

Practical content and lectures

Doctoral Training Centre (MPLS) Resources
/Modules/ 2016-17 / Michaelmas term /
Week 9-10: Foundations of Image Analysis /
day01_practicals

Summary of course: Course-schedule-overview.html

Practicals: day01_practicals/day01-prac-overview.html

Source:



Nanoscopy Oxford



Introduction to Practical with Fiji and iPython

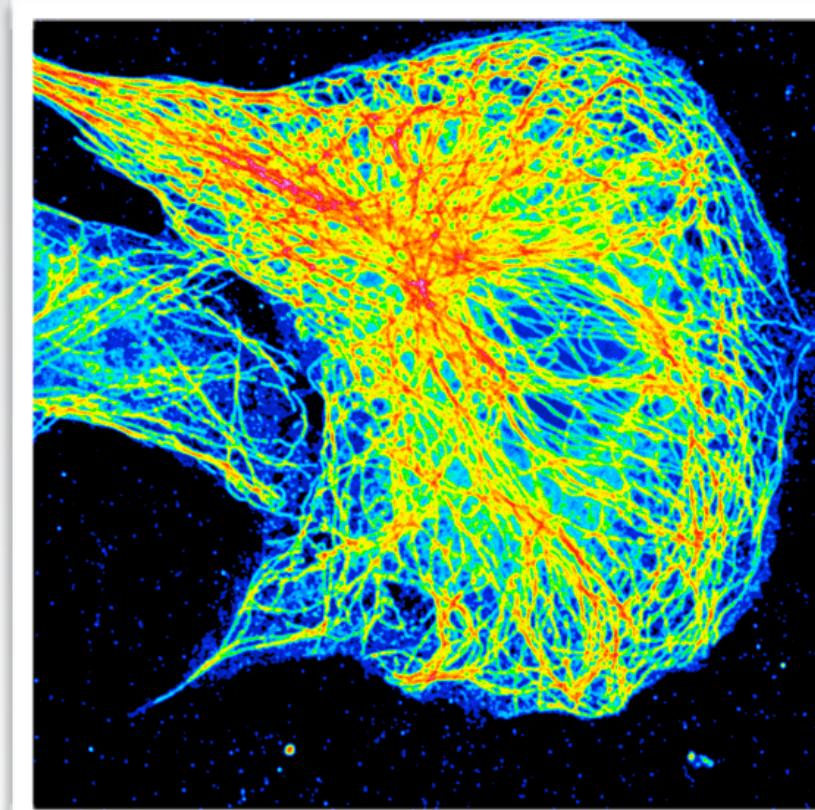
by Dominic Waithe

- Introduction to Applied Image Analysis.
- Image Analysis Software overview.
- Exploring your image.
- Automation
- Conclusions.



The MRC Weatherall Institute of Molecular Medicine is a strategic alliance between the Medical Research Council and the University of Oxford





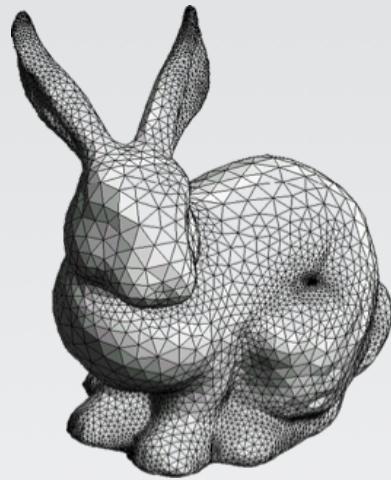
‘Picking the right tool for the job’

Source: STED image of cellular tubulin - Christoffer Lagerholm

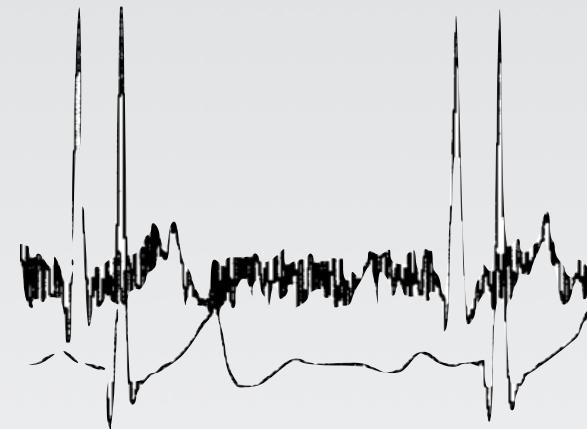
What is image analysis



Pattern Recognition



Discrete Geometry



Signal Processing

- Formal image analysis combines several different fields of mathematics.
- As humans we are much better at it than computers.
- Most if not all image analysis is derived from human intuition.
- Lots of help on the internet to understand the concepts

Source: http://phoenix.fit.edu/~georgio/teaching_pr.php, <http://www.pointwise.com/theconnector/September-2012/Analytic-vs-Discrete-Geometry.shtml>, <http://www.logicaldesigns.com/LDDSP1.htm>

Lots of tools for lots of applications.

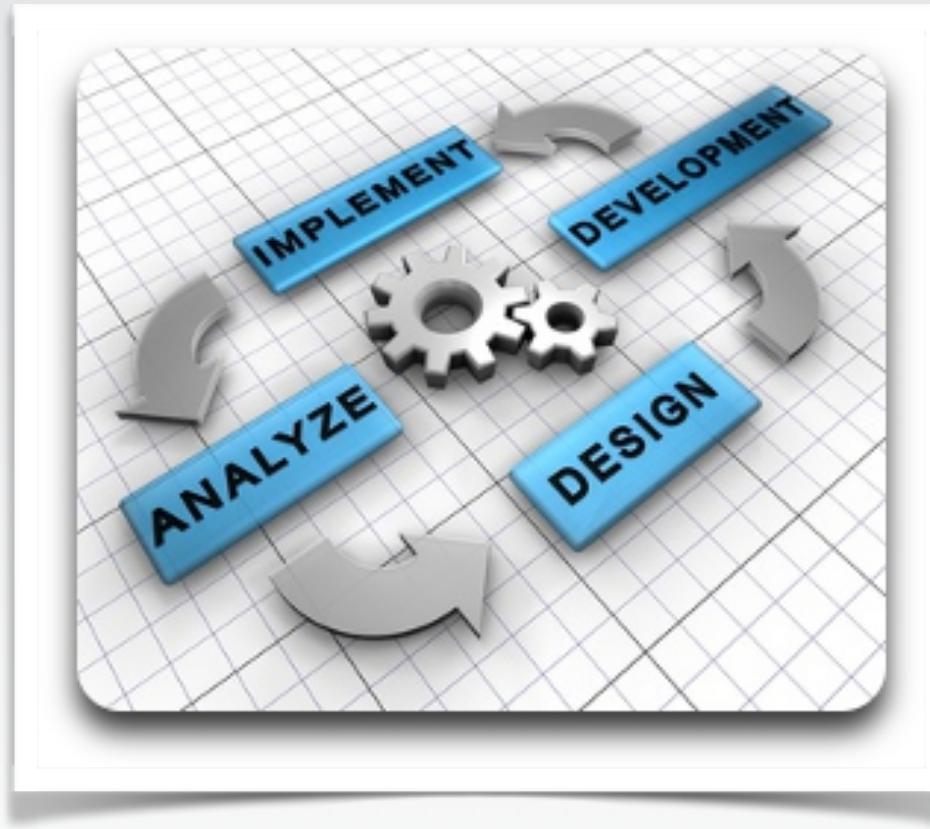
Software

Zen LE software Image-Pro
Volocity ImageJ Oko-Vision
CellProfiler Clemex Vision PE
Fiji IN Cell Investigator Drishti
Omero MetaMorph Image Surfer Imaris
Matlab Photoshop ImageTool
Mathematica MCID ImageTrak
Icy Image Metrology MIATool
SimpleWare ParaView Reconstruct
SoftWoRx FLIMfit Leica LAS Sinema
AutoQuant MeVisLab VIAS Reconstruct
BiolImage XD Voxel2

Disciplines

medicine
microscopy
remote sensing
astronomy
materials science
machine vision
security
robotics
geology
optical character recognition
assay micro-plate reading
metallography
defence
filtering

