

# In Silico Labeling for phase contrast microscopy

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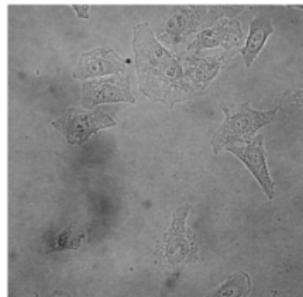
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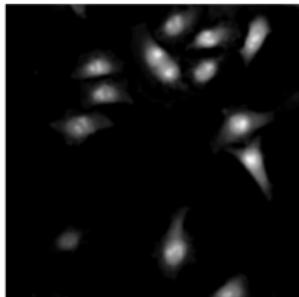
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# Introduction to In Silico Labeling

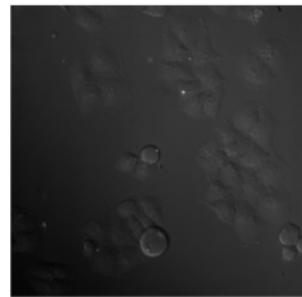
# Transmitted light microscopy



(a) Brightfield



(b) Phase contrast



(c) DIC

Microscopy technique to image morphology of cells and cellular structures

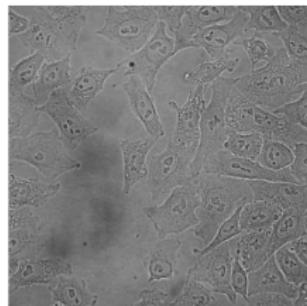
**Advantages** : simple method, modest cost, label-free

**Disadvantages** : low contrast images, no details

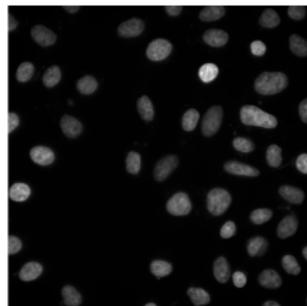
Most simple example of transmitted light microscopy : **Brightfield**

Different variants : **phase contrast** and **DIC** (Differential Interference Contrast)

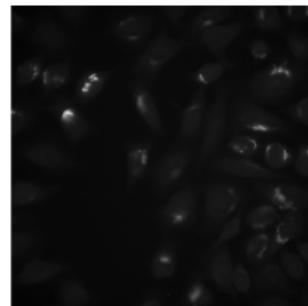
# Fluorescence microscopy



(a) Brightfield



(b) Dapi image



(c) Golgi image

Imaging of structures of interest by fluorescence labeling

Observation of **specific proteins** or **cellular structures** in greater detail

**Advantages** : images with very good signal-to-noise ratio, clarity, more details

**Disadvantages** : invasive, more complex, restricted number of simultaneous fluorescent tags

# In Silico Labeling

**Goal** : develop a method that combines "both" microscopy techniques

**Idea** : design a model able to predict the fluorescent image from its corresponding transmitted light image

**Advantage** : apply ISL model to get fluorescence counterpart of new transmitted light images

Prediction happens "in silico" → ISL method not limited by spectral overlap of fluorophores or experimental issues

# Internship tasks

Three main tasks :

- ① **Nuclei fluorescence prediction** → predict fluorescent labeling of nuclei from Brightfield and phase contrast images
- ② **Cell cycle fluorescence prediction** → predict fluorescence images that label cell cycle stages from DIC images
- ③ **Application of ISL to segmentation tasks** → pre-train a segmentation model on ISL task

# Fluorescence images prediction

# In Silico Labeling task

Supervised learning : ISL is an **image-to-image translation** task.

Develop a model that learns the mapping between a **transmitted light image** (input) and a **fluorescence image** (target).

Define model architecture, training loss, metrics,...

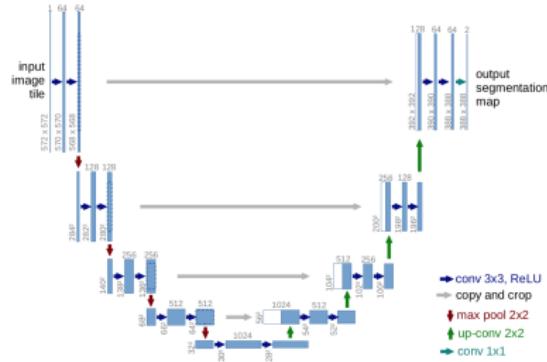
# U-Net architecture

U-Net model developed in 2015 by Ronneberger et al.

