

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

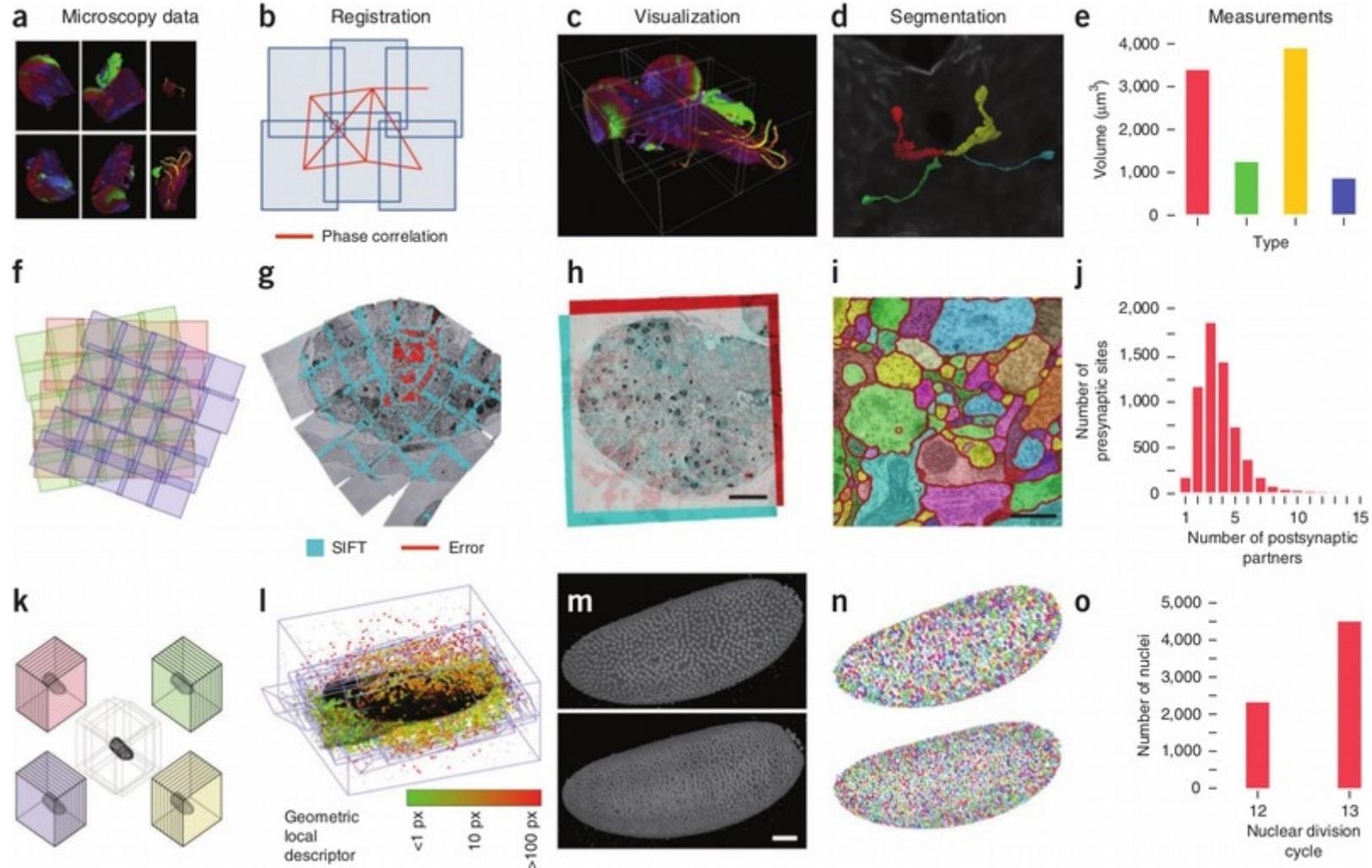
Science = reproducibility

Plugins>Utilities>Make Fiji Package



<http://imagej.net/>

Fiji Is Just ImageJ



<http://imagej.net/Introduction>

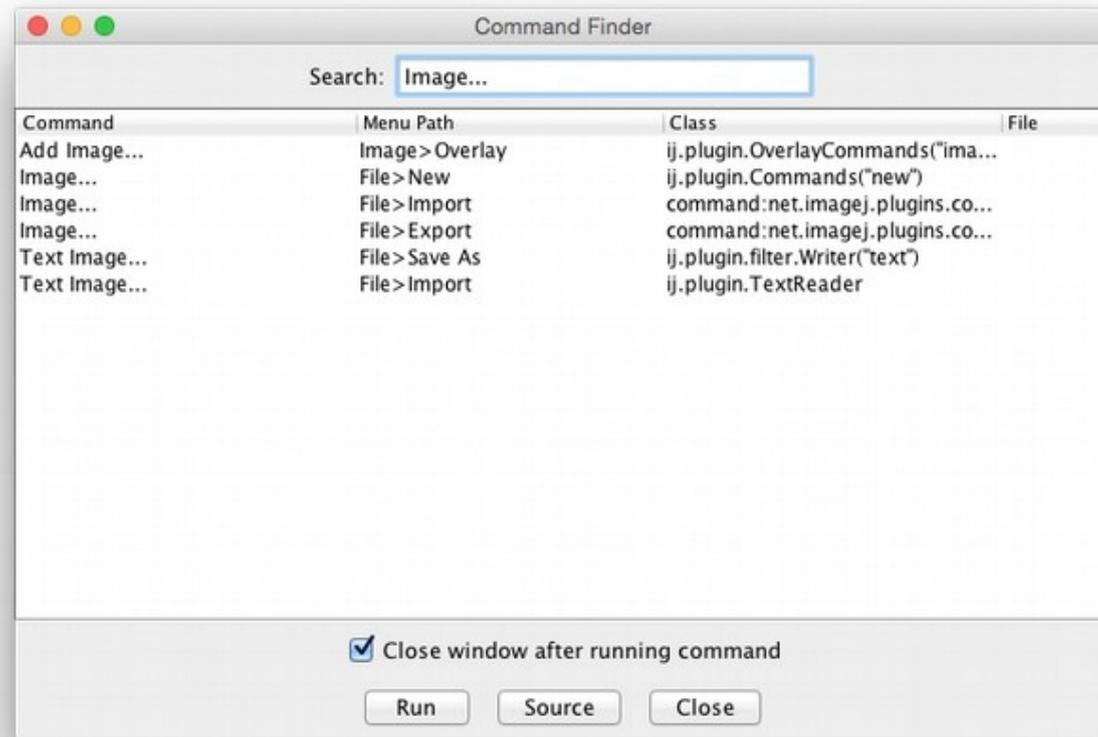
Who uses Fiji?



