Non-parametric change detection methods in fluorescence life cell imaging for sub-cellular trafficking and exocytosis analysis

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Outline

- **Introduction to biological context and motivations**
- *Non-parametric method for sparse event detection in TIRF microscopy*
- *Experimental results and validation*

Transmembrane proteins under study

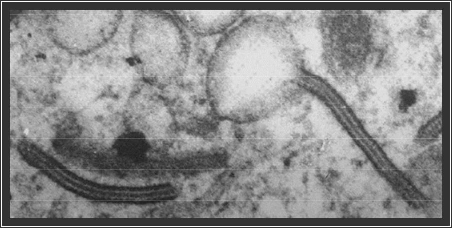
Two constitutively recycling receptors

Transferrin receptor

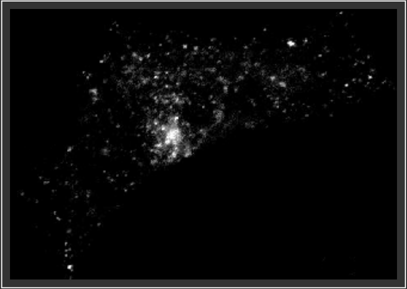
- Well studied canonical recycling transmembrane protein

Langerin

- Major component of Birbeck granules
- C-Type Lectin with carbohydrate recognition domain (CD 207)



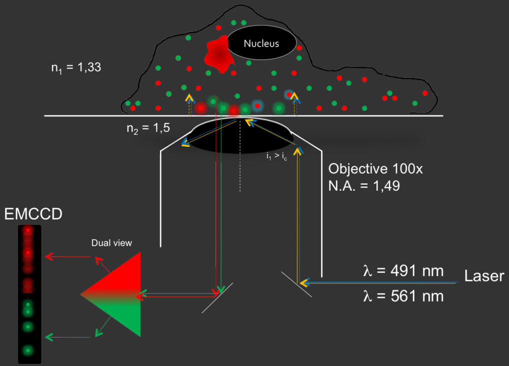
Birbeck granule in EM microscopy [Lipsker 2003]



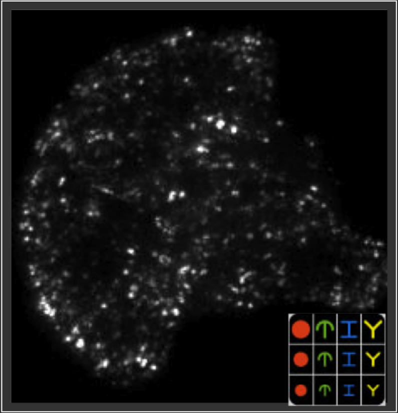
Langerin-YFP in M10 stable cell lines (spinning disk microscopy)

Experimental set-up

Is endocytosis/recycling altered by shape constraints ?
Are events located in specific cell regions ?



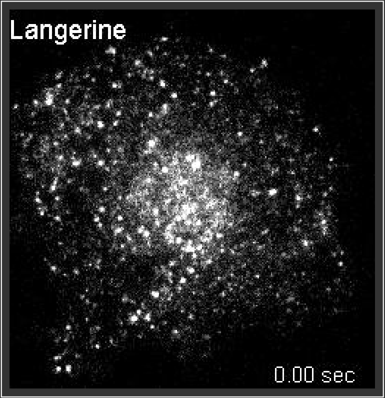
2D+time TIRF microscopy (100ms / frame)

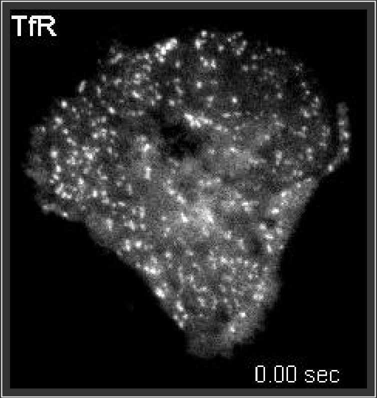


Micro-patterning : shape constraint for variability reduction [Thery 2005]

Comparison of dynamic recycling events

Two independent image sequences and two different micro-patterned cells





Langerin-YFP and TfR-YFP in TIRF microscopy using micro-patterning [Thery 2005]

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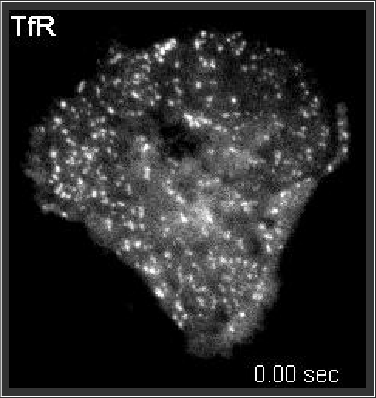

