

Basics of Digital Imaging

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WARWICK

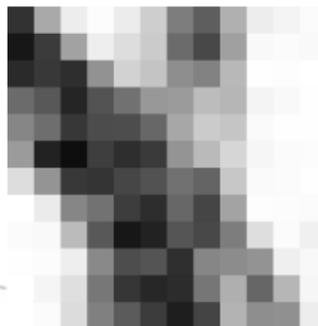
The Warwick University logo, which consists of a stylized blue 'W' shape above the word 'WARWICK' in a blue, sans-serif font.

Outline

- ▶ Images and Pixels
- ▶ Dimensions
- ▶ Types and bit-depths
- ▶ Channels and colours
- ▶ Files and file formats

Images and pixels

- ▶ images are not the data, numbers are the data



```
50 169 237 252 236 204 125 94 176 237 244 251
23 59 162 238 221 203 107 72 160 249 251 249
42 56 46 146 210 196 142 130 184 254 253 255
107 88 37 83 113 152 155 188 182 244 249 254
134 110 55 76 77 103 167 203 197 250 254 254
155 34 14 62 46 58 148 186 214 246 251 252
221 148 56 52 70 82 113 100 199 250 251 254
255 235 134 114 61 45 103 69 163 253 252 251
251 249 187 88 33 34 85 72 125 222 251 240
254 252 238 137 77 84 41 134 140 147 239 248
255 245 223 119 54 39 44 118 175 102 182 246
255 251 220 128 91 60 50 68 179 142 144 237
```

- ▶ Images that look the same can contain different pixel values, images that look different can still contain the same pixel values

ImageJ and Fiji

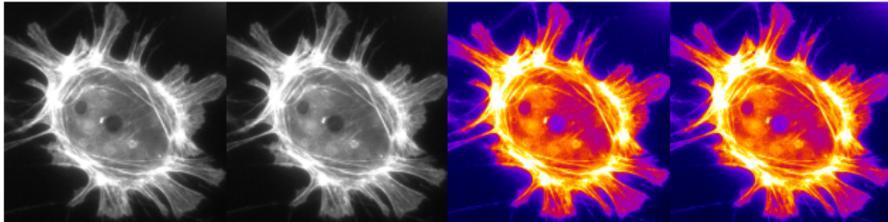
- ▶ Free (good!)
- ▶ Customisable (excellent!)
- ▶ A bit overwhelming (well...)



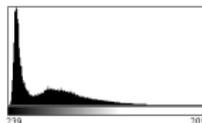
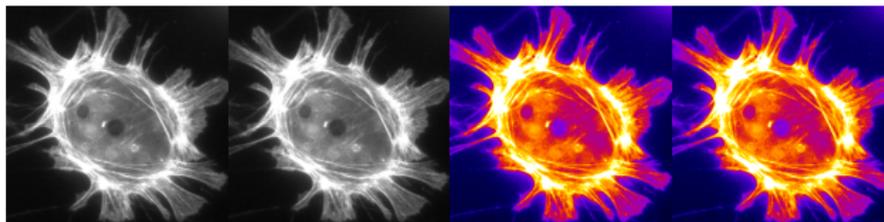
Fiji tips

- ▶ You can drag a file into Fiji to open it!
- ▶ Ctrl+L: command finder
- ▶ Do not rely on undo - duplicate your images!
- ▶ double-clicking/right-clicking on icons reveal more possibilities

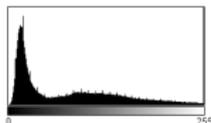
Comparing images



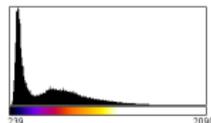
Comparing images



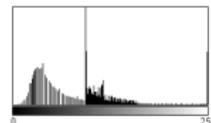
Count 339864 Min: 239
Mean: 591.429 Max: 2090
StdDev: 306.524 Mode: 313 (14617)
Bins: 256 BinWidth: 7.230



Count 339864 Min: 0
Mean: 82.006 Max: 255
StdDev: 71.418 Mode: 255 (10308)



Count 339864 Min: 239
Mean: 591.429 Max: 2090
StdDev: 306.524 Mode: 313 (14617)
Bins: 256 BinWidth: 7.230



Count 339864 Min: 0
Mean: 90.544 Max: 255
StdDev: 56.392 Mode: 95 (21862)

Colour maps

- ▶ Conversion from data to colours
- ▶ exercise time!



