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Detecting Lamellipodia in Epithelial Cell Clusters Using a Fully Convolutional Neural Network for Phase Contrast Microscopy Images

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Abstract: We present an approach for segmenting individual cells and lamellipodia in epithelial cell clusters using fully convolutional neural networks. The method will set the basis for measuring cell cluster dynamics and expansion to improve the investigation of collective cell migration phenomena. The fully learning-based front-end avoids classical feature engineering, yet the network architecture needs to be designed carefully. Our network predicts how likely each pixel belongs to one of the classes and, thus, is able to segment the image. Besides characterizing segmentation performance, we discuss how the network will be further employed.

Keywords: lamellipodia, convolutional neural network, supervised learning, segmentation

1 Introduction

Active migration of cells is an important process in many physiological and pathophysiological situations such as wound healing, development, and cancer spreading. Consequently, many *in-vitro* assays have been developed for studying migration of either single cells or collective cell ensembles [1]. In collective cell migration, cells often form a closed mono-layer in which they interact through cell-cell contacts. The active migration, cell divisions, and cell-cell interactions result in complex collective behavior. For investigating collective cell migration phenomena often an epithelial cell layer is either disrupted by a scratch or the layer is cultured in a geometrical confinement, which can be removed resulting in a continuous spreading of the cell layer into the available culture substrate. In our model experiments, we used photoswitchable surface coatings for initially confining MDCK epithelial cells and releasing them by a short UV light pulse [2]. Cells migrate outwards into uncovered areas, typically guided by so-called leading cells, which show prominent lamellipodia. To understand the complex collective behavior quantification by image processing techniques for microscopy image sequences is essential. Consequently, several methods have been developed previously. One example is the use of particle image velocimetry (PIV)[7, 8].

Some of these methods gain only parts of available information in the images. For example, single cells with their outline are not identified within the ensemble, thus not allowing for morphological analysis and correlating it to the cluster behavior. To address this problem, we use a fully convolutional network to identify lamellipodia (and cells) in a sequence of images or in a video taken by a phase contrast microscope. We will show that a neural network is suitable for distinguishing the lamellipodia from the background and membranes, thus allowing further processing.

2 Pixelwise Segmentation Using Fully Convolutional Neural Networks

Fully convolutional neural networks (FCN) are widely used in the field of semantic segmentation, ranging from classifying images as a whole [5] to pixel-wise labeling [3, 4]. In the biomedical field the chosen form of network depends on the questions that researchers want to answer. In our case, we aim to segment cells, membranes, lamellipodia, and the background within images of cell clusters, taken from a standard phase contrast microscope. Since there are multiple classes to detect in one image we have to assign each pixel in an image to one of the mentioned classes. Therefore, we chose to use a FCN to work with. The architecture of the FCN was implemented in MATLAB. Since we do have the knowledge of an expert to label the data into the corresponding classes we transfer this expert knowledge into our network through supervised learning.

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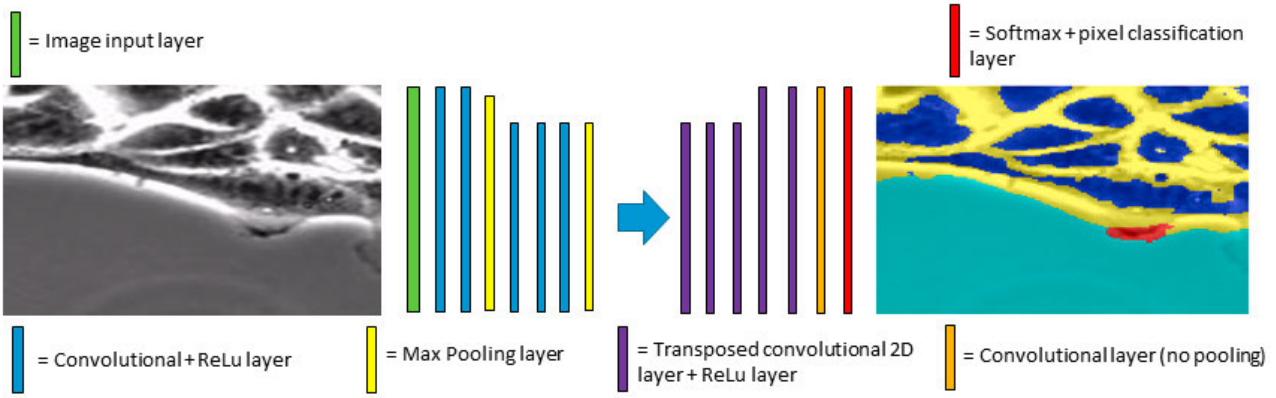


Fig. 1: Architecture of our FCN.

