

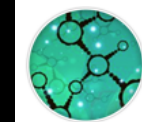
CORRELATIVE MICROSCOPIES: IMAGE REGISTRATION FOR CLEM AND FOR DEEP LEARNING

WHAT CAN BRING ERROR ESTIMATION ?

Perrine.paul-gilloteaux@univ-nantes.fr

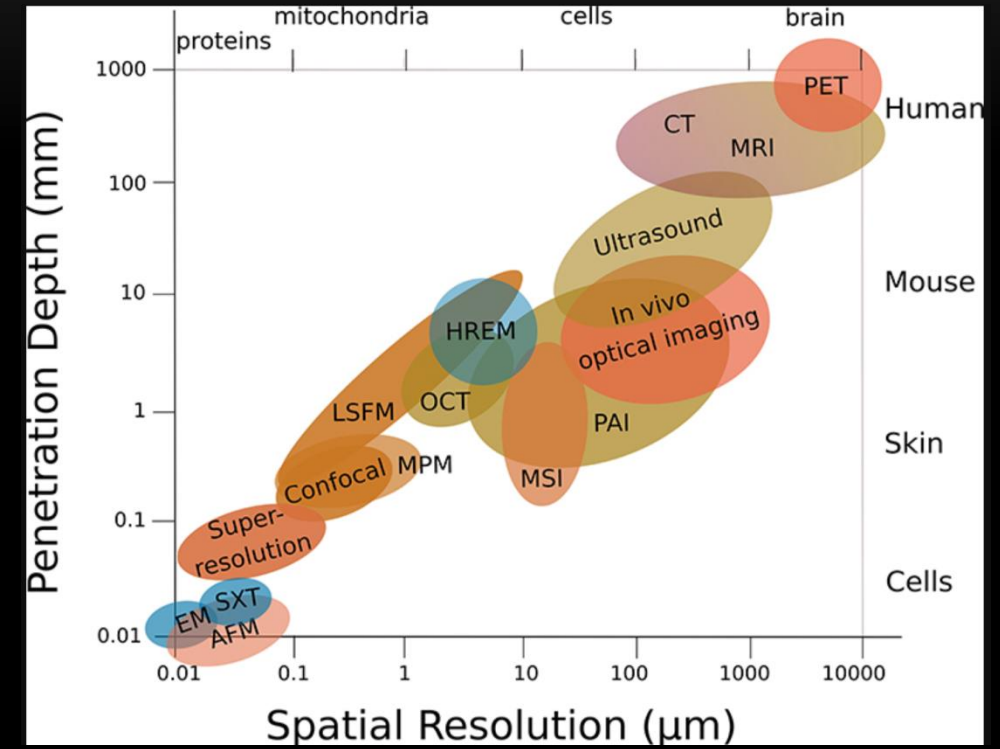
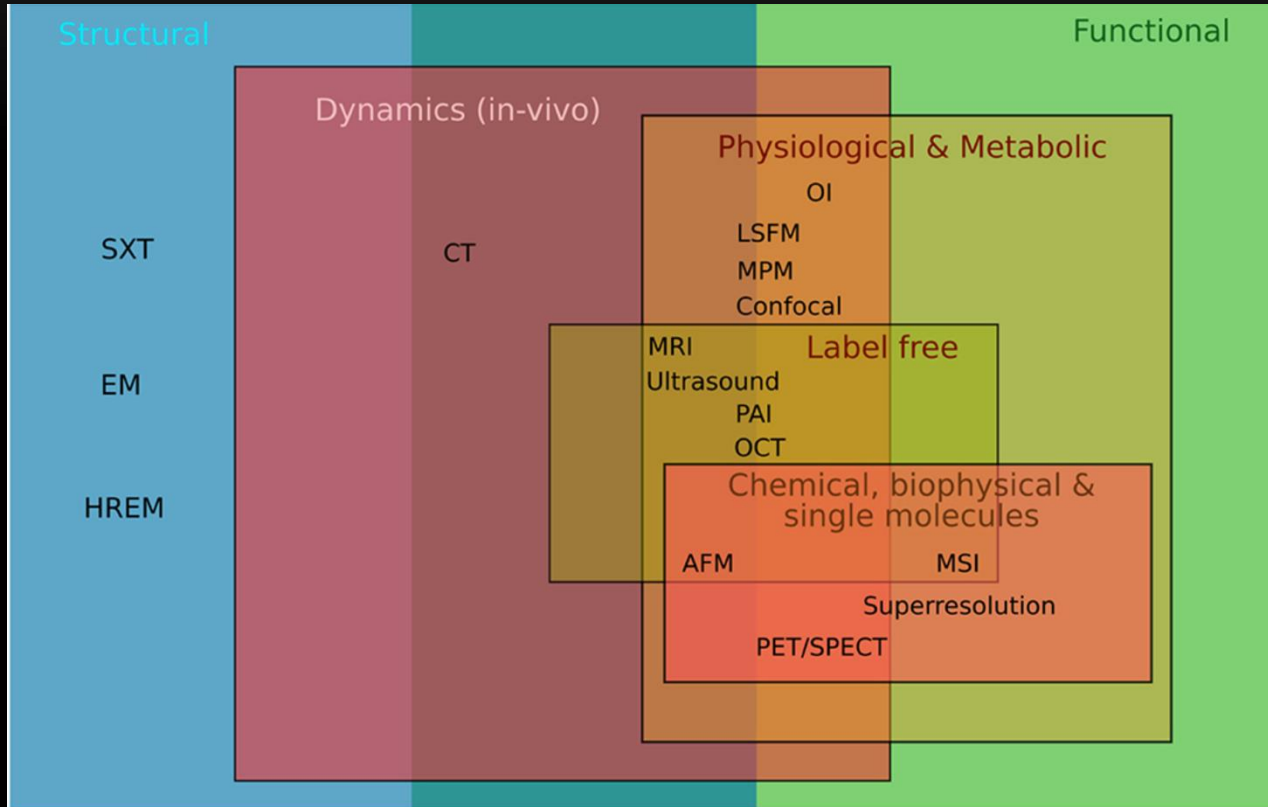


Structure Fédérative
de Recherche - **SANTÉ**
François Bonamy



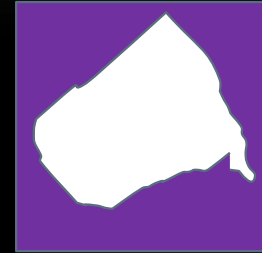
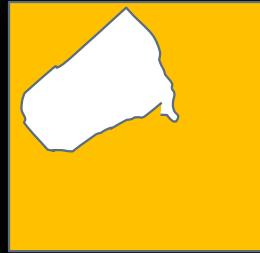
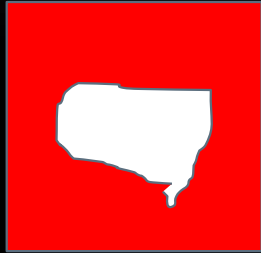
FRANCE-BIOIMAGING

MULTIMODAL IMAGING FOR AN HOLISTIC VIEW



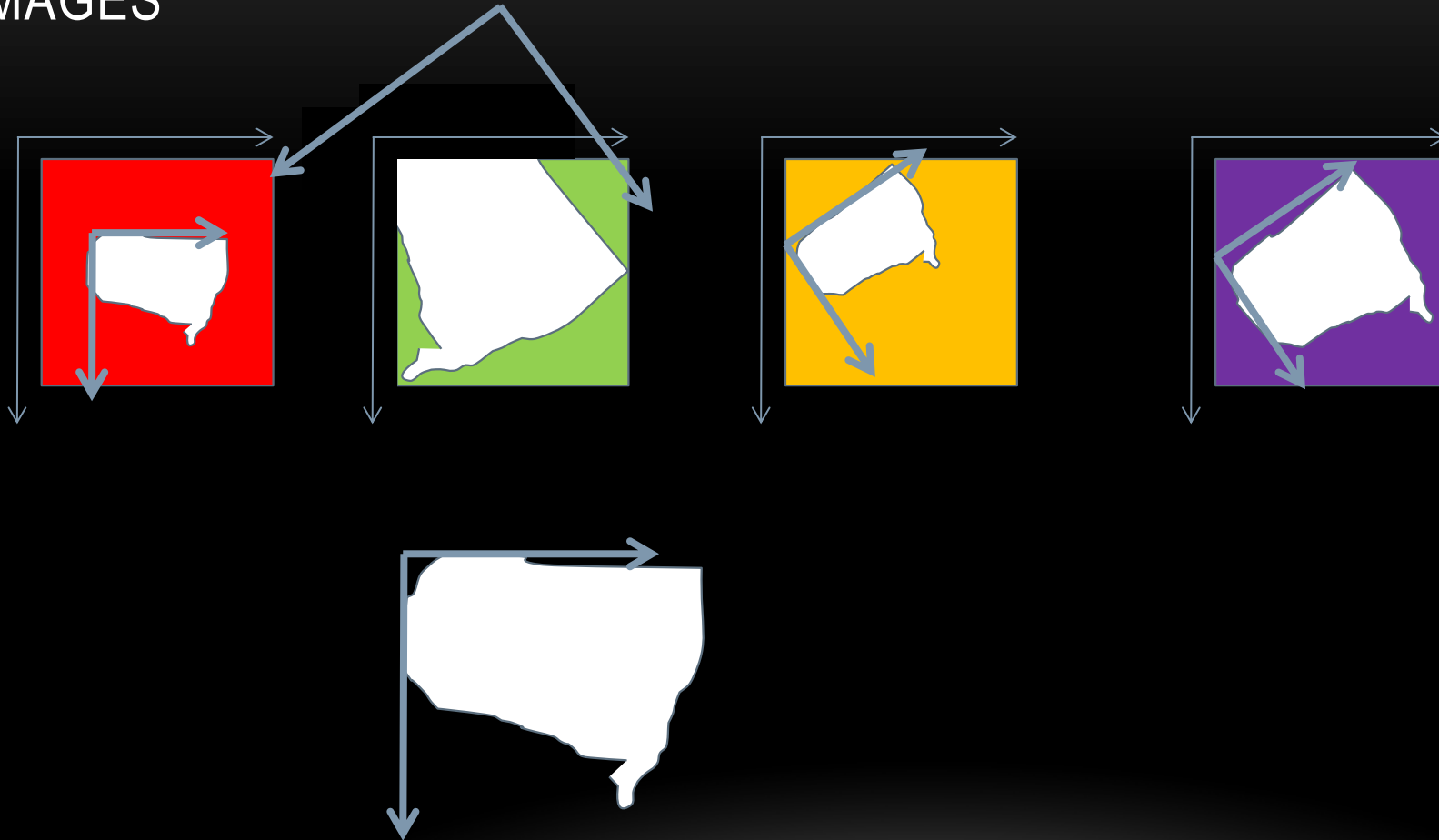
Andreas Walter, Perrine Paul-Gilloteaux, Birgit Plochberger, Ludek Sefc, Paul Verkade, et al..
 Correlated Multimodal Imaging in Life Sciences: Expanding the Biomedical Horizon. *Frontiers in Physics*, Frontiers, 2020, 8, [10.3389/fphy.2020.00047](https://doi.org/10.3389/fphy.2020.00047)

