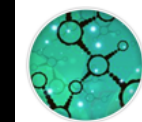


IMAGE PROCESSING AND ANALYSIS IN CLEM: METHODS AND PITFALLS

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



FRANCE-BIOIMAGING

CHALLENGES IN CORRELATIVE IMAGING FOR IMAGE PROCESSING AND ANALYSIS: FOCUS ON REGISTRATION

Very different aspect in
both modalities

Multiscale

Universal solution?

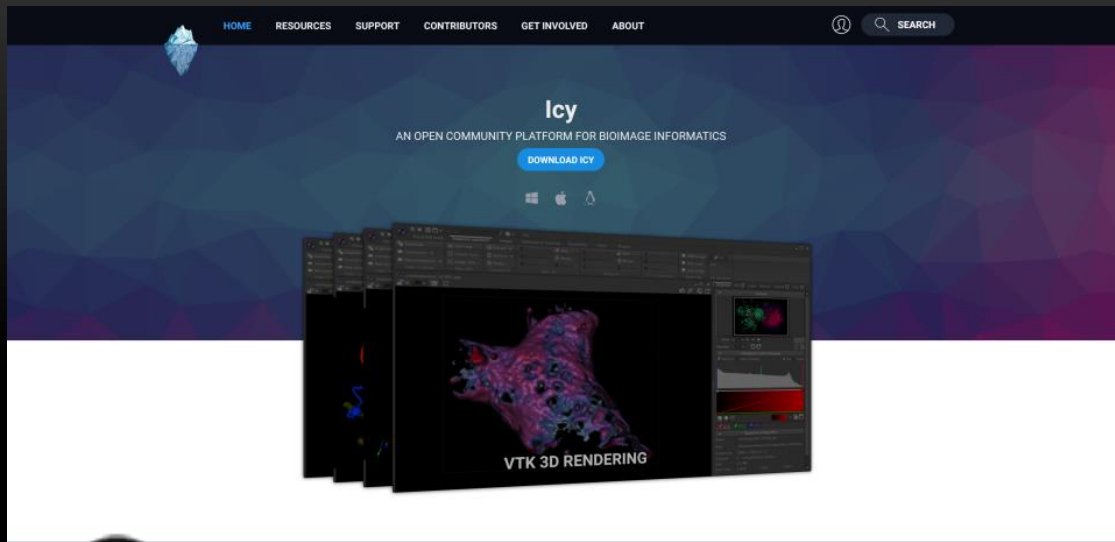
Missing elements

Multidimensional

Easy to use / Guided

Big (size) data

- Workflow definition-> not only two modalities/two acquisition
 - Lack of standards

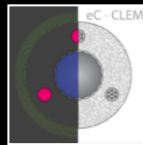


- NEW VERSION !! (beta but usable)
- MUCH MORE FASTER
 - LESS GREEDY in memory
 - New tools for error estimation (colocalization like)
 - New Tools to ease your correlative WF constructions
 - New Tool for visualization

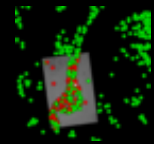
<http://icy.bioimageanalysis.org/>

Ec-clemv2

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**

STAR Protocols

PROTOCOL | FEBRUARY 11, 2022

Sample preparation and image registration for correlative cryo-FM and cryo-FIB-SEM of plunge-frozen mammalian cells

Madav Schar,^{1,4} Katya Reichav,² Perrine Paul-Giloteaux,³ and Ori Avnion^{1,5}

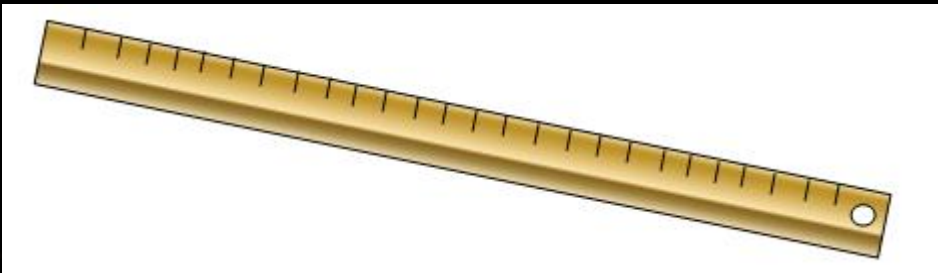
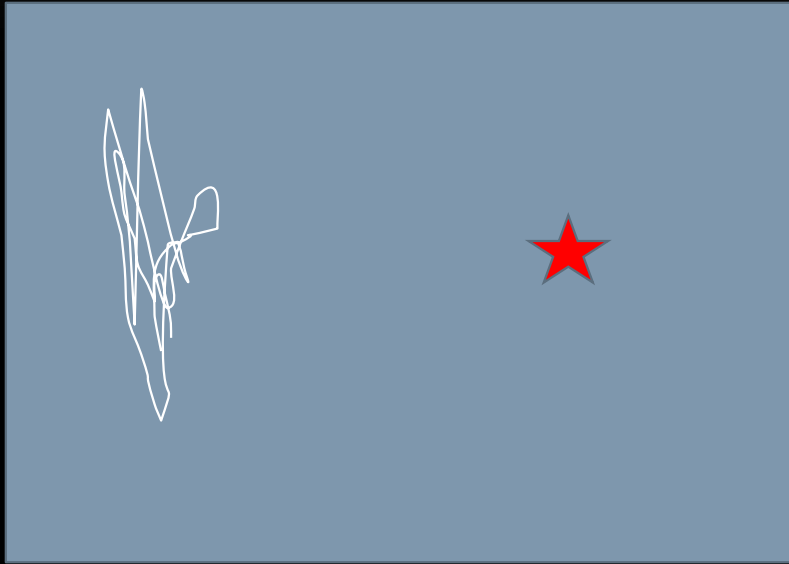
Methods in Cell Biology • Volume 140

CORRELATIVE LIGHT ELECTRON MICROSCOPY III

Publication of correlative soft X-ray tomography and illumination microscopy images

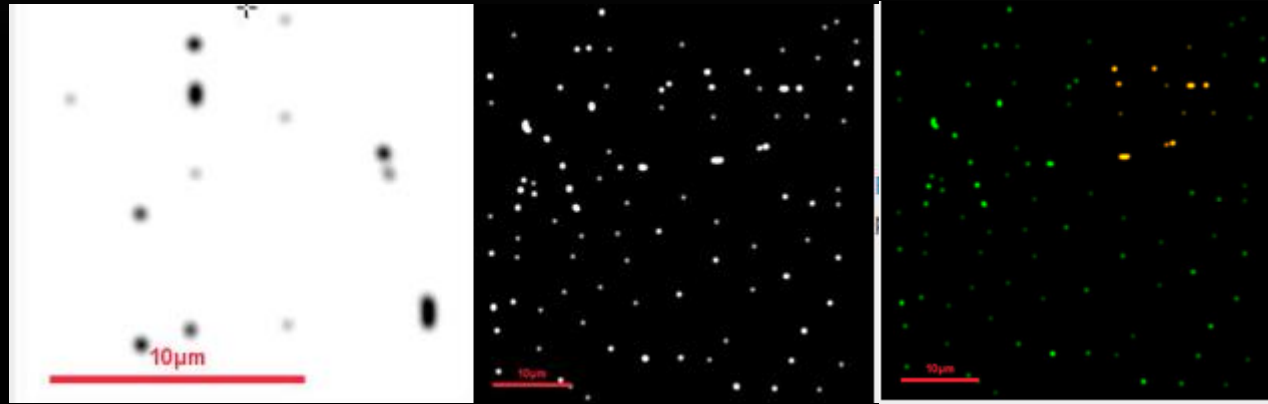
PMCID: PMC8721986 PMID: 36221687

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



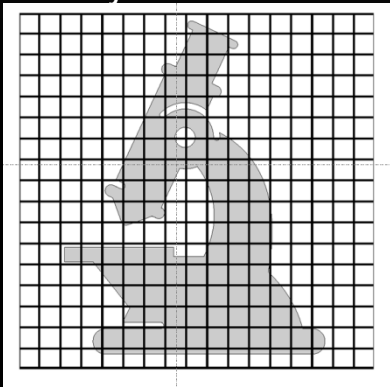
Registration is the computer vision method allowing to compute a TRANSFORMATION from the images content.

Model of transformation

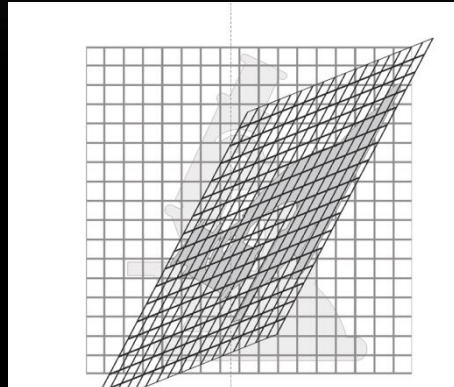
May the sample/specimen transfer/preparation have induced deformation? Is the scale relationship known (pixel size)?

Usually start from a linear transformation -> it may be sufficient and is less prone to errors than non rigid/ non linear.

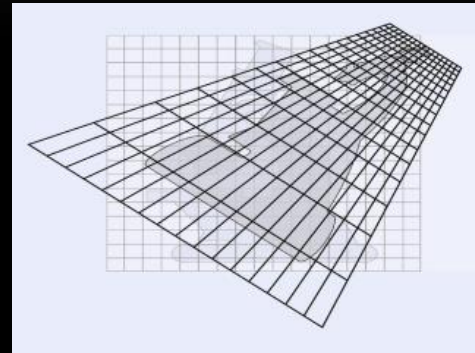
Identity



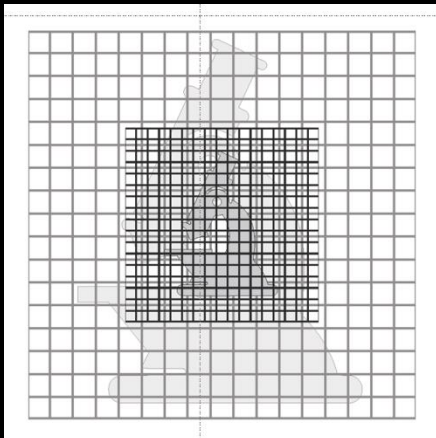
Affine



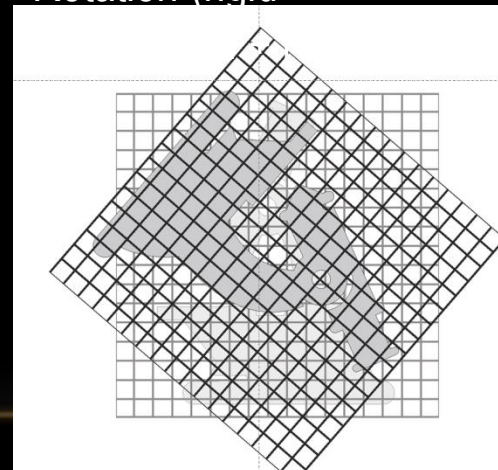
Projection



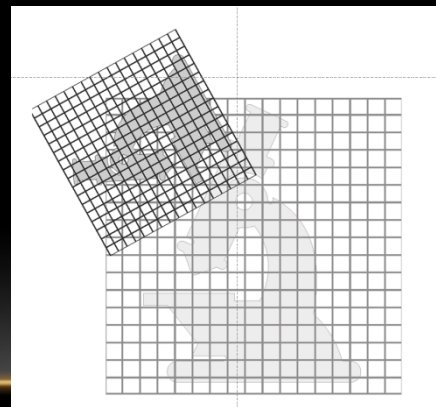
Scaling



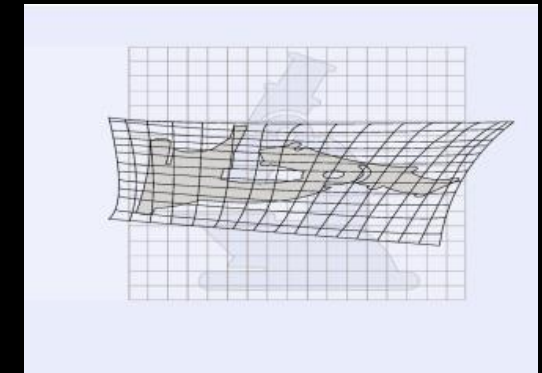
Rotation (rigid)



Similarity (= rigid + scaling)



Splines



Linear: can be represented by a MATRIX, a straight line will stay a straight line

Non Linear

Thousands of models:
Radial basis, Thin Plate Spline, B-splines, Wavelets, Piece-Wise linear transformations, Elastic transformation, Fluid Flows,...

APPROACHES FOR REGISTRATION

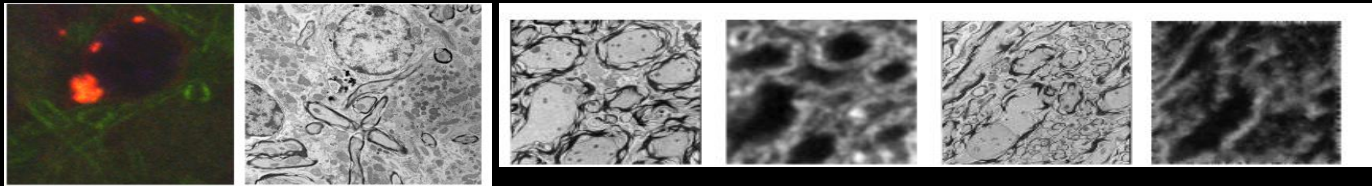
Fiducial= any stuff you or the computer can recognise in both images and that will be used for registration

Not Fiducial= any stuff NOT USED for registration

Intensity based

« Fiducial » based

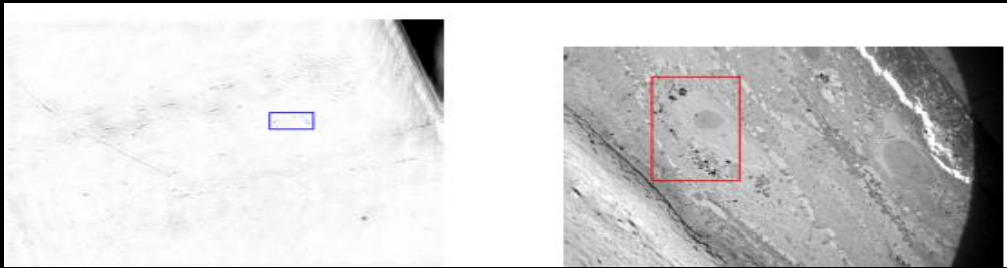
EXTRACTION OF « FIDUCIAL »=
Automatic or manual segmentation



