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Automatic System for Acquisition and Analysis of Microscopic Digital Images Containing Activated Sludge

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ABSTRACT

The article contains the procedure of image acquisition, including sampling of analyzed material as well as technical solutions of hardware and preprocessing used in research. A dataset of digital images containing identified objects were obtained with help of automated mechanical system for controlling the microscope table and used to train the YOLO models. The performance of YOLOv4 as well as YOLOv8 deep learning networks was compared on the basis of automatic image analysis. YOLO constitutes a one-stage object detection model, aiming to examine the analyzed image only once. By utilizing a single neural network, the image is divided into a grid of cells, and predictions are made for bounding boxes, as well as object class probabilities for each box. This approach allows real-time detection with minimal accuracy loss. The study involved ciliated protozoa *Vorticella* as a test object. These organisms are found both in natural water bodies and in treatment plants that employ the activated sludge method. As a result of its distinct appearance, high abundance and sedentary lifestyle, *Vorticella* are good subjects for detection tasks. To ensure that the training dataset is accurate, the images were manually labeled. The performance of the models was evaluated using such metrics as accuracy, precision, and recall. The final results show the differences in metrics characterizing the obtained outputs and progress in the software over subsequent versions of the YOLO algorithm.

Keywords: image acquisition, automatic image analysis, deep learning, YOLO, *Vorticella,* activated sludge.

INTRODUCTION

Machine learning and artificial intelligence are the fields that encompass automatic image analysis; this process is also called computer vision, which entails extracting and processing the image data. Computer vision aims at identification and localization of objects within the image, followed by conveying their assignment to suitable classes. The computer perception of the world differs from that of humans. They analyze images pixels, i.e. arrays of numerical values. However computers can be trained to identify shapes and patterns. The key lies in determining how these numerical values can be used by a computer vision system to identify objects within an image and comprehend their attributes, including colors, edges, sizes, textures, as well as spatial arrangement [1–3].

The development of computer vision began in the mid-90s. One notable event related to the development of artificial intelligence occurred in 1966 when Seymour Papert initiated The

Summer Vision Project [4]. The primary objective was to create a specialized computer system that is able to identify objects within images. To achieve this, programmers manually established the rules for object detection. These rules were often based on specific patterns or features defined by the programmers themselves. Mathematical algorithms or logical conditions were commonly employed in these early computer vision systems to aid in object identification or characterization. Rules could be developed, for example, to identify colors by comparing pixel values to predefined color thresholds or to detect edges by evaluating abrupt changes in pixel intensity values. However, these rules heavily relied on the programmers' knowledge and expertise, demanding substantial manual effort for development and refinement. Nevertheless, the effectiveness using only manually defined rules was constrained due to the variability and complexity of real-world images [5].

During the 1970s and 1980s, researchers dedicated their efforts to creating the algorithms that could identify specific attributes in images, such as textures, corners, and edges. In addition, Witkin developed the idea of scale-space theory in the 1980s, which sought to explain differences in object size and appearance by analyzing images at different scales. This theory helped to develop methods for blob detection such as the difference of gaussians (DoG) and Laplacian of Gaussian (LoG). Neural networks and machine learning became very popular in computer vision as the 1980s and 1990s went on.

Numerous developments and breakthroughs in computer vision occurred between 2000 and 2010. Particularly, advancements in object recognition and detection were made. In 2001, the Viola-Jones algorithm was released, enabling real-time face detection. Furthermore, the development of the histogram of oriented gradients (HOG) feature descriptor improved the ability to identify objects in pictures. Various feature descriptors, like scale-invariant feature transform (SIFT) and speeded-up robust features (SURF), were created to facilitate robust object recognition and matching across various angles and pictures. For image classification tasks, machine learning algorithms – random forests and support vector machines, or SVMs – have become popular. Accurate image classification was greatly improved by combining these algorithms with large datasets such as ImageNet [6–8].

In 2012, the ImageNet image recognition competition was won by the AlexNet convolutional neural network, which was created by Alex Krizhevsky. This significant achievement sparked immense interest and initiated a transformative era in deep network learning. Subsequently, owing to technological advancements and enhanced information accessibility, the precision of object detection models has doubled and continues to undergo further enhancements [9–11].

The initial objective of R-CNN was to take an input picture and output a collection of bounding boxes, each of which would include an item and its category (such as automobile or pedestrian). Recently, R-CNN has been expanded to handle more computer vision jobs [1, 12–15].

