1. Introduction to Image Processing
ImageJ and Plugins: Custom plugins: Fiji: recording Macros

This course will mostly use ImageJ/Fiji because of its ease of use. (I may include a look at MatLab (with the DipImage GUI) and several exercises using it if time permits).

You will need to download a copy on your own computer.

1. Installing ImageJ

(a) ImageJ is distributed as a portable application. That means that you do not have to run an installer; just download, unpack and start it.

Google <ImageJ > and choose the first link to get to
https://imagej.nih.gov/ij/index.html

Have a look at Documentation/Introduction and Introduction.

Click Download at the top. If you have Java pre-installed on your computer download the platform independent zip file (ij152.zip) and then unzip it. (If you don't have Java, choose a download with Java bundled (for Mac or Windows)).

Look at the organization of the sub-directories. Make a shortcut.

Problems: imagej.net may be useful.

(Memory

Use the Edit>Options>Memory command to make more than the default 640MB available to ImageJ. Note that setting the "Maximum Memory" value to more than about 75% of real RAM may result in poor performance due to virtual memory "thrashing". The Edit>Options>Memory command modifies the third line in the ImageJ.cfg file in the ImageJ folder, which must be writable. This is what ImageJ.cfg looks like with "Maximum Memory" set to 1500MB:

```
jre\bin\javaw.exe
-Xmx1500m -cp ij.jar ij.ImageJ
```

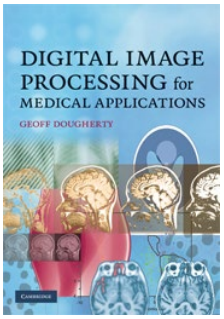
You may get an error message that looks something like this:

```
Unable to update the file "ImageJ.cfg
C:\Program Files\ImageJ\ImageJ.cfg (Access denied)
```

This error is the result of not have write access to the folder containing ImageJ. You need to either run ImageJ as an administrator, or to install it in a folder that you do have write access to, for example the Documents folder.)

(b) Plugins.

ImageJ has some basic plugins (Fiji has more). You can always download more as needed from https://imagej.nih.gov/ij/plugins/index.html . You can read the .java file if it's available, but you must put the compiled (.class) file in the ImageJ Plugins directory. That plugin will then appear in the dropdown menu the next time ImageJ is opened.



There are a number of custom plugins associated with the textbook. Download and unzip them from Textbook Plugins (in this module) and put them in the ImageJ Plugins directory. Open ImageJ to confirm that they appear in the drop down plugins menu.

(You can also get them (and also all the figures used in the book) from the book website at https://www.cambridge.org/ca/academic/subjects/engineering/biomedical-engineering/digital-image-processing-medical-applications?format=HB&isbn=9780521860857 (go to Resources, then plugins, download and unzip, then rename them and put them in your Plugins directory).

(Note for Mac users: When you install ImageJ on the Mac, it creates a folder. In that folder is an icon for ImageJ that you click on to launch ImageJ. Drag that icon completely out of the folder and onto the desktop. Then, drag it right back where it was to begin with.

That's it. It should run and handle plugins correctly now.
On Macs, when you drag and drop, it sometimes "copies" the icon instead of moving it. That won't work. You have to move it. I think holding the Command key down will force a move, but then, I'm not sure because I'm not a Mac person.)

(c) Images and LUTs.
Make a directory called "345 Images" in ImageJ. Populate it with the sample images from https://geoffdougherty.cikeys.com/module-1/.

(d) Fiji is a distribution of ImageJ, bundling a lot of plugins which facilitate scientific image analysis. You should download it to your personal computer.

Go to https://fiji.sc/ then download and chose your operating system. Unzip the downloaded file. (You can also add the book plugins to Fiji).

A Useful site for Fiji:

Google Imagej.net …. Goes to http://imagej.net/Welcome then click “Learn”

2. Digital imaging basics

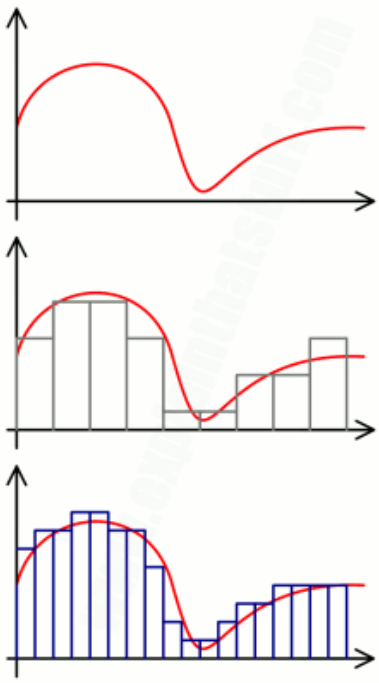
What is the difference between an analog and a digital quantity?

