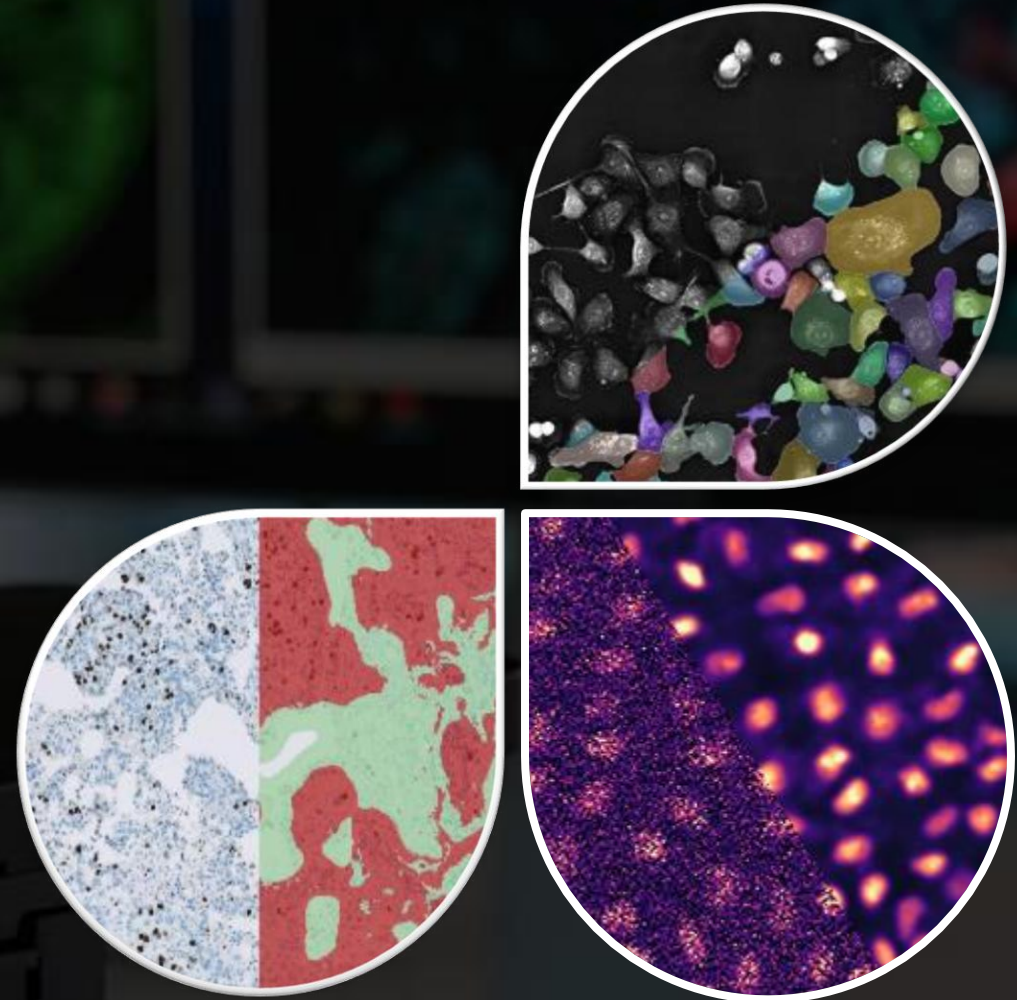


# AI + Microscopy: A Powerful Duo for Biologists



# Cell Imaging platform



Sandra Ormenese  
Flow Cytometry and Cell Imaging Manager



Gaëtan Lefevre  
Cell Imaging & Sample preparation Expert



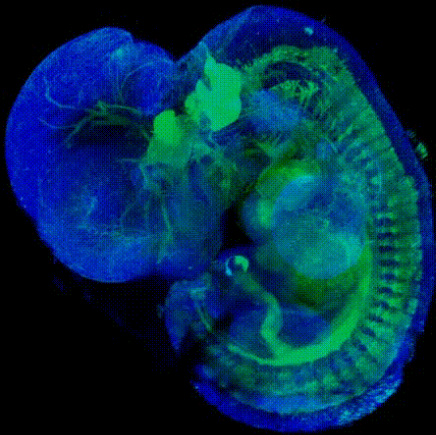
Alexandre Hego  
Cell Imaging & Image Analysis Expert

## 15 microscopes:

Widefield, confocal, high resolution, lightsheet, multiphoton, intravital, live cell automated microscopes, slide scanner, laser microdissection....

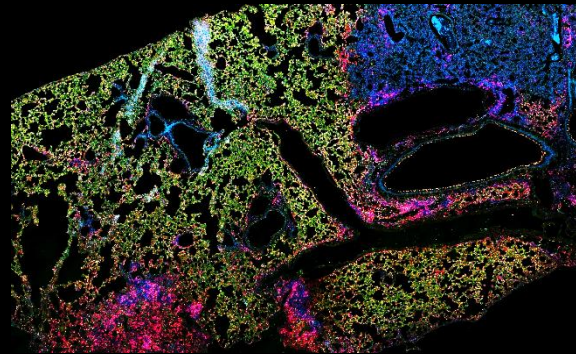
## Examples :

Lightsheet



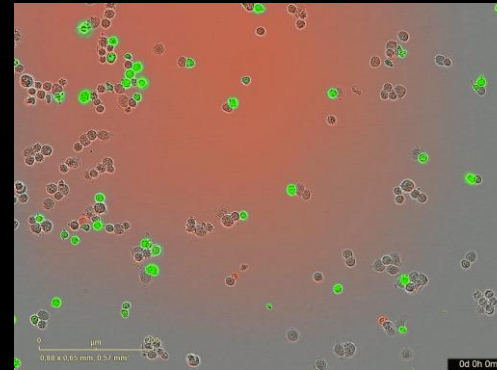
Mouse embryo E11.5 sox10 GFP  
Carla Gomes Da Silva  
GIGA Molecular Regulation of neurogenesis

Slide scanner



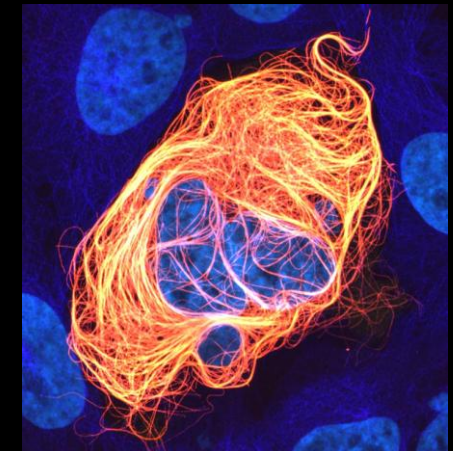
Multiplex 6 colors  
Cecilia Ruscitti  
GIGA Immunophysiology

Live cells imaging



Coculture monocyte  
Clotilde Hoyos  
GIGA cellular and molecular epigenetics

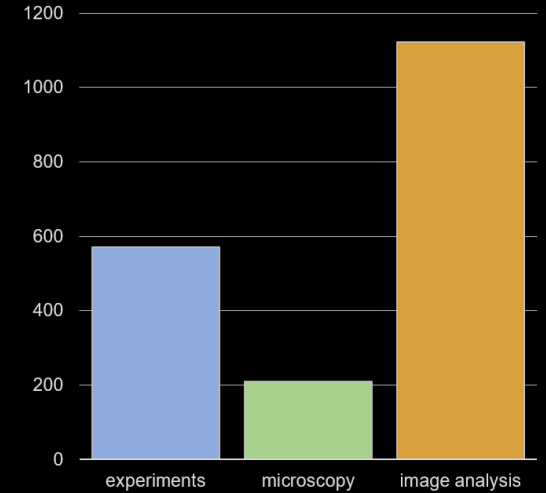
High resolution



Cytoskeleton in osteocarcinoma  
Maxime Gilsoul  
GIGA Molecular Regulation of neurogenesis

# Limitations of classical image analysis

Which step in imaging based research project is the most difficult for you ? Neubias survey 2015 (n=1904)

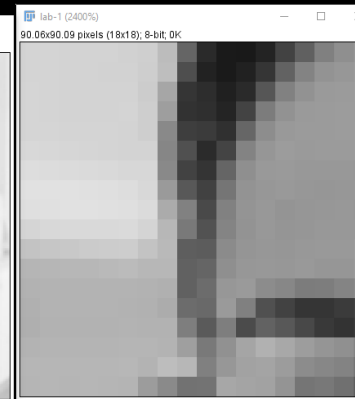
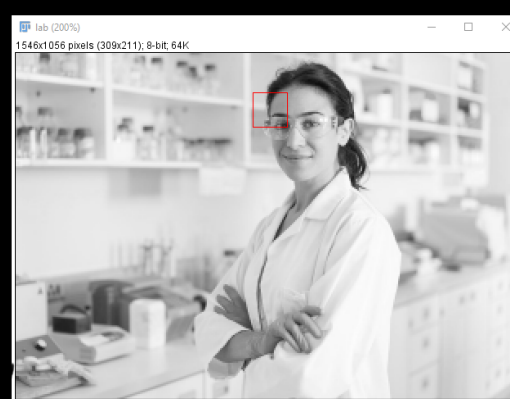