Resources for advice and collaboration

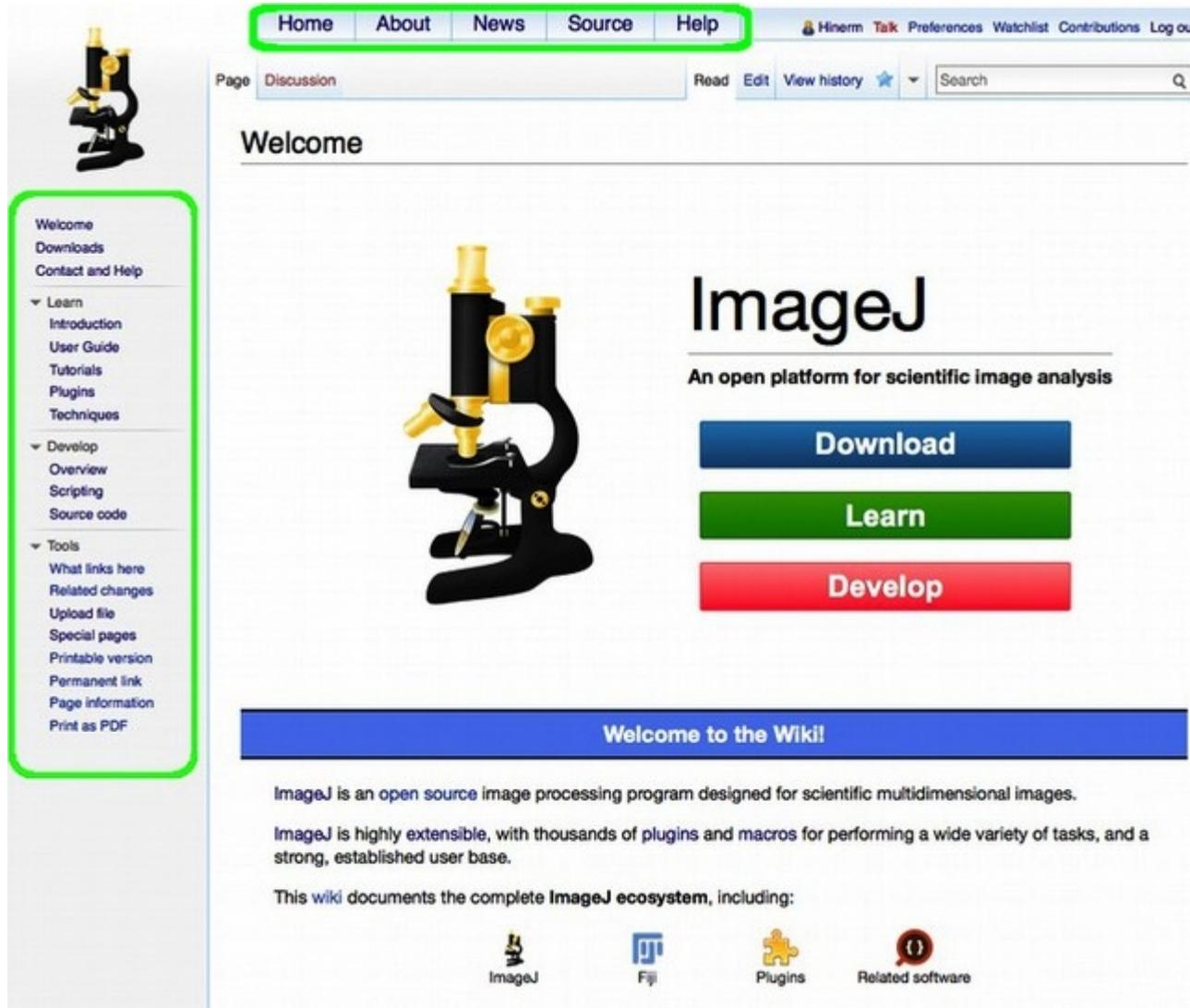
There's a lot of stuff!

Ctrl+L = Command Finder



http://imagej.net/Command_Finder

Learn how to fish:



The screenshot shows the ImageJ website homepage. At the top, a navigation menu contains links for Home, About, News, Source, and Help, which are highlighted with a green border. To the right of this menu are user options: Hinerm, Talk, Preferences, Watchlist, Contributions, and Log out. Below the navigation menu is a search bar and a 'Page' dropdown menu set to 'Discussion'. The main content area features a 'Welcome' message, a large image of a microscope, and the title 'ImageJ' with the subtitle 'An open platform for scientific image analysis'. Three prominent buttons are displayed: 'Download' (blue), 'Learn' (green), and 'Develop' (red). A blue banner below these buttons reads 'Welcome to the Wikil'. The text below the banner states: 'ImageJ is an open source image processing program designed for scientific multidimensional images. ImageJ is highly extensible, with thousands of plugins and macros for performing a wide variety of tasks, and a strong, established user base. This wiki documents the complete ImageJ ecosystem, including:'. At the bottom, four icons represent the ecosystem: ImageJ, Fiji, Plugins, and Related software.

<http://imagej.net/>

Learn how to fish:

Search ImageJ resources

Fix me on GitHub

Category	Main	Additional	Archived
Web site	Website	Wiki Google	
Code + Issues	GitHub	Javadoc Maven	Fiji BugZilla ImageJ Trac
Forum + Lists	Forum ImageJ	scijava OpenSPIM Micro-Manager SLIM-Curve OME	imagej-devel fiji-devel scifio
IRC		#imagejdev #fiji-devel	

<http://search.imagej.net/>

Teach me how to fish!

 FORUM [Sign Up](#) [Log In](#) 

[all categories](#) [all tags](#) **Latest** [Top](#) [Categories](#)

Topic	Category	Users	Replies	Views	Activity
 Welcome to the ImageJ Forum! This is a discussion board for all users and developers of ImageJ and Fiji. The purpose of this ImageJ forum is to: Offer a central place for questions and discussions regarding ImageJ. Include the full breadth of... read more			7	262	9d
Problems with SCIFIO	 Development		7	13	1m
Macro stops running 3D object counter	 Image Analysis		11	122	3m
ImageJ dialog widgets widgets, legacy	 Development		6	65	1h
Can I have imagej-ops repo privileges (at least temporarily)?	 Development		4	17	2h
Host an update site on a free webdav server			3	23	2h
<input checked="" type="checkbox"/> Visualizing velocity fields piv, visualization, vector-field	 Image Analysis		6	79	2h
Problem in area measurement after threshold with Analyze Particles			2	18	7h