What are some examples of analog quantities? Digital quantities?

(Note: you can display analog quantities with analog or digital display, but the quantity itself is still analog.).

How do you convert analog information into digital information?

(i) Take samples (sample-and-hold)



Look at textbook, p.28, fig.2.12 and read p.27-28.

(ii) Assign sampled values to digital values (to be able to store in a computer)

Look at www.falstad.com/fourier Display a sine wave: (re)Sample it and watch the result. Quantize this and watch what happens.

What is the whole process called?

Let's consider 2-D images.

Which is better, an analog image or a digital image? (What are the advantages and disadvantages of a digital image?).

What is the sampling rate (or sampling? What is dpi? Do you prefer small pixels or large pixels? What is the relationship between sampling frequency and pixel size?

What is the spatial resolution of a digital image? (But it might be worse?).

What is it for an analog image? What is the point spread function (PSF) of an imaging instrument? Why is the profile of a blurred point a Gaussian?

How do you store an image in a computer?

How many grays can you get in an 8-bit image? With a 10-bit image?

In 8-bit images, why is black generally coded as 0 and white as 255?

Q. What is the file size of a grayscale digital image of an analog image 11" x 8.5", sampled at 300 dpi? What is the pixel size?

How frequently should you sample an analog image? (Look at textbook, Fig.2.13 which shows sampling and subsequent reconstruction of a simple sine wave). Read textbook, p.29-30. If you don't sample frequently enough, you will get **aliasing** (some frequencies will appear lower than they should). What is the Nyquist Sampling Theorem? Look through the worked example on p.31, in conjunction with Fig.2.15 to see what lower frequencies will result if aliasing occurs. Finish reading to the end of 2.4.1

Read the "What is Sampling?" document.

Can you explain the following terms?

ADC, sampling rate (dpi), spatial quantization, brightness quantization, pixel, spatial resolution, byte, Mbyte, 8-bit deep image, Nyquist sampling criterion, aliasing.

3. A quick look at ImageJ

Note the menu bar, tool bar, and status line. See how to go to the Image website for documentation (within Help).

Download (or drag-and-drop) the color version of Lena (or baboon). This is an RGB image, with 3 different planes – red, green and blue –each 8-bit deep. How many colors can be produced?

Primary colors - https://micro.magnet.fsu.edu/primer/java/primarycolors/additiveprimaries/index.html

Make a composite (Image/Color/Make Composite), the Split Channels to see the individual R, G and B channels.

Using a copy of RGB Lena, change the image to "indexed color" (Image/Type/8 bit color). What differences to you see? Use Image/Color/Edit LUT to see the palette (or color LUT) for this image, which is stored with an indexed color file. (Try looking at the LUT for grayscale and RGB versions of the image).

How many colors in RGB? How many bits/pixel are being used? (What about indexed color? How many colors/how many bits/pixel? Have a look at the LUT. Advantages/disadvantages?).

Change RGB Lena to 8-bit gray (Image/Type/8 bit), and duplicate.

Open Ch 2 Plugins and look at the effect of changing (i) spatial resolution and (ii) brightness resolution. What happens when we reduce the number of brightness levels? (Try Edit/Undo).

Look at File/Import (see how to import LUT, text image or text file (PSF, convolution mask)).

(AuPbSn). Duplicate, Show Info and Properties. Analyze/Tools – scale bar, calibration bar, Analyze/Set Scale, grid.

Line – shift – Analyze/Plot Profile. List/Save. (Average with rectangle).

Lena256 Analyze/histogram (or Ch 5 Plugin – Live (gray-value) histogram). Value/count. Dynamic range. (Open samples - Cell colony and AuPbSn). See textbook, Figs. 5.2 and 5.3, and read 5.1.1

CDF – cumulative distribution function. (Ch.5 plugin). Textbook, Fig.5.1 and read p.124-125.

(Plugins/Utilities – Find Commands).

Image/Adjust – brightness and contrast (*hold down shift to adjust all channels of a composite image*). Slider bars, extreme positions. Binary (B/W) image. Thresholding. Look-Up Table (LUT) – read textbook 5.2. What is the optimum contrast? (Auto, Apply – look at Live Histogram to see that the gray-value histogram has been stretched to the full dynamic range available. This is **linear histogram stretch** (to increase conrast) – read 5.2.1. Why are there missing values?).

