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Deep Learning for Microscopy Image Analysis

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Abstract: Biological Cell images can be produced using microscopy imaging, which is then utilized for cell studies in computational biology research and clinical illness detection. Quantitative analysis of biological cells is helpful for understanding biological activity at the cellular level and is performed by biological cell image analysis techniques such as cell segmentation, classification, tracking, etc. In this article we detail microscopy image processing for biological cell segmentation and tracking from recent literature.

Keywords: Microscopy, Deep Learning, Cell Segmentation, Cell Tracking.

I. INTRODUCTION

The investigation of cell activities by microscopy image analysis of cells is essential for research on embryogenesis [1], tumerogenesis [2], drug development [3], angiogenesis [4] etc.. Image acquisition systems are installed on microscopes in order to capture microscopy images. Massive amounts of data are generated by these investigations, with microscope images containing hundreds or thousands of cells. Microscopy imaging frequently faces a number of difficulties, including clumped cells, low contrast and poor illumination to name a few. Historically, human professionals would painstakingly analyze these images. However, manual analysis has become unfeasible due to the development of high-throughput tests. Furthermore, both intra- and inter-observer variability might occur during manual analysis. Automated or semi-automated examination of microscopy cell pictures has become indispensable because of these factors. For these reasons, a plethora of computer algorithms have been developed, including those for cell segmentation [5], cell identification and counting [6], and cell tracking [7].

Accurate cell segmentation is a crucial task for researching biological processes in general as well as cellular biology and single-cell analysis. The process of dividing a microscopic picture domain into segments that correspond to distinct cell instances is known as cell segmentation. It is considered a cornerstone of image-based cellular research and a basic step in many scientific studies. Cellular morphology is a good way to determine a cell's physiological condition, and a well-segmented image can provide biologically significant morphological details.

Time-lapse microscopy is an important tool to examine biological cell processes and inter cellular dynamics having wide range of applications from cellular and molecular biology to more advanced applications like clinical practice. Time-lapse microscopy is conducted using a microscope system that can acomodate a digital camera with time lapse mechanism. To observe the cell behaviour, living cells of interest are placed in appropriate culture media and then placed under a microscope and images of relevant regions are captured at regular intervals of time. The cells are detected and tracked from the captured images in order to study the spatio-temporal behaviour of cells. These experimental studies are useful in cancer research, cell migration studies, drug discovery process, angiogenesis, mitosis detection, tumoriogenesis to name a few.

Because deep learning has demonstrated potential effectiveness in a variety of domains, we will be reviewing new cell tracking techniques in time lapse microscopy images in this study. The cell tracking challenge shows that, while deep learning is still an emerging subject, there is a lot of room for new approaches and experiments to reach higher benchmarks. Deep learning is worthwhile to investigate because of its outstnading results, even if it demands massive amounts of computer resources and a large amount of data to learn. Tensor Processing Units (TPUs), Amazon Sagemaker, Google Colab, and Graphical Processing Units (GPUs) can now be used to train deep learning models. This work aims to review important advancements in the field of cell segmentation and tracking in microscopy pictures using deep learning techniques.The rest of the article is presented as follows: Section II discusses materials and methods, and section III discusses the evaluation metrics followed by conclusions and then references.

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II. MATERIALS AND METHODS

A. Time Lapse Microscopy and Datasets

The scientific method of using a microscope to examine materials and objects that are invisible to the human naked eye is called microscopy. In these experiments, live cell pictures growing in a culture are captured at regular intervals using microscopes equipped with image or video acquisition equipment. Therefore, by using image processing, computer vision, or deep learning algorithms on the images, the morpho-dynamics of cells can be examined. To extract valuable biological and biochemical insights, computer scientists and life scientists examine these microscopic images. Time lapse microscopy is an extremely useful tool for live cell investigation because it is automatic and non-invasive. There are many different kinds of microscopy techniques. Figure 1 displays a few sample images from the datasets used in the cell tracking challenge [8]. These pictures were produced using a variety of microscopy techniques, including differential interference contrast, phase contrast and fluorescence microscopy.

