

Image processing for correlated and multimodal imaging techniques

# QUANTIFICATION

Extracting quantitative information from correlated multimodal dataset

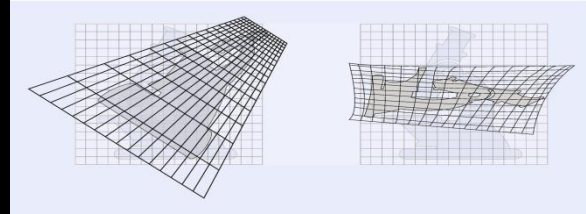
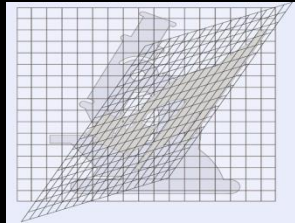
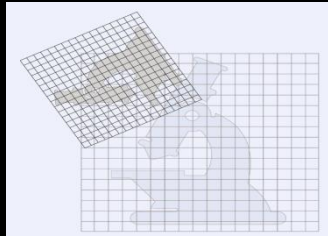
[Perrine.paul-gilloteaux@univ-nantes.fr](mailto:Perrine.paul-gilloteaux@univ-nantes.fr)

# OUTLINE

- Why do you want to fuse multimodal information?  
Once is fused, how do you exploit the spatial correlation?
  - How do you check it is correctly fused?
-

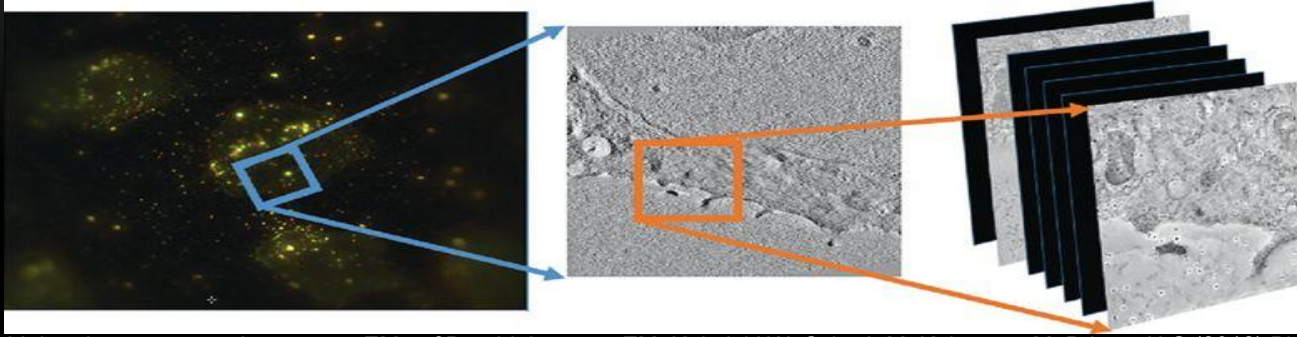
First, some reminder about registration and transformations

- Registration is the process of finding the transformation linking two images/volumes, by optimizing the matching between either intensities, either known matching structures.
- May the sample/specimen transfer/preparation have induced deformation? Is the scale relationship known (pixel size)?



Schorb M., Paul-Gilloteaux P. (2020) Resolving the Process of Clathrin Mediated Endocytosis Using Correlative Light and Electron Microscopy (CLEM). In: Miura K., Sladoje N. (eds) Bioimage Data Analysis Workflows. Learning Materials in Biosciences. Springer, Cham

# WHY DO YOU WANT TO FUSE MULTIMODAL IMAGES?



Schorb M., Paul-Gilloteaux P. (2020) Resolving the Process of Clathrin Mediated Endocytosis Using Correlative Light and Electron Microscopy (CLEM). In: Miura K., Sladje N. (eds) Bioimage Data Analysis Workflows. Learning Materials in Biosciences. Springer, Cham

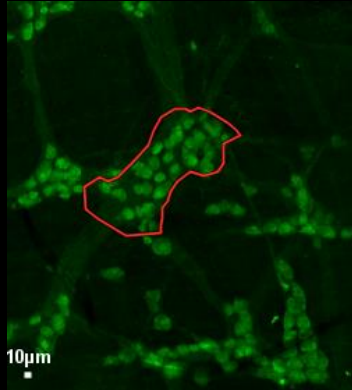
Light microscopy to « low mag » EM to 3D « high mag » EM. Kukulski W, Schorb M, Kaksonen M, Briggs JAG (2012) Plasma membrane reshaping during endocytosis is revealed by time-resolved electron tomography. *Cell* 150(3):508–520.

- **Integrate different source of information about the sample**

Matching structures= beads visible in both modalities

Transformation = Rigid or affine if no deformation,  
spline (non rigid) if sample deformation

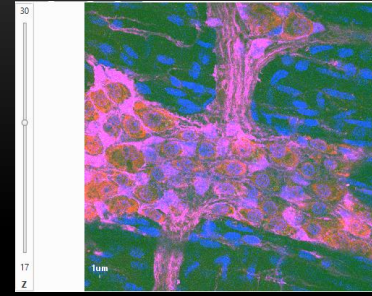
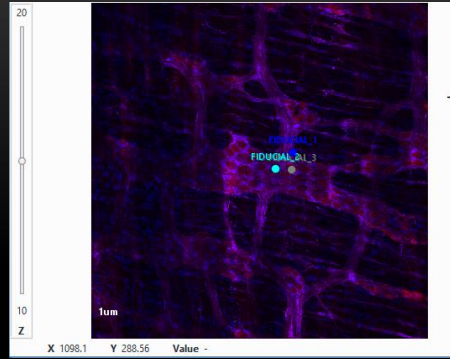
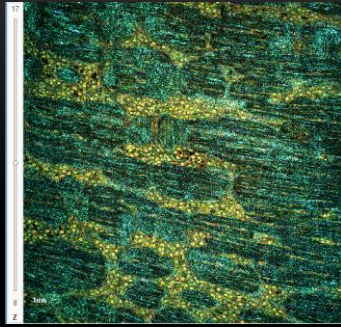
# WHY DO YOU WANT TO FUSE MULTIMODAL IMAGES?



- Integrate different source of information about the sample
- Validate and understand a new modality or a new biomarker/probe/contrast enhancement preparation.

Vibrational OCT (no staining, live tissue)+ fluorescence light microscopy with known staining. Unpublished data, P. Naveilhan/T. Durand/M. Neunlist , Nantes)

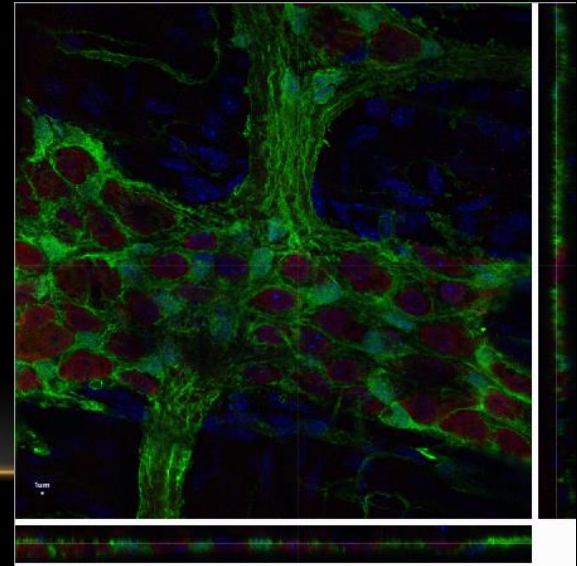
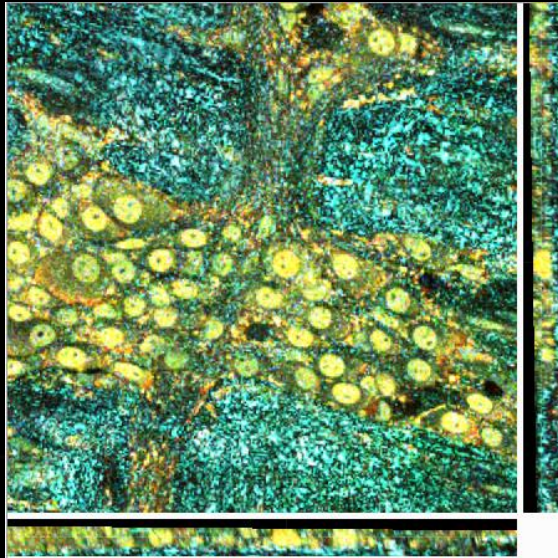
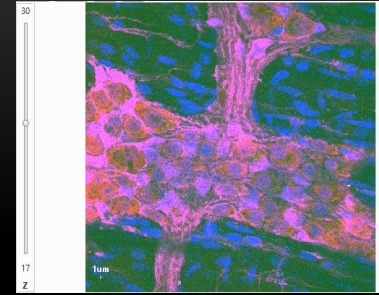
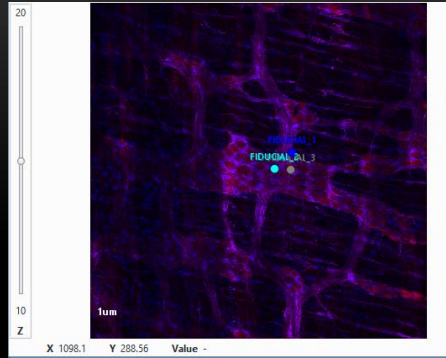
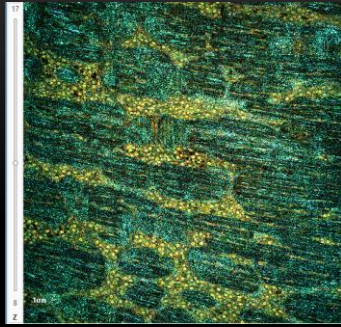
# NO FIDUCIALS: CONSTRAINING THE TRANSFORM TO BE RIGID



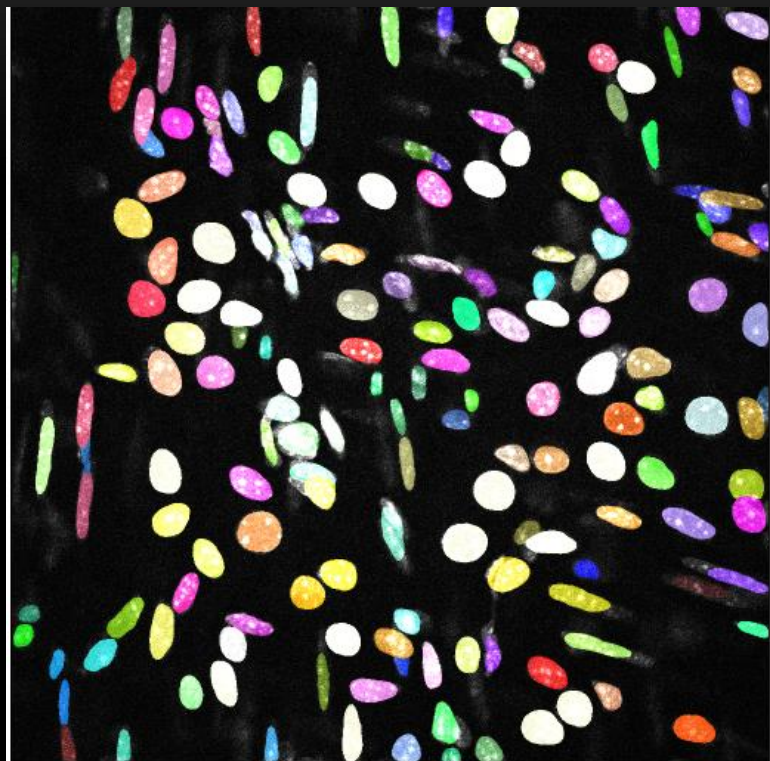
Matching structures= None known, using visible intensity correlation for rough alignment, and center of distinguishable contrasted structures matched with center of nuclei (also centre of cells)

Transformation = Rigid to put a strong constraint and check the validity of the assumption.

