CALT-US

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Platform: Linux

Prerequisites: Python 3

CALT-US: SUMMARY

We used a deep learning approach [1], the latest of our developments built upon our previous works on loss modeling for cell segmentation [2, 3].

CALT-US: PREPROCESSING

For **Fluo-N2DH-GOWT1**, **Fluo-N2DL-HeLa**, and **PhC-C2DL-PSC**, intensity normalization by the 1- and 99-percentile was applied to accommodate for differences in the intensity distribution in the datasets.

CALT-US: SEGMENTATION

Let S be a dataset, where $S = \{(x_i, g_i)\}$ is the subset of images x_i with instance annotation g_i, taken from the available gold truth in the case of **BF-C2DL-HSC** and **BF-C2DL-MuSC**, from both the gold truth and silver truth in the case of **DIC-C2DH-HeLa** and **PhC-C2DH-U373**, and the union of the silver truths of **BF-C2DL-HSC**, **BF-C2DL-MuSC**, **DIC-C2DH-HeLa**, **Fluo-C2DL-MSC**, **Fluo-N2DH-GOWT1**, **Fluo-N2DL-HeLa**, **PhC-C2DH-U373**, and **PhC-C2DL-PSC** in the case of **Fluo-N2DH-GOWT1**, **Fluo-N2DL-HeLa**, and **PhC-C2DL-PSC**. To solve the instance segmentation task, we used a semantic approach by transforming g_i into a semantic annotation h_i of four disjoint classes: *background*, *cell*, *touching cell regions*, and *gap between cells and their parts*. Pixels classified as touching and gap are reclassified into either background or cell in a post-processing stage [1-3], thus obtaining an instance for each single cell.

To create the segmentation y for an image x, we used U-Net [4] with padding 1 for convolutional layers. We applied random rotations, mirroring, warping, gamma correction, and random crops with the size of 512 × 512 as data augmentation strategies. We used the Adam optimizer [5] with initial learning rate R. The training duration was set to N epochs using a mini-batch size of K. The network was initialized using random weights following a normal distribution, as proposed in [6]. In the case of **Fluo-N2DH-GOWT1**, **Fluo-N2DL-HeLa**, and **PhC-C2DL-PSC**, the initial general model was fine-tuned by re-training it separately for each dataset using the available, dataset-specific silver truth.

We used our L_{JC} loss formulation that combines *J* Statistics and Cross Entropy [1]. The general expression for the loss we adopted is

$$L_{JC}(y,z) = -\frac{1}{|\Omega|}\sum_{l=1}^{C}\sum_{p\in\Omega} y_l(p)\cdot\log z_l(p) - \sum_{i=1}^{C}\sum_{k=1}^{C}\lambda_{ik}\log\left(\frac{1}{2}+\sum_{p\in\Omega} z_i(p)\cdot\Delta_{ik}(p)\right)$$

with $\Delta_{ik}(p) = \frac{1}{2}\left(\frac{y_i(p)}{n_i} - \frac{y_k(p)}{n_k}\right)$. In this equation, the first term is the classical Cross Entropy, and the second term is our *J* regularization, after the *J* Statistics introduced in [7]. *y* refers to the one-hot representation of the semantic annotation *h*, and *z* is the computed probability map for all classes, $z(p) = [z_1(p),\ldots,z_C(p)]$, the output of the network followed by a softmax layer. n_i is the number of pixels $p \in \Omega$ that belongs to semantic class *i*, and $C = 4$ is the number of semantic classes. Parameter λ_{ik} was set to 0.5 as proposed in [1].

CALT-US: POST-PROCESSING

After obtaining a final model, we applied a semantic to instance post-processing [3] using Maximum a Posteriori estimation. The strategy uses the same *gap* class assignment from [1]. To potentially minimize the effect of not fully complete edges necessary to separate adjacent cells, a morphological opening with a squared structural element of size s_{open} was applied over pixels within the *cell* semantic class. Pixels removed from the *cell* class were then assigned to the *touching* class. To minimize the effect of small false positive objects, blobs with the area smaller than s_{area} pixels or $w_{\text{area}}\cdot A$, with *A* being the median area of objects in the previous frame, were removed. Instance assignment steps remained the same as in [2, 3].

REFERENCES

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