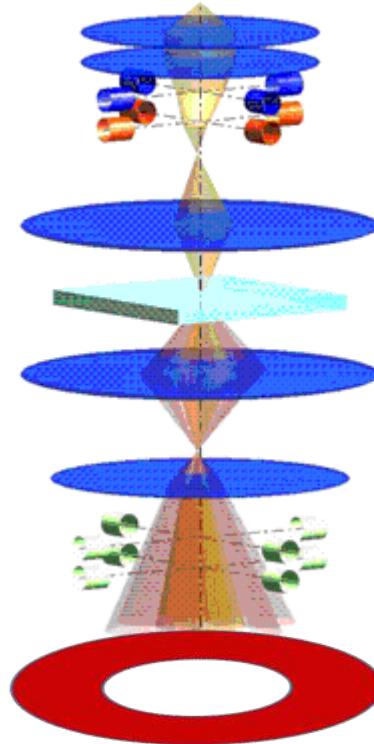
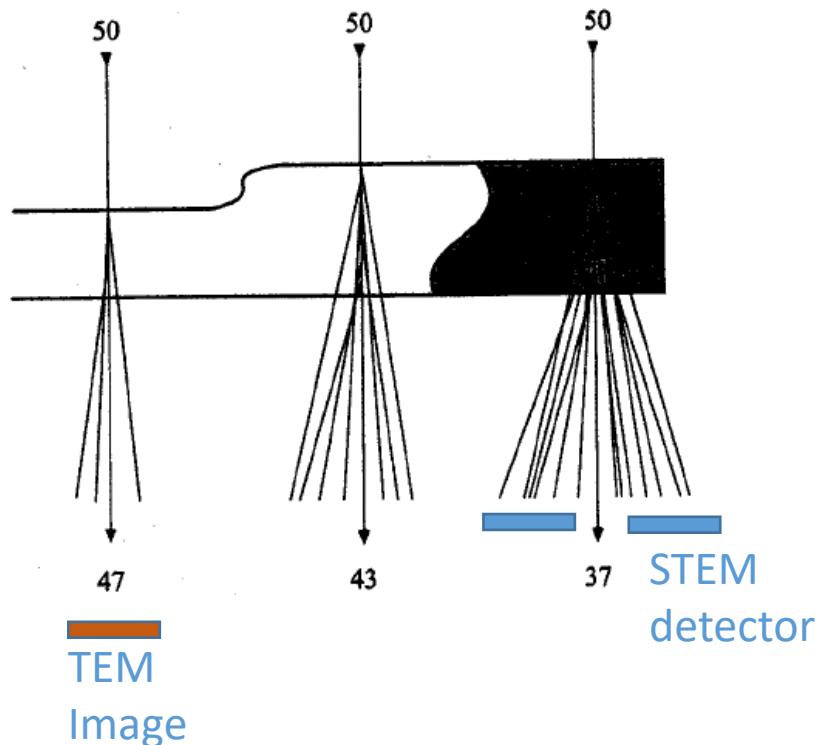
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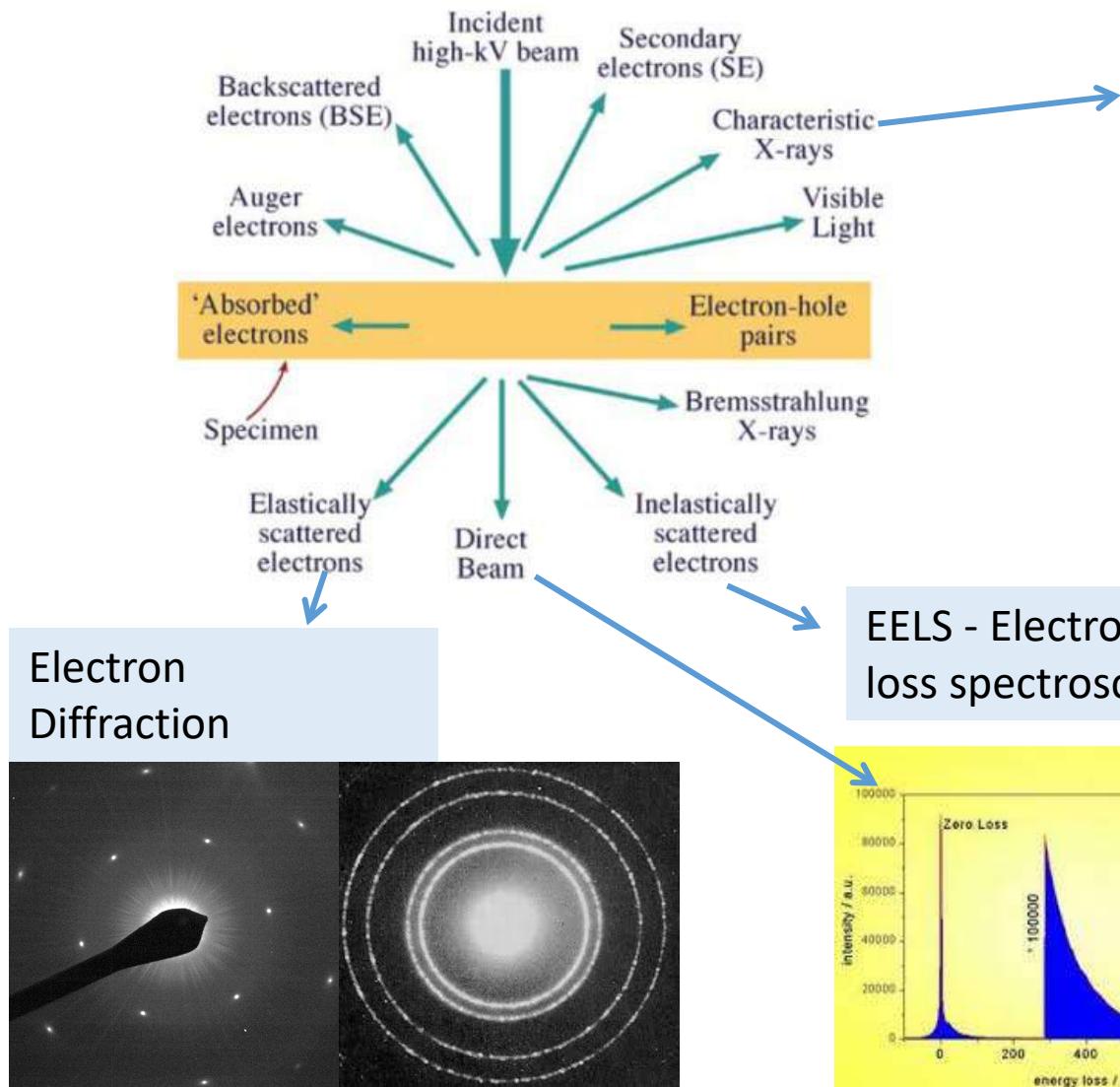


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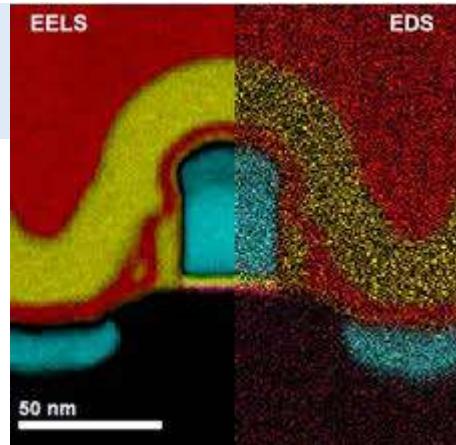
**High - angle annular dark field detector (HAADF)  
is sensitive to the atomic number of atoms**

# The many other signals in the TEM



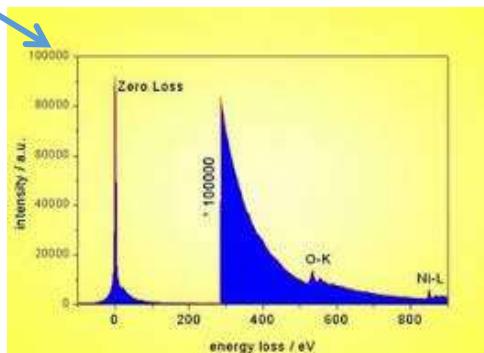
<http://www.agarscientific.com/graphene-oxide-support-films.html>

EDS – energy dispersive x-ray spectroscopy



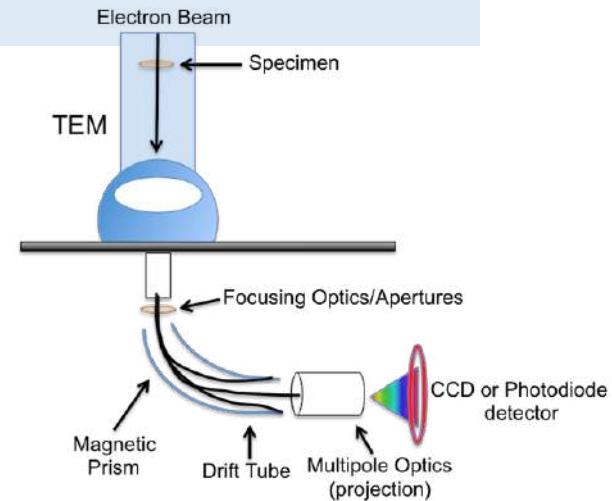
<http://www.gatan.com/techniques/eels>

EELS - Electron energy loss spectroscopy

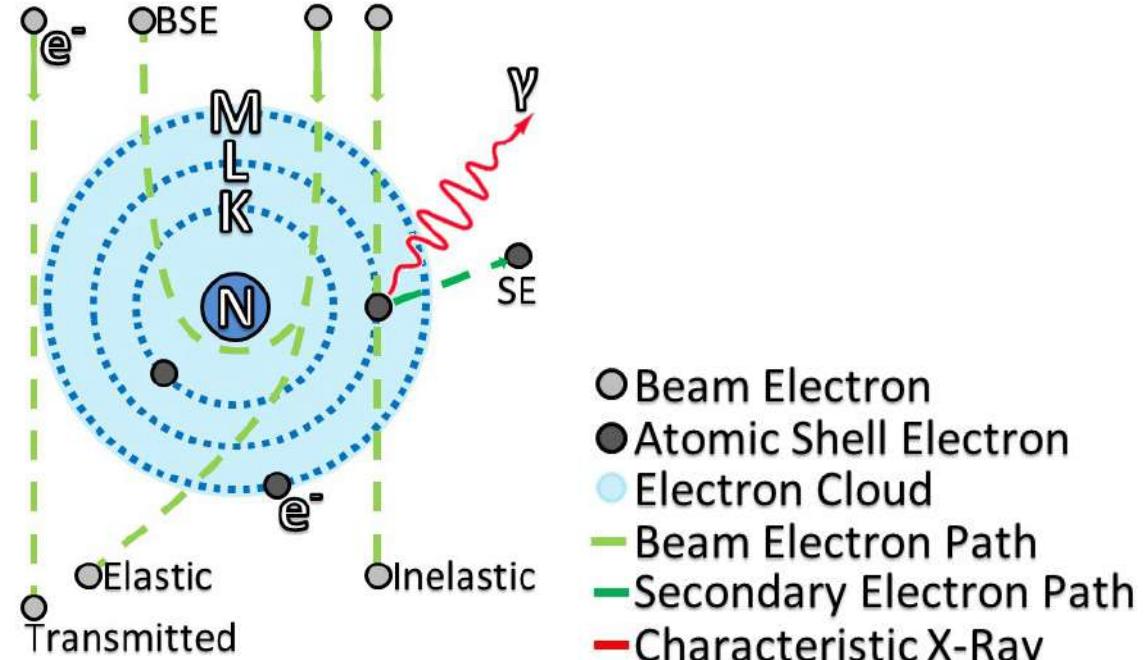
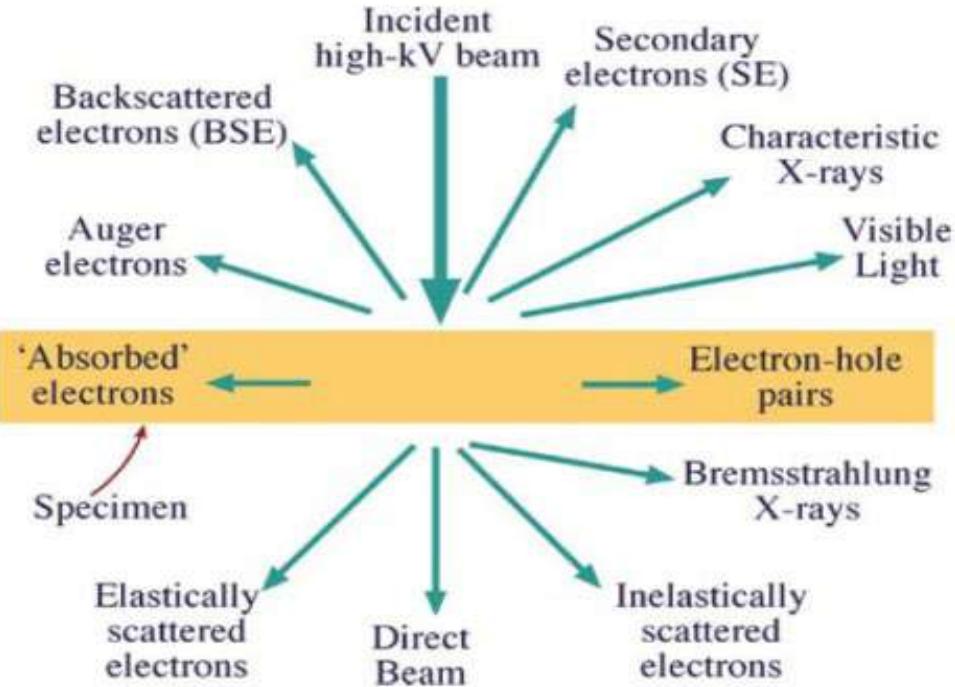


Zero loss vs scattered is a measure of thickness  $t/\lambda_{\text{mfp}}$

Kristian Mølhave krmo@dtu.dk



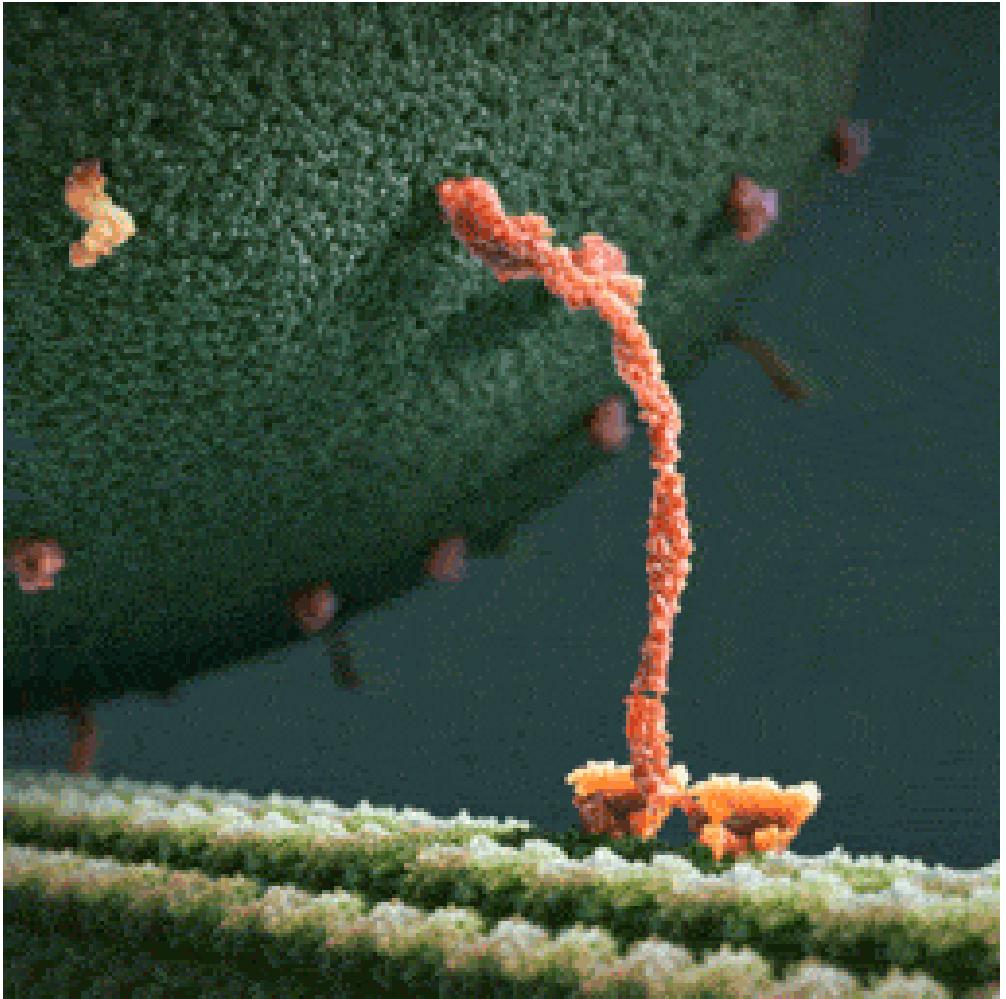
# Electron scattering



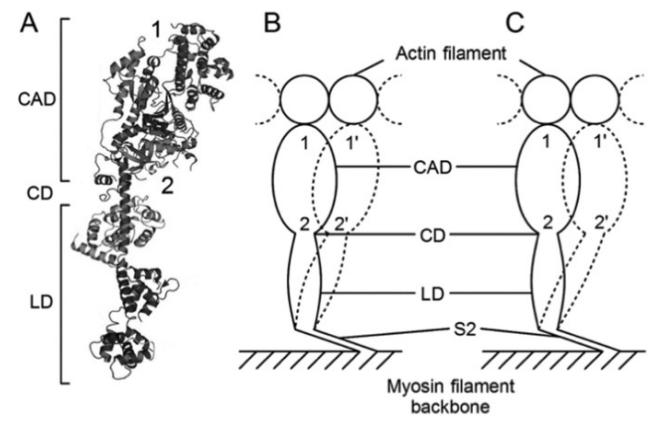
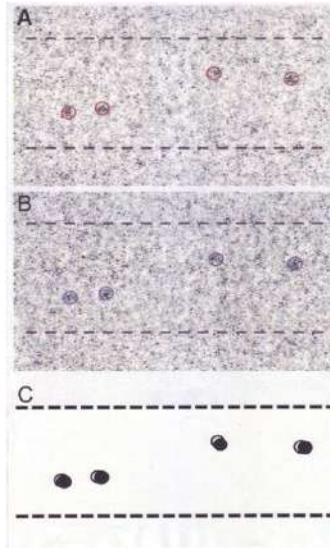
[https://en.wikipedia.org/wiki/Electron\\_scattering](https://en.wikipedia.org/wiki/Electron_scattering)

Transmission Electron Microscopy, A Textbook  
for Materials Science  
By David B. Williams, C. Barry Carter,  
<https://doi.org/10.1007/978-0-387-76501-3>

# Will we ever get a view of this??



Myosin walking - <https://imgur.com/gallery/I1TBI29>



**Few nm steps in**

*Scientific Reports 5,*  
Article number: 15700 (2015)  
<https://www.nature.com/articles/srep15700>

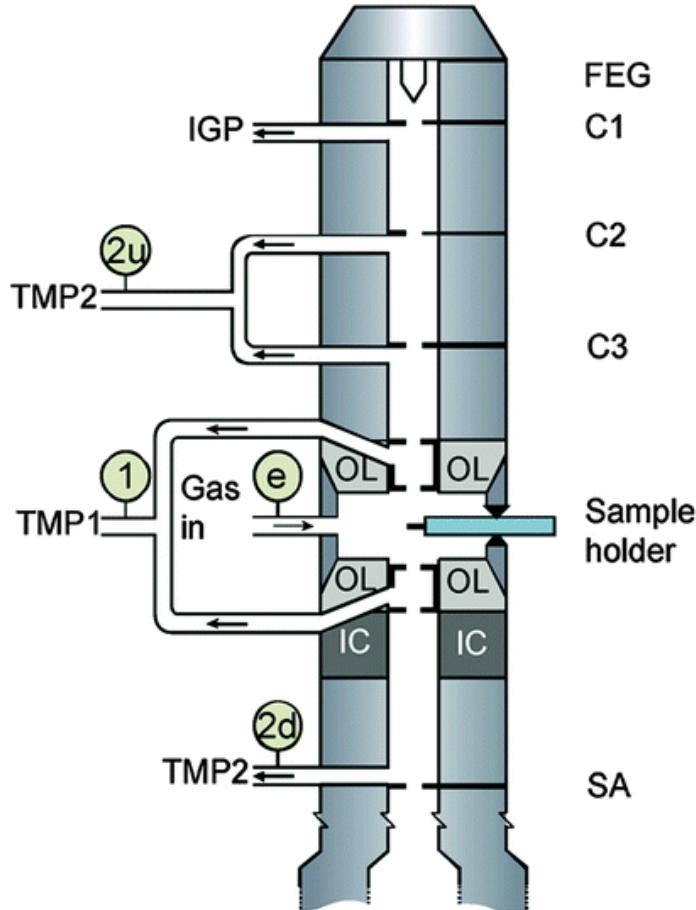
# Environmental TEM

Low gas pressures (<10 mBar) in a region with differential pumping

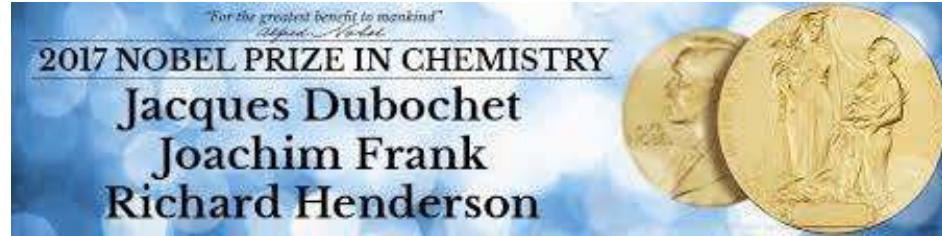
Mainly used for catalysis studies and high temperature processes in gasses

Membrane techniques  
→ 1 bar and 1300 °C

We will talk about this later



# Cryo TEM for nanoscale biology



NEWS AND VIEWS - 21 OCTOBER 2020

## Cryo-electron microscopy reaches atomic resolution

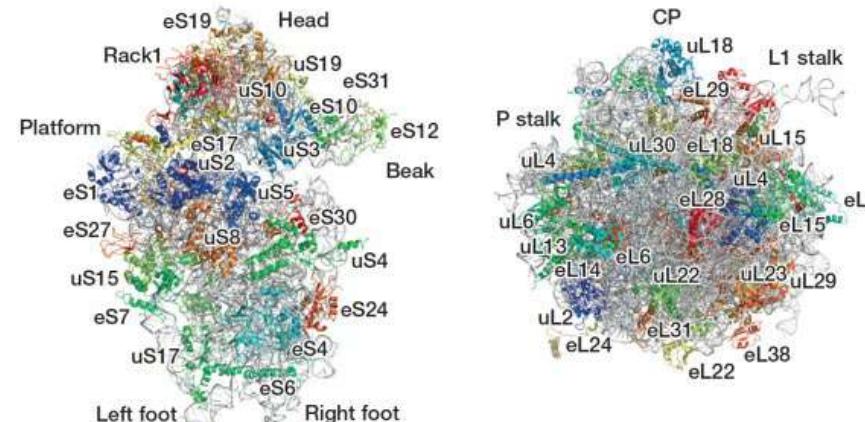
A structural-biology technique called cryo-electron microscopy has attained the ability to locate individual atoms within a protein. What are the implications of this advance?

<https://www.nature.com/articles/d41586-020-02924-y>

CPHU has a new cryofacility recently opened



Prof. Klaus Qvortrup, CPHU



Khatter, H., Myasnikov, A. G., Natchiar, S. K. & Klaholz, B. P.  
Nature 520, 640–645 (2015).

Kristian Mølhave krmo@dtu.dk

Or use X-ray diffraction

What would you tell someone to do if they said:

1. I want to know if this TiO<sub>2</sub> powder is the anatase crystal phase?
2. What is the shape of these 100-1000 nm metal particles?
3. What is the structure of this 100 nm virus particle?
4. Why is it difficult to image bio sample?
5. How can I follow the surface changes of my catalytic particle in gas at 700 C

Next session

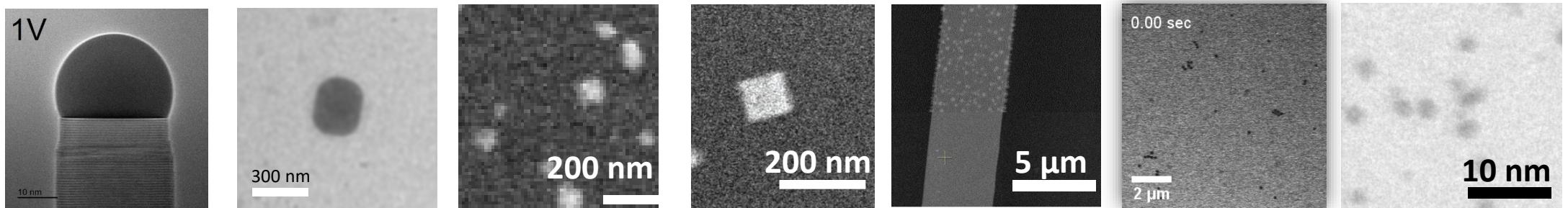
### **3. Liquid phase electron microscopy**

# 3. Liquid phase transmission electron microscopy

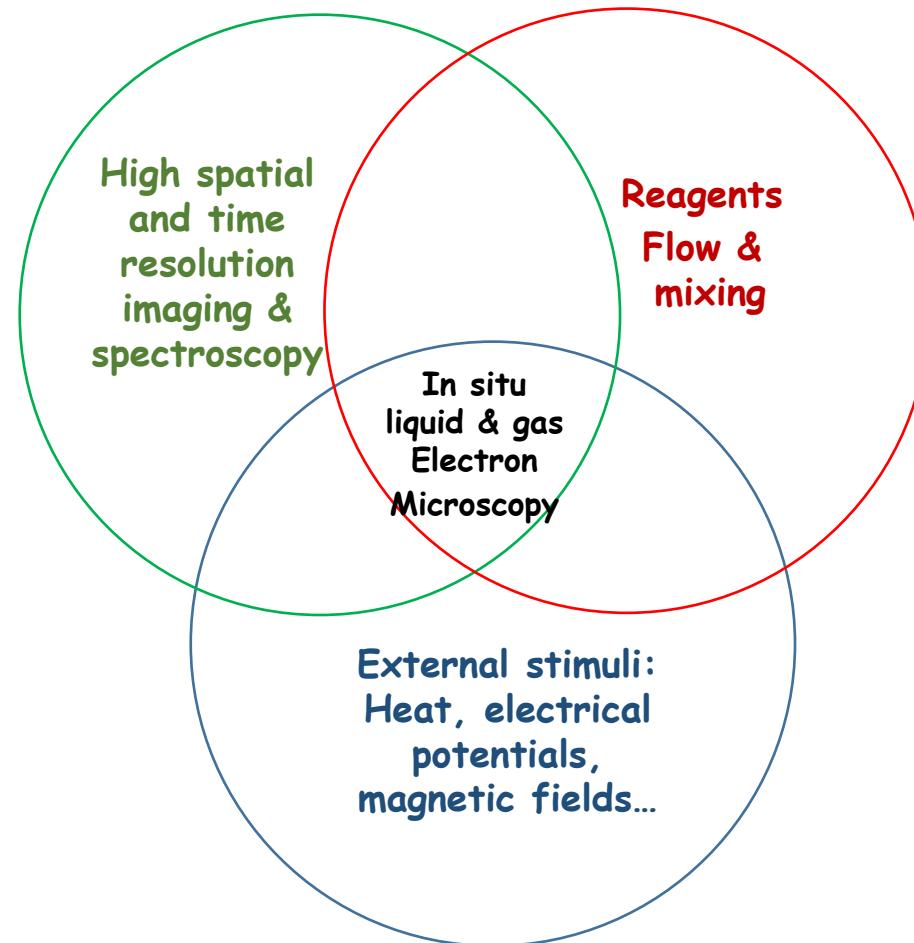
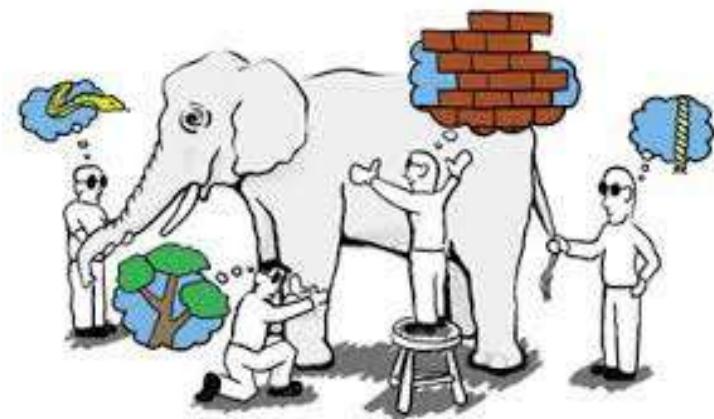
Murat Nulati Yesibolati, Hongyu Sun & Kristian Mølhave

DTU Nanolab

Molecular  
Windows



# The Quest for In situ Liquid Electron Microscopy

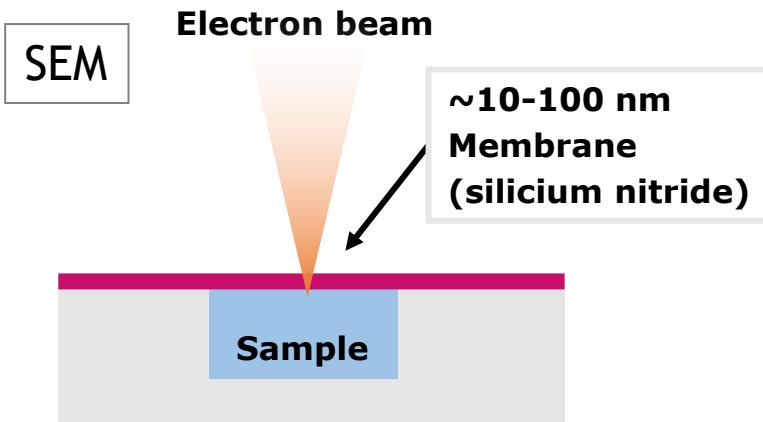


# Discussion

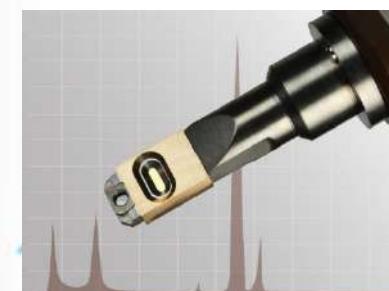
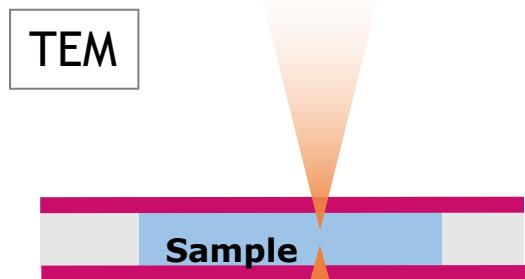
What material do you suggest ?  
or what are the requirements to be a good window materials  
for an electron microscopy liquid cell ?

