

Future challenges in image processing for correlated and multimodal imaging



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[Co-founder of **Cytomine.coop**]



Content & Conflict of Interest Statement

This talk is a mixture of :

- **Overview** of existing works and tools
- **Opinion** about current science practices
- **Challenges and practical perspectives**

I'm co-founder and member of the board of directors of a company providing (selling) software services to > 10K users

- Cytomine software is open-source (cytomine.org)
- Cytomine.coop is a not-for-profit cooperative

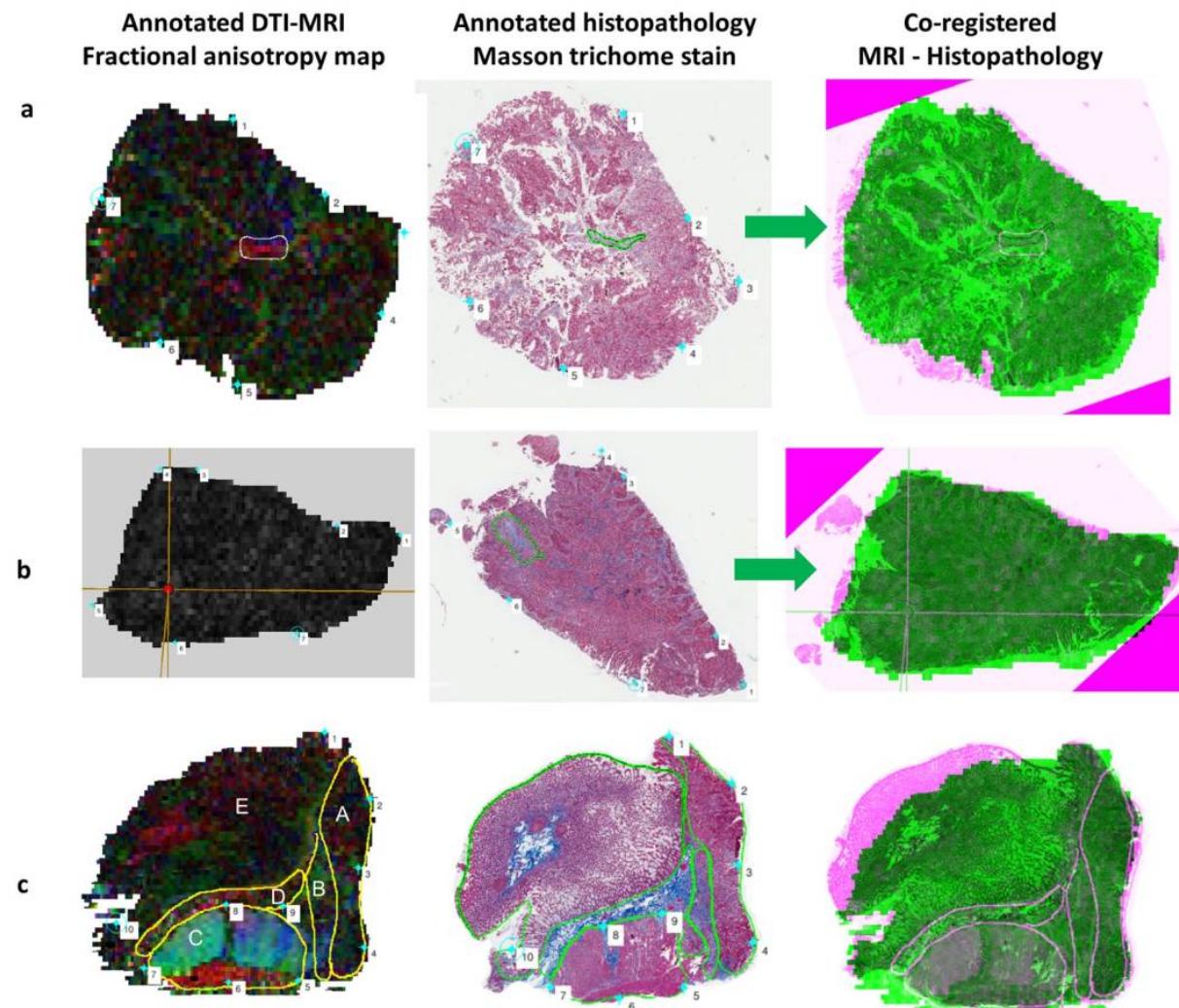


CMI examples (beyond CLEM & PHI)

Correlation of ultra-high field MRI with histopathology for evaluation of rectal cancer heterogeneity

(Scientific Reports, 2019)

Trang T. Pham^{1,2,3}, Timothy Stait-Gardner⁴, Cheok Soon Lee^{2,3,5,6}, Michael Barton^{1,2,3},
Petra L. Graham^{1,2,3}, Gary Liney^{1,2,3}, Karen Wong^{1,2,3} & William S. Price^{1,4,5}



CMI examples (beyond CLEM & PHI)

Correlative x-ray phase-contrast tomography and histology of human brain tissue affected by Alzheimer's disease

(NeuroImage 2020)

Mareike Töpperwien^a, Franziska van der Meer^c, Christine Stadelmann^c, Tim Salditt^{a,b,*}

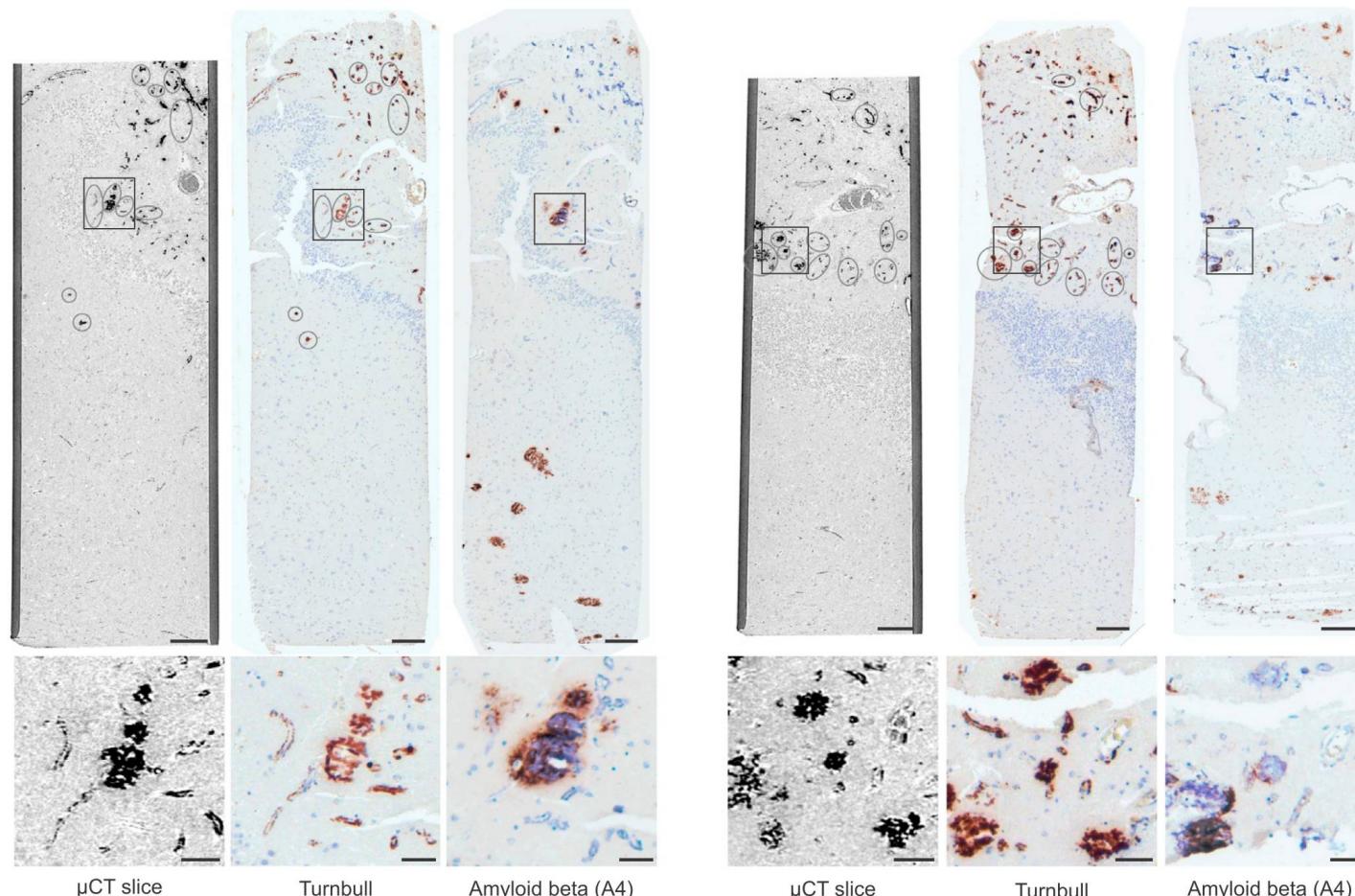


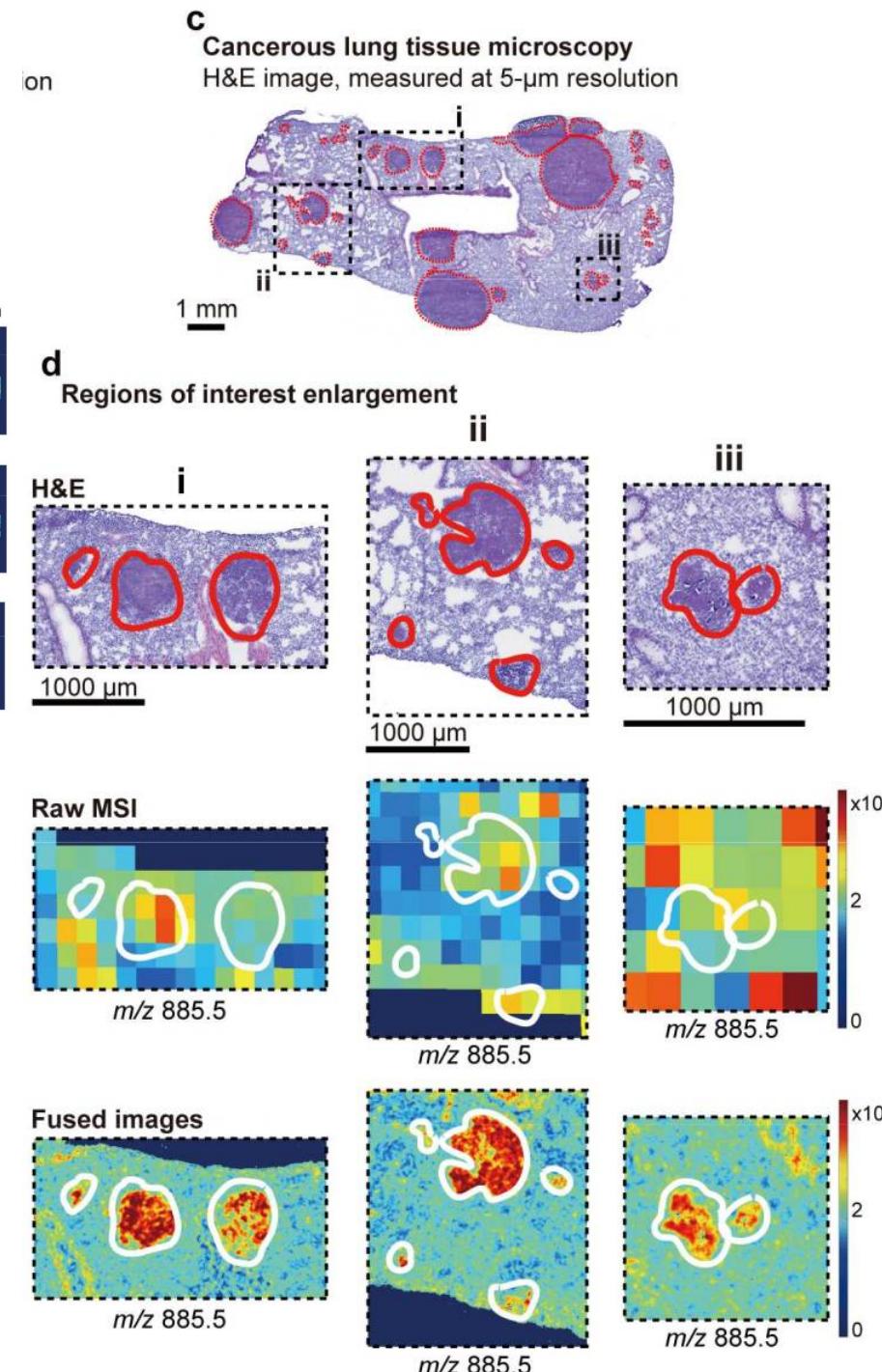
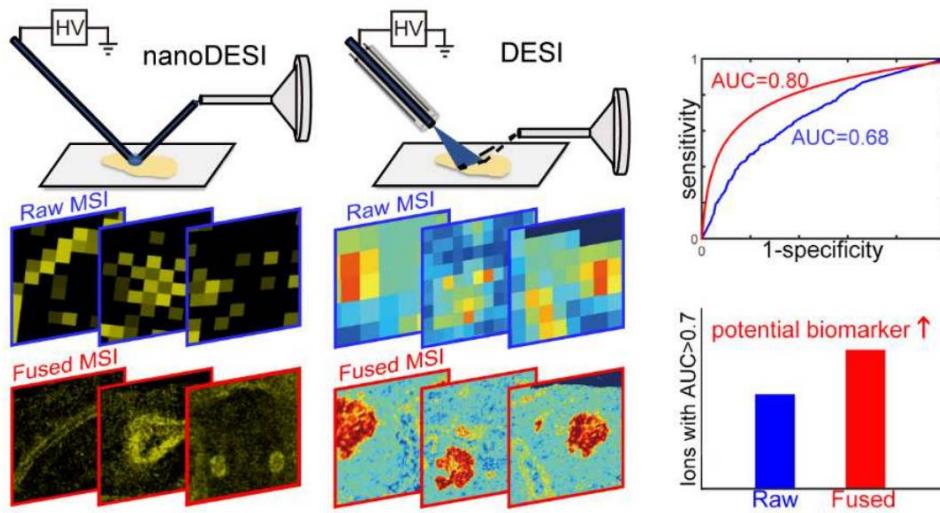
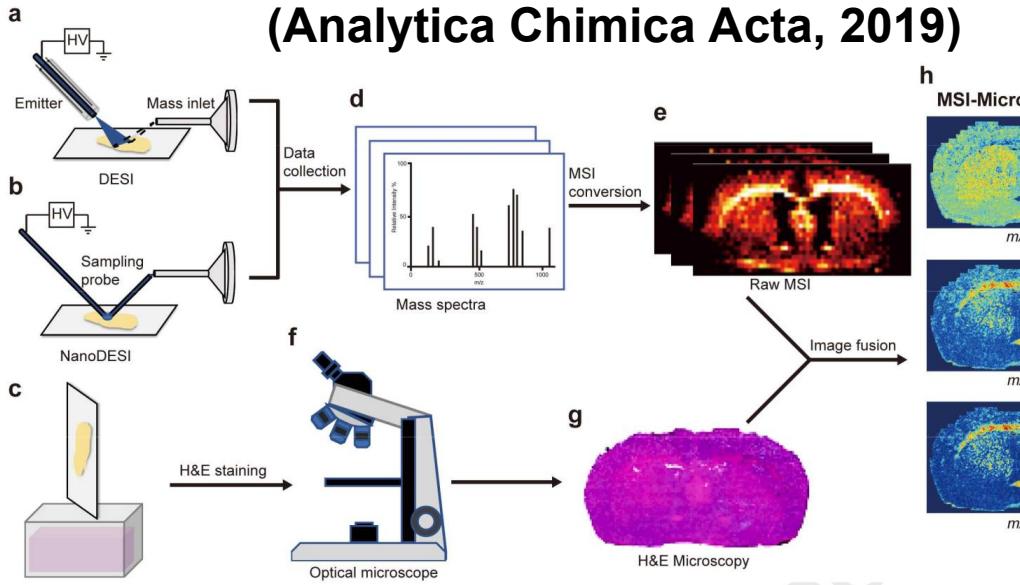
Fig. 6. Comparison between virtual slices through the laboratory μ CT results (left) and histological sections using a β -amyloid (A4) staining protocol (right). As in Figs. 4 and 5, the encircled features in the virtual slice and the histological sections show similarities which indicate that the positions of the virtual slices from the μ CT results and the histological sections approximately coincide. Also in this case, the electron-dense plaque-like structures correspond to features containing iron, as observed in the corresponding histological section stained according to the Turnbull protocol (center). The β -amyloid (A4) together with the hematoxylin stain additionally shows that they consist of calcified structures and β -amyloid, proving that they are indeed mineralized β -amyloid plaques. Note that the contrast in the microscope images of the histology sections has been adjusted to ensure maximum visibility of the features of interest. Scale bars: 200 μm (top) and 50 μm (bottom).