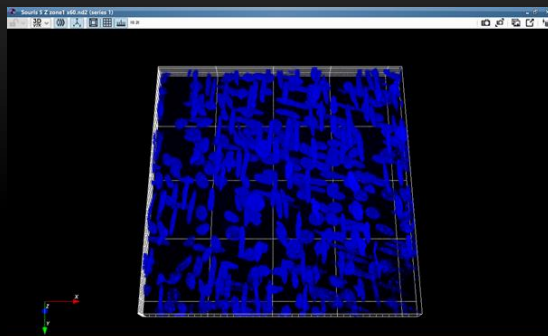
# NO FIDUCIALS: CONSTRAINING THE TRANSFORM TO BE RIGID



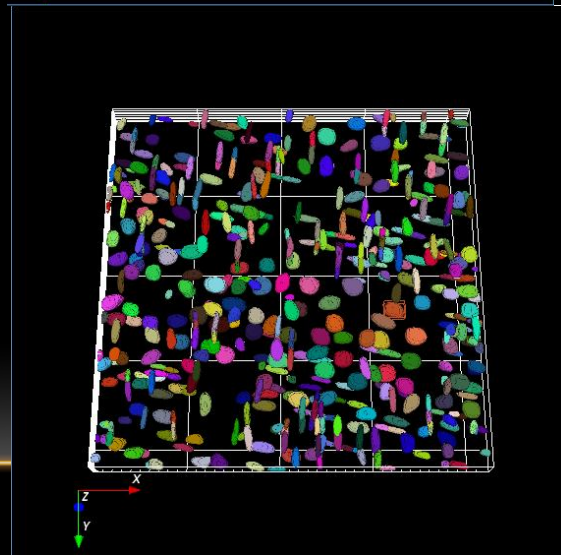
# USING FUSED IMAGES TO CHARACTERISE THE UNKNOWN IMAGING SIGNAL



2D nuclei slices with segmented nuclei overlay



3D nuclei stained



3D segmented nuclei



# 3D INTENSITY MEASURE OF ALL FUSED MULTIMODAL SIGNAL FOR EACH NUCLEUS

The screenshot displays a microscopy software interface with two main image windows and a table on the right. The left window, titled 'Merged', shows a 2D view of a biological sample with numerous colored rectangular regions of interest (ROIs) overlaid. The right window, titled 'C Merge', shows a similar view but with a different color scheme. Both windows include a toolbar with icons for zooming and navigation, and a status bar at the bottom showing coordinates (X, Y, Z) and a value range. The table on the right lists the properties of the detected ROIs, including their names, positions, and colors.

Name	Posit...	Me
ROI Object #...	8	
ROI Object #...	15 79	
ROI Object #...	10 48	
ROI Object #...	27 31	
ROI Object #...	22 41	
ROI Object #...	22 91	
ROI Object #...	8 3.7	
ROI Object #...	12 40	
ROI Object #...	3	
ROI Object #...	15 47	
ROI Object #...	19 47	
ROI Object #...	1	
ROI Object #...	14 40	
ROI Object #...	6 21	
ROI Object #...	15 50	
ROI Object #...	16 64	
ROI Object #...	14 14	
ROI Object #...	27 28	
ROI Object #...	3	
ROI Object #...	2	
ROI Object #...	26 11	

Properties

Color

Stroke

Position

X

Y

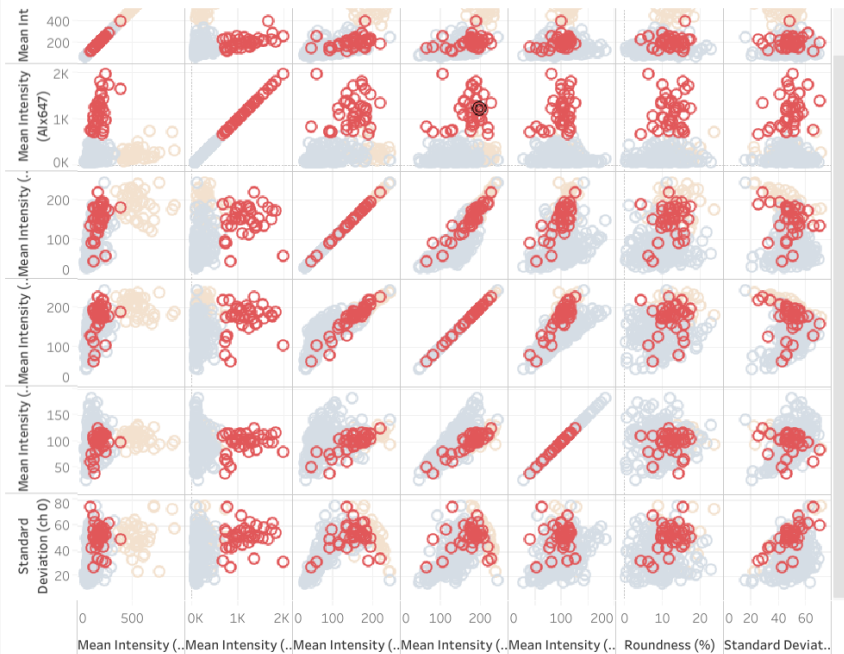
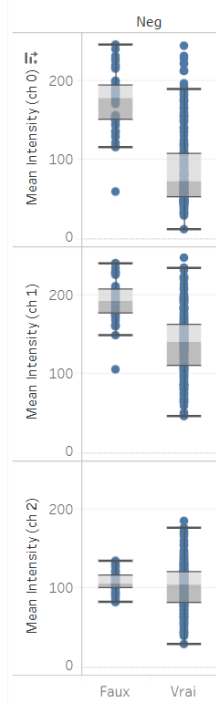
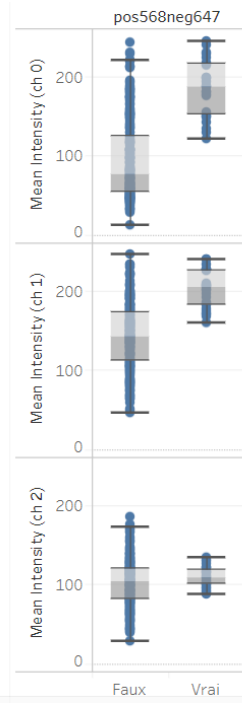
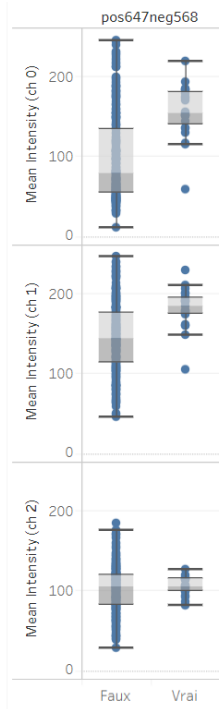
Z

T

C

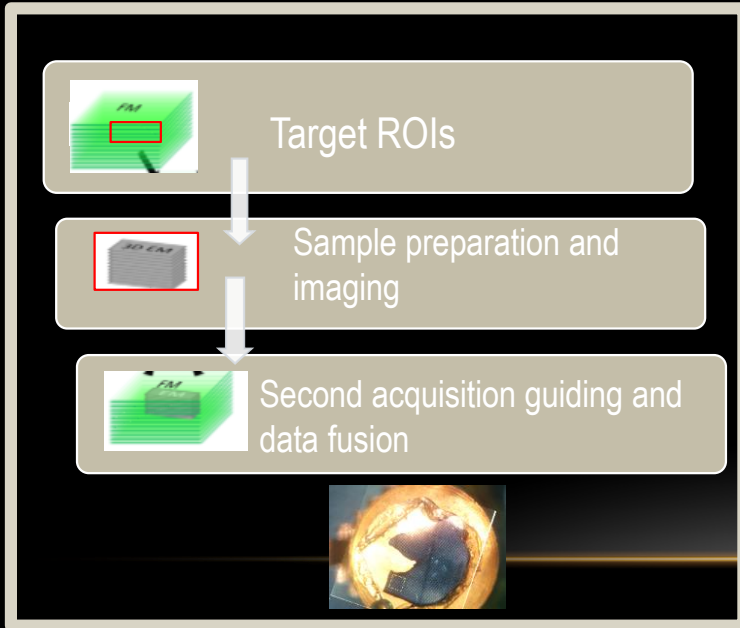
IC4  
Version 2.0.3.0

Based on the known population indicated by the fluorescnet staining, relationship with unknown imaging signal can be established



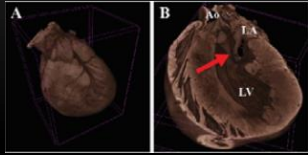
# WHY DO YOU WANT TO FUSE MULTIMODAL IMAGES?

Where am I and where should I acquire the next image? (Margin of error)

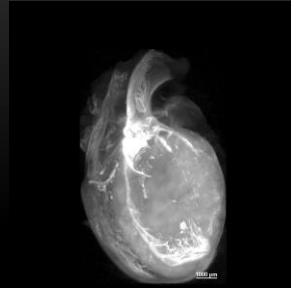


- **Integrate different source of information about the sample**
- **Validate and understand a new modality or a new biomarker/probe/contrast enhancement preparation.**
- **Guide the acquisition**

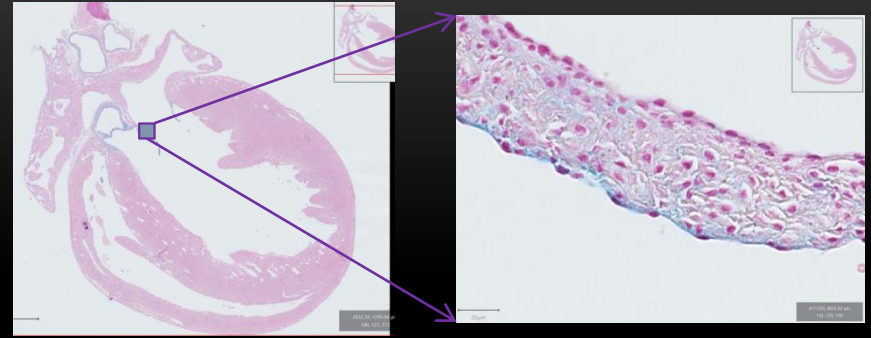
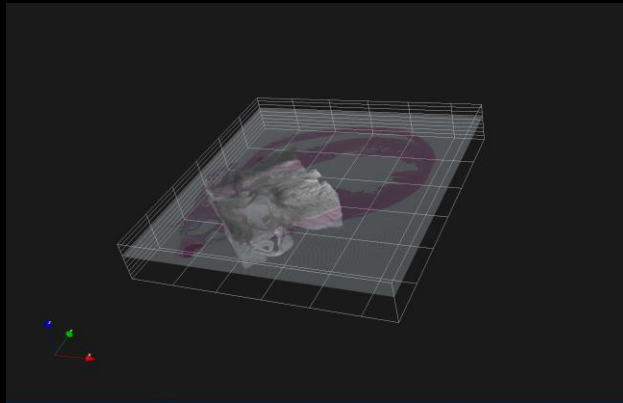
# CORRELATIVES MICROSCOPIES TO GUIDE THE ACQUISITION



microCT, or  
Atlas if  
known

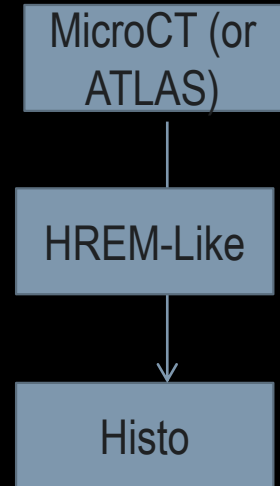


AutoFluorescence on Microtome surface after cut  
-> 3D reconstruction  
3D-3D registration and 2D 2D registration: Use  
shapes.



Histology on microtome slice cuts.