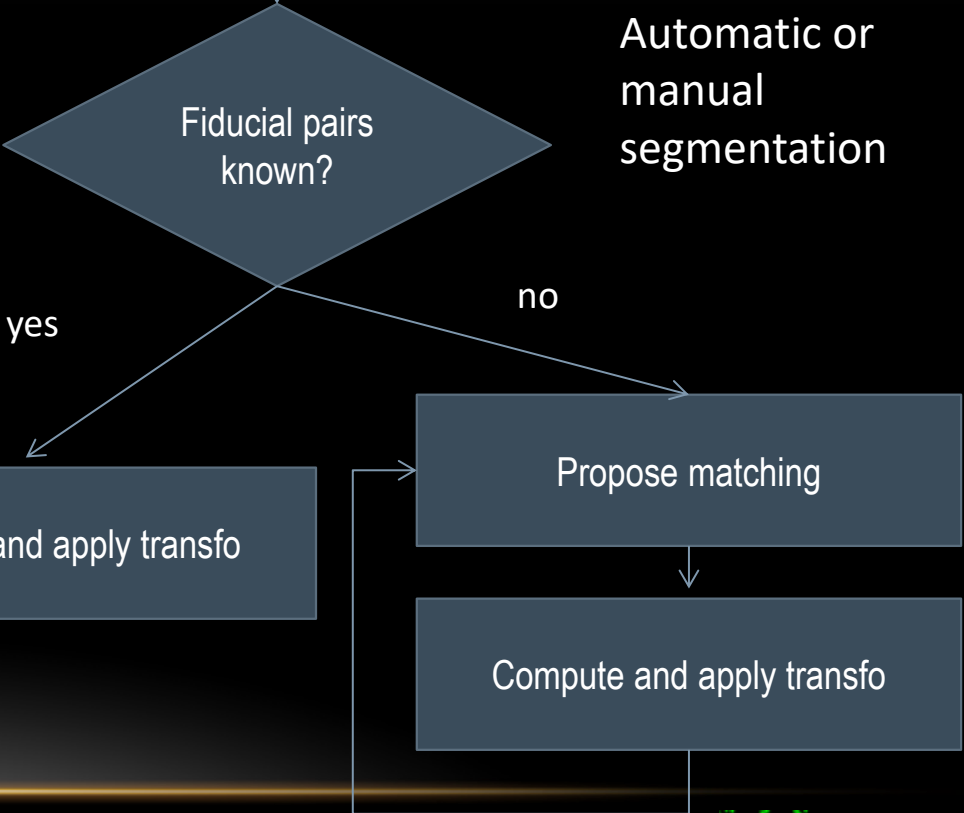
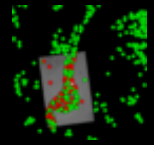
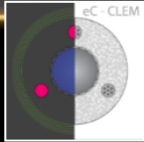
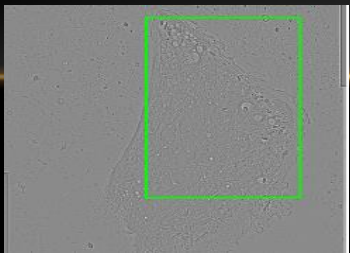
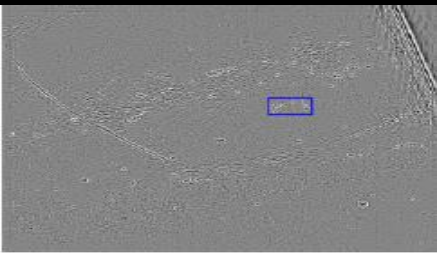
Cao et al . Medical Image Analysis 2014

Training patches TEM et confocal

Input and output Predicted confocal

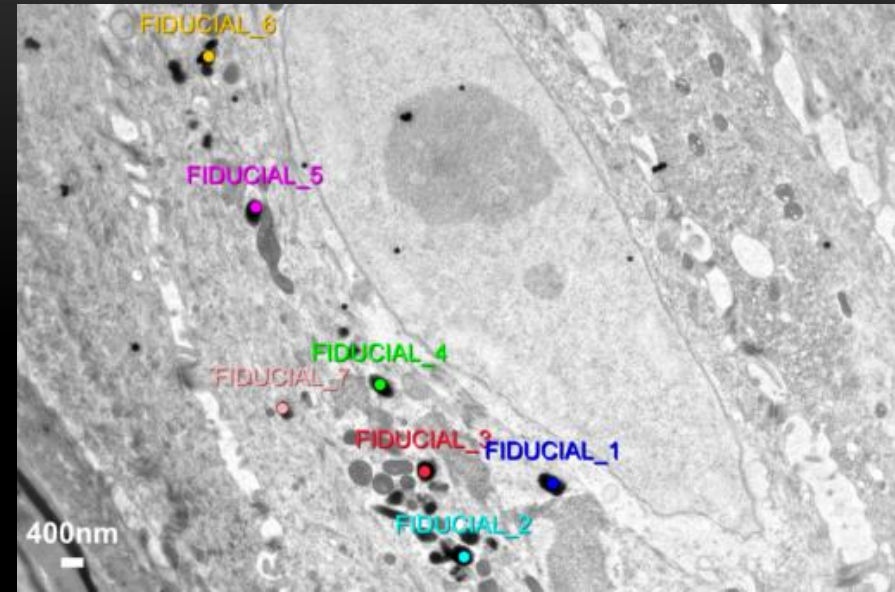
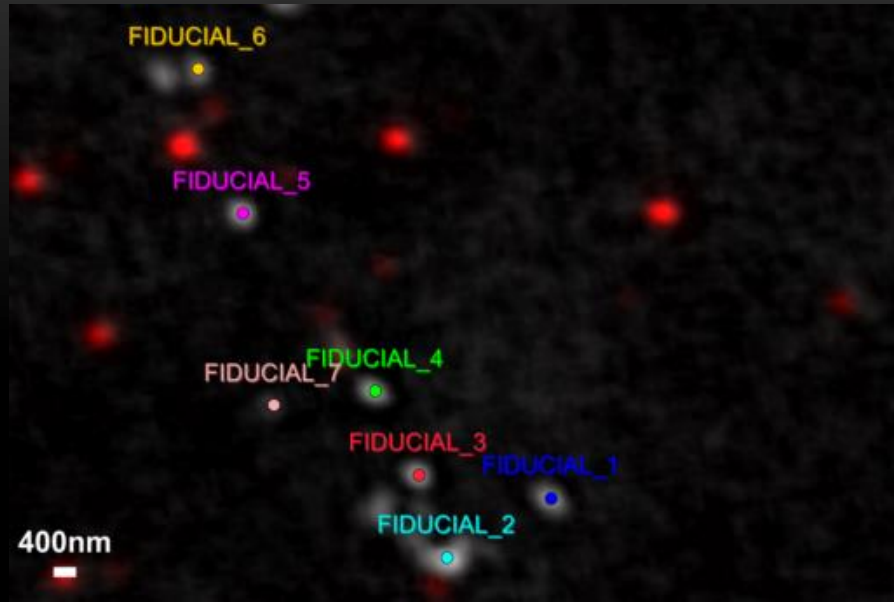


Bouthemy et al ISBI 2016



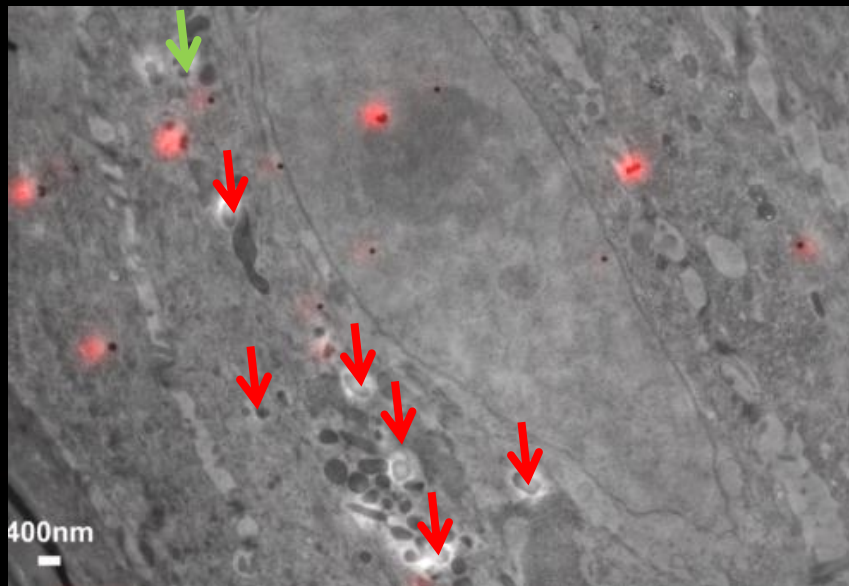
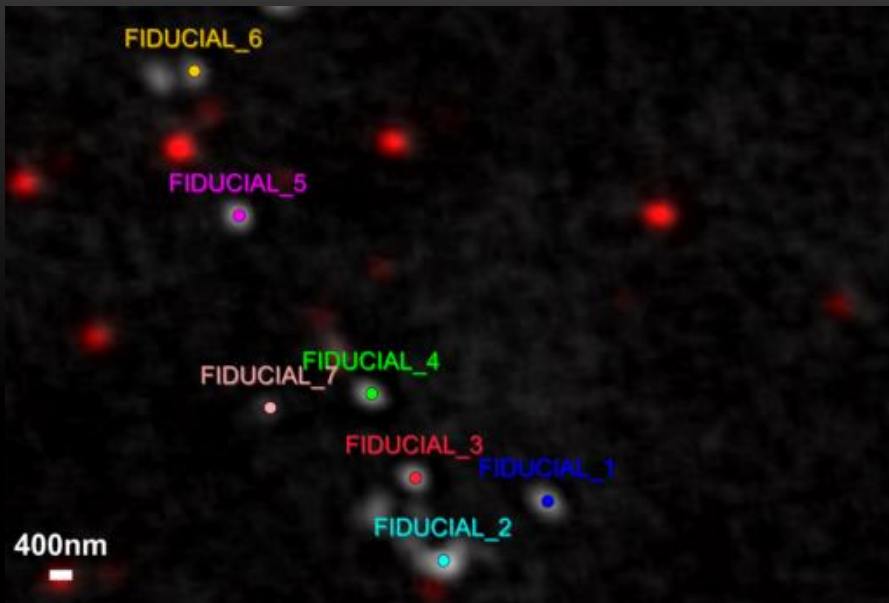
- One thing to know: NOTHING IS PERFECT
 - Because your sample may deform/ evolve between two modalities
 - Because multimodality is usually also multiscale (very different resolution, one pixel can match hundred of pixels in the second modality)
 - Because none modality allows to perfectly locate one 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.

HOW TO ASSESS THE QUALITY OF REGISTRATION?



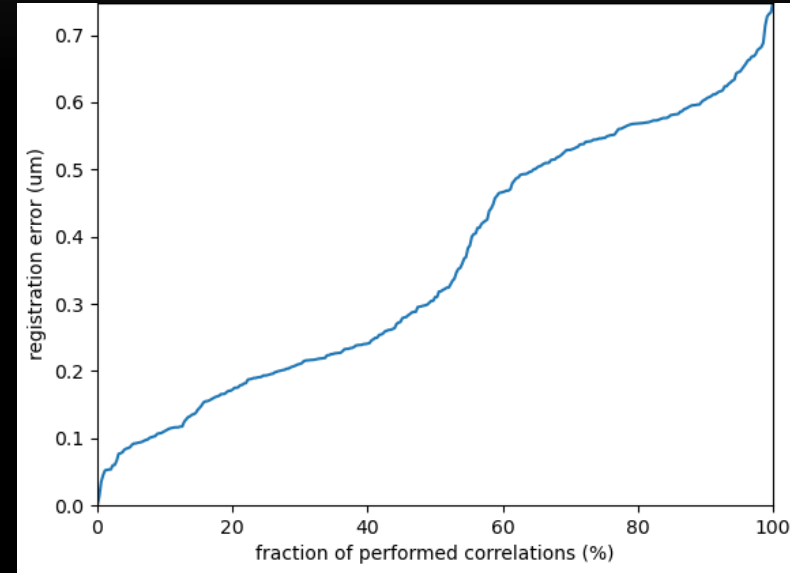
Beads are used here to check how good we are to estimate the error



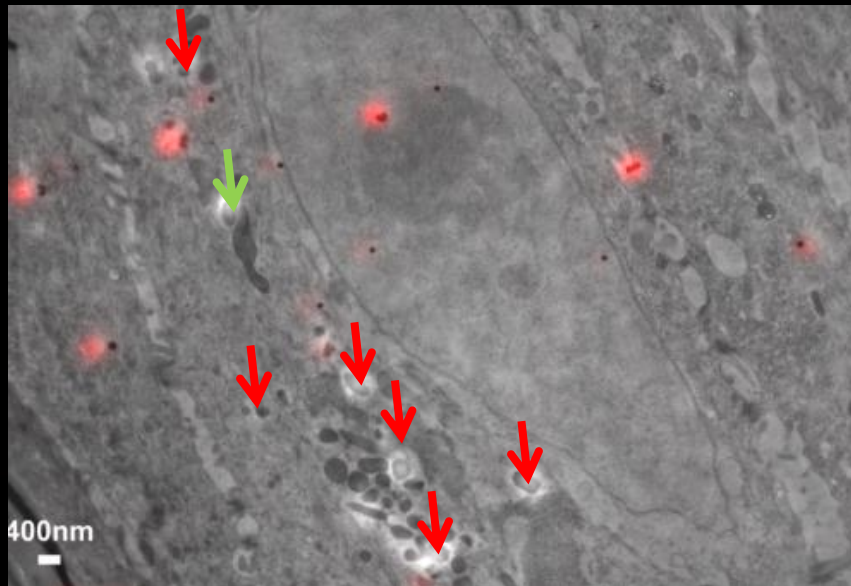
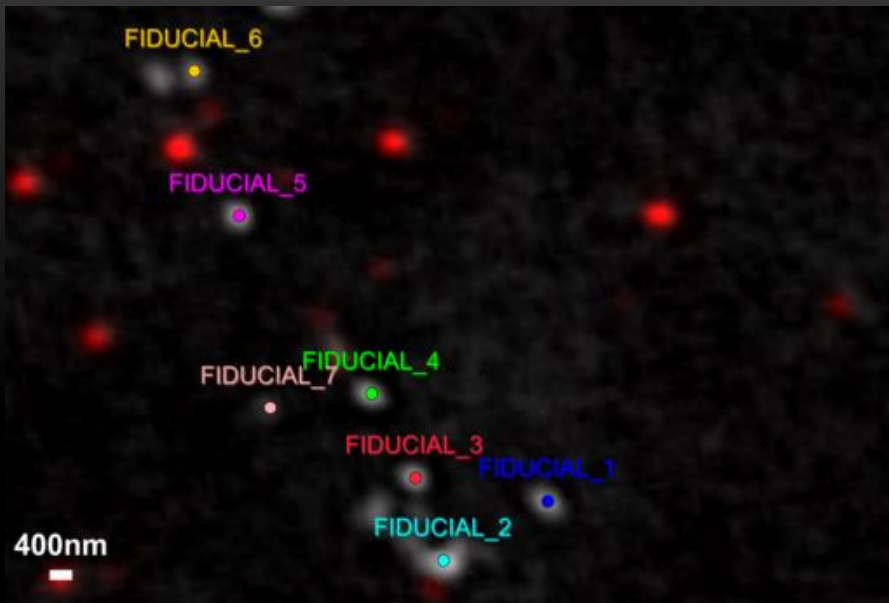