An algorithm that is frequently used for real-time object detection is called YOLO (You Only Look Once). It introduced a unique approach to detection, bringing a significant revolution to the field of computer vision. Unlike traditional methods, e.g. R-CNN, Fast R-CNN, and Faster R-CNN, which involve multiple stages, YOLO performs object detection in a single pass. The input image is divided into a grid by the algorithm, which then forecasts bounding boxes and class probabilities for every grid cell. YOLO forecasts several bounding boxes around each grid cell, enclosing the objects that are contained in that specific cell. The coordinates for the box center (x, y), width (w), height (h), and confidence scores make up these bounding boxes. Additionally, class probabilities are predicted for every object detected in each grid cell, based on the classes predefined during the process of data preparation. To ensure accurate detections, YOLO assigns a confidence score to each predicted bounding box, indicating the model's degree of confidence in the existence of an object inside that box. Next, a threshold with a constant value is used for filtering out low-confidence detections. By using a method known as non-maximum suppression, YOLO removes duplicate and overlapping detections. By calculating the overlap of each detection using intersection-over-union (IoU) calculations, this process eliminates duplicate detections and chooses the most confident detection in each grid cell. YOLO provides the final set of bounding boxes and their corresponding class labels following the application of non-maximum suppression, producing a thorough detection result for the input image [11, 16–18]. YOLO is recognized for its remarkable speed, allowing for real-time object detection across a range of platforms, including drones and embedded systems. This is made possible through the optimization of network architecture and the utilization of methods like anchor boxes. YOLO has progressed through various versions over time, including YOLOv1, YOLOv2 (also referred to as YOLO9000), YOLOv3, YOLOv4, all the way up to YOLOv8. Each iteration introduces improvements as far as speed, accuracy, and architectural advancements are concerned [10, 19].

At present, computer vision is experiencing rapid growth in the artificial intelligence and machine learning domains. One of the main challenges in computer vision is to emulate the amazing powers of the human visual system. This entails understanding and characterizing the entire scene in addition to locating and identifying objects within an image. Automated image analysis algorithms have demonstrated promising outcomes in tasks such as face recognition and autonomous driving. Moreover, they have found applications in various industries including sales, healthcare, and manufacturing. The continuous efforts to enhance computer vision imply that this technology will be capable of performing an even broader range of functions in the future. When combined with artificial intelligence systems, it has the potential to create machines that possess human-like thinking and analytical abilities [20, 21].

The presented study focused on demonstrating the utilization of automatic image analysis to recognize a settled object in activated sludge, using the ciliates from the genus *Vorticella* as a model among the various activated sludge organisms. Ciliates constitute an essential component of activated sludge, which influence the wastewater treatment process efficiency [22–25]. They are also established markers of the quality of activated sludge [26, 27]. *Vorticella* species are almost always present in activated sludge [26–30]; therefore, they can be used as sensitive indicators of the state of activated sludge in a bioreactor, as well as indicators of the effect of wastewater on the ecosystems of receiving water bodies [26, 27, 31, 32]. This makes it important to identify representatives of *Vorticella* using artificial intelligence technologies. The method employed convolutional neural network to classify and localize objects. Automatic analysis holds promise for future applications in evaluating the state of activated sludge and monitoring changes in organism populations, akin to the current practice in assessing flock structure parameters. The algorithm presented is highly efficient at identifying other commonly used bioindicators in activated sludge, as validated by the authors' study [5]. However, the difficulty with supervised machine learning techniques is not only choosing the best algorithm for object recognition but also having an experienced supervisor to help the algorithm distinguish thousands of representatives of different species that are visually similar. In the case of image recognition process of – for example – road signs, the distortions of original images are not crucial. Road signs were designed to help in navigating, so symbols used on them have to be easy to recognize. The number of signs types are also well known. In biological organism communities, the situation is much more complicated. Depending on the angle, a two-dimensional image of a three-dimensional object can look completely different. Also, the differences between individual organisms are not necessarily as clearly visible as in the case of signs. It should also be remembered that the subject of the study corresponds to microorganisms, which seriously limits the possibilities of manipulating them (rotating, changing the shooting angle etc.). In this regard, protozoologist's experience of recognition of ciliates is very important in building automatic recognition system for activated sludge. The ground-truth labeling should be used, because the consequence of incorrectly defining organisms at the learning stage will be their incorrect recognition in the future. Understanding this circumstance, teacher's practice cannot be ignored.