MicroPICell facility and  
Kaerlabs collaboration



# OUTLINE

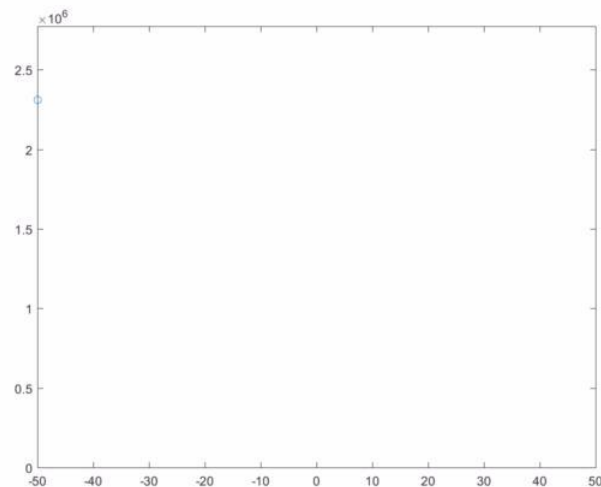
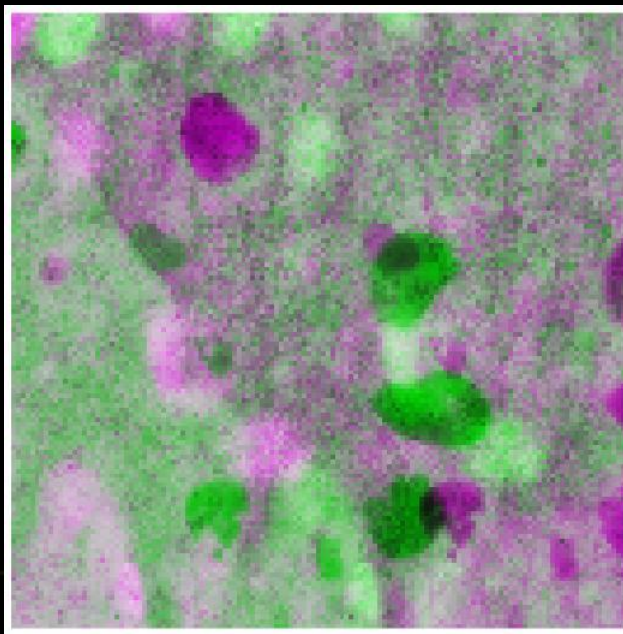
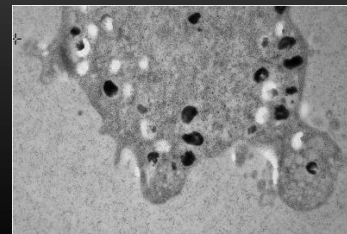
- Why do you want to fuse multimodal information? Once is fused, how do you exploit the spatial correlation?
- How do you check if it is correctly fused?

Multimodal registration validation is known to be one of the most difficult problem in image processing -> there is no ground truth, or only approximation of it (*J .Pluim, International conference of pattern recognition , 2016*)

INTENSITY METRIC SCORING ARE USALLY NOT ADAPTED, some may be very dependent of the common content of your images.

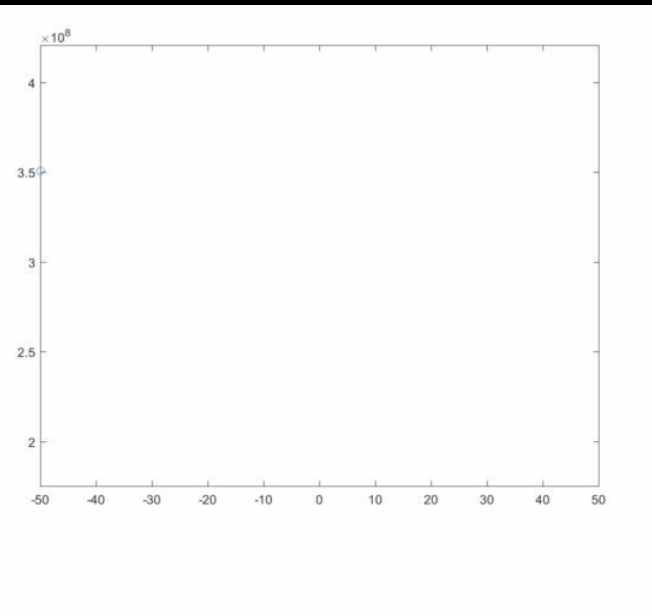
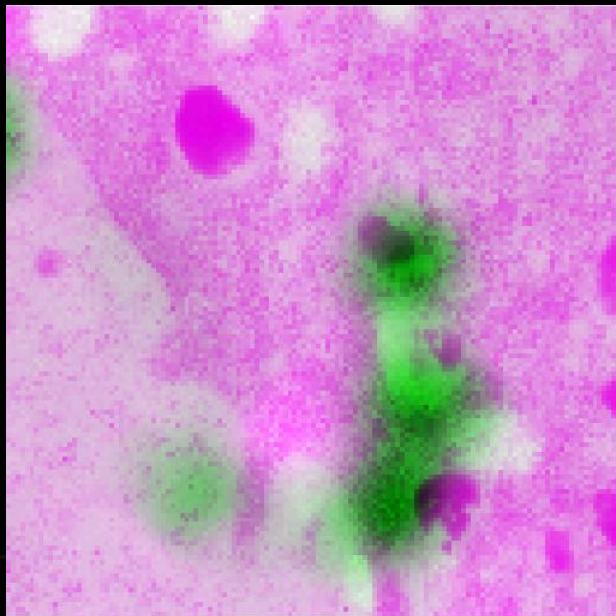
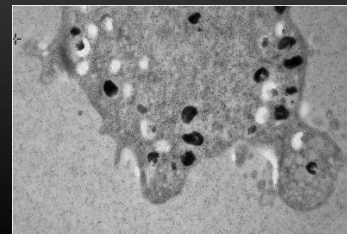
# SUM OF SQUARE DIFFERENCE

$$\sum_{(i,j) \in W} (I_1(i,j) - I_2(x+i, y+j))^2$$



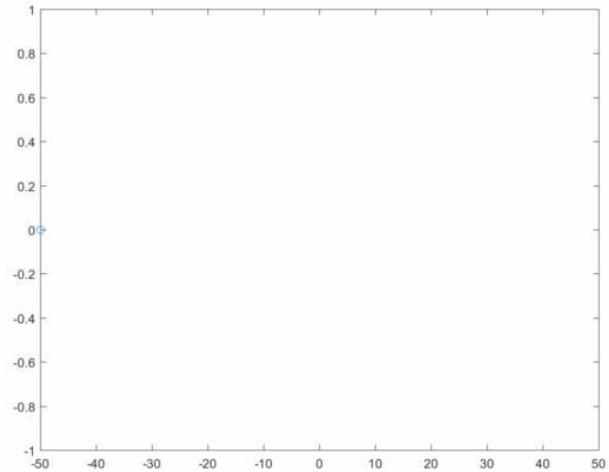
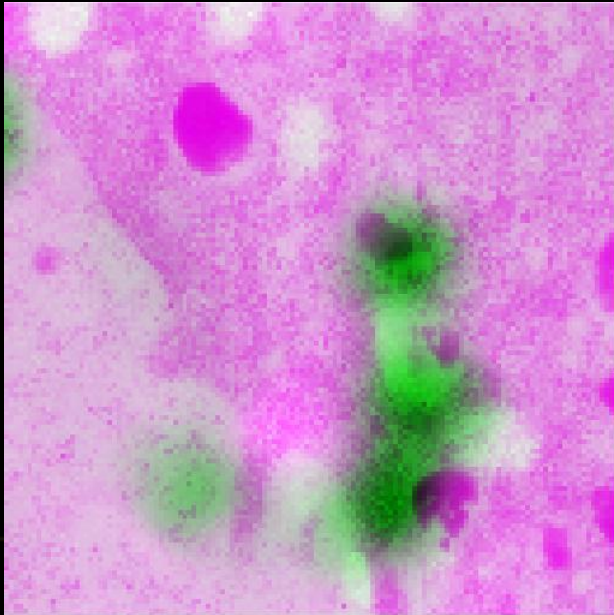
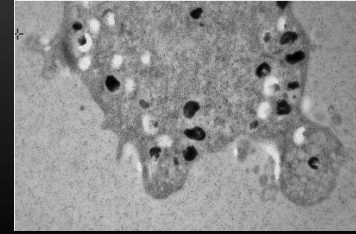
# SUM OF SQUARE DIFFERENCE

$$\sum_{(i,j) \in W} (I_1(i,j) - I_2(x+i, y+j))^2$$



# NORMALIZED CORRELATION

$$\frac{\sum_{(i,j) \in W} I_1(i,j) \cdot I_2(x+i, y+j)}{\sqrt{\sum_{(i,j) \in W} I_1^2(i,j) \cdot \sum_{(i,j) \in W} I_2^2(x+i, y+j)}}$$

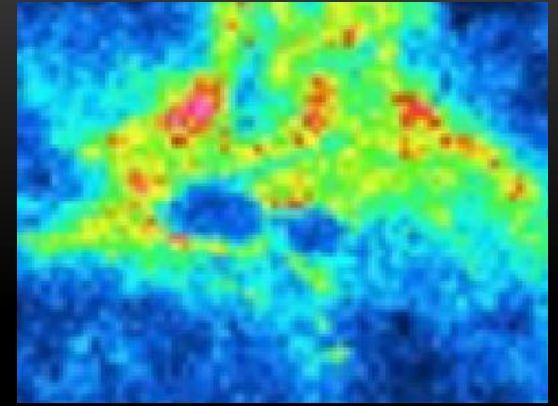
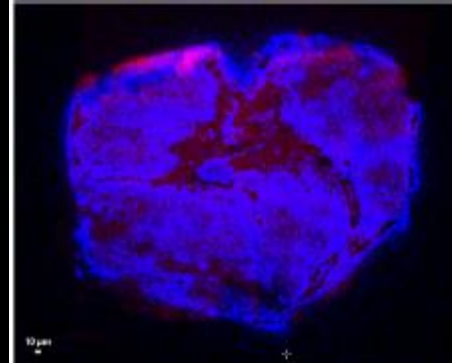
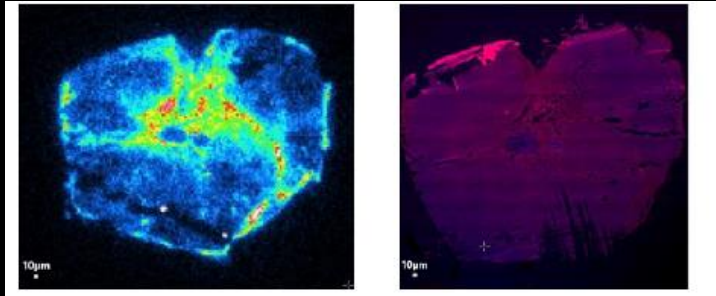




# QUALITATIVE CHECK

Visual inspection using, for instance, side by-side viewing,  
overlays of structures or checkerboard displays

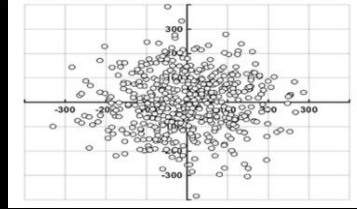
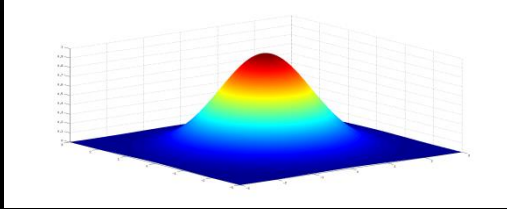
-> warning in particular if you have based your registration  
on the matching of structures or intensity with no constraints on the transformation



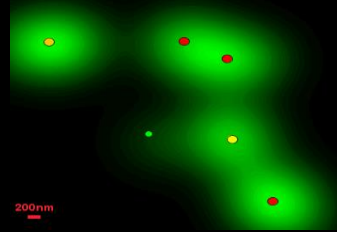
# HOW TO ASSESS THE QUALITY OF REGISTRATION?

- One thing to know: NOTHING IS PERFECT
  - Because your sample may deform/ evolve between two modalities
  - Because multimodality is usually also multiscale (very different resolution, one pixel can match hundred of pixels in the second modality)
  - Because none modality allows to perfectly locate one structure of interest (imaging resolution). Even when the sample did not deform at all , this will create an error you can not avoid, only reduce.

