Segmentation of Living Cells in Microscope Images

CS 585 Final Project
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Problem Definition

The BU Biomedical Image Library (BU-BIL) has collected hundreds of cell images using time-lapse phase-contrast microscopy. This resource includes "ground-truth" segmentations that provide a basis for comparing the effectiveness of proposed segmentation methods. The large volume of data presents a significant challenge for manual interpretation, which is not only labor-intensive and costly but also susceptible to human error. To address this, we propose the development of automated segmentation approaches, including model training and refining connected components.

Method and Implementation

Out of the six datasets available in the BU Biomedical Image Library, encompassing a diverse range of imaging modalities and cell types, we strategically focused our efforts on two distinct sets: datasets 1 and 2, comprising phase contrast microscopy images of rat and rabbit smooth muscle cells, respectively, and datasets 4 and 5, consisting of fluorescence microscopy images of Lu and WM993 melanoma cells. This decision stemmed from our analysis, which revealed shared features between these dataset pairs.

Approach for Datasets 4 and 5 (Fluorescence Microscopy, Lu and WM993 melanoma cells)

1. Contour Detection: Challenges in distinguishing noise contours from target contours.

2. Active Contour: Active contour methods performed adequately for circular shapes but lacked reliability for elongated or elliptical shapes.

3. Connected Components for Segmentation:
    - Thresholding: We applied thresholding to create a binary mask, simplifying the image while retaining relevant features.
    - Connected Components: Employing connected components, we isolated distinct objects within the binary mask.
    - Largest Component Identification: Among the connected components, we identified the largest, assuming it corresponds to the cell of interest.

- Morphology Operations: To refine segmentation, we employed morphology operations to eliminate small objects and further enhance the accuracy of cell identification.

Approach for Datasets 1 and 2 (Phase Contrast Microscopy, Rat and Rabbit smooth muscle cells)

1. Initial Exploration with Handcrafted Methods: Initially, we experimented with various handcrafted methods but encountered limitations in accurately segmenting cells due to the complexity and variability of cell shapes.

2. Machine Learning Integration:
    - Convolutional Neural Network (CNN): Implementing a basic CNN architecture, we observed promising results in localizing cell areas. However, significant noise persisted in the segmented images.
    - Integration of Attention Mechanisms: To address noise issues, we integrated attention blocks into the CNN architecture, enhancing the model's ability to focus on relevant features and reducing noise.
    - Transition to U-Net Architecture: Given the success with attention mechanisms, we transitioned to a U-Net architecture, leveraging its strengths in biomedical image segmentation.
    - Incorporation of Attention Mechanisms in U-Net: Building upon the U-Net framework, we introduced attention layers to further refine segmentation and improve the accuracy of cell identification.

Experiments

Evaluation Metrics

Pixel Accuracy: Measures the percentage of pixels correctly classified in the segmentation mask compared to the gold standard.

Intersection over Union (IoU): Assesses the overlap between the predicted mask and the ground truth, providing insight into the precision and recall of the segmentation method. An IoU of 1 indicates perfect overlap, while an IoU of 0 indicates no overlap.

Experiments for Datasets 4 and 5

Our experiments were conducted on two distinct datasets: Dataset 4, which consists of 61 images, and Dataset 5, comprising 58 images. Each dataset features images of varying sizes. We executed our segmentation code to generate predicted masks, which were then compared against the provided gold standard masks to assess segmentation accuracy. The

Intersection over Union (IoU) and pixel accuracy were calculated to measure the performance of our segmentation approach. These experiments were repeated ten times for each dataset to ensure consistency and reliability of the results.

Experiments for Datasets 1 and 2

We focused on two primary datasets: Dataset 1, which consists of 35 images with dimensions of 1024x811, and Dataset 2, comprising 69 images with a size of 1300x1030.

Models Evaluated:

- Double Convolutional Model: A streamlined version of the U-Net model, which served as a baseline.
- Double Convolutional Model with Attention: The baseline model with the integration of an attention mechanism to refine its focus.
- U-Net: The standard U-Net model, a prevalent architecture in biomedical image segmentation.
- U-Net with Attention: An advanced iteration of U-Net employing an attention mechanism to enhance feature extraction capabilities.

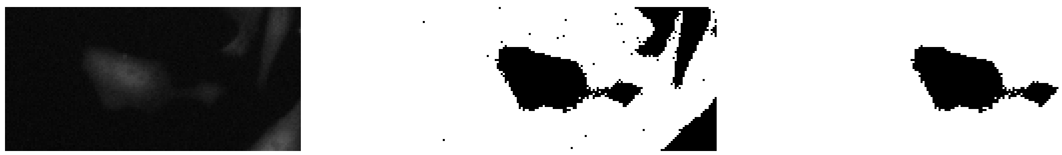
Training Parameters

For each model configuration, we trained over 50 epochs, except in two instances where we extended training to 150 epochs to observe the impact of longer training on model performance.

Results

Results for Datasets 4 and 5

Applying the connected component algorithm to the raw images yielded a promising accuracy of 0.78. Subsequent enhancement through morphological post-processing led to a marked improvement, elevating the accuracy to 0.8774 and the intersection over union (IoU) to a comparable level. Moreover, the consistency of our approach was validated across 10 repeated experiments, each exhibiting negligible variation in results. This consistency underscores the robustness of our method, affirming its reliable performance even under diverse experimental conditions.



