Image Processing and Analysis with FIJI

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Part 1: Basics

ImageJ is open source image processing and analysis software

ImageJ
Image Processing and Analysis in Java

Developed by Wayne Rasband:

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

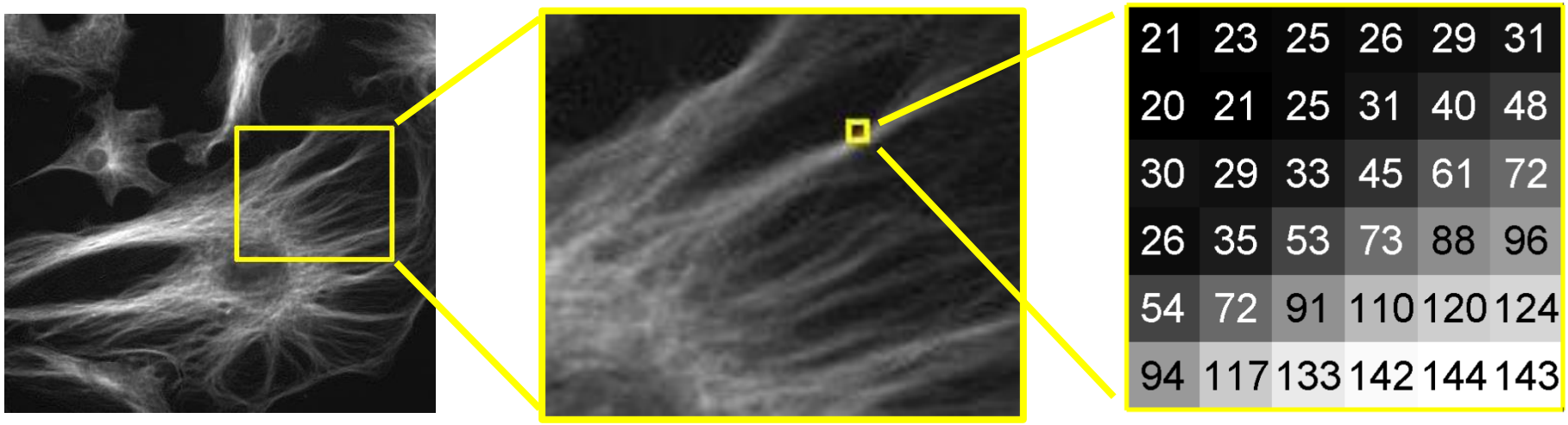
FIJI is a ImageJ distribution with lots of useful plugins pre-installed

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

Digital images are simply arrays of numbers



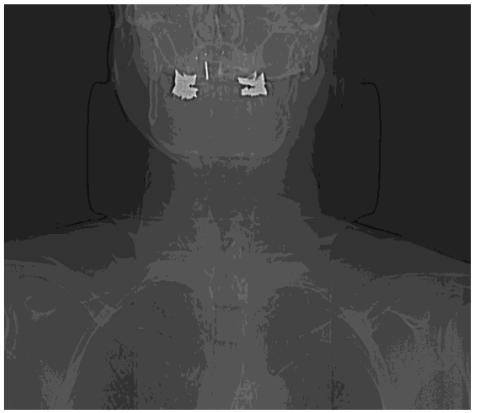
Note pixels are samples of intensity at a spatial point, not little squares!

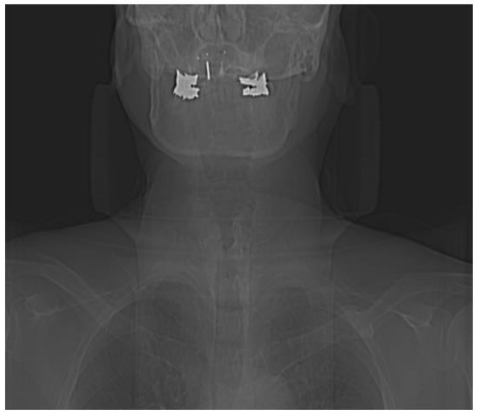
Dynamic range is the number of values each number can take