Perception problem:

- We love images too much, but we forget scientific analysis
- Images are a matrix of numbers and we have to reduce the complexity with measurements



@ Vita Klochko

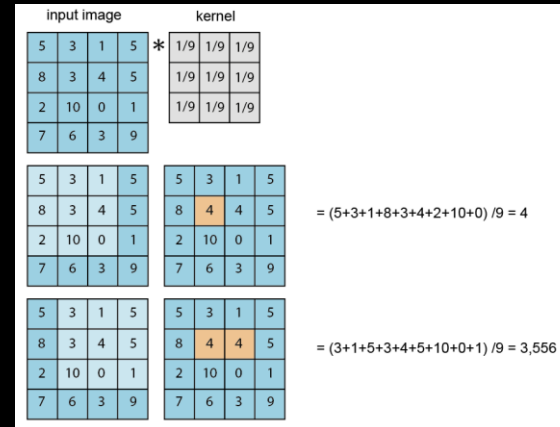
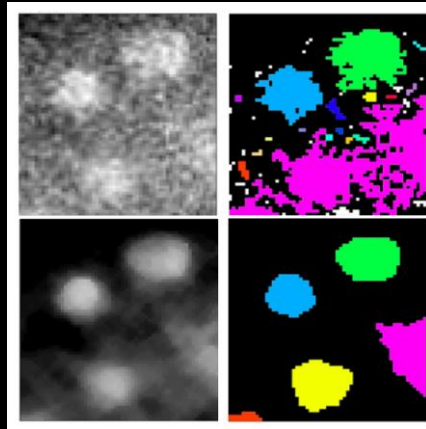


Pixel Values	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160
24	212	212	212	211	211	211	209	207	188	101	42	26	25	35	66	96
25	212	212	212	211	211	211	210	210	206	190	78	34	26	33	53	98
26	212	213	212	211	211	211	211	204	166	53	45	32	42	86	129	146
27	212	213	212	211	211	211	212	206	158	50	47	36	67	124	146	154
28	213	213	213	212	212	212	211	206	137	62	59	48	102	141	153	155
29	216	216	215	214	213	212	211	211	134	80	43	68	131	150	156	154
30	221	221	220	219	219	217	215	215	137	66	52	101	143	153	154	152
31	226	225	225	225	224	223	221	216	154	88	65	117	147	151	153	151
32	224	223	224	225	225	224	223	219	171	129	73	129	148	152	148	150
33	212	213	216	217	217	217	218	212	183	121	82	132	147	152	149	150
34	193	195	198	200	203	204	205	195	185	94	92	139	149	151	150	152
35	178	181	182	183	183	185	187	183	183	97	101	143	151	152	151	153
36	172	178	179	179	179	179	179	178	176	97	108	151	154	140	135	124
37	170	176	179	179	179	179	178	177	172	108	105	154	128	80	66	53
38	169	175	178	178	178	178	179	178	178	126	90	126	75	98	88	72
39	175	179	180	180	180	180	181	181	179	159	121	145	165	177	168	157
40	179	182	182	182	182	182	183	179	189	183	128	151	162	164	156	140
41	179	181	182	181	182	181	180	156	138	114	115	167	164	162	148	117
42	176	180	181	180	181	180	180	136	103	96	97	156	157	158	127	131
43	177	180	180	179	178	177	154	169	110	110	159	166	157	118	93	
44	179	181	180	179	178	177	188	151	136	136	147	161	150	120	91	142

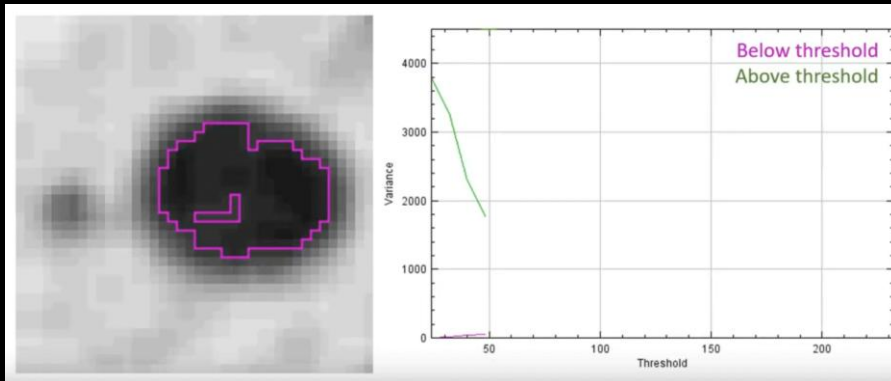
# Limitations of classical image analysis

Traditional workflows problem:

One filter is not sufficient to understand the data. Machine learning will work in multi dimensional space and will take a decision.



Example thresholding with Otsu



Search for a threshold where the variance in both classes (above/below threshold) becomes minimal

$$\text{Mean (Class 1)} = \frac{1}{\text{number of px in class 1}} \sum_{px \text{ class 1}} \text{intensity}(px)$$

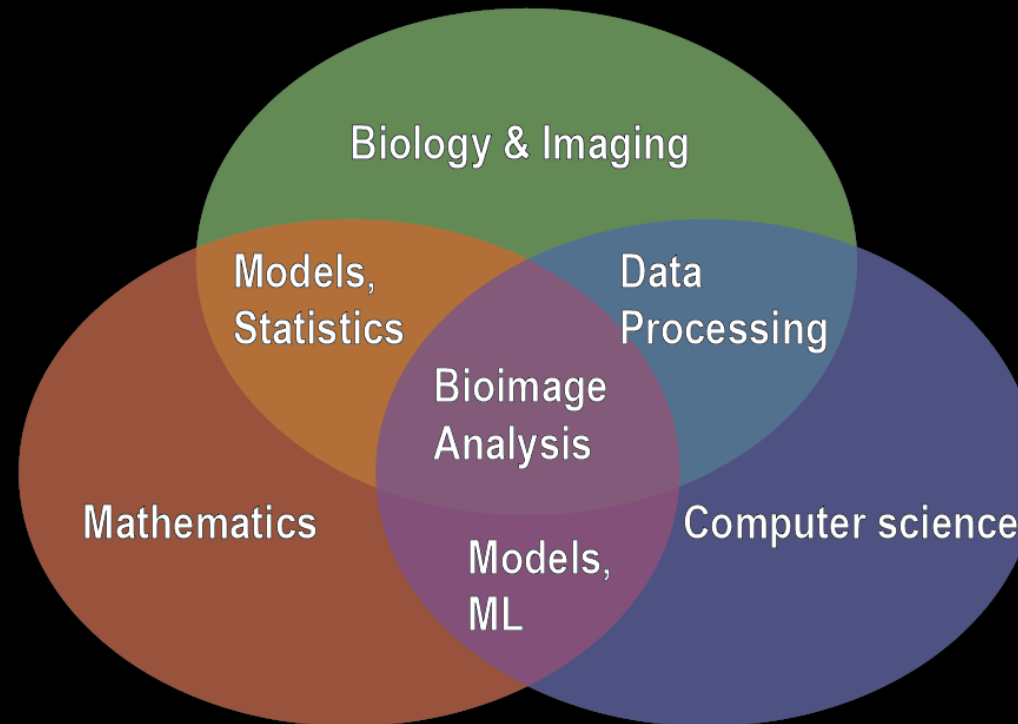
$$\text{Var}(\text{Class 1}) = \frac{1}{\text{nbr of pixel in class 1}} \sum_{px \text{ class 1}} (\text{intensity}(px) - \text{mean in class 1})^2$$

$$\text{Within-Class variance} = \frac{\text{nbr px in class 1}}{\text{nbr px in image}} \text{Var}(\text{class 1}) + \frac{\text{nbr px in class 2}}{\text{nbr px in image}} \text{Var}(\text{class 2})$$

