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Characterization of bio-molecular interactions at surfaces // 5PMBCBM8

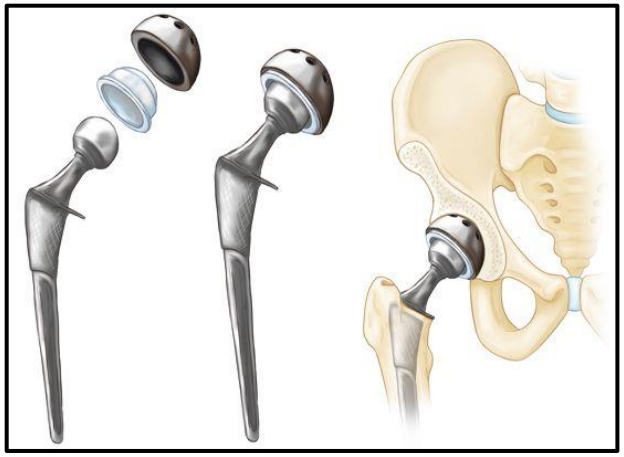
Formation and cross-linking of fibrinogen layers monitored with *in situ* spectroscopic ellipsometry

T.Berlind, M.Poksinski, P.Tengvall, H.Arwin
2010
Colloids and Surfaces B: Biointerfaces

Matteo MARENGO
3A BIOMED – DD M2 N2BIO
2022 - 2023

Biological acceptance / Biocompatibility of medical devices

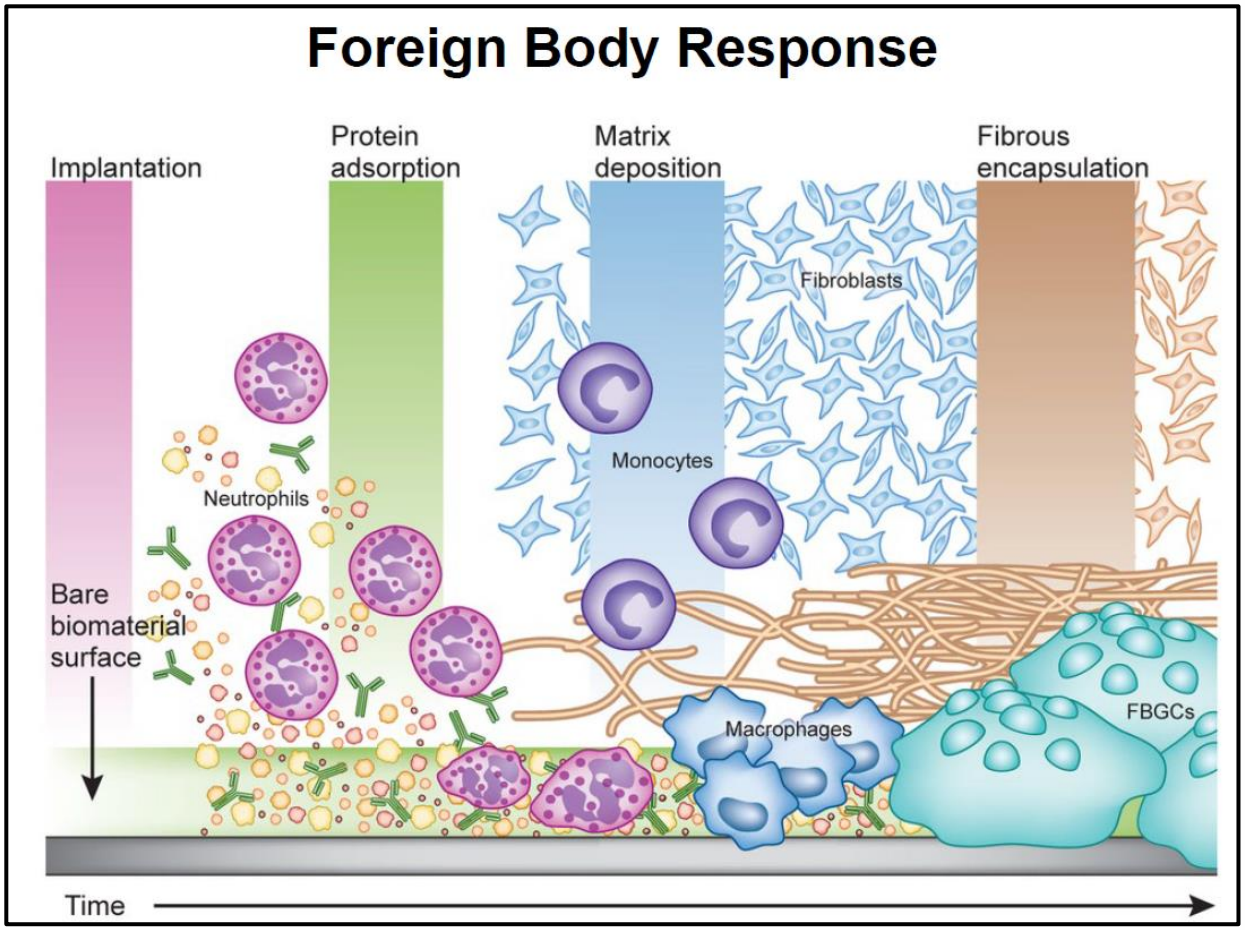
Biocompatible : ability of a material to perform with an appropriate host response in a specific application.



The hip implant

What is the reaction when implanting a foreign-body ?

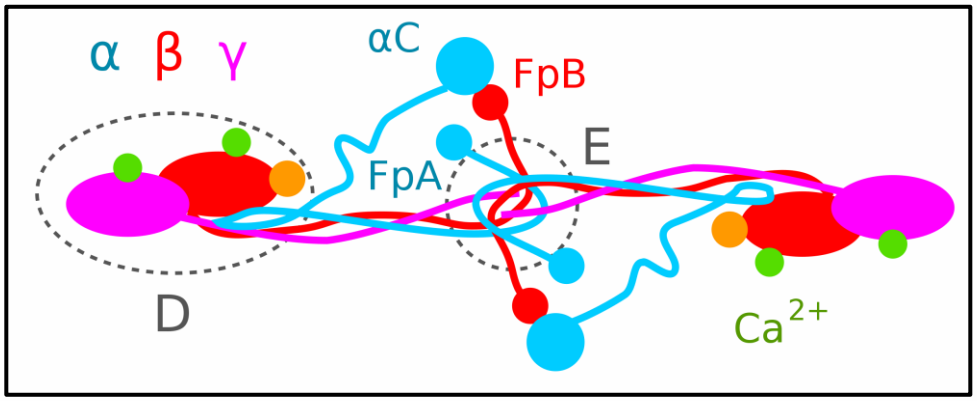
Biological acceptance / Proteins adsorption to the surface



Adapted from Pr. Dankers, Biomaterials, TU Eindhoven, 2022

Vroman effect : protein with highest mobility will adsorb first.

