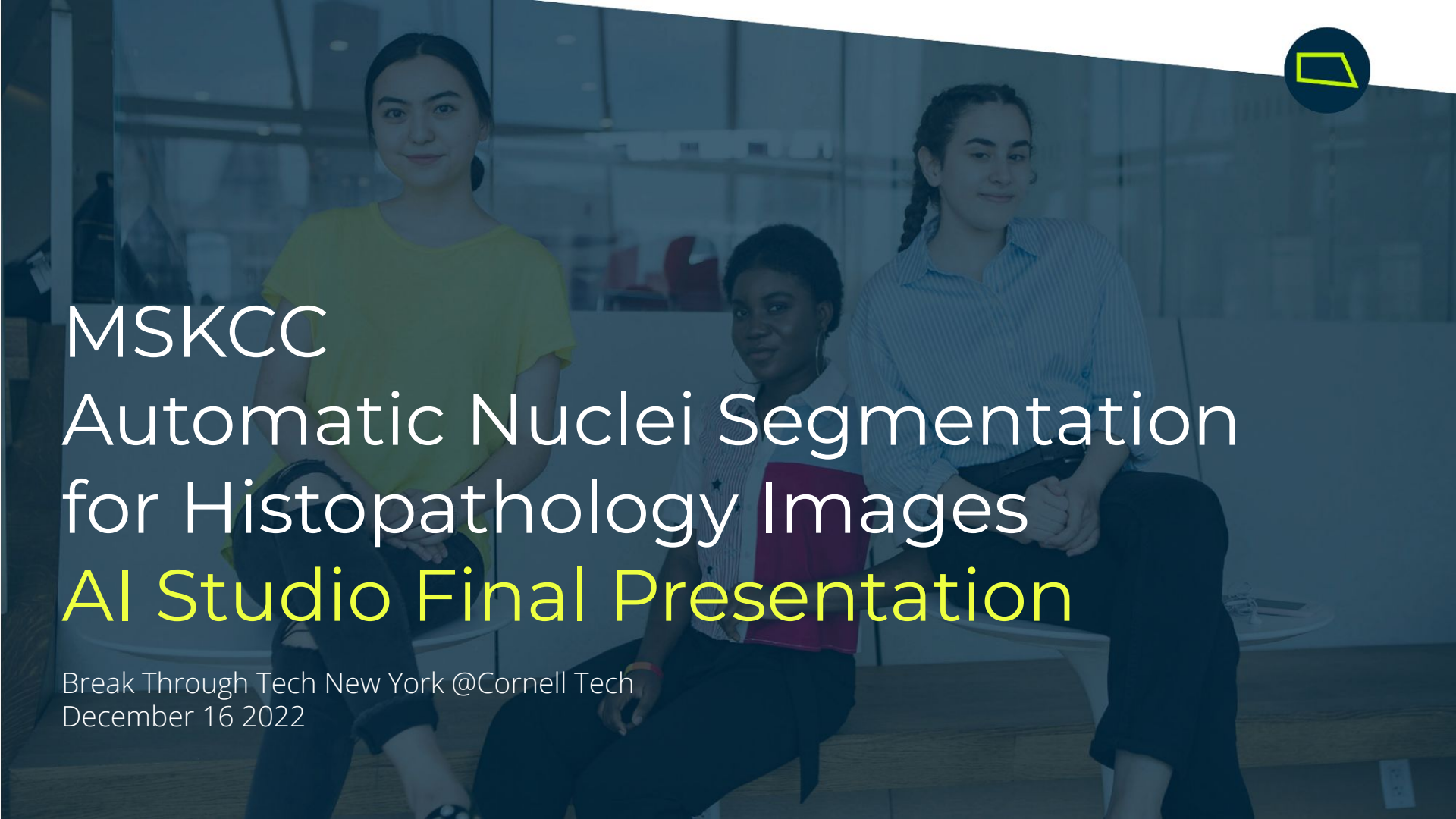

MSKCC
Automatic Nuclei Segmentation for Histopathology Images
AI Studio Final Presentation

