International Journal of Emerging Trends & Technology in Computer Science (IJETTCS)
Web Site: www.ijettcs.org Email: editor@ijettcs.org, editorijettcs@gmail.com
Volume 9, Issue 5, September - October 2020 ISSN 2278-6856

Analysis of Cellular Images using Machine Learning and Image Processing Models

Smruti Karade[1], Dr. S.T. Patil[2]
[1,2]Department of Computer Science and Engineering
Vishwakarma Institute of Technology,
Pune 411037, India.

Abstract: ***Analysis of cellular microscopic images is an essential part of research in medical imaging and is a base for many biomedical applications. The manual inspection for this analysis can be considered as tedious and inaccurate because the microscopic image data recorded consists of hundreds of objects across a large number of images. Cell detection is the first step in the analysis to derive information from its appearance and characteristics. An automated cellular image analysis process would require a lot less human effort resulting in increased productivity and accuracy that can reduce due to human error, judgment differences, fatigue, and other human aspects. The proposed system aims to solve this problem by utilizing the improvements in machine learning and computer vision algorithms that showcased an impressive ability to discern the content within the images.***
Keywords: Cellular image analysis, Image processing, Segmentation, Cell detection.

1. INTRODUCTION

Cell culture is a technique used for studying the behavior of cells in a constraint environment, free of alterations of a system.Various cell cultures are being used for studying basic cell biology and interactions of chemicals and drugs with cells for production of vaccines and proteins etc. Standard image processing techniques are widely being used in biomedical applications for analysis of these cells and tissues by analysis and manipulation of microscopic images.Even though in some use cases where analysing this image data offline is adequate, the ability of computer vision algorithms to generate insights from high content and large datasets show considerable improvements in performance and precision over existing methods.These deep learning algorithms when applied to biological images transform the process of analysis and interpretation of imaging data. These advancements point to render difficult analysis and allow researchers to carry out new experiments that were previously impossible. Cell detection is the starting point for most analyses because it allows researchers to identify every cell sample and measure how they react to various treatments to understand the process.Automated image analysis can speed up research for almost every disease, from rare disorders to the common cold and many lives can be transformed if the cures came faster. This paper is an explanatory research for various methods used in cellular image analysis and the steps required to process the images. The organization of this paper is as follows. Some of the automated techniques that reported a broader perspective in recent years are introduced and analyzed in the survey.The proposed method is discussed in Section 3 followed by the results in Section 4. Section 5 concludes the paper and gives future suggestions.

2. LITERATURE SURVEY

Many works and methods related to cellular image analysis already exist, which have numerous traditional methods based on image sensing techniques. Cellular image analysis can be divided into several steps mainly cell detection, segmentation, feature extraction, feature selection, and classification. As reviewed in [1,3], many living cell experiments have precise interest in tracking and analyzing diverse cell movements. Existing approaches include detection based, model-based, and filter-based approaches.

For multiple object-based tracking, the widely used approach is a filtering based approach in which the current state of object distribution should be known. Also, several techniques are required to reduce the large computational costs. The search for a practically usable and vigorous method for the analysis is still being done as it is a very challenging area due to various complexities and uncertainties present such as noises present in the background, the shape of the cells, conditions of the lighting, etc. The conventional thresholding methods do not perform well due to the existing factors like the distinction between the background and the cell boundary which is highly dependent upon the image capture conditions and the difference in the contrasts occurring due to inconsistent illuminations[4].

Model-based techniques utilize the discrepancies in the shape of the cells under scrutiny by creating and updating the model for each object to be tracked. The model moves inside the object to detect its boundaries, to identify the object, and to describe its shape. An extensively researched model-based approach is the active contour method [11] which uses the object contours of previous frames as initializations in the current frame and although it handles the deformation of cell shapes well, reinitialization is necessary if there is the appearance of new objects in the

frames or large displacements of the objects are seen in the frames.

