STUDY OF PHOSPHOLIPID LAYERS USING FRAPP SETUP

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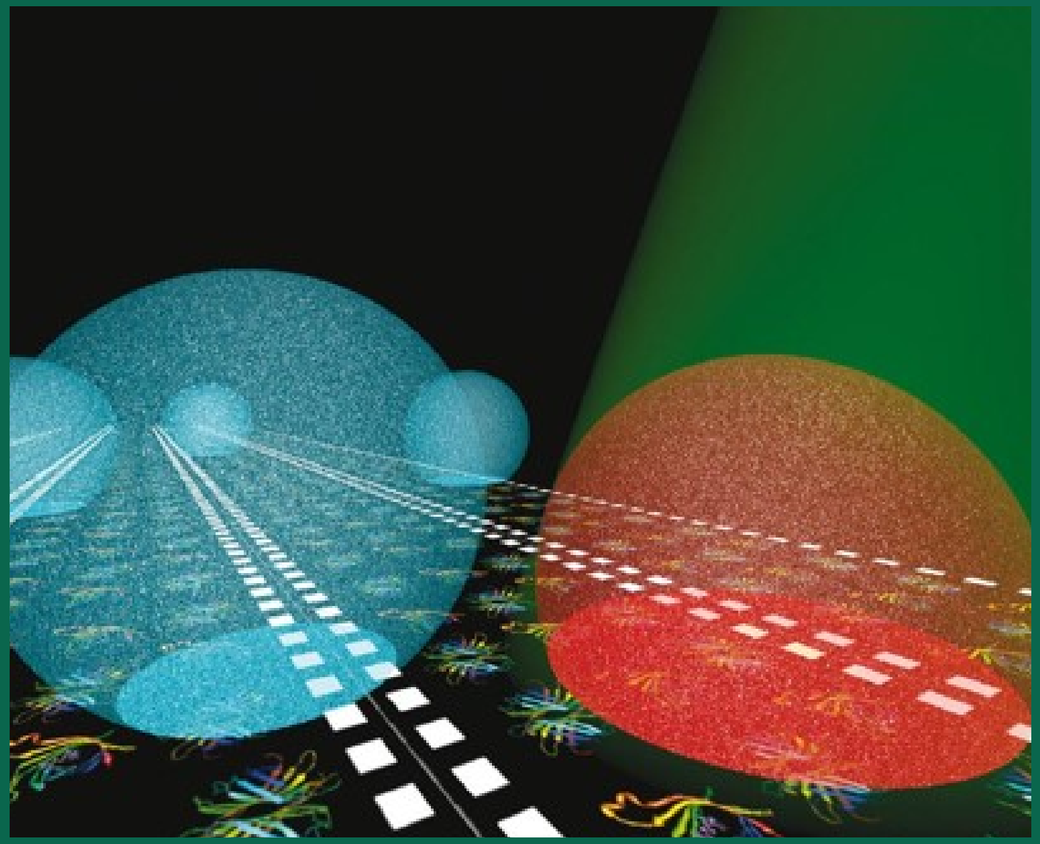
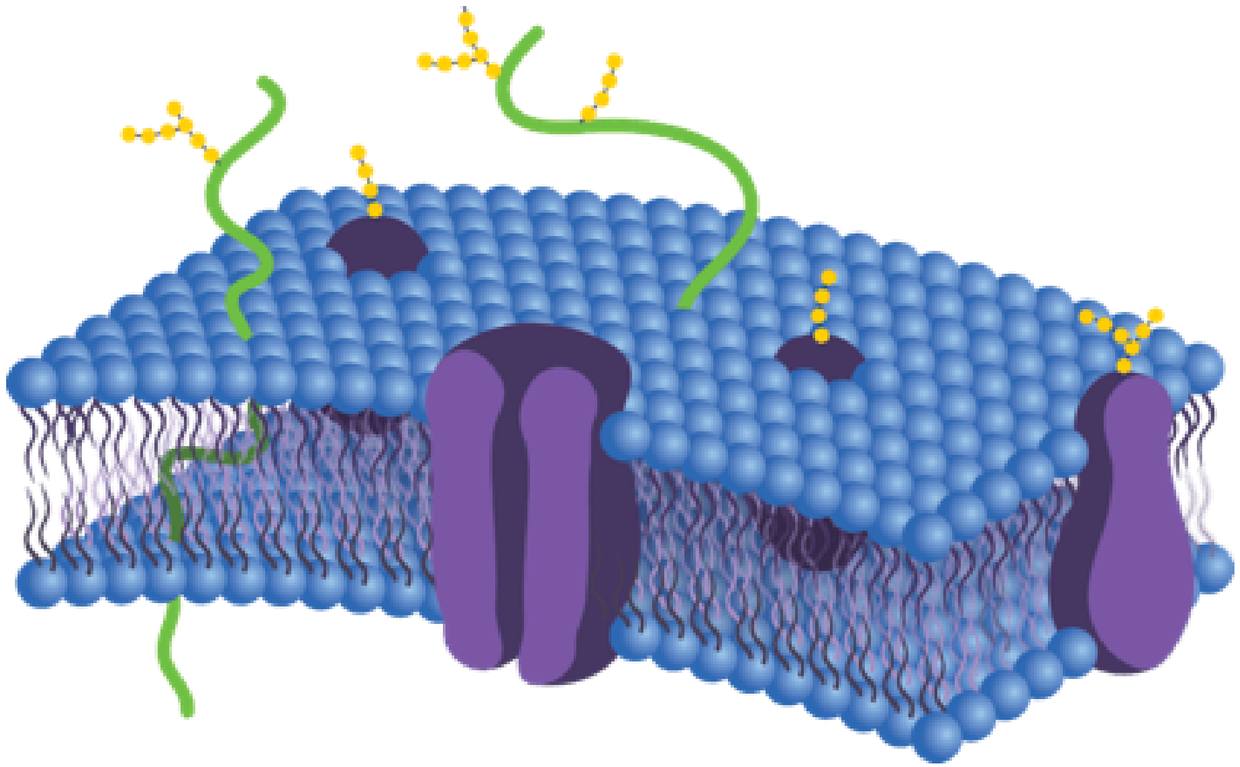


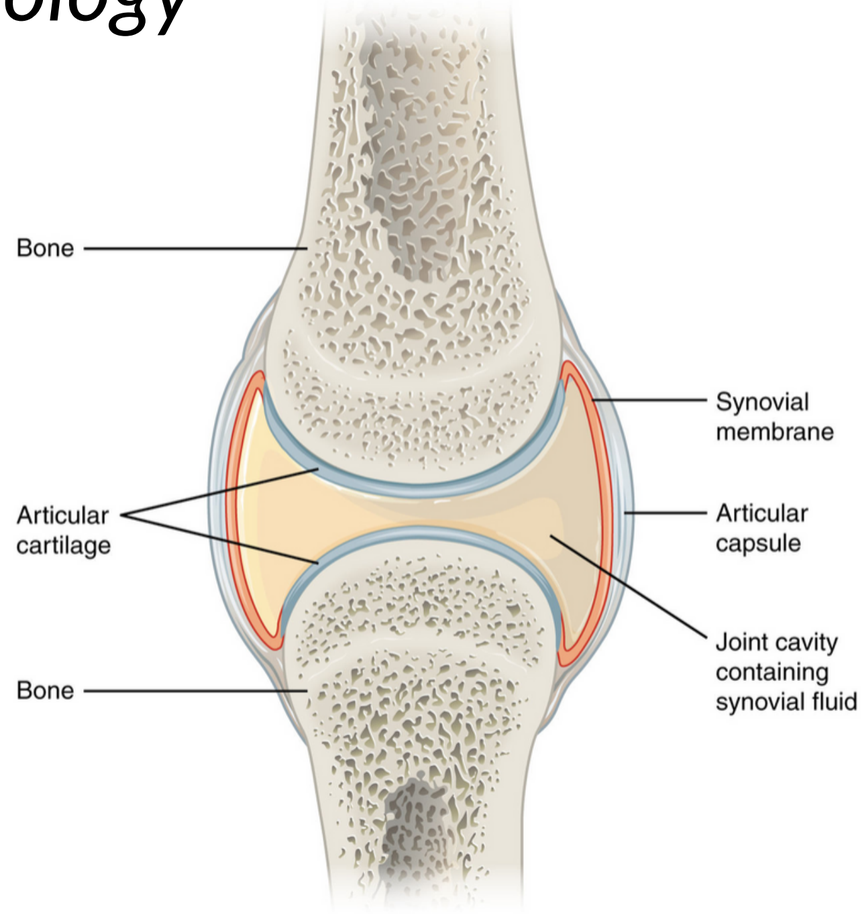
HELSTROFFER SWEN- FESSLER FLORENT

M1 Physics - May 2020

INTRODUCTION : *Lipid bilayers in biology*



Source : https://www.ck12.org/

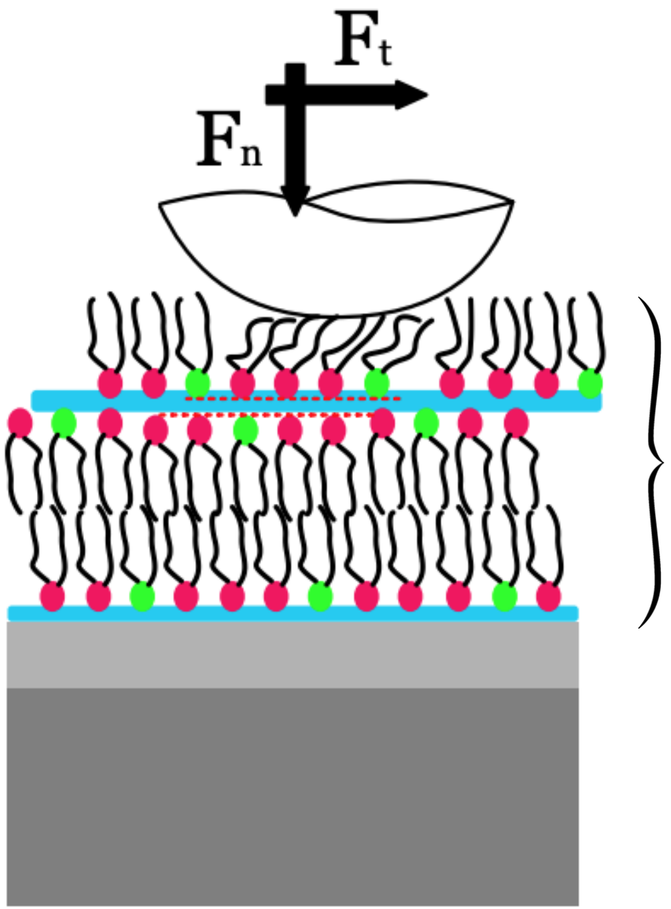


Source : Wikipedia

Fundamental constituents of **biological membranes.**

Interest for physicists to study their ***mechanical, rheological and tribological properties.***