2.1 Architecture of our FCN

The difference between a CNN and a FCN is the use of deconvolutional layers and a softmax function with a pixel classification layer [3]. With these layers it is possible for the FCN to assign each pixel to a learned class instead of assigning the whole image to a learned class. Deconvolutional layers are used to up-sample their respective feature maps and combine them with their corresponding convolutional layers in order to expand from lower-resolution abstract layers towards image space where the final segmentation is taking place. The softmax layer computes the probability of each pixel over the given classes. Using this layer the pixel classification layer assigns each pixel to the class with the highest probability. Our FCN consist of 26 layers in total (c.f. Figure 1) and is similar to U-Net [4]. With this architecture, we achieve an adequate network depth for the task of recognizing membranes, cells, background, and lamellipodia while we are still able to train the network in a reasonable amount of time (around 3 hours). Our hardware setup consists of a TITAN Xp GPU, an Intel Core i7-8700K CPU and 32 GB RAM. The training of the network is performed by the GPU since MATLAB offers support for CUDA enabled GPUs. We used the MATLAB R2018a version for the implementation.

2.2 Generating of the Reference Segmentation

In order to train our FCN a reference segmentation is needed. We generated our reference segmentation from 78 greyscale images with size 120x120px from randomly taken frames of a video showing the growth of an epithelial cell cluster [2] taken with a phase contrast microscope. These images got labeled by hand using the image labeler extension in MATLAB (c.f. Figure 2). Note that in most cases the reference segmentation

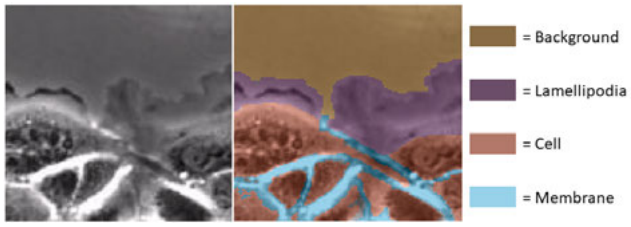


Fig. 2: Reference segmentation for training data.

is called "ground truth", but in our case there is not a 100 percent certain annotation for each pixel due to the uncertainty of the expert who is labeling the training images by hand. A proposed solution for this problem can be found in [9], but since we only have one expert for labeling we do not investigate this issue further in this paper. The classes we aim to detect in our image sequences are cells, membranes, lamellipodia and background. Note that membranes can be distinguished easily in most cases trough the bright appearance. That is not the case for the lamellipodia, their appearance can easily be confused with the background. Since lamellipodia determine the growth orientation they are important to recognize for gaining insight about factors that influence the direction of cell sheet growth. Also, they have to be recognized for analyzing what factors benefit the growth of the lamellipodia itself.

3 Results

The outputs of our net are output maps for each class learned through the labeling to a given image. An output map has the same size as an image and the values range from 0 to 1 for each pixel according to their probability to belong to a given class (c.f. figure 3). Through splitting the segmented image into the different classes the output class can be selected according to the tasks they are needed for. Another advantage is the missing

Fig. 3: The output of the FCN. 1 = resulting segmentation with all classes, 2 = the segmentation for the class "membranes", 3 = class "cells", 4 = class "lamellipodia", 5 = class "background". Each value in a pixel varies between 0 and 1 for their calculated probability to belong to a given class.

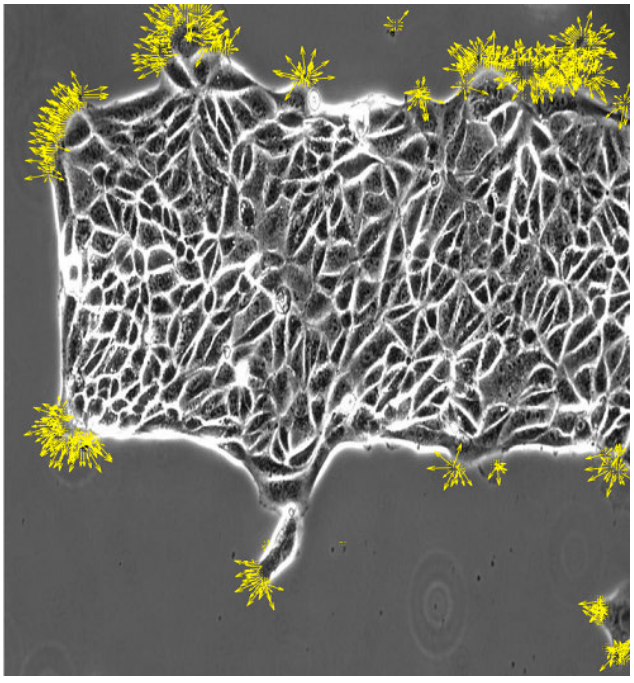
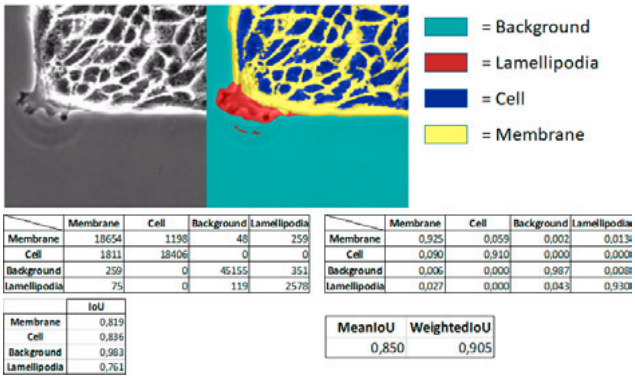


Fig. 4: Representation of the local motion of the lamellipodia. The whole cellcluster is visible for orientation.



