

Collaborative analysis of whole-slide tissue images with Cytomine open-source rich internet application

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[Head of Cytomine R&D @ University of Liège, Belgium]



Conflict of interest statement

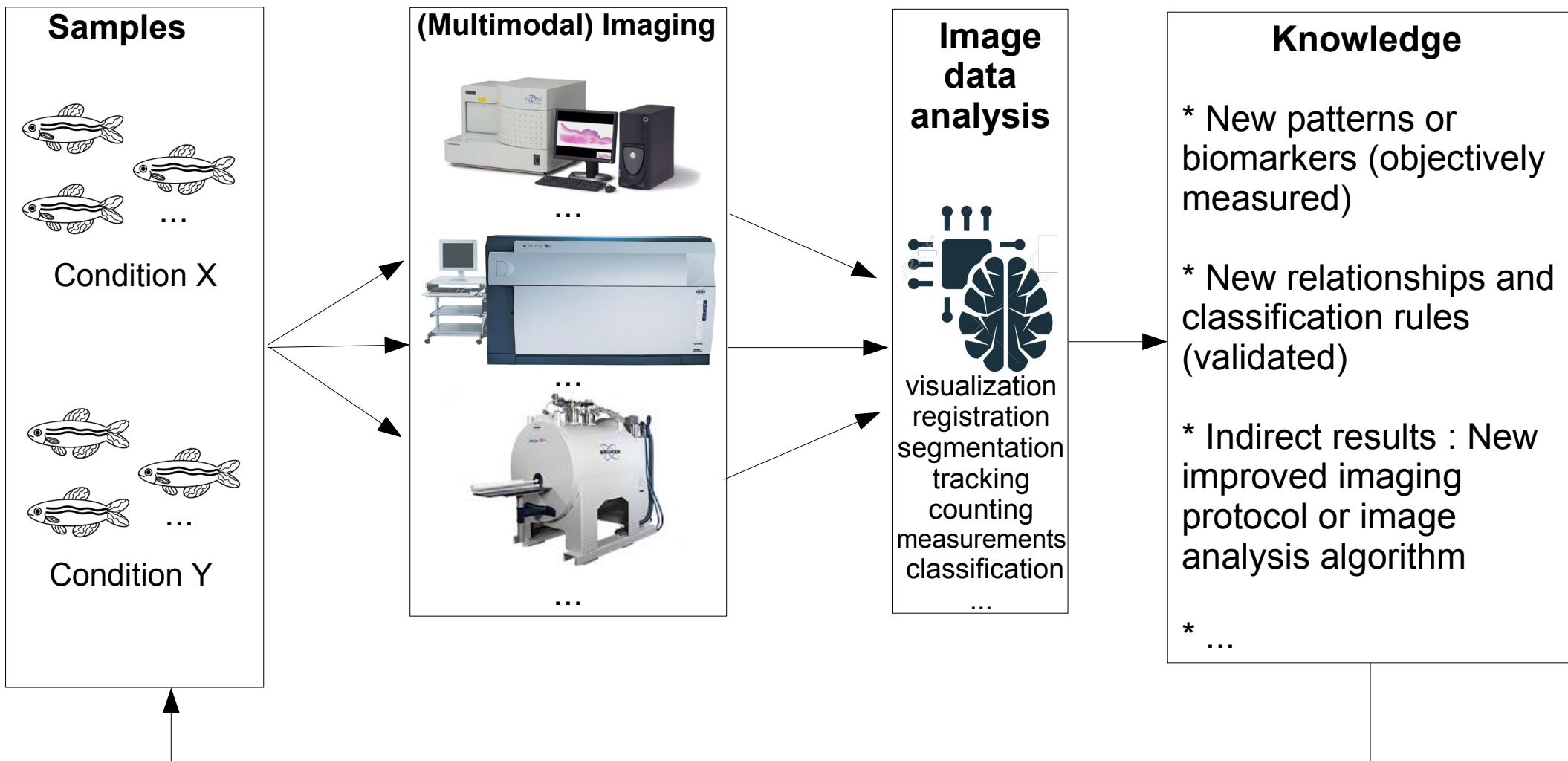
- Co-founder and member of the board of directors of
Cytomine not-for-profit cooperative company



- Another company, *Cytomine corporation* (<https://cytomine.com>), is selling services on top of Cytomine open-source software

Life science research heavily relies on images

(Exploratory analysis / Biomarker discovery / Classification of samples)



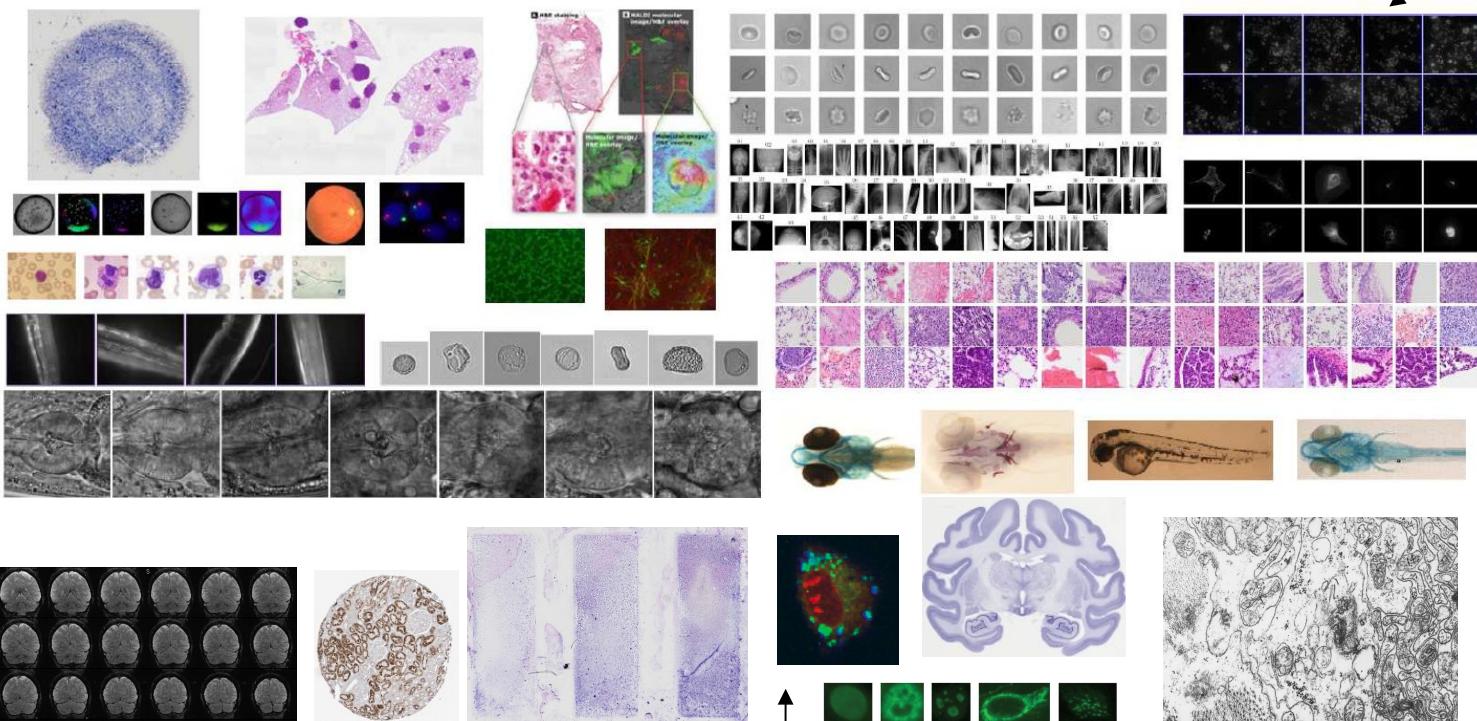
Life science research heavily relies on images



Whole-slide scanners



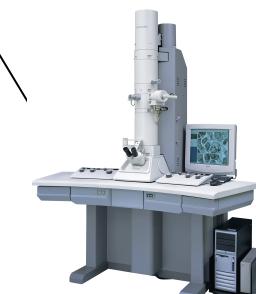
High-content screening



PETscan



Maldi imaging



Electron microscope

Automated tissue bioimaging (digital pathology)

Sample preparation

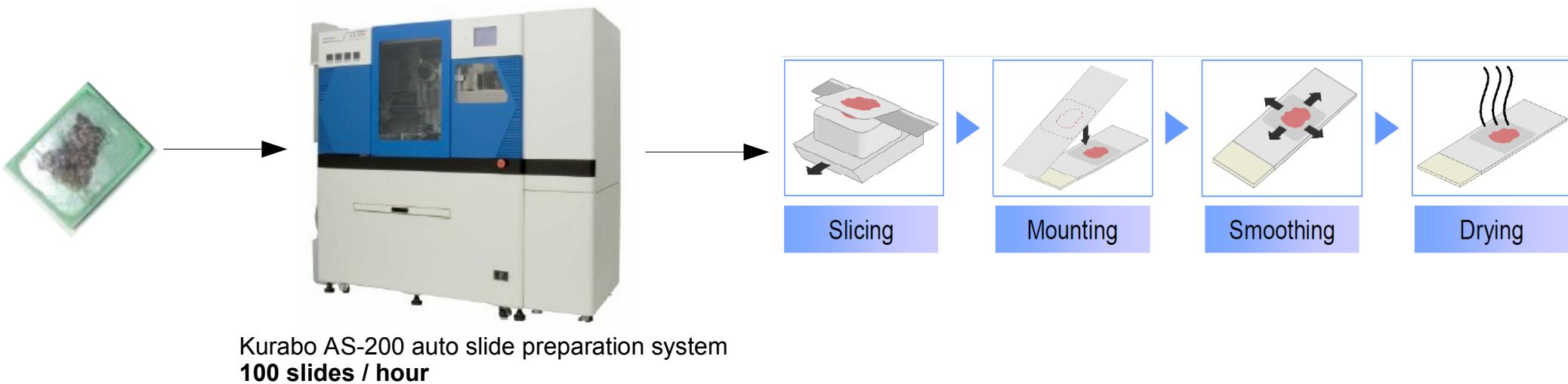
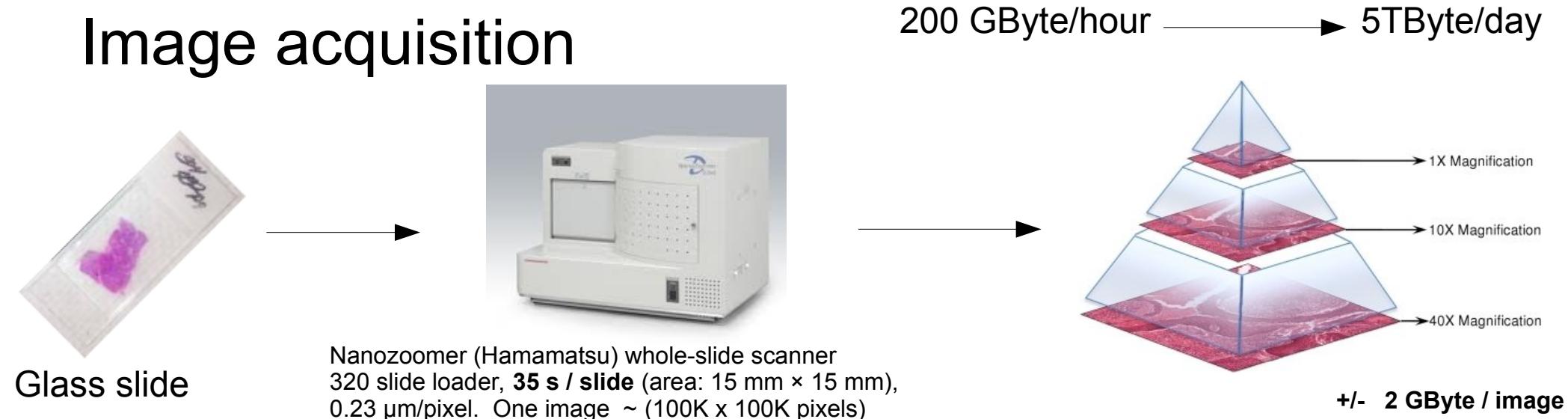