INTRODUCTION : *Long term stakes of the project*



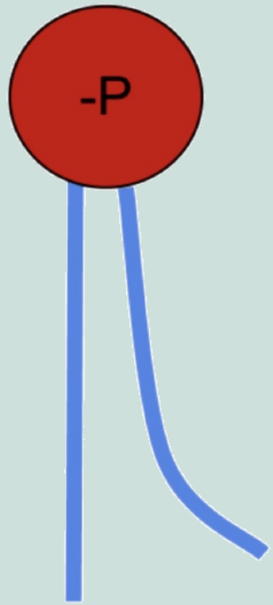
Understand the ***tribological*** and ***rheological*** properties of phospholipid multilayers

PHOSPHOLIPID MULTILAYERS

We focused on the ***velocimetry and diffusion*** parts of the ***NanoTribo-FRAPP*** experimental setup in ICS.

MATERIALS AND METHODS :

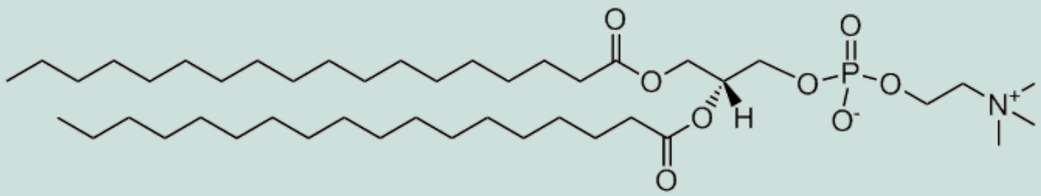
Generalities



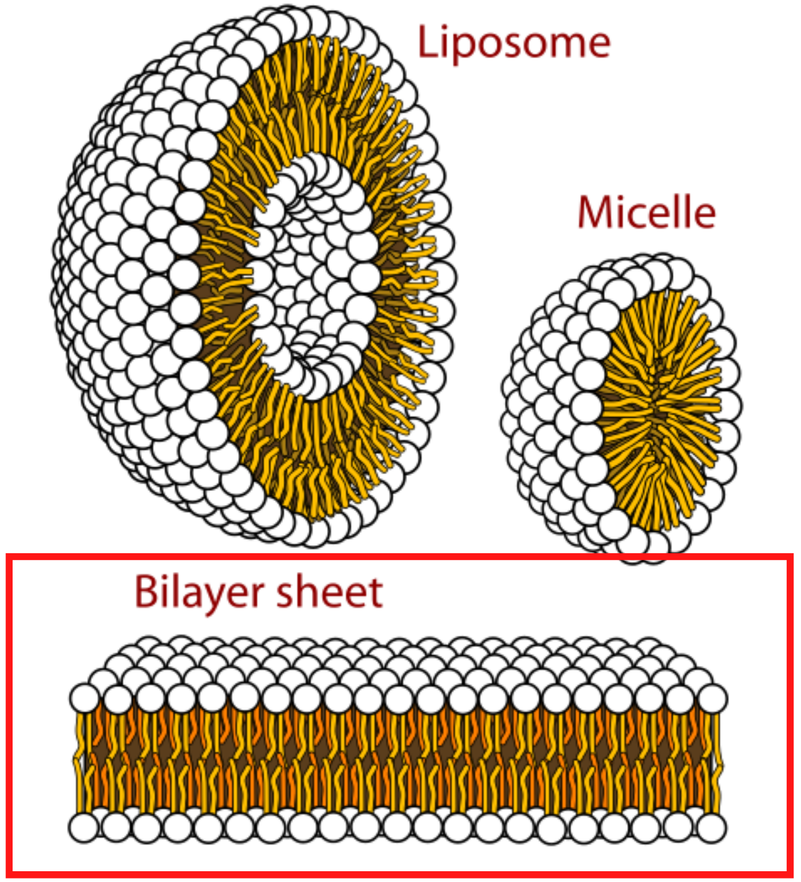
Hydrophilic head

Hydrophobic tail

Example : the **DSPC** molecule :



(1,2-distearoyl-sn-glycero-3phosphocholine)

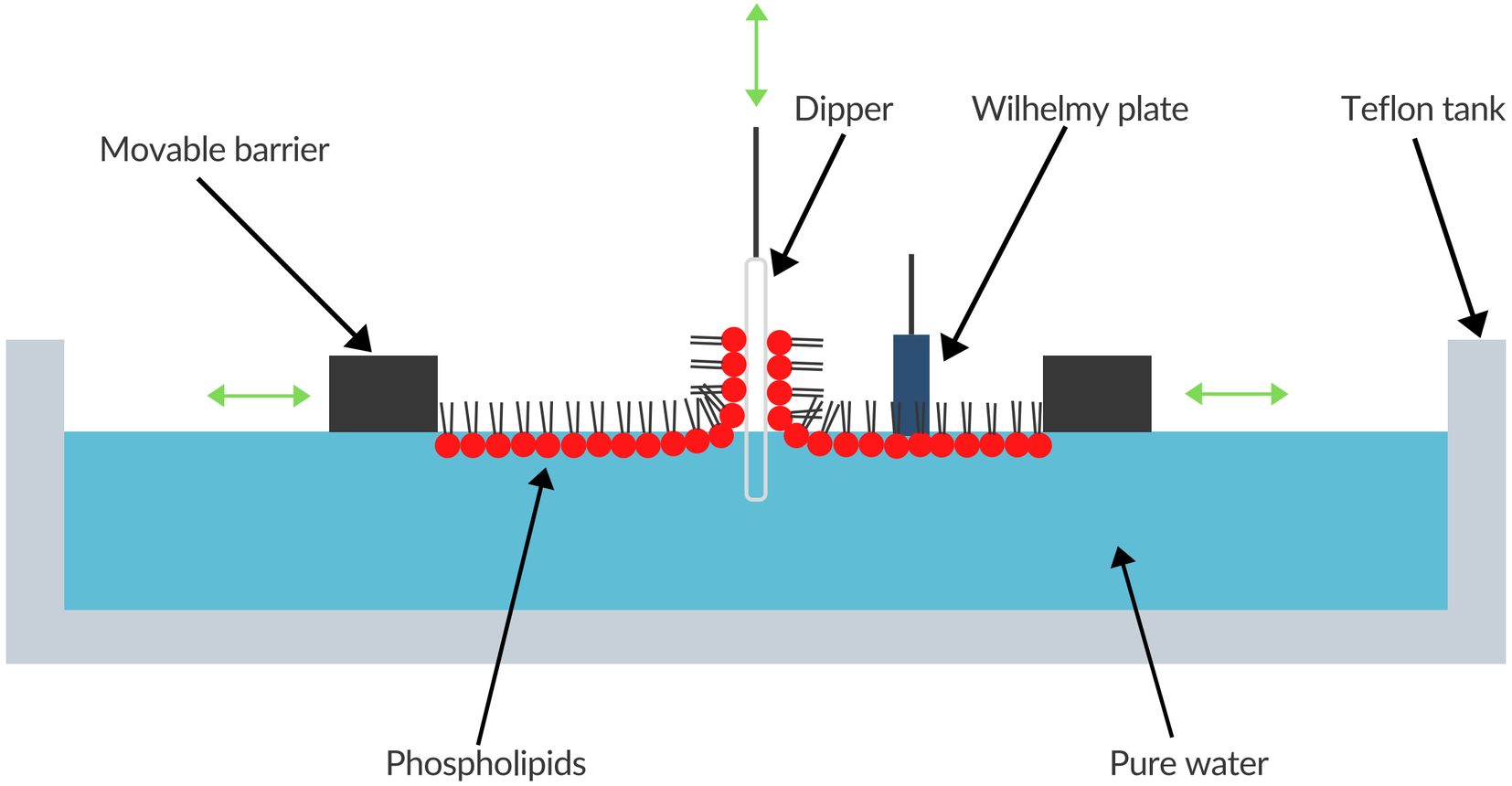


Source : Wikipedia

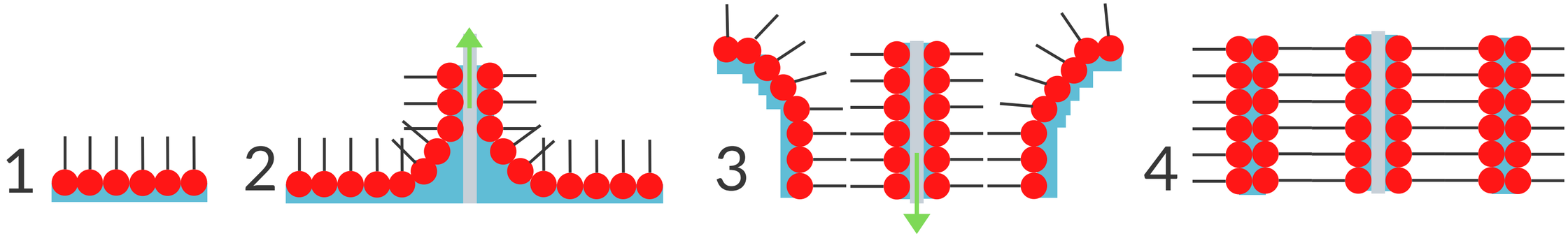
→ **Amphiphilic characteristics** lead to **special conformations** when placed in an aqueous medium.

