Automatic Cell Counting With YOLOv5: A Fluorescence Microscopy Approach

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Received 29 March 2023 | Accepted 24 July 2023 | Published 1 August 2023

Abstract

Counting cells in a Neubauer chamber on microbiological culture plates is a laborious task that depends on technical experience. As a result, efforts have been made to advance computer vision-based approaches, increasing efficiency and reliability through quantitative analysis of microorganisms and calculation of their characteristics, biomass concentration, and biological activity. However, variability that still persists in these processes poses a challenge that is yet to be overcome. In this work, we propose a solution adopting a YOLOv5 network model for automatic cell recognition and counting in a case study for laboratory cell detection using images from a CytoSMART Exact FL microscope. In this context, a dataset of 21 expert-labeled cell images was created, along with an extra Sperm DetectionV dataset of 1024 images for transfer learning. The dataset was trained using the pre-trained YOLOv5 algorithm with the Sperm DetectionV database. A laboratory test was also performed to confirm result's viability. Compared to YOLOv4, the current YOLOv5 model had accuracy, precision, recall, and F1 scores of 92%, 84%, 91%, and 87%, respectively. The YOLOv5 algorithm was also used for cell counting and compared to the current segmentation-based U-Net and OpenCV model that has been implemented. In conclusion, the proposed model successfully recognizes and counts the different types of cells present in the laboratory.

Keywords

Cell Counting, Deep Learning, Microscopic, Yolov5.

DOI: 10.9781/ijimai.2023.08.001

I. Introduction

Scientists have collected large amounts of data thanks to measurement-taking in bioengineering, tissue engineering, regenerative medicine, and biomedical research where microscopy and sample preparation techniques have been able to provide images of different phenomena of study and where the quantification of information plays an essential role for the analysis of more accurate and reliable statistics [1]–[3]. Obtaining useful and accurate information from an image quickly and easily remains a challenge in many research areas. Especially in biology and medicine, it is essential to measure cellular characteristics, such as shape and size, for statistical analysis when comparing different samples or experiments [4]. For this purpose, different methods can be used such as the use of vital dyes, the use of counting chambers, or the use of automatic cytometers. It often involves manual counting of thousands of cells with certain markers or measuring their shape and characteristics [5]. This manual process is tedious and time-consuming, which increases the workload of technicians [6]. Therefore, researchers propose automatic models such as plate counting [7], real-time quantitative PCR [8], hemocytometers [9], automatic cell counting instruments [10], and flow cytometry counting in biological systems. A clear example where the use of automatic counting tools can be beneficial is in the study of leukemia, which is a type of cancer that occurs in the human bone marrow and produces abnormal white blood cells in excess. These white blood cells can vary greatly in number and behavior compared to normal ones, which can indicate that the immune system is failing and that the patient is exposed to antigens. Therefore, white blood cell counts are a quantitative measure of disease progression [11].

It is possible to address the task of cell counting in images using state-of-the-art detection techniques such as YoloV5 [12]. These systems can be trained to adapt autonomously to the task, using data provided by researchers in their laboratories. Although there are several automatic analyzers capable of counting cells and providing

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statistics, these often present limitations in terms of accuracy, speed, and resolution [13]. These factors can hinder the accurate identification of cells, especially when there is overlap, which can negatively affect the quality of counting. Detection-based methods first determine the centroid locations of cells and then count them to estimate the total number of cells. Due to the success of these systems in counting and detection tasks in various areas such as agriculture, urban systems, and driving [14]–[16], it has been shown that the accuracy of these methods is strongly influenced by the accuracy of the detection results. However, in practical applications, such as fruit detection in clusters, where objects are densely concentrated and surrounded by structures that can interfere with detection, cell arrest could positively favor the results [17]. The paper [18] presents a promising approach to cell counting using the YOLOv3 detection technique. While this method has shown significant improvements over manual cell counting methods, it has some limitations that have prompted the need for further research. The performance of YOLOv3 is heavily influenced by the accuracy of the detection results, which can be hindered by overlapping cells and other interfering structures. Moreover, YOLOv3 struggles with handling of small objects and dealing with large variance in object scales. The need for a more efficient and accurate cell counting method is evident, especially in the study of diseases like leukemia where precise white blood cell counts are crucial [11].

