

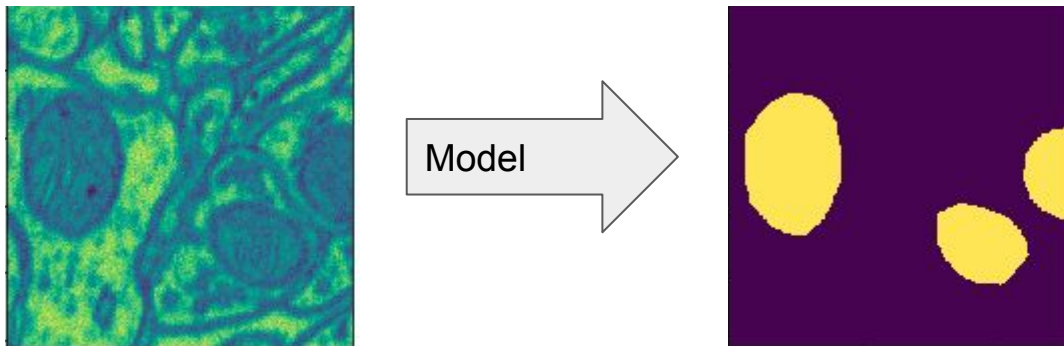
Segmenting Microscopy Images

Client Problem

- Segmenting images by hand is time consuming and laborious
- Microscopy data can be generated faster than it can be analyzed
- Segmentation requires years of experience

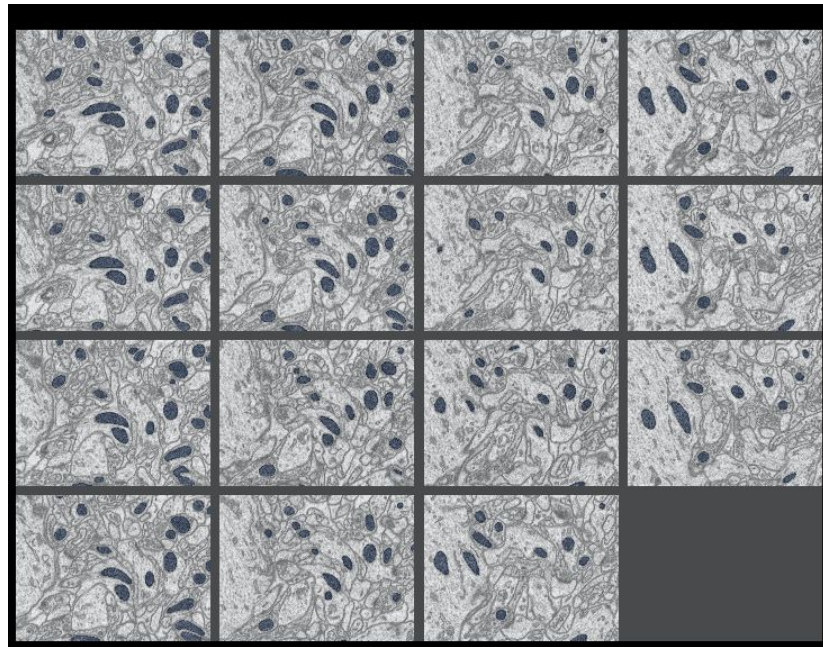
The Data Science Problem

- We would like to generate a binary image from an input image
- A pixel labeled '1' is in the category of interest
- A pixel labeled '0' is not in the category of interest



