

MÉTHODES ET ÉCUEILS DU TRAITEMENT DES IMAGES ISSUES DE MICROSCOPIE CORRÉLATIVE

Perrine.paul-gilloteaux@univ-nantes.fr



Structure Fédérative
de Recherche - **SANTÉ**
François Bonamy



FRANCE-BIOIMAGING

OUTLINE

- Why Correlative Microscopy?
 - How to fuse images ?
 - Once is fused, how do you exploit the spatial correlation?
 - What to be careful about?
-

CORRELATIVE MULTIMODAL IMAGING

- Imaging the same specimen with two or more complementary modalities that – combined – create an informative, composite view of the sample
 - Provides structural, functional, temporal and chemical information
 - Spans all relevant resolution ranges for one sample
 - Provides complementary information due to different contrast mechanisms
 - Identifies regions of interest & rare events for further analysis

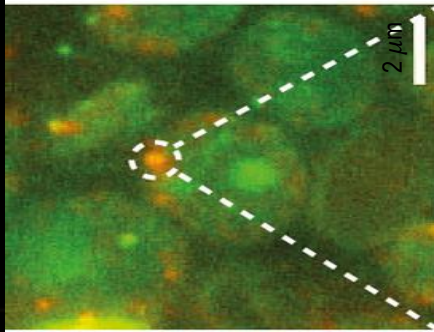
Integrated/hybrid imaging

Correlative microscopy with sample transfer between one or several modalities

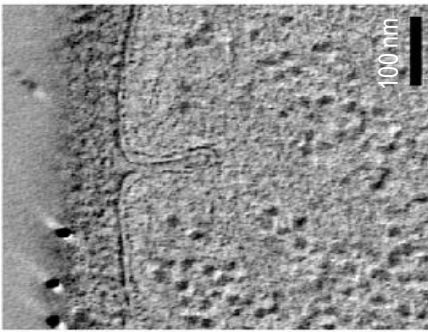
CLEM

- Example: Protein-mediated membrane shape changes during endocytosis
- Correlating ultrastructural changes in membrane curvature with the presence of proteins
- 4D reconstruction of protein-mediated endocytosis with high temporal & spatial resolution

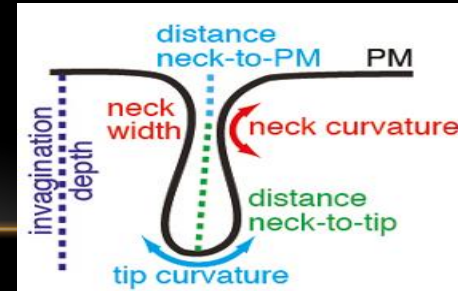
Live-cell imaging to locate
a defined intermediate
stage in endocytosis



Electron microscopy to obtain
high-resolution of membrane
curvature & endocytic



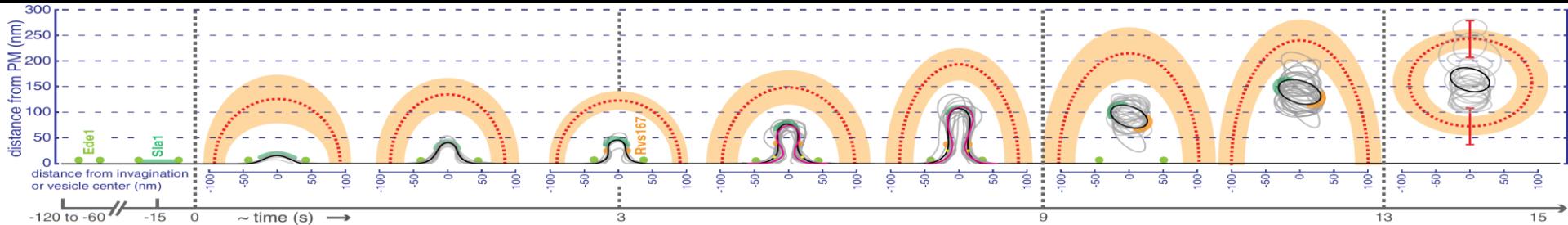
Kukulski/Briggs et al., Cell 2012



CLEM

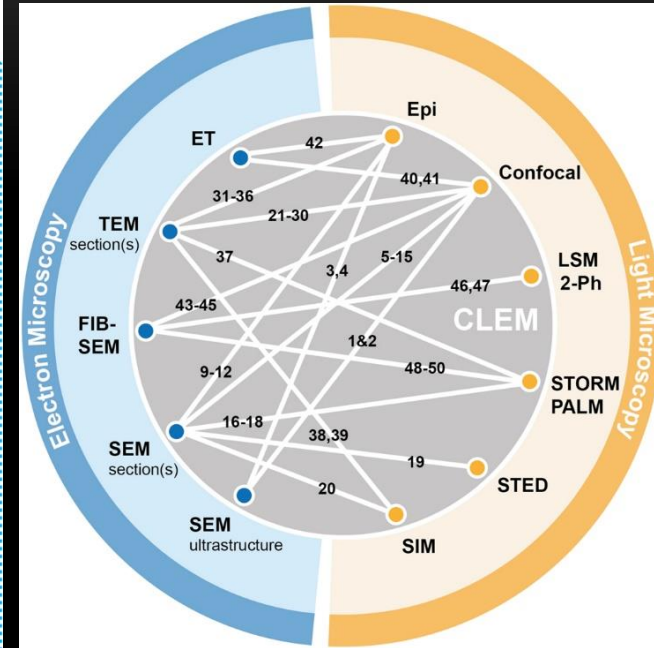
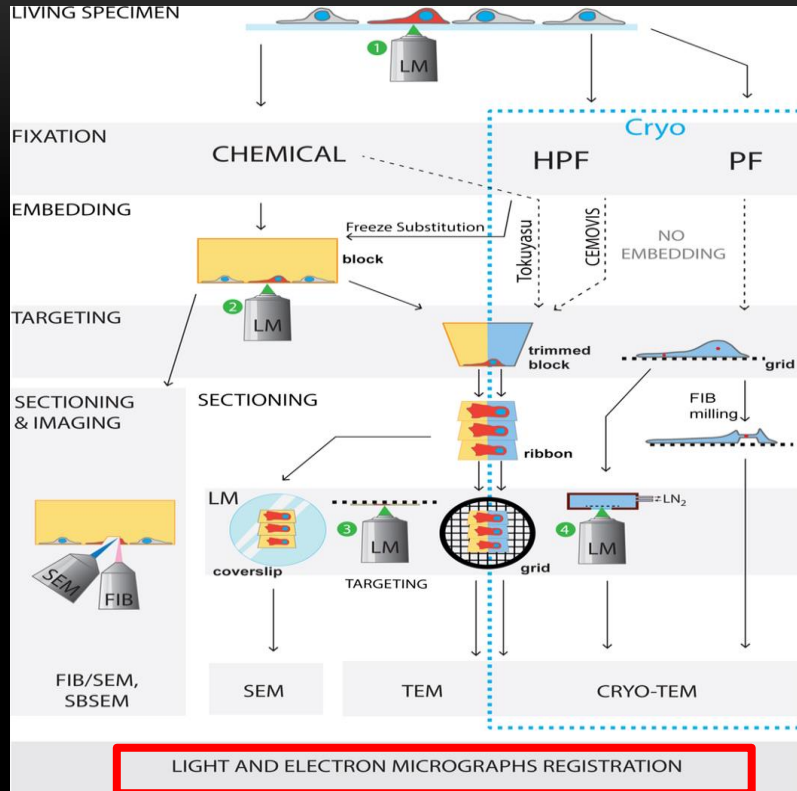
- Example: Protein-mediated membrane shape changes during endocytosis
 - Correlating ultrastructural changes in membrane curvature with the presence of proteins
 - 4D reconstruction of protein-mediated endocytosis with high temporal & spatial resolution

A quantitative 4D model of endocytosis



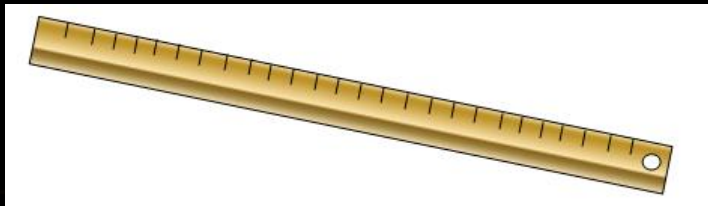
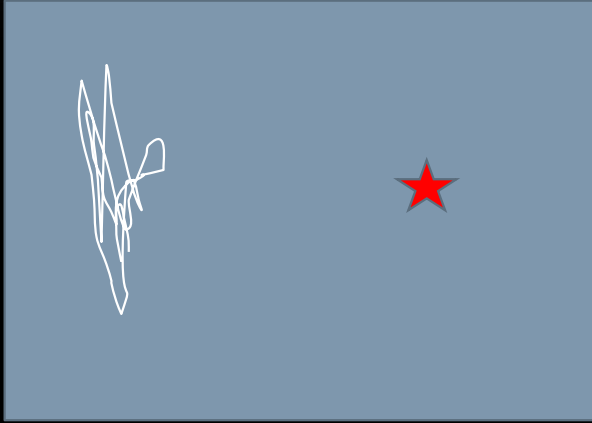
CLEM

- Workflows are complex...



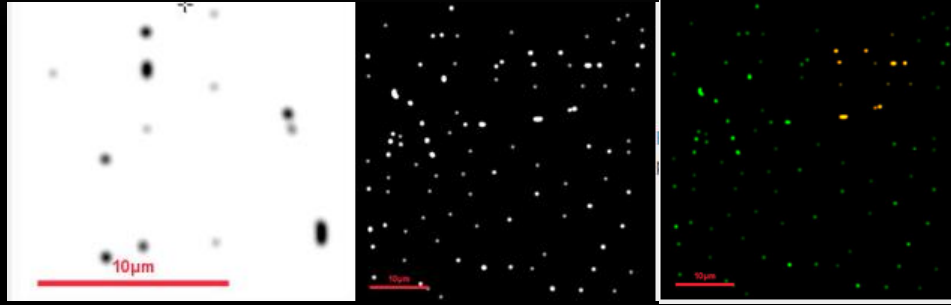
- Begemann I and Galic M (2016) Correlative Light Electron Microscopy: Connecting Synaptic Structure and Function.

RELOCATION VS REGISTRATION



- « Move 5 mm right from the center of the scratch »
- Or Store the position of the stage and transfer coordinates

RELOCATION VS REGISTRATION

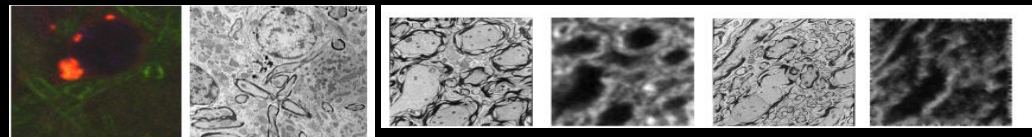


APPROACHES FOR REGISTRATION

Fiducial= any stuff you or the computer can recognise in both images and that will be used for registration

Not Fiducial= any stuff NOT USED for registration

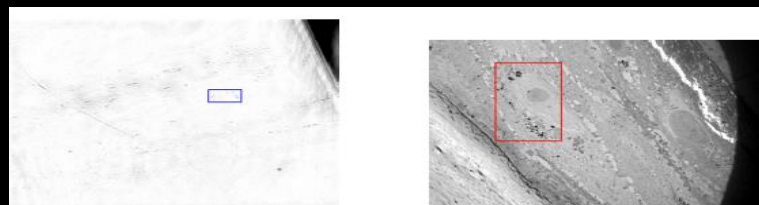
Intensity based



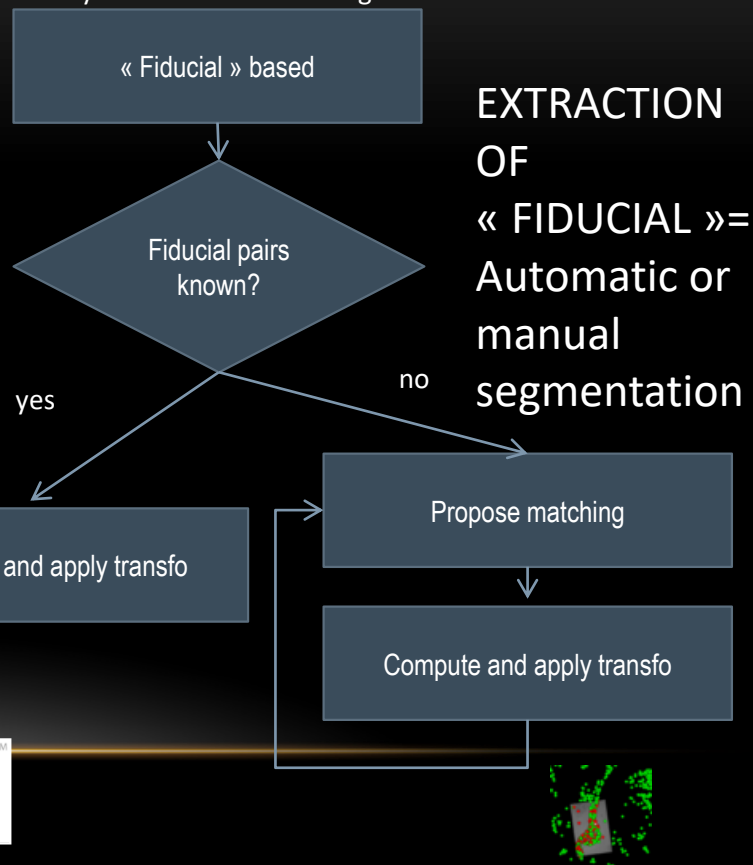
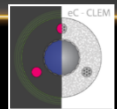
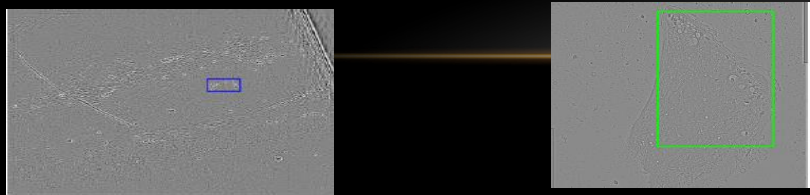
Cao et al . Medical Image Analysis 2014

