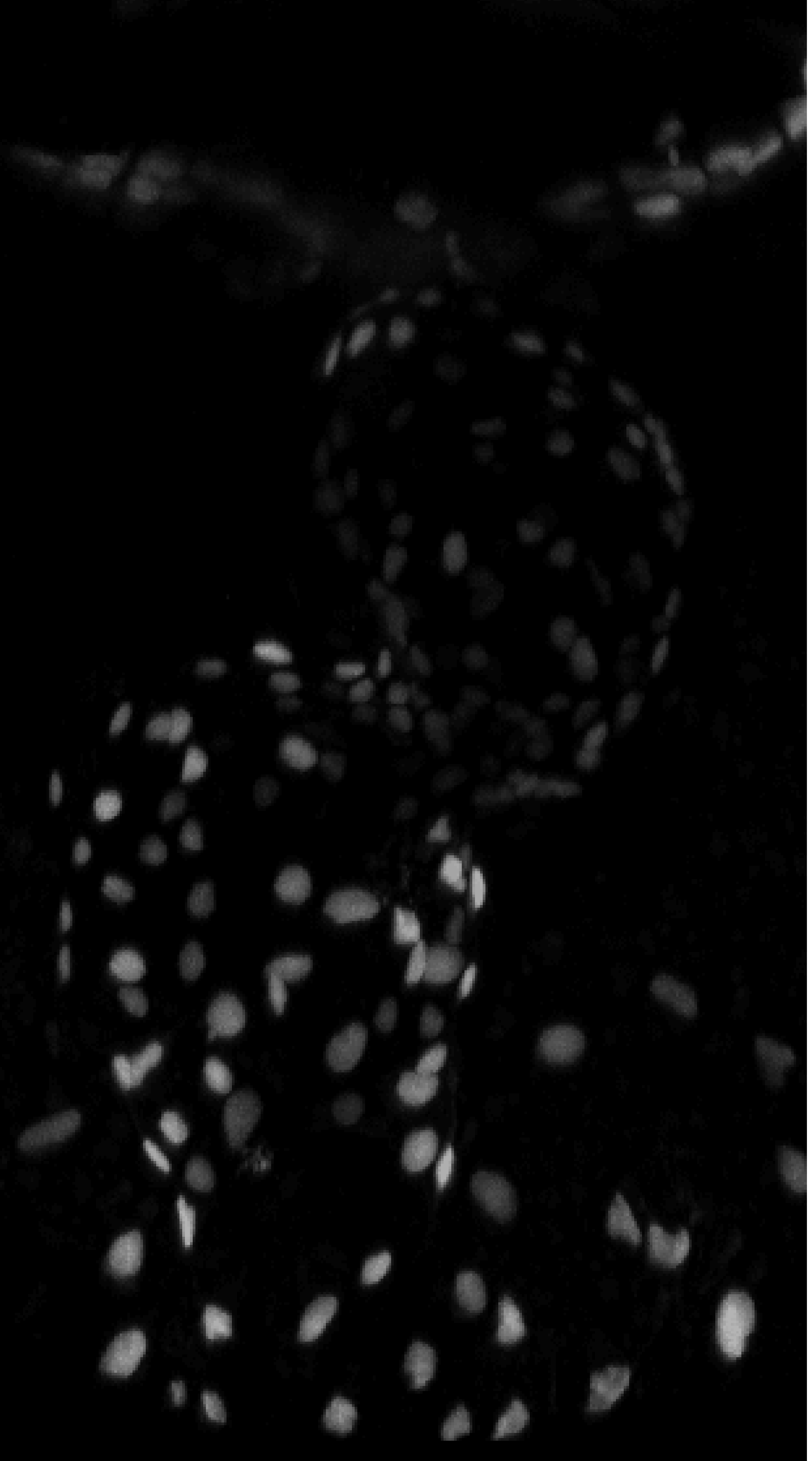


Image processing for cells nuclei tracking using artificial intelligence

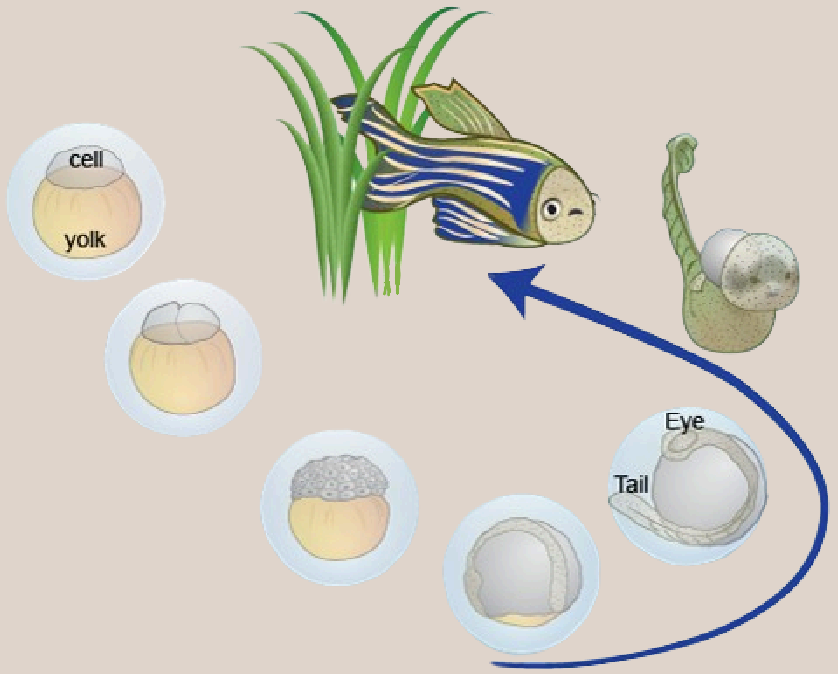
BME PARIS
BioMedical Engineering
MASTER'S PROGRAM

IMPERIAL
VERMOT LAB
Imperial College London

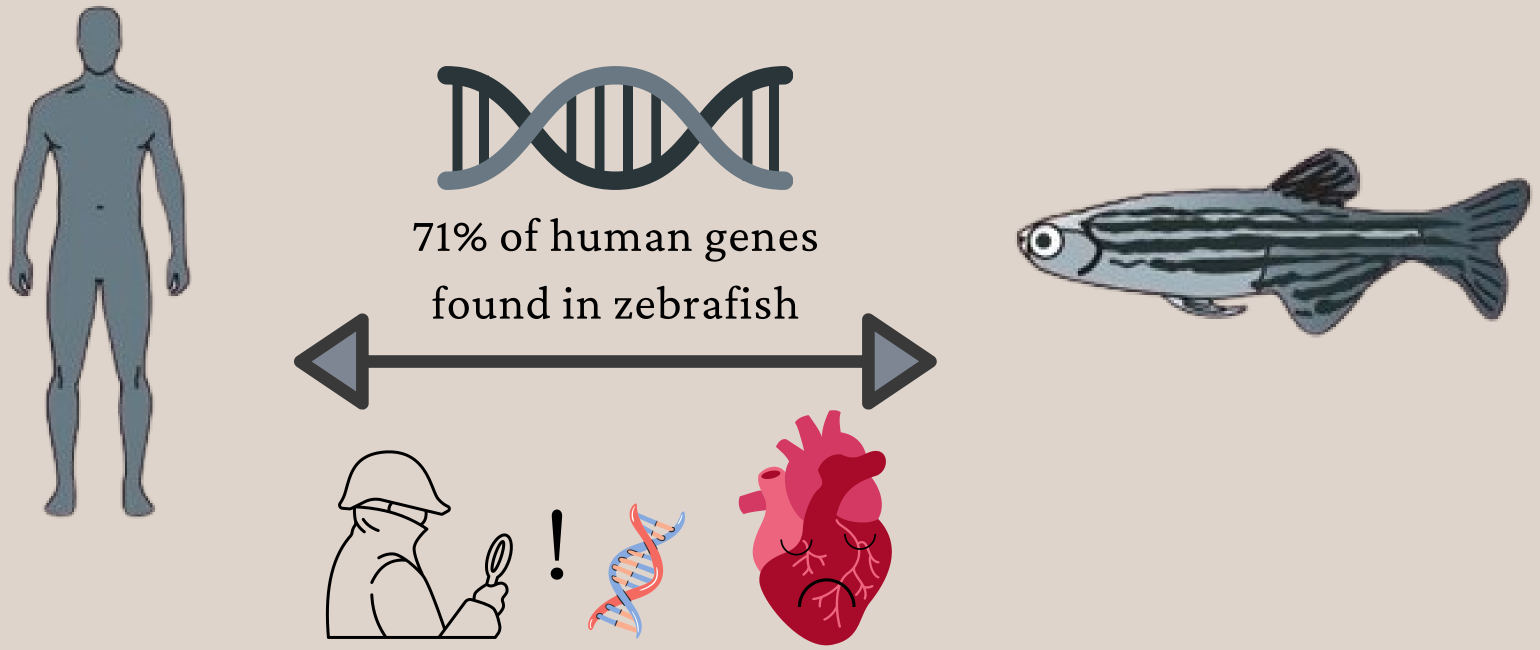
ELSA DOUKHAN
ecd24@ic.ac.uk

I - Purpose of tracking cell nuclei in 3D time lapse in a deforming organ in zebrafish

1) Studying Morphogenesis & understanding Cell Behavior



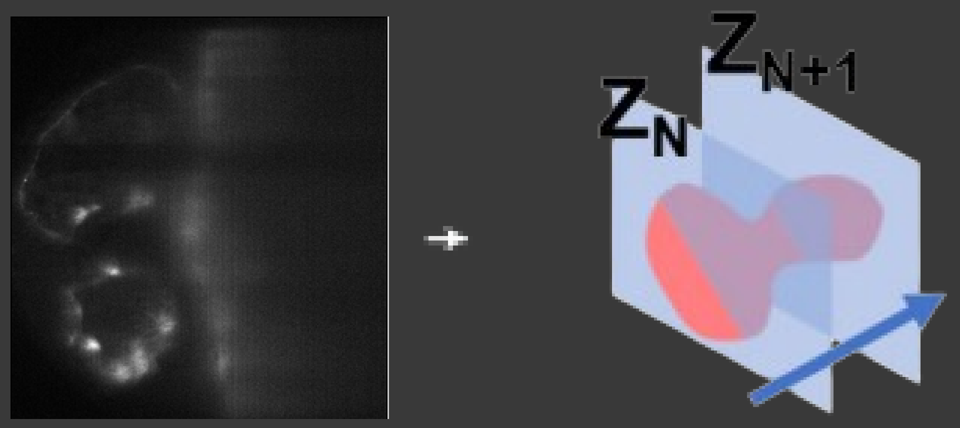
2) Elucidating Developmental Biology & Modeling Diseases