Image acquisition

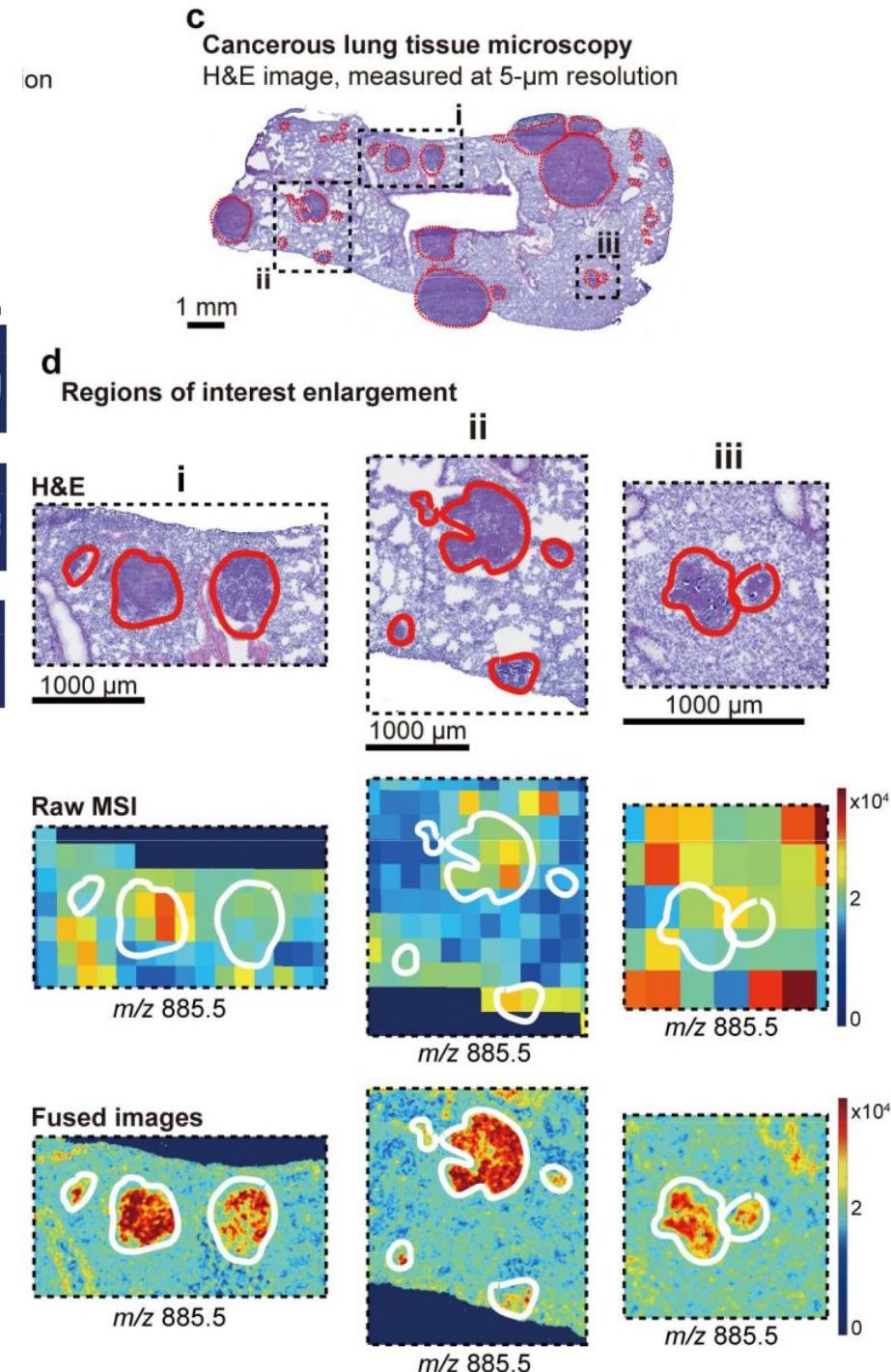
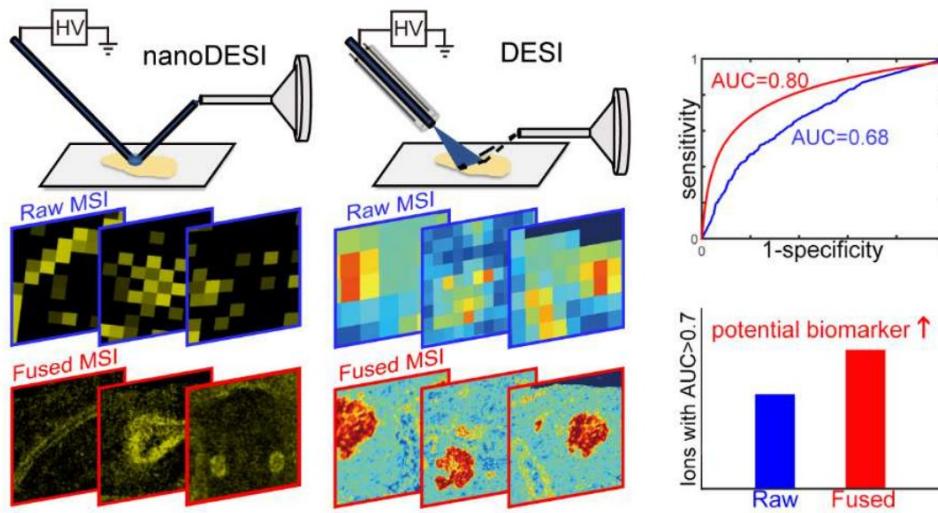
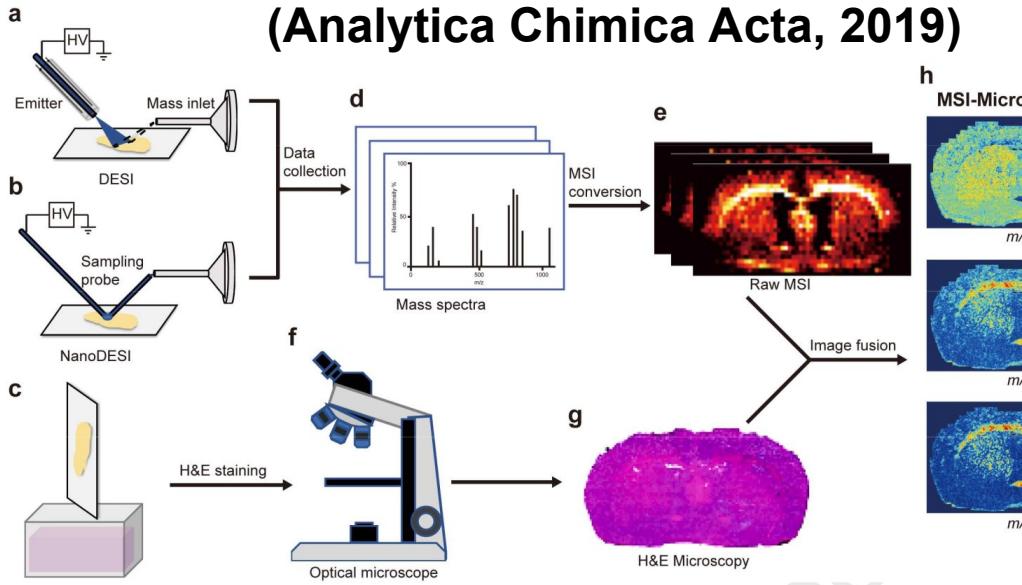


Correlated Multimodal Imaging examples

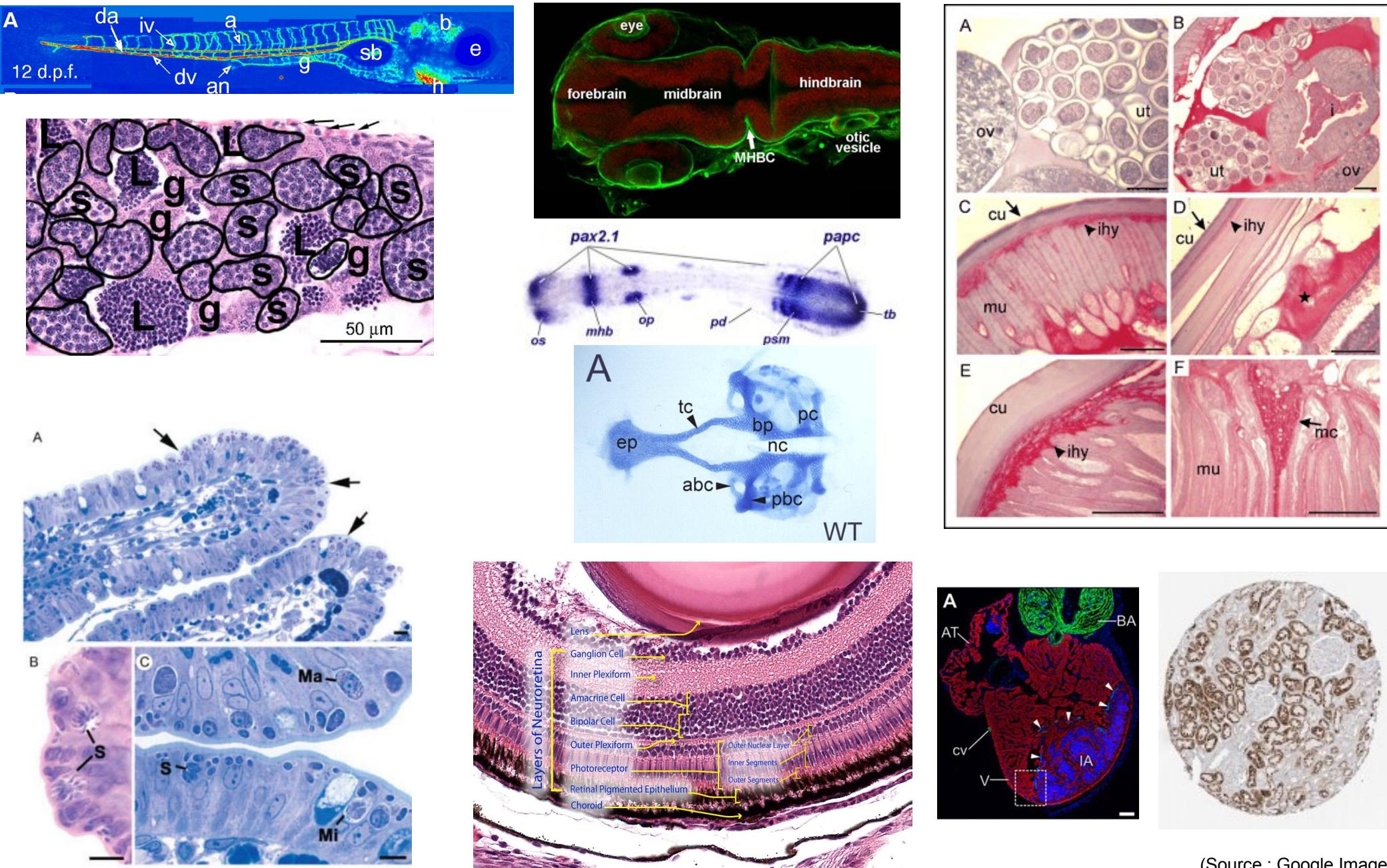
Precision Biomarker Discovery Powered by Microscopy Image Fusion-assisted High Spatial Resolution Ambient Ionization Mass Spec-