Remember last lecture on electron-beam interactions...

# Going Liquid by commercial systems

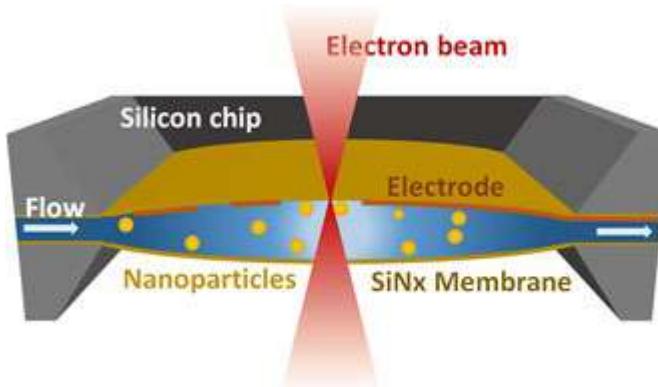


Quantomix WetSEM capsule



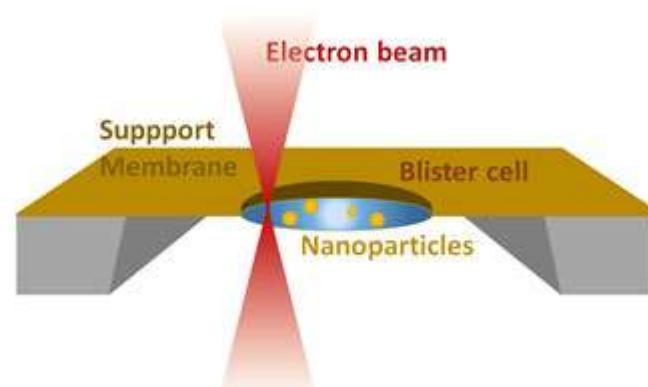
# Main types of TEM liquid cells

'Traditional'  
Clamped Chips



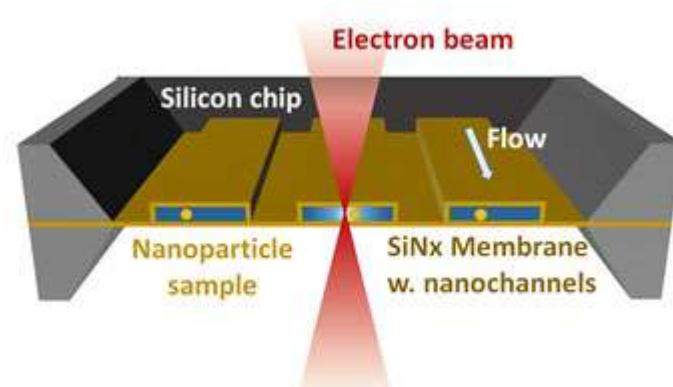
- Varying liquid thickness (0.2 - 4  $\mu\text{m}$ ) gives poor resolution ( $\sim 30 \text{ nm}$ )
- Flow, but diffusion dominated
- Electrochemical electrodes
- Temperature control option
- Plug-n-play commercial systems

'DIY'  
Graphene Blister Cell



- Ultrathin liquid and membrane giving atomic resolution
- No Flow nor Electrodes
- DIY - but very time consuming

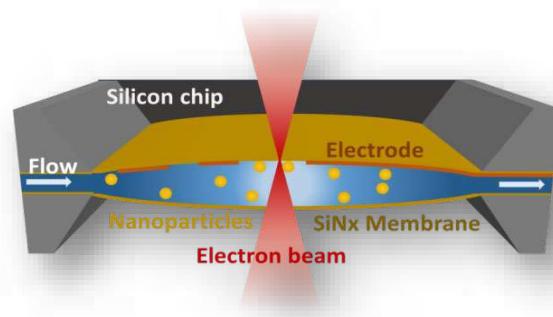
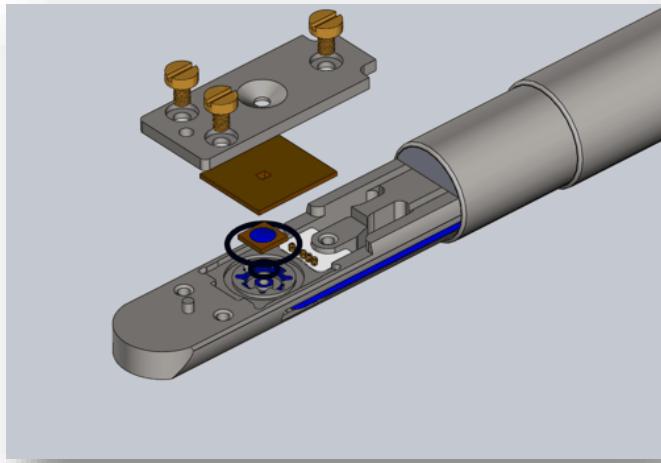
Nanochannel  
system



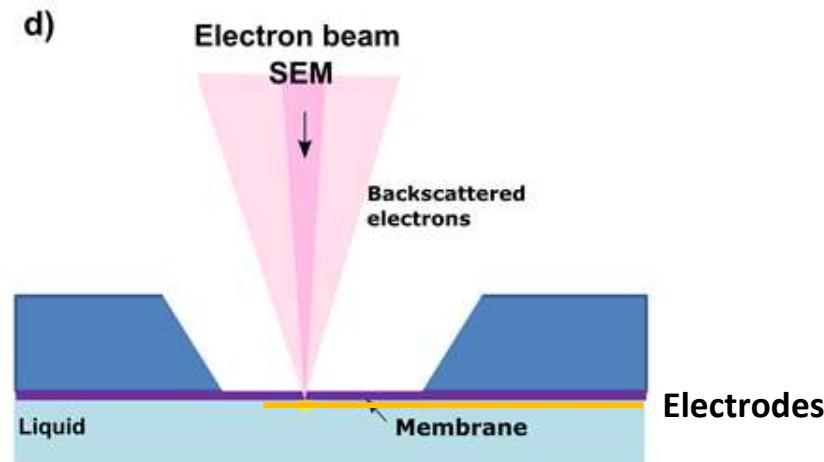
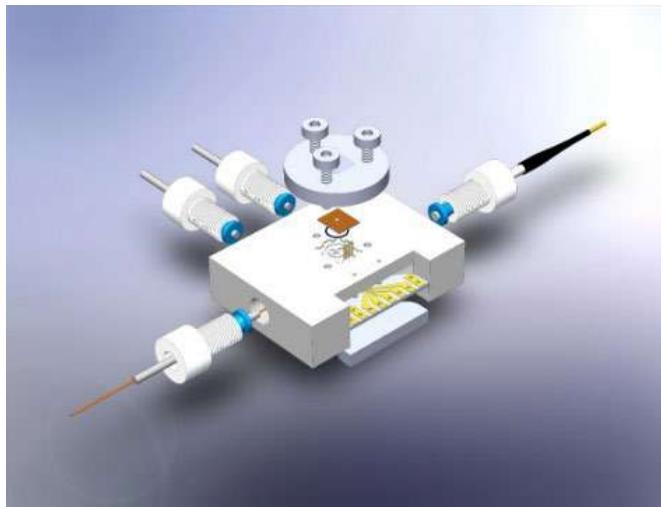
- Well defined thin liquid for high resolution >10 nm nitride
- Flow & Easy sample injection
- Mixing in field of view
- Temperature control up to 200 C
- Electrodes will be developed

# Our Custom built Liquid cells

TEM / STEM

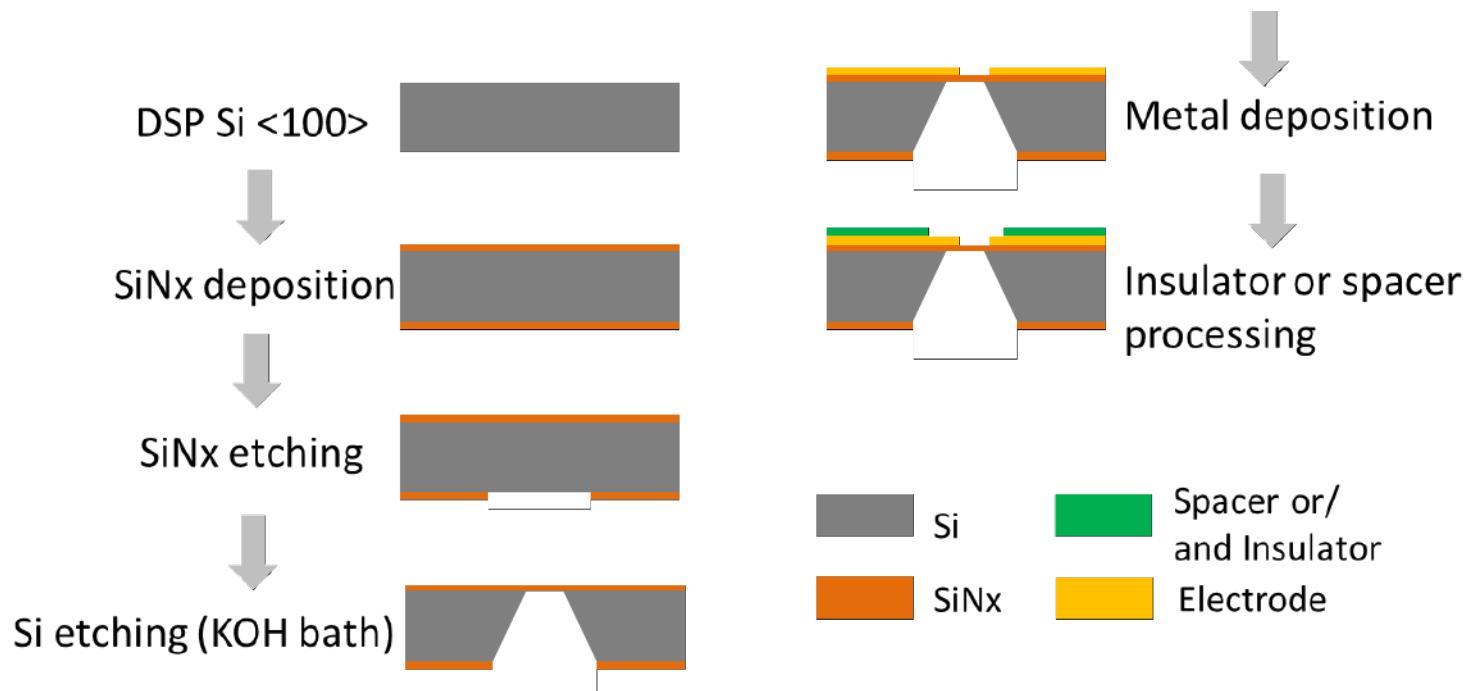


SEM



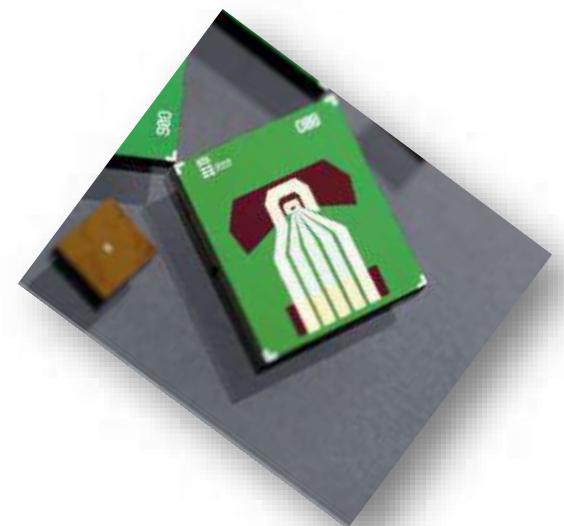
# Chip fabrication

## Microfabrication process

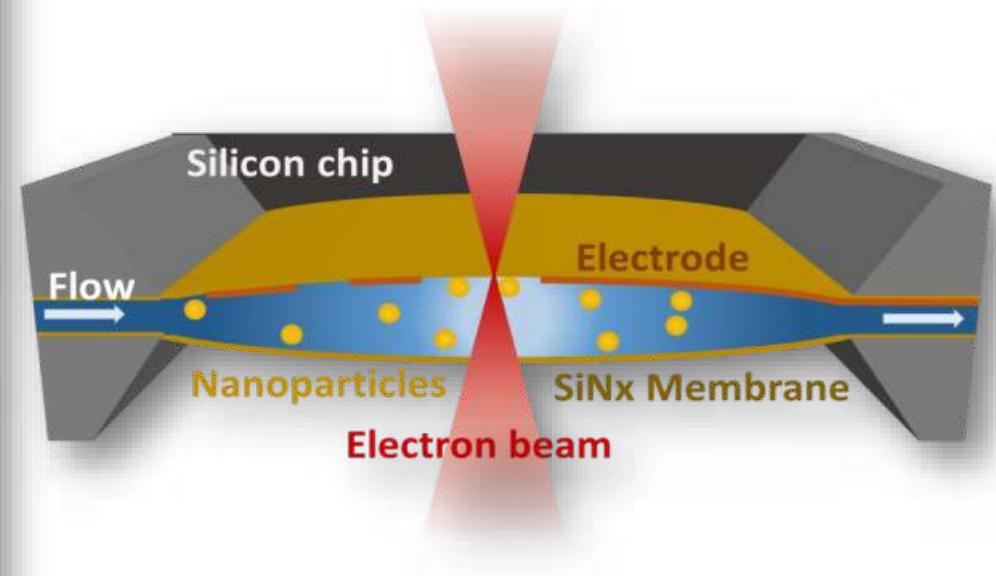
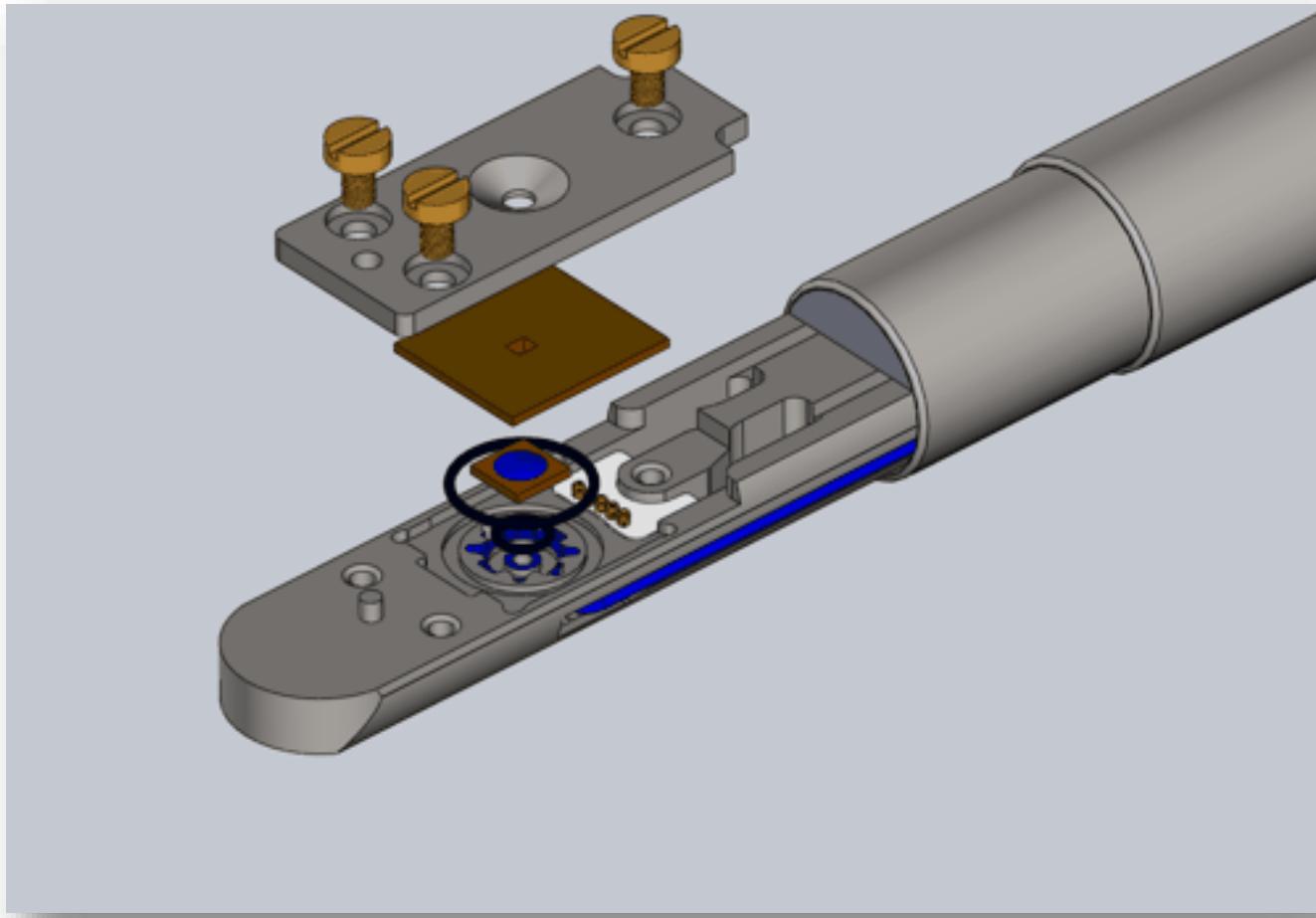


*Eric Jensen et.al. Jensen, E., & Burrows, A. (2012). Engineering Electrochemical Setups for Electron Microscopy of Liquid Processes. DTU Nanotech.*

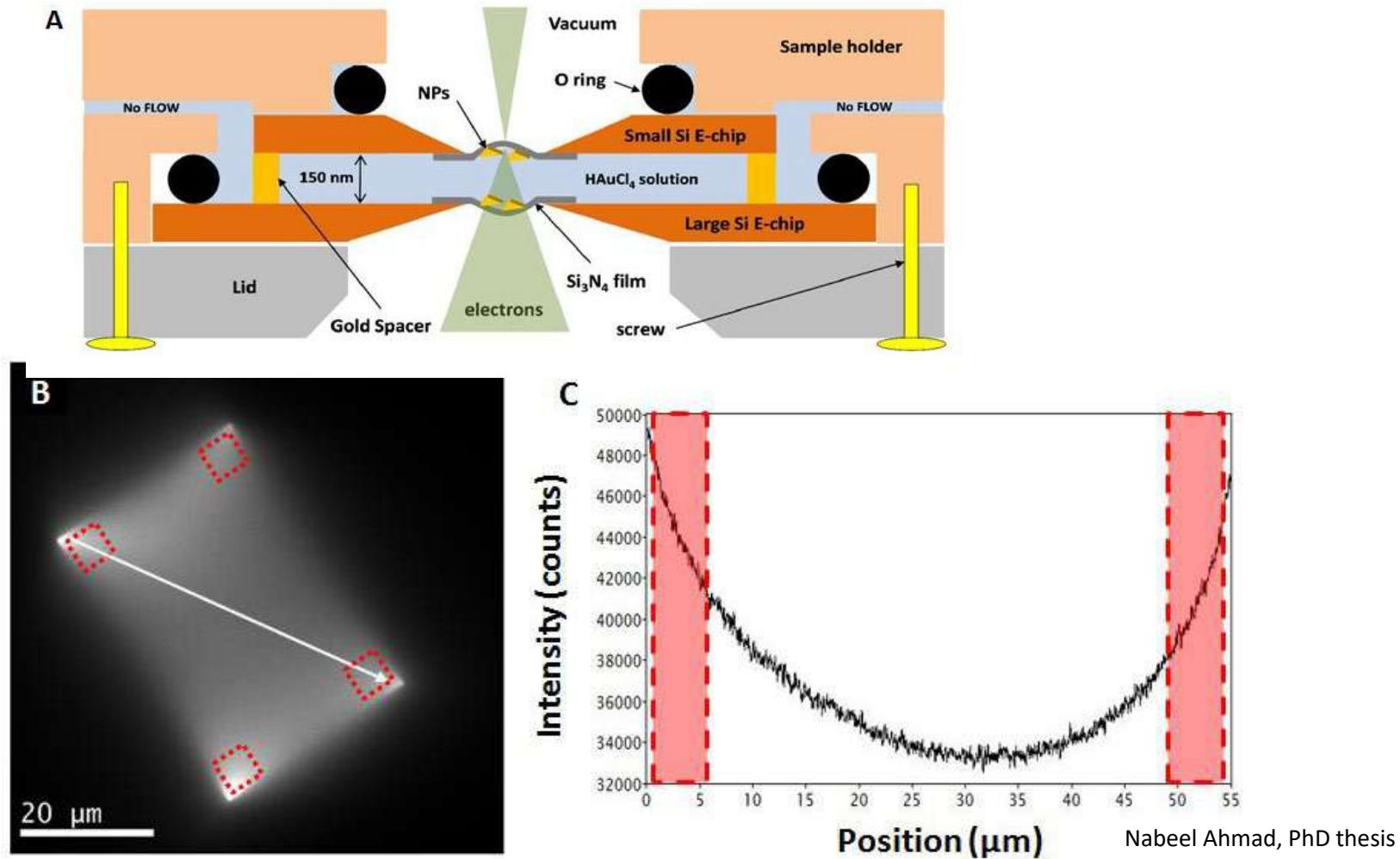
DTU nanolab cleanroom  
Microfabrication



## Assembly

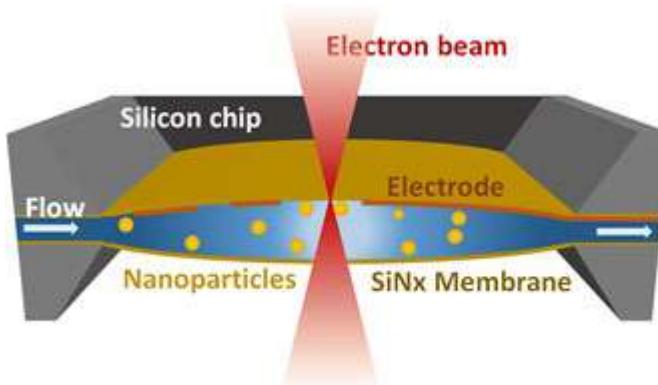


# Bulging membranes in vacuum



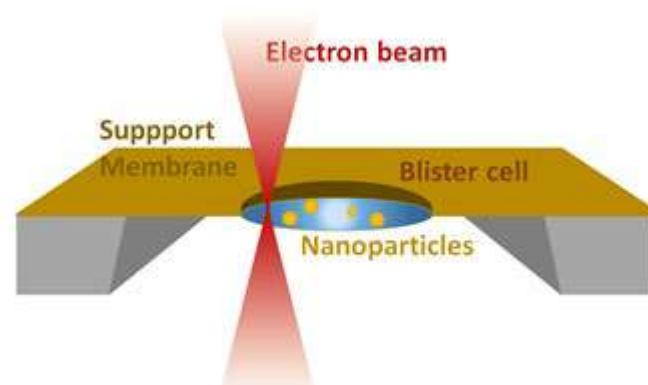
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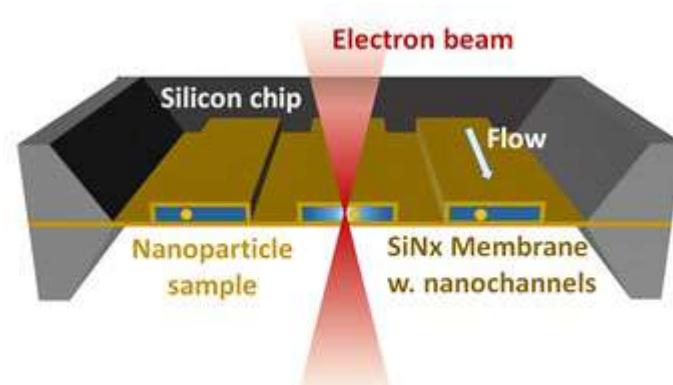
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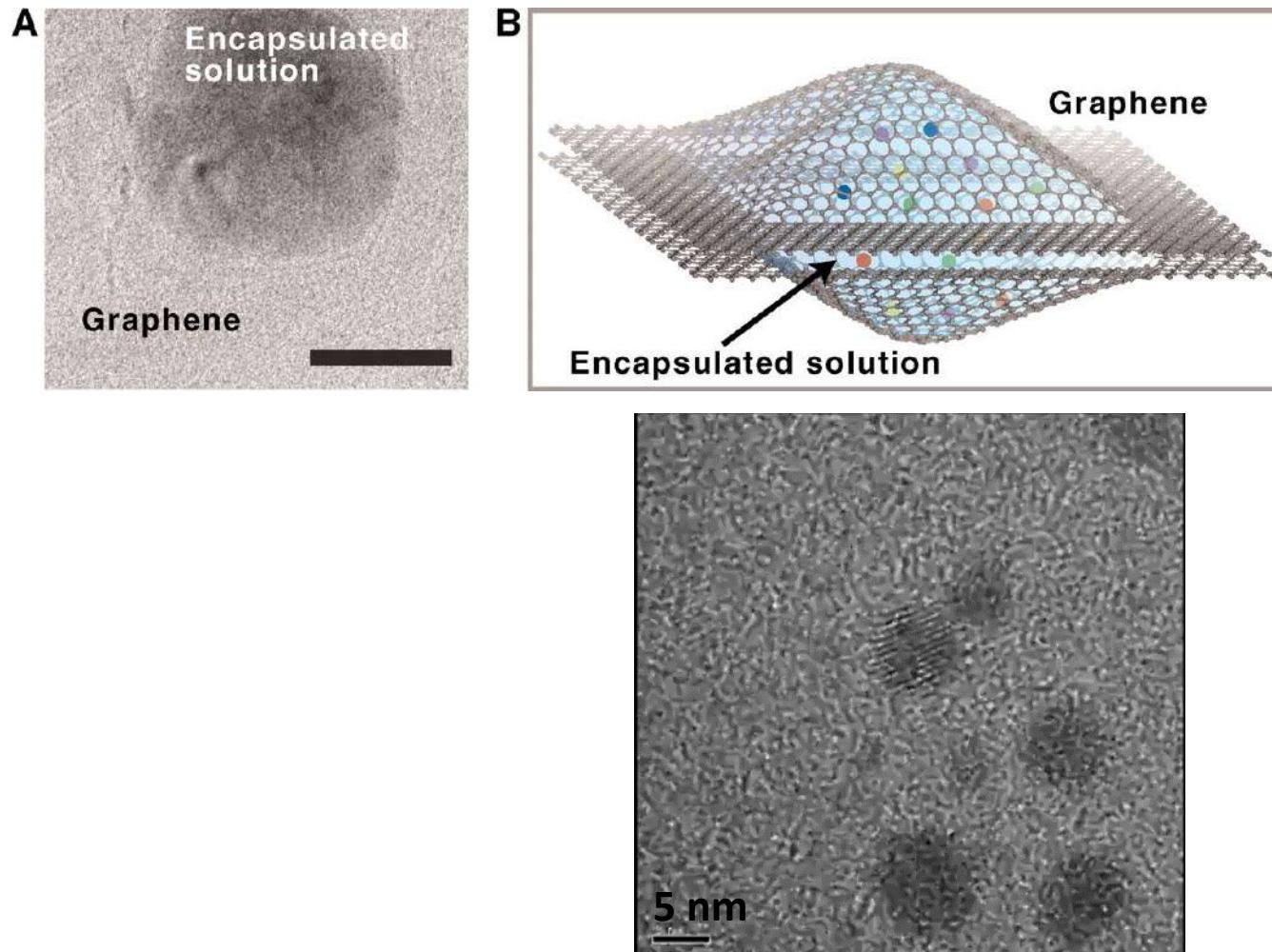
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Nanochannel  
system



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- Flow & Easy sample injection
- Mixing in field of view
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- Electrodes will be developed

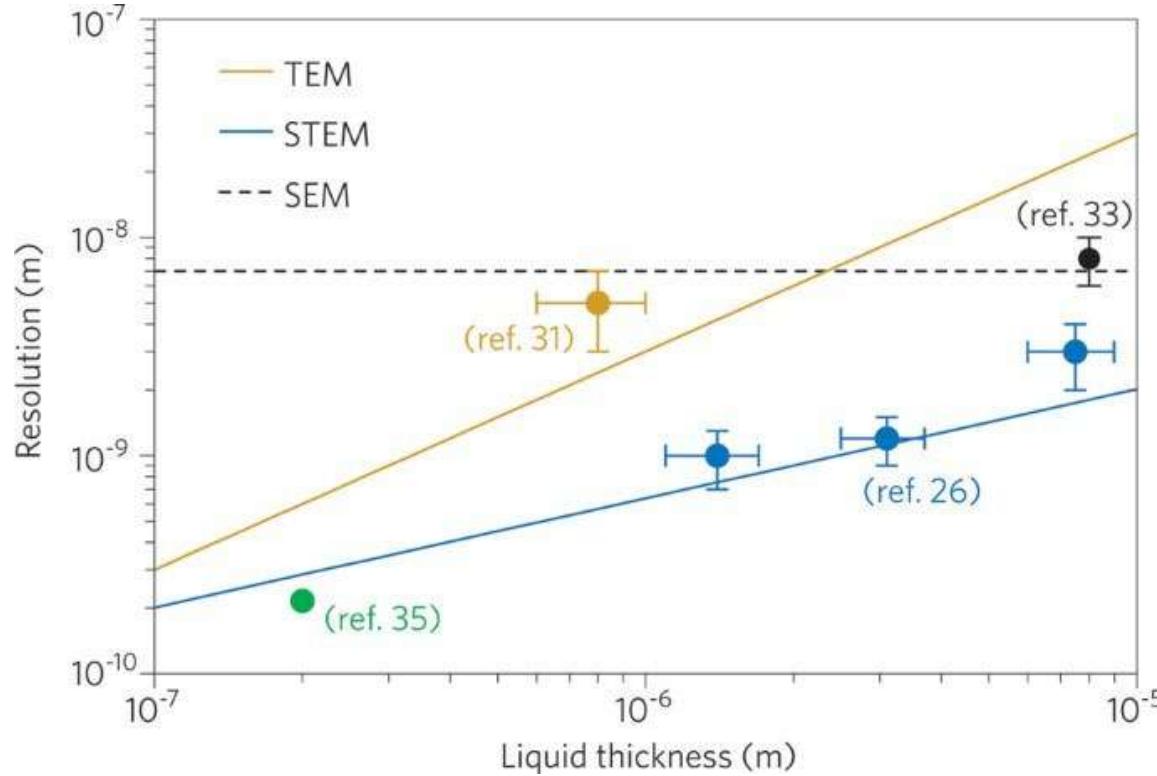
# A Graphene liquid cell alternative



Yuk et al., Science 2012

<http://science.sciencemag.org.proxy.findit.dtu.dk/content/336/6077/61>

# Resolution



Thin liquid layers are essential  
for high resolution!

