

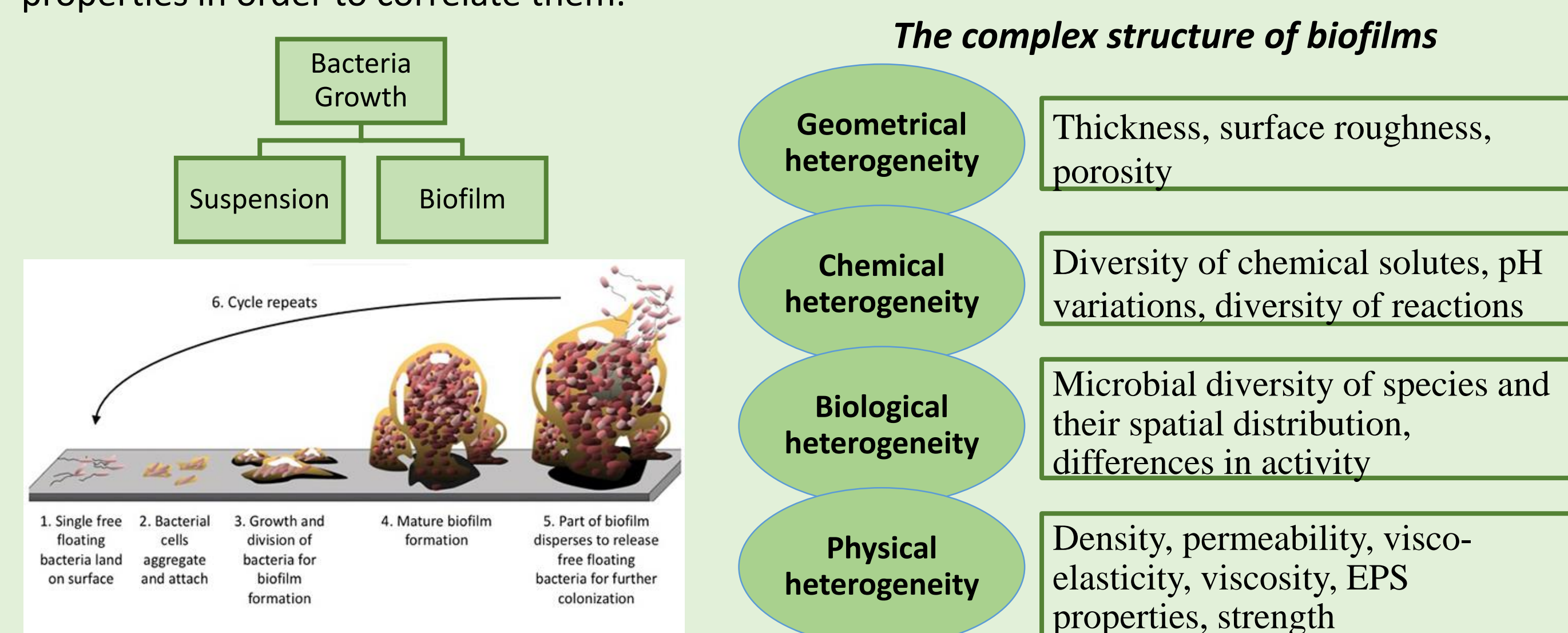
Biofilm investigation by confocal microscopy and atomic force microscopy

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Background

Biofilms represent an important problem in many situations, from hospitals to daily life. Research, at both academic and industrial levels, is looking for systems capable of detecting biospecies around us. This study aims at creating know-how on biofilms, investigating their properties in order to correlate them.



The growth in biofilm form represents a protection, this structure resists very well to external stresses. Planktonic cells land on surface, then they start to aggregate. Next step is the growth and division for the formation of biofilm. When the film is mature, it releases planktonic cells and the cycle starts again. Biofilm is a complex and variable system.

