

UCSB-US

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Platform: Linux

Prerequisites: Python 3 with scipy, skimage, and tensorflow

UCSB-US: SUMMARY

Our cell segmentation algorithm is based on a few key observations of **Fluo-N3DH-CE**: (1) considerable variation in size and staining intensity of the embryonic nuclei, (2) the lack of sufficient gold truth for segmentation, and (3) availability of weakly annotated data for detection. Due to these limitations, standard convolutional neural network (CNN) based methods often lead to lower accuracy results. However, by using traditional image processing methods in combination with CNNs, we expect to observe a higher accuracy in nuclei segmentation. We propose an automated Simple Linear Iterative Clustering (SLIC) based algorithmic pipeline for improving nuclei segmentation of developing embryos in *C.elegans*. It has been shown that the SLIC supervoxel algorithm provides better adherence to cluster boundaries [1]. We present a method of nuclei boundary refinement in our algorithm that takes advantage of this boundary adherence feature in SLIC. Later, to track the segmented nuclei, we propose a graph-based tracking method for linking cell nuclei frame-by-frame. To demonstrate that our algorithmic pipeline can be easily extended, we also experimented with **Fluo-C3DL-MDA231**.

UCSB-US: PREPROCESSING

For **Fluo-N3DH-CE**, we use normalized raw fluorescence images, linearly re-scaled to set the 2nd and 99.5th percentile to 0 and 1, respectively. These 3D intensity images are used as inputs to trained CNNs. For supervoxel segmentation, raw images are processed to remove salt and pepper noise using a median filter of size $1 \times 3 \times 5$.

UCSB-US: DETECTION

We reproduce the detection results using **MPI-GE**. In this algorithm, a 3D U-net has been trained that takes the 3D intensity image as input and predicts a single-channel output image of the same size. We use these detection results for the purpose of segmentation in our algorithm.

UCSB-US: SEGMENTATION

For our segmentation algorithm, we extend **MPI-GE** by adding a branch to generate supervoxels for the input image. The workflow of the proposed algorithm is shown in Figure 1. Given the predicted centroid locations from the detection CNN as seed points to perform a standard watershed segmentation [2], we obtain an instance segmentation of all cell nuclei. We combine intensity and shape characteristics to extract nucleus regions by utilizing the over-segmented output image from SLIC segmentation. The SLIC algorithm generates supervoxels by clustering pixels based on their intensity similarity and proximity. A labeled supervoxel matrix of equal size to the original image is obtained as an output from the SLIC function. The segmented image mask from the previous branch is then applied on the supervoxel matrix to re-label voxels that encounter a supervoxel at the boundary of the mask. A patch is said to be a part of the nuclei region if it has the majority vote among all the labels of the voxels in it. Thus, we refine the boundary for each segmented nuclei to generate the final output images.

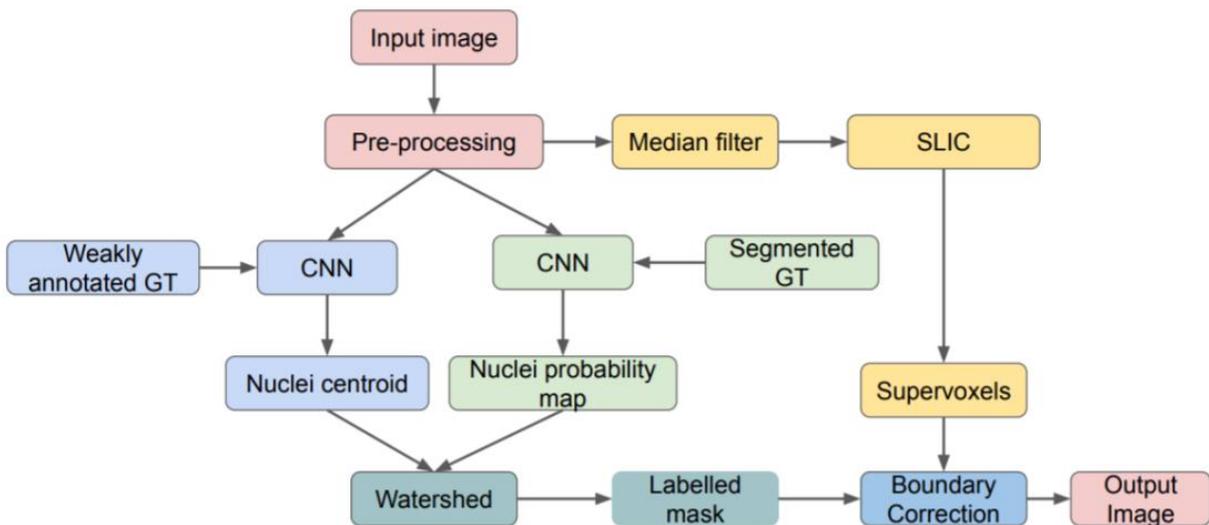


Figure 1. Pipeline for nuclei segmentation.

UCSB-US: TRACKING

We implement a graph-based method to track cell nuclei. An undirected weighted graph is created with each vertex representing a cell nucleus and the weight representing the minimum distance between two cell nuclei. Based on the graph and the segmentation image, we construct a three dimensional feature vector for each cell nucleus. The feature vector for cell nucleus i contains the number of neighboring cell nuclei, the average distance to all other cell nuclei, and the center location. The center location is determined from the segmentation image. For tracking, we link the current frame cell nuclei to the

previous frame cell nuclei using a distance metric. If multiple cell nuclei in the latter frame associate to the same cell nucleus and they are neighbors to each other, then we assume that this is a cell mitosis event. In such cases, we assign a new tracking id to the two daughter cell nuclei in the next frame. We iterate the above tracking procedure on each new frame to generate tracking results.

UCSB-US: POST-PROCESSING

We handle the discrepancy in the segmentation labels obtained as a result of misdetection of cell nucleus by comparing previous and next frames. If the tracking algorithm gives the same label for the two frames, we interpolate the labels to the current frame. We apply the above post-processing only when we get no label at a given time stamp. Since there are other such cases, we are currently exploring how tracking can influence segmentation pipeline.

REFERENCES

1. Kovesi P. Image segmentation using SLIC superpixels and DBSCAN clustering. Technical Report of Image Analysis Group, Centre for Exploration Targeting (2013).
2. Vincent L, Soille P. Watersheds in digital spaces: An efficient algorithm based on immersion simulations. *IEEE Transactions on Pattern Analysis and Machine Intelligence* **6**, 583-598 (1991).