In light of these challenges, this paper proposes a new cell counting method based on the YOLOv5 model, which offers several improvements over YOLOv3. Our proposed model aims to serve as a more accurate and efficient solution to cell counting in real-time in microscopic images, a task challenged by the low quality of visual features and the criticality of accurately locating cells for correct classification. Our initial results suggest that the proposed YOLOv5 model improves the prediction accuracy on a database of images taken by microbiology experts with a CytoSMART Exact FL microscope.

This work is an extended version of a preliminary paper presented in [19]. In this version, we have incorporated a more advanced object detection model, based on a machine-learning method that detects objects without the need for an exhaustive search. The proposed model applies to cell counting in real-time in microscopic images, which is a difficult task due to the low quality of visual features and the importance of locating the desired object for correct cell classification. We have compared our implementation with a method we had worked with previously [19]. The results indicate that the proposed yolov5 model improves prediction accuracy on the database that contains images taken by experts in microbiology with a CytoSMART Exact FL (Fluorescence) microscope that captures cells for counting. Fig 1 shows the components of the cell counting application.

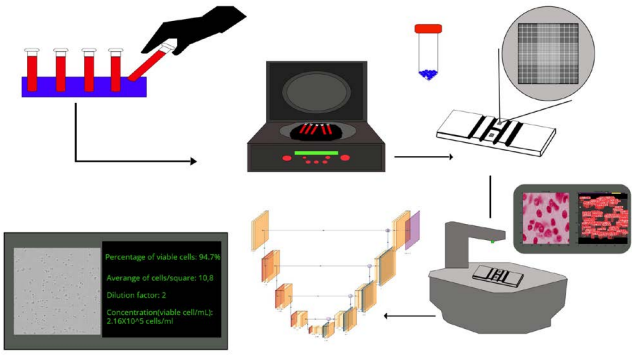

Fig. 1. The figure shows the components of cell counting using YOLOv5 and the CytoSMART microscope. The sample is mixed and placed in the hemocytometer. The sample rests, then is covered and observed under the microscope. YOLOv5 automatically identifies and counts the cells in the large squares. The software calculates the total number of cells. This completes the cell count.

After this introductory section, the remainder of this article is structured as follows: Section II provides a review of related work in the areas of image processing and deep learning. Section III describes the methods and network used in our study, including the handling of the Neubauer Cell Counting Chamber, the CytoSMART Exact FL microscope, the data set used, and the implementation of YOLOv5. Section IV covers our experimental setup and results, with a focus on training validation, materials used, model tuning, and the results obtained. The counting results are discussed in E. The article concludes with Section V, where we summarize our findings and offer some concluding thoughts.

II. Related Work

Cell counting is performed using electronic and optical technologies that analyze images [20], [21]. Previously, it was performed on a cell suspension sample by manually manipulating of the hemocytometer, flow cytometry, and chemical compounds, which was time-consuming and error-prone [22]. However, with the introduction of image analysis, cells can be identified and counted more accurately. Initial studies focus on handcrafted features and use statistical models to detect and classify cells [23], [24]. In recent years, cell counting has been achieved in an automated way thanks to the use of image processing and machine learning techniques [25], [26].

A. Image Processing

Cell counting plays a crucial role in various biomedical applications, such as cancer detection, drug discovery, and toxicity testing. However, traditional manual cell counting methods, performed by skilled workers using microscopes and counting chambers, are labor-intensive, time-consuming, and prone to human error, making standardization and result replication challenging across different samples [27], [28]. Moreover, distinguishing between cells of similar size and shape or cells that cluster together can lead to inaccuracies in cell counts [29]. To address these issues, automated cell counting techniques have been developed. One early approach involved electronic particle counting, which detected cells passing through a small aperture using impedance or light scattering. Although quick and precise, this method failed to differentiate between live and dead cells and required high cell density [30]. These techniques can be categorized into direct and indirect methods. Direct methods involve marking cells with stains or dyes and counting them based on fluorescence or absorbance. Indirect methods rely on analyzing morphological characteristics like size, shape, and texture to identify and count cells in digital images. Automated cell counting techniques can also be classified based on deep learning, machine learning, or image processing approaches. In light of the limitations and advancements in cell counting, the present work aims to propose an improved methodology by building upon the studies conducted by Payasi and Patidar [31], Acharya and Kumar [21], Clarke et al. [32], and Kaur et al. [33]. These studies have contributed valuable insights into counting tuberculosis bacilli, red blood cells, colonies, and platelets, respectively. However, each study has specific limitations related to image preprocessing, segmentation, feature extraction, and counting algorithms, which the present work seeks to address and overcome. By incorporating advancements in image processing, machine learning, and other relevant techniques, the goal is to develop a more accurate and robust automated cell counting method for enhanced biomedical applications.