↓ fiducials

↓ Not fiducials

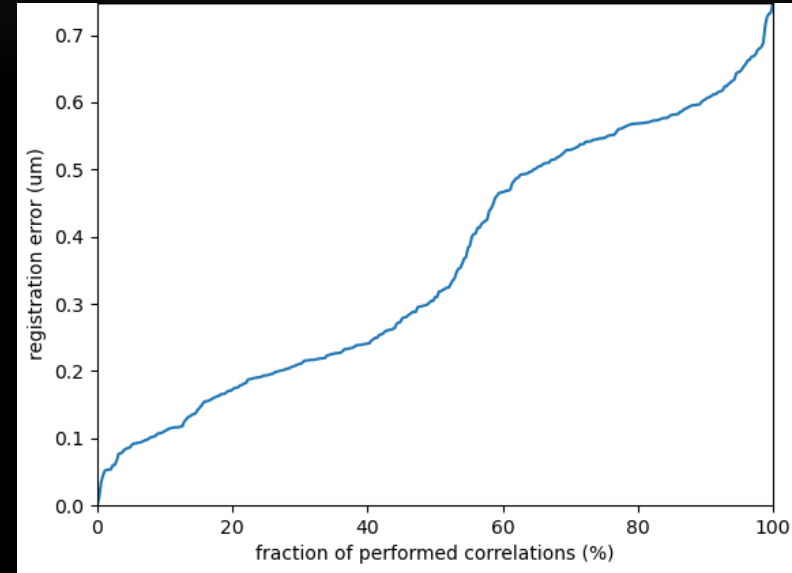


LEAVE-ONE OUT approach: Correlated fluorescence and 3D electron microscopy with high sensitivity and spatial precision Kulkuski, Schorb et al. JCB 2011

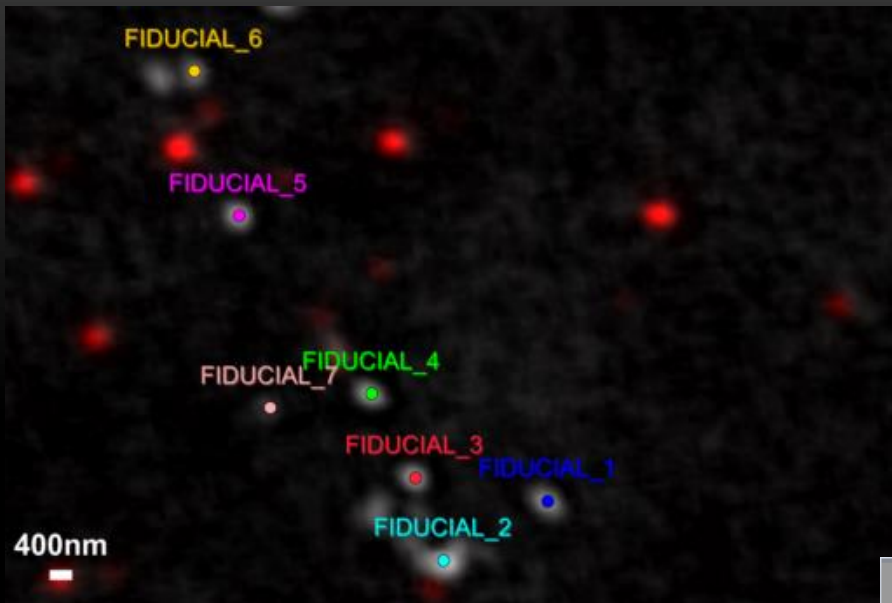


↓ fiducials

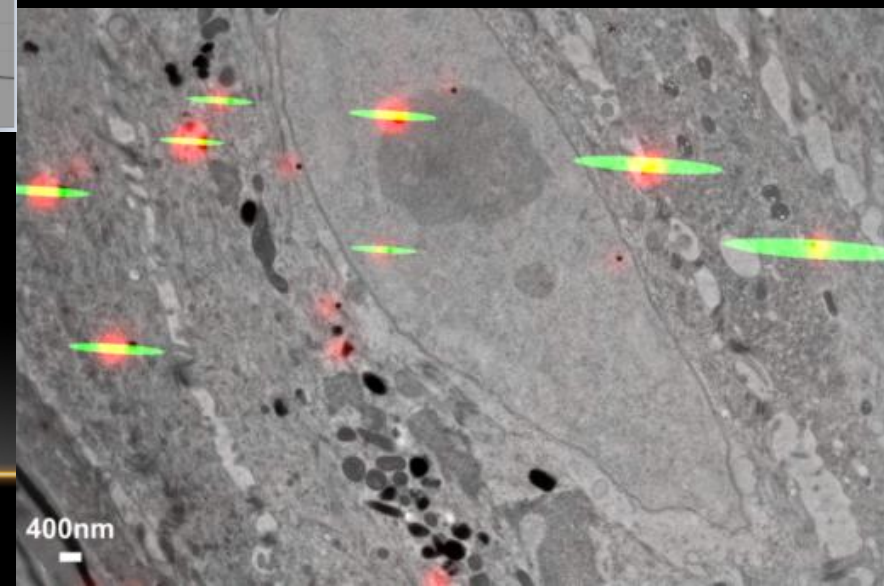
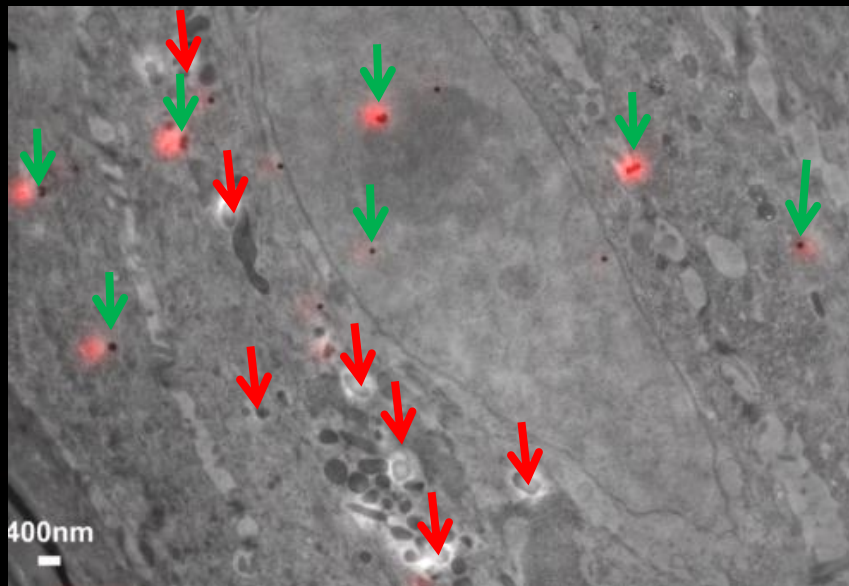
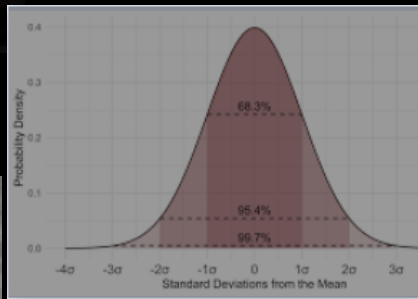
↓ Not fiducials



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

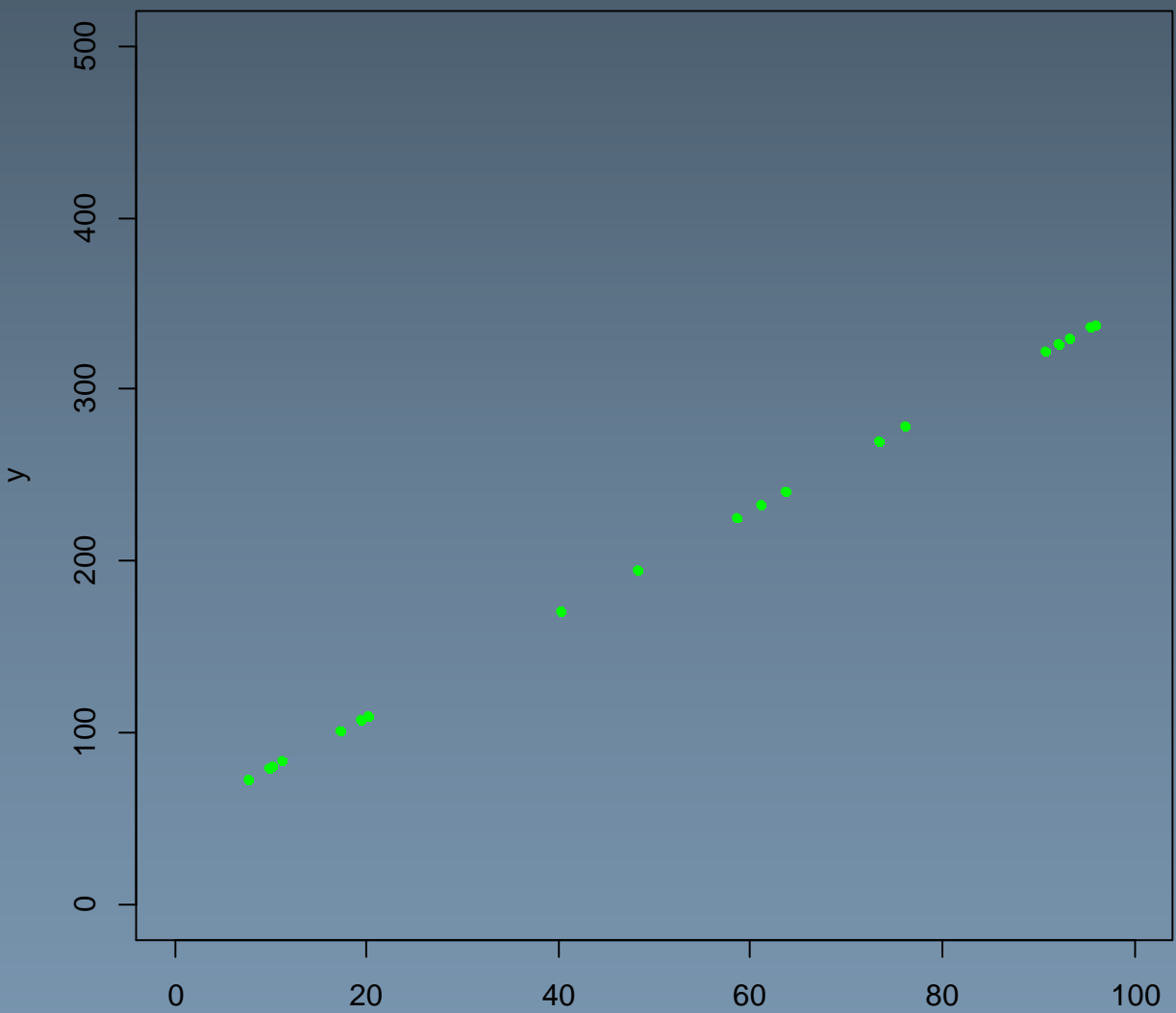


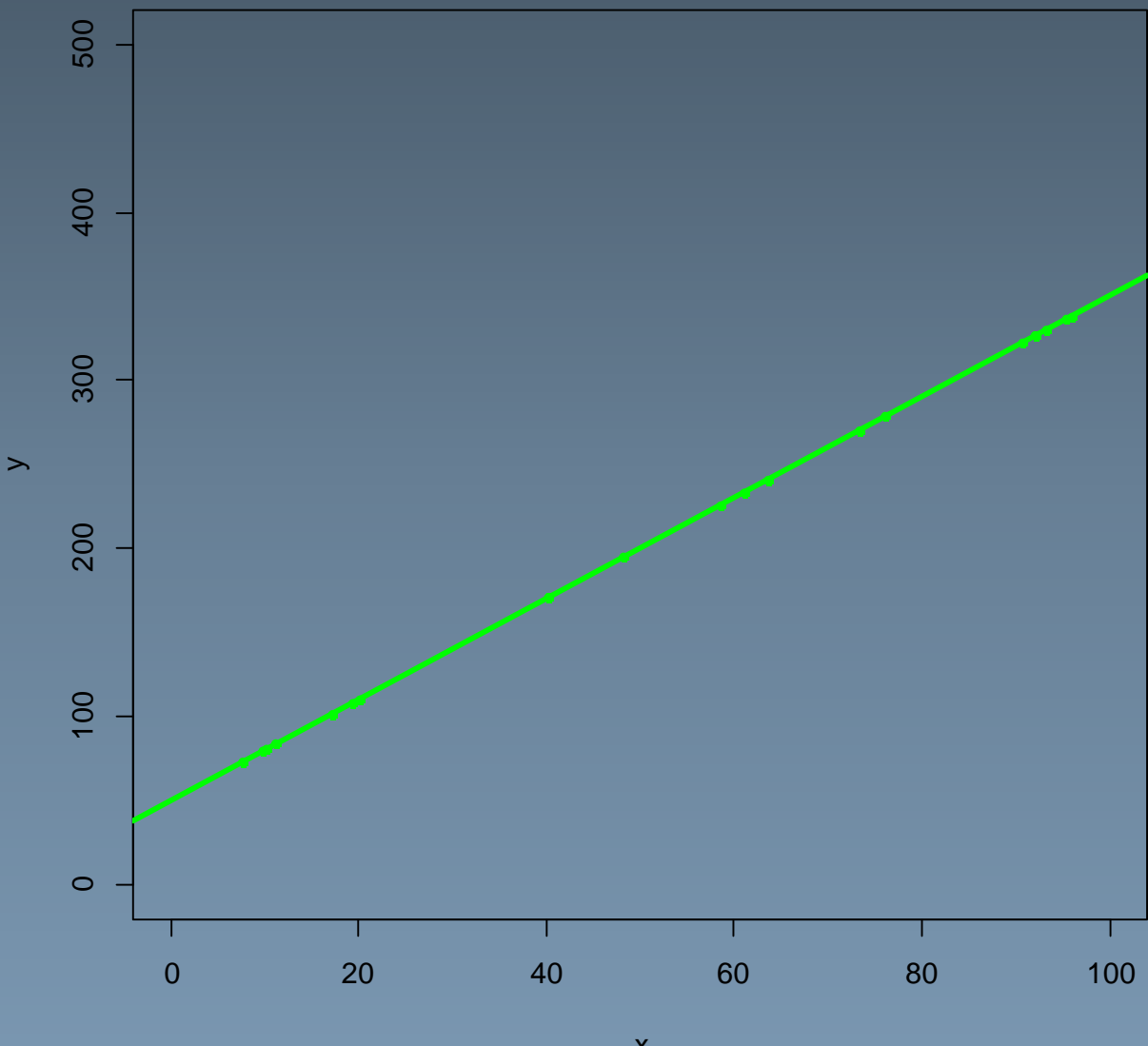
Predicted
Average
accuracy
at each
point