Training patches TEM et confocal

Input and output Predicted confocal



Bouthemy et al ISBI 2016



[Biii.eu](http://biii.eu)

Advanced Search

<http://biii.eu/all-content>

Keywords:

-Image registration

- Correlative Light

and Electron

Microscopy






Content type For which operation?

for which topic? Supported image dimension Type

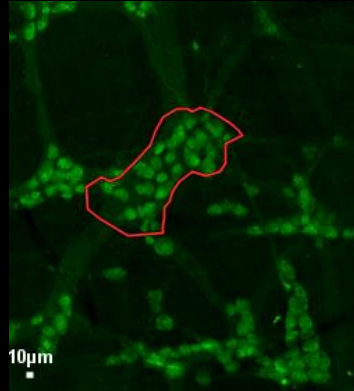
Interaction level License/Openness Programming language

specific biological term

listing content

Name ^	Category	Image	requires	Type	interaction level	License/Openness	has function	has topic
Bigwarp	Software			Component	Manual	Free and open source	Image registration, Deformable registration, Warping	Correlative Light and Electron Microscopy, Electron microscopy
DeepCLEM	Software		Fiji, Register Virtual Stack Slices (Fiji), CSBDeep a toolbox for Content-aware Image Restoration (CARE) in Fiji	Component, Workflow	Automated	Free and open source	Image registration, Affine transformation	Correlative Light and Electron Microscopy, Multimodal imaging
eC-CLEM	Software			Component	Manual, Semi-automated	Free and open source	Alignment construction, Image registration, Affine registration, Rigid registration, Deformable registration	Correlative Light and Electron Microscopy, Multimodal imaging
ec-clem autofinder	Software			Component	Semi-automated	Free and open source	Image registration, Rigid registration, Affine registration	Correlative Light and Electron Microscopy
performing automatic registration for CLEM	Software			Workflow	Automated, Semi-automated	Free and open source	Alignment construction, Image registration, Affine registration, Rigid registration, Spot detection	Correlative Light and Electron Microscopy

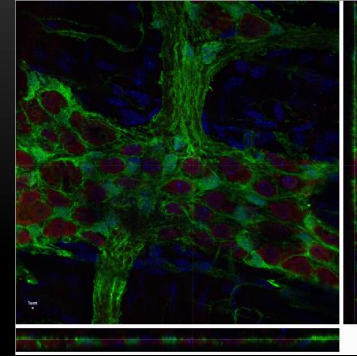
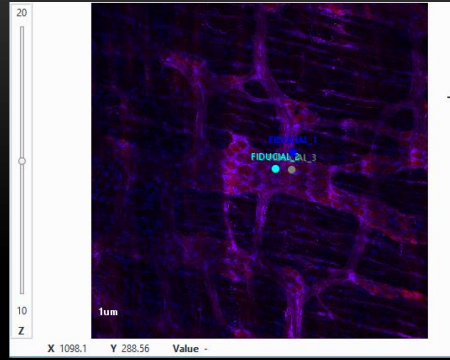
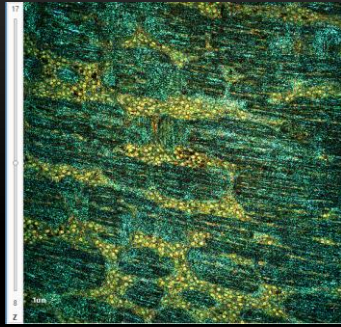
WHY DO YOU WANT TO FUSE MULTIMODAL IMAGES?



- 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)

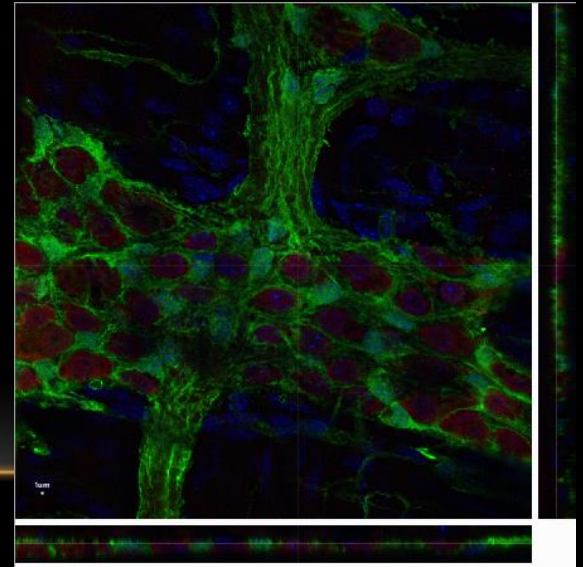
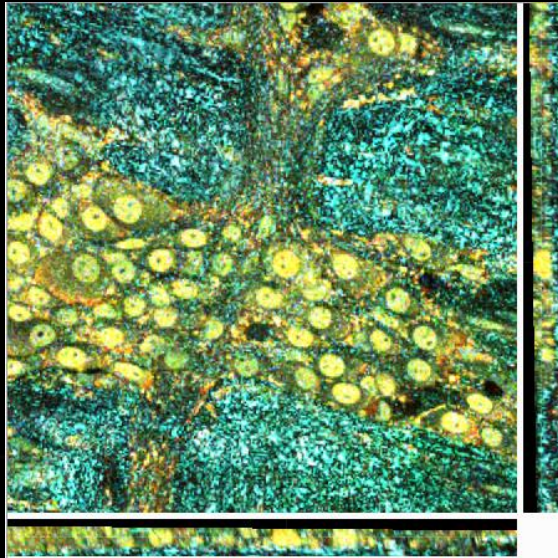
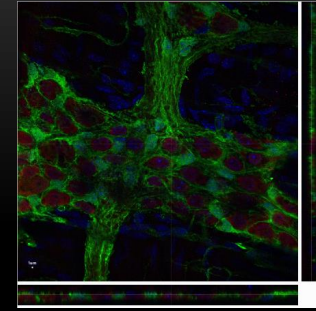
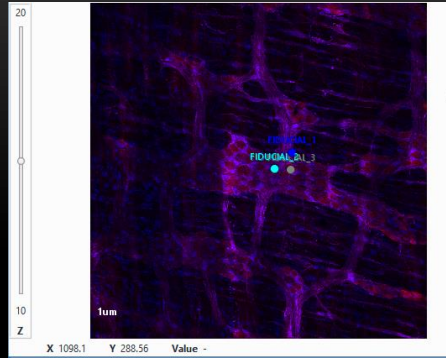
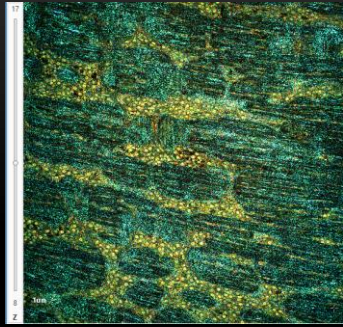
NO FIDUCIALS: CONSTRAINING THE TRANSFORM TO BE RIGID



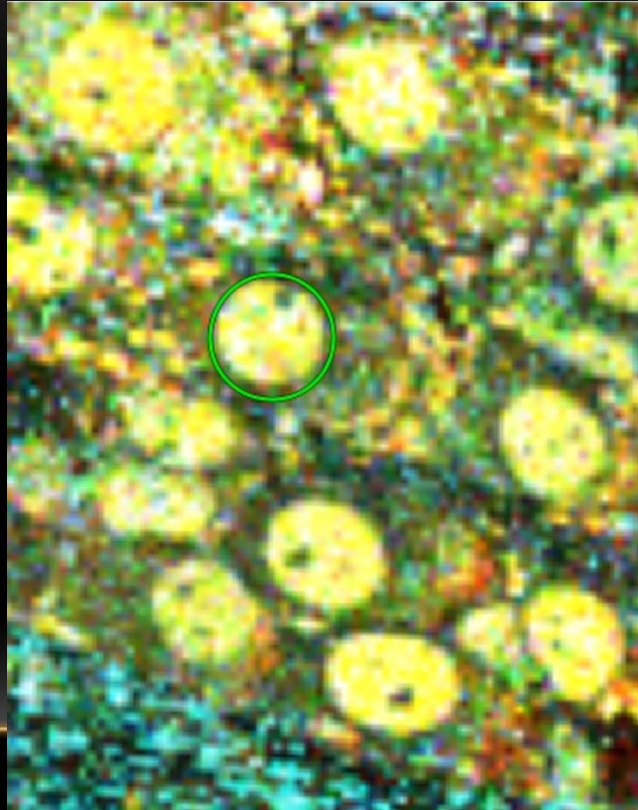
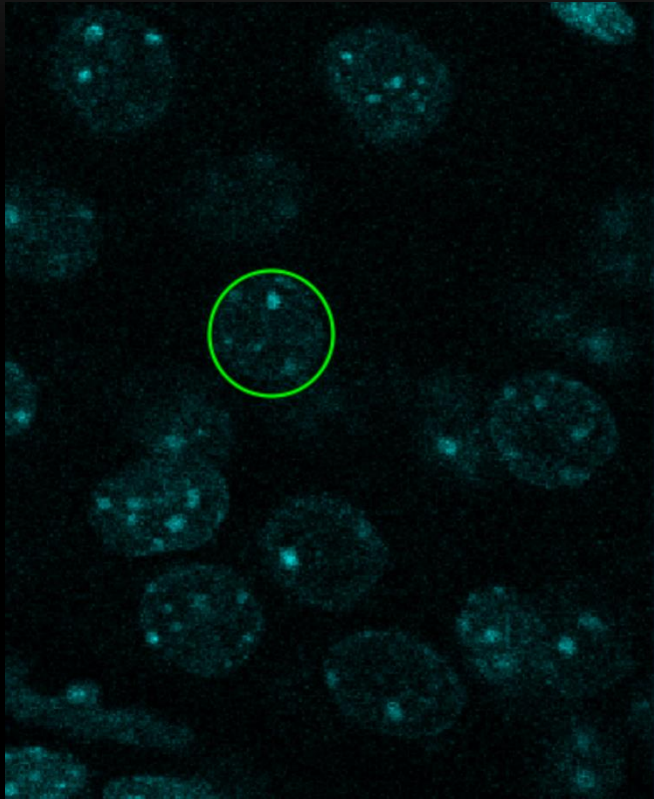
Matching structures= None known, using visible intensity correlation for rough alignment, and center of distinguishable contrasted structures matched with center of nuclei (also centre of cells)

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

NO FIDUCIALS: CONSTRAINING THE TRANSFORM TO BE RIGID



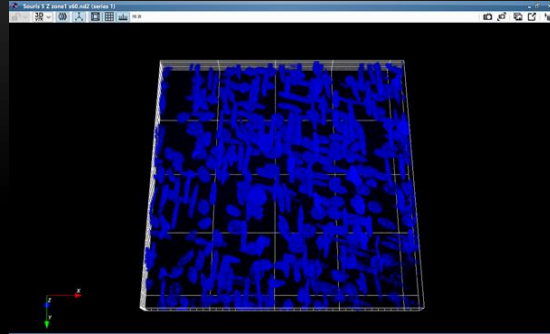
NO FIDUCIALS: CONSTRAINING THE TRANSFORM TO BE RIGID



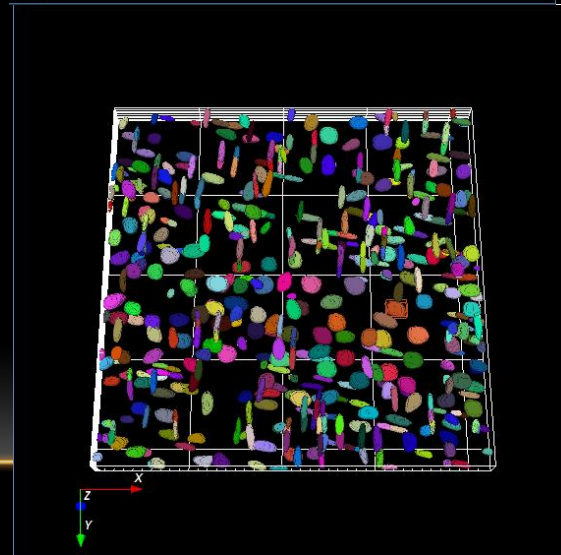
USING FUSED IMAGES TO CHARACTERISE THE UNKNOWN IMAGING SIGNAL



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 a green signal and numerous colored rectangular regions of interest (ROIs). The right window, titled 'C Merge', shows a similar 2D view with a different signal and ROIs. 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' field. The table on the right lists 'ROI Object #' and 'Posit...' (Position) for various objects. The software logo 'ICY' and version 'Version 2.0.3.0' are visible at the bottom right.