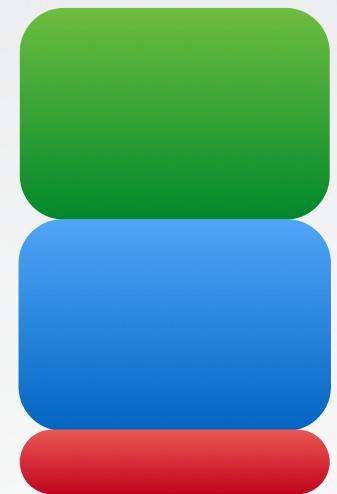
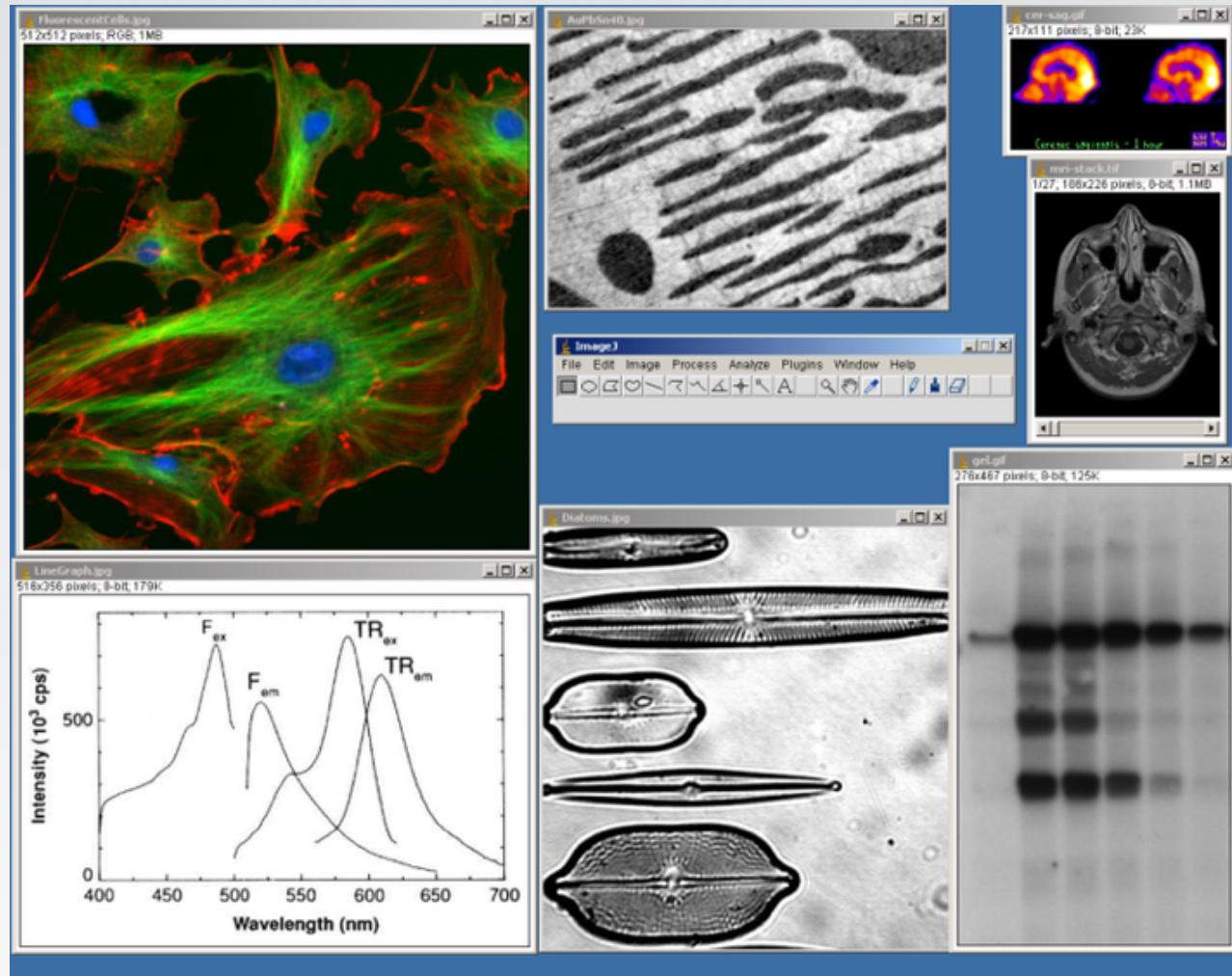
Source: http://en.wikipedia.org/wiki/Image_analysis, <http://www.hsr.it/research-organization/services-open-labs/alembic-advanced-light-and-electron-microscopy-bioimaging-center/9/#fs35>, http://www3.imperial.ac.uk/imagingfacility/links/imaging_software



General image analysis software

Source: www.jerseysoftware.com

General analysis software: Fiji/ImageJ

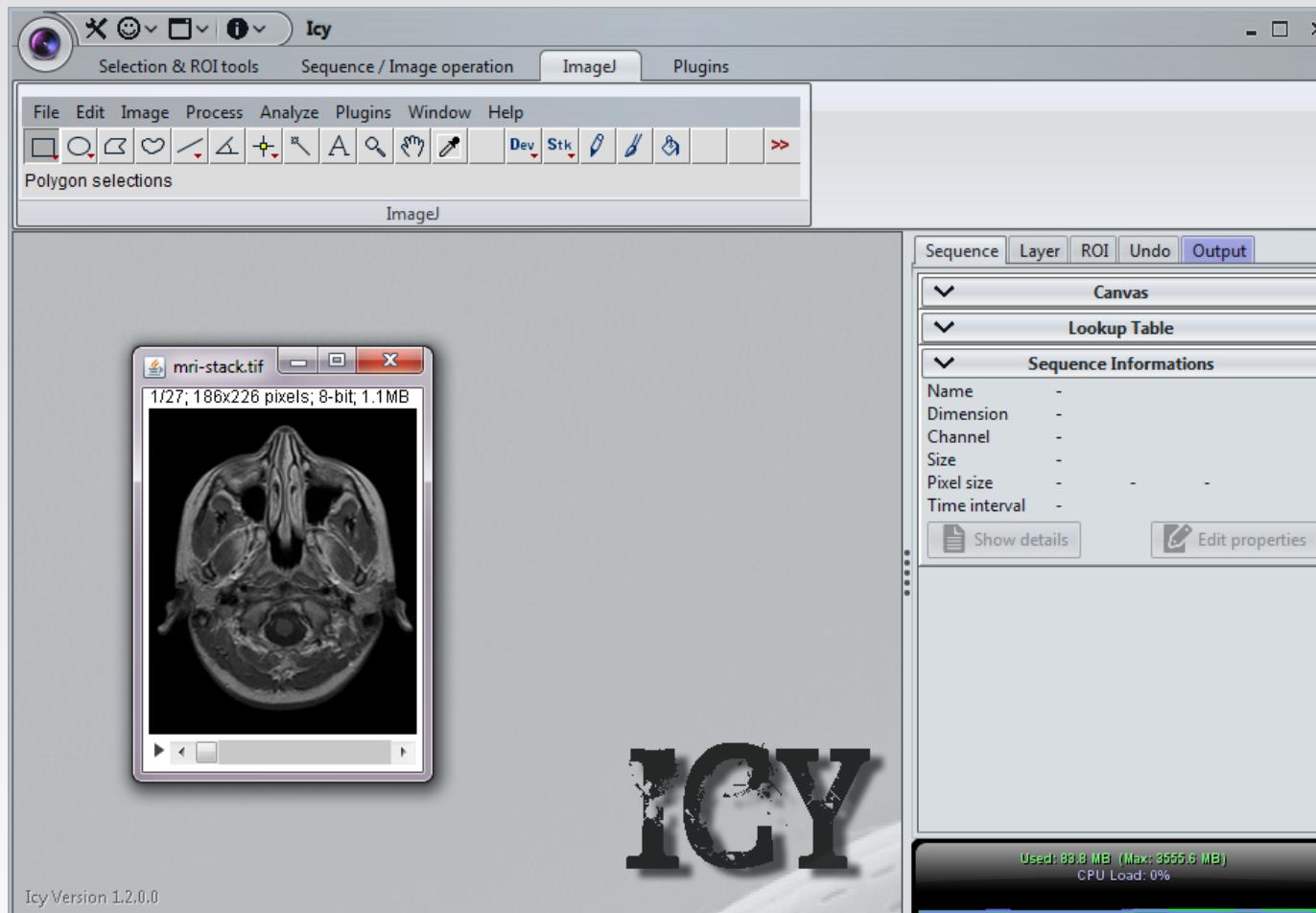


Originally created by Wayne Rasband at the NIH in 1997 as ImageJ.

Free and easy to get running on all systems.