1 bit = 2

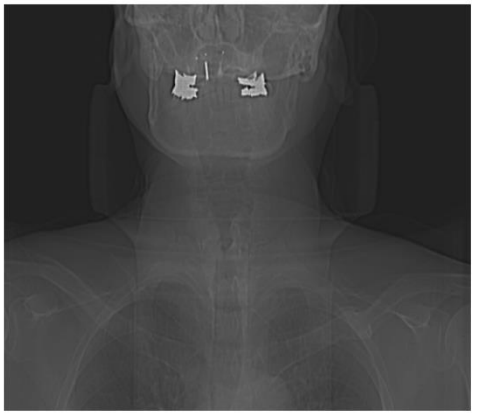
2 bit = 2^2

4 bit = 2^4

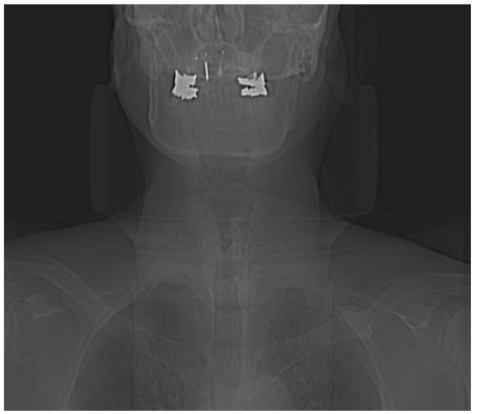




8 bit = 2^8

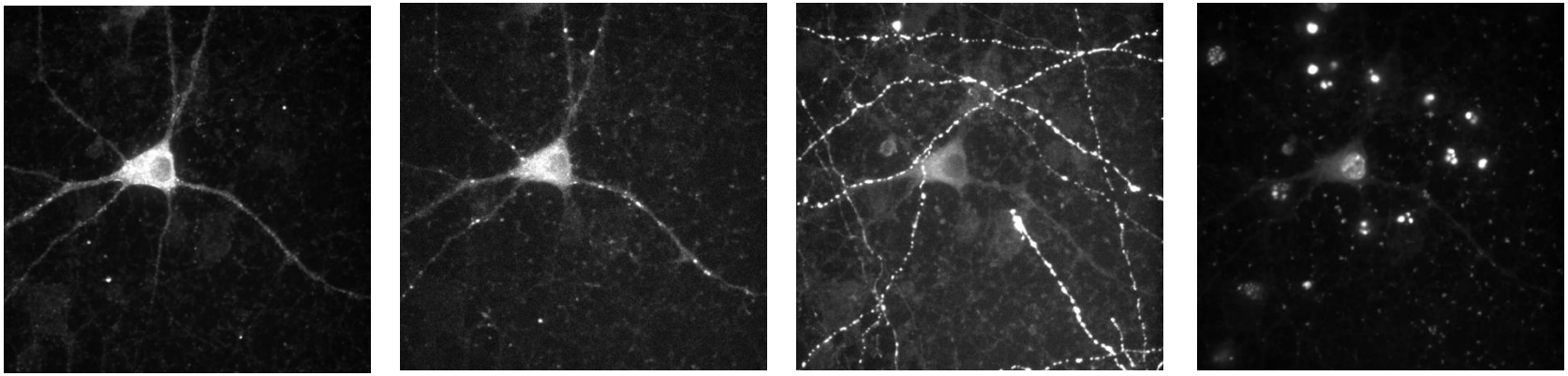


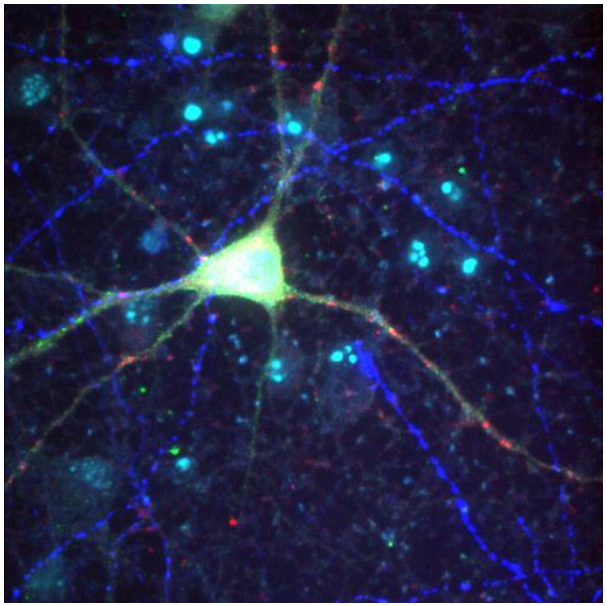
12 bit = 2^{12}



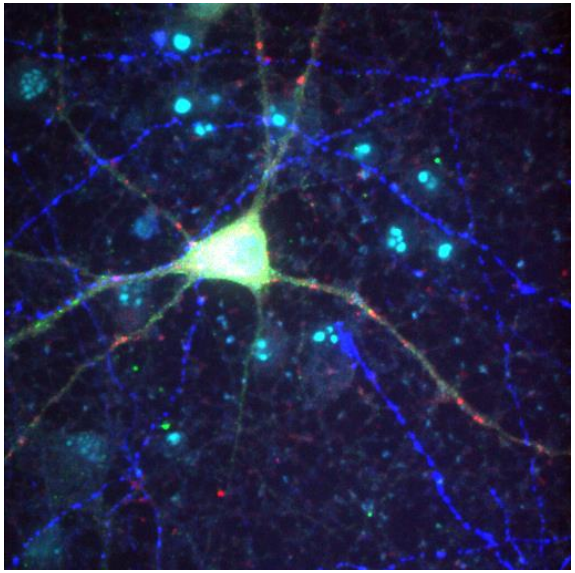
16 bit = 2^{16}

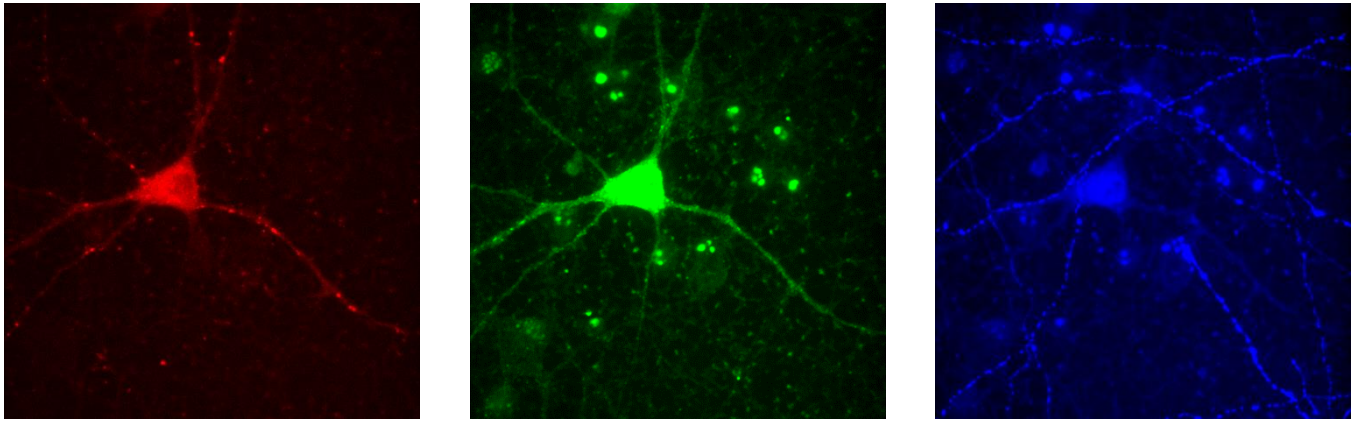
Multi Channel Data



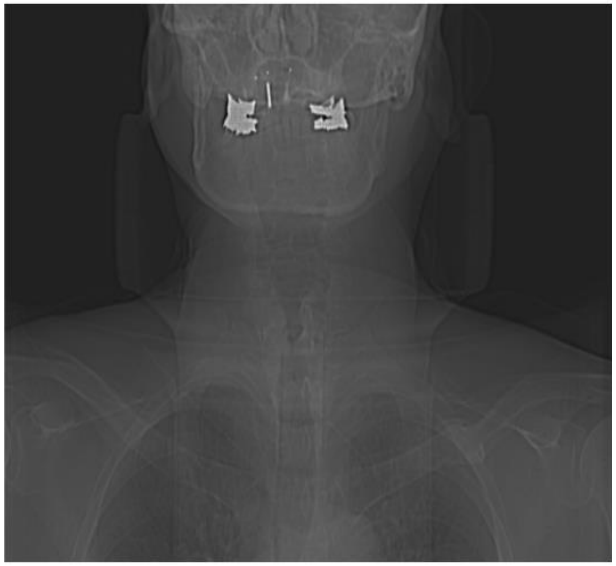


RGB Color Images

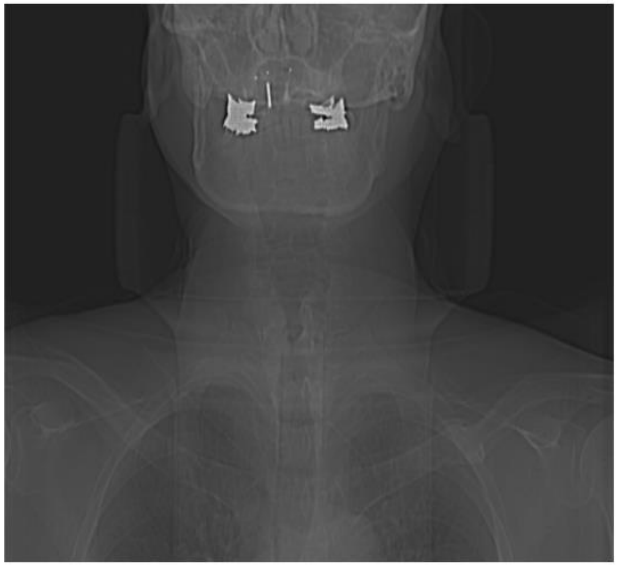




What can we “see” on a monitor



8 bit = 2^8

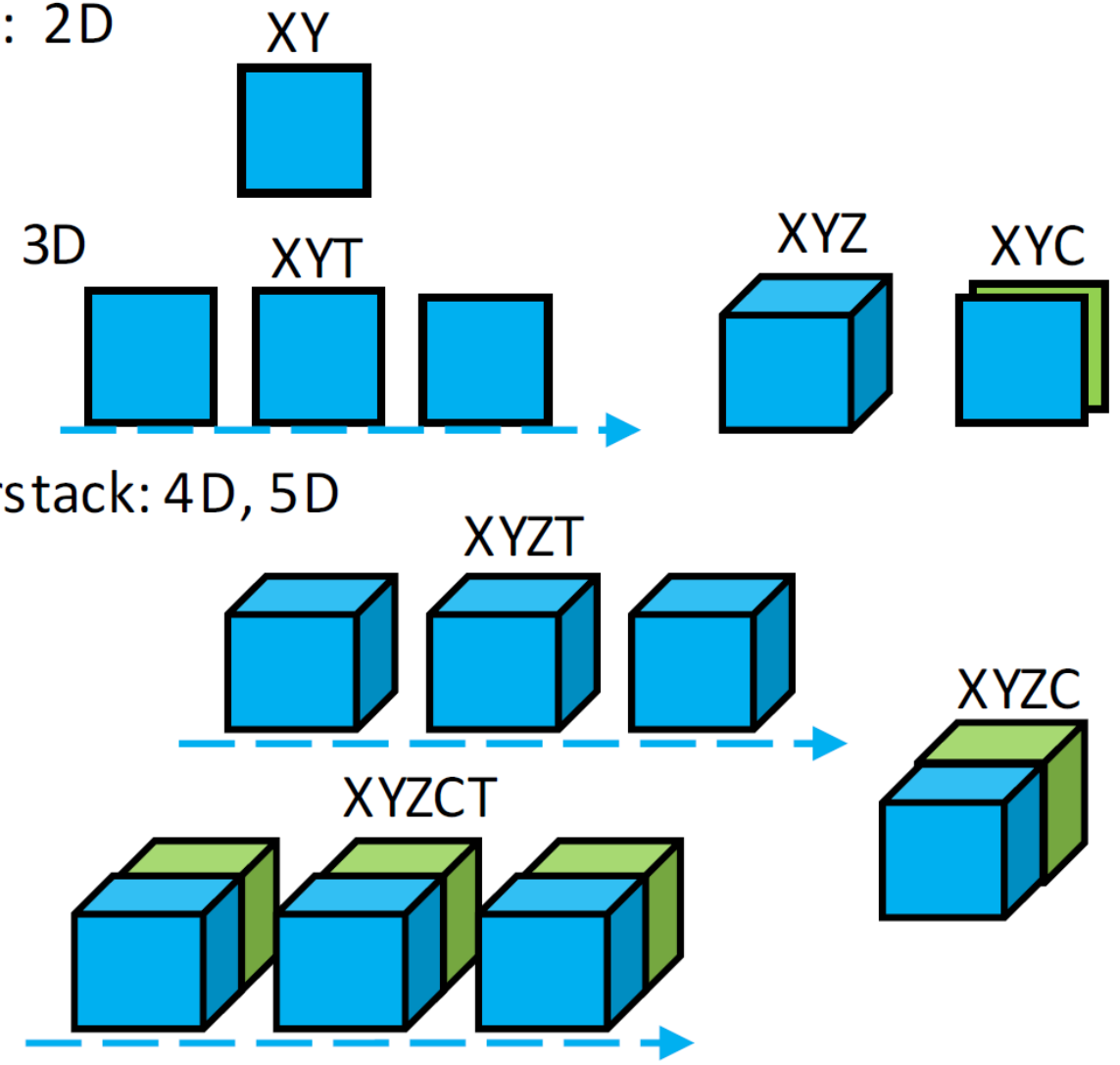


16 bit = 2^{16}

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

Image Stacks

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



