



Correlated Multimodal  
Imaging in Life Sciences



## ***CORRELATED MULTIMODAL IMAGING IN LIFE SCIENCES***

***COMULIS, a new network to bridge (pre) clinical imaging and  
Correlative Light Electron Microscopy***

***-- Focus on multidimensional registration software --***

Perrine Paul-Gilloteaux, CNRS France BioImaging Nantes  
On behalf of COMULIS members and Andreas Walter, Chair of the  
action.

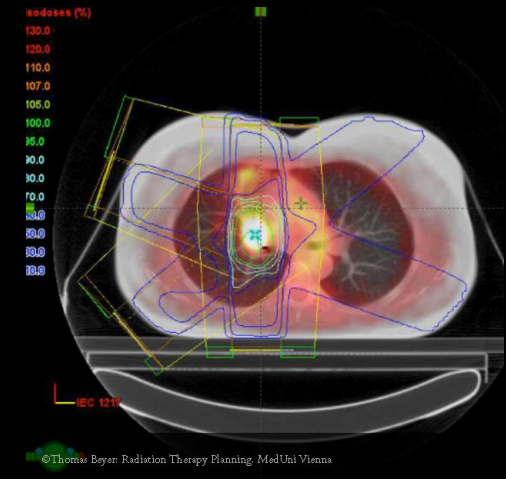
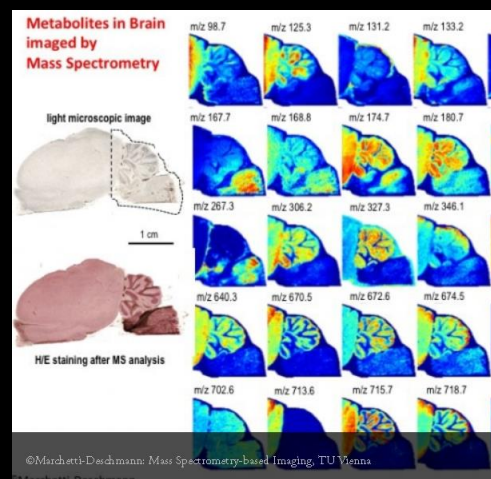
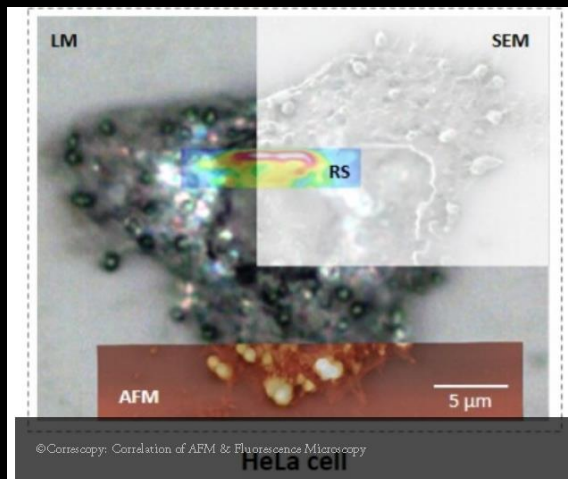
# What is COMULIS?

Correlated Multimodal Imaging : combining 2 or more modalities to gather information about the same specimen

**EU-funded COST Action (11/2018-11/2022)**

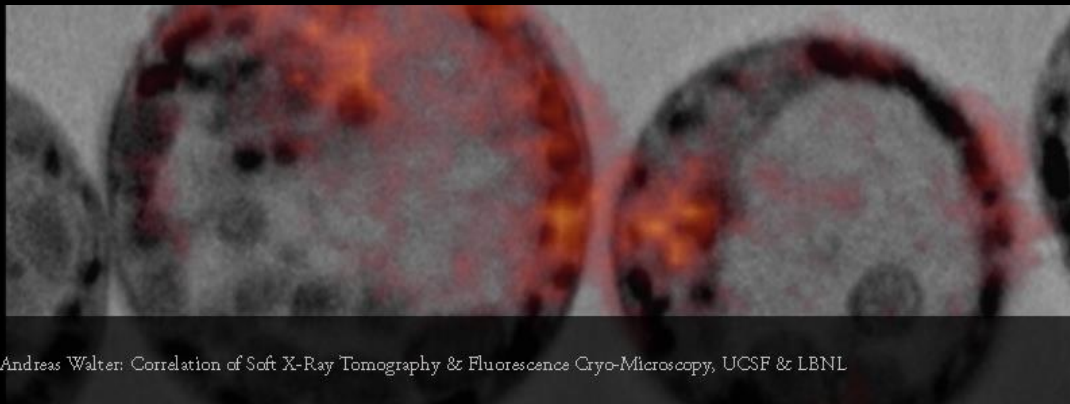
Goals:

- fueling urgently needed collaborations in the field of correlated multimodal imaging
- promoting and disseminating its benefits through showcase pipelines
- paving the way for its technological advancement and implementation as a versatile tool in biological & preclinical research



# Objectives

- **Establishing commonly-accepted protocols & quality standards** for existing CMI approaches;
- **Showcasing novel CMI pipelines** and **bridging the gap between preclinical & biological imaging**;
- **Fostering standardized correlation software** through networking, workshops & open databases;
- **Raising awareness for CMI**, training researchers in multimodal approaches, and working towards a scientific mindset that is enthusiastic about interdisciplinary imaging approaches in life sciences.

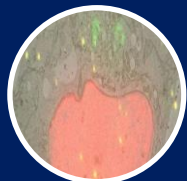


# Organization (work groups)



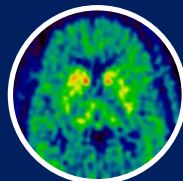
## WG5: Outreach, Dissemination & Partnerships

Foster the recognition of CMI in life sciences



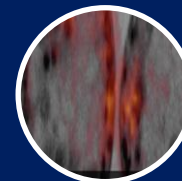
WG1

Correlative Light To Electron  
Microscopy



WG2

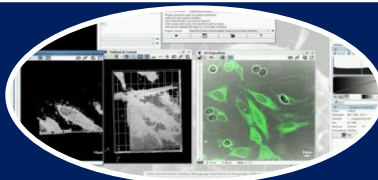
Preclinical Hybrid Imaging



WG3

Novel Correlated multimodal  
imaging Pipelines

Set up standards and best practices, Trigger novel approaches



## WG4: Correlation software

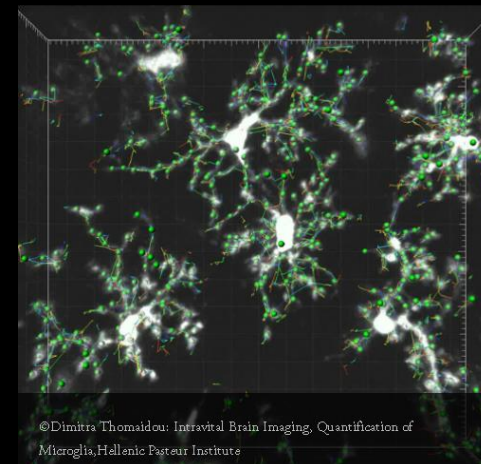
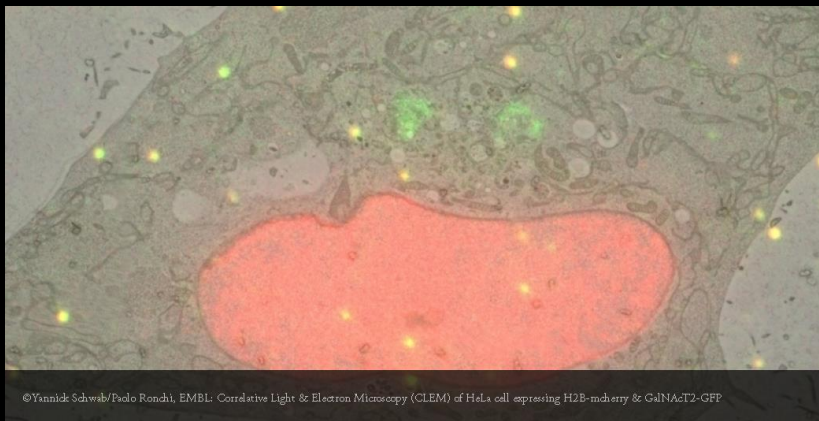
Improve & develop correlation and visualization concerted solutions

- **Meetings & Conferences**

- Next Events working groups meeting 21-22 January in Porto, Portugal
- Focus session at EMIM 2019 European Society for Molecular Imaging, 23 March, Glasgow, UK
- ...