Source: <http://fiji.sc/Fiji>

General analysis software: Icy

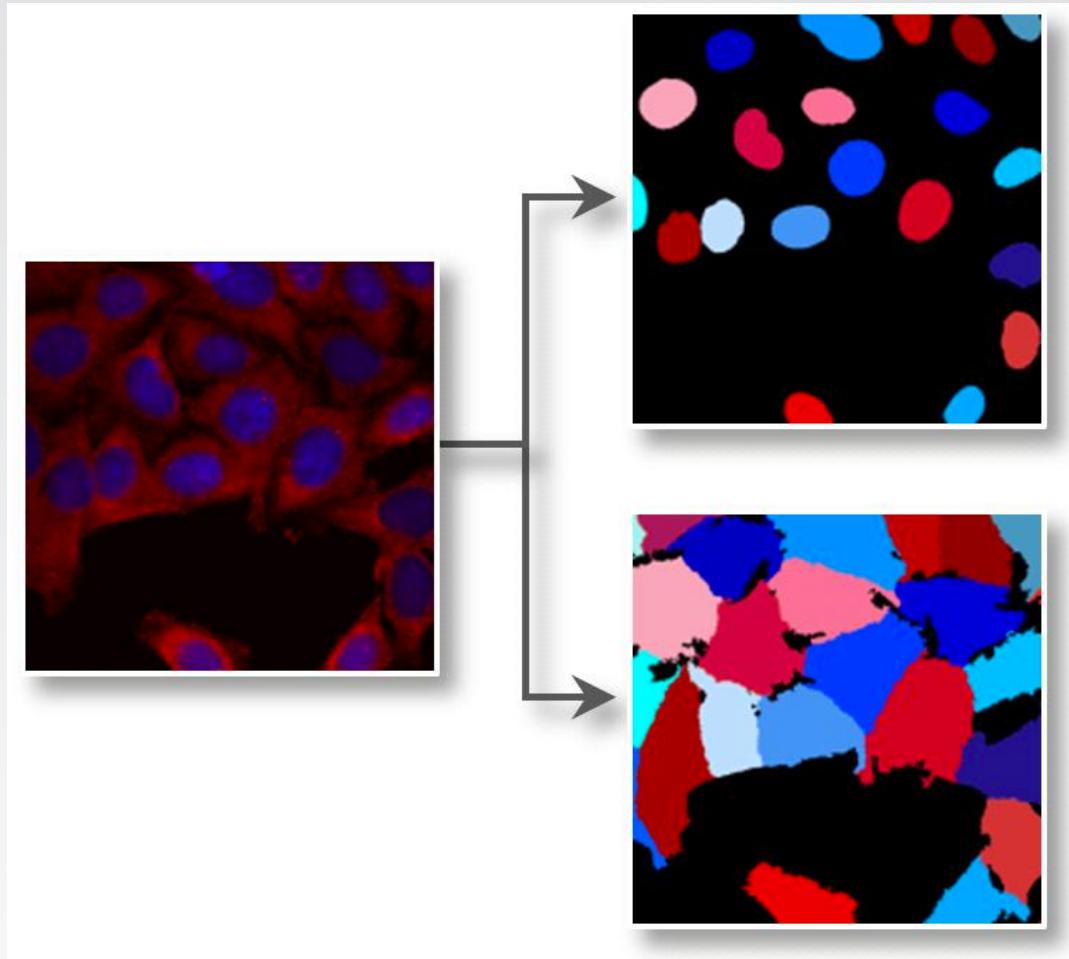


Icy has been created by the Quantitative Image Analysis Unit at Institut Pasteur. Free and easy to get running on all systems.

Source: <http://www.bioimageanalysis.org/>

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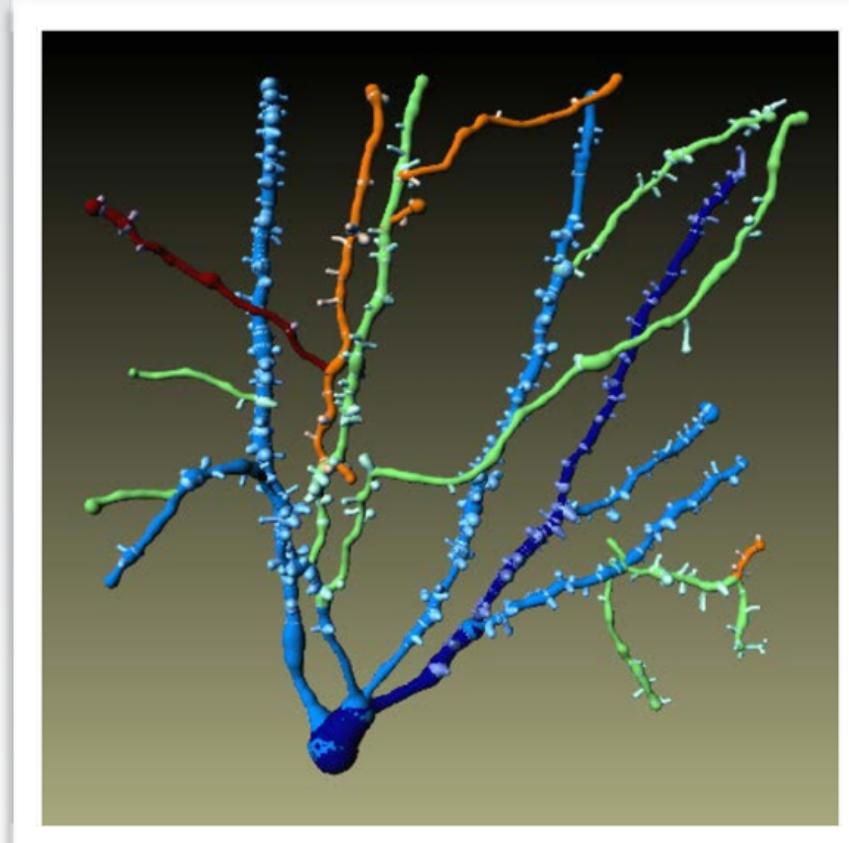
General analysis software: Cell Profiler



CellProfiler is free open-source software designed to enable biologists without training in computer vision or programming to quantitatively measure phenotypes from thousands of images automatically.

Source: <http://www.cellprofiler.org/> started by Anne E. Carpenter and Thouis Jones

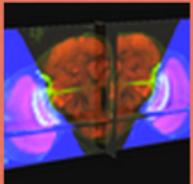
9



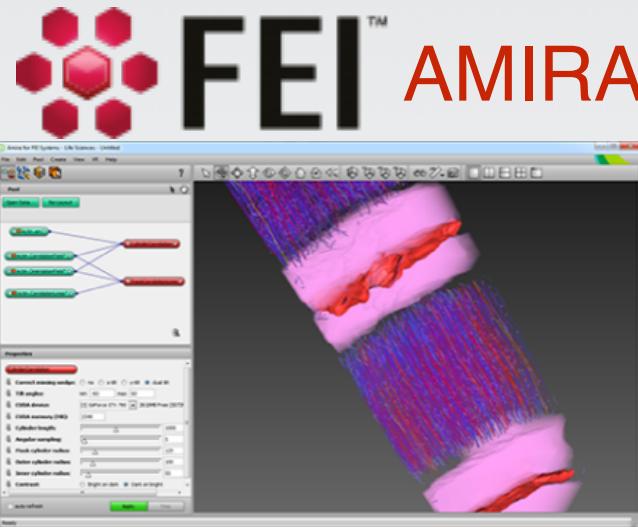
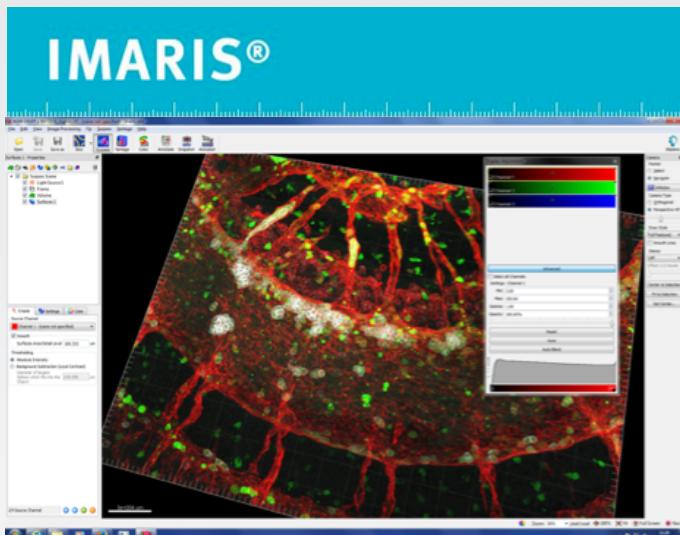
3D datasets

Source: Imaris Filament tracer

3D Software



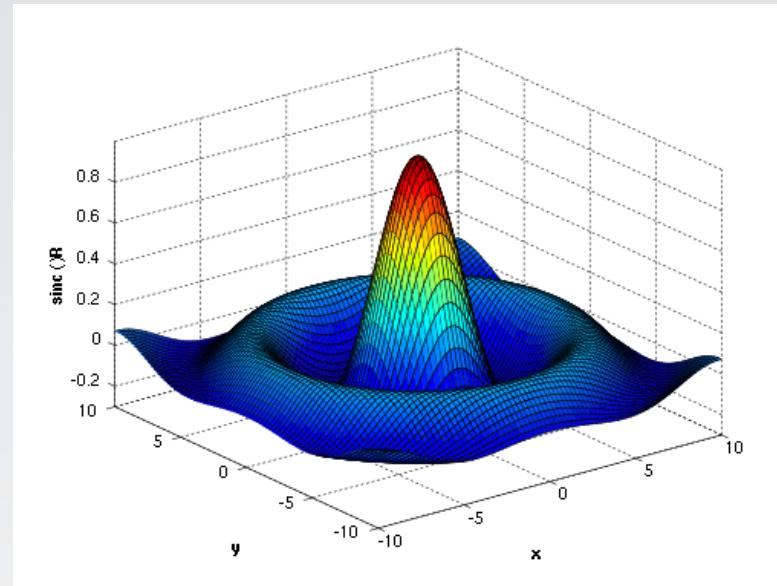
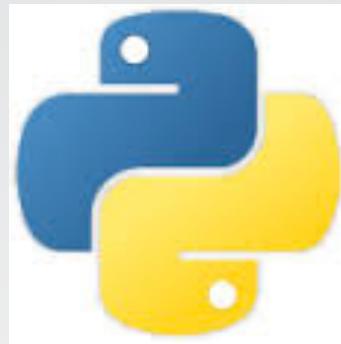
VAA3D 挖三维



- Volume Visualisation
- Automatic and manual identification of objects in 3D
- Tracking and Colocalisation in 3D
- generally expensive

Source: <http://www.fei.com/software/amira-3d-for-life-sciences/> <http://www.bitplane.com/imaris/imaris>, www.vaa3d.org/

Matlab and python



Matlab is popular tool for technical computing. Integrated programming environment. Images are imported as arrays of numbers.

