Image J
FIJI

CIF Workshop
2018

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Who am I?

Cellular Imaging Facility

Bugnon "Technical Manager"

Background in biology, neuroscience

Worked in the CIF platform for 15 years

Developed the basics of the Zeiss LSM reader for Image J

Write plugins for Image J

Job in Training scientists, managing IT, website
Why you’re (probably) here

- You have started working on a microscope
- You have acquired tons of images.
- You are asked to “quantify” the images.
- You don’t know where to start...
- After learning what to do, you have to start again from the beginning
Talk

Image Processing in General

Talk

Study of a use case

Talk

Implementing use case with Fiji

BONUS!

Beyond Fiji
(if there’s time left)
What’s in for you?

Understand an **image processing workflow**

Acquire the **basic knowledge** on how to use **ImageJ**

Understand the **limits and the scope** of image processing

From there, **know where to look** for the specific resources that will apply to your problem.
The image processing workflow
The workflow

Raw Image → ? → Finished Paper
Identify the question to be answered

What’s the scientific question?

What are the features that can help me answer the question?

What can I measure to characterize the features?

What’s the best way to get there?
Features and Measures

Morphology
- Area
- Perimeter
- Length
- Circularity
- 3D
- ...

Location
- Registration
- Coordinates
- Region Overlap
- Scale

Relationship
- Colocalization
- FRET
- ...
- ...

Strength
- Mean Grey Value
- Intensity
- Variations
- Decay
- ...

Nature
- Classification
- Texture
- Skeleton
- ML/IA
- ...

Movement
- Track length
- Velocity
- Expansion
- ...

Occurrence
- Branches
- Count
- Frequency
- ...

And More...
- Anything you turn into a number
Looking for the features

What’s the **right instrument**?

How many **samples** do I need?

What **staining** do I use?

What **controls** do I need?

What **conditions** are relevant?

Complex Setup ≠ Right Tool
Acquire ONLY what you will use

What is the **minimal** information required?

What is the optimal resolution?

Do I need all the **channels**?

Do I really need **stacks**?

Keep in mind **you will have to process** all that data!
Slide scanner

215 Mpx, 32 bit

256 Mb after compression and scaling
Fluorescence microscope

1Mpx, 8 bit
less than 1Mb
Acquire EVERYTHING you will need

Don’t **lose signal by saturating** your images

Use a **higher bit depth** for precise quantification

**Avoid noise** for easier segmentation

Use the right **sampling** (image size, number of z-slices,...) if you intend to use deconvolution, 3D reconstruction
Define the processing protocol

- Pre-processing
- Find what algorithm(s) you need
- Prepare the images for processing*
- Define the steps to avoid the pitfalls*
- Understand the order for each step
- Understand the limitations and pitfalls
Prepare your image for processing

Format the image: resize, crop, downsample,...

Clean the image: remove noise, filter,...

Think of file management: storage location, file and folder naming conventions.
When do I use image J?
The workflow diagram
Image J in the diagram

Acquisition

Algorithm, Filters, ...

Processing

Automation

Analysis

Image J ecosystem

Image J
Basic functions
# Flavors of Image J

<table>
<thead>
<tr>
<th>Name</th>
<th>Author/Maintainer(s)</th>
<th>Description</th>
<th>Initiated</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>ImageJ2</td>
<td>ImageJ developers</td>
<td>A new version of ImageJ targeting scientific multidimensional image data. It is a complete rewrite of ImageJ, but includes ImageJ1 with a compatibility layer, so that old-style plugins and macros can run the same as always. ImageJ2 provides several significant new features, such as an automatic updater, and improved scripting capabilities.</td>
<td>Dec. 2009</td>
<td>Active</td>
</tr>
<tr>
<td>ImageJDev</td>
<td>ImageJ developers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ImageJ1</td>
<td>Wayne Rasband</td>
<td>A stable version of ImageJ which has been in development since 1997. It has a strong, established user base, with thousands of plugins and macros for performing a wide variety of tasks.</td>
<td>1997</td>
<td>Active</td>
</tr>
<tr>
<td>ImageJA</td>
<td>ImageJ developers</td>
<td>ImageJA is a project that provides a clean Git history of ImageJ1, with a proper 'pom.xml' file so that it can be used with Maven without hassles. It is what ImageJ2's legacy support uses at its core.</td>
<td>Jul. 2005</td>
<td>Active</td>
</tr>
<tr>
<td>Fiji</td>
<td>Fiji contributors</td>
<td>Fiji is Just ImageJ, with extras. It is a distribution of ImageJ with many plugins useful for scientific image analysis in fields such as life sciences. It is actively maintained, with updates released often. We recommend Fiji as the preferred version of ImageJ.</td>
<td>Dec. 2007</td>
<td>Active</td>
</tr>
<tr>
<td>ImageFX</td>
<td>Cyril Mongis</td>
<td>ImageFX is a new user interface for ImageJ, built using JavaFX.</td>
<td>2015</td>
<td>Active</td>
</tr>
<tr>
<td>ImageXSM</td>
<td>Steve Barrett</td>
<td>Image XSM is a version of NIH Image that has been extended to handle the loading, display and analysis of scanning microscope images.</td>
<td>May 1993</td>
<td>Active</td>
</tr>
<tr>
<td>AstroImageJ</td>
<td>John Kielkopf</td>
<td>AstroImageJ is ImageJ with astronomy plugins and macros installed.</td>
<td>Unknown</td>
<td>Active</td>
</tr>
<tr>
<td>ImageJ2x</td>
<td>Rawak Software</td>
<td>ImageJ2x is a fork of ImageJ1, modified to use a Swing interface.</td>
<td>Unknown</td>
<td>Active</td>
</tr>
</tbody>
</table>
Beyond image J

