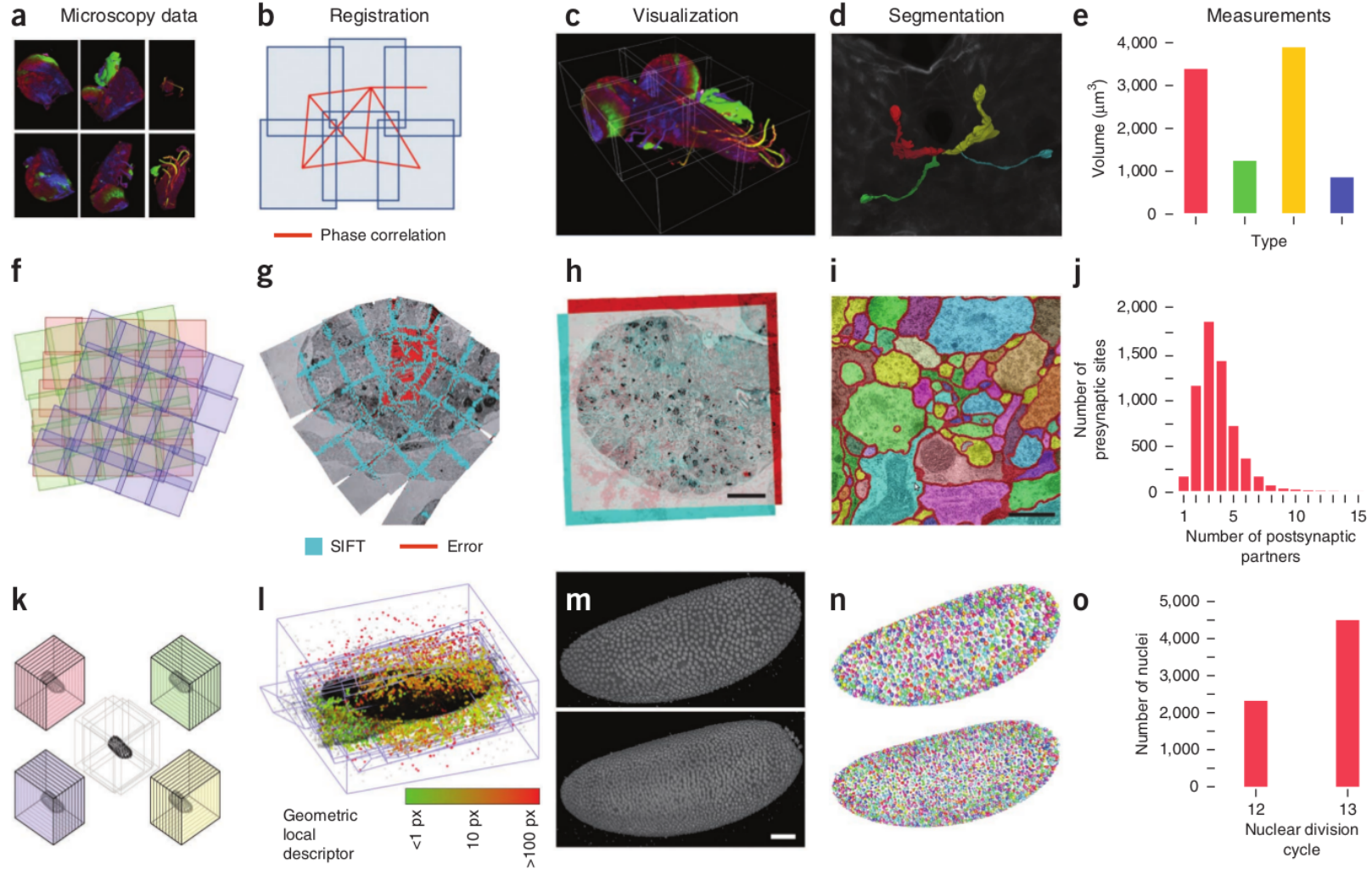


# *Image Analysis with Fiji*



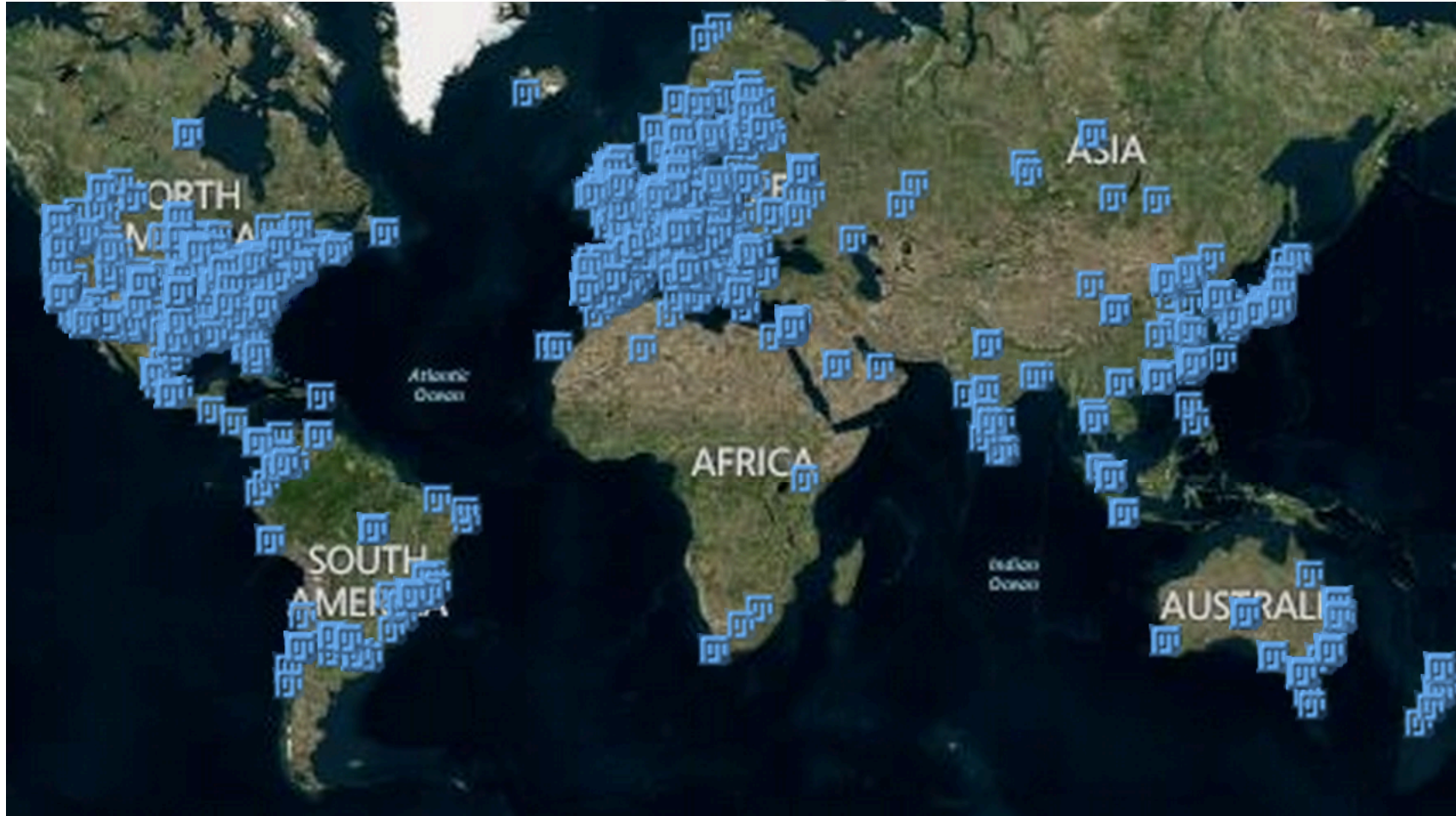
<http://fiji.sc/>

# What is Fiji?



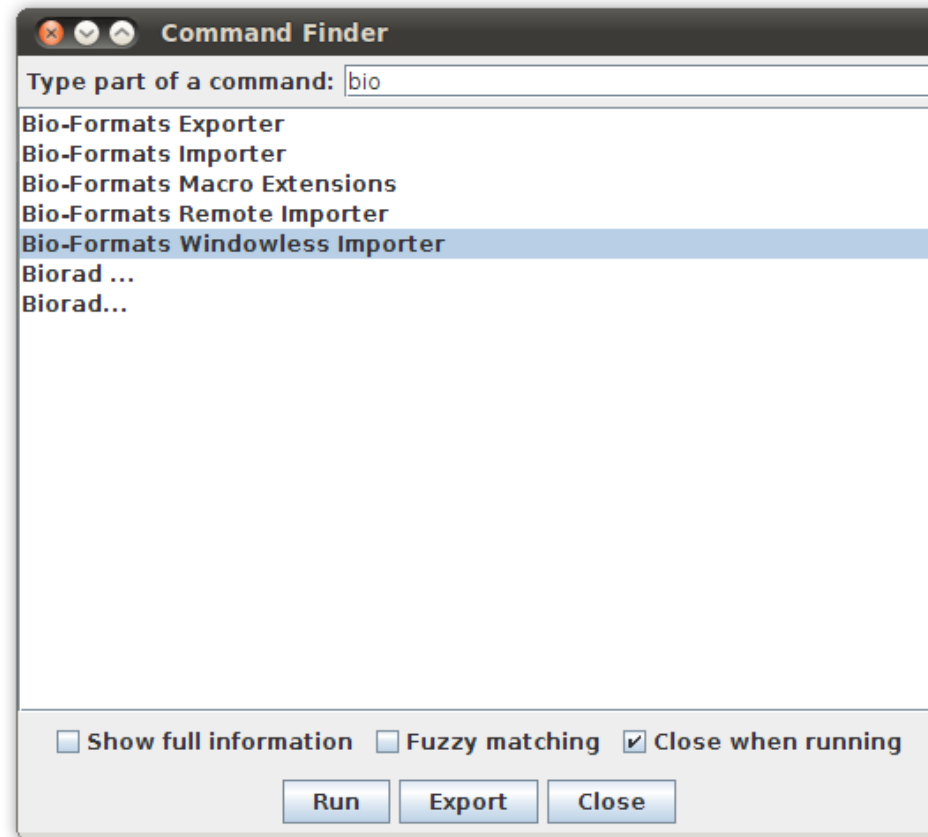
[http://fiji.sc/Fiji\\_Usage\\_weekly](http://fiji.sc/Fiji_Usage_weekly)

# *Who uses Fiji?*



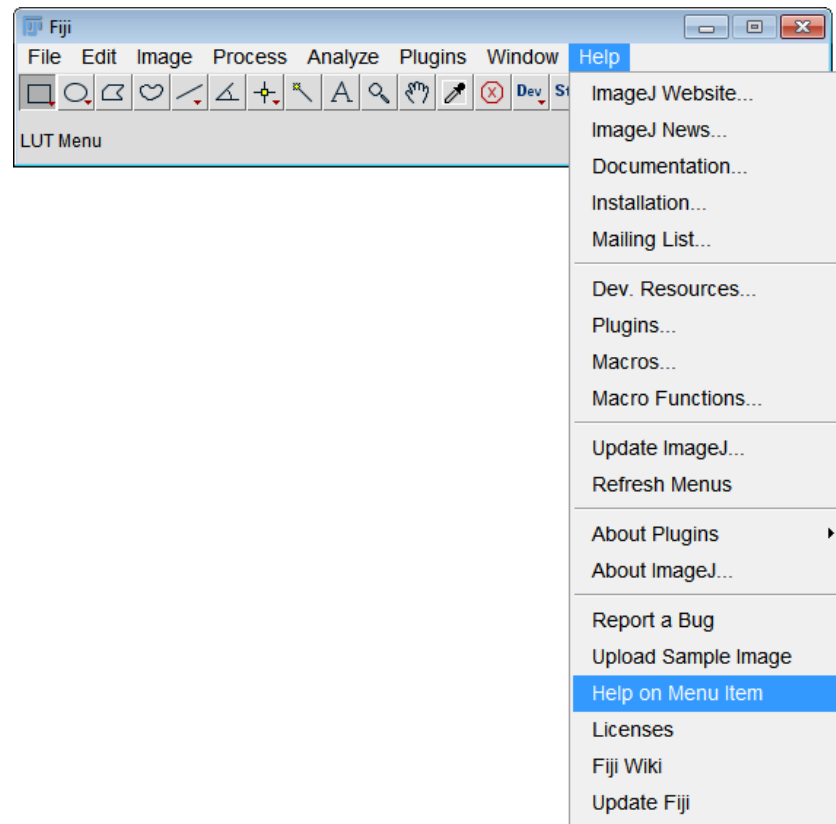
<http://fiji.sc/Usage>

# Teaching how to fish: Command Finder (Ctrl+L)



[http://fiji.sc/Command\\_Finder](http://fiji.sc/Command_Finder)

# *Teaching how to fish: Help on Menu Item*



[http://fiji.sc/Help\\_on\\_Menu\\_Item](http://fiji.sc/Help_on_Menu_Item)

# Teaching how to fish: Help > Fiji Wiki

Navigation Content Highlights Toolbox Login  Search

## Fiji Is Just ImageJ

Like 262 Send

short URL

Fiji is an image processing package. It can be described as a distribution of [ImageJ](#) (and soon [ImageJ2](#)) together with Java, Java 3D and a lot of plugins organized into a coherent menu structure. Fiji compares to ImageJ as Ubuntu compares to Linux.

The main focus of Fiji is to assist research in life sciences.

For users, Fiji is easy to install and has an automatic update function, bundles a lot of plugins and offers comprehensive documentation.

For developers, Fiji is an open source project hosted in a [Git version control repository](#), with access to the source code of all internals, libraries and plugins, and eases the development and scripting of plugins.

[Download Fiji now](#)

### How to cite Fiji?

### News

- 2012-09-14 - The Updater moved
- 2012-07-20 - TrakEM2 published in PLoS ONE
- 2012-06-29 - Fiji papers at Nature Methods
- 2012-06-16 - Elastic registration published in Nature Methods
- 2012-05-10 - New plugin: TrackMate

Subscribe to an RSS or Atom feed of the Fiji news.  
Browse the news archive.

