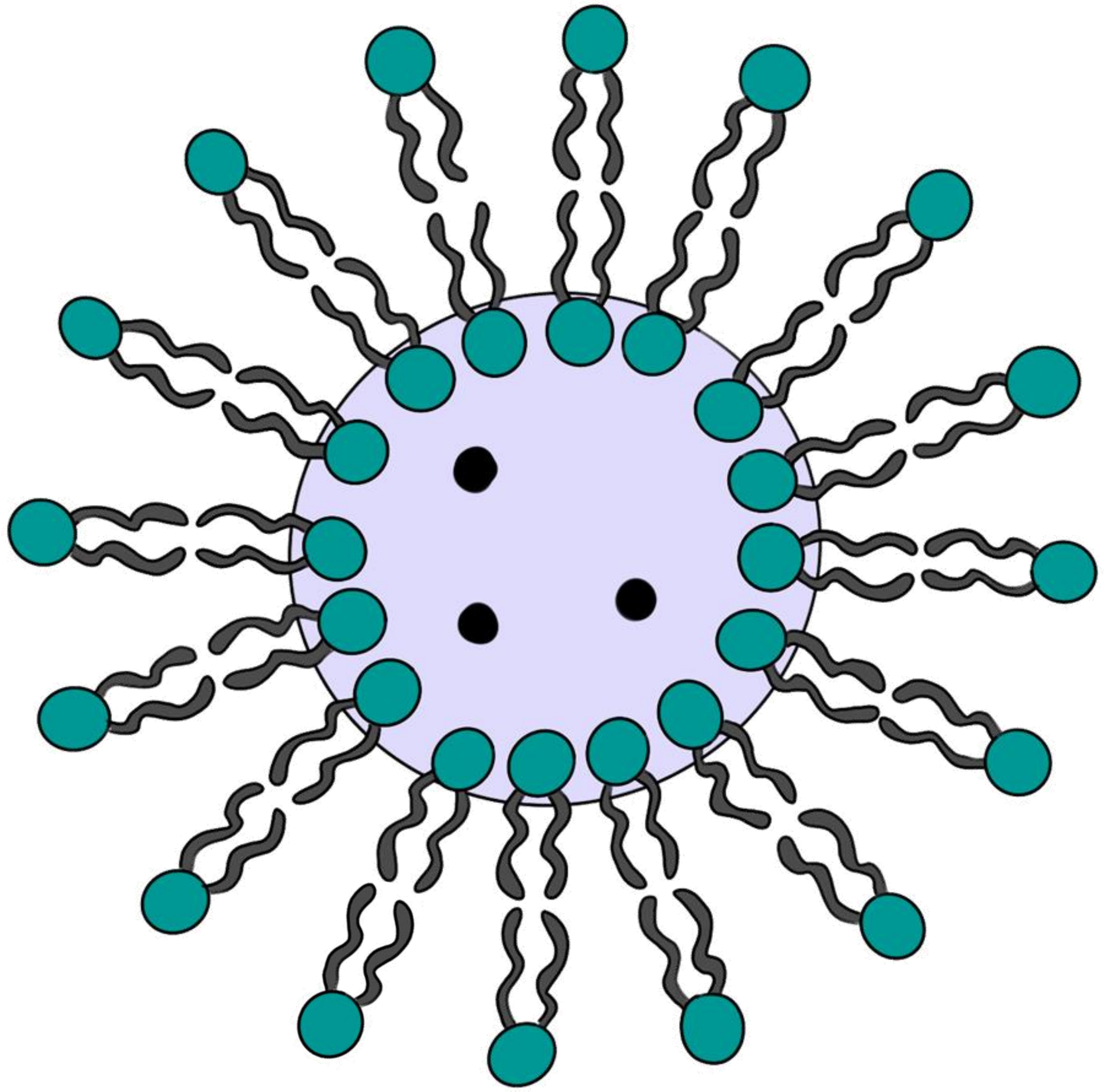
Active biomimetic cell compartments



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Introduction & motivation

I. **Introduction & motivation**

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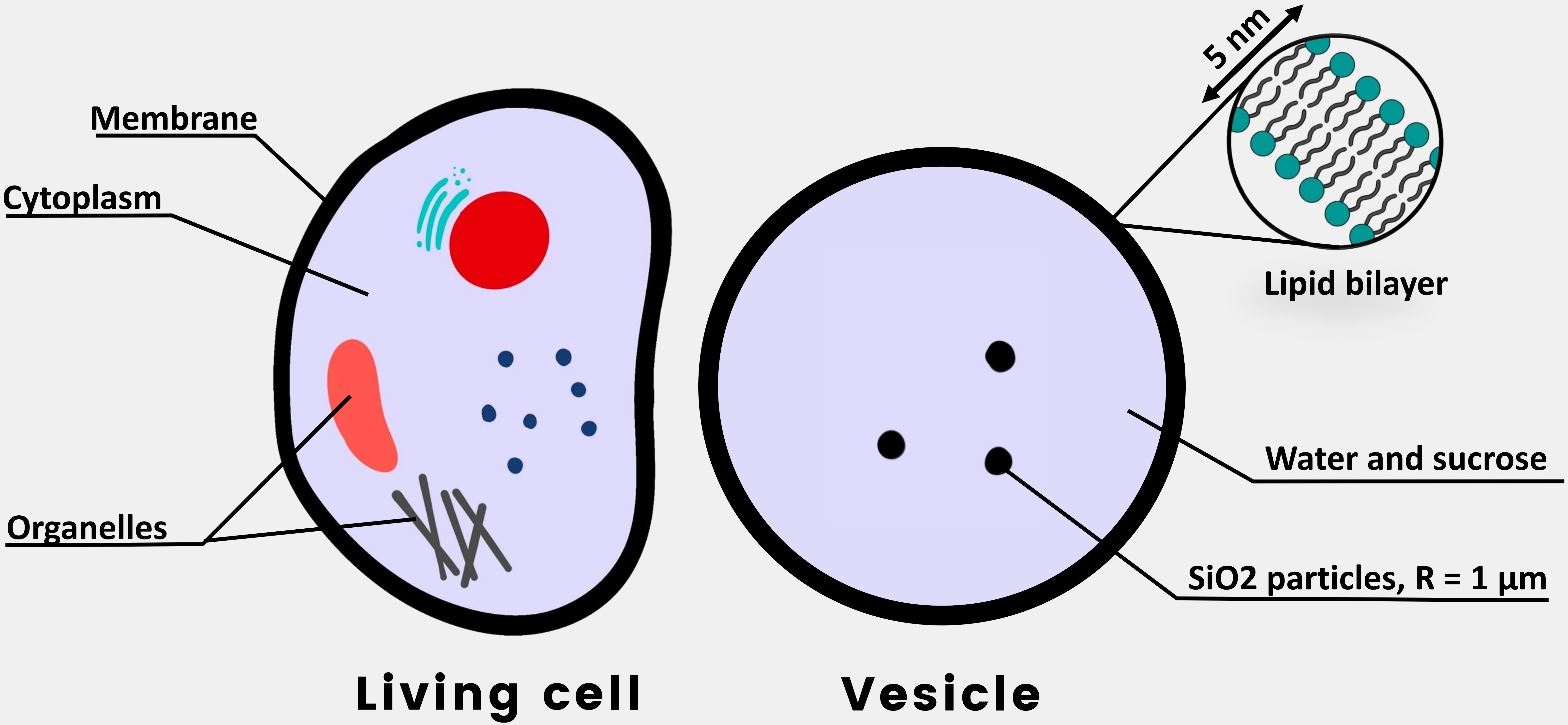
What is a Giant Unilamellar Vesicle ?

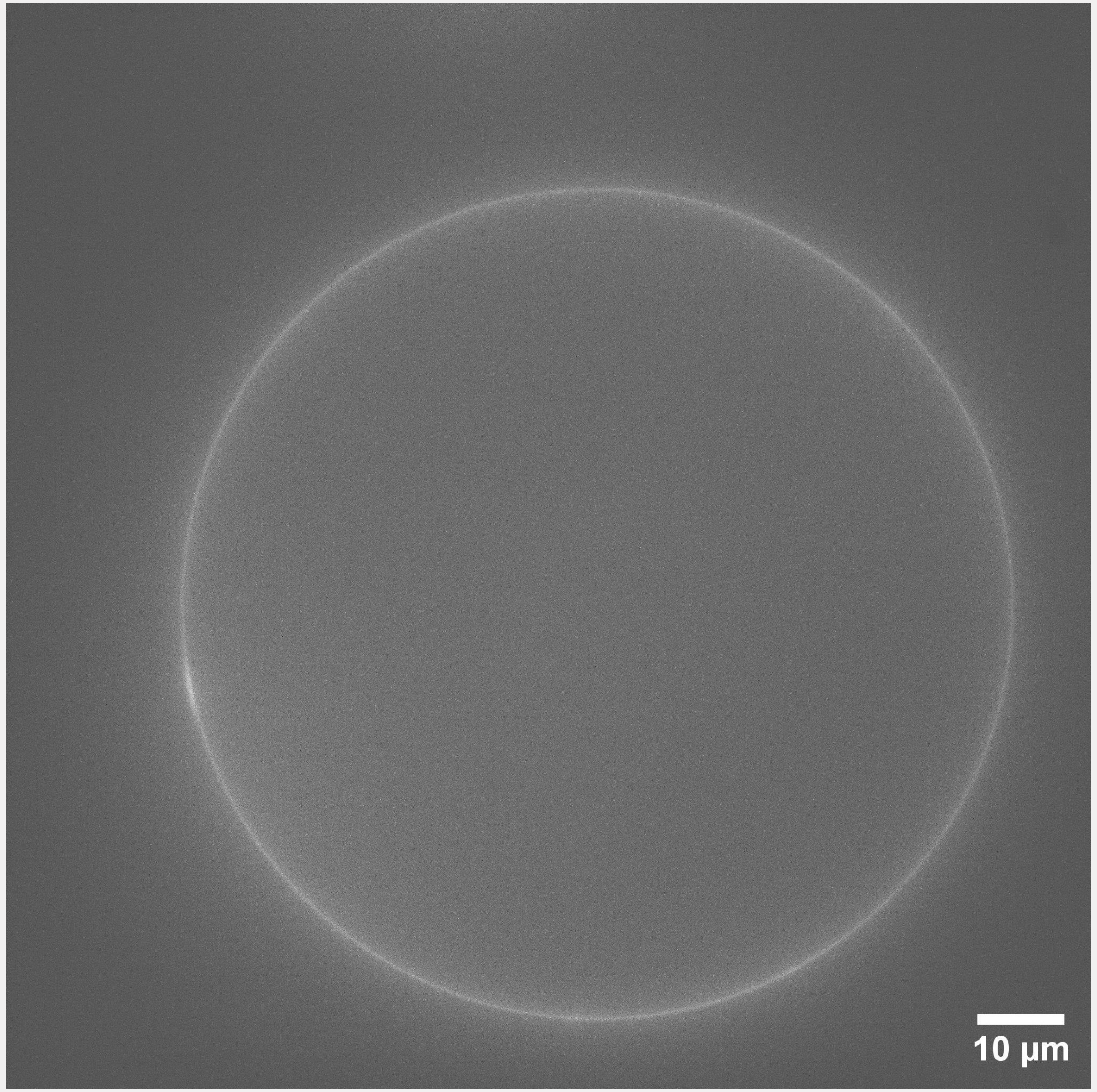


Size of cells / vesicles : a few µm
Diagrams are not to scale

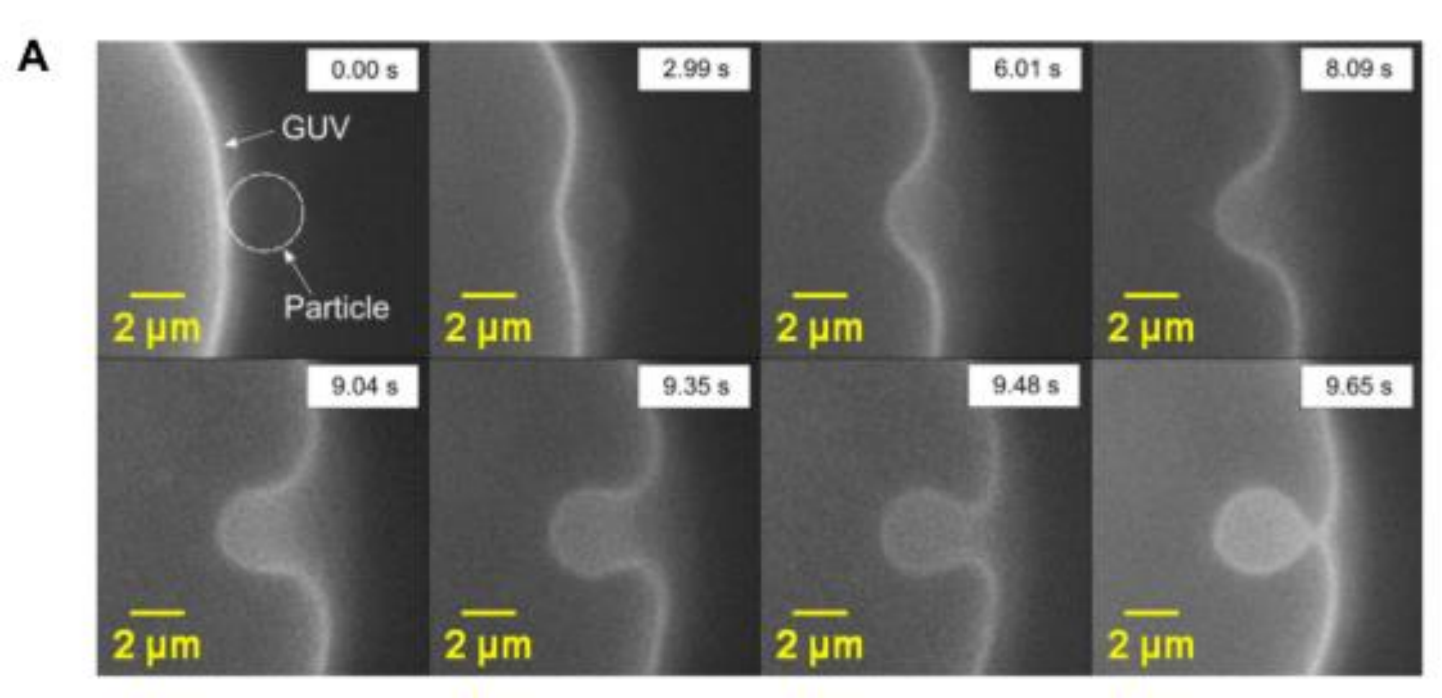
Aim of the internship

- Creating vesicles encapsulating colloidal particles of radius 1 µm
- Studying the physics of the membrane when a particle is pulled trough it
- Relevant quantity for these systems : surface tension
- Motivation : understanding how cells physically work

