Gazi Inkiyad

BME 310 – BC

April 21, 2019

Module 5 – Imaging (Matlab)

Introduction:

The goal of this experiment was to quantitatively count and analyze signals from human mesenchymal stem cells (hMSCs), endothelial cells and stained cells from hemocytometer using MATLAB. Using the data one can quantitatively analyze cell counts, morphology and viability counts.

Materials:

- Image of mesenchymal stem cells (hMSCs)
- Image of endothelial cells
- MATLAB
- Image of stained cells from hemocytometer

Methods:

Filtering Noise: hMSCs and endothelial cells images' contrast was adjusted by histogram equalization using adapthisteq and histeq functions [1]. hMSCs was separated to blue signal (nucleus) and green signal (cell body). To further differentiate cell nucleus for hMSCs and, cell body for both cell types contrasted image was subtracted by blurred image. Blurred image was created by imfilter function.

Binarizing: All images including hemocytometer images were then binarized by using imbinarize function with adaptive and numerical thresholding options [1]. Then additional small spots were removed by bwareaopen function [1]. Cells from hemocytometer were first converted to grayscale and isolated from the background grid by binarizing using numerical thresholding options. To normalize fuzzy dots around cell body imfill, imclose and imopen functions with strel morphological option [1]. For cells in hemocytemeter imerode and imdilate options were used to differentiate dead and alive cells [1].

Masking: hMSCs and endothelial cells were first separated from each other using morphological watershed method. Watershed method is an object separation technique that uses analogy of peaks and valleys. In this case, peaks and valleys were created by using the perimeter and the center of the cells in binary image [1].

Counting and signals: All cells were counted from their masks using bwlabel function. Signals were added by sum function and average was found from quotient of total signal and total number of cells. Standard deviation of signal was estimated by using std function. First, standard deviation was found per pixel of the masked area, then standard deviation was multiplied by ratio between total number of pixels from the masked area and total number of cells.

$$Avg_{\,signal\ per\ cell} = \frac{Total\ Signal}{Number\ of cells}$$

$$Std_{\,signal\ per\ cell} = \frac{Total\ number\ of\ pixels\ in\ mask}{Number\ of\ cells} Std_{\,signal\ per\ pixel}$$

Morphological analysis: Morphology of endothelial cells were analyzed by finding area, perimeter, major and minor axes. Morphological data was obtained using regionprops function.

$$Circularity = \frac{4\pi Area}{(perimeter)^2}$$

$$Aspect\ Ratio = \frac{major\ axis}{minor\ axis}$$

Viability: Viability for the hemocytometer cells were found by separating dead and alive cells. Dead cells did not have a hole which allowed imerode function to separate dead from alive. Then viability % was computed by subtracting dead cell % from 100%.

$$Viability\ \% = 100\ (1 - \frac{dead\ cell\ count}{total\ cell\ count})$$

Results:

hMSCs: Total cell count using the algorithm and manual count were 189 and 191 respectively. The average intensity per cell was 6.22E+05. Standard deviation of intensity per pixel in mask was 43.38 and standard deviation of intensity per cell was 1.69E+05. The coefficient of variability was 0.27.

Endothelial Cells: Total cell count using the algorithm and manual count were 161 and 166 respectively. The average intensity per cell was 3.55E+05. Standard deviation of intensity per pixel in mask was 22.74 and standard deviation of intensity per cell was 7.63E+04. The coefficient of variability was 0.21. Average circularity was 0.68 with a standard deviation of 0.10 and average aspect ratio was 1.48 with a standard deviation

of 0.38. Figure 2 illustrates the cells with outline and figure 3 illustrates a bar graph of morphological stats.

Hemocytometer cells: Using MATLAB algorithm total cell count from full, large and semi grids were 28, 29 and 34. Total dead cell count from full, large and semi grids were 9, 14 and 14. Lastly, cell viability from full, large and semi grids were 67.86%, 51.72% and 58.82%. Manual total cell count from full, large and semi grids were 28, 30 and 33. Manual total dead cell count from full, large and semi grids were 9, 14 and 15. Cell viability using manual technique from full, large and semi grids were 67.86%, 53.33% and 60.60%.