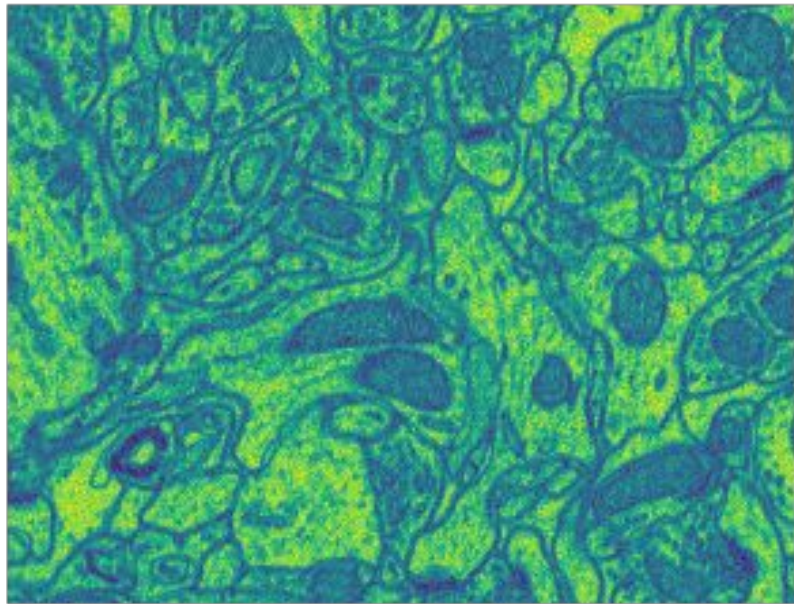
Data Wrangling

- Data took the form of four multi-frame tif files
- 165 images and ground-truths for the training set
- 165 images and ground-truths for the test set
- Both sets are from the same microscope run, but separated by thousands of image slices

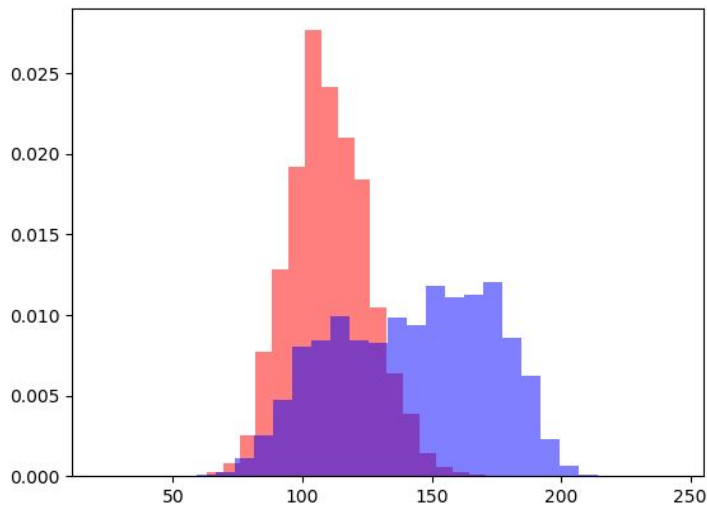


Exploratory Analysis

- Each voxel represents about 5 nm^3 of volume
- Large images, 768×1024 pixels
- On average, mitochondria take up about 13 million nm^3 or a cube about 48 pixels on a side



Exploratory Analysis



- The pixel intensity of mitochondria (red) is lower than on average than non-mitochondria (blue)
- There is a large overlap in intensity between the two regions
- Pixel intensity alone can't be used for segmentation, shape and pattern must also play a role

The Machine Learning Model - Convolutional Neural Network

- Convolutional Neural Nets work well for problems with short range order, like images
- A convolution operation acts on a set of nearby pixels with a filter to search for patterns.

| | | | | | | | |
|----|----|----|----|----|----|----|----|
| 1 | 25 | 23 | 21 | 25 | 23 | 5 | 1 |
| 6 | 25 | 0 | 0 | 0 | 0 | 25 | 6 |
| 25 | 0 | 84 | 82 | 86 | 82 | 0 | 22 |
| 0 | 81 | 0 | 86 | 81 | 0 | 83 | 0 |
| 0 | 81 | 82 | 81 | 79 | 89 | 84 | 0 |
| 24 | 0 | 83 | 0 | 0 | 82 | 0 | 1 |
| 6 | 25 | 0 | 81 | 81 | 0 | 26 | 25 |
| 1 | 6 | 26 | 0 | 0 | 25 | 6 | 6 |