Although automatic image recognition using deep learning of neural networks is the main issue of this study, it is worth mentioning that in order to speed up the work and quickly automatically obtain images, a system for collecting data (digital images) from the surface of the preparation with biological material was developed.

MATERIALS AND METHODS

The procedure of digital image acquisition containing pictures of activated sludge organisms consists of a few stages, including sampling of activated sludge, preparing cover-slips, acquiring images, labeling, training neural network and finally conducting the verification process of training using the images prepared earlier but not used in learning. The research involved the specimens of activated sludge sampled in the bioreactors of the Hajdów treatment plant in Lublin. Using a measuring vessel, 300 ml of activated sludge was poured into each of the plastic containers, up to a maximum of 150 ml per container. To ensure air supply during transportation, half of the volume was left empty. Following preparation, the containers were brought to the laboratory and kept in a refrigerator at 5 °C. Sampling and transportation lasted no longer than an hour, with microscopic examination preparations carried out immediately upon sample delivery. Each activated sludge sample yielded at least three in-vivo slices. An automatic pipette (BIOHIT m 1000) was used to apply the samples to a primary glass. and then a cover glass was placed on top. The obtained preparations were previewed by protozoologist, to determine their suitability for machine learning purposes. It is important to note that some of the samples were included in the next stage even though they did not have significant information about the objects being the subject of research. The image acquisition system was based on an OLYMPUS CX41 trinocular optical microscope with a rotating head equipped with DIN-compliant objective lenses with magnifications ×10, ×40, An external UI-1465LE-C-HQ color digital camera with a resolution of 1280 × 1024 pixels was mounted in the trinocular head via a C-mount connector. The system components shown in Figure 1. The mechanical system for controlling the microscope table consisted

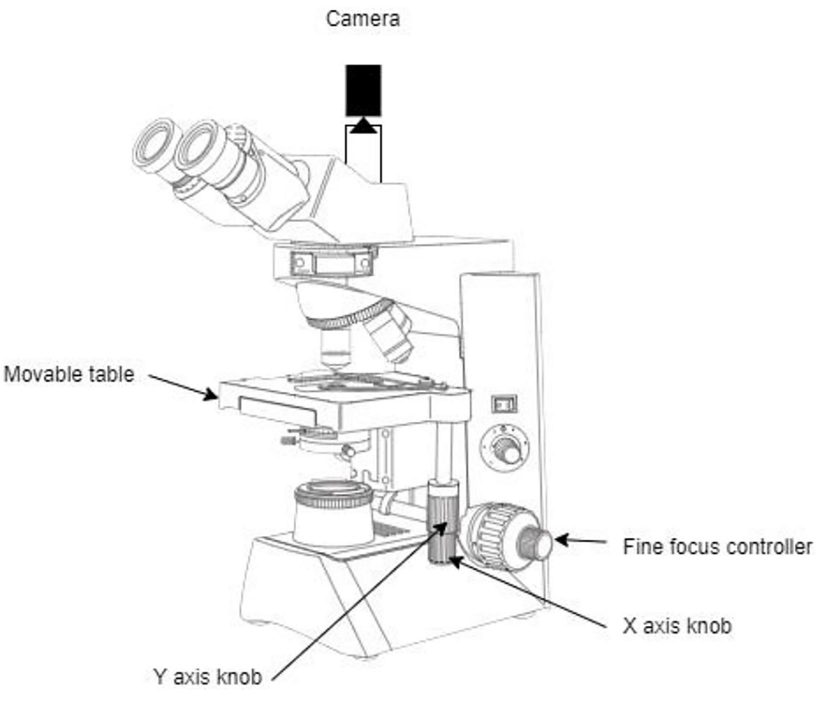

Figure 1. Acquisition system components

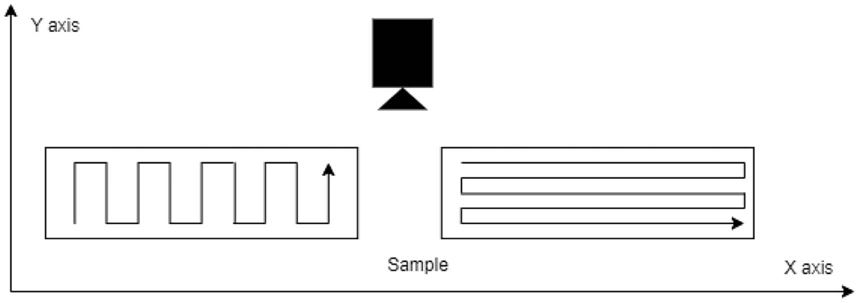

Figure 2. Movement of points located on slices according to optical path with digital camera