SAMPLE TO BE TRACKED FROM ONE IMAGING MODALITY TO ANOTHER

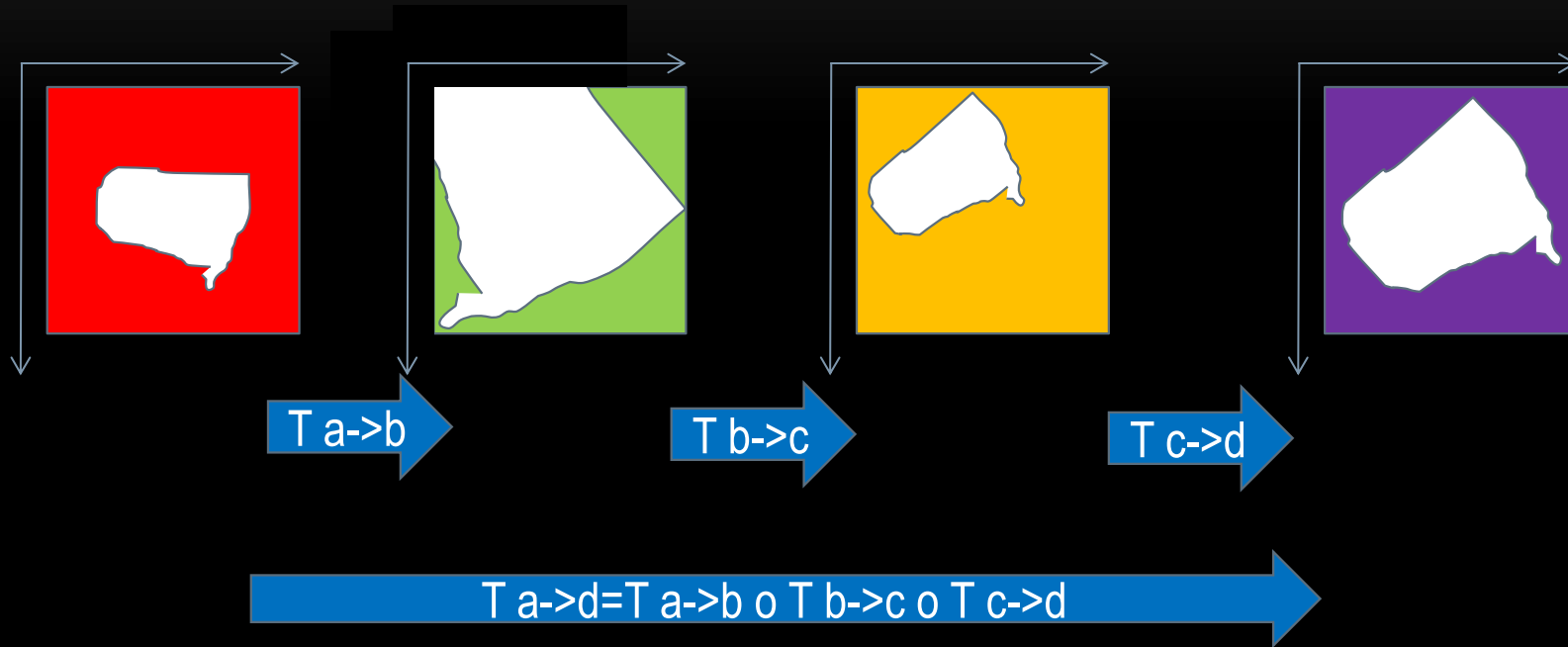


Sometimes additional modalities will help

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



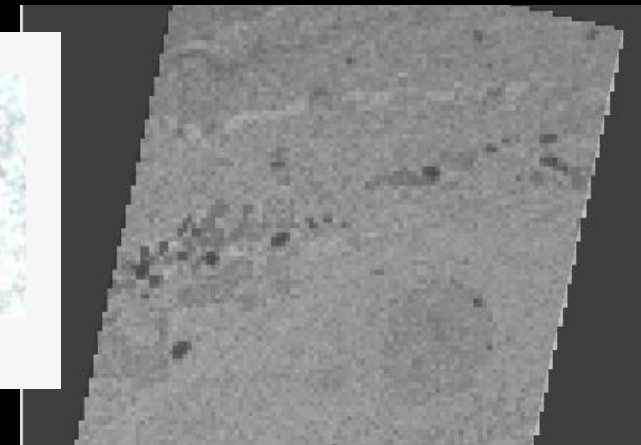
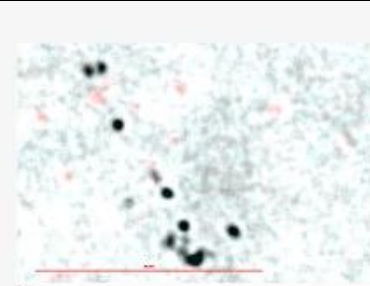
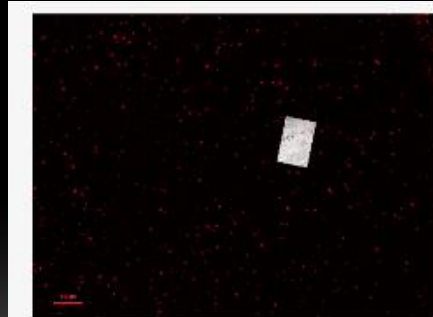
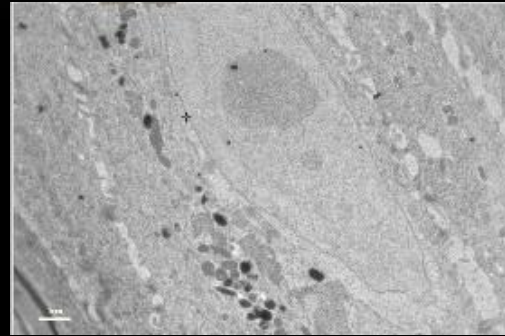
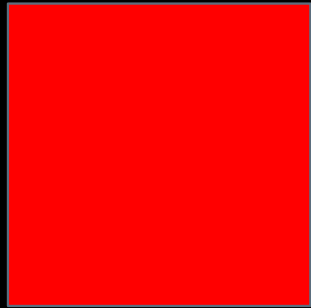
TRANSFORMATION CAN BE RIGID OR NON RIGID, BUT THEY CAN ALWAYS BE CASCADED



HOW TO EXPLOIT THE TRANSFORMATION COMPUTED FOR QUANTIFICATION?

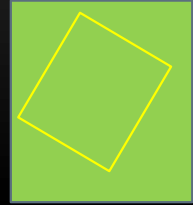
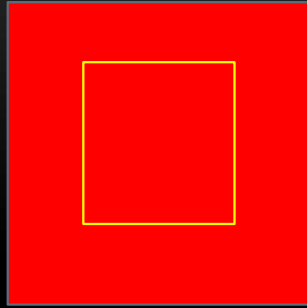
COMPUTE THE TRANSFORMATION
LINKING **source** and **target**

Ecclem Apply transform



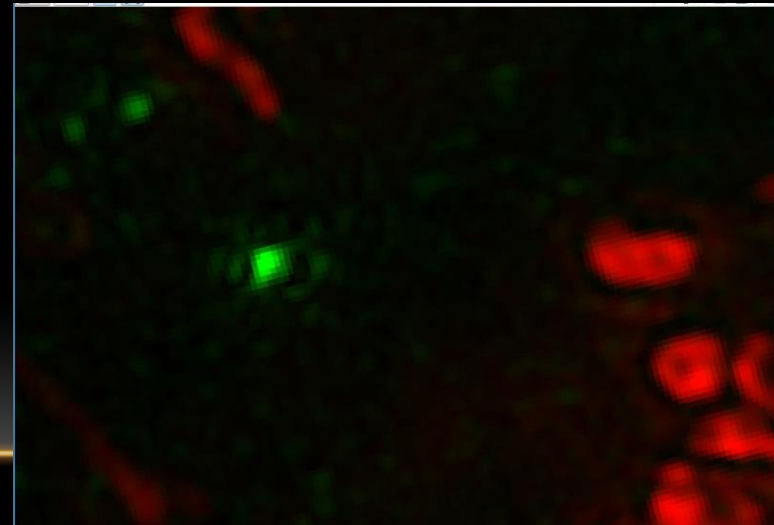
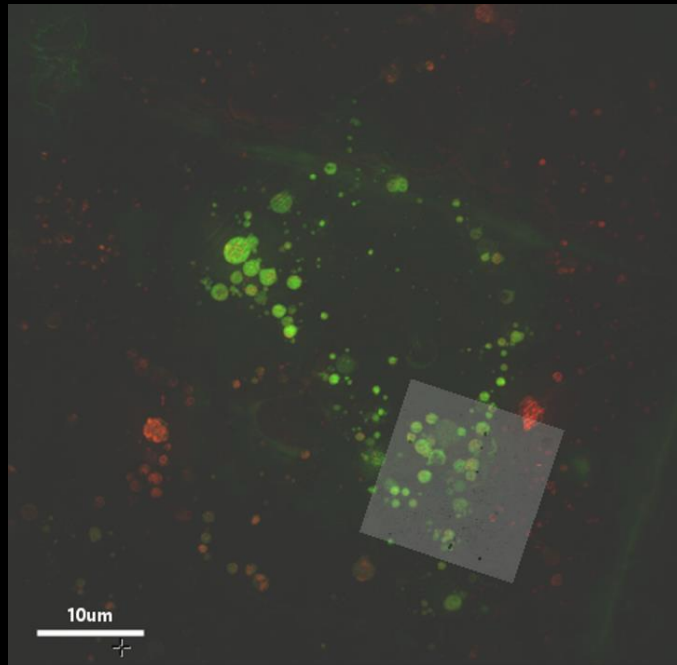
Overlay: one image got **interpolated** (new pixels) or **reduced in quality** to create a new merged dataset.

HOW TO EXPLOIT THE TRANSFORMATION COMPUTED FOR QUANTIFICATION?

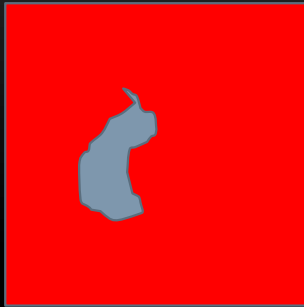


Transform ONLY
the DISPLAY
(coordinate
system of arrival
= screen)

Ecclem CorrelativeView

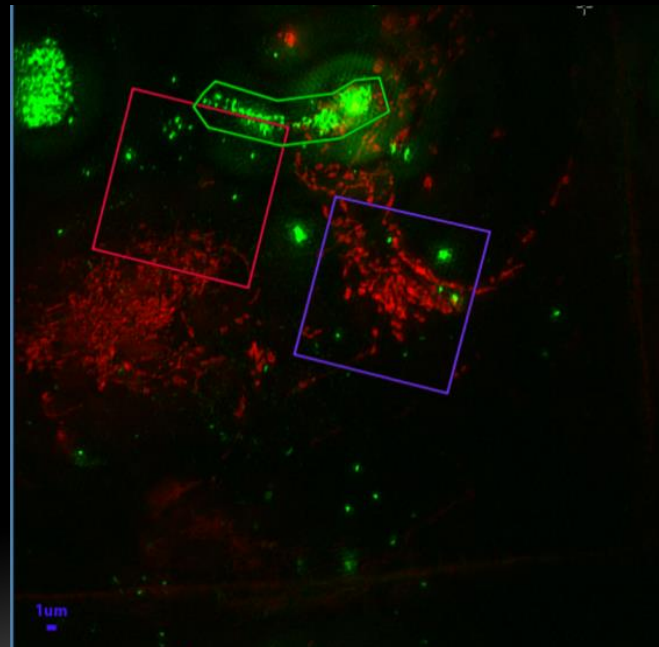
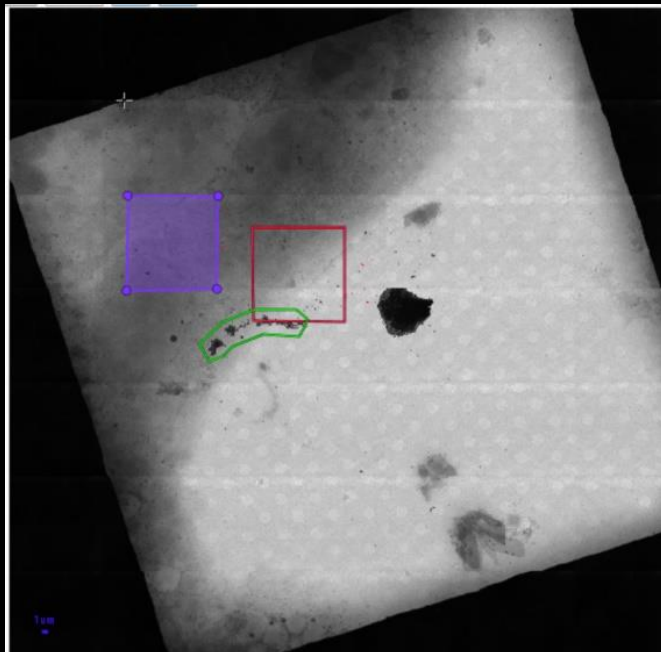


HOW TO EXPLOIT THE TRANSFORMATION COMPUTED FOR QUANTIFICATION?



Region of interest transformed

Ecclem Apply transform to ROI

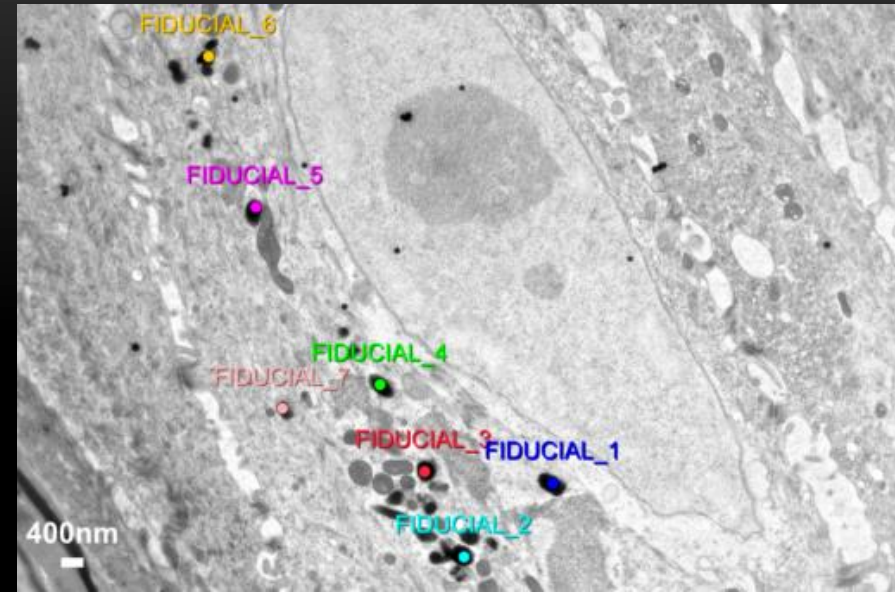
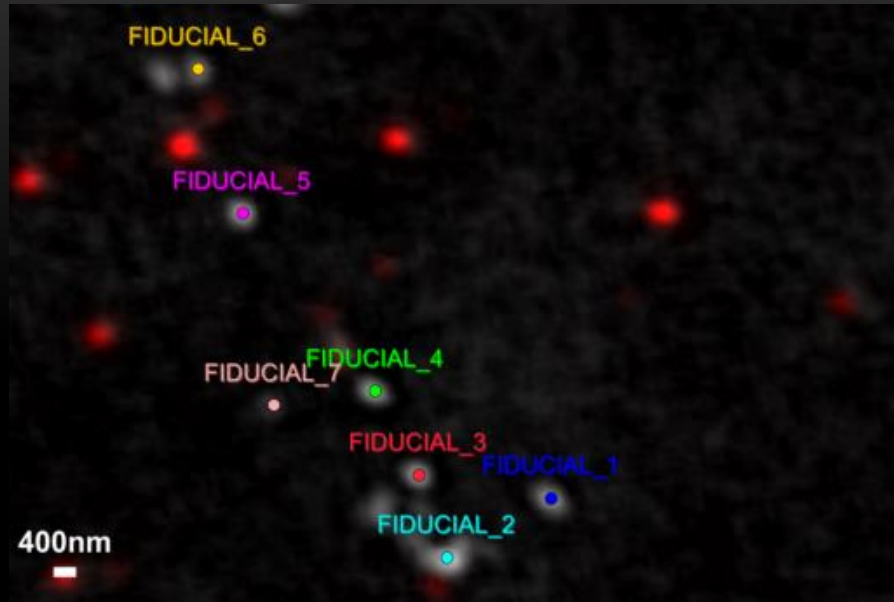


	Name	Conto...	Interior (px)	Area (u...	Mean Inte...	Mean Int...	Max Inte...	Max Intens...	Texture contr...	Texture contrast ...	Texture homogeneity (c...	Texture homogeneity (c...
	Polygon2D	710.08	20316.5	79.361	13492.1	358.91	154107.3	8919.14	37.886	37.532	0.63182	0.59267
	Polygon2D	1015.9	64424.5	251.66	7813.26	68.502	124714.1	3707.97	14.366	1.9072	0.78402	0.85863
	Polygon2D	1011.11	63754	249.04	20290.6	58.312	231600.6	3159.71	66.098	0.99523	0.53865	0.88852