The Machine Learning Model - Convolutional Neural Network

- This filter looks for dark diagonal lines

| | | |
|----|----|----|
| 1 | 1 | 1 |
| -1 | -1 | -1 |
| 1 | 1 | 1 |

| | | | | | | | |
|----|----|----|----|----|----|----|----|
| 1 | 25 | 23 | 21 | 25 | 23 | 5 | 1 |
| 6 | 25 | 0 | 0 | 0 | 0 | 25 | 6 |
| 25 | 0 | 84 | 82 | 86 | 82 | 0 | 22 |
| 0 | 81 | 0 | 86 | 81 | 0 | 83 | 0 |
| 0 | 81 | 82 | 81 | 79 | 89 | 84 | 0 |
| 24 | 0 | 83 | 0 | 0 | 82 | 0 | 1 |
| 6 | 25 | 0 | 81 | 81 | 0 | 26 | 25 |
| 1 | 6 | 26 | 0 | 0 | 25 | 6 | 6 |

The Machine Learning Model - Convolutional Neural Network

- This filter looks for dark diagonal lines

| | | |
|----|----|----|
| 1 | 1 | 1 |
| -3 | -3 | -3 |
| 1 | 1 | 1 |

- Multiplying this group of pixels gives -7, a low score

| | | | | | | | |
|----|----|----|----|----|----|----|----|
| 1 | 25 | 23 | 21 | 25 | 23 | 5 | 1 |
| 6 | 25 | 0 | 0 | 0 | 0 | 25 | 6 |
| 25 | 0 | 84 | 82 | 86 | 82 | 0 | 22 |
| 0 | 81 | 0 | 86 | 81 | 0 | 83 | 0 |
| 0 | 81 | 82 | 81 | 79 | 89 | 84 | 0 |
| 24 | 0 | 83 | 0 | 0 | 82 | 0 | 1 |
| 6 | 25 | 0 | 81 | 81 | 0 | 26 | 25 |
| 1 | 6 | 26 | 0 | 0 | 25 | 6 | 6 |

The Machine Learning Model - Convolutional Neural Network

- This filter looks for dark diagonal lines

| | | |
|----|----|----|
| 1 | 1 | 1 |
| -3 | -3 | -3 |
| 1 | 1 | 1 |

- Multiplying this group of pixels gives -321, a high score

| | | | | | | | |
|----|----|----|----|----|----|----|----|
| 1 | 25 | 23 | 21 | 25 | 23 | 5 | 1 |
| 6 | 25 | 0 | 0 | 0 | 0 | 25 | 6 |
| 25 | 0 | 84 | 82 | 86 | 82 | 0 | 22 |
| 0 | 81 | 0 | 86 | 81 | 0 | 83 | 0 |
| 0 | 81 | 82 | 81 | 79 | 89 | 84 | 0 |
| 24 | 0 | 83 | 0 | 0 | 82 | 0 | 1 |
| 6 | 25 | 0 | 81 | 81 | 0 | 26 | 25 |
| 1 | 6 | 26 | 0 | 0 | 25 | 6 | 6 |

The Machine Learning Model - Convolutional Neural Network

- This filter looks for dark diagonal lines

| | | |
|----|----|----|
| 1 | 1 | 1 |
| -3 | -3 | -3 |
| 1 | 1 | 1 |

- Each filter passes the entire image

| | | | | | | | |
|----|----|----|----|----|----|----|----|
| 1 | 25 | 23 | 21 | 25 | 23 | 5 | 1 |
| 6 | 25 | 0 | 0 | 0 | 0 | 25 | 6 |
| 25 | 0 | 84 | 82 | 86 | 82 | 0 | 22 |
| 0 | 81 | 0 | 86 | 81 | 0 | 83 | 0 |
| 0 | 81 | 82 | 81 | 79 | 89 | 84 | 0 |
| 24 | 0 | 83 | 0 | 0 | 82 | 0 | 1 |
| 6 | 25 | 0 | 81 | 81 | 0 | 26 | 25 |
| 1 | 6 | 26 | 0 | 0 | 25 | 6 | 6 |

