Segmentation of U2OS cell nuclei using Cellpose tool

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1 Objective

In this project I aim to test the Cellpose[1] software on a publicly available dataset of fluorescent microscopy images and to evaluate the precision of the base model provided by the authors.

2 Dataset

For my project I have chosen the image set BBBC039v1 Caicedo et al. 2018 from the Broad Bioimage Benchmark Collection.[2]

The image set originates in a hight-throughput chemical screening of U2OS cells and contains a single DNA channel of a single field of view per compound. The whole dataset was manually annotated to establish the ground truth, so that the dataset can be used for benchmarking nuclei segmentation algorithms. The ground truth is provided in form of single channel images containing masks of the detected nuclei.

The U2OS cells is a homo sapiens cancer derived cell line often used in study of bone growth and bone tumors.[3]

3 Workflow

After obtaining and decoding the input images and masks, the input was sorted into training, validation and testing sections based on provided metadata file. In my project I have used only the testing set containing 50 images to reduce the computational time complexity.

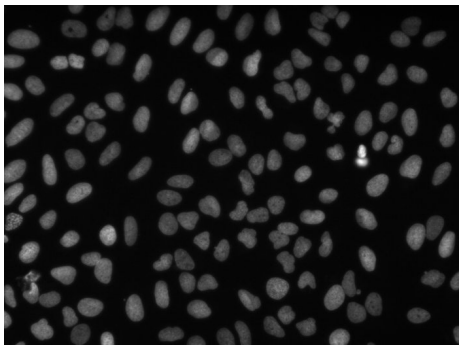

Figure 1: Example input image.

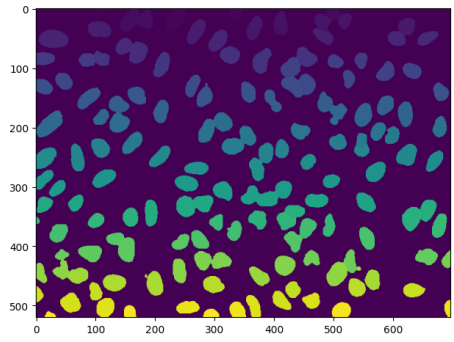

Figure 2: Corresponding mask.

The mask image was created using the Matplotlib[4] python package. Then using the Cellpose in python enviroment the segmentation was performed through a python script. The output of the segmentation was the generated mask in same shape as the ground truth files.

1. Carsen Stringer et al., "Cellpose: a generalist algorithm for cellular segmentation," *Nature methods*, 2021,

2. Vebjorn Ljosa, Katherine L Sokolnicki, and Anna E Carpenter, "Annotated high-throughput microscopy image sets for validation," *Nature methods*, 2012,

3. Katerina M Niforou et al., "The proteome profile of the human osteosarcoma U2OS cell line," *Cancer genomics proteomics*, 2008,

4. John D. Hunter, "Matplotlib: A 2D Graphics Environment," *Computing in Science Engineering*, 2007,

Further to quantify the accuracy of base Cellpose model, I have prepared a script that quantifies the rate of agreement between generated mask and provided ground truth in form of percentage of correctly assigned pixels. Each pixel corresponding to a nuclei is set to have a non-zero value while empty parts of the image are set to have the value of zero.

4 Results

The Cellpose base model was evaluated on 50 test images using pixel-wise comparison against the manually annotated ground-truth masks.

I have used the following metrics for evaluation: Dice coefficient, Intersection over Union (IoU), precision, recall, F1-score, and percentage of correctly classified pixels. The Dice coefficient quantifies overlap between predicted and ground-truth masks, ranging from 0 (no overlap) to 1 (perfect match). Intersection over Union (IoU) similarly measures overlap as the ratio of the intersection to the union of predicted and true masks. Precision represents the fraction of predicted nucleus pixels that are correct (low false positives), while recall captures the fraction of true nucleus pixels that were successfully detected (low false negatives). Across all images the model achieved high values, following are the mean values.

Mean Dice coefficient: 0.96 (range 0.93 – 0.98)

Mean IoU: 0.93

Mean precision: 0.97

Mean recall: 0.95

Mean pixel accuracy: 0.99

These values indicate that the predicted nuclei masks overlap strongly with the reference masks and that false positives and false negatives are rare.

5 Discussion

The consistently high Dice and IoU scores demonstrate that Cellpose's pretrained model generalizes well to this fluorescent U2OS dataset without any fine-tuning.

Precision values near 0.97 show that very few background pixels were misclassified as nuclei, while recall around 0.95 indicates only a small fraction of true nuclei pixels were missed.

The narrow spread of metrics across all 50 images suggests stable performance even with the natural variation in nuclear size, shape, and density present in the BBBC039v1 collection.

Minor performance differences across images can likely be attributed to challenging cases such as densely packed or overlapping nuclei, where accurate instance separation is inherently difficult, or the wrongly assigned pixels correspond to border sections of nuclei and as such these have only a small impact on the results.

Further improvements could come from training a custom model on this dataset, using more sophisticated post-processing (e.g., watershed refinement), or leveraging instance-level rather than purely pixel-wise evaluation.

Overall, these results confirm that the out-of-the-box Cellpose model provides highly reliable nuclei segmentation for high-throughput fluorescent microscopy experiments and can serve as a strong baseline for future methodological comparisons.

The scripts used in this analysis are available at following Github repository: https://github.com/FKloda/Microscop

References

Hunter, John D. "Matplotlib: A 2D Graphics Environment." *Computing in Science Engineering*, 2007.

Ljosa, Vebjorn, Katherine L Sokolnicki, and Anna E Carpenter. "Annotated high-throughput microscopy image sets for validation." *Nature methods*, 2012.

Niforou, Katerina M, Athanasios K Anagnostopoulos, Konstantinos Vougas, Christos Kittas, Vassilis G Gorgoulis, and George T Tsangaris. "The proteome profile of the human osteosarcoma U2OS cell line." *Cancer genomics proteomics*, 2008.

Stringer, Carsen, Tim Wang, Michalis Michaelos, and Marius Pachitariu. "Cellpose: a generalist algorithm for cellular segmentation." *Nature methods*, 2021.