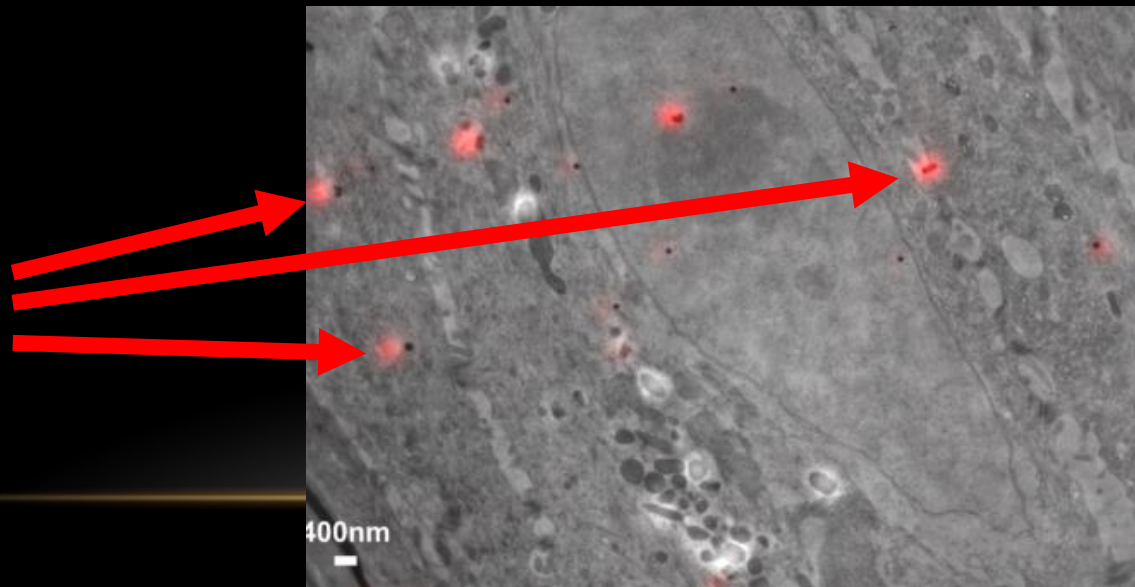
- One thing to know: Matching is NEVER PERFECT
 - Because your sample may deform/ evolve between two modalities
 - Because multimodality is usually also multiscale (very different resolution, one pixel can match hundred of pixels in the second modality)
 - Because none modality allows to perfectly locate one structure of interest (imaging resolution). Even when the sample did not deform at all , this will create an error you can not avoid, only reduce.

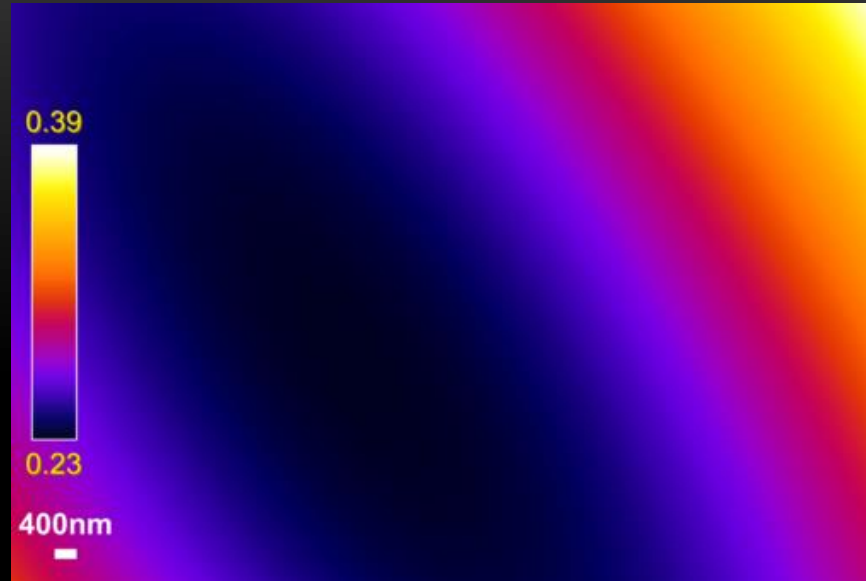
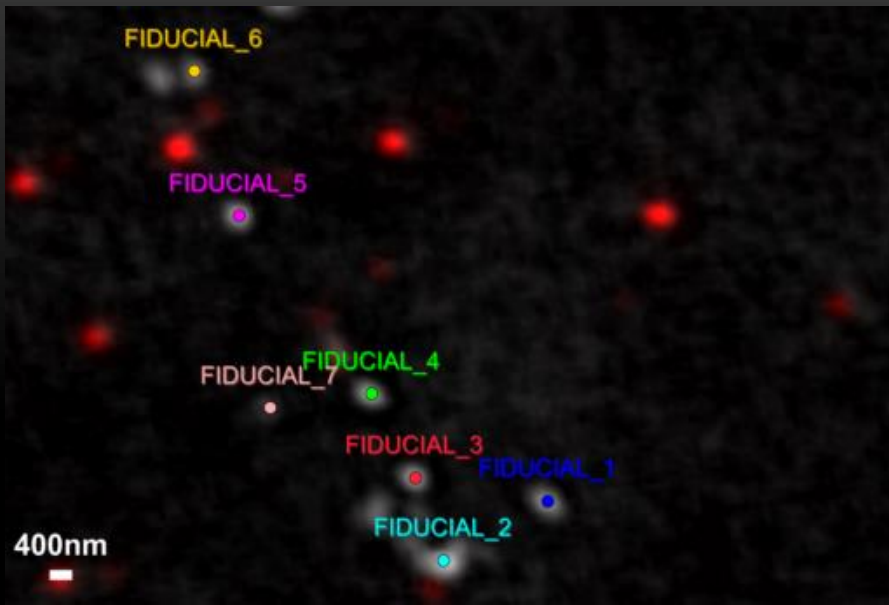
WE HAVE DEVELOPED A METHOD TO SPATIALLY ASSESS THE QUALITY OF REGISTRATION WITH NO GROUND TRUTH (NO OTHER INPUT THAT POINTS (OR SURFACE) USED FOR REGISTRATION: LOCALISATION NOISE AND ERROR REGISTRATION ARE ESTIMATED).

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.

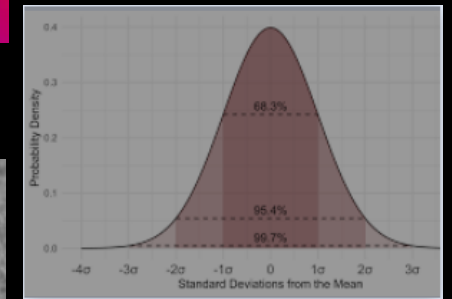
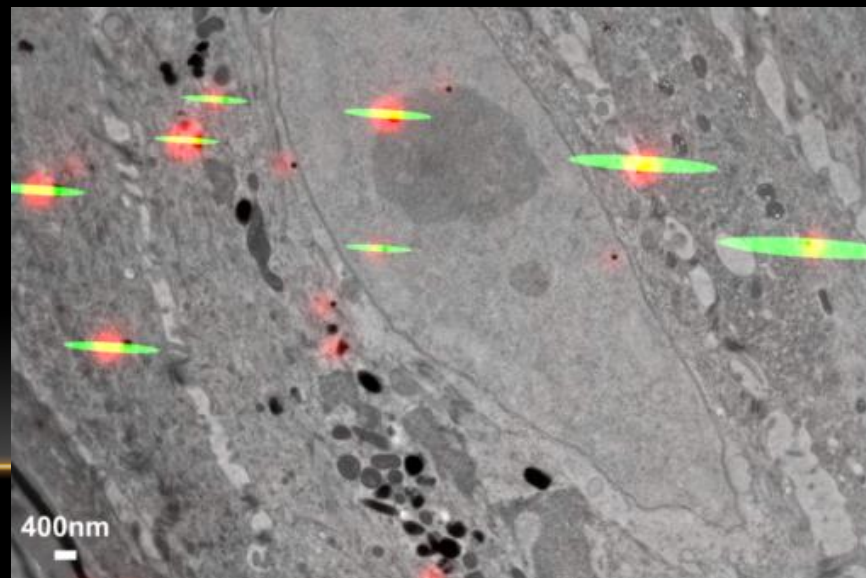
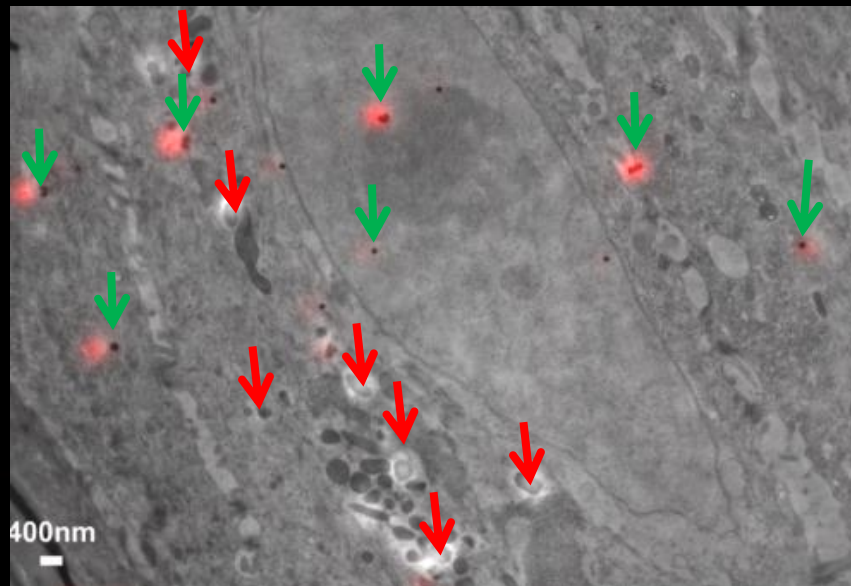


Beads are used here to check how good we are to estimate the error





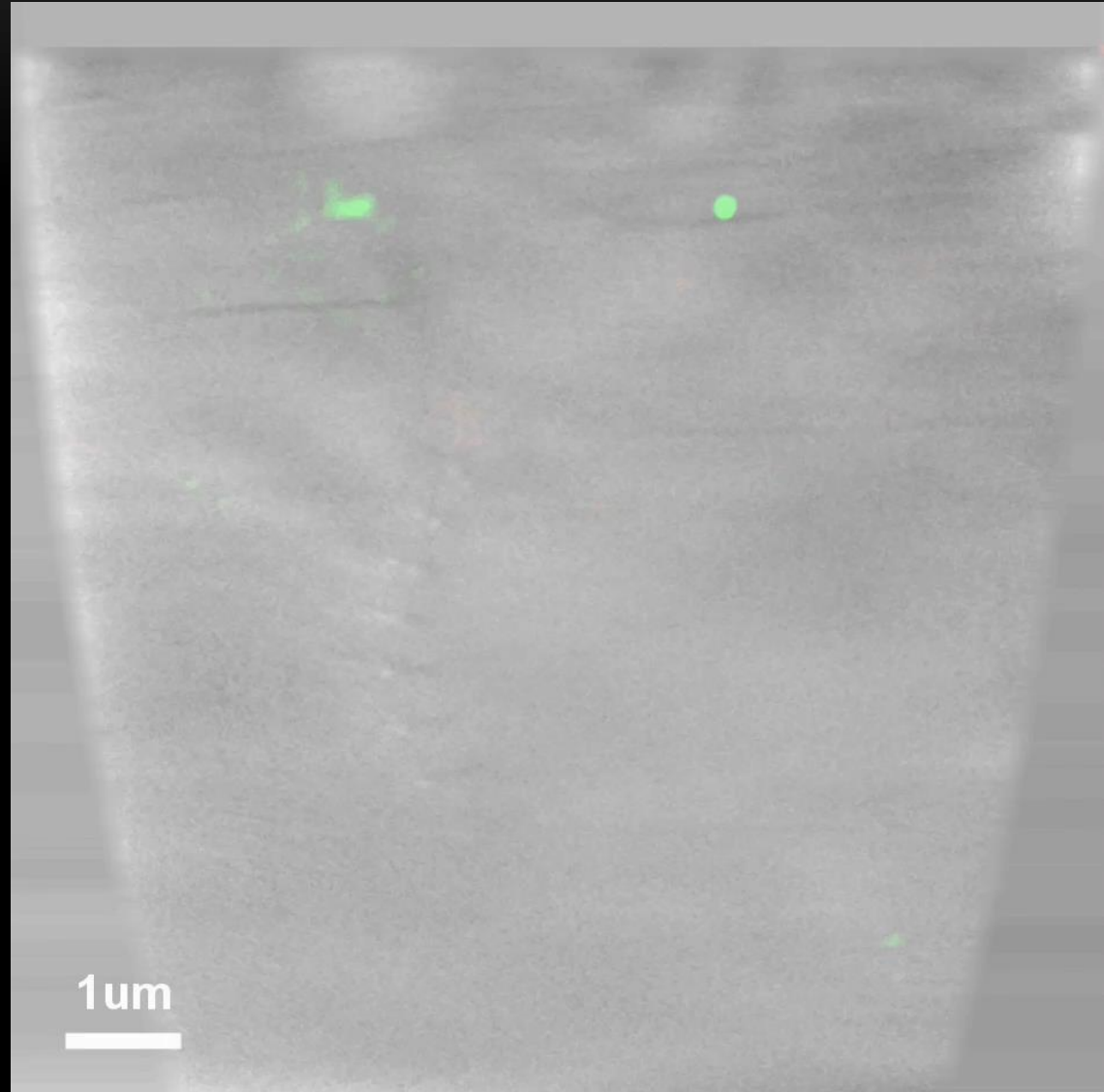
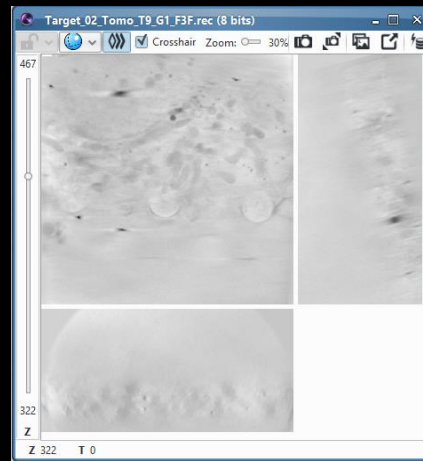
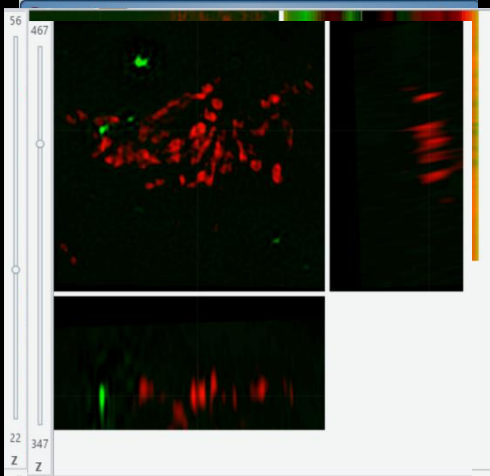
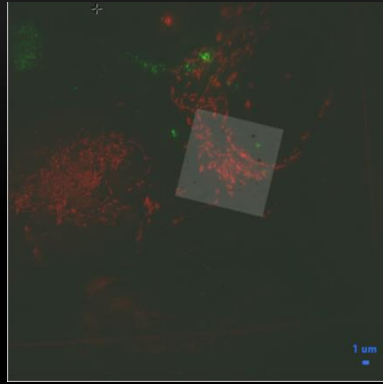
Predicted Average accuracy at each point