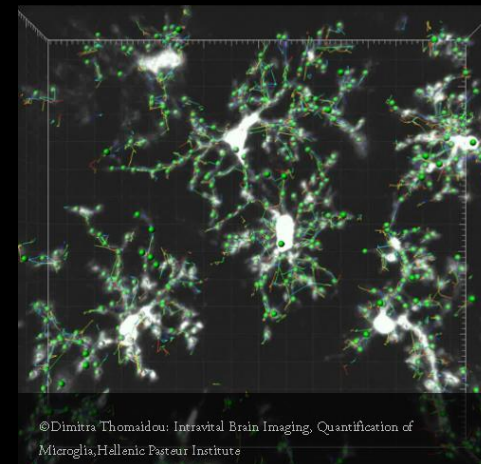
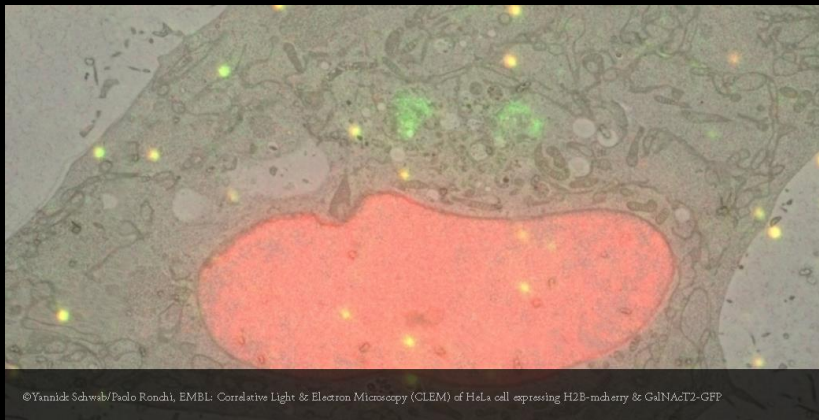
- **Dissemination Activities**

- ASCB-EMBO meeting Focus group , San Diego USA
- Analytix 2019, Singapore

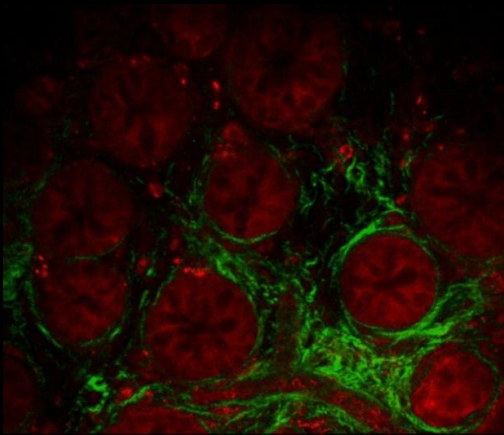




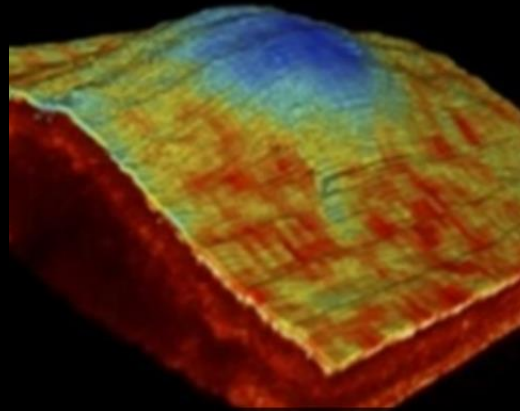
- **Training**
  - Training schools in PHI
  - Training schools in CLEM
  - All with image analysis and software training



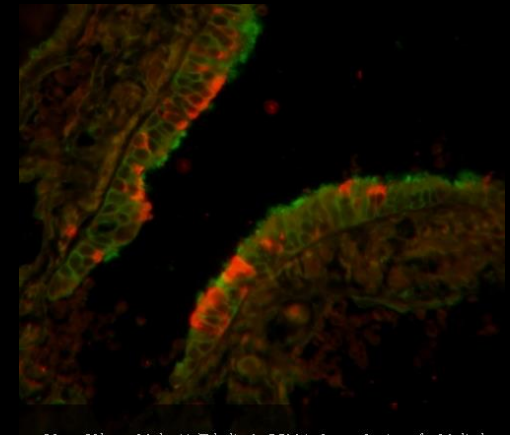
- **Mobility Grants (Short-Term Scientific Missions )**
  - cover scientist from research labs & core facilities (EU) to visit an host lab (EU or World Wide) to perform a scientific project on correlative multimodal imaging
  - between 1 week to 6 months.
- **Conference grants**
  - Cover Early Career Investigator for a specific list of country to attend any conference in the field multimodal imaging/ image analysis



©Sanja Despotovic: Combined SHG and two-photon excited fluorescence, Institute of Histology and Embryology, Faculty of Medicine, University of Belgrade



©Dror Fixler: OCT FLIM, Bar-Ilan University

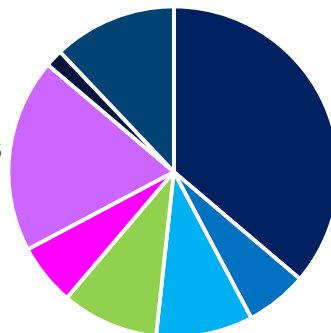


©Ivana Vrhovac Madunic: Tubulin & CC10 in Lungs, Institute for Medical Research and Occupational Health

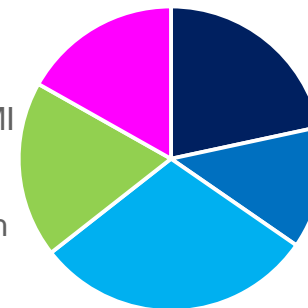
# Who is COMULIS?

- 118 members for now. Scientific Background Working groups Participants

- Biology
- Chemistry
- Computer Sciences
- Engineering
- Medicine
- Physics
- Biophysics
- Other..



- WG1 CLEM
- WG2 PHI
- WG3 New CMI
- WG4 soft
- WG5 outreach



- Management committee: 2 representatives per country



1st COMULIS MC Meeting at Brussels.



# Who is COMULIS?

- Core group:



Andreas Walter (Austria) -  
Chair COMULIS



Perrine Paul-Gilloteaux  
(France) - Vice Chair  
COMULIS



Paul Verkade (UK) -  
Leader of WG1 (CLEM)



Ludek Sefc (Czech  
Republic) - Leader of  
WG2 (Preclinical Hybrid  
Imaging)



Yannick Schwab  
(Germany) - Leader of  
WG3 (Novel Correlated  
Multimodal Imaging  
Pipelines)



Natasa Sladoje (Serbia) -  
Leader of WG4  
(Correlation Software)



Paula Sampaio (Portugal)  
- Leader of WG5  
(Dissemination, Outreach  
& Partnerships)



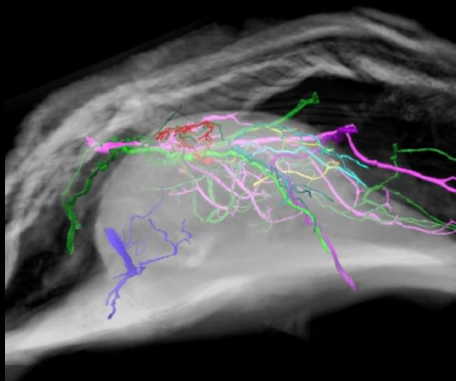
Julia Fernandez-Rodriguez  
(Sweden) - STSM  
Coordinator



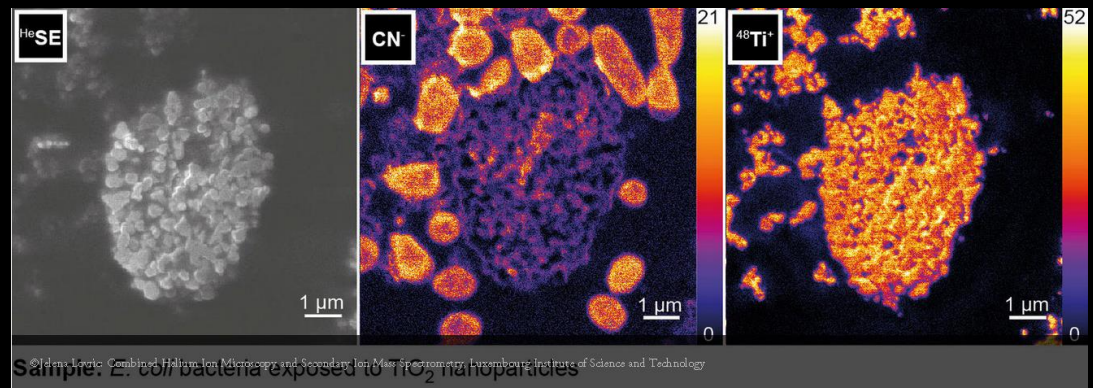
Martina Marchetti-  
Deschmann (Austria) -  
Science Communication  
Manager

# How to join?

- Any **individual** - researcher, engineer, or scholar - in universities, research centers, public and private organizations, and whose expertise is relevant to the Action, is welcome to join.
- See <https://www.comulis.eu/registration/> for the full pathway , do not hesitate to ask further details!



©Wolfgang Weninger: High Resolution Episcopic Microscopy (HREM), Segmented Blood Vessels, MedUni Vienna, BioImaging Austria-CMI



# Correlation software

**Deliverable 1:** Create compilations of existing software used in correlative multimodal imaging.

[WWW.BIIL.EU](http://WWW.BIIL.EU)

> 1000 software and workflows curated by and for the community of bioimage analysis

**Segmentation**  
**Registration**  
**Visualization**



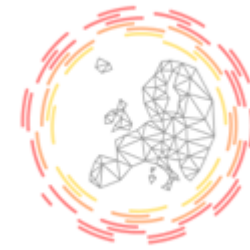
## Finding and Reusing specific content

In this demo, we show how we can use the Bioimaging EDAM ontology to filter specific tools from the Neubias catalog. COMULIS is a scientific community interested in the field of correlative multimodal imaging. Several image processing and analysis tasks are involved such as image registration, segmentation or visualization.