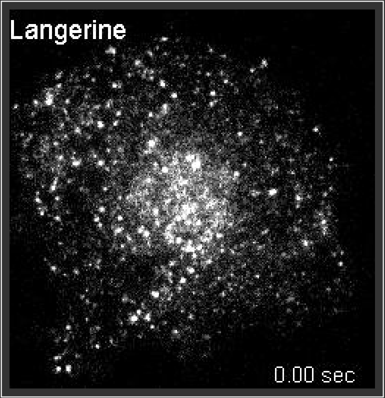

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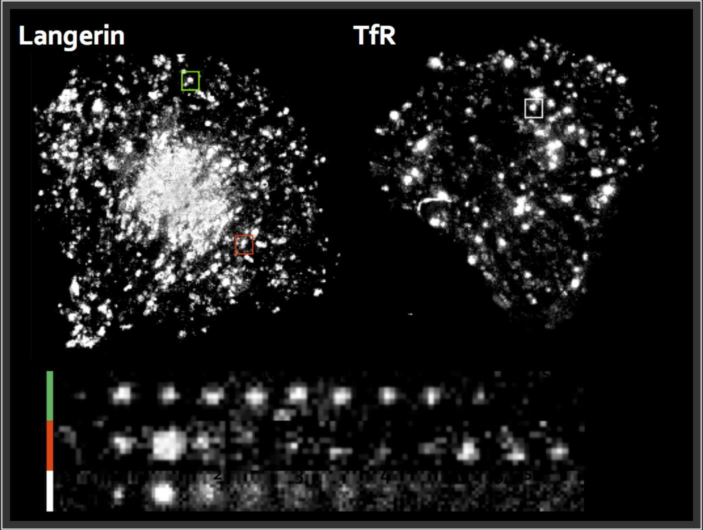
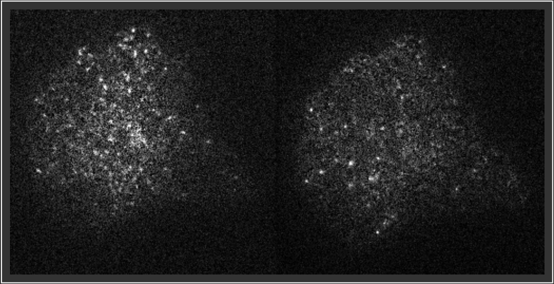


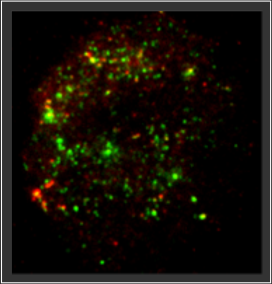
Langerin-YFP and TfR-YFP in TIRF microscopy

Langerin's recycling is Rab11 dependent ...

Dual view of a single micro-patterned cell / two synchronized TIRF image sequences



Rab11 (L) and Langerin (R) in TIRF microscopy



Langerin / Rab11 colocalization

- Space-time organization and coordination of membrane trafficking
- Biogenesis of the recycling compartment and related ultra-structures
- Molecular events and recycling scenarii
- Specificity of addressing mechanisms

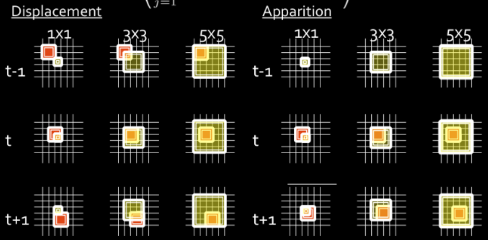
Outline

- *Introduction to biological context and motivations*
- **Non-parametric method for sparse event detection in TIRF microscopy**
- *Experimental results and validation*

Framework for detection in independent sequences: *"Automated Event Detection"*

Non-parametric detector: Probability of finding no similar patch within a neighborhood in the subsequent images is very low

$$P_{H_0}\left(\sum_{j=1}^{N} \mathbf{1}[|X_i - X_j| \geq \tau_i] = N\right) \leq \varepsilon$$