# FIDUCIAL LOCALIZATION ERROR



Individual points

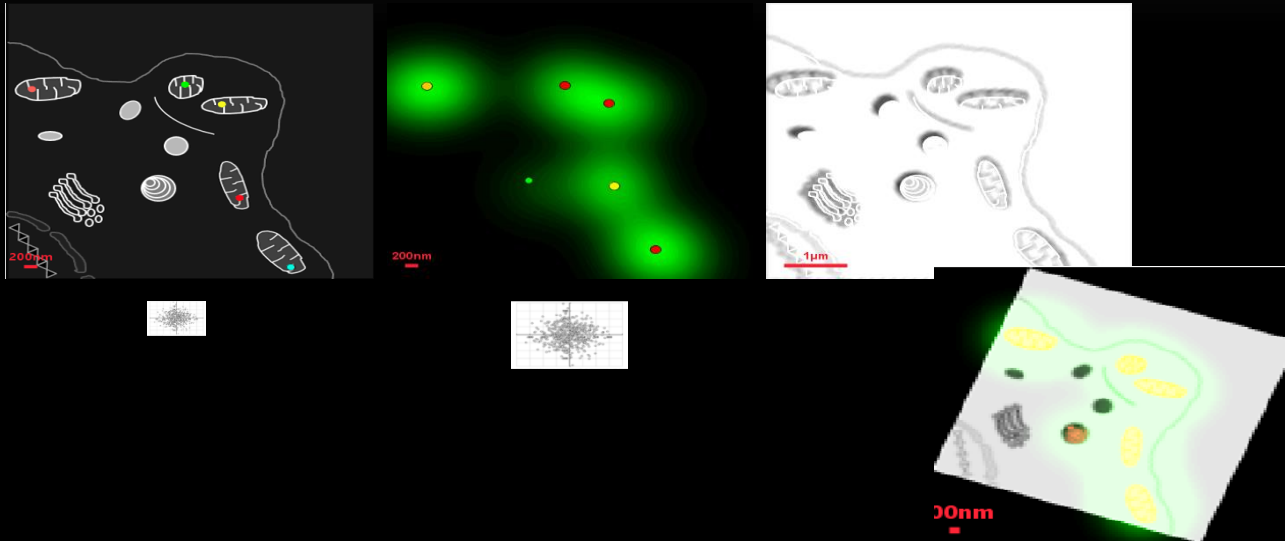


Fiducials can be:

Beads,

Structures of interest (in life sc: point sampled on membrane, nuclei centroids, stretch...)

# REGISTRATION ERROR DUE TO FIDUCIAL LOCALIZATION ERROR



# SO HOW DO YOU CHECK QUANTITATIVELY THE REGISTRATION ACCURACY?

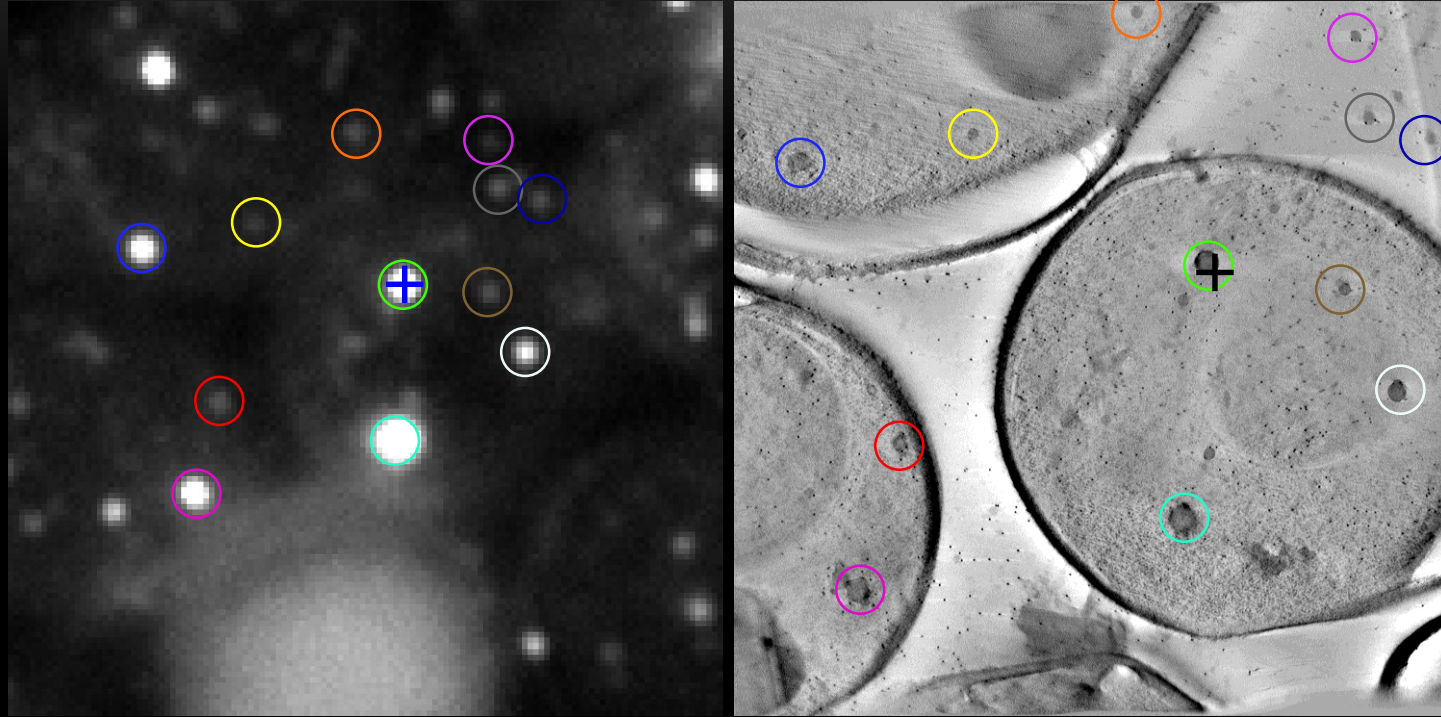
- Check on simulated data (but likely bias in the simulation)
- Apply a known deformation and perform your workflow (like a known compression) to check your approach can recover it
- **Use objects that can be seen in both modalities, and DO NOT USE ALL :**
  - Either Fiducials (points)
  - Either boundaries of known object visible in both modalities, even scratch or others..

# LEAVE ONE OUT METHOD

- How good it is elsewhere, in area /points you did not try to match explicitly by registration?

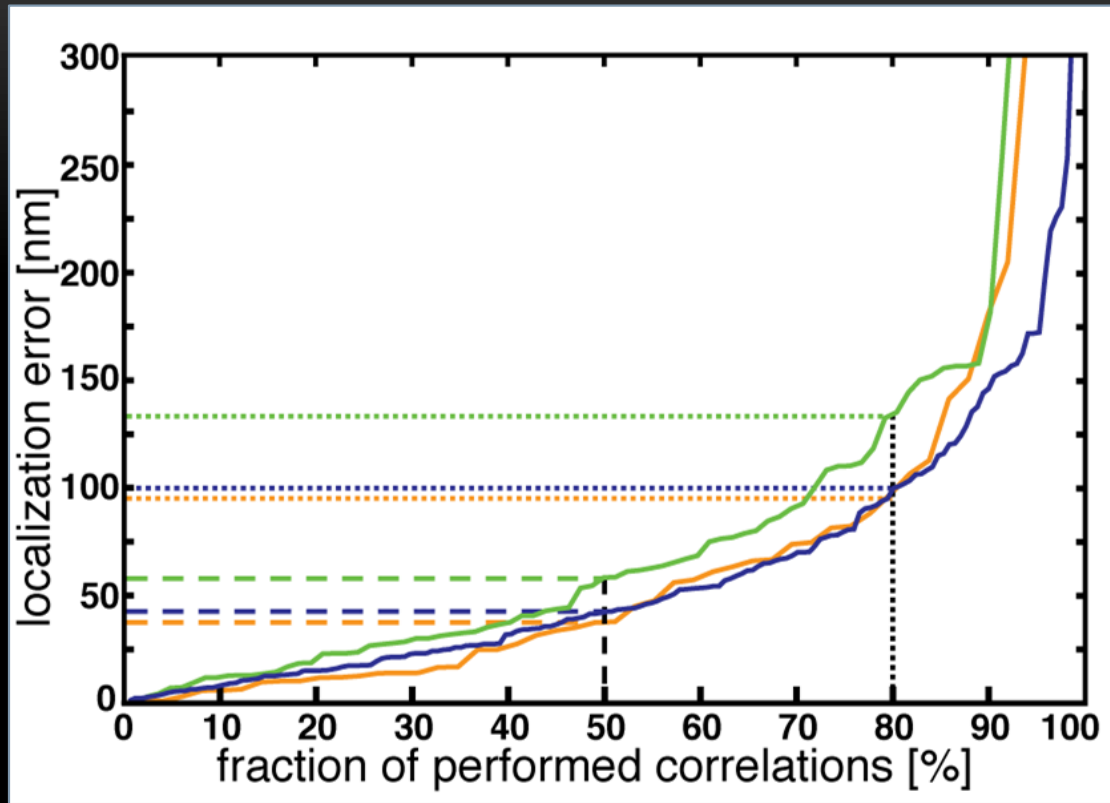
# Error assessment

(prediction of accuracy on non-fiducial part of the image)



**LEAVE-ONE OUT approach: Correlated fluorescence and 3D electron microscopy with high sensitivity and spatial precision Kulkuski, Schorb et al. JCB 2011**

accuracy

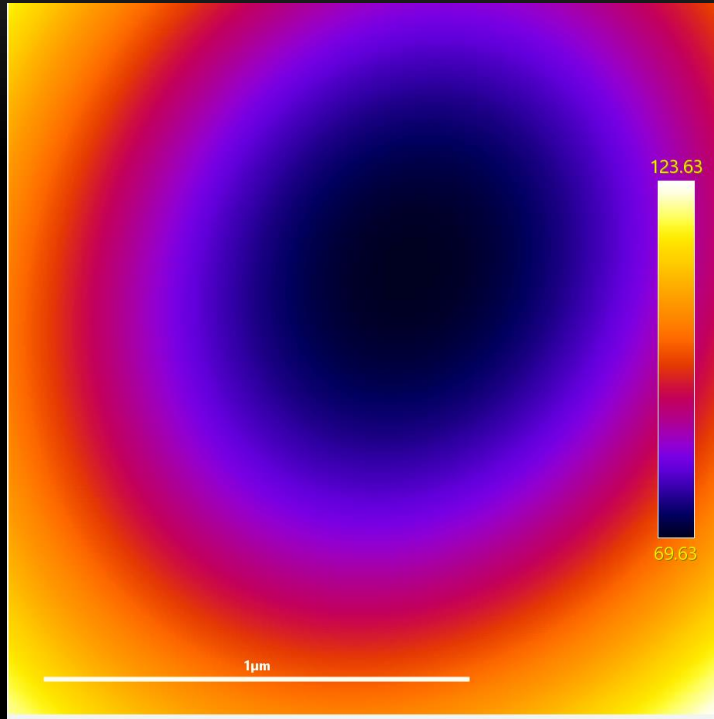


- *S. pombe* dataset
- *S. cerevisiae* dataset
- MDCK dataset

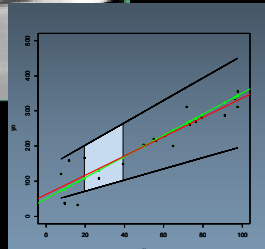
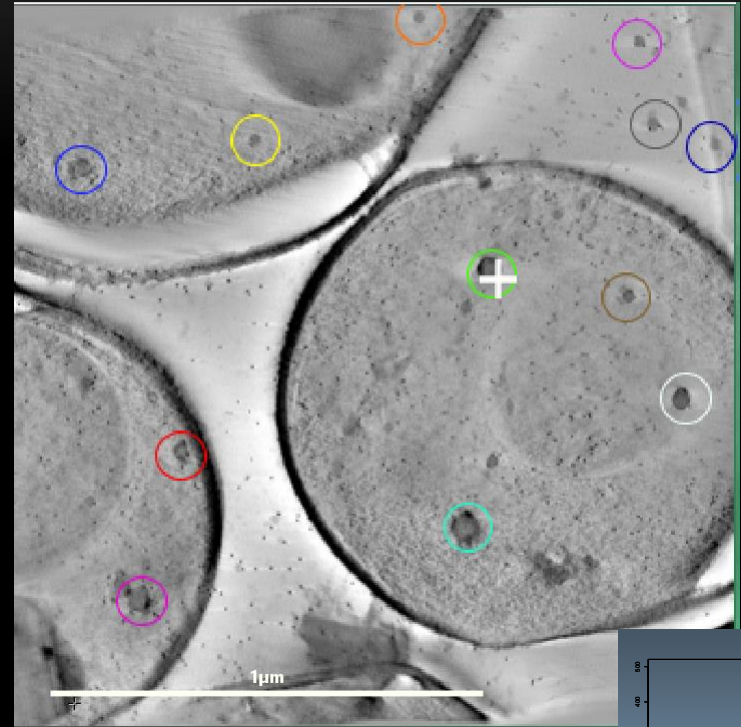
**LEAVE-ONE OUT approach: Correlated fluorescence and 3D electron microscopy with high sensitivity and spatial precision Kulkuski, Schorb et al. JCB 2011**



