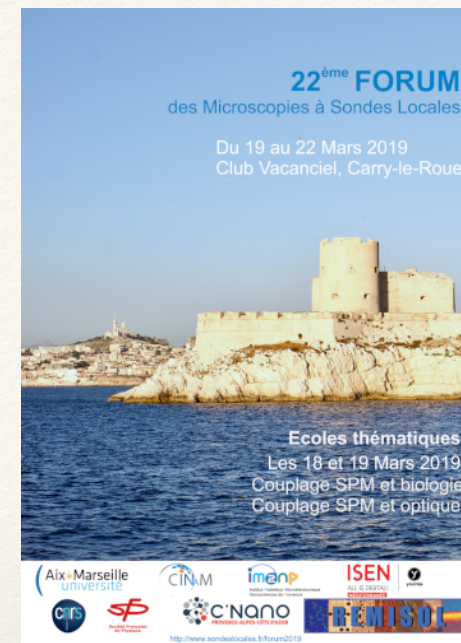


*Journée thématique: application des techniques de
microscopie à sondes locales à la biologie*



Cellular Microbiology
and Physics of Infection

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**Utilisation de la microscopie à force atomique
en corrélation avec les microscopies photonique et électronique
appliquée au domaine biomédical**

Plan

- ❖ Introduction
 - ❖ What are we talking about?
 - ❖ Why performing correlative studies?
 - ❖ Which modality for what purpose?
- ❖ How to proceed?
- ❖ Examples...
- ❖ Future

Introduction

❖ **What are we talking about?**

Correlative *Microscopy* techniques

- Photonique incl. Modalities (FCS, super-resolution...-

- Electronique (TEM, SEM)

... to analyse biomedical samples

i.e. very soft material most often in liquid

❖ Fluo + AFM: detection of baits

Detection of HSP60 on the membrane surface of stressed human endothelial cells by atomic force and confocal microscopy

Gerald Pfister^{2,*}, Cordula M. Stroh^{1,*}, Hannes Perschinka², Michaela Kind³, Michael Knoflach³, Peter Hinterdorfer^{1,‡} and Georg Wick³

¹Institute for Biophysics, University of Linz, A-4040 Linz, Austria

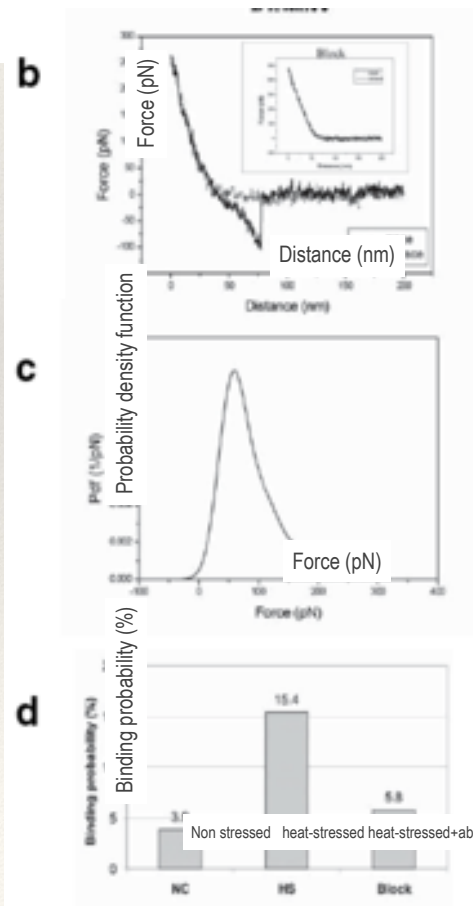
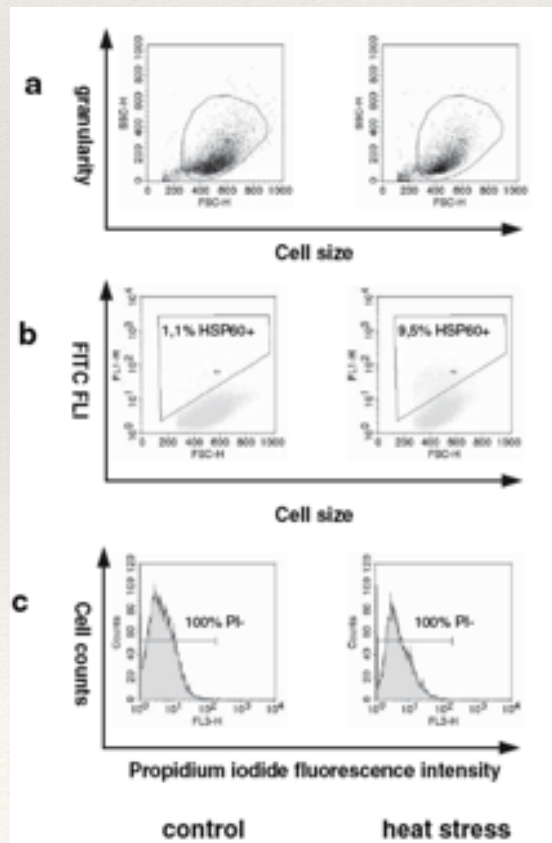
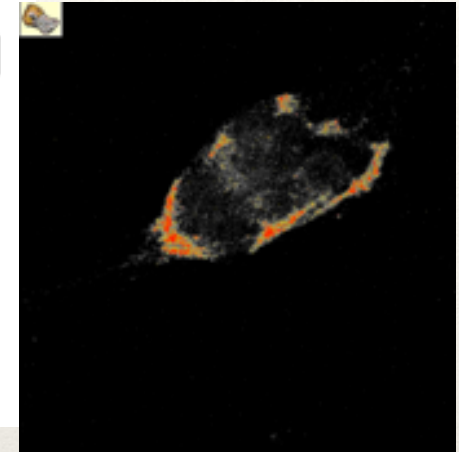
²Institute for Biomedical Aging Research, Austrian Academy of Sciences, and ³Institute of Pathophysiology, University of Innsbruck, Medical School, A-6020 Innsbruck, Austria

*These authors contributed equally to this work

‡Author for correspondence (e-mail: peter.hinterdorfer@jku.at)

Accepted 26 January 2005

Journal of Cell Science 118, 1587-1594 Published by The Company of Biologists 2005



❖ Fluo + AFM: detection of bait

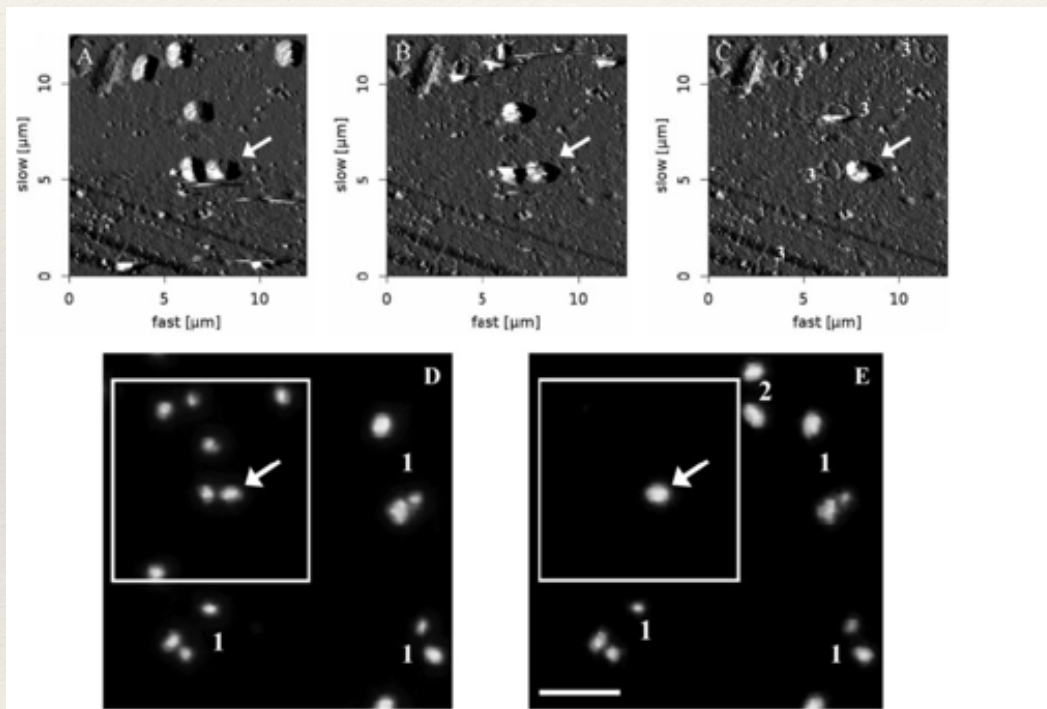
APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 2008, p. 410-415
0099-2240/08/\$08.00+0 doi:10.1128/AEM.01112-07
Copyright © 2008, American Society for Microbiology. All Rights Reserved.

Vol. 74, No. 2

Novel Combination of Atomic Force Microscopy and Epifluorescence Microscopy for Visualization of Leaching Bacteria on Pyrite[▽]

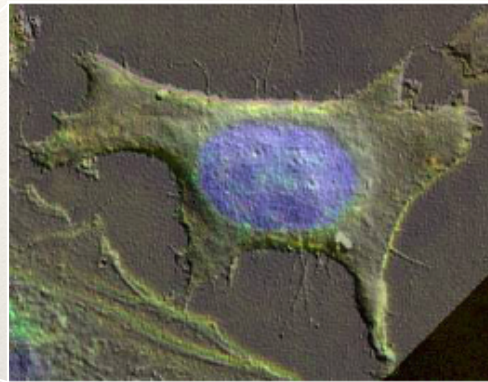
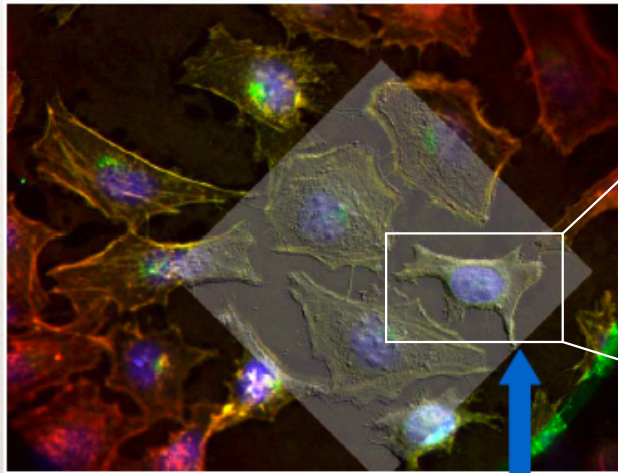
Stefanie Mangold,^{1,2} Kerstin Harneit,¹ Thore Rottwerder,¹ Günter Claus,² and Wolfgang Sand^{1*}

*University of Duisburg-Essen, Biofilm Centre, Aquatic Biotechnology, Gabelstr. 41, 47057 Duisburg, Germany,¹ and
Mannheim University of Applied Sciences, Institute of Technical Microbiology,
Windeckstrasse 110, 68163 Mannheim, Germany²*



❖ Fluo + AFM: correlation on the same sample

Wide field fluorescence

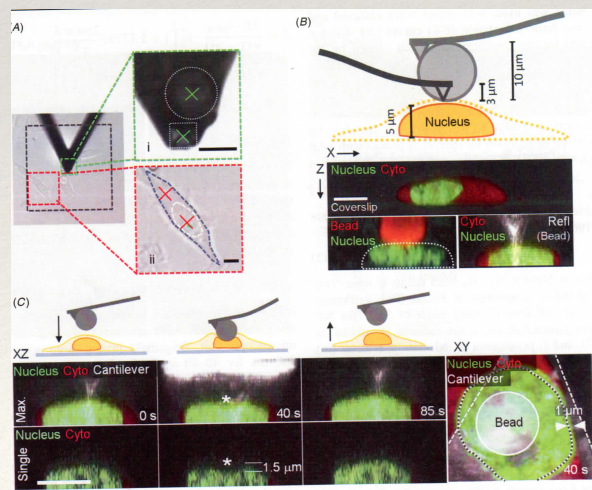


Veeco Appl. Note. Lafont and Berquand

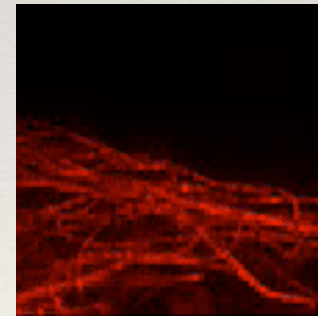
3-fold-fluorescence markers

- * GM130-GAM Alexa488
- * Phalloidin RITC
- * DAPI

63x objective, oil immersion
 150µm x 200µm section
 100µm x 100µm square for AFM



Krause et al. Phys Biol 2013

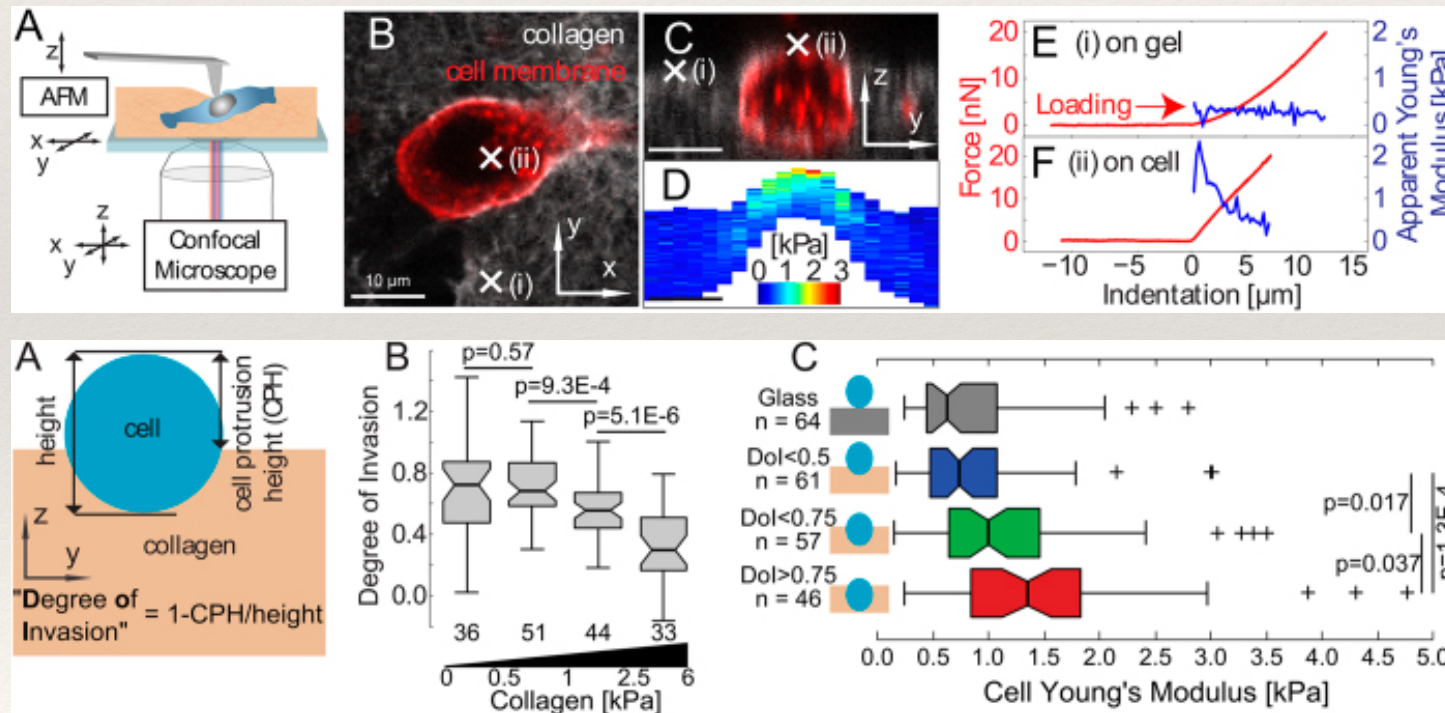


Discher & Coll

- ❖ Fluo + AFM: correlation on the same sample

Confocal microscopy

Breast cancer cells (pleural effusion) MDA-MB-231 cells (mb red) embedded in collagen (white) matrices of different stiffnesses



❖ Quality of the sample

Atomic force microscopy of BHK-21 cells: an investigation of cell fixation techniques

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^a Department of Biological Sciences, Cork Institute of Technology, Bishopstown, Cork, Ireland

^b Department of Microbiology, National University of Ireland Galway, Ireland

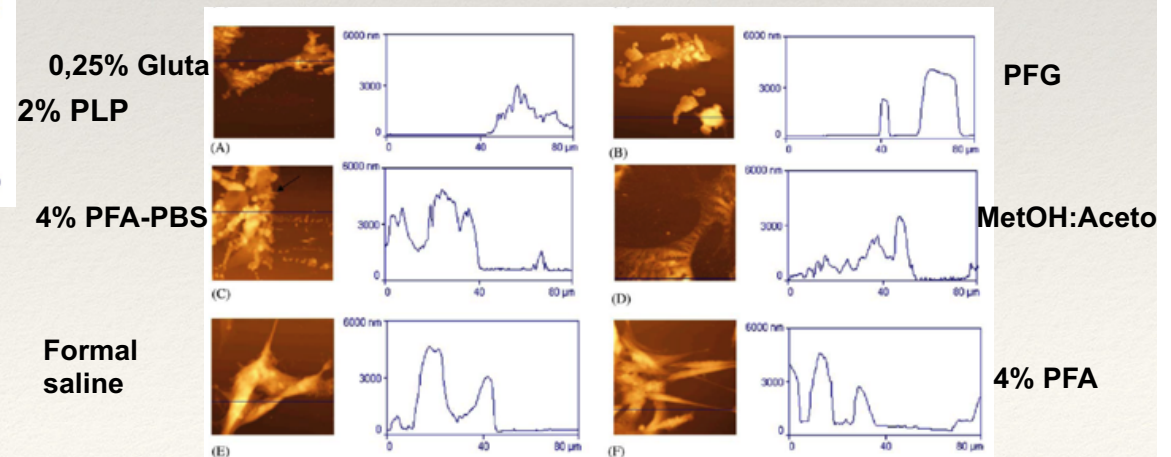
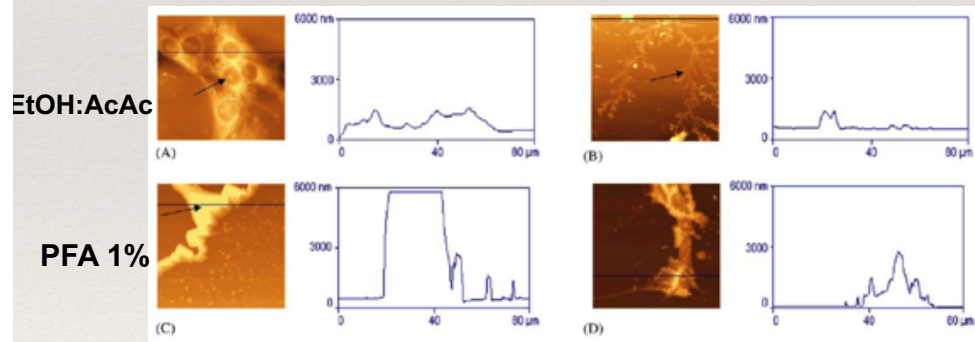
^c Department of Applied Physics and Instrumentation, Centre for Surface and Interface Analysis, Cork Institute of Technology, Bishopstown, Cork, Ireland

Ultramicroscopy 100 (2004) 153-

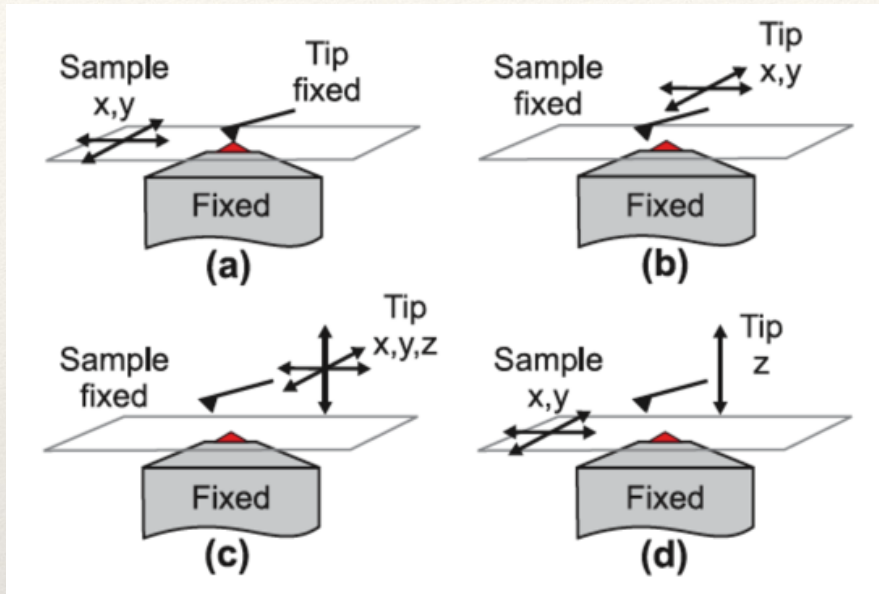
Table 2
Fixative efficacy assessment of duplicate fixation trials

Fixative	Debris	Streaking artifact	Coating artifact	Depression artifact	Fibroblastic morphology	Saturation constraint	Overall result
2% PLP	o	-	+	+	++	+	++
0.25% Glutaraldehyde	-	+	+	+	+	+	+
PFG	-	+	+	+	o	-	-
4% Phosphate-buffered formal saline	-	-	+	+	o	+	-
1% Formaldehyde	-	+	+	+	+	-	o
Methanol:acetone	o	-	+	-	++	+	+
Formal saline	o	+	+	+	+++	+	+++
4% Paraformaldehyde	+	+	+	+	+++	+	+++
Ethanol:acetic acid	+	+	-	-	+	+	+

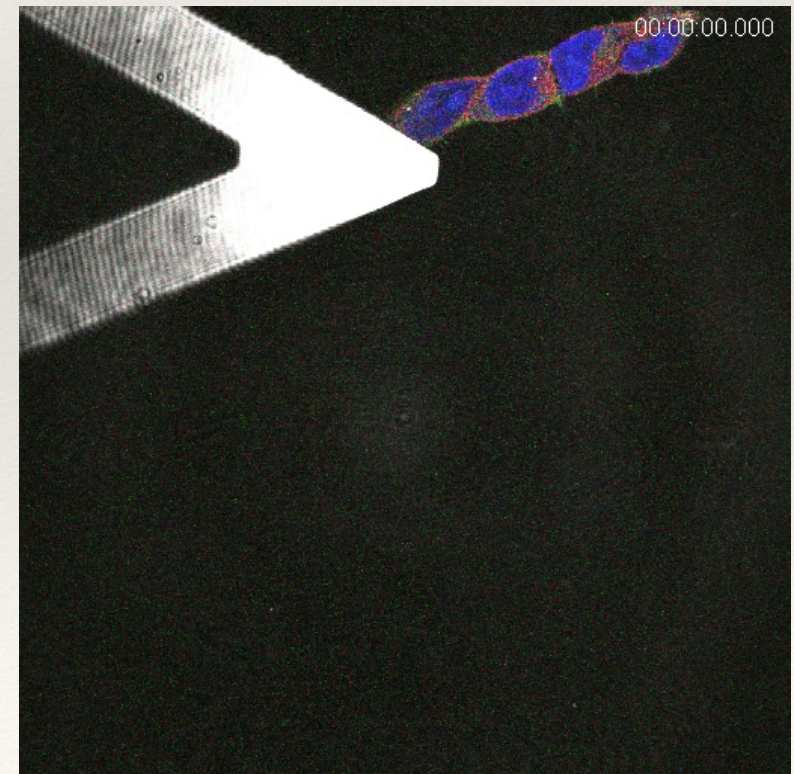
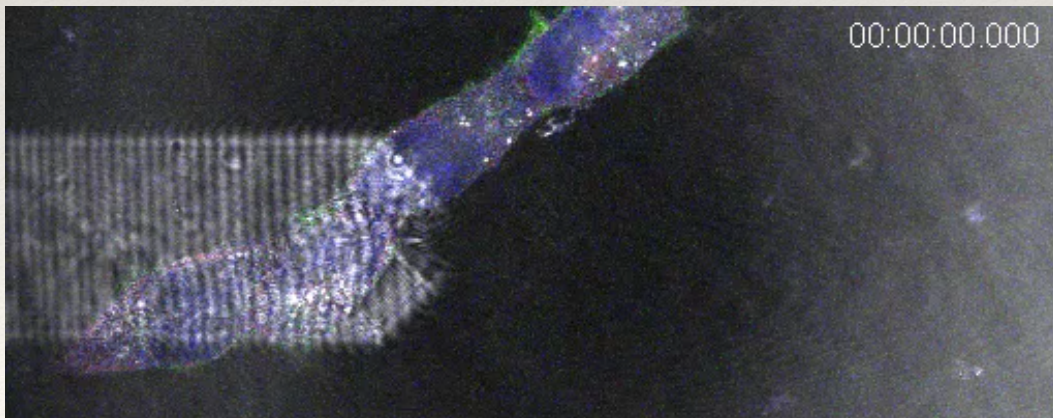
Fixatives were assessed as follows: Debris: + if absent, o if small amount present, - if significant amount present. Streaking, coating, and depression artifacts and saturation constraint: + if absent, - if present. Fibroblastic morphology and overall result: +++ if excellent; ++ if good; + if average; o if fair; - if poor.



