**MATLAB For Scientists**

**HW8**

**Instructions**

* Complete each question below in an **individual** script or function. Each individual script should be named according to: HW#Q#\_script.m, or HW#Q#\_fun.m where <#> indicates the corresponding HW number or question. These are the scripts that will be considered for grading.
* Submit all files (**including** the provided data, but **excluding** any tables/files that are saved as a result of the HW question) as a compressed .zip folder with the name **<HW#\_YourCodeName.zip>.**
* Each script **must** run entirely through without error. If you did not finish part of a question, comment it out so it runs.
* Suppress all intermediate outputs other than your answer to the question; only the answer should display on the command line.
* Absolutely no hard coding beyond the minimum specified.
* **All plotting should now conform to our guidelines of publication quality figures**
* **Everything must be commented. Uncommented codes get zero credit!**

**Problems**

**Question 1 (4 pt)**

High pixel intensity corresponds to areas within the image of high bacterial growth. Clearly, however, pixel intensity data is noisy. To identify trends in noisy data, moving averages are often performed in complex algorithms to smooth curves, which reduces noise and amplifies signal. The function CalculateSlidingAvg.m has been provided for you, which takes the following syntax: svec = CalculateSlidingAvg(vec,n), where vec is the vector you are looking to smooth, n is the width of the moving average window, and svec is the output which is a smoothed vector. Use the grayscale still frame called HW8Q1\_gray.tif to write a script that does the following:

1. Defines a vector vecX that corresponds to the pixel intensity across the horizontal midpoint of the image and plot it as a function of distance (e.g., the x coordinates where x = 0 corresponds to the left-most pixel).
2. Smooths vecX by looping through sliding windows of 2, and up to 65, at increments of 5, and make a new figure that contains all smoothed vectors as a function of distance, including the original vector.
3. Plots the vector that you decide (from manually inspecting the results of (b)) best smooths the data such that the regions of the chamber are most clearly defined on a new figure.
4. Marks the two boundaries of the growth chamber by placing markers at each index on the figure generated in (c). Hint: This can be done in many ways that we have learned, such as diff(x).

**Question 2 (5 pt)**

Using the RGB image called HW8Q2\_rgb.tif, write a script to do the following:

1. Visualize the image using imshow

Ideally, intensity outside the growth chamber should be 0, since signal here represents fluorescence that is noise or coming from a different chamber, and not from the cells themselves. Clearly, in this image, background is too high. Removing this is called background subtraction.

1. Background subtracts by (1) converting the image to grayscale, (2) calculating the background using a representative region on the image and subtract this value from the entire grayscale image, and (3) contrast-adjusting the image with one of the functions we learned.
2. Plots a 1x3 subplot with the original image on the left, the background-subtracted image in the center, and the grayscale contrast-adjusted image on the right. Hint: Convert the background into uint8 to keep units consistent.
3. Generates and plots a mask for the chamber in the image using the equation of a circle: (x - centerX).^2 + (y - centerY).^2 = r^2. Assume centerX = centerY = 512, and r = 400, where x and y are pixel indices (how would you determine the center and radius without hard-coding?). The mask of the image should consist of the value 1 for any pixel that is located within the circle (less than r2), and 0 for any pixel located outside of the circle (greater than r2) equals 0. To plot the mask, it must be converted to uint8.
4. Plots a new figure of the contrast-adjusted image that is filtered by this mask.
5. Calculates and reports the average intensity of the background-subtracted image inside of the masked region in a complete sentence.

**Question 3 (5 pt)**

Choose one of the video files you were assigned and do the following:

1. Import the video and loop through the time points starting at the first one, to the 100th one, at increments of 20 (if images are taken 5 minutes apart, and image 1 is at time 0, how many hours is the 100th image?). Show the image at each of these time points using a subplot.

Hint: dvread outputs a cell where each element is an individual uint16 image at one time point. To visualize, this must be converted into uint8.

1. Define a mask to hide everything outside of the microchamber based on a frame at a sufficiently late time point when the chamber is full. Use this mask to filter the 1st time frame (corresponding to time = 0). Calculate the total intensity of cells at time = 0 and report it in a complete sentence.
2. Use imbinarize to binarize the filtered image at time = 0. Calculate the total number of pixels at this time point and report it in a complete sentence. Note: fluorescence intensity and pixel number are both examples of different ways to define cell density. Can you think of any others?