In detection-based approach, the object is first located and segmented first and then is associated across frames.Some examples include edge or gradient detection, morphological operations, and watershed algorithms. In digital image processing, mathematical morphology is among the basic theories and helps extract different shape- based features by using structuring elements in many small images. This method is very efficient and is used for many computer vision tasks like detection of contours, removal of noise, and image enhancement[15]. Cell localization calculates the position of the cell and is widely used because it enables better accuracy. When the starting position is known, it is easy to perform many high-level tasks like segmentation to get the representation of the shape. That is if there is no complex issue associated with the position in which case the output is satisfactory. However, in cases where there is very little or almost no existence of separation in between two cells, an approximate location of the cell is identified. For particle detection, a technique based on machine learning that uses Haar based methods to extract features was proposed in [16]. Moreover, the automatic determination in multiple cells is somewhat complex because traditional methods like edge detection and operations cannot be applied. Besides, qualitative and quantitative characterization of cell images is important. In [12] the authors proposed an edge-based approach to find a single global threshold to analyze gradient magnitude images instead of thresholding the intensities of original images.The idea is to morphologically process the result by detecting the pixels that have large gradient magnitude on the bright side of the edges and process the result to get the final segmentation. In [4] the authors proposed a method for segmentation and color coding based on nucleus to cell Ratio in which they detected the nucleus using k-means color clustering algorithm and minimum intensity thresholding technique. The authors in [13] summarised various segmentation techniques to be used for the analysis and region based segmentation techniques showcase promising results as compared to clustering, thresholding or edge based segmentation.The authors also discuss several features used for cell identification from the images such as size and structure, texture and colour. For Feature extraction the study finds that the SVM is better to be used for iterative improvements in the training data. The authors in [14] present a network and training strategy that uses data augmentation to efficiently use that available samples.This network can be trained on a very few images and perform better when compared to the sliding window convolutional network. The input images and their segmentation maps are used to train the network with the stochastic gradient descent implementation.However the output image can be smaller than the input due to unpadded convolutions. They demonstrate the application of the network on three different segmentation tasks for multiple datasets and achieved an IOU (intersection over union) from 70-80% which is significantly better than the prior algorithm.The architecture can also be easily applied to many more segmentation tasks as it only requires very few annotated images and less training time.

3 METHODOLOGY

To address the drawbacks of the previous approaches, we introduce an algorithm for cell image analysis that automatically segments the cells using neural networks for further analysis. Figure 1. shows various steps to be taken for cellular image processing.

Algorithm :

1. Acquisition of microscopic cellular images of cell cultures.
2. The images are preprocessed for every cell giving enhanced cell images as result.
3. Enhanced cell images undergo the segmentation process to highlight the shapes of the cells.
4. Extraction of features like texture, geometric appearance and property, co-occurrence matrix from the segmented image.
5. Selection of features which contribute the most to the use case and prediction variables so as to get most accuracy on relevant features.
6. Statistical and quantitative analysis based on features selection on whether the cell is suitable or not to be used for the use case.
7. Using classification algorithms to divide the cells on whether they follow a given rule so as to further divide them.
8. Evaluation of cell images after classification and bifurcation of images.

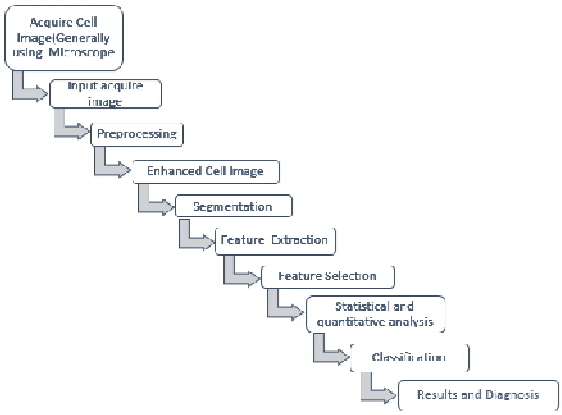


Figure 1 Various Steps in cellular image processing

Figure 2 shows U-Net architecture which is a special type of architecture for image segmentation purposes.U-Net architecture is designed for semantic segmentation in which every pixel is painted corresponding to cells either to be the object or the background. The U-Net architecture is an extension of a fully convolutional network[7] and has been modified so that it produces more precise segmentations

with very few training images.

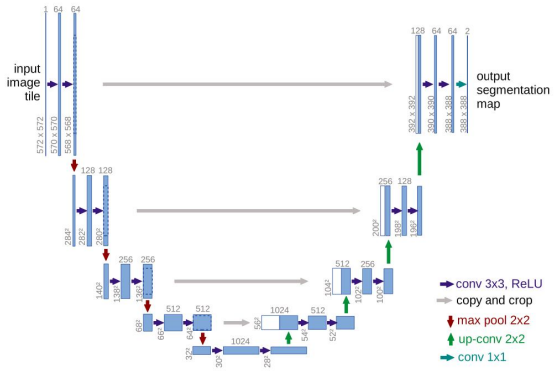


Figure 2 U-net architecture [14]