Python is free and very versatile scripting language growing in popularity.

Matlab and python has many tools used for segmentation and analysis of data.

Both include visualisation tools for end-2-end analysis.

Source: en.wikipedia.org/wiki/MATLAB

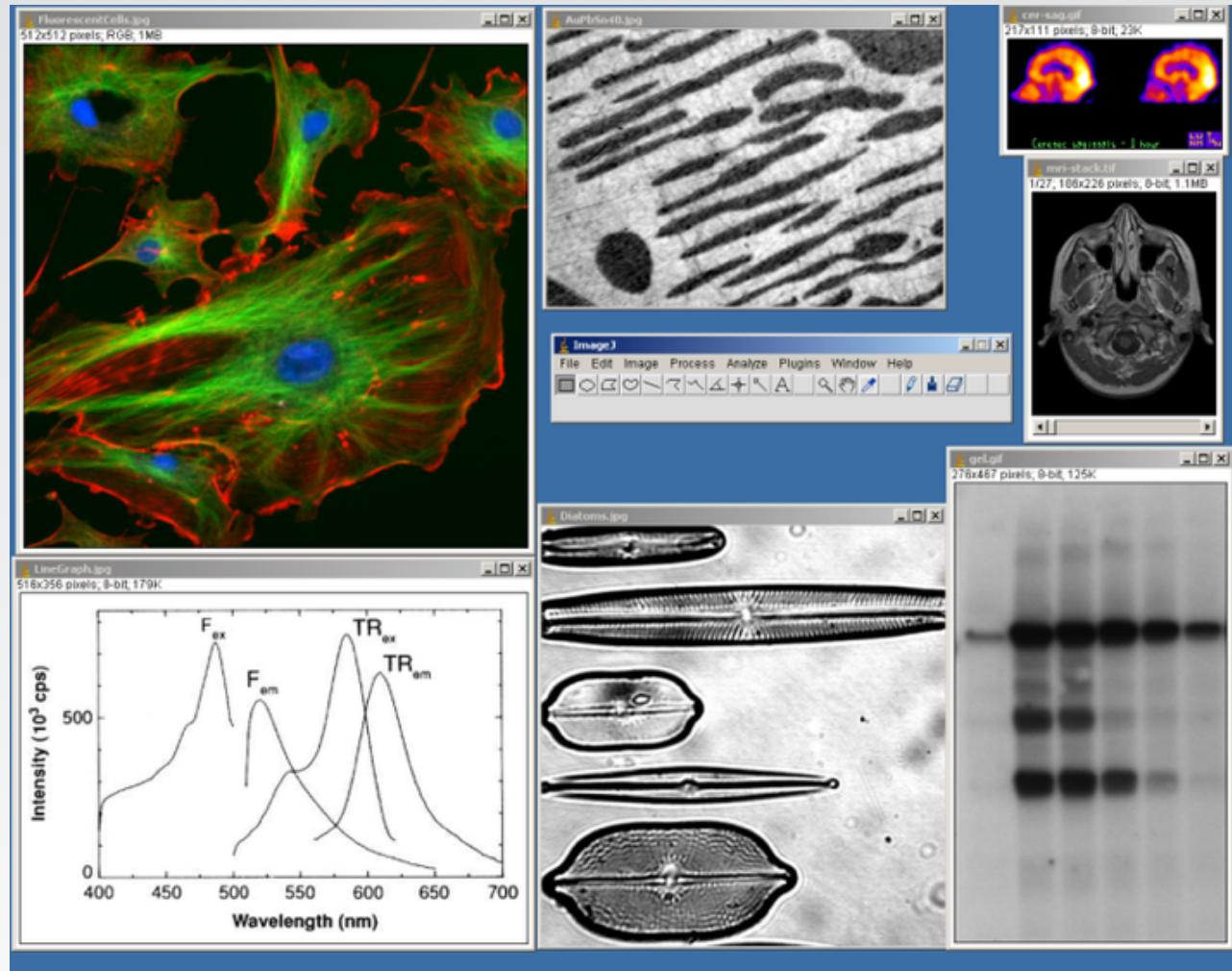
Fiji
ImageJ

Imaris,
Huygens

Matlab, Mathematica, Python

CellProfiler

ImageJ and FIJI



Originally created by Wayne Rasband at the NIH in 1997 as ImageJ.

Free and easy to get running on all systems.

Source: <http://fiji.sc/Fiji>

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Image representation: Grayscale

