

CORRELATIVE MICROSCOPIES: IMAGE REGISTRATION FOR CLEM AND FOR DEEP LEARNING

WHAT CAN BRING ERROR ESTIMATION ?

Perrine.paul-gilloteaux@univ-nantes.fr

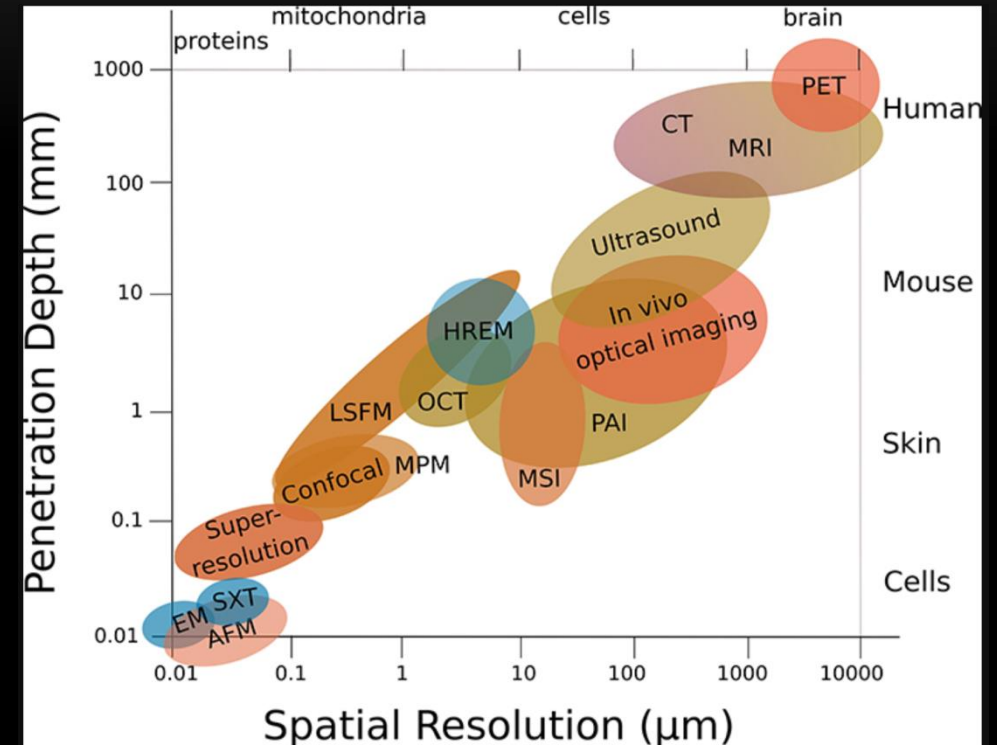
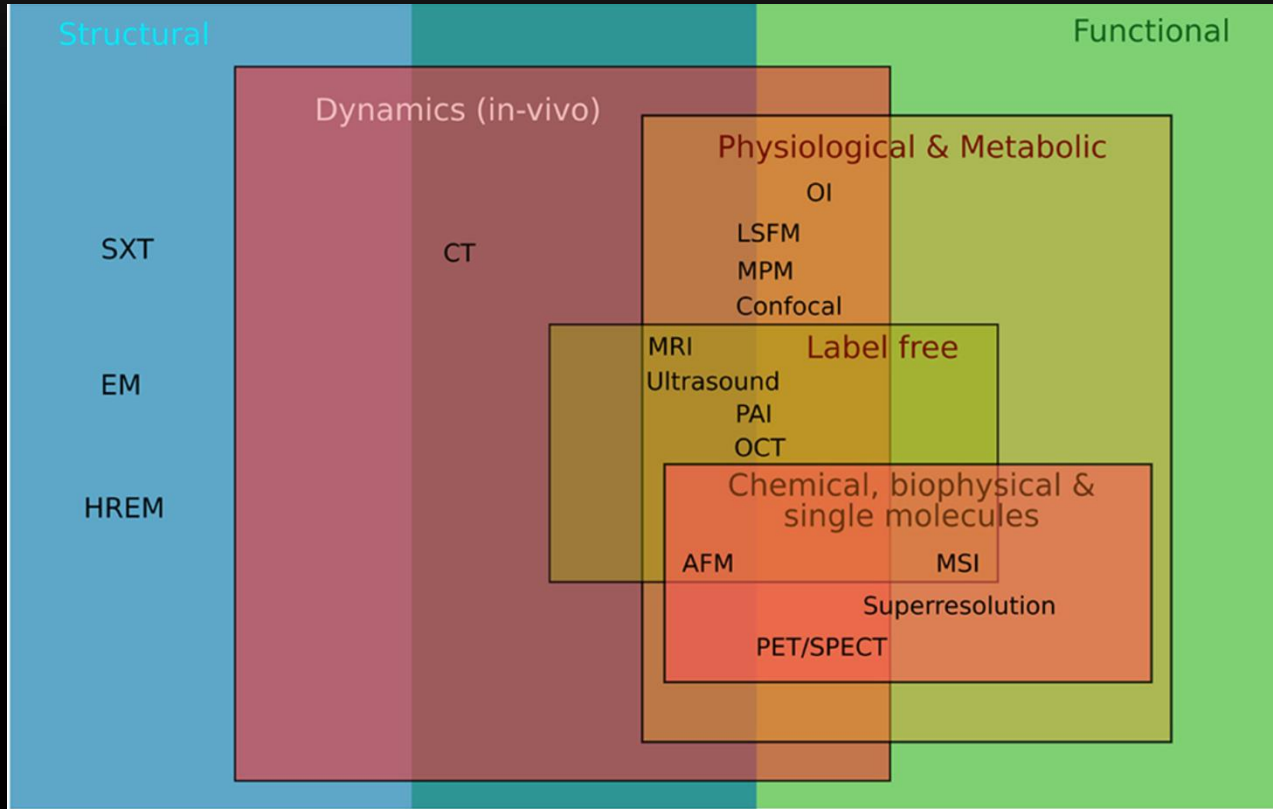
Nantes Université, CHU Nantes, CNRS, Inserm, BioCore, SFR Bonamy, Nantes, France

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



WHAT IS CORRELATIVE MICROSCOPIES?

MULTIMODAL IMAGING FOR AN HOLISTIC VIEW



Walter, Paul-gilloteaux 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)

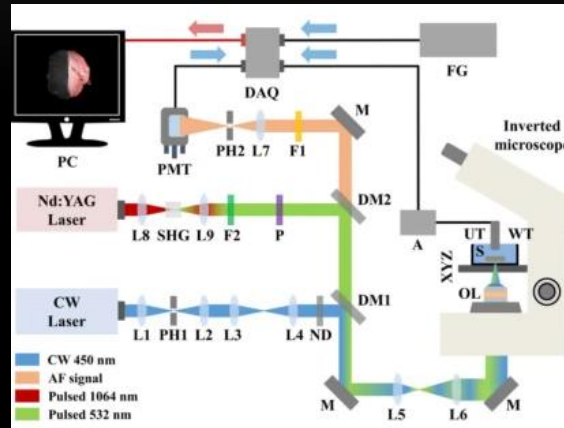
THEN COME THE NATURAL NEED TO FUSE THEM

- 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)

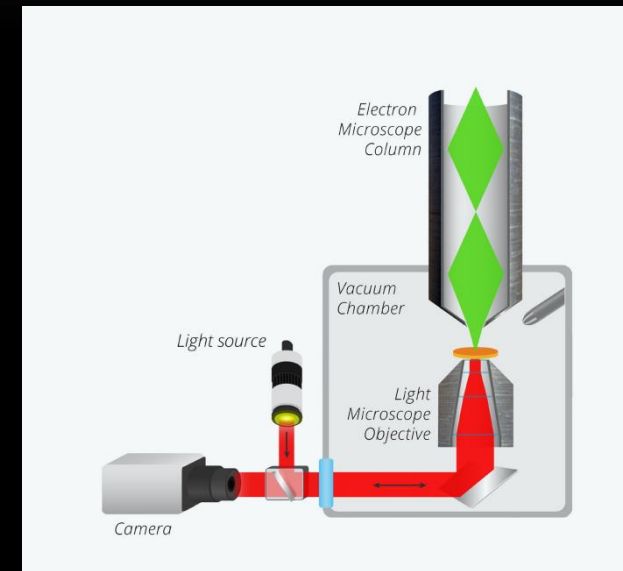


Cal-Gonzales et al. Front. Phys., 18 May 2018
 Sec. Medical Physics and Imaging
<https://doi.org/10.3389/fphy.2018.00047>



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

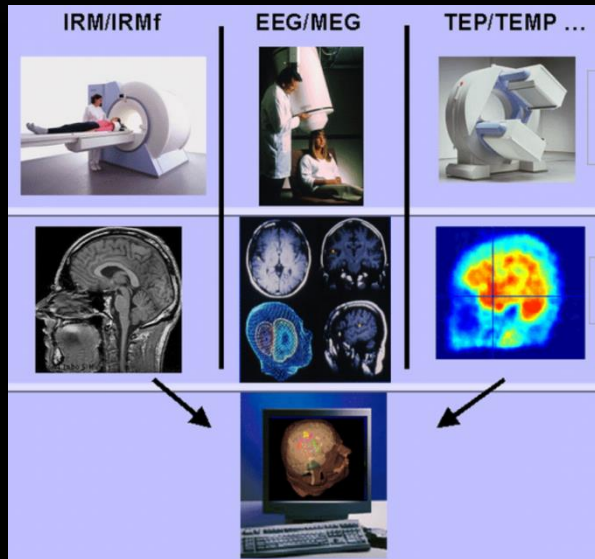
Confocal fluorescence
 + Photo Acoustic



SECOM from DELMIC integrated CLEM

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

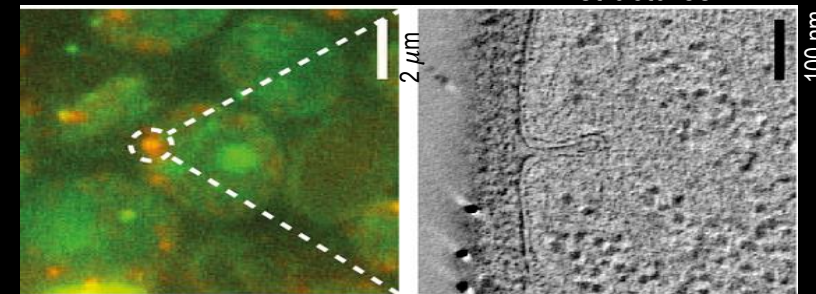
SOLUTION 2 : CORRELATIVE MICROSCOPY WITH SAMPLE TRANSFER BETWEEN ONE OR SEVERAL MODALITIES



