MU-Wa-US

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Platform: Linux

Prerequisites: Python 3.8 with PyTorch

MU-Wa-US: SUMMARY

The proposed cell detection and segmentation pipeline consisted of three main steps: preprocessing, deep cell detection and segmentation, and post-processing. Mask R-CNN [1] network with a ResNet-50 [2] backbone was used for cell detection and segmentation.

MU-Wa-US: PREPROCESSING

A two-step pre-processing was applied to all training and testing images before feeding them to the deep network. The first pre-processing step normalized the input images, the second step applied adaptive histogram equalization.

Image Normalization. Different microscopy image datasets consisted of images with largely varying pixel intensity ranges (i.e., 8-bit vs. 16-bit images). Variations in pixel intensity ranges make it harder for the deep learning network to converge. To tackle this problem, we normalized the input image I_0 using:

$$I(x,y) = 0.5 \cdot \left(\left[\frac{I_0(x,y) - \mu(I_0)}{\sigma(I_0)} \right] + 1 \right)$$

where $\mu(I_0)$ and $\sigma(I_0)$ is respectively the mean and standard deviation of intensities in I_0, and $[\cdot]$ limits the intensities to the [-1, 1] range.

Adaptive Histogram Equalization (AHE). The processed images contained cells with varying appearances including some with low contrast compared to the background. We applied AHE to the normalized images I in order to improve their contrast. Unlike the ordinary histogram equalization that uses a single histogram for the entire image, AHE computes several local histograms to improve the local contrast in an image. Since the *equalize_adapthist* function in the *skimage* library requires input images with pixel intensity ranges in [0, 255], normalized images were multiplied with 255 prior to AHE.

MU-Wa-US: SEGMENTATION

For our current results, we used Mask R-CNN [1, 2] networks with a ResNet-50 [3] backbone. The networks were trained on the full image with the provided silver truth segmentation masks with ten frames held out for validation and after training using the unseen gold truth segmentation masks for testing. To prevent over-fitting, a common deep learning problem, we applied data augmentation during training. For **Fluo-N2DH-GOWT1** and **Fluo-N2DL-HeLa**, we only applied random horizontal/vertical flip and random scaling. For **Fluo-C2DL-MSC** and **Fluo-N3DH-CHO**, we applied random horizontal and vertical flips, random scale, and random affine transform. Each of these random transformations is randomly selected. Mask R-CNN network predicts class label, segmentation mask, and bounding box for each of the cells. We only kept the segmentation masks as the final outputs. Each of the cell datasets was trained separately, with only two classes: foreground/cell and background, resulting in four different network weights respectively. Binary cross-entropy [4] was used as the loss function, and Stochastic gradient descent (SGD) was used for the optimization. Learning rate was initialized at 0.005 and was divided by 10 every 20 epochs. Batch size was set to 8 for all the datasets.

MU-Wa-US: POST-PROCESSING

For some cases of adjacent cells or cells undergoing mitosis, Mask R-CNN predicts multiple overlapping masks. For example, the system predicts three masks for two adjacent cells, one predicted mask for each touching cell, and another mask for the union of two cells considering them as a single cell. The post-processing step was used to remove duplicate masks. We compared each bounding box with all the others and computed the intersection over union (IoU) of the bounding box pairs. For all the pairs of overlapping bounding boxes *A* and *B* with IoU equal or greater than *T*, if the size of *A* was greater than *B*, then *A* was ignored. Otherwise, *A* was kept. This post-processing step was applied to all the datasets to remove overlapping union masks.

REFERENCES

1. He K, Gkioxari G, Dollár P, Girshick R. Mask R-CNN. In *Proceedings of the IEEE International Conference on Computer Vision*, 2961-2969 (2017).
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