

# CORRELATIVE MICROSCOPIES: COMULISGLOBE AND NEWS FROM EC-CLEM

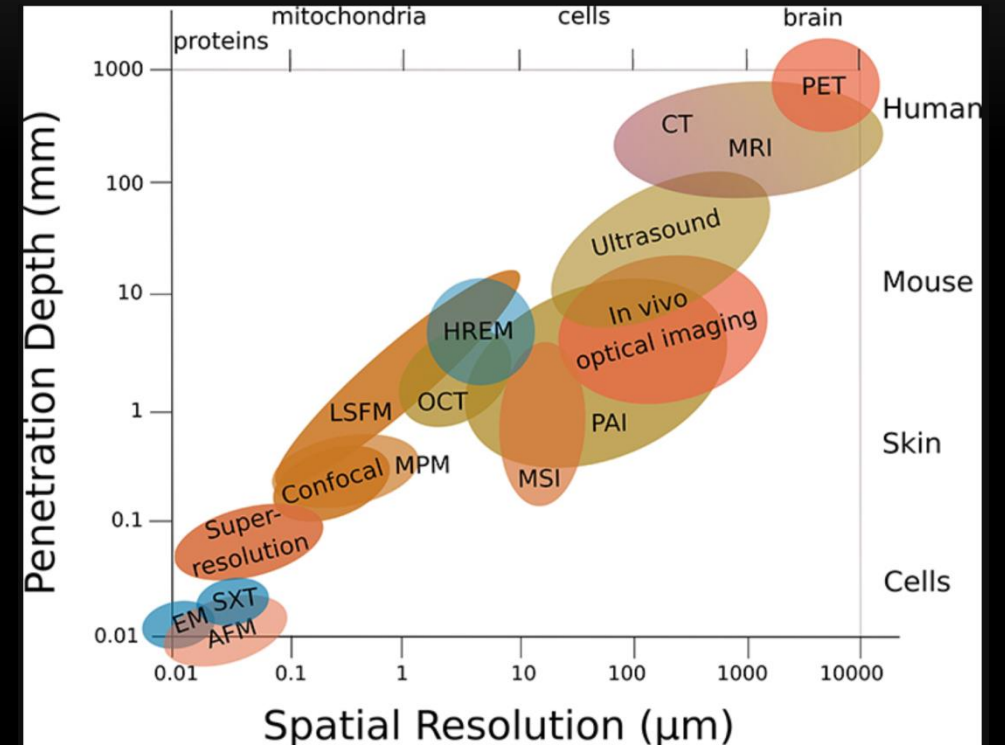
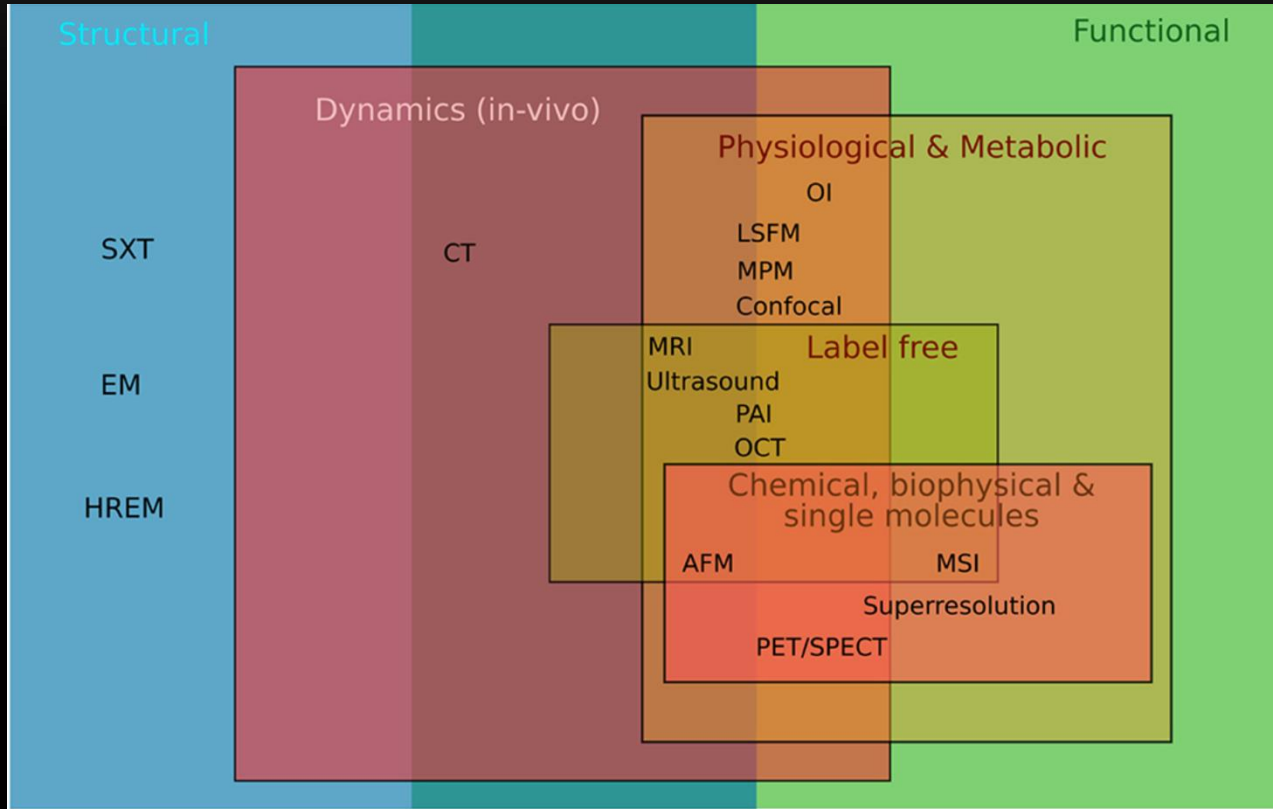
[Perrine.paul-gilloteaux@univ-nantes.fr](mailto:Perrine.paul-gilloteaux@univ-nantes.fr)



Structure Fédérative  
de Recherche - SANTÉ  
François Bonamy



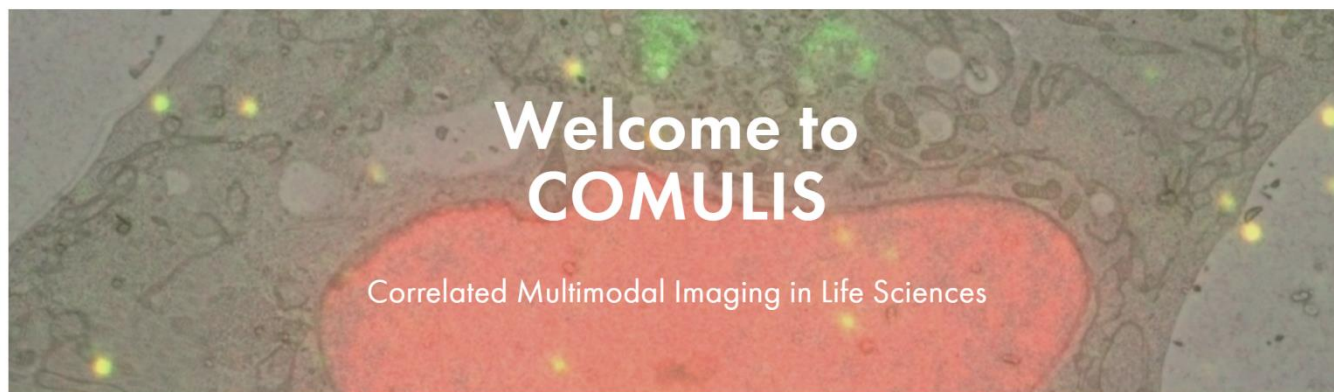
# MULTIMODAL IMAGING FOR AN HOLISTIC VIEW



Andreas Walter, Perrine Paul-Gilloteaux, Birgit Plochberger, Ludek Sefc, Paul Verkade, et al..  
 Correlated Multimodal Imaging in Life Sciences: Expanding the Biomedical Horizon. *Frontiers in Physics*, Frontiers, 2020, 8, [10.3389/fphy.2020.00047](https://doi.org/10.3389/fphy.2020.00047)



**WELCOME** ABOUT CHALLENGES EVENTS GRANTS CAREER RESOURCES CONTACT & JOIN



Stay Updated:  
[www.comulis.eu](http://www.comulis.eu)

**Register to the mailing list!!!**

# COMULIS: CORRELATED MULTIMODAL IMAGING IN LIFE SCIENCES to bridge CLEM and PHI

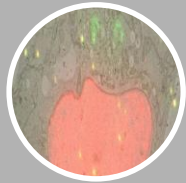


Oct 2018-  
Oct 2022

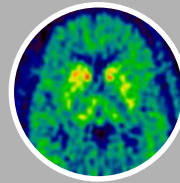


Outreach, Dissemination & Partnerships

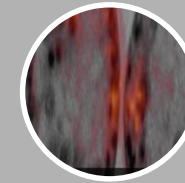
**Foster the recognition of CMI in life sciences**



Correlative Light To Electron  
Microscopy

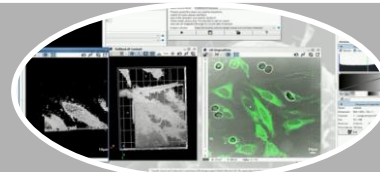


Preclinical Hybrid Imaging



Novel Correlated multimodal  
imaging Pipelines

**Set up standards and best practices, Trigger novel approaches**



Correlation software

**Improve & develop correlation and visualization concerted solutions**

Correlated Multimodal  
Imaging in Life Sciences

2.0

C O  
M U L  
I S

M u l t E M P l e x

## MultEMplex - COST INNOVATORS GRANT (CIG)

We are happy to announce that COMULIS was awarded a COST Innovators Grant (CIG) for the MultEMplex project, as an extension of the COST action to develop commercial applications based on our scientific expertise.

Faites Vos Jeux!

Moving high-resolution in cellulo structural biological of infectious processes  
from the roulette table to the chess board.

The COST Innovators Grant (CIG) is an initiative that aims to build bridges between research and take up at market, product, service, or societal level. CIGs aim to enhance the pace and success of research breakthroughs and as such, offers ending Actions the possibility to create additional impact during the year after the end of the Action.

The MultEMplex CIG is active until October 2023 and can provide the same networking activities available to COST Actions such as Virtual Mobility Grants, Short Term Scientific Missions (STSMs) or Dissemination Conference Grants.



HORIZON EUROPE

cost  
EUROPEAN COOPERATION  
IN SCIENCE & TECHNOLOGY

Oct 2022-  
Oct 2023

Core team:

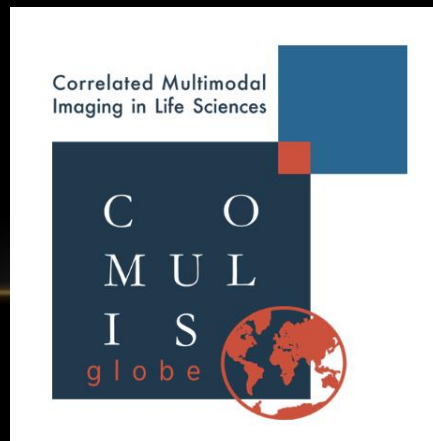
Matthijn Vos/ Anna Sartori/ Andreas Walter/ Erin Tranfield/ Paul Verkade/ Luc Tallini

# COMULISGLOBE (CZI GRANT 23-25)

Chan  
Zuckerberg  
Initiative 

March 2023-  
March 2025

- Management and communication:
  - Foundation of the COMULISGlobe Society (likely Portugal Based)
  - Industry board setting
  - Conferences
  - Joint User meeting with EUroBioimaging + with ESUO (Synchrotron EU) and LEAPS ()



- |  |   |
|--|---|
| ▶ <b>Andreas Walter</b><br>Aalen University                    | ▶ <b>John Eriksson</b><br>Euro-Biolmaging                       |
| ▶ <b>Julia Fernandez-Rodriguez</b><br>University of Gothenburg | ▶ <b>Jan Grimm</b><br>Memorial Sloan Kettering Cancer<br>Center |
| ▶ <b>Lize Engelbrecht</b><br>Stellenbosch University           | ▶ <b>Claudia Kuntner-Hannes</b><br>Medical University of Vienna |
| ▶ <b>Kirsi Lorentz</b><br>The Cyprus Institute                 | ▶ <b>Perrine Paul-Gilloteaux</b><br>University of Nantes        |
| ▶ <b>Paula Sampaio</b><br>University of Porto                  | ▶ <b>Nataša Sladoje</b><br>Uppsala University                   |
| ▶ <b>Paul Verkade</b><br>University of Bristol                 |   |



# COMULISGLOBE (CZI GRANT 23-25)

- Scientific actions:
  - Showcase projects (publications of on-going + definition of 3 new)
  - Image registration challenge and hackatons.
  - Repositories/Ressources set up
- Training/ Mobility/Access
  - 2 training schools (global)
  - Mobility fellowship with Eurobioimaging and synchrotrons.

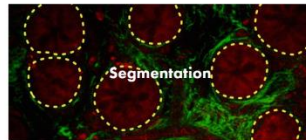
Stay Updated:

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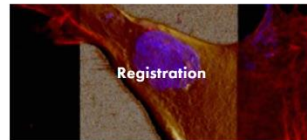
**Register to the mailing list!!!**

Total budget: 350keuros (Including one project officer + 0.5 Technical officer)

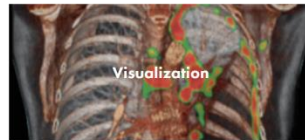
Use the buttons below to search the **Biolmage Informatics Index** for correlation software — for *segmentation*, *registration*, and *visualization*, spanning both biological and preclinical imaging.