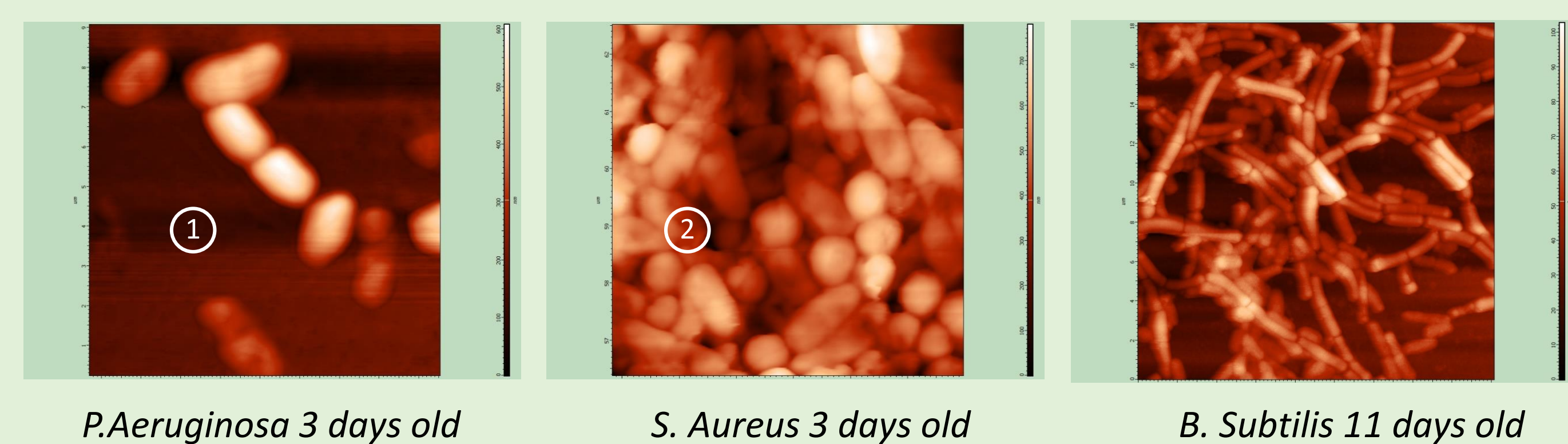
Our work is structured as follows:

- Growth of biofilm (*P. Fluorescens*, *P. Aeruginosa*, *B. Subtilis* and *E. Coli*) on different substrates (glass, steel and polypropylene) under controlled conditions to obtain reproducible biofilms ;
- Biofilm study: by confocal microscopy (CLSM) and by AFM (morphological study);
- Measurement of the elastic properties of biofilm on glass by AFM.