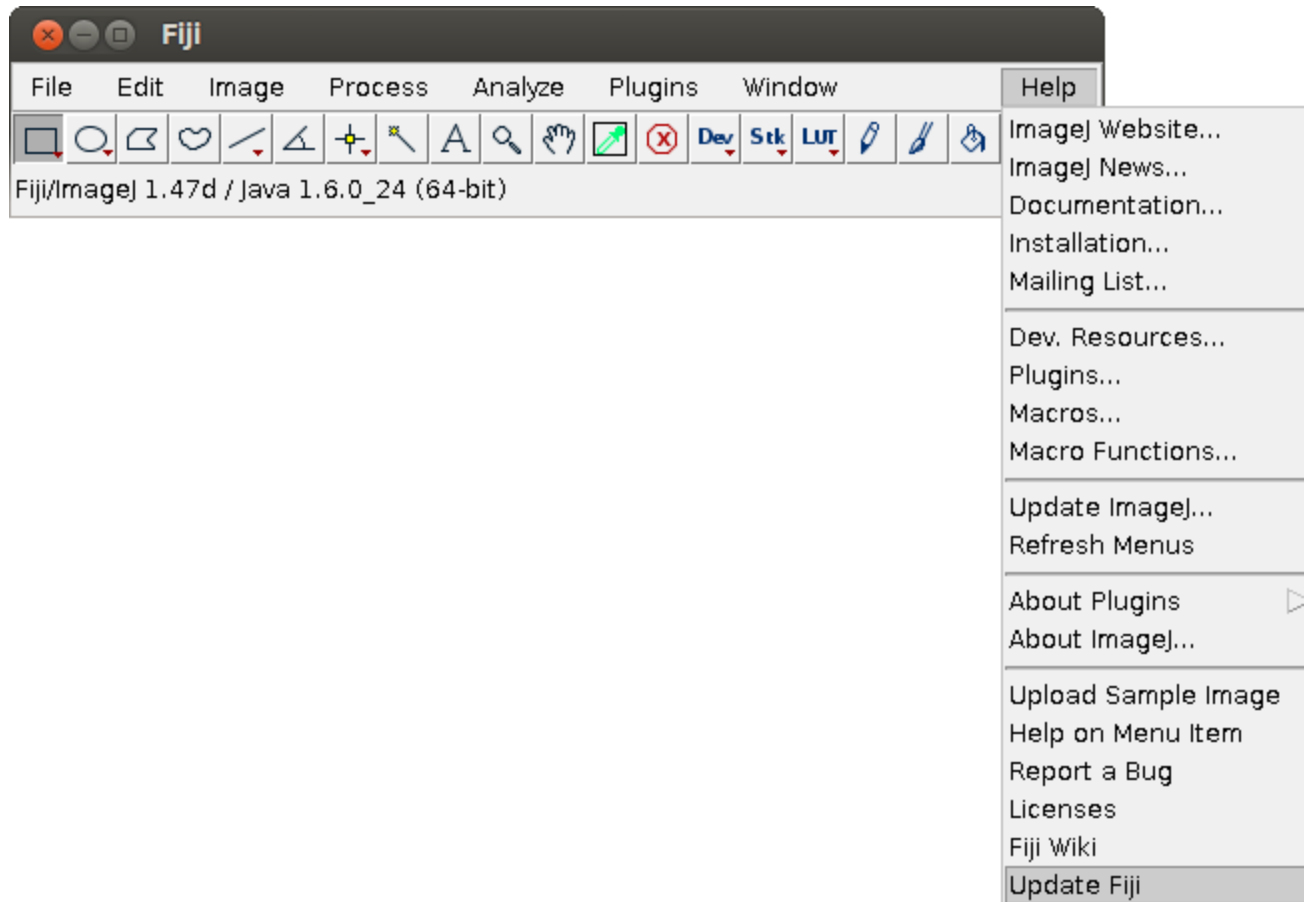
### Collaboration

The Fiji project is driven by a strong desire to improve the tools available for life sciences to process and analyze data. To this end, Fiji collaborates closely with the following projects:

ImageJ2 Bio-Formats OME µManager KNIME

<http://fiji.sc/>

# Staying up-to-date



<http://imagej.net/Updater>

# *Fiji's main window*

Menubar

Tools

Status Bar



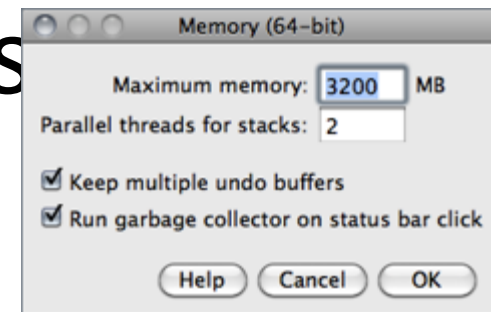
Tip: click on the status bar

[http://fiji.sc/Getting\\_started](http://fiji.sc/Getting_started)

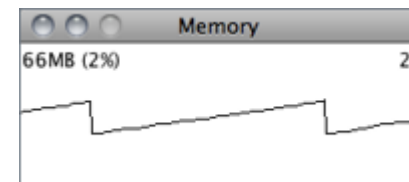


# Memory management

*Edit>Options>Memory & Threads*



*Plugins>Utilities>Monitor Memory:*

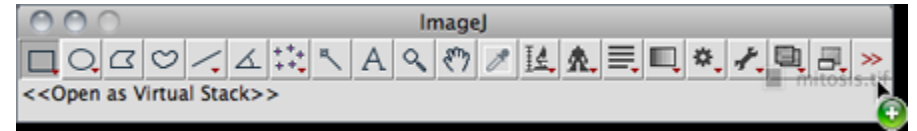


<http://imagej.net/docs/guide/>

# Opening images

Drag & Drop (even from web browser!)

