Image Analysis with Fiji

http://imagej.net/Presentations

What is ImageJ?

A tool for ***scientific*** image analysis

Open source

- You wouldn't write:
  Added 500mg of unknown chemical.
- So don't put your data in a black box!

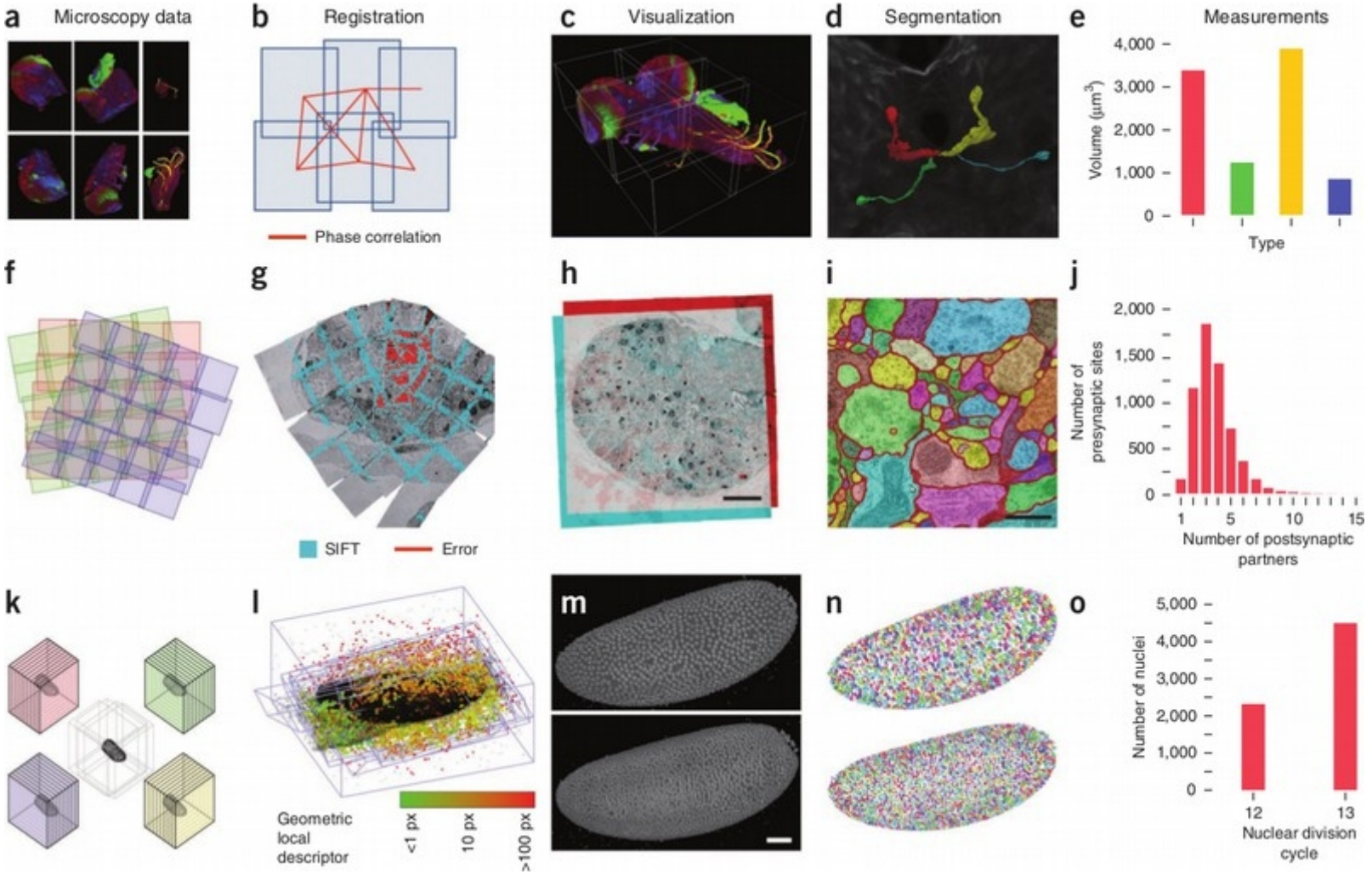
Rich ecosystem: thousands of plugins

Science = reproducibility

Plugins>Utilities>Make Fiji Package

http://imagej.net/

Fiji Is Just ImageJ



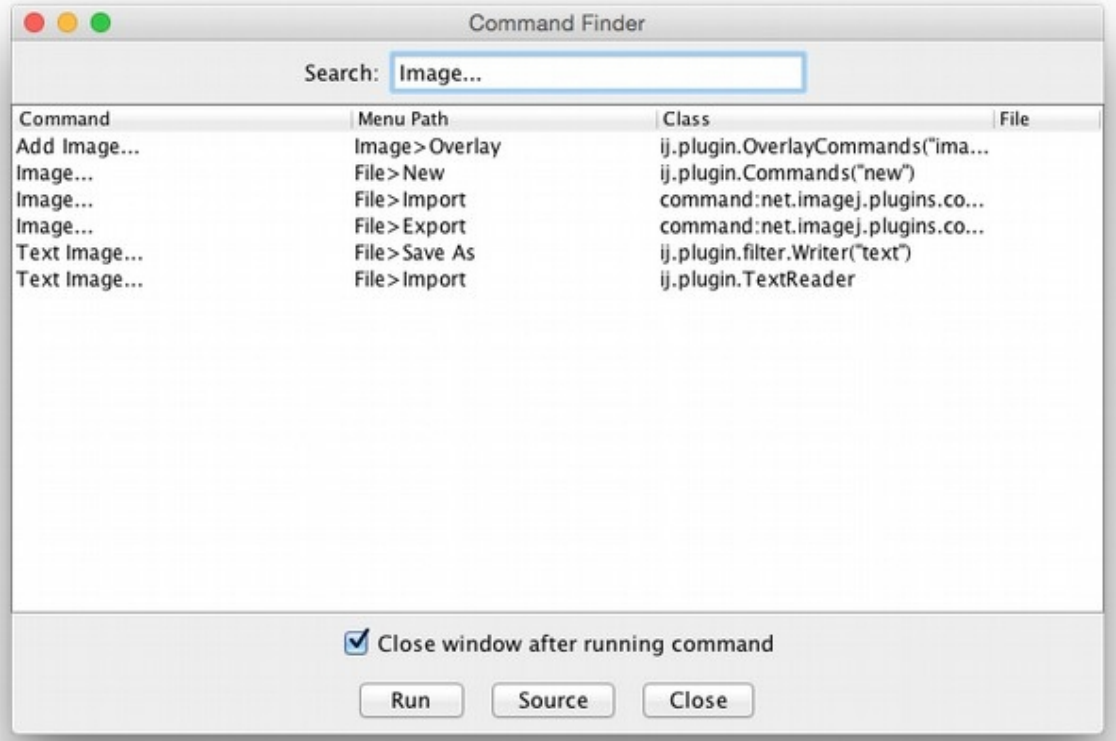
http://imagej.net/Introduction

Who uses Fiji?