# Limitations of classical image analysis

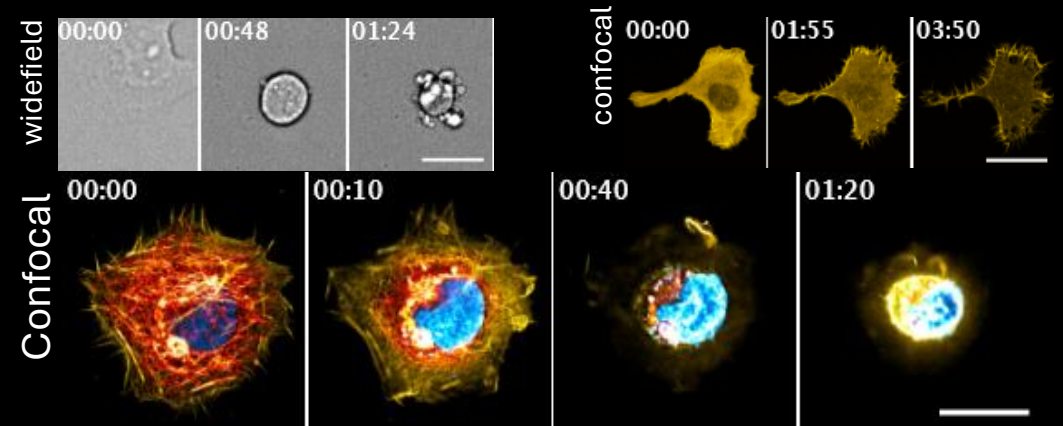
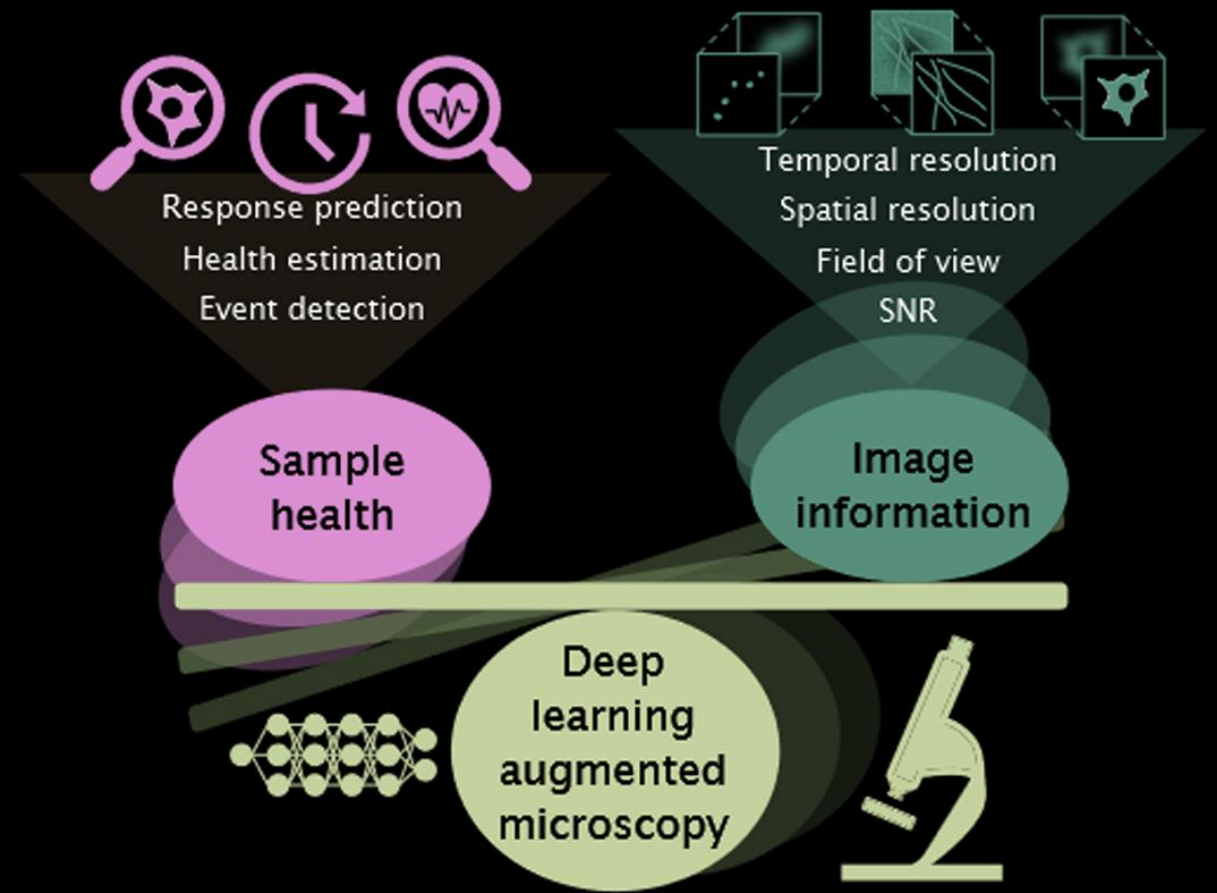
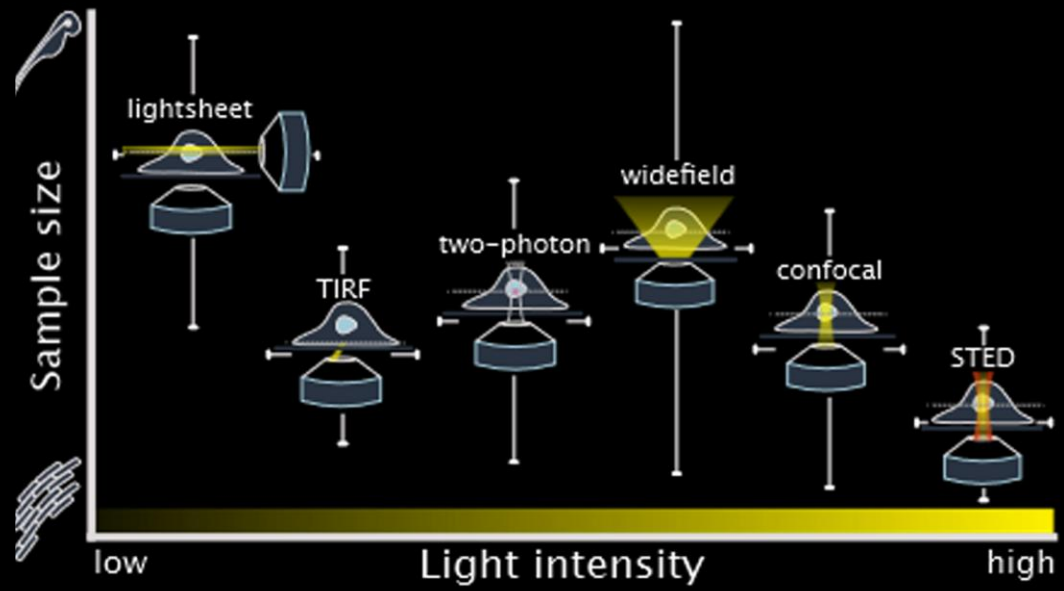
Bioinformatic problem:

- Computational science and biology have different vocabularies
- Traditional methods need notion in a lot of domains
- Expert in bio-images analysis bridge the gap between domains



# Limitations of Fluorescence Microscopy

Light alter biological process under observation and promote **photobleaching**, **photodamage** and **phototoxicity**



Henriques et al. AI-enabled Live imaging 2023

Henriques et al. AI-enabled Live imaging 2023

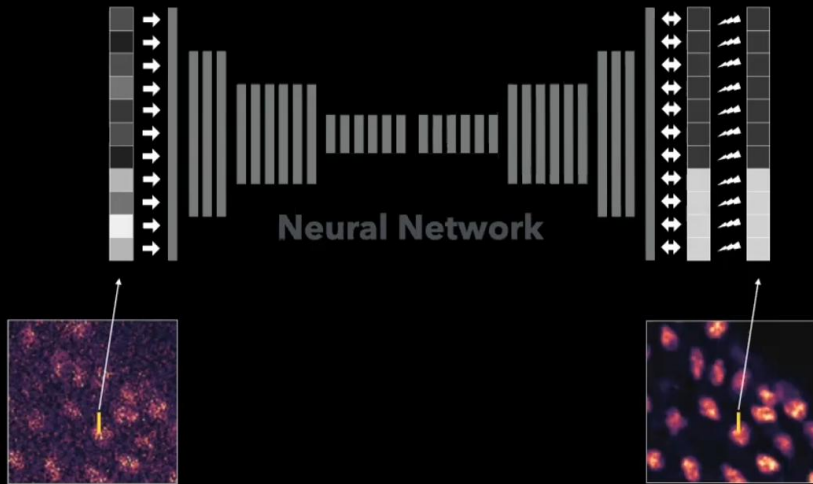
# Denoising by Deep learning: CARE

## Pro

- + One of the best denoising methods
- + Can resolve complicated restoration task
- + Available in the platform with Python script