MATERIALS AND METHODS : *Sample preparation*

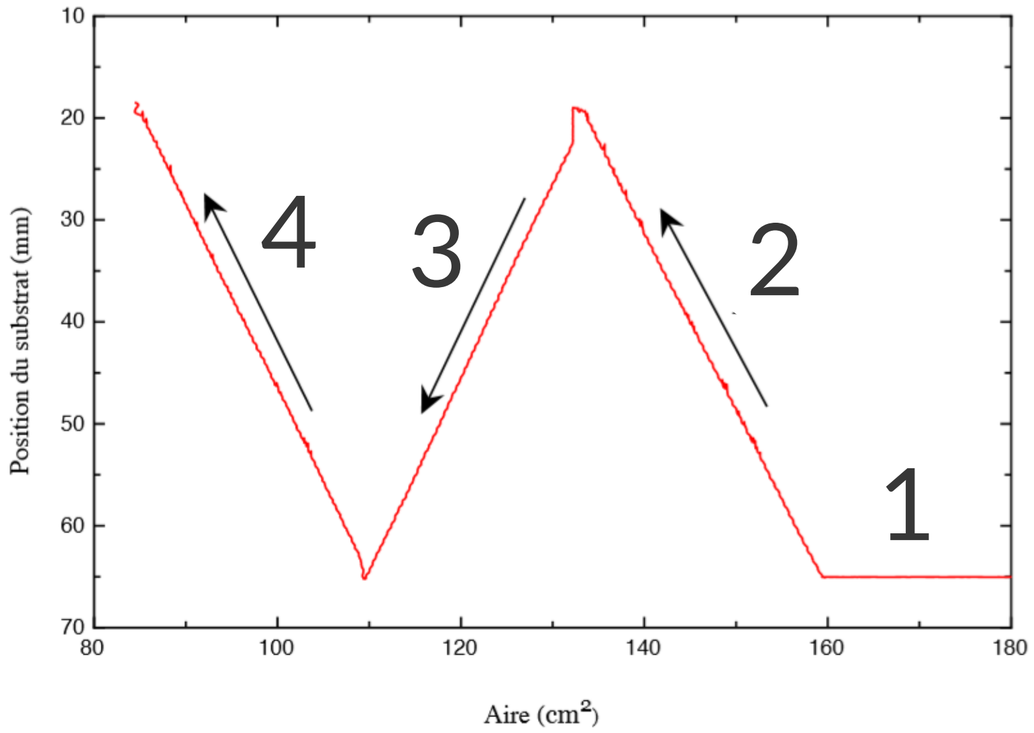
The ***deposition of a molecularly thin film on a solid substrate.***
Depending on the number of dippings, one obtains a succession of layers.



