Quantitative In-situ Image Analysis in Highly Multiplexed Fluorescence IHC Image Datasets of Rat Brain

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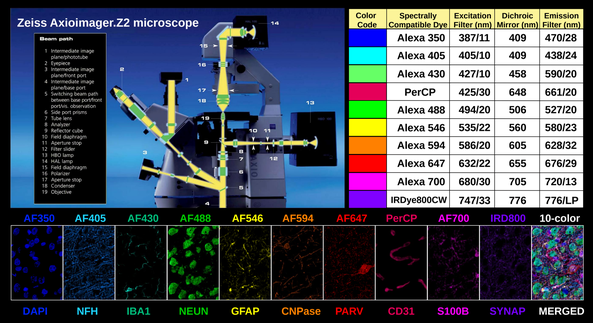
INTRODUCTION

Development of novel image analysis methods and robust machine learning pattern recognition techniques can provide powerful new tools to efficiently and accurately investigate large and multidimensional image datasets sourced from highly complex and integrated tissues such as the brain, and facilitate deep mining of the resultant data to better identify altered signaling pathways and/or vulnerable cell populations that could be targeted for interventional treatment in different types of brain disorders and dysfunction.



Color Code	Spectrally Compatible Dye	Excitation Filter (nm)	Dichroic Mirror (nm)	Emission Filter (nm)
	Alexa 350	387/11	409	470/28
	Alexa 405	405/10	409	438/24
	Alexa 430	427/10	458	590/20
	PerCP	425/30	648	661/20
	Alexa 488	494/20	506	527/20
	Alexa 546	535/22	560	580/23
	Alexa 594	586/20	605	628/32
	Alexa 647	632/22	655	676/29
	Alexa 700	680/30	705	720/13
	IRDye800CW	747/33	776	776/LP

Figure 1. 10 color multi-spectral fluorescence imaging captures high content information for image segmentation or deep learning techniques.

Using multiplexed histological immunostaining (MP-IHC) and multi-spectral imaging techniques [1] to screen for a multitude of biomarkers related to cytoarchitecture, cell proliferation, cell death and intracellular or intercellular signaling functions, we are able to discover and profile for the first time the unique seminal biological properties of individual cells and whole populations of relevant cell types in the context of complex microenvironments, as well as specific anatomical regions in the brain.

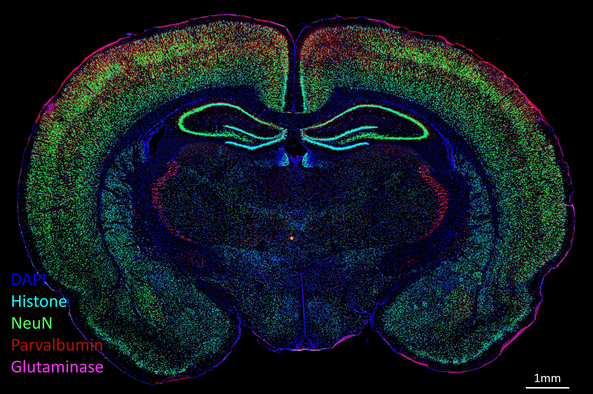


Figure 2. 5 color composite image of a normal rat brain showing all nuclei (DAPI, Histone), classification of nuclei using a pan-neuronal marker (NeuN) and sub-classification of neurons to inhibitory (Parvalbumin) and excitatory (Glutaminase) sub-types.

Due to the extensive heterogeneity of cell types in brain tissues, traditional masking and segmentation methods cannot capture all the spatial information of cells or handle the high variability in cells. Deep learning methods, on the other hand, learn similar features between intra-class samples and discriminative features between inter-class samples. These methods provide a deep characterization of the cell morphology, molecular composition, and spatial distribution of each marker mapped to individual cells. They are able to utilize multi-channel information to cope with discretely distributed staining patterns (such as cytoplasmic but not nuclear staining). Deep learning has been widely used in detection, segmentation, and analysis of large imaging datasets. In this study, we define precisely the learning task of detecting the cell nuclei in whole slice images of rat brain as a quantitative analysis of image datasets. We show how Convolutional Neural Network (CNN) outperforms currently available conventional segmentation methods to detect cells by learning the abstract representation of each cell, while at the same time maintaining the flexibility to deal successfully with cell variability in tissues through sampling of large training image datasets representing different parts of the brain.

METHODS

1. Automatic Nucleus Segmentation

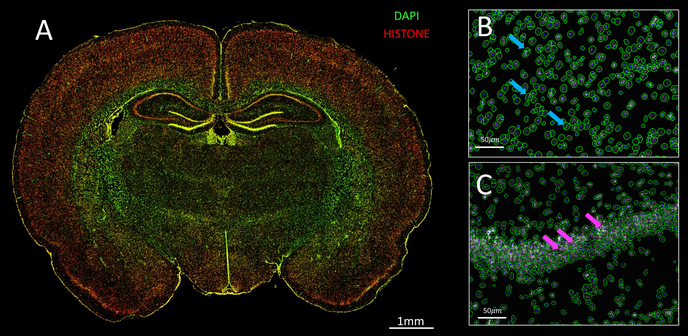


Figure 3. Whole brain automatic nucleus segmentation using DAPI and Histone.

Automatic nucleus segmentation [2] has been applied on a 16-bit gray scale whole brain compound image of DAPI + Histone with the size of 29,200×42,900 pixels. Two samples of cropped segmentation results (B,C) show that the automatic segmentation method fails on regions where the nuclei are not compactly located resulting in over-segmentation (blue arrows) or in regions forming large connected components resulting in under-segmentation (pink arrows) errors.

2. Manual Bounding Box Correction

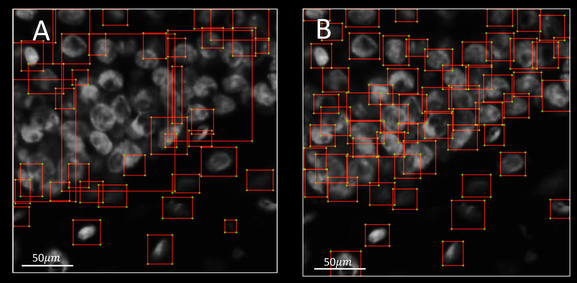


Figure 4. Manual correction of bounding boxes generated by automatic segmentation method.

Generated bounding boxes from automatic nucleus segmentation (A) are corrected (B) manually using labelImg software [3] to be used as training set in the deep learning model. 220 crops (each with size of 300×300 pixels) were corrected and added to the training set.