AFM analysis – the very first results

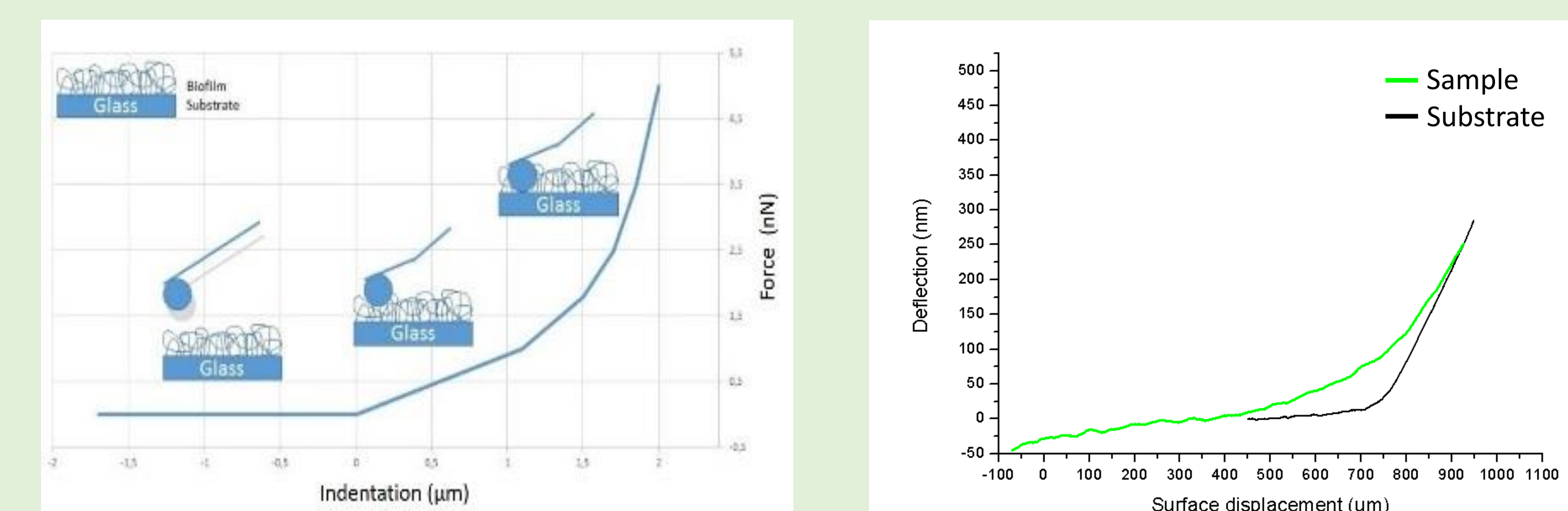
Morphology

Morphology studies were done using the AFM. The samples were biofilms grown on glass for 3 days, and then dehydrated at 60°C for 60 minutes. A pyramidal tip was used and the analysis was performed in air.



Mechanical properties – work in progress

Using the AFM it is possible to investigate the elastic properties of the biofilms of different ages. We started performing studies using silica-spherical probe in liquid environment (home made and commercial probe, diameter ~ 5µm to 20µm).



$$p = \frac{1}{1 + \frac{k_s}{k_t}} \quad k_s = G \cdot \Phi$$

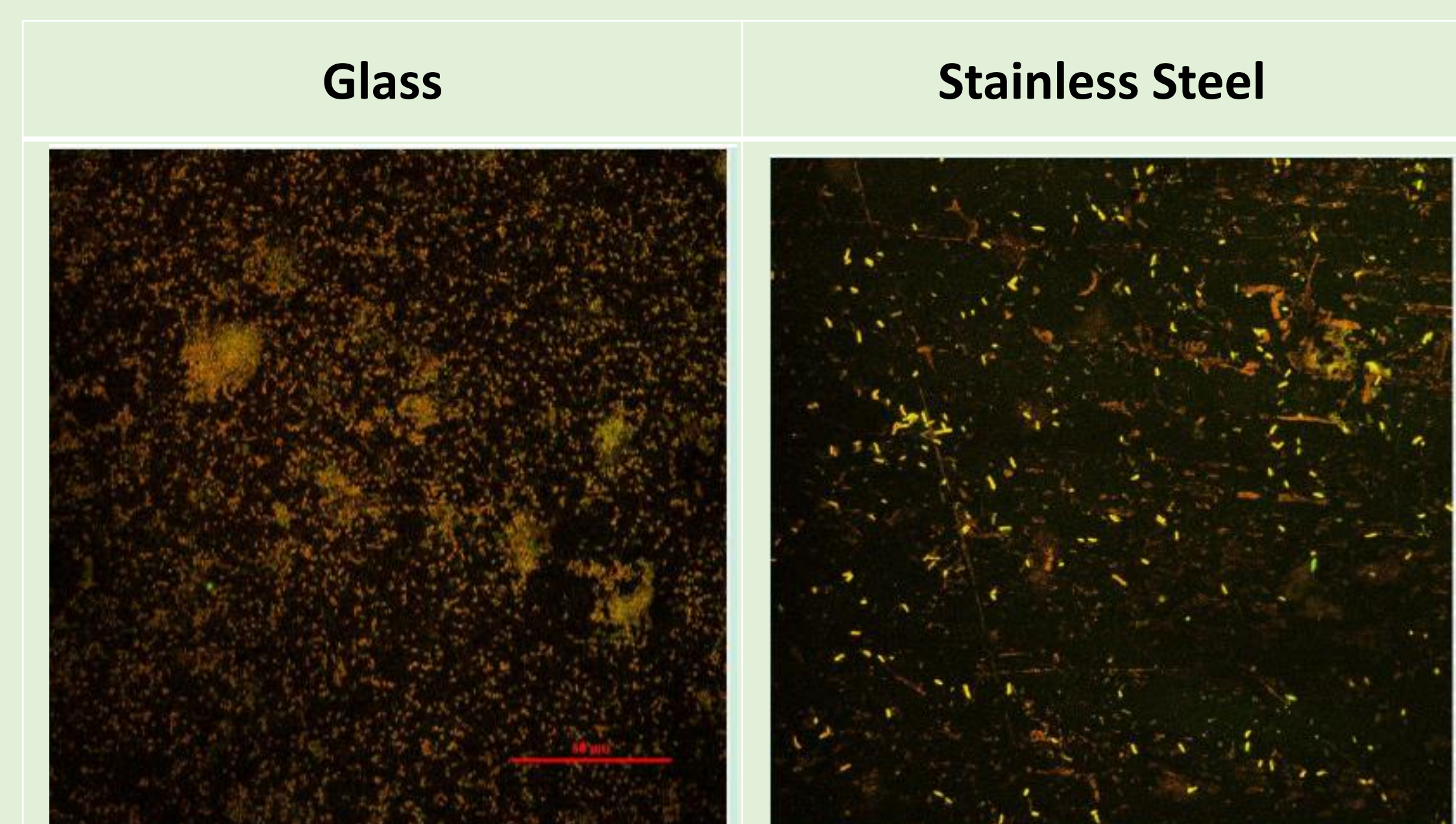
p: Force curve slope
k_s: Sample stiffness
k_t: Tip stiffness
G: Sample elastic modulus