trometry Imaging

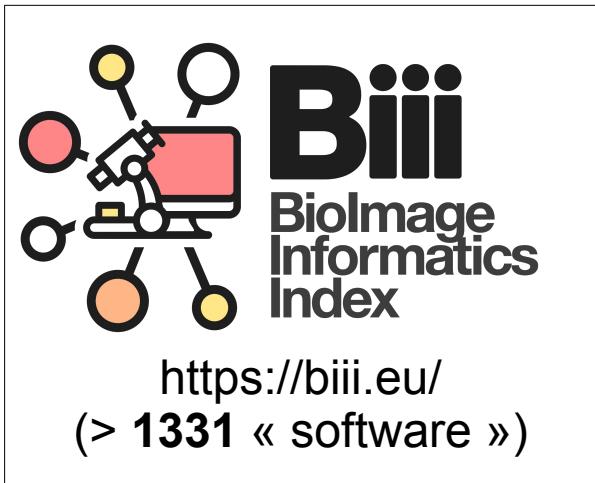
(*Analytica Chimica Acta*, 2019)



A multiplicity of image analysis tasks



A plethora of image analysis tools



In the field of « visual proteomics » :

QuPath (Bankhead et al., 2017) ; ec-CLEM (Paul-Gilloteaux, 2017) ; HistoCat (Schapiro 2017) ; Basis (Veselkov et al., 2018) ; OpenMSI & BASTet (Rübel et al., 2018) ; Starfish (Ganguli et al., 2018) ; ImaCyte (Somarakis 2019) ; Giotto (Dries et al., 2020) ; TissUUmaps (Solorzano 2020) ; SpaCell (Tan et al., 2020) ; BIRDS (Wang et al., 2021) ; CloudReg (Chandrashekhar et al., 2021)...

<http://spatialomics.net/>

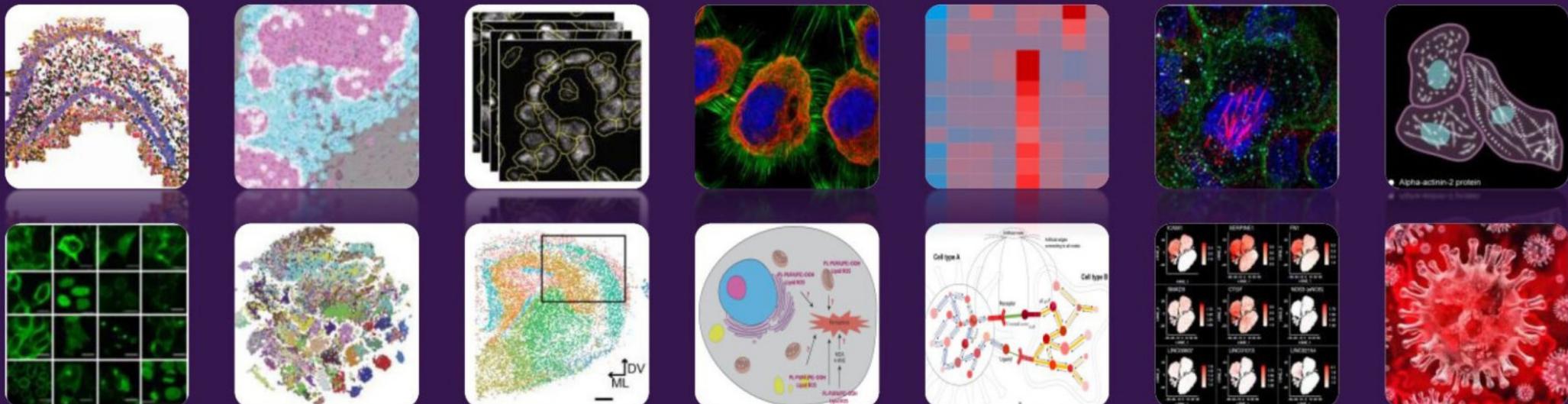


Image analysis practices are not ideal

Challenges for biomedical researchers:

- « Not easy to reuse previous work on my images because ... »
- ... many nice biology papers do not come with a tool or too few details
 - ... 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 or proprietary)
 - ... large scale computing resources are not available in the lab
 - ... publish or perish ... (a lot of work is lost after publication e.g. data, image analysis results & tuned methods)...

Challenges for computer scientists / AI developers:

- « Not easy to design generic methodologies because ... »
- ... so many imaging techniques & combinations
 - ... few annotated (ground-truth) datasets available (proprietary formats)
 - ... meaningful results ? expert's proofreading ?
 - ... publish & perish... validation only on 1-2 (small) dataset(s) : ad hoc

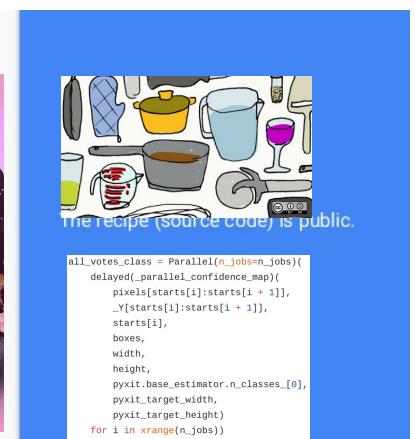
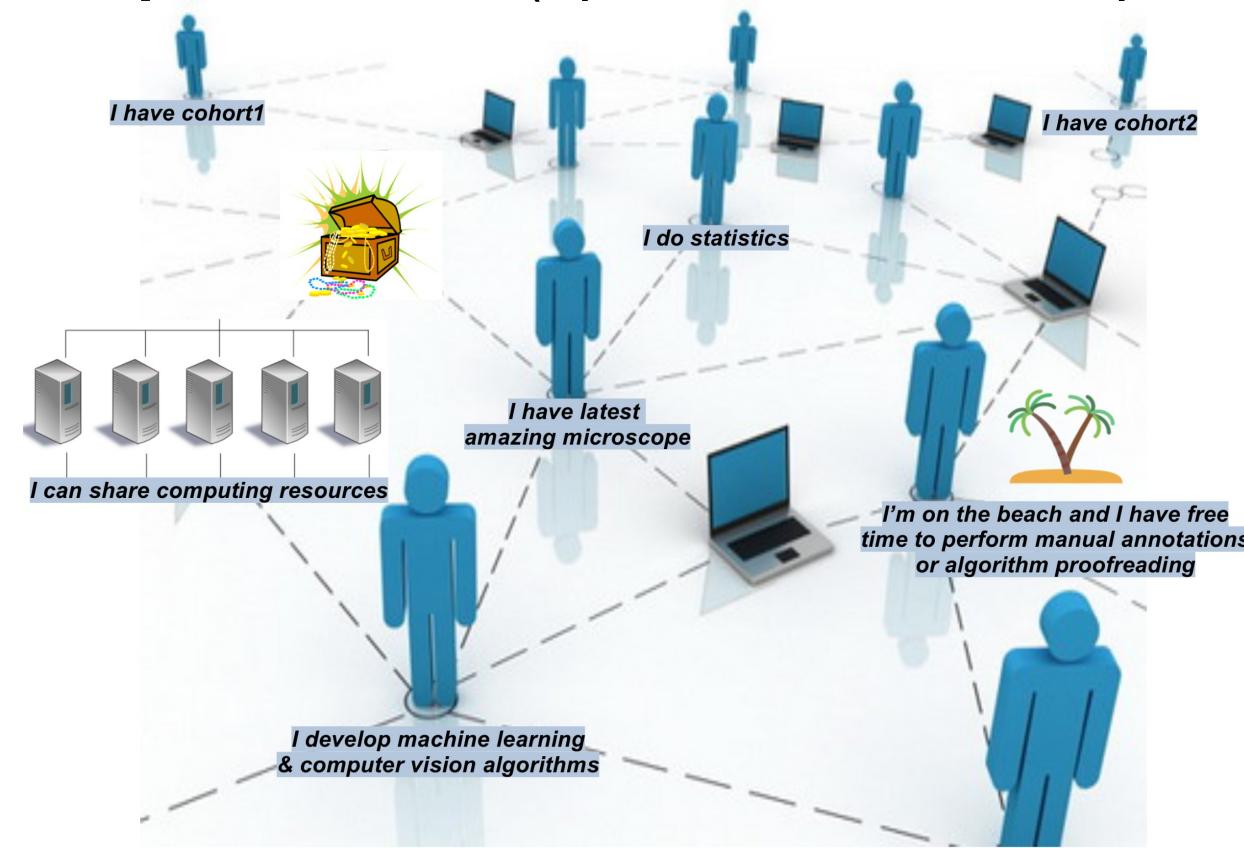
→ **suboptimal science practices** (reproducibility issues, waste of time, ...)

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

Let's try to **collaborate** more effectively towards **more generic** and **reproducible** image analysis workflows and biomedicine research.

