

Beyond the paradigm of nanomechanical measurements on cells using AFM: an automated methodology to rapidly analyse thousands of cells

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November the 23rd 2021

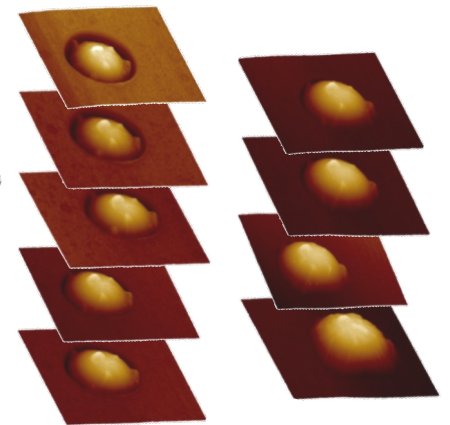
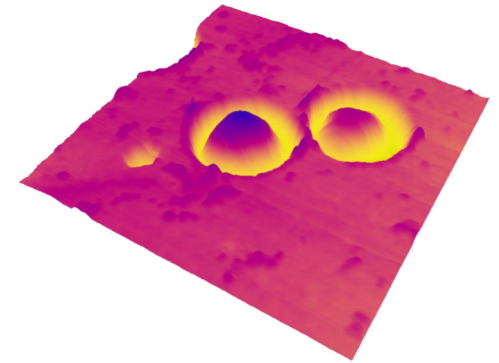
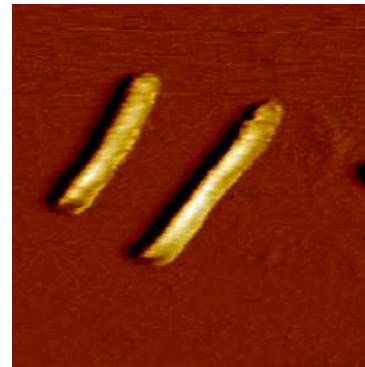
Immobilize without distorting!

2007-2008

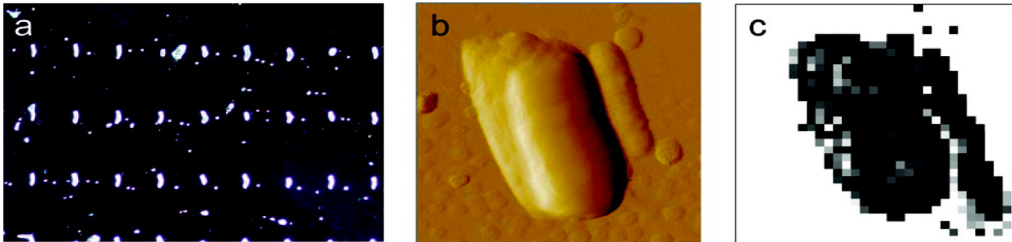


Immobilize without distorting!

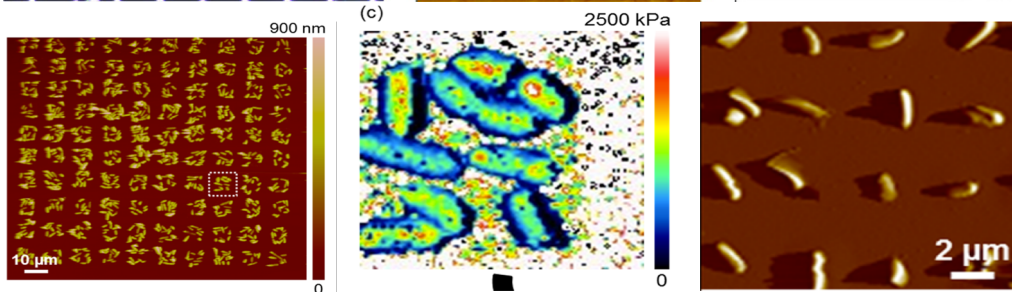
2007-2008



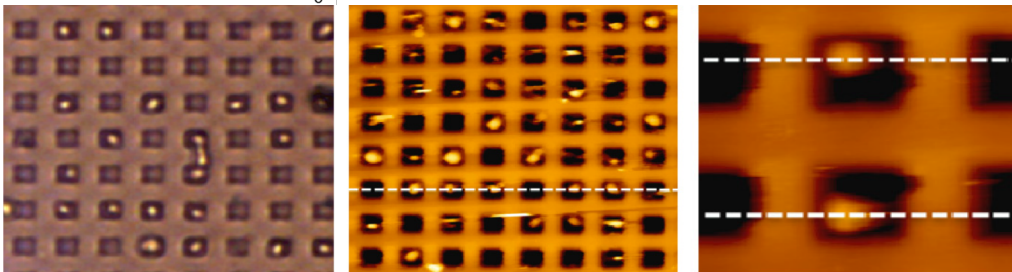
Technology: directed immobilization of microorganisms



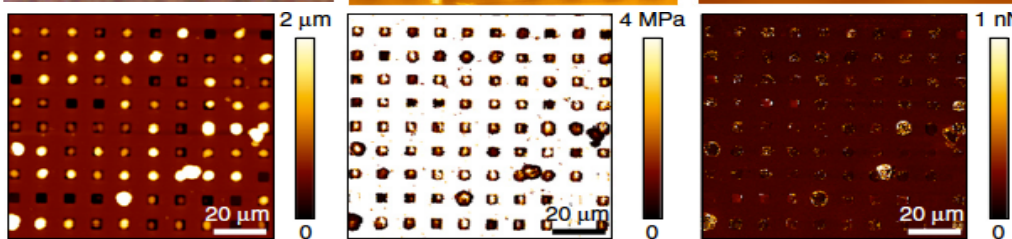
Micro contact printing of adhesive and antiadhesive molecules, Cerf et al., Langmuir 2009



Polycation deposition after surface structuring by nanoxerography, Jauvert et al., ACS Applied materials and interfaces 2014