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

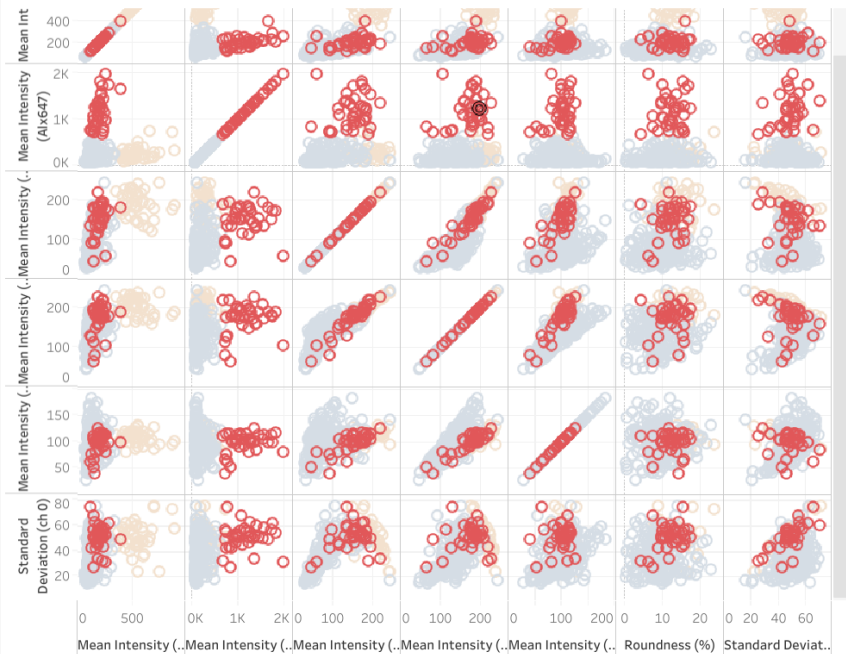
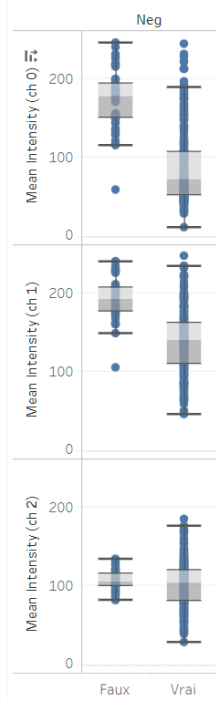
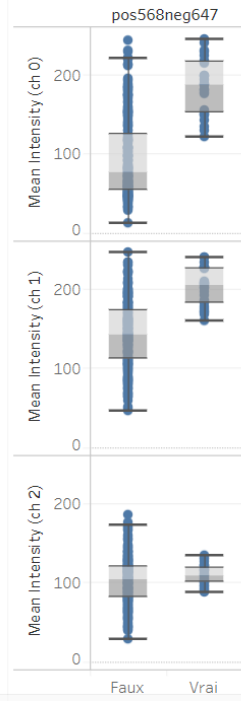
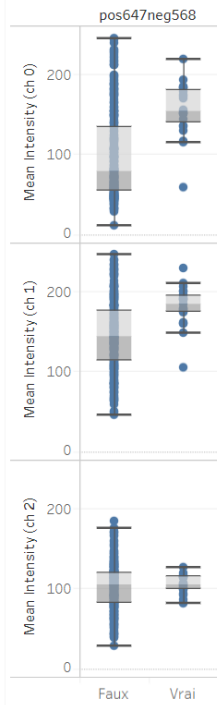
Z

T

C

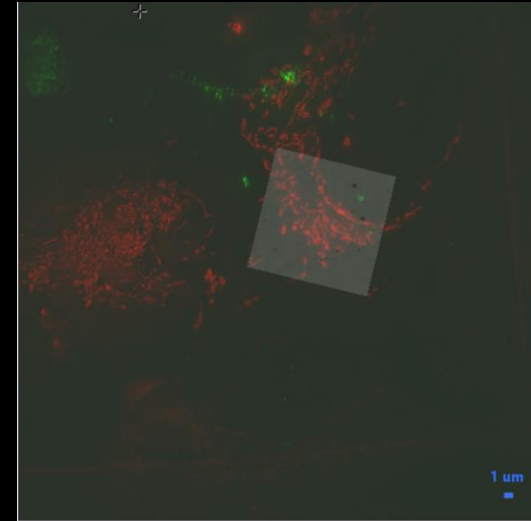
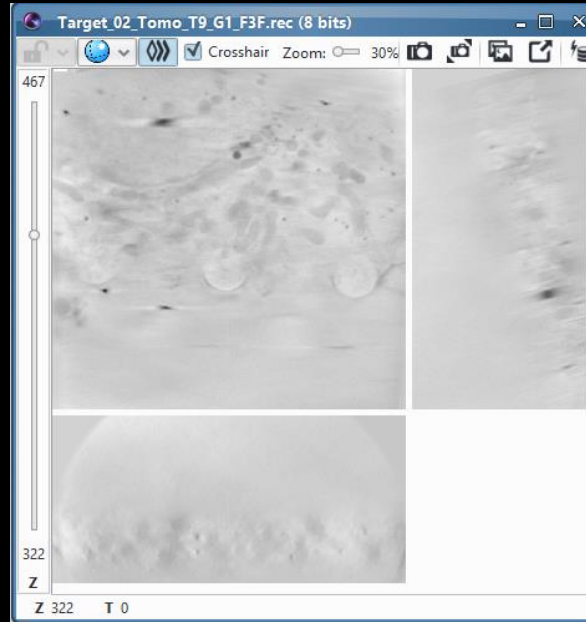
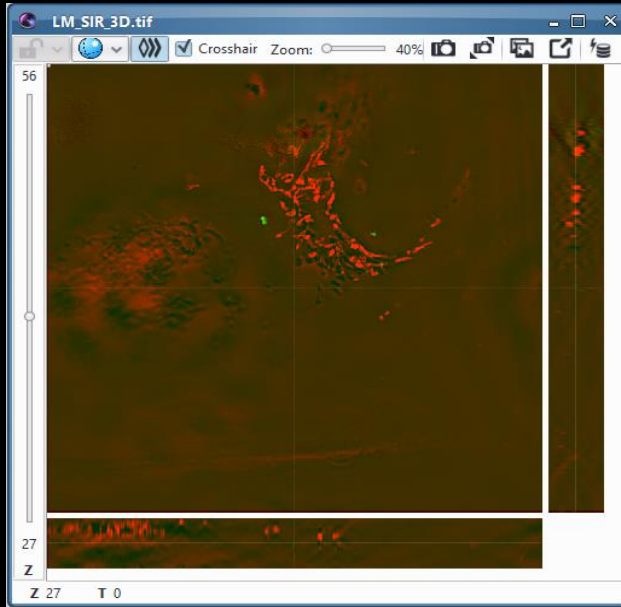
ICY
Version 2.0.3.0

