

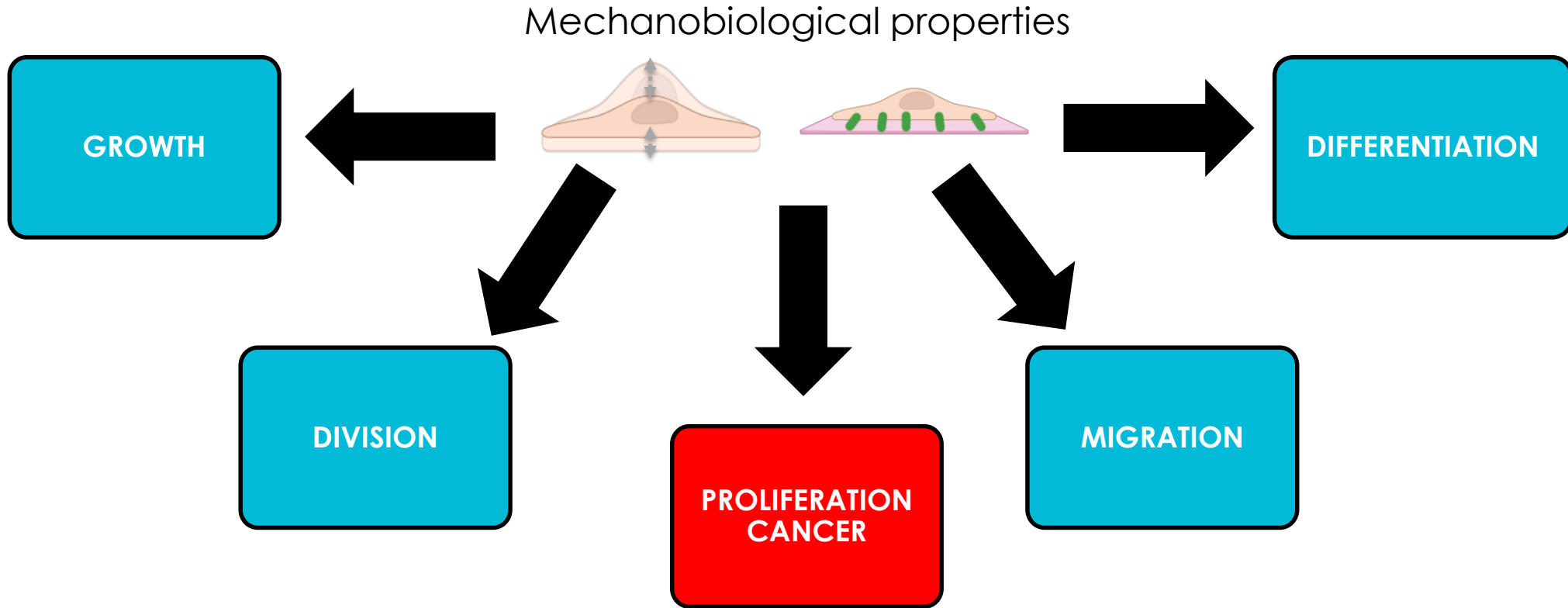
AutoBioTip: Towards automation of mechanobiological measurements by AFM

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Childéric Séverac (RESTORE Institute)
& Emmanuelle Trévisiol (TBI)



Mechanobiological properties of cells

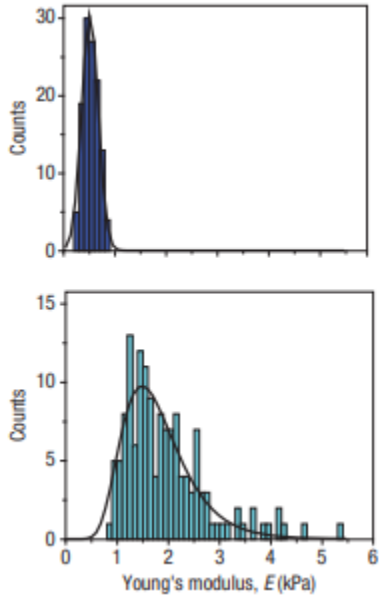


- Lower stiffness
- Better deformability

Lekka *et al.* European Biophysics Journal (1999)

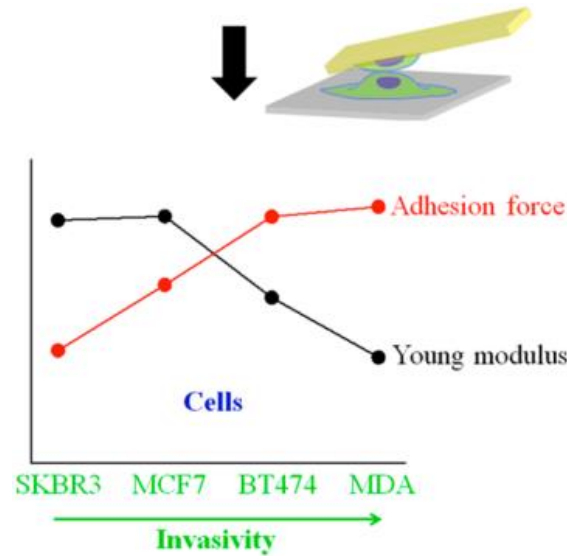
Explore the mechanobiology of living cells

1st study with AFM-cancer
Lower stiffness for cancer cells



Cross *et al.*
Nature nanotechnology (2007)

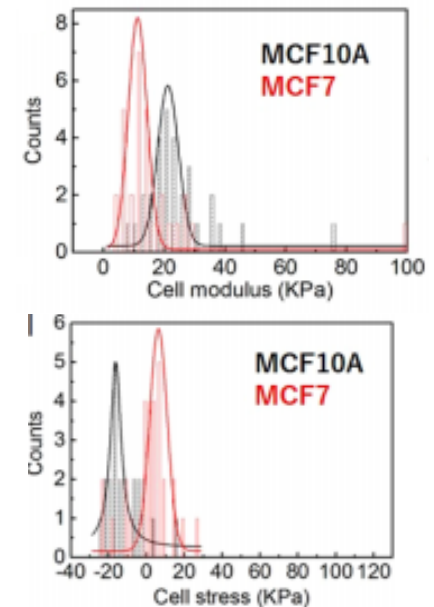
Lower Young's modulus for invasive cells
2 parameters:
Young's modulus + Adhesion force



Smolyakov *et al.*
ACS Appl. Mater. Interfaces (2016)

Lower elastic modulus for cancer cells
2 parameters:
Young's modulus + Prestress

Mechanome



Wang *et al.* *Nano Lett.* (2021)

$n_{\text{cells}} = 5-40$
with **thousands** of force curves per cell



Can AFM be used for clinical application?