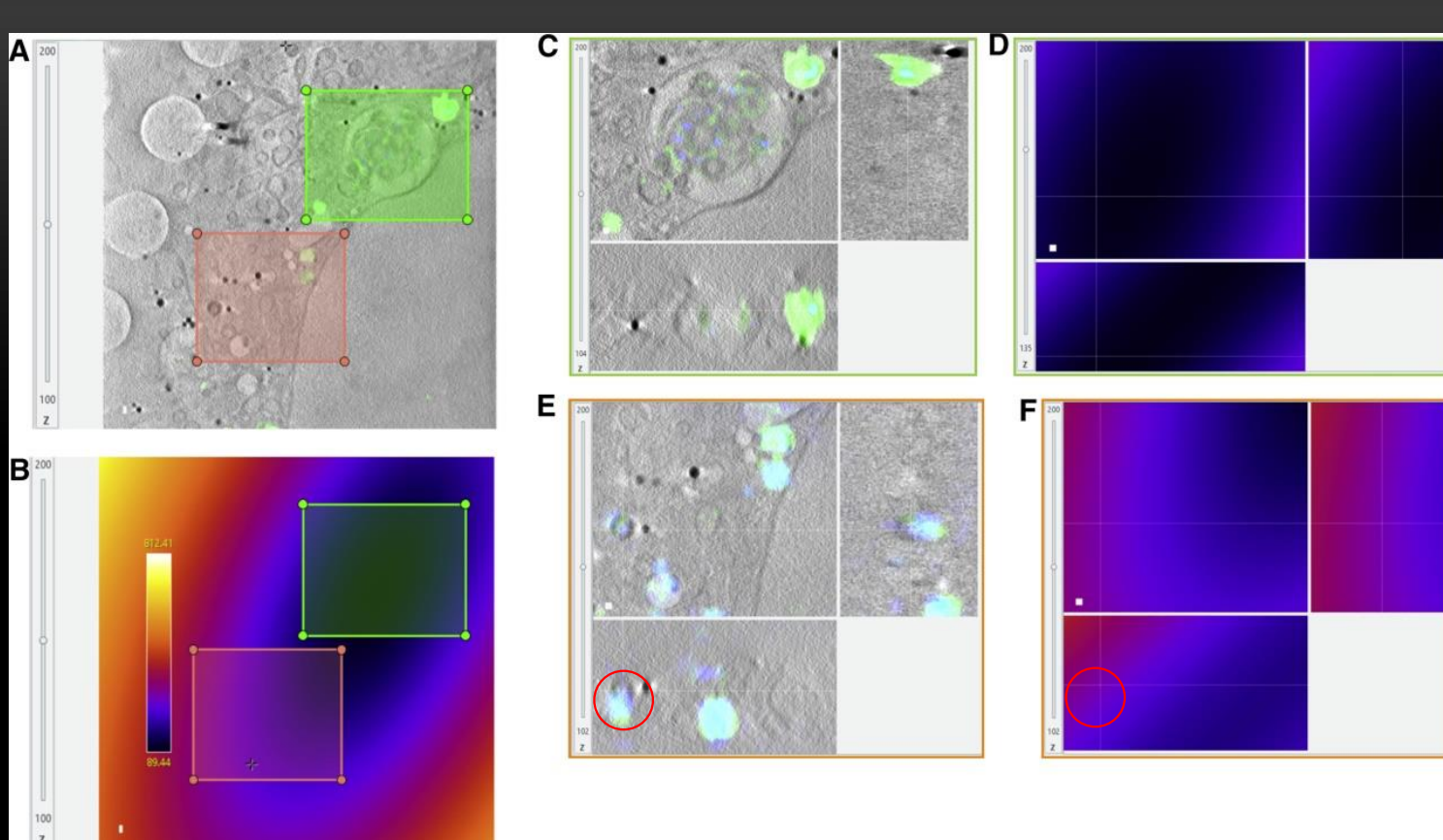
New in ecclem:
95% accuracy prediction

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

Kounatidis I, Stanifer ML, Phillips MA, Paul-Gilloteaux P, et al. 3D Correlative Cryo-Structured Illumination Fluorescence and Soft X-ray Microscopy Elucidates Reovirus Intracellular Release Pathway. Cell. 2020



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

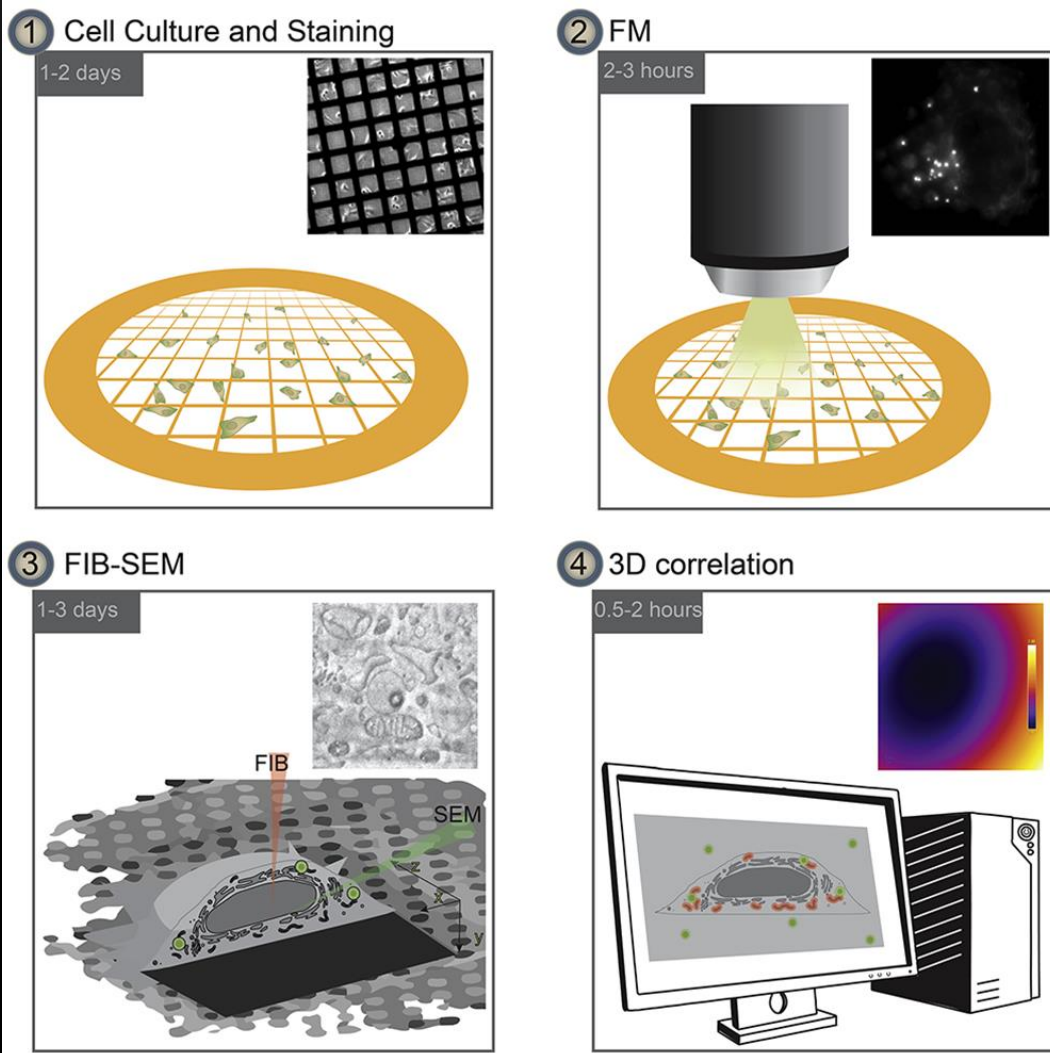


Kounatidis I, Stanifer ML, Phillips MA, Paul-Gilloteaux P, et al.
 3D Correlative Cryo-Structured Illumination Fluorescence and
 Soft X-ray Microscopy Elucidates Reovirus Intracellular Release
 Pathway. Cell. 2020

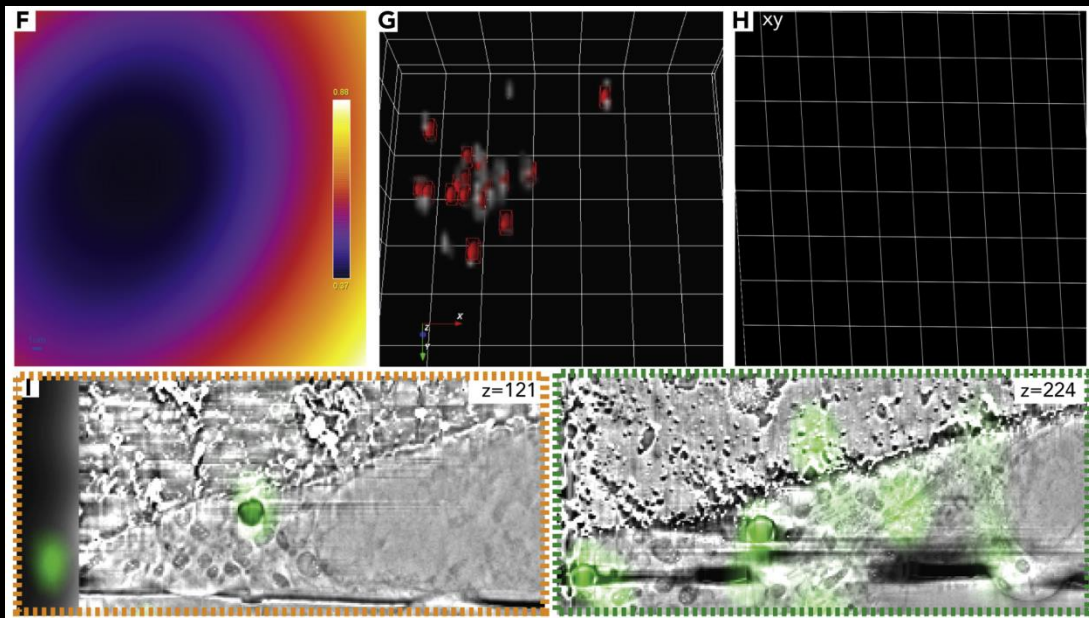
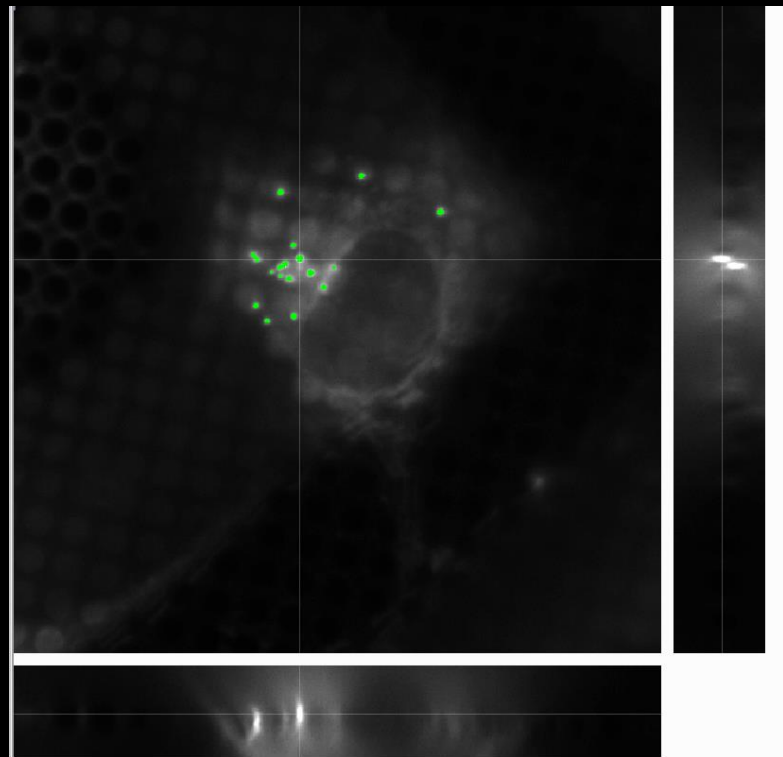
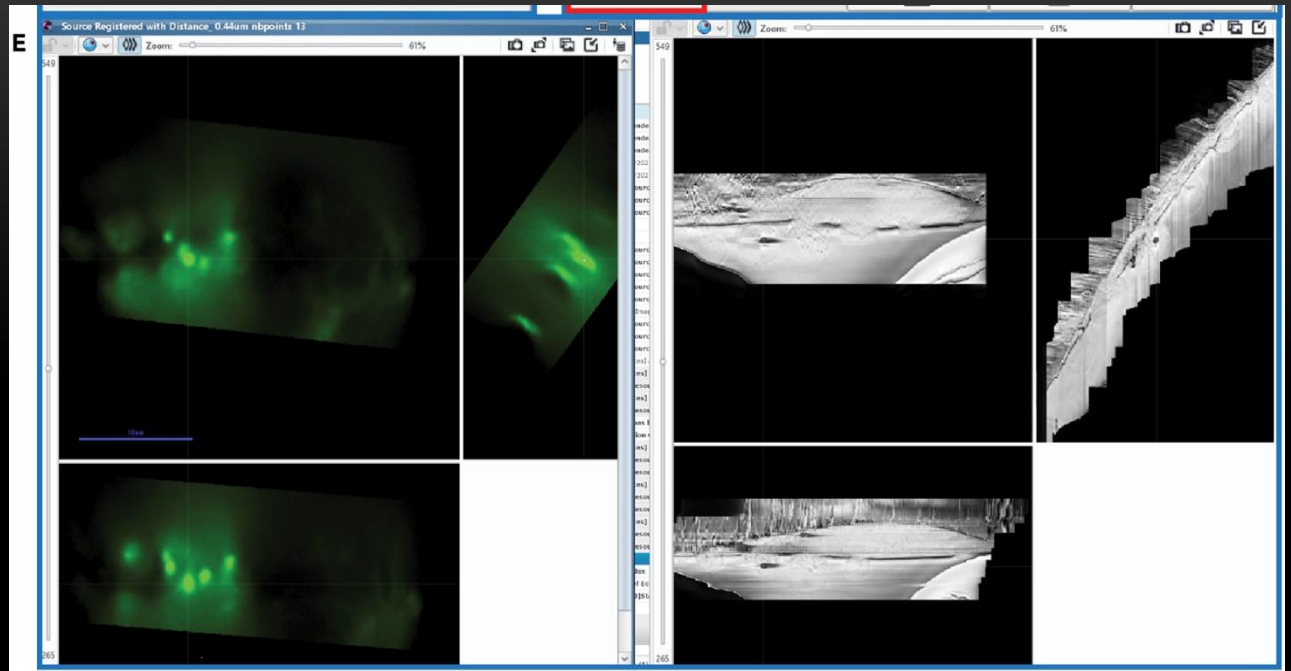
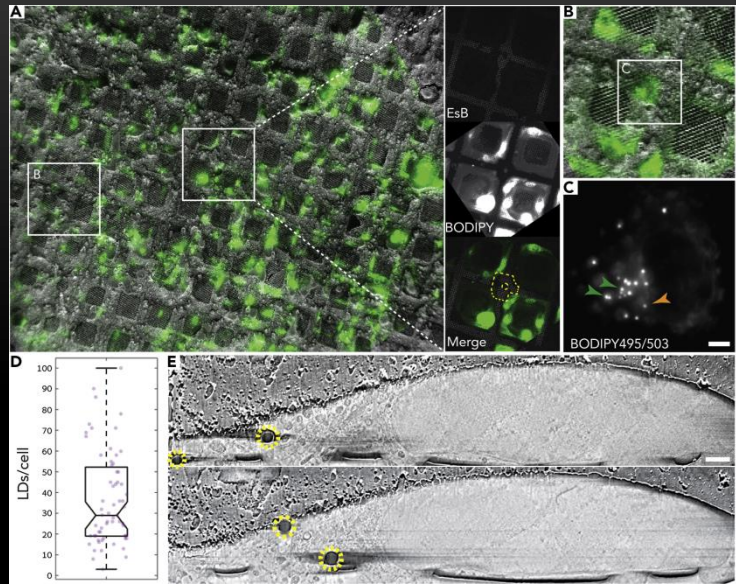
Assessing that it is likely to be
 at the same position rather
 than warping.