Grayscale Computer images are 2D arrays of numbers:

```
[ 93 ], [ 23 ], [ 23 ], [155 ], [155 ], [155 ]  
[107 ], [198 ], [198 ], [140 ], [140 ], [140 ]  
[121 ], [ 11 ], [ 11 ], [ 7 ], [ 7 ], [ 7 ]  
[135 ], [235 ], [235 ], [198 ], [198 ], [198 ]  
[149 ], [114 ], [114 ], [213 ], [213 ], [213 ]  
[163 ], [187 ], [187 ], [ 9 ], [ 9 ], [ 9 ]  
[ 8 ], [ 80 ], [ 80 ], [150 ], [150 ], [150 ]  
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[ 16 ], [165 ], [165 ], [111 ], [111 ], [111 ]  
[158 ], [ 15 ], [ 15 ], [ 34 ], [ 34 ], [ 34 ]  
[200 ], [120 ], [120 ], [ 69 ], [ 69 ], [ 69 ]  
[  ], [  ], [  ], [  ], [  ], [  ]
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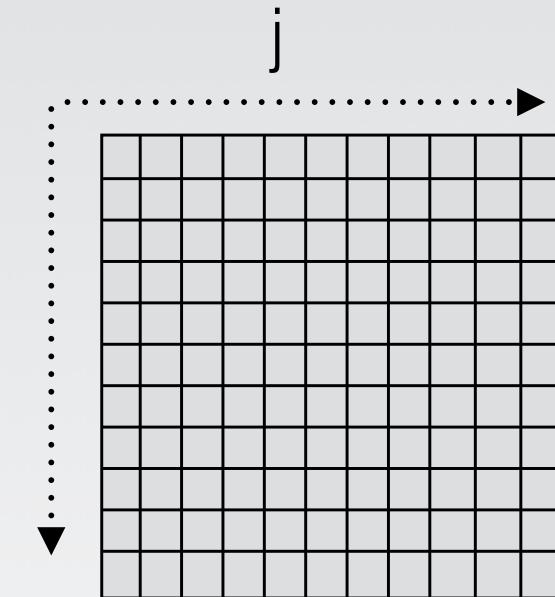
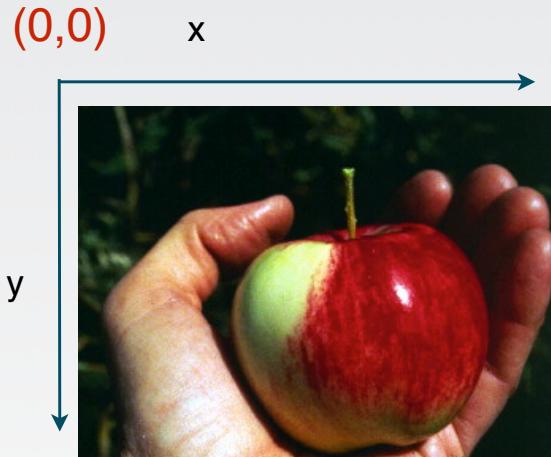


Image spatial quantisation



Digital representation.
Co-ordinate system
of image.



Resolution (no. pixels x,y).
Spatial quantisation



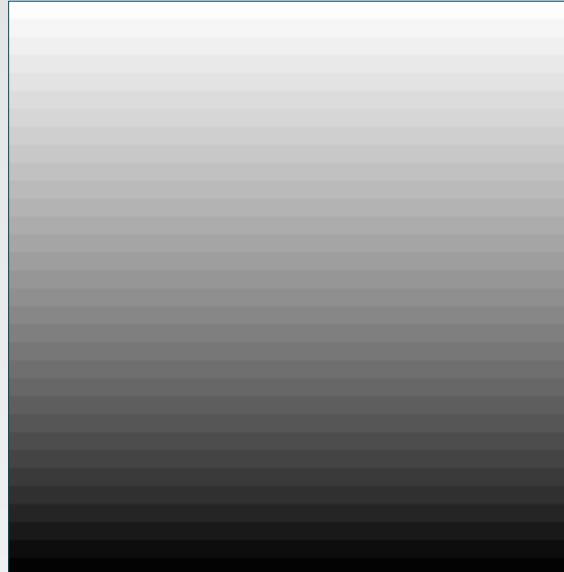
Source: <http://en.wikipedia.org/wiki/File:95apple.jpeg>

Image intensity quantisation

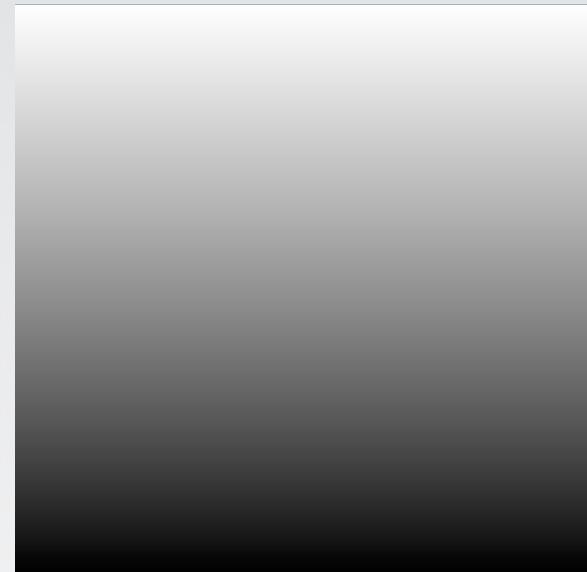
3-bit (2^3) 8 levels



5-bit (2^5) 32 levels

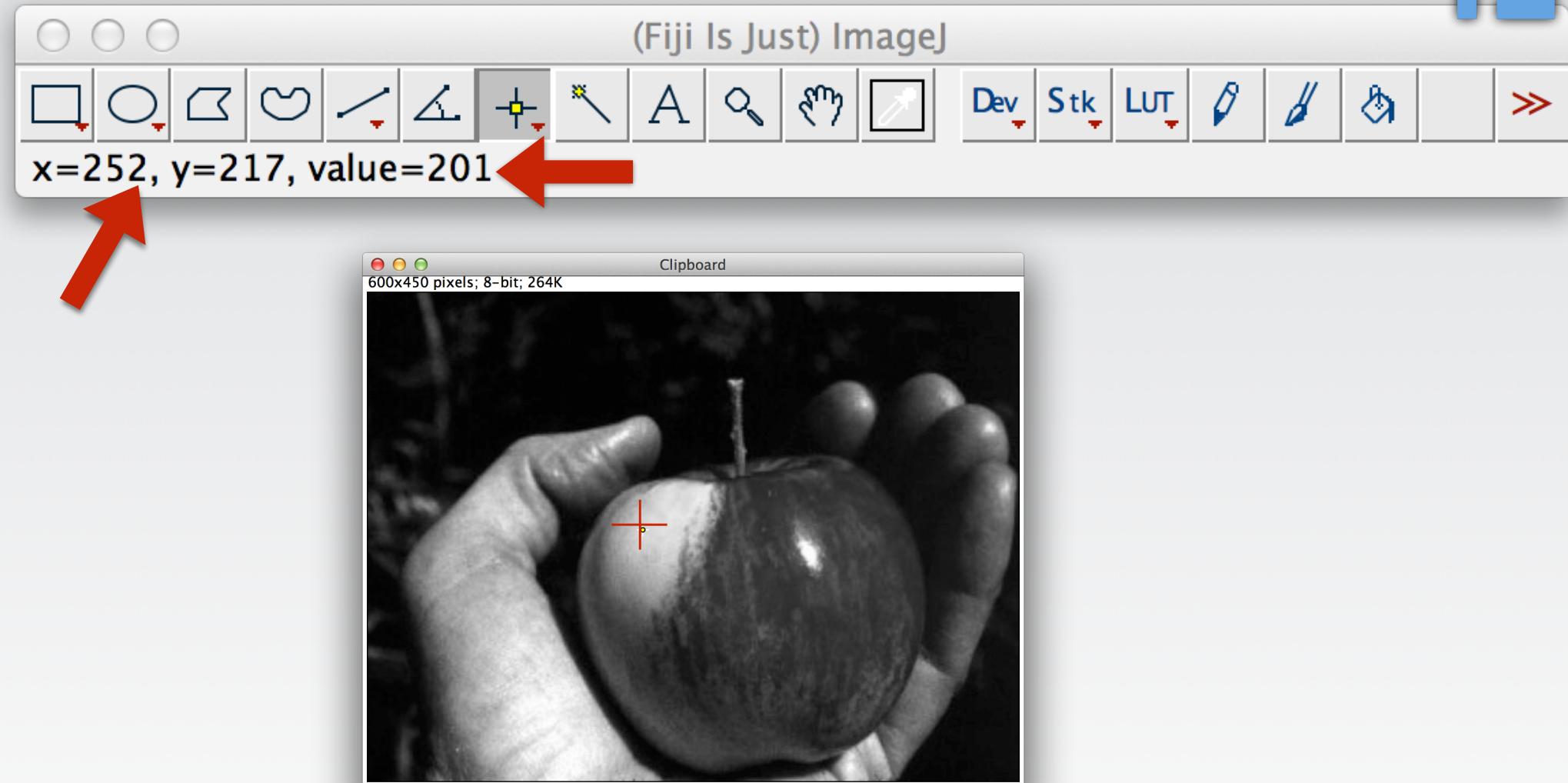


8-bit (2^8) 256 levels