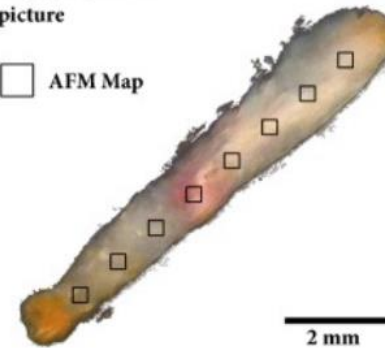
Paradigm shift to perform statistical experiments with AFM

One strategy

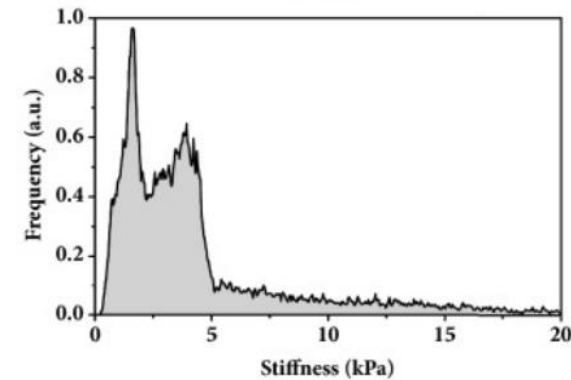
Native biopsies
Clinical tool
Diagnosis of breast cancer

Biopsy top-down picture

□ AFM Map



Biopsy wide stiffness histogram



Zanetti-Dällenbach *et al.* *Biomed Res Int.* (2018)

AUTOBIOTIP

Paradigm shift:

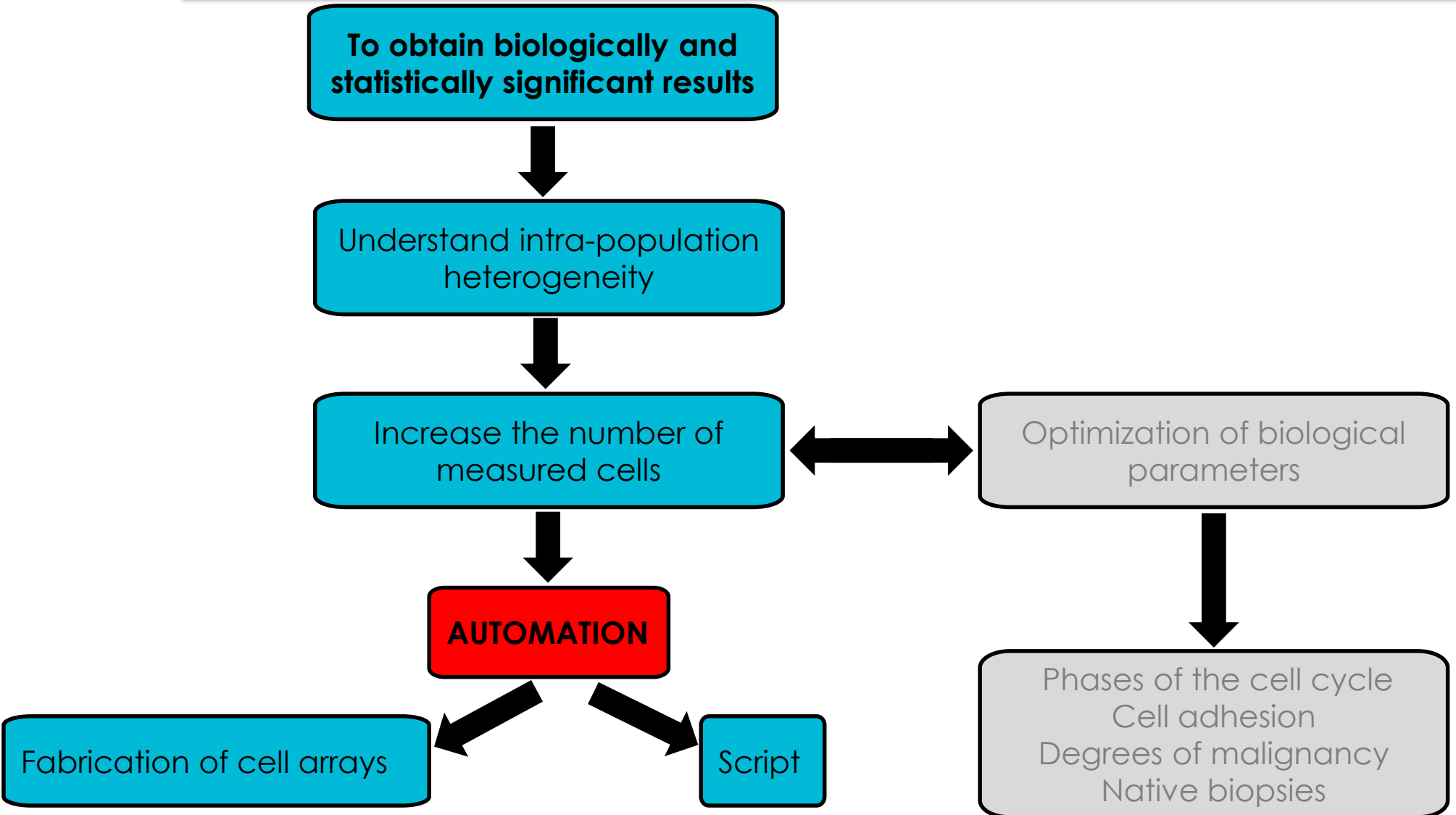
- Fewer force curves
- More cells



Hundreds of cells or more

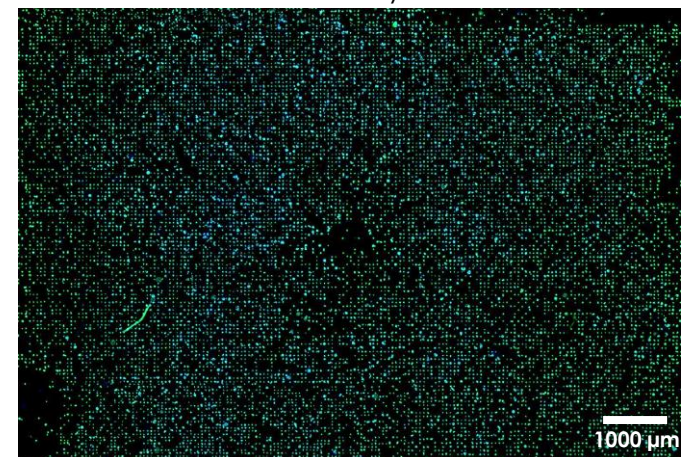
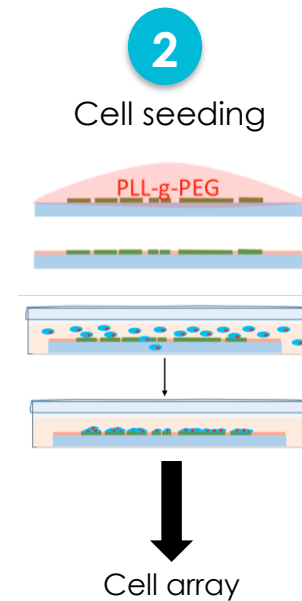
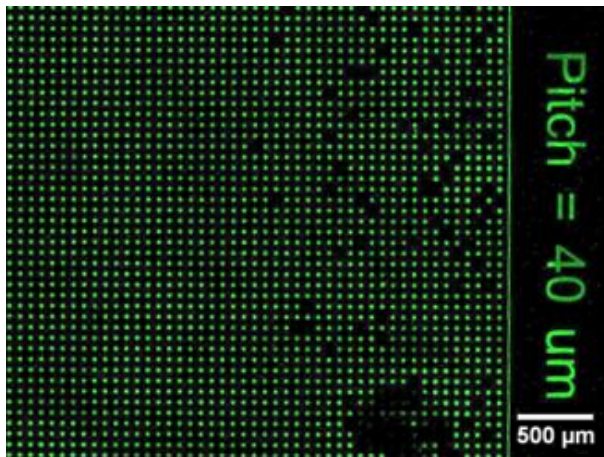
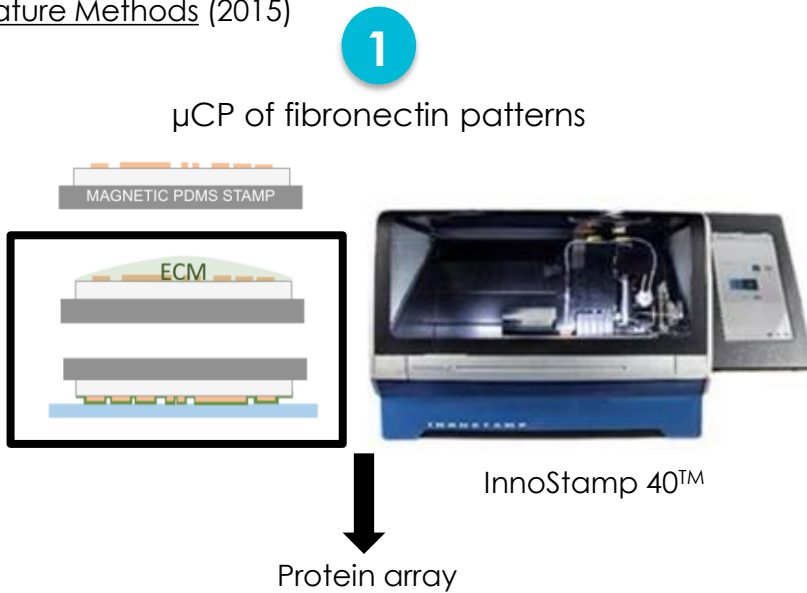
- To provide statistically relevant experiments
- To be applied in medical field

Automation to provide statistically relevant experiments



Fabrication of mammalian cell arrays for AFM automation

Lagraulet et al. *Nature Methods* (2015)



Automation on mammalian cells is possible

Automation script for mammalian cells

```

ForceSpectroscopy.setPosition(P[0], P[1])
print "g: " + str(g) + "P: " + str(P[0]) + ", " + str(P[1])
if g > 0:
    #Engaging MotorStage
    MotorizedStage.engage()

    #Moving the MotorStage to a particular coordinate
    MotorizedStage.moveToAbsolutePosition(MSCoord[g].x, MSCoord[g].y, 40e-6)

    #logfile.write('Motor stage current coordinate '+ str(g)+' :'+str(MSCoord[g].x)+str(MSCoord[g].y) + "\n")
    #logfile.close()
    MotorizedStage.disengage()
    print "Motor stage current coordinate "+ str(g)+' :'+str(MotorizedStage.getPosition().getX()+str(MotorizedStage.getPosition().getY()))
    #MotorizedStage.disengage()
    #calculatePoints(ws,angle,fileName, fineCoord)
    MotorizedStage.disengage()
MotorizedStage.disengage()
MotorizedStage.disengage()
Scanner.approach()
Snapshotter.saveOpticalSnapshot(path + "Cell" + str(g) + ".png") # rajout pour mesurer les erreurs de déplacements
print "snapshot"
ForceMapping.activateGUIMode()
print "GUI"
ForceMapping.Autosave.on()
print "Autosave"
now = datetime.datetime.now()
print "datetime"
timestamp = str(now.strftime("%Y%m%d_%H%M%S"))
print "timestamp"
ForceMapping.setOutputDirectory(path+"Cell"+str(g) + '--' + str(timestamp)) # we change time.asctime() with timestamp to get rid off ":" in the directory name
print "directory"
#ForceMapping.setScanSize(ScanSize[0],ScanSize[1])
#print "Scansize"
#ForceMapping.setScanPixels(ScanPixels[0], ScanPixels[1])
#print "Scanpixel"
#ForceMapping.setScanOffset(ScanOffsetCenter[0], ScanOffsetCenter[1]) #coordinates where the force map is going to be obtained
#print "scanoffset"
ForceMapping.setScanAngle(angle) # &&&&doit on utiliser cet angle ici?
#print "scanangle"
ForceMapping.startScanning(1) #Number of force maps you want from this region

Scanner.retract()
print "retract_center"
#ForceMapping.setOutputDirectory(path+"Cell"+str(g) + "Corner" + '--' + str(timestamp)) #
#ForceMapping.setScanOffset(ScanOffset[0], ScanOffset[1])
#Scanner.approach()
#ForceMapping.startScanning(1) #Number of force maps you want from this region
#Snapshotter.saveOpticalSnapshot(path + "Cell" + str(g) + "Corner" + ".png") # rajout pour mesurer les erreurs de déplacements