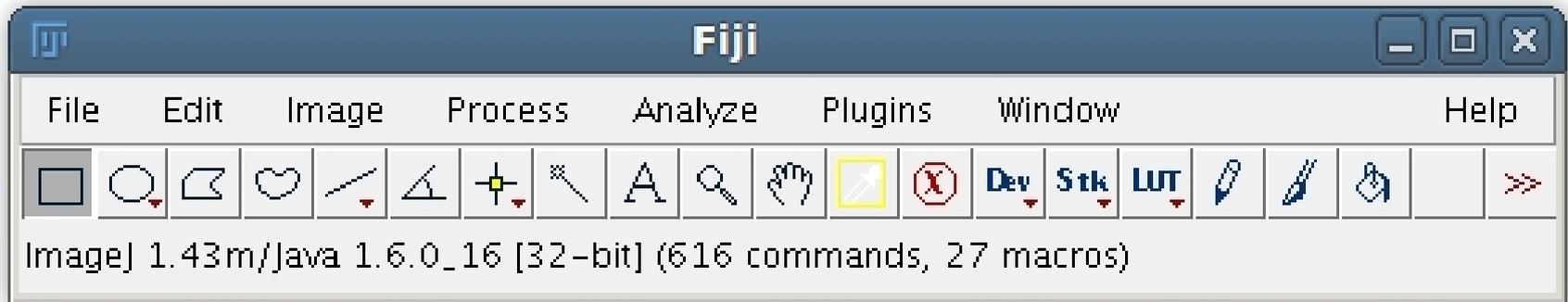
<http://forum.imagej.net/>

The main window

Menubar

Tools

Status Bar

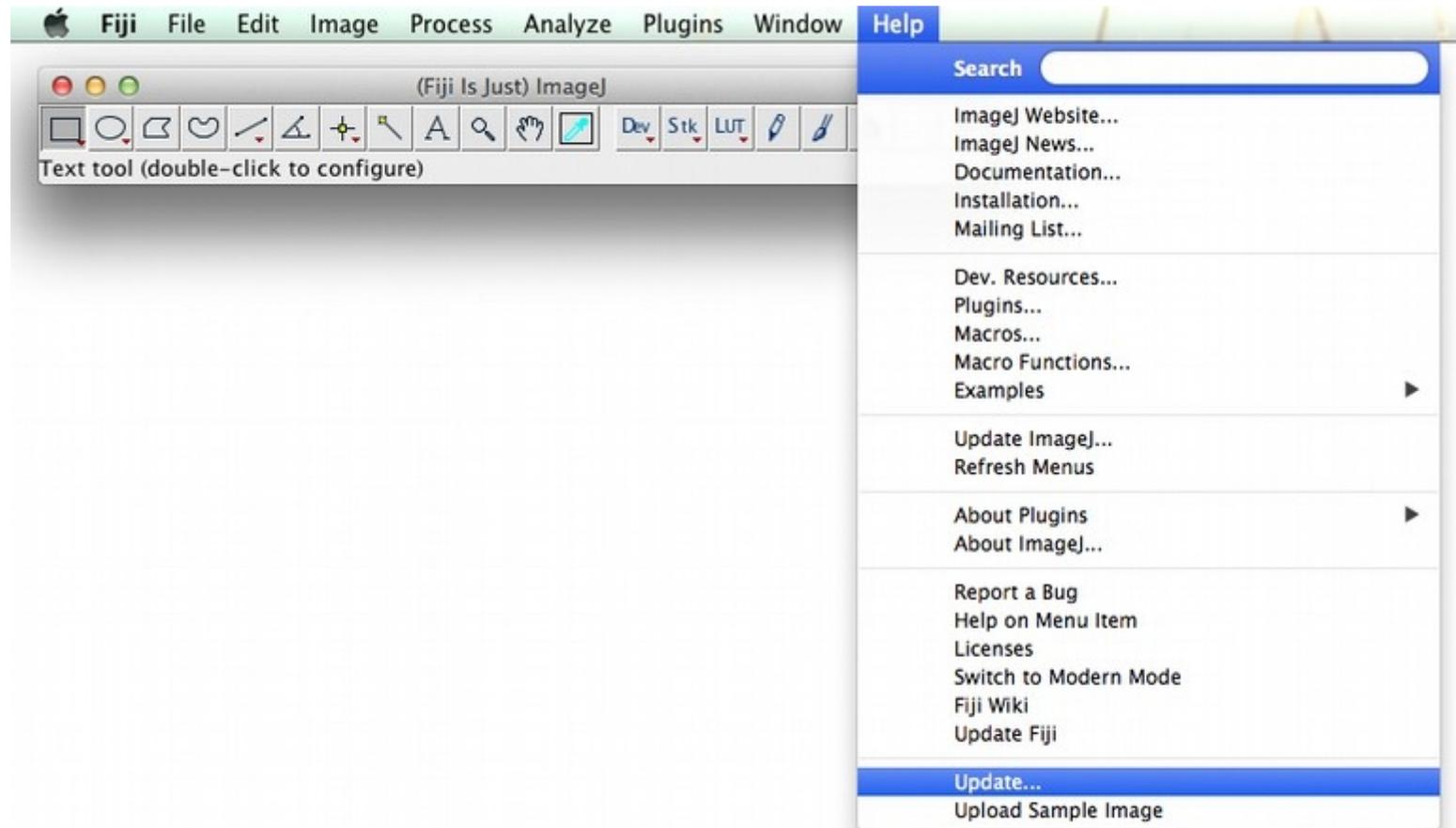


Tip: click on the status bar

Tip: right / double-click on Tools

http://imagej.net/Getting_started

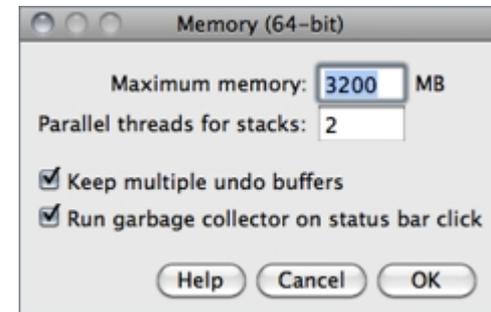
Staying up-to-date



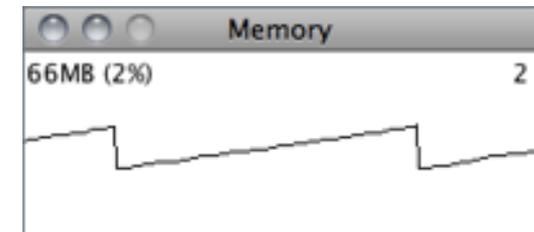
<http://imagej.net/Updater>

Memory management

Edit>Options>Memory & Threads



Plugins>Utilities>Monitor Memory

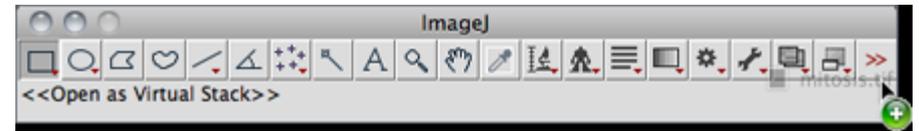


<http://imagej.net/guide/146-27.html>

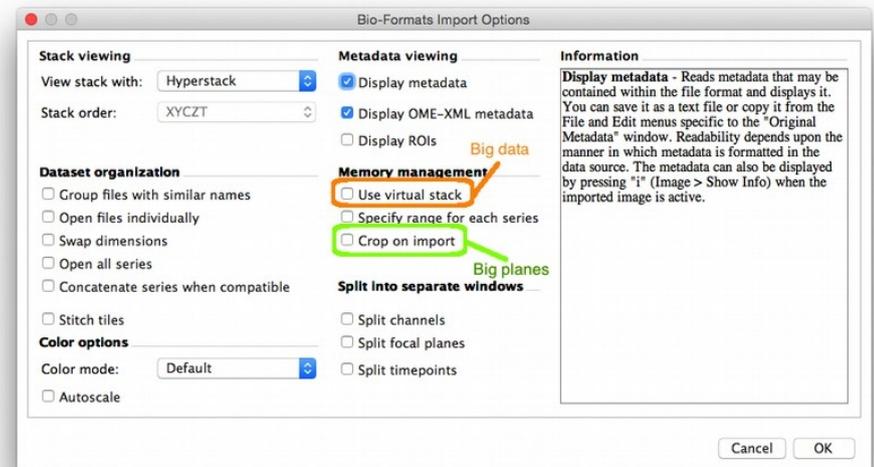
Opening images

Drag & Drop

File>Open