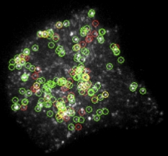
PATCH-BASED ALGORITHMS

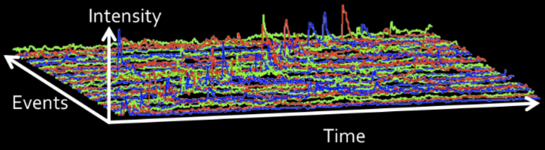
✓ **DENOISING**

C. Kervrann & J. Boulanger, IEEE IP, 2006, Boulanger et al., IEEE PAMI, 2007; C. Kervrann & J. Boulanger, IJCV, 2008; J. Boulanger et al., IEEE TMI, 2010; Carlton et al., PNAS, 2010: Saliba et al., PNAS, 2010; Dokudovskaya et al., Mol Cell Prot, 2011…

✓ **EVENT DETECTION**

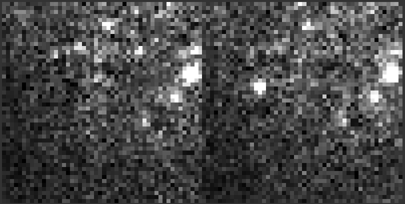
J.Boulanger et al., PLoS One, 2010; A. Chessel et al., Proc. of IEEE ISBI'10; C. Kervrann et al., SIAM MMS, 2011



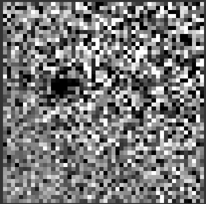


How to detect ONE “meaningful” event on average in an image pair ?

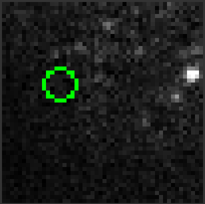
extract of an image pair



difference image



“meaningful” change



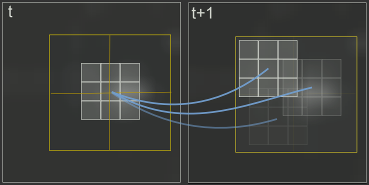
Lateral movement of vesicles (trafficking) and global movement of the cell prevent the application of usual “motion detection” algorithms.

- **Things to do :**
  - Track all the vesicles as much as possible
  - Extract the beginning and ending of the trajectories...
- **Problems :**
  - Isn’t it an overkill ?
  - Robustness of the tracking method $\rightsquigarrow$ over-detection ?
  - How to control the number of false alarms ?

Image redundancy and notations

- **Definition and notations :** Let $u = (u(x))_{x\in\Omega}$ and $v = (v(x))_{x\in\Omega}$ be an image pair defined over a bounded domain $\Omega \subset \mathbb{R}^2$.

- **Non-parametric detection :**
  - A change occurs at pixel $x \in \Omega$ if we find no match between a n-dimensional patch $\underline{u}(x)$ from u and patches $\underline{v}(x)$ from v.
  - We consider a fixed size search window $B(x) \subset \Omega$ (semi-local neighborhood) where $N = |B(x)|$ is the number of tested patches.



Local scores and collective decision fusion

- **Step 1 :** For each pixel $y \in B(x)$, we compute a score (dissimilarity measure) between patches :

  $$\Phi(\underline{u}(x), \underline{v}(y))$$

  and we compare the score to a threshold $\tau(x)$ (e.g. $\Phi(\cdot) = \| \cdot \|^2$).

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and we compare the score to a threshold $\tau(x)$ (e.g. $\Phi(\cdot) = \| \cdot \|^2$).

- **Step 2 :** At each pixel x, we count the total number of positive decisions and we define $D(x) \in \{0, 1\}$ as :

$$D(x) = \begin{cases} 1 & \text{if } \sum_{y \in B(x)} 1[\Phi(\underline{u}(x), \underline{v}(y)) \geq \tau(x)] = N \\ 0 & \text{otherwise} \end{cases}$$

... a change occurs at location x if all the scores are higher than $\tau(x)$ (maximum vote).

Advantages and difficulties...

The setting of thresholds $\tau(x)$, the search window size N and the patch size n must be addressed to make the procedure successful !

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1. Motion (traffic) in the background is not well defined
2. Collaborative neighborhood-wise decisions (space and scale) is attractive for implicit spatial regularization (patch overlapping) [Boulanger 2010]
3. Performance analysis of detectors in terms of false alarm rates
4. No energy minimization procedure
5. No optical flow estimation

Analysis of background motion amplitude

- **Local detection threshold :** Given $B(x)$, $\tau(x)$ is defined as the highest score computed from the reference image u :

$$\tau(x) \stackrel{\triangle}{=} \sup_{y \in B(x)} \Phi(\underline{u}(x), \underline{u}(y))$$

- **Size of the search window $B(x)$:** The choice of N depends on the motion amplitude we do not want to detect... The value $\frac{\sqrt{N}}{2}$ is related to the expected amplitude of background dynamics [1].

