The use of convolutional neural networks to identify artifacts of cells micrographs in biomedical research

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Abstract. The work is devoted to the use of artificial neural networks to solve the problem of recognition and isolation of objects (cells) in digital micrographs used in the practice of microbiological research. This task is relevant due to a combination of two factors: a large amount of data (hundreds and thousands of photographs, hundreds of objects on each) arising from such studies, as well as the high complexity of manual image processing and the risk of operator errors. To solve the problem of recognition and separation of objects (cells) in digital biomedical micrographs, artificial convolutional neural networks are used. However, in most cases, the use of artificial neural networks requires selection of the network architecture and its training for each task individually. Each time when solving the problem of training the network, it is necessary to collect huge training samples, the more complex the more complex the architecture. The task of cell isolation was solved using a convolutional neural network. The neural network architecture of Mask R-CNN Facebook Research was used. We used a pre-trained neural network, retrained in digital micrographs obtained and marked out by the authors. The solution is implemented in Python using TensorFlow, an open source machine learning software library developed by Google. As a result of testing the system, on the available data, the correctness of cell recognition in microphotographs was more than 95%, despite the small size of the digital images used. The applied approach definitely showed its efficiency on the available experimental data and has development prospects in the direction of increasing the size of processed images, increasing recognition accuracy, expanding the composition of secreted objects, working not only with cells, but also with structures in tissues.

1. Introduction
Currently, there is the problem of analyzing micrographs of biological objects. Namely, the recognition of cells and cellular structures, since the main key moment in the recognition of objects is a set of various signs (categories) of objects and their description. Moreover, there are a large number of methods for calculating the total number of cells (objects), measuring cell sizes, intracellular structures, tissue formations [1], [2], [3], [4], [5]. However, they are based on direct work with a large number of individual images and the measurement of each object by the researcher, which is very time-consuming, and also requires a lot of time and increased attention [6], [7]. During the experiments, the researcher receives a large amount of data (hundreds and thousands of photographs, hundreds of objects on each),
which explains the complexity of data processing. Moreover, the results of the work are quite subjective in nature, which is associated with the experience and state of health, the degree of researcher fatigue, equipment features, and photo quality. In general, this increases the number of errors and reduces the repeatability of the results.

In recent years, in biomedical research and medical practice, in practice, only digital optical microscopy has been used, with the possibility of obtaining digital photographs. Subsequently, mathematical processing of these images is required. Digital and micrographs have found application in practical medicine: surgery, oncology, ophthalmology, hematology and microbiological research, as well as in biomedical research.

Given the development of immunocytic (histo) chemical studies, molecular diagnostics, when not only the cells as a whole, but also various individual molecules, receptors, organelles, and intercellular contacts in the same image are identified, which are marked with different fluorochromes, the task of recording and analyzing the material obtained is complicated [8].

Currently, various manufacturers of microscopy equipment (Carl Zeiss, Leica, Olympus, Nikon, etc.) provide their own software packages that automate, simplify or conduct the initial, often minimal, process of morphometry and image analysis. At the same time, software is quite expensive, often the cost is greater than or equal to the cost of the equipment itself, and data storage and processing takes place in its own formats (*.oif, *.nef, etc.) and does not answer all the tasks set by the researcher .

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The main key point in the recognition of objects is a set of various features (categories) of objects and their description. This requires a set of reliable and repeatable parameters (descriptors) that are obtained from test images. Actually, object recognition is a process of comparing certain characteristics and the ability to attribute an object to one or another mutually exclusive class [9], [10]. Those recognition of images and objects is one of the varieties of classification, and in cases where the class contains only one object, classification is identification, that is, assigning a unique name to the object under consideration. However, in complex systems, such as tissues or organs, there are quite a lot of different objects (cells) that have both the same type and individual characteristics. In other words, obtaining certain characteristics in the image will allow further classification of the objects in question. Currently, there are three main areas in the field of recognition: 1) image matching with the standard; 2) image recognition by characteristic points (the method of obtaining data of characteristic points may vary); 3) recognition through artificial neural networks (ANN).

A crucial issue in machine learning is the problem of processing large amounts of data, i.e. training sample. Many modern algorithms with good generalizing ability have high computational complexity, and therefore they cannot be used to solve massive problems. In addition, many algorithms generally do not have freely distributed implementations comparable to advanced commercial software, which makes it difficult to use algorithms in applications. Another serious problem that arises in machine learning is the violation of the principle of independence of observations in the training sample, at a time when most algorithms assume this principle to be observed.

Images obtained with microscopes during microbiological experiments require processing of millions of cells. Complex cellular phenotypes are characterized by a high density of contents. Most well-proven visualization tools are not suitable for displaying data with such a density, which led to the development of a new visualization methodology. The review presented in [1] discusses methods of data visualization both in classical studies and in studies in the field of high-content microscopy (microscopy with high data density), as well as the advantages and disadvantages of various visualization methods.
PhenoPlot visualization tool was presented in [2], which presents quantitative data of high-content images with easy-to-interpret glyphs. It is shown how PhenoPlot can be used to improve the study and interpretation of complex phenotypes of breast cancer cells.