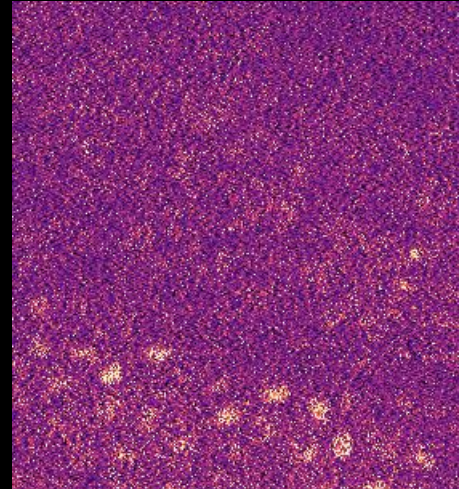
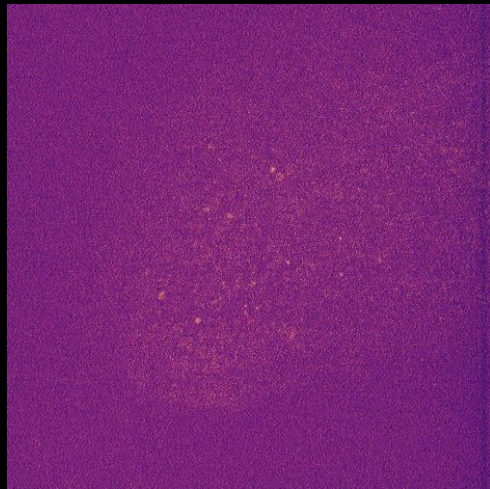
## Con

- Need a lot of data for the training more than 200 paired images
- Can be hard to image high quality paired training data (ex: if particles moved significantly between the two acquisitions)

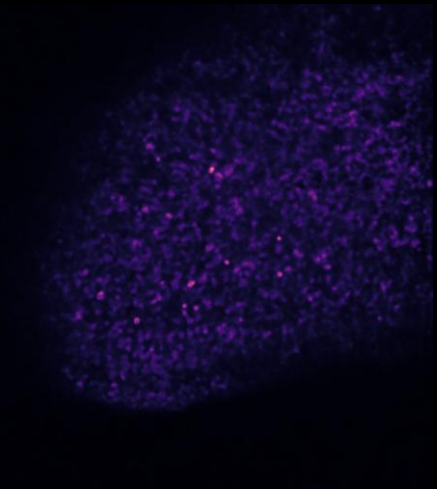
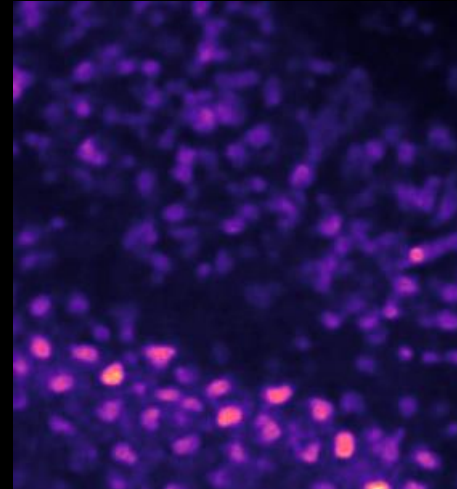


Martin Weigert et al. Nature Methods (2018)

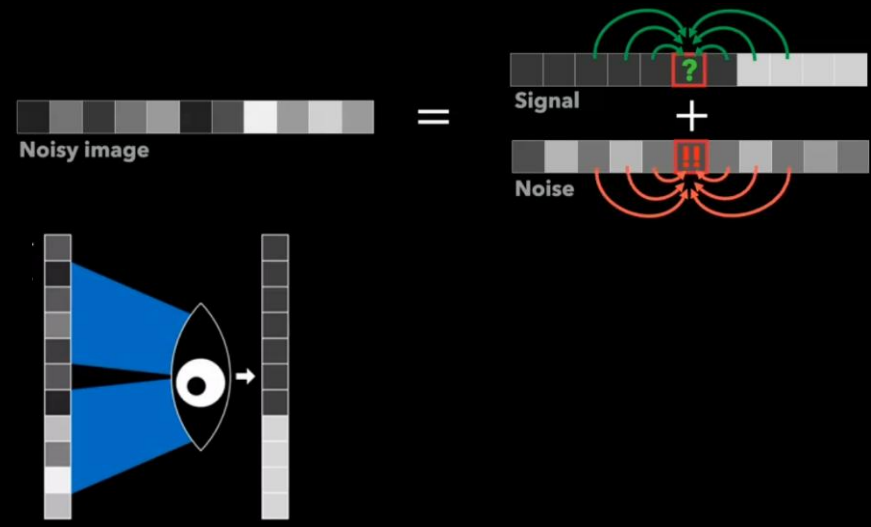
RAW data



CARE output



# Denoising by Deep learning : N2V



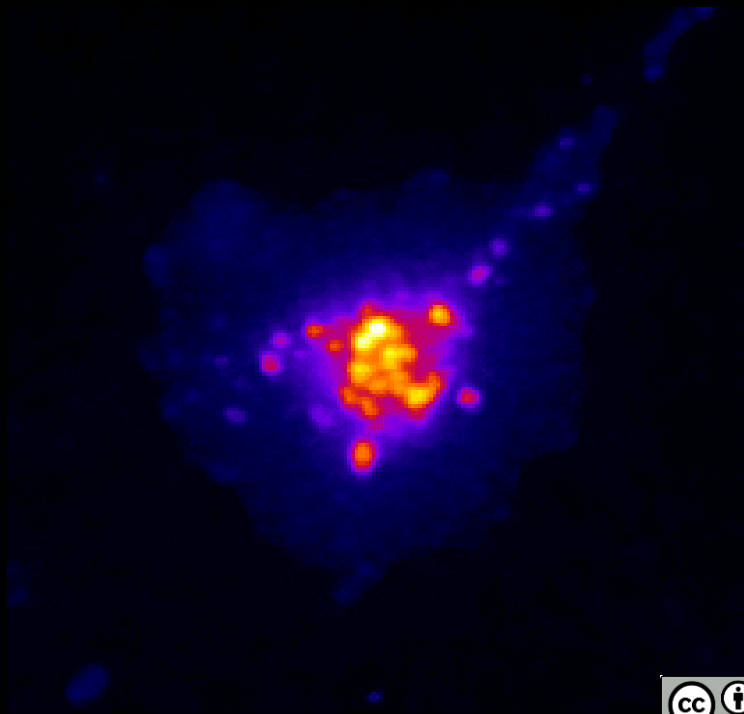
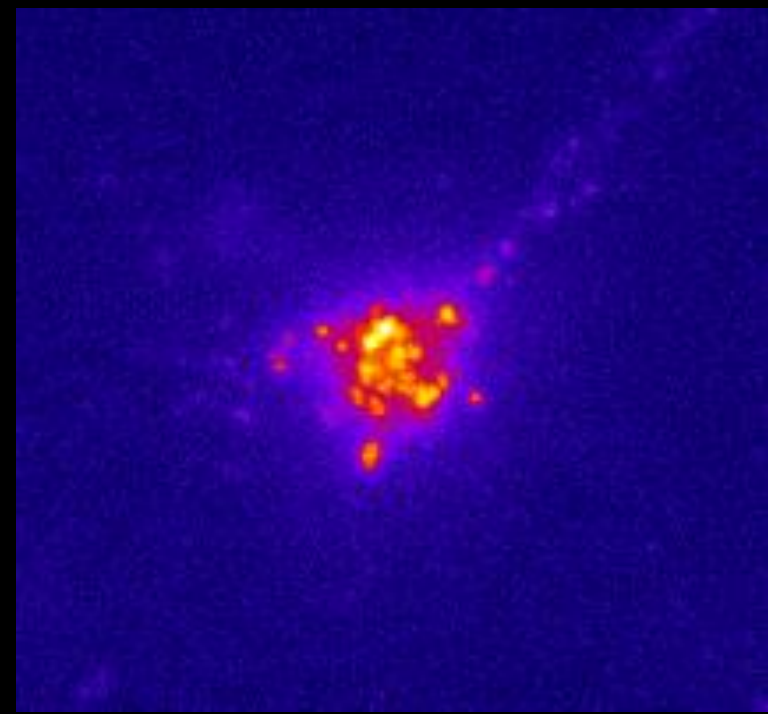
Alexander Krull et al. computer vision and pattern recognition (2019)

## Pro

- + Don't need a lot of dataset (< 20 images)
- + Plugin Fiji work on CPU or GPU (5x faster available in the platform)