LUTs. Open grayscale Lena, and view her LUT using Image/Colors/Edit LUT and Show LUT.

Import `log LUT.txt` as a LUT, show it as a 2D plot and as a palette. Import it as a text file to see the contents. Do Activities 5.7 and 5.8 from the textbook. (Note: the pixel values are not changed, only the gray values).

See Fig. 5.15 (and read 5.2.5).

Look at Process/Math for permanent changes to the pixel values. Try several, including square-root (then adjust brightness/contrast – note posterization).

Can you explain the following terms?

False contours (posterization), RGB image, indexed color image, look up table (LUT), palette, pixel value histogram, gray value histogram, profile, brightness, contrast, linear contrast stretch, binary image, thresholding, cumulative distribution function (CDF), signal-to-noise ratio (SNR). (Use the Index in the textbook if you need to).

4. More ImageJ

False color (pseudocolor)

ImageJ: Gray image –e.g. `brainpathology` - Add false color. (Image/LUTs: also >> to put LUT Menu into the Tool Bar …. Also put in Line Profile).

Stacks and DICOM images

Open DICOM stacks (from Samples) `MRI` and `T1 Head`, and `MR0001` and `NM0001` (from Canvas/Files). Scale up `NM001`.

Start animation (>), and right-click > to adjust speed.

(Note: ImageJ can't open compressed images: use Radiant or ezDicom).

DICOM images

Open Sample `CT`, move the cursor around – what do you see? Check Image/Properties and Show Info.

This image is 16-bits deep (2^{16} gray levels), but most monitors only show 256 grays. What do do? (Either merge gray levels, or use "windowing"). With DICOM images, ImageJ sets the initial display range based on the Window Center (0028, 1050) and Window Width (0028, 1051) tags. Click Reset and it sets the display range to the minimum and maximum pixel values.

As an example, the File>Open Samples>`CT`. Image/Show Info (or just press 'i') – the image has a Window Center of 50 and Window Width of 500, so the display range is set to -200 to 300 (center-width/2 to center+width/2). (Also look at Image/Properties).

Go to Image/Adjust/Window Level. Click Reset and WL is set to 342 and WW to 2121(the display range is set to -719 to 1402). Press 'h' (Analyze>Histogram) and you will see that the minimum pixel value in the image is -719 and the maximum is 1402. Press 'r' (File>Revert) to revert to the initial display range.

DICOM Stack

Unzip `CT Stack.7z` and drop `CT Stack` into ImageJ.

Move the cursor around in the black background. What values do you see? Can you explain?

Look at Image/Show Info. How many bits/pixel? What are the values of WC (or WL) and WW? Therefore, what HU values does the window span? What is the image resolution?

From the image info, what is the display range?

Go to Histogram and check the display range. Hit list.

Go to Image/Adjust/Window/Level to confirm the window span. In WL, hit Reset. Explain the appearance of the image stack.

5. Record macros

Use Plugins/Macros/Record and later Run.

Unzip Exercise 1 the Basics to get 3 images (put them in 345 Images) and a pdf file, exer1.pdf. Work through exercise 1-1 (Macro recording) and exercise 1-3 (LUTs). (Optionally: do exercise 1-4).

(i) If you need to do something custom for each run of the macro (i.e. adjust a threshold manually) there is a waitForUser(" ") command. This will pause the macro indefinitely and let you do what you want. The macro resumes when you hit "OK."

(ii) getNumber("prompt", defaultValue)
Displays a dialog box and returns the number entered by the user. The first argument is the prompting message and the second is the value initially displayed in the dialog. Exits the macro if the user clicks on "Cancel" in the dialog. Returns defaultValue if the user enters an invalid number.

```
// This macro demonstrates how a macro can display a
// data input dialog box. The dialog it creates contains
// one string field, one popup menu, two numeric fields,
// and one check box.

  title = "Untitled";
  width=512; height=512;
  Dialog.create("New Image");
  Dialog.addString("Title:", title);
  Dialog.addChoice("Type:", newArray("8-bit", "16-bit", "32-bit", "RGB"));
```

```
Dialog.addNumber("Width:", 512);
Dialog.addNumber("Height:", 512);
Dialog.addCheckbox("Ramp", true);
Dialog.show();
title = Dialog.getString();
width = Dialog.getNumber();
height = Dialog.getNumber();;
type = Dialog.getChoice();
ramp = Dialog.getCheckbox();
if (ramp==true) type = type + " ramp";
newImage(title, type, width, height, 1);
```