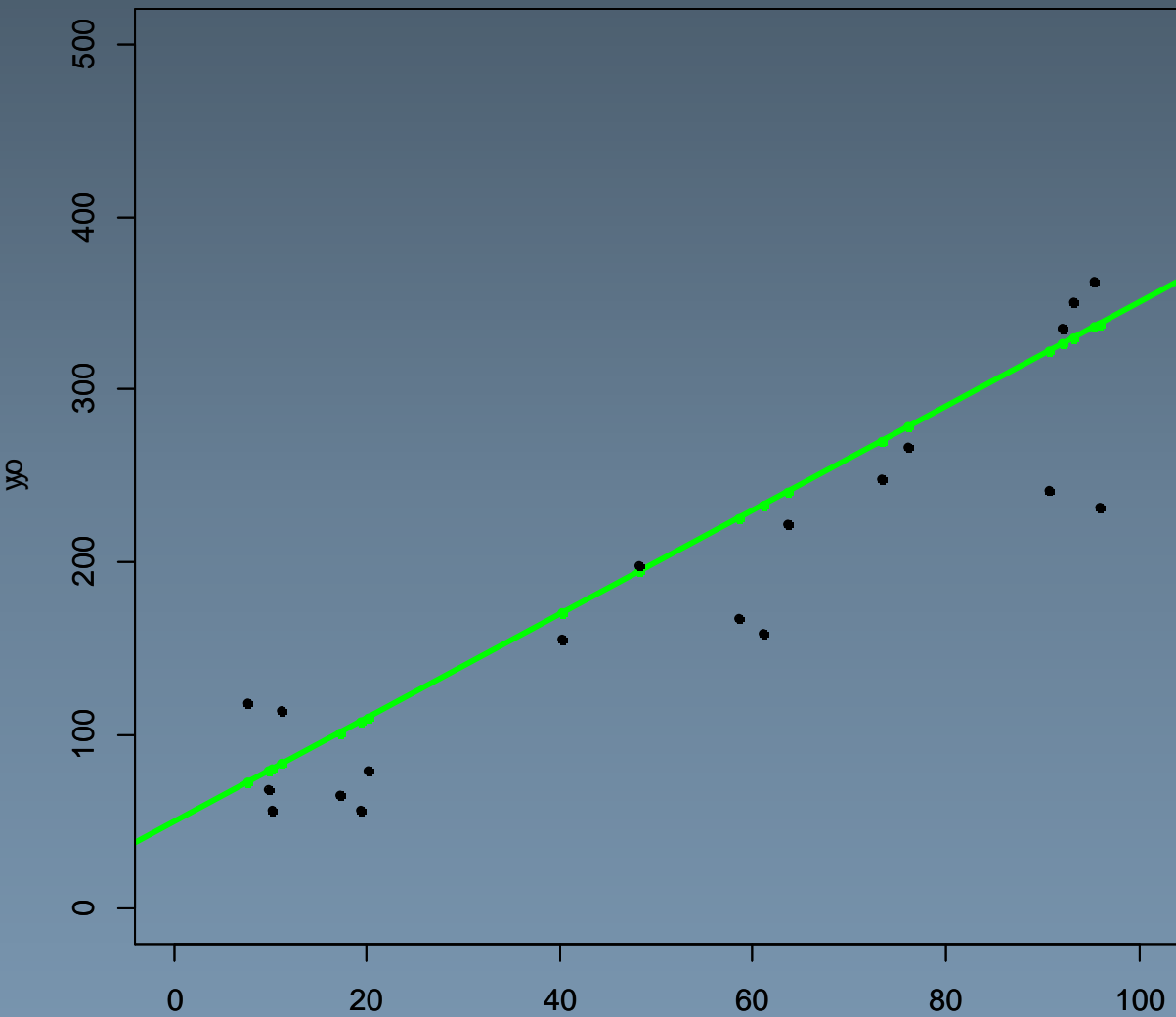
New in
ecclem:
95%
accuracy
prediction

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





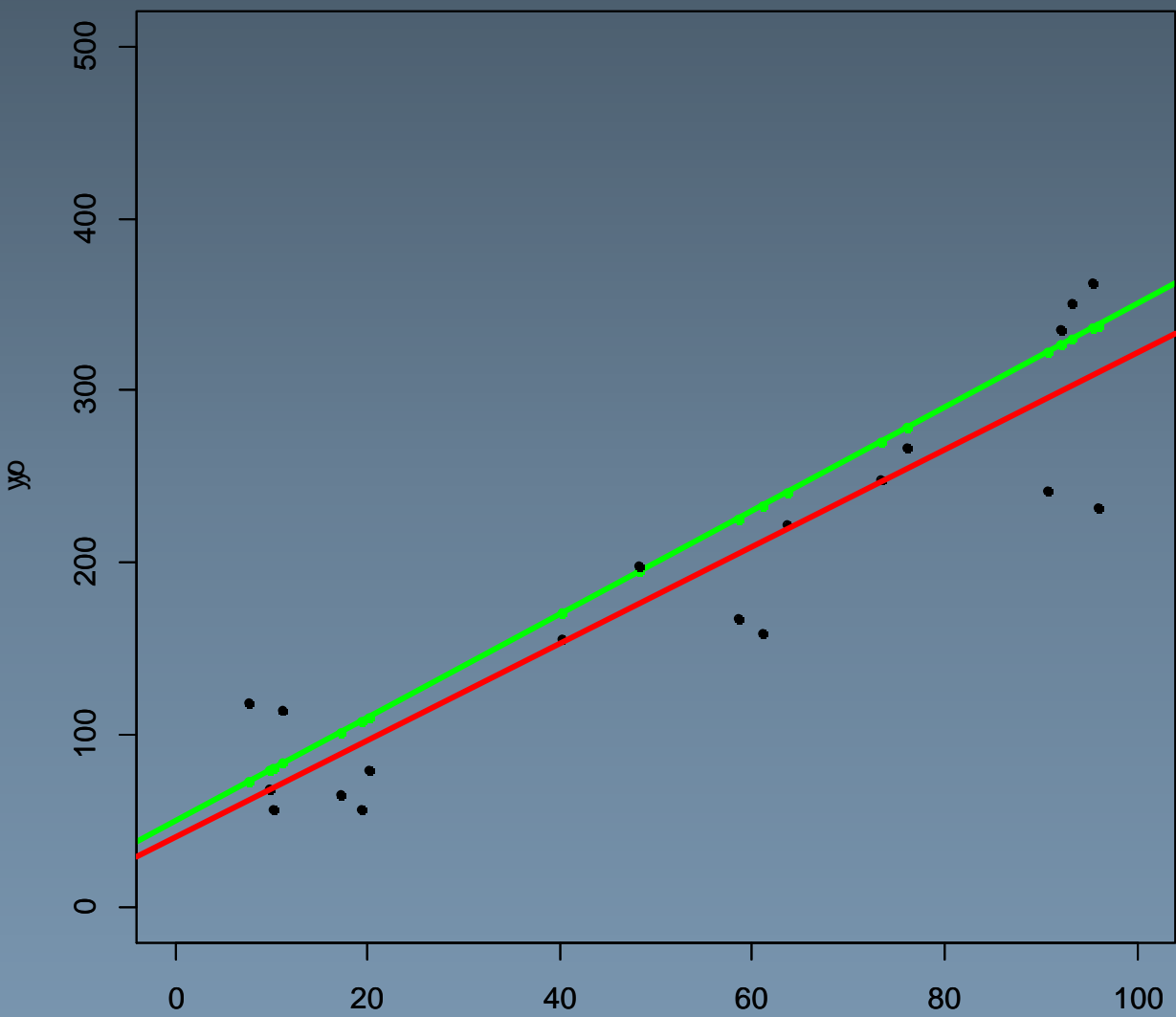
Seeing the transformation
computation as a regression problem:
example with a linear relationship
(**RIGID** i.e rotation + translation)
Find R and T such as:
 $y = Rx + T$



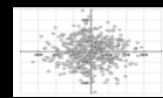
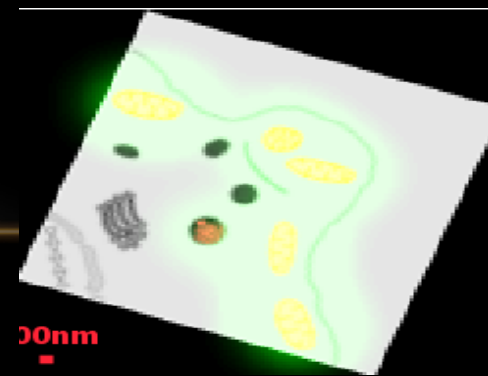
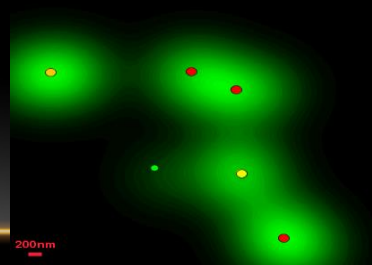
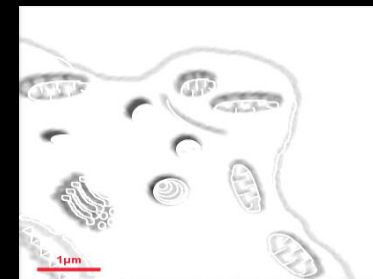
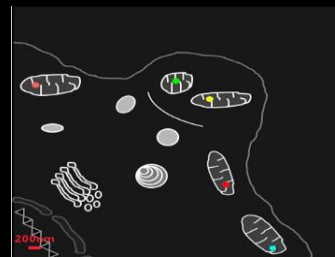
More realistic situation: « noise »
Typically the error in positioning the fiducial
(RESOLUTION!!)

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

$x_o = x + \text{noise}$

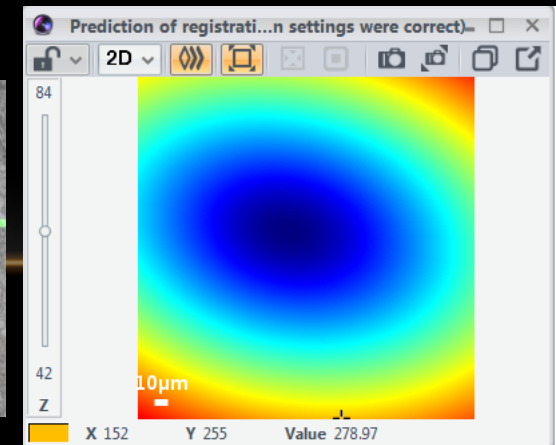
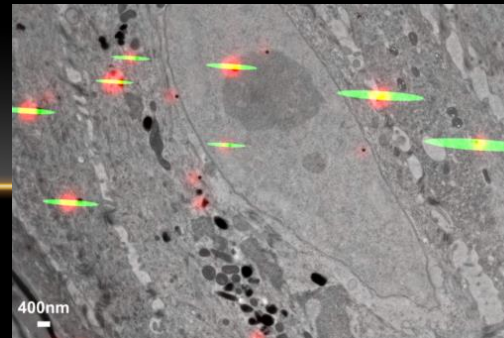
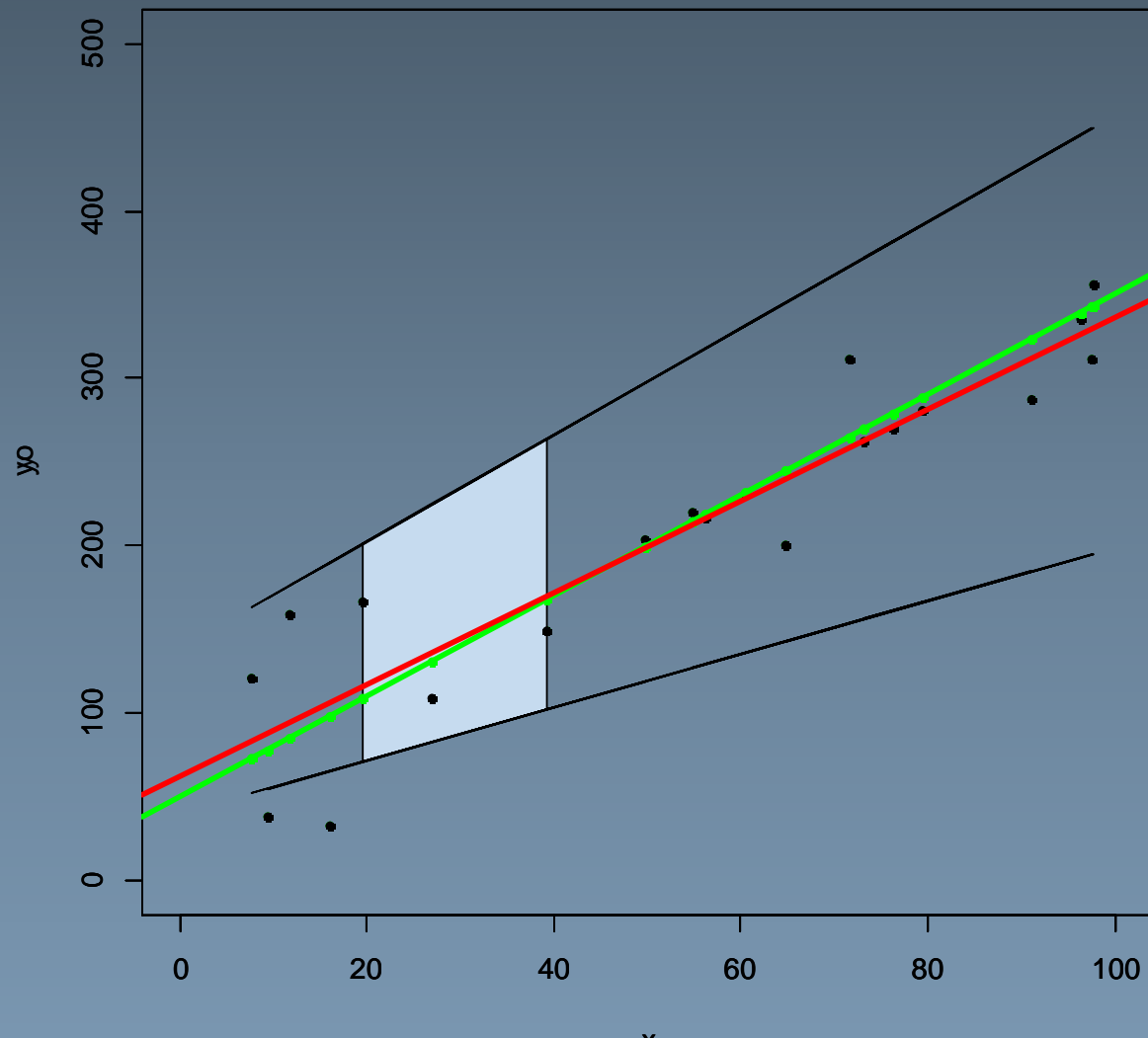