STEM generally  
better than TEM

Both better than SEM  
for nanoscale imaging

<https://www.nature.com/articles/nnano.2011.161>

# Liquid thickness and resolution

## TEM resolution

$$d_C = \alpha C_C \Delta E / E,$$

$$d_{C, \text{TEM}} = 6 \times 10^{12} \alpha C_C T / E^2.$$

with E in electron energy, T is sample thickness.  $\alpha$ ,  $C_C$  are microscope parameters.

For pure water, neglecting the enclosing window(s), and using typical experimental parameters, say  $\alpha = 10$  mrad,  $C_C = 2$  mm,  $E = 200$  keV, and  $T = 1$   $\mu\text{m}$ , we obtain  $d_C, \text{TEM} = 4$  nm.

## STEM resolution

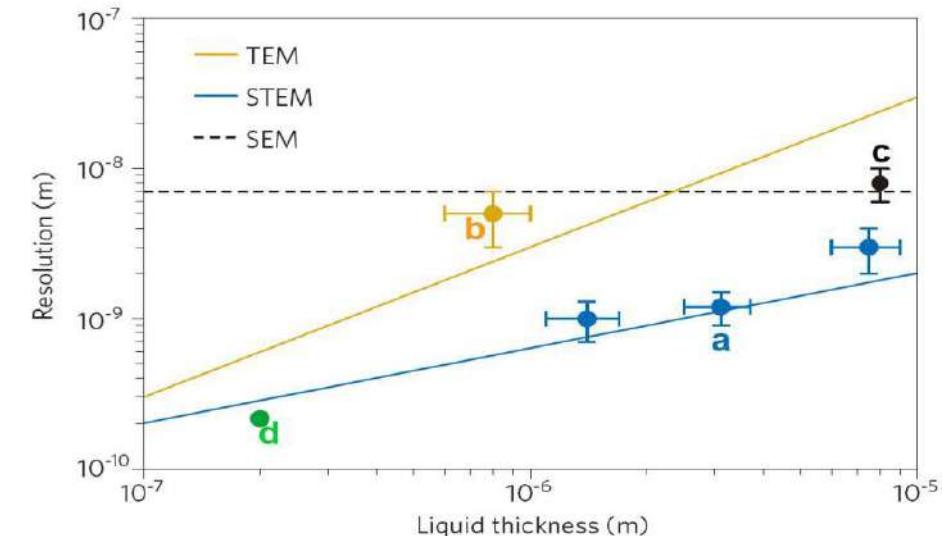
(Probe size is about 0.1-1 nm)

$$d_{\text{STEM}} = 5l_{\text{particle}}(T/l_{\text{water}}N_0)^{1/2}$$

$l$ : mean free path for elastic scattering (depends on electron energy, and materials)  
 $T$ : liquid thickness

$N_0$ : electrons in the primary beam in each pixel (a value depending on the probe current and the dwell time)

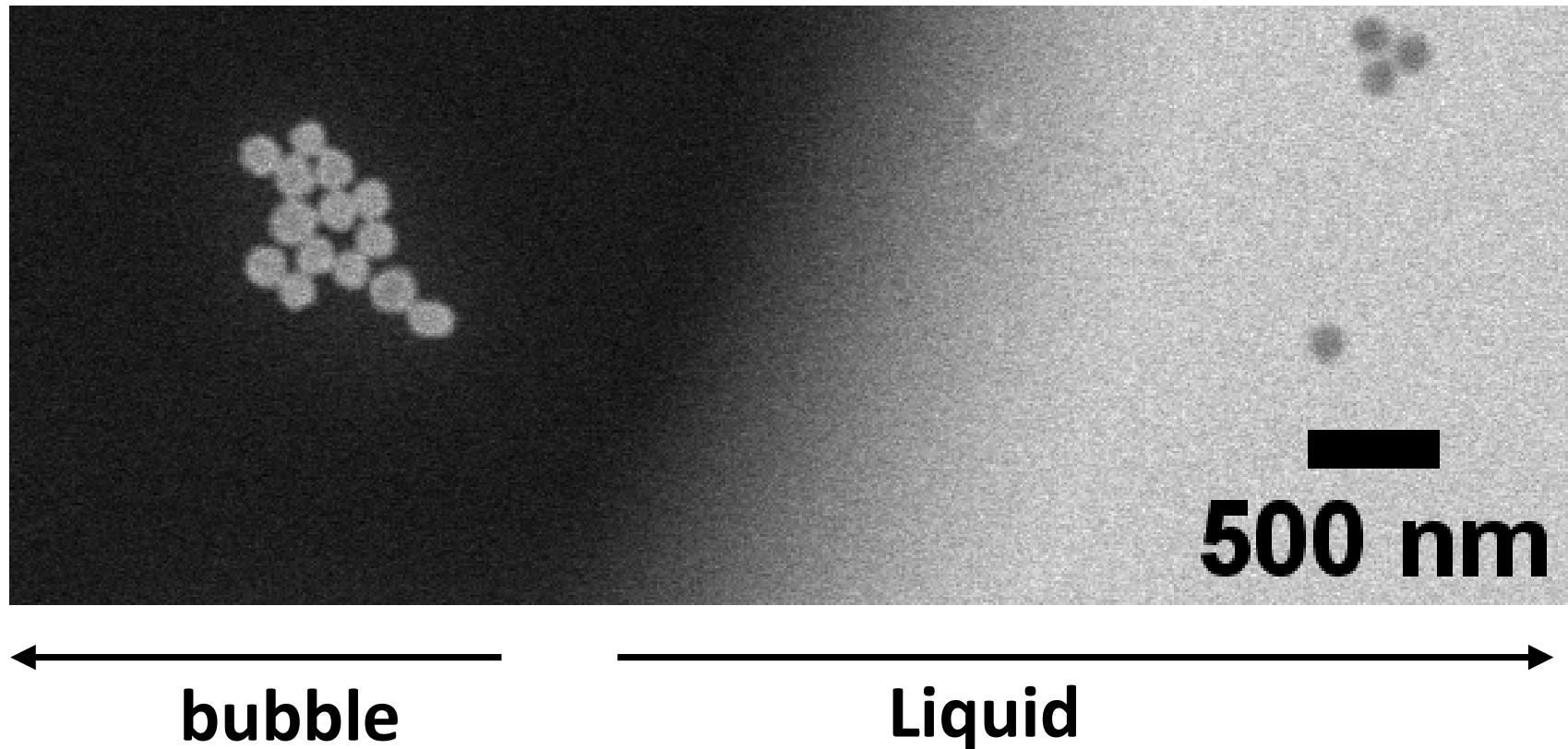
Can resolve 1.4 nm Au, at  $t=5$   $\mu\text{m}$  using a probe current of 0.5 nA and a pixel dwell time of 10  $\mu\text{s}$ .



Dots are reported experiment data

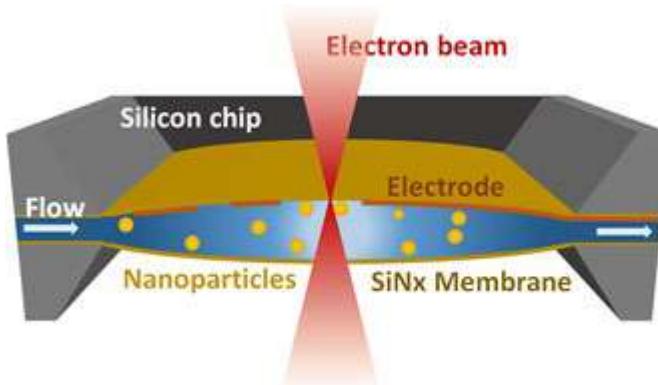
# Contrast inversion...

Thick liquid layer – poor resolution



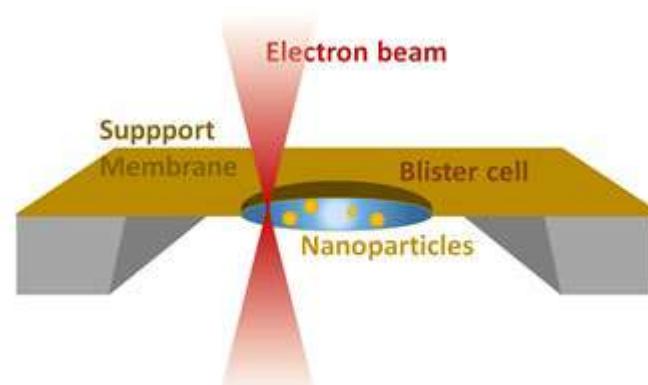
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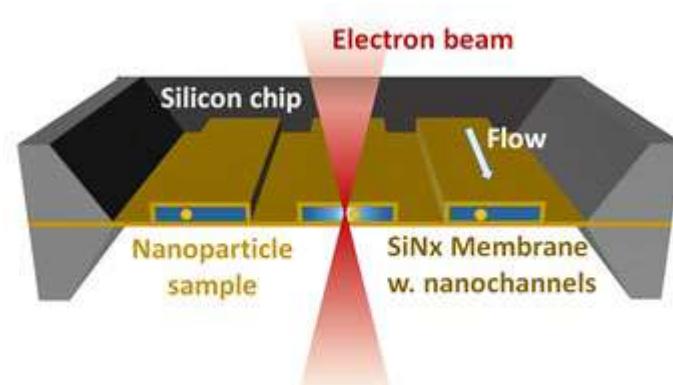
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Nanochannel  
system



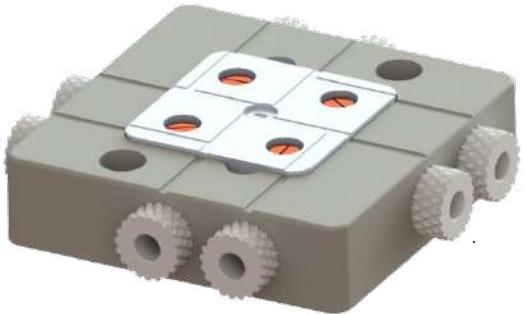
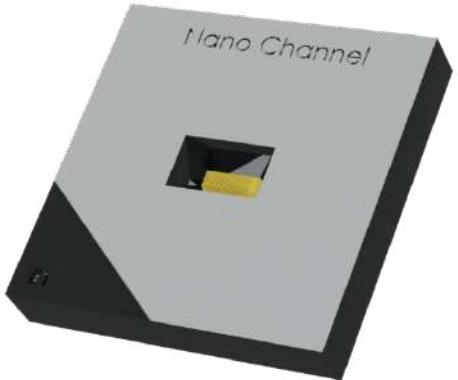
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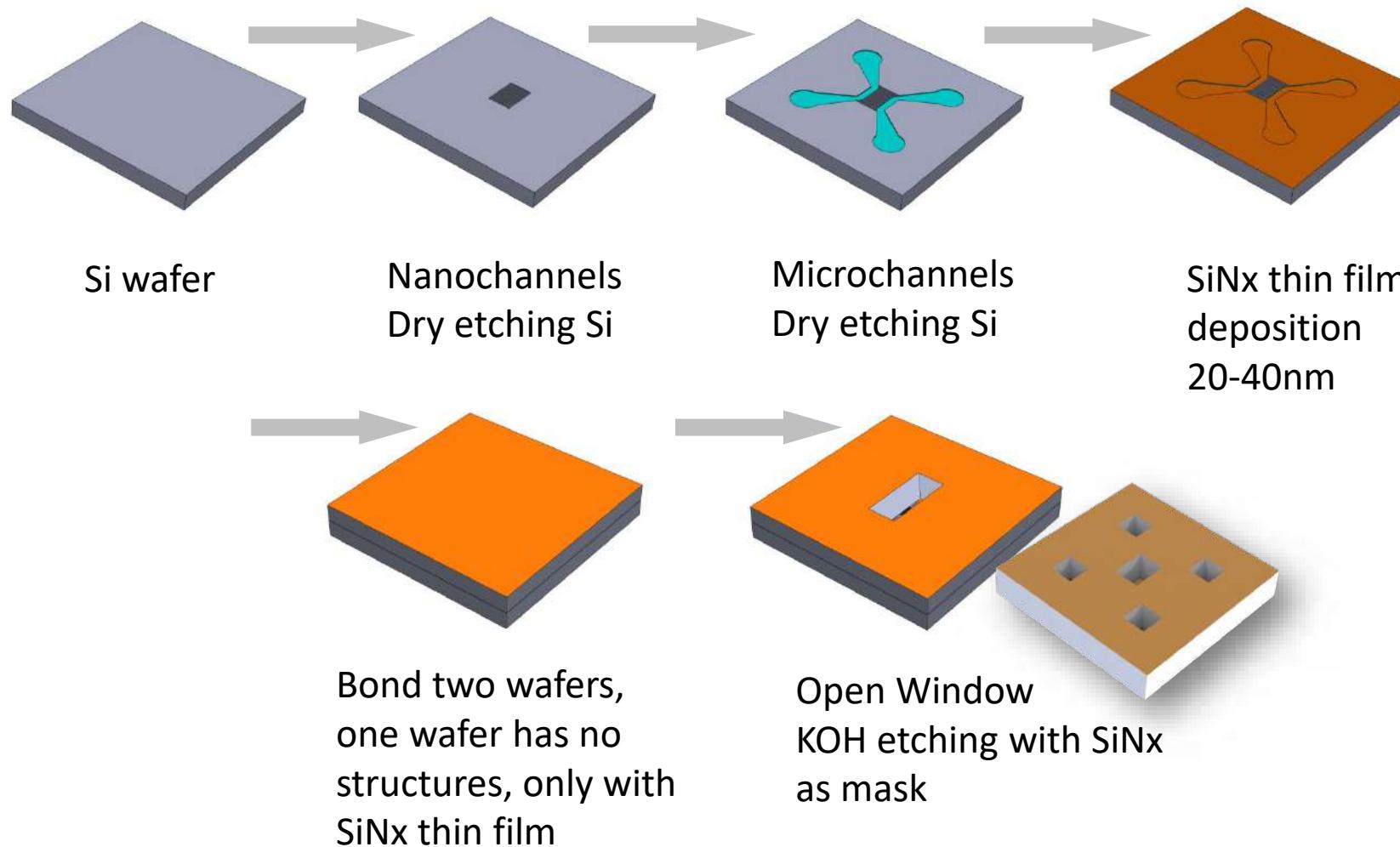
# INSIGHT CHIPS

PROVIDING ELECTRON MICROSCOPY SOLUTIONS

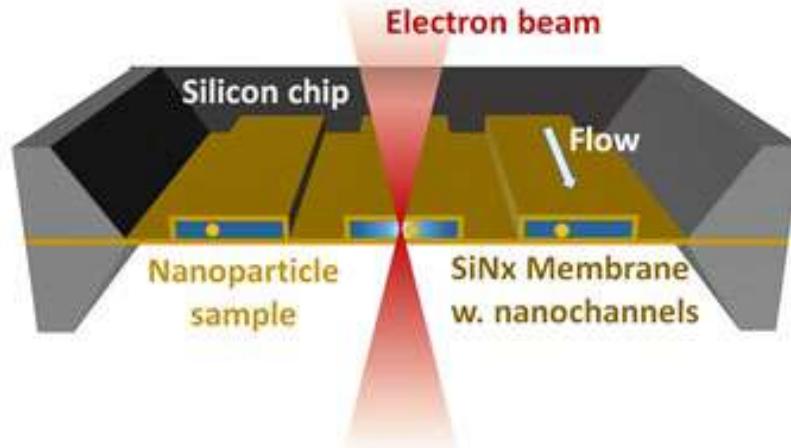
A rapidly growing DTU Nanolab start up!



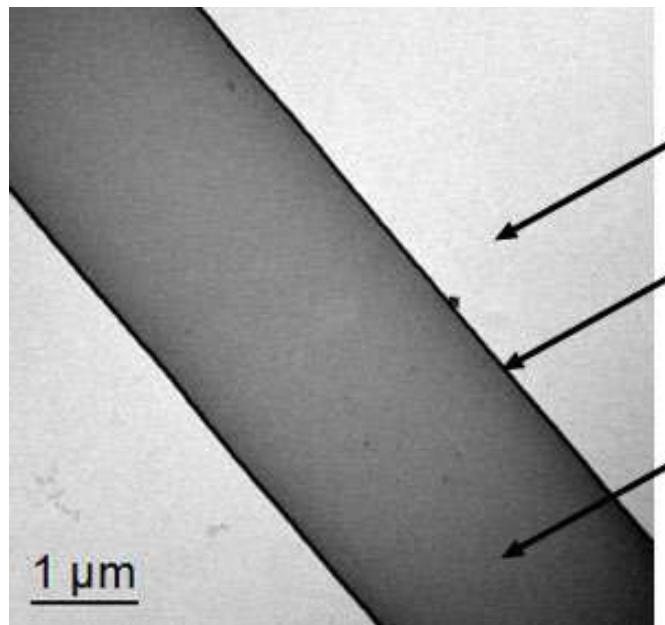
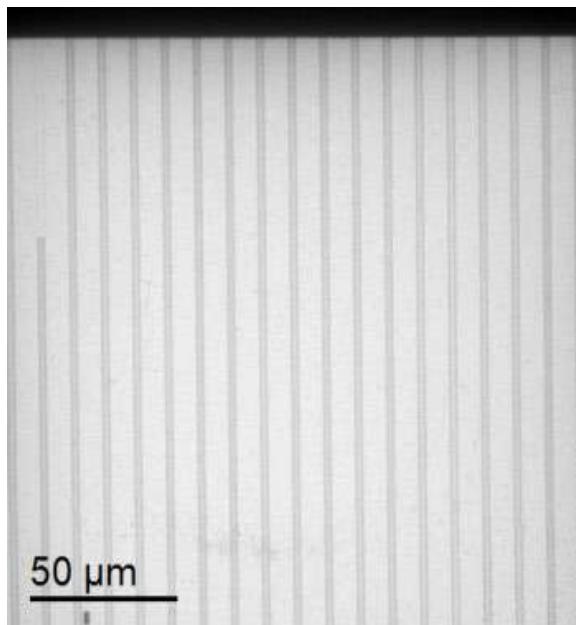
# Nanochannel TEM Microfabrication



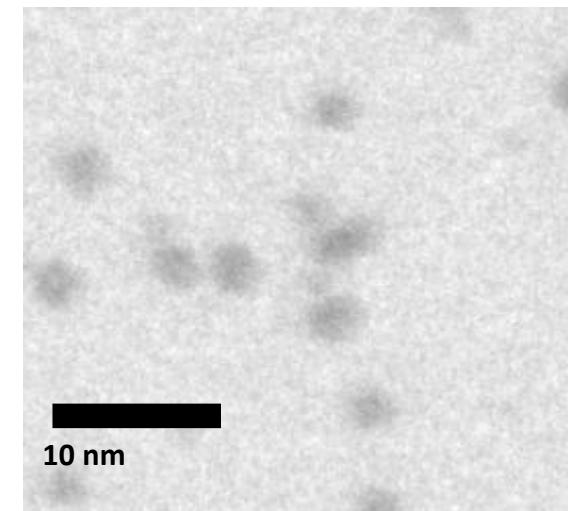
# Nanofluidic LPTEM



TEM of Membrane with suspended channels



Bonded region  
Nanochannel sidewall  
Nanochannel + liquid

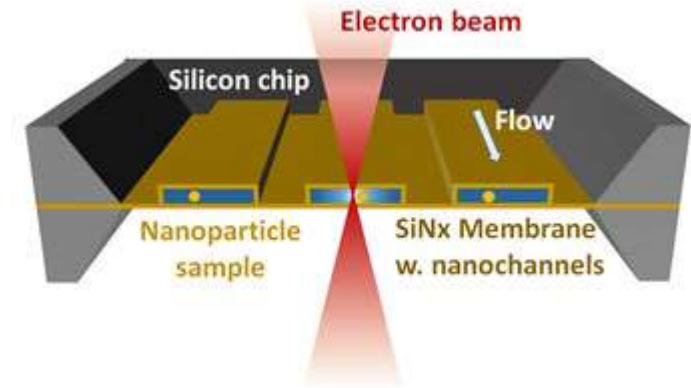
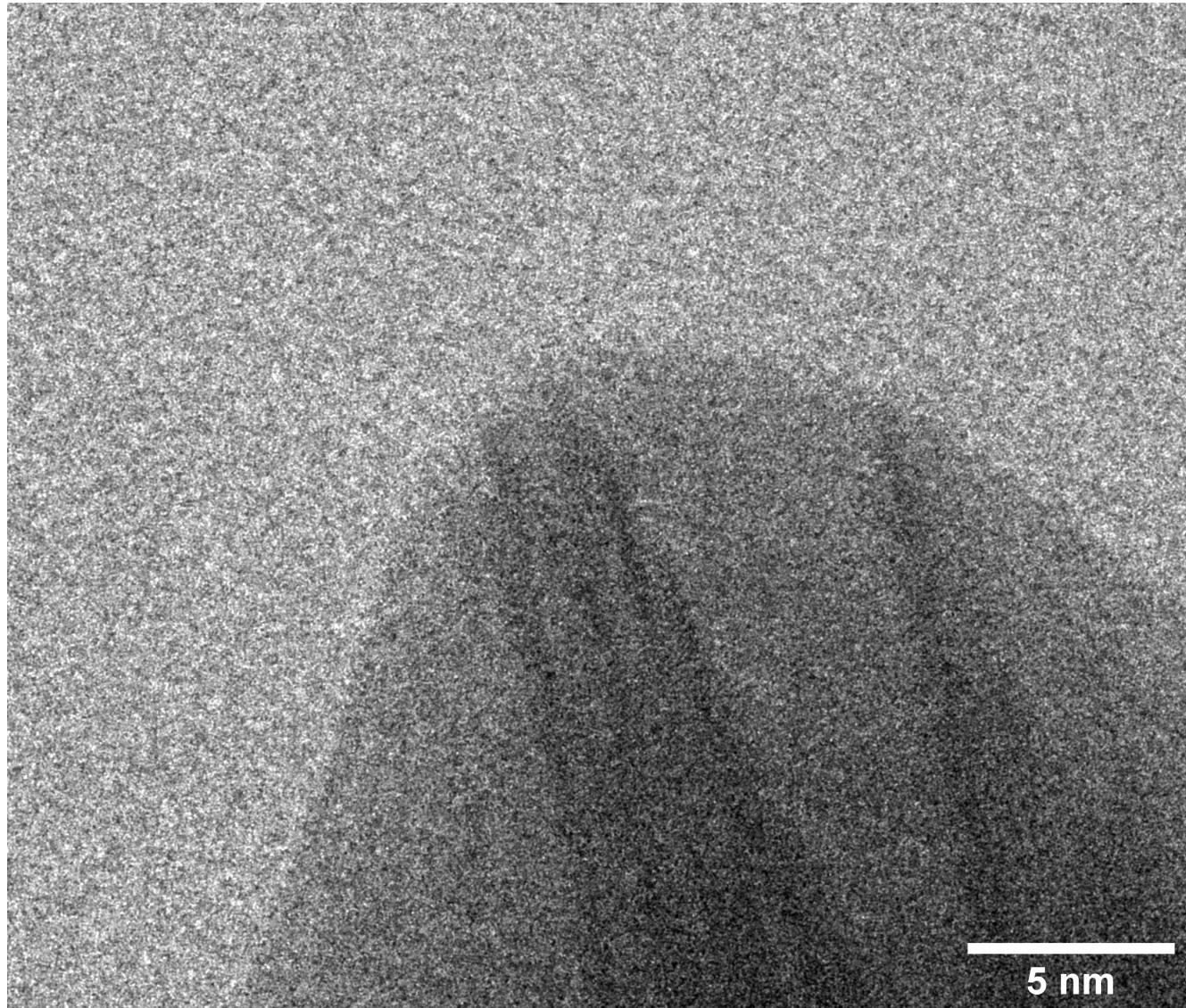


Au nanoparticles formation from HAuCl<sub>3</sub> Solution  
Particles' spacing >1.5 nm is just a few water molecules  
200 kV Tecnai.

Lagana et al.  
Microelectronic Engineering 2017

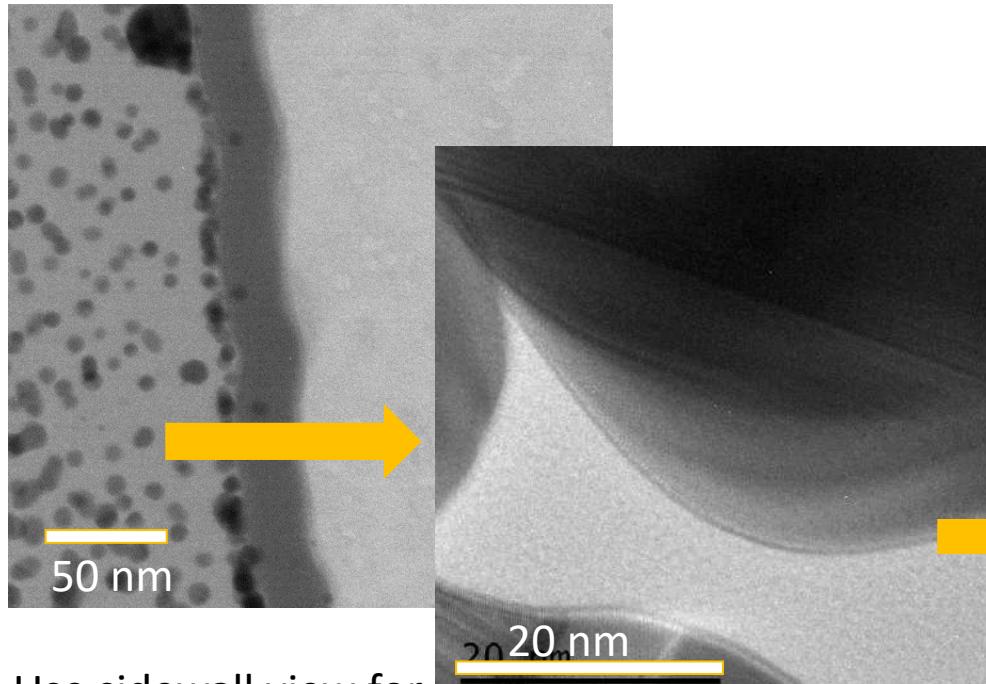
# Nanochannels: Atomic scale resolution + flow

0.5 mM HAuCl<sub>4</sub>  
60 nm liquid  
2x10 nm SiNx  
200 kV Tecnai T20  
G2 at 1Mx mag.