B. Deep Learning

In the field of automated cell counting, two primary methodologies are employed: detection-based and regression-based methods. Detection-based methods, which aim to identify and count cell centers, are instrumental in locating individual cells and their precise positions,

fitting specific lab conditions [18], [34], [35]. Conversely, regression-based techniques are more suitable for assessing cell sample density and conditions associated with cell dispersion, rather than individual or clustered cells [31], [32], [36]. In related work, Kumaar et al. [37] proposed a novel approach for brain tumor classification using a pre-trained Auxiliary Classifying Style-Based Generative Adversarial Network, demonstrating the broader applicability of machine learning techniques in the medical field.

In the context of automated cell counting and medical imaging, another study worth mentioning uses deep learning for detecting Ventricular Septal Defects in ultrasound images. Chen et al. (2021) proposed a modified YOLOv4-DenseNet algorithm for this purpose. They found the algorithm to be effective, and it outperformed other methods such as YOLOv4, YOLOv3, YOLOv3–SPP, and YOLOv3–DenseNet in terms of the mAP-50 metric. This study demonstrates the applicability of deep learning methods like YOLOv4-DenseNet in medical imaging and could provide insights for enhancing automated cell counting techniques [38].

The limitations of the aforementioned methods illustrate the challenges inherent in cell counting and analysis. These techniques, while effective in their specific applications, illustrate the need for a more versatile approach that can account for the diversity of cell types and variations in cell morphology.

Kaur et al.'s method [39], for instance, uses the circular Hough transform to count platelets in blood images. This method is effective due to the size and shape characteristics of platelets. However, when applied to cells of different sizes and shapes, its effectiveness may decrease.

The machine learning approach proposed by other researchers [22] employs the YOLO object detection and classification algorithm to identify and count three types of blood cells. This method is innovative in its use of machine learning for cell counting, but its generalizability to other cell types may be limited.

In a subsequent study, an algorithm using YOLOv3 for counting red and white blood cells was introduced [18]. This method relies on image density estimation for counting grouped red blood cells, which may lead to inaccuracies due to variations in cell grouping and distribution.

Single-stage detector methods, such as YOLO [40], are pivotal tools in cell counting due to their speed, efficiency, and accuracy. They are typically faster than two-stage detection methods, like R-CNN (Region with Convolutional Neural Networks), which is a critical advantage in healthcare applications where time can be essential, such as in disease diagnosis and treatment. These methods are capable of detecting and classifying objects (in this case, cells) in a single pass through the network, which can be more efficient in terms of computational resources than methods requiring multiple passes [41]. Although single-stage detection methods may not be as precise as some two-stage detection methods, their performance is often sufficient for many applications, including cell counting. In summary, single-stage detection methods offer a balance between speed, efficiency, and accuracy that makes them valuable for cell counting and analysis.

These methods exhibit the complex challenges associated with cell counting and analysis. They underscore the need for a method that is not only effective with a specific type of cell or under specific conditions but can also adapt to different cell types and conditions. This study aims to address these challenges by developing a more versatile and accurate approach to cell counting and analysis.

III. Methods Network

The proposed method consists of three elements: image capture using a CytoSMART Exact FL microscope with open API for cells in Neubauer plates, labeling and cell detection and counting. Due to the existence of several types of Neubauer plates, an additional database was searched to strengthen the model and then tests were performed with images under laboratory conditions.

