Deep Learning-Based Nuclei Segmentation of Cleared Brain Tissue

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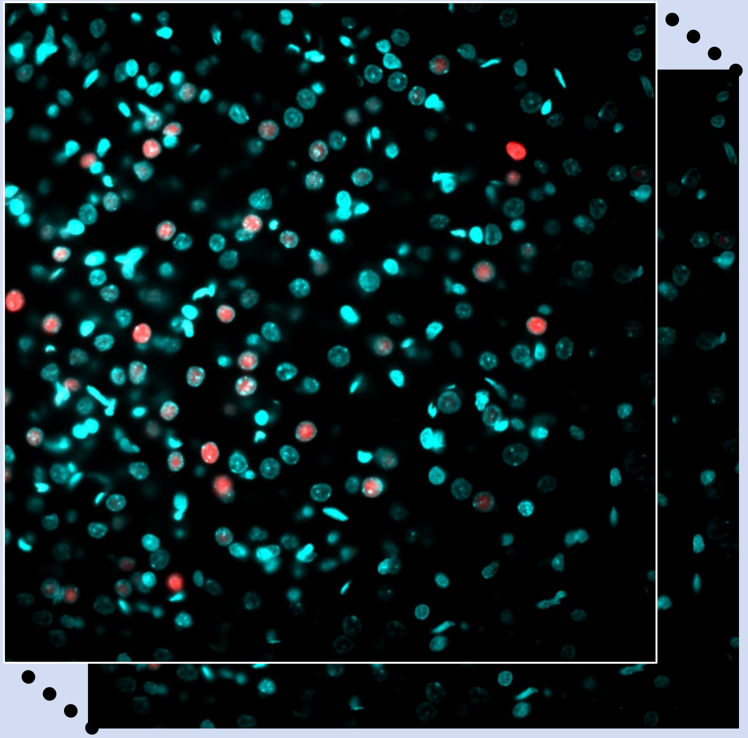
Program Goal, Challenges, and Proposed Approach

Light-sheet Microscopy Images



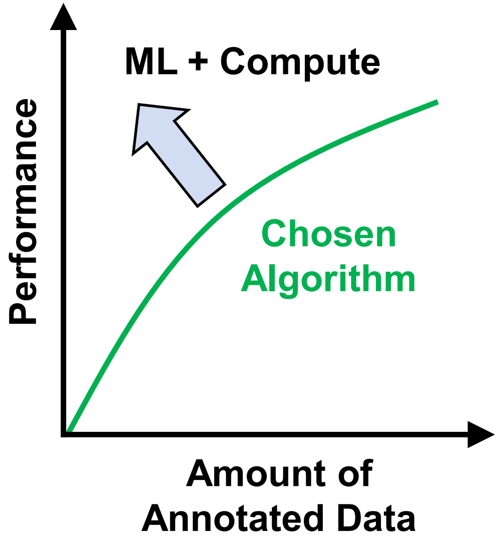
Goal is to map neuron connectivity

Applications

- 3D Nuclei Segmentation
- Centroid Detection
- Axon Tracing
- ⋮

Challenges: Difficult to process large-scale, densely populated data automatically with high accuracy