Figures and Tables:

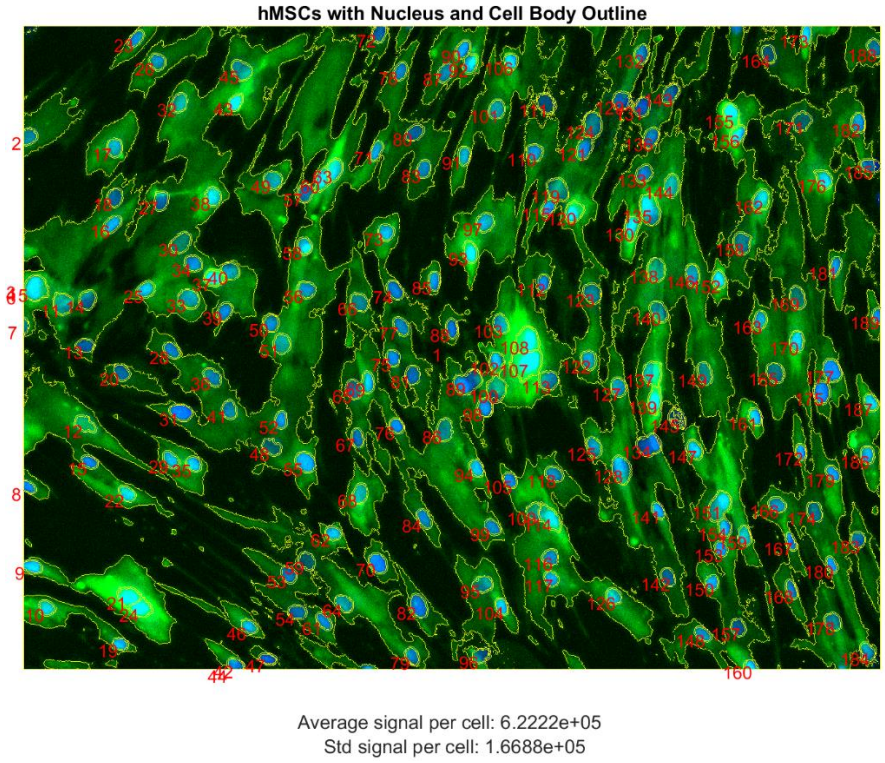

Figure 1: hMSCs image analysis with cell numbers.

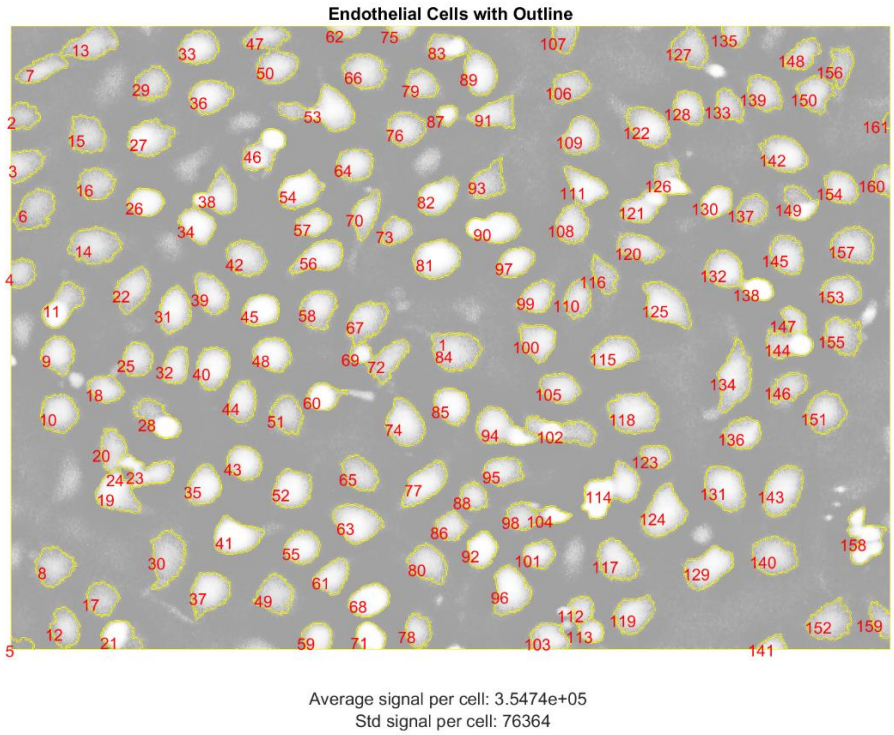

Figure 2: Endothelial cells image analysis with cell numbers.