A. Handling of the Neubauer Cell Counting Chamber

The counting chamber system involves placing a small amount of the cell suspension to be counted in the center of a special slide called a counting chamber. This slide has a known surface pattern and a fixed height. Next, the chamber is covered with a coverslip that rests on pillars that determine the volume of the suspension between the slide and the coverslip. Then, the chamber is observed under a microscope, and the cells or particles that are found within the areas marked by the pattern, are counted. Finally, the concentration of cells or particles in the suspension is calculated using the number of cells counted, the area, and the volume of the chamber. This system is mainly used in blood analysis, counting bacterial, sperm, and fungal cells [42].

The microscope was used to capture the information presented on CytoSMART Exact FL neubauer cameras. Using the 6.4 MP CMOS camera combined with 10x magnification, the CytoSMART Exact FL can view and count cells down to 4 µm in diameter [43].

B. CytoSMART Exact FL Microscope

The CytoSMART Exact FL microscope is a key tool in biological research due to its advanced, integrative features. Unlike other microscopes, it combines high-resolution imaging with cloud-based analysis and automated cell counting, offering a comprehensive solution for cellular studies. Its fluorescence capabilities allow visualization and quantification of fluorescently labeled cells, crucial for various forms of research. The cloud-based platform facilitates collaboration and remote analysis, fitting well with the modern trend of remote work. Despite its advanced features, the CytoSMART Exact FL is user-friendly, making it accessible to a wide user base. Its compact design further enhances its practicality in various lab settings. In essence, the CytoSMART Exact FL microscope, with its unique combination of features, provides convenience, efficiency, and accuracy, making it indispensable in cellular research [44].

C. Data Set

This research was based on the Sperm DetectionV4 Image Dataset [45], which consists of a total of 1024 images. Of these images, 820 were used for training with pixel-level annotations, 104 for testing, and 100 for validation. For our case study, we needed data on cells obtained through CytoSMART's Neubauer Exact FL cameras. Since object detection methods require object position data, we needed to create our own labels for the data. We used an annotation tool that exports boxes as coordinates that will be used later for training. This tool allowed us to locate the cells within a rectangle, generating a specific label for each patch. All of this was done through the LabelImg program, as shown in Fig 2. In total, this image set consists of 16 training images and 5 validation images.

D. YOLOV5

YOLOv5, the base of our proposed method, employs advanced modules such as Mosaic, Focus, BottleneckCSP, SPP, and PANet to enhance object detection performance [46]. Its architecture is composed of three key parts: a backbone network, a detection neck, and three detection heads.

The training images, denoted as I with dimensions $H \times W \times C$ (height, width, and number of channels, respectively), first undergo mosaic processing before being fed into the backbone network. This backbone network, consisting of convolutional layers, extracts features at multiple scales, transforming the input image into a set of feature maps, $F = F_1, F_2, ..., F_n$, where each F_i has size $H_i = W_i \times C_i$.

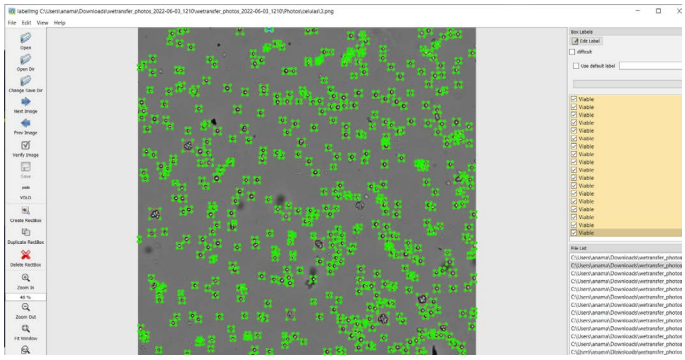

Fig. 2. The figure shows the annotation of cell images using a labeling program. The user loads an image, selects cells by clicking or drawing rectangles, the program assigns unique labels, and saves coordinate data and cell sizes. This process is repeated on multiple images to create a training data set that trains a neural network to automatically detect cells.

Following feature extraction, these maps are then processed by the detection neck, which applies a series of $F_i' = T(F_i)$, where T is the transformation operation, and F_i' is the transformed feature map.

The detection heads make final predictions for objects of varying sizes. Each detection head outputs a tensor, $D = D_1, D_2, ..., D_m$, where D_i represents a detected object and consists of the object's category c_i, confidence score s_i, and bounding box position $b_i = (x_i, y_i, w_i, h_i)$ (with xi, yi being the coordinates of the box's center, and wi, hi being the width and height of the box, respectively).