❖ Tip scan vs. Sample scan



<https://youtu.be/1IszD5CneQQ>



❖ Electron microscopy: what we are NOT talking about...

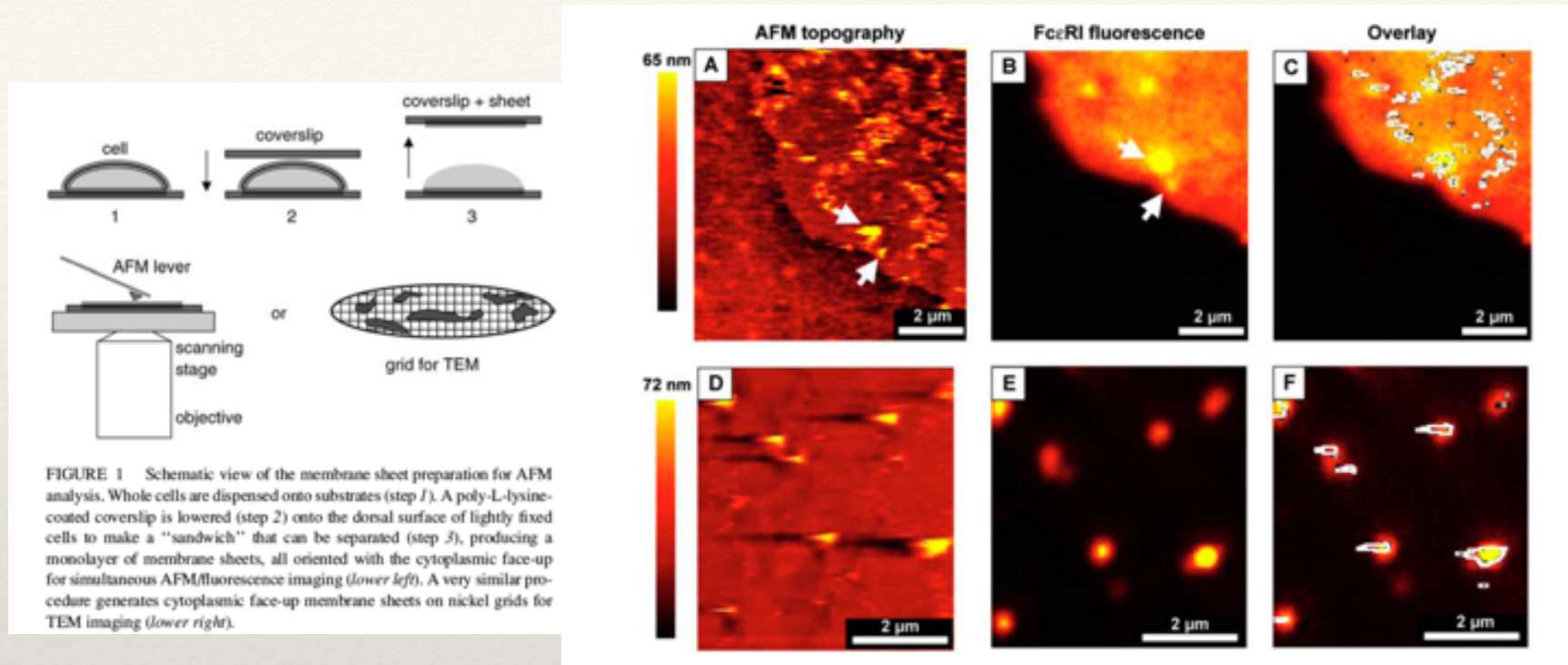


<https://www.youtube.com/watch?v=yvZleHfF364>



<https://www.youtube.com/watch?v=0xx8GCcrLPg>

❖ Electron microscopy correlative not extemporaneously...



2404

Biophysical Journal Volume 90 April 2006 2404-2413

Revealing the Topography of Cellular Membrane Domains by Combined Atomic Force Microscopy/Fluorescence Imaging

D. J. Frankel,^{*} J. R. Pfeiffer,[†] Z. Surviladze,[‡] A. E. Johnson,[§] J. M. Oliver,[†] B. S. Wilson,[†] and A. R. Burns^{*}

^{*}Biomolecular Materials and Interfaces Department, MS1413 Sandia National Laboratories, Albuquerque, New Mexico 87186; [†]Department of Pathology and Cancer Research and Treatment Center, University of New Mexico, Albuquerque, New Mexico 87131; and [‡]School of Medicine, Texas A&M University, College Station, Texas 77843

... But with cells !!!

Introduction

- ❖ **Why performing correlative studies?**
 - ➔ Fluorescence provides IDENTIFICATION of constituents (molecules, cellular compartments, ...)
 - ➔ AFM provides HEIGHT (nm) & FORCE data
 - ➔ Electron microscopy provides ULTRASTRUCTURAL information incl. of non labelled structures

Live material

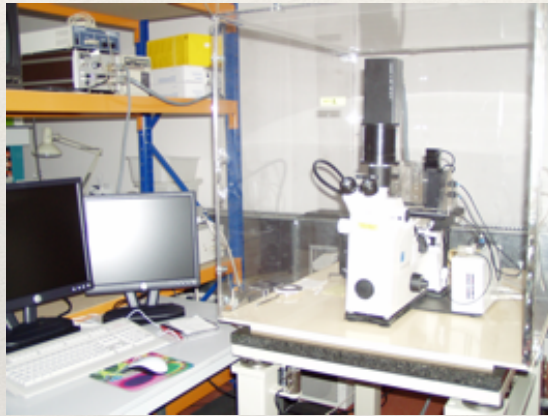
Fixed material

❖ AFM Measurements of identified cellular structural components

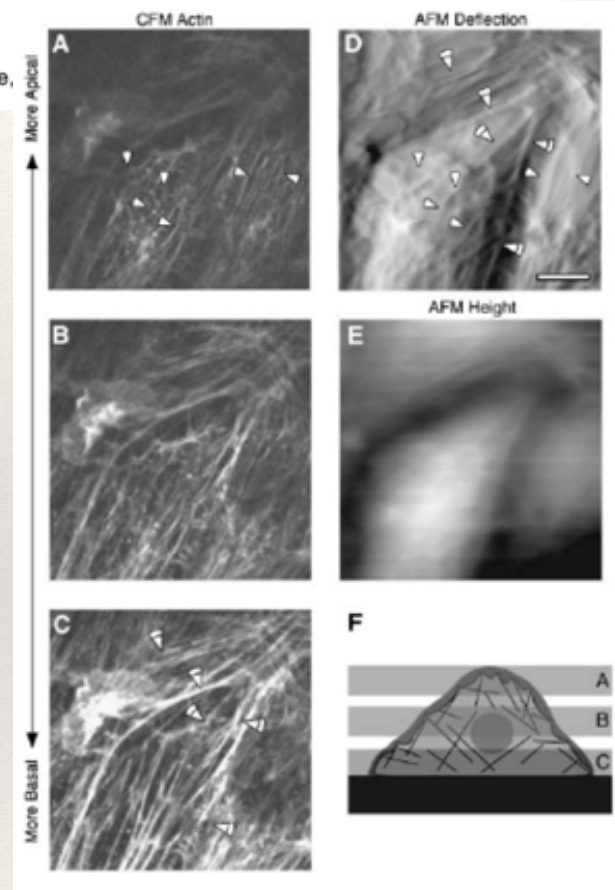
670 Biophysical Journal Volume 88 January 2005 670–679

Micromechanical Architecture of the Endothelial Cell Cortex

Devrim Pesen and Jan H. Hoh
Department of Physiology, Johns Hopkins University School of Medicine, Baltimore,



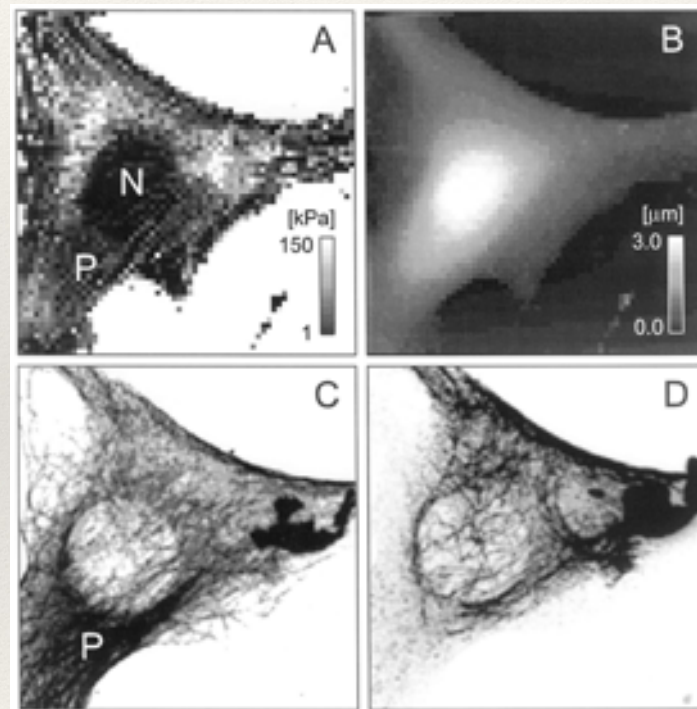
Correlated AFM Confocal



Elasticity mapping of living fibroblasts by AFM and immunofluorescence observation of the cytoskeleton

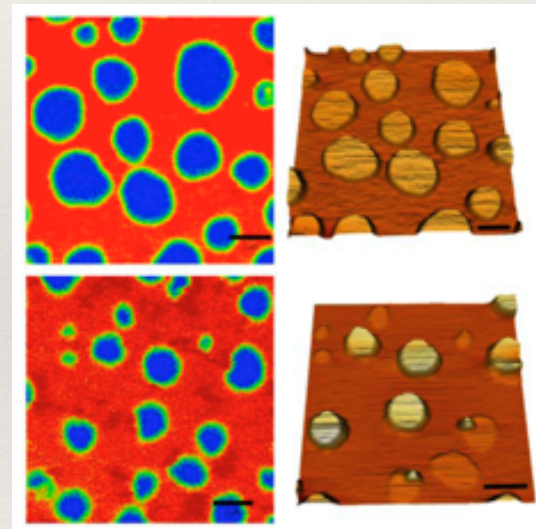
Hisashi Haga^{a,*}, Shigeo Sasaki^a, Kazushige Kawabata^a, Etsuro Ito^b,
Tatsuo Ushiki^c, Takashi Sambongi^a

Ultramicroscopy 82 (2000) 253–258



Effects of Ceramide on Liquid-Ordered Domains Investigated by Simultaneous AFM and FCS

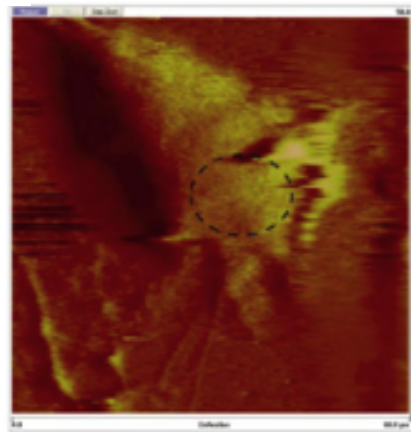
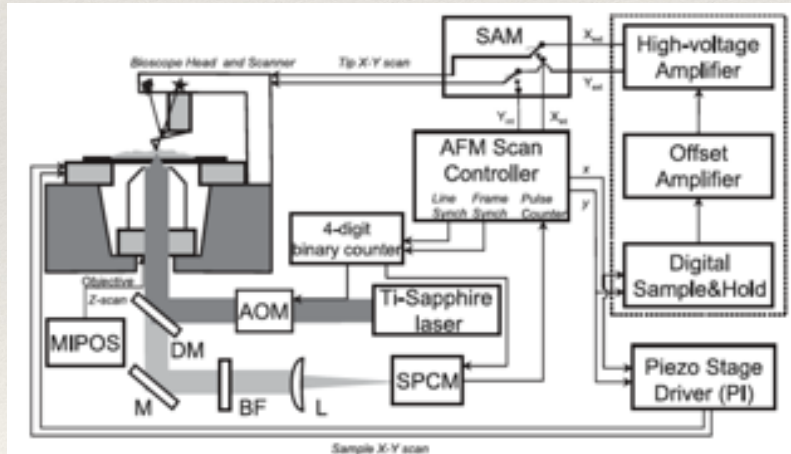
Salvatore Chiantia, Nicoletta Kahya, Jonas Ries, and Petra Schwille
Biotechnologisches Zentrum, Dresden University of Technology, Tatzberg, Dresden, Germany



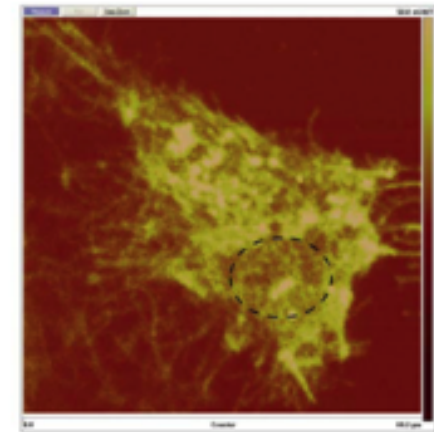
Simultaneous atomic-force and two-photon fluorescence imaging of biological specimens in vivo

Claudiu C. Gradinaru¹, Peter Martinsson, Thijs J. Aartsma, Thomas Schmidt*

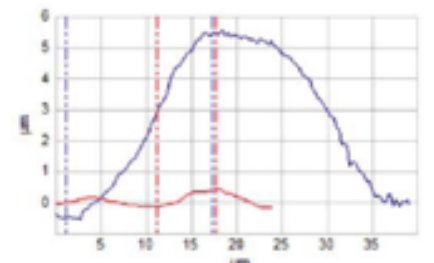
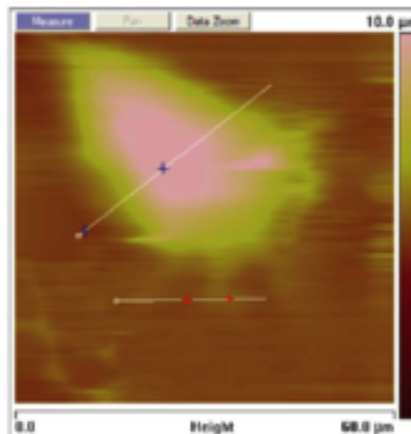
Department of Biophysics, Leiden University, Nieuwe Boeleaan 7, 2311 CA Leiden, The Netherlands



(A)



(B)



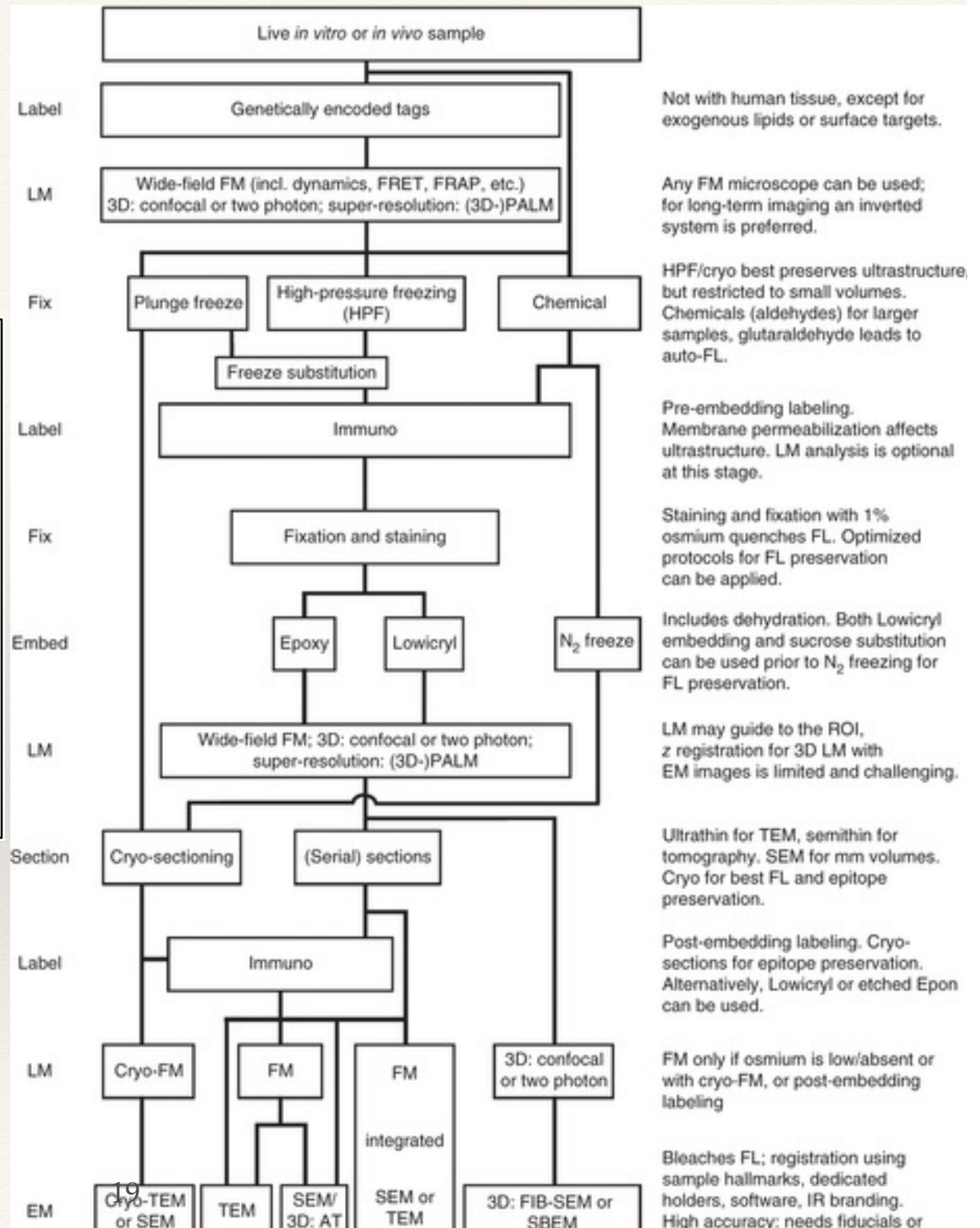
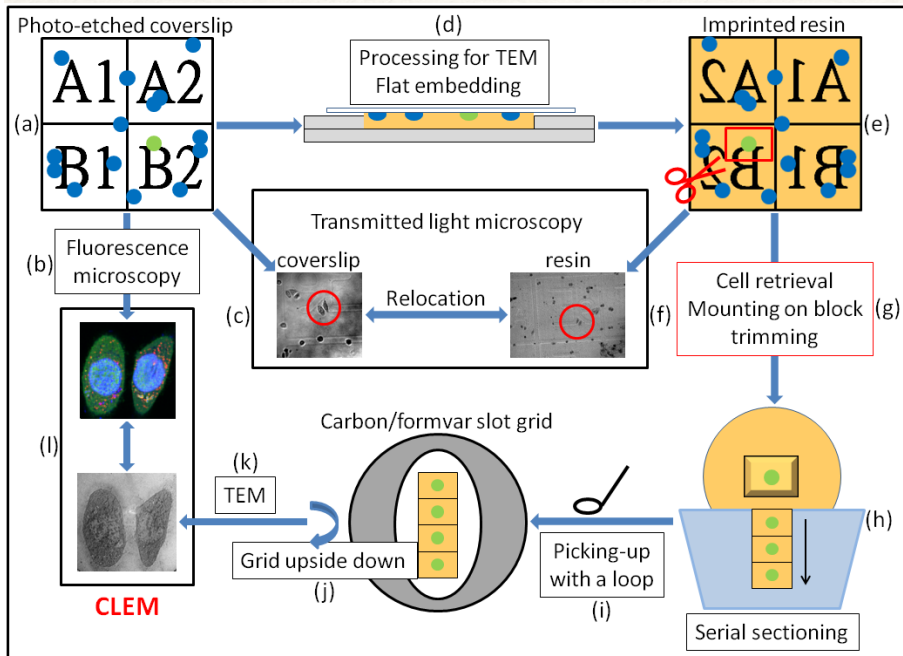
Introduction

- ❖ **Which modality for what purpose?**
 - ➔ Photonic μ : Wide field, confocal, SR, FCS...
 - ➔ AFM: intermittent mode, contact mode, fast modes
 - ➔ Electron microscopy: Transmission, Scanning
 - ➔ Correlative: CLEM, CLAM, CLAFEM...