### Segmentation softwares

EImage	<a href="#">High content screening</a> <a href="#">Bioimage informatics</a>	<a href="#">Cell segmentation</a>
2D brain slice region annotation: SliceMap	<a href="#">Widefield microscopy</a>	<a href="#">Image segmentation</a>
2D cell tracking and analysis of morphological dynamics		<a href="#">Active contours</a> <a href="#">Cell segmentation</a>
ANTs: Advanced Normalization Tools	<a href="#">Bioimage informatics</a>	<a href="#">Image segmentation</a>
APP (All-path pruning)	<a href="#">Confocal microscopy</a>	<a href="#">Image thresholding</a>
Adaptive Thresholding (EImage)		<a href="#">Cell segmentation</a> <a href="#">Adaptive thresholding</a>
Adipocyte quantification ImageJ by Baecker	<a href="#">Digital histology</a>	<a href="#">Cell segmentation</a>
Adipocyte quantification MATLAB	<a href="#">Digital histology</a>	<a href="#">Cell segmentation</a>
Adiposoft	<a href="#">Digital histology</a>	<a href="#">Watershed</a> <a href="#">Cell segmentation</a> <a href="#">Image thresholding</a>
Avia	<a href="#">Machine learning</a>	<a href="#">Cell segmentation</a> <a href="#">Image segmentation</a>
Arabidopsis Seedlings Tool	<a href="#">Bioimage informatics</a>	<a href="#">Image thresholding</a>
Blob segmentation		<a href="#">Image segmentation</a>
Blood vessel segmentation and network analysis		<a href="#">Image segmentation</a>
Cell or particle counting and scoring the percentage of stained objects	<a href="#">Fluorescence microscopy</a>	<a href="#">Cell segmentation</a>
Cell segmentation and quantification with CellK	<a href="#">Brightfield microscopy</a> <a href="#">Phase contrast microscopy</a>	<a href="#">Image segmentation</a>
Cell segmentation in phase contrast images	<a href="#">Phase contrast microscopy</a>	<a href="#">Active contours</a> <a href="#">Cell segmentation</a>
CellProfiler Examples - Speckle Counting		<a href="#">Image segmentation</a>
Counting Clumped Cells		<a href="#">Cell segmentation</a>
DNA MicroArray Image Processing Case Study		<a href="#">Image segmentation</a>
FibrilTool	<a href="#">Statistics</a> <a href="#">Microscopy</a>	<a href="#">Interactive segmentation</a>
Find nuclei on nuclear and		<a href="#">Active contours</a>

# Correlation software



Correlated Multimodal  
Imaging in Life Sciences

## BIII.EU




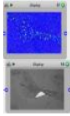
### Tag Registration

### Tag Registration

Also tagged with the topic  
CLEM

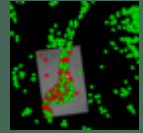
Registration softwares

2D brain slice region annotation: SliceMap	Widefield microscopy	Image registration
ANTs: Advanced Normalization Tools	Bioimage informatics	Affine registration, Rigid registration, Image registration, Deformable registration
Align slices in stack		Image registration
BigDataViewer	Light-sheet microscopy, Imaging	Affine registration, Rigid registration, Image registration
BigStitcher		Affine registration, Rigid registration, Image registration
Bigwarp	Correlative Light and Electron Microscopy, Electron microscopy	Image registration, Deformable registration
CMTK	Statistics, Bioimage informatics	Affine registration, Image registration, Deformable registration
CellProfiler Align		Rigid registration
FMRIb's Linear Image Registration Tool FLIRT	Magnetic resonance imaging	Image registration
FNIRT	Magnetic resonance imaging	Image registration, Deformable registration
Fiji CMTK GUI	Imaging	Affine registration, Image registration, Deformable registration
FreeD	Bioimage informatics	Object registration, Deformable registration
ITK		Image registration
Image Stabilizer		Image registration
ImageJ Align RGB planes		Rigid registration
KNIME align image		Image registration
MIDAV	Computerized tomography, Bioimage informatics, Magnetic resonance imaging, Imaging	Image registration
Manual Frame Drift Registration		Image registration
MorphoLeaf	Bioimage informatics	Image registration
Multiview Reconstruction	Light-sheet microscopy	Affine registration, Rigid registration, Image registration
Register Virtual Stack Slices		Image registration
Spark Stitcher	Light-sheet microscopy, Light microscopy	Image registration
Template Matching and Slice Alignment--- ImageJ Plugins		Image registration
Tera Stitcher		Rigid registration, Image registration
ec-CLEM	Correlative Light and Electron Microscopy	Affine registration, Deformable registration
ec-clem autofinder	Correlative Light and Electron Microscopy	Affine registration, Rigid registration, Image registration, Deformable registration
elastik		Affine registration, Rigid registration, Deformable registration
performing automatic registration for CLEM	Correlative Light and Electron Microscopy	Affine registration, Rigid registration, Image registration
pystackreg	Fluorescence microscopy, Electron microscopy	Affine registration, Rigid registration, Image registration
simpleElastik		Affine registration, Rigid registration

Name ▲	Category	Image	requires	Type	interaction level	License/Openness	has function	has topic
Bigwarp	Software			Component	Manual	Free and open source	Image registration, Deformable registration, Warping	Correlative Light and Electron Microscopy
ec-CLEM	Software			Component	Manual, Semi-automated	Free and open source	Affine registration, Deformable registration, Image registration	Correlative Light and Electron Microscopy
ec-clem autofinder	Software			Component	Semi-automated	Free and open source	Image registration, Rigid registration, Affine registration	Correlative Light and Electron Microscopy
performing automatic registration for CLEM	Software			Workflow	Automated, Semi-automated	Free and open source	Image registration, Affine registration, Rigid registration, Alignment construction	Correlative Light and Electron Microscopy



# Ec-clem and EC-clem Autofinder in ICY



Open source tools for:

- **user guided registration** (2D/2D/ 3D/3D 2D/3D 3D/2D)
- **automatic registration workflows** construction (2D/2D/ 3D/3D 2D/3D 3D/2D)
- automatic **assessment of error**
- **testing different configurations** of beads/landmarks : fiducials

With or  
without  
deformations  
(rigid or non  
rigid)





# eC-CLEM : a simple user interface



The screenshot displays the icy software interface with several windows and panels:

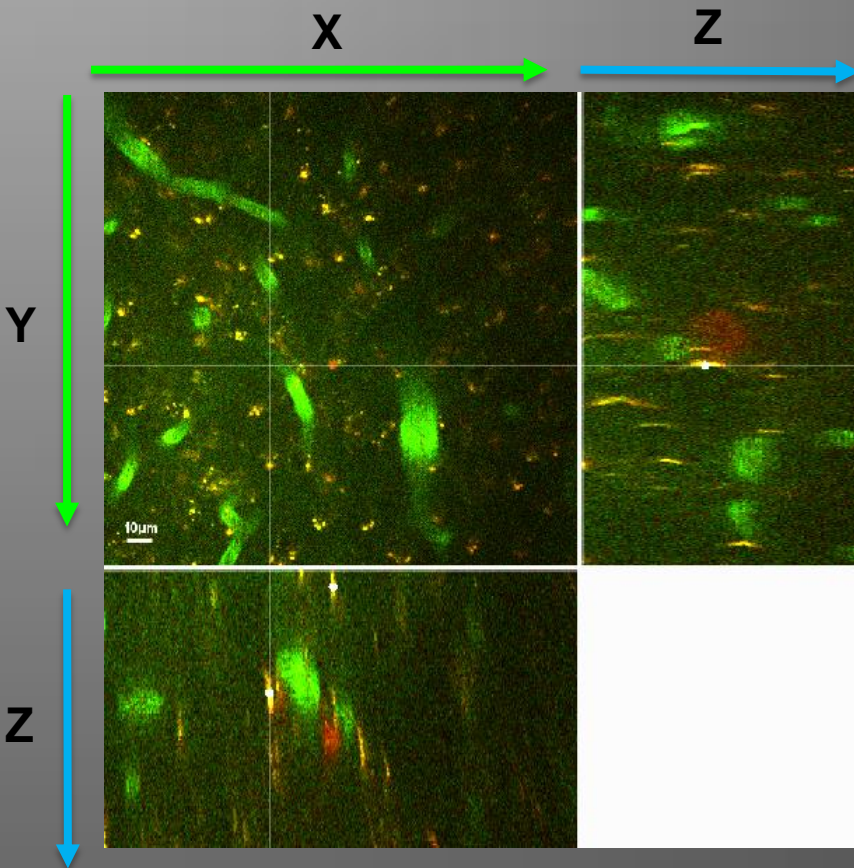
- Windows:**
  - FullStack.tif (resized):** Shows a 3D volume rendering of a sample with a white grid overlay.
  - LM-OriginalData (Ch1):** Shows a 2D fluorescence image of the sample with a green signal and a white grid overlay.
- Right Panel:**
  - Canvas:** Includes settings for Background color, Interpolation (Linear), Blending (Composite), Sample (Auto), and Shading (0.2, 0.4, 0.4).
  - Histogram and colormap:** Includes checkboxes for Refresh and Auto bounds, and options for log and linear scales.
  - Sequence Properties:**

Name	LM-OriginalData (Ch1)		
Dimension	1024 x 1024 x 16 x 1		
Channel	1 - unsigned byte (8 bits)		
Size	16.0 MB		
Pixel size	0.22µm	0.22µm	0.4µm
Time interval	1sec		
- Bottom Panel:** Displays system information: Memory: 1.1 GB (Max: 26 GB), CPU: 35%.

