PURD-US

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Platform: Linux (tested on Centos 7.7)

Prerequisites: Python 3.8 with PyTorch

PURD-US: SUMMARY

Our approach is an deep-learning based method with minor modifications of the U-Net architecture [1].

PURD-US: PREPROCESSING

In order to apply the same training protocol across datasets, all raw 8-bit and 16-bit images are converted to float data type with intensities normalized to the range of [0, 1], for both training and testing datasets. This was done by looking at each pixel locations across the temporal sequence.

PURD-US: SEGMENTATION

We use the PyTorch framework for implementation of the U-Net architecture. We use 5 layers of U-Net blocks, with 64 filters in the first block. We added occasional skip connections to facilitate gradient flow during back-propagation. All weights of the network were obtained by optimization of the pixel-wise cross-entropy loss, thus we do not use any hand-tailored parameters. For a particular dataset, two training sets are blended together to train the network as a whole. When provided, the silver segmentation truth was used as the training set, while the gold segmentation truth was used as the validation set for model recommendation. If the silver segmentation truth was not provided, about 10% of the gold truth was randomly selected as the validation set, and the remaining 90% of the total frames were used as the training set. All trainings are carried out for 1500 epochs. Semantic segmentation masks are further converted to instance masks by simple connected component analysis.

PURD-US: TRACKING

Segmented cells are tracked by the multi-parametric tracking method originally designed for particle tracking velocimety, as described in [2], which has also been used for tracking fibronectin-expressing mesenchymal tumor cells [3]. The instance segmentation masks are re-numbered by the track numbers across frames, and no additional modification was performed to the masks.

PURD-US: POST-PROCESSING

We performed simple post-processing to suppress false positives by removing cells that are either too small or too large (for example smaller than 10 pixels, or larger than 1000 pixels). These thresholds are not optimal, and were chosen empirically from observation.

REFERENCES

1. Ronneberger O, Fischer P, Brox T. U-net: Convolutional networks for biomedical image segmentation. In *Proceedings of Medical Image Computing and Computer-Assisted Intervention*, 234-241 (2015).
2. Guo T, Ardekani AM, Vlachos PP. (2019). Microscale, scanning defocusing volumetric particle-tracking velocimetry. *Experiments in Fluids* **60**, 89 (2019).
3. Jun BH, Guo T, Libring S, Chanda MK, Paez JS, Shinde A, Wendt MK, Vlachos PP, Solorio L. Fibronectin-expressing mesenchymal tumor cells promote breast cancer metastasis. *Cancers* **12**, 2553 (2020).