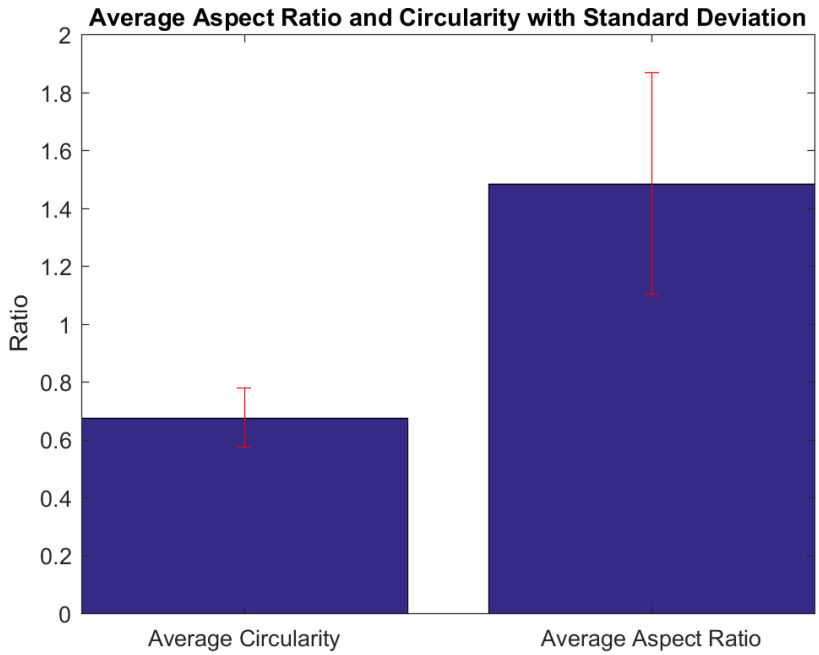

Figure 3: Morphological analysis of endothelial cells from figure 2.

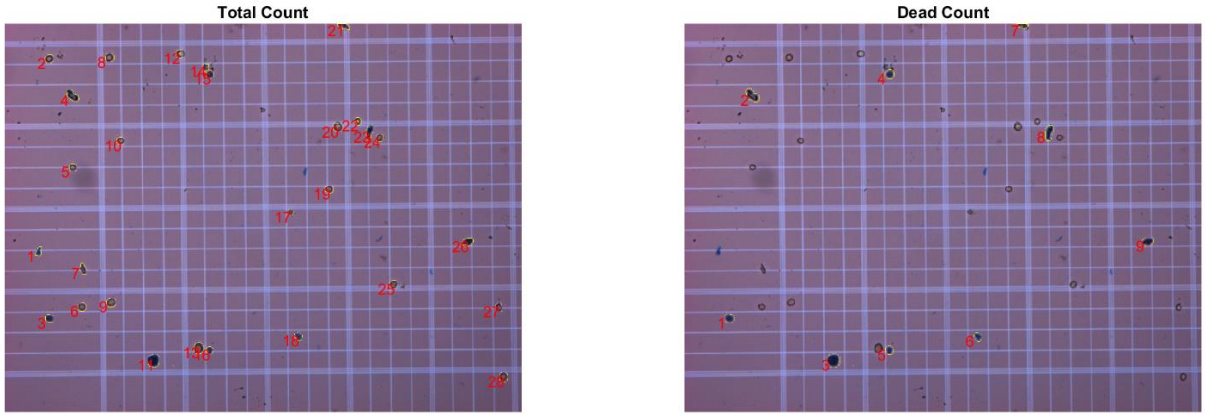

Figure 4: Total and dead cell count from hemocytometer image with full grid. Red markers indicate counted cells.