of adapters printed from ABS using 3D printing to adjust the x and y axes. For precise focusing, an adapter was modeled, printed, and then attached to the motor shaft using a flexible coupling. Optimal focusing was performed through algorithms implemented in the motor controller. The stepper motors of each axis were controlled by an EasyDriver microcontroller using an Arduino UNO connected to a PC. Figure 2 shows the camera movement according to the paths controlled by the algorithm.

The samples were placed into the described acquisition system and a set of images were gathered from every preparation slice. The collected images were saved on attached computer hard disk for labeling tasks and further works.

Next step involved labeling, which is crucial for machine learning. Labeling allows algorithms to learn how to recognize objects by giving feedback, where the mentioned object exactly is placed in image. In the presented work, the Label-Studio platform was used, which is very flexible in labeling tasks. Label Studio is fully prepared for group work, what makes the system more complex, but provides more work opportunities. Label Studio works as a web browser application, which allows users to use their favorite browser during work. The application is designed to set many instances of projects, in which different group of users can be included. The process of labeling in this software is available using different templates, depending on type of objects or user habits. In computer vision, there are available templates for polygon marking, masking, bounding boxes, key points, ellipses and more. In the described case, bounding boxes were used. The labeling process was carried out by protozoologist, because it is the key task for the best possible results of the further research.

The dataset comprised a total of 990 images with labeling notes that were split into training (70%), validation (20%) as well as test (10%) subsets – as a preparation for machine learning task using YOLO v4 and v8 networks.

In YOLO architectures, the terms "head", "backbone" and "neck" refer to different network components that fulfill particular roles during the object detection procedure. The backbone network, which usually consists of several convolutional layers arranged in a deep neural network structure, is responsible for extracting high-level features from the input image. From low-level features like edges and textures to higher-level semantic features, this backbone network learns to capture and represent different levels of visual information. The backbone network forms the foundation for feature extraction in YOLO architectures. By combining features from various scales, the neck network serves as a mediator between the head and backbone networks, improving feature representation. Combining feature maps from various core stages allows neck, also referred to as a feature extractor, to capture data at various scales. By capturing multi-scale information, the neck network aids in detecting objects of varying sizes, incorporating top-down and bottom-up pathways, feature fusion modules, and lateral connections. The neck network in YOLO architectures, like PANet (Path Aggregation Network), integrates multi-scale features to improve object detection accuracy. The final predictions for object detection, including bounding box coordinates, class probabilities and objectness scores, are generated by the head network. To produce the detection results, it processes the features that the neck network and backbone network extracted and refined. In YOLO architectures, the

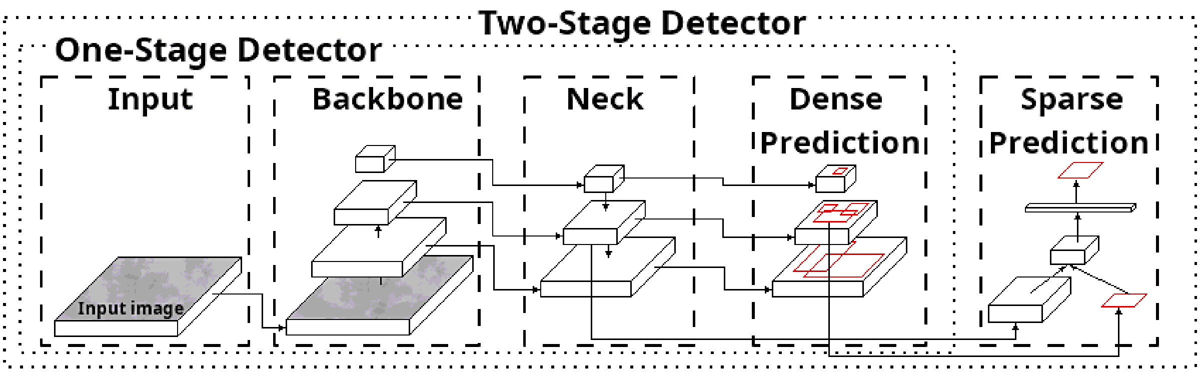

Figure 3. YOLOv4 network architecture [13]

