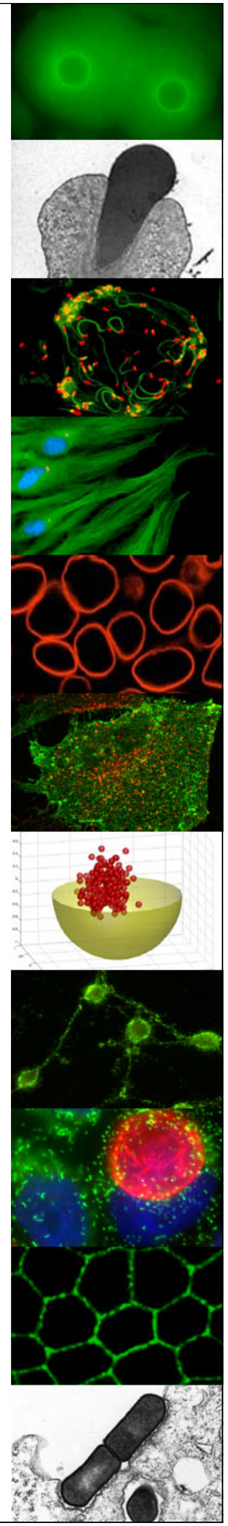
Collège de France Symposium
Seeing is Believing:
Imaging Infectious Processes *in vitro* and *in vivo*

The Future of Imaging:
Less Photons, More Numbers

Jean-Christophe Olivo-Marin
Unité d'Analyse d'Images Quantitative
Département de Biologie Cellulaire et Infection
Institut Pasteur, Paris

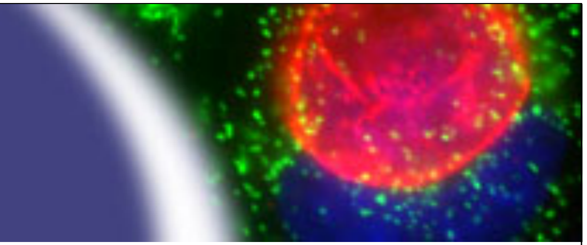
INSTITUT PASTEUR

INSTITUT CARNOT Pasteur MI



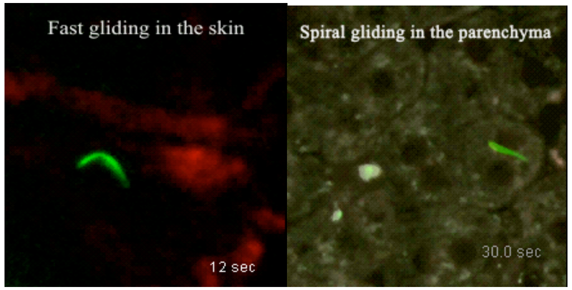
Biological Imaging

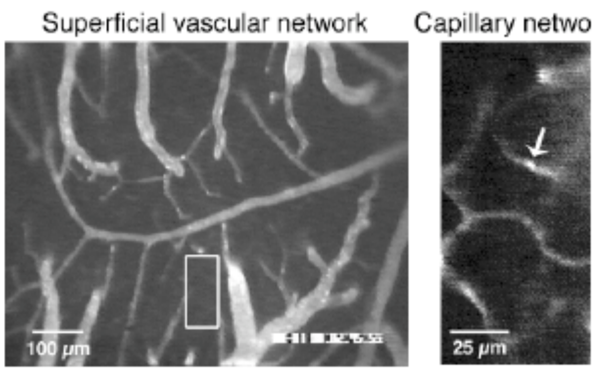
New microscopies open new frontiers in biology



Organism/Tissue

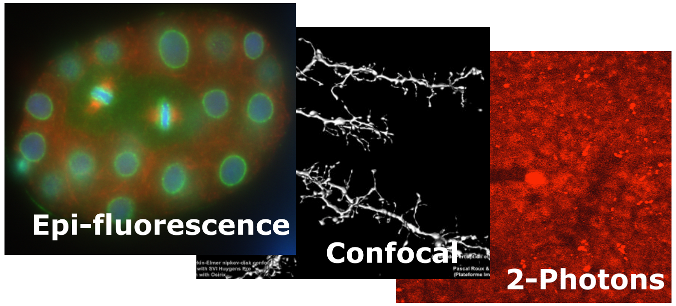
Intravital imaging

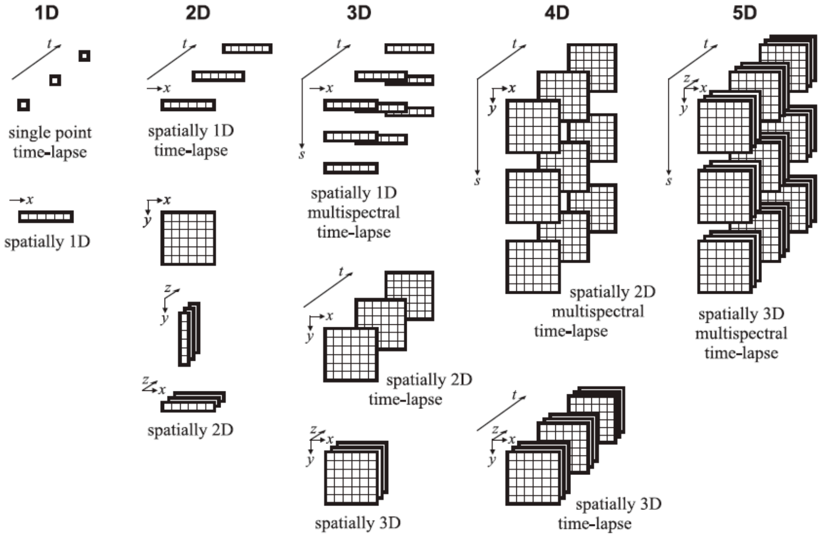




Cellular

Multi-D fluorescence





Molecular

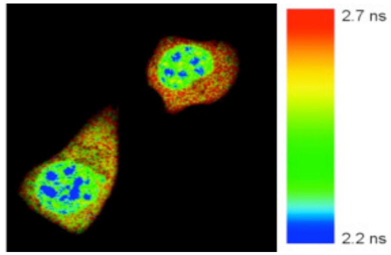
FRET/FLIM/FCS/FRAP

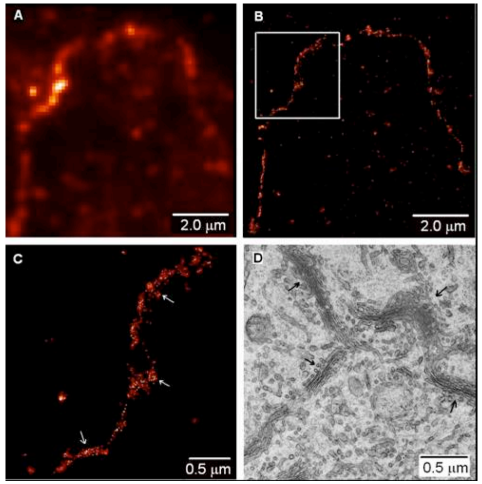
Molecular imaging

+FRET

+FLIM

+FRAP





PALM, STORM, STED

Macro — Micro — Nano

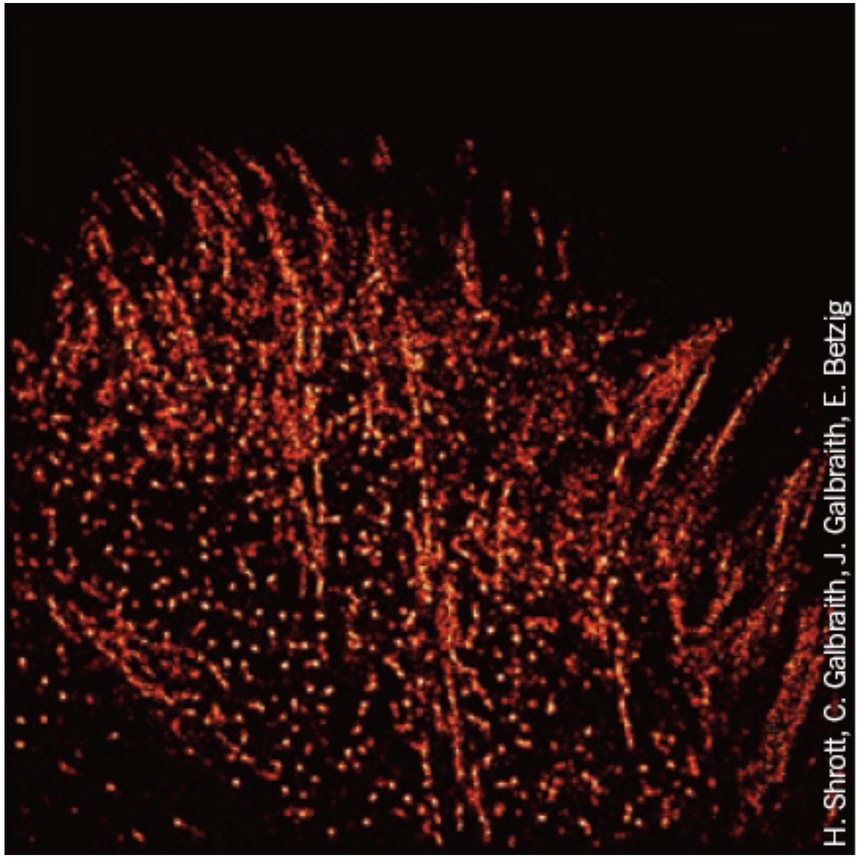
Biological Imaging
Imaging cells in fluorescence microscopy

Current trends and requirements in microscopy include:

- long acquisition times
- high spatial resolution
- high temporal resolution
- multiwavelength imaging
- high depth imaging
- single molecule detection

Resulting bottlenecks:

- reduced number of photons
- photo-bleaching of fluorophores
- low signal to noise ratio (SNR)
- huge amounts of data

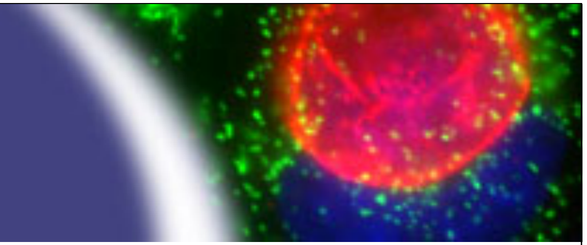


Fluorescein image sequence, 200 images acquired each 20 ms (total time = 4 s).

Noisy image

Biological Imaging

Imaging cells in fluorescence microscopy



Standard solutions:

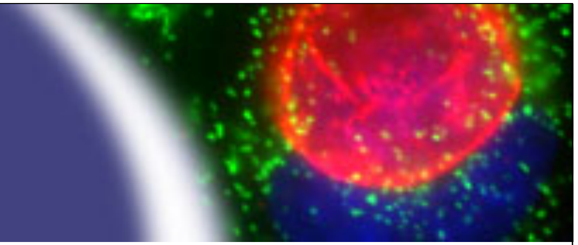
low nb of photons → **increase camera gain** → increased electronic noise

photo-bleaching → **reduce exposure time** → increased photonic noise

low SNR images → **denoising (e.g. Total Variation (*TV*), wavelets, etc.), averaging** → complete acquisitions

Future solutions:

- **mathematical imaging**
- **integration of physics and chemistry**
- **improve the quality of microscopy by using computational techniques during the acquisition**
- **optimize the acquisition sampling**
- **discriminate signal and noise**



Marcio Marim

Our approach

Compressive Sensing (CS) Framework*

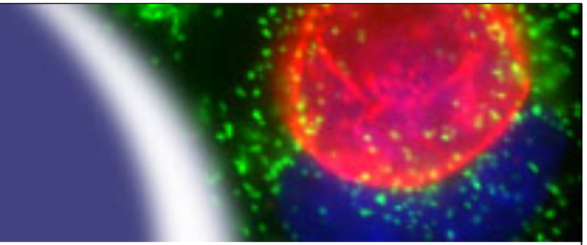
Image acquisition → **Optimized sampling:**

- preserve photons

Image reconstruction → **Recovery algorithm with noise and signal discrimination:**

- SNR improvement

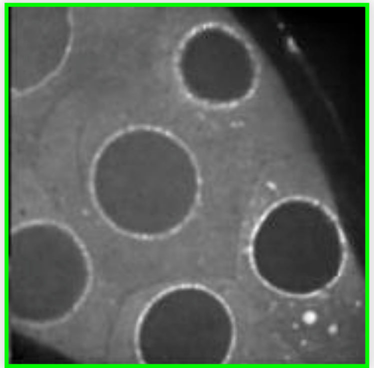
* CS has been developed by several teams including:
  - D. Donoho, E. Candès, J. Romberg (UCLA, Caltech)
  - J. Fadili, J.L. Starck, G. Peyré (ENSICaen, CEA, ENS)