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1. Let $s(x) \in \mathbb{R}^2$ be a 2D brownian motion. If $u \in \mathbb{R}^\Omega$ is K-Lipschitz ($|u(x) - u(y)| \leq K\|x - y\|$), then

$$\mathbf{P}\left(\|s(x)\| \geq \frac{\sqrt{N}}{2}\right) \leq \frac{nK^2\mathbb{E}[\|s(x)\|^2]}{\tau(x)}.$$

From local to global decisions

- **Motivation :** We need to make a pointwise decision from large spatial and multiscale contexts... detecting unusual events (one spot on average) when comparing two images.

- **Multiscale modeling :**
  1. Let $\{D_1(x), \cdots, D_L(x)\}$ be the set of binary decisions at pixel x, where $D_\ell(x)$ is the decision made for a given patch size $n_\ell = (2\ell + 1)^2$, $1 \leq \ell \leq L$ and L is the number of sizes considered at each location.
  2. The binary Bernouilli variables $D_\ell(x)$ at pixel x are correlated because the patches with different sizes are nested.

Patch-space framework

- **Property :** Let X be a random variable defined as the sum of L correlated Bernouilli variables. X is known to tend to a Poisson law in distribution as $L \to \infty$ (Chen-Stein method [Arratia 1989]).

- **Multiscale decision :** The probability of false alarm $\mathrm{P}_{FA}(x)$ is given by the Poisson tail with parameter λ. A change occurs at pixel x if $(S_L(x) = \sum_{\ell=1}^{L} D_\ell(x))$:

$$\begin{aligned} \mathrm{P}_{FA}(x) &\triangleq \mathbf{P}\left(X \geq S_L(x) | H_0\right) \approx 1 - \sum_{k=0}^{S_L(x)} (\lambda)^k \frac{e^{-\lambda}}{k!} \\ &\leq \frac{\varepsilon}{|\Omega|} \end{aligned}$$

... on average 1 pixel is falsely detected if $\varepsilon = 1$ [Desolneux, Moisan and Morel, 2000].

A practical algorithm ...

Kervrann et al., SIAM J. Multiscale Modeling & Simulation, 2011

Let us consider 3×3 search windows $B(x)$ and L patch sizes.

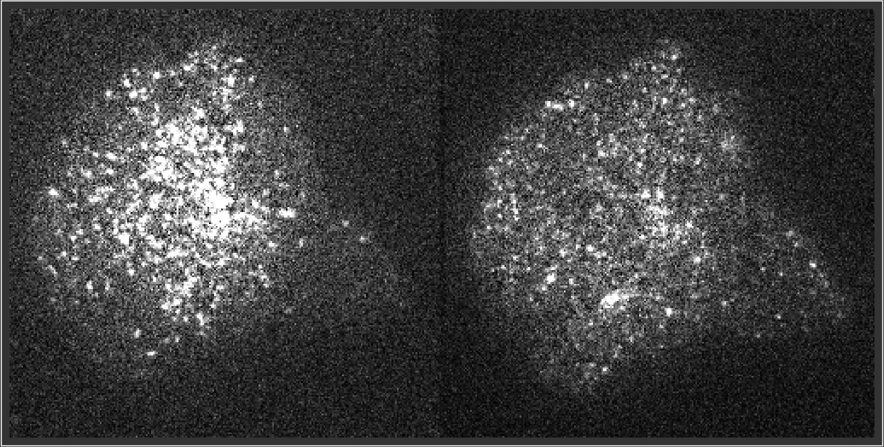
1. For $\ell = 1 \cdots L$
   - For each pixel $x \in \Omega$ compute $\tau_\ell(x) = \sup_{y \in B(x)} \Phi(\underline{u}_\ell(x), \underline{u}_\ell(y))$
   - For each pixel $x \in \Omega$ compute $D_\ell(x) \in \{0, 1\}$:

$$D_\ell(x) = 1\left[\sum_{y \in B(x)} 1[\Phi(\underline{u}_\ell(x), \underline{v}_\ell(y)) \geq \tau_\ell(x)] = N\right]$$

2. Compute $\lambda = \frac{e^{-N}}{|\Omega|} \sum_{\ell=1}^{L} \sum_{y \in \Omega} e^{\sum_{z \in B(y)} 1[\Phi(\underline{u}_\ell(x), \underline{v}_\ell(y)) \geq \tau_\ell(x)]}$