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

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



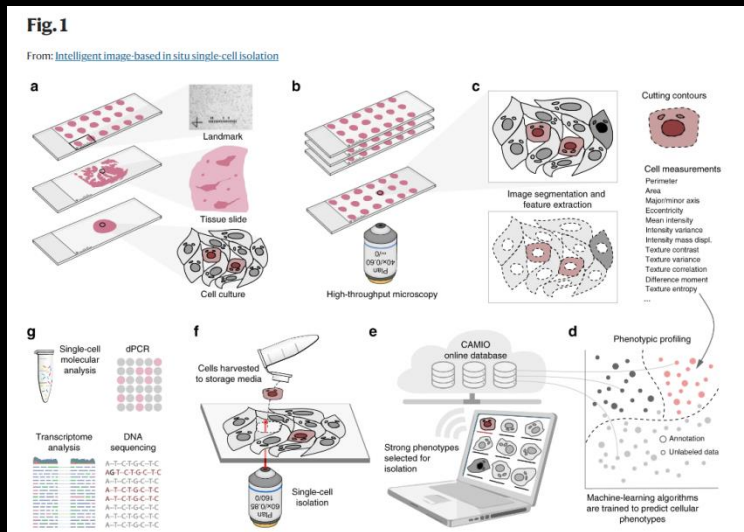
Scher, N., Rechav, K., Paul-Gilloteaux, P., and Avinoam, O. (2021). In situ fiducial markers for 3D correlative cryo-fluorescence and FIB-SEM imaging. *iScience* 24, 102714. <https://doi.org/10.1016/j.isci.2021.102714>



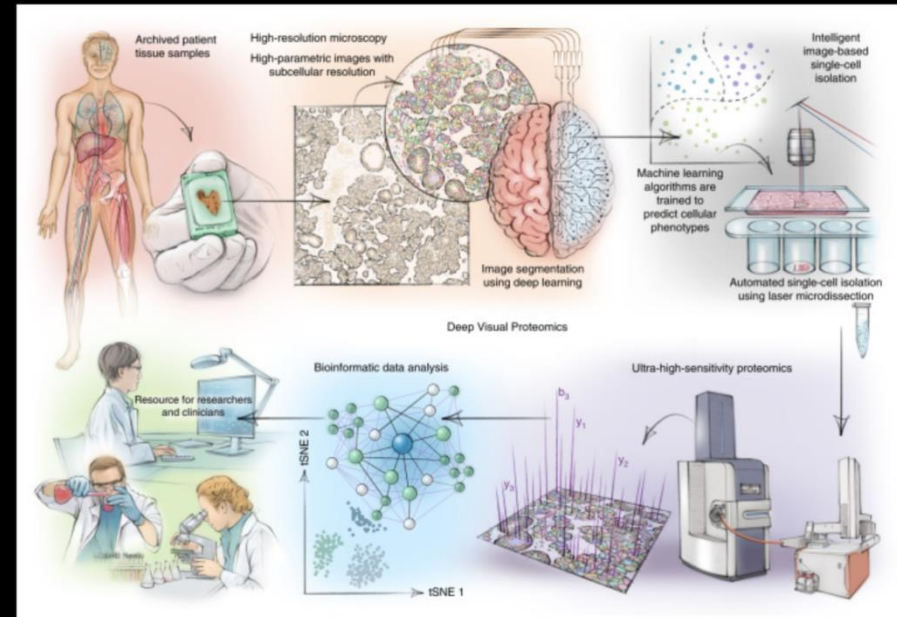
OTHER USAGES OF REGISTRATION IN MULTIMODAL MICROSCOPIES: SMART MICRODISSECTION FOR SPATIAL OMICS

Brasko, C., Smith, K., Molnar, C. *et al.* Intelligent image-based in situ single-cell isolation. *Nat Commun* **9**, 226 (2018). <https://doi.org/10.1038/s41467-017-02628-4>

Mund A, Coscia F, Kriston A, et al. Visual Proteomics defines single-cell identity and heterogeneity. *Nat Biotechnol.* 2022 Aug;40(8):1231-1240. doi: 10.1038/s41587-022-01302-5. Epub 2022 May 19. PMID: 35590073; PMCID: PMC9371970.

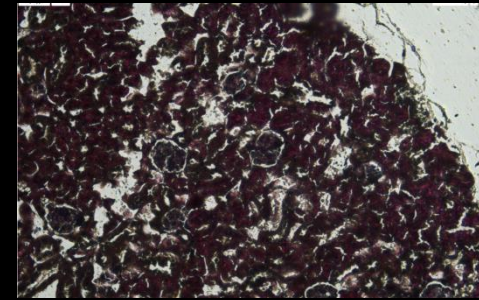
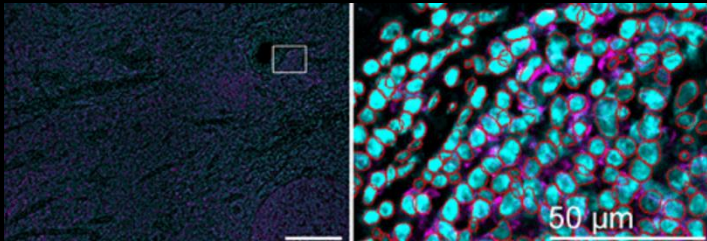
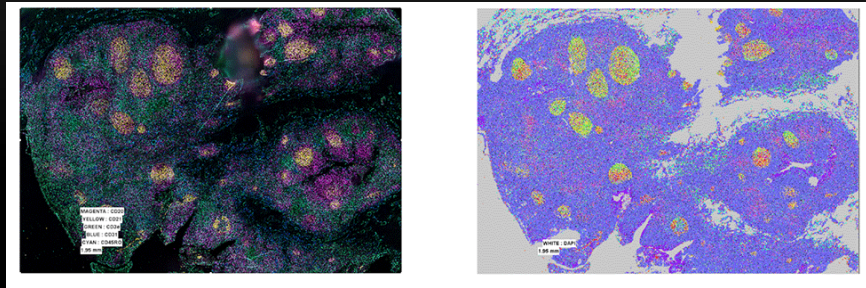


Scratch registration based



Style transfer based for identification of Cell/Area of interest

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



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



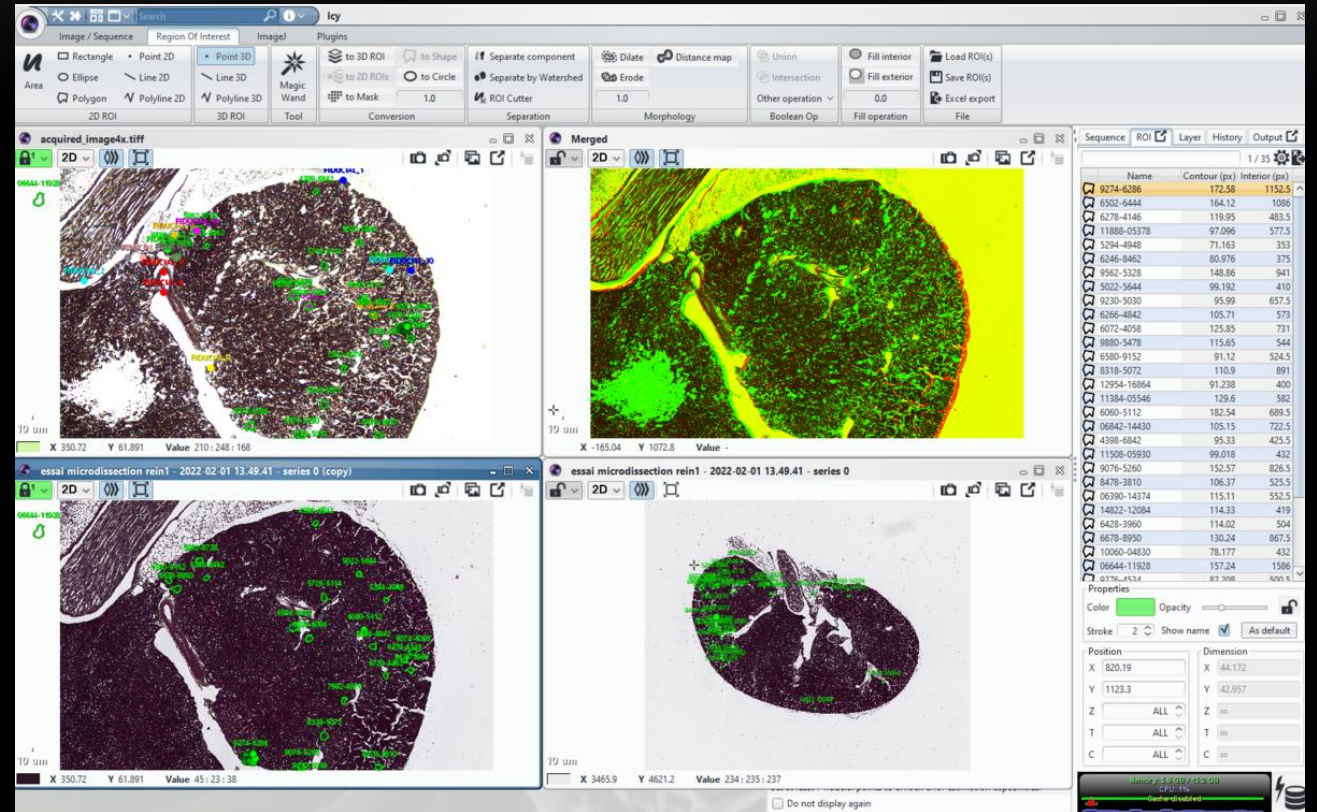
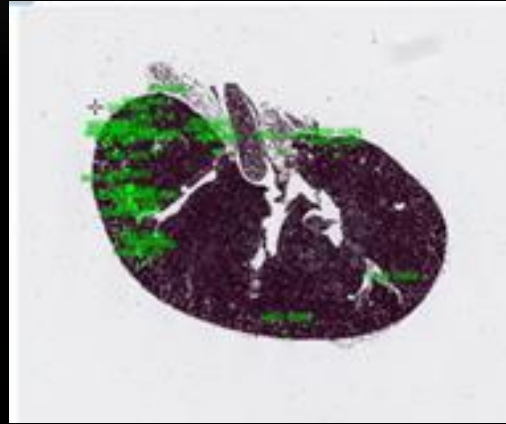
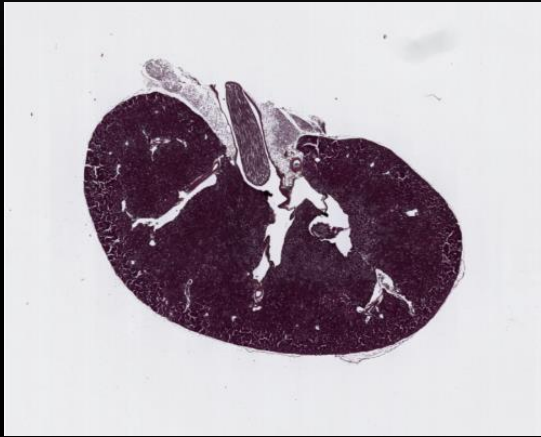
Phenocycler/Codex for fluorescence hyperplexing



