

Cellpose 2.0 and TrackMate:

A powerful combination for
effortless and accurate analysis of
live cells in phase and fluorescent
microscopy

Bibliography

I would like to thank Carsen Stringer, her lab, and her collaborators



Stringer C, Wang T, Michaelos M, Pachitariu M. **Cellpose: a generalist algorithm for cellular segmentation**. Nat Methods. 2021 Jan;18(1):100-106. doi: 10.1038/s41592-020-01018-x. Epub 2020 Dec 14. PMID: 33318659.
(<https://www.nature.com/articles/s41592-020-01018-x>)

Pachitariu M, Stringer C. **Cellpose 2.0: how to train your own model**. Nat Methods. 2022 Dec;19(12):1634-1641. doi: 10.1038/s41592-022-01663-4. Epub 2022 Nov 7. PMID: 36344832; PMCID: PMC9718665.
(<https://www.nature.com/articles/s41592-022-01663-4>)

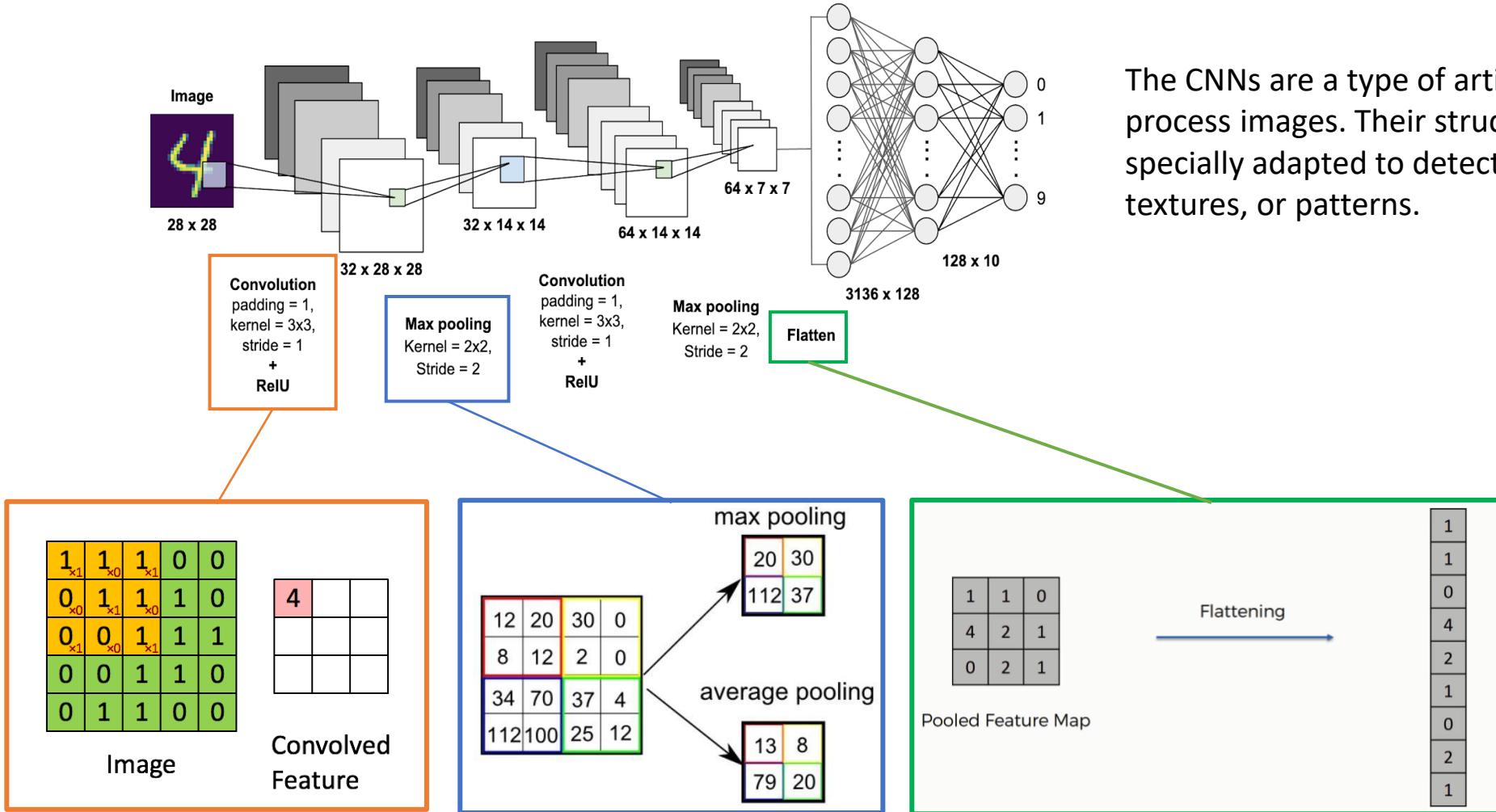
I would like to thank Jean-Yves Tinevez, his lab, and his collaborators



Ershov, D., Phan, M.-S., Pylvänen, J. W., Rigaud, S. U., Le Blanc, L., Charles-Orszag, A., ... Tinevez, J.-Y. (2022). **TrackMate 7: integrating state-of-the-art segmentation algorithms into tracking pipelines**. Nature Methods, 19(7), 829–832. <https://doi.org/10.1038/s41592-022-01507-1> (<https://www.nature.com/articles/s41592-022-01507-1>)

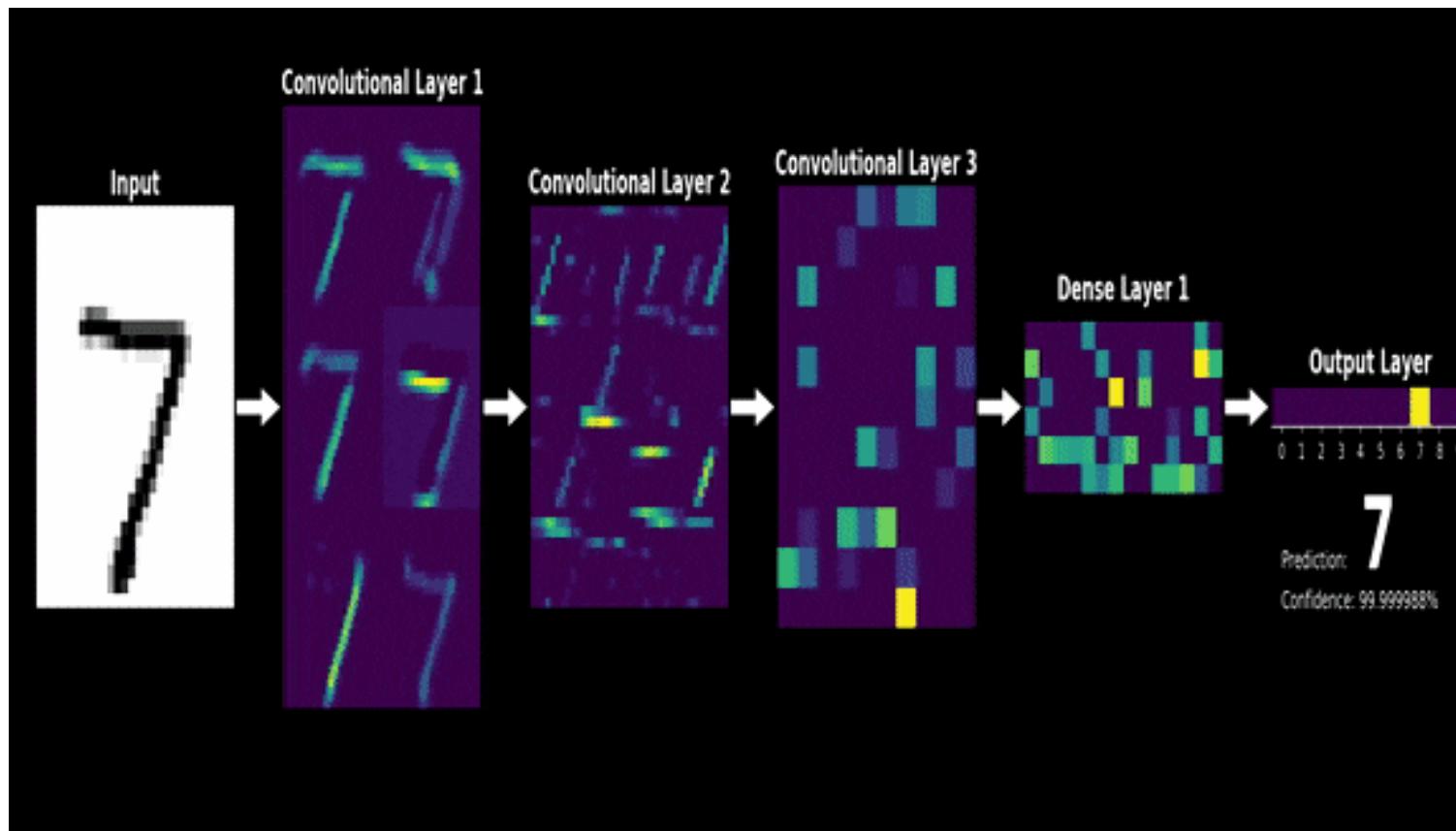
Jean-Yves Tinevez, Nick Perry, Johannes Schindelin, Genevieve M. Hoopes, Gregory D. Reynolds, Emmanuel Laplantine, Sebastian Y. Bednarek, Spencer L. Shorte, Kevin W. Eliceiri, **TrackMate: An open and extensible platform for single-particle tracking**, Methods, Available online 3 October 2016, ISSN 1046-2023,
<http://dx.doi.org/10.1016/j.ymeth.2016.09.016>
(<http://www.sciencedirect.com/science/article/pii/S1046202316303346>)

CNN : Convolutional neural network



The CNNs are a type of artificial neural network designed to process images. Their structure consists of layers of neurons specially adapted to detect visual features, such as edges, textures, or patterns.

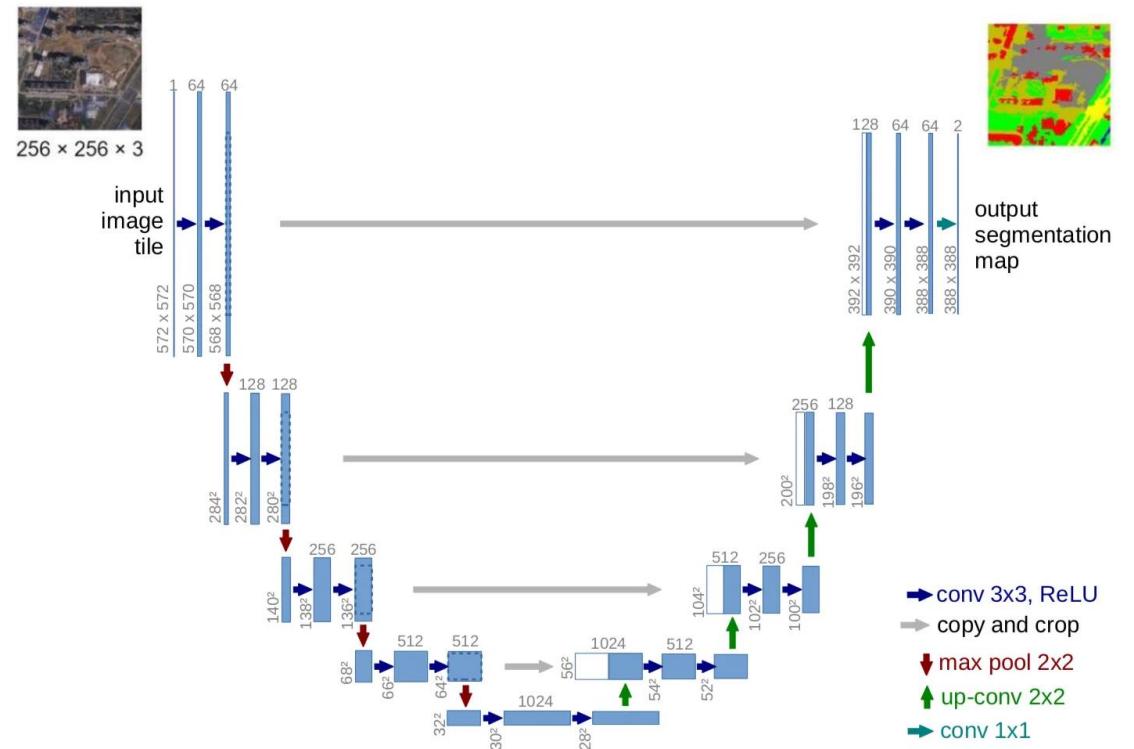
CNN : Convolutional neural network



U-net

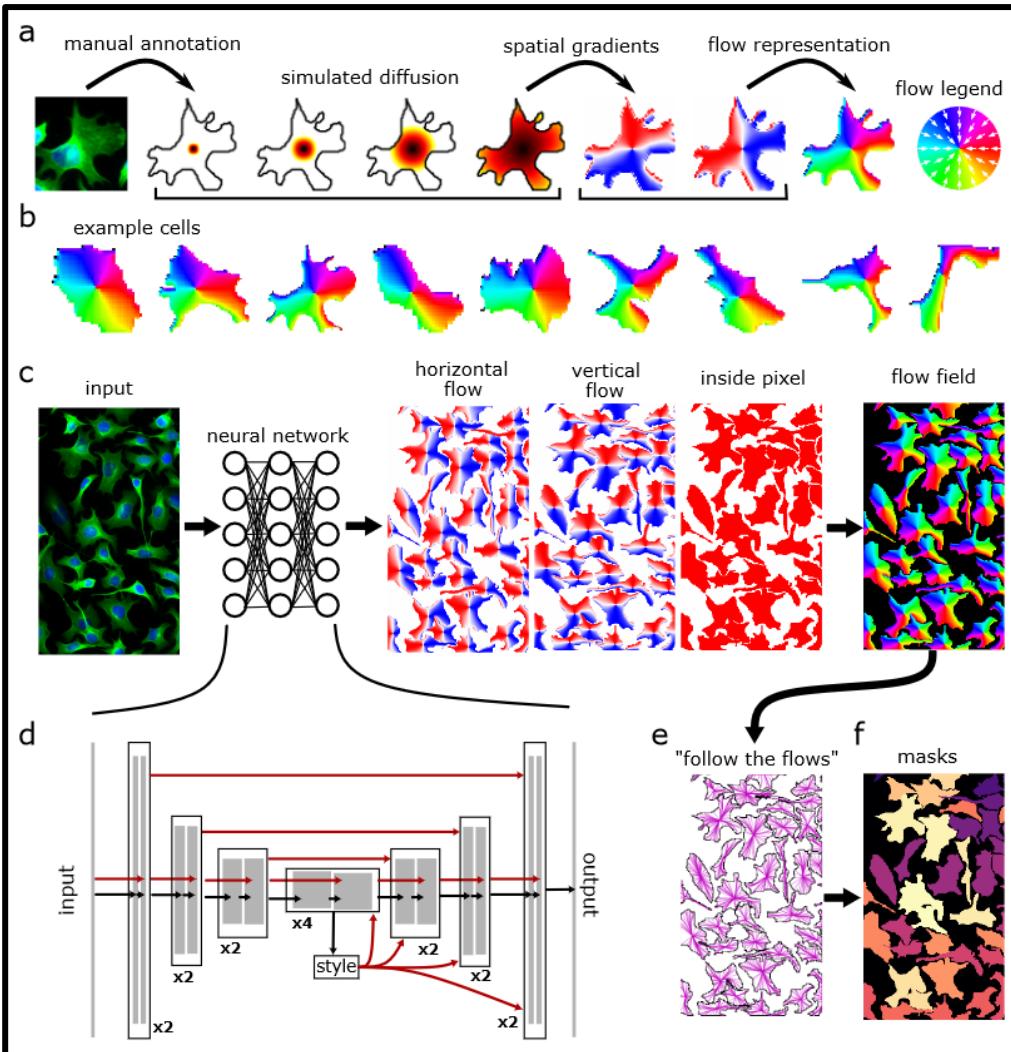
The U-Net is a particular type of CNN (Convolutional Neural Network) known for its ability to segment complex structures while preserving details.