Microstructuring of a PDMS stamp, Jauvert et al., Nanotechnology 2012

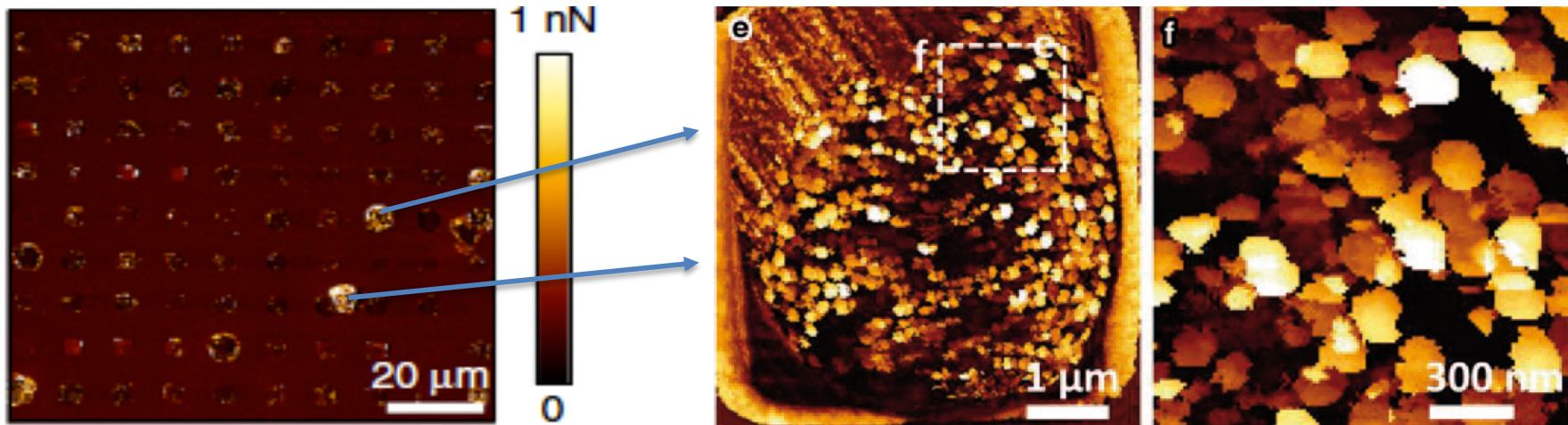


Optimization by coupling the convective capillary deposit, Formosa et al., Nature Protocols 2015

Model microorganism = *Candida albicans*

- Responsible for opportunistic infection, present in 80% of the population
- But also fatal infection in immunocompromised people
- 1.5 million deaths per year worldwide due to fungal infection, 43% are candidiasis.

Adhesion map



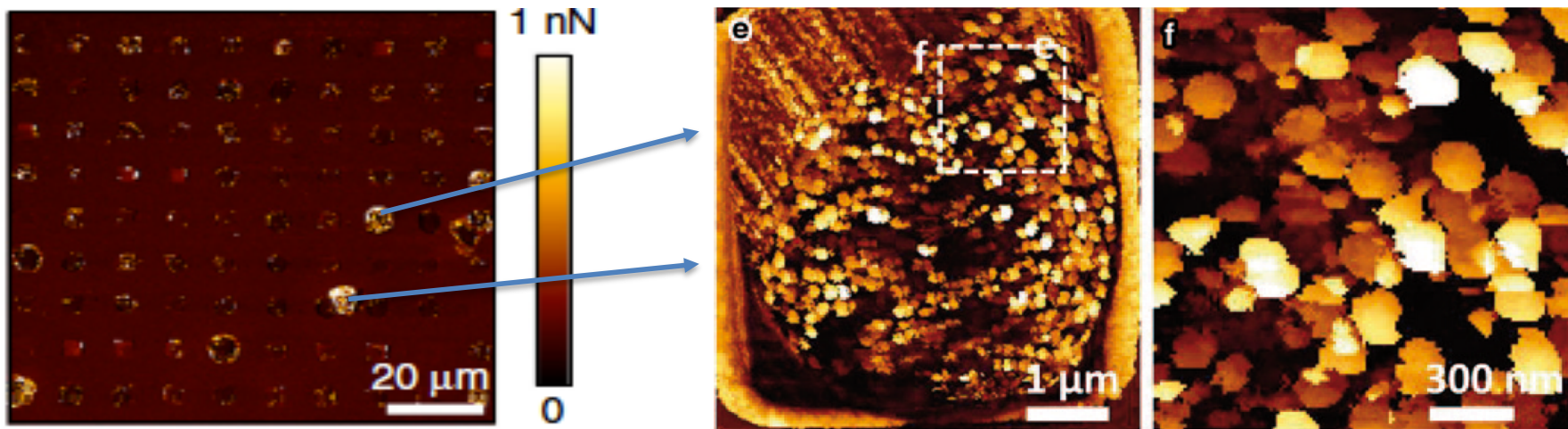
- Nanomedicine, Nanotechnology Biology and Medicine 2015
- Antimicrobial Agents and Chemotherapy 2015

Detection of nanodomains of adhesins and amyloid aggregation on the surface of *Candida albicans*