The main architecture has two paths, a contraction path(also known as the encoder path) and the expansion path(also known as the decoder path). The contracting network is added in every successive layer wherever the upsampling operators replace the pooling operators. As a result, the convolutional layers increase the resolution of the output. The upsampled output is then integrated with the high-resolution features from the contracting path. Concatenation of these feature maps is the reason why we get localized information. The successive convolution layer then learns to assemble more precise output based on the input information. In the upsampling (2x2) part, a large number of feature channels that have been added allow the network to transmit the output information to higher resolution layers.As a result, the expansive path is symmetric to the contracting path and therefore generates a u-shaped architecture. The network only uses the valid part of each convolutional layer and does not have any fully connected layers meaning, the segmentation map only contains the pixels. This allows the smooth segmentation of large images. The missing context is extrapolated to predict the pixels in the border region of the image i.e it adds extra pixels to the image so that the output image is of the same dimensions as the input image. Otherwise depending upon the kernel size used the output image might be larger or smaller than the input image. The background between the touching cells obtain a large weight in the loss function and this resolves the challenge of separation of touching objects in the image[14]. Depending upon the datasets being used certain parameters can be modified to be suitable for the images.

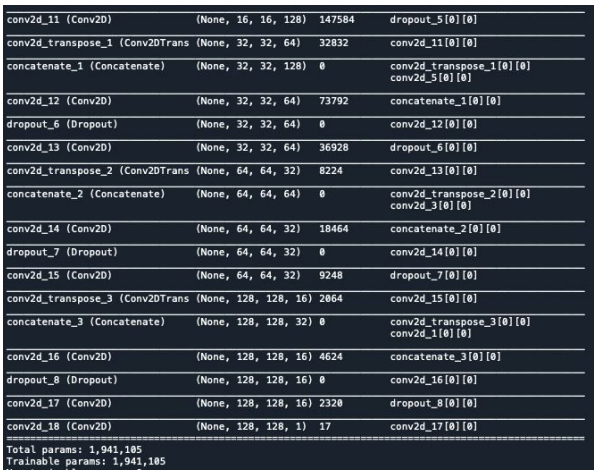
A CNN is a combination of many types of layers. Input layer is used to input image data by pixel. There are many hidden layers included such as fully connected (dense) layer, pooling layer etc. Convolutional layers : In convolution, we have a kernel or filter (3x3 Relu is used as the activation), and this kernel has some value which is applied on a sub-region of the original image to perform some mathematical operations. Depending upon the stride the kernel convolves over the image to perform operations. At first the feature space of 16 is used which doubles in the successive convolutional layer since the kernel size is of stride 2.

There are three main layers in the architecture (1) convolutional layers, (2) max pooling layers, and (3) fully connected (dense) layers.

1. **Input layer:** This layer loads images and produces outputs that are then fed to the first convolutional layer. The input image dimensions used are 128x128x3. The images are resized into 128x128 and 3 indicating that the images are coloured images.
2. **Max Pooling layer (2x2)** stride = 2**:** The objective of Max pooling layer is to output the maximum pixel value within whatever the kernel size is being used and replace the 2x2 matrix with that value.This is done to help overfitting and to provide an abstract form of representation. Max filter is used on sub regions that are non-overlapping on the input.
3. **Fully Connected:** In a fully connected layer neurons are connected to all the activities done in the previous layer like regular neural networks. This is the reason their activities are computable through matrix multiplication.
4. **Dropout:** Dropout deals with dropping out some neurons during forward and backward passes. This is done during the training between the convolutional steps so as to prevent the neural network from over-fitting.

4 RESULTS AND CONCLUSIONS

Implementation of the network showcases an impressive performance on image segmentations. The model is able to localise the cell borders because it classifies every image pixel by pixel predicting whether the pixel belongs to the object or the background.The CNN model prepared is trained on CPU for approximately 6 hours for a number of images.The dataset used was divided for training, validation and testing purpose for which result is as shown below:



conv2d_11 (Conv2D)	(None, 16, 16, 128)	147584	dropout_5[0][0]
conv2d_transpose_1 (Conv2DTrans	(None, 32, 32, 64)	32832	conv2d_11[0][0]
concatenate_1 (Concatenate)	(None, 32, 32, 128)	0	conv2d_transpose_1[0][0] conv2d_5[0][0]
conv2d_12 (Conv2D)	(None, 32, 32, 64)	73792	concatenate_1[0][0]
dropout_6 (Dropout)	(None, 32, 32, 64)	0	conv2d_12[0][0]
conv2d_13 (Conv2D)	(None, 32, 32, 64)	36928	dropout_6[0][0]
conv2d_transpose_2 (Conv2DTrans	(None, 64, 64, 32)	8224	conv2d_13[0][0]
concatenate_2 (Concatenate)	(None, 64, 64, 64)	0	conv2d_transpose_2[0][0] conv2d_3[0][0]
conv2d_14 (Conv2D)	(None, 64, 64, 32)	18464	concatenate_2[0][0]
dropout_7 (Dropout)	(None, 64, 64, 32)	0	conv2d_14[0][0]
conv2d_15 (Conv2D)	(None, 64, 64, 32)	9248	dropout_7[0][0]
conv2d_transpose_3 (Conv2DTrans	(None, 128, 128, 16)	2064	conv2d_15[0][0]
concatenate_3 (Concatenate)	(None, 128, 128, 32)	0	conv2d_transpose_3[0][0] conv2d_1[0][0]
conv2d_16 (Conv2D)	(None, 128, 128, 16)	4624	concatenate_3[0][0]
dropout_8 (Dropout)	(None, 128, 128, 16)	0	conv2d_16[0][0]
conv2d_17 (Conv2D)	(None, 128, 128, 16)	2320	dropout_8[0][0]
conv2d_18 (Conv2D)	(None, 128, 128, 1)	17	conv2d_17[0][0]

Total params: 1,941,105
Trainable params: 1,941,105

Figure 3 Model Summary

Figure 3 shows a model summary which displays various parameters used in each and every layer of the network. It also displays the total number of parameters to be calculated by the network (weights & biases).

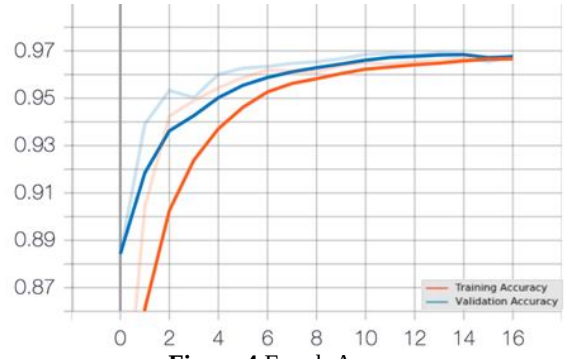


Figure 4 Epoch Accuracy

Figure 4 shows accuracy comparison between the training and validation of the model. Initially the model gained the epoch accuracy and after the 12th epoch the model stabilised and achieved an accuracy of around 97%.

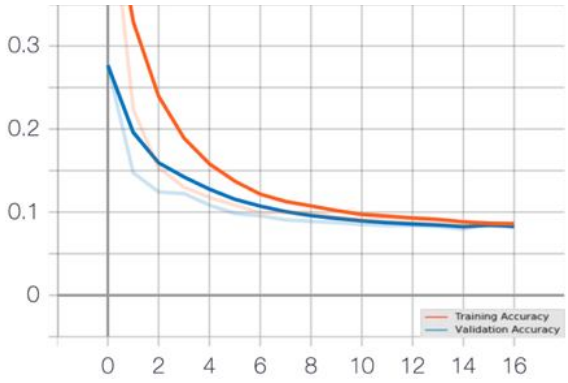


Figure 5 Epoch Loss

Figure 5 shows comparison between the training and validation loss. After the model stabilised the validation loss reduced to 0.08%.

The proposed system presents an effective way to analyze cellular images using various techniques in image processing.Different algorithms that can be used for analysis have been studied and compared. Various steps in cellular image processing have been covered for example: cell localization, segmentation, feature extraction and selection, classification, estimation, shape analysis etc. Cellular image analysis plays a crucial role in biomedical image analysis and an automated cellular image application can have multiple applications in this research domain.

An automated cell culture analysis system will enable the processing and analysis of these cultures in a more productive and accurate way. Although Human experts finally carry out the image analysis, image processing is necessary to extract the data these images contain. This would result in reduction of uncertainty in the characterization of these cell cultures depending upon the test cases, and reduce costs and time needed for testing.

References

[1] A. Hand *et al.*, “Automated Tracking of Migrating Cells in Phase-contrast Video Microscopy Sequences using Image Registration” , Journal of Microscopy, 234: 62-79, 2009.