P Jannin, Visages team 2005

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

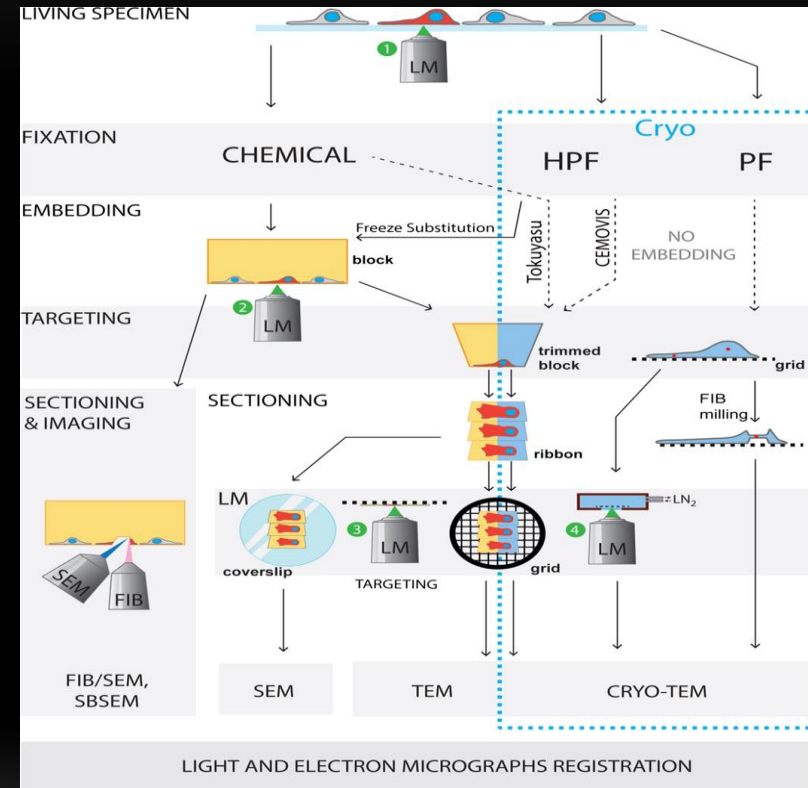
SOLUTION 2 : CORRELATIVE MICROSCOPY WITH SAMPLE TRANSFER BETWEEN ONE OR SEVERAL MODALITIES

+ limited by your imagination

- (and a few technical details)

Example in CLEM:

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



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

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

APPROACHES FOR AUTOMATIC 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?

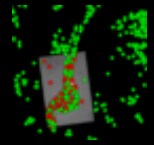
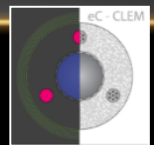
yes

no

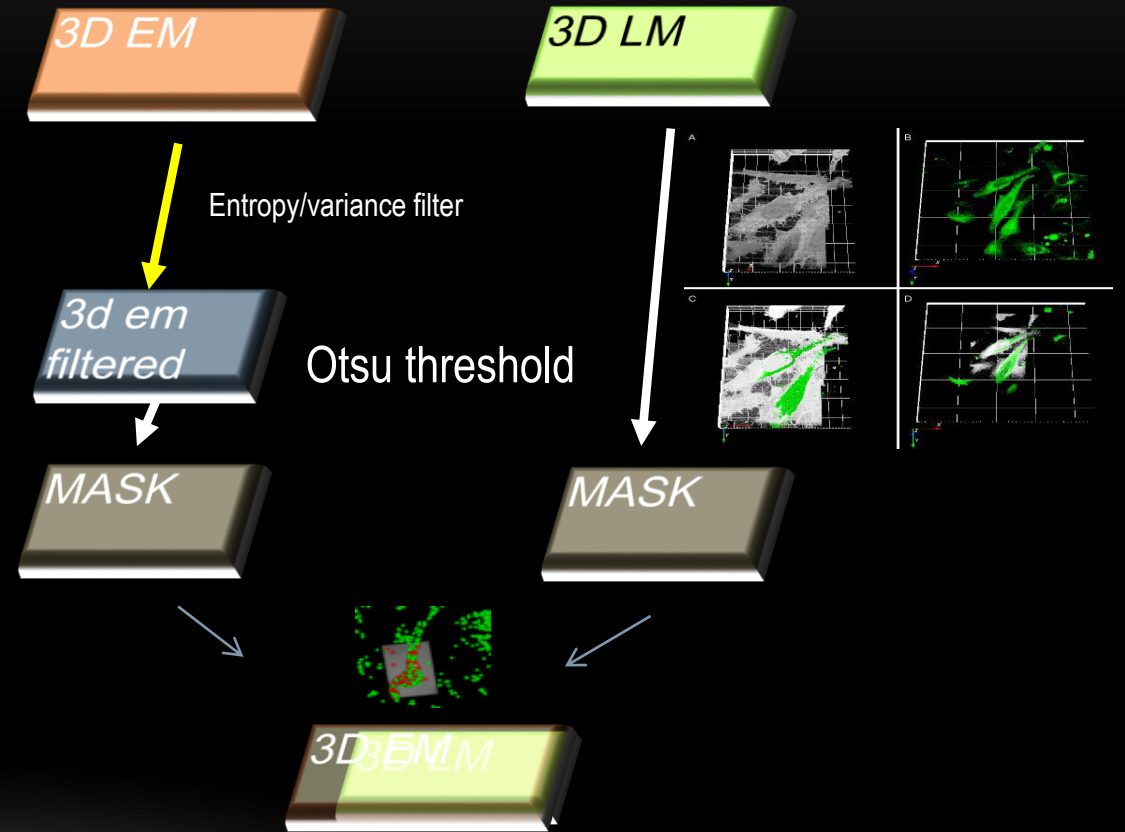
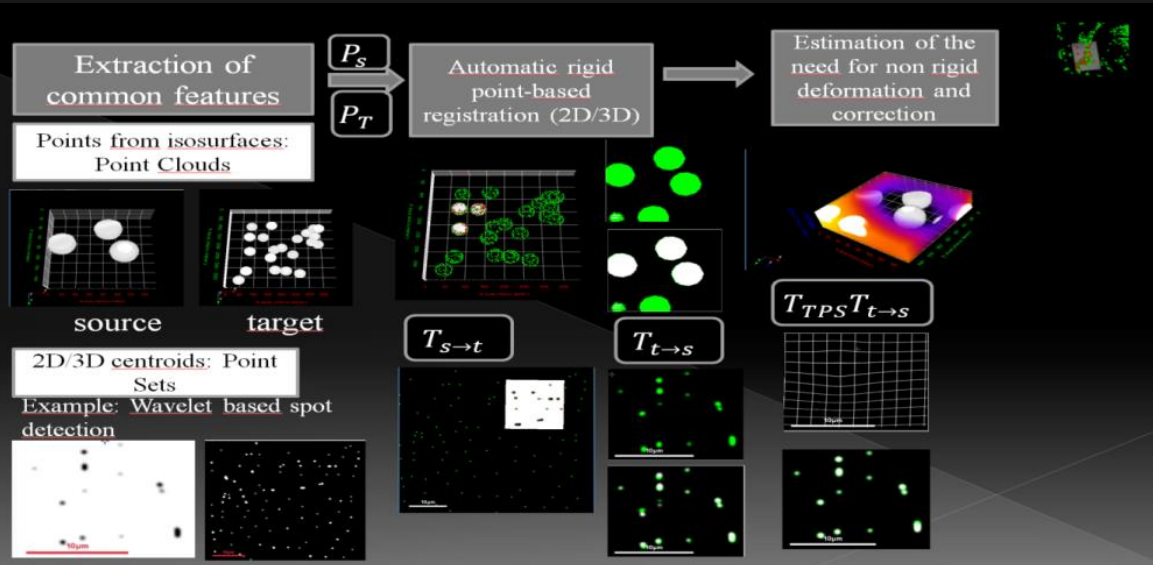
Compute and apply transfo

