HD-Wag-GE

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Platform: Linux

Prerequisites: Python 3

HD-Wag-GE: SUMMARY

We use an efficient deep learning model with an encoder-decoder architecture as core of our segmentation method. Our method is designed for volumetric segmentation of cells in fluorescence microscopy images. We employ context aware pseudocoloring to preserve spatial context while performing volumetric cell segmentation slice-wise.

HD-Wag-GE: PREPROCESSING

3D images are first converted to stacks of 2D slices by slicing them in *z*-direction. We then use context aware pseudocoloring to add information of adjacent slices to all 2D slices. For each 2D slice, two pseudocolor channels are added, which combine information from the current slice with information from the previous (*z*-1) and the following (*z*+1) slice. During this process, we use Contrast Limited Adaptive Histogram Equalisation (CLAHE) filters and thresholding to extract regions of interest (ROIs). Furthermore, the data type is changed from unsigned 16-bit integer values to 32-bit floating point values. The 2D slices of **Fluo-C3DL-MDA231** and **Fluo-C3DH-A549-SIM** are resized to 384 × 384 pixels. For **Fluo-N3DL-TRIC**, our method is applied using a sliding window scheme with six square patches, which are also resized to 384 × 384 pixels.

HD-Wag-GE: SEGMENTATION

Segmentation is performed by an encoder-decoder model with an EfficientNet-based encoder. We use six stages of the EfficientNet architecture as encoder and four U-Net-like decoder blocks [1, 2]. We utilize transfer learning using an encoder that is initialized with weights learned from ImageNet. We use all available reference annotations per dataset, and perform data augmentation by random horizontal and vertical flipping as well as cropping. During training, we use Adam [3] as optimizer and reduce the learning rate at plateaus. For all considered datasets, we train separate models to perform semantic segmentation. For **Fluo-C3DL-MDA231**, we additionally train a second model to predict heatmaps for the

cell centers. The semantic segmentation models are trained with Dice loss [4], and the cell center heatmap model is trained with focal loss [5].

HD-Wag-GE: POST-PROCESSING

Since **Fluo-C3DH-A549-SIM** has only two labels, the output of the semantic segmentation model is converted to a binary mask via thresholding. **Fluo-N3DL-TRIC** contains many round and oval cells. Therefore, we first apply thresholding to the semantic segmentation result and then use a standard distance-based watershed algorithm to label individual cells. **Fluo-C3DL-MDA231** contains many irregularly shaped cells that are difficult to distinguish using a standard distance-based watershed algorithm. Hence, we apply max-pooling to the heatmaps for the cell centers and use the results as markers in a marker-based watershed algorithm to label individual cells. Finally, the data type of all segmentation masks is converted from 32-bit floating point values to unsigned 16-bit integer values.

REFERENCES

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