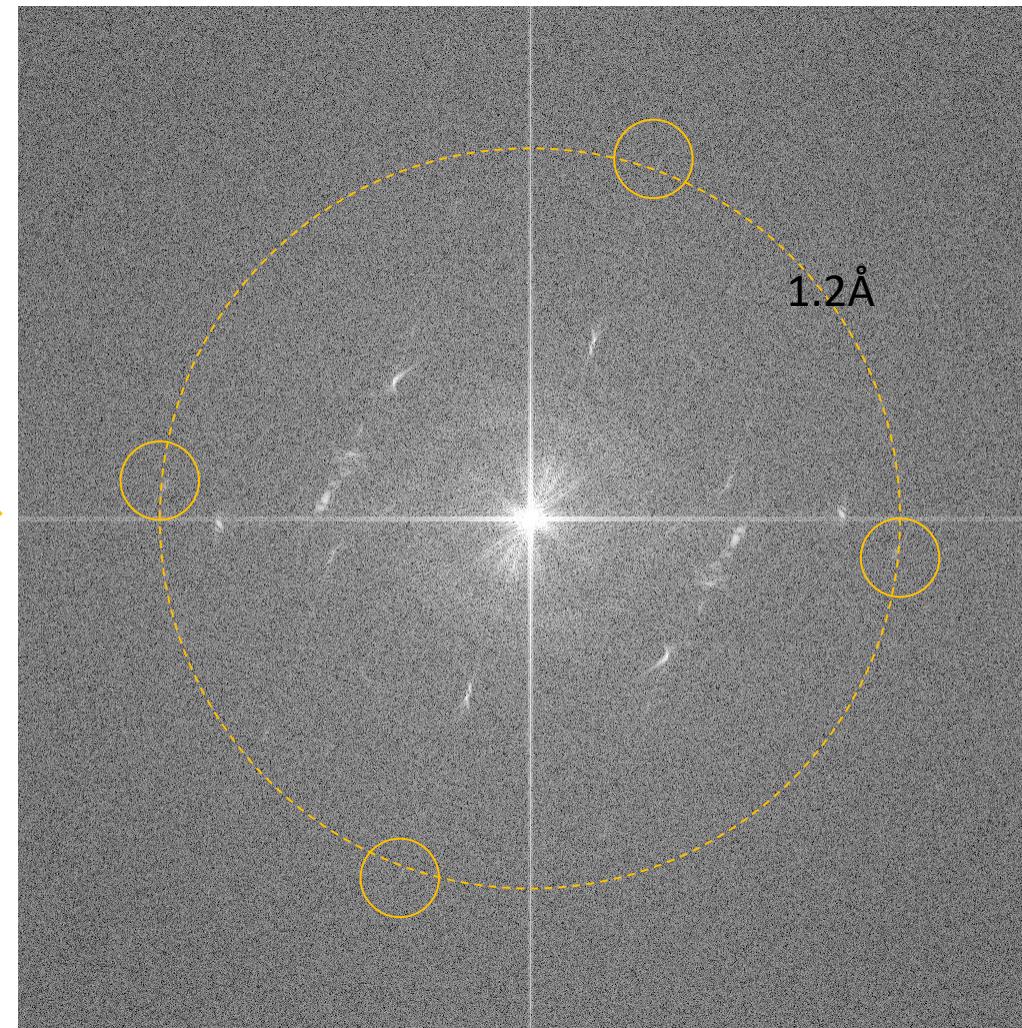
- Well defined thin liquid for high resolution
- Flow & Easy sample injection
- Mixing in field of view

# Au at 1.2 Å resolution



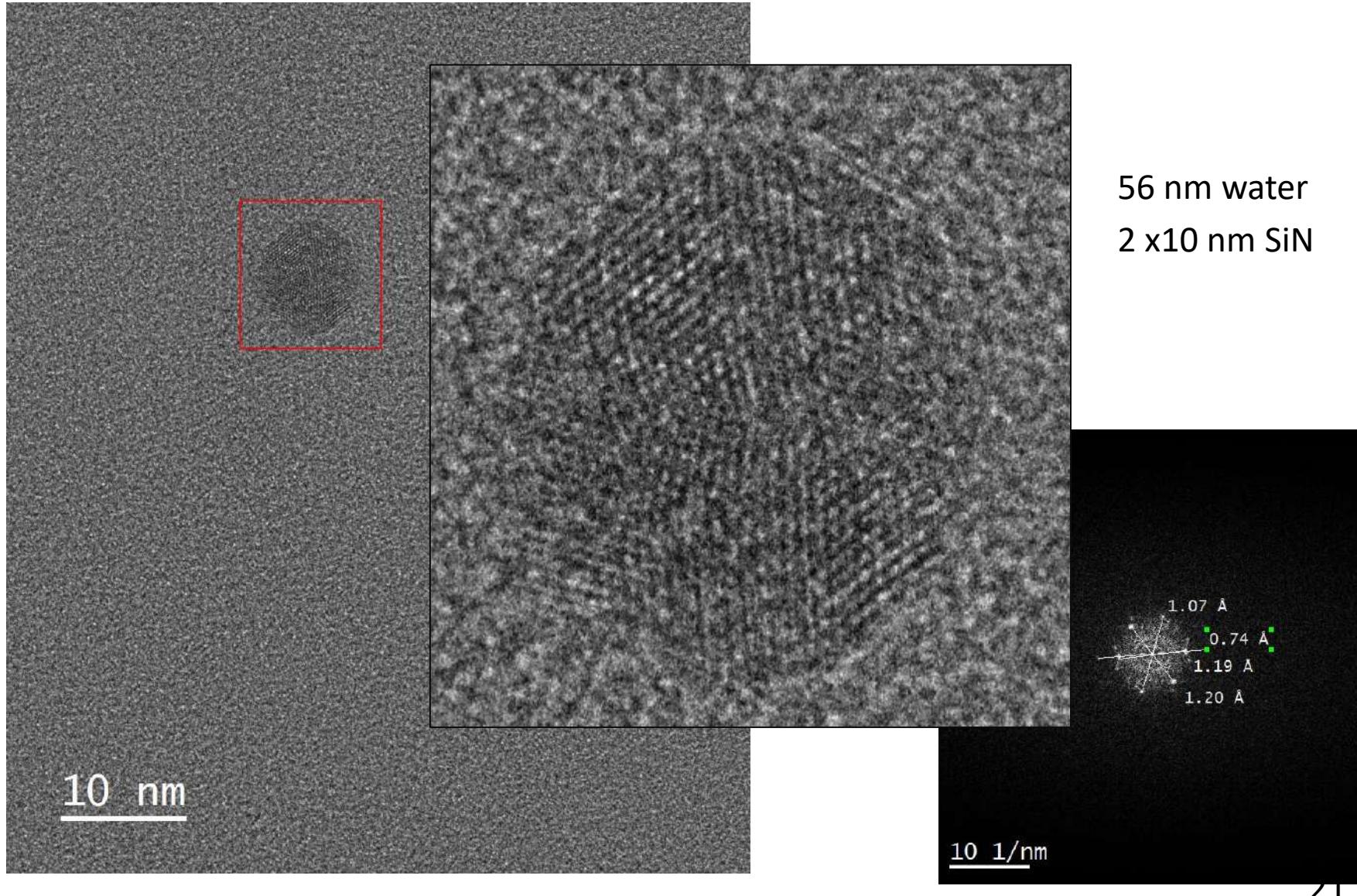
Use sidewall view for  
3<sup>rd</sup> dimension

10 nm SiN, 60 nm liquid, EBID of Au



David Mücke  
Central Facility of Materials Science Electron Microscopy,  
Universität Ulm, Germany

# 5 nm Au Nanoparticle



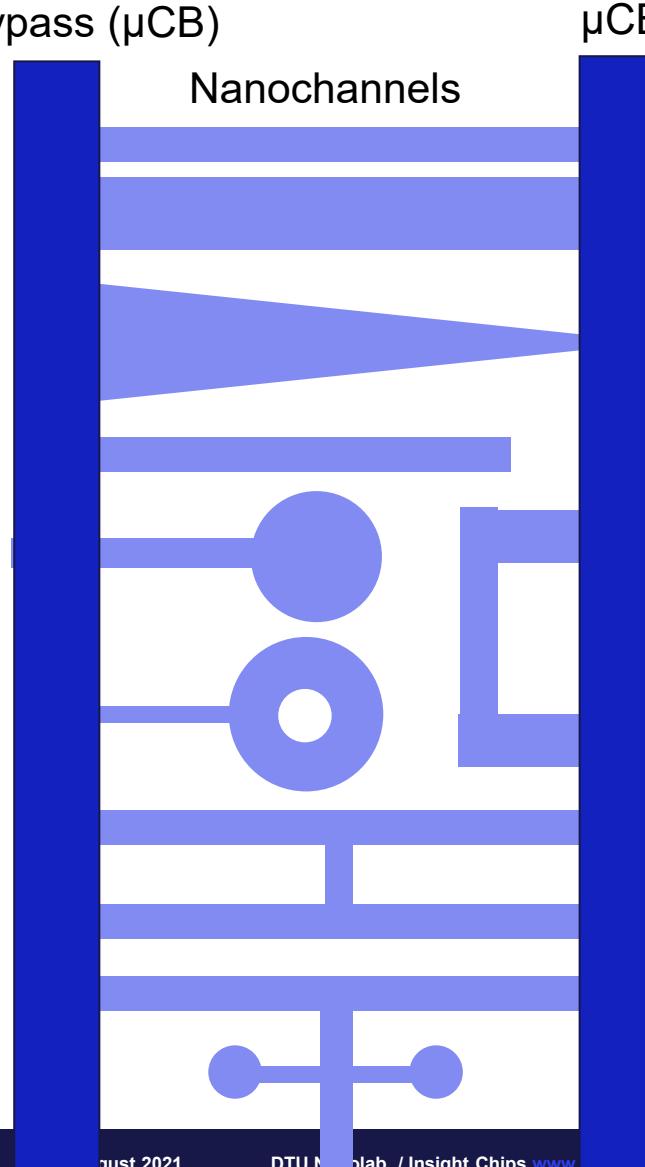
By Sofie Tidemand  
TEM @ TOPSOE with  
Stig Helveg

# Nanochannel TEM flow geometries - Overview

Microchannel

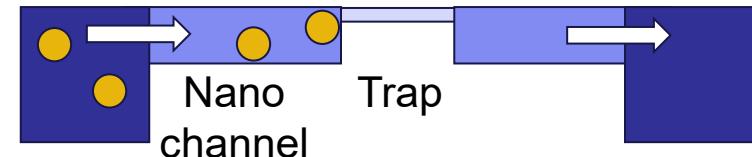
Bypass ( $\mu$ CB)

$\mu$ CB



- **Parallel Channels:** Reproduce experiments, flush samples in
- **Conical channels:** New levels of diffusiophoretic control, and a gradient of flow velocity in the channel
- **Dead end channels:** No flow. Filling as “sqrt Dt” with new species flown by. Capillary pressure dissolve trapped bubbles (slowly)..
- **Reservoirs:** color is a pressure indicator. Can trap particle and show reactions with no flow as different reagents flow in  $\mu$ Ch.
- **U-channels:** A fraction of the  $\mu$ -channel flow rate.
- **H and T-channels and mixers:** combine reagents in a mixing stream.
- **Droplet generation**

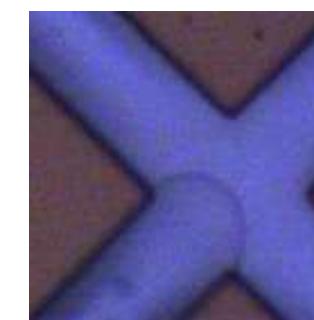
Side View of trap chip channels



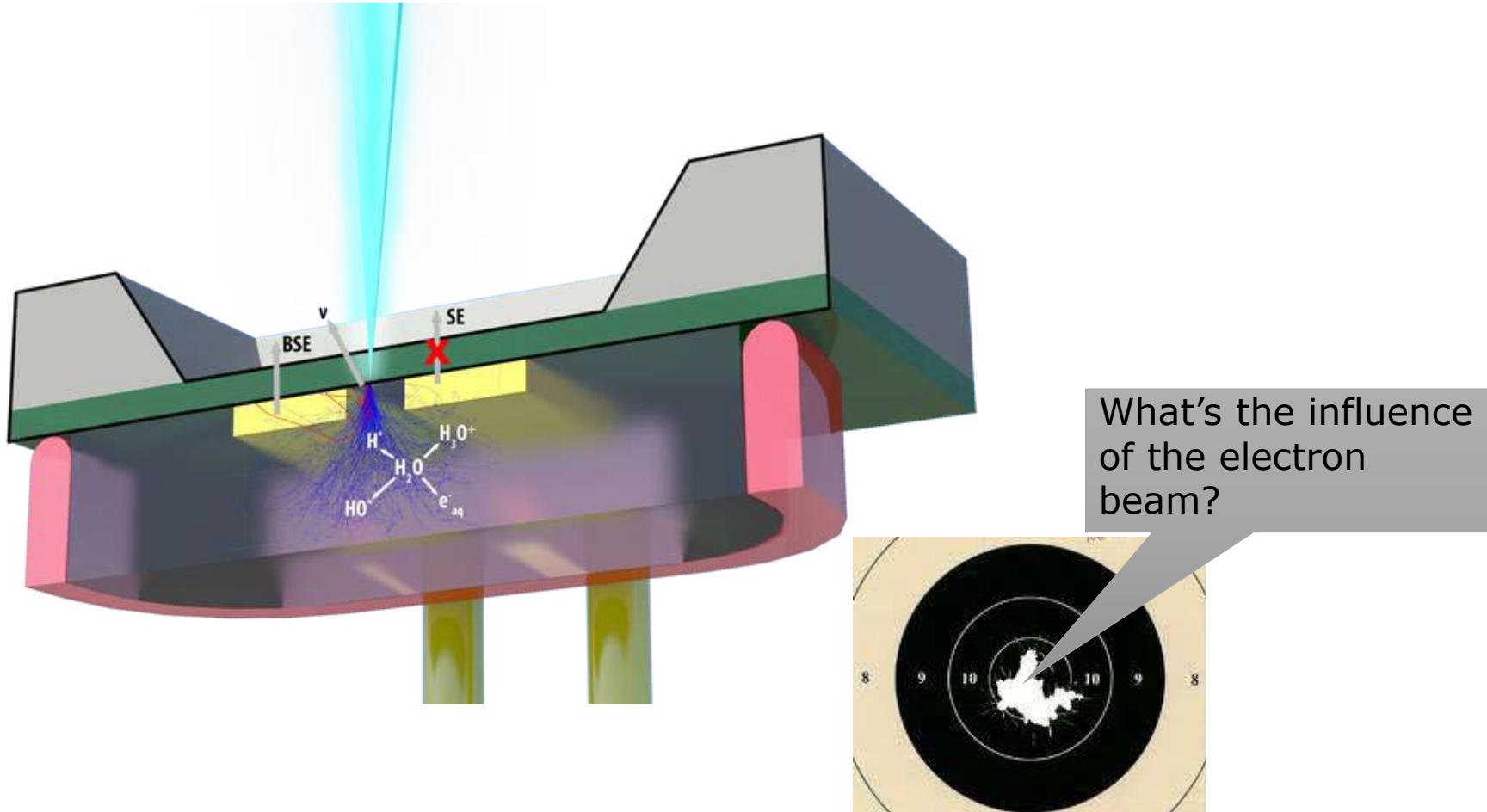
Micro  
channel

## Traps for

- Regions with higher resolution due to thinner liquid.
- Filter to trap many particles for population statistics
- Single particles for catalytic studies



# In-situ Electrochemistry: A crossed beam & current experiment??



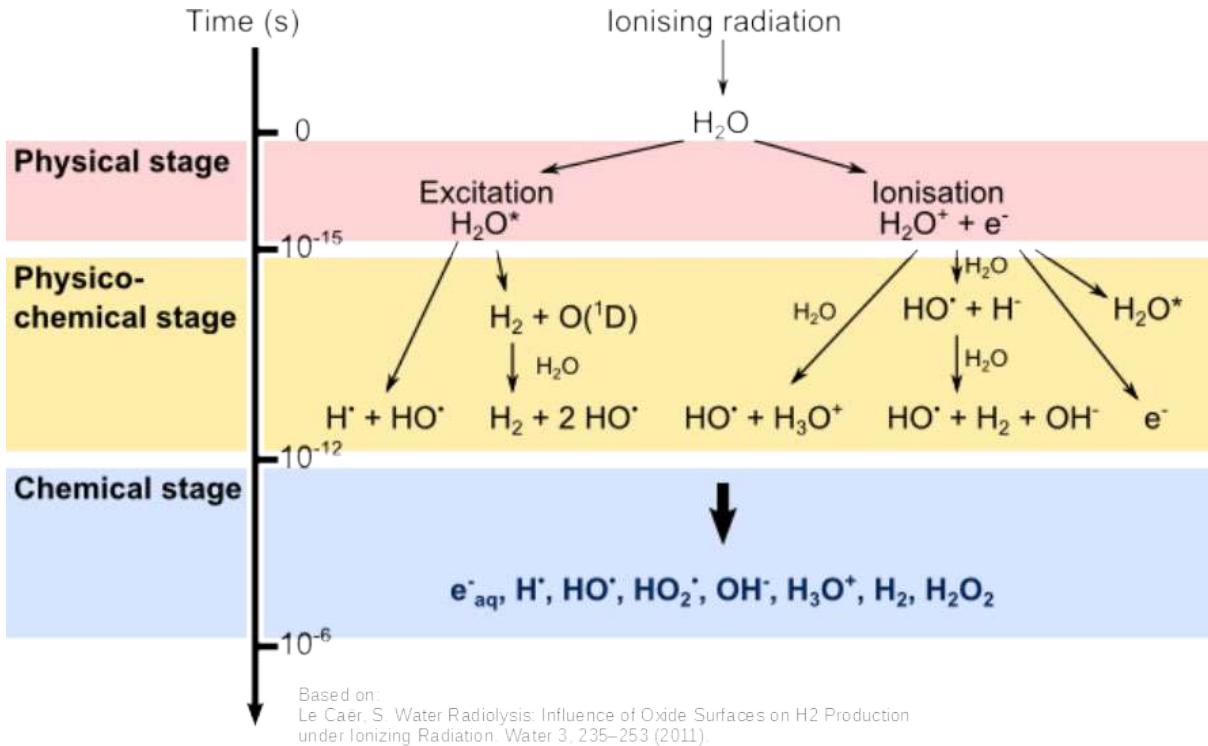
# Don't cross the beams!



Ghostbuster, 1984

# The complexity of radiolysis

#	Reaction	Rate constant (dm <sup>3</sup> mol <sup>-1</sup> s <sup>-1</sup> ) <sup>a</sup>
Acid-base reactions associated with pK <sub>a</sub> 's		
8	H <sup>+</sup> + OH <sup>-</sup> → H <sub>2</sub> O	1.4 × 10 <sup>-11</sup>
9	H <sub>2</sub> O → H <sup>+</sup> + OH <sup>-</sup>	$k_2 \times K_2 / [H_2O]$ <sup>b</sup>
10	H <sup>+</sup> + O <sub>2</sub> <sup>-</sup> → HO <sup>·</sup> + H <sup>+</sup>	$k_1 \times K_1 / [H_2O]$ <sup>b</sup>
11	H <sup>+</sup> + HO <sup>·</sup> → H <sub>2</sub> O + H <sup>+</sup>	2.3 × 10 <sup>-10</sup>
12	HO <sup>·</sup> + OH <sup>-</sup> → HO <sub>2</sub> <sup>·</sup> + H <sub>2</sub> O	1.3 × 10 <sup>-10</sup>
13	HO <sub>2</sub> <sup>·</sup> + H <sub>2</sub> O → H <sub>2</sub> O <sub>2</sub> <sup>·</sup> + OH <sup>-</sup>	$k_2 \times K_2 / K_3 \times [H_2O]$
14	e <sub>n</sub> + H <sub>2</sub> O → H + OH <sup>-</sup>	1.9 × 10 <sup>-61</sup>
15	H + OH <sup>-</sup> → e <sub>n</sub> + H <sub>2</sub> O	2.2 × 10 <sup>-63</sup>
16	H <sup>+</sup> → e <sub>n</sub> <sup>+</sup>	2.3 × 10 <sup>-59</sup>
17	e <sub>n</sub> + H <sup>+</sup> → H <sup>+</sup>	1.0 × 10 <sup>-10</sup>
18	OH + OH <sup>-</sup> → H <sub>2</sub> O	4.0 × 10 <sup>-10</sup>
19	O <sup>·</sup> + H <sub>2</sub> O → OH + OH	$k_3 \times K_3 / K_2 \times [H_2O]$
20	OH <sup>-</sup> → O <sup>·</sup> + H <sup>+</sup>	$k_2 \times K_2 / K_3$
21	O <sup>·</sup> + H <sup>+</sup> → OH + OH <sup>-</sup>	1.0 × 10 <sup>-11</sup>
22	HO <sub>2</sub> <sup>·</sup> → O <sub>2</sub> <sup>·</sup> + H <sup>+</sup>	$k_2 \times K_2 / K_3$
23	O <sub>2</sub> <sup>·</sup> + H <sup>+</sup> → HO <sub>2</sub> <sup>·</sup>	5.0 × 10 <sup>-10</sup>
24	HO <sub>2</sub> <sup>·</sup> + OH <sup>-</sup> → O <sub>2</sub> <sup>·</sup> + H <sub>2</sub> O	5.0 × 10 <sup>-10</sup>
25	O <sub>2</sub> <sup>·</sup> + H <sub>2</sub> O → HO <sub>2</sub> <sup>·</sup> + OH <sup>-</sup>	$k_2 \times K_2 / K_3 \times [H_2O]$
Chemical reactions		
26	e <sub>n</sub> + OH → OH <sup>-</sup>	3.0 × 10 <sup>-60</sup>
27	e <sub>n</sub> + H <sub>2</sub> O <sub>2</sub> → OH <sup>-</sup> + OH <sup>-</sup>	1.1 × 10 <sup>-60</sup>
28	e <sub>n</sub> + H <sub>2</sub> O → OH <sup>-</sup> + OH <sup>-</sup>	1.3 × 10 <sup>-60</sup> / [H <sub>2</sub> O] <sup>c</sup>
29	e <sub>n</sub> + H <sub>2</sub> O <sub>2</sub> → OH <sup>-</sup>	2.0 × 10 <sup>-60</sup>
30	e <sub>n</sub> + O <sup>·</sup> → O <sub>2</sub> <sup>·</sup>	1.9 × 10 <sup>-60</sup>
31	O <sup>·</sup> + e <sub>n</sub> + 2H <sub>2</sub> O → H <sub>2</sub> + OH <sup>-</sup> + OH <sup>-</sup>	5.5 × 10 <sup>-60</sup> / [H <sub>2</sub> O] <sup>d</sup>
32	e <sub>n</sub> + H <sub>2</sub> O <sub>2</sub> → H <sub>2</sub> + OH <sup>-</sup>	2.5 × 10 <sup>-60</sup> / [H <sub>2</sub> O] <sup>e</sup>
33	e <sub>n</sub> + HO <sup>·</sup> → O <sup>·</sup> + OH <sup>-</sup>	3.5 × 10 <sup>-60</sup>
34	e <sub>n</sub> + O <sup>·</sup> + H <sub>2</sub> O → OH <sup>-</sup> + OH <sup>-</sup>	2.2 × 10 <sup>-60</sup> / [H <sub>2</sub> O] <sup>f</sup>
35	e <sub>n</sub> + O <sup>·</sup> + H <sub>2</sub> O → OH <sup>-</sup> + OH <sup>-</sup>	1.8 × 10 <sup>-60</sup> / [H <sub>2</sub> O] <sup>g</sup>
36	e <sub>n</sub> + O <sup>·</sup> → OH <sup>-</sup>	3.6 × 10 <sup>-60</sup>
37	H + H <sub>2</sub> O → H <sub>2</sub> + OH	1.1 × 10 <sup>-60</sup>
38	H + O <sup>·</sup> → OH <sup>-</sup>	1.0 × 10 <sup>-60</sup>
39	H + HO <sub>2</sub> <sup>·</sup> → OH <sup>-</sup> + OH <sup>-</sup>	9.0 × 10 <sup>-67</sup>
40	H + O <sub>2</sub> <sup>·</sup> → OH <sup>-</sup> + O <sub>2</sub>	1.0 × 10 <sup>-68</sup>
41	H → H <sub>2</sub>	7.7 × 10 <sup>-68</sup>
42	H + OH → H <sub>2</sub> O	7.0 × 10 <sup>-69</sup>
43	H + H <sub>2</sub> O <sub>2</sub> → OH <sup>-</sup> + H <sub>2</sub> O	9.0 × 10 <sup>-69</sup>
44	H + O <sub>2</sub> <sup>·</sup> → H <sub>2</sub> O <sub>2</sub>	2.1 × 10 <sup>-69</sup>
45	H + HO <sub>2</sub> <sup>·</sup> → H <sub>2</sub> O <sub>2</sub>	1.8 × 10 <sup>-69</sup>
46	H + O <sub>2</sub> <sup>·</sup> → OH <sub>2</sub> <sup>·</sup>	1.8 × 10 <sup>-69</sup>
47	H + O <sub>3</sub> → HO <sub>2</sub> <sup>·</sup>	3.8 × 10 <sup>-69</sup>
48	OH + OH → H <sub>2</sub> O <sub>2</sub>	3.6 × 10 <sup>-69</sup>
50	OH + HO <sub>2</sub> <sup>·</sup> → OH <sub>2</sub> <sup>·</sup> + O <sub>2</sub>	6.0 × 10 <sup>-69</sup>
51	OH + O <sub>2</sub> <sup>·</sup> → OH <sub>2</sub> <sup>·</sup> + O <sub>2</sub>	8.2 × 10 <sup>-69</sup>
52	OH + H <sub>2</sub> → OH <sup>-</sup> + H <sub>2</sub> O	4.3 × 10 <sup>-69</sup>
53	OH + H <sub>2</sub> O <sub>2</sub> → HO <sub>2</sub> <sup>·</sup> + H <sub>2</sub> O	2.7 × 10 <sup>-69</sup>
54	OH + O <sup>·</sup> → OH <sub>2</sub> <sup>·</sup>	2.5 × 10 <sup>-69</sup>
56	OH + HO <sub>2</sub> <sup>·</sup> → OH <sub>2</sub> <sup>·</sup> + OH <sup>-</sup>	7.5 × 10 <sup>-69</sup>
57	OH + O <sub>2</sub> <sup>·</sup> → OH <sub>2</sub> <sup>·</sup> + OH <sup>-</sup>	2.55 × 10 <sup>-69</sup>
58	OH + O <sub>3</sub> → OH <sub>2</sub> <sup>·</sup> + O <sub>2</sub> <sup>·</sup> + H <sup>+</sup>	5.92 × 10 <sup>-69</sup>
59	HO <sub>2</sub> <sup>·</sup> + O <sub>2</sub> → HO <sub>2</sub> <sup>·</sup> + O <sub>2</sub>	8.0 × 10 <sup>-69</sup>
60	HO <sub>2</sub> <sup>·</sup> + HO <sub>2</sub> <sup>·</sup> → H <sub>2</sub> O <sub>2</sub> + O <sub>2</sub>	7.0 × 10 <sup>-69</sup>
61	HO <sub>2</sub> <sup>·</sup> + O <sup>·</sup> → O <sub>2</sub> <sup>·</sup> + OH <sup>-</sup>	6.0 × 10 <sup>-69</sup>
62	HO <sub>2</sub> <sup>·</sup> + H <sub>2</sub> O <sub>2</sub> → OH <sup>-</sup> + O <sub>2</sub> + H <sub>2</sub> O	5.0 × 10 <sup>-1</sup>
63	HO <sub>2</sub> <sup>·</sup> + HO <sub>2</sub> <sup>·</sup> → OH <sup>-</sup> + O <sub>2</sub> <sup>·</sup> + OH <sup>-</sup>	5.0 × 10 <sup>-1</sup>
64	HO <sub>2</sub> <sup>·</sup> + O <sub>2</sub> <sup>·</sup> → OH <sup>-</sup> + O <sub>2</sub> <sup>·</sup> + OH <sup>-</sup>	6.0 × 10 <sup>-1</sup>
65	HO <sub>2</sub> <sup>·</sup> + O <sub>3</sub> → OH <sup>-</sup> + O <sub>2</sub> <sup>·</sup>	5.0 × 10 <sup>-1</sup>
66	O <sup>·</sup> + O <sub>2</sub> <sup>·</sup> + 2H <sub>2</sub> O → HO <sub>2</sub> <sup>·</sup> + O <sub>2</sub> + 2OH <sup>-</sup>	1.0 × 10 <sup>-92</sup> / [H <sub>2</sub> O] <sup>d</sup>
67	O <sub>2</sub> <sup>·</sup> + O <sup>·</sup> + H <sub>2</sub> O → HO <sub>2</sub> <sup>·</sup> + OH <sup>-</sup> + OH <sup>-</sup>	6.0 × 10 <sup>-92</sup> / [H <sub>2</sub> O] <sup>e</sup>
68	O <sup>·</sup> + HO <sub>2</sub> <sup>·</sup> → OH <sup>-</sup> + O <sub>2</sub> <sup>·</sup> + OH <sup>-</sup>	1.3 × 10 <sup>-1</sup>
69	O <sup>·</sup> + HO <sub>2</sub> <sup>·</sup> → O <sup>·</sup> + O <sub>2</sub> <sup>·</sup> + OH <sup>-</sup>	1.3 × 10 <sup>-1</sup>
70	O <sup>·</sup> + O <sup>·</sup> + H <sub>2</sub> O → O <sub>2</sub> <sup>·</sup> + O <sub>2</sub> + 2OH <sup>-</sup>	1.0 × 10 <sup>-92</sup> / [H <sub>2</sub> O] <sup>f</sup>
71	O <sup>·</sup> + O <sup>·</sup> + O <sub>2</sub> → O <sub>2</sub> <sup>·</sup> + O <sub>2</sub>	1.3 × 10 <sup>-92</sup> / [H <sub>2</sub> O] <sup>g</sup>
72	O <sup>·</sup> + O <sub>2</sub> → HO <sub>2</sub> <sup>·</sup> + OH <sup>-</sup>	1.0 × 10 <sup>-92</sup> / [H <sub>2</sub> O] <sup>h</sup>
73	O <sup>·</sup> + O <sub>2</sub> → O <sub>2</sub> <sup>·</sup>	3.6 × 10 <sup>-98</sup>
74	O <sup>·</sup> + H <sub>2</sub> → OH <sup>-</sup>	8.0 × 10 <sup>-97</sup>
75	O <sup>·</sup> + H <sub>2</sub> O <sub>2</sub> → O <sub>2</sub> <sup>·</sup> + OH <sup>-</sup>	5.0 × 10 <sup>-97</sup>
76	O <sup>·</sup> + HO <sub>2</sub> <sup>·</sup> → O <sub>2</sub> <sup>·</sup> + OH <sup>-</sup>	4.0 × 10 <sup>-97</sup>
78	O <sup>·</sup> + O <sub>2</sub> <sup>·</sup> → O <sub>2</sub> <sup>·</sup>	7.0 × 10 <sup>-98</sup>
79	O <sup>·</sup> + O <sub>3</sub> → O <sub>2</sub> <sup>·</sup> + O <sub>2</sub>	5.0 × 10 <sup>-98</sup>
80	O <sup>·</sup> + O <sub>2</sub> <sup>·</sup> → O <sub>2</sub> <sup>·</sup>	3.0 × 10 <sup>-98</sup>
81	O <sup>·</sup> + H <sup>+</sup> → O <sup>·</sup> + OH	9.0 × 10 <sup>-98</sup>
82	HO <sub>2</sub> <sup>·</sup> → OH + OH	1.1 × 10 <sup>-98</sup>
83	H <sub>2</sub> O <sub>2</sub> <sup>·</sup> → OH + OH	> 4.4 × 10 <sup>-9</sup>
84	HO <sub>2</sub> <sup>·</sup> → O <sup>·</sup> + OH	> 1.0 × 10 <sup>-3</sup>

