Couplages de microscopies photoniques et à force atomique en biologie : applications

Sébastien JANEL

Simone BOVIO

Elisabeth WERKMEISTER

Antonino BONGIOVANNI

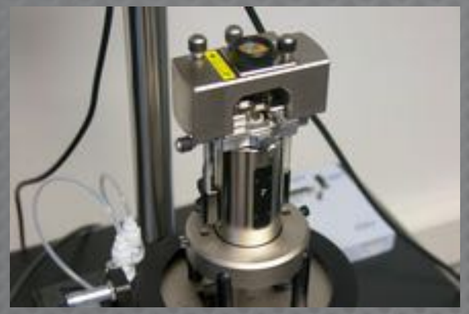
Frank LAFONT

Outline

- AFM facility at Institut Pasteur Lille
- Nanoguided photonic measurements
- Adhesion measurements
- Sensor tool
- 3D elasticity measurements
- Confocal/AFM and STED/AFM coupling

Atomic Force Microscopy facility at Institut Pasteur Lille

Standalone AFM



MultiMode 8

FastScan Bio

AFM + « standard » photonics

Bruker Catalyst + TIRF @L2 lab

JPK NWIII + CellHesion + BioMat station

AFM + Super-resolution

Bruker Resolve + Zeiss PALM/STORM

JPK NWIIIUltra + Abberior STED/RESOLFT

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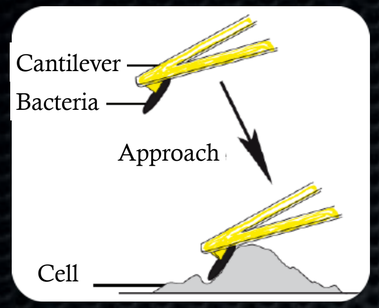
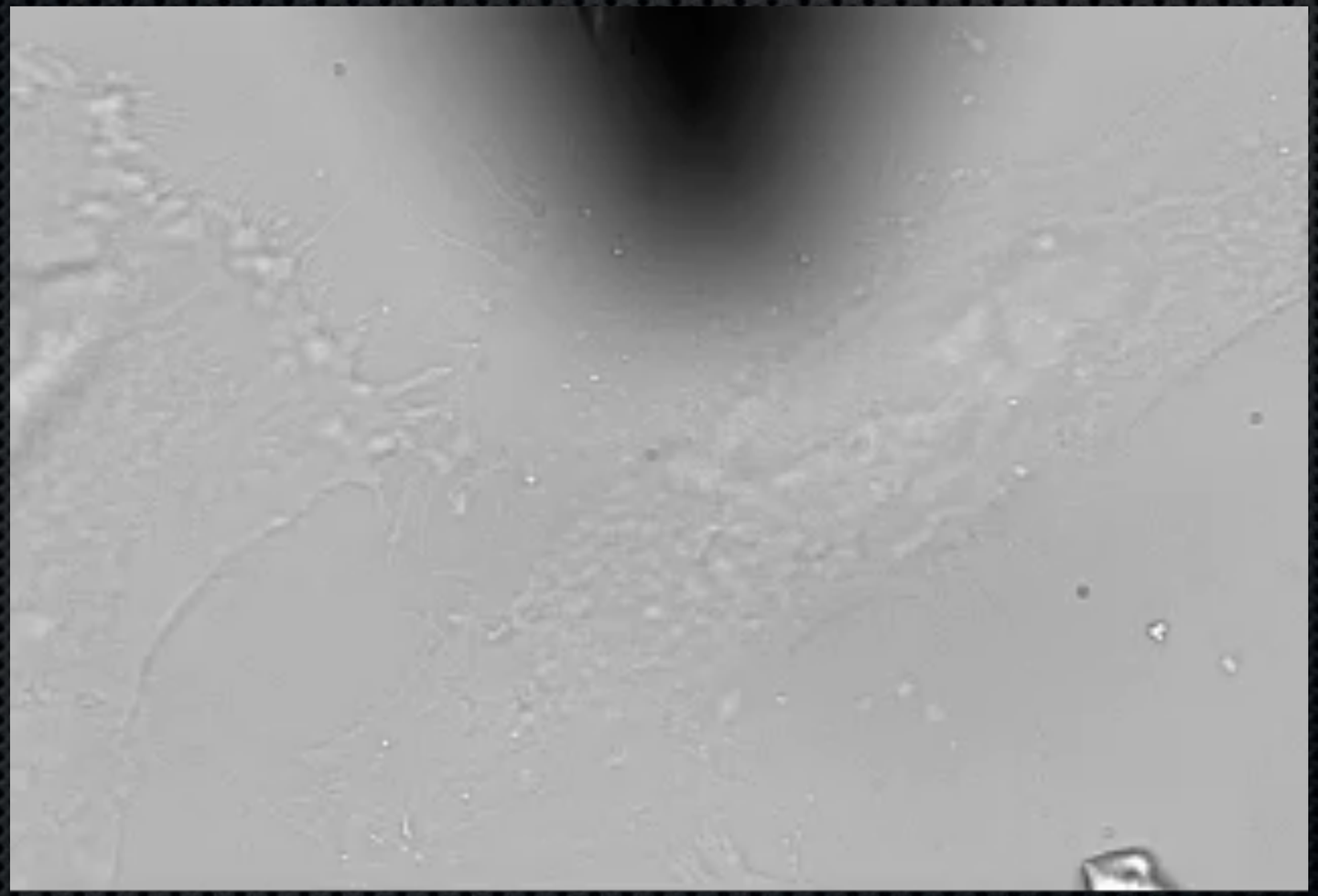
1. AFM-guided photonics measurements



→ **Uncoupling adhesion and entry to follow the recruitment of signalling molecules**

GPI-AP domains on HeLa cells

Y pseudotuberculosis



Ciczora et al. submitted

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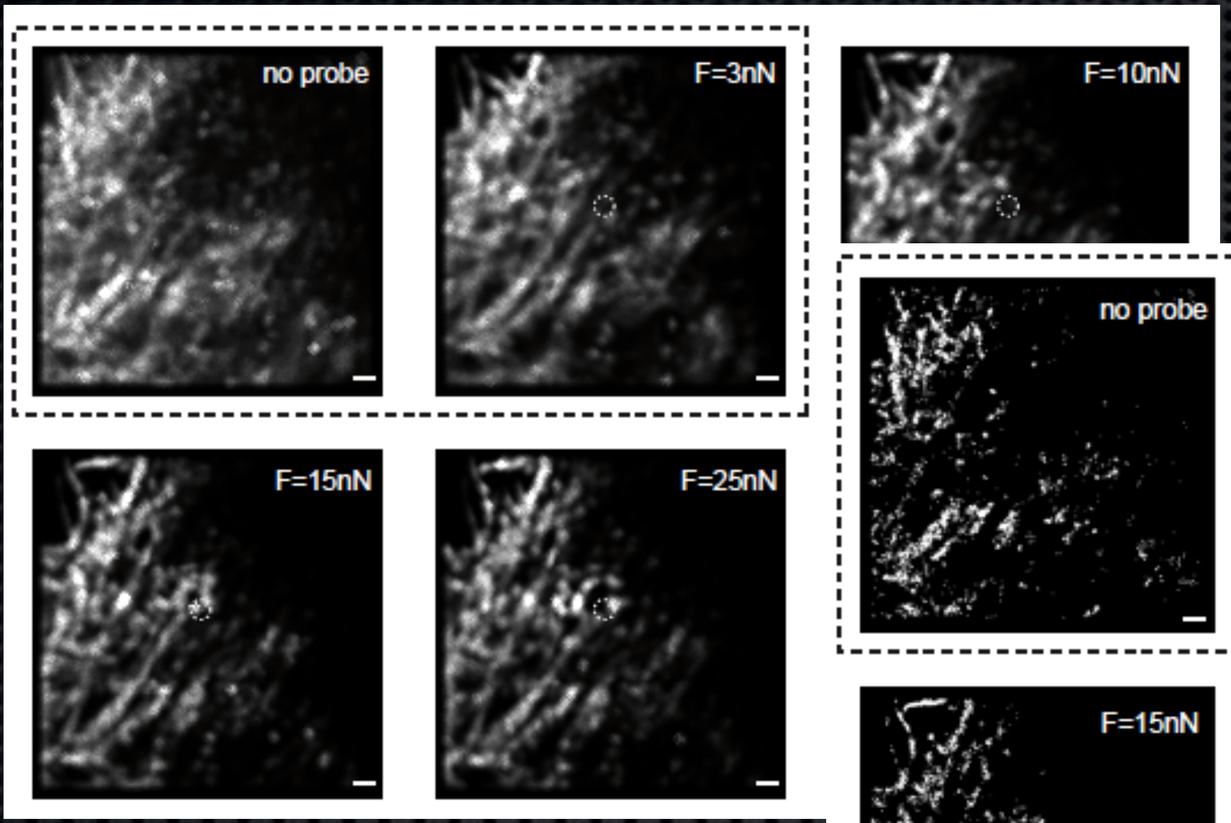
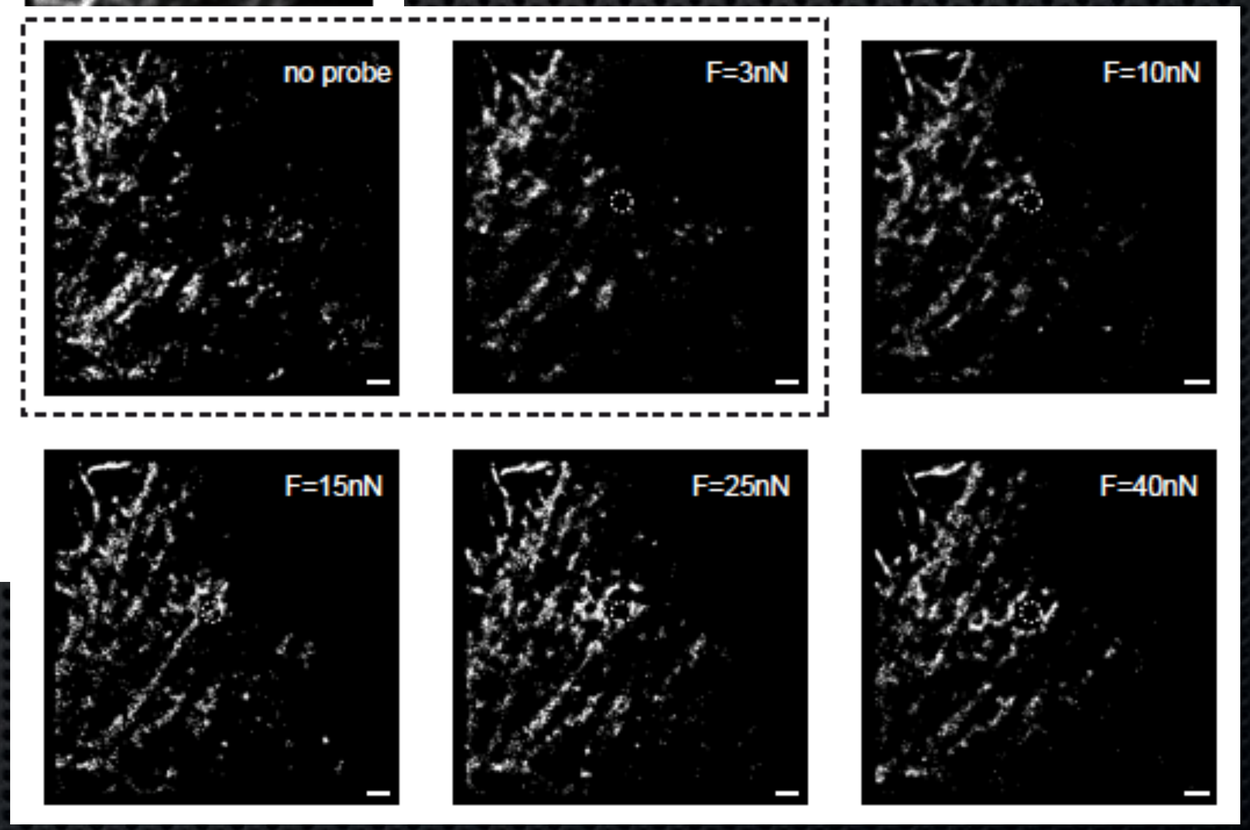
1. AFM-guided photonics measurements