Segmentation



Registration



Visualization

In computer vision, image segmentation is the process of partitioning a digital image into multiple segments (sets of pixels, also known as image objects). The goal of segmentation is to change the representation of an image into something that is more meaningful and easier to analyze.

Image registration is the process of transforming different sets of data into one coordinate system. Data may be multiple images, data from different sensors, times, depths, or viewpoints.

Visualization is a group of techniques for creating images, diagrams, or animations to communicate a message. These techniques are of huge importance in the field of correlation software, as usually there's a need to mix different kinds of images

LIST SEGMENTATION SOFTWARE FROM BIII.EU

LIST REGISTRATION SOFTWARE FROM BIII.EU

LIST VISUALIZATION SOFTWARE FROM BIII.EU

17 multimodal (70 other) results



**Image registration in Matlab with Image Processing Toolbox**

Align two images using intensity correlation, feature matching, or control point mapping Together, Image Processing Toolbox™ and Computer Vision Toolbox™ offer four image registration solutions: inte...



**BIRL**

BIRL stands for "Benchmark on Image Registration methods with Landmark validation". BIRL is a cross-platform framework for comparison of image registration methods with landmark validation (registrati...



**Registrationshop**

It is an interactive front-end visualization for registration software based on Elasis (VTK/ITK)...



**simpleElastix**

quote: > Elastix cite{Klein2010} is an open source, command-line program for intensity-based registration of medical images that allows the user to quickly configure, test, and compare different ...



**eC-CLEM**

This plugin allows to compute a similarity (translation/rotation/scaling and flipping) transform from pair of points. It is updating the transformed image interactively such that the user get immediat...



**CMTK**

A software toolkit for computational morphometry of biomedical images, CMTK comprises a set of command line tools and a back-end general-purpose library for processing and I/O...



**Fiji CMTK GUI**

Working version of a simple GUI frontend for CMTK image registration tools in Fiji...



**Correlia**

Correlia is an open-source ImageJ/FIJI plug-in for the registration of 2D multi-modal microscopy data-sets. The software is developed at ProVIS - Centre for Correlative Microscopy and is specifically ...



**MSRC Registration Toolbox**

This python toolbox performs registration between 2-D microscopy images from the same tissue section or serial sections in several ways to achieve imaging mass spectrometry (IMS) experimental goals. ...



**elastix**

Elastix is a toolbox for rigid and nonrigid registration of (medical) images. Elastix is based on the ITK library, and provides additional algorithms for image registration. The software can be run...



**DeepCLEM**

This Fiji plugin is a python script for CLEM registration using deep learning, but it could be applied in principle to other modalities. The pretrained model was learned on chromatin SEM images and fl...



**Bigwarp**

Bigwarp is a tool for manual, interactive, landmark-based deformable image alignment. It uses the BigDataViewer for visualization and navigation, and uses a Thin Plate Spline implemented in Java to bu...



**VIBE-Z**

The Virtual Brain Explorer (ViBE-Z) is a software that automatically maps gene expression data with cellular resolution to a 3D standard larval zebrafish (Danio rerio) brain. It automatically detects ...



**Landmark Correspondences**

The plugin Landmark Correspondences calculates a transformation between two corresponding landmark clouds and renders a transformed image. The landmarks are read from point selections over two images....



**Amira**

Amira is 3D visualization and analysis software for life sciences. " Amira software is a powerful, multifaceted 3D platform for visualizing, manipulating, and understanding life sciences data from ...



**Epina Image Lab**

Epina ImageLab is a Microsoft Windows-based multisensor imaging tool for processing and analyzing hyperspectral images. It is a modular system consisting of a basic engine, a graphical user interface....



**Imaris**

Imaris is a software for data visualization, analysis, segmentation and interpretation of 3D and 4D microscopy images. It performs interactive volume rendering that lets users freely navigate even ver...



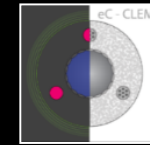
# EC-CLEM: a plugin of ICY

NEW VERSION !! (beta but usable)

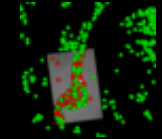
- MUCH MORE FASTER
- LESS GREEDY in memory
- New tools for error estimation (colocalization like)
- New Tools to ease your correlative WF constructions
- New Tool for visualization

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

Ec-clemv2

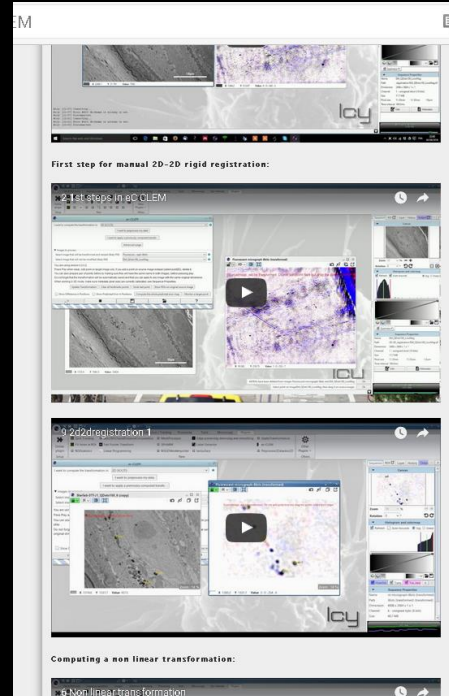


Ec-clem



Ec-clem  
AutoFinder

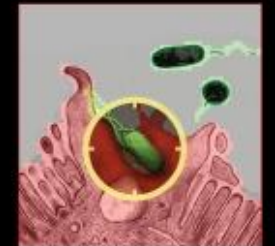
Online Tutorials on Icy Bio Image Analysis website (look for ec-clem and ec-clem autofinder)



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

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

Methods in Cell Biology • Volume 140  
**CORRELATIVE LIGHT  
AND ELECTRON MICROSCOPY III**



Edited by  
Thomas Müller-Reichert & Fritz Jockusch



# AVAILABLE VIDEO TUTORIALS

[Introduction to Icy for Ec-Clem \(new users of ICY\)](#)

Registration in 2D, [rigid](#) or [non rigid](#)

Registration of [a slice to a volume](#) (2D to 3D) (same principle for 3D to 2D, just inverse source and target)

[Registration in 3D](#)

[Pre registration by 3D volume rotation](#) from 3D viewer.

[Reapply a previously computed transform](#) (tutorial in 2D, but exact same procedure for 3D)

Monitoring [accuracy](#)

Integrated [preprocessing](#) (such as flattening options or denoising)

Register [using LINES](#) rather than points (actually using [AUTOFINDER](#))

*List of pdf tutorials:*

[2D-2D step by step PDF](#)

[3D-3D step by step PDF](#)

[Preprocessing step by step PDF](#)

[Register a reconstructed tomogram to 2D fluo in 2D and then merge in 3D](#)

<http://icy.bioimageanalysis.org/plugin/ec-clem/>

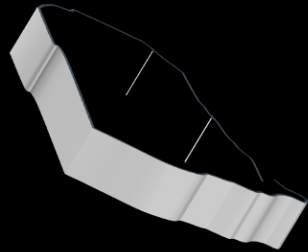
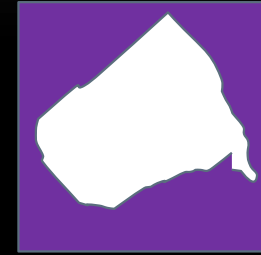
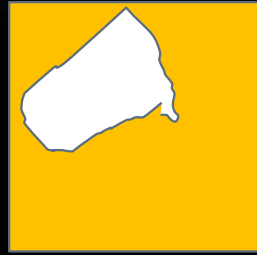
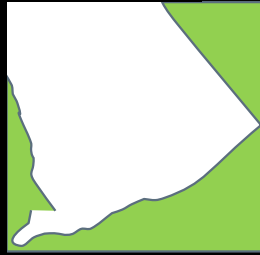
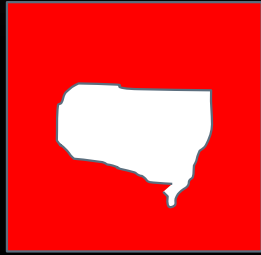
[anrcrocoval/ec-clem \(github.com\)](https://github.com/anrcrocoval/ec-clem)

## Documentation

Online tutorials are available on the icy webpage <http://icy.bioimageanalysis.org/plugin/ec-clem/> Further tutorials are available here:

- on error estimation at 95% of confidence [https://youtu.be/Rz1\\_MLqn6-k](https://youtu.be/Rz1_MLqn6-k)
- on creating a cascaded transform <https://youtu.be/5yNuP8hQuJM>
- on applying a transformation such as the one created by cascaded transforms <https://youtu.be/ZVB1mUZXA4>
- to visualize both registered images without any interpolation or resampling of the data <https://github.com/anrcrocoval/CorrelativeView>

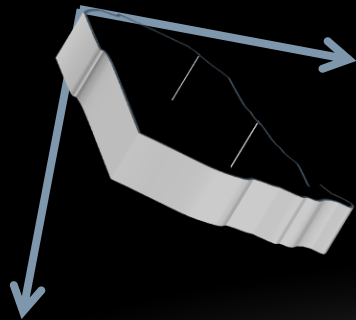
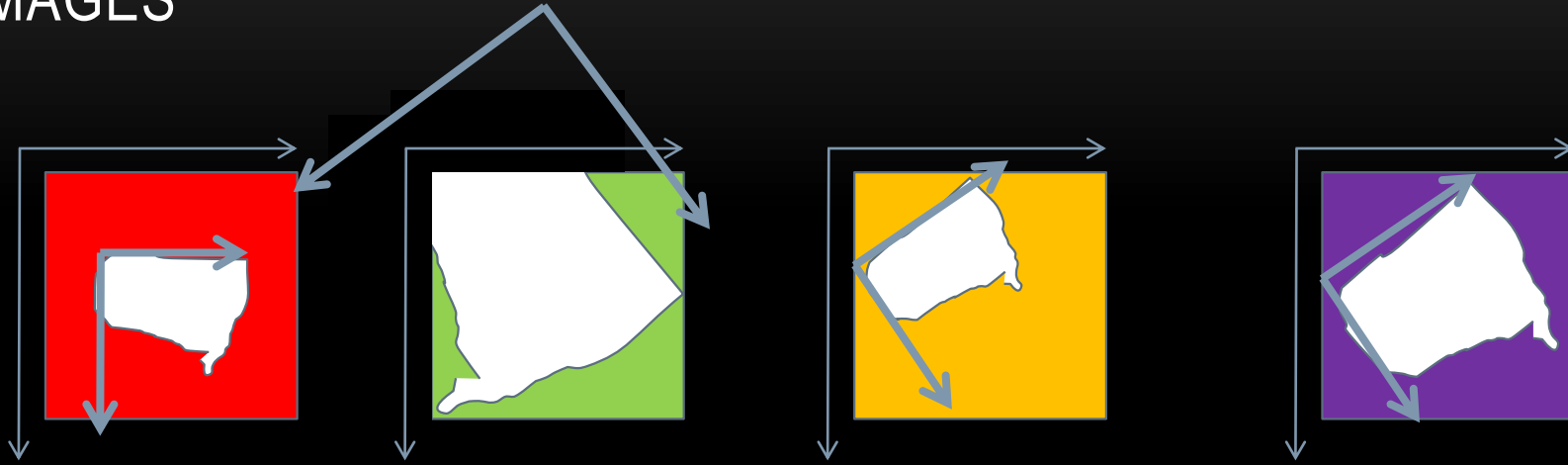
# SAMPLE TO BE TRACKED FROM ONE IMAGING MODALITY TO ANOTHER



Sometimes additional modalities will help

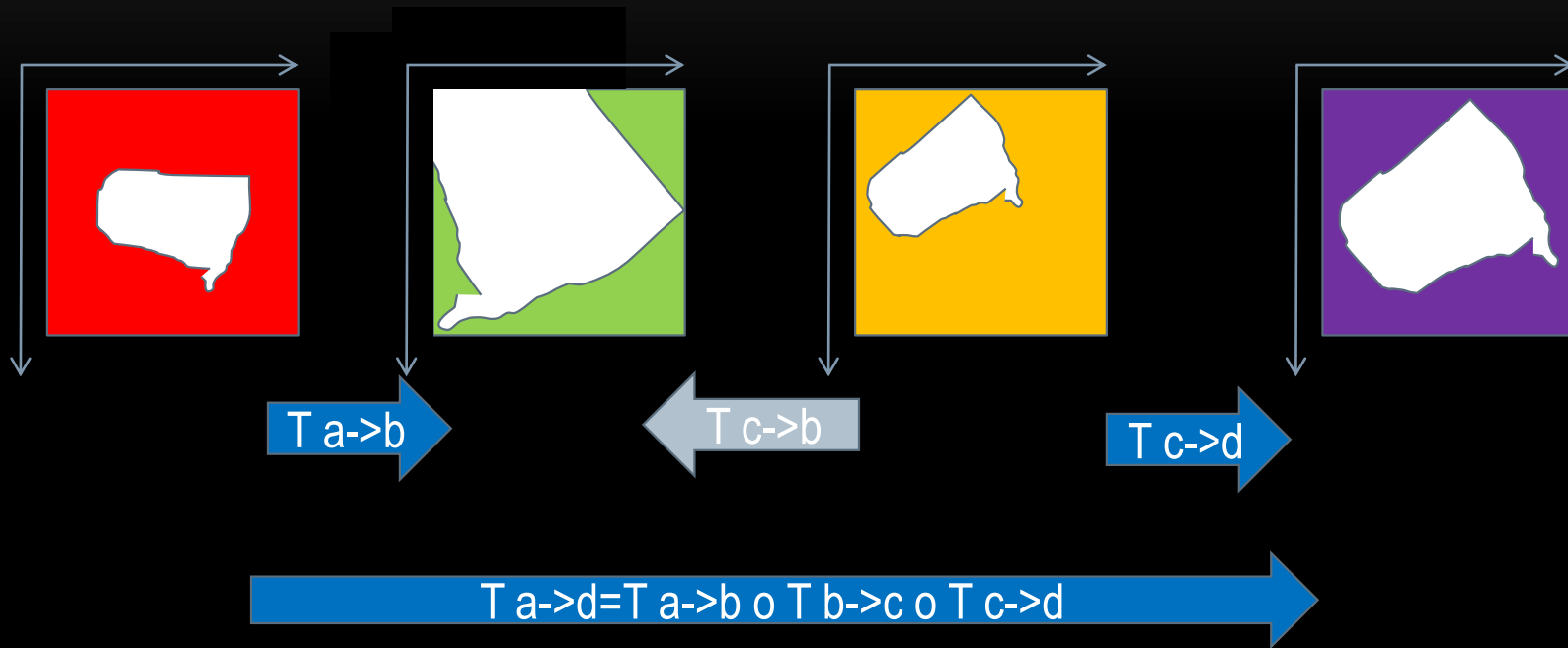
Ecclem Cascaded  
transform/ Invert Transform