Slide Scanner for fast full FOV

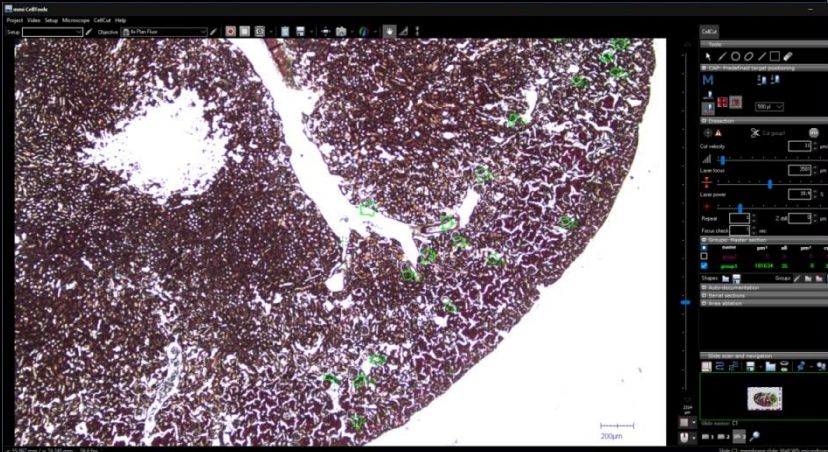
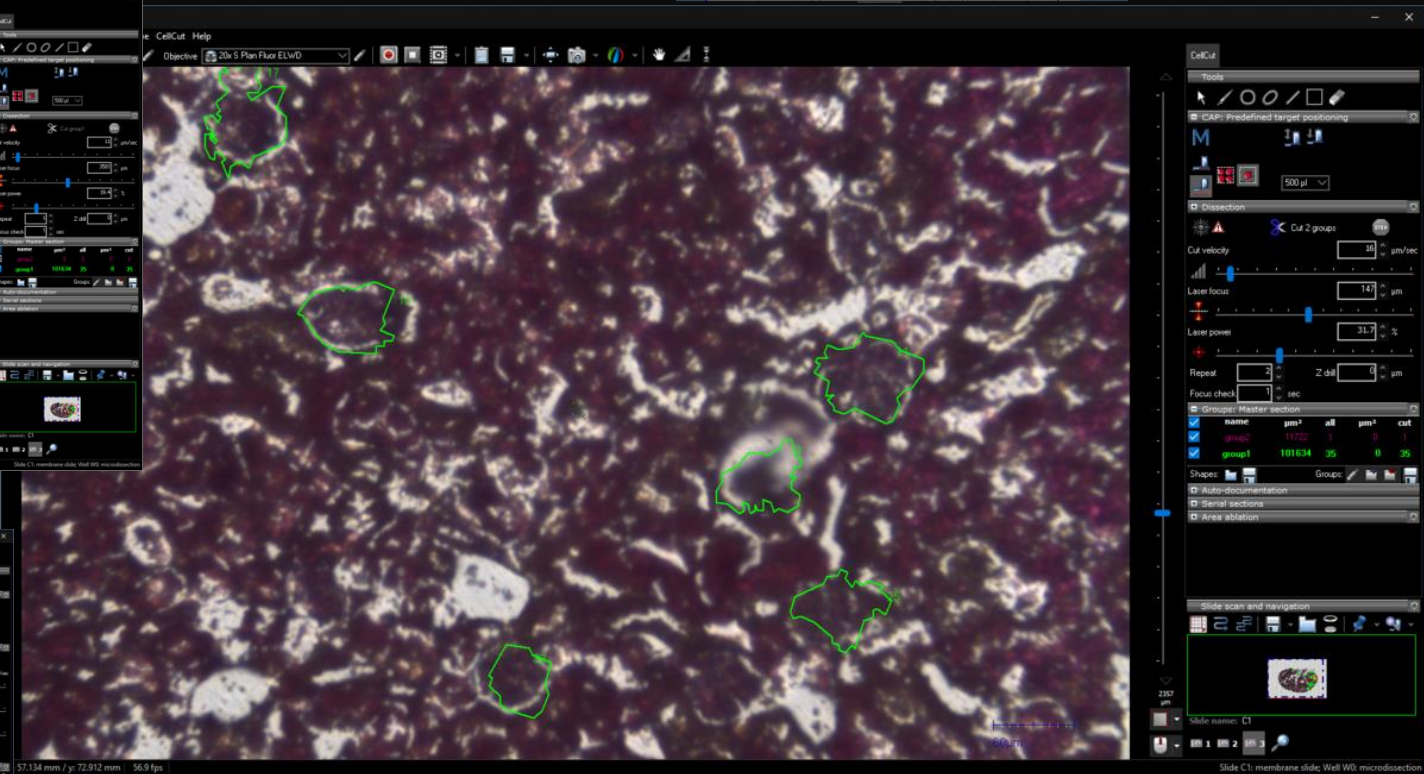
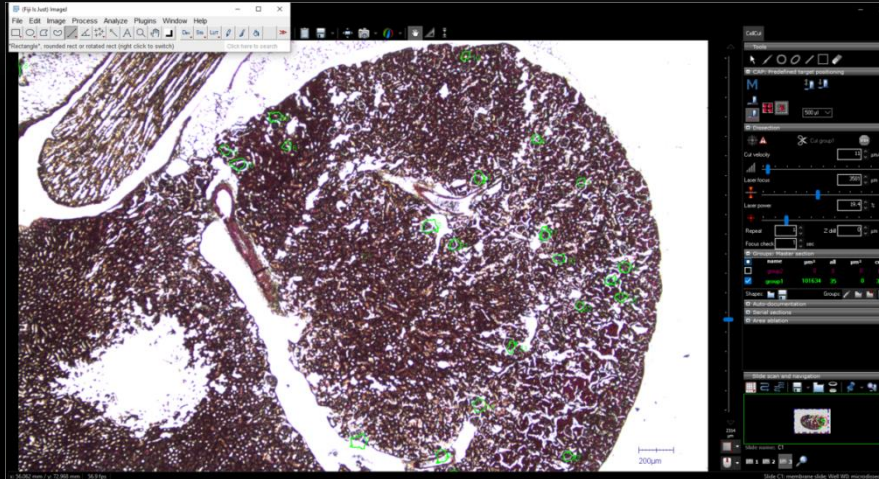


Microdissector (MMI cellcut)



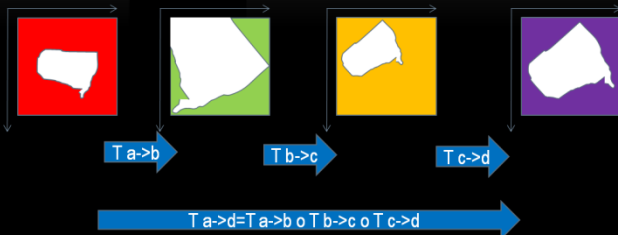
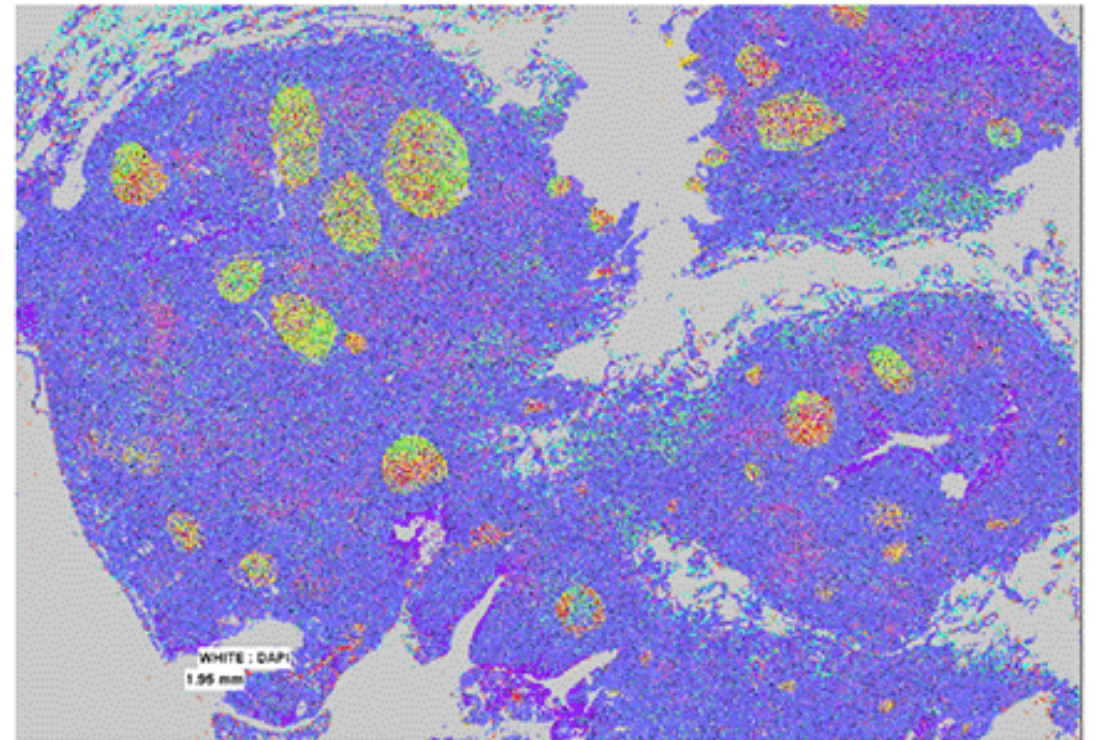
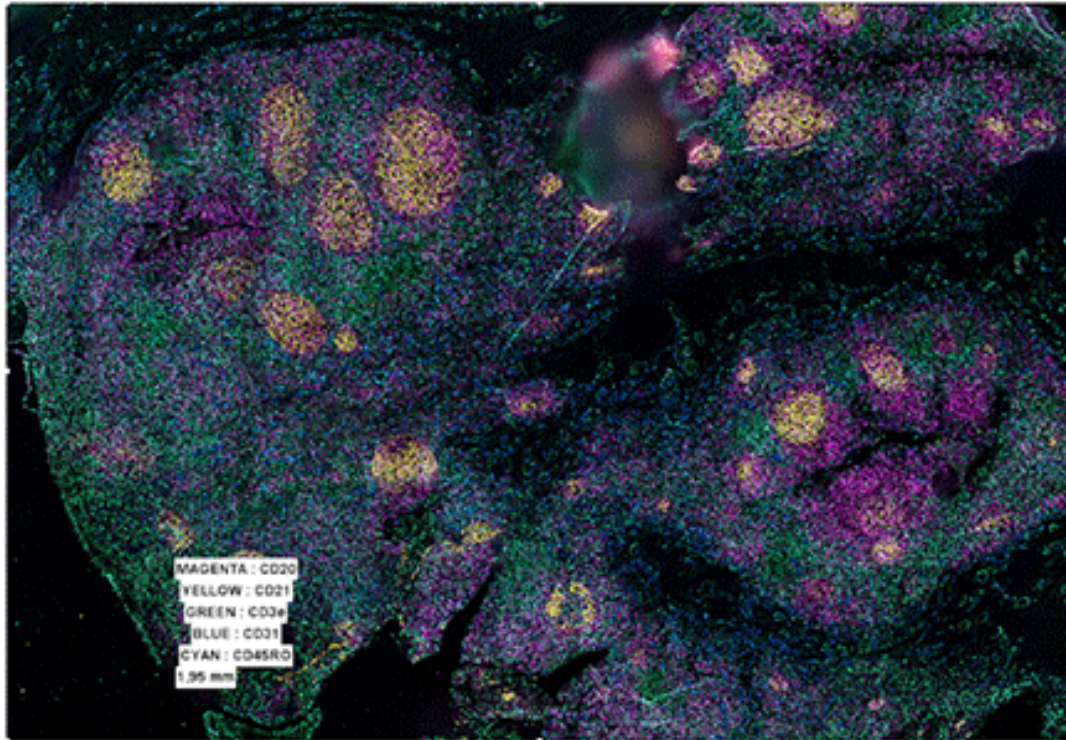
Area of interest (to be microdissected) are identified on the whole scan.