How to proceed?

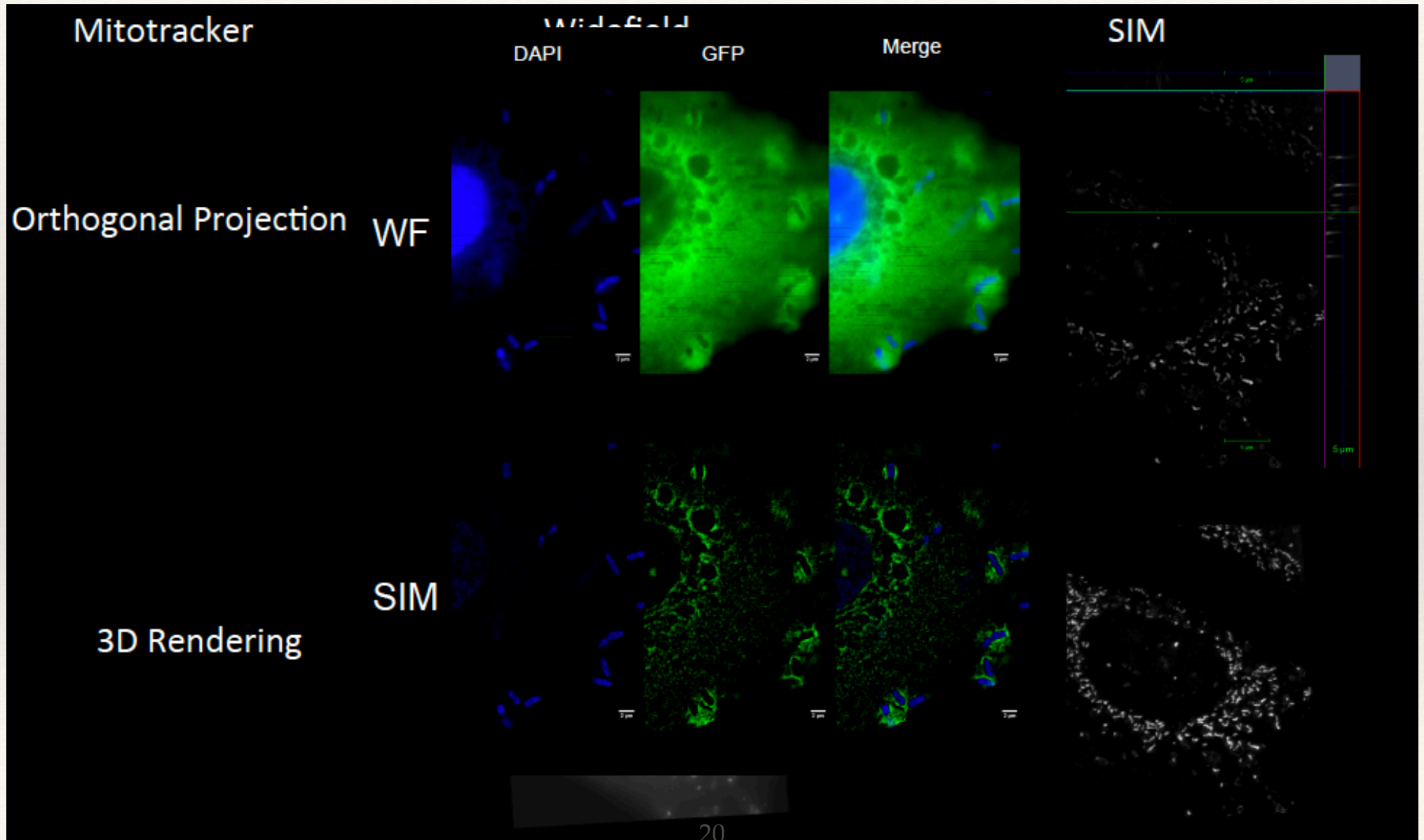
- CLEM principles
- CLAFEM

❖ CLEM



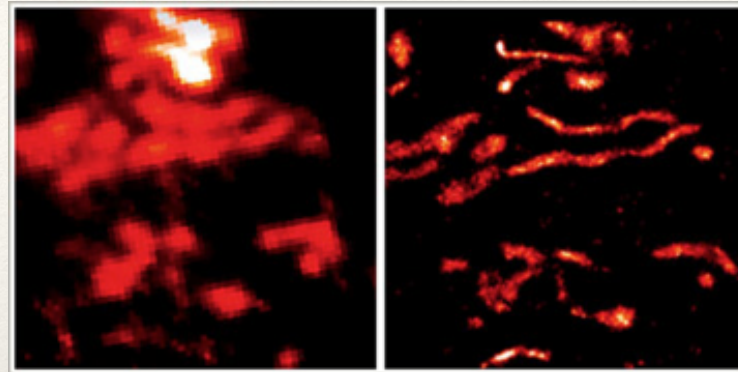
❖ Toward super-CLEM

Structured illumination microscopy

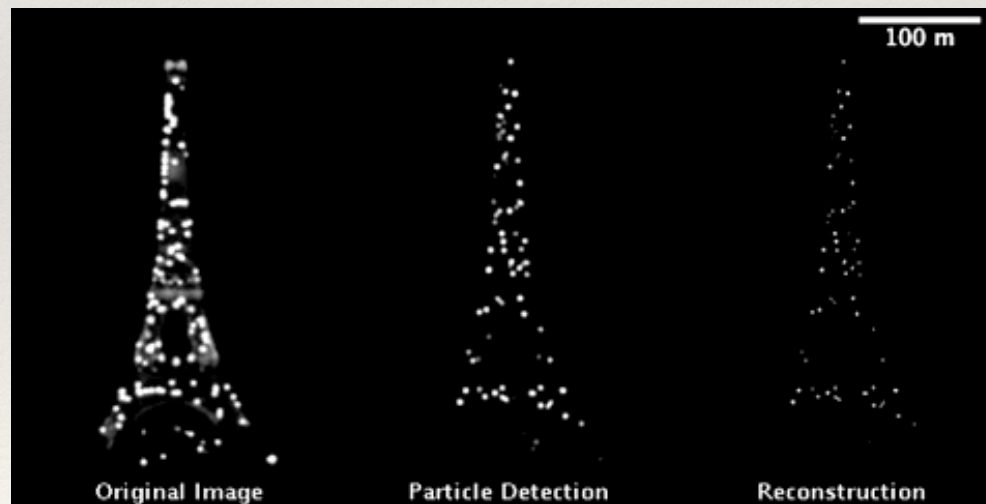
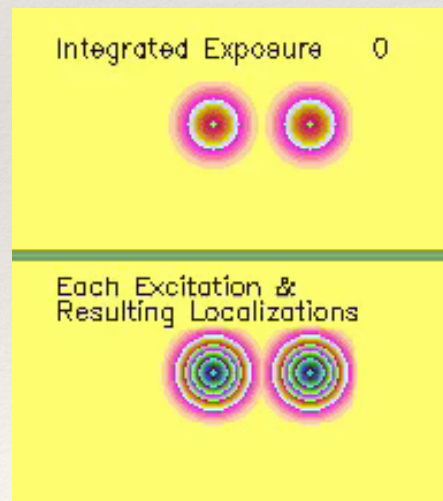


❖ Toward super-CLEM

PhotoActivated Light Microscopy

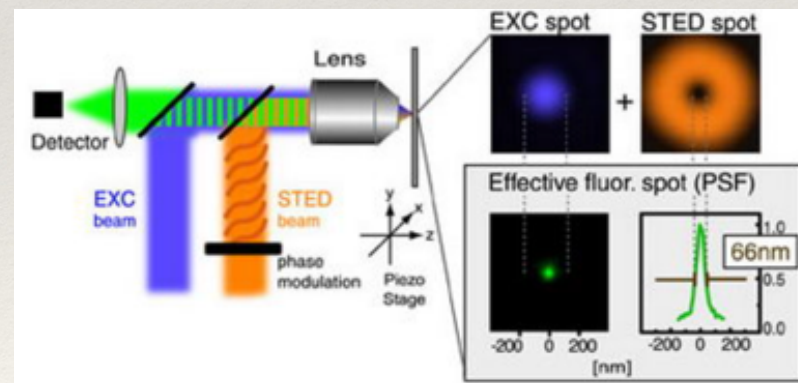
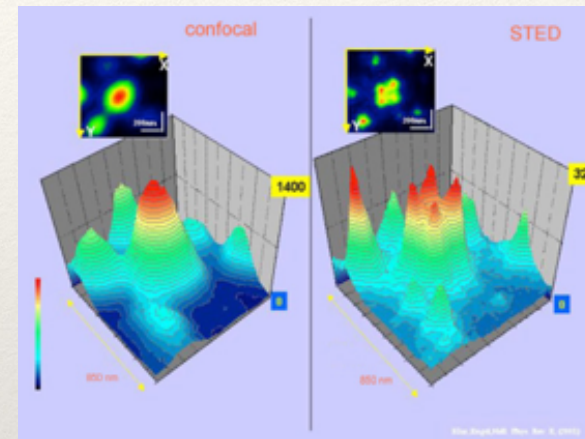
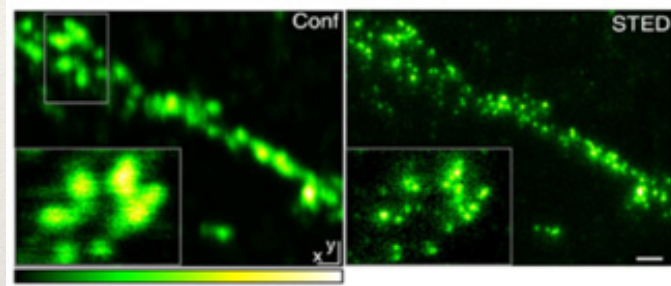


Eric Betzig, George H. Patterson, Rachid Sougrat, O. Wolf Lindwasser, Scott Olenych, Juan S. Bonifacio, Michael W. Davidson, Jennifer Lippincott-Schwartz, Harald F. Hess, *Science* 2006 Sep15; 313(5793):1642-5.



❖ Toward super-CLEM

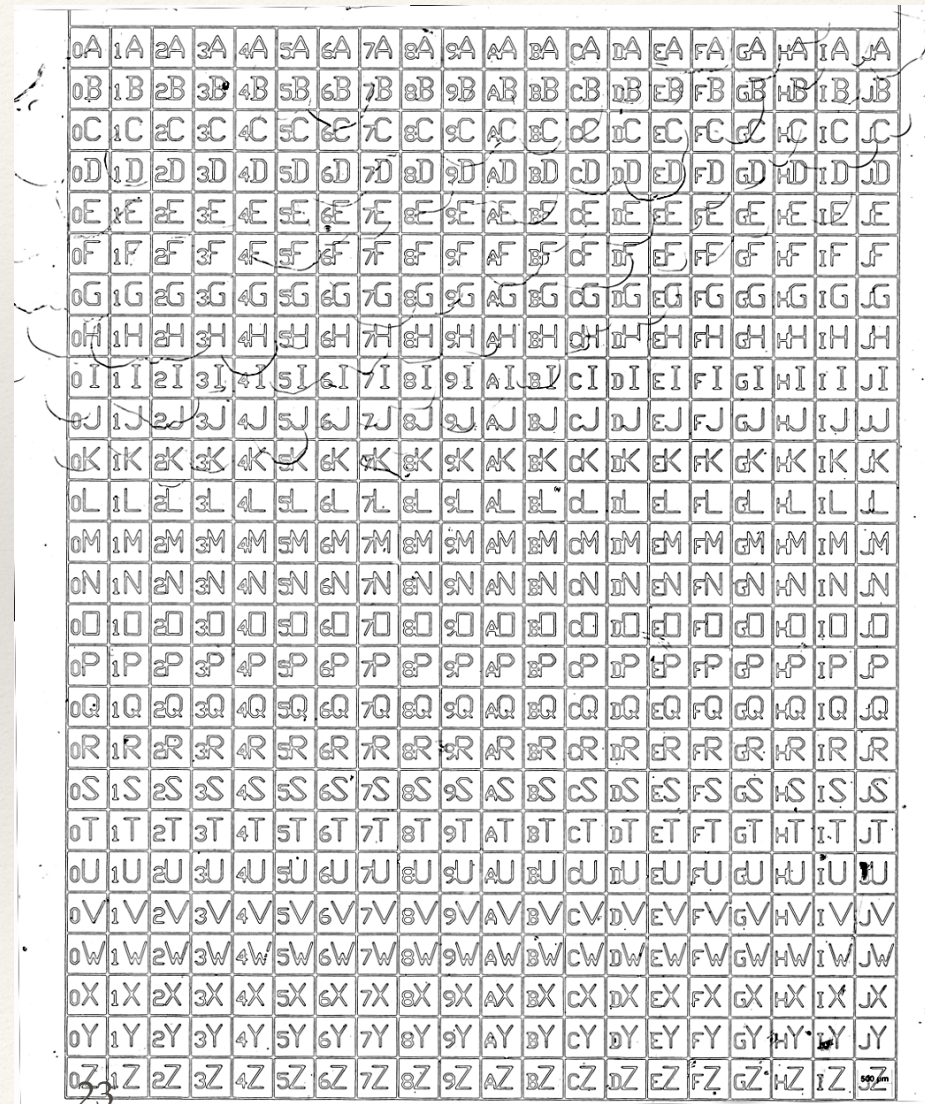
STimulated Emission Depletion



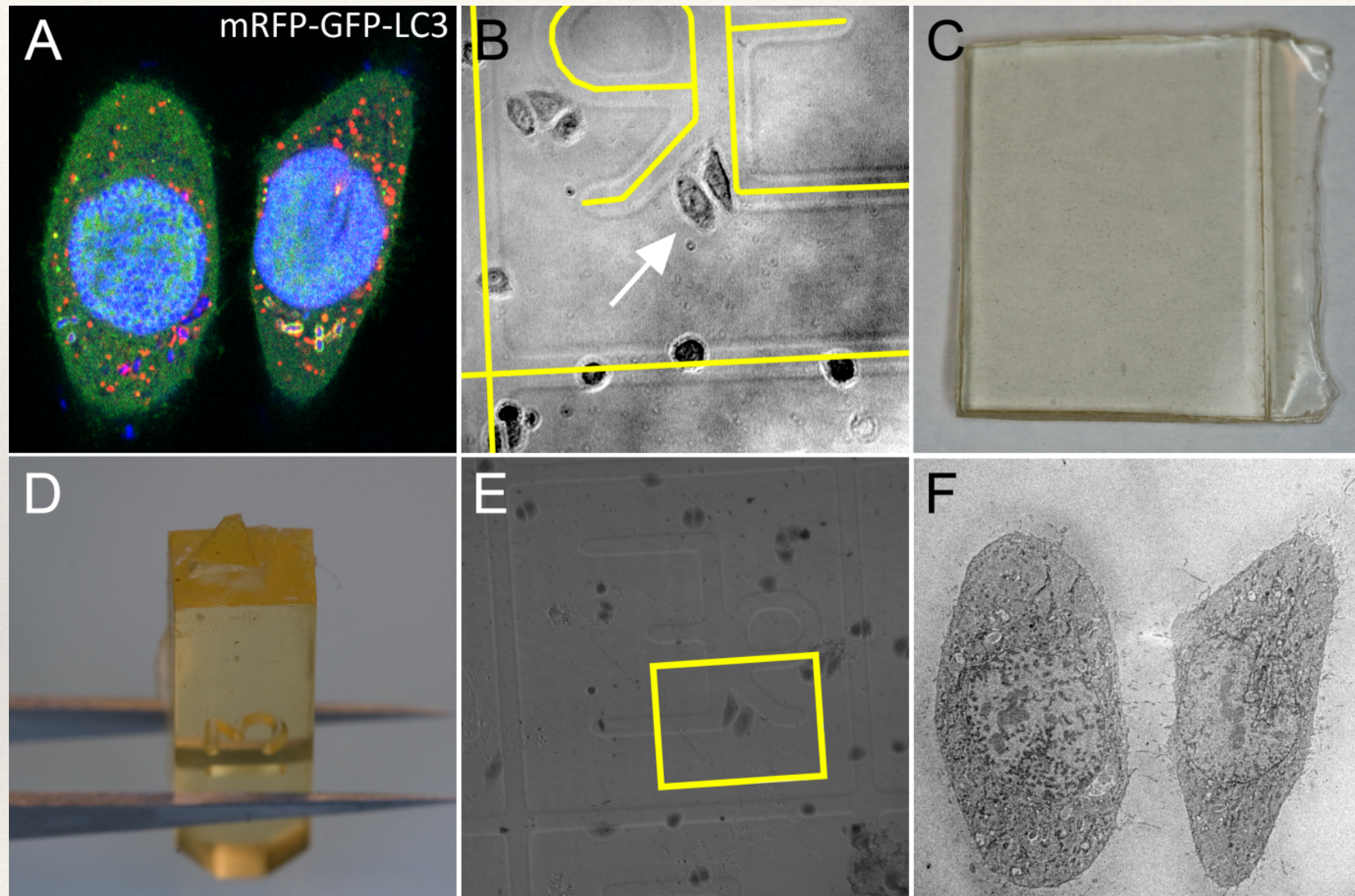
Willig KI, Rizzoli SO, Westphal V, Jahn R, Hell SW.
STED microscopy reveals that synaptotagmin remains clustered after synaptic vesicle exocytosis.
Nature. 2006 Apr 13;440(7086):935-9.

❖ Correlative microscopy principle

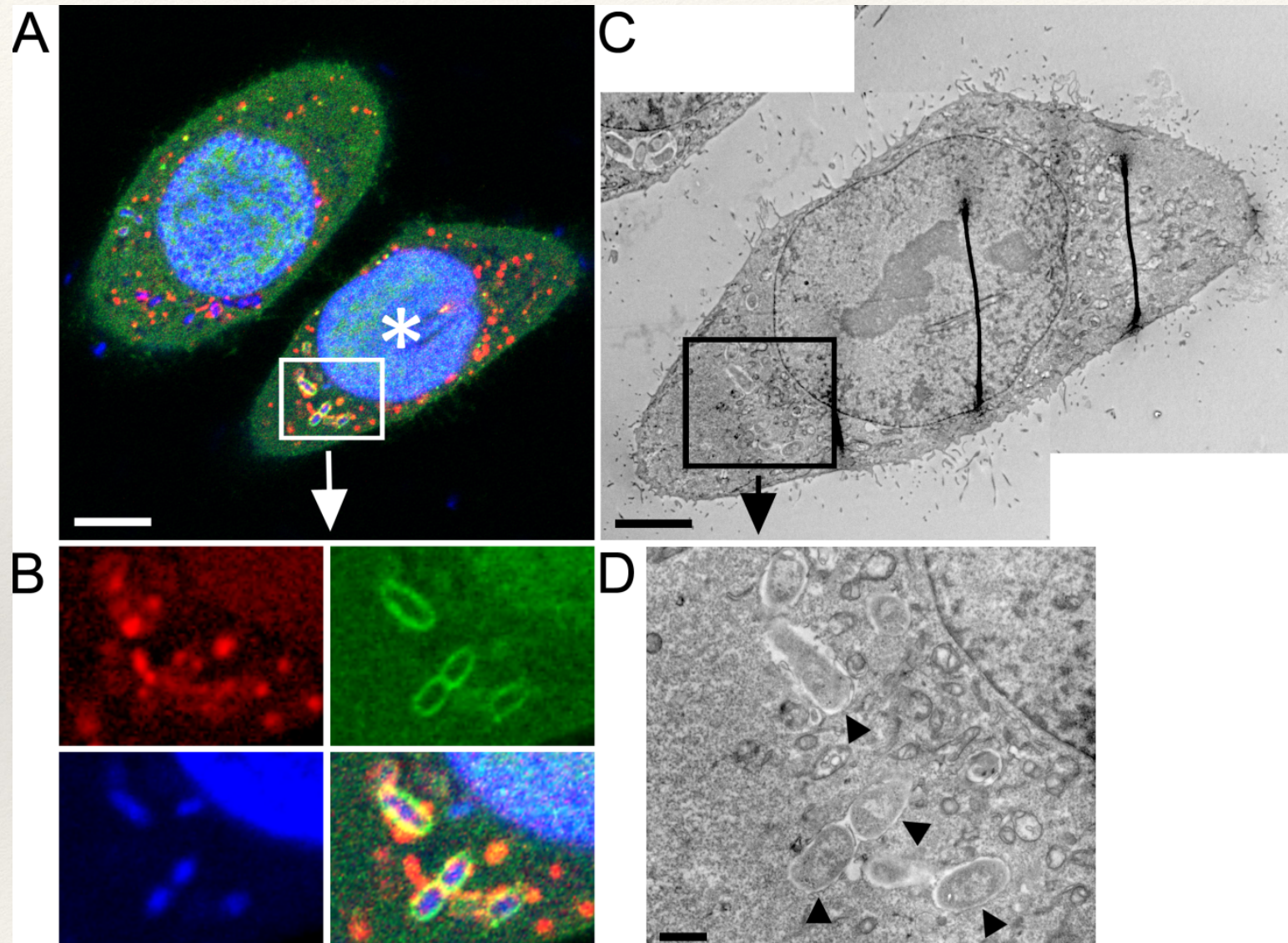
- To find back to zone of interest, a registration system is required
 - Glass coverslip with coordinates engraved at the surface