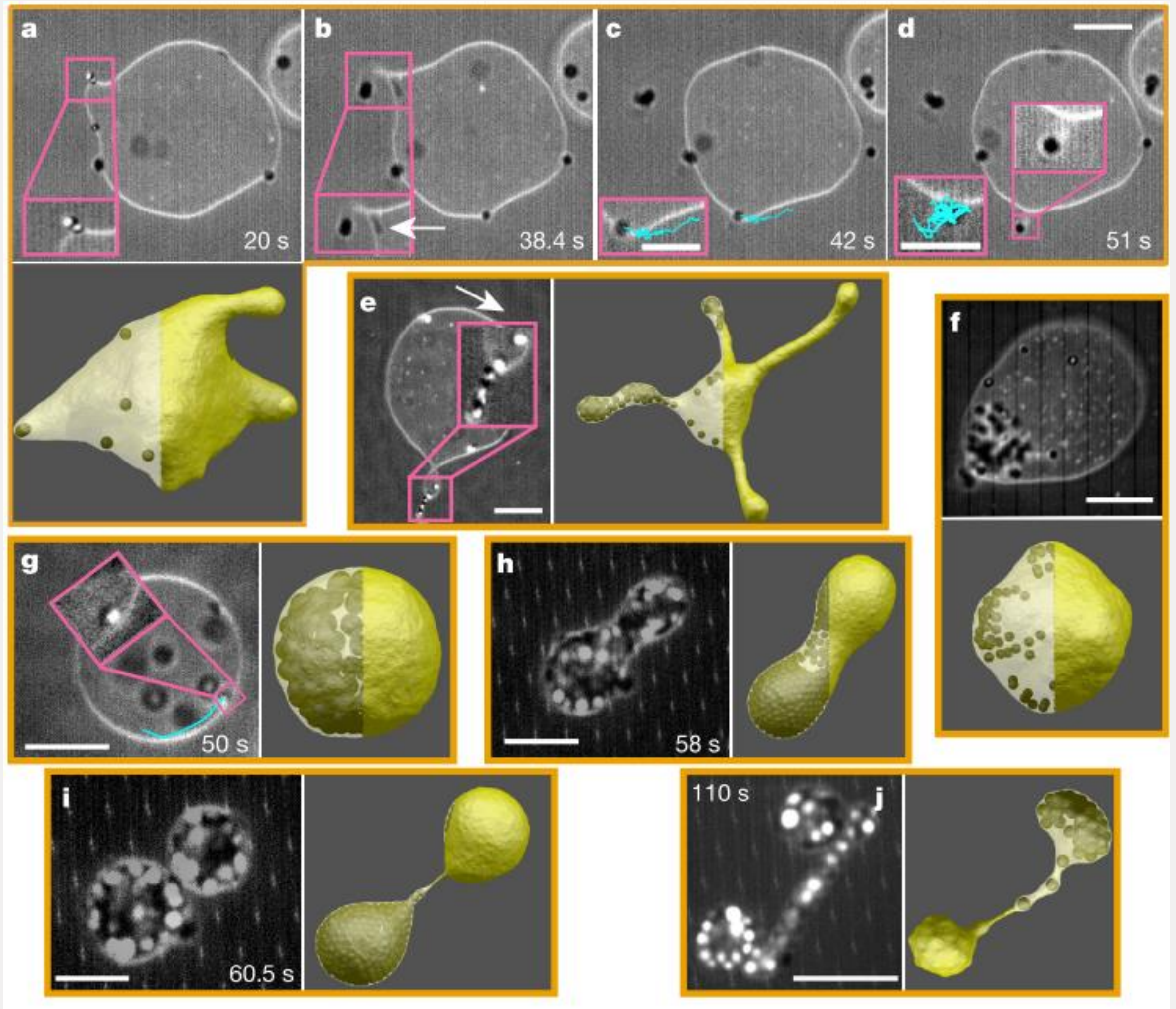
With Thomas Dartige (M2 Cell Physics)



Interactions and deformations of vesicle- particles systems



[1] Fessler F., Sharma V., Muller P., & Stocco A. (2023). *Entry of Microparticles into Giant Lipid Vesicles by Optical Tweezers*. *Phys Rev E.*



[2] Vutukuri, H. R., Hoore, M., Abaurrea-Velasco, C., van Buren, L., Dutto, A., Auth, T., Fedosov, D. A., Gompper, G., & Vermant, J. (2020). *Active particles induce large shape deformations in giant lipid vesicles. Nature*, *586*(7827), 52–56.

These experiments are performed at a low surface tension regime $(\sim 10^{-8} N \cdot m)$ so that the forces are of the order of a pN.

Fabrication of Giant Unilamellar Vesicles

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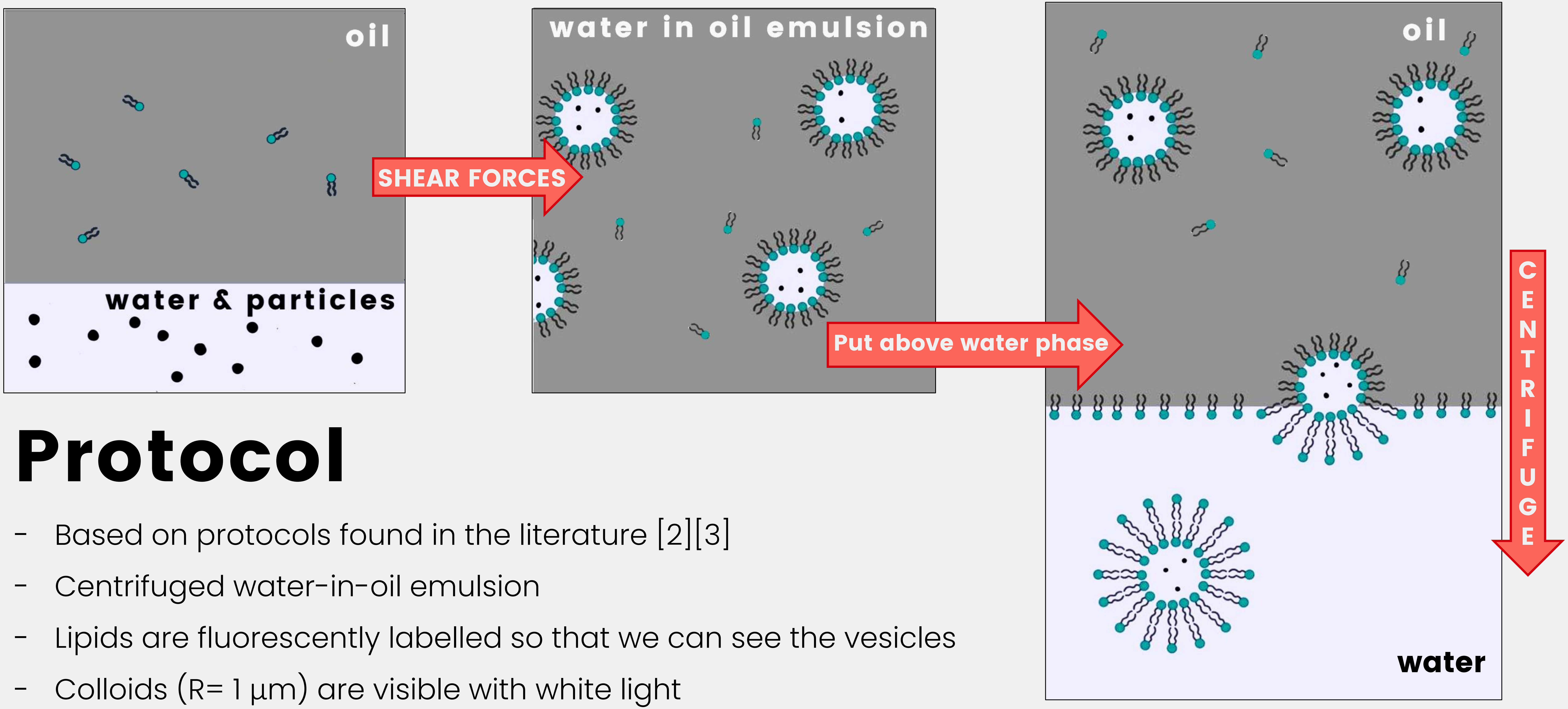
II Fabrication of GUVs



Protocol

- Based on protocols found in the literature [2][3]
- Centrifuged water-in-oil emulsion
- Lipids are fluorescently labelled so that we can see the vesicles
- Colloids (R= 1 µm) are visible with white light

Forces on the vesicle during centrifugation

Centrifugation force

$$F_C = \frac{4}{3}\pi R^3 \times \Delta\rho \times a$$

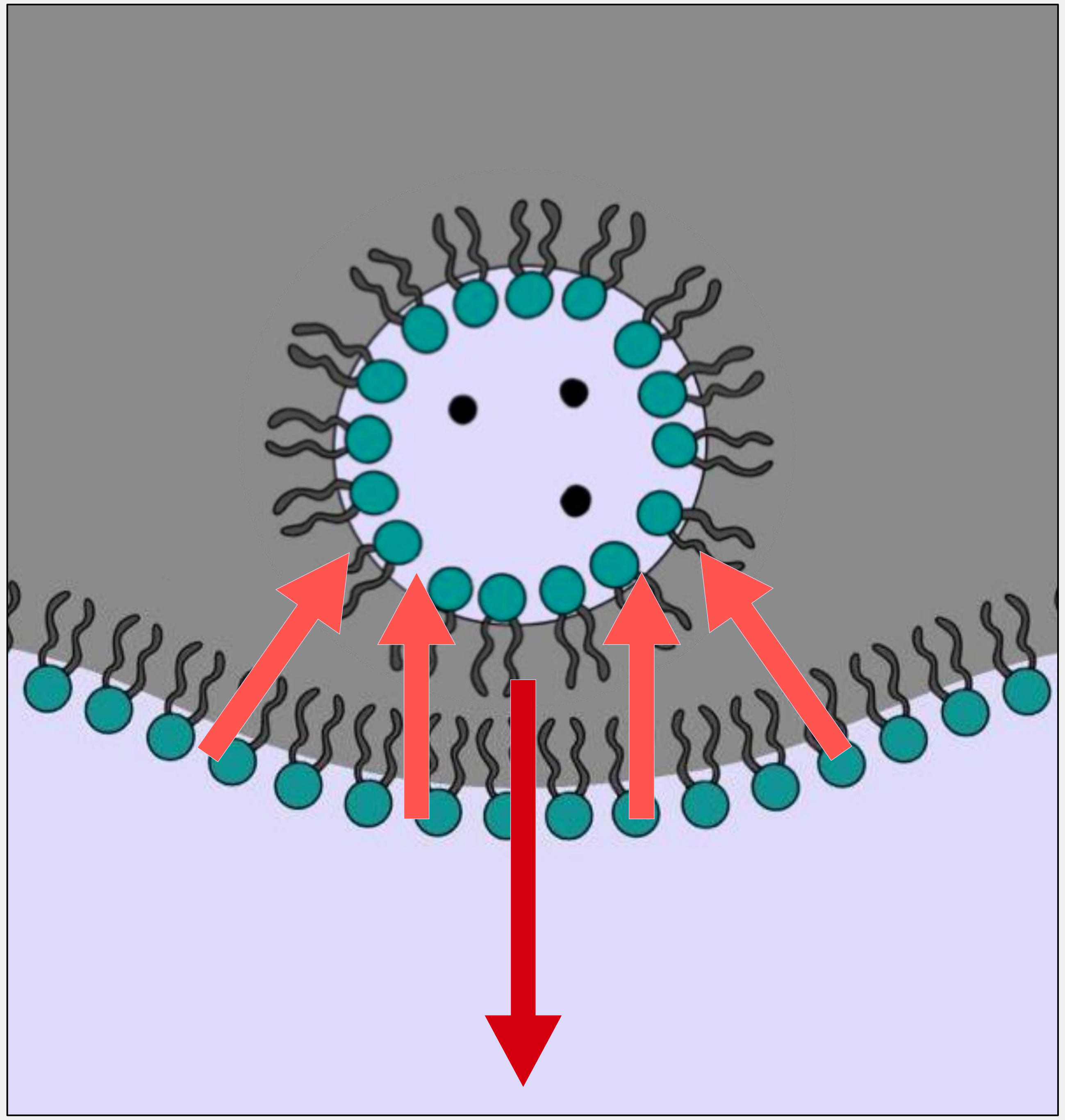
$R =$ radius
$\Delta\rho =$ difference of density between oil and water,
$a =$ acceleration

$$10^{-10}N < F_C < 10^{-7}N$$

Surface tension

$$\gamma \approx 10^{-3}\mathrm{N}\cdot\mathrm{m}^{-1}$$

$$F_{surface\ tension} \approx 10^{-8}N$$



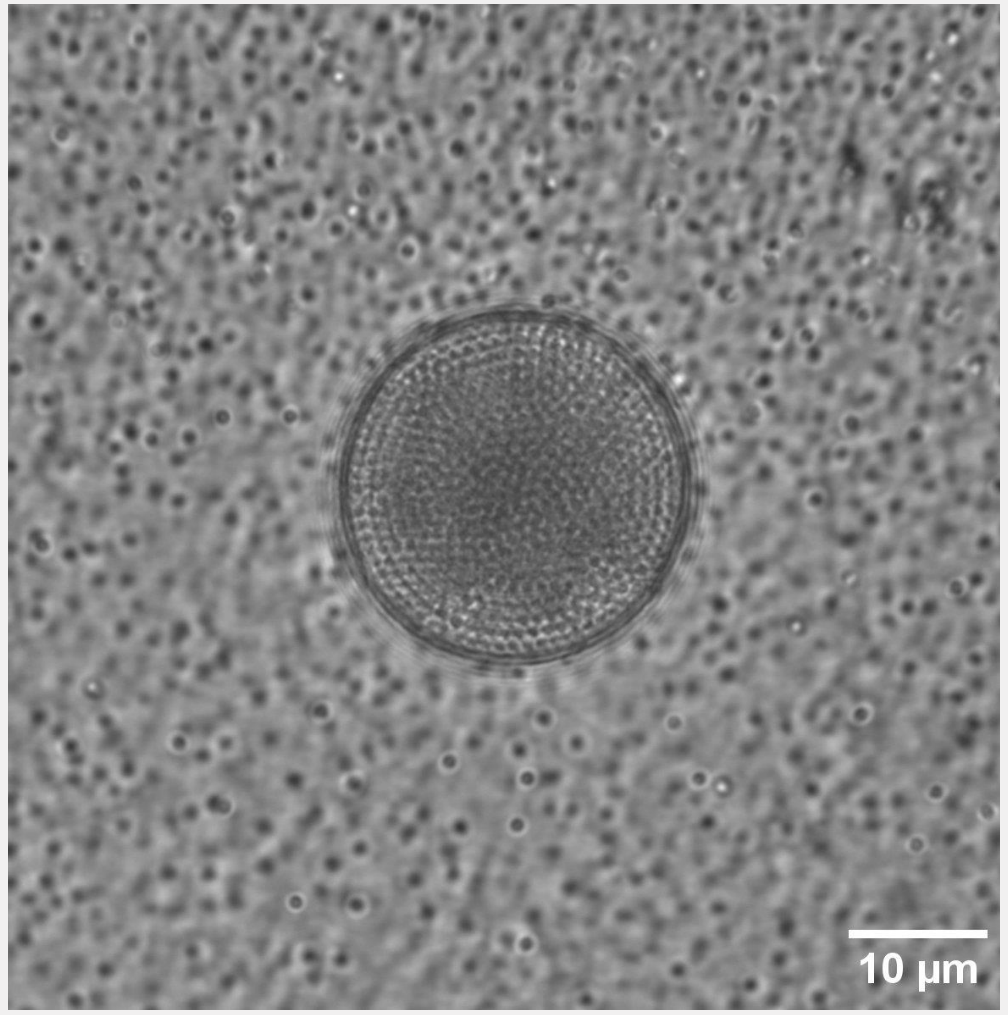
Challenges

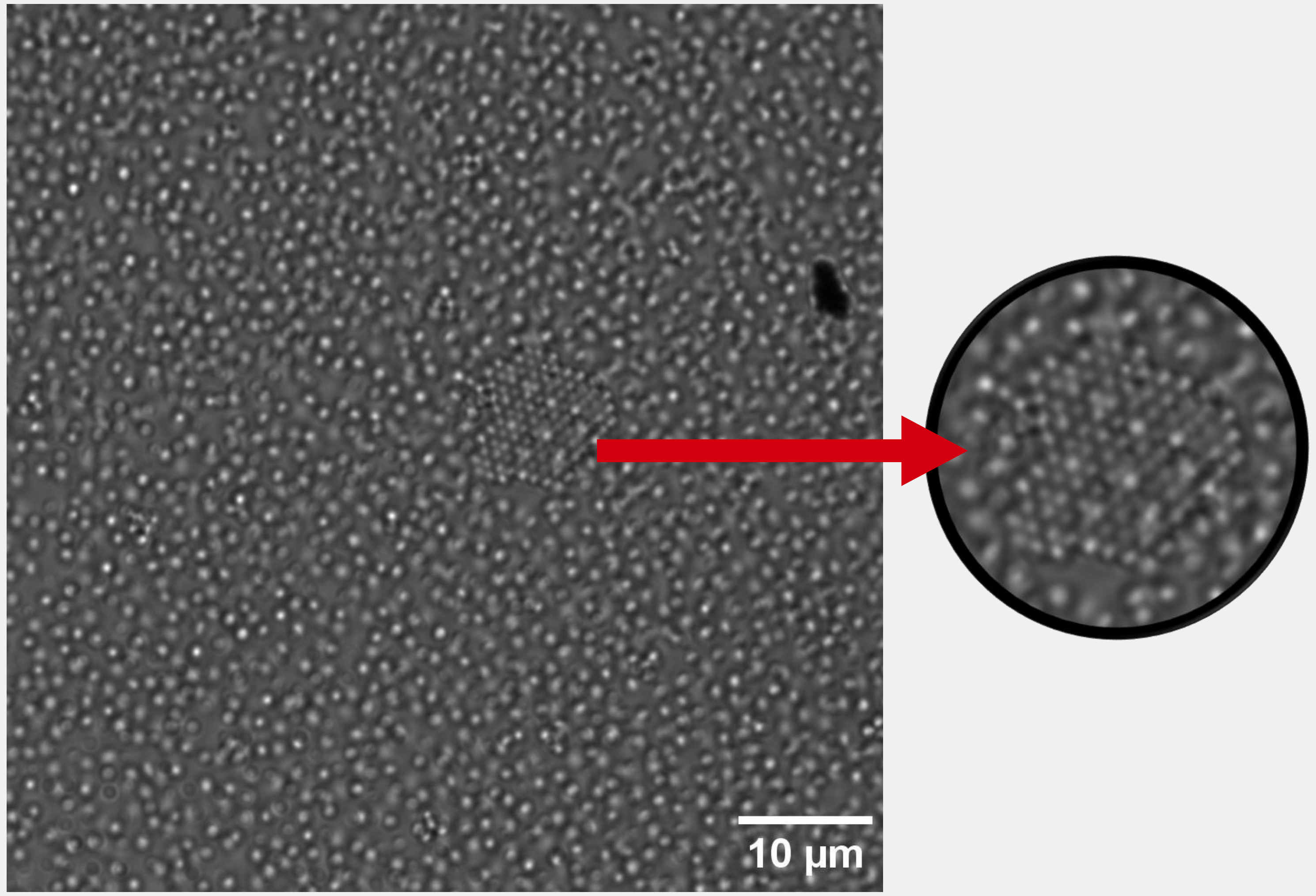
We had to tune every experimental conditions :