IN ADDITION TO COORDINATES TRANSFER BETWEEN IMAGING MODALITIES,  
IMAGE REGISTRATION WILL HELP TO FIND THE RELATIONSHIP BETWEEN  
IMAGES



Ecclem Cascaded  
transform/ Invert Transform

# TRANSFORMATION CAN BE RIGID OR NON RIGID, BUT THEY CAN ALWAYS BE CASCADED



Ecclem Cascaded  
transform/ Invert Transform

# EXAMPLE OF USE OF CASCADED TRANSFORM



Cell

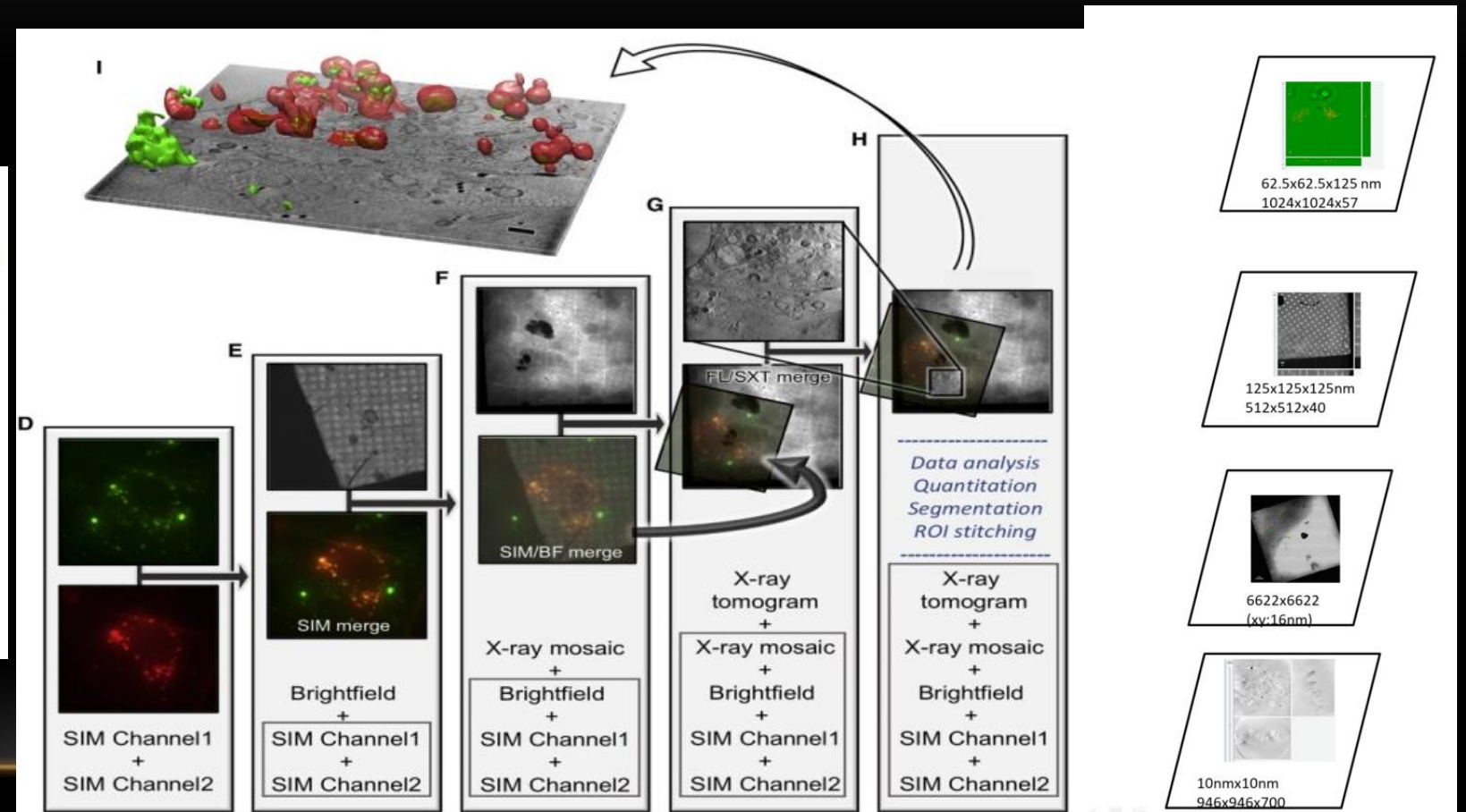


Volume 182, Issue 2, 23 July 2020, Pages 515-530.e17

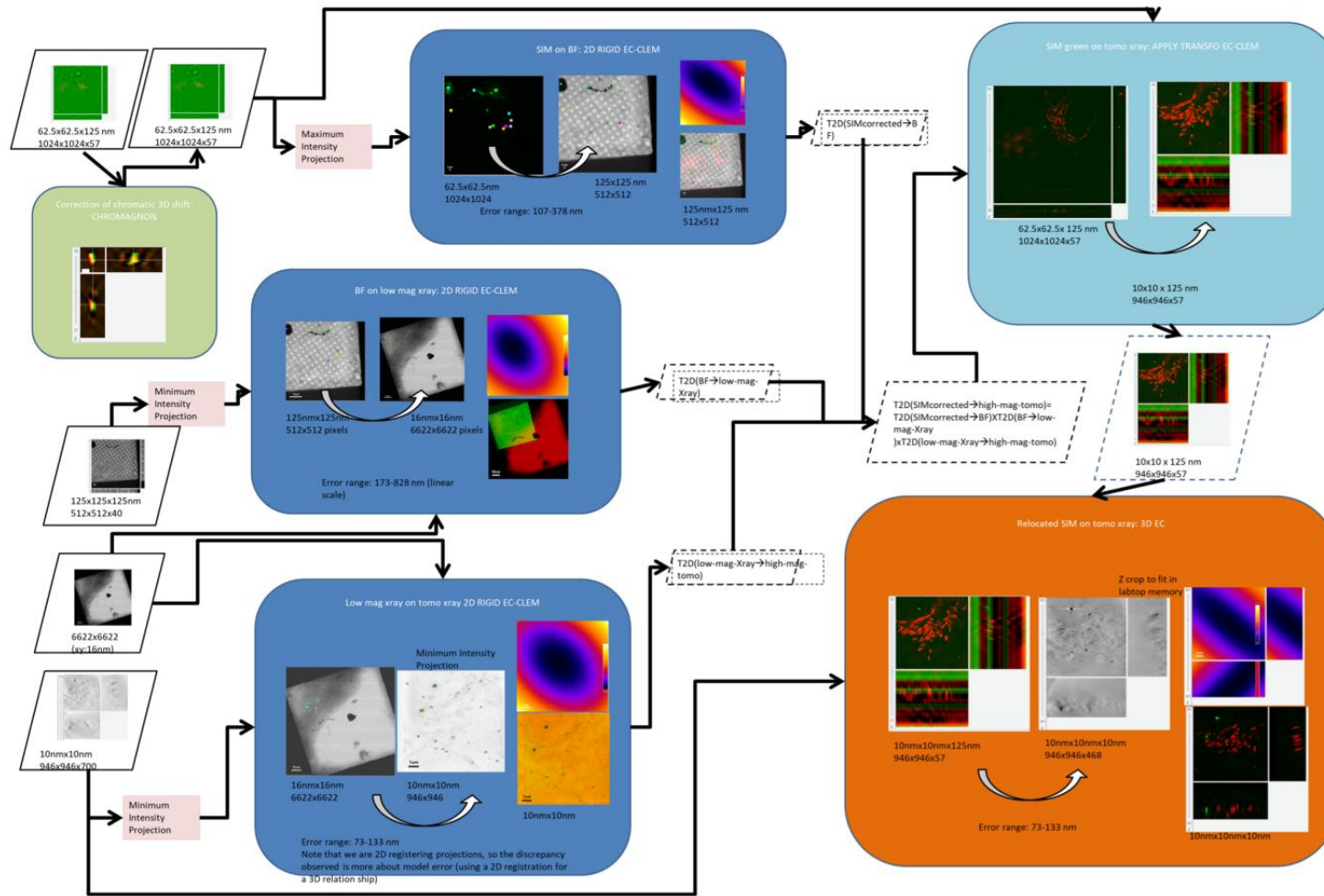
Resource

## 3D Correlative Cryo-Structured Illumination Fluorescence and Soft X-ray Microscopy Elucidates Reovirus Intracellular Release Pathway

Ilias Kounatidis<sup>1 10</sup>, Megan L. Stanifer<sup>2 10</sup>, Michael A. Phillips<sup>1 3 4 10</sup>, Perrine Paul-Gilloteaux<sup>5 6</sup>, Xavier Heiligenstein<sup>7</sup>, Hongchang Wang<sup>1</sup>, Chidinma A. Okolo<sup>1</sup>, Thomas M. Fish<sup>1</sup>, Matthew C. Spink<sup>1</sup>, David I. Stuart<sup>1 4</sup>, Ilan Davis<sup>3</sup>, Steeve Boulant<sup>8 9</sup>, Jonathan M. Grimes<sup>1 4</sup>, Ian M. Dobbie<sup>3</sup>, Maria Harkiolaki<sup>1 11</sup>  



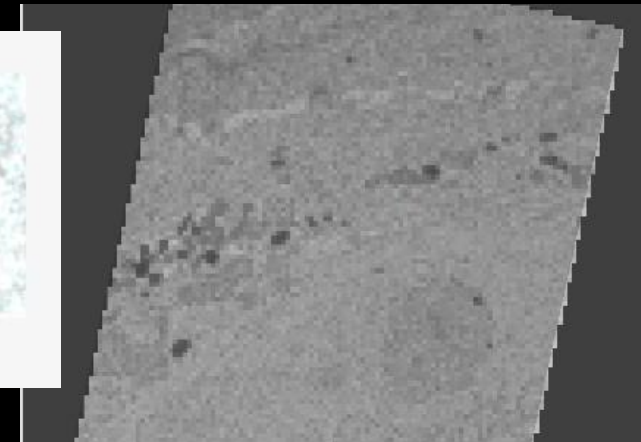
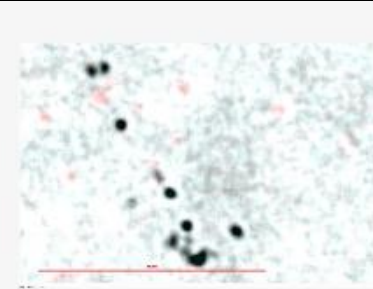
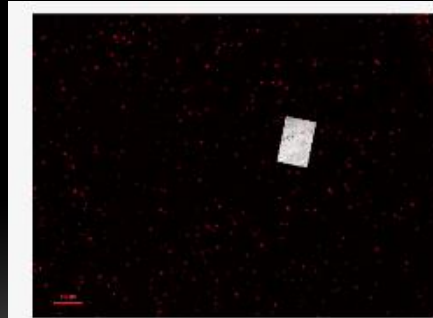
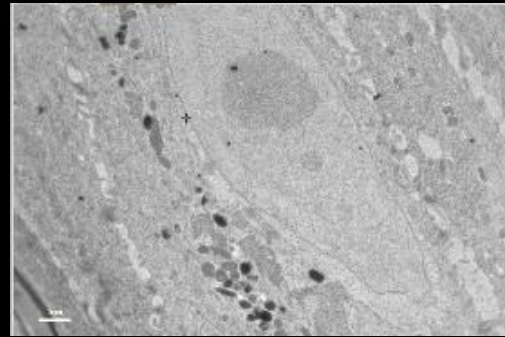
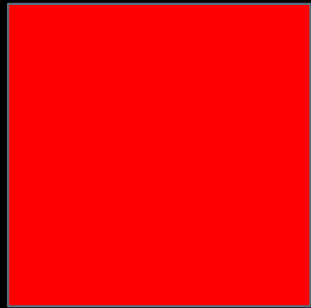




# HOW TO EXPLOIT THE TRANSFORMATION COMPUTED FOR QUANTIFICATION?

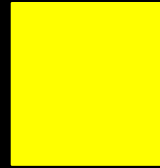
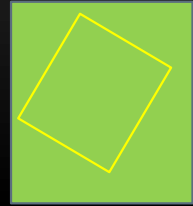
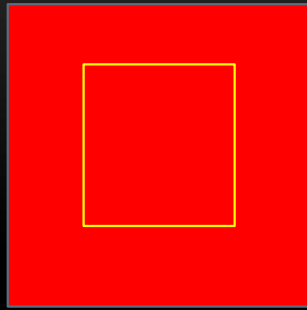
COMPUTE THE TRANSFORMATION  
LINKING **source** and **target**