Resources for advice and collaboration

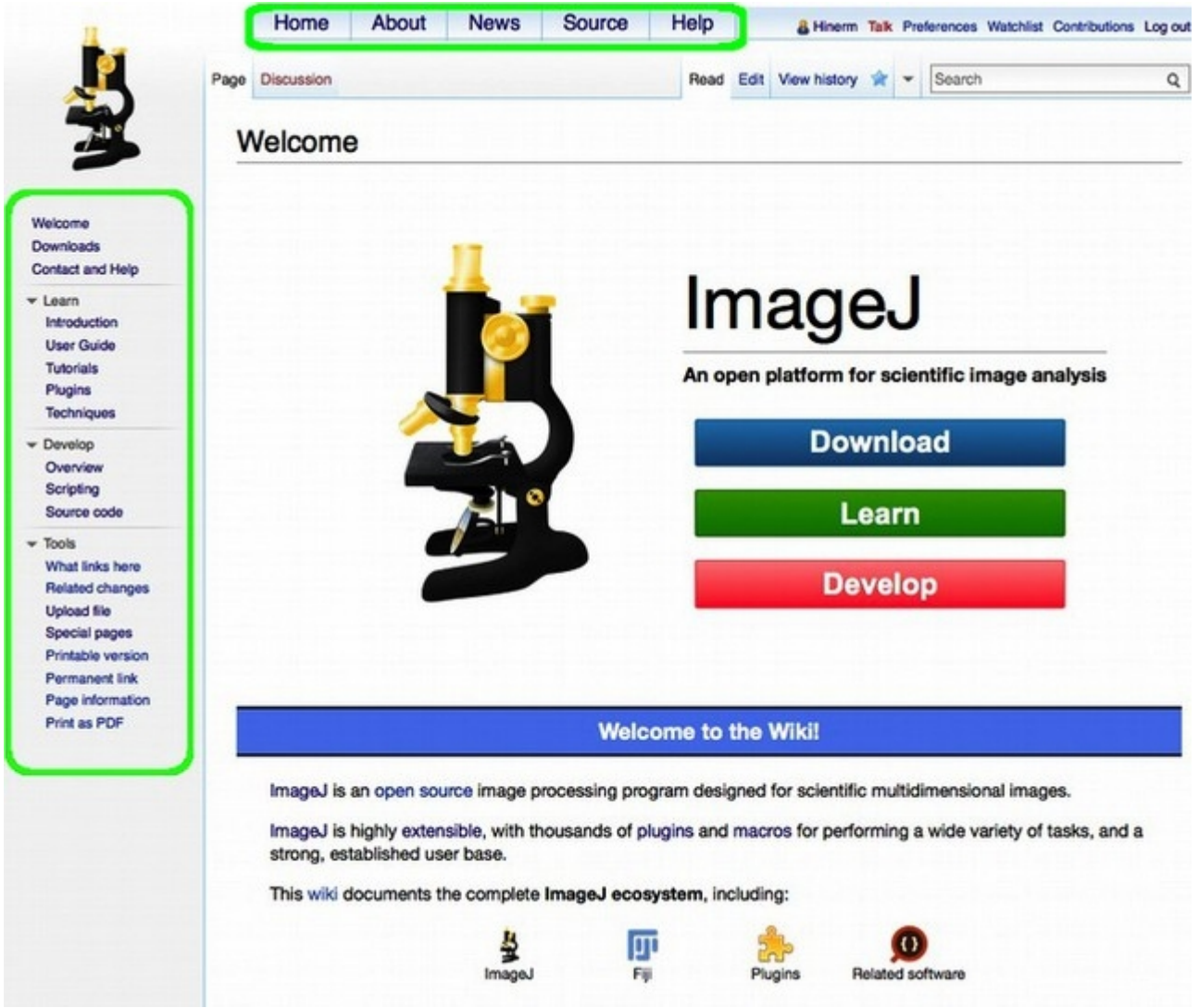
There's a lot of stuff!

Ctrl+L = Command Finder



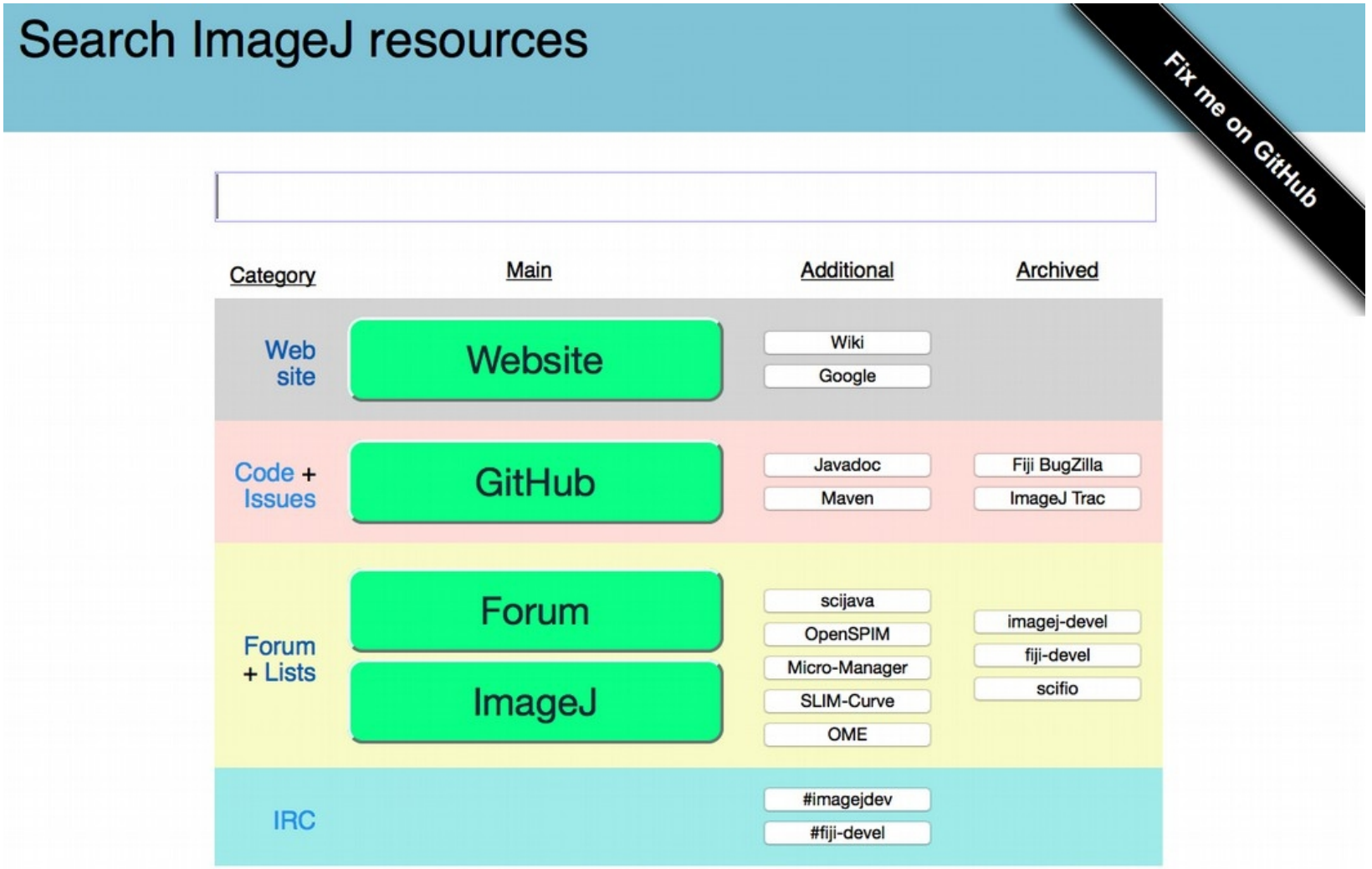
http://imagej.net/Command_Finder

Learn how to fish:



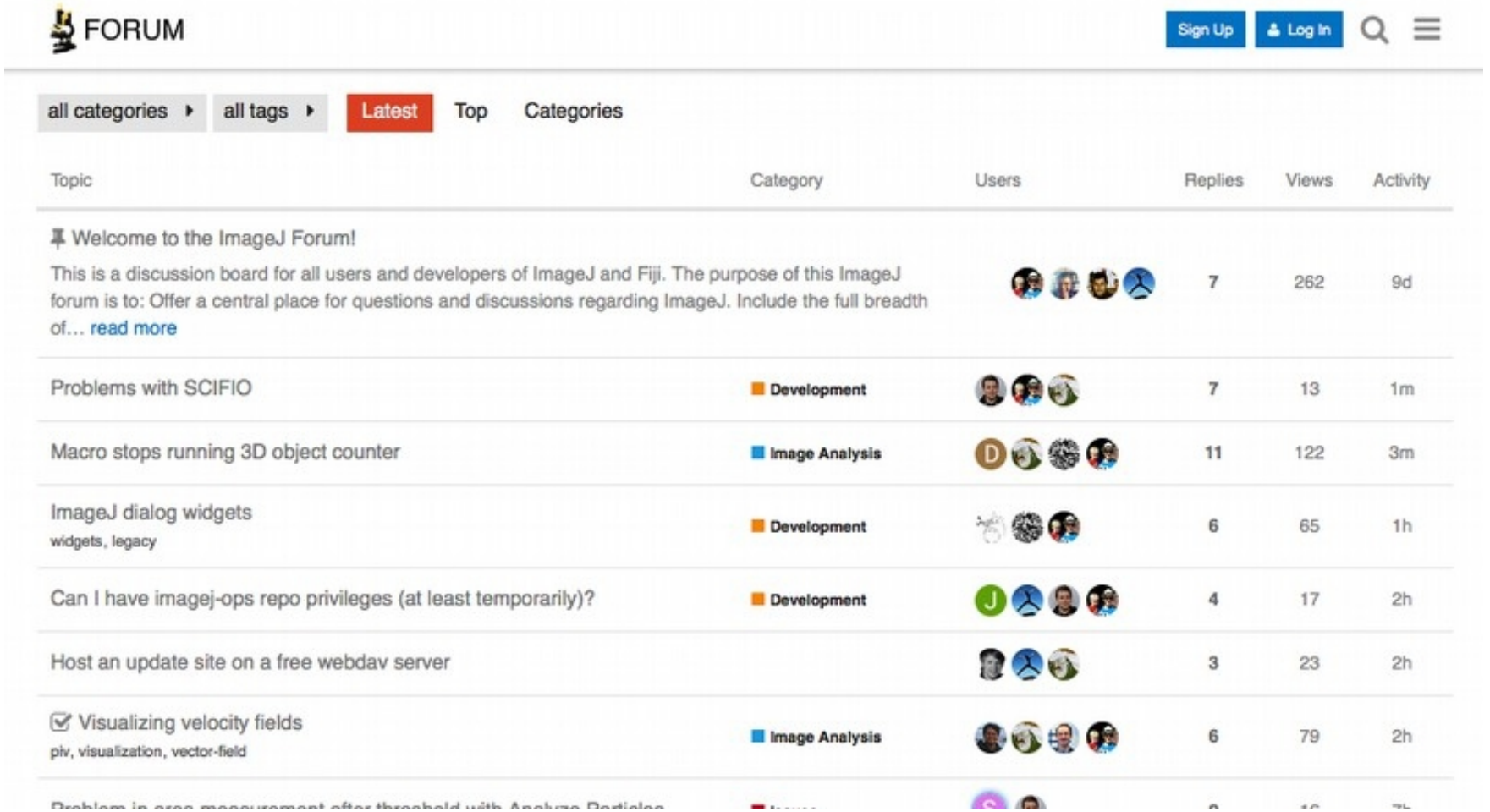
http://imagej.net/

Learn how to fish:



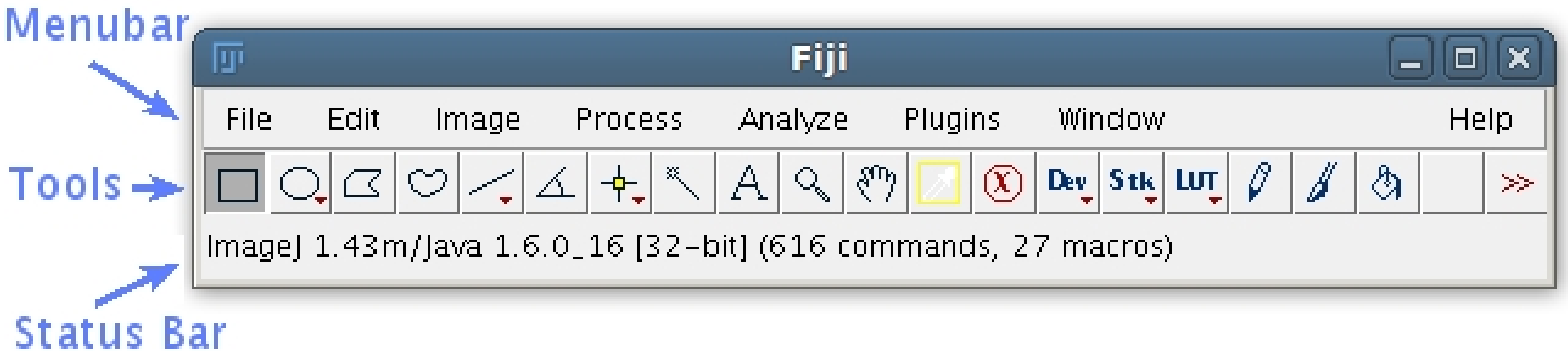
http://search.imagej.net/

Teach me how to fish!



