Statistical Consulting

Segmentation of Myotubes and Myoblasts

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Abstract

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1 Background and Project Goal

Myogenesis is the process that describes the formation, growth, development as well as regeneration of muscle tissues in the body. In detail, mononucleated, partially differentiated precursor cells, also called myoblasts, proliferate, migrate, differentiate, align, fuse to ultimately form multinucleated myotubes. In vivo, myotubes mature into myofibers that stretch along the whole skeletal muscles. ref(s) missing 1
Several diseases such as sarcopenia, muscle dystrophy, obesity, or diabetes severely affect the physiological homeostasis and lead to a loss of skeletal muscle mass and function (muscle atrophy). In contrast, physical activity dows not just lead to an increase of muscle mass and function (muscle hypertrophy), but also reduces glucose levels and lipid accumulation. Treatment of diseases that lead to muscle atrophy often requires a multifactorial approach. Therefore, candidate compounds, genes, proteins or metabolites are initially analyzed in large-scale in vitro experiments utilizing myoblasts cell lines such as murine C2C12 cells. ref(s) missing 2
Given the spatiotemporal nature of myogenesis, it is crucial to identify and quantify individual myoblasts and fused myotubes in microscopy images to evaluate the effect of each compound or biological molecule on myoblast fusion or atrophy/hypertrophy. Other than simply counting the number of each cell type, the creation and morphological quantification of individual myotube masks enables a thorough analysis of several other biological relevant parameters such as the differentiation state/extend or myotube area and diameter in addition to interaction of different myoblasts. Unfortunately, the manual creation of individual cell masks in large-scale experiments is tremendously time consuming and might take months to finish. Therefore, this project intends to obtain such quantitative statistics without using heuristics by utilizing two different instance segmentation models for cell nuclei and myotubes.
These models need to overcome two hurdles. First and foremost, it must be able to differentiate between overlapping instances. Besides coincidence, these overlaps might be caused by myogenesis itself or due to the threedimensional nature of the cells caused by acutal overlapping myotubes. Secondly, it should be robust in its predictions. Many times, microscopy images require preprocessing that can create artifacts by amplify noise or blur small scale structures. Therefore, the instance segmentation should aim to be as independent of the preprocessing as possible.
Taken together, the goal is not only to speed up otherwise time-consuming analyses, but also to improve reproducibility and eliminate all human bias within the process of instance segmentation. Human bias can occur at various points. Many times, it is difficult to distinguish whether a continuously bright region is one object or several ones. Furthermore, the quality of preprocessing can influence the number of counted cells because some instances may be too dim too spot with the naked eye.

2 Segmentation using Unsupervised Methods

In the beginning, established, unsupervised methods were used to segment the images in order to gain a better understanding of the data. Unsupervised learning includes algorithms which intend to find regularities, structures, or patterns within unlabelled datasets. As they require no ground truth labelled data and are not too computationally expensive, they are a great fit for exploratory analysis. The results of two classical methods (watershed and tbd) applied to the given images is discussed in the following.

2.1 Watershed

Little math is necessary to understand how segmentation using watersheds functions. First, the image needs transformed to grayscale because the resulting single channel needs to be thought of as the a third dimension defining a height profile or topography. In case of a uint8 encoding, the height may take values between 0 and 255. Each pixel can be either of the three following types: a (regional) minimum, a catchment basin or watershed of that minimum, or watershed lines. The first type of pixel is self-explainatory. Continuing with the metaphor, a pixel of second or third type can be thought of in the following manner: picture the position and intensity of the pixel as defining the starting point on the 3d topography defined by the grayscale image. Placing a drop of water on this location can either have it run down (second type) or stay put (third type). All the points where water would run downhill are known as watersheds. All the other points that are not minima define crests, which are the divide (or watershed) lines, beyond which water would not move at all. Iterating over possible intensities starting from the lowest one in the image, or, by analogy, flooding the 3d landscape by poking a hole in the minimum, defines connected areas, or collections of water within a basin, around every regional minimum. Continued flooding will have the water level rise until the first two connected areas merge into one. To prevent that, a dam, whose locations define the pixels of the watershed lines, would need to be built. How to properly construct such dams by means of morphological operations cite is thoroughly explained in [1] ref Gonzales + Couprie/Bertand; overlap cannot be resolved The resulting watershed lines are then interpreted as the boundaries of an instance.
Based on this intuition, two observations can be made. Firstly, on first glance a catchment basin can have the shape of a myotube or cell nuclei making watershed a sensible segmentation method. Secondly, this method requires the instances to have low grayscale values. This implies that images need to be processed before applying the watershed algorithm since cells are accumulations of high intensity areas. The most naive approach would be a simple inversion of the image. But this can lead to oversegmentation due to noisy sections. More sophisticated approaches either are based on image gradients or a distance transform applied to a binary representation of the original image. The latter approach is used in this report and will be concretized before long.
Just from these theoretical discussions alone, it becomes evident that the algorithm will have a hard time differentiating between merging nuclei because they presumably will be interpreted as one single catchment basin due to their intesities being similar. from these theoretical considerations: cannot resolve overlaps; include results