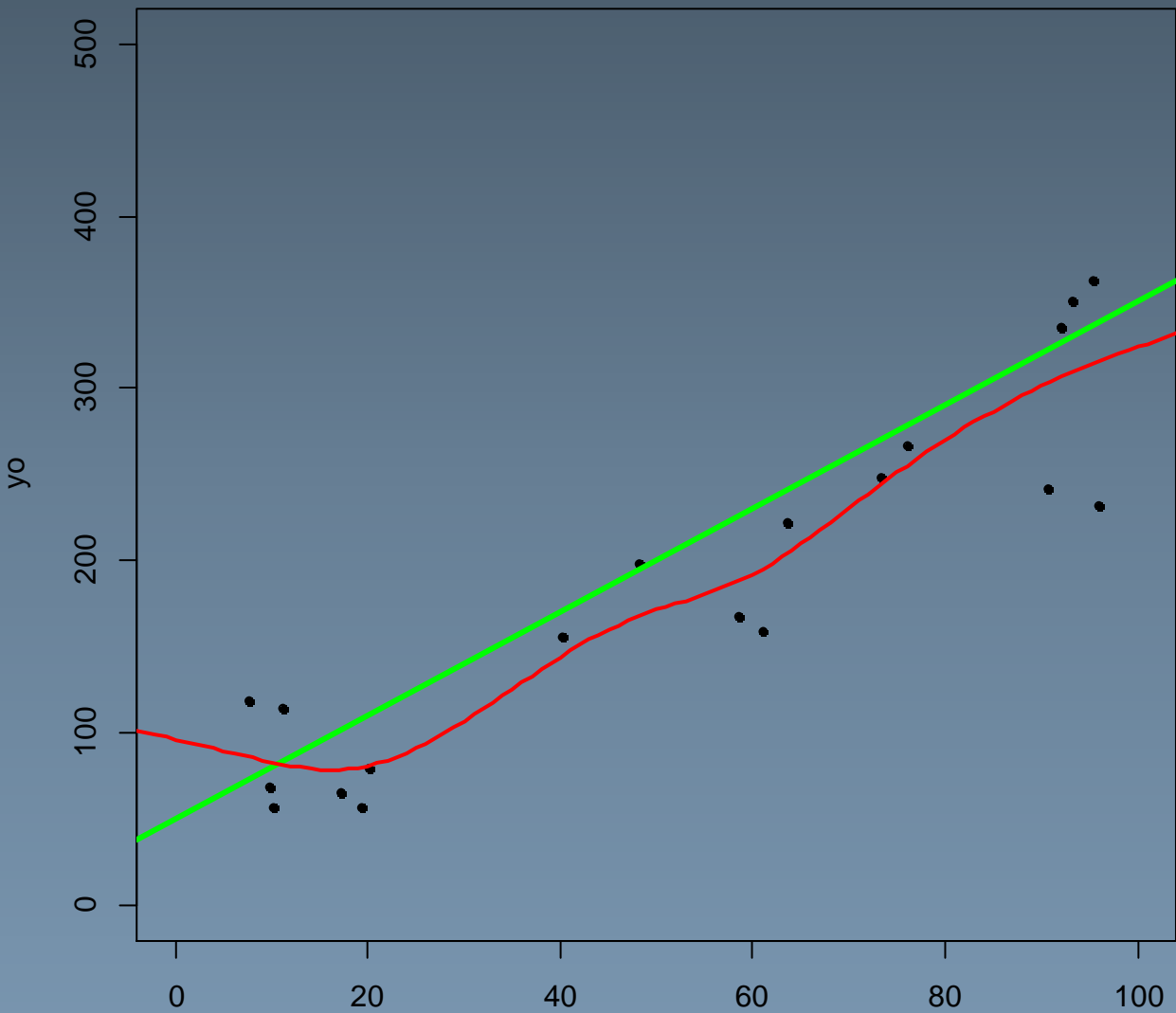
Estimated transform
 $Y_0 = R X_0 + T$



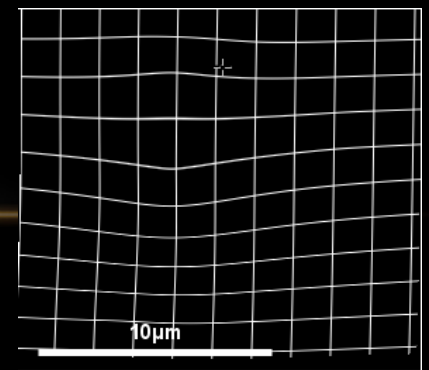
We have statistical tools to predict the confident interval (here 95 %) for any new x!

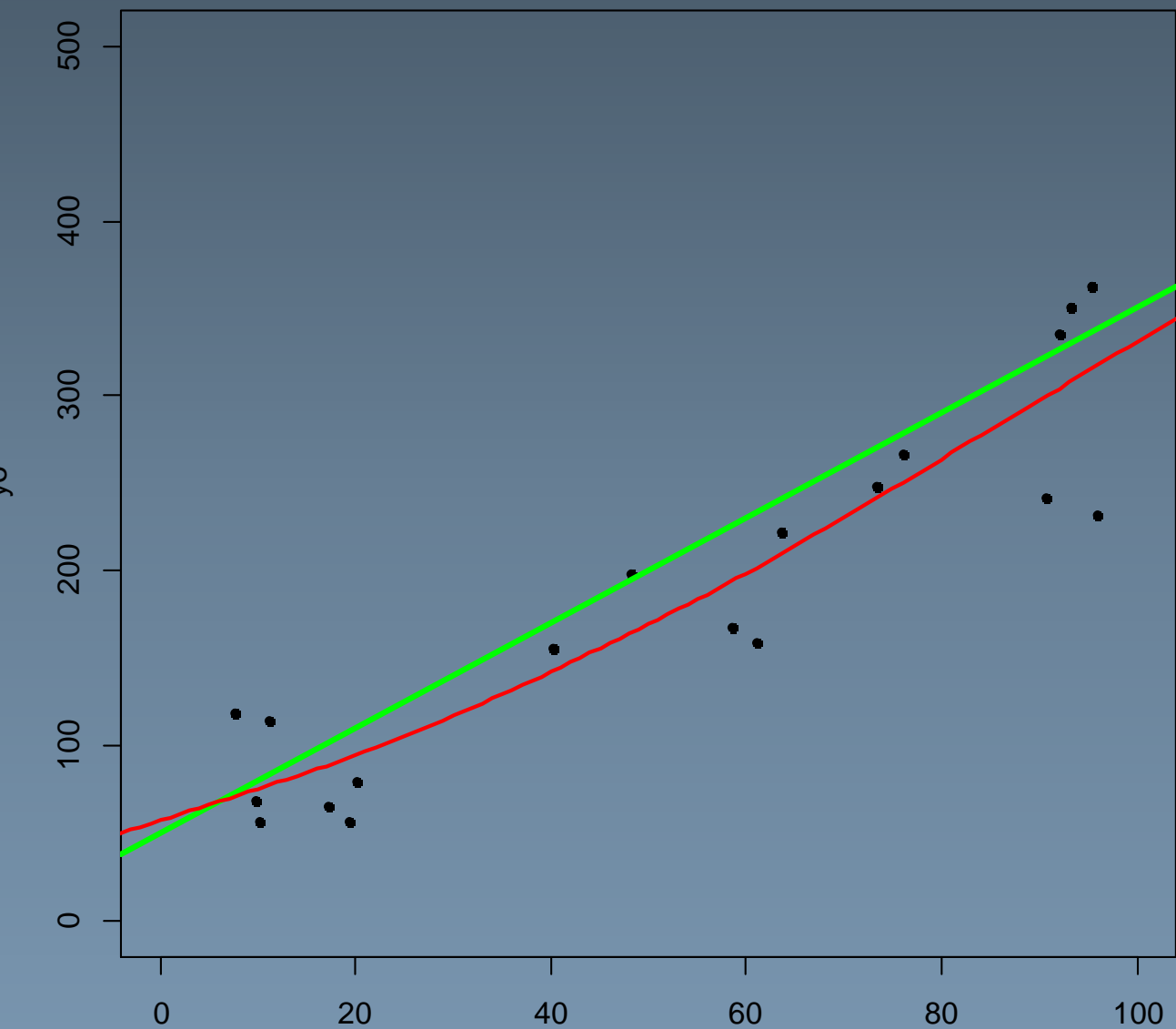
we only have an estimation of the transform because of the « noise » on the position of fiducials **BUT AT LEAST we know how accurate we are**



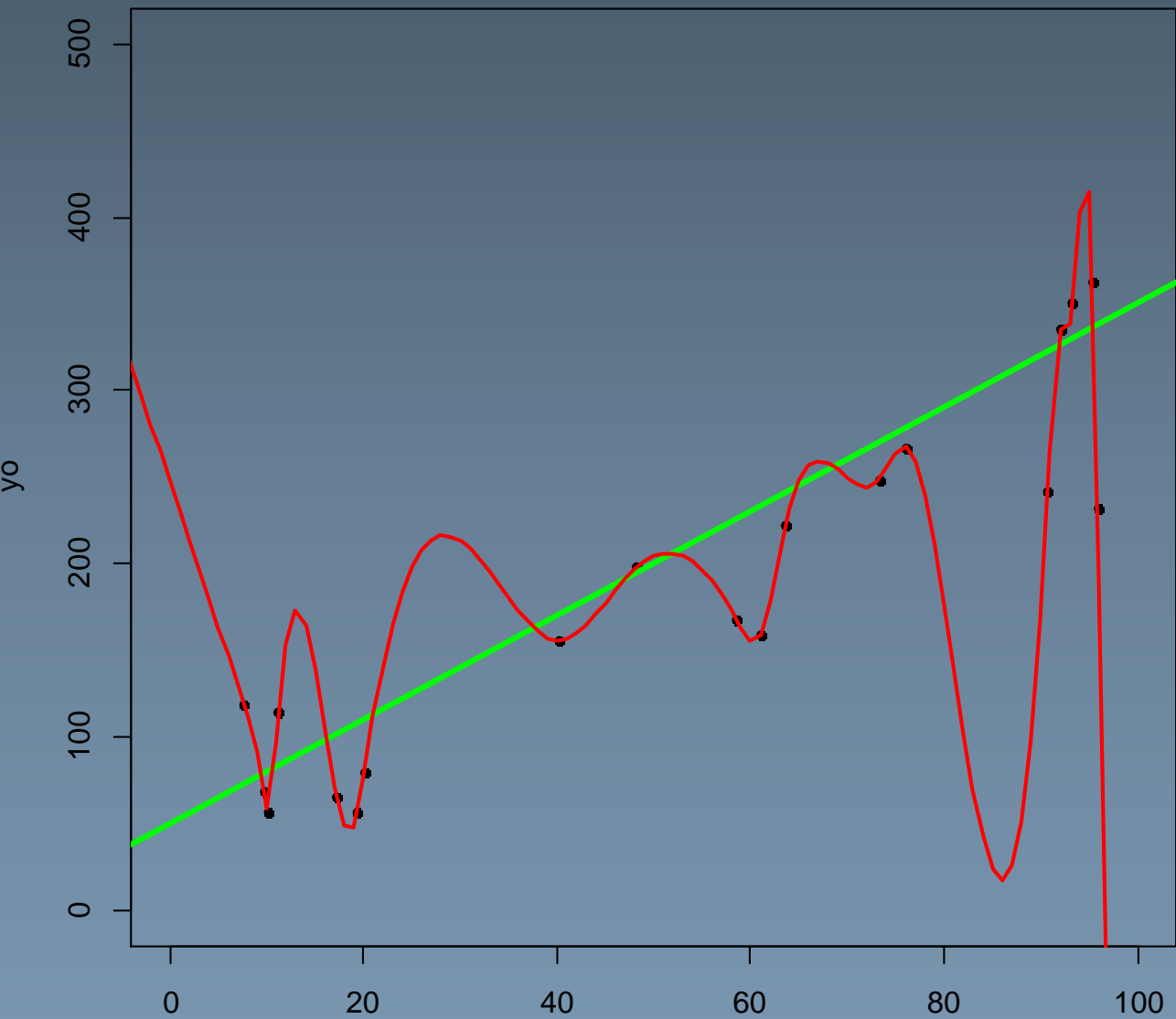


What about changing the basis of
the transform? **NON RIGID**
Cubic spline degree of freedom
(nodes)=5





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

APPLICATION TO CONSTRUCT CORRELATIVE WFS

- Cryo Structured Illumination Microscopy with Cryo Soft X-Ray (B24 beam line Diamond Synchrotron) -> based on cascaded transformations and rigid transformation assumption. (Collaboration with Diamond , Oxford UK-Heidelberg , Germany)
- Cryo WF microscopy with Cryo FIB-SEM -> based on lipid droplets as fiducials (Collaboration with Weizmann Institute Israel)

 **STAR Protocols**

STAR Protoc. 2021 Jun 18; 2(2): 100529. PMID: PMC8121986
Published online 2021 May 6. doi: [10.1016/j.xpro.2021.100529](https://doi.org/10.1016/j.xpro.2021.100529) PMID: [34027487](https://pubmed.ncbi.nlm.nih.gov/34027487/)

Protocol for image registration of correlative soft X-ray tomography and super-resolution structured illumination microscopy images

Nina Vyas,¹ Stephan Kunne,² Thomas M. Fish,¹ Ian M. Dobbie,³ Maria Harkiolaki,^{1,6,*} and Perrine Paul-Gilloteaux^{2,4,5,**}