On the microdissector, a 10x image is acquired, and the transformation is computed, and applied to Area of interest



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

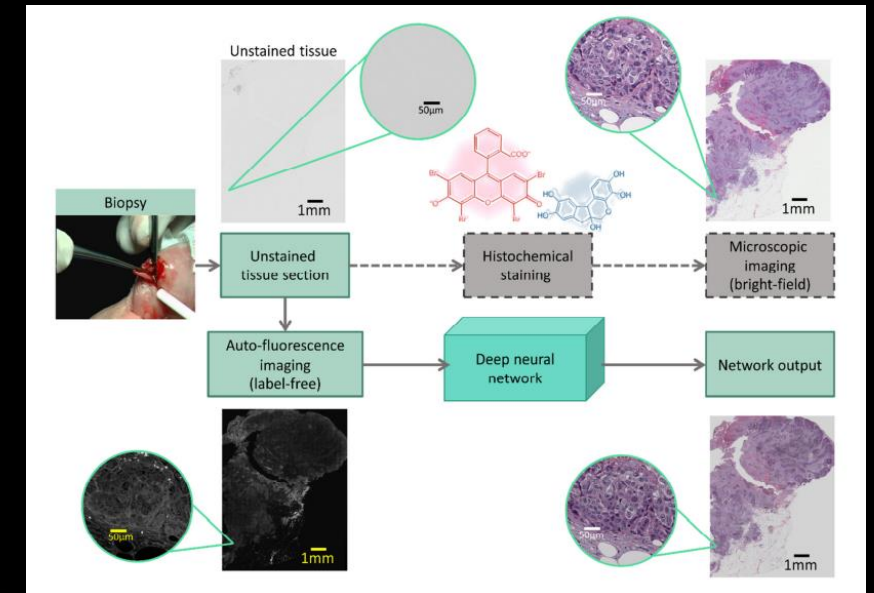
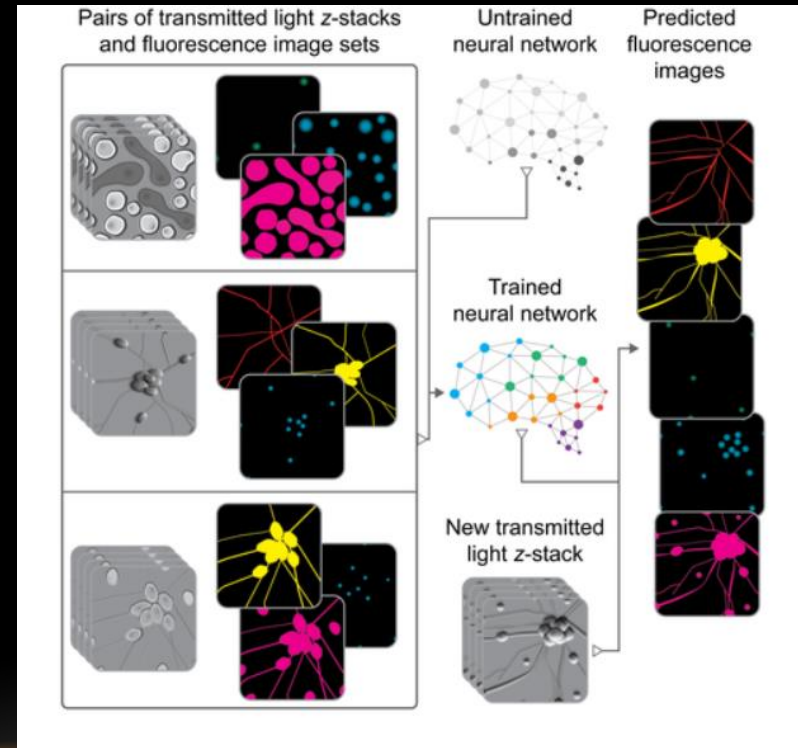
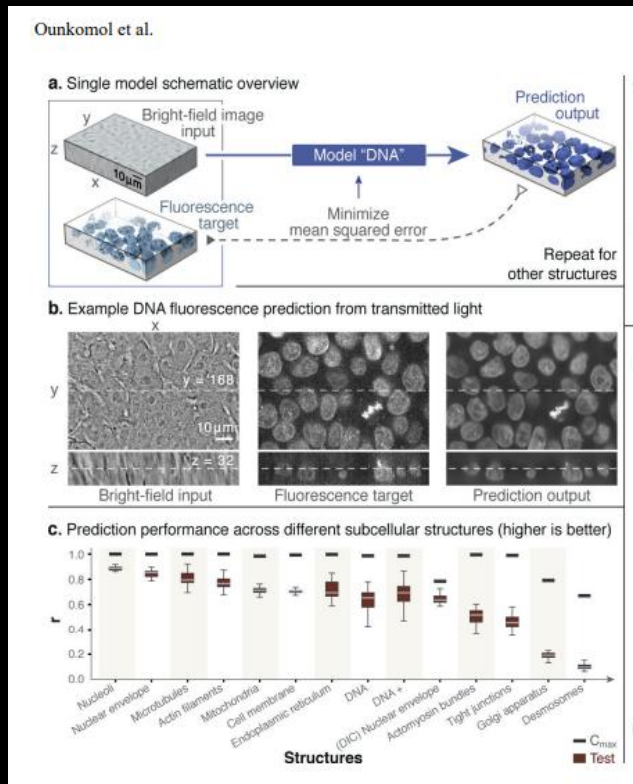
OTHER USAGES OF REGISTRATION IN MULTIMODAL MICROSCOPIES: SMART MICRODISSECTION FOR SPATIAL OMICS



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

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

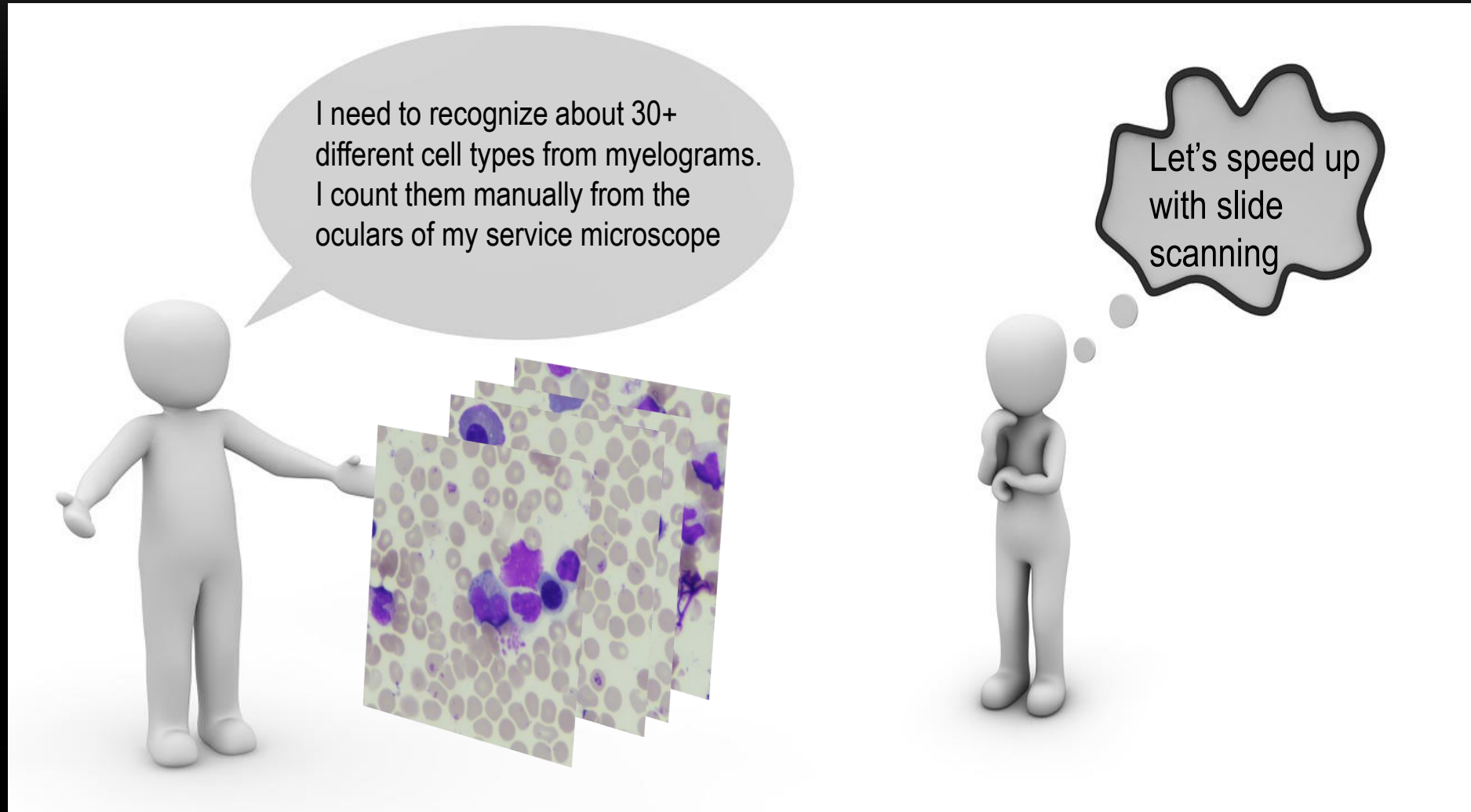
Christiansen et al. Cell. 2018
Apr 19;173(3):792-803.e19.
doi: 10.1016/j.cell.2018.03.040

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
- Instance segmentation: automatic or manual labeling from another modality

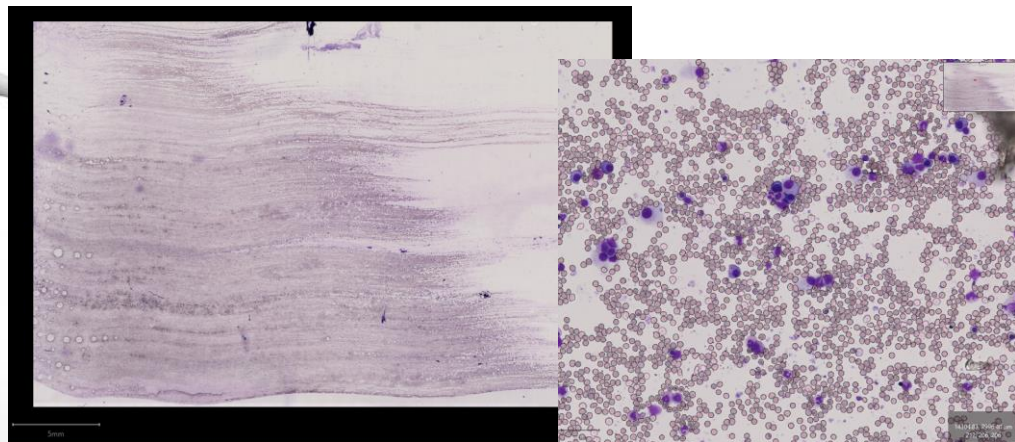
EXAMPLE OF NEED OF DIFFERENT MICROSCOPES FOR DEEP LEARNING



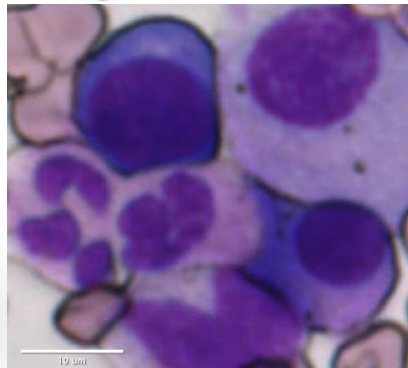
Cedric Almeida Braga
PhD on Multiple
Myeloma
Diagnosis

How wonderful!

Great! Go and annotate,
here is the annotation protocol!



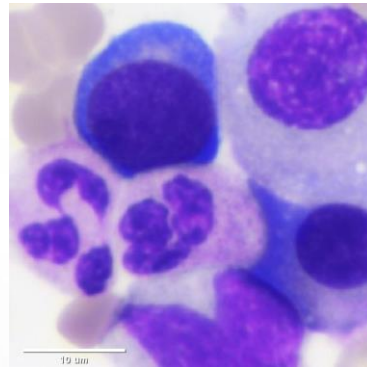
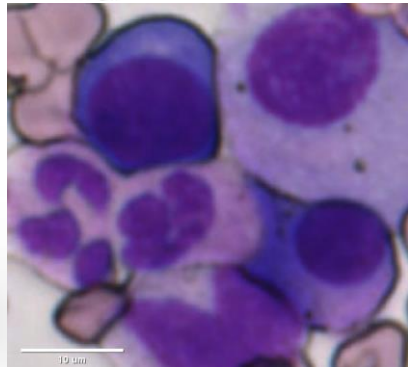
I can not recognize my cell types
at 40x, i need my immersion oil
and at least 50x



Can't you say
from the start...



I can not recognize my cell types
at 40x, i need my immersion oil
and at least 50x

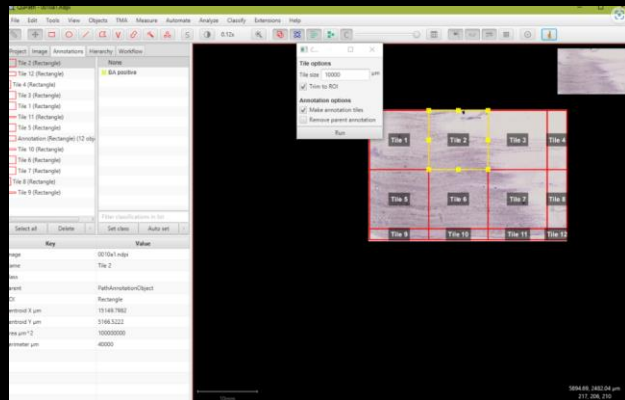
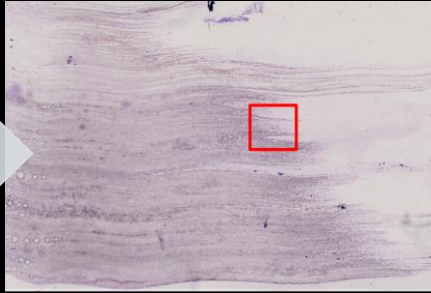


ok...