```

AFM stage displacement

Indentation

300 lines of code

Tip displacement and indentation for mammalian cells

In the Jython script, implement:

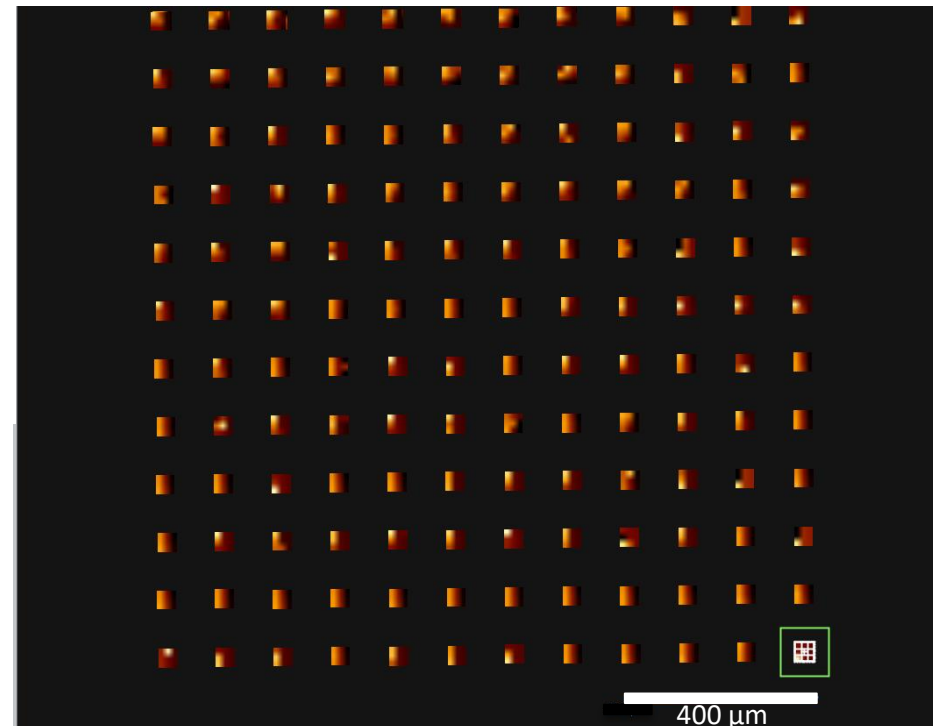
- Coordinates of the center of the 1st and the last cell
- Number of cells between the 2



Calculate the distance between each cell

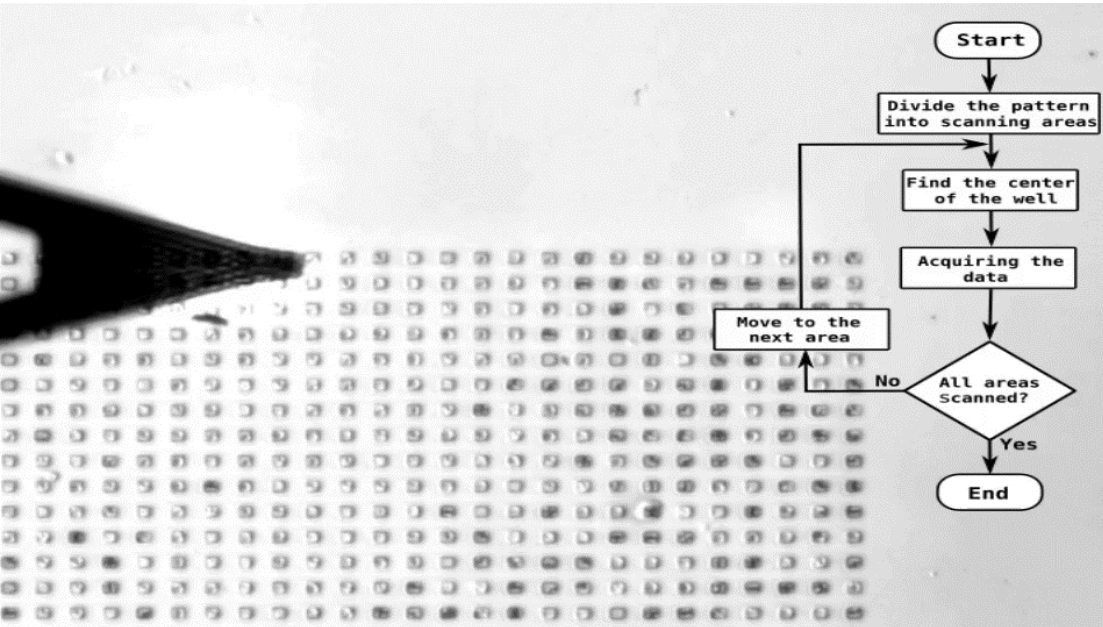
Tip movement:

- 1) Indent the 1st cell
- 2) Record force curves
- 3) Move to next cell
(etc.)