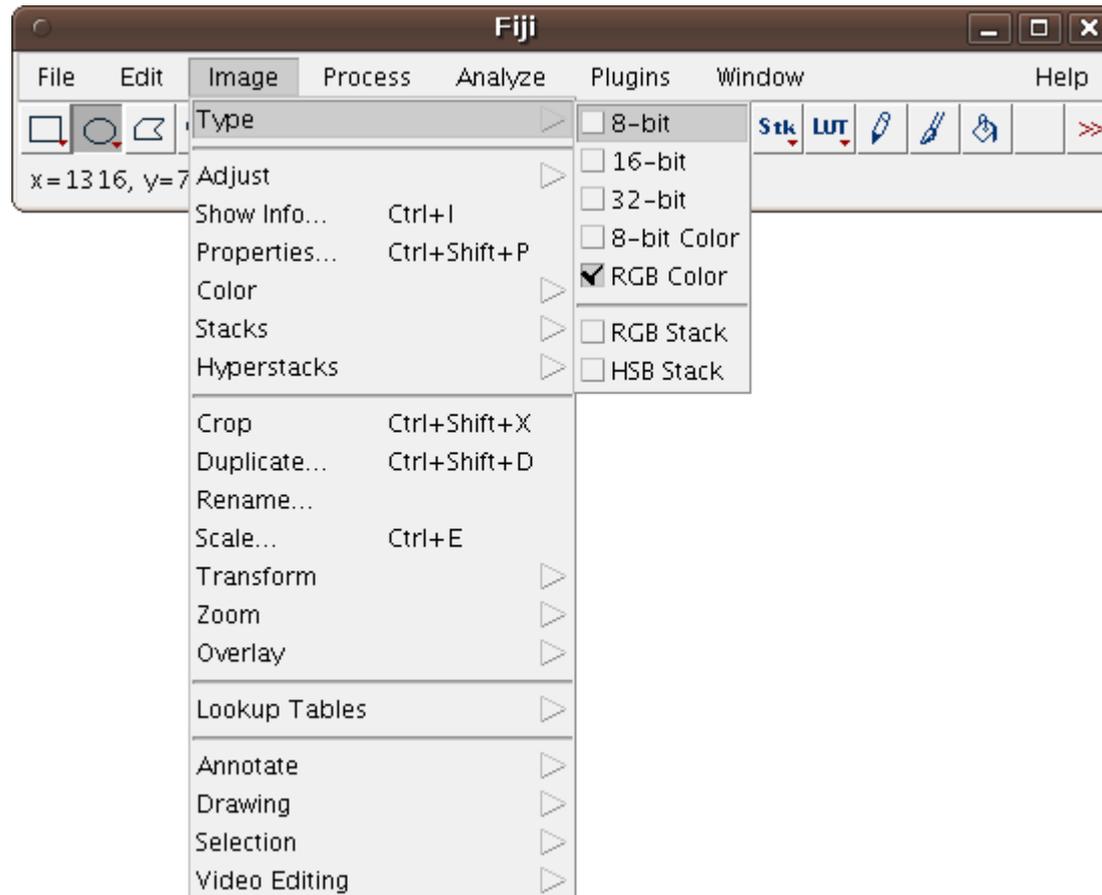


Plugins>Bio-Formats>
Bio-Formats Importer



http://imagej.net/Importing_Image_Files

Pixel types

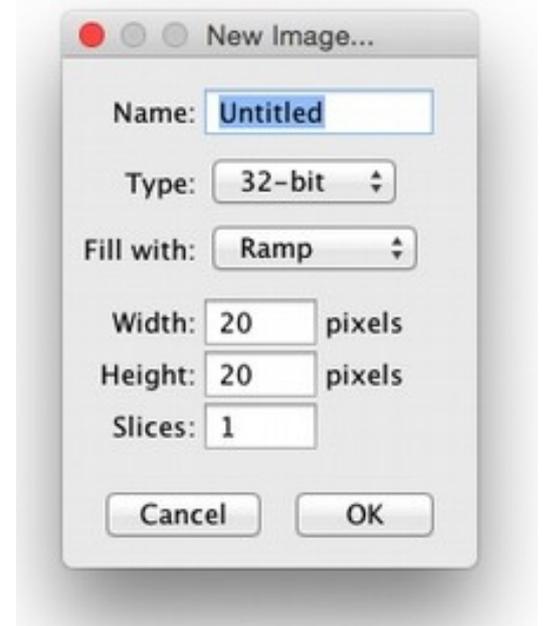


<http://imagej.net/docs/guide/146-7.html>

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?

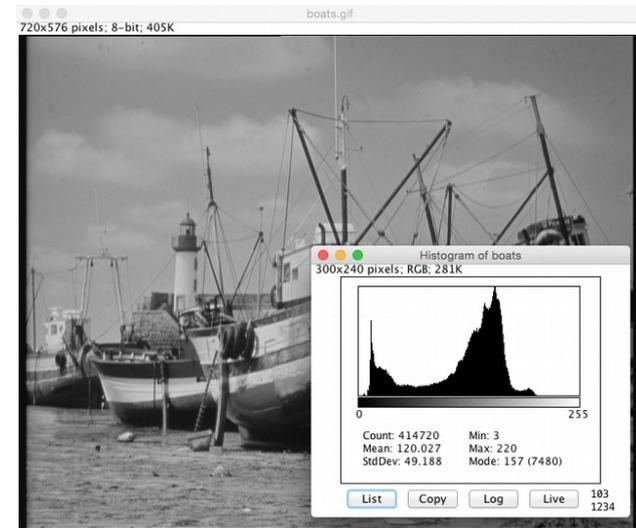
http://imagej.net/IP_Principles

Get to know your data

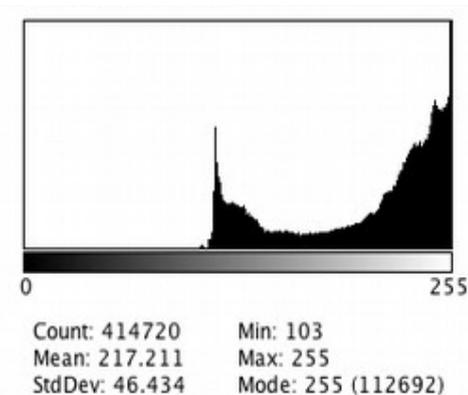
- *File>Open Samples>Boats*
- *Analyze>Histogram*

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*

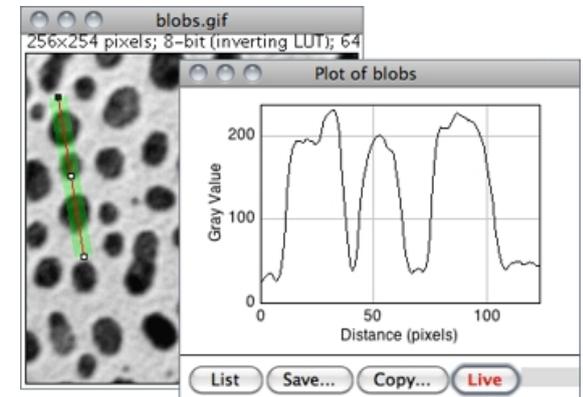
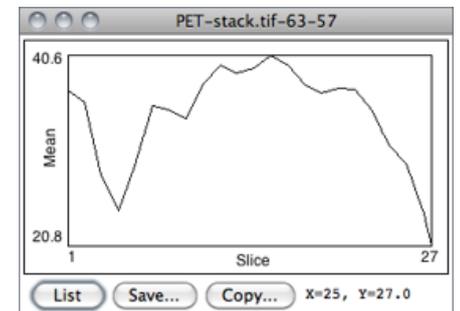


Image > Stacks > Plot Z-Axis Profile...