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

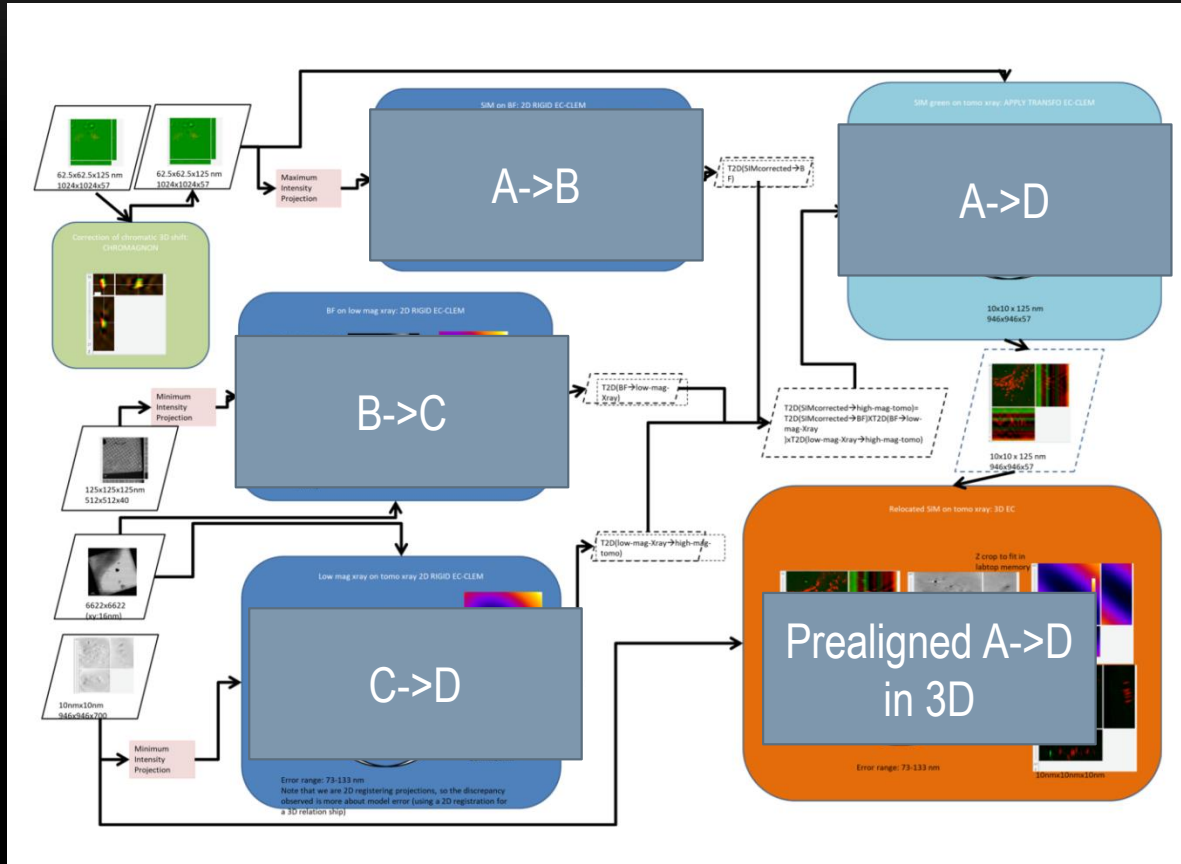


USUALLY IN 3D AND USUALLY IN NEED OF INTERMEDIATE IMAGING

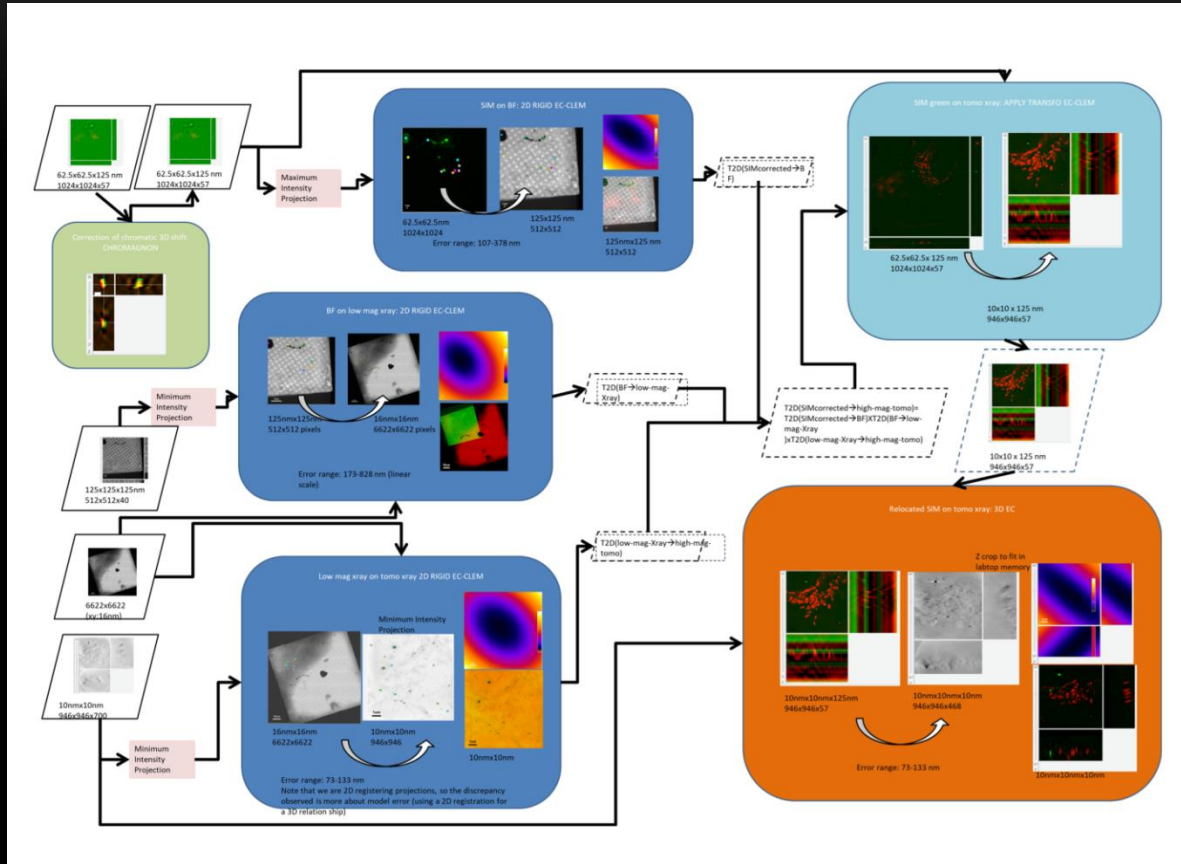
Kounatidis I, Stanifer ML, Phillips MA, Paul-Gilloteaux P, et al.
3D Correlative Cry-Structured Illumination Fluorescence and
Soft X-ray Microscopy Elucidates Reovirus Intracellular
Release Pathway. Cell. 2020



USUALLY IN 3D AND USUALLY IN NEED OF INTERMEDIATE IMAGING



USUALLY IN 3D AND USUALLY IN NEED OF INTERMEDIATE IMAGING

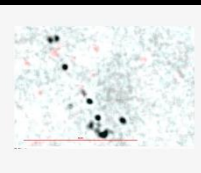
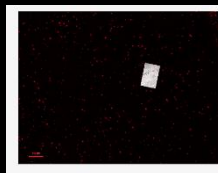
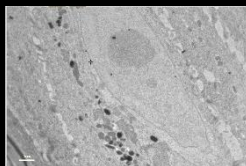
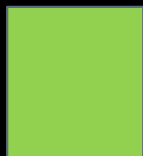
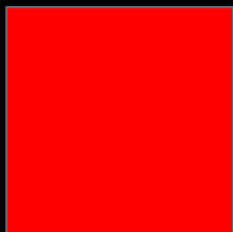


HOW TO EXPLOIT THE TRANSFORMATION COMPUTED FOR QUANTIFICATION?

COMPUTE THE
TRANSFORMATION LINKING

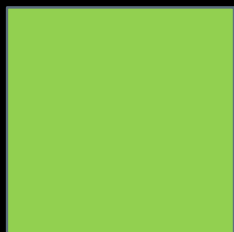
source and target

Ecclem Apply
transform

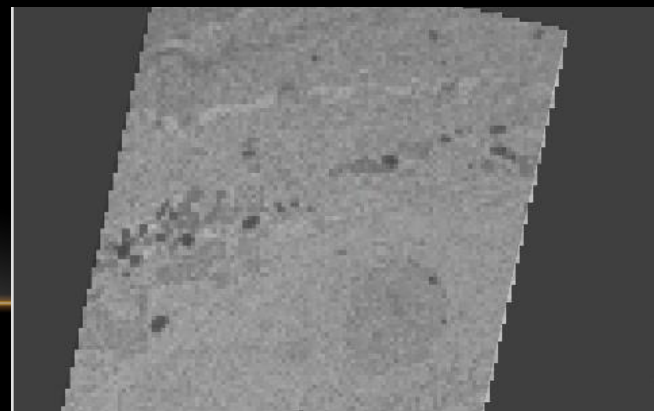


FROM THE
TRANSFORMATION LINKING

source and target

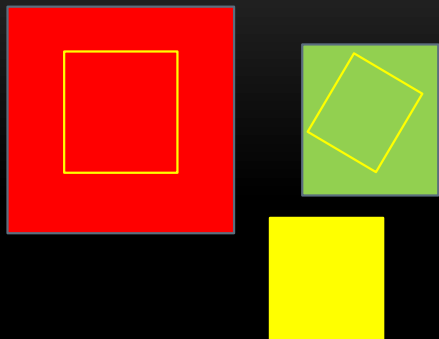


Interpolation

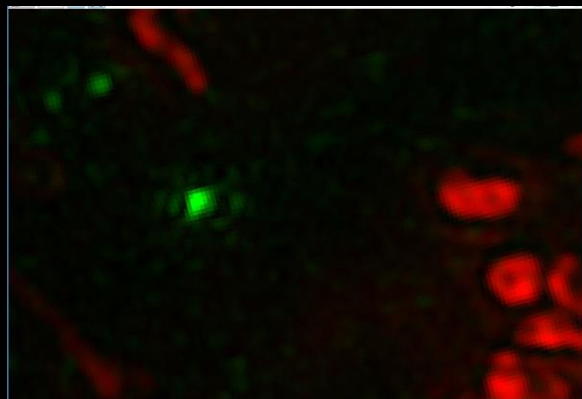


Overlay: one image got **interpolated** (new pixels) or **reduced in quality** to create a new merged dataset.

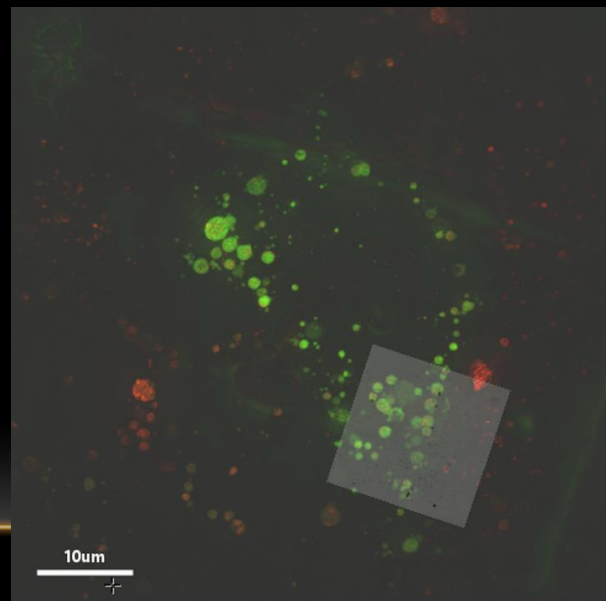
HOW TO EXPLOIT THE TRANSFORMATION COMPUTED FOR QUANTIFICATION?



Transform
ONLY the
DISPLAY
(coordinate
system of
arrival =
screen)



Ecclem
CorrelativeView

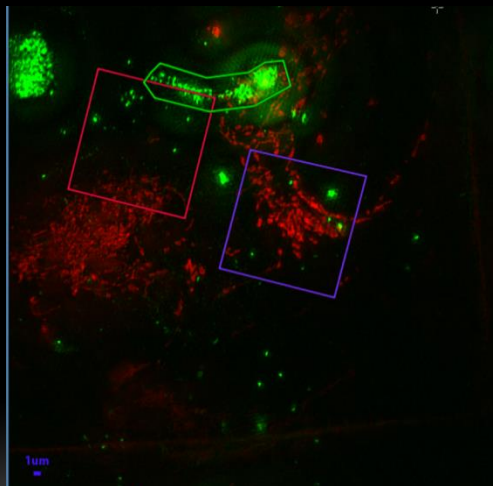
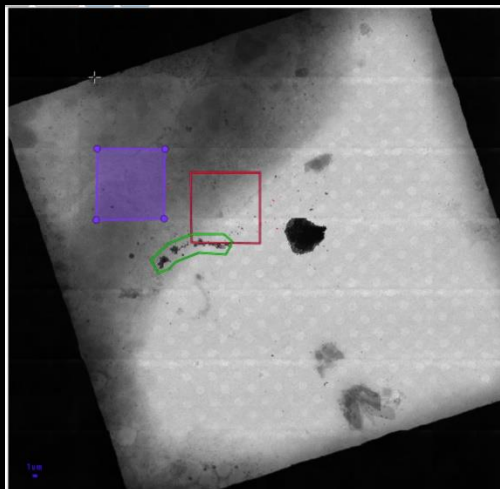


HOW TO EXPLOIT THE TRANSFORMATION COMPUTED FOR QUANTIFICATION?



Region of interest transformed

Ecclem Apply transform to ROI



	Name	Conto...	Interior (px)	Area (u...	Mean Inte...	Mean Inte...	Max Inte...	Max Intens...	Texture contr...	Texture contrast ...	Texture homogeneity (c...	Texture homogeneity (c...
	Polygon2D	710.08	20316.5	79.361	13492.1	358.91	154107.3	8919.14	37.886	37.532	0.63182	0.59267
	Polygon2D	1015.9	64424.5	251.66	7813.26	68.502	124714.1	3707.97	14.366	1.9072	0.78402	0.85863
	Polygon2D	1011.11	63754	249.04	20290.6	58.312	231600.6	3159.71	66.098	0.99523	0.53865	0.88852

HOW DO YOU CHECK IF IT IS CORRECTLY FUSED?

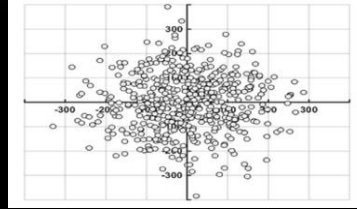
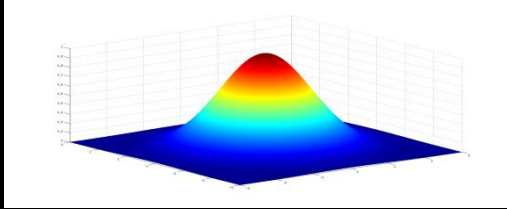
« Multimodal registration validation is known to be one of the most difficult problem in image processing -> there is no ground truth, or only approximation of it » (*J .Pluim, International conference of pattern recognition , 2016*)

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

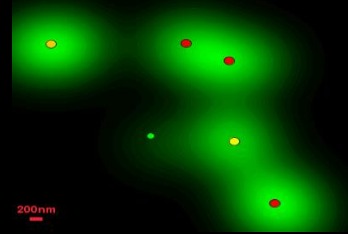
HOW TO ASSESS THE QUALITY OF REGISTRATION?