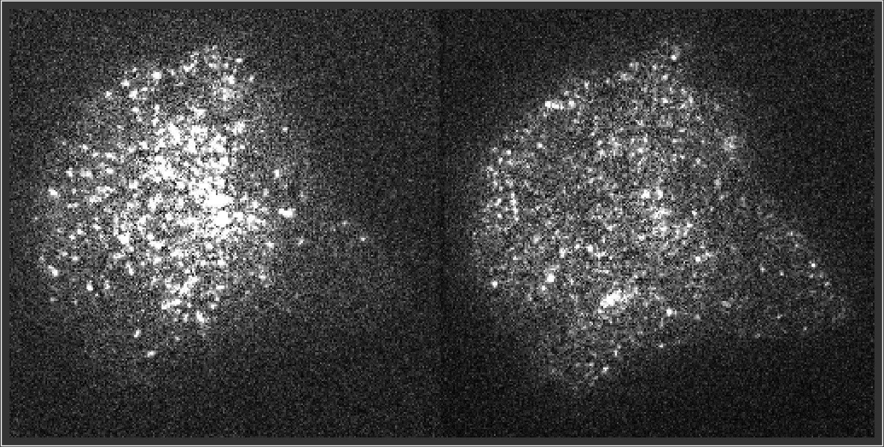
3. A “meaningful” change occurs a location $x \in \Omega$ if $\mathrm{P}_{FA}(x) \leq \frac{\varepsilon}{|\Omega|}$.

A first demonstration...



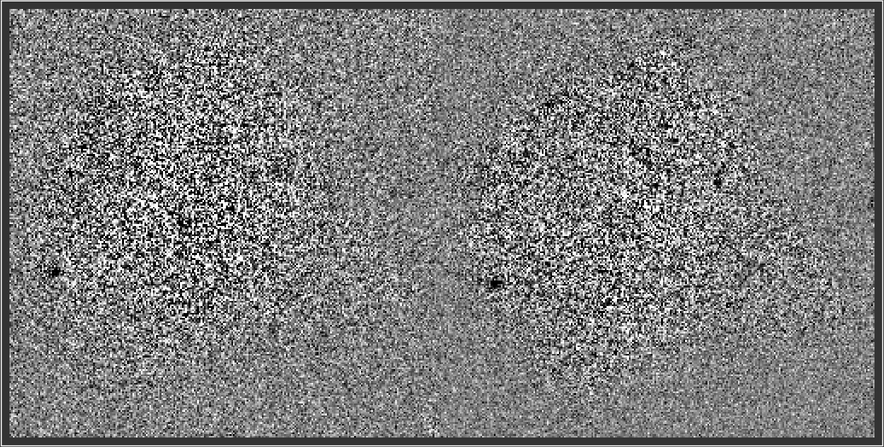
Rab11 (L) and Langerin (R) in TIRF video-microscopy (frame # 192)

A first demonstration...



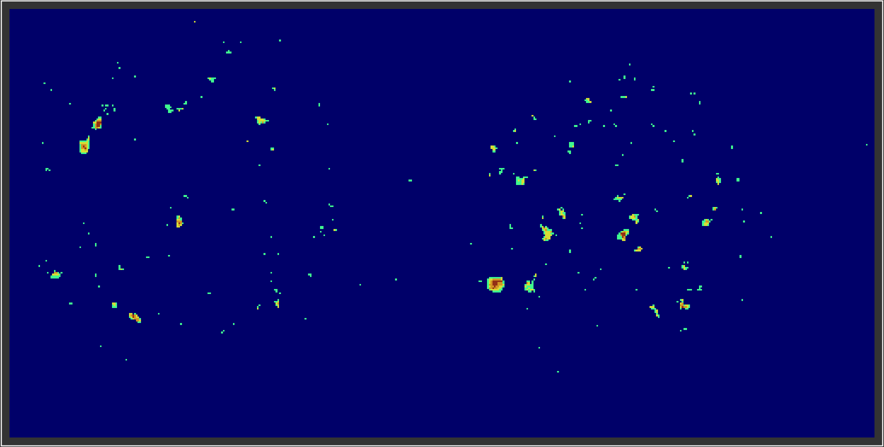
Rab11 (L) and Langerin (R) in TIRF video-microscopy (frame # 193)

A first demonstration...



