AUTOMATIC MULTI CLASS ORGANELLE SEGMENTATION FOR CELLULAR FIB-SEM IMAGES

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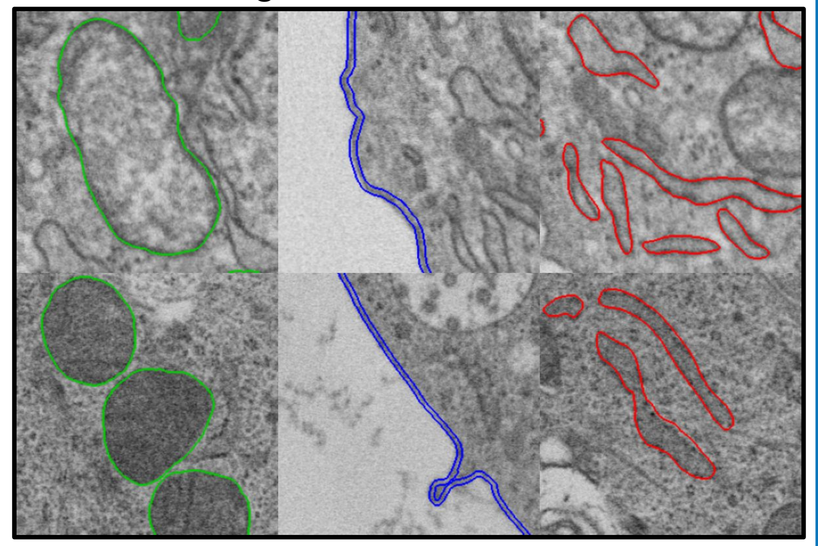
INTRODUCTION

- Focused Ion Beam milling combined with Scanning Electron Microscopy (FIB-SEM) technique is an electron microscopy imaging method that offers the possibility of acquiring 3D isotropic images of biological structures at the nanometric scale
- Cell compartments and organelles annotation is crucial to extract quantitative information such as size, distribution and morphology
- Information could be useful for medical analysis at the cell scale
- Automation of the segmentation step is required for analysis of huge image stacks and to save time consuming manual intervention.

Example of FIB-SEM image
6 patches of size 256 x 256

3 annotated classes :
- mitochondria in green
- cell membrane in blue
- endoplasmic reticulum in red

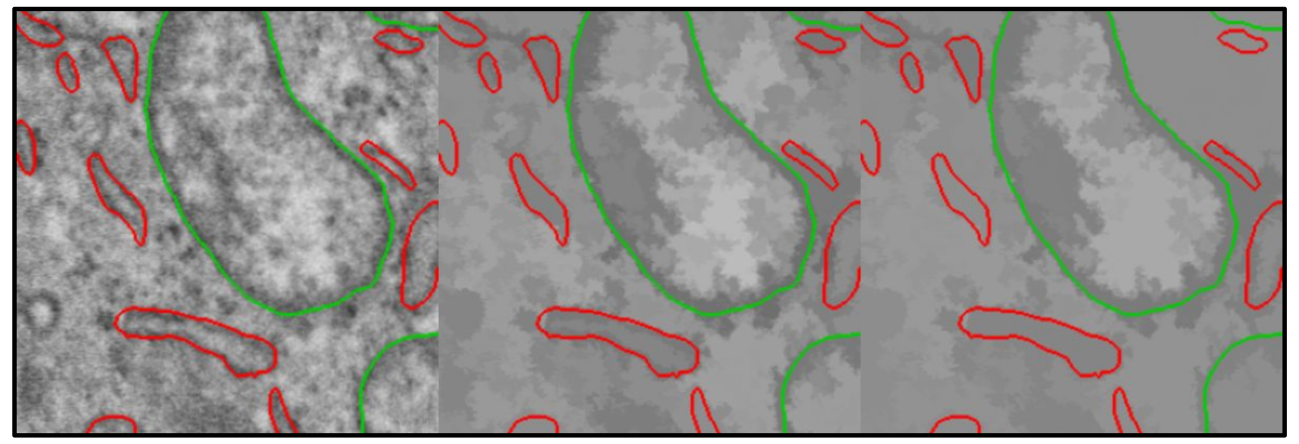


METHOD

- Convolutional Neural Network for semantic segmentation
  - UNet : usual contracting/expanding network with skip connection
  - EfficientUNet : UNet architecture with an EfficientNet contracting network
- Multiclass dice loss, with X_i the label for class i and Y_i the output given for class i

$$\mathrm{Loss} = 1 - \frac{1}{c}\sum_{i=1}^{c} \alpha_i \times \mathrm{Dice}(X_i, Y_i)$$

- Grain filter preprocessing from mathematical morphology



Example of filtered patches with threshold value λ of 650 (middle) and 3200 (right)