head network combines features from different scales to produce accurate object detection predictions. In YOLO architectures, the head, neck, and backbone components work together to improve representation, extract features, and produce predictions for object detection tasks. YOLOv4 was created to detect objects with the best possible speed and accuracy. The YOLOv4 architecture includes CSPDarknet53 as the core, PANet as the neck, and YOLOv3 as the detection head. This design allows YOLOv4 to perform object detection at notable speeds, enabling its use in real-time applications. The architecture of YOLOv4 is depicted in Figure 3 [12, 13].

YOLOv8, created by Ultralytics, is an upgraded version of the popular YOLOv5 model, incorporating several architectural enhancements and improvements in developer experience. One notable feature is its anchor-free methodology, as opposed to depending on an offset from a fixed anchor box, predicts an object's center directly. YOLOv8 also includes user-friendly features like a simple command-line interface (CLI) and a neatly organized Python package. These upgrades are designed to offer developers a more convenient and effective experience when working with the YOLOv8 model. YOLOv8 architecture can be roughly split into three primary parts:

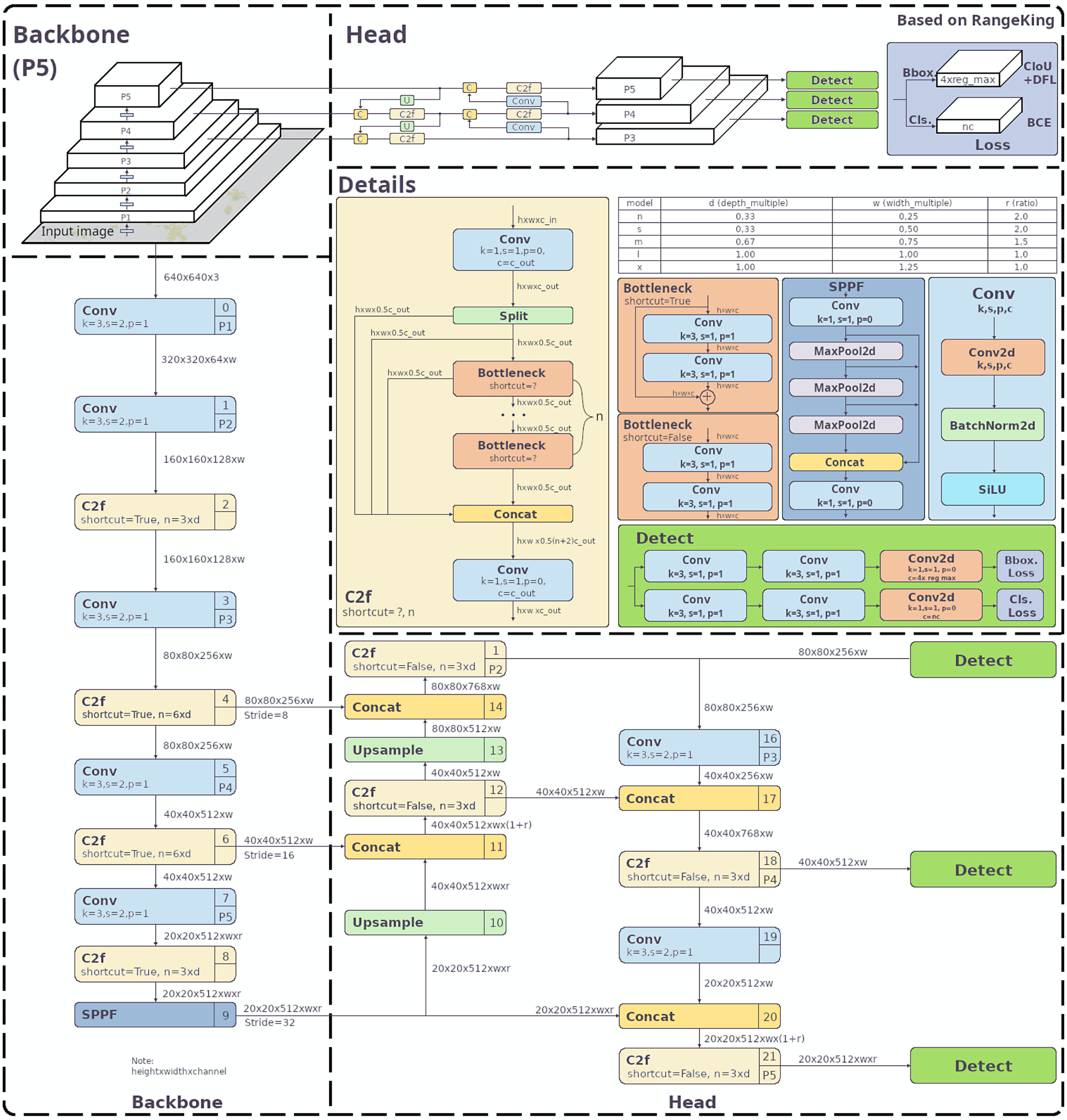

Figure 4. YOLOv8 architecture [33]

1) Neck uses the new C2f module rather than the conventional Feature Pyramid Network (FPN);
2) Core (backbone) uses a custom CSPDarknet53 core that uses partial connections at various stages to improve information flow between layers and increase accuracy. Improved detection accuracy, particularly for small objects, is achieved by combining high-level semantic features with low-level spatial information in this module;
3) Head of YOLOv8 employs multiple detection modules that predict bounding boxes, objectivity scores, and class probabilities for each grid cell in the feature map. The final detections are then obtained by averaging these predictions. The architecture of YOLOv8 is shown in the Figure 4 [33, 34].