*File>Open*



*File>Import>Bio-Formats*

*File>Import>Image Sequence...*



Tip: virtual stacks

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

# *Saving images*

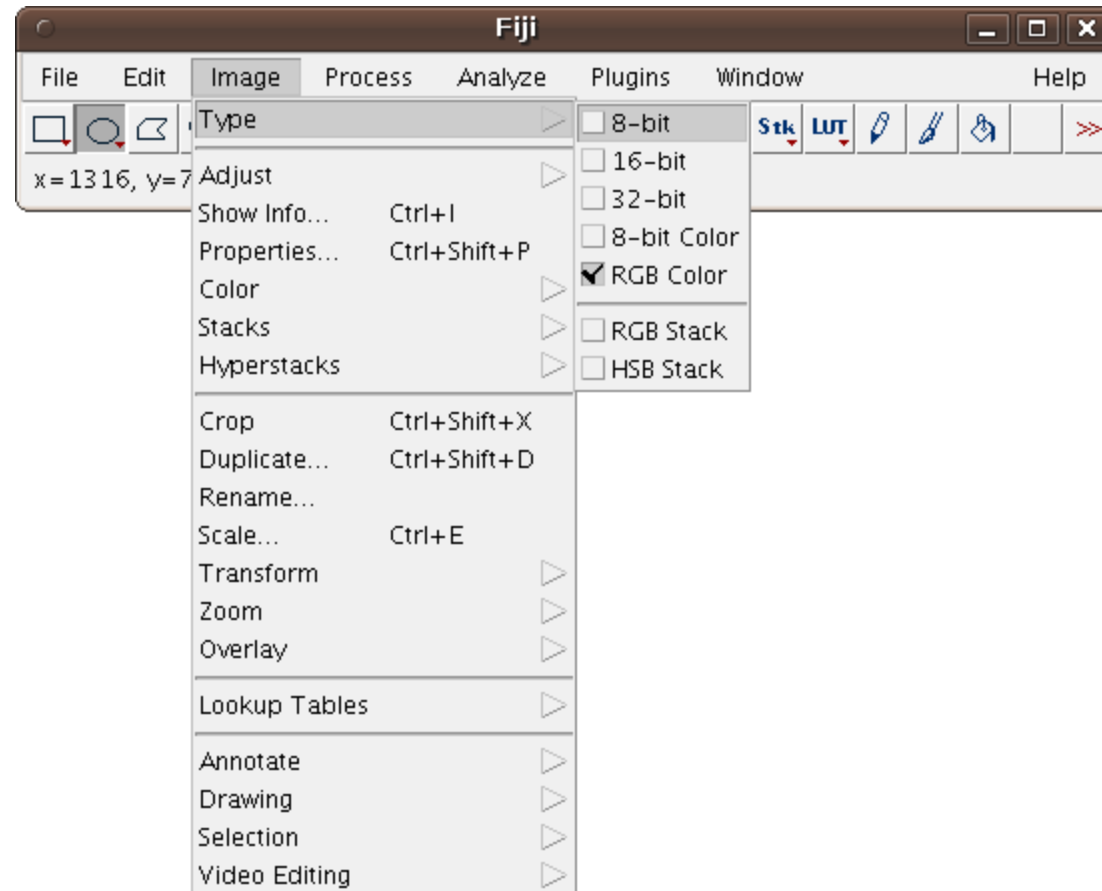
*File > Save As*

*Plugins > LOCI > Bio-Formats Exporter*



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

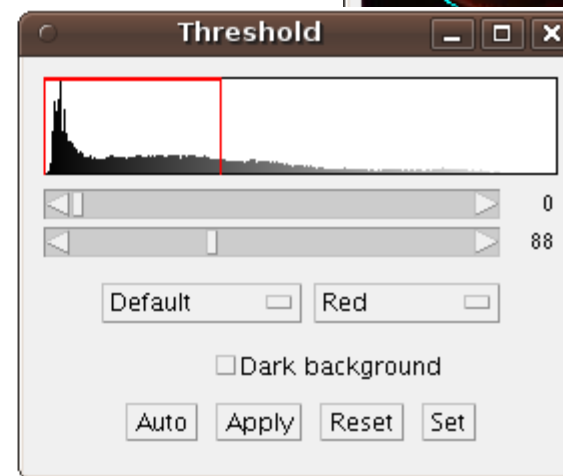
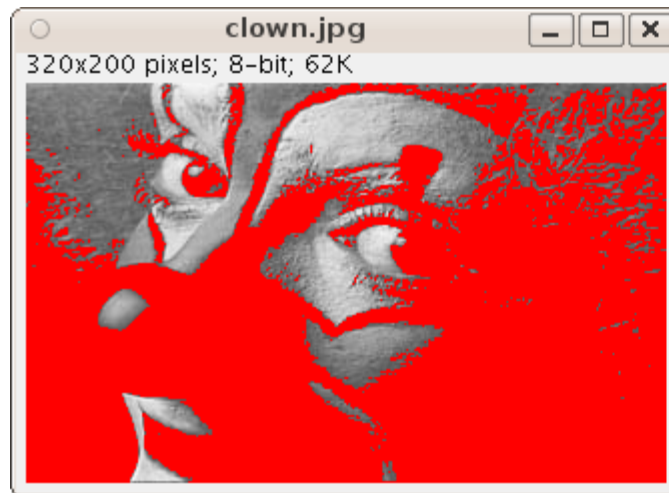
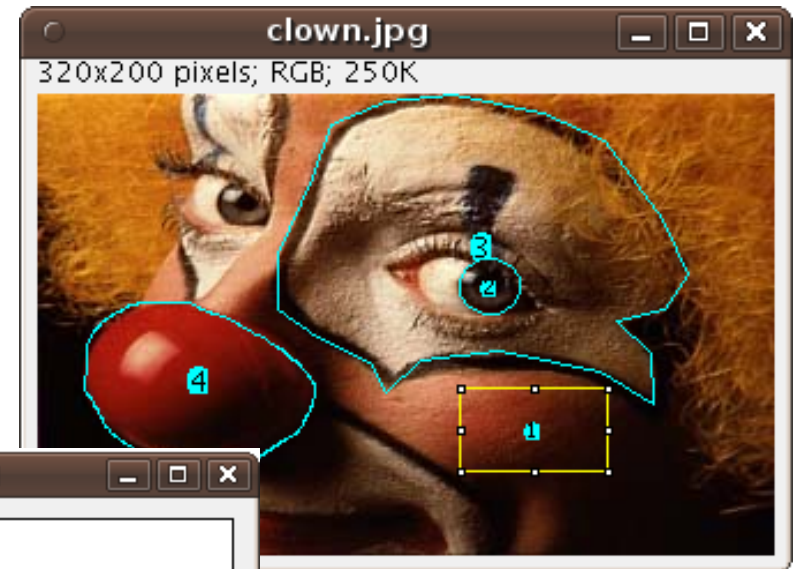
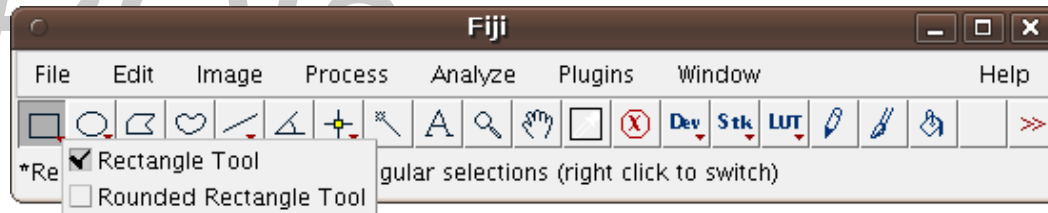
# Pixel types



[http://fiji.sc/Getting\\_started](http://fiji.sc/Getting_started)

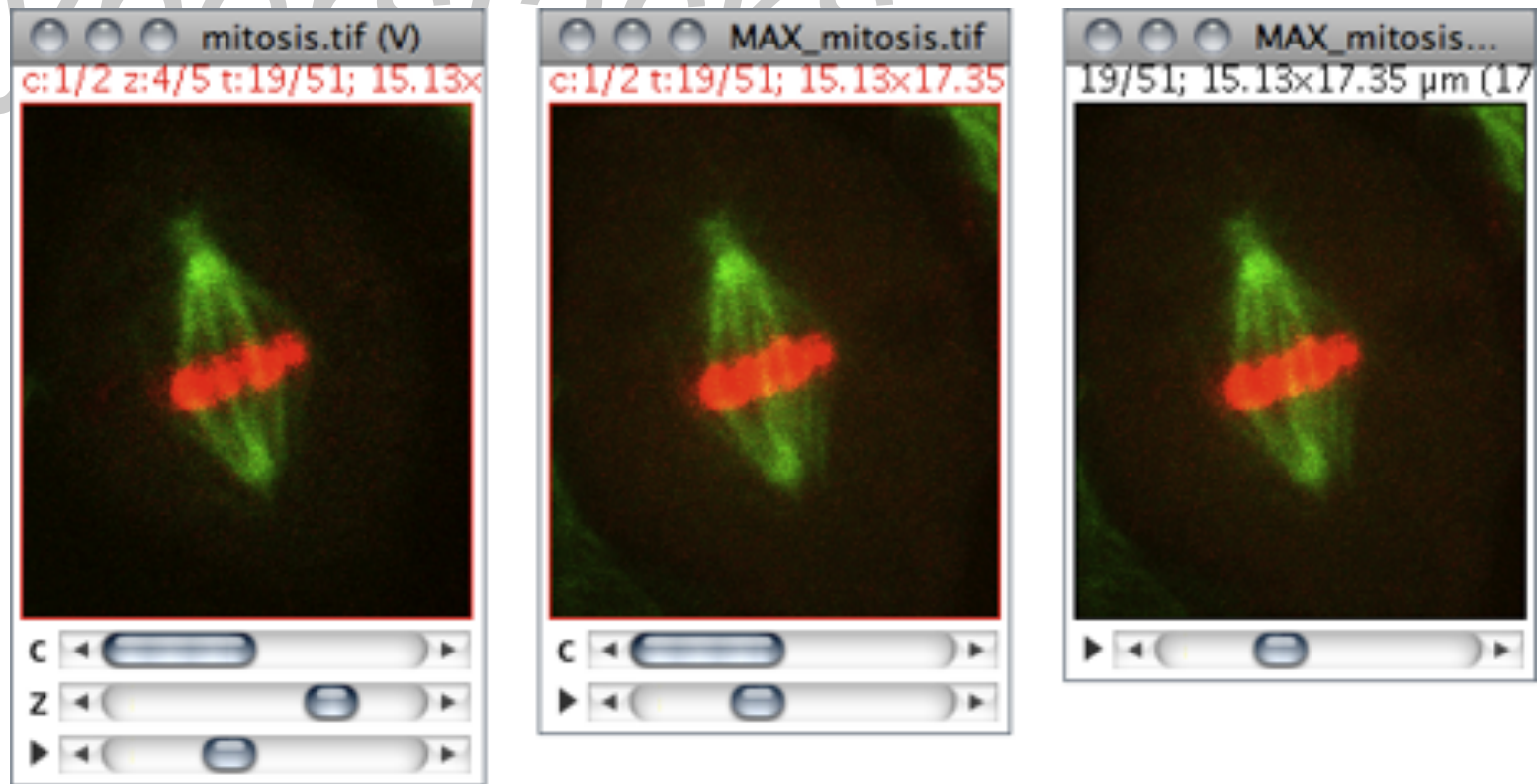
# Regions of interest:

## ROI



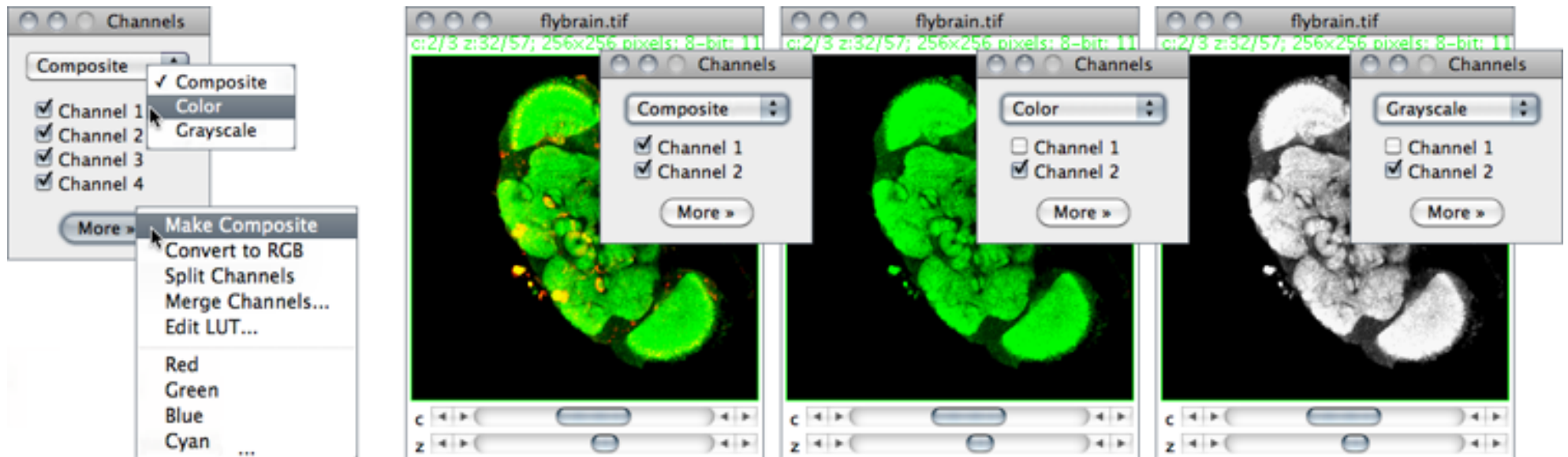
[http://fiji.sc/Getting\\_started](http://fiji.sc/Getting_started)

# Beyond 3D: Hyperspectroscopy



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

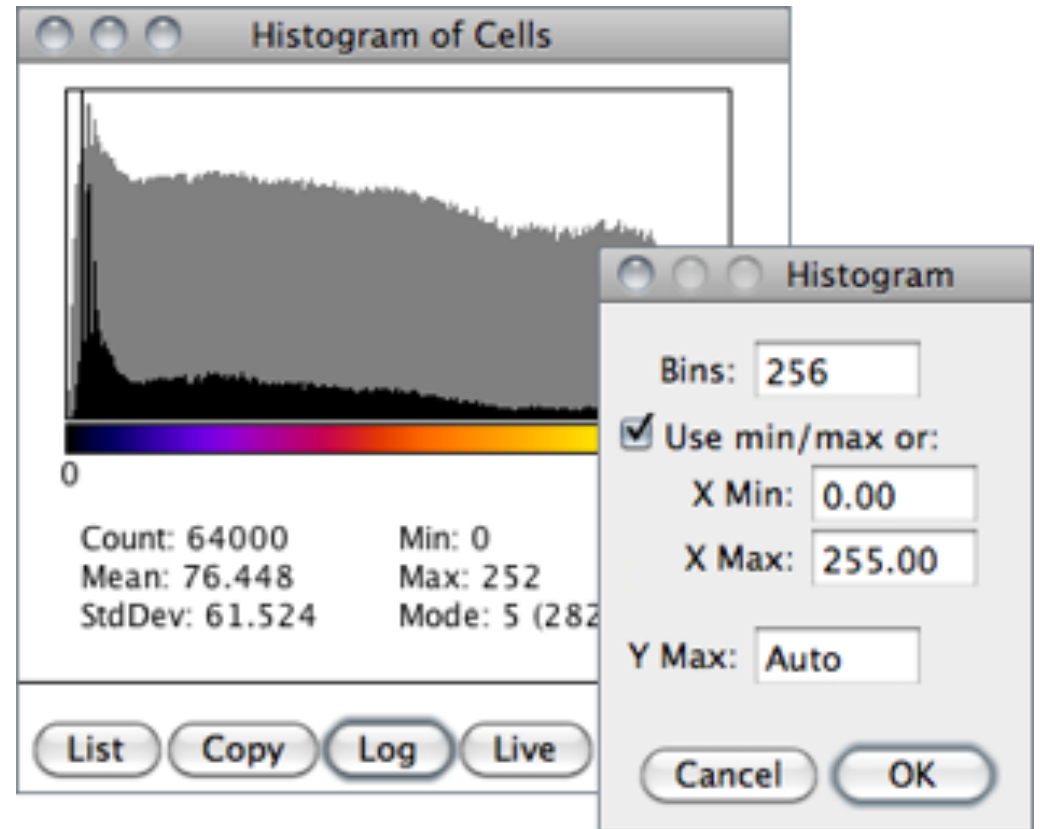
# Color Modes



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

# Histogram

*Analyze > Histogram*



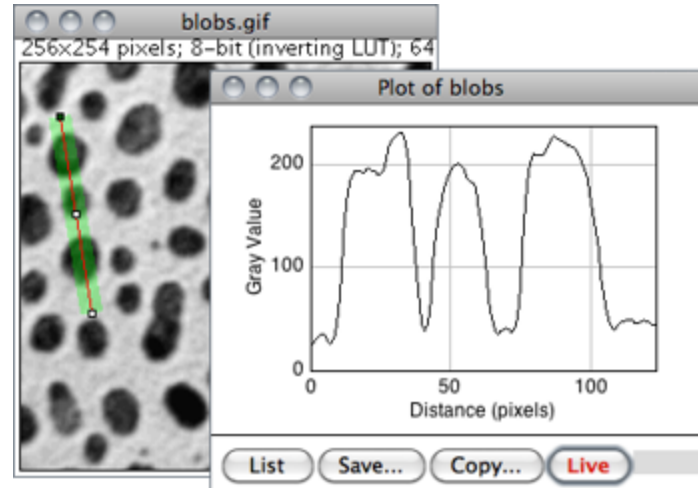
Exercise: What is wrong with  
*File > Open Samples > Blobs*

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

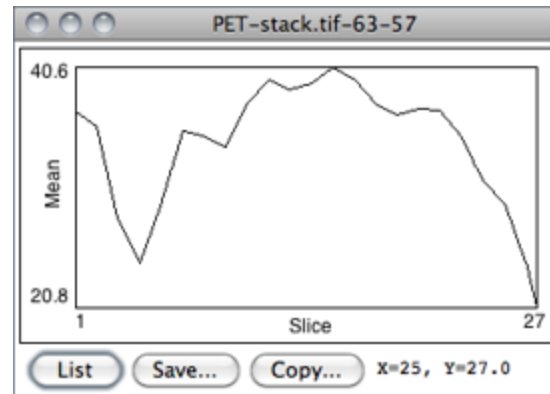


# Profile Plots

*Analyze>Plot Profile*

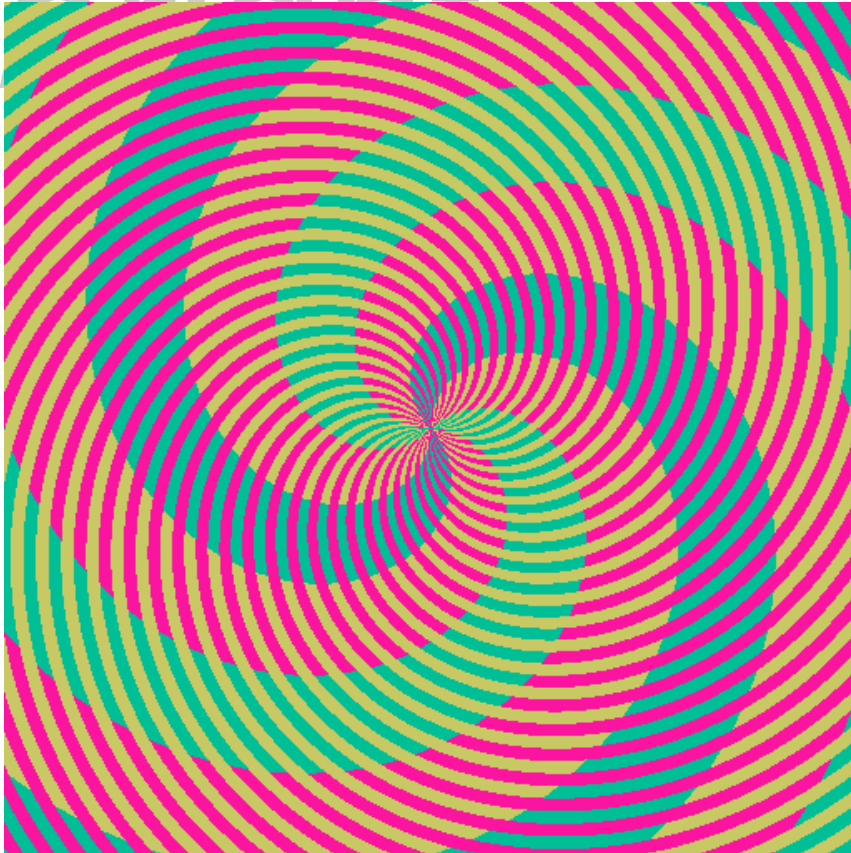


*Image>Stacks>Plot Z-Axis Profile...*



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

# Red/Green is not bright



Tip: *Image > Adjust > Brightness/Contrast...*

# *Information flow*



# *Information loss*



# Pitfalls

- Quantitative = numbers,  
qualitative = eyeballing it
- Don't trust color:  
*File>Open Samples>Spirals*
- Don't trust intensity:  
*File>Open Samples>Adelsons Squares*
- Don't trust lossy compression!
  - *Image>Lookup Tables>glasbey*
  - *File>Open Samples>Boats*
  - *File>Save As>JPEG*