http://forum.imagej.net/

The main window

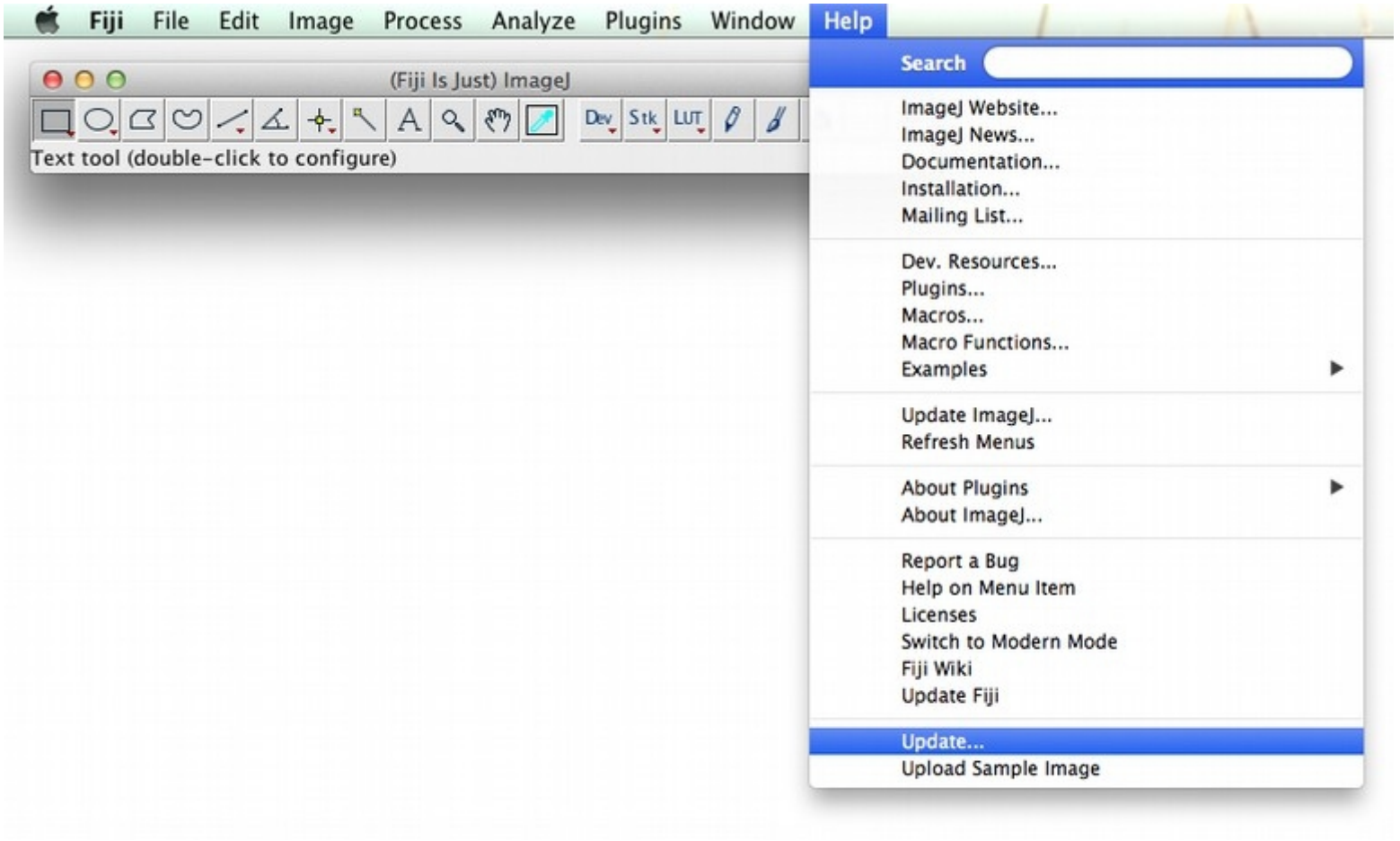


Tip: click on the status bar

Tip: right / double-click on Tools

http://imagej.net/Getting_started

Staying up-to-date

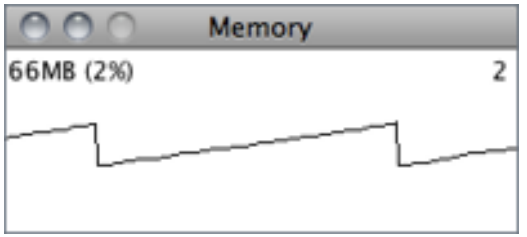


http://imagej.net/Updater

Memory management

Edit>Options>Memory & Threads

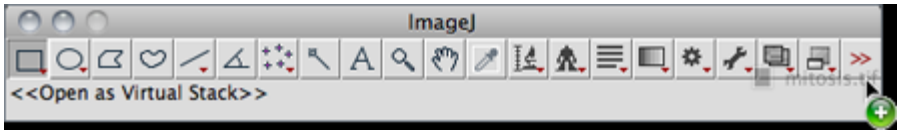
Plugins>Utilities>Monitor Memory



http://imagej.net/guide/146-27.html

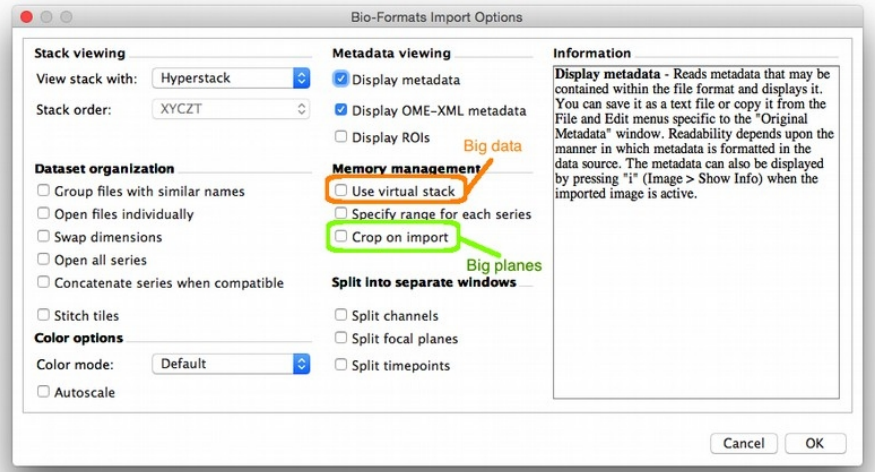
Opening images

Drag & Drop



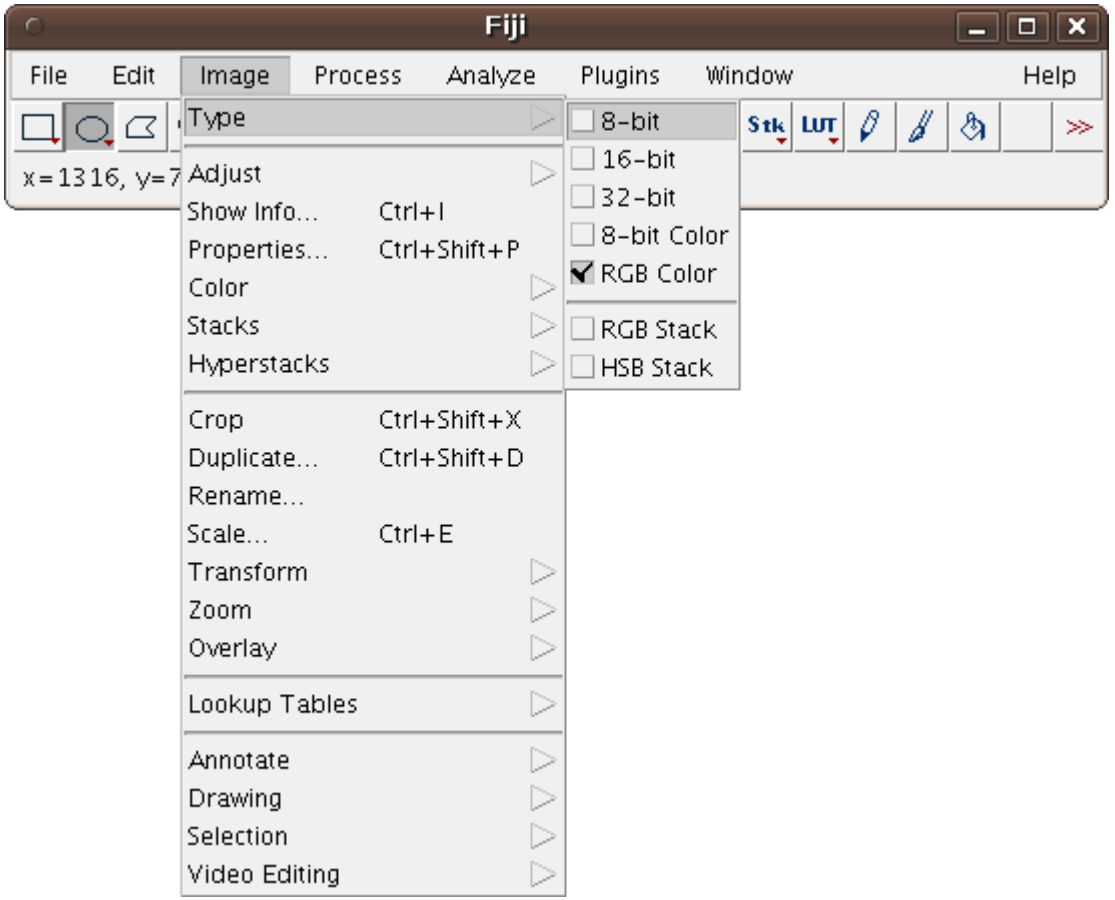
File>Open

Plugins>Bio-Formats>
Bio-Formats Importer



http://imagej.net/Importing_Image_Files

Pixel types

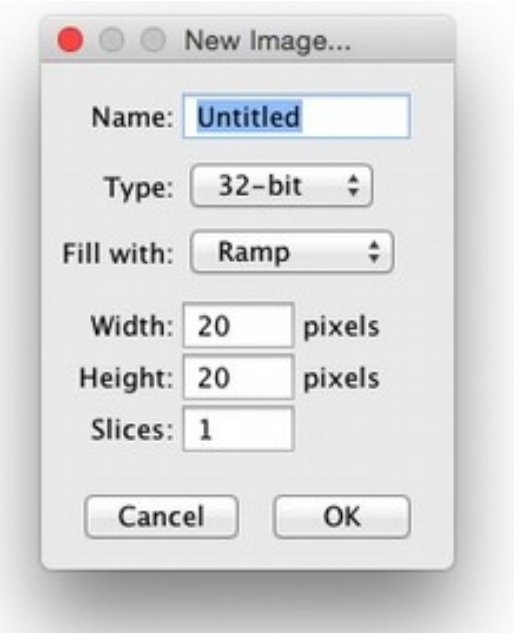


http://imagej.net/docs/guide/146-7.html

Pixel type pitfalls

Know the limitations of your data

- *File>New>Image...* (32-bit, ramp, 20x20)
- *Process>Math>Multiply...* : 100,000,000