A number of significant advancements made by YOLOv8 are responsible for its outstanding performance. One such innovation is a spatial attention mechanism that concentrates on pertinent areas of the image to improve object localization. By efficiently combining low-level spatial information with high-level semantic features, the C2f module increases the precision of small object detection. The CSPDarknet53 core's bottles lower computational complexity without sacrificing accuracy. To further enhance detection performance, the spatial pyramid pooling fast (SPPF) layer additionally records features at various scales. The model evaluation involved the calculation of accuracy, precision, and recall. The metrics were computed using the following formulas:

$$Accuracy = \frac{TP+TN}{TP+TN+FP+FN} \quad (1)$$

$$Precision = \frac{TP}{TP+FP} \quad (2)$$

$$Recall = \frac{TP}{TP+FN} \quad (3)$$

where: *TN* – stands for true negative instances,
TP – for true positive instances.

Similarly, FN denotes false negative instances and FP denotes false positive instances. Version 3.9.13 of the Python programming language was used for both model training and prediction. The procedure made use of the ultralytics package. Figures 5a and 5b depict the selected *Vorticella* ciliates which were chosen for research and analysis purposes. These ciliates belong to a group that is relevant in evaluating the stability of treatment plants with biological reactors, particularly in terms of removing organic carbon in colloidal and suspension form [35]. Their distinct appearance, abundant population, and sedentary lifestyle make them easily observable and countable under a microscope. Consequently, they serve as an model subject for the conducted detection task [24, 30].

RESULTS

Figures 6a to 6d depict the chosen YOLO network predictions. The image analysis model successfully identifies *Vorticella* in the image, providing bounding boxes and labeling them.

A significant performance enhancement can be observed with YOLOv8. YOLOv4, which was trained for 3200 epochs, yielded inferior results compared to YOLOv8 trained for 100

a) b)

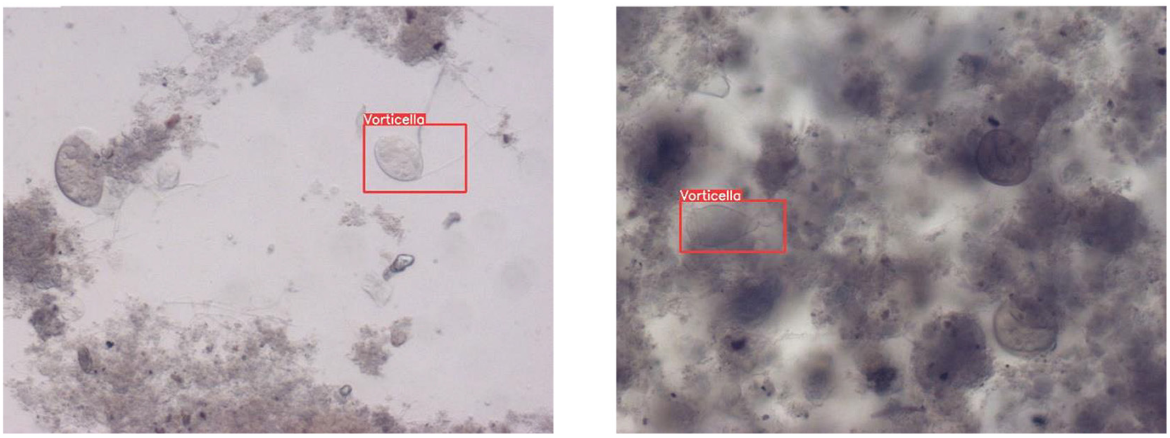

Figure 5. Visible *Vorticella* ciliates

a) b) c) d)