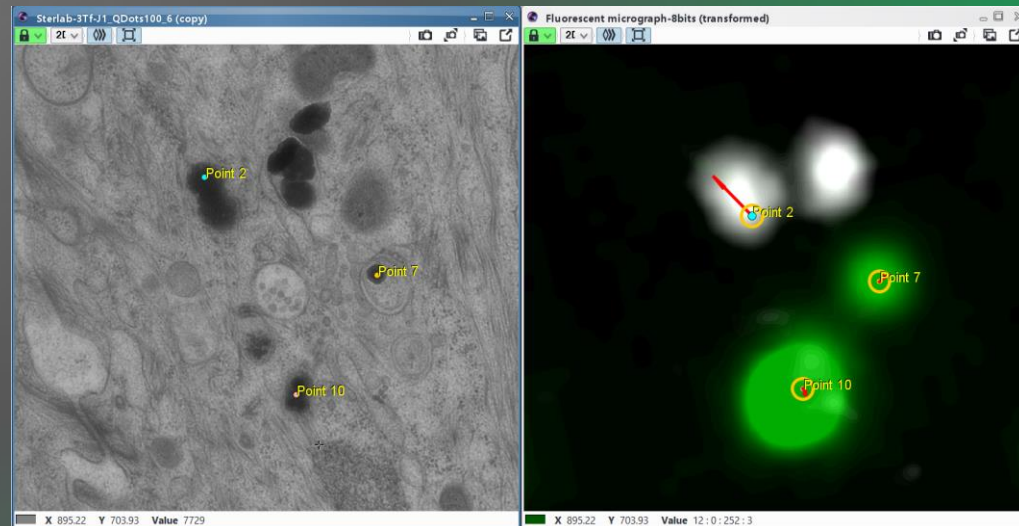
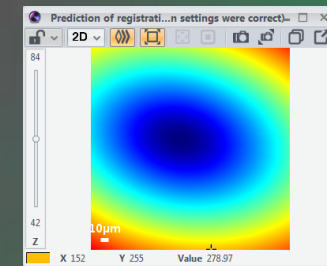
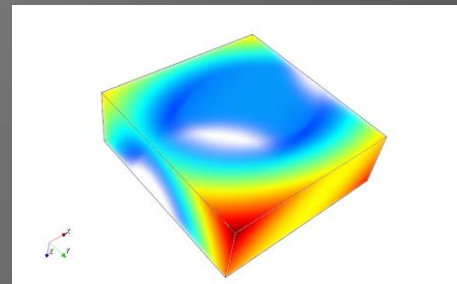
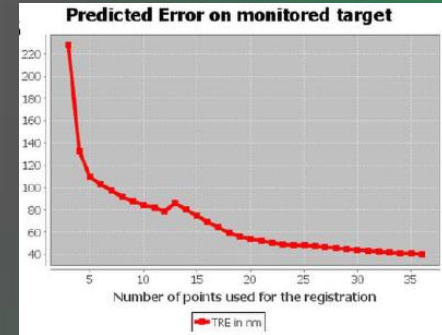
icy  
Version 1.9.0.1

# Convenient tools for registration and error assessment

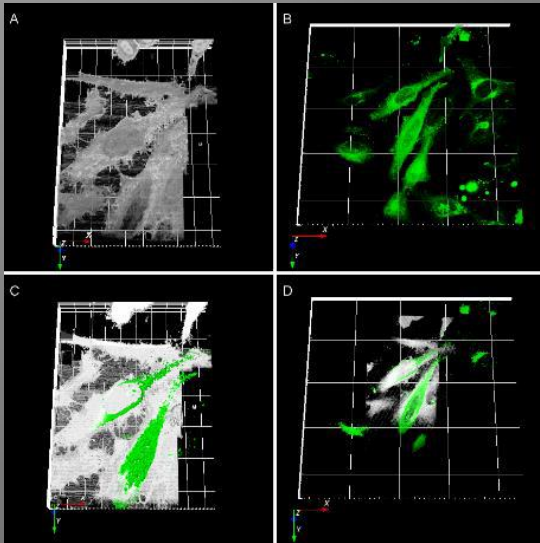
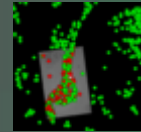
Place points in 3D in orthoviews



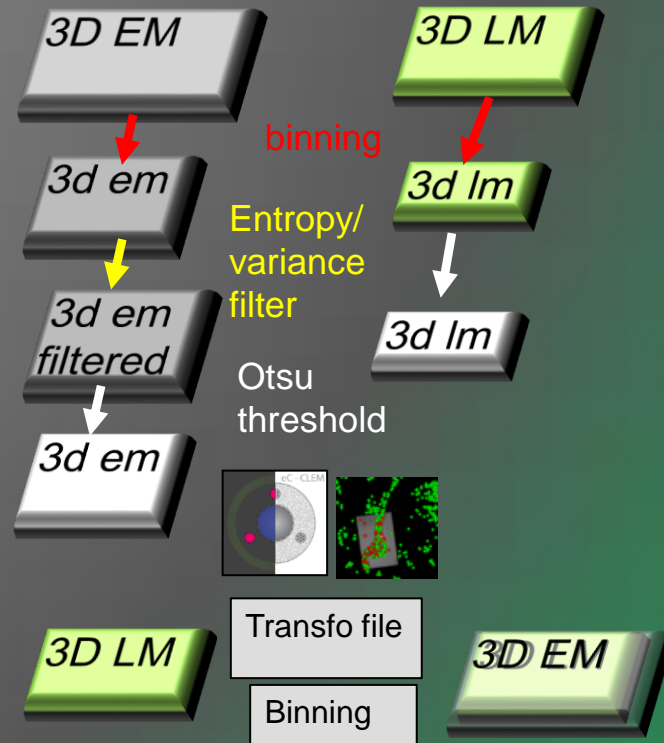
Predict error when no ground truth, and measure it when available



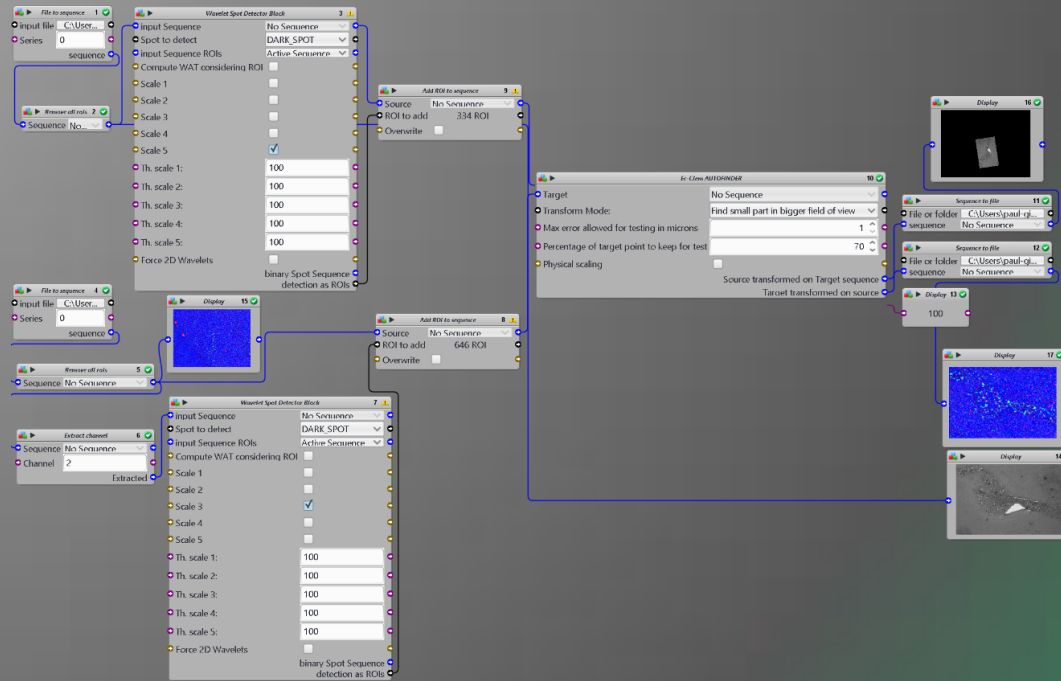
# Example of automatic workflow



(A) Preprocessed EM volume. Grid space: 10 microns (B) preprocessed LM volume. Grid space: 100 microns. (C) Automatically registered binary LM on EM. (D) Binary LM on binary EM.



# Construct your own automatic registration workflow by visual programming



Press me!