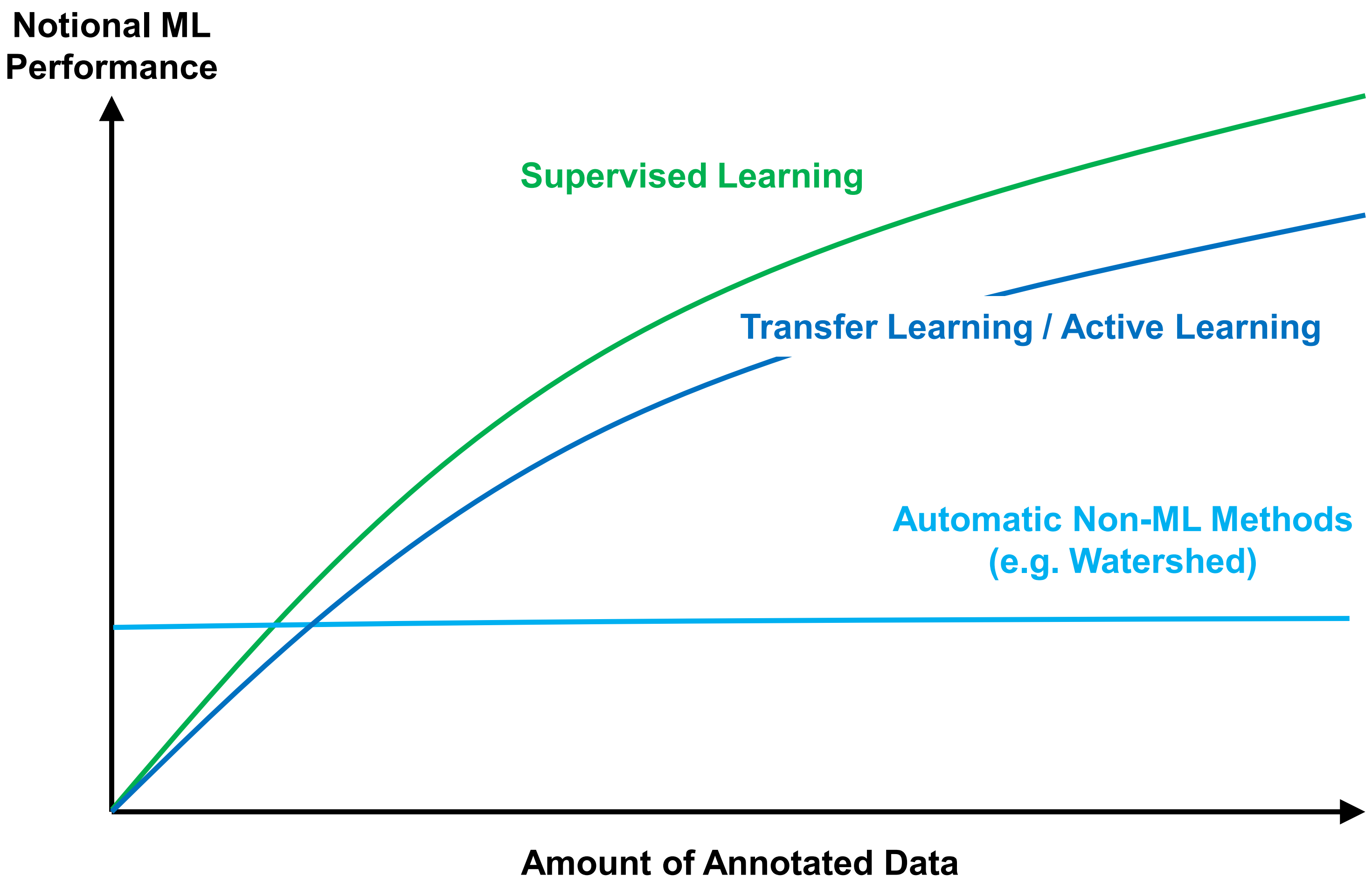
Evaluation



Propose Approach: Develop HPC-based ML algorithms

ML: Machine Learning

Concept Overview



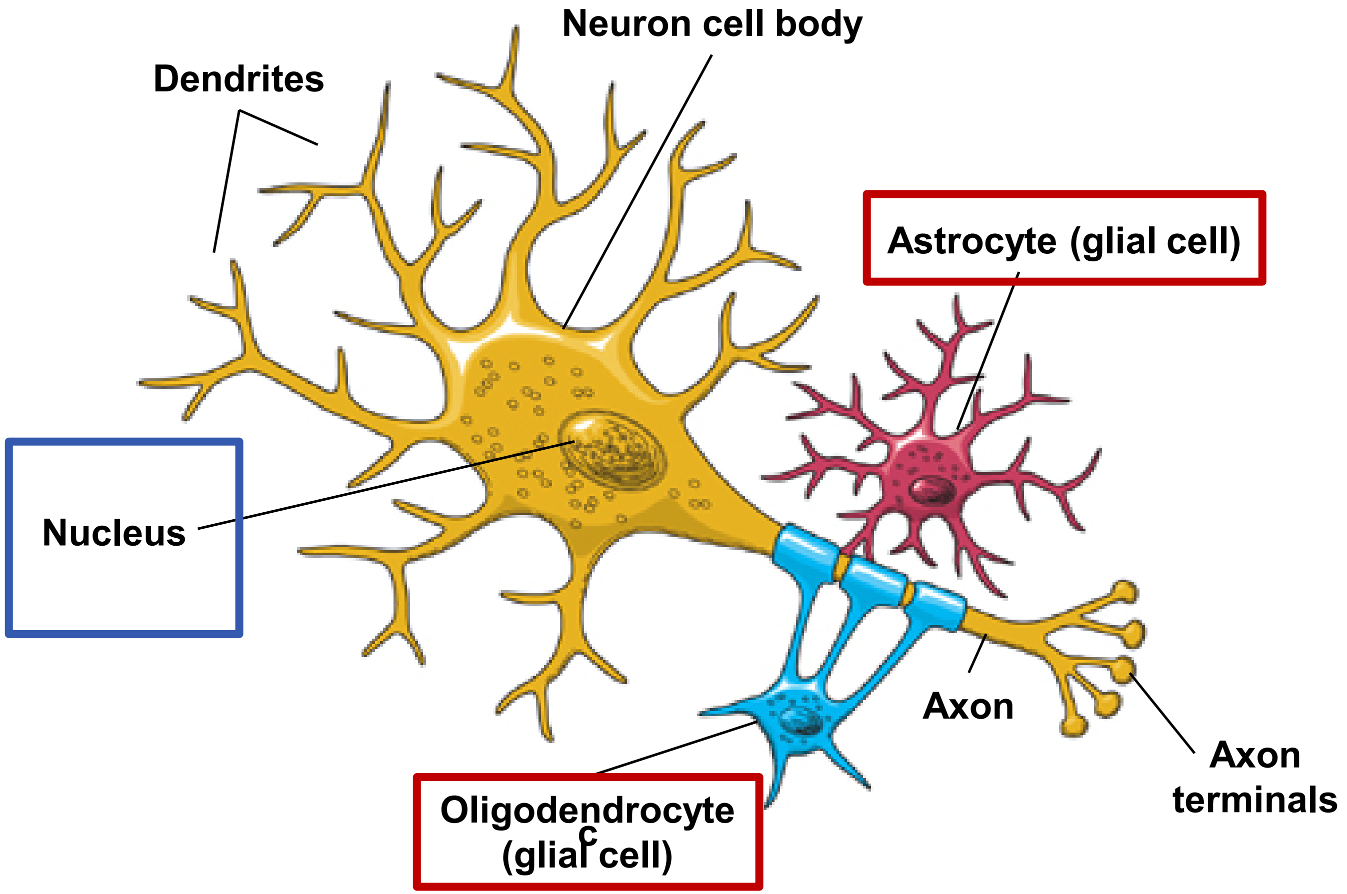
Available Approaches

- Traditional Approach
  - Collect and annotate more data
  - Challenge: time+resource intensive
- Lincoln Approach
  - Leverage automatic and/or semi-automatic algorithms to obtain comparable accuracy with less annotation

ML: Machine Learning

Targets of Interest

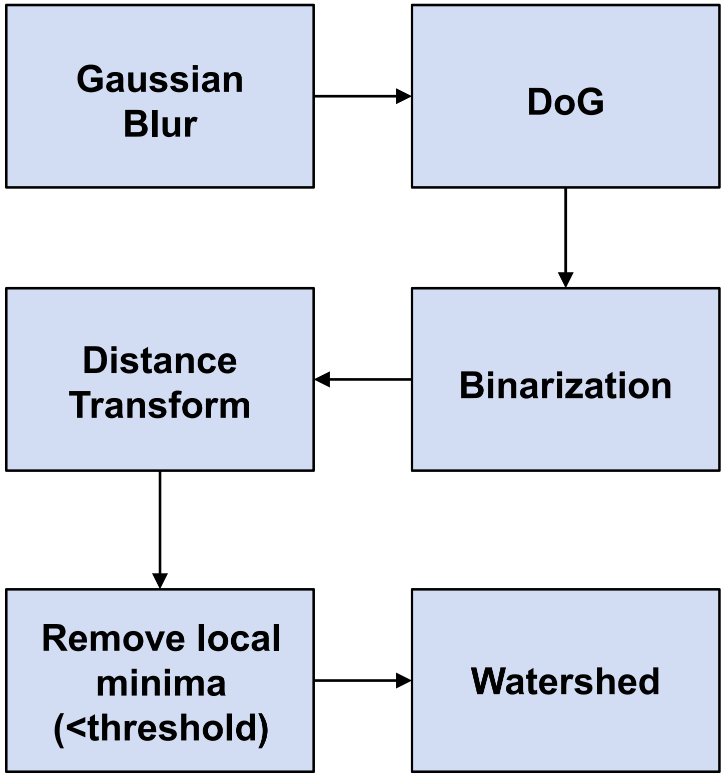
- Neurons (nuclei)
  - Receive stimuli
  - Conduct action potentials
- Glial cells
  - Supporting functions
  - Hold neurons close together



