

17/03/2023

IMAGE ANALYSIS TOOLS AND APPLICATIONS FOR CORRELATIVE MICROSCOPY

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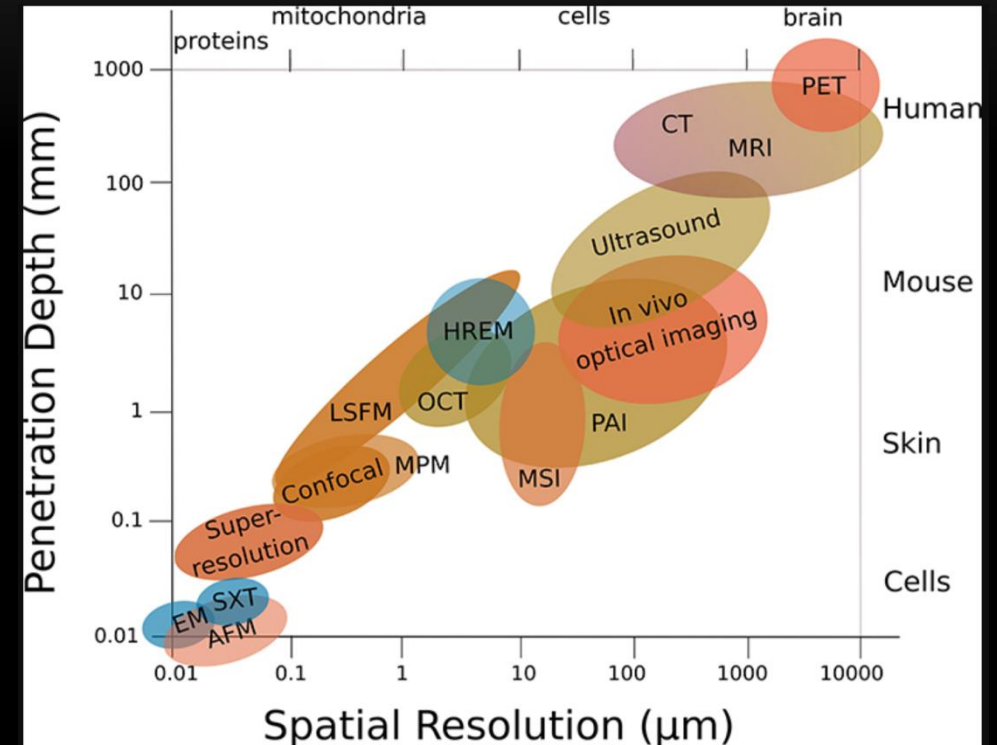
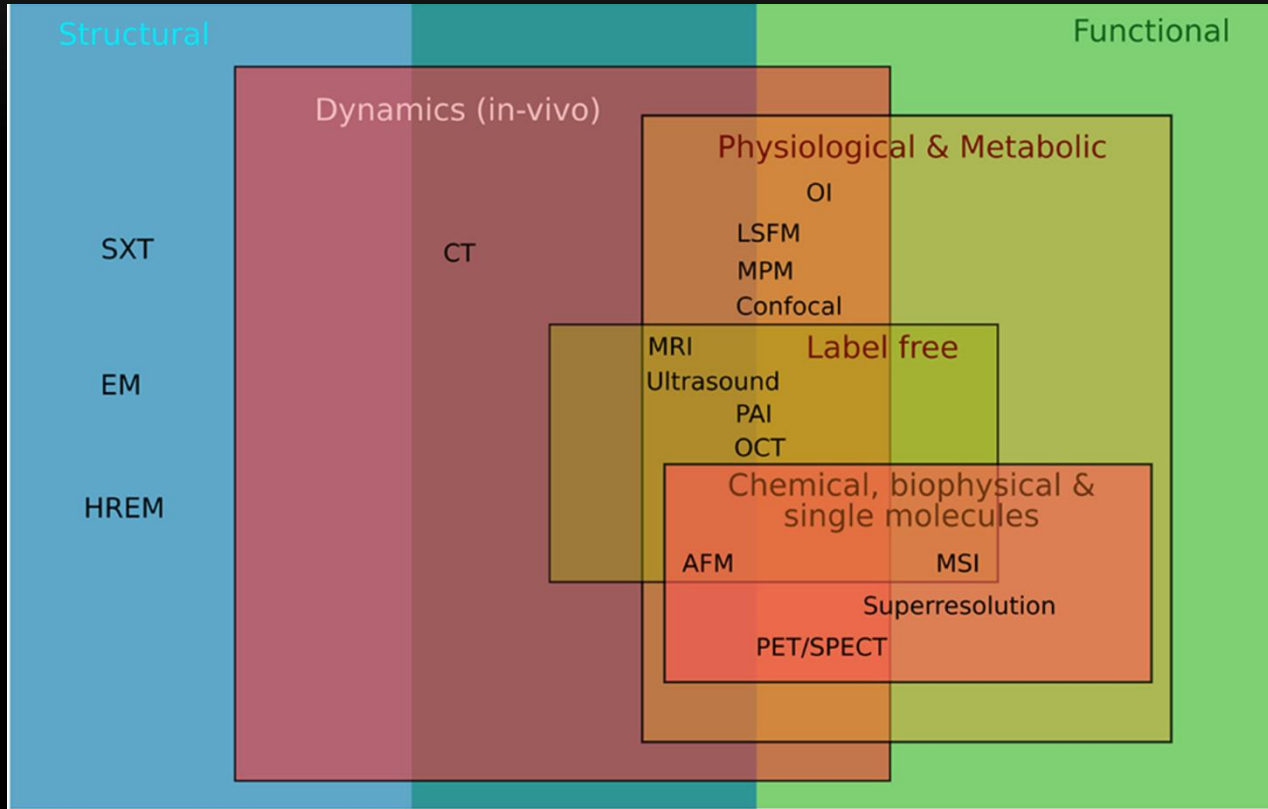
Nantes Université, CHU Nantes, CNRS, Inserm, BioCore, SFR Bonamy, Nantes, France

Nantes Université, CNRS, INSERM, l'institut du thorax, Nantes, France.



WHAT IS CORRELATIVE MICROSCOPIES?

MULTIMODAL IMAGING FOR AN HOLISTIC VIEW



Walter, 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)

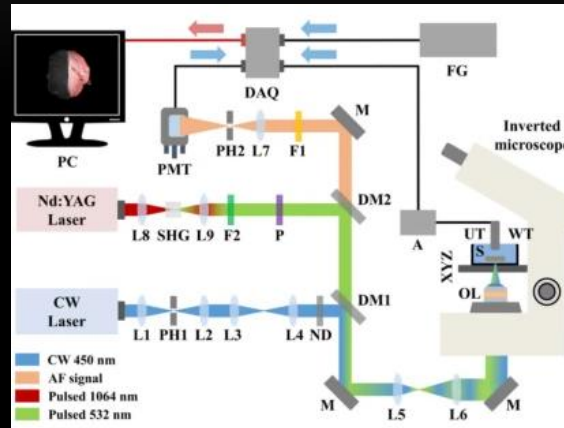
THEN COME THE NATURAL NEED TO FUSE THEM

- Imaging the **exact same specimen** with **two or more complementary modalities**
 - 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
-

SOLUTION 1: USE SYSTEM INTEGRATING SEVERAL MODALITIES (HYBRID IMAGING)

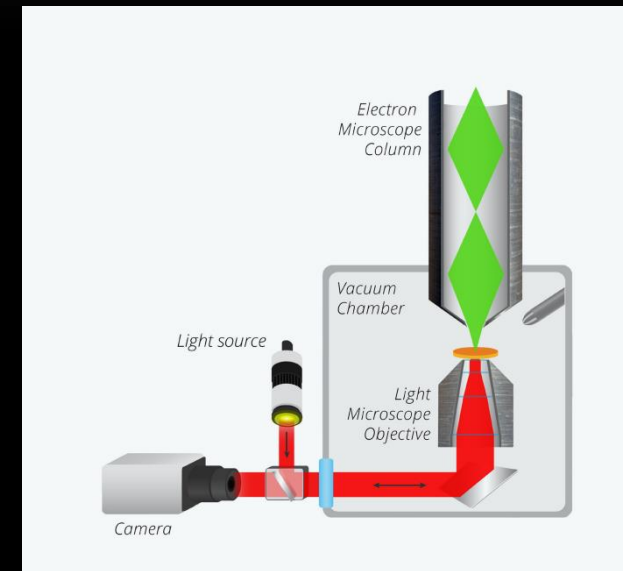


Cal-Gonzales et al. Front. Phys., 18 May 2018
 Sec. Medical Physics and Imaging
<https://doi.org/10.3389/fphy.2018.00047>



Tserevakis et al. Scientific reports, 03 May 2022
 Sec. Medical Physics and Imaging
<https://doi.org/10.3389/fphy.2018.00047>

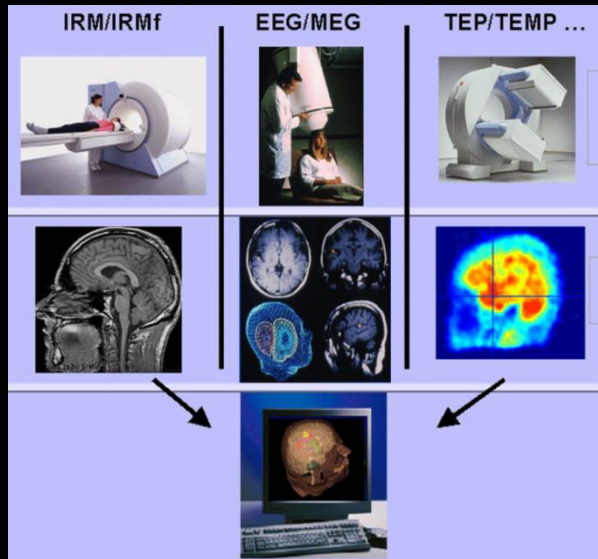
Confocal fluorescence
 + Photo Acoustic



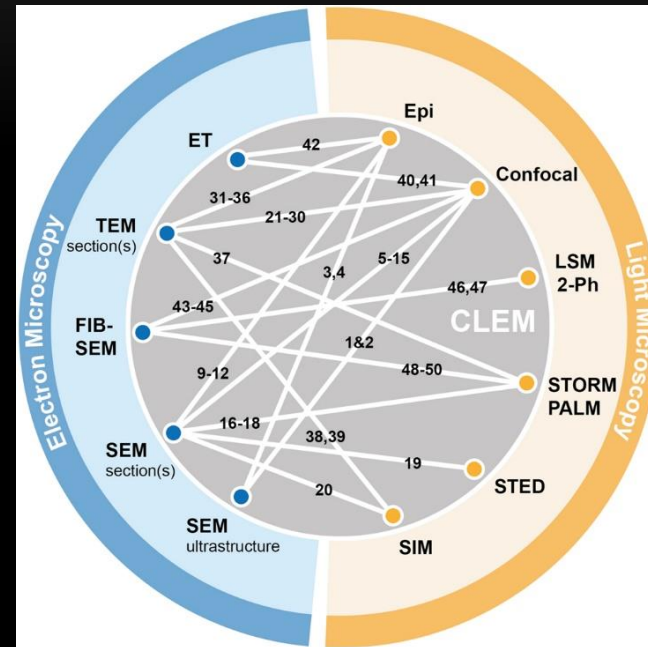
SECOM from DELMIC integrated CLEM

- + Ready to use, no sample deformation
- Limited to the combination proposed: new information new machine?

SOLUTION 2 : CORRELATIVE MICROSCOPY WITH SAMPLE TRANSFER BETWEEN ONE OR SEVERAL MODALITIES



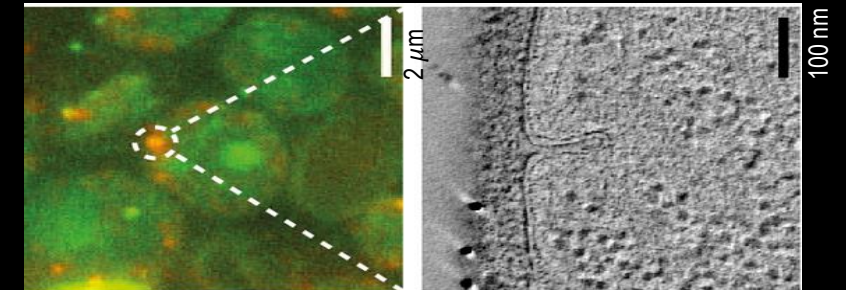
P Jannin, Visages team 2005



Begemann I and Galic M (2016)
 Front Synaptic Neurosci. doi:
 10.3389/fnsyn.2016.00028

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

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



Kukulski/Briggs et al., Cell 2012

CLEM -> correlative light electron microscopy

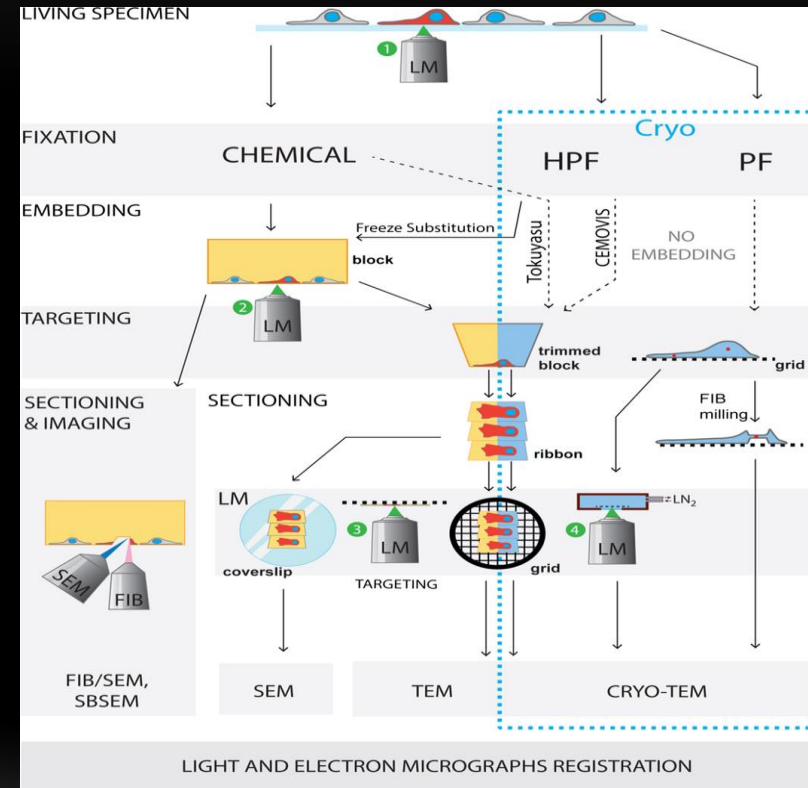
SOLUTION 2 : CORRELATIVE MICROSCOPY WITH SAMPLE TRANSFER BETWEEN ONE OR SEVERAL MODALITIES

+ limited by your imagination

- (and a few technical details)

Example in CLEM:

- Preserving fluorescence during fixation
- Dealing with sample transformation
(or finding protocols/systems allowing good imaging quality even in Cryo for instance)
- Multimodal probes visible in different modalities
- Targeting the right area (the needle in the hay problem)
- Merging dataset



Bykov et al., FEBS Letters 2016 doi: 10.1002/1873-3468.12153

**MERGING DATASETS: COMPUTING THE LINK
BETWEEN IMAGES/VOLUMES**

APPROACHES FOR AUTOMATIC AND SEMI 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.

Fiducial pairs known?

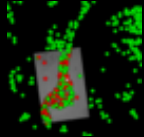
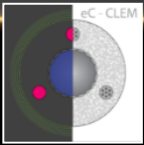
yes

no

Compute and apply transfo

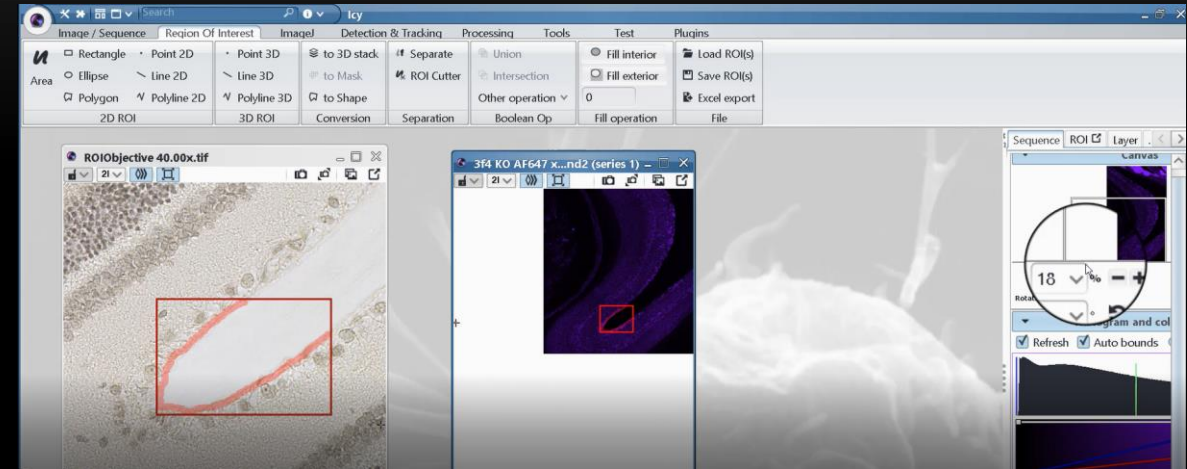
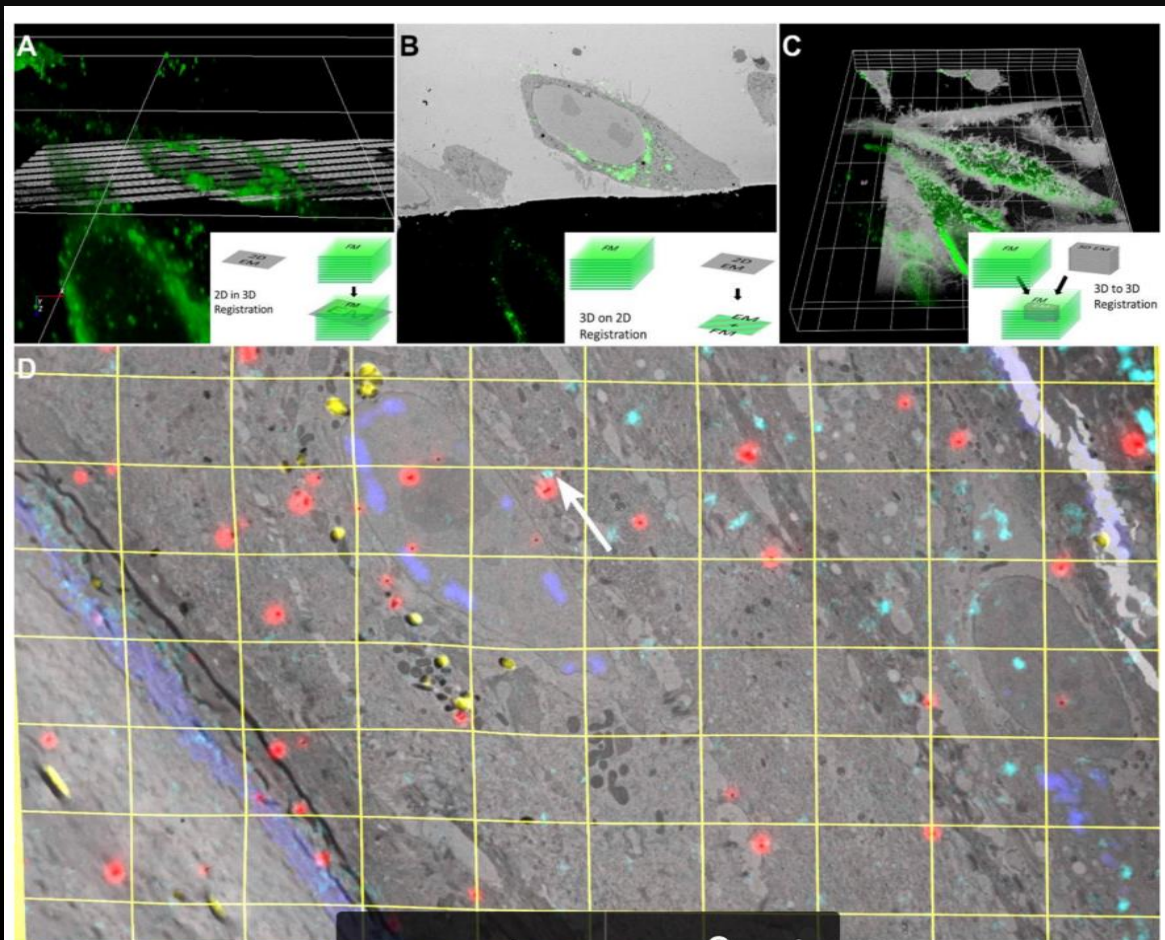
Propose matching