Ecclem Apply transform



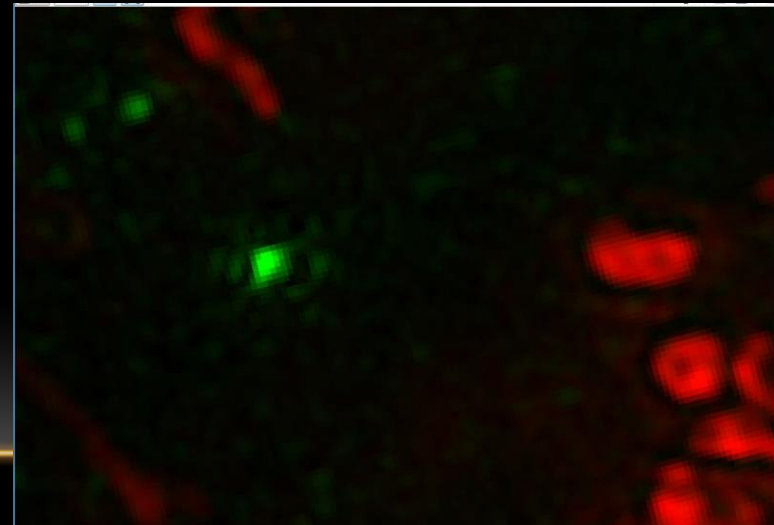
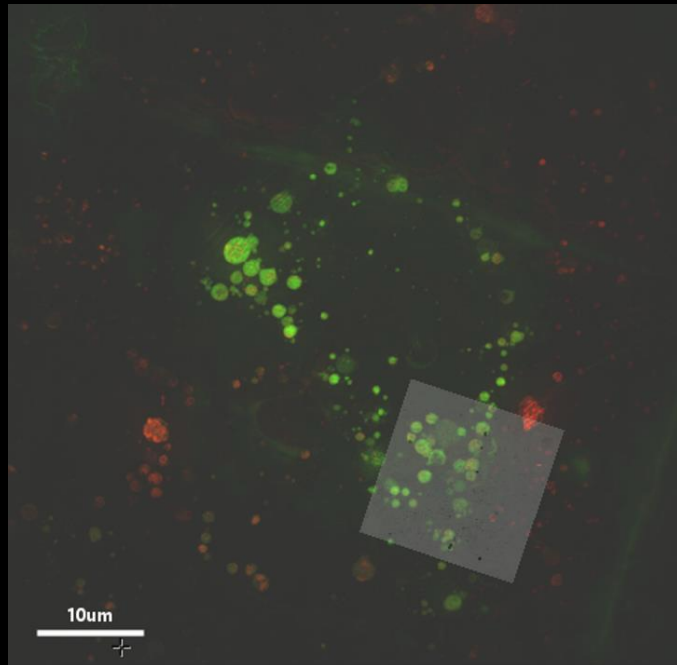
Overlay: one image got **interpolated** (new pixels) or **reduced in quality** to create a new merged dataset.

# HOW TO EXPLOIT THE TRANSFORMATION COMPUTED FOR QUANTIFICATION?

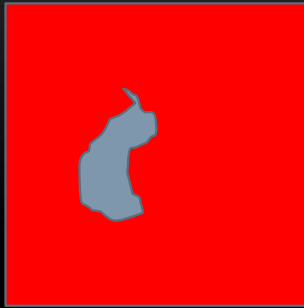


Transform ONLY  
the DISPLAY  
(coordinate  
system of arrival  
= screen)

Ecclem CorrelativeView

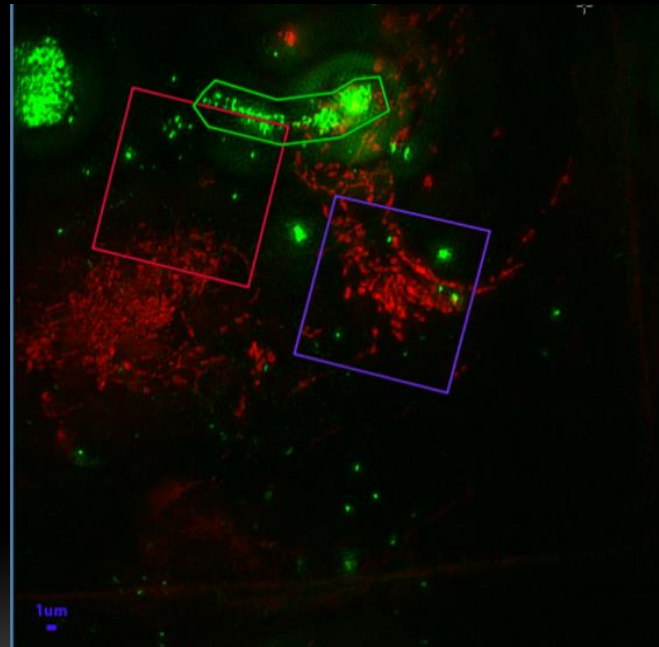
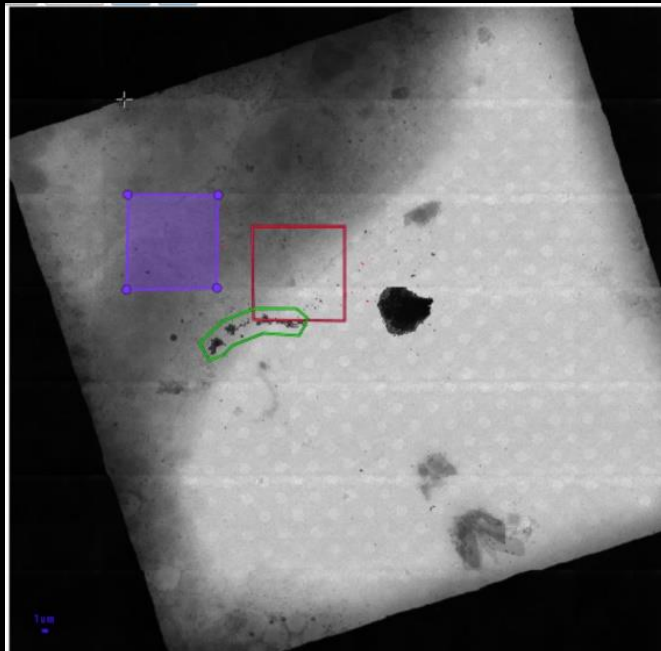


# HOW TO EXPLOIT THE TRANSFORMATION COMPUTED FOR QUANTIFICATION?



Region of interest transformed

Ecclem Apply transform to ROI



	Name	Conto...	Interior (px)	Area (u...	Mean Inte...	Mean Int...	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

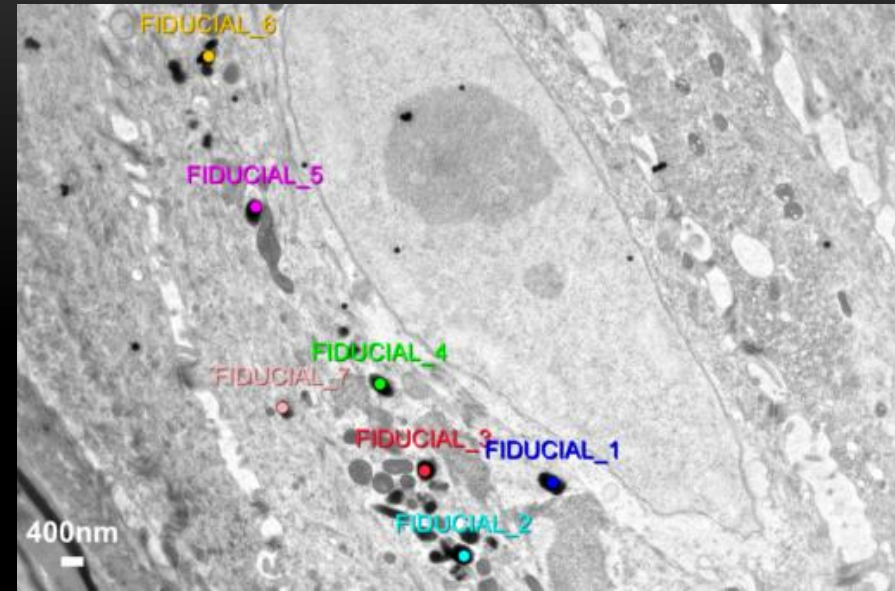
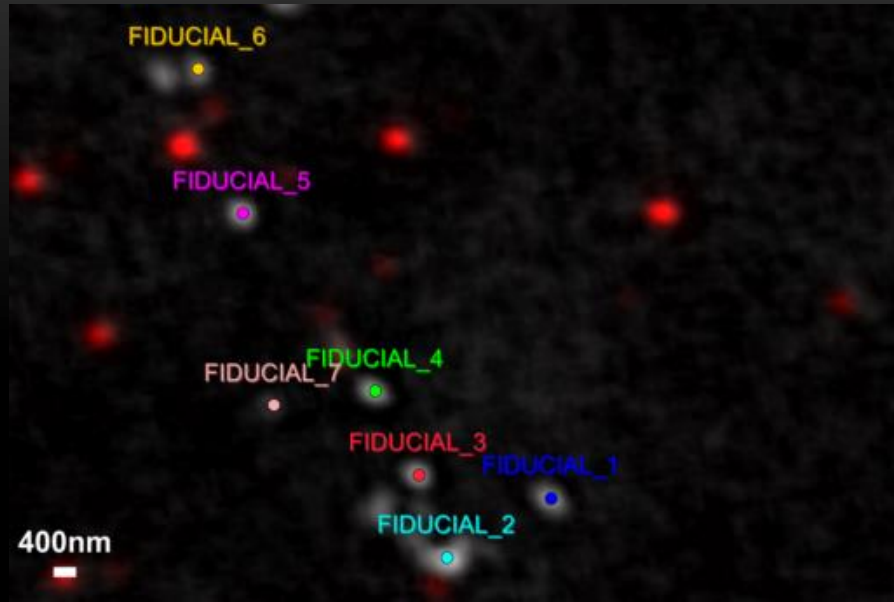
- One thing to know: Matching is NEVER PERFECT
  - Because your sample may deform/ evolve between two modalities
  - Because multimodality is usually also multiscale (very different resolution, one pixel can match hundred of pixels in the second modality)
  - Because none modality allows to perfectly 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.

**WE HAVE DEVELOPED A METHOD TO SPATIALLY ASSESS THE QUALITY OF REGISTRATION WITH NO GROUND TRUTH (NO OTHER INPUT THAT POINTS (OR SURFACE) USED FOR REGISTRATION: LOCALISATION NOISE AND ERROR REGISTRATION ARE ESTIMATED).**

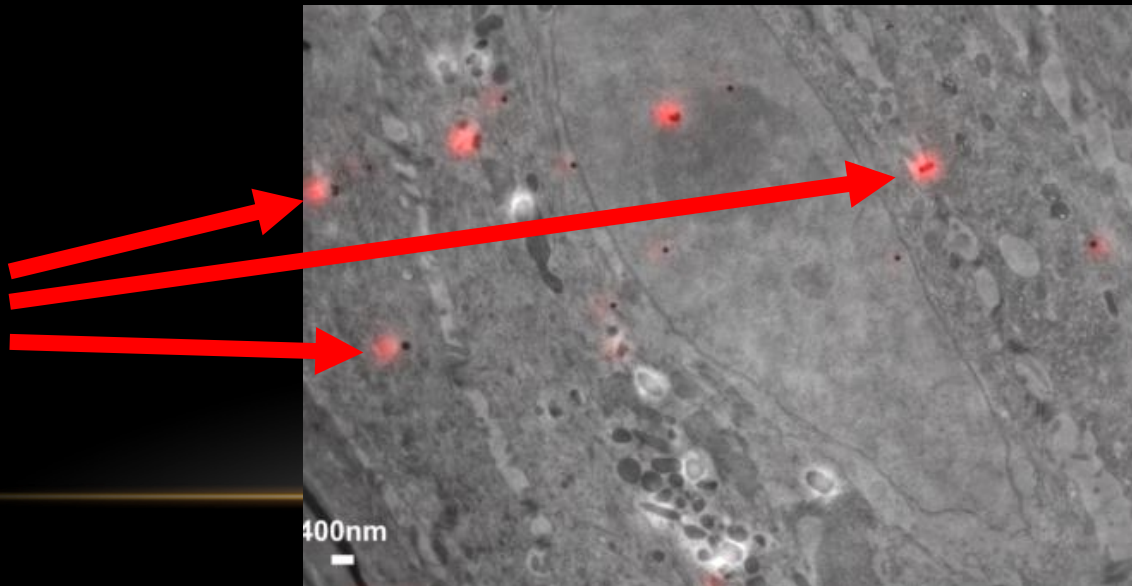
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G. Potier, F. Lavancier, S. Kunne and P. Paul-Gilloteaux, "A Registration Error Estimation Framework for Correlative Imaging," *2021 IEEE International Conference on Image Processing (ICIP)*, 2021, pp. 131-135, doi: 10.1109/ICIP42928.2021.9506474.