[http://imagej.net/IP\\_Principles](http://imagej.net/IP_Principles)

# Pitfalls

- Pixels are not little squares
  - See Alvy Ray Smith's article for details:  
[http://alvyray.com/Memos/CG/Microsoft/6\\_pixel.pdf](http://alvyray.com/Memos/CG/Microsoft/6_pixel.pdf)
  - Can think of detectors as sampling a Gaussian (approximately)
  - *Edit>Options>Appearance*



[http://imagej.net/IP\\_Principles](http://imagej.net/IP_Principles)

# Pitfalls

- Math with ints and floats has limitations
  - *File>New>Image...* (float, ramp)
  - Multiply by 10,000,000
  - Add 1
  - Probe values;  
Do you see what is wrong now?

[http://imagej.net/IP\\_Principles](http://imagej.net/IP_Principles)

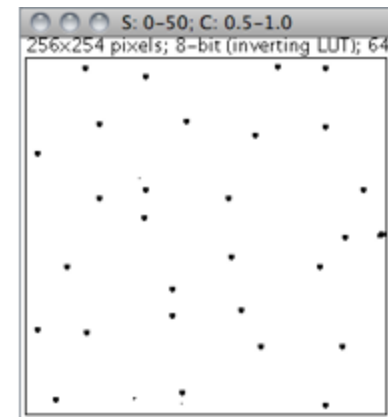
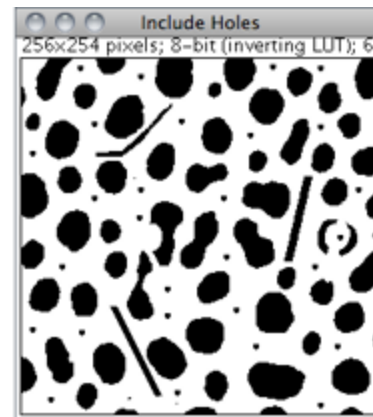
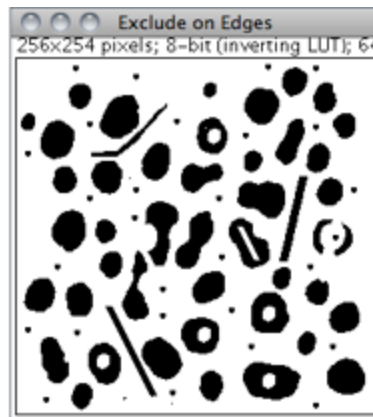
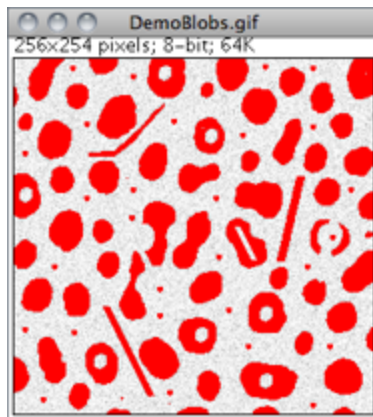
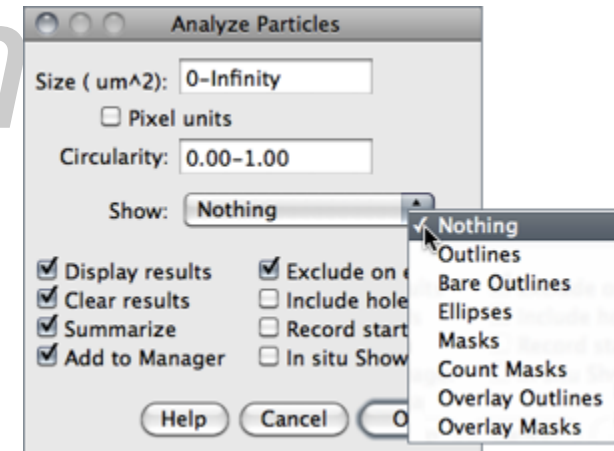
# *3D Viewer*



[http://fiji.sc/3D\\_Viewer](http://fiji.sc/3D_Viewer)

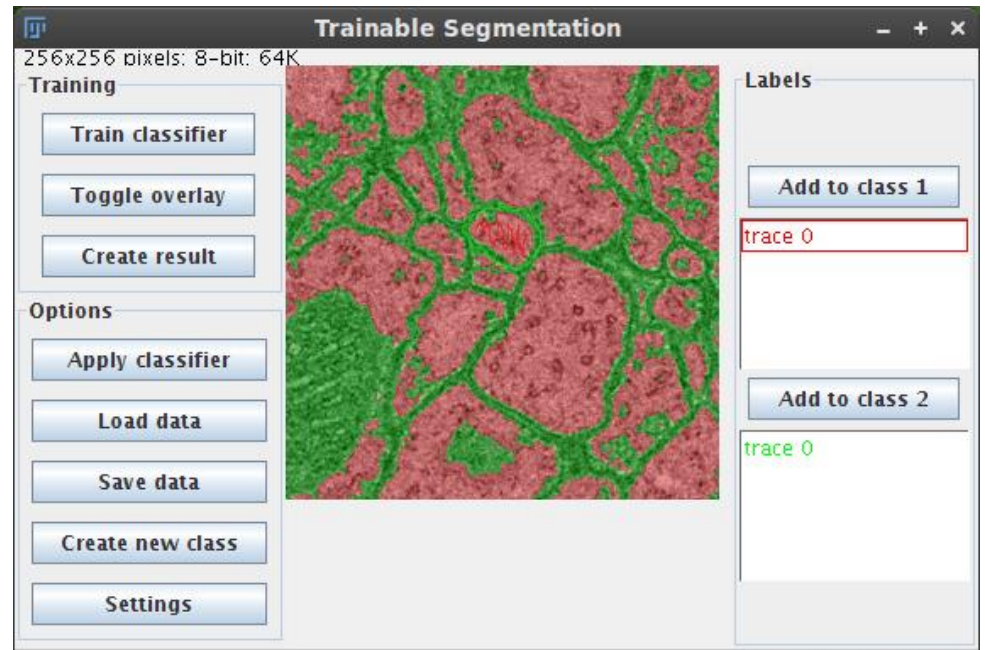
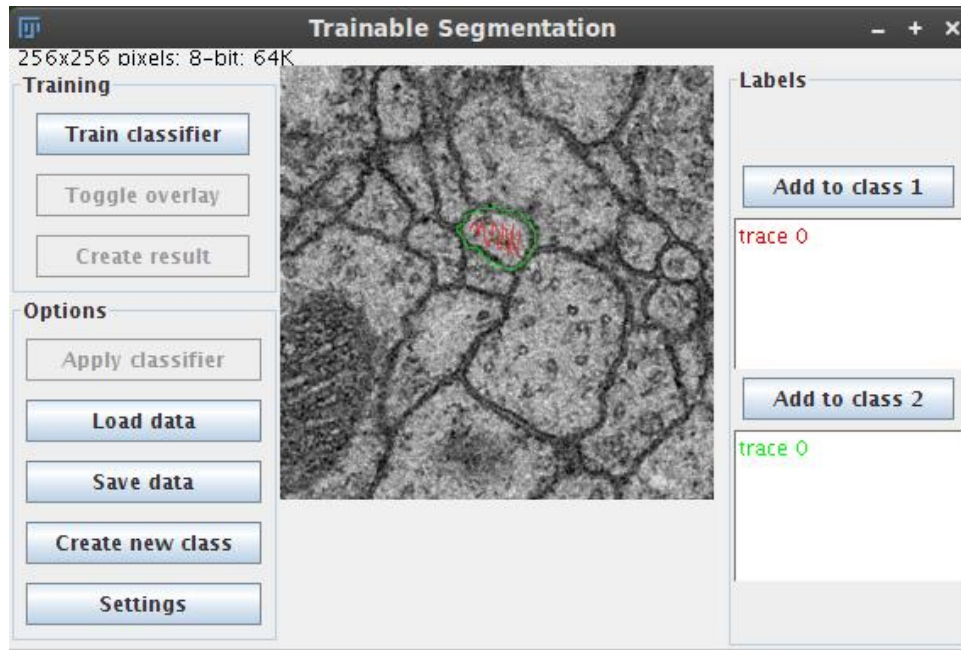