Compute and apply transfo





EC-CLEM : A SIMPLE USER INTERFACE



2D/3D manually paired fiducial based registration
Line registration
Linear and non linear
Error assessment
construct your automatic registration workflow

Published: 31 January 2017

eC-CLEM: flexible multidimensional registration software for correlative microscopies

[Perrine Paul-Gilloteaux](#), [Xavier Heiligenstein](#), [Martin Belle](#), [Marie-Charlotte Domart](#), [Banafshe Larjani](#), [Lucy Collinson](#), [Graça Raposo](#) & [Jean Salamero](#)

Nature Methods 14, 102–103 (2017) | [Cite this article](#)

NEW VERSION !! (beta but usable)

-MUCH MORE FASTER

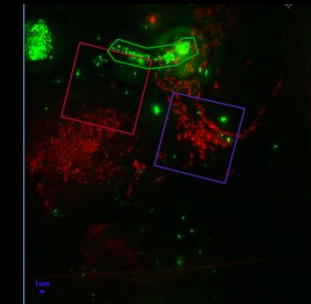
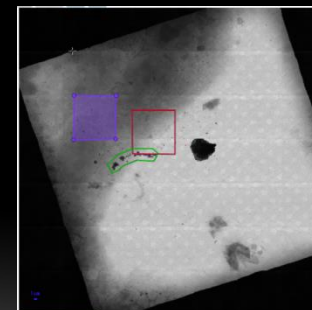
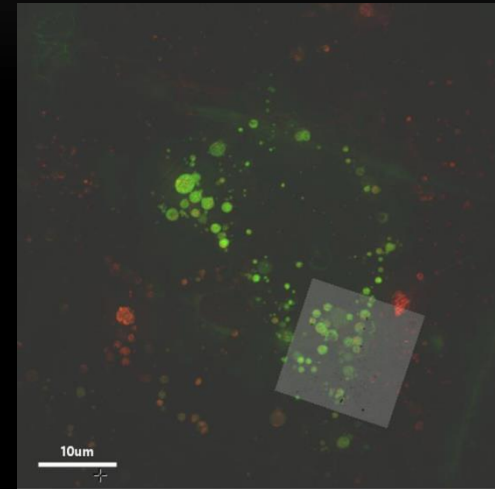
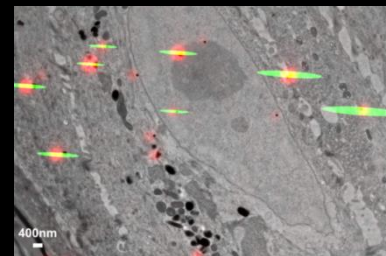
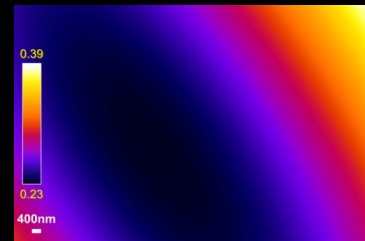
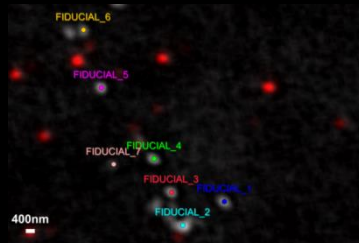
- LESS GREEDY in memory

- New tools for error estimation (colocalization like)

- New Tools to ease your correlative WF constructions (export)

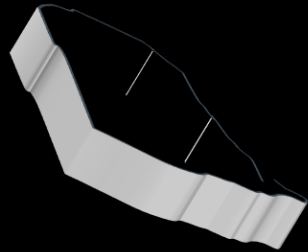
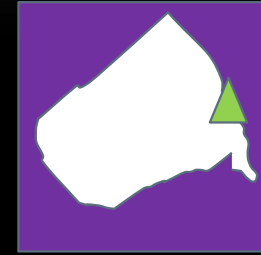
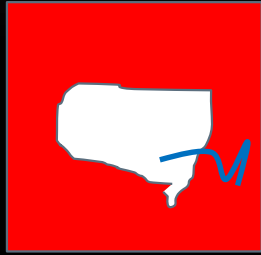
- New Tool for visualization

Ec-clemv2 another plugin of ICY for the new version



	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

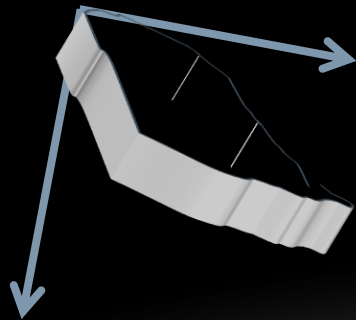
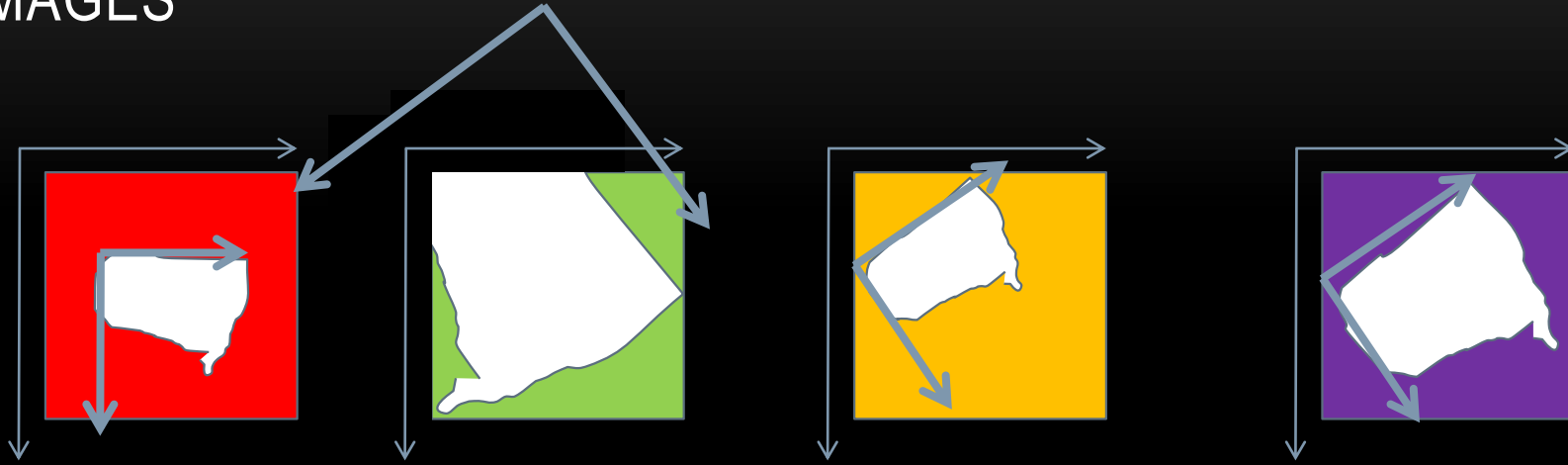
SAMPLE TO BE TRACKED FROM ONE IMAGING MODALITY TO ANOTHER



Sometimes additional modalities will help

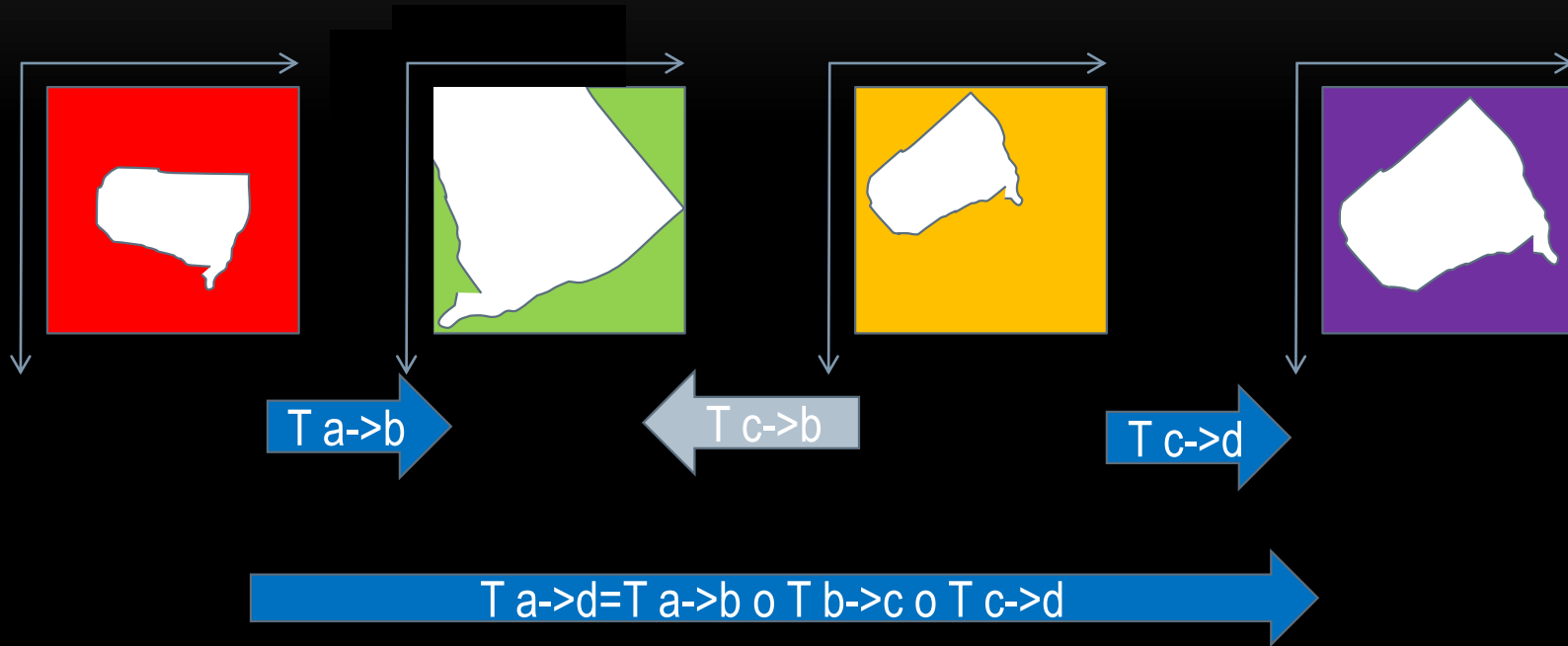
Ecclem Cascaded
transform/ Invert Transform

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

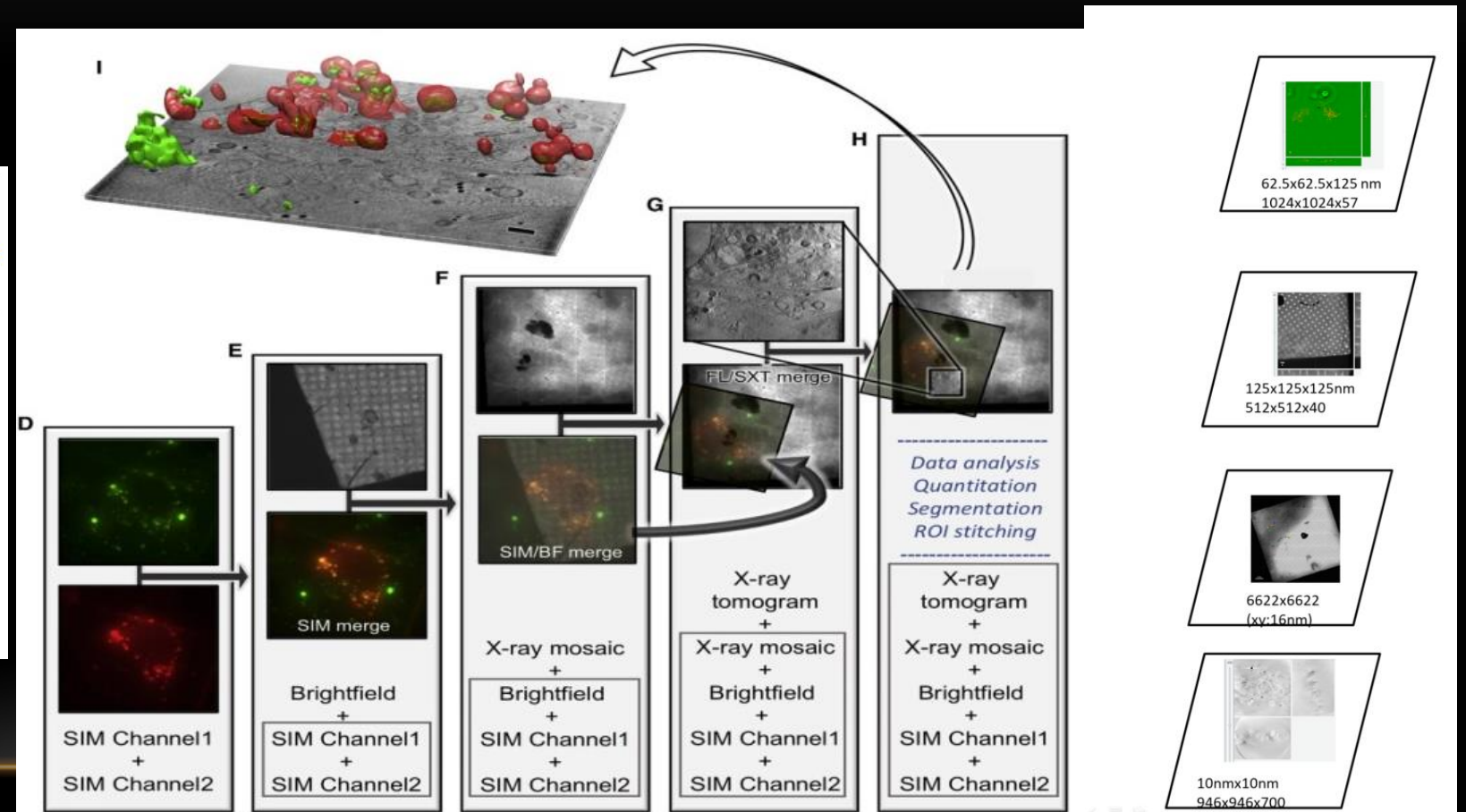


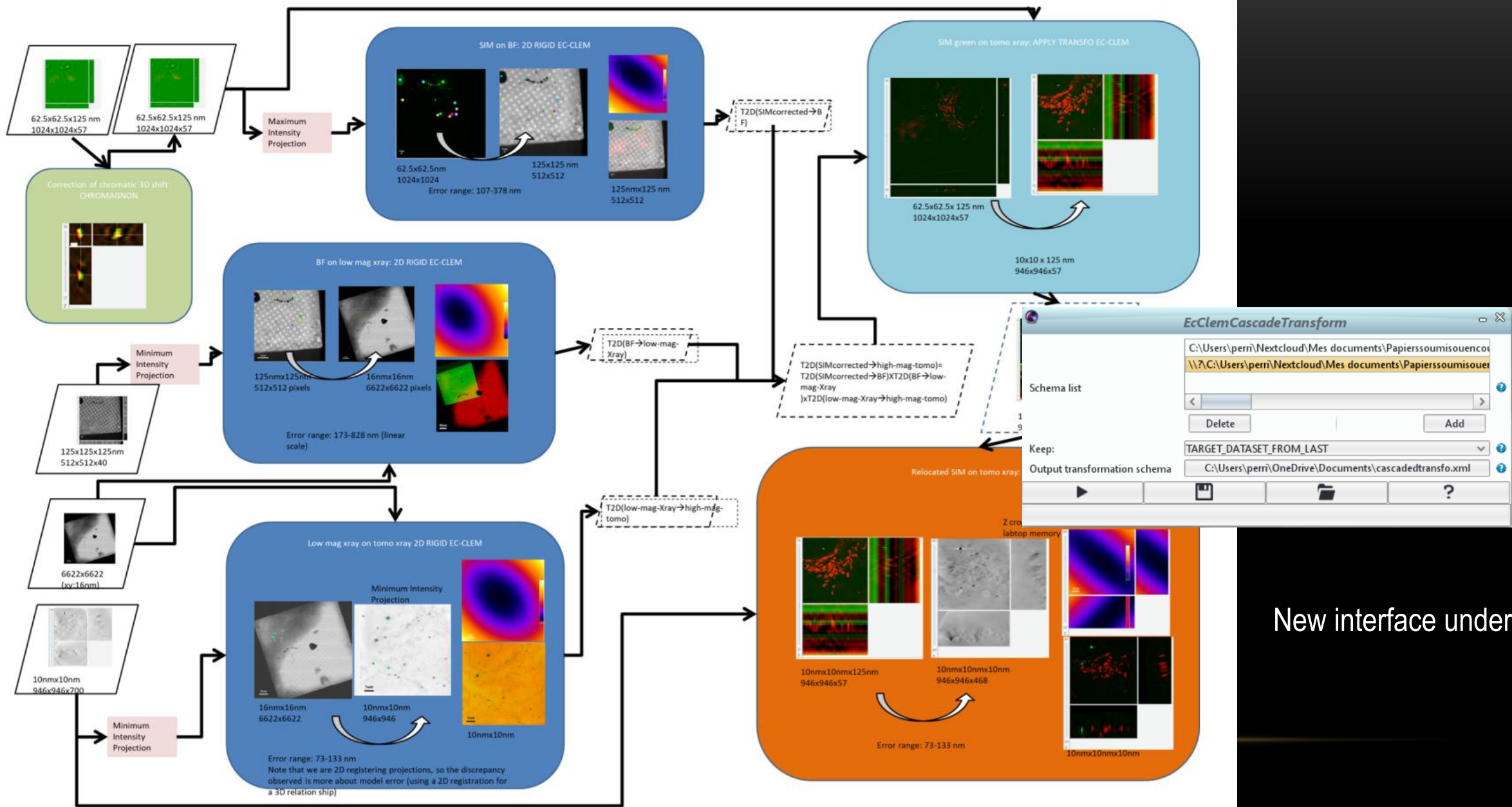
Volume 182, Issue 2, 23 July 2020, Pages 515-530.e17