YOLOv5 utilizes the FPN-PAN structure, CSP2 structure from CSPNet, and PANet as the neck for feature aggregation, improving the detection of objects of varying scales. The model employs a new FPN structure in the feature extractor, and the PAN structure helps transfer robust localization features from lower to higher feature maps, improving low-level feature propagation and enhancing the feature fusion capability of the Neck network [47].

The loss function for YOLOv5 was changed from binary cross-entropy to focal loss. This can be explained as:

Binary cross-entropy loss:

$$Loss_{clc,obj} = -\frac{1}{N}\sum_{i=1}^{N} y_i \log(p(y_i)) + (1 - y_i)\log(1 - p(y_i)) \quad (1)$$

(1) Binary cross-entropy is used to judge the difference between the predicted result of a classification model and the true value. If the predicted value $p(y_i)$ is closer to 1, then the value of the loss function should be closer to 0, that is, the smaller the difference between the predicted result and the true value, the smaller the value of the loss function.

Focal loss:

$$Loss_{fl} = -\frac{1}{N}\sum_{i=1}^{N} \alpha_{y_i}(1 - p(y_i)^{\gamma})\log(p(y_i)) + (1 - \alpha)(1 - y_i)p(y_i)^{\gamma}\log(1 - p(y_i)) \quad (2)$$

(2) where $Loss_{fl}$ is the Focal loss function, the α weight factor is used to regulate the balance between positive and negative samples, the γ weight factor is to regulate the weight balance between difficult samples. y_i is the true value of the tag, 1 is a positive sample and the rest is a negative sample, $p(y_i)$ is the predicted value output by the network model.

Compared to the binary cross-entropy loss, the focal loss dynamically scales the loss contribution from easy samples and focuses on hard samples. By down-weighting easy examples and emphasizing hard examples, the focal loss accelerates model convergence and improves accuracy -which is important for object detection tasks like in YOLOv5.

For transfer learning, YOLO-v5 used pre-trained weights from 70 epochs trained on the COCO dataset. The model was trained for a maximum of 20 trials with a patience level of 100, meaning that training would stop after 20 consecutive trials without improvement. The image resolution was set to 640 px by 640 px, and the batch size was 32. Model performance during training was evaluated using visual analysis of the training loss and validation curves. To test the model's performance, a set of images with correct and faulty states were processed from the camera.

IV. Experimental Setup and Results

This section presents the experiments that were carried out to evaluate the effectiveness of the proposed approach. First, the data set used in the research is described, then the performed experiments are analyzed, and the results obtained with the proposed approach are compared with other competitive approaches.

A. Data Augmentation

In the context of cell counting using the YOLOv5 object detection model, data augmentation is applied to the microscopy image data of the cells. Techniques include image translation, where images are shifted horizontally or vertically. This can help the model generalize to scenarios where the cells may not be perfectly centered in the field of view. Rotation or scaling of images can help the model learn to recognize cells in various orientations and sizes. Flipping images horizontally or vertically can assist the model in recognizing cells that can appear in different orientations within a biological sample. Adjusting the brightness and contrast of images can help the model generalize to different lighting conditions that can occur during microscopic imaging. Lastly, image cropping can create 'new' images by focusing on different parts of the original image, which can help the model learn to recognize cells even when only a part of them is visible. This is particularly useful in scenarios where cells may be partially obscured by other biological material.

B. Training Validation

The YOLOv5-based network was pre-trained using the sperm detection database, and the obtained weights were saved for future use. The appropriate number of epochs to train a new dataset of molds was determined by selecting the 205-epoch model, which took approximately 40 minutes to complete. During the model training process, the training and validation dataset was used, while an additional test dataset of 5 images was presented to independently evaluate the model performance.

C. Materials

After 50 epochs, the YOLOv5 model demonstrated good performance. However, as the epochs were increased, all losses including classification loss, box loss, and objectness loss increased, resulting in a decrease in the model's performance. The YOLOv5 model was used to detect cells under various microscope imaging conditions after creating a labeled dataset to achieve optimal cell detection. During the model training, several image resolutions were used, but an appropriate image resolution of 500×500 pixels was chosen.

