Nuclear segmentation and gene expression quantification
in mouse brain in situ hybridization image data
(Computer Vision)

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1 Introduction

Experimental access to specific neuronal classes and types is critical for understanding their role in the functional connectivity of the brain. Marker genes, which have been identified for various cell types via integrative analysis of transcriptomic data from mouse primary visual cortex, are being used to develop viral genetic tools to access specific cell populations. The goal of this project is to validate these viral tools - are the viruses targeting the intended cell type, how specific and complete is that viral labeling within the cell type? Based on co-labeling or the absence of co-labeling of probe genes, using RNAscope assay, with viral labeling, we can determine how well the virus is targeting the intended cell type (Fig 1).

Historically, these images are annotated by hand for analysis, which creates a bottleneck as data collection scales. From these images, the goal is to segment expression spots in order to characterize RNA expression within each cell, providing validation of these viral tools more quickly.

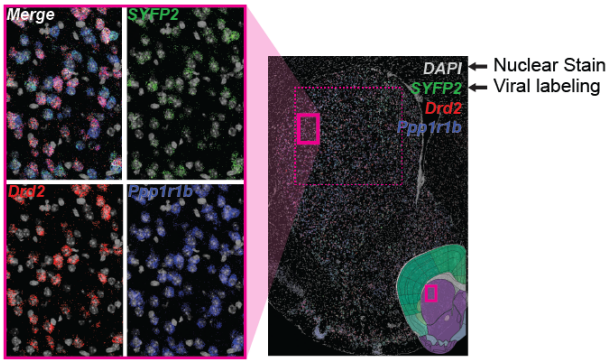


Fig 1. Targeting medium spiny neurons (MSN) in the mouse striatum. This image shows an example of the composite image of the RNAscope assay - the DAPI signal in gray stains nuclei, and the other channels include expression of SYFP2 (pan-MSN viral labeling), and Drd2 & Ppp1r1b, which are genes that are expressed in certain MSNs, there should be co-labeling of Drd2 & Ppp1r1b in all nuclei that show labeling from the virus.

2 Details on the Dataset

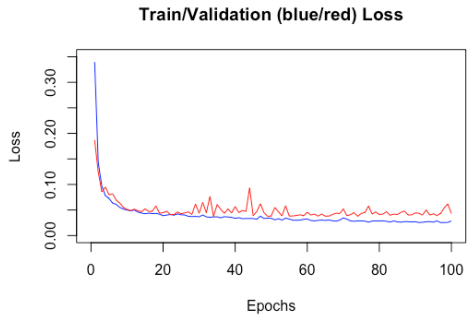
The dataset consists of 50 images of mouse brain RNAscope assay sections, each image consisting of 4 channels (Fig 1). This model will be trained on the three uncorrelated/unrelated expression spot channels, which can be considered as a total of 150 separate images. Ground truth coordinates were generated using a variety of segmentation algorithms from the CellProfiler[1] tool, to find a method that best labels expression spots for each section. These coordinates have been inspected and validated by multiple lab members and are considered accurate representations of the biology. During training 260x260 px tiles will be randomly generated from these much larger brain section images, and will undergo random transformations in order to augment the training set. Currently, I have collected 260x260 px tiles to initially make sure that a model is being training without errors.

3 Approach

I have initiated a simplified baseline Unet model, with two contraction blocks and two expansion blocks, instead of three in the original paper[2], in order to make sure that I can generate a minimum viable model for this milestone. I also decided to change the activation function, ReLU in the original paper, to leaky ReLU, based on suggestions from lecture to give this a try, and that it may work better than ReLU, despite

it not being used as much in practice. In iterations moving forward. I have chosen to use the Adam optimizer, based on class discussion regarding general success with this optimizer, and am calculating loss with the cross-entropy loss function. This beginning code is established/modified from a Satellite Image segmentation project[3].

I've begun some initial training, and although the validation loss is decreasing with more passes through the dataset/epochs and is not extremely higher than the training loss, it is not as smooth as the training loss, and does seem to stop decreasing after 50/60 epochs (see Train/Validation plot below). I am hoping that including all training data with augmentation will help the model to improve in the next iterations.



[1] McQuin C, Goodman A, Chernyshev V, Kamentsky L, Cimini BA, Karhohs KW, Doan M, Ding L, Rafelski SM, Thirstrup D, Wiegraebe W, Singh S, Becker T, Caicedo JC, Carpenter AE (2018). CellProfiler 3.0: Next-generation image processing for biology. PLoS Biol. 16(7):e2005970 / doi. PMID: 29969450

[2] Ronneberger, Olaf; Fischer, Philipp; Brox, Thomas (2015). "U-Net: Convolutional Networks for Biomedical Image Segmentation". arXiv:1505.04597

[3] 38-Cloud-Simple_Unet: https://www.kaggle.com/cordmaur/38-cloud-simple-unet