The Machine Learning Model - Convolutional Neural Network

- This filter looks for dark diagonal lines

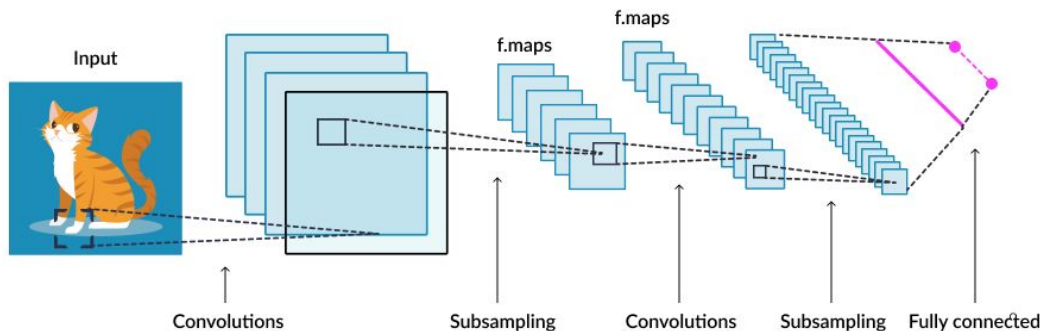
| | | |
|----|----|----|
| 1 | 1 | 1 |
| -3 | -3 | -3 |
| 1 | 1 | 1 |

- Each filter passes the entire image
- This picks out specific features in the image for each filter

| | | | | | | | |
|----|----|----|----|----|----|----|----|
| 1 | 25 | 23 | 21 | 25 | 23 | 5 | 1 |
| 6 | 25 | 0 | 0 | 0 | 0 | 25 | 6 |
| 25 | 0 | 84 | 82 | 86 | 82 | 0 | 22 |
| 0 | 81 | 0 | 86 | 81 | 0 | 83 | 0 |
| 0 | 81 | 82 | 81 | 79 | 89 | 84 | 0 |
| 24 | 0 | 83 | 0 | 0 | 82 | 0 | 1 |
| 6 | 25 | 0 | 81 | 81 | 0 | 26 | 25 |
| 1 | 6 | 26 | 0 | 0 | 25 | 6 | 6 |

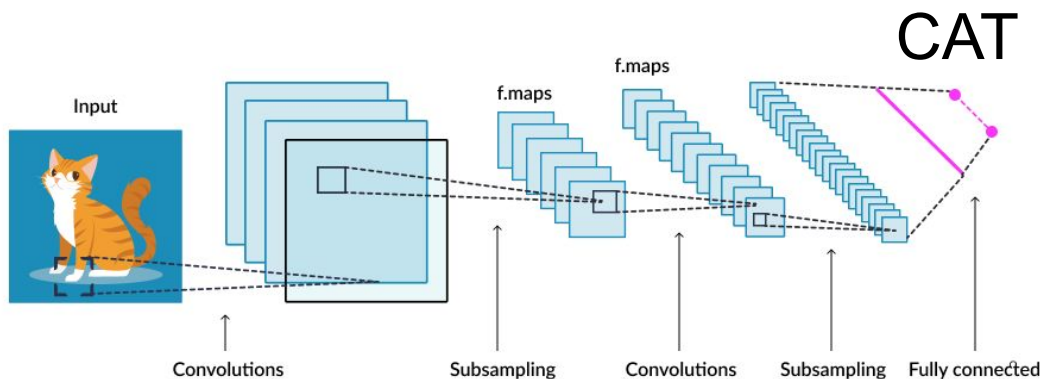
Convolutional Neural Nets

- Convolutional neural nets use a convolution operation instead of fully connected layers
- The weights that the model learns in this case are the values for the many filters involved in the convolutions
- Each layer of the net searches for more complicated patterns