Propose matching

Compute and apply transfo

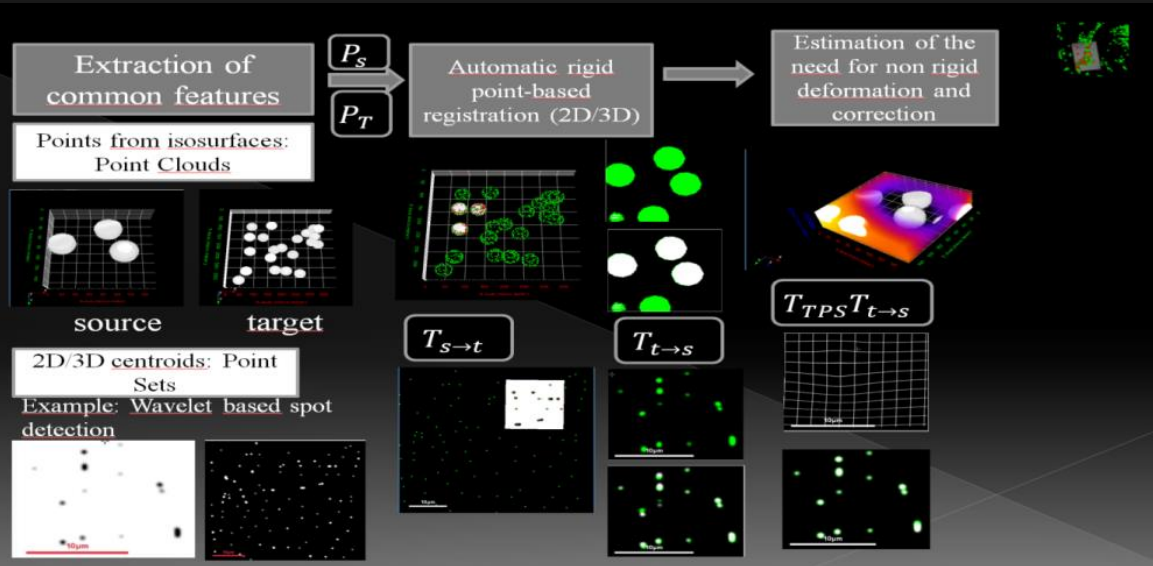


GENERIC APPROACH FOR AUTOMATIC REGISTRATION



Feature extraction

GENERIC APPROACH FOR AUTOMATIC REGISTRATION



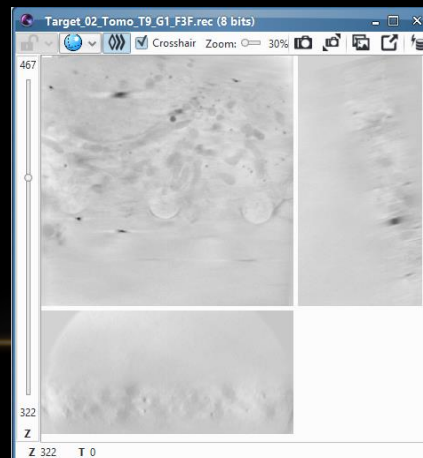
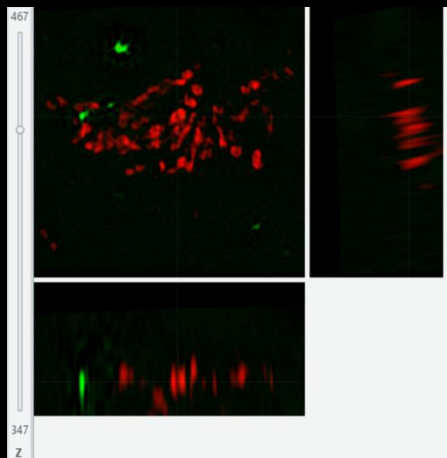
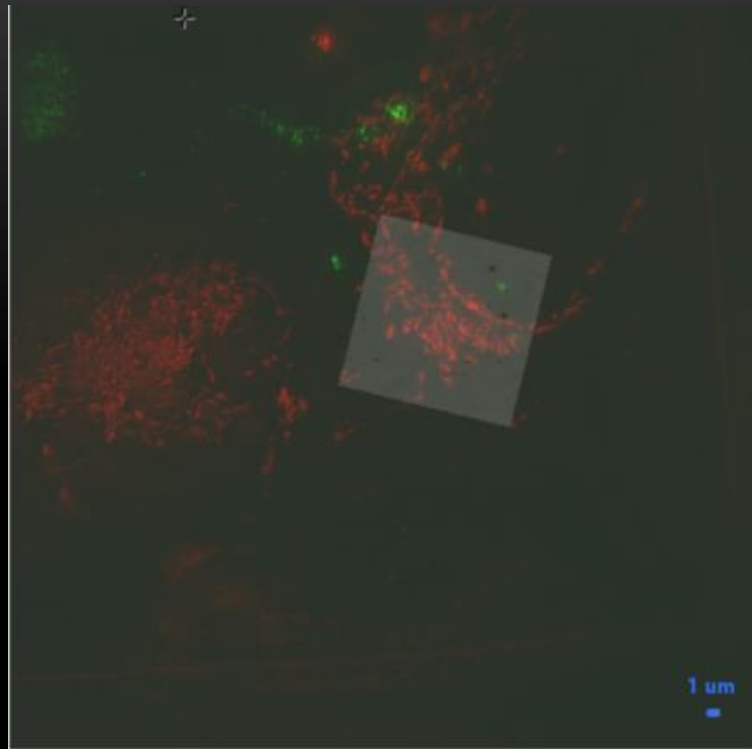
3D EM

3D LM

Wavelet Spot
detection

Wavelet Spot
detection

3D₃EMLM



Cell

CellPress
OPEN ACCESS

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 Hellgenstein,⁷ Hongchang Wang,¹ Chidinma A. Okolo,¹ Thomas M. Fish,¹ Matthew C. Spink,¹ David I. Stuart,^{1,4} Ilan Davis,³ Steeve Boulant,^{8,9} Jonathan M. Grimes,^{1,4} Ian M. Dobbie,⁹ and Maria Harkiolaki^{1,11,*}

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⁵Université de Nantes, CNRS, INSERM, l'institut du thorax, Nantes, France

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⁷CryoCapCell, 155 Boulevard de l'hôpital, 75013 Paris, France

⁸Department of Infectious Diseases, Virology, Heidelberg University Hospital, 69120 Heidelberg, Germany

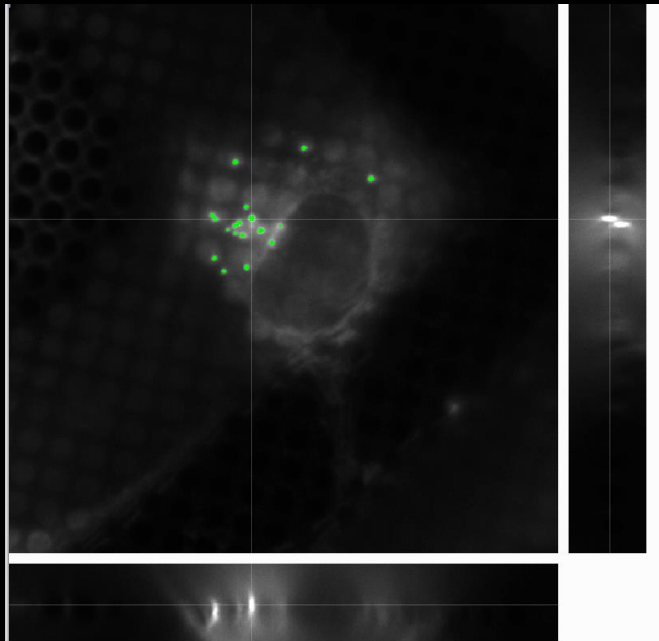
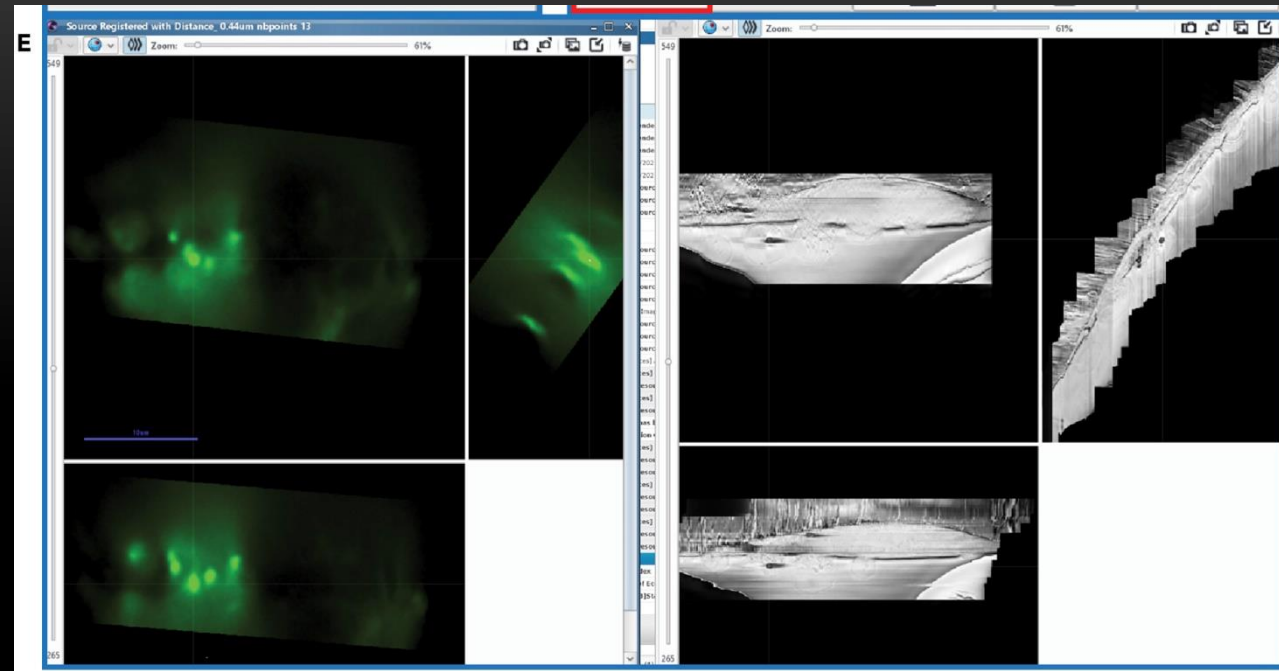
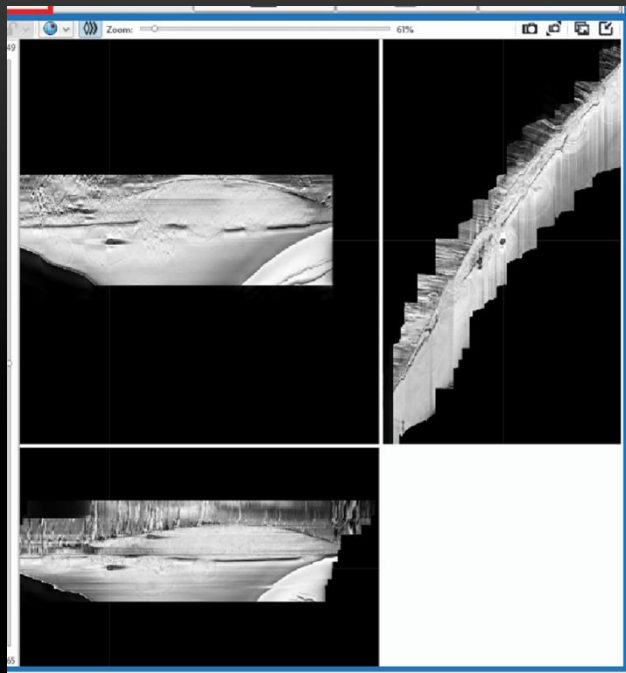
⁹Research Group "Cellular polarity and viral infection," German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany

¹⁰These authors contributed equally

¹¹Lead Contact

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<https://doi.org/10.1016/j.cell.2020.05.051>