Control : applied force and actin organization using inert bead



TIRF

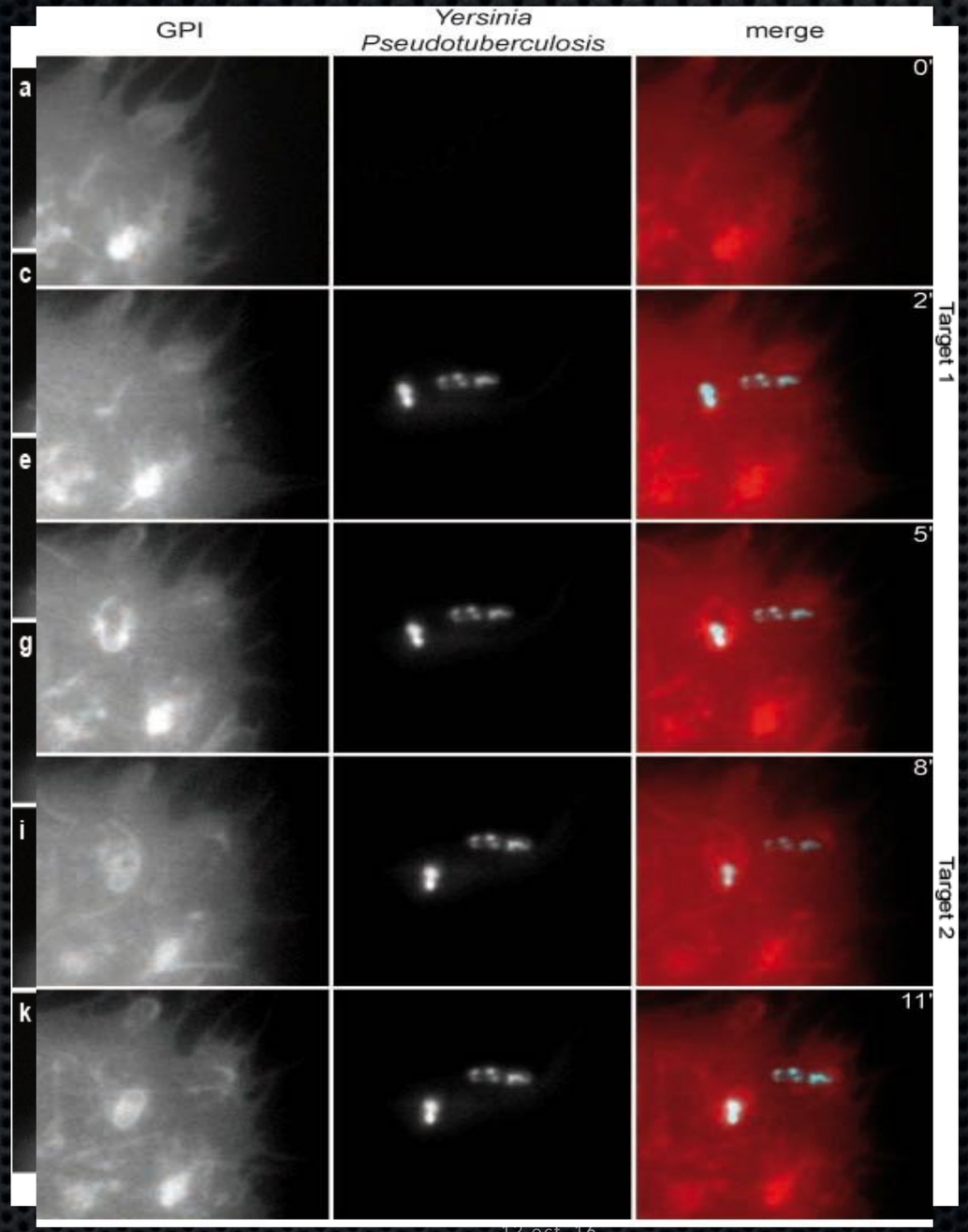


PALM

Ciczora et al. submitted

1. AFM-guided photonics measurements

Possibility of several engagements with each time recruitment of the GPI-GFPs



→ only living bacteria recruit GPI-GFPs

Ciczora et al. submitted

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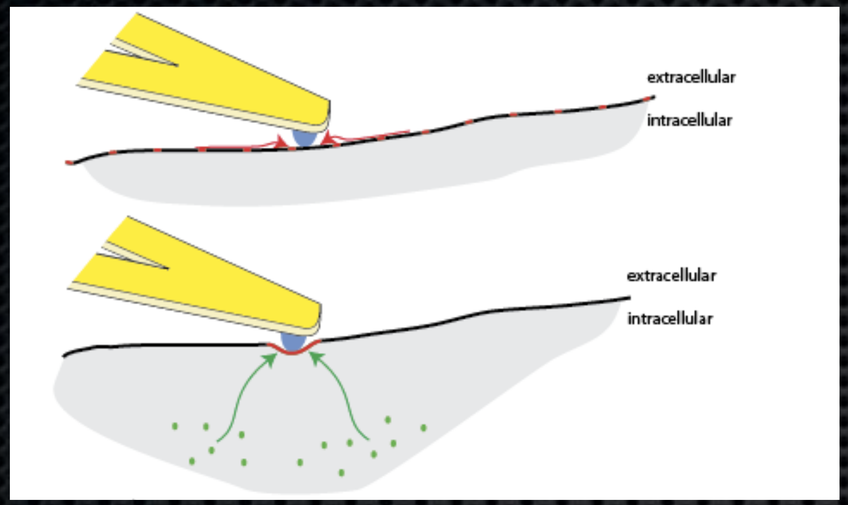
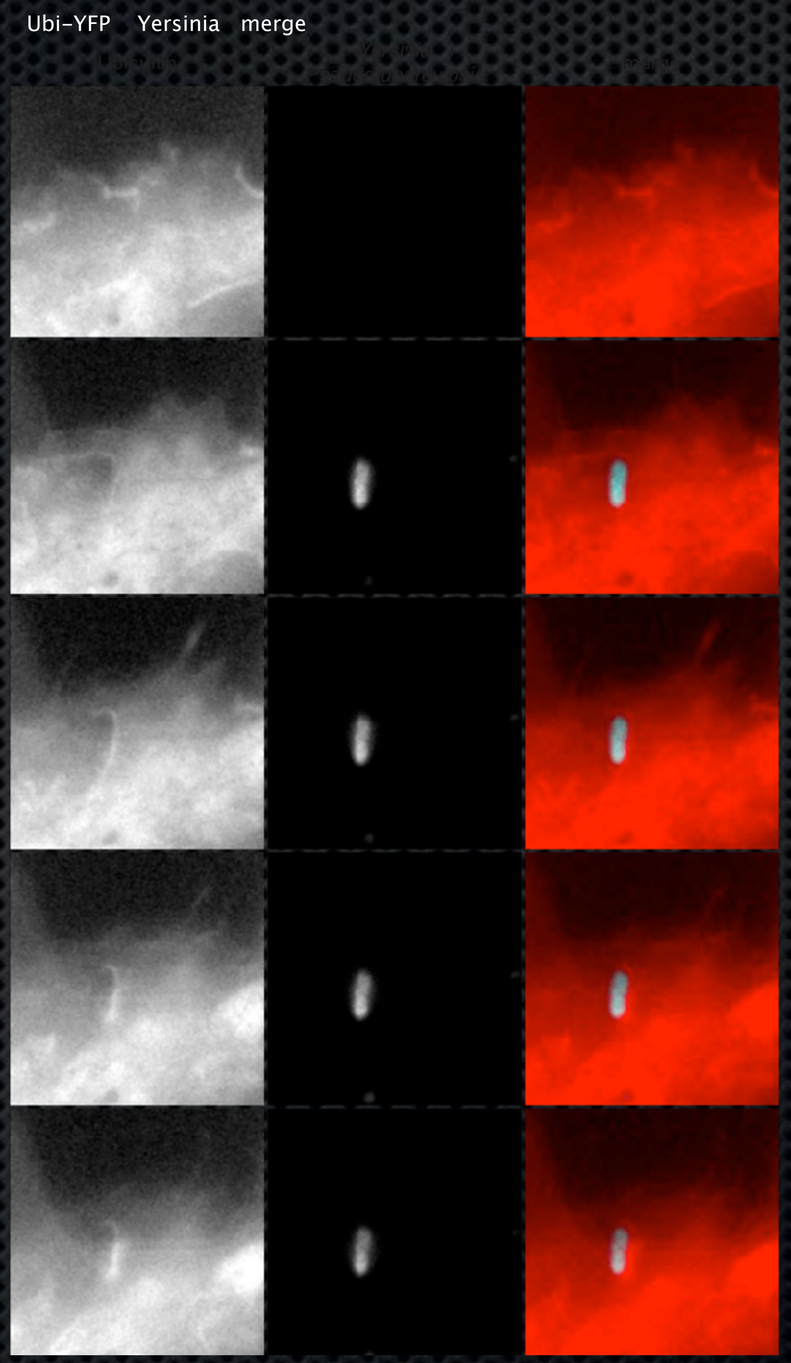
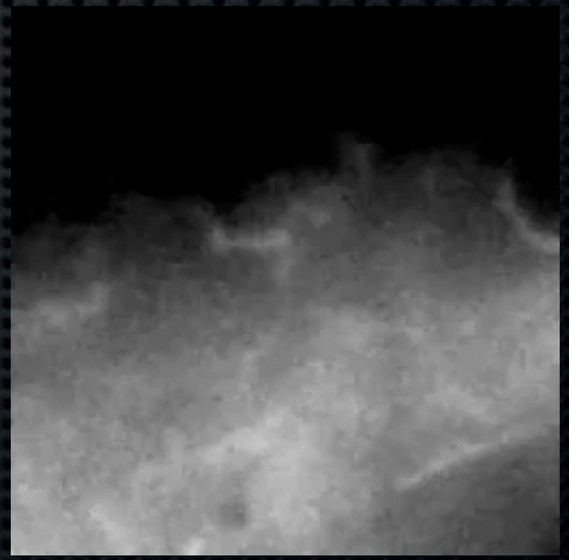
1. AFM-guided photonics measurements