- 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

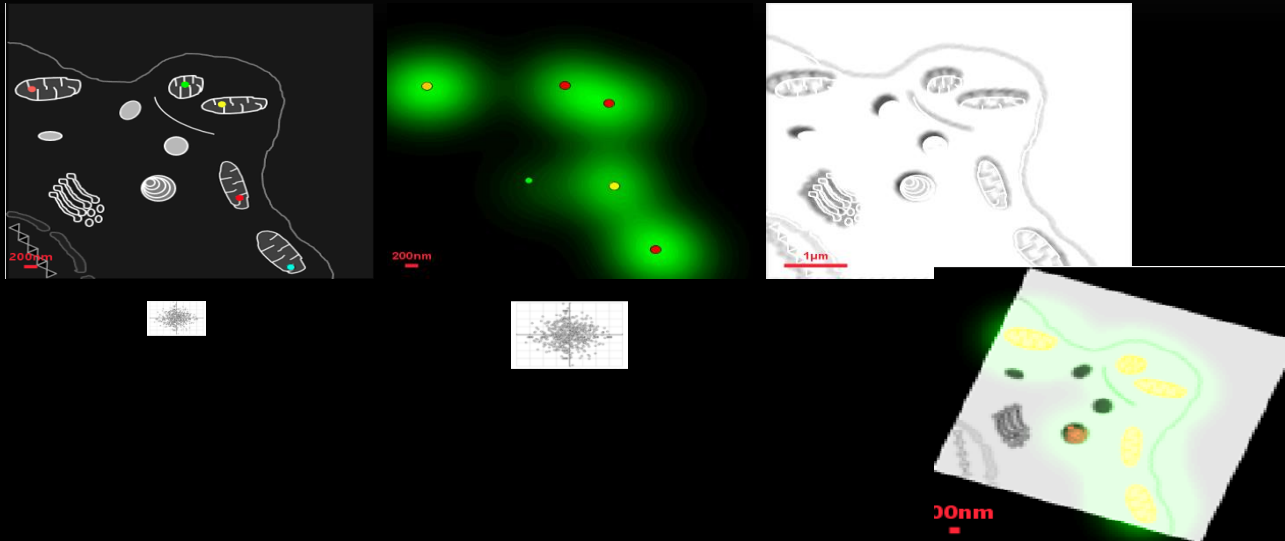


Fiducials can be:

Beads,

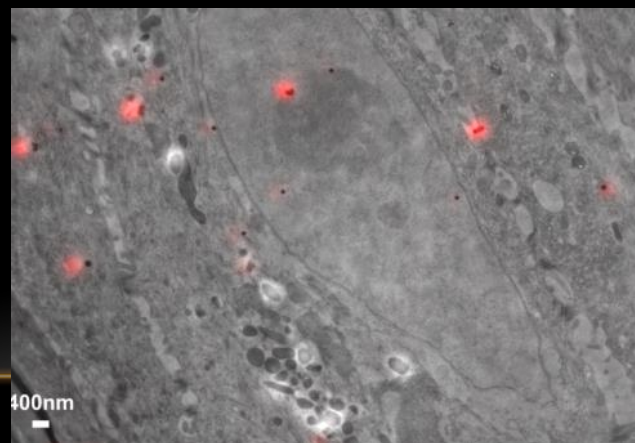
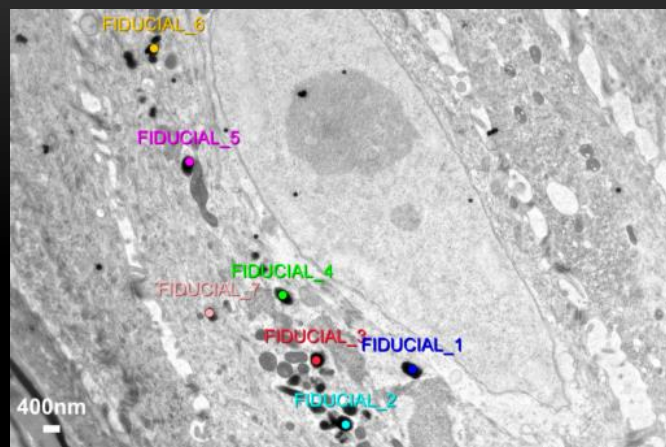
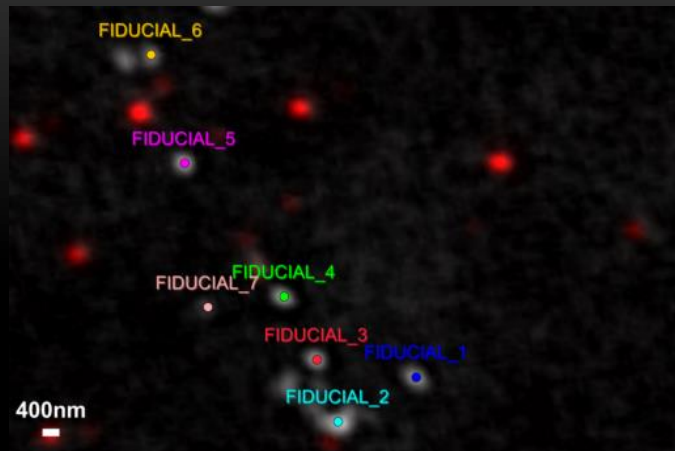
Structures of interest (in life sc: point sampled on membrane, nuclei centroids, stretch...)

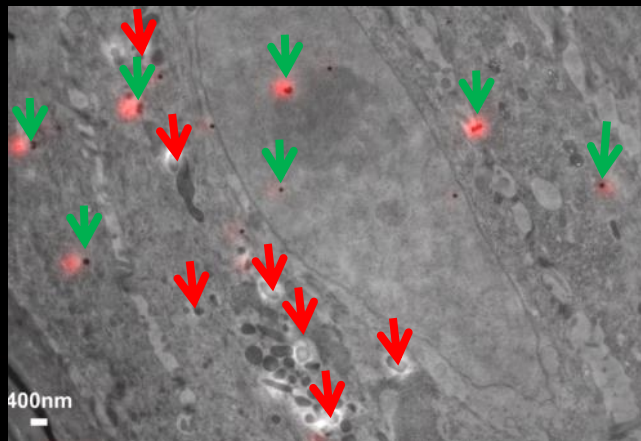
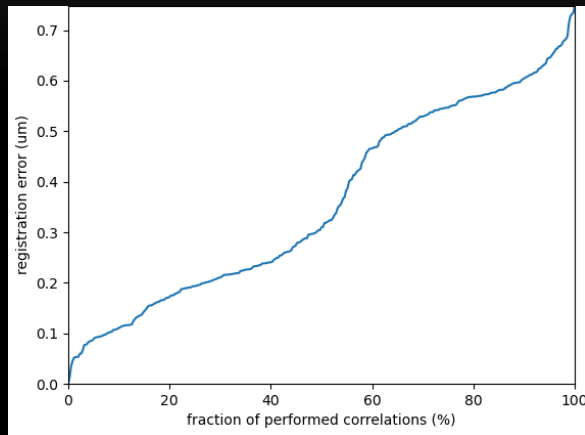
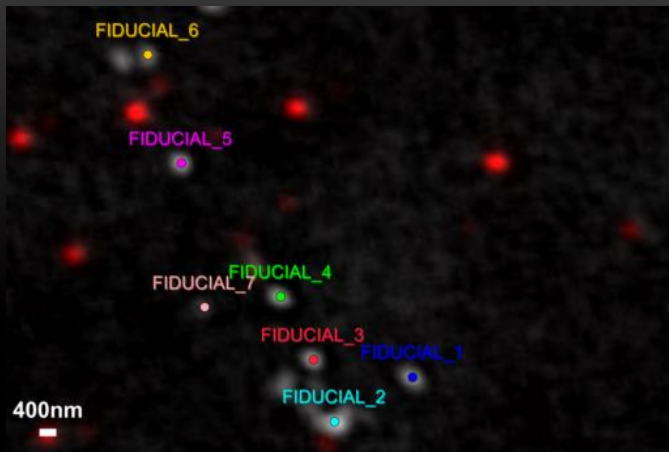
REGISTRATION ERROR DUE TO FIDUCIAL LOCALIZATION ERROR



SO HOW DO YOU CHECK QUANTITATIVELY THE REGISTRATION ACCURACY?

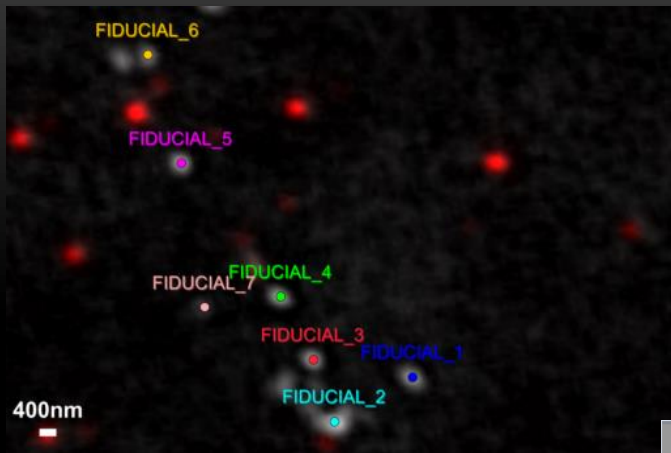
- Use objects that can be seen in both modalities, and DO NOT USE ALL :
 - Either Fiducials (points)
 - Either boundaries of known object visible in both modalities, even scratch or others..



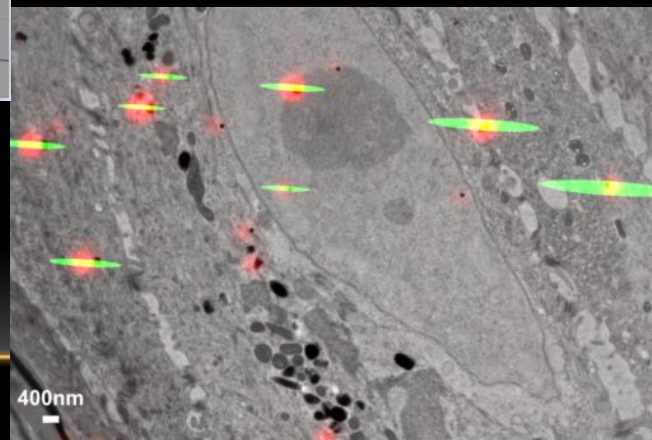
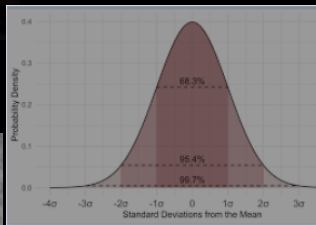
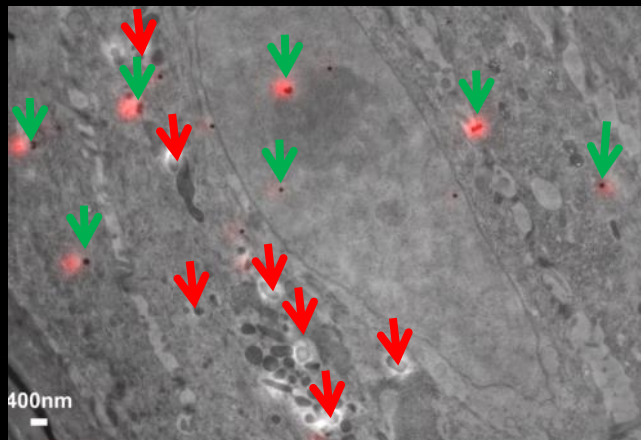