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

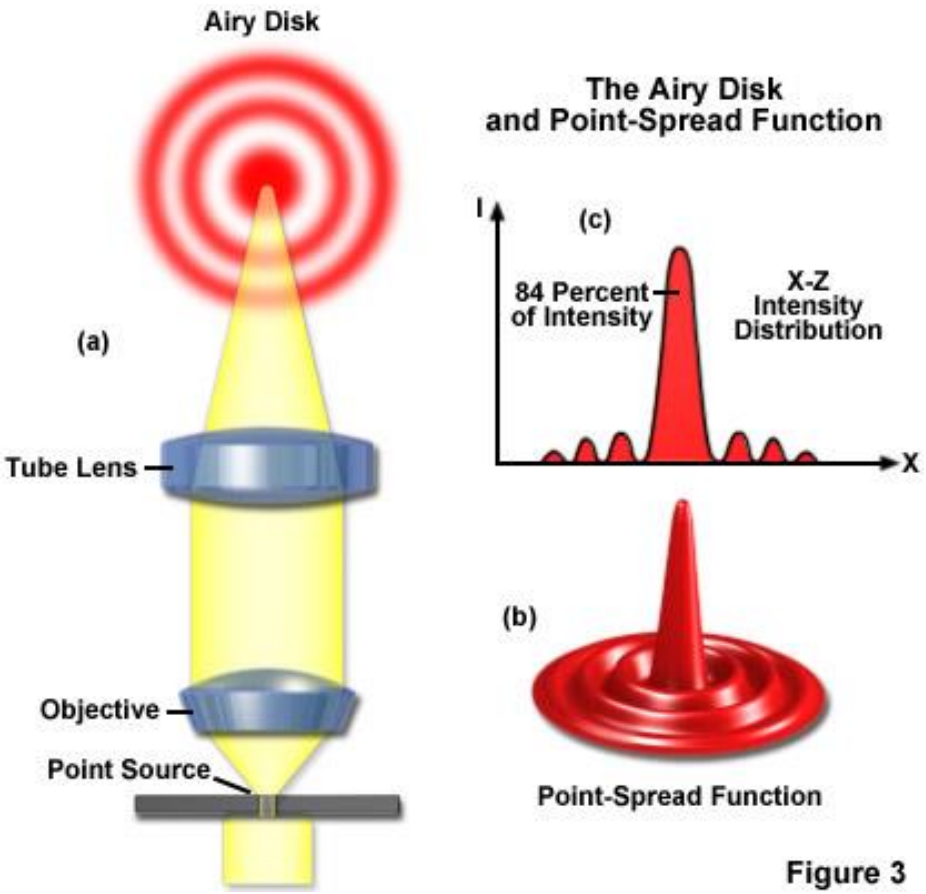


Figure 3

"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

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

What determines resolution 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

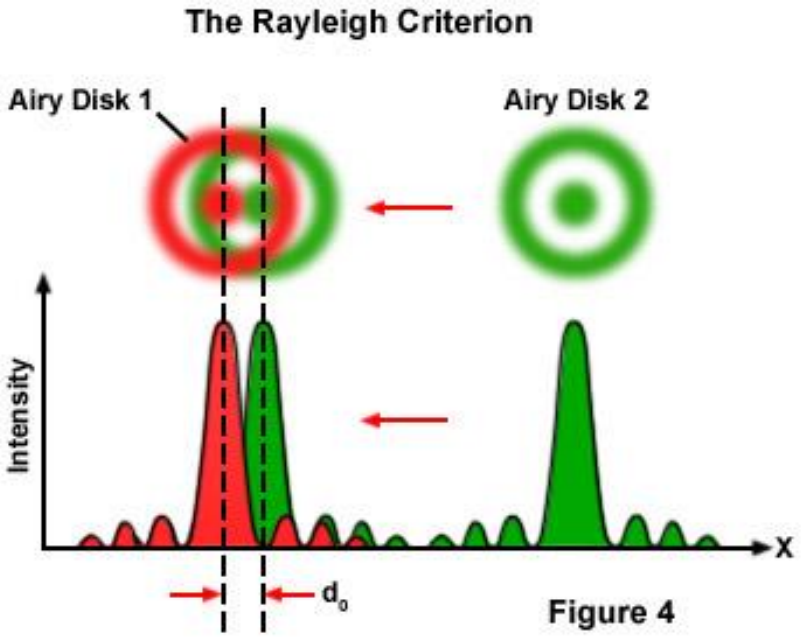


Figure 4

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

Part 2: Introduction to Image Processing and Analysis

Image Processing:

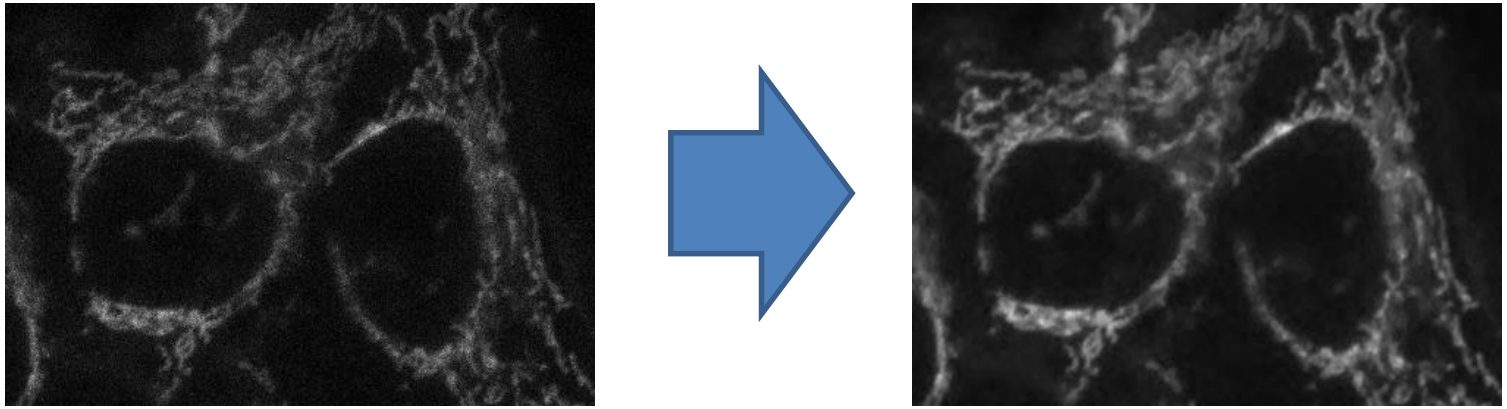
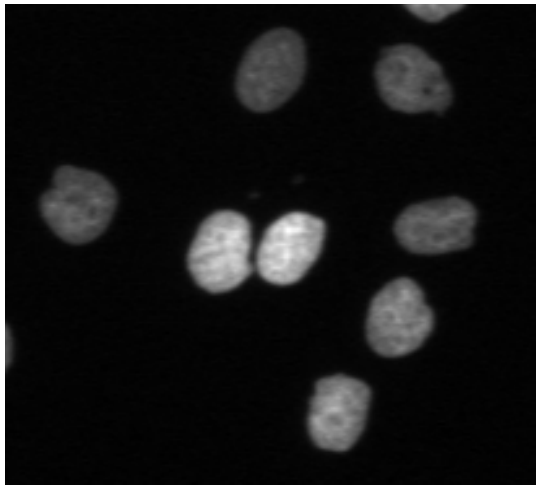
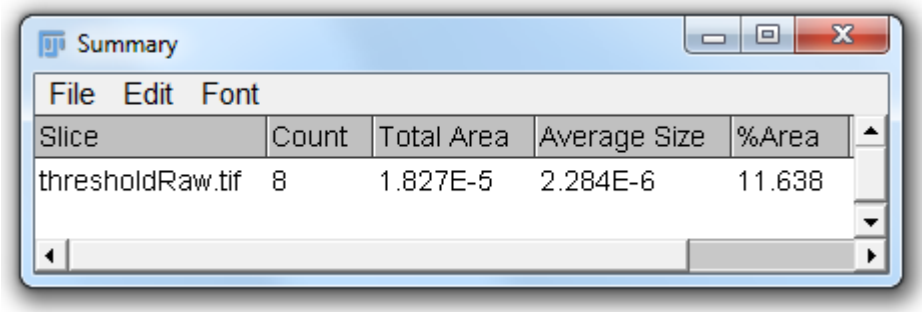


Image Analysis:



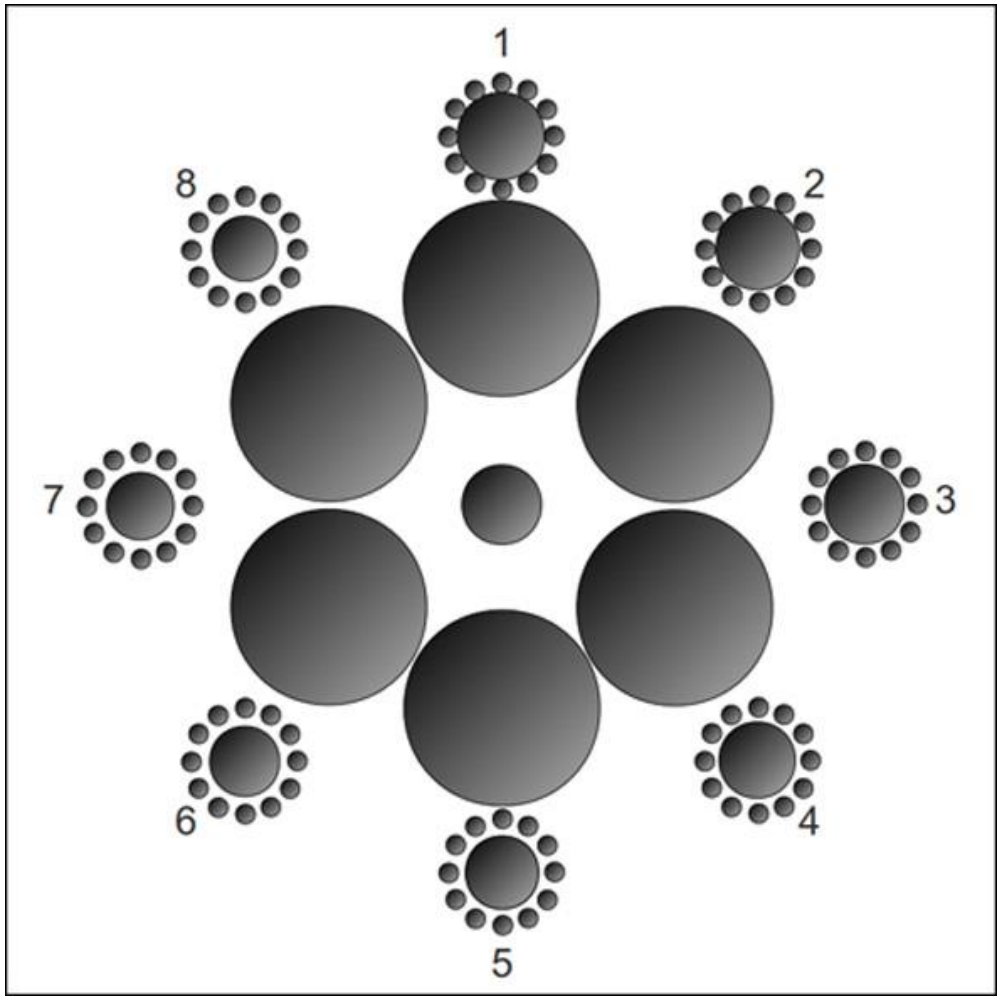


Why do Computational Processing and Analysis:

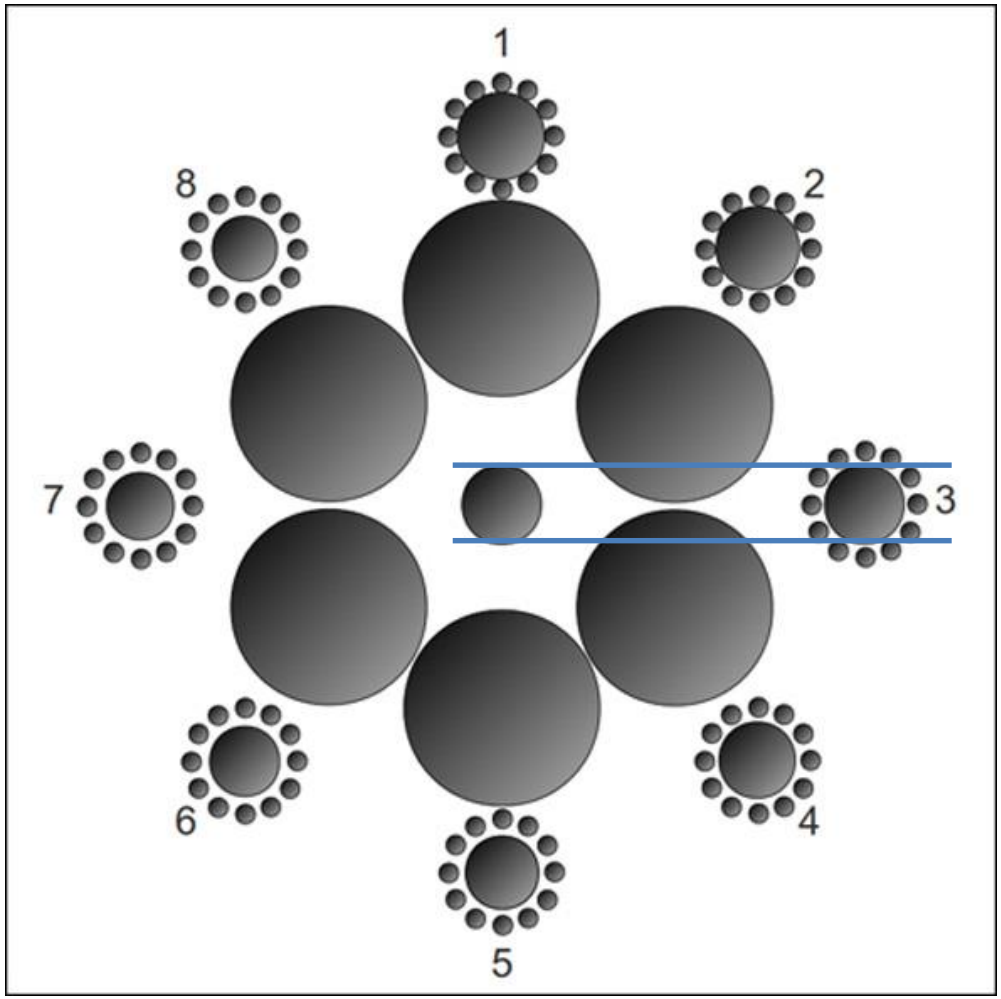
- Its quantitative
- Its unbiased
- Can enhance understanding of the data
- Can be automated for processing of large datasets

"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

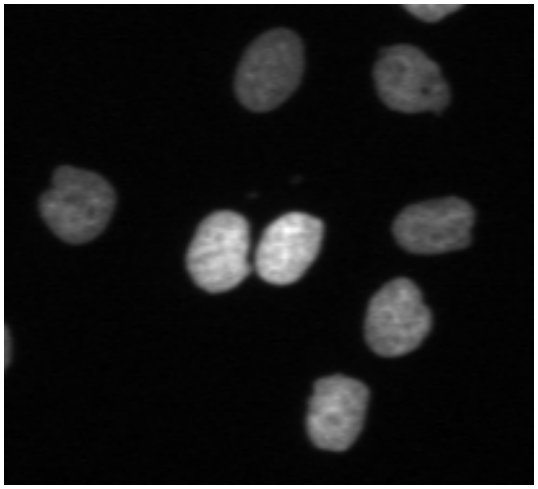
Which outer circle is the same size and the central circle?

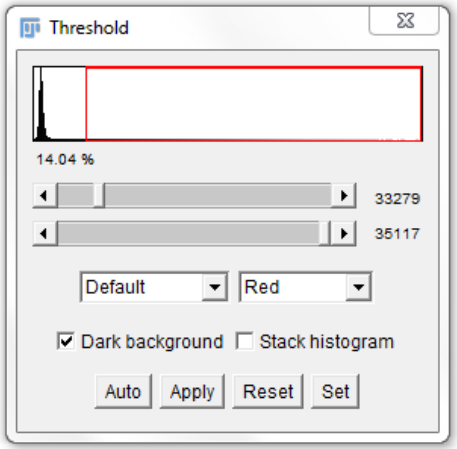


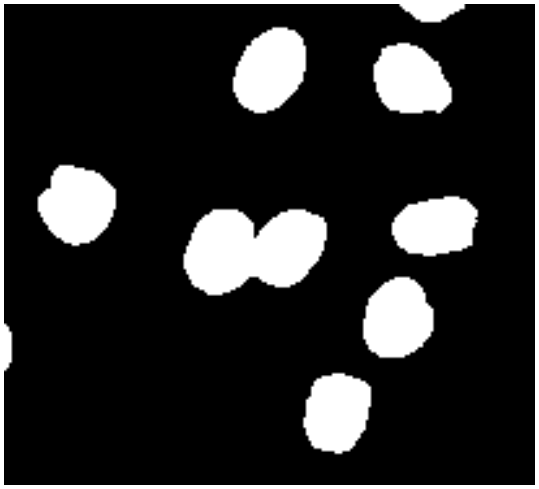
Which outer circle is the same size and the central circle?



Intensity thresholding to segment objects

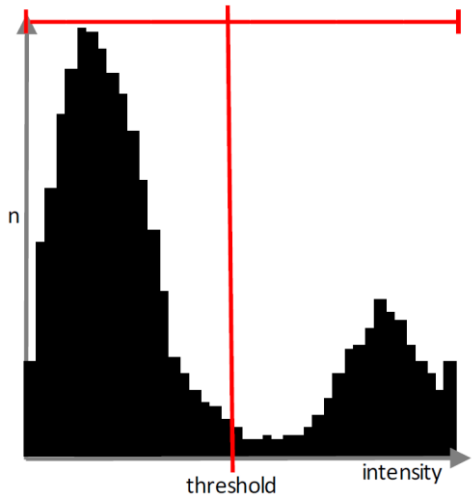






Automated threshold values are preferable to manual selection

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

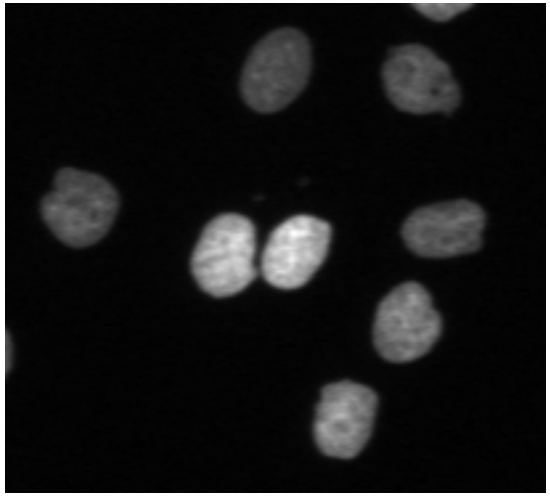


Otsu, N (1979), "A threshold selection method from gray-level histograms", *IEEE Trans. Sys., Man., Cyber.* **9**: 62-66.