 **STAR Protocols**

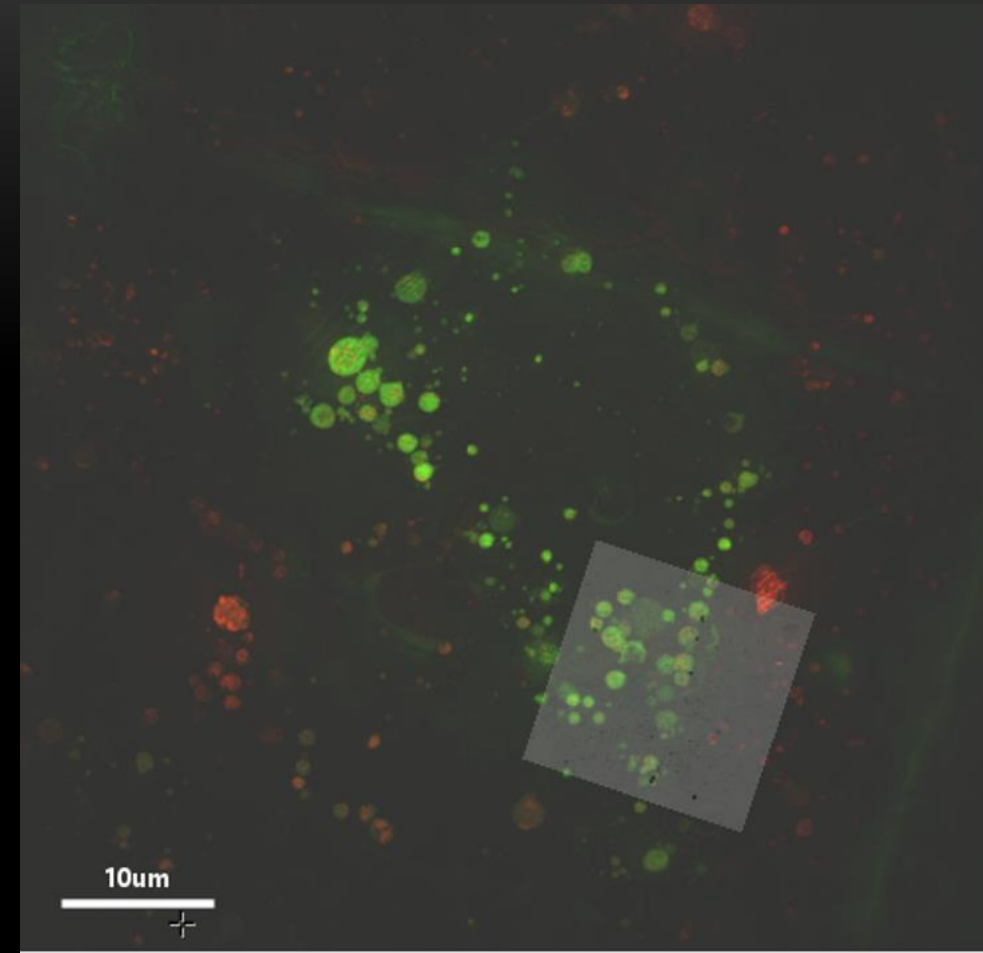
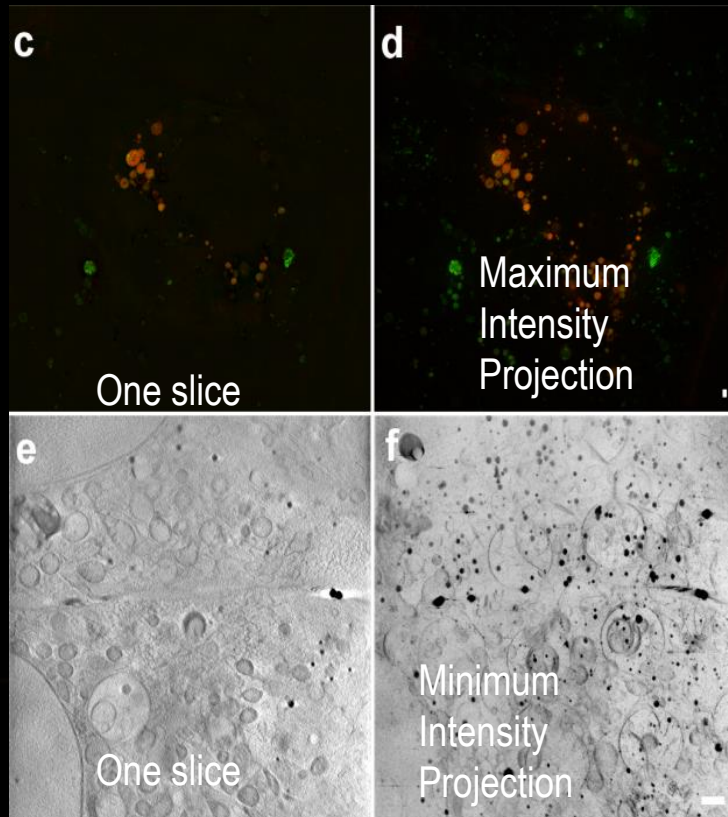
PROTOCOL | FEBRUARY 11, 2022

Sample preparation and image registration for correlative cryo-FM and cryo-FIB-SEM of plunge-frozen mammalian cells

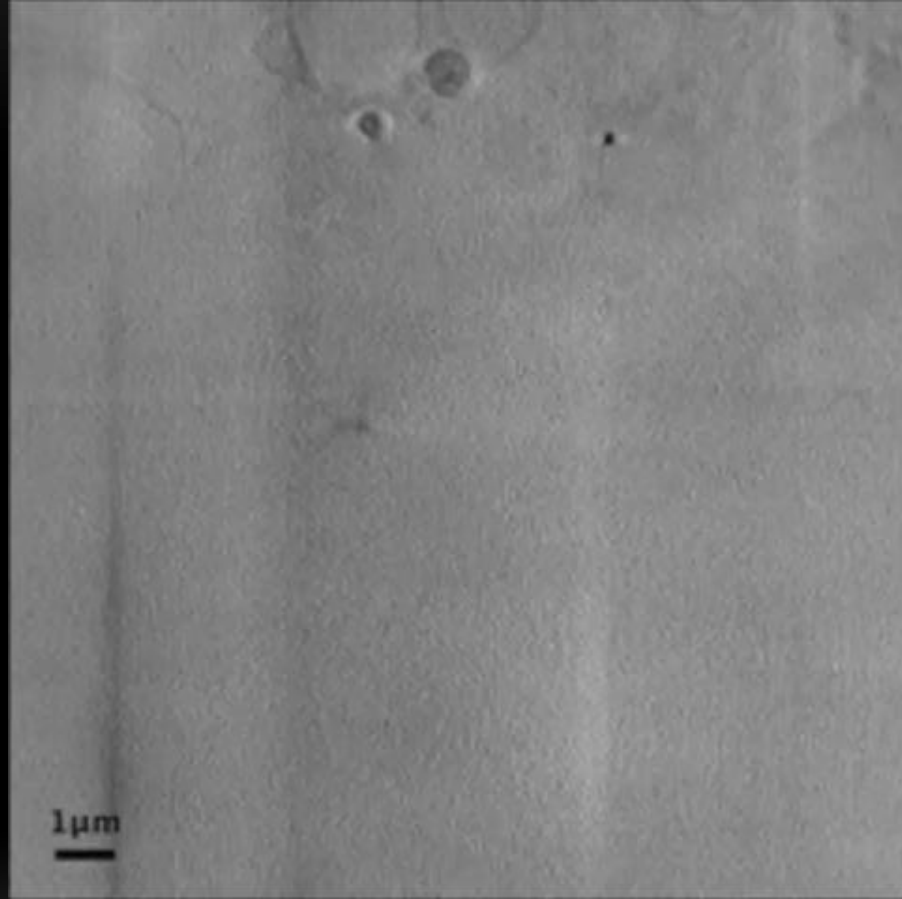
Nadav Scher,^{1,4} Katya Rechav,² Perrine Paul-Gilloteaux,³ and Ori Avinoam^{1,5,*}

FIDUCIAL FREE Approach

- U2OS cells stably expressing red fluorescence (mCherry-Galectin-3), indicators of endosomal membrane disruption
- infected by a fluorescent reovirus green fluorescence (Alexa647-reovirus)
- sample vitrification: frozen in liquid nitrogen-cooled liquid ethane

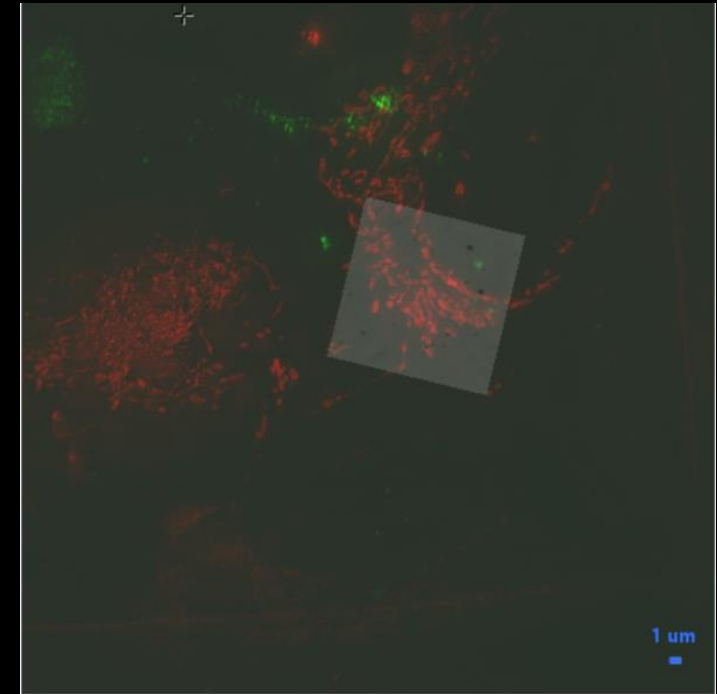
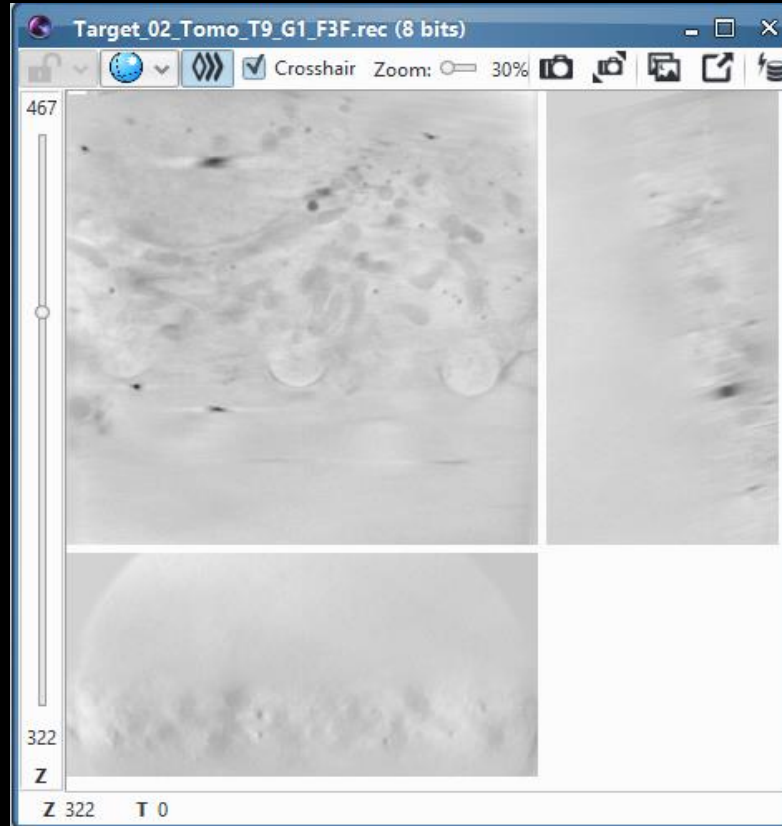
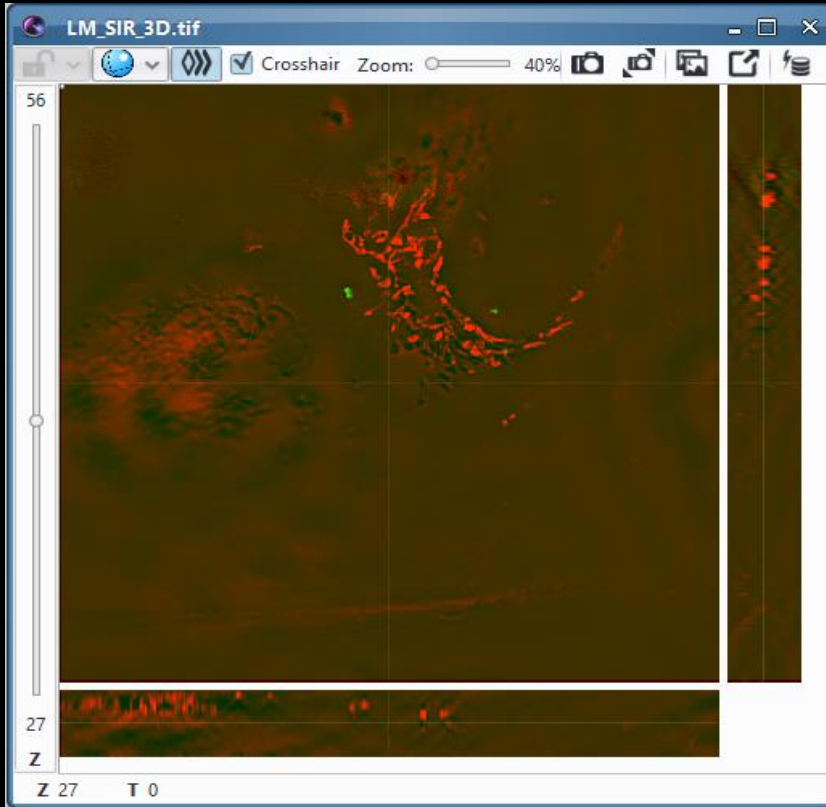


Kounatidis I, Stanifer ML, Phillips MA, Paul-Gilloteaux P, et al. 3D Correlative Cryo-Structured Illumination Fluorescence and Soft X-ray Microscopy Elucidates Reovirus Intracellular Release Pathway. Cell. 2020