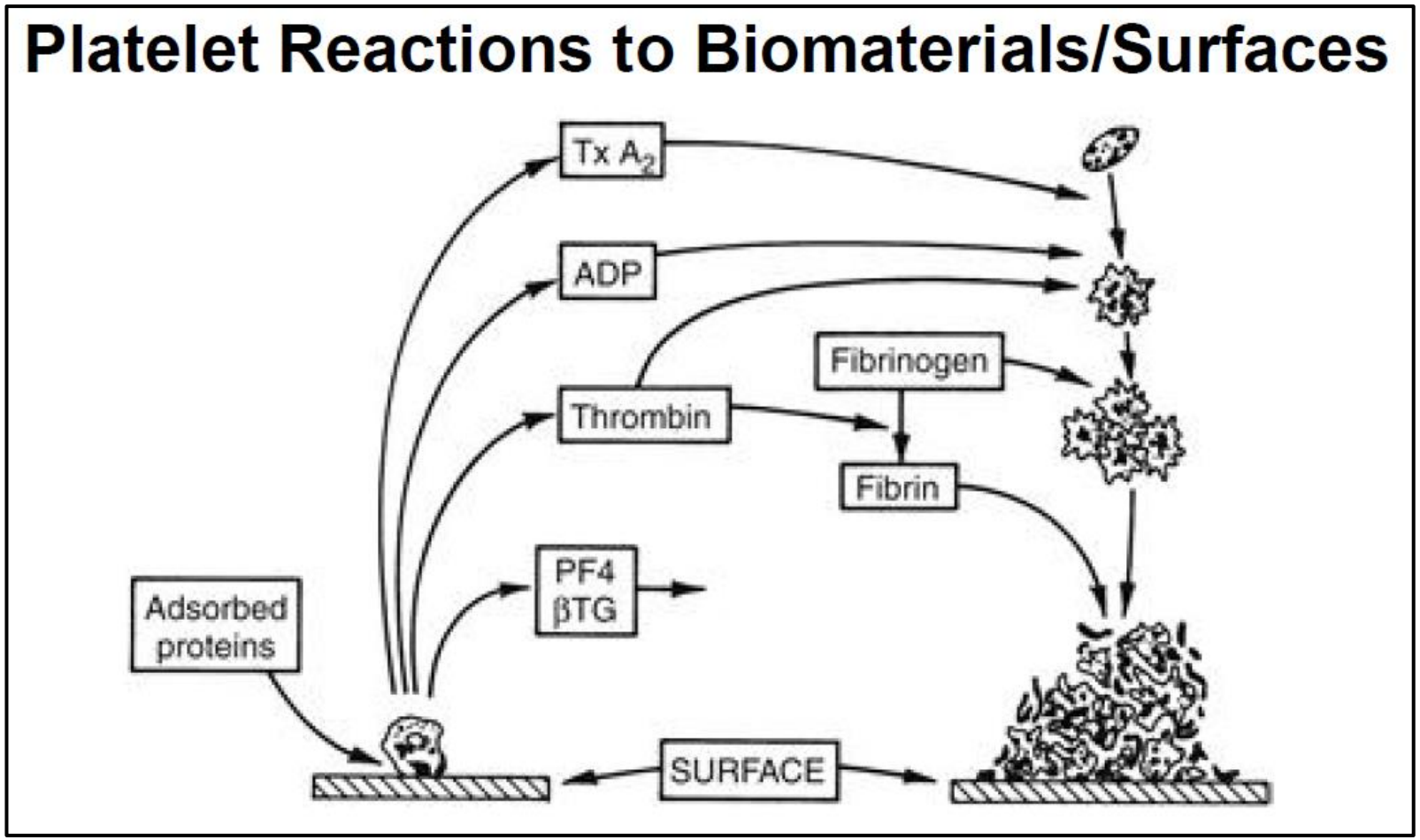
Fibrinogen : Chemistry and Action in the clotting process



Fibrinogen is a glycoprotein complex.
Converted by thrombin to fibrin.

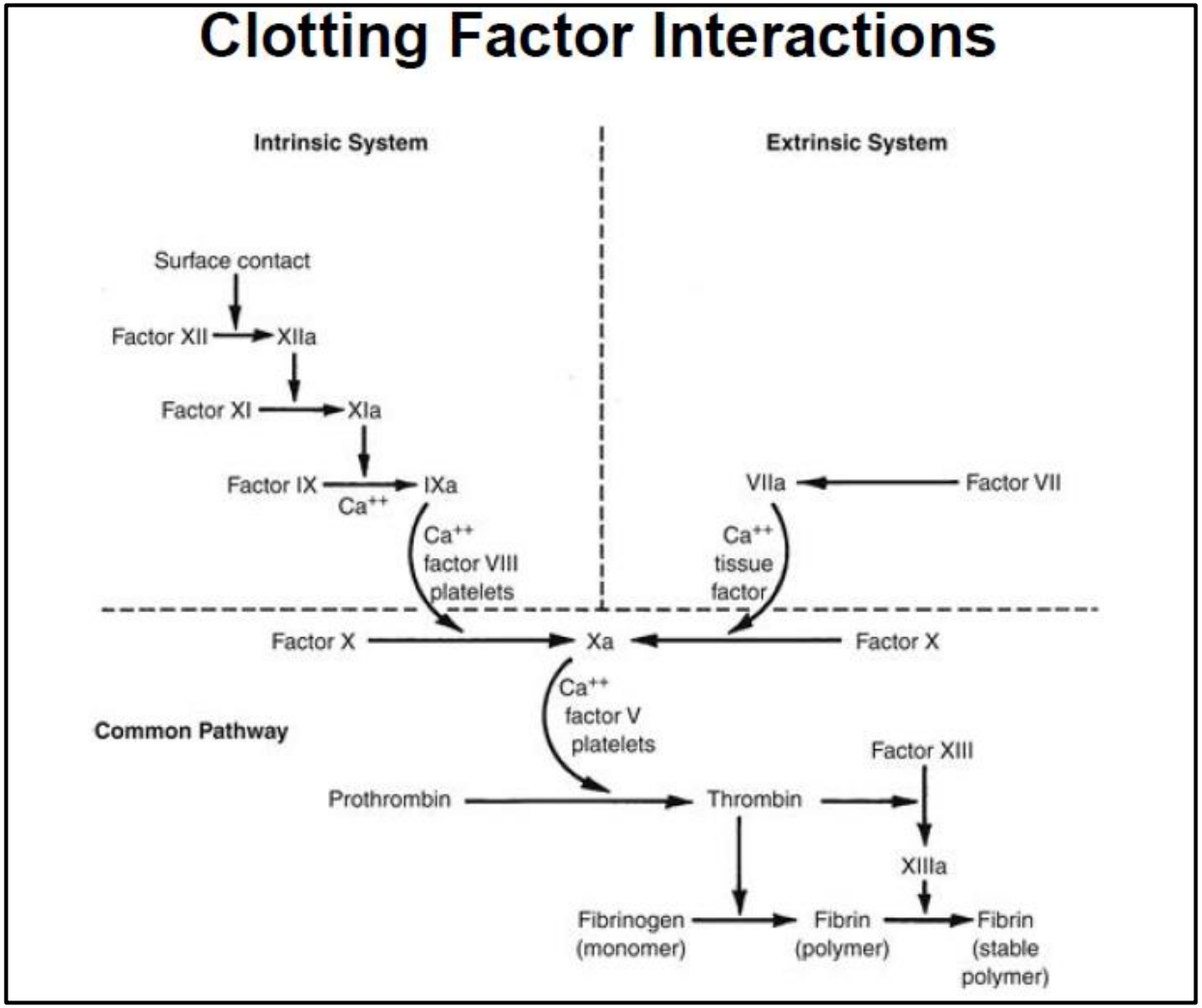
What is the role played by fibrin in the clotting process ?

Fibrinogen : Chemistry and Action in the clotting process



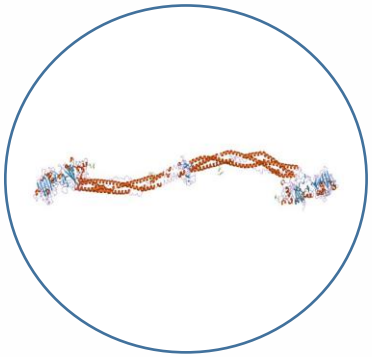
Adapted from Pr. Dankers, Biomaterials, TU Eindhoven, 2022

Fibrinogen : Chemistry and Action in the clotting process

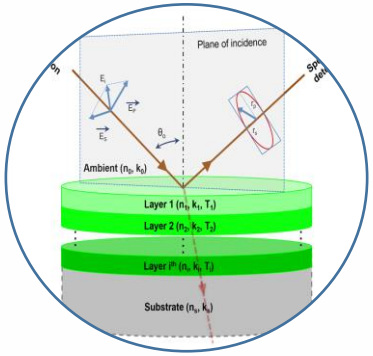


Adapted from Pr. Dankers, Biomaterials, TU Eindhoven, 2022

Goal of the paper : study formation and cross linking of fibrinogen layers



Preparation of thick protein layers



Determine with SE
- Surface mass density
- Refractive index
- Thickness

Incorporate drug into the matrix

Investigation of the dynamics → structure of the layer / orientation of the protein molecules

Goal of the paper : study formation and cross linking of fibrinogen layers

Doxycycline is a matrix metalloproteinase inhibitor.

