

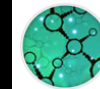
DEVELOPMENT OF DATA CORRELATION SOFTWARE FOR CLXT

Example of 3d correlative cryo-structured illumination fluorescence and soft x-ray microscopy to elucidate reovirus intracellular release pathway

Perrine.paul-gilloteaux@univ-nantes.fr



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



FRANCE-BIOIMAGING

TWO IMAGING SYSTEMS FOR CRYO-FIXED CELL IMAGING

Correlative cryo-imaging at beamline B24 of the UK synchrotron, Diamond Light Source.

Step 1:
Cells grown on a flat grid and captured in vitreous ice at a near native state



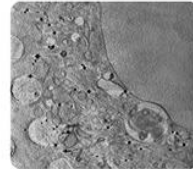
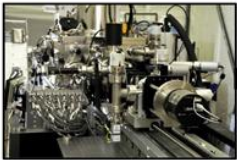
Step 2:
Conventional imaging and sample mapping

**Fluorescence imaging:
cryoSIM**



Structured Illumination

**X-ray absorption imaging:
cryoSXT**



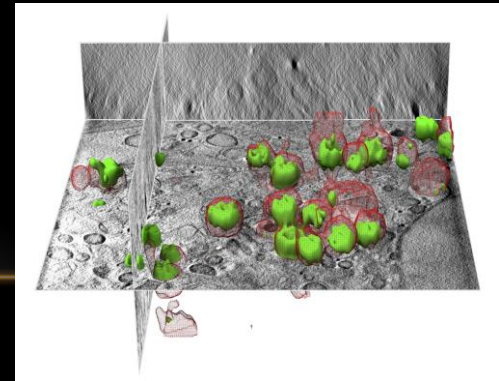
Soft X-ray Tomography

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

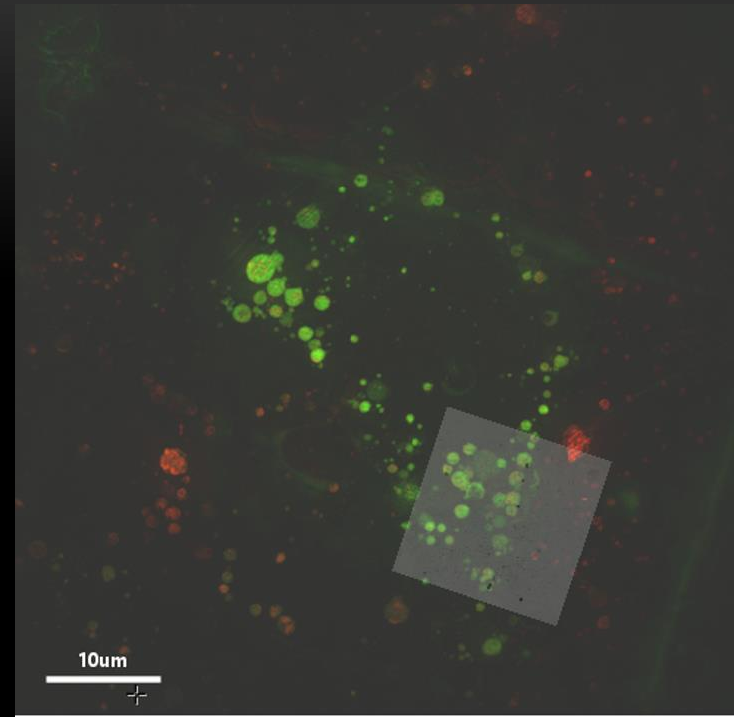
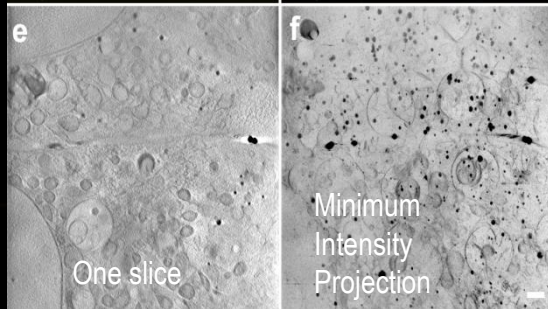
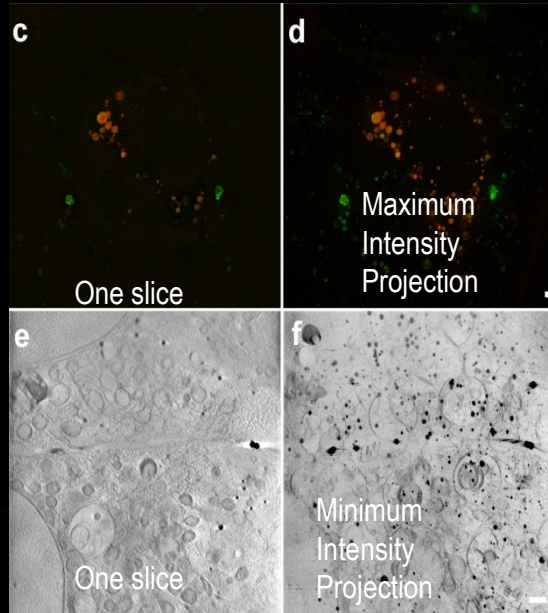
Ilias Kounatidis, Megan L. Stanifer, Michael A. Phillips, Perrine Paul-Gilloteaux, Xavier Heiligenstein, Hongchang Wang, Chidinma A. Okolo, Thomas M. Fish, Matthew C. Spink, David I. Stuart, Ilan Davis, Steeve Boulant, Jonathan M. Grimes, Ian M. Dobbie, Maria Harkiolaki

Cell

DOI: 10.1016/j.cell.2020.05.051

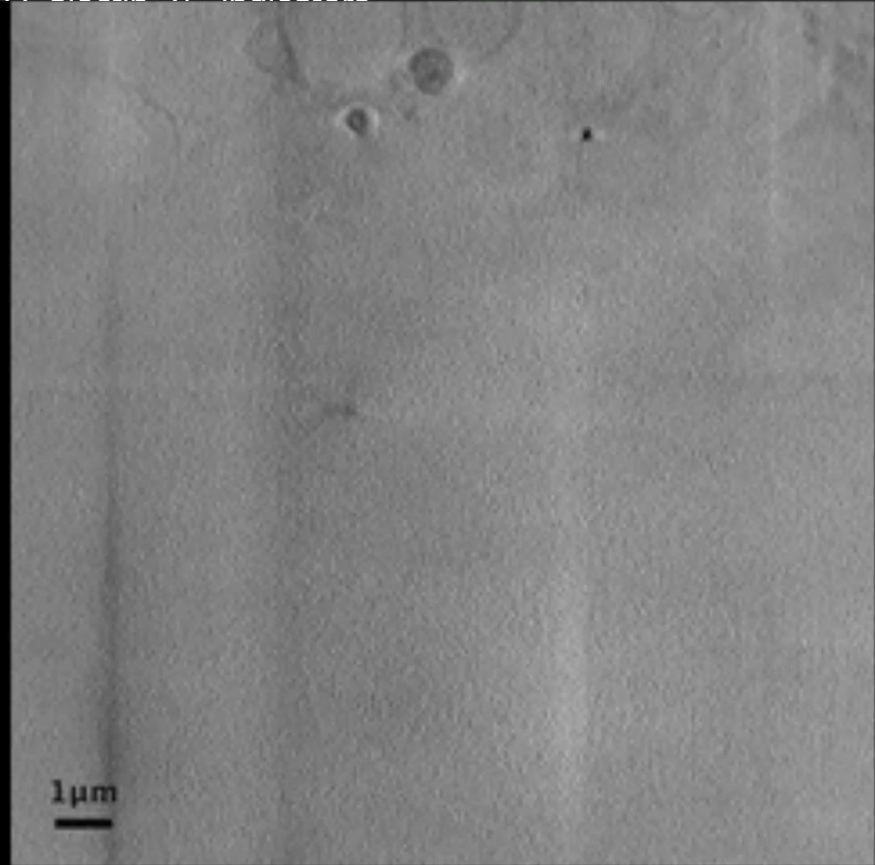


- U2OS cells stably expressing red fluorescence (mCherry-Galectin-3) , indicators of endosomal membrane disruption
- infected by a fluorescent reovirus green fluorescence (Alexa647-reovirus)
- sample vitrification: frozen in liquid nitrogen-cooled liquid ethane