CMI examples (beyond CLEM & PHI)

Precision Biomarker Discovery Powered by Microscopy Image Fusion-assisted High Spatial Resolution Ambient Ionization Mass Spec-

trometry Imaging

(*Analytica Chimica Acta*, 2019)



CMI examples (beyond CLEM & PHI)

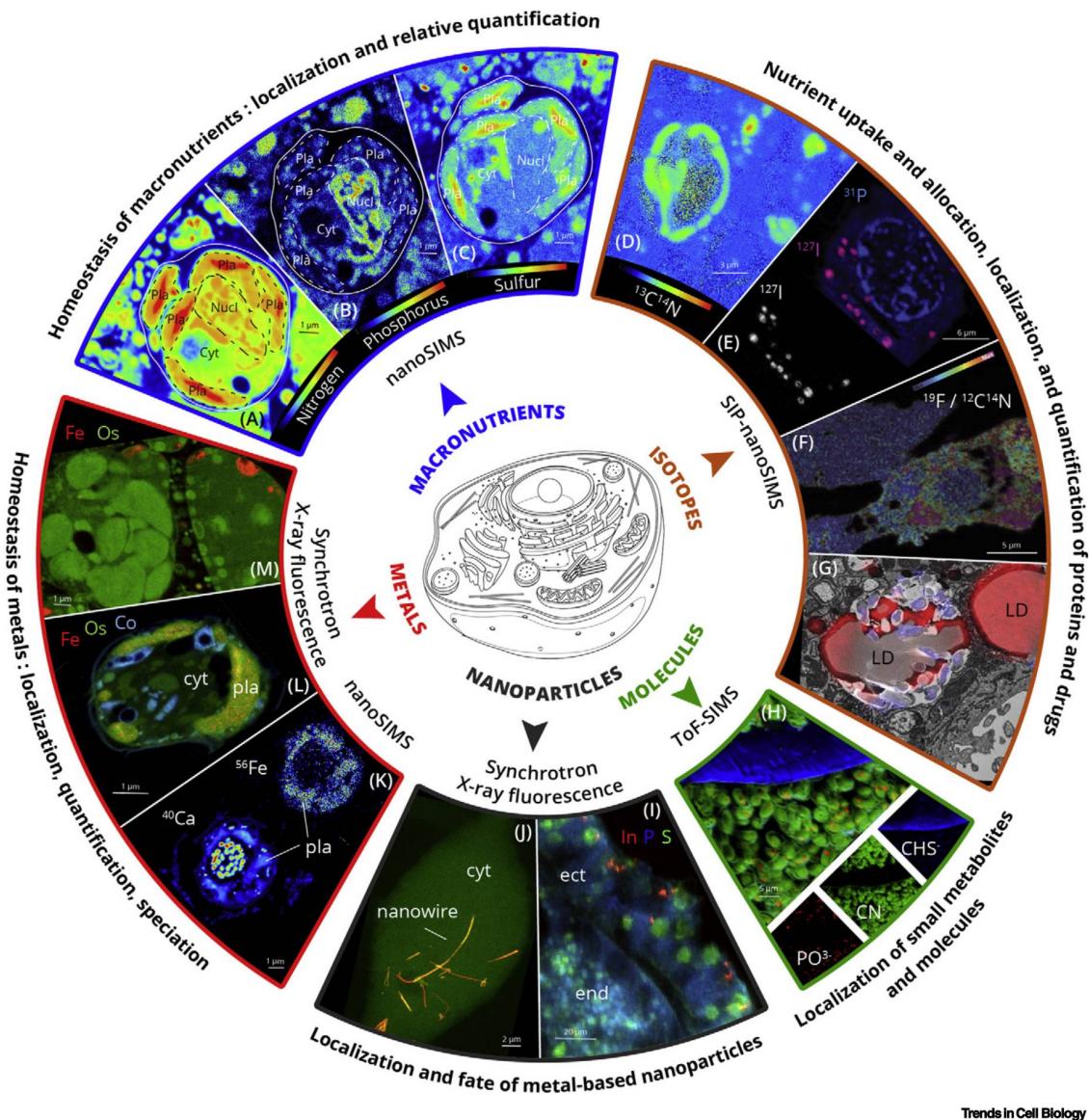


Figure 2. The Potential of Chemical Imaging to Unveil the Chemical Landscape of a Cell: Composition and Distribution of Elements, Isotopes, and Molecules at the Nanoscale. (A–C) Nano-secondary ion mass spectrometry (nanoSIMS) images showing the distribution of the macronutrients nitrogen [^{14}N], phosphorus [^{31}P], and sulfur [^{32}S] in a cell. (D–F) Correlation between electron microscopy (EM) and chemical imaging. (G) Correlation between TEM-EDS and EFTEM. (H) Correlation between SEM and ToF-SIMS. (I) Correlation between nanoSIMS and X-ray fluorescence tomography. (J) Correlation between nanoSIMS and X-ray phase-contrast tomography.

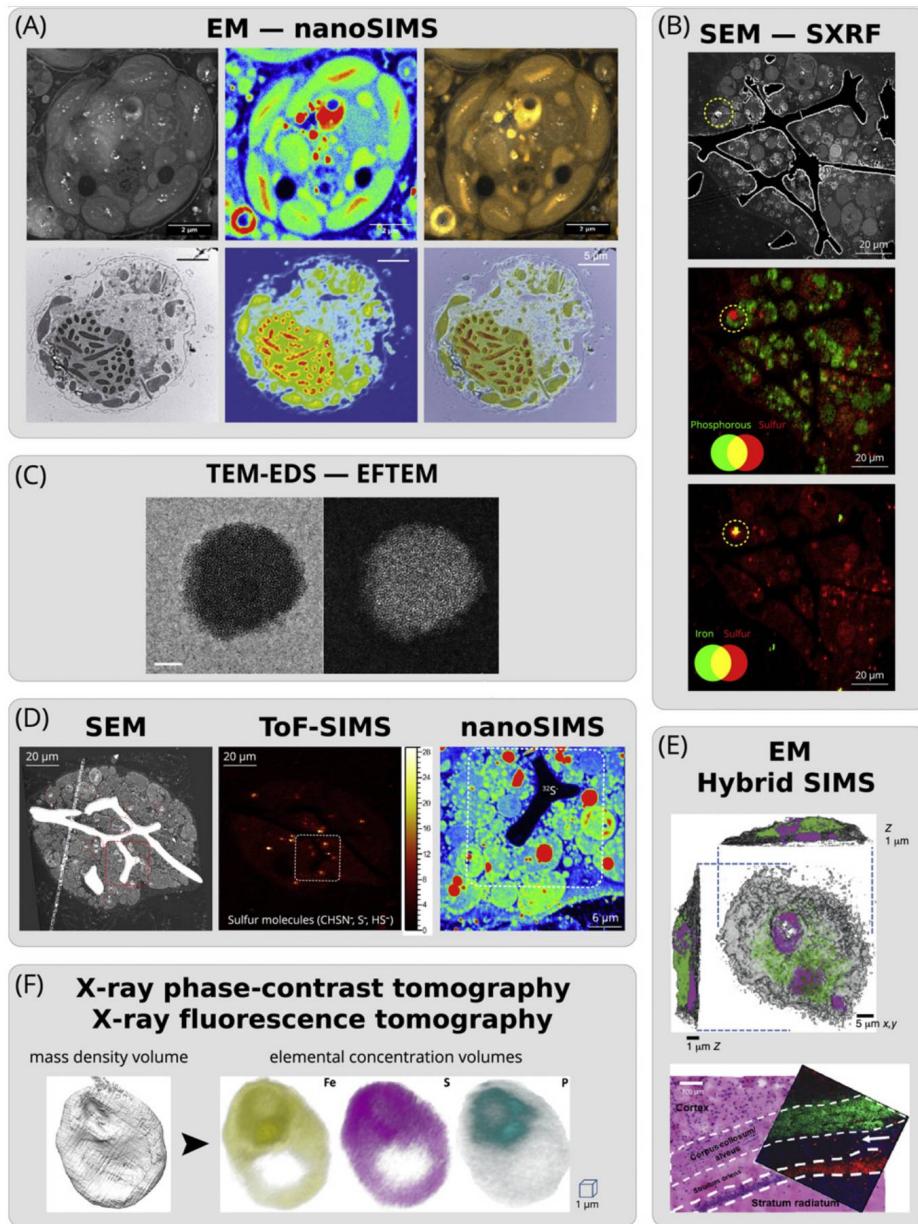
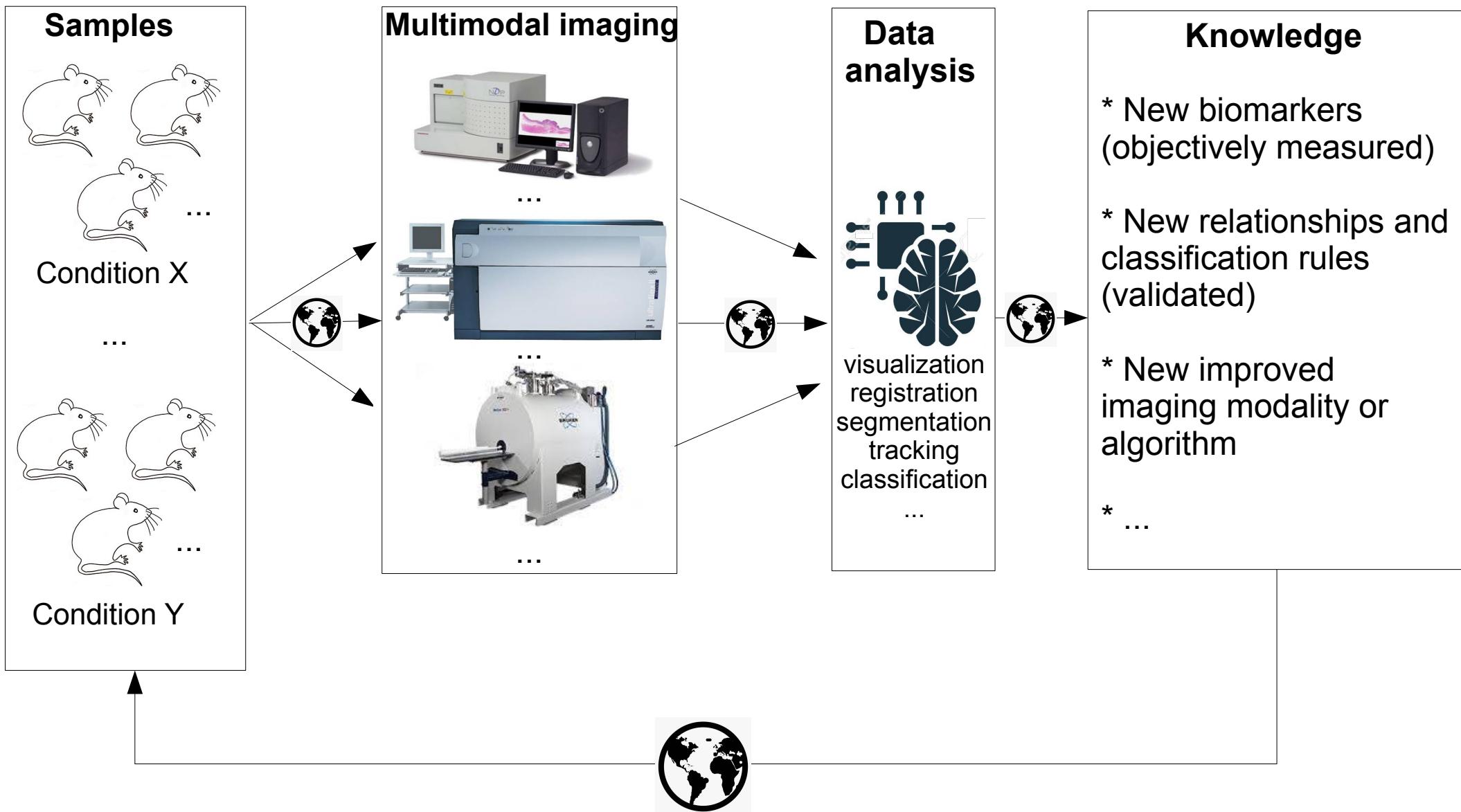


Figure 3. Examples of Correlated Electron Microscopy (EM) and Chemical Imaging. (A) Correlation between EM and nanoSIMS. (B) Correlation between SEM and SXRF. (C) Correlation between TEM-EDS and EFTEM. (D) Correlation between SEM and ToF-SIMS. (E) Correlation between EM and Hybrid SIMS. (F) Correlation between X-ray phase-contrast tomography and X-ray fluorescence tomography.