- 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



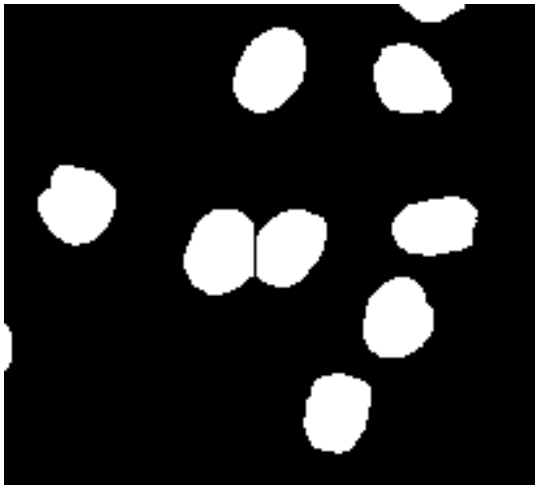
Raw Data

Otsu Threshold

Li Threshold

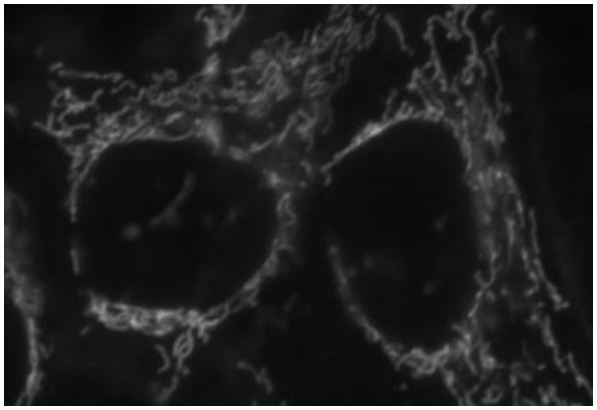
Watershed transformation to separate touching objects

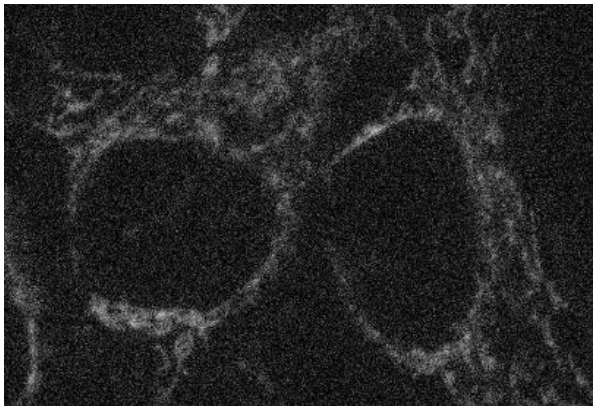




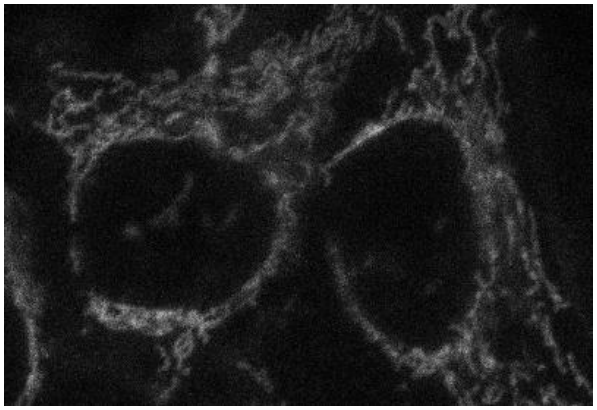
- Seeds put a minimum of distance map and dilated
- This can be visualised as flooding the distance map