- U2OS cells stably expressing red fluorescent (mCherry-Galactin 2) indicators

- o
- ir
- fl
- s
- c
- c

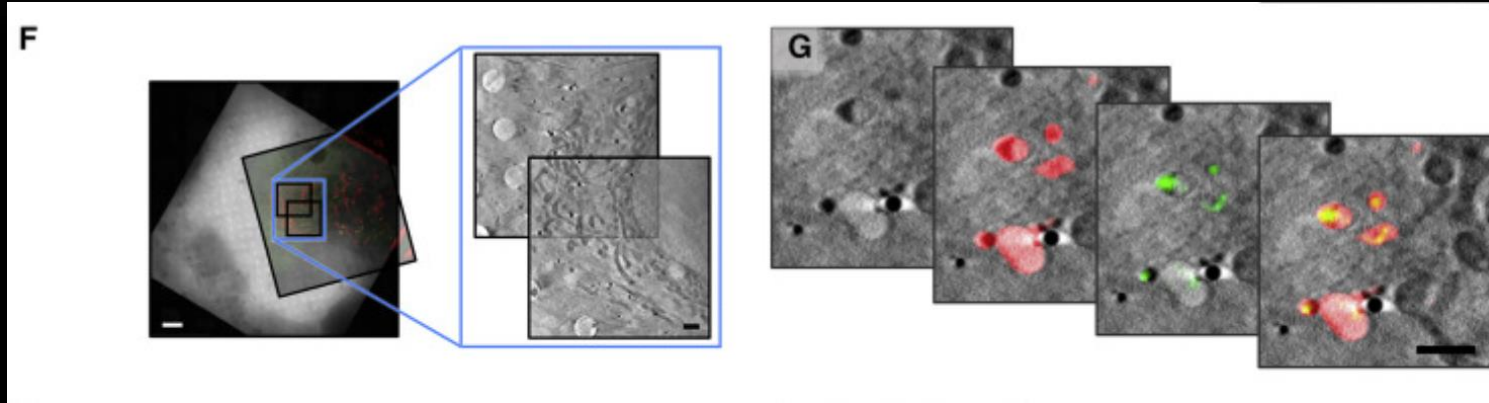


c
e

One slice Intensity Projection



CORRELATION RESULTS

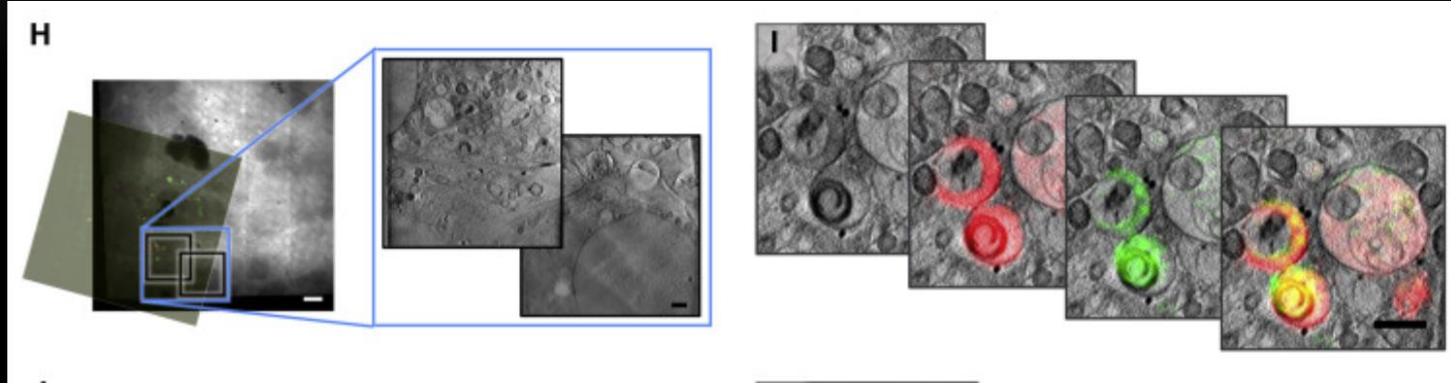


Reovirus (RV)-infected U2OS cells

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

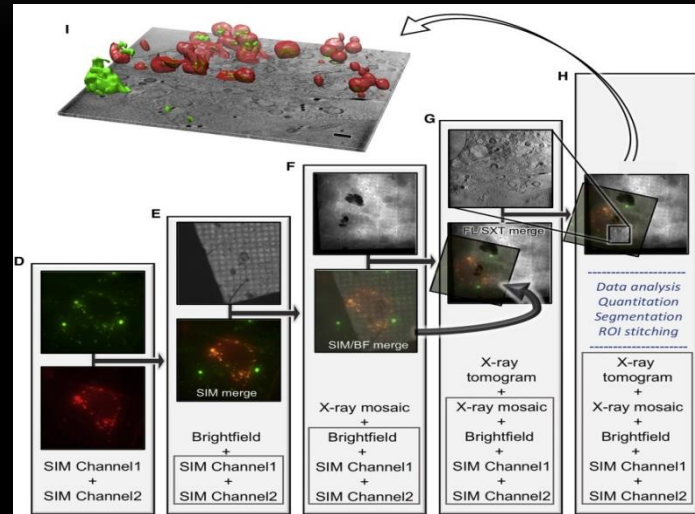
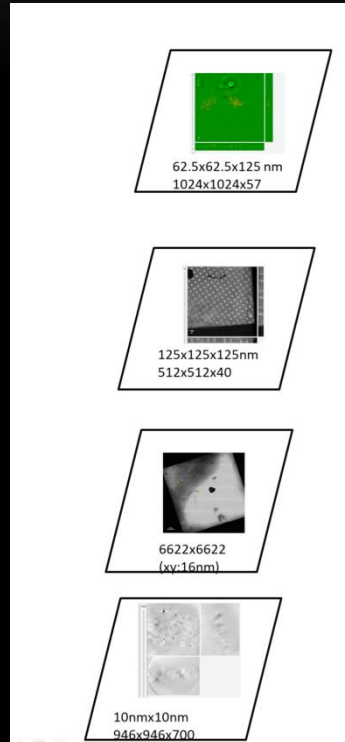
Black bars are 1 μm and white bars 10 μm .

CORRELATION RESULTS

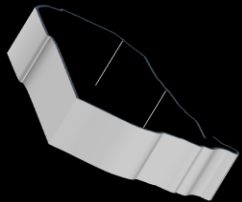


2h post infection : smaller endosomes have now merged to give rise to MVBs that have distinct compartments infused with Gal3 while others have not been similarly compromised (presumably arising from concatenation of membranes from MRV-free vesicles).

HOW WAS THE REGISTRATION PERFORMED?