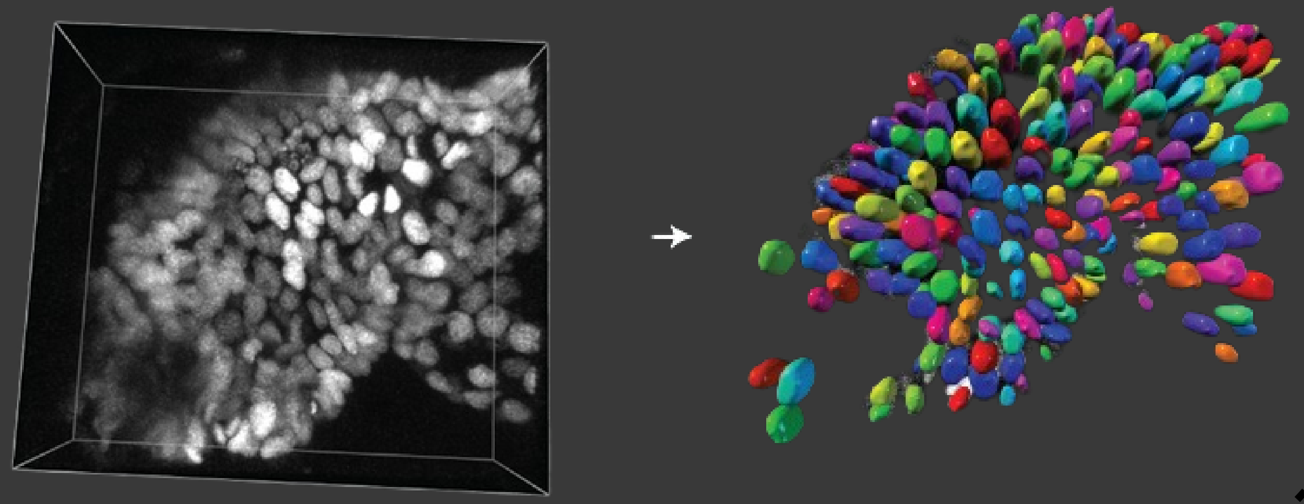
→ Understanding normal development at the cellular level provides a baseline for identifying abnormalities that lead to diseases

II - Methods

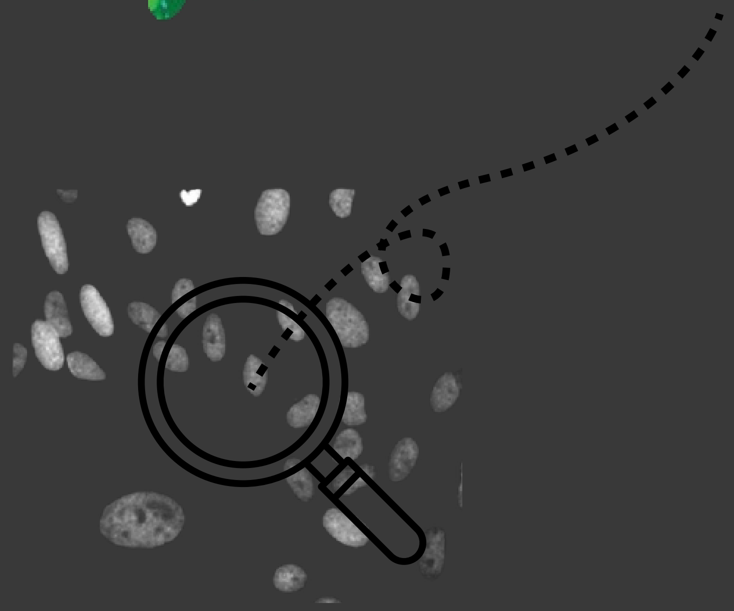
→ 1) Building a 3D+Time image of a beating heart



→ 2) Segmenting and tracking nuclei in our 3D time-lapse image

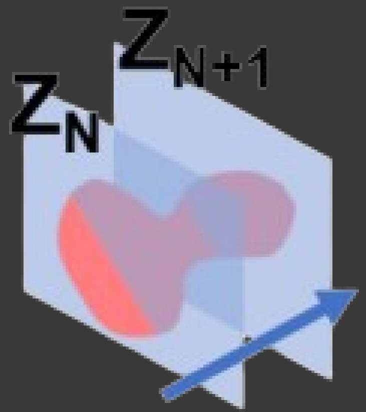


→ 3) - Analysing of the performaces of 3DeeCellTracker on our image
- Analysing movement and the speed of nuclei during an entire beat

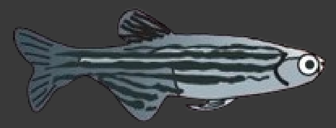


II - Methods

→ 1) Building a 3D+Time image of a beating heart



Materials : Beating heart slices obtained with LSFM



Wildtype 48 hpf

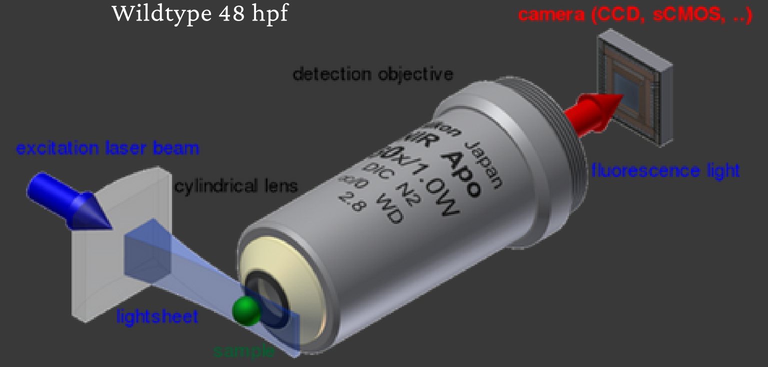
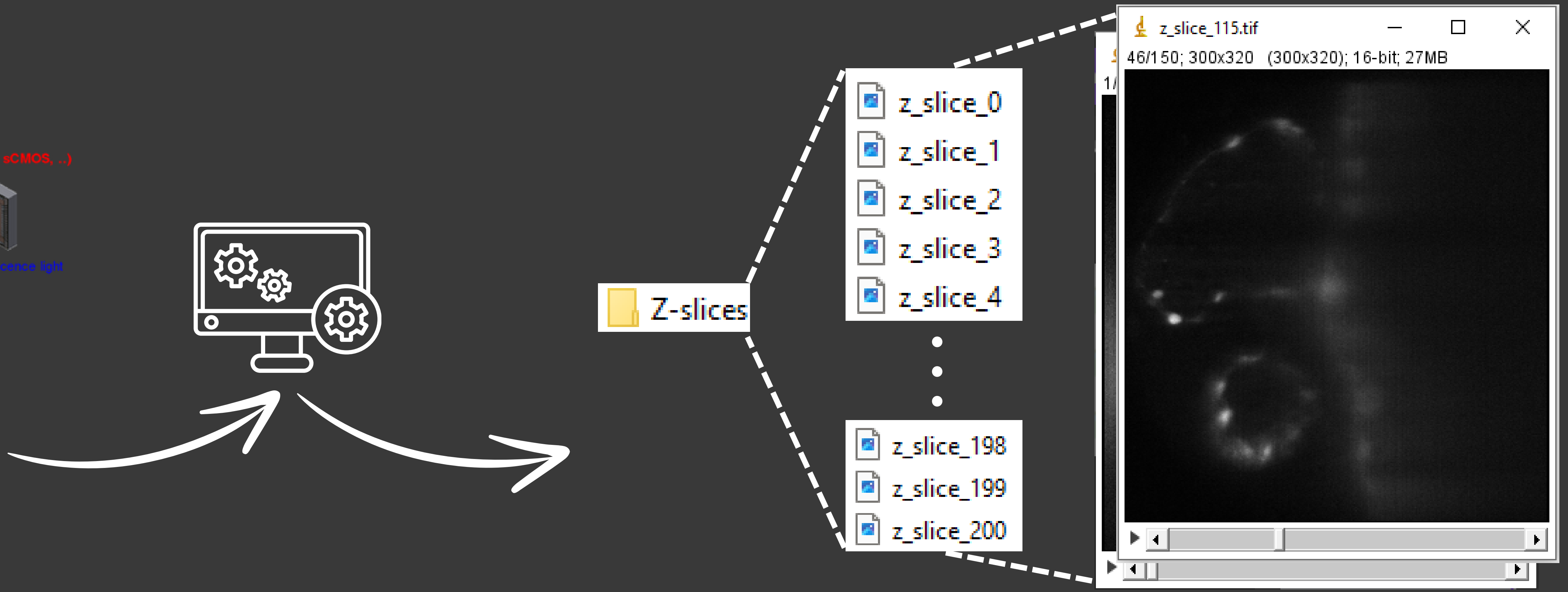


image capture frequency : 150Hz



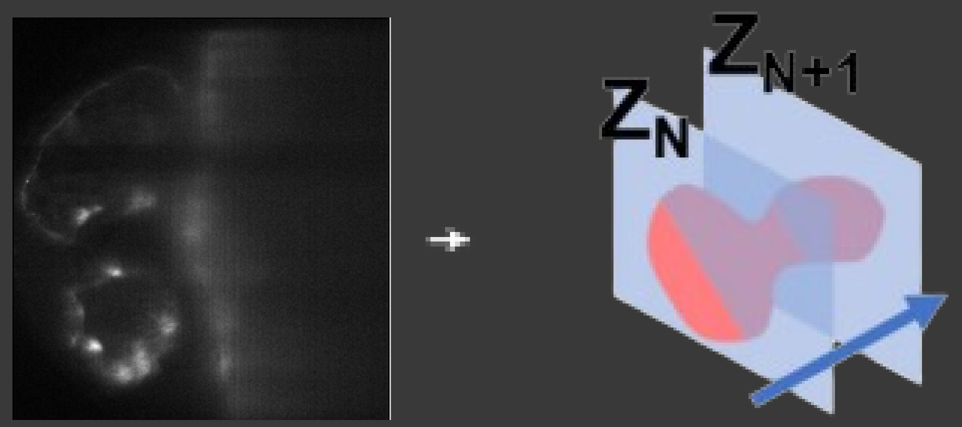
150 images/z-slices
<=> video of 1 sec

201 * 150 = 30 150 images

Tools : various Python libraries and ImageJ

II - Methods

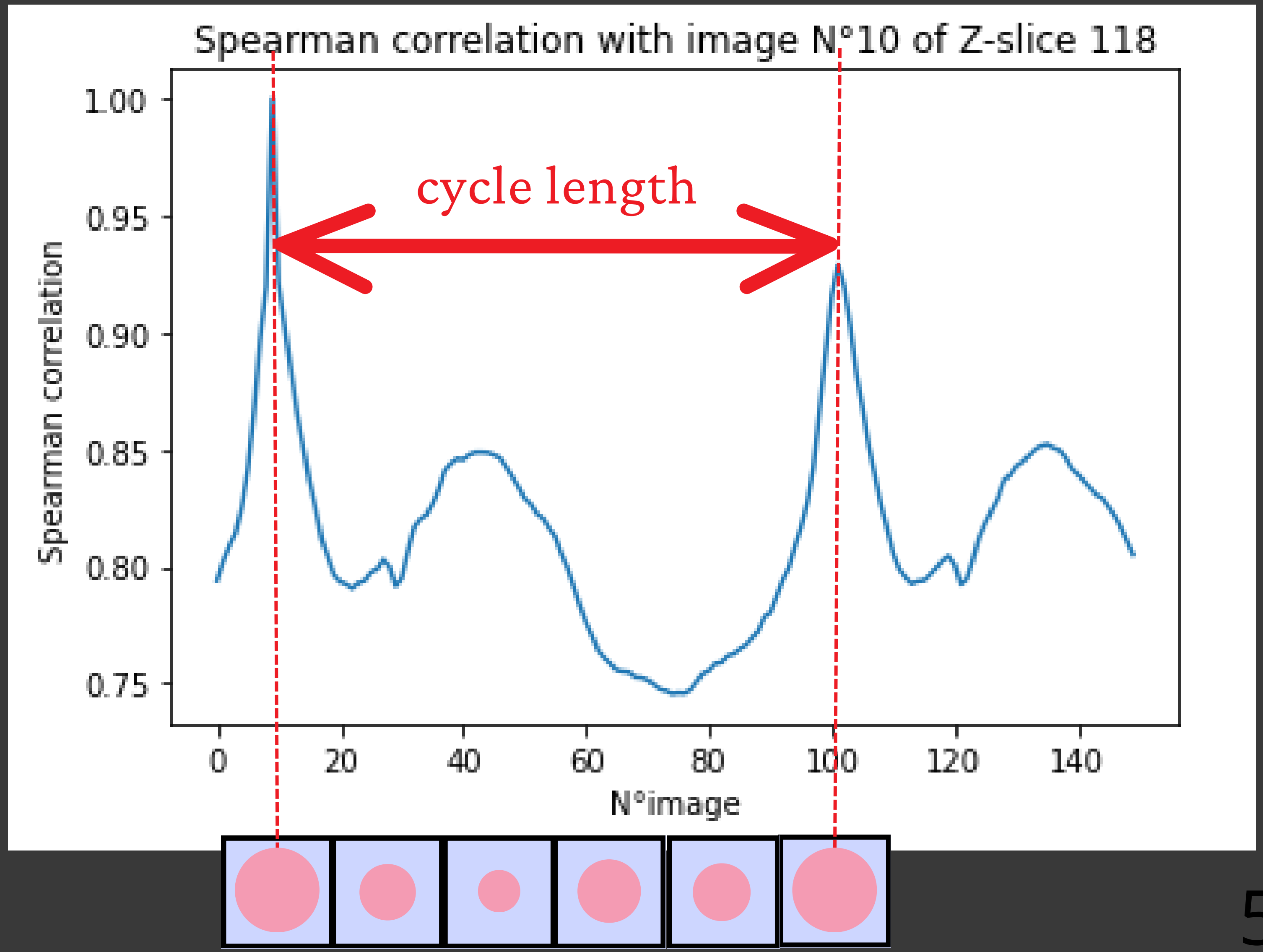
→ 1) Building a 3D+Time image of a beating heart