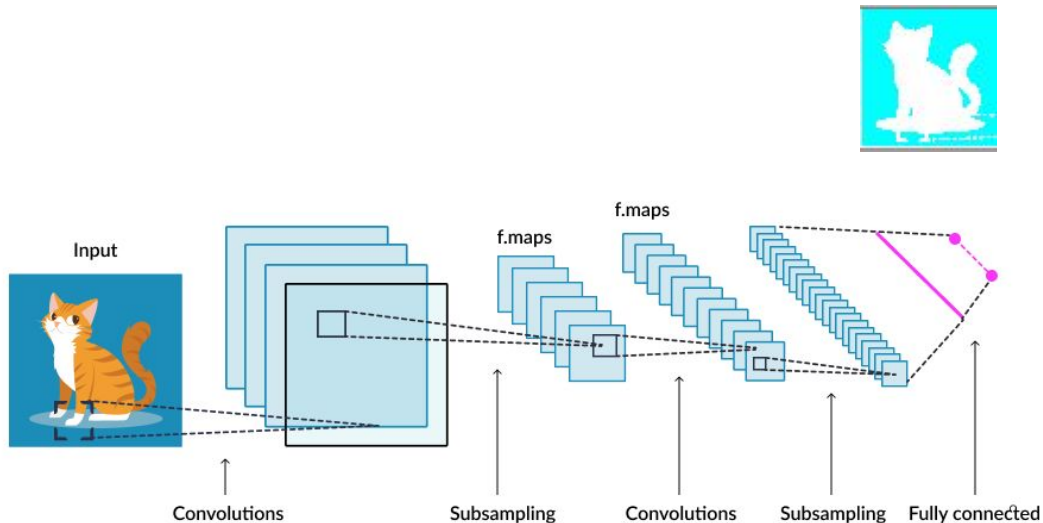
Convolutional Neural Nets

- Many CNN's will follow a pattern of convolution followed by downsampling, increasing the number of filters with each step
- This would end with a large number of filters representing a few pixels, with an output to a fully connected layer

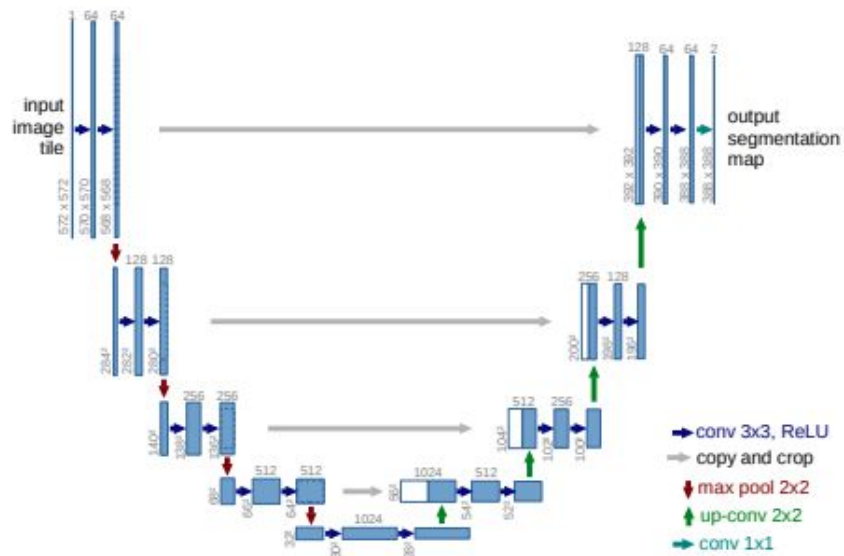


Convolutional Neural Nets

- Many CNN's will follow a pattern of convolution followed by downsampling, increasing the number of filters with each step
- This would end with a large number of filters representing a few pixels, with an output to a fully connected layer
- But we want the output to be a binarized image