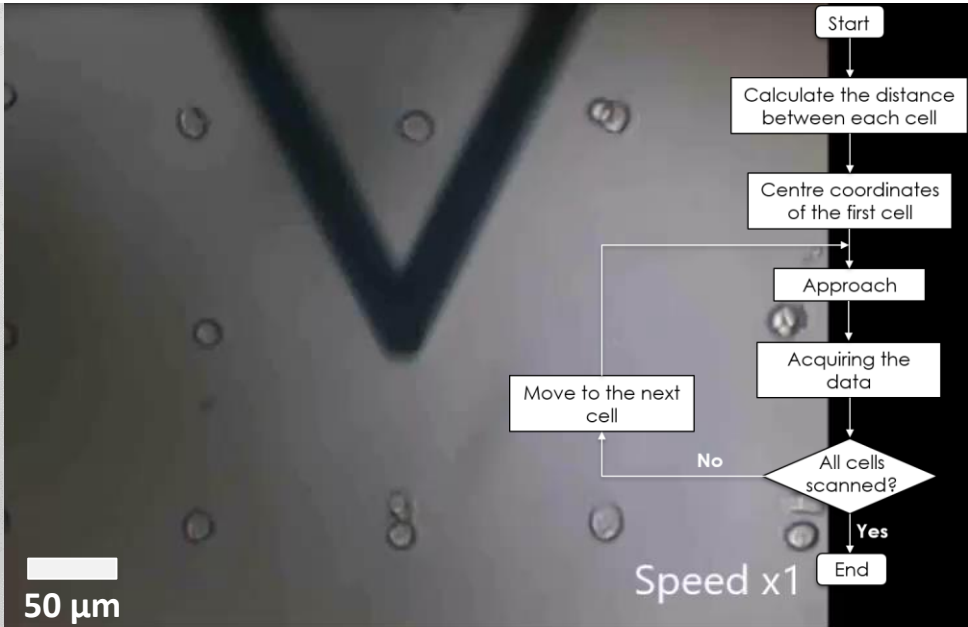


Video of automated AFM movement on mammalian cells

Displacement of AFM tip



Displacement of AFM stage

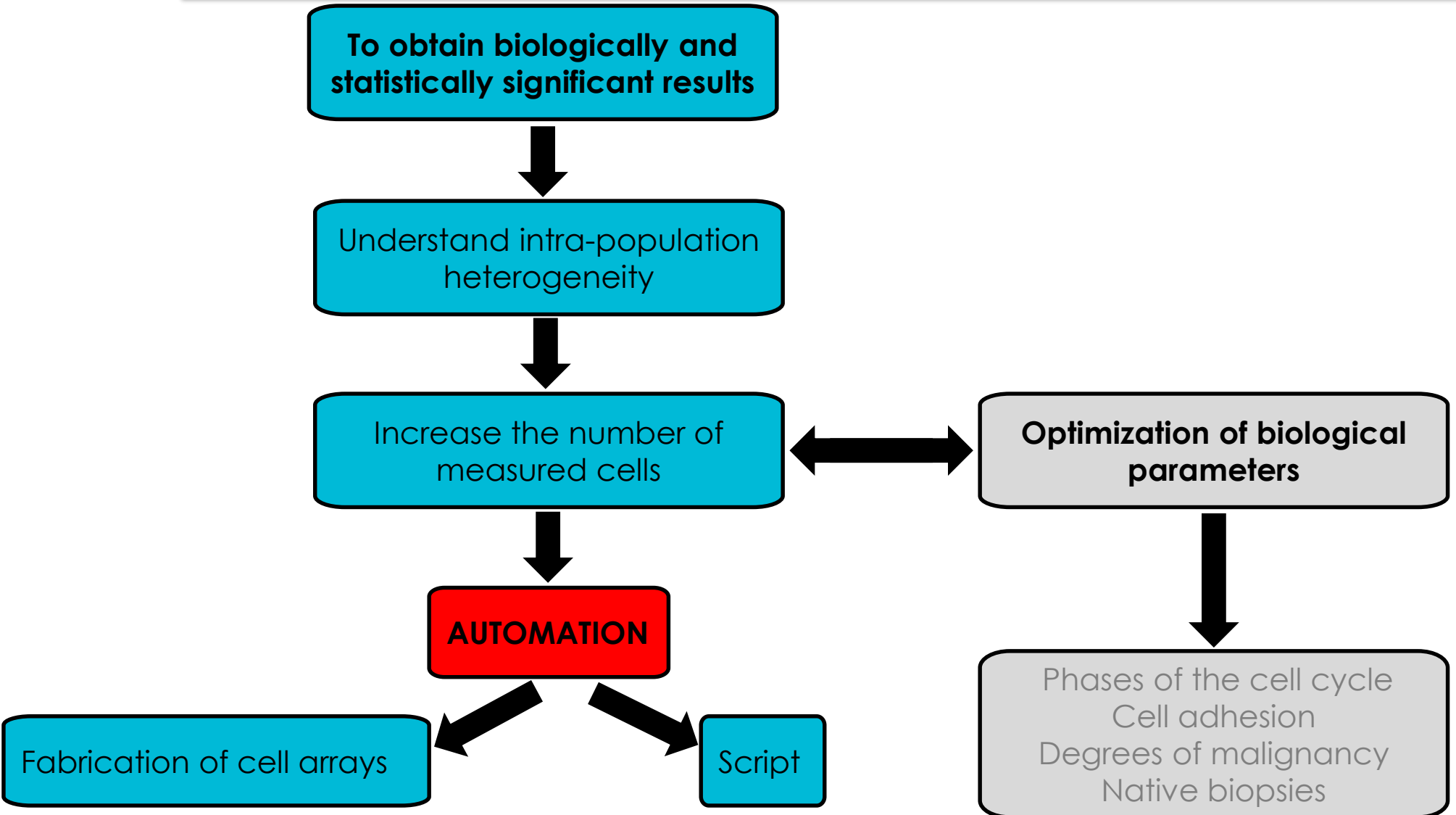


Séverac *et al.* Jove (2020)
Proa-Coronado *et al.* Nanoscale Horizons (2020)