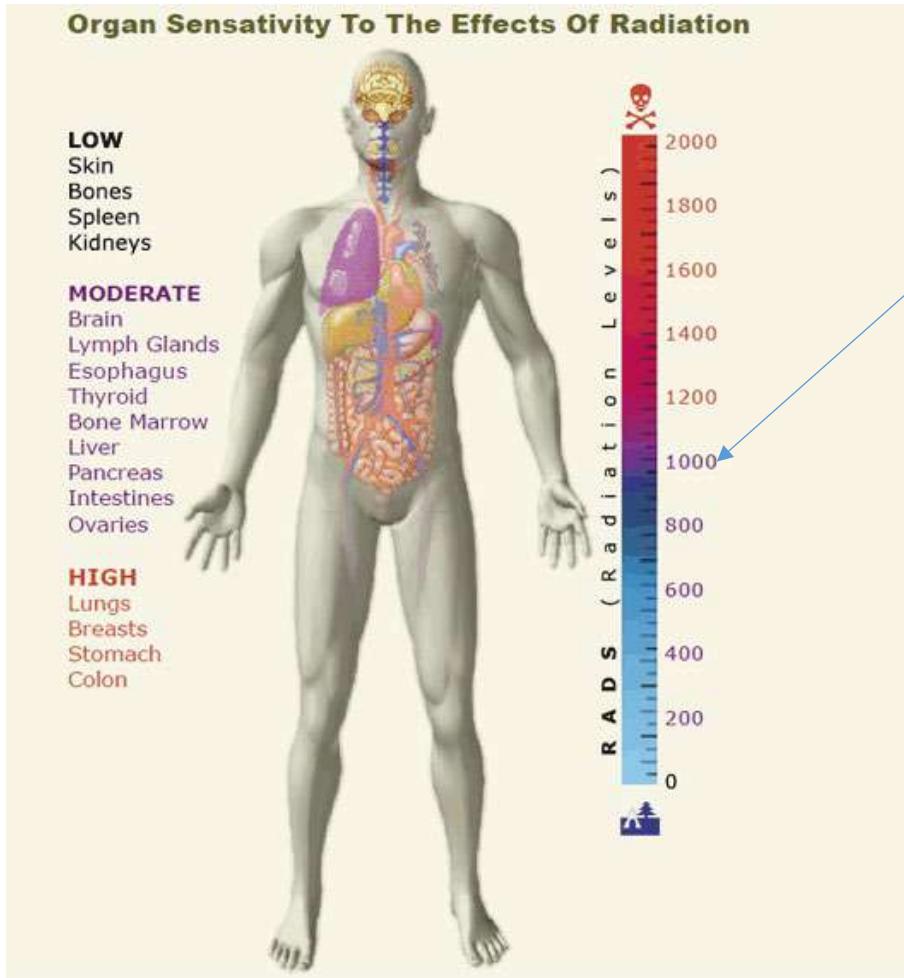


...80 coupled reactions

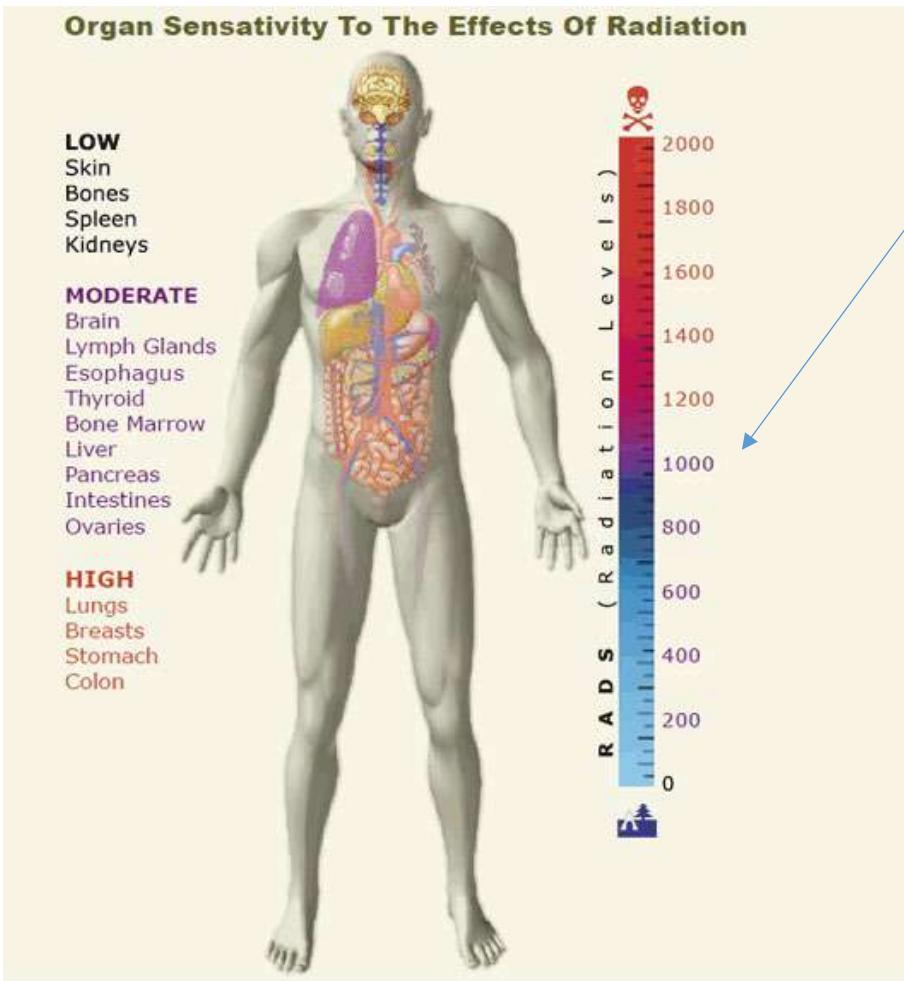
... equations to be solved in time and space

- over many orders of magnitude considering the time scale and length scales

# Radiation dose



# Radiation dose



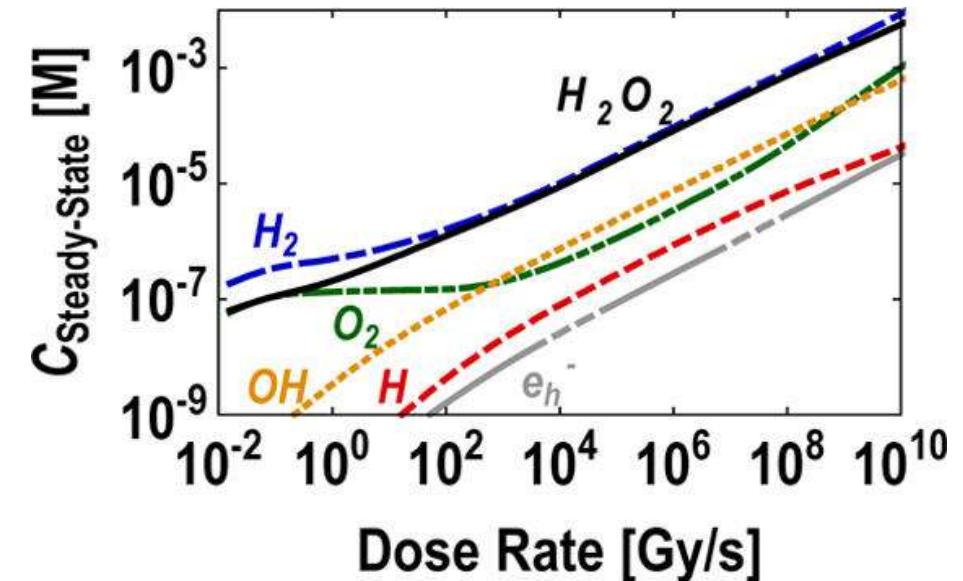
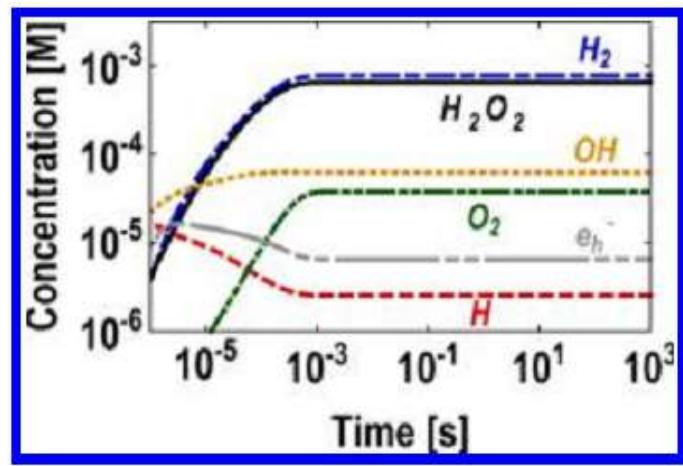
**TEM 2  $\mu\text{m}$  diameter beam of 1 nA at  
300 kV  $\rightarrow$  75 Giga Gy/sec**



# FEM Simulation study of Radiolytic Products

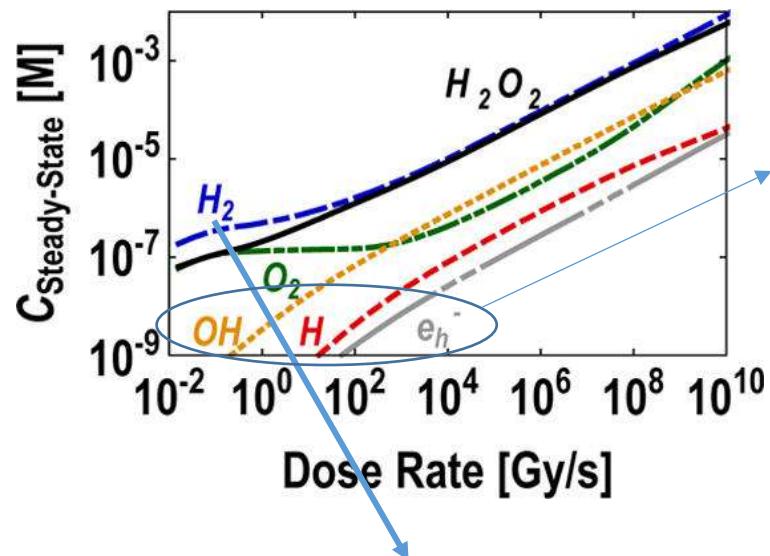
Schneider et al. *The Journal of Physical Chemistry C* 118.38 (2014): 22373-22382:

- Finite element simulation of ~80 coupled reactions in time and space
- Extrapolating reaction constants about  $10^7$  to TEM conditions



**Figure 1.** Concentrations of  $e_h^-$ ,  $H^\bullet$ ,  $H_2$ ,  $H_2O_2$ ,  $OH^\bullet$ , and  $O_2$  as functions of time. Initially neat, deareated water is irradiated continuously at a dose rate of  $7.5 \times 10^7$  (Gy/s).

# Radiolytic bubble formation



Bubble formation  
when saturated at  
around 1mM H<sub>2</sub>

Reducing and oxidative etching processes  
- all depending on beam conditions and position



**Why is it moving towards right?**

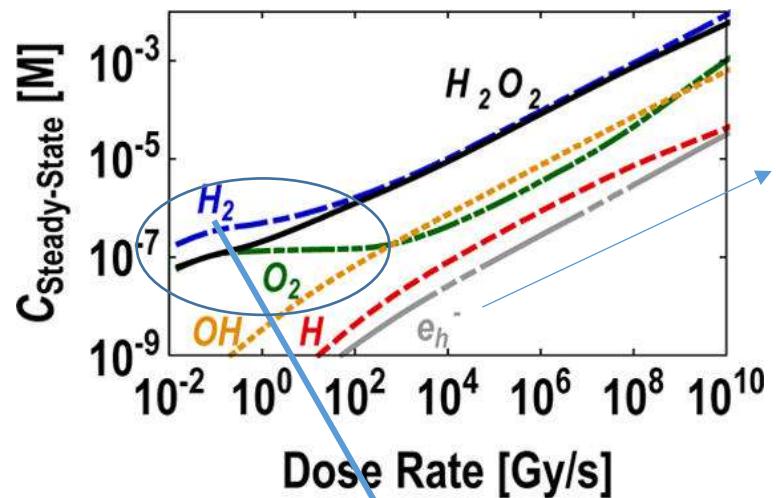
Movie 3 on

<http://pubs.acs.org.proxy.findit.dtu.dk/doi/suppl/10.1021/nl404169a>

*Nano Lett.*, 2014, 14 (1), pp 359–364

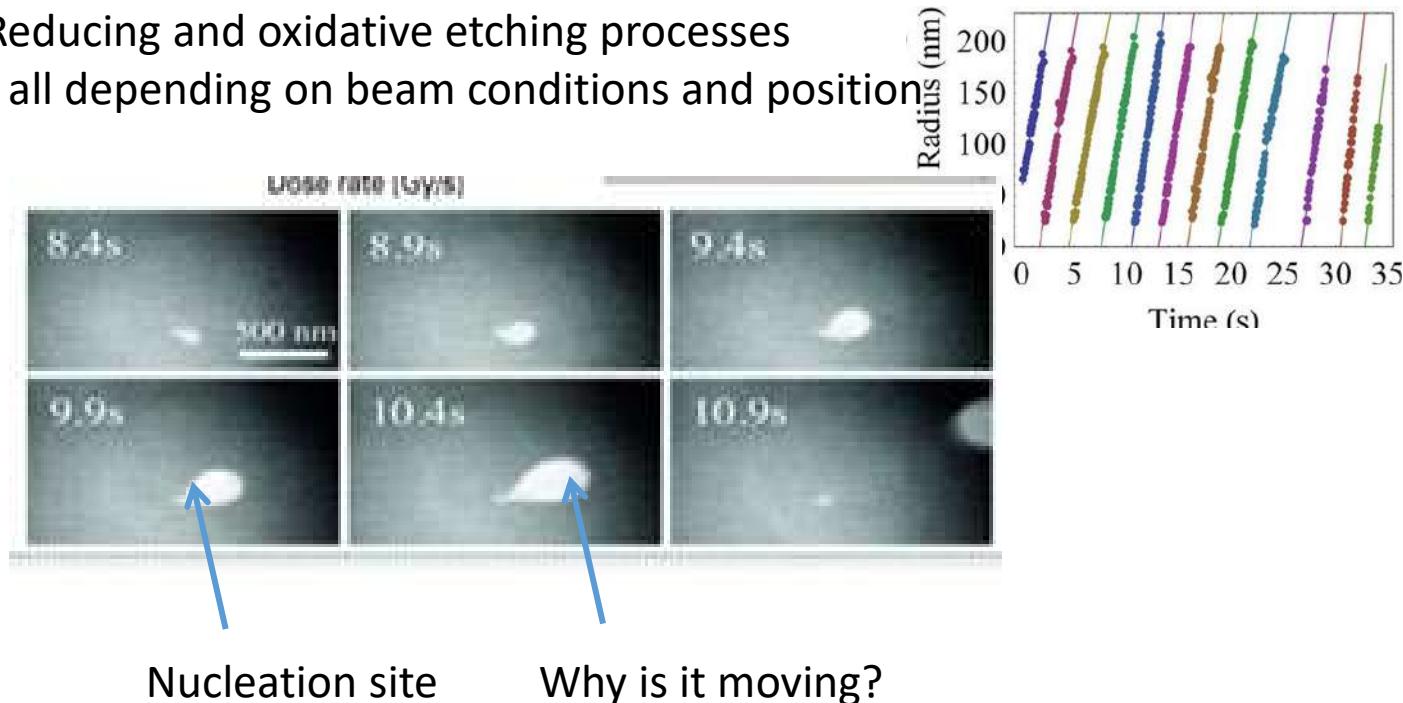
DOI: 10.1021/nl404169a

# Radiolytic bubble formation



Bubble formation  
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Reducing and oxidative etching processes  
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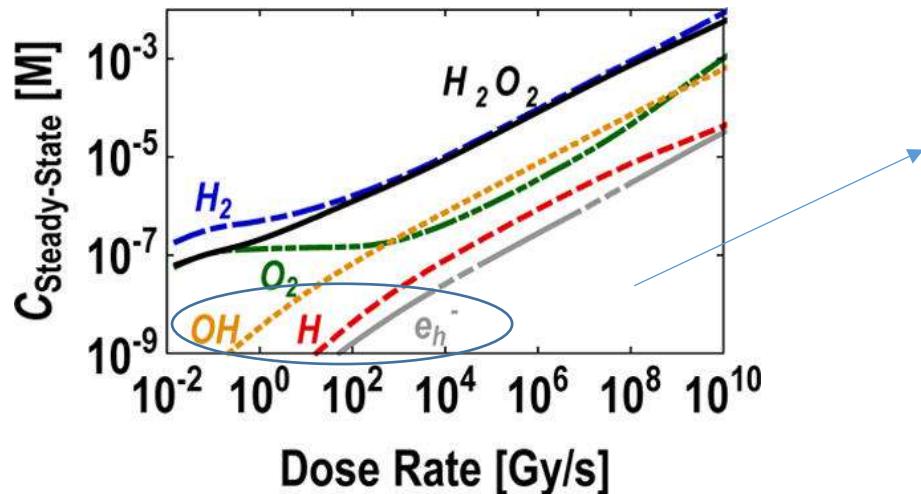
Moves with bulging membrane



Movie 3 on  
<http://pubs.acs.org.proxy.findit.dtu.dk/doi/suppl/10.1021/nl404169a>

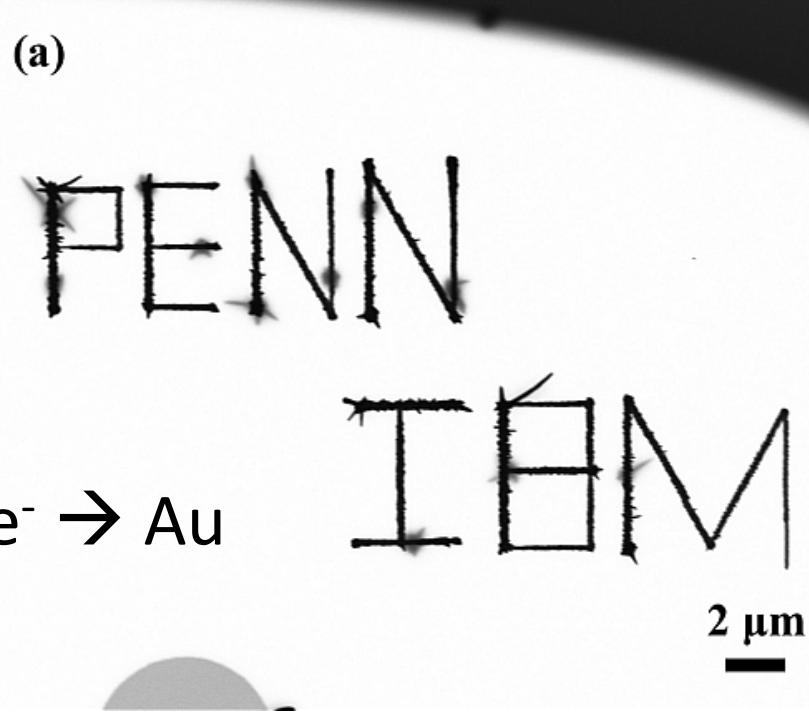
*Nano Lett.*, 2014, 14 (1), pp 359–364  
DOI: 10.1021/nl404169a

# Beam induced nucleation



Reducing and oxidative etching processes  
- all depending on beam conditions and position

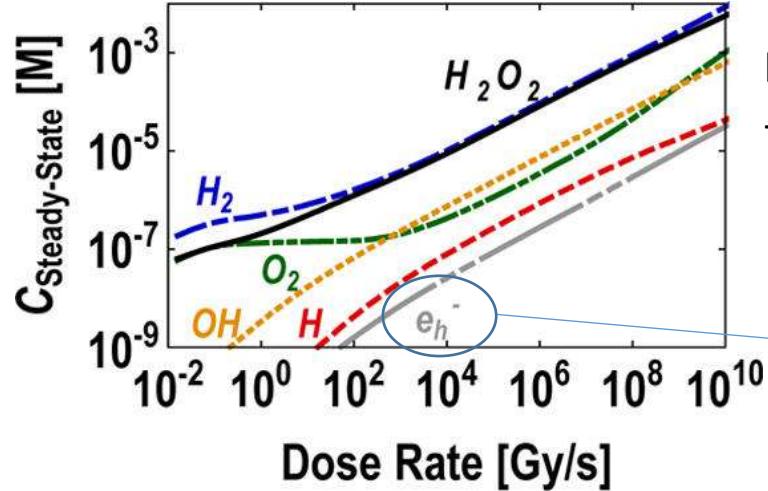
(a)



Gold structures written from a Aurochloric acid solution by moving the electron beam

Many papers use beam induced deposition...  
- Would you trust this?

# Radiolytic Radical chemistry



Reducing and oxidative etching processes  
- all depending on beam conditions and position

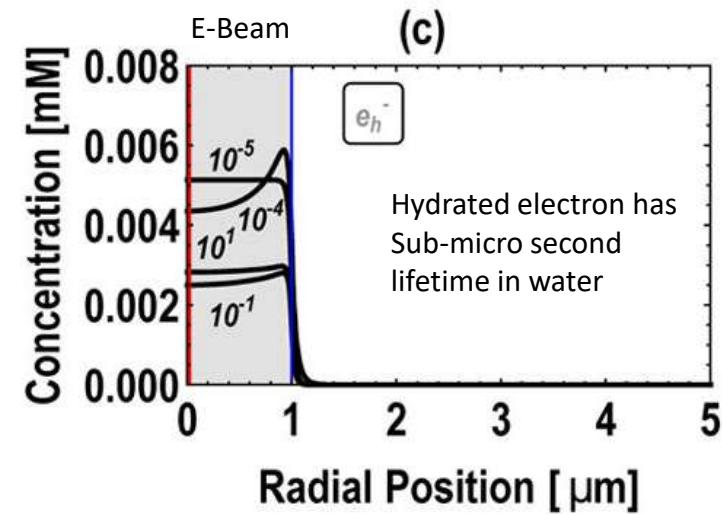
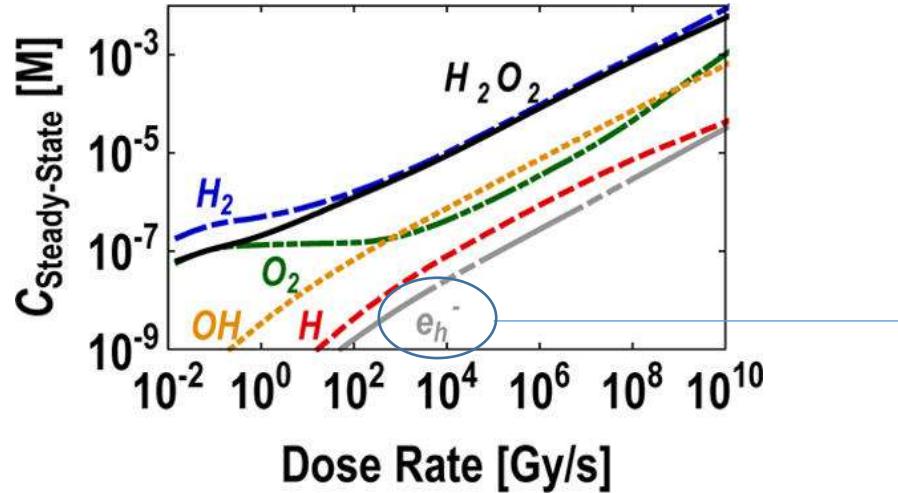
What causes beam induced deposition?

Hydrated electron has  
Sub-micro second  
lifetime in water

Can it diffuse out and  
make reactions all over?

How about  $\text{H}_2$  Hydrogen or Oxygen  
molecules  $\text{O}_2$ ?

# Radiolytic Radical chemistry



Different chemical conditions  
inside and outside the beam

# Radiolysis exercise

1. How reliable do you think LPTEM is to make conclusions about nanoscale processes?  
How would you make the observations most reliable?
2. How could the different liquid cell designs influence radiolysis if you consider
  1. Diffusion / Flow
  2. Liquid layer thickness
  3. Encapsulation thickness- And how would these factors influence image resolution?
3. Some say that deposition of Gold from gold ion solutions in regions micrometers away from the beam is due to hydrated electrons. Do you agree?
4. Search the net: Can you find the radiation dose rate near a nuclear bomb?

# How to make useful LPTEM observations - and not just image beam effects

1. Always compare to ex-situ experiments – optical, x-ray etc
2. Try comparing to Cryogenic TEM – it gives a snapshot of a process
3. Study process with beam off, and then only a glash occassinally to see the process
4. Work at very low dose rates ( $<1 \text{ e- pr } \text{\AA}^2 \text{ per second}$ ) with pulsed beams
5. Study you process as function of TEM dose rate and extrapolate to zero
6. Flush radicals away
7. Add radical scavengers to the solution
8. Use conductive encapsulation (graphene seems to help)

# Work with high energy electrons

- higher IMFP gives less deposited energy

Beer-Lambert law of exponential intensity decay of transmitted beam with distance

Probability of not scattering:

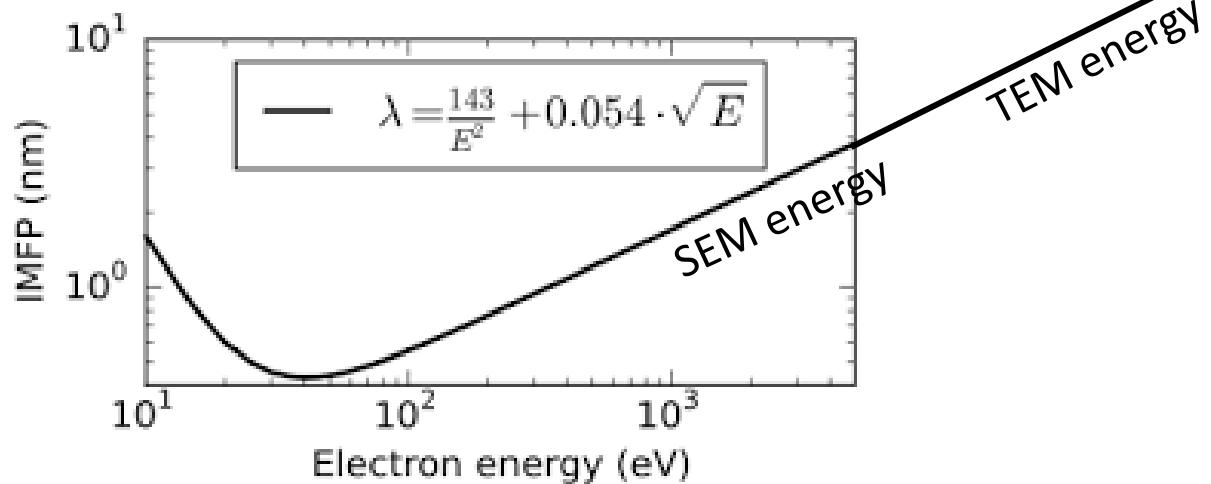
$$P(z, n = 0) = e^{-\frac{x}{\lambda_{IMFP}}}$$

Higher E longer IMFP

At 300 kV ca 200 nm...