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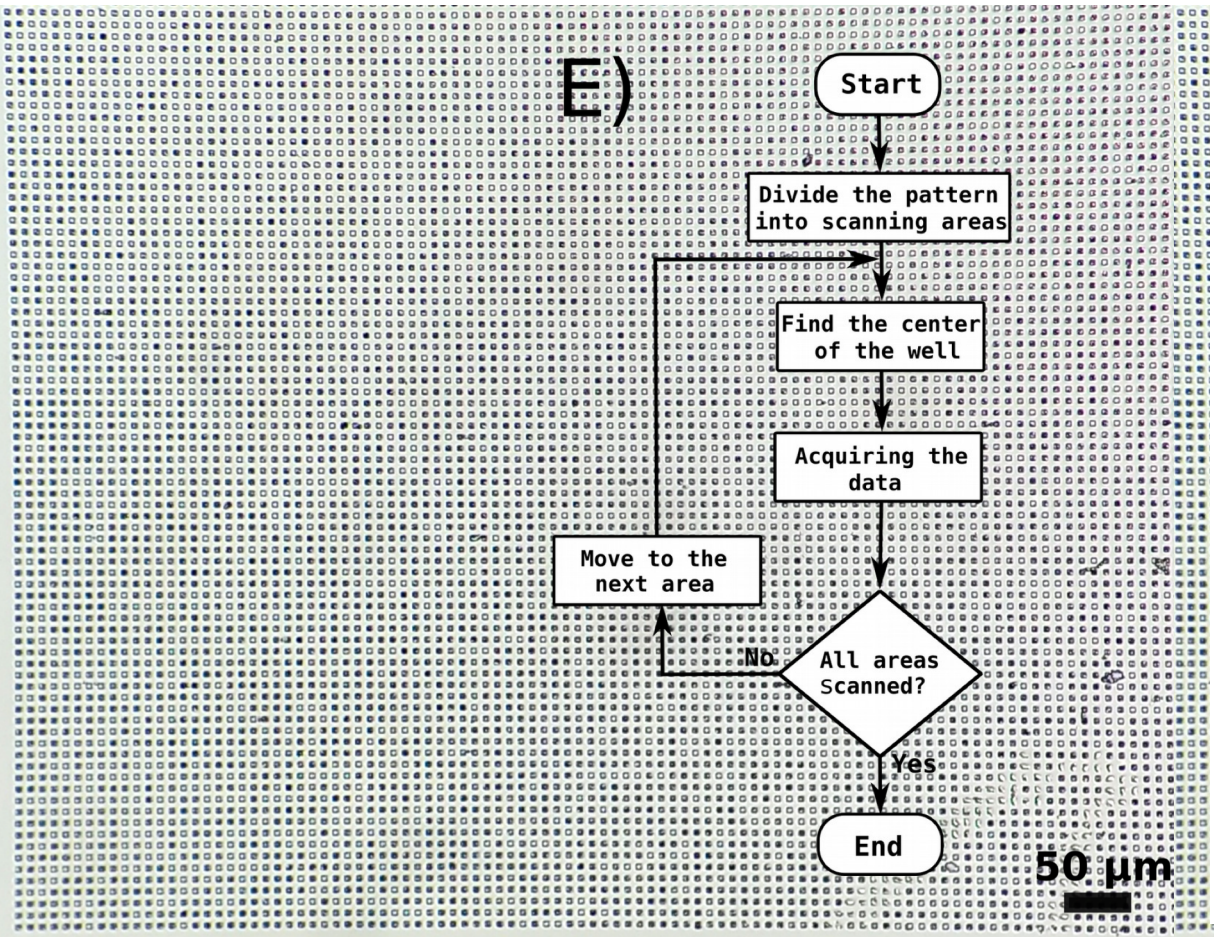
Adhesion map



**Identification of adhesin nanodomains on the surface of
a subpopulation of cells**

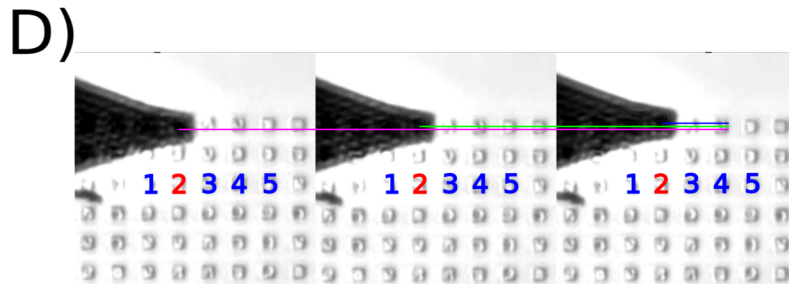
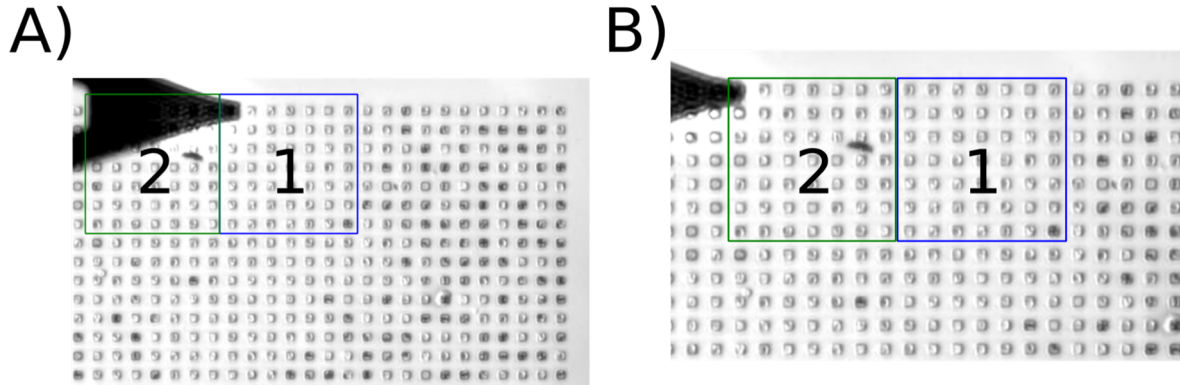
Automation of Bio-AFM measures

- Hypothesis: perform thousands of CFs on a few cells = **ignore the heterogeneity** of the samples and therefore part of the information
- Objective: **measure 1000 cells in 2 hours** = transition to a diagnostic tool



1. Organize cells on the support:
Microstructured PDMS Stamp; LAAS-Clean Room
2. Automate AFM movements between cells
Collaboration Mexico, Institut Polytechnique National Centre d'investigation en Computation; ECOS Nord contract
- Doctoral student in international co-supervision S. Proa
3. Analyze the data generated by automatic learning,
Supervised, Unsupervised (DISCO team collaboration)

Proof of concept on microbial arrays



The program have to

- find the coordinates of the first well
- move from well to well
- move from scanning area to scanning area
- record force curves in each well
- store, organize and analyze the data