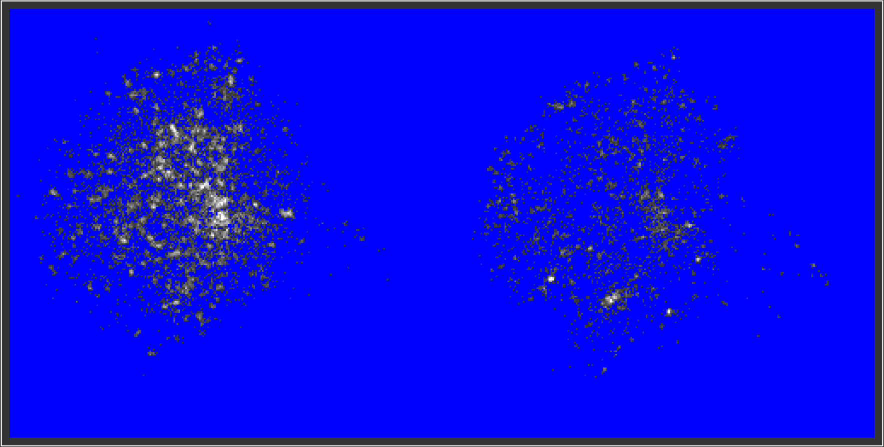
Difference image (Rab11 (L) and Langerin (R))

A first demonstration...



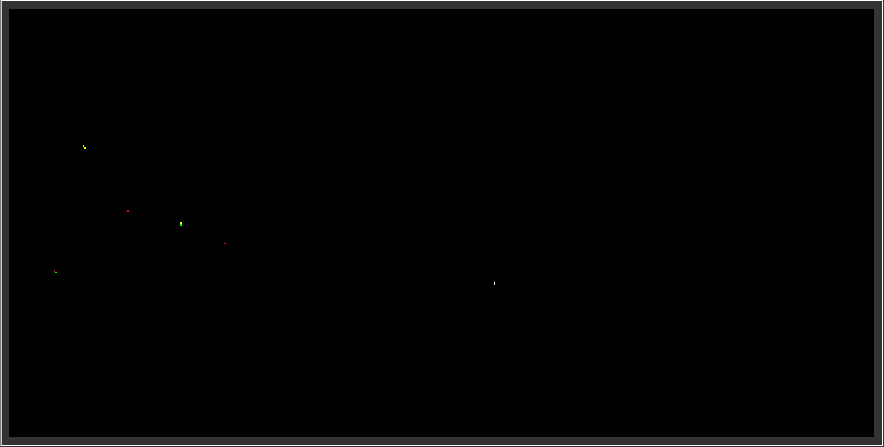
Probabilities of false alarms (regions of interest are in red)

A first demonstration...



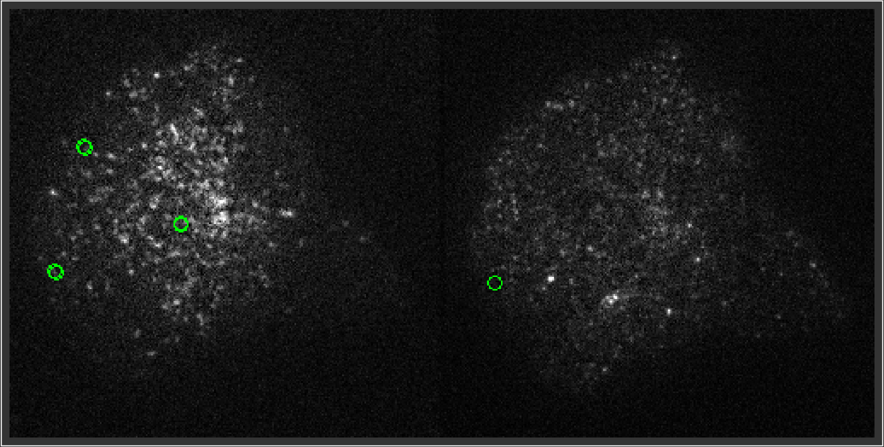
Analysis on significant fluorescence signals

A first demonstration...



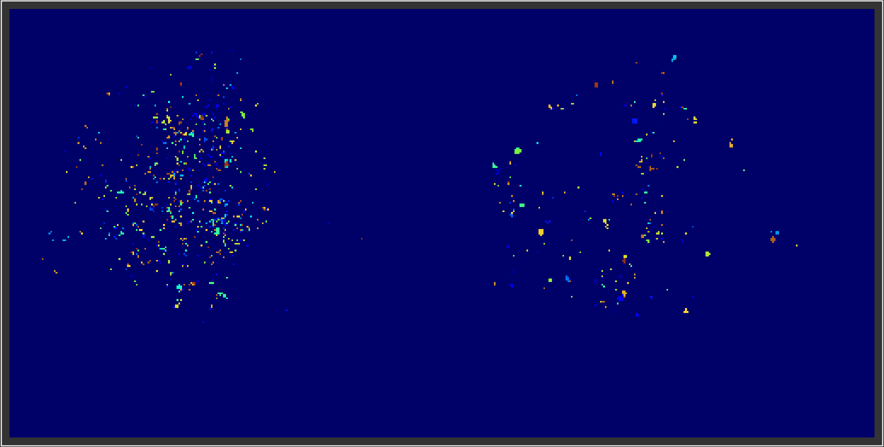
Probabilities of false alarms from significant fluorescence signals

A first demonstration...