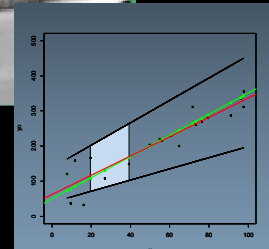
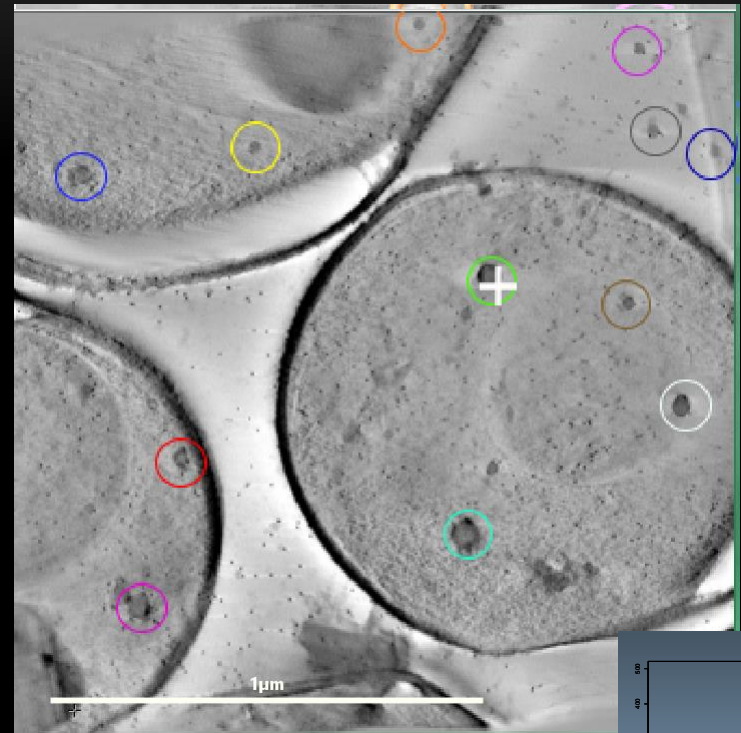
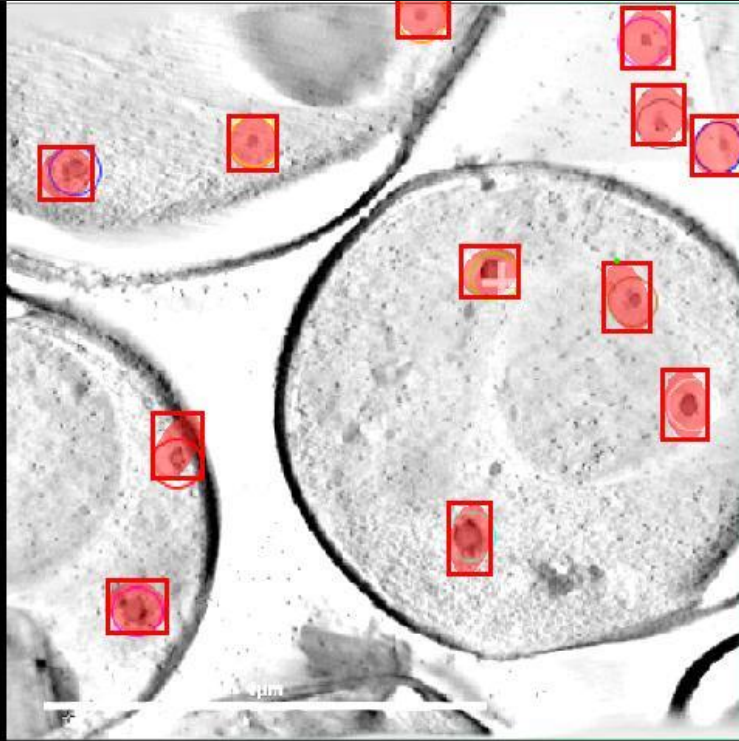
# OUR APPROCH (BASED ON STATISTICS: NOT ON MEASURING ANYTHING ON THE IMAGE)



Color coded average spatial error expectation in nanometers



# OUR APPROCH (BASED ON STATISTICS: NOT ON MEASURING ANYTHING ON THE IMAGE)



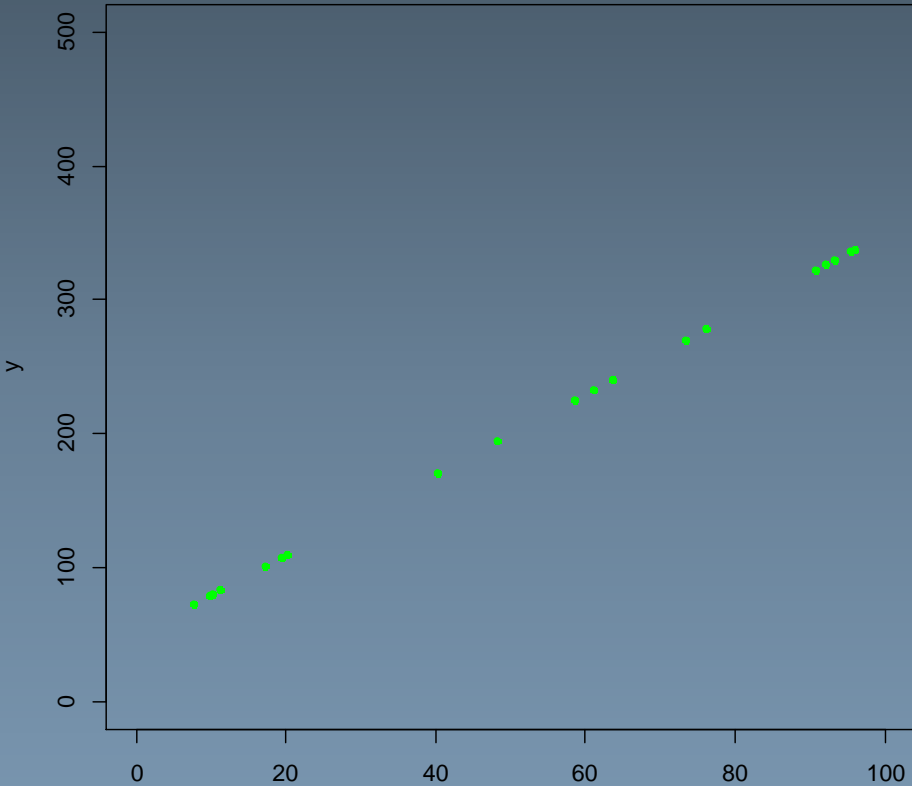
Ellipse giving the 95% interval (somehow the maximum error possible)

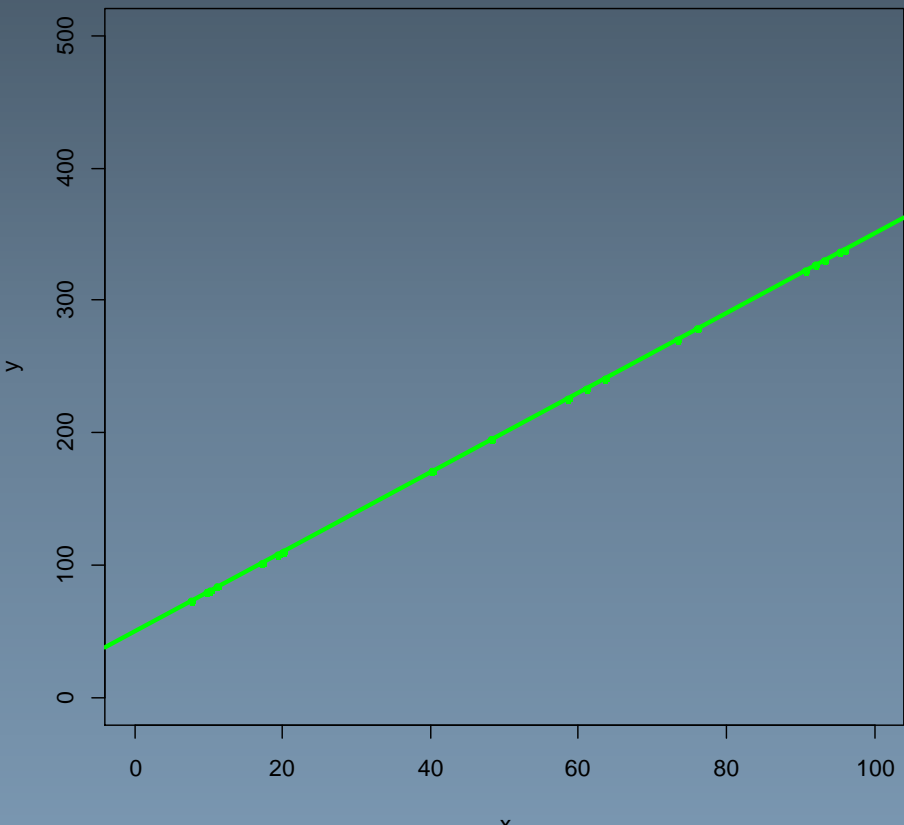
# STATISTICAL METHODS: ASSESSING REGISTRATION QUALITY WITHOUT GROUND TRUTH

- How does it work?

Think registration as a linear regression.

Purpose : compute the transformation linking x  
he position of a point in an image and y its  
position in another image : predict the position  
new y of new x

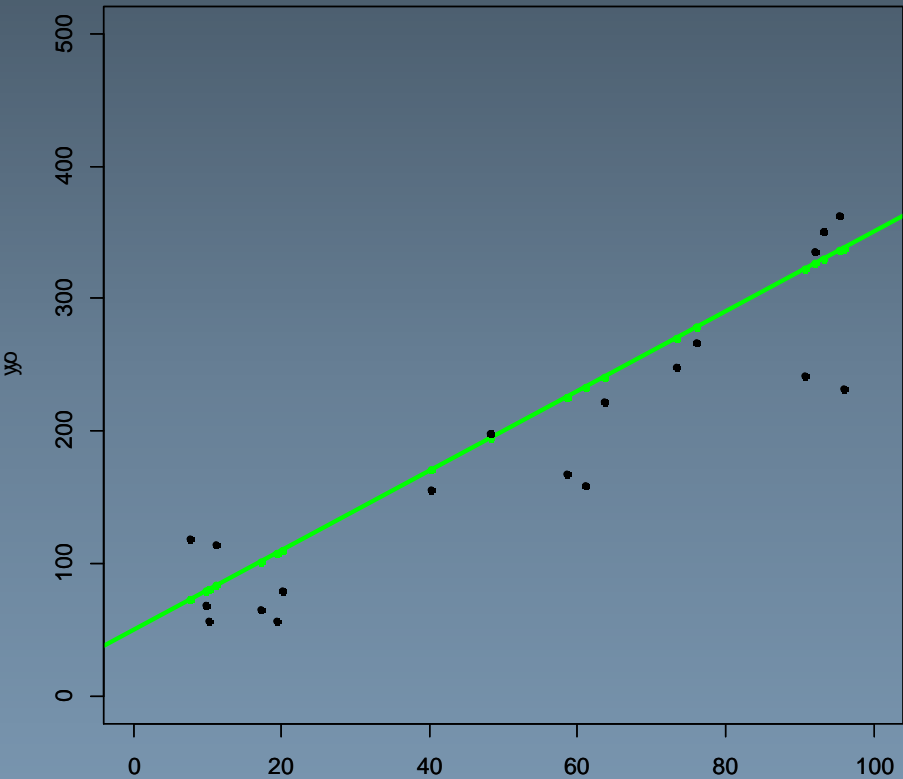




Seeing the transformation computation as a regression problem: example with a linear relationship (**RIGID** i.e rotation + translation)

Find  $R$  and  $T$  such as:

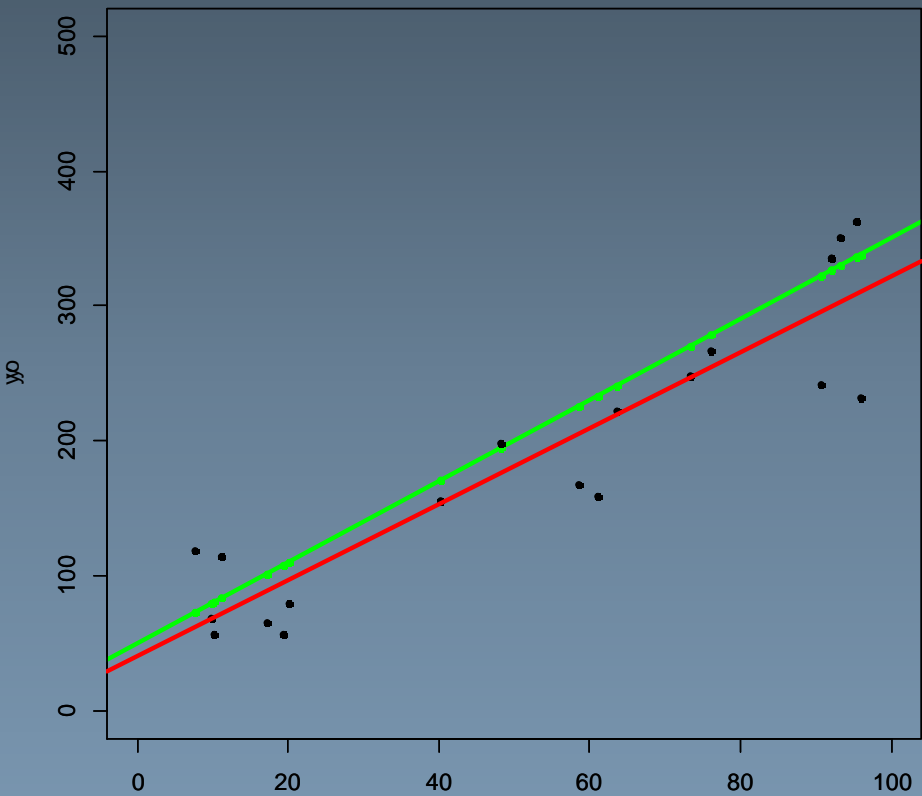
$$y = Rx + T$$



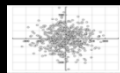
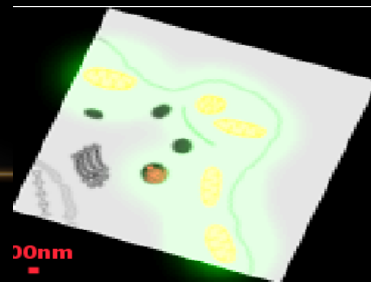
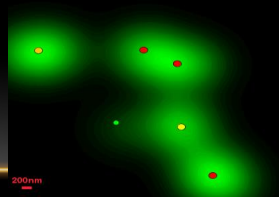
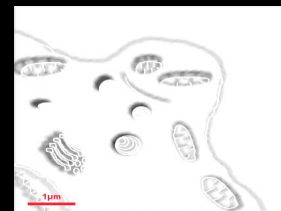
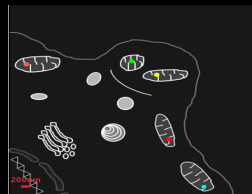
More realistic situation: « noise »  
Typically the error in positioning the fiducial  
(RESOLUTION!!)

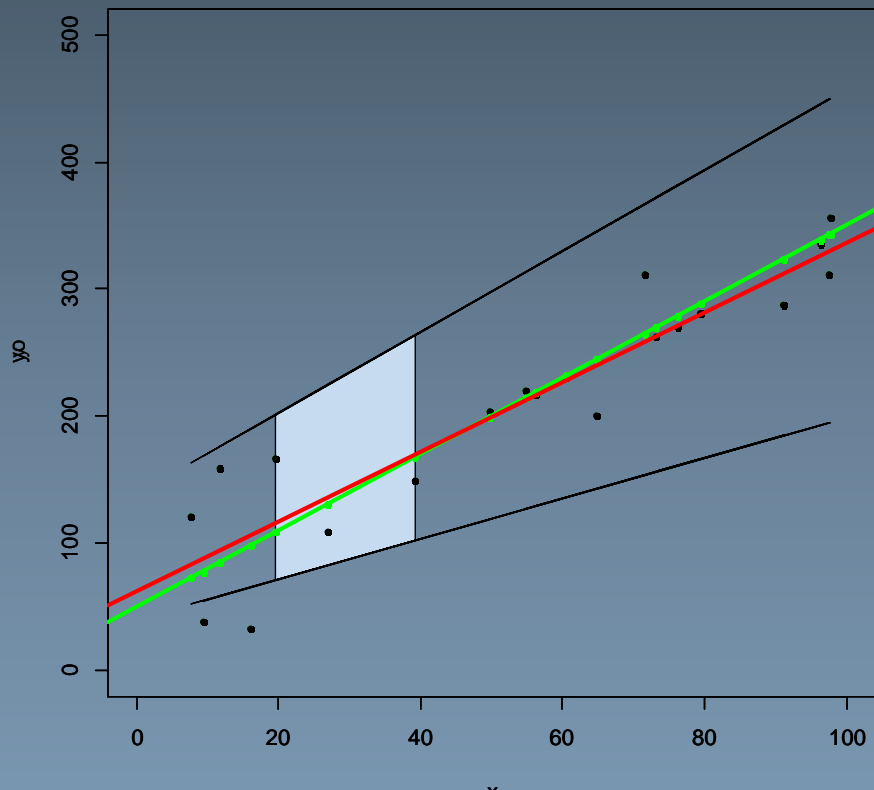
$y_0 = y + \text{noise}$  in localisation (measure)

$x_0 = x + \text{noise}$



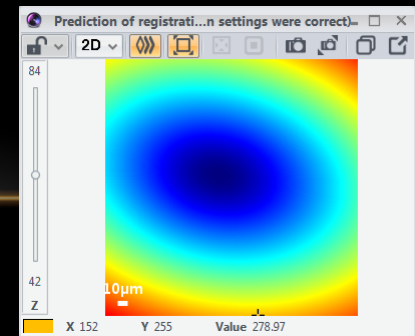
Estimated transform  
 $Y_0 = R X_0 + T$



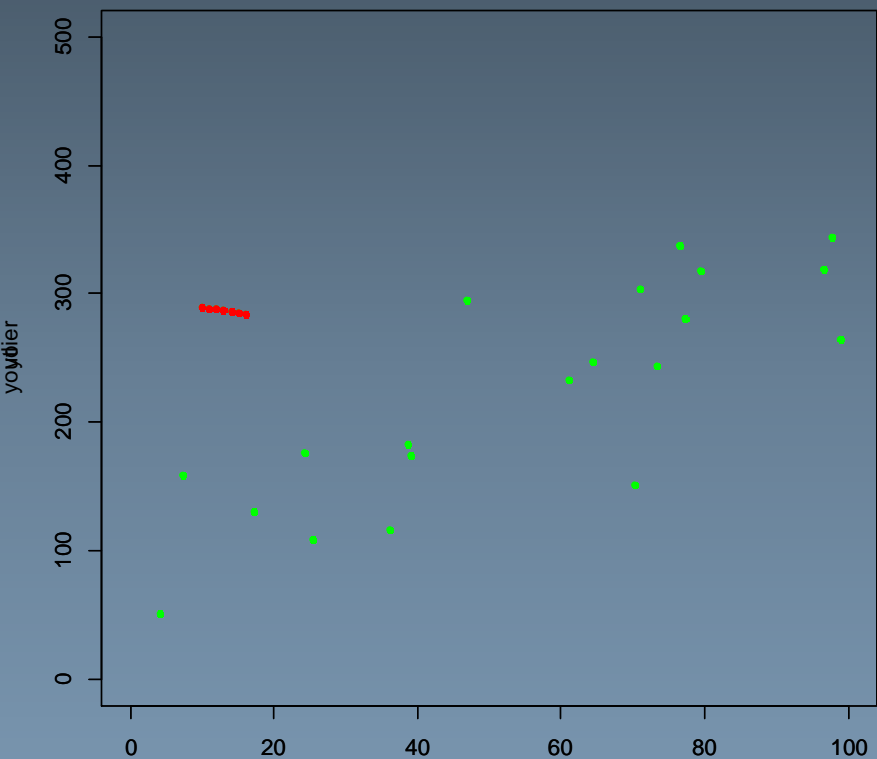


We have statistical tools to predict the confident interval (here 95 %) for any new  $x$ !

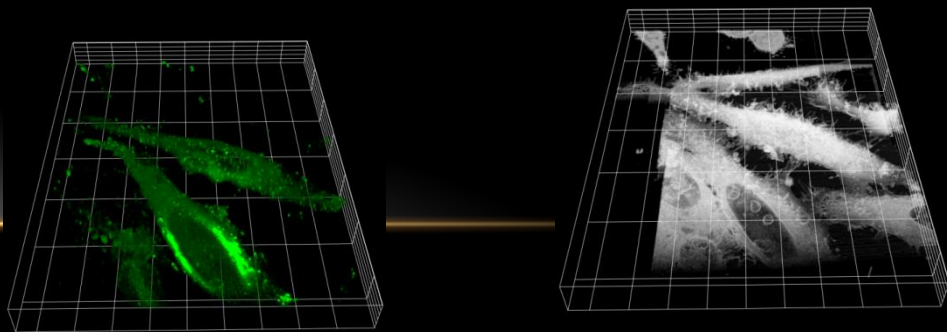
we only have an estimation of the transform because of the « noise » on the position of fiducials **BUT AT LEAST we know how accurate we are**

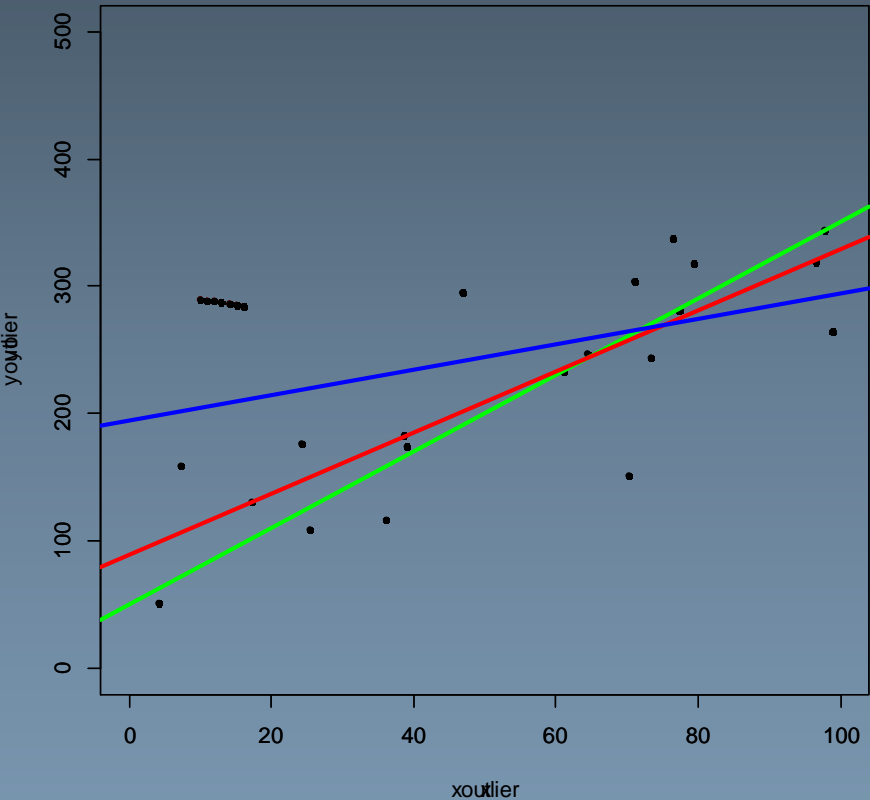






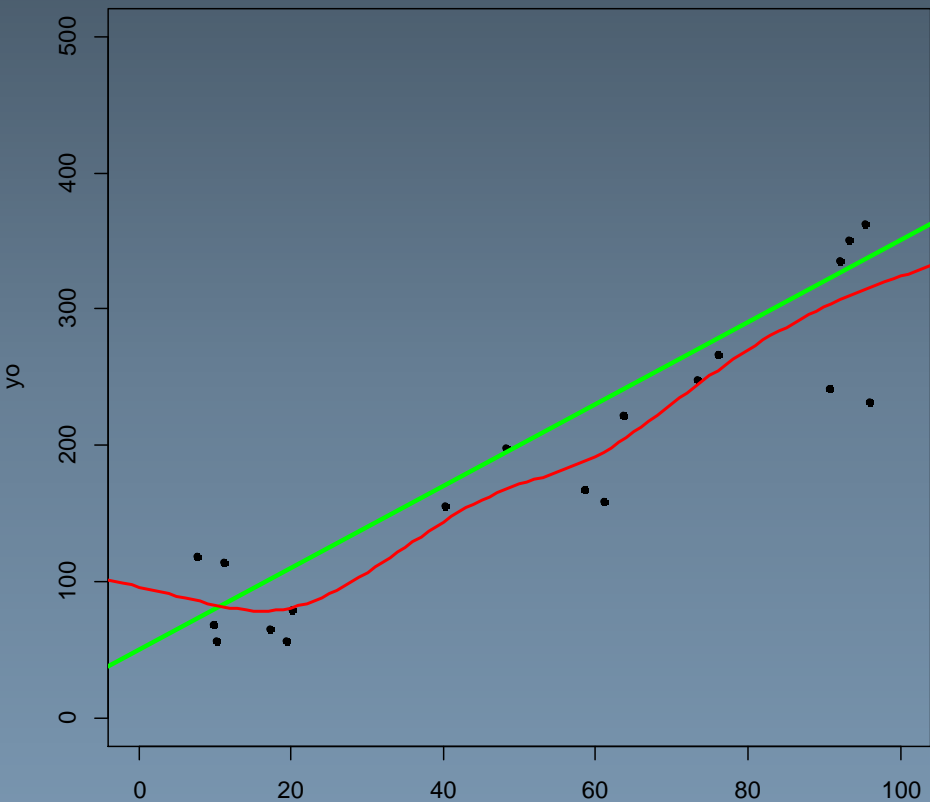
We can have outliers (e.g. bad matching, object not existing in the two modalities, bad segmentation of fiducials...)





