Predicting subcellular organelle localization from label-free brightfield microscopy images

Anjali Bisaria, Yilin Fan, Nalin Ratnayeke
{abisaria, yilinfan, nalinr}@stanford.edu

Motivation

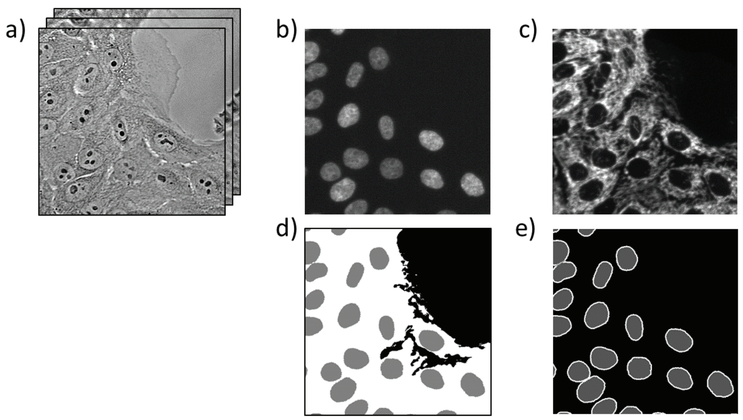
Combining fluorescence imaging with orthogonal imaging modalities, such as brightfield imaging, can give information on cell shape and subcellular structure without using molecular probes or taking up a fluorescence channel. Here we implement a deep learning-based segmetation pipelinewhich uses brightfield images to predict sub-cellular organelle location (nuclear and cytoplasm). We use a U-net CNN architecture for semantic segmentation to predict if each pixel is in one of three classes: background, nuclear, or cytoplasmic. The algorithm was trained on masks that were generated using automated pixel-wise ground-truth annotation using gold-standard chemical probes

Data

To generate our training set, we simultaneously imaged brightfield images and fluorescence images of nuclear stain Hoechest 33342 (for idenityding nucleus) and mitochondrial stain MitoTracker (for identifying cytoplasm). In total, we acquired 1,920 sites using a 20x (0.75 NA) objective on a digital camera capturing 16-bit 2160x2160 images. Ground truth masks were generated by through traditional segemntation methods of the fluroescent stains.

Features

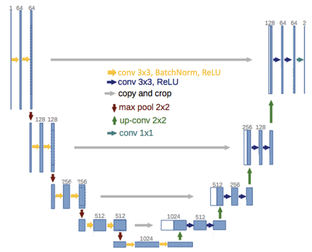
We first trained models on ground truth masks from the 3-class labeling scheme (background-nucleus-cytoplasm). To evaluate our segmentation models, we used pixel-level intersection-over-union (IoU) and F1 scores (harmonic mean of precision and recall) as primary metrics.



a) input brightfield images b) ground truth nuclear marker (Hoechst) c) ground truth cytoplasmic marker (Mitrochondria) d) Segmentation of nucleus (gray), cytoplasm (white) and background black for taining) e) nuclear mask with border pixels separated