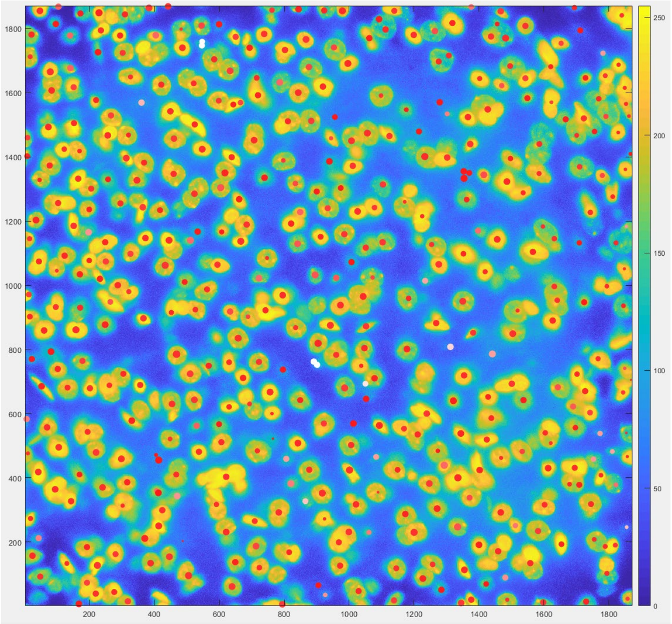
Source: NIH Institute of Neurological Disorders and Stroke

Automatic Approach
Conventional Image Processing on LLSC

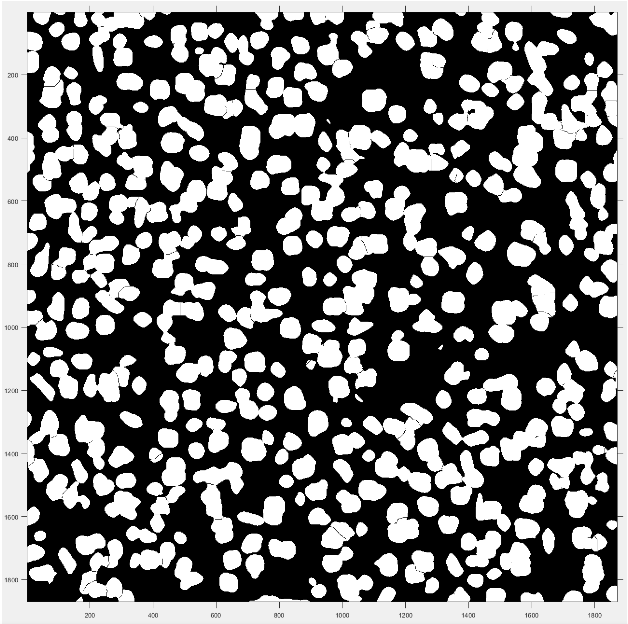
Difference of Gaussian (DoG), Thresholding, and Watershed Segmentation



Centroid Detections



Binary Mask



Strength
- No need for annotation
- Results can be fed to ML algorithm as weakly annotated data

Weakness
- Bulk blob detection method does not distinguish between different cell types (i.e. neurons and glia)

LLSC: Lincoln Laboratory Supercomputing Center

Proposed Technical Approach
Reduce costly human annotation

Existing model

U-Net Model on nuclei segmentation

Transfer Learning

Our datasets

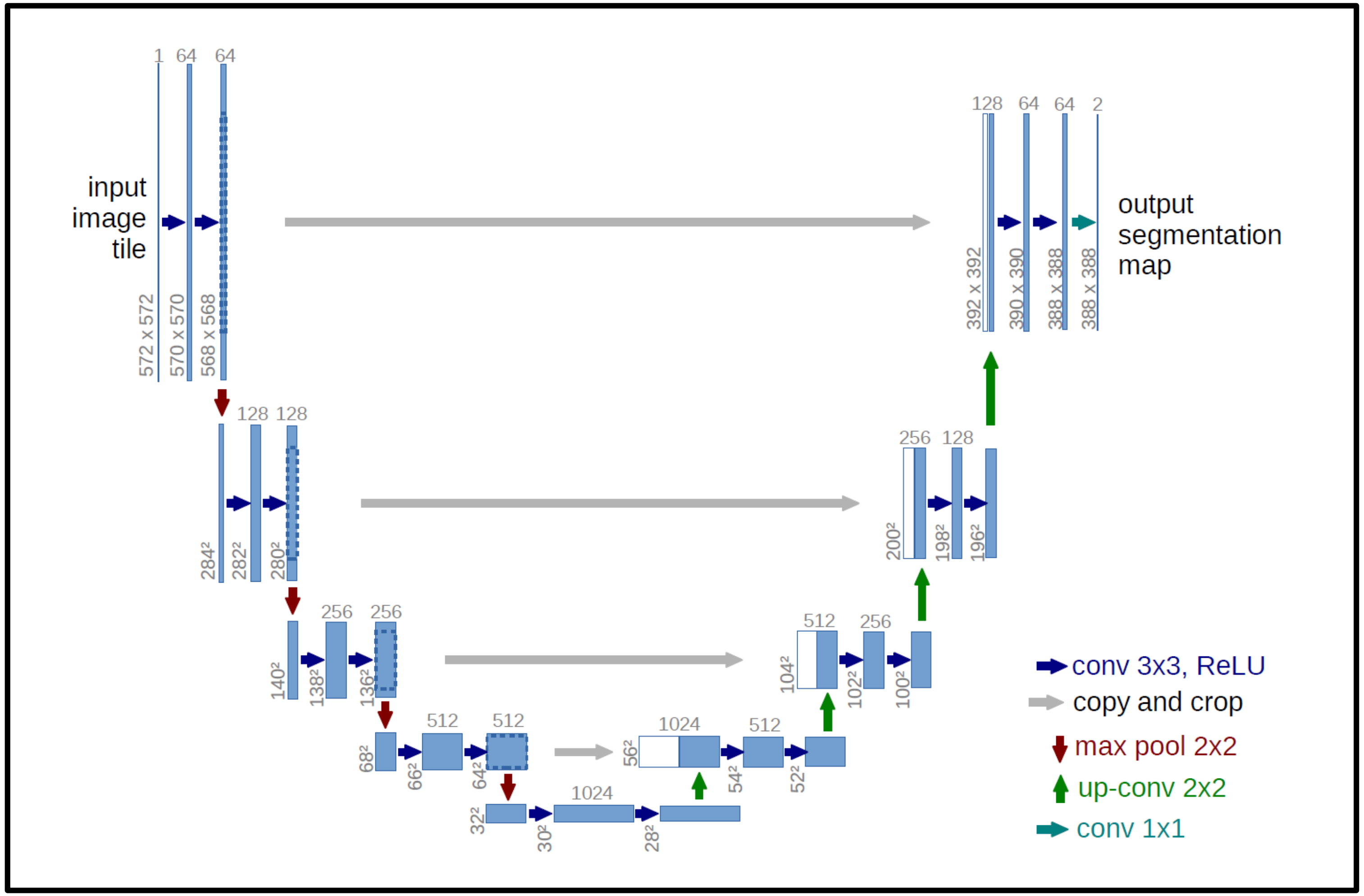
- **Large amount of unlabeled nuclei data**
- **Small amount of labeled nuclei data**