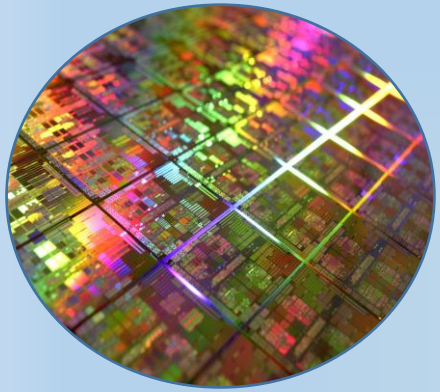
Comparison *in situ* vs *ex situ* of previous published results.

What are the challenges of these experiments ?

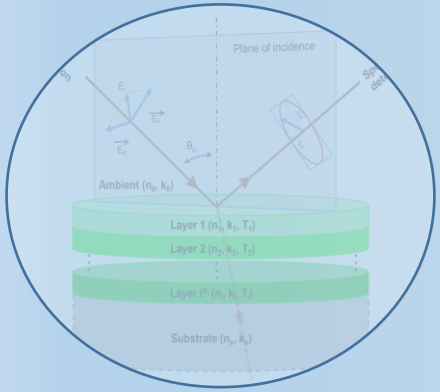
Challenges of the paper

1. Surface preparation / Protein incubation
2. Spectroscopic ellipsometry
3. Do the data processing and analysis

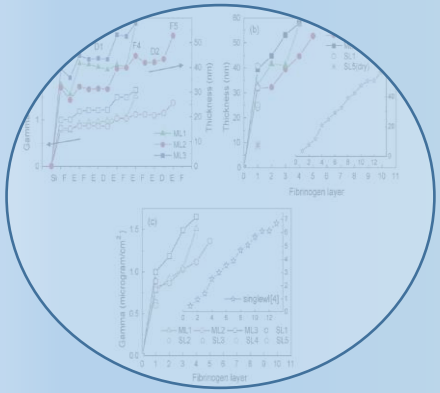
PLAN



Surface preparation / Protein incubation

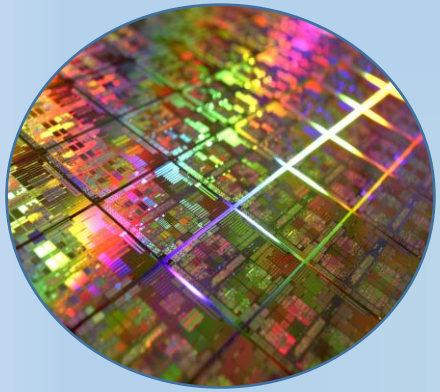


Spectroscopic ellipsometry



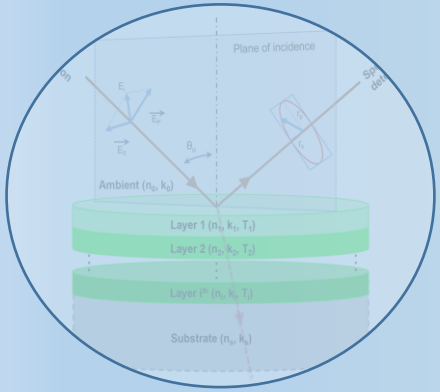
Results & Discussion

PLAN



Surface preparation / Protein incubation

a) Surface preparation



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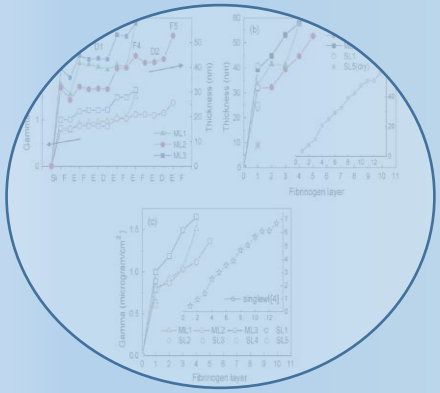
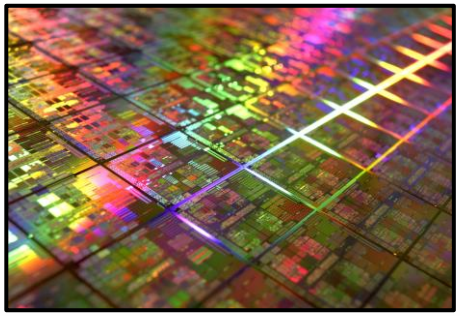
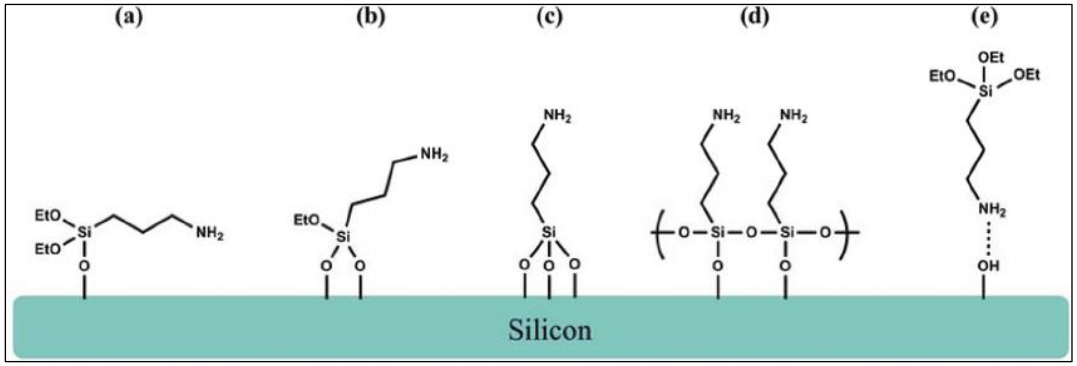