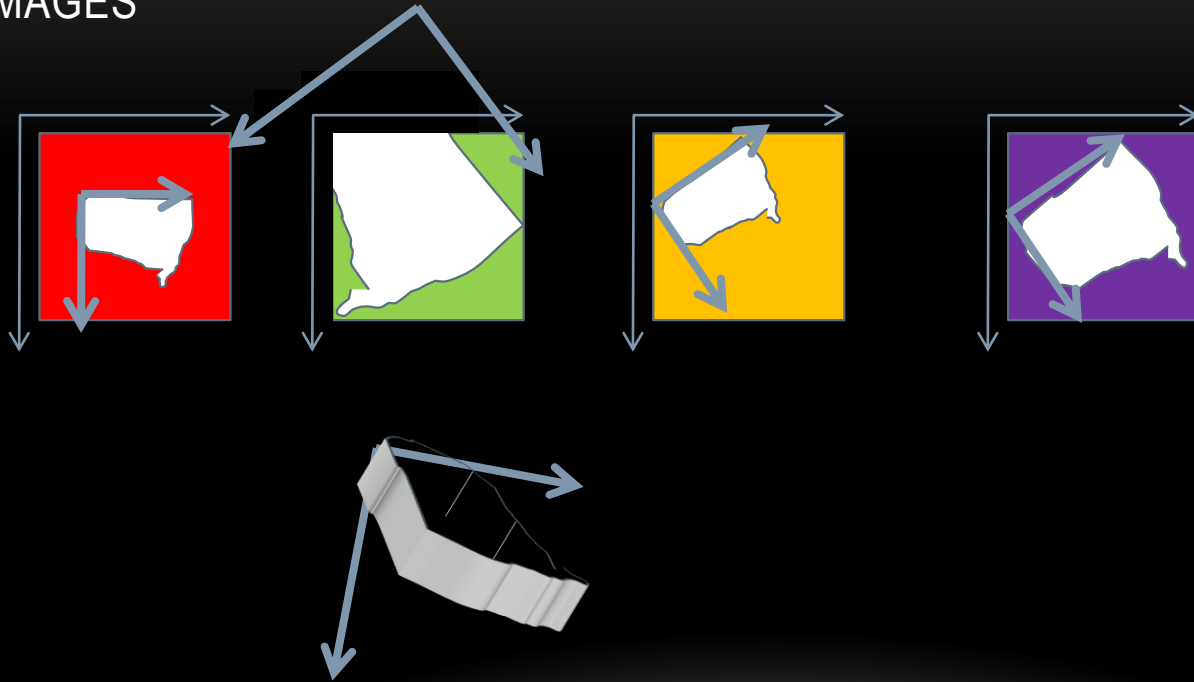


SAMPLE TO BE TRACKED FROM ONE IMAGING MODALITY TO ANOTHER

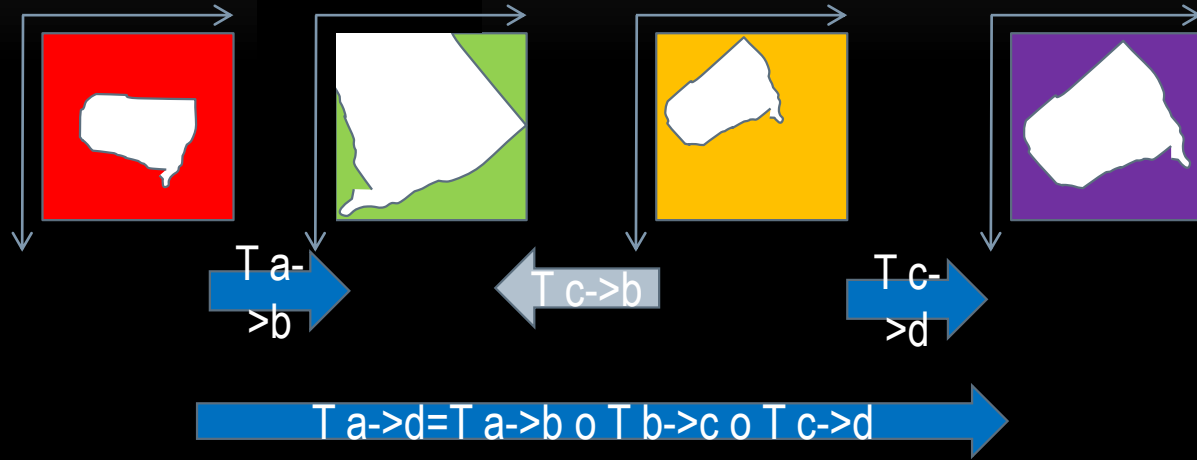


Sometimes additional modalities
will help

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



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

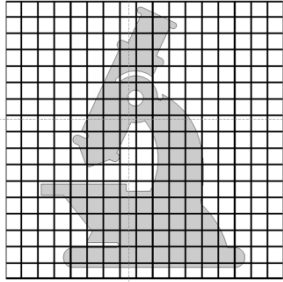


model of transformation

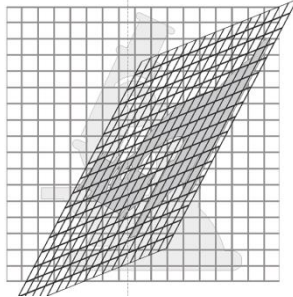
May the sample/specimen transfer/preparation have induced deformation? Is the scale relationship known (pixel size)?

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

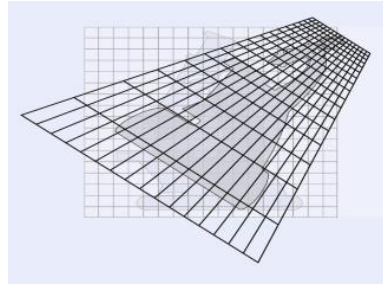
Identity



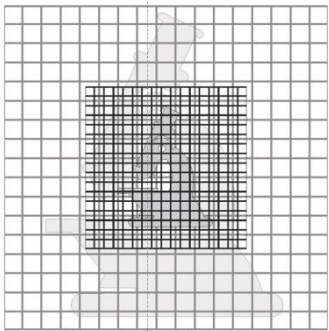
Affine



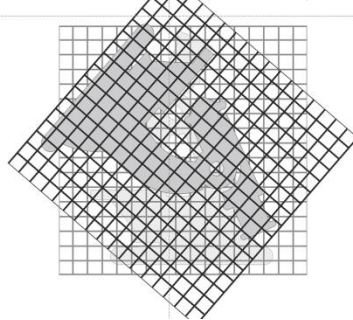
Projection



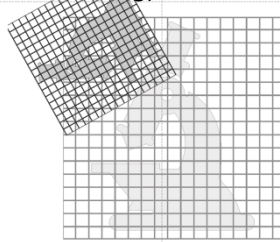
Scaling



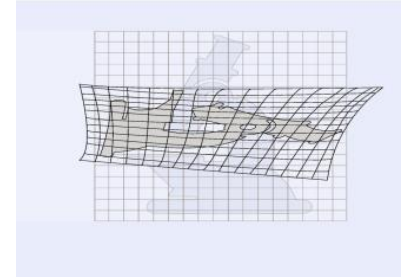
Rotation (rigid
=translation + rotation)



Similarity (= rigid
+scaling)



Splines



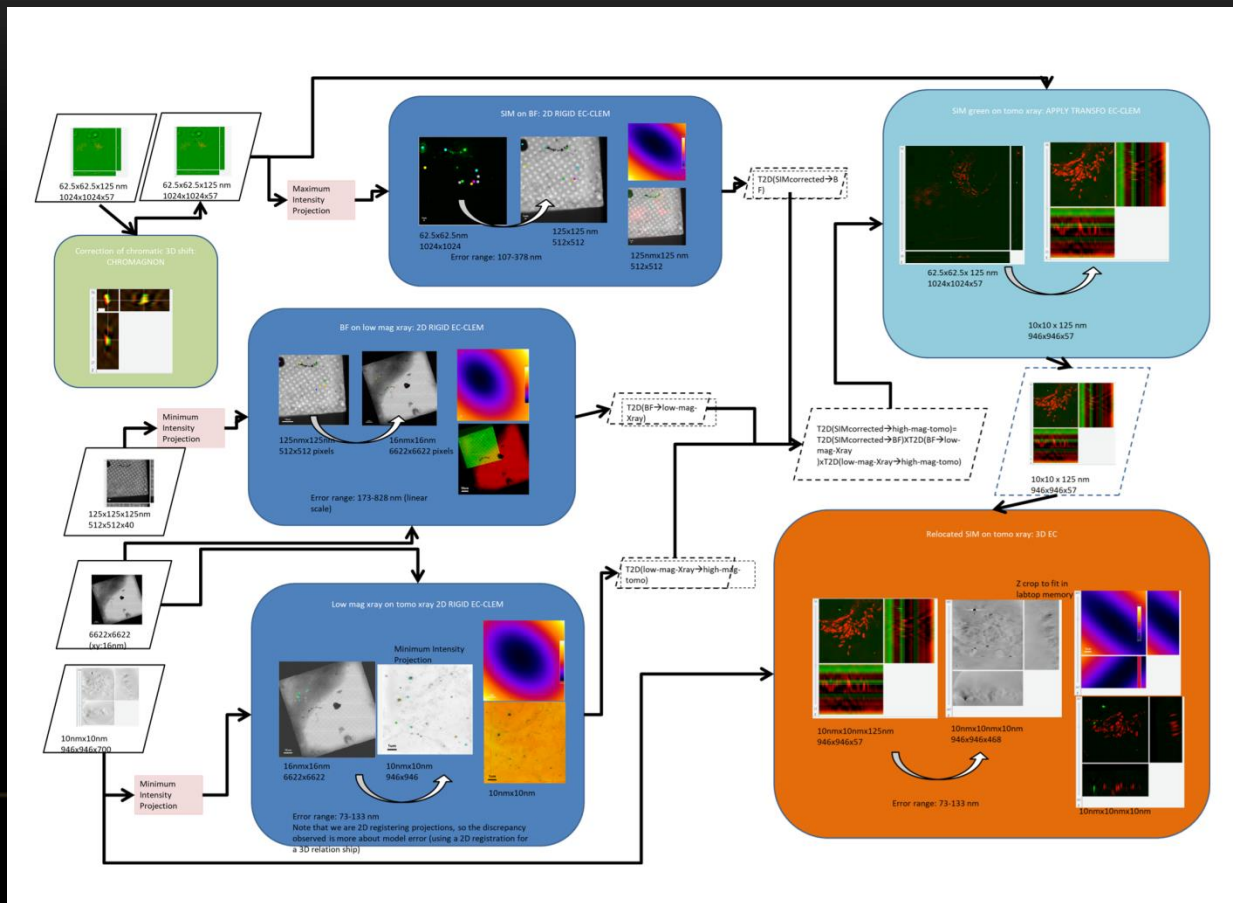
Non Linear

Thousands of models:

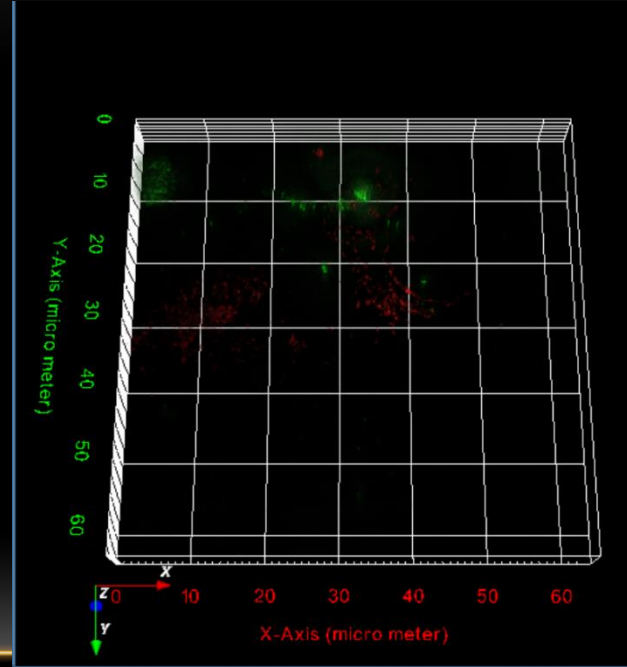
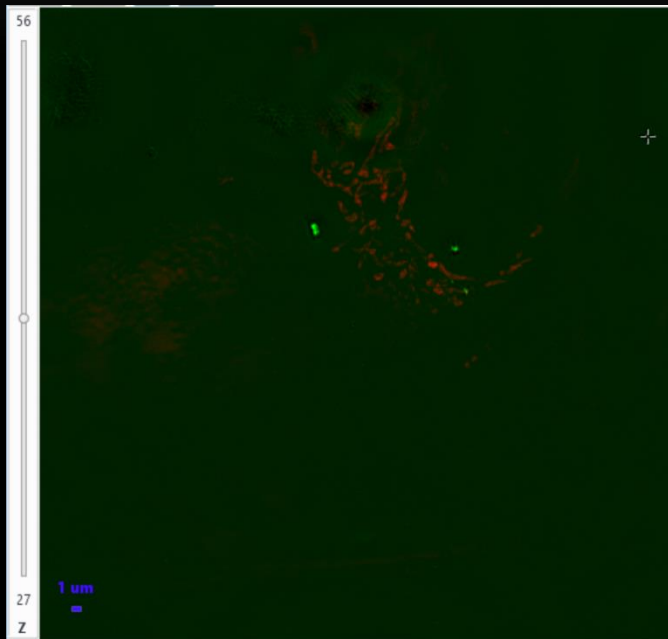
Radial basis, Thin Plate Spline, B-splines, Wavelets, Piece-Wise linear transformations, Elastic transformation, Fluid Flows, ...

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

FULL WORKFLOW

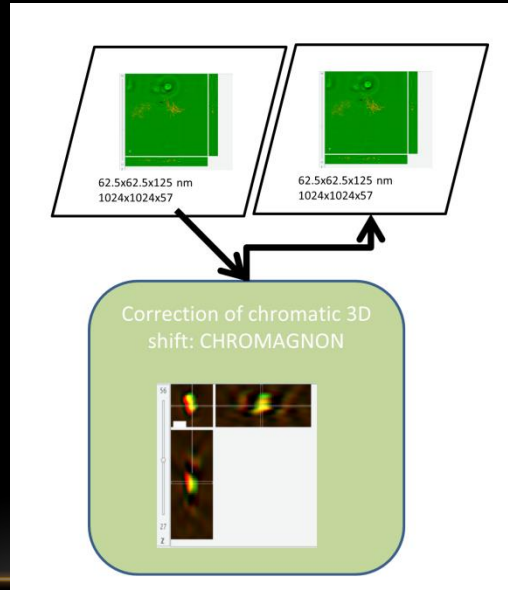


EXAMPLE ON MITOCHONDRIA WITH GREEN BEADS TO HAVE GROUND TRUTH



Green : 150 nm beads coated with Alexa488; Red mitochondria with MitoTracker

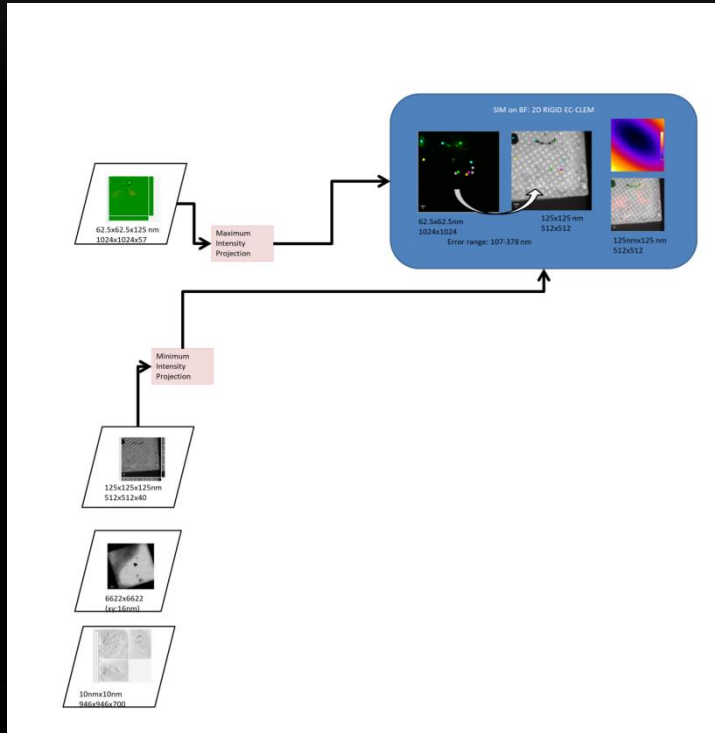
SIM DATA NEED TO BE REALIGNED BY APPLYING A 3D CHROMATIC SHIFT COMPUTED ON ANOTHER SLIDE WITH BEADS EMITTING IN RED AND GREEN.



Matsuda et al., Accurate and fiducial-marker-free correction for three-dimensional chromatic shift in biological fluorescence microscopy. *Sci. Rep.* 2018

<https://github.com/macronucleus/chromagnon>

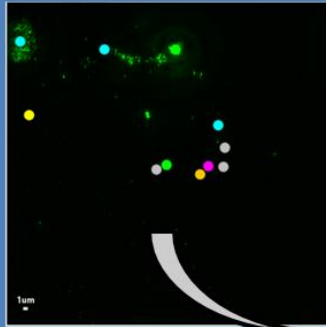
2D ALIGNMENT OF BRIGHT FIELD AND SIM PROJECTION



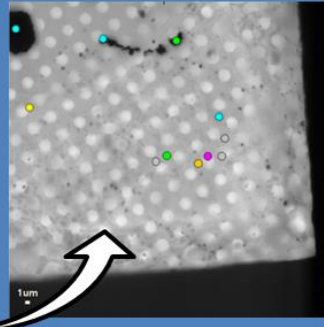
- SIM nb pixels is twice the BF one
- some small drift in subsequent acquisition on the same microscope.
- We need the transformation matrix at the same format

REGISTER USING IMAGE FEATURES

SIM on BF: 2D RIGID EC-CLEM

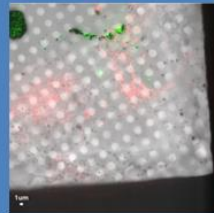
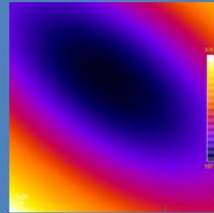


62.5x62.5nm
1024x1024



125x125 nm
512x512

Error range: 107-378 nm



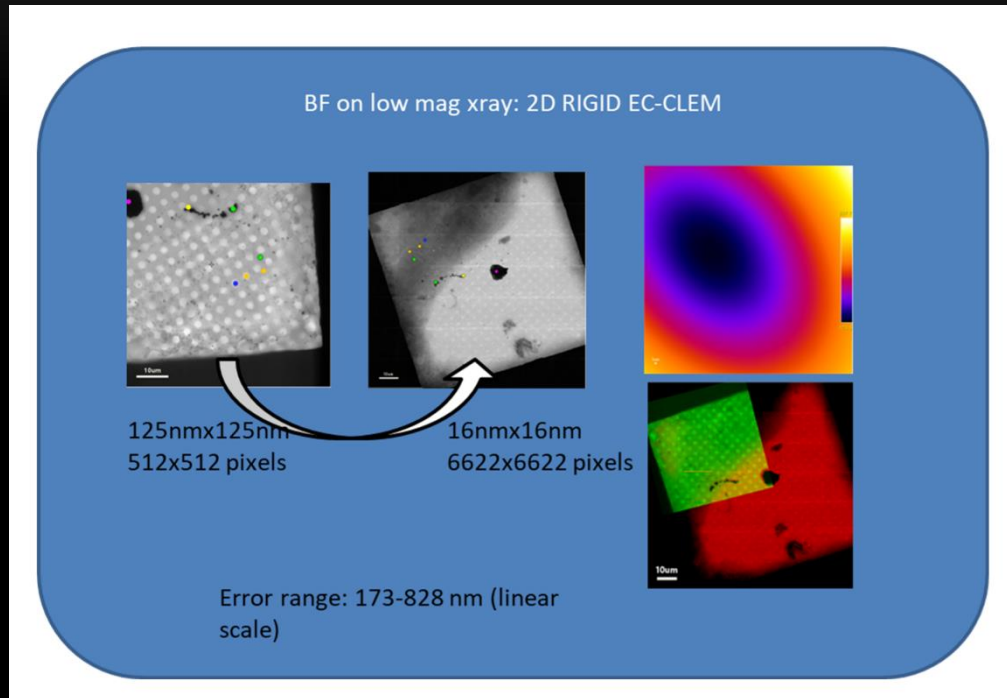
125nmx125 nm
512x512

Example of image features :
Fiducial markers e.g. gold nanoparticles.
High contrast cell features e.g. nuclei or lipid droplets.
Sample damage e.g. cracks in ice or foreign objects.
Holes in the EM grid support.