## Con

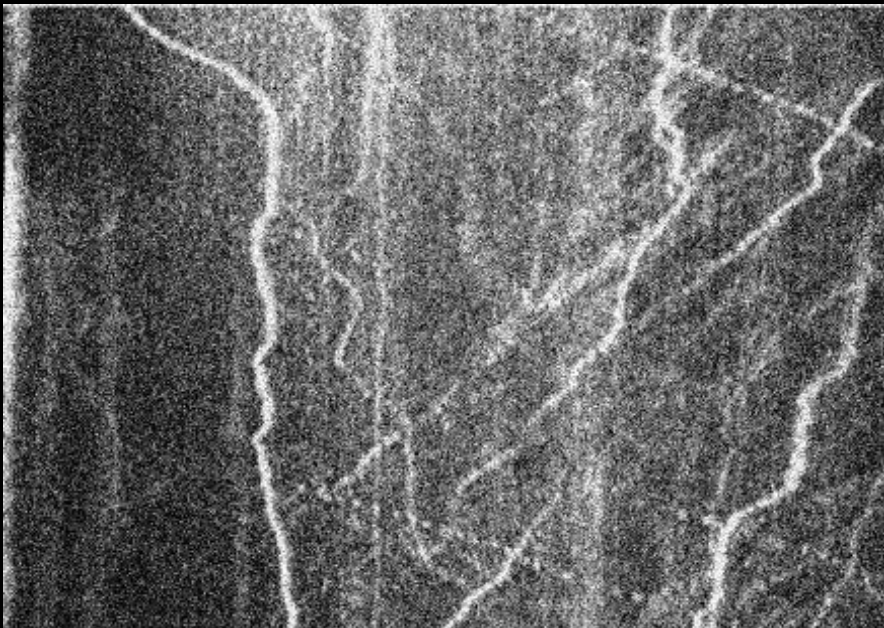
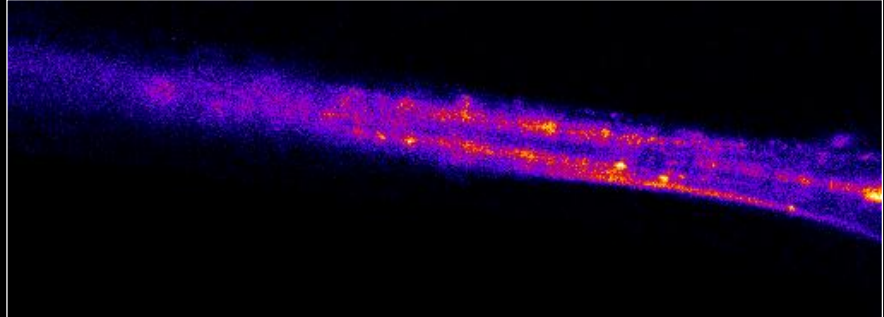
- May not work if the noise is not pixel independent and present patterns.



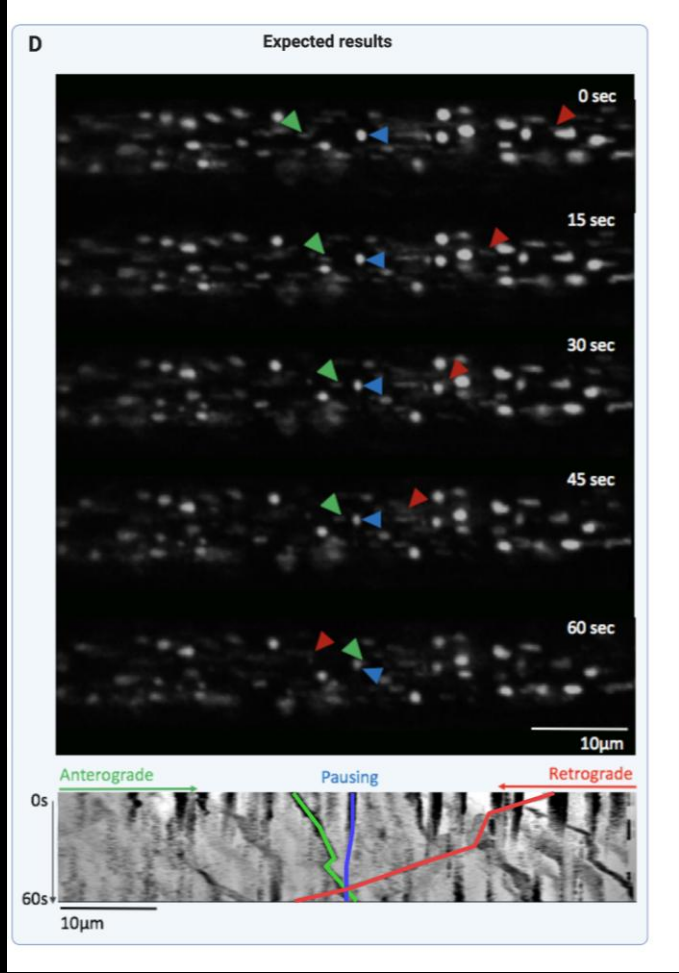
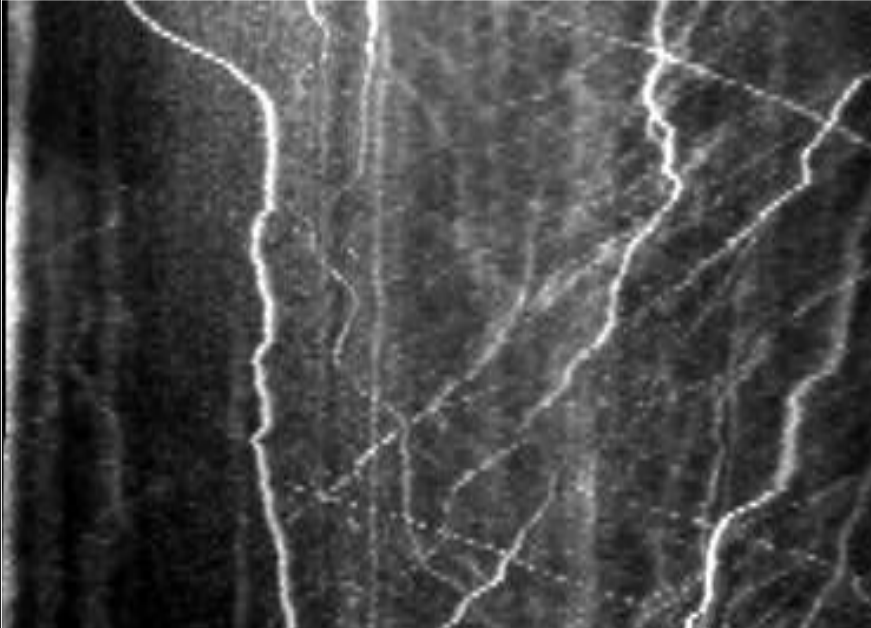
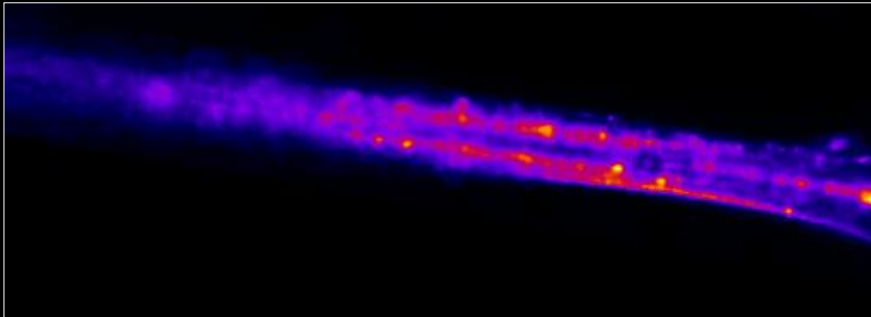


# Example desoising for GIGA

Raw data



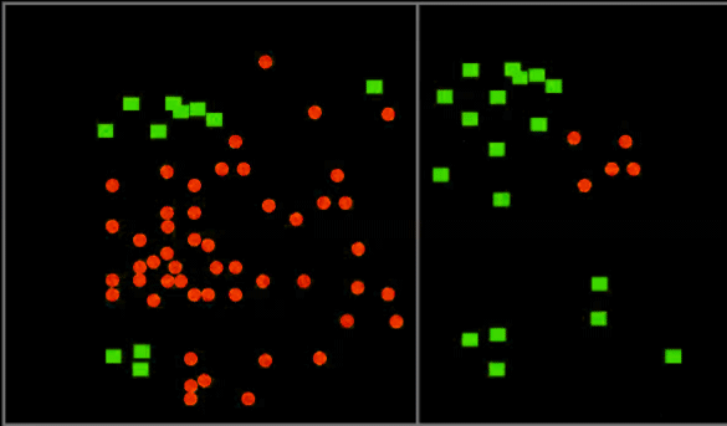
Noise2Void



# Machine learning : Decision tree

$$x_1 > s_1$$

$s_1$



## Pro

- + Simple to interpret
- + Require little data preparation
- + Easy to process data with high dimension
- + Extremely Fast

## Con

- Overfitting may happen (learn by heart) often with large (deep) tree
- A tiny change in the training data can cause large changes in the final result