Results & Discussion

Surface preparation : three steps

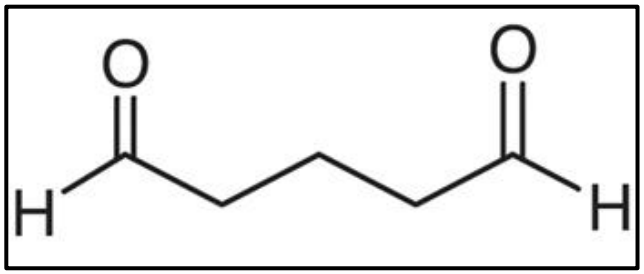


1) Clean the Silicon wafers



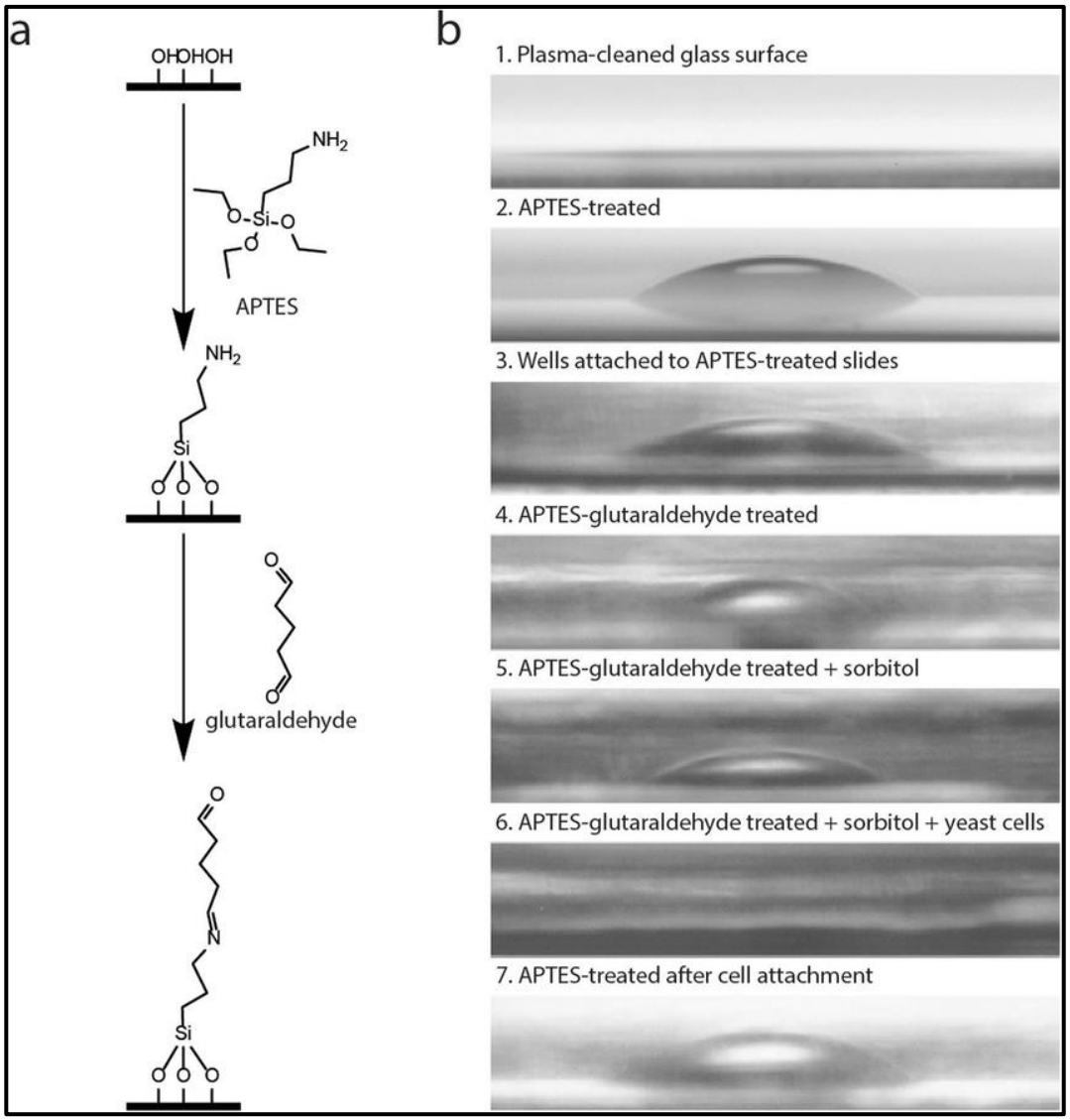
2) Functionalized in APTES

Adapted from Khodakov, 2012



3) Incubated in Glutaraldehyde (GA)

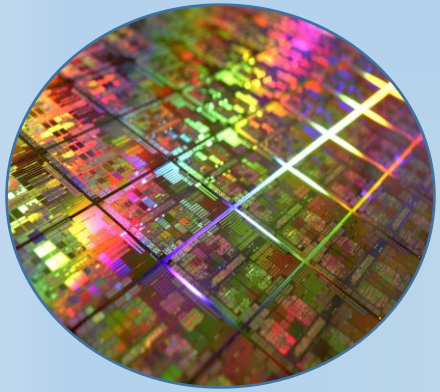
Surface preparation : three steps



An idea of the whole process

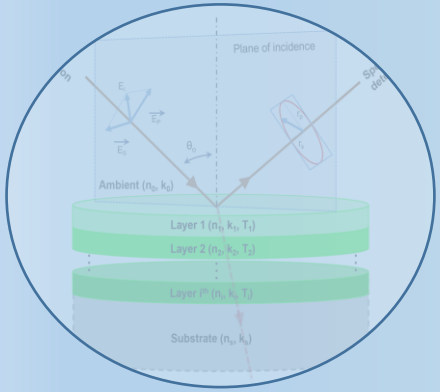
Adapted from Syga, 2018

PLAN

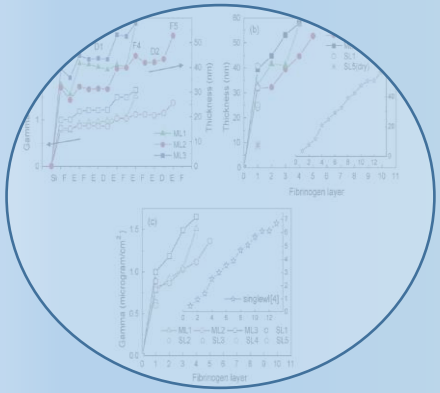


Surface preparation / Protein incubation

b) Protein incubation

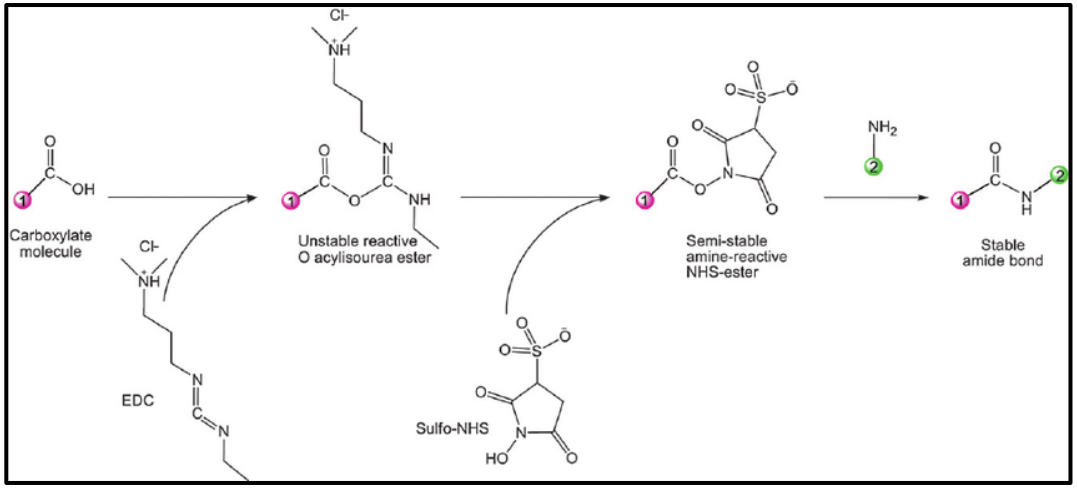


Spectroscopic ellipsometry



Results & Discussion

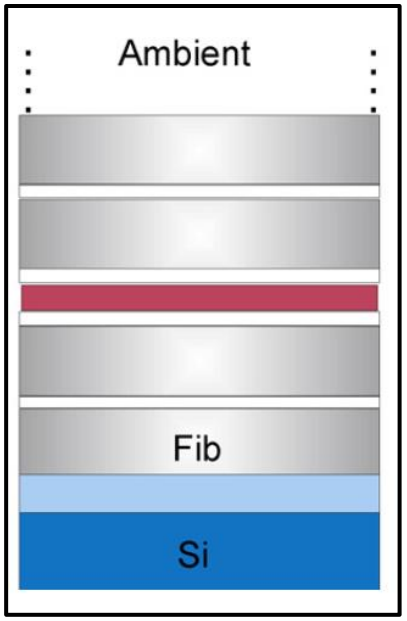
Protein incubation procedure



EDC/NHS incubation for cross-linking and surface activation.