Resource

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

Ilias Kounatidis^{1 10}, Megan L. Stanifer^{2 10}, Michael A. Phillips^{1 3 4 10}, Perrine Paul-Gilloteaux^{5 6}, Xavier Heiligenstein⁷, Hongchang Wang¹, Chidinma A. Okolo¹, Thomas M. Fish¹, Matthew C. Spink¹, David I. Stuart^{1 4}, Ilan Davis³, Steeve Boulant^{8 9}, Jonathan M. Grimes^{1 4}, Ian M. Dobbie³, Maria Harkiolaki^{1 11}

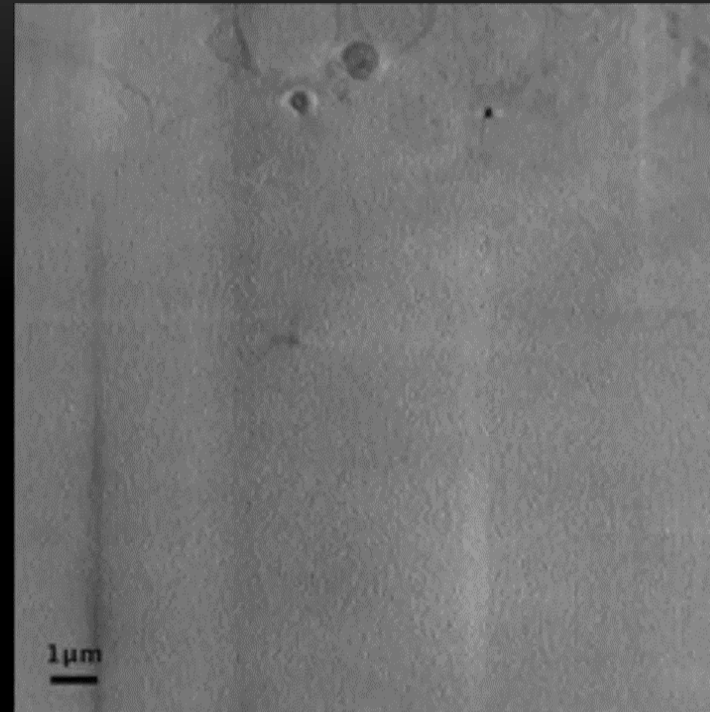
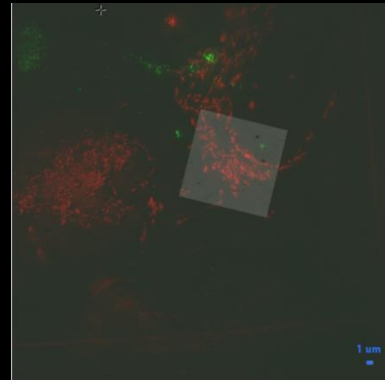
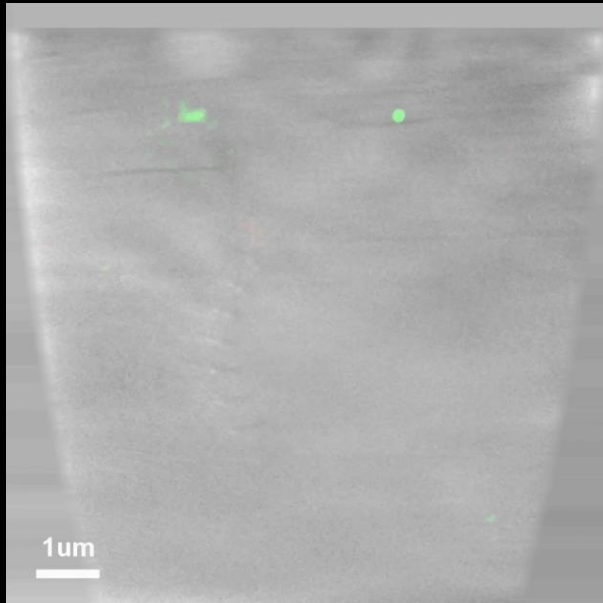




New interface under dev

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



Data
available
on
ZENODO

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

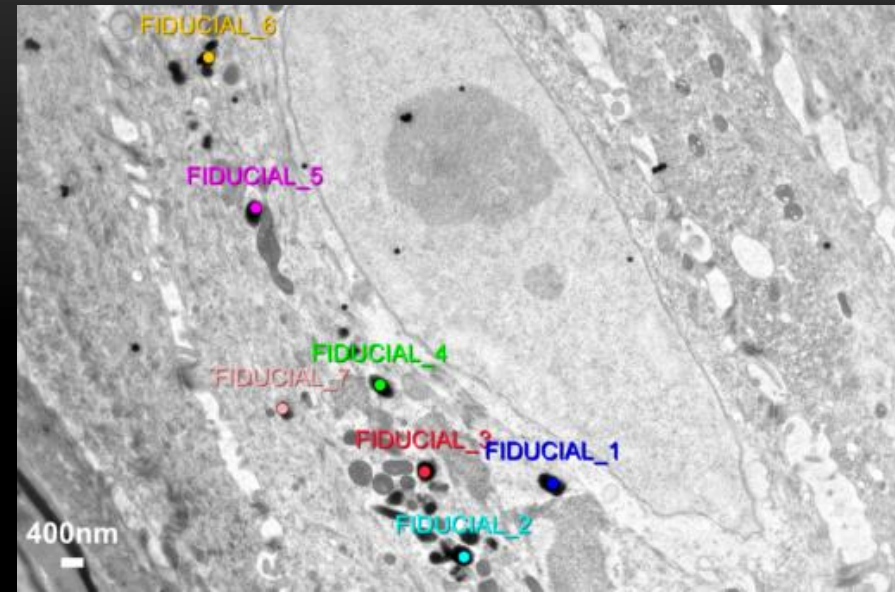
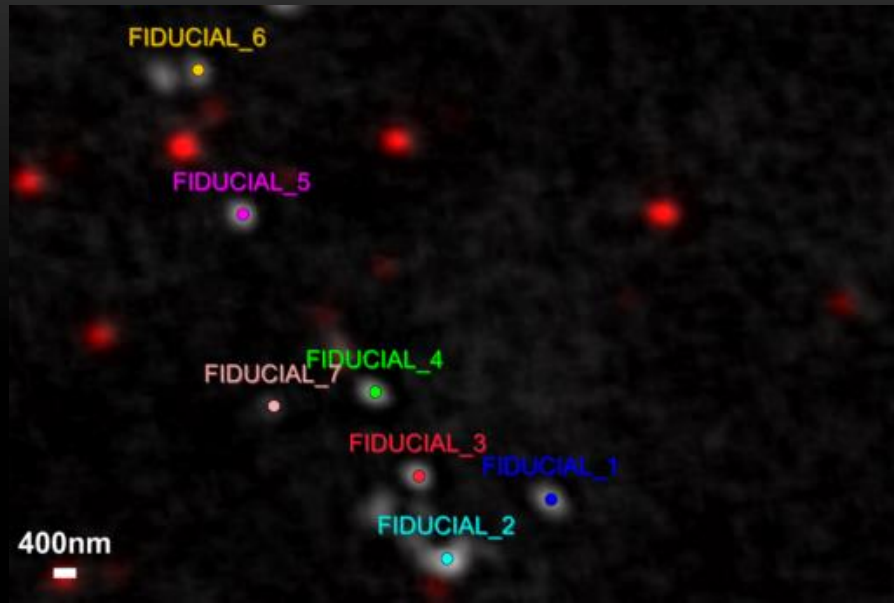
- U2OS cells stably expressing red fluorescence (mCherry-Galectin-3) , indicators of endosomal membrane disruption
- infected by a a fluorescent reovirus green fluorescence (Alexa647-reovirus)
- sample vitrification: frozen in liquid nitrogen-cooled liquid ethane

COMPUTING AND USING ERROR IN REGISTRATION

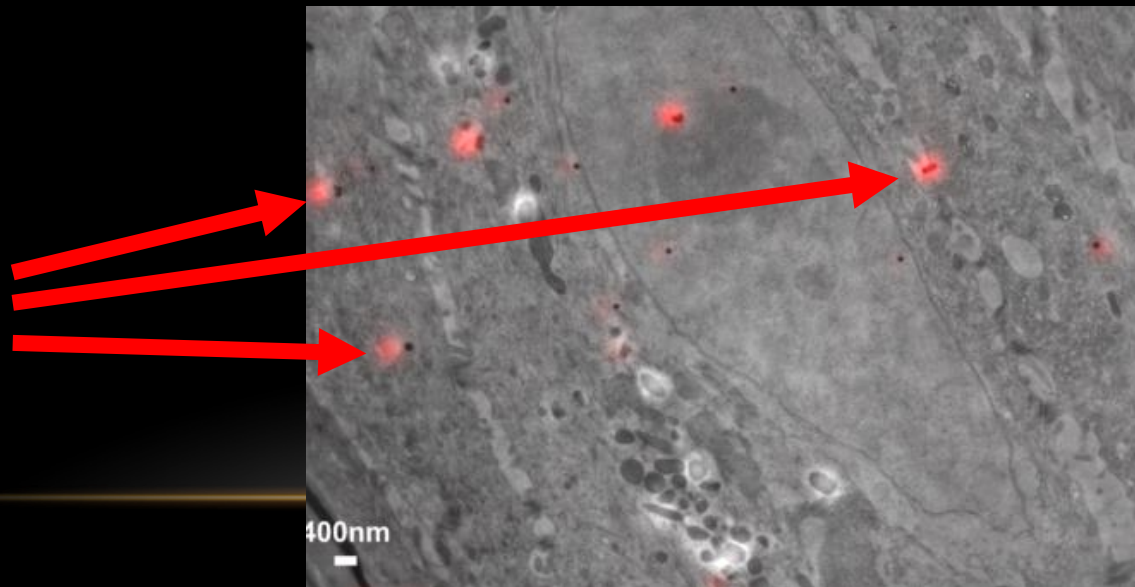
- One thing to know: Matching is NEVER PERFECT
 - Because your sample may deform/ evolve between two modalities
 - Because multimodality is usually also multiscale (very different resolution, one pixel can match hundred of pixels in the second modality)
 - Because none modality allows to perfectly locate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

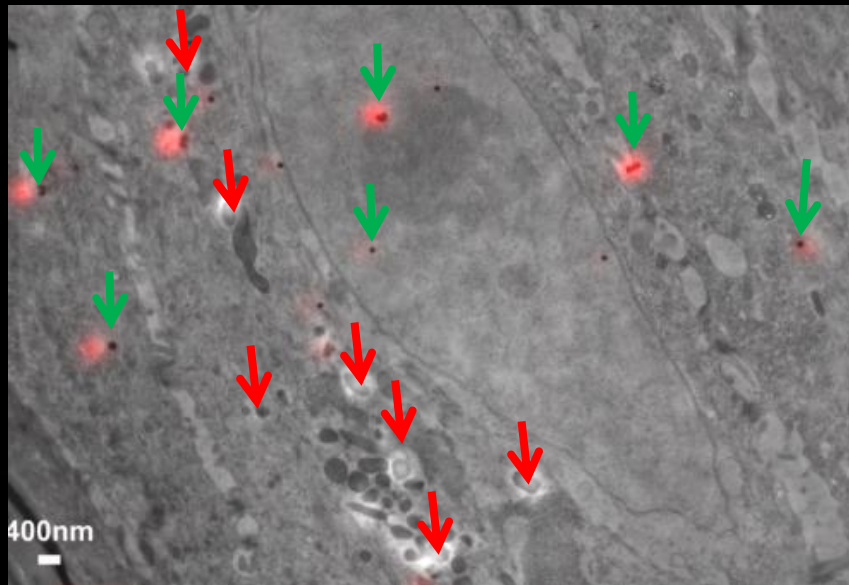
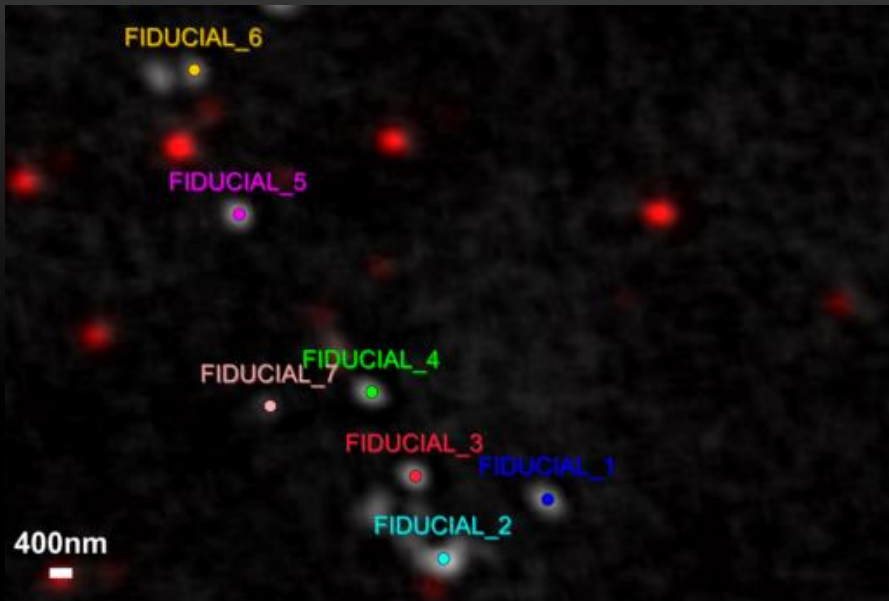
WE HAVE DEVELOPED A METHOD TO SPATIALLY ASSESS THE QUALITY OF REGISTRATION WITH NO GROUND TRUTH (NO OTHER INPUT THAT POINTS (OR SURFACE) USED FOR REGISTRATION: LOCALISATION NOISE AND ERROR REGISTRATION ARE ESTIMATED).

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.



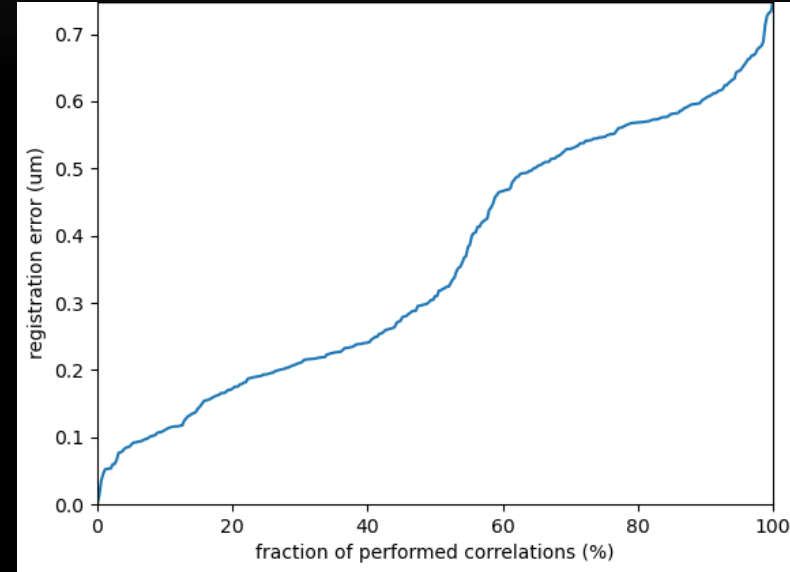
Beads are used here to check how good we are to estimate the error



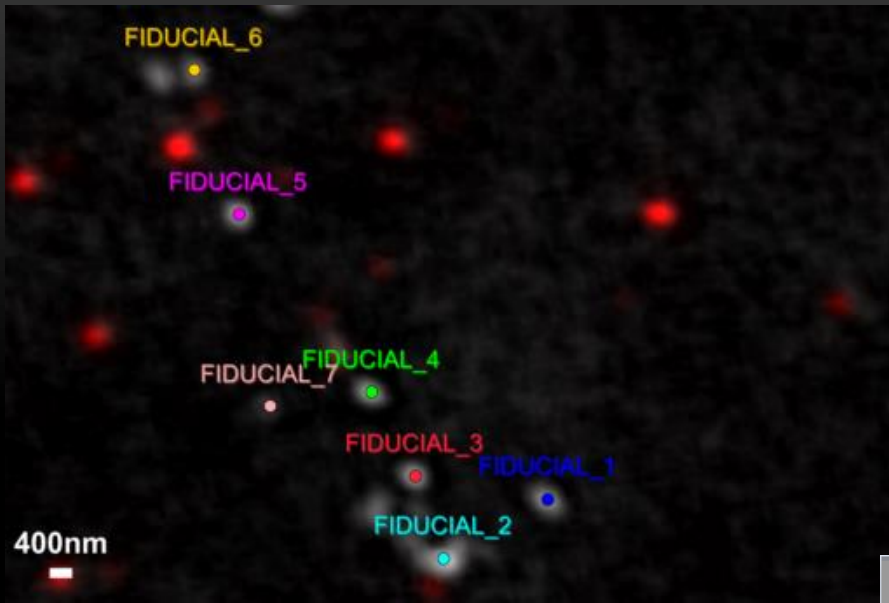


↓ fiducials

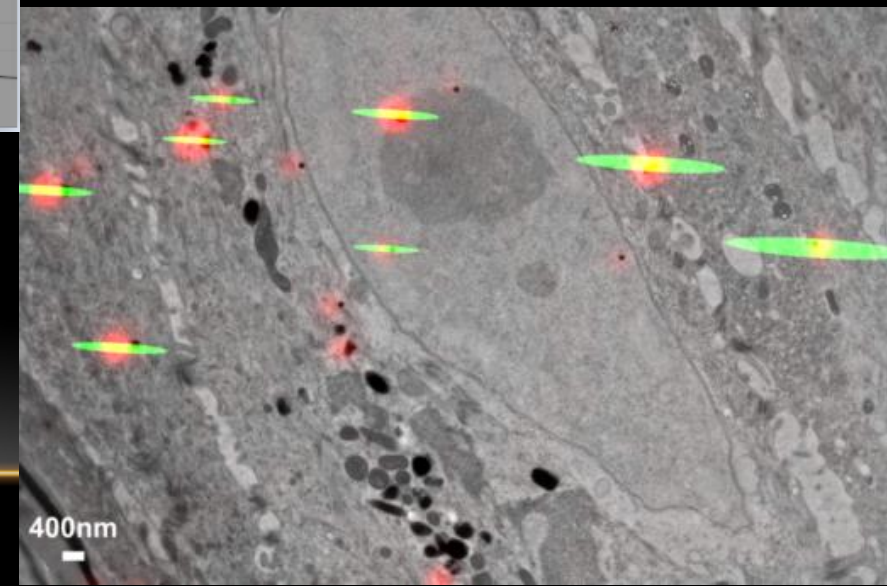
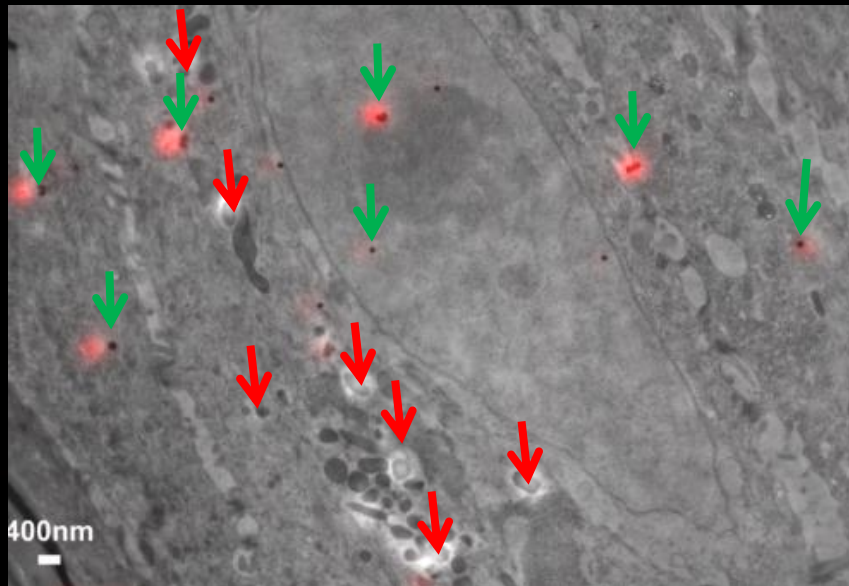
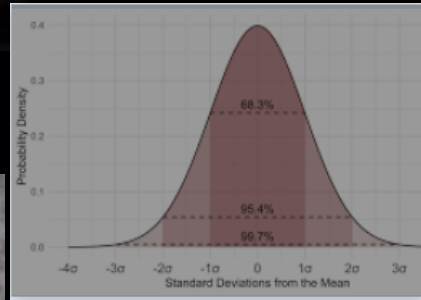
↓ Not fiducials



