

Detection of organelles in FIB-SEM microscopy images

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Abstract

This paper presents a novel application of the YOLOv8 model for addressing the task of detecting organelles in Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) biological images. The motivation behind this research stems from the need for accurate and efficient detection techniques to identify complex microstructures in biological material samples. The primary objective of this project involves fine-tuning of YOLOv8, a state-of-the-art object detection model, for semantic detection on FIB-SEM images. The study involves retraining the YOLOv8 model to suit the unique characteristics of FIB-SEM data, optimizing its performance for precise delineation of cellular structures. Experimental results showcase the model's performance in effectively detecting intricate features while maintaining computational efficiency, enabling in-depth analysis and understanding of diverse microscopy samples in scientific research and industrial applications.

1 Introduction

Focused Ion Beam Scanning Electron Microscope (FIB-SEM) Images are captured using an ion beam to directly interact with sample surfaces [1]. The process involves removing a thin surface layer, exposing a new surface that is imaged by an optical sensor. The process is repeated for the entire three-dimensional sample, producing a stack of slices. Typically, these images have nanometer resolution, with around 2000 pixels on the longer side. They are often stored in a format that permits stacking for easy random access to regions of the image, which proves advantageous for FIB-SEM images and 3D visualisation of the analysed material. Due to their very large resolution, detection in FIB-SEM images is a time-consuming task, being desirable to have computerised tools to automate the process. Unfortunately, FIB-SEM images are quite noisy and current detection algorithms often fail to identify small objects. In this work, we propose using YOLOv8 to perform FIB-SEM biological image detection, retraining the model using a proprietary FIB-SEM dataset.

2 Literature review

Aswath et al. [2] reviewed 38 research papers surrounding the theme of using Deep-Learning techniques applied to FIB-SEM images, mainly covering Convolutional Neural Networks (CNN), resulting in a table comparing how they were acquired for analysis, the region from where they were taken, the number of pixels per nanometer, the size of the dataset in pixels, the labeled (sub) cellular structures to be detected in the images and the paper's source. They conclude that Fully Convolutional Networks (FCN) and U-NET are the most notable backbone networks due to their use of deeper contextual network architectures, which are essential for 2D prediction and subsequently 3D reconstruction. Rosebrock [3] has published a book detailing several ways AI behaves with different datasets for certain purposes, while accompanying the concepts with Python code to better condense the learning. From how images are composed in an RGB matrix and operate them with Python libraries, to studying libraries and packages used in Deep Learning and image analysis, to developing and training the user's first CNN and saving its results, this book can provide assistance to both newcomers to the technology as well as to experts.

3 Architecture and implementation

The chosen model was YOLOv8 [4] due to already having a pre-trained model for object detection, lacking only the dataset and the labels to fine-tune it for the task at hand. In addition, the creation of the labels was relatively easy, abiding by the structure "[class] [x] [y] [width] [height]" for every sample to be detected in training, with "class" advertising to the class number attributed to the sample. For example, an algorithm to detect animals would have "0" for dogs, "1" for cats, etc., "x" and "y" refer to the position in the image where the sample is found, and "width" and "height" refer to the dimensions the sample occupies in the image. Note that "x" and "width" must be ratioed according to the number of pixels in the image on the x axis and "y" and "height" to the number of pixels in the y axis. The resulting numbers must be ratios between 0 and 1 [5].

3.1 Dataset

The dataset consisted of a FIB-SEM sample with 1776 slices, divided into a training set (1066 images), a validation set (355 images) and a test set (355 images), all provided by Prof. Dr. Andreas Walter from the Zentrum für Optische Technologien at Aalen University, Germany. As stated above, FIB-SEM images are saved such that one file stores 1776 images separated by page numbers. A Python program was used to convert each page into a .png file, a file extension accepted by YOLOv8. With the images, the model still required labels for each object to detect, which were created with Python and Sherlock [6]. Since the dataset was split into masks for every sample for better management of classes, resulting in 5 masks, Sherlock was a good choice. This is because it would be possible to actively see what was detected by the software and consequently added to the labels by establishing a threshold, finding white blobs with area greater than a given number, and thus obtaining the values required and writing them into a .txt file with in-program Javascript. Python was then used for masks where the sample was presented in a way Sherlock couldn't work with it. With each image and associated labels (each image and associated label file must have the same name) the last step before the training would be creating a .yaml file that contains information about the images and labels such as the relative location of the training set, the test set and the validation set (the images are to be located in one folder and the labels in another inside each set location, so the training set would have training/images/imgxx.png and training/labels/imgxx.txt), and the names of the classes in numeric order. This .yaml file would then be located next to the folders containing the sets.

3.2 Training

The neural network model used, YOLOv8, has the function to identify the cell's nuclei, lysosomes, early-endosomes, late-endosomes and mitochondria. To train the YOLOv8 neural network, we used the model's default method for object detection, using its auto-batch feature to define the batch size. The neural network was trained for 100 epochs, with an initial learning rate of 0.01 (model's default) and a momentum of 0.937 (model's default), using an image size of 2023*846 pixels (original image size). The model also used the validation set after the training period.

4 Results and evaluation

After the training and validation processes, the model presented the following confusion matrix (Figure 1). The results show a good performance overall, specially with predicting the nuclei (biggest structure in the image). Mitochondria is the most difficult to identify due to its similarity with the cell's cytoplasm.