- Concentration of lipids
- How to prepare emulsion
- How fast should it spin
- How long should we wait between each step

…

Challenges :
Choice of particles concentration

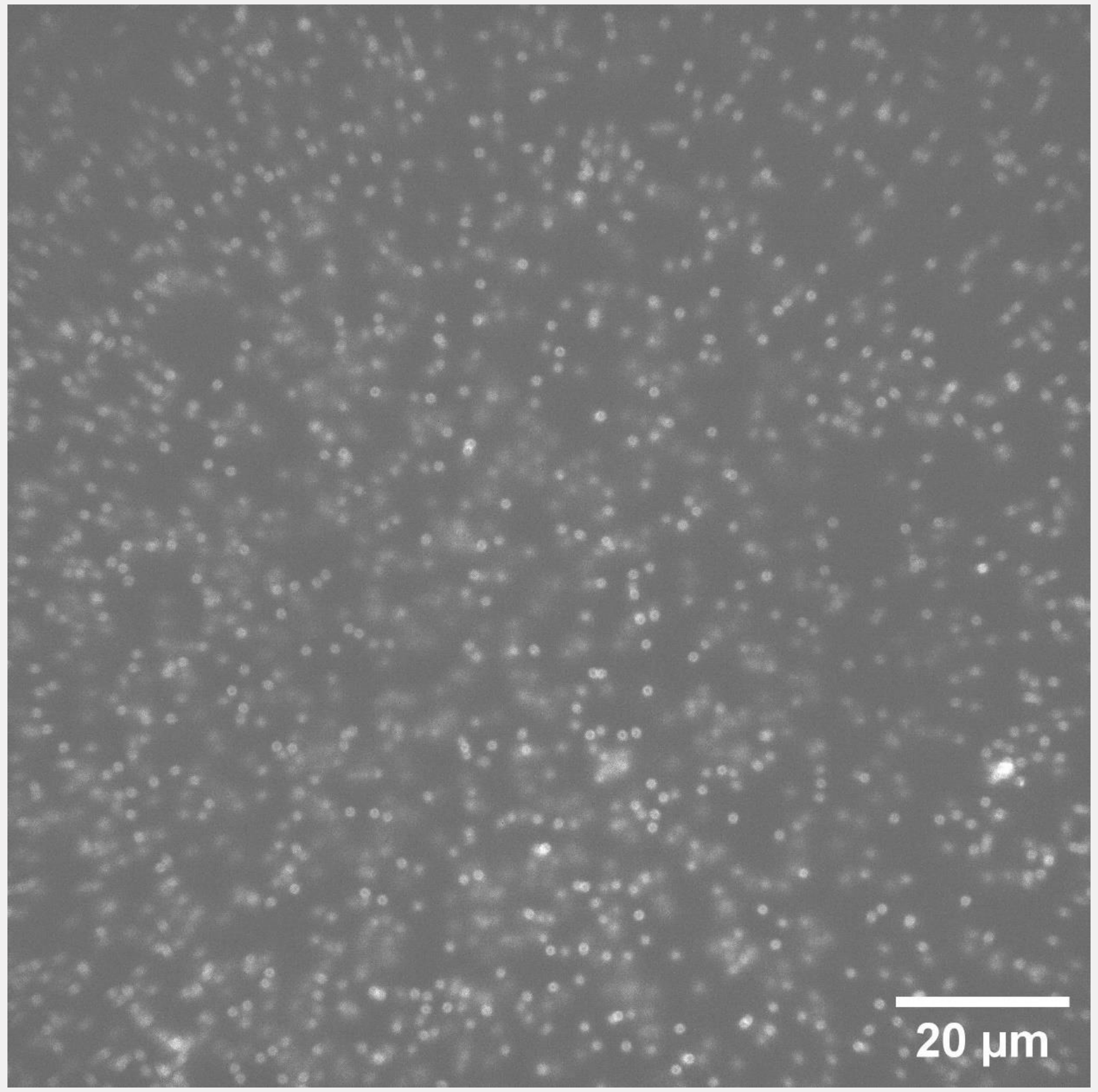




Those colloidal crystals are observed when particles are adsorbed on a membrane.

They are a subject of interest for some physicists [4].

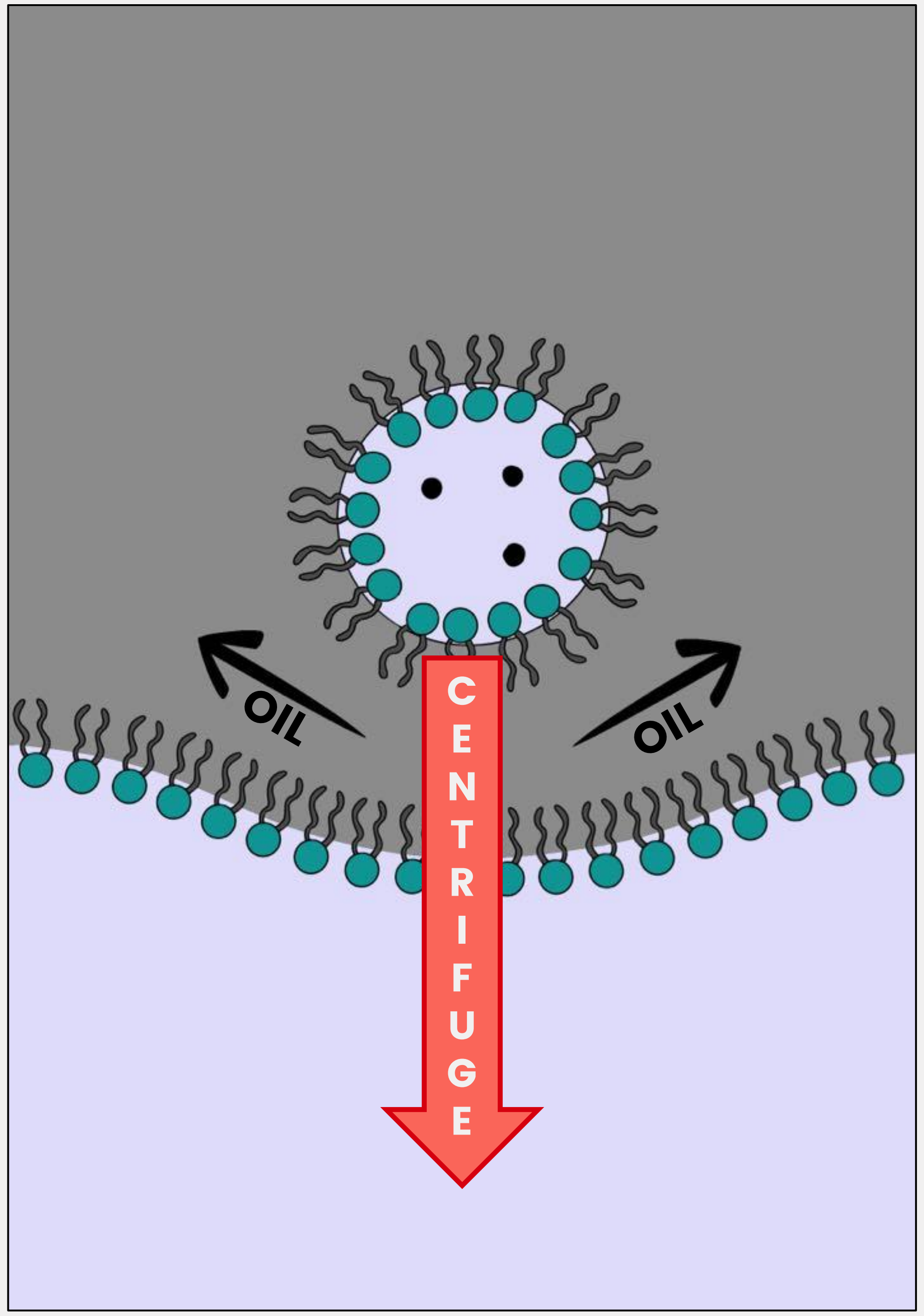
Challenges : Centrifugation force



If the sample is accelerated too much it can cause the particles (heavier part of the sample) to cross the interface alone.

As they are more accelerated than the surrounding water, they escape the vesicle and are covered with lipids, therefore visible with fluorescent light.

Challenges : Centrifugation duration

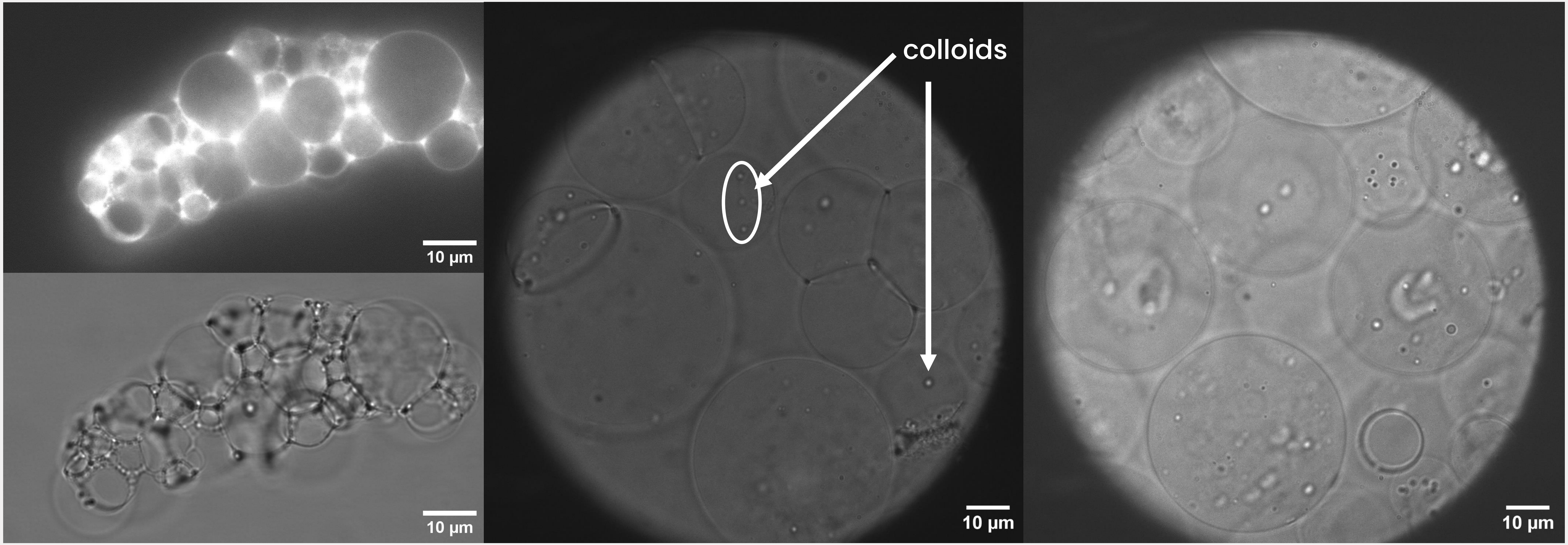


The time and the force of centrifugation are key elements for the vesicle to cross the interface. The force felt by the vesicle is :

$$F_{centrifugation} = \frac{4}{3}\pi R^3 \times \Delta\rho \times a$$

With R the radius, $\Delta\rho$ the difference of density between oil and water, and a the acceleration. It has to overcome the surface tension force, which is of the order of $10^{-8}N$.

Examples of GUVs



Ideal parameters : centrifugation at 500g $(\sim 10^{-8}\mathrm{N})$ during 30 minutes

Optical Tweezers

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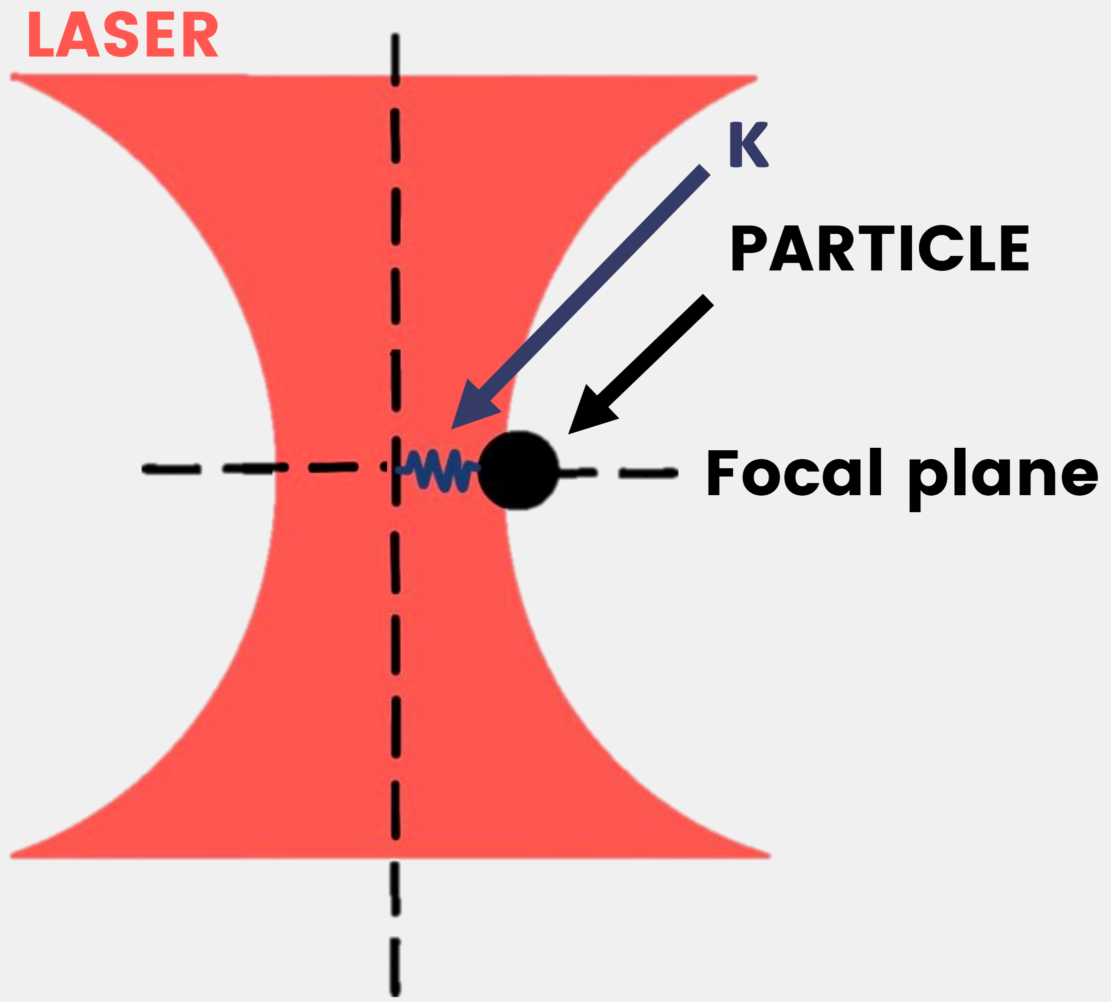
Optical tweezers