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

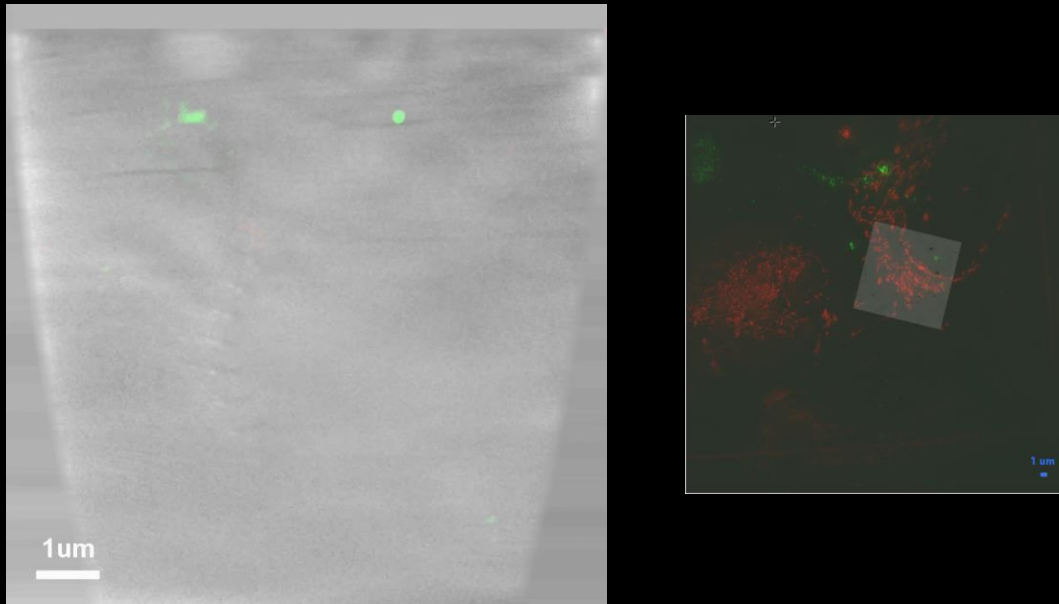


New in
ecclem:
95%
accuracy
prediction

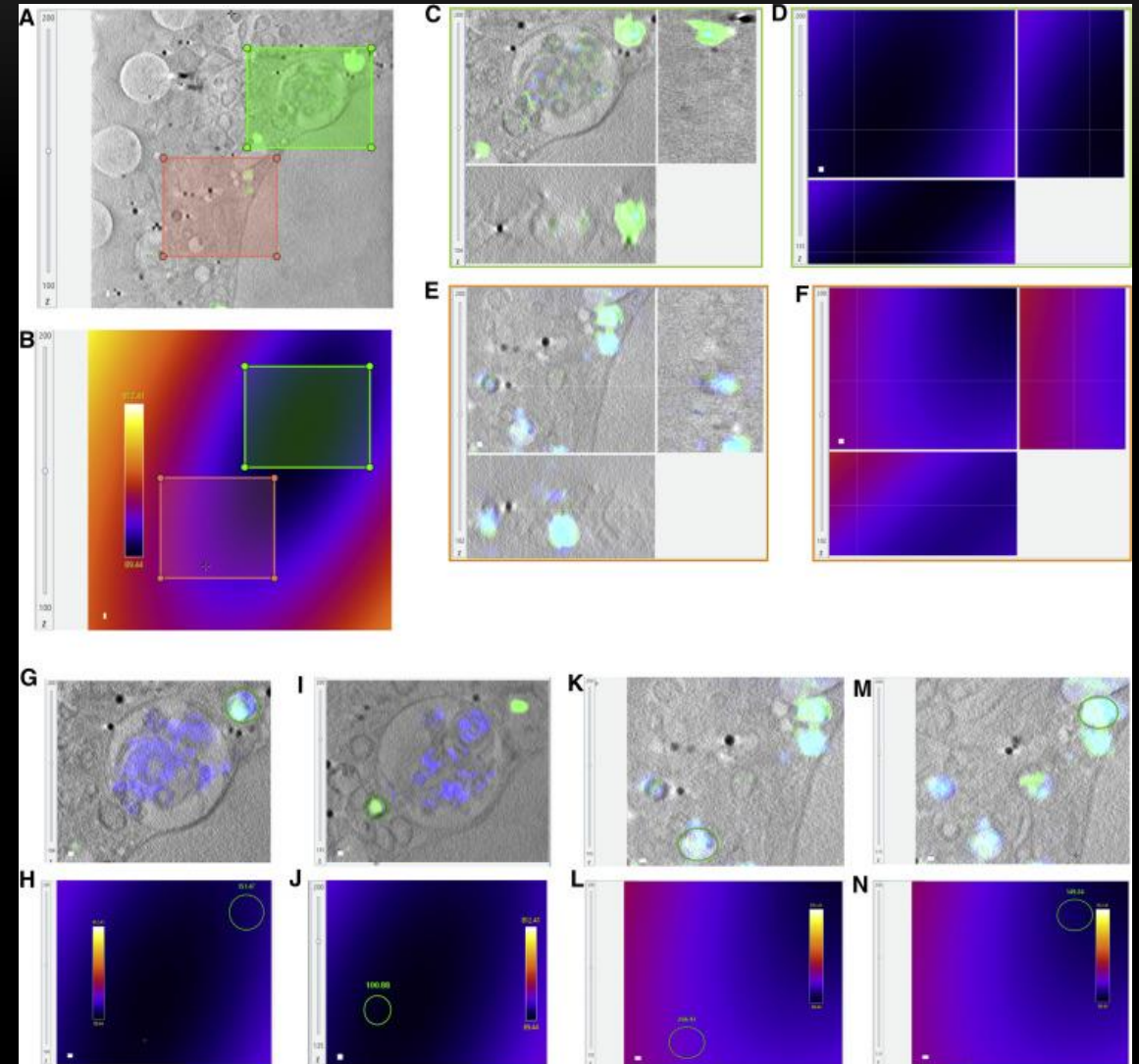
APPLICATIONS

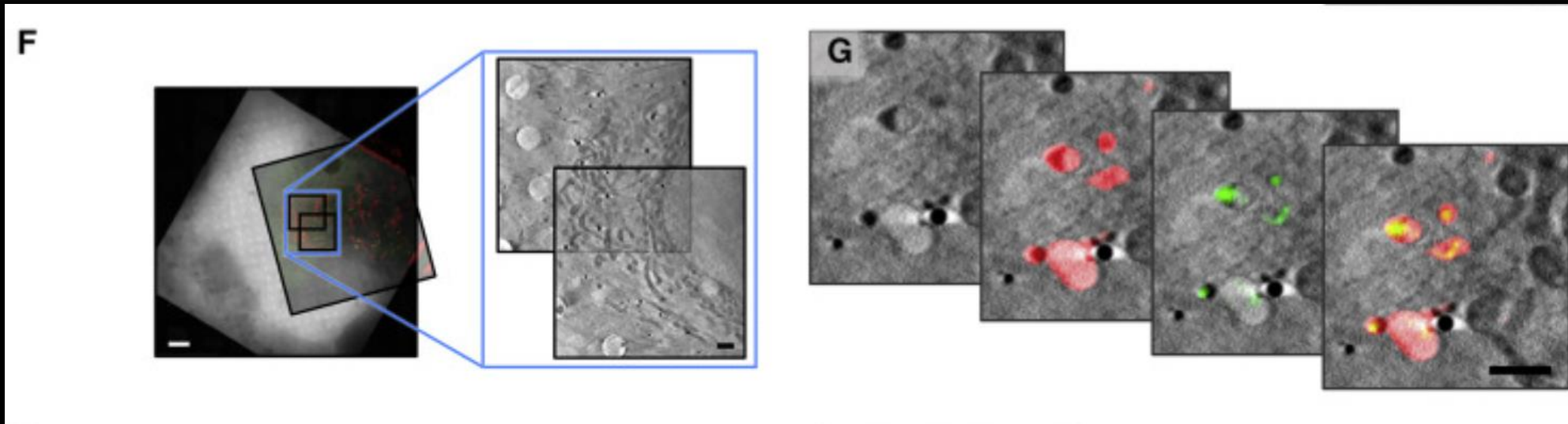
EXAMPLE FOR CRYO-SIM AND SOFT X-RAY CORRELATION WORKFLOW

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



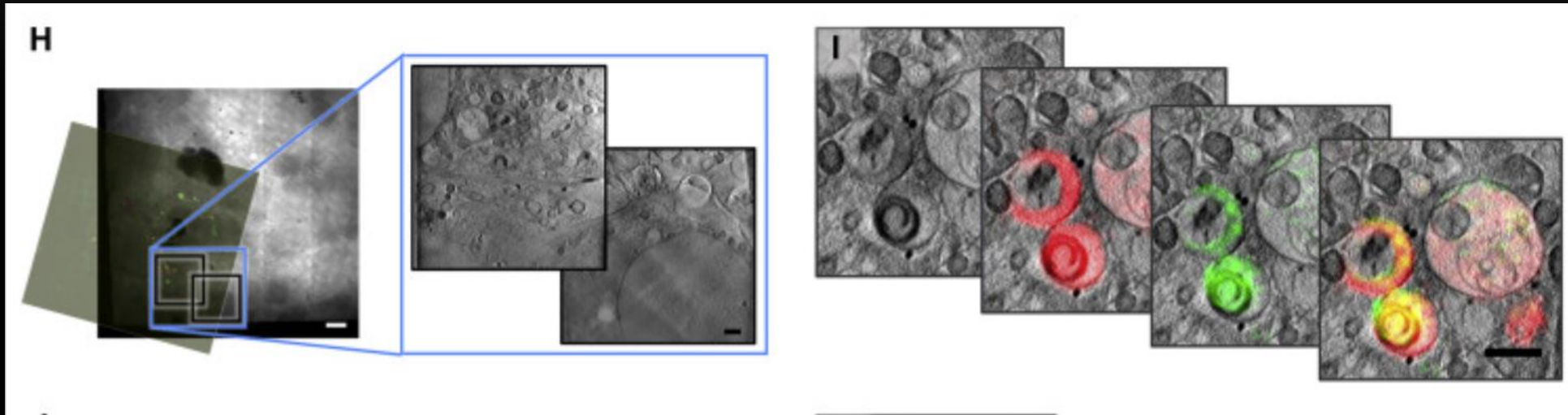
Test on mitochondria stained cells : here we could conclude that there were no deformation in the correlative imaging process: all discrepancy was in the expected range of rigid transformation



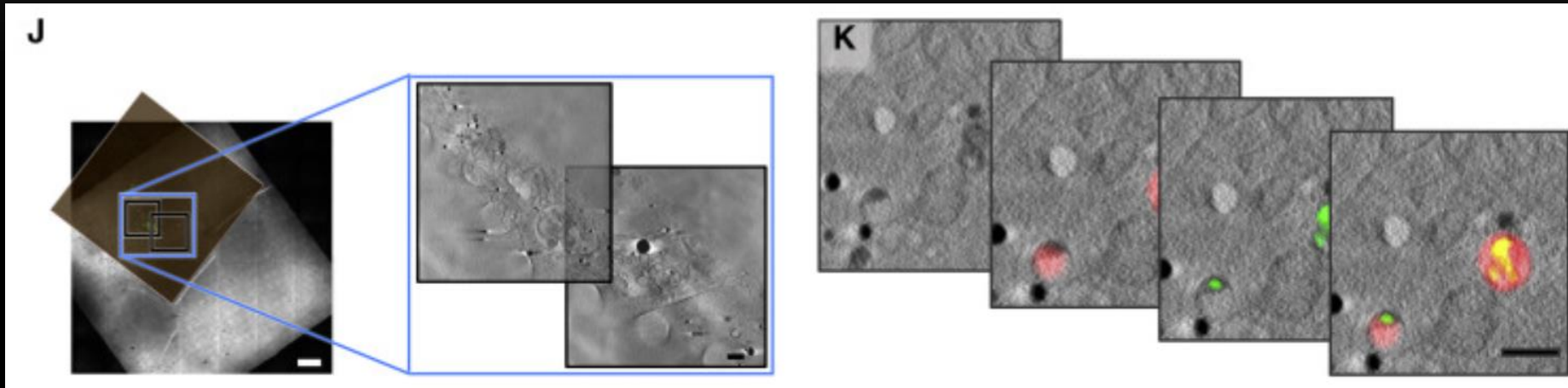


reovirus-infected U2OS cells

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



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). Carbon-rich virus-induced domed structures are also seen in single vesicles.

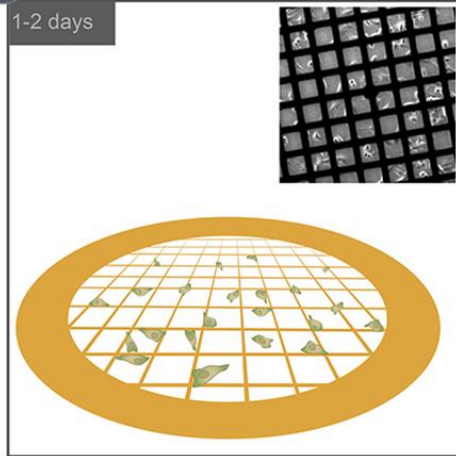


4 hours post infection Fluorescence is shown in smaller vesicles alongside large vesicle superstructures

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

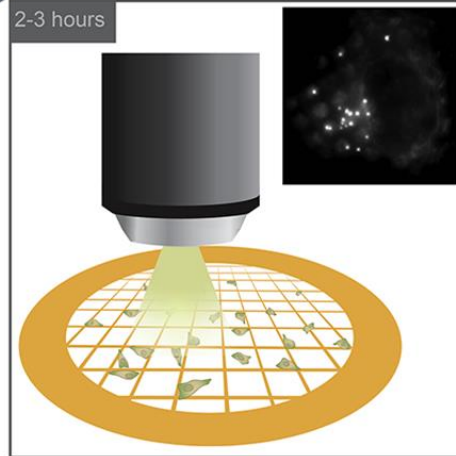
1 Cell Culture and Staining

1-2 days



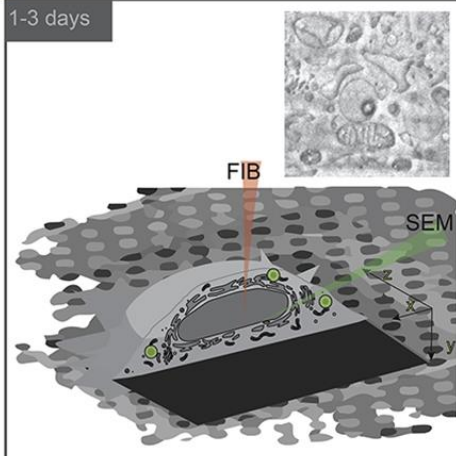
2 FM

2-3 hours



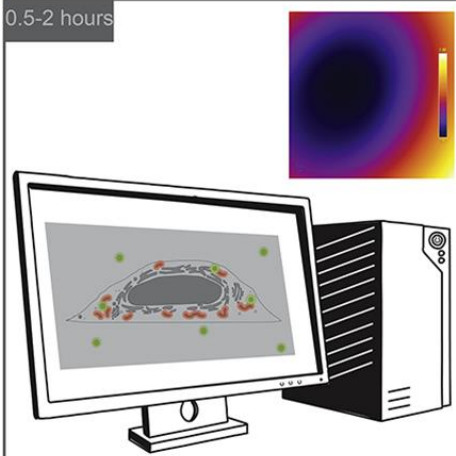
3 FIB-SEM

1-3 days



4 3D correlation

0.5-2 hours



iScience



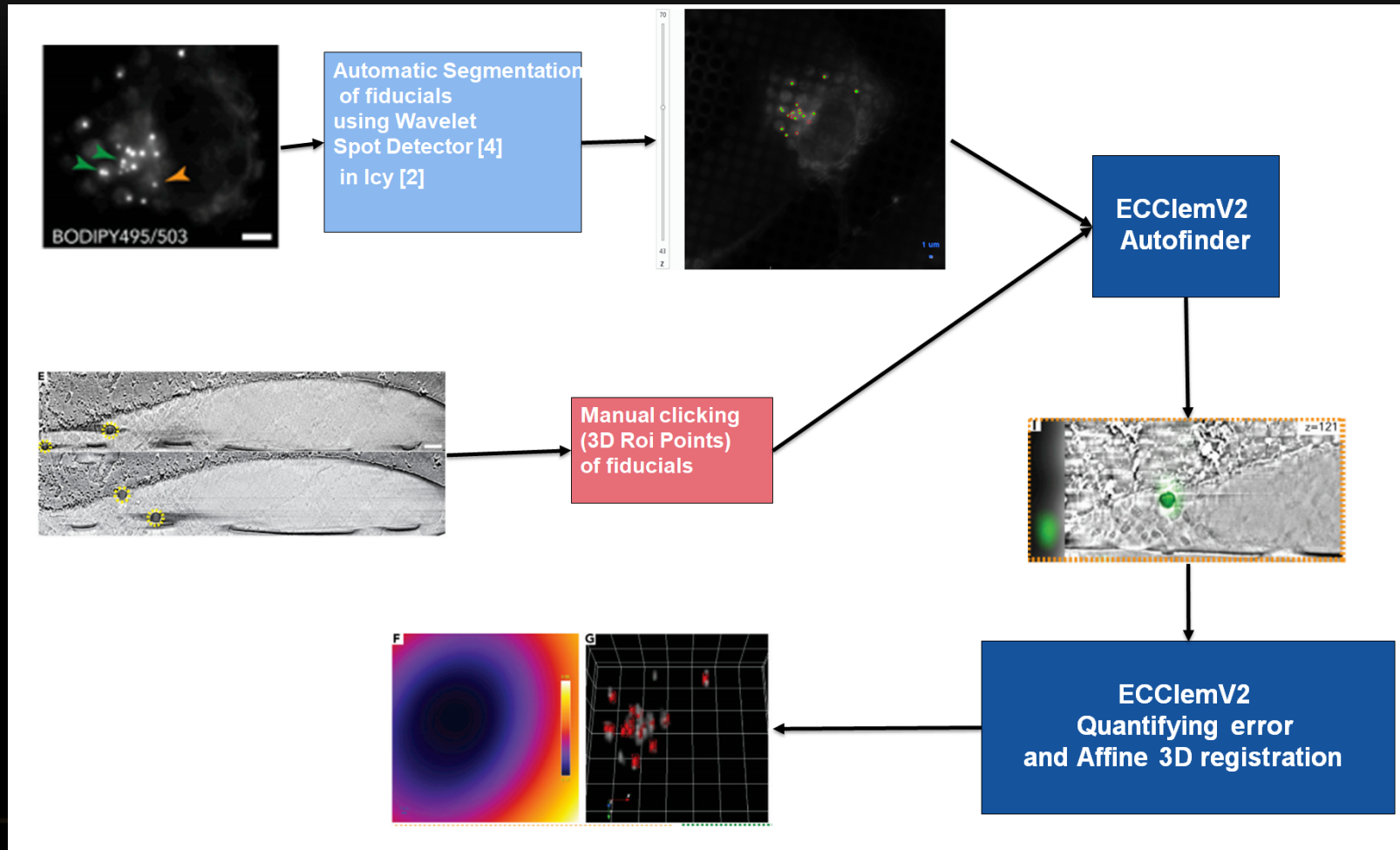
Volume 24, Issue 7, 23 July 2021, 102714