iScience



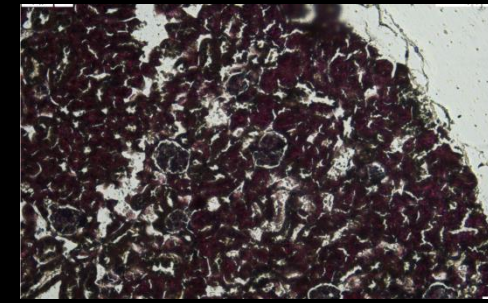
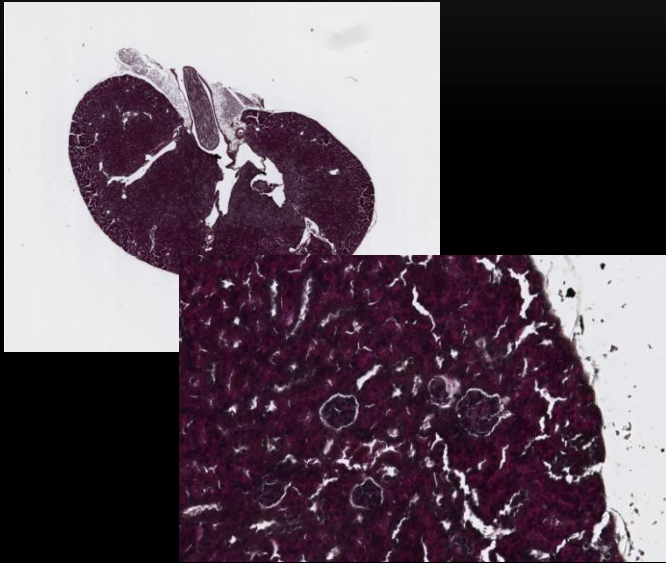
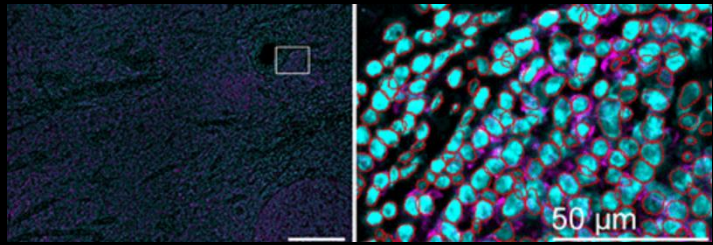
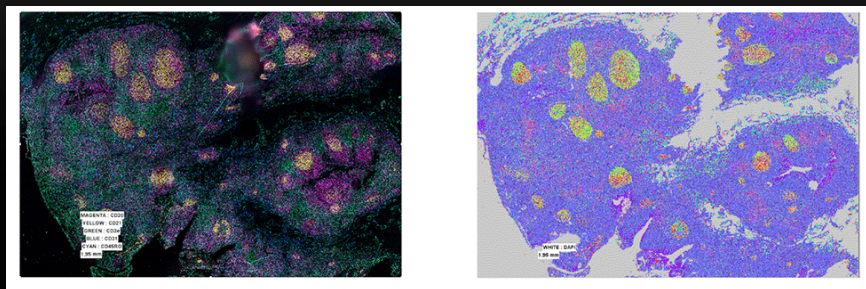
Volume 24, Issue 7, 23 July 2021, 102714

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} [ORCID](#) [Email](#)

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



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

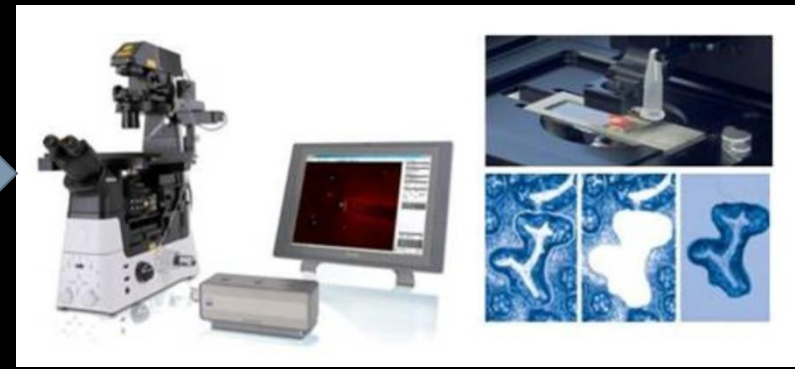
PCR
Sequencing
etc...



Phenocycler/Codex for fluorescence hyperplexing

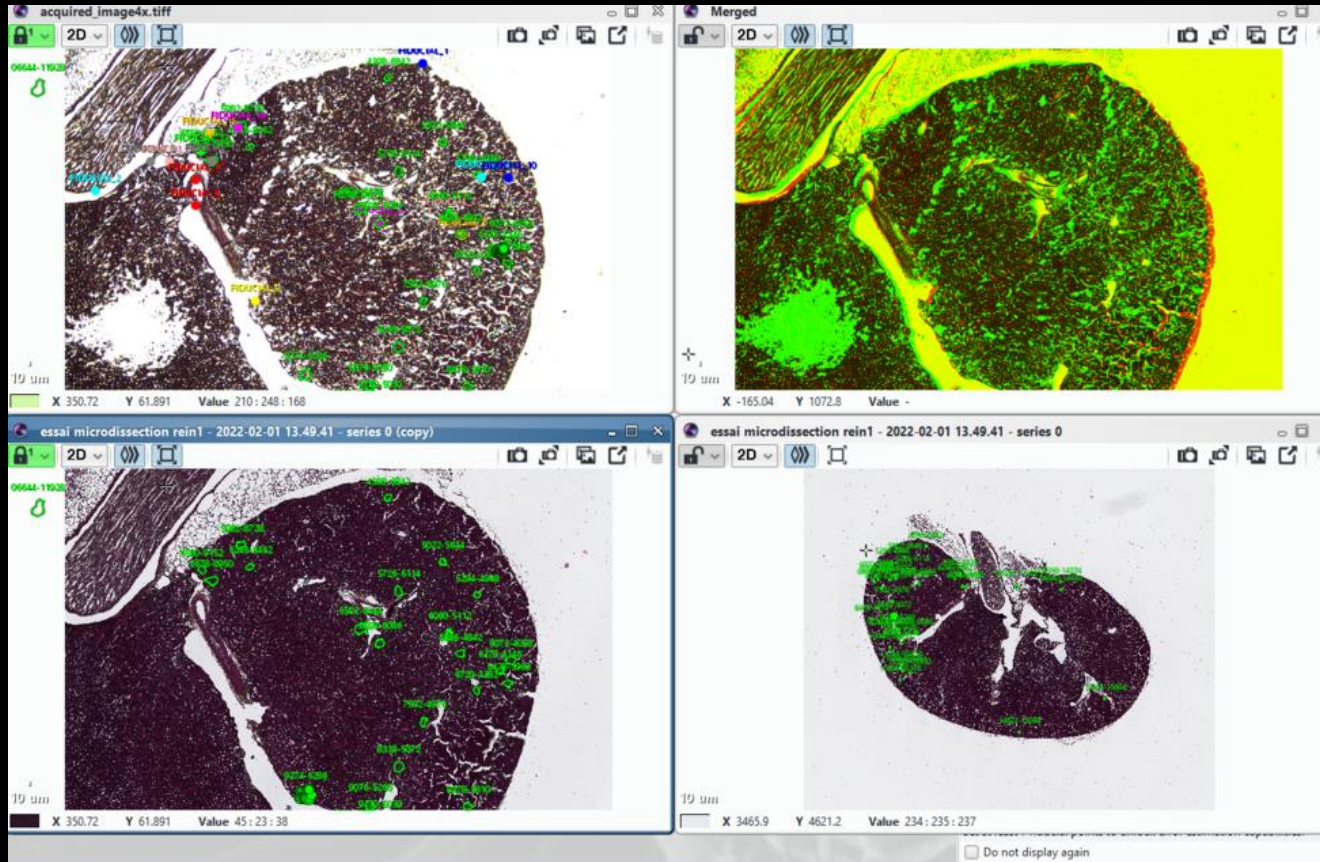


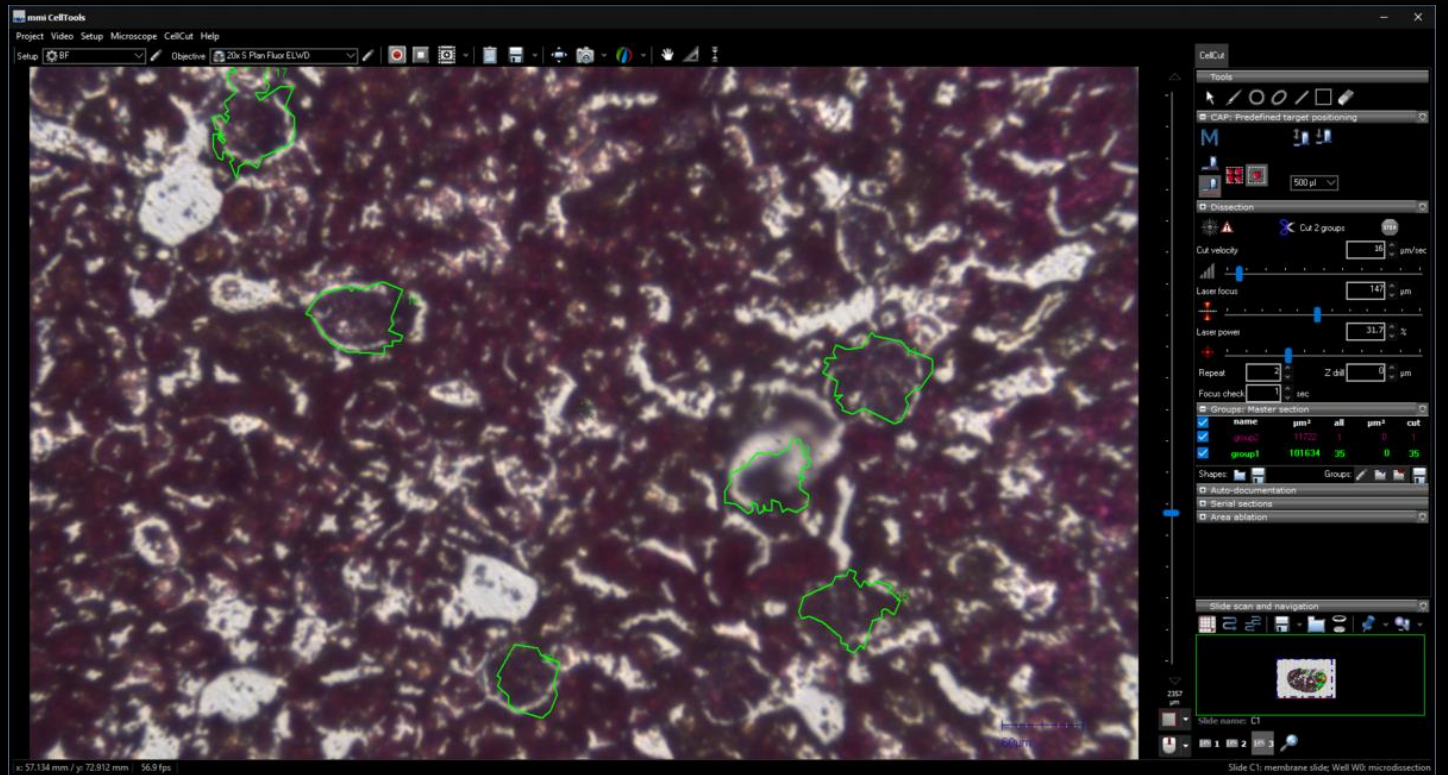
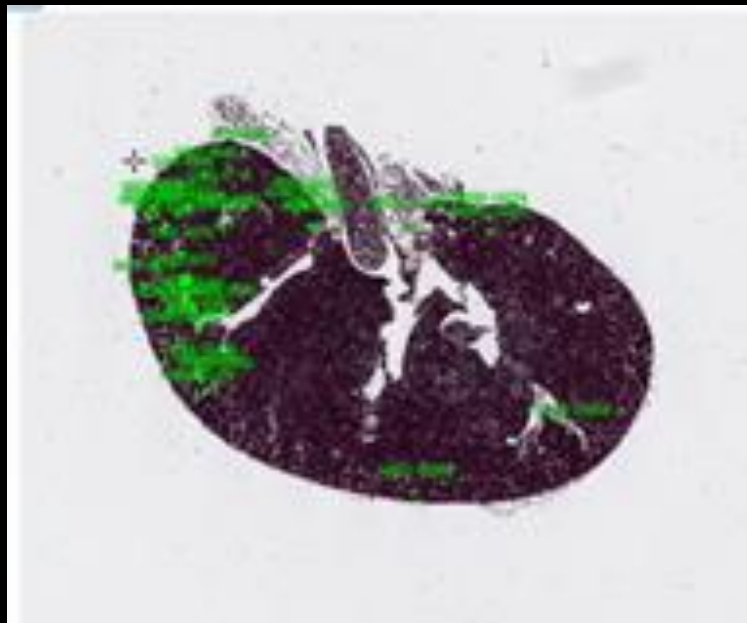
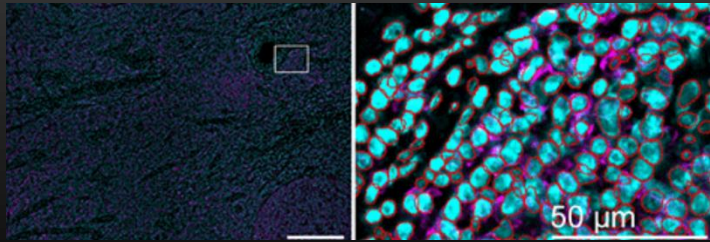
Slide Scanner for fast full FOV