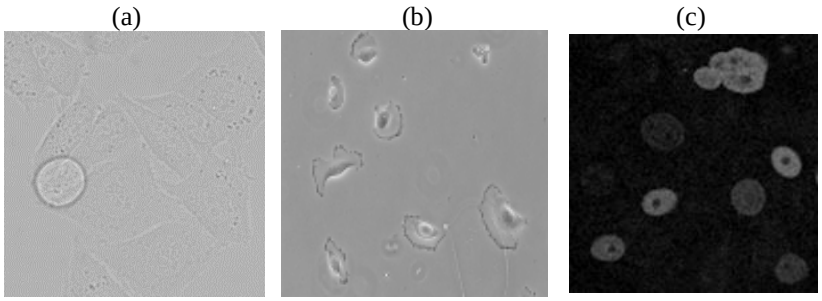

Fig 1: (a) DIC_C2DH_HELA (b) Phc-C2DH-U373 (c) Fluo-N2DH-GOWT1

Some of the microscopy datasets which were used for different studies from the literature is shown in the Table 1.

Table I

Task/	**Datasets/ Reference**	**Microscopy**	**Links/ Reference**
Cell Segmentation + tracking	ISBI Cell Tracking Challenge	Fluorescence microscopy Phase contrast microscopy Differential Interference contrast (DIC) microscopy	http://celltrackingchallenge.net/
Image Segmentation	NIH 3T3 Fibroblast cells	Phase contrast microscopy	https://isg.nist.gov
Image Segmentation	Broad Bioimage Benchmark Collection	Fluorescence microscopy Differential Interference contrast (DIC) microscopy Bright field microscopy	https://bbbc.broadinstitute.org/image_sets
Image Segmentation +cell tracking	DeepCell Datasets	Fluorescence microscopy Phase contrast microscopy	http://www.github.com/vanvalenlab/deepcell-tf, http://simtk.org/projects/deepcell

B. Cell Segmentation using Deep Learning

U-Net [9] architecture is the most successful deep learning network for biomedical image segmentation giving outstanding segmentation accuracy for the segmentation of cytoplasm in Cell tracking challenge. From then on researchers across the globe have adapted U-Net with various extensions and modifications to further enhance its performance and widen its applications. U-Net architecture has “U” shape and is a symmetrical deep learning network and is divided into three parts: the contracting path, the bottleneck, and the expansive path. The contracting path consists of convolutional layers, ReLu Activation and maxpooling operations. The bottommost or bottleneck layer uses convolutional layers followed by upconvolution layer and acts as bridge between contracting and expansive paths. The

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expansive path includes convolutional layers and upsampling layers. Also, the feature maps of the corresponding contracting path are appended to the expanding path which enables the network to use the complex features that are learnt in the contracting path for reconstruction and localization. Their unique way of training the network using a weight map resulted in separation of touching cells which is very crucial in evaluating segmentation accuracy in the presence of cluttered cells. One more highlight of their network is they have done data augmentation thus requiring very less images to effectively train a deep learning model. Thus, U-Net model soon became the benchmark for image segmentation not only in medical domain but also in several other domains. Among other famous CNN networks for segmentation of cells are Mask R-CNN [10] and DeepCell [11]. Mask R-CNN is a state-of-the-art model for instance segmentation, which extends the model Faster R-CNN [12]. This framework for instance segmentation extends Faster R- CNN by adding an additional branch for predicting segmentation masks on each Region of Interest, in parallel to the existing branch for classification and bounding box regression, and generates a superior segmentation mask for each instance. Instance segmentation can be considered as a subtype of image segmentation where each instance of each object is identified within the image at the pixel level. Another subtype of image segmentation is known as semantic segmentation in which every pixel in an image is classified into a specific class. In cellular microscopic images since the cell instances may overlap with each other, for detecting individual cell instances, instance segmentation would be preferred. However semantic segmentation in this context may also be used with additional care while training and using postprocessing techniques to detect the cluttered cells. DeepCell uses deep convolutional neural networks for classifying each pixel into three classes based on whether the pixel belongs to the cell boundary, cell interior, or the background (cell exterior). They showed that the image segmentation of single cells in microscopy images can be converted to an image classification problem. Several others have proposed some CNN architectures [13,14] for segmentation. However, the widely used deep learning architecture for medical image segmentation is U-Net and its variants