Paul-Gilloteaux P. et al
eC-CLEM: flexible multidimensional registration software for correlative microscopies. *Nat. Methods.* 2017

<https://github.com/anrcrocoval/ec-clem>

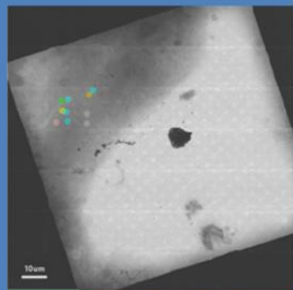
REGISTER BRIGHT FIELD PROJECTION TO 2D MOSAICK LOW MAGNIFICATION X-RAY



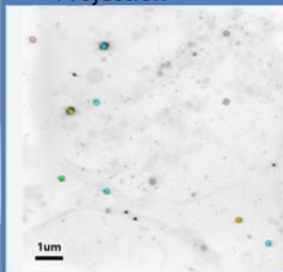
Note that because the sample are captured in vitreous ice, we **constrain ALL transformations to be RIGID** (no scaling as pixel size is known in the microscope metadata). Only flip allowed (in case of upside down sample).

REGISTER RIGIDLY XRAY MOSAICH TO 2D PROJECTION OF X-RAY TOMOGRAPH

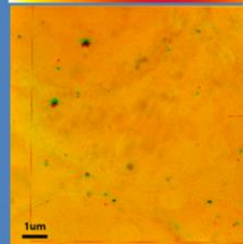
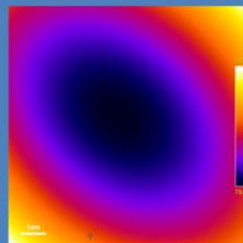
Low mag xray on tomo xray 2D RIGID EC-CLEM



16nmx16nm
6622x6622



10nmx10nm
946x946

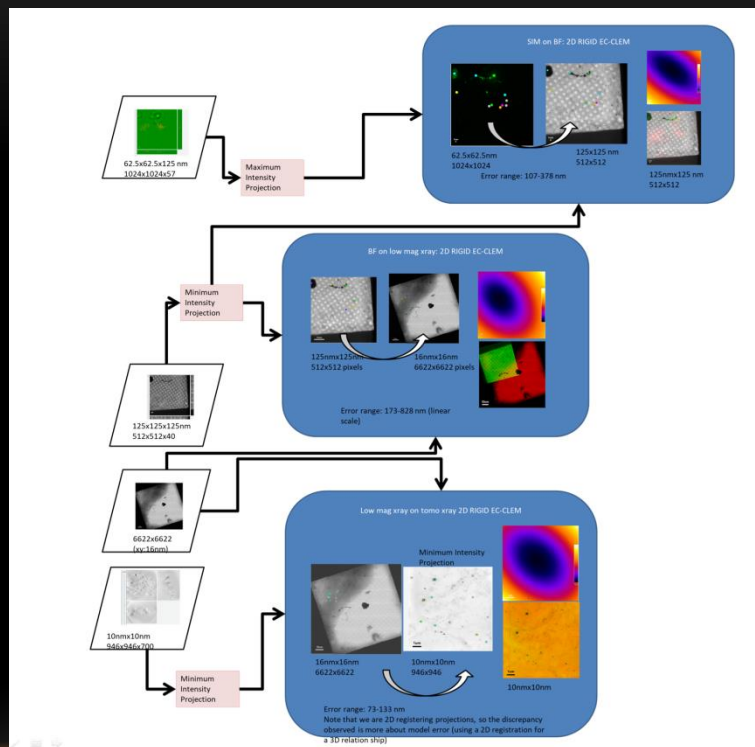


10nmx10nm

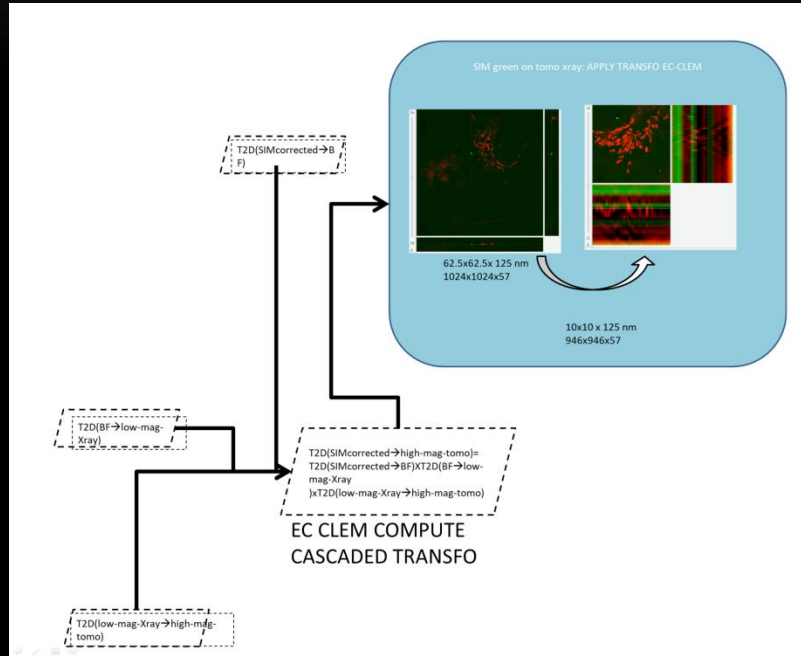
Error range: 73-133 nm

Note that we are 2D registering projections, so the discrepancy observed is more about model error (using a 2D registration for a 3D relationship)