Localization of "meaningful" appearances

A first demonstration...

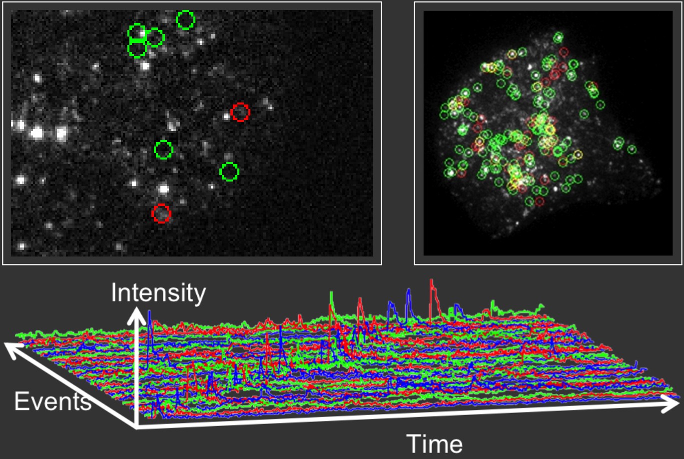


Cumulative change detection map

Outline

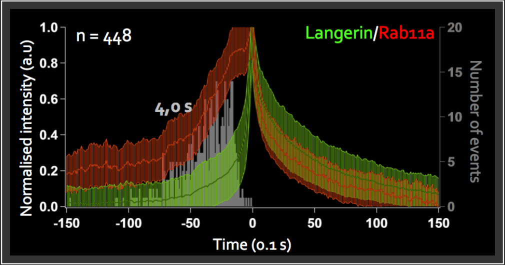
- *Introduction to biological context and motivations*
- *Non-parametric method for sparse event detection in TIRF microscopy*
- **Experimental results and validation**

Patch-Based Event Detection (PBED) algorithm

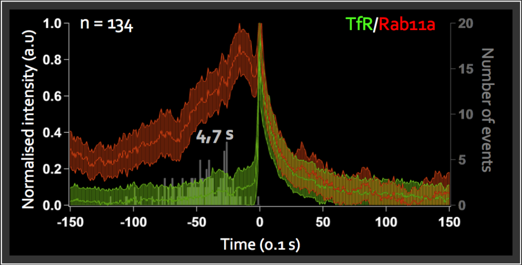


Detection of of appearances (green) and disappearances (red) for YFP-Langerin in M10 stable cell lines acquired in TIRFm (100ms / frame)