- Identifying cardiac cycle LENGTH with the Spearman correlation coefficient on the z-slices 116 to 120

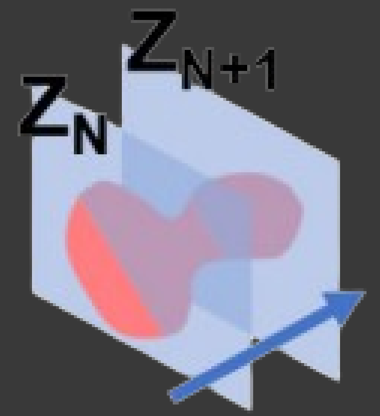
Z-movies


```
scipy.stats.spearmanr(list_px_referrence_image, list_px_current_image)
```

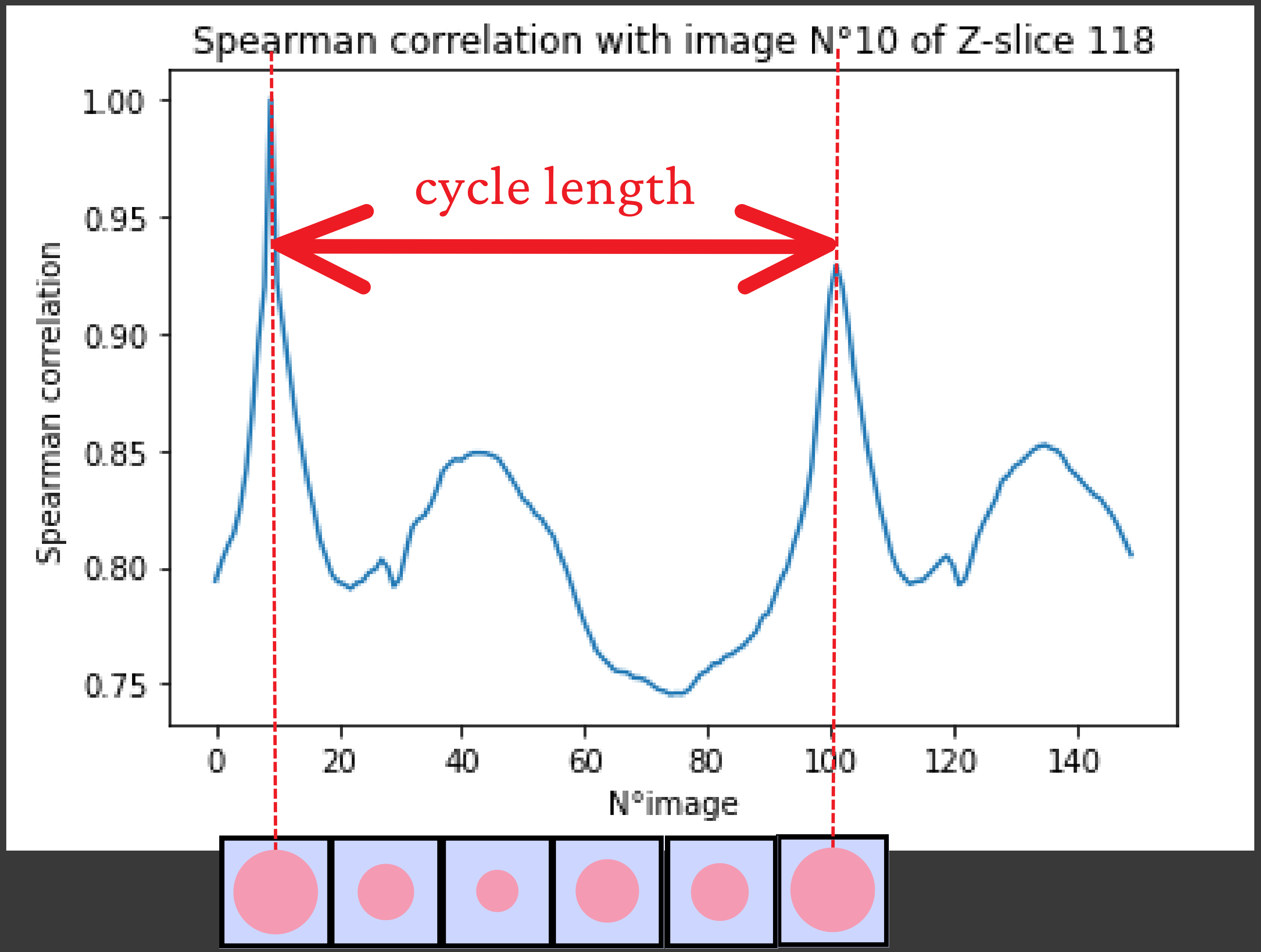


II - Methods

→ 1) Building a 3D+Time image of a beating heart

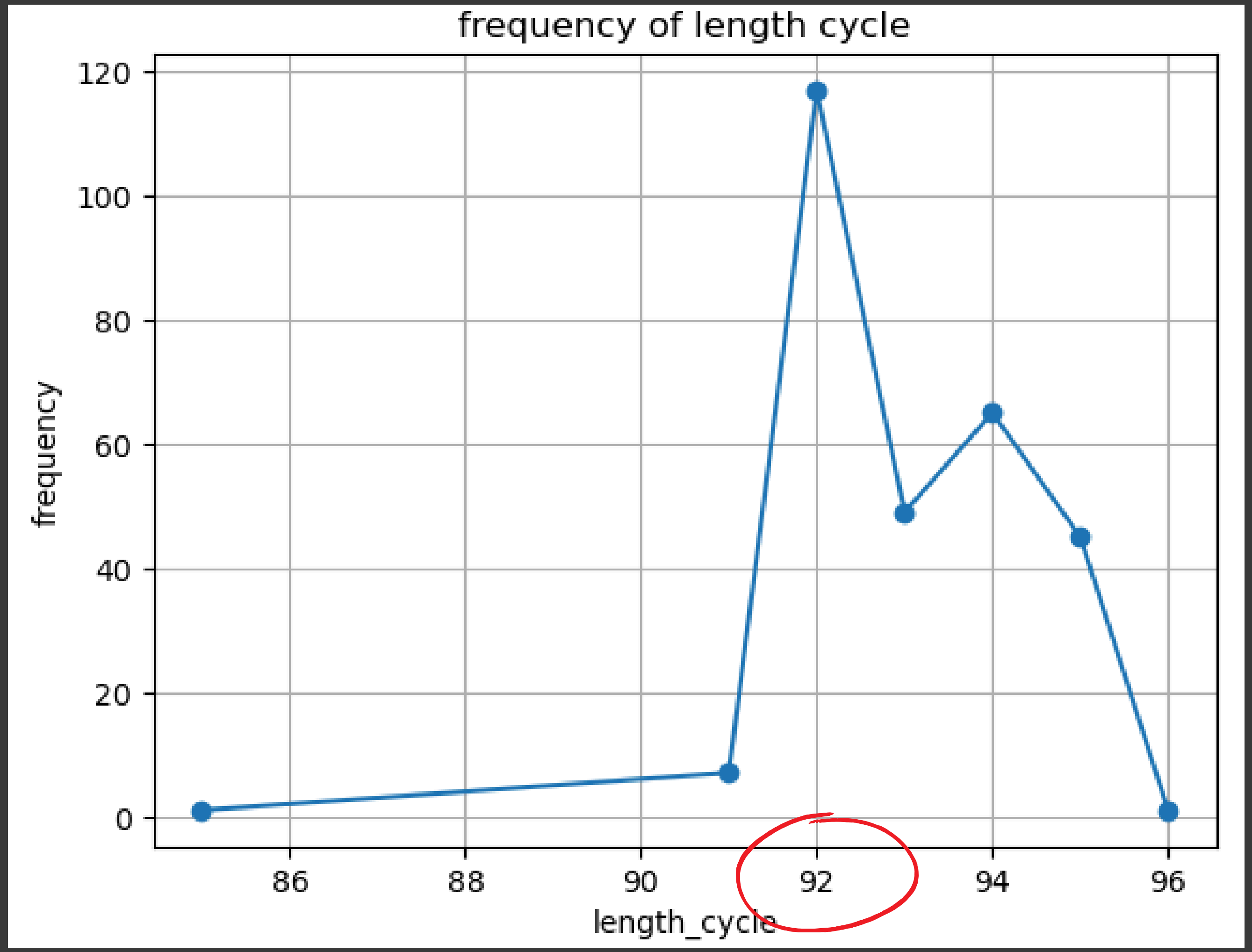


- Identifying cardiac cycle LENGTH with the Spearman correlation coefficient on the z-slices 116 to 120