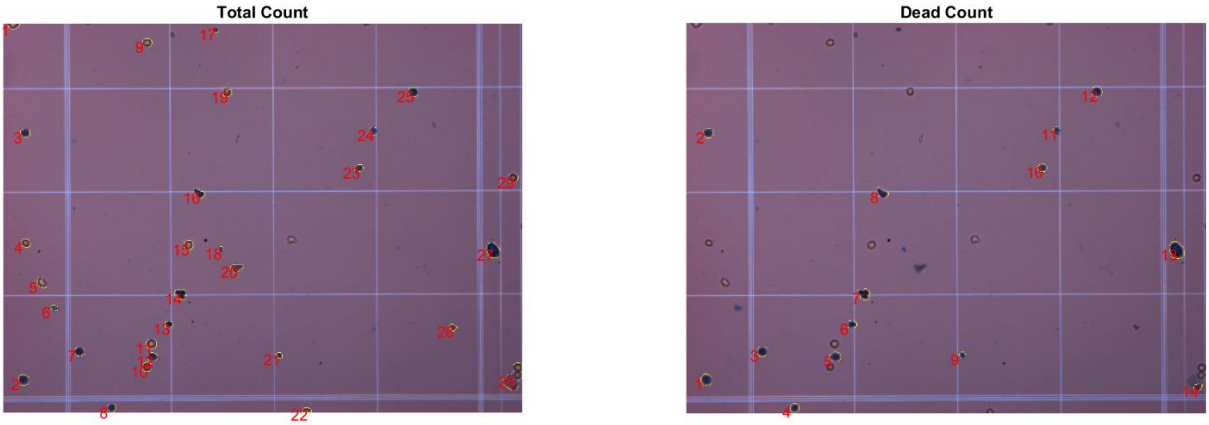

Figure 5: Total and dead cell count from hemocytometer image with large grid. Red markers indicate counted cells.

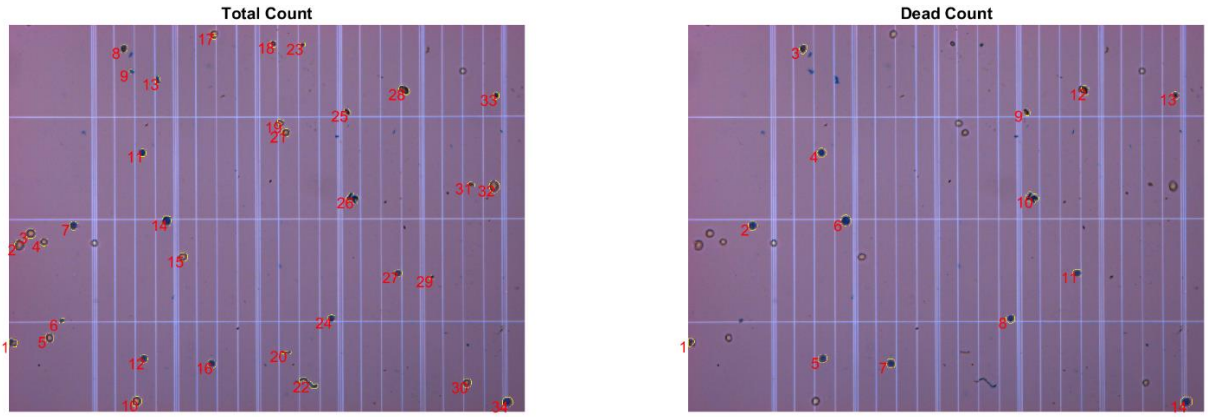

Figure 6: Total and dead cell count from hemocytometer image with semi grid. Red markers indicate counted cells.

Table 1: Cell viability analysis using MATLAB.

Grid	Total Count	Dead Count	Viability %
Full	28	9	67.86
Large	29	14	51.72
Semi	34	14	58.82

Discussion:

Overall, the algorithm for all three types of cell image analysis were accurate. For the hMSCs image analysis only two nuclei were mistaken as a single nucleus twice. According to figure 1 cell number 89 and cell number 134 consist of two nuclei. Hence cell count for hMSCs image was off by 2. Additionally, the algorithm did not pick some of the cell body signal due to higher binarizing threshold.

For the endothelial cells the cell count was off by 5 cells after utilization of histogram equalization function. Without equalization, total cell count was about 147 but after equalization effect total cell count was 161. The cells were also somewhat circular

with average circularity of 0.676 with a standard deviation of 0.1. Under static condition endothelial cells usually have a circularity value around 0.8 [2]. The cells were more circular compared to hMSCs from imageJ. Average circularity for hMSCs was 0.48 with a standard deviation of 0.12.

Lastly, the MATLAB algorithm gave an accurate viability for three types of grid. Full grid had identical cell viability and other grids had <2% difference in cell viability between automated and manual counts. Machine learning algorithms can be used to further improve the automated algorithm to differentiate cells.

Reference:

1. Image Filtering and Enhancement. (n.d.). Retrieved from https://www.mathworks.com/help/images/image-enhancement-and-restoration.html

2. Ye, M., Sanchez, H. M., Hultz, M., Yang, Z., Bogorad, M., Wong, A. D., & Searson, P. C. (2014). Brain microvascular endothelial cells resist elongation due to curvature and shear stress. Scientific Reports, 4(1). doi:10.1038/srep04681