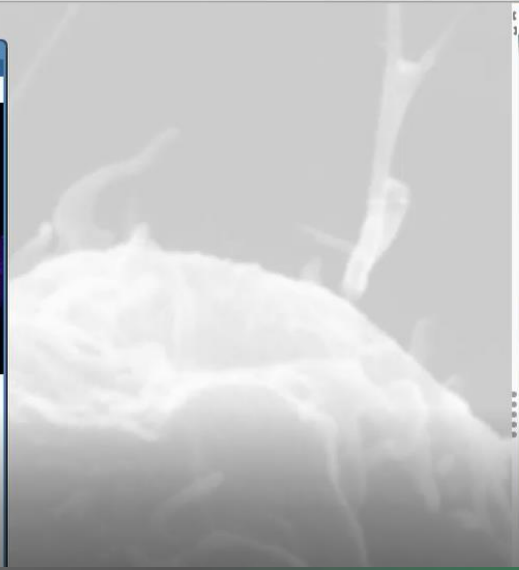
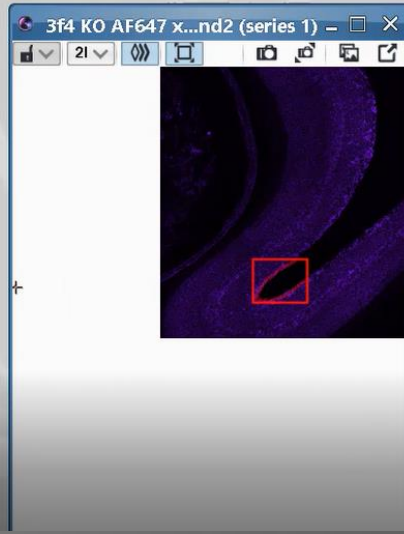


Image / Sequence    Region Of Interest    ImageJ    Detection & Tracking    Processing    Tools    Test    Plugins

Area

- Rectangle
- Point 2D
- Point 3D
- to 3D stack
- Separate
- Union
- Fill interior
- Load ROI(s)
- Ellipse
- Line 2D
- Line 3D
- to Mask
- ROI Cutter
- Intersection
- Fill exterior
- Save ROI(s)
- Polygon
- Polyline 2D
- Polyline 3D
- to Shape
- Other operation
- 0
- Excel export

2D ROI    3D ROI    Conversion    Separation    Boolean Op    Fill operation    File



Sequence ROI Layer

Canvas

18 %

Refresh Auto bounds

Ec-Clem AUTOFINDER

Version 1.0.0.2

Select Source Image with Rois on it: ROIObjective 40.00x.tif

Select Target Image with Rois on it: 3f4 KO AF6...(series 1)

▼ Metadata to check

Pixel size x,y of source in nanometers: 228

Slice spacing of source in nanometers: 1000

Pixel size x,y of target in nanometers: 420

Slice spacing of target in nanometers: 999,94

Transform Mode: Already in the same orientation

Also show the transformed target on source:

Export results for further analysis in ec-dem:

Max error allowed for testing in microns: 10

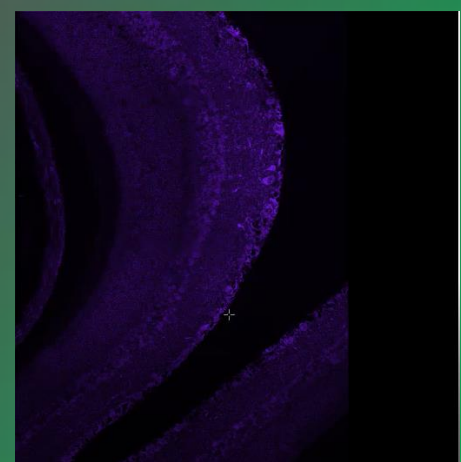
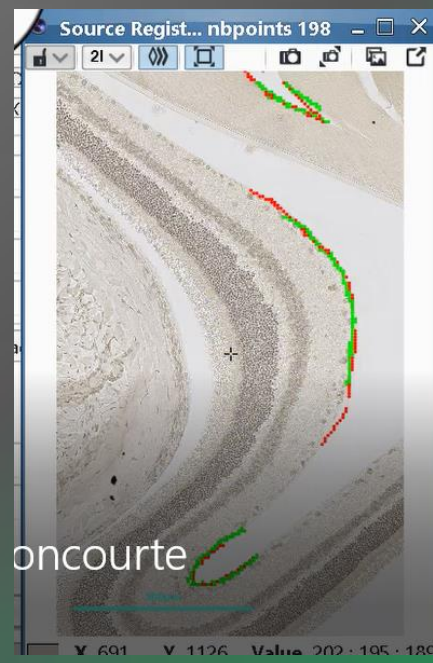
Percent point to keep for test: 70

scaling

Register from Lines Versioncourte

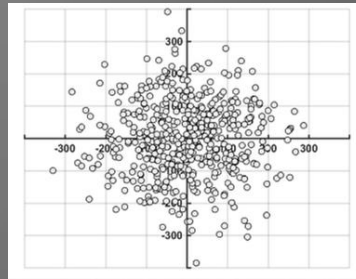
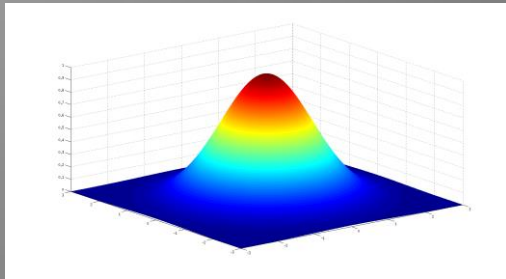
Running