Same test on every images from z-slices 116 to 120

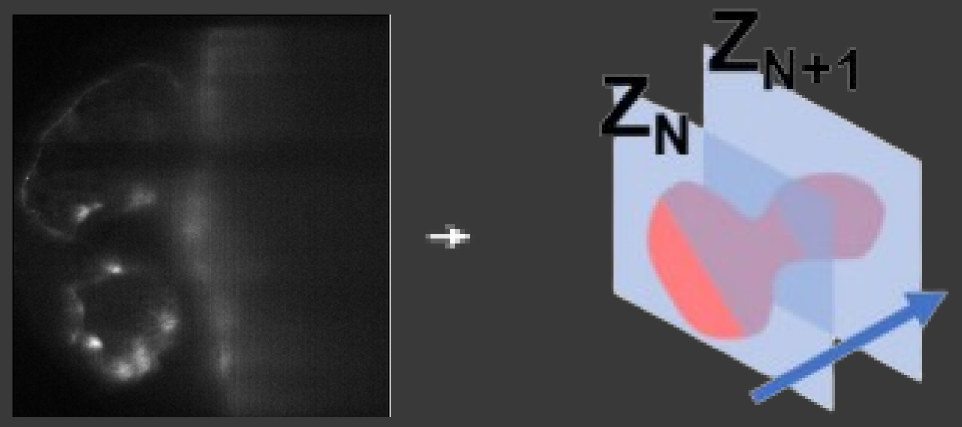
150*5 = 600 tests



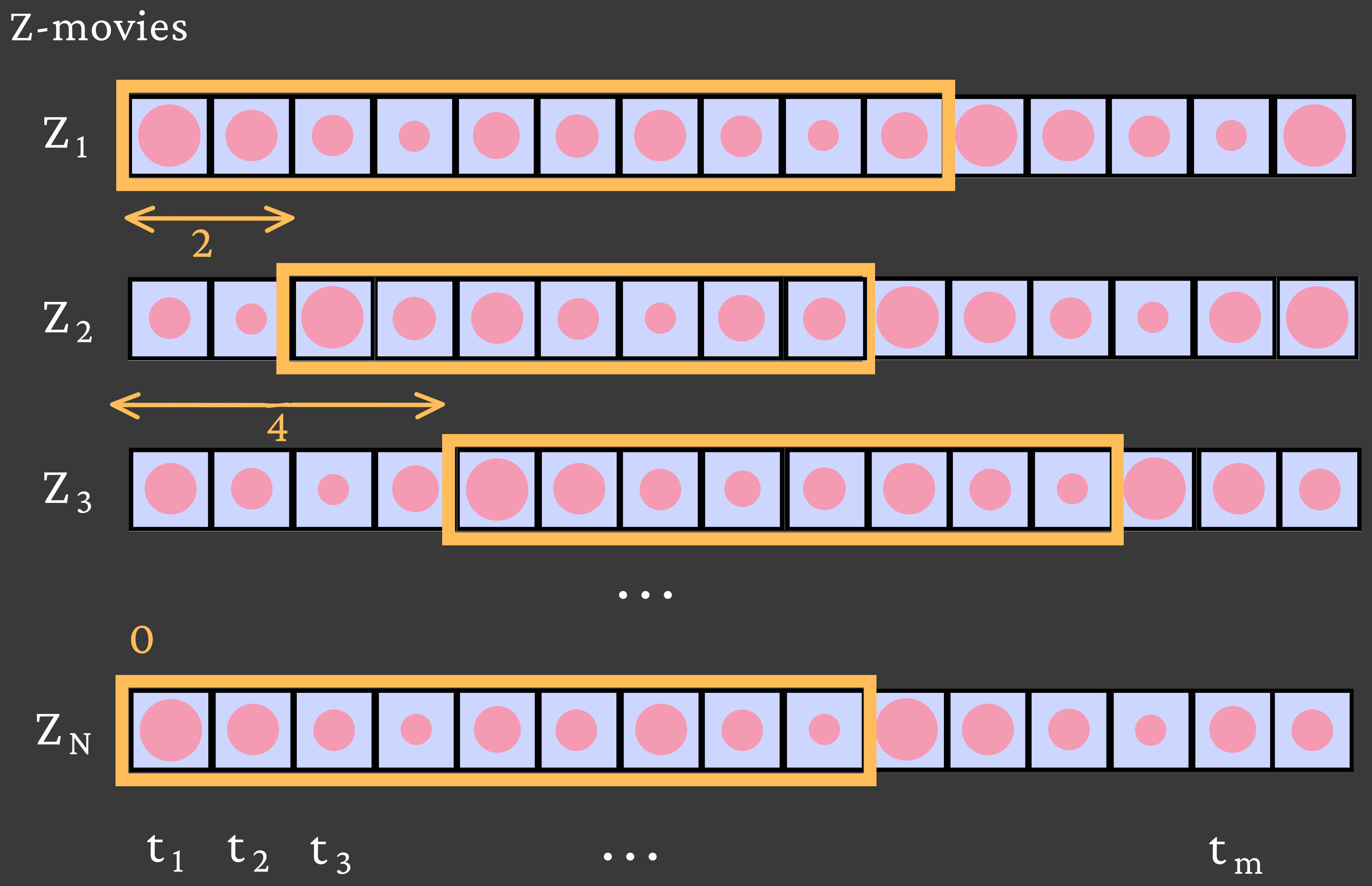
cardiac cycle length estimated at **92 images**

II - Methods

→ 1) Building a 3D+Time image of a beating heart



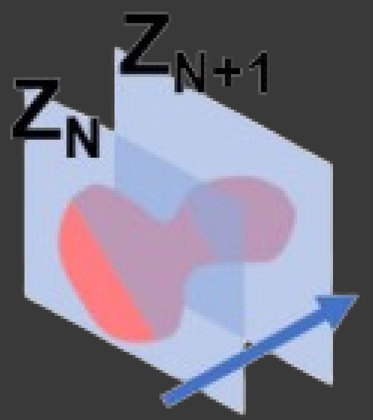
- Identifying cardiac cycle START with the Spearman correlation coefficient on the z-slices 116 to 120



list_shift_with_max_freq : [38, 38, 40, 38, 38, 36, 38, 38, 39, 37, 40, 40, 36, 35, 35, 34, 36, 36, 36, 35, 36, 35, 36, 38, 36, 36, 37, 36, 36, 35, 35, 35, 36, 38, 35, 34, 35, 34, 34, 34, 36, 35, 36, 36, 34, 35, 34, 35, 36, 33, 34, 34, 34, 34, 32, 34, 35, 35, 34, 35, 33, 33, 35, 34, 34, 36, 35, 34, 33, 34, 33, 33, 34, 33, 32, 36, 35, 33, 33, 33, 38, 35, 34, 35, 38, 37, 34, 34, 36, 35, 35, 36, 35, 36, 38, 36, 37, 37, 38, 39, 36, 38, 35, 34, 38, 37, 37, 39, 37, 36, 35, 38, 38, 35, 39, 35, 35, 36, 36, 39, 35, 36, 38, 38, 37, 37, 37, 38, 37, 39, 39, 37, 38, 36, 38, 40, 39, 40, 38, 38, 37, 37, 37, 35, 35, 37, 37, 39, 37, 38, 41, 41, 39, 38, 39, 38, 38, 39, 38, 39, 37, 39, 41, 39, 41, 39, 39, 39, 40, 39, 42, 42, 38, 41, 38, 39, 40, 39, 39, 40, 40, 39, 43, 39, 40, 43, 44, 42, 38, 41, 42, 38, 41, 41, 38, 38, 42, 47, 41, 46]

II - Methods

→ 1) Building a 3D+Time image of a beating heart

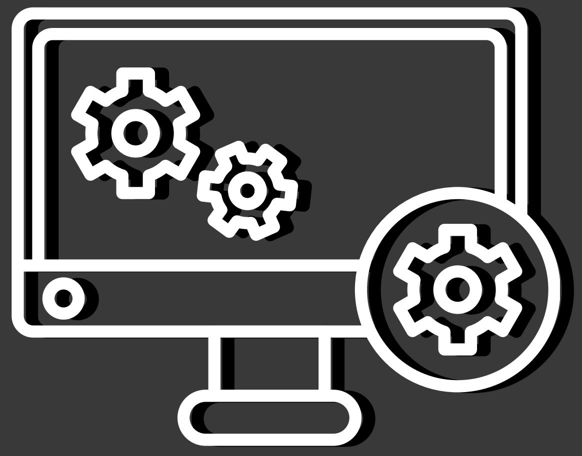


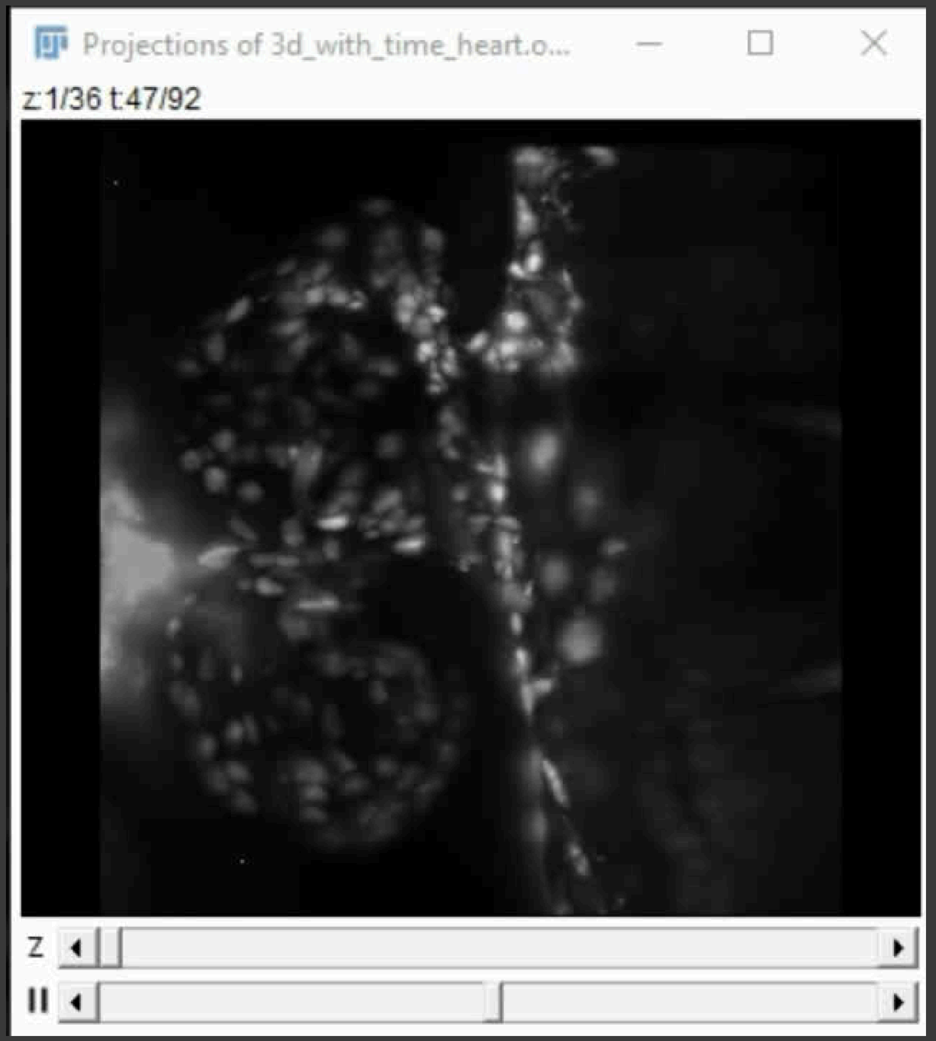
- Result :

cardiac cycle length : 92 images

list_shift_with_max_freq : [38, 38, ..., 41, 46]

z_slice_0
z_slice_1
z_slice_2
z_slice_3
z_slice_4
⋮
z_slice_198
z_slice_199
z_slice_200





II - Methods

→ 2) Segmenting and tracking nuclei in our 3D time-lapse image

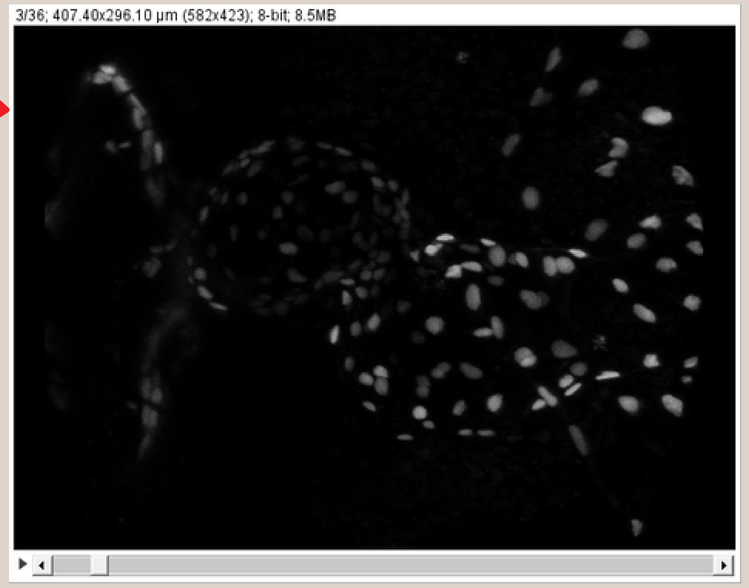
Materials: Dataset of 3D raw images and their associated manual segmentations (ground truths)