16-bit (2^{16}) 65536 levels



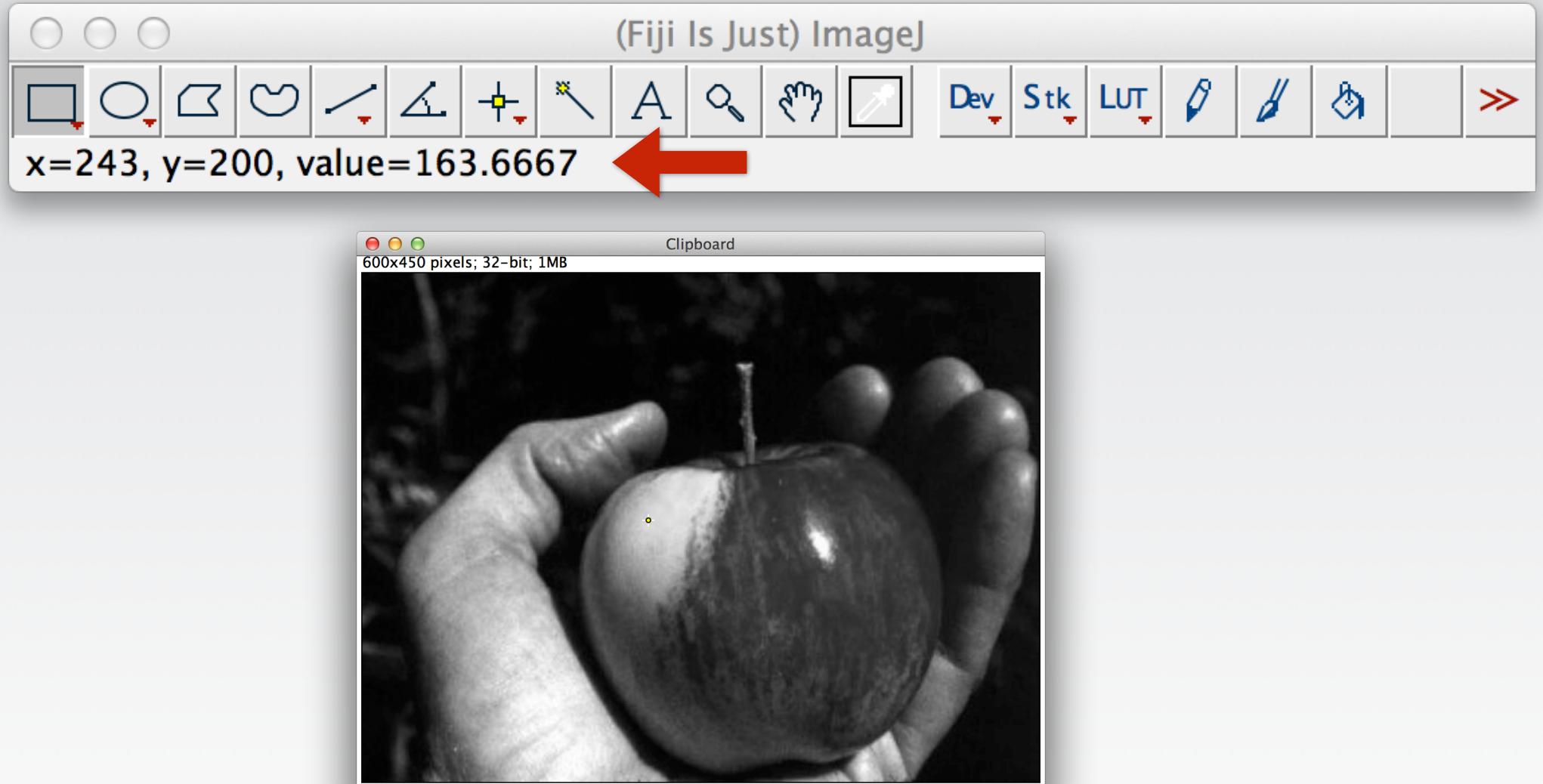
In reality most digital monitors can only show up to 8-bit.
This is why 8-bit and 16-bit images appear the same on a monitor.

Image representation



Inspecting your images is quick and easy using ImageJ/Fiji

Floating point numbers



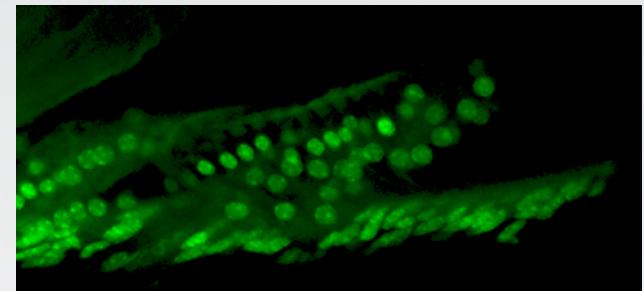
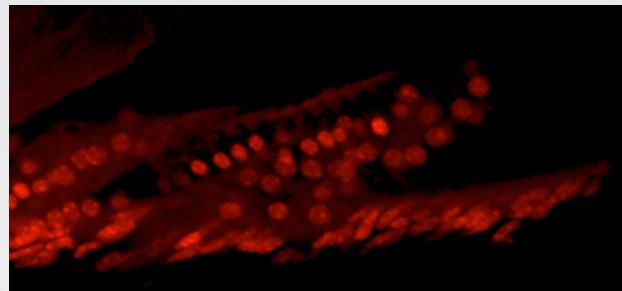
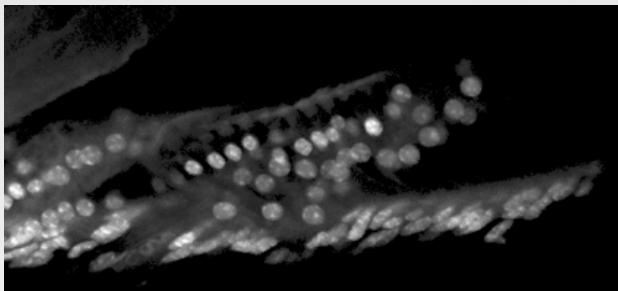
32-bit images represent floating point numbers. Useful for image maths.

Sourc

Image intensity

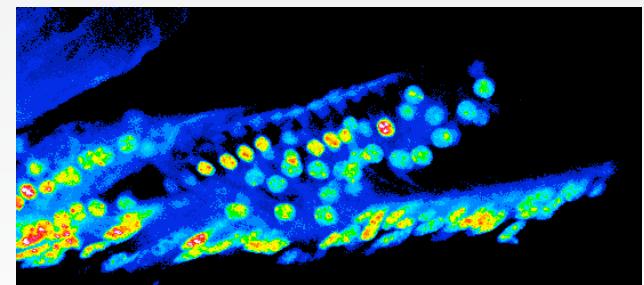
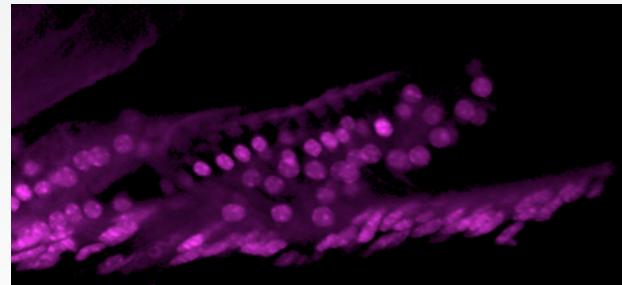
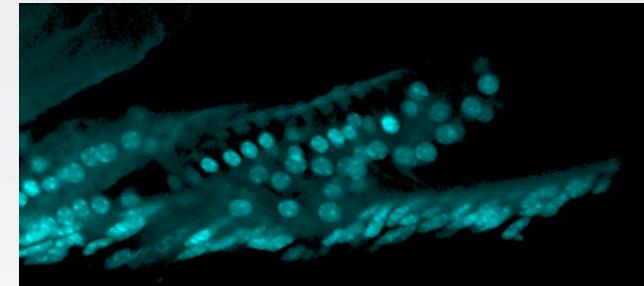
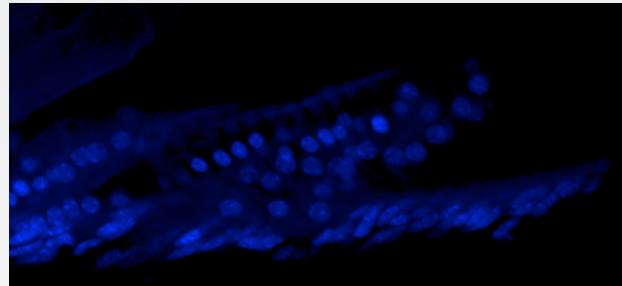


Pixel values when visualised on a screen can be coloured in a number of different ways, independent of the actual pixels values.



The colour pixels appear on a screen is defined by the look-up-table.

Any grayscale value can be mapped to any tone.

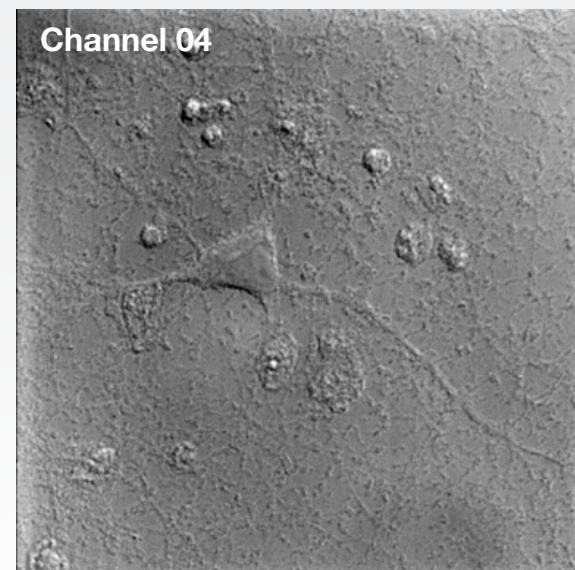
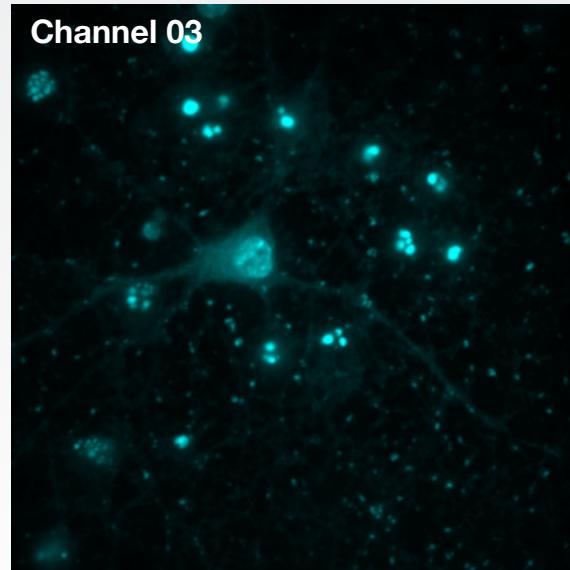
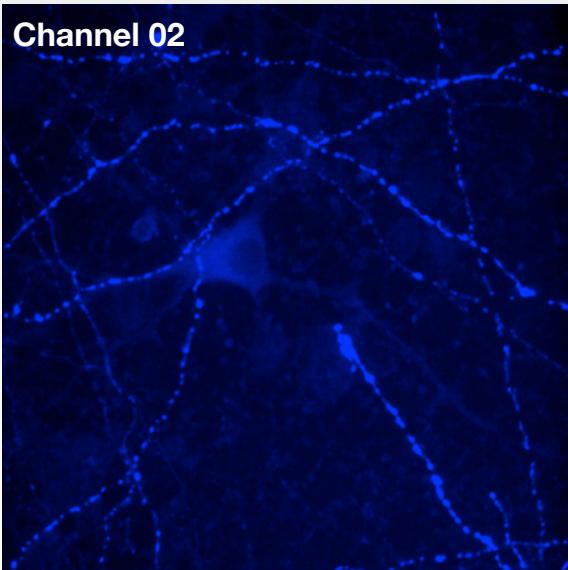
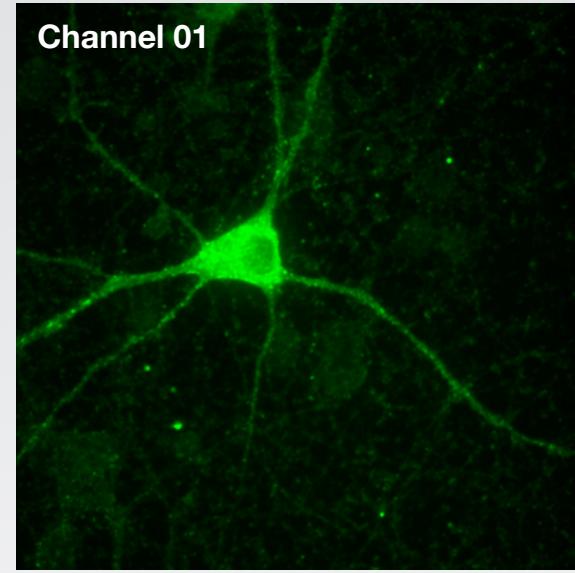
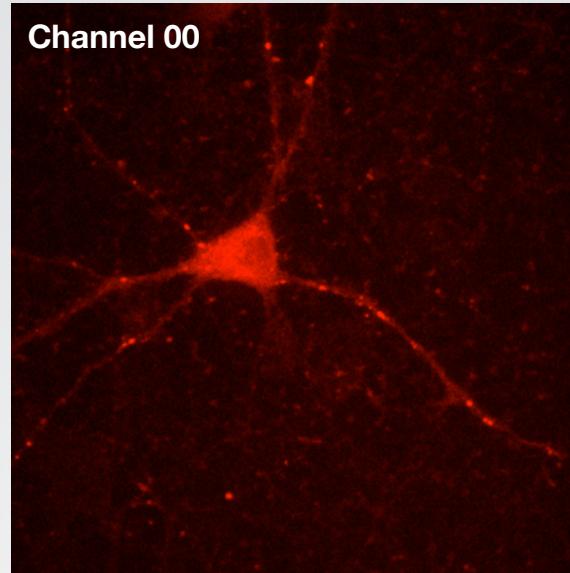