# Machine learning ex: Random forest

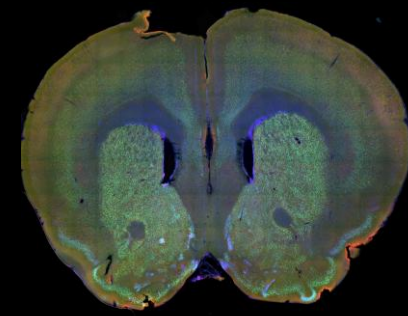
## Pro

- + Use a large number of small trees (reduce variance)
- + Use majority rules = Robust and closer the true answer

## Con

- Slower than one decision tree
- The more trees you have, the slower the process
- The more features you have, the slower the process (closer to the features / parameters if you don't need it)
- Difficult to interpret

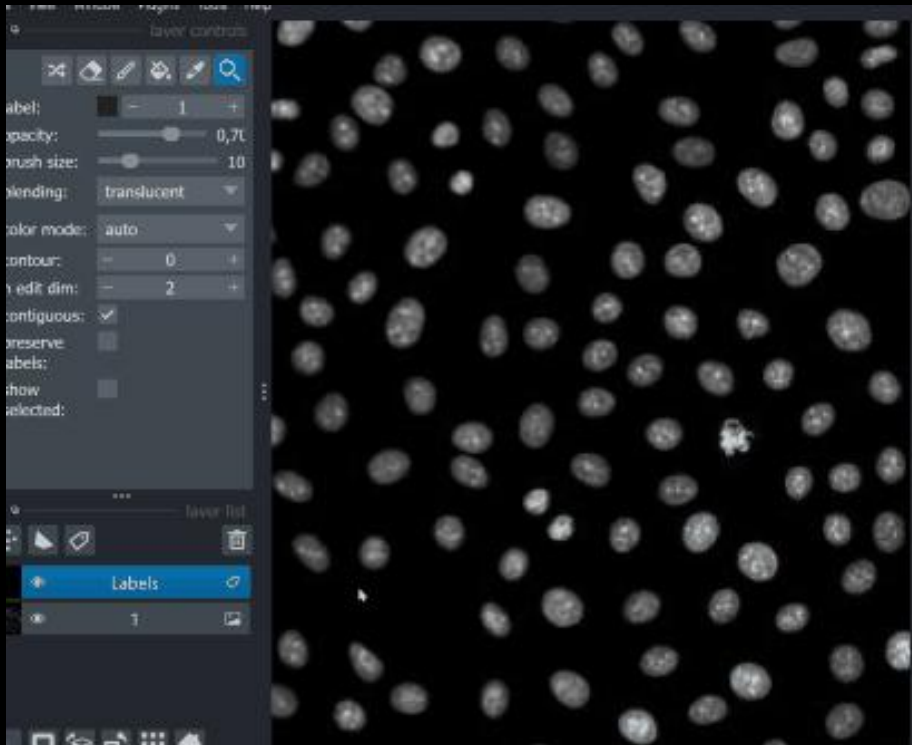
# Machine learning ex: Random forest



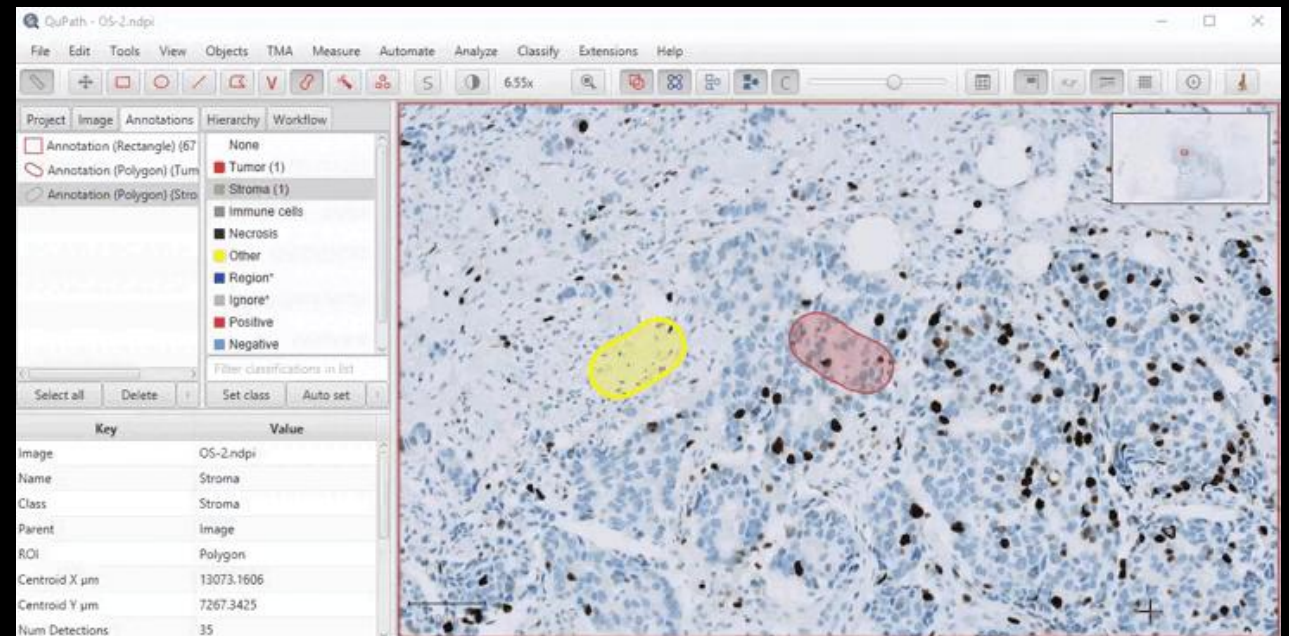
Machine learning algorithms can classify pixels and object to detect specific tissue, cells or staining.

Only one skill is needed “drawing” / “painting” annotations

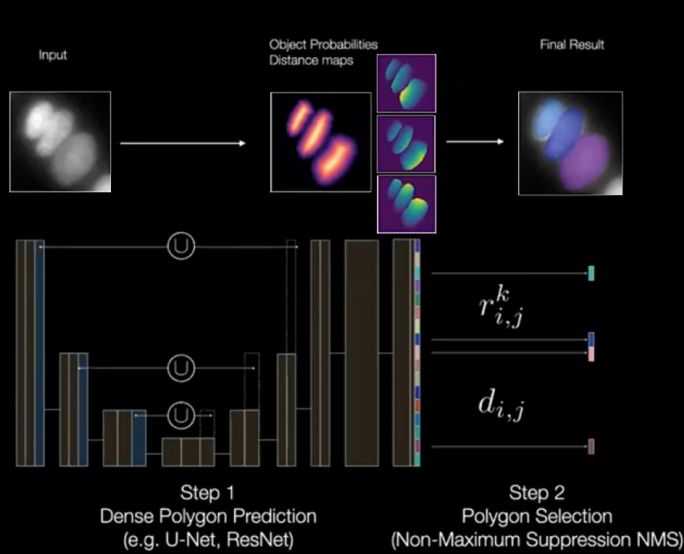
Pixel classifier for cells detection



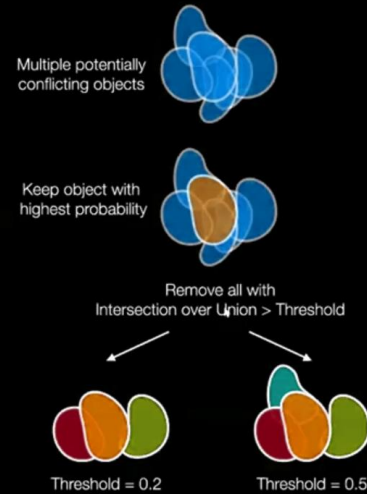
Object classifier for specific objects detection