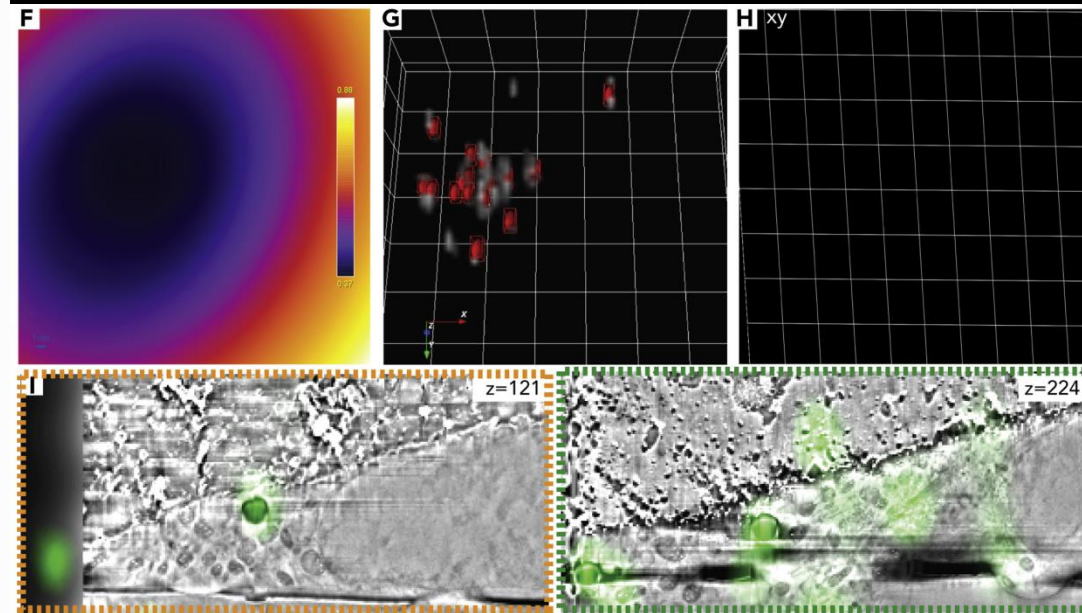
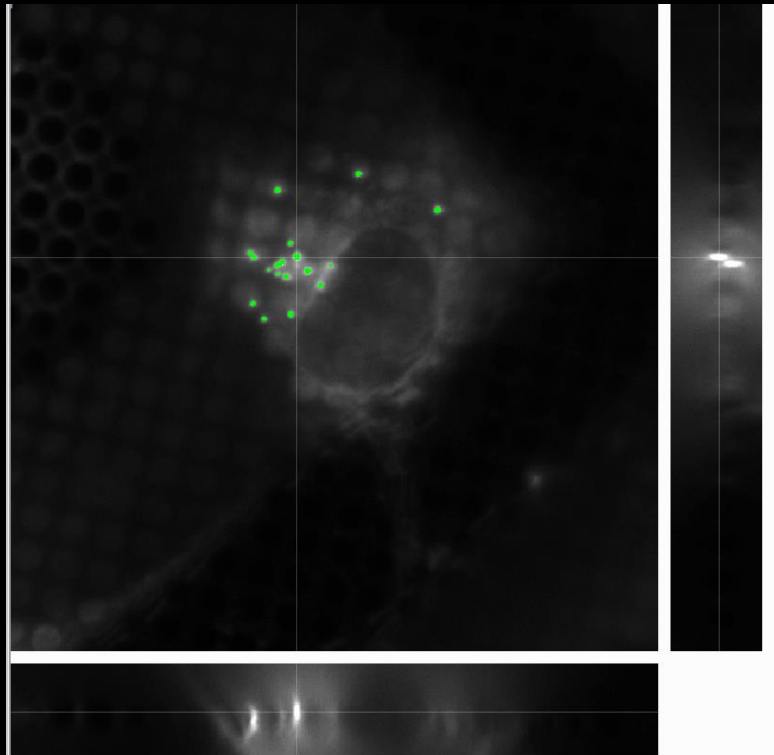
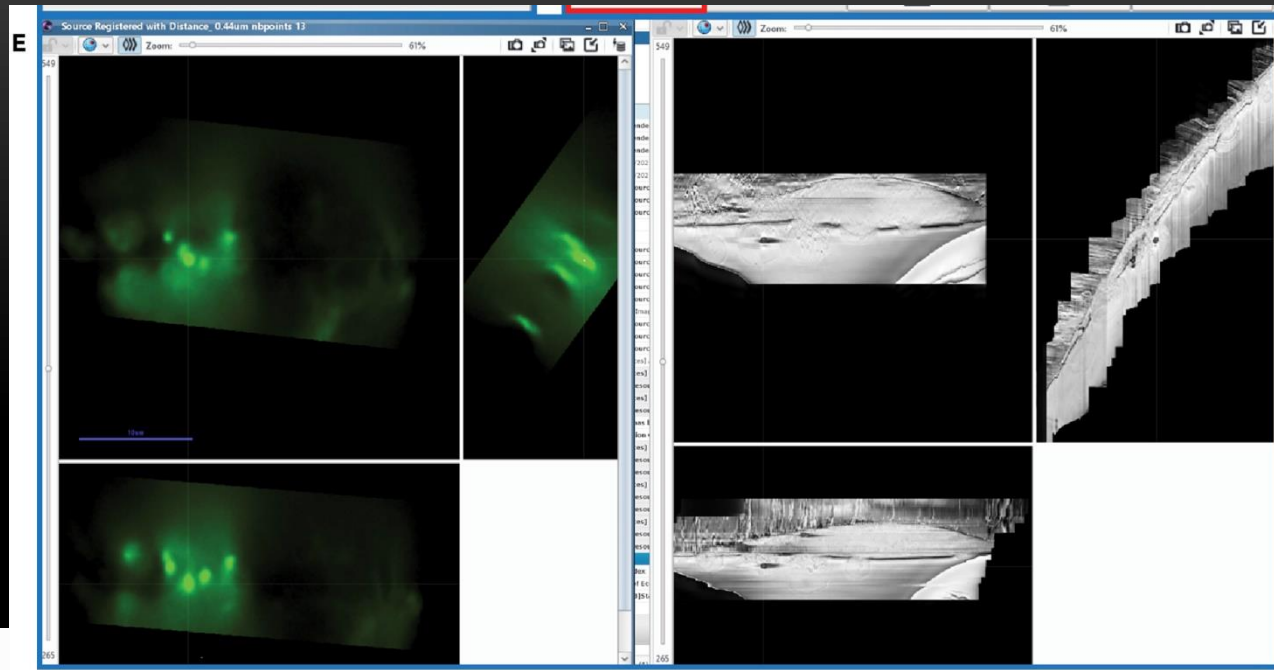
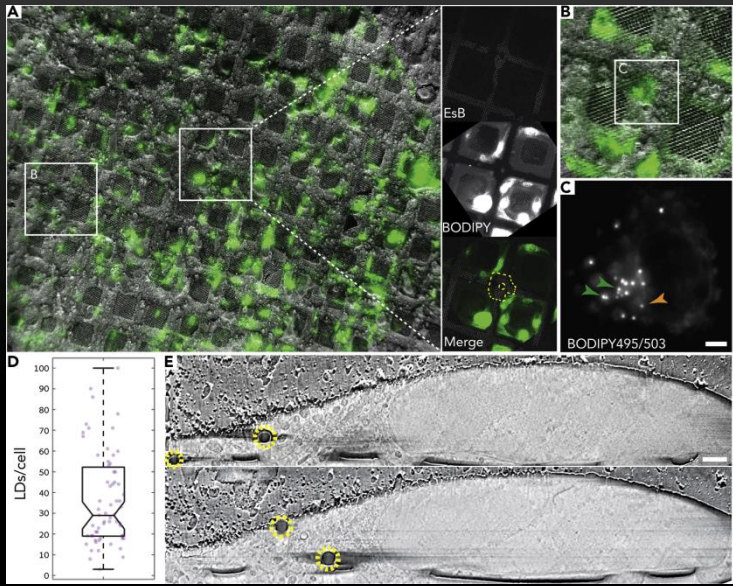
Article

In situ fiducial markers for 3D correlative cryo-fluorescence and FIB-SEM imaging

Nadav Scher¹, Katya Rechav², Perrine Paul-Gilloteaux³, Ori Avinoam^{1,4}  

EXAMPLE OF CREATED WORKFLOW: CRYO FIB SEM AND CRYO WF





STAR Protocols



Volume 3, Issue 1, 18 March 2022, 101142

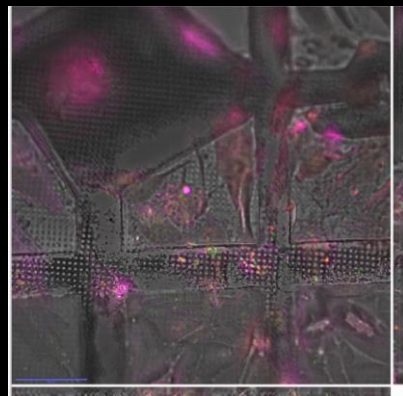
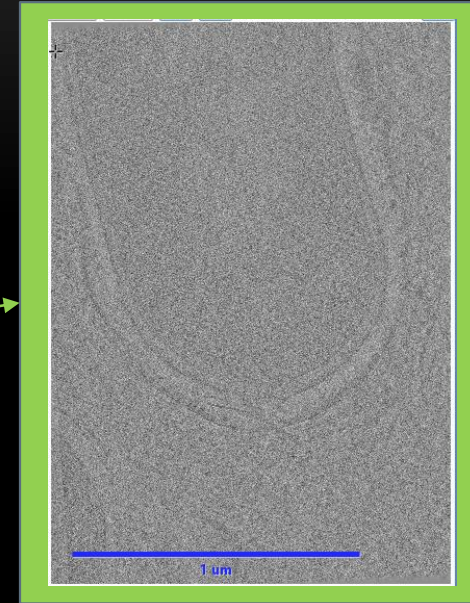
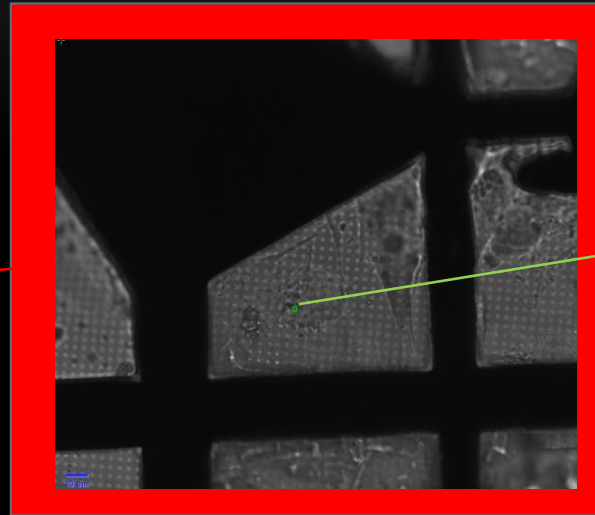
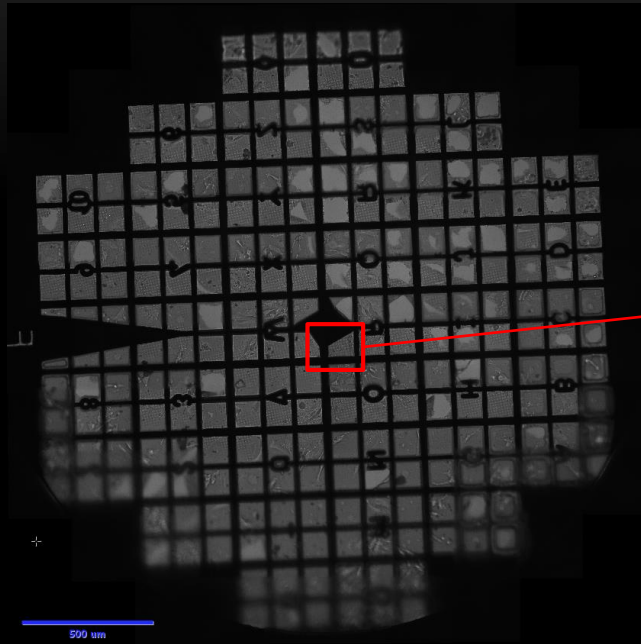
Protocol

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

Nadav Scher^{1,4}, Katva Rechav², Perrine Paul-Gilloteaux³, Ori Avinoam^{1,5}

Test affine transform:
Deformation in one axis.

IN SITU LAMELLA



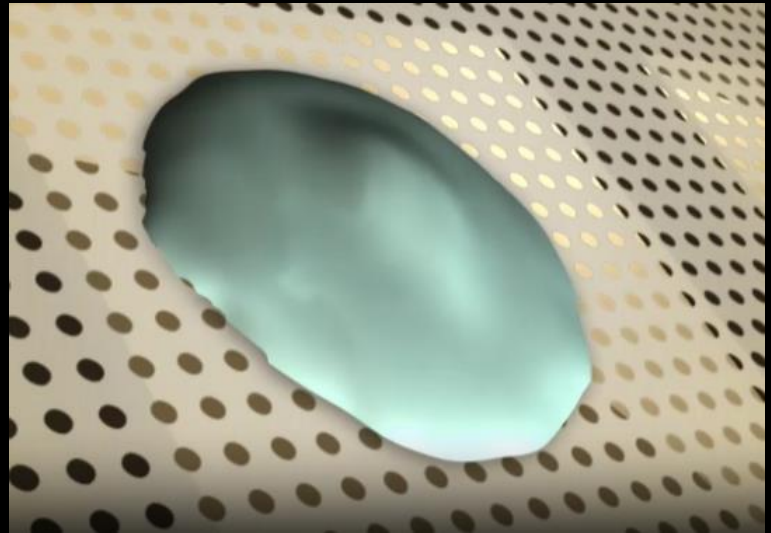
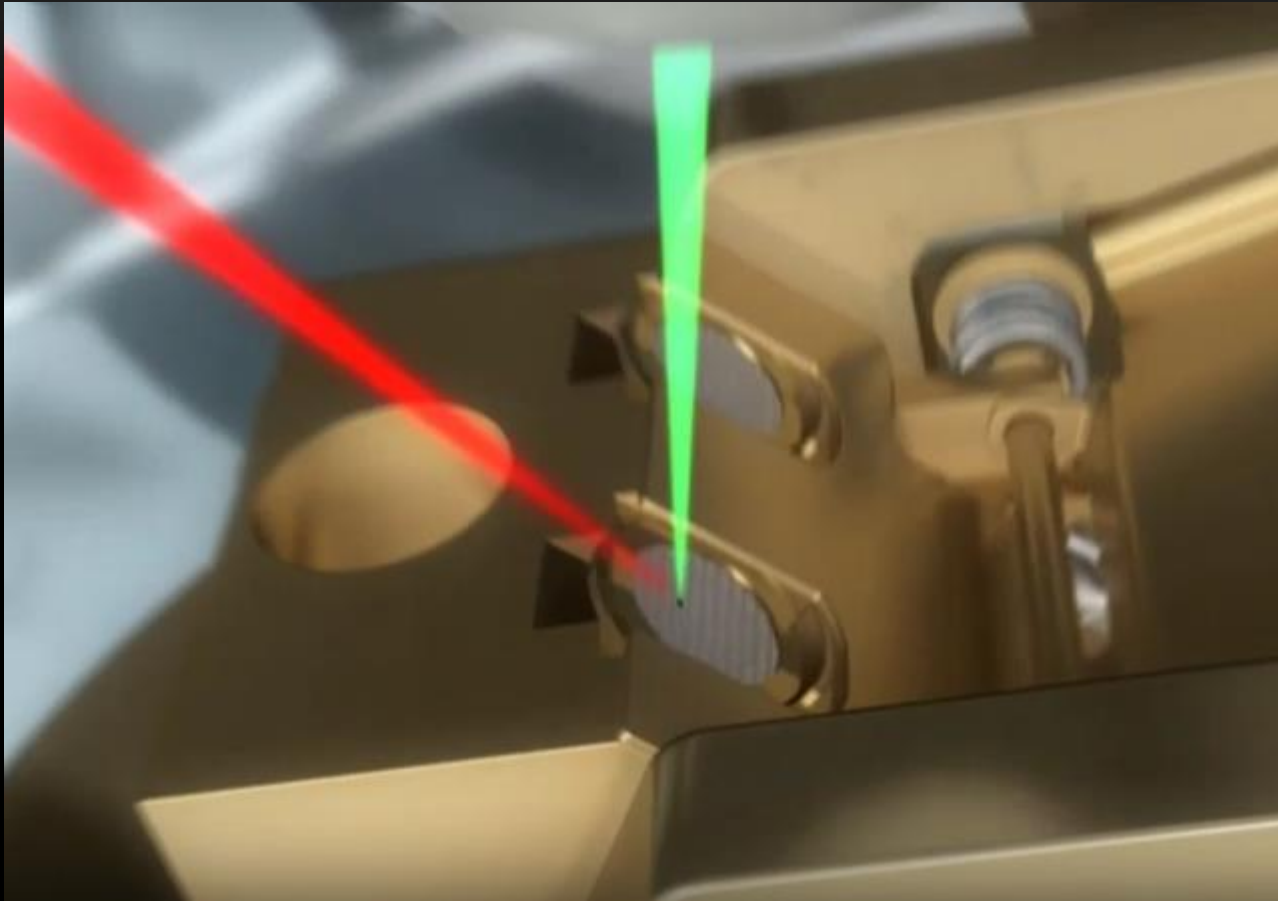
Acquisition:

Get Fluorescent Stack of area with bacteria :

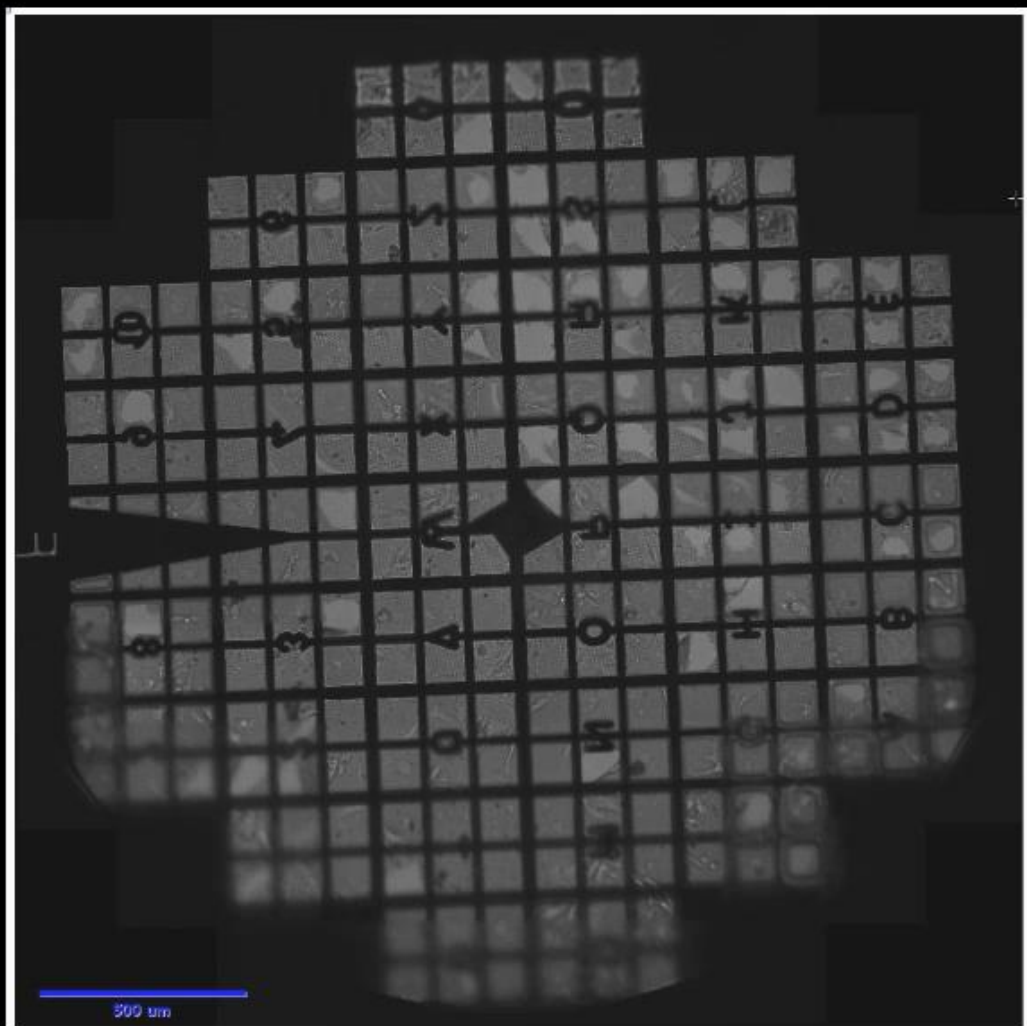
Target Area on the grid to make multiple lamella: link known

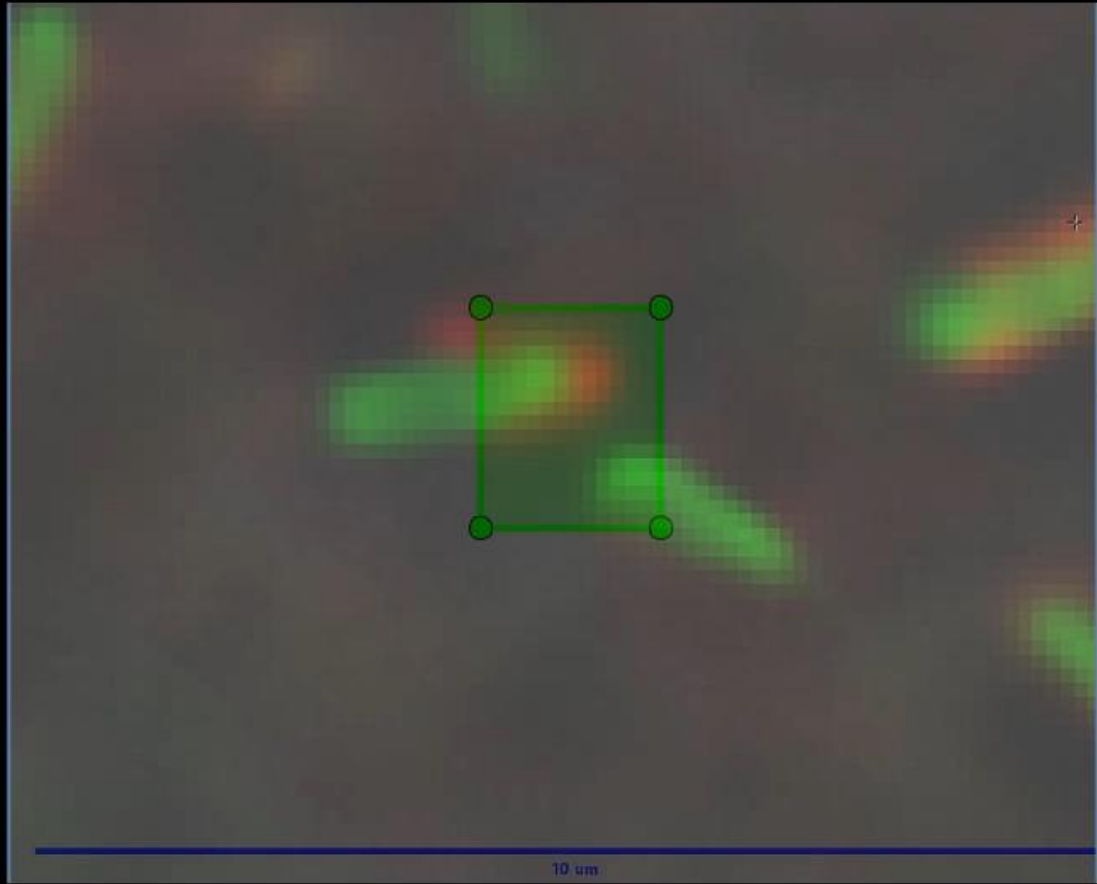
Pick all Bacteria that can be found on the lamellae for Cryo-TEM:
link lost

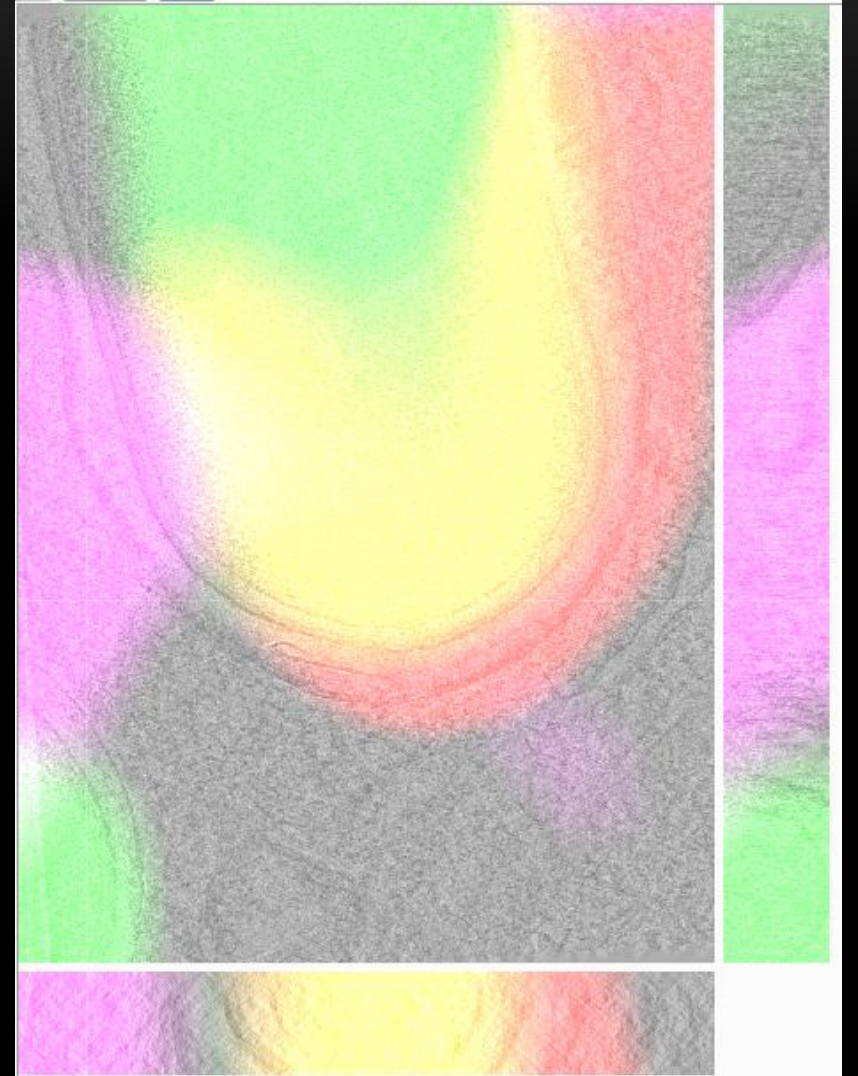
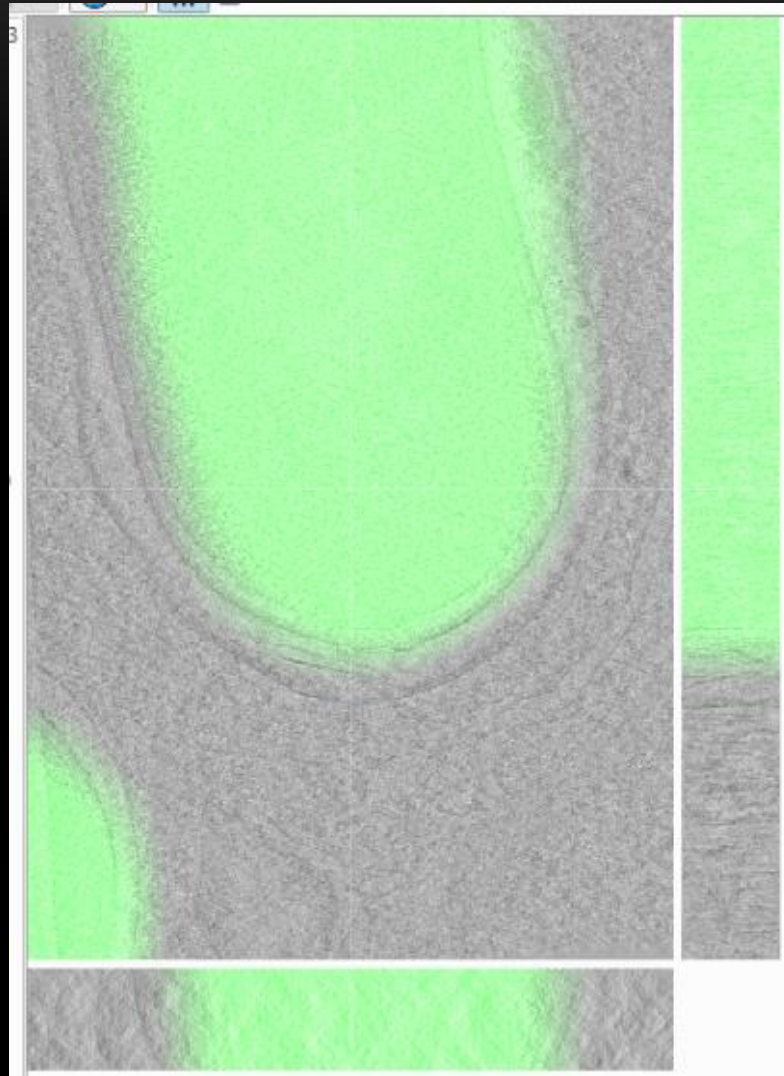
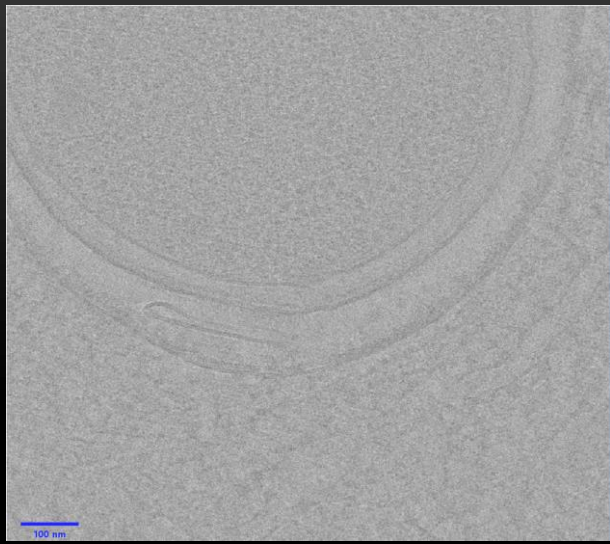
-> Need for automation of Tomograph-Fluo matching



Screenshots from ThermoFisher Ditactic Video for Cryo TEM on lamella



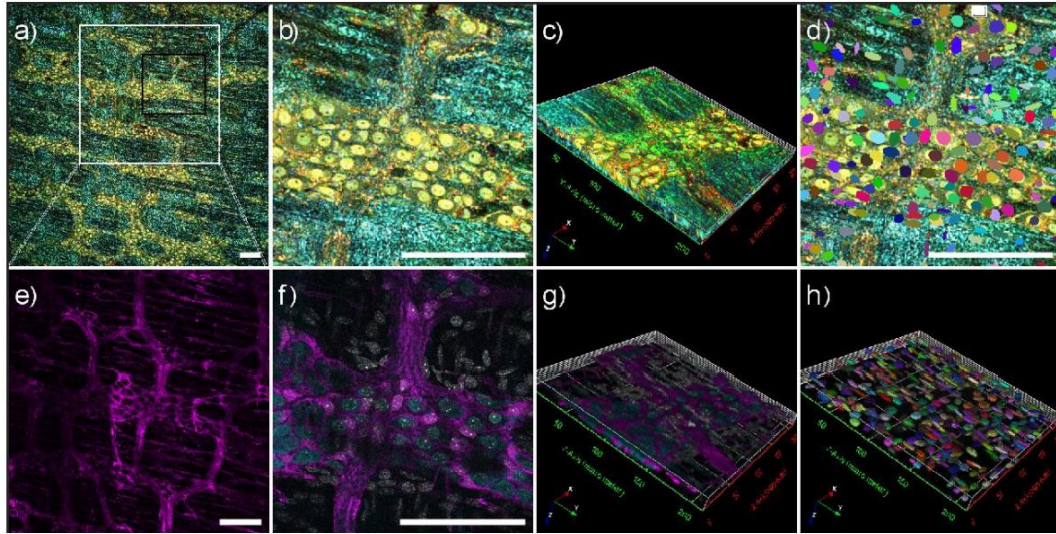




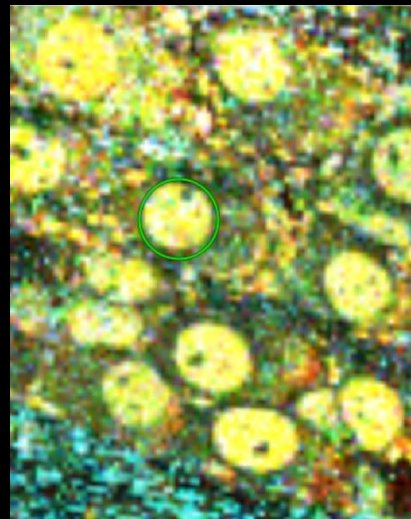
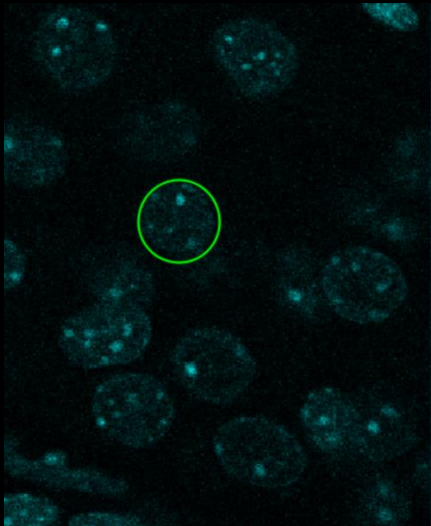
CORRELATIVE MICROSCOPY

Beyond CLEM

MORE MULTIMODAL (WHY MULTIMODAL?)