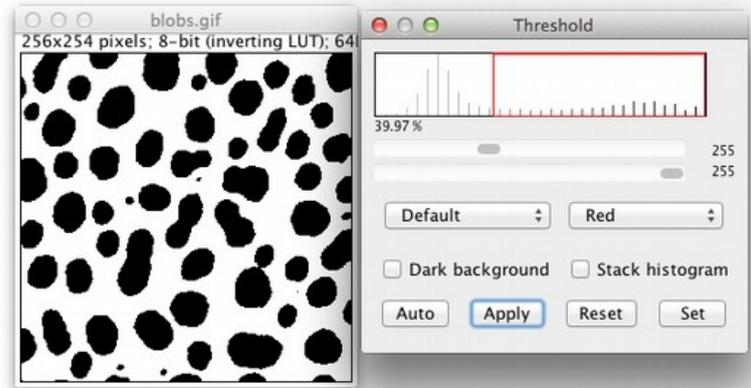


<http://imagej.net/docs/guide/146-28.html>

Thresholding

Isolate **values** of interest

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



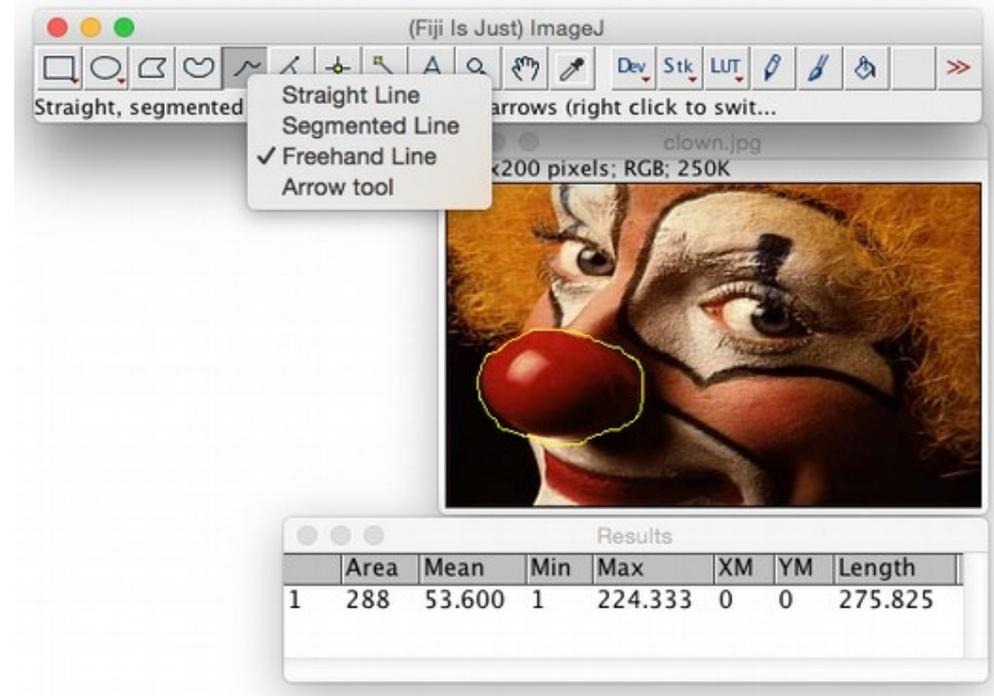
Which method is best?

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*

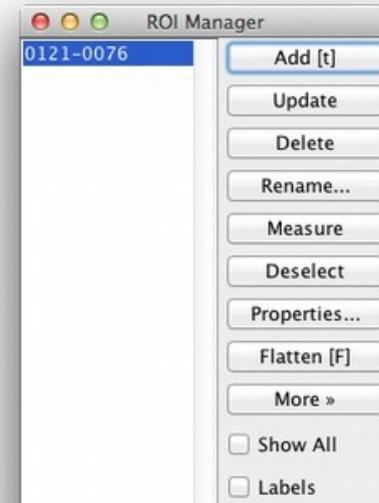


Can you draw the same circle on a new clown?

<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*

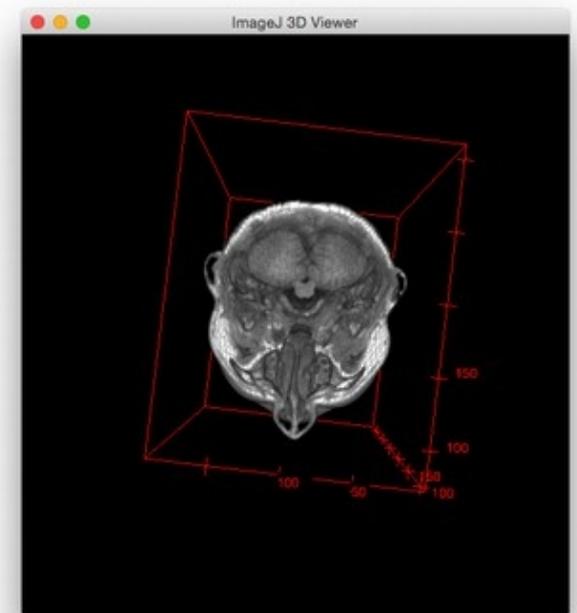
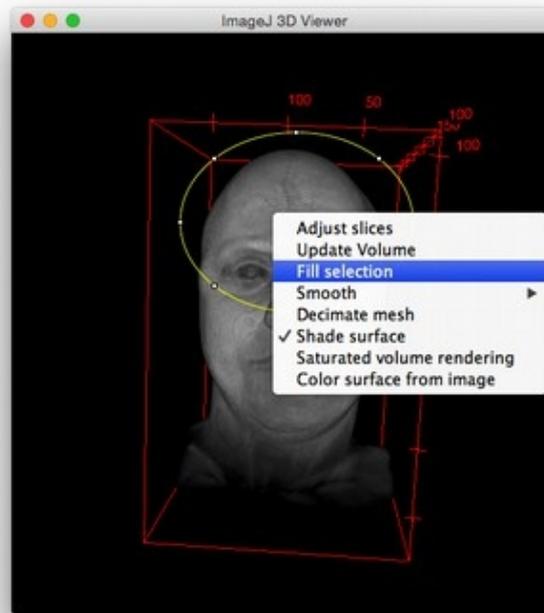
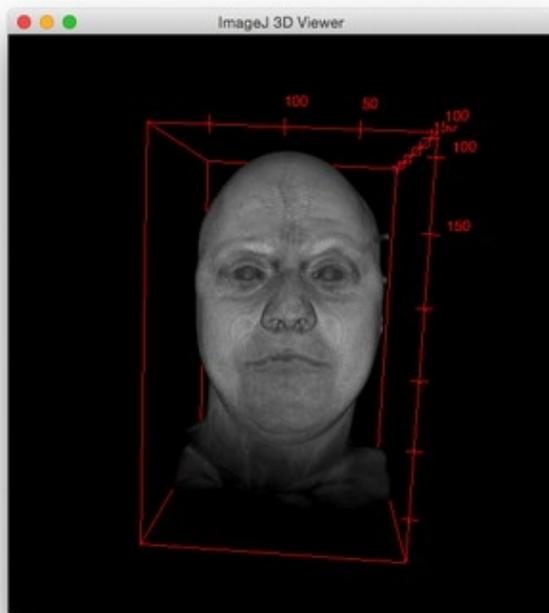
<http://imagej.net/docs/guide/146-10.html>