Microdissector (MMI cellcut)







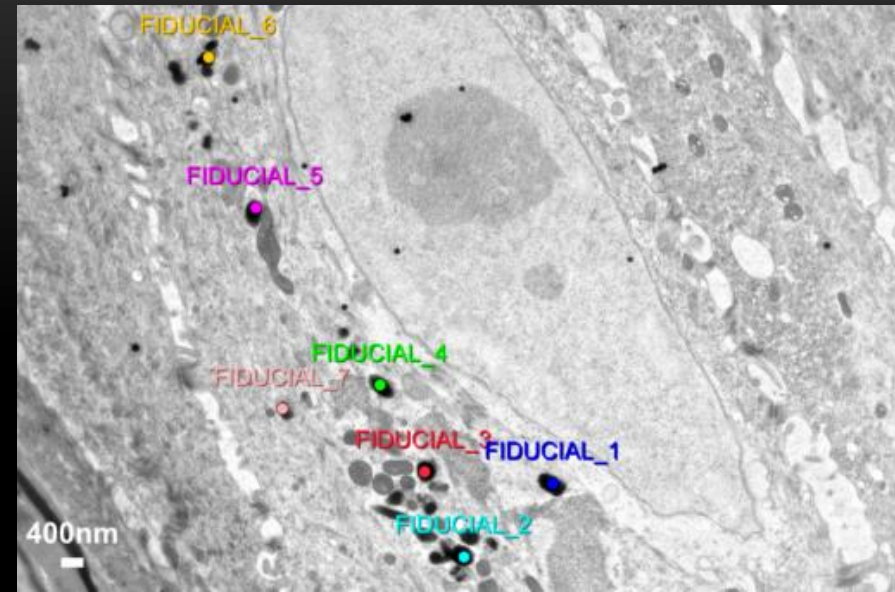
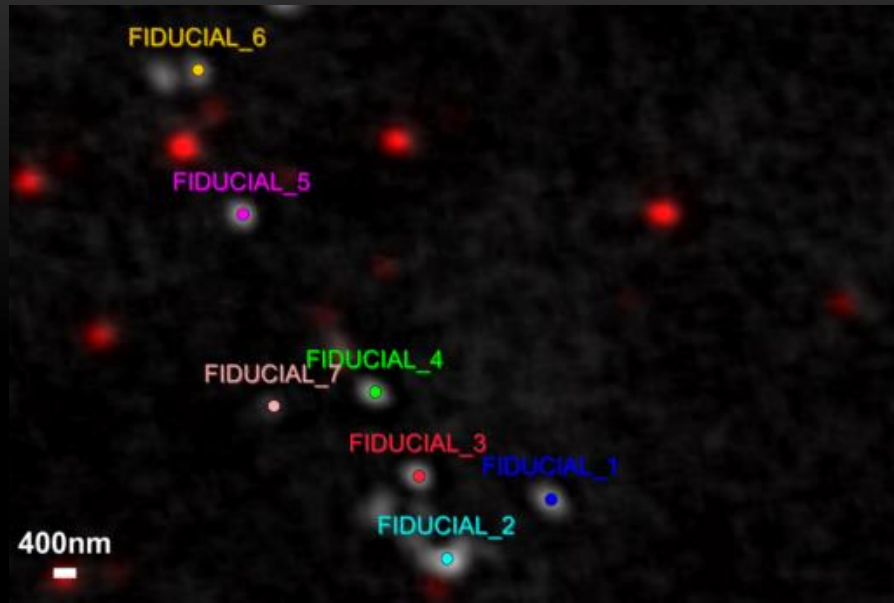
A home made interface (collaboration with MMI) allow to import these area of interest as s=« shape to be cut at the right position, at once.

POINT [-CLOUD] BASED REGISTRATION
ERROR

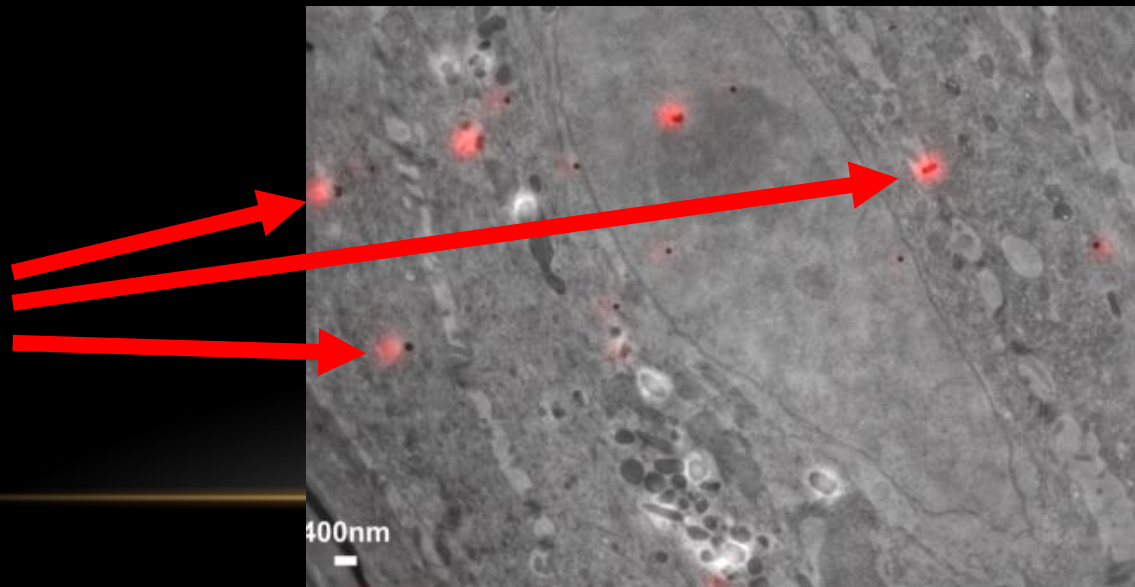
- One thing to know: Matching is NEVER PERFECT
 - Because your sample may deform/ evolve between two modalities
 - Because multimodality is usually also multiscale (very different resolution, one pixel can match hundred of pixels in the second modality)
 - Because none modality allows to perfectly locate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

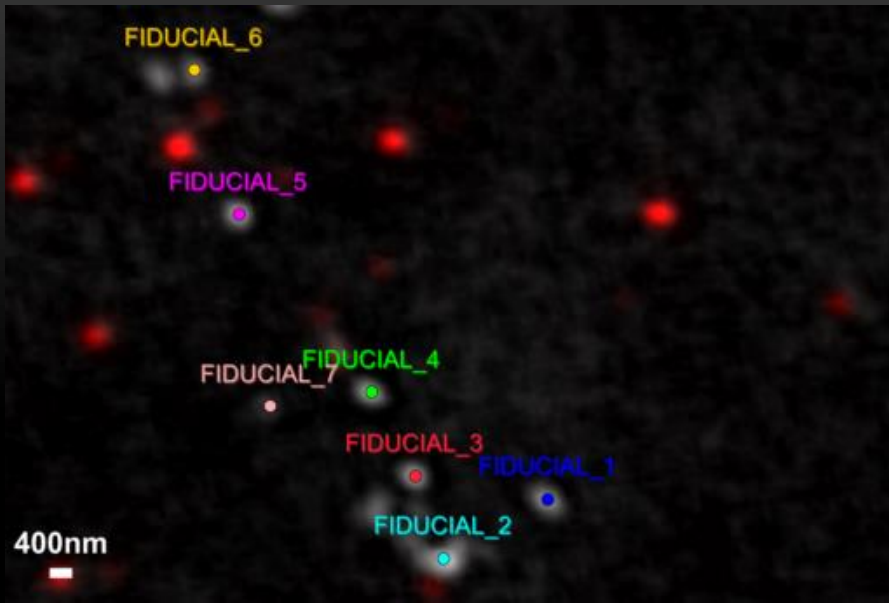
WE HAVE DEVELOPED A METHOD TO SPATIALLY ASSESS THE QUALITY OF REGISTRATION WITH NO GROUND TRUTH (NO OTHER INPUT THAT POINTS (OR SURFACE) USED FOR REGISTRATION: LOCALISATION NOISE AND ERROR REGISTRATION ARE ESTIMATED).

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.