Confocal laser microscopy

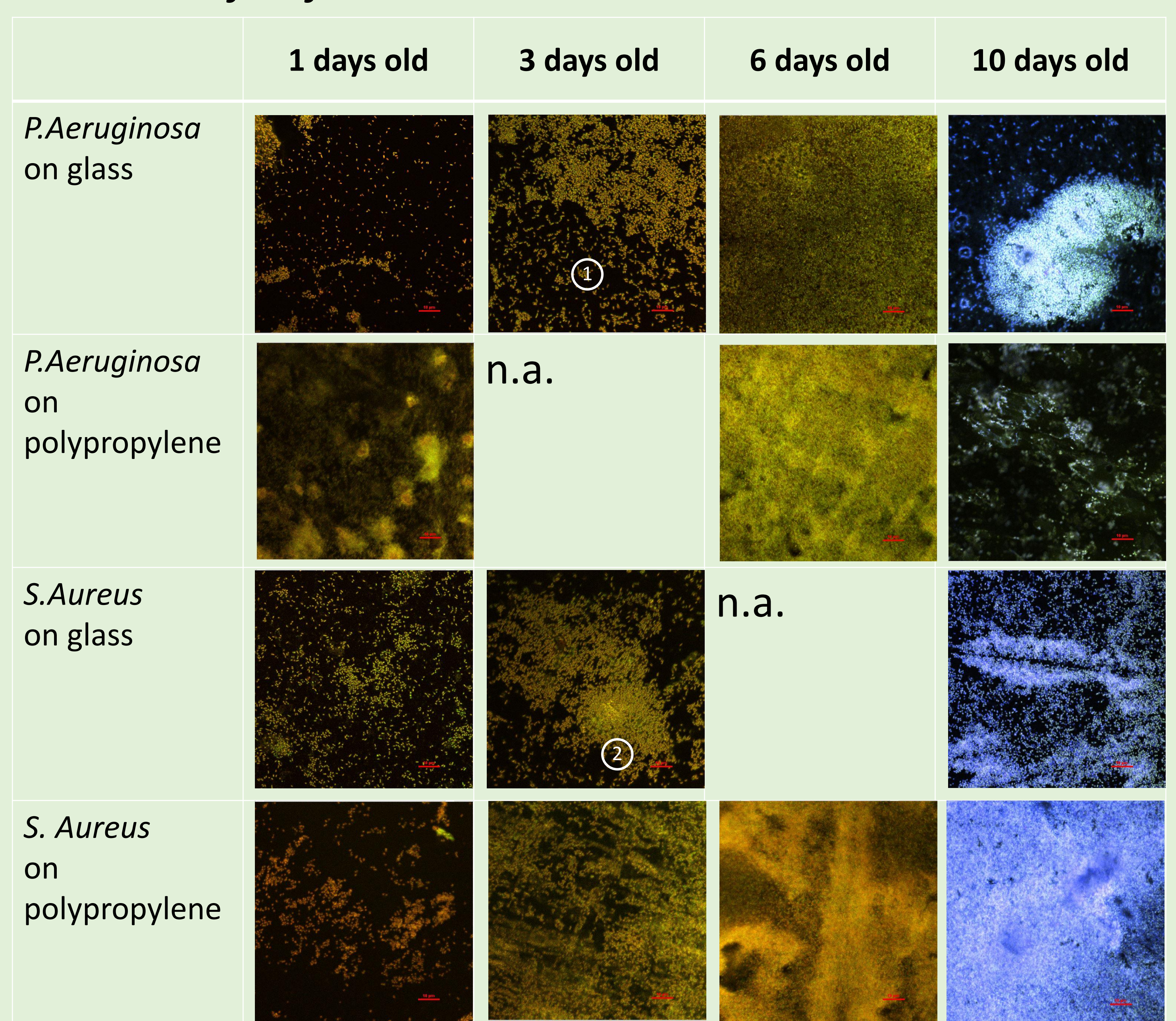
The samples were dehydrated at 60°C for 60 minutes and then labeled using acridine orange. The analysis was performed using the Nikon Eclipse C1si and 60x and 40x magnifications. The area acquired was 100µm x 100µm. Tests have been done on stainless steel, glass and polypropylene.