Subcellular Chemical Imaging: New Avenues in Cell Biology (CellPress Reviews, 2020)

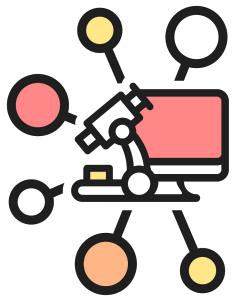
Ideal correlative/multimodal workflow

(Exploratory analysis / Biomarker discovery / Classification of samples)



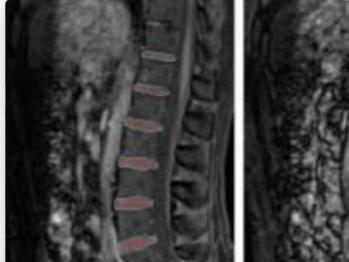
A plethora of image analysis tools

Image registration : « *literally hundreds of different registration algorithms exist* »,
(Borovec et al., IEEE TMI 2020)



BiiII
Biolimage
Informatics
Index

<https://biii.eu/>
(> 1331 « software »)



Medical Image Segmentation

57 benchmarks

113 papers with code

<https://paperswithcode.com/>
(> 11000 ML papers with code)

openneuroscience
<https://open-neuroscience.com>

 **Quantitative Plant**

<https://www.quantitative-plant.org/>

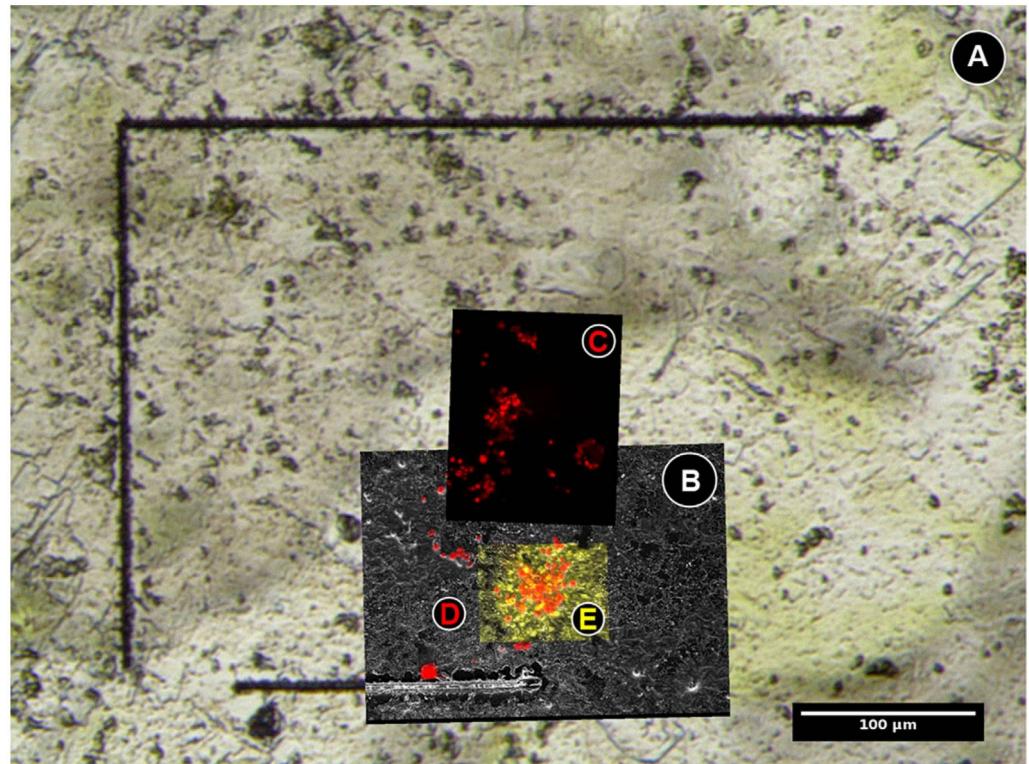


Fig. 2. A correlative data set measured on an algal biofilm visualised by Correlia: The light microscopy image (A) serves as background and is overlaid with a scanning electron micrograph (B, opaque) and two images of the red fluorescence of the algae (C, opaque and D, red). The chemical map of the phosphorous distribution measured by energy-dispersive X-ray spectroscopy (E, yellow) is co-registered on top (Moreno Osorio et al.).

(Correlia, Rohde et al., Journal of Microscopy, June 2020)

CMI data analysis is not ideal ... Why ?

Challenges for biologists:

« Not easy to reuse previous work on my data because ... »

... many nice biology papers do not come with a tool

... which algorithm to choose in the zoo ? How can I trust it ?

... best algorithms might not be implemented in user-friendly software

... many algorithms are not interpretable (black box)

... too much data, too few computer resources

... publish or perish ... (a lot of work is lost after publication e.g. data, image analysis results & tuned methods)...

Challenges for computer scientists :

« Not easy to design generic methodologies because ... »

... so many imaging techniques & combinations (very high resolution !)

... few datasets available (and proprietary formats)

... even less annotated datasets (ground-truths) available

... meaningful results ?

... publish & perish... validation only on 1-2 (small) dataset(s) : ad hoc

It is probably better to think about it now, before the imaging data deluge...

e.g. in MALDI-MSI imaging

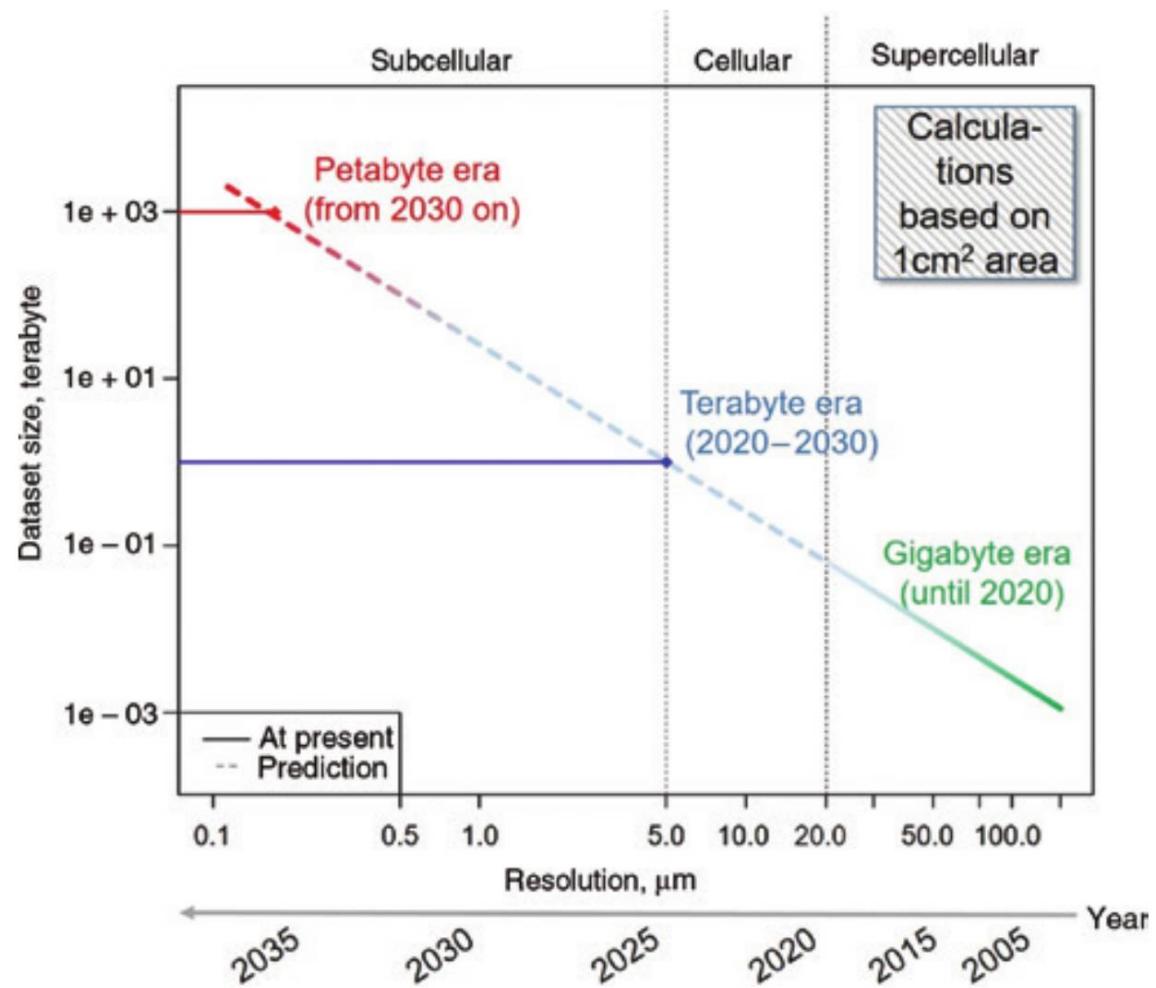


Figure 6: Prediction of data size growth for routine MALDI-MSI measurements in the next decade when spatial resolution reaches submicron levels.
All axes are log-scaled.

Can we solve this by tomorrow? No, but...

*Let's try to **collaborate** more effectively towards **more generic** and **reproducible** correlative/multimodal image analysis workflows.*

Practical suggestions to improve our situation :

- Image data **sharing** : what/how/where to share ?
- Image data **annotation (ground truths)**: what/how/where to annotate ?
- **Quantitative and reproducible** image data analysis : which method to choose and how to publish it ?
- **Use of and contribution to collaborative and community tools**
 - biaflows.neubias.org ; Biii.eu ; Image.sc ; ...

Image data sharing : what/where ?

What ? Image and metadata in non-proprietary formats (TIFF, OME-TIFF, hdf5, imzml, DICOM, BIDS, ...). Metadata should includes details about samples, preparation protocols, instruments, ...

Where ? Centralized repositories



www.ebi.ac.uk/bioimage-archive/



www.oasis-brains.org/



www.cancerimagingarchive.net/

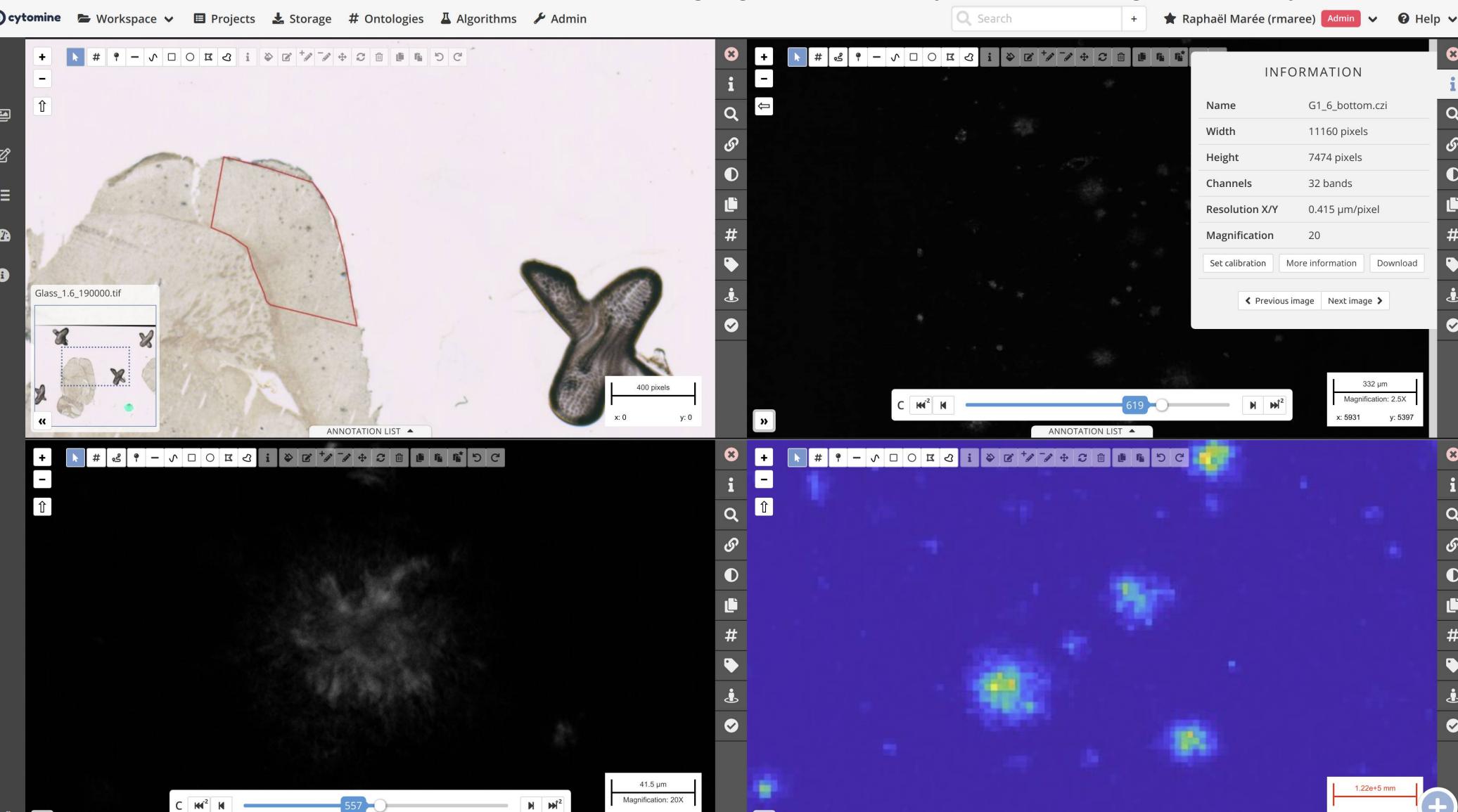


... or setup your own server !

