Image Processing and Analysis with FIJI
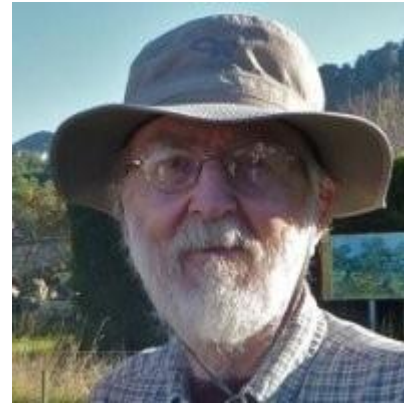
Jeremy Pike

www.lightmicroscopy.cruk.cam.ac.uk/
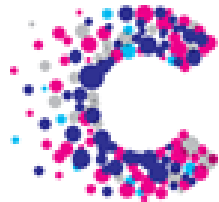
With slides from Richard Butler

imaging.gurdon.cam.ac.uk/

ImageJ is open source image processing and analysis software

Developed by Wayne Rasband:

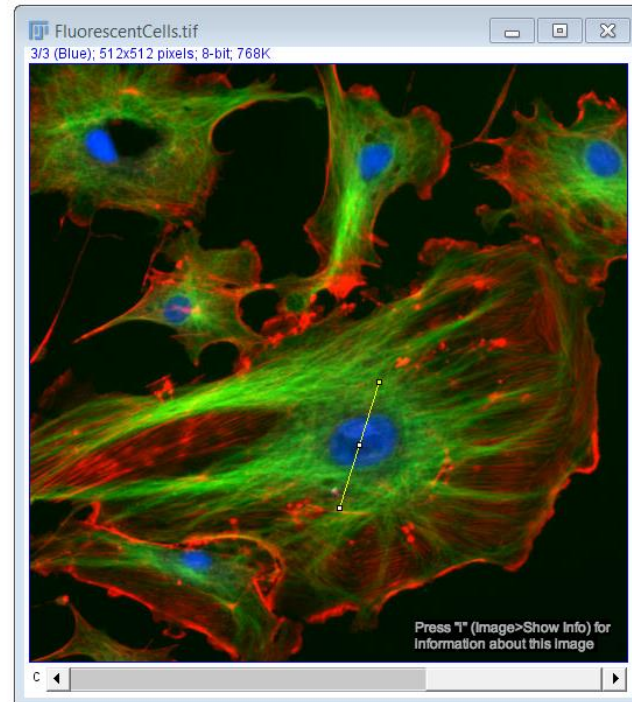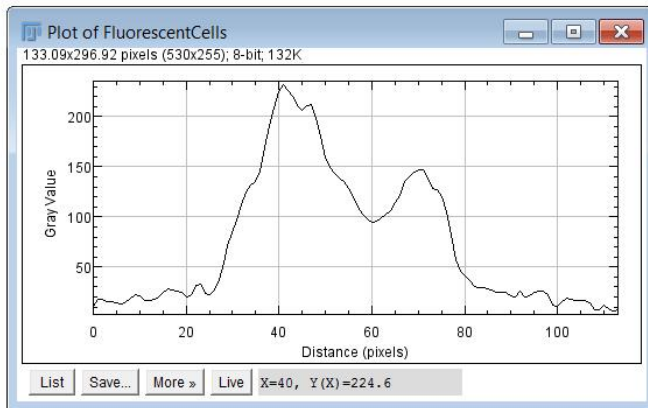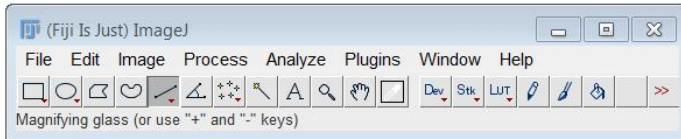*Schneider, C. A.; Rasband, W. S. & Eliceiri, K. W. (2012), Nature methods **9(7)**: 671-675*.

FIJI is a ImageJ distribution with lots of useful plugins pre-installed

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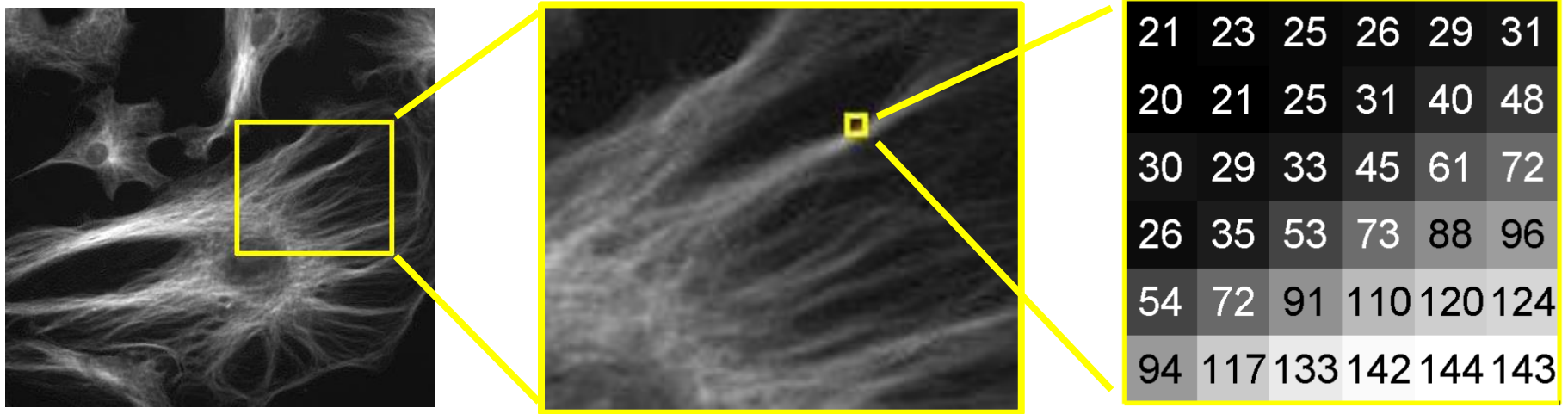
Why use FIJI?

- Open source and popular!

- User-written plugins make it possible to solve almost any image processing or analysis problem

- Great for beginners all the way to developers

- Macros for easy automation

Part 1: Basics

Digital images are simply arrays of numbers

21	23	25	26	29	31
20	21	25	31	40	48
30	29	33	45	61	72
26	35	53	73	88	96
54	72	91	110	120	124
94	117	133	142	144	143

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

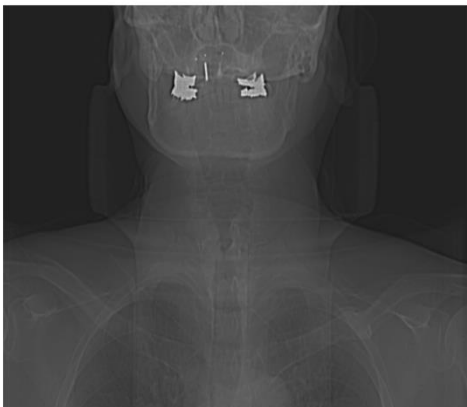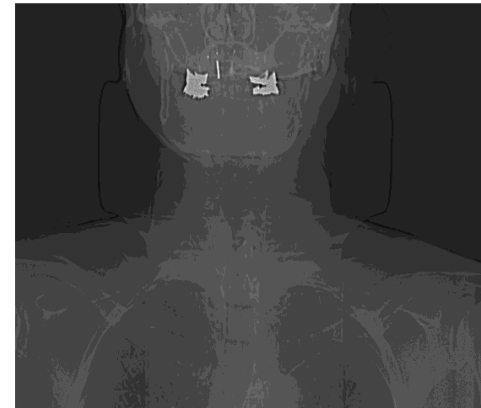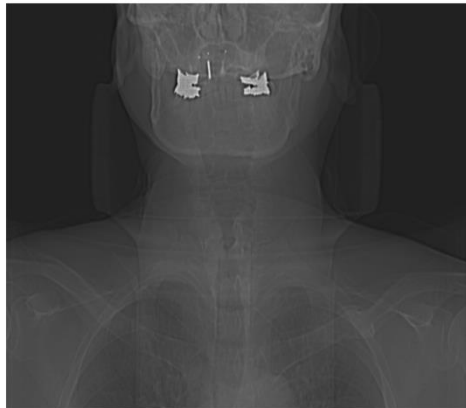# Dynamic range is the number of values each number can take
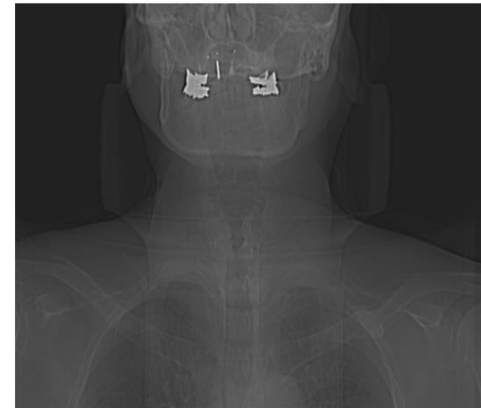
1 bit = 2	2 bit = 2^2	4 bit = 2^4

8 bit = 2^8	12 bit = 2^{12}	16 bit = 2^{16}

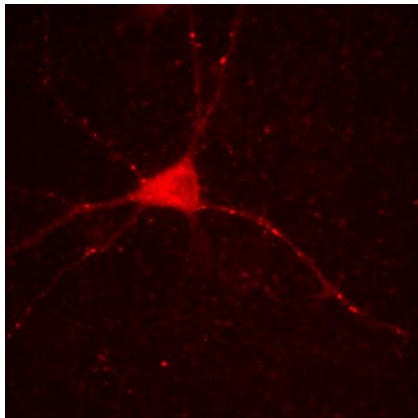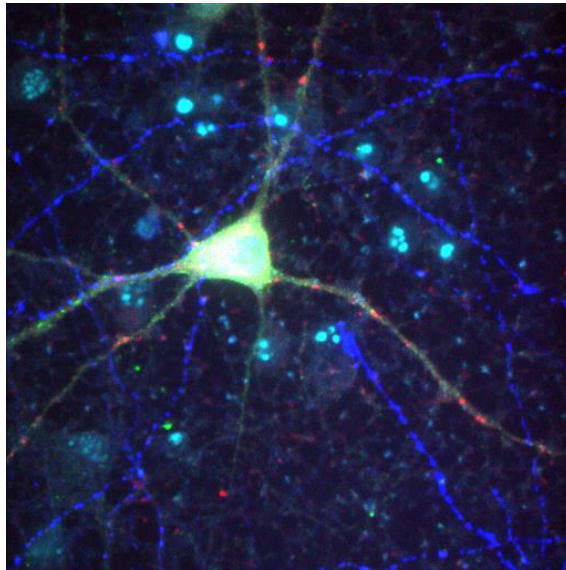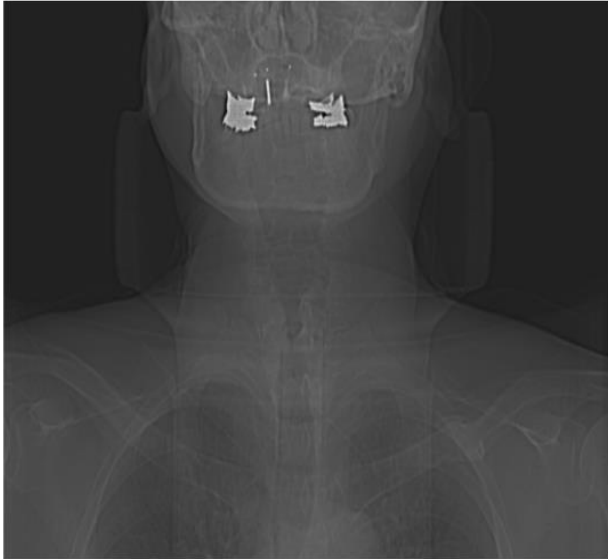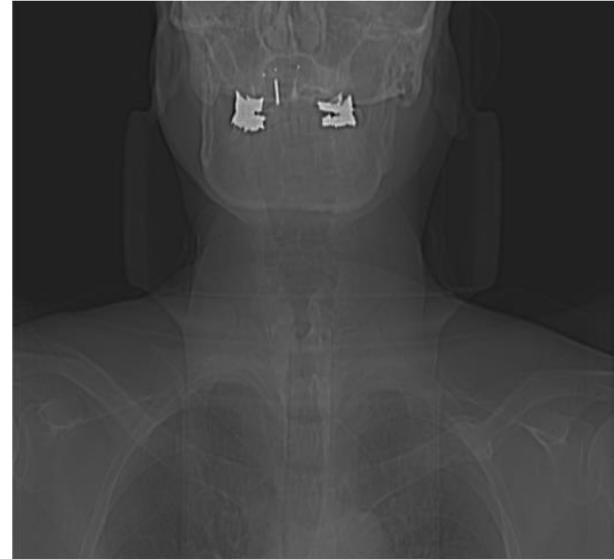# Multi Channel Data
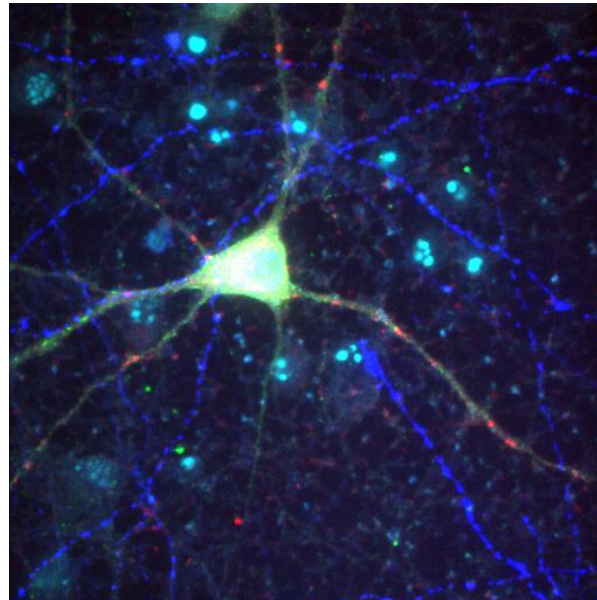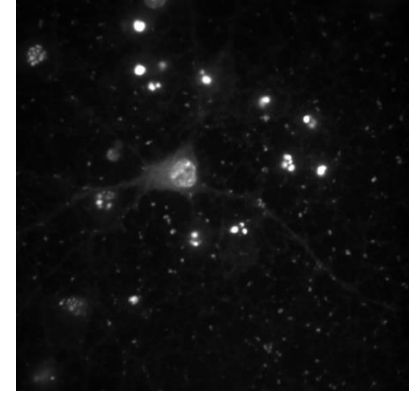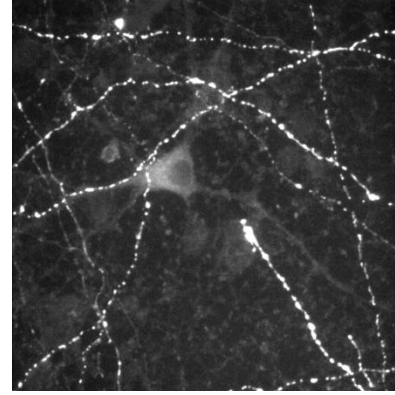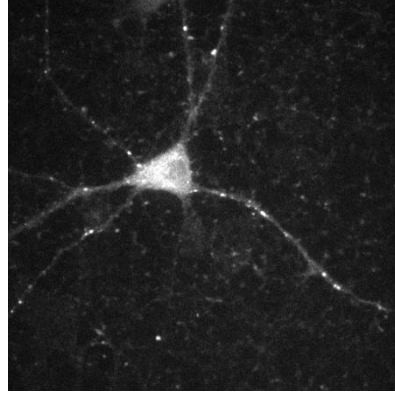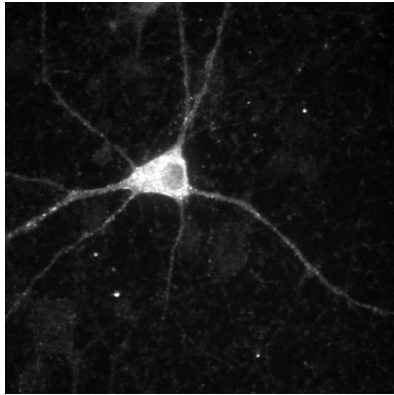
RGB Color Images
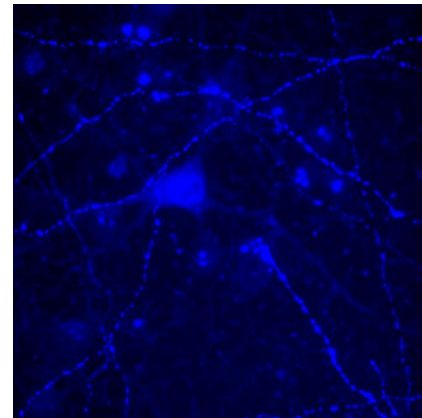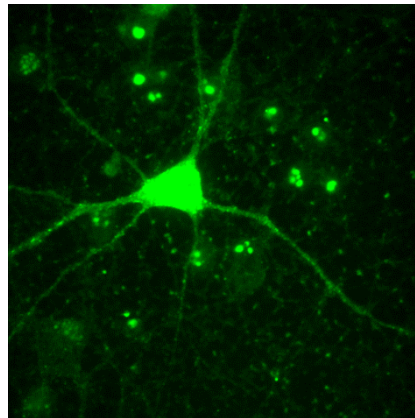
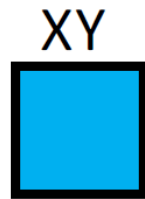# What can we "see" on a monitor
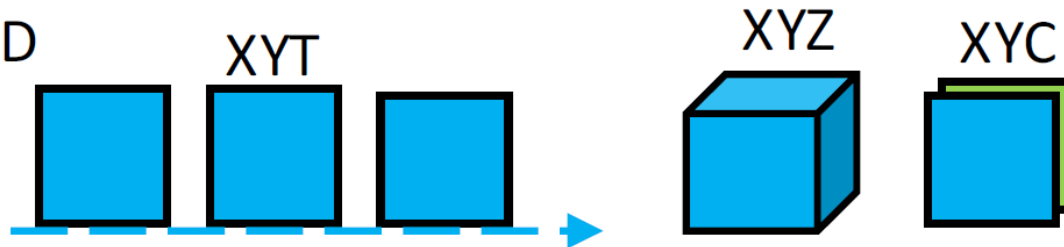

8 bit = 2^8

16 bit = 2^{16}

- 8 bit display range

- 3 x 8bit RGB for color display

- Human eyes cant distinguish between more than approximately 900 grey levels
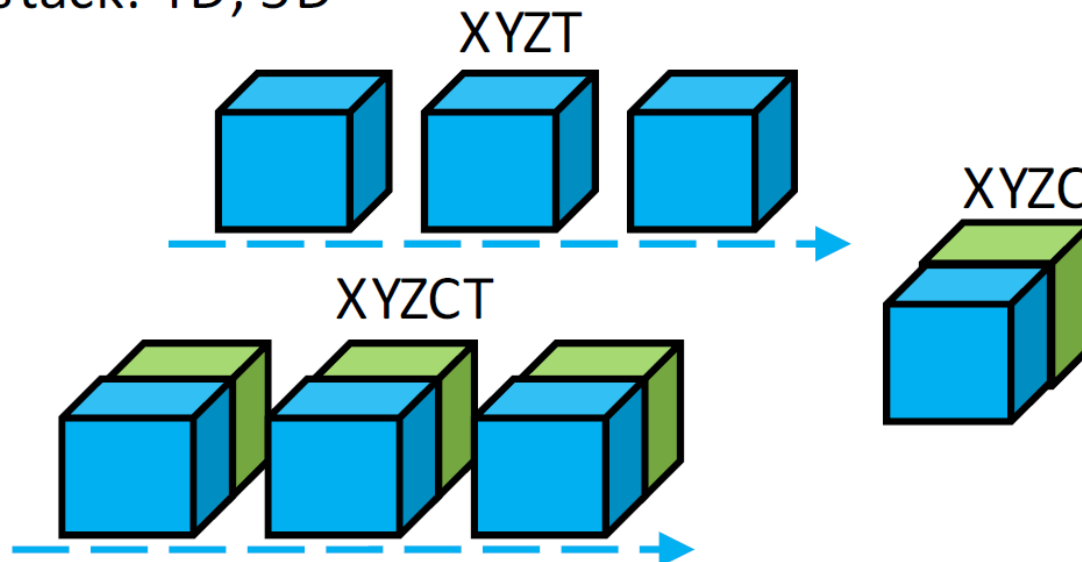
# Image Stacks

- Image: 2D

  XY

- Stack: 3D

  XYT   XYZ   XYC

- Hyperstack: 4D, 5D

  XYZT

  XYZCT   XYZC

Common file types:

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

- **Proprietary formats** (Eg. lif, oib) are good if you can open them
  – Often just a TIFF wrapper
  – Encode everything about the image

- **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 artifacts
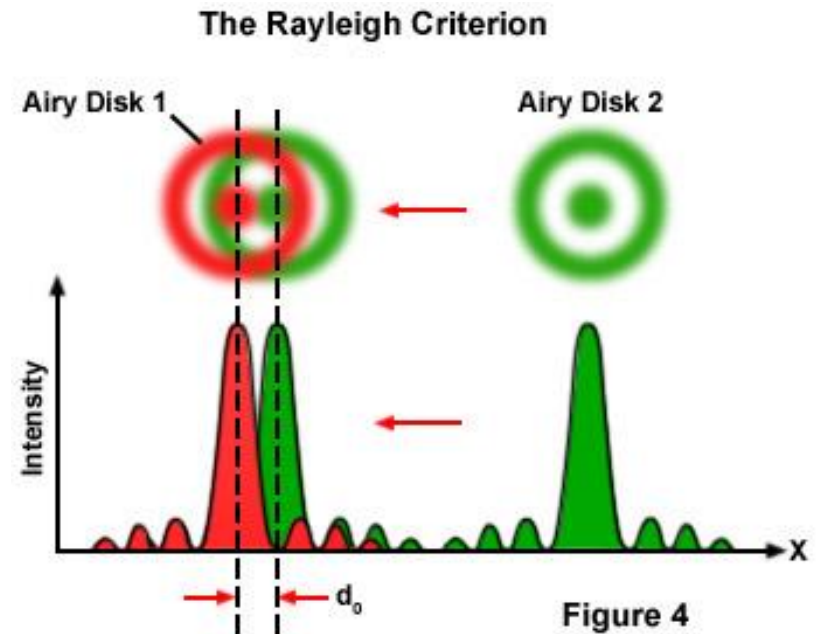
An image is the sum of its point spread functions
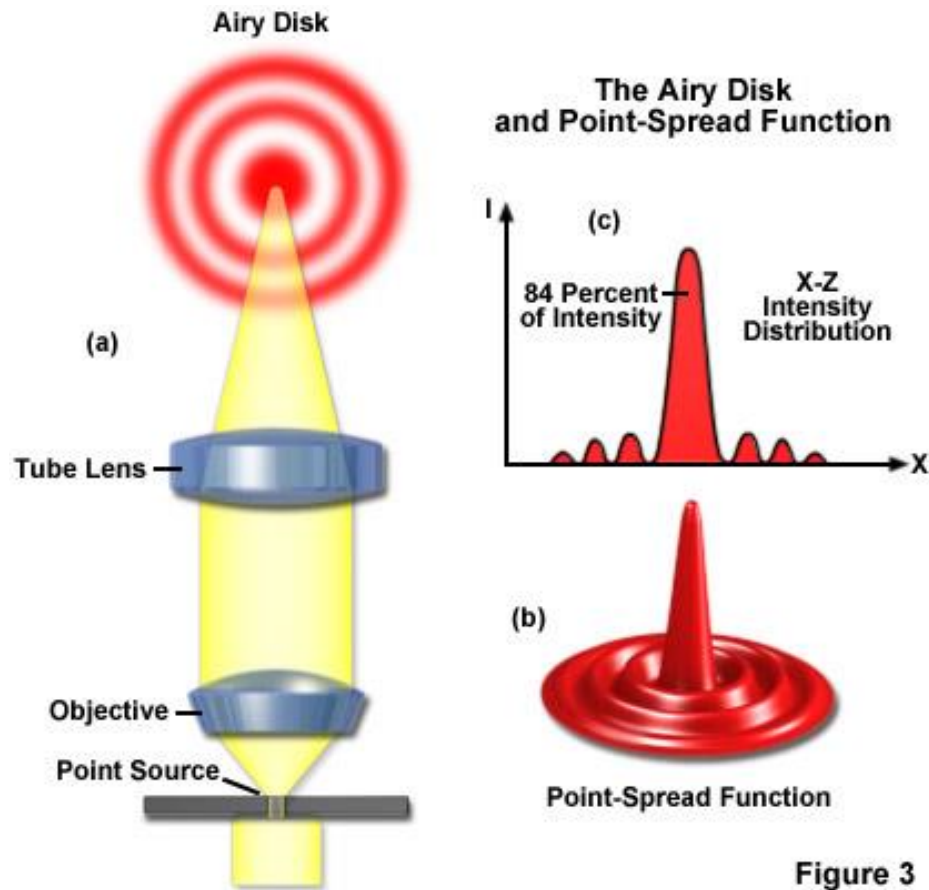
"*Each element of the primary image is a small diffraction pattern, and the actual image, as seen by the eyepiece, is only the ensemble of the magnified images of these patterns*"

Born and Wolf, Principles of Optics

What determines resolution limit in 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

The Rayleigh Criterion

Figure 4

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

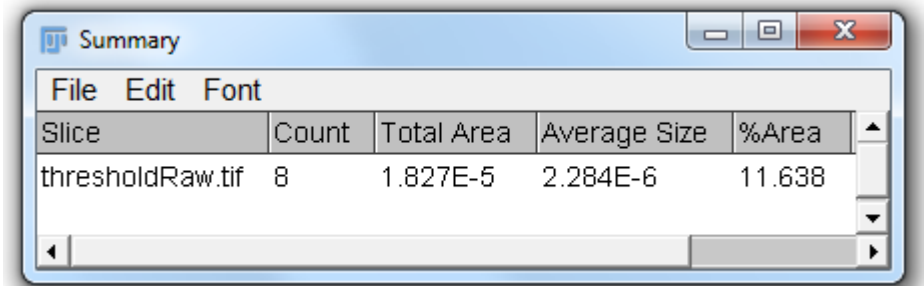# Part 2: Introduction to Image Processing and Analysis

# Image Processing:
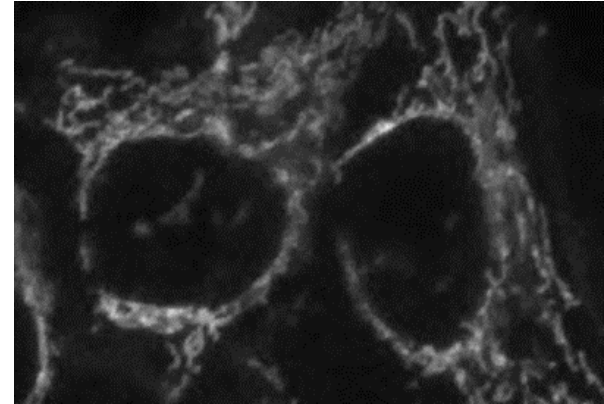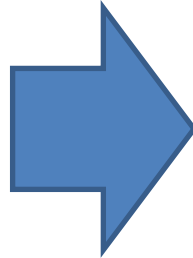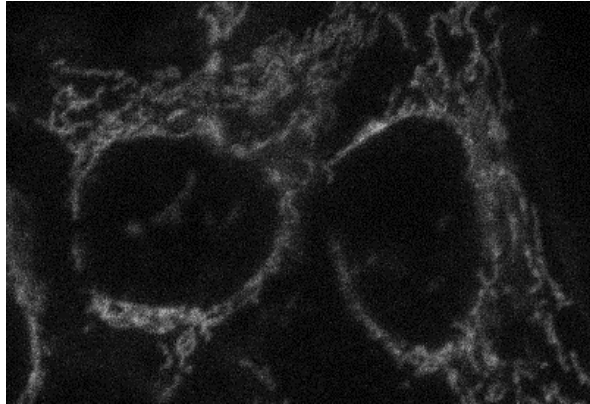
Image Analysis:
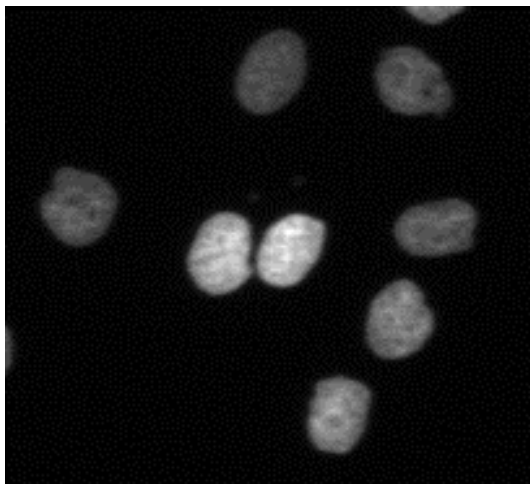
Why do computational processing and analysis:

- 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."
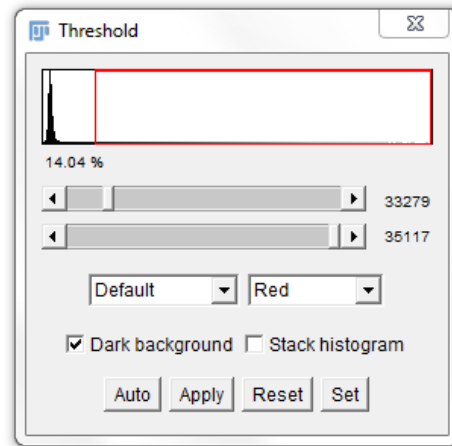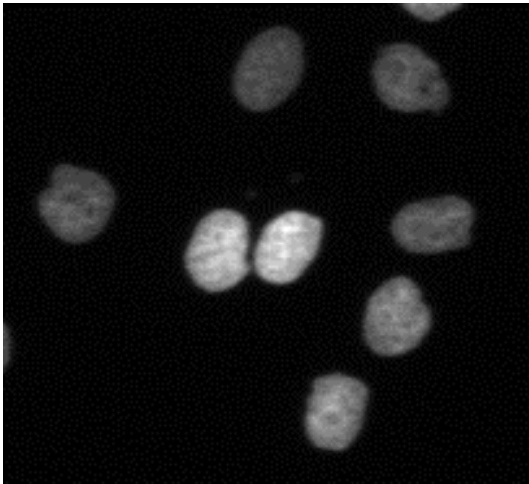***Richard Feynman***

Which outer circle is the same size and the central circle?
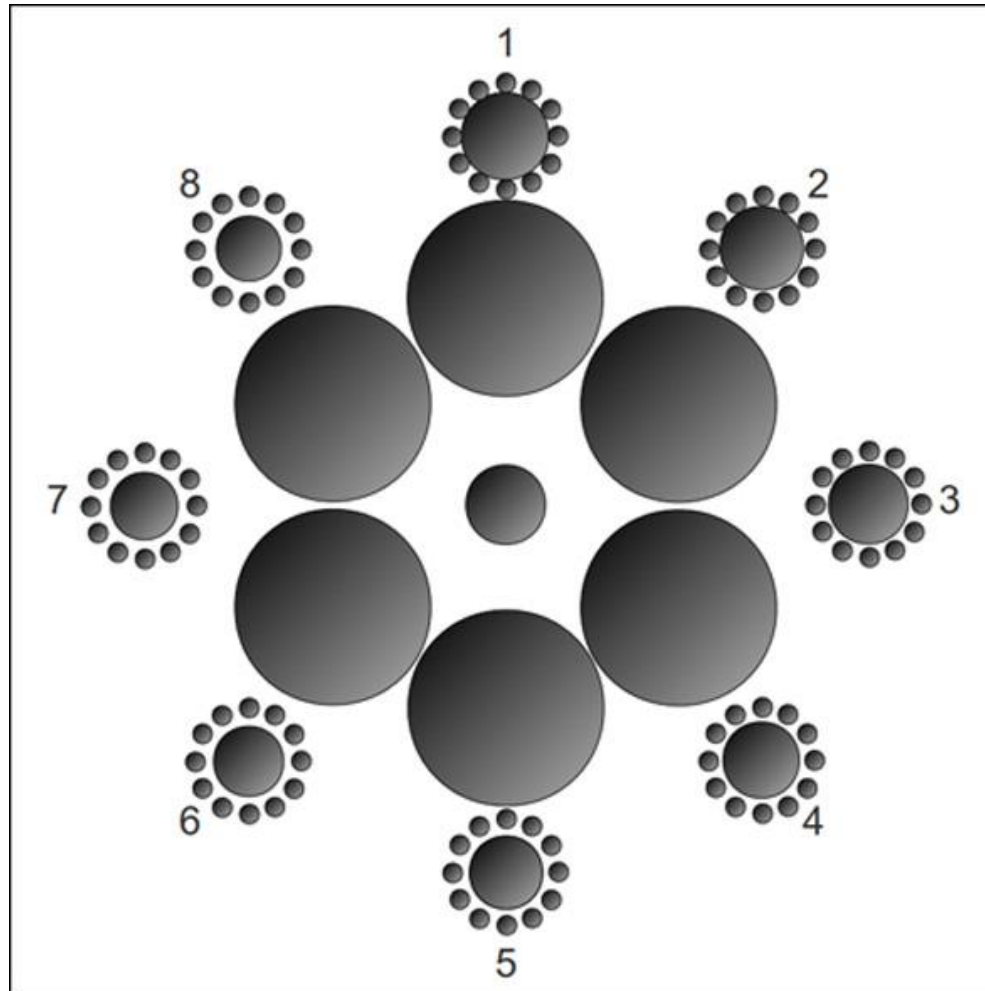
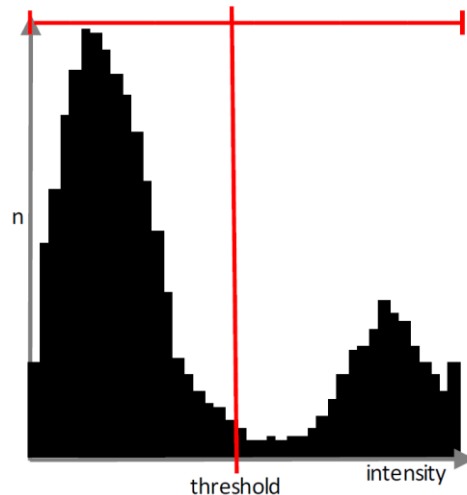# Which outer circle is the same size and the central circle?
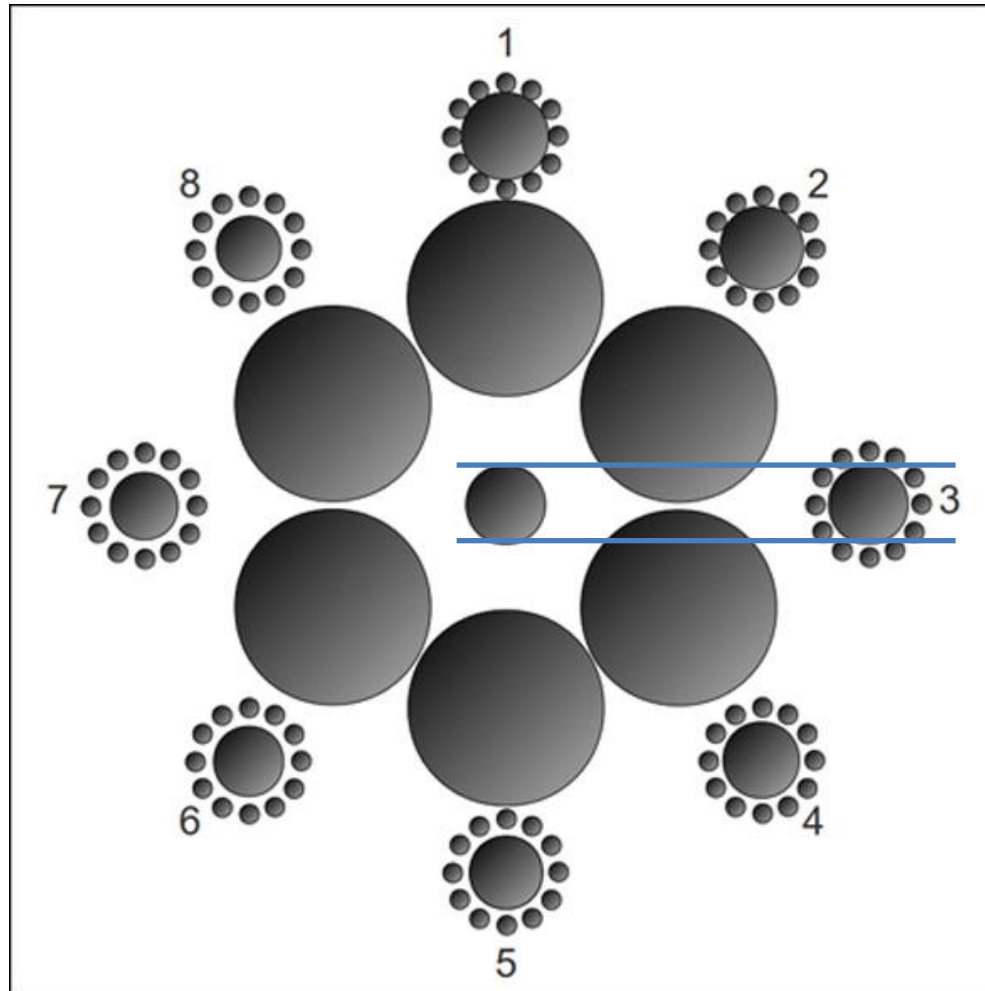
Intensity thresholding to segment objects

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), "A threshold selection method from gray-level histograms", *IEEE Trans. Sys., Man., Cyber.* **9**: 62-66.
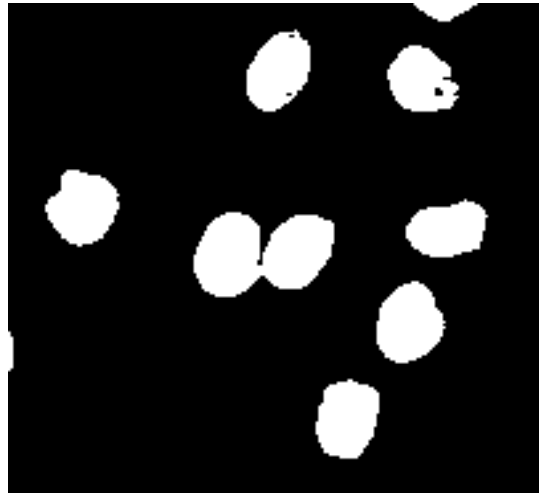
- Li thresholding minimises the cross entropy between the original and segmented images.

Li, CH & Tam, PKS (1998), "An Iterative Algorithm for Minimum Cross Entropy Thresholding", *Pattern Recognition Letters***18(8)**: 771-776
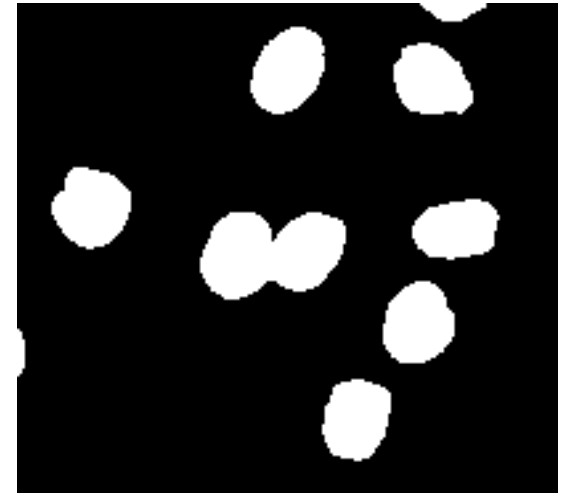
Automated threshold values are preferable to manual selection
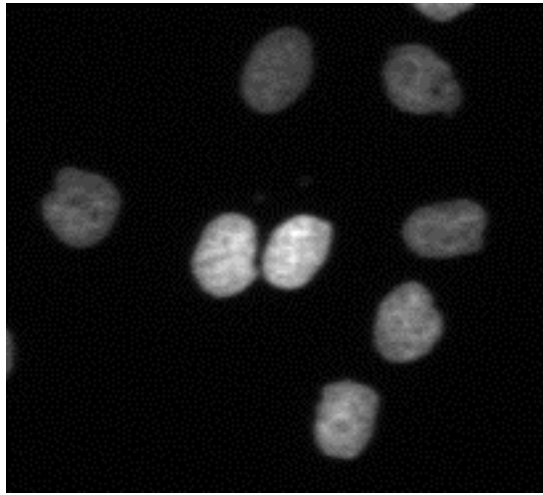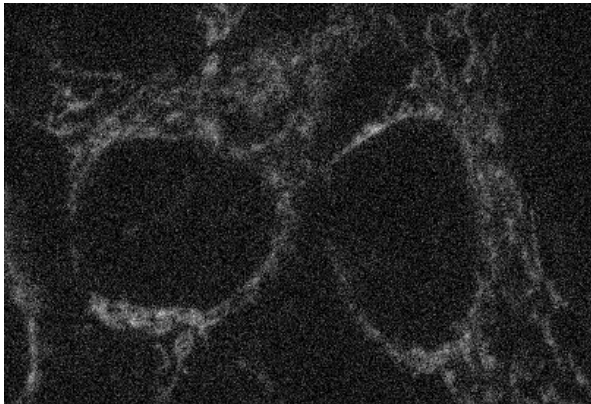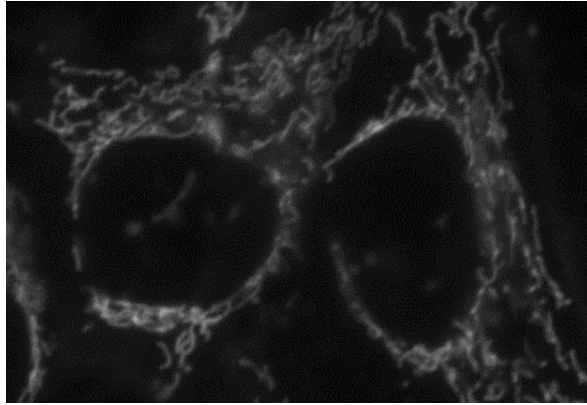

Raw Data

Otsu Threshold

Li Threshold

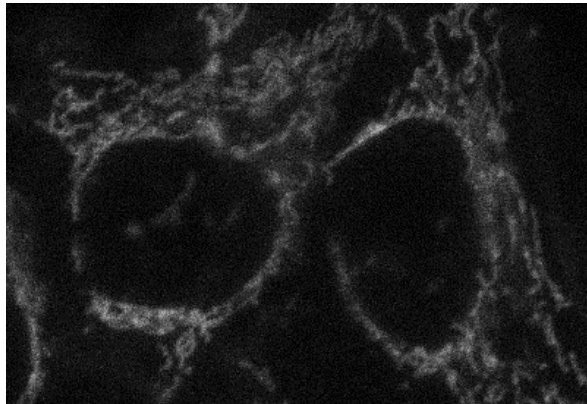# Watershed transformation to separate touching objects

- Seeds put a minimum of distance map and dilated

- This can be visualised as flooding the distance map

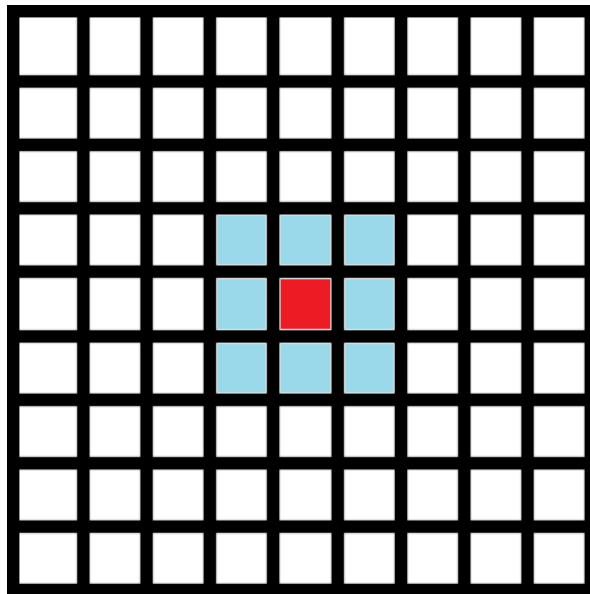# Noise is image corruption from the acquisition process
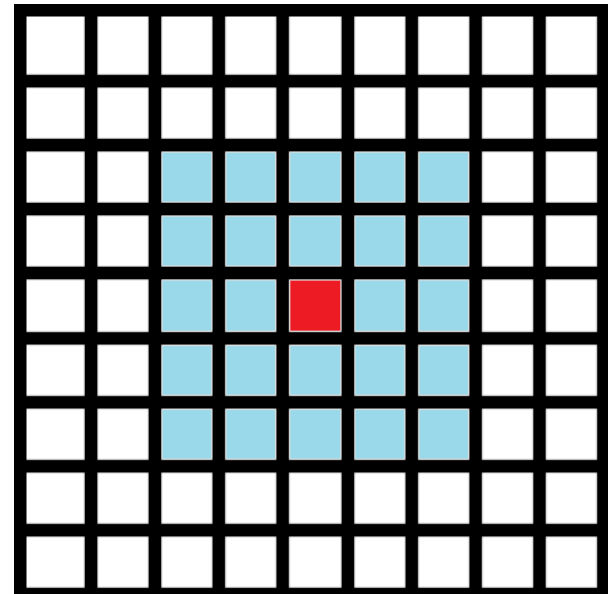

Gaussian

Poisson

Impulse

Image filters and convolution

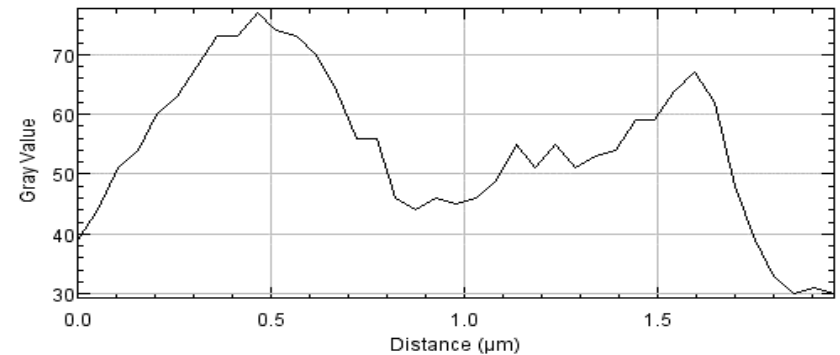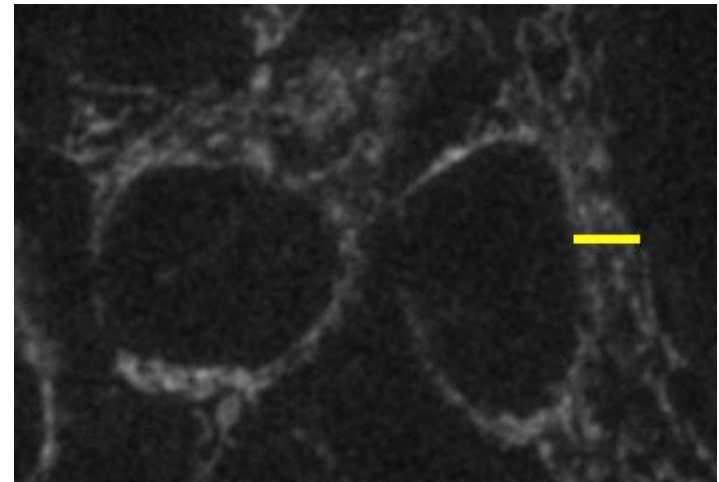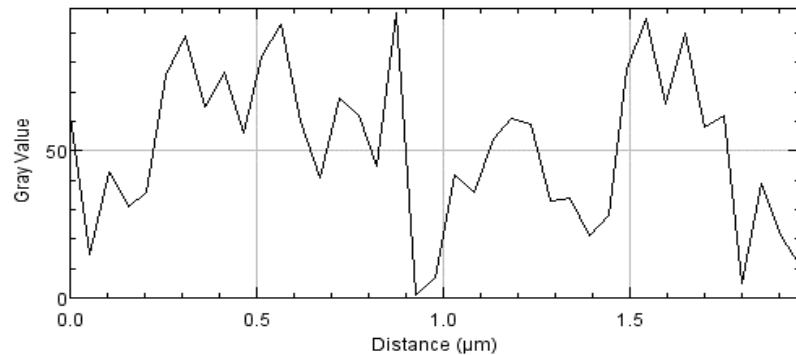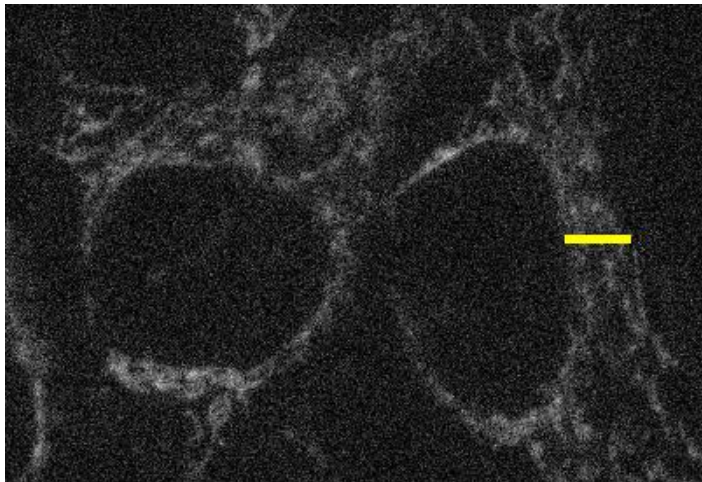The intensity of pixels in the filtered image is dependent on the intensities of neighbouring pixels

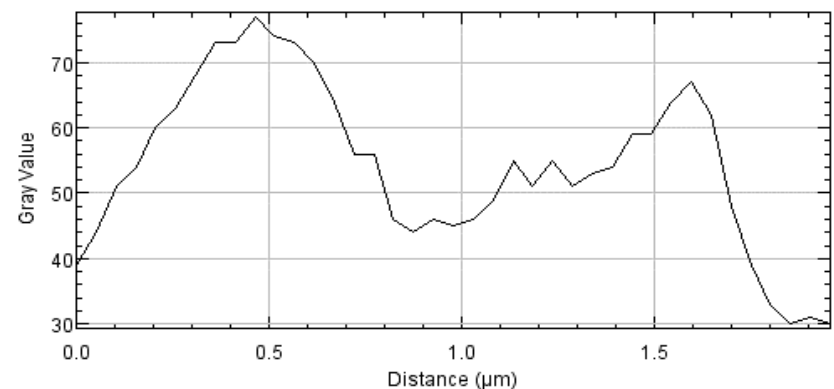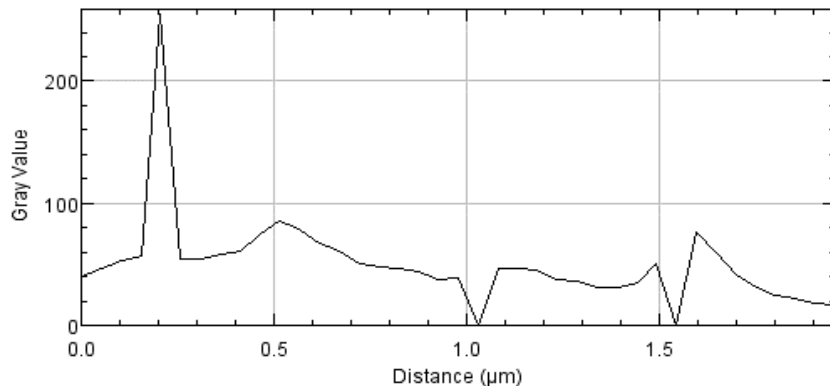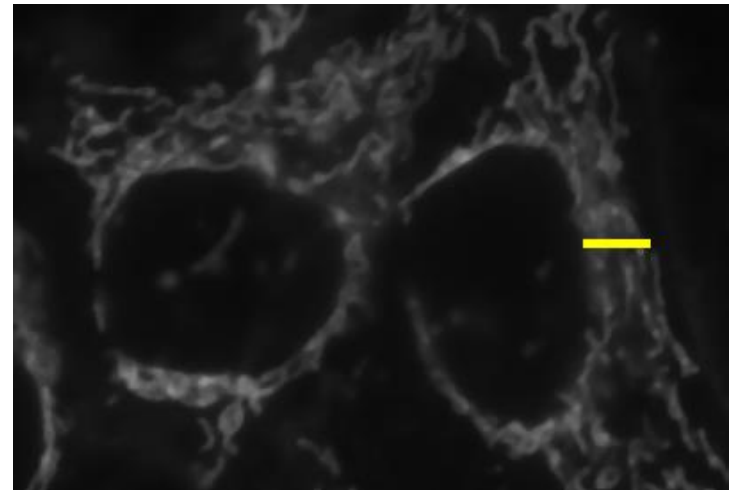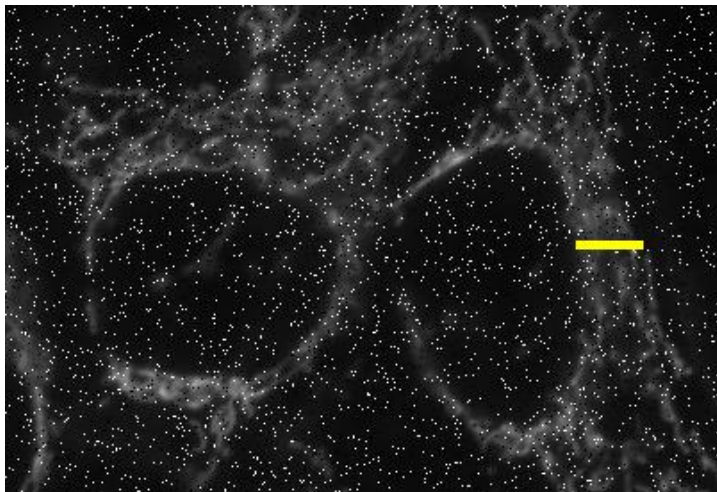3x3 Neighbourhood

5x5 Neighbourhood

Mean filter

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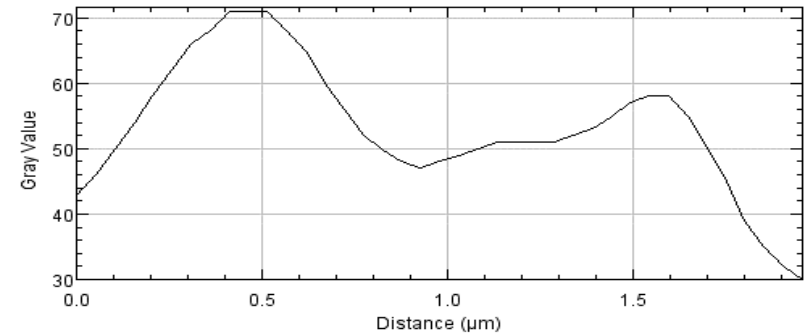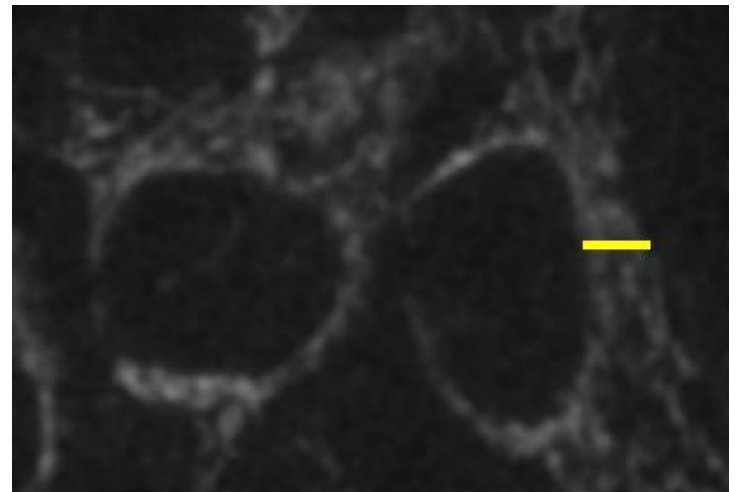
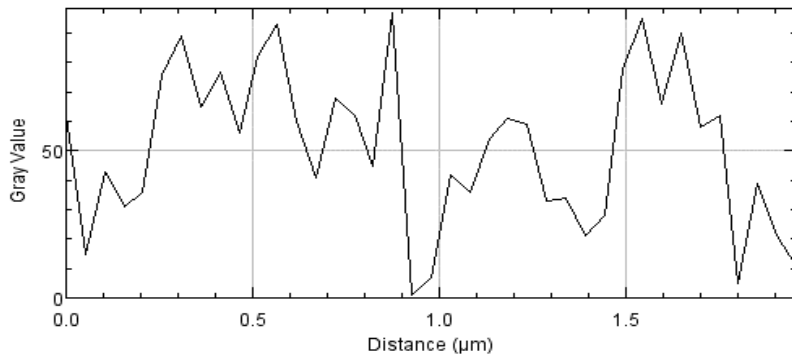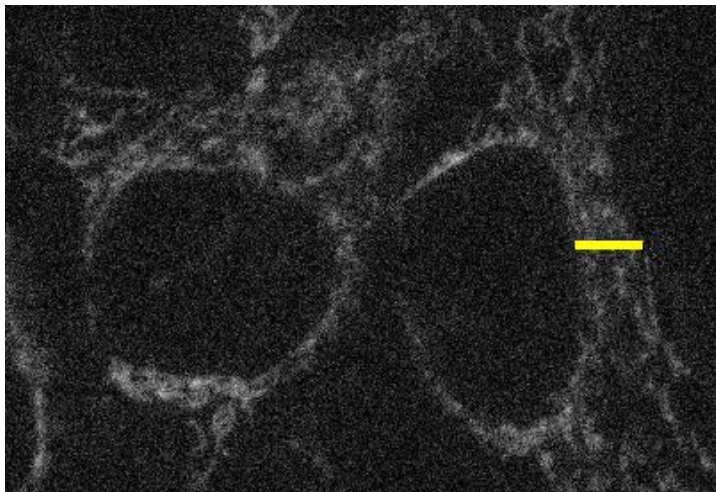- Removal of Gaussian and Poisson Noise

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

- Removal of Poisson noise

- Sophisticated research plugin but estimated parameters from image

F. Luisier, C. Vonesch, T. Blu, M. Unser, Signal Processing, vol. 90, no. 2, pp. 415-427, February 2010.
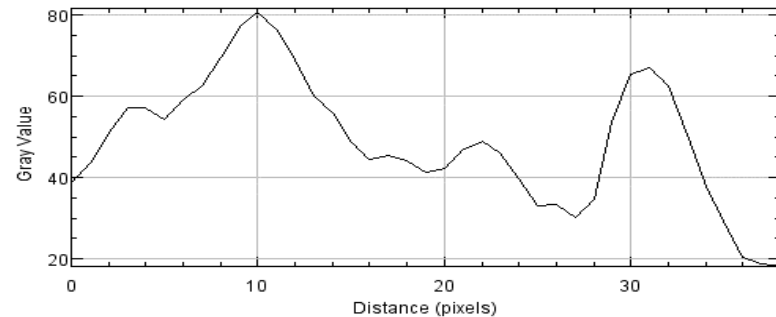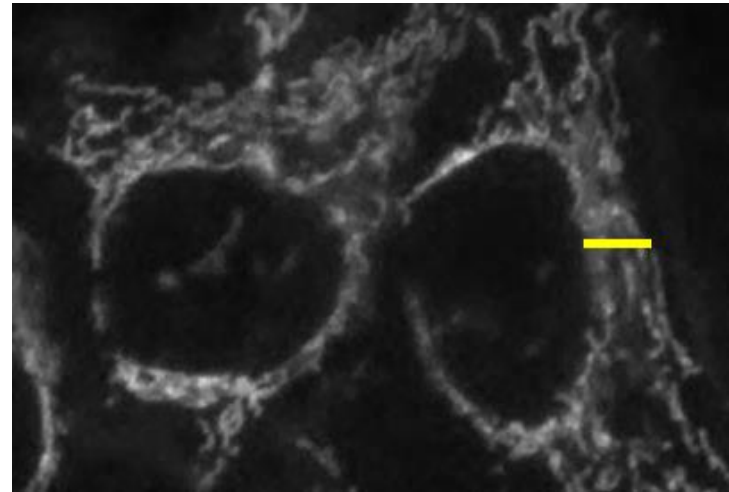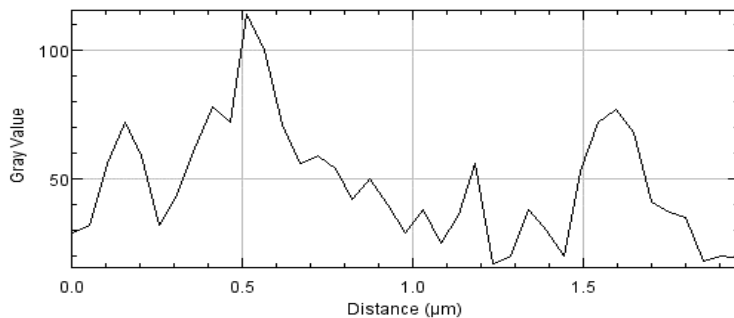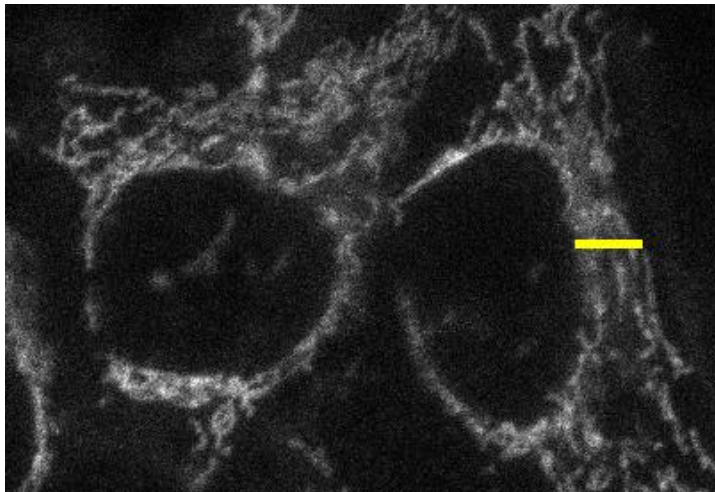
Laplacian of Gaussian filter is great for spot detection

Rolling Ball Background Subtraction

Part 3: Workflow Automation with Recorded Macros

Why automate a workflow?