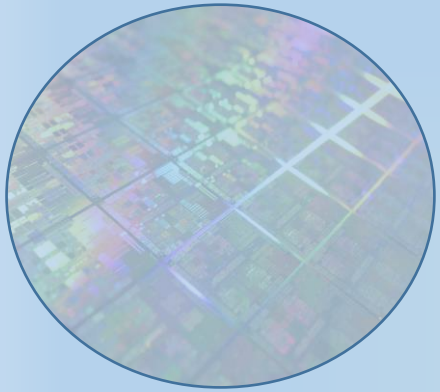
Adapted from Udomsom, 2021

Two incubations of Fib
Adsorption of Doxycycline
Two incubations of Fib

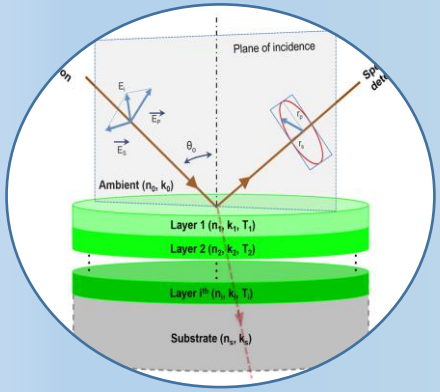


Adapted from Arwin, 2010

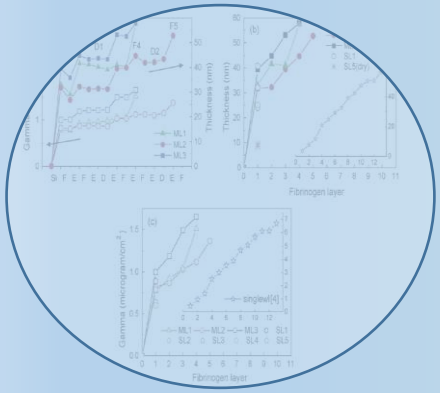
PLAN



Surface preparation / Protein incubation

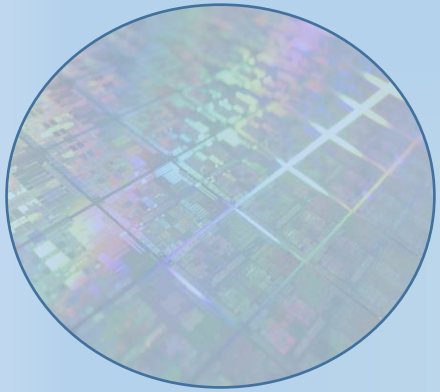


Spectroscopic ellipsometry

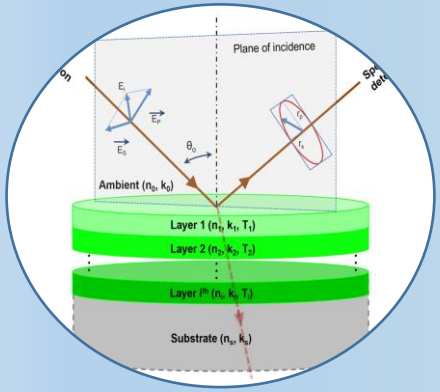


Results & Discussion

PLAN

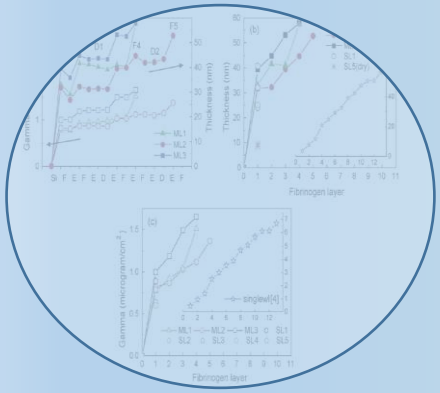


Surface preparation / Protein incubation



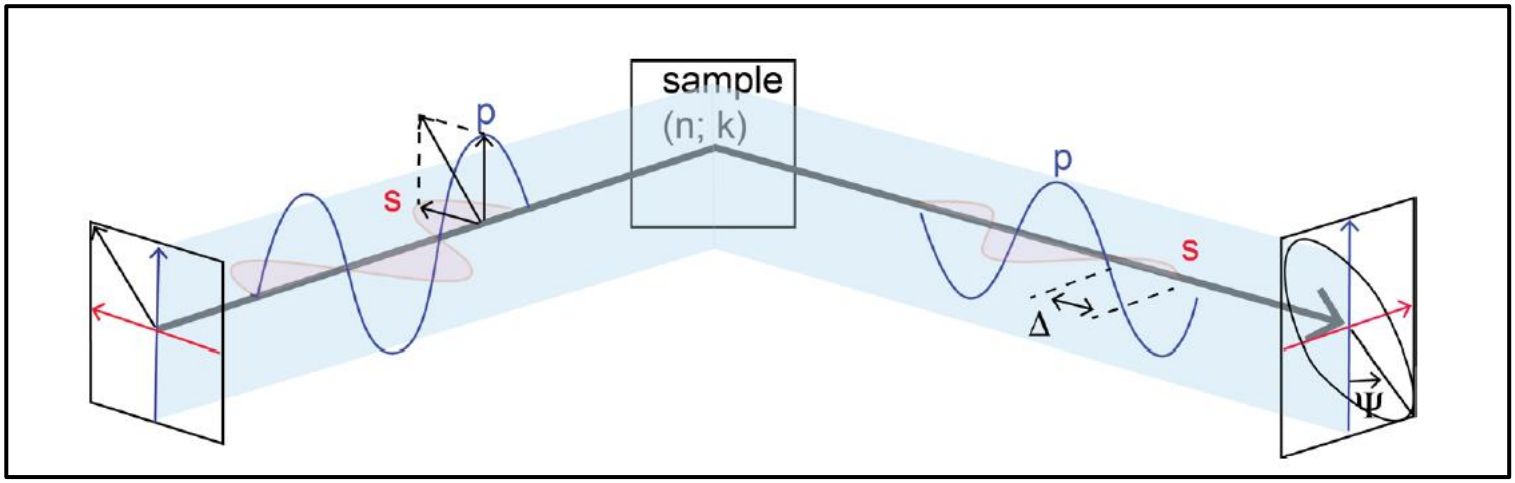
Spectroscopic ellipsometry

a) Theoretical explanation



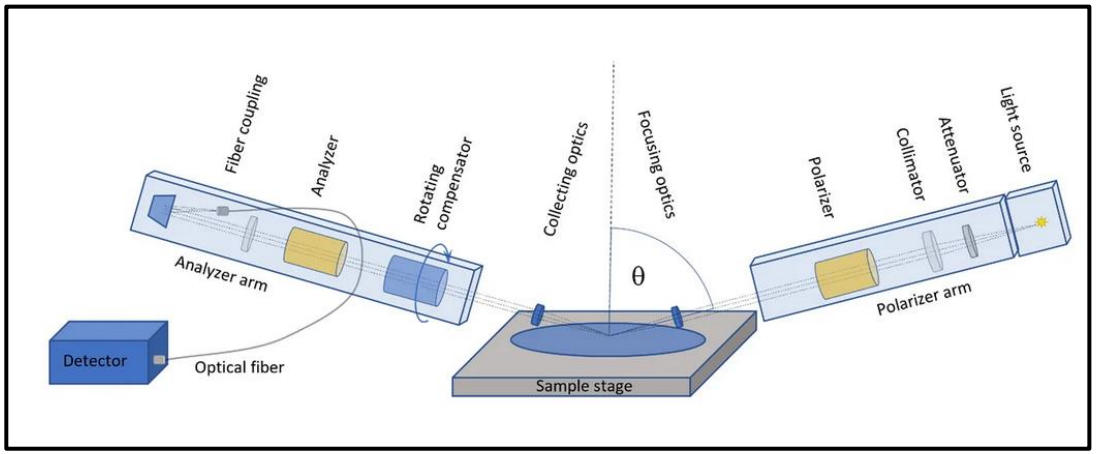
Results & Discussion

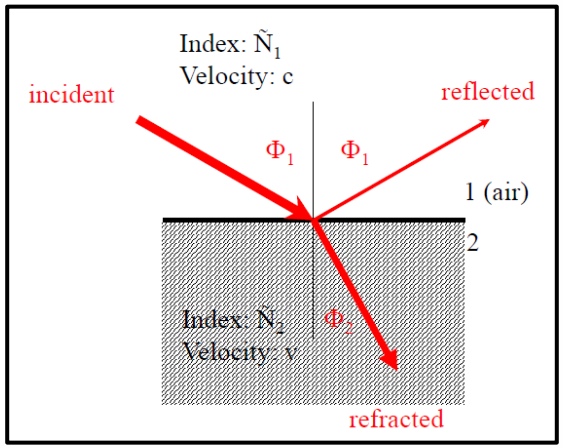
SE : Polarization of the light



The **phase differences** of p and s-polarized light → **Δ**
the **amplitude ratio** → **ψ**

Adapted from E. Migliorini, Spectroscopic Ellipsometry, *2022*





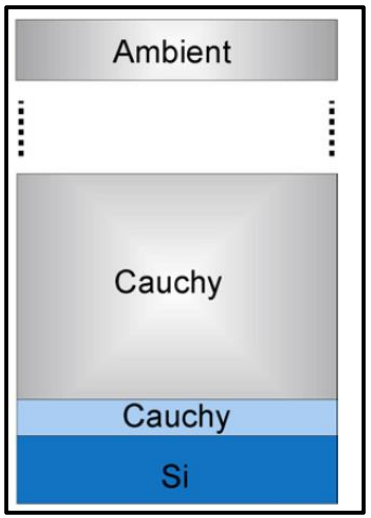
SE : Cauchy Model

- for transparent materials (k=0)

$$\Delta_{measured}(\lambda_i,\varphi_j)=\Delta_{simulated}(\lambda_i,\varphi_j,n_{substrate}(\lambda_i),k_{substrate}(\lambda_i),n_{film}(\lambda_i),k_{film}(\lambda_i),d_{film},\ldots)$$
$$\Psi_{measured}(\lambda_i,\varphi_j)=\Psi_{simulated}(\lambda_i,\varphi_j,n_{substrate}(\lambda_i),k_{substrate}(\lambda_i),n_{film}(\lambda_i),k_{film}(\lambda_i),d_{film},\ldots)$$