❖ (super) Correlative microscopy principle

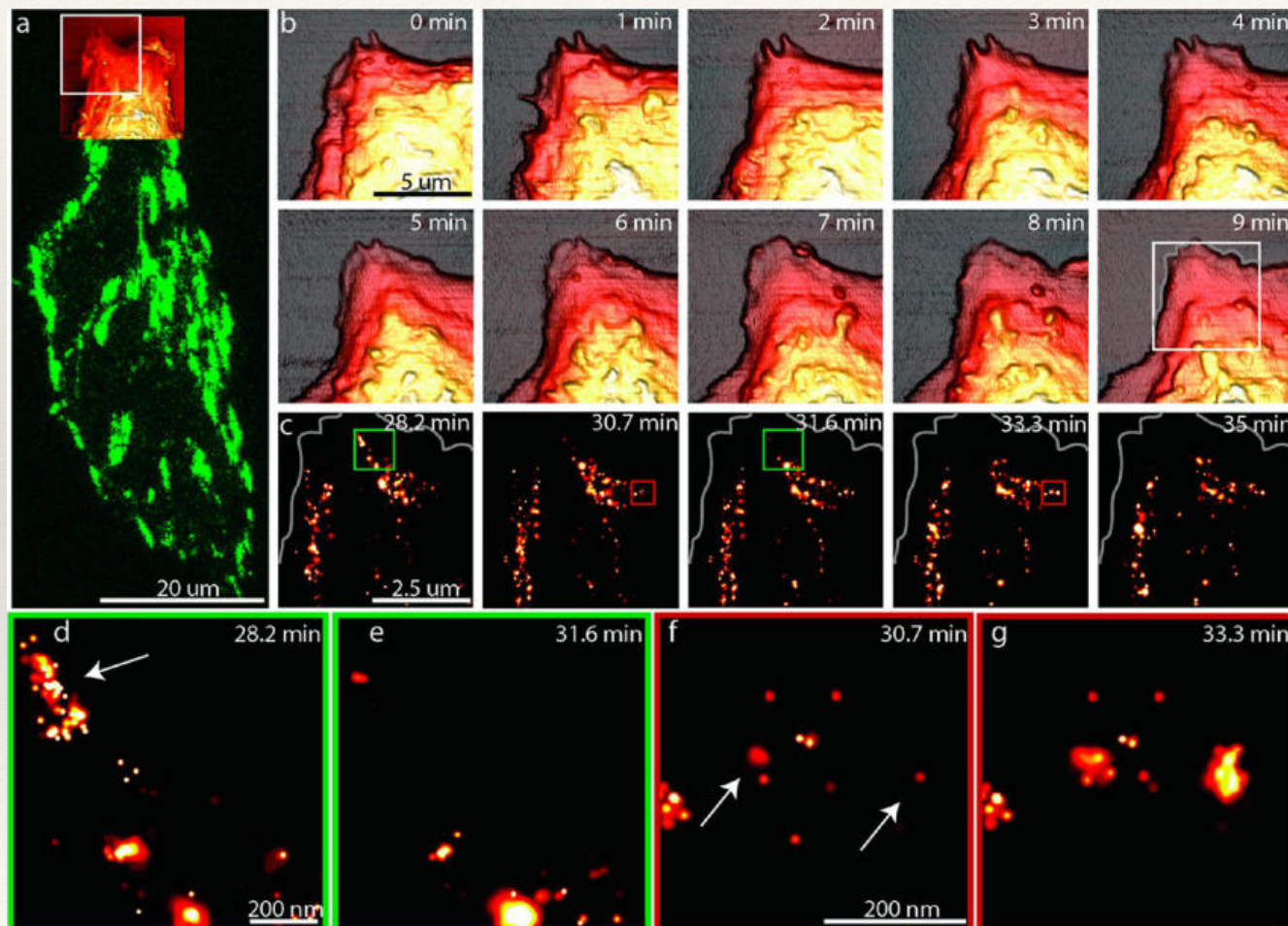


❖ (super) Correlative microscopy principle



❖ Correlative Light Atomic Force microscopy (CLAM)

Photoactivated localisation microscopy (PALM)

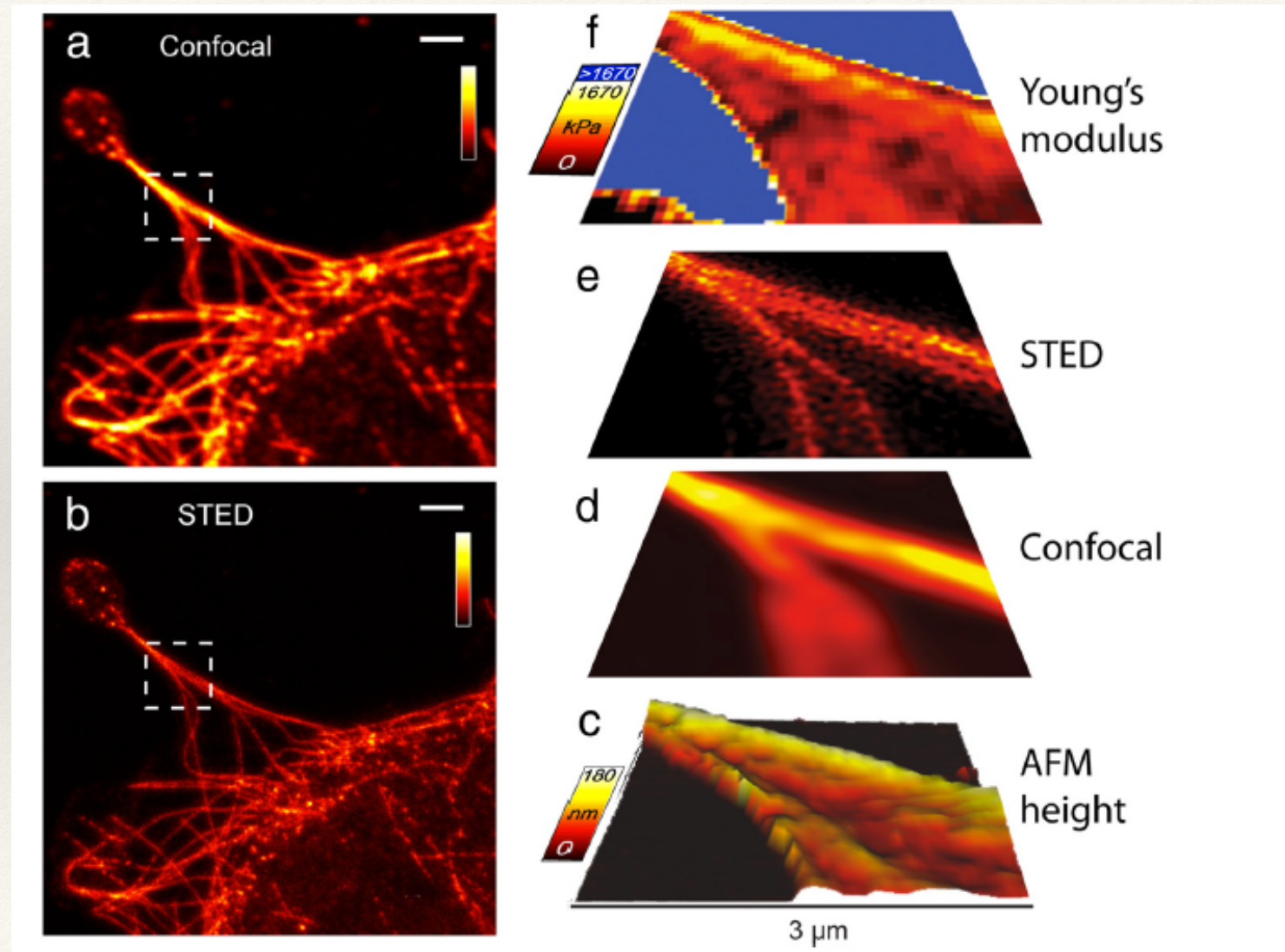


Odematt et al ACS Nano 2015

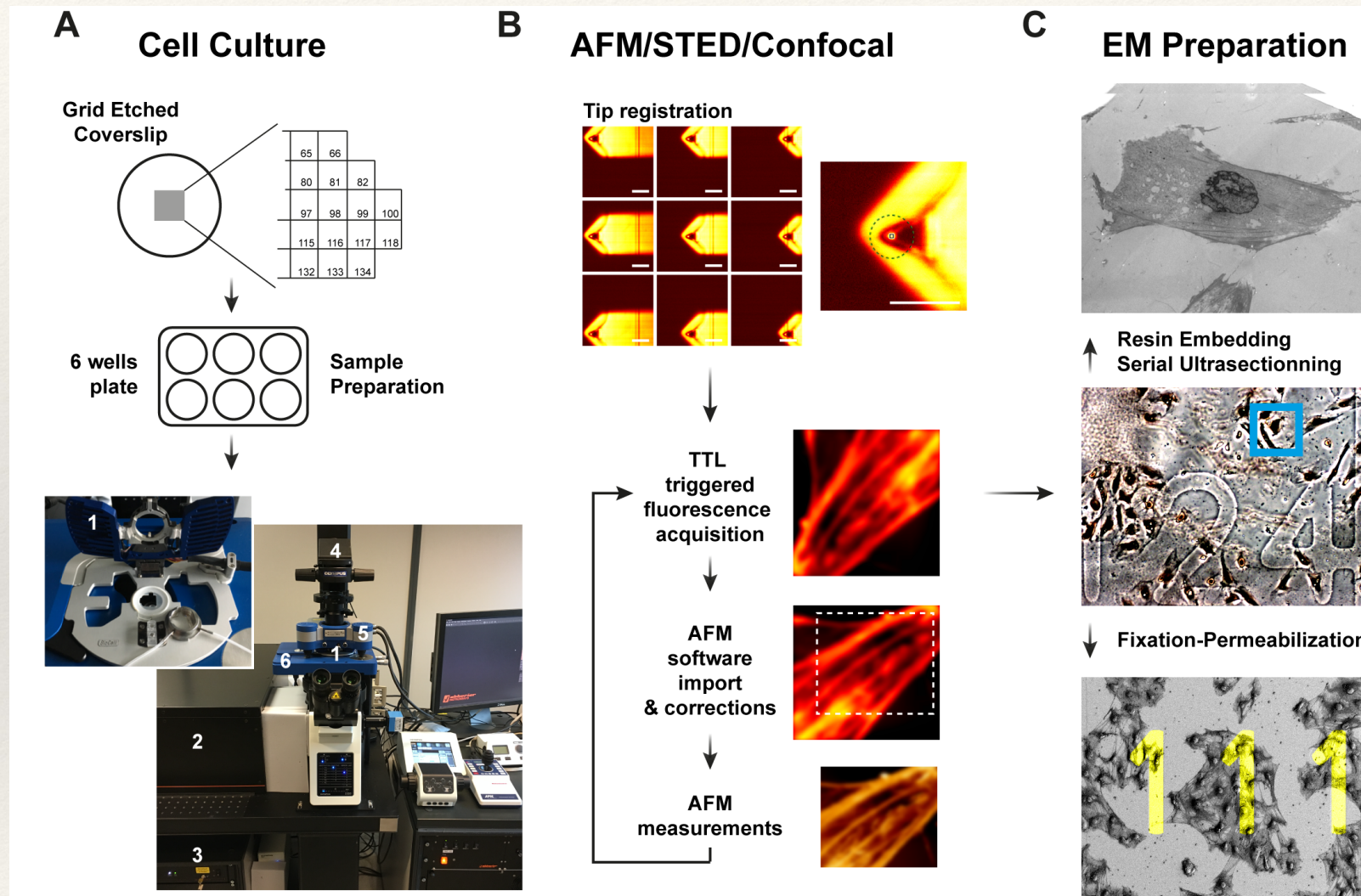
❖ Correlative Light Atomic Force microscopy (CLAM)

Stimulated emission depletion microscopy (STED)

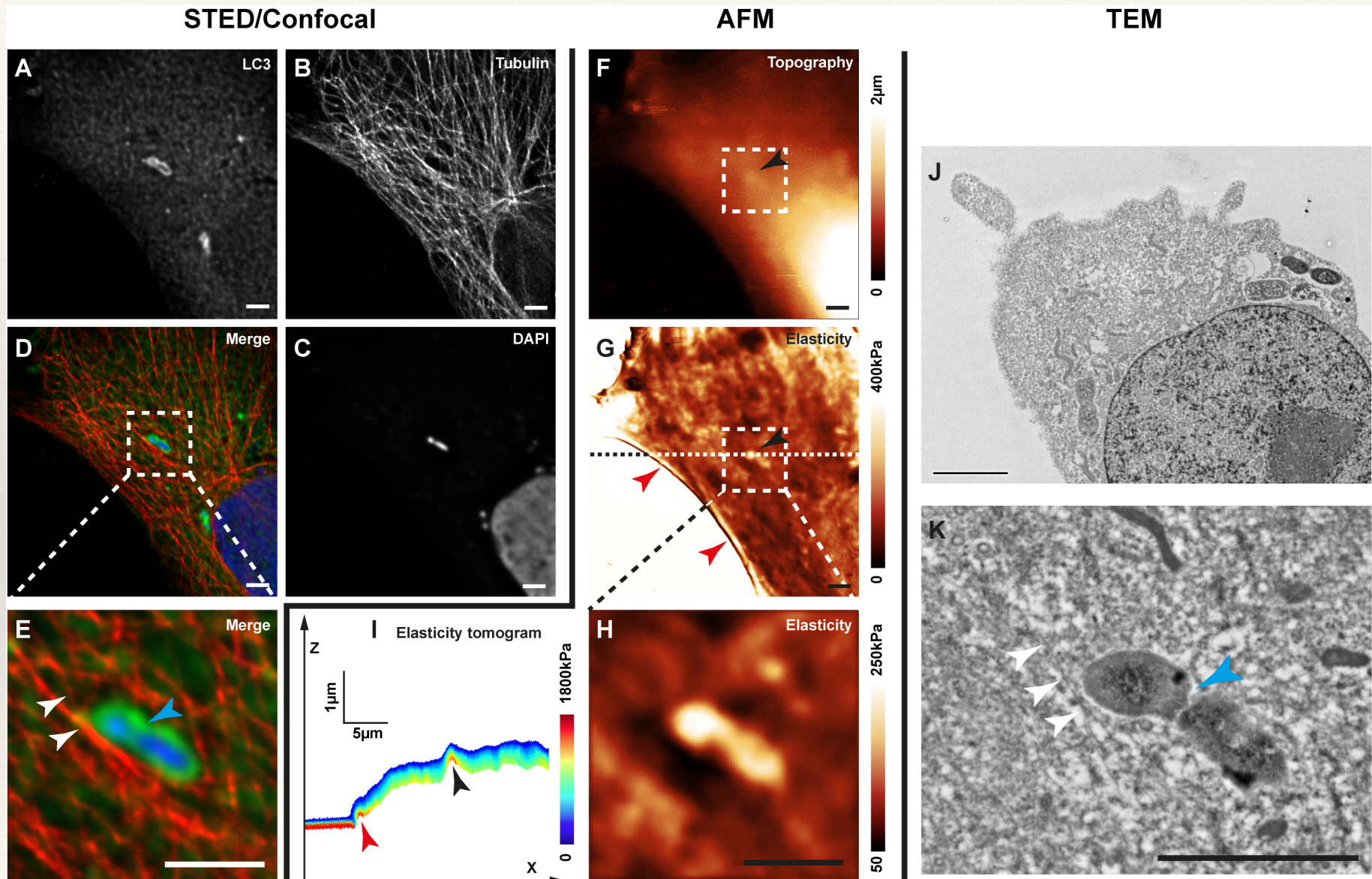
sequential



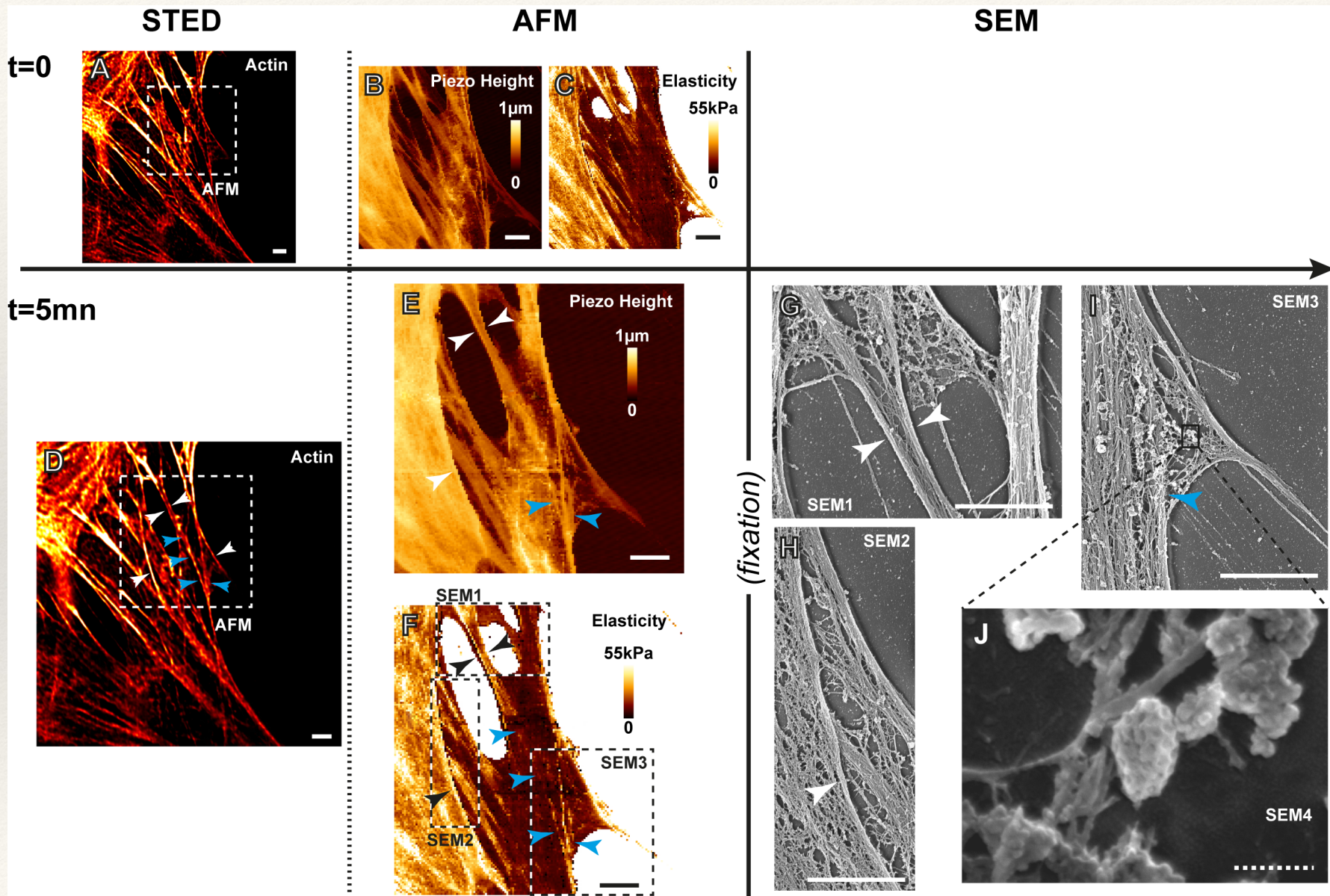
❖ Correlative Light Atomic Force Electron microscopy (CLAFEM)



❖ Correlative Light Atomic Force Electron microscopy (CLAFEM)

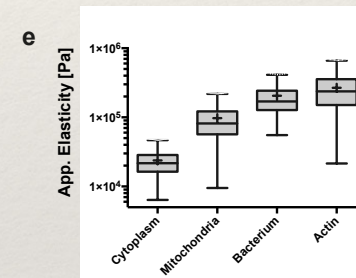
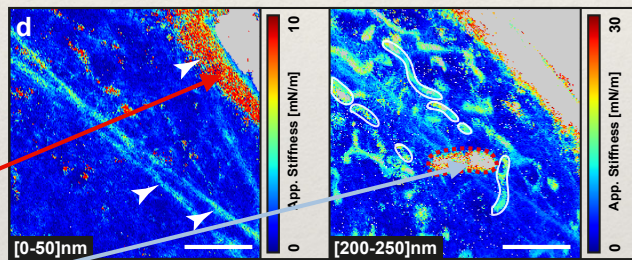
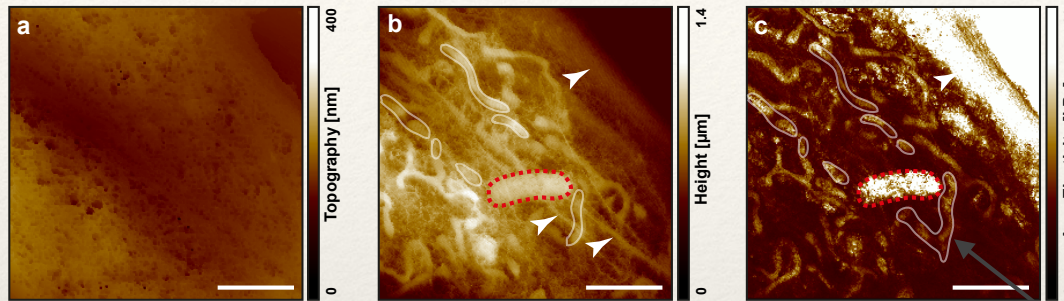


❖ Correlative Light Atomic Force Electron microscopy (CLAFEM)



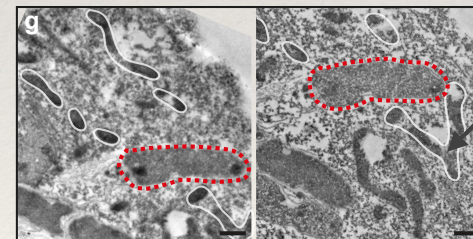
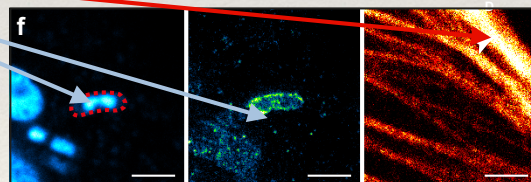
❖ Correlative Light Atomic Force Electron microscopy (CLAFEM)

Fluorescence provides identity of the stiff material analysed by AFM
 EM can give that of the non-fluorescently labeled material