RESULTS

Experiments details

Data

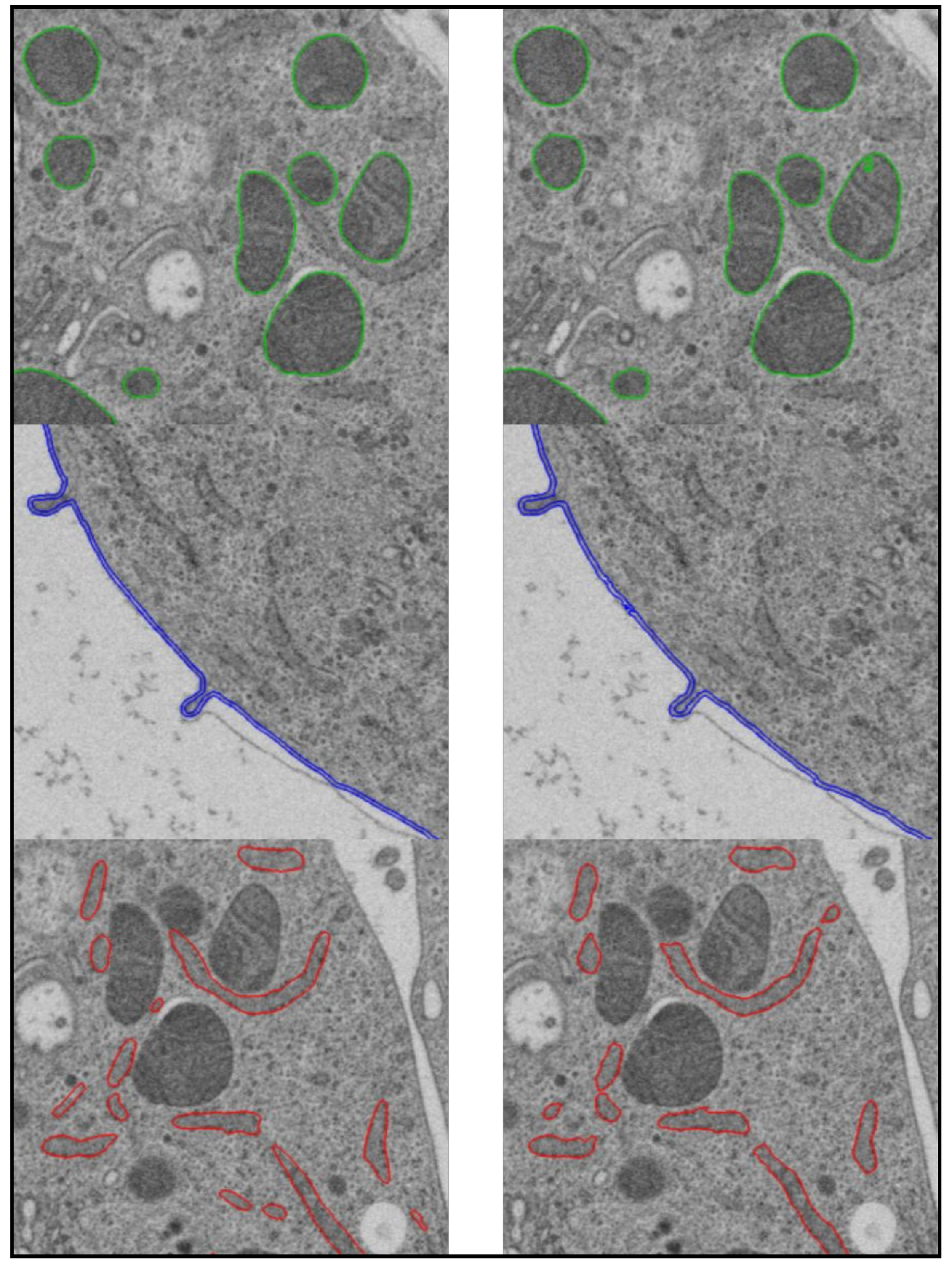
- Two different FIB-SEM images annotated by two experts
- FS-1
  - annotated slices : 79
  - resolution : 5nm x 5nm x 20nm
- FS-2
  - annotated slices : 80
  - resolution : 7.5nm x 7.5nm x 15nm
- 3 classes
  - mitochondria
  - cell membrane
  - endoplasmic reticulum