Break Through Tech New York @Cornell Tech
December 16 2022

Introductions

Our Team

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Presentation Agenda

1. Introductions
2. Project Overview
3. Data Preprocessing
4. Model Selection and Evaluation
5. Final Thoughts

AI Studio Project Overview

Create a deep learning model that can accurately segment nuclei and separate overlapping or touching nuclei in H&E stained tissue sections

Our Goals

- Learn more about deep learning, computer vision, and image processing techniques
- Understand how to work with a small data set
- Differentiate between the various types of image segmentation
- Create a neural network that can accurately segment nuclei and separate overlapping and touching cells in H&E-stained tissue sections using Deep Learning.

Business Impact

- Accurately segmented nuclei provide important insights for pathologists:
  - Physical characteristics of nuclei (size, shape)
  - Spatial distribution
- Reduces need for manual annotations of nuclei
- Manual separation of images is tedious and time consuming
- Automated nuclei segmentation is important to gaining further insight into cell features and functionality

Our Approach

Step 1

Data Understanding and Choosing Augmentation Techniques

Clean + preprocess the images (normalization, tiling), and apply augmentation techniques

Step 2

Semantic Segmentation

Train a U-Net model to semantically segment nuclei and evaluate using Dice Score

Step 3

Instance Segmentation

Train a Mask RCNN model to segment the nuclei cells and identify overlapping cells. Evaluate with Jaccard Index.

Step 4

Improving our Models

Modifying our models to produce better evaluations(training for longer epochs, using more augmentation techniques)

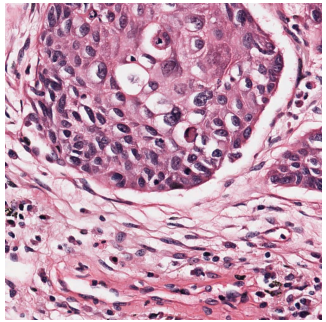
Resources We Leveraged

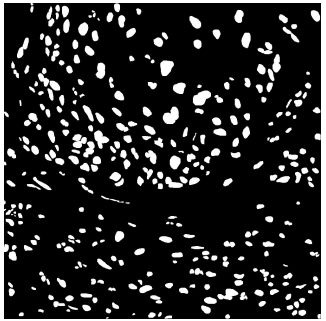
Data Preprocessing

The Dataset

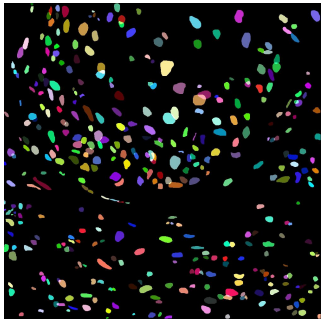
- **MoNuSeg dataset**
  - Public domain data
  - Obtained by annotating tissue images of several patients with tumors of different organs at multiple hospitals
  - Tissue images across 30 patients and 7 different organs
- 44 H&E stained tissue images, each 1000x1000
  - 30 for training, 14 for testing

