Homework Set 3

Due by 12:00 noon on Friday, Nov. 3, 2006

This is a hybrid lab/homework set. It will count as a homework, and be graded accordingly, but most of the work should be done in the lab. Feel free to work outside the lab, if you have access to MATLAB with the image processing toolbox. However, you will find it useful to get help and advice from instructors. ***Definitely don't put off working on this until the night before – you will not be able to finish.***

1   Objectives

1. Learn to use MATLAB to manipulate image files in a variety of formats, sizes, and resolutions.

2. Explore several different image processing techniques, and learn to improve images with them.

3. Extract quantitative data from images.

4. Prepare some analysis/processing algorithms for future experimental modules.

2   Image Files

All the image files you will use in this lab can be found on the lab PCs in the `C:\BioInst\imgproc` directory. They are of different sizes, file formats, and aspect ratios. Here we will only work with grayscale files (one channel of intensity data), but the techniques generalize for working with color (three or four channels).

3   Guidelines and Hints for Image Processing in MATLAB

As usual in MATLAB, your best resource is its Help function. Specifically for this lab, you'll want to start by opening Matlab Help (Help → Matlab Help), and browsing through the Image Processing Toolbox help files.

3.1   Image Input/Output and Manipulation

Below are a some commands for opening, displaying, and writing image files, for which Help will provide the details. Pay attention to the numerical data type of these images when MATLAB imports them (the "workspace" window will be key here) – you may need to inter-convert between types.

`imread` is used to load image data from a file. The basic syntax is `A = imread('filename')`, with the image data from the file ending up stored in matrix `A`.

`imshow` displays the data of an image file in a MATLAB figure window. You can do it either without loading it into the MATLAB workspace, or when it's present in the workspace and assigned to a variable.

`imwrite` writes image data to a file, in a format that you specify. The basic syntax is `imwrite(A,'filename')`.

`aviinfo` returns the header information about a movie file in .avi format

`aviread('filename', 1)` reads one frame of the avifile into a Matlab movie structure. If `f` is the movie structure, you can assign the image data of that frame to a matrix `A` using the command `A = f.cdata`.

3.2   Other Useful Commands

The details of how these work will be important for you to explore on your own, with the help of MATLABand the instructors. Be aware that the list below is by no means exhaustive or exclusive – you may not use every command on the list, and you should explore other commands you find to be relevant that may not be listed here.

```
fft, fftshift, imhist, histeq, imadjust, conv, conv2
```

4   Image Processing Problems

1. Optimize the contrast for visualization of the microscope image on the left below, (`dark_actin.jpg`) taken at too low a light level.


2. Create a 3×3 and a 5×5 low pass filter. Apply these filters to remove noise from the cell image on the right above (`noisy_actin.tif`). Can you make a 7×7 filter also?

3. Generating a long integration image using a CCD camera sometimes produces large intensity spikes due to cosmic rays. Try to remove the noise from the image `spiky_actin1.tif`. Can you develop a different method to remove the spikes if you have two pictures taken one after the other (`spiky_actin1.tif`, `spiky_actin2.tif`)?
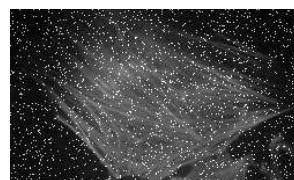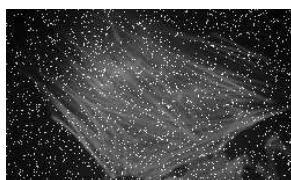

4. Perform a segmentation to isolate the region corresponding to the actin stress fibers in the image `actin_f.tif`. Also perform a segmentation to isolate the region corresponding to clustered actin in a cell with a chemically disrupted cytoskeleton `actin_treated.tif`. The output of both tasks should be binary images corresponding to the desired regions.

    (*BONUS*: can you devise a quantitative metric to quantify the degree of fiber-ness of the cytoskeleton?)
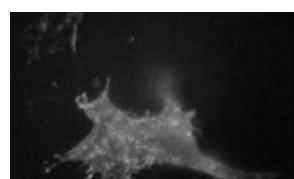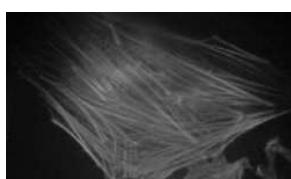
5. You have two images of a "test pattern" consisting of light and dark line pairs with a spacing of 18 line-pairs per millimeter (`HiMag.bmp` and `LowMag.bmp`). This test pattern was imaged with a home-built microscope at two different magnifications. Calculate the magnification difference between these two images.
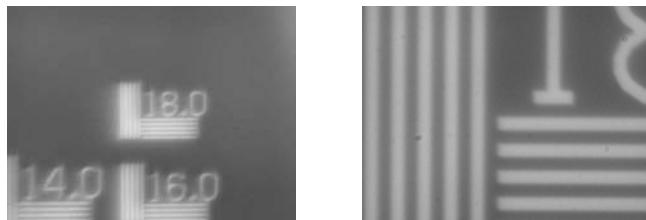
6. Periodic noise often occurs in biological imaging — `high_noise.tif` (below, left) & `low_noise.tif` (below, center). Can you remove the noise if you know the (normalized) noise characteristics (`noise.tif` - below, right)?
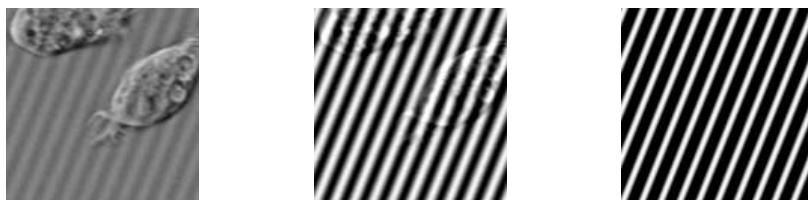
Consider a case in which you do not know the noise characteristics. Can you clean up the image below (`ecoli_noisy.jpg`)? (For bonus credit, feel free to work on any AFM images of your own that you'd like to similarly clean up.)
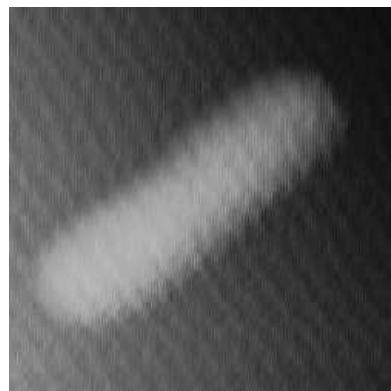
7. You are given a movie file (`beadtest.avi`) of a fluorescent bead in the cytoskeleton of the cell. Choose any bead in the image. Can you find the centroid of the bead as a function of frame number?

Note: If you are having trouble opening the bead movies on your home computer and need a codec, it can be downloaded from the LEAD Multimedia Website (http://www.leadcodecs.com/Download/eval.htm). Select the "LEAD MCMP/MJPEG Video Codec" on the left column and install with the evaluation version.