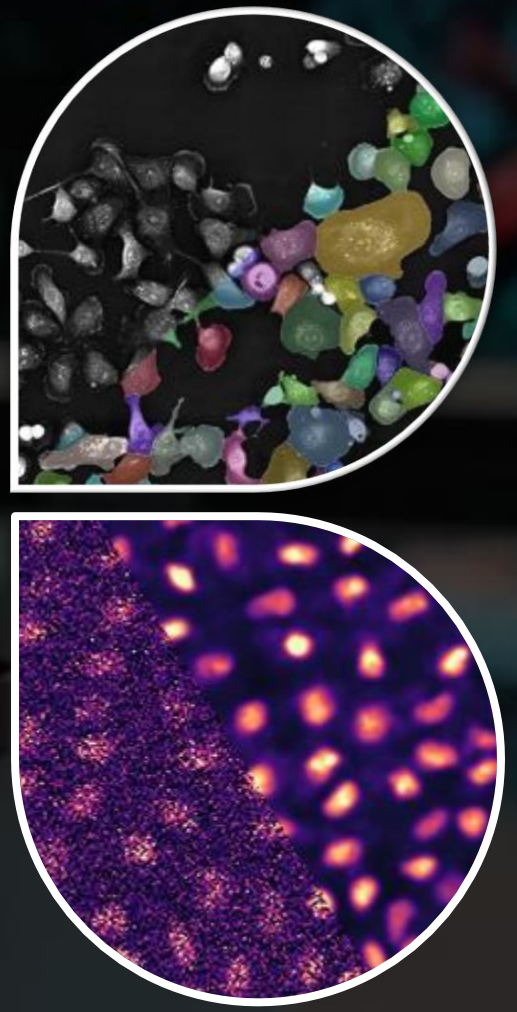
LIÈGE université
GIGA
Cell Imaging

AI + Microscopy:

A Powerful Duo for Biologists



Alexandre Hego GIGA Cell Imaging – Uliege

Cell Imaging platform

Sandra Ormenese

Flow Cytometry and Cell Imaging Manager

Gaëtan Lefevre

Cell Imaging & Sample preparation Expert

Alexandre Hego

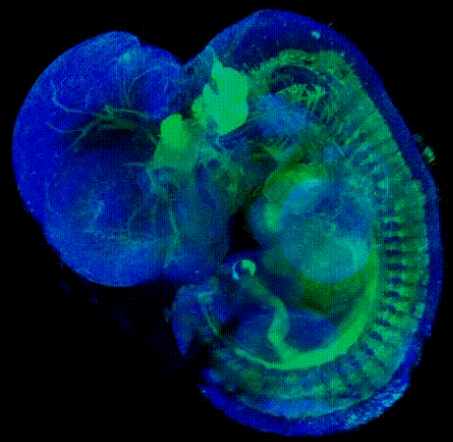
Cell Imaging & Image Analysis Expert

15 microscopes:

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

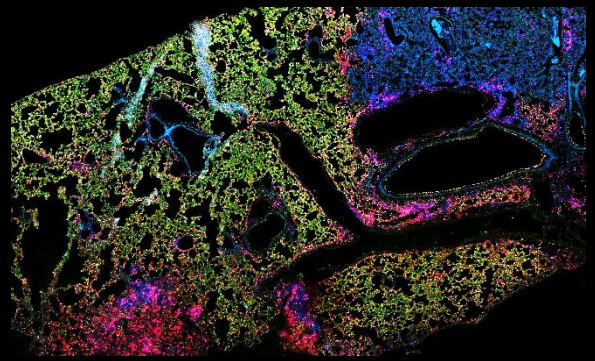
Examples :

Lightsheet



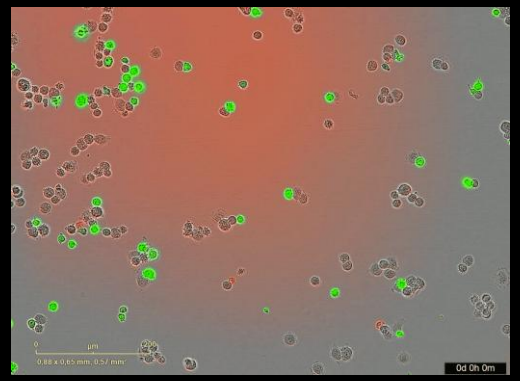
Mouse embryo E11.5 sox10 GFP
Carla Gomes Da Silva
GIGA Molecular Regulation of neurogenesis

Slide scanner



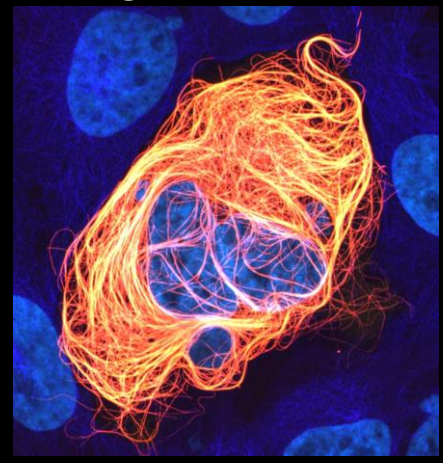
Multiplex 6 colors
Cecilia Ruscitti
GIGA Immunophysiology

Live cells imaging



Coculture monocyte
Clotilde Hoyos
GIGA cellular and molecular epigenetics

High resolution



Cytoskeleton in osteocarsoma
Maxime Gilsoul
GIGA Molecular Regulation of neurogenesis

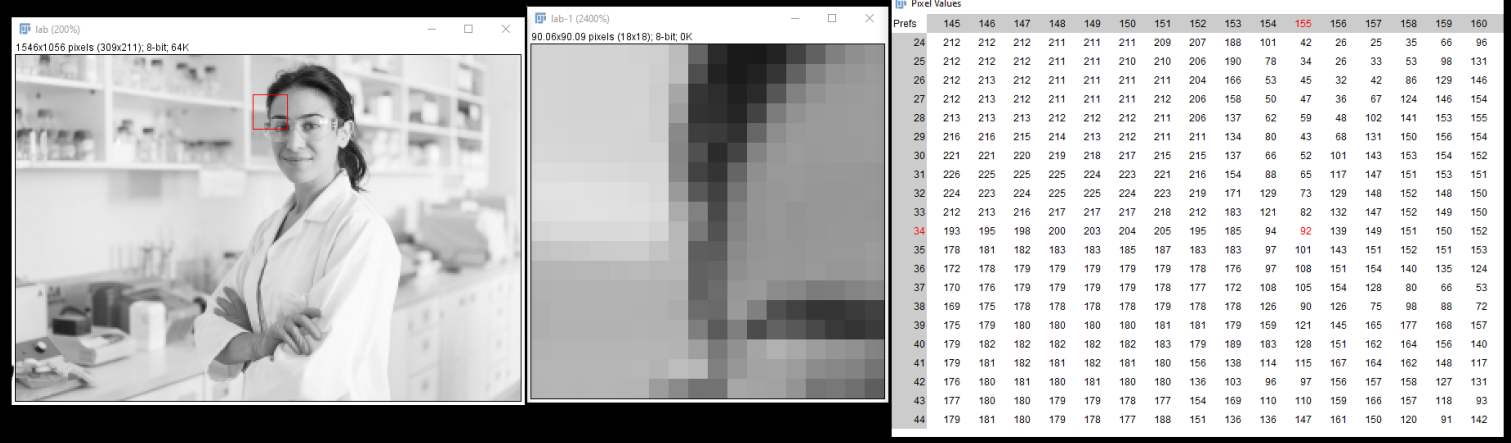
Limitations of classical image analysis

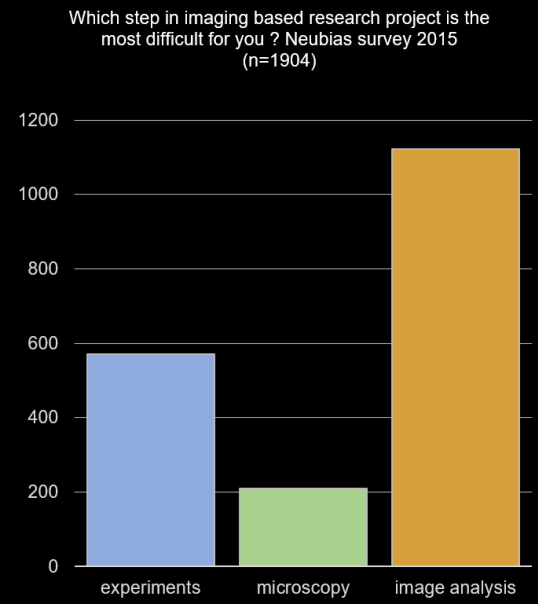
Perception problem:

- 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

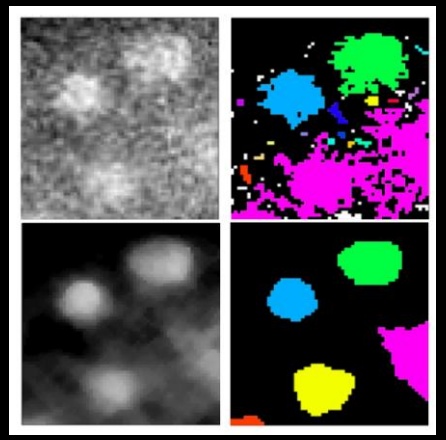


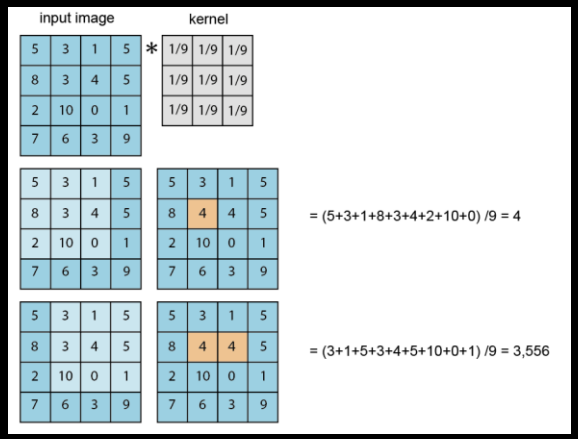


Limitations of classical image analysis

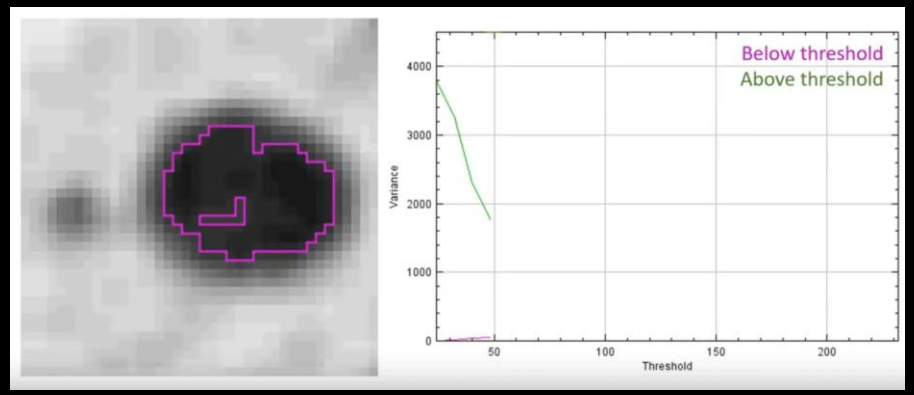
Traditional workflows problem:

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





Example throsholding with Otsu



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

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

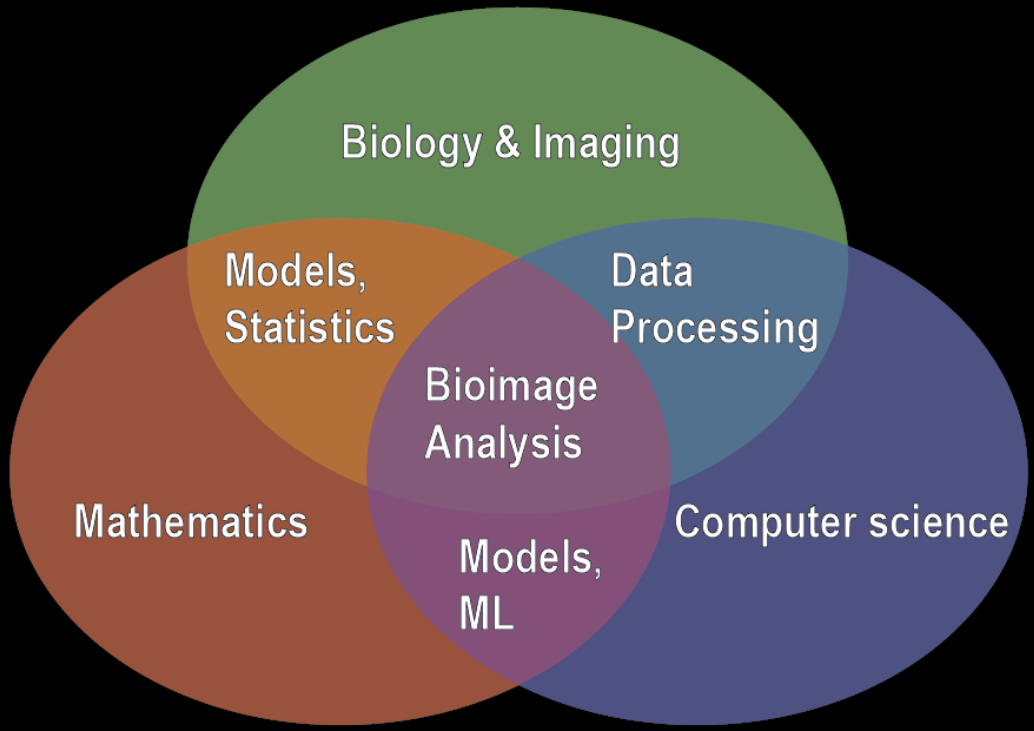
$$\text{Var(Class 1)} = \frac{1}{\text{nbr of pixel in class 1}} \sum_{px\ class1} (\text{intensity(px)} - \text{mean in class1})^2$$

$$\text{Within-Class variance} = \frac{\text{nbr px in class1}}{\text{nbr px in image}} \text{Var(class1)} + \frac{\text{nbr px in class 2}}{\text{nbr px in image}} \text{Var(class2)}$$

Limitations of classical image analysis

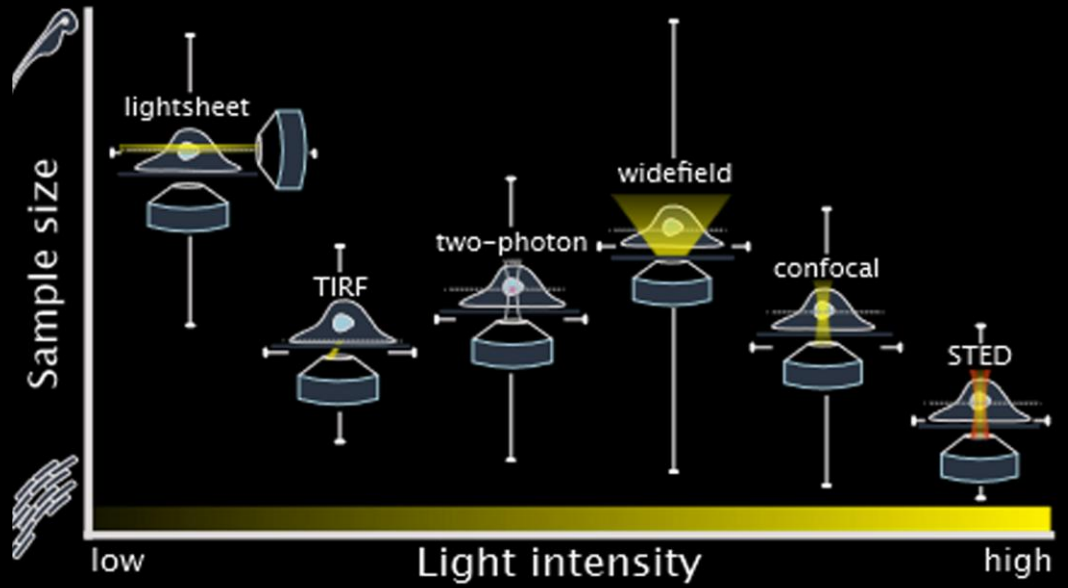
Bioinformatic problem:

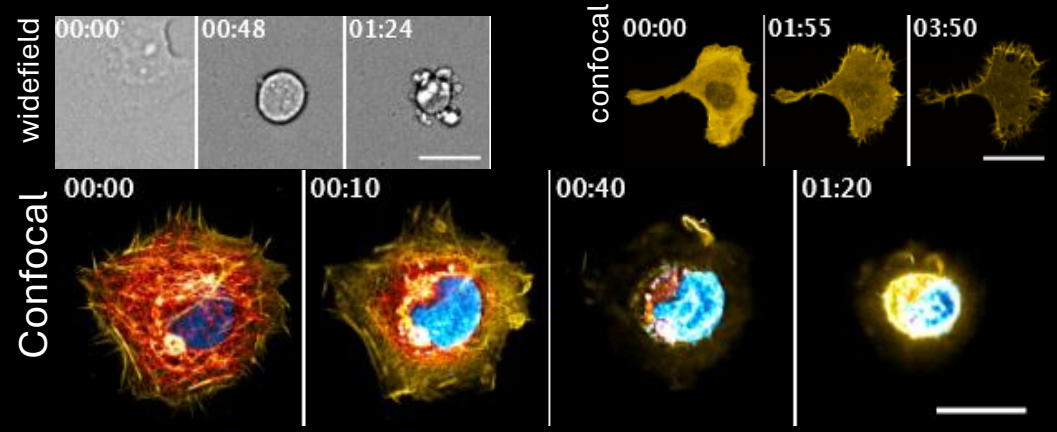
- Computational science and biology have different vocabularies
- Traditional methods need notion in a lot of domains
- Expert in bio-images analysis bridge the gap between domains



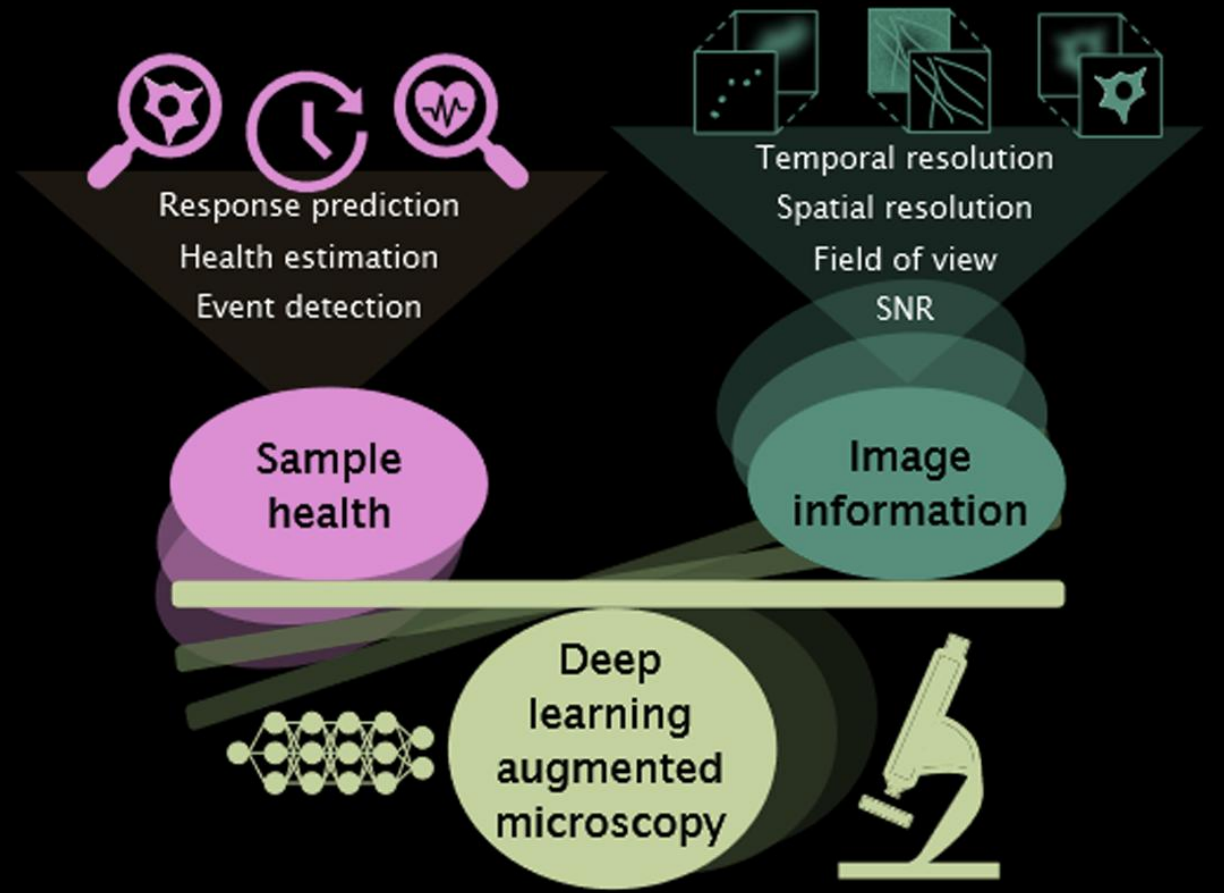
Limitations of Fluorescence Microscopy

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



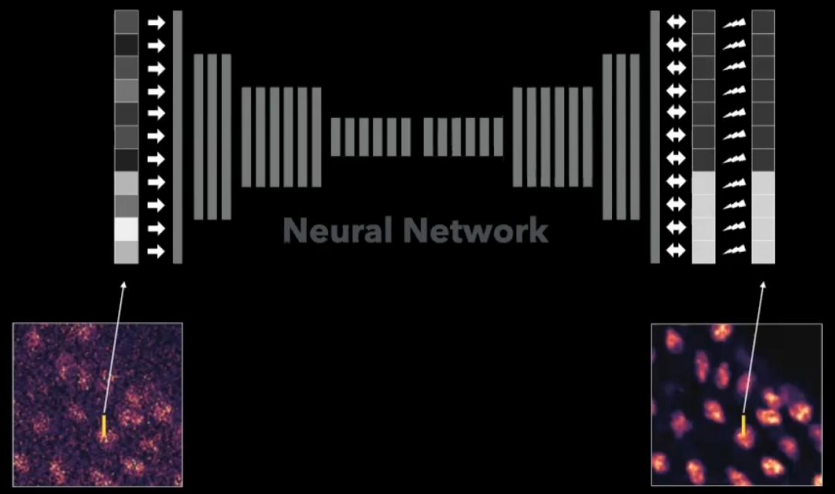


Henriques et al. AI-enabled Live imaging 2023



Henriques et al. AI-enabled Live imaging 2023

Denoising by Deep learning: CARE



Martin Weigert et al. Nature Methods (2018)

Pro

+ One of the best denoising methods

+ Can resolve complicated restoration task

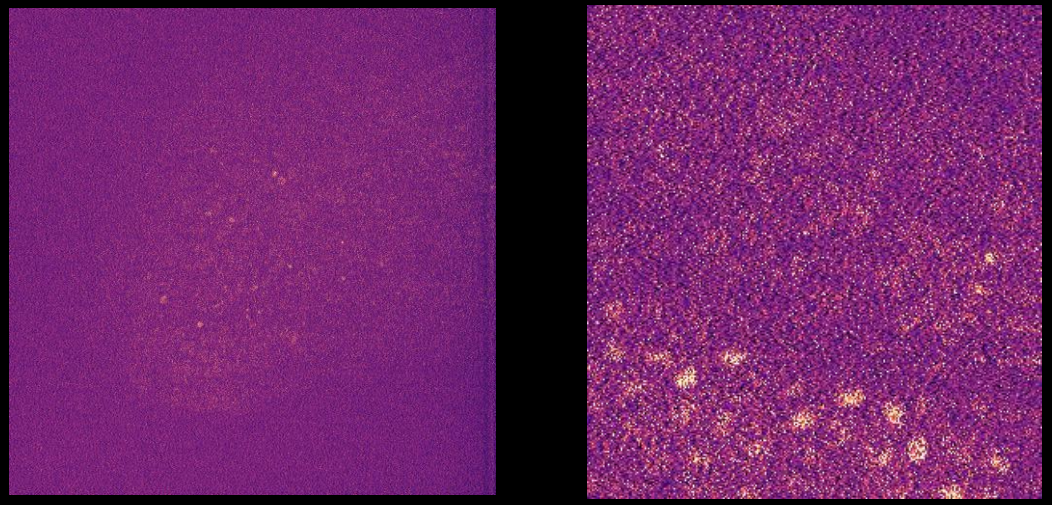
+ Available in the platform with Python script