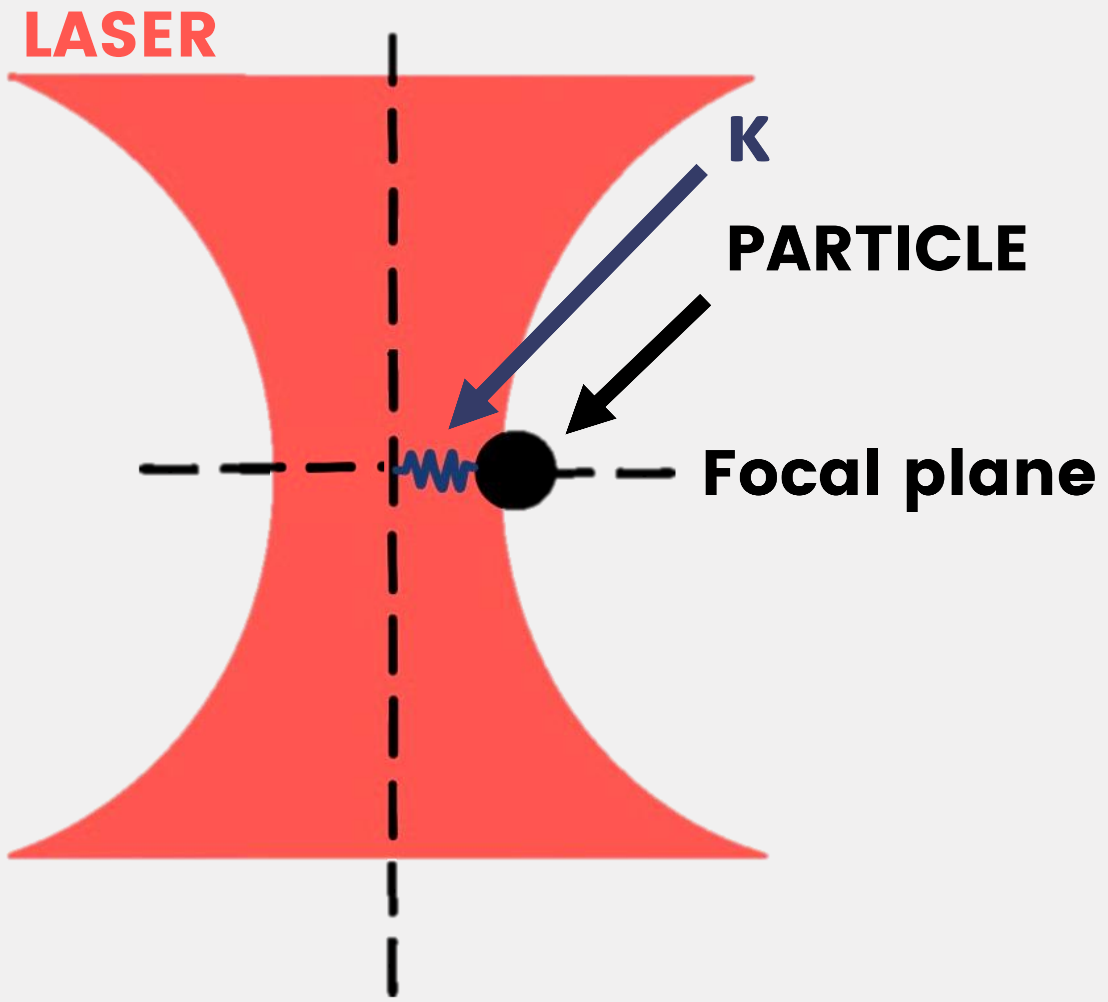


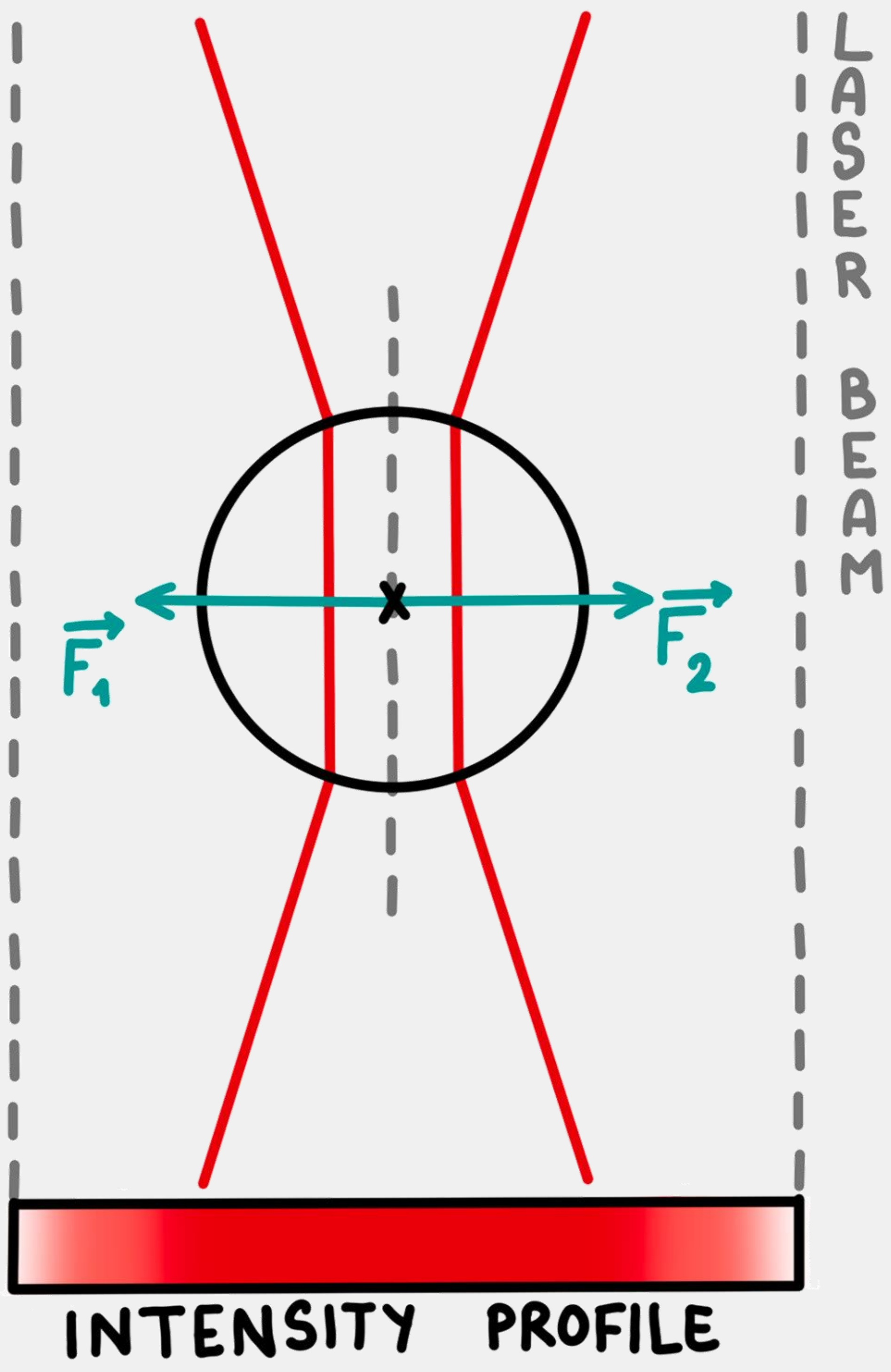
- An infrared laser creates a trap for the particles.
- The potential induced by the laser is a harmonic potential. The resulting force is :

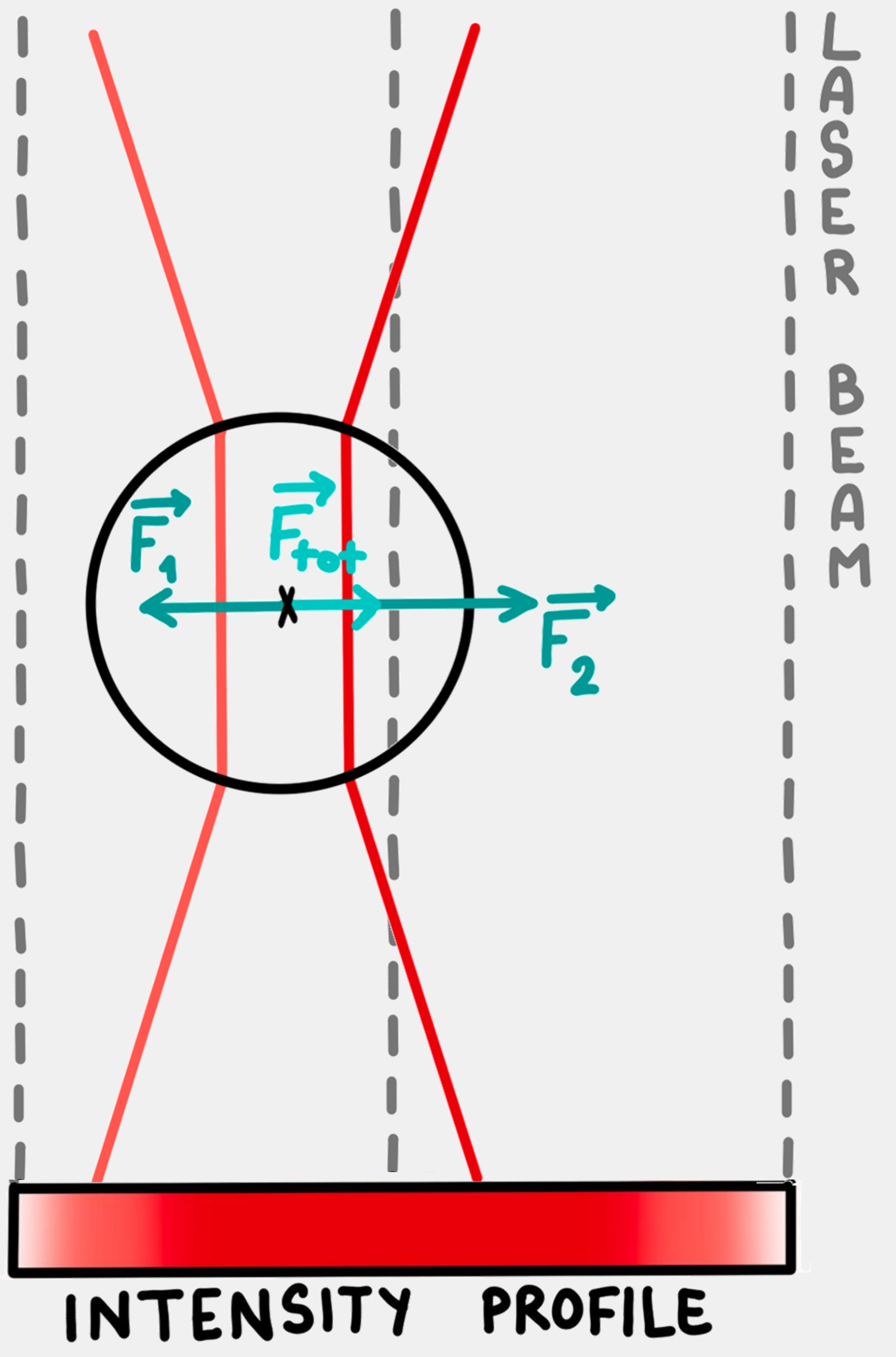
$$\vec{F} = -\kappa(\vec{x} - \vec{x}_0)$$

- Location of the trap = focal plane = equilibrium position

Optical tweezers







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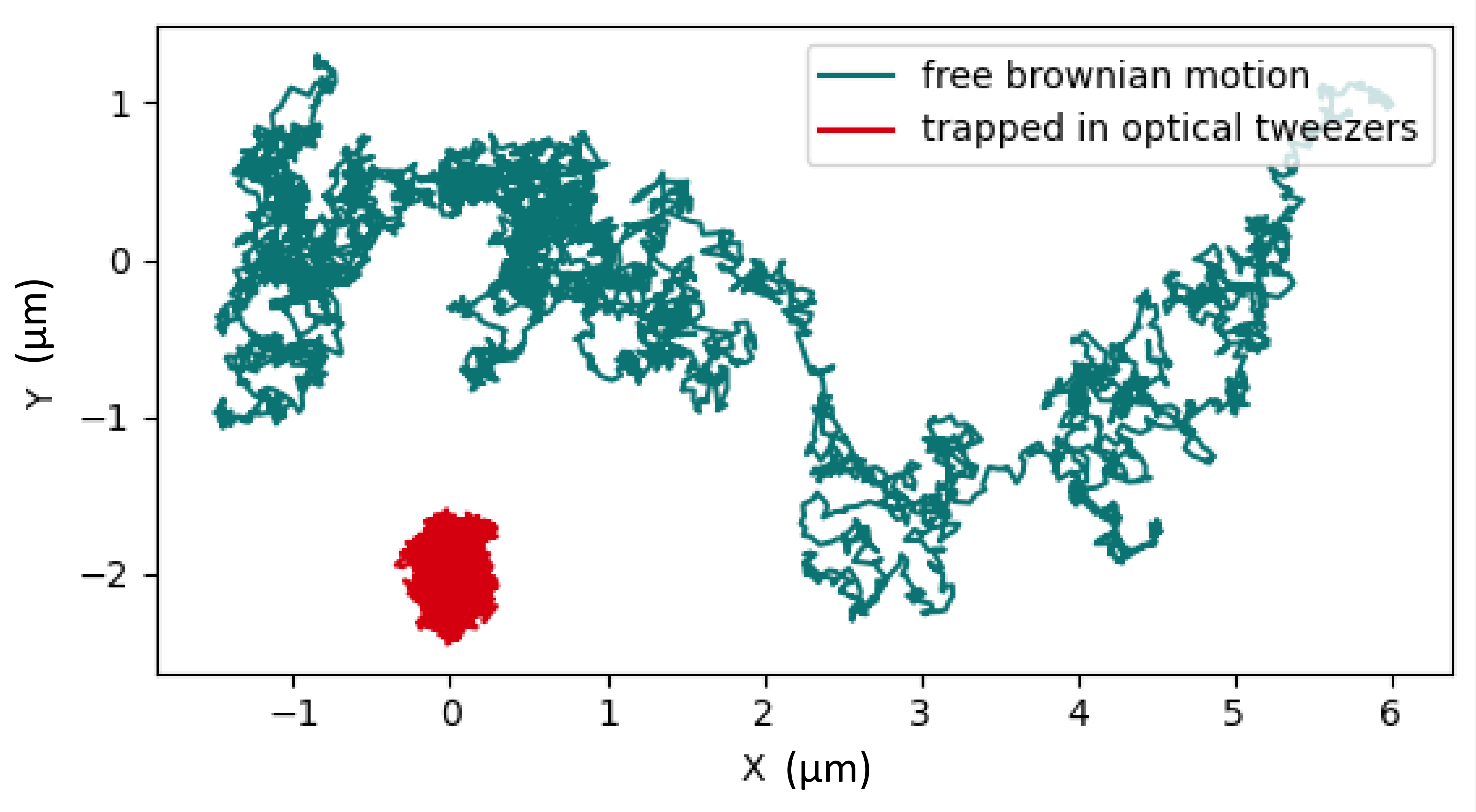
- Location of the trap = focal plane = equilibrium position