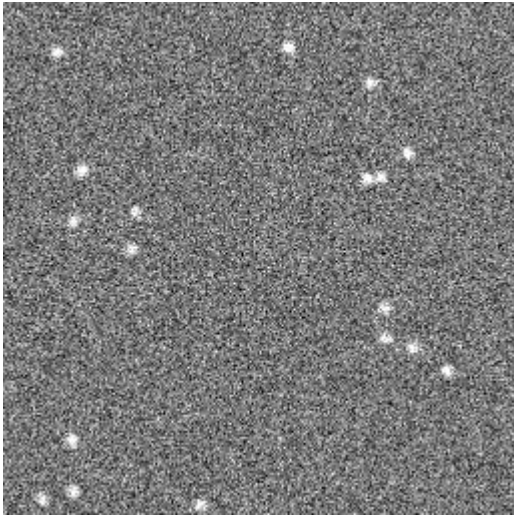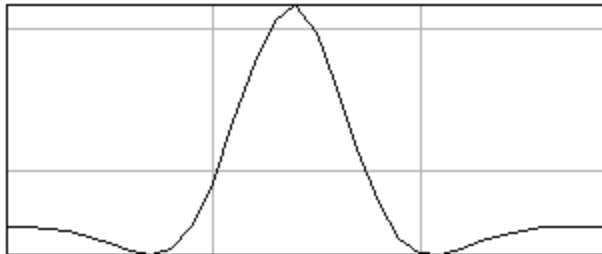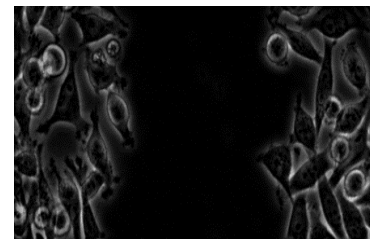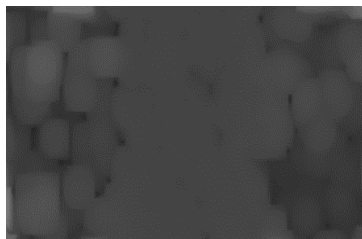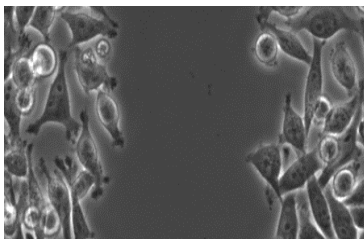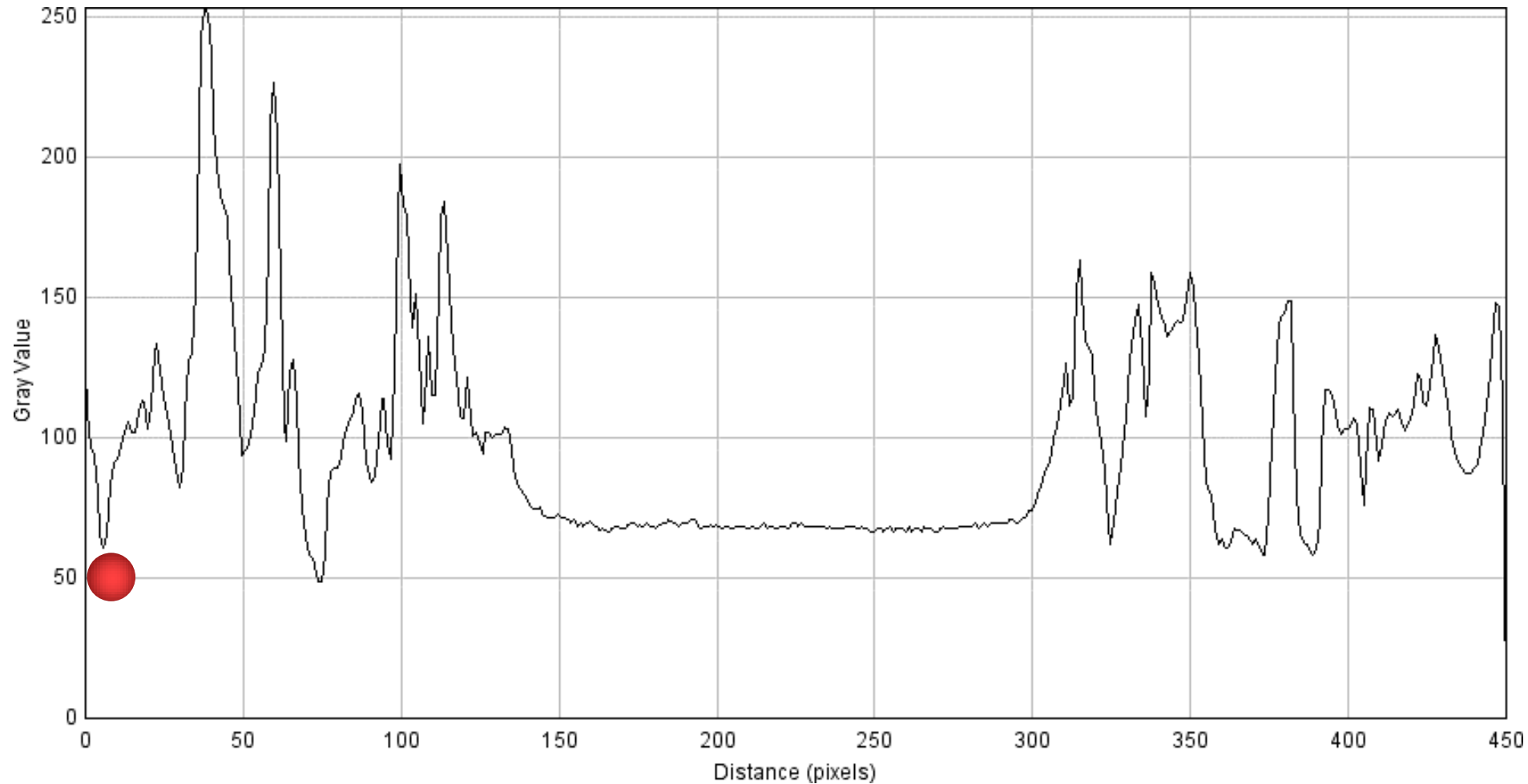
- Save time and eliminate user mistakes

- Unbiased and consistent approach

- Have a record off what you have done

A macro is a simply series of ImageJ commands

- Simple, easy to learn language

- Calls predefined ImageJ and Java functions

- Any built-in ImageJ menu command can be run in one simple line of code

- Lots of online tutorials examples and resources including:
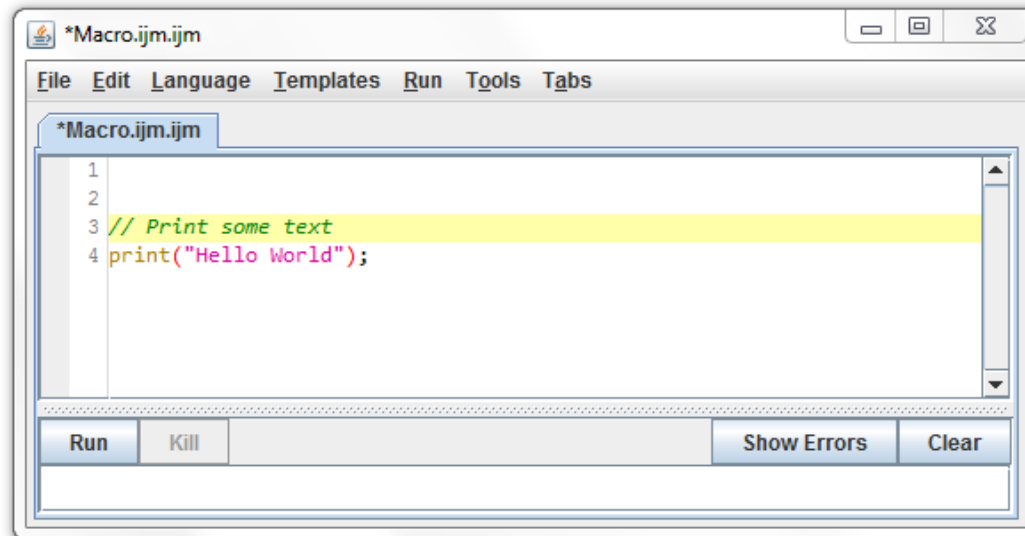
    http://fiji.sc/Introduction_into_Macro_Programming

    http://rsbweb.nih.gov/ij/docs/macro_reference_guide.pdf

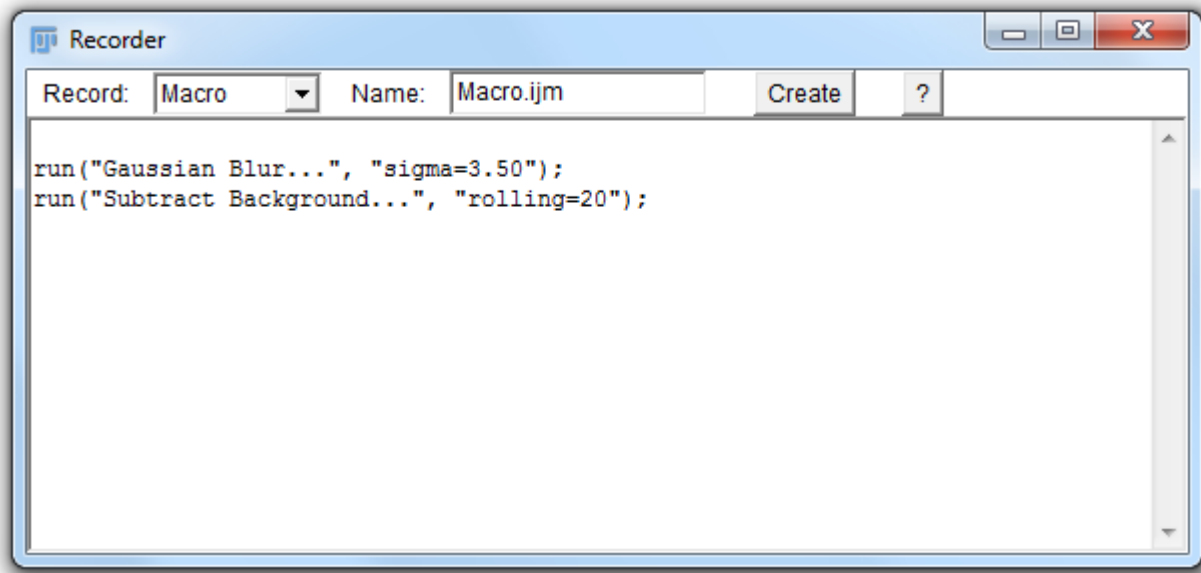# The FIJI Script Editor

Plugins -> New -> Macro

- Has syntax highlighting

- File and Edit menus have useful standard text editor commands

The Command Recorder
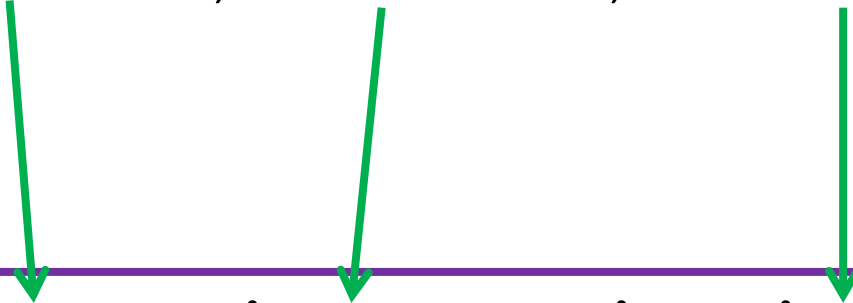
Plugins -> Macros -> Record…

- A real simple way to automate a work flow and make a Macro

- Simple perform the analysis on one image and click create!

For Loops

- An iteration statement that executes a code block a specific number of times
- For (initialisation; condition; increment){

    do something

  }

For(starting with this variable; while this is true; increment the variable){

    do this

}

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

Acknowledgments

Mark Dunning
Anne Pajon
Gabriella Rustici

Stefanie Reichelt
Heather Zecchini
Lorraine Berry

Richard Butler
Alex Sossick