Dispersion, Cauchy

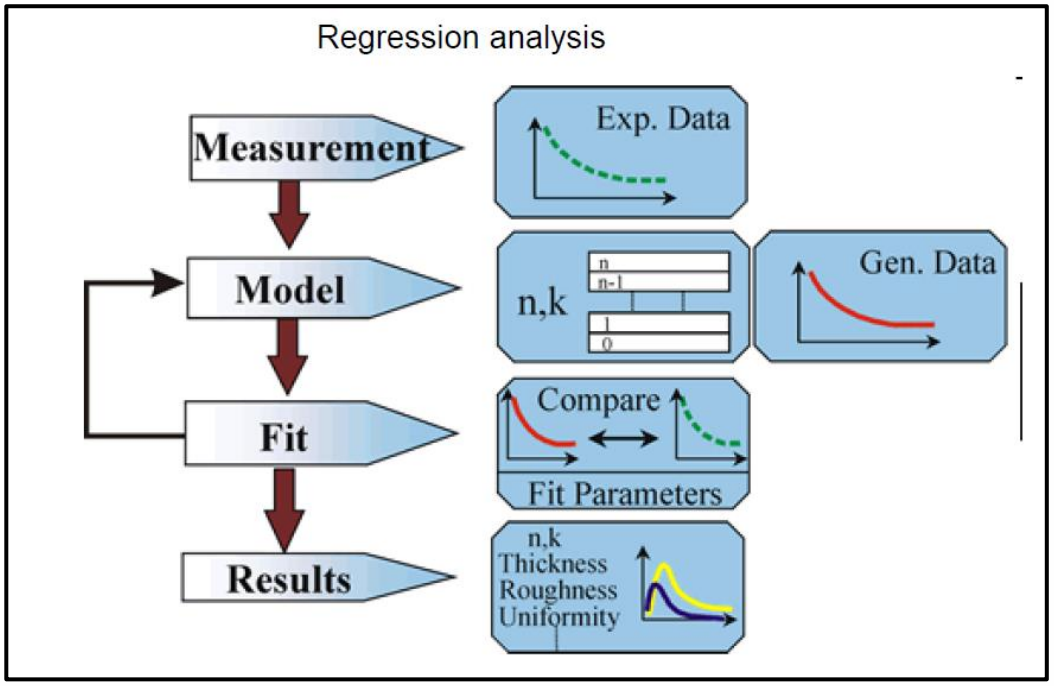
$$n(\lambda)=A+B/\lambda^2+C/\lambda^4$$



Adapted from E. Migliorini, Spectroscopic Ellipsometry, *2022*

Adapted from Arwin, 2010

SE : How to obtain film properties



Adapted from E. Migliorini, Spectroscopic Ellipsometry, *2022*

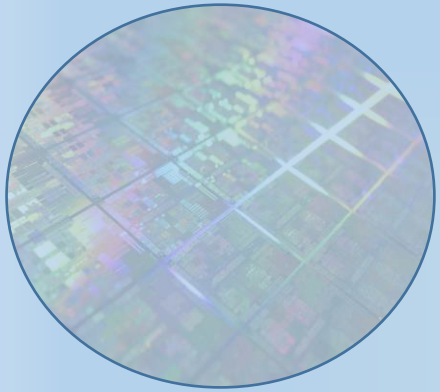
$$\Gamma = \frac{d_f(n - n_0)}{dn/dc}$$

n_0= refractive index of the ambient

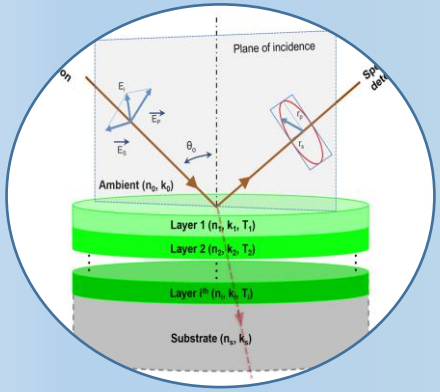
dn/dc = **refractive index increment of molecules in the layer** (known parameter)

- Thickness (d)
- Refractive index (n)
- Mass adsorption (Γ)