Noise is image corruption from the acquisition process

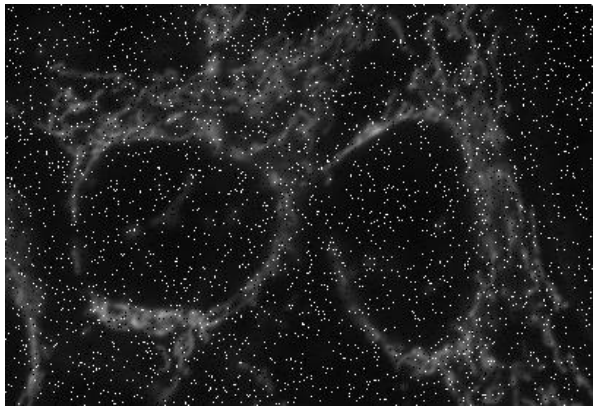




Gaussian



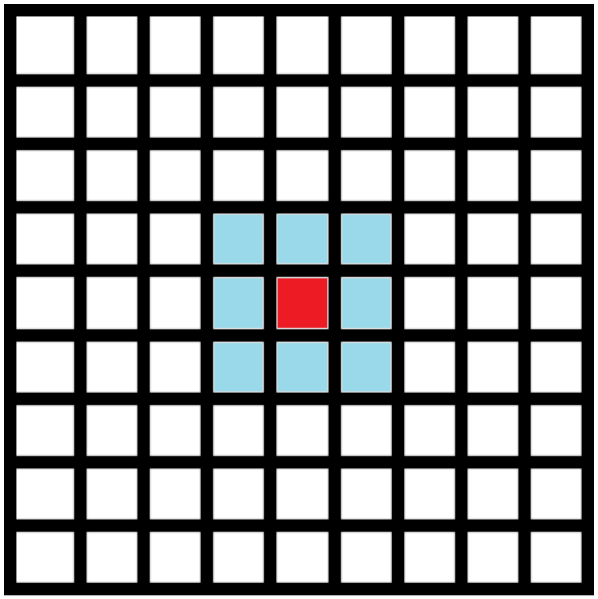
Poisson



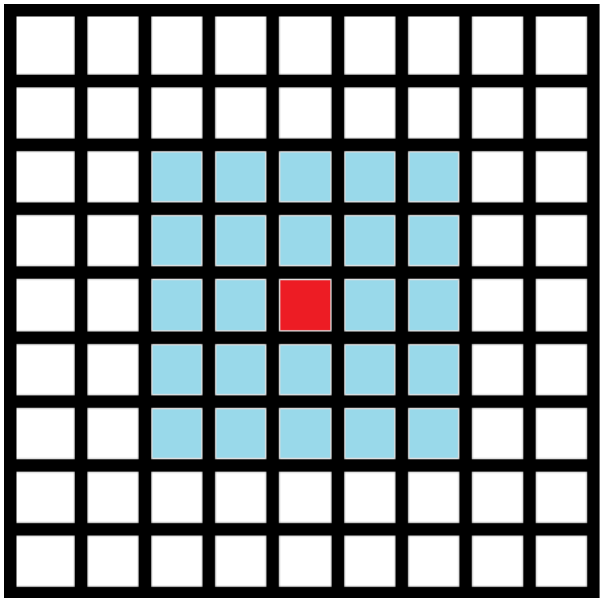
Impulse

Image Filters and Convolution

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



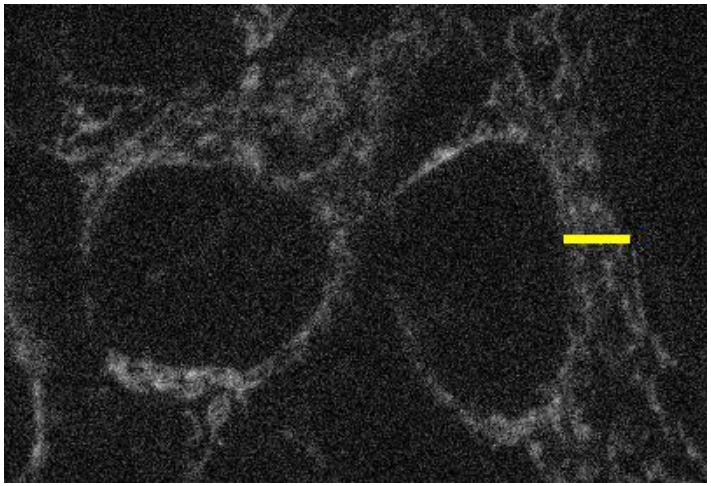
3x3 Neighbourhood

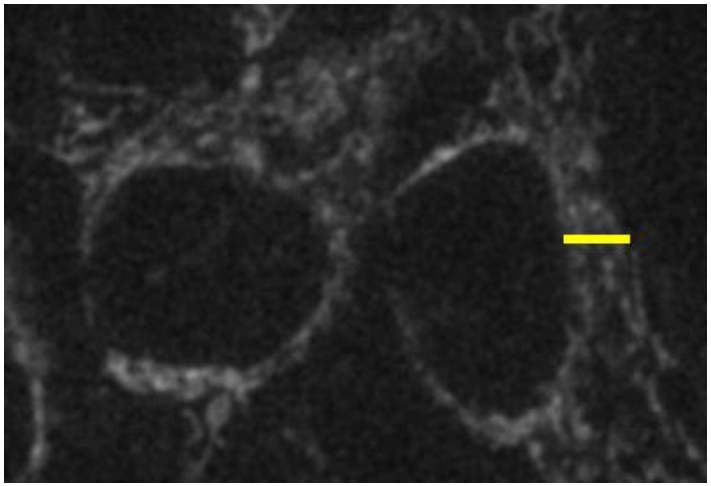


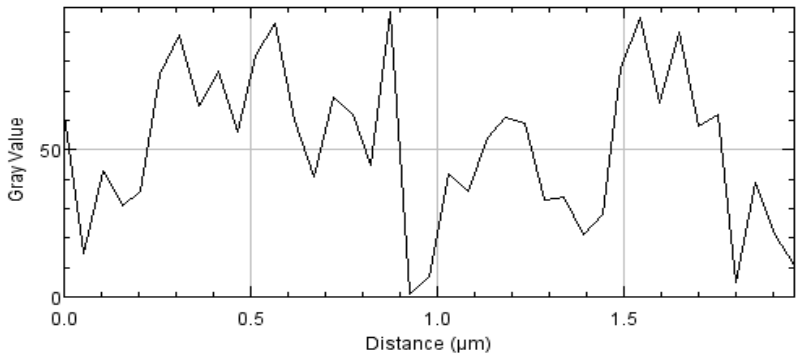
5x5 Neighbourhood

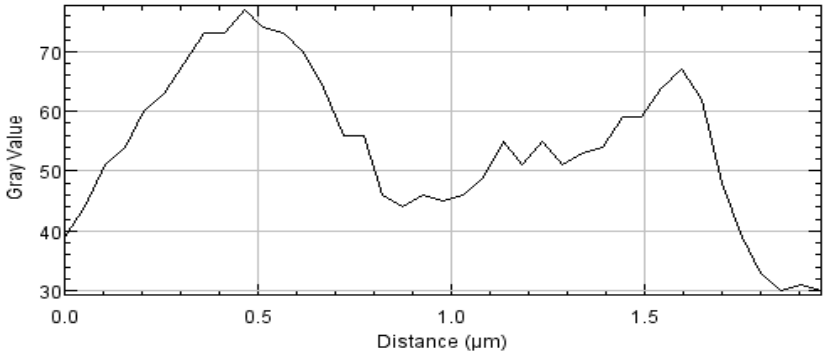
Mean Filter

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



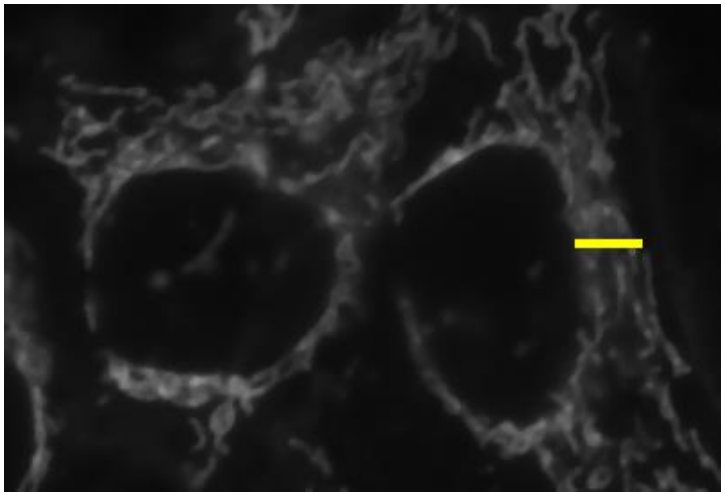


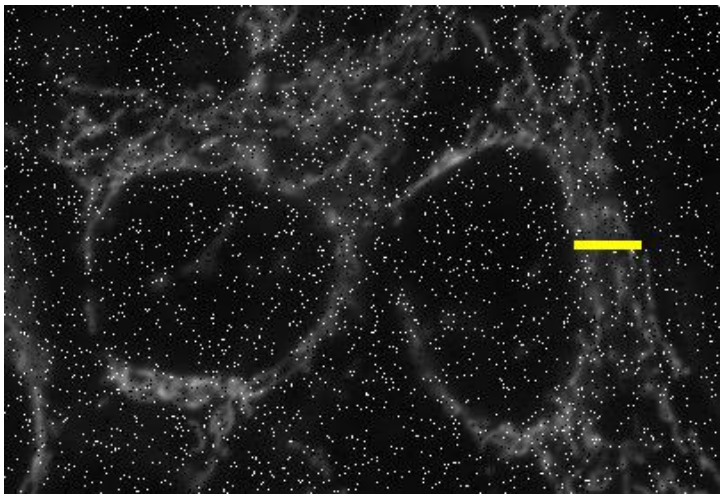


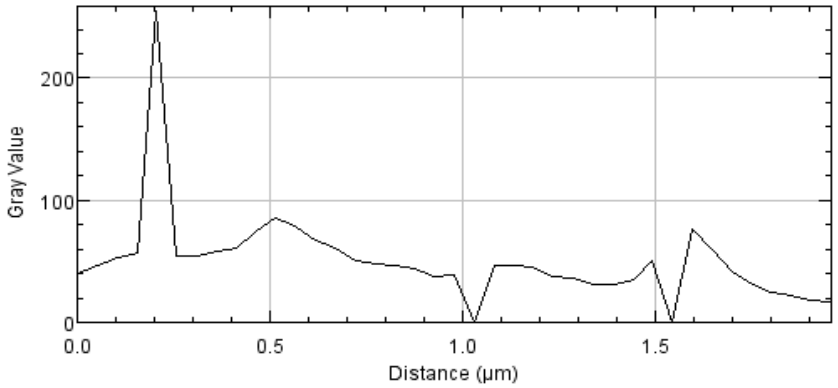


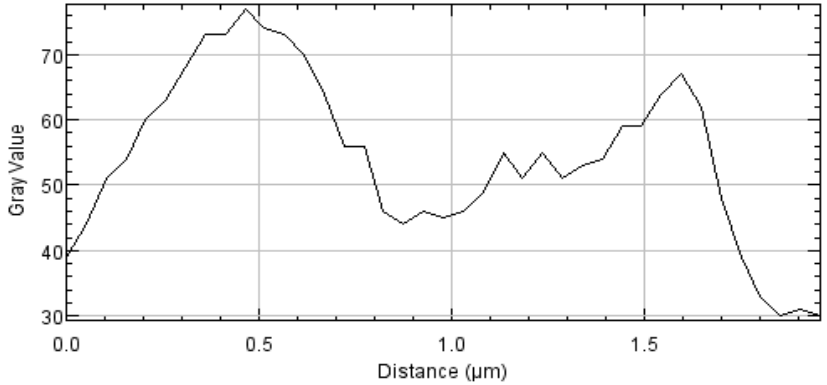
Median Filter

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



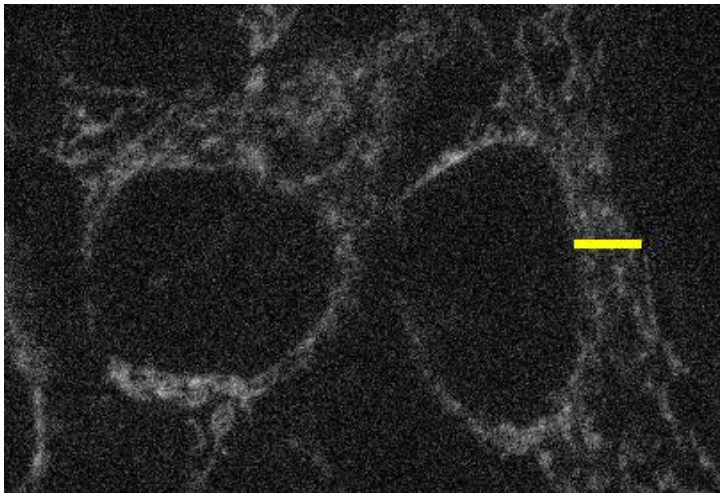


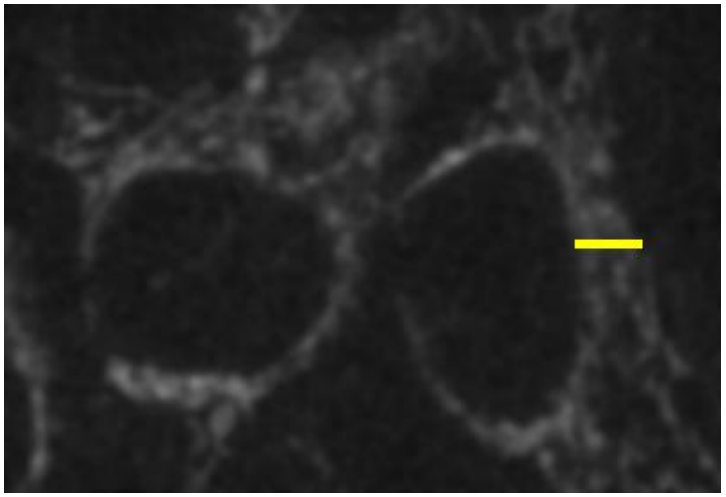


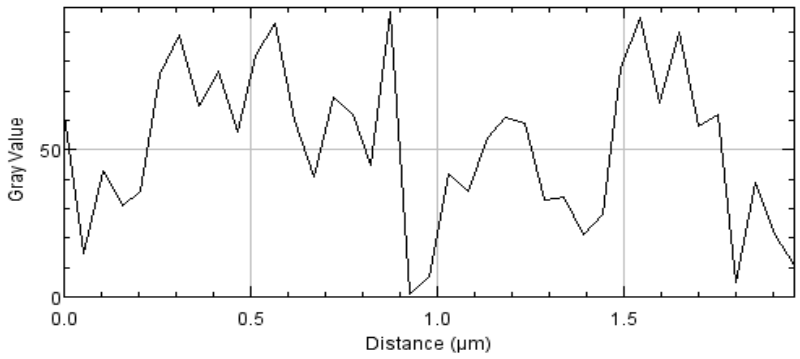