In [11], the authors, using standard computer gaming equipment, developed an application that can be run in the laboratory to speed up the execution time of iterations of biological experiments. Uncontrolled image analysis algorithms have been combined with interactive visualization of the results. The authors believe that their tool is the first to combine all these aspects using the synergy obtained by using verification information from stereo-visualization to improve methods for solving low-level image processing problems.

The authors of [12] discuss several important image processing functions, including noise reduction, contrast enhancement, and feature extraction. They believe that no matter what the ultimate goal, understanding the nature of the acquisition and digitization of the image and the subsequent mathematical manipulations with this digitized image are important. The paper discusses the basic mathematical and statistical processes that are usually used in microscopy for the daily production of high-quality digital images and the extraction of key characteristics of interest, using various tools for extracting and determining thresholds.

The current stage of the development of the theory and practice of ANN in terms of working with images is characterized by the fact that they began to recognize images better than a person does with the naked eye. This result was achieved through the use of convolutional neural networks and deep learning algorithms. Work on the use of artificial neural networks (ANNs) for image processing in medicine and biology is being carried out in several directions.

The article [13] considers the algorithm for finding metastases in the body using the convolution neural network Inception-v3. The model accurately identifies areas with metastases. This solution can be used in the analysis and final diagnosis.

In [14], as the assessment metric, scientists chose the Area Under the Curve (AUC). The neural network was trained to isolate and label soft tissue lesions and compaction. An image with a mammogram was fed to the input of the system, and at the output, the system showed a degree of confidence that the patient was ill with cancer, ranging from 1 to 10. Each training sample included images, an estimate of the likelihood of the disease made by the radiologists, and the patient’s actual diagnosis. The total training sample amounted to 189,000, of which 9,000 were positively diagnosed.

The article [15] describes the process of learning a convolutional neural network to determine the structure of a protein. It is noted that after the successful construction of a 3d model of protein, it will be possible to use it in the study of diseases such as cystic fibrosis, Alzheimer's, Parkinson's and Huntington's. A generative adversarial neural network was used to create new fragments and assess the quality of the structure of the new protein. The process of adjusting the synapse weights of the neural network was performed by optimizing the error functional by the gradient descent method.

The solution closest to the proposed topic of the project is described in [16]. The process of training a neural network that tracks the connections between neurons is described. Methods are being developed for staining bundles of neurons that can be used to convert samples of brain tissue into three-dimensional electron microscopic images. The objective of the SyCon solution described in the work is to study the biological neural network, predicting the connections of neurons in the brain.

In a study [17], the authors propose a deep learning model that automatically classifies the histological patterns of lung adenocarcinoma on surgical resection slides. Their model uses a convolutional neural network to identify areas of tumor cells, and then aggregates these classifications to infer prevailing and minor histological patterns for any given image of the entire slide.

The above review allows us to draw the following conclusions:

- The task of processing digital micrographs in modern medical and microbiological research is of high relevance;
- A promising and relevant solution for identifying objects (living cells) in microphotographs is the use of convolutional artificial neural networks.
2. Methods
In this work, micrographs of a monolayer of cerebral endothelium obtained from laboratory rats at the age of 14 days were used. Cells were cultured for 72 hours in an incubator under standard conditions.

To solve the problem of cell nuclei recognition, the architecture of the mask convolutional neural network Mask R-CNN [18] was proposed by Facebook Research, one of the divisions of Facebook specializing in research in the field of artificial intelligence. This architecture develops the ideas of Faster R-CNN by adding a branch that predicts the position of the mask, which allows you to outline the found object. The network diagram is shown in figure 1.

![Figure 1. Architecture of Mask R-CNN (see article [18]).](image)

The authors of the architecture conditionally divide the developed architecture into a convolutional neural network to extract image features, which they call the backbone, and head (which is the union of the parts responsible for predicting the area of an object, its classification and determining its mask), a schematic image of which is shown in figure 2.

![Figure 2. The architecture of the head (see article [18]).](image)

Here, a combination of ResNet101 and FPN is used as the backbone. The FPN architecture allows combining the advantages of large and small dimension feature cards (the former have high resolution, but low generalizing ability, and the latter - vice versa) obtained by ResNet101. For this, the map of each overlying layer is enlarged to the size of the underlying one and their contents are added element by element. In the final predictions in the head, the resulting maps of all levels are used. Head consists of:

- RPN, which divides the image into areas and determines in which objects are present and in which background;
- ROI classifier (areas of interest generated by RPN);
- A convolutional network that calculates masks for detected and classified objects.
The presented training model was chosen because of its flexibility. It provides an opportunity for a researcher to independently choose the backbone and head components, and also supports the ability to work with video files, which in the future will expand the functionality of the system to work with dynamic images.

The training of this neural network was carried out on a pre-trained model, where the data was taken from a similar architecture of a sample from the Data Science Bowl 2018 competition [19] (more than 33 thousand images), the task of which is to recognize the nuclei of cell photos taken with a microscope. The sample for retraining contains 50 marked images.