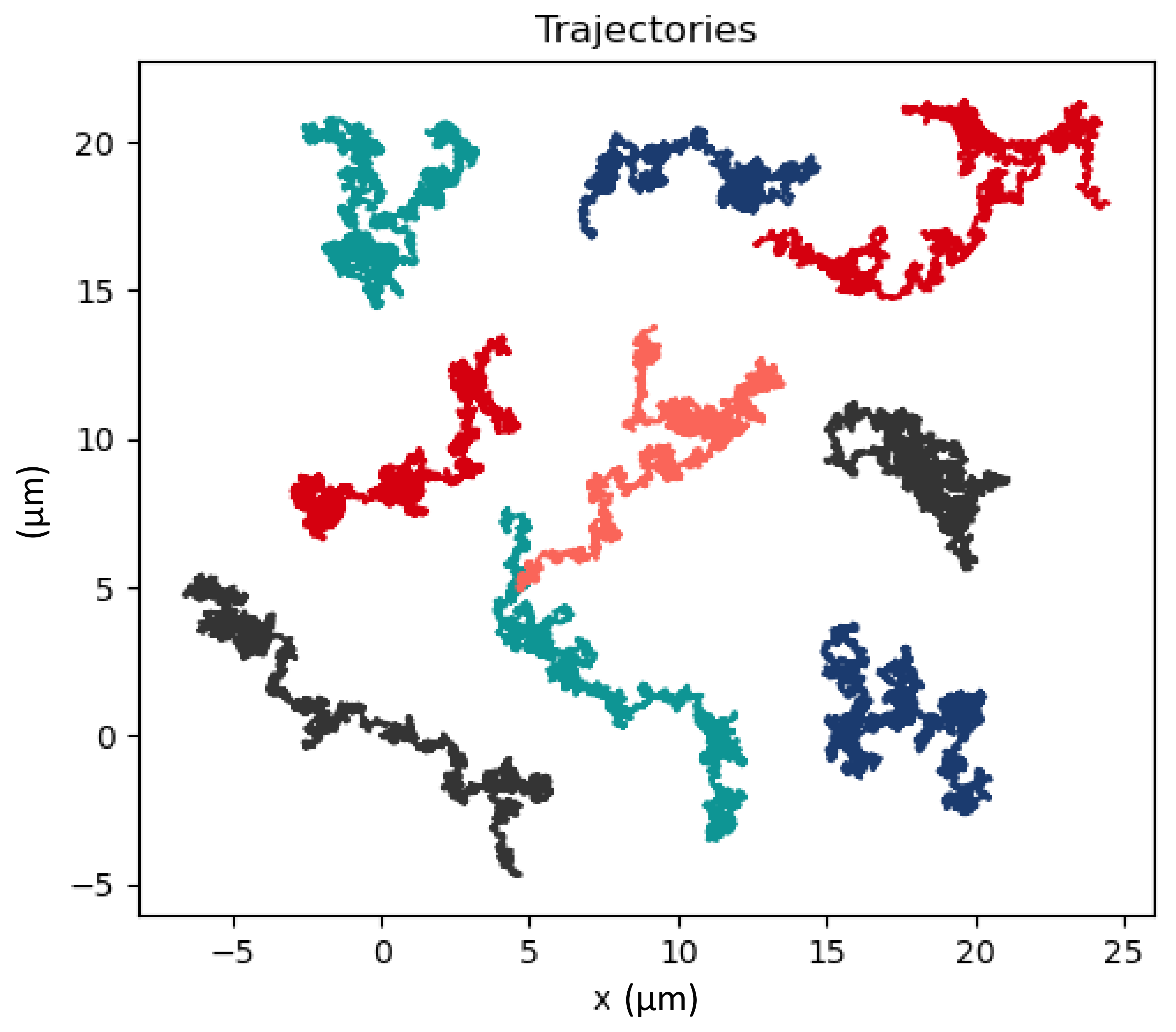
Comparison of the trajectories



Comparison of the 2D trajectories of two particles during 19 seconds.
Those were obtained using a tracking algorithm on videos of the particles.

Free particles : brownian motion

When colloids are free, they have a pure Brownian motion [6][7].
Here, our colloids are in water (viscosity $\eta \approx 10^{-3}\mathrm{Pa.s}$).
Even though they have a radius of 1 µm, they have a Brownian motion.



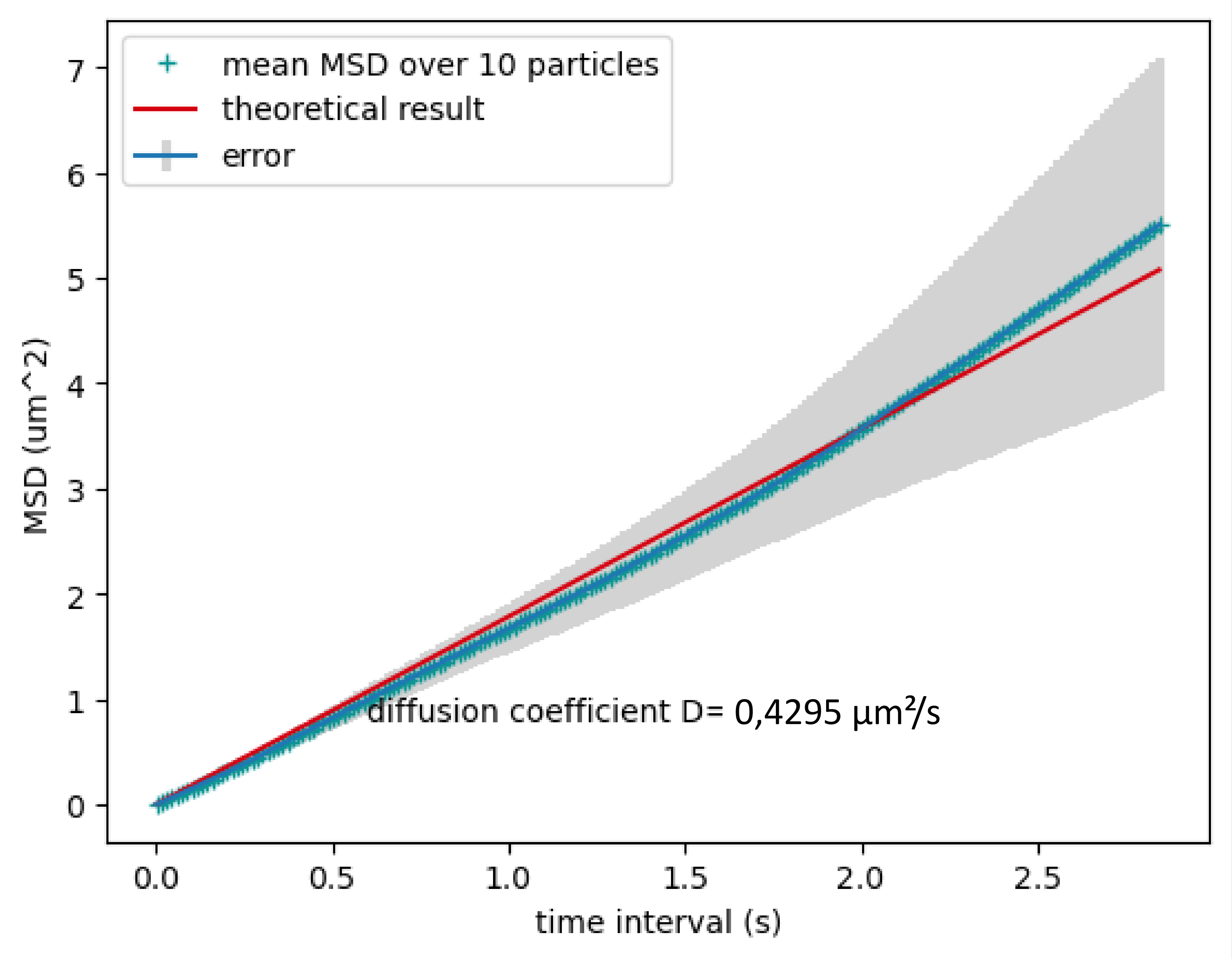
2D Trajectories of several particles
(19 seconds of motion)

Mean Square Displacement (MSD)

- MSD = mean squared distance travelled by a particle in a time interval.
- Brownian motion [7] :

$$\langle (x(t) - x_0)^2 \rangle = 2Dt$$

x_0 = initial position of the particle
D = diffusion coefficient
t = time



Mean Square Displacement (MSD)

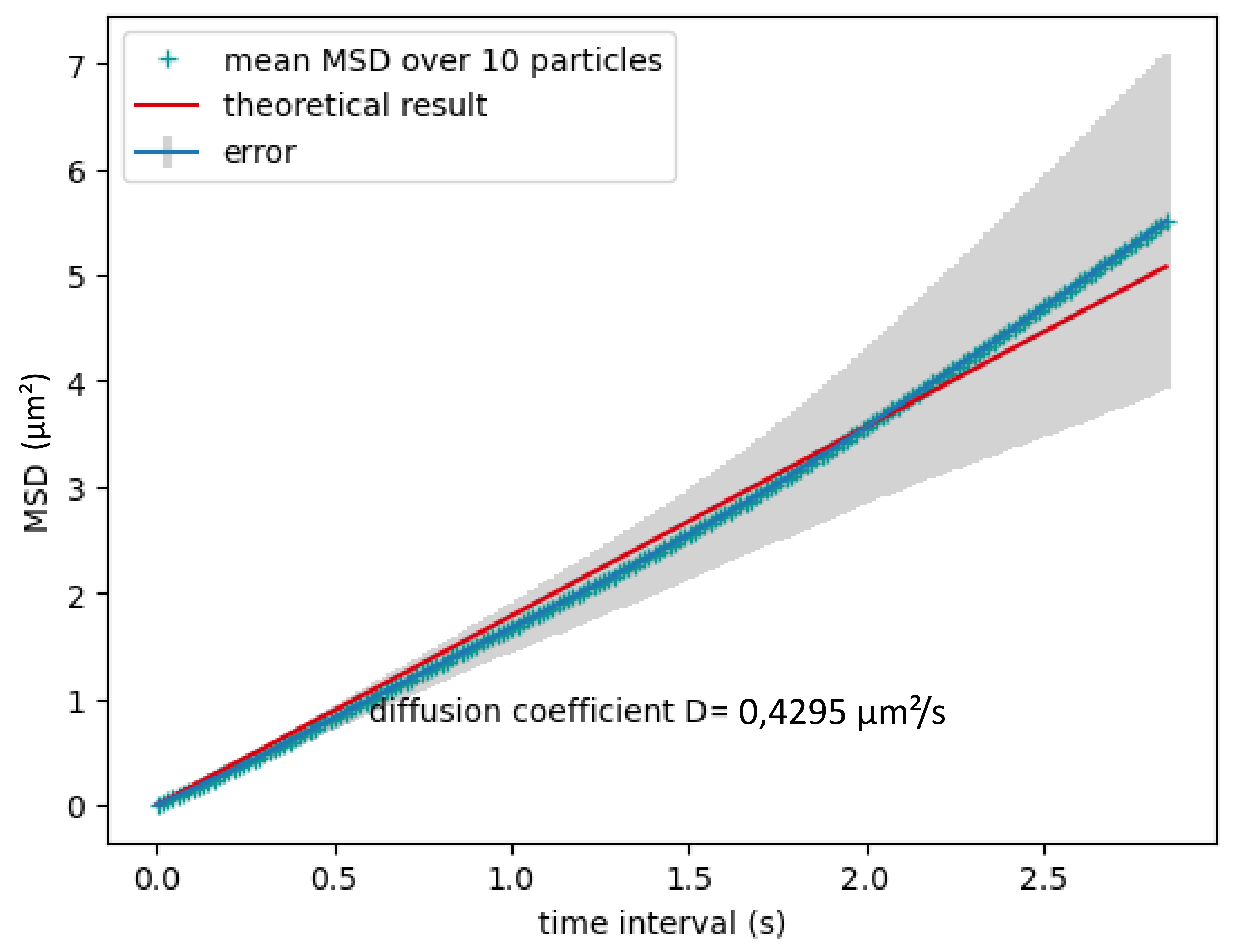
In a fluid, the theoretical value of D reads :

$$D_{th} = \frac{k_B T}{6\pi R\eta}$$

k_B = Boltzmann constant
T = temperature
R = radius of the particle
η = viscosity of the fluid

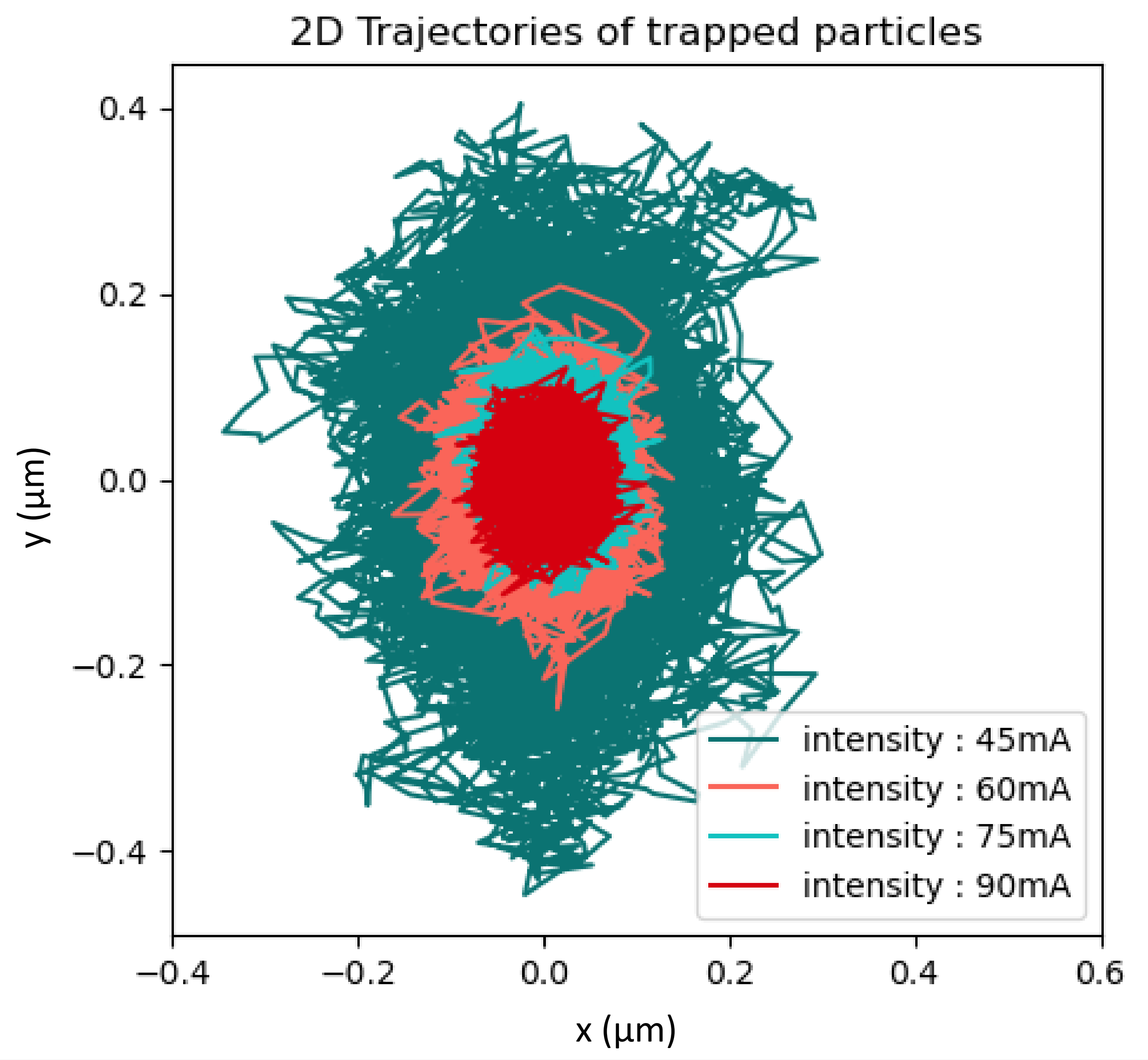
$$D_{th} \approx 4{,}46 \times 10^{-1}\ \mu m^2/s$$

$$D_{exp} \approx (4{,}30 \pm 0{,}29) \times 10^{-1}\ \mu m^2/s$$



Influence of the laser power

- The more current in the laser, the stronger the trap gets;
- The power of the laser and the intensity are linearly related; those intensities correspond to a 0-50 mW range of power
- The maximum power of the laser is about 1W
- The laser beam is not perfectly circular in the trapping plane, leading to anisotropic trajectories.