Results

- **Qualitative results**
- **Quantitative results**

Transfer Learning Approach
U-Net

- **U-Net [1] is a popular type of "fully-convolutional" neural network**
- **It is comprised of a contracting path (left) and an expanding path (right)**
  - **Expanding path uses information from contracting path (via "copy and crop" operations)**
- **Contains 23 convolutional layers in total**



[1] - O. Ronneberger, P. Fischer, and T. Brox, "U-net: Convolutional networks for biomedical image segmentation," in International Conference on Medical image computing and computer-assisted intervention. Springer, 2015, pp. 234–241.

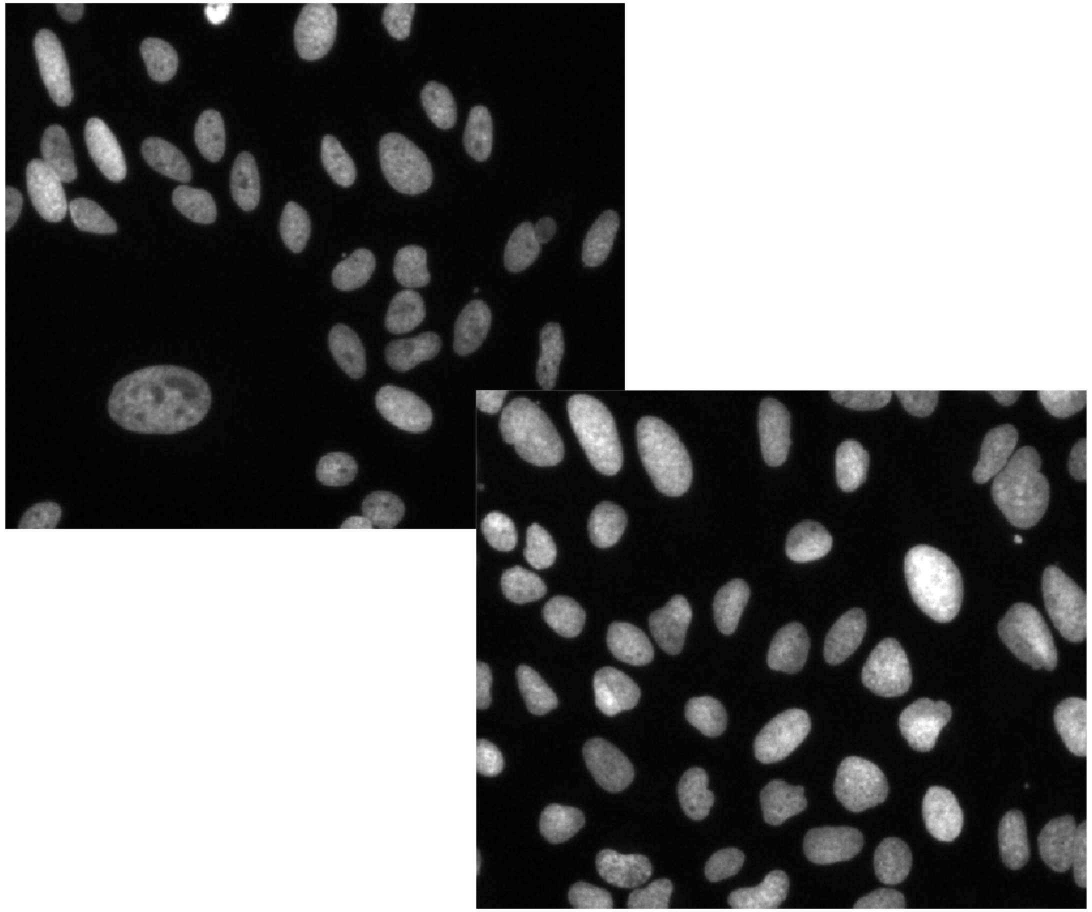
Proxy Dataset

Used nuclei segmentation dataset prepared by the Broad Institute (BBBC039) [1, 2]

200 images collected – fluorescence microscopy

Around 23,000 nuclei annotated

Image dimensions: 520 x 626 pixels



[1] - V. Ljosa, K. L. Sokolnicki, and A. E. Carpenter, "Annotated high-throughput microscopy image sets for validation." Nature methods, vol. 9, no. 7, pp. 637–637, 2012.
[2] - https://data.broadinstitute.org/bbbc/BBBC039/