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

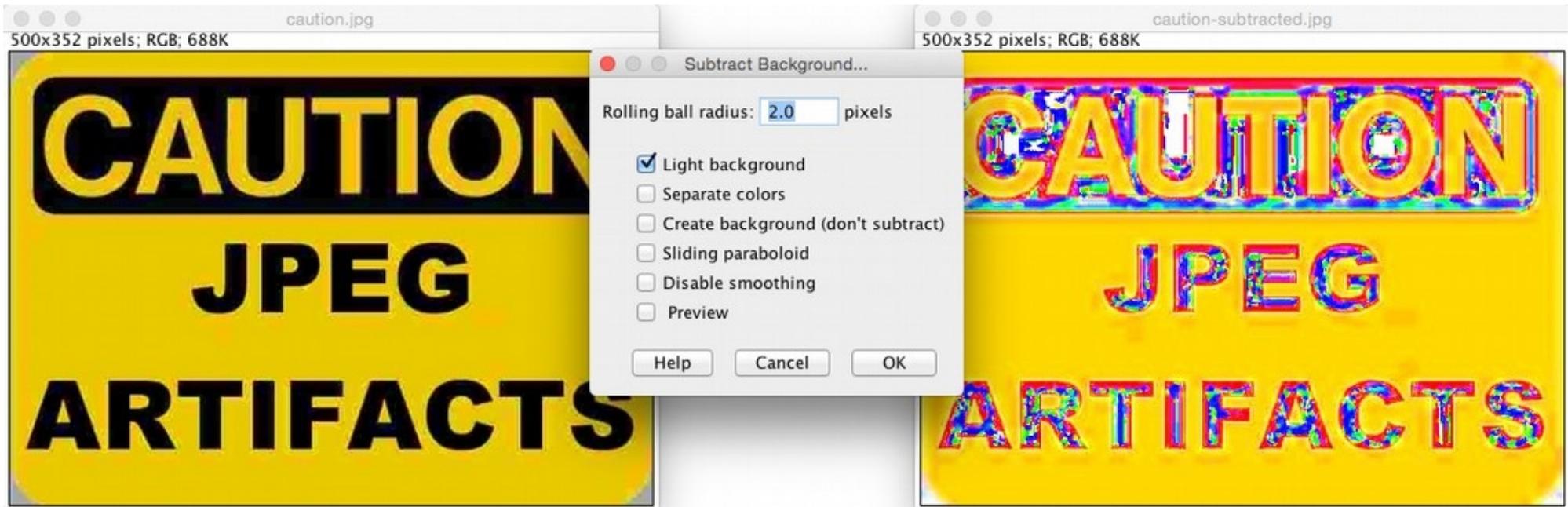


Image formats are not created equal!

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

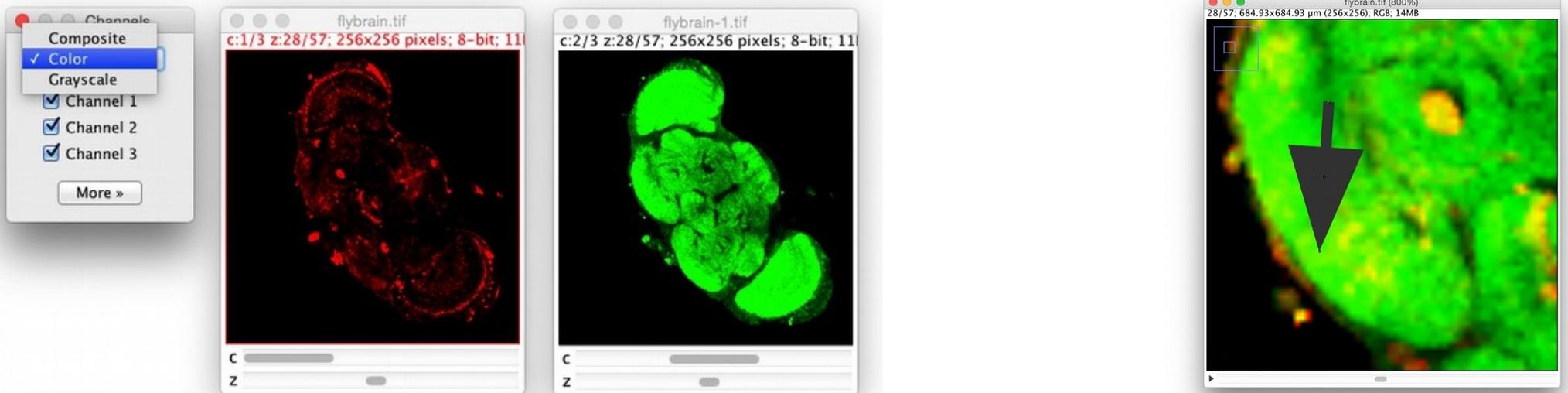
*Edit>Options>Appearance...>
Interpolate zoomed images*



http://imagej.net/IP_Principles

Eyes? More like LIES!

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



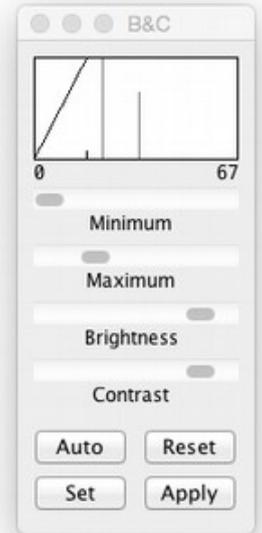
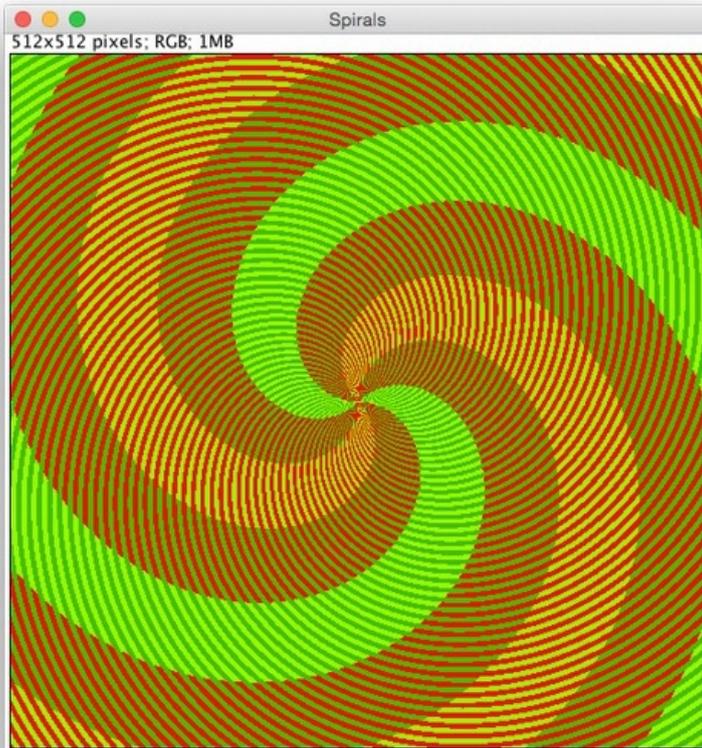
Can “*Composite*” mode show colocalization?

<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?

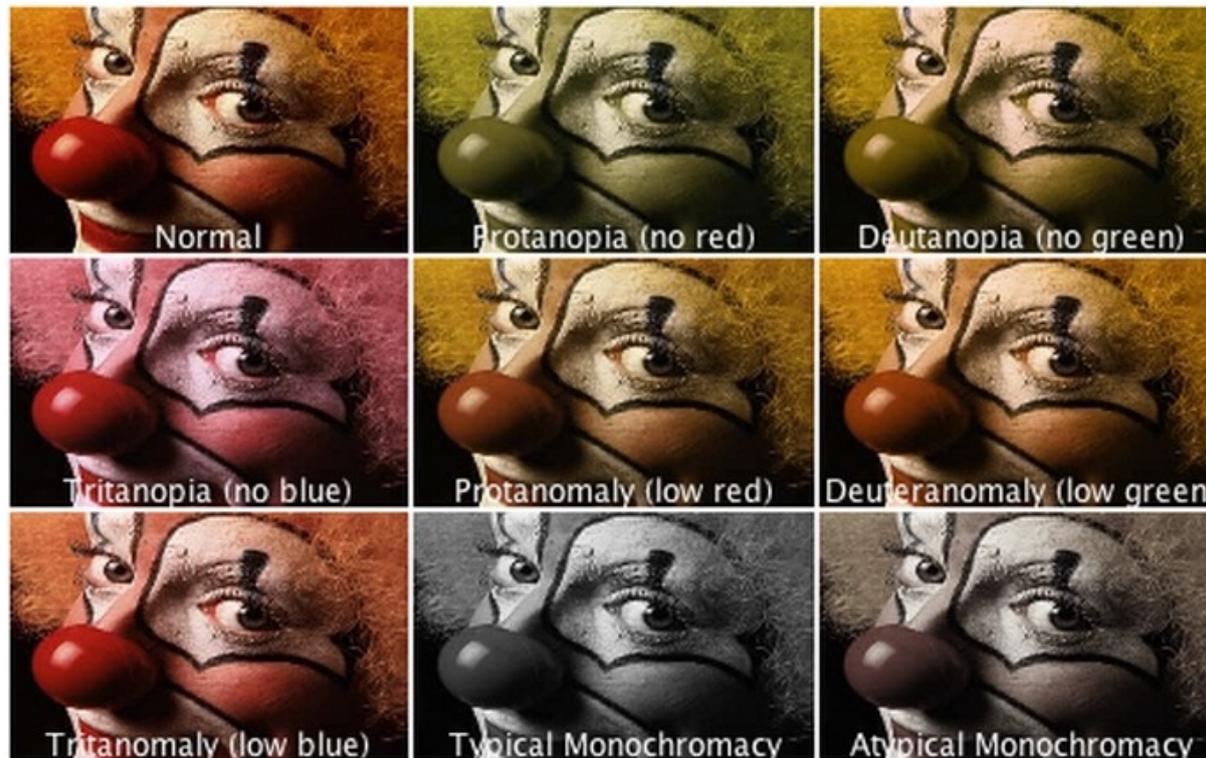
Image>Type>8-bit color

Image>Lookup Tables>glasbey

... zoom in (+) and probe?

Eyes are not all equal

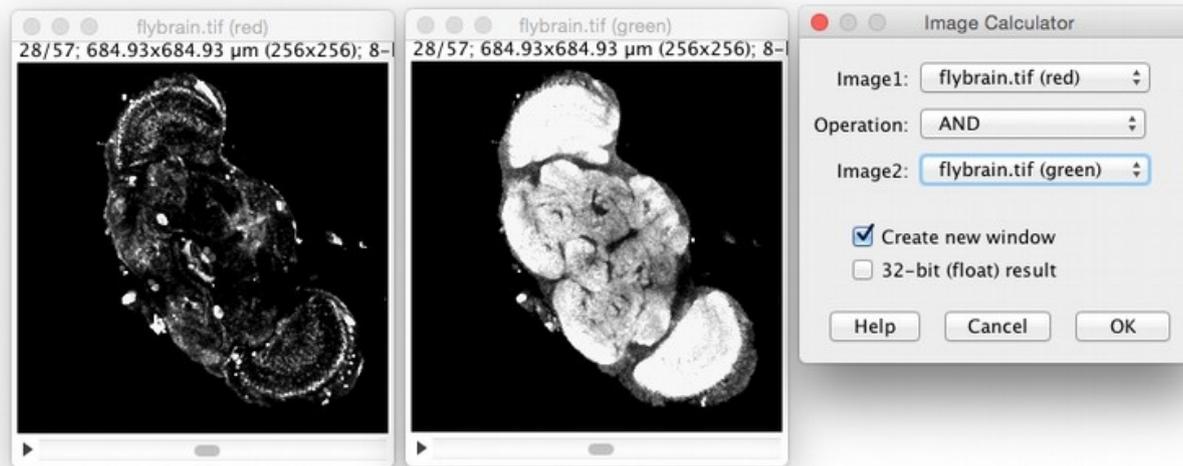
Image > Color > Simulate Color Blindness



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

So what CAN we do?

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



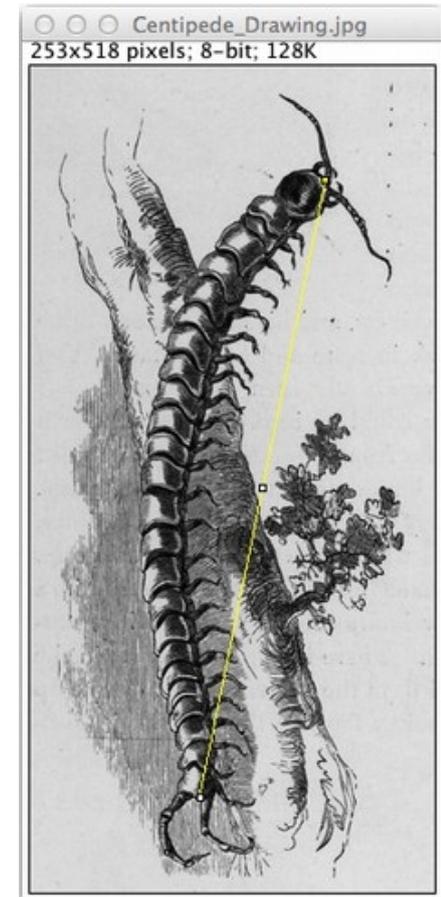
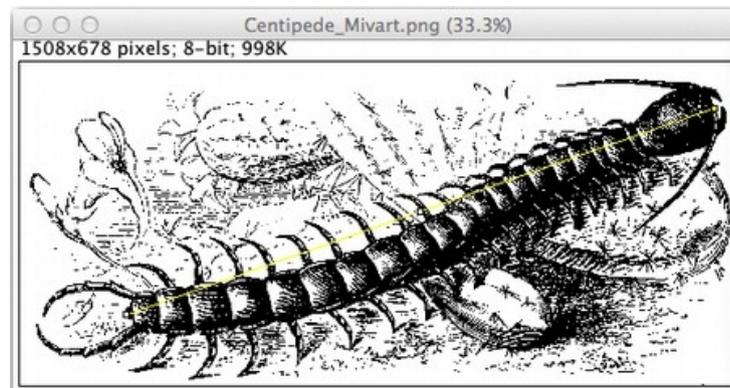
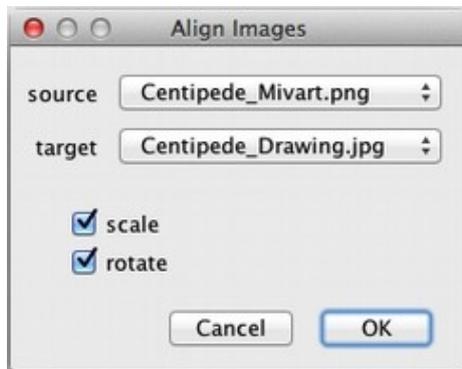
- **But...** what question have we answered?

<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*

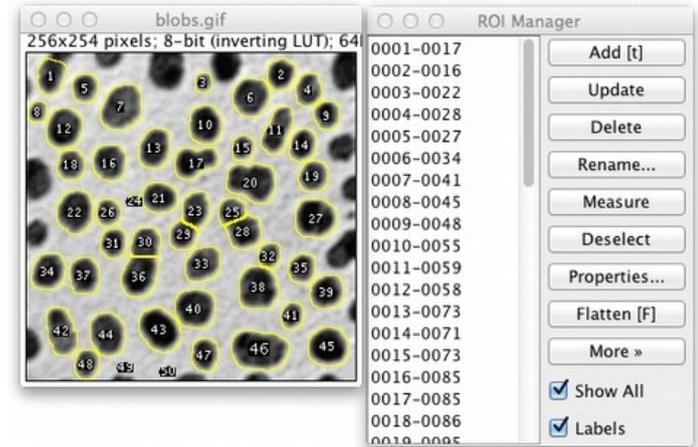
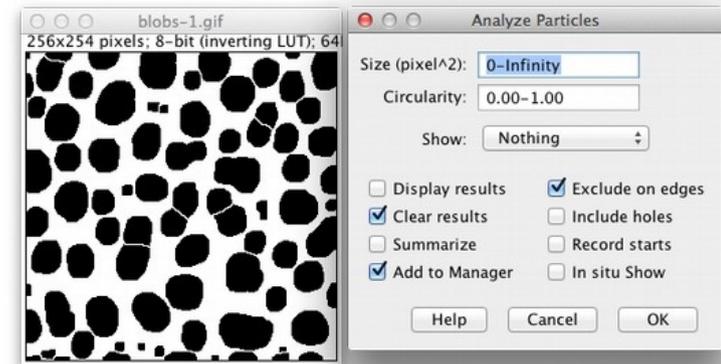


http://imagej.net/Image_Stitching

Segmentation

Identify blobs of interest

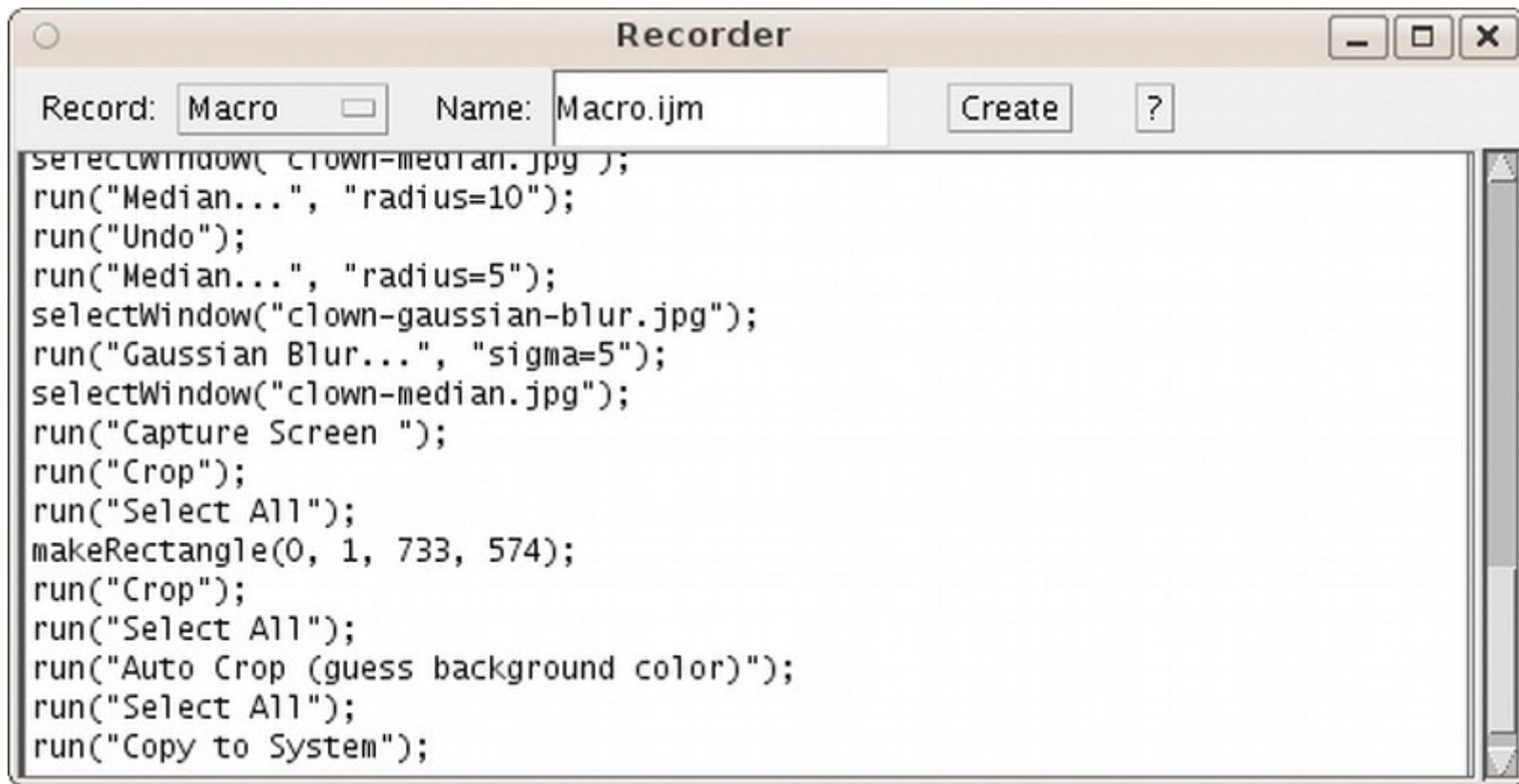
- *File>Open Samples>Blobs (25k)*
- *Image>Duplicate...*
- *Image>Adjust>Auto Threshold*
- *Process>Binary>Dilate (x2)*
- *Process>Binary>Watershed*
- *Analyze>Analyze Particles...*



<http://imagej.net/Segmentation>

Macros: Never Forget

Plugins > Macros > Record...



```
selectWindow('clown=median.jpg');
run("Median...", "radius=10");
run("Undo");
run("Median...", "radius=5");
selectWindow("clown-gaussian-blur.jpg");
run("Gaussian Blur...", "sigma=5");
selectWindow("clown-median.jpg");
run("Capture Screen ");
run("Crop");
run("Select All");
makeRectangle(0, 1, 733, 574);
run("Crop");
run("Select All");
run("Auto Crop (guess background color)");
run("Select All");
run("Copy to System");
```

<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>