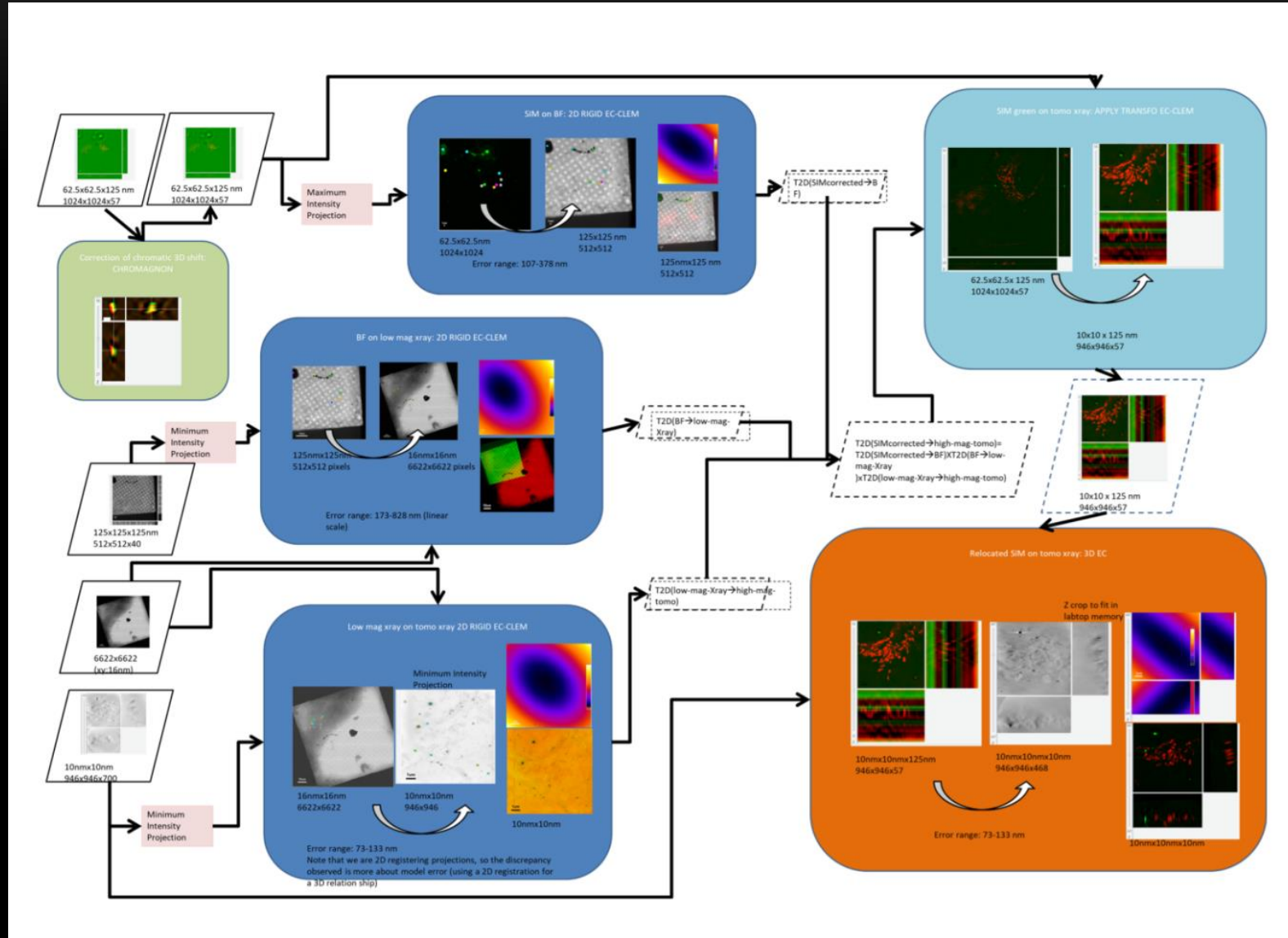
APPROACH WITHOUT FIDUCIALS

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

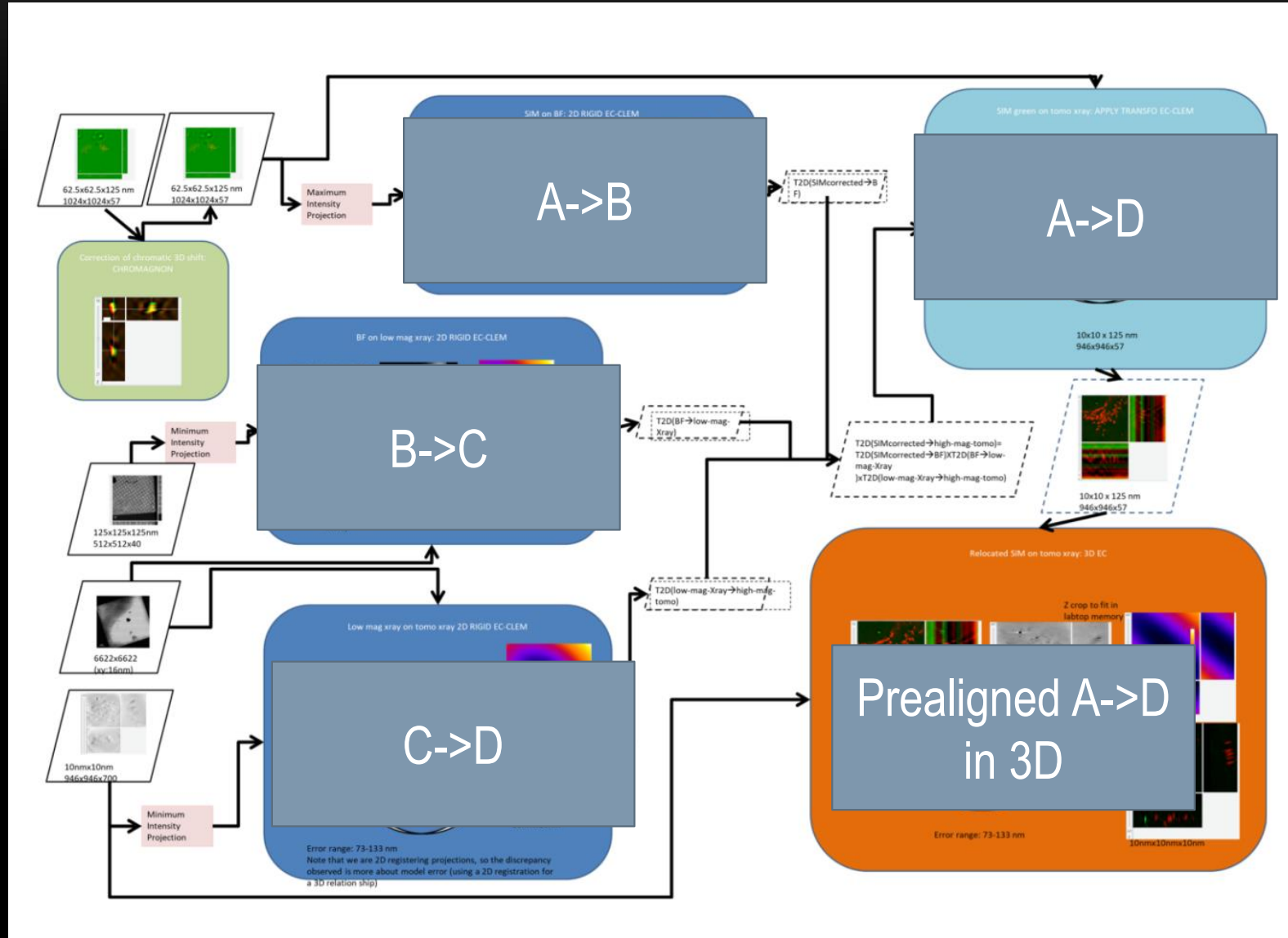


Test

USUALLY IN 3D AND USUALLY IN NEED OF INTERMEDIATE IMAGING

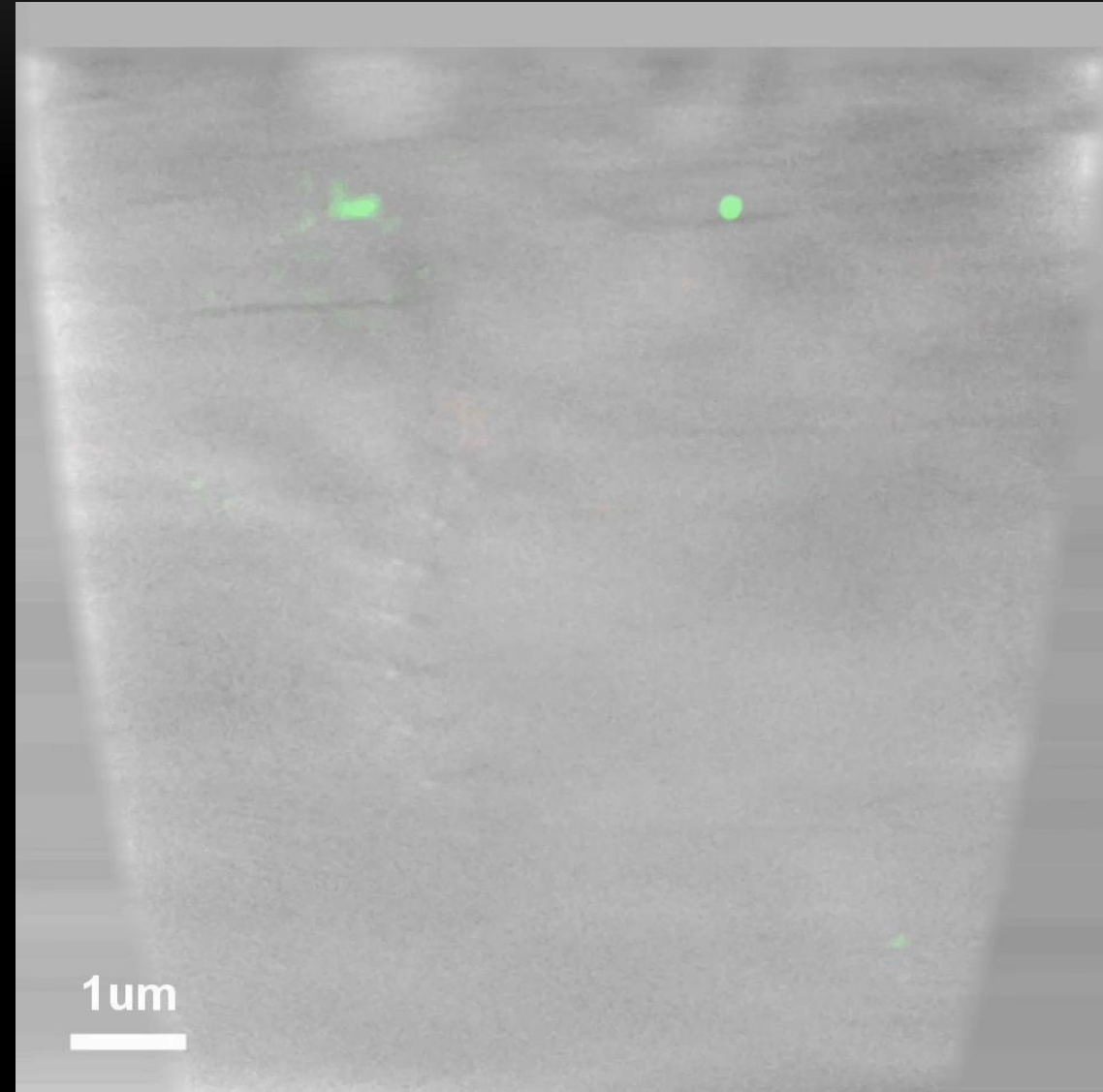
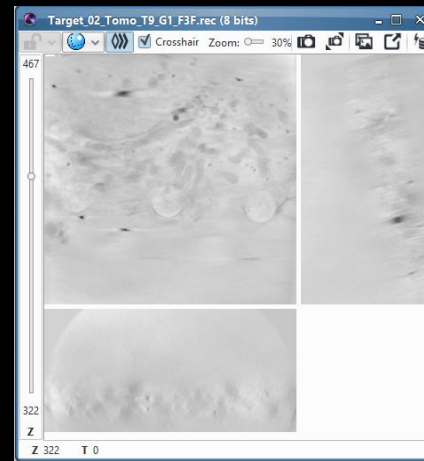
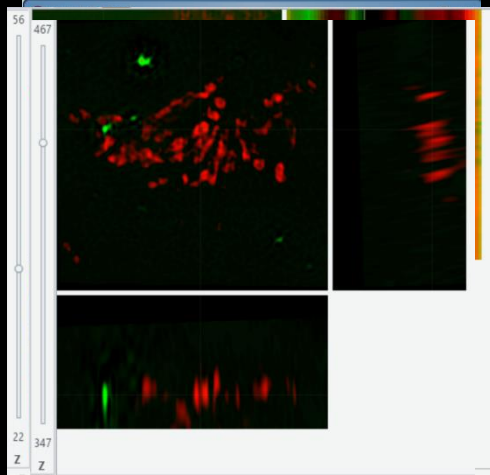
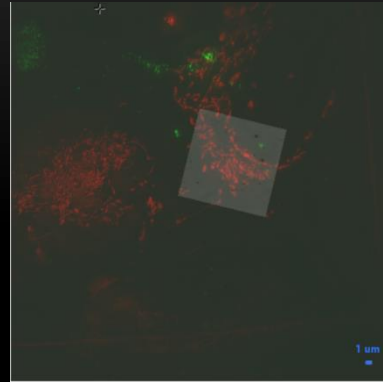


USUALLY IN 3D AND USUALLY IN NEED OF INTERMEDIATE IMAGING



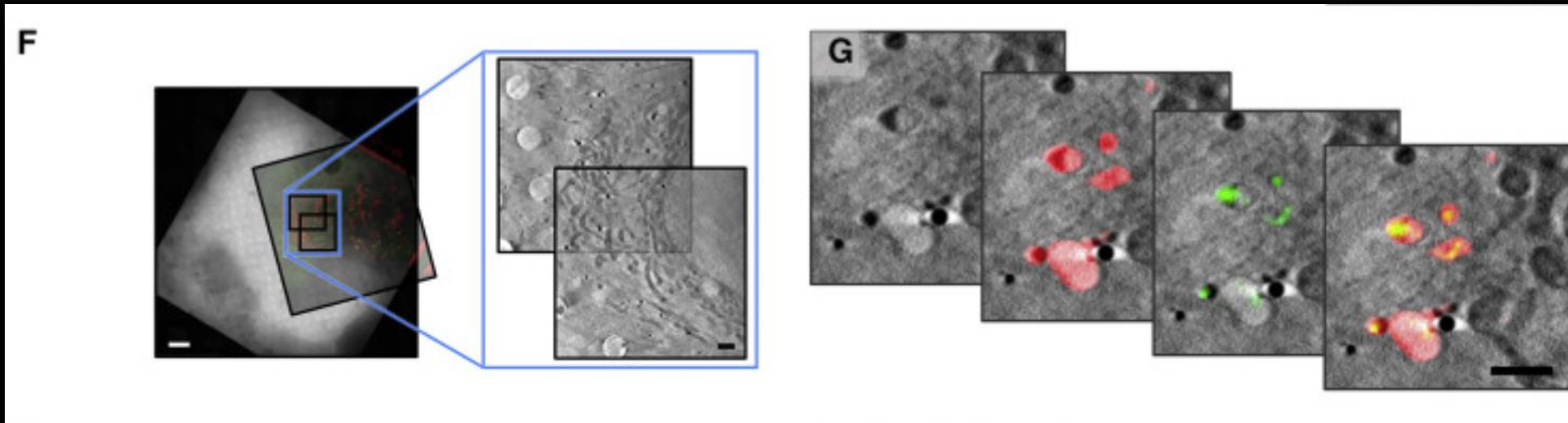
USUALLY IN 3D AND USUALLY IN NEED OF INTERMEDIATE IMAGING

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



Test on mitochondria stained cells : here we could conclude that there were no deformation in the correlative imaging process: all discrepancy was in the expected range of rigid transformation