Patent WO2019112414 (A1)—2019

Program overview

```

17 # Function to calculate angle
18 def calculateAngle(A = [], B = []):
19     """ Given two points (x,y) an angle is calculated
20     """
21     Time_angleS = time.time()
22     if A[0] != B[0]:
23         print 'angle calculated: ' + str(math.atan((B[1] - A[1])/(B[0]-A[0])))
24         Time_angleF = time.time()
25         print'Time for calculate angle: ' + str(Time_angleF - Time_angleS)
26         return math.atan((B[1] - A[1])/(B[0]-A[0]))
27

```

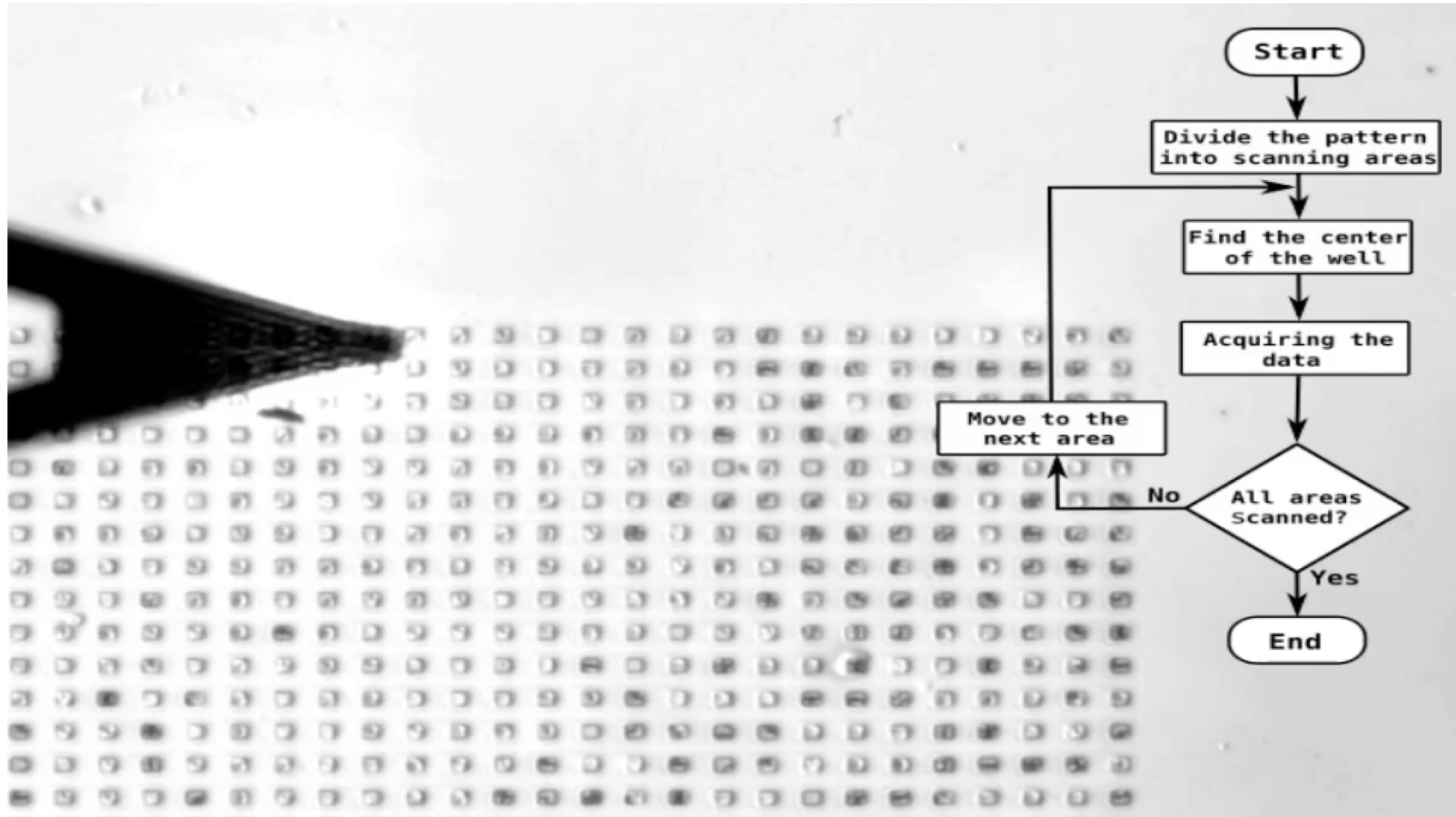
34 # Function to determine plane equations
46 # Function to recalculate points 1, 2 and 3.
61 # Variables to determine weights ...
...
367 # Iterating through the MotorStage coordinates
407 # Calculating Well positions
435 # activate autosaving
444 # Iterating through the Wellgrid ...
511 # Retract the piezo after finish

- 515 code lines, described function by function to record the force curves
- a python script to organize the data
- data analysis on classic AFM softwares
- an R script to present and analyze the results (histograms and anova tests)

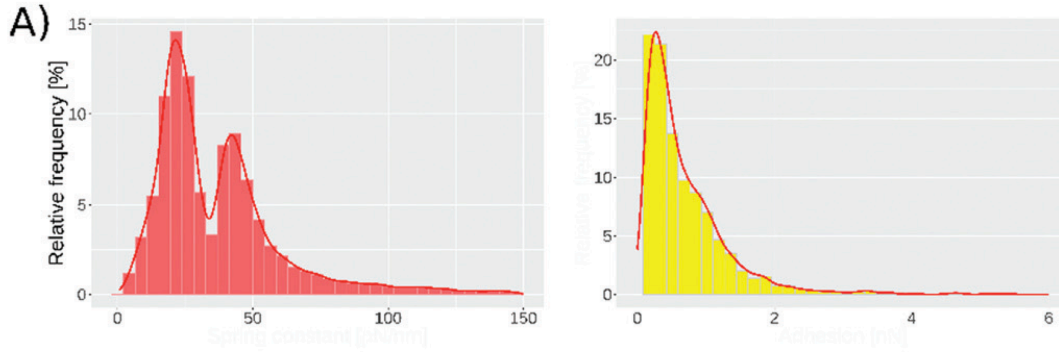
available in a JoVE publication,

Severac C., et al. **2021**, Automation of bio-atomic force microscope measurements on hundreds of *C. albicans* cells **Journal of Visualized Experiments** 170, e61315, [doi:10.3791/61315](https://doi.org/10.3791/61315)