- red arrow fiducials
- green arrow Not fiducials

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

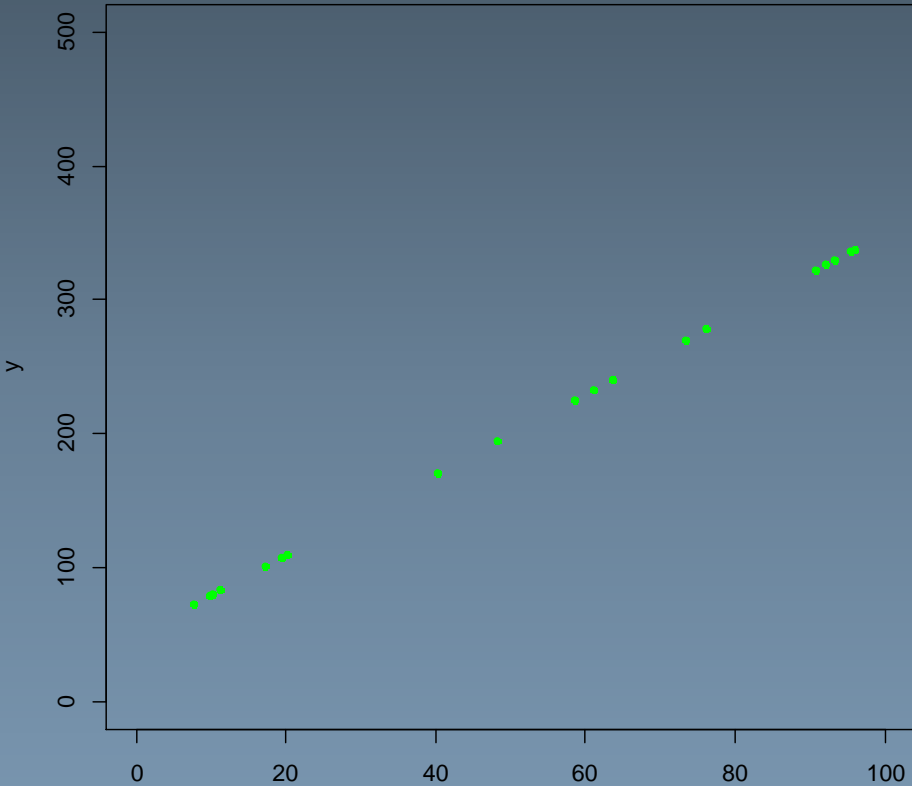


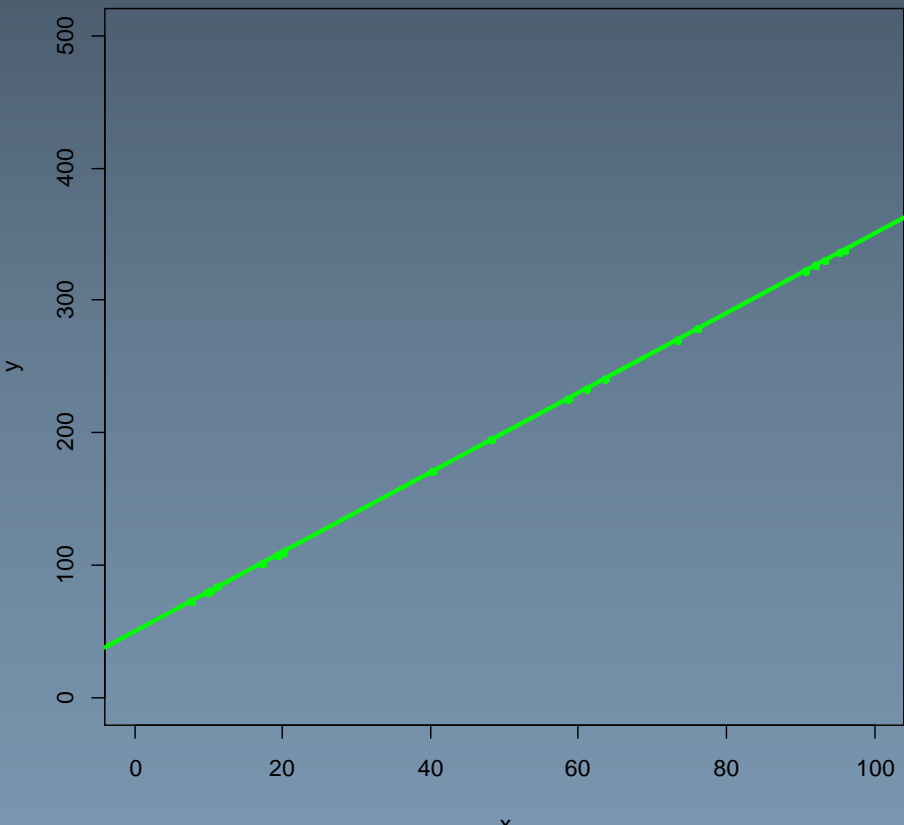
Predicted
Average
accuracy
at each
point



New in
ecclem:
95%
accuracy
prediction

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

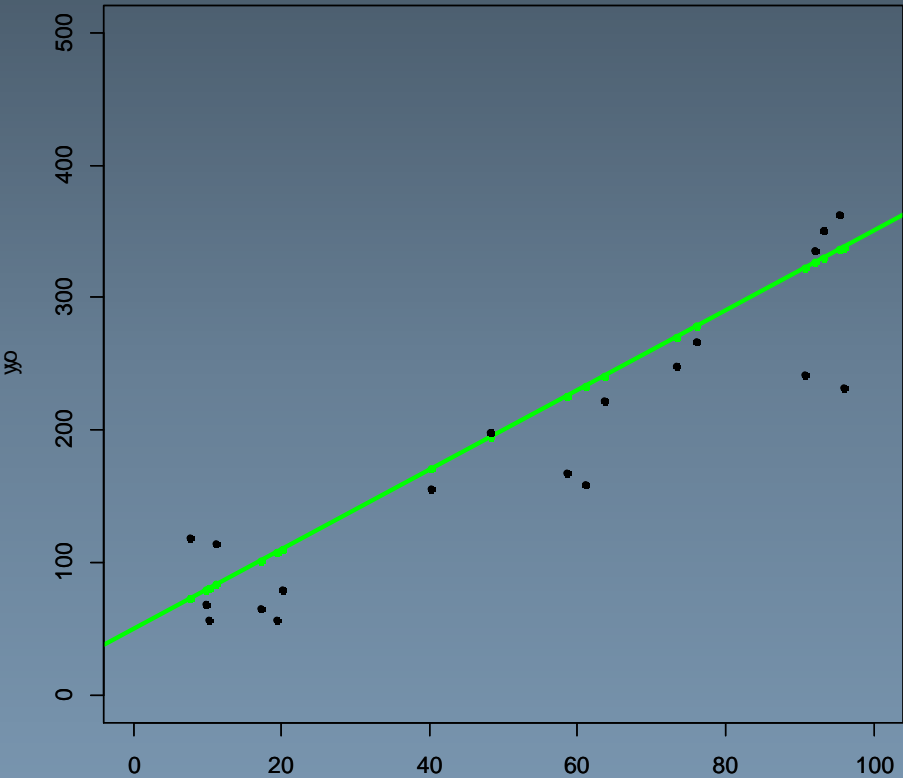




Seeing the transformation computation as a regression problem: example with a linear relationship (**RIGID** i.e rotation + translation)

Find R and T such as:

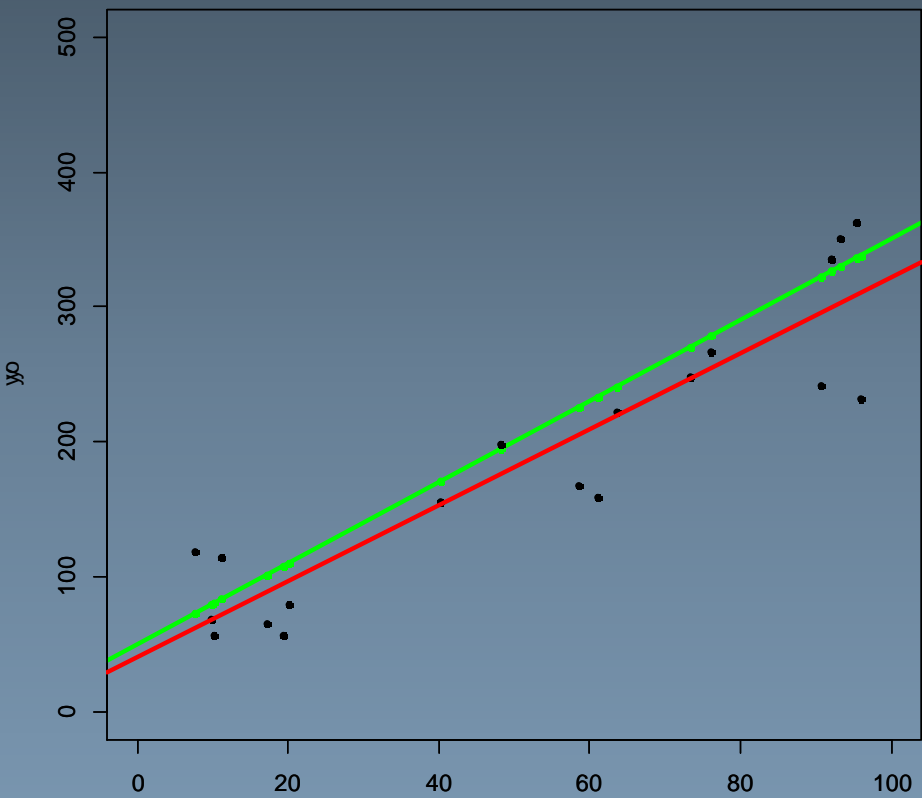
$$y = Rx + T$$



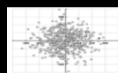
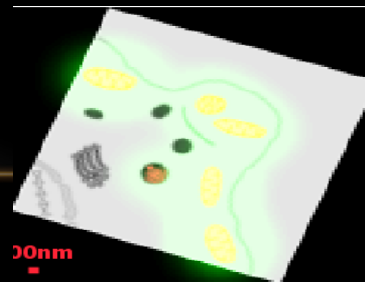
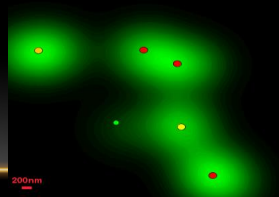
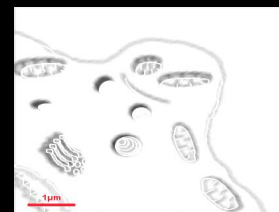
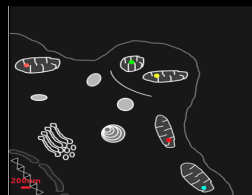
More realistic situation: « noise »
Typically the error in positioning the fiducial
(RESOLUTION!!)

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

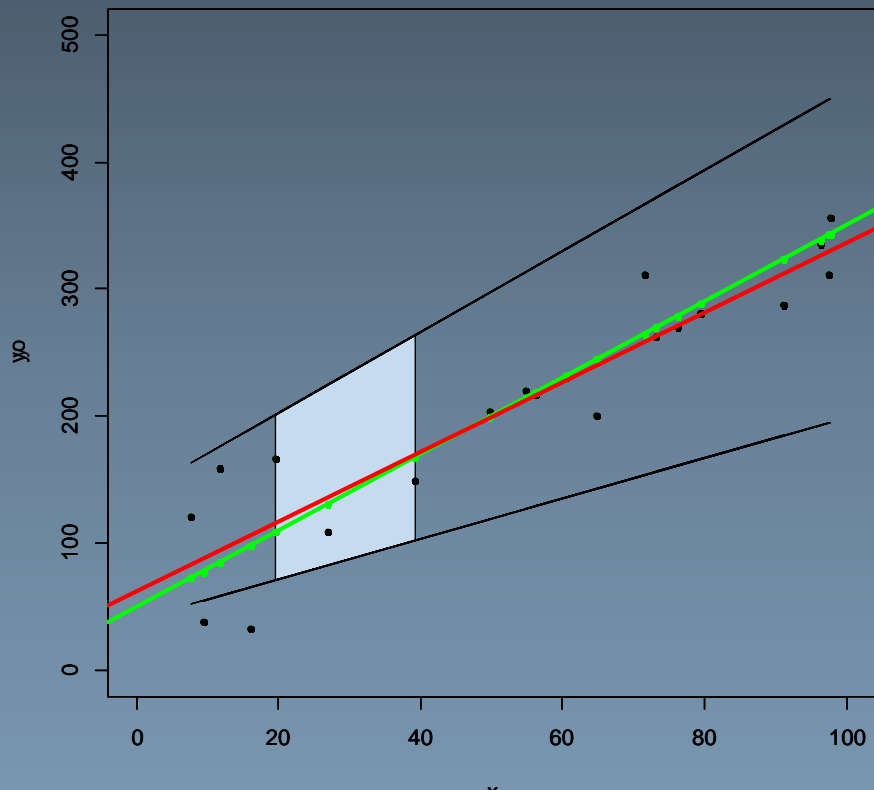
$x_0 = x + \text{noise}$



Estimated transform
 $Y_0 = R X_0 + T$

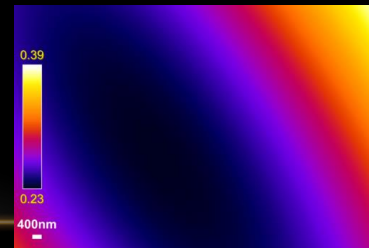
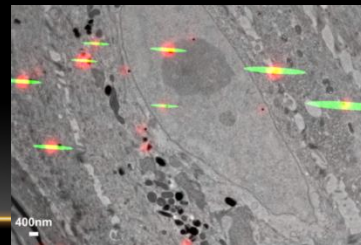