Only  $\sim 2000$  atoms thick

IMFP Universal curve approximation



# LPTEM Literature - If you want to know more:

Science Review paper: **Opportunities and challenges in liquid cell electron microscopy**

<http://science.sciencemag.org.proxy.findit.dtu.dk/content/350/6267/aaa9886>

Book: **Liquid cell electron microscopy**

<https://www.cambridge.org/core/books/liquid-cell-electron-microscopy/A668214DAFA539E0682ADF8672FE8C6C>

More broad in-situ TEM electrical measurement review book chapter

[https://link-springer-com.proxy.findit.dtu.dk/chapter/10.1007/978-3-319-22988-1\\_10](https://link-springer-com.proxy.findit.dtu.dk/chapter/10.1007/978-3-319-22988-1_10)

Next session

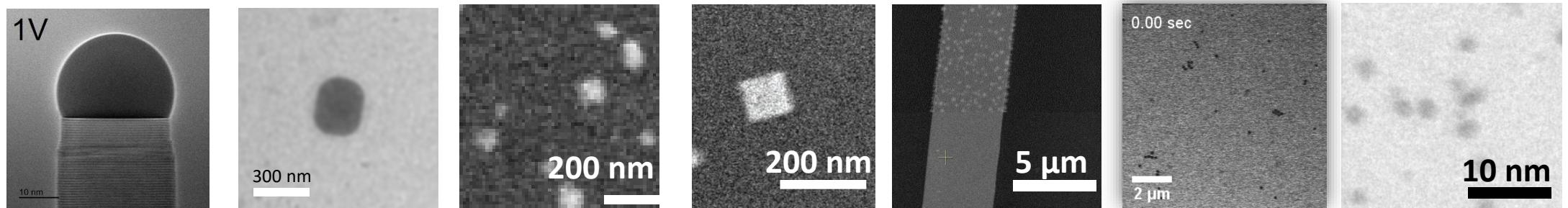
## **4. Introduction to Solution Synthesis Mechanisms - with a Direct View to the Processes**

## 4. Introduction to Solution Synthesis Mechanisms - with a Direct View to the Processes

Murat Nulati Yesibolati, Hongyu Sun & Kristian Mølhave

DTU Nanolab

Molecular  
Windows



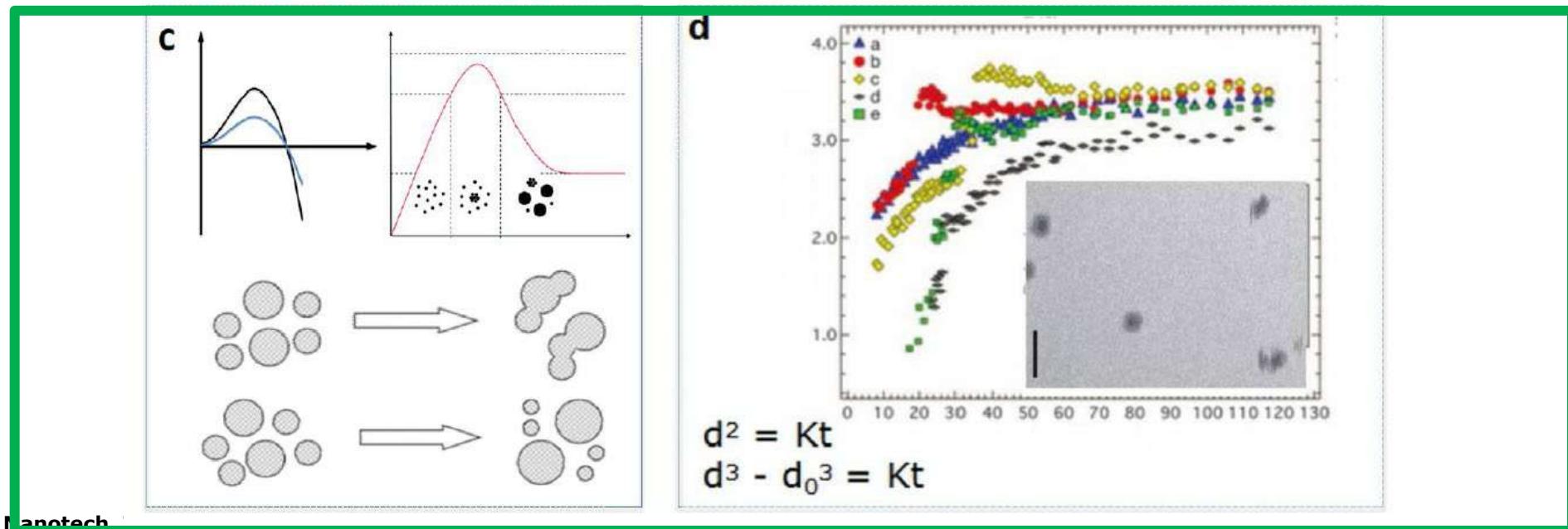
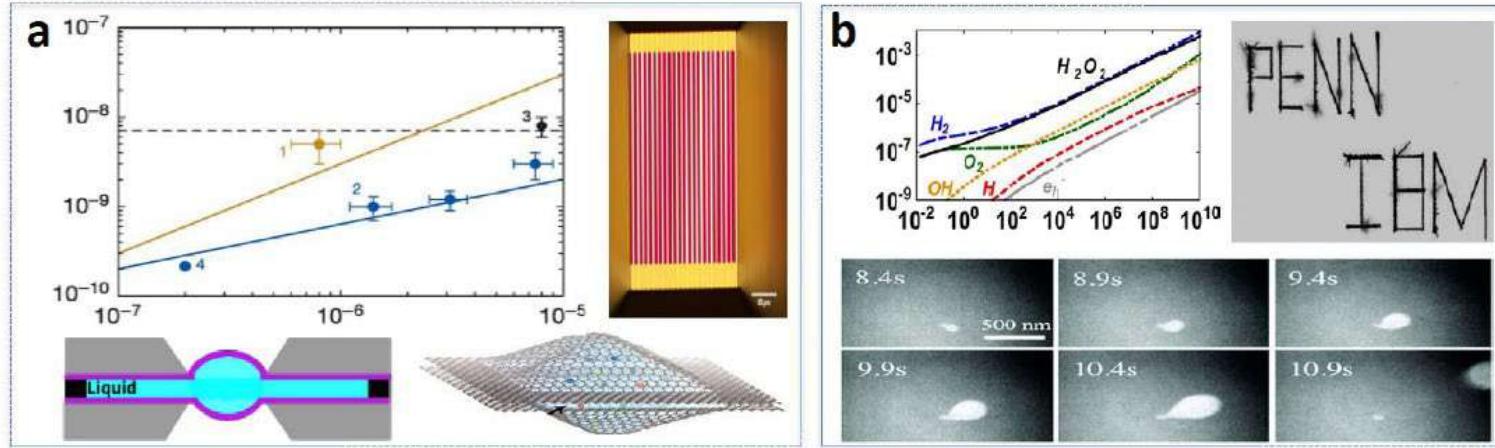
## Today's program

1. 8:00-8:45 : Nucleation processes
2. 9:00-9:45 : Growth processes
3. 9:45-11:15: Group presentations preparation
4. 11:15-12:00: Group presentations 3 x 15 minutes

# Reading Materials

- **Book: Physical Metallurgy** (Fourth, Revised and Enhanced Edition), CHAPTER 8: SOLIDIFICATION, Edited by. Robert W. Cahn,, Peter Haasen. © 1996 Elsevier Science B.V.  
**Standard text in physical metallurgy**
- **Book: Materials Science and Engineering: An Introduction**, 9th Edition William D. Callister, David G. Rethwisch © Wiley. **A good introductory textbook on materials science and engineering**
- **Review paper:** Nguyen T. K. Thanh, N. Maclean, and S. Mahiddine. Mechanisms of Nucleation and Growth of Nanoparticles in Solution. Chem. Rev., 2014, 114, 7610–7630 **A review paper on classical nucleation and growth, and analysis different materials system**
- **Review paper:** Younan Xia, Yujie Xiong, Byungkwon Lim, and Sara E. Skrabalak. Shape-Controlled Synthesis of Metal Nanocrystals: Simple Chemistry Meets Complex Physics? Angew. Chem. Int. Ed. 2009, 48, 60-103 **A comprehensive review of recent research activities on the controllable synthesis of metal nanocrystals**

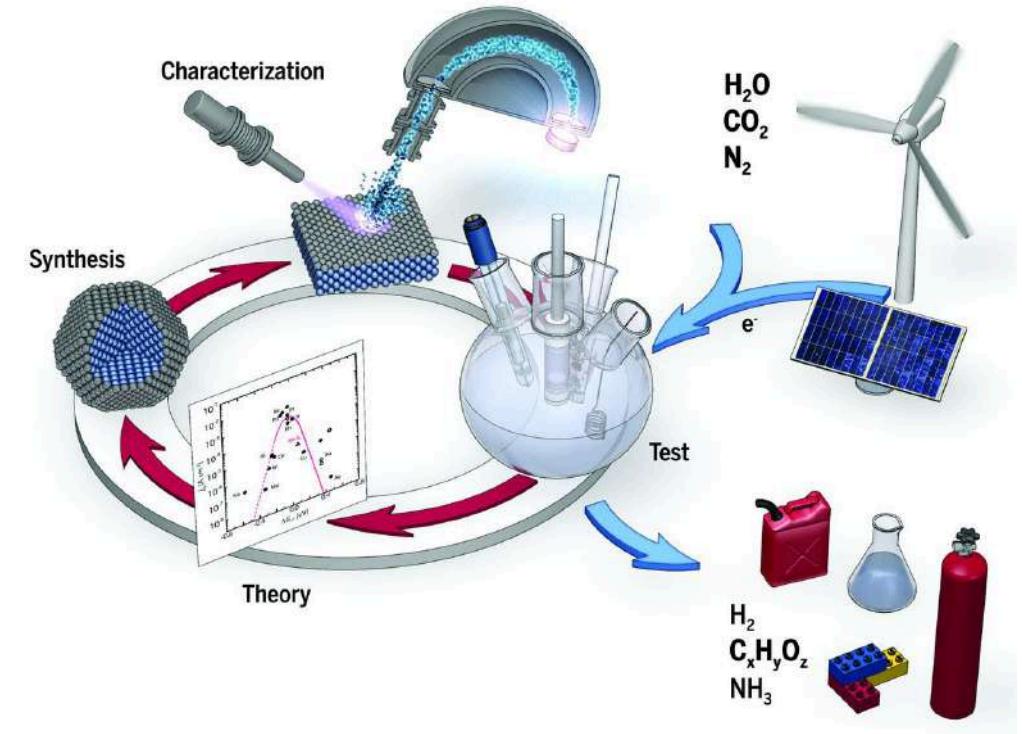
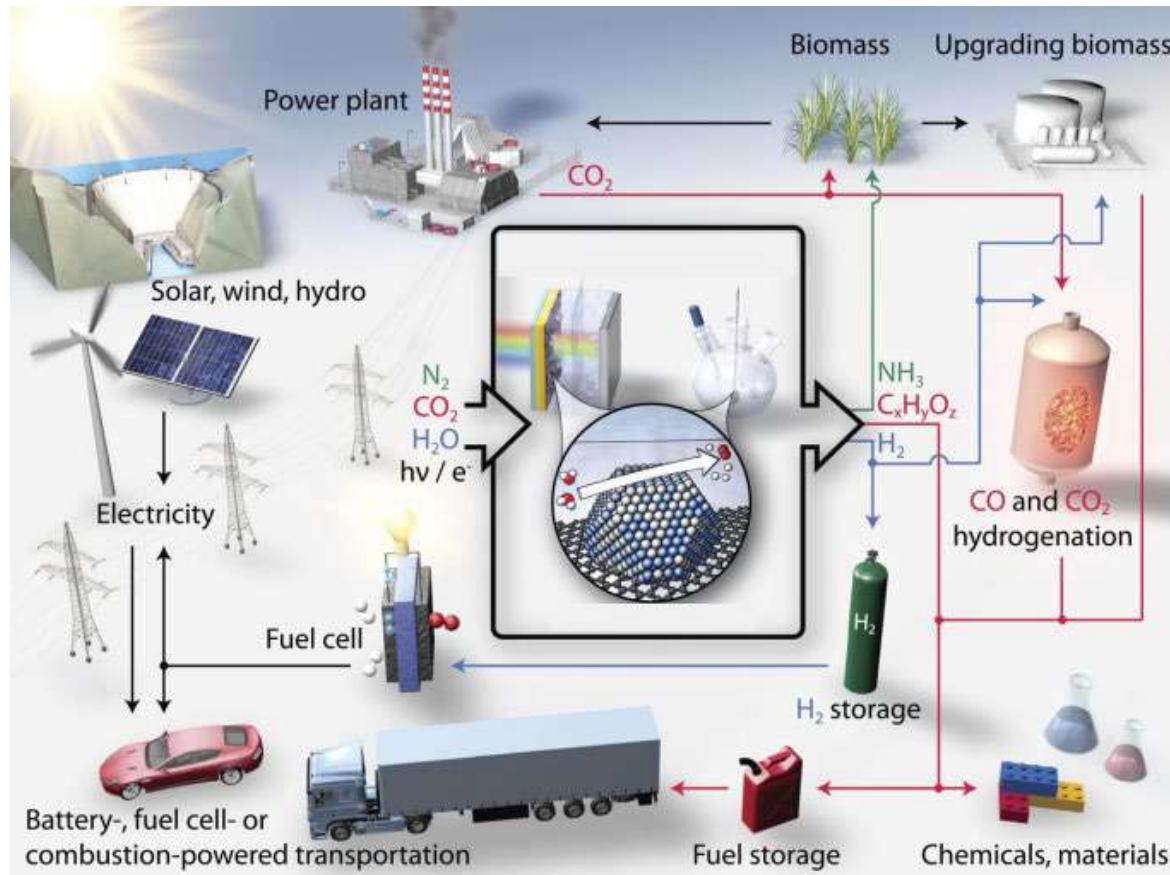
# What's in this case? (exam slide)



Today

## **After this lecture you should be able to:**

- Account for difference btw. homogeneous and heterogeneous nucleation
- Interpret growth models
- Discuss diffusion and growth measurements in LPTEM results



Combining theory and experiment in electrocatalysis: Insights into materials design, Science 2017, 355, eaad4998

# There are two synthesis strategies

- ❑ **Top Down:** Start with the bulk material and sculpt away material to make what you want
  - the “physical” way



- ❑ **Bottom Up:** Building what you want by assembling it from building blocks (atoms, molecules, etc)
  - The “chemical” way



# **Can you list some synthesis methods?**

## **pros and cons?**

# Can you list some synthesis methods?

## pros and cons?

### Physical routes:

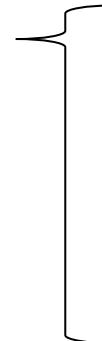
- High energy ball milling
- Lithography
- Vapor deposition (sputtering; electron beam; laser; vacuum arc)

### Biological routes:

- Biogenesis (microorganisms, bio-templates assisted)

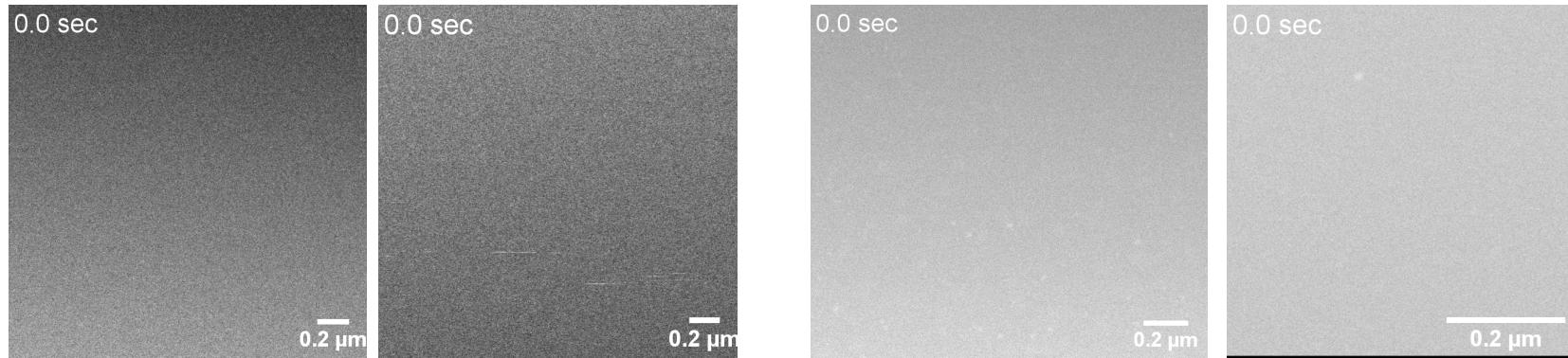
### Chemical routes:

- Sol-gel
- Microemulsion
- Hydrothermal/solvothermal
- CVD

- 
- Low cost in energy consumption (< 200 °C)
  - Low equipment costs (no high vacuum etc)
  - Easier scalability to larger volumes
  - Ease of handling in a variety of processes

**There are two process during synthesis:  
nucleation + growth**

**Can you give examples?**



Influence of Cetyltrimethylammonium Bromide on Gold Nanocrystal Formation Studied by In Situ Liquid Cell Scanning Transmission Electron Microscopy. K.Mølhave et al. J. Phys. Chem. C 2018, 122, 4, 2350–2357

# Nucleation processes

- Homogeneous nucleation – in the solution
- Heterogeneous nucleation – on an interface

Can you give examples?

# Examples

- Dew condenses on things – it doesn't rain down.
- Clouds are actually heterogeneous condensation on nanoparticles in the atmosphere
- Soda and beer bubbles form on the glass not in the middle of the liquid
- Freezing ice cubes start on the container not in the liquid



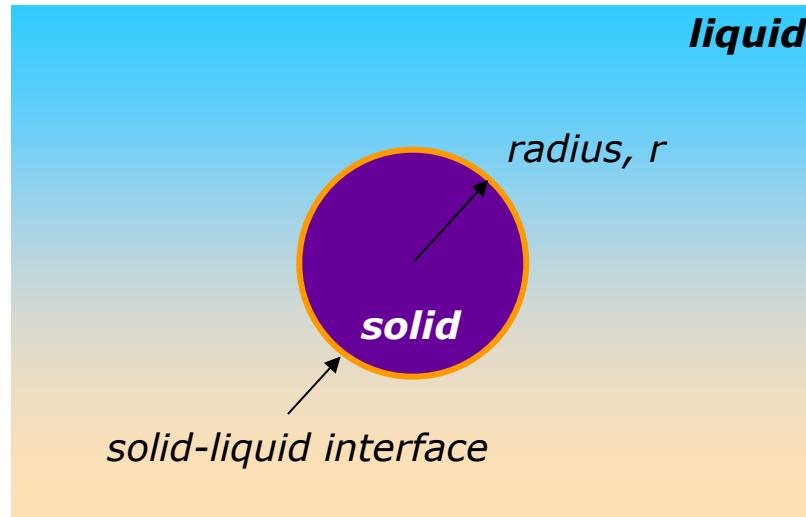
# Describe homogeneous nucleation

**The total Gibbs free energy change=**

**The volume free energy + surface energy**

$$\Delta G = V \cdot (-\Delta G_V) + A \cdot \gamma$$

↓      ↓      ↓      ↓  
volume    bulk free energy    surface area    surface energy

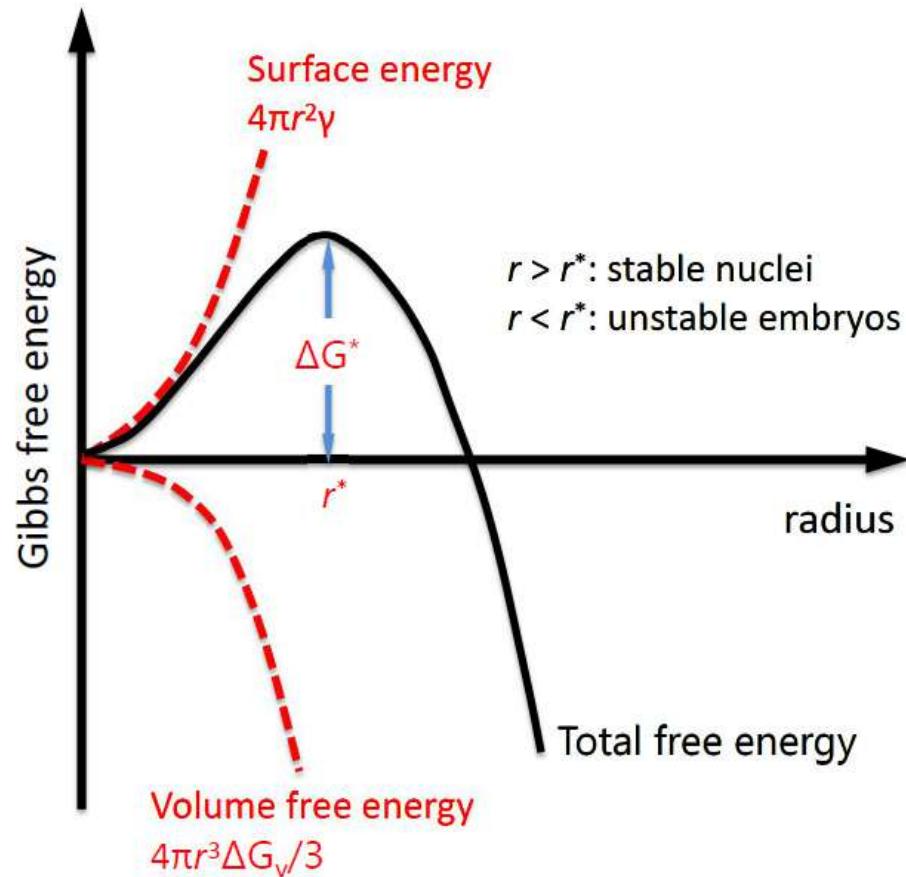


For simplicity, consider a spherical nucleus with a radius of  $r$

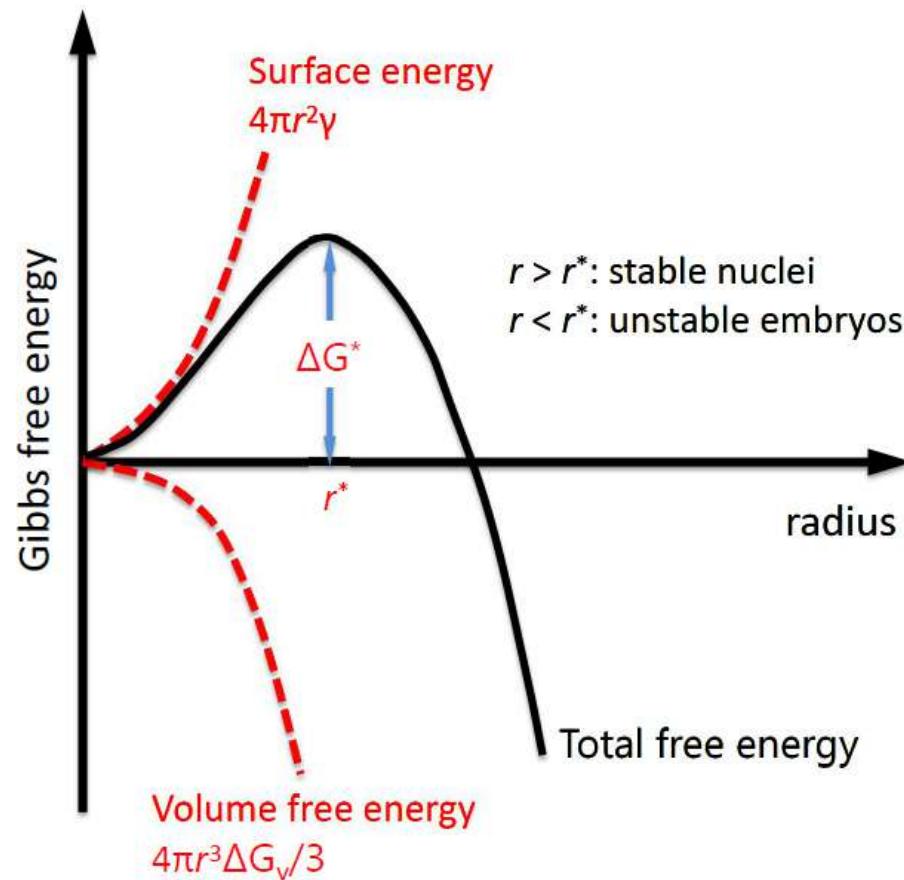
$$V = \frac{4}{3}\pi r^3, \quad A = 4\pi r^2$$

$$\Delta G = \frac{4}{3}\pi r^3 \cdot (-\Delta G_V) + 4\pi r^2 \cdot \gamma \quad \text{There are both negative and positive terms!}$$

$$\Delta G = \frac{4}{3} \pi r^3 \cdot (-\Delta G_V) + 4\pi r^2 \cdot \gamma$$



- Nuclei of radius  $r < r^*$  cannot grow as it will lead to increase in the free energy of the system !
- Nuclei of radius  $r > r^*$  will grow
- $r^*$  is known as the critical radius of homogeneous nucleation



### The determination of $r^*$

$$\frac{\partial \Delta G}{\partial r} \Big|_{r=r^*} = 0$$

$$\Delta G = \frac{4}{3} \pi r^3 \cdot (-\Delta G_V) + 4\pi r^2 \cdot \gamma$$

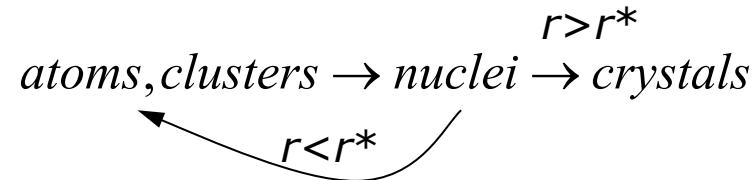
$$r^* = \frac{2\gamma}{\Delta G_V}$$

Giving critical free energy barrier

$$\Delta G^* = \frac{16\pi\gamma^3}{3(\Delta G_V)^2}$$

# Conclusions of homogeneous nucleation

1. Process depends on size relative to  $r^*$



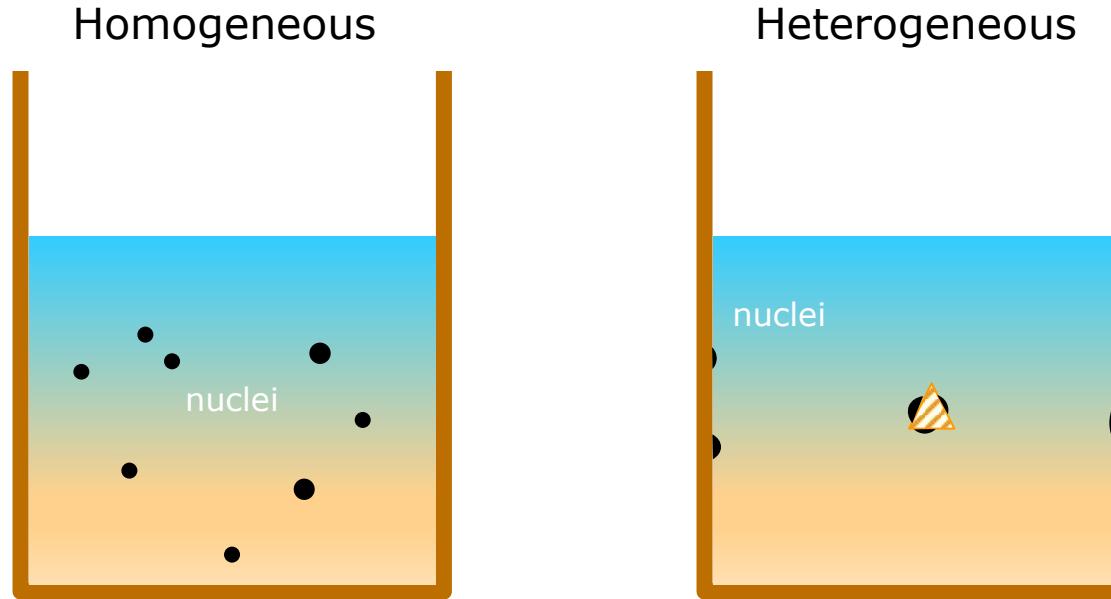
2. The smaller  $\gamma$  or bigger  $\Delta G^*$ , the easier nucleation can occur

$$r^* = \frac{2\gamma}{\Delta G_V} \quad \Delta G^* = \frac{16\pi\gamma^3}{3(\Delta G_V)^2}$$

3.  $\gamma$  and  $\Delta G^*$  is governed by the magnitude of surface energy and volume free energy. So any process that modifies these values will have an influence on the possible viability of the nucleation process.

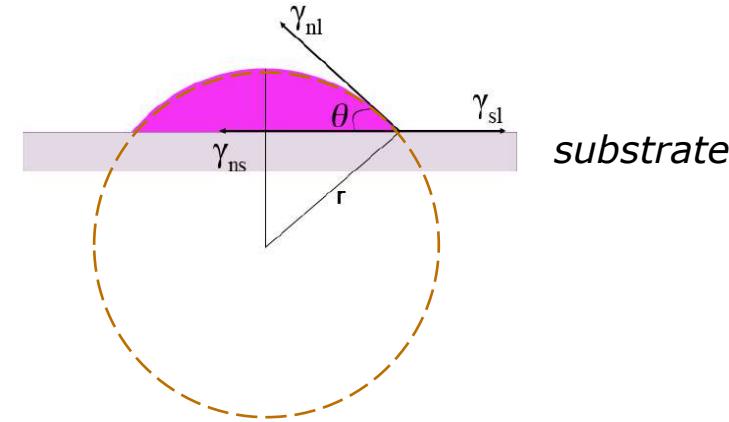
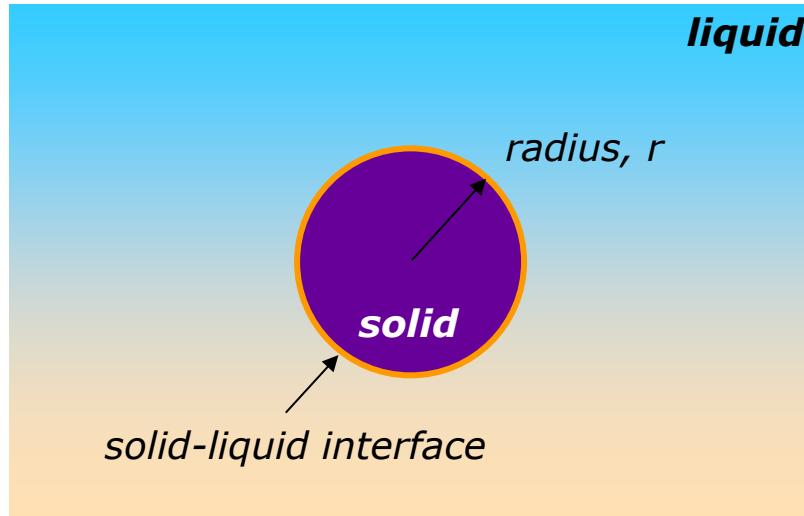
Change solvent, add surfactants, etc...

# Describe heterogeneous nucleation



If other nucleation surfaces/centers (impurities, walls, bubbles, drops, etc.) present in the solution, the nuclei are formed on the already existing surface of a foreign body. This process is called **heterogeneous nucleation**.