PLAN

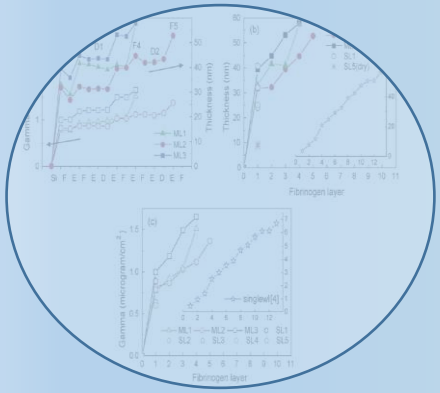


Surface preparation / Protein incubation



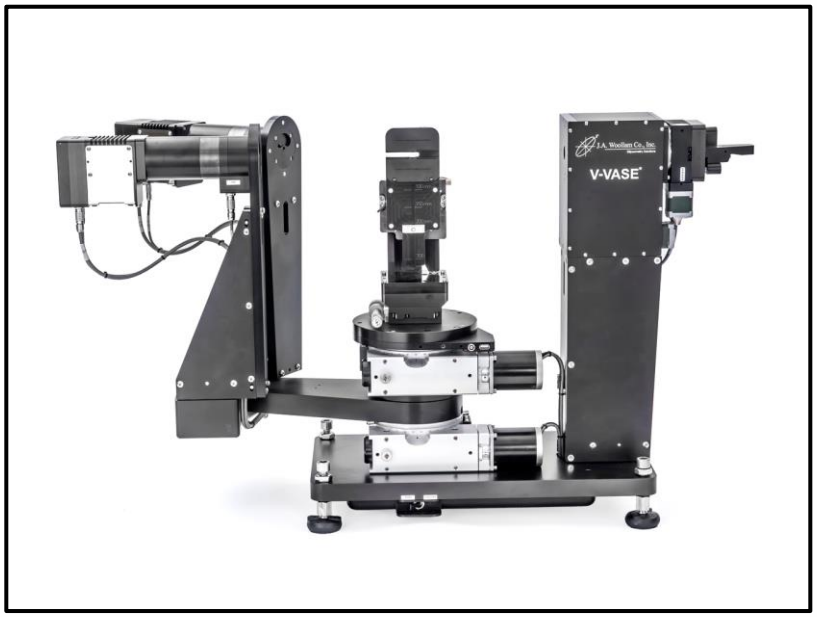
Spectroscopic ellipsometry

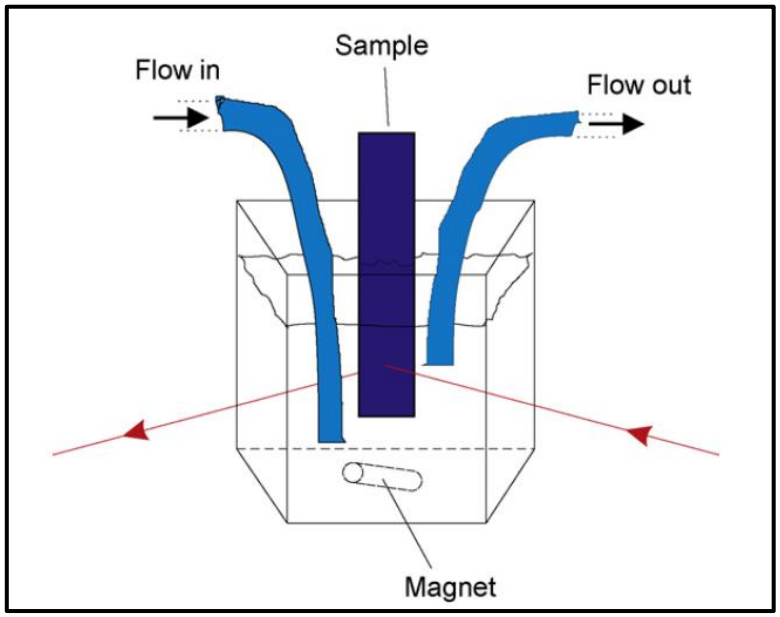
b) The use in the paper



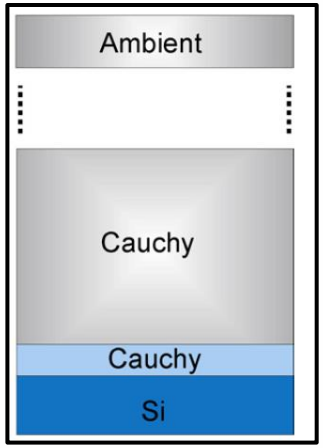
Results & Discussion

SE : Set-up for in-situ measurements



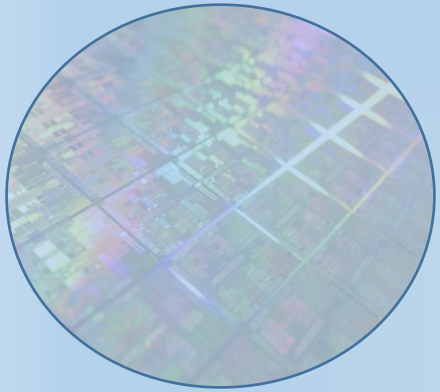


- Bottom layer : silicon dioxide including APTES and GA.
- Top layer : all cross-linked layers.



Adapted from Arwin, 2010

PLAN



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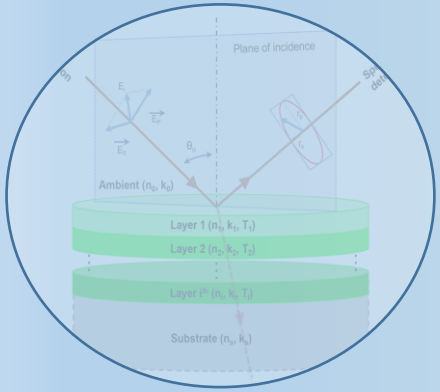
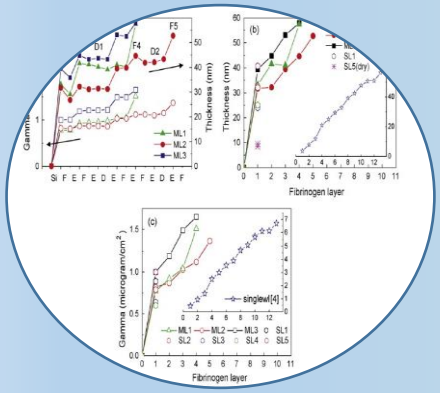
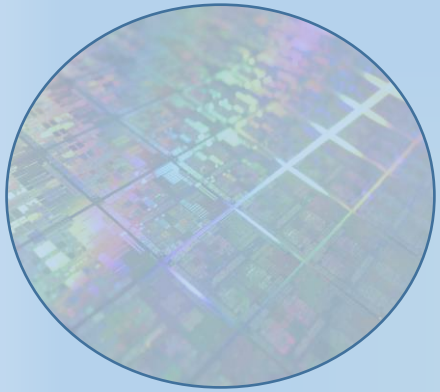