Fiji  BoneJ  Bio7

Image J "distributions"
Beyond Image J

Image J related applications

BoneJ, CellProfiler, Cytomine, Fiji, ilastik, ImageJ, NEUBIAS, OpenSPIM, scikit-image, SLIM Curve, Alida, MiToBo, Bio-Formats, Bio7, μManager, KNIME, OMERO, VCELL, Icy
Close Cousins

ICY All-in-One Package

New plugins: How to install a plugin

Label Extractor
ALEXANDRE DUFOUR
TAG: ROI
Extracts labeled objects from a binary or labeled image into ROI using connected component analysis
not rated yet

Repository Generator
STEPHANE DALONGEVILLE
This plugin allows you to create a private repository to distribute your plugins and workspaces from your own server.
not rated yet

DcmReceiver
WILL OLIVAN
A simple DICOM/DICONDE receiver used to receive, store and open dcm images. This plugin is a part of EVA project. http://owenay.github.io/EVA/.
not rated yet

Most popular plugins:

Protocols
ALEXANDRE DUFOUR
TAG: PROTOCOL
Visual programming environment, letting you develop image processing protocols graphically. No programming skills required!
(5)

Intensity Projection
ALEXANDRE DUFOUR
TAG: PROTOCOL
Intensity projection along depth or time with multiple algorithms: mean, max, median, variance, standard deviation, saturated sum. Projection can be restricted to ROIs.
(4)

Active Cells
BIOMEDICAL IMAGING GROUP
TAG: SEGMENTATION
This plug-in implements fast active contours for image segmentation. Their representation in terms of spline curves allows for a natural and intuitive manipulation of the active co...
(4)

ImageBrowser
NICOLAS HERVÉ
TAG: IMAGE
Browse files in directories with thumbnails view
(5)

Spot Detector
FABRICE DE CHAUMONT
TAG: SPOT COUNT
Spot detector detects and counts spots. - Detects spots in noisy images 2D/3D. - Depending on objective, spots can be nuclei, nucleus or cell - Versatile input: sequence or batch...
(8)

Script Editor
THOMAS PROVOST
TAG: SCRIPT
Create powerful scripts to implement what's missing with plugins. Syntax Color and Autocomplete features implemented (still need testing). Should be used with Icy-Master on github...
(4)
Close Cousins

High Throughput

CellProfiler

Load an example CellProfiler pipeline, a series of image-processing modules

Adjust the settings to measure the phenotypes of interest in your images

Process images automatically - even millions

Export your data to a spreadsheet or database

Explore your data and classify complex or subtle phenotypes using machine learning in CellProfiler Analyst
So what about Fiji?
FIJI Is Just ImageJ
Fiji Is more than ImageJ

Many, many more Plug-Ins
Fiji Is also ImageJ 2
Practical considerations
Installing Image J - Fiji

**SYSTEM REQUIREMENTS**

ImageJ will run on any system that has a Java 8 (or later) runtime installed. This includes, but is not limited to:

1. Windows XP, Vista, 7 or 8 with Java installed from java.com
2. Mac OS X 10.8 “Mountain Lion” or later with Java installed from java.com
3. Ubuntu Linux 12.04 LTS or later with OpenJDK 8 installed

**INSTALLATION**

Caution: “Program Files” not recommended!