MATERIALS AND METHODS : *Trilayer preparation*

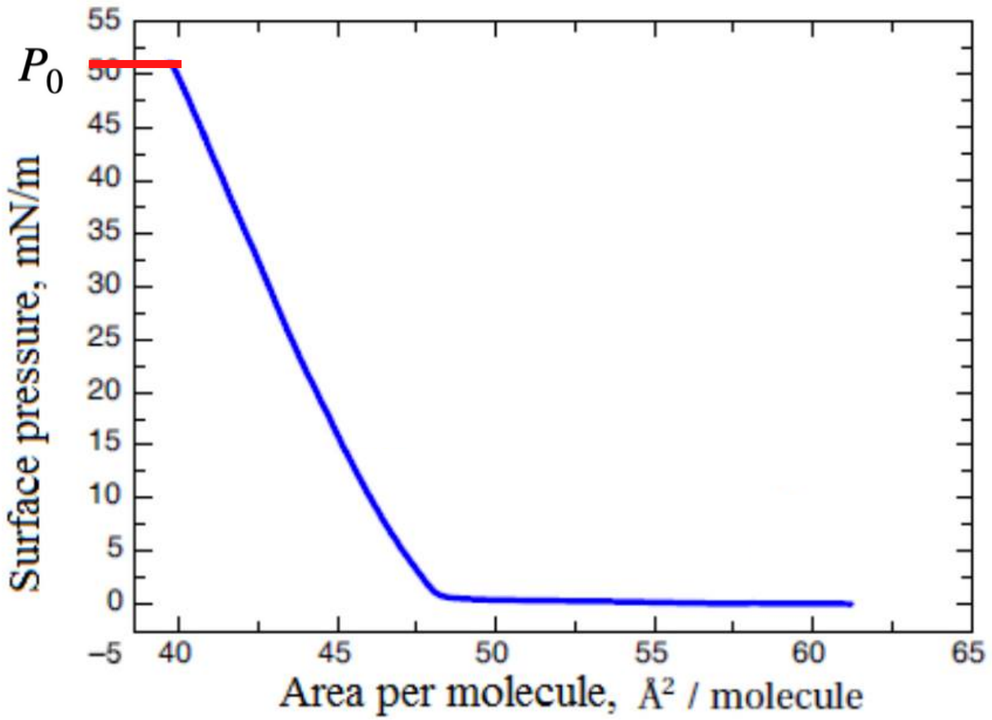


Position of the dipper as a function of area



Source : L. Fu Phd thesis

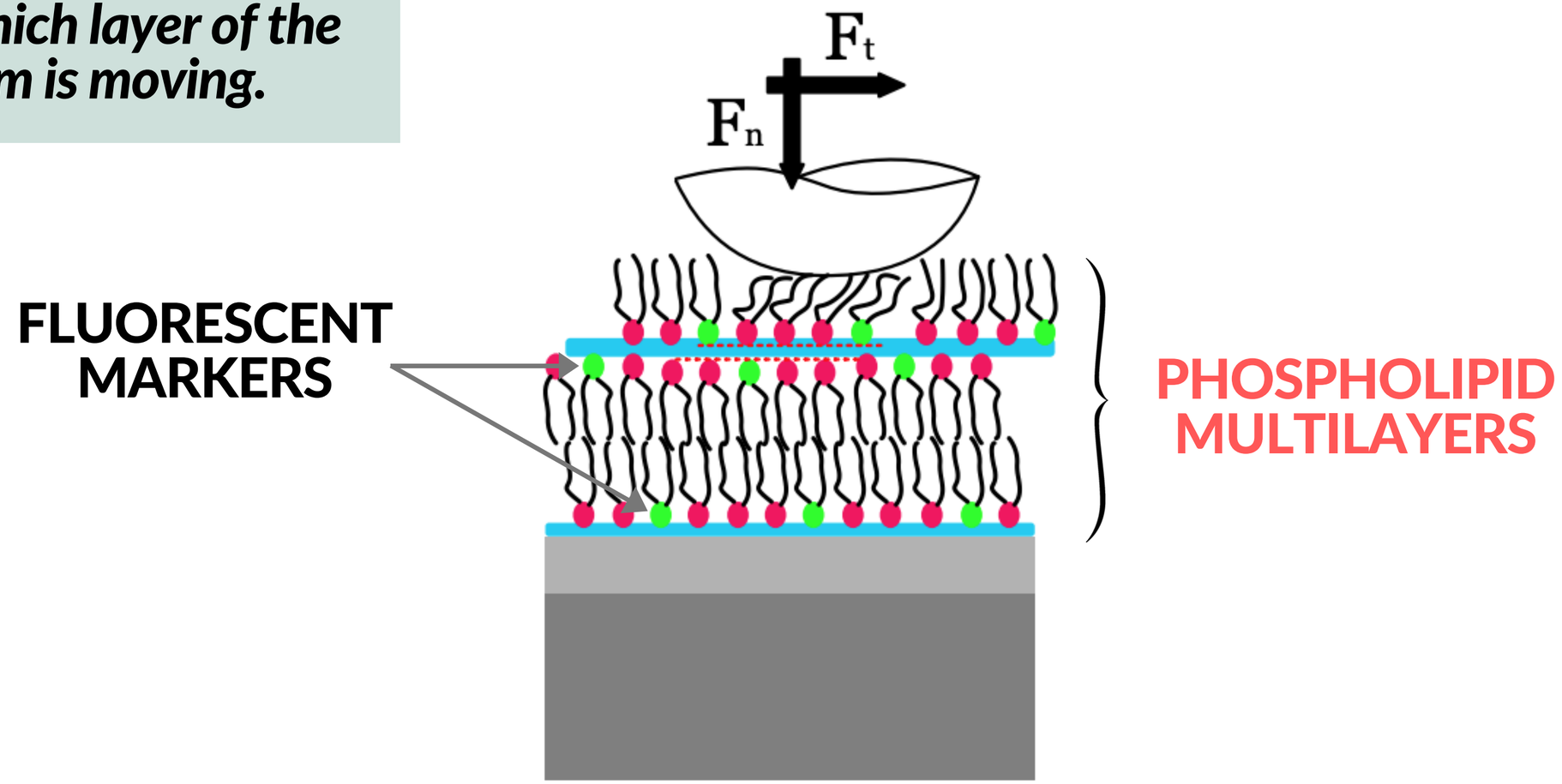
Control of the surface pressure



Source : L. Fu Phd thesis

*From **sample preparation** to **FRAPP experiment***

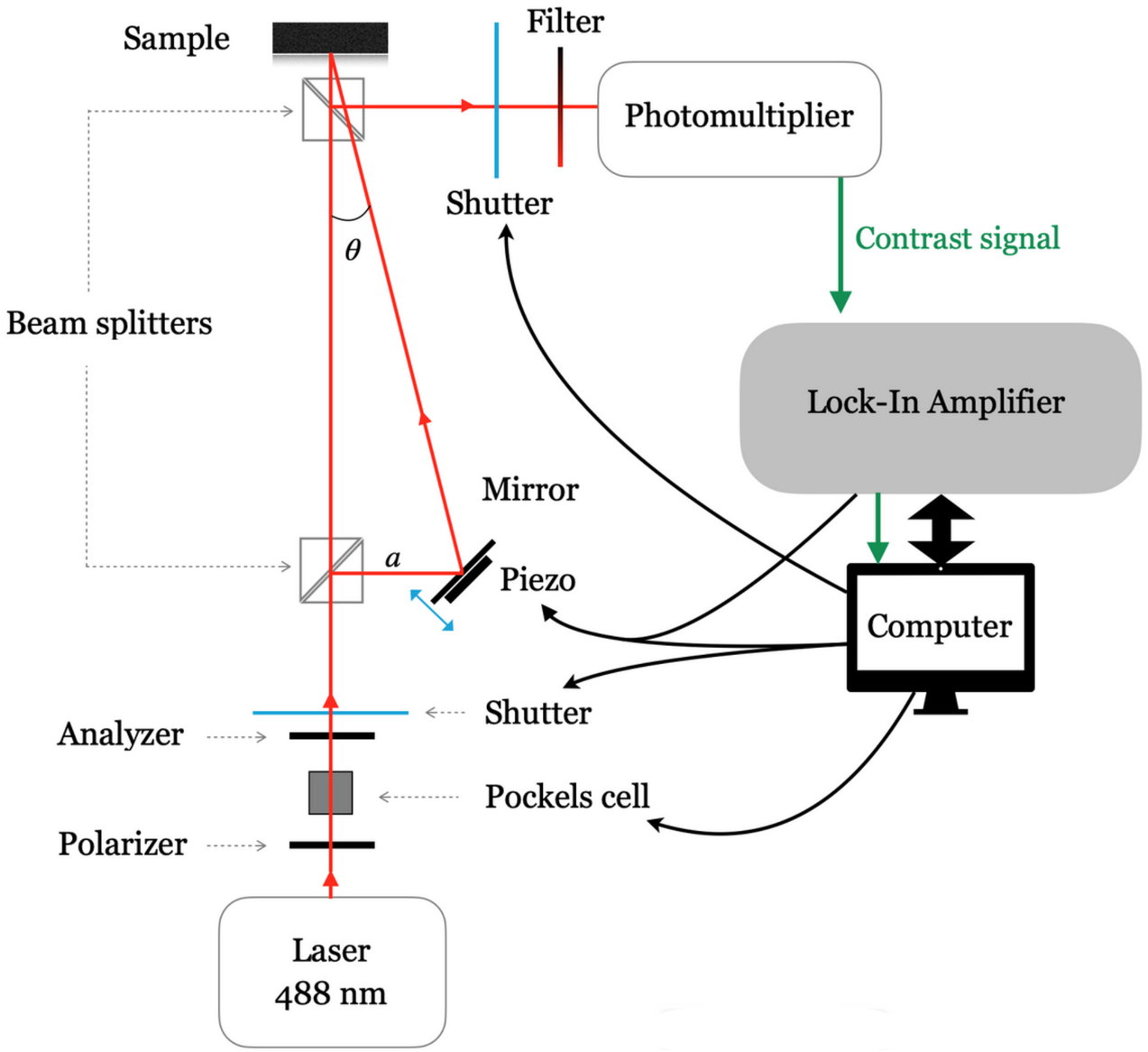
Know which layer of the system is moving.



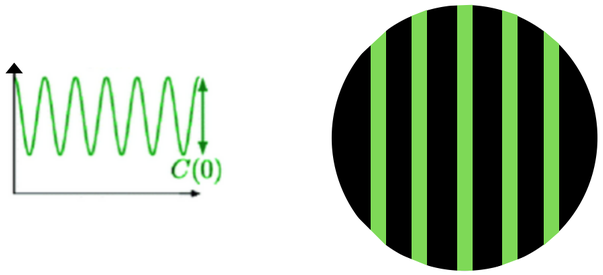
MATERIALS AND METHODS : *Principle of the FRAPP setup*

How does it work ?

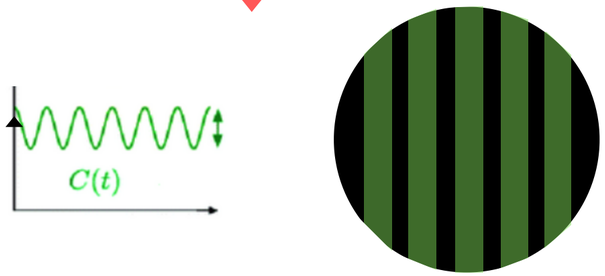
What informations about the sample does it allow to access ?



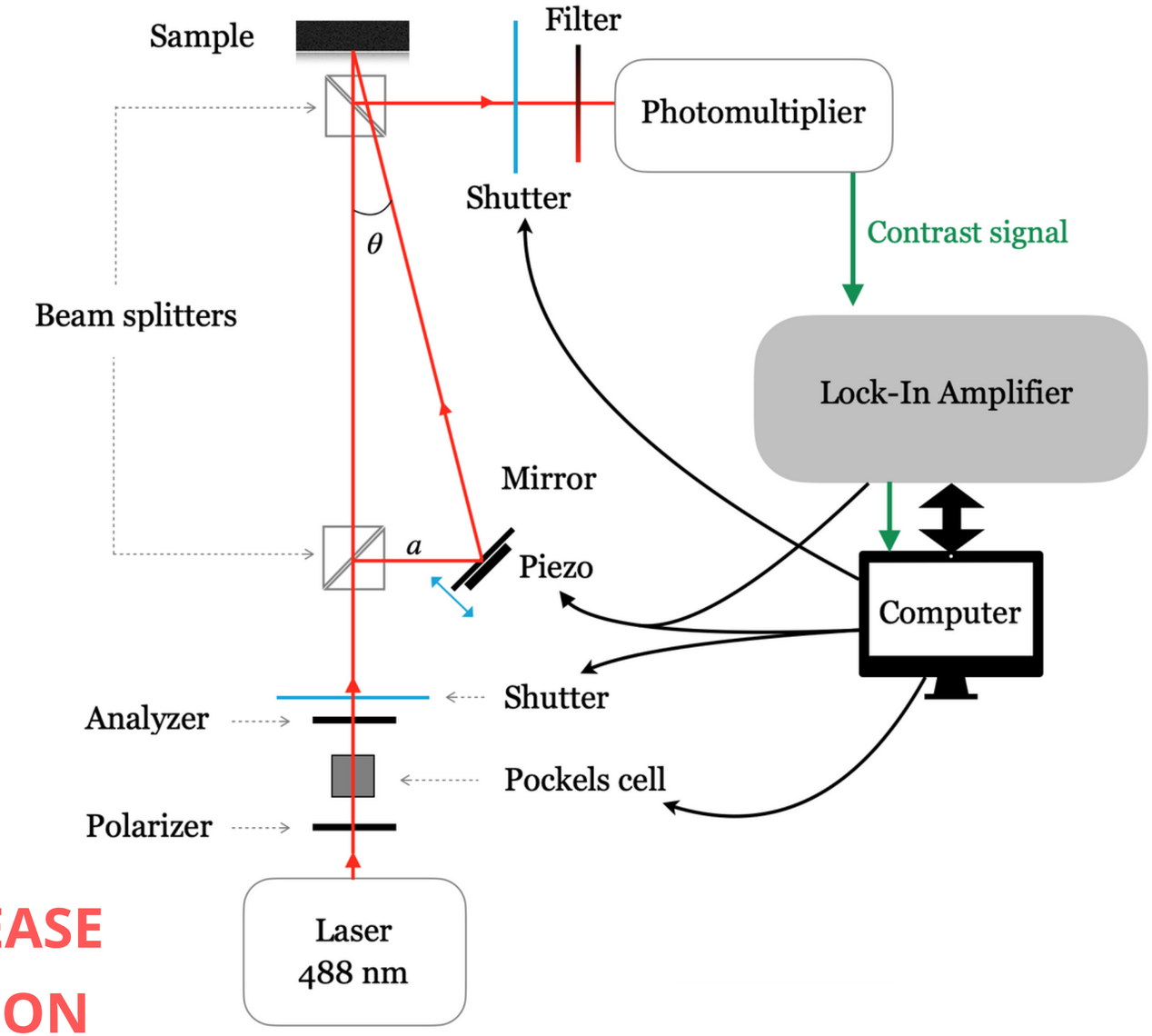
MATERIALS AND METHODS : *Principle of the FRAPP setup*



Photobleaching of the markers in the sample, following an interference **fringe pattern**.



Photomultiplier records the **contrast** between the bleached pattern and a monitoring beam with the *same fringe pattern* modulated at a certain **frequency**.