Decentralized image data sharing : give access to your own server

Any institute can install a **cytomine** server and start sharing data (within a day with the help of your institute IT: <https://doc.cytomine.org/>)

(data: Centre for Cellular Imaging Core Facility, Gothenburg University)



Decentralized image data sharing:

cytominne web API to import/export data or use them in your tools

e.g. get Spectra and maximum projection of a subwindow in the image:

<https://research.cytomine.be/api/imageinstance/153042325/window-3000-3000-512-512.png?projection=max>

Python:

```
i = ImageInstance().fetch(153042325)
i.window(3000,3000,512,512,projection='max', dest_pattern="mywindow.png"
```

```
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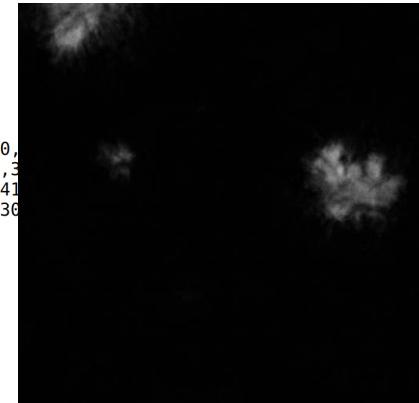


Image annotations : what/how

Use annotation tools to ease the annotations and store them in **computer-readable formats** for analysis or further development of more accurate annotation algorithms

The figure consists of four panels arranged in a 2x2 grid, each showing a screenshot of the AnnotatorJ software interface. The top row shows the initial steps of creating a contour, while the bottom row shows refining and accepting the annotation.

- 1. initialize contour**: Shows the initial step where a user has drawn a rough outline around a cell nucleus. The software's 'Contour assist' feature is active, as indicated by a checked checkbox in the toolbar and a callout arrow. A small inset shows a zoomed-in view of the red outline being refined.
- 2. suggested contour**: Shows the software's 'Contour assist' feature suggesting a more accurate, smooth red outline around the cell nucleus. The 'AnnotatorJ' toolbar is highlighted with a blue arrow.
- 3. refine contour**: Shows the user refining the suggested contour by adding more points to it. The 'Smooth' checkbox in the toolbar is checked, and a blue arrow points to the toolbar.
- 4. accept**: Shows the final annotated cell with a red outline. The 'Accept' button in the toolbar is highlighted with a blue arrow. The status bar at the bottom of the window says 'press \'q\''. To the right, a list of labeled regions is shown, with the first entry '0001' highlighted with a blue arrow.

(AnnotatorJ,
MBoC, 2020)

Semi-automated annotations of cellular compartments (desktop tool)

Image annotations : what/how

« Point » annotations for image registration, landmark-based measurements, cell counting
(data : ANHIR challenge)

cytominē

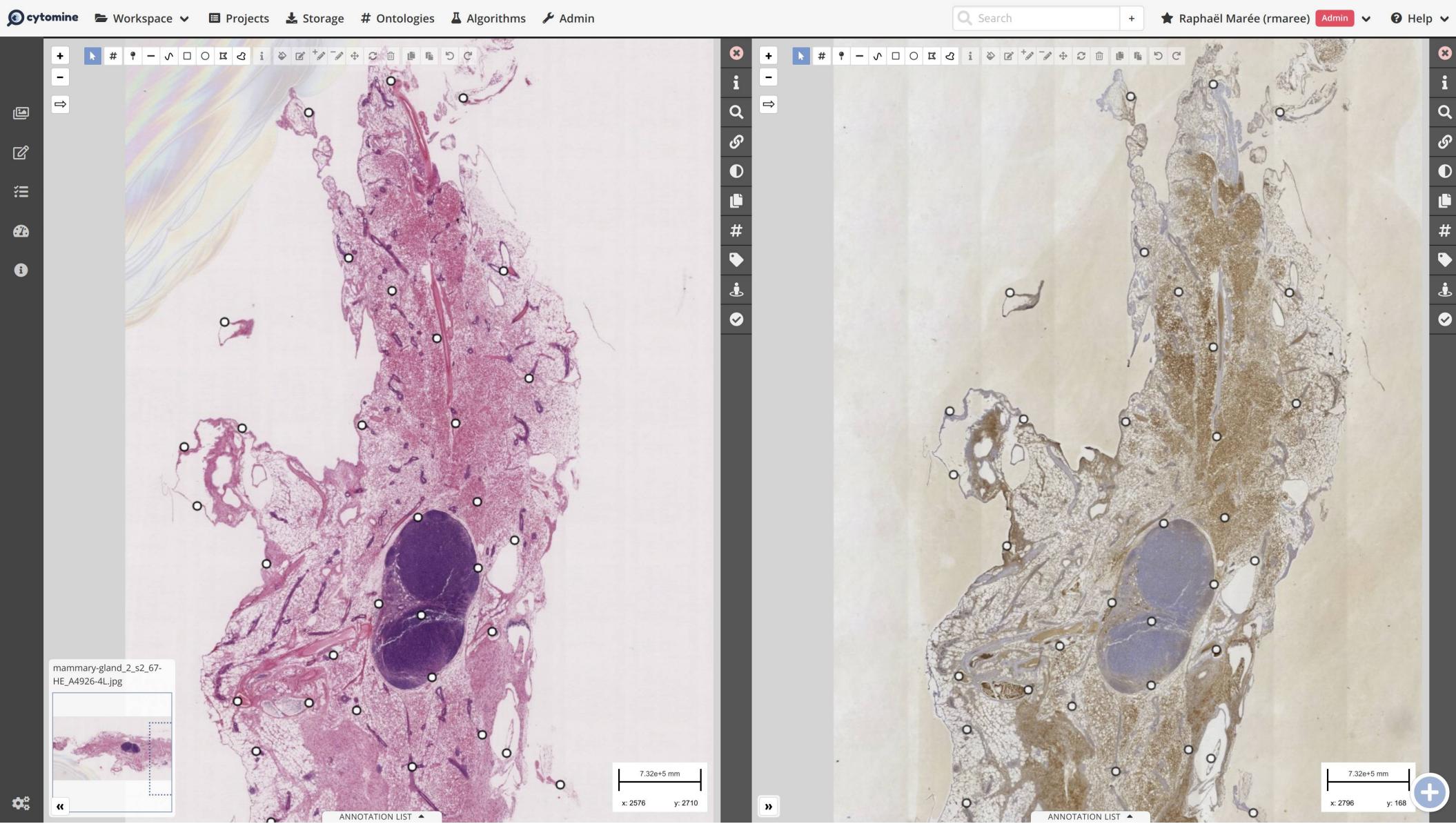


Image annotations : what/how

« Point » annotations for image registration, landmark-based measurements, cell counting

cytominE

(data : GIGA, UPMC, LESA, Rekomitjie+ Stellenbosch University)