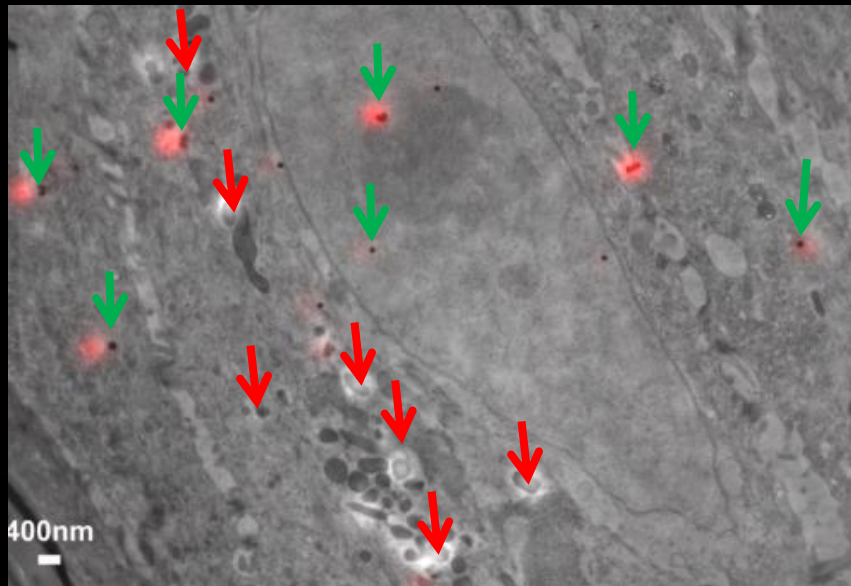
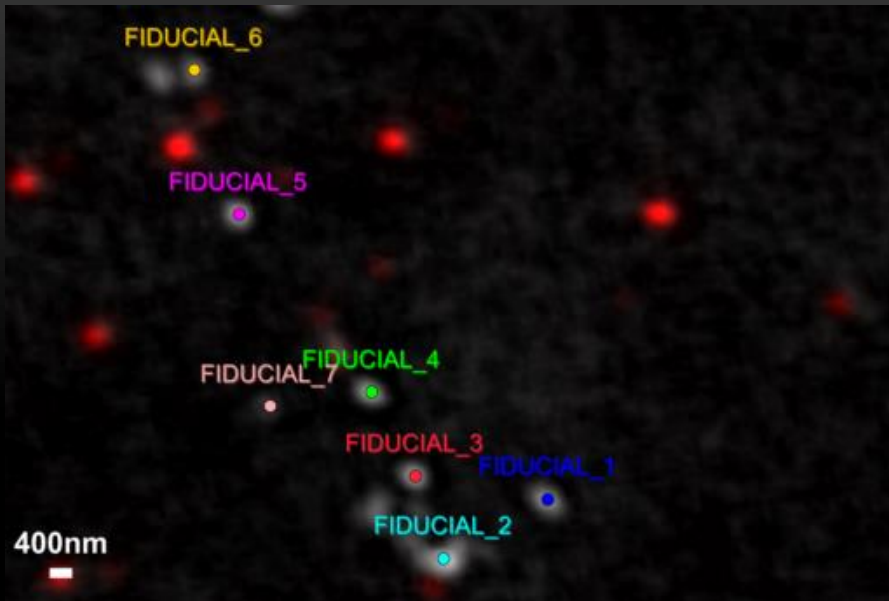




Beads are used here to check how good we are to estimate the error

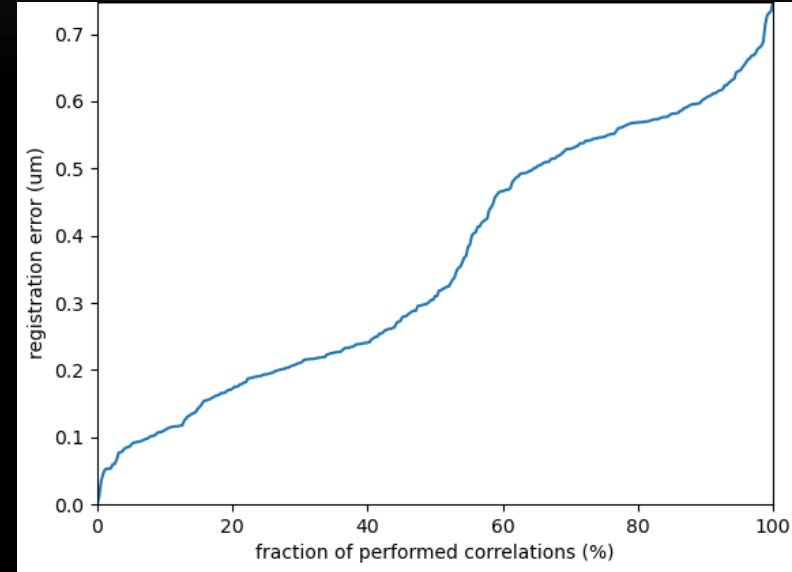




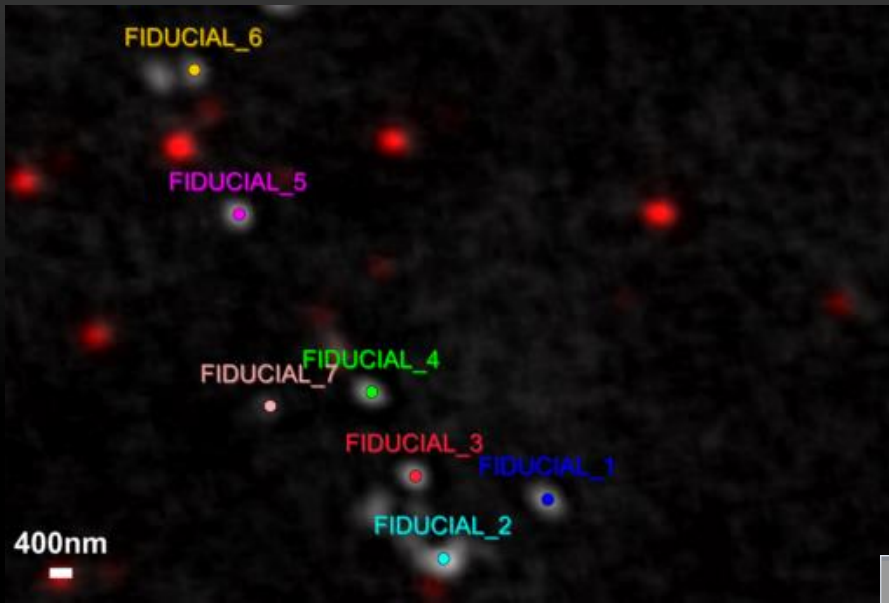


↓ fiducials

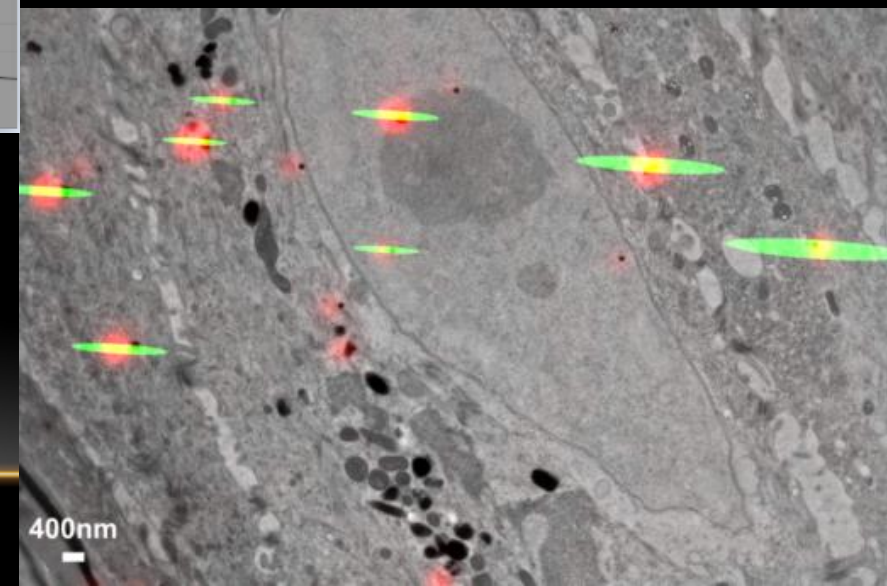
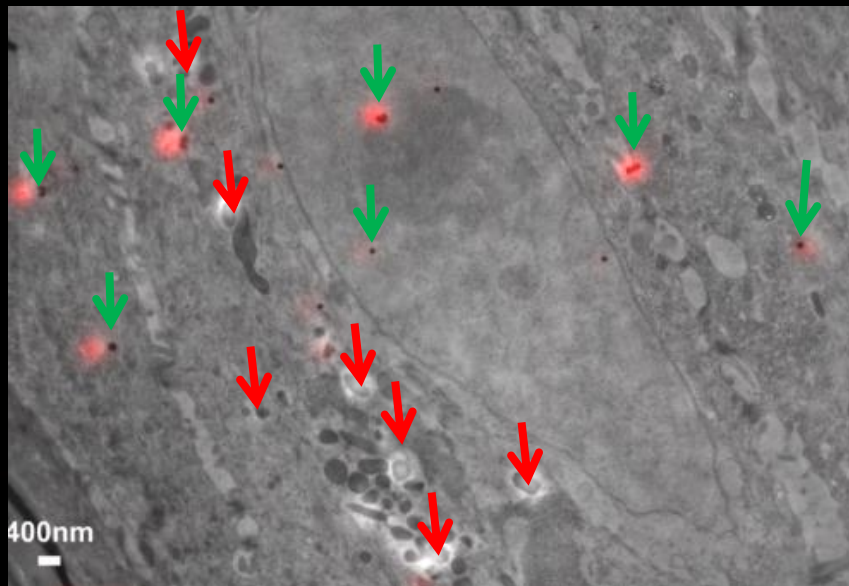
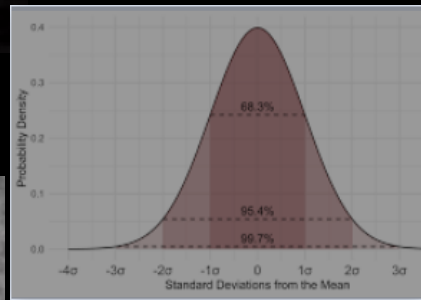
↓ Not fiducials



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



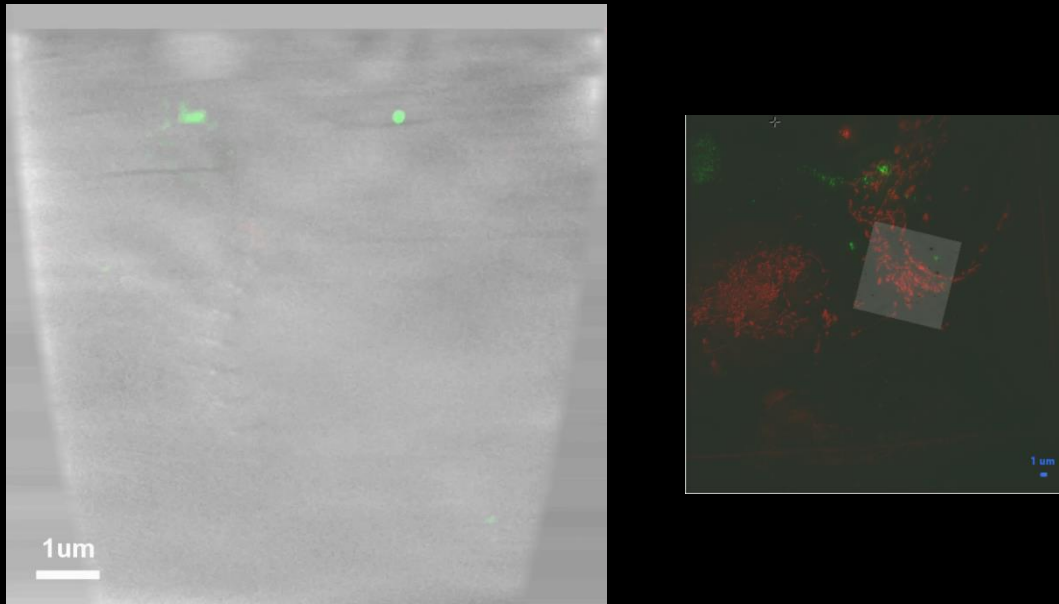
Predicted  
Average  
accuracy  
at each  
point



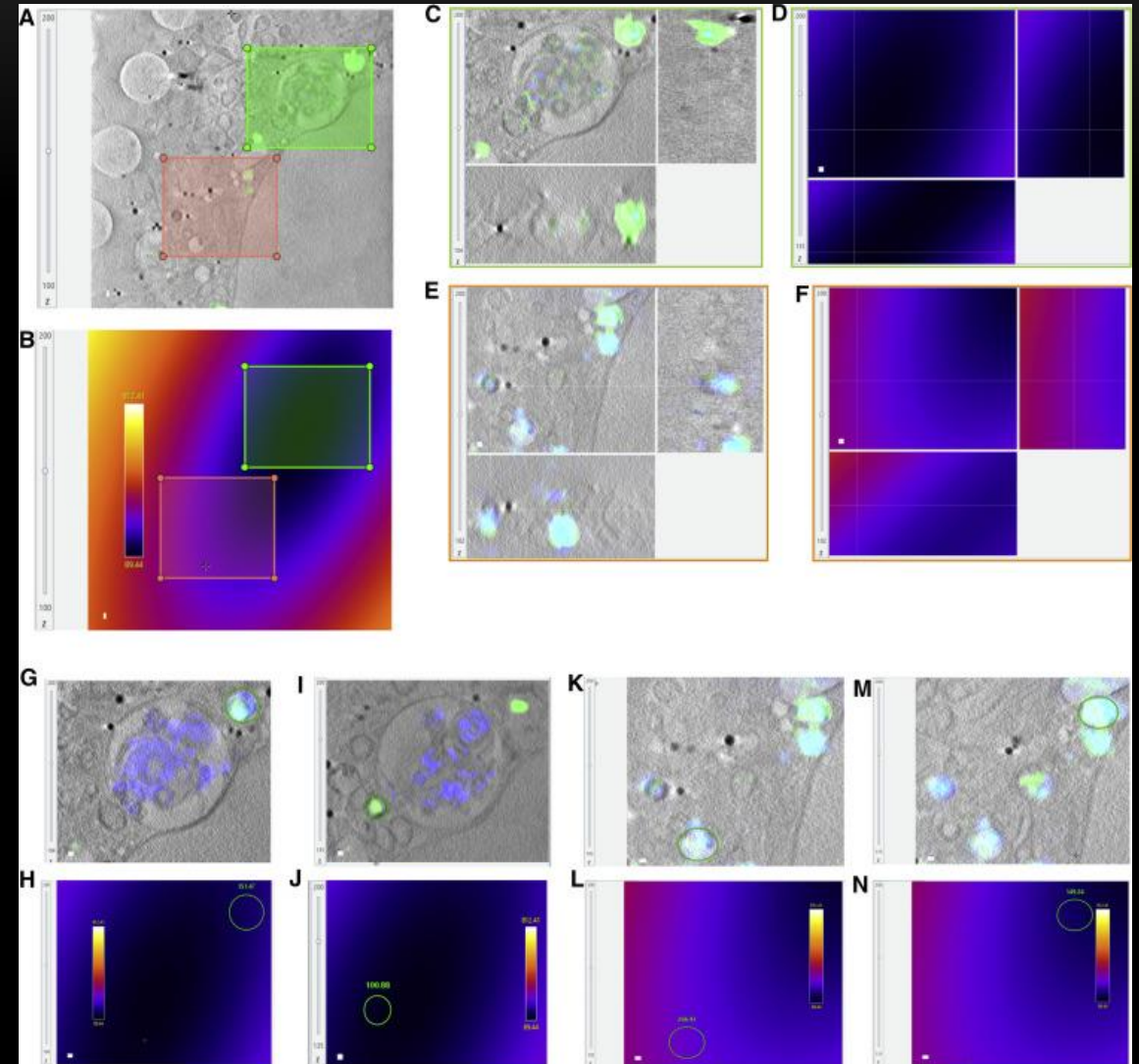
New in  
ecclm:  
95%  
accuracy  
prediction