DIFFUSION → **CONTRAST DECREASE**

DRIFT → **CONTRAST MODULATION**

MATERIALS AND METHODS : *Theory of the FRAPP signal*

FLUORESCENCE SIGNAL

$$c(q,t) = c_0 A_n(K,0)\exp(-Dn^2q^2t)$$

$$F(t) = \int c(q,t)I(-q)\ d^3\vec{q}$$

$$I(\vec{r}) = I_0\left[1 + cos(\phi(t) + q_0Vt)\right]$$

$$\rightarrow F(t) = c_0 I_0[A_0 + A_1(K,0)e^{-Dq_0^2t}\cos(\phi(t) + q_0Vt)]$$

$$\phi(t) = usin(\omega t) + \phi_0(t)$$

$$\begin{aligned} F(t) = C\big[&A_0 + A_1(K,0)e^{-Dq_0^2t}cos(\phi_0 + q_0Vt) \\ &+2A_1(K,0)J_1(u)e^{-Dq_0^2t}sin(\phi_0 + q_0Vt)sin(\omega t) \\ &+2A_1(K,0)J_2(u)e^{-Dq_0^2t}cos(\phi_0 + q_0Vt)cos(2\omega t)\big] \end{aligned}$$

MATERIALS AND METHODS : *Theory of the FRAPP signal*

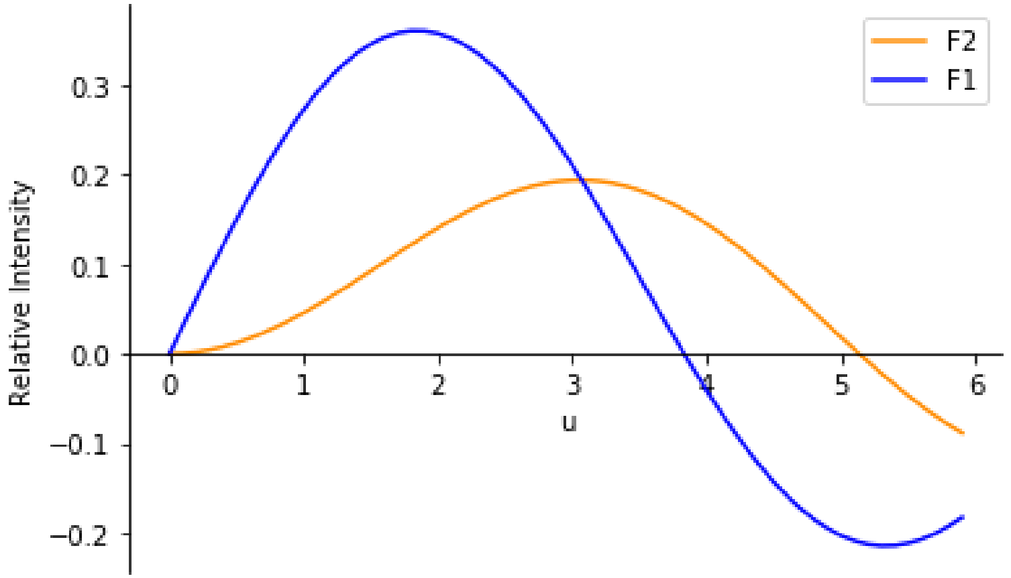
- All harmonics follow exponential law : $e^{-Dq_0^2t}$

→ We can get the **diffusion coeficient D** by fitting harmonics.

- All harmonics contain a speed modulation term $\sin(\phi_0 + q_0 Vt)$

→ Access to ***velocimetry*** informations.

- A precise oscillation amplitude **u** of the piezoelectric mirror maximizes each harmonic.



Amplitude of 1rst and 2nd harmonics as a function of the ocsillation amplitude of the miror.

$$F(t) = C\Big[A_0 + A_1(K,0)e^{-Dq_0^2t}cos(\phi_0 + q_0Vt) + 2A_1(K,0)J_1(u)e^{-Dq_0^2t}sin(\phi_0 + q_0Vt)sin(\omega t) + 2A_1(K,0)J_2(u)e^{-Dq_0^2t}cos(\phi_0 + q_0Vt)cos(2\omega t)\Big]$$

RESULTS · *Optimization : pockels cell*

Goal:

- Optimize the ***voltage settings*** (applied to the pockels cell) to ***maximize*** the ratio :

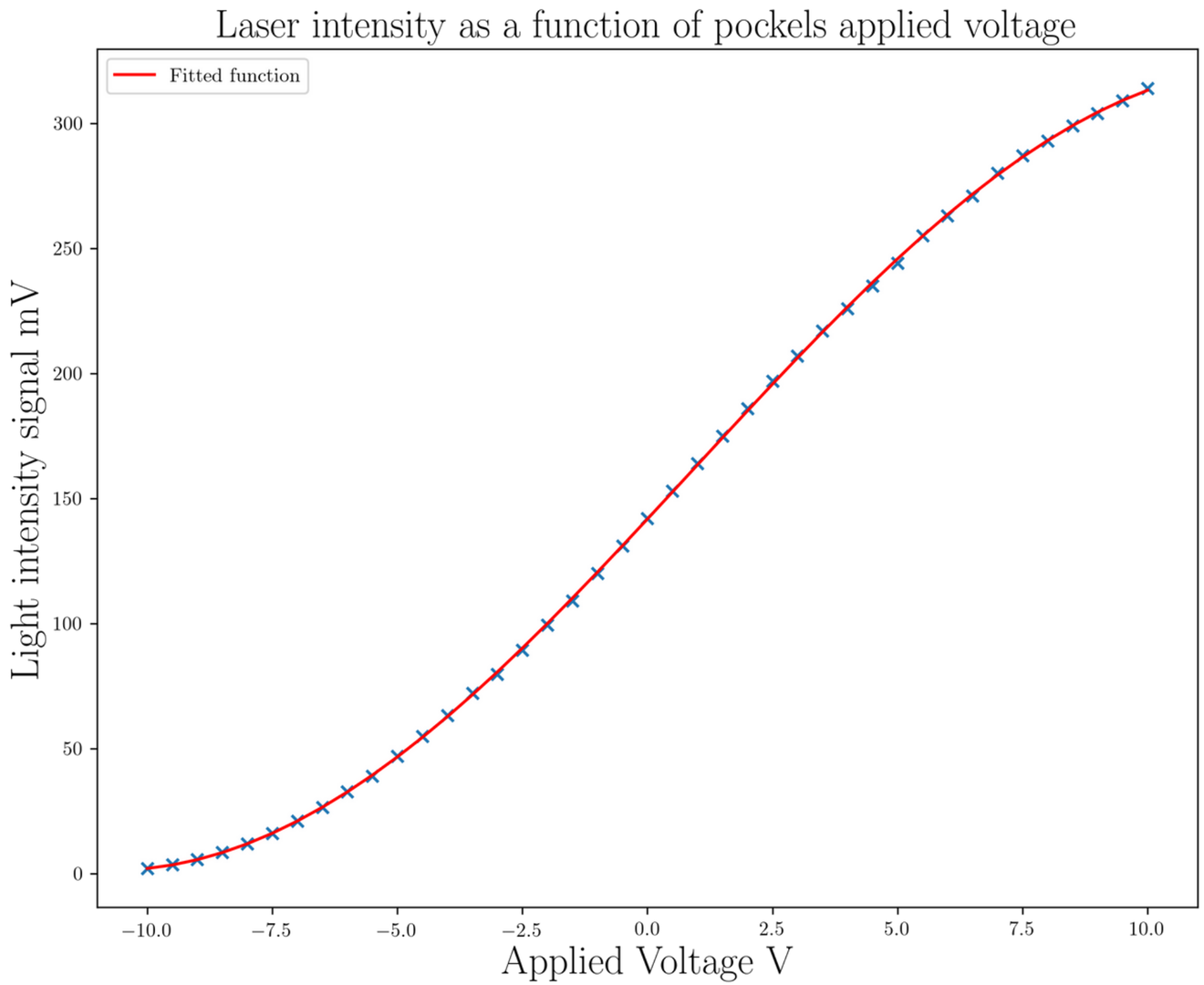
$$\frac{\textit{Bleaching phase beam intensity}}{\textit{Reading phase beam intensity}}$$

We recover Malus's Law !

$$I(V) \propto \alpha \ \sin^2(\beta V + \phi)$$

→ *Optimal voltage settings :*

- -10 V for the reading phase.
- 10 V for the photobleaching phase.



RESULTS • *Optimization : mirror amplitude*

Does the optimization amplitude depend on the interfringe ?
How does it behave ?

Epoxy resin (with fluo. markers) on microscope slides.

▼

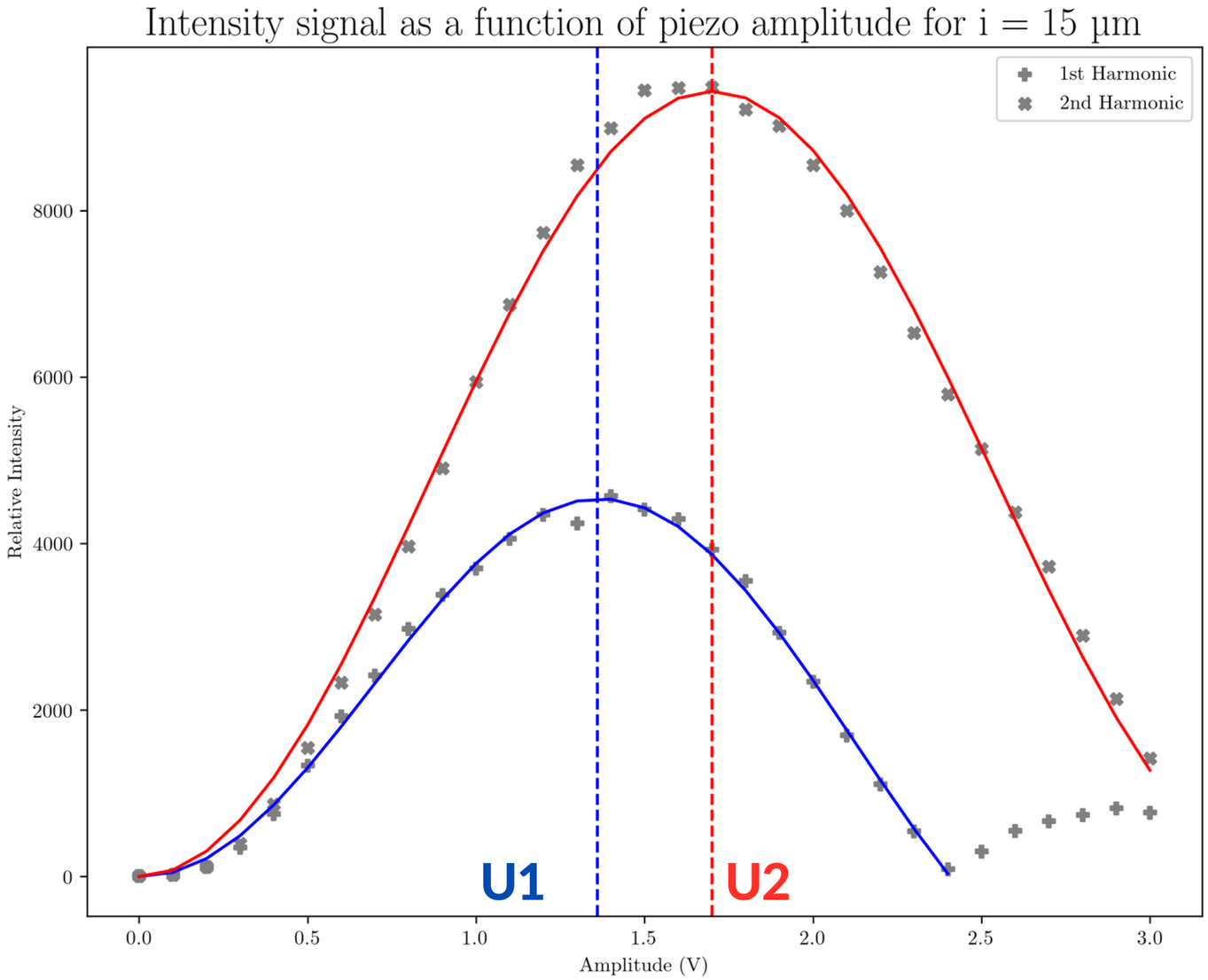
No diffusion of the particles in the sample.