Possibility of recording the recruitment of cytosolic proteins







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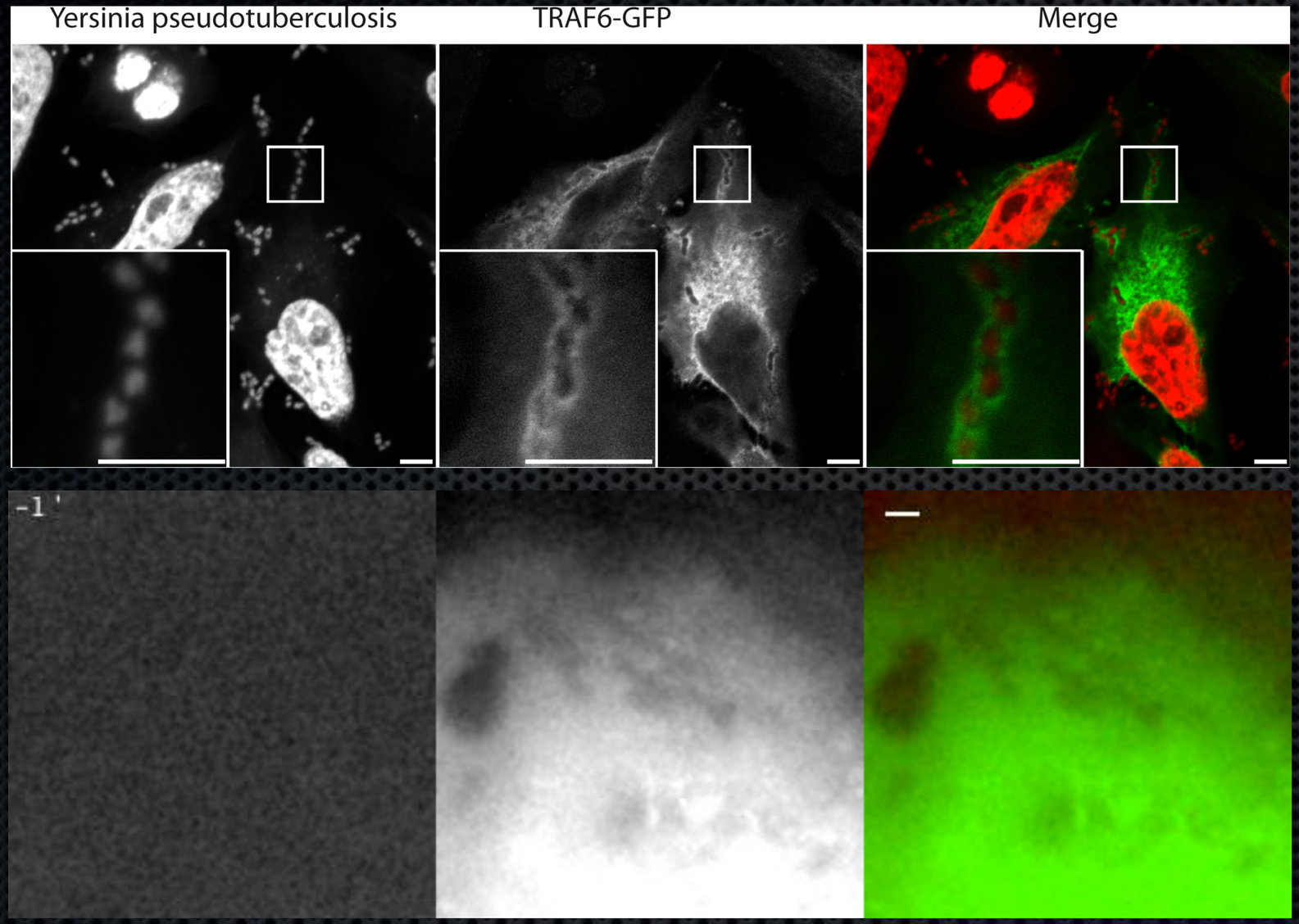
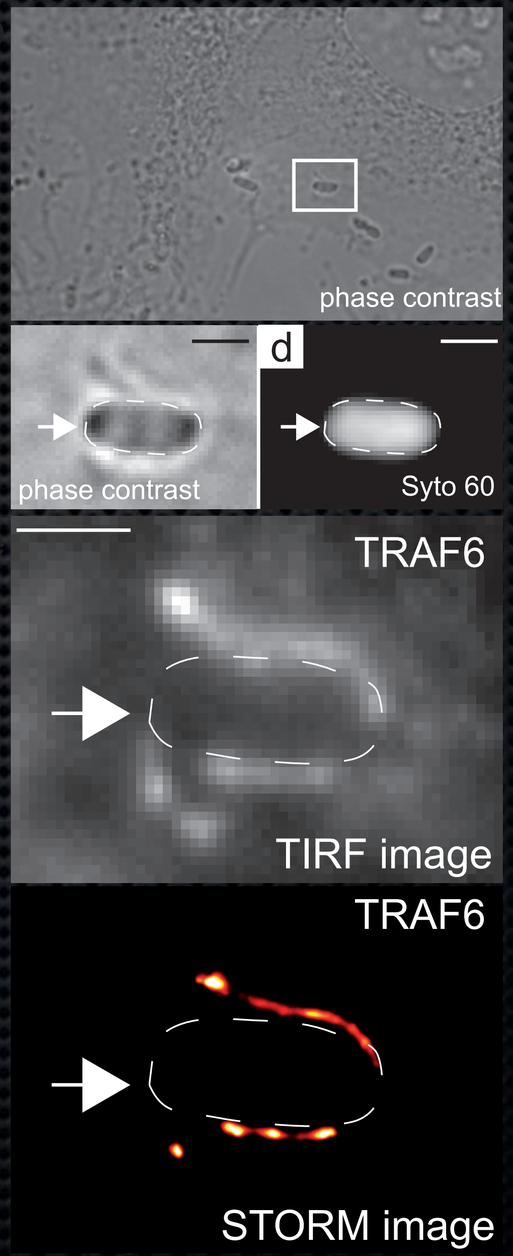
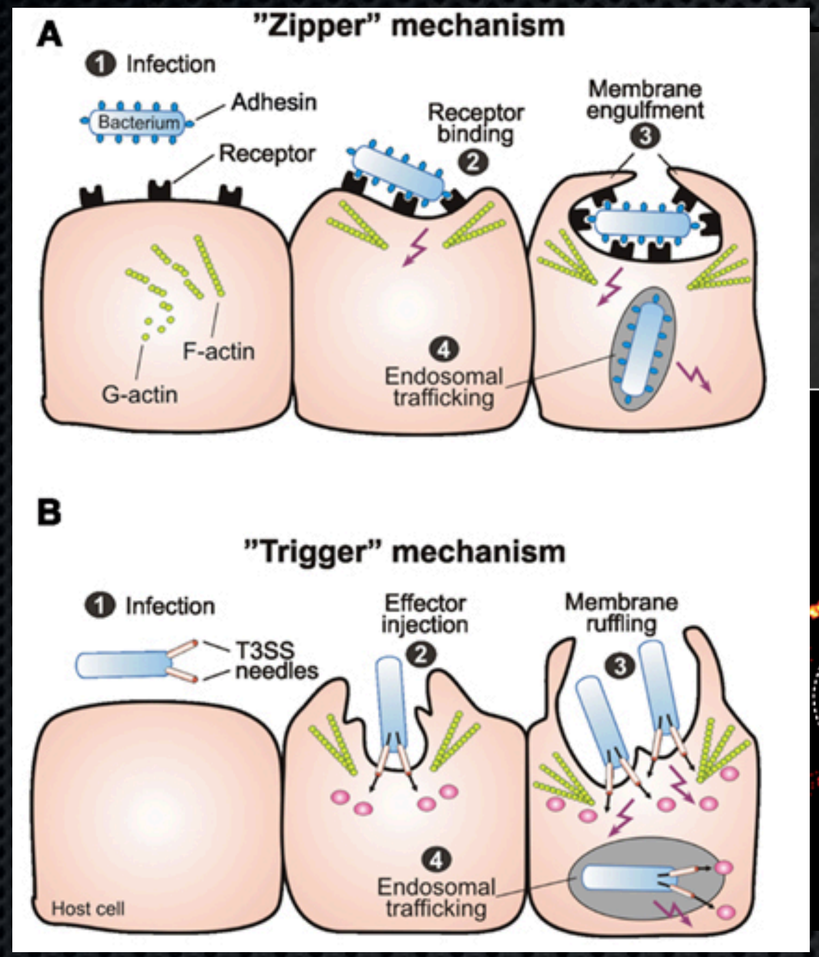