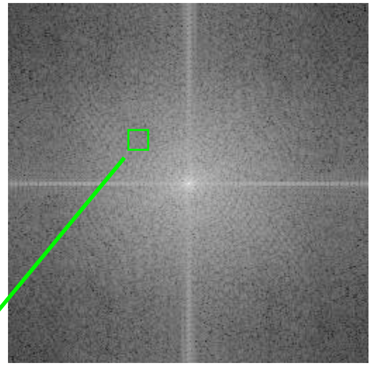
Reconstructing signals

1. Signal projection and random under-sampling

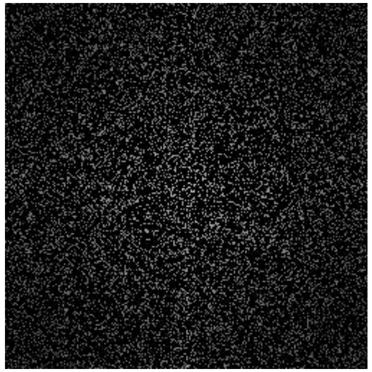
Drosophila Oocytes



Optical Fourier Transform



Random and incomplete measurements

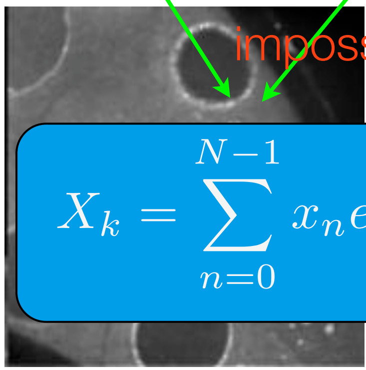


2. Sparsity prior

possible

In the Fourier domain each value is a projection of the

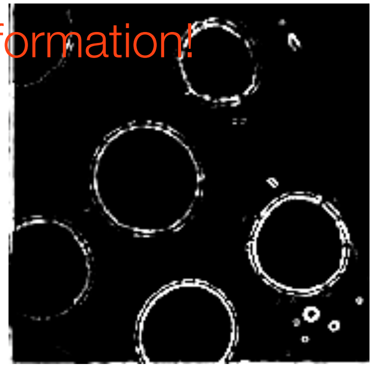
impossib... information!



$$X_k = \sum_{n=0}^{N-1} x_n e^{\frac{-}{N} kn}$$

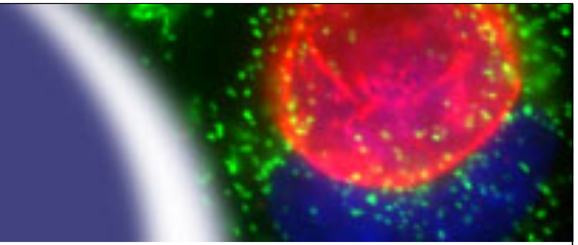
$$TV(x) = \| \nabla x \|_{\ell_1}$$

...me signal encoded differently



Biological Imaging

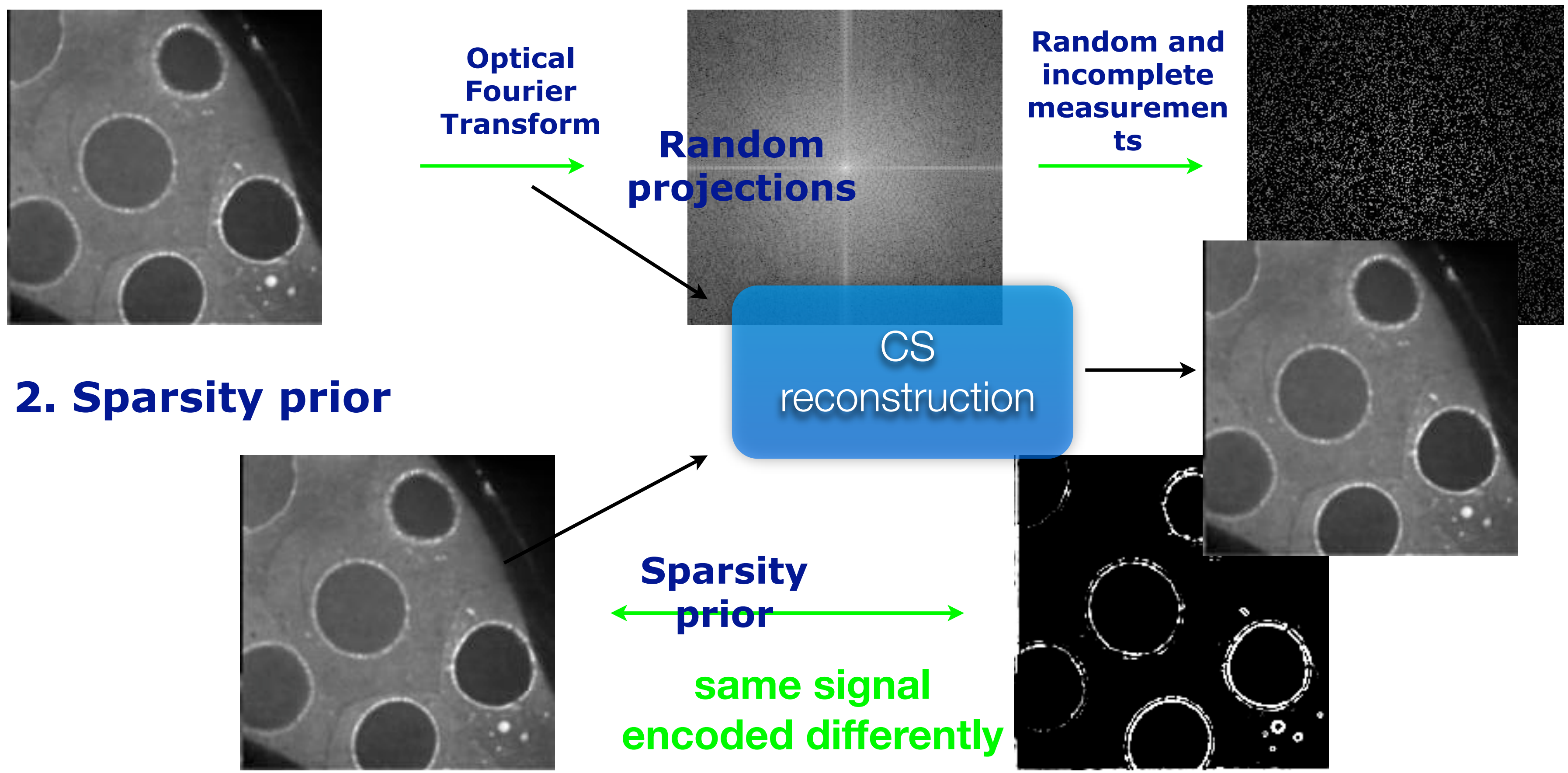
Compressive sensing in fluorescence microscopy



Reconstructing signals

1. Signal projection and random under-sampling

Drosophila Oocytes.



2. Sparsity prior

The recovery algorithm

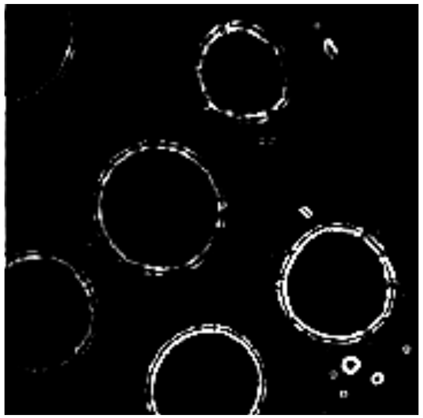
The signal reconstruction consists in solving:

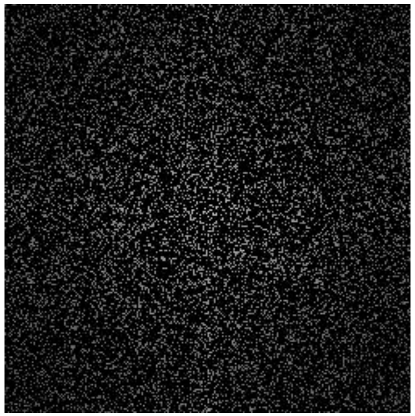
$$\hat{x} = \arg \min_{x \in \mathbb{R}^N} \| \Psi x \|_{\ell_1} \quad s.t. \quad y = \Phi x$$