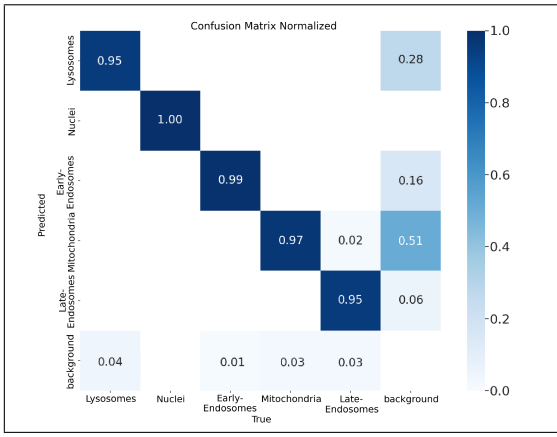


Figure 1: Normalised confusion matrix

The relation between the F1 score and the confidence, in addition to the relation between precision and confidence, help to consolidate the previous observations about the nuclei and the mitochondria identification, as shown in Figure 2 and Figure 3.

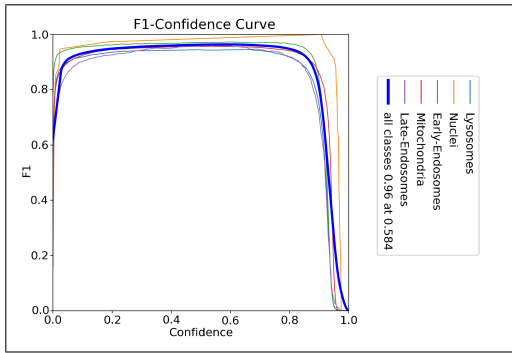


Figure 2: F1-Confidence relation

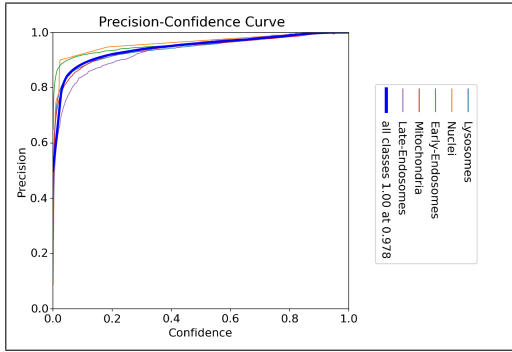


Figure 3: Precision-Confidence relation

We proceeded to use the best parameters (selected by YOLOv8 during the training epochs) to apply on the test dataset. After comparing the results with the original labels, we obtained the results presented in Table 1. These results are slightly higher than the ones presented in the literature for similar objectives. However, in our experiments, the images used to train and test the neural network came from a single dataset.

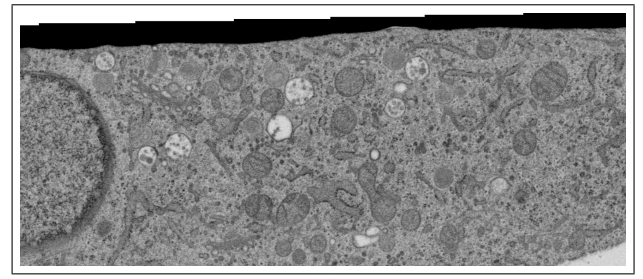
Table 1: Test set results

Recall	Precision	F1	IoU
0.938	0.908	0.922	0.857

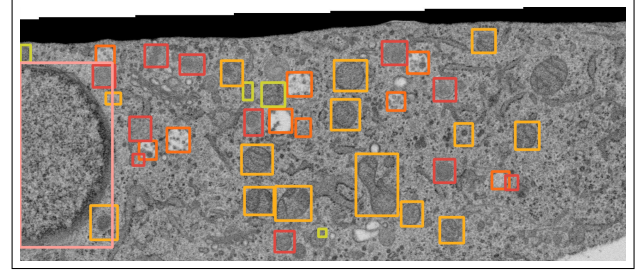
Visual results representing identification examples of organelles with nuclei, mitochondria, early-endosomes, lysosomes and late-endosomes, together with associated accuracy, can be observed in Figure 4.

5 Conclusions and future work

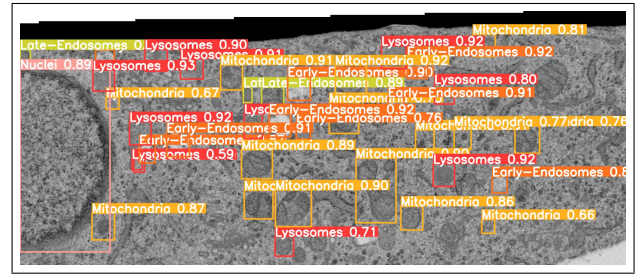
The obtained results reflect a high Recall (how much of the object was correctly detected, from 0 to 1), high Precision (how much of the detected content belongs to the sample, from 0 to 1), high F1 score (the harmonic mean between Precision and Recall) and high IoU (Intersection over Union - relation between the correctly detected and the incorrectly detected/not detected).



(a)



(b)



(c)

Figure 4: (a): Original image to be analysed; (b): Location of the organelles with nuclei in pink, mitochondria in yellow, early-endosomes in orange, lysosomes in red and late-endosomes in green; (c): Prediction made by YOLOv8 to detect said organelles and respective accuracy

This means that, after fine-tuning, the AI-based detection model (YOLOv8) performed well despite the noisy characteristics and high resolution of the images in the test dataset.

FIB-SEM image detection with Deep Learning is a technology still in development and needing improvements. Inaccuracies in the results such as false positives (the detector incorrectly attributing a class to an object with a different class/no class) and false negatives (failure to detect a classified object) need to be minimized. A possible improvement may involve the use of preprocessing to diminish the effect of image noise which is quite high in these images. Future work will target the improvement of the detection task performance and modifying the model for the task of pixel-level semantic segmentation.

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