If you are installing ImageJ on Windows, we strongly recommend that you store your ImageJ.app directory somewhere in your user space (e.g., "C:\Users\[your username]\ImageJ.app") rather than in "C:\Program Files" or other system-wide directory. If you move ImageJ.app to such a directory, certain versions of Windows will deny ImageJ write permission to its own directory structure, preventing it from being able to update. See also imagej/imagej#72.

ImageJ is distributed as a portable application. That means that you do not have to run an installer; just download, unpack and start it.
Installing Image J - Fiji

~ Download Fiji for your OS ~

64-bit  macOS  64-bit

Other downloads

32-bit  No JRE  32-bit
Online and offline resources
Strong and active community

https://forum.image.sc/tags/imagej
### Worldwide conferences and events

<table>
<thead>
<tr>
<th>Date</th>
<th>Event Details</th>
<th>Location</th>
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<tbody>
<tr>
<td>October 16-19, 2018</td>
<td>Biologic Analysis Schools: Early Career Investigators (life scientists), Facility staff (imaging &amp; analysis staff from core-facilities)</td>
<td>Edinburgh, Scotland</td>
</tr>
<tr>
<td>October 22-26, 2018</td>
<td>13th LFD Workshop in Advanced Fluorescence Imaging and Dynamics</td>
<td>Laboratory for Fluorescence Dynamics, University of California, Irvine, CA USA</td>
</tr>
<tr>
<td>November 14-15, 2018</td>
<td>Fiji Basics</td>
<td>MIAP, Freiburg, Germany</td>
</tr>
<tr>
<td>December 6-8, 2018</td>
<td>From Images to Knowledge with Fiji &amp; Friends (i2K)</td>
<td>EMBL, Heidelberg, Germany</td>
</tr>
<tr>
<td>December 17-18, 2018</td>
<td>ImageJ/Fiji introduction and macro workshops</td>
<td>University of Leicester, UK</td>
</tr>
<tr>
<td>February 2-8, 2019</td>
<td>NEUBIAS Symposium &amp; Conference (TS10 for Early Career Investigators, TS11 for Biologic Analysis)</td>
<td>Luxembourg</td>
</tr>
</tbody>
</table>

*Feel free to edit and update this thread with upcoming conferences, meetings, symposia, and workshops.*
Learning to code

https://studio.code.org/courses

https://www.codecademy.com/

https://www.freecodecamp.org/

https://www.edx.org/course/introduction-to-java-programming-starting-to-code-with-java

https://www.lynda.com/Programming-Languages-training-tutorials
Books

http://imagej.net/User_Guides

- **Fiji Cookbook**: Provides an analysis-oriented introduction and user guide to ImageJ.
- **Analyzing Fluorescence Microscopy Images with ImageJ**: Provides detailed information on image analysis using ImageJ.
- **Digital Image Processing**: A textbook covering many aspects of digital image processing, with code examples built with ImageJ.
- **Bioimage Data Analysis**: A textbook for constructing bioimage analysis workflows using ImageJ, macro, Matlab, and R, to extract numbers out of image data. Freely downloadable from Wiley site.
- **Fiji Training Notes**: Including demo images from Monash University. Detailed screenshot and explanation steps on basic analysis through to macro code.
Working on a use case
The case

Cleaved-Caspase 3 staining on B16 tumors (courtesy of Dr. T. Santoro)
Prep the images
Select a good candidate image

Keep it **simple:**
- no artifacts, no border effects, no debris,

Use a **small sample**
- to minimize the development time

Work for the **ideal case**
- first
The Good

Foreign object

Empty area

Good candidate

Object to characterize
The Bad and the Ugly

Complex background
Smaller objects, mixing with background
Debris
Object to characterize
Foreign structure close in color or shape

Bad candidate #1
Bad candidate #2
Implement the processing protocol

Is the output what you expected?

If not, rethink your processing protocol or improve pre-processing.
Test your processing on your candidate image
Test your processing on a worst-case scenario image

- Good Candidate
- Bad Candidate #1
- Bad Candidate #2

- ≈500 cells
- ≈50 cells
- ≈230 cells
Automate the processing

Macro recorder

Script Editor
Test the automation on a sample set of images
Process the whole data set
Format and analyze the results

Consistent naming scheme

Outputs for each steps

Raw results

Formatted tables

Format and analyze the results

Consistent naming scheme

Outputs for each steps

Raw results

Formatted tables
Answer the question

Population #1

Population #2
Demo time!