Determination of the spring constant

$$\vec{F} = -\kappa(\vec{x} - \vec{x}_0)$$

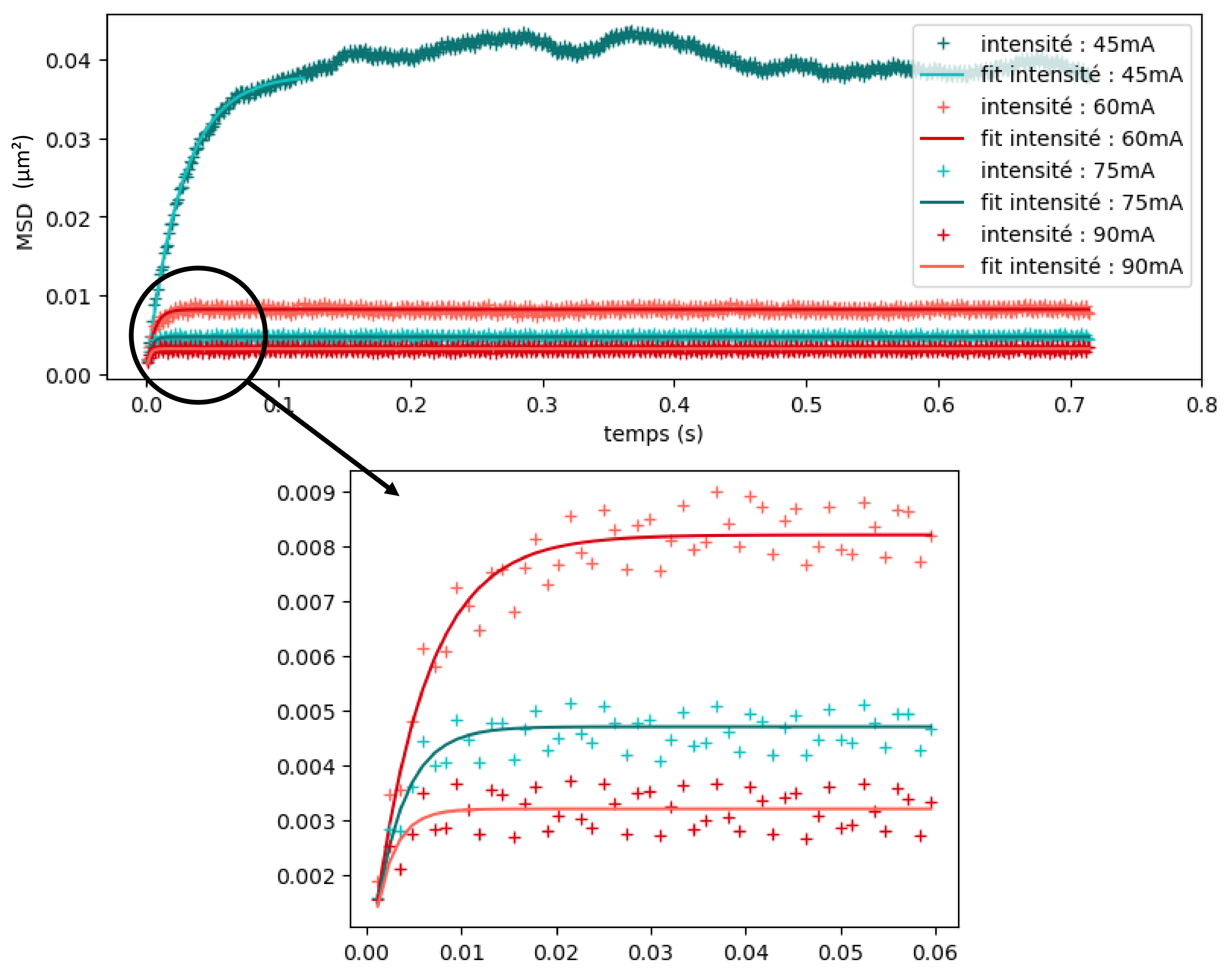
- Trapped particles MSD is different [6] :

$$MSD = \frac{2k_BT}{\kappa}\left(1 - \exp(-\kappa t/\gamma)\right)$$

Where κ is the spring constant, γ is the friction coefficient $(D = k_BT/\gamma)$.

- Eventually, it reaches a plateau as the particle is trapped
- The plateau gives the stiffness constant of the trap :

$$\text{MSD} = \frac{2k_BT}{\kappa} \quad \text{for} \quad t \gg 1$$

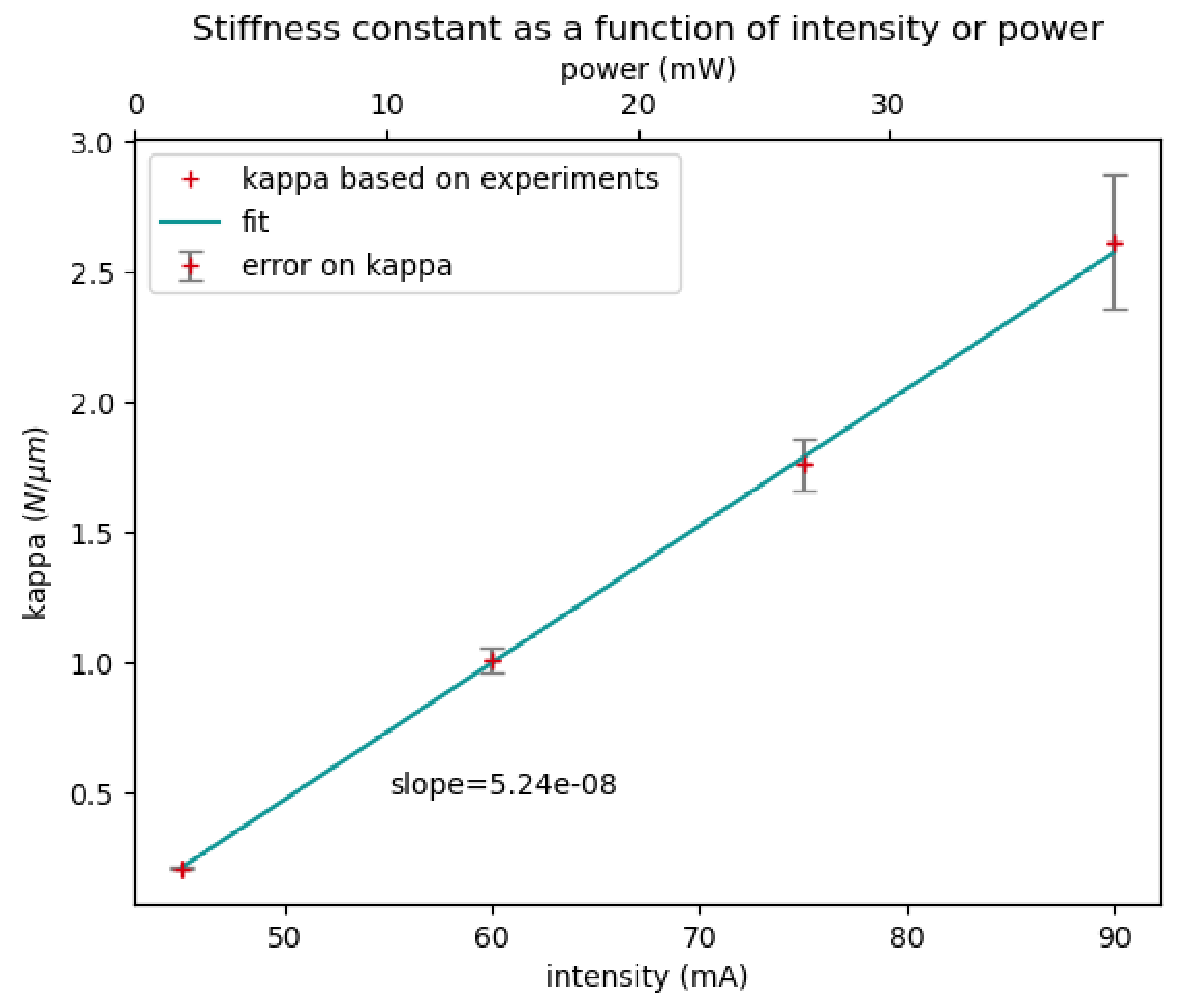


Determination of the spring constant

$$\vec{F} = -\kappa(\vec{x} - \vec{x}_0)$$

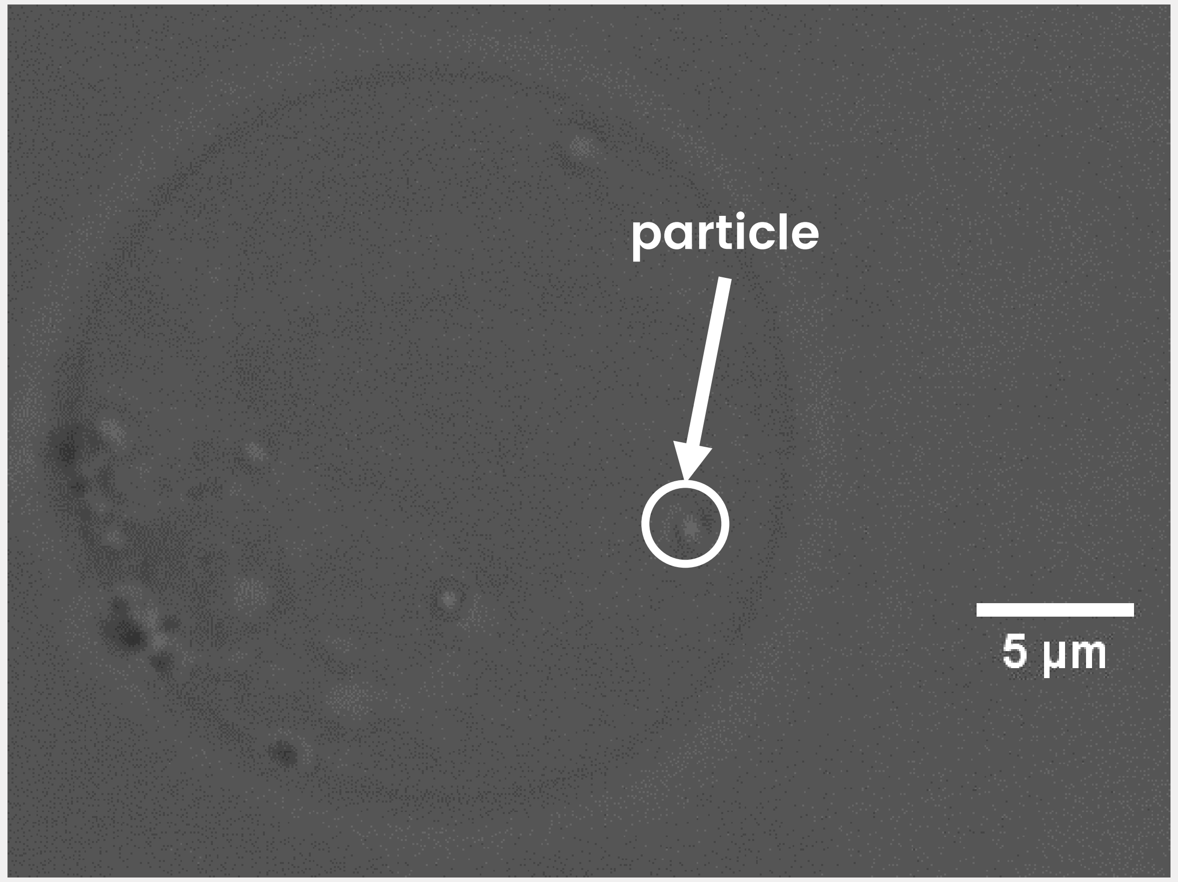
- The spring constant κ grows linearly with intensity or power.

- Calibration of the optical tweezers; now, when we use the laser with a given intensity, we know how strong the trap is.