[2] Chen S, Zhao M, Wu G, Yao C, Zhang J. Recent advances in morphological cell image analysis. *Comput Math Methods Med*. 2012;2012:101536. doi:10.1155/2012/101536

[3] J. Rittscher, “Characterization of Biological Processes through Automated Image Analysis,” Annual Review of Biomedical Engineering, 12: 315-344, 2010.

[4] J. John, Madhu S. Nair and Wilscy M. , “Cell Image Segmentation and Colour coding based on Nucleus to Cell Ratio” Engineering in Medicine and Biology Society, 36th Annual International Conference of the IEEE, 26-30 Aug 2014, pp. 6302-6307.

[5] Lorenzo-Ginori, Juan & Orozco, Ruben. (2013). Cell Microscopy Imaging: A Review on Digital Image Processing Applications.

[6] Kong J, Cooper L, Kurc T, Brat D, Saltz J. Towards building computerized image analysis framework for nucleus discrimination in microscopy images of diffuse glioma. *Conf Proc IEEE Eng Med Biol Soc*. 2011;2011:6605-6608. doi:10.1109/IEMBS.2011.6091629

[7] Kung-Shiuh Huang, C. B. Kuznia, B. K. Jenkins and A. A. Sawchuk, "Parallel architectures for digital optical cellular image processing," in Proceedings of the IEEE, vol. 82, no. 11, pp. 1711-1723, Nov. 1994, doi: 10.1109/5.333749.

[8] Long, J., Shelhamer, E., Darrell, T.: Fully convolutional networks for semantic segmentation (2014), arXiv:1411.4038 [cs.CV]
[9] M. Gamarra, E. Zurek and H. S. Juan, "Cell identification using image analysis: A literature survey," 2017 12th Iberian Conference on Information Systems and Technologies (CISTI), Lisbon, 2017, pp. 1-7, doi: 10.23919/CISTI.2017.7975973.
[10] M. Roy et al., "Cellular image processing using morphological analysis," 2017 IEEE 8th Annual Ubiquitous Computing, Electronics and Mobile Communication Conference (UEMCON), New York, NY, 2017, pp. 237-241, doi: 10.1109/UEMCON.2017.8249037.
[11] O. Dzyubachyk *et al*., "Advanced Level-Set-Based Cell Tracking in Time-Lapse Fluorescence Microscopy," IEEE Trans. on Medical Imaging, 29(3):852-867, 2010.
[12] Oei RW, Hou G, Liu F, et al. Convolutional neural network for cell classification using microscope images of intracellular actin networks. *PLoS One*. 2019;14(3):e0213626. Published 2019 Mar 13. doi:10.1371/journal.pone.0213626
[13] R. M. Thomas and J. John, "A review on cell detection and segmentation in microscopic images," 2017 International Conference on Circuit ,Power and Computing Technologies (ICCPCT), Kollam, 2017, pp. 1-5, doi: 10.1109/ICCPCT.2017.8074189.
[14] Ronneberger O., Fischer P., Brox T. (2015) U-Net: Convolutional Networks for Biomedical Image Segmentation. In: Navab N., Hornegger J., Wells W., Frangi A. (eds) Medical Image Computing and Computer-Assisted Intervention – MICCAI 2015. MICCAI 2015. Lecture Notes in Computer Science, vol 9351. Springer, Cham.
[15] S. Hore, S. Chakraborty, S. Chatterjee, N. Dey, A. S. Ashour, L. V. Chung, and D. N. Le. "An Integrated Interactive Technique for Image Segmentation using Stack based Seeded Region Growing and Thresholding" International Journal of Electrical and Computer Engineering, Vol 6, no. 6, 2016.
[16] S. Jiang, X. Zhou, T. Kirchhausen, and S. T. C. Wong, "Detection of molecular particles in live cells via machine learning," Cytometry Part A, vol. 71, no. 8, pp. 563–575, 2007.
[17] T. Kanade et al., "Cell image analysis: Algorithms, system and applications," 2011 IEEE Workshop on Applications of Computer Vision (WACV), Kona, HI, 2011, pp. 374-381, doi: 10.1109/WACV.2011.5711528.
[18] Xiaobo Zhou and S. T. C. Wong, "High content cellular imaging for drug development," in IEEE Signal Processing Magazine, vol. 23, no. 2, pp. 170-174, March 2006, doi: 10.1109/MSP.2006.1598095.