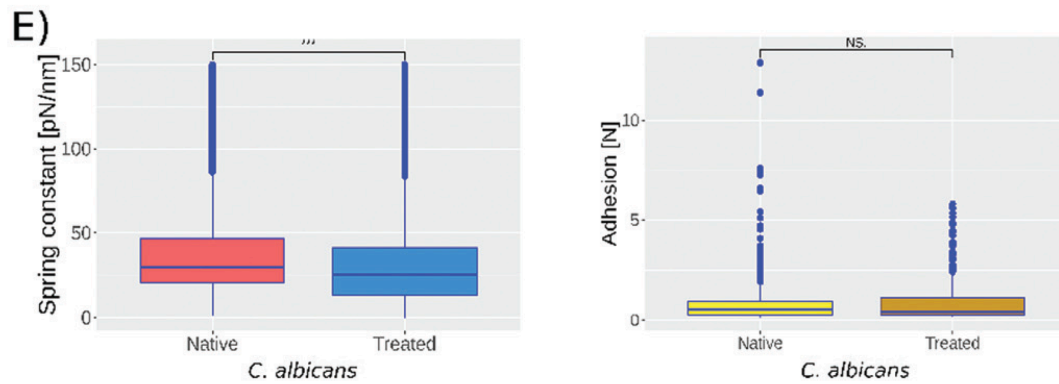
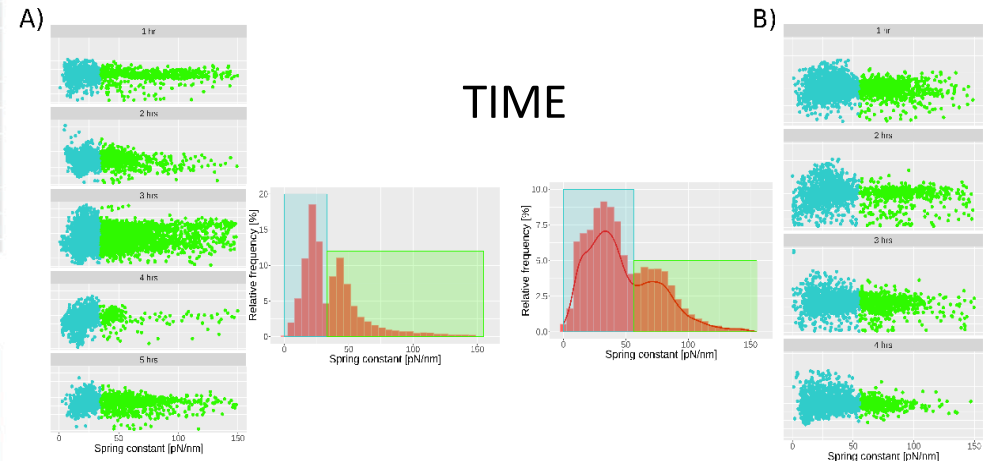
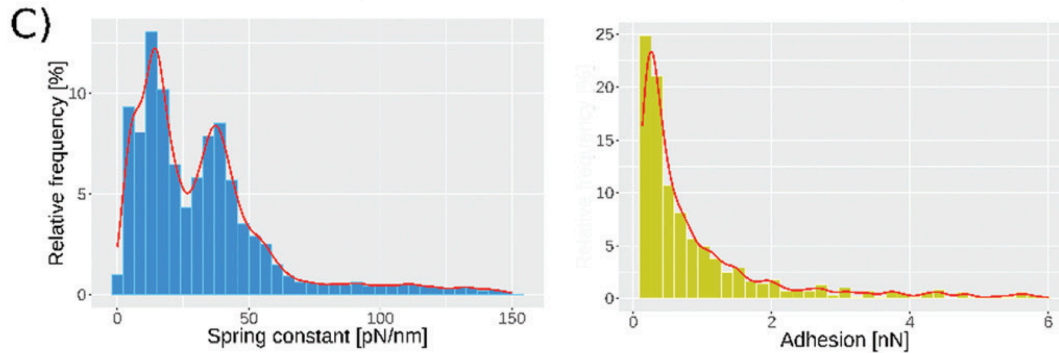
Automated AFM



Subpopulations and effect of caspofungin



- 2 sub population based on spring constant in native cultures
- Unpredictable adhesion properties in between cultures
- Decrease of spring constant in caspofungin treated cells
- no link with the time nor with the tip position



> Fabrication of eukaryotic cell chips : BioSoft:
LAAS-Innopsys

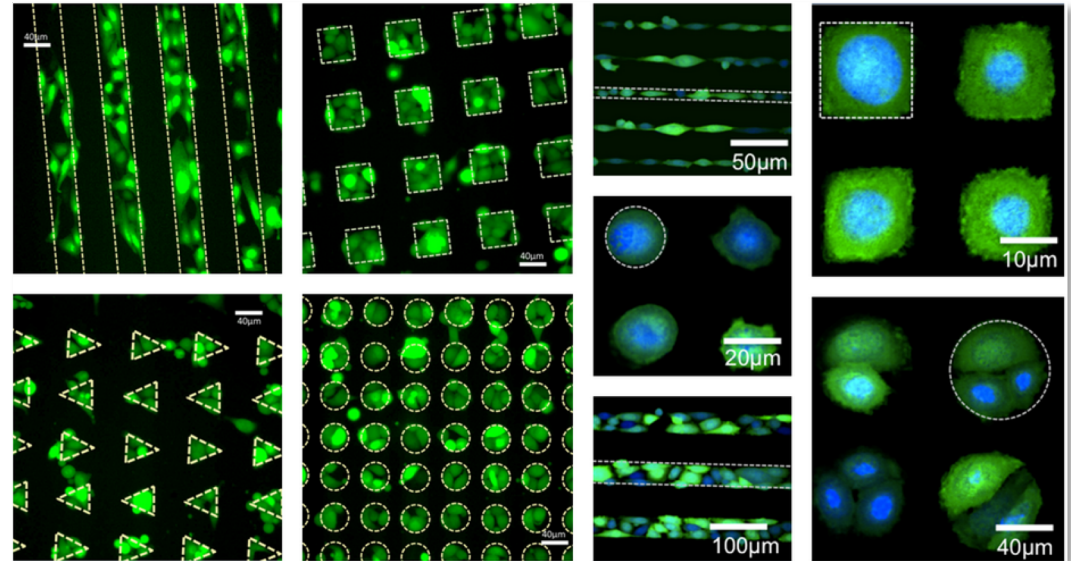
- Microfabrication of molds
- White room LAAS
- Automated Micro Contact Printing of fibronectin
- Innostamp 40