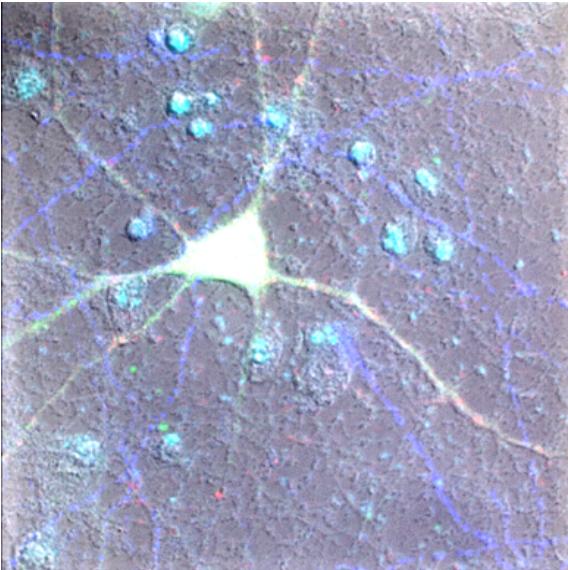


Source: Fiji, organ-of-corti.tif

Image representation



Composite representation

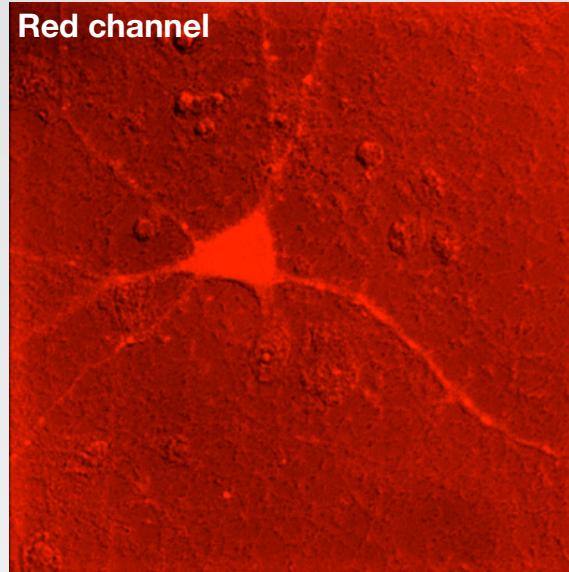
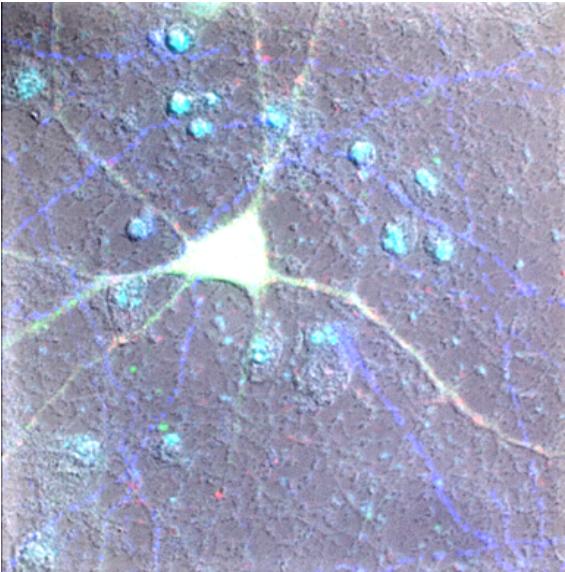


Source: ImageJ Rat_Hippocampal_Neuron.tif.

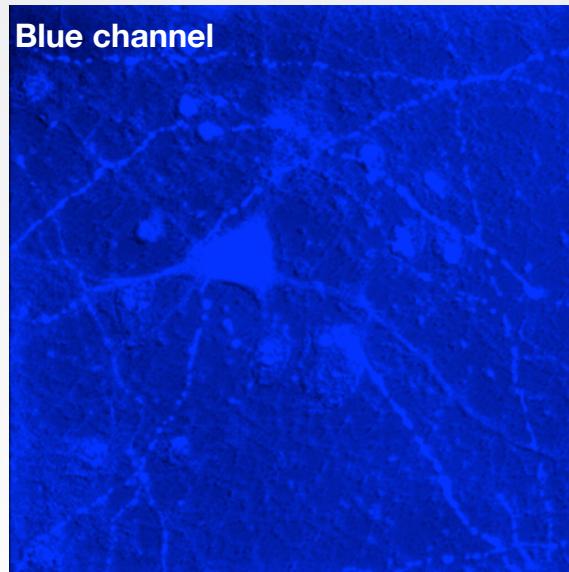
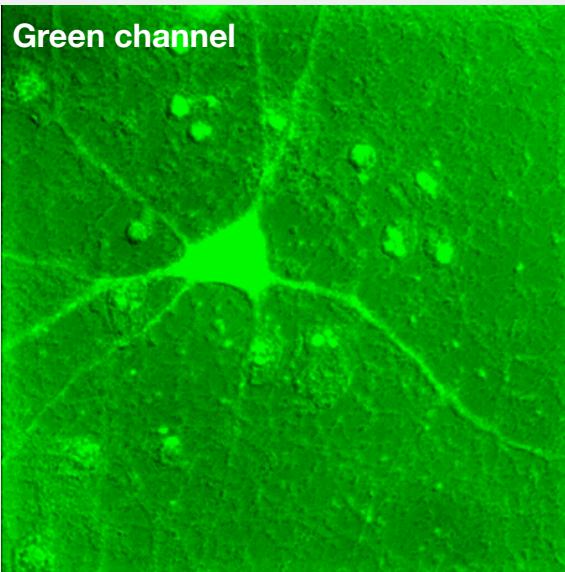
Image representation



RGB representation



If saved as a conventional image file (e.g. png or jpeg). The image intensity values are distributed amongst the red green and blue channels.



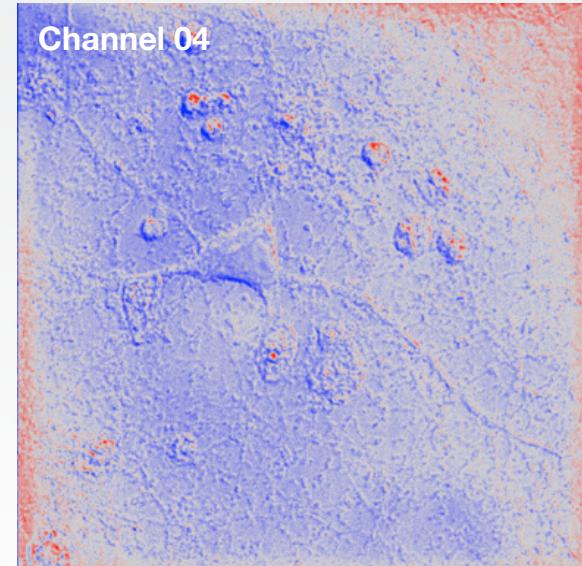
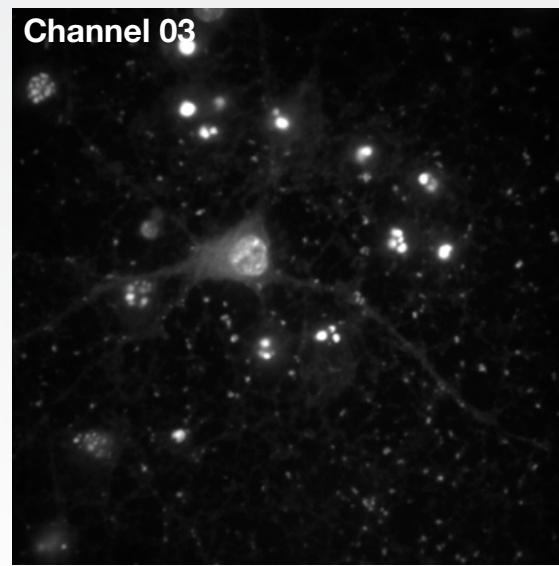
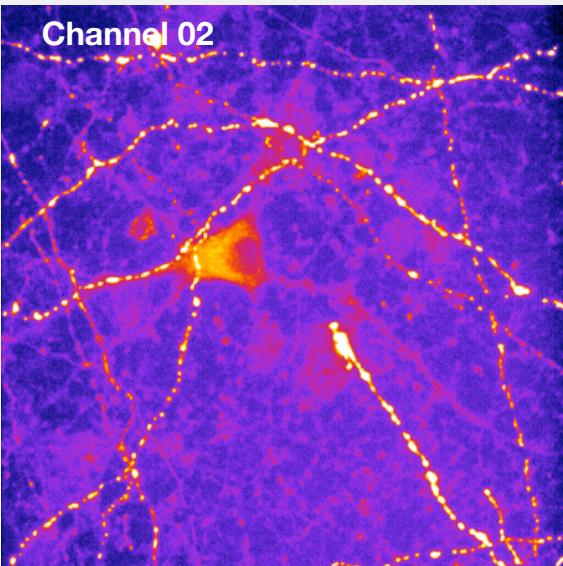
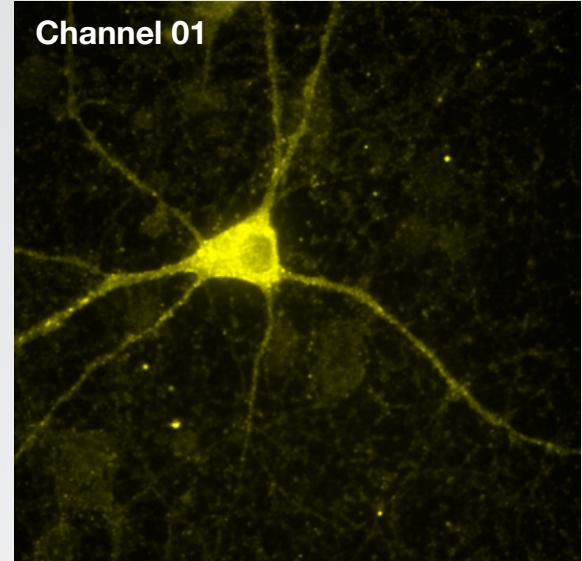
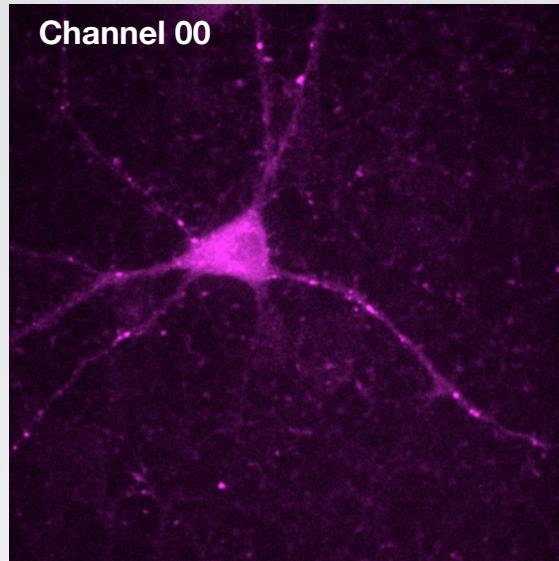
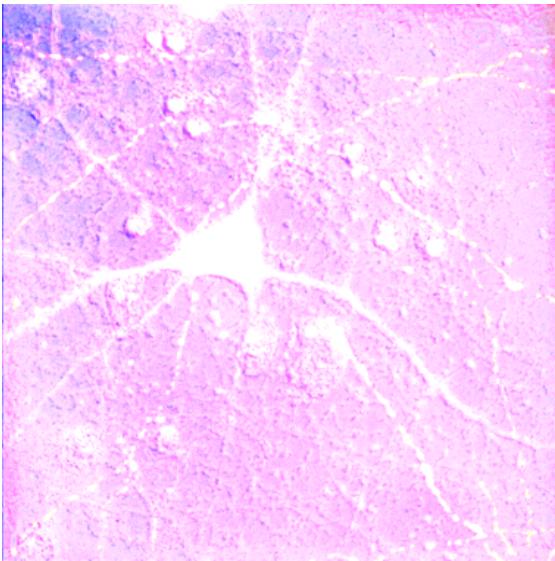
The intensity attributed to each channel is defined by the look-up-table. This is fine for making a picture or figure but bad for analysis as information is mixed and lost.

Source: ImageJ Rat_Hippocampal_Neuron.tif.

Image representation



Composite representation



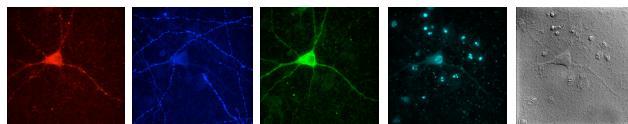
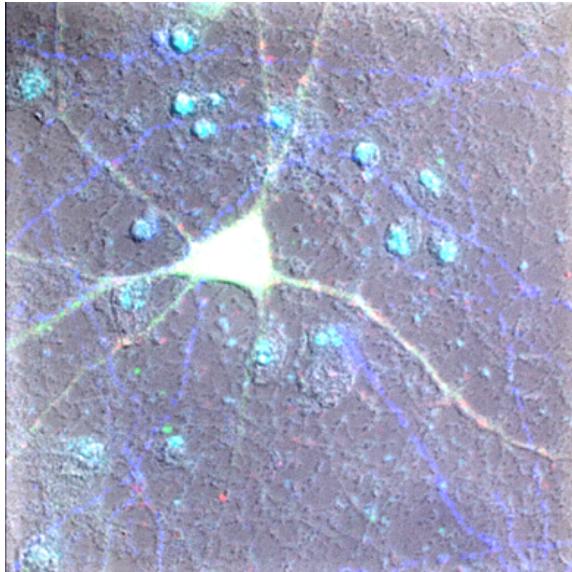
Source: ImageJ Rat_Hippocampal_Neuron.tif.

Image inspection