X 712.2 Y -217... Val

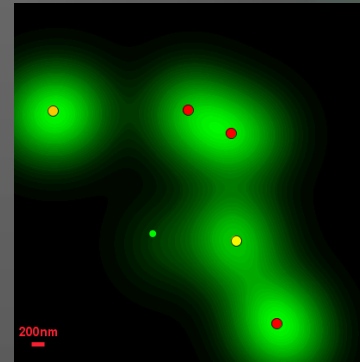




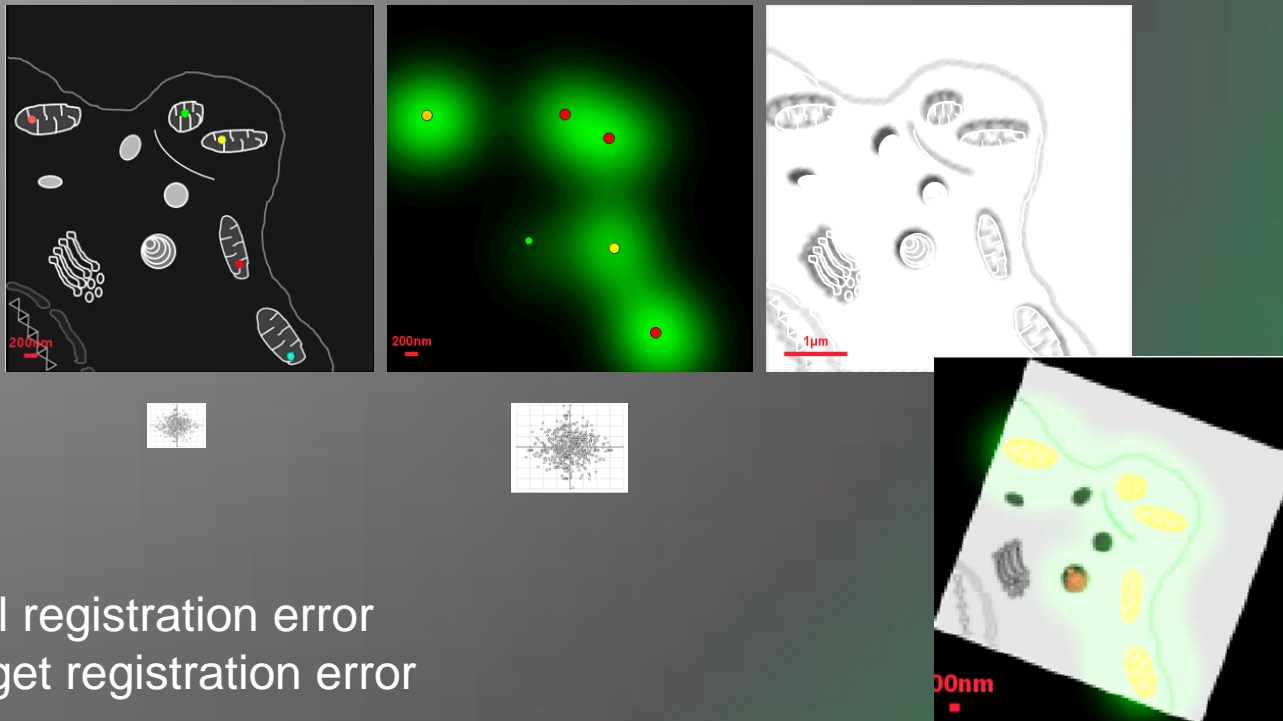
# Fiducial Localization Error



Individual points

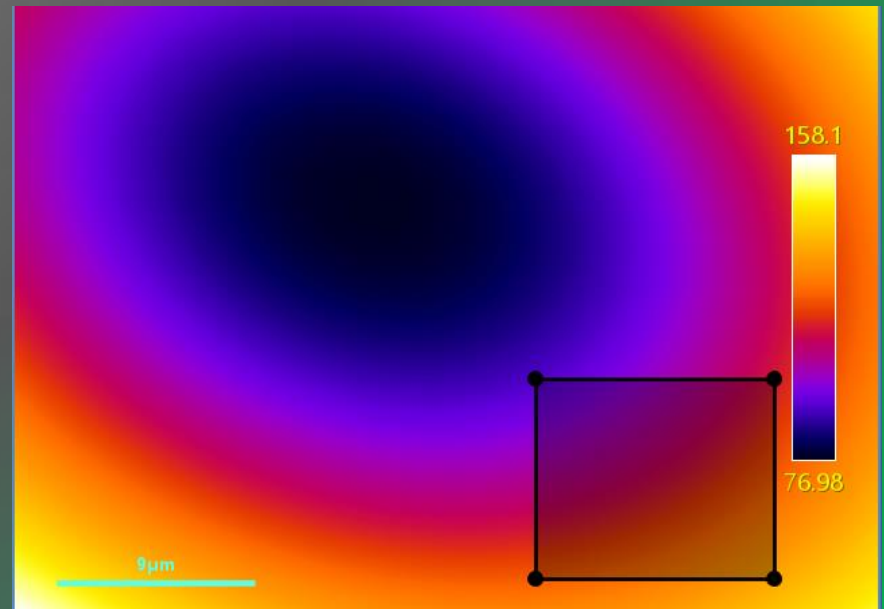
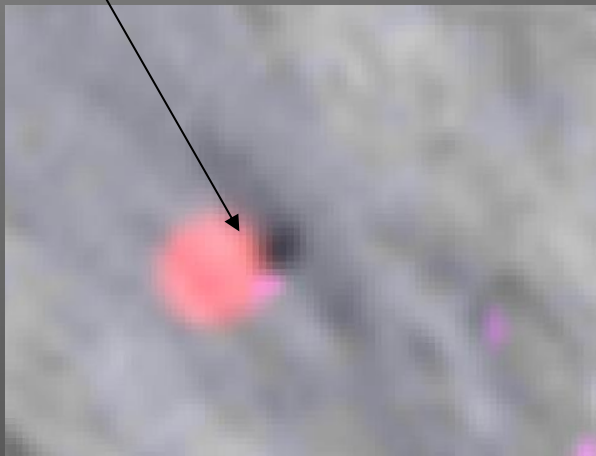
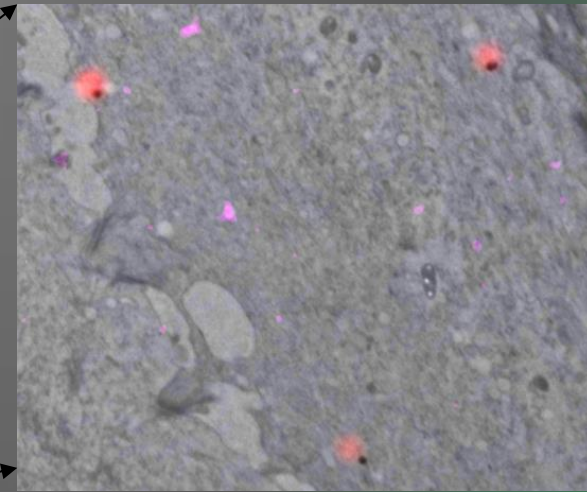
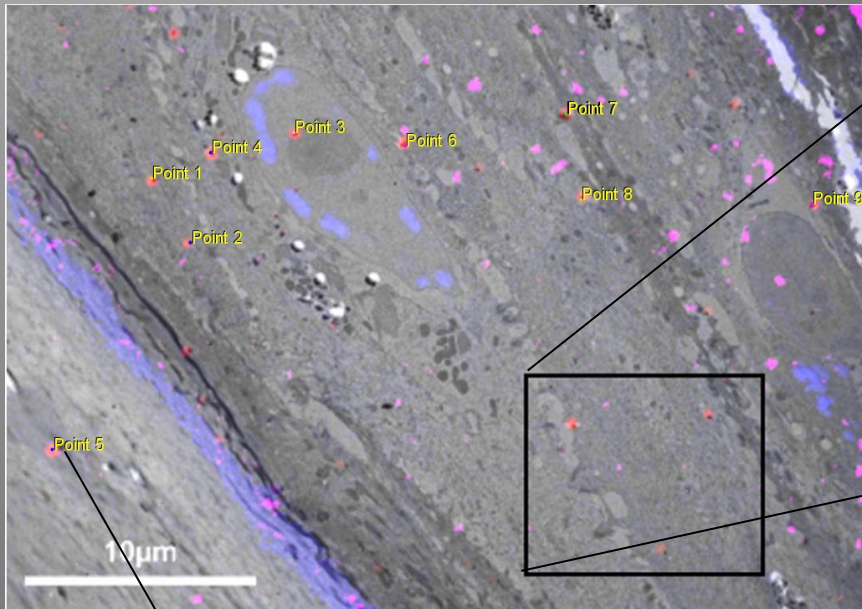


# Registration Error due to Fiducial localization error

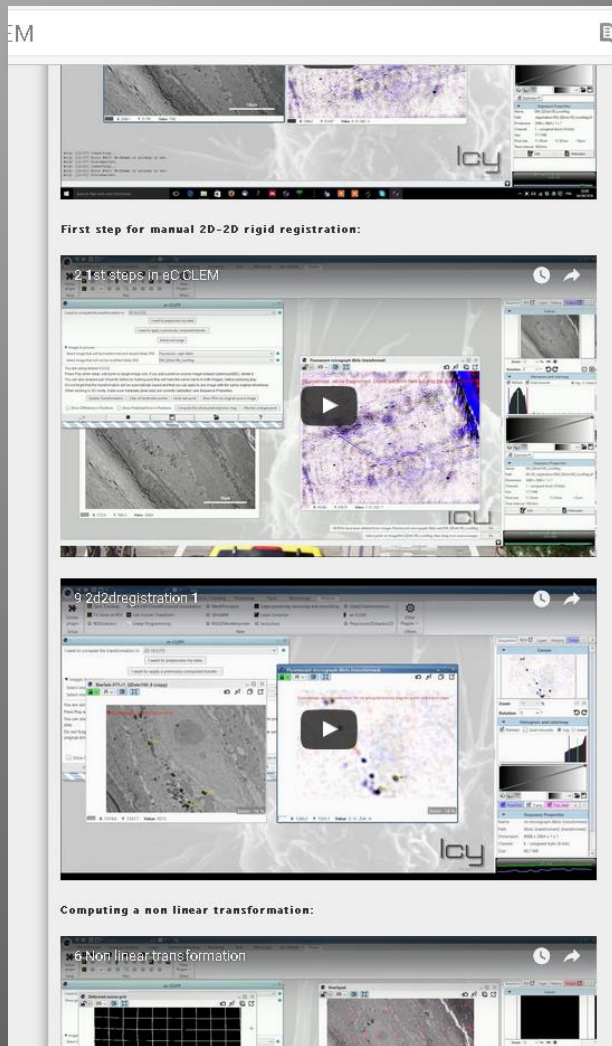


Fiducial registration error  
Vs Target registration error

Errors can be predicted and does not mean a non rigid deformation is needed.

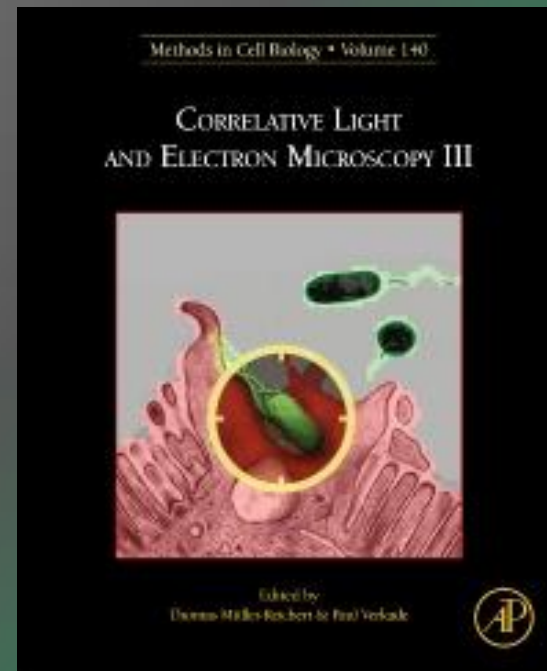


# Online Tutorials on Icy Bio Image Analysis website (look for ec-clem and ec-clem autofinder)



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

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



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



HIRING in Nantes, Pays de la Loire, France!!  
Statistics  
Computer Vision  
Perrine.paul-gilloteaux@univ-nantes.fr



### Micropicell

Manon Leroy  
Steven Nedellec  
Philippe Hulin  
Stéphanie Blandin  
Marin Mallocci  
Magalie Feyeux