# Segmentation by Deep learning: Stardist



## Non-Maximum-Suppression (NMS)



## Pro

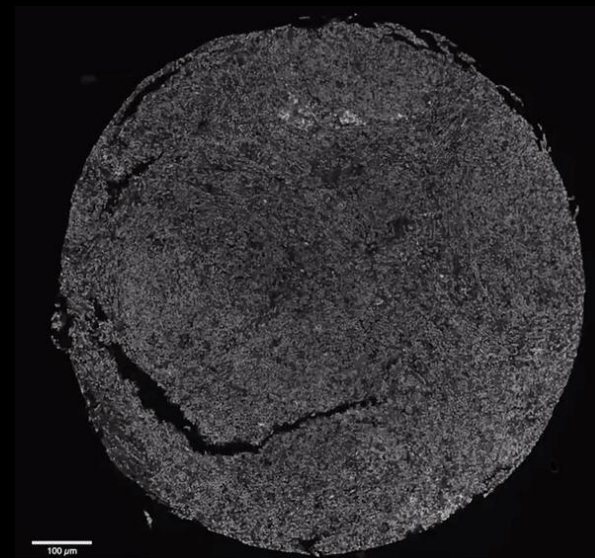
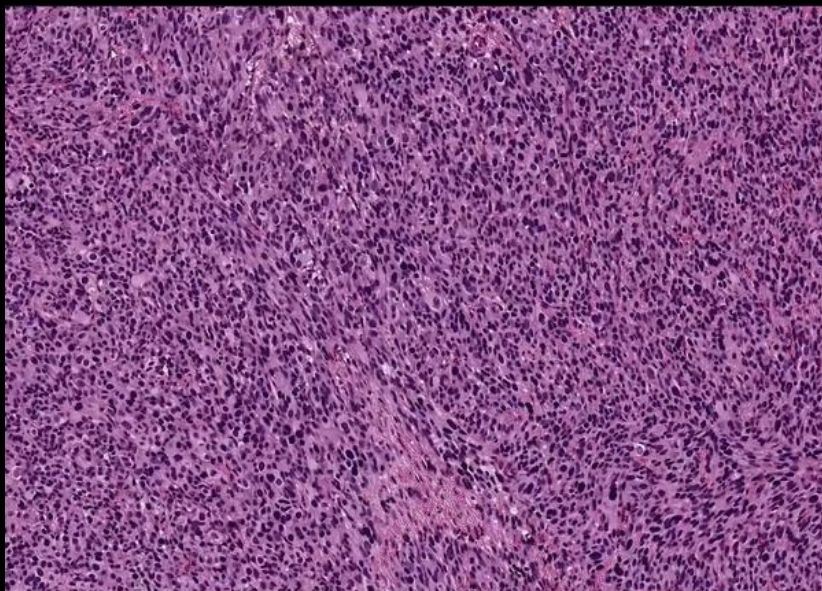
- + Work on 2D, 3D dataset
- + Work with fluorescence, Brightfield, or HE
- + Existing model work well on a lot of data
- + Available in the platform on Fiji and QuPath
- + Easy to train on personal annotations with QuPath

## Con

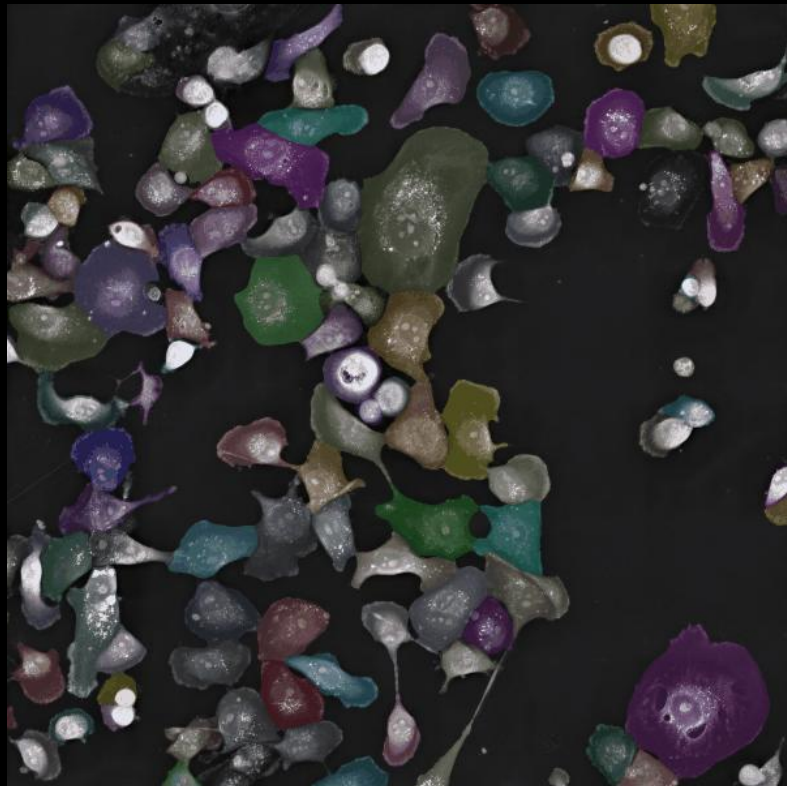
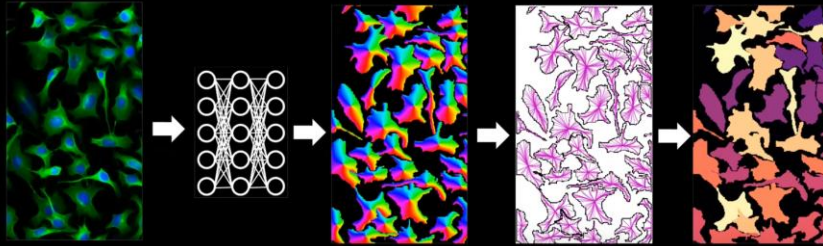
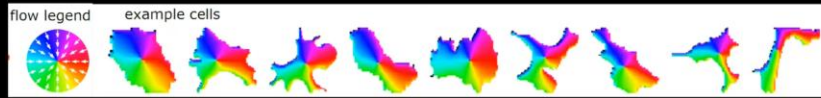
- Need cells with convex shape
- Can't help for the morphology (see cellpose)

## Applications

- Fluorescence measurements
- Counting
- Clustering
- Spatial omics



# Segmentation by Deep learning: Cellpose



## Pro

- + Work on 2D or 2D + T dataset
- + Work with fluorescence, Brightfield, or HE
- + Existing model work well on a lot of data
- + work on all cell morphology
- + Easy to train on personal annotations with the GUI and QuPath
- + Available in the platform on Fiji and QuPath and Napari

## Con

- Very difficult to use on 3D dataset (need a lot of VRAM) because the predictions are computed 3 times in XY, XZ, and YZ directions

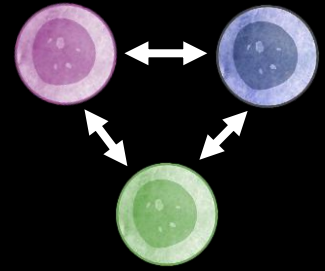
## Applications

- Fluorescence measurements
- Counting
- Clustering
- Spatial omics
- Morphology

# Clustering

Multiplexing analysis with more than 40 markers

NN Self Organisation MAP

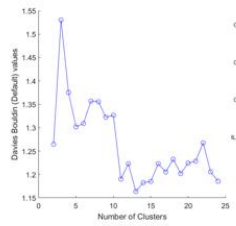


## Analyse 3

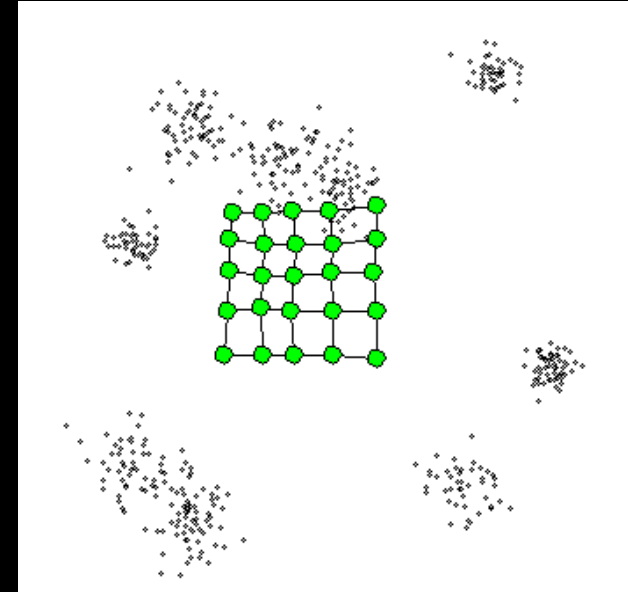
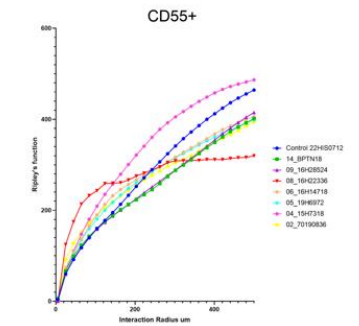
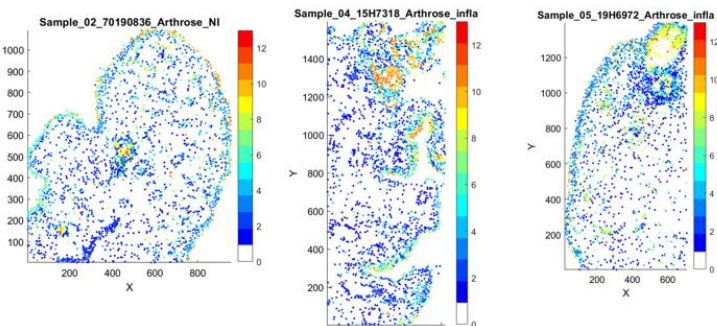
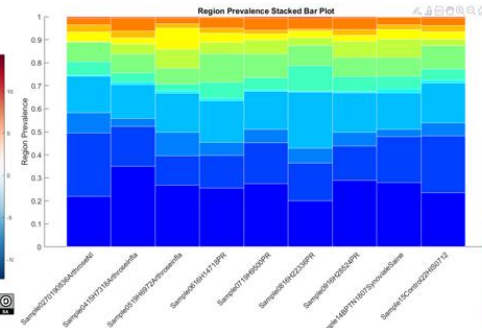
Utiliser les images 2, 4, 5, 6, 7, 8, 9, 14, 15 et clusteriser marqueurs CD55, CD34, CD68, HLADR, CD14