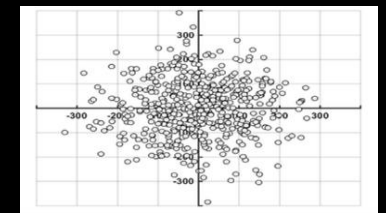
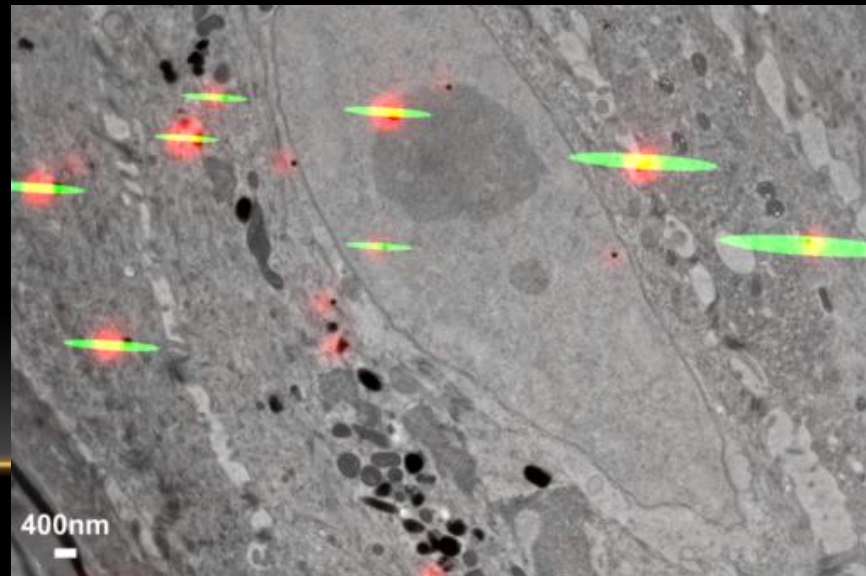
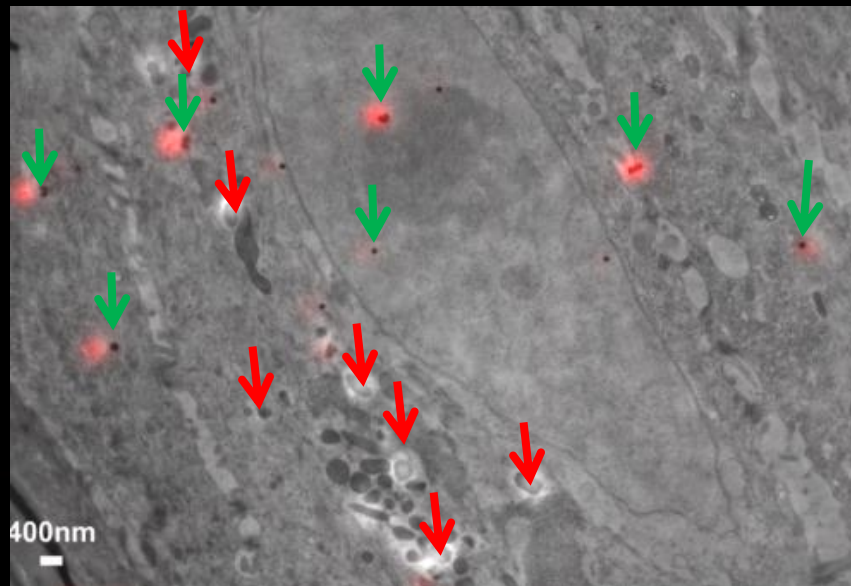


Beads are used here to check how good we are to estimate the error





Expectation of the distribution of accuracy at each point

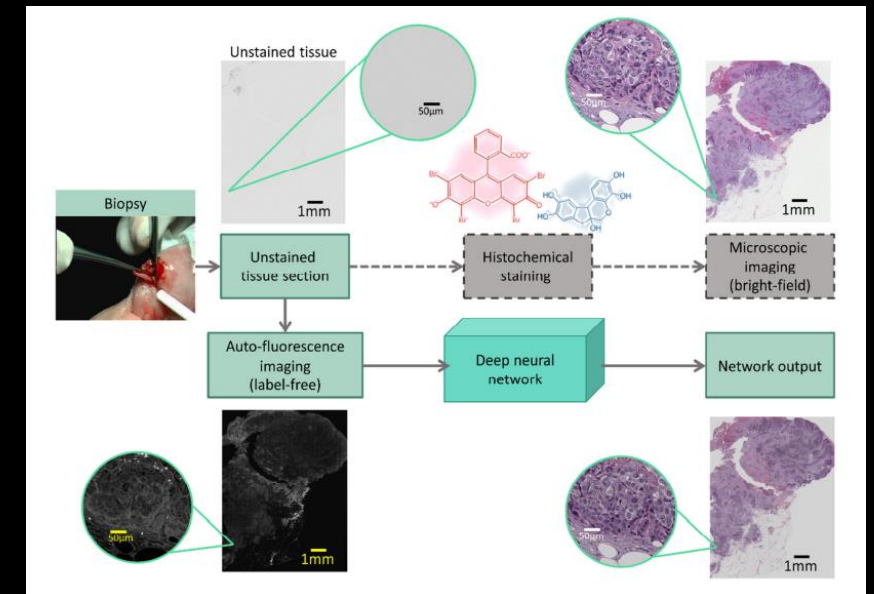
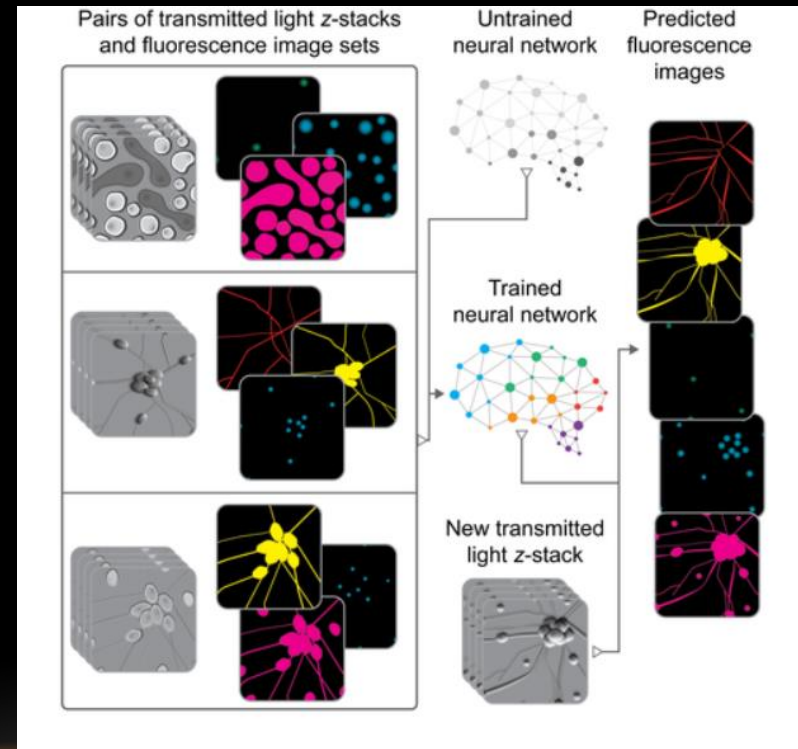
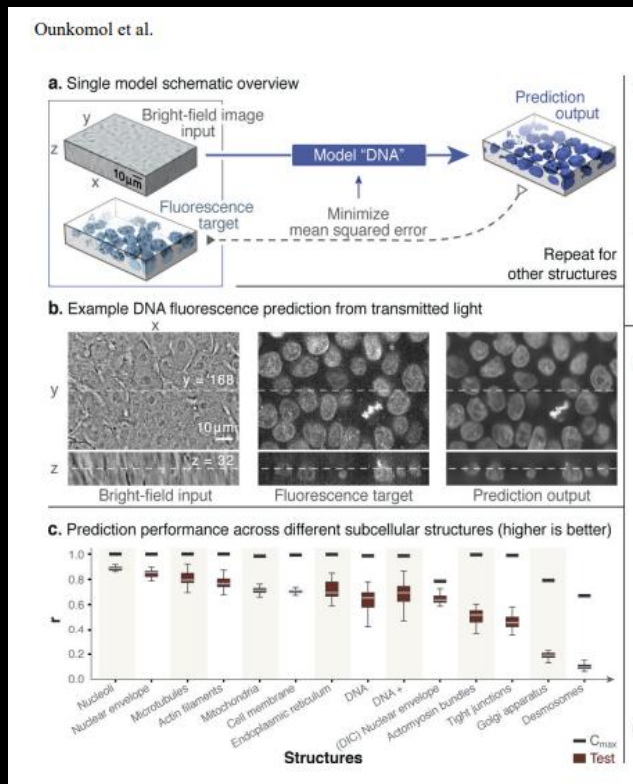


Computed confidence ellipses at 95% (Fisher)

USING REGISTRATION AND ERROR ESTIMATION IN DEEP LEARNING

DIFFERENT APPROACHES USE MULTIMODALITY AND REGISTRATION TO LEVERAGE THE BURDEN OF ANNOTATIONS

- In silico labelling



Ounkomol et al. Nat Methods. 2018
November ; 15(11): 917–920.
doi:10.1038/s41592-018-0111-2

Christiansen et al. Cell. 2018
Apr 19;173(3):792-803.e19.
doi: 10.1016/j.cell.2018.03.040

Rivenson et al. Nat Biomed Eng 3, 466–477
(2019). <https://doi.org/10.1038/s41551-019-0362-y>

DIFFERENT APPROACHES USE MULTIMODALITY AND REGISTRATION TO LEVERAGE THE BURDEN OF ANNOTATIONS

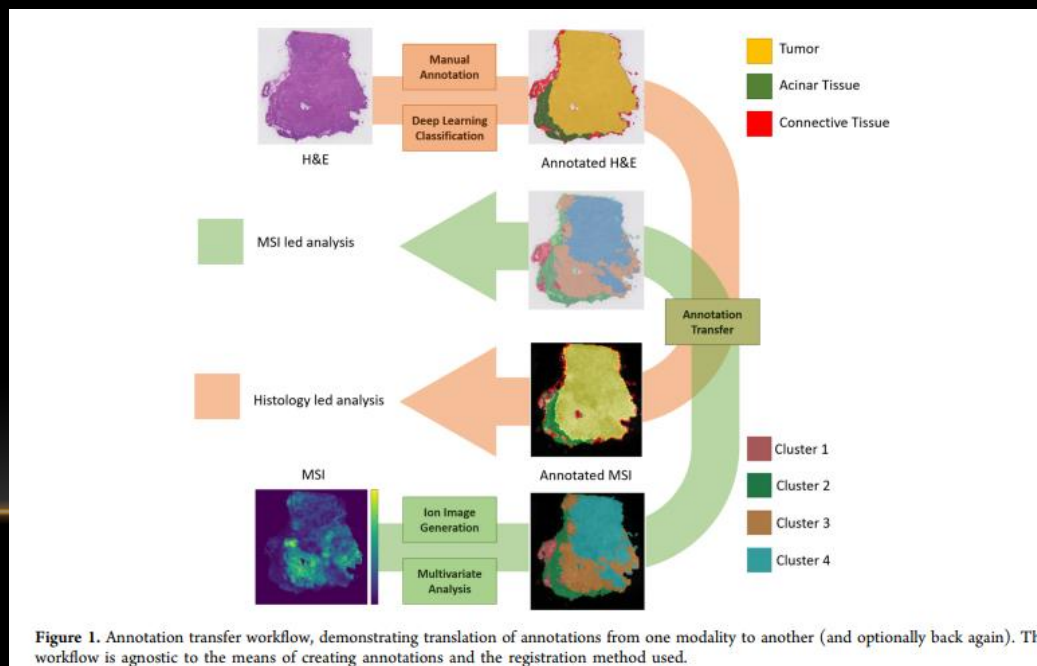
- In silico labeling
 - Denoising/ image restoration (usually from the same microscope, using low and high exposure time for example)
 - Instance segmentation: automatic or manual labeling from another modality
 - Classification
-