THREE INDEPENDANT TRANSFORMATIONS IN 2D



COMBINING THE TRANSFORMATION (CASCADED TRANSFORMATION TO OBTAIN ONE 2D TRANSFORM FROM SIM TO X-RAY TOMOGRAPH

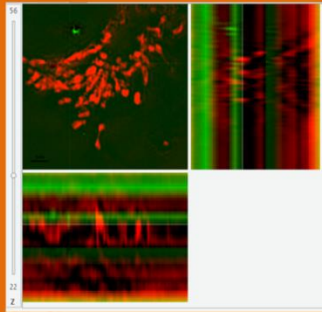


This 2d transform is applied to the chromatic-shift corrected SIM 3D volume

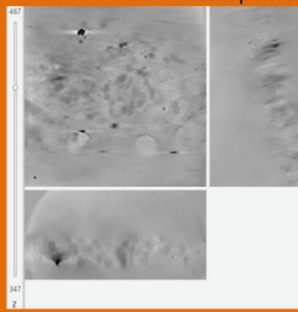
3D RIGID REGISTRATION OF THE RELOCATED 3D SIM TO 3D TOMOGRAPH

Relocated SIM on tomo xray: 3D EC

Z crop to fit in
labtop memory



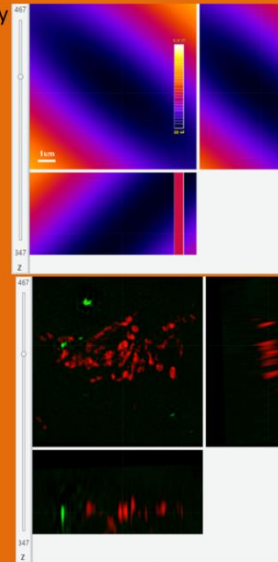
10nmx10nmx125nm
946x946x57



10nmx10nmx10nm
946x946x468

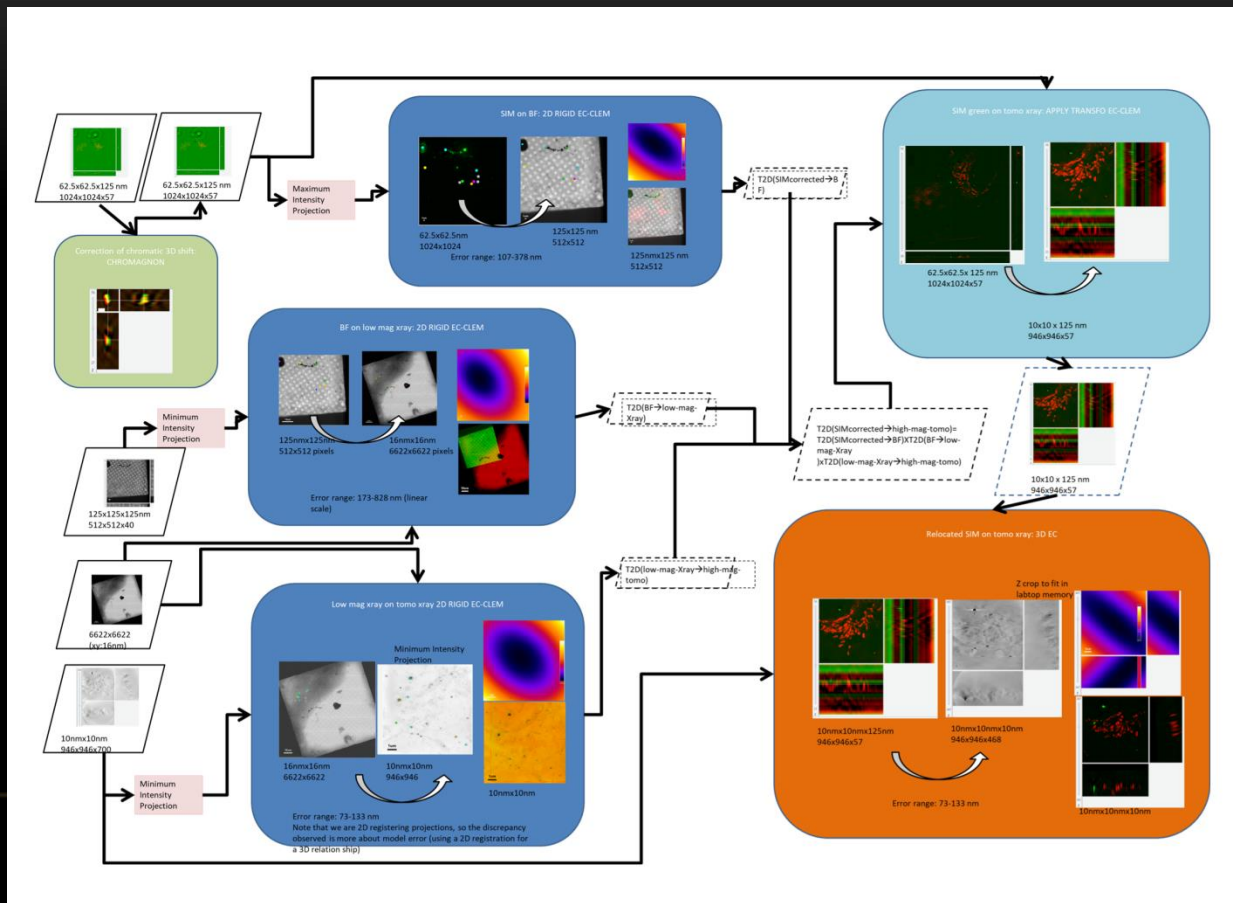


Error range: 73-133 nm

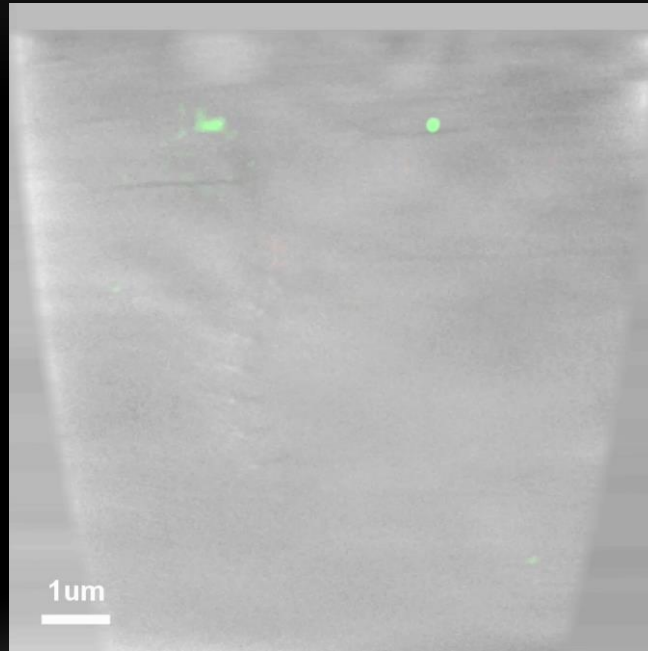


10nmx10nmx10nm

FULL WORKFLOW

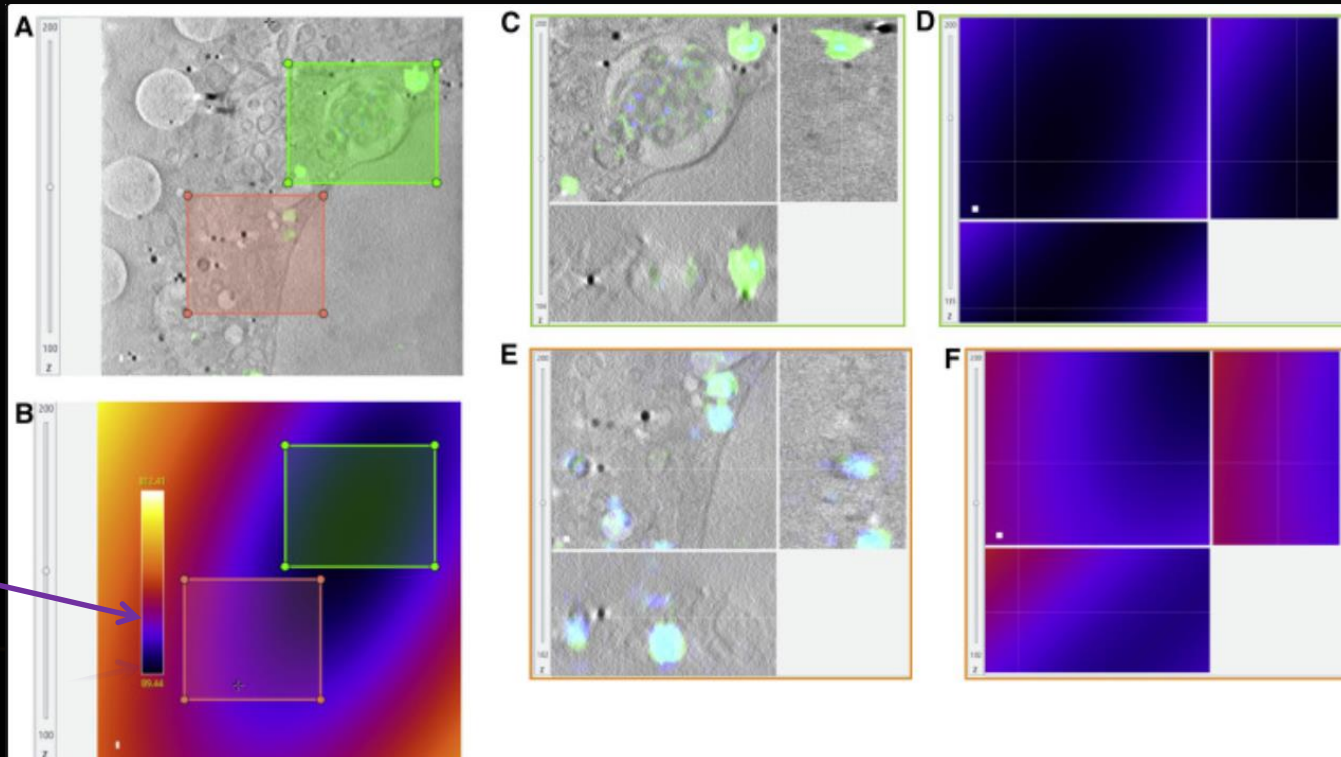


FULL WORKFLOW



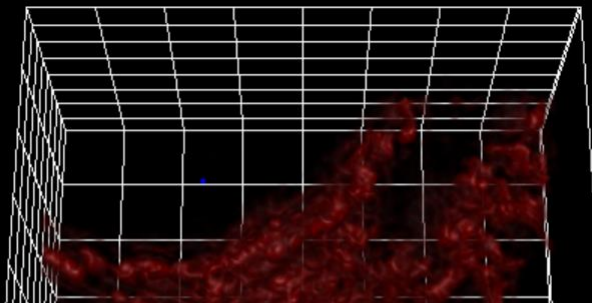
Video2

ESTIMATING THE ACCURACY OF RIGID REGISTRATION

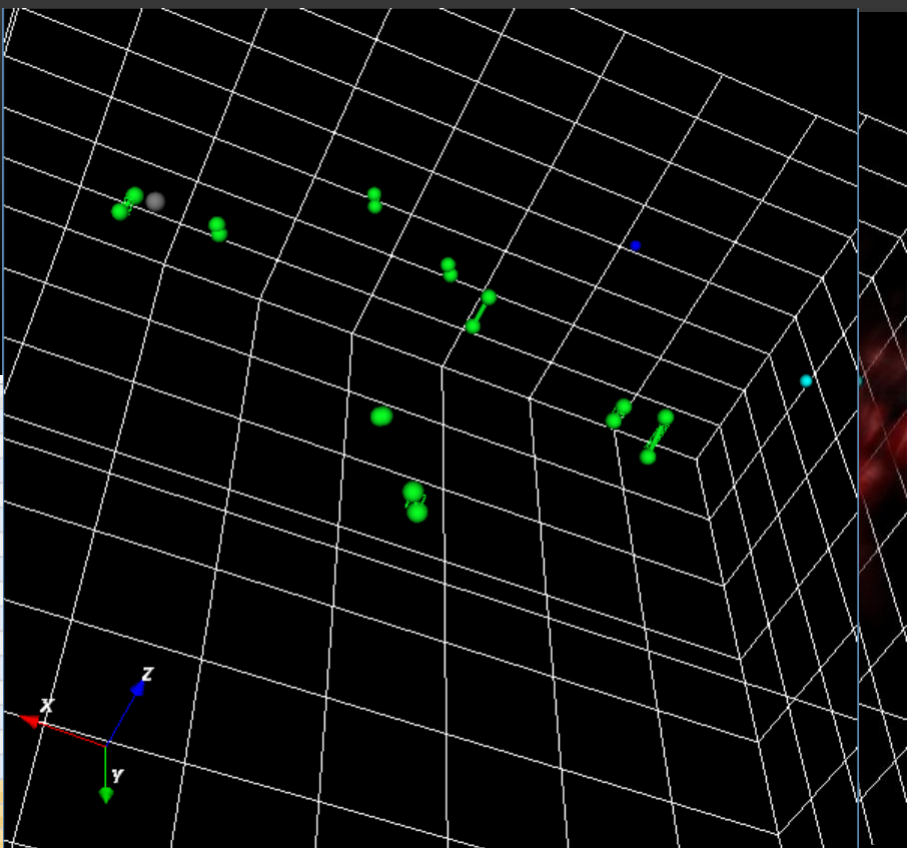


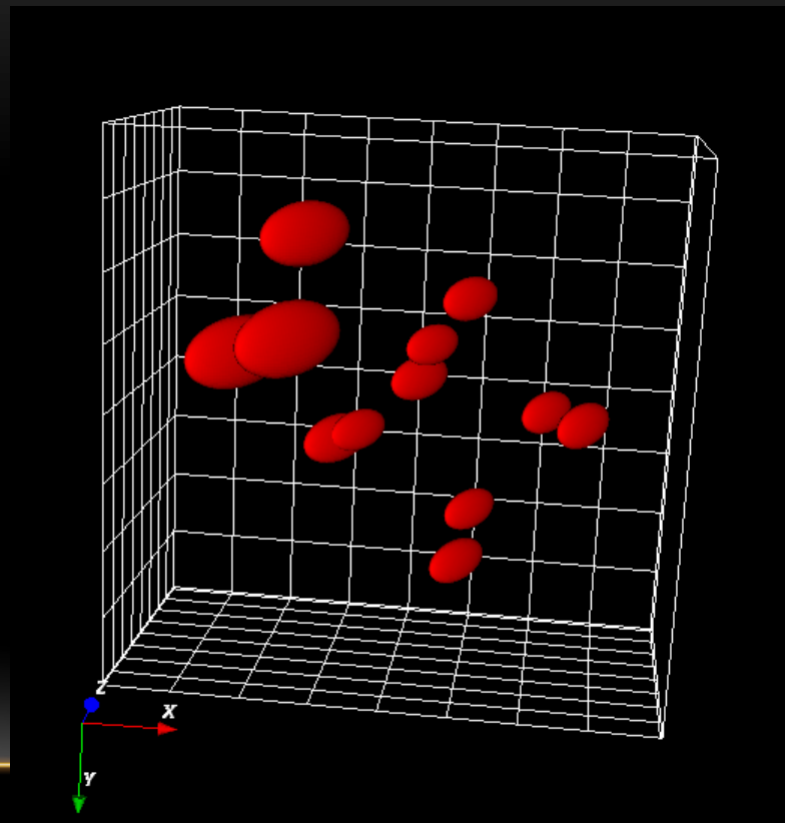