# EXAMPLE FOR CRYO-SIM AND SOFT X-RAY CORRELATION WORKFLOW

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

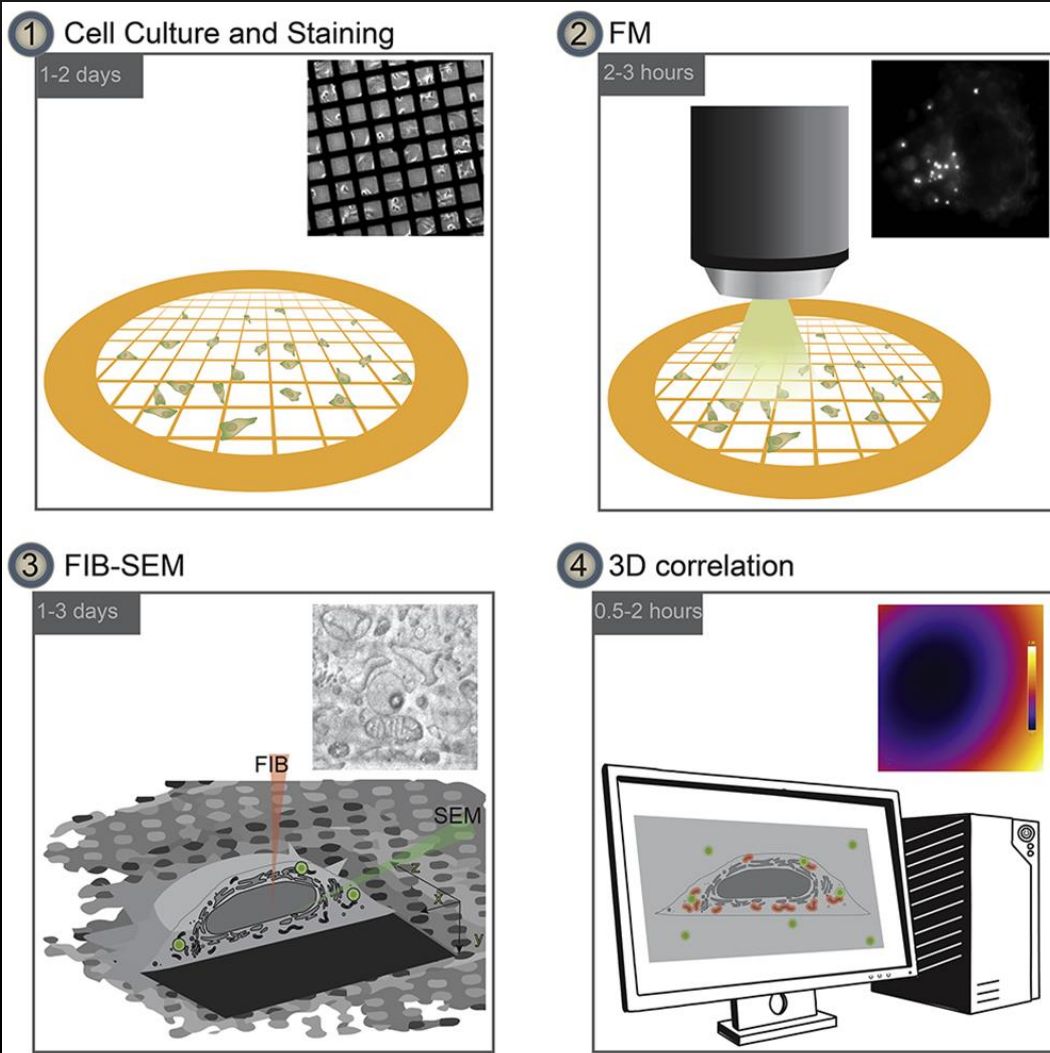


Test on mitochondria stained cells : here we could conclude that there were no deformation in the correlative imaging process: all discrepancy was in the expected range of rigid transformation



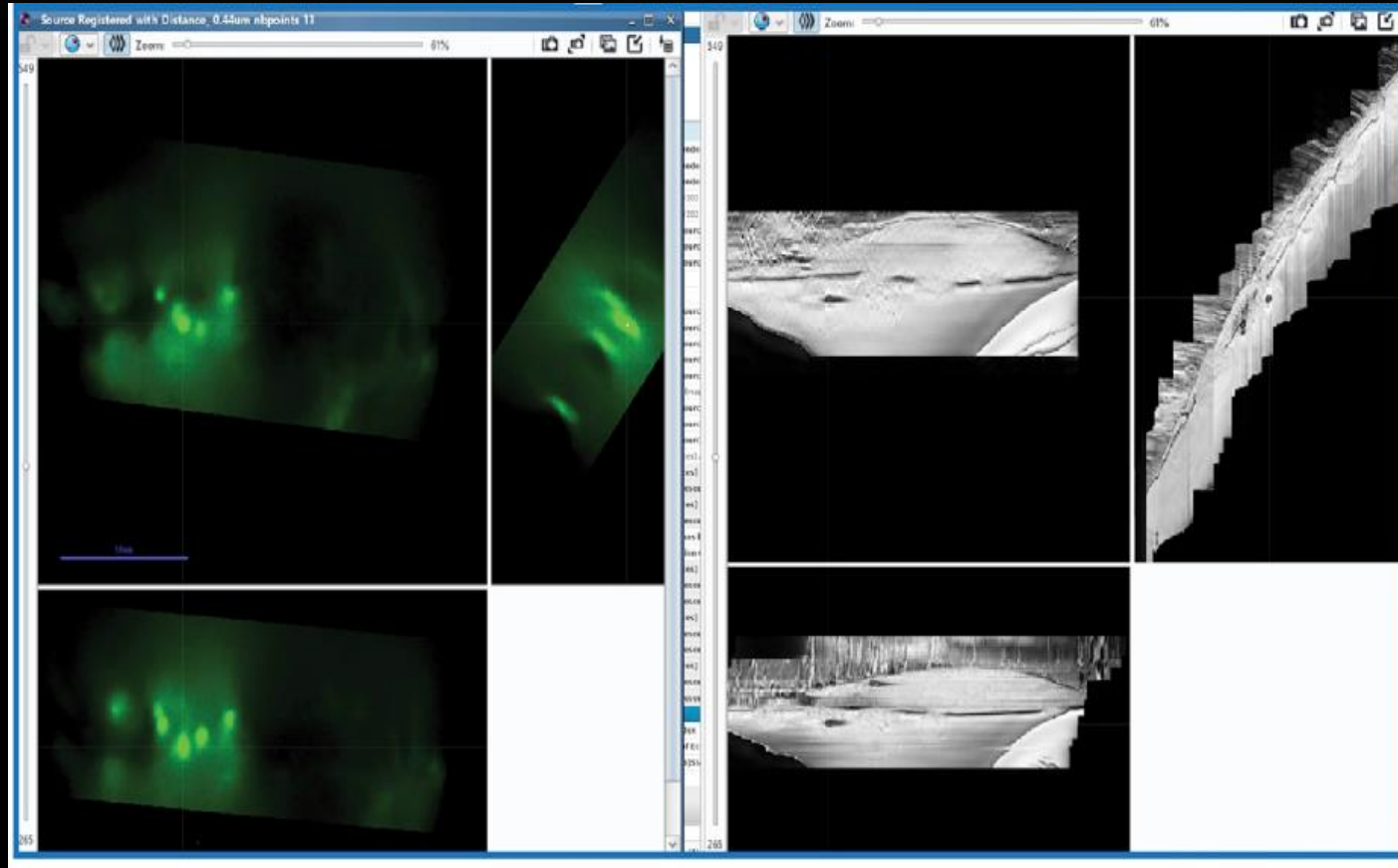


# CRYO FIB-SEM/ FM EXAMPLE WITH LIPID DROPLETS AS FIDUCIALS



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>

# 3D AUTOFINDER AND MANUAL REGISTRATION



Data in D: Scher

**STAR Protocols**

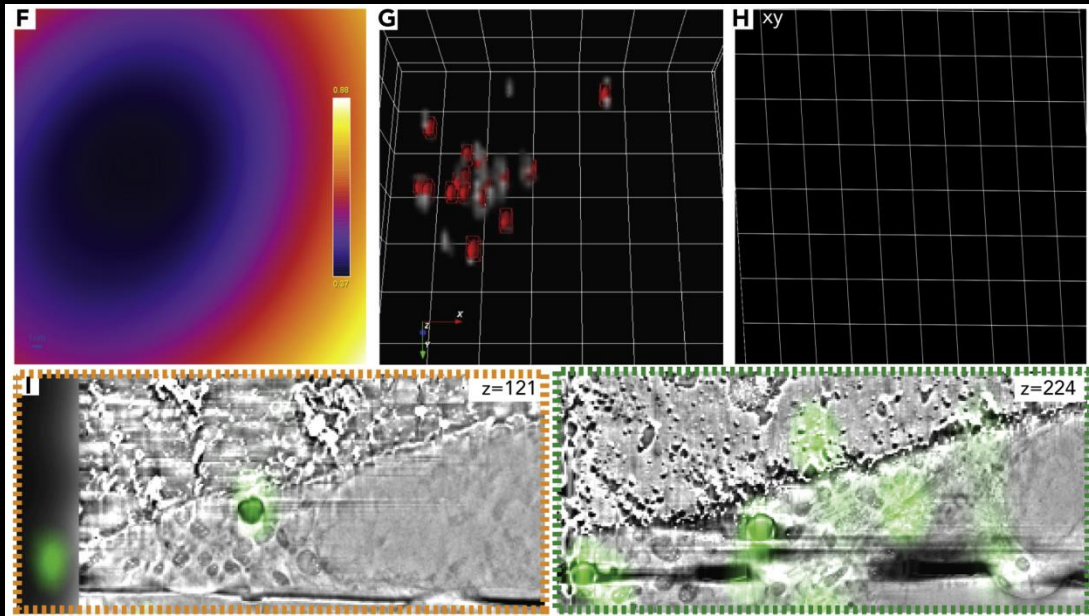
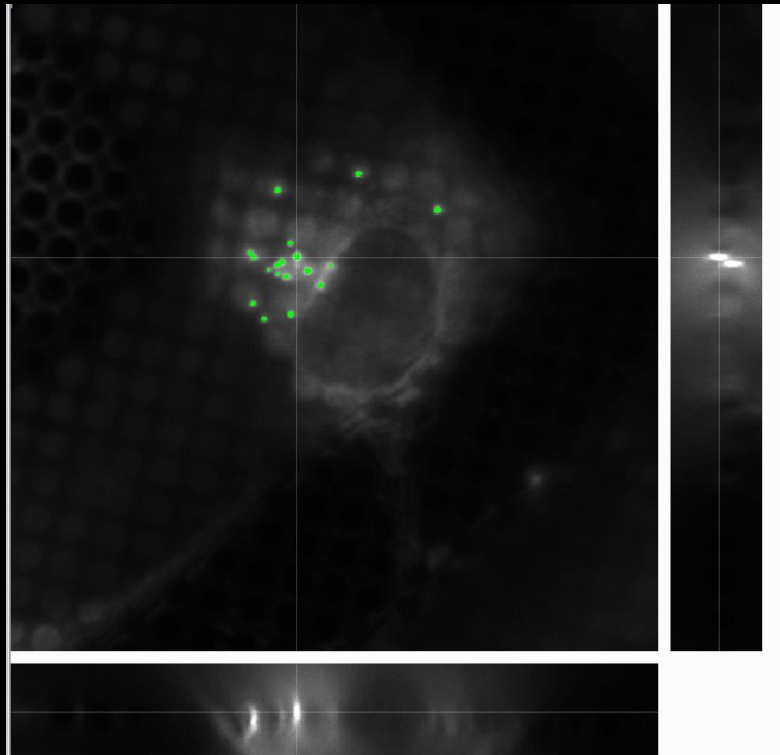
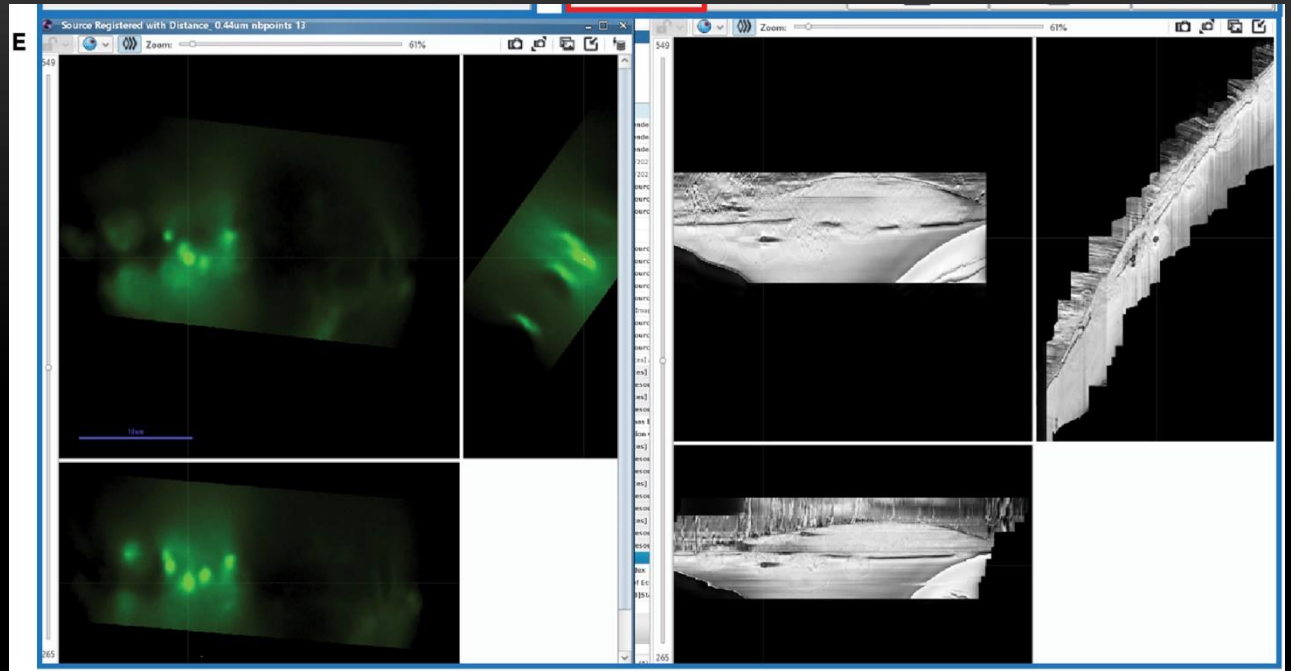
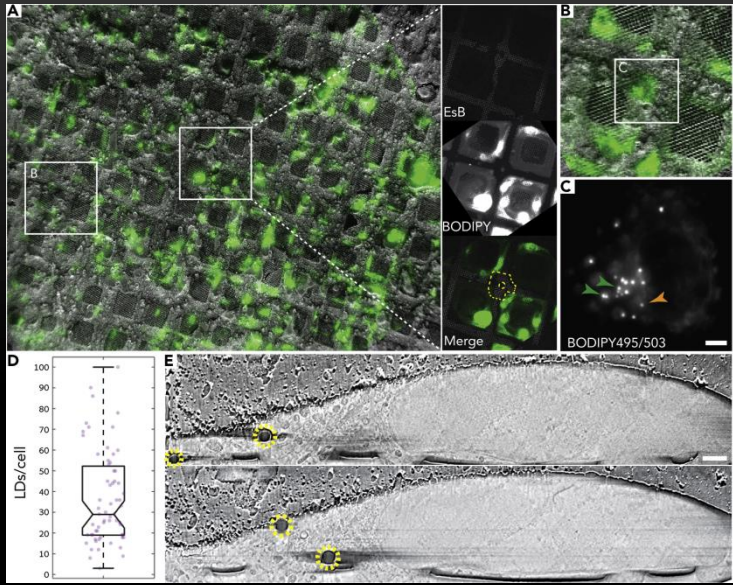