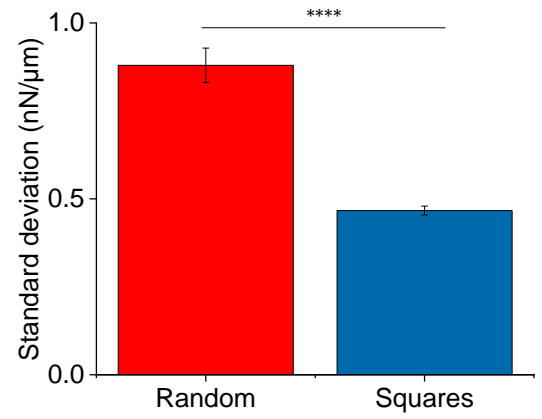
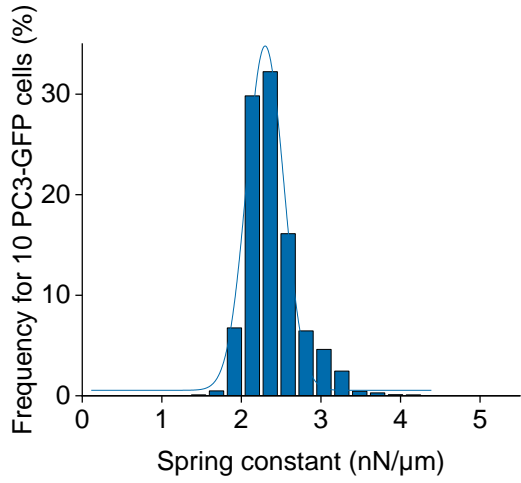
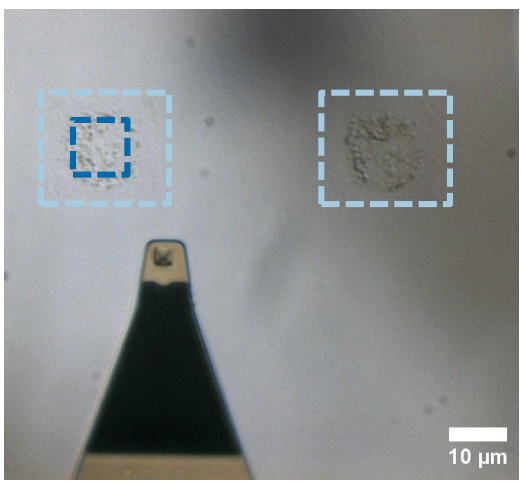
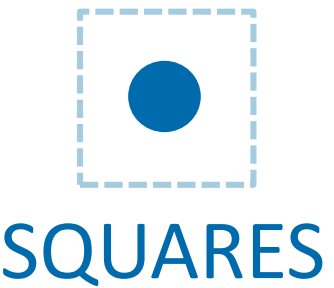
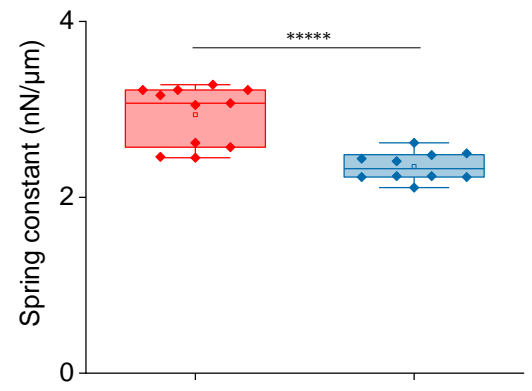
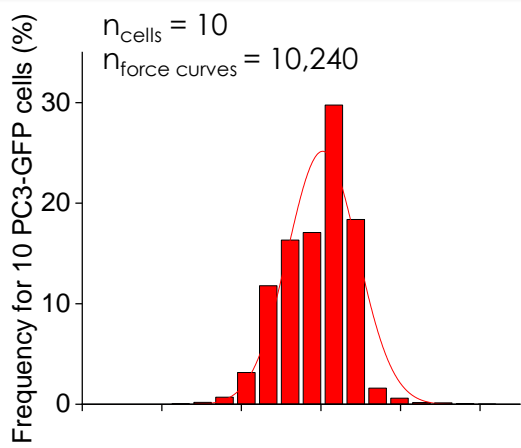
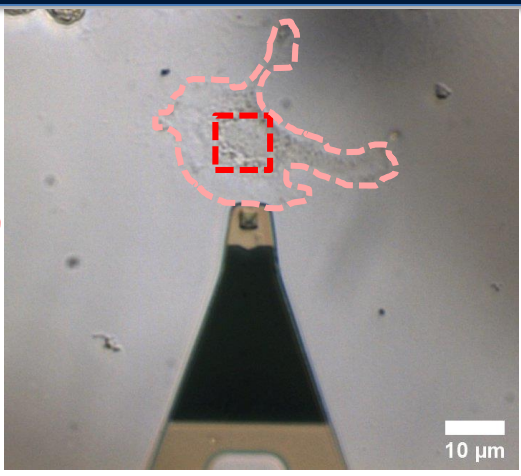
376 positions
in 1h30'52''

Ability to probe a large number of cells in a controlled time

Automation to provide statistically relevant experiments



One way to reduce the measured dispersion is to constrain the cells to patterns



Lower distribution with patterns

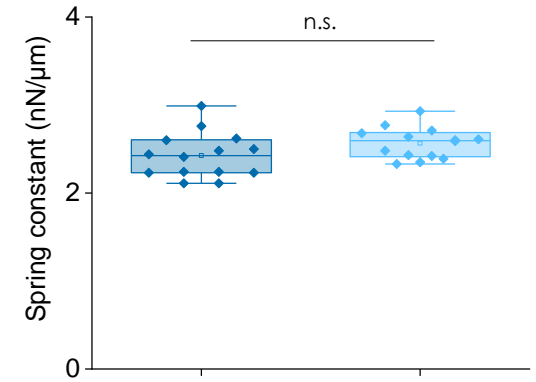
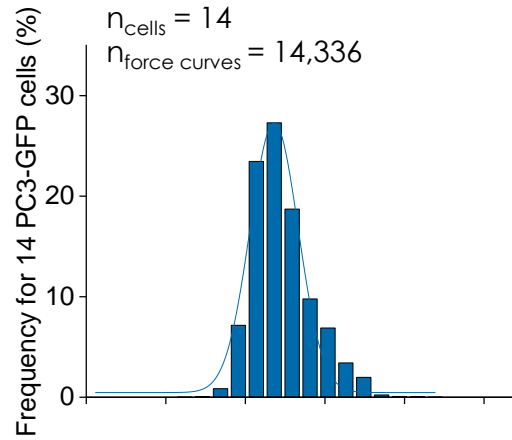
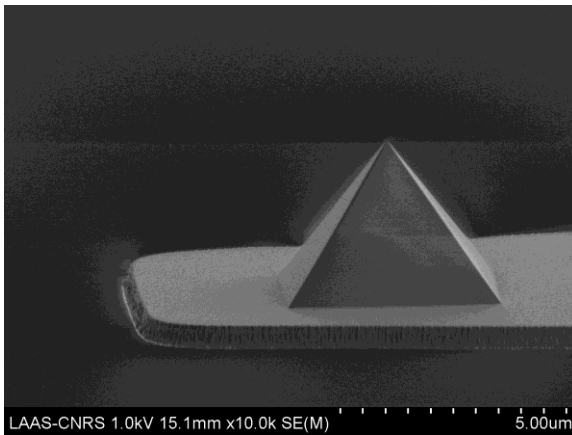