Practical suggestions to improve Biomedical & AI research :

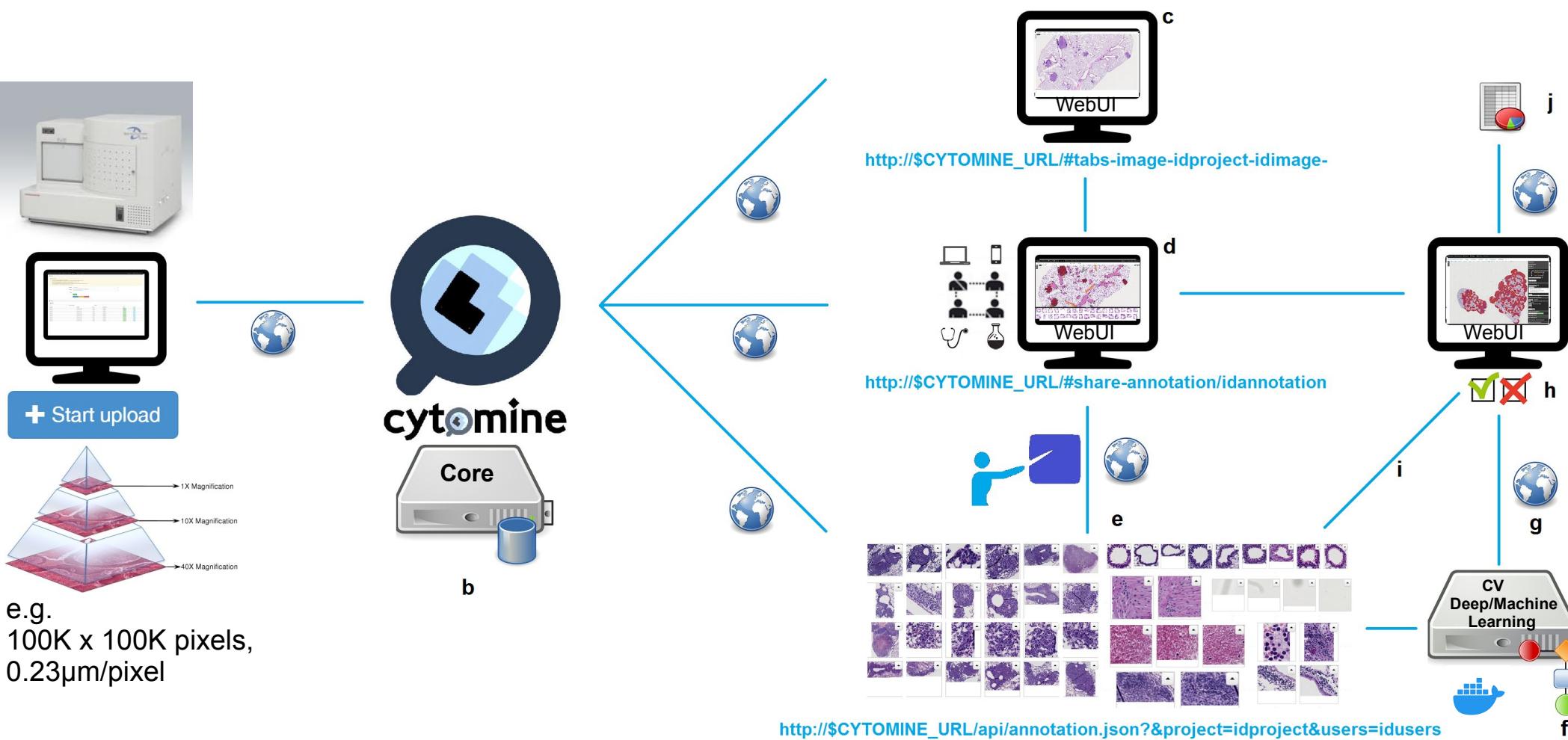
- **Image sharing**
- **Image annotation (ground truths) sharing**
- **Image analysis workflows sharing and benchmarking**
- **Open science** (open data access, open source code, ...)



→ Freedom to install, inspect, extend, improve, reproduce, redistribute

cytominne enables collaboration through the web (Sharing of images, annotations, algorithms, results)

(Marée et al., Bioinformatics 2016 ; Rubens et al., Proteomics Clin Appl. 2019 ; Rubens et al., Cell Patterns, 2020)



Note : Cytomine-Core can be installed on Linux-based servers or on a desktop/laptop (but then without collaborative features)
<https://doc.cytomine.org/>

cytominē users

Numerous server installations around the world (> 20000 users)



BIGPICTURE
European Digital Pathology Platform



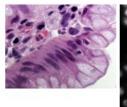
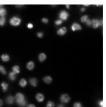
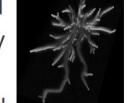
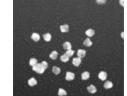
EU IMI project
2021-2027-... :



cytominE: organize your images, securely, on the web

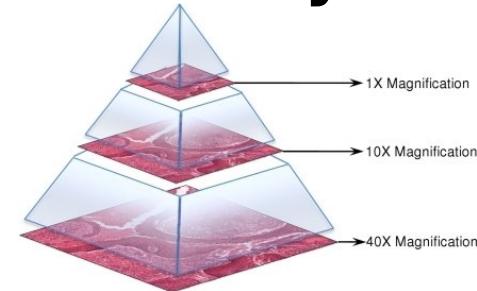
Create and manage multiple **projects**:

- Support **various digital pathology, microscopy & other image formats**
- Users with **authentification** (e.g. LDAP), **access rights**, and **roles**

Name ↑	Description	Members ↓	Images ↓
➤ DATA-SCIENCE-BOWL-2018	Heterogeneous collection of 2D images used to illustrate nuclei segmentation. Includes stage1_test image set from BBC038v1 , 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 Vaa3d . The masks were then convolved by a synthetic PSF (Born & Wolf) 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

cytominE : visualize large images, remotely

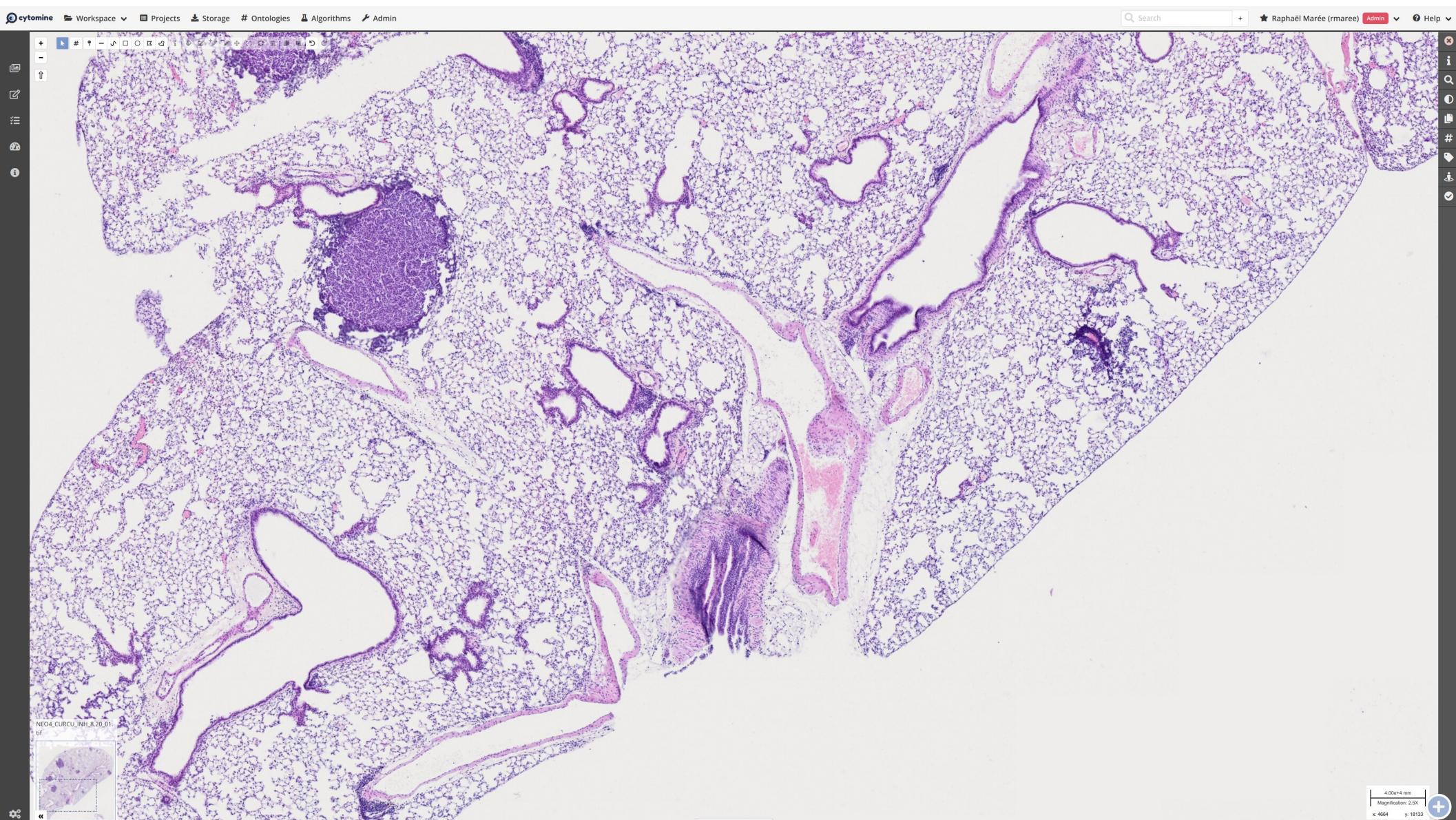
- Explore **large** (multi-gigapixel) images at multiple resolutions, **remotely**
- OpenStreetMaps browsing style (zoom in/out, pyramid tile-based)



The screenshot shows the cytominE software interface. At the top, there is a navigation bar with links for 'cytominE', 'Workspace', 'Projects', 'Storage', 'Ontologies', 'Algorithms', and 'Admin'. On the right side, there is a user profile for 'Raphaël Marée (rmaree)' with an 'Admin' button. Below the navigation bar, there is a search bar and a help icon. The main area displays a large tissue slice image with purple-stained nuclei and pinkish-red cytoplasmic areas. A text overlay on the left side of the image states: 'e.g. one tissue slice = 40000 x 30000 pixels (0.23µm/pixel)'. In the bottom left corner, there is a small thumbnail image labeled 'PGP POUMON PBSS 1 - 2012-08-07 11.39.07.jp2'. The bottom right corner shows a zoomed-in view of the tissue slice, highlighting cellular details.

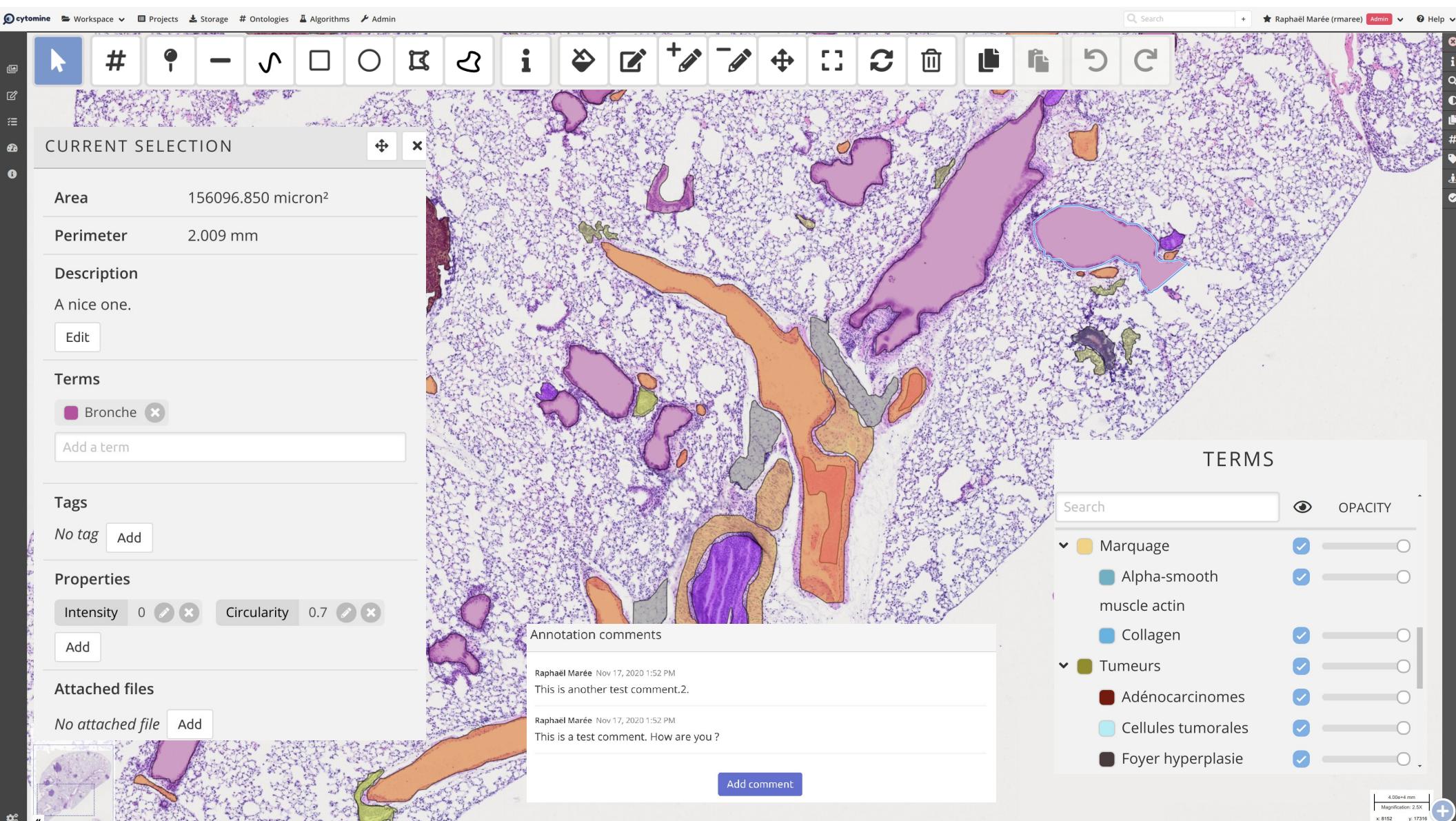
cytominE : annotate semantically, collaboratively