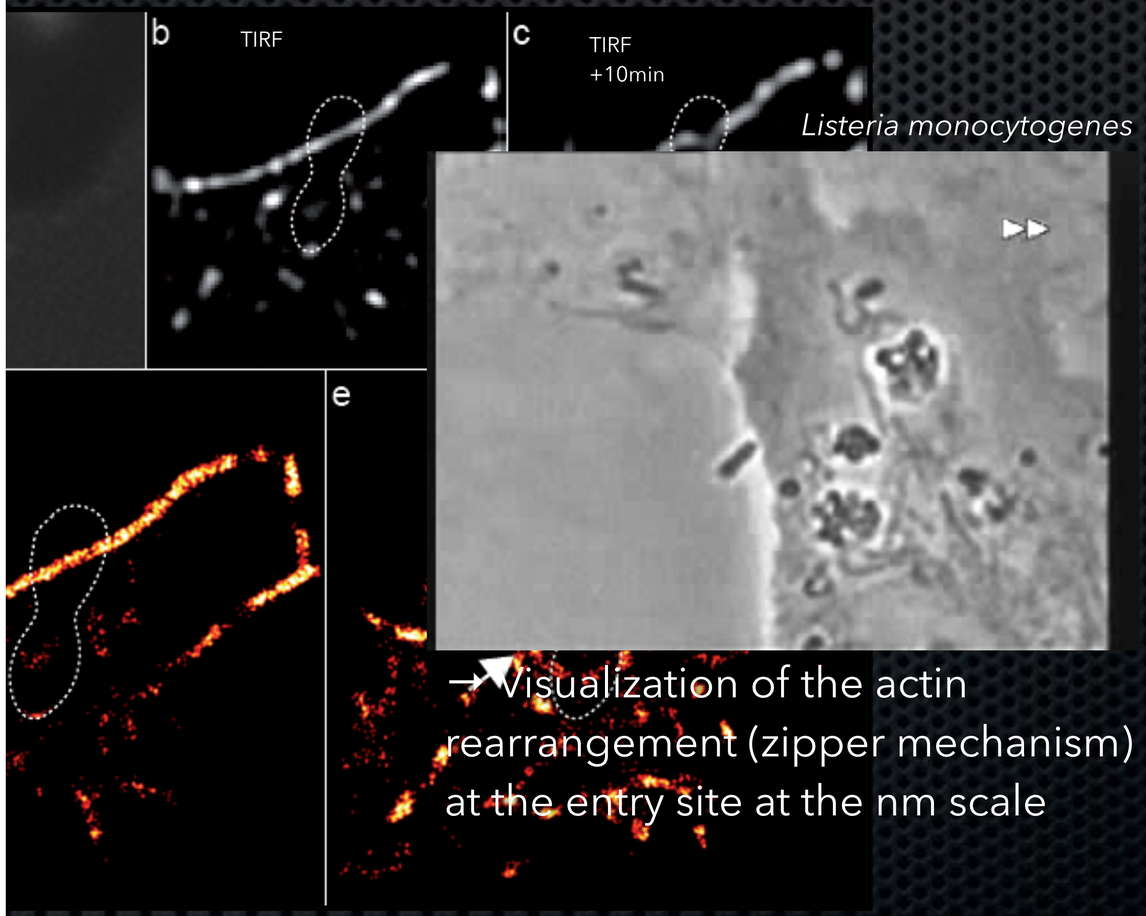


Ciczora et al. submitted

1. AFM-guided photonics measurements

Cell infection Correlative microscopy: AFM & *super resolution* fluorescence





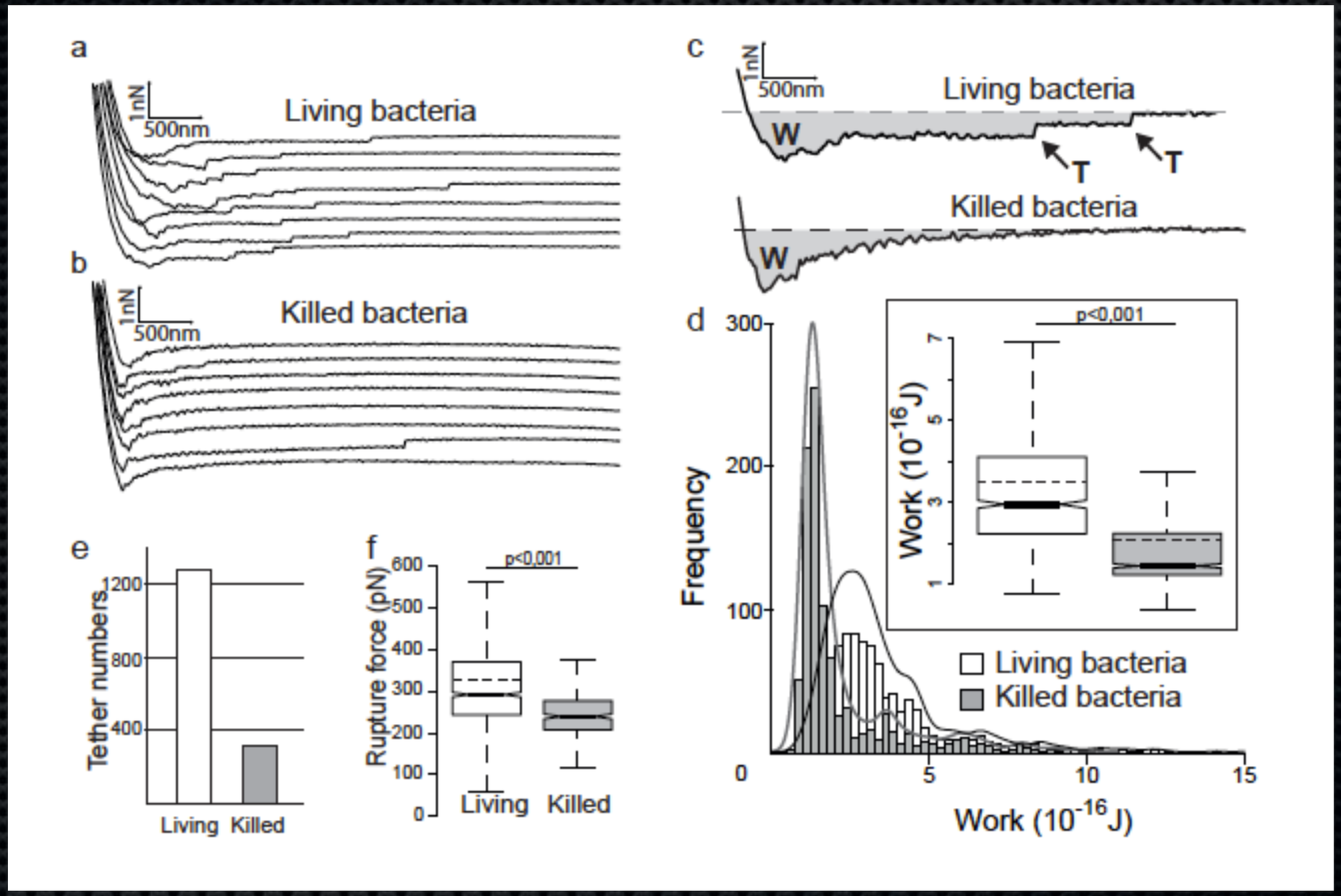
Listeria monocytogenes

→ Visualization of the actin rearrangement (zipper mechanism) at the entry site at the nm scale

Ciczora et al. submitted

1. AFM-guided photonics measurements

→ Multiparametrical measurements of adhesion and interaction forces



Ciczora et al. submitted

1. AFM-guided photonics measurements

What about the integration of the 2 techniques ? ✗

✓ Need for **advanced video recording** : # channels (not available in AFM sw)

✓ Need for **automation** in photonic microscope

✓ Need for **AFM manipulation and measurements**

- No need for precise x-y landing of the cantilever

⇒ AFM and Video have to be handled by their own software until and AFM company releases a video microscope...

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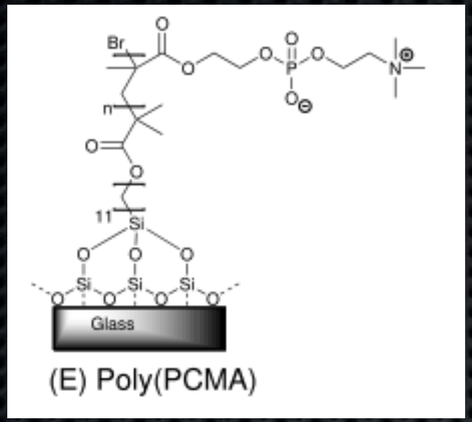
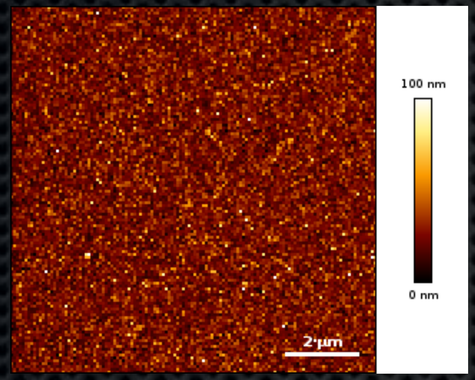
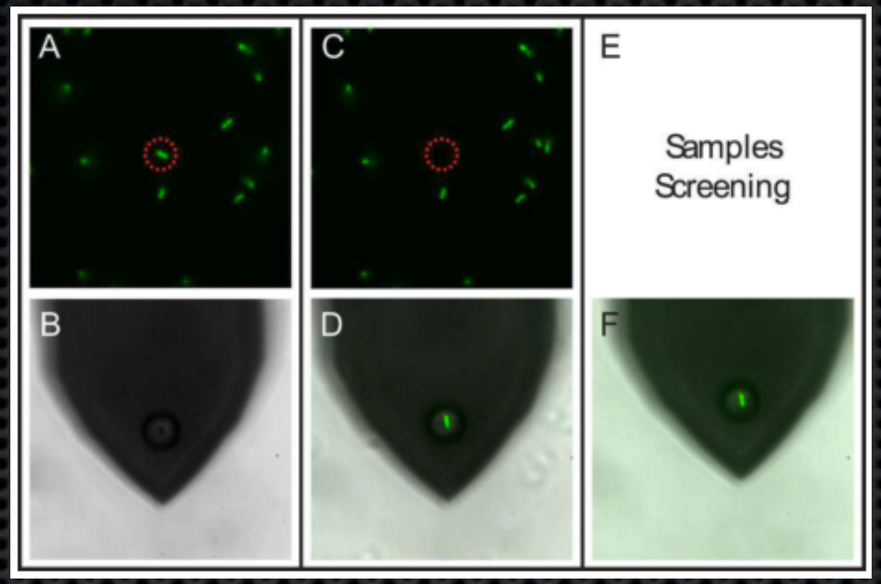
2. Adhesion measurements

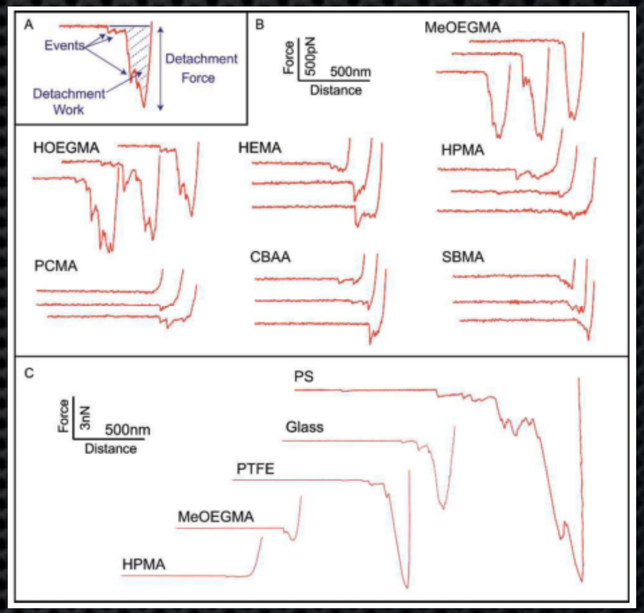
Antifouling polymers

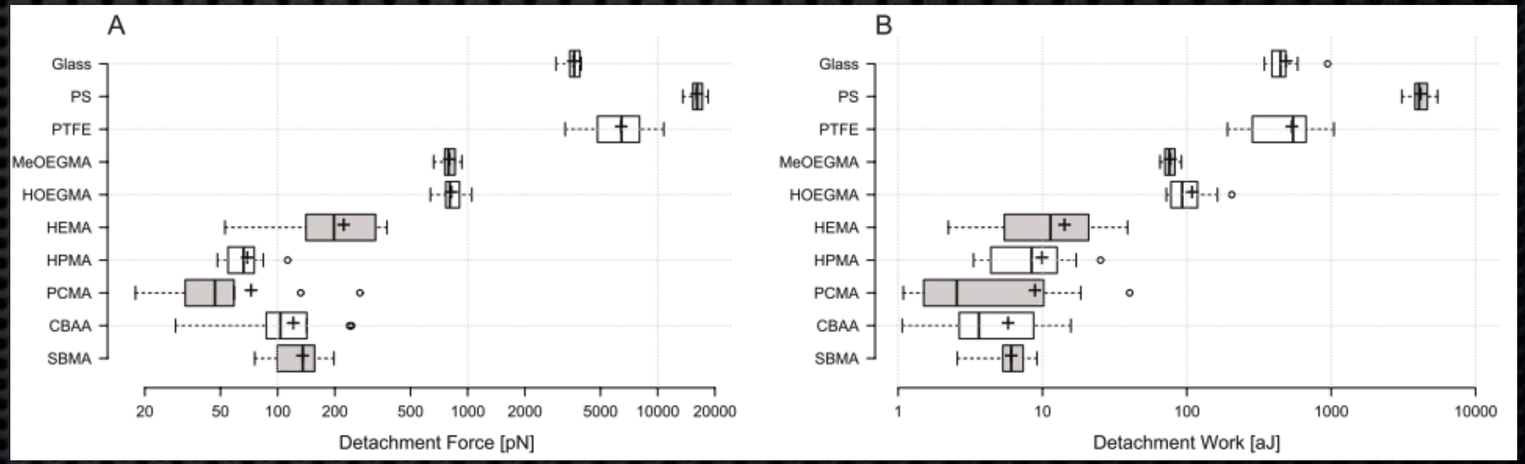




Yersinia pseudotubersulosis







Rodriguez-Emmenegger Polym. Chem 2015

2. Adhesion measurements

What about the integration of the 2 techniques ?

✗

✓ Need for ~~advanced~~ video recording (not **really** available in AFM softwares)

✓ Need for **simple** automation in photonic microscope (µmanager…)