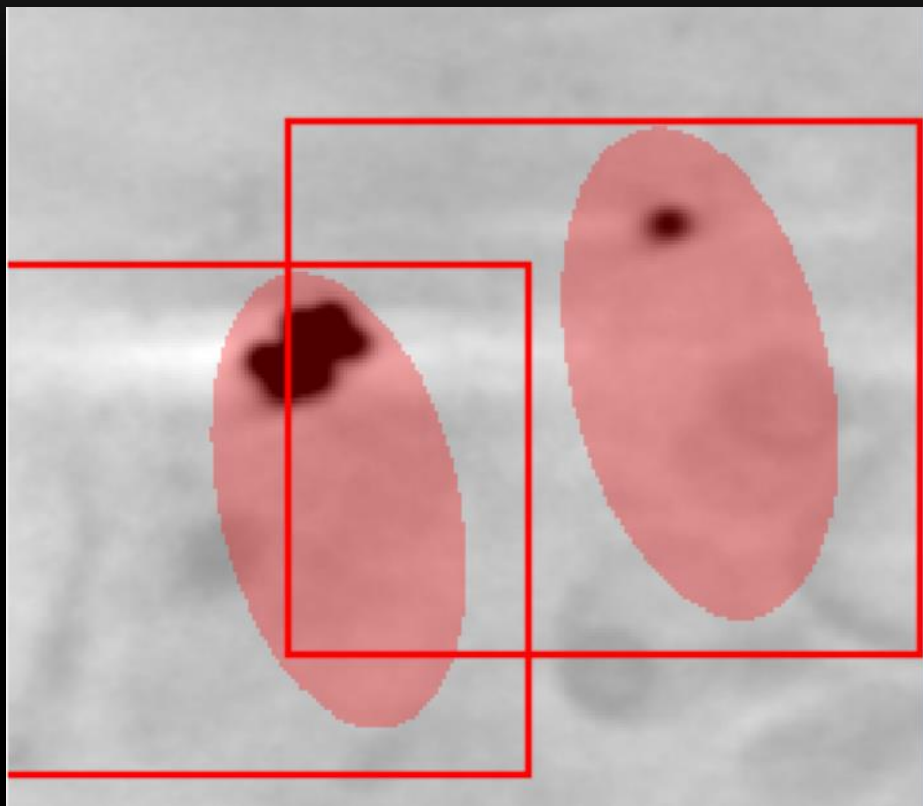
89 to 150 nm
of registration
error due to
the
localisation
error

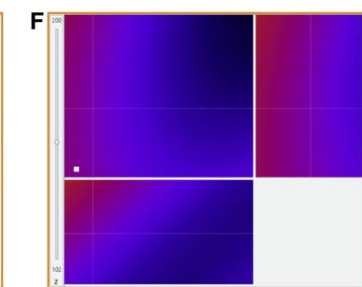
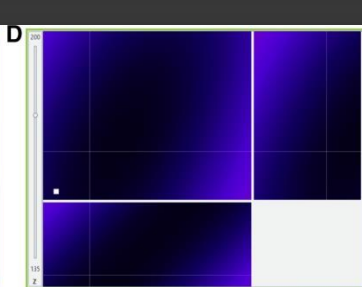
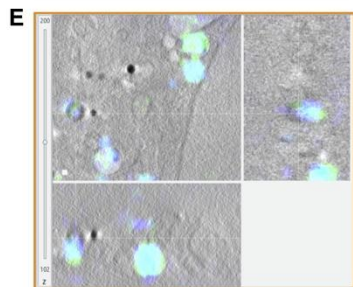
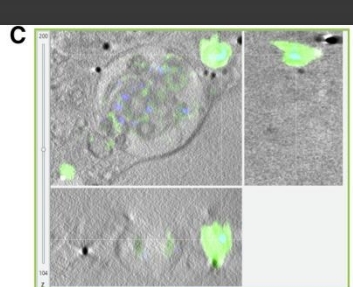
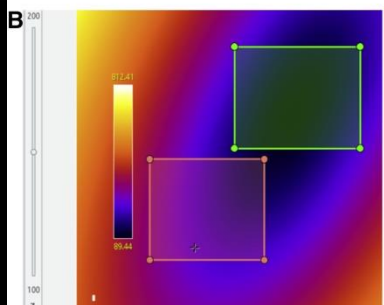
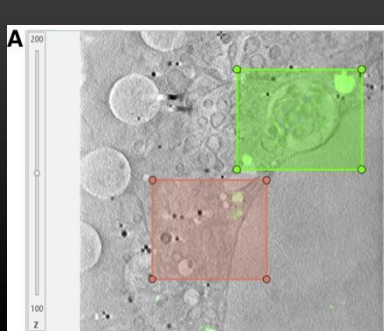
Scale bar:
White bar 100
nm



Name	Perimeter (um)
ROI PREDICTED_ERROR_FIDUCIAL_8	
ROI PREDICTED_ERROR_FIDUCIAL_9	
• FIDUCIAL_1	0
• FIDUCIAL_2	0
• FIDUCIAL_3	0
• FIDUCIAL_4	0
• FIDUCIAL_5	0
• FIDUCIAL_6	0
• FIDUCIAL_7	0
• FIDUCIAL_8	0
• FIDUCIAL_9	0
• NOT_FIDUCIAL_1	0
• NOT_FIDUCIAL_2	0
• NOT_FIDUCIAL_3	0
• NOT_FIDUCIAL_4	0
MEASURED_ERROR_FIDUCIAL_1	0.038618
MEASURED_ERROR_FIDUCIAL_2	0.25281
MEASURED_ERROR_FIDUCIAL_3	0.28128
MEASURED_ERROR_FIDUCIAL_4	0.11922
MEASURED_ERROR_FIDUCIAL_5	0.50807
MEASURED_ERROR_FIDUCIAL_6	0.60297
MEASURED_ERROR_FIDUCIAL_7	0.25436