# Segmentation



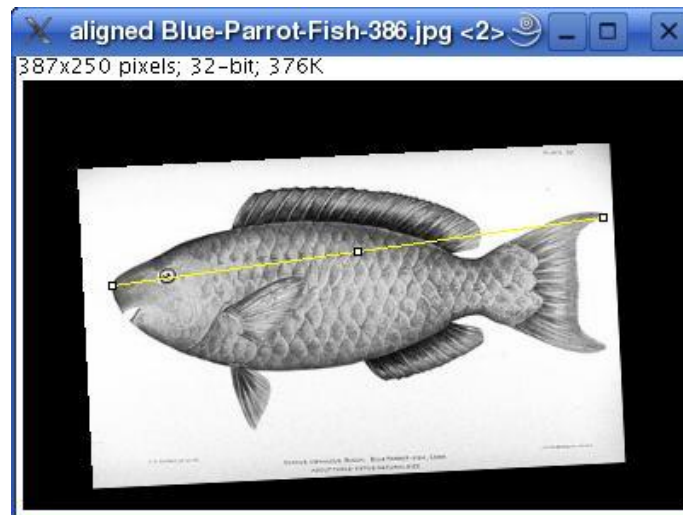
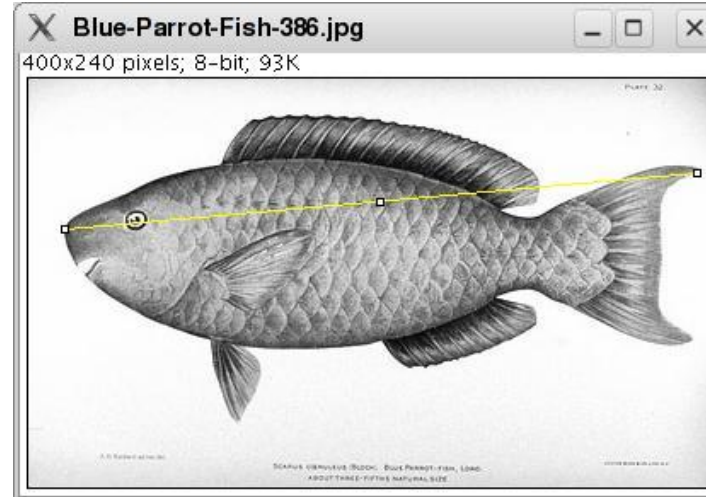
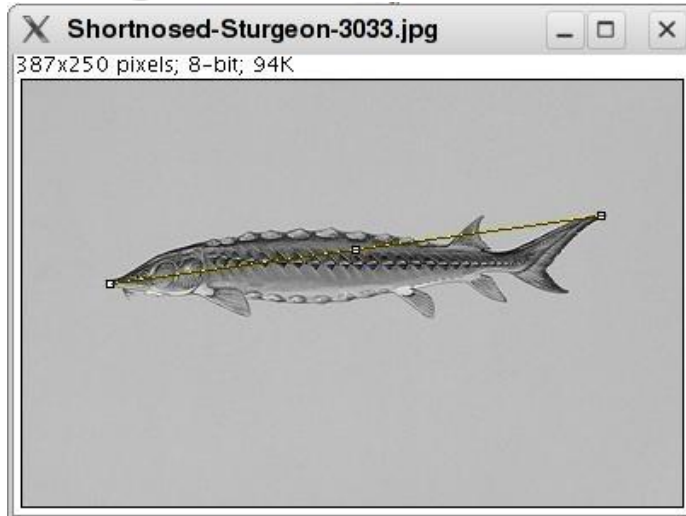
<http://imagej.net/docs/guide/>

# Segmentation



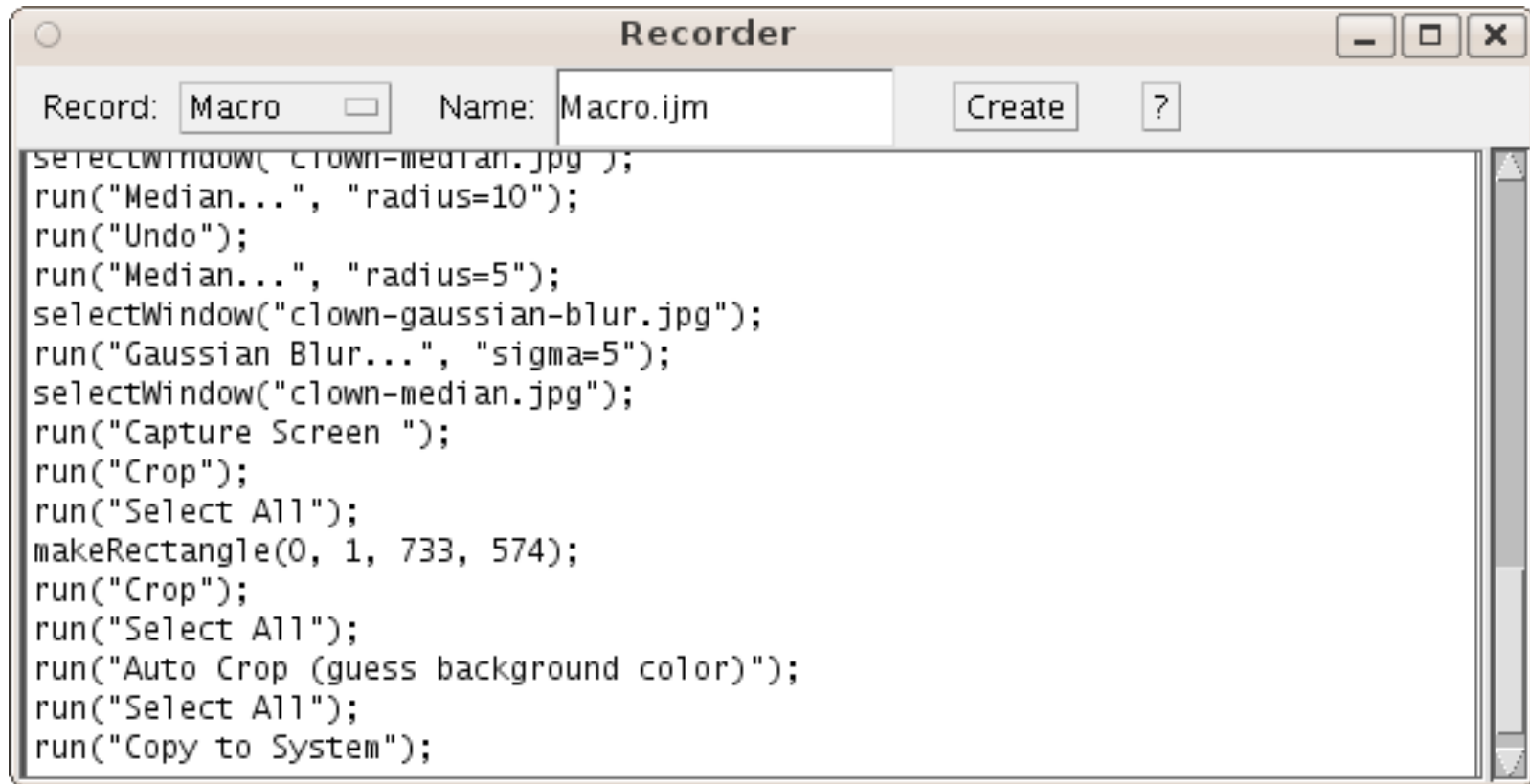
[http://fiji.sc/Trainable\\_Segmentation](http://fiji.sc/Trainable_Segmentation)