✓ Need for AFM manipulation and measurements

- No need for precise x-y landing of the cantilever

⇒ AFM and Video **could** be handled by AFM software

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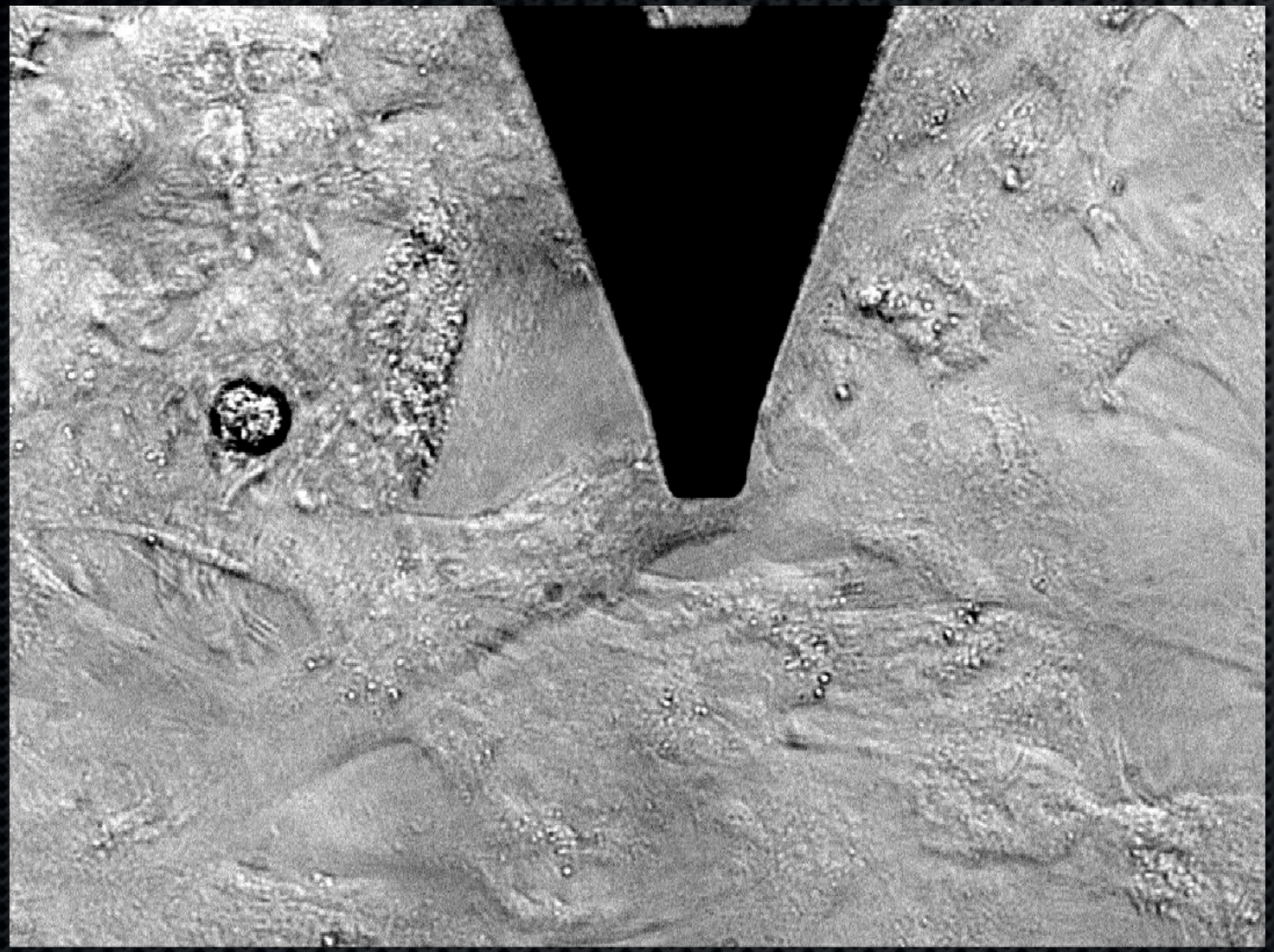
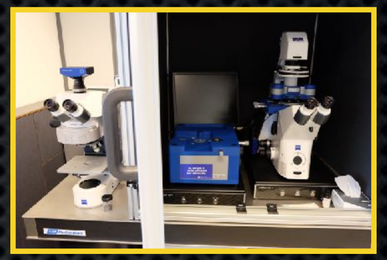
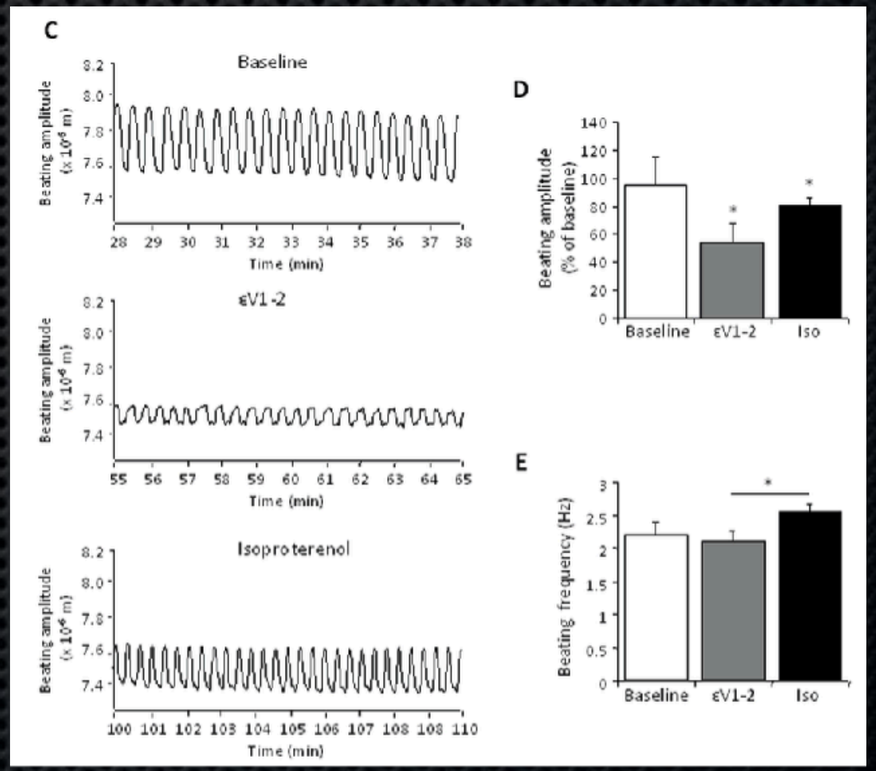
3. AFM as a sensor tool for cardiomyocytes contractility

Constant force







Dubois-Deruy et al. Cardio. research 2015

3. AFM as a sensor tool for cardiomyocytes contractility

What about the integration of the 2 techniques ?

✓ Need for ~~advanced~~ video recording (not really available in AFM softwares)

✓ ~~Need for automation in photonic microscope~~

✓ Need for AFM manipulation and measurements (**close loop**)

- No need for precise x-y landing of the cantilever

⇒ AFM and Video **could** be handled by AFM software

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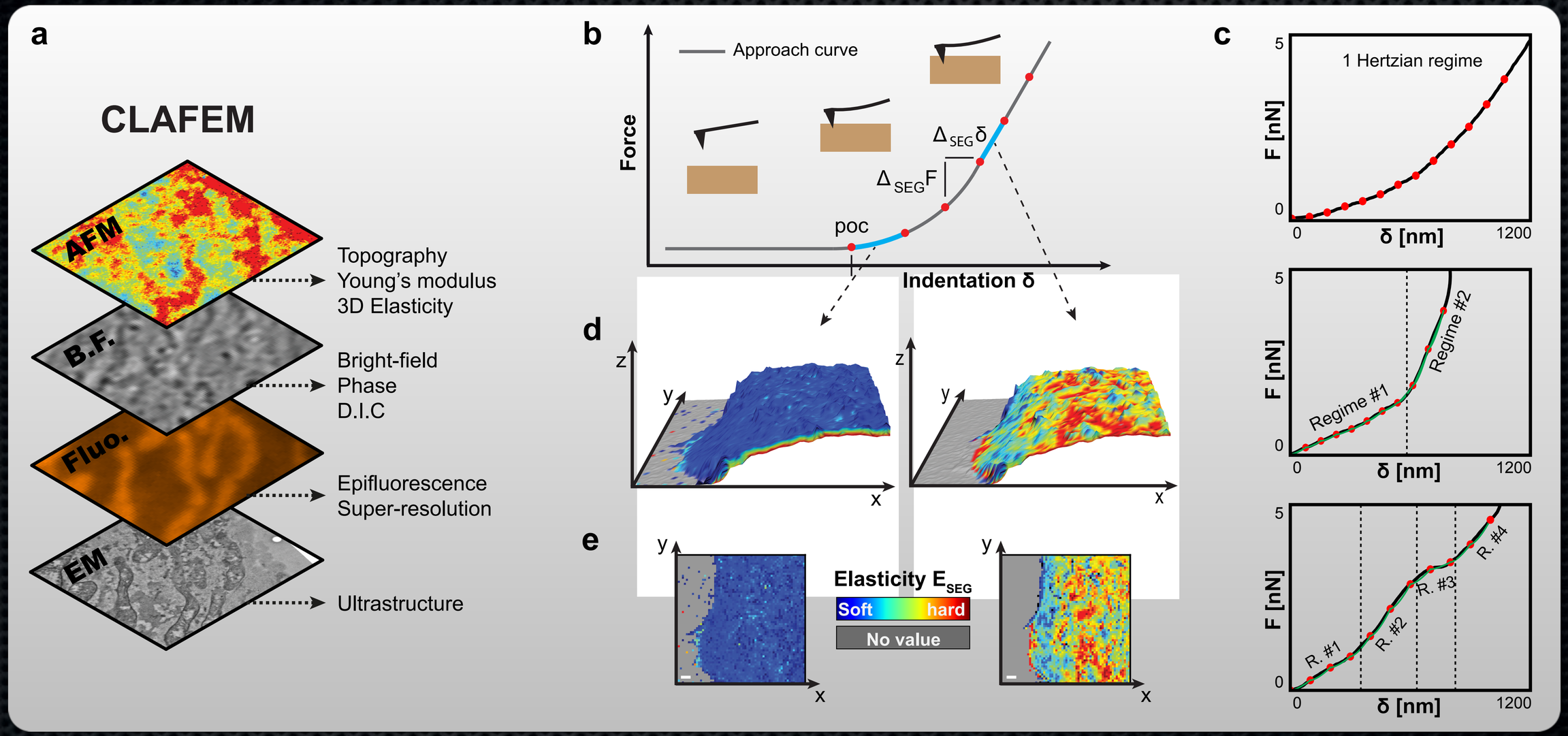
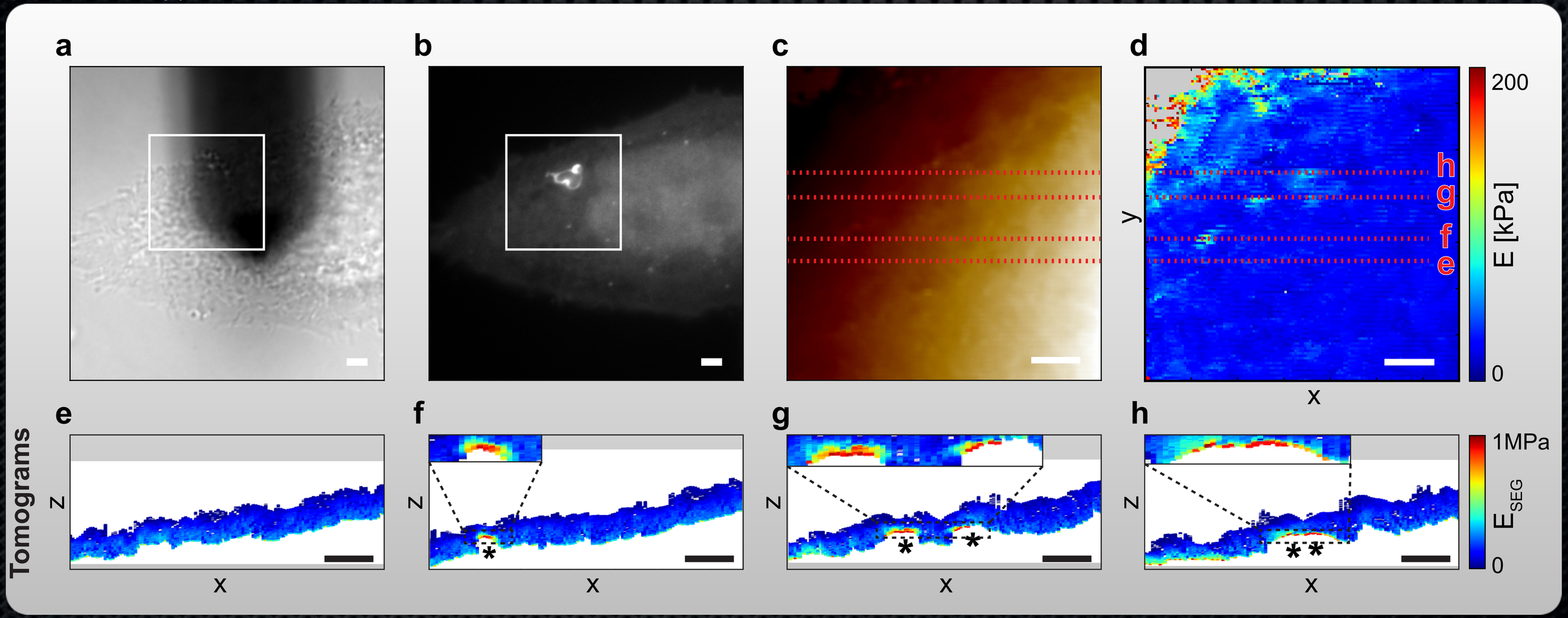
4. 3D elasticity & CLAFEM principles



