This document describes how to run the colonycounting\_v2 code. If you run “example.m” it will run the entire pipeline on the example data.

This code was generated by Lauren in July 2018 as an update to Rohit’s colonycounting repo.

**Step 0: Data Organization and Analyzing Multiple Folders at Once**

The first thing to know is that the code was written so that it could run on multiple folders or a single folder. Each folder can contain raw images from more than one scan. The scans in a folder can have different sizes, channels, and alignments.

To analyze multiple folders at once, you must pass (to each of the three functions) a cell array of paths to the folders.

>> paths = {‘\*\*path\_to\_folder\*\*’, ‘’};

Alternatively, if you do not provide any input to the functions, MATLAB will assume the current working directory is the folder you wish to analyze.

**Step 1: Stitch the Scans**

1. Run the “stitch\_all\_scans” function.

>> colonycounting\_v2.stitch\_all\_scans(paths);

OR

>> colonycounting\_v2.stitch\_all\_scans;

1. You will be prompted to
2. You will be prompted to
3. You will be prompted to

**Step 2: Segment the Well and Colonies**

1. Run the “segment\_all\_scans” function.

>> colonycounting\_v2.segment\_all\_scans(paths);

OR

>> colonycounting\_v2.segment\_all\_scans;

1. A window will pop up with displaying the stitch of the first scan.
2. Outline the well.
   1. To add a segmentation click “Add” and draw the shape on the image. When you are done, double-click within that shape to save the shape. To add another segmentation, click “Add” again.
   2. To delete a segmentation click “Delete” and click within the shape you want to remove. To add another segmentation, click “Delete” again.
   3. Click “Done” to when you are finished.
3. Outline the colonies.
   1. Repeat 3a – 3c.
4. Repeat steps 2 – 4 for the remaining scans.

NOTES:

* If there is only one channel in the scan, that channel will be used. If there is more than one channel, the code uses the dapi channel.
* The code should allow you to be able to re-run the analysis without re-doing the work. For example, imagine that the first time you segmented the colonies you accidentally included an extra colony in just 1 of the scans. If you re-run that step, the colonies you originally segmented will be displayed and you can edit them only as needed.

**Step 3: Count the Cells**

>> colonycounting\_v2.count\_cells\_all\_scans(paths);

OR

>> colonycounting\_v2. count\_cells\_all\_scans;

DOES IT WORK FOR MULTIPLE MAGNIFICATIONS??

SAVE SCALE FACTOR

**How Does the Stitching Work?**

The code to generate the stitched images is a little nuanced. Hopefully, this description of how it works will save someone the headache in the future.

If we look at the stitched scan made by Metamorph, we can see that there is overlap between the columns and the rows. Further, both overlaps have both an x and a y component to them. It is hard to see the y component of the column overlap and the x component of the row overlap in these images, but they do exist (and treating them as non-zero makes the stitched image better).

We need to figure out how much to shift each column (in x and y) and each row (in x and y). In order to properly arrange the images each pixel will need to be shifted once for the column row and once for the row shift. Each of those shifts will involve an x and y component.