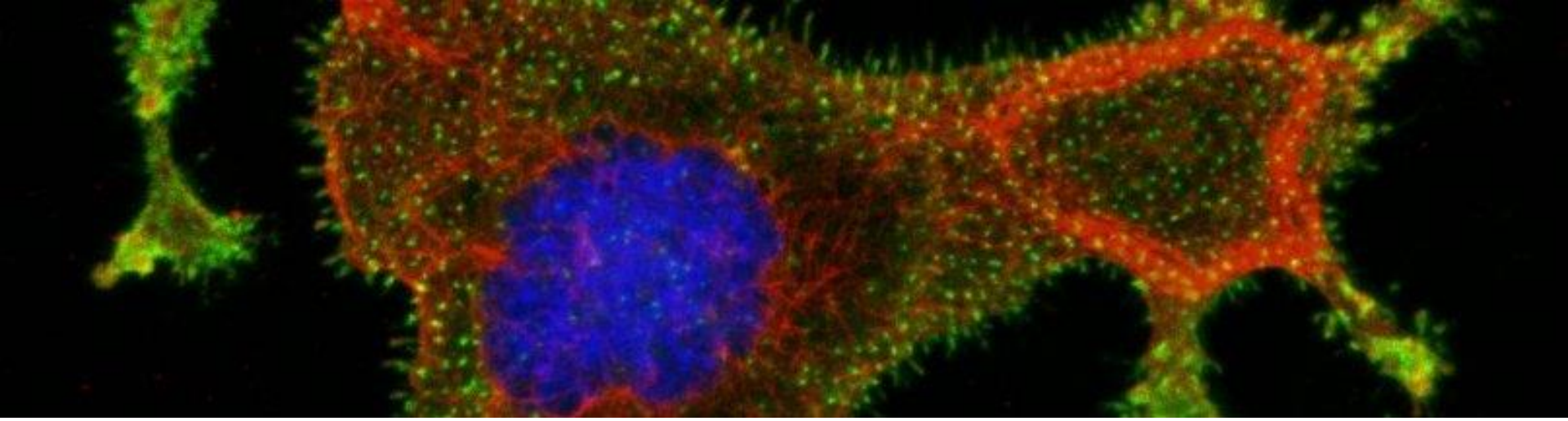

Image Analysis with Fiji

An introductory course for biologists

Jeremy Pike, Image Analyst for COMPARE

Course website:
https://jeremypike.github.io/image-analysis-with-fiji/

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Fiji installation and course material

1. Download Fiji:

http://imagej.net/Fiji/Downloads

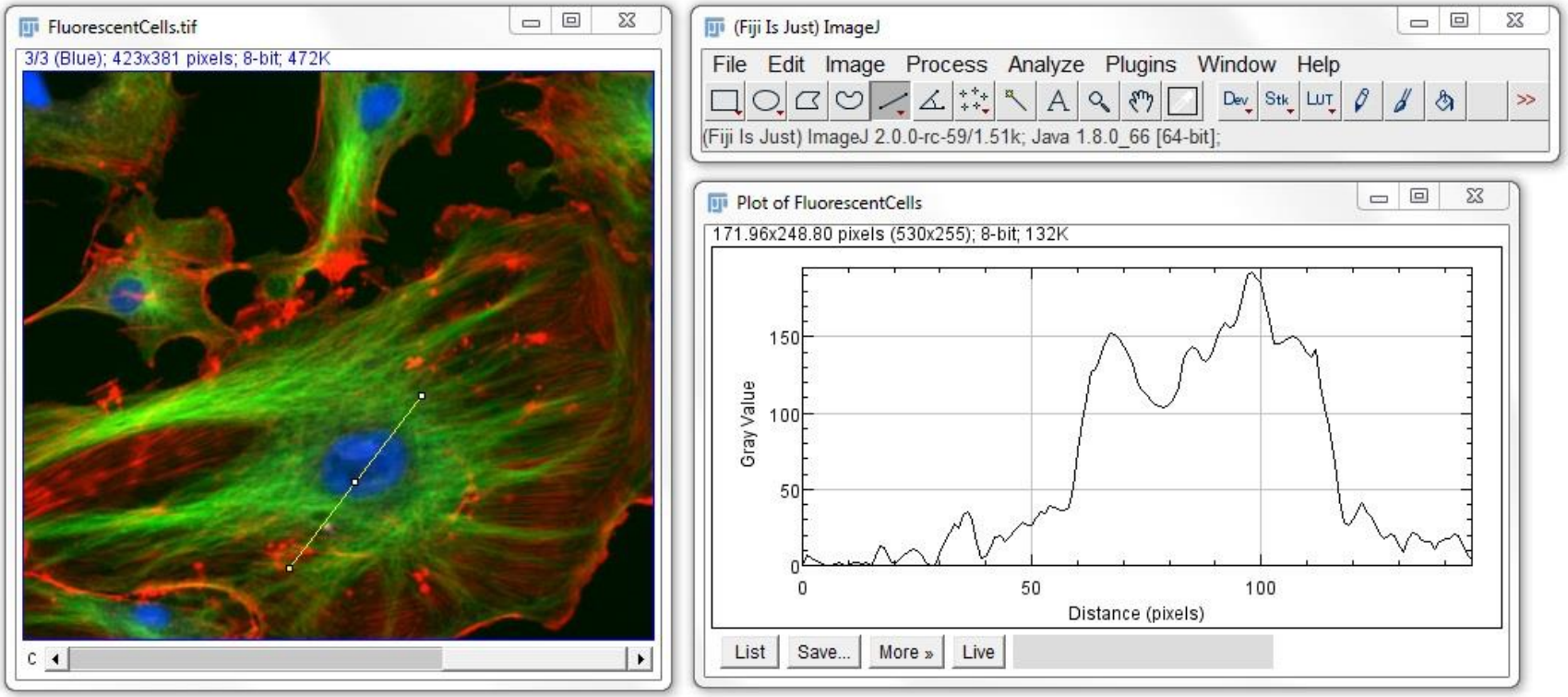
You don’t need to install Fiji, just unpack and start ☺

2. Download the course sample data:

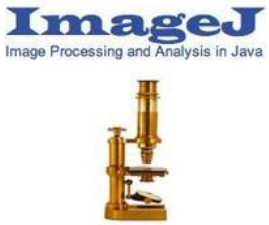
https://jeremypike.github.io/image-analysis-with-fiji

Click sample data zip file, and unpack. You can also get copies of the exercises, slides and macros if you want.

Part 1: Basics



ImageJ, Fiji and ImageJ2 …



ImageJ is an open source image processing and analysis software application

Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. (2012), Nature methods 9(7): 671-675.

Fiji is a distribution of ImageJ with loads of really useful plugins pre-installed

Schindelin, J., Arganda-Carreras, I. & Frise, E. et al. (2012), Nature methods 9(7): 676-682.

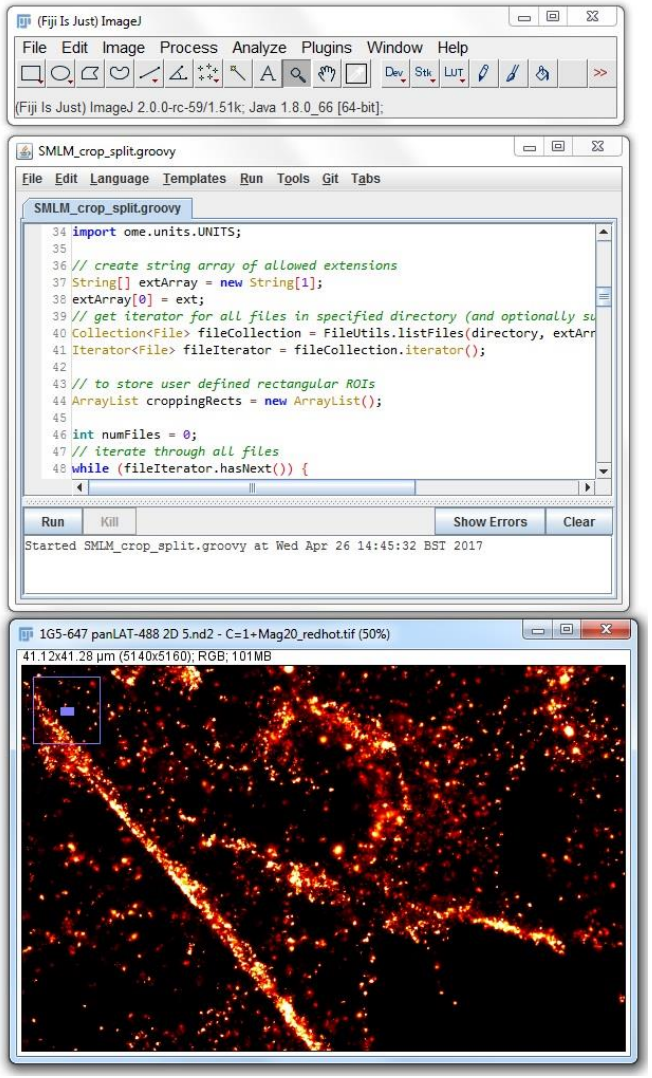
ImageJ2 is a complete rebuild of ImageJ, it is built into Fiji

Rueden, C. T., et al. (2017), preprint arXiv:1701.05940.

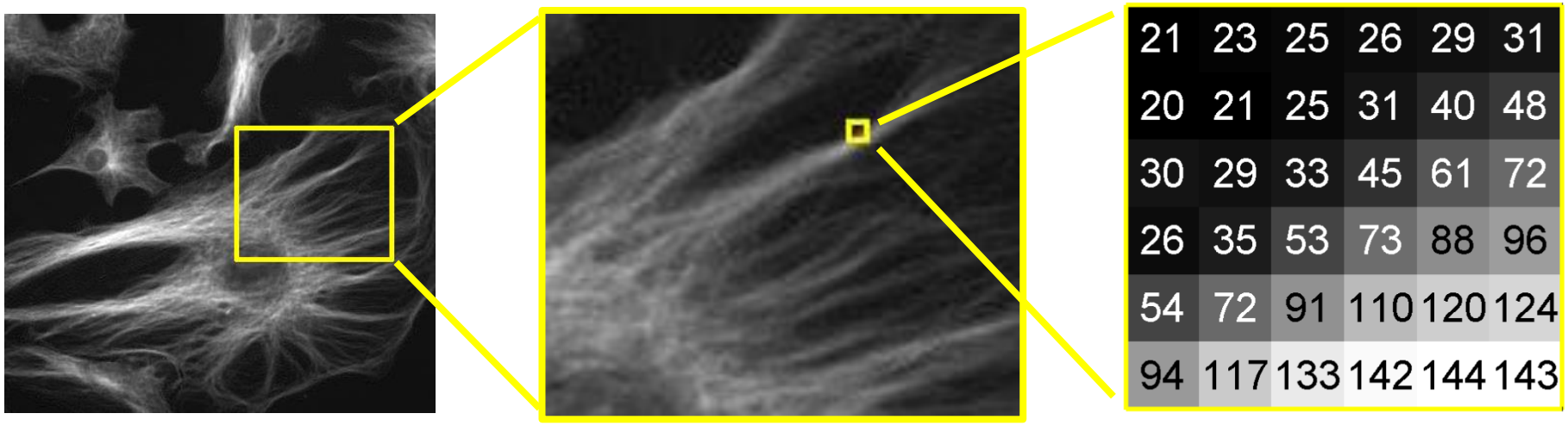
If in doubt get Fiji!

Why use Fiji?

- Open source
- Very popular
- Wide range of sophisticated user-written plugins
- Great for beginners all the way to developers
- Macros and scripting for easy automation
- Interoperability with other software (e.g. KNIME)



Digital images are simply arrays of numbers



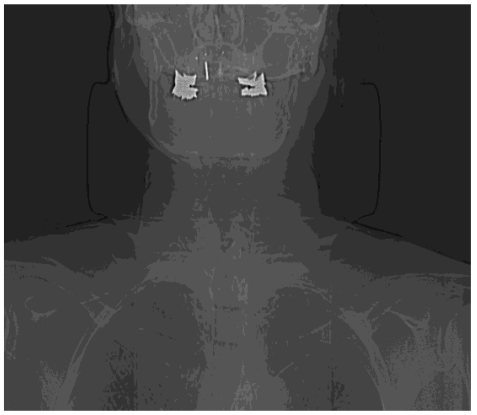
Note pixels are samples of intensity at a spatial point, not little squares!

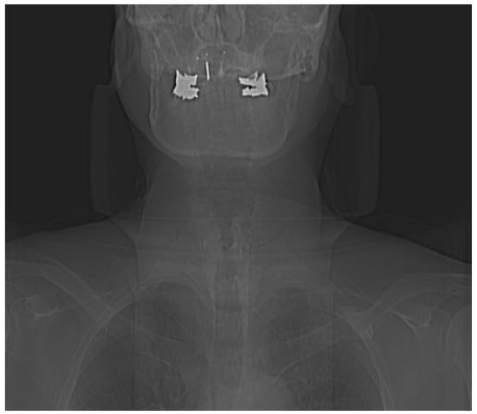
Dynamic range is the number of values each number can take

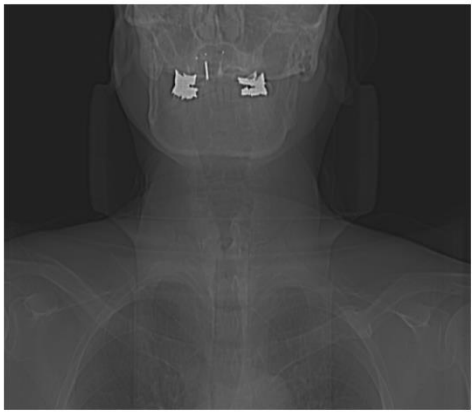
1 bit = 2

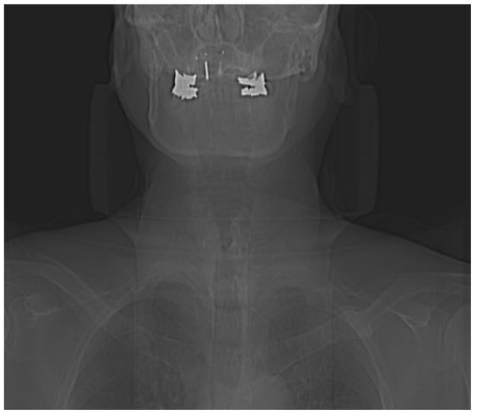
2 bit = 2^2

4 bit = 2^4







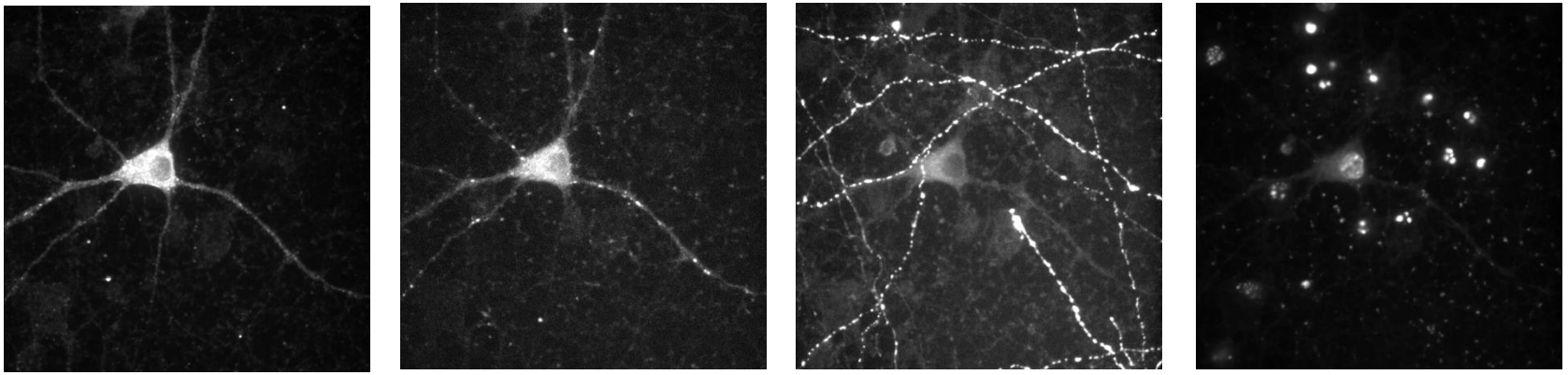


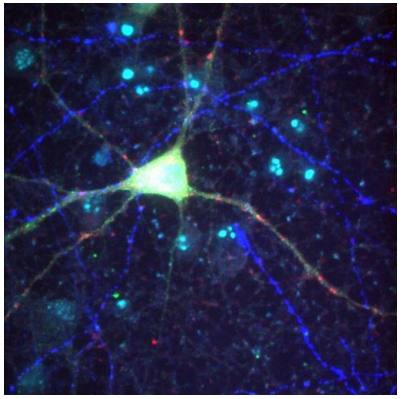
8 bit = 2^8

12 bit = 2^{12}

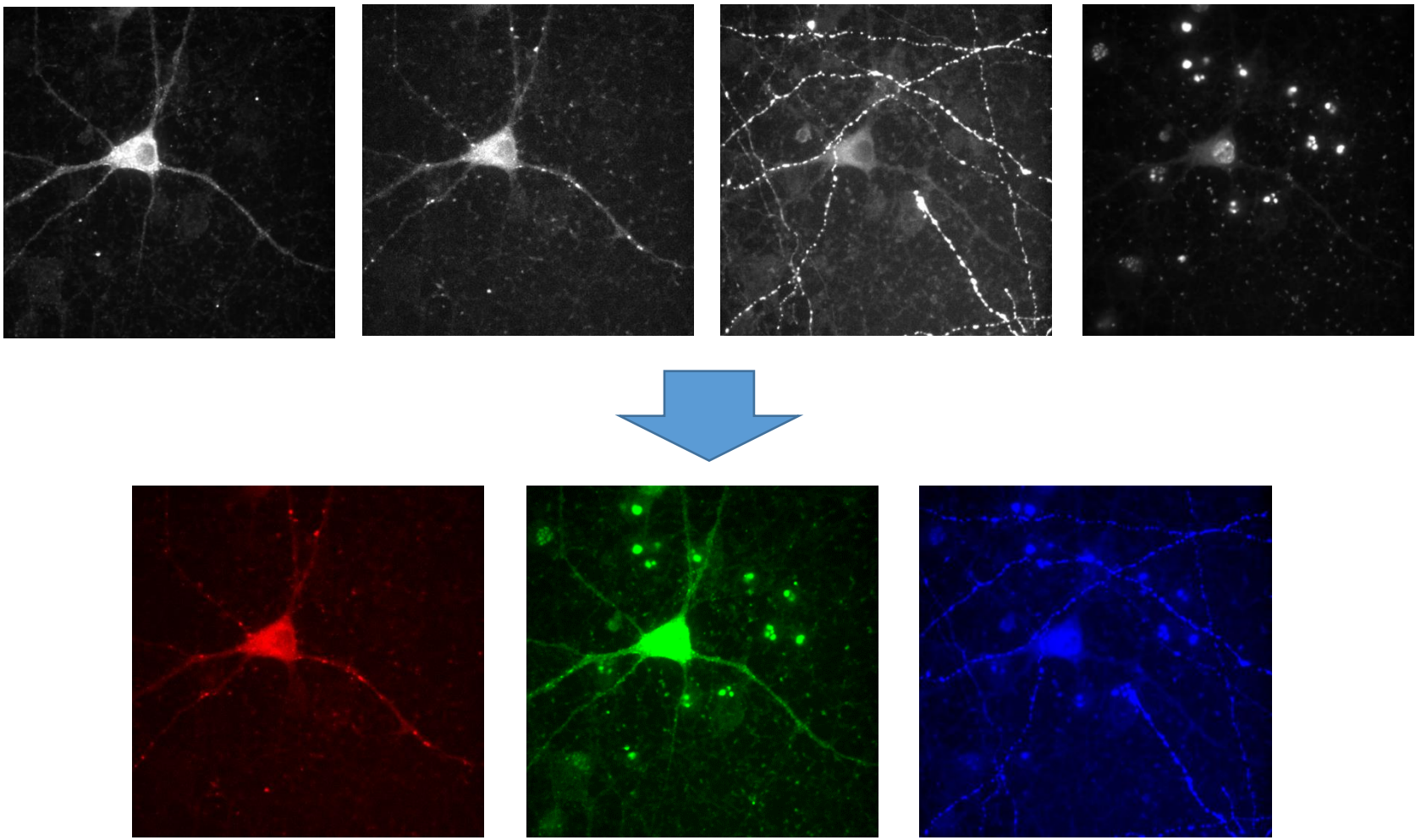
16 bit = 2^{16}

Multi Channel Data

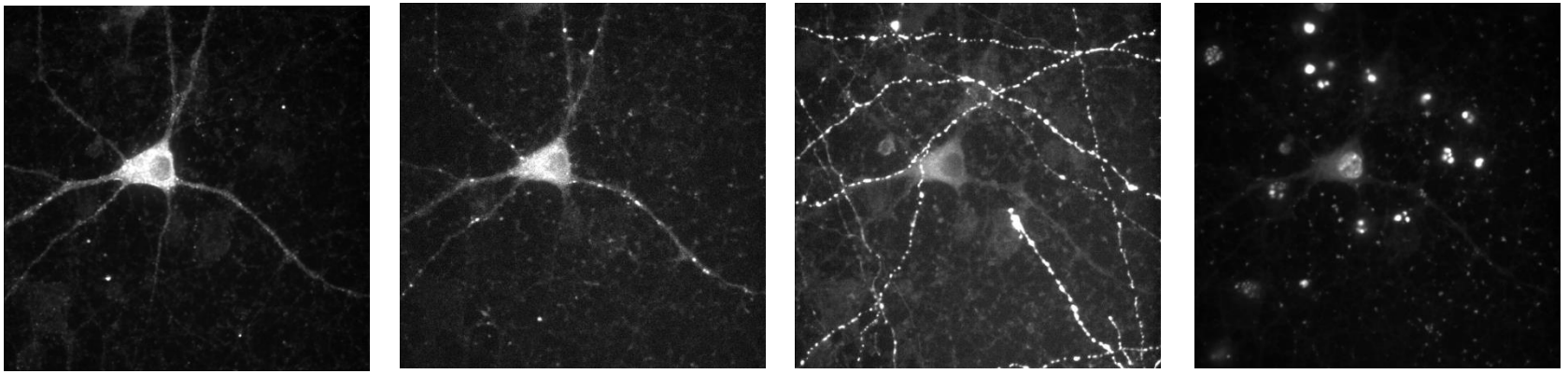


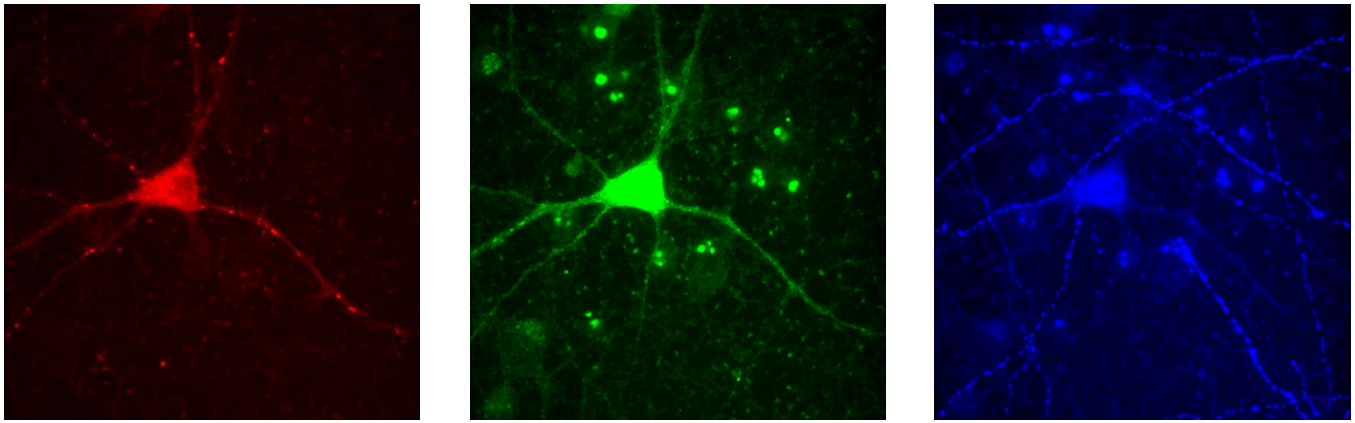


RGB Colour Images

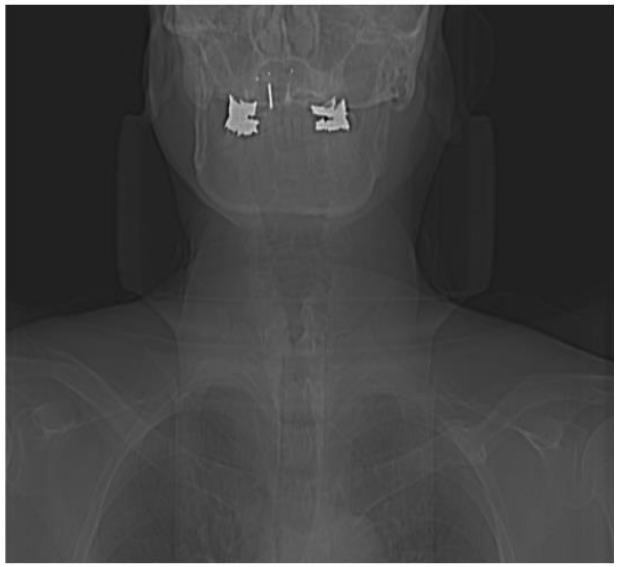


RGB Colour Images

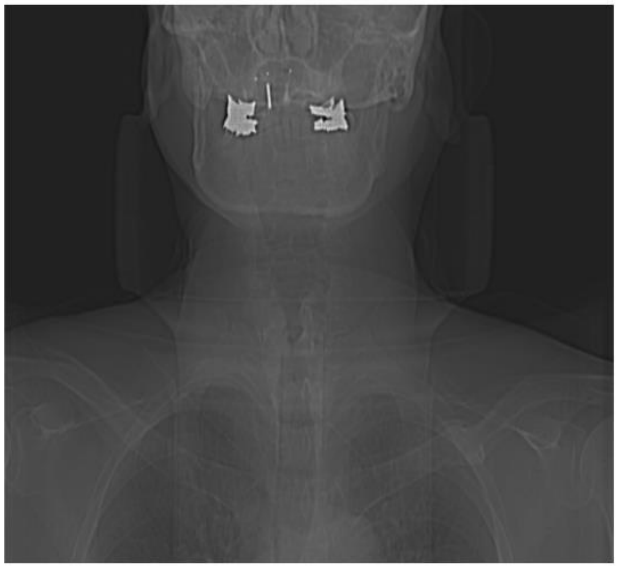




What can we “see” on a monitor?



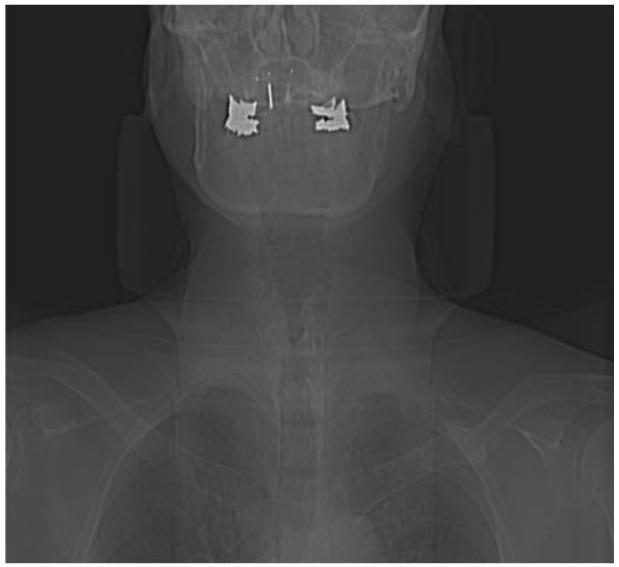
8 bit = 2^8



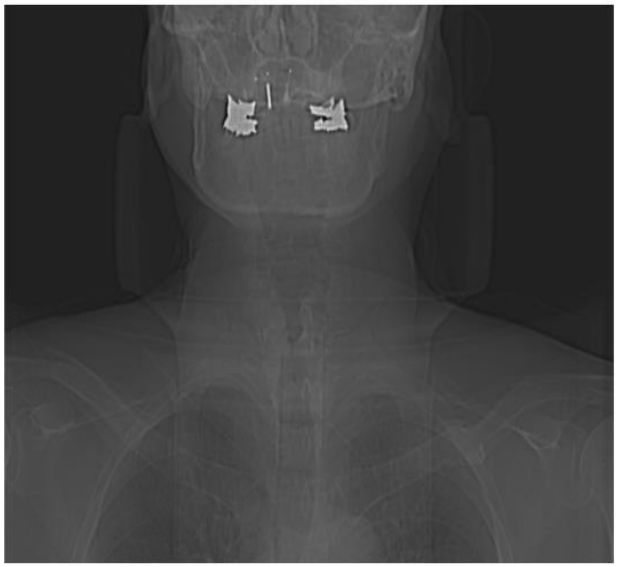
16 bit = 2^{16}

Can you tell the difference?

What can we “see” on a monitor?



8 bit = 2^8

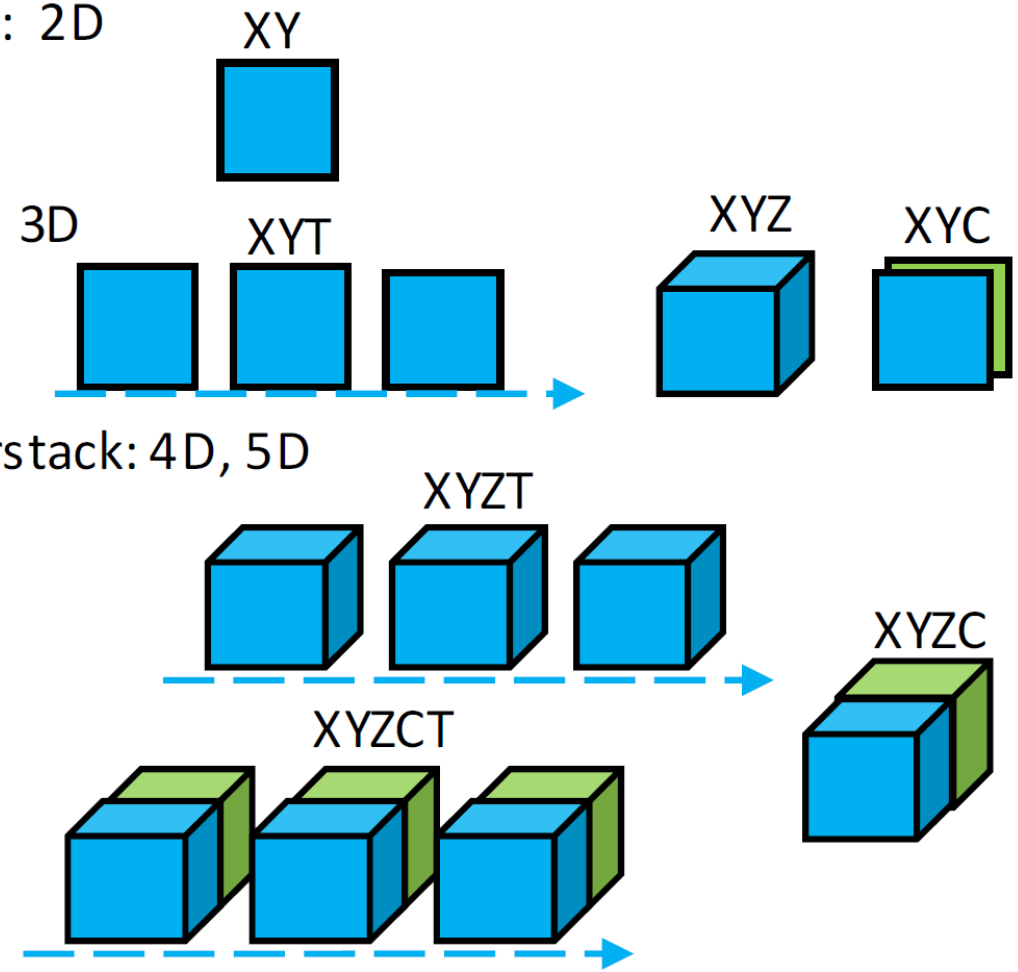


16 bit = 2^{16}

- 8 bit display range
- 3 x 8bit RGB for colour display
- What bit-depth can our eyes detect?

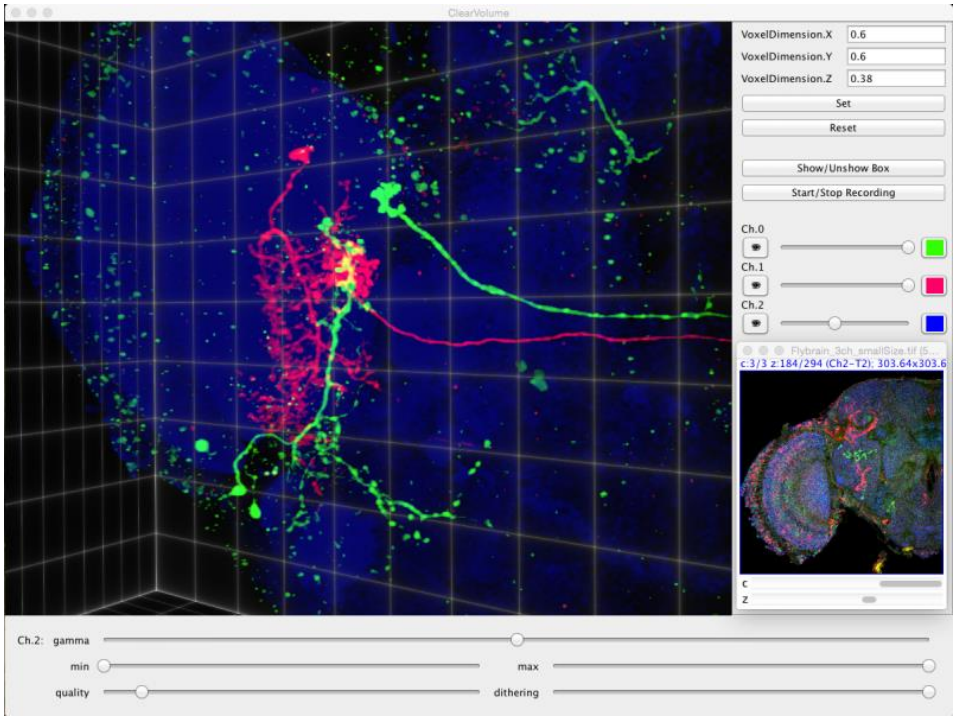
Microscopy data

- Image: 2D
- Stack: 3D
- Hyperstack: 4D, 5D



Volume rendering in Fiji

We recommend using ClearVolume
http://imagej.net/ClearVolume



Royer, L. A., Weigert, M. & Günther, U. et al. (2015), Nature Methods 12(6): 480-481.