Popoff & Janel et al. submitted

4. 3D elasticity measurements on infected cells

Salmonella Typhimurium in a HeLa GFP-LC3 cell



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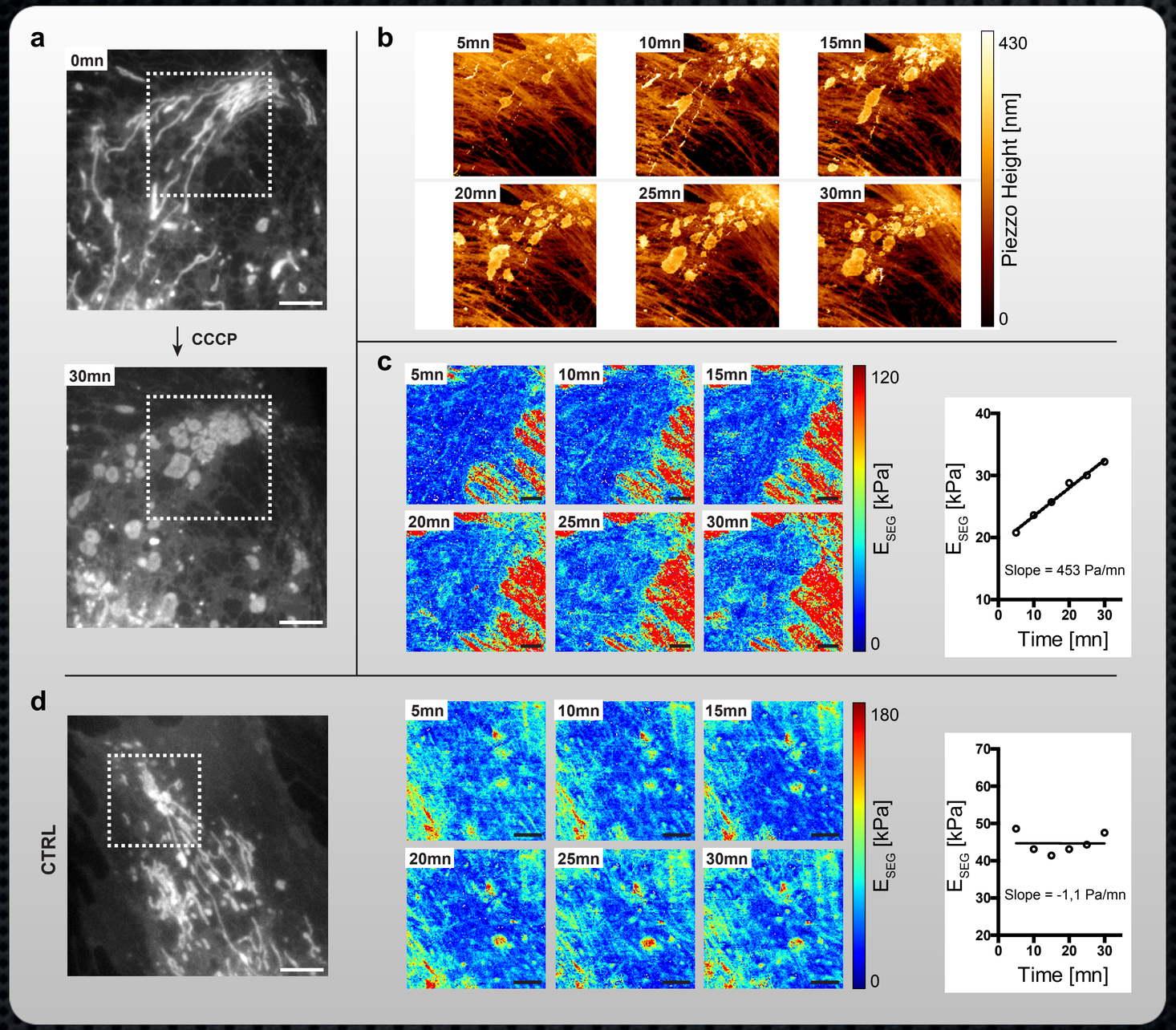
Popoff & Janel et al. submitted

4. 3D elasticity measurements on stressed mitochondria



Mitochondria in PtK_2 cells
Mitotracker red
CCCP treatment

Popoff & Janel et al. submitted

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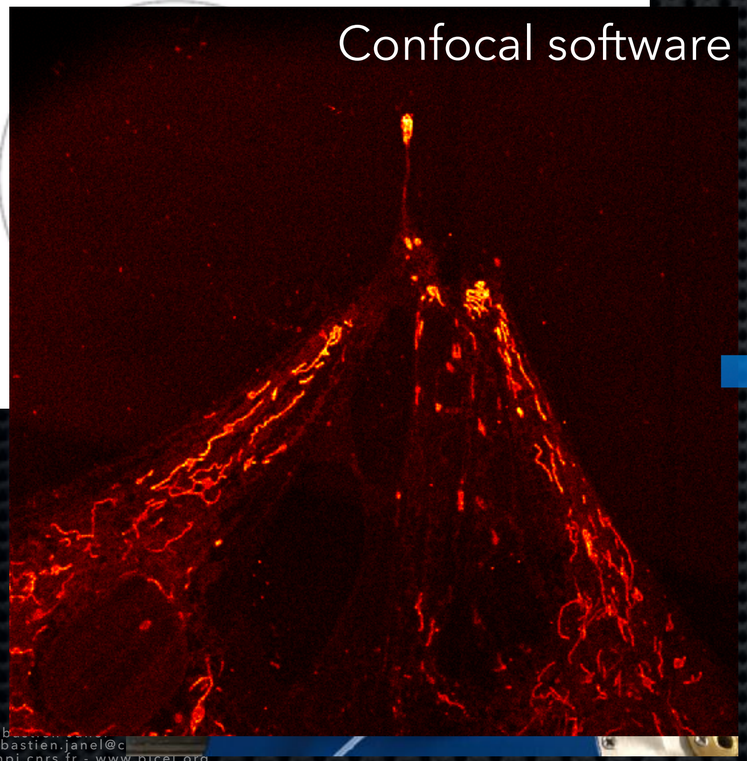
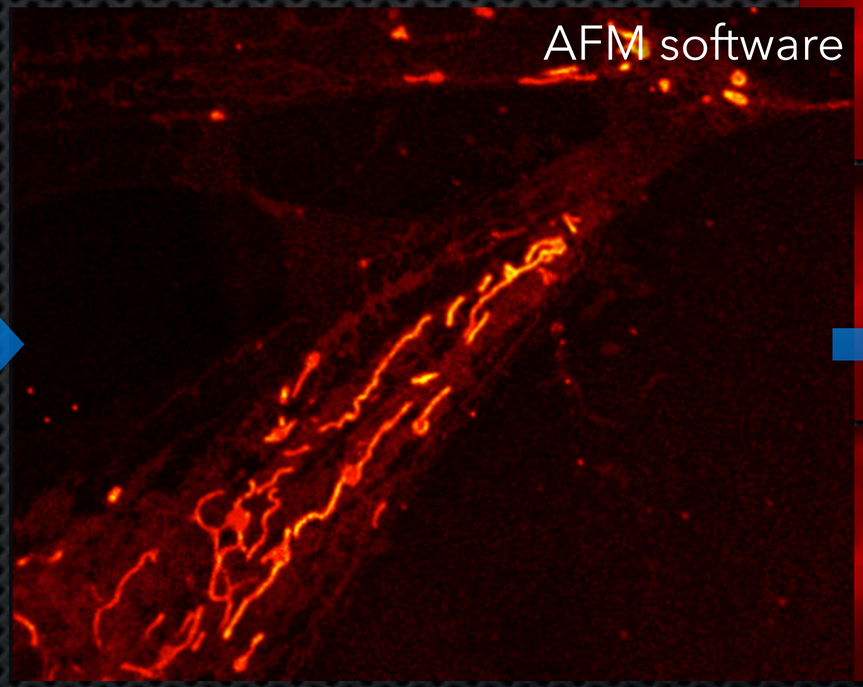
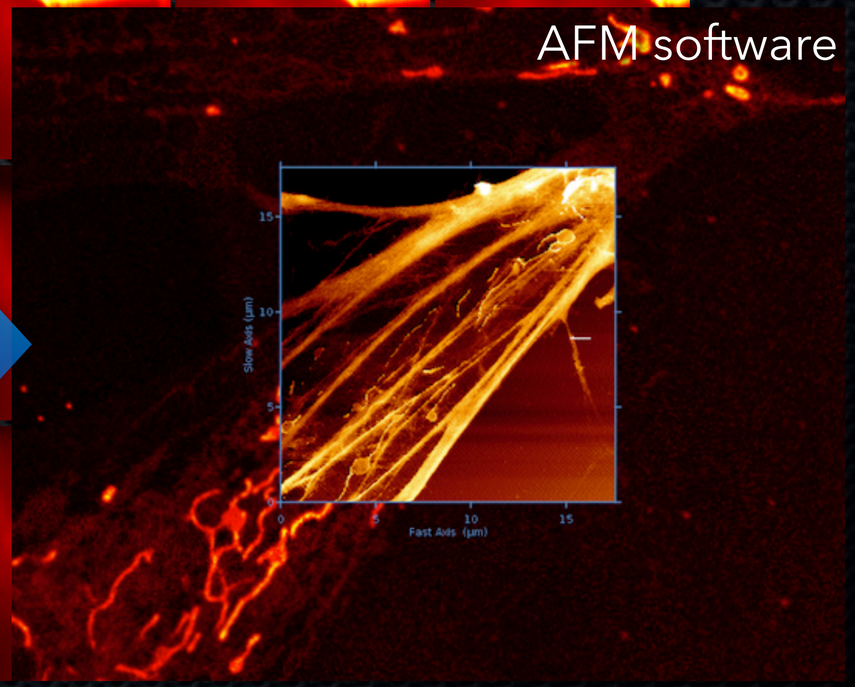
5. Confocal-STED/AFM coupling & integration

- Keyboard/Mouse shared by Synergy software (linux/windows)
- Data folder shared on the network for auto import of fluo images in AFM software
- Controllers connected through digital connection for triggering fluorescence acquisition
- Bellco photoetched 25mm glass coverslip (see « CNRS GT re positionnement »)
- TetraSpeck 100nm fluorescent bead for correlation (need AFM pixel size <75nm)
- Tip calibration using piezzo matrix (3x3) and PMT reflection detector using final image size
- Acquire fluorescence image and start AFM measurements
- Align images offline if necessary (ec-CLEM plugin under Icy software,…)