Actin

Bacteria
In
LC3+ compartment

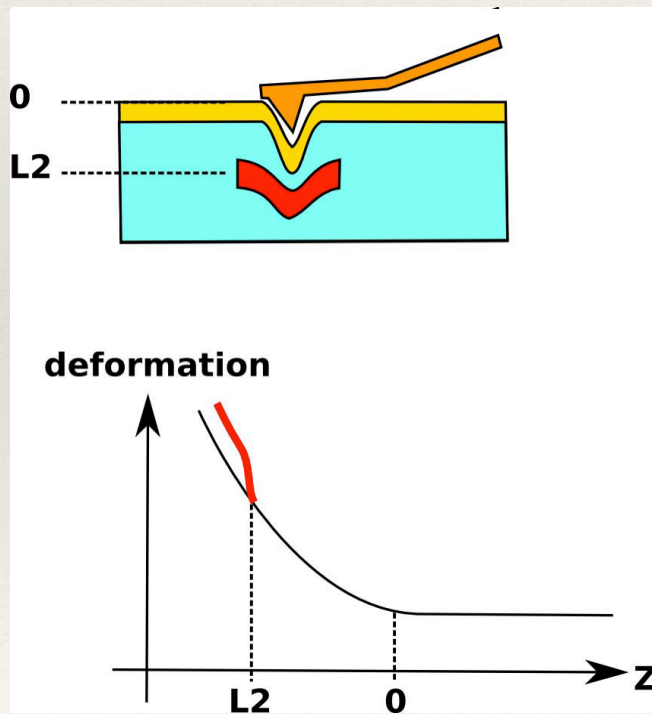


Mitochondria

Examples

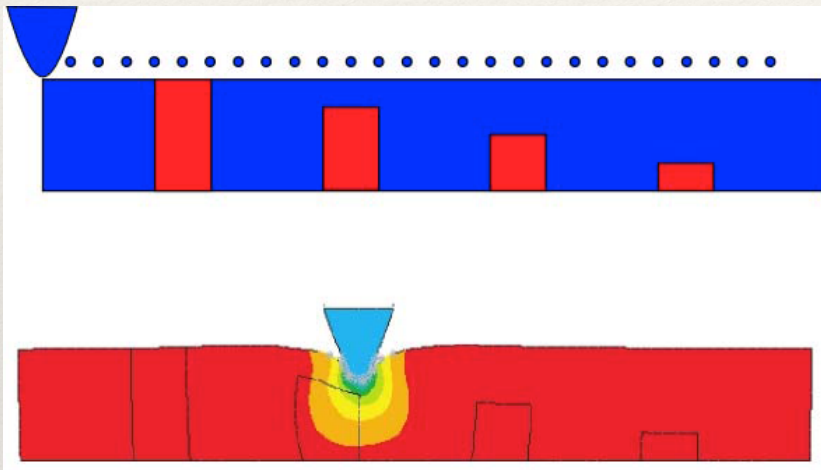
- CLAFEM and Stiffness tomography

Stiffness tomography



- Ideal case
- Fc change due to a soft inclusion
- The deformation on the Fc depends on the position of the inclusion within the cell
- Fc change due to a stiff inclusion
- The deformation on the Fc depends on the position of the inclusion within the cell

Stiffness tomography



- **Finite element method**

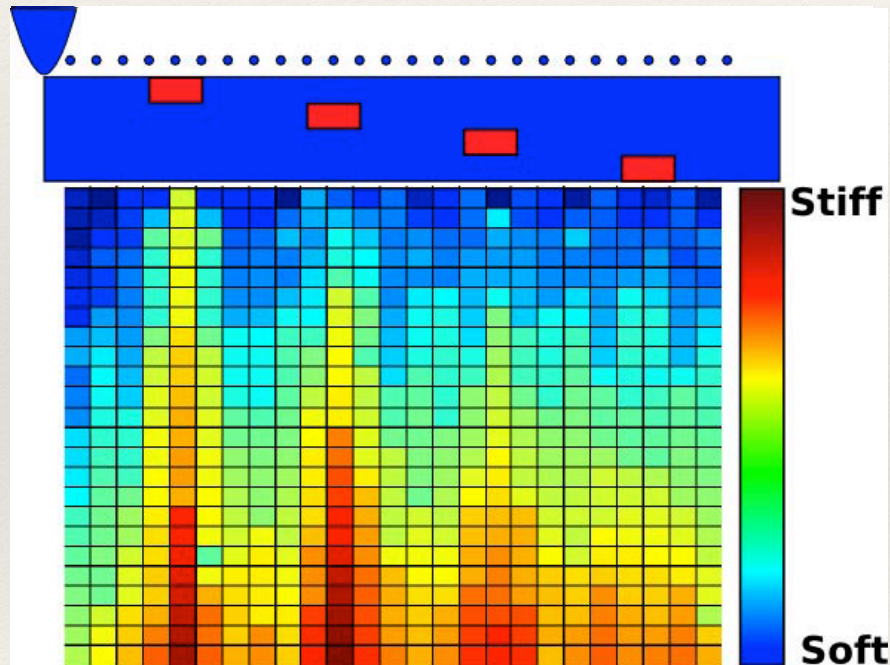
- **Homogenous sample with inclusion**

- **Finite elements simulate the tip indentation**

- **Cantilever deformation simulated with a spring**

Roduit et al., Biophys J 2008

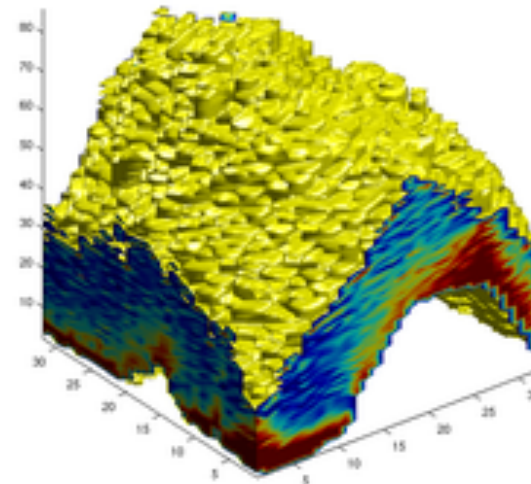
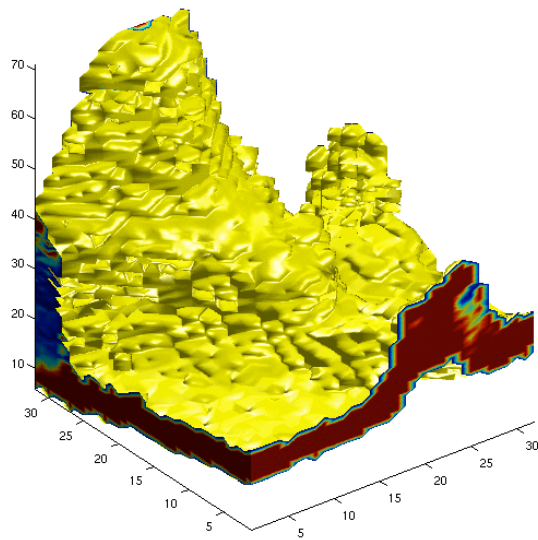
Stiffness tomography



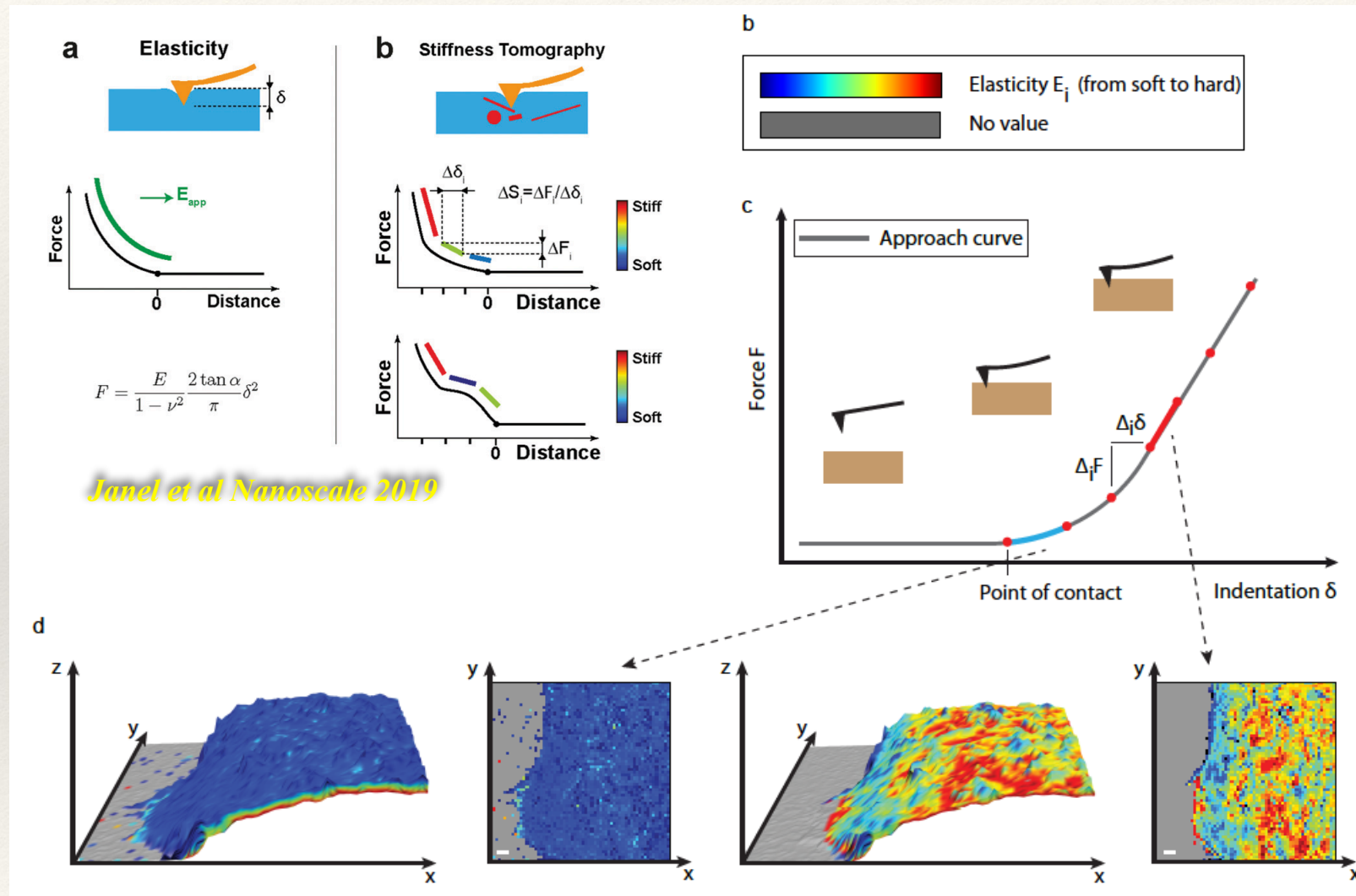
- **Columns hindered within the sample are detected**
- **Platforms are less contrasted**
- **Stiffer platforms (x10) are detected**
- **Thicker platforms are detected**

Stiffness tomography

- Stiffness tomography of a living cell
- High quality Fc are required
- **Contrasts are detected within the sample**



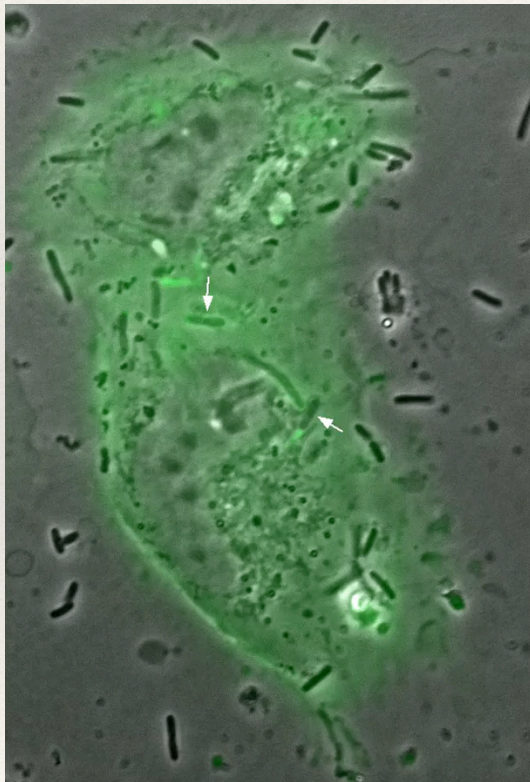
- CLAFEM and Stiffness tomography



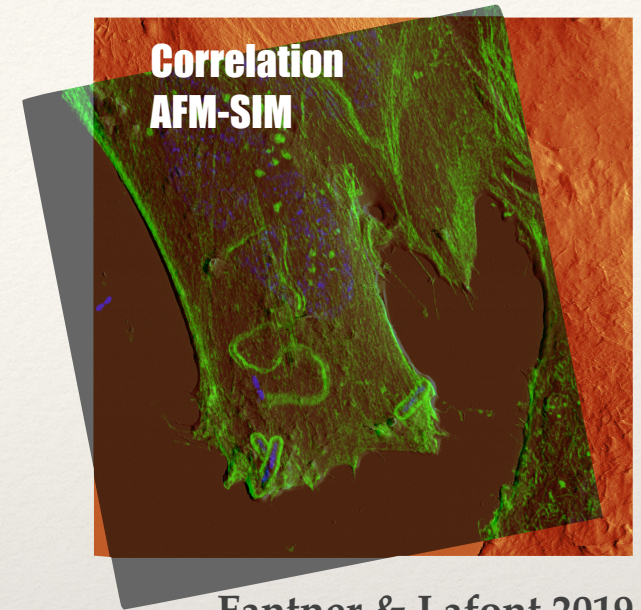
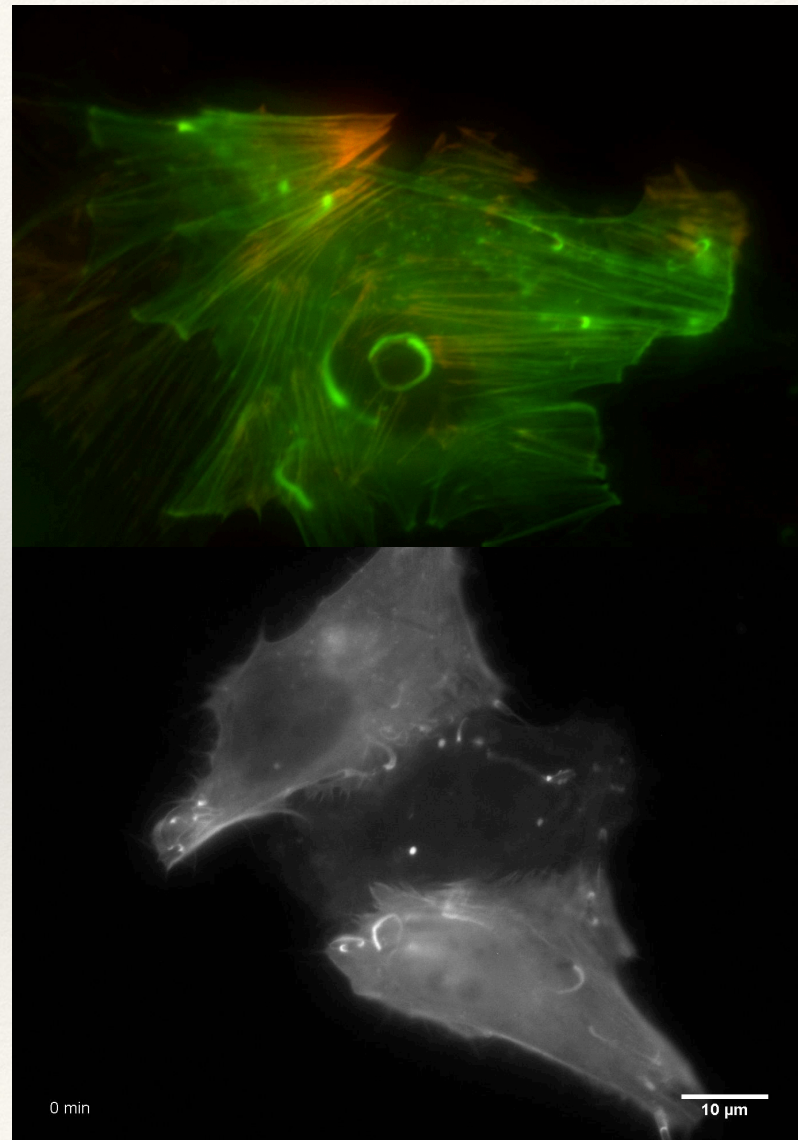
Janel et al Nanoscale 2019

- CLAFEM and Stiffness tomography

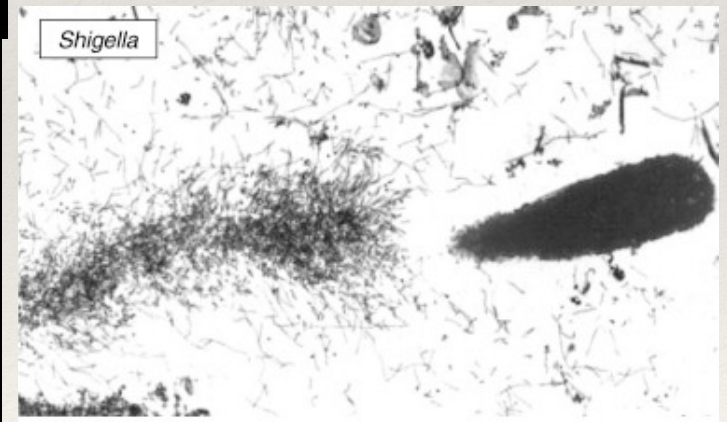
Correlative microscopy: Actin tail comets



CMPI movies

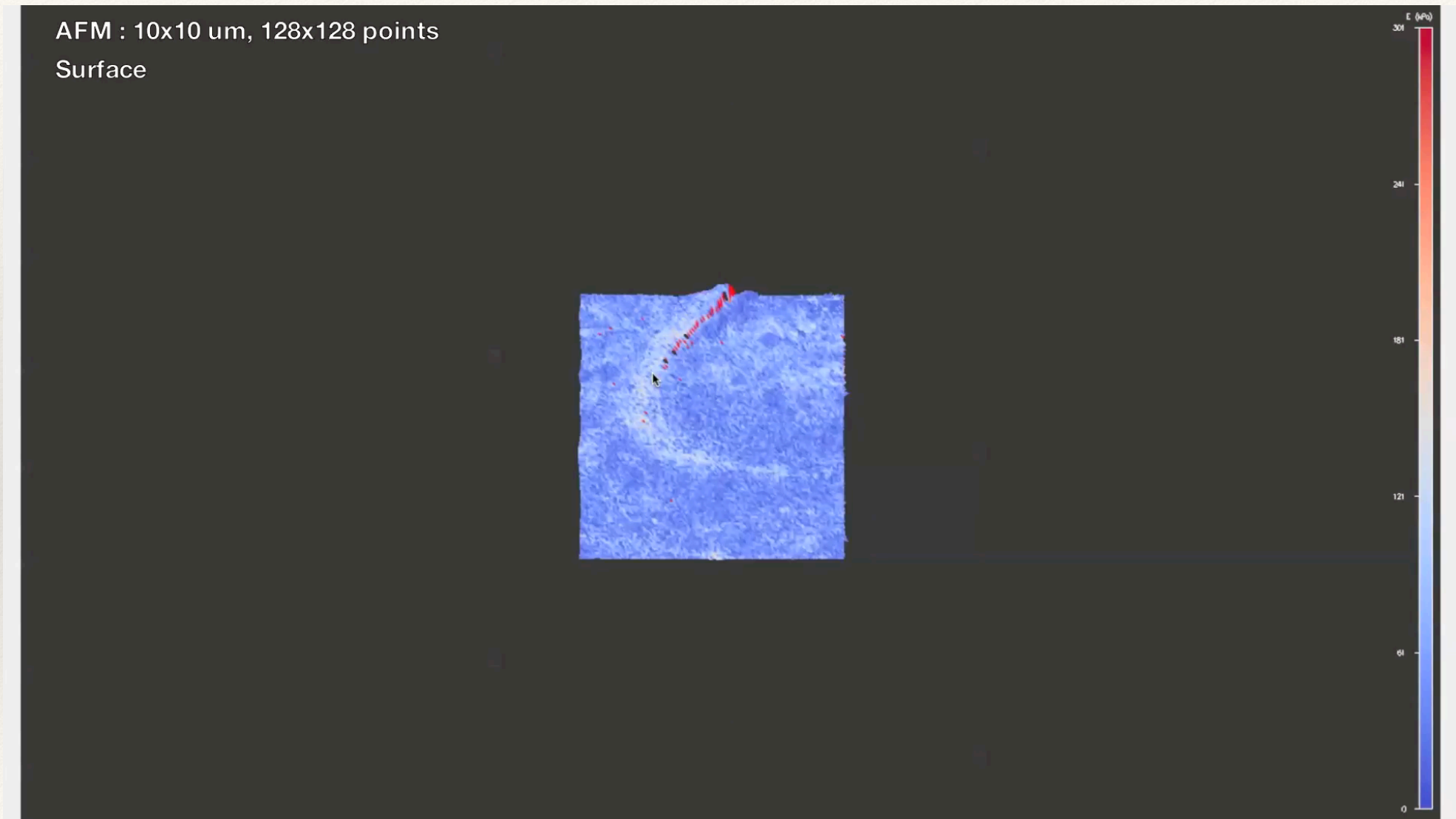


Fantner & Lafont 2019



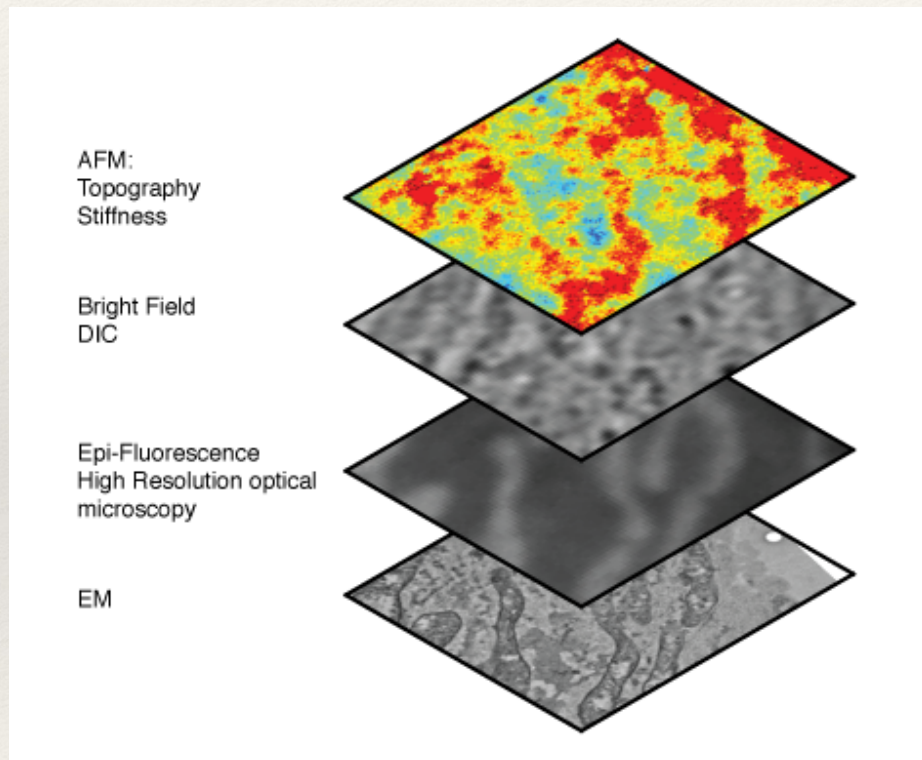
Gouin et al JCSi 1999

- CLAFEM and Stiffness tomography



- CLAFEM and Stiffness tomography

using CLAFEM and stiffness tomography
to « sense » stressed organelles



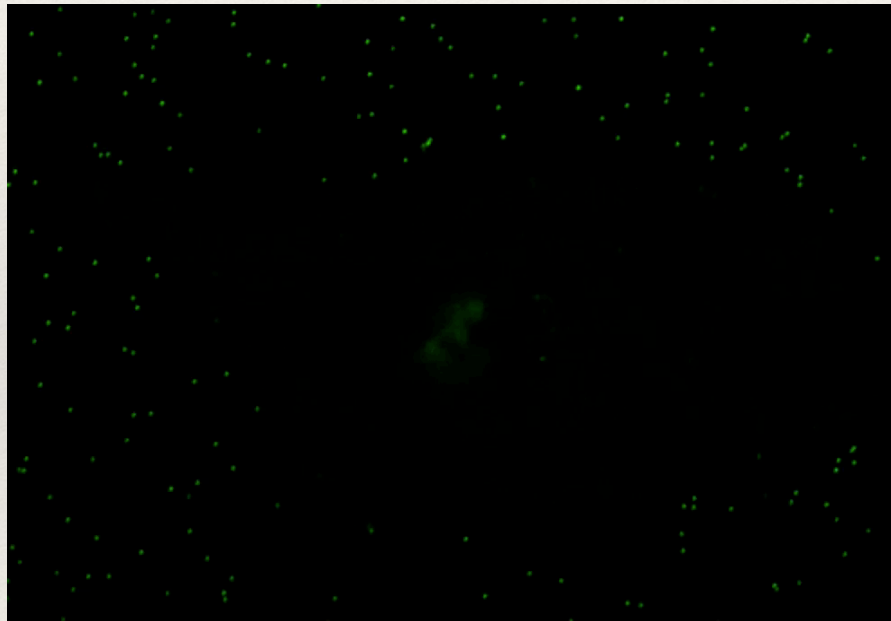
Fantner & Lafont 2019

- CLAFEM and Stiffness tomography

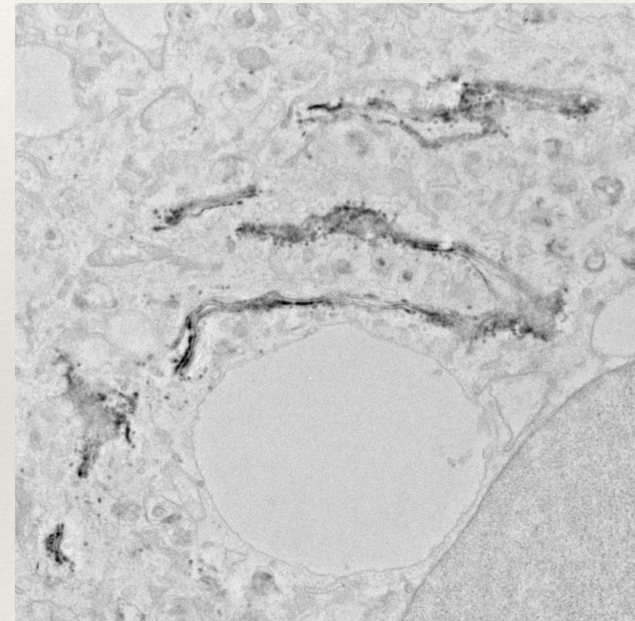
Cross linked Golgi Apparatus

Golgi Apparatus : modifies, packages and sorts the proteins before their distribution to the cell.