- Annotate images using various **drawing tools**, with **user-specific layers**
- Describe ROIs **semantically** with (user-defined) **ontology terms**
- Associate any **key-value properties, tags, file, or text description**



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cytominE for linking images & annotations

- Create « **Image Groups** » (e.g. multiple images describing the same sample)
- Copy, paste, **link annotations** between multiple images



(image data: ANHIR challenge)

The image displays four separate windows of the cytominE software interface, each showing a histological image of a tissue section. A specific annotation, represented by a blue polygonal shape, is present in all four images, indicating a linked set of annotations across different views or crops of the same sample. The software's toolbar and various annotation tools are visible along the top and sides of the windows.

CURRENT SELECTION

- Properties: No property / Add
- Attached files: No attached file / Add
- Linked annotations: Shows three previous linked annotations with checkboxes. Buttons for < Previous link, Next link >, Show all linked annotations, Center view on this annotation, View crop, Copy URL, Comments (0), and Delete.
- Created by: Raphaël Marée (rmaree)
- Created on: May 3, 2021

INFORMATION

Name	mammary-gland_2_s2_66-CNEU_A4926-4L.jpg
Image group	mammary-gland
Width	9412 pixels
Height	2946 pixels
Resolution X/Y	1828800.000 µm/pixel
Magnification	Unknown

Annotations

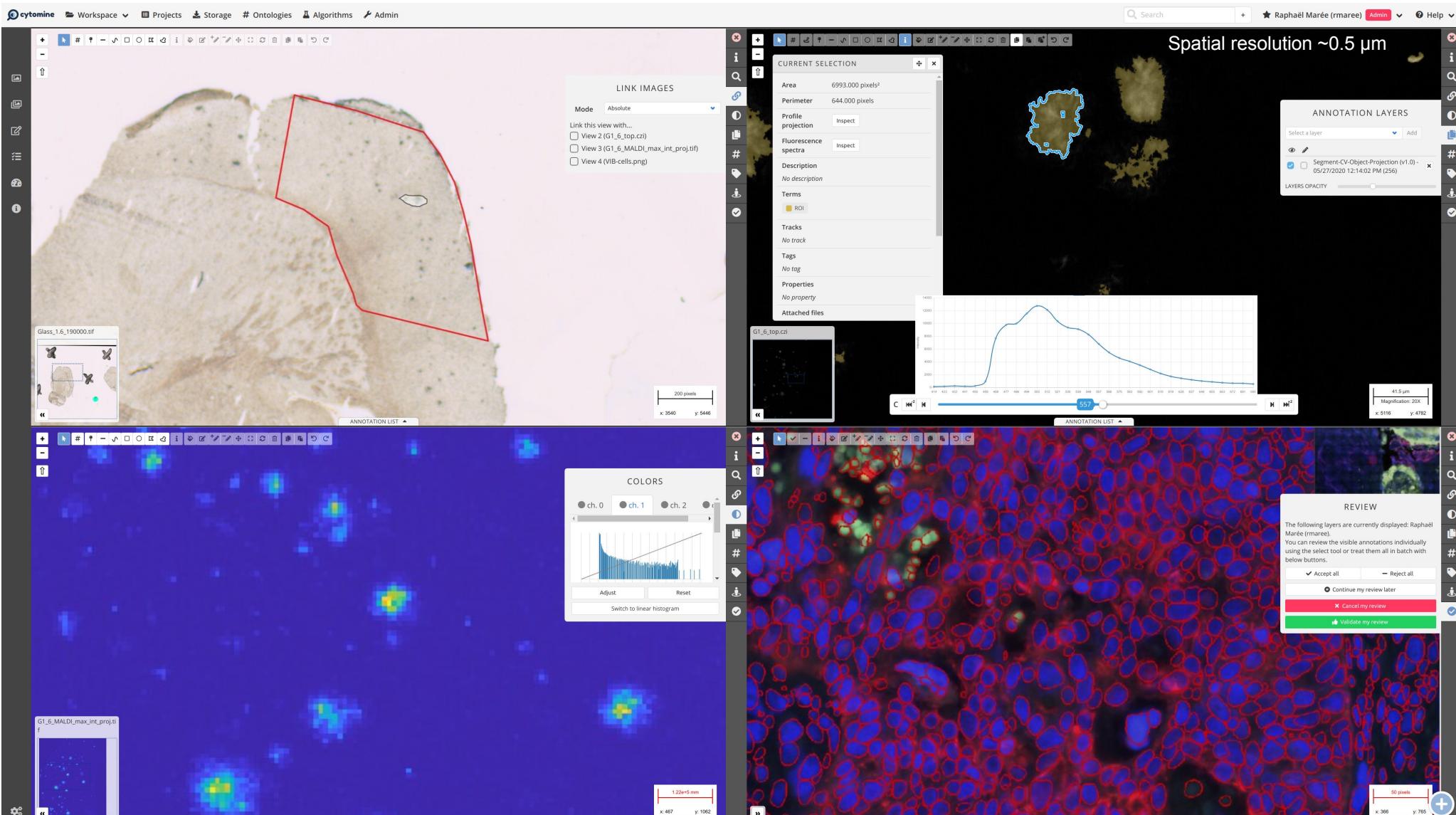
- Annotation 1: A blue polygonal shape highlighting a large, dark brown, irregularly shaped area within a tissue section. It is labeled with a dimension of 3.66e+5 mm and coordinates x: 3693, y: 1481.
- Annotation 2: A blue polygonal shape highlighting a similar large, dark brown area in another section. It is labeled with a dimension of 3.66e+5 mm and coordinates x: 4139, y: 736.
- Annotation 3: A blue polygonal shape highlighting a large, dark brown area in a third section. It is labeled with a dimension of 3.66e+5 mm and coordinates x: 3693, y: 1481.
- Annotation 4: A yellow polygonal shape highlighting a large, dark brown area in a fourth section. It is labeled with a dimension of 3.66e+5 mm and coordinates x: 4139, y: 736.

cytominé for multidimensional, multimodal images

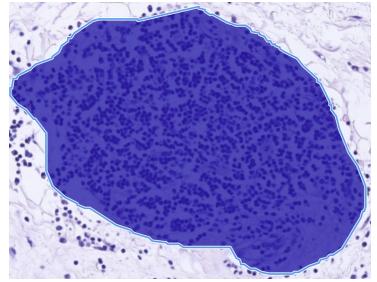
- Explore **2D+c+z+t slices**
- Explore **spectral profiles** (currently based on HDF5, Zarr under development)
- Side-by-side comparison of multiple views



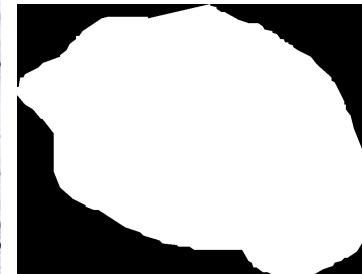
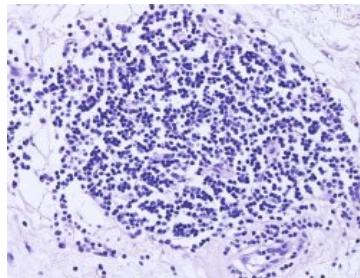
(data: VIB & Centre for Cellular Imaging Core Facility, Gothenburg University)



cytominE is highly interoperable : API (web services) to import/export all data



Web API →



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

```
{"class":"be.cytomine.ontology.UserAnnotation","id":26675587,"created":"1508877897417","updated":null,"deleted":null,"slice":146609783,"image":24935900,"project":22706,"location": "POLYGON ((40164 22674, 40320 22710, 40320 22706, 40338 22702, 40338 22698, 40348 22690, 40356 22690, 40392 22670, 40416 22662, 40440 22662, 40452 22654, 40456 22646, 40476 22642, 40476 22638, 40488 22634, 40494 22622, 40504 22622, 40508 22614, 40516 22614, 40516 22618, 40528 22606, 40528 22602, 40540 22594, 40572 22578, 40581 22558, 40624 22526, 40624 22518, 40632 22514, 40656 22466, 40656 22458, 40660 22458, 40660 22446, 40672 22438, 40680 22398, 40704 22338, 40728 22302, 40748 22250, 40748 22198, 40744 22186, 40748 22186, 40736 22170, 40688 22090, 40664 22074, 40656 22074, 40648 22066, 40632 22062, 40628 22054, 40664 22046, 40574 22030, 40564 22038, 40560 22026, 40562 22026, 40480 22030, 40464 22038, 40452 22038, 40436 22050, 40428 22026, 40424 22062, 40416 22066, 40496 22094, 40284 22094, 40284 22094, 40268 22102, 40240 22156, 40236 22166, 40280 22110, 40192 22118, 40152 22130, 40144 22138, 40168 22102, 40236 22166, 40280 22110, 40192 22118, 40152 22130, 40144 22138, 40168 22156, 40214 22194, 40628 22194, 40628 22192, 40608 22206, 39976 22222, 39944 22250, 39956 22294, 39968 22610, 39964 22622, 39984 22636, 39992 22630, 40000 22642, 40448 22674, 40664 22679, 40164 22678, 40164 22674)"}, {"x": 40315, "y": 73937196692, "w": 22371.35279565198}, {"area": 91131.05480176363, "areaUnit": "micron", "perimeter": 1.2823776183507887, "perimeterUnit": "mm", "term": "[25635608]_nbComments:0", "cropURL": "https://research.cytomine.be/api/userannotation/26675587/crop.png", "smallCropURL": "https://research.cytomine.be/api/userannotation/26675587/crop.png?maxSize=256", "url": "https://research.cytomine.be/#/userannotation/26675587/crop.png", "imageURL": "https://research.cytomine.be/#/project/24796414/image/24935900/annotation/26675587", "reviewed": false}
```