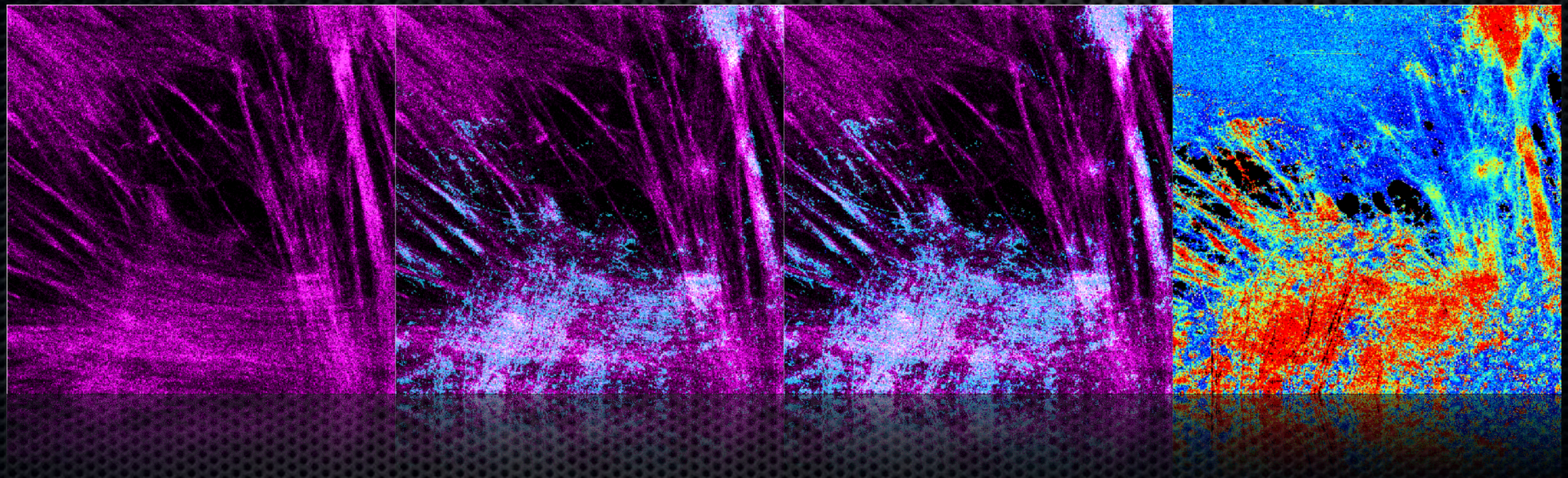




5. STED/AFM correlation of cell cytoskeleton

Precise localisation

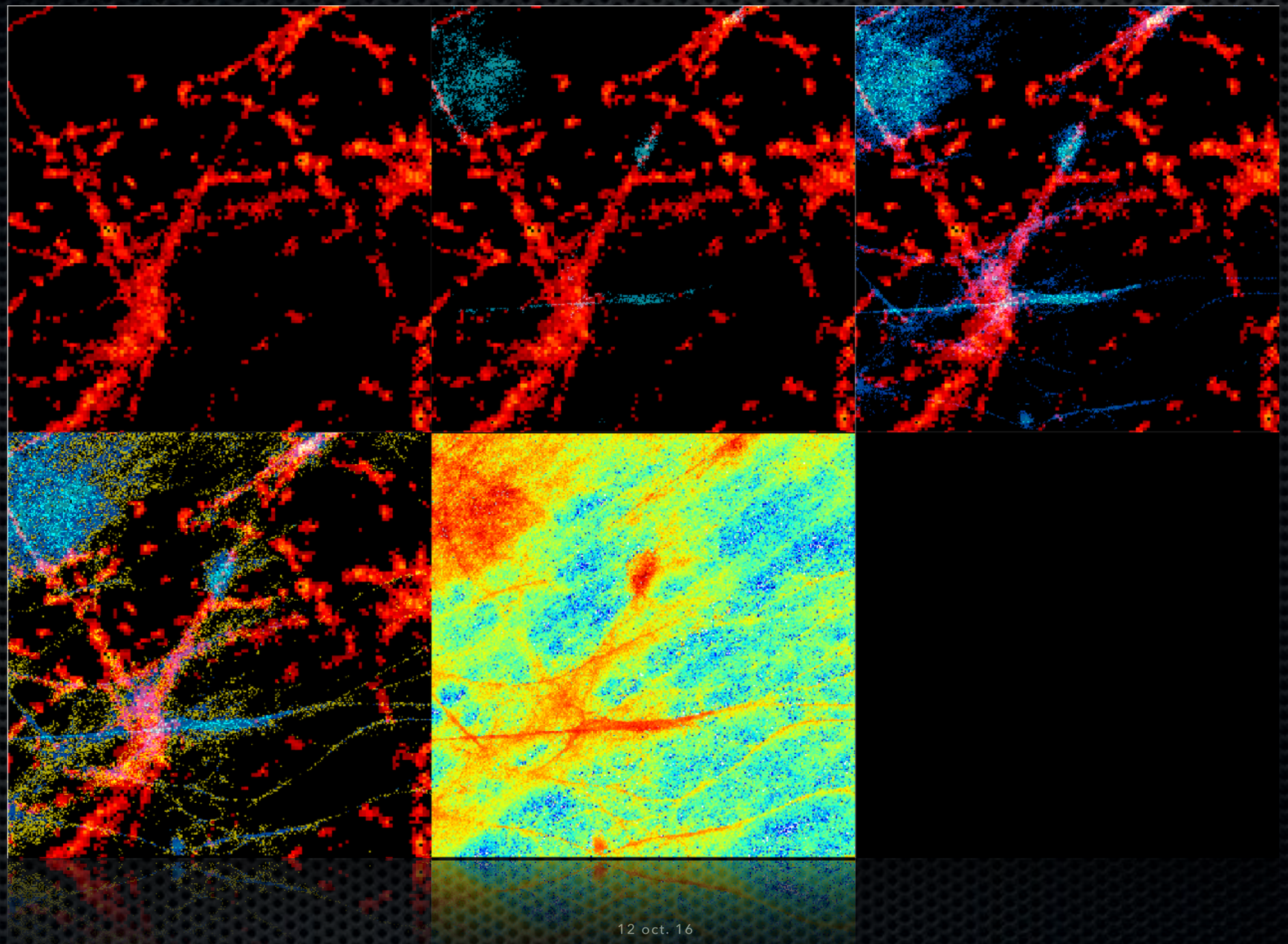
PtK2 fixed cells, siR actin frfp, 20µm, 40nm pixel size



Bovio S.

5. STED/AFM correlation of cell cytoskeleton

PtK2 fixed cells, tubulin, 5µm, 15nm pixel size



Bovio S.

5. Confocal-STED/AFM coupling & integration

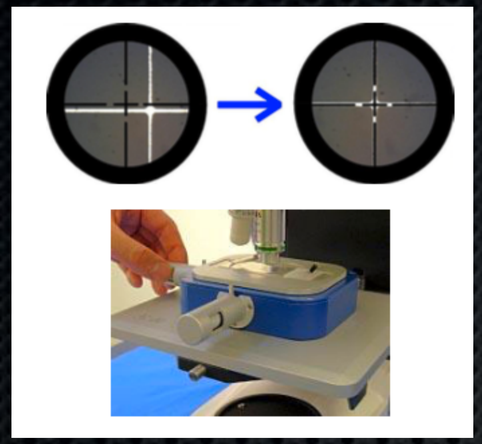
What about the integration of the 2 techniques ?

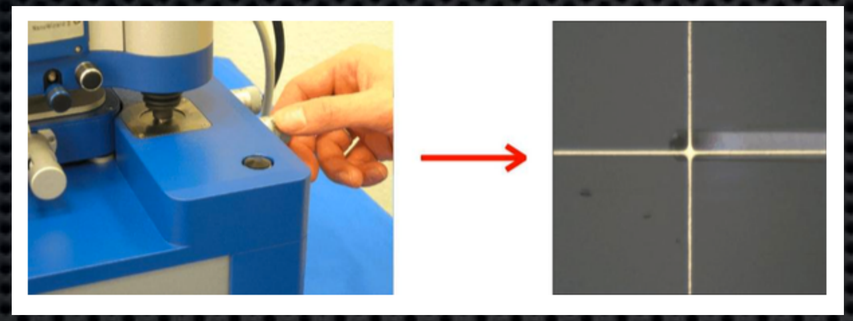
- ✓ Need for advanced video recording (not available in AFM softwares)
- ✓ Need for automation in photonic microscope
- ✓ Need for AFM manipulation and measurements
- ✓ **Need** for precise x-y landing of the cantilever

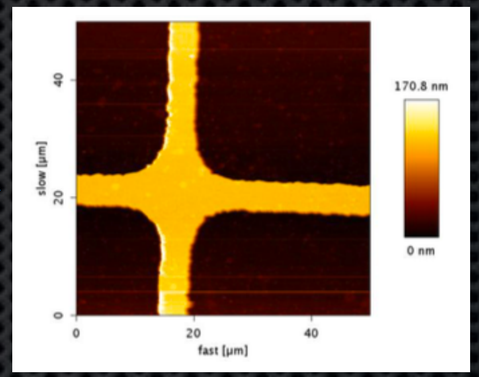
⇒ AFM and Video **have to** be handled by their own software

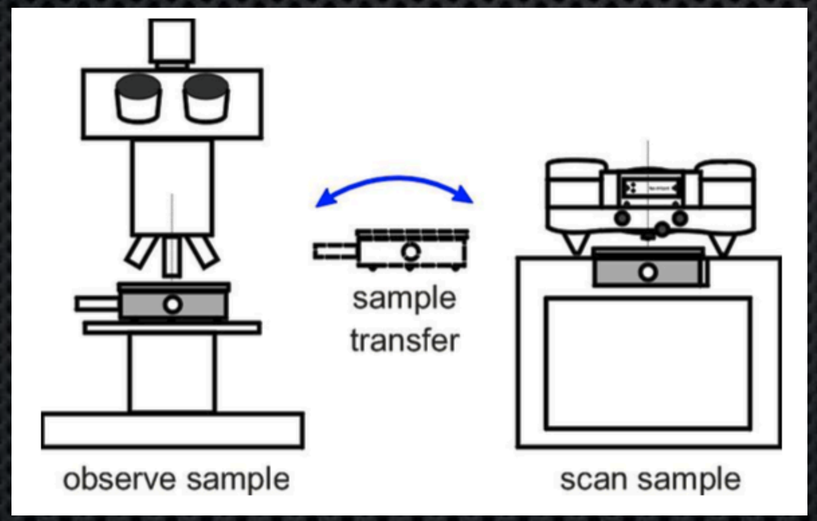
(Image is acquired on photonics PC/Software and **automatically transferred** to AFM)