Stable HeLa cell line ManII – HRP



DAB : 3,3' Diaminobenzidine
HRP : Horseradish peroxidase



DAB + H₂O₂ + HRP

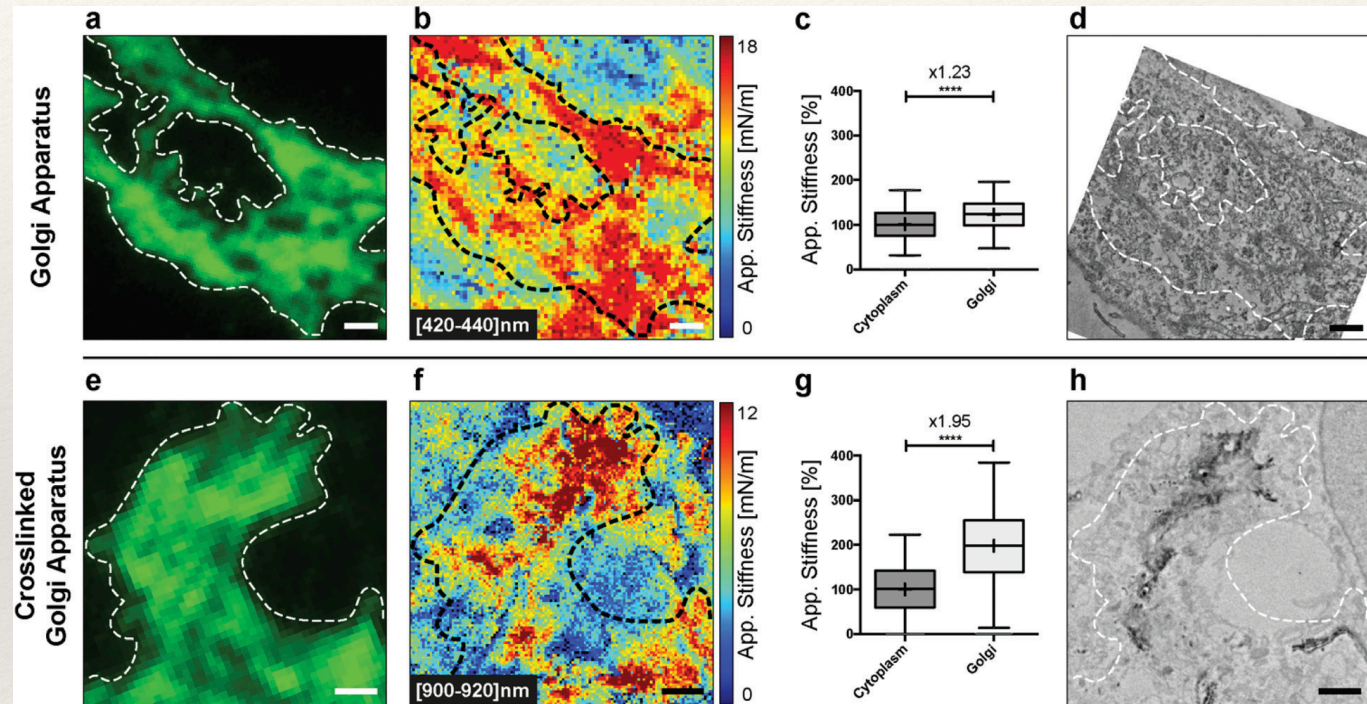


H₂O + DAB (precipitate)

Analysis of De Novo Golgi Complex Formation after Enzyme-based Inactivation

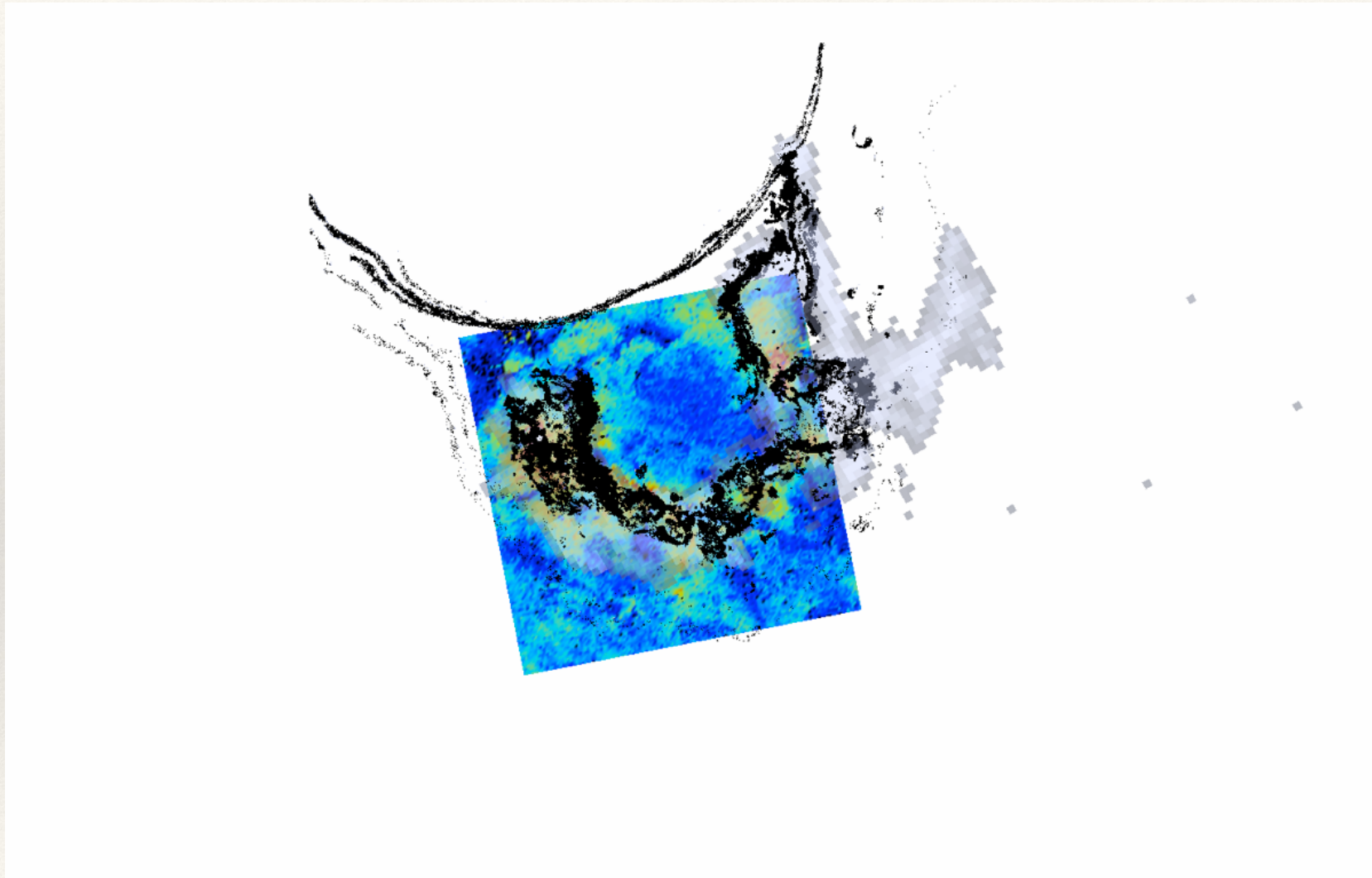
Florence Jollivet, Gracia Raposo, Ariane Dimitrov, Rachid Sougrat, Bruno Goud, and Franck Perez
Molecular Biology of the Cell, Vol. 18, 4637–4647, November 2007

- CLAFEM and Stiffness tomography



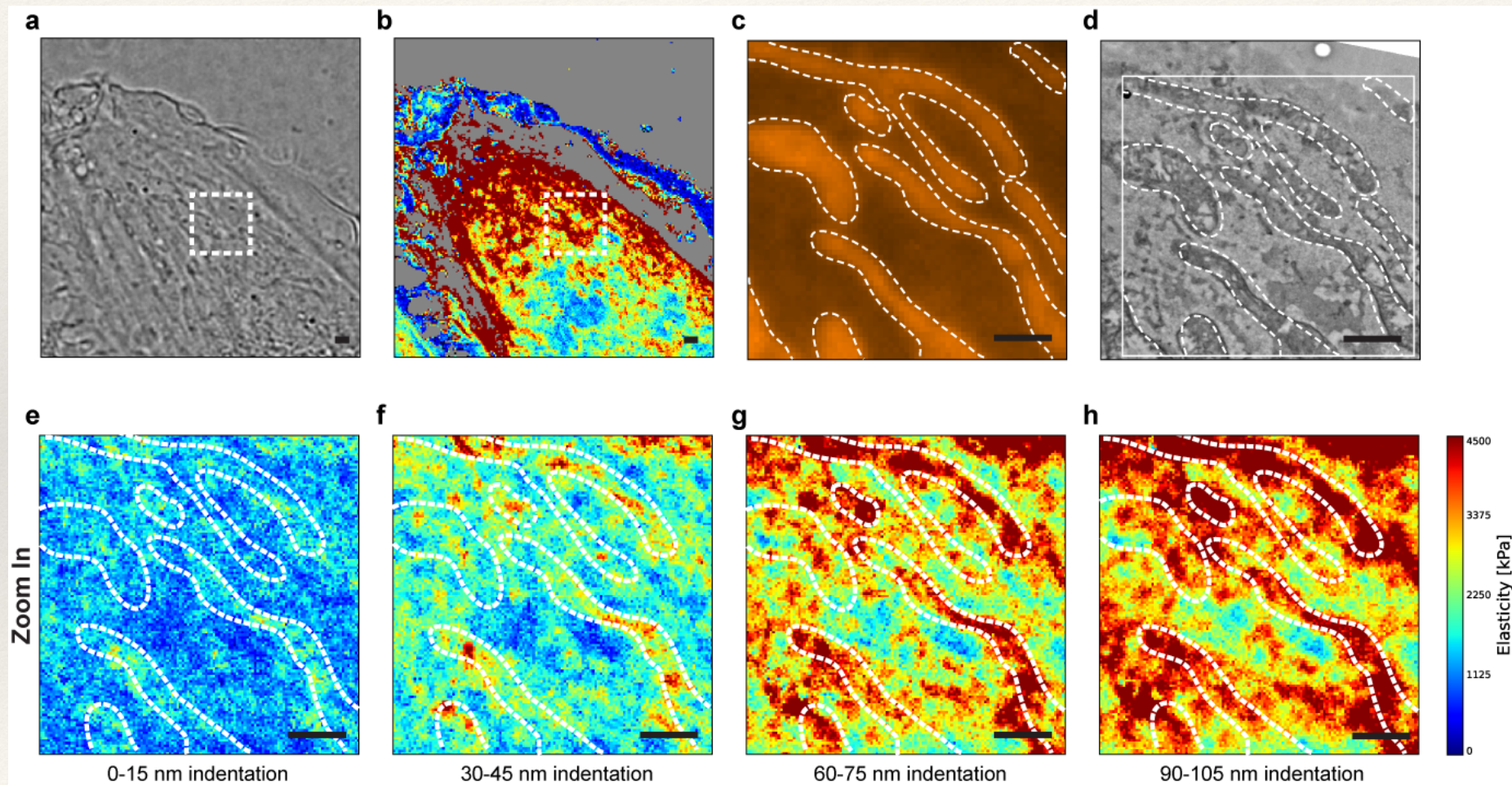
Janel et al. Nanoscale 2019

- CLAFEM and Stiffness tomography



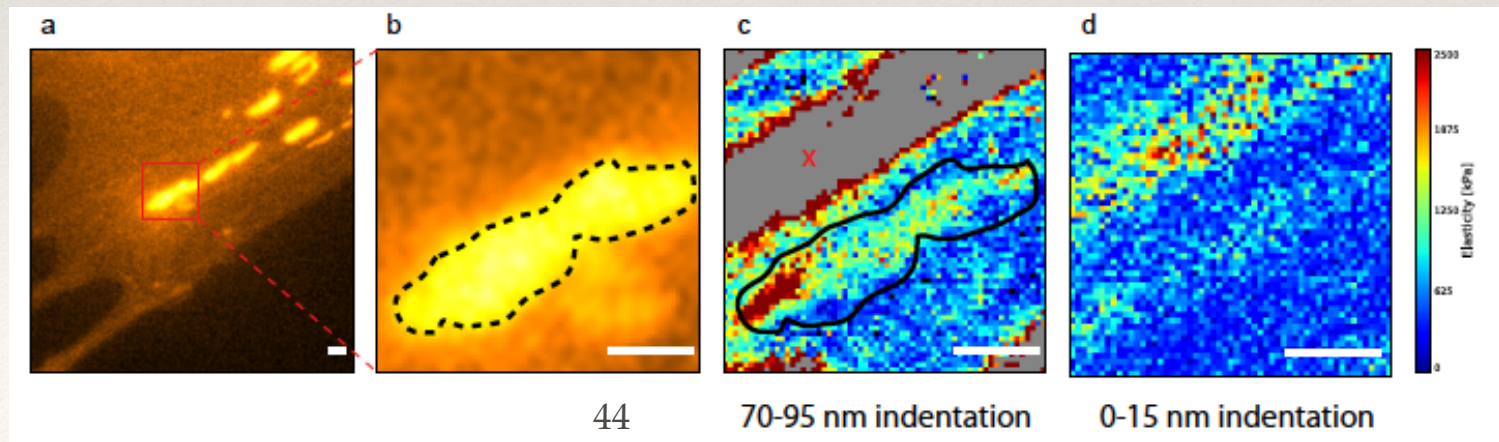
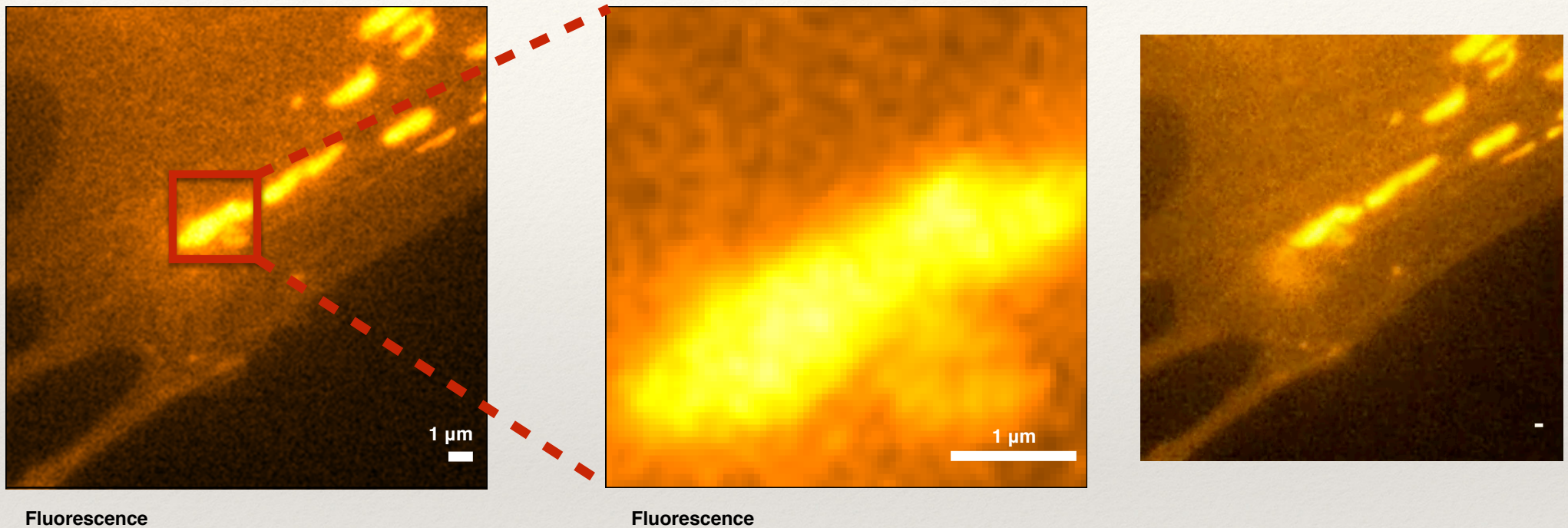
- CLAFEM and Stiffness tomography

Correlative microscopy in fixed cells: mitochondria



- CLAFEM and Stiffness tomography

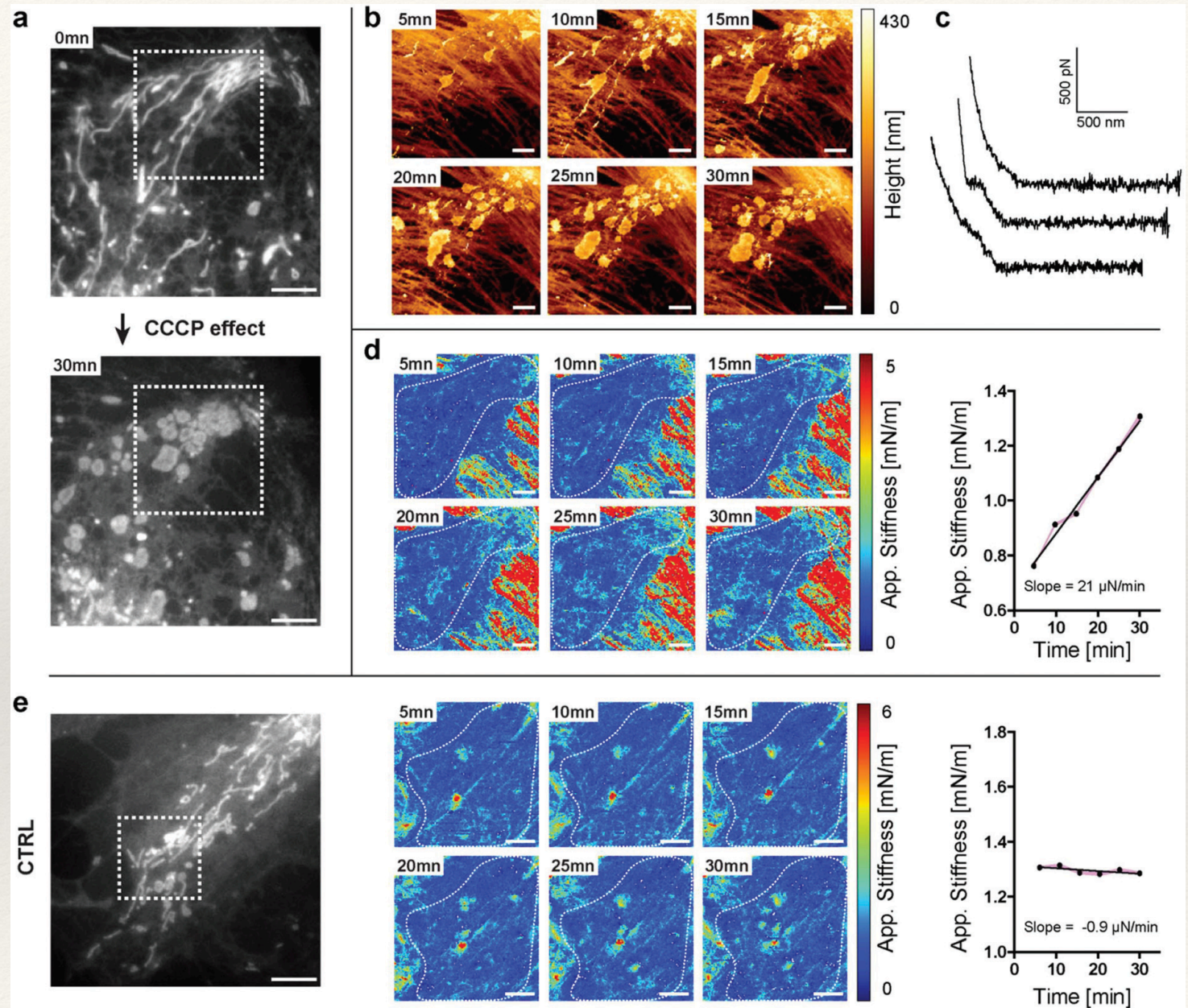
Mitochondria in living cells



INTEREST

Effects of drugs, cytoskeleton, diet, lipid loading
on organelles biophysical features

Comparing intracellular membrane compartments in
healthy cells vs. cells from patients suffering from
diabetes, lysosomal storage diseases...