Developed for biomedical segmentation but can also be applied to other image-to-image translation tasks.

The model consists in :

- **a contraction path** → encodes the input image as a feature vector
- **an expansion path** → decodes the encoded vector as an output image.



# U-Net on Steroids

Developed in 2018 for the fourth Kaggle Data Science Bowl.

Based on **transfer learning**.

U-Net architecture with a **pre-trained encoder**.

Here, DenseNet121 trained on ImageNet database for image classification  
→ rich image feature representations.

**Idea** : pre-trained encoder learns better features from input images  
→ improved results.

## L1 loss

Initial naive approach : train U-Net model with **L1 loss**

$$\mathcal{L}_{L1} = \frac{1}{n} \sum_{i=1}^n |\hat{y}_i - y_i|$$

Euclidean distance between predicted and ground truth pixels

U-Net trained as a **pixel-wise regressor**

# Pix2pix model

Developed by Isola et al. in 2018 to solve image-to-image translation tasks.

Model based on **Conditional GANs**.

Generator receives an input image  $x$  and learns to predict the corresponding output image  $y$ .

**Goal** : to fool the Discriminator + to generate an image that matches the ground truth → L1 component added to its objective loss

We used our U-Net based model for the generator architecture

# Perceptual loss for image-to-image translation

**Other approach:** train our U-Net model to minimize a perceptual loss  
→ loss based on feature representations learnt by pre-trained networks

**Motivation :** perceptual losses capture image similarities more robustly than per pixel losses

Here, we use the **content loss** defined by Gatis et al.

$$\mathcal{L}_{content}(\hat{y}, y, l) = \frac{1}{2} \sum_{i,j} (\hat{F}_{i,j}^l - F_{i,j}^l)^2$$

where  $\hat{F}^l$  and  $F^l$  are respectively the feature representations of the predicted fluorescence image  $\hat{y}$  and its ground truth  $y$  in layer  $l$  of pre-trained VGG19

**High layers** in VGG network capture the image content without its precise appearance (Gatis et al.)

# Experiments

# Datasets and pre-processing

Two different datasets :

- **First dataset** : 960 images (2160x2160 size)  
→ phase contrast + brightfield + nuclei fluorescence images (DAPI)
- **Second dataset** : 100 images (1024x1024 size)  
→ DIC + nuclei fluorescence images (Hoechst) + cell fluorescence images (Cy5) + cell cycle fluorescence images (Cy3 + GFP)

**Pre-processing** : data augmentation + image normalization (z-score) + crops (512x512 size)

# Results : Nuclei prediction

## Results : Nuclei prediction from Brightfield and phase contrast images

**Task** : to predict DAPI images from Brightfield + phase contrast images.

**First approach** : U-Net model trained with L1 loss on 5540 crops and tested on 1850 crops.

Metric used : **Pearson correlation coefficient** (PCC) → measures linear correlation between two images.

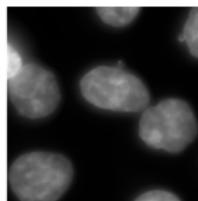
**Problem** :

- very low mean PCC ( $\approx 0.512$  on test set)
- very high std PCC ( $\approx 0.435$  on test set)

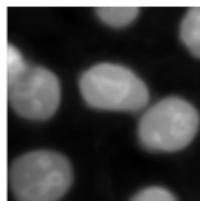
→ model performs well on certain images and poorly on others.