- Probe values
- *Process>Math>Add...* : 1
- Probe values

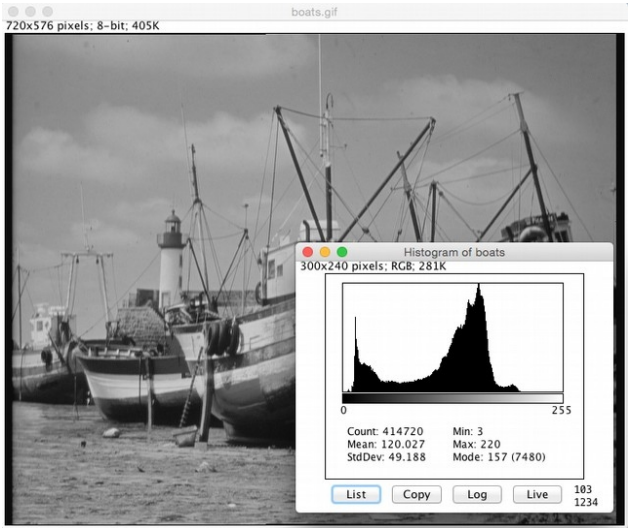


Can you find any problems?

http://imagej.net/IP_Principles

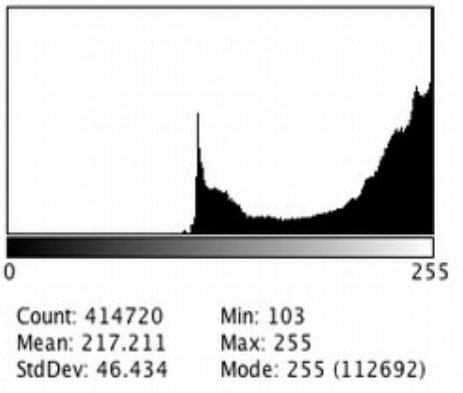
Get to know your data

- *File>Open Samples>Boats*
- *Analyze>Histogram*



Compare histograms:
File>Open Samples>Blobs

What would cause this histogram:

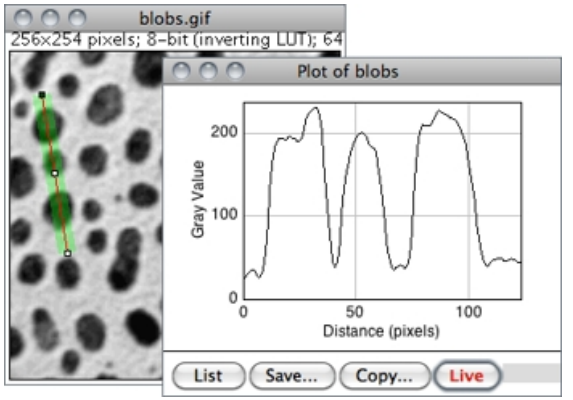


http://imagej.net/docs/guide/146-30.html

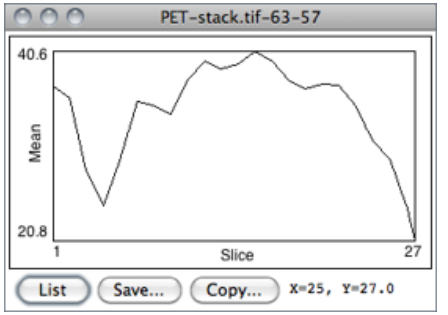
Profile Plots

Qualitative observations → Quantitative data

- Open Blobs *(Shift + B)*
- Use any *Line* tool
- *Analyze>Plot Profile*



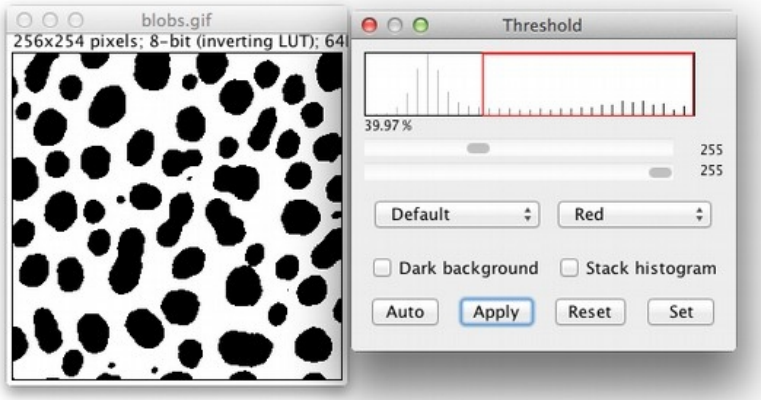
Image>Stacks>Plot Z-Axis Profile...



http://imagej.net/docs/guide/146-28.html

Thresholding

Isolate **values** of interest



- Open Blobs *(Shift + B)*
- *Image>Adjust>Threshold…*
- Use *Profile Plot* to guide threshold