- **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](#)

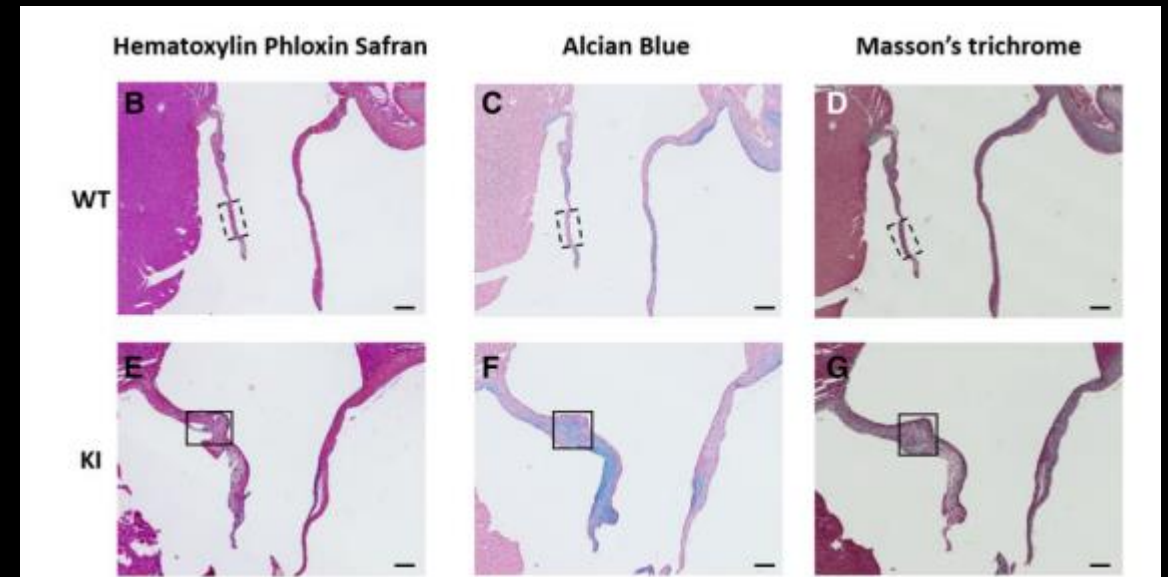
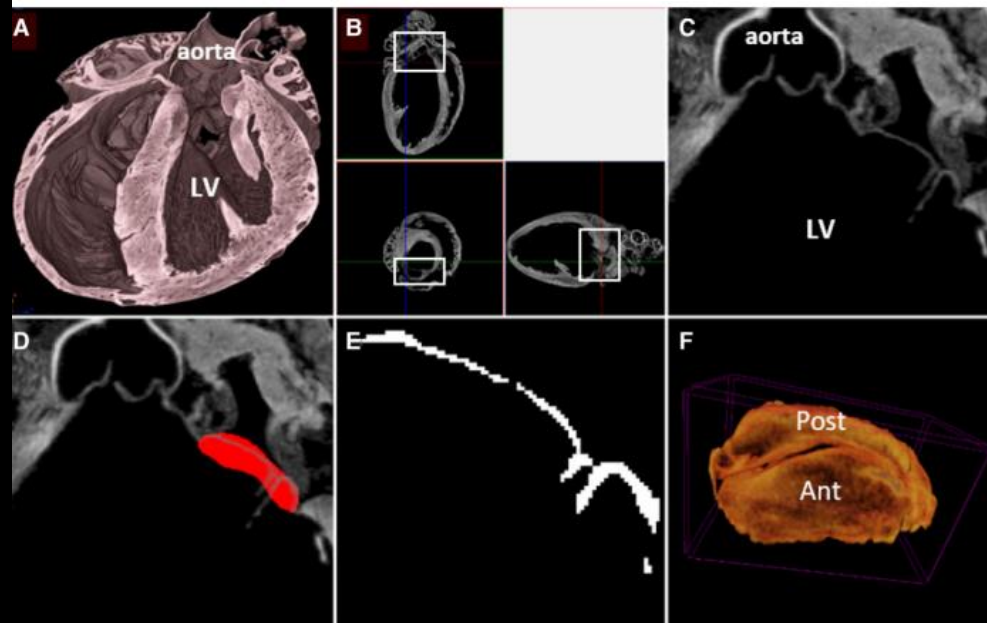
HELP TO LINK MULTIMODALITIES

Multimodality imaging and transcriptomics to phenotype mitral valve dystrophy in a unique knock-in Filamin-Arat model [Get access >](#)

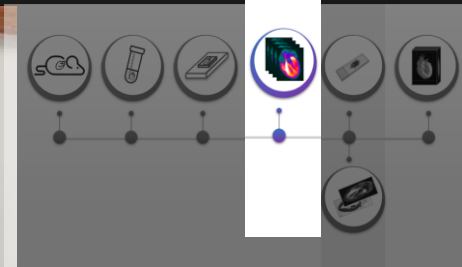
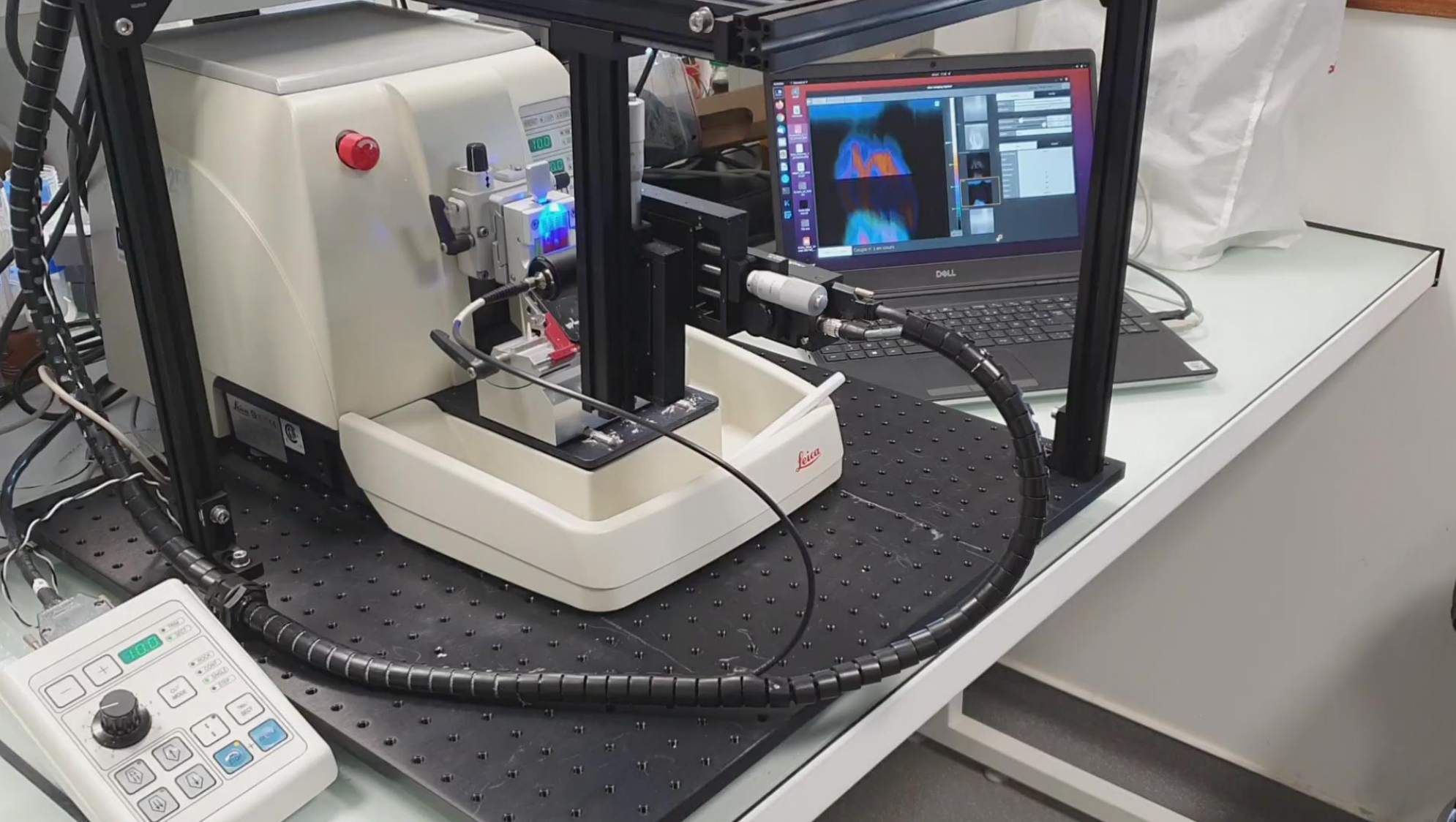
Constance Delwarde, Claire Toquet, Pascal Aumond, Amir Hossein Kayvanjoo, Adrien Foucal, Benjamin Le Vely, Manon Baudic, Benjamin Lauzier, Stéphanie Blandin, Joëlle Véziers, Perrine Paul-Gilloteaux, Simon Lecoïnte, Estelle Baron, Ilaria Massaiu, Paolo Poggio, Séverine Rémy, Ignacio Anegon, Hervé Le Marec, Laurent Monassier, Jean Jacques Schott, Elvira Mass, Julien Barc, Thierry Le Tourneau, Jean Merot ✉, Romain Capoulade ✉

Cardiovascular Research, cvac136, <https://doi.org/10.1093/cvr/cvac136>

Published: 24 August 2022 [Article history](#) ▾



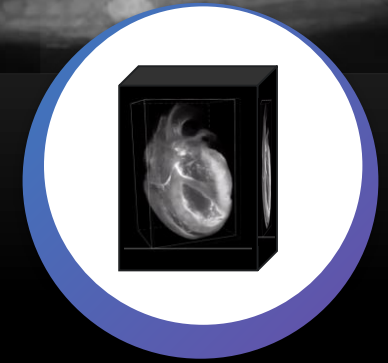
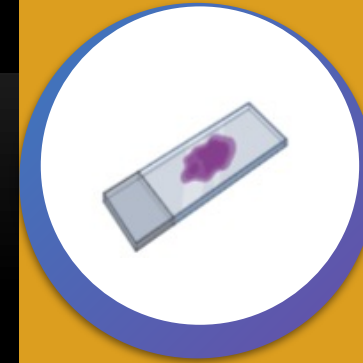
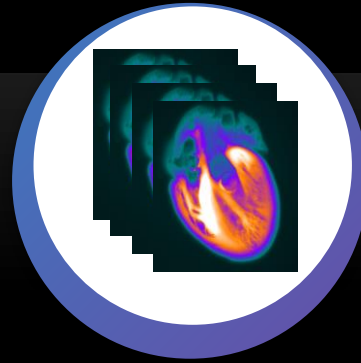
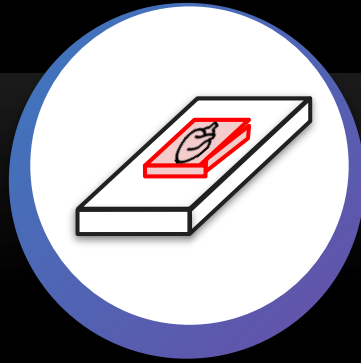
Kratoscope: Serial sectioning and imaging



KAER LABS



Serial block face imaging : principle



1

Harvesting

2

Fixation
Dehydration

3

Paraffin
embedding

4

Kratoscope
serial sectioning
and imaging

4b

OPTIONAL



Correlative
image analysis

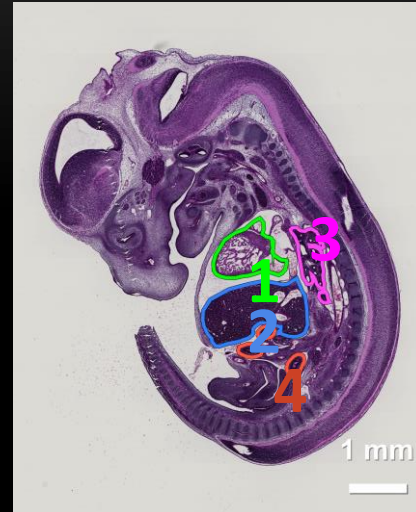
5

Image
analysis and
3D
rendering

Applications: Correlative imaging



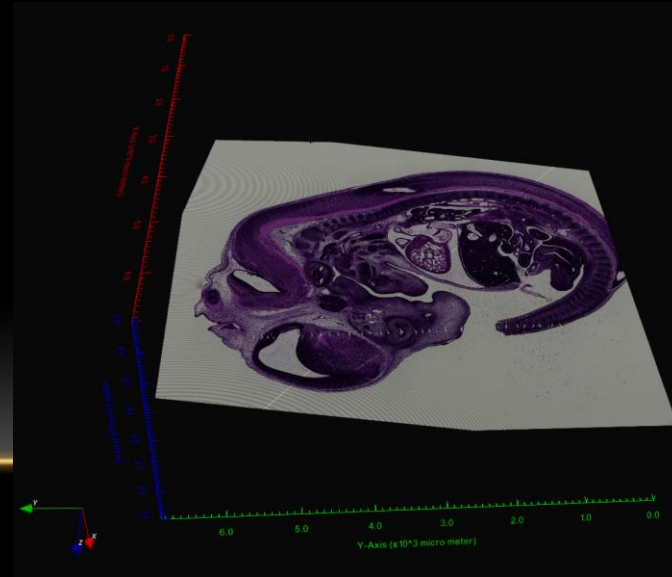
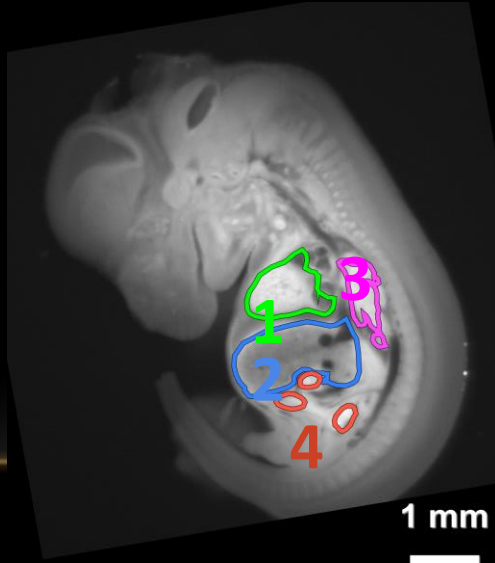
3D reconstruction from serial block face imaging



HPS staining on collected sections and segmentation of main organs
1/Heart, 2/Liver, 3/Lung, 4/Gut

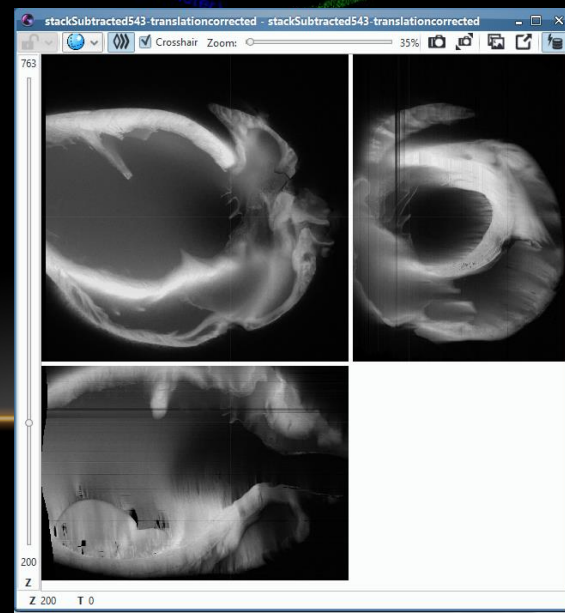
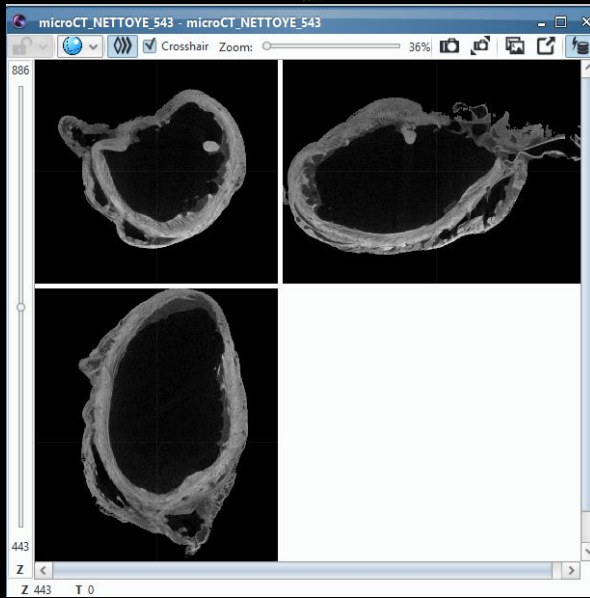
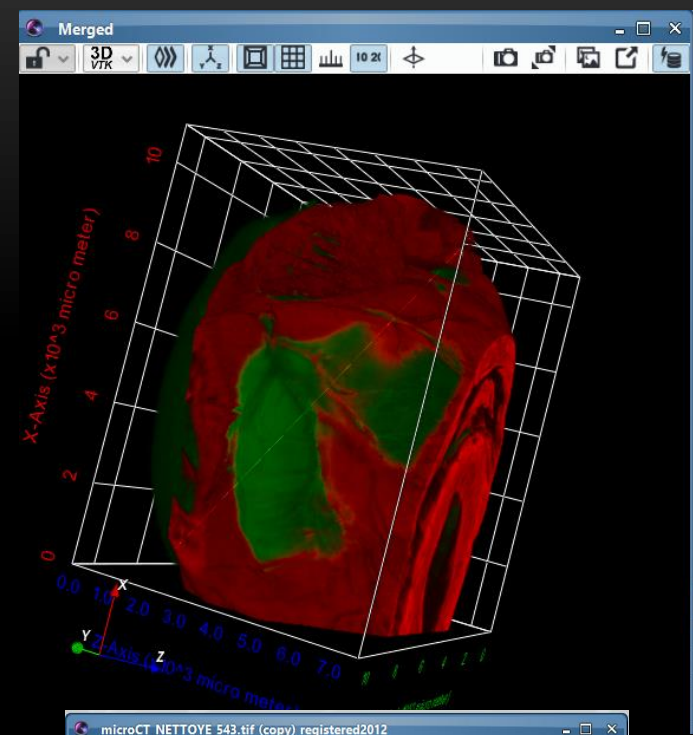
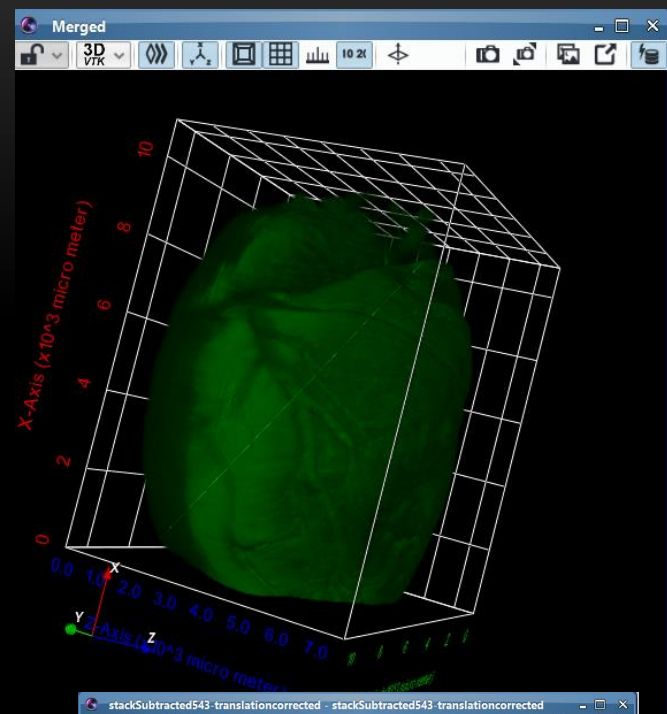
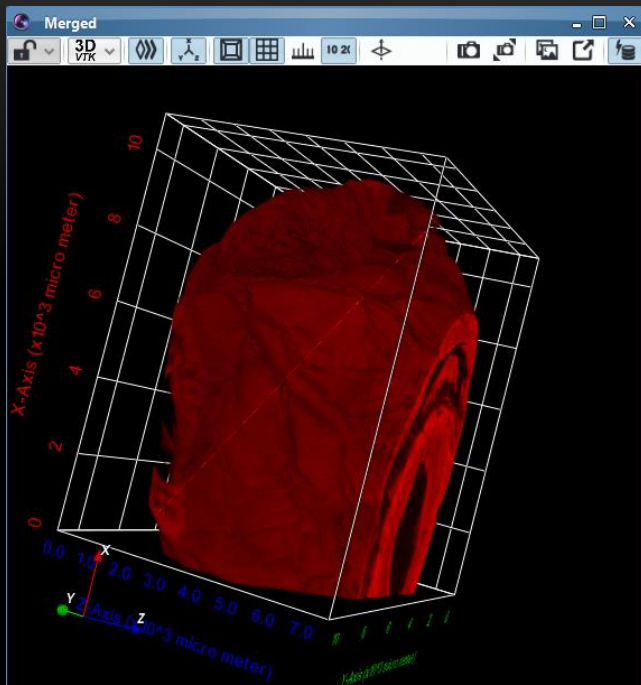