The effect of substrate – *P. Fluorescens* 3 days old

The growth of bacteria film depends on various conditions, the substrate influence very well the formation of the biofilm.



Formation of biofilm

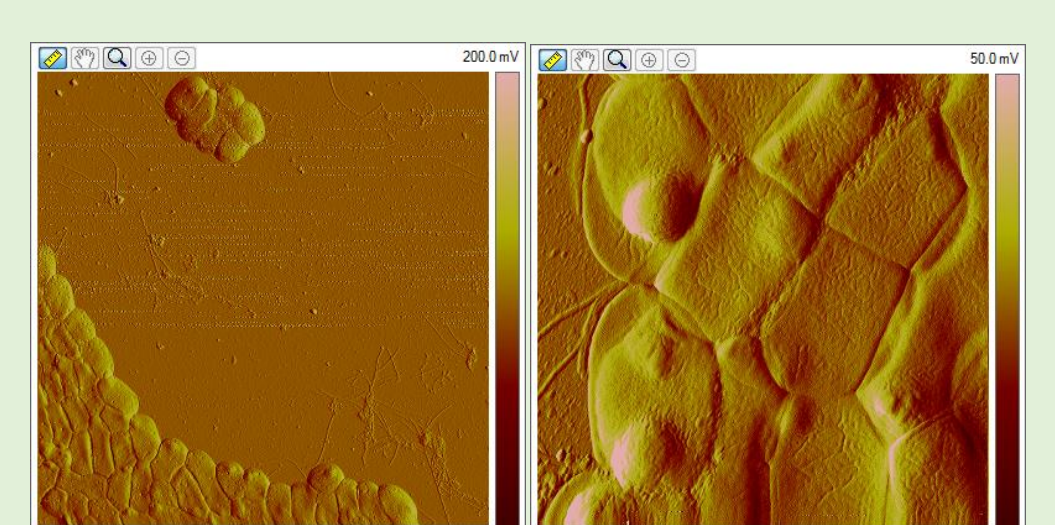


The results obtained by CLSM showed that stainless steel substrate does not favor the formation of the biofilm. On the other hand, glass and polypropylene substrates have proven to be good substrates.

The difficulties in using biofilms

When the cells are not well attached to the substrate, the biofilm undergoes fundamental changes in morphology, and it does not resist to the contact with the tip during mechanical tests.

E. Coli 24h old



From our first results:

- Confocal microscopy** is useful to understand how the substrate influences the biofilm formation and consequently how to choose the substrate of interest.
- Using **AFM** is possible to visualize the morphology of the biofilm and its elasticity, and correlate these properties to the age and the thickness.

It appears, from our first results, that investigation of biofilm using different techniques, especially confocal microscopy and atomic force microscopy, is a promising way to understand the properties of these complex structure and then to monitor their presence.