# Results

Ground truth dapi

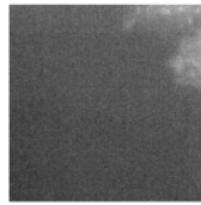


Predicted dapi

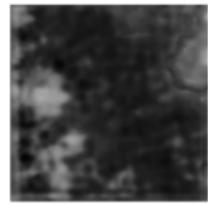


Pearson coeff : 0.952

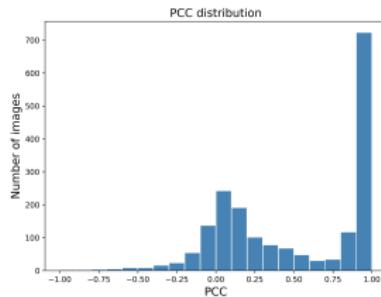
Ground truth dapi



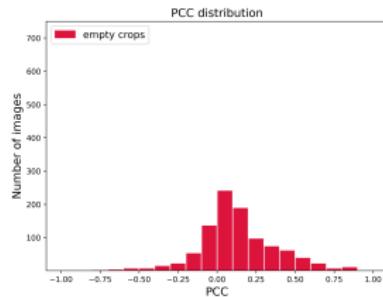
Predicted dapi



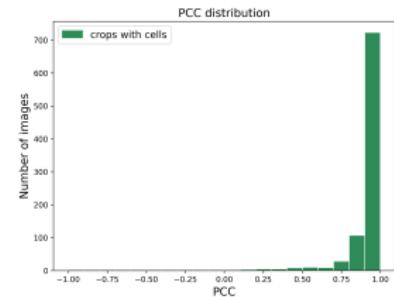
Pearson coeff : 0.145



(a) PCC on the whole test set



(b) PCC on empty test crops



(c) PCC on non-empty test crops

# Results

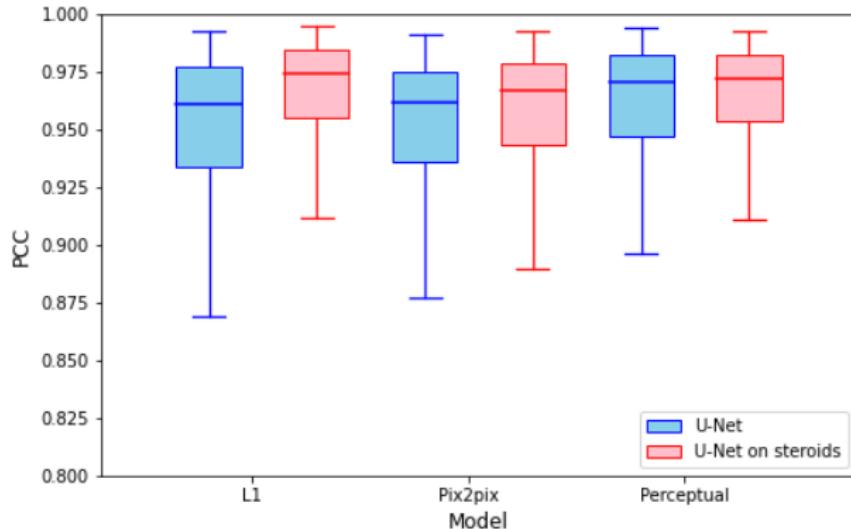
Solutions tested :

- ① Pre-processing → change the image normalization
- ② Weight our model loss
- ③ Question the metric choice

→ Not the main interest

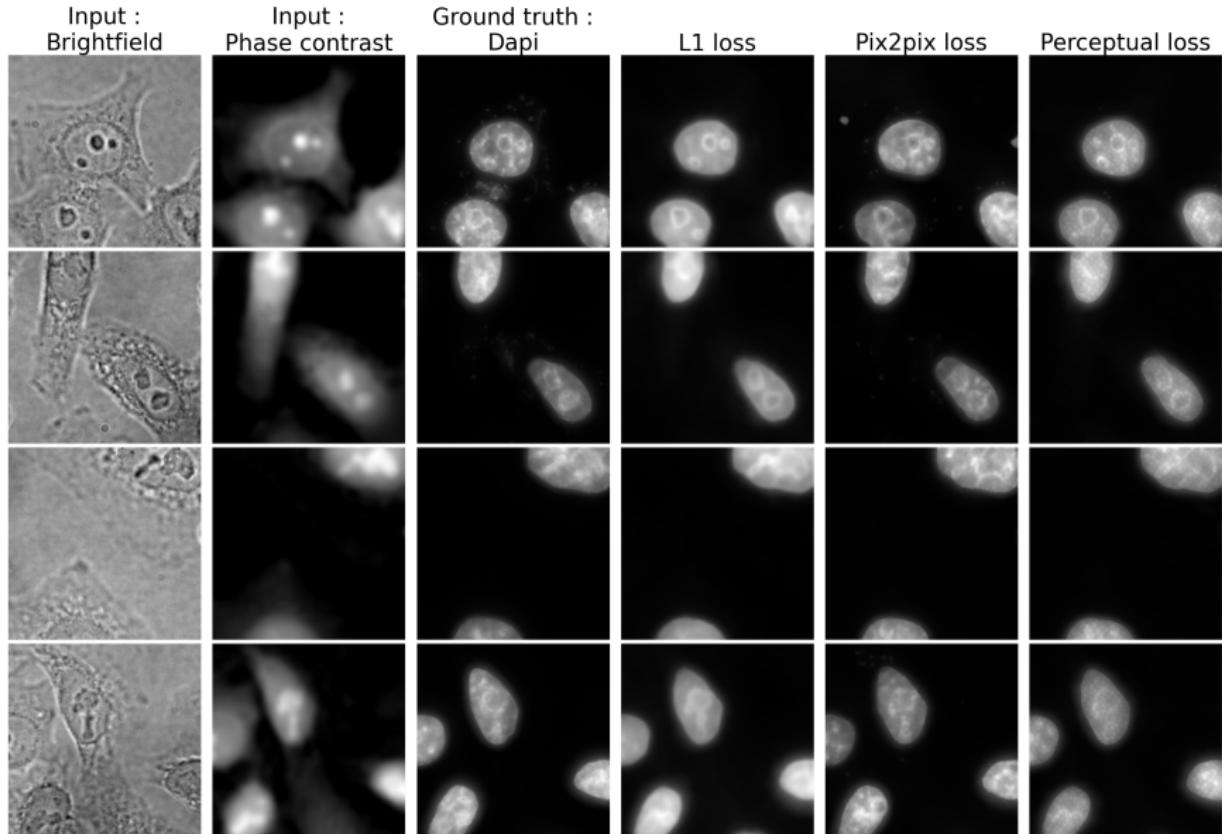
→ Models trained on 2626 non-empty crops and tested on 880 non-empty crops