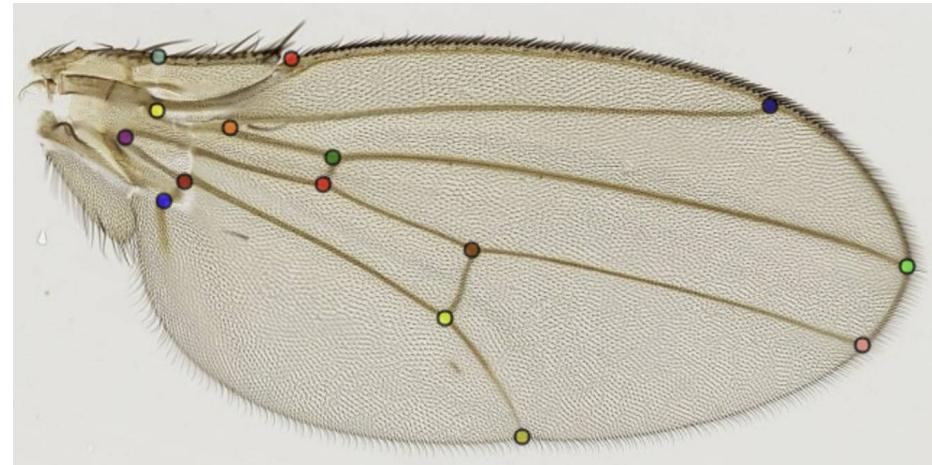
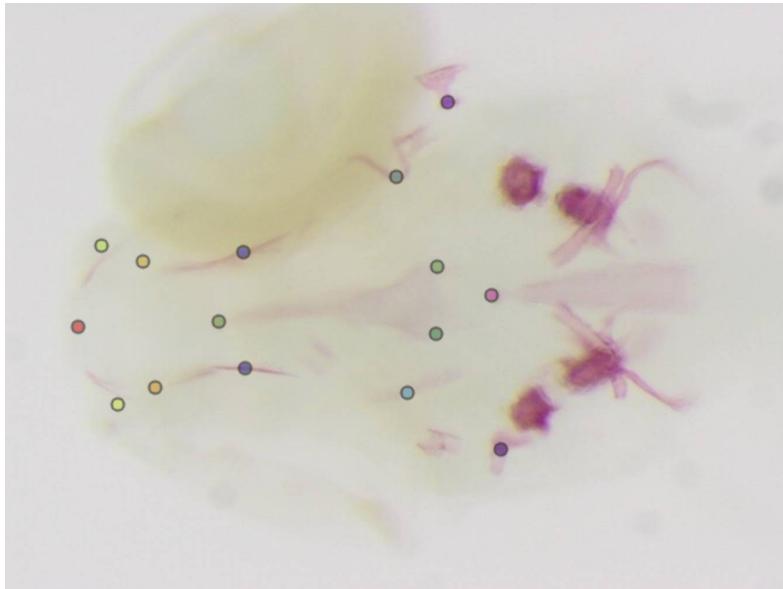


Image annotations : what/how

« Point » annotations for image registration, landmark-based measurements, cell counting

(data : GIGA)

cytominē

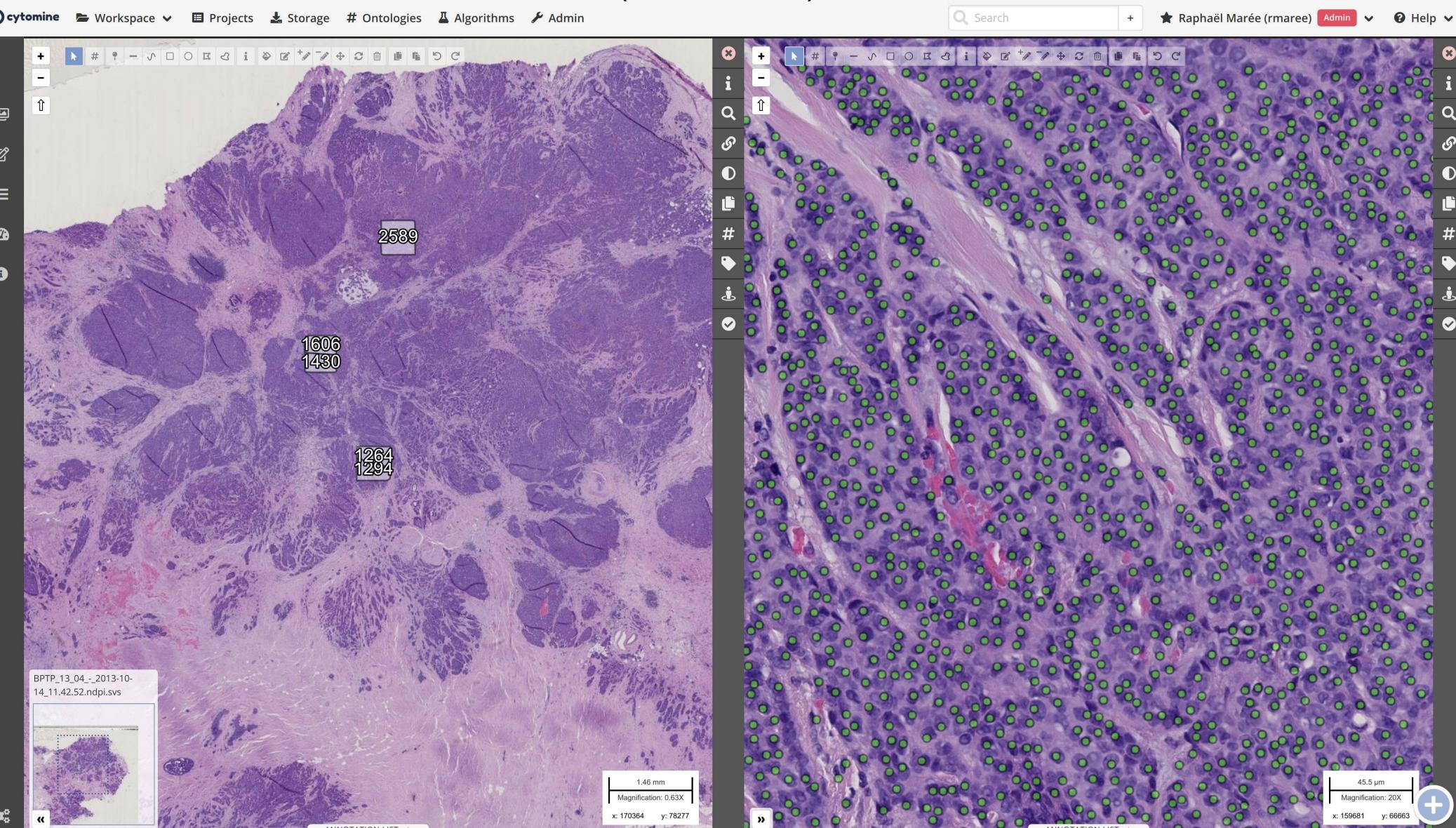


Image annotations : what/how

« Polygon » annotations for segmentation and measurements

(data : GIGA, BioMedAQu)

cytominē

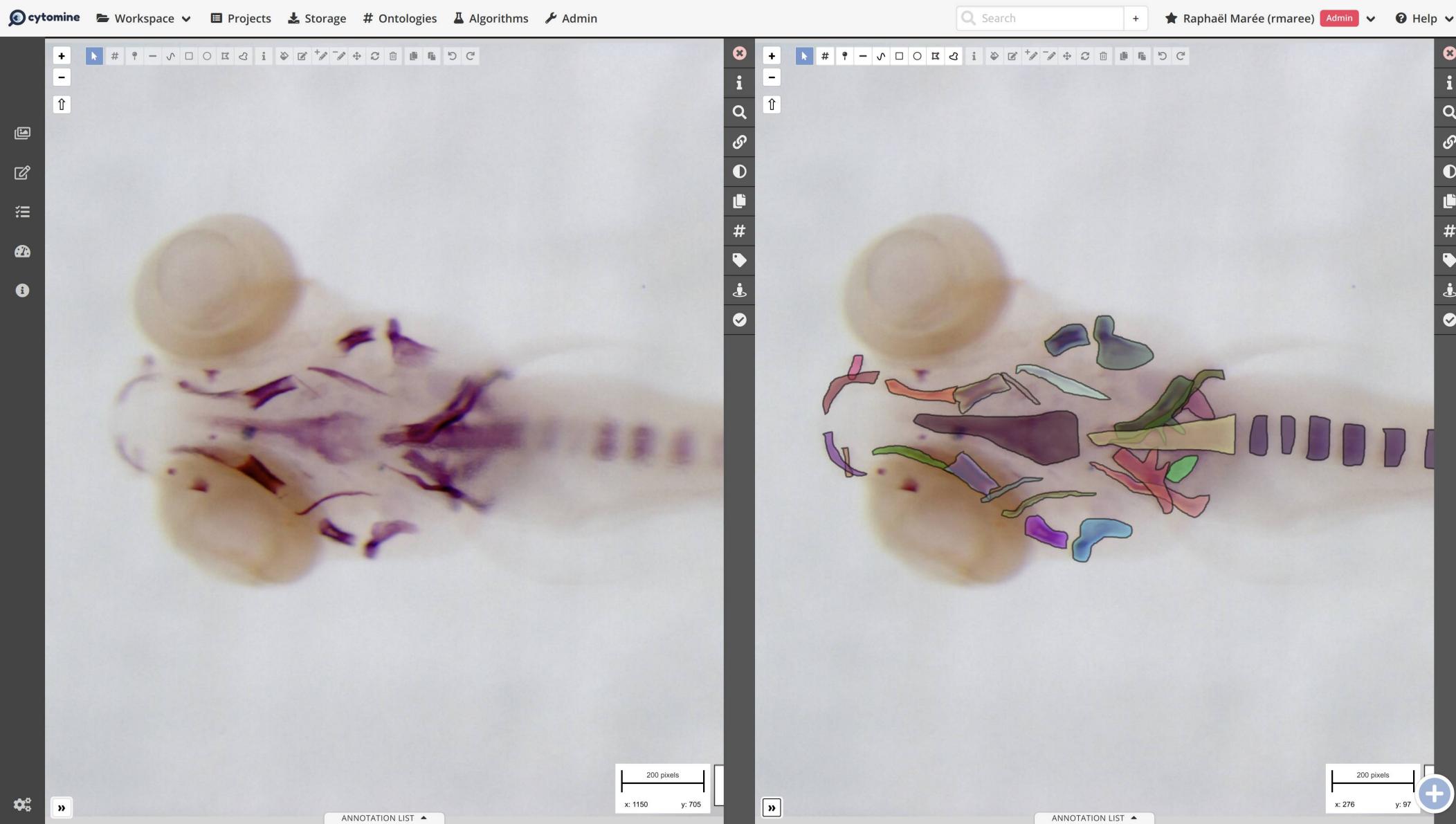


Image annotations : what/how

« Polygon » annotations for segmentation and measurements

(data : CCMR, BioMedAQu)

cytominē

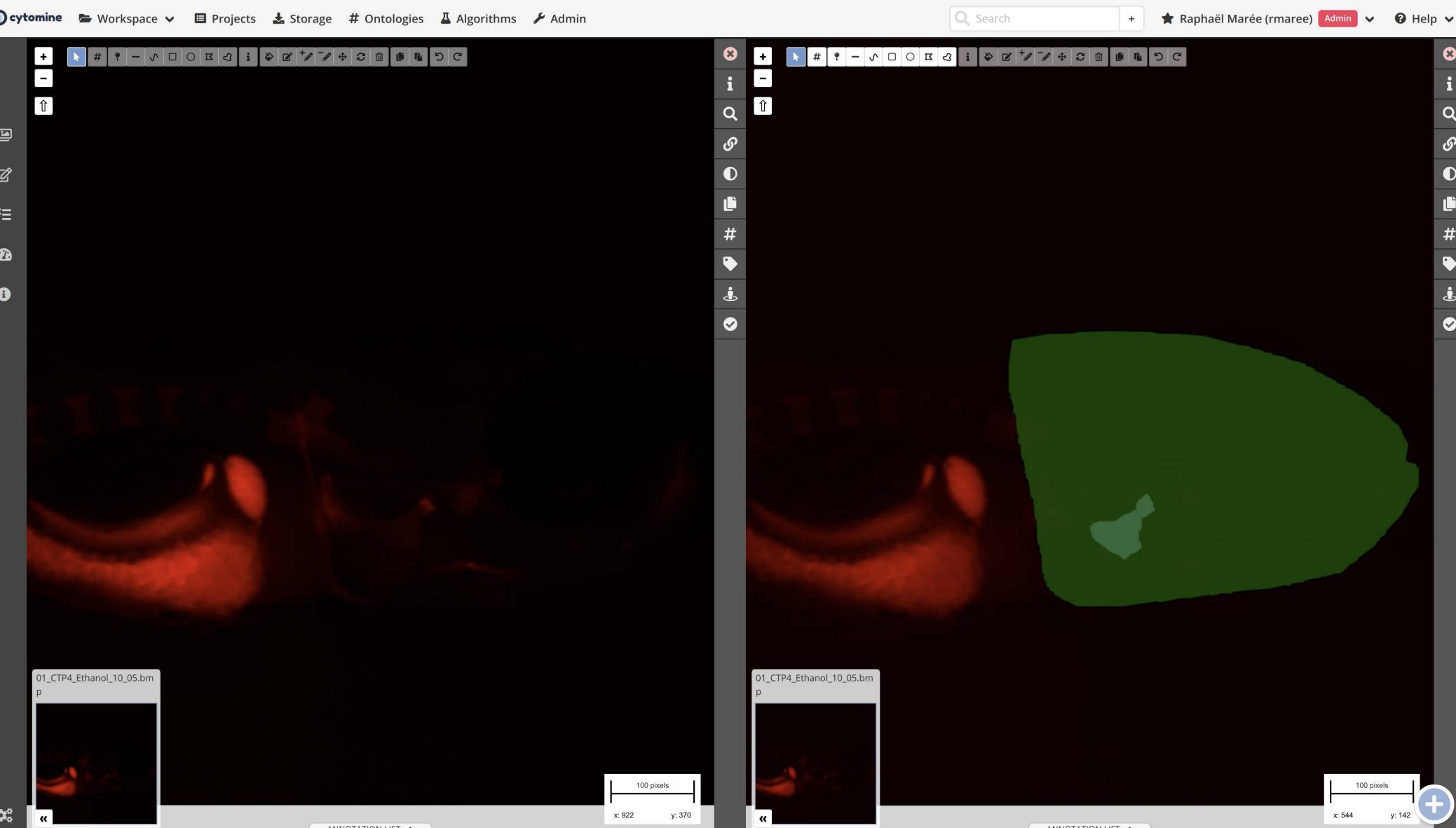
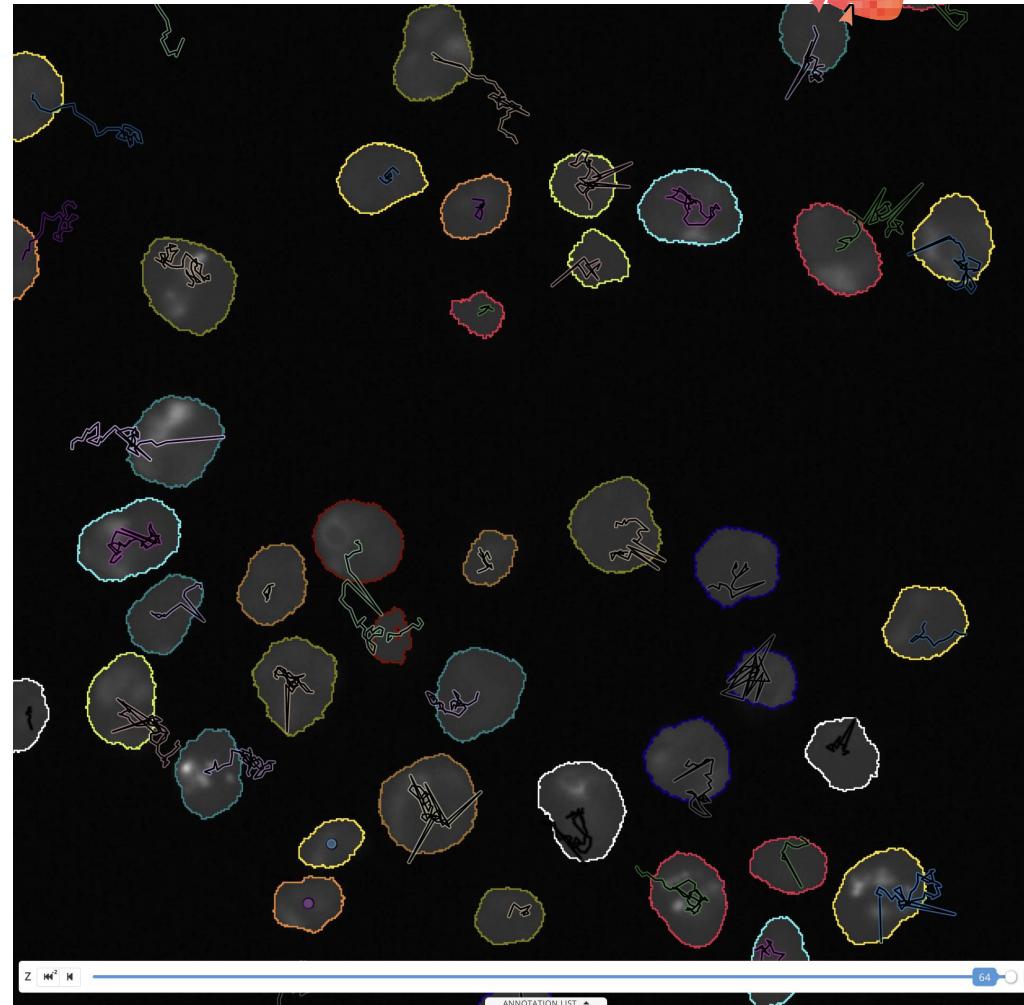
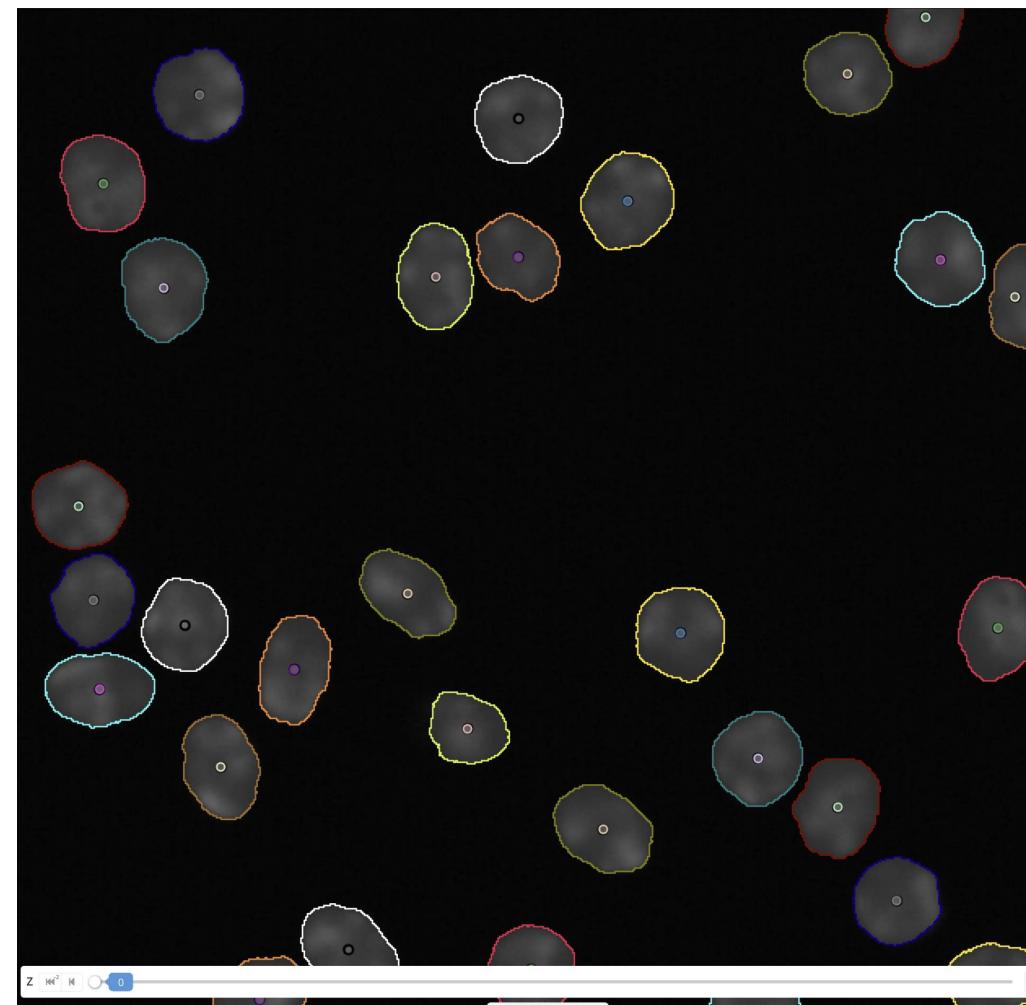


Image annotations : what/how

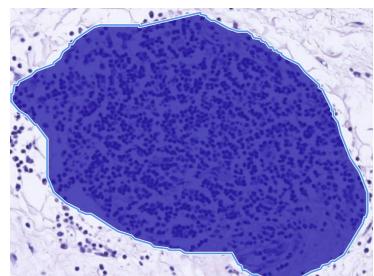
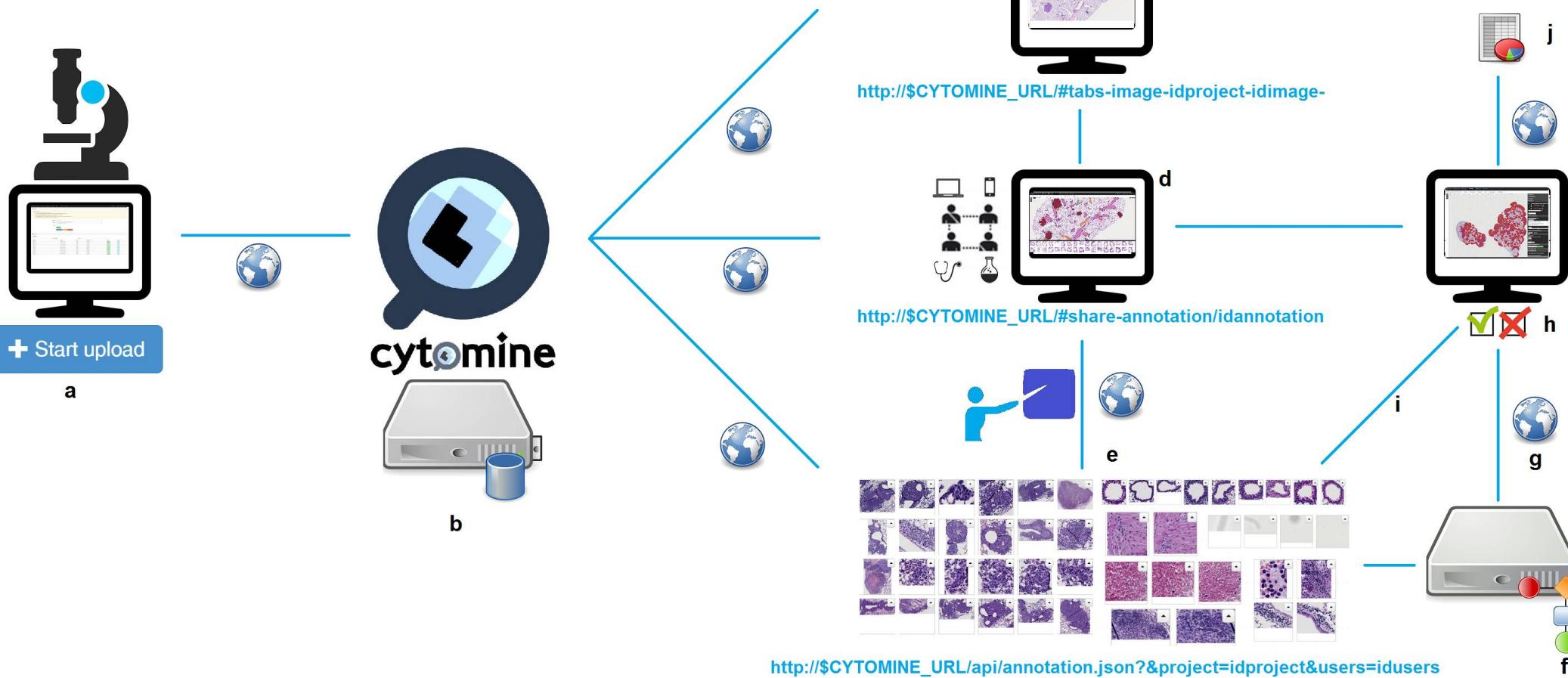
« Coordinates over time » annotations for tracking

(data : CellTracking Challenge)

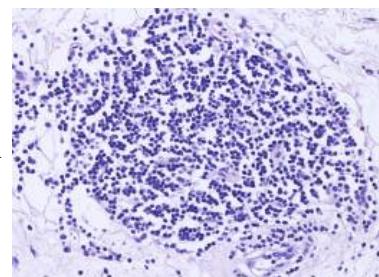


Sharing of image annotations : where

On your own server :



Web API



<https://research.cytomine.be/api/userannotation/26675587/crop.png?mask=true>

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```

Sharing of image annotations : where

Challenges are ways to attract more computer vision researchers to work on difficult tasks

Grand Challenge

grand-challenge.org

The screenshot displays a grid of eight challenge cards:

- MitoEM Challenge**: Large-scale 3D Mitochondria Instance Segmentation. Includes two microscopy images of mitochondria.
- Apples-CT**: Includes two images of apples.
- LoDoPaB-CT**: Includes a diagram of a 3D coordinate system with axes labeled "Detector", "X-ray source", and "S".
- QUBIQ**: Includes a microscopy image of a brain slice with colored segmentation masks.
- Pathology Visual Question Answering**: Includes a microscopy image of tissue cells.
- The PANDA challenge**: Includes a diagram of a brain with segmented regions.
- CADA - Aneurysm Segmentation**: Includes a 3D rendering of a brain with an aneurysm highlighted.
- CADA - Rupture Risk Estimation**: Includes a 3D rendering of a brain with a different segmentation.

Each card includes a summary, a "Members" count (0-5), and a "Images" count (5-130).



biaflows.neubias.org

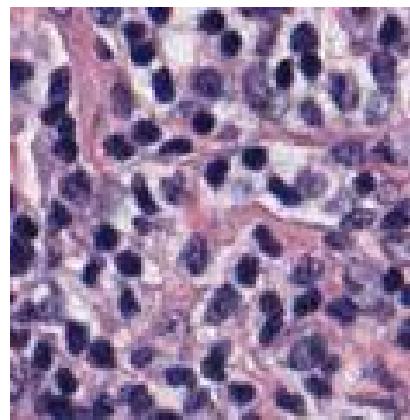
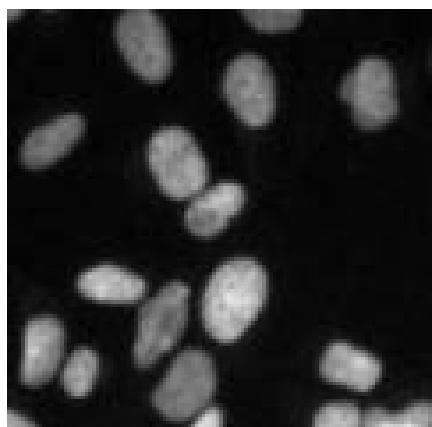
Name ↑	Description	Members	Images
DATA-SCIENCE-BOWL-2018	Heterogeneous collection of 2D images used to illustrate nuclei segmentation. Includes stage1_test image set from BBB038v1, available from the Broad Bioimage Benchmark Collection [Ljosa et al., Nature Methods, 2012].	5	130
GLAND-SEGMENTATION-TEST	The aim of this problem is to classify pixels belonging to glands in histopathology images cropped out from images of 2015 MICCAI challenge of gland segmentation (GlAs 2015).	5	480
