MU-Lux-CZ

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Platform: Linux

Prerequisites: None

MU-Lux-CZ: SUMMARY

The method combines deep learning with watershed segmentation. Due to the combination, the method is suitable for segmentation cell clusters even in difficult modalities. For each frame, the convolutional neural network of U-Net shape [1] detects all cells by markers and recognizes the foreground and the background of the frame. Then, the final segmentation is generated by a *Marker-Controlled Watershed* transformation [2].

MU-Lux-CZ: PREPROCESSING

We enlarged the training dataset by data augmentation. We used *Random Distortion* [3] to simulate variations in cell shapes. Because cells have no fixed orientation, we generated training data also by geometrical transformations such as *rotation*, *mirroring*, and *scaling*. For all these operations, the data outside the image domain were mirrored. Before neural network training, each training sample is transformed into four different grayscale images: *normalized input*, *cell markers*, *cell mask*, and *weight map*. Each input image is normalized by the histogram equalization [4] (HE) or median normalization (MN). There are two ways, how to define *cell markers*: First, the marker is a full cell mask eroded by an elliptic structuring element of radius *b* pixels. Second, in the case of circular cells, it is a cell detection marker. The *cell mask* image distinguishes the area of cells from the background. The *weight map* image W reflects the importance of each pixel for a good prediction; it is defined by a map of real values greater than one, where a higher value means higher pixel importance. Let Φ be a set of all cells φ in the given frame. The weight $w_i \in W$ of pixel p_i is computed by the formula:

$$w_i = 1 + a \sum_{\varphi \in \Phi} \max(b - \|p_i, \varphi\|, 0)$$

where $\|p_i, \varphi\|$ denotes the Euclidean distance between the pixel p_i and the cell φ. The map magnitude can be regulated by setting the parameter $a \in R^+$.

MU-Lux-CZ: SEGMENTATION

To predict cell markers and masks, we use a convolutional neural network of the U-Net topology [1]. In contrast to the original U-Net, our network has twice fewer feature maps in each layer. Next, it produces two outputs at once; the first one we call *Predicted Markers* (Ω_1), the second one we call *Predicted Cell Boundaries* (Ω_2). The prediction error of each of them we computed by weighted *Cross Entropy*:

$$E(\Omega) = -\sum_{i \in \Omega} w_i \log\left(p_{i,l(i)}\right).$$

The loss is computed as an average error of both layers normalized by the sum of all weights:

$$L = \frac{1}{2} \cdot \frac{E(\Omega_1) + E(\Omega_2)}{\sum_{i \in \Omega_1} w_i + \sum_{j \in \Omega_2} w_j}.$$

We trained the neural network from scratch for 320 epochs with 40 mini-batches of size 8. For training, we used a machine with the GPU NVIDIA Quadro P6000. We stored a model at every tenth epoch then we picked the one with the best performance.

MU-Lux-CZ: POST-PROCESSING

The neural network does not predict the final segmentation directly; it only predicts inputs for the marker-controlled watershed segmentation. The network outputs are two proximity maps that can be seen as grayscale images with values in the range from 0 to 1. The image of *Predicted Cell Markers* is thresholded by t_m to a binary image. In the case of convex and circular cells, markers are refined by *h-dome* transform with the parameter *h*. The result is also cleaned from small particles by a *Morphological Opening* operation using a disk structuring element of diameter *e* pixels. In the watershed *Segmentation function*, cell markers are minima-imposed into *Predicted Cell Boundaries*. The image background is extracted from boundaries prediction by thresholding by the value of t_b. It is masked out from the watershed result. The last step is to remove all objects that are closer than *B* pixels from the image border.

MU-Lux-CZ: TRACKING

In the end, we label cell masks in the whole sequence according to the tracking procedure. Frames are processed in order of acquisition time. Labeling of each cell mask *c* follows three rules:

- If the cell mask covers at least 15% of any cell in the previous frame, then it is the same cell.
- If more than one cells are covering at least 15% of some cell in the previous frame, then all of them are daughters.
- In all other cases, the cell *c* is a new cell with no parent cell.

REFERENCES

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