Volume 3, Issue 1, 18 March 2022, 101142

Protocol

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

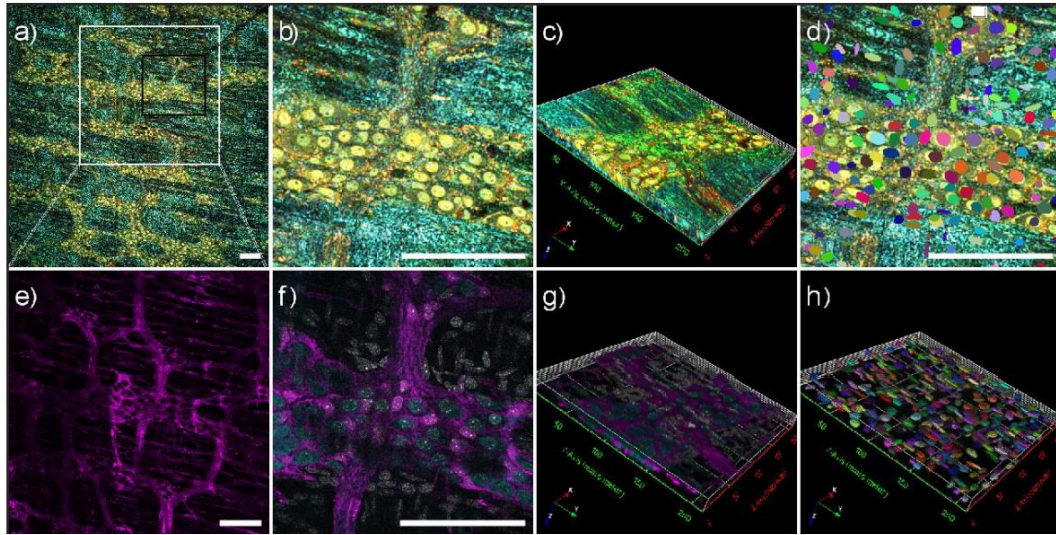
Nadav Scher<sup>1,4</sup>, Katya Rechav<sup>2</sup>, Perrine Paul-Gilloteaux<sup>3</sup>, Ori Avinoam<sup>1,5</sup>  



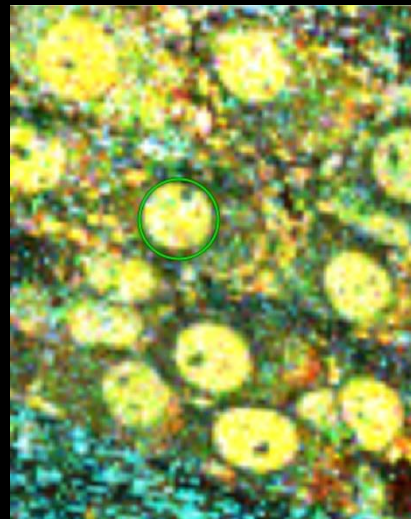
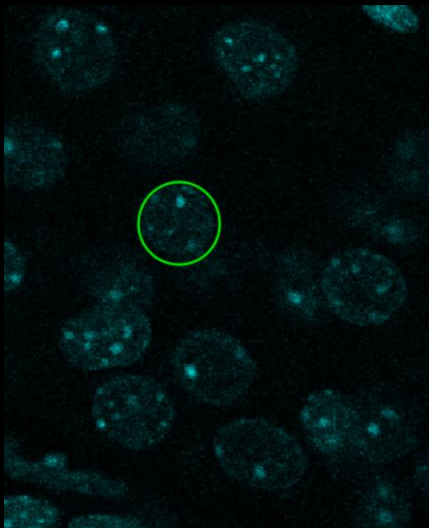
Test affine transform:  
Deformation in one axis.



# MORE MULTIMODAL (WHY MULTIMODAL?)



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



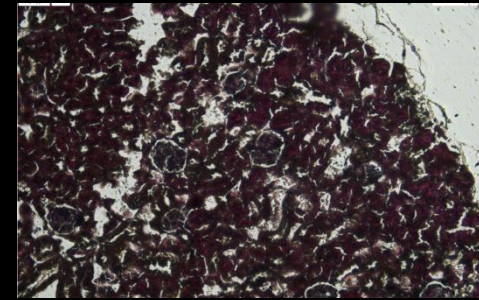
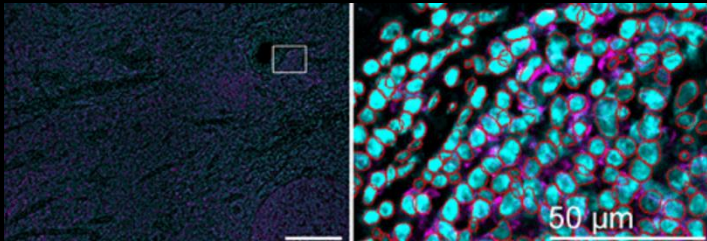
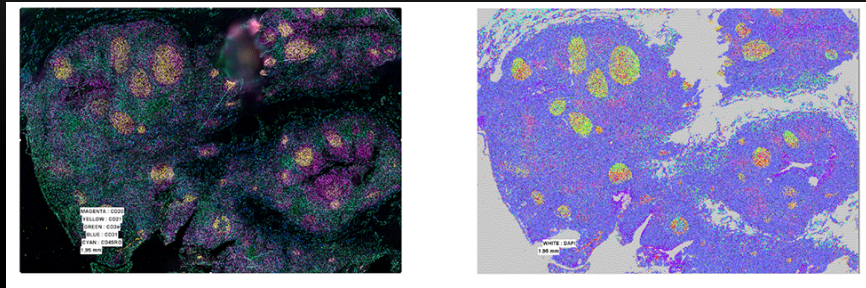
Article | [Open Access](#) | [Published: 02 March 2023](#)

## Visualizing enteric nervous system activity through dye-free dynamic full-field optical coherence tomography

[Tony Durand](#), [Perrine Paul-Gilloteaux](#), [Michalina Gora](#), [Lara Laboudie](#), [Emmanuel Coron](#), [Isabelle Neveu](#), [Michel Neunlist](#) & [Philippe Naveilhan](#)

[Communications Biology](#) **6**, Article number: 236 (2023) | [Cite this article](#)

# OTHER USAGES OF REGISTRATION IN MULTIMODAL MICROSCOPIES: SMART MICRODISSECTION FOR SPATIAL OMICS



10x, 20x or 40x (1 micrometer of cutting accuracy at 20x)

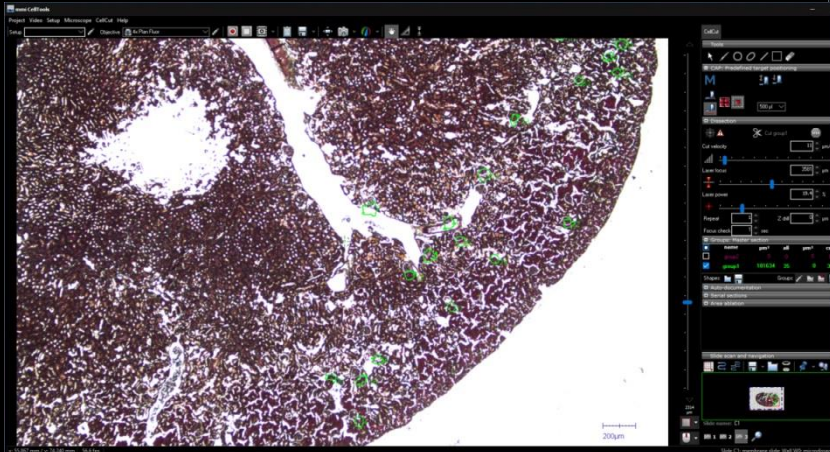
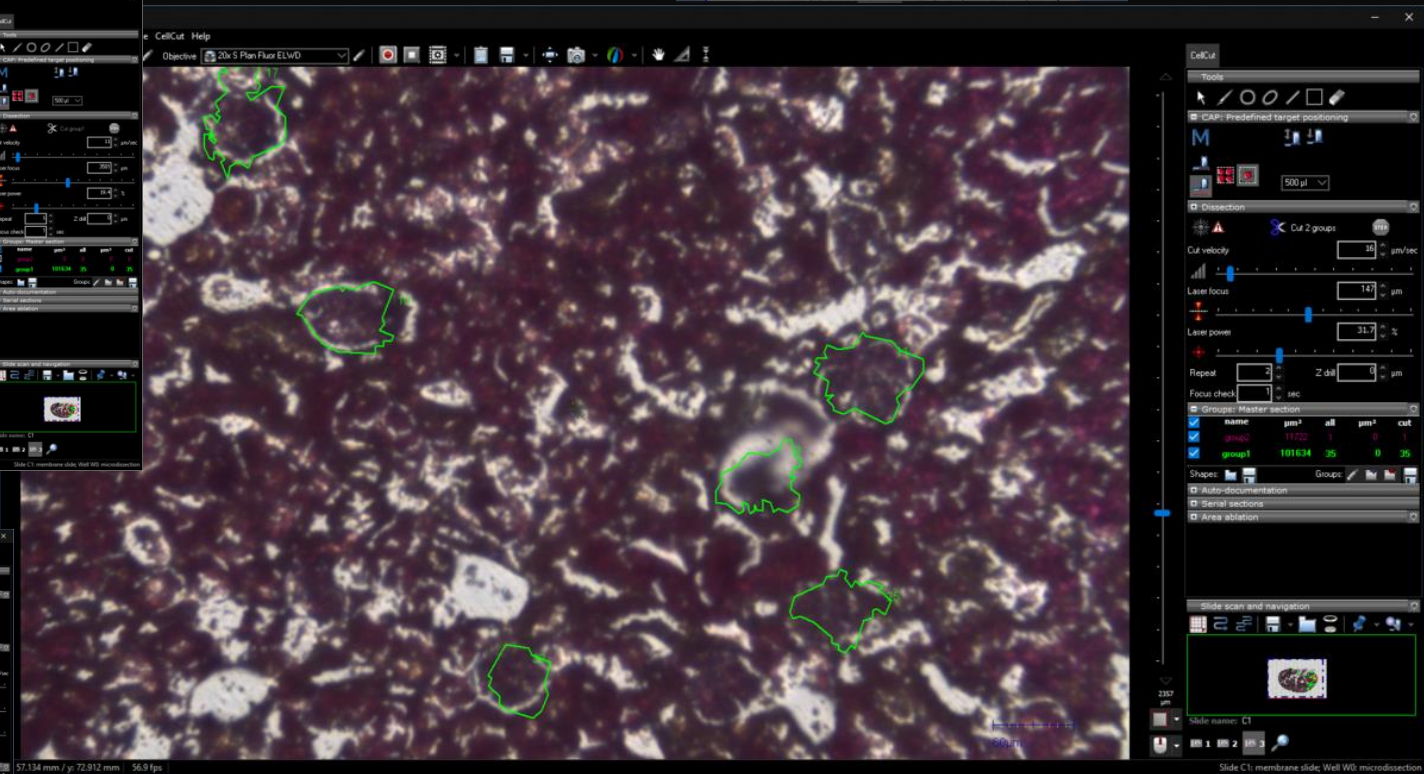
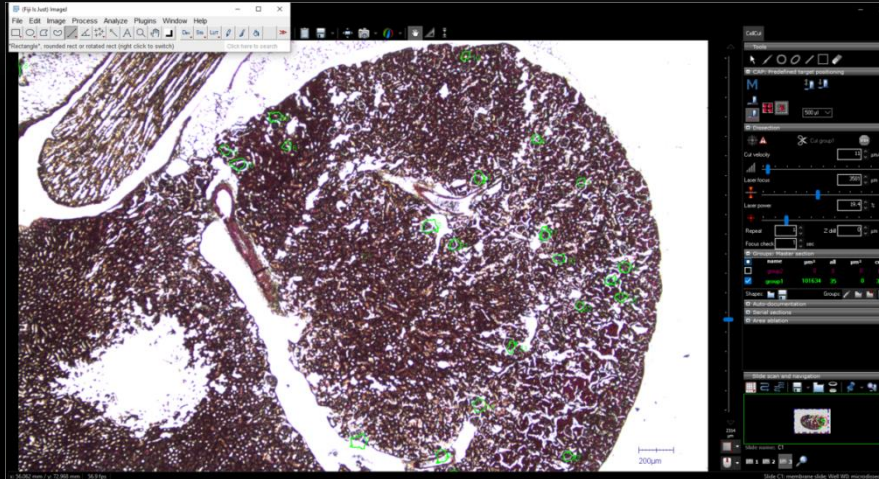