→ Probe, for several interfringes, which tension amplitude will optimize the response.

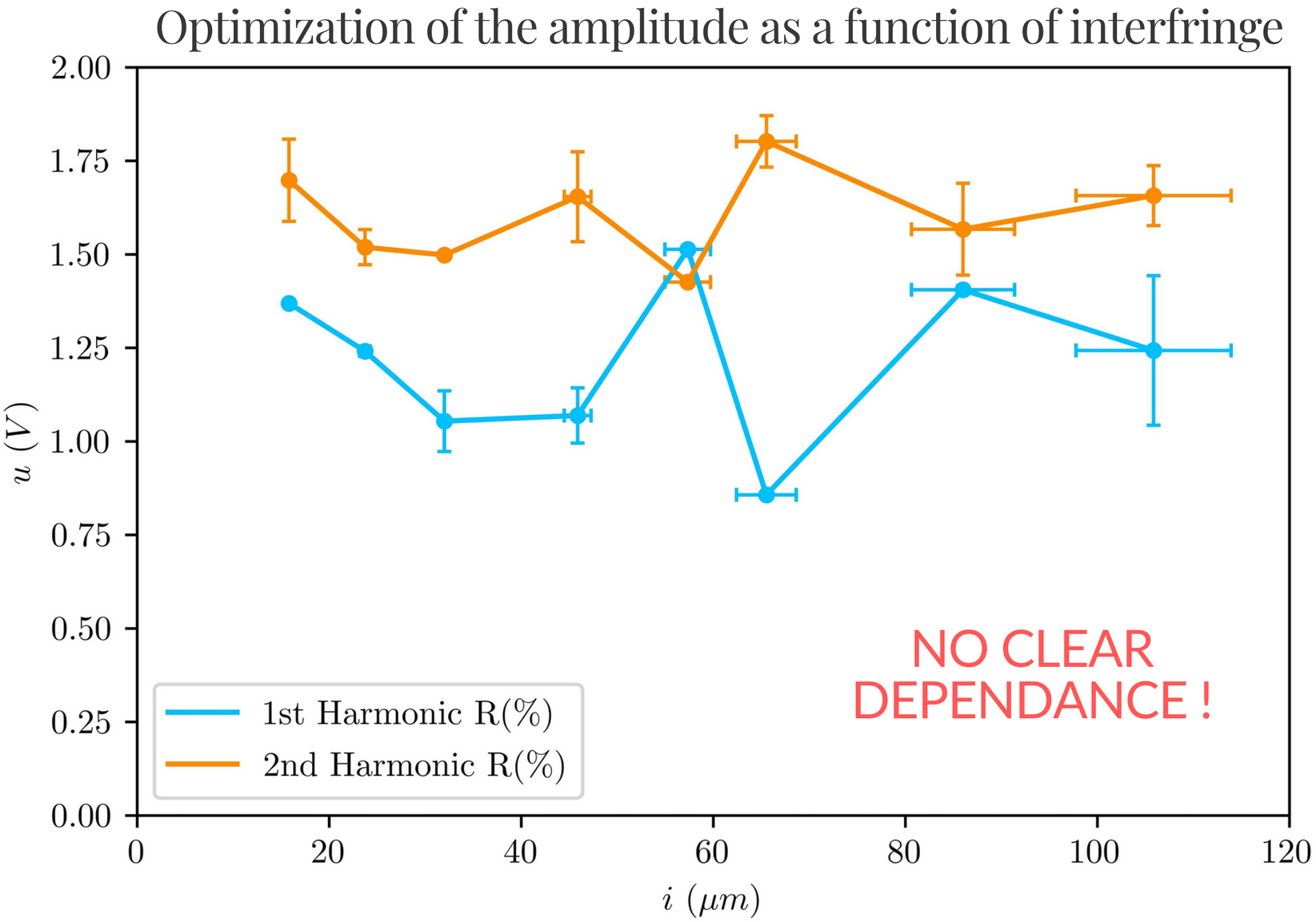
Here :

U1 = 1.36 V

U2 = 1.70 V



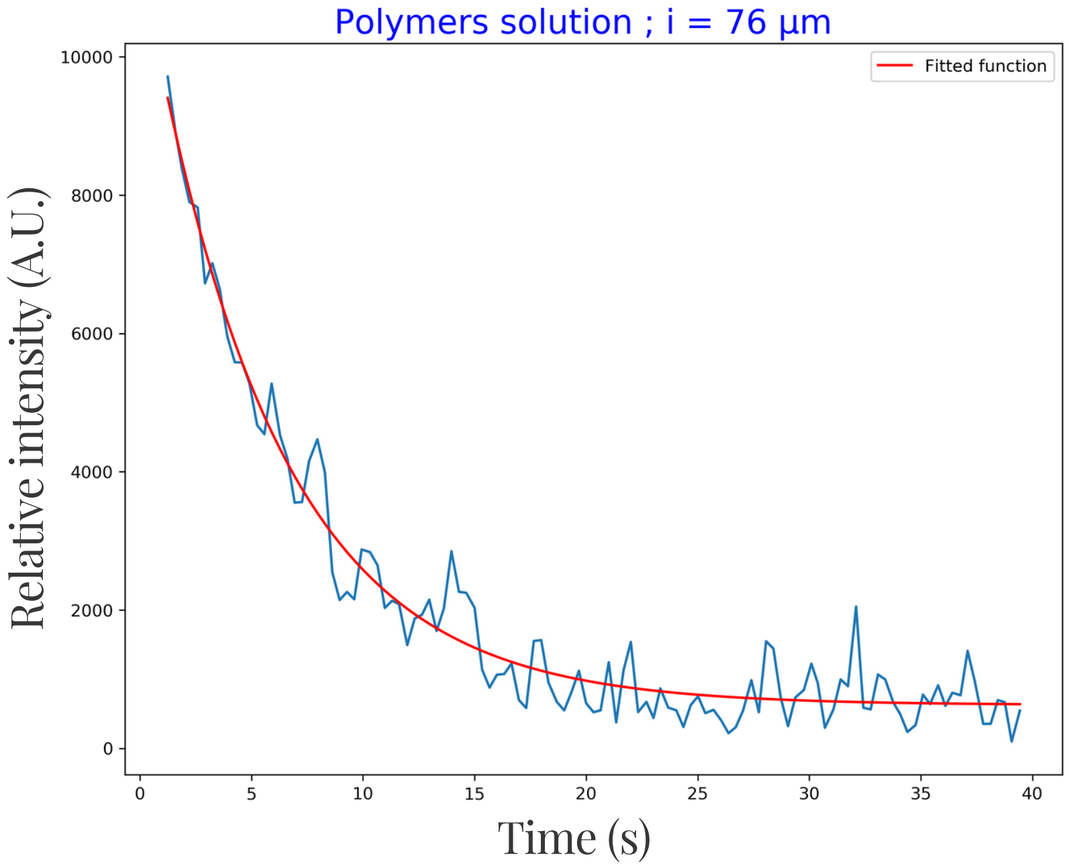
RESULTS · *Optimization : mirror amplitude*