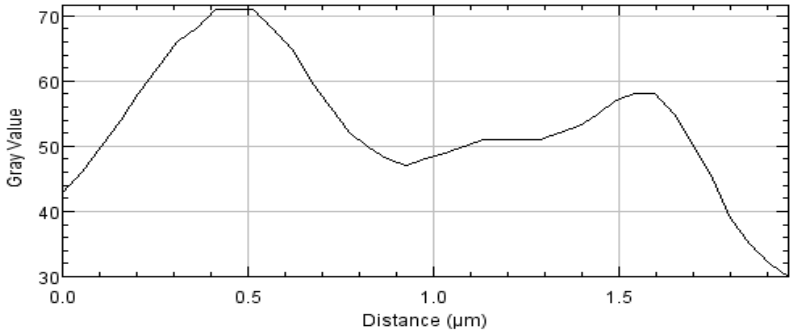
Gaussian Filter

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





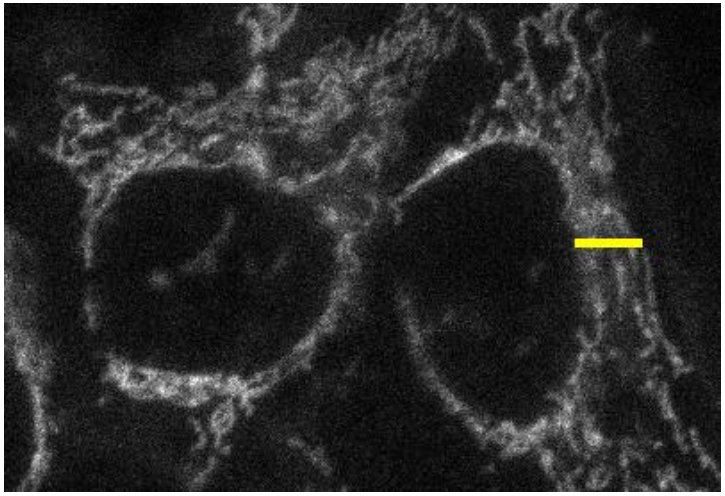


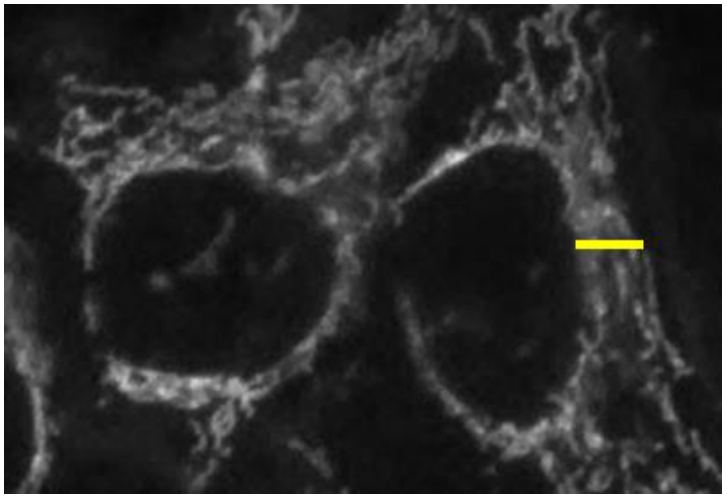


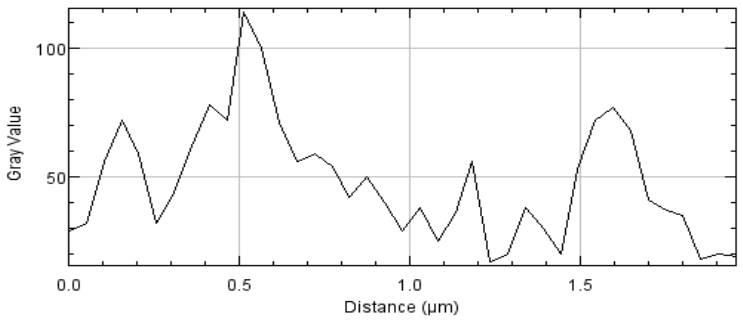
The PureDenoise Plugin

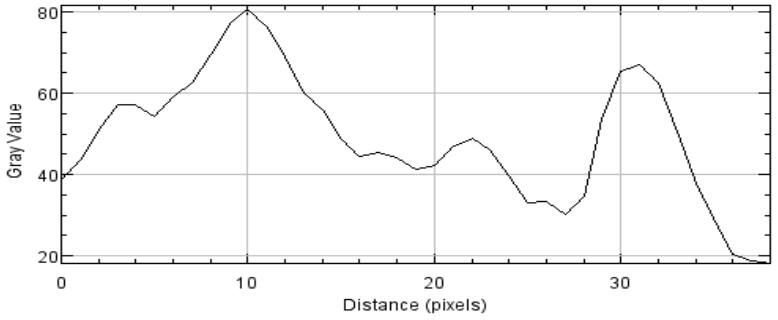
- Removal of Poisson noise
- Sophisticated research plugin but estimated parameters from image

F. Luisier, C. Vonesch, T. Blu, M. Unser, Signal Processing, vol. 90, no. 2, pp. 415-427, February 2010.

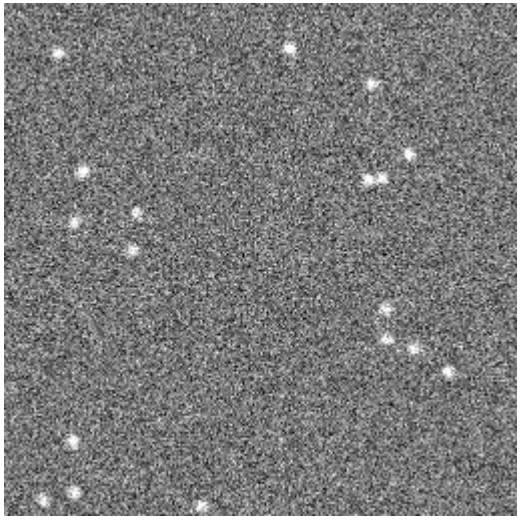


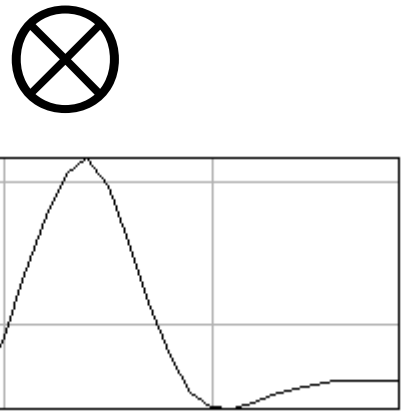






Laplacian of Gaussian Filter for Spot Detection





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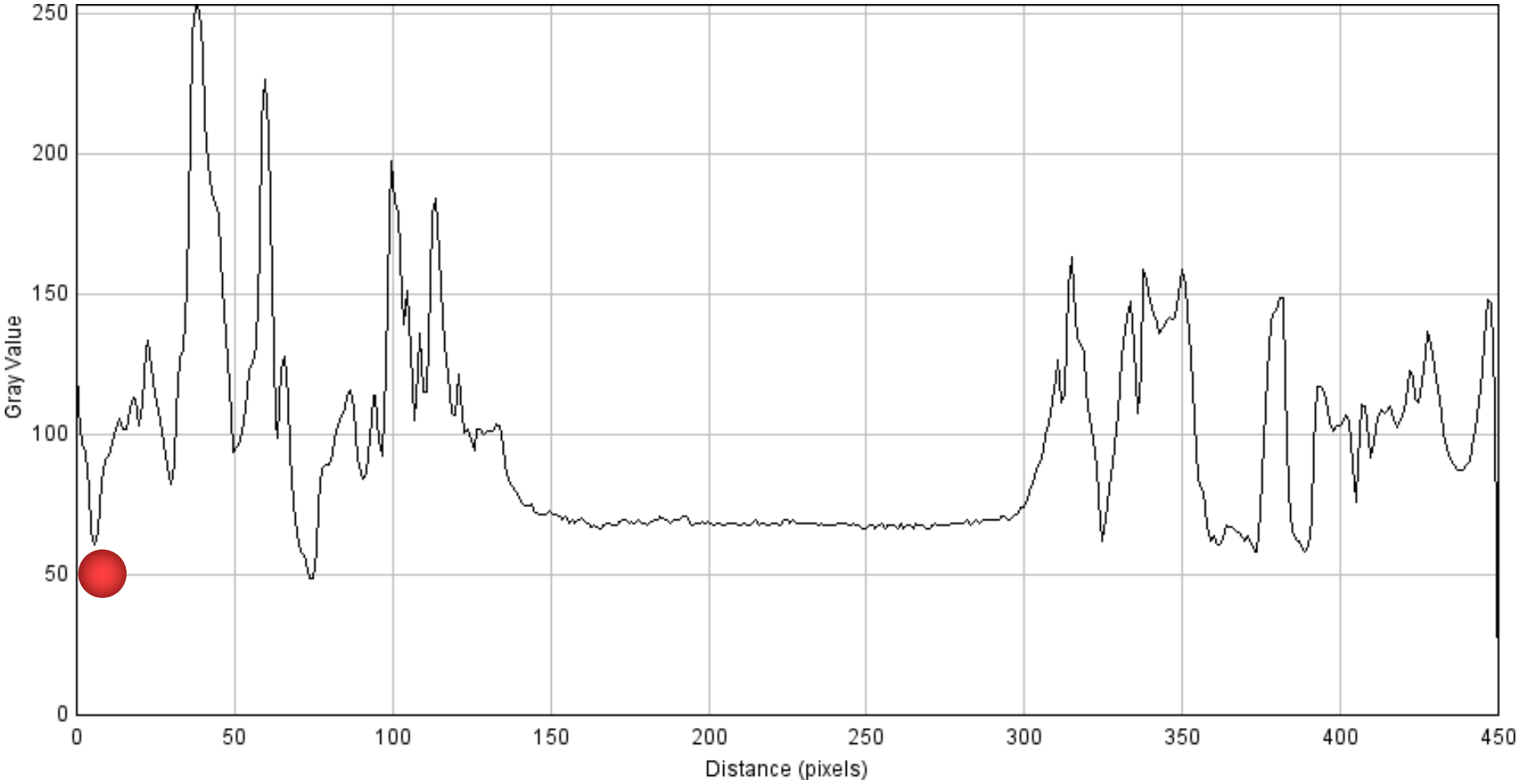
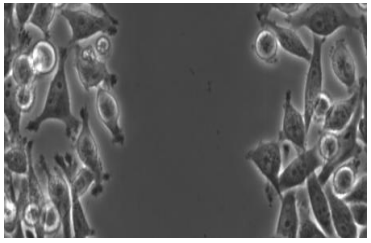
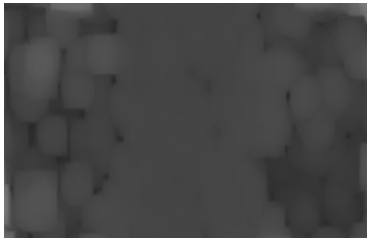
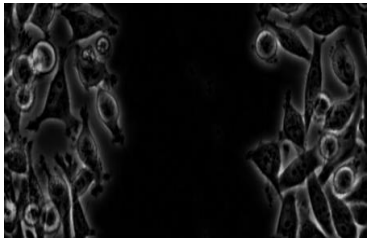
Rolling Ball Background Subtraction