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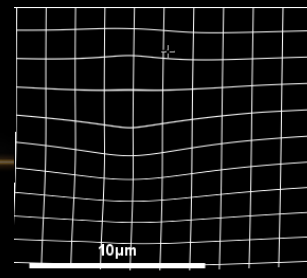
**we have ROBUST methods, able to detect and ignore outliers**

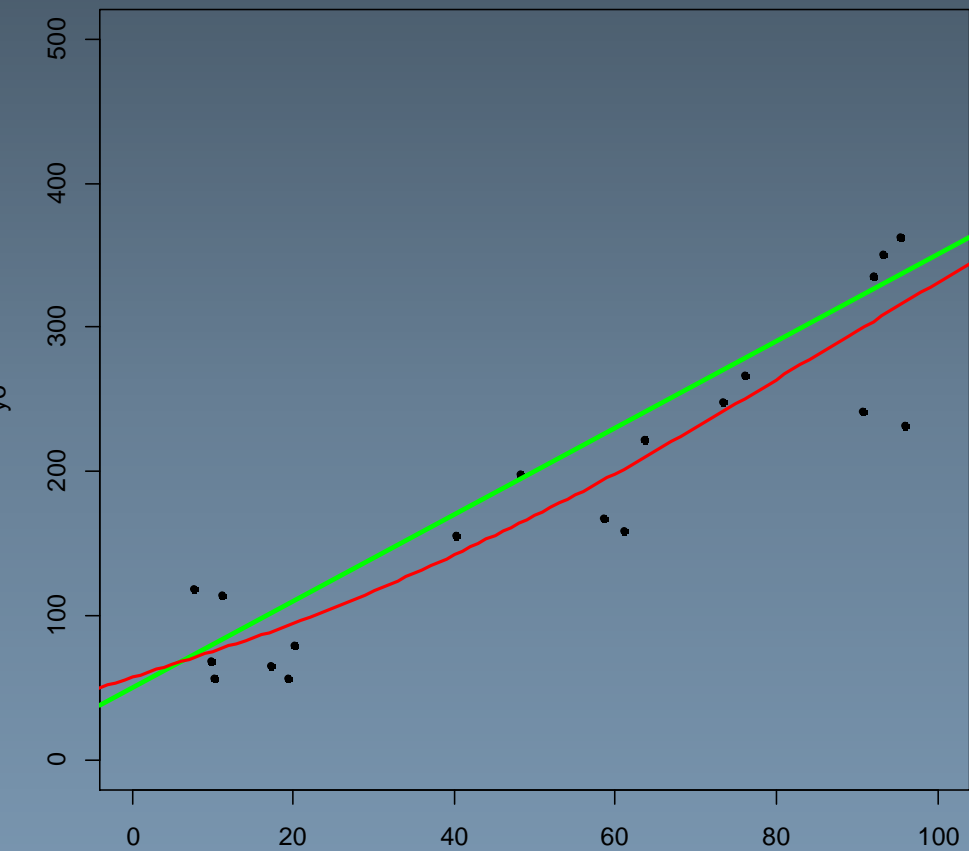


What about changing the  
basis of the transform?

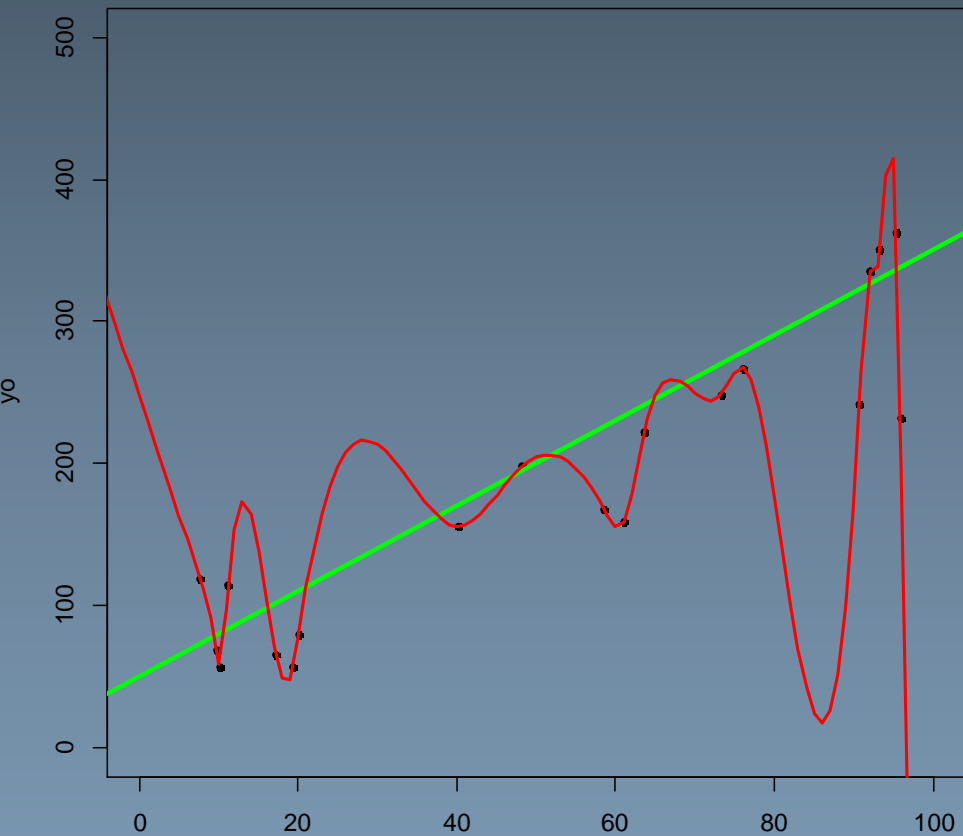
**NON RIGID**

Cubic spline degree of  
freedom (nodes)=5





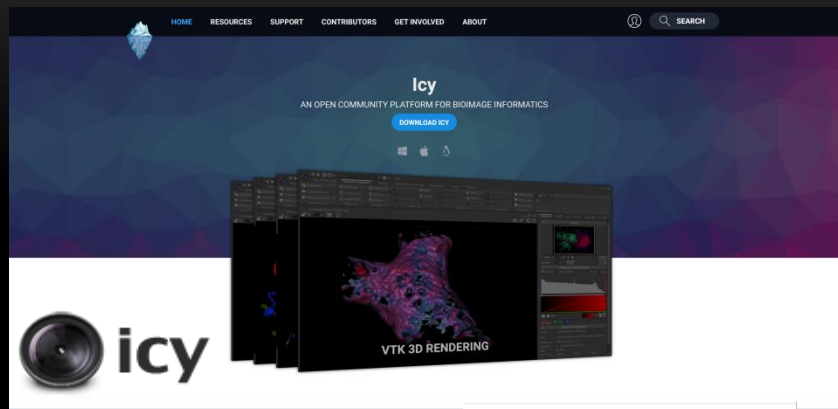
What about changing the  
basis of the transform?  
Cubic spline degree of  
freedom (nodes)=3



What about changing the  
basis of the transform?  
Cubic spline degree of  
freedom (nodes)=20

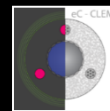
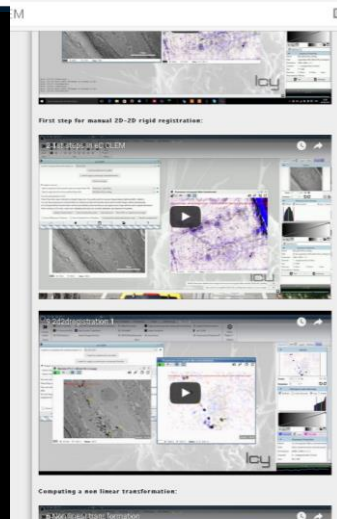
**when there is deformation, selecting  
the good transformation model is  
not obvious and can have big  
consequences**

# FOR THIS WE PROVIDE A SET OF PLUGINS UNDER ICY, AN OPEN SOURCE AND FREE IMAGE ANALYSIS PLATFORM

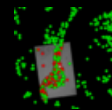


<http://icy.bioimageanalysis.org/>

Online Tutorials on Icy Bio Image Analysis website (look for ec-clem and ec-clem autofinder)



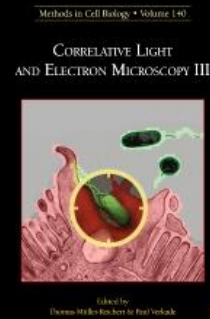
Ec-clem



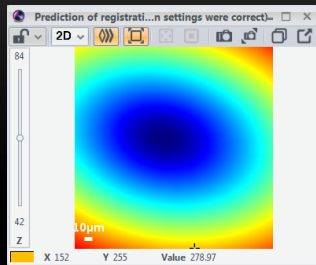
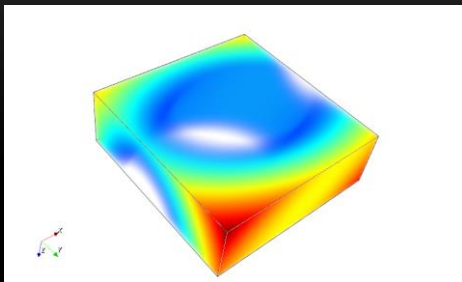
Ec-clem  
AutoFinder

**eC-CLEM: flexible multidimensional registration software for correlative microscopies**

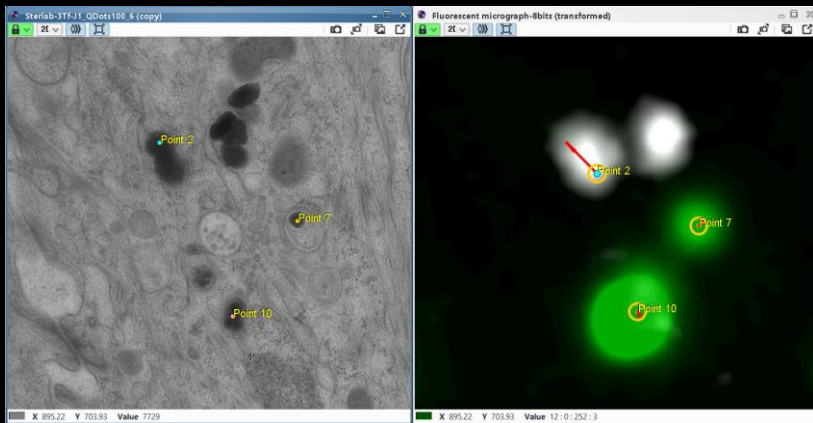
102 | VOL.14 NO.2 | FEBRUARY 2017 | **NATURE METHODS**



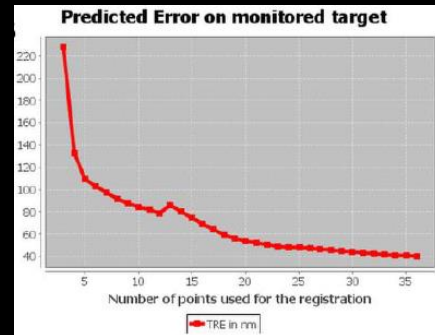
# ERROR ASSESSMENT



Construct error map reflecting error of registration in any point of your image/volume