Advantage of **patterns** to better discriminate between different cell populations by decreasing the measure dispersion

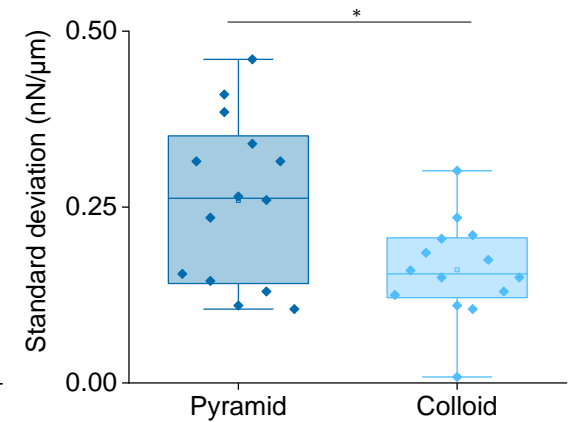
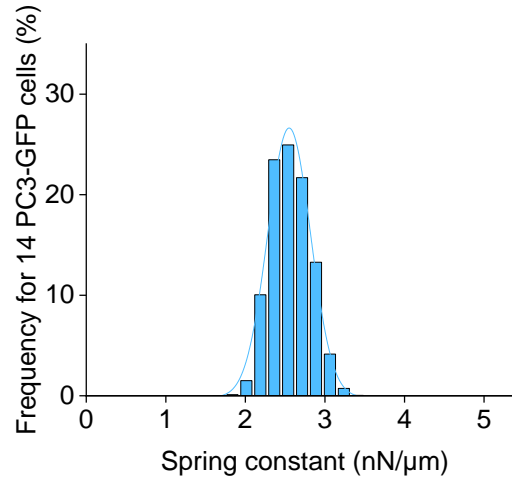
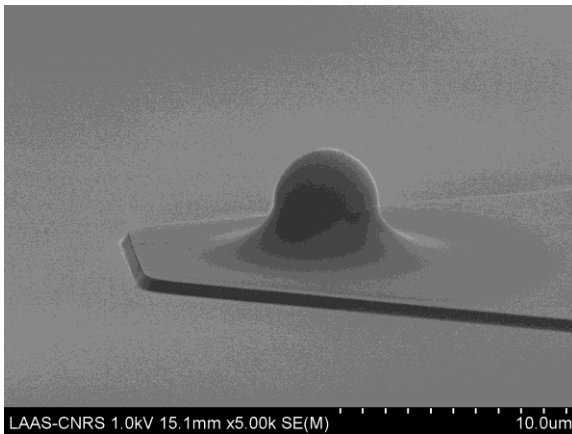
Another way to reduce the measured dispersion is to use a colloidal probe



PYRAMID



COLLOID



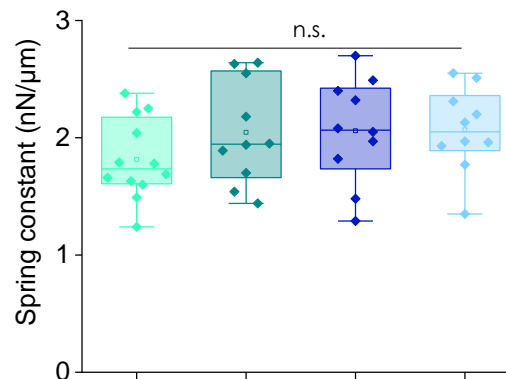
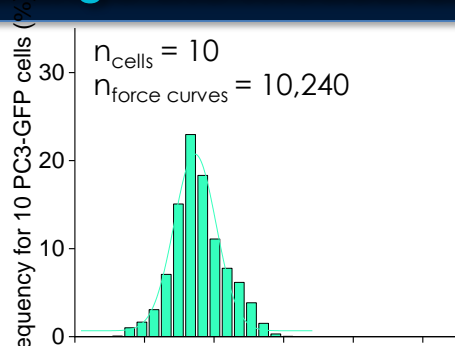
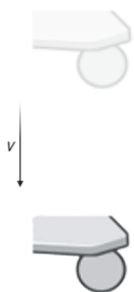
Lower distribution with colloids



Advantage of **colloids** to better discriminate between different cell populations by decreasing the measure dispersion

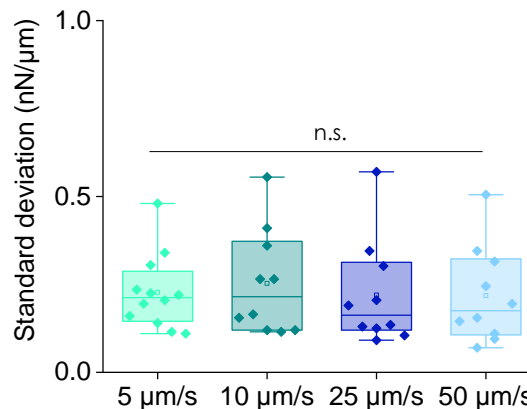
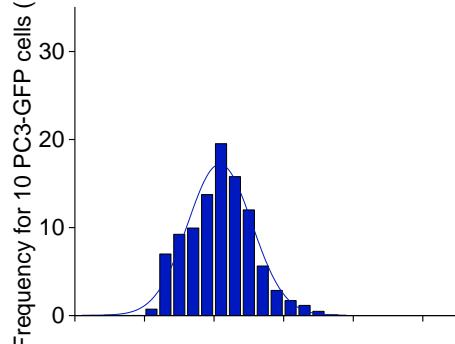
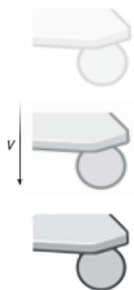
To increase the number of cells: Increase the velocity Does this change the measurement?