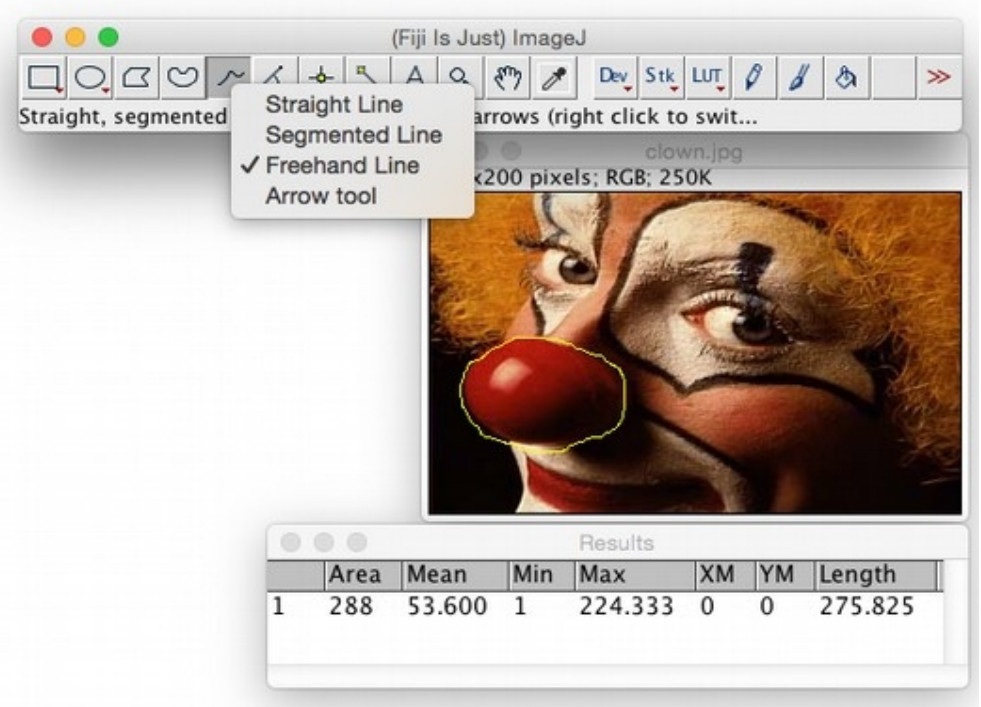
Which method is best?

Image>Adjust>Auto Threshold>Try All

http://imagej.net/docs/guide/

Regions of interest: ROIs

- *File>Open Samples>Clown*
- *Freehand Line*
- Circle the clown nose
- *Analyze>Measure*



Can you draw the same circle on a new clown?

http://imagej.net/docs/guide/146-10.html

ROI Manager

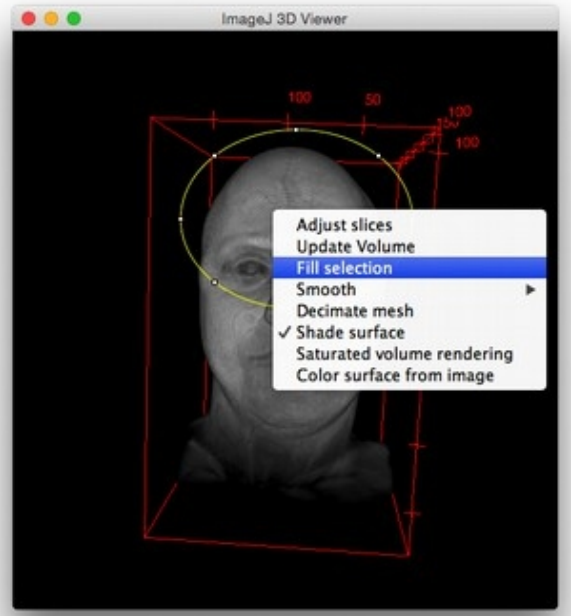
Two clowns, one measurement

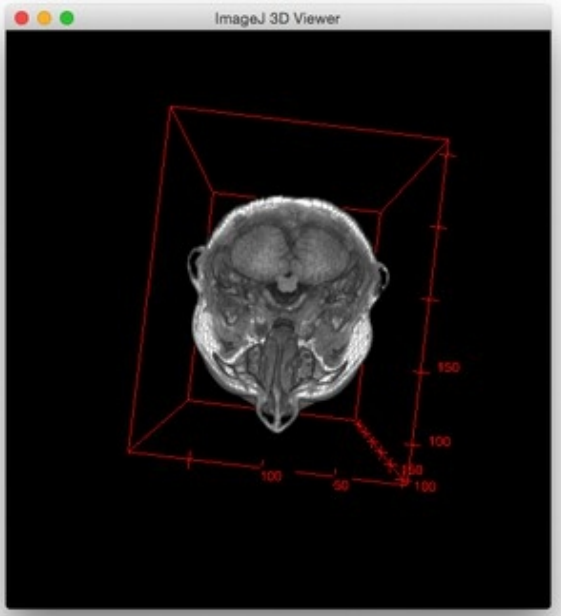
- Select a nose
- Press *t* – **or** *Analyze>Tools>ROI Manager*
- Select other clown
- Click ROI in manager – **or** *Edit>Selection>Restore Selection*

http://imagej.net/docs/guide/146-10.html

Qualitative Observations

File>Open Samples>T1 Head (2.4M, 16-bits)
Image>Type>8-bit
Plugins>3D Viewer (Resampling Factor: 1)





http://imagej.net/3D_Viewer

Information flow

Starts at image acquisition!

What does this image tell us
about the volume of this pipette?

Information loss

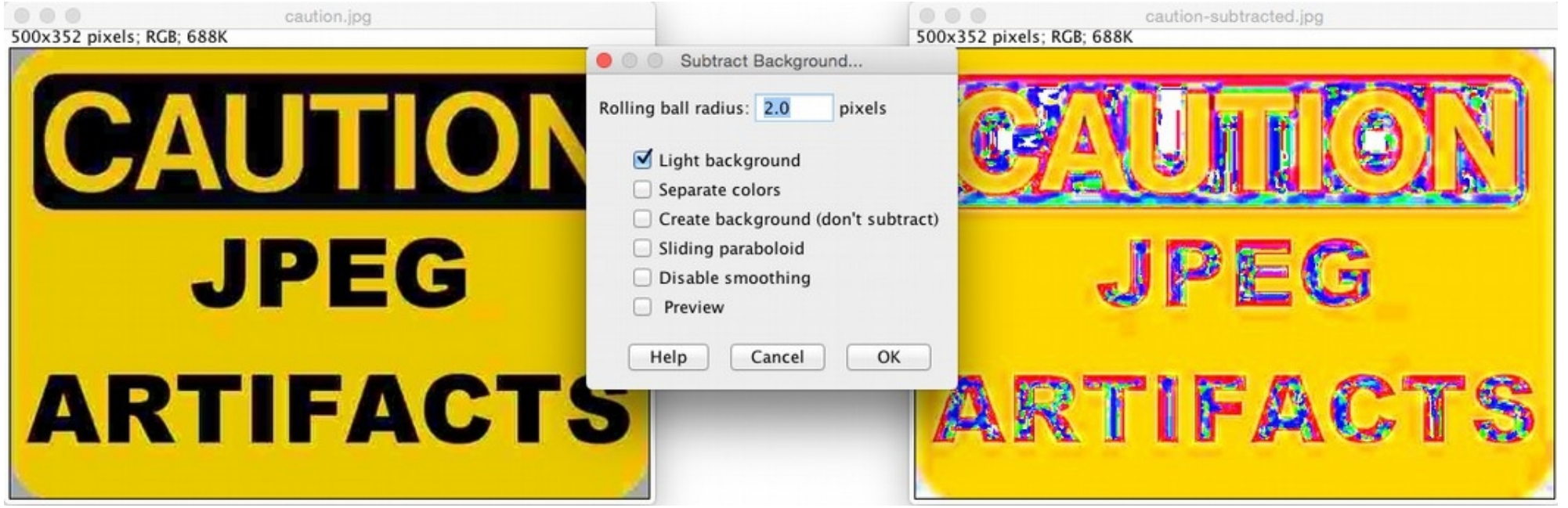
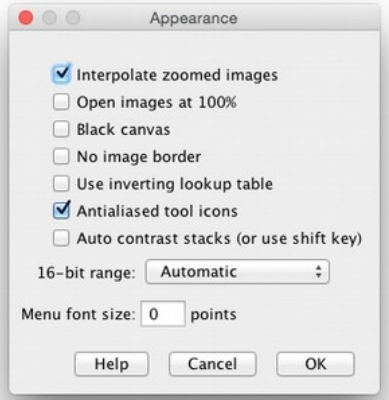


Image formats are not created equal!