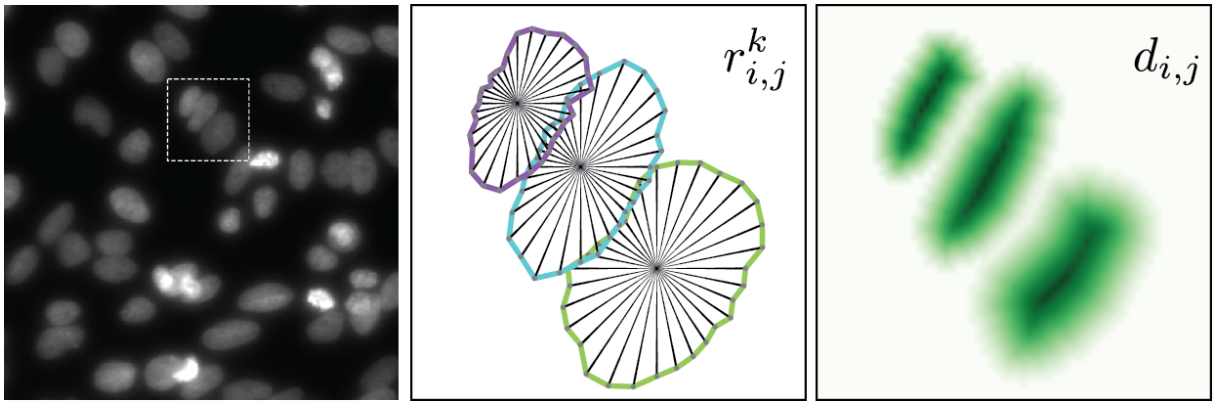

Figure 3.1: Example of an area that is complicated to segment due to overlaps. `StarDist` creates the segementation by forming star-convex polygons and computing the probability to belong to said instance. Source: [2].

3 Myoblast Segmentation using Stardist

Typical instance segmentation methods also suffer from the suppresion of valid objects or the merging instances given overlapping instances. In order to better segment the myoblasts, a cell detection method called `StarDist` [2, 3] is used. It was developed with the intent to segment microscopy data like the nuclei found in this project.
In brief, a convolutional neural network is trained to predict polygons imitating typical cell shapes for every pixel of the image. More concretely, it predicts a star-convex polygon for every (non-background) pixel. Intuitively speaking, a star-convex set $\mathcal{S}$ is one where there exists one point s_0 such that for every point $s \in \mathcal{S}$ the line segment connecting s_0 to s is element of $\mathcal{S}$. For every pixel such a shape can be approximated by following n predefined radial directions for a distance of $\{r^k_{ij}\}^n_{k=1}$ starting from the pixel parametrized by i, j serving as s_0. Furthermore add d_{ij}, add reffig, add discussion of loss and weights
In `StarDist`, the `U-Net` architecture [4] is used as the backbone and is slightly modified by adding another 128-channel 3x3-convolutional layer with ReLu activation to the `U-Net` output. This output, in turn, is fed into two other convolutional layers. The first one is a single channel convolutional layer with sigmoid activation meant learn object probabilities. The second one has a linear activation and as many channels as there are radial directions.

4 Segmentation of Myotubes with MyoSAM

5 Results

6 Conclusion

References

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A Appendix