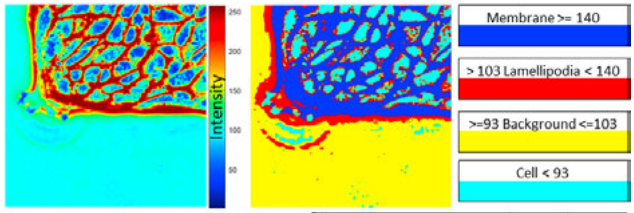
	Membrane	Cell	Background	Lamellipodia
Membrane	18654	1198	48	259
Cell	1811	18406	0	0
Background	259	0	45155	351
Lamellipodia	75	0	119	2578

	Membrane	Cell	Background	Lamellipodia
Membrane	0,925	0,059	0,002	0,013
Cell	0,090	0,910	0,000	0,000
Background	0,006	0,000	0,987	0,008
Lamellipodia	0,027	0,000	0,043	0,930

	IoU
Membrane	0,819
Cell	0,836
Background	0,983
Lamellipodia	0,761

MeanIoU	WeightedIoU
0,850	0,905

Fig. 5: Test image (top left) with segmented classes from network(top right). Absolute confusion matrix with pixel count (left) and normalized confusion matrix (right) underneath the images. IoU for each class and weighted- meanIoU for the whole image.



	Membrane	Cell	Background	Lamellipodia
Membrane	20026	0	5	128
Cell	2176	10493	2073	5475
Background	206	842	40303	4414
Lamellipodia	886	399	174	1313

	Membrane	Cell	Background	Lamellipodia
Membrane	0,993	0,000	0,000	0,006
Cell	0,108	0,519	0,103	0,271
Background	0,005	0,018	0,881	0,096
Lamellipodia	0,320	0,144	0,063	0,474

	IoU
Membrane	0,961
Cell	0,519
Background	0,880
Lamellipodia	0,414

MeanIoU	WeightedIoU
0,694	0,802

Fig. 6: Greyscale image with heatmap according to intensity on the left. Segmentation based on threshold on the right.

pre- and postprocessing with morphological operations. Each of these output maps can directly be used for other operations since they are already a stable and normalized outcome.

A problem with the cells, however, is their natural behavior. Cell division challenges the tracking of individual cells since the object changes appearance during division and the number of objects change over time. Therefore, we initially focus on analyzing lamellipodia since they do not show such complex behavior over time. Data about spatial and temporal appearance and further dynamics of lamellipodia, like their velocity, are key information about the cell sheet behavior in such in-vitro experiments done by cell biologists. Through the independent outputs of the resulting segmentation, we can observe and process the classes in each combination we desire. As shown it can be favorable to use the classes of membranes in combination with the lamellipodia or just the cells. Another suitable method of tracking and, in this case, possibly predicting the cell growth is the use of the Horn-Schunck optical flow method for analyzing the local motion of observed particles in an image sequence. Since the output of the Horn-Schnunck method is the orientation and the magnitude of movement for the observed particles (in this case the lamellipodia) we should be able to measure the direction and velocity of cell growth and further generate a model for prediction. A visualization of the local motion of the lamellipodia can be seen in Figure 4.

3.1 Evaluation

For evaluating the result of the output maps we use the confusion matrix and the weighted intersection over union (IoU) [6] due to the imbalance of the class sizes. With the confusion matrix, we can evaluate our overall precision of the segmentation as well as identify potential inaccuracies for our network. As can be seen in Figure 5 we use a test image which contains all four classes for the evaluation. This image has been segmented by hand from an expert and the result of the network has been compared to the expert's segmentation. The confu-

sion matrix demonstrates minor shortcomings in distinguishing the background and the membrane from the lamellipodia. The cause might be that even for an expert the difference of where the lamellipodia originates and in which pixels they actually end is not easy to spot. Note the falsely segmented area in the background, here we have a dark ring which is confused with lamellipodia. For countering this phenomenon we apply an area threshold to isolate the correct detections from the false positives. Another finding of the evaluation is the relatively (compared to the other classes) low IoU of the lamellipodia. This observation might be due to the problem of that their occurrence is blended over other classes. To demonstrate this hypothesis we also segmented the test image based on the greyscale. This effect can be seen in Fig. 6. The threshold for the classes has been selected by hand from an expert, note that the membrane scores are better with this approach while the result for cells and especially lamellipodia is worse. Even if the threshold is adjusted, there is no threshold that seperates the lamellipodia from the background or cells. Thus morphological filtering is not suitable for detecting lamellipodia.

4 Further Work

For further work, we aim to track lamellipodia and the individual cells in the cluster over time in order to quantify collective phenomena over different length scales in the ensemble. The basis for this step is the result of the FCN networks segment predictions in combination with for example the Horn-Schunck method (c.f. figure 4) or a recurrent neural network for measuring the local motion. With the use of the local motion we should be able to establish a tracking method for each class given by the output maps (i.e. lamellipodia or cells). The evaluation suggests that the output maps are suitable for further processing.

Author Statement

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