sparsity term

data attachement term

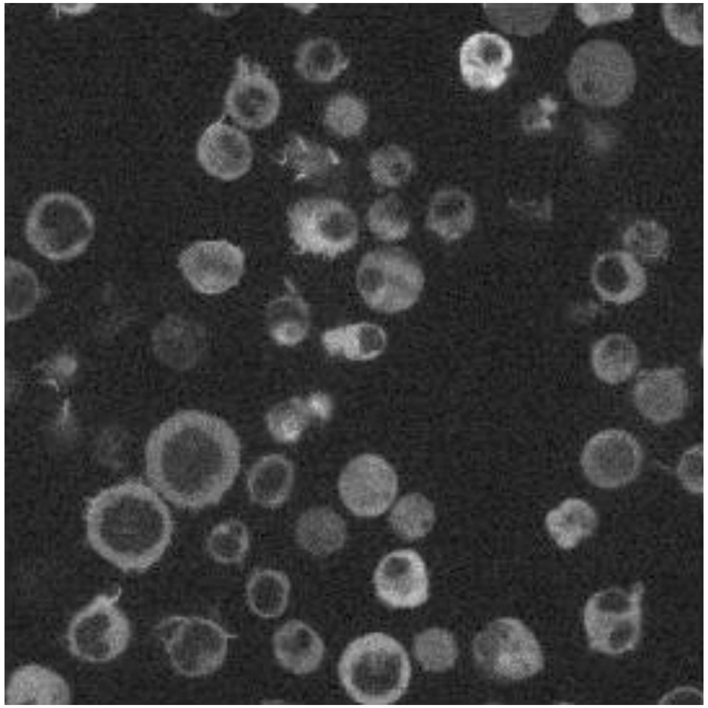
$$TV(x) = \| \nabla x \|_{\ell_1}$$



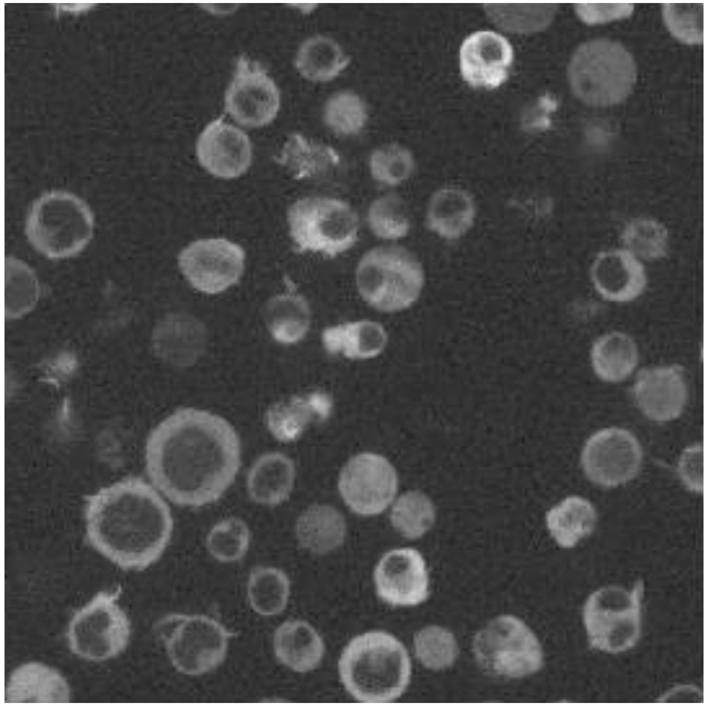


random and incomplete measurements

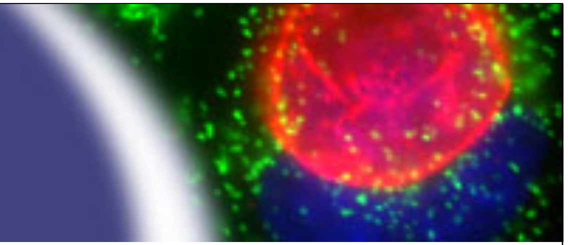
Example of reconstruction



Original image 512x512 pixels



CS recovery with 5% of measurements in the Fourier domain



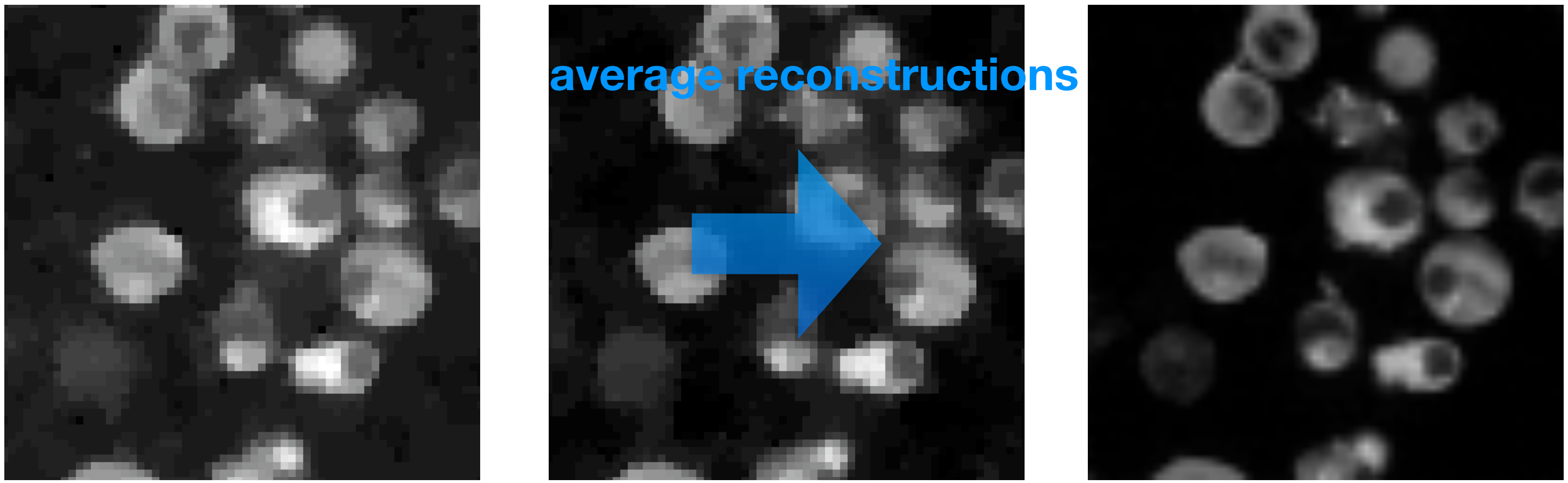
CS-based denoising

CS-based algorithm with multiple reconstructions:

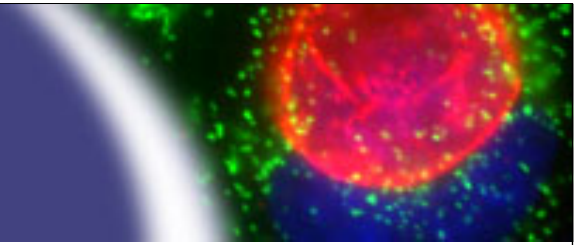
$$\hat{x}_i = \underset{x \in \mathbb{R}^N}{\arg\min} \parallel \Psi x_i \parallel_{\ell_1} \quad s.t. \quad \parallel y_i - \Phi_i x_i \parallel_{\ell_2} \leq \delta$$

minimize sparsity ⟷ relax data attachment

for $i = 1, ...k.$

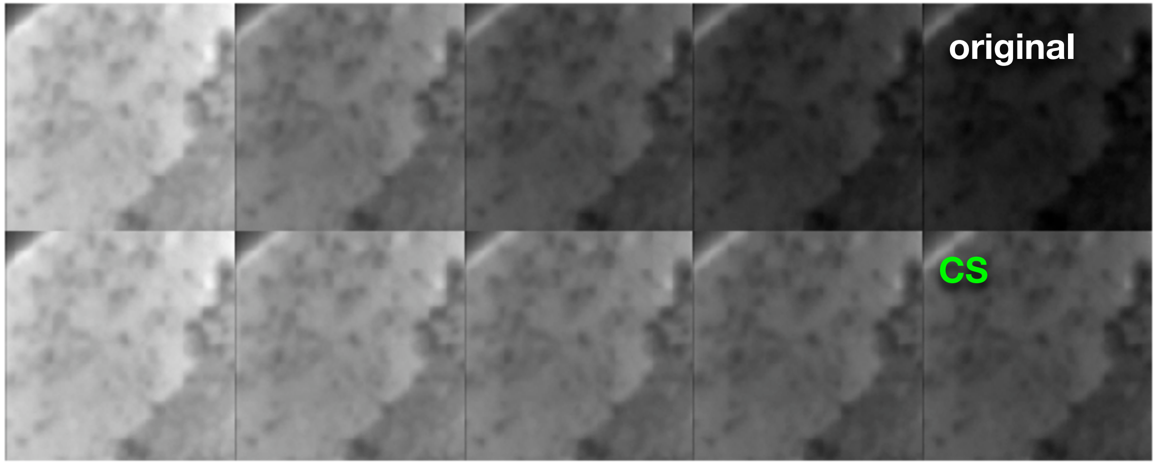


Denoised image



Reducing photobleaching with CS

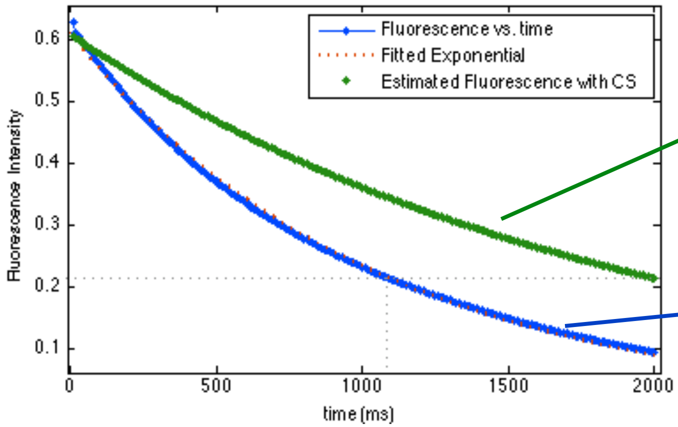
time - photo-bleaching



Fluorescein images.

Top: Original image, acquired at t = 0; 500; 1000; 1500; 2000 ms. Exposition time = 20 ms.

Bottom: Results using CS denoising. Exposition time = 8 ms.

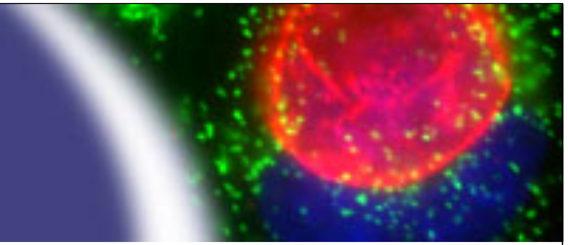


Estimated photo-bleaching using CS

Original photo-bleaching

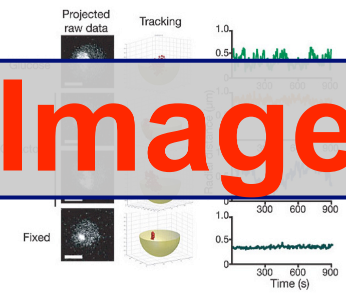
Biological Imaging

Extraction and analysis of visual information



Sample Preparation

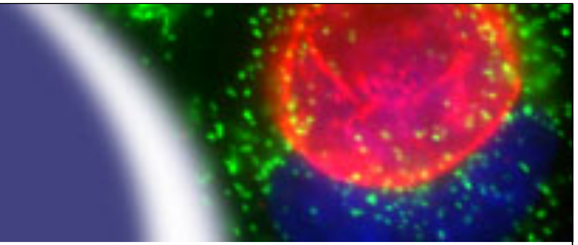
Physical Instrumentation



Quantitation

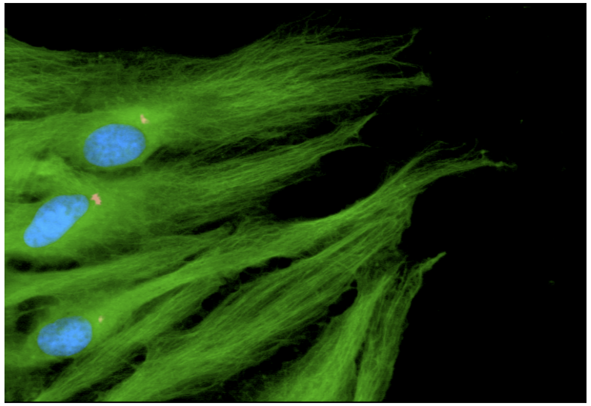
- **Automation**
- **Exhaustive extraction of data**
- **Individual/collective information**
- **Statistical quantification**
- **Modelisation**
- **Intelligent information storage and annotation**

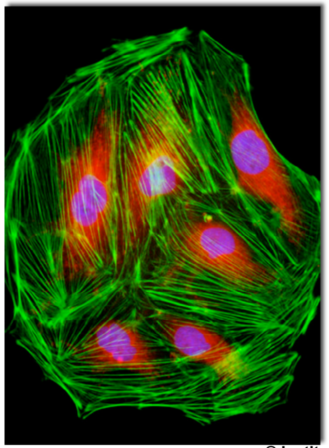
Image Processing and Analysis

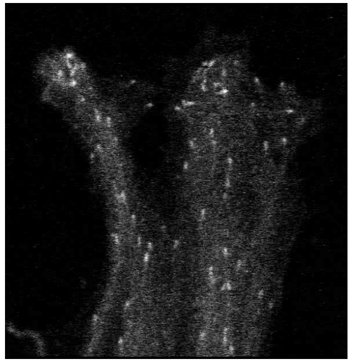


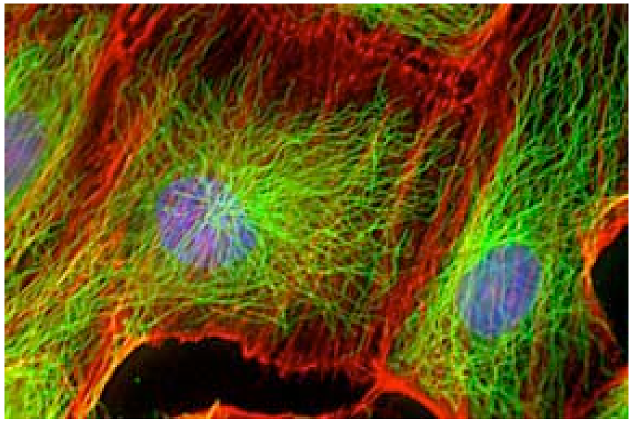
Topics

- Deconvolution
- Inverse problems
- Denoising
- Registration
- Detection
- Segmentation
- Particle tracking
- Deformable objects in 2D/3D+t
- Cell shape and motility
- Tissue architecture
- Data fusion and correlation
- Classification
- Modeling
- Computer vision
- Image databases



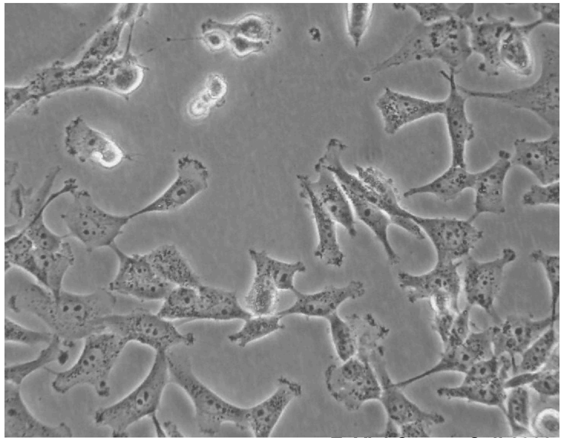




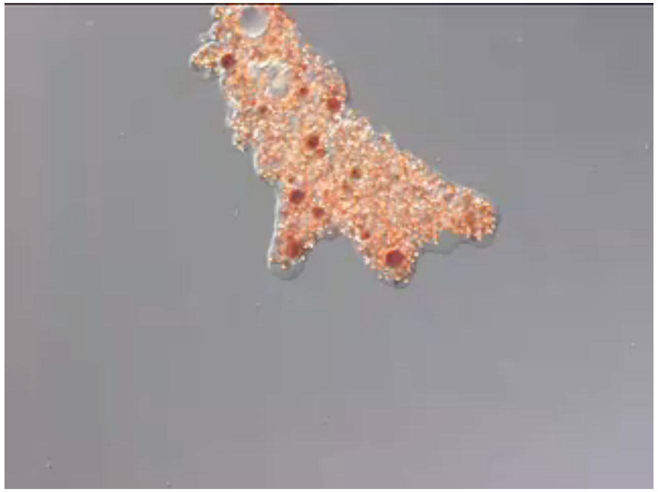


Cell shape and motility

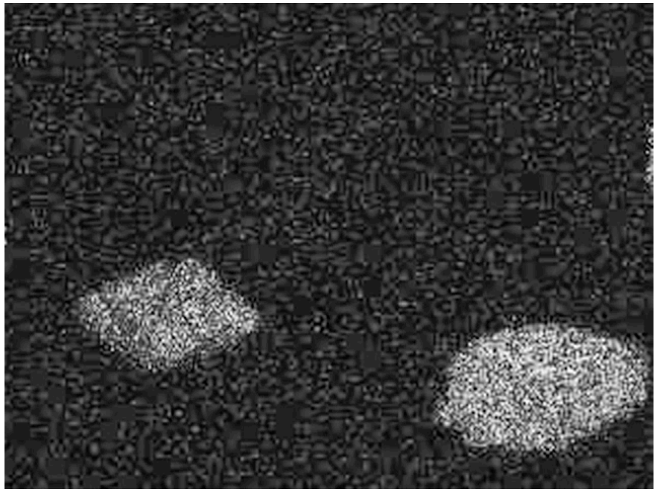
- **Pathogen virulence**
  - **shape and movement are major factors of parasite virulence**
  - **alter motility to alter virulence**
- **Molecular mechanisms of:**
  - **cytoskeleton dynamics**
  - **extension processes**
  - **adherence**
  - **protrusion**
  - **deformation**
- **Cancer**
  - **viral origin**
  - **metastasis**