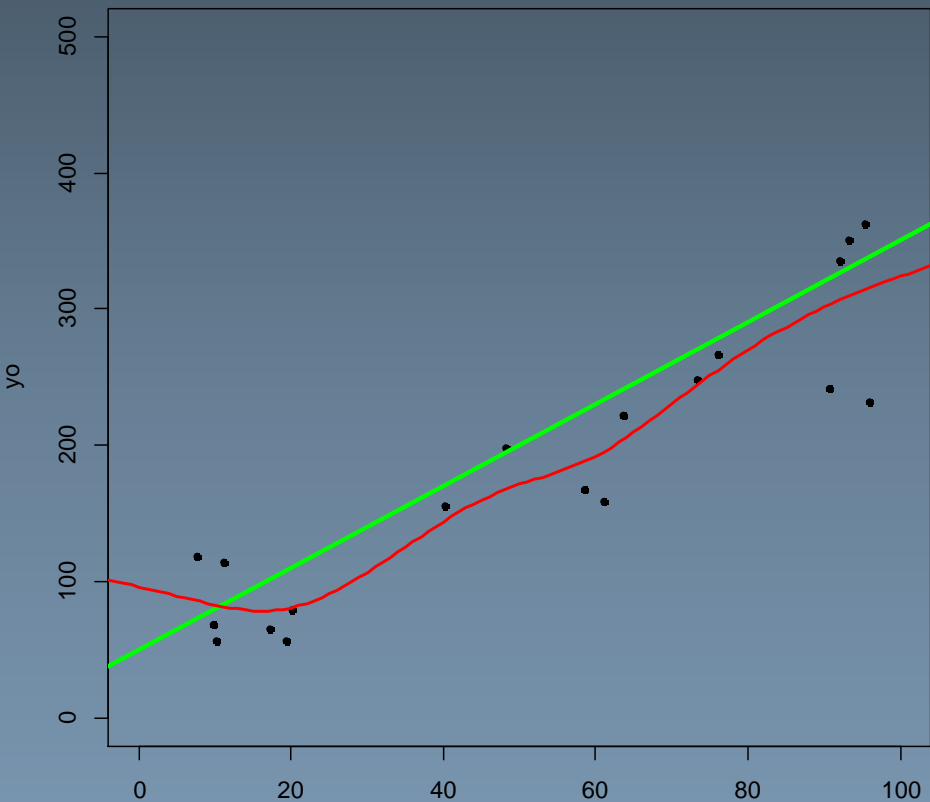
200nm



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**

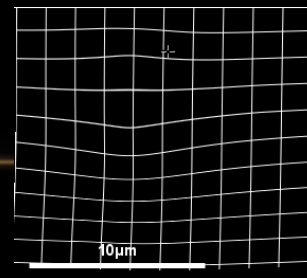


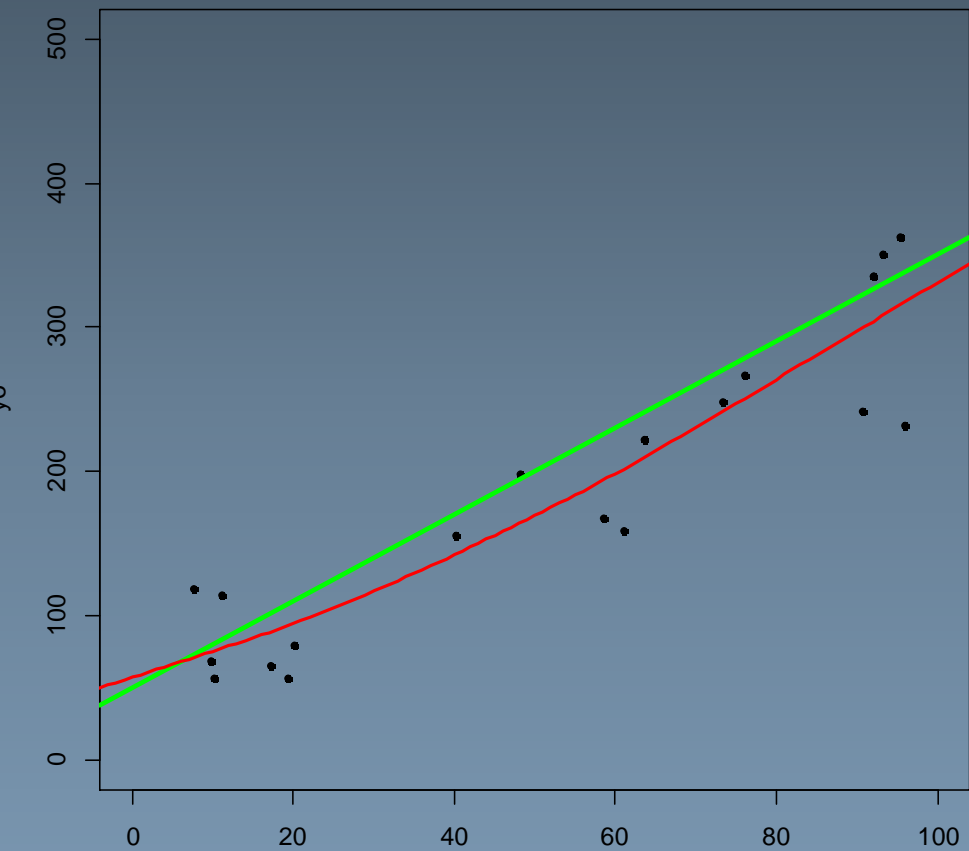


What about changing the
basis of the transform?

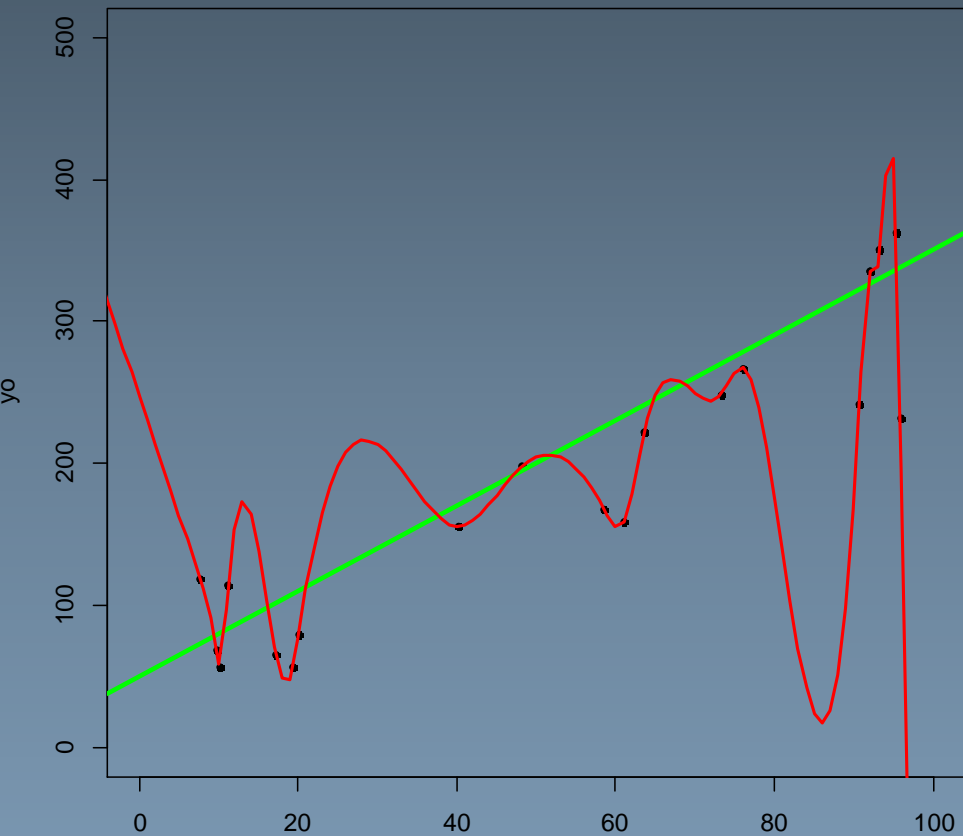
NON RIGID

Cubic spline degree of
freedom (nodes)=5





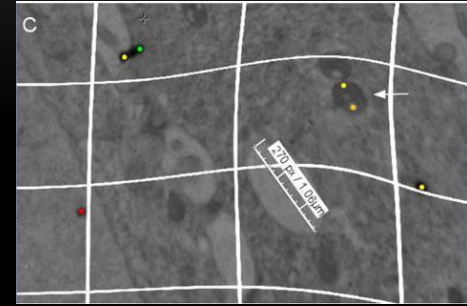
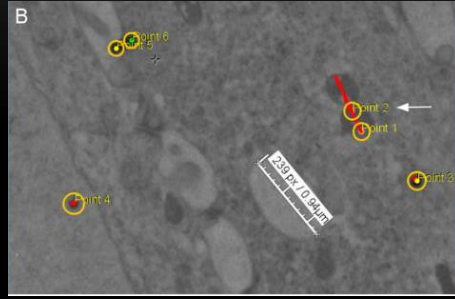
What about changing the basis of the transform?
Cubic spline degree of freedom (nodes)=3



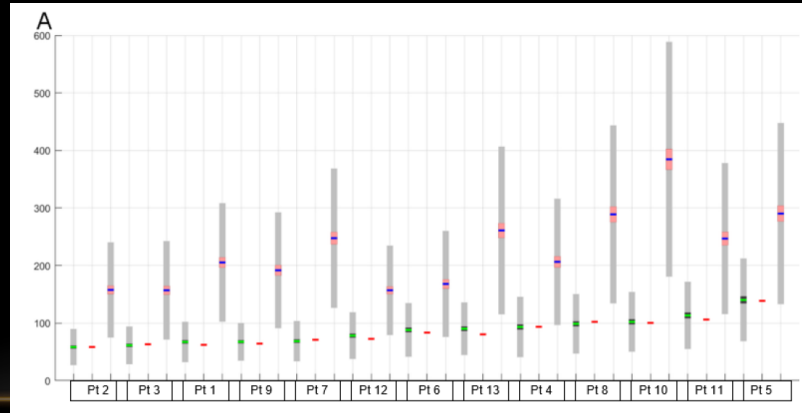
What about changing the
basis of the transform?
Cubic spline degree of
freedom (nodes)=20

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

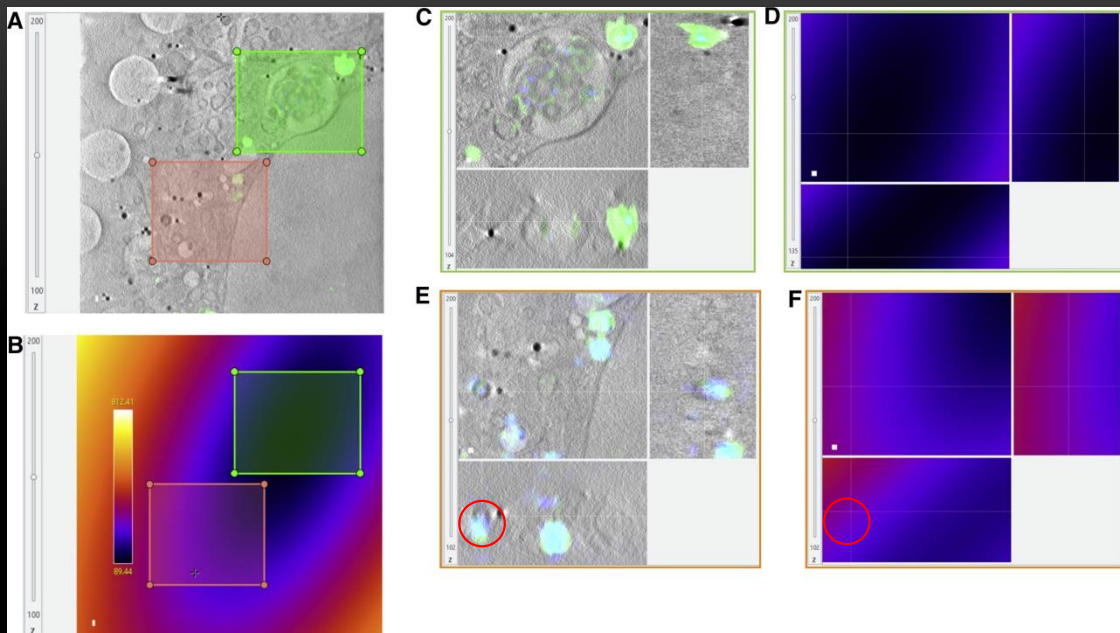
WARNING WITH NON RIGID REGISTRATION



Error
in nm



Point not used for the registration
(ordered by their distance to fiducial set)

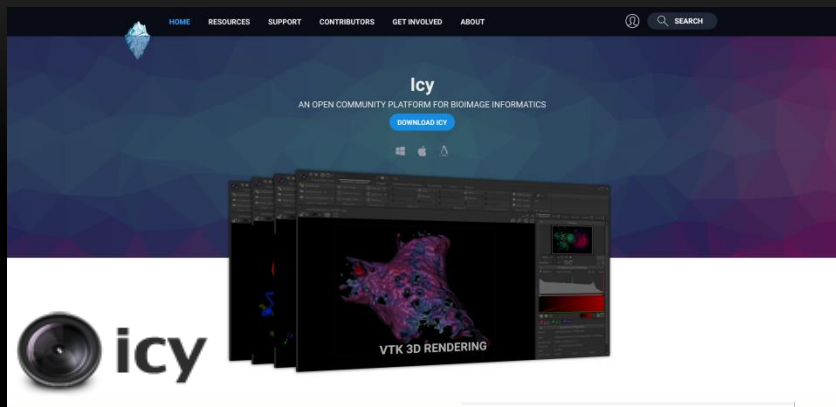


Kounatidis I, Stanifer ML, Phillips MA, Paul-Gilloteaux P, et al.
 3D Correlative Cryo-Structured Illumination Fluorescence and
 Soft X-ray Microscopy Elucidates Reovirus Intracellular
 Release Pathway. Cell. 2020

Assessing that it is likely to be
 at the same position rather
 than warping.