# Results

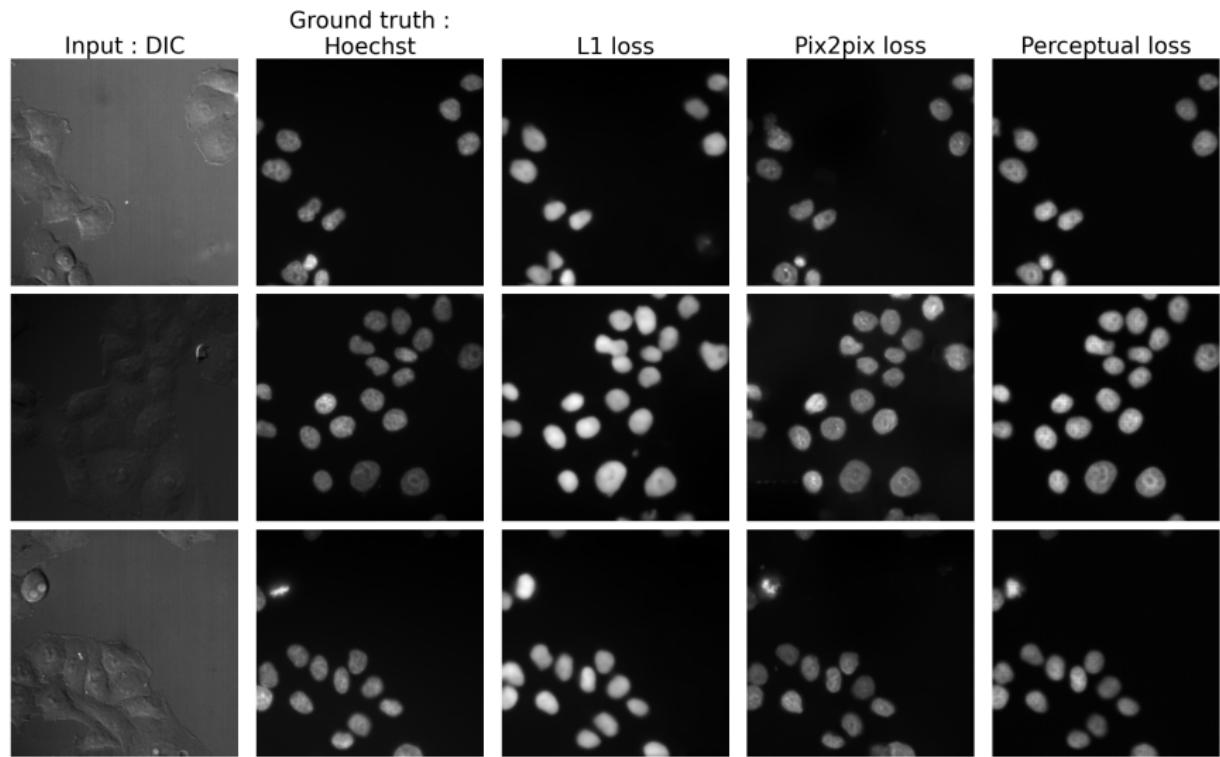


**Figure:** PCC for nuclei fluorescence prediction with both U-Net and U-Net on steroids, measured over 880 test crops.

# Results



# Results on DIC dataset : nuclei prediction



# Results : Cell cycle prediction

## Results : Cell cycle fluorescence prediction

**Task** : predict the fluorescent labeling of cells that undergo interphase (ie. that are in G1 or in S/G2 phases of the cell cycle).