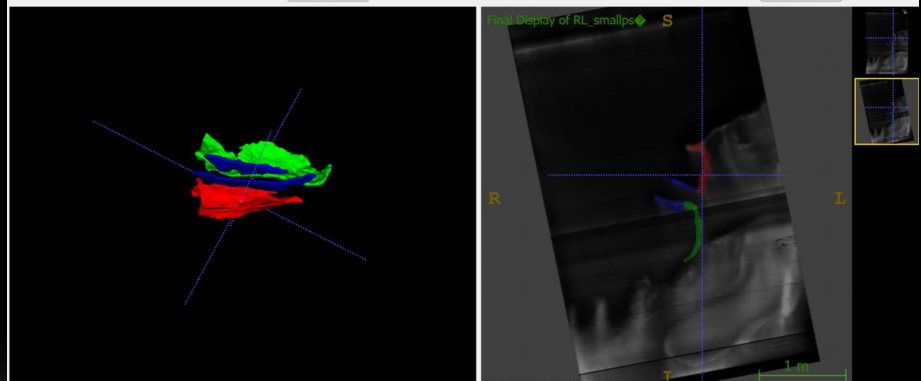
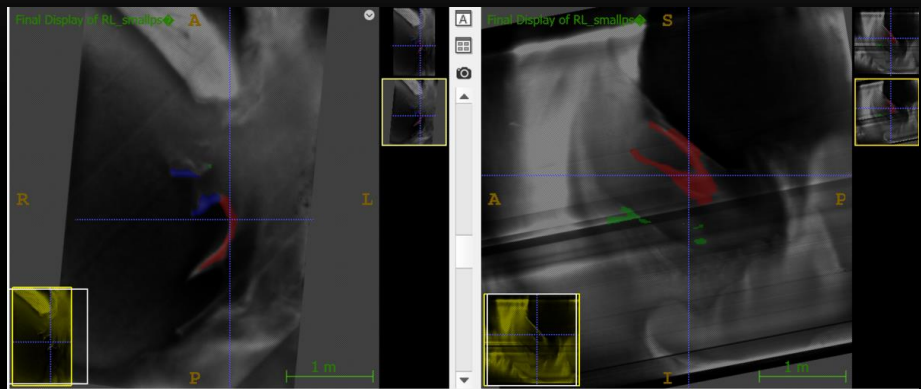
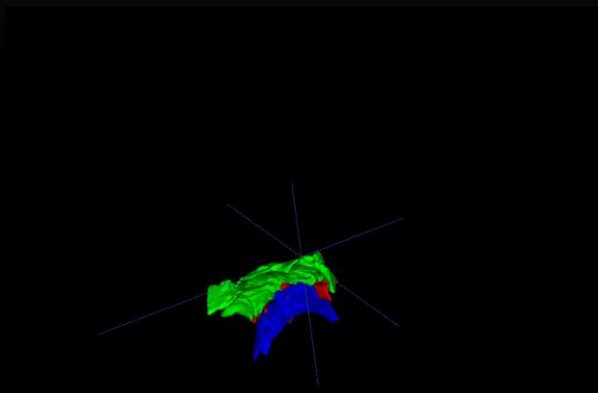
Segmentation correlated to the corresponding slice of the serial block face imaging

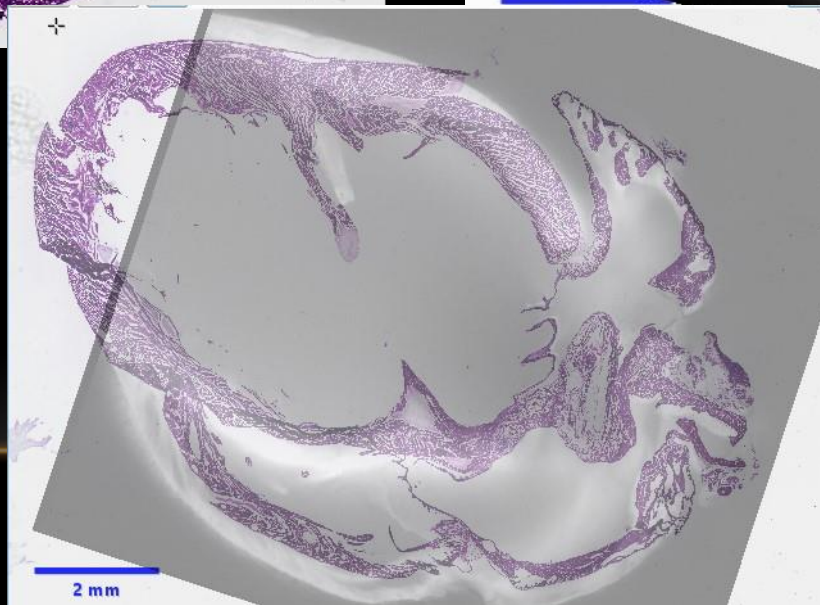
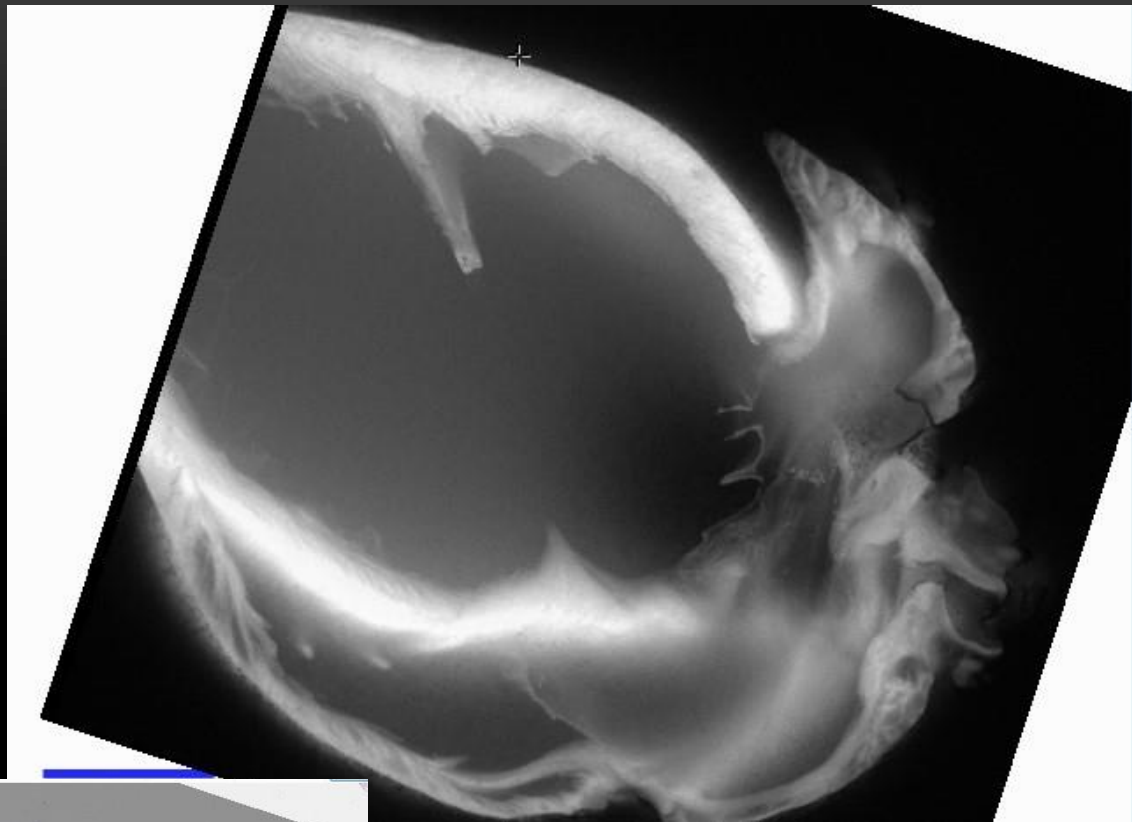


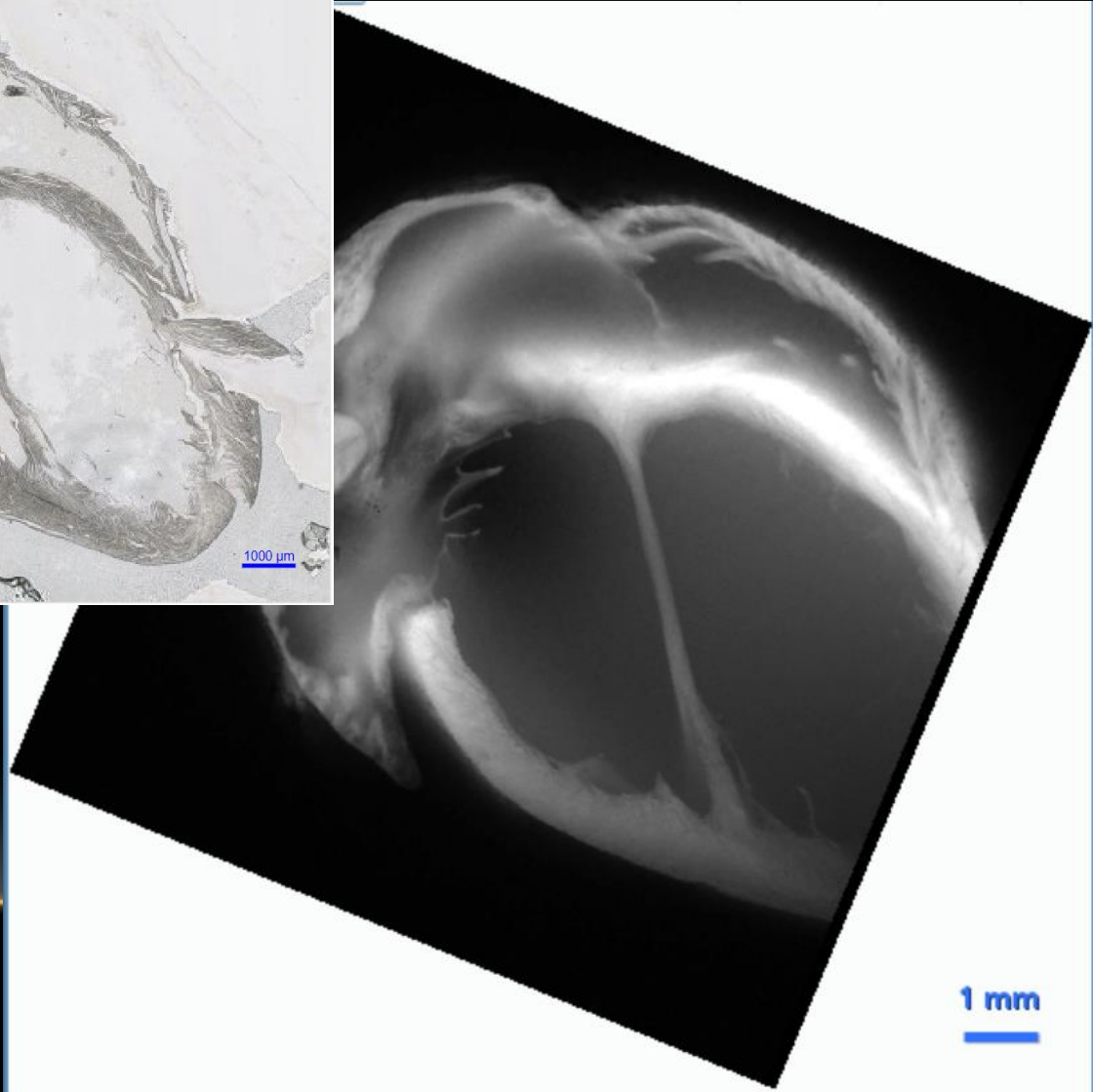
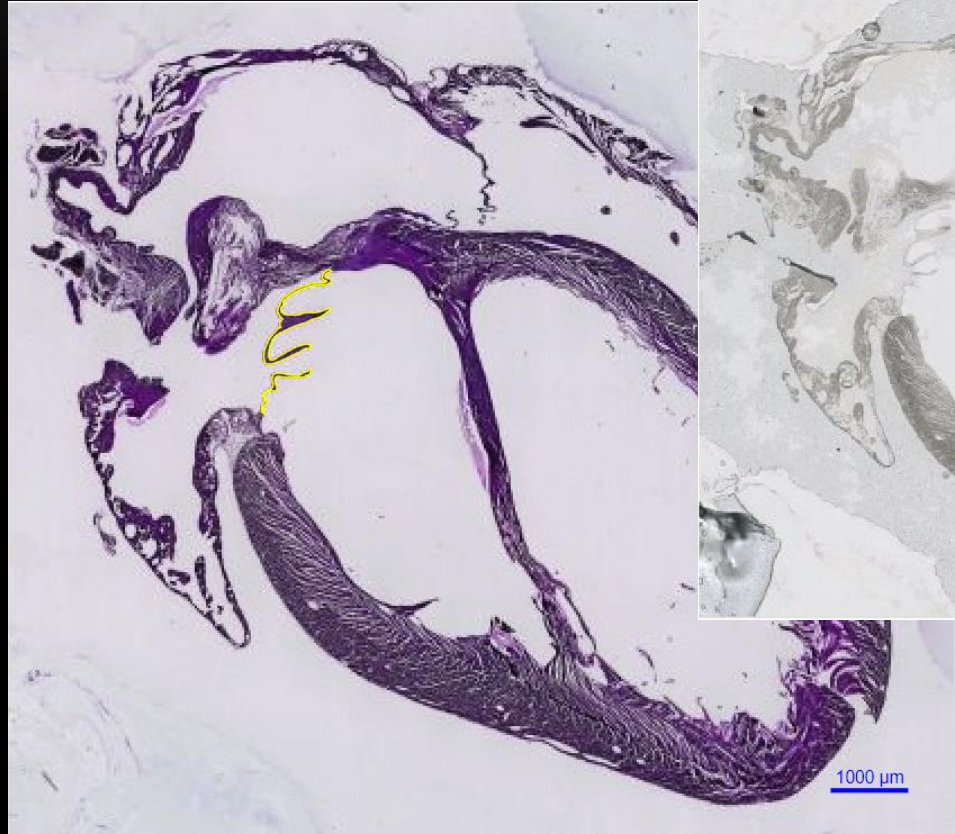
2D section registered in the 3D volume and 3D segmentation of the heart was performed (ICY)
Heart volume : 0.746 mm³.





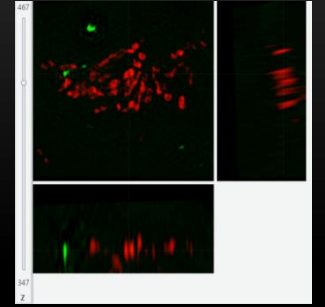
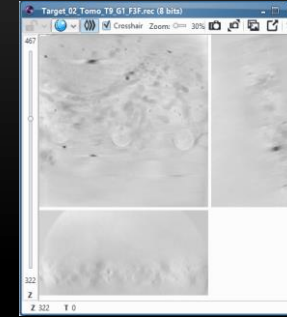
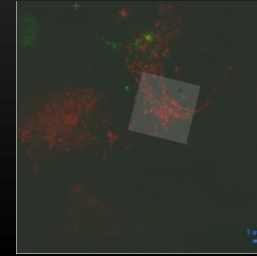






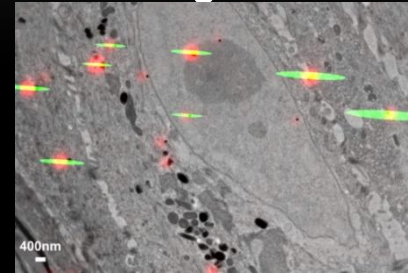
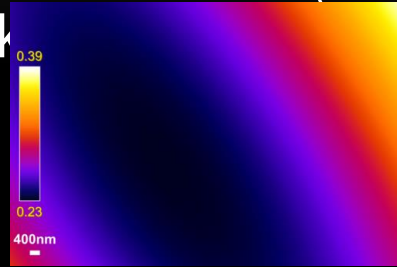
CONCLUSION

- Registration allow to link multimodal data through a transformation, which can be cascaded
- Example of applications in
 - Cryo Xray and cryo SIM
 - Cryo FIB SEM and cryo WF
 - In situ Cryo Tem of lamellae
 - FFOCT and Confocal
 - Histology and MicroCT

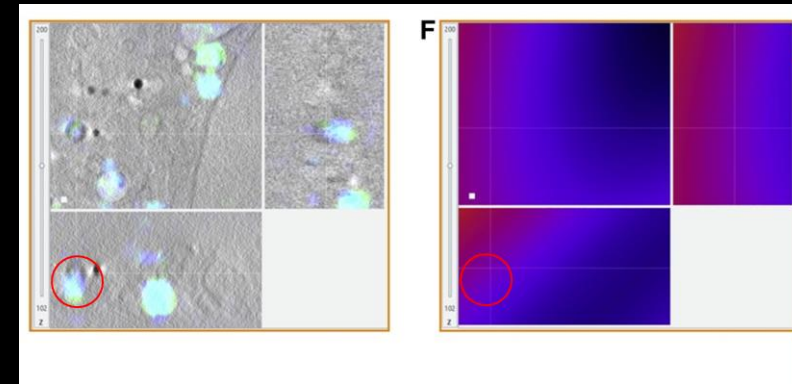


CONCLUSION

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



- This has multiple purposes:
 - Target area accuracy (Adding error margin for Example)
 - Multimodal colocalisation
 - Model selection (to be deformed or not to be deformed)





ANR-18-CE45-0015

Acknowledgements

EC-CLEM error estimation

ITX Nantes

Guillaume Potier

Stephan Kunne

Laboratoire de Mathématiques J. Leray

Frédéric Lavancier

Institut Pasteur

Lea Swistak

Jost Enninga

Anna Sartori

Marvin Albert

EC-CLEM workflows

Weizmann Institute

Nadav Scher

Katya Rechav

Ori Avinoam

Diamond Light Source

Maria Harkiolaki

Nina Vyas

All team

L'Institut du Thorax Nantes

Jean Mérot

Pascal Aumond

Constance Delwarde

Romain Capoulade

Laetitia Rialland

MicroPICell

Anabelle Justin

Aurélie Doméné

Stéphanie Blandin

Philippe Hulin

Magalie Feyeux

Steven Nedellec

Anne Gaignerie

Marianne Guisset

Héloïse Duluc

Kaerlabs

Perrine de Villemagne

Pierre-Alix Dancer

Guillaume Schmid

Paul Dorval



K A E R L A B S

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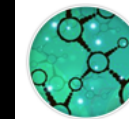
Philippe Naveilhan

Tony Durand

Michel Neunlist



Structure Fédérative
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François Bonamy



FRANCE-BIOIMAGING