Future

- Towards automation from the sample perspective
- Towards automation from the analysis perspective

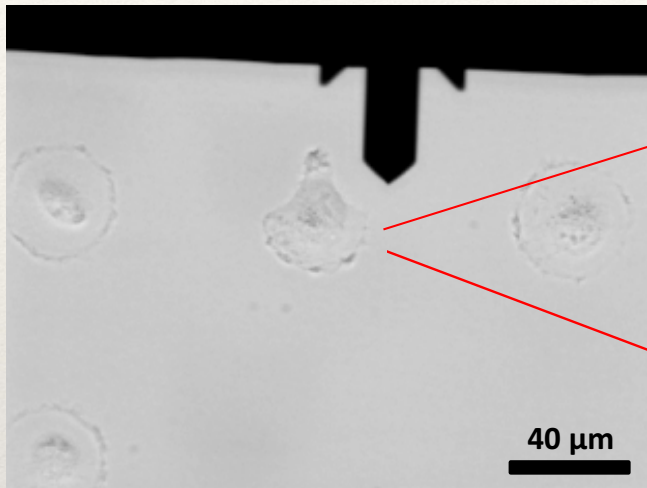
Grey : tubulin

Red : vimentin

Green : actin

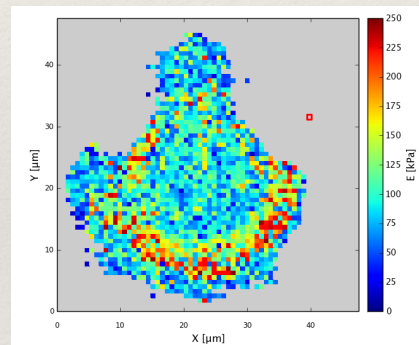
Blue : nuclei

Future

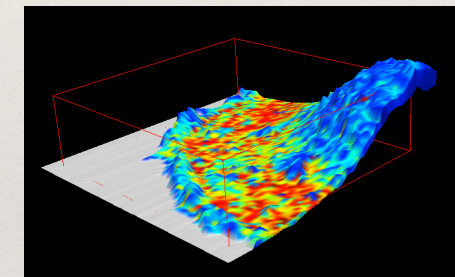
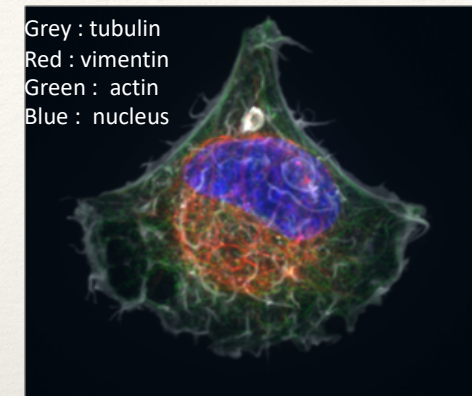


RPE 1 cells

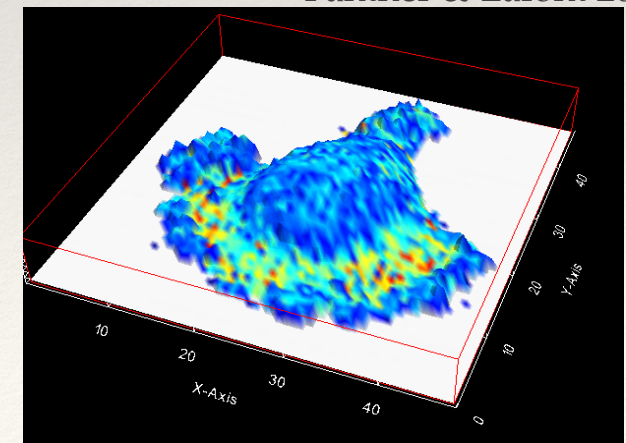
E (kPa)
Sneddon model



Elasticity map



Fantner & Lafont 2018



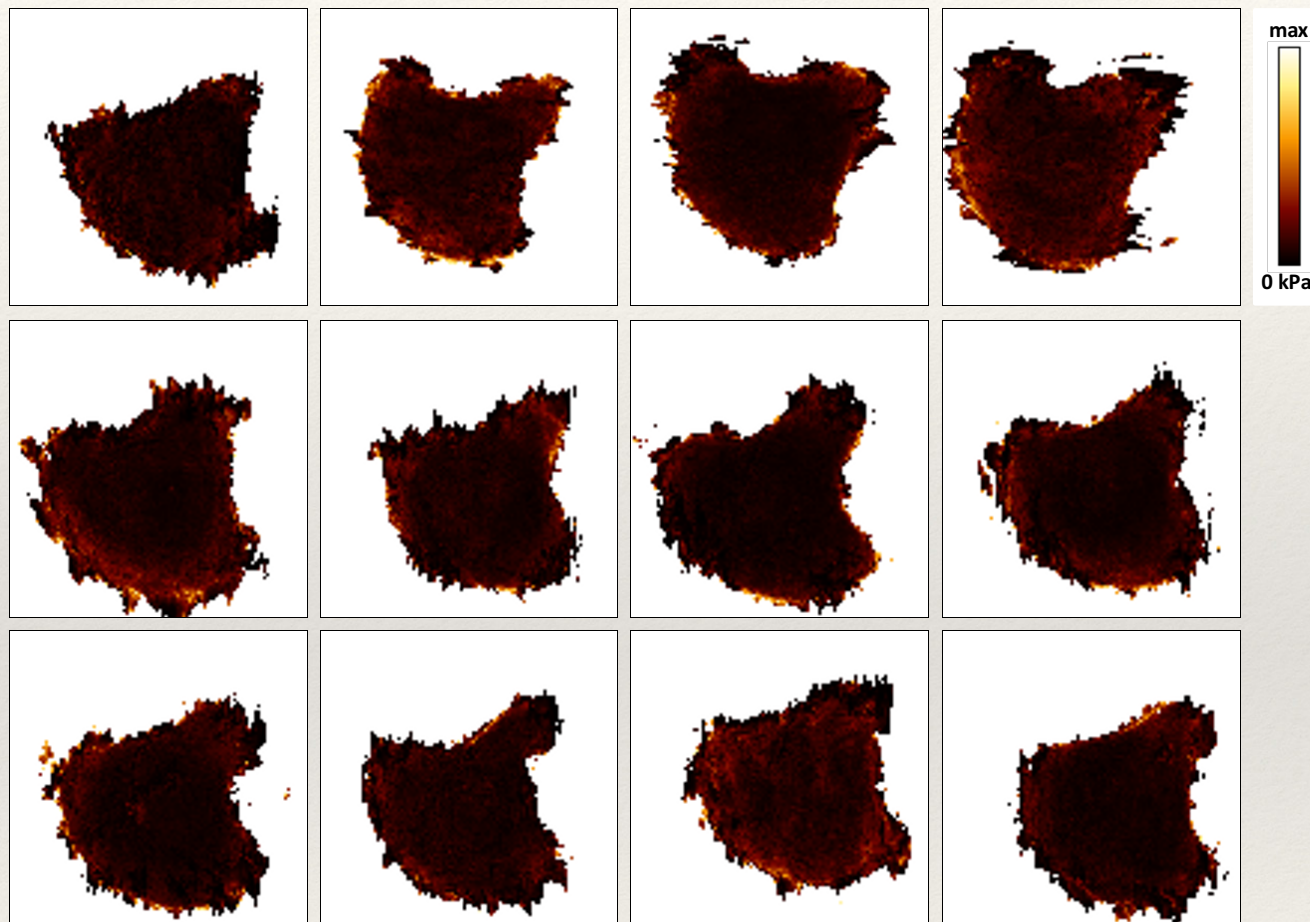
Quantitative imaging mode: 100 x 100 px (50m²)

Tips: biolever mini, PFQNM-LC , threshold 0.5nN

Treatment and analyse using in-house pyA software (Sneddon model)

- Automation

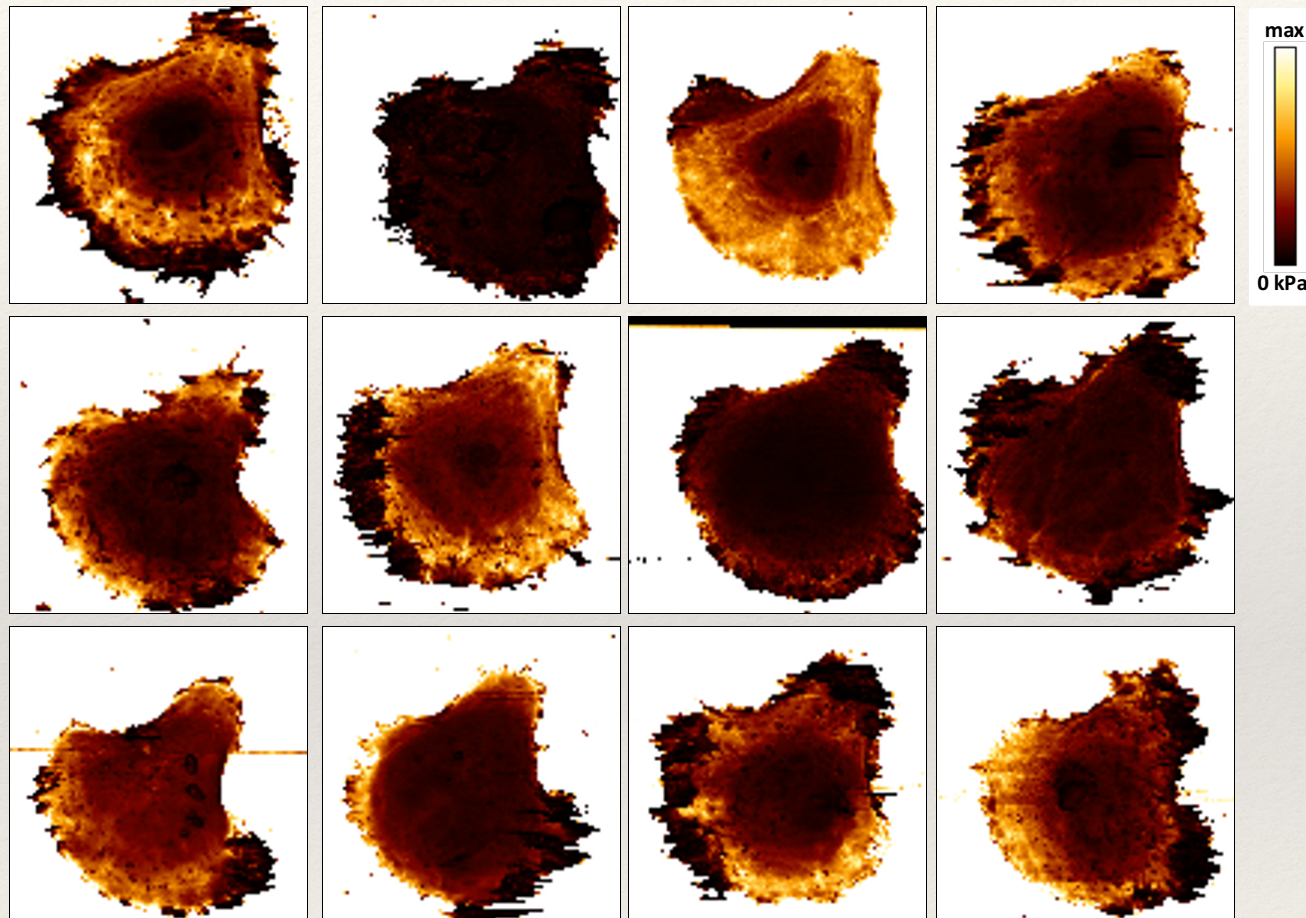
Without rapamycin



Dupres et al. in preparation

- Automation

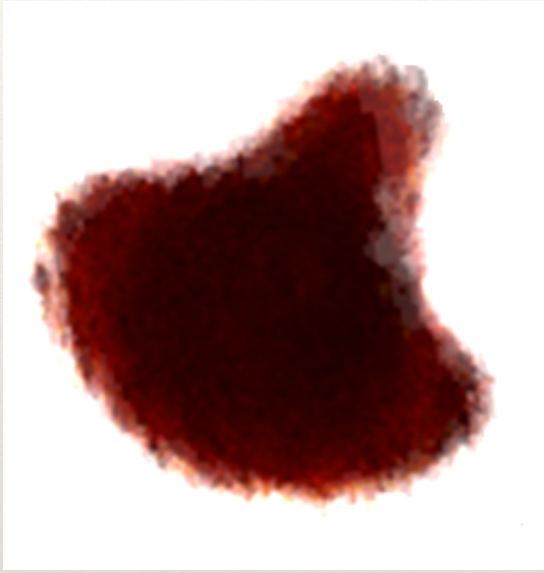
With rapamycin



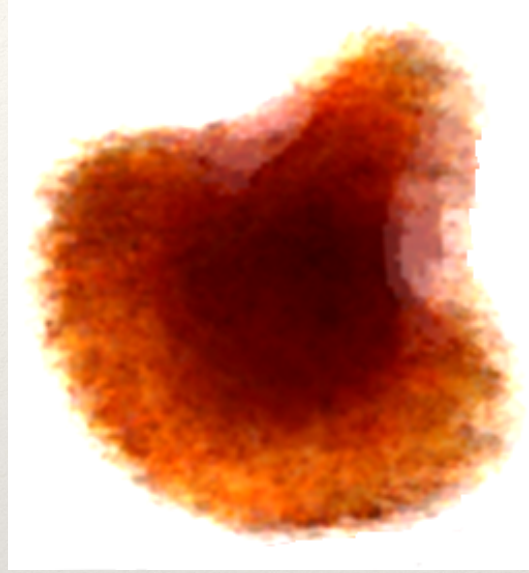
Dupres et al. in preparation

- Automation

Average elasticity maps

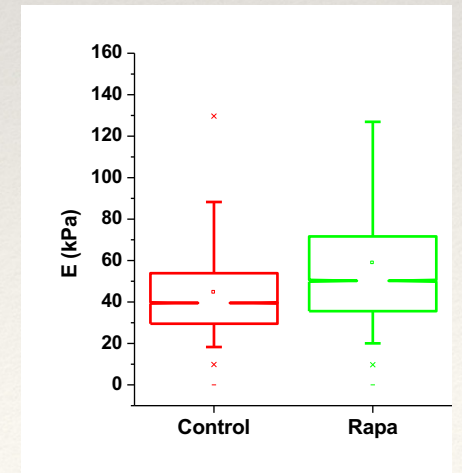
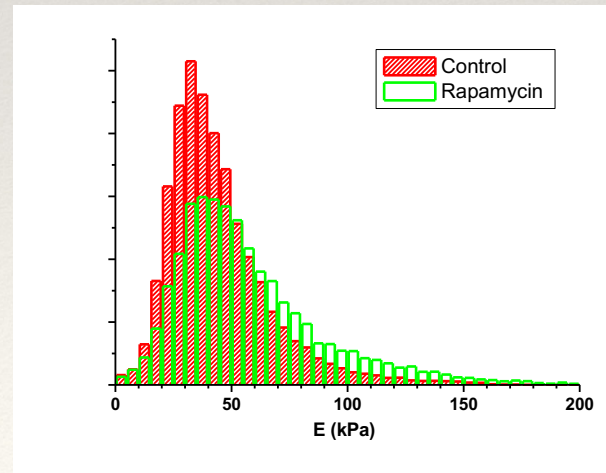


- rapa



+ rapa

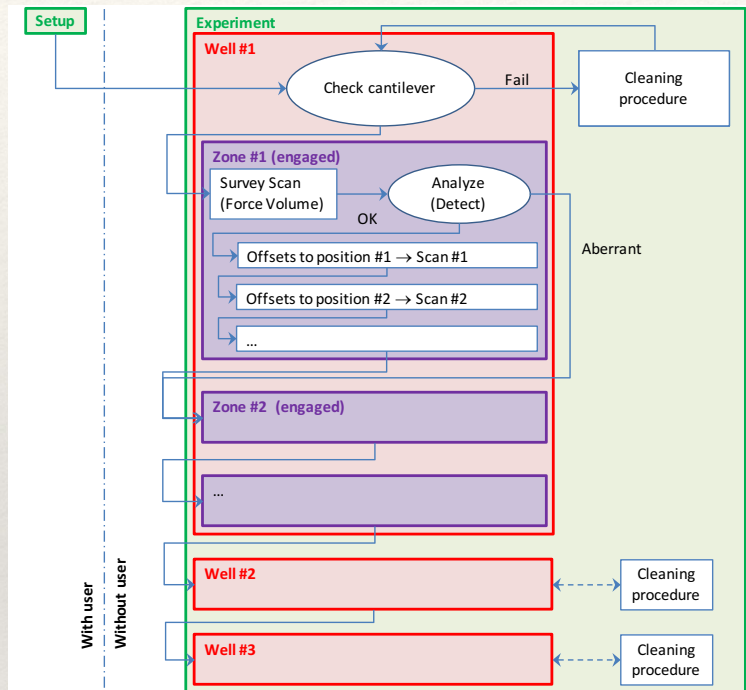
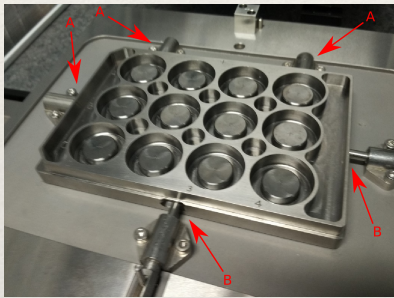
Elastic properties



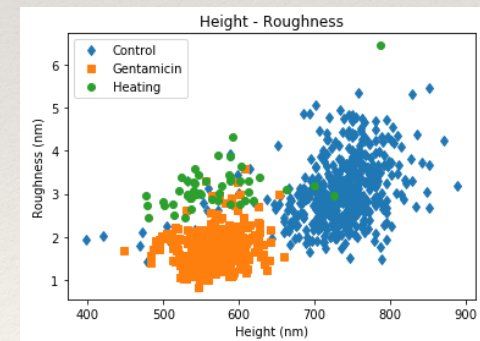
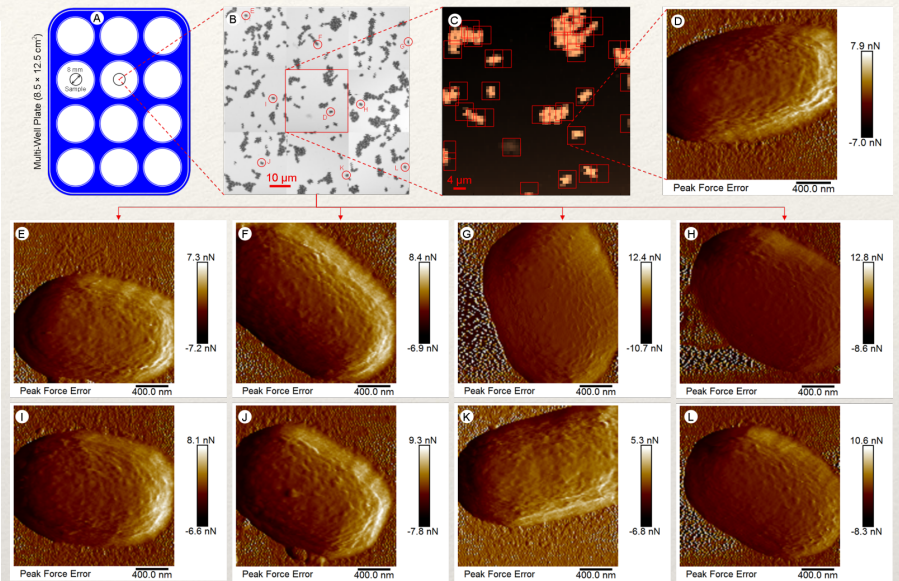
Dupres et al. in preparation

- Automation

Controlled physical constraint & cell responses



Dujardin et al submitted

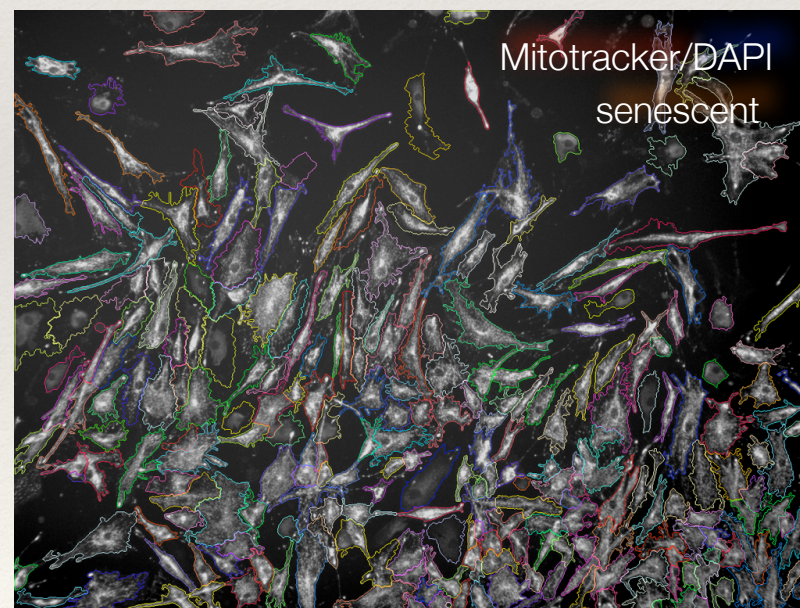
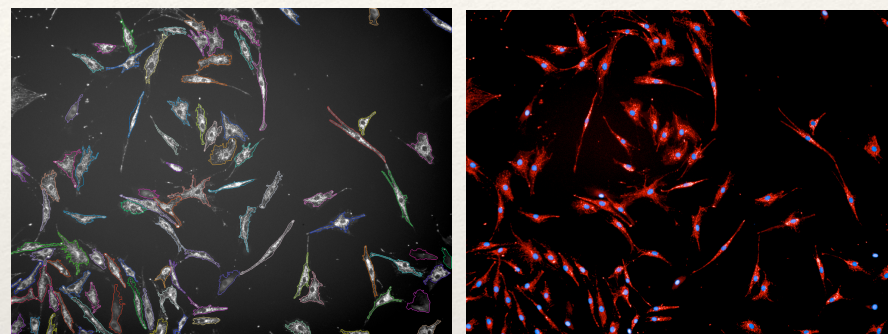
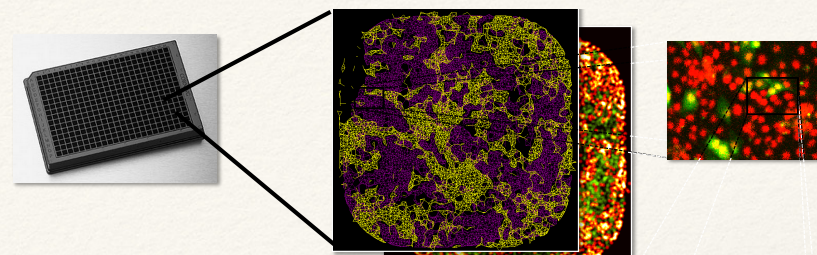