Cells in G1 phase labeled by Cy3 red fluorescent dye.

Cells in S/G2 phases labeled by GFP green fluorescent molecule.

Models trained to predict both **Cy3** and **GFP** channels **together** from DIC images.

# Results : Cell cycle fluorescence prediction

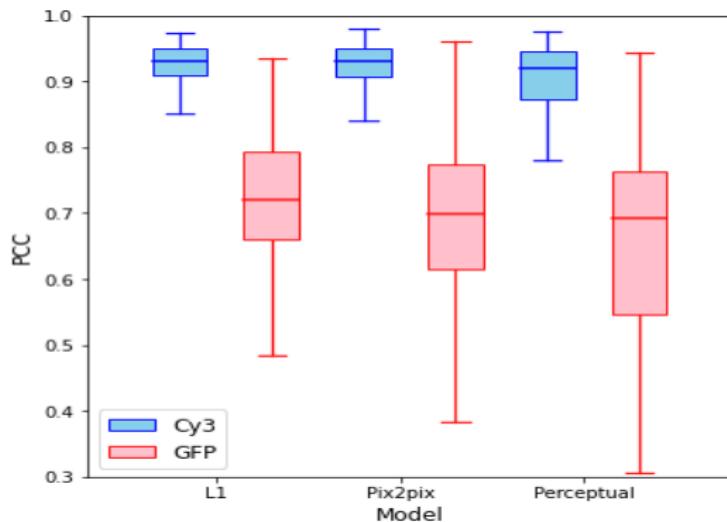
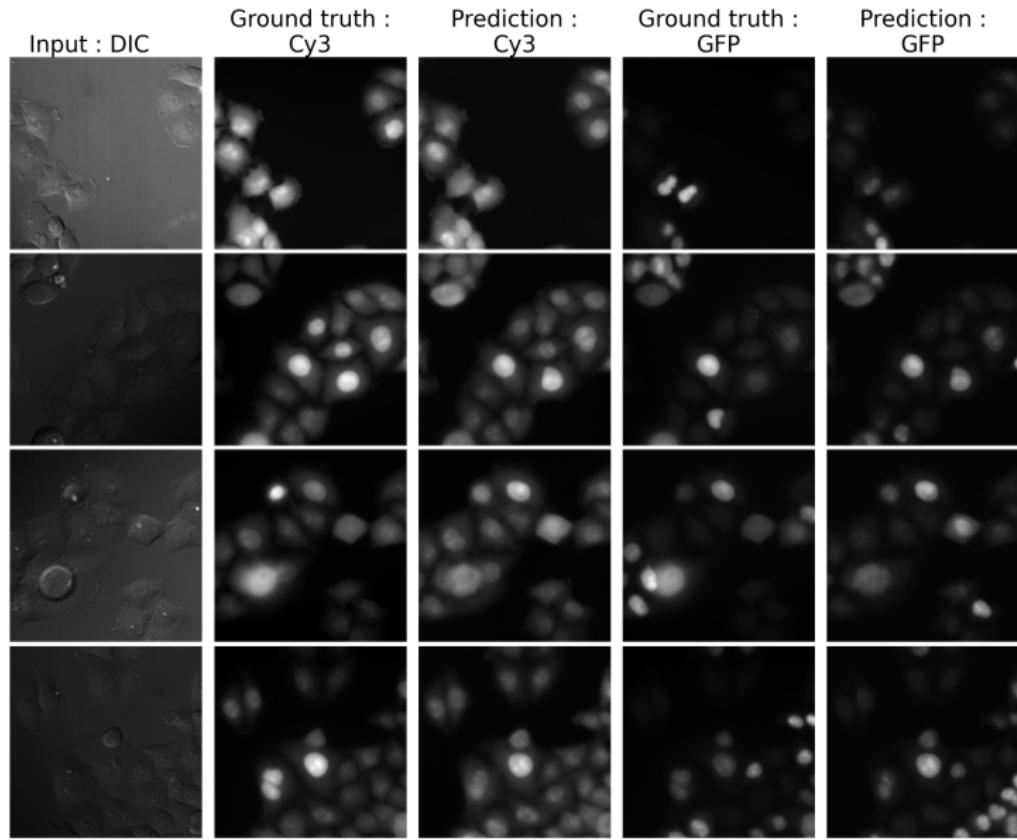


