Deep Learning for Segmentation and Counting within Microscopy Data

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Motivation
Cell counting is a ubiquitous, yet tedious task that would greatly benefit from automation. From basic biological questions to clinical trials, cell counts provide key quantitative feedback that drive research. Unfortunately, cell counting is most commonly a manual task and can be time-intensive. The task is also made more difficult due to overlapping cells, existence of multiple focal planes, and poor imaging quality, among other things. Here, we attempt to automate both the segmentation and counting of cells for a given microscopy image. We hope to create a useful tool for biologist and ultimately expedite research.

Previous Work
At the moment there is no state-of-the-art convolutional neural network (CNN) architecture for counting objects in an image, much less counting cells. However, there is quite a bit of ongoing research in the field of image segmentation using CNNs on which we based our methodology:


There has also been previous work done in the task segmenting and classifying cell types using CNNs:

- DeepCell (https://doi.org/10.1371/journal.pcbi.1005177)

Dataset
We used the BBBC005 dataset from the Broad Institute’s Bioimage Benchmark Collection. This dataset is a collection of 9,600 simulated microscopy images of stained cells. These images were simulated for a given cell count with a clustering probability of 25%. Focus blur was simulated by applying Gaussian filters to the images. Each image is 696 x 520 pixels in 8-bit TIFF format (eventually converted to JPEG and scaled down to 256 x 192 pixels), with cell areas matched to the average cell areas of human U2OS cells. Of the 9,600 images, 600 images have a corresponding foreground mask. For each experiment, we used an 80/20 split for training and validation.

Cell Segmentation

Cell Counting

Experiments
Pre-trained FPN on 480 images with known foregrounds:

- Backprop on L1 (w/ aleatoric uncertainty) and TV losses for each output mask using ADAM optimizer
- Converged validation MSE on 120 validation images after 50 epochs

Trained VGG-11-based network on 7,680 FPN-generated masks:

- Backprop on L1 (w/ aleatoric uncertainty) loss with cell counts using ADAM optimizer
- Converged validation MSE between counts on 1,920 validation images after 50 epochs

Results
After 50 epochs of training, our best model is able to achieve an $R^2$ value of .987. 80% of the time, the ground truth falls within the predicted 95% confidence interval.

Failure Cases
We find that our method systematically fails in a number of cases:

- Lots of Overlapping Cells
- Oddly Shaped Cells
- Bad Focal Planes

Conclusion
We show that it is possible to design and train a CNN architecture to count cells in microscopy images with relatively good accuracy. While there are a number of failures cases that arise, we believe that better training data might help to overcome these. Specifically, the dataset used lacked foreground masks for out of focus images, which could have helped greatly in improving performance.

We also demonstrate a good use-case for aleatoric losses in estimating uncertainty in cell counting. As the eventual goal is to creating a scientific tool, generating error bounds makes improves the statistical power of our method and the ability to form hypotheses based on its results.

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All code (as well as this poster and a manuscript) will be made publicly available on GitHub (https://github.com/cxhernandez/cellcount).