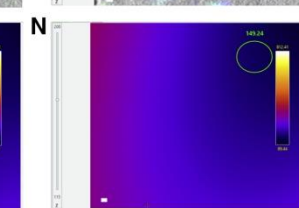
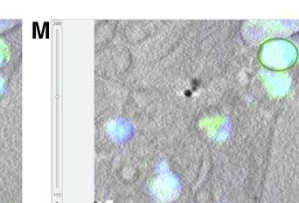
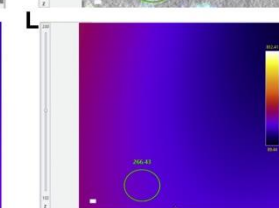
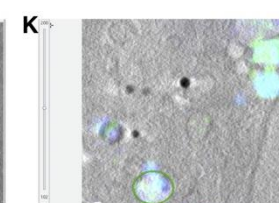
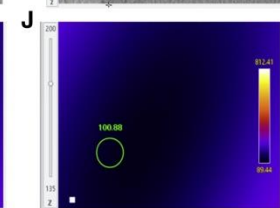
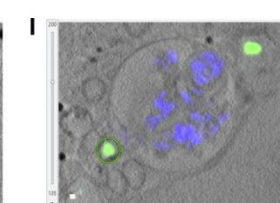
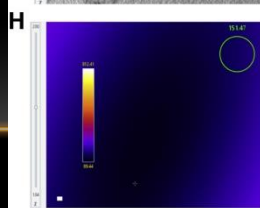
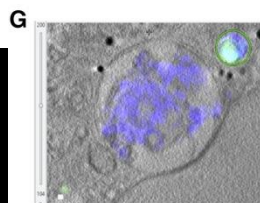






Matching structures= beads visible in both modalities

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



HOME RESOURCES SUPPORT CONTRIBUTORS GET INVOLVED ABOUT
🔍 SEARCH

PLUG-IN

ec-CLEMv2

Perrine Paul-Giboteaux

Publication ID: 1074244

SHORT DESCRIPTION

DOCUMENTATION

REVIEW THIS

DEPENDENCIES

CHANGELOG

Short Description

in beta test. Further info on anrcrocoval/ec-clem (github.com)

CATEGORIES #3-D #3D #ALIGNMENT #CLEM #CORRELATION #DEFORMABLE_REGISTRATION #DISPLACEMENTS
#NON_RIGID_REGISTRATION #REGISTRATION

Team: ANR Crocoval
Institution: Inserm CNRS Univ-Nantes
Website: <https://anrcrocoval.github.io/>
GIT HUB: <https://github.com/anrcrocoval/ec-clem>

Documentation

Online tutorials are available on the icy webpage <https://icy.bioimageanalysis.org/plugin/ec-clem/>, which are basically all valid. No right click needed any more to validate, and you can place your point on source or target image it doesn't matter. The main difference is that it will decide for you if it is 2D or 3D, and you can apply a 2D transform on a 3D dataset (See cascaded transform demo for example), and for now it give you more liberty on the choice of transformation on the constraint (further doc to come). Some 3D Further tutorials are available here specific to this new version:

- on error estimation at 95% of confidence https://youtu.be/Rz1_MLq6-k
- on creating a cascaded transform <https://youtu.be/5yNuP8QuJM>
- 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>
- Usage example of the incorporated autofinder: <https://youtu.be/zwiEAEJG4>

Icy

AN OPEN COMMUNITY PLATFORM FOR BIOIMAGE INFORMATICS

DOWNLOAD FOR WINDOWS

STAR Protocols

PROTOCOLS | 14th 11 2021

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

Nina Vasi,¹ Saphir Kumar,² Thomas M. Fish,¹ Ian M. Dobbs,² Maria Haskova,^{1,3*} and Perrine Paul-Giboteaux^{1,4*}

*Technical contact

*Lead contact

Correspondence: maria.haskova@lshtm.ac.uk #

Correspondence: perrine.paul-giboteaux@inserm.fr #

Show Affiliations

DOI: 10.1038/s41598-021-11032-9

Summary

Before you begin

Key resources

Table

Step-by-step method details

Expected outcomes

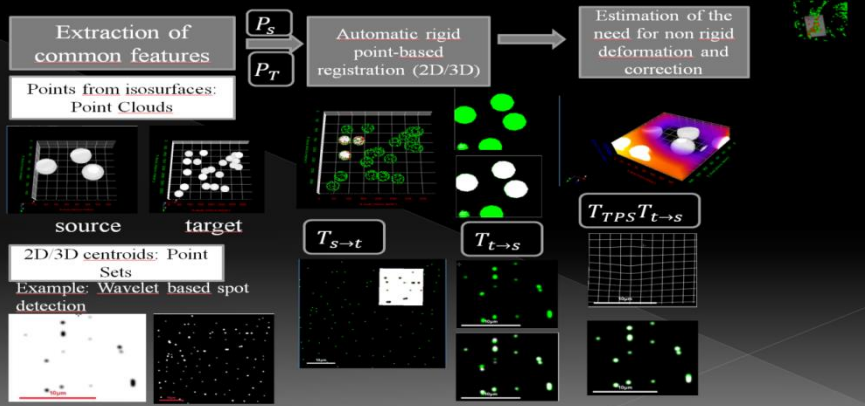
Limitations

Summary

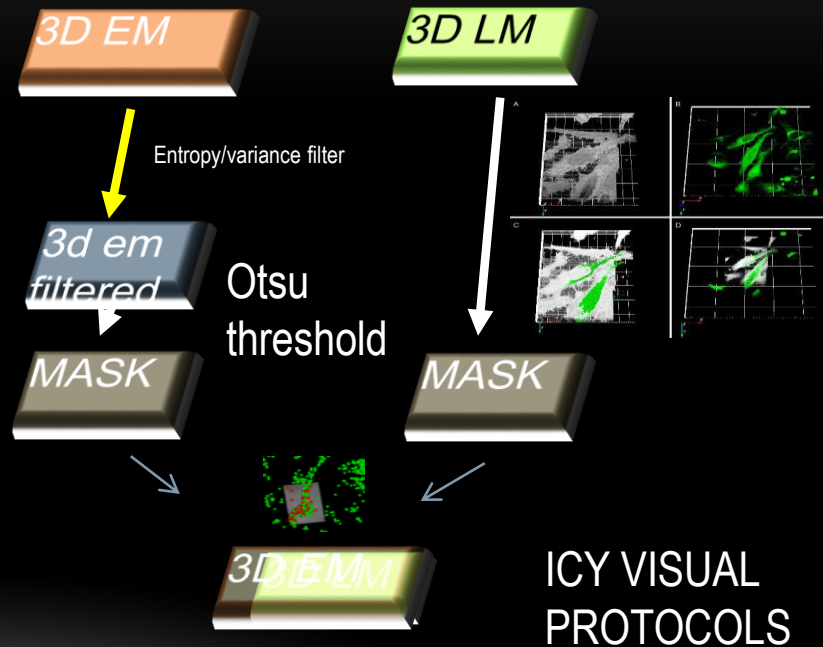
Correlation of 3D images acquired on different microscopes can be a daunting prospect even for experienced users. This protocol describes steps for registration of images from soft X-ray absorption contrast imaging and super-resolution fluorescence imaging of hydrated biological materials at cryogenic temperatures. Although it is developed for data generated at synchrotron beamlines that offer the above combination of microscopes, it is applicable to all analogous imaging systems where the same area of a sample is examined using successive non-destructive imaging techniques.

For complete details on the use and execution of this protocol, please refer to Kounatidis et al. 2021.

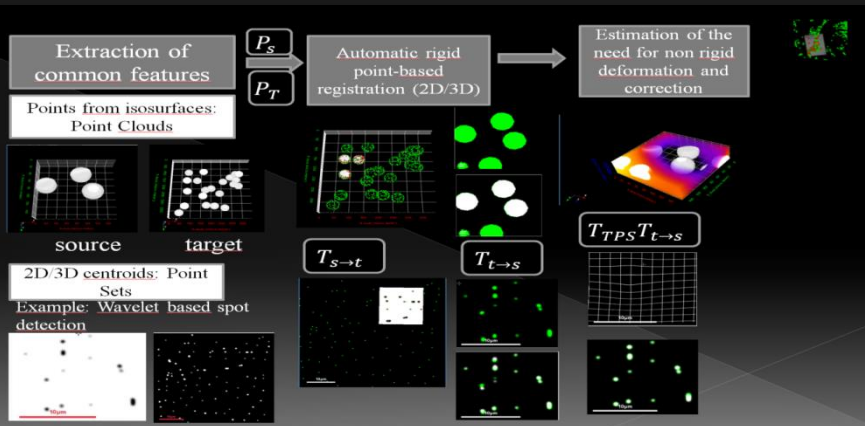
GENERIC APPROACH



Ecclem AUTOFINDER



GENERIC APPROACH



Ecclem AUTOFINDER

3D EM

3D LM

Wavelet
Spot
detection

Wavelet
Spot
detection

3D EMM

ICY VISUAL
PROTOCOLS



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