DIFFERENT APPROACHES USE MULTIMODALITY AND REGISTRATION TO LEVERAGE THE BURDEN OF ANNOTATIONS

- In silico labeling
- Denoising/ image restoration (usually from the same microscope, using low and high exposure time for example)
- Instance segmentation: automatic or manual labeling from another modality
- Classification

Deep Learning-Based Annotation Transfer between Molecular Imaging Modalities: An Automated Workflow for Multimodal Data Integration

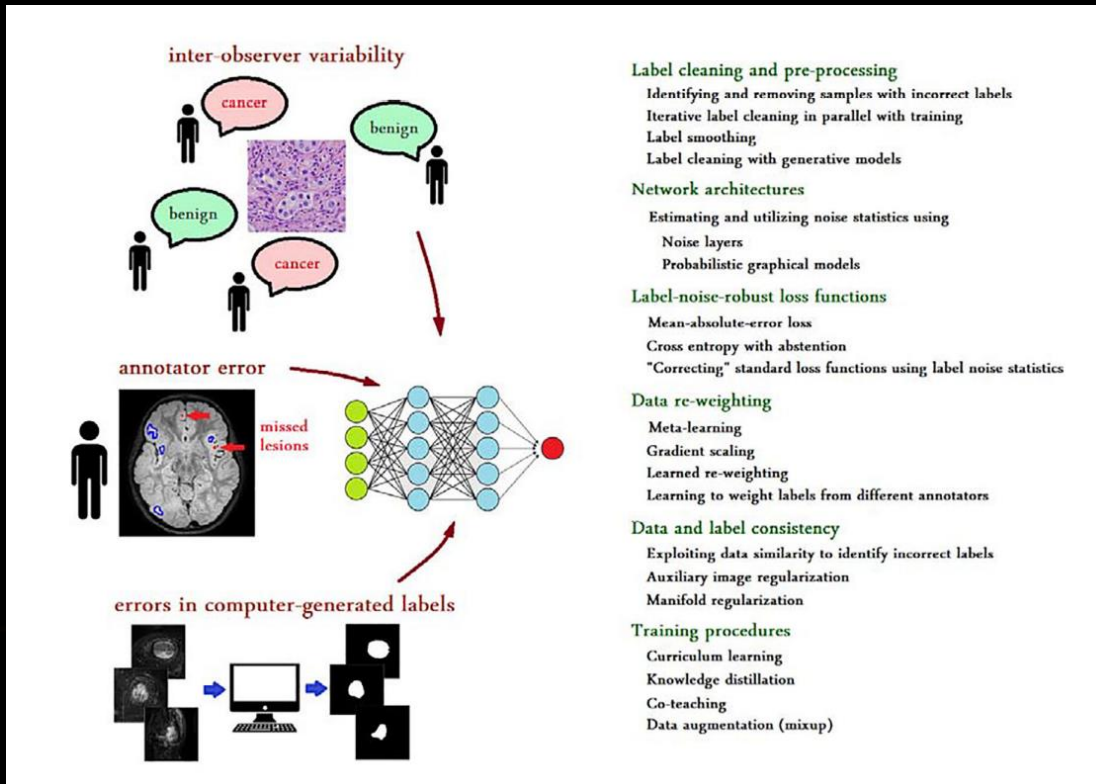
Alan M. Race, Daniel Sutton, Gregory Hamm, Gareth Maglennon, Jennifer P. Morton, Nicole Strittmatter, Andrew Campbell, Owen J. Sansom, Yinhai Wang, Simon T. Barry, Zoltan Takáts, Richard J. A. Goodwin, and Josephine Bunch
Analytical Chemistry **2021** 93 (6), 3061-3071
DOI: 10.1021/acs.analchem.0c02726



CAN WE USE REGISTRATION UNCERTAINTY TO HELP DEEP LEARNING?

Deep learning with noisy labels: exploring techniques and remedies in medical image analysis. Karimi et al. Medical Image Analysis. 2020. <https://doi.org/10.1016/j.media.2020.101759>

“[this] review shows that recent progress on handling label noise in deep learning has gone largely unnoticed by the medical image analysis community.”



CAN WE USE REGISTRATION UNCERTAINTY TO HELP DEEP LEARNING?

Label cleaning and pre-processing

Identifying and removing samples with incorrect labels

Network architectures

Estimating and utilizing noise statistics using

Noise layers

Probabilistic graphical models

Label cleaning and pre-processing

Identifying and removing samples with incorrect labels

Iterative label cleaning in parallel with training

Label smoothing

Label cleaning with generative models

Network architectures

Estimating and utilizing noise statistics using

Noise layers

Probabilistic graphical models

Label-noise-robust loss functions

Mean-absolute-error loss

Cross entropy with abstention

"Correcting" standard loss functions using label noise statistics

Data re-weighting

Meta-learning

Gradient scaling

Learned re-weighting

Learning to weight labels from different annotators

Data and label consistency

Exploiting data similarity to identify incorrect labels

Auxiliary image regularization

Manifold regularization

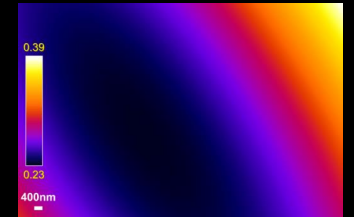
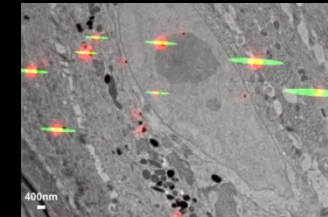
Training procedures

Curriculum learning

Knowledge distillation

Co-teaching

Data augmentation (mixup)

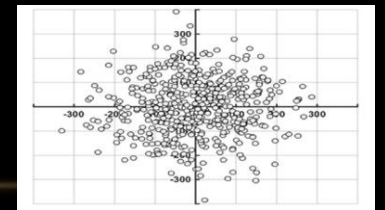


Label-noise-robust loss functions

Mean-absolute-error loss

Cross entropy with abstention

"Correcting" standard loss functions using label noise statistics



Training procedures

Curriculum learning

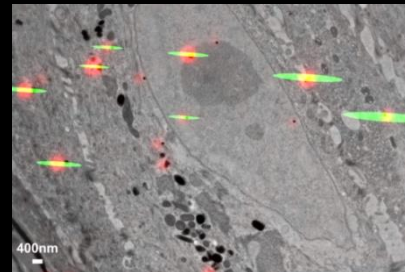
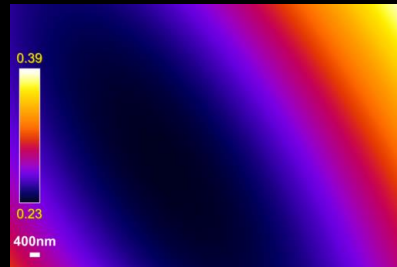
Knowledge distillation

Co-teaching

Data augmentation (mixup)

CONCLUSION

- Registration uncertainty with no other information that proposed point matching can be estimated (accurate for rigid and affine registration for now-> local affine ok as well)

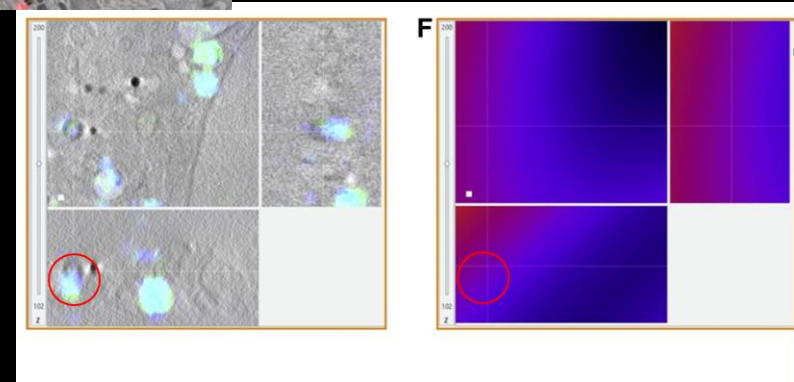


- This has multiple purposes:

- Target area accuracy (Adding error margin for Example)

- Multimodal colocalisation

- And likely and supported by the literature but not yet in our field for better accuracy with less data of deep learning models





ANR-18-CE45-0015

Acknowledgements

EC-CLEM error estimation

ITX Nantes

Guillaume Potier

Stephan Kunne

Laboratoire de Mathématiques J. Leray

Frédéric Lavancier

cryo-CLEM workflows

Diamond Light Source

Maria Harkiolaki

Nina Vyas

All team

EC-CLEM microdissector

MicroPICell

Anabelle Justin

Aurélie Doméné

Stéphanie Blandin

Philippe Hulin

Magalie Feyeux

Steven Nedellec

Marianne Guisset

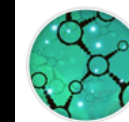
Héloïse Duluc

INCA PLAN

CANCER



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



FRANCE-BIOIMAGING

- **Goals:**

- Foster dialogue between scientific disciplines in interaction with AI;
- Establish radically new modes of collaboration between scientific fields far beyond the mere assimilation of AI methods;
- Address domain-specific strategic questions such as explainability, expert knowledge, robustness, ethics, frugality, etc.
- Address new scientific questions and accelerate scientific discovery in sciences (physics, chemistry, materials, biology, humanities, etc).
- Build ties with other similar centers abroad (e.g., Canada).

- **Scientific events:**

- Thematic trimesters on multidisciplinary subjects (phystat-AI (2022), Causality in AI (2023), others are to come);
- Incubator for projects: fellowships, visiting researchers, project funding, AI engineers in multidisciplinary projects;
- Training sessions related to AI for researchers from different communities (e.g., Les Houches Summer School of Physics, 2022);
- Workshops, webinars, or hackathons;
- Host multidisciplinary teams;
- Outreach: AI and society.

- **Governance:**

- **International Scientific Advisory Board:** 10 internationally renowned experts spanning all scientific spectrum of CNRS.
- **Executive Committee:** 1 director, 1 deputy director and 1 project manager.
- **Steering Committee:** 12 members representative of all CNRS institutes.