H&E stained tissue

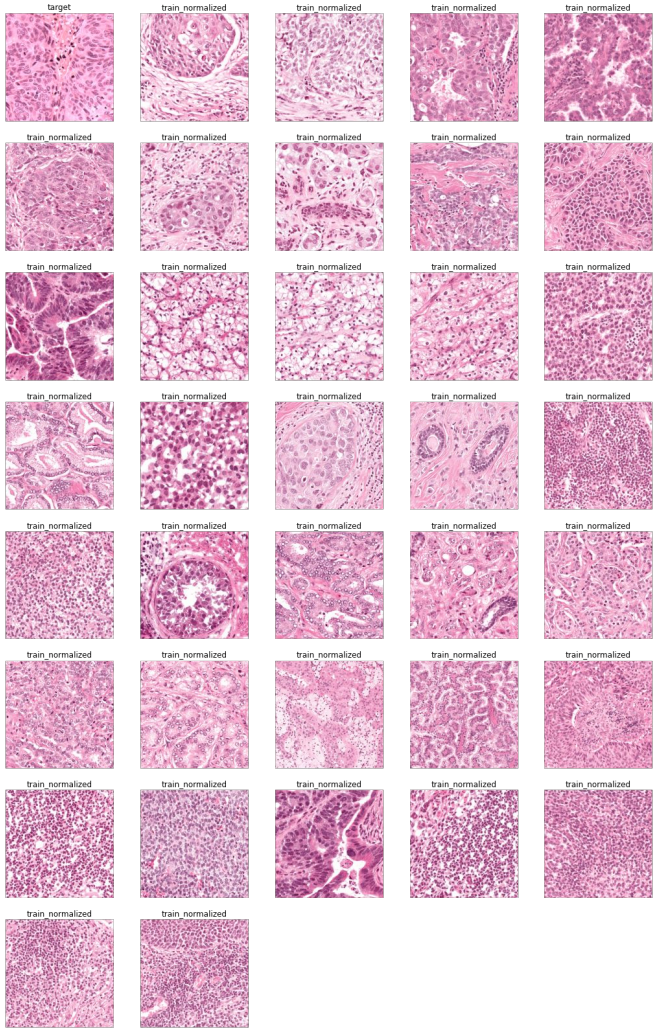




Ground-Truth Binary Mask



Ground-Truth Color Mask



Stain Normalization

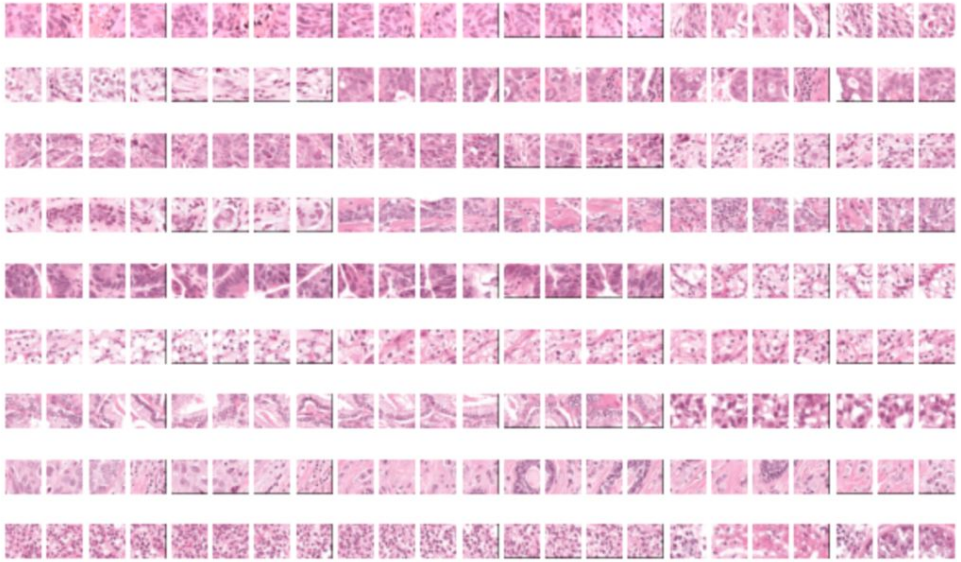
- Every image in the training and test dataset was normalized according to one random image from the dataset
  - Normalizes based on stain color and hue
  - Transforms all features to be on a similar scale
- Improves model training and performance
- Tools used: **StainTools** library (https://staintools.readthedocs.io/en/latest/)

Image Tiling

- Each 1000x1000 image + mask was tiled into 16 256x256 tiles
- Benefits of tiling:
  - Faster training and performance
  - Increases batch diversity
- Tools used: **tiler** library (https://pypi.org/project/tiler/)