Its name comes from its U-shaped architecture, which resembles the letter “U”.



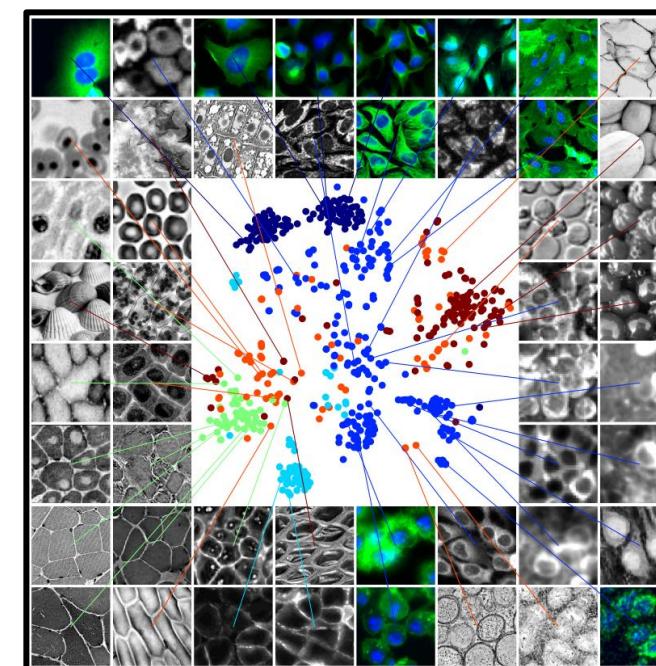
Ronneberger, O., Fischer, P., Brox, T. (2015). U-Net: Convolutional Networks for Biomedical Image Segmentation. In: Navab, N., Hornegger, J., Wells, W., Frangi, A. (eds) Medical Image Computing and Computer-Assisted Intervention – MICCAI 2015. MICCAI 2015. Lecture Notes in Computer Science(), vol 9351. Springer, Cham. https://doi.org/10.1007/978-3-319-24574-4_28

Cellpose



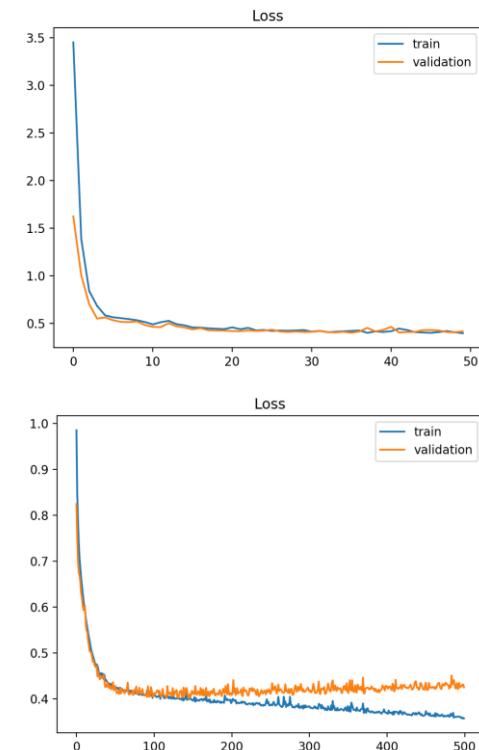
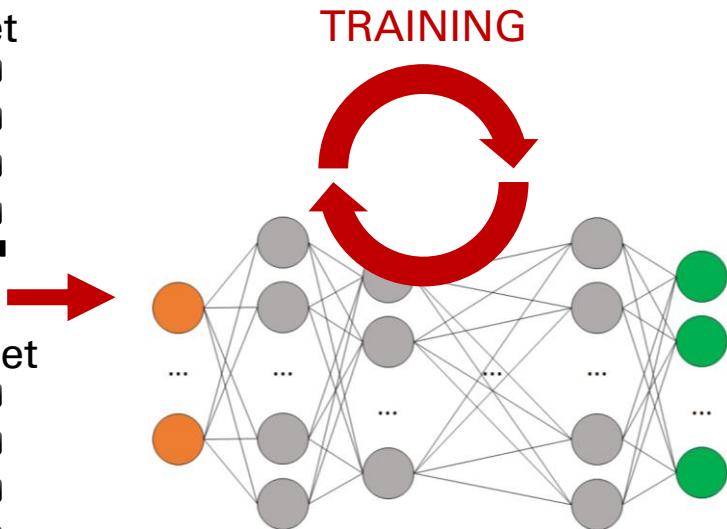
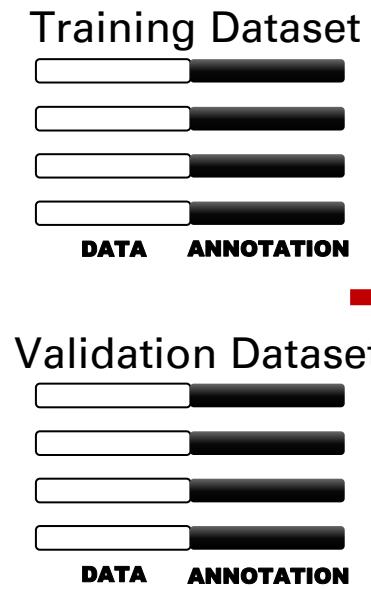
Cellpose is a deep learning method dedicated to the instance segmentation of biomedical images.

Based on a U-Net network, Cellpose includes the estimation of horizontal and vertical gradients to identify pixel membership to an object

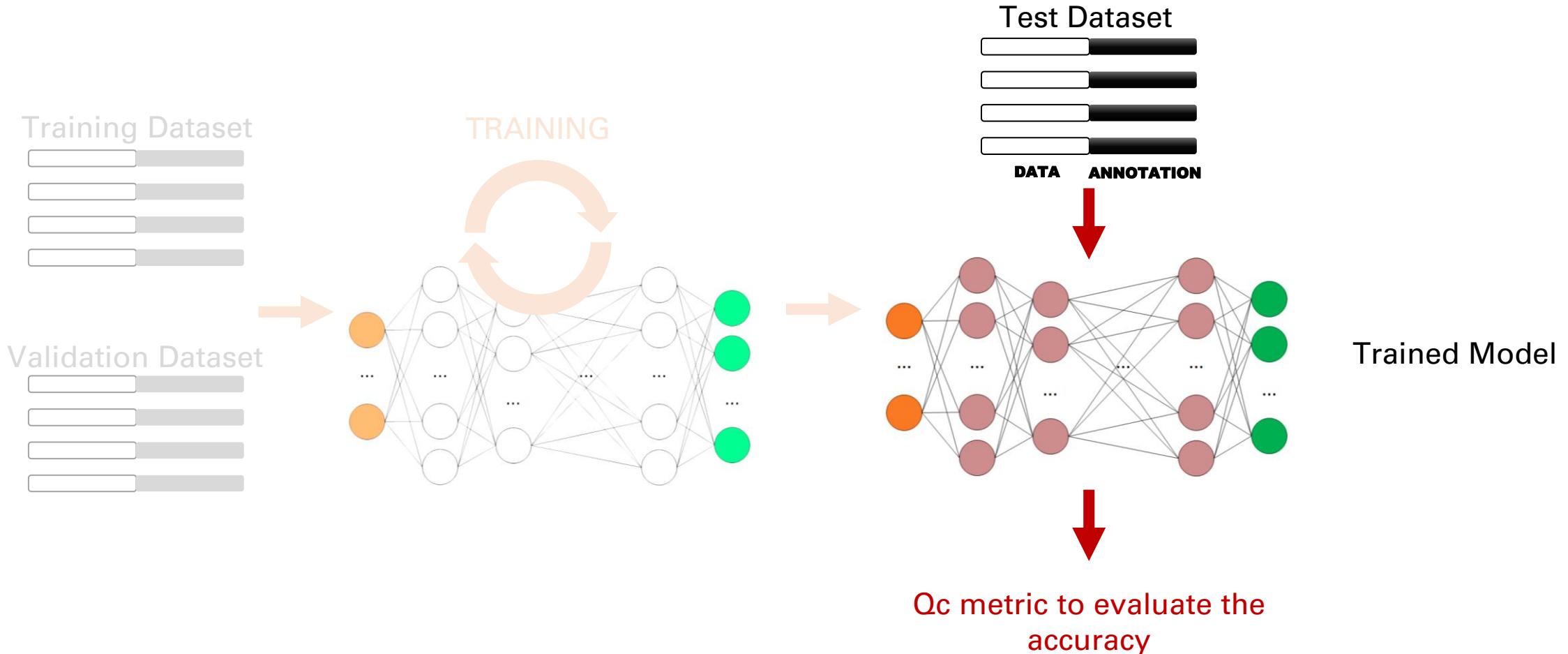


Cellpose has been trained on a large dataset consisting of a wide variety of cell images

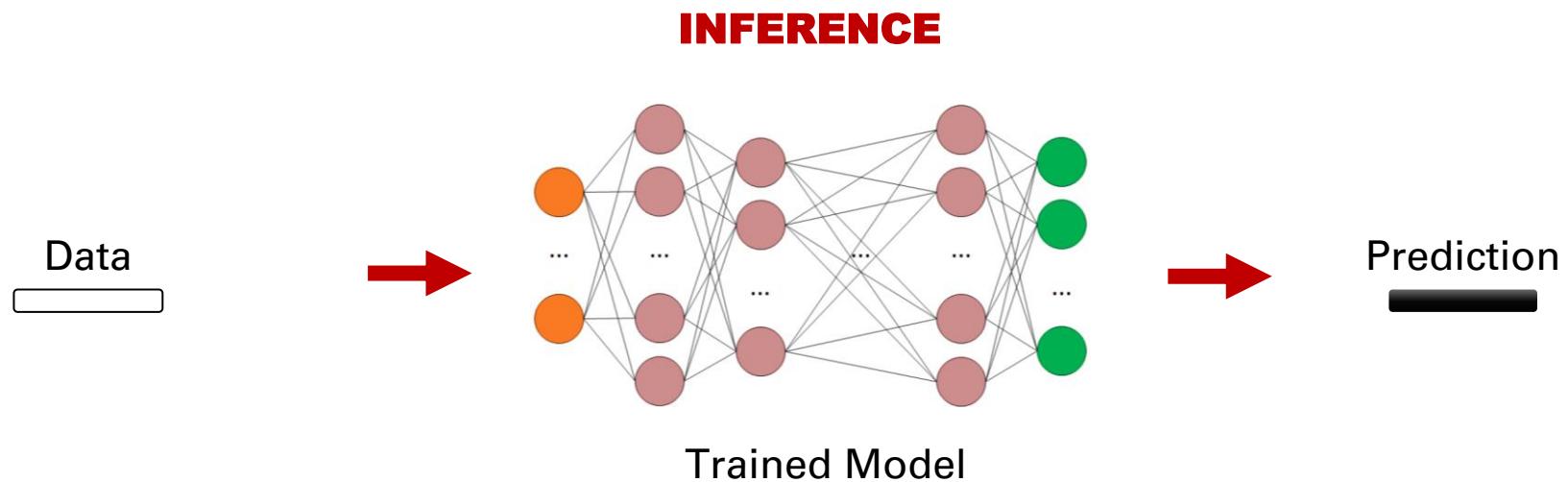
Training neural network



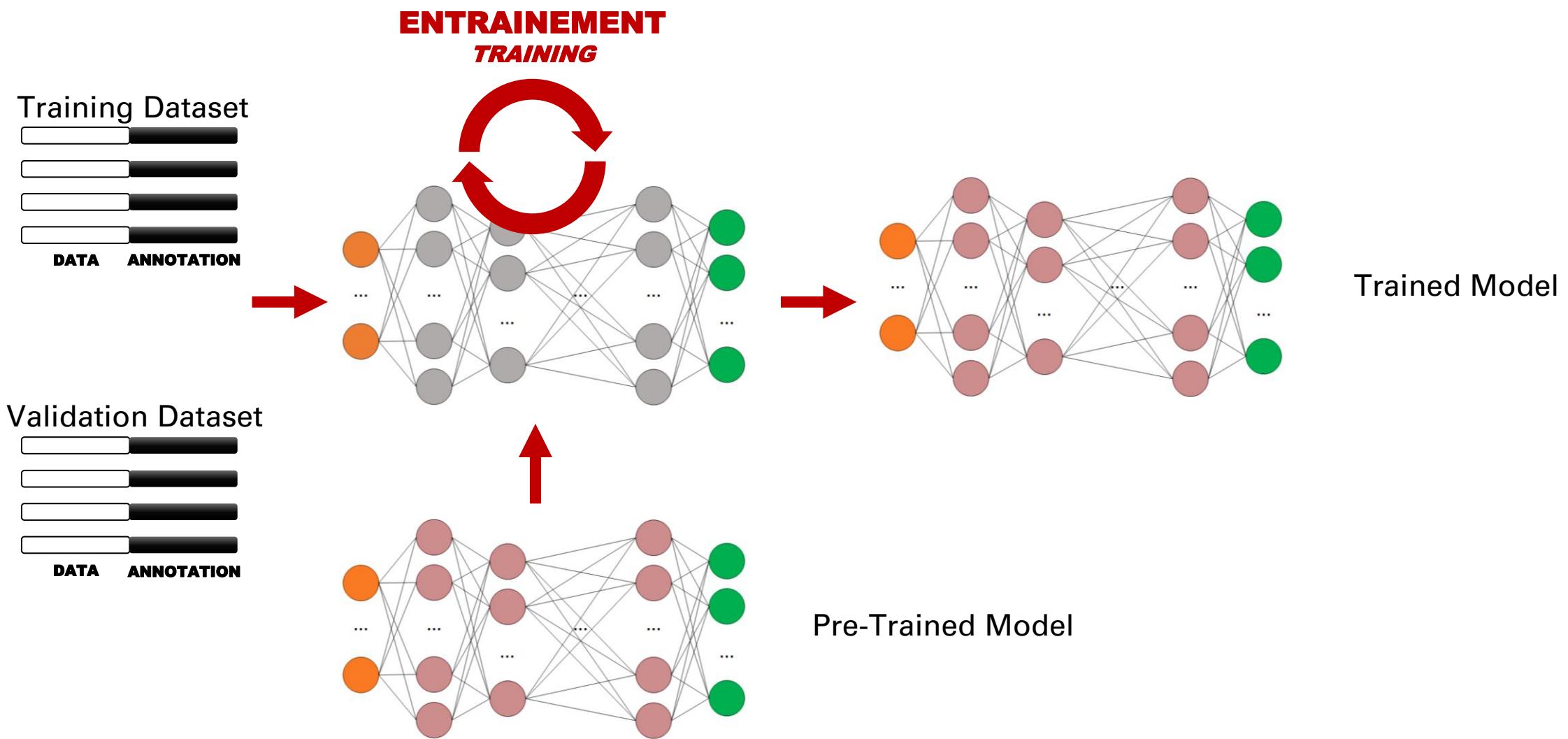
Evaluation of the model



How do we use the model



What is transfer learning?