Future

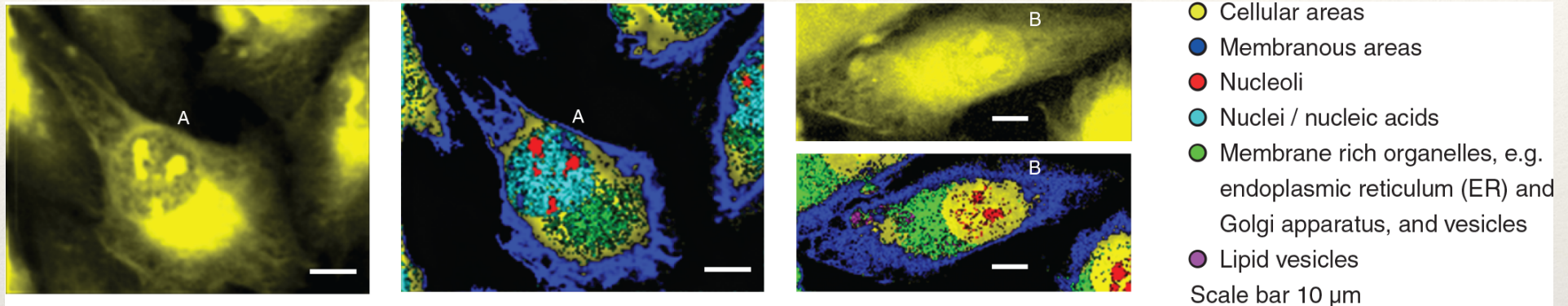
Grey : tubulin
Red : vimentin
Green : actin
Blue : nuclei

- Towards automation from the sample perspective
- Towards automation from the analysis perspective
- Development of other correlative approaches relevant for BioMed applications

- Automation

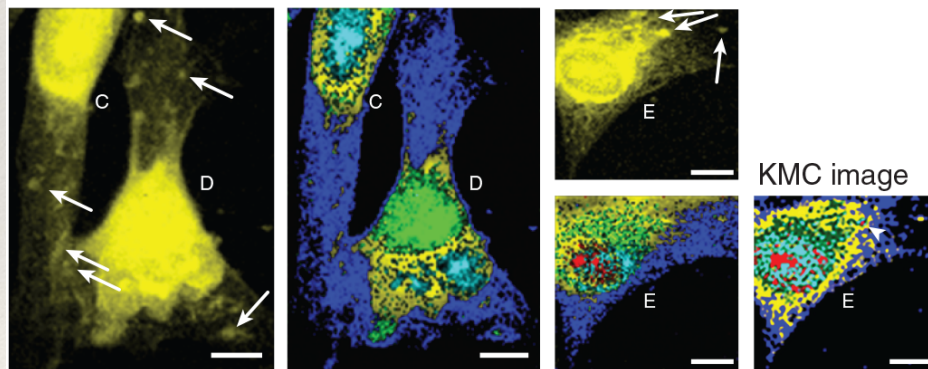


• RAMAN



Raman images of autophagic MG-63 cells generated by PCA (all except labelled) and KMC

Lau et al Renishaw



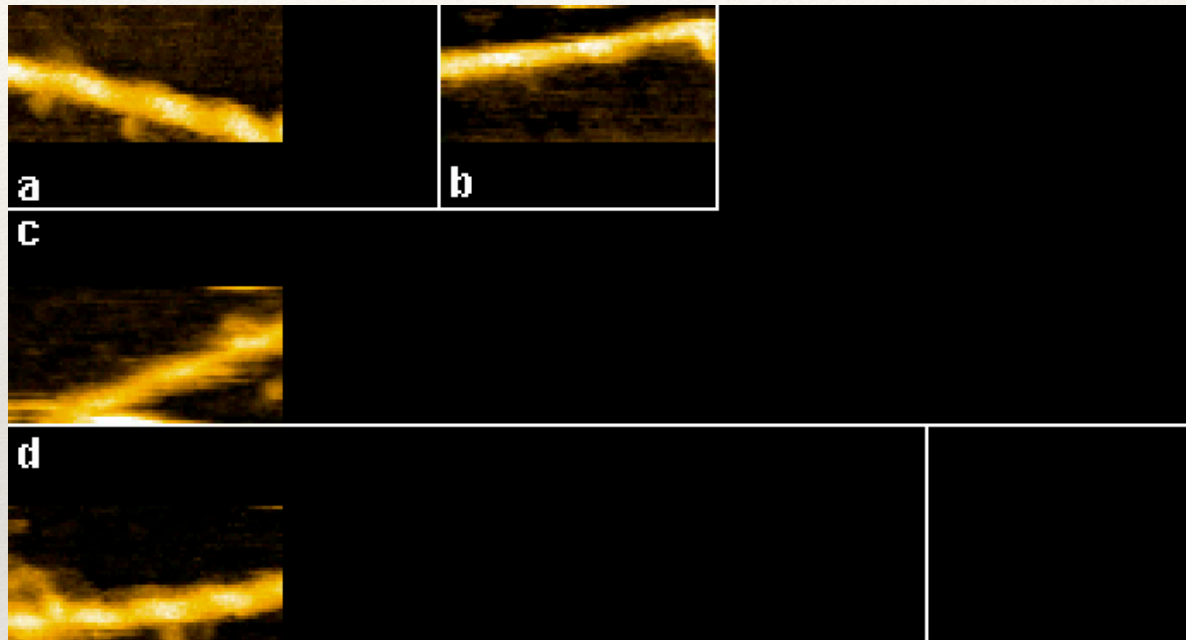
Chemical information from the Raman spectra:

- The nucleus was clearly defined in Cell A; whereas in Cell B the nucleus emitted weaker Raman signals and was not defined as a component in PCA.
- Cell B: phosphatidylcholine (PtlCho) and phosphatidylinositol were found in the green region; only PtlCho was detected in the magenta domains.
- More vesicles were present in the autophagic cells, especially in the membranous areas (arrows).
- Cell C displayed a contained nucleus, whereas in Cell D there was dispersed DNA.
- An DNA-containing autophagosome was observed in Cell E (arrowhead). DNA sequestration has been linked to the control of aneuploidy in these cells.^{iv}
- Other putative autophagosomes contained lipids and proteins.

- HS-AFM

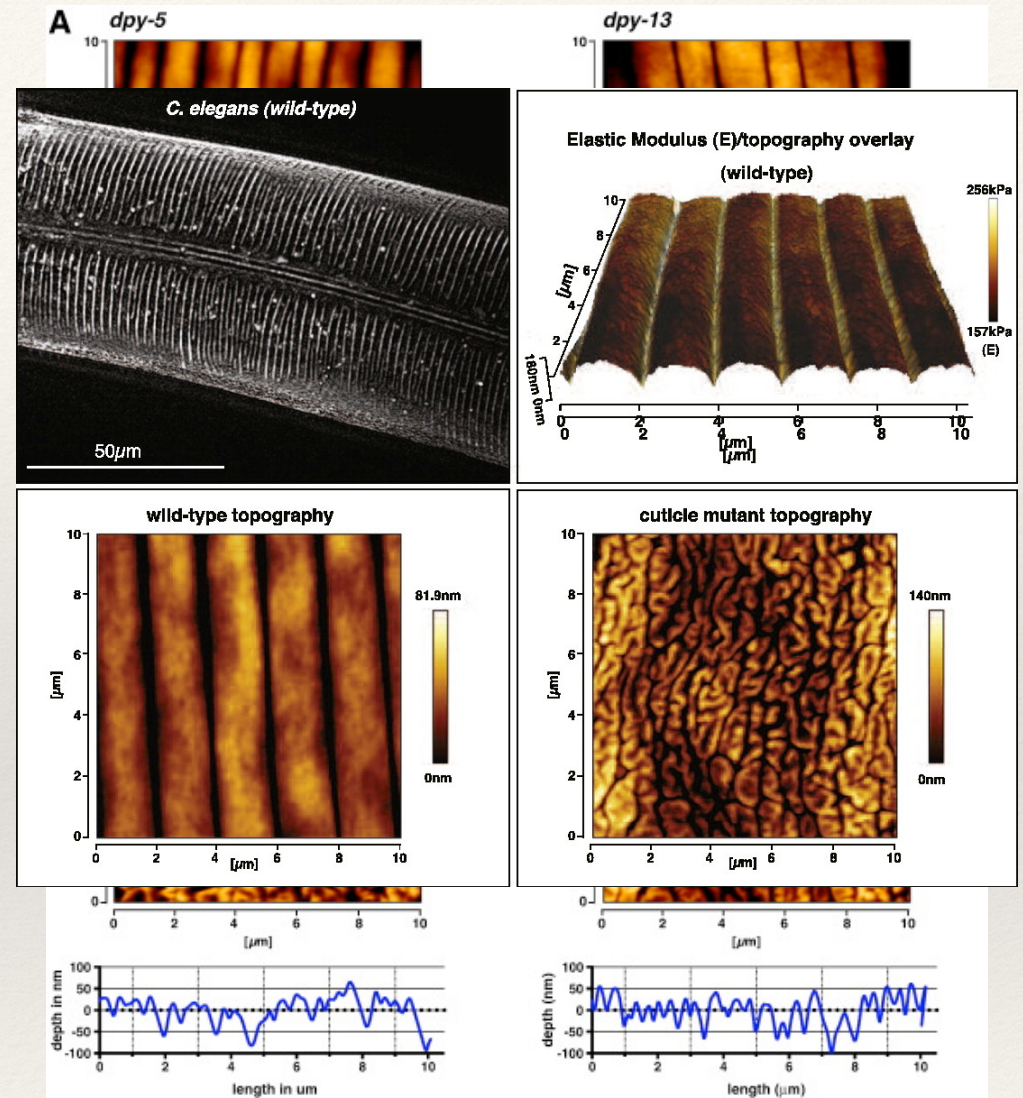
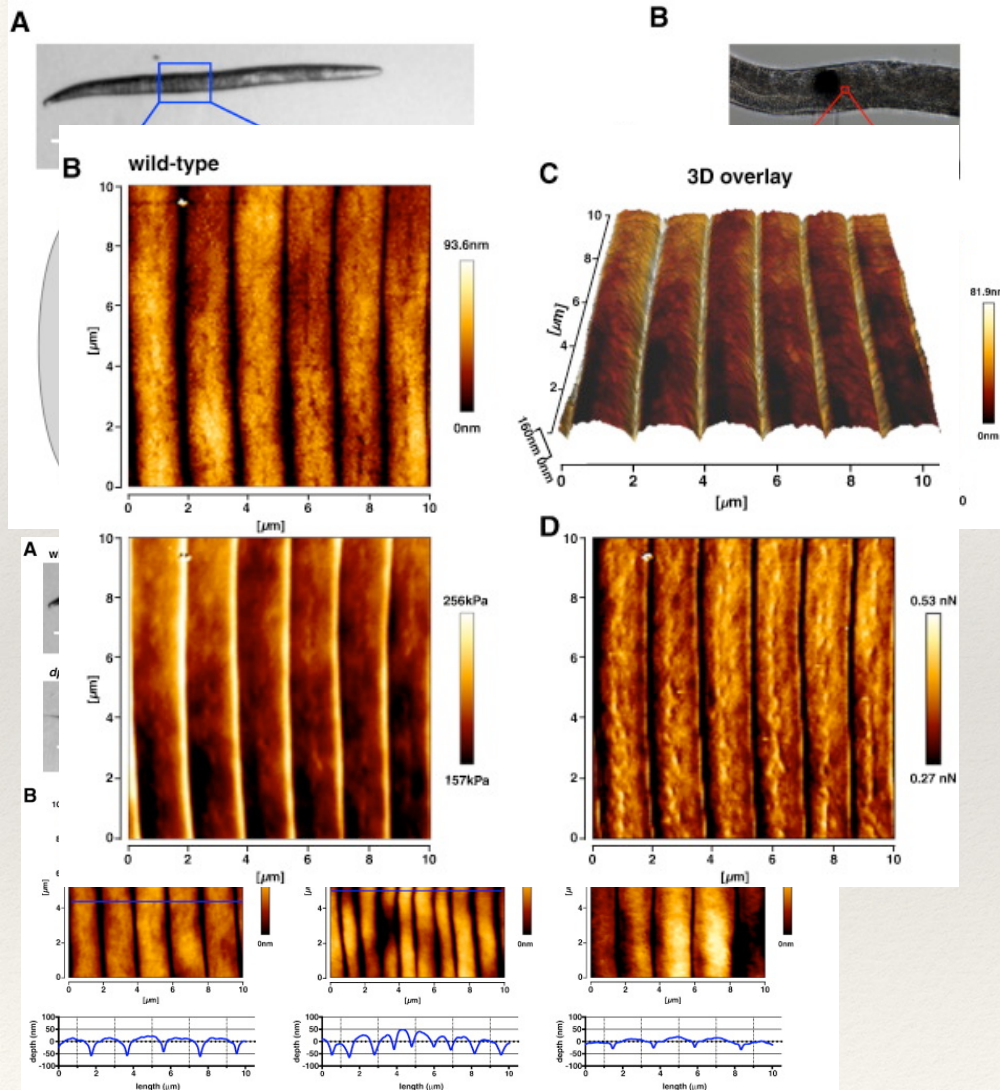


T Ando



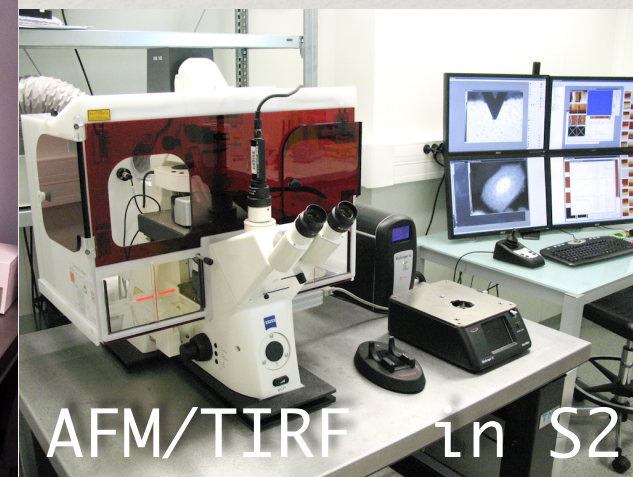
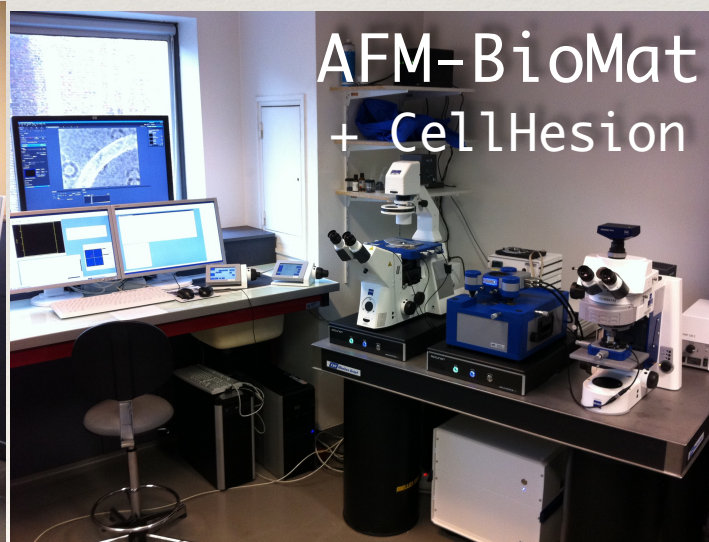
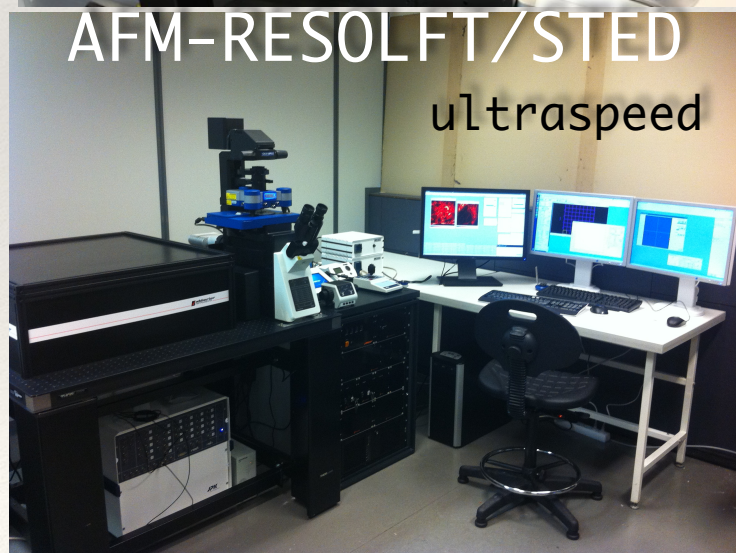
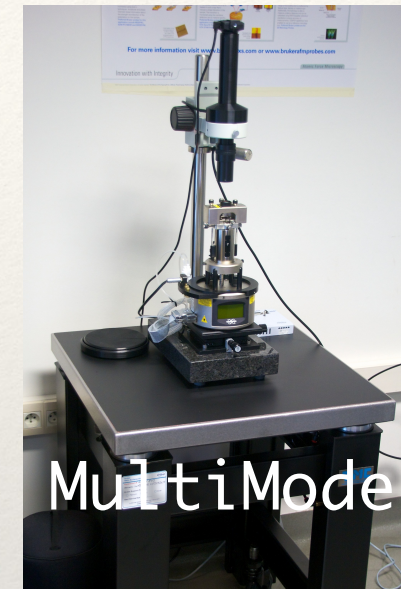
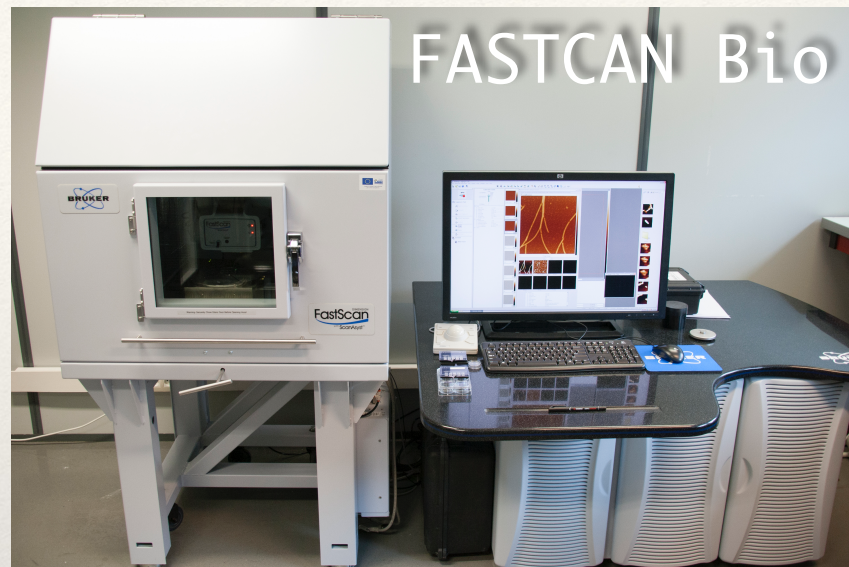
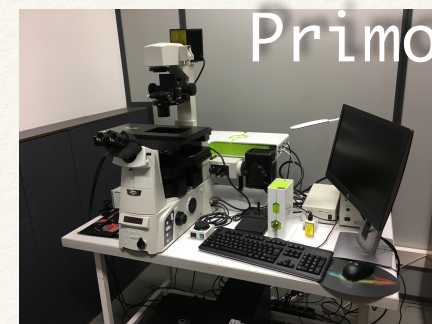
Kodera et al Nature 2010

- AFM on total organism



Essmann et al. Nanomedicine. 2017

AFM@ Pasteur Lille





[HTTP://WWW.CMIP.CNRS.FR](http://www.cmip.cnrs.fr)



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2 Open PhD positions



1 Open Post-doc position



Collaborations: Autophagy



C Abbadie (UMR8161)



P Sansonetti



P Chaminade, A Tfayli (Lip(sys)²)



T Galli (U950)



**V Pierrefite-Carle (INSERM U638)
B Antony (IPMC)**



T Proikas-Cezanne (MPG Tuebingen)



V Bragua (Imperial College London)



T Yoshimori (Osaka Univ.)



MI Colombo (Univ. Mendoza)



S Tooze (Francis Crick Institute London)



A Alonso (Univ Bilabo)



H Stenmark



J McKenzie

Collaborations: Physics



**F Pinet (INSERM U744)
P Brodin C Locht S Tomavo M Gissot S Marion (CILL)
T Melin, A Vlandas**



J Boskaert



**P. Cossart A. Scherf JP Latge T Wollert
R Brosch E Lemichez I Guijarro O Schwartz**



G Tran van Nhieu (CNRS UMR7241)



C Guilhot



G Bourdin (LERPé)



P Schmitz (LISBP)



T Galli (U894)



**S Bourdoulous, S Marulo, C Lavazec
(CNRS UMR8104 INSERM U565 UP V)**



**F Brochard-Wyart (UMR168)
P Bassereau (UMR168)
F Perez (CNRS UMR144)
K Schauer (CNRS UMR 144)**



J Rothman (Yale Univ, USA & UCL UK)



P Leclère (Chemistry of Novel Material)



Y Dufrene (Unité Chimie des Interfaces)



**G Dietler-S Kasas (Complex Matter Inst.)
P De Los Rios (Physics Theroy Inst.)
F Fantner (Inst Bioeng.)**



S Hell (MPI für Biophysical chemistry)



D Collard (CNRS & Tokyo Univ)

THANK YOU FOR YOUR ATTENTION