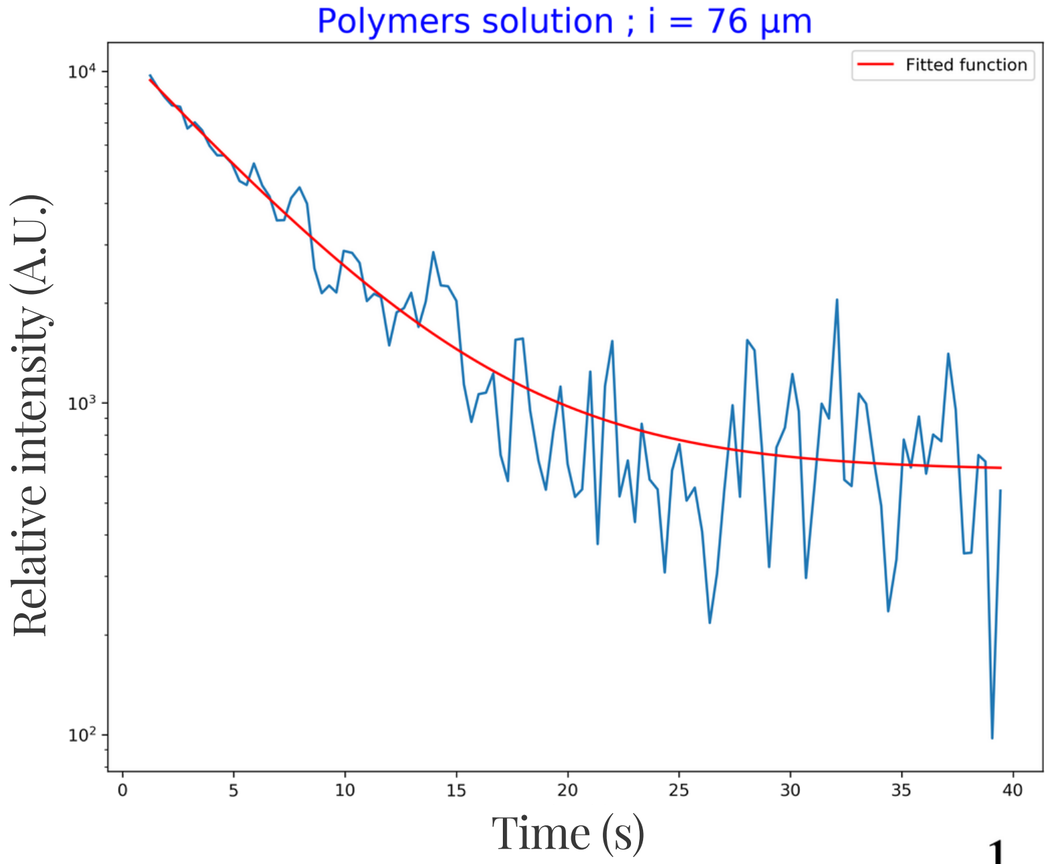


RESULTS AND DISCUSSION : *Diffusion of polymers in solution*

- Diluted solution of PEG (Polyethylene glycol).

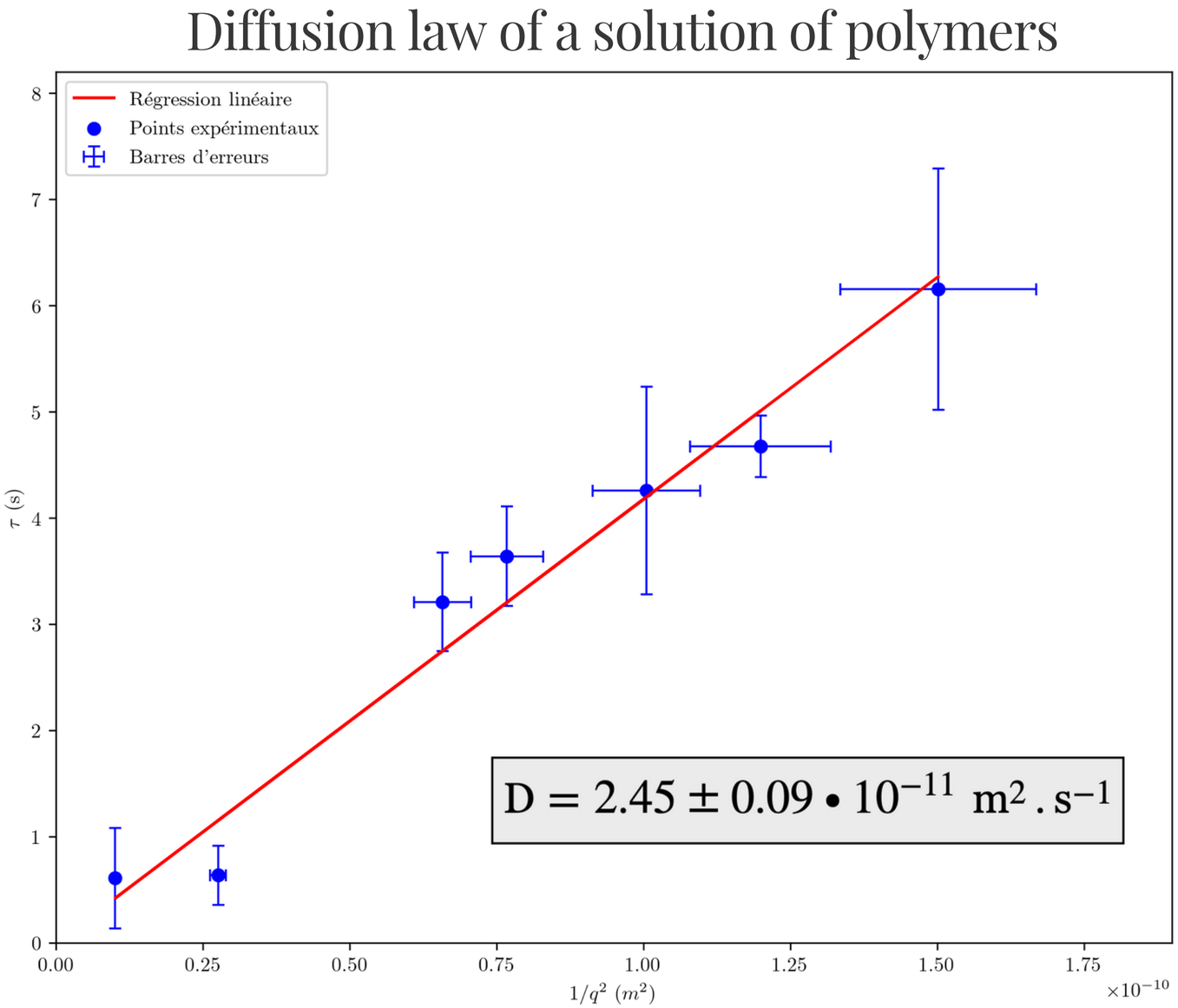
→ By fitting with a characteristic decay time for different interfringes value, one can recover the diffusion constant of the polymers solution.





Example of 2nd harmonic signals collected on the polymers solution, used to extract a ***characteristic time*** *:* $\tau_{q_0} = \frac{1}{Dq_0^2}$

RESULTS AND DISCUSSION : *Diffusion of polymers in solution*

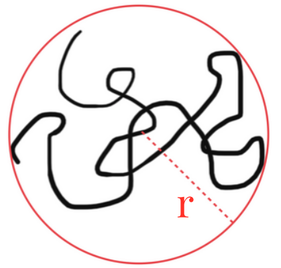


If we plug this result in the **Stokes-Einstein** relation :

$$D = \frac{k_B T}{6\pi\eta r}$$

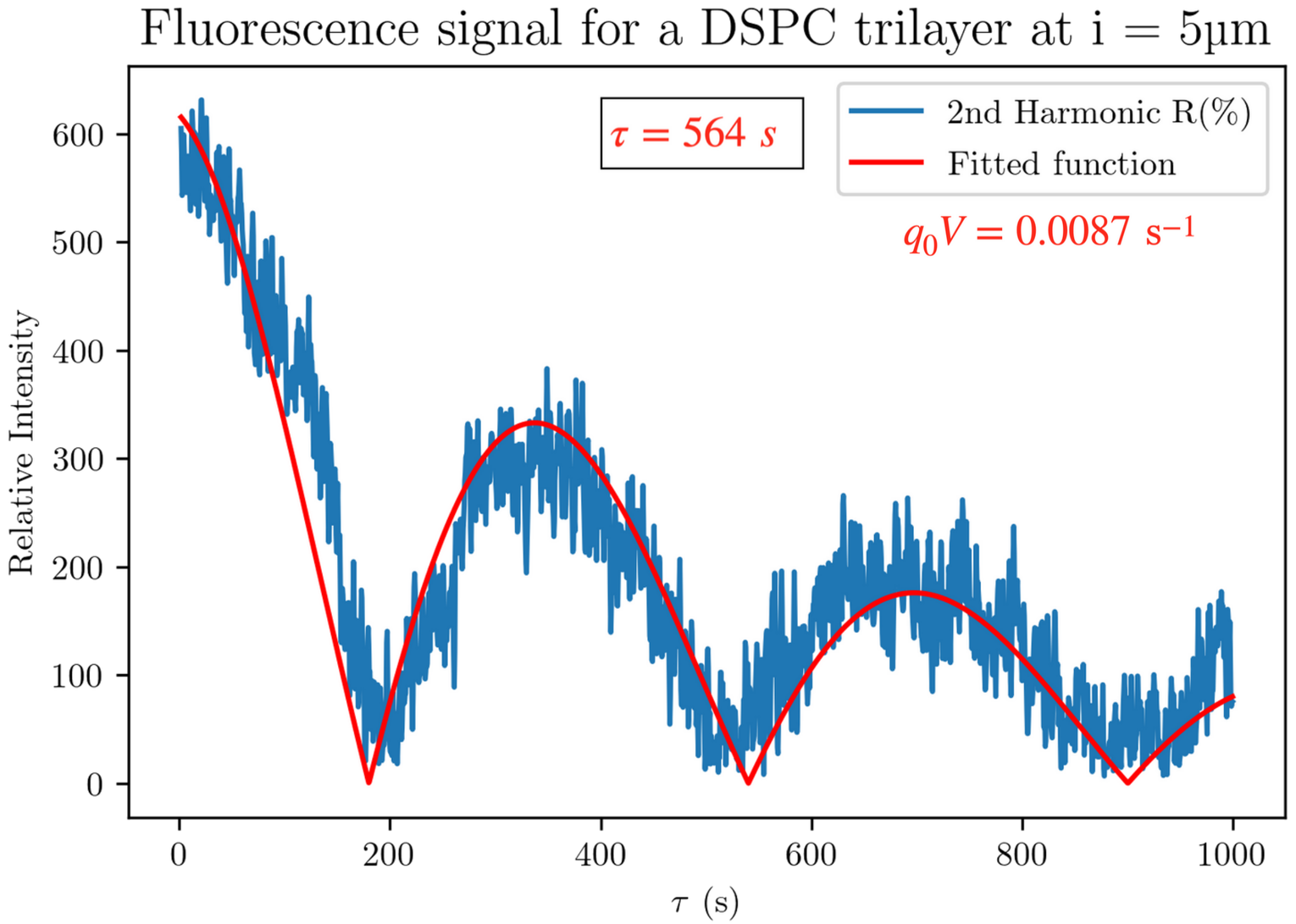
With :