Test 1 : Dalvies Bouldin + NN Self-Organizing Map

12 Clusters identifiés

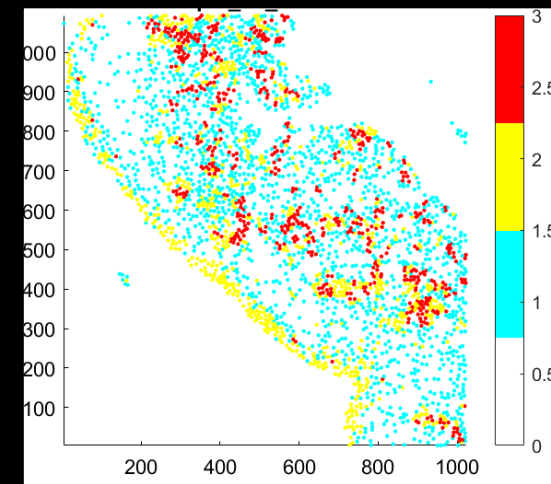
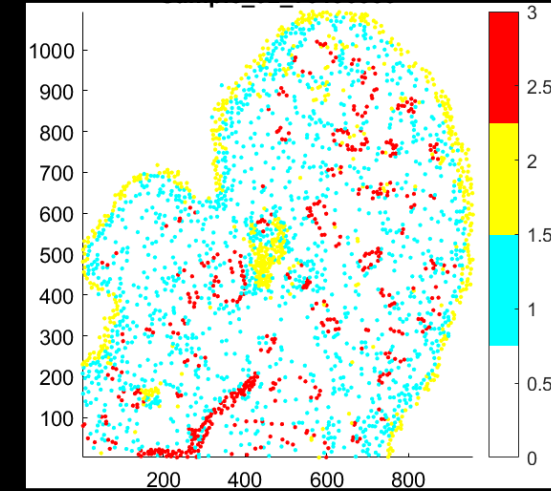


	1	2	3	4	5	6	7	8	9	10	11	12	13
CD34 Cytosolem Mean	0.1419	0.2851	0.3389	0.2259	0.3704	0.3382	0.3305	1.089	0.8016	0.4839	0.5044	0.4437	14.24
CD14 Cytosolem Mean	0.327	0.826	0.8204	0.7744	0.9581	0.9068	1.388	0.8806	0.883	1.5	2.149	1.146	0.9008
CD55 Cytosolem Mean	0.257	0.5475	1.818	0.3783	3.906	0.8272	0.5723	0.6386	0.5015	0.9914	1.023	0.9314	0.6887
CD68 Cytosolem Mean	0.1876	0.3074	0.6416	0.5988	0.622	1.882	0.7443	0.5158	0.7979	3.501	1.227	1.188	0.5985
HLADR Cytosolem Mean	0.18	0.1806	0.3874	0.4501	0.4872	0.7425	0.6565	0.3309	1.722	1.483	1.329	4.449	0.2589
Percentage	26.51	18.38	6.173	17.42	0.907	0.907	4.977	3.996	2.187	4.136	0.5285	0.02119	



1 2 3

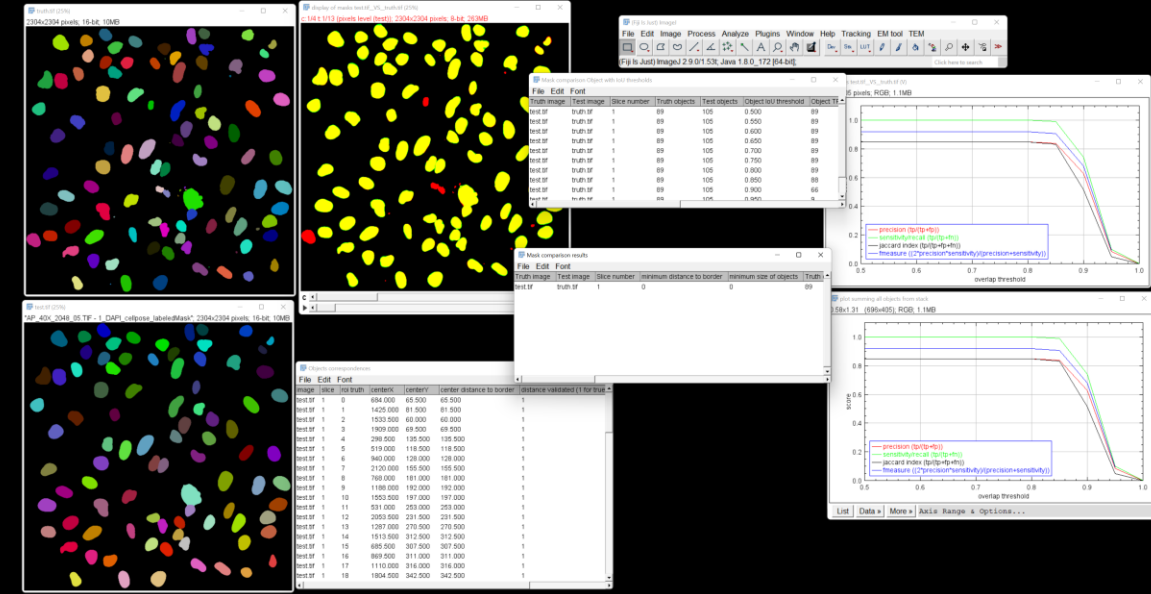
	1	2	3
CD11c Membrane Mean	1.156	0.9807	0.8634
CD34 Membrane Mean	0.6343	1.373	0.9925
CD38 Membrane Mean	0.6121	1.379	1.009
Ki67 Membrane Mean	0.6636	1.455	0.8816
CD14 Membrane Mean	0.7727	1.535	0.928
CD30 Membrane Mean	0.342	0.9993	2.029
CD45 Membrane Mean	0.6729	1.703	0.8245
CCR7 Membrane Mean	0.5964	1.126	1.278
Foxp3 Membrane Mean	0.6296	1.307	1.063
CD4 Membrane Mean	0.6852	1.673	0.6415
CD1c Membrane Mean	0.626	1.365	0.9987
CD68 Membrane Mean	0.7407	1.822	0.4374
CD20 Membrane Mean	0.5445	1.98	0.4754
CD8a Membrane Mean	0.6201	1.702	0.5975
CD138 Membrane Mean	0.4764	1.712	1.7411
MPO Membrane Mean	0.6071	1.94	1.053
PD1 Membrane Mean	0.6985	1.513	0.8882
CD56 Membrane Mean	0.6755	1.268	1.056
Icos Membrane Mean	0.6281	1.333	1.041
CD3 Membrane Mean	0.5956	1.6	0.8944
FAP Membrane Mean	0.807	1.48	0.7131
CCR2 Membrane Mean	0.865	1.28	1.055
HLDR Membrane Mean	0.6771	1.077	0.3887
CD19 Membrane Mean	0.8032	1.681	0.8555
Podoplanin Membrane Mean	0.7337	1.215	1.051
CD15 Membrane Mean	0.5778	0.878	1.534
Timbo Membrane Mean	0.5804	0.9896	1.43
SMA Membrane Mean	0.3445	0.4878	2.168
Percentage	56.41	28.19	15.4



Quality control:

Mask instant Comparator (MiC) compare segmentation masks from the prediction and the ground true

- Precision defined as  $\frac{TP}{TP+FP}$
- Recall (or sensitivity) defined as  $\frac{TP}{TP+FN}$
- Jaccard index (or global perrecision) defined as  $\frac{TP}{TP+FP+FN}$
- F1-measure (or Sorensen Dice Coefficient - DSC) defined as  $\frac{2TP}{2TP+FP+FN}$



Misconduct:

not publishing image analysis scripts, data, and sharing raw images.

For scripts : Github, Gitlab

For images : BioImage Archive, Image Data Ressource, Figshare, Zenodo



BioImage Archive

zenodo

figshare