CORRELATION RESULTS

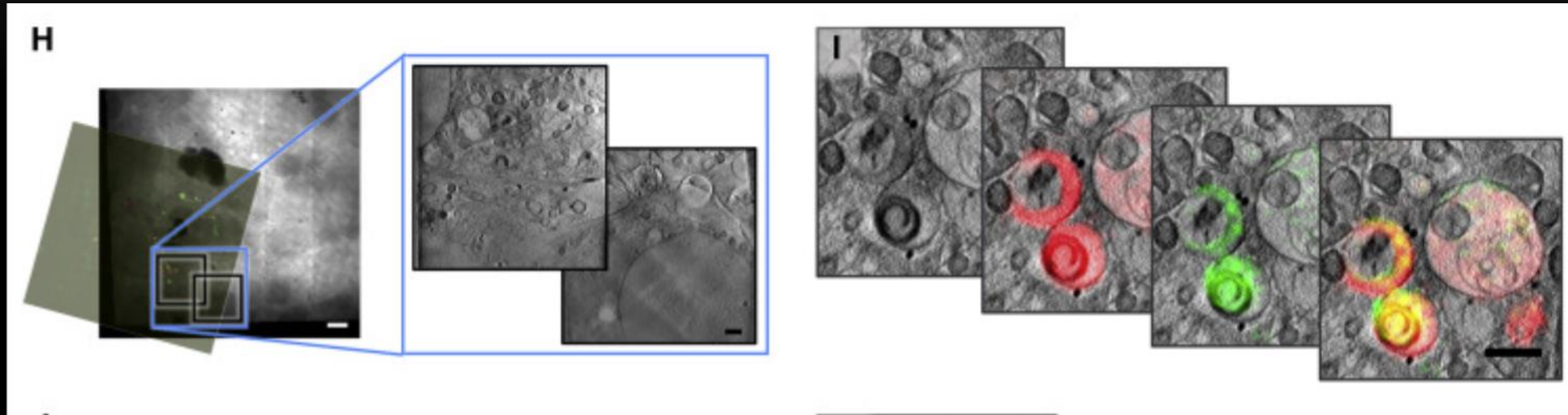


Reovirus (RV)-infected U2OS cells

1h after infection, a number of small but distinct vesicles are both Gal3 and RV positive

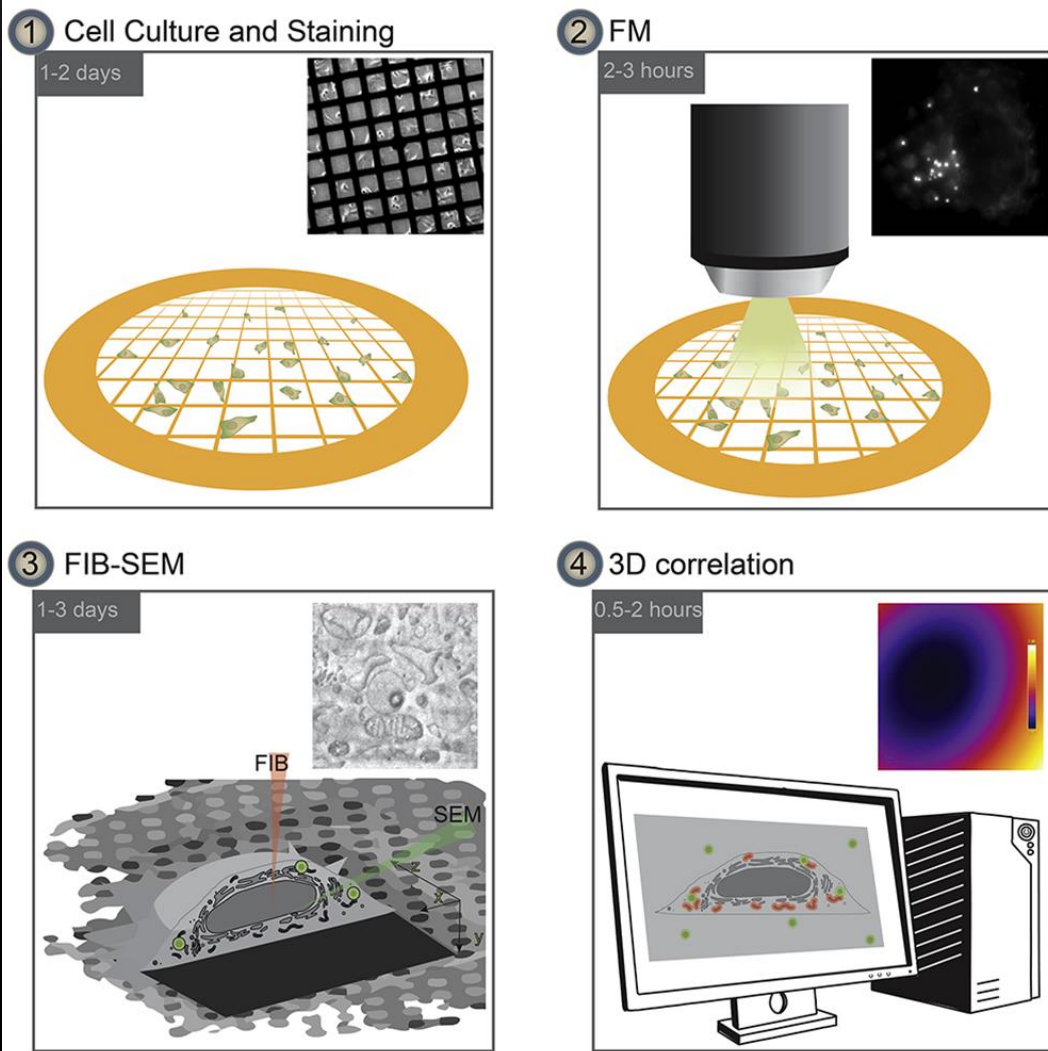
Black bars are 1 μm and white bars 10 μm .

CORRELATION RESULTS

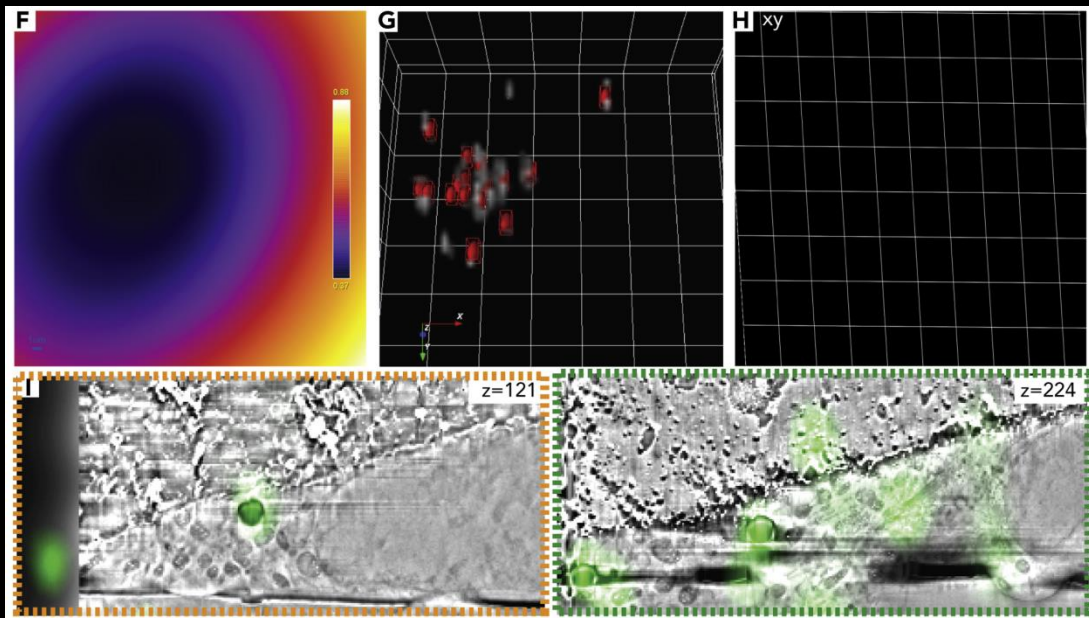
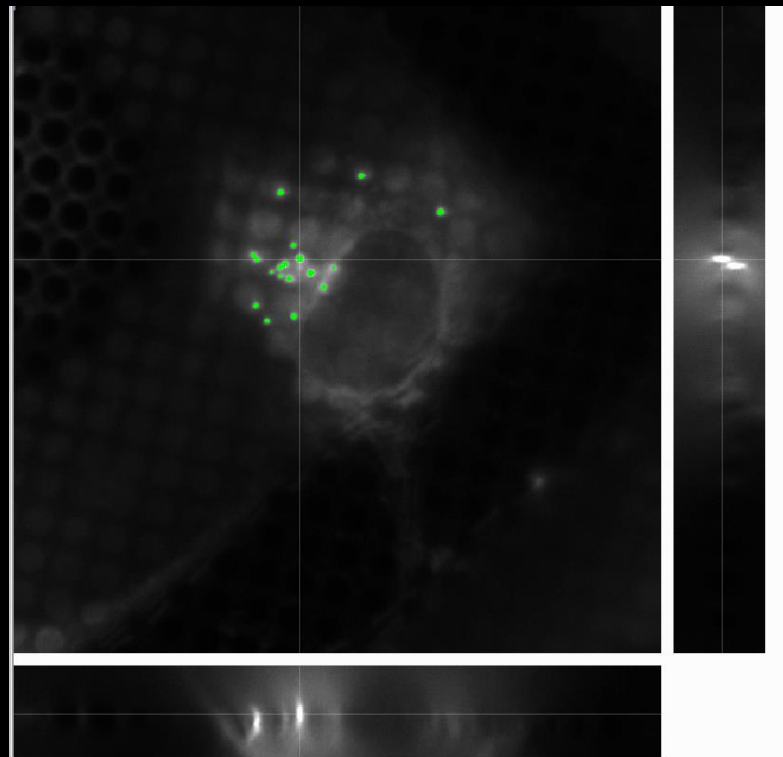
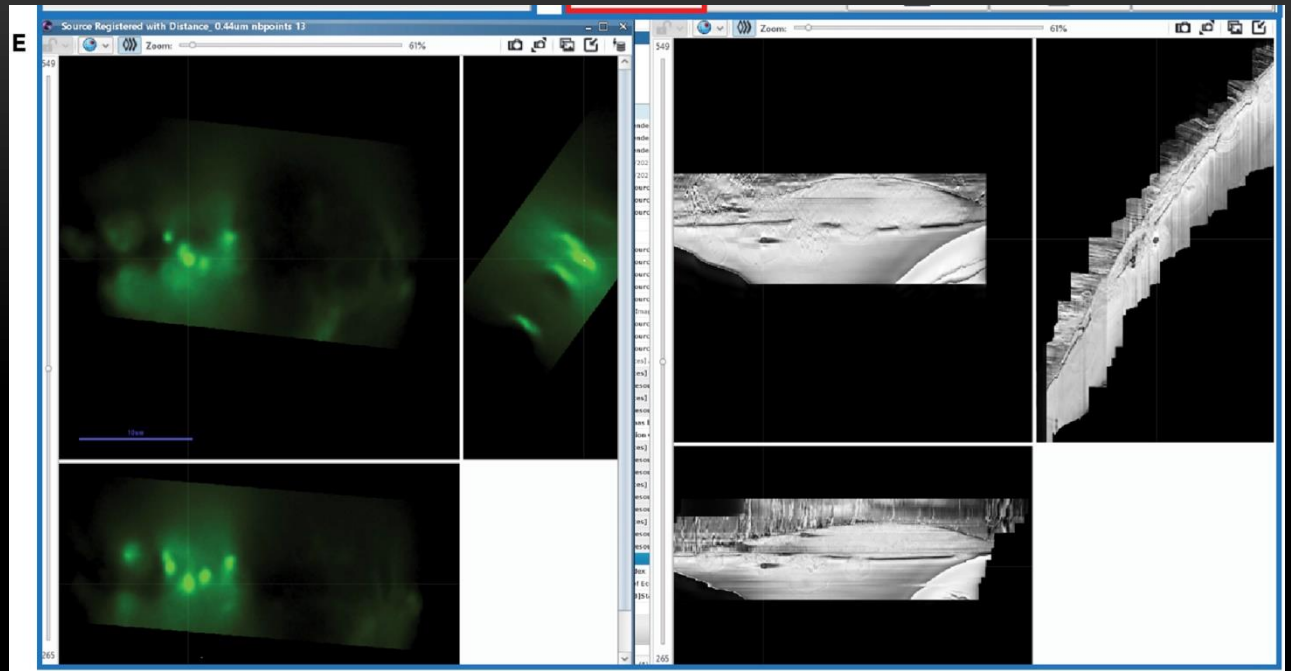
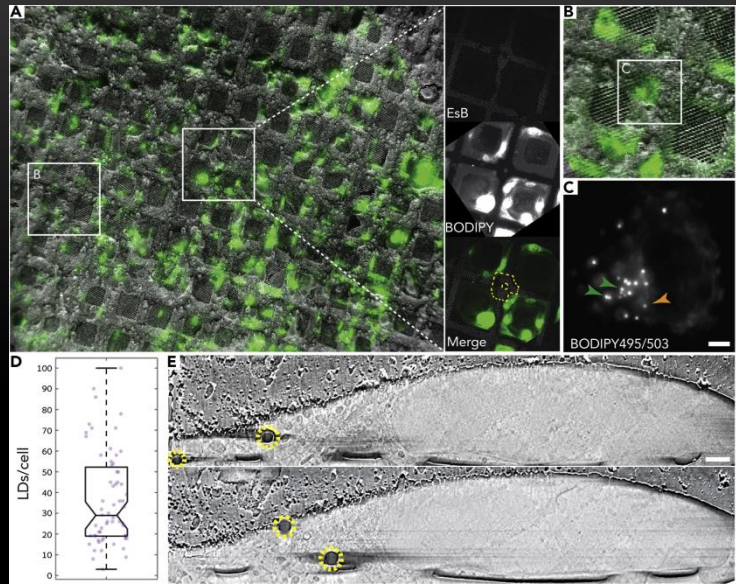


2h post infection : smaller endosomes have now merged to give rise to MVBs that have distinct compartments infused with Gal3 while others have not been similarly compromised (presumably arising from concatenation of membranes from MRV-free vesicles).

CRYO FIB-SEM/ FM EXAMPLE WITH LIPID DROPLETS AS FIDUCIALS



Scher, N., Rechav, K., Paul-Gilloteaux, P., and Avinoam, O. (2021). In situ fiducial markers for 3D correlative cryo-fluorescence and FIB-SEM imaging. *iScience* 24, 102714. <https://doi.org/10.1016/j.isci.2021.102714>



TAKE HOME MESSAGES

- Choose carefully what will be used for computing the transformation
 - Choose carefully the model of transformation
 - Cascaded transformation can be useful to ease the correlation process
 - Think about the way the accuracy of the correlative workflow will be estimated.
 - Ecclem software provides tools to help to construct these workflows.
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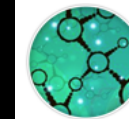
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