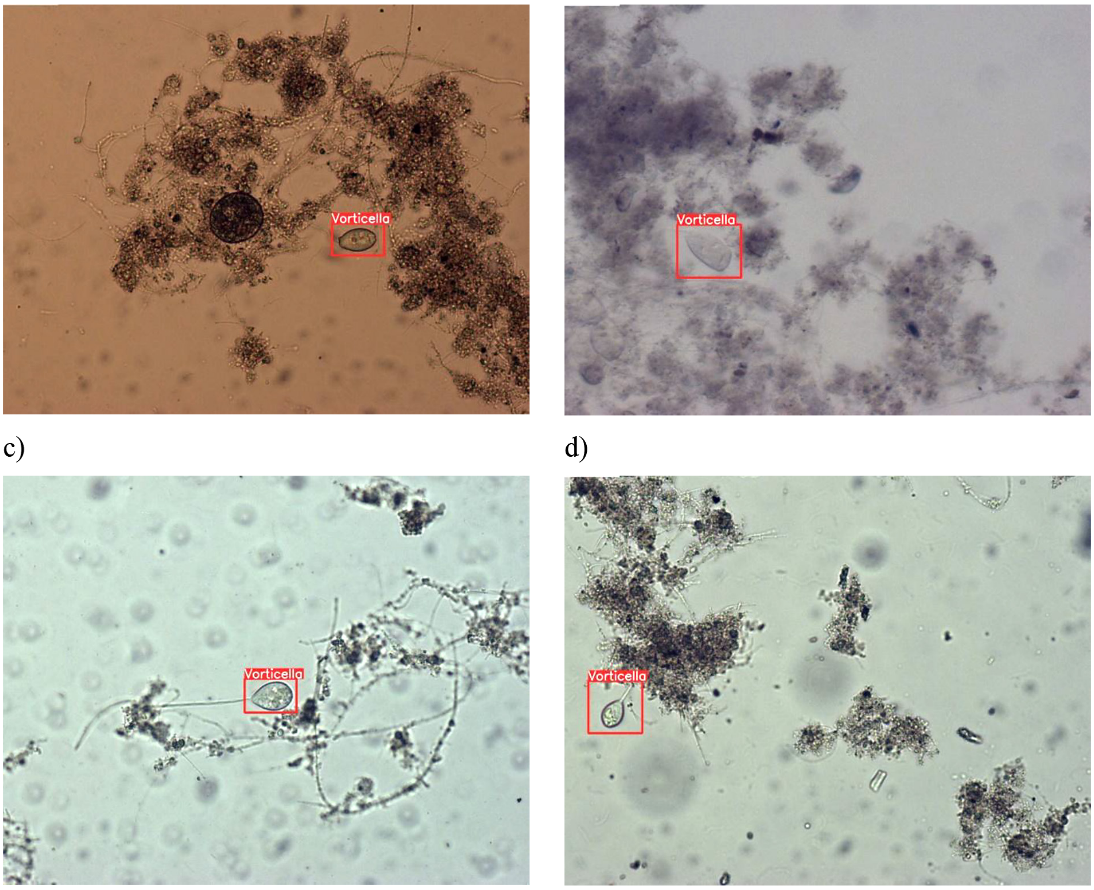

Figure 6. Detected *Vorticella* instances

Table 1. Comparison of object detection quality measures on the test set

Model	Epochs	Training time [h]	Precision	Recall	Accuracy
YOLOv4	3200	20.67	0.75	0.6	0.79
YOLOv8	100	16.41	0.875	0.7	0.86