# Homogeneous vs. Heterogeneous



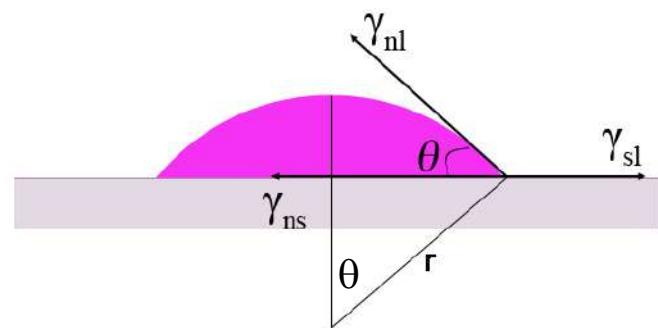
**Heterogeneous nucleation takes less material at a given  $r$  !**

**The total Gibbs free energy =**

**The volume free energy + surface energy**

$$\Delta G = V \cdot (-\Delta G_V) + A \cdot \gamma$$

↓      ↓      ↓      ↓  
volume   bulk free energy   surface area   surface energy



$$A \cdot \gamma = S_{ns} \cdot (\gamma_{ns} - \gamma_{sl}) + S_{nl} \cdot \gamma_{nl}$$

$$S_{nl} = 4\pi r^2 (1 - \cos \theta)$$

$$S_{ns} = \pi r^2 \sin^2 \theta$$

$$V = \frac{4}{3}\pi r^3 (2 - 3\cos \theta + \cos^3 \theta)$$

Surface tension

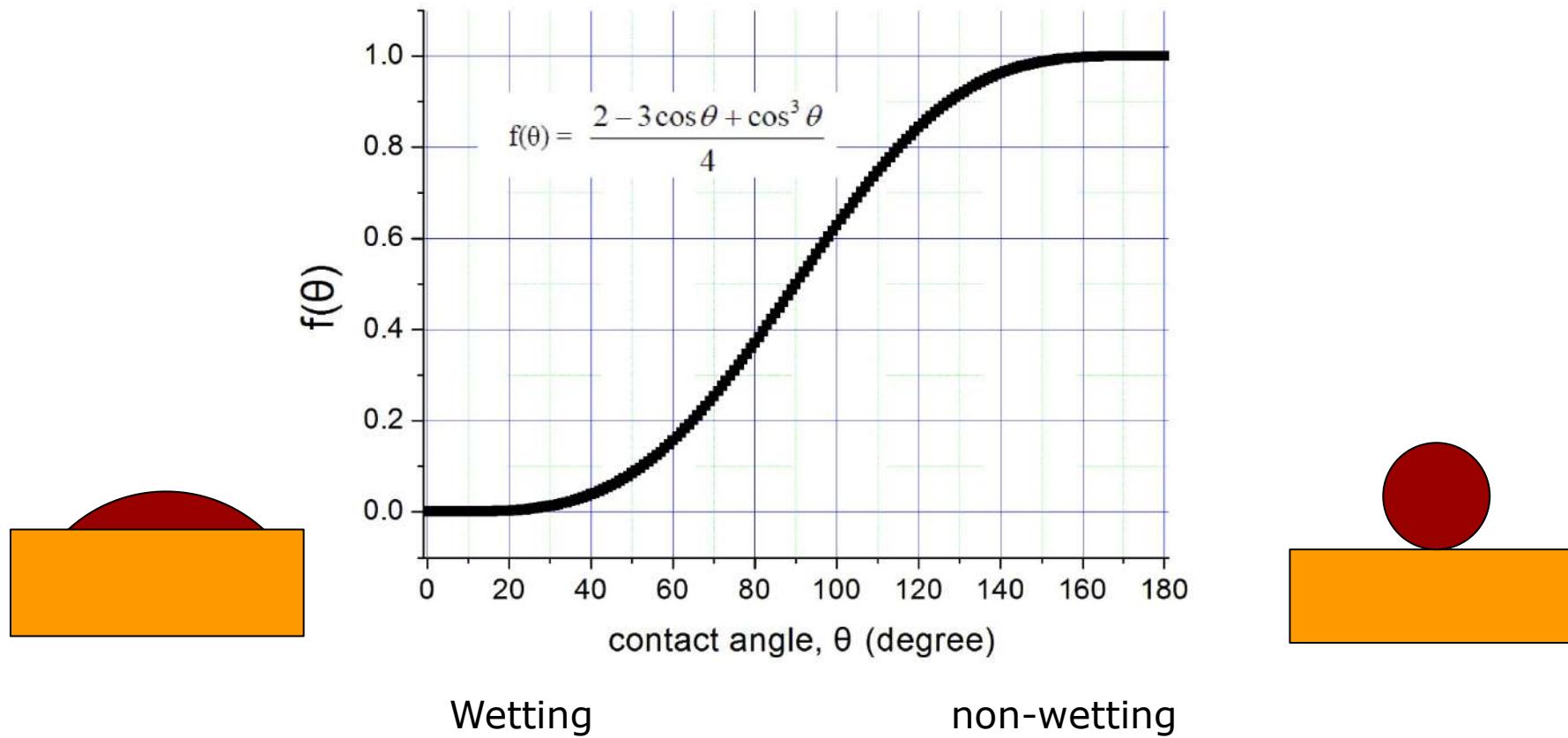
$$\gamma_{ns} - \gamma_{sl} = -\gamma_{nl} \cos \theta$$

$$\Delta G_{\text{het}} = \left( \frac{4}{3}\pi r^3 \cdot (-\Delta G_V) + 4\pi r^2 \cdot \gamma_{nl} \right) \cdot \frac{2 - 3\cos \theta + \cos^3 \theta}{4}$$

$$\Delta G_{\text{hom}}$$

$$\Delta G_{\text{het}} = \Delta G_{\text{hom}} \cdot f(\theta)$$

$$f(\theta) = \frac{2 - 3 \cos \theta + \cos^3 \theta}{4}$$

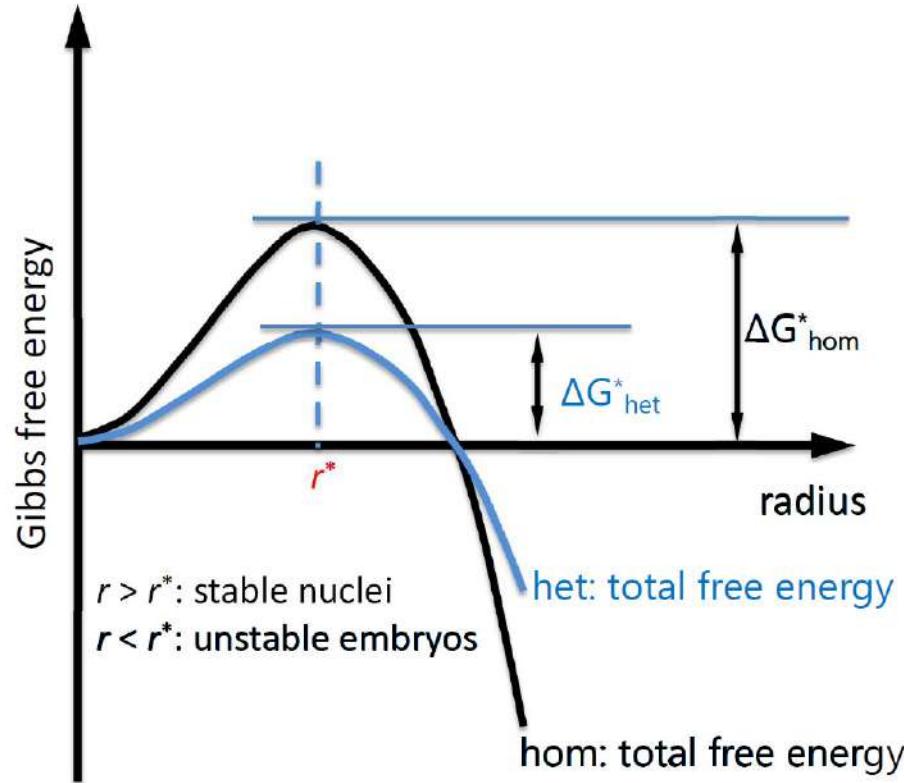


# Conclusions of heterogeneous nucleation

$$r^*_{het} = -\frac{2\gamma}{\Delta G_V} = r^*_{hom}$$

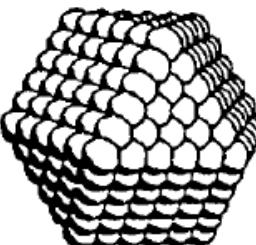
$$\Delta G^*_{het} = \frac{16\pi\gamma^3}{3(\Delta G_V)^2} f(\theta) = \Delta G^*_{hom} f(\theta)$$

1. The critical radius  $r^*$  is unchanged for heterogeneous nucleation and homogeneous nucleation
2. on a substrate the volume of critical nucleus can be significantly less for heterogeneous nucleation than for homogeneous nucleation.
3. The critical free energy can be significantly lower for heterogeneous nucleation due to the wetting effect of the substrate



# Not so simple I:

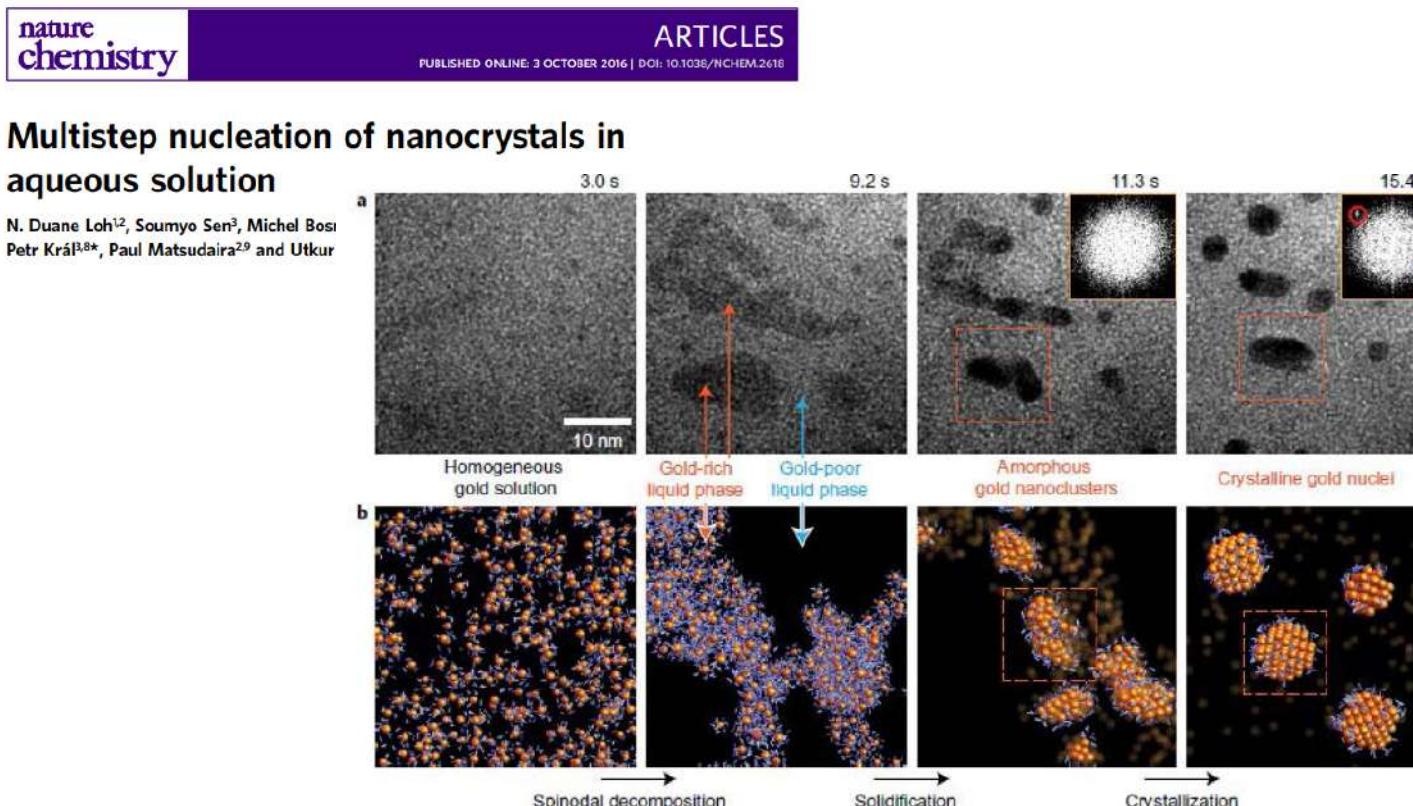
The nuclei in solution phase synthesis maybe not be spherical, often clusters have a magic number of atoms

Full-shell “magic number” clusters					
Number of shells	1	2	3	4	5
Number of atoms in cluster	13	55	147	309	561

Idealized representation of full-shell metal clusters with “magic numbers” of atoms, which are built upon the densest sphere packing.

The magic number clusters display shapes remarkably similar to those that typify the stable and observable seeds from which metal nanocrystals are known to grow.

There is a lower energy for these than the continuous model spherical particles.

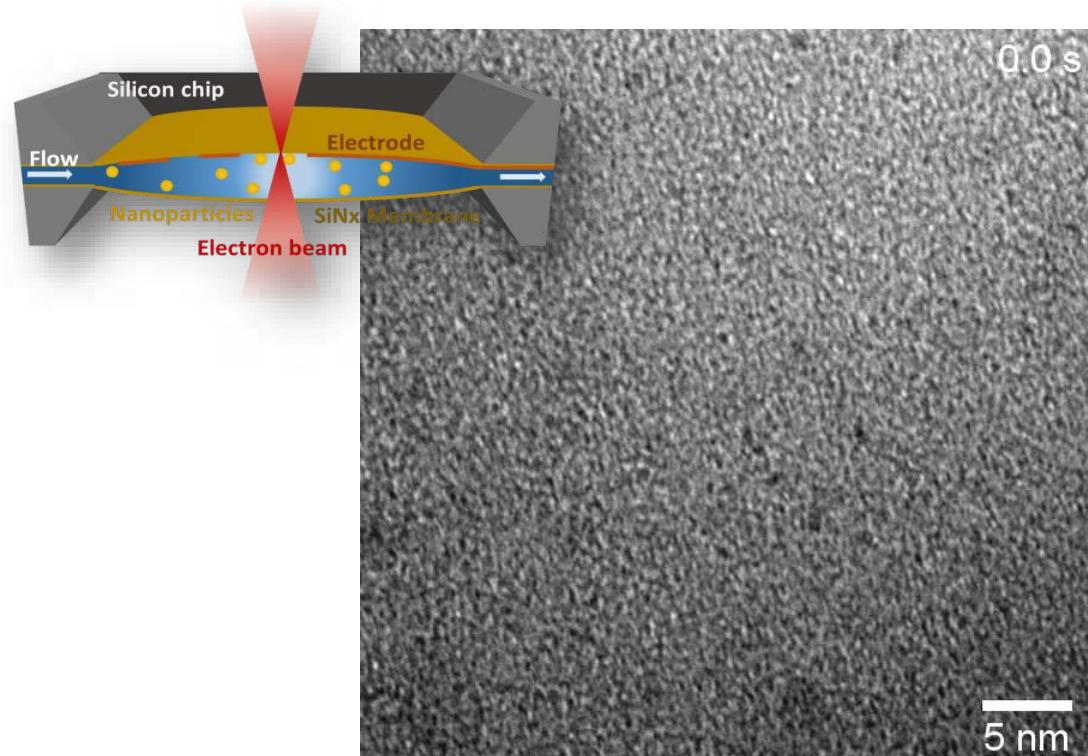


The solution separates into solute-rich and solute-poor liquid phases

nucleation of amorphous nanoclusters within the metal-rich liquid phase

crystallization of these amorphous clusters

The above discussions on hom. and het. nucleation are in a single step.  
Actual case is more complex.



1. spinodal decomposition (i.e. separation) into solute-rich and solute-poor liquid phases
2. nucleation of amorphous nanoclusters within the metal-rich liquid phase
3. crystallization of these amorphous clusters.

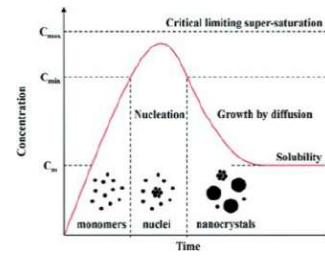
**LPTEM provides useful information to enrich the traditional nucleation theory !**

<https://www.nature.com/articles/nchem.2618#supplementary-information>

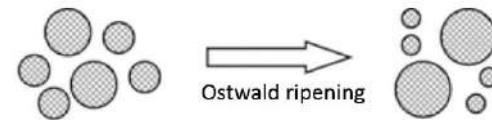
Nature Chemistry 9, 77–82 (2017)

# Next lecture : different growth models

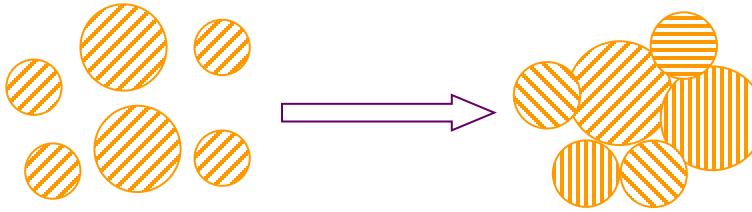
## 1. LaMer



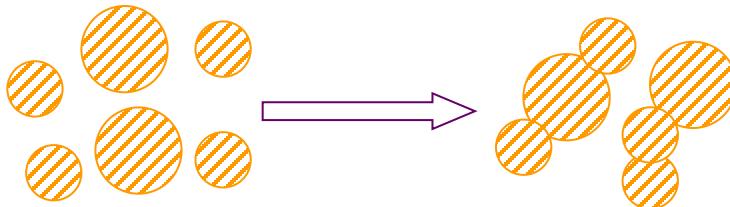
## 2. Ostwald ripening



## 3. Coalescence



## 4. Oriented attachment (OR)

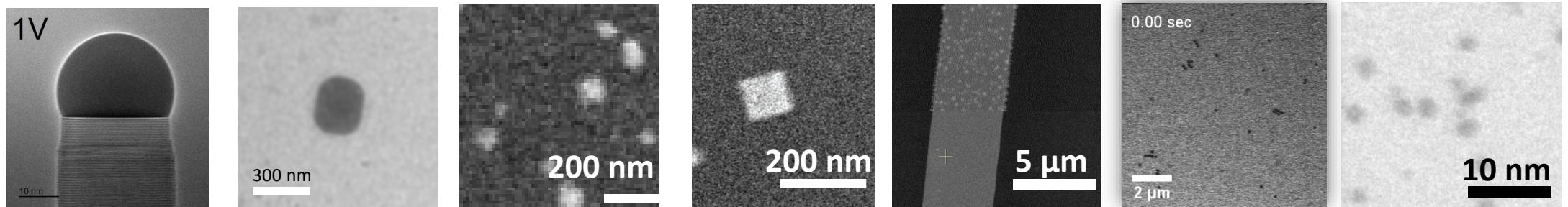


## 5. Growth Models

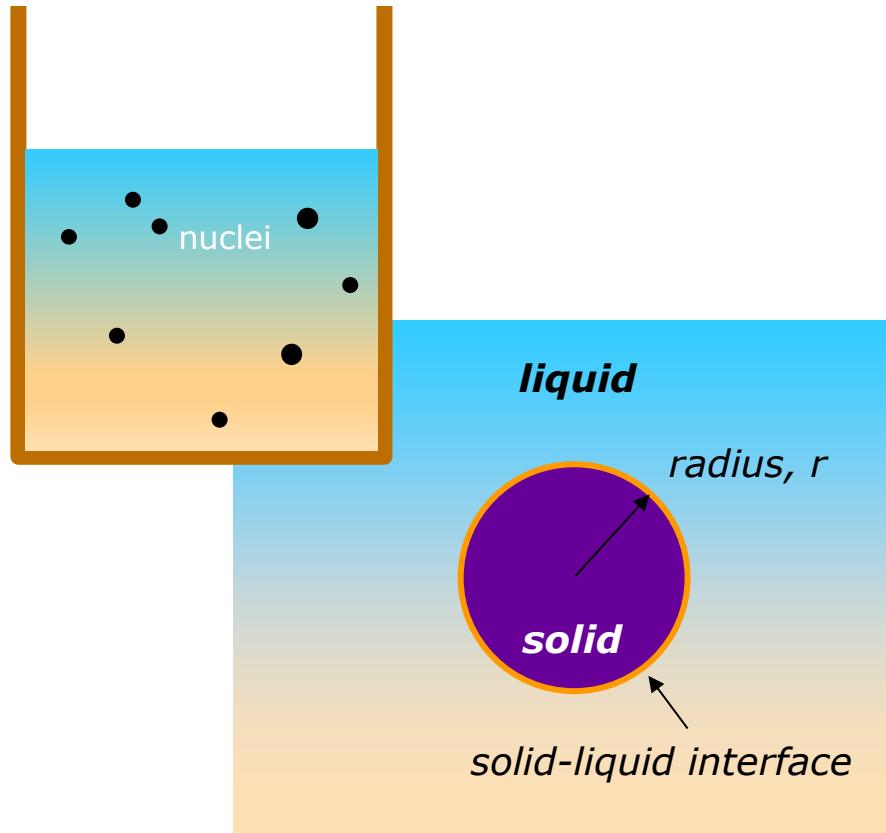
Murat Nulati Yesibolati, Hongyu Sun & Kristian Mølhave

DTU Nanolab

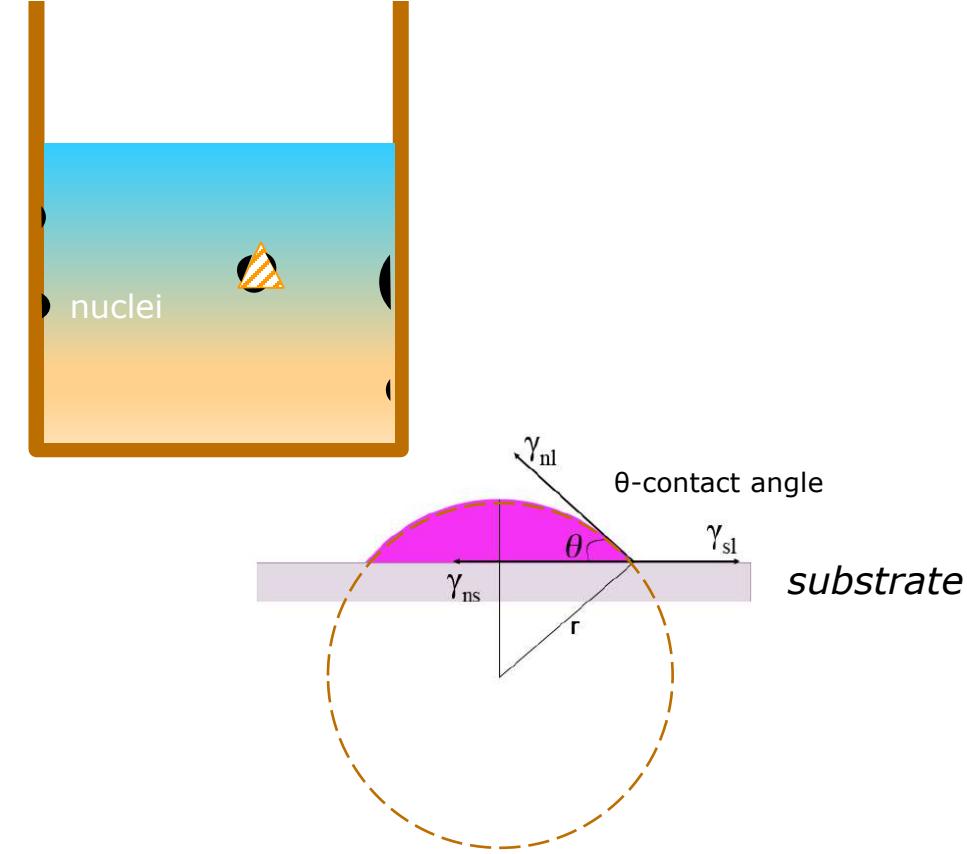
Molecular  
Windows



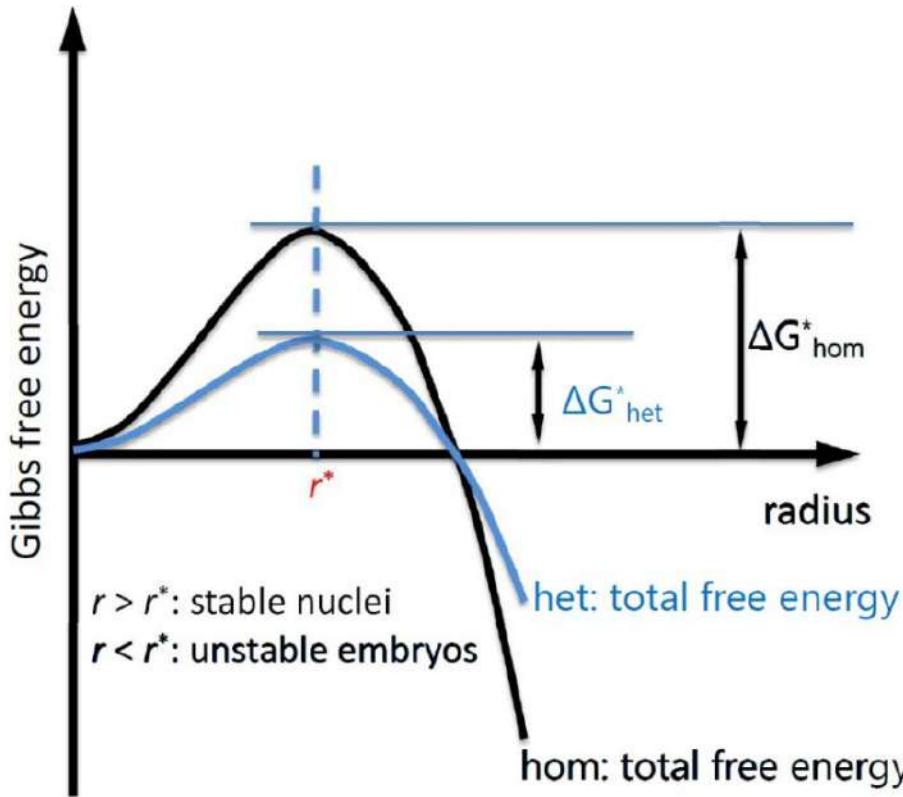
# Homogeneous vs. Heterogeneous nucleation



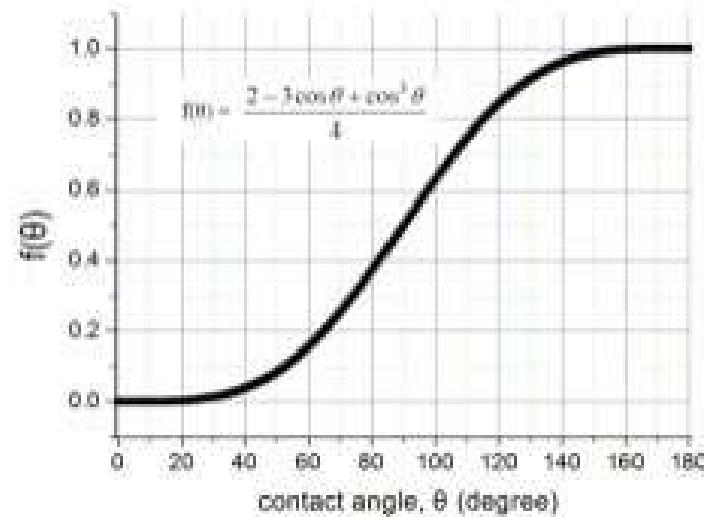
**Total Gibbs free energy =  
Volume free energy + surface energy**



**critical radius  
critical free energy barrier**

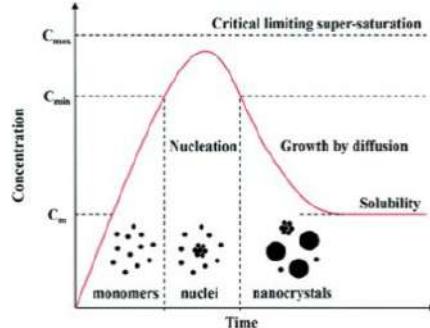


	$r^*$	$\Delta G^*$	volume of critical nucleus
hom.	$-\frac{2\gamma}{\Delta G_V}$	$\frac{16\pi\gamma^3}{3(\Delta G_V)^2}$	large
het.	$-\frac{2\gamma}{\Delta G_V}$	$\frac{16\pi\gamma^3}{3(\Delta G_V)^2} f(\theta)$ $(0 \leq f(\theta) \leq 1)$	small

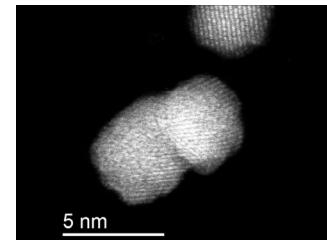
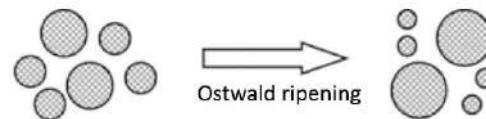


# After nucleation - Growth models

## 1. LaMer

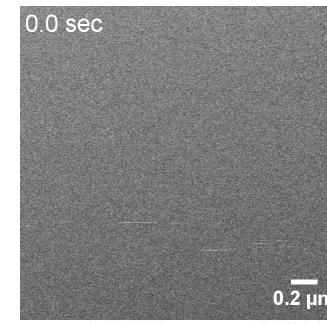
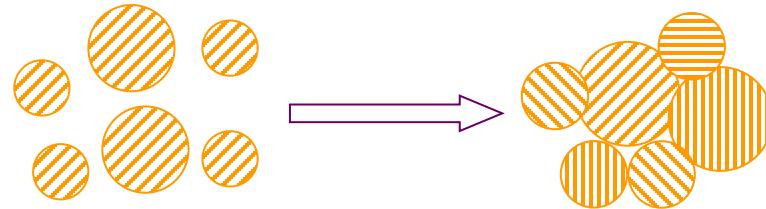


## 2. Ostwald ripening

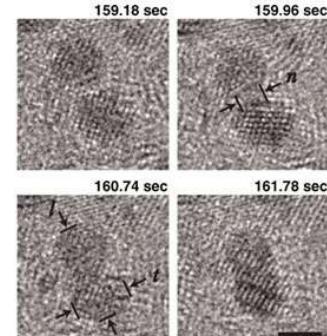
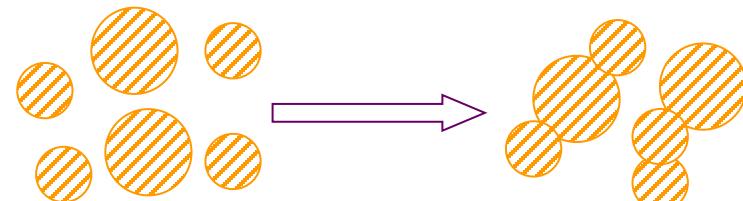