Con

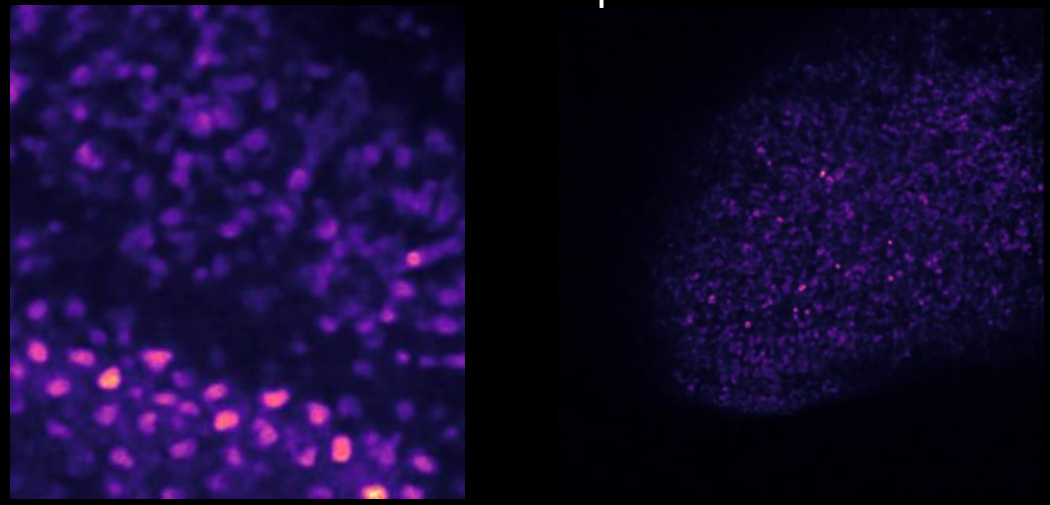
- Need a lot of data for the training more than 200 paired images

- Can be hard to image high quality paired training data (ex: if particles moved significantly between the two acquisitions)

RAW data

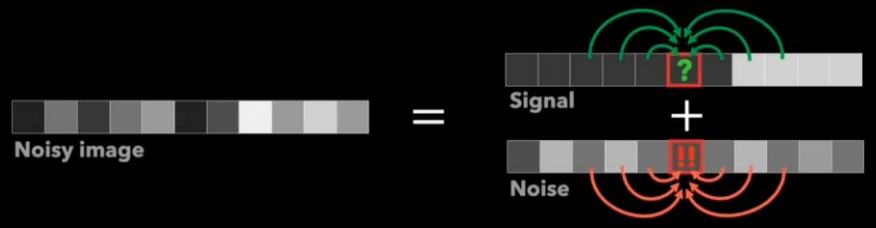


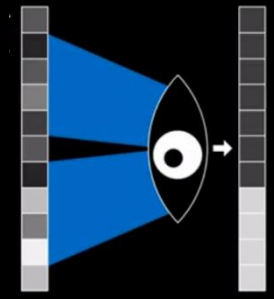
CARE output



Thank to Rink lab, Planaria sample (Schmidtea mediterranea) at 0,5% laser and 10 ms per plane, low SNR and high noise

Denoising by Deep learning : N2V





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

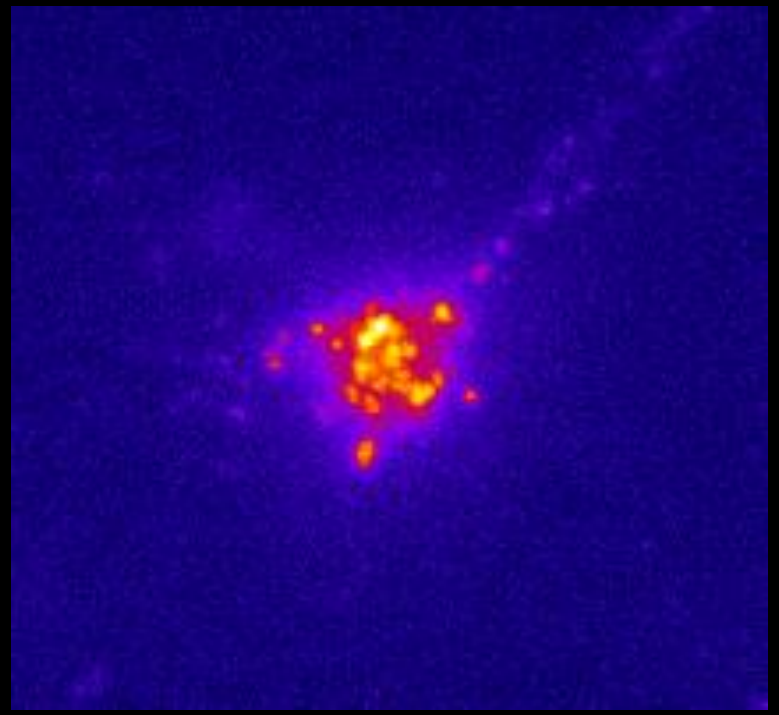
Pro

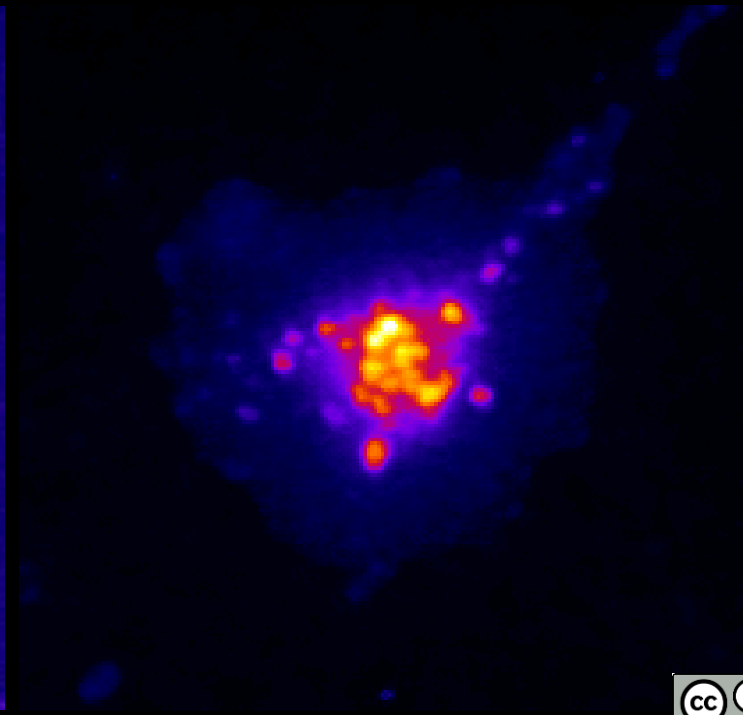
\+ Don’t need a lot of dataset (< 20 images)

\+ 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.