Figure: ISL for cell cycle fluorescence prediction from DIC images : U-Net on steroids with Perceptual loss

Good results on Cy3 prediction (**mean PCC 0.92**)

More difficulty to predict the GFP channel (**mean PCC 0.72**)

# Results : Cell cycle fluorescence prediction



# ISL for segmentation

# Application of ISL to segmentation : motivation

**Image segmentation** in bioimaging → very useful but complex task.

Development of deep learning models to solve these tasks.

But deep learning → large number of **annotated data**.

Idea : investigate the use of **ISL** for **pre-training** segmentation networks.

Example : nuclei segmentation.

# Application of ISL to segmentation : task

Comparison of two nuclei segmentation models (U-Net architectures with sigmoid activation in the last layer) :

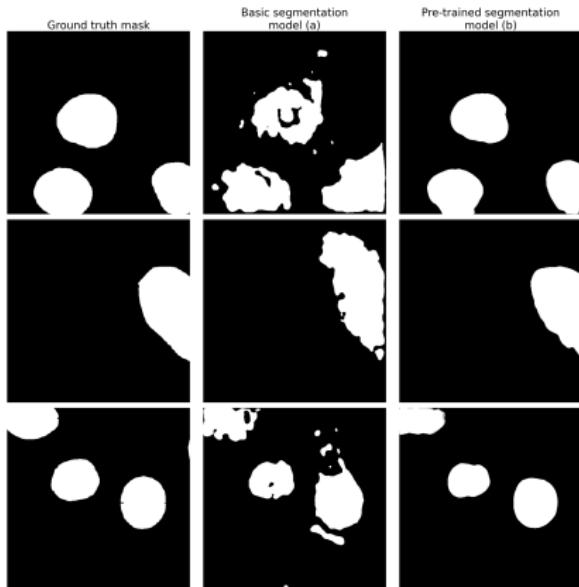
- a model initialized randomly,
- a model pre-trained on the prediction of fluorescence nuclei images (ISL task).

Training set with **increasing** number of images, fixed test set (185 images).

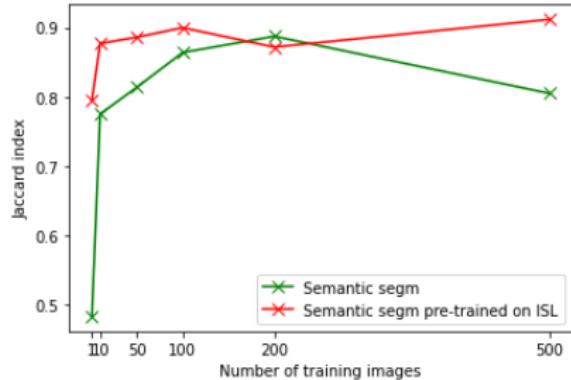
Metric used : **Jaccard index** (IoU) ; Training loss : **Jaccard Distance**.

$$IoU = \frac{|target \cap prediction|}{|target \cup prediction|} \quad \text{and} \quad \text{Jaccard Distance} = 1 - IoU$$

# Results



(a) Results on semantic segmentation : training on 1 image. (a) Basic segmentation and (b) segmentation pre-trained on ISL.



(b) Evolution of the jaccard metric on the test set in function of the number of training images.

# Conclusion

# Conclusion and future work

ISL performs well to predict **cell structures** as nuclei

Deceiving results on **cell cycle** fluorescence prediction → complex task

ISL can be used as a **pretext task** for segmentation

Future work :

- Try to generalize ISL as a pretext task for segmentation of other cellular structures (ie. cell segmentation)  
→ powerful tool to reduce the problem of massive annotation in Deep learning

# Thanks for listening !