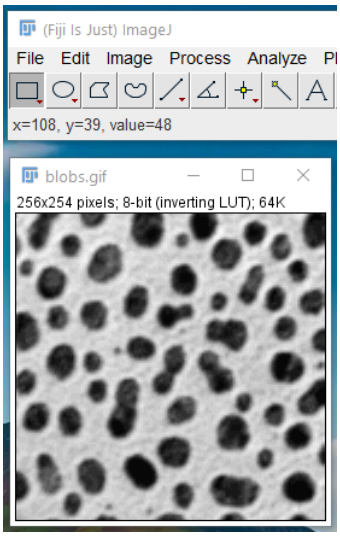
Exercises Session 1 - Digital Images

Exercise 1) Images are just numbers (5-10 mins)

- Open the sample image Blobs
- ***File / Open Samples… / Blobs (25K)***. If you've got no internet it is also in the course files 'Exercise Images' folder. (NOTE- ***Bold Italics*** denotes an ImageJ Menu command)
- ***File / Save as > text image…*** (NOT 'text…' make sure it's 'text image…')
- Drag the .txt file to Fiji to open it.
- Make the original Blobs image binary (Process / Binary > Make Binary)
- ***File / Save as > text image…*** call it blobsBinary.txt
- Drag the blobsBinary.txt file to imagej



Why are the two text files different?

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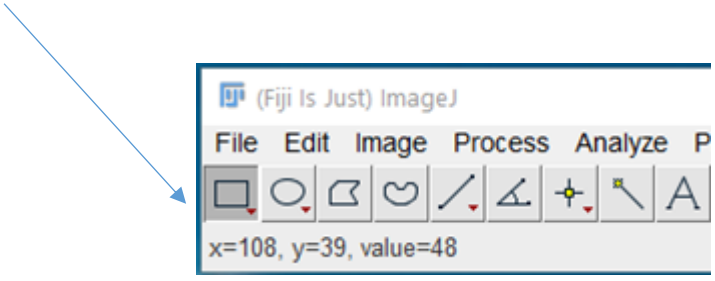
- Import both text files as image files (***File / Import > Text Image…***)

Why are the images the wrong way around? (white and black are reversed – Hint: LUT)

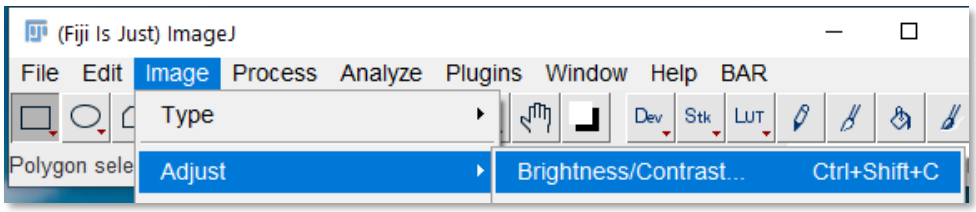
- Examine image co-ordinates and pixel values…

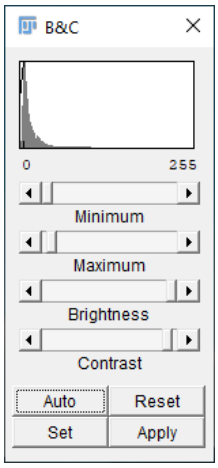
Move your cursor over the images, see the pixel coordinates and values are displayed in the Fiji control panel.



Exercise 2) Bit depth display and Image Histogram (5-10 mins)

- Open Dapi_WhatBitDepth.tif from the Exercise Images folder (I sent a link to download it).
- Open the image Histogram (***Analyze / Histogram*** – or Ctrl H). Press Log.
- Click on the image again (to make it the active image) & open a second histogram window. Press Log and Live. (We will compare the histograms as we make changes).
- What is the likely bit depth? (8 bit 0-255, 12 bit 0-4095, 16 bit 0-65535)
- Open the brightness & contrast control window (***Image / Adjust > Brightness/Contrast…***)

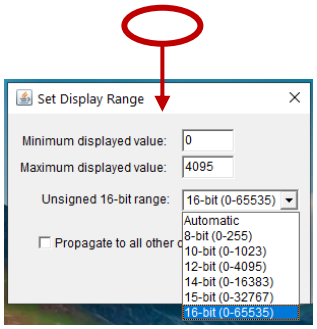




- You can move the sliders in the B&C window… watch the image change & the live histogram also.
- You can press Auto or Reset.
- Press SET, then in the 'unsigned 16-bit range:' menu choose the correct bit depth.

How has the histogram window changed?

- Click in the image (to activate it) then set the B&C range to 16 bit.
- Press Reset

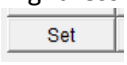


Where did the image go?

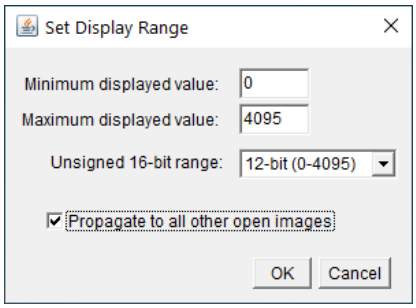
- Press Auto – what changes? (Toggle between Auto & Reset to see what changes)

It is important to display an image at its correct bit depth.

Exercise 3) (Optional / Homework) Changing bit depth. (20 minutes)

- Open '03-BitDepth&Brightness' from Exercise Images folder.
- Open Brightness and Contrast window (***Image / Adjust > Brightness/Contrast***)
- Press 

& Set Unsigned 16-bit range to "12-bit". Tick 'Propagate to all other open images' then Press OK.
- Now change the Unsigned 16-bit range to "Automatic". Press OK. (Automatic allows you to change the bit depth display settings).
- Try Reset & Auto buttons. Image display changes but pixel values remain the same (put the mouse over a pixel to see it's value)



- Use '***Process / Enhance Contrast…***' Set Saturated pixels to 0.3%. Press OK.
- Save the image as a Jpeg. '***File / Save As…Jpeg…'*** and re-open the new .JPG file. (Drag it into the Fiji window)
- Press H (***Analyze / Histogram***) for each image (Click Image, then press Ctrl H). Press Log.

Note the histograms: one has 255 levels of brightness, the other 4095.

- Use the Line tool to draw a line through the brightest spot on the original image.
- Press K (Analyze / Plot profile).
- Activate the Jpeg image (Click on it) , press Shift+E (Restore selection), press K.

Why are the graphs different? (Bit depth!)

- On the original image (click it to select it) set the display range to **12-bit** (in Brightness and contrast window, the SET button). Then press Reset. The image looks less bright (but the data in the image is unchanged, it is just displayed less brightly).
- Save this image as a new Jpeg (with a different name).
- Open the new Jpeg image, press 'Shift E' the 'K'. It's fixed it!
- Close the three profile plots.
- Make a histogram from the new 8-bit image. Compare the 3 histograms… especially the Log view.

Why is the new profile no longer clipped (the log histogram shows many pixels with a value of 255)?

Answer: The computer display is 8 bit, the data is 12 bit, converting to 8 bit within ImageJ takes values from the displayed image, not the underlying data. BE CAREFUL: Decreasing bit depth reduces image intensity detail and may crop data. (If you save as a JPG, BMP, PNG, GIF you will convert to 8-bit).