- 13022023_aqp8_OE_control_fish1220230214_121341 PM_w0002
- 14022023_marckl1_mutants_fish320230214_33435 PM_C2_NS
- C1-fish1-sih+++-_30012020
- C1-fish9-sih--_30012020
- fish1-48HPF-nuclei_NS
- fish2-30HPF-nuclei_NS
- fish2-36HPF-nuclei
- wt-heart 2 dpf-e1_25

⋮

34 stacks of different sizes

“+++” for wildtype and “sih” for silent heart



“aqp8” for aquaporin mutant which shows valve elongation defects

Tools : softwares for segmentation

itk-SNAP

Zebrasegtool

3DeeCellTracker

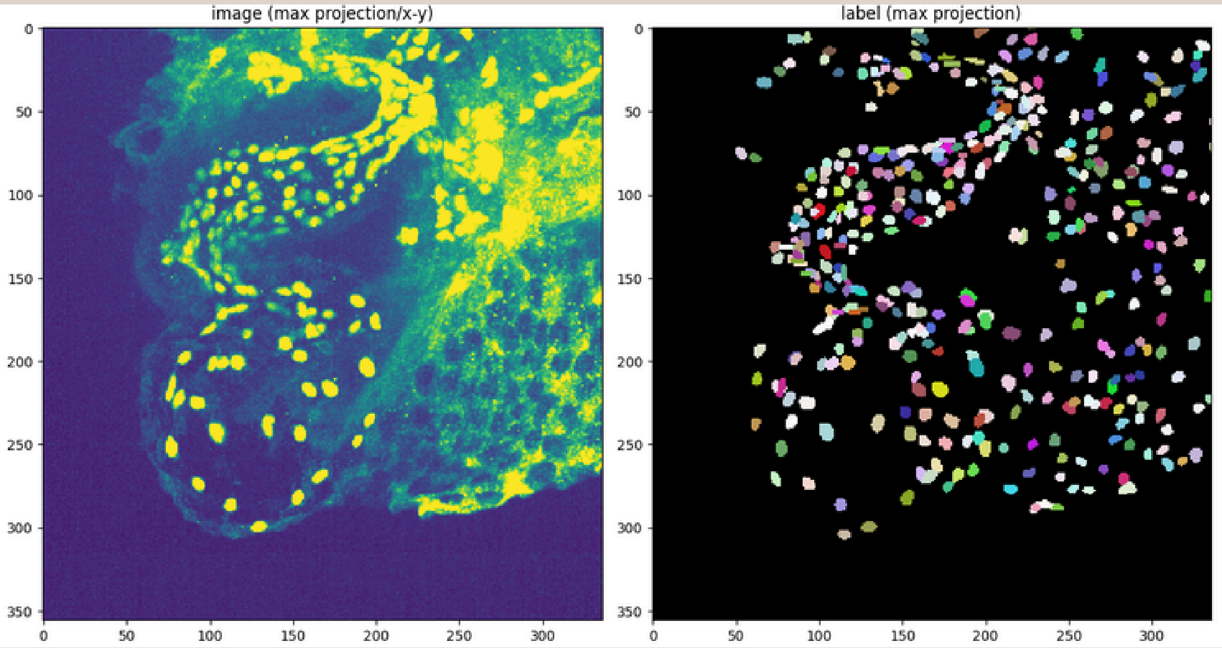
manual segmentation

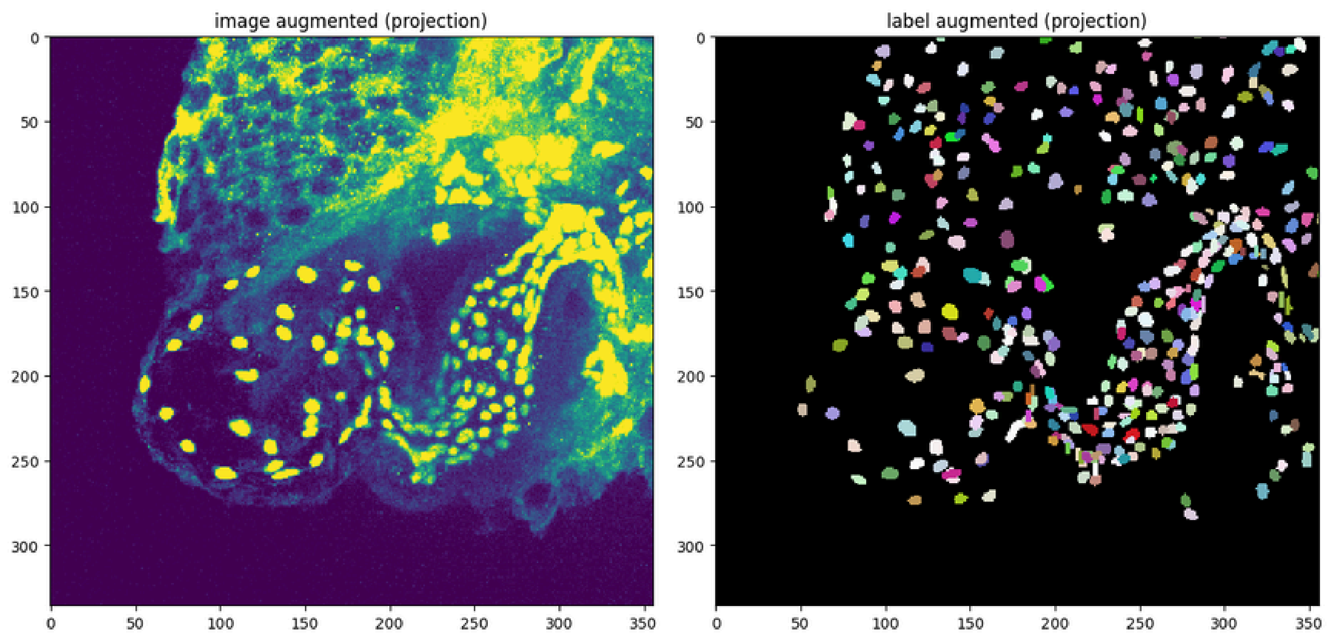
automatic segmentation

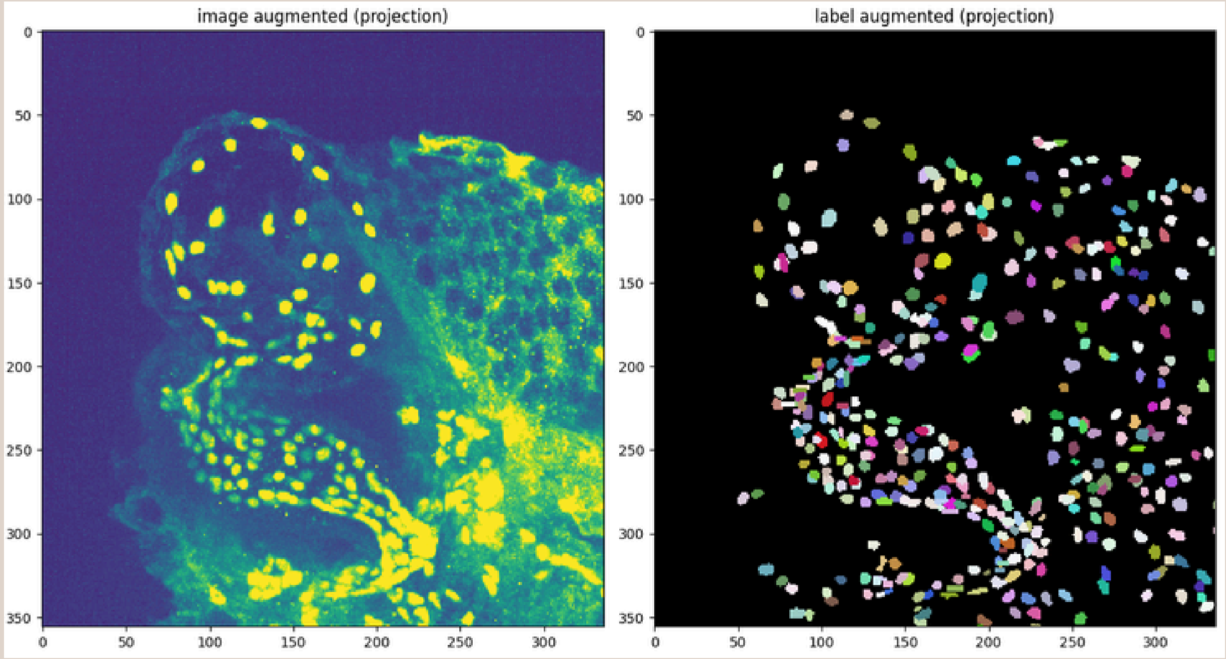
II - Methods

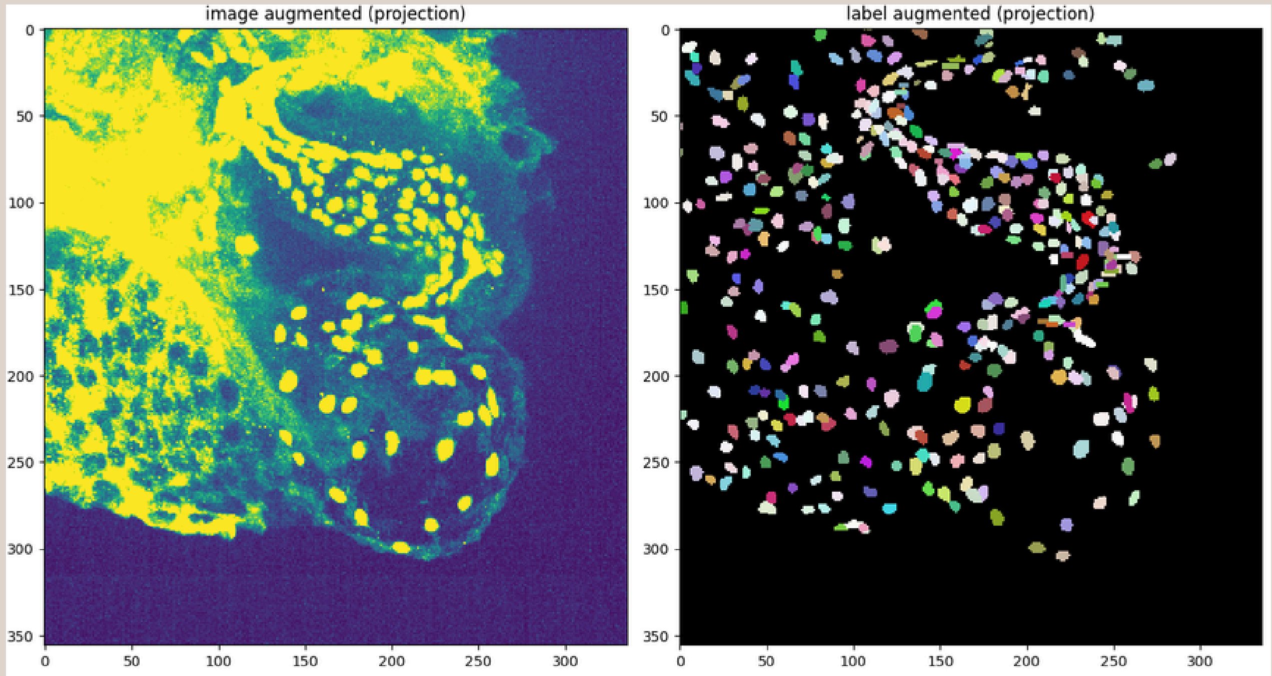
→ 2.1 - Train Stardist model

- Data augmentation









- Flipping
- zoom in
- crop

II - Methods

3DeeCellTracker

→ 2.1 - Train Stardist model

- Train StarDist3D

```
epochs=500

model.train(X_trn, Y_trn, validation_data=(X_val,Y_val), augmenter=sdw.augmenter, epochs=500)
```

```
Epoch 1/500
100/100 [==============================] - 60s 542ms/step - loss: 0.6121 - prob_loss: 0.0975 - dist_loss: 2.5726 - prob_kld: 0.0809 - dist_relevant_mae: 2.5724 - dist_relevant_mse: 11.5007 - dist_dist_iou_metric: 0.1929 - val_loss: 0.3870 - val_prob_loss: 0.0572 - val_dist_loss: 1.6486 - val_prob_kld: 0.0503 - val_dist_relevant_mae: 1.6482 - val_dist_relevant_mse: 4.5222 - val_dist_dist_iou_metric: 0.3881
Epoch 2/500