GLAND-SEGMENTATION-TRAIN	The aim of this problem is to classify pixels belonging to glands in histopathology images cropped out from images of 2015 MICCAI challenge of gland segmentation (GlAs 2015). These images were used to train the machine learning classifiers available in GLAND-SEGMENTATION-TEST.	5	671
LANDMARKS-DROSO	Landmark detection in Drosophila wings, data from UPMC [Vandaele et al., Nature Scientific Reports, 2018].	5	60
NEURON-TRACING-3D	Neuron tracing from 3D images. The images are from the DIADEM challenge (olfactory bulb projection fibers labeled with GFP) and were acquired by confocal microscopy (40x, NA = 1.3).	5	4
NEURON-TRACING-TREES-3D	Neuron tracing in 3D images. The ground truth trees were generated by TREES Toolbox as SWC files and transformed into binary masks by Vaad3d. The masks were then convolved by a synthetic PSF (Born & Wollf generated by ImageJ PSF Generator) and some noise was added with ImageJ Random.	5	2
NUCLEI-SEGMENTATION	Nuclei segmentation from 2D images. The images were generated by SIMCEP, a widefield fluorescence microscopy biological images simulator.	5	30
NUCLEI-SEGMENTATION-3D	Nuclei segmentation from 3D images. The images were generated by CytoPaca, a fluorescence microscopy simulator of biological objects.	5	4
NUCLEI-TRACKING-3D	Tracking of nuclei from 3D images. The images were generated by CytoPaca, a microscopy image simulator of biological objects. Note: No workflow yet available for this problem.	5	37

(Call for datasets in Nov. 2020)

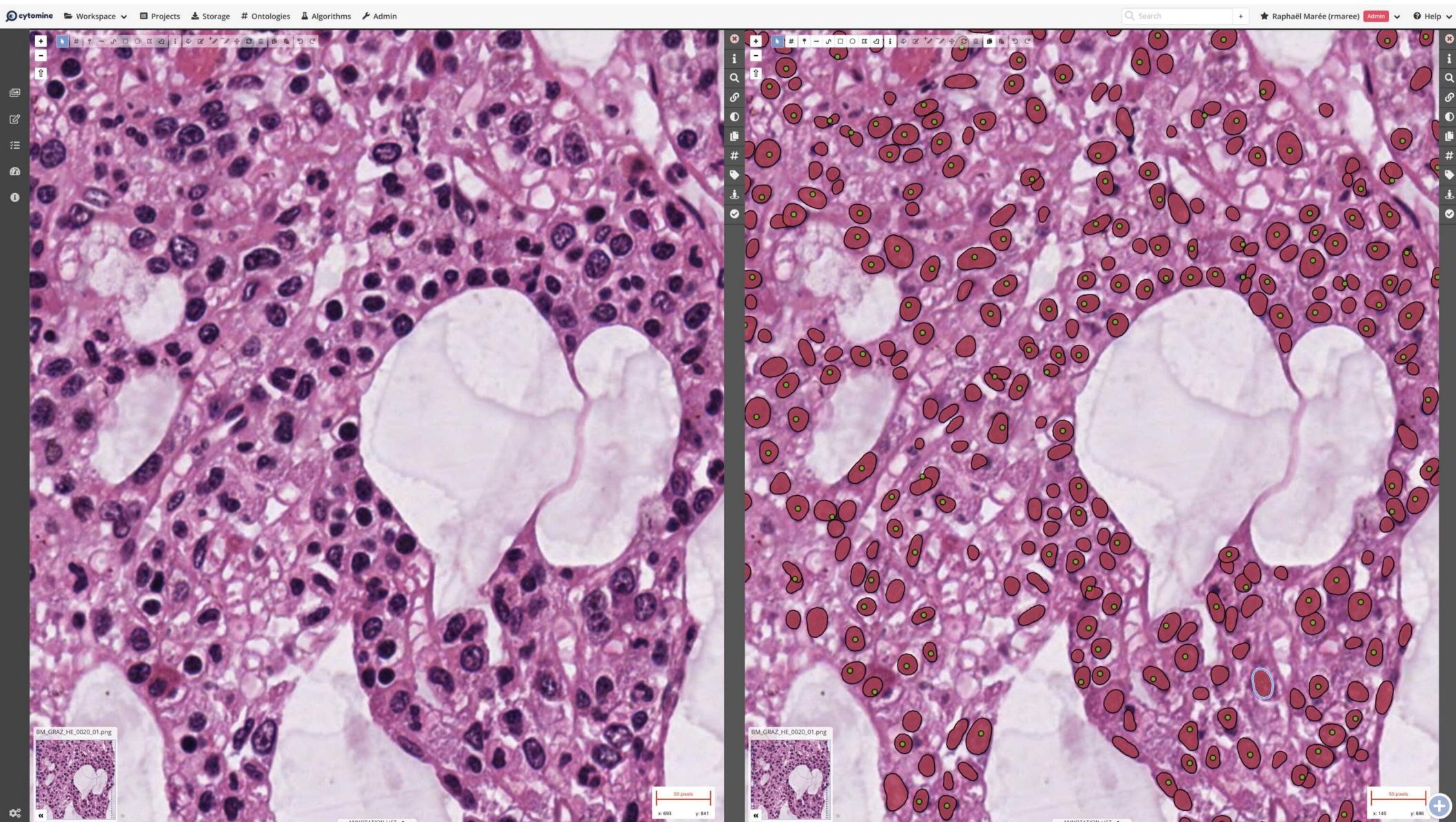
An example of the potential benefits of an open/sharing approach

StarDist cell segmentation algorithm (Schmidt et al., 2018)

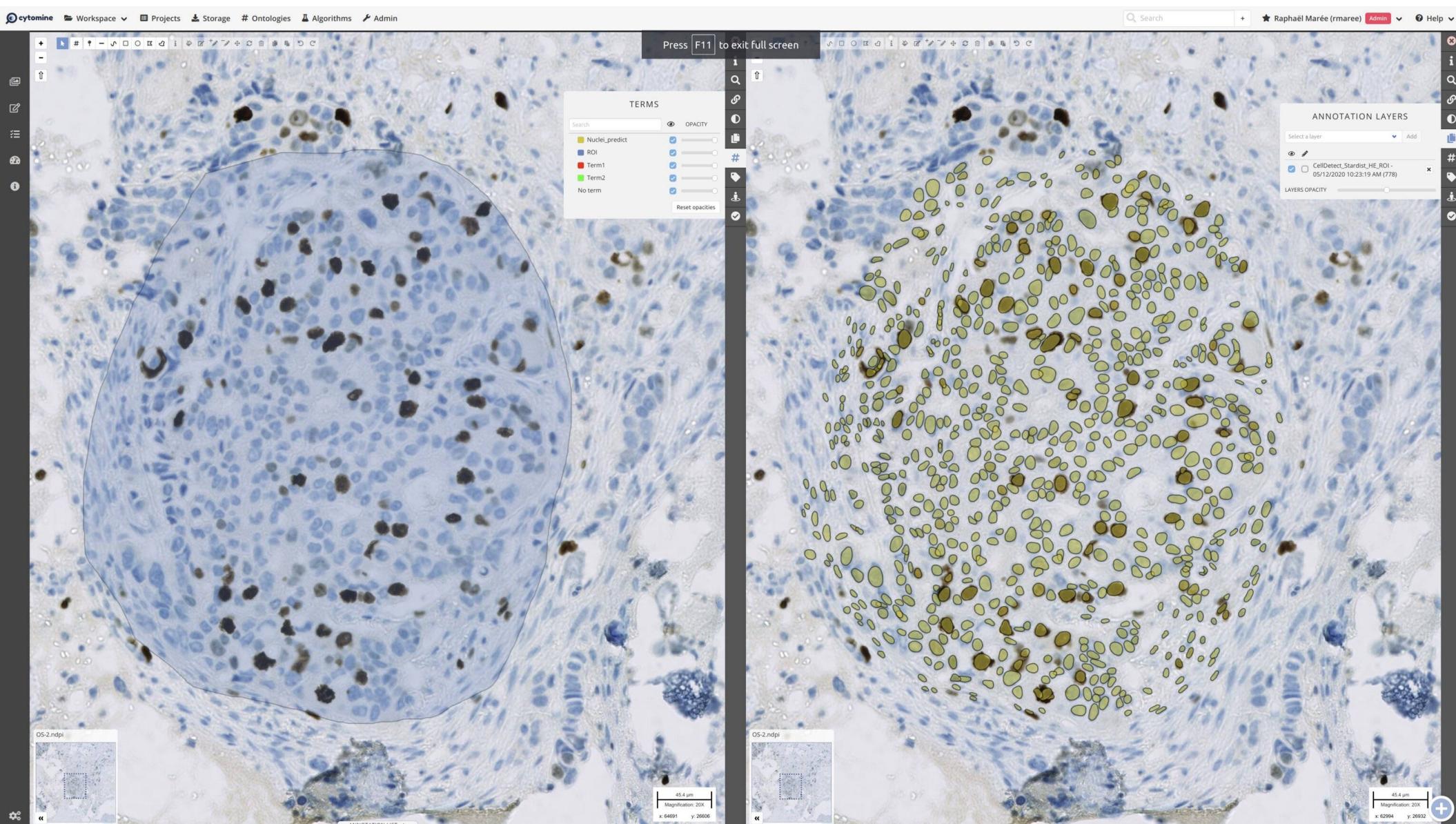
- Was trained on public datasets (images+ground-truths) : TCGA, DSB 2018 and Monuseg 2018 challenges
- It is **open-source** (<https://github.com/mpicbg-csbd/stardist>) and can be easily integrated into other software tool
- It is a promising « generic » candidate (it works for **multiple imaging modalities**, originally tested on fluorescent nuclei and H&E)



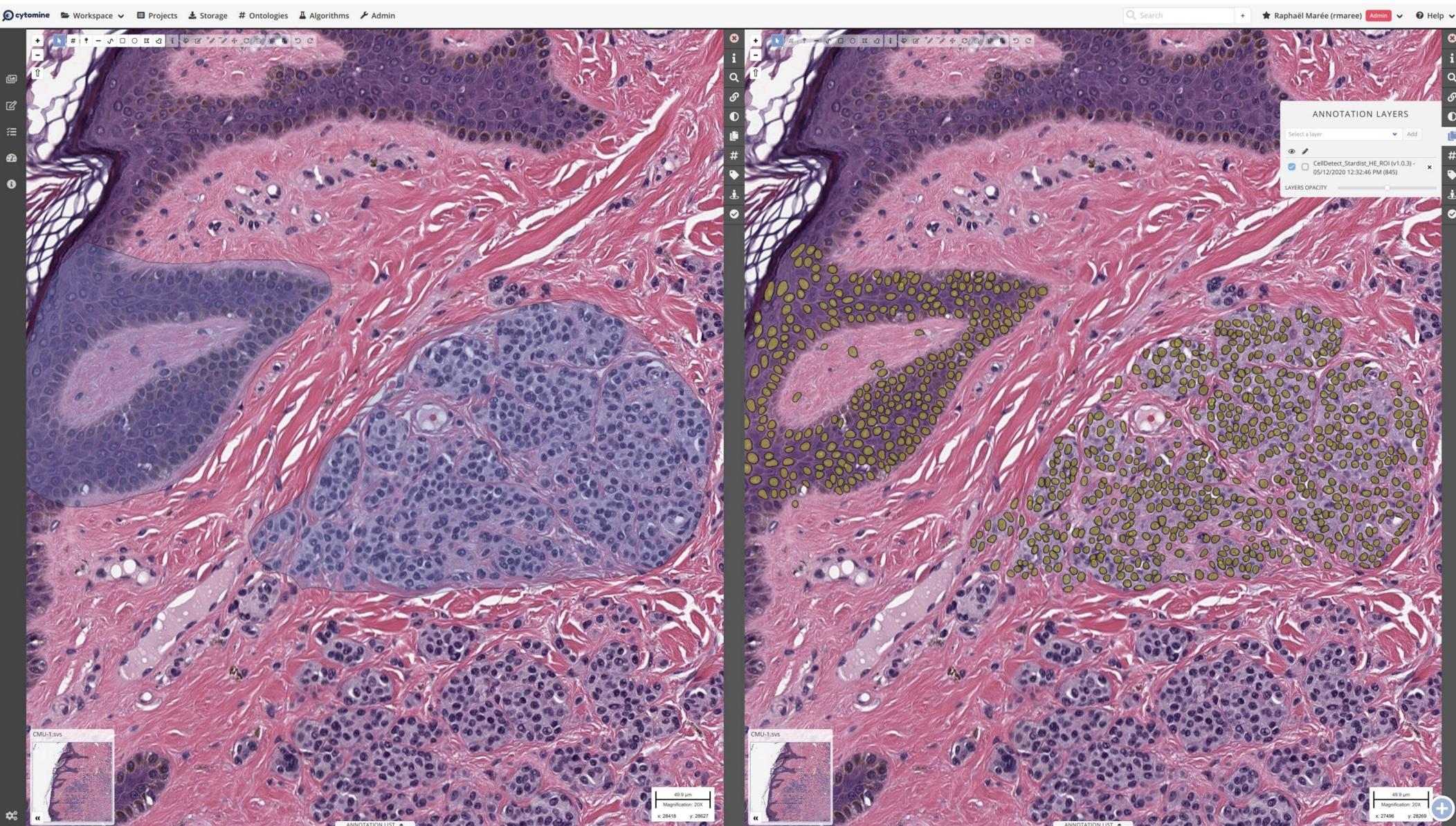
An example of the potential benefits of an open/sharing approach



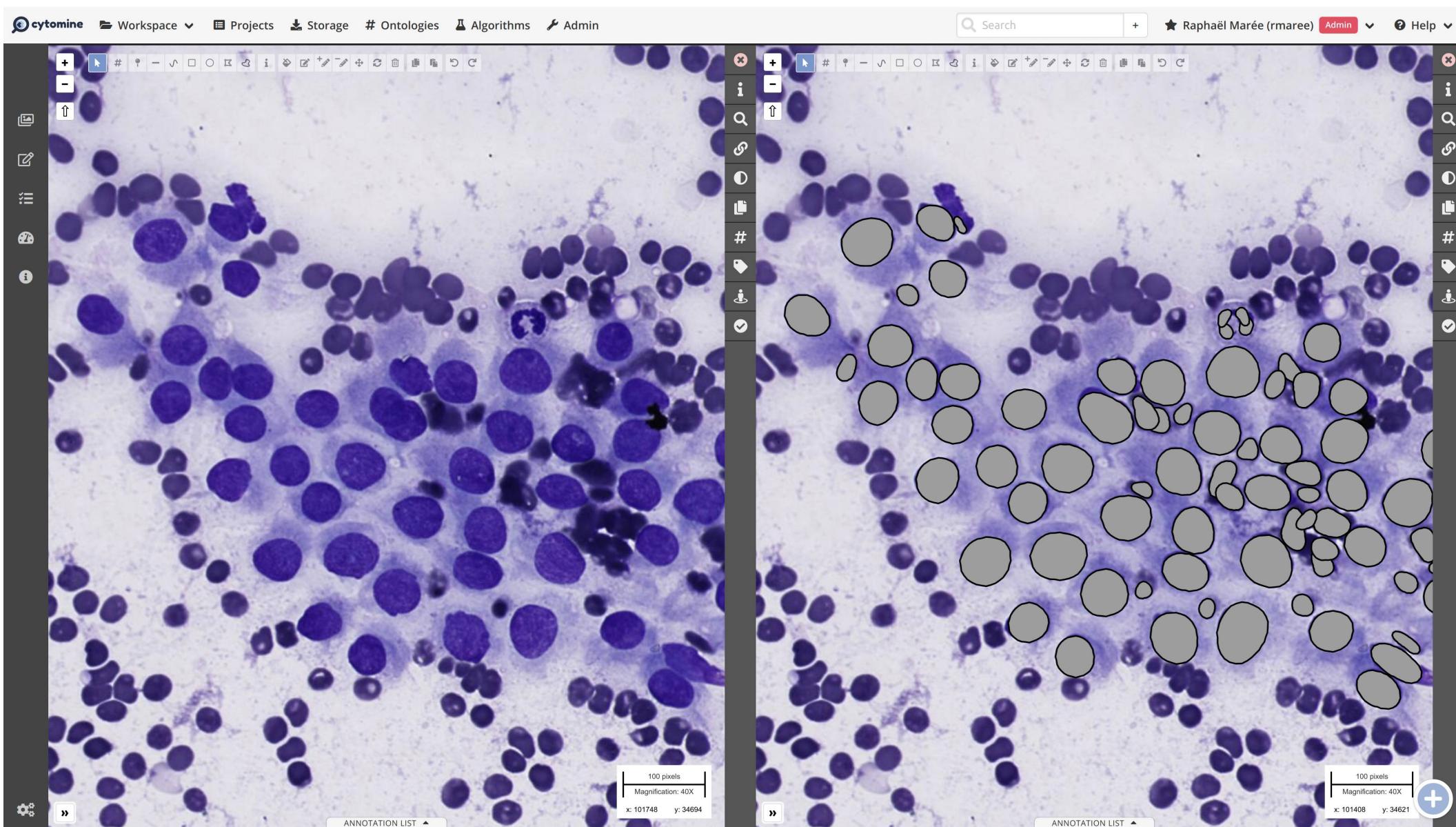
An example of the potential benefits of an open/sharing approach



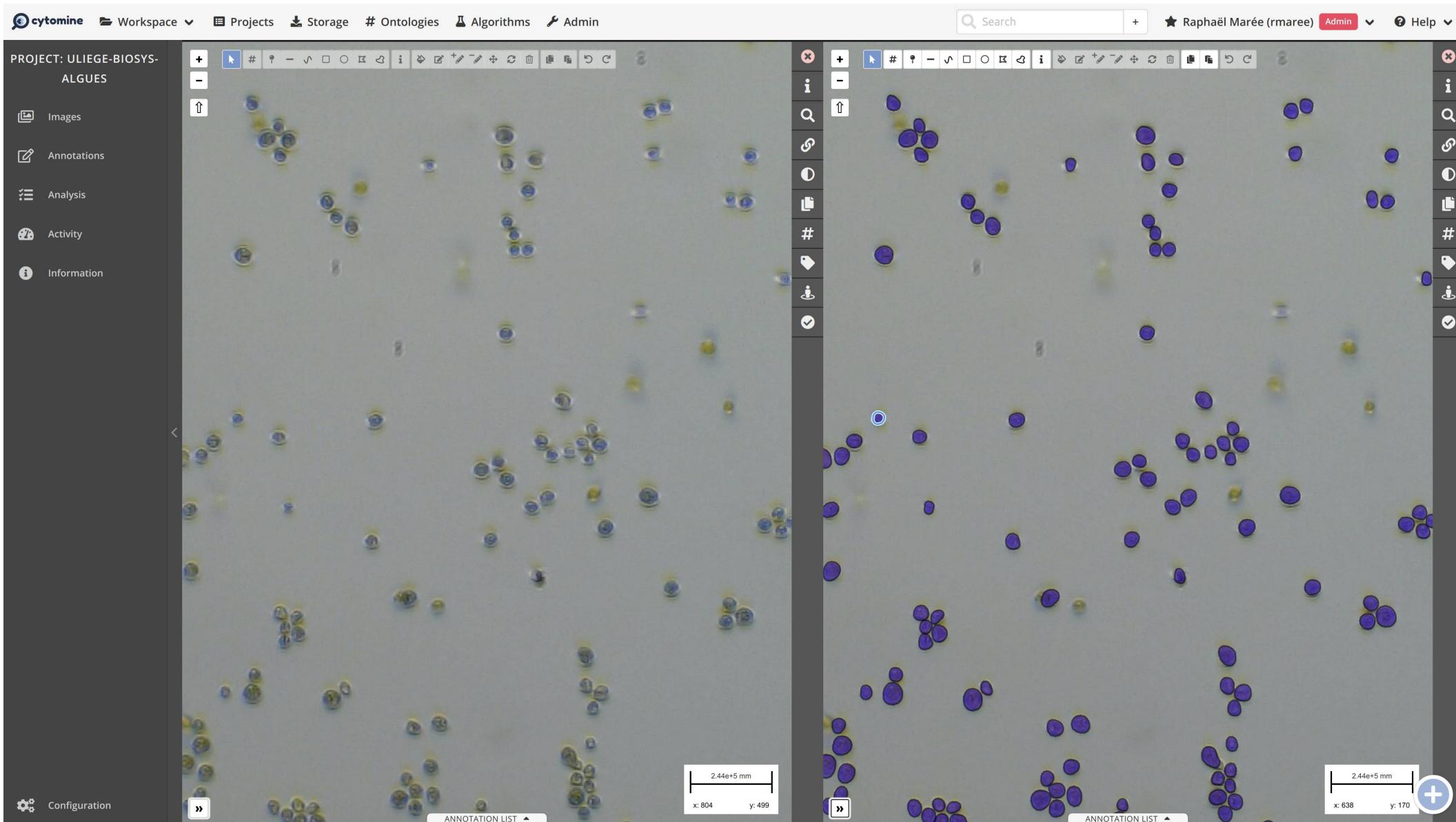
An example of the potential benefits of an open/sharing approach



An example of the potential benefits of an open/sharing approach



An example of the potential benefits of an open/sharing approach



Qualitative vs Quantitative evaluation