After training the model, the precision, recall, and average precision (AP) of the detected objects were calculated and compared with other models [46].

$$A = \frac{TP + TN}{TP + TN + FP + FN} \quad (3)$$

$$Precision = \frac{TP}{TP + FP} \quad (4)$$

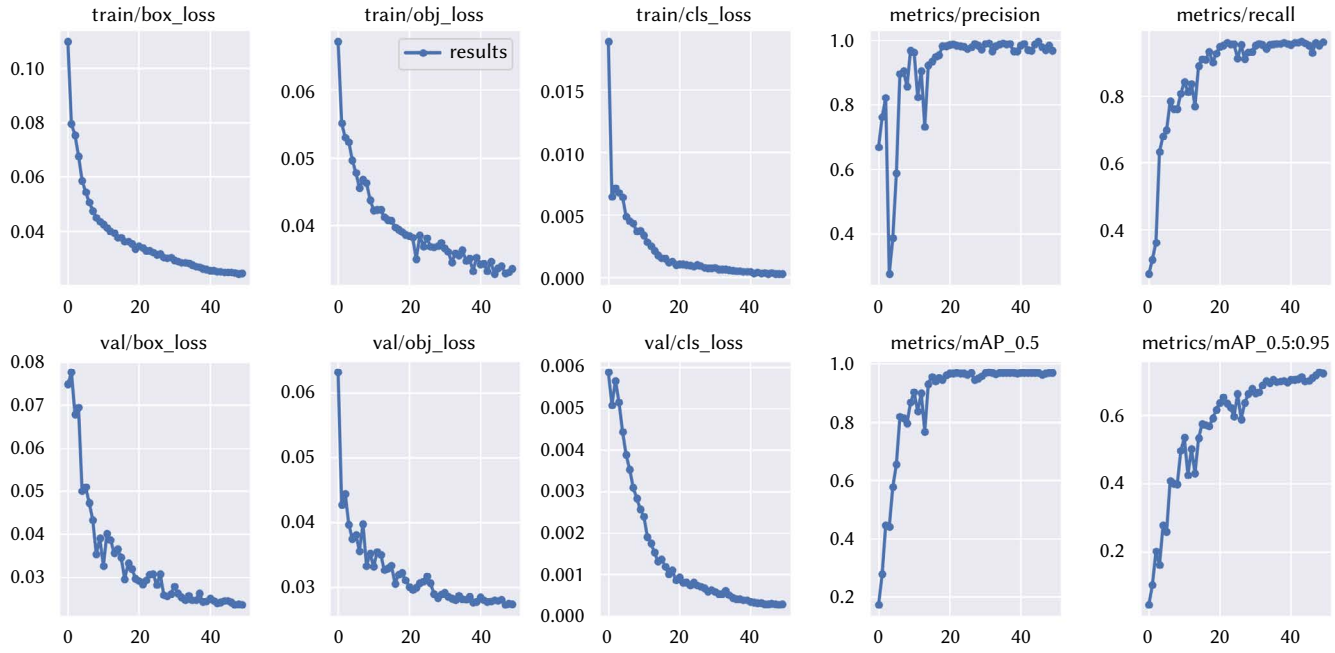

Fig. 3. YOLOv5: (top row) training, and (bottom row) validation graphs.

$$Recall = \frac{TP}{TP + FN} \tag{5}$$

$$F1Score = \frac{2 \times Precision \times Recall}{Precision + Recall} \tag{6}$$

D. Model Tuning

The study was conducted on a local machine that included a 16 GB NVidia RTX2080 GPU, 32 GB main memory, 1.9 GHz CPU and SSD hard disk. cuDNN 10 was used to run YOLOv5 on this GPU. The YOLOv5 architecture was tuned and configured to fit the sperm detectionV4 image dataset by using transfer learning. Previously pre-trained weights were used, which were trained on the sperm detectionV4 dataset. The last three YOLOv5 and convolutional layers were adjusted to match the number of classes present in the dataset.