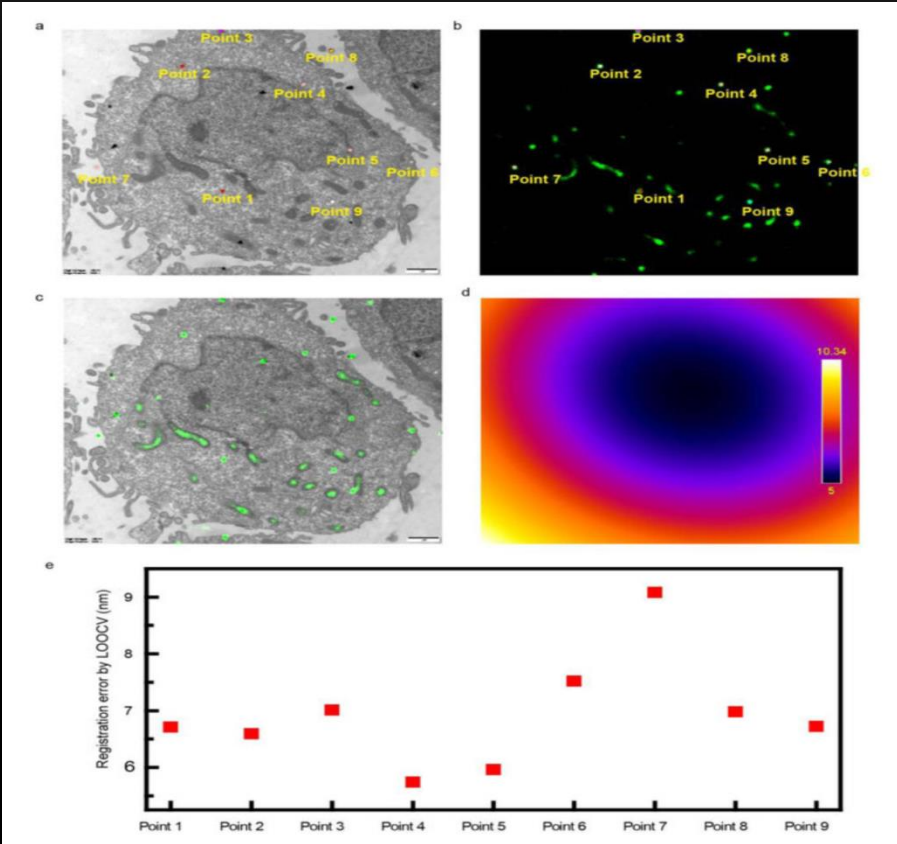
Easily detect any bad matching and/or deformations



Monitor the accuracy on your Region of Interest while you are adding manual features

# ERROR ASSESSMENT

Construct error map reflecting error of registration in any point of your image/volume



Fu, Z., Peng, D., Zhang, M. *et al.* mEosEM withstands osmium staining and Epon embedding for super-resolution CLEM. *Nat Methods* (2019)



# TAKE HOME MESSAGES

- Multimodality use can serve several purposes (complementarity/ validation/ guiding)
- Purpose will define the needed accuracy , and visual check/assessment is usually not sufficient.
- There is no such thing perfect registration
- Estimating the confidence in pixel matching is important, it usually requires to put in purpose some object visible in both modalities to check the quality of matching, but some statistical method are also available for it.
- Think twice about the registration method you use
- Always think about how to prove the correct fusion, and the fact that even two pixels not perfectly aligned can be at the same location in reality.

## TENS Nantes

Michel Neunlist  
Philippe Naveilhan  
Tony Durand

## L'Institut du Thorax Nantes

Guillaume Potier  
Stephan Kunne  
Romain Capoulade  
Jean Mérot

## CRiCNA

Sébastien Gouard  
Michel Cherel



**ANR-18-CE45-0015**

## MicroPICell

Stéphanie Blandin  
Philippe Hulin  
Magalie Feyeux  
Steven Nedellec  
Marine Malloci

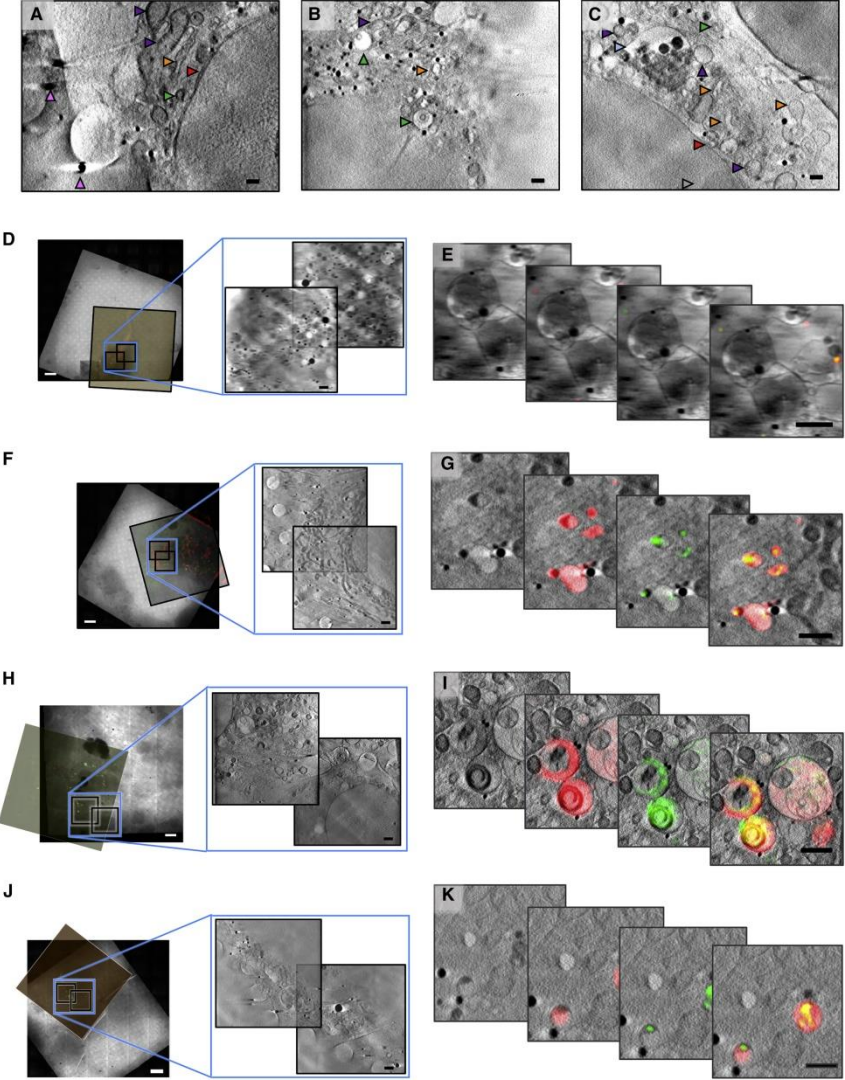
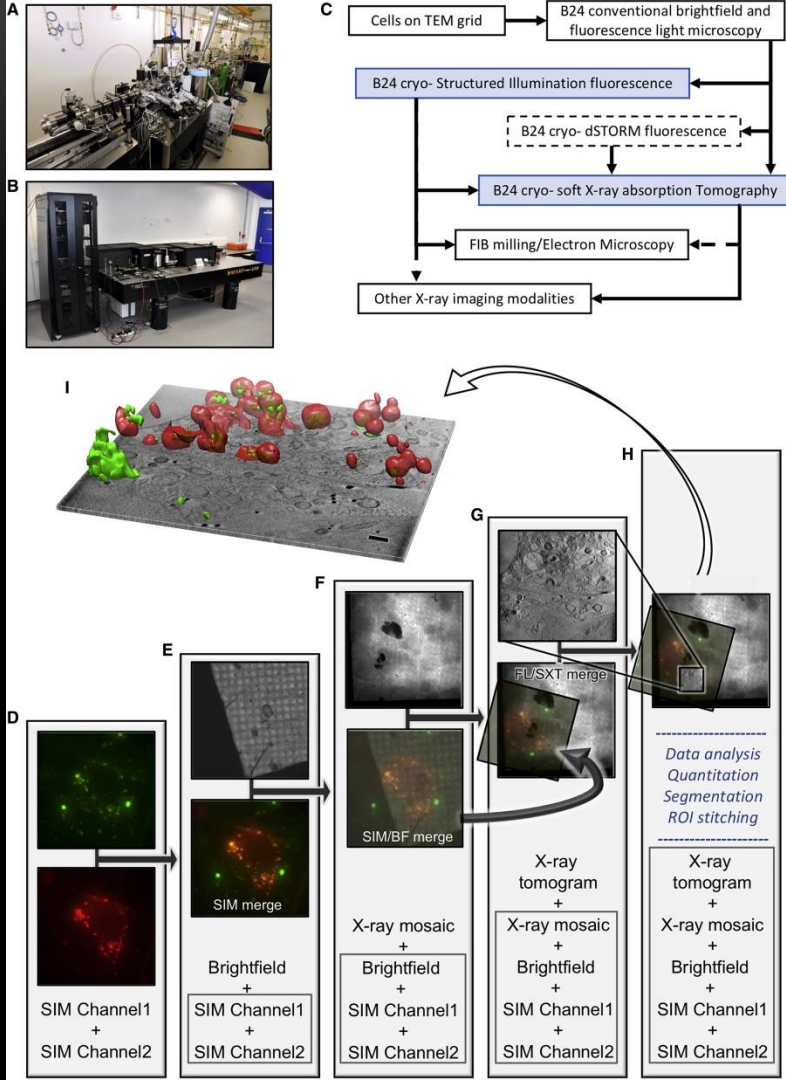
## Institut Curie

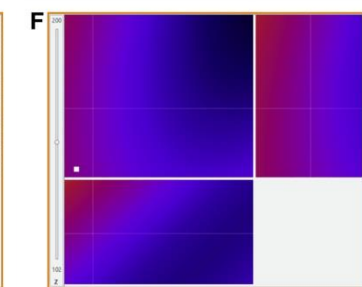
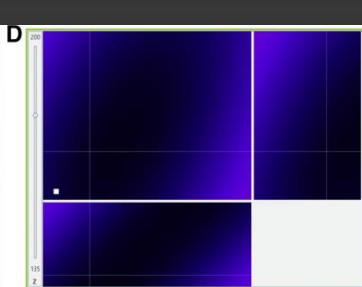
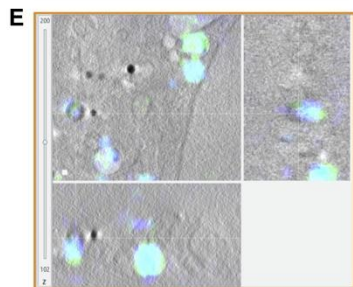
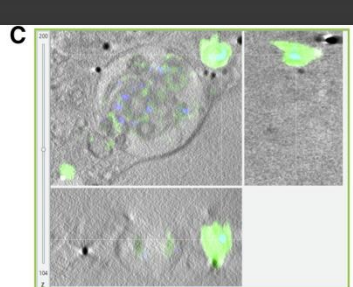
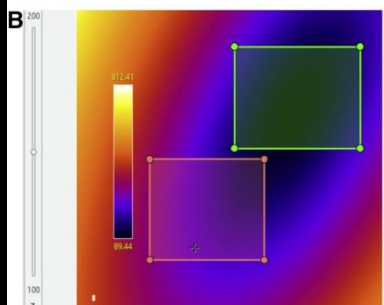
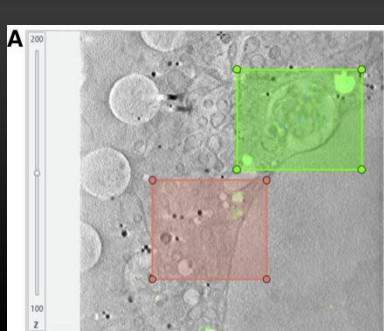
Xavier Heiligenstein (CryoCapcell)  
Jean Salamero  
Graca Raposo

## Diamond Light Source

Maria Harkiolaki  
Nina Vyas

QUESTIONS?





Matching structures= beads visible in both modalities

Transformation = Rigid because sample were cryogenized and exactly the same sample with no further sample prep between the two steps