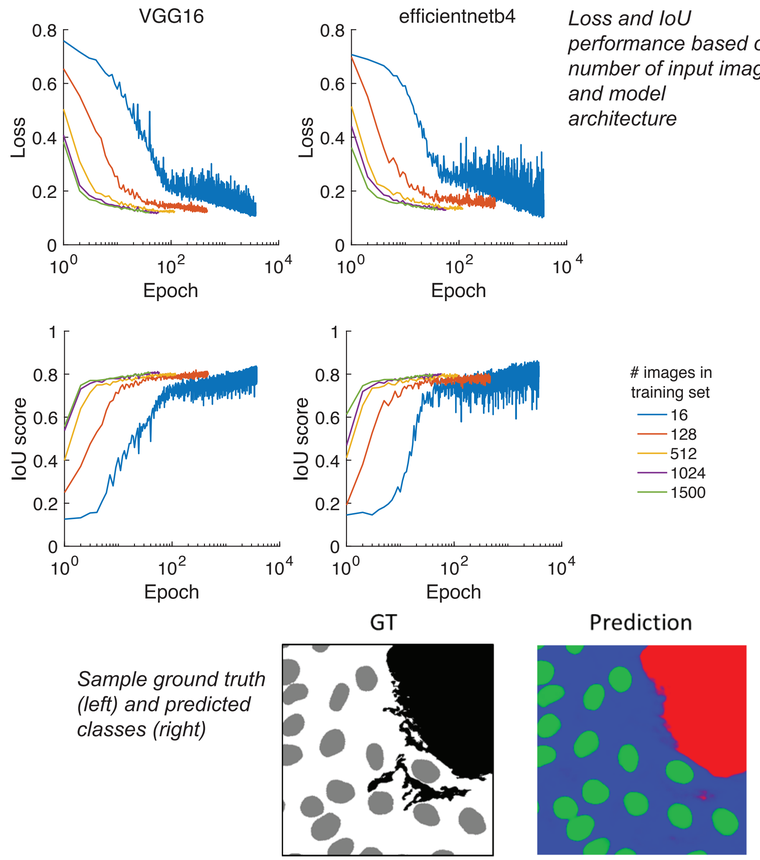
U-Net Image Classification

The U-Net is a fully convolutional network which is made up of a series of encoding convolutional layers followed by decoding layers which upsamples the output to the same size as the input. Skip connections between the encoding and decoding layers allow small-scale information to pass to the output of the network. In this way, a segmentation map comprised of pixel-wise classifications can be output



Results

We first trained models on ground truth masks from the 3-class labeling scheme (background-nucleus-cytoplasm). To evaluate our segmentation models, we used pixel-level intersection-over-union (IoU) as the primary metric given different numbers of training images or model architecture.



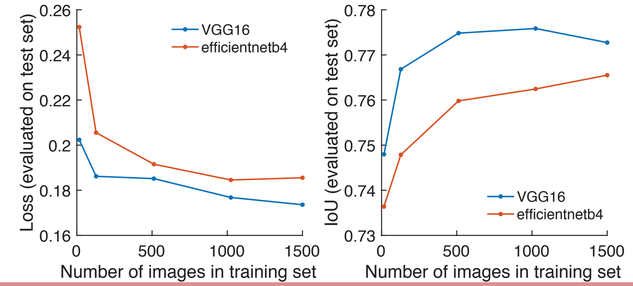
Loss and IoU performance based on number of input images and model architecture

Sample ground truth (left) and predicted classes (right)

Table 1: Model metric evalutated on Test set

Schemes	Loss	IoU (average over classes)	F1 (average over classes)	IoU (nucleus only)	F1 (nucleus only)
Different imaging modes					
Three-plane images	0.08477	0.77114	0.84612	0.78615	0.87966
Single-plane images	0.082248	0.77612	0.84943	0.79262	0.88383
Different loss functions					
Hybrid loss	0.085647	0.78086	0.85228	0.80887	0.89389
Dice loss	0.080889	0.76105	0.83958	0.78483	0.87895
Focal loss	0.001512	0.77823	0.85296	0.78522	0.87921
Different model architectures					
VGG16	0.085647	0.78086	0.85228	0.80887	0.89389
efficientnetb4	0.092493	0.76835	0.84596	0.77549	0.87266

Test set Performance



Discussion

We found that U-net implementions were able to reproduce high fidelity nuclear masks using brightfield imaging, though this wasn't that unexpected given other work in the field. We were surprised to find that training with a small number of images (<500) were able to achieve high IoU, though required more epochs. This means we might be able to train new models for different acquisition settings. VGG16 had better IoU and Loss and would be the preferred architeture in the future.

Future Directions

While examining ground truth and predicted labels, we noticed that the model performs poorly on mitotic cells, which comprise a small fraction of total cells (~5%) and display a distinct morphology. We propose to manually annotate mitotic cells based on brightfield and Hoechst stain, and include them as an additional label class.

References

[1] Ronneberger, Olaf, Philipp Fischer, and Thomas Brox. "U-net: Convolutional networks for biomedicalimage segmentation".International Conference on Medical image computing and computer-assisted intervention. Springer. 2015. 234–241
[2] Sadanandan, Sajith Kecheril, et al. "Automated training of deep convolutional neural networks forcell segmentation".Scientific Reports (2017): 1–7.
[3] .Christiansen, Eric M, et al. "In silico labeling: predicting fluorescent labels in unlabeled images".Cell173.3 (2018): 792–803
[4]Ounkomol, Chawin, et al. "Label-free prediction of three-dimensional fluorescence images fromtransmitted-light microscopy".Nature Methods 15.11 (2018): 917–920.
[5]Fishman, Dmytro, et al. "Segmenting nuclei in brightfield images with neural networks".bioRxiv(2019): 764894.
[6] Guo, Syuan-Ming, et al. "Revealing architectural order with quantitative label-free imaging and deepneural networks".BioRxiv(2019): 631101.
[7] Moen, Erick, et al. "Deep learning for cellular image analysis".Nature methods(2019): 1–14.