+ évaluation <https://www.ins2i.cnrs.fr/fr/cnrsinfo/appel-projets-pour-beneficier-de-laccompagnement-dingenieurs-en-intelligence-artificielle>

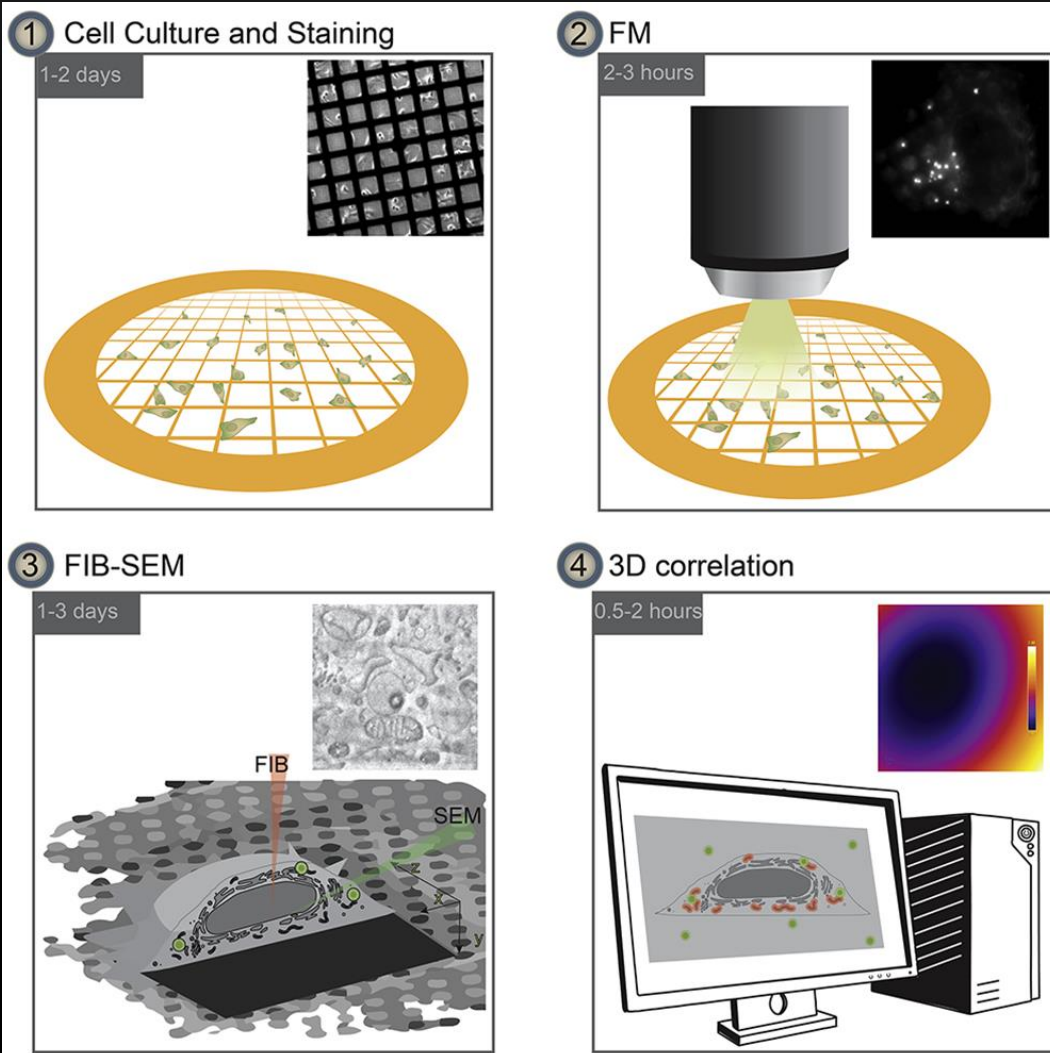
AFFINE MODEL

- Find the affine transformation in the general case by **casting the problem as a multivariate linear regression**
- Derive the prediction error covariance matrix using the linear regression theory
- Construct a confidence area enclosing the registration error at a given point

$$\begin{aligned} & (y - \hat{\beta}' z_0)' \left(\frac{n}{n - r - 1} \hat{\Sigma} \right)^{-1} (y - \hat{\beta}' z_0) \\ & \leq (1 + z_0' (Z' Z)^{-1} z_0) \left(\frac{m(n - r - 1)}{n - r - m} \right) F_{m, n-r-m}(1 - \alpha), \end{aligned}$$

Compared to Cohen and Ober who had also developed an affine framework, we do not require noise estimation of localisation. It will be estimated as well. Compared to Fitzpatrick (TRE/FLE), in addition no affine development was proposed.

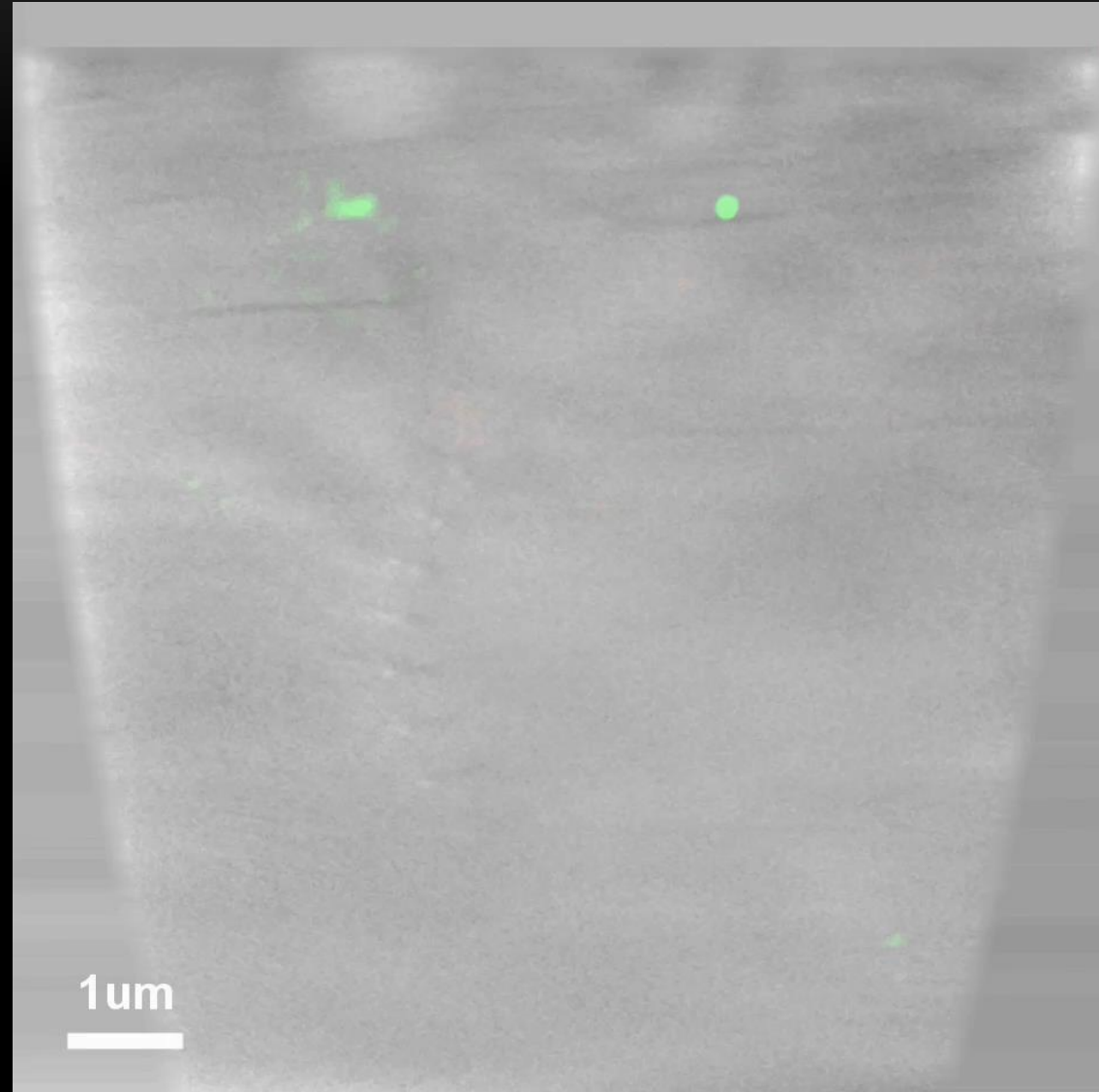
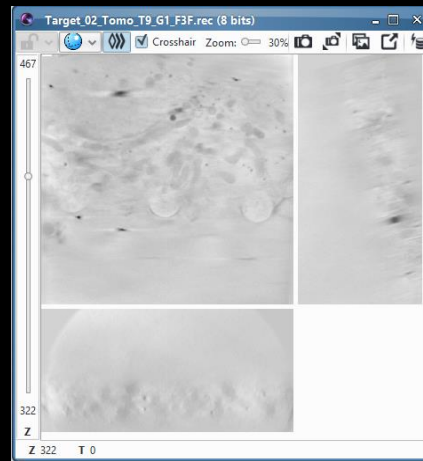
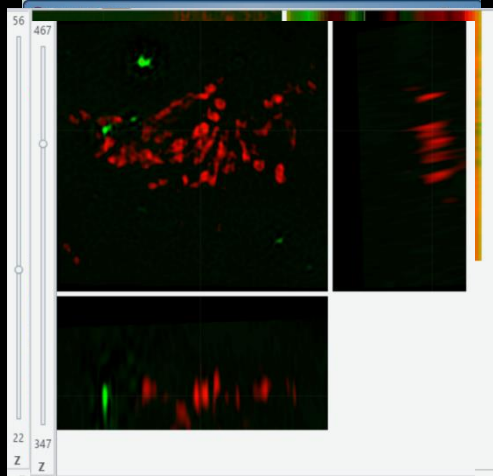
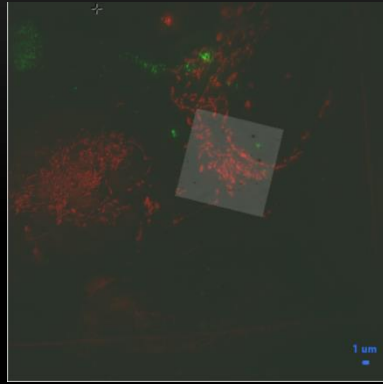
CRYO FIB-SEM/ FM EXAMPLE WITH LIPID DROPLETS AS FIDUCIALS



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.i sci.2021.102714>

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

RIGID MODEL

- Find the rigid transformation in the general case using a numerical solver
- Use the Cramer-Rao asymptotic lower bound to get an estimation of the covariance matrix of the prediction error
- Construct an asymptotic confidence area enclosing the registration error at a given point

AFFINE MODEL

- Find the affine transformation in the general case by **casting the problem as a multivariate linear regression**
- Derive the prediction error covariance matrix using the linear regression theory
- Construct a confidence area enclosing the registration error at a given point