E. Vial Cancer Cell 2003



E. histolytica © Institut Pasteur

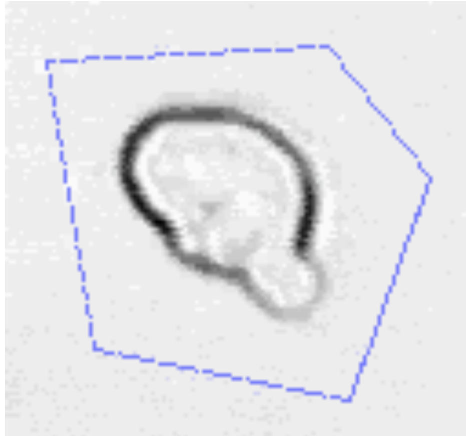


E. histolytica © Institut Pasteur

Biological Image Analysis
Active contours

- **Principle: attract a curve *C* towards an object by minimizing an energy that depends on the image and on *C***
- **Parametric curve x(s) that evolves under the constraint of internal and external forces to minimise the total energy**

$$E_{\mathrm{tot}} = \int_0^1 \left[E_{\mathrm{int}}(\mathbf{x}(s)) + E_{\mathrm{ext}}(\mathbf{x}(s)) \right] ds$$



- **Internal forces: ensures the curve is regular by minimising:**

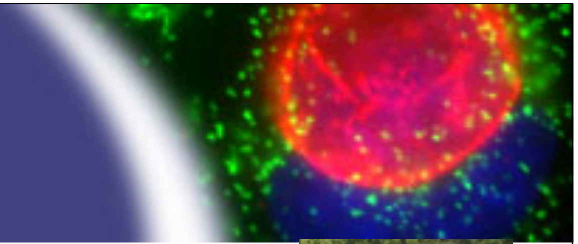
$$E_{\mathrm{int}}(\mathbf{x}(s)) = \underbrace{\alpha \left| \frac{d\mathbf{x}}{ds} \right|^2}_{\text{elasticity}} + \underbrace{\beta \left| \frac{d^2\mathbf{x}}{ds^2} \right|^2}_{\text{rigidity}}$$

- **External forces: deform the curve towards the boundaries of the objet by minimising:**

$$E_{\mathrm{ext}}(\mathbf{x}) = -\left| \nabla \underbrace{I}_{\text{Image intensity}}(\mathbf{x}) \right|$$

- **Steady state: corresponds to the contour**
- **Regional energy: Mumford-Shah formulation** (Chan&Vese, TIP, 2001)

$$E(\mathbf{C}, c_{\mathrm{in}}, c_{\mathrm{out}}) = \lambda_{\mathrm{in}} \iint_{\mathrm{inside}(\mathbf{C})} (I - c_{\mathrm{in}})^2 \, dx\, dy + \lambda_{\mathrm{out}} \iint_{\mathrm{outside}(\mathbf{C})} (I - c_{\mathrm{out}})^2 \, dx\, dy + \tfrac{1}{2} \int_0^1 \alpha \left[\left| \frac{\partial \mathbf{C}}{\partial s} \right|^2 + \beta \left| \frac{\partial^2 \mathbf{C}}{\partial s^2} \right|^2 \right] ds$$



Handling touching cells

Alexandre Dufour

- **Presence of multiple objects**
  - **with different mean intensities**
  - **with multiple contacts**

- **One level set function for *each* object with a penalty for overlaps**

$$E(\phi_1,\ldots,\phi_n,c_O,c_{I,1},\ldots,c_{I,n}) = \iiint_\Omega \sum_{i=1}^{n} \Bigg[\alpha g \delta(\phi_i)\,|\nabla \phi_i|$$

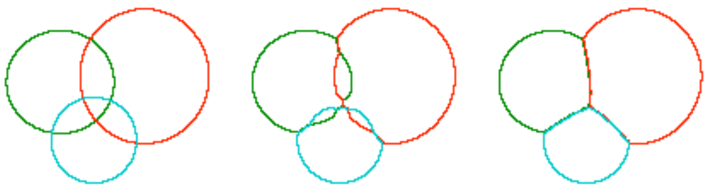
$$+\lambda_I H(\phi_i)(I-c_{I,i})^2$$

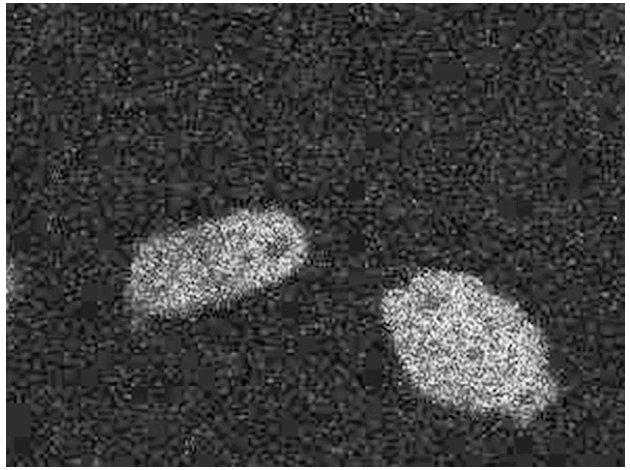
$$+\frac{\lambda_O}{n}\prod_j (1-H(\phi_j))(I-c_O)^2$$

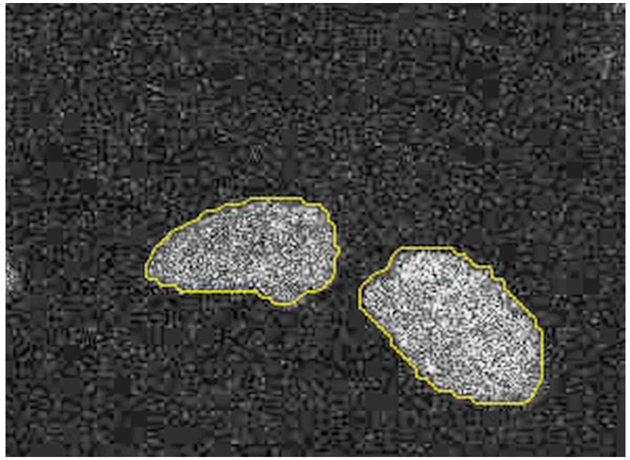
$$\boxed{+\gamma \sum_{i<j} H(\phi_i)H(\phi_j) \Bigg]}\,dxdydz$$

Penalty for surface overlaps

- **Objects are repulsively coupled**
- **Each object is individualised**



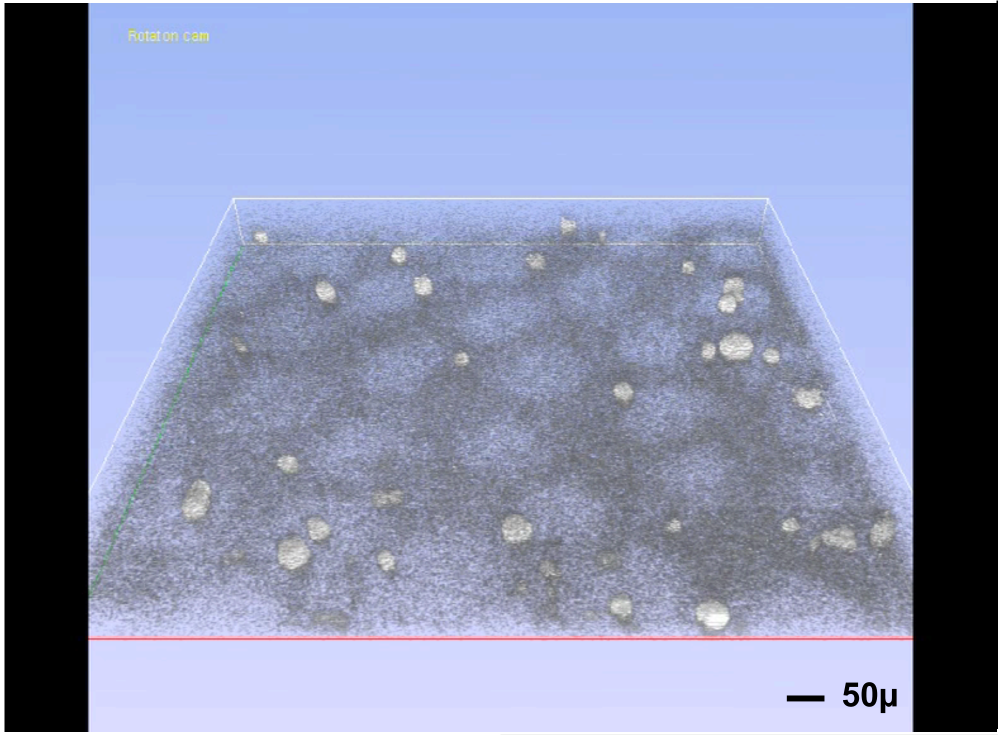


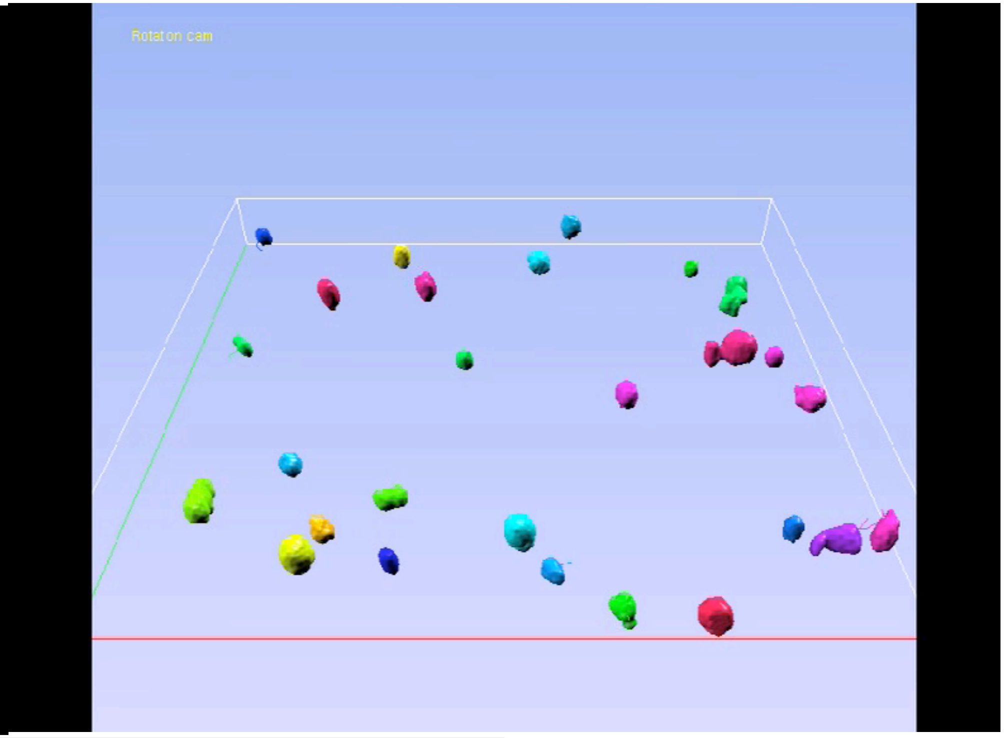


C. Zimmer and J.-C. Olivo-Marin, *IEEE Trans. Pattern Analysis Machine Intell.* (2005)

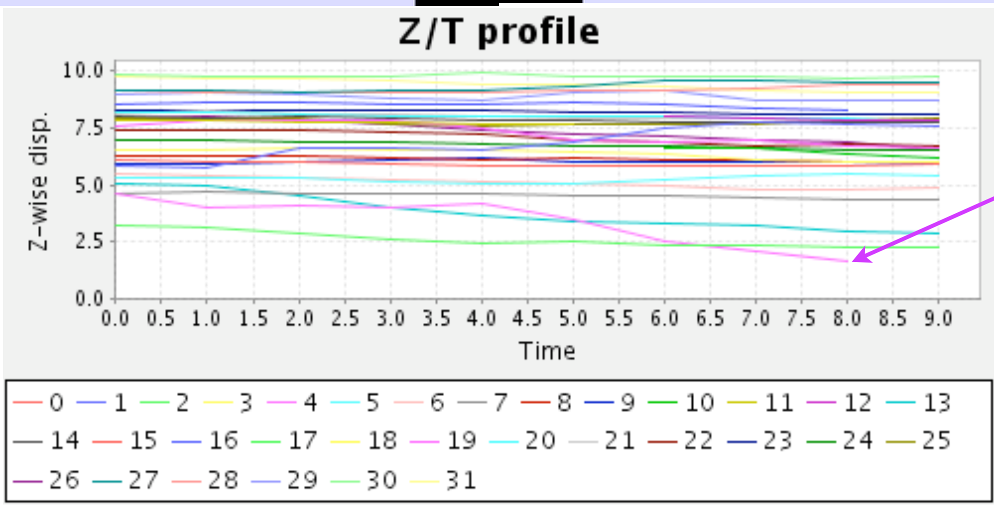
Dufour, A., Tashbacsh, S., Guillén, N., Olivo-Marin, J.-C., Zimmer, C., *IEEE Trans. Image Processing* (2005)