The original pre-trained YOLOv5 model was trained on 2 classes, so we reconfigured it to a single "valid" class to address the sparseness of our dataset. To further address data sparsity and cover semantic variations, we applied several data augmentation techniques before and during YOLOv5 training. Data augmentation parameters were tuned to generate multiple images from a single image and enrich the training data. Additionally, we set the number of batches to 6 to increase model robustness and better fit GPU memory, and the number of training epochs to 50, at which point the model stabilized. Other hyperparameters were kept at default values [48].

Finally, we trained and tested YOLOv5 on our local machine using the laboratory's dataset. We trained YOLOv5 for 50 iterations, saving weights every 10 iterations. We then plotted mean average precision (mAP) vs. a number of iterations at four different saved weight points to analyze performance over training.

E. Results

Fig 3 shows how the YOLOv5 model performs as it is trained. The top row shows the results of the model using the training set, while the bottom row shows the results of the model using the validation set. It can be seen that the accuracy of the model in detecting drone objects improved significantly after 50 epochs, reaching a loss of less than 0.03. To avoid overtraining, the early stopping technique was used, which means that the training process is stopped when no noticeable improvements in performance are observed. In Fig. 3, some fluctuations in the signals can be seen, which are common during the training process and are due to divergent weights.

Table I compares the approach proposed in this research for the object detection task with other competing methods in the literature. As shown in the table, the best results are achieved with the yolov5 model. As the dataset used in this research contains small objects, such as cells, the accurate detection of these objects is a critical challenge for object detection models. In this context, the yolov5 model has proven to be an effective choice, as it achieves the best results for the cell detection task.

TABLE I. The Testing Results of Different Object Detection Algorithms. Accuracy. F1: F1-score. FPS: Frames Per Second; FPS Represents the Detection Speed of the Algorithm Under CPU Computing Conditions, Respectively

Model	Accuracy	Precis	Recall	F1Score	FPS
Yolov4	0.90	0.80	0.89	0.84	30.85
Yolov5	0.92	0.84	0.91	0.87	35.86

Despite the cell model's commendable detection rate and satisfactory loss value outcomes, there remain instances where the test set images display errors. These errors primarily arise from excessive occlusion and light interference that confound the localization and classification modules, as depicted in Fig 4.

Simultaneously, the functionality of our cell detection system is vividly illustrated. The system's efficiency and precision come to the fore through a microscopic view of a cell sample, where bounding boxes produced by our system are prominently displayed. Each of these boxes encapsulates a single cell, thereby underlining the system's adeptness in accurately identifying and isolating individual cells within the sample. This integration of the two paragraphs provides a balanced view of the system's capabilities and areas for improvement.

The application of deep learning models in cell counting has shown promising results, improving accuracy and efficiency in biological research. This article focuses on the evaluation of YOLOv5 in comparison with its predecessor, YOLOv4, for automatic cell counting using fluorescence microscopy.

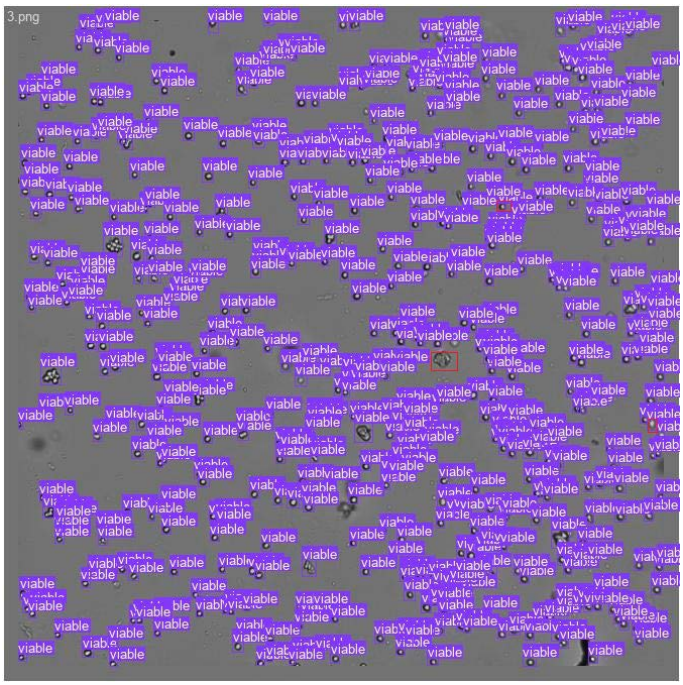

Fig. 4. Visual Representation of the YOLOv5 Model Detecting Minute Cells in a Functional System.