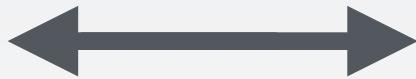
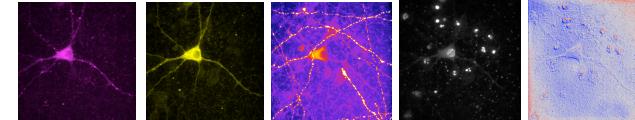
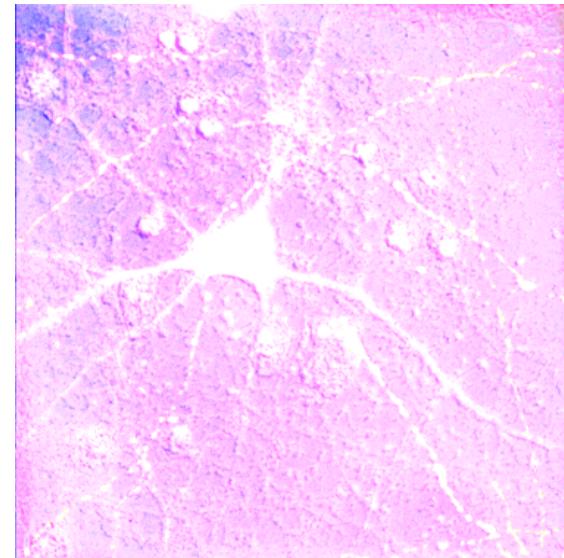


Same information just represented with different colours

Composite representation



Composite representation



Remember to save in TIFF format if you have many channels (multi-page format).
Don't be tricked by the colour of something. Use Fiji to inspect.

Source: <http://fiji.sc/Fiji>

Image -> color -> Channels Tool

Imaging pitfalls: Compression

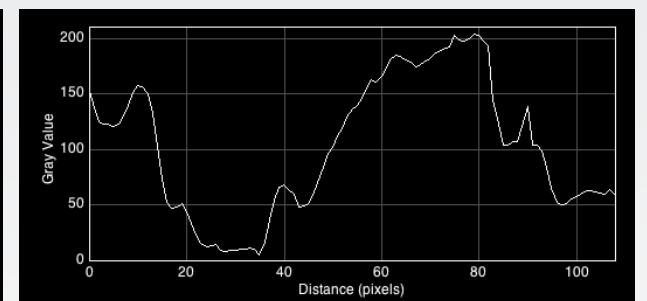
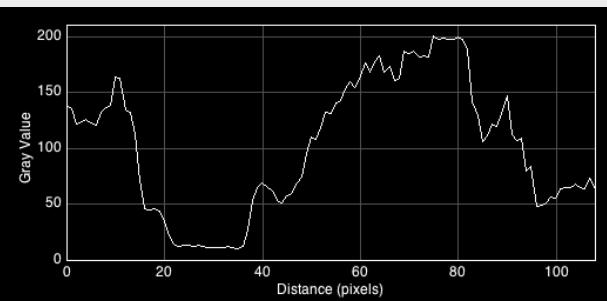
no Compression



medium Compression



max Compression



JPEG, movie formats = LOSSY & BAD (BMP, GIF, PNG also not great)
Compression also reduces certain colours more than others (e.g. blue tones).
TIFF is good, especially OME-TIFF

Source: http://upload.wikimedia.org/wikipedia/commons/c/ce/Quality_comparison_jpg_vs_saveforweb.jpg

Practical content and lectures

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/Modules/ 2016-17 / Michaelmas term /
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Summary of course: Course-schedule-overview.html

Practicals: day01_practicals/day01-prac-overview.html

Source: