Disclaimer

Too slow, too boring

Always better experts, resources

Too fast, too complex

Specific questions in mind

Know better, different ways
Talk

Image Processing in General

Study of a use case

Implementing use case with Fiji

Demo

BONUS!

Weka?
PART I

Image Processing in General
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
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

Image acquired

Published 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 a good **processing** to get me there?
Features and Measures

Morphology
- Area
- Perimeter
- Length
- Circularity
- same in 3D
- Scale

Location
- Registration
- Coordinates
- Region Overlap

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**?
- What **staining** do I use?
- How many **samples** do I need?
- What **controls** do I need?
- What **conditions** are relevant?

**Most 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
... but also make sure to acquire EVERYTHING that is needed

Make sure you 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

1. Find the algorithm(s) you need
2. Understand the order for each step
3. Understand the limitations and pitfalls
4. Define the steps to avoid the pitfalls
5. Prepare the images for processing
6. Check and if not enough

* see next slide

= Pre-processing
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?
A workflow diagram
Image J in this diagram

Acquisition

Algorithm, Filters, ...

Processing

Automation

Analysis

Image J ecosystem

Image J ecosystem
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></td>
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</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.</td>
<td>Dec. 2007</td>
<td>Active</td>
</tr>
<tr>
<td>ImageJFX</td>
<td>Cyril Mongis</td>
<td>ImageJFX 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>
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</table>
Beyond image J

Fiji  BoneJ  Bio7

Image J "distributions"
Beyond Image J

Image J related applications
**Close Cousins**

**ICY**

**All-in-One Package**

**New plugins:**

- **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**
  - Stéphane Dallongeville
  - This plugin allows you to create a private repository to distribute your plugins and workspaces from your own server.
  - Not rated yet

- **DicmReceiver**
  - Wido Olwanic
  - A simple DICOM/DICONDE receiver used to receive, store and open dcm images. This plugin is a part of EVA project, http://owenow.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)

- **ImageBrowser**
  - Nicolas Hervé
  - **Tag:** GUI
  - Browse files in directories with thumbnails view
  - (5)

- **Intensity Projection**
  - Alexandre Dufour
  - **Tag:** GEPLUG
  - 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**
  - **Tag:** SEGMENTATION
  - Biomedical Imaging Group
  - This plugin 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)

- **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 ba....
  - (8)

- **Script Editor**
  - Thomas Provoost
  - **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...
Close Cousins

High Throughput

Cell Profiler

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\[you]\Applications\ImageJ.app”) rather than in “C:\Program Files” or other system-wide directory. If you move ImageJ.app to such a directory, then 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 ~

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<tr>
<td>64-bit</td>
<td>macOS</td>
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Other downloads

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</tr>
<tr>
<td>32-bit</td>
<td>No JRE</td>
<td>32-bit</td>
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Online and offline resources
Strong and active community

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

<table>
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<td>October 16-19, 2018</td>
<td>NEUBIAS</td>
<td>Workshops</td>
<td>Biobioage 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>Workshop</td>
<td>This workshop outlines advanced concepts of fluorescence imaging techniques and instrumentation, including advanced theoretical lectures, computer based training on data analysis and simulations, and hands on laboratory training using the fluorescence microscopy instrumentation of the LFD facility.</td>
<td>Laboratory for Fluorescence Dynamics University of California, Irvine Irvine, CA USA</td>
</tr>
<tr>
<td>November 14-15, 2018</td>
<td>Fiji Basics</td>
<td>Workshop</td>
<td>A basic workshop for life science students, Ph.D students, postdocs and technicians covering basic methods of image analysis with ImageJ / Fiji</td>
<td>MIAP Freiburg, Germany</td>
</tr>
<tr>
<td>December 6-8, 2018</td>
<td>From Images to Knowledge with ImageJ &amp; Friends (i2K)</td>
<td>Conference</td>
<td>A forum for discussing forward looking strategies for dealing with the ever increasing flood of large and content rich microscopy imagery.</td>
<td>EMBL Heidelberg, Germany</td>
</tr>
<tr>
<td>December 17-18, 2018</td>
<td>ImageJ/Fiji introduction and macro workshops</td>
<td>Workshop</td>
<td>For the 15th time I will organize two 1-day workshops with a mixture of lectures and hands-on sessions. During the workshops you will get an introduction into the use of ImageJ/Fiji for digital image processing focused mostly on biological examples. I will also try to answer specific questions from participants concerning image analysis of their own data. No prior knowledge of ImageJ, Fiji or macro coding is required, only basic use of a laptop.</td>
<td>University of Leicester, UK</td>
</tr>
<tr>
<td>February 2-8, 2019</td>
<td>NEUBIAS Symposum, TS10 and TS11</td>
<td>Workshops &amp; Symposium</td>
<td>Symposia and Biobioage Analysis Schools (TS10 for Early Career Investigators, TS11 for Biobioage Analysts)</td>
<td>Luxembourg</td>
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</table>

Feel free to edit and update this thread with upcoming conferences, meetings, symposia, and workshops.
"Official" user guides

http://imagej.net/User_Guides
Recommended Guides

More Advanced Topics

http://wiki.cmci.info/documents/ijcourses

Bioimage Data Analysis
(Use case and examples using FIJI/Matlab)

Basic Image Processing and Analysis

Macro Programming in Image J

Kota Miura
CMCL
General Imaging

What can you do and what can't do in image processing (ethics)

https://ori.hhs.gov/education/products/RIandImages/default.html

The Image Processing Handbook, Seventh Edition

John C. Russ, F. Brent Neal

ISBN-10: 149874026X
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
PART II

Study of a use case
The case

Cleaved-Caspase 3 staining on B16 tumors (courtesy of Dr. T. Santoro)
Prepare the images

Slide → Scanned slide → Cropped ROI → Exported ROI → 240 1000px tiles → 2.8 Mb TIFF
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
Good candidate

Foreign object

Empty area

Object to characterize

Good candidate
Bad and terrible candidates

- Complex background
- Smaller objects, mixing with background
- Debris
- Foreign structure close in color or shape

Object to characterize

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 small subset of images

Automation result
Process the whole data set
Format and analyze the results

- Consistent naming scheme
- Outputs for each steps
- Raw results
- Formatted tables
Answer the question

Population #1

Population #2
PART III

Demo time