5 $\mu\text{m/s}$



No significant difference
from 5 to 50 $\mu\text{m/s}$

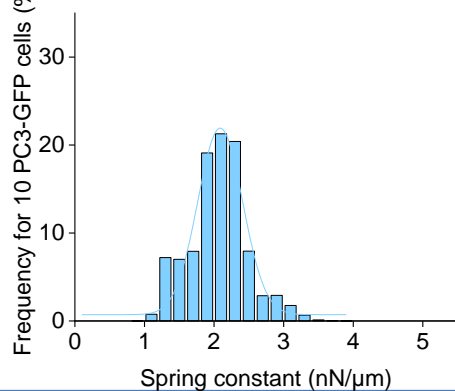
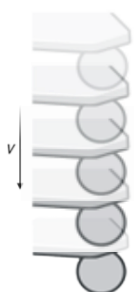
25 $\mu\text{m/s}$



Advantage of 50 $\mu\text{m/s}$
to measure more cells

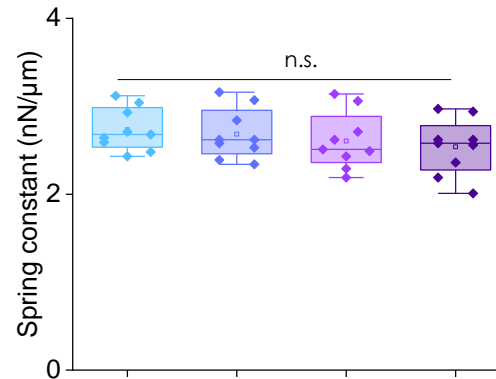
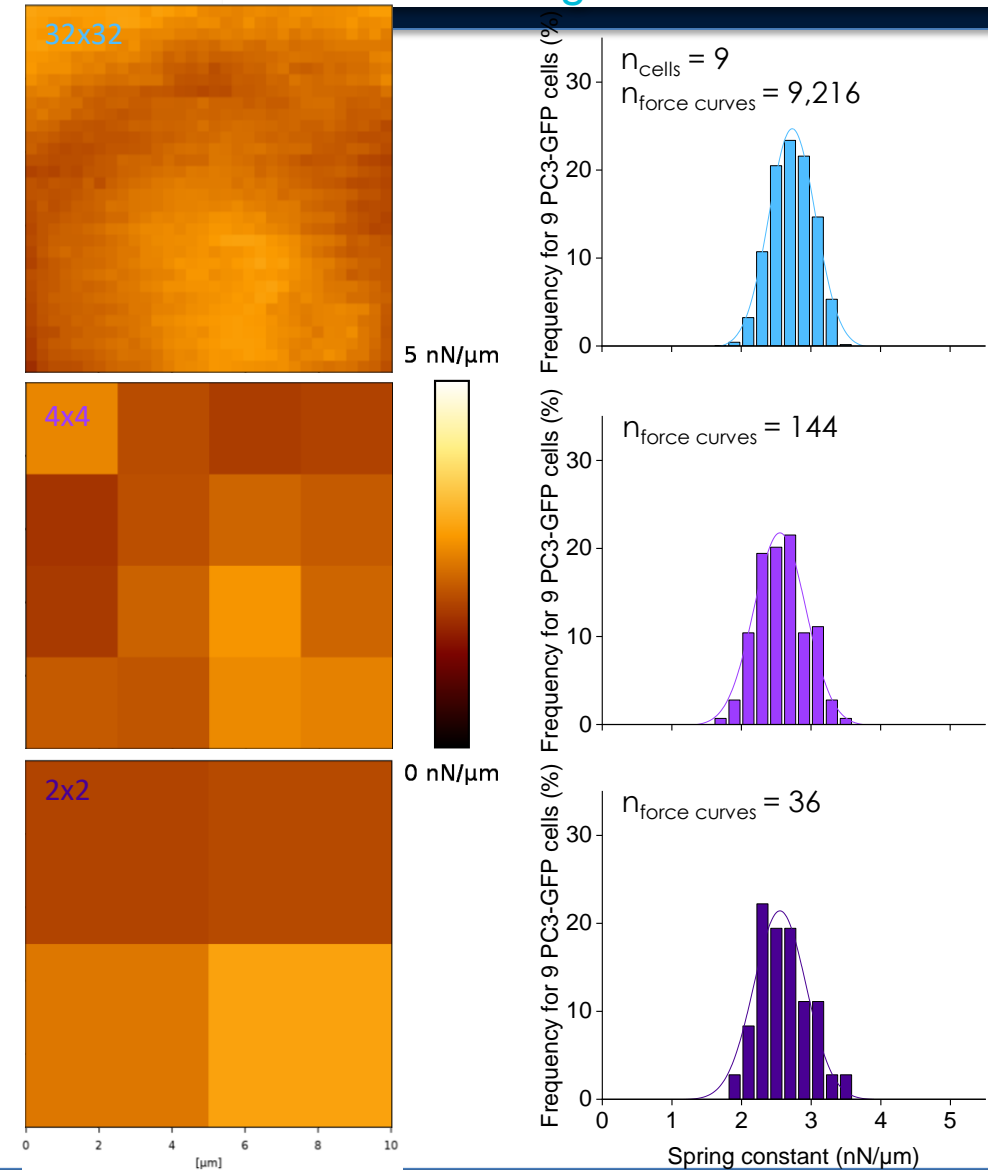


50 $\mu\text{m/s}$

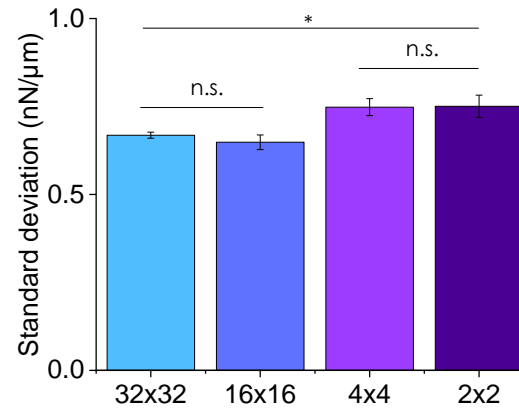


Spring constant (nN/μm)

To increase the number of cells: Reduce the number of indentations per cell Does this change the result?



No significant difference from 32x32 to 2x2



Advantage of 2x2 to measure more cells

AUTOMATION

OPTIMIZATION

Influence of pattern
 Influence of indenter geometry
 Influence of indentation velocity
 Influence of surface chemistry
 Influence of substrate (hydrogels)

SAMPLING

4 indentions per cell
 More than 100 cells
 Location (nucleus, cytoplasm)

NEXT STEPS

Optimization of biological parameters

COMPARISON

Different degrees of malignancy
 Different phases of the cell cycle
 Different molecules on cells
 Cell adhesion on different hydrogels

MACHINE LEARNING

Elasticity
 Adhesion
 Work of adhesion
 etc.

Mechanome



Thank you for your attention