Biological Image Analysis
3D Active meshes



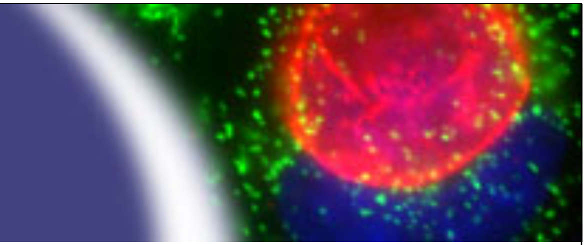


Amiba in intestinal tissue imaged in 2-photon microscopy



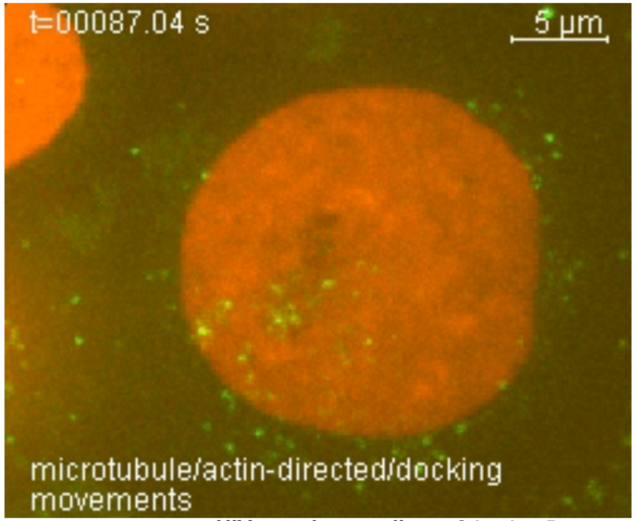
amiba traversing the mucosa

A. Dufour and E. Labruyere

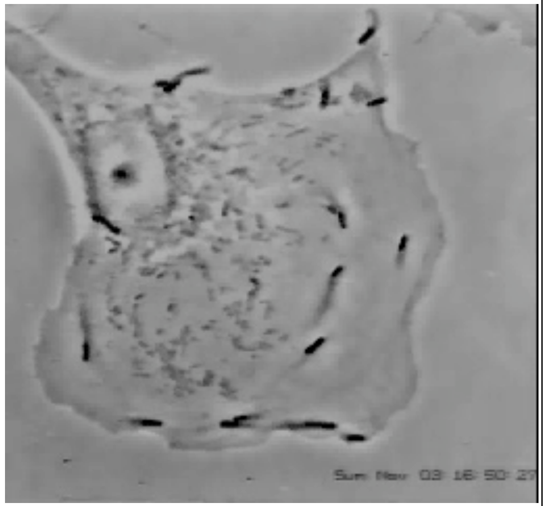


Tracking

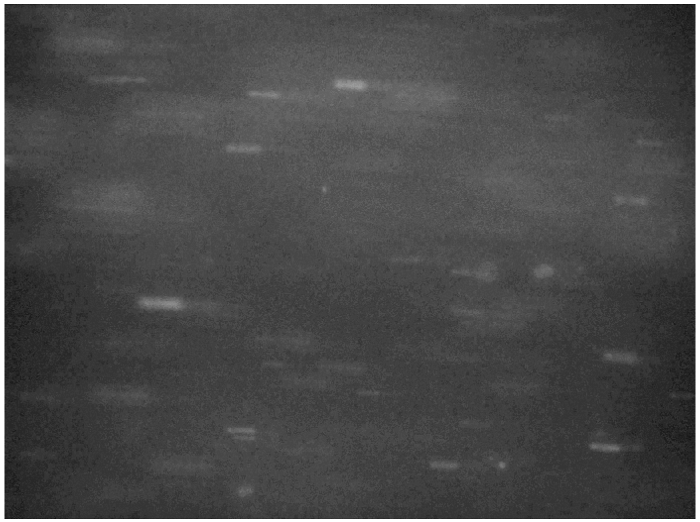
- Virus and bacteria invasion of cells
  - have to enter or target host cells
  - impair invasion to impair replication
  - impair motility to impair spread
- Molecular mechanisms of:
  - transport
  - organelle dynamics
  - endo-(exo-)cytosis
  - vesicle movement
  - pathogen movement
  - pathogen adhesion



HIV entering a cell © Institut Pasteur

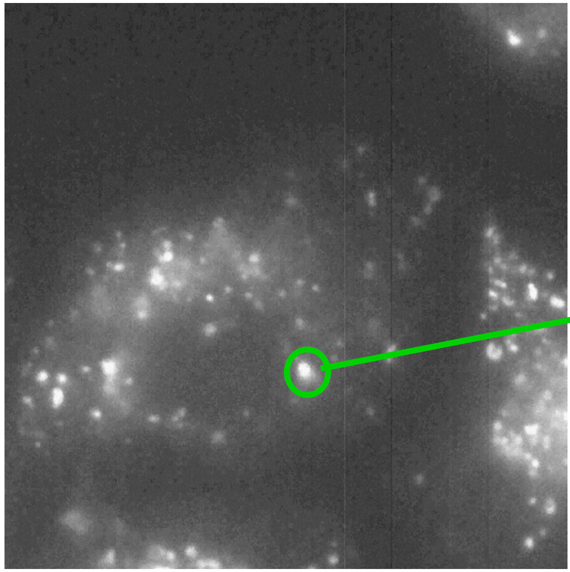


Listeria moving in a cell © J. Thériot

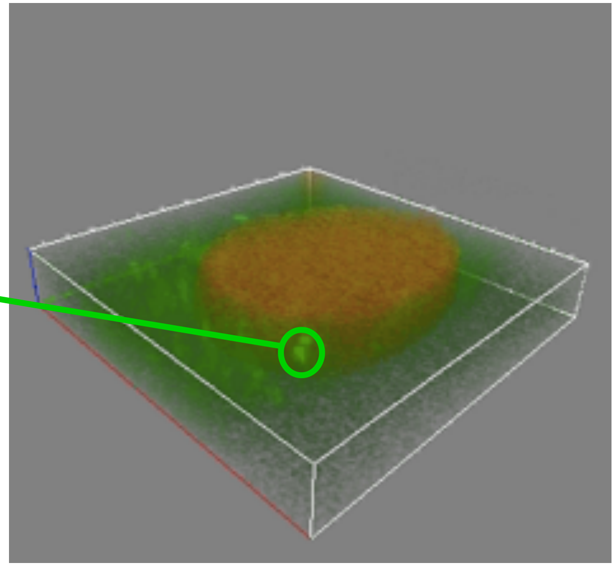


N. menengiditis in a flow © Institut Pasteur

Biological Image Analysis
Multi Particle Tracking



$$m_t = \begin{bmatrix} x_t \\ y_t \\ z_t \\ v_t \\ i_t \end{bmatrix}$$



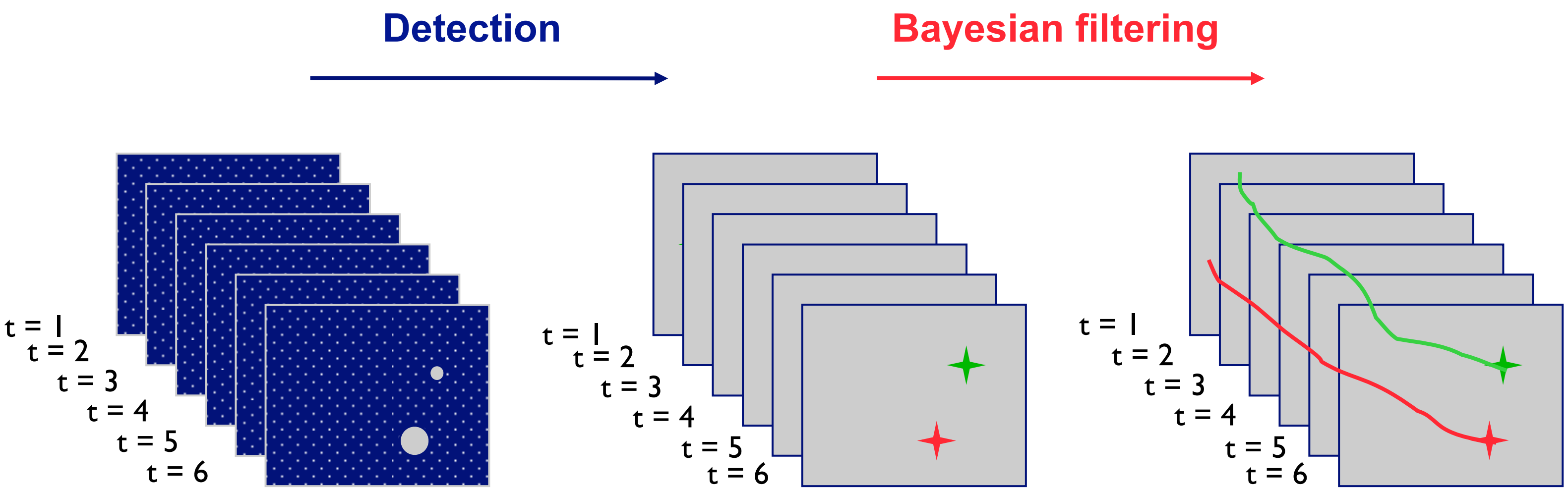


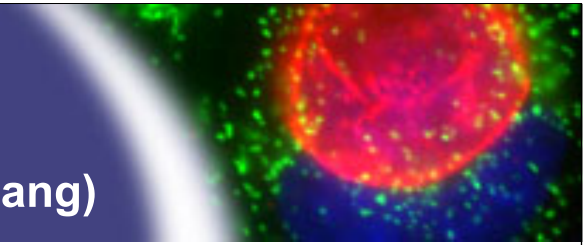
Image sequence

Coordinate sequence

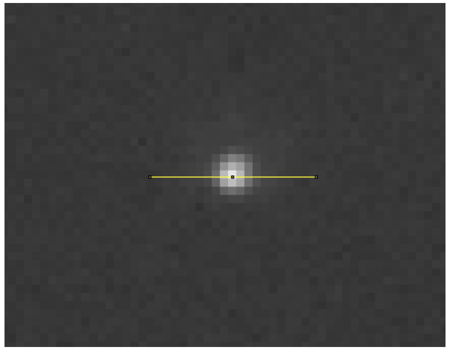
Trajectories

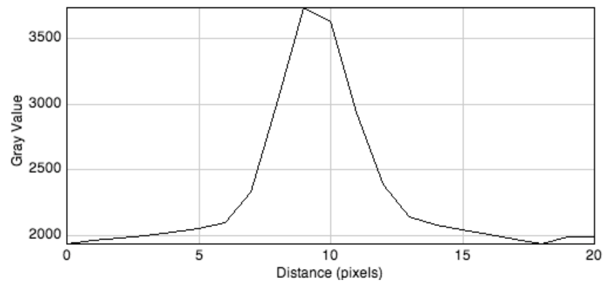
Biological Image Analysis
Object modeling