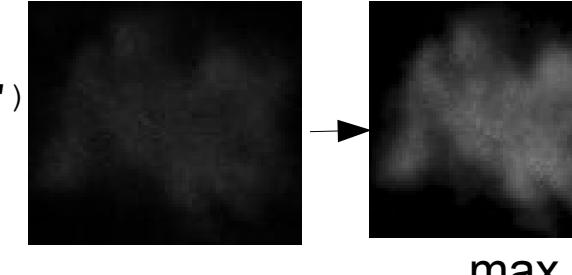
Get maximum projection of an annotation as an image:

<https://research.cytomine.be/#/project/146747628/image/153042325/annotation/156138240>

<https://research.cytomine.be/api/annotation/156138240/profile/max-projection.png>

Python:

```
a = Annotation().fetch(156138240)
a.profile_projection(projection='max', dest_pattern="myimage.png")
```



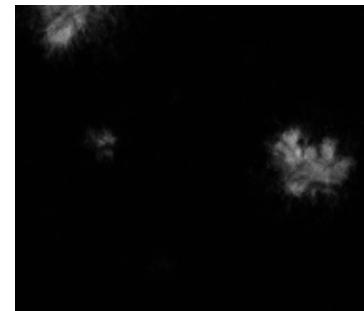
max

Get 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")
```



cytominne includes various monomodal algorithms

e.g. ROI detection using maximum intensity projection in hyperspectral images
(10K x 7K x 32 bands)

Reproducibility : source code and containerized software environment

https://github.com/Cytomine-ULiege/S_Segment-CV-Object-Projection + database with parameter values for each execution

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

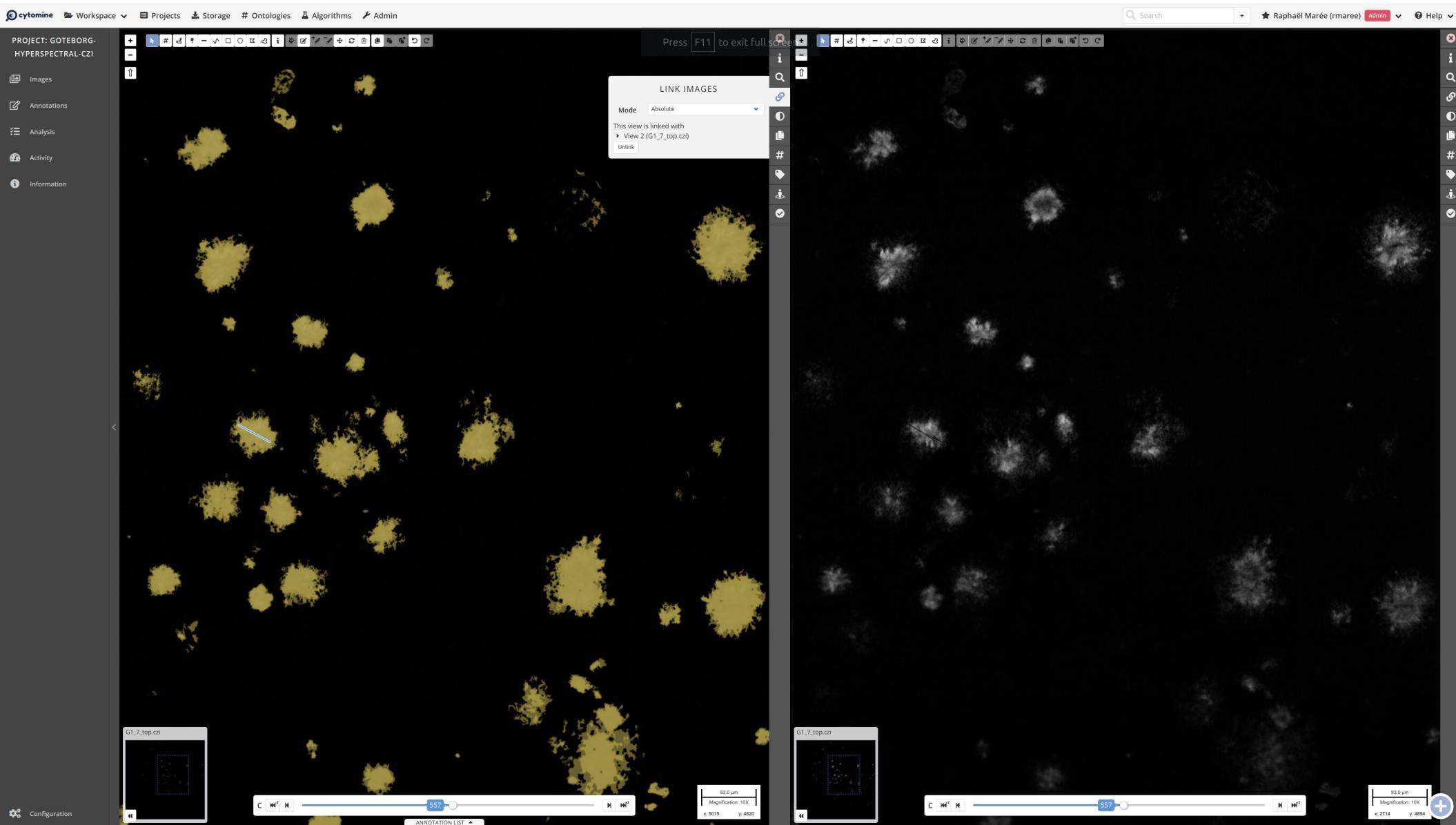
Cancel Launch new analysis

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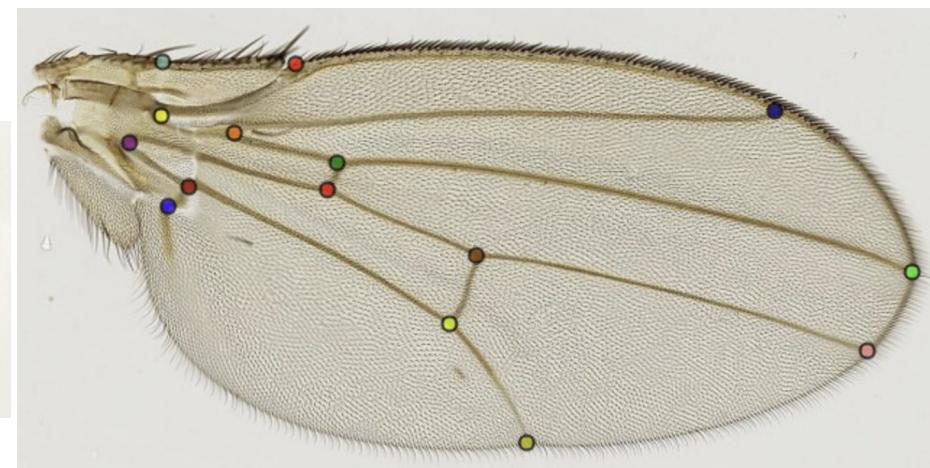
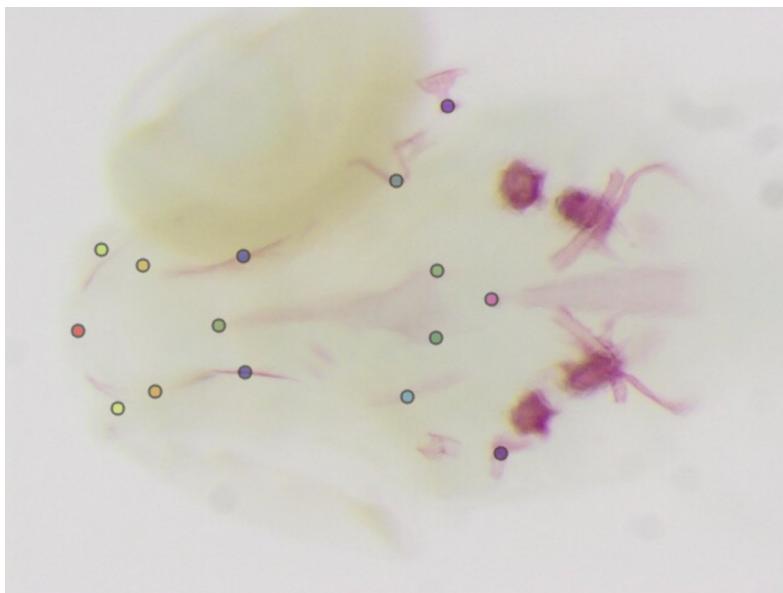
https://github.com/Cytomine-ULiege/S_Segment-CV-Object-Projection + database with parameter values for each execution



Applications : Landmark detection

cytominē

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

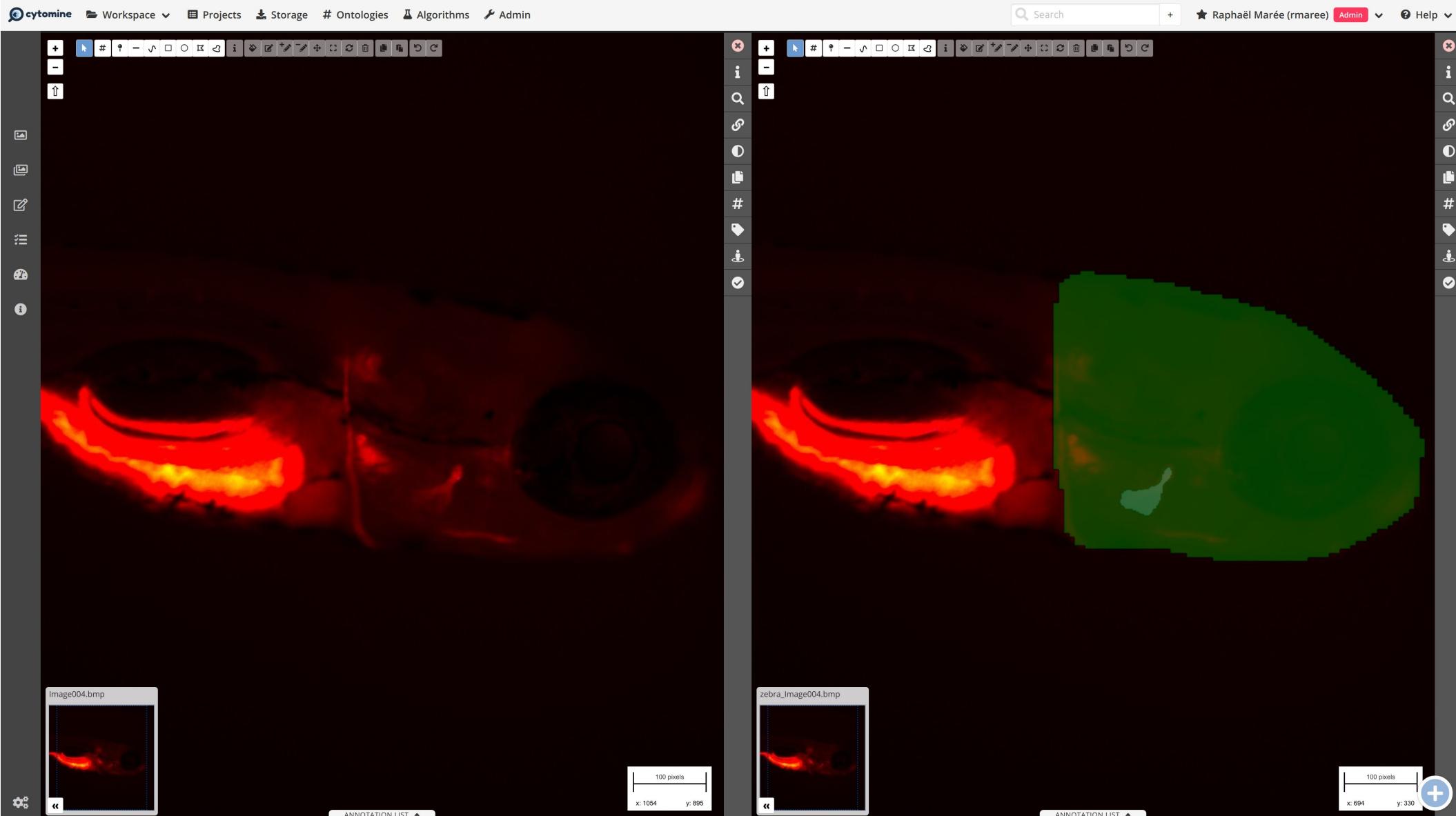


(Vandaele et al., Scientific Reports, 2019)

Applications : tissue quantification

(data : CCMR, BioMedAQu)

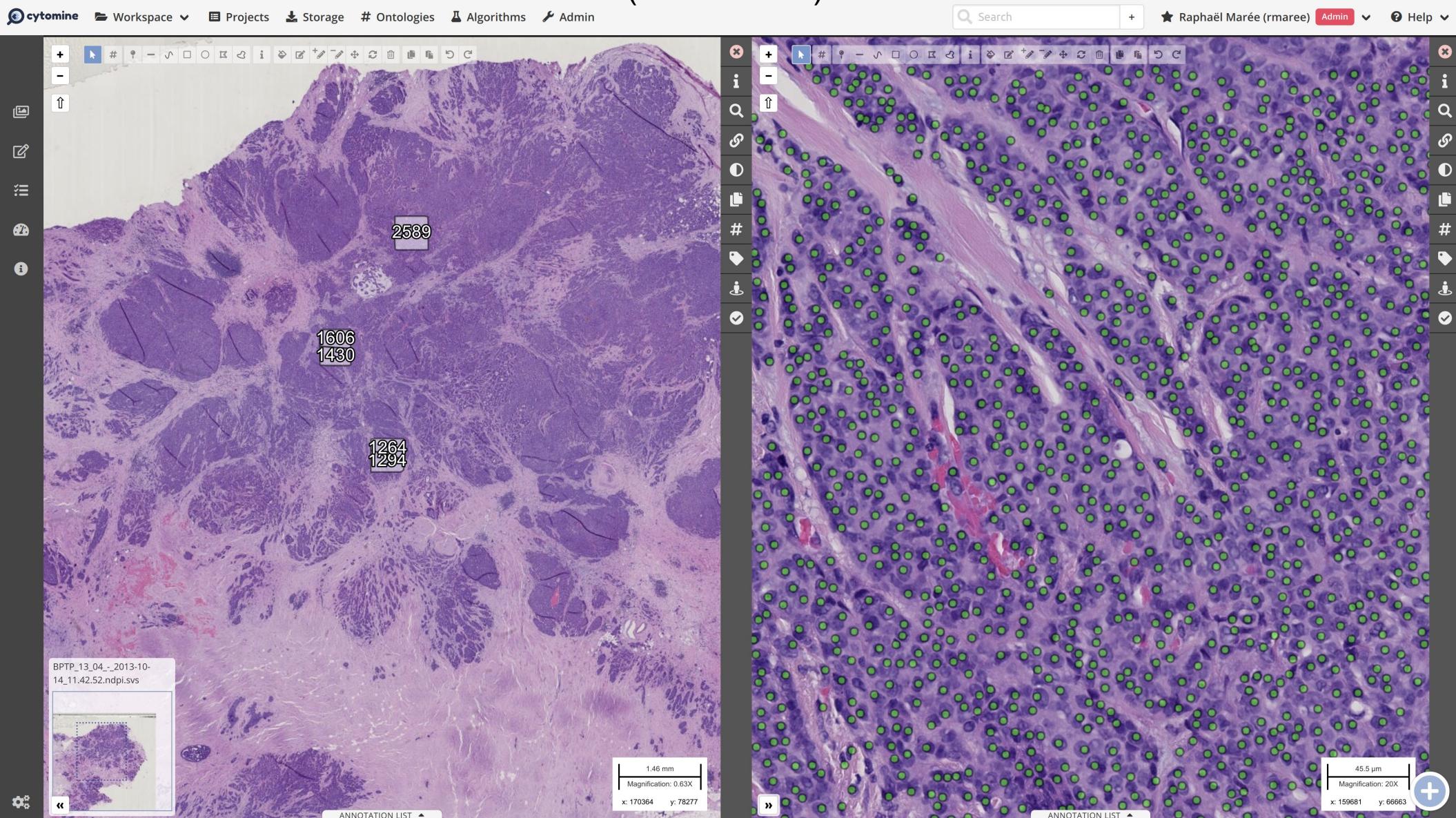
cytominne



Applications : cell counting

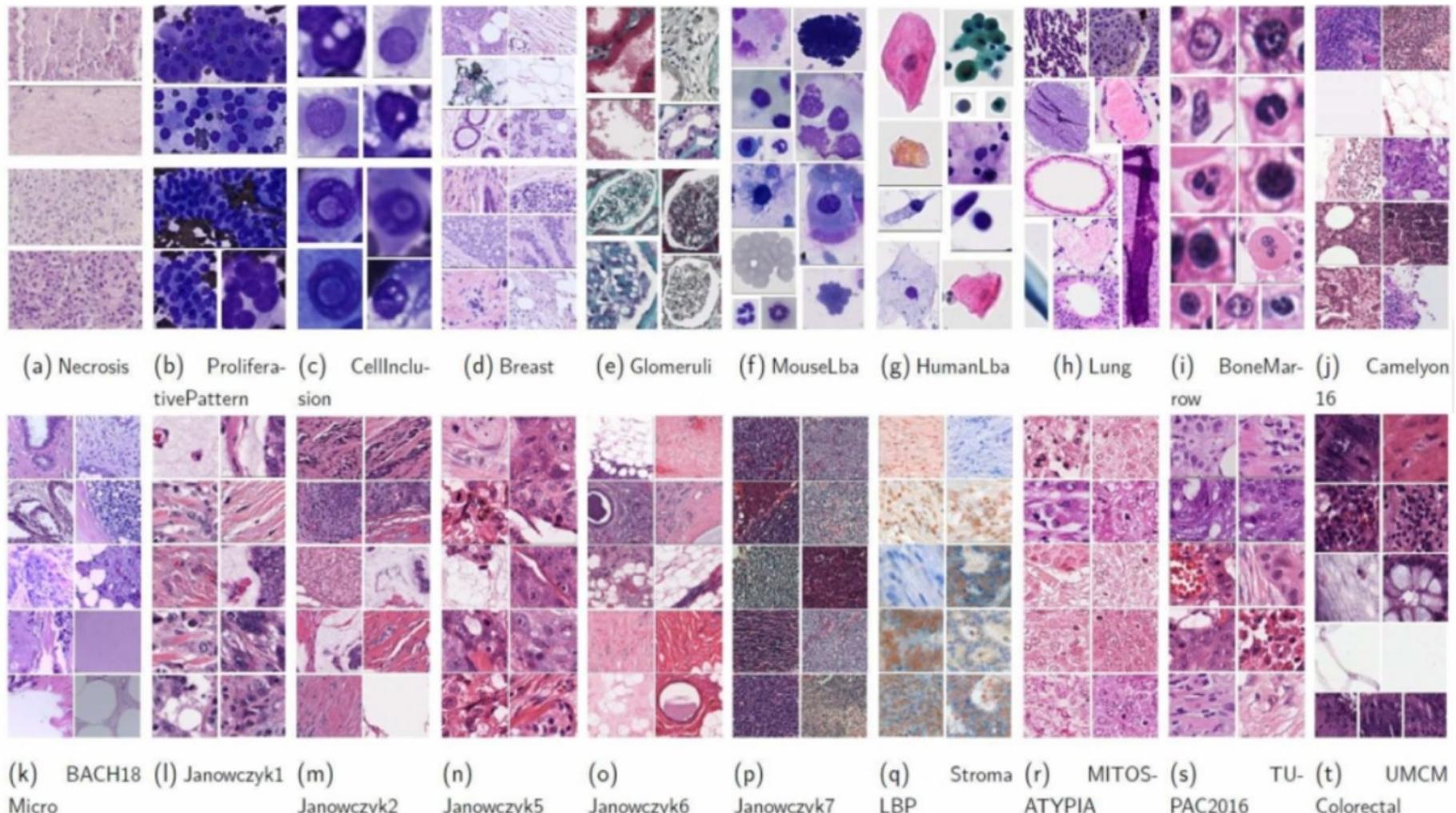