# Registration



[http://fiji.sc/Align\\_Image\\_by\\_line\\_ROI](http://fiji.sc/Align_Image_by_line_ROI)

# Macros



The Recorder window displays the following recorded actions:

```
selectWindow( crown=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>

# Macros

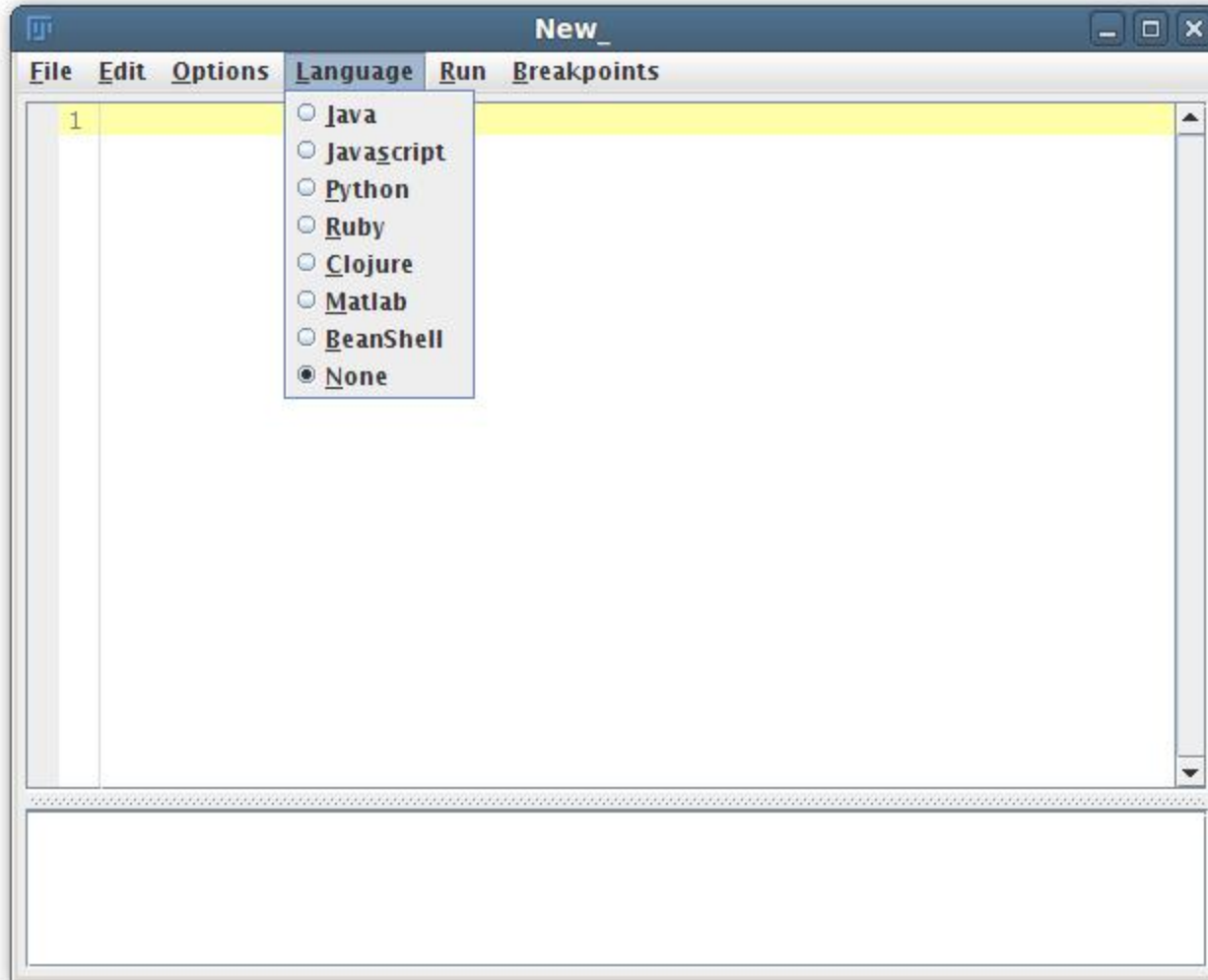
## Exercise: record a macro

- either using the Command Finder!
- or clicking the Dev icon, then Record...
- or with Plugins>Macros>Record...

Suggested workflow: open the Blobs sample, set default threshold, make mask, dilate, make selection, invert selection, close mask, restore selection, set to 0, select none, analyze particles

<http://imagej.net/Macros>

# Macros



- Comments
- Variables
- Functions
- String manipulation
- Conditionals
- Loops

[http://imagej.net/Script\\_Editor](http://imagej.net/Script_Editor)

# Macros: comments

```
// This is a comment trying to help you to remember  
// what you meant to do here:  
value = 2;
```

```
// Code can be disabled by commenting it out  
// x = y * 2;
```

<http://imagej.net/Macros>

# Macros: variables (1/2)

```
intensity = 255;
```

```
a = exp(x * sin(y)) + atan(x * y - a);
```

```
title = "Hello, World!";
```

```
text = "title";
```

```
text = title;
```

<http://imagej.net/Macros>



# Macros: variables (2/2)

// after this, y will have the same value as x

```
y = x;
```

// now, x will be assigned a new value, but y will stay the same

```
x = y * y - 2 * y + 3;
```

// the variable is assigned after the expression is evaluated

```
intensity = intensity * 2;
```

<http://imagej.net/Macros>

# Macros: functions

```
print("Hello, world!");
```

```
// functions can return values
```

```
number = getNumber("Type in a number!");
```

```
// the "run" function is the most important one
```

```
run("Duplicate...", "title=New");
```

```
run("Duplicate...", "title=[with spaces]");
```

```
// Try Tools>Help on Macro Functions...
```

```
// then select a function name, such as "print" and try again
```

<http://imagej.net/Macros>

# Macros: strings

```
number = 1;
```

```
// you can concatenate strings, and strings and numbers
```

```
text = "The number is " + number;
```

```
// why does this not work?
```

```
run("My plugin", "does_not_work=number");
```

```
run("My plugin", "this_works=" + number);
```

<http://imagej.net/Macros>

# Macros: conditionals

```
if (getBoolean("Is Curtis going too fast?")) {  
    hint = "Tell him!";  
} else {  
    hint = "Try to modify the code, play with it...";  
}  
  
showMessage(hint);
```

<http://imagej.net/Macros>

# Macros: loops

```
for (i = 1; i <= 10; i++) {  
    print("Counter: " + i);  
}
```

```
while (getBoolean("Are you sick of my questions yet?")) {  
    print("You know, I really have all day to keep asking...");  
}
```

<http://imagej.net/Macros>

# Macros: tying it together

```
// this example makes a stack of blurred versions of the  
// current slicewith a range of radii.
```

```
radius = getNumber("Maximal radius?");
```

```
title = "Blurred stack of " + getTitle();
```

```
run("Duplicate...", "title=[" + title + "]");
```

```
run("Select All");
```

```
run("Copy");
```

```
for (i = 1; i <= radius; i++) {
```

```
    run("Add Slice");
```

```
    run("Paste");
```

```
    run("Gaussian Blur...", "radius=" + radius);
```

```
}
```

# *Further reading*

Thorough guide for beginners:

<http://nic.uni-hd.de/>

Thorough, up-to-date manual on ImageJ:

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

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

[\*\*http://fiji.sc/Cookbook\*\*](http://fiji.sc/Cookbook)

Help from the community—ImageJ mailing list! ~2000 members:

[\*\*http://imagej.net/Help\*\*](http://imagej.net/Help)