Exercise!



Investigate pixel values, LUTs, brightness and contrast



Colour maps

- ▶ LUTs can match light colour or just be used to distinguish values
- ▶ keep the raw data raw - you saw what happened when you pressed "apply"!
- ▶ Scientific analysis is not the same as photo editing - if you want it to "look good", you need to do it without changing the data!



Properties/pixel size

- ▶ Pixel have dimensions that are "physical"
- ▶ Pixels are not squares - they are point values!
- ▶ Check whether metadata is correct in properties
- ▶ Smaller pixel = more resolution (in general)



Dimensions

- ▶ Dimensionality = number of coordinates to identify pixel
- ▶ Microscopy data is typically 2D to 5D
- ▶ 2D is not necessarily x/y (kymographs, for example!)
- ▶ Stacks (z-stacks or videos), hyperstacks (up to 5d) - why should we care about stacks, then?
- ▶ only one 2d slice is ever active at one time in Fiji (sliders at the bottom)
- ▶ exercise time!



Exercise!

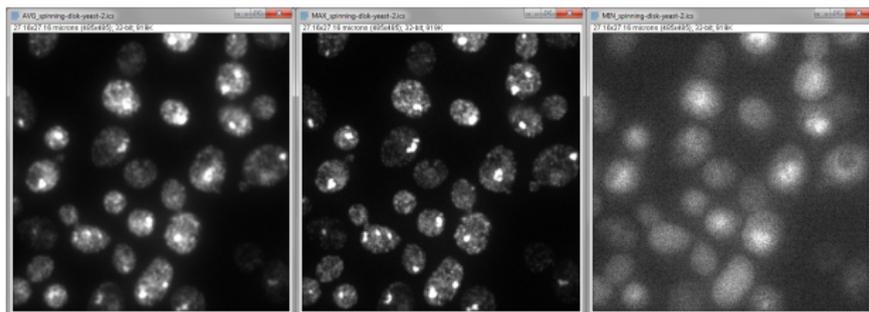


Can you create a hyperstack that makes sense?



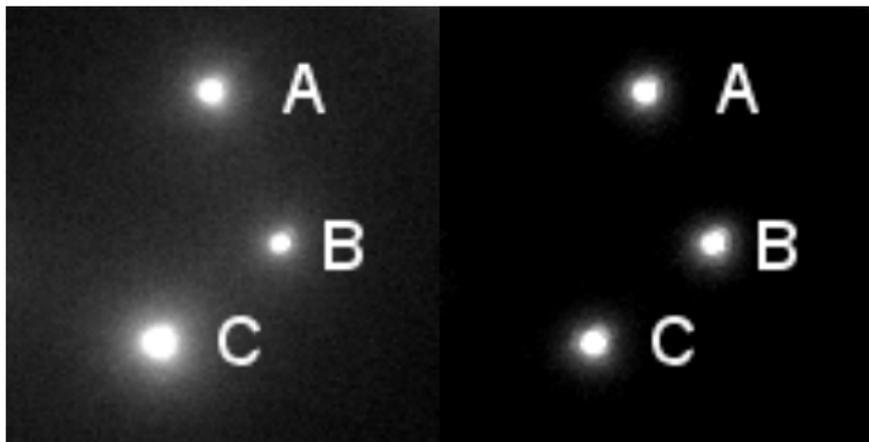
Presenting dimensions

- ▶ Viewing angles, 3d viewer, orthogonal views
- ▶ Z-projections (sum, max, min) and its possible pitfalls



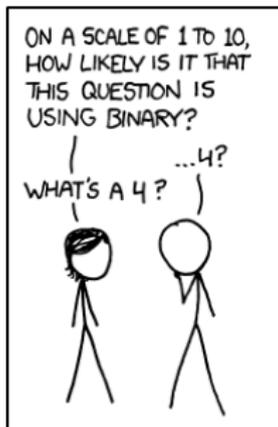
Question!

Which one is sum and which one is maximum? Which one would you use to determine the distance between beads?