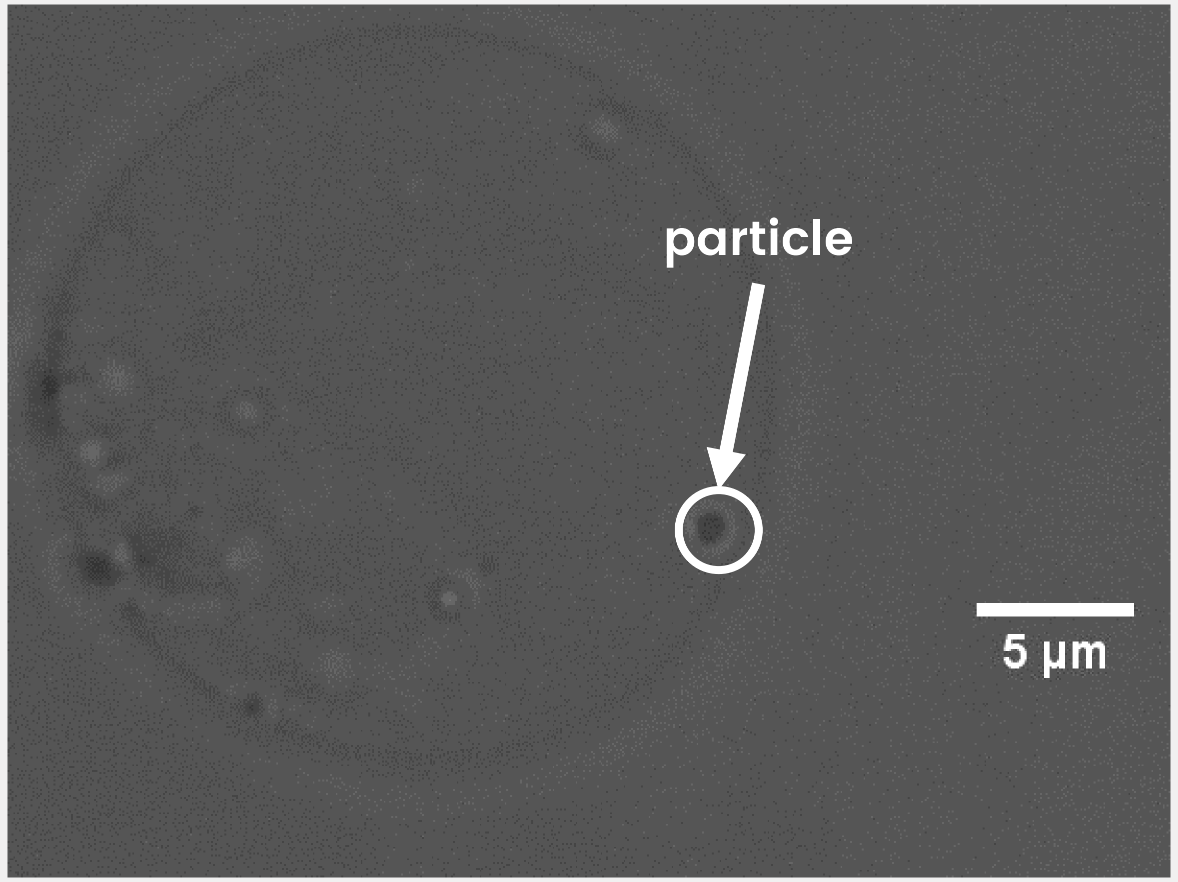


Results & Discussion

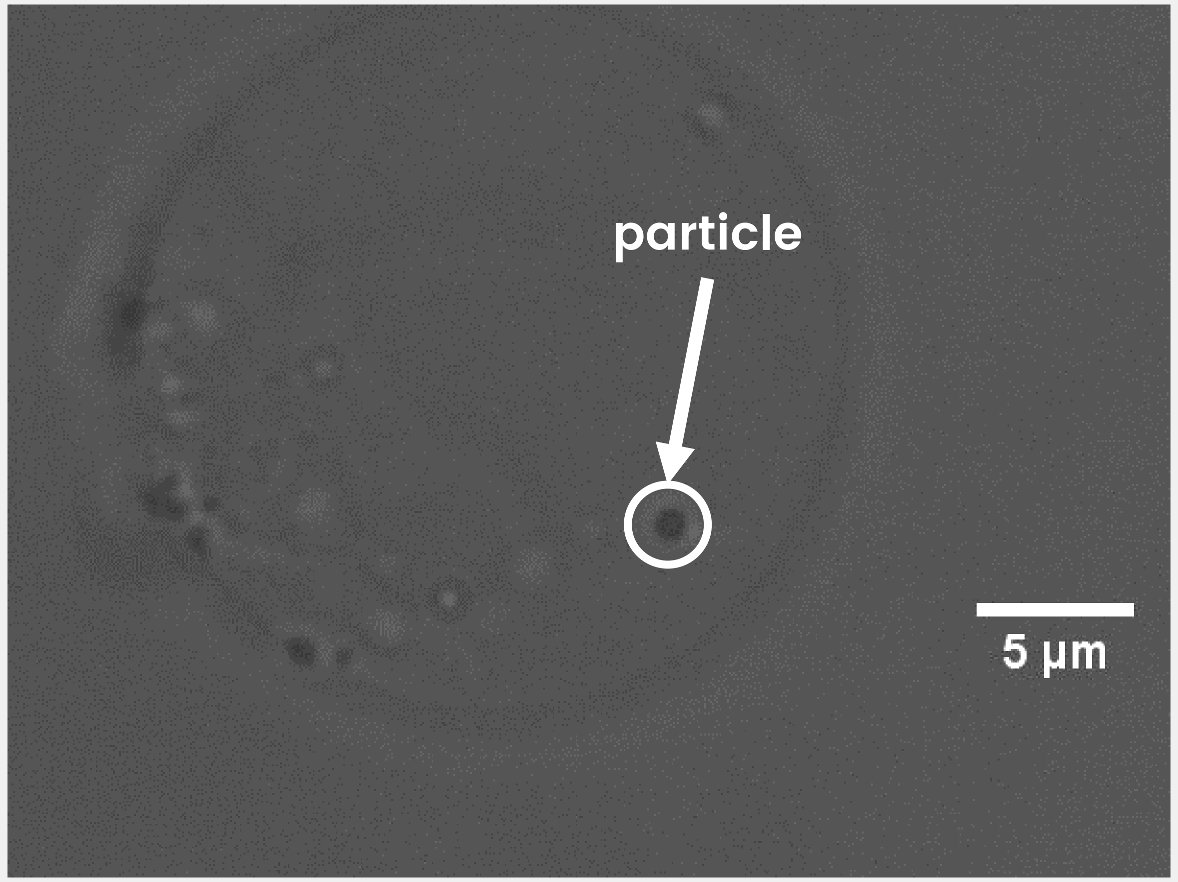
GUVs encapsulating particles



We trap the particle with optical tweezers

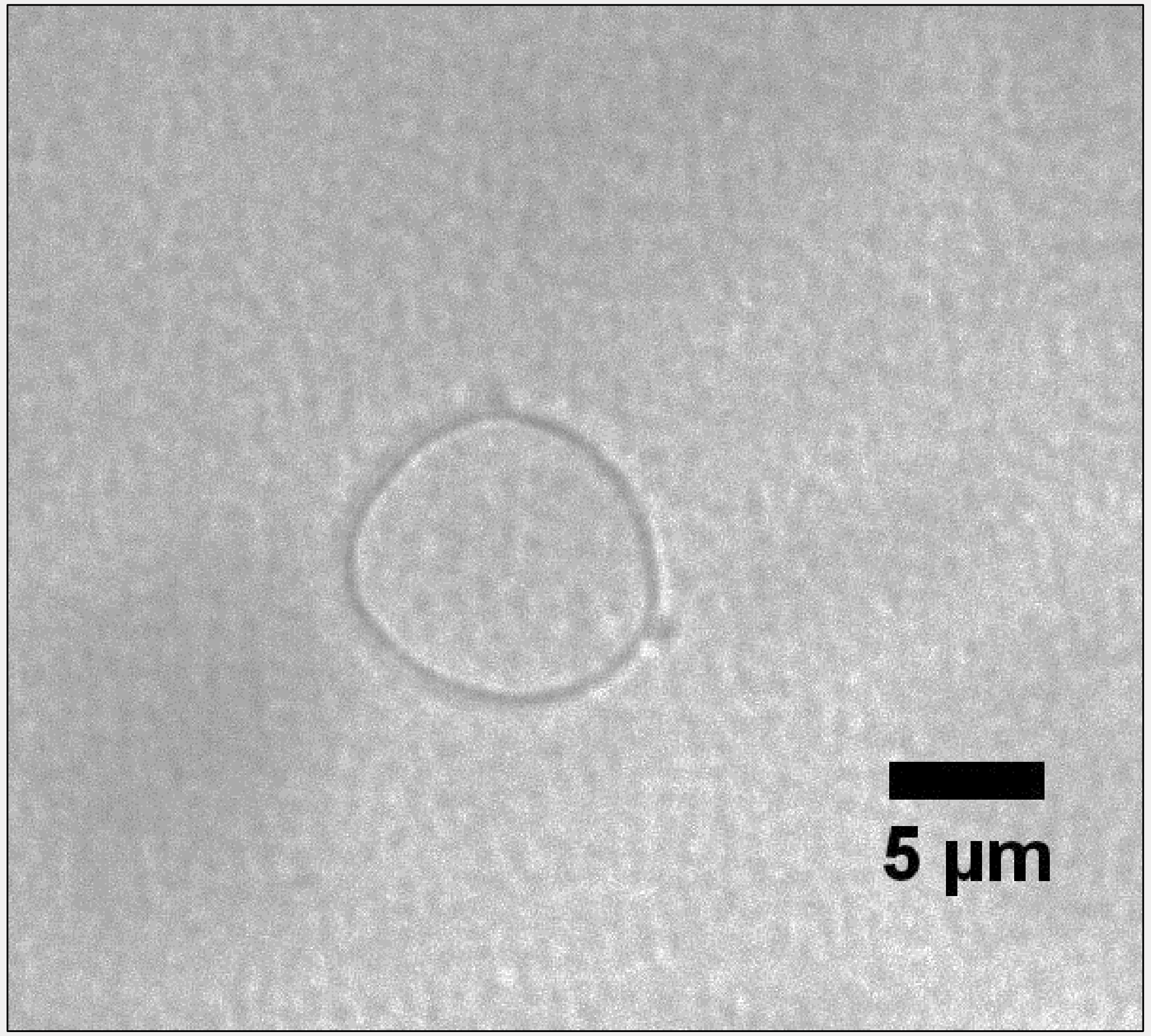


We move it towards the membrane and we push it



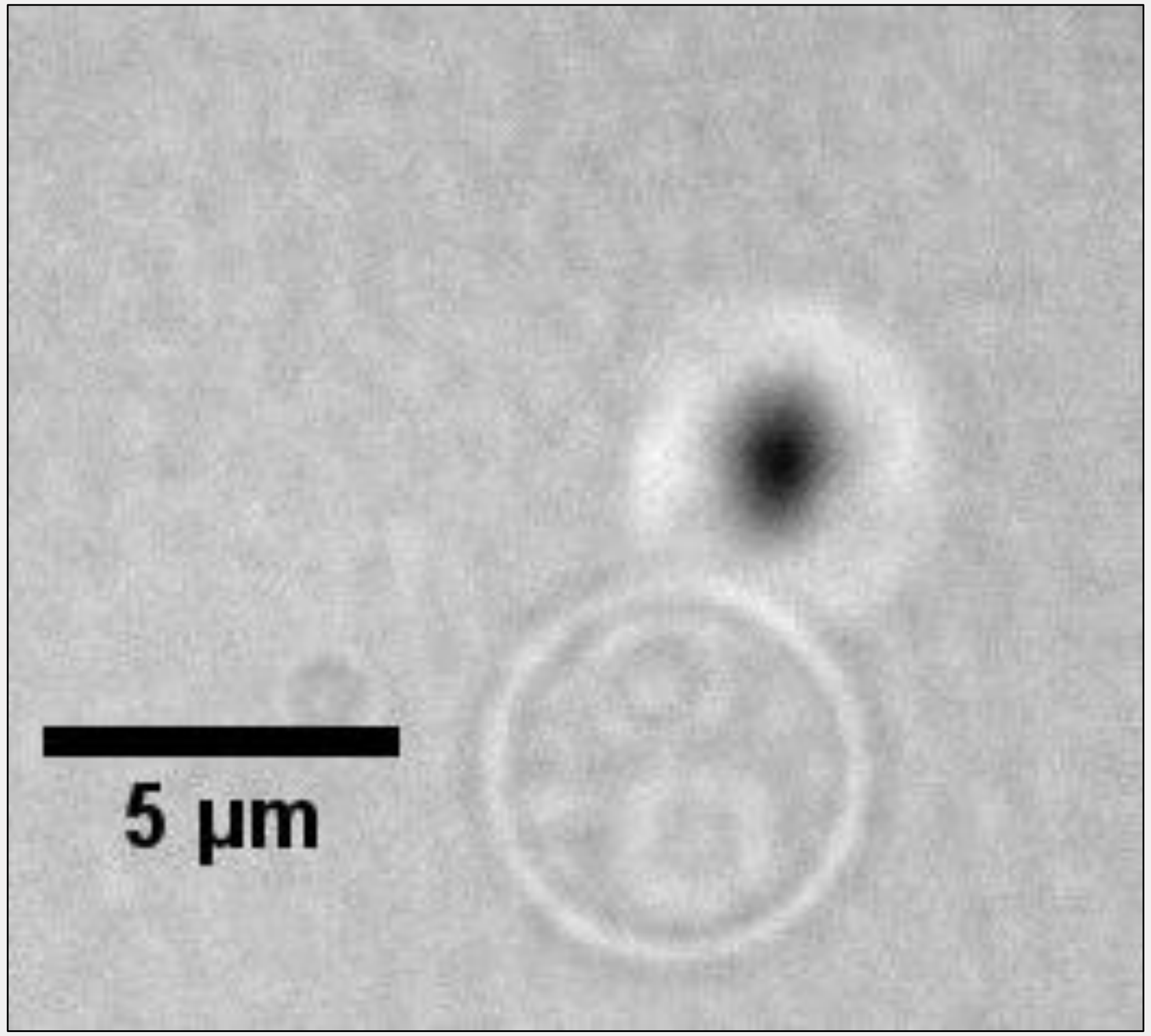
The membrane does not deform. The vesicle is moved by the kick given by particle.

GUVs are not “floppy” enough



"Floppy" GUV

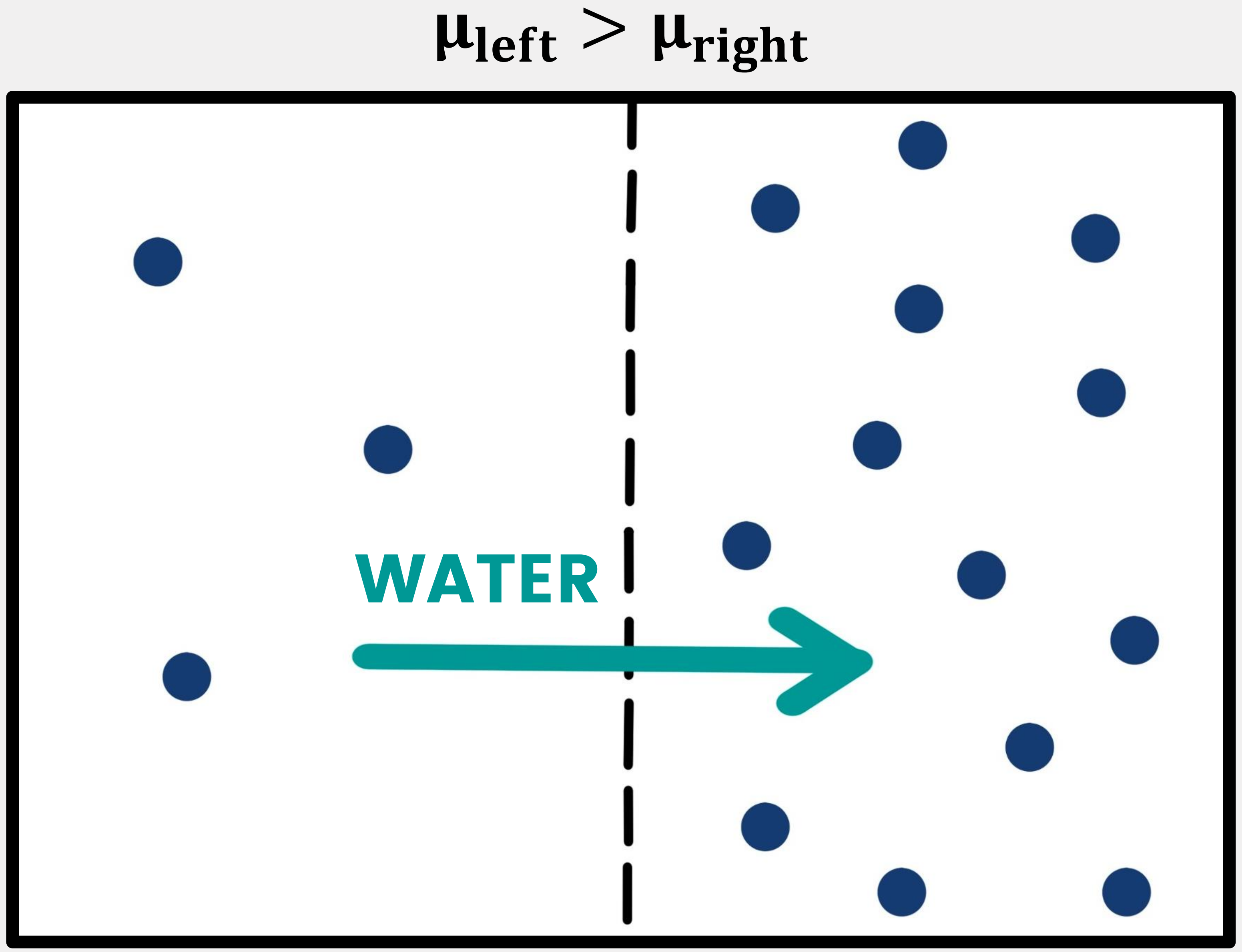
Membrane tension typically $< 10^{-7} N/m$



"Stiff" GUV

Membrane tension typically $> 10^{-7} N/m$

Solution : osmotic pressure ?



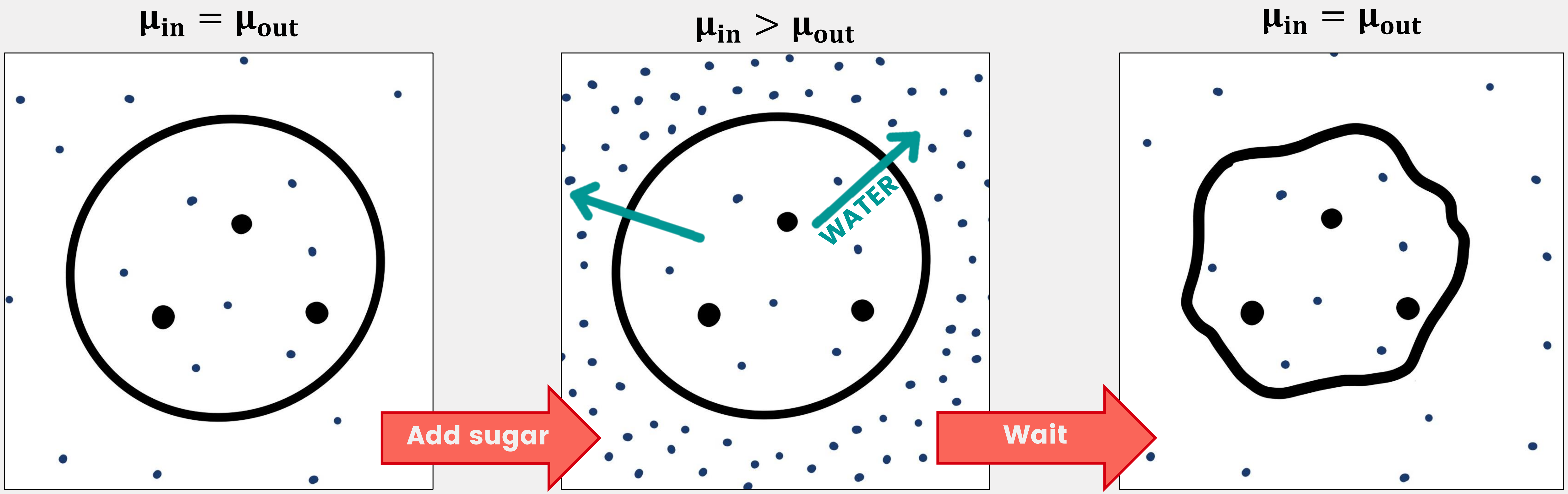
– – – Porous membrane

● Sugar

μ = chemical potential

If the membrane can let water trough, the water will move to balance the chemical potential.

Solution : osmotic pressure ?