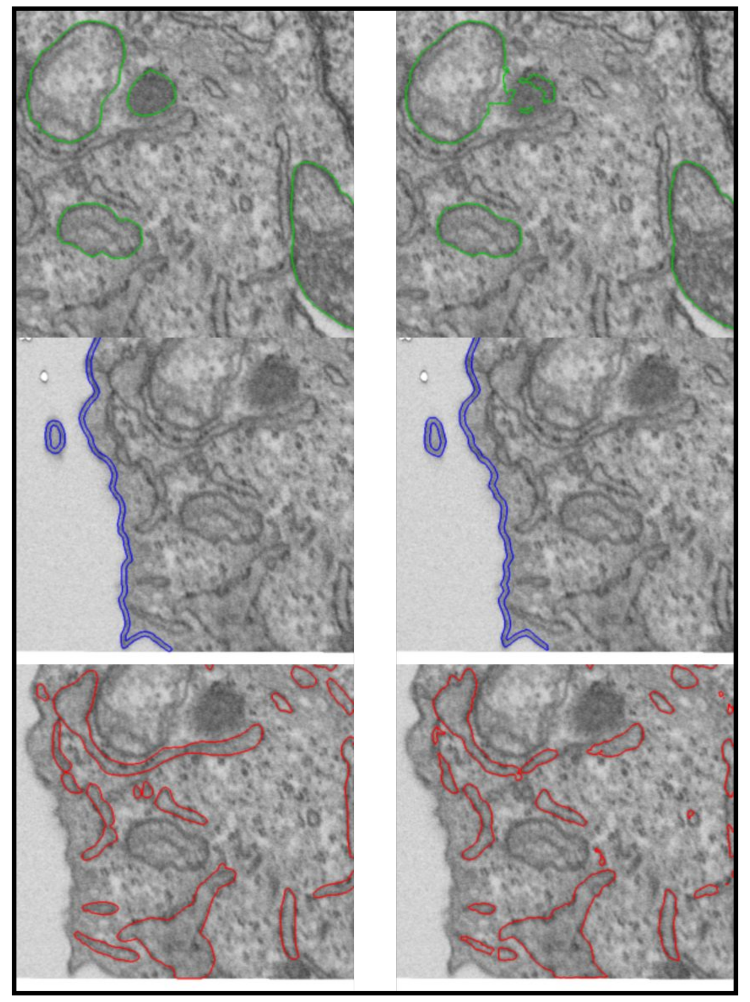
Methodology

- 256 x 256 patches
  - oversampling patches containing annotation
- Class weight : 0.1 (bg), 0.7 (mito), 1.0 (mem), 0.9 (endo)
- Grain filter preprocessing
  - λ = 650 and λ = 3200

Implementation

- TensorFlow, Keras and Higra





Comparison on 500 × 500 subsections of image ground truth on the left, our method on the right
FS-2 on the left, FS-1 on the right

Method	F1 Class 1	F1 Class 2	F1 Class 3
	FS-1 image		
UNet	0.937 (0.009)	**0.806 (0.012)**	0.724 (0.010)
UNet + Filter	0.939 (0.013)	**0.800 (0.019)**	0.731 (0.012)
EUNet	**0.951 (0.008)**	**0.764 (0.036)**	0.733 (0.015)
EUNet + Filter	**0.947 (0.011)**	**0.757 (0.040)**	**0.739 (0.011)**
Ilastik	0.185	0.672	0.112
2nd expert	0.941	0.751	0.740
	FS-2 image		
UNet	**0.955 (0.002)**	**0.754 (0.005)**	0.727 (0.003)
UNet + Filter	**0.950 (0.002)**	**0.742 (0.009)**	0.716 (0.005)
EUNet	**0.957 (0.001)**	**0.752 (0.009)**	**0.739 (0.007)**
EUNet + Filter	**0.955 (0.002)**	**0.748 (0.009)**	0.729 (0.008)
Ilastik	0.872	0.520	0.216
2nd expert	0.949	0.593	0.783

F1 score on test set, mean (standard deviation)

Method	True Positive	False Negative	Under Detected	True Positive	False Negative	Under Detected
	Class 1 (mitochondria)			Class 3 (endoplasmic reticulum)		
	FS-1 image					
EUNet + Filter	**92.7% (179/193)**	4.7% (9/193)	2.6% (5/193)	**46.9% (485/1034)**	19.1% (198/1034)	33.9% (351/1034)
Ilastik	0% (0/193)	49.2% (95/193)	50.8% (98/193)	0% (0/1034)	82.2% (850/1034)	17.8% (184/1034)
2nd expert	**100% (10/10)**	0% (0/10)	0% (0/10)	23.6% (13/55)	**9.1% (5/55)**	**67.3% (37/55)**
	FS-2 image					
EUNet	**89.0% (323/363)**	6.1% (22/363)	5.0% (18/363)	**56.1% (824/1468)**	21.5% (316/1468)	22.3% (328/1468)
Ilastik	62.8% (228/363)	4.7% (17/363)	32.5% (118/363)	0% (0/1468)	60.6% (889/1468)	39.4% (579/1468)
2nd expert	**100% (21/21)**	0% (0/21)	0% (0/21)	26.5% (18/68)	**19.1% (13/68)**	**54.4% (37/68)**

Percentage of detected component on test set

CONCLUSIONS

We proposed a deep learning based method, experimented a grain filter preprocessing and two different model architectures. The grain filter preprocessing does not consistently improve results despite noticeable improvement for the human perception. The EfficientUNet model slightly improves the results.

Our method achieves results **close to inter-expert variability**.

Our method has the advantage of being **generic** to multi-class organelles segmentation, allowing the addition of new classes easily.

Adding more organelles to the segmentation task, such as the golgi apparatus, endosome, and the nucleus with its heterochromatin and euchromatin or nucleolar compartments or the nuclear envelope will be an interesting challenge.

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https://github.com/Cyril-Meyer/NeNISt