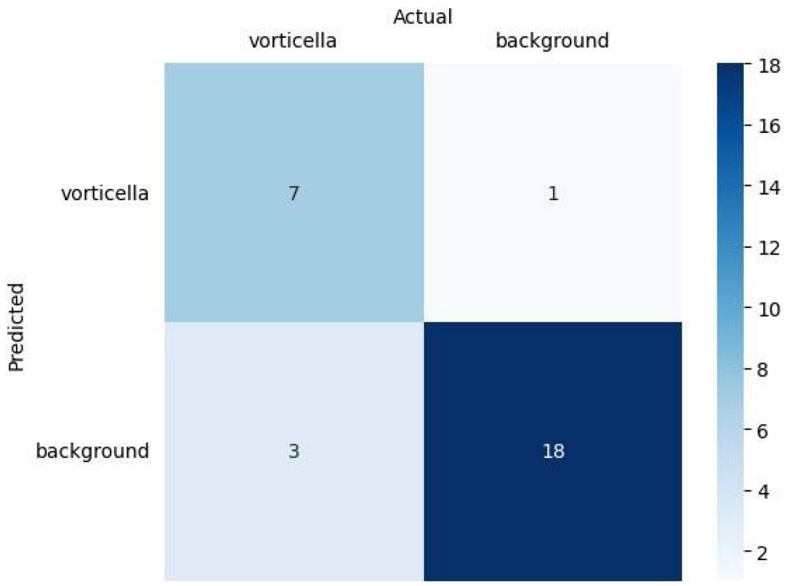

Figure 7. Confusion matrix for the YOLOv8 algorithm

epochs (Table 1). The precision metric for YOLOv4 stands at 0.75, whereas for YOLOv8 it equals 0.875. Similarly, the recall values are 0.6 for YOLOv4 and 0.7 for YOLOv8. The accuracy raises from 0.79 to 0.86. Referring to the authors' previous research, it should be stated that the YOLOv4 and YOLOv8 models were characterized by a similar behavior of characteristic parameters set for the indication and determination of shell amoebae – *Arcella vulgaris* – which are also biomarkers of the functioning of activated sludge bioreactors [30]. However, for amoebae, the number of epochs in YOLOv4 was much smaller and amounted to 1500, while in YOLOv8 it was 100, which is the same as for *Vorticella*. A similar relationship concerning two applied models can be observed when analyzing Training time, where YOLOv4 required 20.85 hours and YOLOv8 10.40 hours. The precision of Arcella determination was higher than *Vorticella* and amounted to 0.94 for YOLOv4 and 0.95 for YOLOv8. The situation was similar with recall and accuracy in detection of Arcella 0.94 and 0.88 for YOLOv4 when 0.95 and 0.97 for YOLOv8 [5]. It can therefore be concluded that the correct identification of sedentary ciliates *Vorticella* in the same sets of digital images is a more difficult task for both networks than the identification of shell amoeba Arcella vulgaris. Figure 7 shows the YOLOv8 confusion matrix – correctly and incorrectly detected individuals. Three *Vorticella* organisms set by human were omitted by the algorithm. Also, prediction of an instance of *Vorticella* was found in an image not labeled by protozoologist.

CONCLUSIONS

This study was aimed at conducting a performance evaluation between the YOLOv4 and YOLOv8 deep learning networks used as a core in system of an automated image acquisition and analysis. The analysis focused on identifying and classifying a specific group of activated sludge organisms, namely *Vorticella,* using digital photos of biological samples containing different organisms from the bioreactors of treatment plant. It is important to note that the network architectures created for the detection task were kept generic, meaning that the layer and filter structures were not impacted by the particular use of these models. Hence, given the universal structure of the models, the results in terms of precision and quality of classification may be deemed highly satisfactory. This implies that the overall framework of the YOLO networks is applicable for particular assignments like identifying settled ciliates. Nevertheless, YOLOv8 showcased a notable enhancement in performance – requiring fewer epochs, it offered superior object detection accuracy compared to the previous iteration, YOLOv4. It is also worth considering labeling organisms using other methods, as the bounding box labeling is probably not the optimal option for elipsoid objects with stalks.

The organism identification model used for the Peritrichia group (*Vorticella*) from images has the potential to expand its capabilities to classify their a wider range. This enhanced model could be utilized in the future for automated analysis of activated sludge conditions, monitoring changes in organism populations, and assessing bioreactor stability based on technical treatment stages and treated wastewater samples. Furthermore, this tool could prove valuable in evaluating surface water quality, particularly in the areas affected by polluted water discharges, such as stormwater from urbanized catchments.

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