▾ Tiling

```
[55] # tiling tissue images
     tile_dataset(data)
```

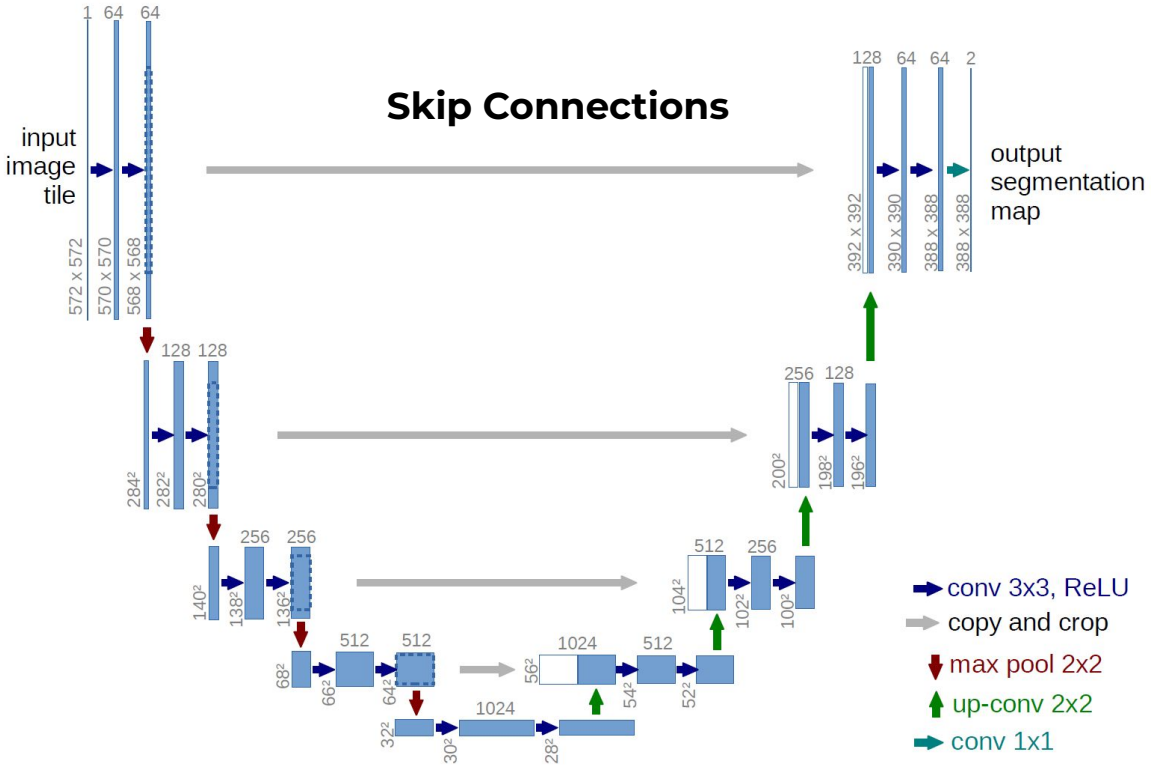


Preprocessing Object

```
[ ]  class PreProcessing:
       def __init__(self, path_to_training, path_to_testing):
         self.training_imgs_path = path_to_training
         self.testing_imgs_path = path_to_testing

         # names of images
         self.train_names = np.sort(np.array([file_name for file_name in listdir(self.training_imgs_path) if isfile(join(self.training_imgs_path, file_name))]))
         self.test_names = np.sort(np.array([file_name for file_name in listdir(self.testing_imgs_path) if isfile(join(self.testing_imgs_path, file_name))]))

         # normalize images
         self.training_imgs = []
         self.testing_imgs = []

         # normalized and tiled images
         self.training_imgs_tiled = []
         self.testing_imgs_tiled = []

         # normalized, tiled and augmented training images
         self.training_imgs_aug = []
```