cytominē

(data : GIGA)



(Longuespée et al., Methods 2016) (Rubens et al., Proteomics: Clinical Applications, 2019)

Applications : object classification in digital pathology



882K manually annotated images, > 80 classes of « objects » (data : GIGA, PASTEUR, ULB, GRAZ, RUMC,...)

→ Combination of multi-task learning and transfer learning with fine-tuning yield better recognition rates than ImageNet models

Reproducible, online, benchmarking



biaflows.neubias.org

NUCLEI-TRACKING-DIVISION	This project illustrates the 2D tracking of cell nuclei. The time-lapses are derived from Fluo-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.	

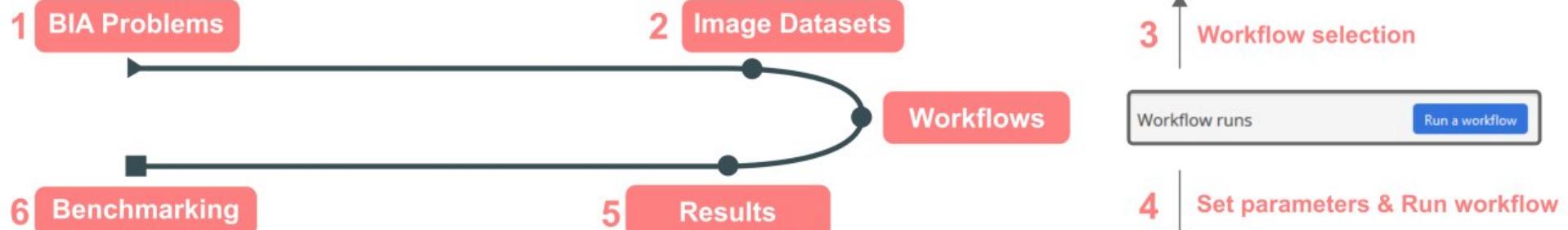


New workflow run

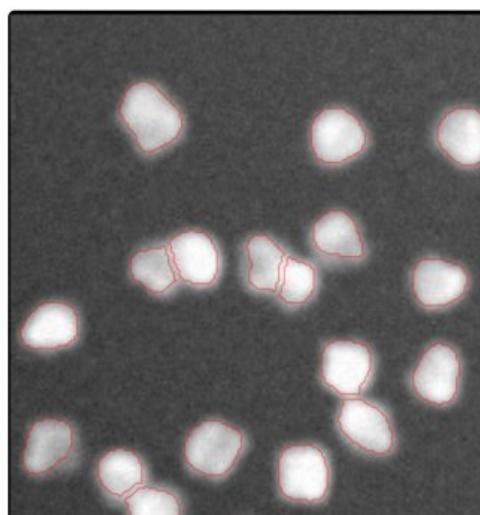
Workflow

Select options

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



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



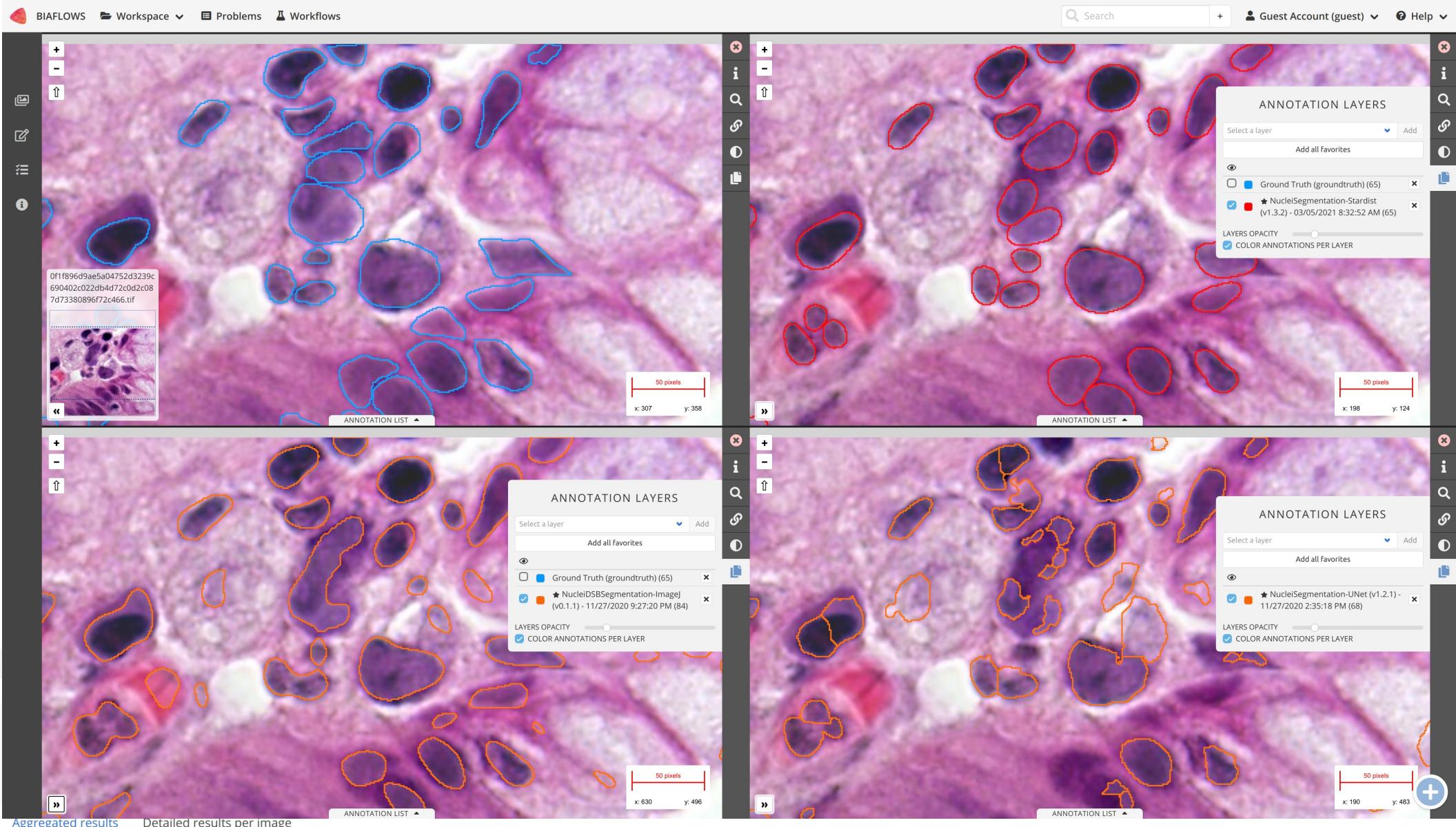
New workflow run

Workflow

NucleiSegmentation-ImageJ (v1.10.1)

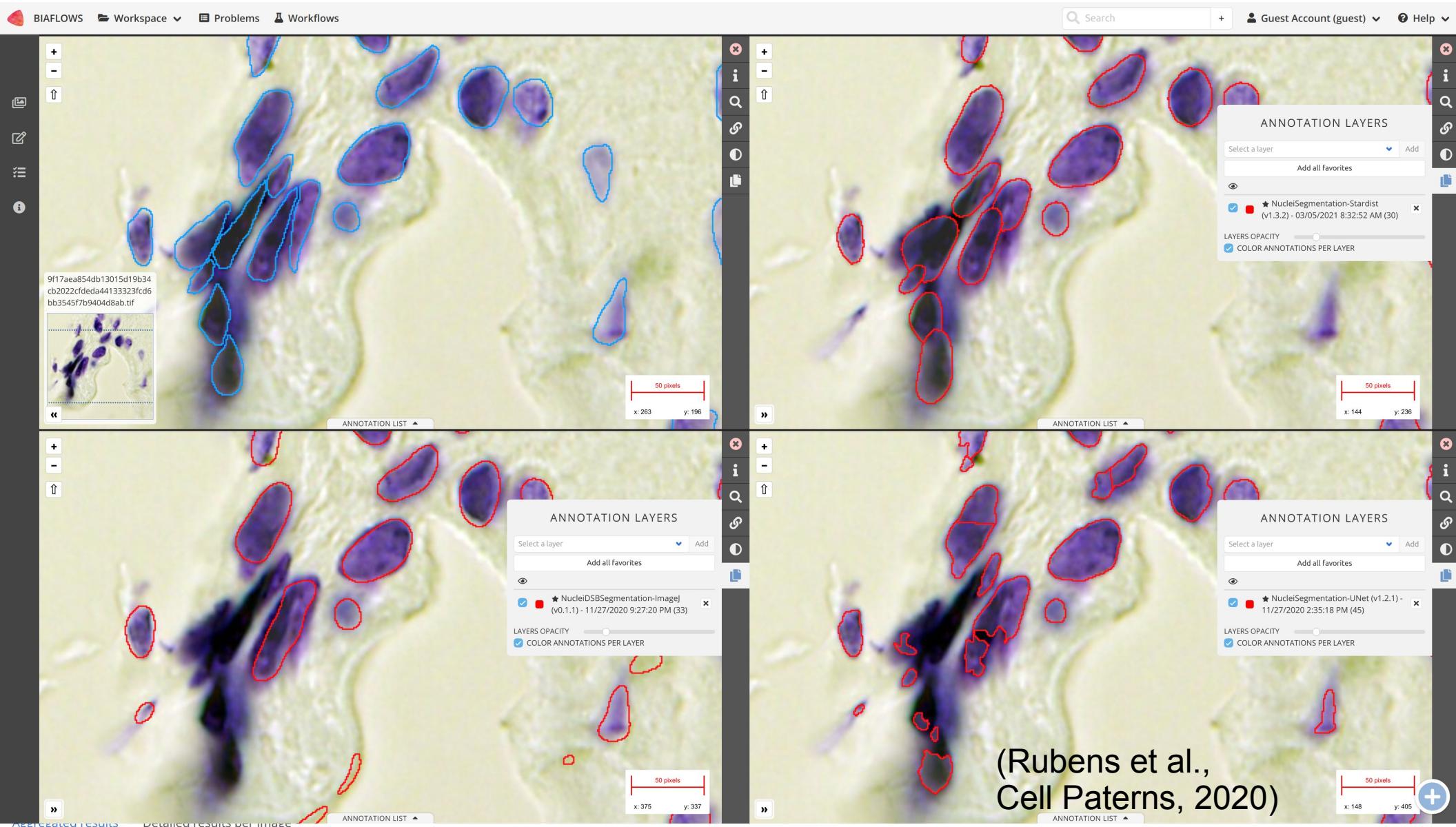
Name	Value
Radius	5
Threshold	-0.5

Cancel Run a workflow



Workflow run

	Mean Average Precision [Main metric]	Dice coefficient	Average Hausdorff distance	Fraction Overlap Pred
★ NucleiSegmentation-Cellpose (v1.2.2) #1 on Mar 5, 2021 8:08 PM	AVG	AVG	AVG	AVG
★ NucleiSegmentation-Stardist (v1.3.2) #2 on Mar 5, 2021 8:32 AM	0.312	0.701	10.545 *	0.553
★ NucleiSegmentation-ilastik (v1.4.2) #1 on Nov 28, 2020 11:52 AM	0.397	0.845	1.584	0.701
★ NucleiSegmentation-MaskRCNN (v1.5.4) #1 on Nov 28, 2020 11:35 AM	0.208	0.725	3.843	0.502