Types, bit depth and so on

- ▶ Possible/impossible pixels - bit depth
- ▶ basic binary encoding

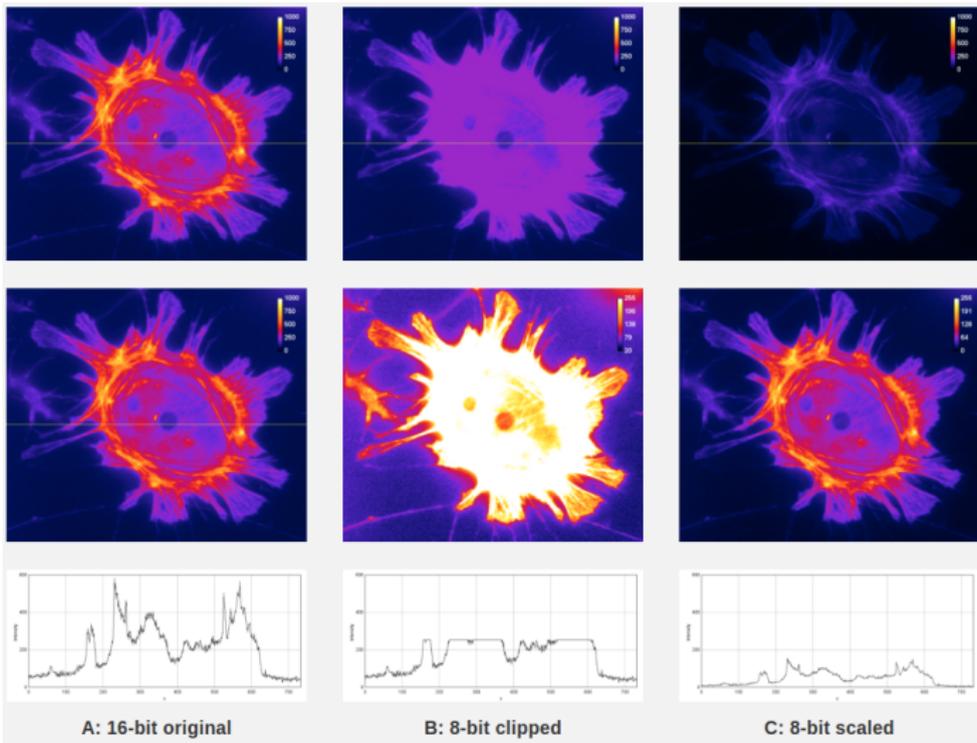


Types, bit depth and so on

- ▶ Possible/impossible pixels - bit depth
- ▶ basic binary encoding
- ▶ 8-bit encoding and its significance, 16-bit
- ▶ floating point representation
- ▶ Clipping/saturation and rounding - losses of information
- ▶ range of possible values depend on bit depth of image AND acquisition equipment



Types, bit depth and so on



Rounding, converting

- ▶ Rounding errors tend to be small at acquisition level, but can be problematic at filtering/ analysis
- ▶ More bits are usually better (except for file sizes and when they're not actually needed)
- ▶ downsampling in fiji takes brightness/contrast into account, so be aware



Exercise!

Can you destroy an image by increasing and decreasing bit depth?



Multichannel and RGB images

- ▶ Multichannel: original pixel values, channel as dimension (split/merge channels)
- ▶ RGB can only have 3 8-bit channels (but it's very compatible with stuff, and what you're seeing on screen is always RGB)
- ▶ Red/Green images are common but think of the poor colorblind people!



File formats

- ▶ Image data x metadata
- ▶ Good for analysis x good for display
- ▶ First group is normally proprietary, which is a pain
- ▶ Fiji uses LOCI Bioformats plugin - good, but not perfect. Keep the originals and confirm metadata!
- ▶ Metadata landscape is slippery and complicated - OME-TIFF might be a good idea whenever possible



A healthy approach to metadata



File formats

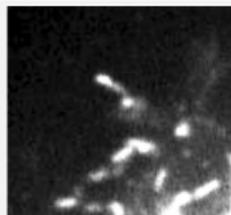
- ▶ Original metadata can be wrong too!
- ▶ (i.e. magnification that needs manual input, etc etc.)
- ▶ Lossy vs lossless compression
- ▶ Be wary of lossy compression, it throws any analysis out the window
- ▶ for publication: vector vs bitmap



File formats



A: Uncompressed TIFF



B: Compressed JPEG



C: Subtracted TIFF



D: Subtracted JPEG



Exercise!



Hints:

The file contains only a single image, and a single channel.

The dimensions (width and height) of the image are each a multiple of 100 pixels.

The data is in 8, 16 or 32-bit format (signed or unsigned).

There are no offsets or gaps included.

The total size of the file is 400 000 bytes.