ACQUISITION WORKFLOW

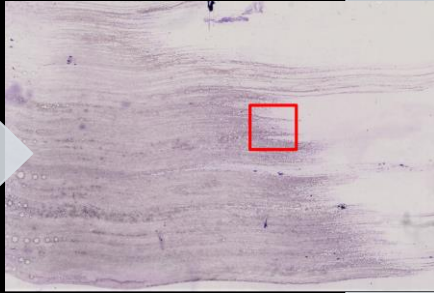
- Slide scanning
- Reading area Annotated by hematologist



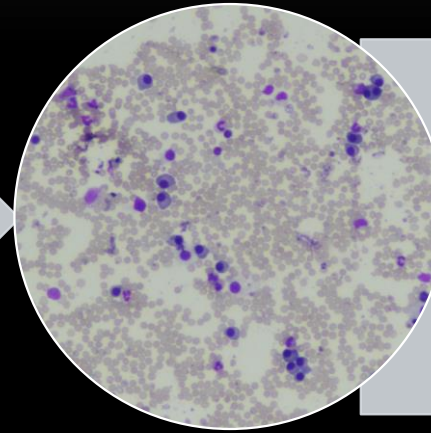
QuPath Script for
Tiles and Reading
area coordinates

ACQUISITION WORKFLOW

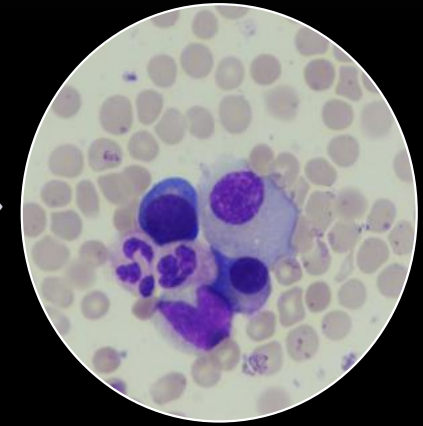
- Slide scanning
- Reading area Annotated by hematologist



- Acquisition 10x
Air wherever
- Automatic registration



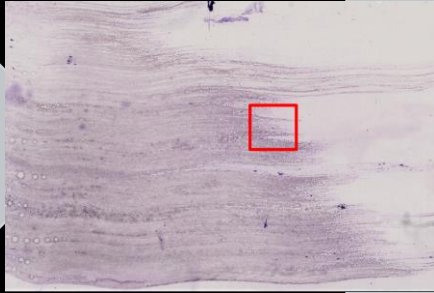
- Automatic acquisition of Region of interest at 60x oil



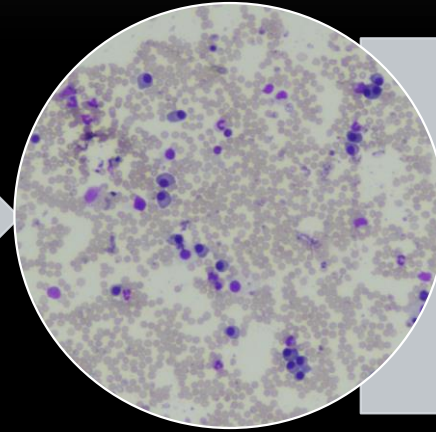
Registration using a portable python on a usb stick on the microscope -> Open CV (create same res images in greyscale, Blur, then Max normalized cross correlation)

Automatically create the list of multiple positions to be acquired to cover the reading area with autofocus

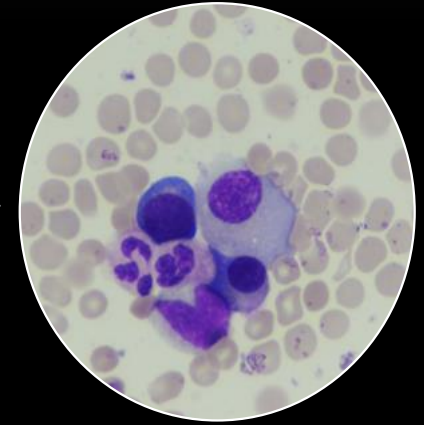
ACQUISITION WORKFLOW



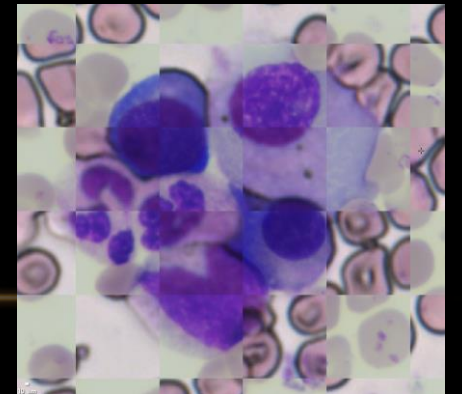
- Acquisition 10x Air
- Automatic registration



- Automatic acquisition of Region of interest at 60x oil

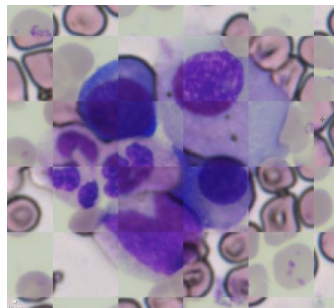
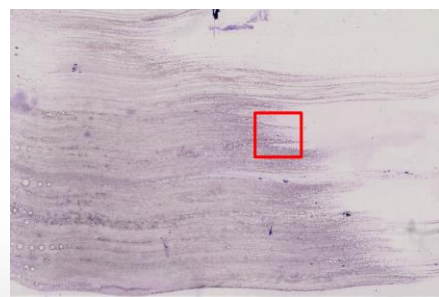


All data are directly linked with the virtual slide



How wonderful!

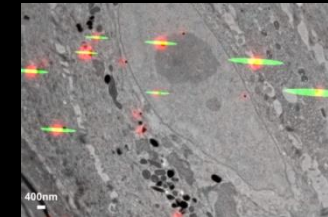
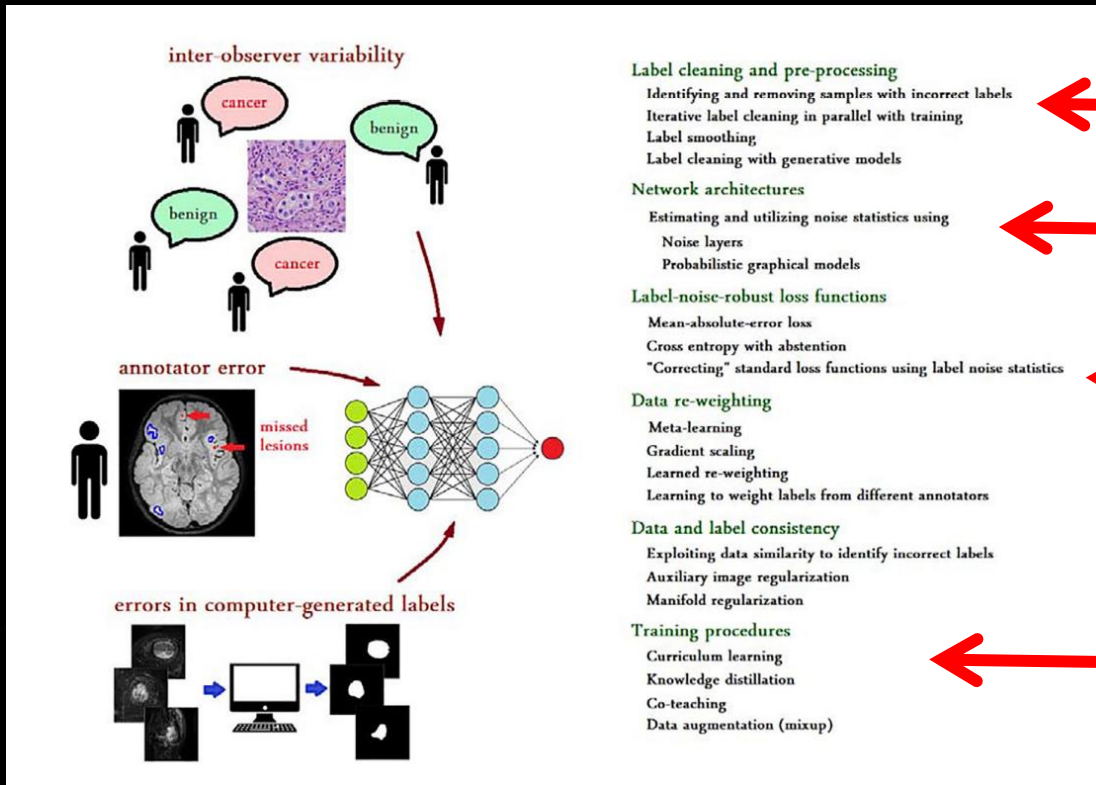
Great!
Go and annotate,
here is the annotation
protocol.



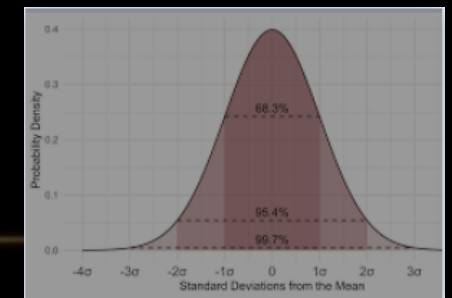
CAN WE USE REGISTRATION UNCERTAINTY TO HELP DEEP LEARNING?

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.”



Yes!
(but not sure yet how better it will be)



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

Smoothing

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

Weighting

Learning

Entropy scaling

Adaptive re-weighting

Learning to weight labels from different annotators

Data and label consistency

Exploiting data similarity to identify incorrect labels

Adaptive image regularization

Global regularization

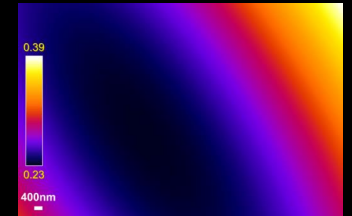
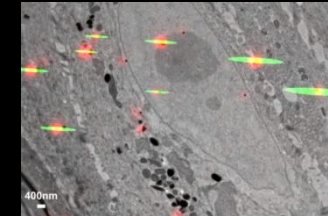
Training procedures

Curriculum learning

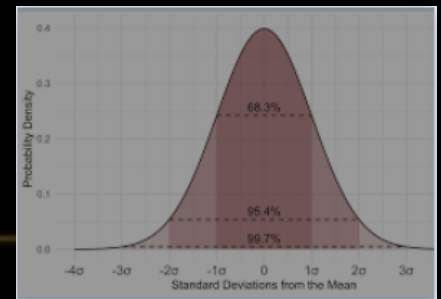
Knowledge distillation

Co-teaching

Data augmentation (mixup)

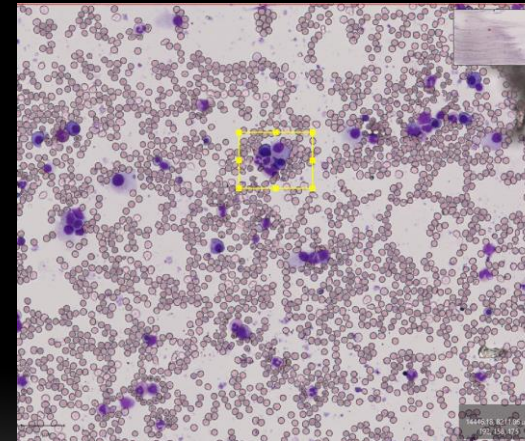
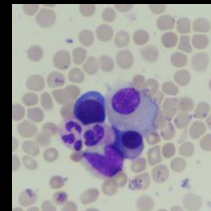
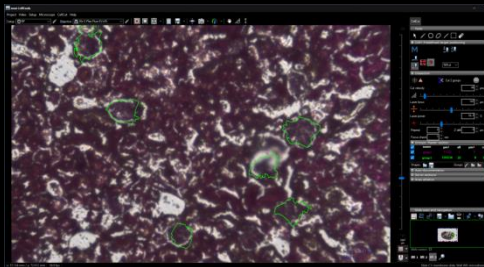
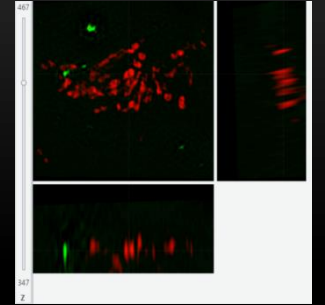
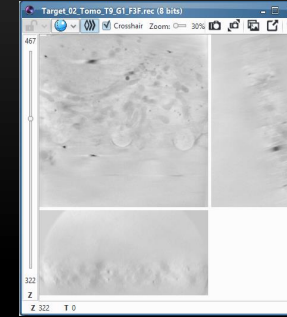
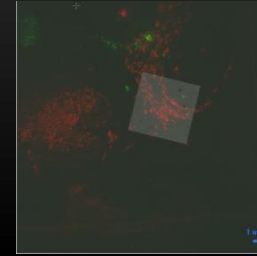


Yes!
(but not sure yet how
better it will be)



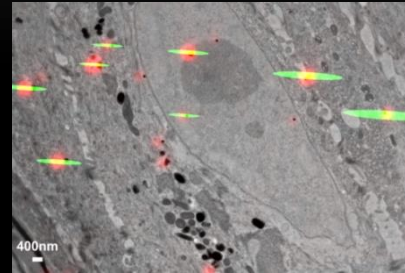
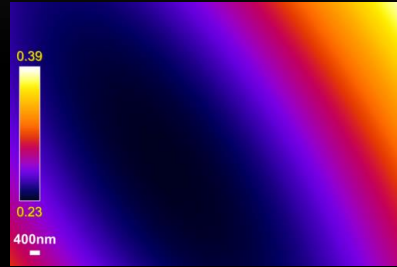
CONCLUSION

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



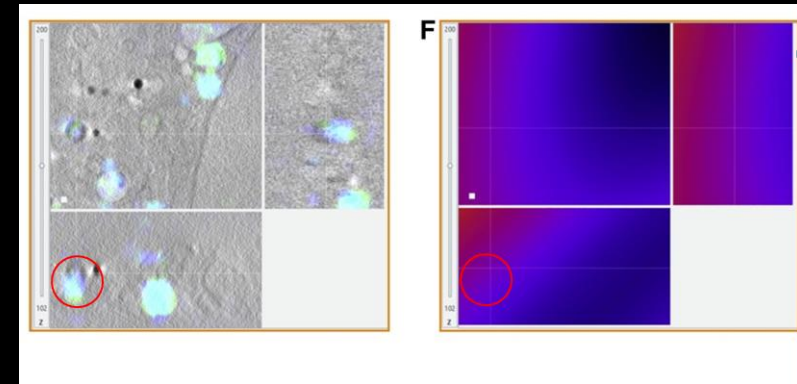
CONCLUSION

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



- This has multiple purposes:

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



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



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Aurélie Doméné

Stéphanie Blandin

Philippe Hulin

Magalie Feyeux

Steven Nedellec

Marianne Guisset

Héloïse Duluc

INCA PLAN

CANCER

Multiple Myeloma project

Marion Eveillard (Cr2INA)

Cedric Almeida (LS2N)

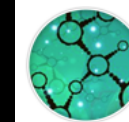
Nicolas Normand (LS2N)



QUESTIONS?



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



FRANCE-BIOIMAGING