> Cell culture

> PF cell culture LAAS

> Python script driving the AFM from cell to cell

- find the coordinates of the 1st cell
- move from cell to cell
- correct step motor errors
- record the force curves on each cell



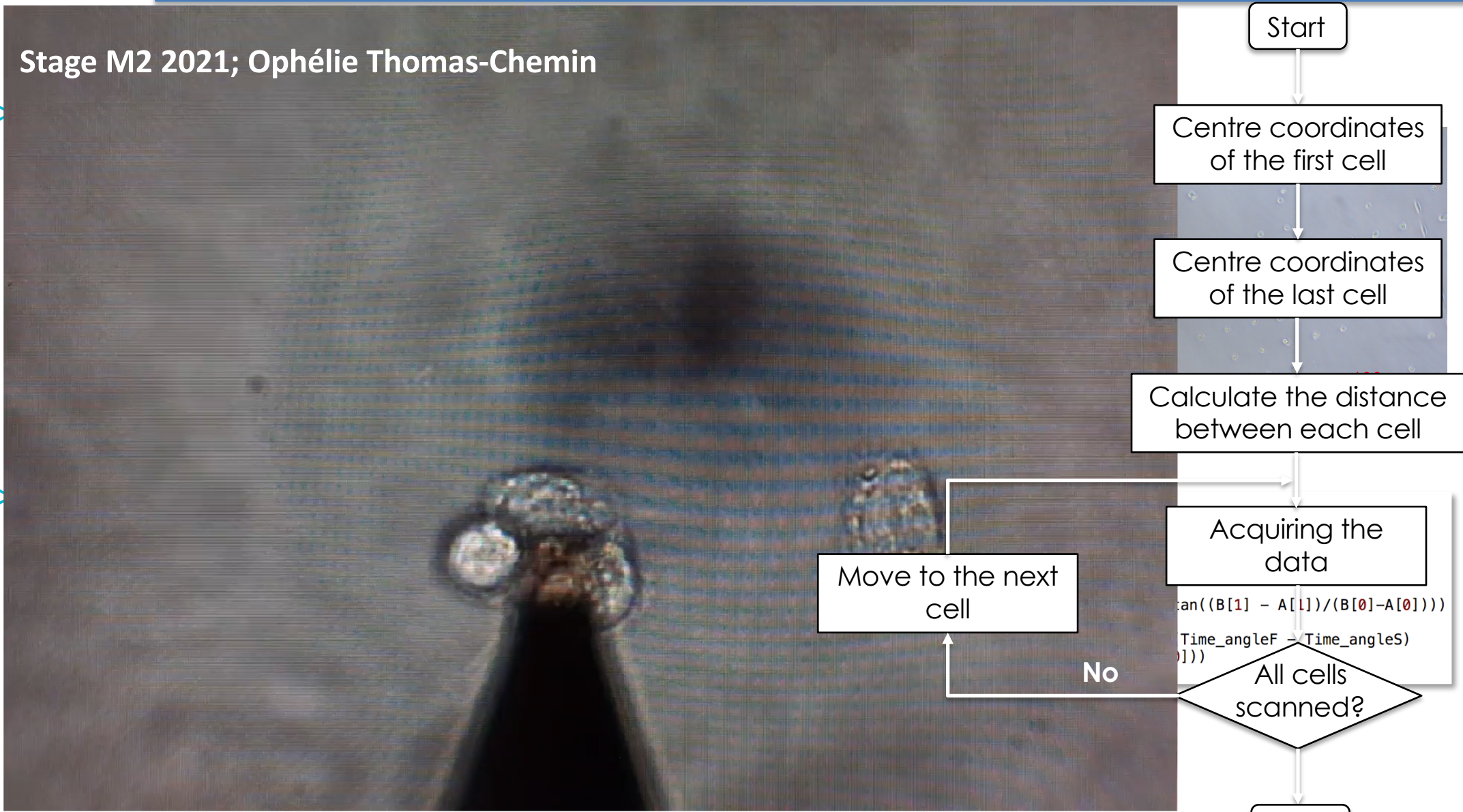