(B. Zhang)

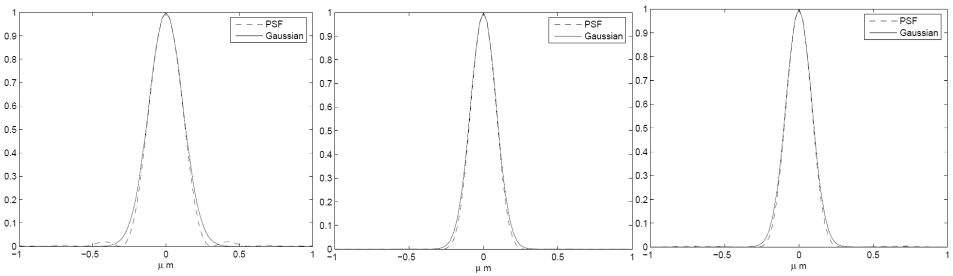


- **For a subresolution source (Dirac source):**
  - **profile = Point Spread Function (PSF) of the microscope**
  - **matched filter = wavelet**

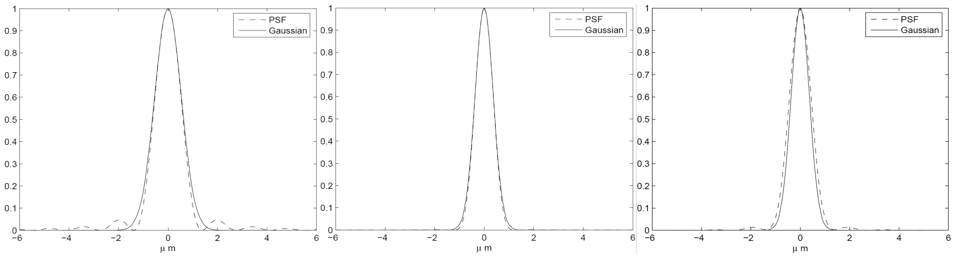




Point Spread Function approximation

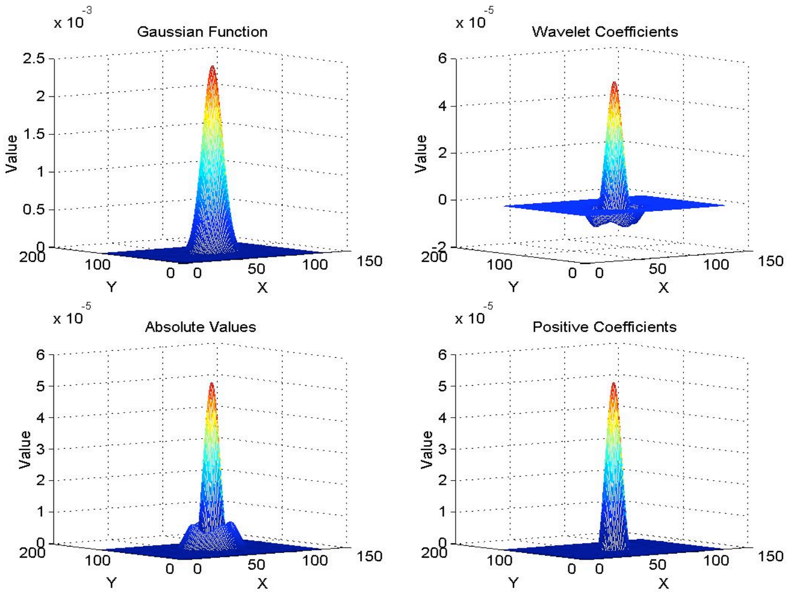


WFFM (Lateral) LSCM (Lateral) DSCM (Lateral)



WFFM (Axial) LSCM (Axial) DSCM (Axial)

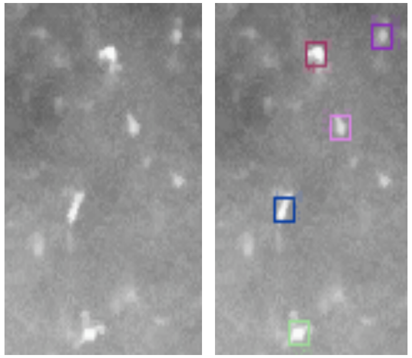
PSF approximation with B3 Wavelet

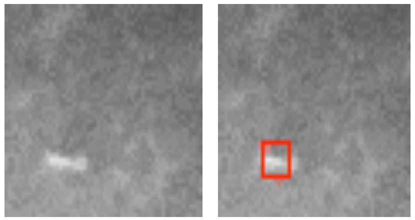


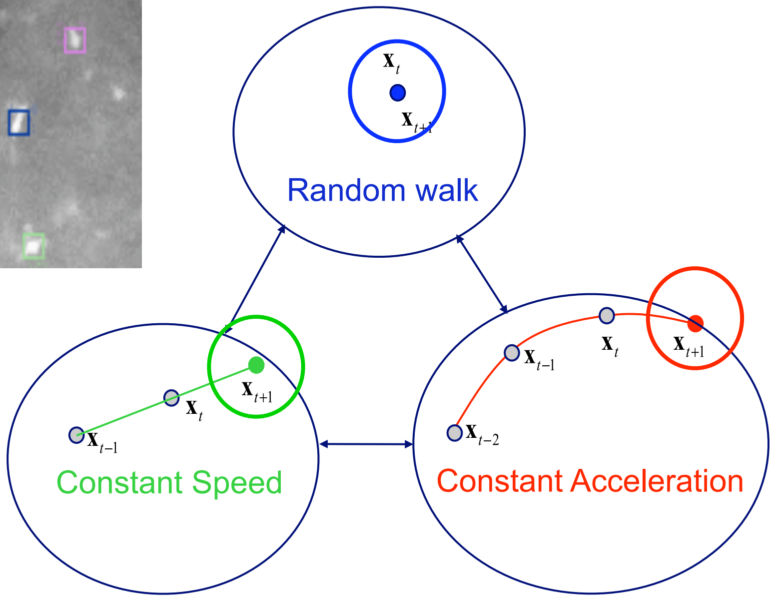
Zhang, B., Zerubia, J. & Olivo-Marin,J.-C., *Applied Optics* (2007)

Bayesian Filtering

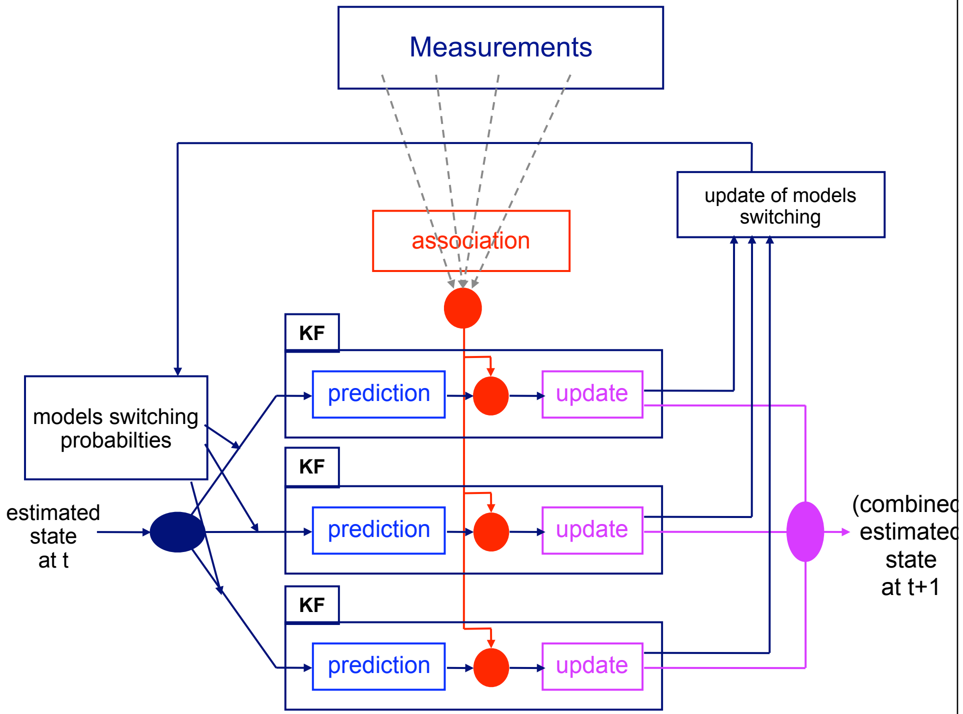
Dynamic models







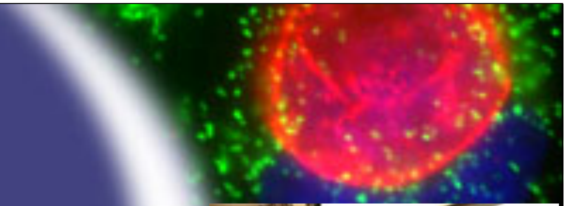
Interacting multiple models



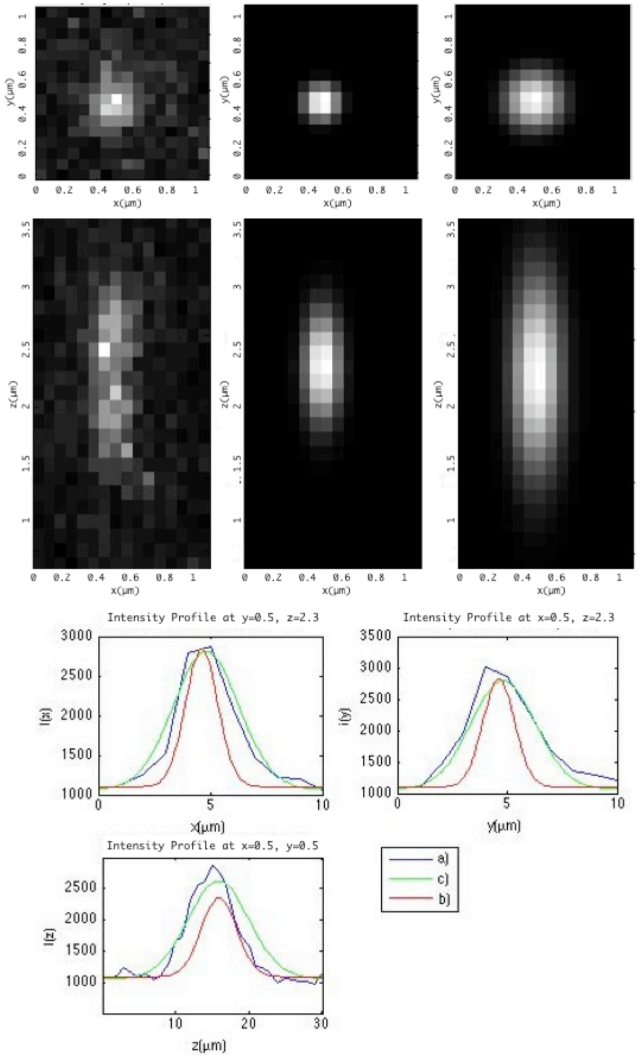
A. Genovesio, V. Emiliani, W. Parack, M. Coppey,J.-C. Olivo-Marin, *IEEE Trans. Image Processing* (2006)