Choosing an algorithm (e.g. for cell segmentation) only by **visual examination** on a small subset of images is **unsafe**

- Bias, artefacts, ...

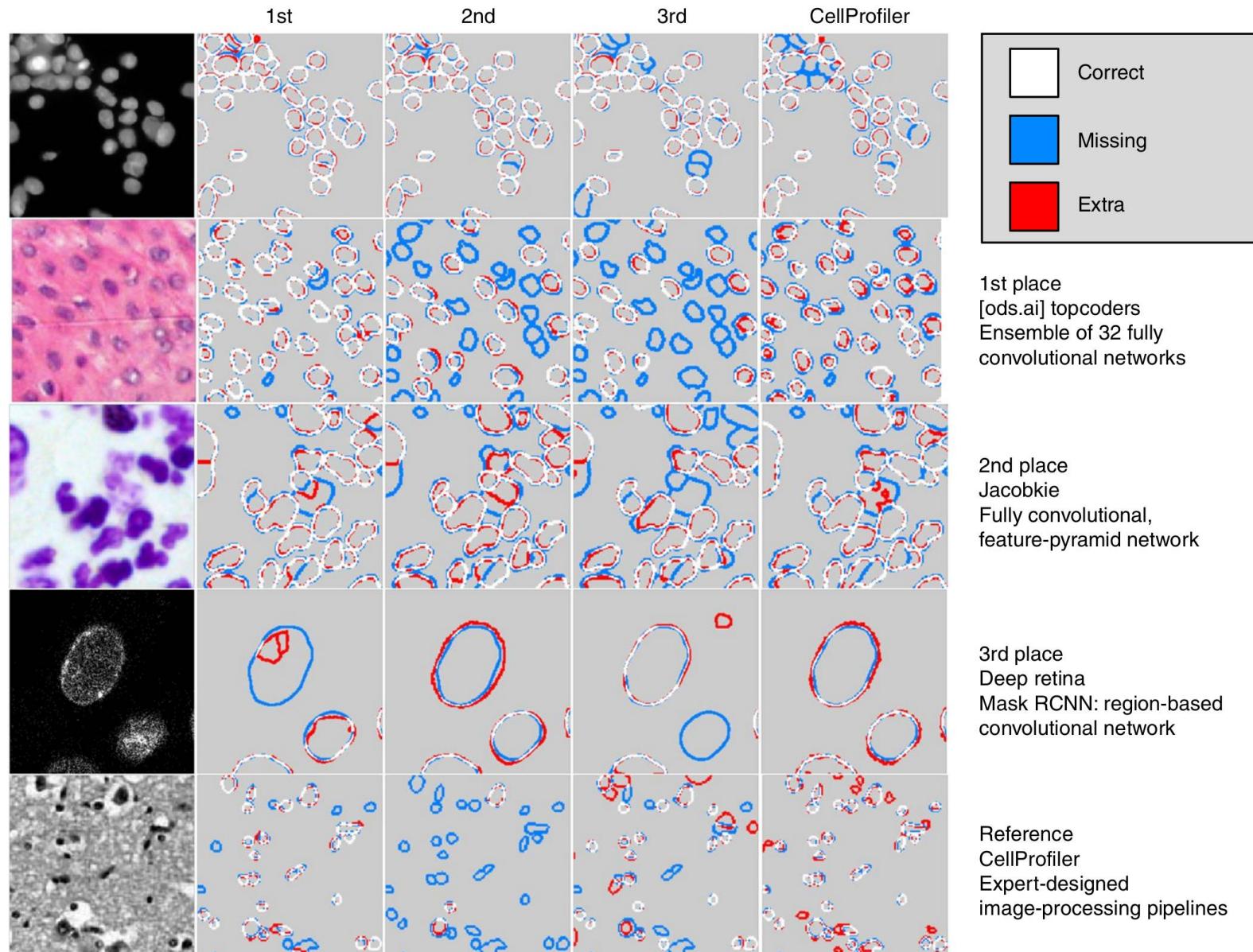
Ideally, **benchmarking** (quantitative evaluation) should drive image analysis method choice :

- Preselects methods that have good results on « similar », large datasets
- Evaluate quantitatively and tune methods on your own images + ground truths, proofread results

(see also Perrine Paul-Gilloteaux's talk)

Benchmarking enables continuous progress in image analysis

(Nucleus segmentation across imaging experiments: the 2018 Data Science Bowl
Caicedo et al., Nature Methods 2019)



Benchmarking enables continuous progress in image analysis

(Nucleus segmentation across imaging experiments: the 2018 Data Science Bowl
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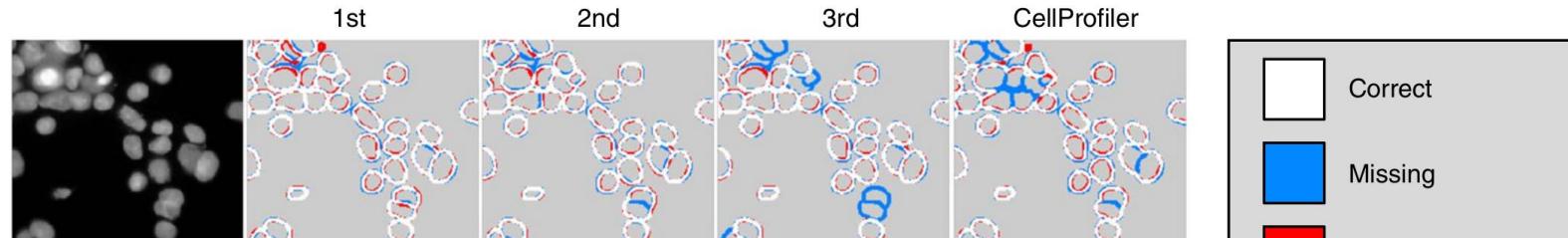
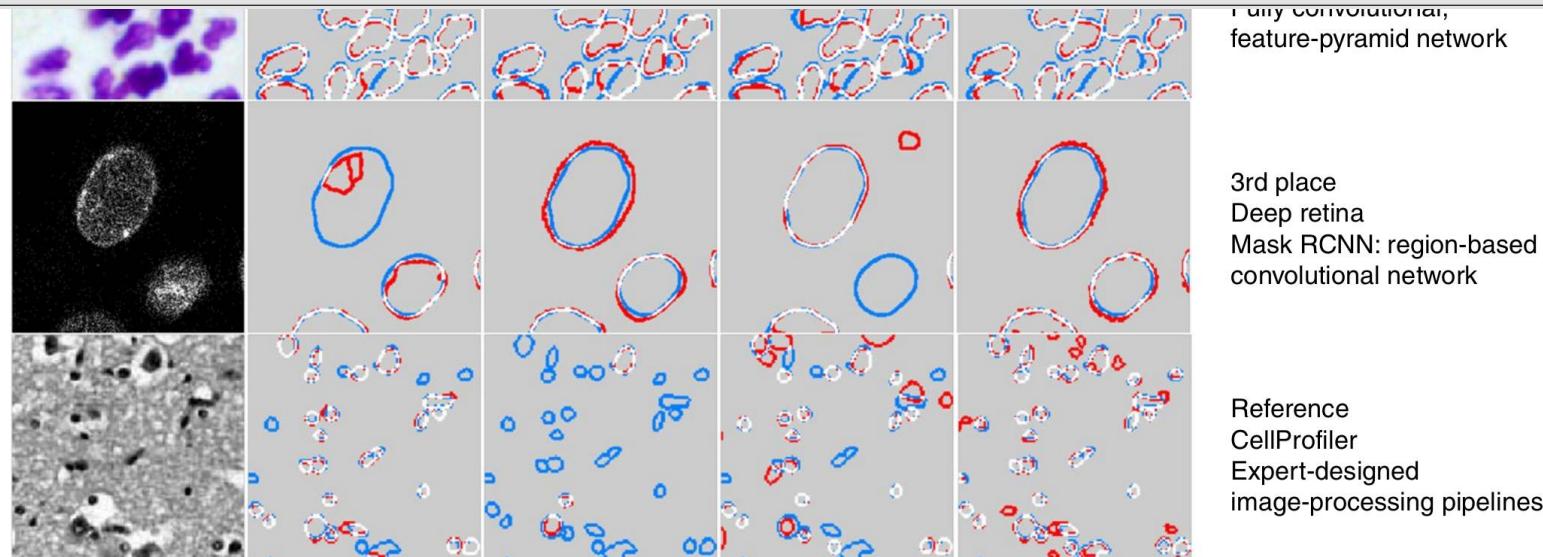


Table 1 | Comparison of performance of the top three methodologies

Team	Core model	Competition score	Average F1	Recall at 0.7 IoU (%)	Missed at 0.7 IoU (%)	Extra at 0.7 IoU (%)
[ods.ai] topcoders	32x U-Net/FPN	0.6316	0.7120	77.62	22.38	14.55
Jacobkie	1x FC-FPN	0.6147	0.6987	69.14	30.86	15.04
Deep Retina	1x Mask-RCNN	0.6141	0.7008	68.07	31.93	10.90
CellProfiler ^a	-	0.5281	0.6280	59.35	40.65	39.55



Sharing reproducible workflows

Ideally, we should **publish image analysis results** and **image analysis methods in full details** (not only « *quantitative analysis was performed with ImageJ* ») ie. with source code, parameter values, software environment. It **maximizes reusability and reproducibility** incl. for further benchmarking and refinement

Tools to ease online publication of data, results, and reproducible image analysis methods:



Reproducible, online, image analysis

ROI detection using maximum intensity projection in 10K x 7K x 32 bands hyperspectral images

(data: Centre for Cellular Imaging Core Facility, Gothenburg University) 

Launch new analysis

Algorithm

Segment-CV-Object-Projection (v1.0)



Name

Value

Images to process

G1_7_top.czi



Optional parameters Hide

 Term to predict

ROI



Pre-filled parameters Hide

 Projection

max

 Thresholding filter

otsu

 Tile size

1024

 Tile overlap

32

 Minimum Object Area

100

 Slices to use for annotations

median

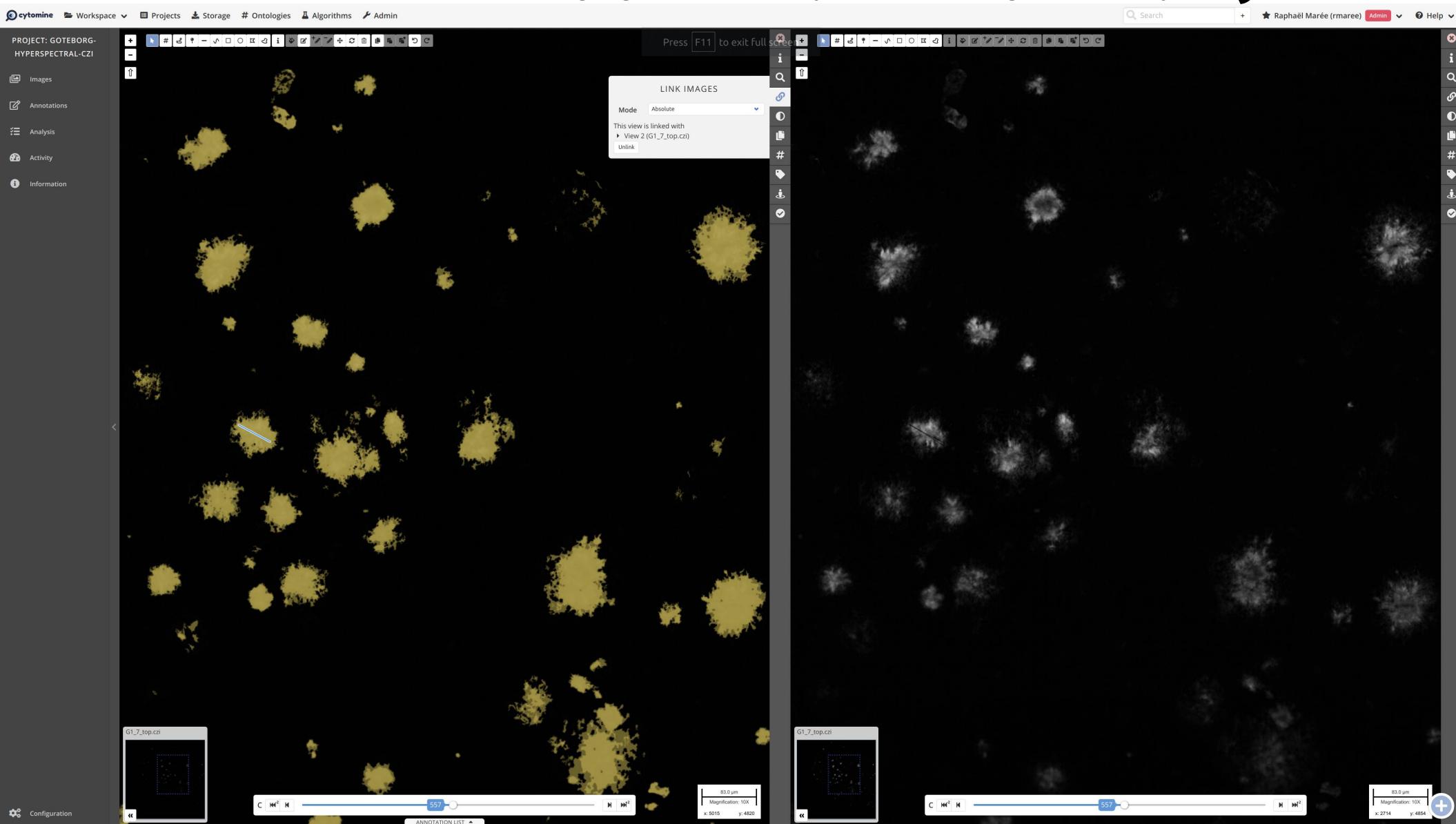
Cancel

Launch new analysis

Reproducible, online, image analysis

ROI detection using maximum intensity projection in 10K x 7K x 32 bands hyperspectral images

(data: Centre for Cellular Imaging Core Facility, Gothenburg University)



Reproducible, online, benchmarking



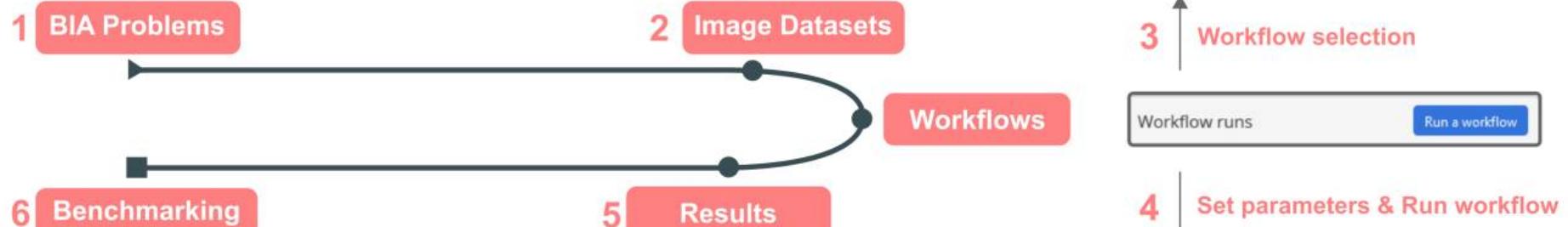