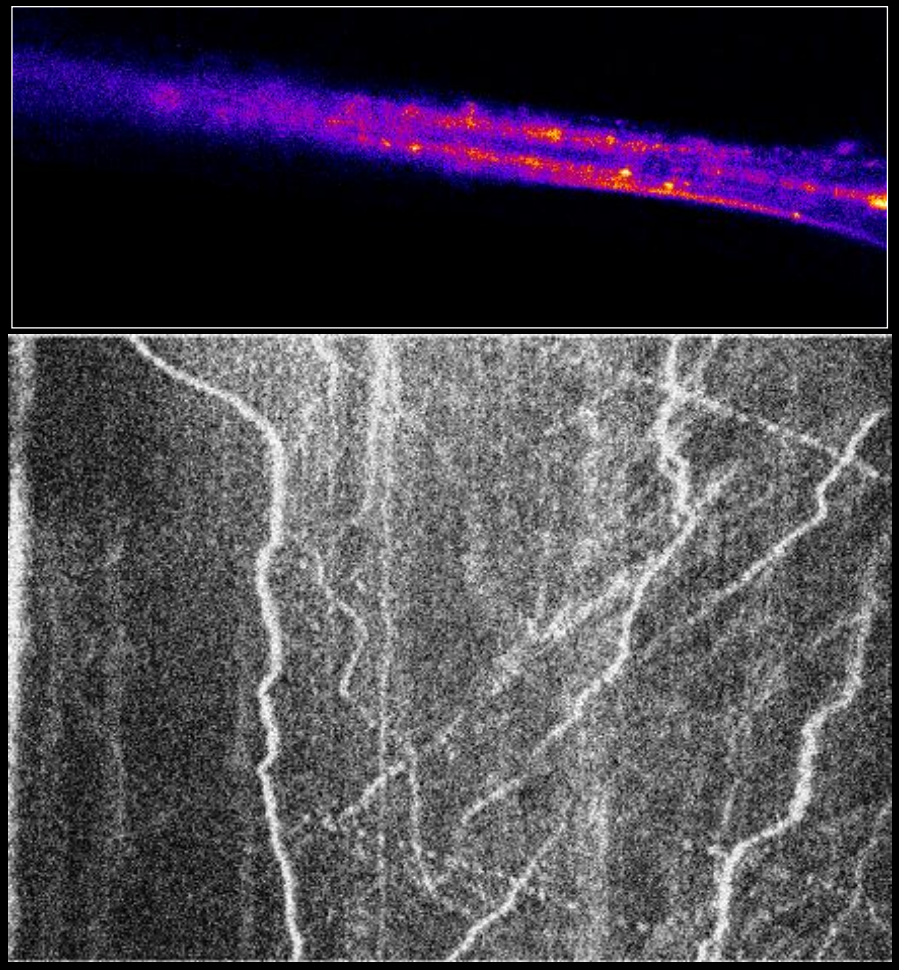




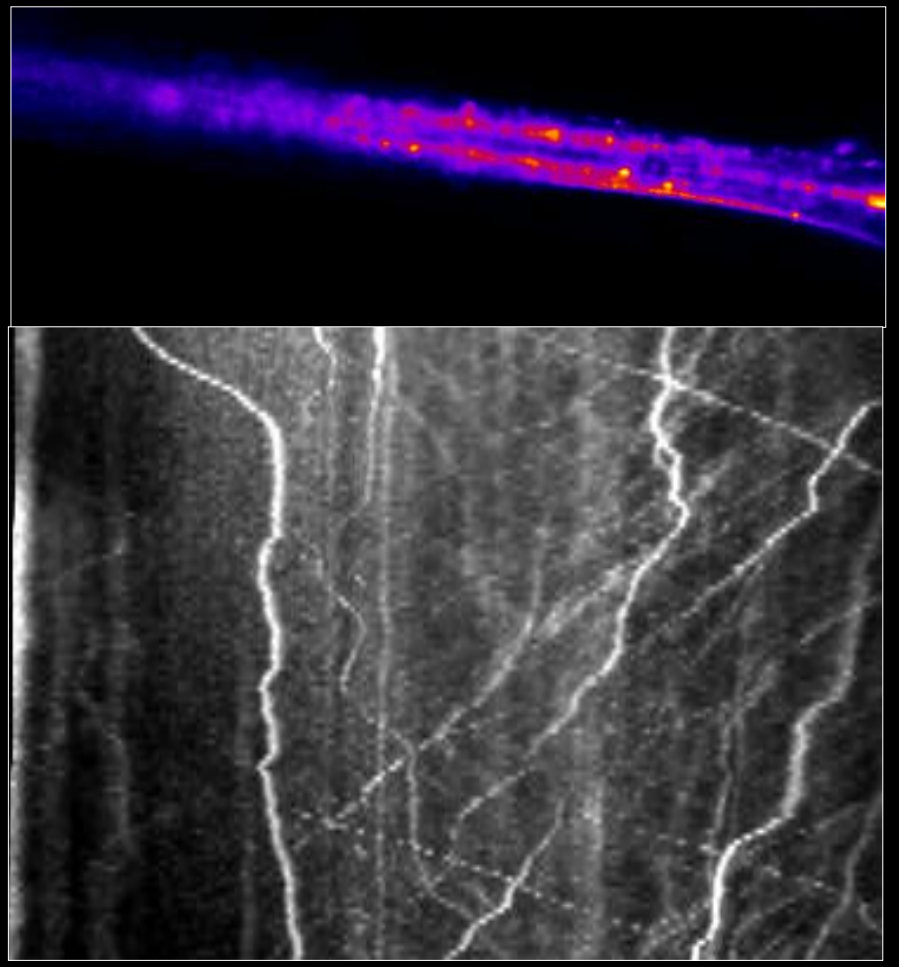
Silvia Turchetto, GIGA Molecular Regulation of neurogenesis

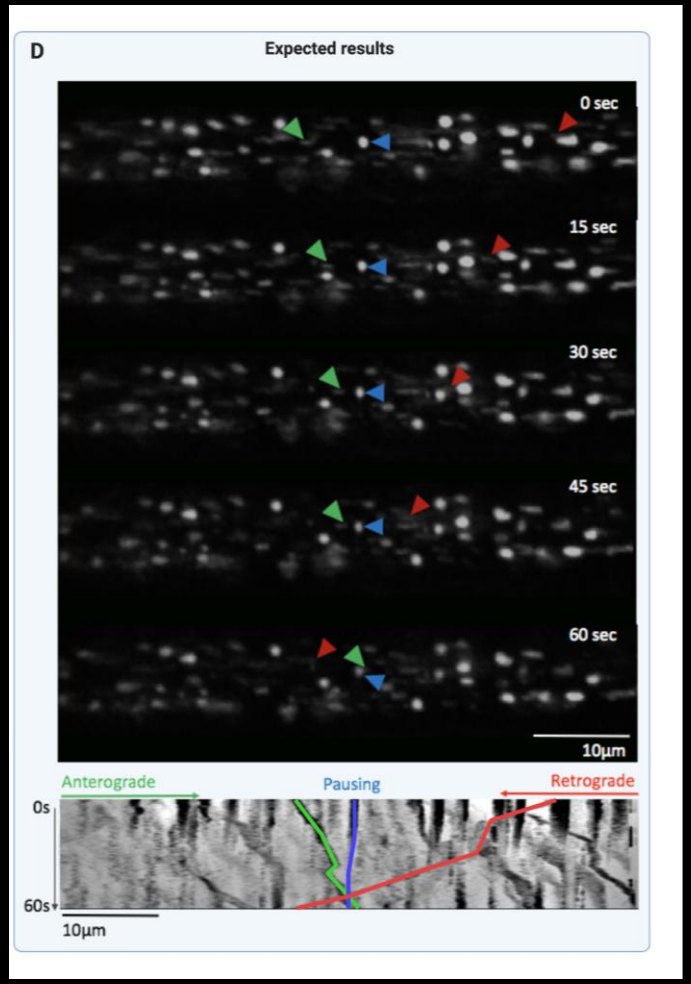
Example desoising for GIGA

Raw data

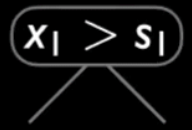


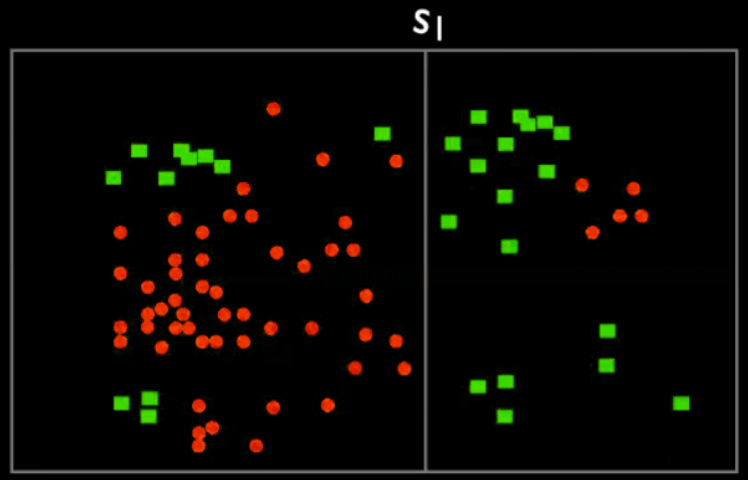
Noise2Void





Machine learning : Decision tree





Pro

+ Simple to interpret

+ Require little data preparation

+ Easy to process data with high dimension

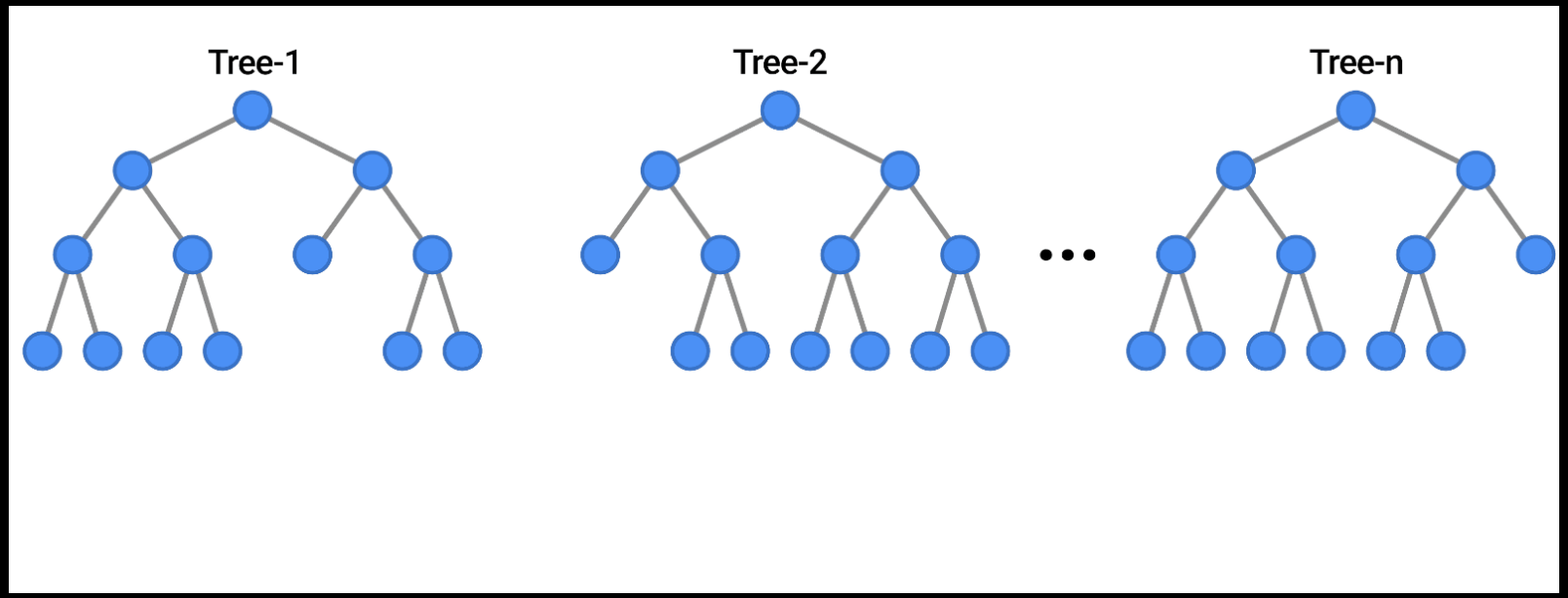
+ Extremely Fast

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

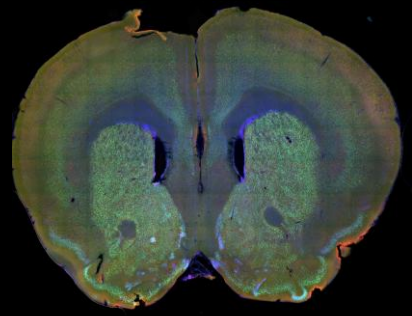
- Slower than one decision tree

- The more trees you have, the slower the process

- The more features you have, the slower the proceclosserl the features / parameters if you don’t need it)

- Difficult to interpret

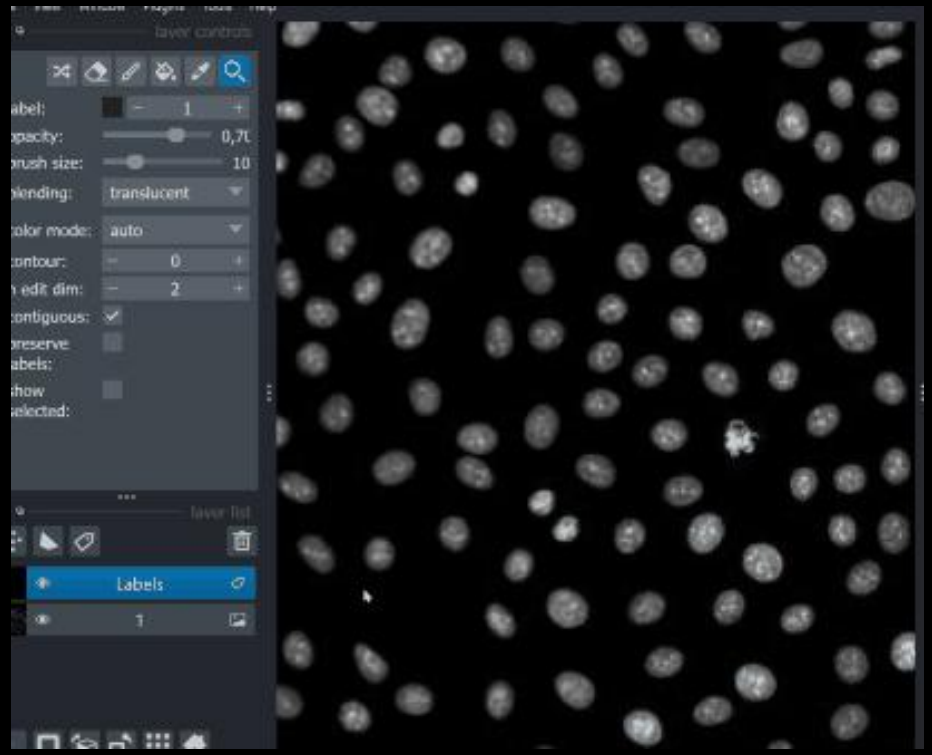
Machine learning ex: Random forest



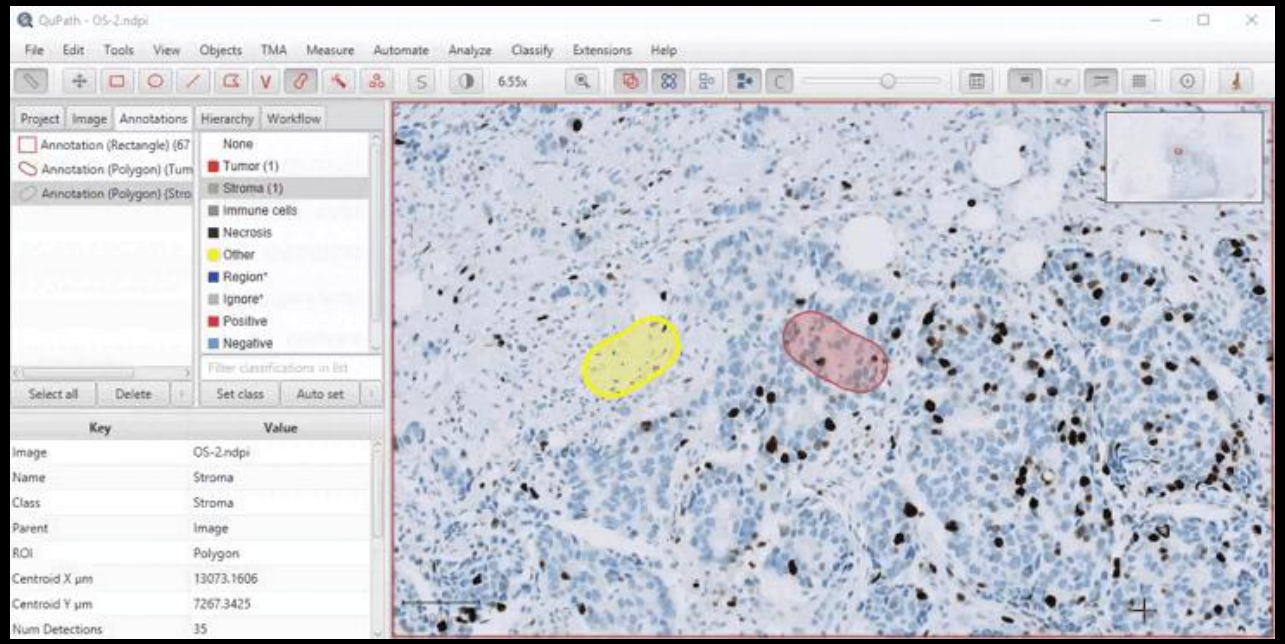
Machine learning algorithms can classify pixels and object to detect specific tissue, cells or staining.

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

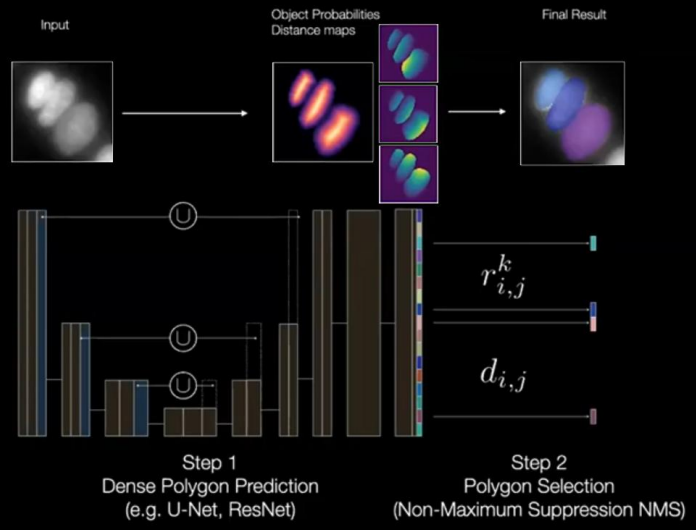
Pixel classifier for cells dectection

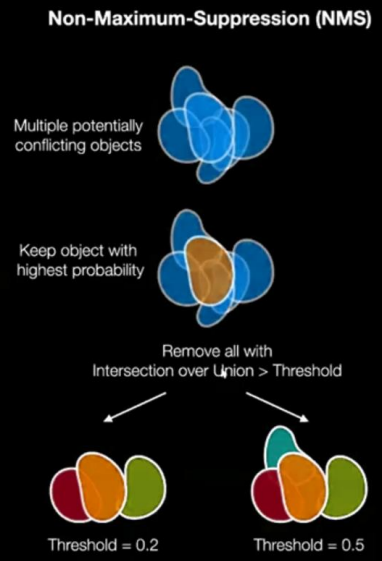


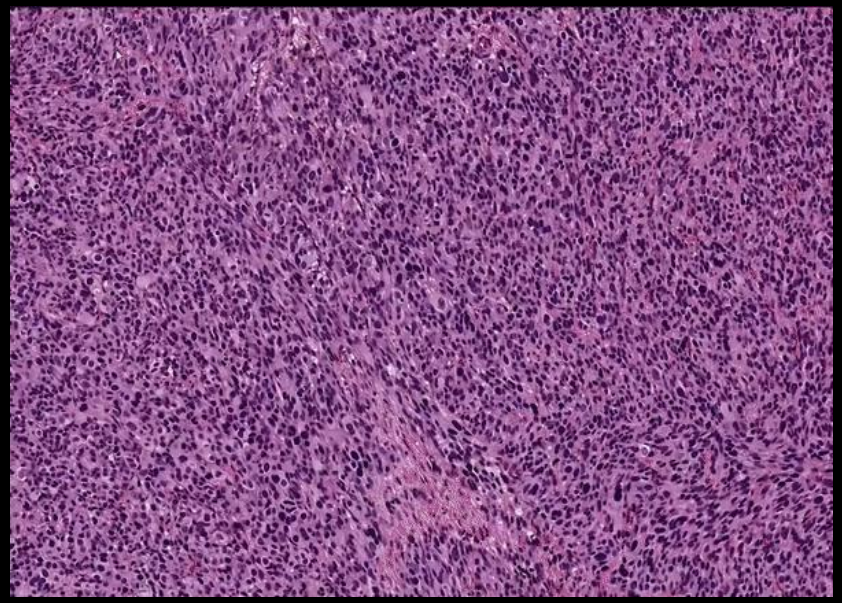
Object classifier for specific objects detection

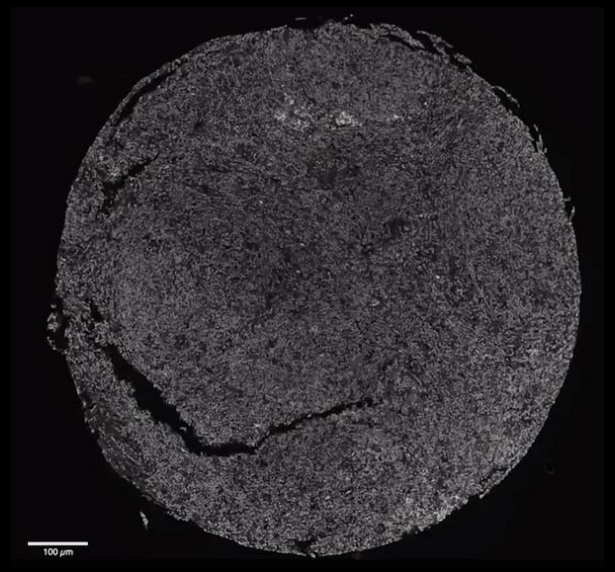


Segmentation by Deep learning: Stardist









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

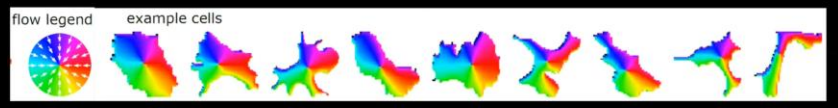
- Need cells with convex shape
- Can't help for the morphology (see cellpose)

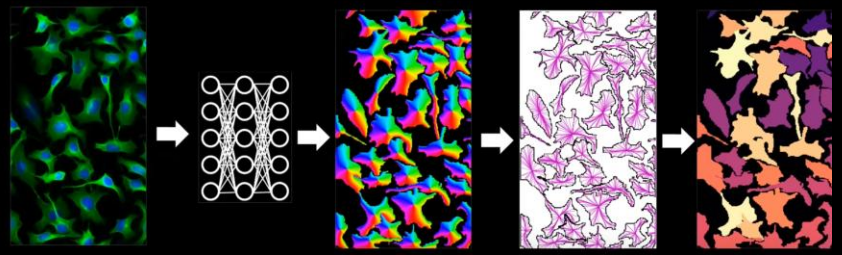
Applications

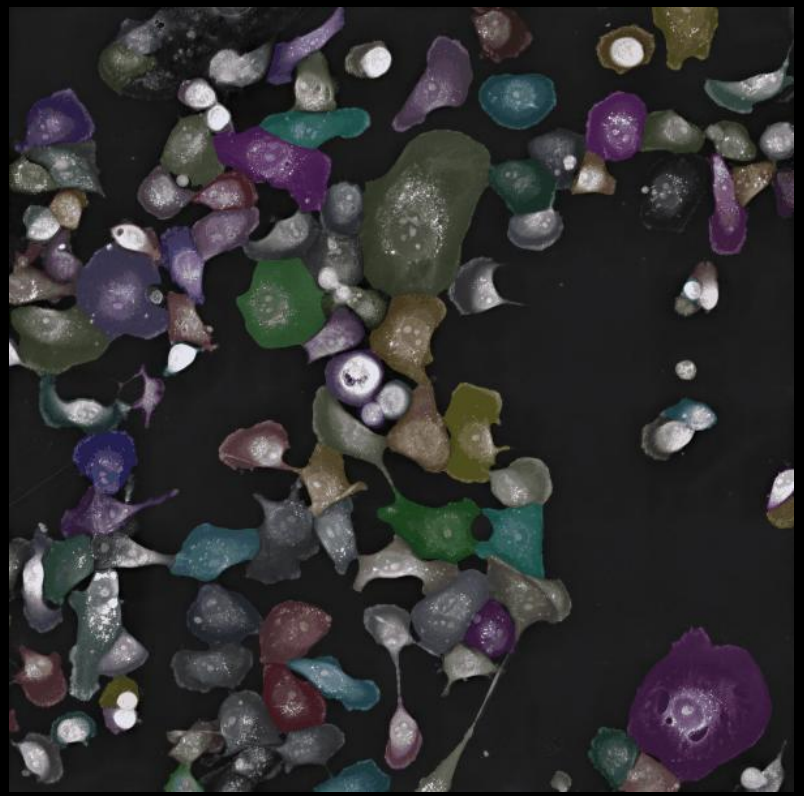
Fluorescence measurements
Counting
Clustering
Spatial omics

Uwe Schmidt et al. computer vision and pattern recognition (2018)

Segmentation by Deep learning: Cellpose







Erik Maquoi, GIGA Cancer

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

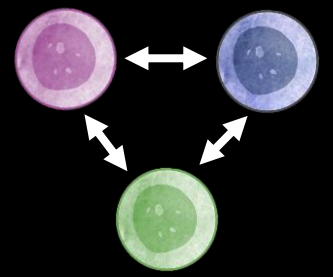
- Very difficult to use on 3D dataset (need a lot of VRAM) because the predictions are computed 3 times in XY, XZ, and YZ directions

Applications

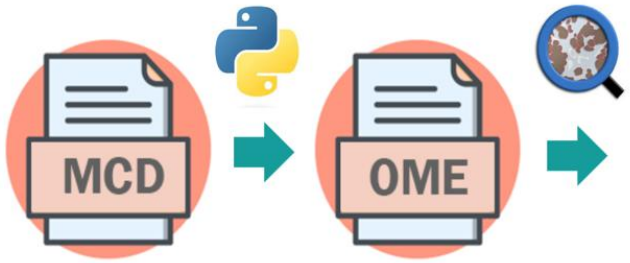
Fluorescence measurements
Counting
Clustering
Spatial omics
Morphology

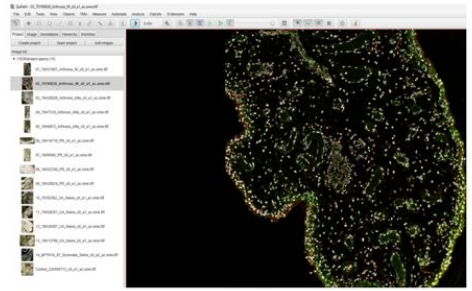
Stringer, C., et al (2021). Cellpose: a generalist algorithm for cellular segmentation. *Nature methods*

Clustering



Multiplexing analysis with more than 40 markers



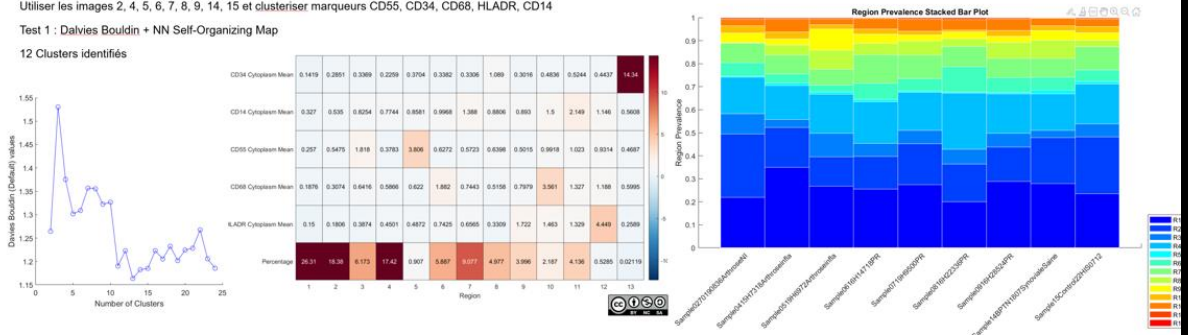


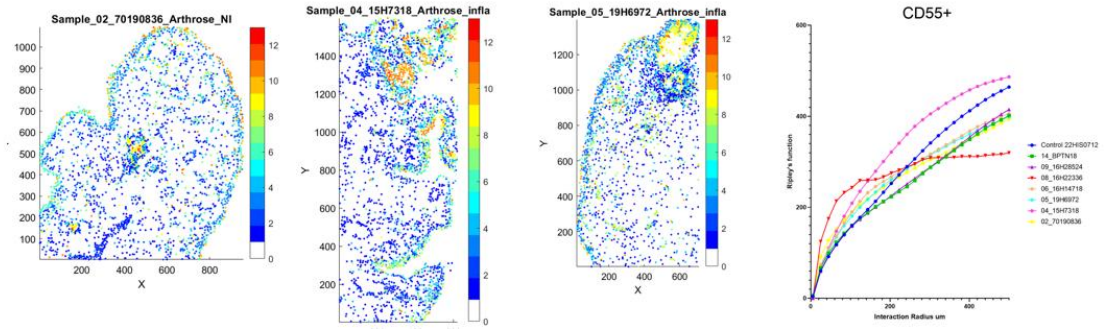
Analyse 3

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

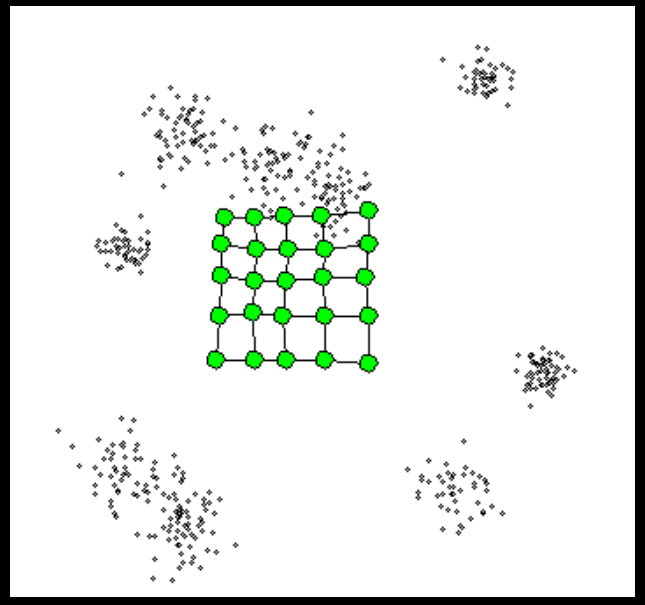
Test 1 : Dalvies Bouldin + NN Self-Organizing Map

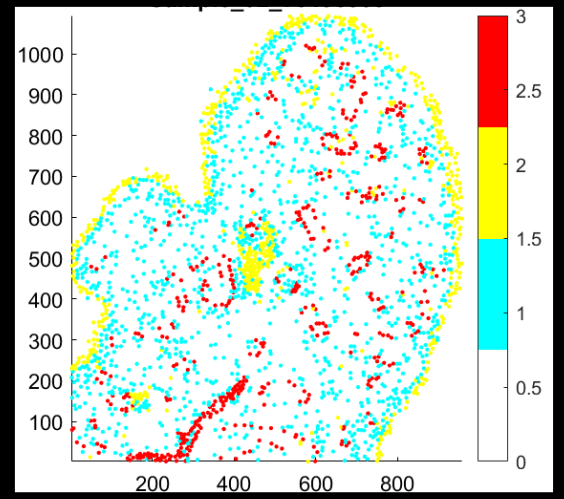
12 Clusters identifiés

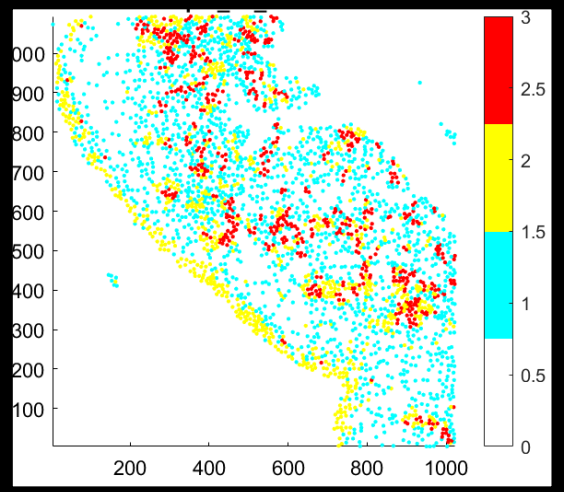




NN Self Organisation MAP







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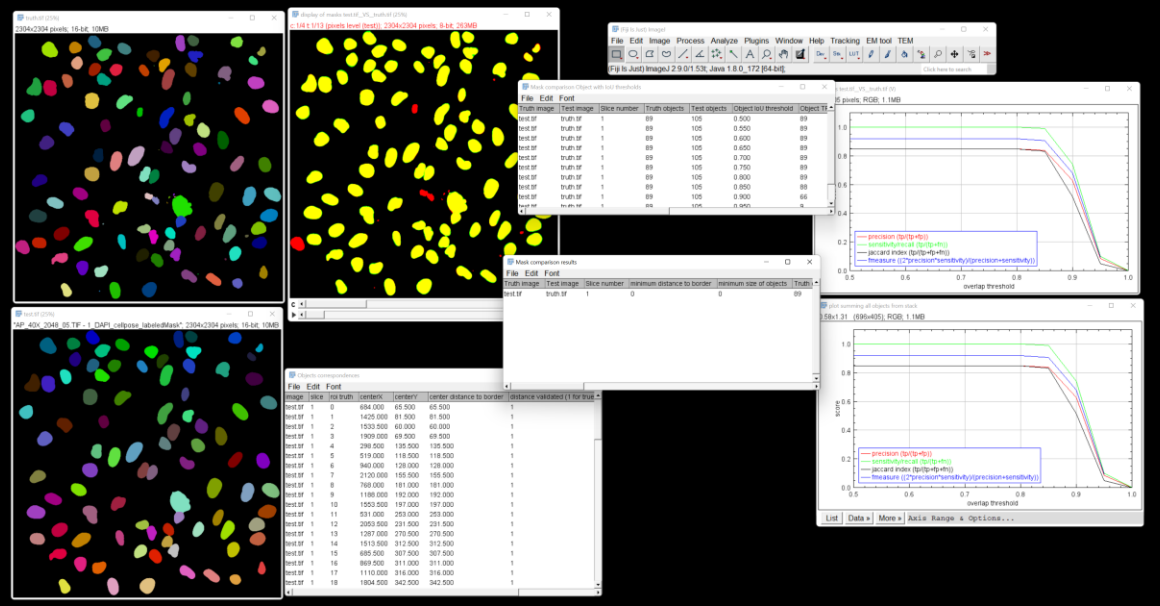
Quality control & Misconduct

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 perecision) 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