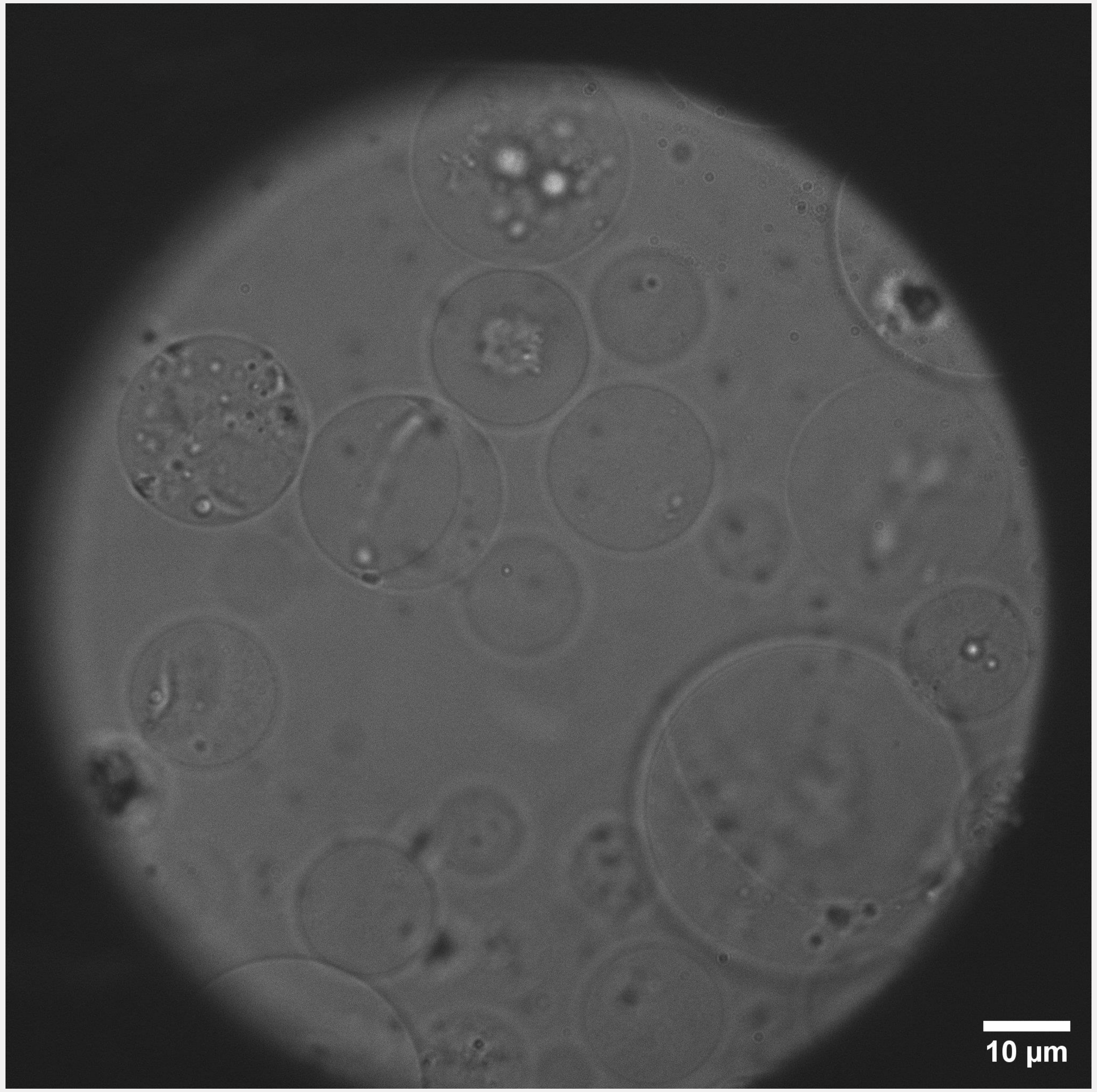


Initial state : the membrane is too stretched and we can’t pull a particle out of it.

The addition of sugar outside of the vesicle causes the water to go out.

Equilibrium is restored; the vesicle contains less water and is therefore “floppy”.

Solution : osmotic pressure ?



After adding a lot of sugar (+30%) and letting it rest all night, we did not see any progress

CONCLUSION & ACKNOWLEDGEMENTS

And it was already the end…

What we did :

- GUVs with a controlled number of colloids
- Optical tweezers calibration
- Even though there was too much tension, we could move the vesicles with the optical tweezers

What's next :

- Fabrication of low tension vesicles
- Measurement of the physical properties of the membrane (bending modulus, tension, …)
- Compare with theoretical models [8][11] and understand shape transition

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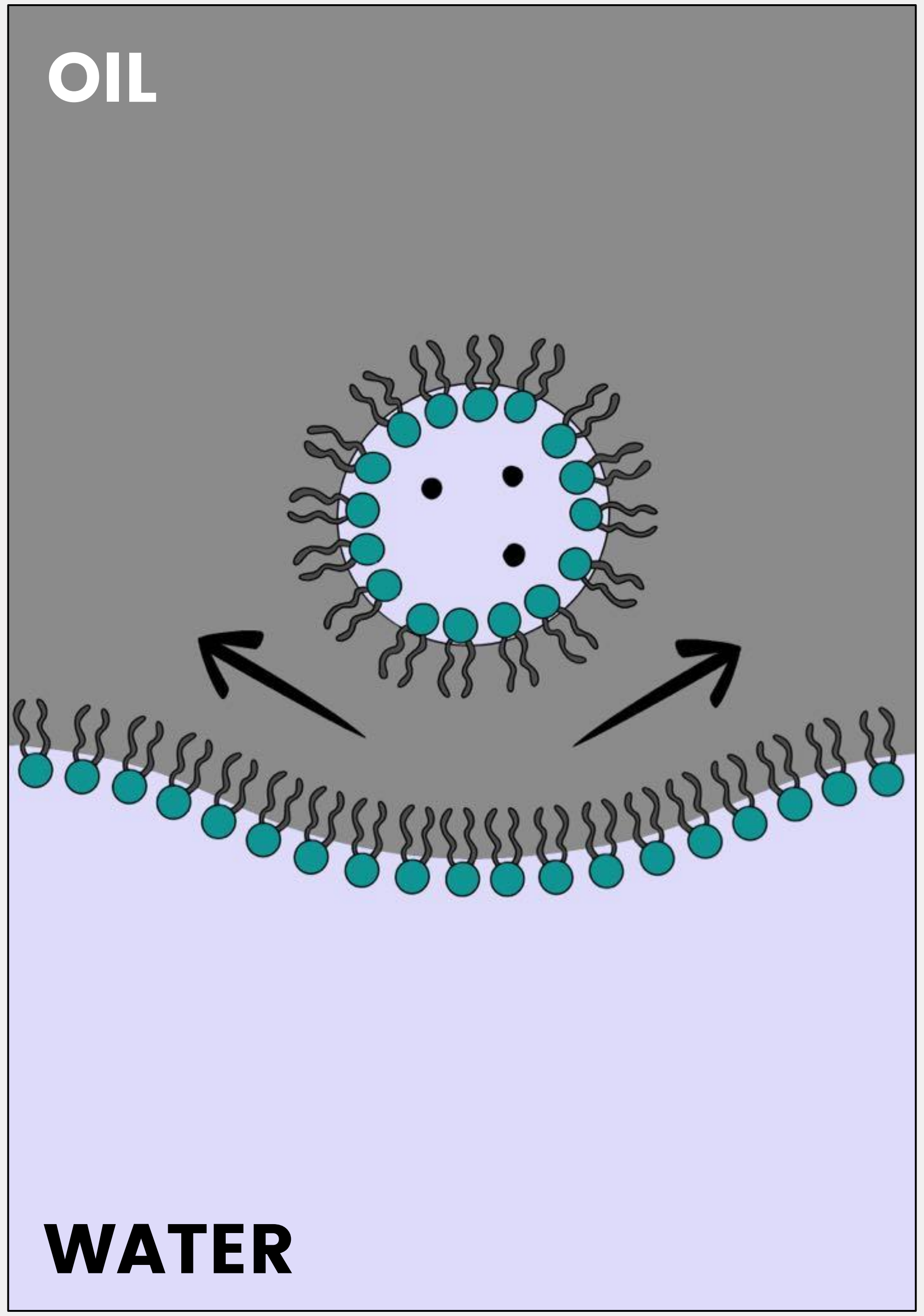
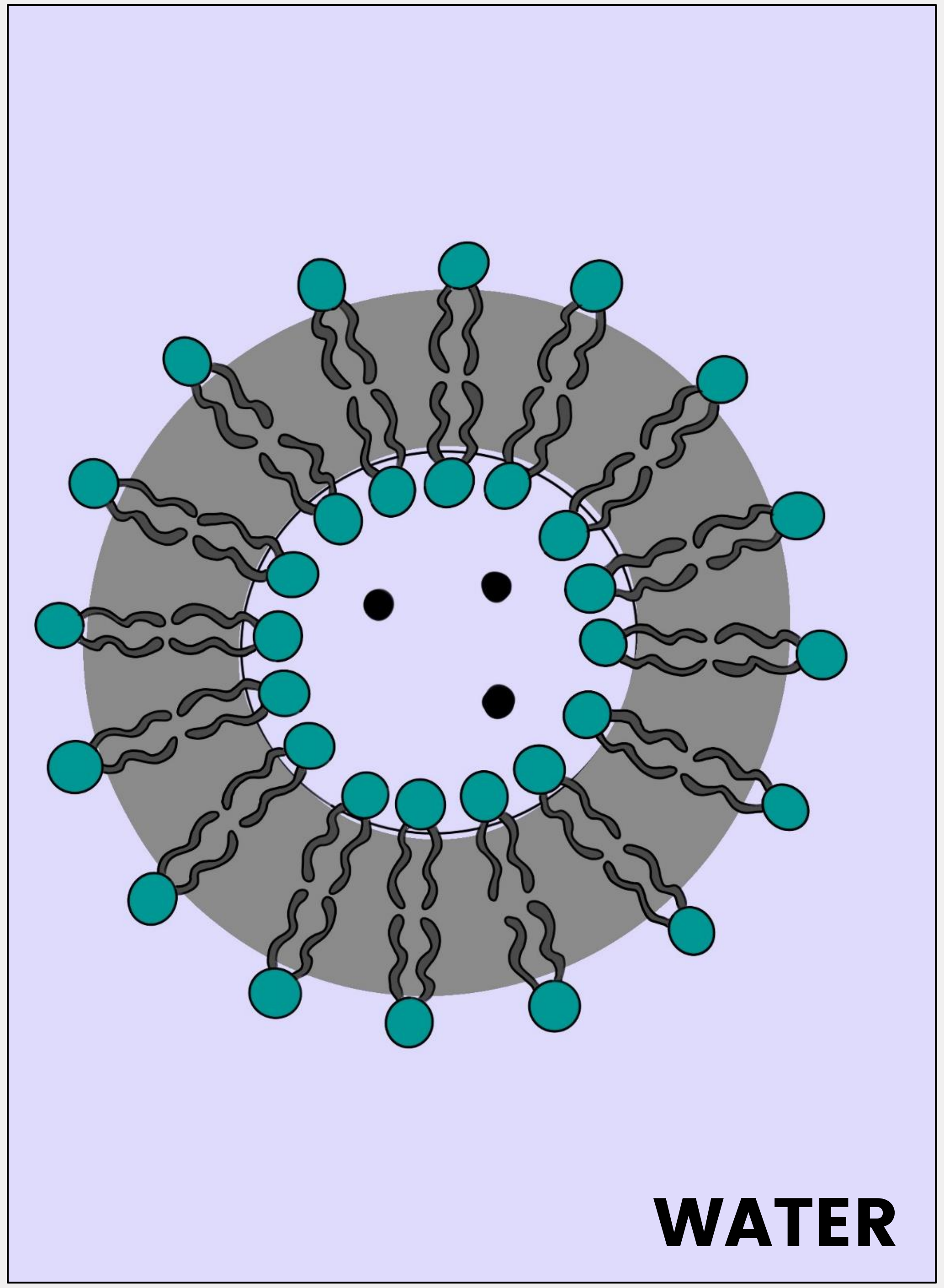
Thank you for listening

ANNEX

Solution : osmotic pressure ?





Mean Square Displacement (MSD)

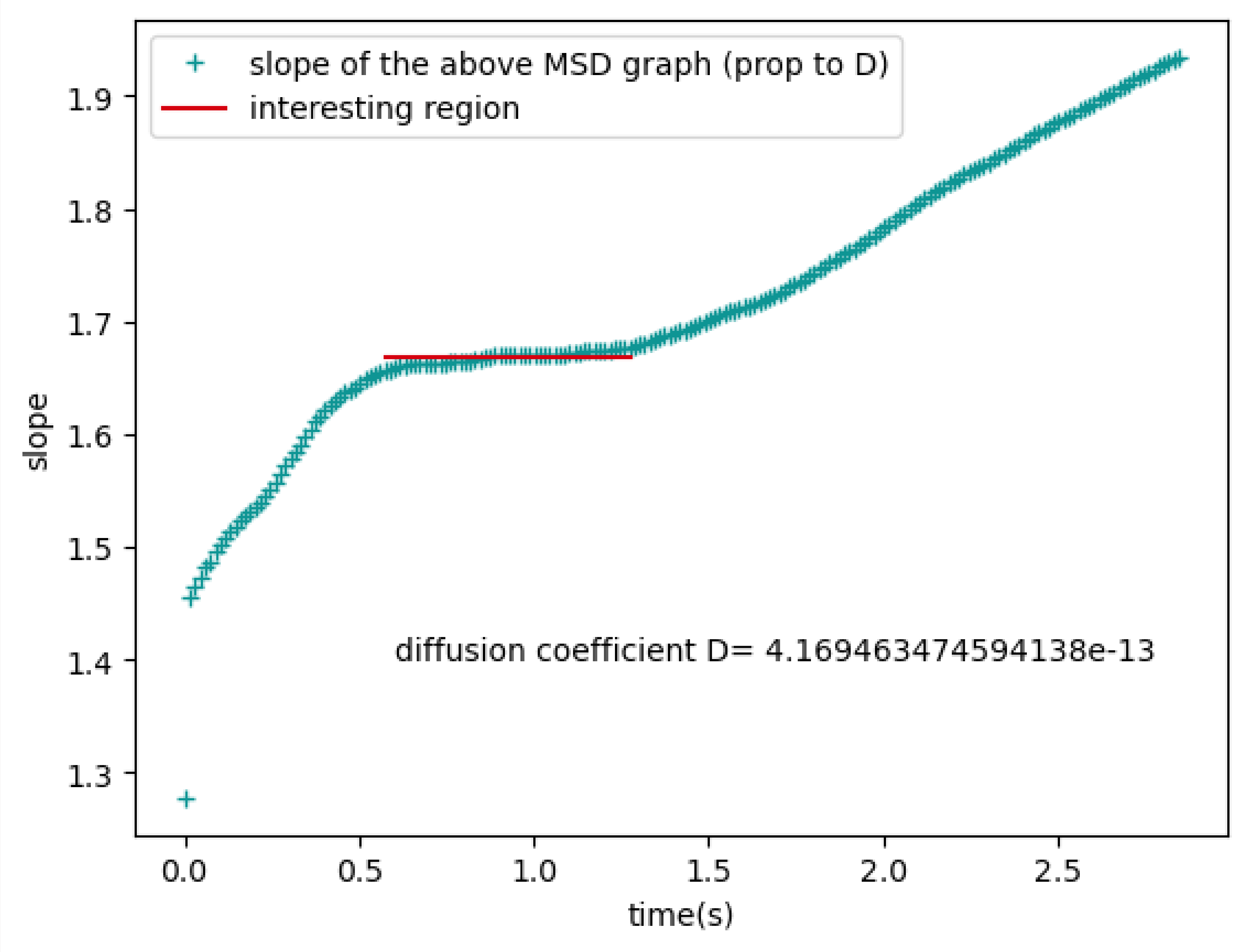
- The experimental value found before is not reliable, because it is the mean slope over all points.

- Small time interval : not enough precision, long time slot between two frames

- Long time interval : drift, currents, …

- More reliable value if we look at the region where D is constant

$$D_{exp} \approx 4{,}17 \times 10^{-13}\ m^2/s$$



Solution : micropipette ?