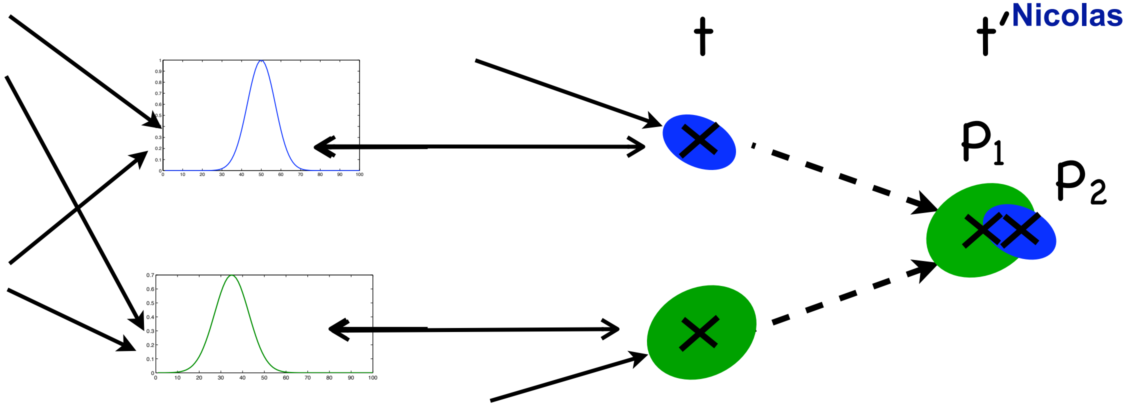
Biological Image Analysis

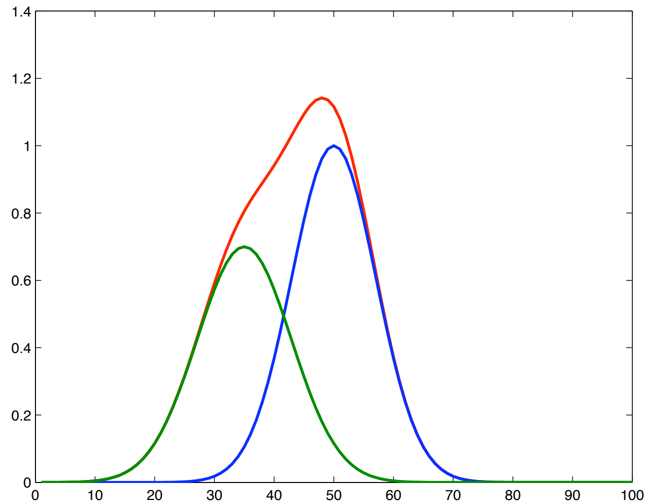
Profile Adapted Multitarget Tracking

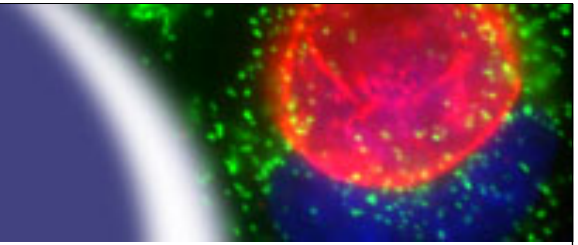


Nicolas Chenouard

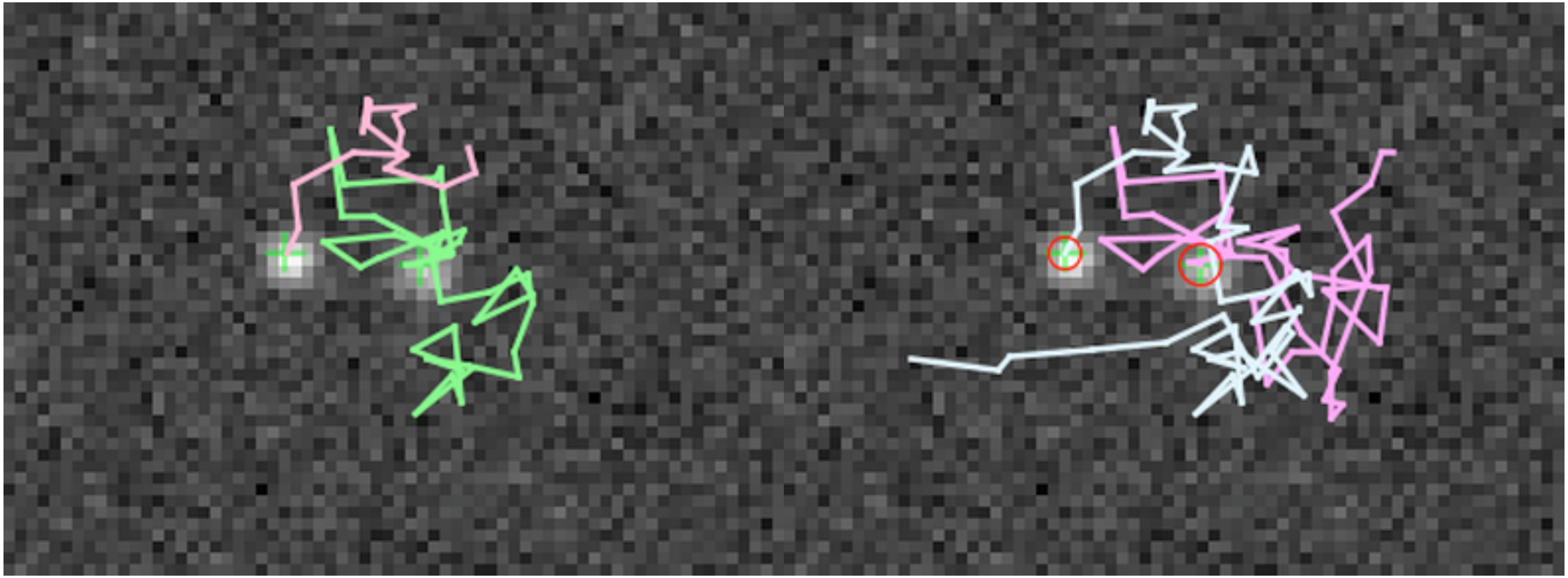








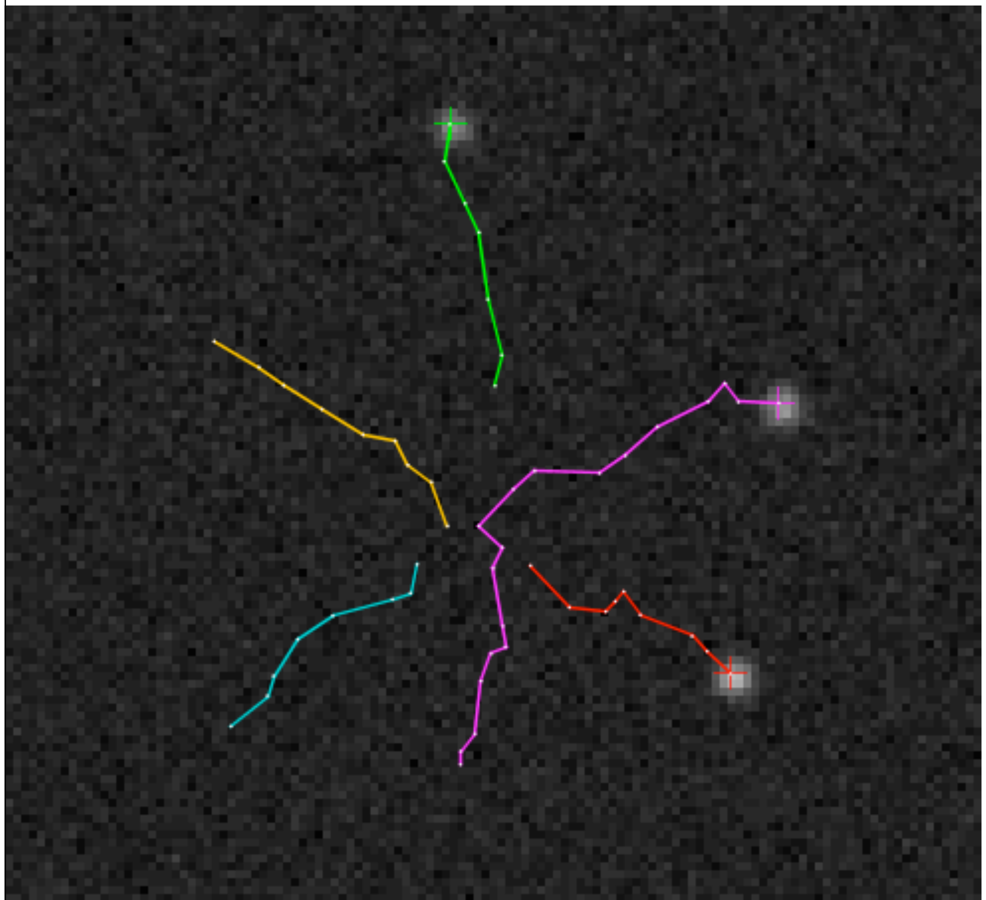
Feature aided tracking



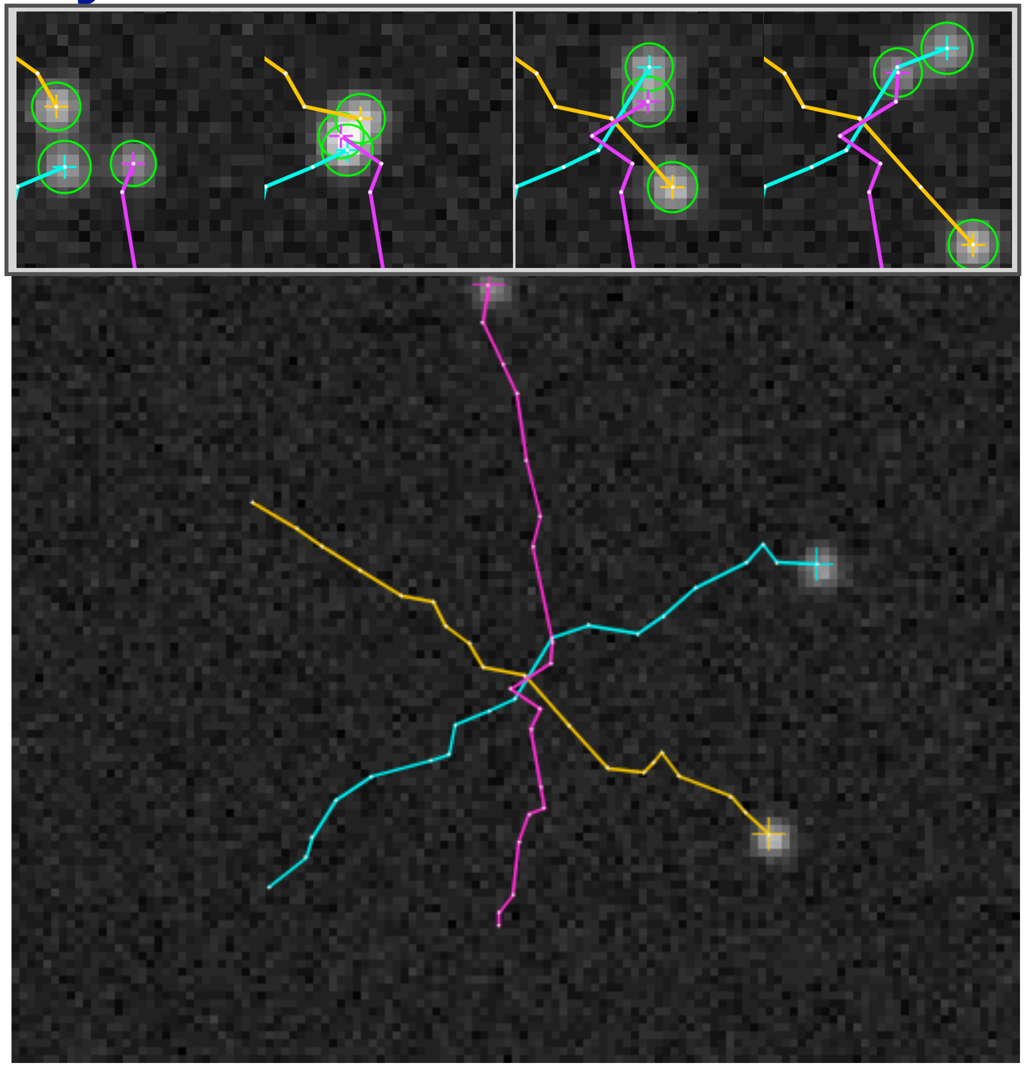
without profile information

with profile information

Tracking of synthetic beads



Kinetic only tracker

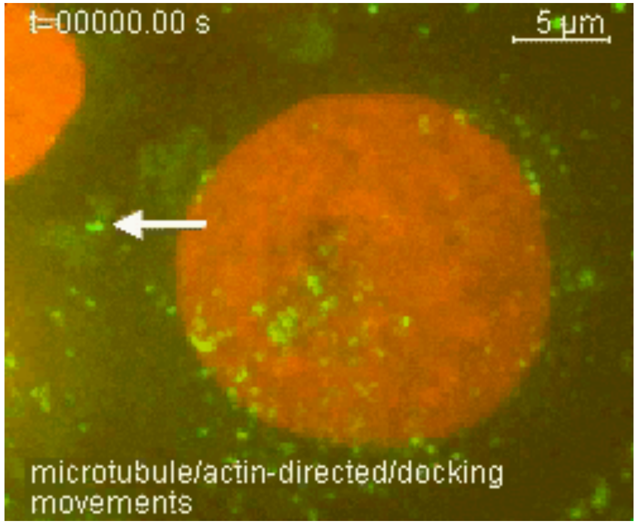


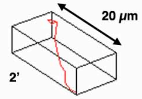
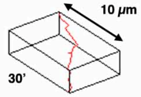
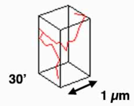
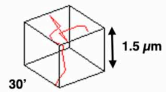
Combined kinetic and feature tracker