C. Tracking Cell Using Deep Learning

Junjie et.al. [15] performed cell tracking by "tracking by detection" method. First the U-Net segmentation technique is implemented to detect all cells in each frame. Thereafter a cell time-series model is constructed by employing a discrete -time Markov process and Kalman filter is designed to predict the cell states which is used to formulate the cell association as a linear assignment problem. Cell association is solved by using a deep reinforcement learning (DRL) framework which uses a residual CNN neural network. The data association may be enhanced to deal with one to many and many to one cell association. Panteli et. al. [16] performed the initial segmentation using U-Net model and employed Siamese tracker which matches each cell in the temporal axis. The raw images were first preprocessed using histogram processing and then normalization before feeding into the U-Net model and postprocessed to find the cell centroids. These cell centroids are used as seeds for a random walker algorithm to produce individual cells. These detected individual cells in every frame are fed to a Siamese tracker [17] for cell linking. Tracking is done in both forward and backward direction to predict the cell location accurately. Their work modelled cell collision, cell mitosis and cell apoptosis. They have also incorporated re-segmentation using watershed deconvolution [18] in the case of collision of two or more cells, thereby enhancing the accuracy. Christian payer et.al [19] have proposed a modified recurrent fully convolutional network architecture in order to track instance segmentations over a period of time. They used Convolutional gated recurrent units (ConvGRU) into a stacked hourglass network for utilizing the temporal information. Further, they trained the network using a novel embedding loss based on cosine similarities, so that unique embeddings for every instance are predicted by the network, throughout the frames inspite of the presence of dynamic topological changes of cells. For instance tracking, they have shown that the prediction of embedding vectors for instance segmentation can be successfully combined with incorporating temporal information as recurrent networks. DeLTA [20] uses two U-Net models for segmentation and tracking each, and they claim that U-Net model was used for tracking cells for the first time. They have done single cell, non-dividing cell tracking after segmenting the cells. Further their method may be improvised by using a single U-Net model for both segmentation and tracking.

III. RESULTS

A. Evaluation Metrics

Dice Index (DI) and Jaccard Index (JI) is utilized to measure the segmentation performance metrics. Dice Index is given by Eq. 1

$$DI = \frac{2 * (GT \cap PM)}{|GT| + |PM|} \quad (1)$$

where, GT stands for Ground Truth, PM stands for Predicted mask. DI value lies between 0 and 1, higher value of DI indicates that the ground truth and the prediction mask are more similar. Jaccard Index (JI) is is given by Eq. 2

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$$JI = \frac{|\ GT \cap PM\ |}{|\ GT \cup PM\ |} \qquad (2)$$

Tracking accuracy measure (TRA): gives the normalized weighted distance between the reference tracking ground truth and the tracking solution by an algorithm [8].

IV. CONCLUSION

An overview of some of the most recent deep learning-based cell tracking methods is provided in this article. The study of cell segmentation and tracking is a crucial field. Even with a wealth of prior research, several problems remain to be solved, such as improving cell connection in unclear circumstances, accurately separating clumped cells, and segmenting cytoplasm images. Deep learning models that are trained on certain datasets typically do not generalize well to diverse cell types, and as a result, they may not produce correct findings for other datasets. In future research novel deep learning models which are trained on heterogeneous data may be developed for more generalizability.

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