biaflows.neubias.org

NUCLEI-TRACKING-DIVISION
This project illustrates the 2D tracking of cell nuclei. The time-lapses are derived from Fluor-N2DH-SIM+ datasets from [Cell Tracking Challenge](#).

GLAND-SEGMENTATION-TRAIN
The images are crops of histopathology slides taken from the [2015 MICCAI challenge of gland segmentation](#) (GLaS 2015). The aim of the problem is to classify pixels as belonging to a gland or not. These images were used to train machine learning based workflows.

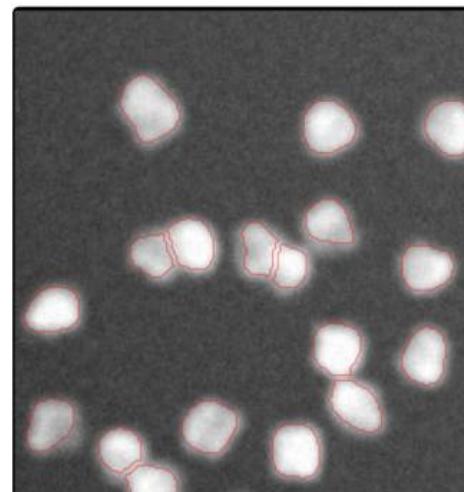
LANDMARKS-DROSO
Landmark detection in Drosophila wings, data from UPMC ([Vandaele et al., Nature Scientific Reports, 2018](#)).

VESSEL-TRACING-3D
This project illustrates the 3D tracing of blood vessels. The images were generated by [VascuSynth ITK](#), a biological image simulator, and some artificial noise was added.



Aggregated results Detailed results per image

Workflow run	Dice coefficient		
	MIN	MAX	AVG
★ NucleiSegmentation-ilastik (v1.0) #1 on Mar 25, 2019 1:19 PM	0.58	0.637	0.614
★ NucleiSegmentation-MaskRCNN (v1.3) #1 on Mar 25, 2019 9:16 AM	0.587	0.649	0.633
★ NucleiSegmentation-ImageJ (v1.10.1) #2 on Mar 19, 2019 10:37 AM	0.613	0.67	0.641
★ NucleiSegmentation-Python (v1.1) #6 on Mar 18, 2019 4:10 PM	0.554	0.613	0.586
★ NucleiSegmentation-CellProfiler (v1.4.1) #2 on Mar 11, 2019 9:22 AM	0.558	0.637	0.595



New workflow run

Workflow: Select options

- NucleiSegmentation-Python (v1.1)
- NucleiSegmentation-CellProfiler (v1.4.1)
- NucleiSegmentation-ImageJ (v1.10.1)
- NucleiSegmentation-MaskRCNN (v1.3)
- NucleiSegmentation-ilastik (v1.0)

Workflow runs [Run a workflow](#)

3 Workflow selection

4 Set parameters & Run workflow

New workflow run

Workflow: NucleiSegmentation-ImageJ (v1.10.1)

Name	Value
Radius	5
Threshold	-0.5

[Cancel](#) [Run a workflow](#)

Summary

Imaging data deluge in correlative multimodal imaging
(beyond CLEM & PHI)

Thousands of image analysis algorithms

- It is not easy to choose among them
- A lot of approaches tend to be *ad hoc* so they are not easily applicable in correlative/multimodal context

We suggest to improve the situation by sharing everything
(images, annotations, workflows,...)

... with the hope to design more generic approaches
hence getting closer to the ideal correlative/multimodal
workflow

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Neubias network : Sebastien Tosi, Benjamin Pavie, Volker Backer [neubias.org]

Training school organizers : Saskia Lippens, Sebastian Munck, Joke Baute, Geneviève Sachem, ...



Further reading / References

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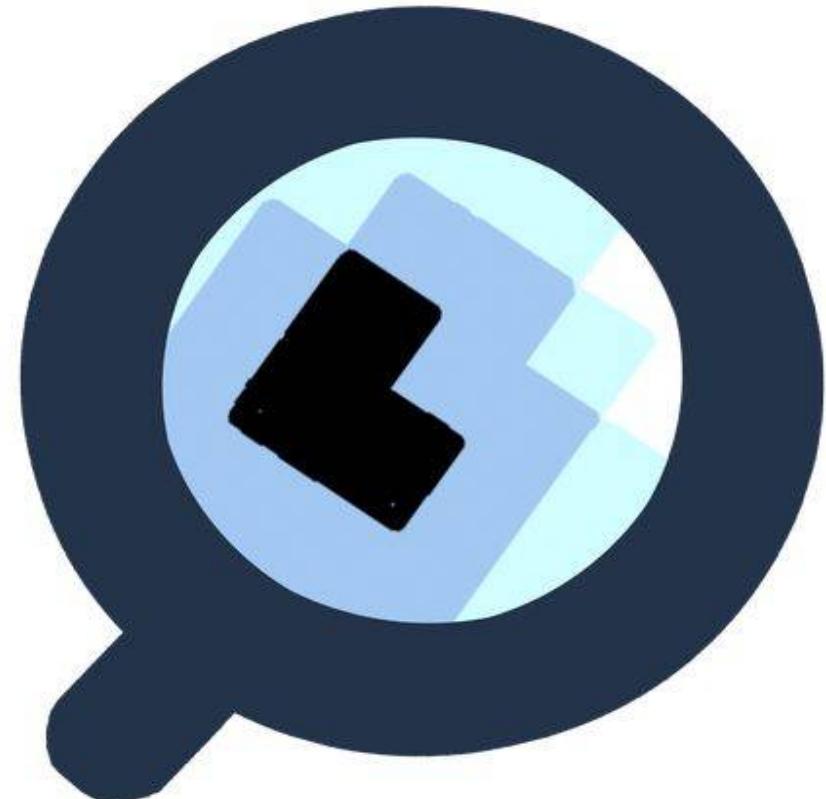
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