Raw Connected components Post-processed

Fig 1. BU-BIL_Dataset4/000152.png

Results for Datasets 1 and 2

Models trained on Dataset 1 for 50 epochs demonstrated progressive improvement in IoU when comparing the simple Convolutional Neural Network (CNN) (IoU: 0.38) to the more complex U-Net with Attention (IoU: 0.56). Extending the training duration to 150 epochs substantially improved the IoU for U-Net with Attention on Dataset 1, achieving an IoU of 0.85. On Dataset 2, the U-Net with Attention trained for 150 epochs achieved an IoU of 0.77, reinforcing the model's effectiveness across different datasets.

In addition to IoU, we qualitatively assessed model performance. A case in point is image B23 from Dataset 1 (Fig 2), where our best model achieved near-perfect segmentation with an IoU of 0.99. Conversely, we also observed instances of poor performance, such as the model failing to locate the target cell among many cells in the screen, resulting in an IoU of 0. This is probably caused by our assumption that the target cell is always the largest cell member on the screen.

Raw Predicted Post-processed

Fig 2. BU-BIL_Dataset1/B23.png

Moreover, our exploration uncovered that integrating an attention layer into the U-Net architecture could inadvertently heighten susceptibility to distraction from surrounding noise. However, intriguingly, as we prolonged the training duration, we observed a mitigated effect of this distraction, suggesting that extended epochs play a role in refining the model's ability to focus on pertinent features amidst noise interference. This finding underscores the dynamic interplay between model complexity, training duration, and performance outcomes in image segmentation tasks, warranting further investigation into optimizing attention mechanisms for enhanced noise resilience.

These experiments elucidate the capabilities of attention-augmented U-Net architectures in cellular segmentation tasks and suggest that extended training periods can lead to significant performance gains.

Model	Dataset	Epoch	IoU
Double Conv	1	50	0.38
Double Conv + Attention	1	50	0.41
U-net	1	50	0.56
U-net + Attention	1	50	0.55
U-net + Attention	1	150	0.85
U-net + Attention	2	150	0.77

Table 1. Results for Datasets 1 and 2

Discussion

Method Efficacy and Outcomes

In our research, we compared different image segmentation techniques, using both conventional algorithms and neural network models. The connected component algorithm was effective on clean data, and its performance was improved with morphological post-processing, as reflected by consistent IoU scores. Neural networks, like the U-Net architecture, showed promise in identifying complex patterns, especially when given enough time to train.

Strengths and Limitations

The strength of the connected components algorithm lies in its consistent performance on clean data, while the U-Net models, especially with attention layers, excel in learning complex patterns given sufficient epochs. However, the U-Net's performance was initially constrained by limited data, with the attention variant initially amplifying noise. Additionally, the variation in image quality due to different microscopes presented a challenge in data consistency.

Success and Expectations

Our methods were effective, especially when we increased the training time, which significantly boosted the IoU scores. This was somewhat expected, though restricted by the quality and amount of data. The conventional algorithm-based method worked well for clear data but struggled with noisy images. This challenge led to the integration of additional post-processing steps to improve its accuracy.

Directions for Future Research

To advance our methodologies, future work could delve into:

Data Augmentation: To mitigate the data insufficiency issue, synthetic data generation and augmentation strategies would be a priority.

Hybrid Techniques: Combining the reliability of algorithmic approaches with the adaptiveness of neural networks could offer a more balanced solution.

Extended Training Regimens: Further exploration of training durations and regularization strategies would help refine the neural networks, particularly the attention-based models.

Uniform Data Preprocessing: Standardizing image data from different sources would likely improve the neural networks' ability to generalize across diverse datasets.

In conclusion, while our methods showed promise, especially with extended training periods, the limitations highlighted by data insufficiency and variability present avenues for future enhancement. Continued exploration in these areas could lead to the development of more robust and generalizable image segmentation techniques.

Conclusions

This study's findings highlight the efficacy of U-Net models in the automated segmentation of cells in microscope images. Enhanced by attention mechanisms, our models showed substantial improvements in segmentation accuracy, especially after extended training periods. However, the challenges posed by data variability and noise remind us of the necessity for robust preprocessing and the standardization of imaging data.

Moving forward, blending traditional algorithmic approaches with advanced neural network techniques holds promise for overcoming current limitations. By refining these computational tools, we aim to provide robust and efficient solutions for the analysis of biomedical images, assisting researchers in the swift and accurate interpretation of cellular imagery.

Credits and Bibliography

[1] D. Gurari, D. Theriault, M. Sameki, B. Isenberg, T. A. Pham, A. Purwada, P. Solski, M. Walker, C. Zhang, J. Y. Wong, and M. Betke. "How to Collect Segmentations for Biomedical Images? A Benchmark Evaluating the Performance of Experts, Crowdsourced Non-Experts, and Algorithms." *Winter conference on Applications in Computer Vision* (WACV), 8 pp, 2015. [In Press].

[2] O. Oktay et al., "Attention u-net: Learning where to look for the pancreas," in Proceedings of MIDL'18, Amsterdam, The Netherlands, July 4-6, 2018.

[3] O. Ronneberger, P. Fischer, and T. Brox, "U-Net: Convolutional Networks for Biomedical Image Segmentation," arXiv:1505.04597 [cs.CV], May 18, 2015. [Online]. Available: https://doi.org/10.48550/arXiv.1505.04597

[4] ChatGPT was used to check for grammatical issues in the report.