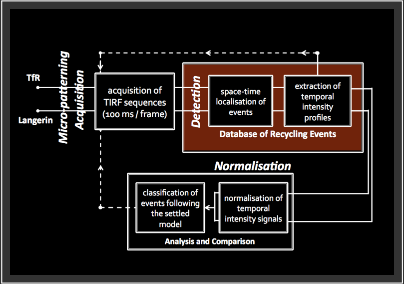
Basic statistical analysis

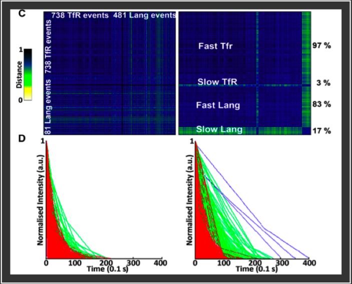


Rab11a escorts the vesicles until the fusion with the plasmic membrane



Workflow from photons to signal clusters

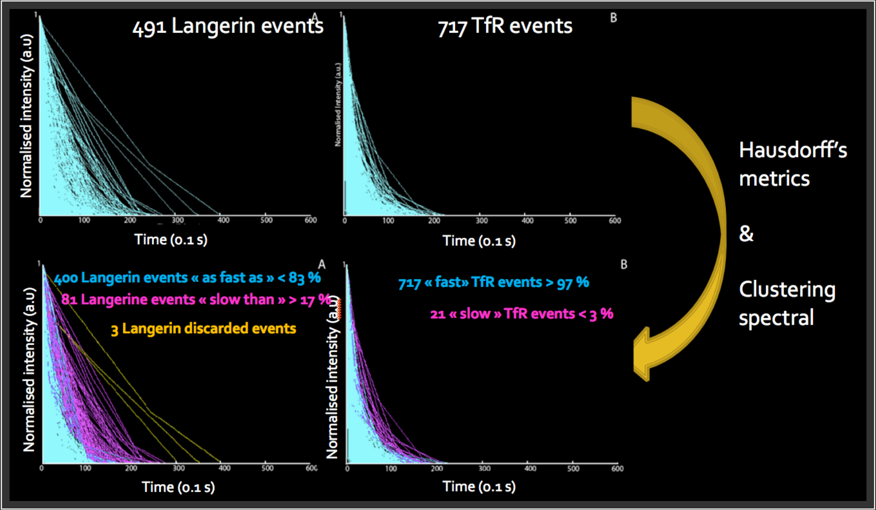




- **Sparse detection of ”meaningful” events**
- Signal descriptors : *convex hulls (computational geometry and alpha-shapes / ”Hullkground” algorithm*[2] *)*
- Event classification : *Hausdorff’s distance and spectral clustering*

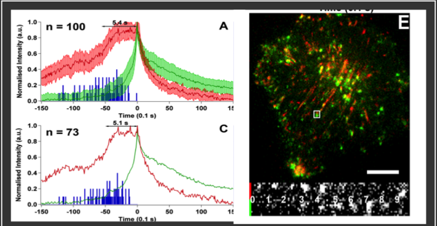
2. A.Chessel et al., Proc. of SSVM’09 ; Proc of IEEE ISBI’10

Classification of two cargo proteins



- Quantification of the heterogeneity of Langerin recycling behaviors
- Measurement of "docking-fusion" times of vesicles

Specific regulation of the Langerin cargo recycling by the Rab11A/Rab11FIP2/MyoVb platform [3]



Conditions	Number of detected events	% of coloc.	Number of treated events	$t_{1/2}$ (s)	Slow Events (%)
Langerine (5)	481	Ø	481	1.04	17
RTf (7)	738	Ø	738	0.75	3
Lang/ Rab11a (12)	1210	92	448	1.78	21
RTf/ Rab11a (4)	350	70	134	0.81	4
Lang/ Rab11FIP2 (4)	352	80	100	2.58	40
RTf/ Rab11FIP2 (4)	370	80	81	0.67	3
Lang/ Rab11FIP2-RBD	499	82	191	0.92	5

- MyoVb plays a crucial role
- Rab11A dissociates concomitantly to the docking/fusion process
- Rab11FIP2 is a sensor for regulation of fusion
- Cargo specificity

3. Boulanger et al, PLoS ONE 2010 ; Gidon et al., Traffic 2012 ; Chessel, Cinquin et al., in revision

PBED : General tool for endo-exocytosis event analysis

- Automatic detection of docking/membrane fusion events
- Quantification of space-time interactions

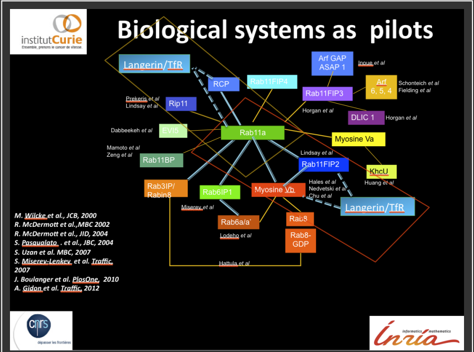
Change detection algorithm :

1. Patch-based image representation
2. Intuitive algorithm parameters and implicit regularization
3. Collaborative neighborhood-wise decisions (space and scale)
4. Performance analysis (false alarm probabilities)
5. No energy minimization procedure and no optical flow estimation

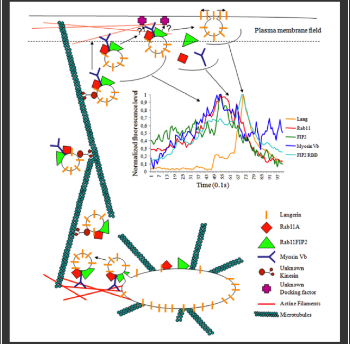
$\sim$ 85% **of success rate wrt manual labeling (several hundred image pairs) !**

Space-time coordination of membrane trafficking

Langerin’s recycling is Rab11 dependent ...



Platforms and molecular complexes



Traffic overview

- Biogenesis of the recycling compartment and related ultra- structures
- Molecular events and recycling scenario
- Specificity of addressing mechanisms