★ NucleiDSBSegmentation-ImageJ (v0.1.1) #1 on Nov 27, 2020 9:27 PM	0.394	0.798	2.702	0.625
★ NucleiSegmentation-UNet (v1.2.1) #1 on Nov 27, 2020 2:35 PM	0.235	0.734	3.57	0.528
	0.282	0.754	8.485	0.542



Workflow run

	Mean Average Precision [Main metric]	Dice coefficient	Average Hausdorff distance	Fraction Overlap Pred
★ NucleiSegmentation-Cellpose (v1.2.2) #1 on Mar 5, 2021 8:08 PM	0.312	0.701	10.545 *	0.553
★ NucleiSegmentation-Stardist (v1.3.2) #2 on Mar 5, 2021 8:32 AM	0.397	0.845	1.584	0.701
★ NucleiSegmentation-ilastik (v1.4.2) #1 on Nov 28, 2020 11:52 AM	0.208	0.725	3.843	0.502
★ NucleiSegmentation-MaskRCNN (v1.5.4) #1 on Nov 28, 2020 11:35 AM	0.394	0.798	2.702	0.625
★ NucleiDSBSegmentation-ImageJ (v0.1.1) #1 on Nov 27, 2020 9:27 PM	0.235	0.734	3.57	0.528
★ NucleiSegmentation-UNet (v1.2.1) #1 on Nov 27, 2020 2:35 PM	0.282	0.754	8.485	0.542

Summary

Current situation : thousands of image analysis algorithms

- It is not easy to choose among them
- A lot of approaches tend to be *ad hoc* (not easily reusable)

We suggest to improve the situation by conducting
collaborative and open research (sharing everything :
images, ground-truth annotations, workflow code & results,
...), and by performing **quantitative benchmarking**, using
our open-source Cytomine web software tools

... with the hope to design more generic and efficient tools

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Funding sources since 2010 :



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Two webinars (May 2021):

https://www.youtube.com/results?search_query=neubias+academy+cytomine