Pixel Problems

Pixels are not little squares

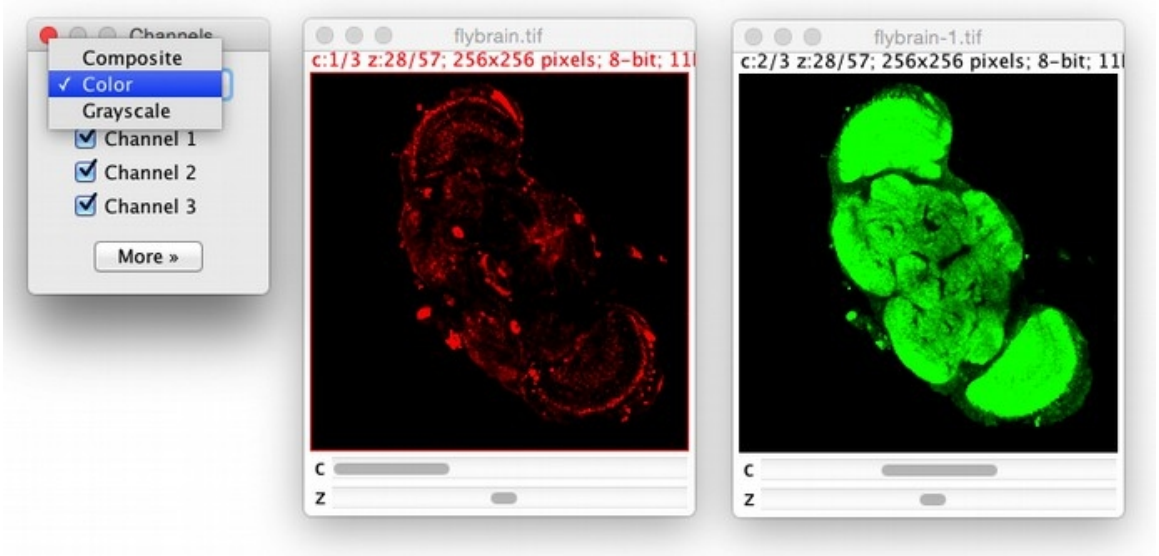
- See Alvy Ray Smith's article for details:
  http://alvyray.com/Memos/CG/Microsoft/6_pixel.pdf
- Can think of detectors as sampling a Gaussian
  Edit>Options>Appearance...>
  Interpolate zoomed images

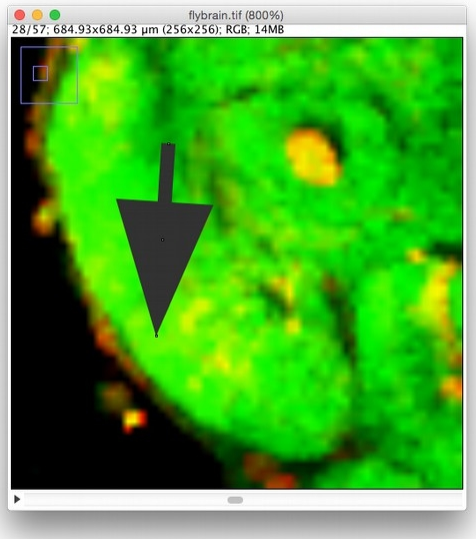


http://imagej.net/IP_Principles

Eyes? More like LIES!

- *File>Open Samples>Fly Brain*
- *Image>Color>Channels Tool…*



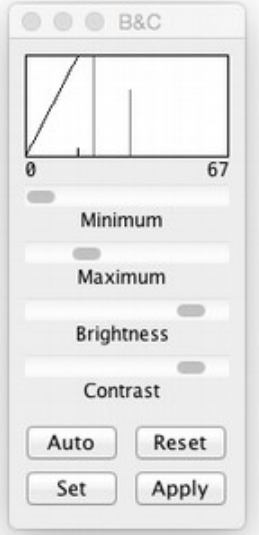


Can *"Composite"* mode show colocalization?

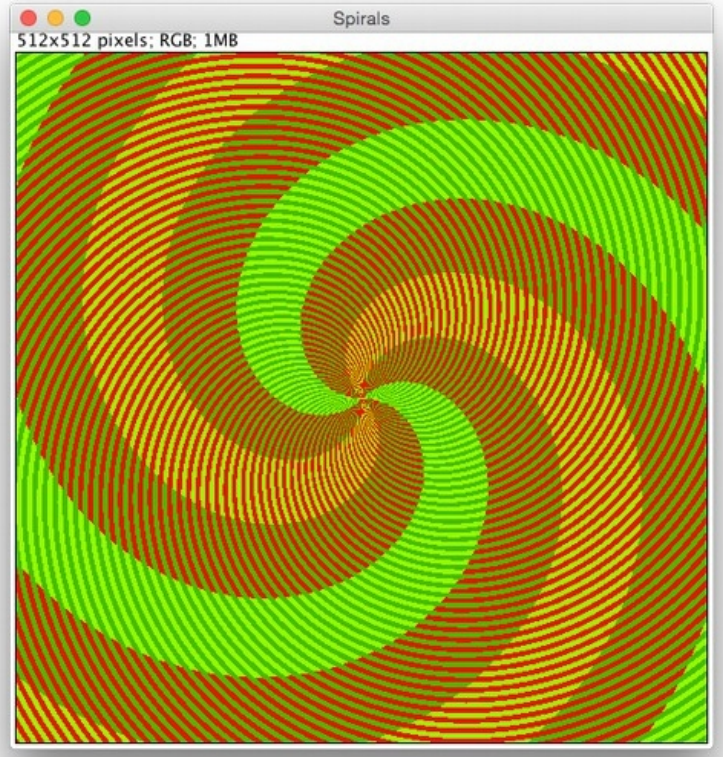
http://imagej.net/docs/guide/146-28.html

"Color" is not "signal"

File>Open Samples>Spirals



How many colors in the spiral below?



What if we:

Image>Adjust>Brightness/Contrast

… change colors?

Image>Type>8-bit color
Image>Lookup Tables>glasbey

… zoom in (+) and probe?

Eyes are not all equal

Image>Color>Simulate Color Blindness

https://nei.nih.gov/health/color_blindness

So what CAN we do?

- *Image>Color>Split channels*
- *Process>Image calculator (AND)*

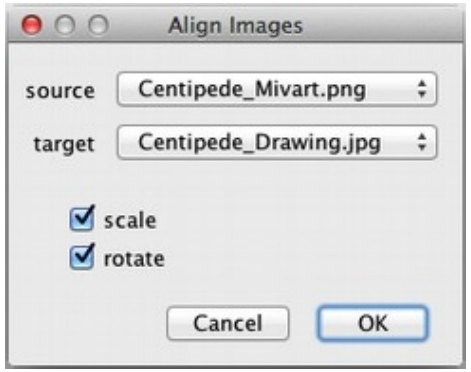
- **But…** what question have we answered?