Model Selection and Evaluation

U-Net Architecture

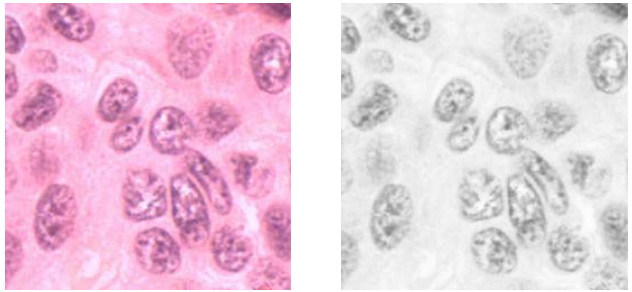


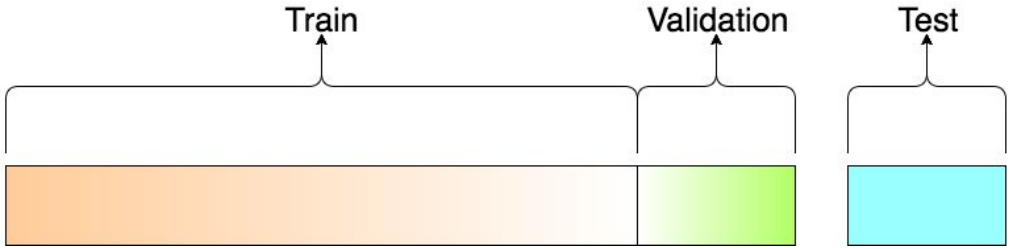
Example Code

```
#Contraction/Encoder path (going down the U) - this is made up of convolutional layers (c1, c2, etc)
# feature space = 16; apply 3x3 convolutions; padding = same to keep output width and height same as input
c1 = Conv2D(16, (3, 3), activation='relu', kernel_initializer='he_normal', padding='same')(s)
c1 = Dropout(0.1)(c1)
c1 = Conv2D(16, (3, 3), activation='relu', kernel_initializer='he_normal', padding='same')(c1)
p1 = MaxPooling2D((2, 2))(c1)
```

- Scaled down feature space dimensions by a factor of 8
- For the outputs, we used a sigmoid activation function to ensure that the mask pixels are in a [0, 1] range

Training Process





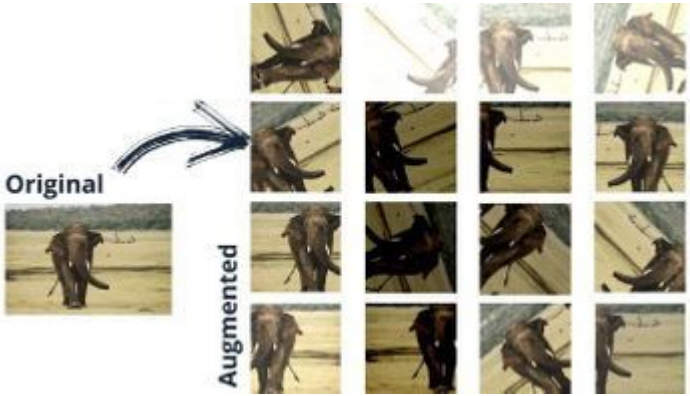
Stage 1: When we first started training the U-Net model, we converted the images to grayscale
Best Accuracy Score: ~20%

Stage 2: Added a validation set that was 10% of the training data

Best Accuracy Score: ~40%

Stage 3: Data Augmentation (on-the-fly)

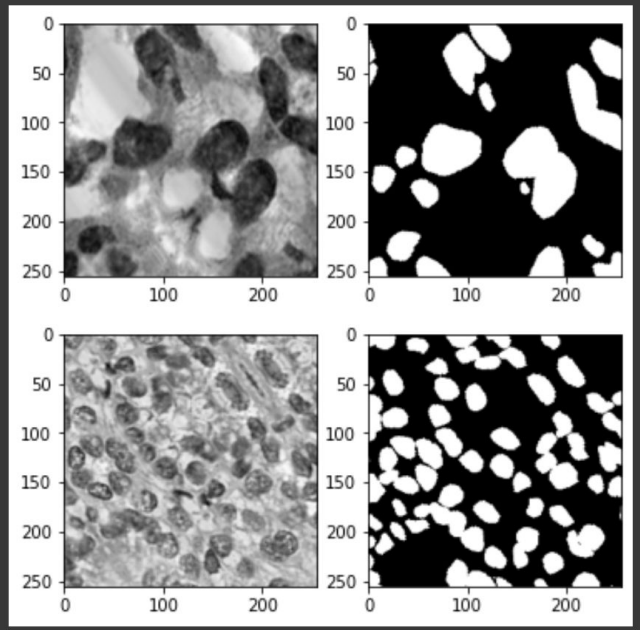
```
img_data_gen_args = dict(rotation_range=90,
                         width_shift_range=0.3,
                         height_shift_range=0.3,
                         zoom_range=0.3,
                         horizontal_flip=True,
                         vertical_flip=True,
                         fill_mode='reflect')
```