Spectroscopic ellipsometry

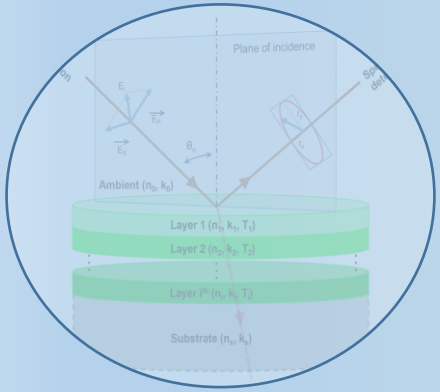


Results & Discussion

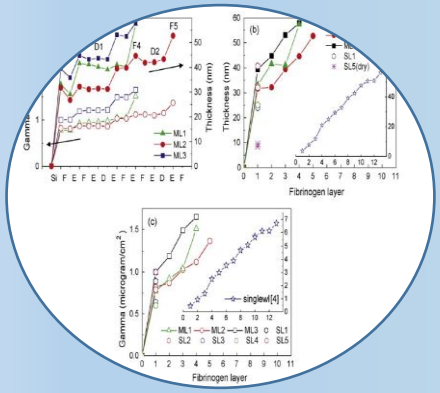
PLAN



Surface preparation / Protein incubation



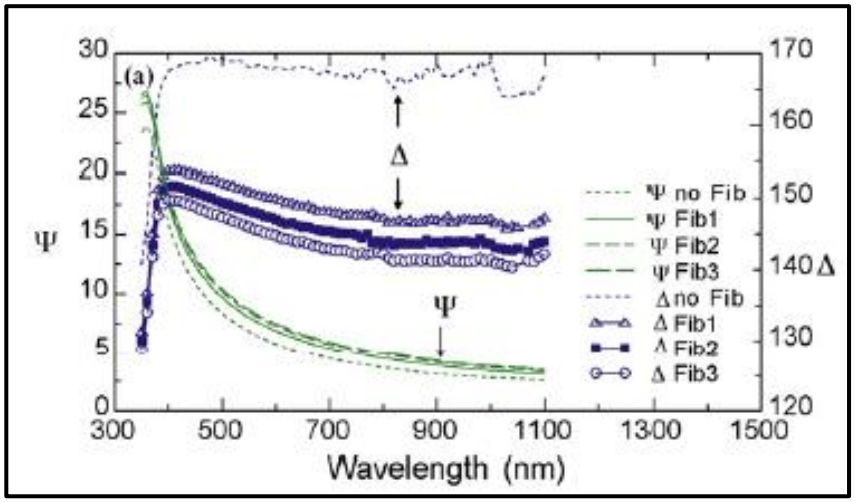
Spectroscopic ellipsometry



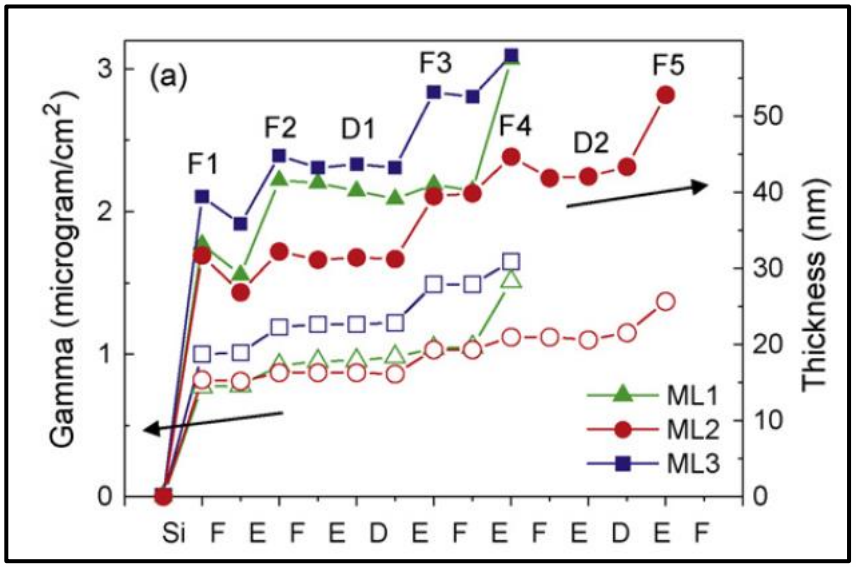
Results & Discussion

a) Results

Results



Display of the spectroscopic scans

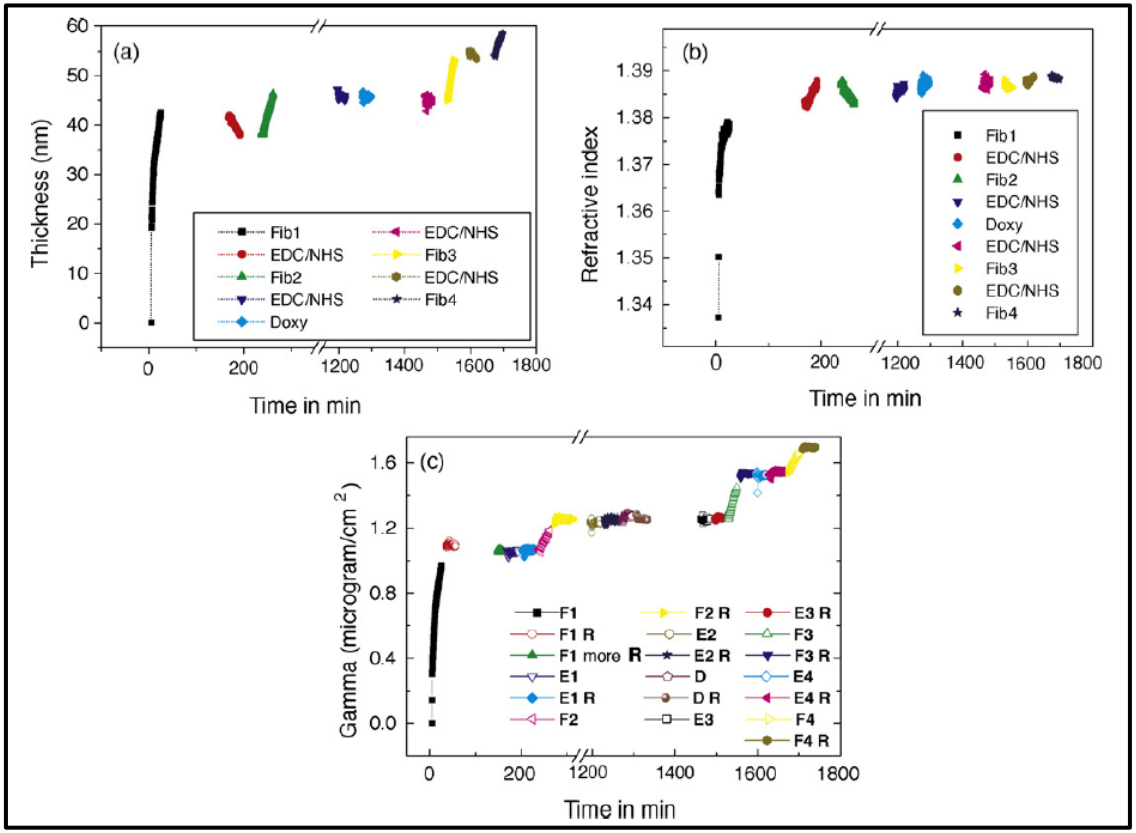


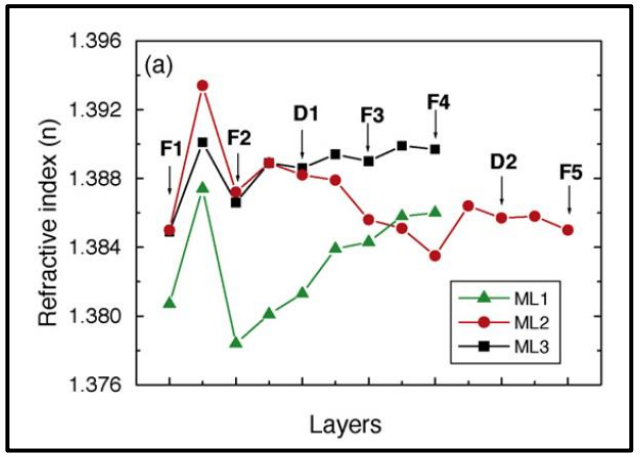
Display of **Γ** and **d**

Adapted from Arwin, 2010

Results

Display of the refractive index

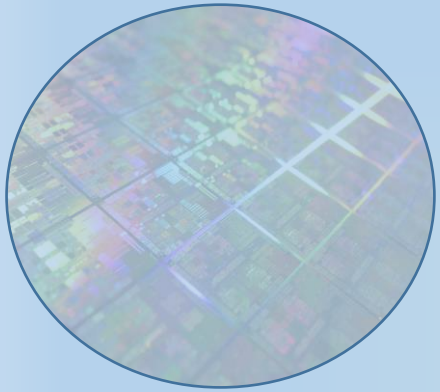




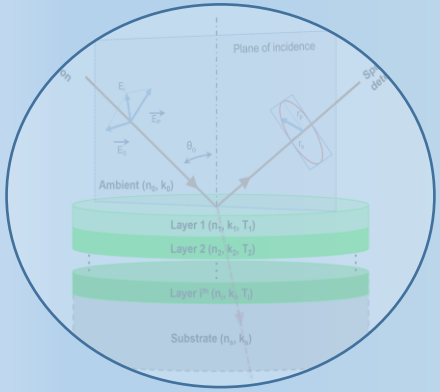
Display of the dynamics

Adapted from Arwin, 2010

PLAN



Surface preparation / Protein incubation



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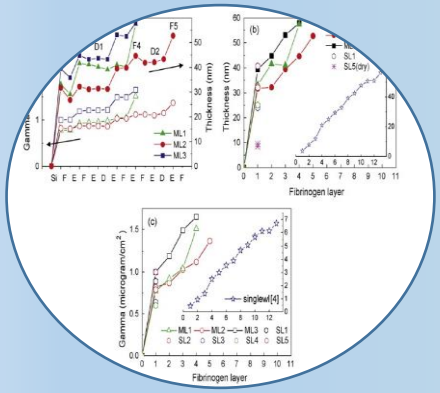
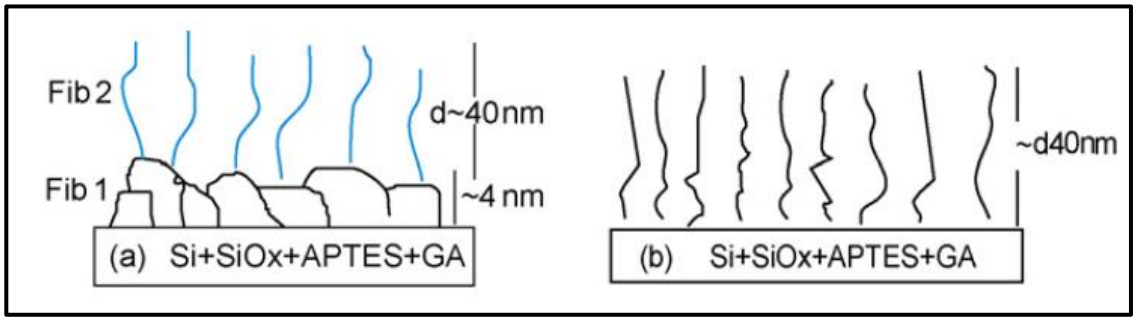


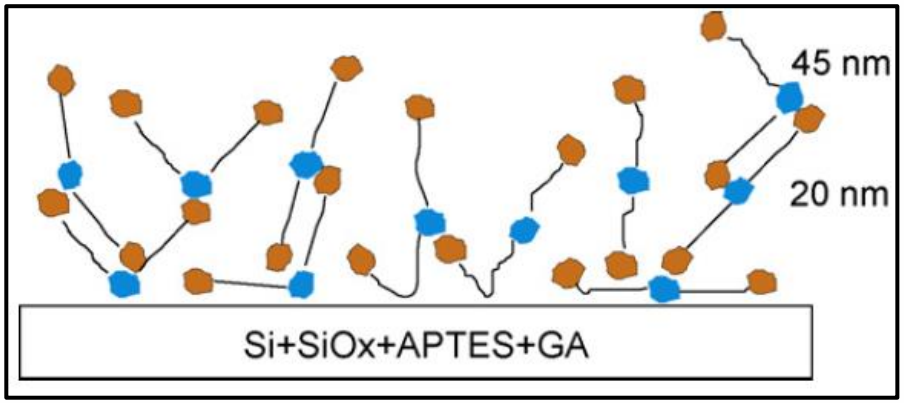
Results & Discussion

b) Discussion

Discussion : *in situ* vs *ex situ*



Drying step of the surface in *ex situ* → collapsed layer



Mixture of covalent bonds and hydrophobic interactions

Adapted from Arwin, 2010

TAKE-HOME MESSAGES

- Differences between protein matrices prepared *in situ* and protein matrices *ex situ* has been demonstrated.

- Spectral ellipsometry data provides enhanced resolution and allows a separation of layer thickness and index.

- The adsorption process is a complicated molecular organization with a mixed set-up of molecular interactions.

- Contributions to the development of a model for layered growth of fibrinogen have been provided.