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→

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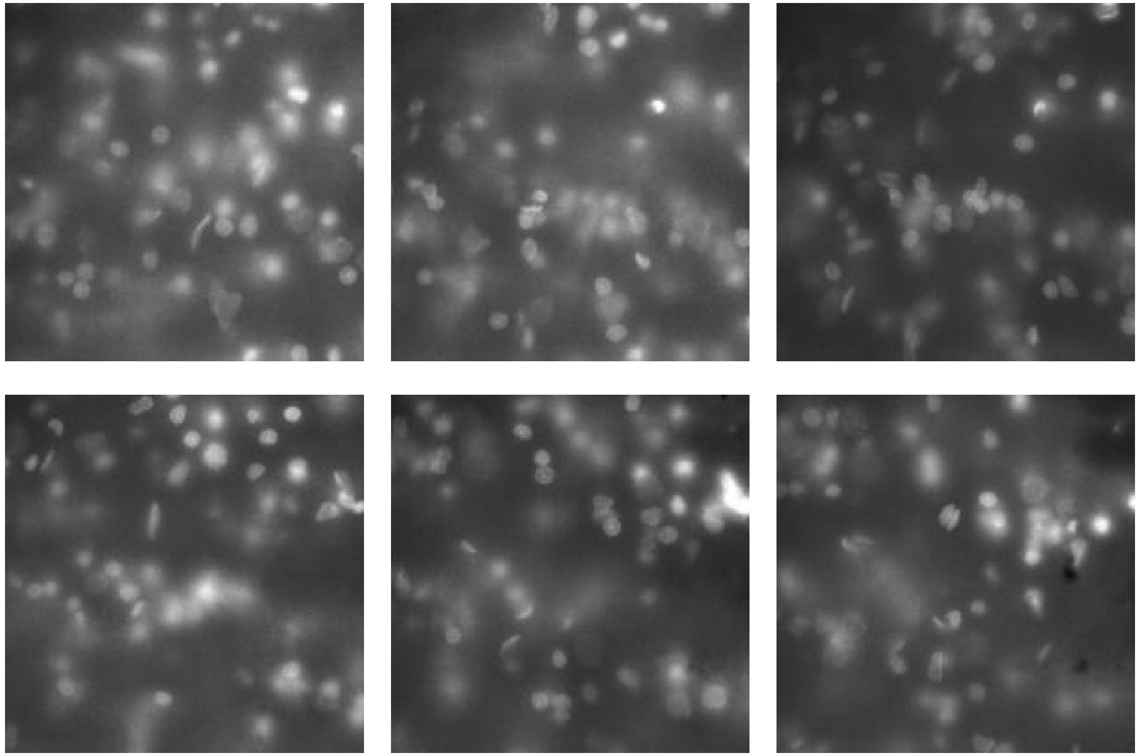
Unlabeled Data (DAPI)

- Rat brain tissue sample of the nucleus tractus solitarus region
- Prepared using CLARITY tissue clearance [1]
- Applied a fluorescent DNA stain, diamidino-2-phenylindole (DAPI)
- Image acquired using a light-sheet microscope (ZEISS Lightsheet Z.1)
- Resolution:
  - 1920 x 1920 x 650 voxels
  - 0.2µm x 0.2µm x 1µm



Examples of DAPI slices

[1] - K. Chung and K. Deisseroth, “Clarity for mapping the nervous system,” Nature methods, vol. 10, no. 6, p. 508, 2013.

U-Net Training Details

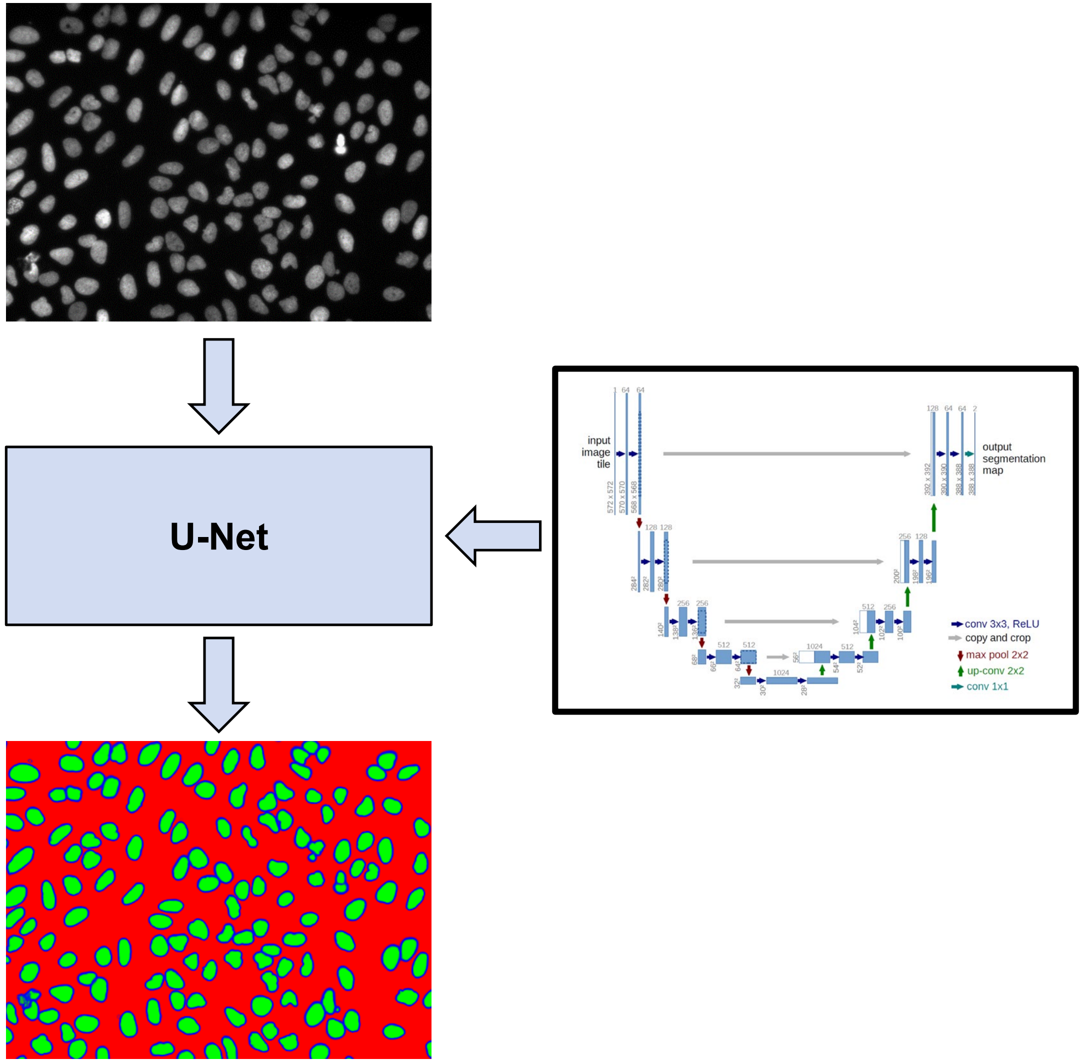
Trained U-Net on BBBC039 dataset using code/library associated with [1]

- Model outputs predictions as being one of three classes:
  1. Background
  2. Nuclei Interior
  3. Nuclei Boundary

Model was trained using Keras with Tensorflow backend on Nvidia Tesla K40

Used MapReduce on LLSC to apply trained model to DAPI slices

- Processed 20 blocks with ~650 slices each
- Watershed Segmentation Approach was also run on LLSC