Python was used as a programming language for implementing this solution using TensorFlow [20], an open source machine learning software library developed by Google.

This trained model supports instance segmentation, which eliminates object overlap. The disadvantage of this solution is the size of recognizable photos 256x256 pixels.

For ease of use, a user interface has been developed that allows you to upload photos and calculate the number of cores on it.

3. Results
Figures 3-8 show the results of the work of the developed neural network.

![Figure 3. Images for recognition No. 1.](image1)

![Figure 4. The result of recognition and counting cells in the image No. 1.](image2)
Figure 5. Images for recognition No. 2.

Figure 6. The result of recognition and counting cells in the image No. 2.

Figure 7. Images for recognition No. 3.

Figure 8. The result of recognition and counting cells in the image No. 3.
It can be seen that in most cases (on average, 20 out of 21), the cells in the micrograph are marked and counted correctly.

4. Discussion
As a result of testing the system on the available data, the correctness of cell recognition in microphotographs was not less than 95%. This indicator is not inferior to the results obtained by live operators during the operation of cell counting. Confidently separated cells that met the Intersection over Union (IoU) criterion of about 61%. A limitation of the proposed solution is the small size of the applied digital images (256 * 256 pixels).

The applied approach, despite the limitations, definitely showed its efficiency on the available experimental data.

5. Conclusions
Thus, the task set in this work - the creation of an artificial neural network that allows to select cells in digital micrographs, is solved with an acceptable level of accuracy.

The resulting solution allows to automate the operation of cell counting in microphotographs, removing from experimenters the most time-consuming and least creative work in the process of microbiological experiments. In addition, the use of the developed system eliminates the subjectivity factor in the analysis of experimental results.

The development of the resulting system is possible in the following directions.

- Increasing the size of processed images;
- Increasing recognition accuracy to at least 85%;
- Expansion of the composition of secreted objects, work not only with cells, but also with structures in tissues.

References
[1] Sailem H Z, Cooper S and Bakal C 2016 Visualizing quantitative microscopy data: History and challenges Critical Reviews in Biochemistry and Molecular Biology pp 96–101
[2] Sailem H Z, Seru J E and Bakal C 2015 Visualizing cellular imaging data using PhenoPlot Nature Communications
[3] Popkov VM, Ponukalin A N et al 2010 Application of automated morphometric method of estimation of expression of immunohistochemical markers in diagnostics of prostate and bladder cancer Saratov Journal of Medical Scientific Research 6 4 845-9
[4] Lee H W, Arif E, Altintas M M et al 2018 High-content screening assay-based discovery of paullones as novel podocyte-protective agents American Journal of Physiology-Renal Physiology 314 2
[5] Shishkin A V, Ovchinina N G and Bessmeltsev S S 2011 Novel approach for detection of cell antigen coexpression using immunological biochip Oncohematology 6 2 23-30
[6] Doronicheva A V and Savin S Z 2014 Methods of medical images recognition in computer aided diagnostics Modern problems of science and education
[7] Doronicheva AV and Savin S Z 2015 Method of segmentation of medical images Fundamental research 5 294-8
[8] Astakhov A S and Bumagin V V 2017 Analysis of the efficiency of the image processing algorithms for biological micro objects recognition in the histological sections Engineering Journal of Don 4
[9] Khotilin M I, Paringer R A, Rystsarev I A and Kravtsova N S 2018 Development and analysis of methods for selecting objects in an image The IV International Conference on Information Technology and Nanotechnology (ITNT-2018), Samara pp 3581-9
[10] Myasnikov V V Description of images using model-oriented descriptors Computer Optic 41 6 888–96
[11] Wait E, Winter M et al 2014 Visualization and correction of automated segmentation, tracking and lineaging from 5-D stem cell image sequences *BMC Bioinformatics* **114** 285-315
[12] Liu Y, Kohlberger T et al Artificial Intelligence–Based Breast Cancer Nodal Metastasis Detection *Archives of Pathology & Laboratory Medicine* In-Press
[13] Rodriguez-Ruiz A, Lang K et al 2019 Stand-Alone Artificial Intelligence for Breast Cancer Detection *JNCI J Natl Cancer Inst.*
[14] Evans R, Jumper J et al. 2018 AlphaFold: Using AI for scientific discovery *Thirteenth Critical Assessment of Techniques for Protein Structure Prediction*
[15] Dorkenwald S, Schubert PJ et al 2017 Automated synaptic connectivity inference for volume electron microscopy *Nature Methods* **14** 435–42
[16] Wei J W, Tafe L J et al 2019 Pathologist-level classification of histologic patterns on resected lung adenocarcinoma slides with deep neural networks *Scientific Reports* **9**
[17] He K, Gkioxari G et al 2018 Cornell University https://arxiv.org/abs/1703.06870
[18] Mirzaev I 2018 5th place solution (based only on Mask-RCNN) *2018 Data Science Bowl* https://www.kaggle.com/c/data-science-bowl-2018/discussion/56326 (2018)
[19] An end-to-end open source machine learning platform 2019 *TensorFlow* https://www.tensorflow.org/