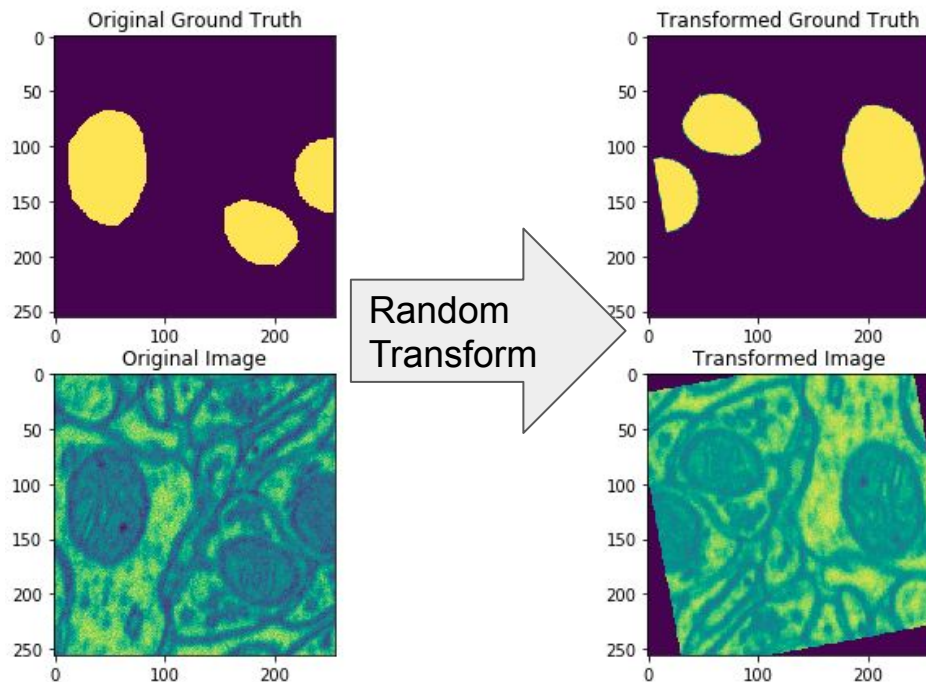


U-Net



- The solution is U-Net, which takes a the usual series of convolution and downsample operations, and then reverses them
- The upsampling layers concatenate the output of each downsampling layer is also concatenated with the input of an upsampling layer
- The final output sends each pixel to a sigmoid activation function so that the result is a segmentation map of the original resolution

Data Augmentation



- Another important technique used here is data augmentation
- A lot of times data is more scarce than what is necessary to get a good fit.
- We can create more data by applying random transformations such as rotation, flips, random noise, etc.
- In this case it is very important to transform the ground-truth images exactly the same way as the original image
- It improves both the training loss and leads to better performance on unseen data

Hyper-parameter tuning

- Neural Nets have an exceptional number of hyper-parameters
- The long training time can also mean that it is difficult to explore hyperparameters in a gridsearch fashion
- In the end, systematic searches of parameters must be combined with an understanding of different hyperparameter properties to reach an optimal solution