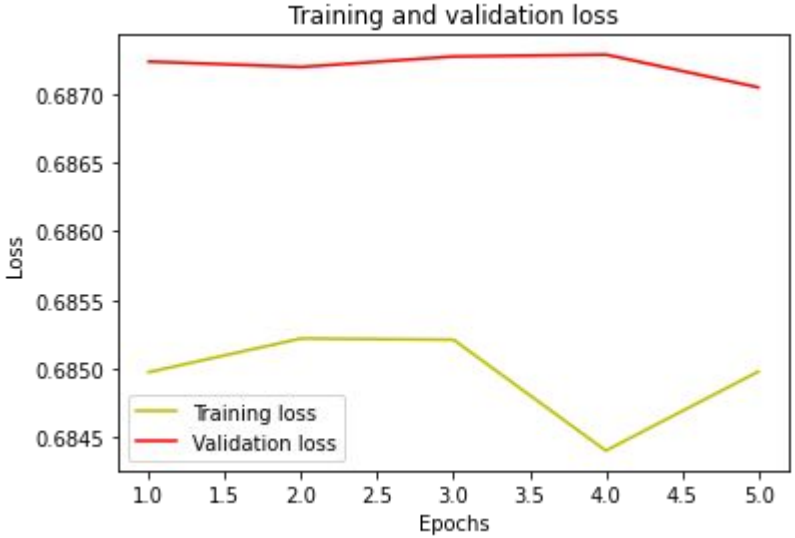
Stage 3: Data Augmentation increases the amount of data needed to train robust AI models. Applied to both images and masks.
Best Accuracy Score: ~60%

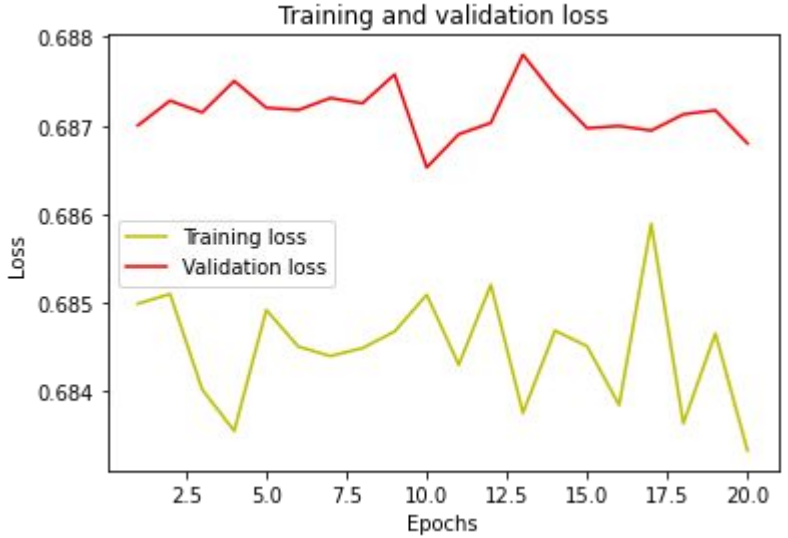
On-the-fly data augmentation via the keras Image Data Generator ensures that the augmented images are matched up with their corresponding masks

```
[57]  x = image_generator.next()
      y = mask_generator.next()
      for i in range(0,2):
          image = x[i]
          mask = y[i]
          plt.subplot(1,2,1)
          plt.imshow(image[:,:,0], cmap = 'gray')
          plt.subplot(1,2,2)
          plt.imshow(mask[:,:,0], cmap = 'gray')
          plt.show()
```