100/100 [==============================] - 12s 122ms/step - loss: 0.3853 - prob_loss: 0.0315 - dist_loss: 1.7688 - prob_kld: 0.0264 - dist_relevant_mae: 1.7685 - dist_relevant_mse: 5.7550 - dist_dist_iou_metric: 0.3563 - val_loss: 0.3377 - val_prob_loss: 0.0356 - val_dist_loss: 1.5109 - val_prob_kld: 0.0287 - val_dist_relevant_mae: 1.5105 - val_dist_relevant_mse: 3.7088 - val_dist_dist_iou_metric: 0.4276
```

Loss ↘

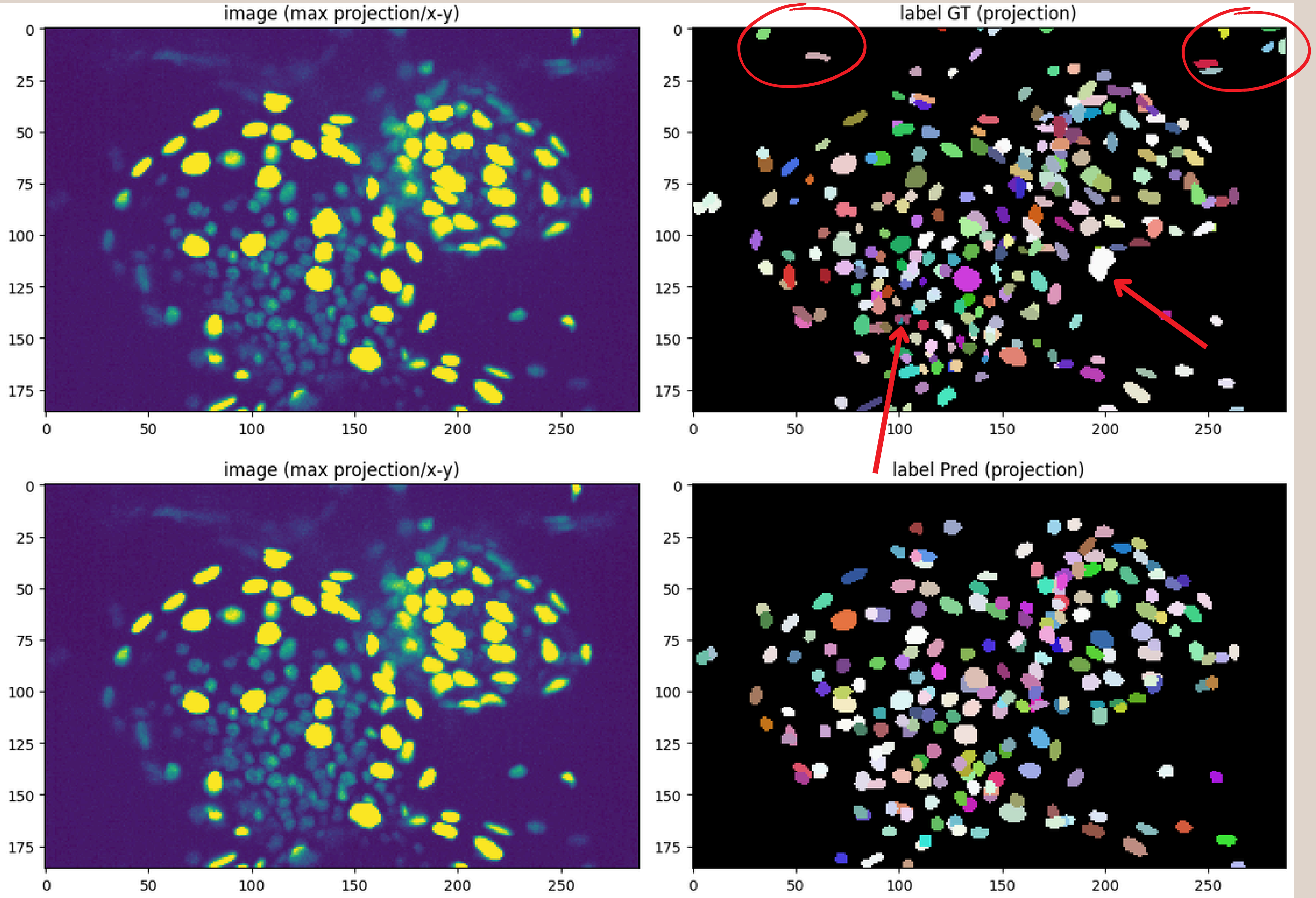
```
Epoch 474/500
100/100 [==============================] - 11s 110ms/step - loss: 0.1341 - prob_loss: 0.0077 - dist_loss: 0.6319 - prob_kld: 0.0023 - dist_relevant_mae: 0.6317 - dist_relevant_mse: 0.9412 - dist_dist_iou_metric: 0.7410 - val_loss: 0.1197 - val_prob_loss: 0.0102 - val_dist_loss: 0.5474 - val_prob_kld: 0.0034 - val_dist_relevant_mae: 0.5472 - val_dist_relevant_mse: 0.6210 - val_dist_dist_iou_metric: 0.7550
Epoch 475/500
 19/100 [====>.........................] - ETA: 8s - loss: 0.1321 - prob_loss: 0.0067 - dist_loss: 0.6270 - prob_kld: 0.0023 - dist_relevant_mae: 0.6268 - dist_relevant_mse: 0.9337 - dist_dist_iou_metric: 0.7240
```

Loss: Difference between the predicted segments and the actual labeled segments.
If the model's predictions are close to the actual segments, the loss is small.

II - Methods

→ 2.1 - Train Stardist model

- Threshold optimization & confirmation of the segmentation results with trained model

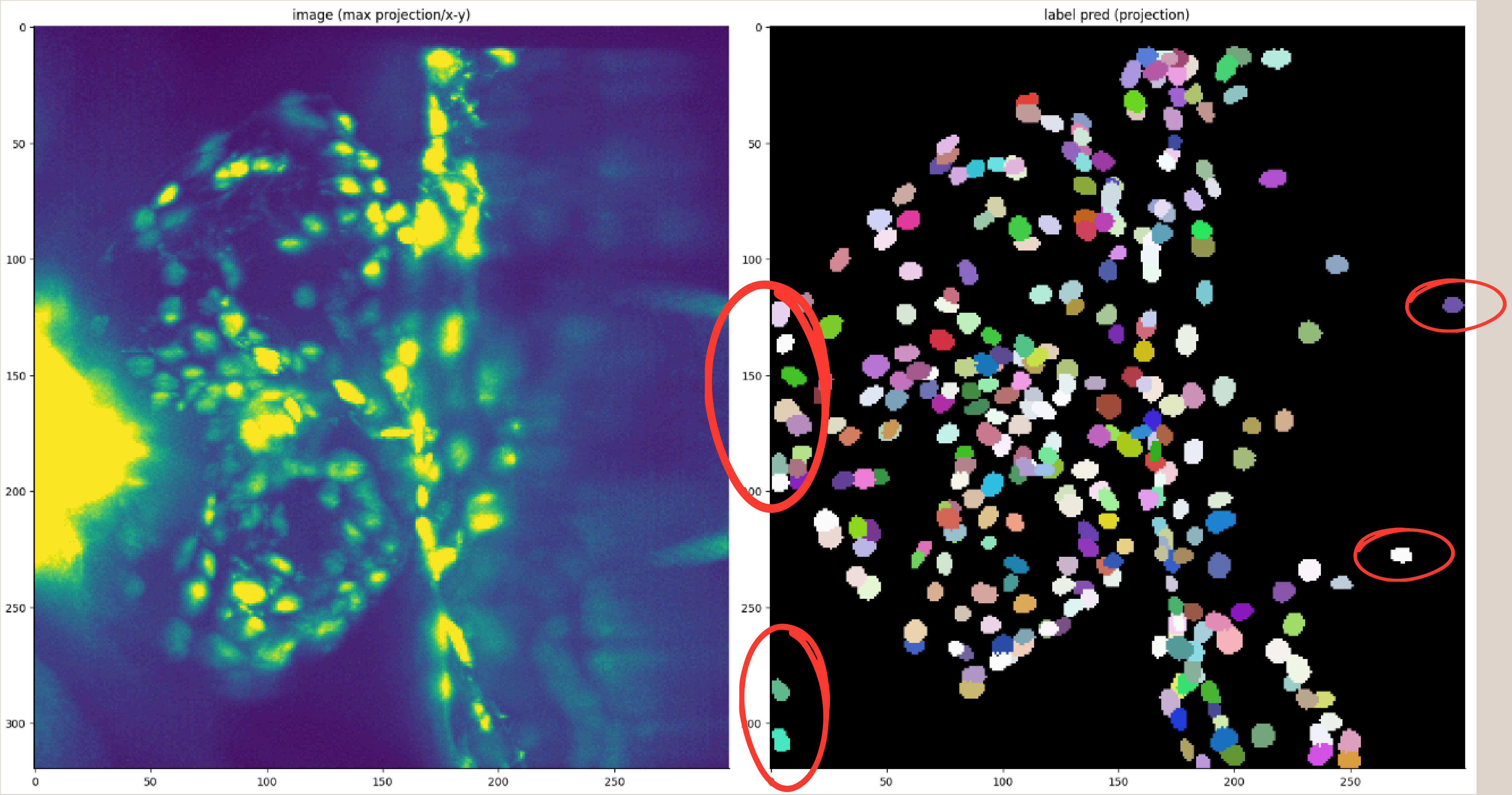


3DeeCellTracker

II - Methods

→ 2.2 - Use Stardist model

- Stardist segmentation of all the volumes of our image from t=0 to t=91 (**first volume showed**)