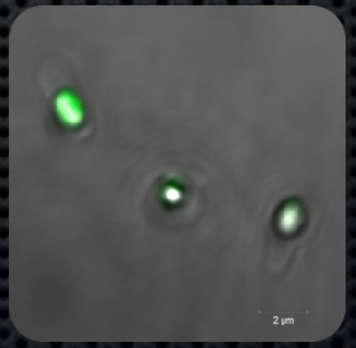
In the case of opaque samples



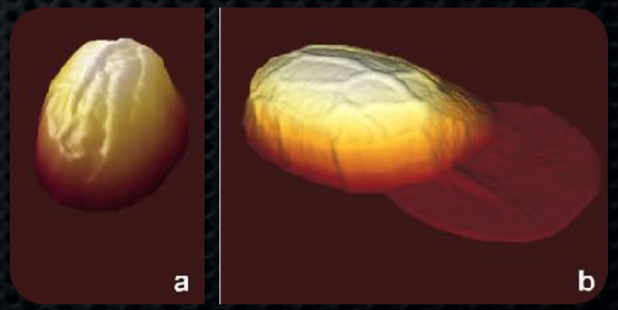






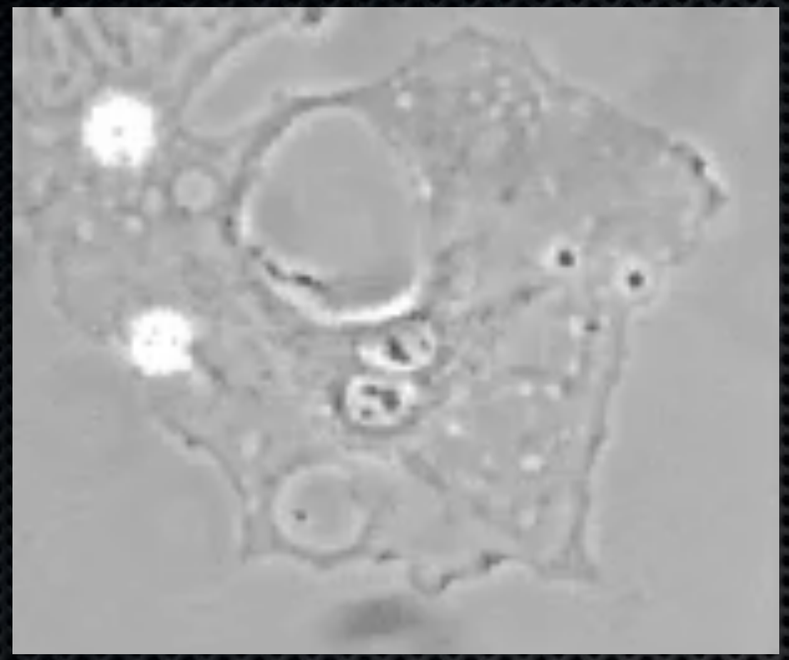


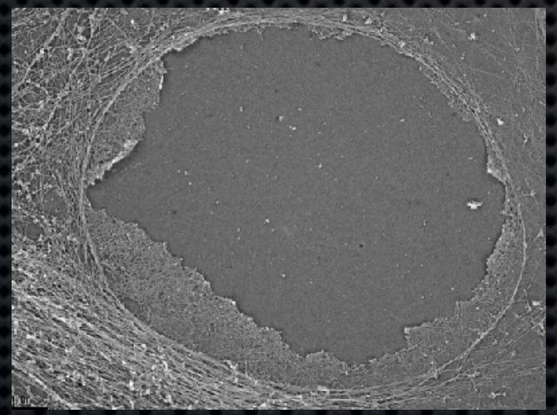
Bacillus spores on stainless steel

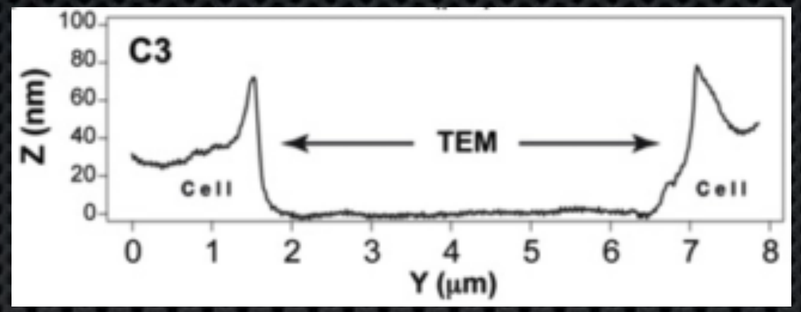


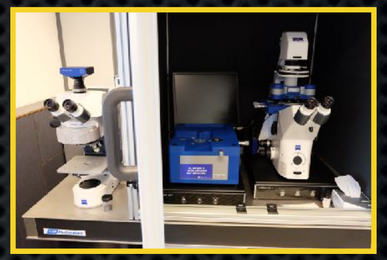
Fast biological processes

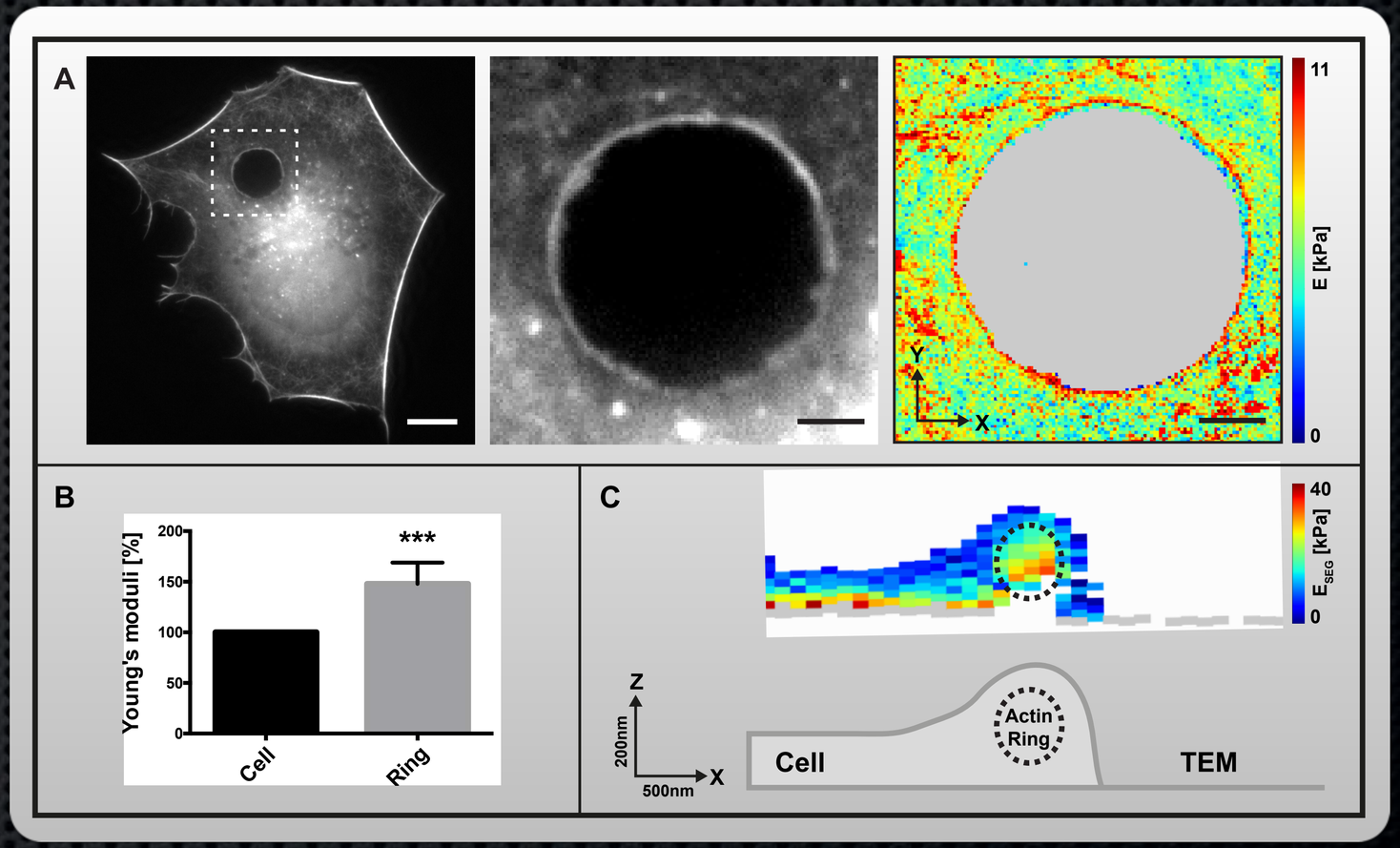
TransEndothelial Macroapertures by bacterial toxins (Anthrax, Edin)











Maddugoda et al. cell host & Microbes 2011, Gonzalez-Rodriguez et al. PRL 2012, Stefani et al. submitted

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Fast biological processes

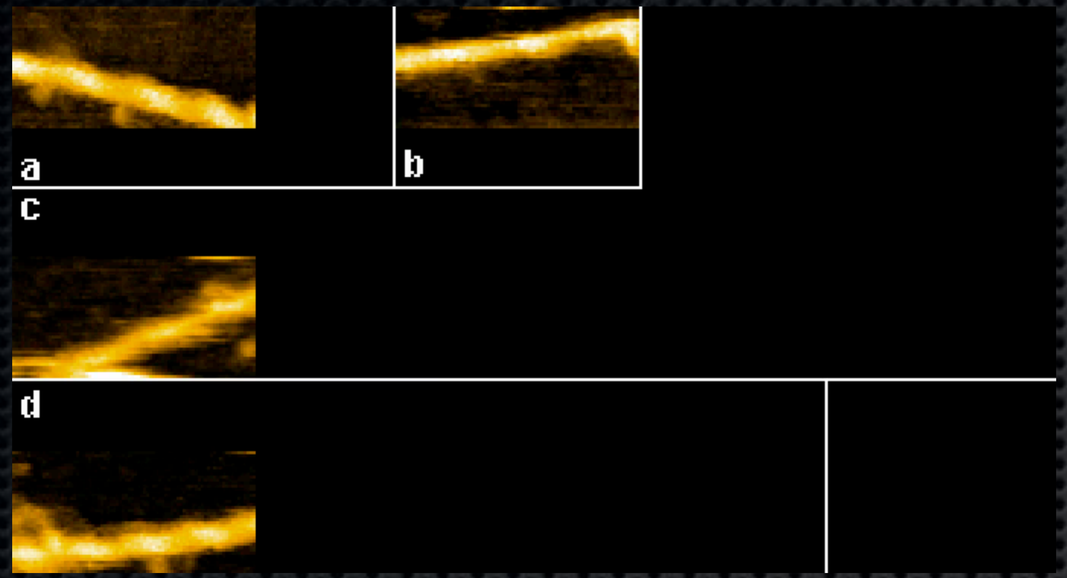
What about the integration of the 2 techniques ? ✗

- ✓ Need for **fast** video recording (not available in AFM softwares)
- ✓ Need for automation in photonic microscope
- ✓ Need for **fast** AFM manipulation and measurements
- ✓ **Need** for precise x-y landing of the cantilever

⇒ AFM and Video have to be handled by their own software until and AFM company releases a video microscope...

High-Speed AFM

Already available - AFM frame rate : 25fps



Mikihiro Shibata et al (2015),
Scientific Reports,
DOI: 10.1038/srep08724

In terms of coupling with photonics :

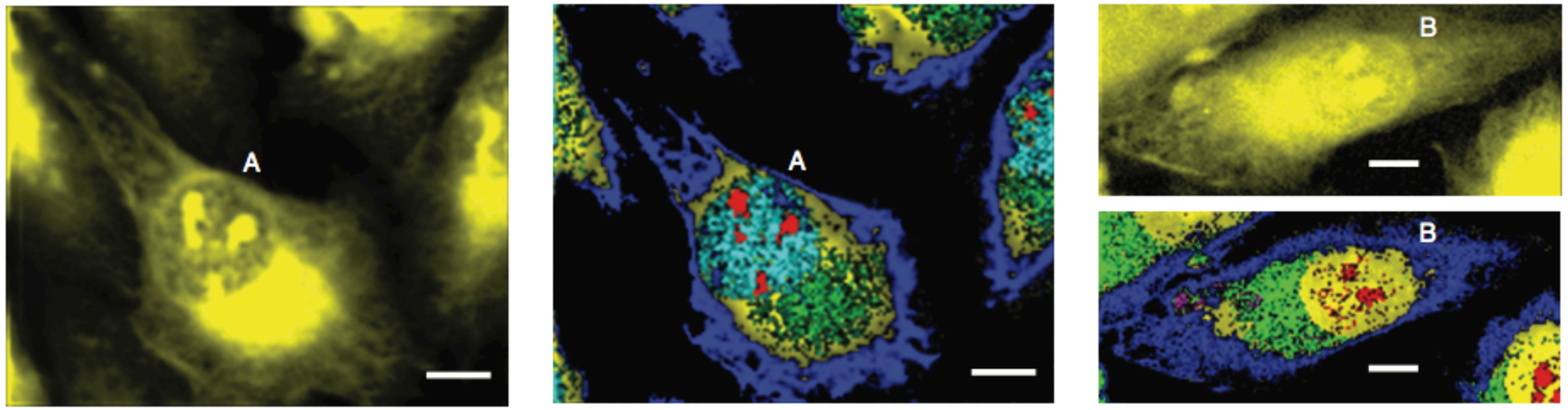
- ✓ Already in development in few labs on existing high-speed setups.
- ✓ T. Ando & Takeyasu labs working on implementing on inverted microscopes (first coupling in 2013)
- Sample 1mm^2, 4µm^2 max scan size

➡ Lot of data

➡ Synchronization issues

AFM/Raman coupling for cellular applications (No TERS)

Raman images of normal MG-63 cells generated by PCA



- Cellular areas
- Membranous areas
- Nucleoli
- Nuclei / nucleic acids
- Membrane rich organelles, e.g. endoplasmic reticulum (ER) and Golgi apparatus, and vesicles
- Lipid vesicles

Scale bar 10 µm

Remerciements

Cellular Microbiology and Physics of Infection
cmpi.cnrs.fr

BioImaging Center Lille Nord de France
www.bicel.org

Tutelles

Réseaux

Soutiens

LASER

Nicolas Barois
Hélène Bauderlique
Antonino Bongiovanni : STED, PALM
Simone Bovio : STED/AFM
(Yann Ciczora) : Nanoguided AFM
Antoine Dujardin
Vincent Dupres
Frank Lafont
Sebastien Janel
Marie-Christine Renaud
Sophie Salomé-Desnoulez
(Michka Popoff) : CLAFEM, pyAF
Magali Soyer
Joëlle Warein
Elisabeth Werkmeister STED, PALM