Cellpose installation in french (GT MAIIA)

<https://forgemia.inra.fr/gt-maiia/kit-du-debutant/-/wikis/Home>

The screenshot shows the GitHub repository page for 'Cellpose' on the 'gt-maiia' wiki. The repository has 13 stars and 1 branch. The README file contains instructions for Windows and Linux installations, mentioning Anaconda3 and Miniconda3. It also includes sections for prerequisites, usage, and documentation. A 'Clone repository' button is visible.

Installation Cellpose via Anaconda3 - Alex & Nicolas

Téléchargement d'Anaconda3

Vous le trouverez à cette adresse : <https://www.anaconda.com/download#downloads>

Installation d'Anaconda3

1. Installation Documentation
2. Installation Anaconda dans : C:\Users\ votre_nom_d_utilisateur\anaconda3
3. Lors de l'installation : cocher la case "add anaconda/miniconda to my PATH environment"

Miniconda3 py39_4.10.3 (64-bit) Setup

ANACONDA Customize how Anaconda integrates with Windows

Advanced Options

- Add Miniconda3 to my PATH environment variable
- Register Miniconda3 as my default Python 3.9

Anaconda, Inc. < Back Install Cancel

GUI - Alex

Ouvrir Cellpose avec anaconda ou le raccourci que l'on a créé lors de l'installation de Cellpose sur Anaconda

1. Principaux contrôles de l'interface utilisateur graphique

- Déplacer = clic gauche + glisser
- Zoom = molette de défilement (ou +/- et boutons -)
- Vue complète = double clic gauche
- Sélectionner un masque = clic gauche sur le masque
- Supprimer un masque = Ctrl (ou Commande sur Mac) + clic gauche
- Fusionner les masques = Alt + clic gauche (fusionne les deux derniers)
- Commencer à dessiner un masque = clic droit
- Terminer le dessin du masque = clic droit, ou revenir au cercle initial
- Sauvegarder les annotations = ctrl + S

Les chevauchements de masques ne sont PAS autorisés. Si vous dessinez un masque par-dessus un autre masque, il sera coupé de manière à ne pas se chevaucher avec l'ancien masque. Les masques en 2D doivent être des traits simples (si "single_stroke" est activé).

2. Utilisation d'un modèle déjà entraîné

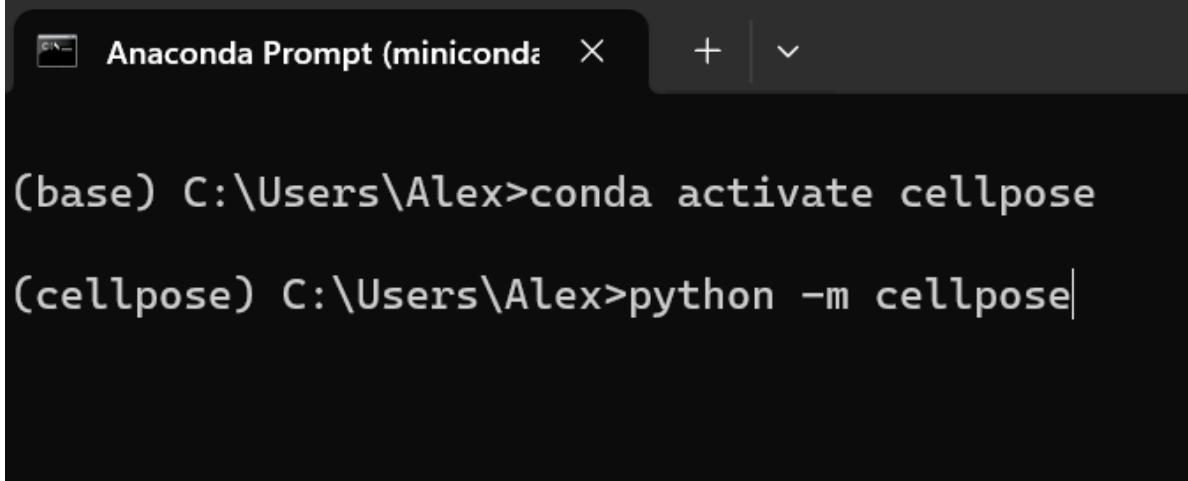
- Glisser-déposer vos images (.tif, .png, .jpg, .gif) dans l'interface de Cellpose (GUI)
- TAILLE : vous pouvez entrer manuellement le diamètre approximatif de vos cellules, ou appuyer sur "calibrer" pour laisser le modèle l'estimer. La taille est représentée par un disque en bas de la fenêtre d'affichage (vous pouvez désactiver ce disque en décochant "activer l'échelle du disque").
- GPU : activez la carte graphique pour gagner du temps.
- MODÈLE : il existe un modèle de cytoplasme et un modèle de noyaux, choisissez ce que vous souhaitez segmenter.
- CHAN À SE6 : il s'agit du canal dans lequel se trouvent le cytoplasme ou les noyaux.
- CHAN2 (OPT) : si le modèle de cytoplasme est choisi, choisissez ensuite le canal nucléaire pour cette option.
- run segmentation : pour lancer la prédiction

The GUI interface shows a heatmap of cell segmentation results. The top right panel displays a grid of heatmaps with various color overlays. The bottom right panel shows a zoomed-in view of a single cell with a red circle highlighting its boundary. A scale bar labeled "scale disk of size 'cell diameter'" is visible. The left side of the interface contains various control buttons and sliders, including "logistic RGB and color to help segment multi-channel images", "ROI drawing auto-ends when selecting ROI", "To show the segmentation", "Calibrate the ROI diameter with the 'cyto' model or enter it manually", "nuclear channel for cyto (CP) and nucleoplasm (NP) models", "Run a model from the dropdown menu", "Run a custom model, add or train a custom model from file/drop menu", and "Image saturation controls".

Your feedback is welcome

Start Cellpose

You can start the GUI with conda or the shortcut on the Desktop



```
Anaconda Prompt (miniconda) + | ▾  
(base) C:\Users\Alex>conda activate cellpose  
(cellpose) C:\Users\Alex>python -m cellpose|
```

Cellpose GUI controls

Pan = left-click + drag

Zoom = scroll wheel (or +/- and - buttons)

Full view = double left-click

Select mask = left-click on mask

Delete mask = Ctrl (or Command on Mac) + left-click

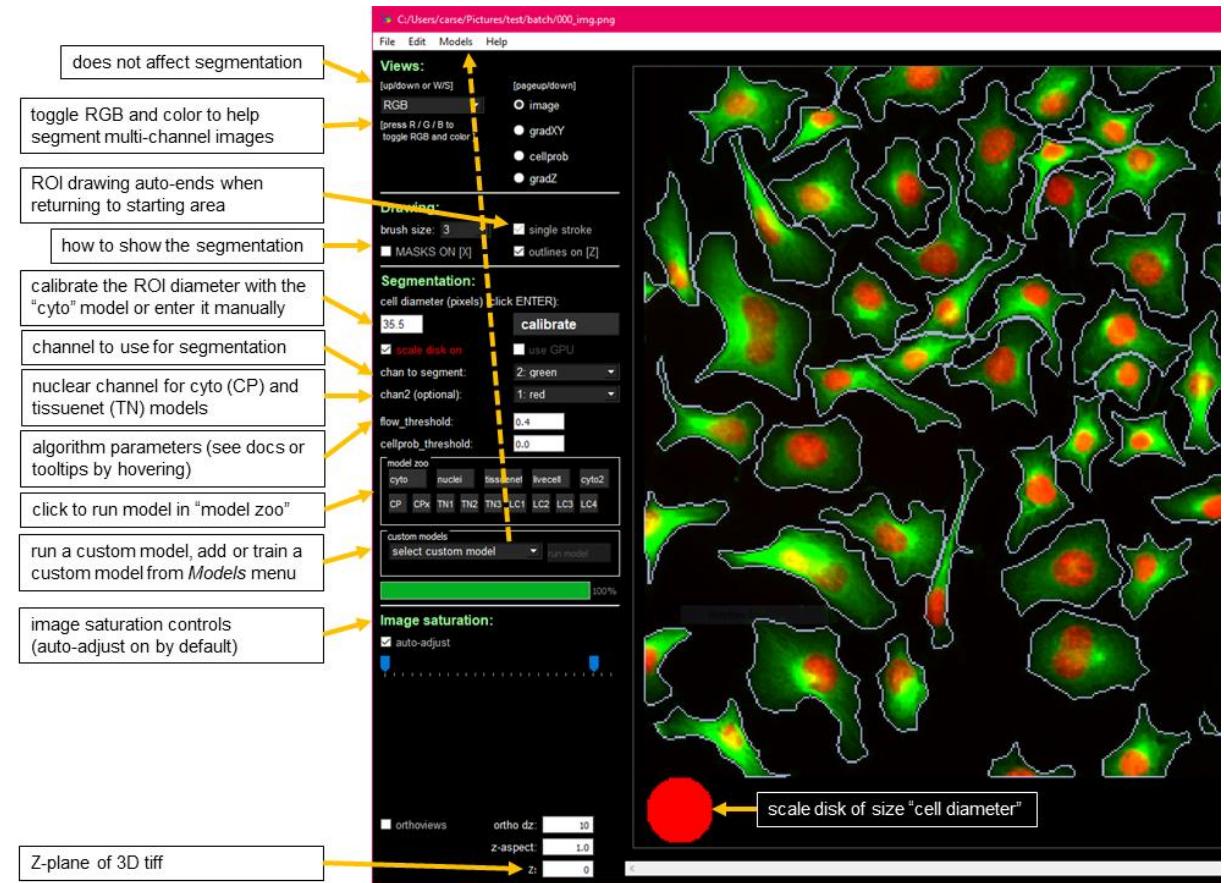
Merge masks = Alt + left-click (will merge last two)

Start draw mask = right-click

End draw mask = right-click, or return to circle at beginning

Save mask = Ctrl + S

Note: Overlaps in masks are NOT allowed. If you draw a mask on top of another mask, it is cropped so that it doesn't overlap with the old mask. Masks in 2D should be single strokes (if single_stroke is checked).



Cellpose prediction with pretrained model

The screenshot shows the Cellpose segmentation interface with several input fields and options:

- Segmentation:** use GPU
- cell diameter (pixels) (click ENTER):** 50 **calibrate**
- chan to segment:** 0: gray
- chan2 (optional):** 0: none
- flow_threshold:** 0.4
- cellprob_threshold:** 0.0
- stitch_threshold:** 0.0
- model zoo**: cyto, nuclei, tissue, livecel, cyto2, CF, TN, LC
- compute style and run suggested model**
- custom models**: model_mifobio **run model**

Options:

- flow_threshold : maximum allowed error for each mask (default = 0,4)
- Cellprop_threshold : decrease this value if cellpose isn't returning enough ROI (default = 0)
- Stitch_threshold : if > 0 masks are stitched in 3D to return volume segmentation

Select the model for the prediction in model zoo or « run suggested model »

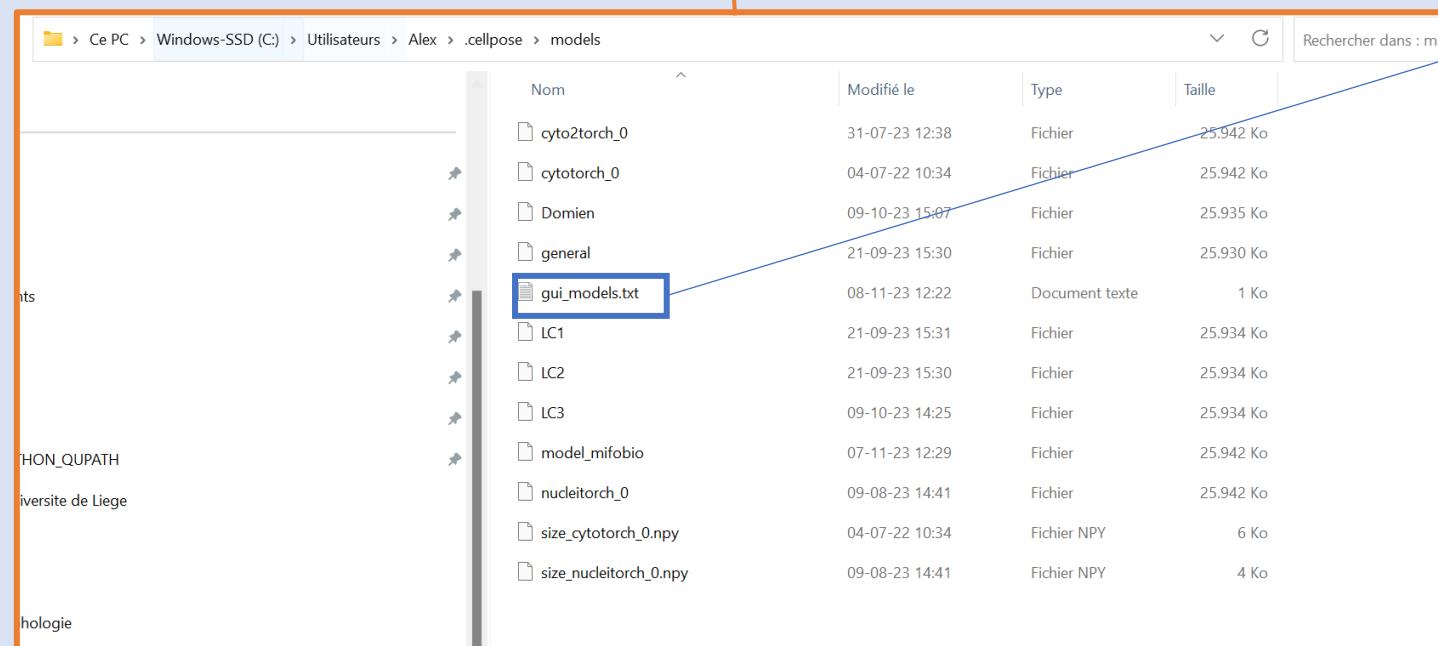
100% **82 ROIs**

Cellpose model that was shared by a coworker

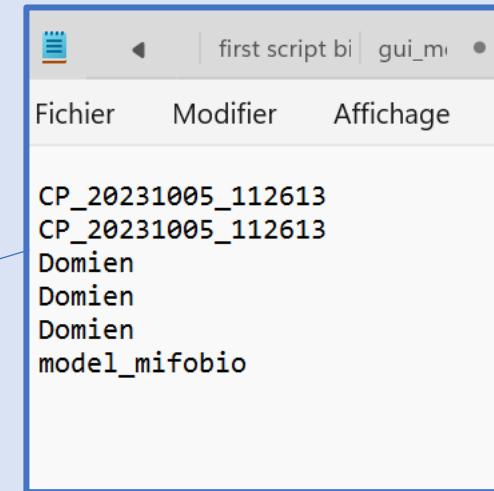
For me the path is

C:\\Utilisateurs\\Alex\\.cellpose\\models

Edit the file `gui_models.txt` and add the name of the model



Nom	Modifié le	Type	Taille
cyt2torch_0	31-07-23 12:38	Fichier	25.942 Ko
cytotorch_0	04-07-22 10:34	Fichier	25.942 Ko
Domien	09-10-23 15:07	Fichier	25.935 Ko
general	21-09-23 15:30	Fichier	25.930 Ko
gui_models.txt	08-11-23 12:22	Document texte	1 Ko
LC1	21-09-23 15:31	Fichier	25.934 Ko
LC2	21-09-23 15:30	Fichier	25.934 Ko
LC3	09-10-23 14:25	Fichier	25.934 Ko
model_mifobio	07-11-23 12:29	Fichier	25.942 Ko
nucleitorch_0	09-08-23 14:41	Fichier	25.942 Ko
size_cytotorch_0.npy	04-07-22 10:34	Fichier NPY	6 Ko
size_nucleitorch_0.npy	09-08-23 14:41	Fichier NPY	4 Ko

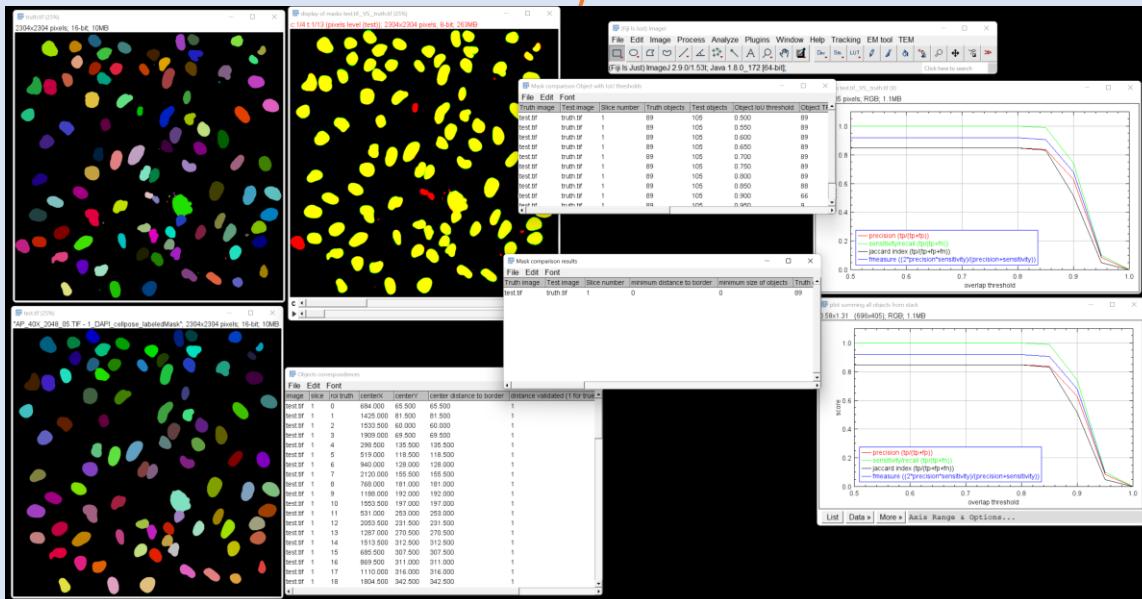


```
CP_20231005_112613
CP_20231005_112613
Domien
Domien
Domien
model_mifobio
```

Cellpose model evaluation

Quality control:

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



<https://github.com/MultimodalImagingCenter/MiC>

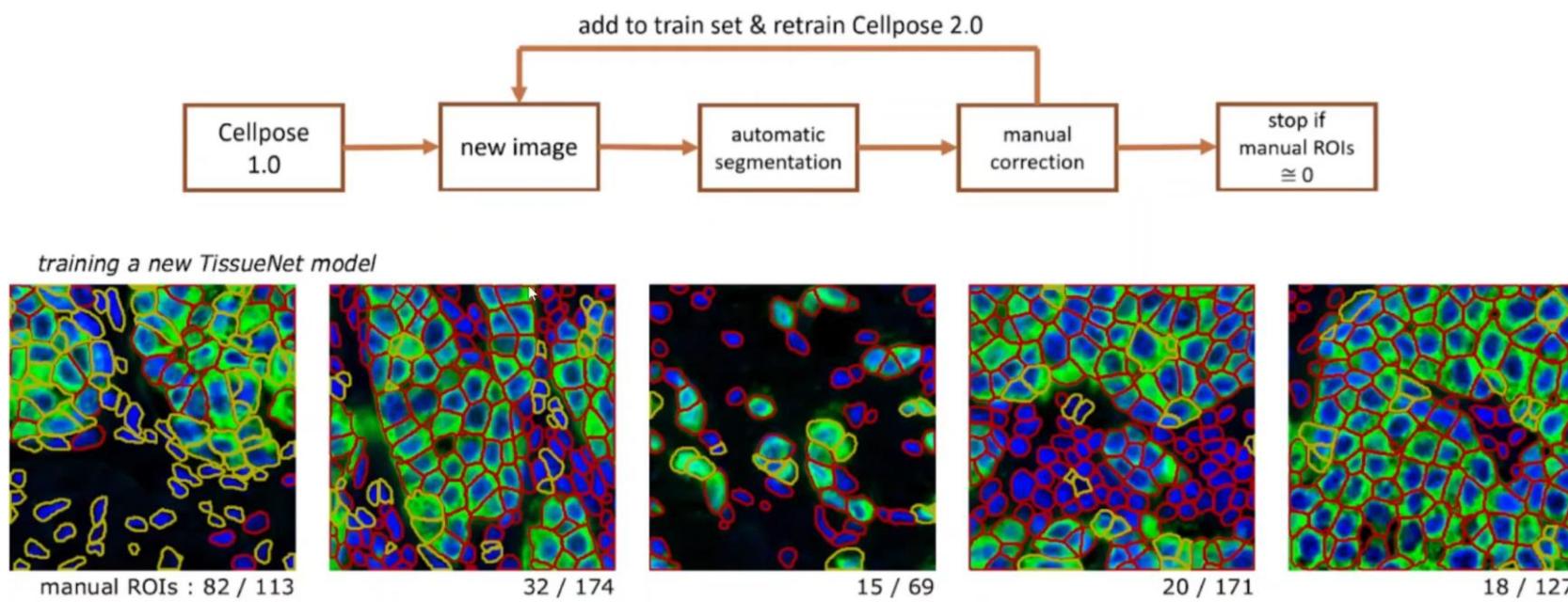
- Precision defined as $\frac{TP}{TP+FP}$
- Recall (or sensitivity) defined as $\frac{TP}{TP+FN}$
- Jaccard index (or global precision) defined as $\frac{TP}{TP+FP+FN}$
- F1-measure (or Sorenson Dice Coefficient - DSC) defined as $\frac{2TP}{2TP+FP+FN}$

When to retrain a model ?

1. Does the model I am currently using provide me with segmentation results that are roughly correct but still of insufficient quality for my research question?
2. Do I have time to dedicate to retraining it?
3. Do I have a large number of images to segment?
4. Are the objects to be segmented clear enough to avoid differences in interpretation between individuals and/or days of manual annotation during the annotation process?

Cellpose : human in the loop methods

- 1) Predict using a pretrained model on an image
- 2) Use the GUI to edit those segmentation masks. Because the pretrained model hasn't seen your exact data before, it will likely be a little off.
- 3) After adjusting some of the masks, you train a new model starting from the one that you just used to predict the masks.
- 4) Do it until you have a correct model



Cellpose GUI annotations

Pan = left-click + drag

Zoom = scroll wheel (or +/- and - buttons)

Full view = double left-click

Select mask = left-click on mask

Delete mask = Ctrl (or Command on Mac) + left-click

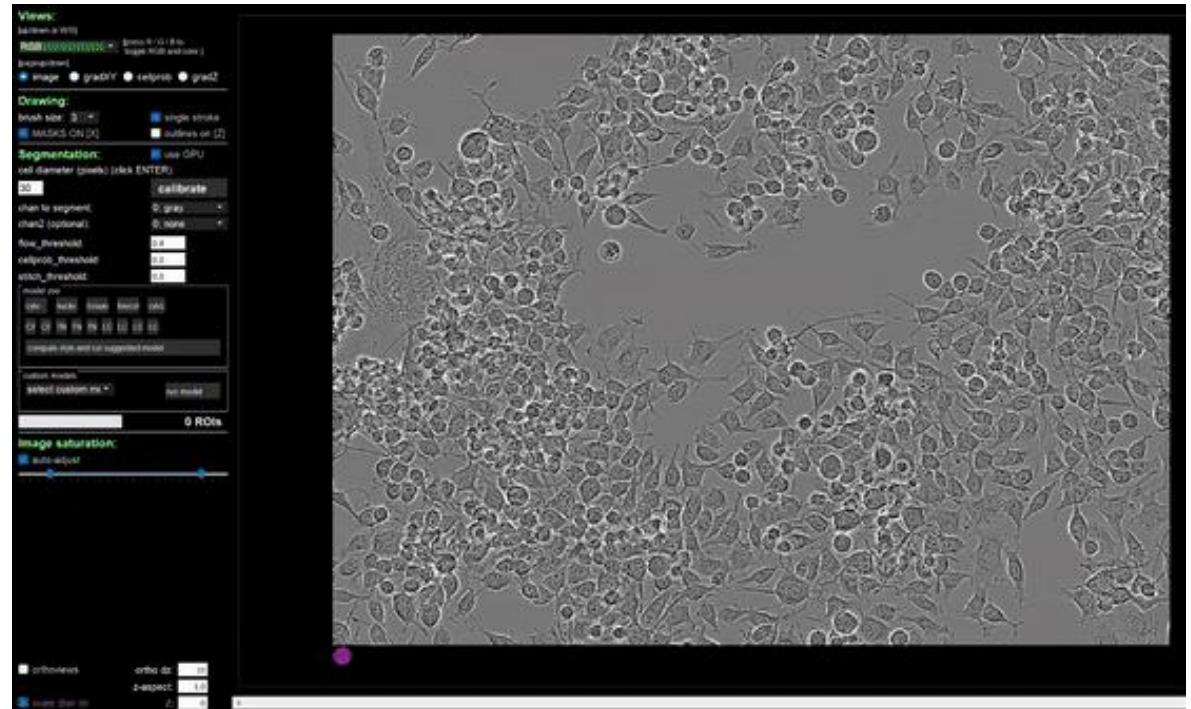
Merge masks = Alt + left-click (will merge last two)

Start draw mask = right-click

End draw mask = right-click, or return to circle at beginning

Save mask = Ctrl + S

Note: Overlaps in masks are NOT allowed. If you draw a mask on top of another mask, it is cropped so that it doesn't overlap with the old mask. Masks in 2D should be single strokes (if single_stroke is checked).



Cellpose transfert learning

Training = Ctrl + T

Choose the initial model with the best accuracy on your dataset

Select the channel to segment

Options:

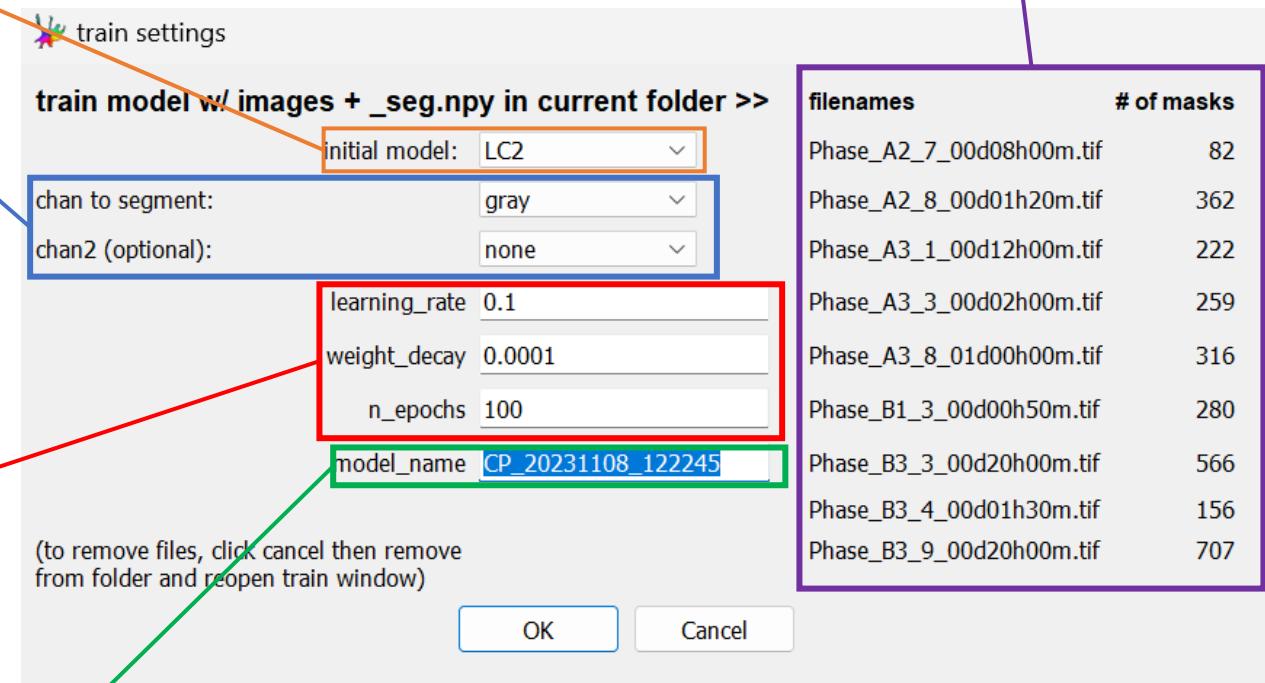
Learning_rate : corresponds to the learning speed. We recommend leaving it at 0.1

Weight_decay : is a regularization technique that is used to regularize the size of the weights of certain parameters. Leave it at (0,0001)

n_epochs : is a network training (or learning) cycle during which the network sees the training data set once.

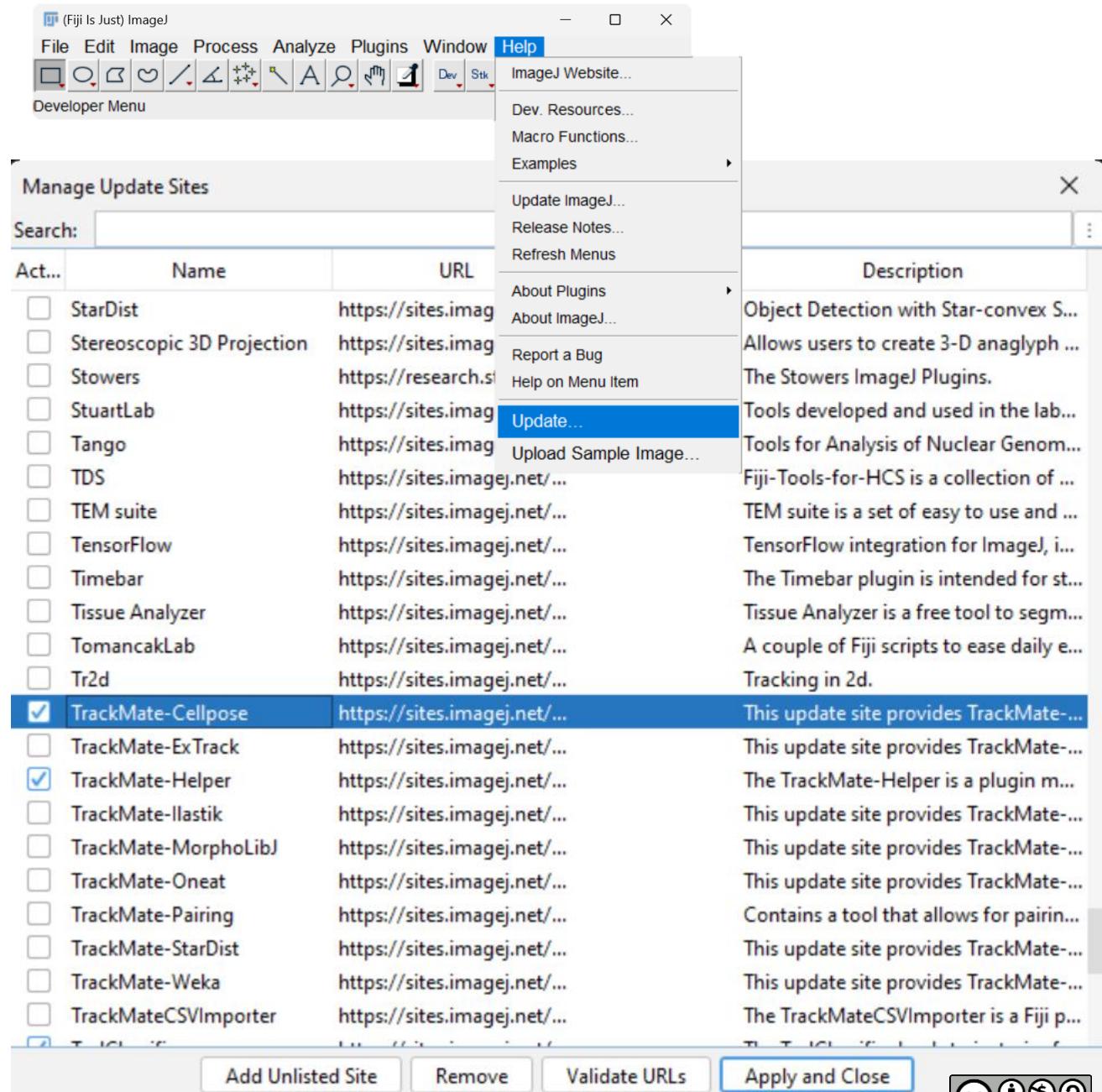
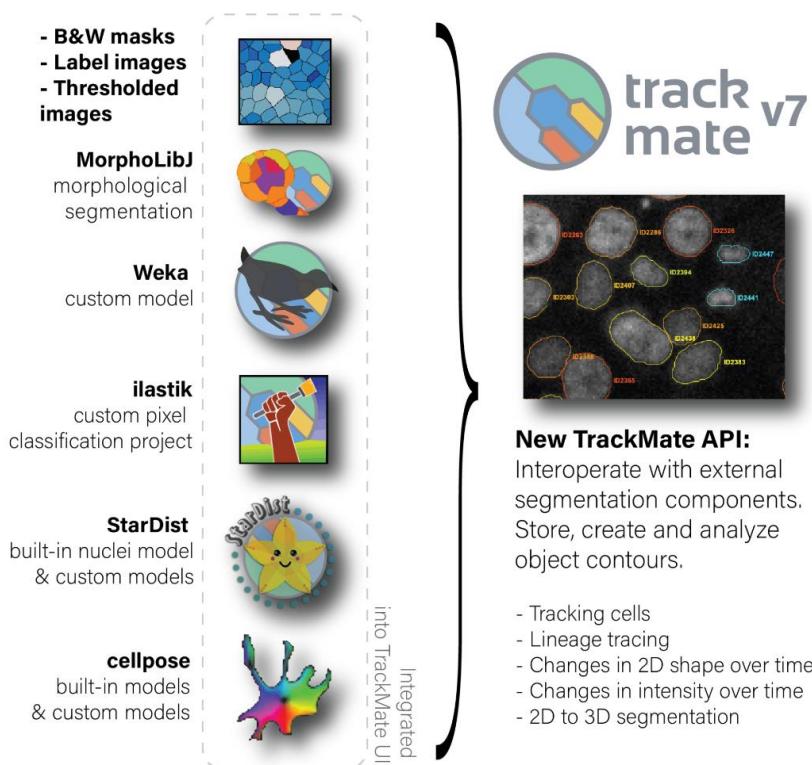
model_name : chose the name of your model

The GUI will automatically find the mask



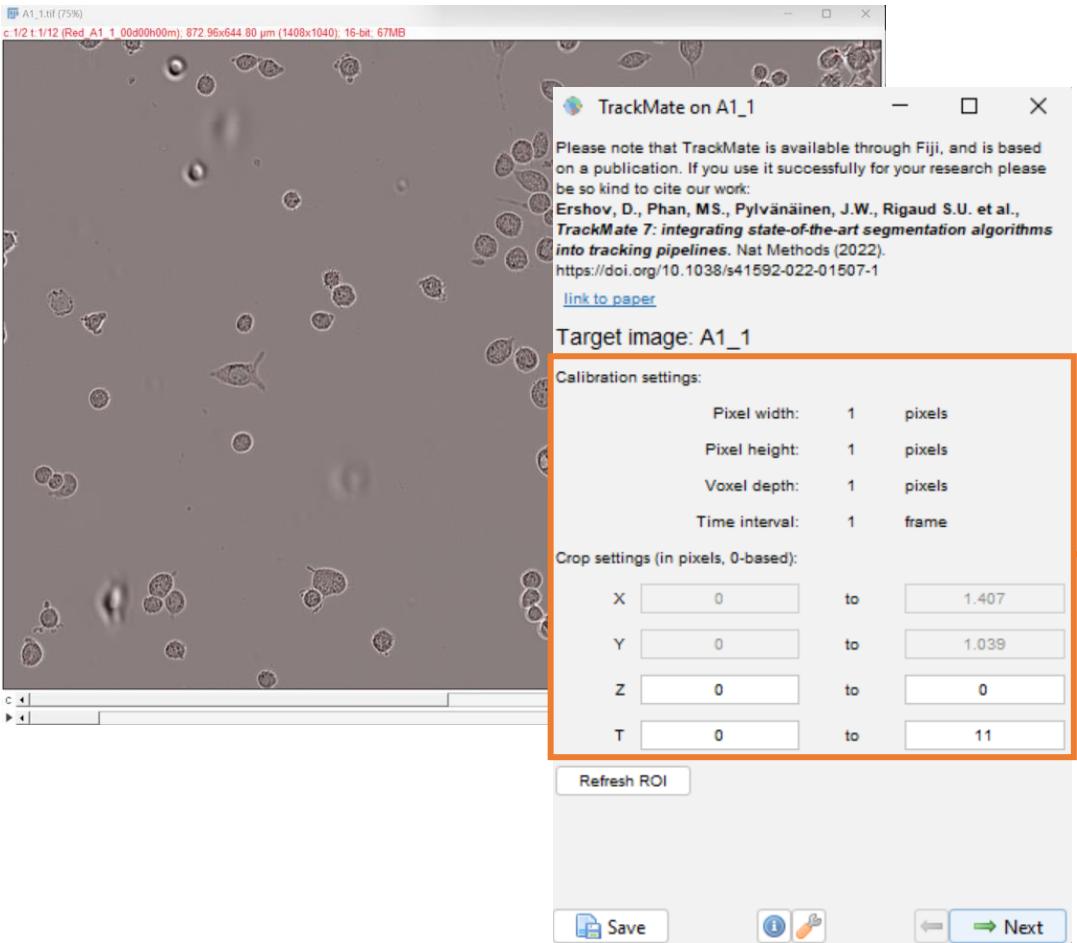
TrackMate v7

Fiji will install all the libraries needed for deep learning and machine learning segmentation

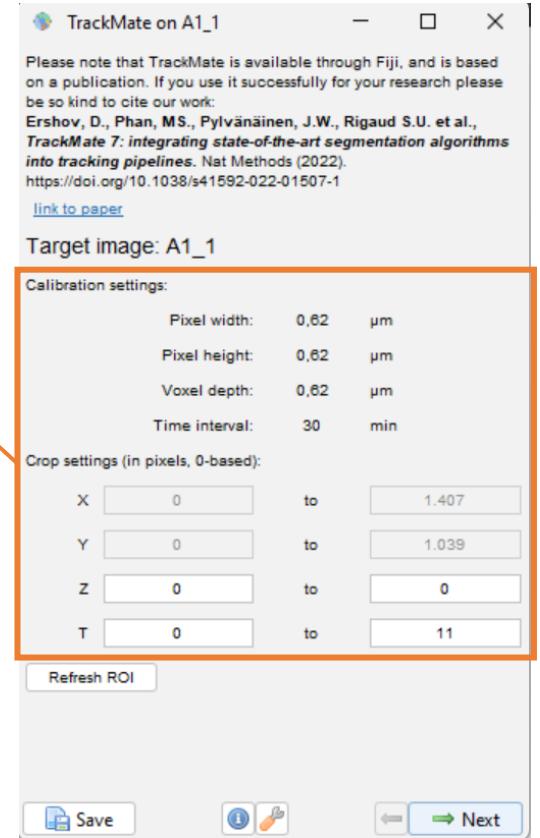


Set up data

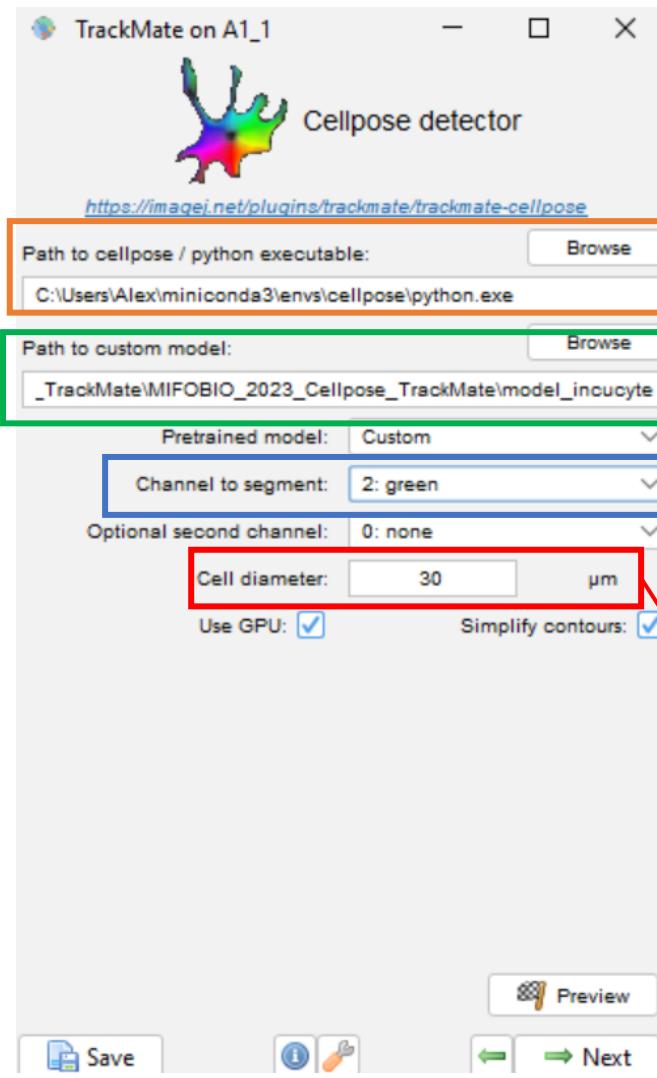
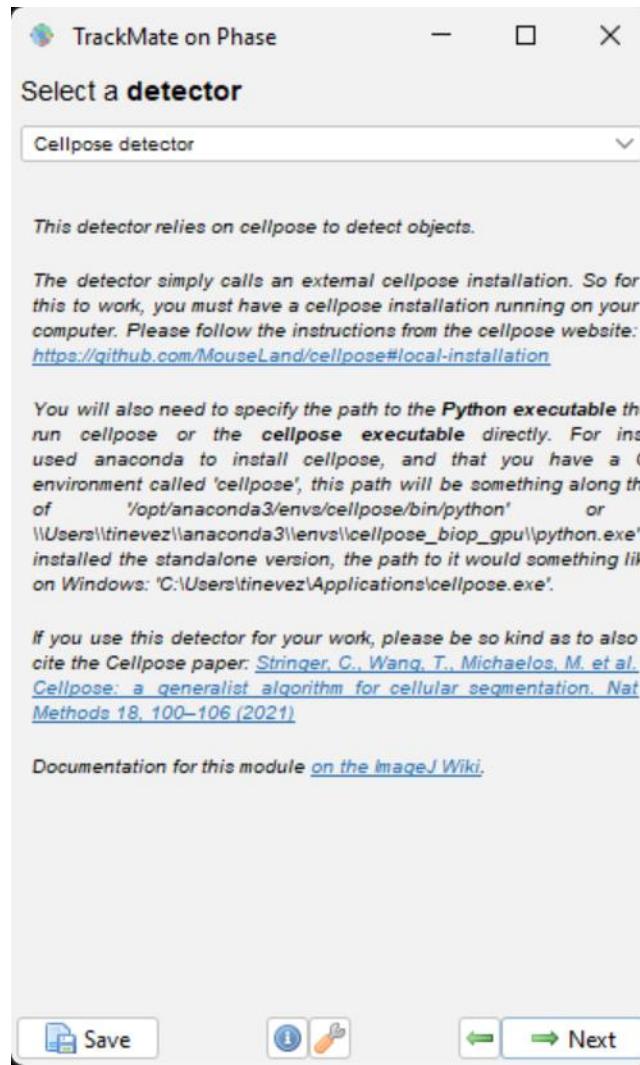
Open an hyperstack in Analyse folder and then start TrackMate



If you don't have the correct pixel size or time interval, you can modify the image properties by going to
"image > properties."



Select a detector



If you have Cellpose installed via conda, DON'T specify the path to Cellpose; instead, provide the path to Python within your Cellpose environment

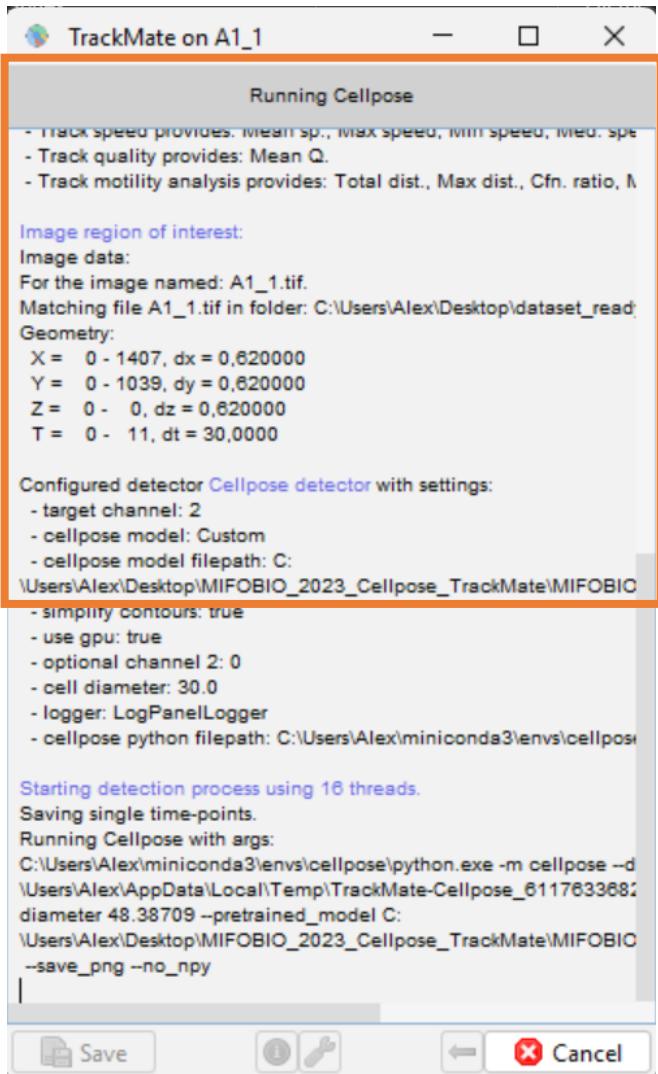
Path to your model !

Select channel 2 because today the phase channel is in the second position

Select the cell diameter you chose during the training of Cellpose

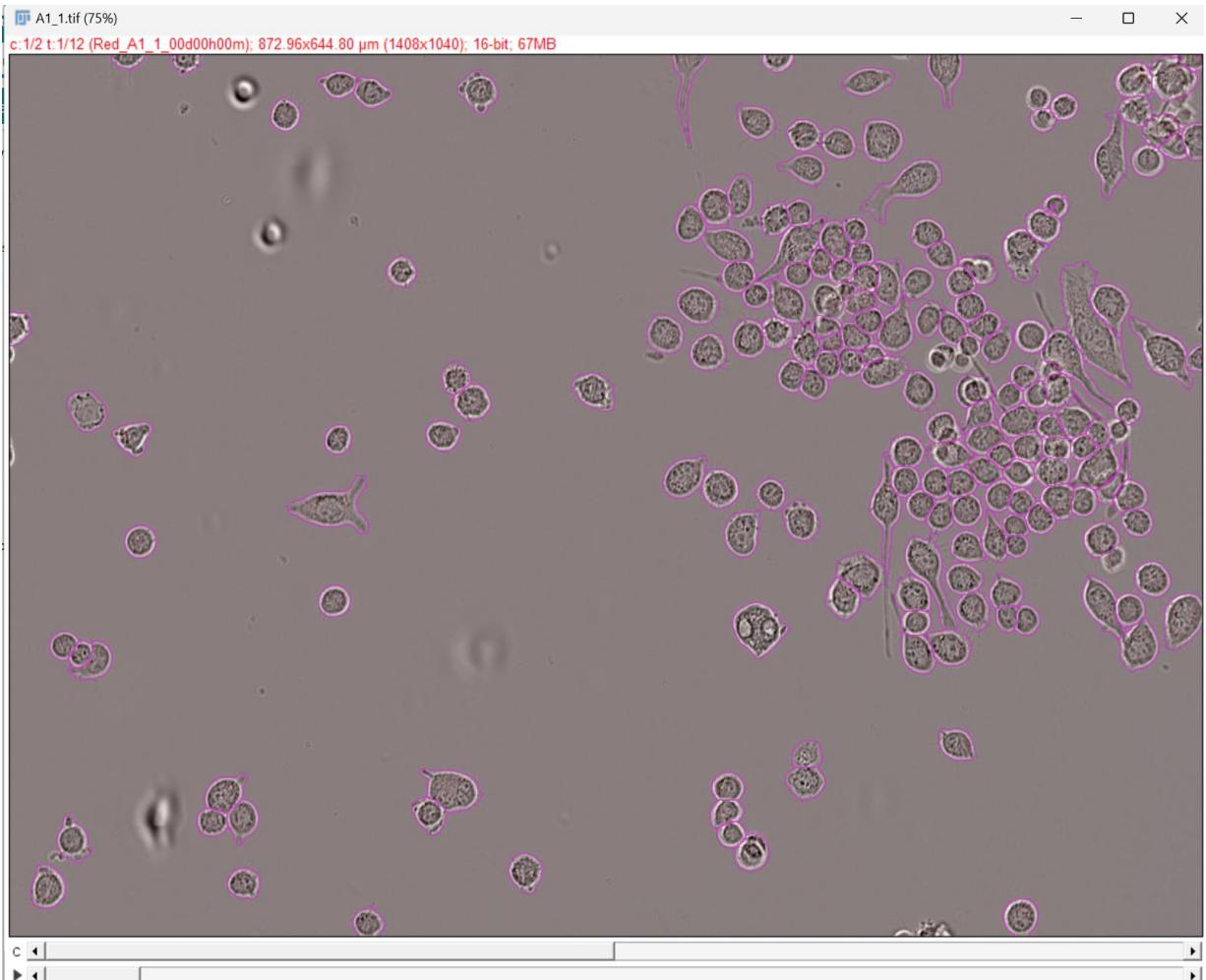
If you use the mifobio_model please use 30

Cellpose prediction

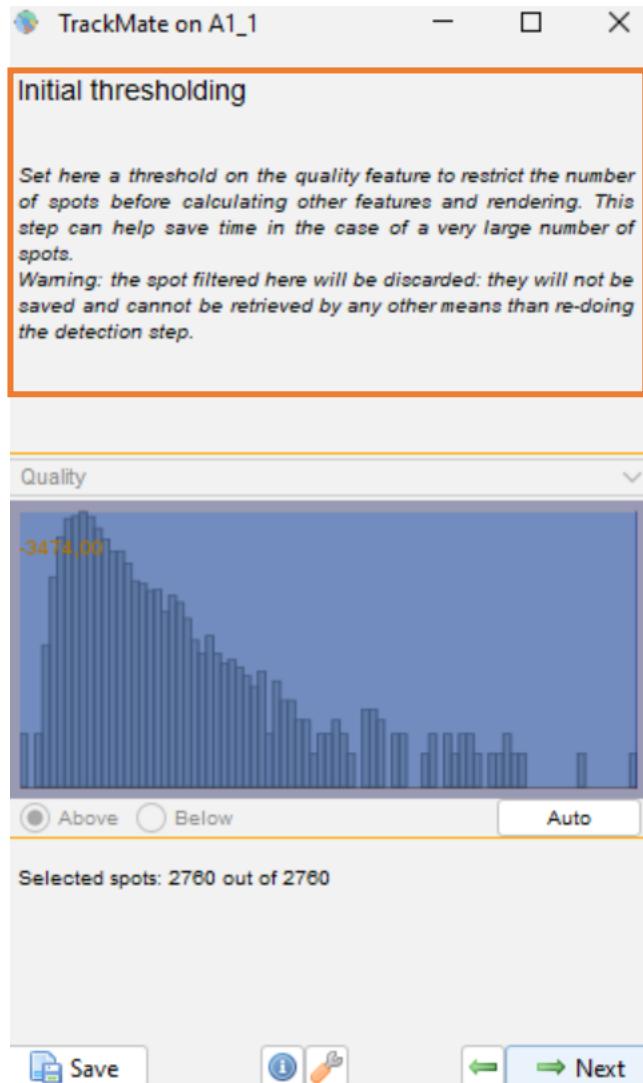


Cellpose prediction may take some time, especially if you are using a CPU or a low-power GPU. Please be patient

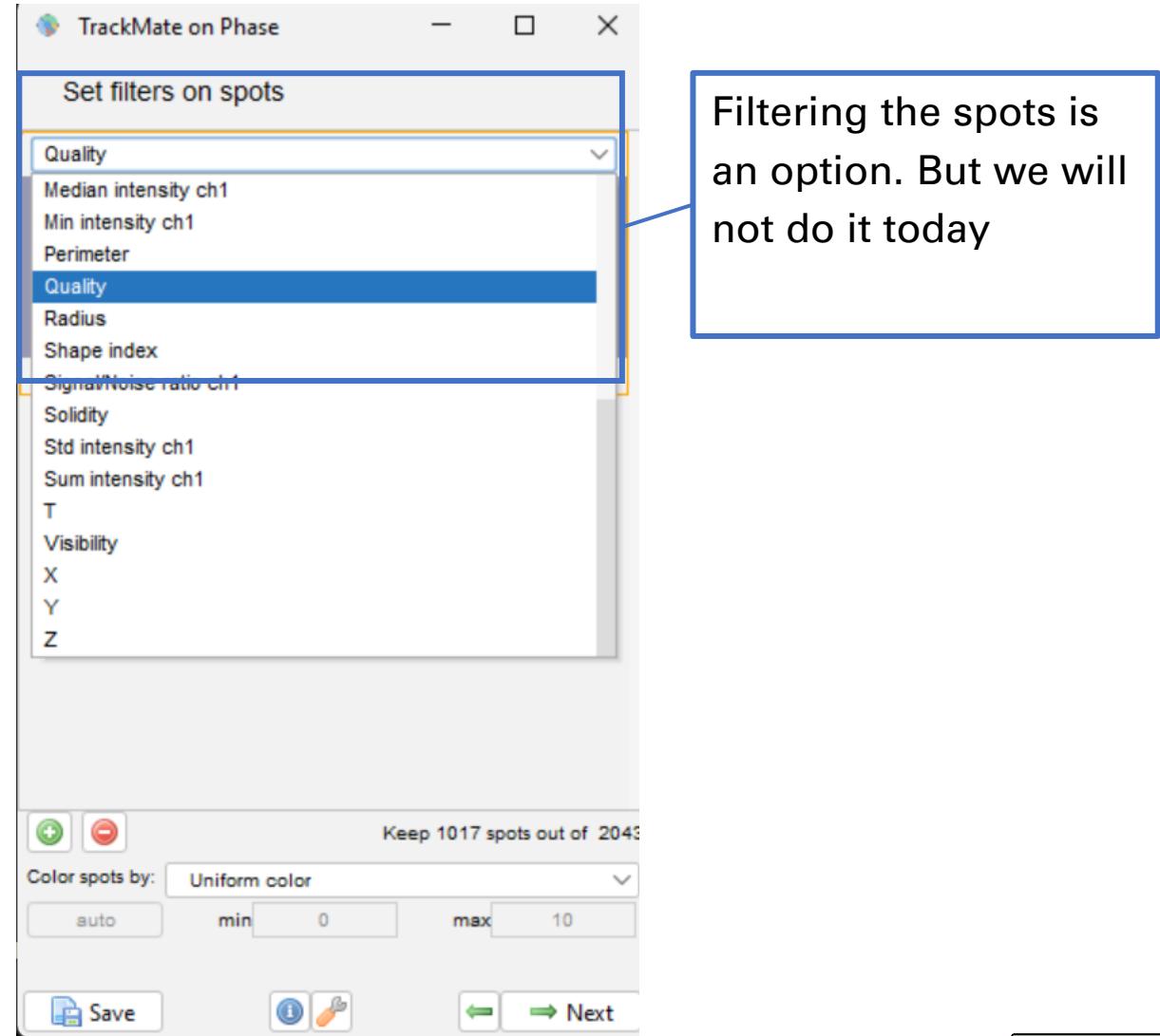
The absence of any error messages is good news



Initial thresholding and filtering

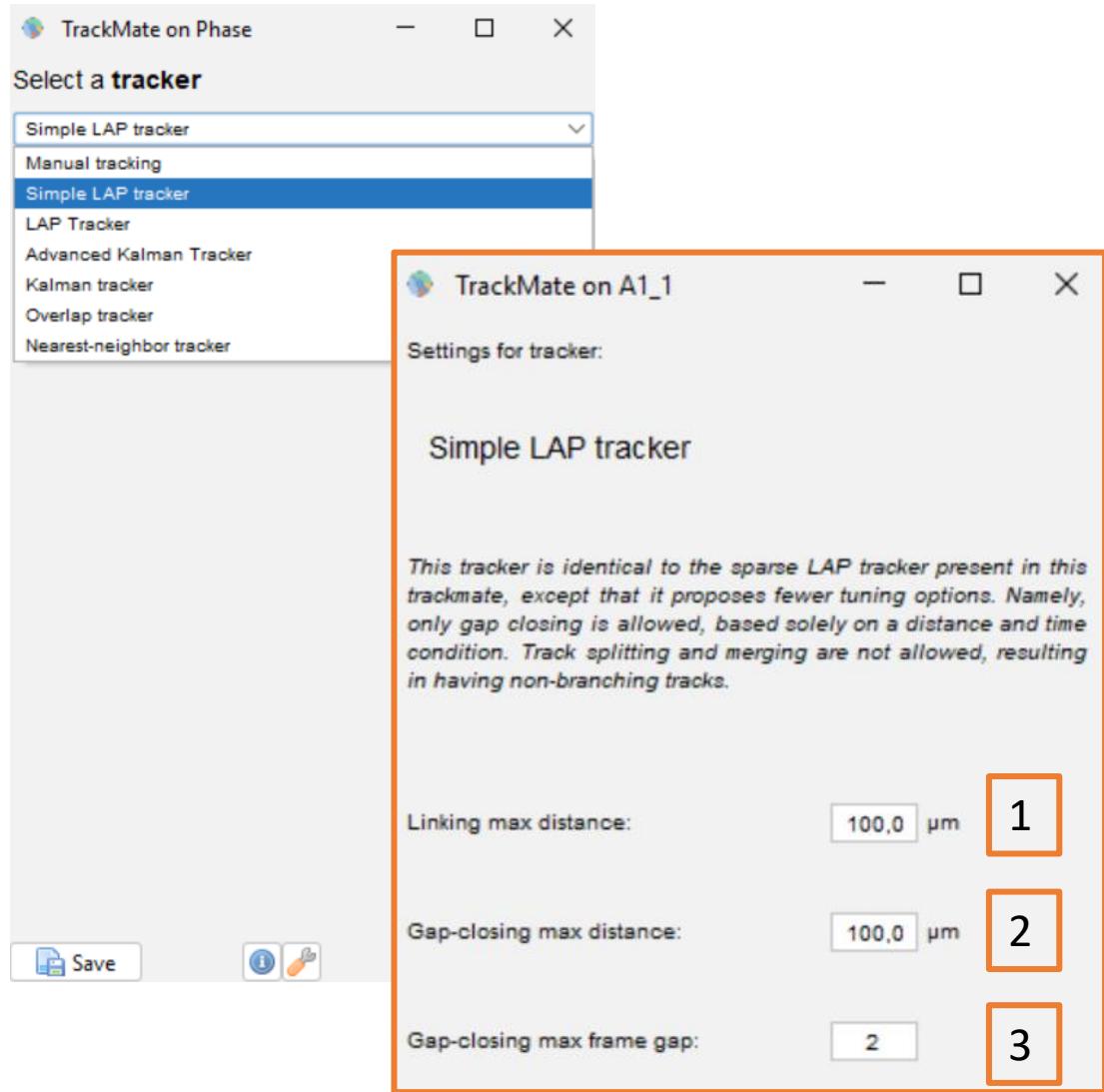


It doesn't make sense to limit the number of objects since they were generated by Cellpose



Filtering the spots is an option. But we will not do it today

Tracker : LAP tracker

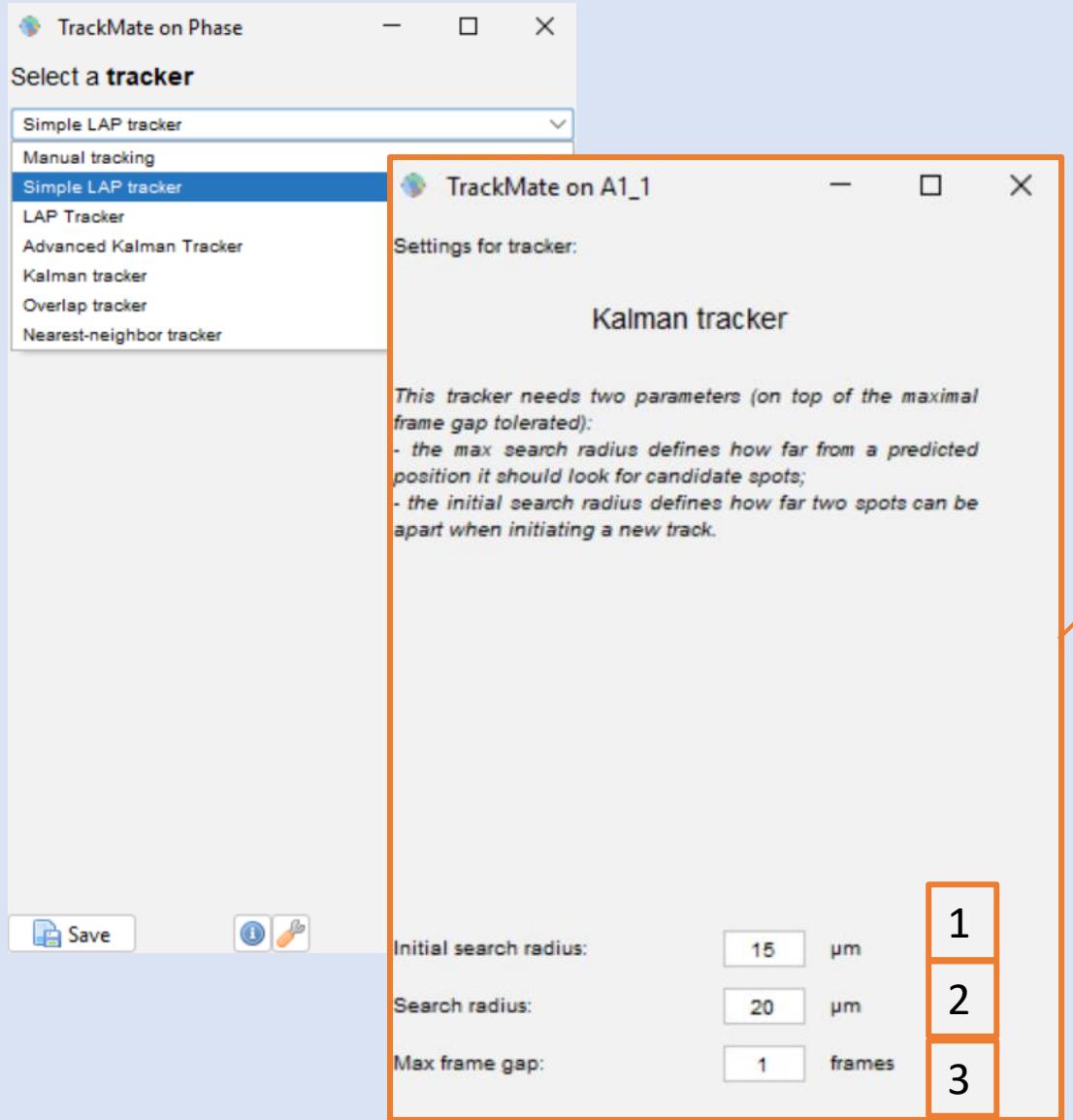


Simple LAP tracker or LAP tracker will work 75% of the case. It's a very robust algorithm.

Define :

- 1) The maximum distance between spots in consecutive time points to be considered as the same spot
- 2) The maximum distance between spots that are absent in the subsequent time points to be considered as the same spot
- 3) The number of time points in which the spots are not present

Tracker : Kalman tracker



Kalman tracker is a new tracker that can deal specifically with linear motion, or particle moving with a roughly constant velocity. This velocity does not need to be the same for all particles

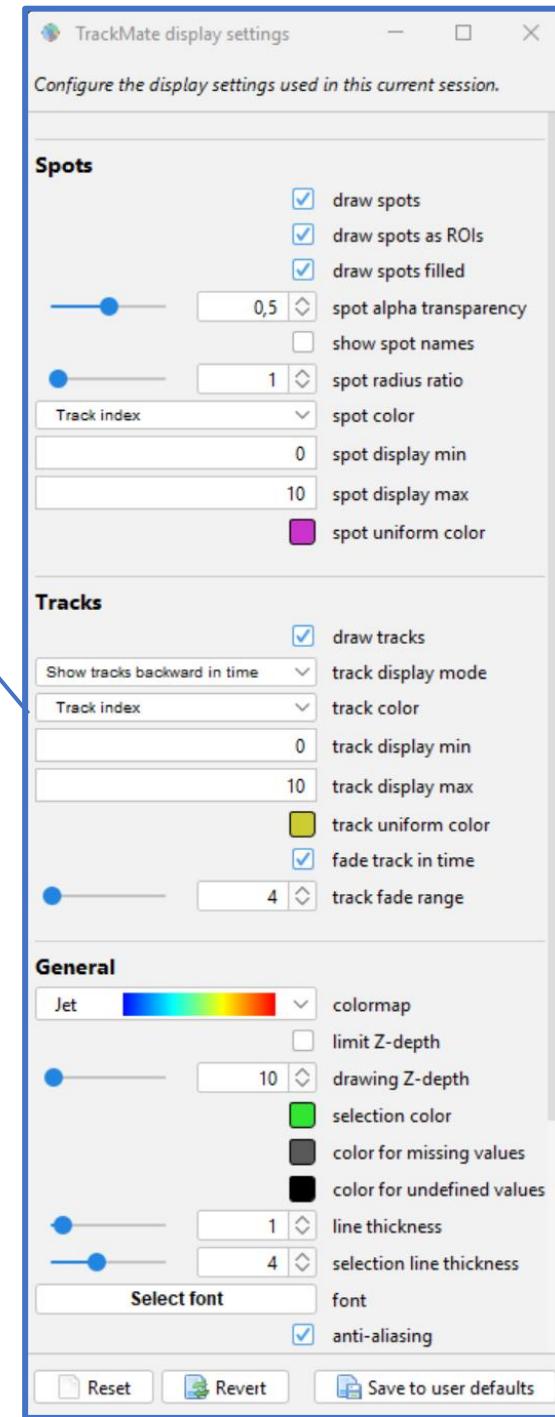
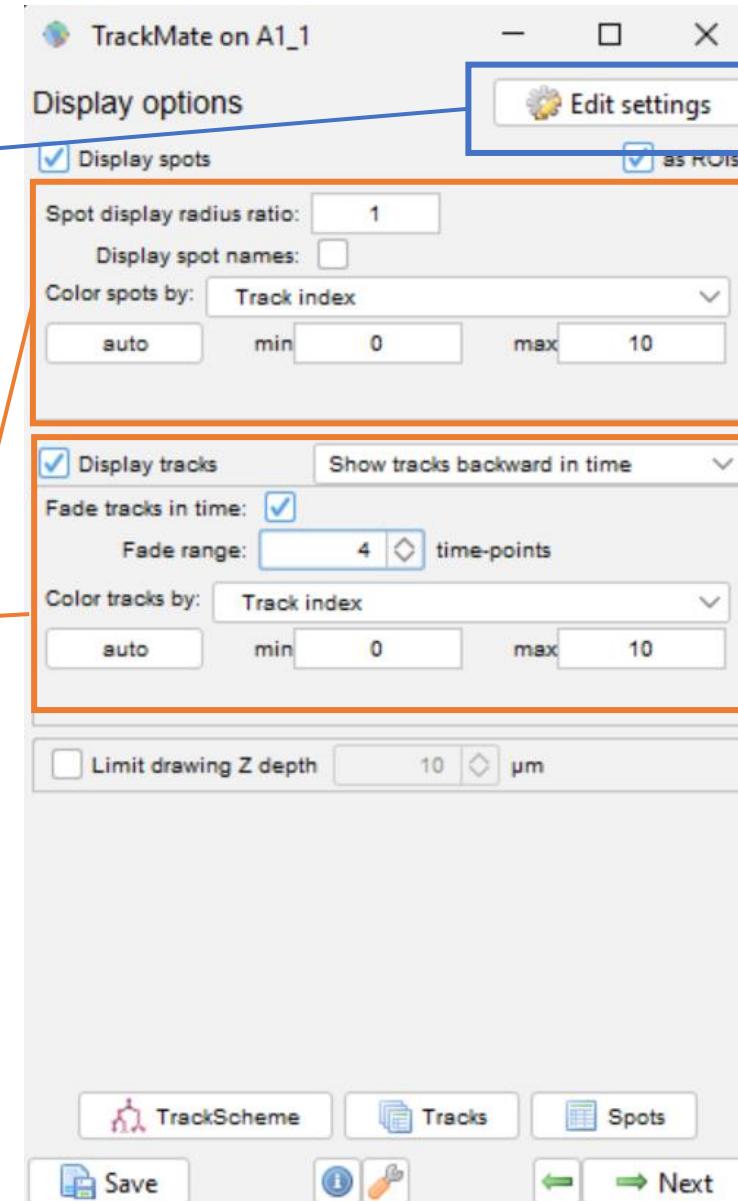
Define :

- 1) maximal distance allowed for the initial search
- 2) how far can be an actual position from a predicted position for linking
- 3) The number of time points in which the spots are not present

Display options

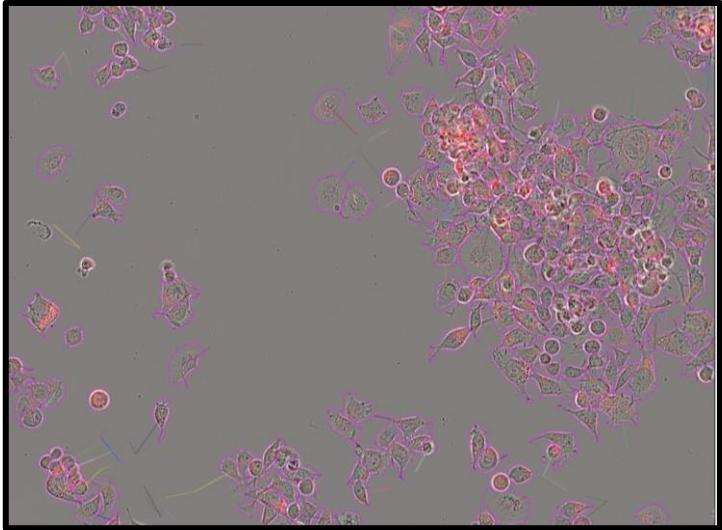
Edit settings will let you access to advance settings

You can change the color spots/ tracks to highlight specific features such as MFI, Shapes, Speed etc..

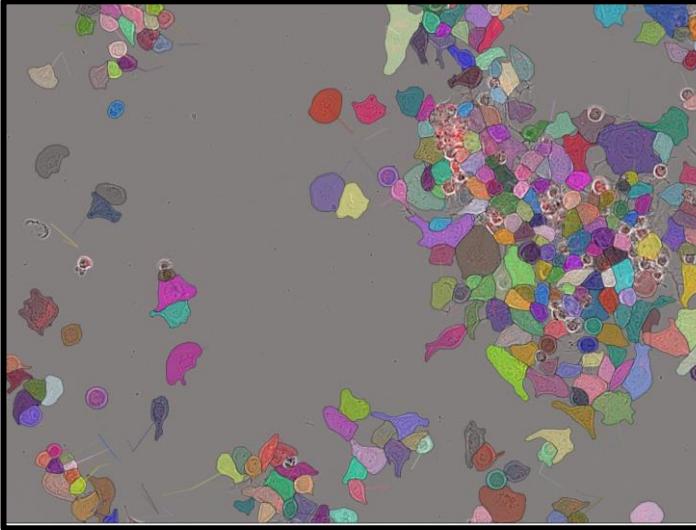


Display options

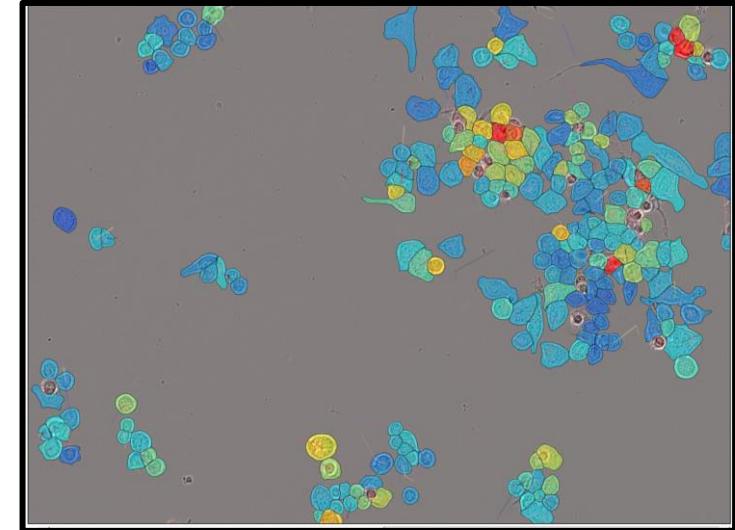
Default



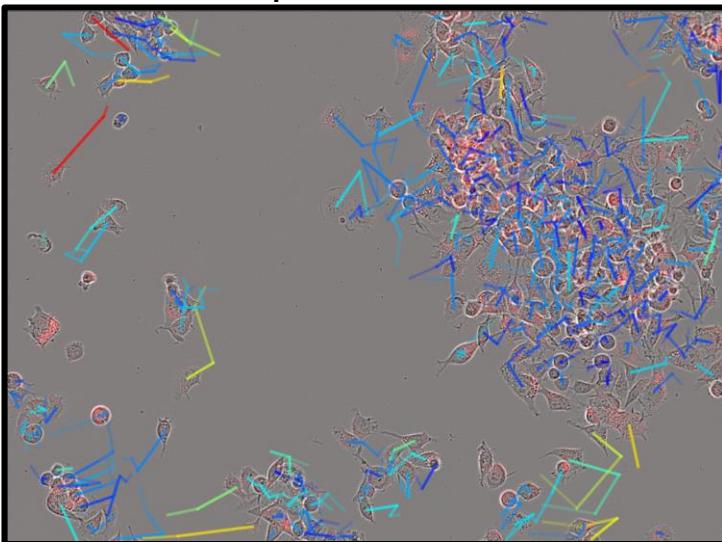
Track Index



MFI channel 1

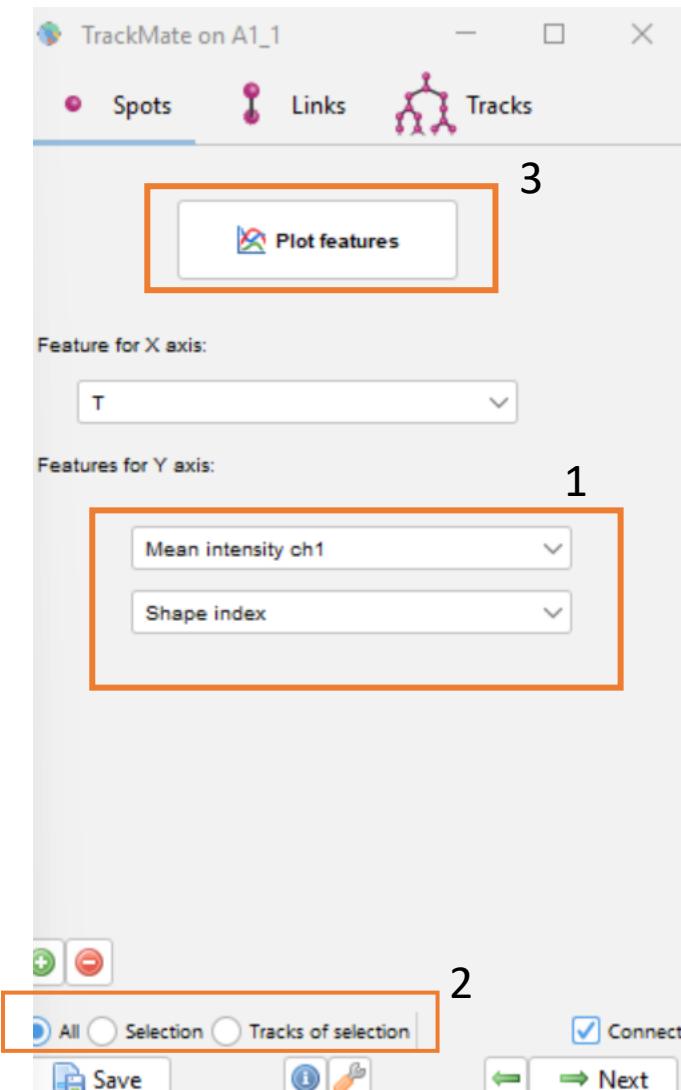


Track Mean speed

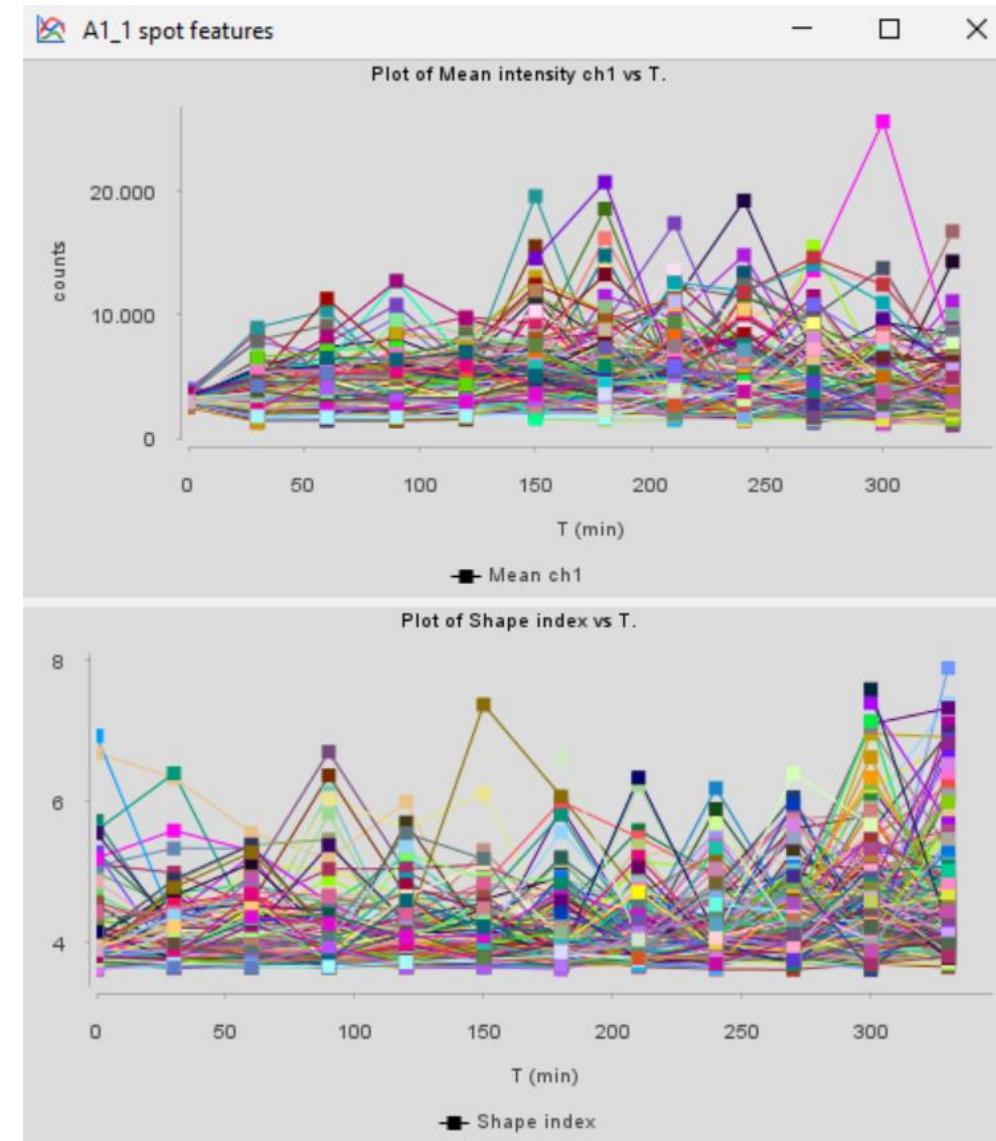


Let's play with it ..

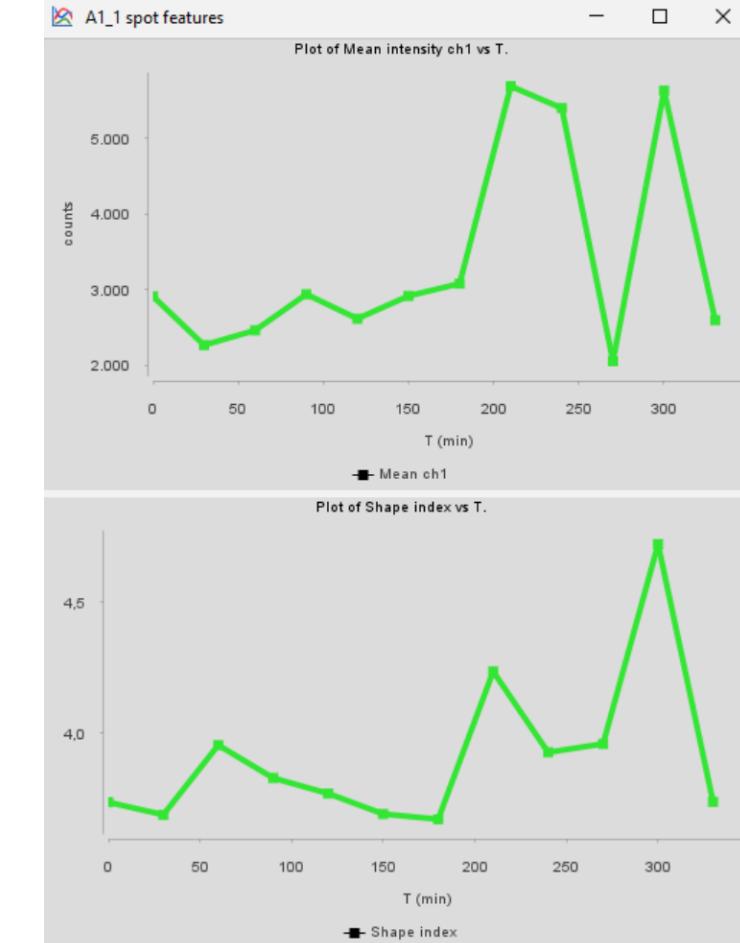
Graphs



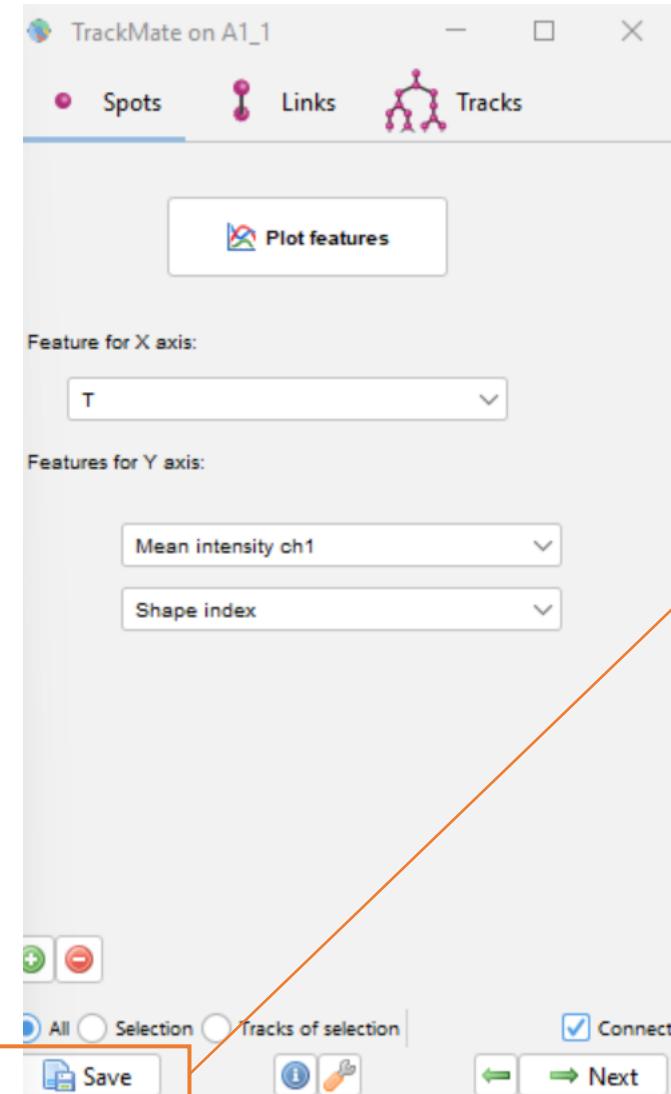
We can plot all the data



Or only selected event or tracks



Save .xml file



```
- merging feature penalties:  
- splitting max distance: 15.0  
- blocking value: Infinity  
- allow gap closing: true  
- allow track splitting: false  
- allow track merging: false  
- merging max distance: 15.0  
- splitting feature penalties:  
- cutoff percentile: 0.9  
- gap closing feature penalties:  
  
Starting tracking process.  
Tracking done in 0.2 s.  
Found 316 tracks.  
- avg size: 8.4 spots.  
- min size: 2 spots.  
- max size: 12 spots.  
  
Calculating features done in 0.1 s.  
  
Performing track filtering on the following features:  
No feature threshold set, kept the 316 tracks.  
Saving data...  
Computing edge features:  
- Directional change in 4 ms.  
- Edge speed in 4 ms.  
- Edge target in 4 ms.  
- Edge location in 8 ms.  
Computation done in 20 ms.  
Computing track features:  
- Branching analyzer in 4 ms.  
- Track duration in 2 ms.  
- Track index in 0 ms.  
- Track location in 5 ms.  
- Track speed in 2 ms.  
- Track quality in 3 ms.  
- Track motility analysis in 3 ms.  
Computation done in 21 ms.  
Saving aborted.  
Saving data...  
Computing edge features:  
- Directional change in 2 ms.  
- Edge speed in 4 ms.  
- Edge target in 5 ms.  
- Edge location in 5 ms.  
Computation done in 16 ms.  
Computing track features:  
- Branching analyzer in 2 ms.  
- Track duration in 3 ms.  
- Track index in 0 ms.  
- Track location in 2 ms.  
- Track speed in 1 ms.  
- Track quality in 2 ms.  
- Track motility analysis in 3 ms.  
Computation done in 14 ms.  
Added 10...
```

The XML file is a large document containing measurements for all spots, tracks, and instructions for performing the analysis

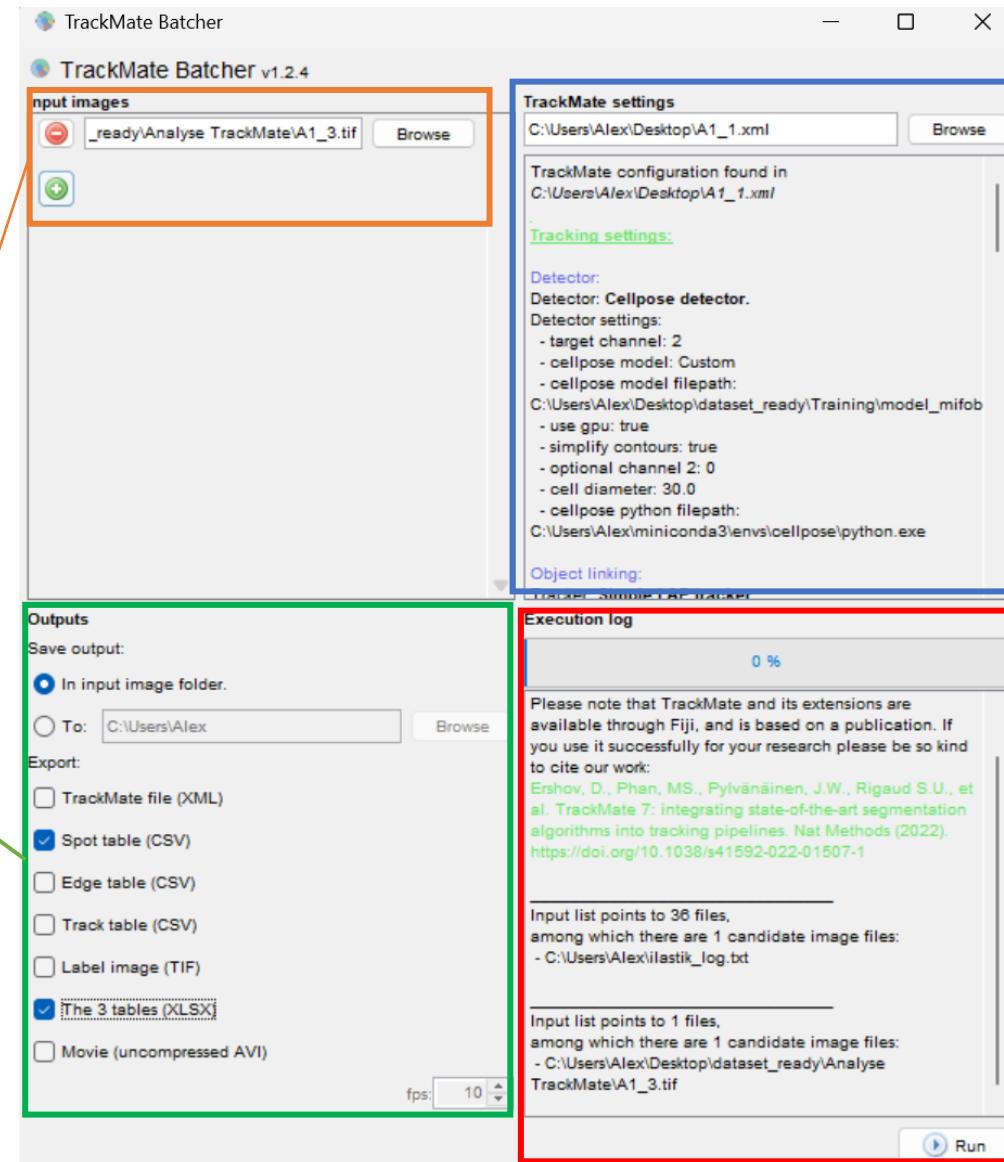
TrackMate Batcher

the paths to the input images.

You can drag and drop folder and files

If you add a folder, all the images in it will be analyzed

You can export CSV tables, an Excel table, and AVI movies as well as the TrackMate file for each of the input images



browse or drag and drop to TrackMate the XML file

Log of the process and Run

Next ? CellTracksColab



CellTracksColab simplifies the journey from data compilation to analysis.

Built on the Google Colaboratory framework, it provides a cloud-based solution accessible with just a web browser and a Google account.

Key Features

- **Holistic View:** Comprehensive analysis across fields of view, biological repeats, and conditions.
- **User-Centric:** Intuitive GUI designed for all users.
- **Visualization:** Track visualization and filtering.
- **Analysis:** Deep-dive into track metrics and statistics.
- **Reliability:** Check experimental variability using hierarchical clustering.
- **Advanced Tools:** Harness the power of UMAP and HDBSCAN.
- **Flexibility:** Tailor and adapt to your needs.

Guillaume Jacquemet. (2023). CellTracksColab—A platform for compiling, analyzing, and exploring tracking data. *bioRxiv*.
<https://doi.org/10.1101/2023.10.20.563252>