The choice to compare YOLOv5 with YOLOv4 is deliberate. YOLOv4 is a previous version of the You Only Look Once (YOLO) series of object detection models. Comparing YOLOv5 with YOLOv4 allows us to highlight the improvements and advancements in the latest version, demonstrating why YOLOv5 is a more suitable choice for our study.

F. Counting Result

The cell counting results from the computer vision models are summarized in Table II. The YOLOv5 model achieved a mean relative error of 1.84% on the cell counting task, significantly outperforming the U-Net model which attained an error of 39.9%. This substantial discrepancy in performance can be attributed to the superior ability of the YOLOv5 framework to handle the complexity and nuances of the cell counting environment. In particular, the YOLOv5 model can efficiently detect objects amid semantic clutter and occlusions, enabling it to generate more accurate cell counts than the U-Net approach which struggles with such challenging conditions.

TABLE II.: Microscope Cell Count Results With the Yolov5 and Semantics Technique, Where N Tests Is the Number of Tests, R Error Is the Relative Error, and A RE Error Is the Average Relative Error

Techniques	Exp	N Test	Re Error	A R Error	FPS
Yolo V5	1	201/201	0%	1.84%	28
	2	150/148	1.3%		36
	3	522/500	4.21%		30
	4	323/317	1.86%		35
U-Net [49]	1	201/124	38.30%	39.9%	28
	2	150/86	42.66%		36
	3	522/284	45.59%		30
	4	323/216	33.12%		35

In the domain of automated cell counting, various models exhibit different levels of precision and operational efficiency. This work examines and contrasts two methodologies: YOLOv5, U-Net, and OpenCV. Table II presents experimental results using these techniques, comparing their relative errors and processing speed (FPS). The evaluation includes U-Net, a deep-learning model previously used in our laboratory, and YOLOv5, the most recent model adopted for cell detection and counting.

OpenCV is a programming function library used alongside U-Net for cell counting after segmentation. Performance metrics, namely relative error and FPS, provide a comprehensive evaluation of each model's capabilities. Relative error quantifies the deviation between expected and actual cell counts, whereas FPS measures the speed of processing frames, thereby demonstrating each model's efficiency.

V. Conclusion

In this work, we demonstrate the application of a deep learning system for cell counting. The proposed YOLOv5 model, applied to CytoSMART Exact FL microscope images, enables a customized tool for the specific use case of counting different cells studied in the laboratory. Our model adopted object detection and multi-object tracking technology to achieve feasible cell detection and counting. The proposed architecture was compared with a segmentation-based method, which yielded promising results by outperforming the current method implemented in the laboratory. The introduction of semantic expert context labels improves the detection of clustered or overlapping cells. Automating cell counting could save time spent on this tedious and time-consuming task, freeing workers to focus on other important tasks and reducing costs and workload. The network used transfer learning to adapt network weights from a Sperm DetectionV4 database. To verify the effectiveness of the algorithm, a dataset of cell count use cases obtained in the laboratory was used to train and test the algorithm. Experimental results indicate that, compared to the original U-Net segmentation-based network implemented in the laboratory, the improved network achieves faster image processing, averaging 32.25 fps versus 0.95 fps per image for the previous system. The accuracy, precision, recall and F1 score for detection between YOLOv4 90%, 80%, 89% and 84%, and YOLOv5 reached 92%, 84%, 91% and 87%, respectively. Counting performance had a mean relative error of 1.84% for YOLOv5 versus 39.9% for U-Net, demonstrating considerable improvement.

As future work it is necessary to expand the database to include a larger number of events for which the current algorithm may not be prepared, this could give a better perspective if there are changes in scale or new cells are brought into the laboratory.

Acknowledgements

This work has been supported by the project "XAI -Sistemas Inteligentes Auto Explicativos creados con Módulos de Mezcla de Expertos", ID SA082P20, financed by Junta Castilla y León, Consejería de Educación, and FEDER funds.

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