Evaluation: The Dice Coefficient

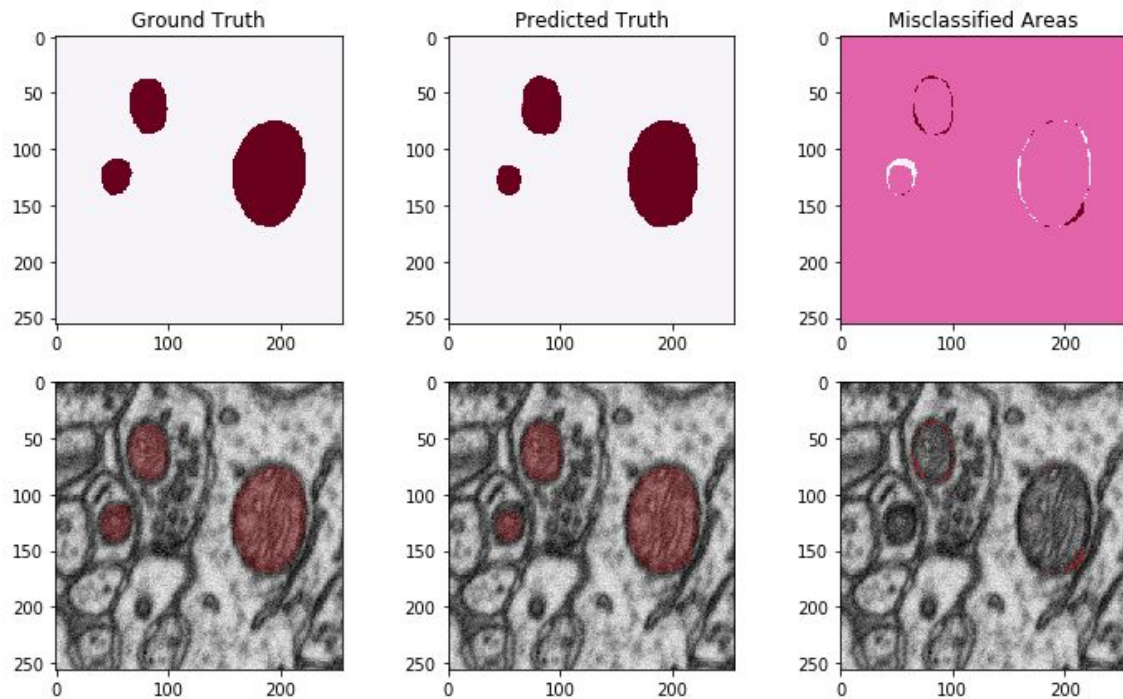
- The Dice coefficient measures the overlapping pixels between the predicted and true values, then divides by the total number of values that are true

$$\textit{Dice Coefficient} = \frac{2 \cdot \Sigma X \cap Y}{\Sigma X \cup Y}$$

- This is a common measure for how well two binary images match
- It lies between 0 and 1
- This can easily be turned into a loss function so that :