U-Net Results

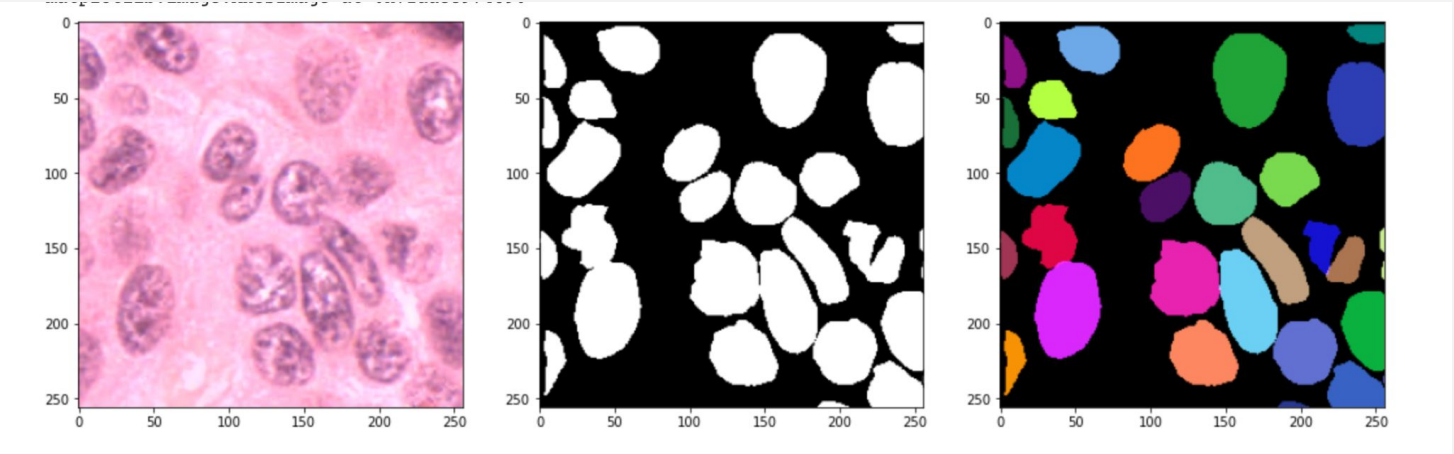




Stage 4: Fix mask and image tile error (some were not matched up)
Best Accuracy Score: ~75%

```
[64] loss = history.history['loss']
     val_loss = history.history['val_loss']
     epochs = range(1, len(loss) + 1)
     plt.plot(epochs, loss, 'y', label='Training loss')
     plt.plot(epochs, val_loss, 'r', label='Validation loss')
     plt.title('Training and validation loss')
     plt.xlabel('Epochs')
     plt.ylabel('Loss')
     plt.legend()
     plt.show()
```

Mask R-CNN for Instance Segmentation



Model Descriptions

Model Name	Description	Results	Why we chose it?
U-Net	**Semantic segmentation** - segments objects in image from background	A binary mask (black and white)	Gold standard for biomedical image segmentation problems
Mask R-CNN	**Instance segmentation** - combines image segmentation with object detection to segment objects from each other	A mask that shows each distinct nuclei cells	Proven to be a well performing model for instance segmentation

Final Thoughts

Obstacles

- We had to ensure that the dimensions of the input images for the U-Net matched up
- The mrcnn package for the Mask RCNN model is only compatible with a certain version of tensorflow which forced us to use virtual machines
- Using virtual machines on Google Cloud allowed us to run our U-Net model for more epochs to get a more accurate model

Insights and Key Findings

- On the fly augmentation removes the problem of having to match up masks and the predicted masks
- Converting the training images to grayscale may simplify computations and reduce time
    - However, training with RGB images provides information in all three channels and improves model performance
- The Dice Score and Jaccard Index are similar metrics for evaluating model performance, but the Dice Score provides more nuance in comparing pixel similarity

Potential Next Steps

- Get more training data
- Experiment with U-Net architecture
- Implement advanced techniques for hyperparameter tuning
    - Learning Rate Scheduler
- Watershed segmentation to improve accuracy


Questions?