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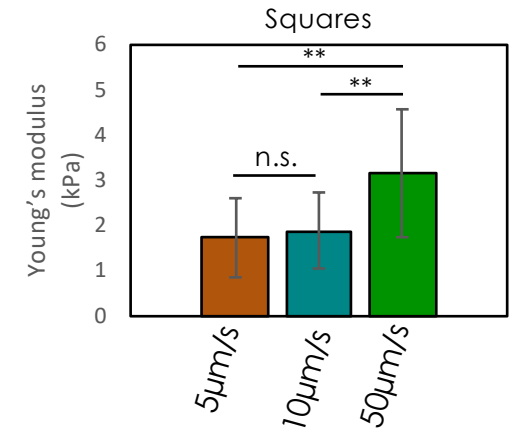
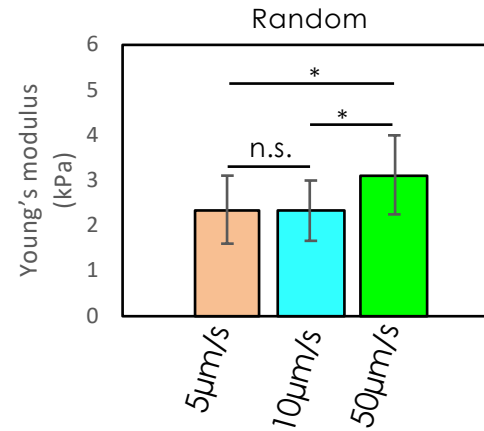
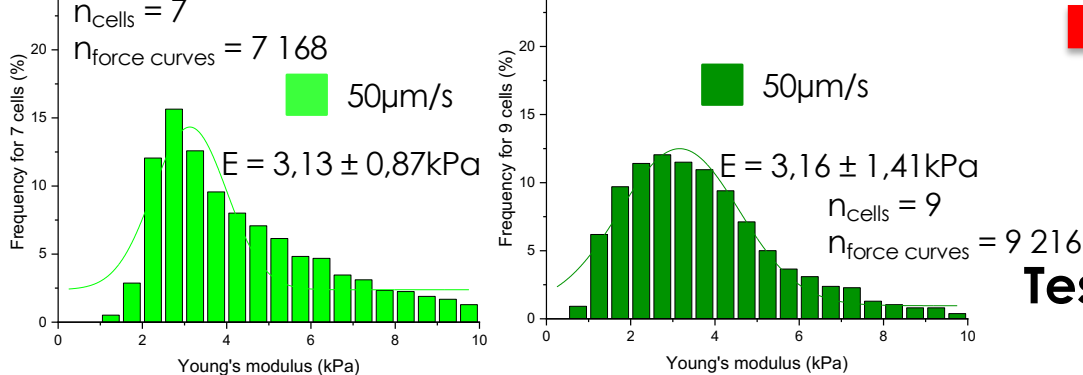
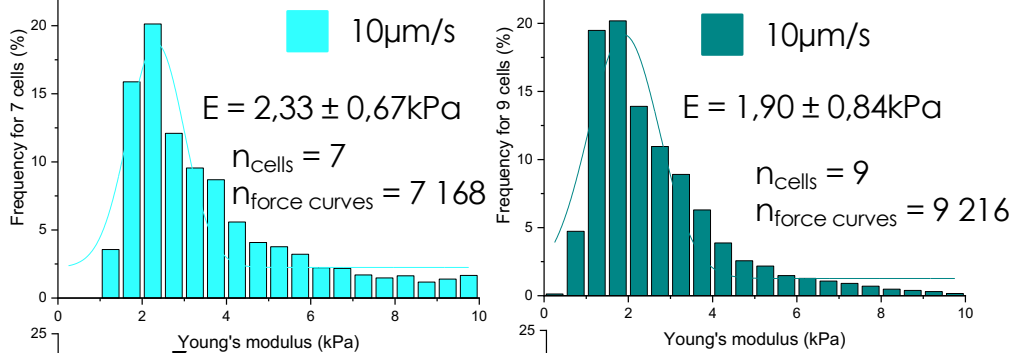
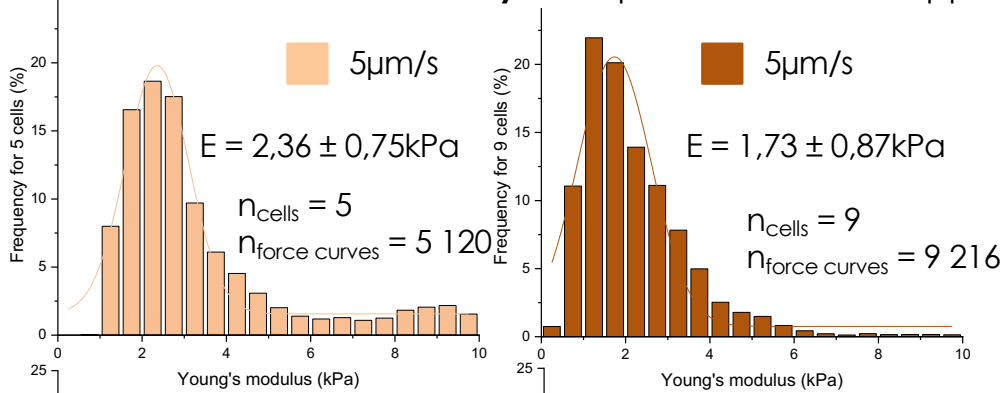
Video of AFM automated motion on mammalian cells