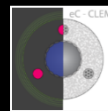
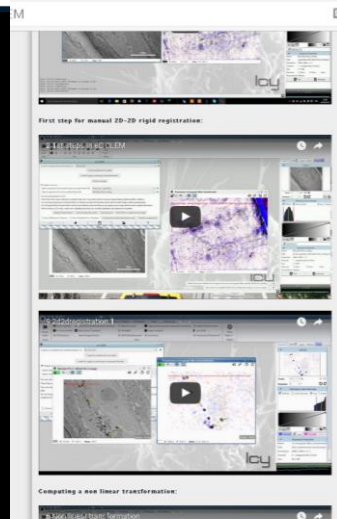
Transformation = Rigid because sample were
 cryogenized and exactly the same sample
 with no further sample prep between the two
 steps

EC-CLEM IS A SET OF PLUGINS UNDER ICY, AN OPEN SOURCE AND FREE IMAGE ANALYSIS PLATFORM

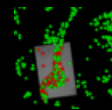


<http://icy.bioimageanalysis.org/>

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**



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.
- Think about what could happen to your sample during sample prep, how you will ensure to find back the same area. Think 3D for the registration if needed, think intermediate images
- There is no such thing perfect registration: Estimating the confidence in pixel matching is important, it usually requires to add in purpose some object visible in both modalities to check the quality of matching, but some statistical method are also available for it.
- Ec-CLEM is a free software especially developed for Correlative Microscopies

Acknowledgements

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Marie-Charlotte Domart

MicroPICell

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Philippe Hulin
Magalie Feyeux
Steven Nedellec
Marine Malloci

Institut Curie

Xavier Heiligenstein (CryoCapcell)
Jean Salamero
Graca Raposo

Diamond Light Source

Maria Harkiolaki
Nina Vyas

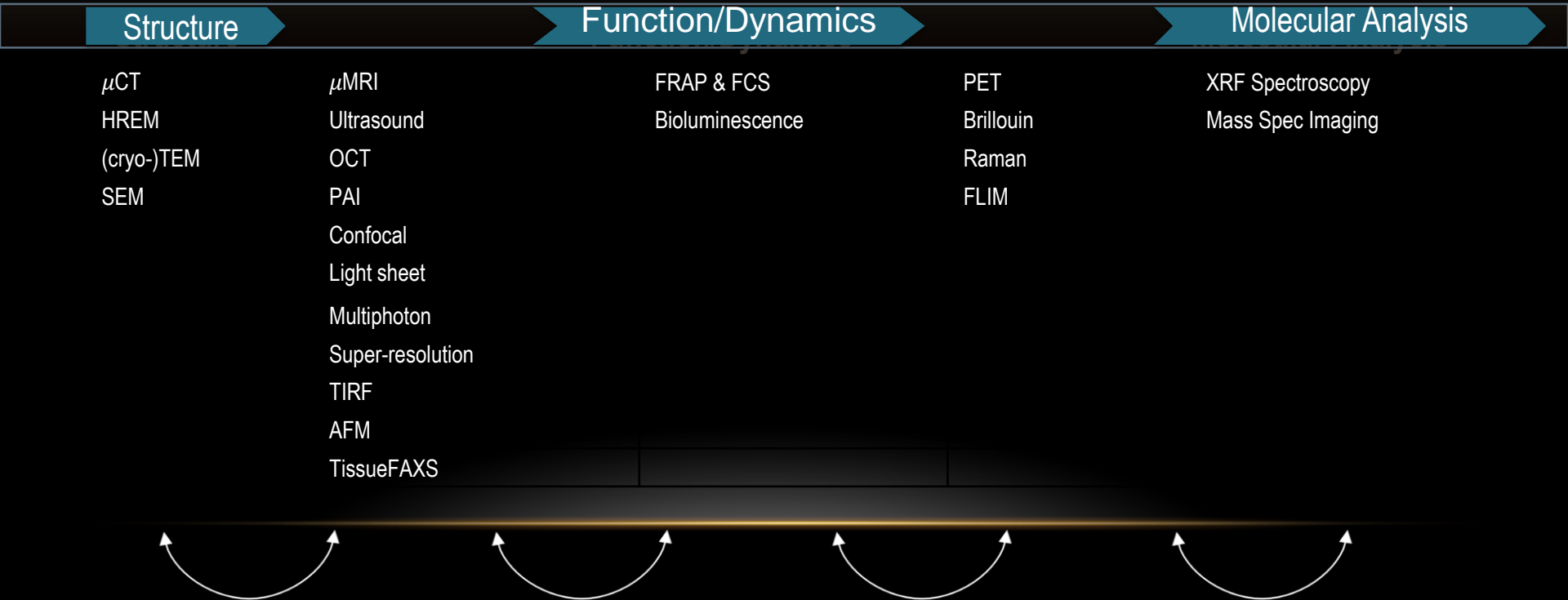
EMBL

Martin Schorb
Christian Tisher

QUESTIONS?

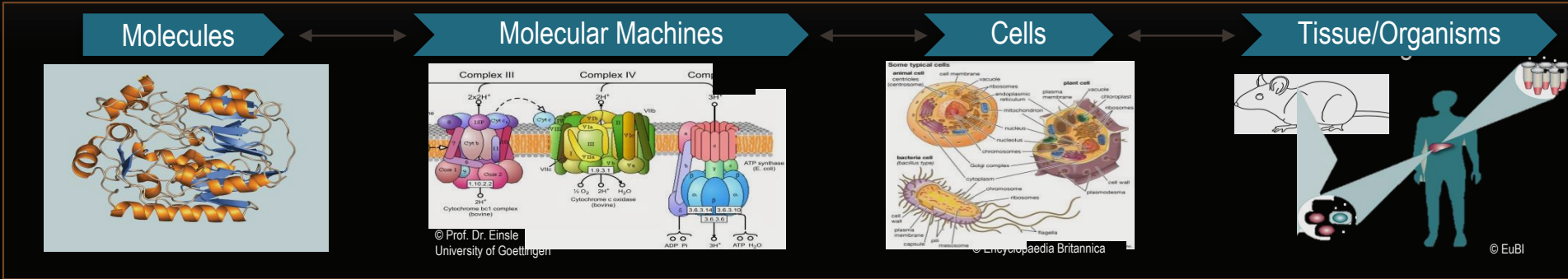
Background: Correlated Imaging

- **Gaining Holistic Information**

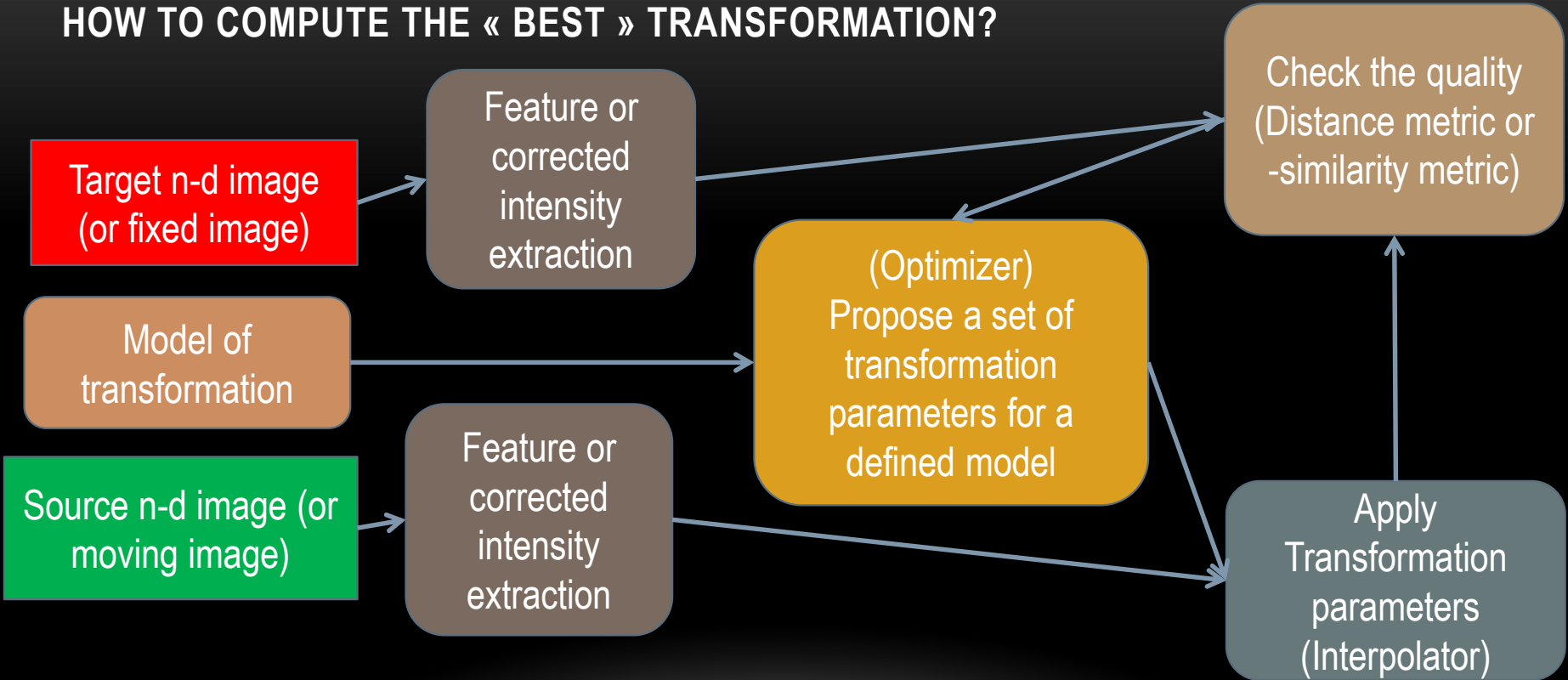


Background: Correlated Imaging

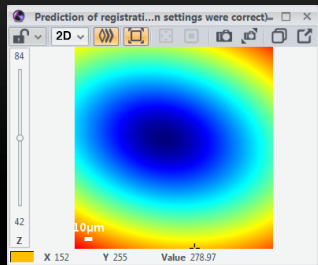
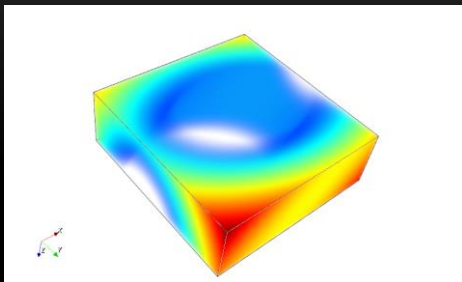
- Gaining Multiscale Information



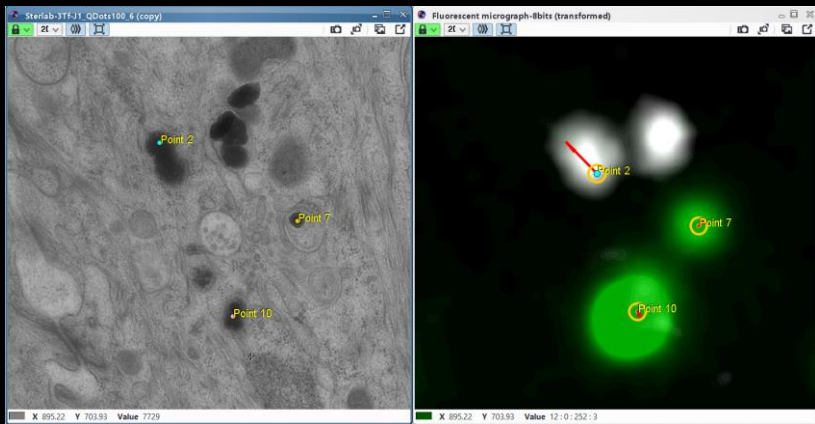
HOW TO COMPUTE THE « BEST » TRANSFORMATION?



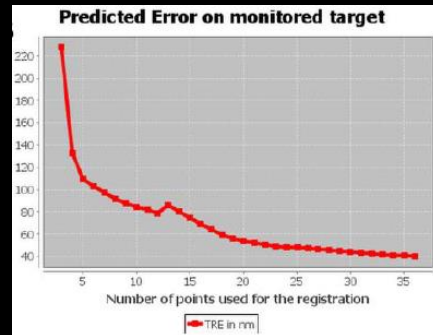
ERROR ASSESSMENT



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