3. Learning Corrected Samples with Deep Learning Model

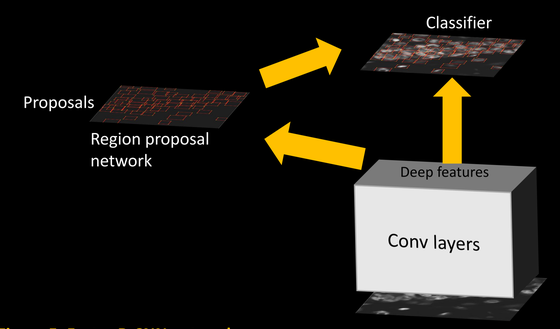
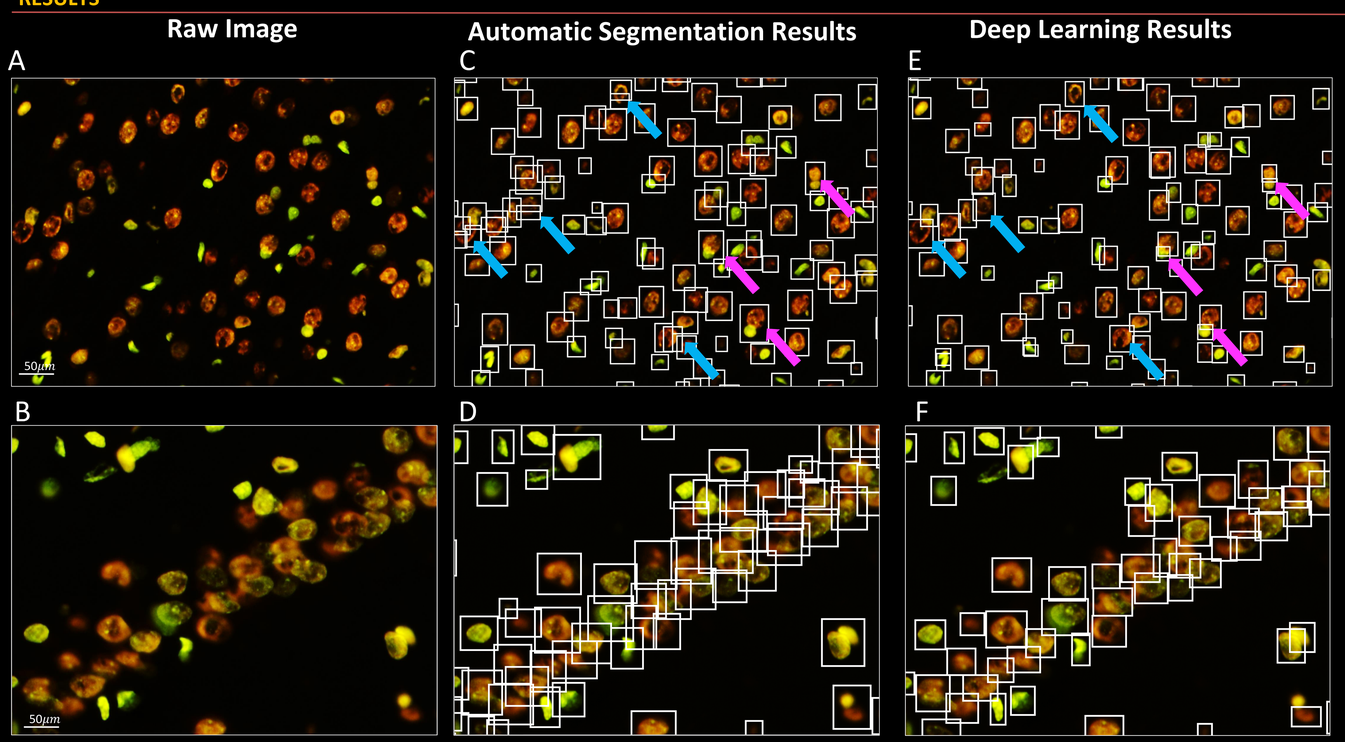


Figure 5. Faster R-CNN network.

Faster RCNN [4] is used as the state-of-the-art object detection network to detect cells in images. This network extracts deep features using fully convolutional layers and proposes regions with region proposal network. The proposed regions are classified as cell (+) or non-cell (-) events using deep features extracted from convolution layers.

RESULTS



	Automatic segmentation	Deep learning detection
Time	9 hours	3 hours
Number of detected cells	194,074	208,836

Table 1. Time and counting results on whole brain image.

	Method	Precision TP/(TP+FP)	Recall TP/(TP+FN)
A	Automatic segmentation	87.25	95.69
	Deep learning detection	98.03	96.15
B	Automatic segmentation	74.13	82.62
	Deep learning detection	98.30	84.31

Table 2. Performance comparison of methods shows great increase in precision and recall of deep learning method.

Precision and recall reports the correct location and the ratio of the missed cells of the detected boxes over the ground truth boxes respectively. Deep learning method increases the precision and recall by >20% and 1% respectively.

Figure 6. Comparison of cell detection results between automatic segmentation and deep learning methods.

Detection results were validated one selected region from motor cortex (A) and hippocampus (B). Automatic segmentation results (C,D) show several over-segmentation (blue arrows) and under-segmentation (pink arrows) that are resolved using deep learning method (E,F).

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[3] Tzutalin. LabelImg. Git code (2015). https://github.com/tzutalin/labelImg

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CONCLUSIONS

Accurate cell detection is the first step in any computational image analysis pipeline. Traditional nucleus and cell segmentation algorithms are limited to the quality of the image, homogeneity of cells in the image and tuning different hyper-parameters. These methods do not have the capacity to be generalized on heterogeneous samples. In comparison, deep learning can take advantage of existing segmentation algorithms as initial training sets and by adding corrected samples we can increase the performance of the detection drastically.

Deep learning techniques make it feasible to develop state-of-the-art methods in the field of computational biology to obtain comprehensive quantitative methods for studying complex systems biology of the entire brain, which is essential to get a better understanding of the normal and abnormal brain function.