### **Institut Pasteur JC Olivo Marin group**

Stephane Dallongeville  
Fabrice de Chaumont  
Alexandre Dufour

**Yannick Schwab Team**  
**EMBL, Heidelberg Germany**  
Neeraj Prabhakar

**Lucy Collinson group**  
Francis Crick Institute

**Diamond synchrotron X-Ray line**  
Maria Harkiolaki  
MRC UK Rob Parton

**Abo university Turku**  
Neeraj Prabhakar

### **INSTITUT Curie**

**Raposo Group , Salamero group & Institut Curie**  
Biolmaging Center  
**PICT-IBiSA**

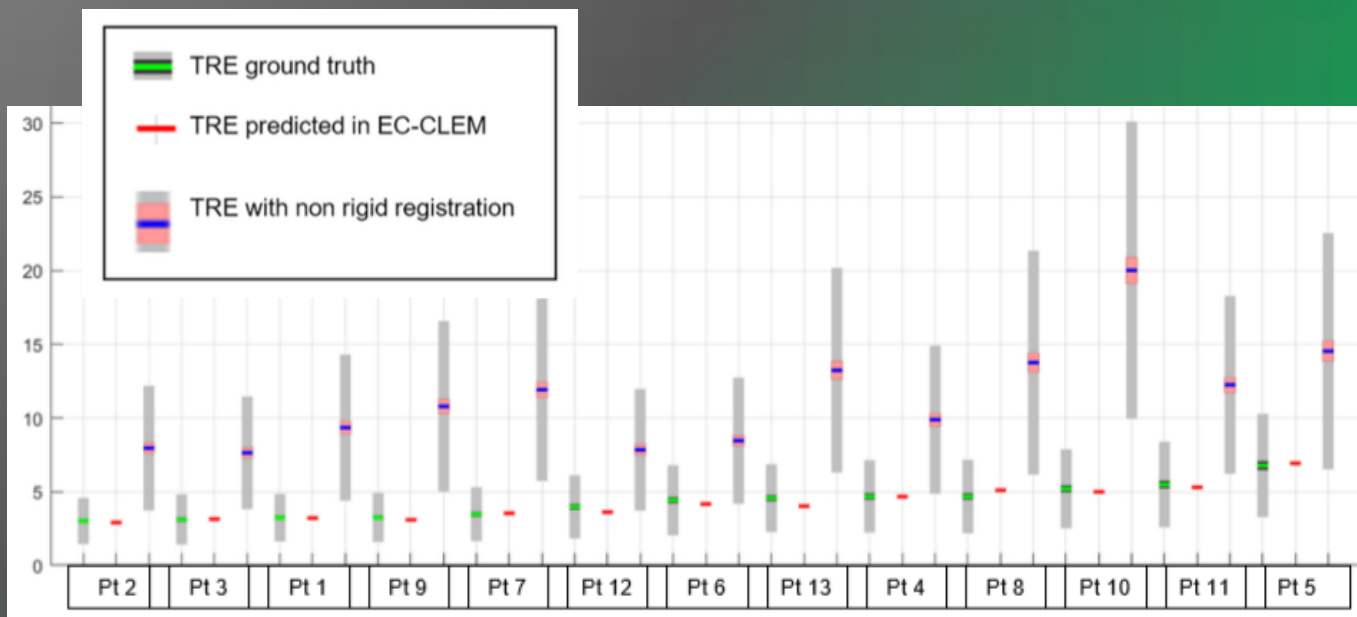
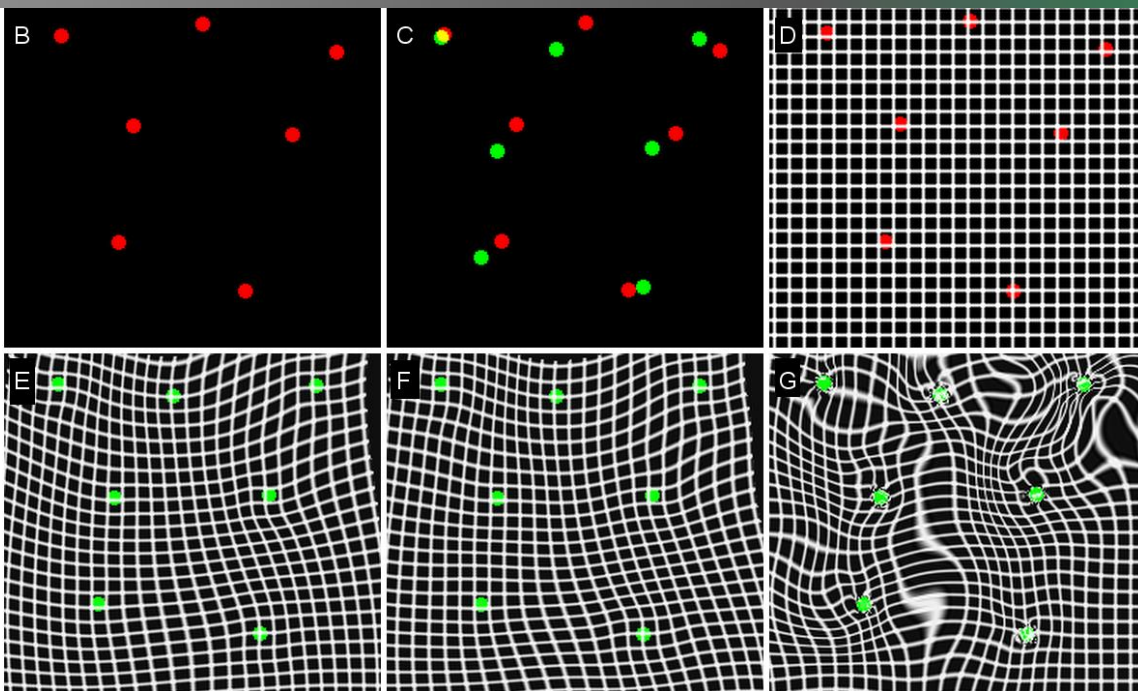
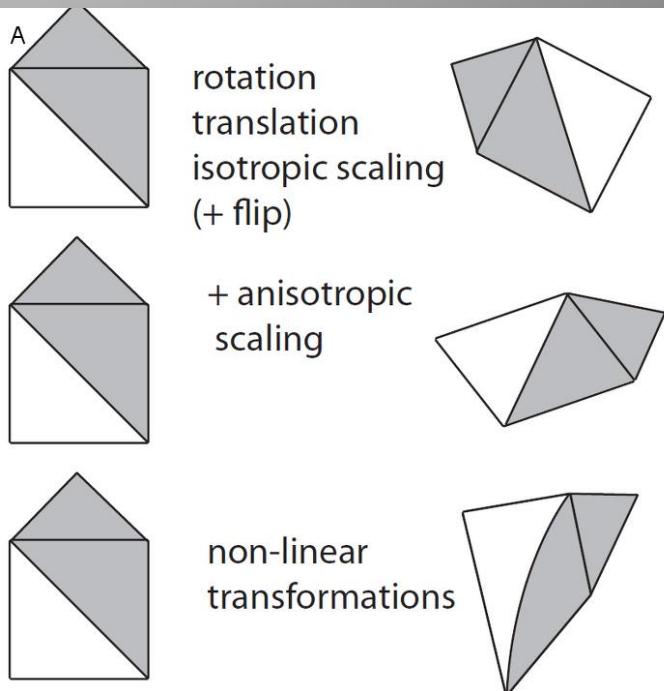
**C Kervrann group Inria rennes**

**Graça Raposo , Jean Salamero**

**Xavier Heiligenstein**

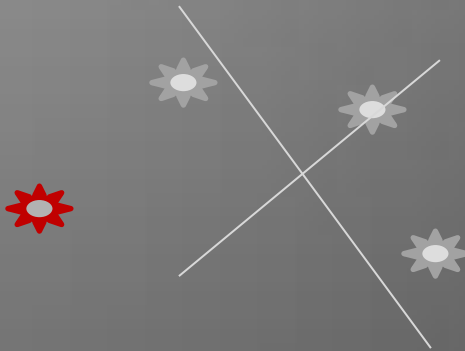






# Predicting error in any point from fiducial configuration and localization error

$$\langle TRE^2(r) \rangle = \langle FLE^2 \rangle \left( \frac{1}{N} + \frac{1}{3} \sum_{i=1}^3 \frac{d_i^2}{f_i^2} \right)$$



f : mean of squared distance from fiducials to each main axis

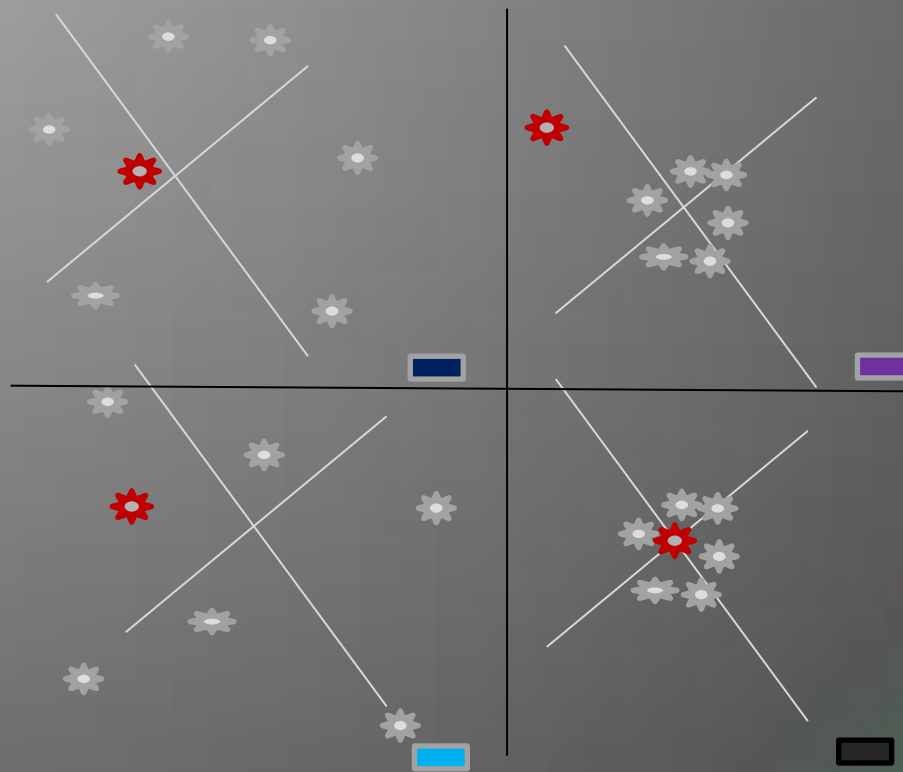
d : distance from target to each main axis