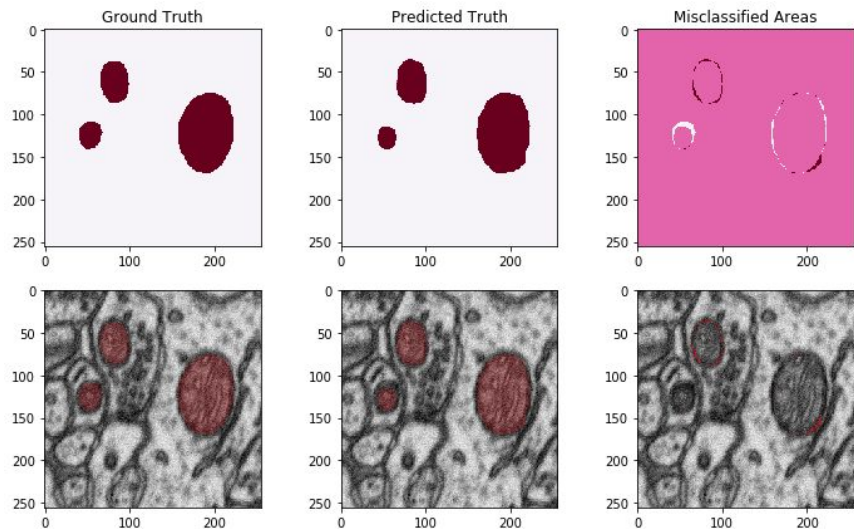
Dice Loss = $\log(1 - \text{D.C.})$

Results - Segmented Images



Dice Coefficient - 0.89

Results - Segmented Images

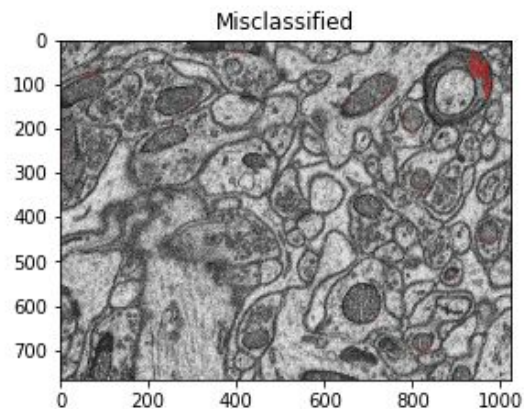
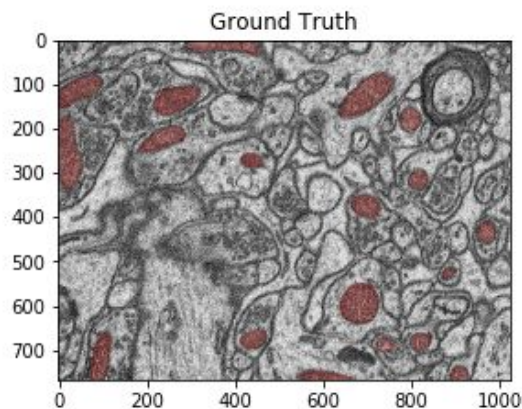
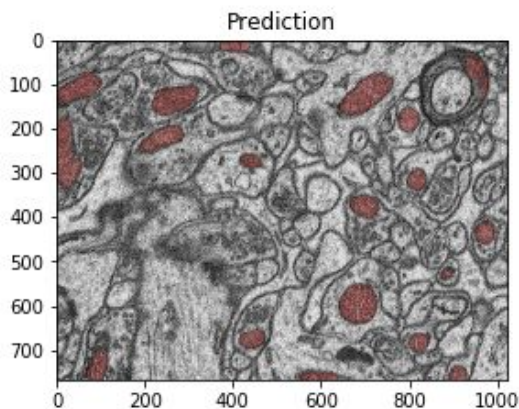


Dice Coefficient - 0.89

- After hyper-parameter tuning and data augmentation, a dice coefficient of 0.89 was achieved on the test set
- Inspection of various individual images demonstrate that edges are difficult to correctly categorize the very edge of mitochondria
- From a practical standpoint, this is not a huge issue, as it may not be important to outline the mitochondria perfectly as to correctly label as many mitochondria as possible

Results - Segmented Images

- This image was created by scanning a smaller window across the large image to generate many predicted values that were averaged together
- For this image the only major misclassification is in the upper right, the rest are on the edges of correctly classified mitochondria



Client Recommendations - Data acquisition

- Acquiring more data is essential to improve the model, as the current network was trained from only one microscope run
- Standard practices could be adopted to insure uniformity of images, for example standard magnification settings, or at least recording the magnification
- With proper data collection, the technique can easily be extended to any number of biological image segmentation tasks

Client Recommendations - Metrics and explanations for researchers

- The Dice coefficient is not particularly useful for deciding whether or not the algorithm produces false negatives or false positives
- A method of counting the number of mitochondria correctly identified
- Translate into precision and recall of individual mitochondria
- Particularly important when application tests for presence or absence of disease, ethical and legal concerns require a quantification of the error in the algorithm that fits in with standard practices

Client Recommendations - Metrics and explanations for researchers

- Applications may also require that researcher or doctor can ask to see how the algorithm arrived at its answers
- The filters in the net most responsible for the identification can be generated
- This would constitute a type of 'explainable AI'
- Significant investment in this aspect of the algorithm may be necessary to gain trust with doctors and researchers who are used to relying on their intuition

Future Work

- Three dimensional convolutions
 - Make use of truly 3D data
 - Would have less training examples, but may reveal better patterns in each example
- Focus on data augmentation
 - Not all transformations are independent
 - Some transformations clip pixels, and therefore some sections of the image are underrepresented
- More computing power
 - GPUs
 - Google Cloud
 - AWS
 - Microsoft Azure