Common file formats

• **TIFF** is a good choice
– Lossless storage of data
– Header tags for metadata

• **Proprietary formats from microscope vendors** (e.g. lif, nd2, czi)
– Often just a TIFF wrapper
– Easy handling or 5D data, and lots of metadata added automatically
– The Bio-Formats plugin will load most formats

• **PNG** should only be used for transfer and display
– Lossless compression
– No metadata
– RGB only

• **JPEG** should not be used for scientific images
– Lossy compression discards information and causes artefacts

An image is the sum of many point spread functions

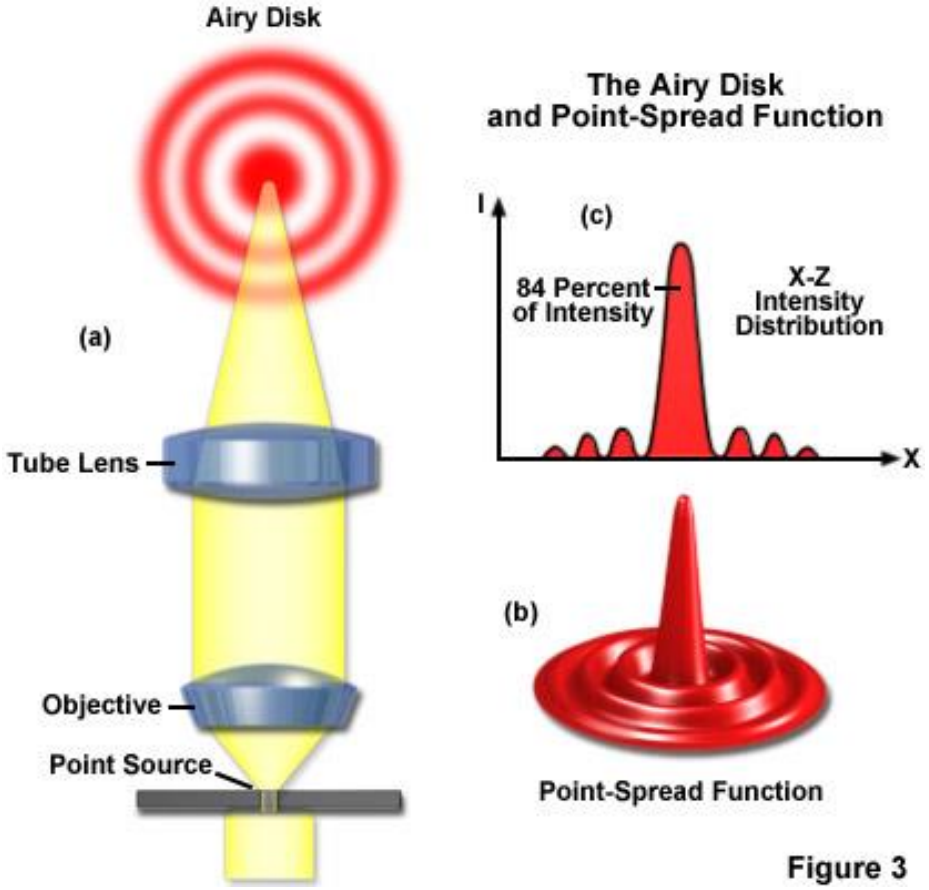


Figure 3

"Each element of the primary image is a small diffraction pattern, and the actual image, as seen by the eyepiece, is only the ensemble of the magnified images of these patterns"

Born and Wolf, Principles of Optics

What determines the resolution limit in light microscopy

- ~~The number of pixels in an image~~
- ~~The magnification of the objective lens~~
- The width of the point spread function
  - Numerical aperture of objective lens
  - Wavelength of light
  - Refractive index of immersion medium

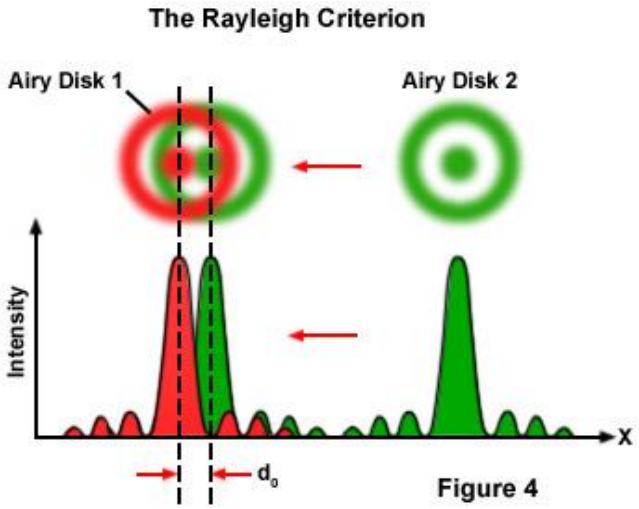


Figure 4

http://zeiss-campus.magnet.fsu.edu/articles/basics/resolution.html

Part 2: Introduction to Image Processing and Analysis

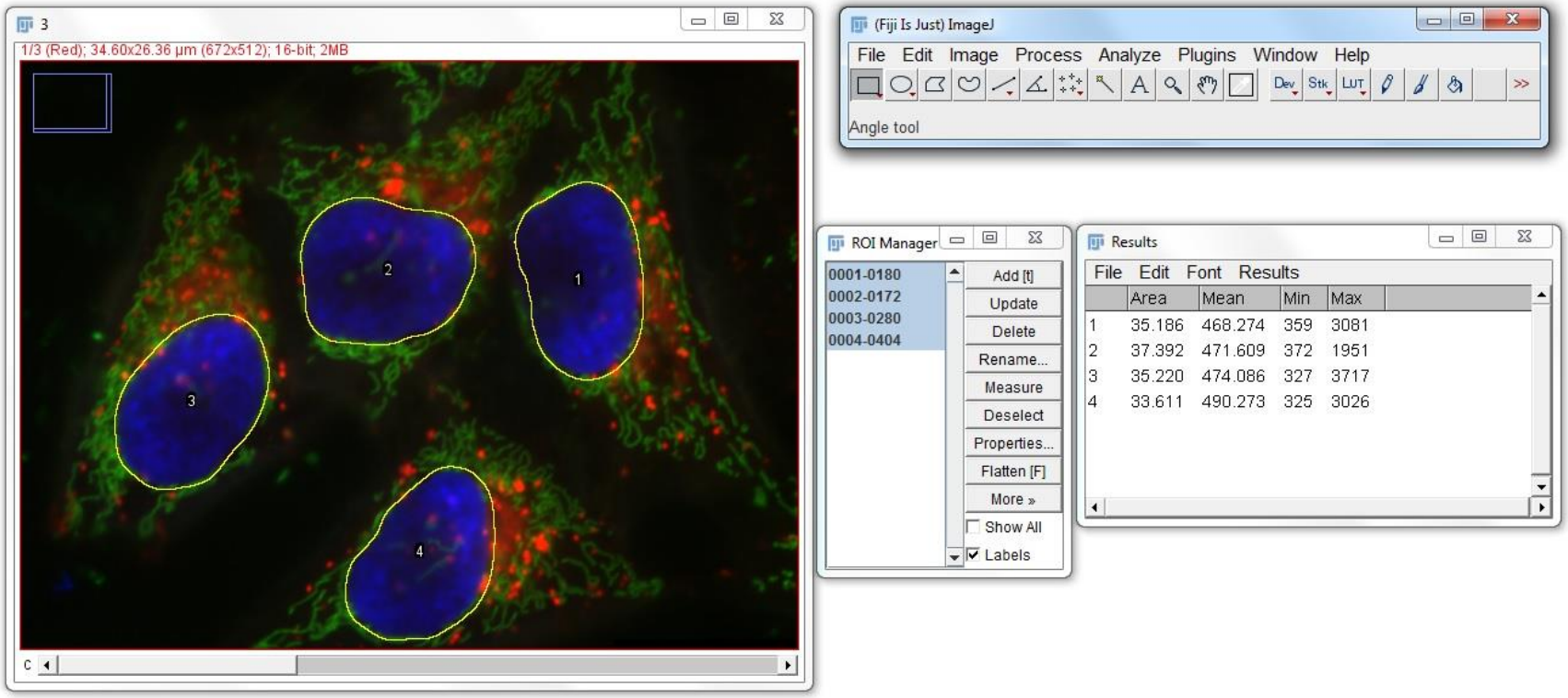


Image Processing:

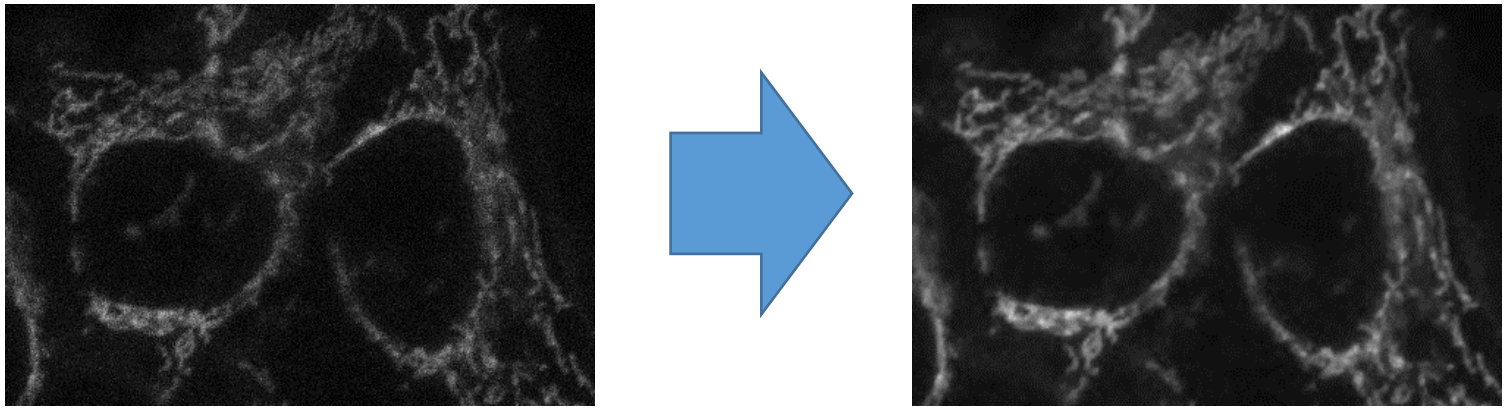
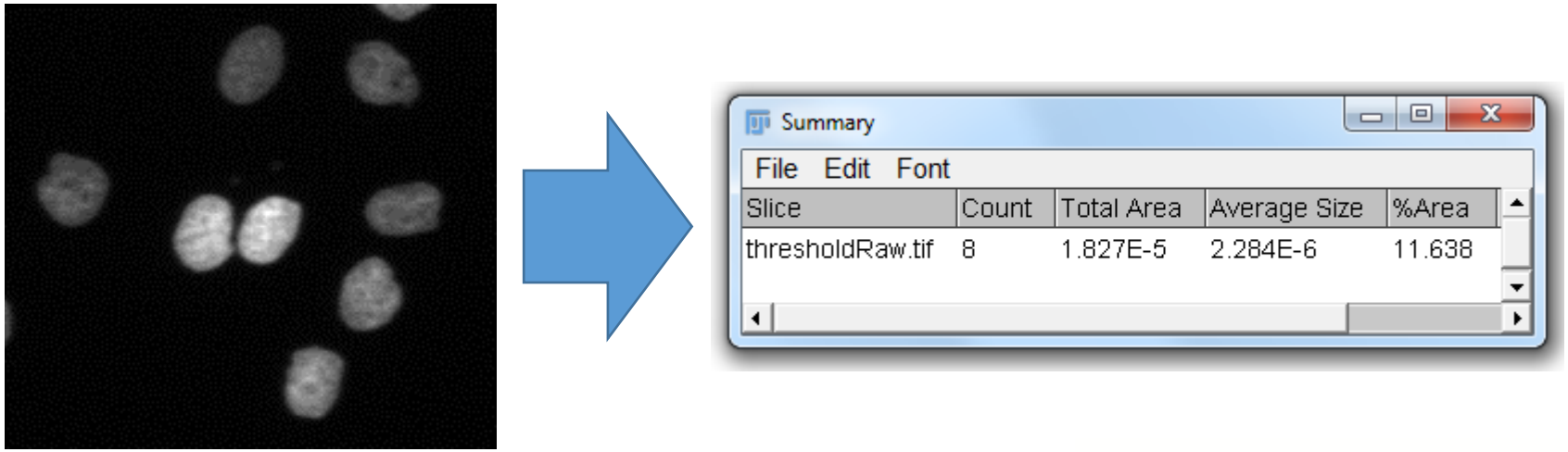