FLE: Fiducial Localization Error

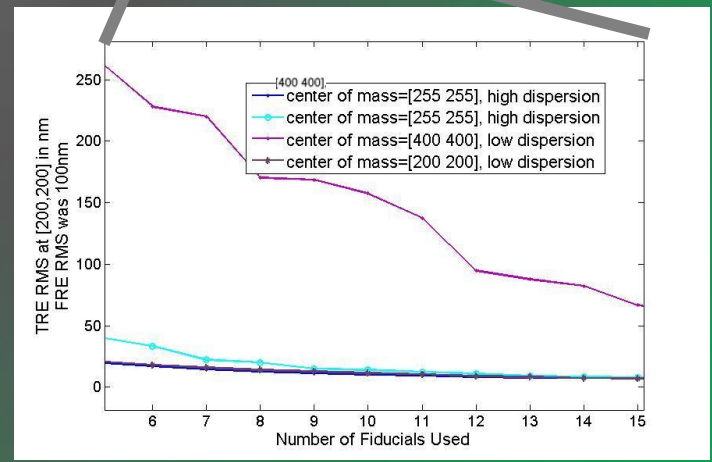
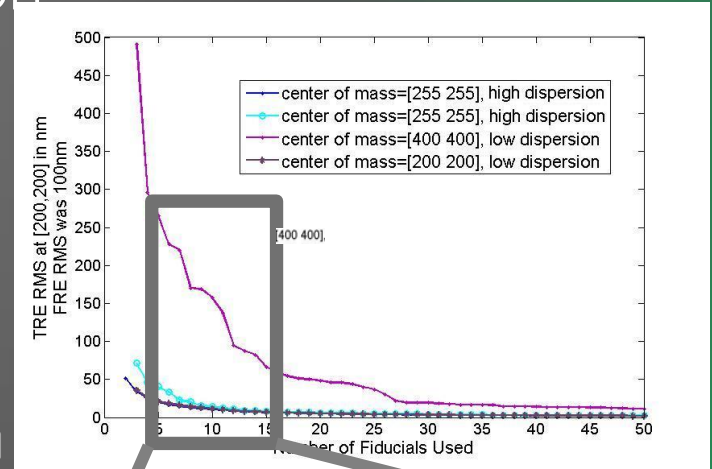
TRE: Target Registration Error

N: Number of fiducials

# Analysis of fiducial configuration

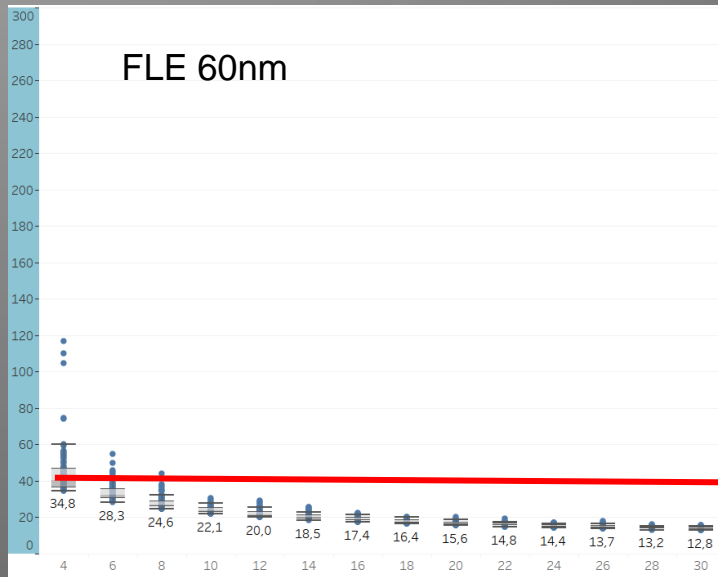
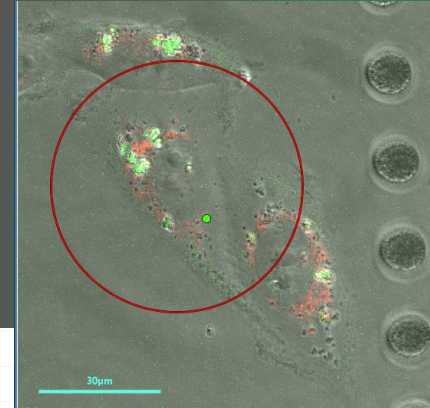


eC-CLEM > advanced usage > Study Errors

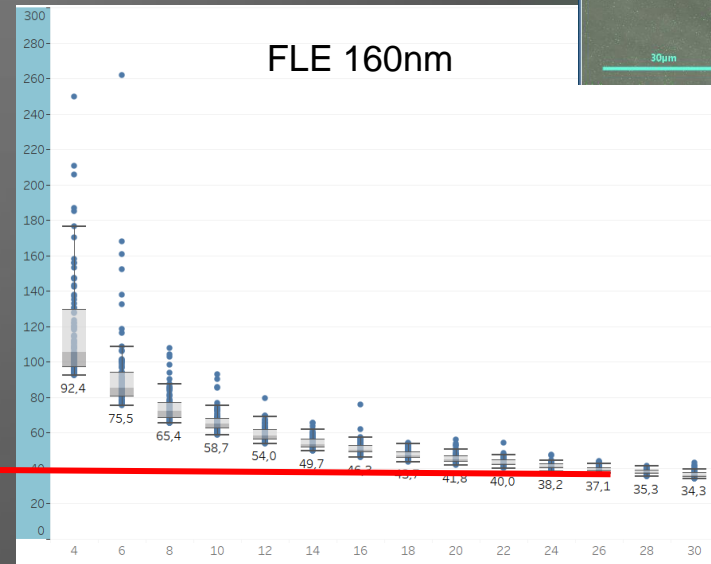


# What can simulation bring to your experiment? Optimizing fiducial choice!

- Nearly ideal case: widely distributed fiducials
- Simulate (Plugin StudyLandmarksConf againstN) different N and different FLE, 100 random configurations.



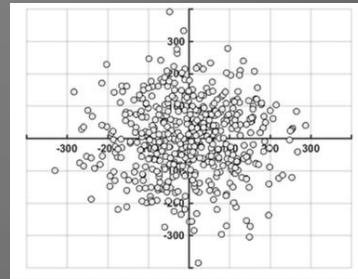
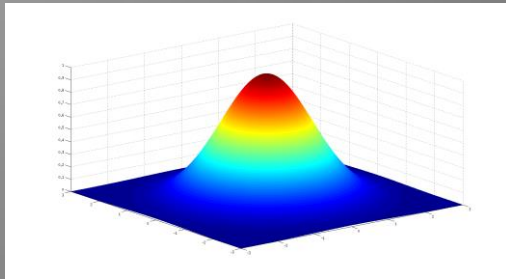
The better the accuracy of localization, the better the accuracy of the registration



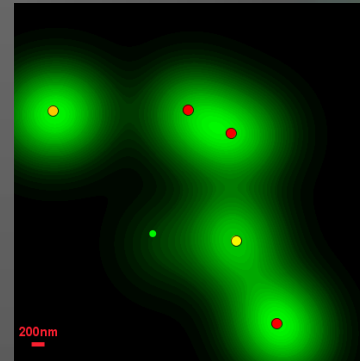
But... A big number of fiducial can compensate for a bad localisation accuracy

**TRY IT! EC-CLEM/ADVANCED/STUDY ERRORS**

# Fiducial Localization Error

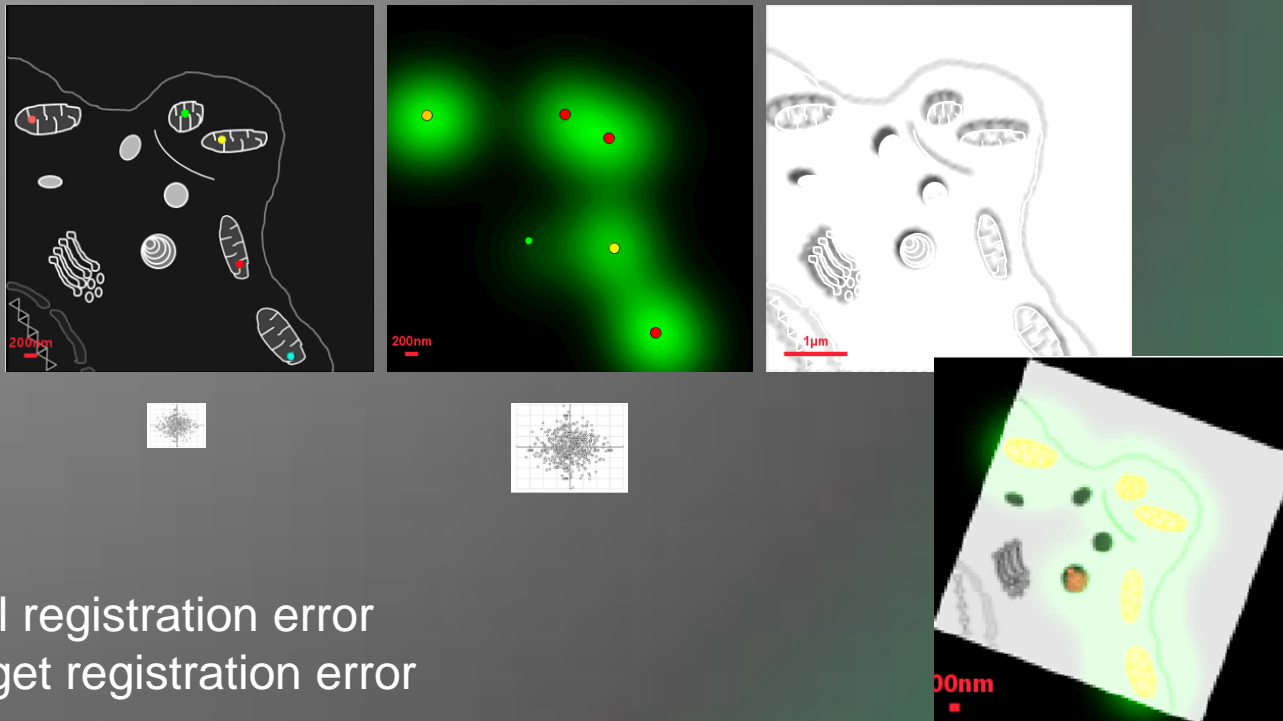


Individual points





# Registration Error due to Fiducial localization error



Fiducial registration error  
Vs Target registration error