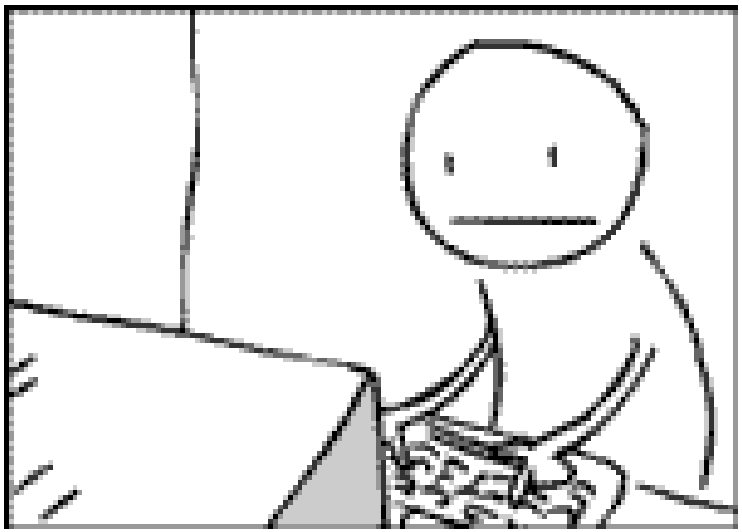






Part 3: Workflow Automation with Recorded Macros

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 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:

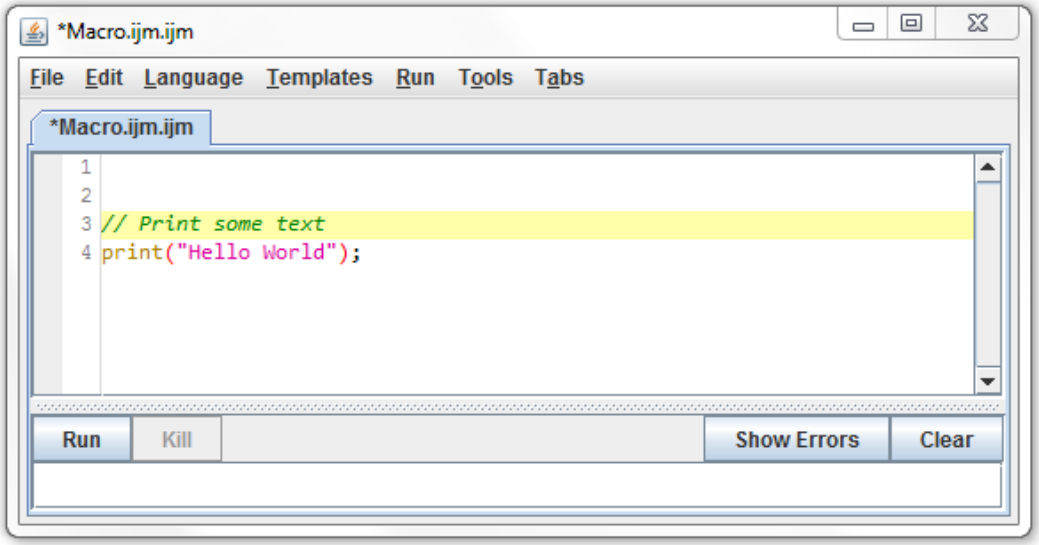
  http://fiji.sc/Introduction_into_Macro_Programming

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

The FIJI Script Editor

Plugins -> New -> Macro

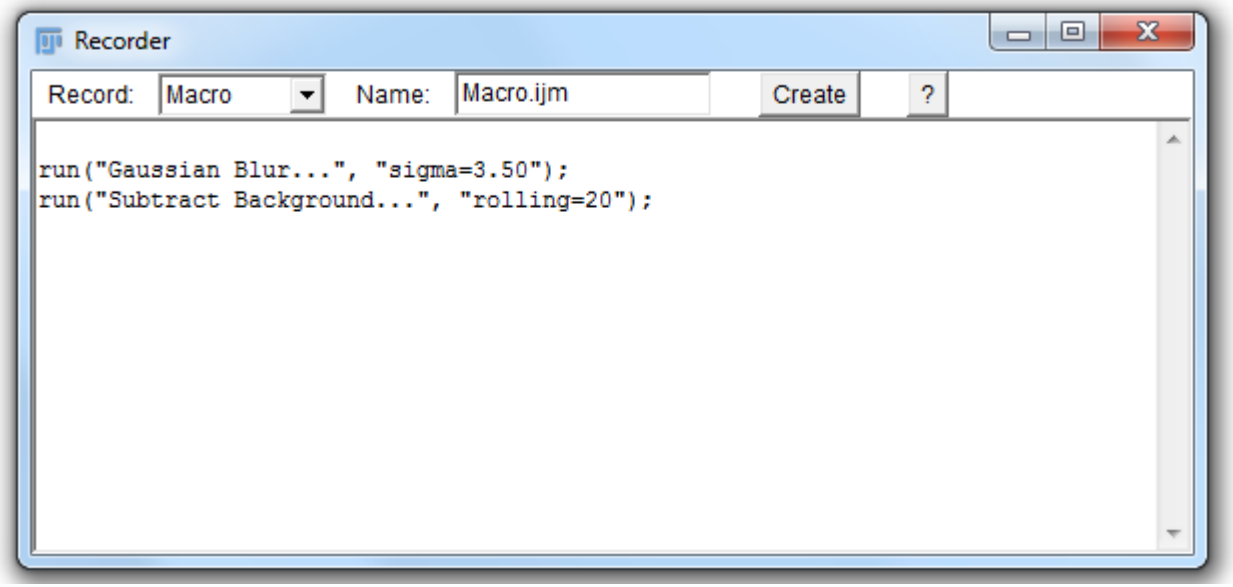
- Has syntax highlighting
- File and Edit menus have useful standard text editor commands



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){

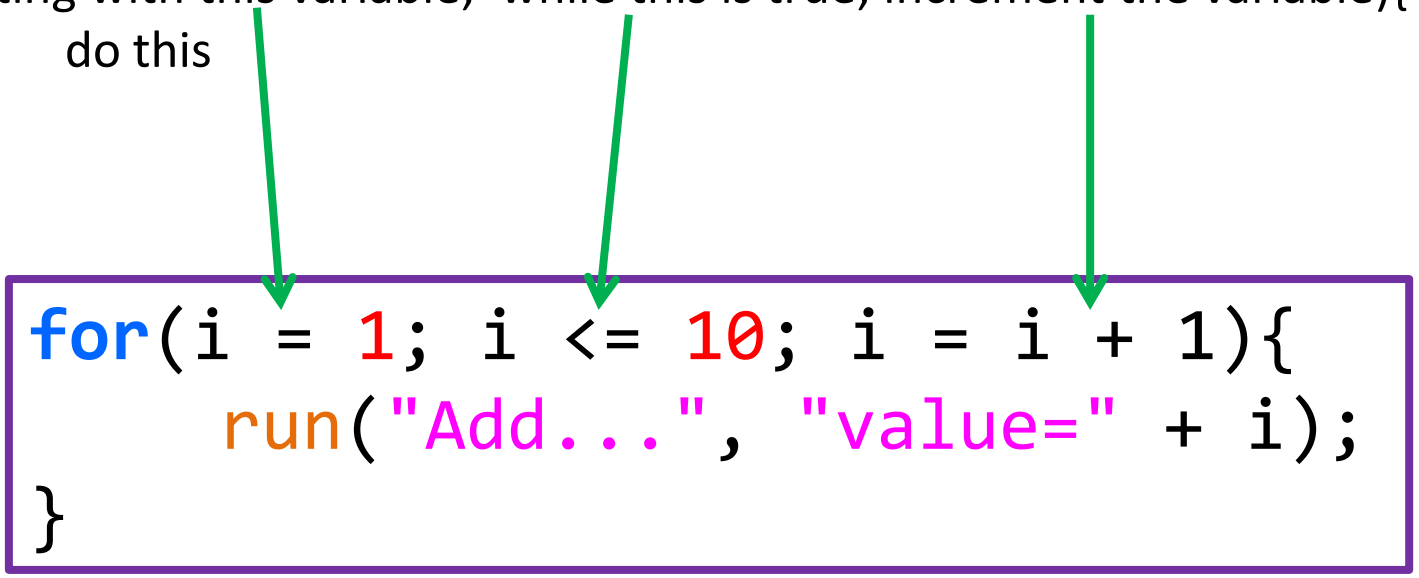
  do something

  }

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);
}
```

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