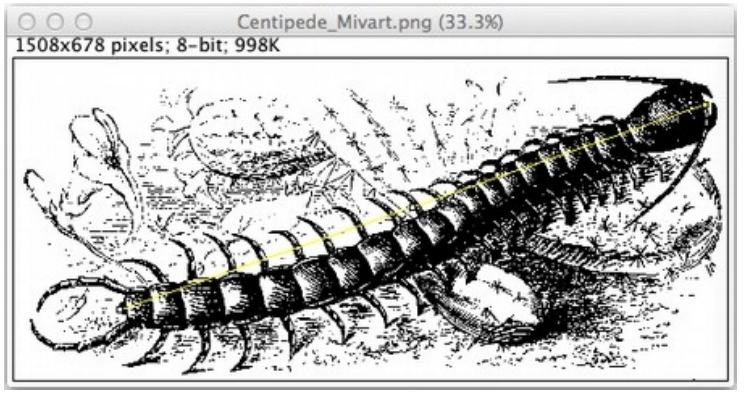
http://imagej.net/docs/menus/process.html

Registration

Unify coordinates of 2+ images

- *File>Open Samples>Centipede Drawing*
- *File>Open Samples>Centipede Mivart*
- Draw lines between equivalent points
- *Plugins>Registration>Align Image by line ROI*



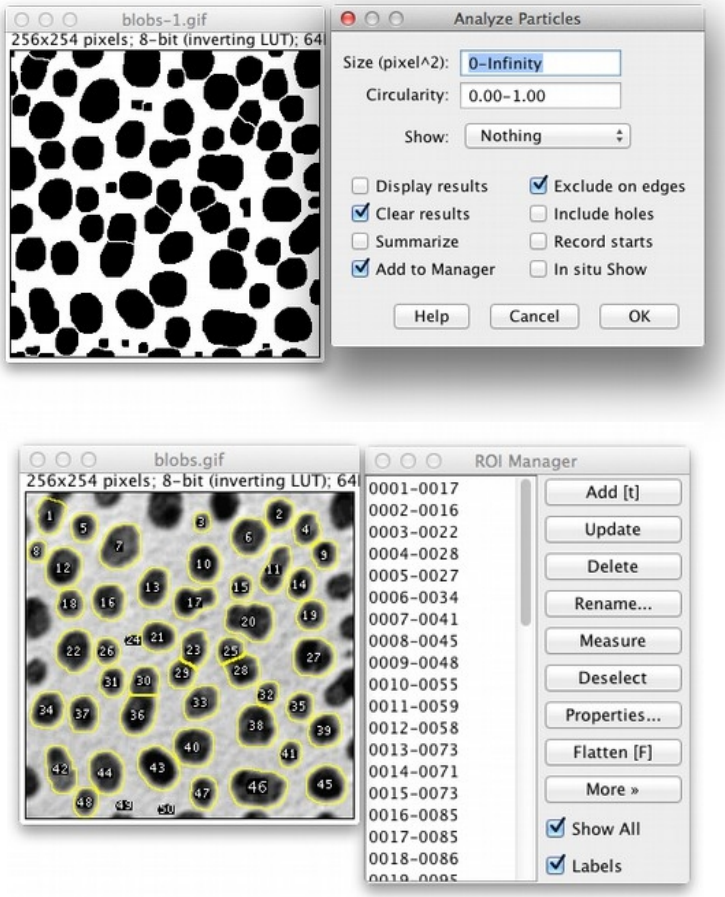


http://imagej.net/Image_Stitching

Segmentation

Identify blobs of interest

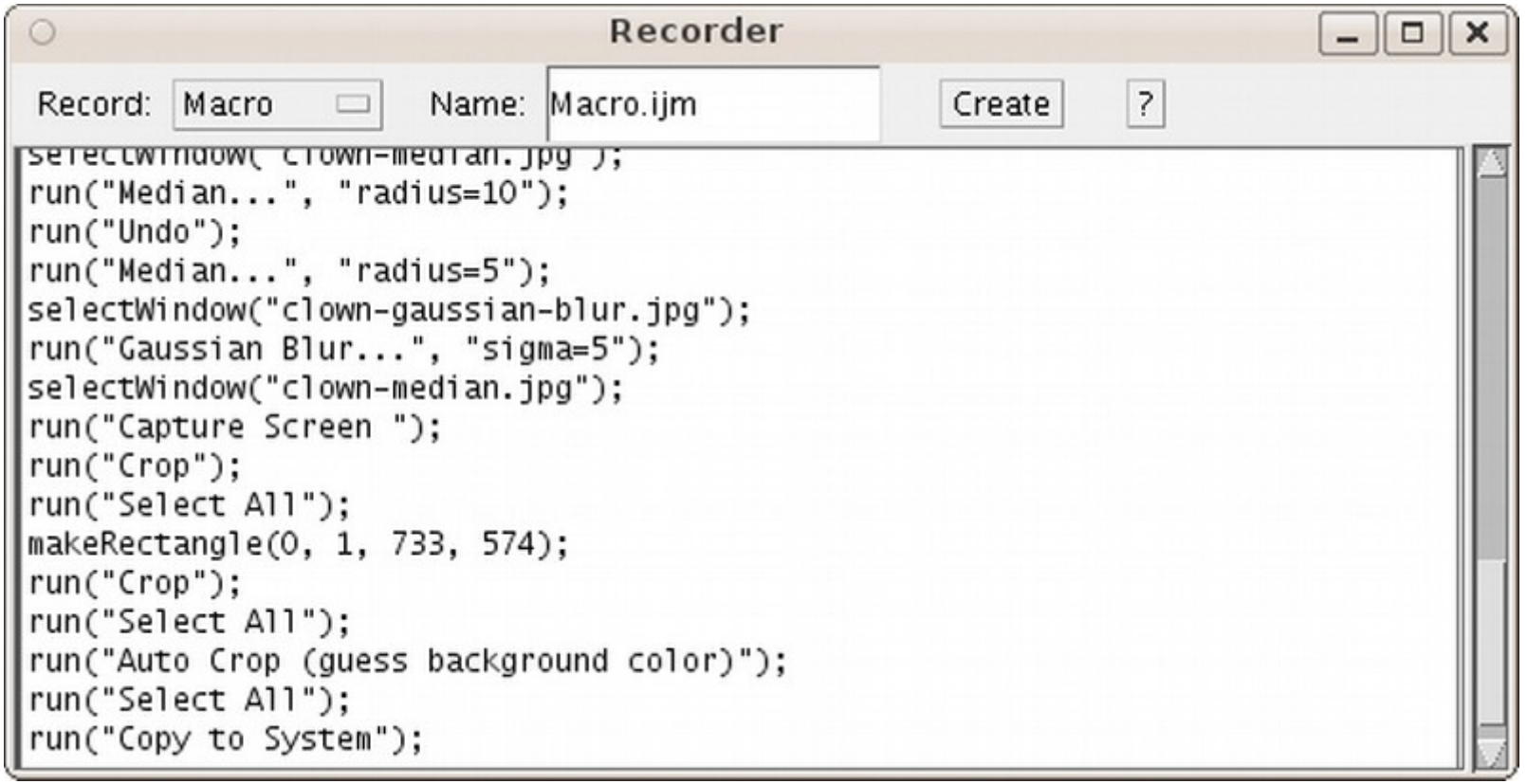
- *File>Open Samples>Blobs (25k)*
- *Image>Duplicate…*
- *Image>Adjust>Auto Threshold*
- *Process>Binary>Dilate* ***(x2)***
- *Process>Binary>Watershed*
- *Analyze>Analyze Particles...*



http://imagej.net/Segmentation

Macros: Never Forget

Plugins>Macros>Record...



http://imagej.net/Macros

Further reading

The community—forum and mailing list! ~2000 members:

http://imagej.net/Help

The ImageJ manual:

http://imagej.net/docs/guide/

The Cookbook, a collection of image analysis “recipes”:

http://imagej.net/Cookbook

A thorough Fiji tutorial for beginners:

http://nic.uni-hd.de/analysis.html

Additional workshops and presentations:

http://imagej.net/Presentations