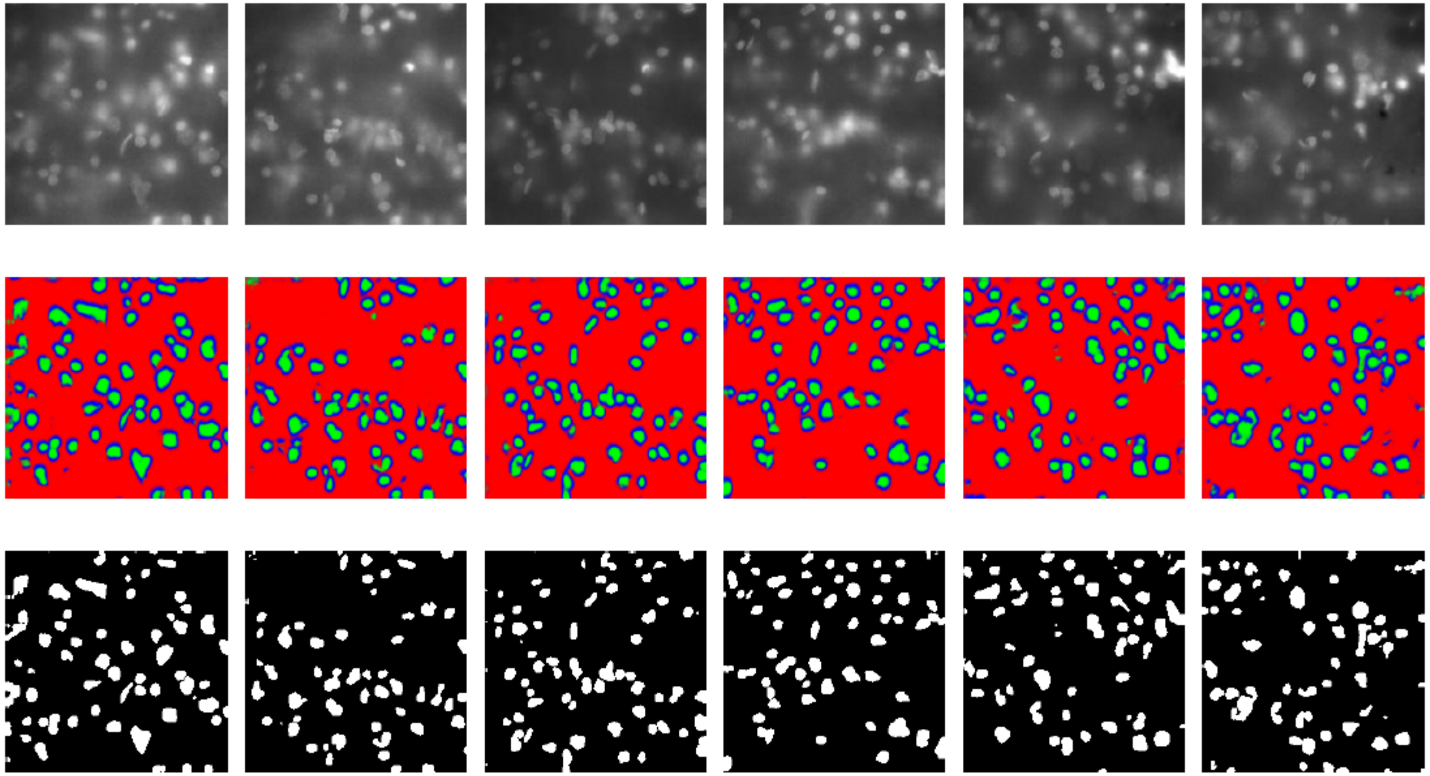
[1] - J. C. Caicedo et al., "Evaluation of deep learning strategies for nucleus segmentation in fluorescence images," BioRxiv, p. 335216, 2019.

Qualitative Results

DAPI Images

Prediction Output
Red: Background
Green: Cell/Nuclei Interior
Blue: Cell/Nuclei Boundary

Binary Map
(Interior + Boundary Classes)



U-Net model generates very reasonable segmentation masks using transfer learning

Proposed Technical Approach
Reduce costly human annotation

Existing model

U-Net Model on nuclei segmentation

→ **Transfer Learning** →

Our datasets

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- Small amount of labeled nuclei data

→

Results

- Qualitative results
- Quantitative results

Small Labelled Dataset

15 hand-annotated images (LL15)

Annotated with 3 classes:

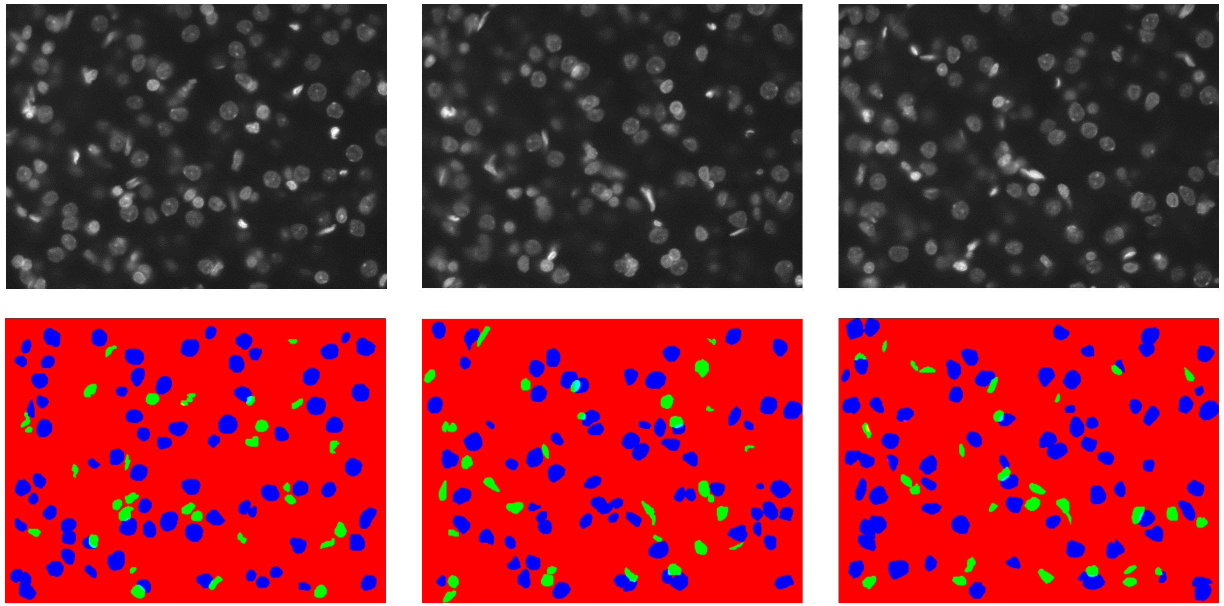
1. Background
2. Glial cell
3. Nuclei

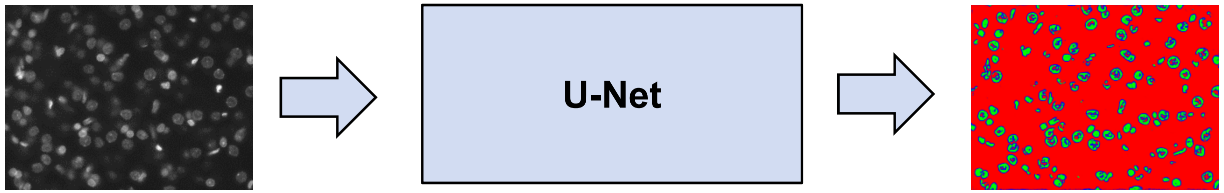
Directly apply U-Net trained on BBB039 dataset on the hand-annotated images

– Combined pixels classified as cell interior and cell boundary to make binary mask

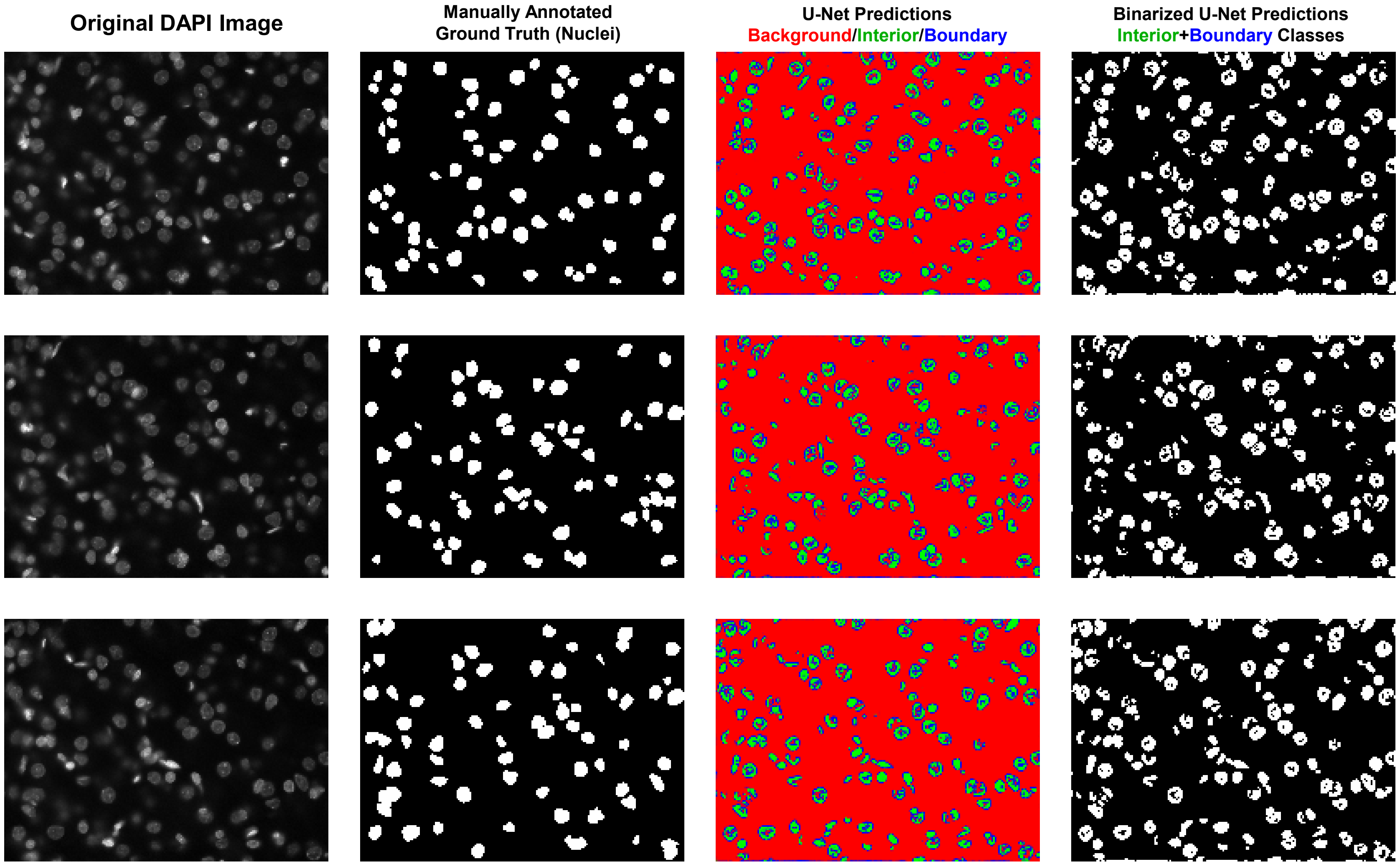
Compute DICE score

– Average over 15 images





Pretraining Results



$$DICE\ Score = \frac{2 \cdot TP}{2 \cdot TP + FP + FN}$$

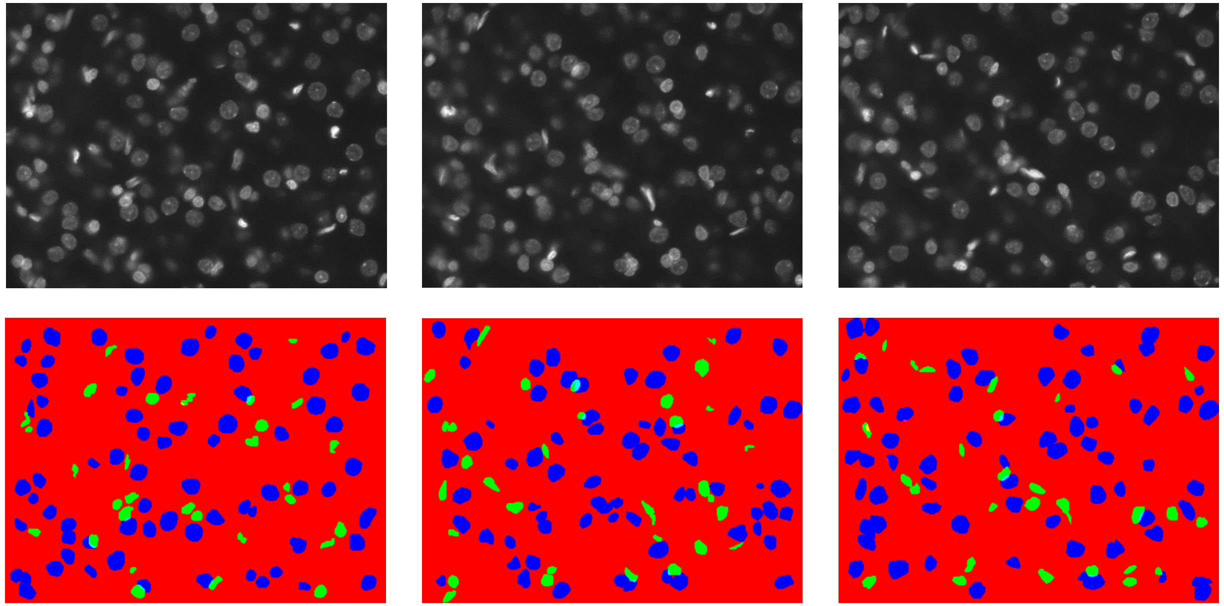
Approach	DICE Score
Automatic (Watershed Segmentation)	0.561
Transfer Learning	0.655