Phenocycler/Codex for fluorescence hyperplexing

Slide Scanner for fast full FOV

Microdissector (MMI cellcut)

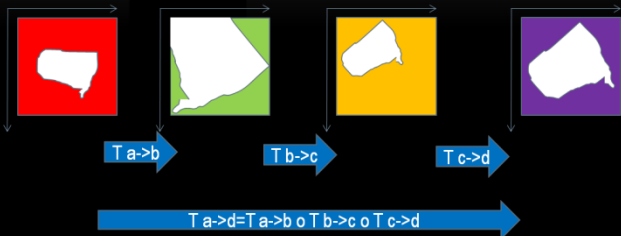
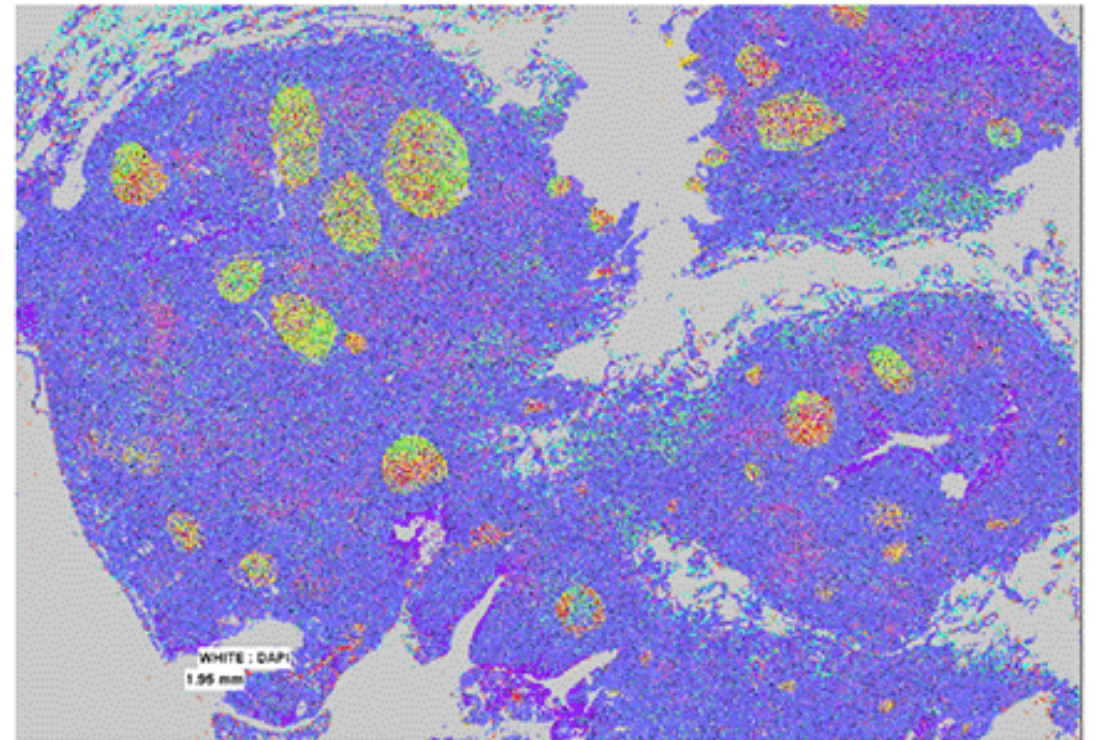
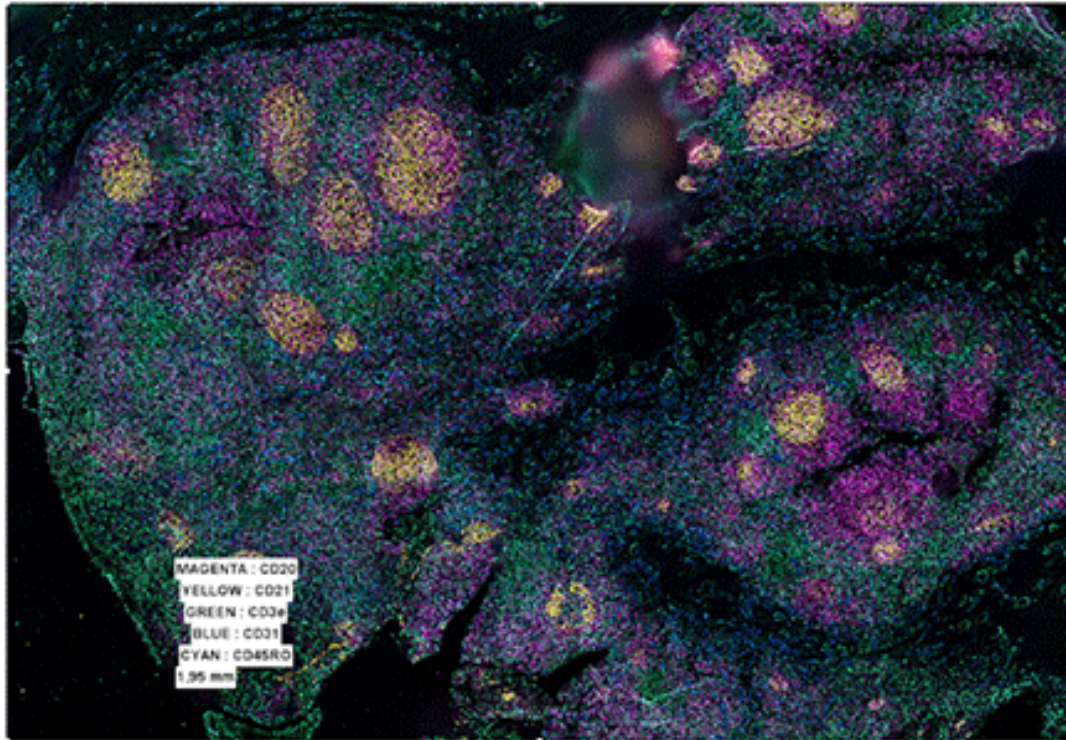




A home made interface (collaboration with MMI) allow to import these area of interest as s=« shape to be cut at the right position, at once.



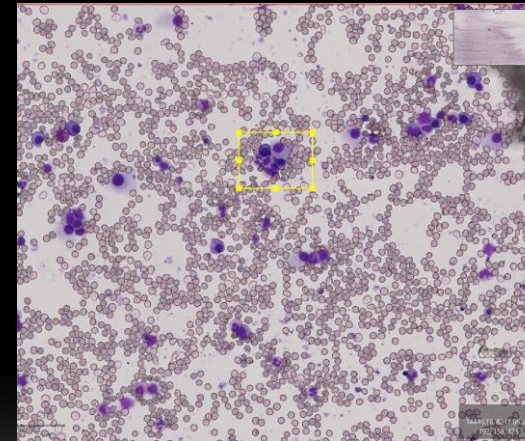
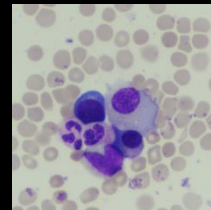
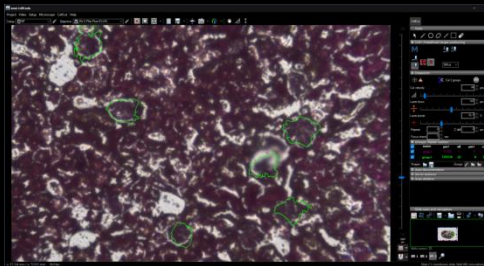
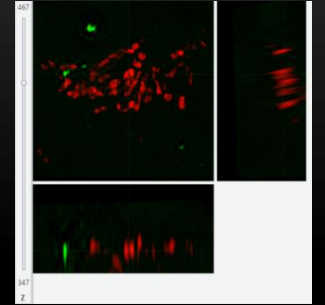
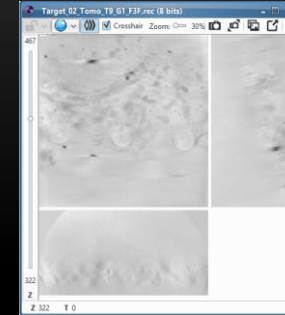
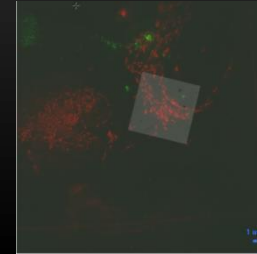
# OTHER USAGES OF REGISTRATION IN MULTIMODAL MICROSCOPIES: SMART MICRODISSECTION FOR SPATIAL OMICS



(not the same sample yet, here tonsil vs kidney used in faisability test)

# CONCLUSION

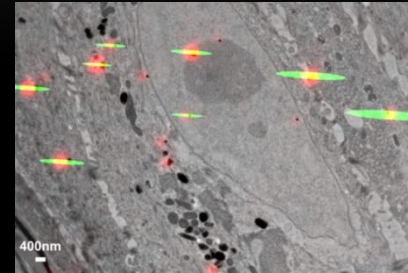
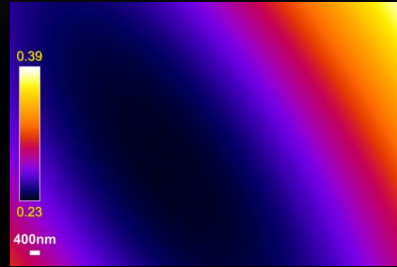
- Registration allow to link multimodal data through a transformation
- This transformation allows to link areas, but also ROIs





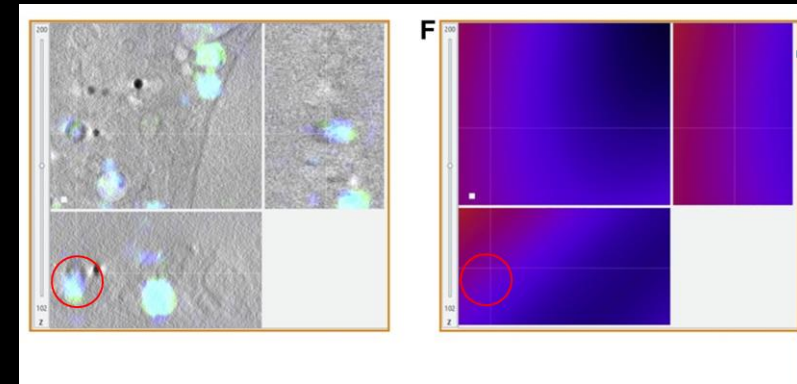
# CONCLUSION

- Registration uncertainty can be estimated (accurate for rigid and affine registration for now-> non rigid local affine ok as well)



- This has multiple purposes:

- Target area accuracy (Adding error margin for Example)
- Multimodal colocalisation



- And likely and supported by the litterature but not yet in our field for better accuracy with less data of deep learning models (for example in loss function similarly to bayesian approaches)





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Katya Rechav

Ori Avinoam

Diamond Light Source

Maria Harkiolaki

Nina Vyas

All team

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*Héloïse Duluc*

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CANCER**

**D-FFOCT**

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Philippe Naveilhan

Tony Durand

Michel Neunlist

CryoCapCell

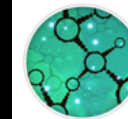
Xavier Heiligenstein



QUESTIONS?



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



**FRANCE-BIOIMAGING**