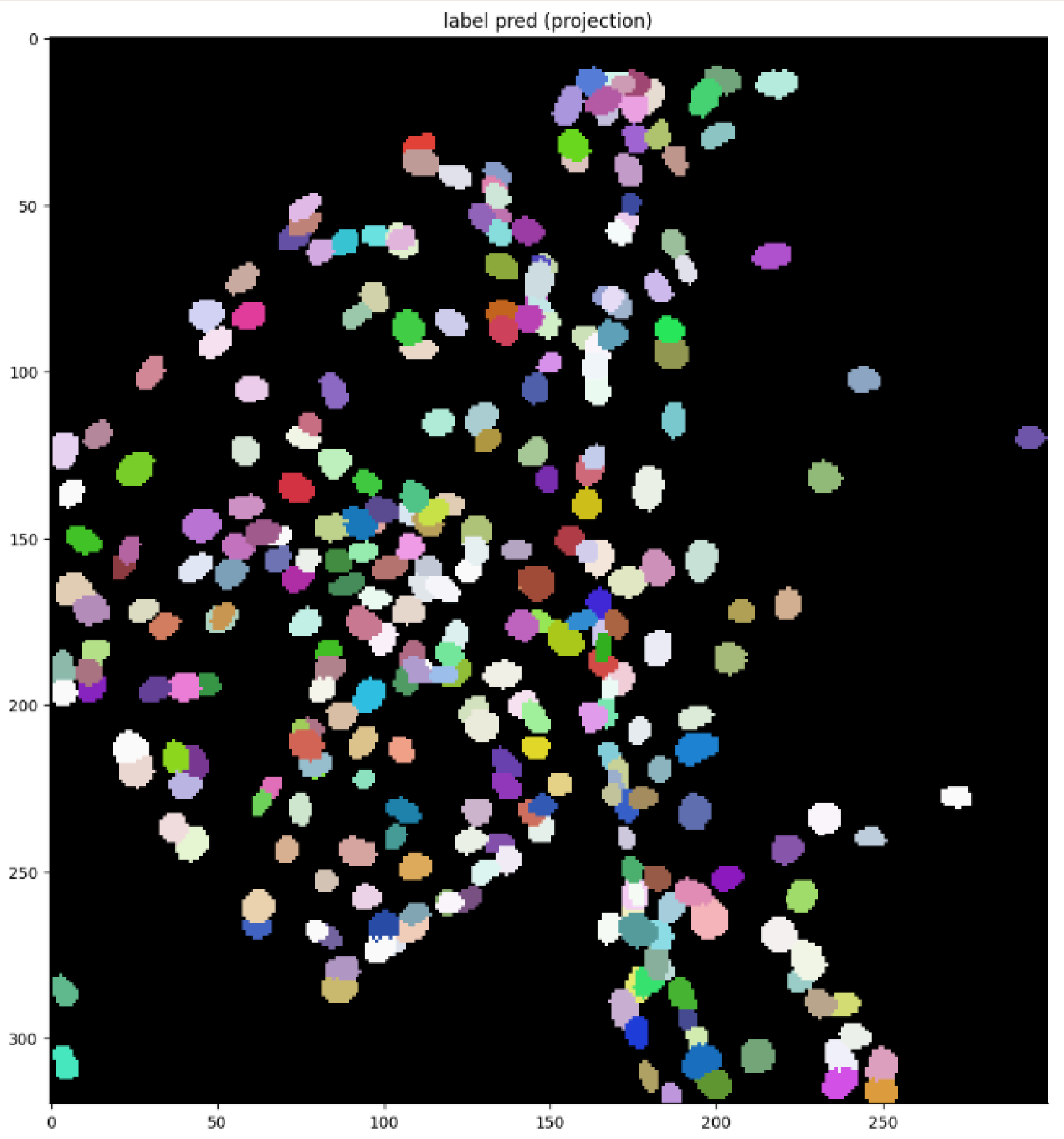
→ Noises that has been identified as cells and has to be removed manually with ITK-SNAP

3DeeCellTracker

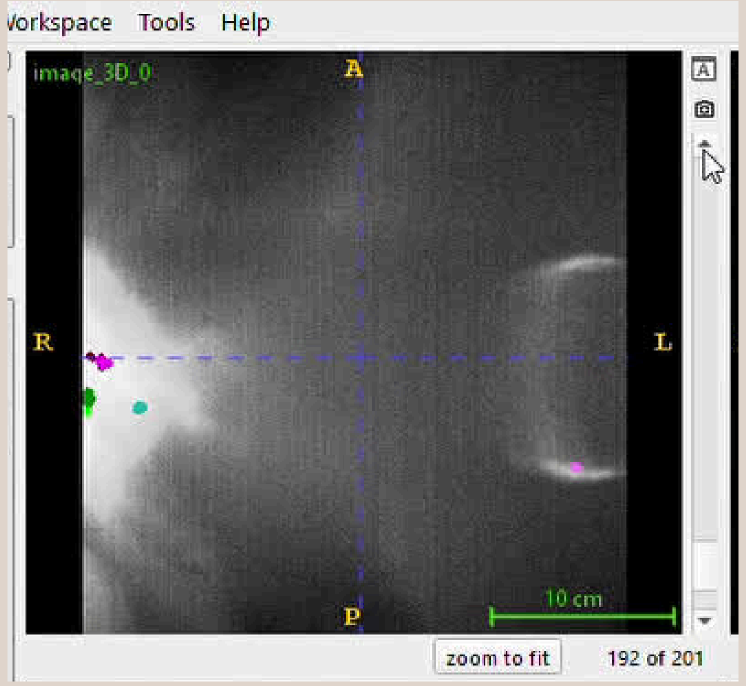
II - Methods

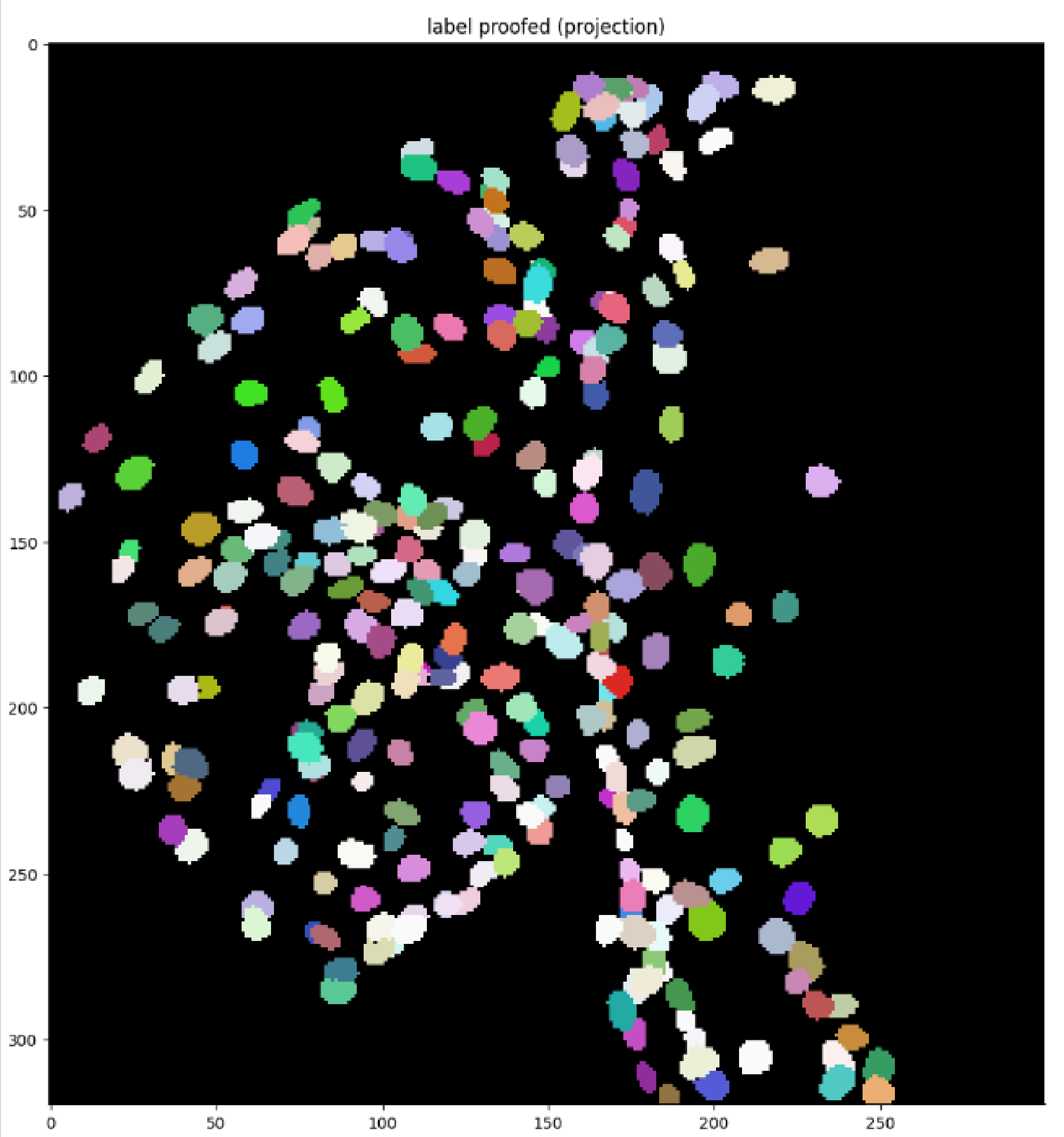
→ 2.2 - Use Stardist model

- Manual segmentation of the first volume and interpolation

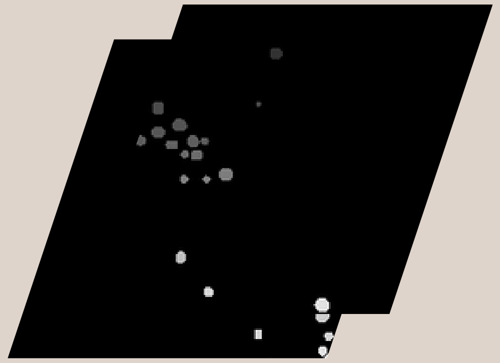


15 labels removed

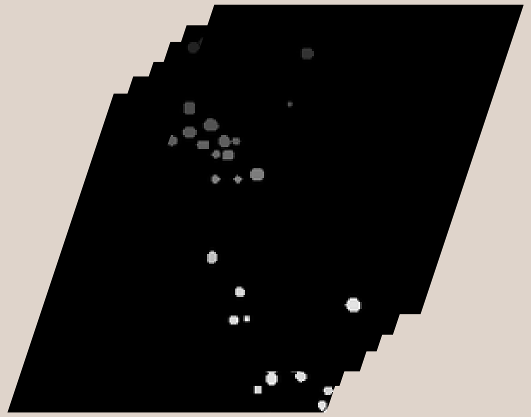




Interpolation :



x 10 : ↗ accuracy of the estimated cell positions

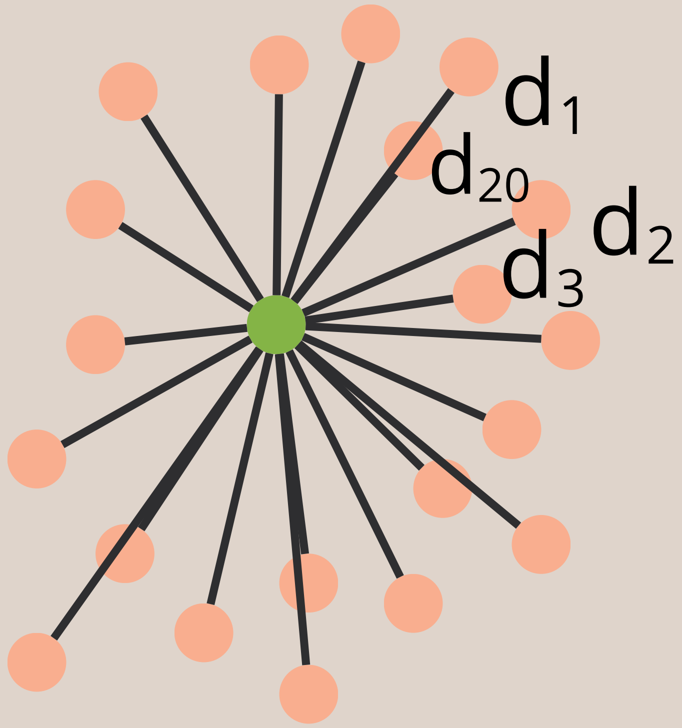


II - Methods

→ 2.2 - Tracking cells

- FeedForward Network (FFN)