Finetuning Experiment Setup

Can we leverage the labels given in the 15 annotated images to improve performance?

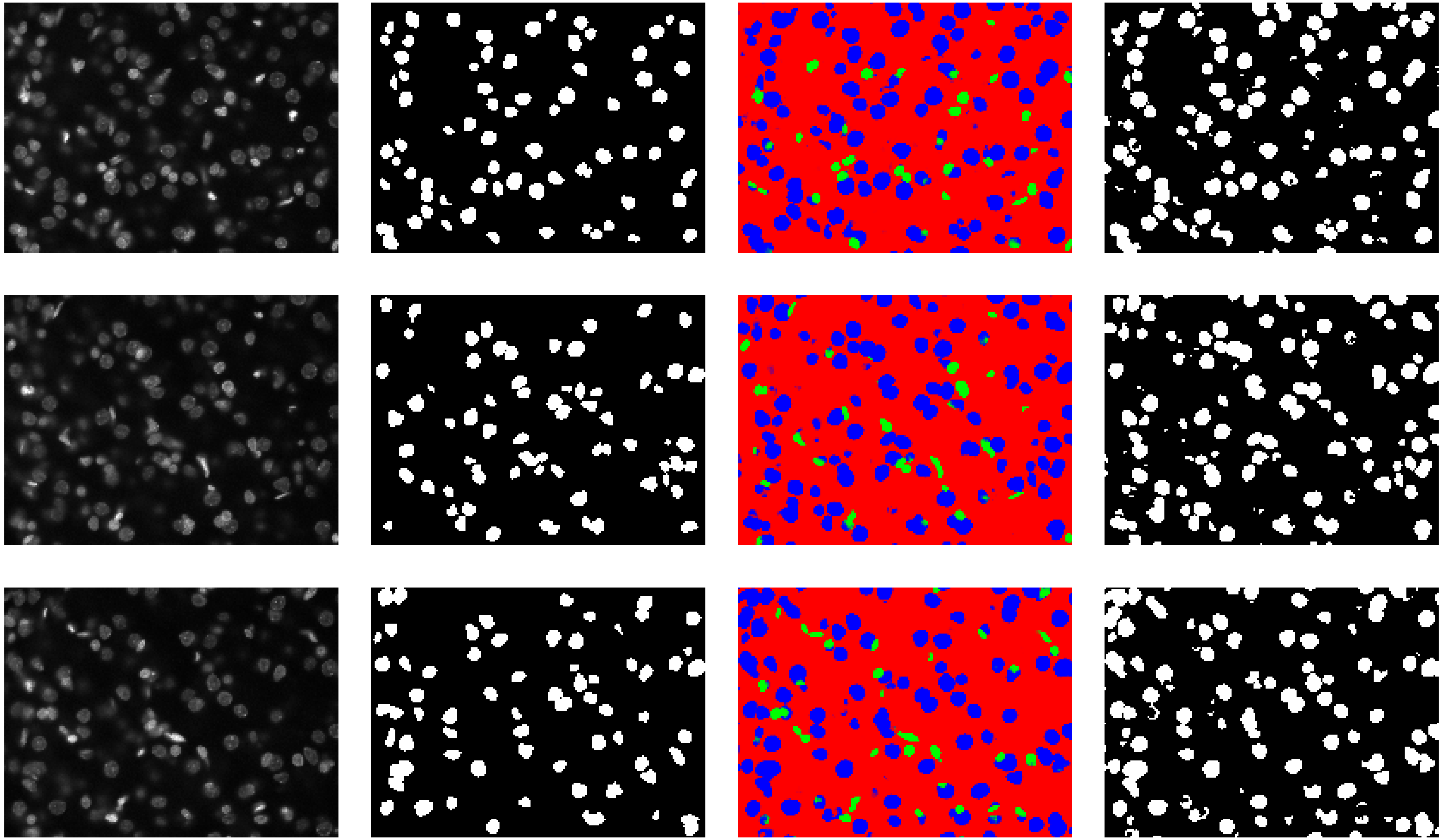
Procedure:

- We perform leave one image out cross validation (train: 14 images, test: 1 image)
- Initialize Model with parameters learned on BBBC039 dataset
- Train for 5 epochs
- Data Augmentation is used
  - Crops, flips, rotations, illumination changes
- Compute DICE score
  - Average across all 15 images



Improved Results

Original DAPI Image | Manually Annotated Ground Truth (Nuclei) | U-Net Predictions Background/Glial/Nuclei | Binarized U-Net Predictions Nuclei Class



$$DICE\ Score = \frac{2 \cdot TP}{2 \cdot TP + FP + FN}$$

Approach	DICE Score
Supervised Learning (Baseline)	0.710
Transfer Learning + Supervised Learning	0.733

Comparison of Our Approaches

Approach	Algorithm	Train Dataset	DICE Score
Automatic	Watershed	N/A	0.561
Transfer Learning	U-Net	Broad Institute Dataset (BBBC039)	0.655
Supervised Learning	U-Net	LL15	0.710
Transfer Learning + Supervised Learning	U-Net	Broad Institute Dataset (BBBC039) -> LL15	0.733
Transfer Learning	Mask R-CNN	Kaggle 2018 Science Bowl (BBBC038v1)	0.722

Summary and Future Work

- Automated, high-performance computing approaches are needed to map the brain connectivity
  - Deep learning on brain microscopy data has shown promise
- Human annotation is costly and requires domain expertise
- Develop scalable, learning-based methods
  - Transfer learning on nuclei segmentation shows promising results
  - Future work will make use of conventional image processing results as weakly annotated data
  - Extend to detecting additional classes (e.g., different cell types)