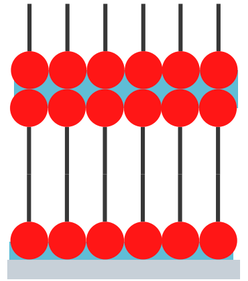
η	The dynamic viscosity (Pa.s)
T	Temperature (K)
k_B	Blotzmann constant



→ $r = 8.7 \pm 0.3$ nm

RESULTS AND DISCUSSION : *Study of a DSPC trilayer*





We have to perform much **longer** experiments because of the **slow diffusion.**

Using this fitting model :

$$A_0 \,.\, \exp(-\frac{t}{\tau}) \,|\cos(q_0 V t)\,|$$

$D = 8 \bullet 10^{-16}\ \mathrm{m^2\,.\,s^{-1}}$

$V = 6.9 \bullet 10^{-9}\ \mathrm{m\,.\,s^{-1}}$

RESULTS AND DISCUSSION : *Study of a DSPC trilayer*

A priori, there is **no macroscopic speed** of the sample.
Why do we have results as if there was one ?

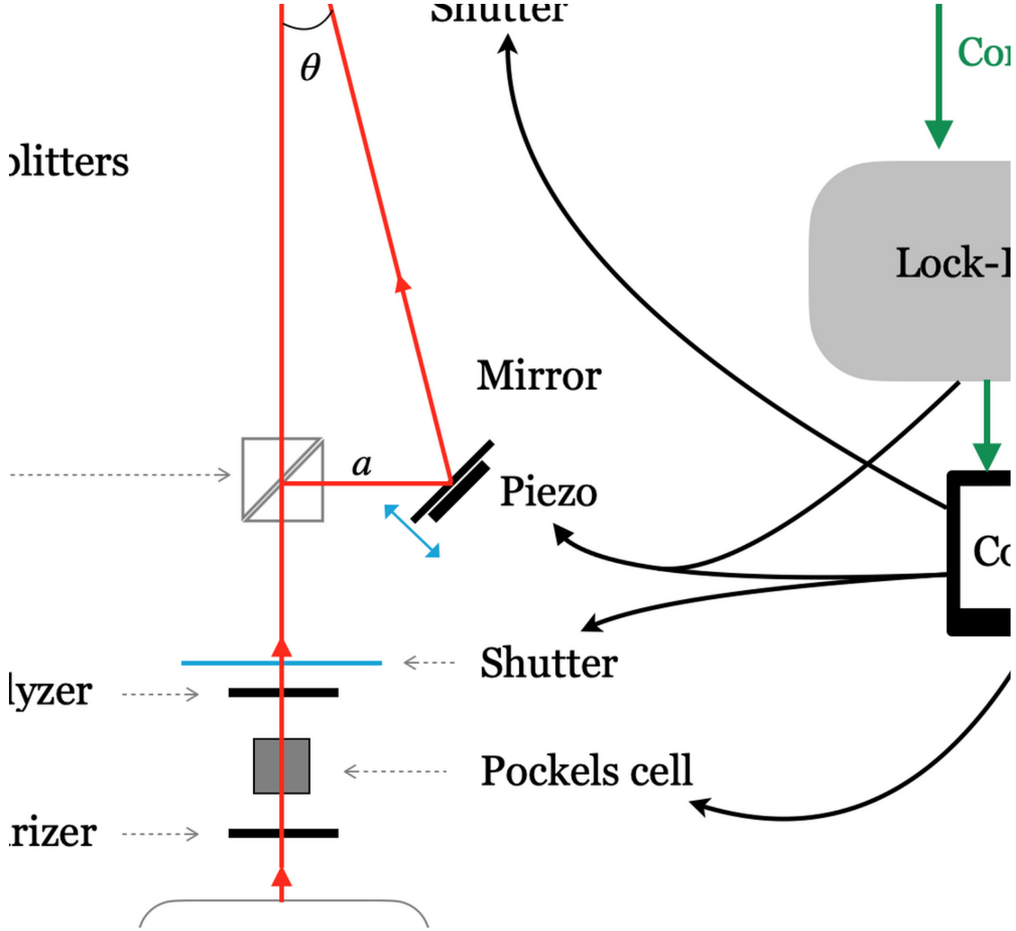
Probably mechanical drifts of the setup !

Drift of the piezoelectric cell, adding a non constant term in the phase shift ?

$$\phi(t) = usin(\omega t) + \phi_0(t)$$

Problem due to the heating of the mediums where laser beam passes ?

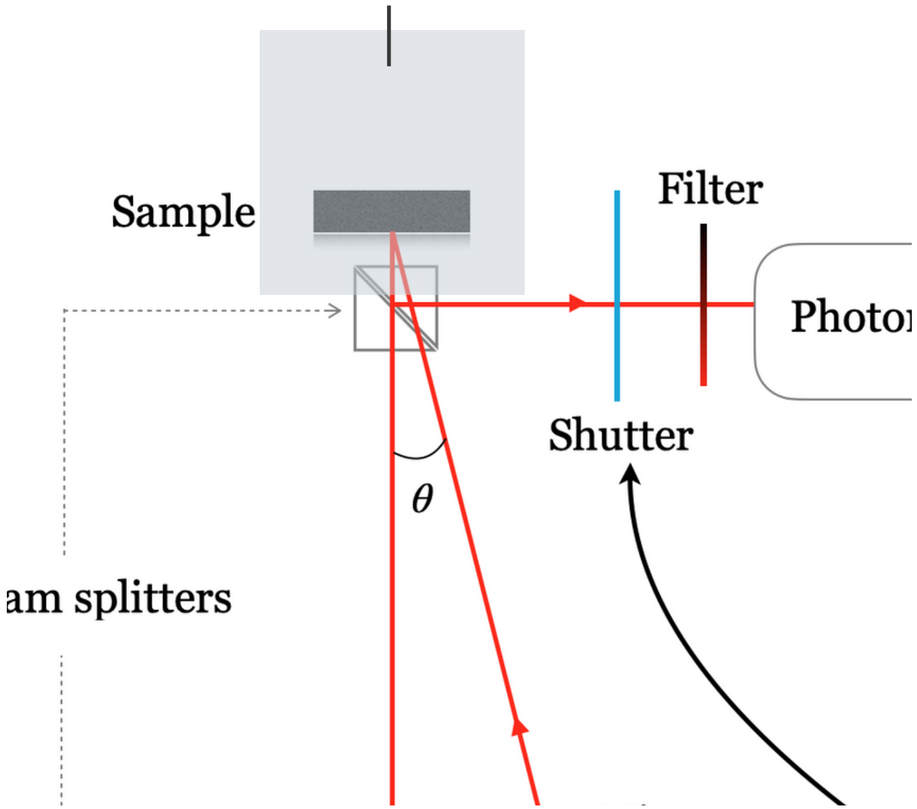
Symmetrize the 2 beams paths to avoid these problems.



RESULTS AND DISCUSSION : *Perspectives and next steps*

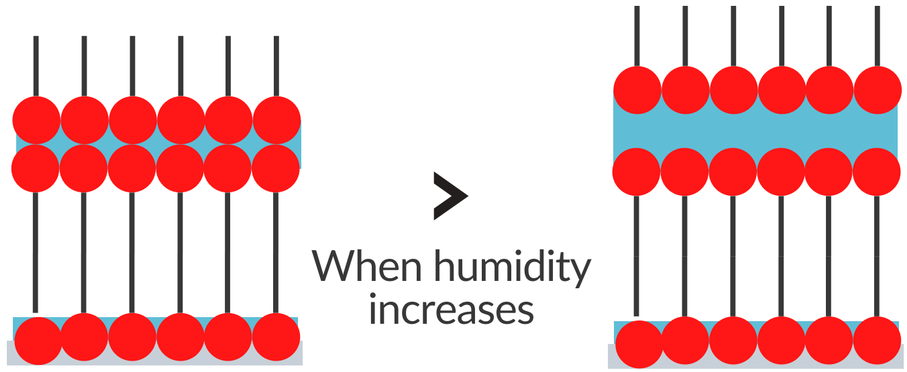
- Same experiment but varying **humidity** and **temperature**.

Hermetic box allowing to control temperature and humidity.



- Reach a **liquid phase** of the bilayer instead of a gel phase

- See how the *diffusion constant* changes according to **humidity variations**.



CONCLUSION AND OUTLOOK

- *The FRAPP setup is a very **powerful** yet **sensitive** experimental setup providing **diffusion** and **velocimetry** informations about phospholipid multilayers.*

- *We tried to **optimize** the FRAPP setup and were able to perform experiments on systems of interest.*

- *Still, we acknowledged the limits and weaknesses of the setup.*

→ *How to modify the setup in order to **avoid mechanical drifts** ?*

Bibliography

[1] Li Fu. *Rhéologie des polymères dans les contacts confins: tribologie des interfaces tudies par un nouveau dispositif couplant FRAPP et nano-tribologie*. PhD thesis, 2015.

[2] Jean Davoust, Philippe F.Devaux, and Liliane Leger *Fringe pattern photobleaching, a new method for the measurement of transport coefficients of biological macromolecules*. 1982.

[3] L. Fu, D. Favier, T. Charitat, C. Gauthier, and A. Rubin. *A new tribological experimental setup to study confined and sheared monolayers*.