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
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/
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

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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 \texttt{t} – or \texttt{Analyze}>\texttt{Tools}>\texttt{ROI Manager}
- Select other clown
- Click ROI in manager – or \texttt{Edit}>\texttt{Selection}>\texttt{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!

Leci n’est pas une pipette.

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盲ness
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