image at t = 0

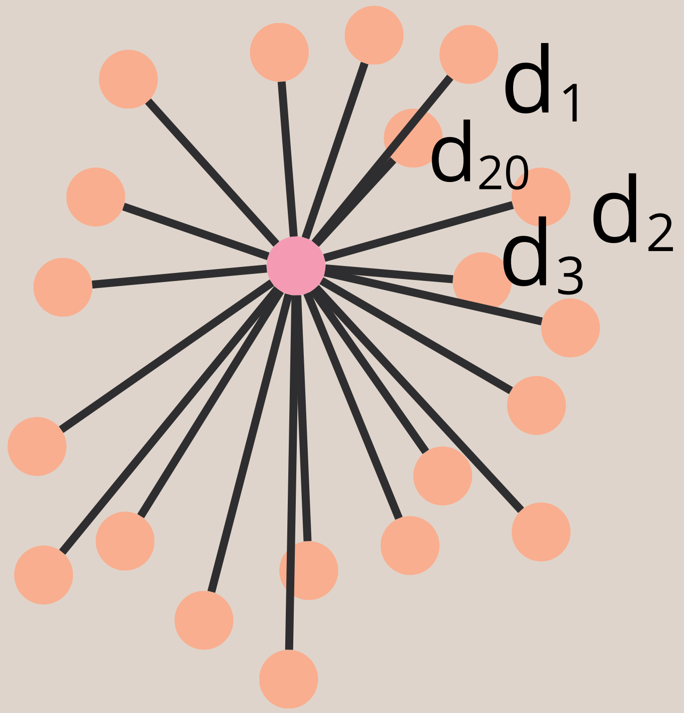


= f(d1/d,...d20/d, t=0)

= f(d1/d,...d20/d, t=1)

d = (d1 + ... + d20) /20

image at t = 1



FFN generates a similarity score indicating the likelihood that these points represent the same cell

→ =

The FFN was trained using over 500,000,000 synthetic training data points generated by simulating cell movements.

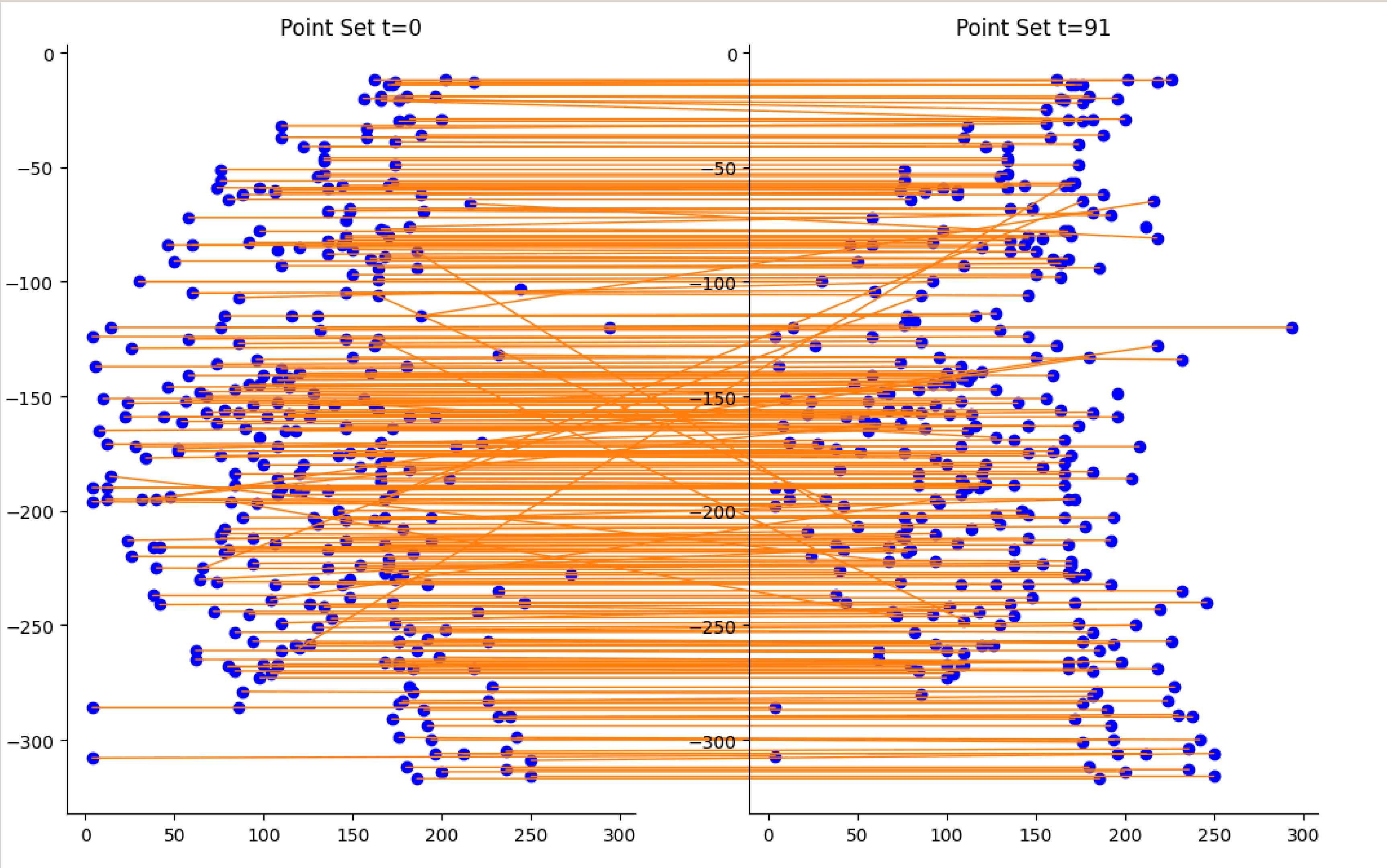
II - Methods

→ 2.2 - Tracking cells

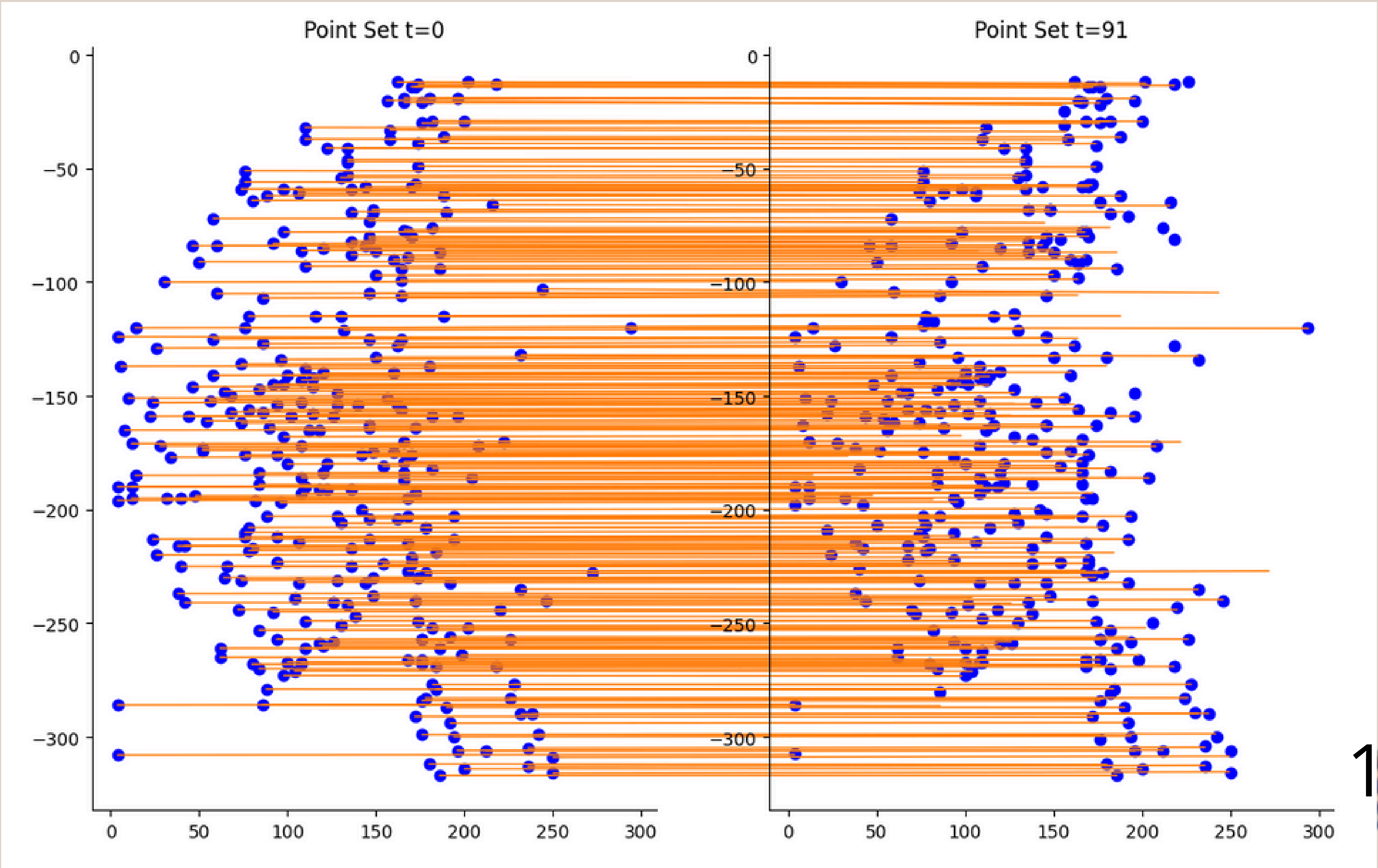
- PR-GLS

To improve the initial matching, a non-rigid point set registration method (PR-GLS) is used to generate a coherent transformation, that is, neighboring cells should have similar movements

Initial matching by Feed Forward Network



Coherent transformation by PR-GLS after FFN



II - Methods

→ 3 - Analysing the results

- Evaluate the performaces of 3DeeCellTracker

IMARIS
3D/4D Visualization & Analysis Software

To superimpose images of the tracked labels onto the raw images, and then visually checked the tracking results of each cell individually in 3D mode.

- Analysing the movement & speed of nuclei during an entire beat

PyVista

Utilize the coordinates of each cell over time to plot their movement and speed in an interactive 3D graph.

III - Expected results

- Evaluate the performaces of 3DeeCellTracker

Around 70% of the nuclei successfully tracked

- Analysing the movement & speed of nuclei during an entire beat

diastole : $V_{\text{ventricular cells}} > V_{\text{atrial cells}}$
systole : $V_{\text{atrial cells}} > V_{\text{ventricular cells}}$

VI - Perspectives for a future PhD student

- Enhancing tracking accuracy
- Analyzing cell surface/volume deformation
- Conducting comparative assessments across diverse transgenic fish samples
- ...

Thank you for your attention !