<https://www.youtube.com/watch?v=5LkjaaH3Q>

## 3. Coalescence



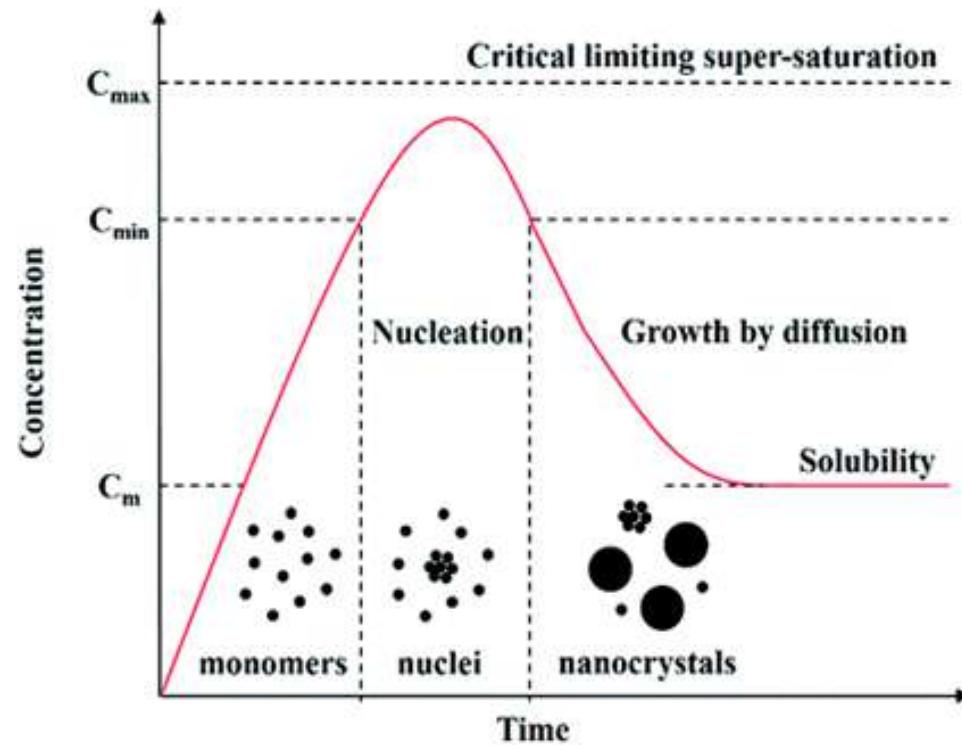
## 4. Oriented attachment



# Describe growth process

Growth: Increase in the size of a product particle after it has nucleated

Models: growth of nanoparticles in solution



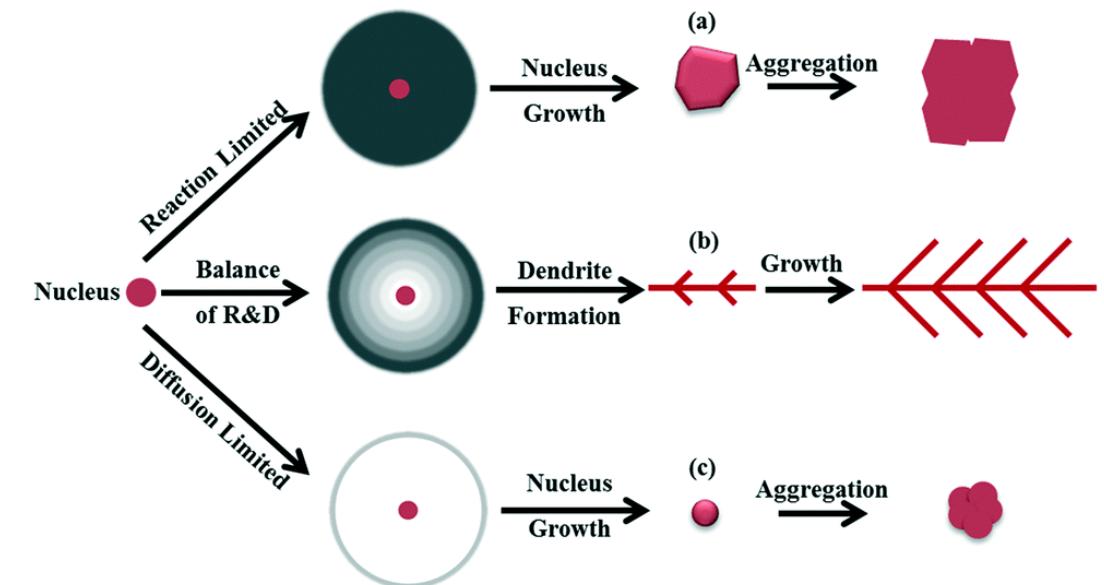
## 1. La Mer mechanism:

- (I) A rapid increase in the concentration of free monomers in solution  
e.g. a reaction creates them, or addition, or light or electrons...
- (II) the monomer undergoes 'burst nucleation' which significantly reduces the concentration of free monomers in solution
- (III) following nucleation, the growth occurs under the control of the diffusion of the monomers through the solution leading to adsorption on the particles' surface.

## Diffusion or reaction limited Growth

**Diffusion Limited Growth:** particle growth is controlled by the diffusion of the monomers to the surface

$$\frac{dr}{dt} = \frac{r^2}{K}, r^3 - r_0^3 = Kt \quad K=\text{constant}$$



**Reaction-Limited Growth:** growth rate is limited by the surface reaction of the attaching monomers

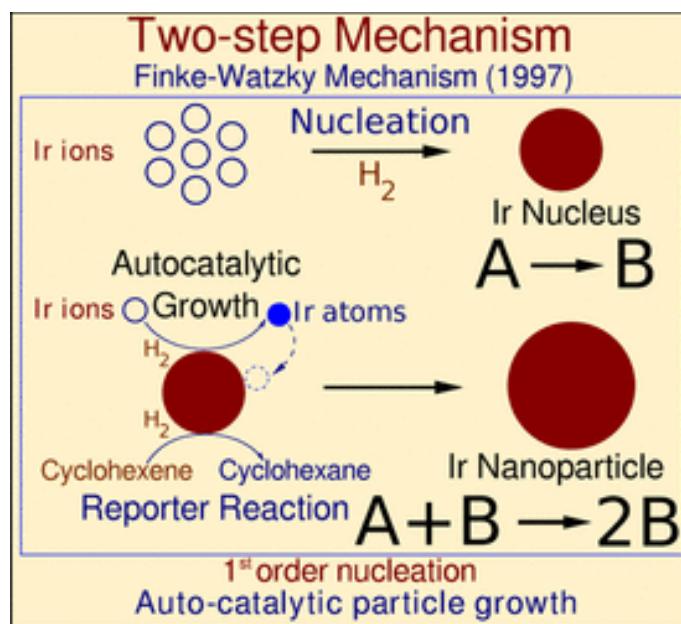
$$r^2 = K_R t \quad K_R=\text{constant}$$

DOI: [10.1039/C6CE02068B](https://doi.org/10.1039/C6CE02068B) (Paper) *CrystEngComm*, 2017, **19**, 72-79

## Further complications...

### Finke-Watzky two step mechanism

Firstly a slow continuous nucleation occurs ( $A \rightarrow B$ ), the newly formed nuclei will further catalyze the surface growth which is not diffusion controlled ( $A + B \rightarrow 2B$ ).



<https://pubs.acs.org/doi/pdf/10.1021/la503199m>

preparation is often small; hence, each new colloidal particle requires what is tantamount to a new synthetic strategy. It is also now believed that the LaMer mechanism is, as a mechanistic chemist would expect, rigorously appropriate only to the system it was developed for: sulfur sols, and other, closely analogous systems.<sup>13</sup> This can explain why others have referred to the LaMer mechanism as “overcited”<sup>7a</sup> (perhaps much fairer to LaMer’s pioneering work would be the term “inappropriately cited”), a phenomenon which really only points to the dearth of new, broadly applicable and kinetically verified alternative mechanisms in the intervening nearly 50 years. If one adds

Finke and watzky

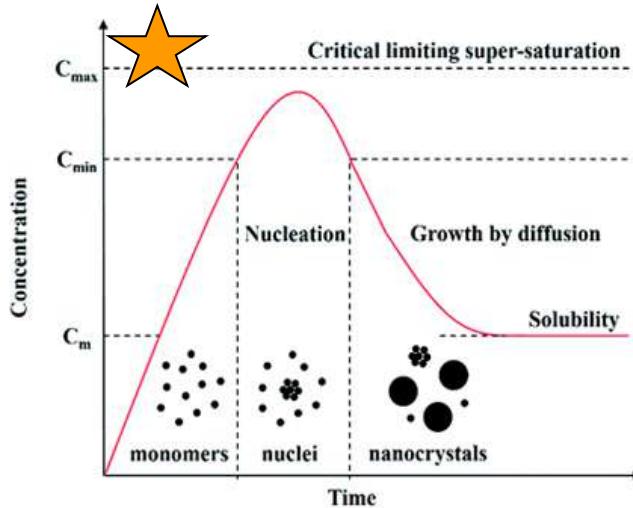
<https://pubs.acs.org/doi/10.1021/ja9705102>

claimed to work, *vide infra*. It is now abundantly clear that CNT is misapplied if used beyond weakly bonding (“weakly associating”) systems, an example of a weakly bonding system being hydrocarbon (RH) aggregation in the gas phase where CNT has some of its best success as discussed more in a moment. Relevant here is that applying classic theories far beyond their original, intended applications and beyond the limits of their assumptions is a documented, more general problem in chemical science as described in a highly recommended paper by B. Peters.<sup>18</sup> Additionally, the dominant effect

<https://pubs.rsc.org/en/content/articlehtml/2021/ma/d0ma00439a>

# Growth models: growth of nanoparticles in solution

Instantaneous creation of monomers

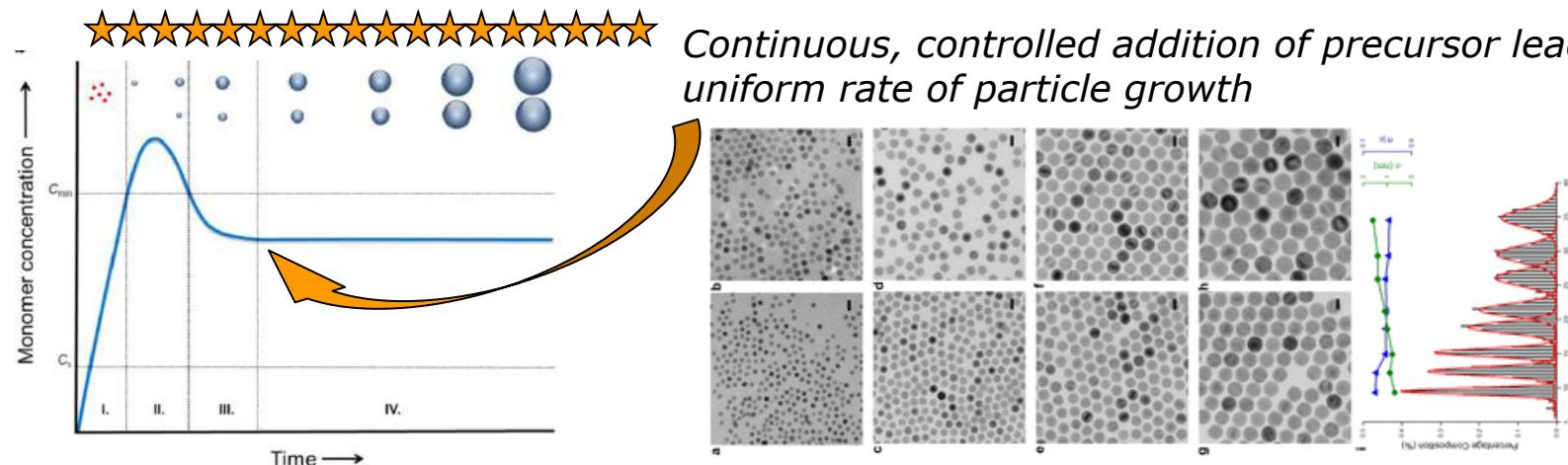


## Discussion:

1. Describe this model
2. How to synthesize nanoparticles with uniform size

Solubility  $M(s) \leftrightarrow M(aq)$  at a solubility product

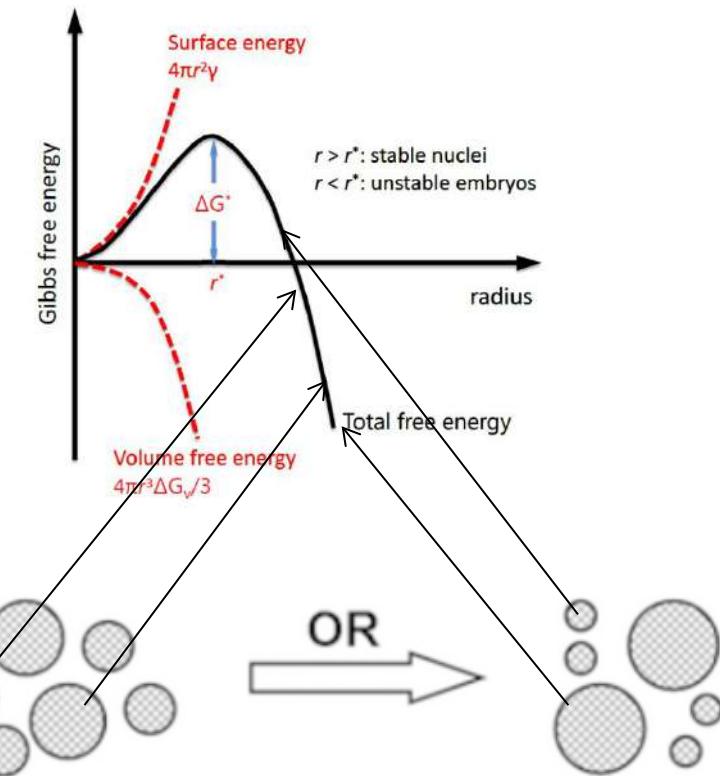
Continuous Creation of monomers



## 2. Ostwald ripening (OR)

Larger particles are more stable (lower energy) and hence will grow faster.

Both large and small particles are in equilibrium with monomer concentration, with atoms adding to or being removed from the particles. More being removed from small ones and more being added to larger ones



Mathematical description of Ostwald ripening within a closed system is described by Lifshitz and Slyozov and Wagner (LSW theory)

**Discussion:**

1. Can you think of examples of Ostwald ripening in daily life?

## Examples

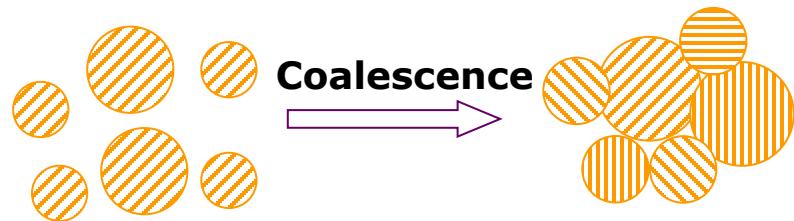


- Beer foam where the larger bubble grow and small bubble disappear over time.
- Condensed water droplets where larger grow and small disappear over time

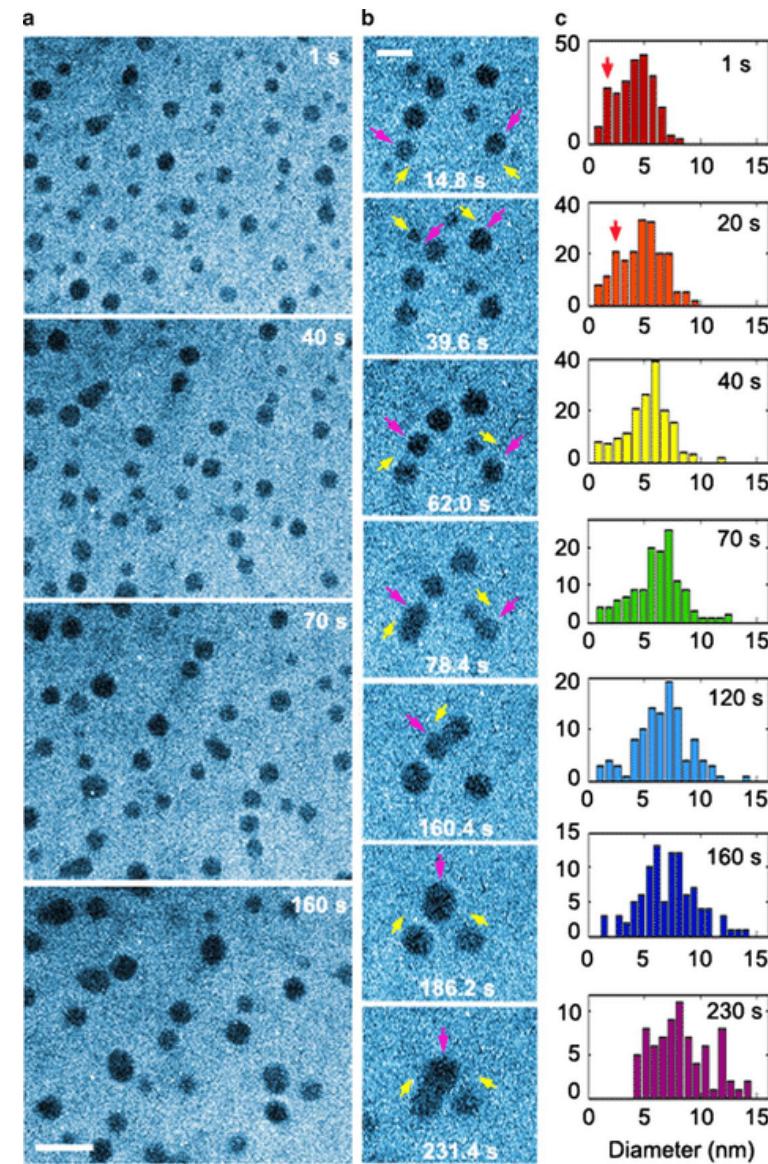


- Small ice crystals disappear and larger grow with time, making stored icecream less smooth and yummy.

### 3. Coalescence

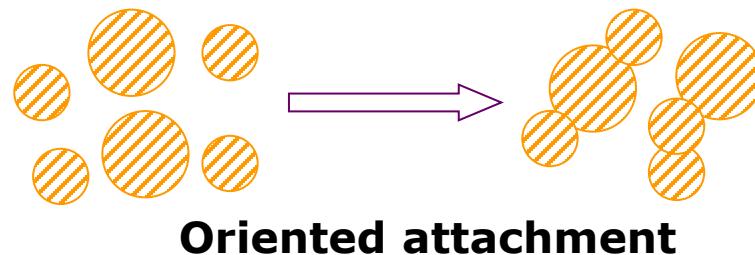


**Coalescence: the lattice planes are randomly oriented between particles**

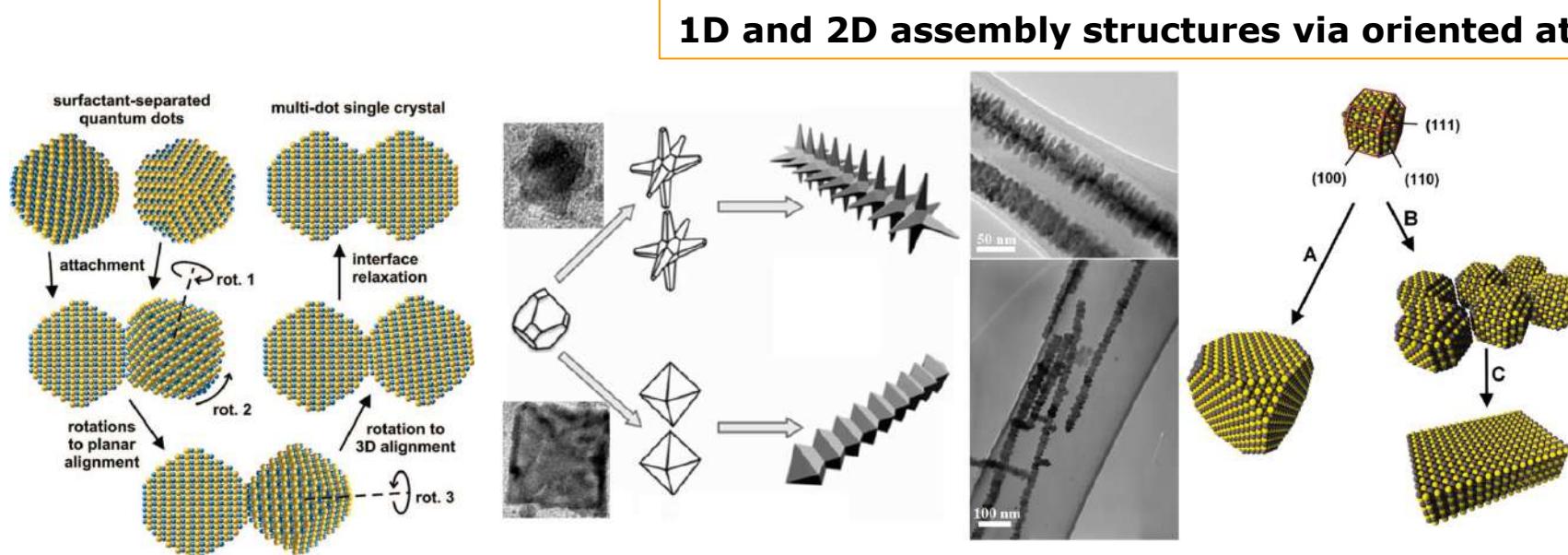


DOI: <https://doi.org/10.1017/S1431927614000282>

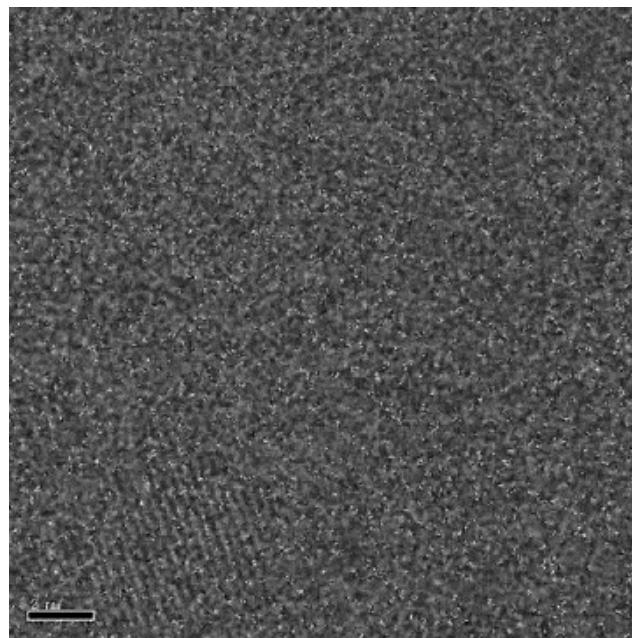
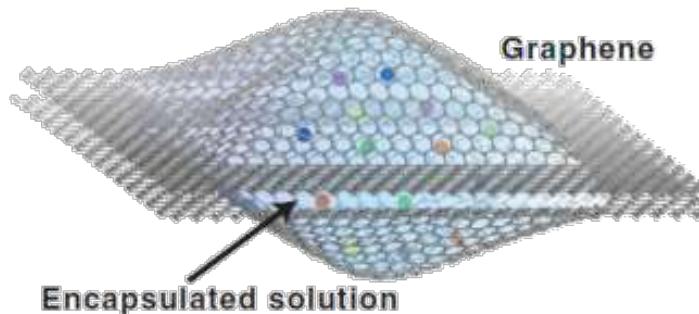
## 4. Oriented attachment



**Oriented attachment: there is a perfect alignment of the planes.**



# Nanoparticle nucleation and interaction



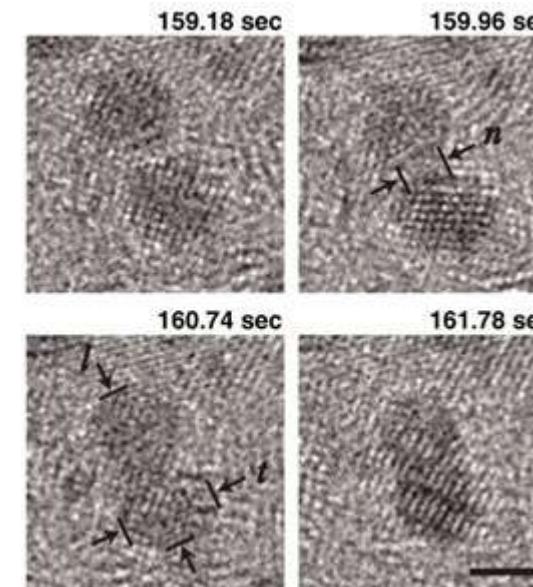
Decoding the movie: What do you see?

Small particles appearing growing while diffusing

Some disappearing?

Some merging?!

Even Oriented  
Attachment to  
Make crystal  
lattices match



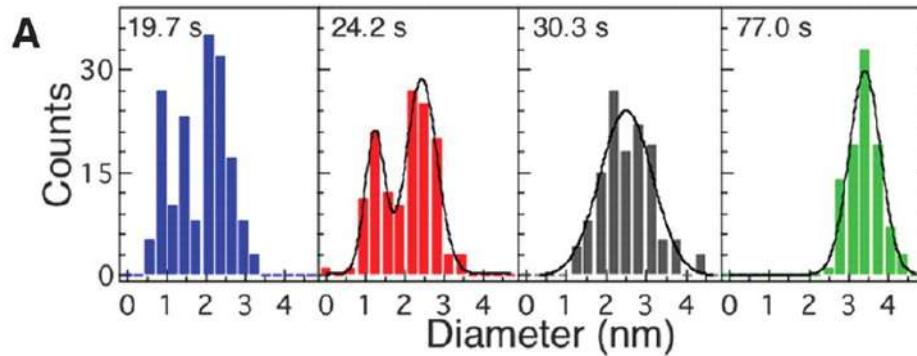
<https://science.sciencemag.org/content/336/6077/61>

# Example of growth of Pt nanoparticles

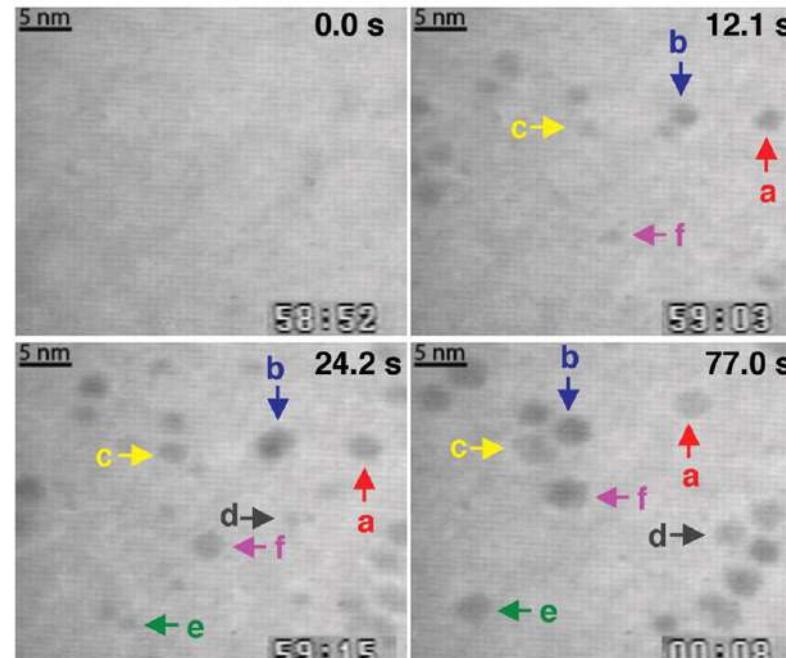
<https://www.youtube.com/watch?v=K8JuIyNzS1A>



# Growth kinetics of Pt nanoparticles: LPTEM studies



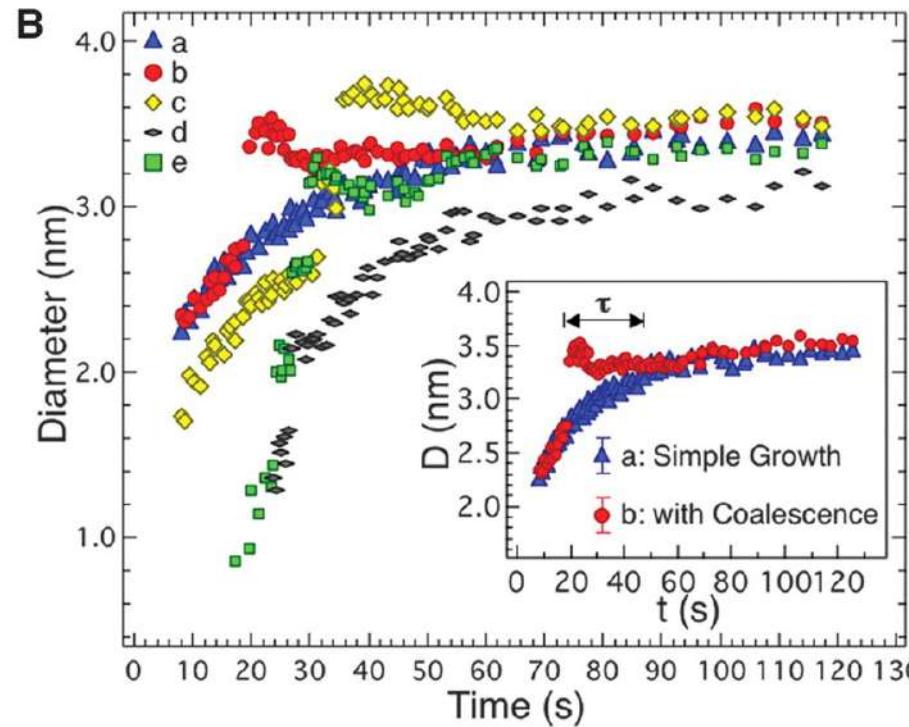
The histograms of size distribution at different stages of growth



Specific particles are labeled with arrows. The growth trajectories of these individual particles reveal the multiple pathways.

# Growth kinetics of Pt nanoparticles: LPTEM studies

<https://www.youtube.com/watch?v=K8JuIyNzS1A>



The size of the particles ( $d$ ) is proportional to growth time ( $t$ ),  $d^3 \sim t$

*The direct observations of single-particle growth trajectories with LPTEM method provide important insights into nanocrystal growth mechanisms, which are not accessible with a conventional analysis on the basis of the ensemble.*