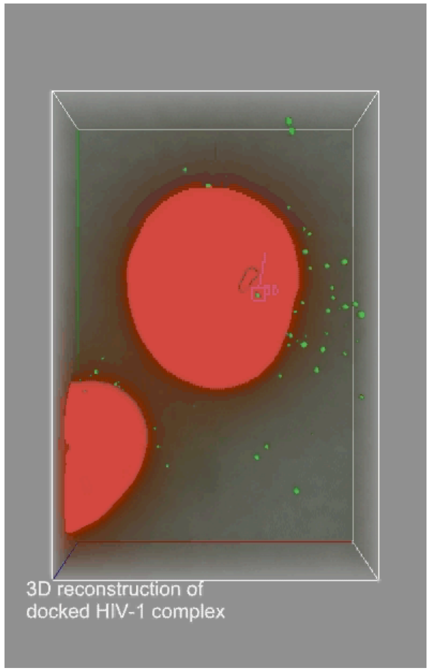
Biological Image Analysis
Multitarget Tracking Applications

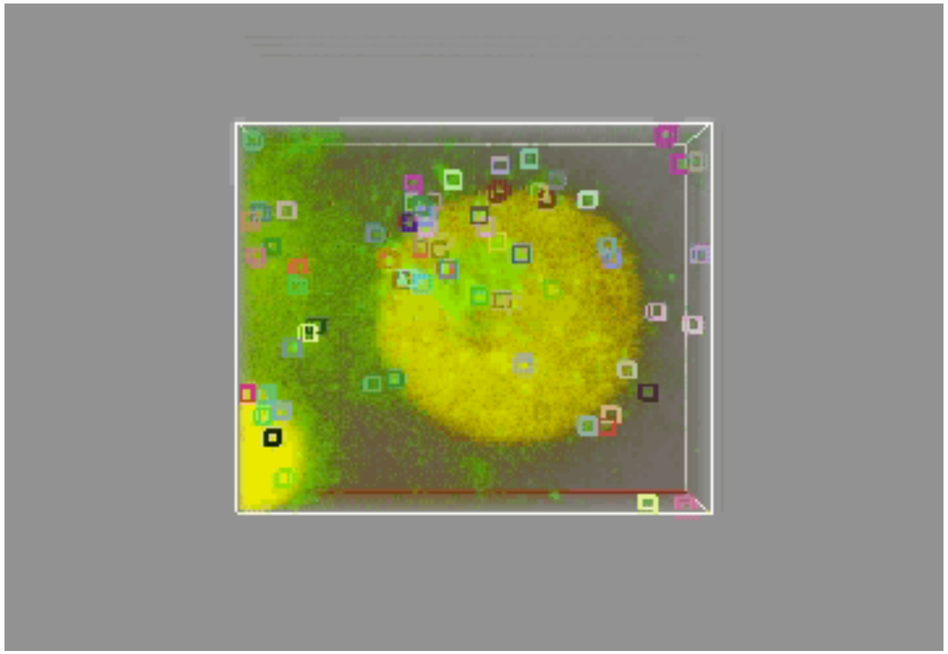
A. Genovesio

HIV dynamics in HeLa cells



	Type	Velocity	3D tracks	Movement characteristics
Movement I	Microtubule-directed	peaks at 0.1 to 1 µm/s		Directed
Movement II	Actin-directed	under 0.03 µm/s		Directed
Movement III	Docking at the nuclear membrane	under 0.03 µm/s		Confined
Movement IV	Intranuclear movement	under 0.005 µm/s		Diffusive



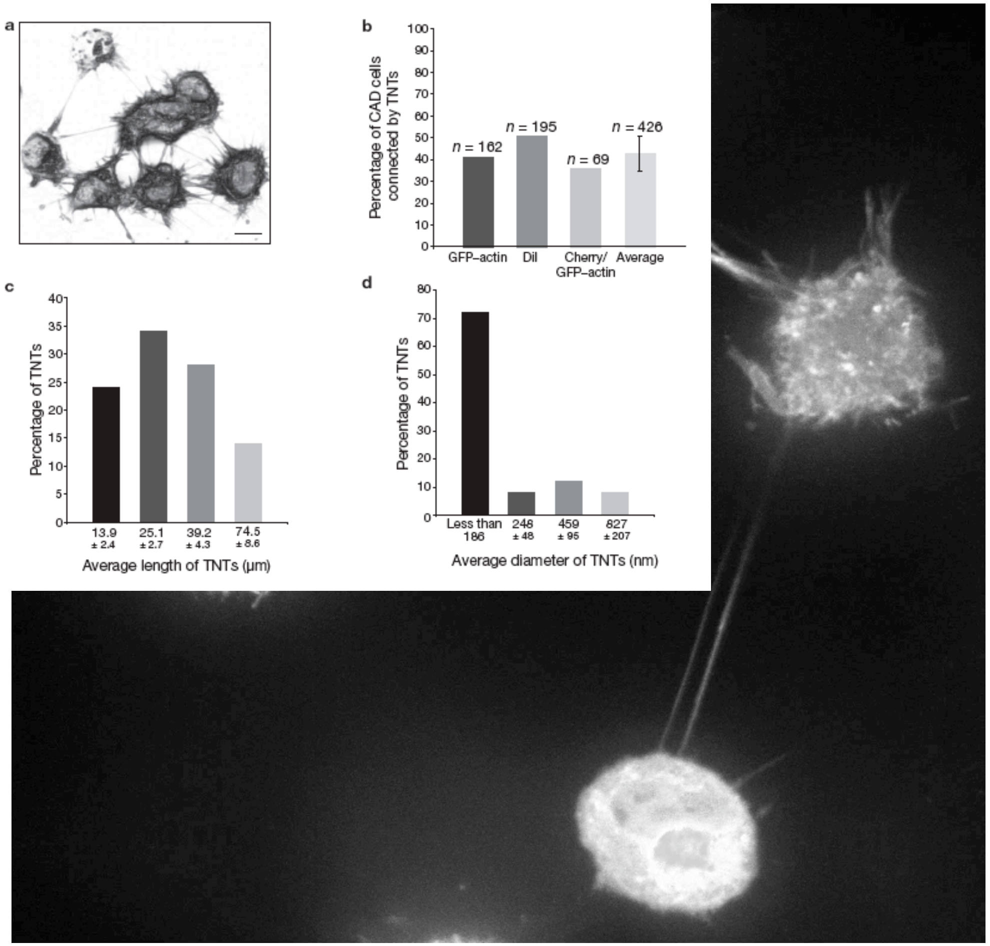


N. Arhel, A. Genovesio, J.-C. Olivo-Marin, S. Shorte, P. Charneau, *Nature Methods* (2006)

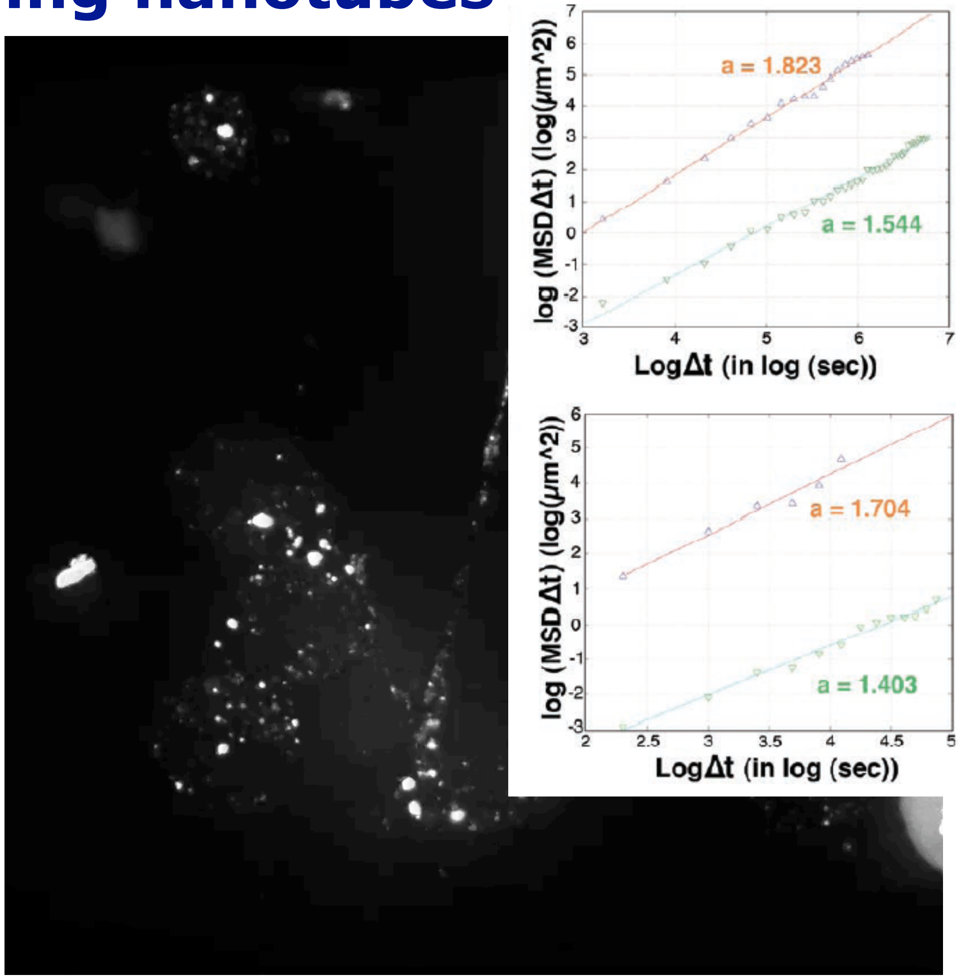
Biological Image Analysis
Multitarget Tracking

F. deChaumont

Prions on tunneling nanotubes



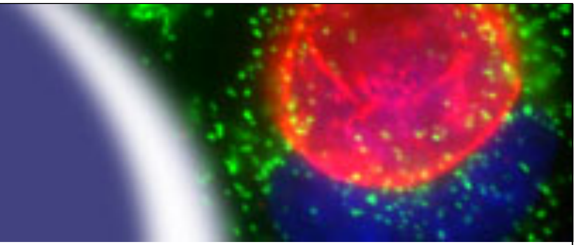
Intercellular TNTs dynamics



Prions moving on TNTs

Gousset K., et al.,, *Nature Cell Biology* (2009)

Summary



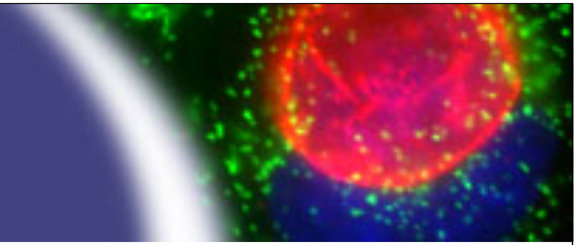
Present

- Image acquisition can be optimized thanks to mathematics
- Sophisticated image analysis are required to fully exploit imaging
- Analysis of image is one of the major bottlenecks in biological imaging
- Methods ought to be fully automatic and provide comprehensive data
- Handle the high variability of biological data
- Provide robust, quantitative and reproducible results

What's next:

- Integration of nanoscale physics
- Development of sophisticated mathematical imaging
- Predictive modeling of biological functions

Quantitative Image Analysis Unit



Members

M. Blanchard
N. Chenouard
F. de Chaumont
A. Dufour
M. Marim
V. Meas-Yedid
A. Servais
I. Szléliga

Alumni

L. Ait-Ali
S. Berlemont
Z. Belhassine
S. Carme
S. Cousquer
F. Coutier
G. Cuartero
V. DerApramian
M. Féral
A. Genovesio
E. Glory
J. Leduigou
F. Ollivier
L. Pénard
S. Tilie
S. Vaie
B. Zhang
C. Zimmer

Support

CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

AGENCE NATIONALE DE LA RECHERCHE ANR

NanoSciences ILE-DE-FRANCE

INSTITUT CARNOT Pasteur MI

Collaborators

Institut Pasteur

A. Alcover
P. Bastin
J.P. Bourgeois
P. Charneau
P. Cossart
A. Dautry
V. Galy
M.L. Gougeon
S. Granon
N. Guillén
E. Labruyère
P.M. Lledo
U. Nehrbass
J.F. Nicolas
A. Phalipon
P. Roux
P. Sansonetti
N. Sauvonnet
S. Shorte
S. Tashbacsh
G. Tran Van Nhieu
C. Zurzolo

External

E. Angelini (Paris)
L. Blanc-Féraud (Sophia-A.)
I. Bloch (Paris)
B. Dubertret (Paris)
F. Frischknecht (Heidelberg)
A. Feuer (Haifa)
A. Guichet (Paris)
Z. Kam (Rehovot)
M. Mhlanga (Johannesburg)
E. Morélon (Lyon)
I. Texier-Nogues (Grenoble)
E. Thervet (Paris)
N. Dey (Sophia-Antipolis)
J. Zérubia (Sophia-Antip.)
A. Ziljstra (Tennessee)