Machine Learning for Brain Histological Image Analysis

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**Abstract**

Manual image analysis for immunohistochemistry (IHC) images is time consuming and quantification results vary between researchers. This project experimentally determines which machine learning model gives the best results when automating this task, and implements that model as software to analyze IHC images. These images are of mice brain slices which have been stained for different neural and neuropathological processes. Software will allow for more efficient cellular-level quantification and potentially results that are as reliable as manual techniques. The software will be trained on sample images from Dr. Jamie Near’s lab, then tested on the remaining images and optimized to provide accurate results for different stains.

**Introduction**

Neuropsychiatric disorders are studied using a variety of research methods including behavioural tests, assays of brain structure and function, and histological and immunohistochemical (IHC) analyses of brain tissue. IHC combines the immune response of various cells in the brain, including glial cells and neurons, with the visibility of histochemistry (“Immunohistochemistry / IHC Antibody-Brain Tissue”). Immuno-stains produce a variety of different IHC results depending on the immune response that stain causes. Different stains cause immune responses in different types of cells. Immuno-stained slices of brain tissue can be photographed under a microscope with or without fluorescence, depending on the stain. Once the tissue has been photographed, the IHC image can be analyzed to determine the number of cells stained.

Analysis of IHC images is typically done manually, and while manual segmentation and cell counting provide accurate results, the process can be time consuming and subject to rater bias. Multiple raters can reduce analysis time; however, this adds variance to the data due to inconsistent inter-rater reliability (LaCroix-Triki et al. 1). Automating this process would benefit researchers who use IHC imaging by reducing image analysis time and variability.

Previous research has implemented machine learning for image analysis of microscopy data, including IHC images. A variety of machine learning models have been used, from simple to complex as outlined in the literature review section. Complex models require large amounts of data for training. Simple methods often achieve accuracy close to that of complex neural networks.

The purpose of this research project is to design and implement software that uses machine learning to perform analysis on IHC images. Multiple machine learning models will be studied and tested to determine which has the highest performance on the sample images provided by Dr. Jamie Near. Once the model is trained and optimized on that data set, extending the model will be investigated. Ideally, the software will perform well on multiple types of immuno-stains to provide the maximum value for researchers. The goal for this project is to produce a software system where researchers can upload their IHC images and tell the program which stain(s) were used, and the software reports the positive cell count in a precise and accurate manner.

**Literature Review**

A variety of approaches have been used to automate cell counting in immunohistochemistry images. This automation started by using algorithms based on intensity thresholding, edge detection, template matching, and active shape models (Pham et al. 842).

As machine learning became more popular, different models have been trained for IHC image analysis. Machine learning approaches began with basic algorithms including support vector machines (SVM), random forests (RF), K-means clustering, and fuzzy c-means (Pham et al. 842).  K-means and fuzzy c-means algorithms are more useful for pathologies where cells appear in clusters.

Areta et al. explored the use of SVM for cell detection in microscopy images. Their algorithm was trained with simple dot annotation on sample images. Their use of SVM achieved state-of-the-art accuracy on hematoxylin and eosin (H&E)-stained images (1). H&E staining reveals a considerable amount of microscopic anatomy, and is used to diagnose a range of histopathological conditions. Mualla et al. studied RF for cell detection in bright-field microscope images (1). Bright field microscopy is for unstained images, not those produced with IHC, but their method could be extended to IHC. Pham et al. experimented with SVM and RF for cell counting and segmentation of IHC images in the spinal cord. They performed preprocessing for both algorithms, and their RF contained 200 bagged classification trees (843).

In recent years, deep learning using neural networks has become popular. Pham et al. also explored these techniques, using U-nets and fully convolutional networks (FCN) (843). There are a variety of neural network architectures explored by different research groups. Convolutional neural networks (CNNs) were explored by Swiderska-Chadaj et al. for cell detection in IHC images. They implemented four different CNN methods, and found the best results using U-nets. Fully convolutional networks (FCNs) are CNNs where the final layer has been replaced with a convolutional layer to capture the global context of the image (Araujosantos). FCNs were explored by Sheikhzadeh et al. for automatic labelling of biomarkers in IHC images. Their approach uses CNNs and FCNs, and allows multiple types of biomarkers to be labelled by the same algorithm (1). This approach requires large amounts of data and computational complexity (3). Fully convolutional regression networks (FCRNs) were used by Xie et al. to perform cell counting and detection. CNNs are used to regress a cell spatial density map across an image, and the need for large amounts of training data is satisfied by using synthetic data to train the model. They showed that this model trained entirely on synthetic data generalizes well to real world data, and is useful even in the case of overlapping cells (1).

**Methods**

**Dataset:**

The data to be used for this project are from the lab of Dr. Jamie Near, and are IHC images of TgF344-AD rat brains, an Alzheimer’s disease model. This dataset may be supplemented with images from the Master’s project of Chloe Anastassiadis in Mallar Chakravarty’s laboratory to facilitate training on the model on multiple IHC stains. Different IHC stains are used to visualize different cell responses in a brain slice, including multiple types of glial cells and their activation, neurons, and leukocytes. The images will be split into training and testing sets, and the training set will be manually tagged with cell counts and positive areas. The models will be evaluated based on recall and precision as compared to manual analysis as the ground truth. K fold cross validation will be used when comparing the performance of different models.

**Models:**

This project will follow the structure of the study by Pham et al. who trained simple and complex models on IHC image analysis. The simple models used in this project will be SVM and RF. Different preprocessing options will be tuned, including histogram equalization, convolution and intensity thresholding. Features including shape, texture, and histogram gradients will be extracted from the images and provided as input to the models. For the RF model, different numbers of bagged classification trees will be tested to see where optimal accuracy is achieved. The simple models may be supplemented with K-means clustering and fuzzy c-means models if this improves performance on stains with more clustering.

The complex models will be U-nets and a multi-scale network. The implementation of the U-nets will closely follow that of Ronneberger et al., which implements a network and training strategy to reduce the amount of data required to train the model (234). The multi-scale network is a network of FCNs and the implementation will follow that of Pham et al. Preprocessing is not required on the complex models, so raw images can be used as input to the model (843). In a paper by Janowczyk and Madabhushi, a tutorial on using deep learning for digital image pathology is presented, and this will serve as a starting point for building the model architecture.

Once the models have been trained on the data from Dr. Jamie Near’s lab, they will be generalized to work with multiple stains. The use of synthetic data as described in Xie et al. may help with this process (1).

**Software:**

Once the models have been evaluated, the best performing model will be packaged as software for use by researchers. In previous studies, simple models tend to perform as well or better than complex ones, and they require much smaller datasets for training and less computational power.

**Work Plan**

Fall Semester (October - December) 2019:

* Image pre-processing for simple models
* Follow Janowczyk and Madabhushi tutorial to set up architecture for complex models
* Begin implementing the four models

January/February 2020:

* Tune models to optimize performance
* Generalize models to work on multiple stains
* Progress report on the performance of each model
* Select best performing model

March/April 2020:

* Refine implementation of model
* Write software packages to facilitate use by researchers
* Final report

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