Image Analysis:



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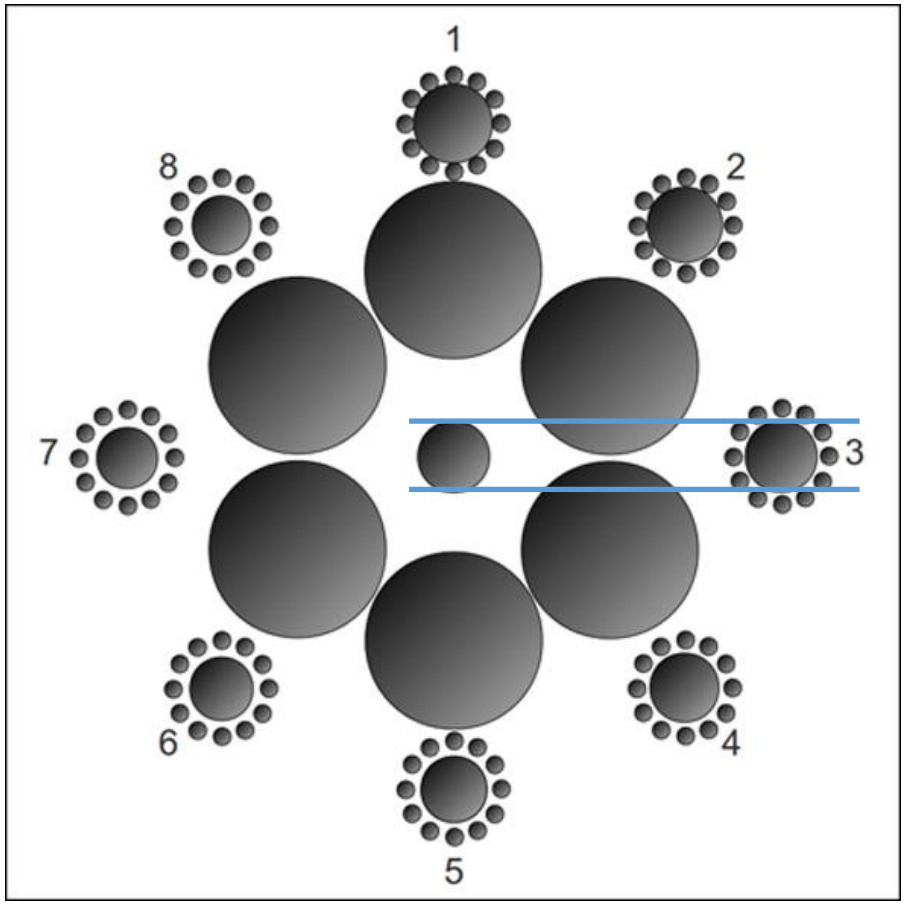
Why do computational processing and analysis?

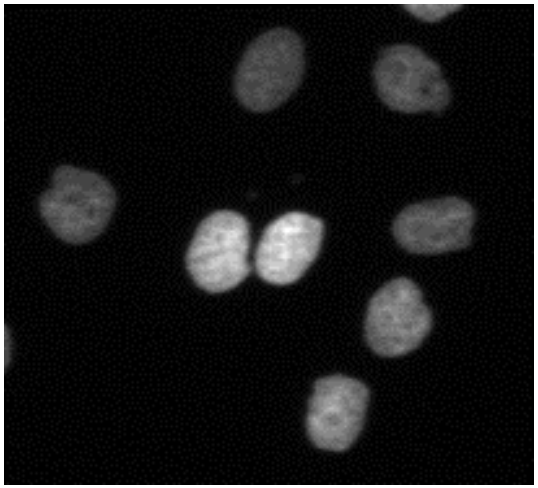
- Its quantitative
- Its unbiased
- Can enhance understanding of the data
- Can be automated for processing of large datasets

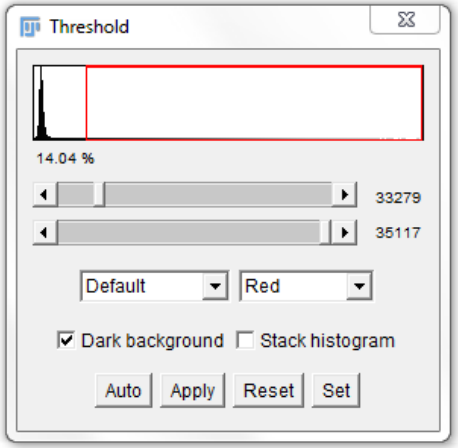
"The first principle is that you must not fool yourself - and you are the easiest person to fool. So you have to be very careful about that. After you've not fooled yourself, it's easy not to fool other scientists. You just have to be honest in a conventional way after that."
Richard Feynman

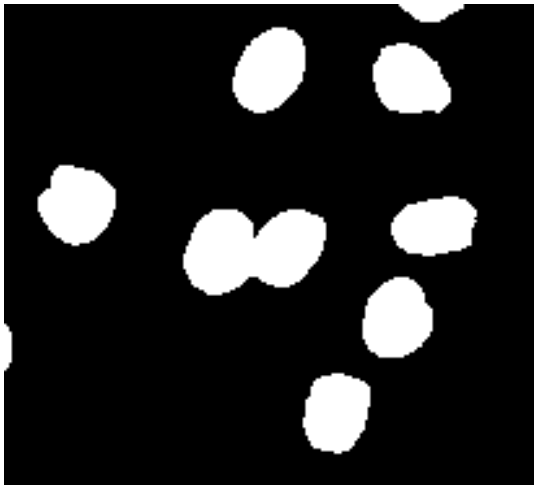
What outer circle is the same size and the central circle?



Intensity based thresholding to segment objects

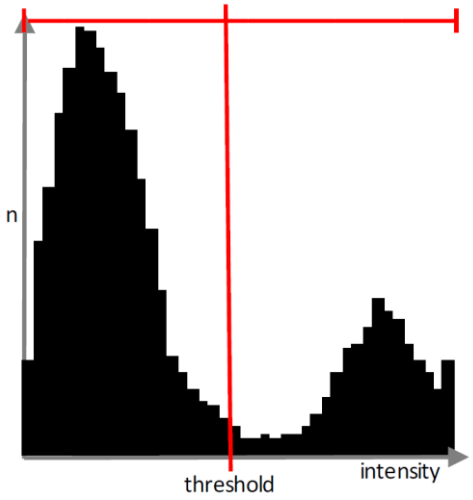






Automated threshold values are preferable to manual selection

- Otsu thresholding assumes there are two classes (signal and background) and maximises the intra-class variance.

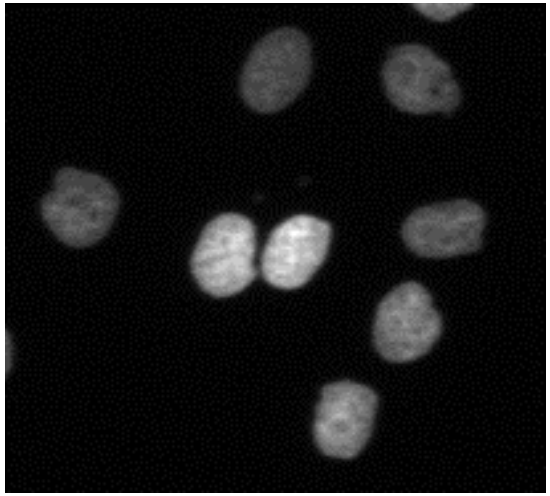


Otsu, N (1979), IEEE Trans. Sys., Man., Cyber. 9: 62-66.

- Li thresholding minimises the cross entropy between the original and segmented images.

Li, CH & Tam, PKS (1998), Pattern Recognition Letters18(8): 771-776

Automated threshold values are preferable to manual selection

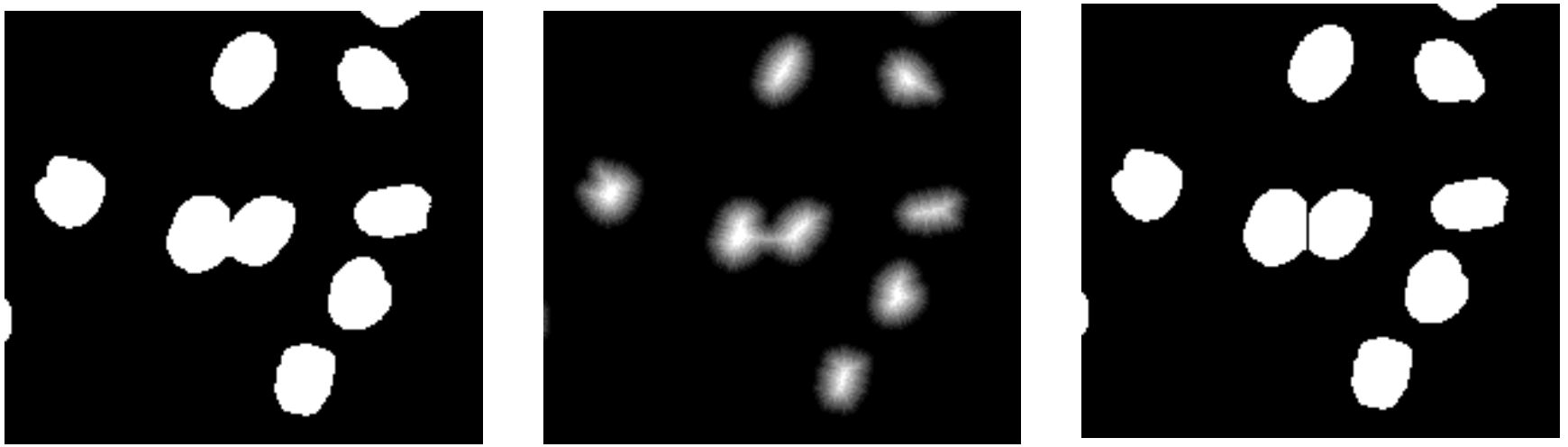


Raw Data

Otsu Threshold

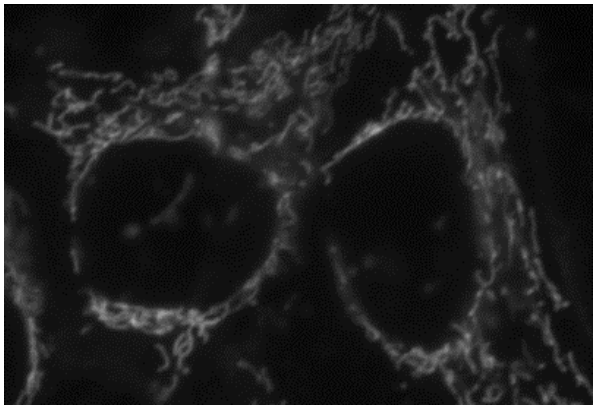
Li Threshold

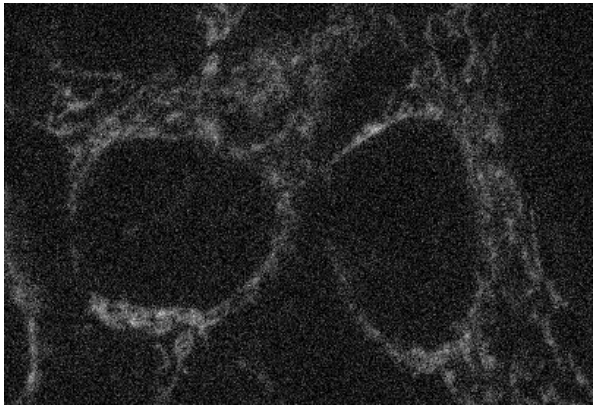
Watershed transformation to separate touching objects



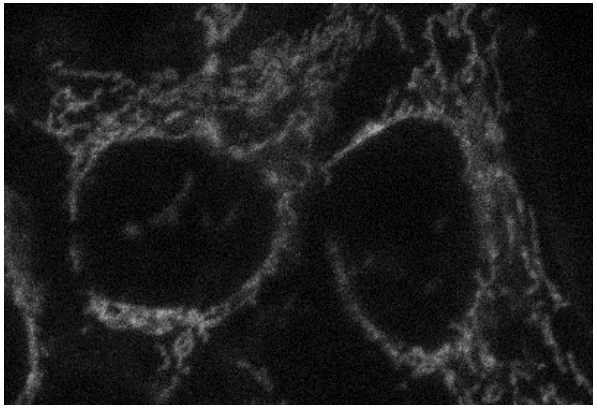
- Seeds placed at local minima of distance map and dilated
- This can be visualised as flooding the distance map

Noise is image corruption from the acquisition process

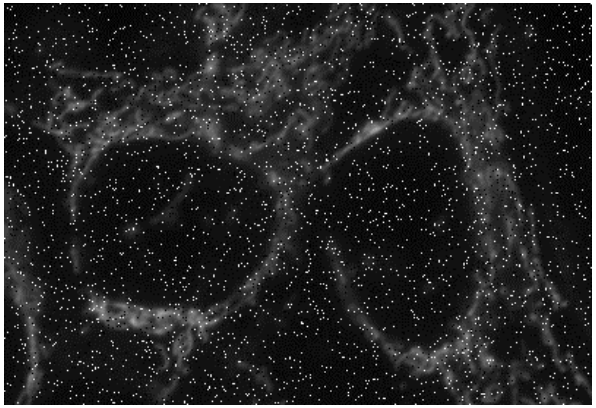




Gaussian



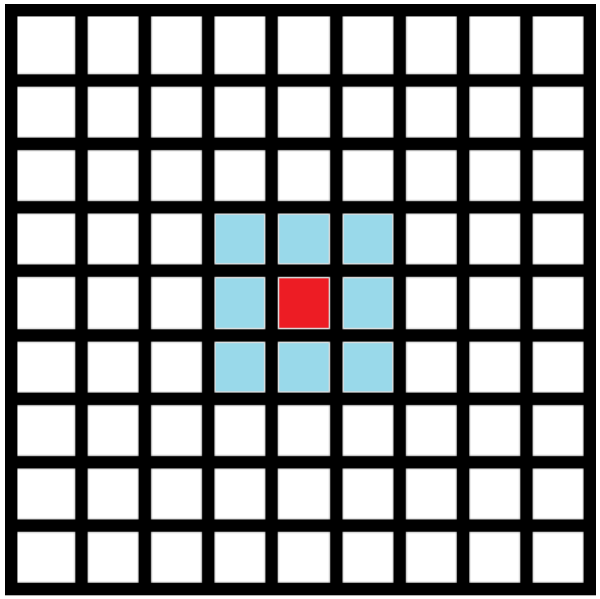
Poisson



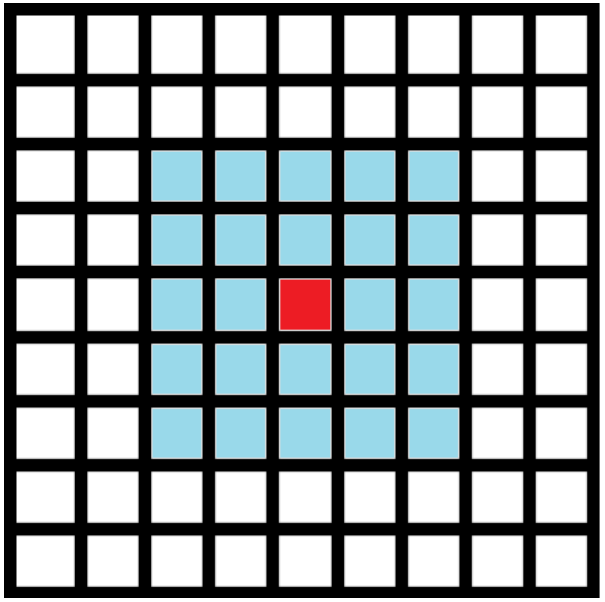
Impulse

Image filters and convolution

The value for a pixel in the filtered image is dependent on pixels in the local neighbourhood



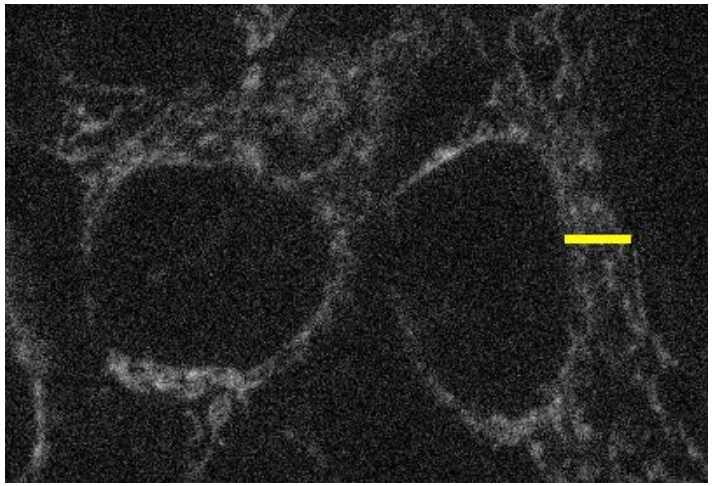
3x3 Neighbourhood

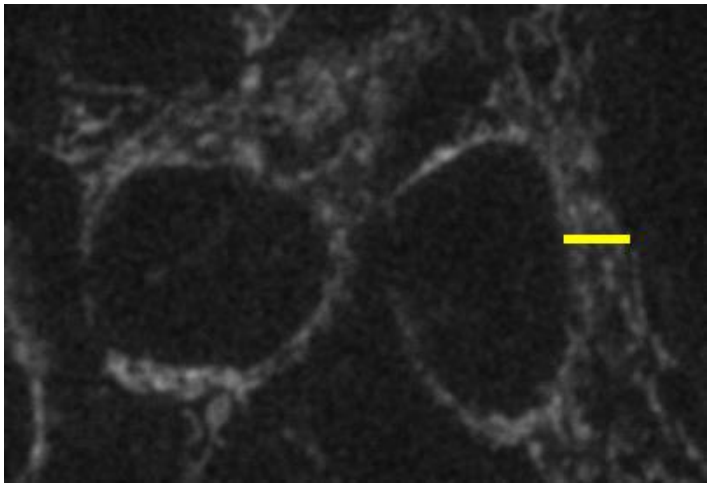


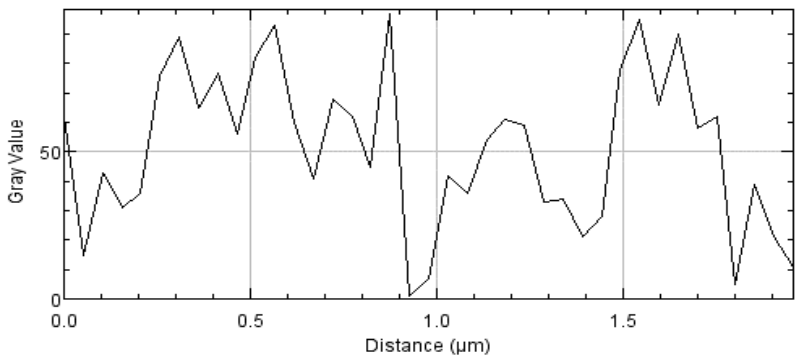
5x5 Neighbourhood

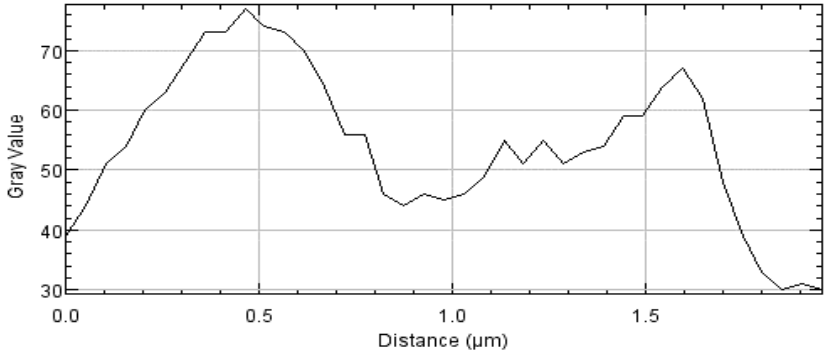
Mean filter

- Pixel values given by mean over neighbourhood
- Removal of Gaussian and Poisson Noise



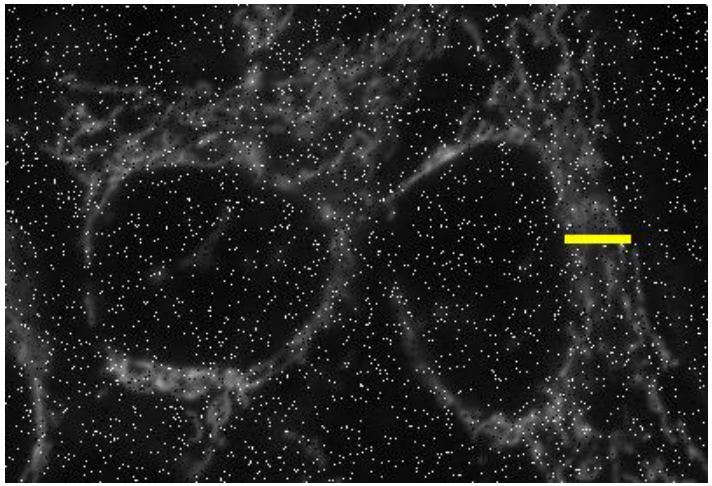


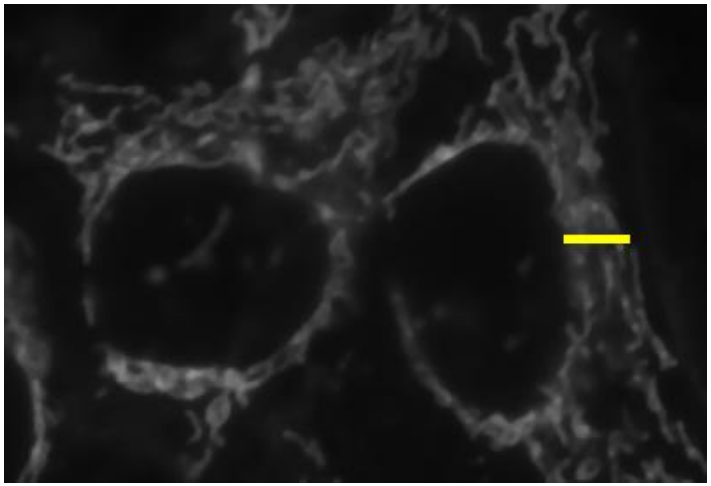


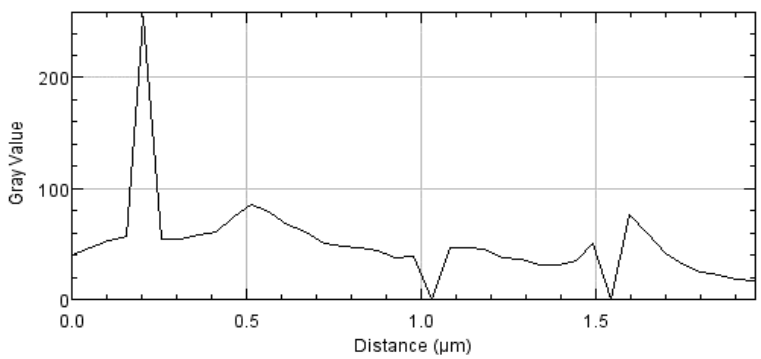


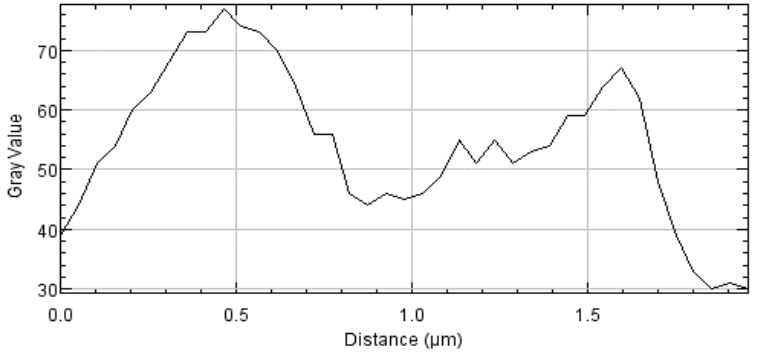
Median filter

- Pixel values given by median over neighbourhood
- Removal of salt and pepper (impulse) noise



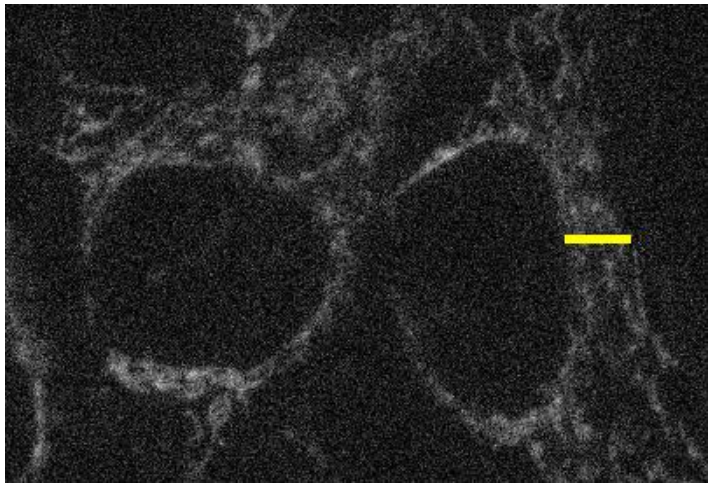


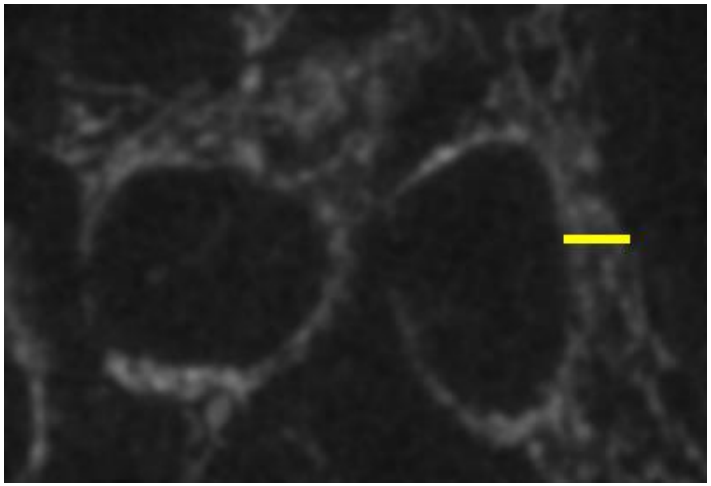


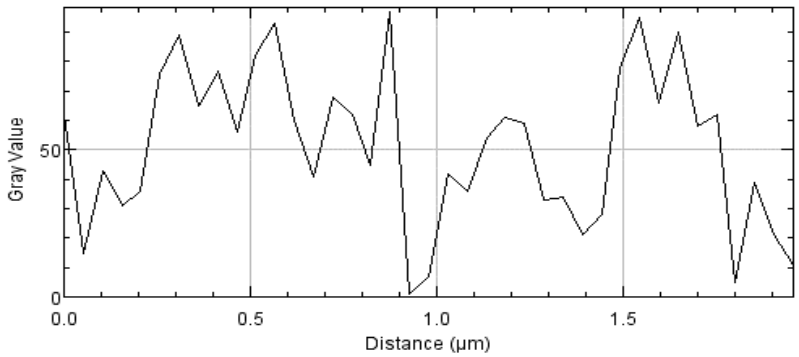


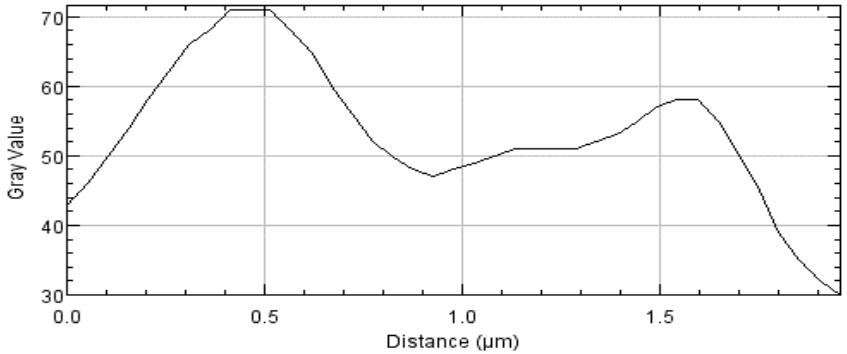
Gaussian filter

- Contribution of neighbourhood pixels weighted by Gaussian profile
- Removal of Gaussian and Poisson Noise





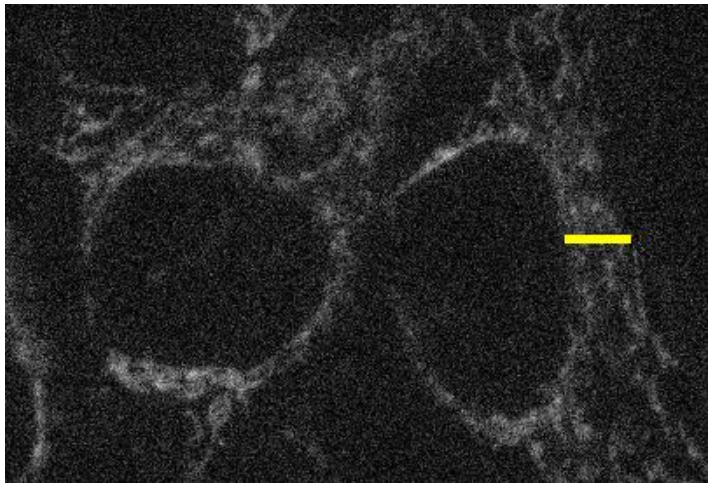


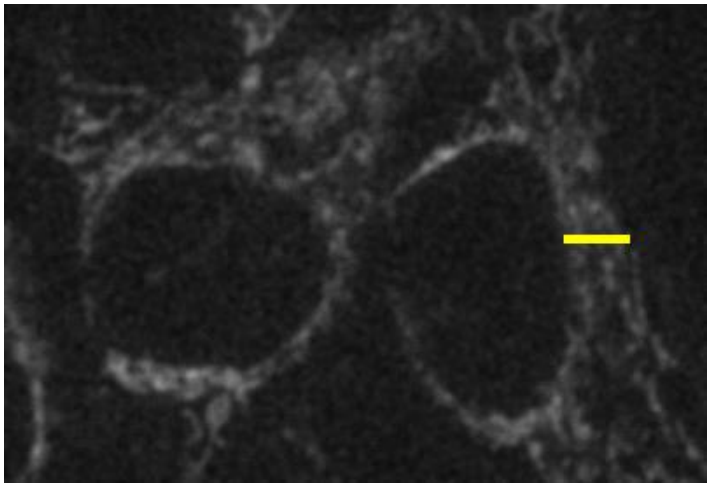


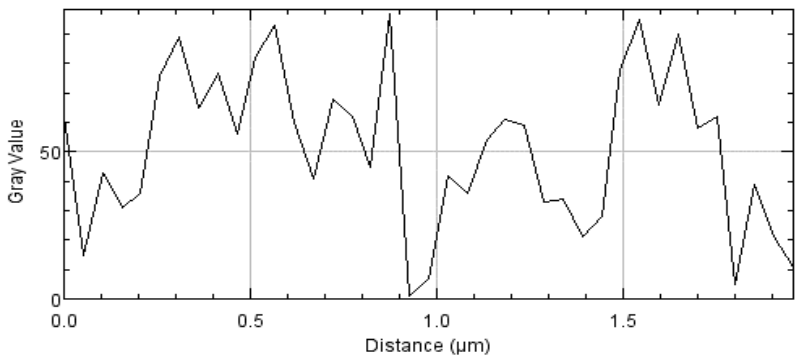
The PureDenoise plugin

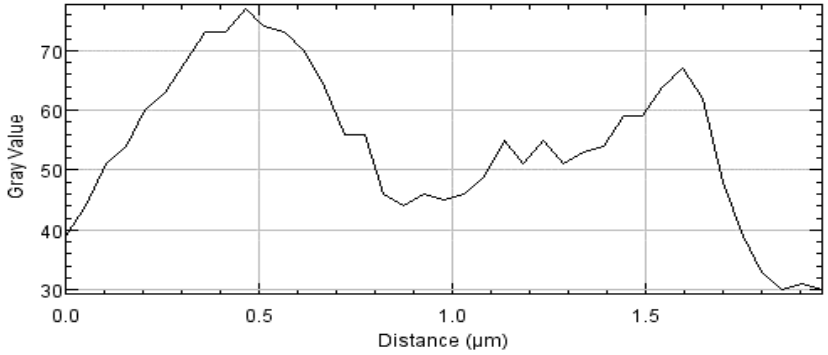
- Sophisticated plugin for removal of Poisson noise

F. Luisier, C. Vonesch, T. Blu, M. Unser (2010). Sig. Process., 90, 2, 415-427.





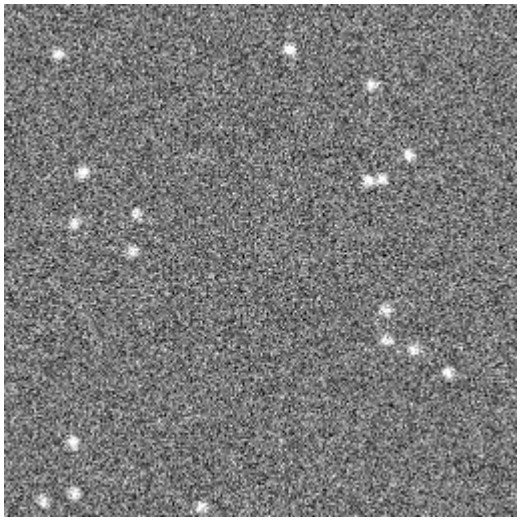


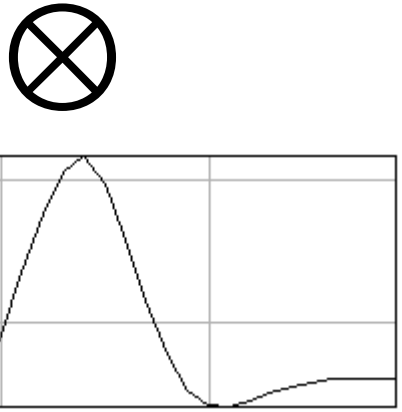


IN PARTNERSHIP:
The Universities of Birmingham and Nottingham

COMPARE
CENTRE OF MEMBRANE PROTEINS AND RECEPTORS

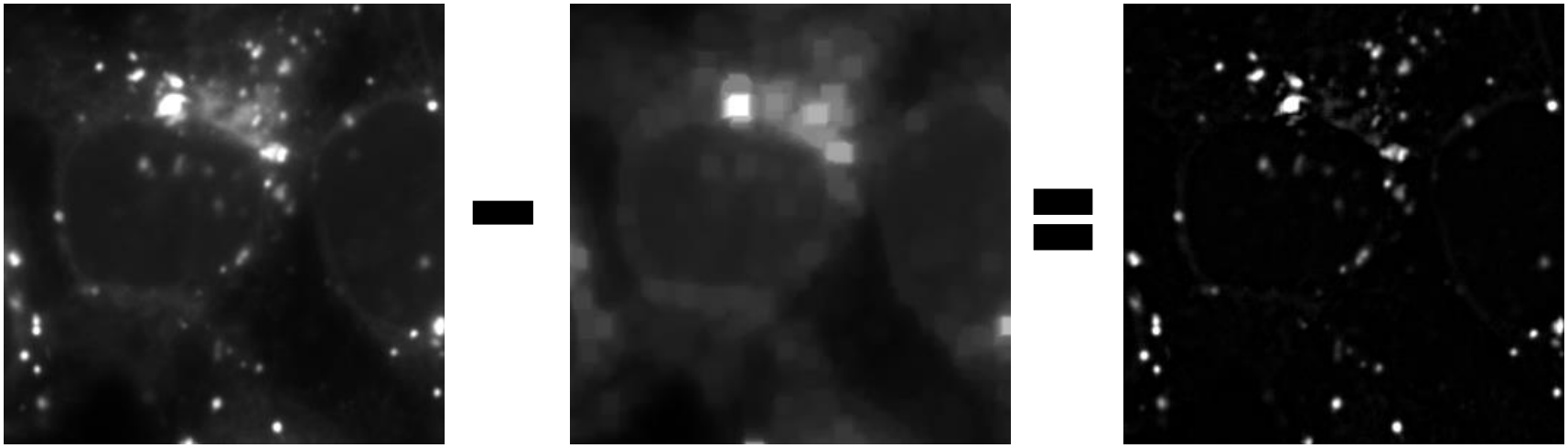
Laplacian of Gaussian filter for spot detection



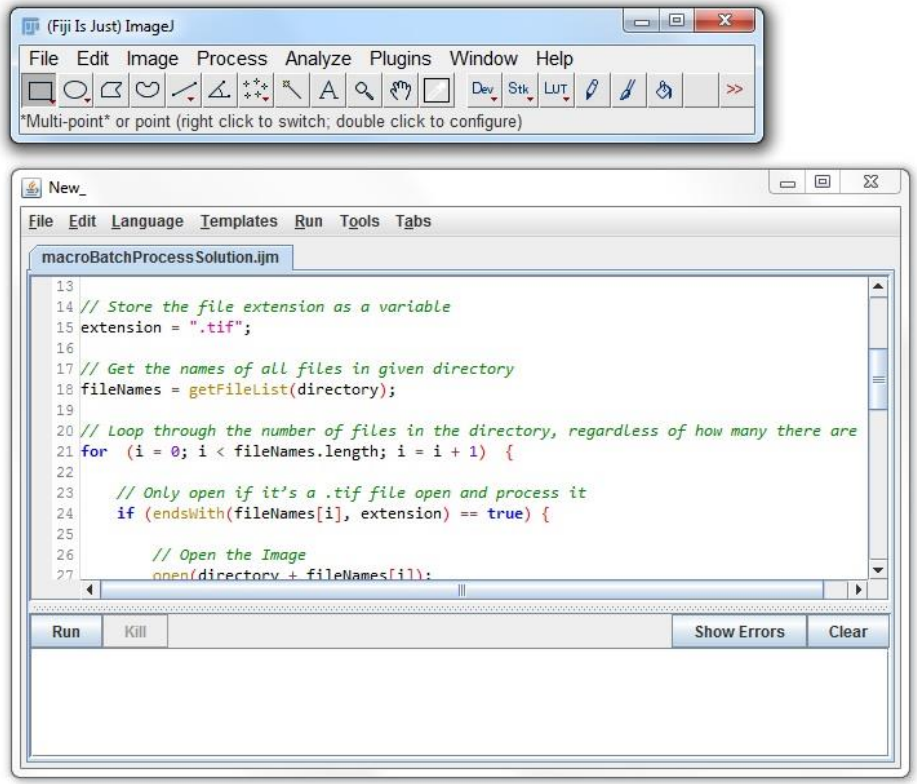


Rolling ball background subtraction

- Background calculated using the mean of a circular local neighbourhood
- The radius of the “ball” should be at least as large the radius of the largest target



Part 3: Introduction Macros and Workflow Automation



Common file formats

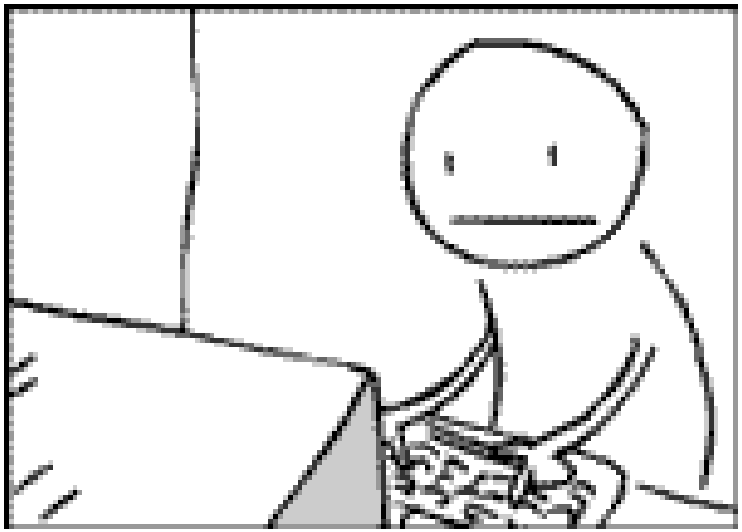
• **TIFF** is a good choice
– Lossless storage of data
– Header tags for metadata

• **Proprietary formats from microscope vendors** (e.g. lif, nd2, czi)
– Often just a TIFF wrapper
– Easy handling or 5D data, and lots of metadata added automatically
– The Bio-Formats plugin will load most formats

• **PNG** should only be used for transfer and display
– Lossless compression
– No metadata
– RGB only

• **JPEG** should not be used for scientific images
– Lossy compression discards information and causes artefacts

Why automate a workflow?



- Save time and eliminate user mistakes
- Unbiased and consistent approach
- Have a record off what you have done

A macro is just a sequence of ImageJ commands

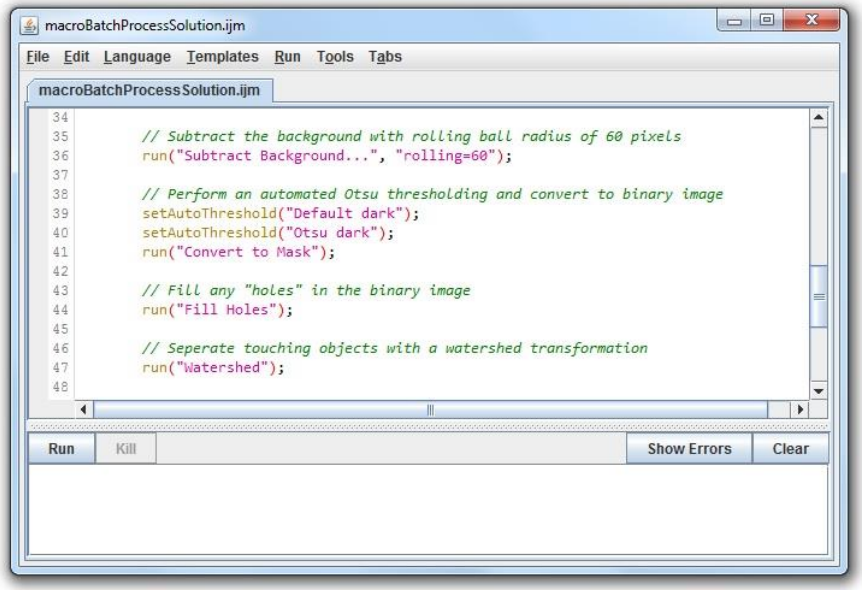
- Simple, easy to learn language
- Calls predefined ImageJ and Java functions
- Any ImageJ menu item can be called with a macro command
- Lots of online tutorials examples and resources including:

http://fiji.sc/Introduction_into_Macro_Programming

The Fiji script editor

Plugins -> New -> Macro (or press {})

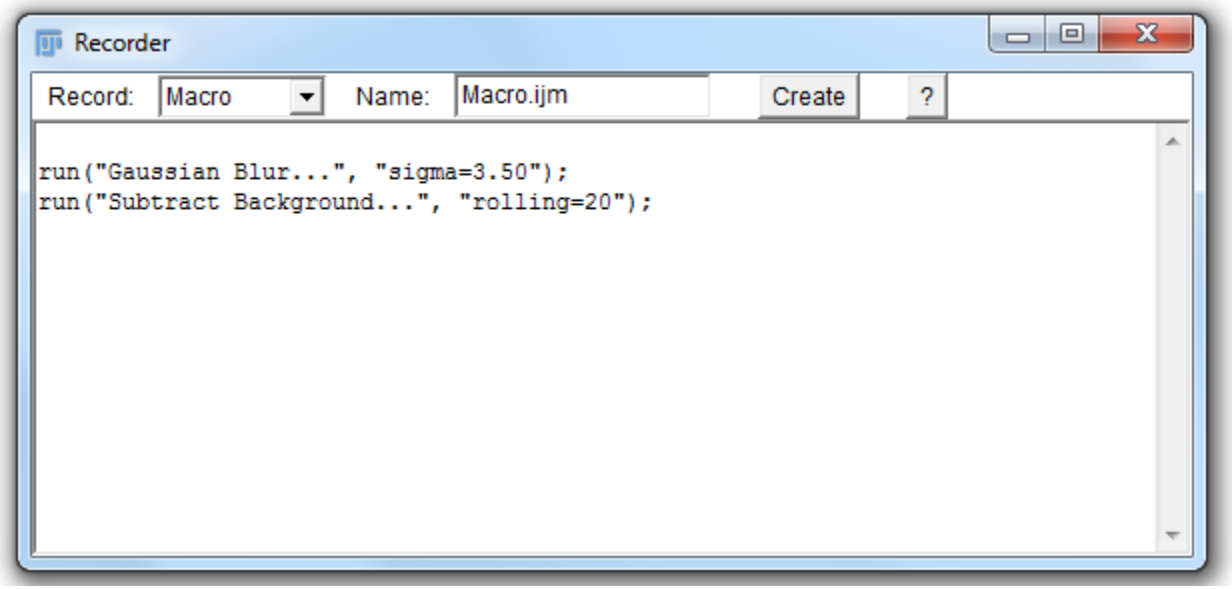
- Syntax highlighting
- Templates menu contains some useful examples



The Command Recorder

Plugins -> Macros -> Record…

- A really simple way to automate a workflow and make a Macro
- Simply perform the analysis on one image and click create!



For loops

An iterative statement that executes a block of code a specified number of times.

```
for (initialisation; stop condition; increment) {
        do something
    }
```

```
for (i = 1; i <= 10; i = i + 1) {
        run("Add...", "value=" + i);
}
```

Conditional statements

if (condition) {do something}
else if (other condition) {do this instead}
else {do the alternative}

```
if(nImages() == 1) {
      print(getTitle() + " is open.");
}
else if(nImages() > 1) {
      print(nImages() + " images are open.");
}
else {
      print("No images are open.");
}
```

Whats next?

- COMPARE will be organising further courses covering:
  - Segmentation, deconvolution, tracking, colocalization etc
  - Analysis and visualisation of light sheet and SMLM datasets
  - Any suggestions?

- Loads of online resources for further study:
  - ImageJ website
  - ImageJ forum
  - Open source image analysis textbook

- I can work with you on collaborative projects.
  Email (j.a.pike@bham.ac.uk) for enquires.

Acknowledgments

This course was organised and run by the Centre of Membrane Proteins and Receptors (COMPARE), a partnership between the Universities of Birmingham and Nottingham.

http://www.birmingham-nottingham.ac.uk/compare/

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Richard Butler

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Mark Dunning
Stefanie Reichelt