Stage M2 2021; Ophélie Thomas-Chemin



Influence of patterning and of the indentation velocity on the measured Young's modulus

Influence of indentation velocity. Comparison between approach speeds of 5µm/s ; 10µm/s ; 50µm/s.



No significant difference between 5 and 10µm/s
Measured Young's modulus higher for 50µm/s

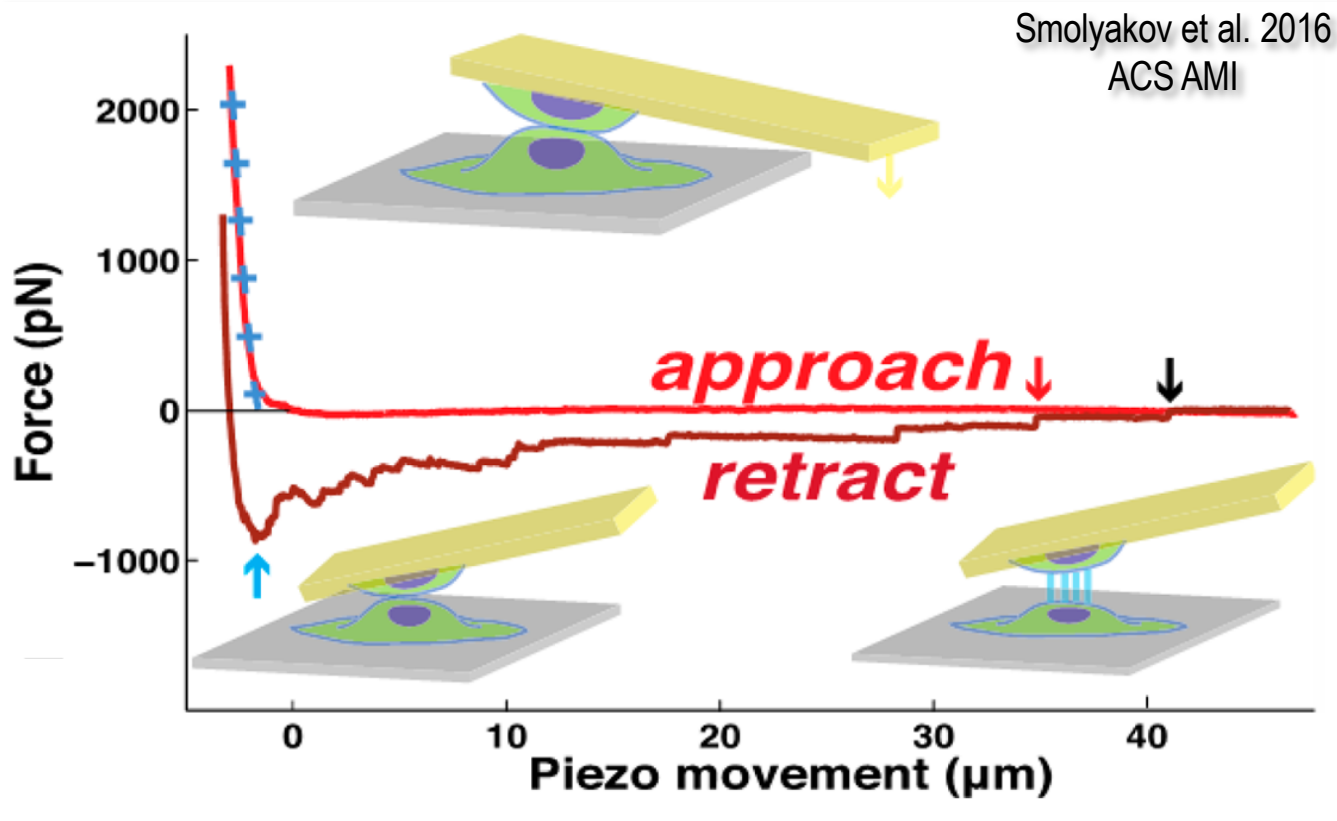


Square patterning does not change the measured Young's modulus

Indentation velocity influences the measured Young's modulus from 50µm/s

**Test other pattern shapes (circles, triangles)
indenter shapes (spheres, pyramids)**

Question: which mechanobiological markers?



- Approach curve :

- Stiffness, Elasticity
- Pre Stress
- Jumping on approach
- Repulsive interaction

- Retract curve

- Adhesion
- Number of nanotubes
- Length of nanotubes
- Breaking force of the nanotubes
- Adhesion work
- Difference approach/retraction
- Dissipated energy
- Viscoelasticity as a function of the stress regime

Several mechanobiological markers allow the discrimination of breast cancer lines

Towards a multi-marker mechanobiological analysis

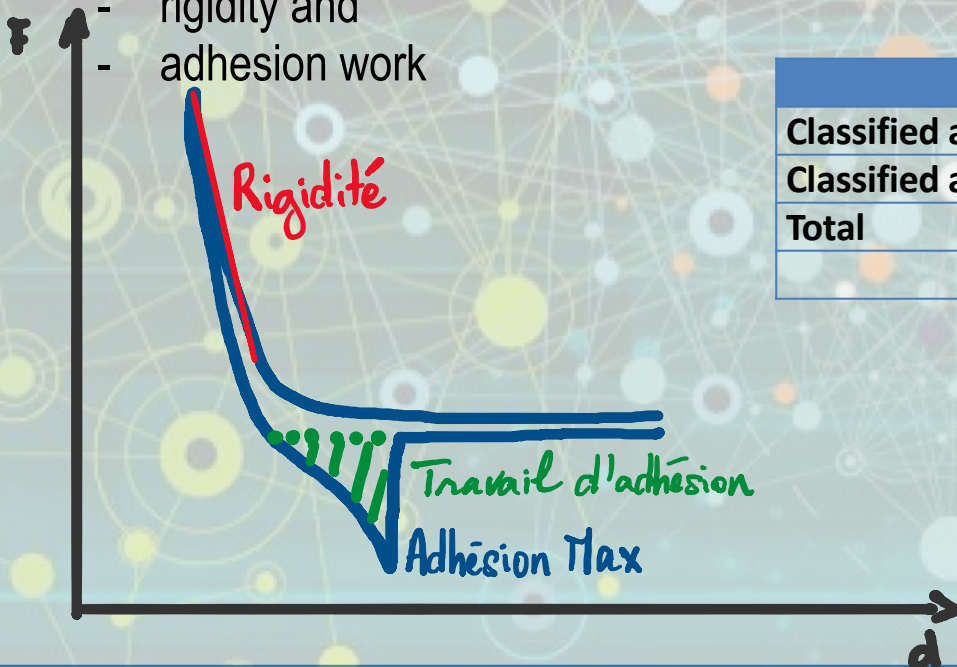
- Introduction de la notion de mécanome par Wang et al. Nanoletters 2021

$$F = F_s + F_c = 4/3 E_c R^{0.5} d^{1.5} + 2 \pi^2 R d \sigma \cos[\tan^{-1}(2 R / d)]$$

E_c : Elasticity, σ : prestress 2 markers calculated on approach curve

Their combination allows to better discriminate the more or less invasive cell lines

- 85% efficient supervised machine learning by combining 3 markers
- maximum adhesion,
- rigidity and
- adhesion work



	Cellules traitées	Cellules non traitées	Total
Classified as Treated	540	187	727
Classified as Native	35	770	805
Total	575	957	1532
		Correct classification rate	85.5 %

Collaboration équipe DISCO MV Lelann

Conclusions

- A paradigm shift
 - Thousands of curves per cell on a few cells ;
 - a few curves per cell on thousands of cells
- Requires a combination of surface patterning, automation and Bio-AFM expertise
- Generate large amounts of data compatible with machine learning useful for classification
- Need to advance fundamental knowledge in biophysics to know which markers to analyze.

Acknowledgements

- > Childéric Severac RESTORE
- > Emmanuelle Trévisiol TBI Biosoft
- > Adrian Martinez-Rivas CIC IPN Mexico
- > Sergio Proa-Coronado, Ph D Conacyt
- > Ophélie Thomas Chemin, PhD ANR in Progress



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