ImageJ / FiJi Workshop 2012

Niko Ehrenfeuchter

Outline

Part I – The Basics

- ImageJ's history
- Technical details, FiJi
- The ImageJ user interface
 - Mem, options, pointer, tools & variants
- Basic ImageJ tools and features
 - B&C, histogram, threshold, measure, de-noise / smooth
- Short-cuts & Usability
 - Command Finder, Control Panel, Action Bar
- Advanced tools
 - Watershed, shading, stitching?

History

ImageJ: successor of a software from the National Institutes of Health called *NIH Image* (Pascal-based, Mac only, early 90's)

- Developed by Wayne Rasband, started 1997
- Public Domain \rightarrow source code available
- Java based \rightarrow Win, Mac, Linux, ...
- Macros & plug-ins
- Huge potential, very active community
- Technical design limitations
 - 15 yrs. old, usability somewhat "aged"

 \rightarrow ImageJ basically can do everything, it's just sometimes very hard to find out how...

History

ImageJ from the technical / administrative view

- Requires a Java runtime installed
- Can update *itself* only
 - Java?
 - Plugins?

- Shipped with small, generic set of Plugins & Macros

Distributions showed up to address this:

- MBF ImageJ (MacBiophotonics) "IJ for Microscopy"
 - Plugin collection
 - Abandoned
- Since 2007 FiJi is the don't-worry, ready-to-run solution!

http://developer.imagej.net/history

FiJi is just ImageJ – Batteries included

FiJi is an ImageJ distribution intended for Life sciences:

- JRE with Java3D included
- Huge set of Plugins, e.g. LOCI Bio-Formats
- Powerful update mechanism
 - Non-intrusive
 - Cares about Java, the Plugins, ...
 - Customizable
- Extensive documentation
- Easy to install
- But: shares ImageJ's limitations

FiJi is actively co-developed with ImageJ2 to reduce redundant work and share good ideas and mechanisms.

FiJi vs. ImageJ continued

ImageJ

- Generic image processing framework unfocused
- Single "lead" developer W.Rasband

FiJi

- Aimed for Life Sciences
- Emphasis on Registration, Segmentation & Volume Data
- Community development effort
- Quality mechanisms for plugins
 - Interoperability & code checks
 - Proper documentation
 - Maintainers for plugins
- Support for more additional languages: Python, Ruby, ...
- Script editor to develop plugins and macros

\rightarrow Use FiJi and add the plugins you need! (e.g. from MBF)

Resources

"The Bible" - ImageJ user's guide: http://rsbweb.nih.gov/ij/docs/guide/

ImageJ dokuwiki: <u>http://imagejdocu.tudor.lu/</u>

ImageJ macros: http://imagej.nih.gov/ij/macros/

ImageJ mailing list: http://rsb.info.nih.gov/ij/list.html

FiJi download: <u>http://fiji.sc/wiki/index.php/Downloads</u>

FiJi wiki containing documentation for plugins, tutorials, etc: <u>http://fiji.sc/wiki/</u>

FiJi – getting started

First installation on Windows:

- Download from http://fiji.sc/
- If possible, use 64bit version
- Choose a writeable location
 - NOT "C:\Program Files\..." !
 - NOT on a network share (works, but slow!)
 - e.g. "D:\Tools\"
- Unzip
- Start & run updater

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Updating FiJi

Sometimes the updater has a problem, just delete the folder "update" inside the Fiji folder.

× Update the updater
? There is an update available for the Fiji Updater. Install now?
<u>O</u> K <u>A</u> bbrechen

× – 🗆 ImageJ Updater

Name	Status/Action
fiii-linux64	Update it
plugins/3D Blob Segmentation	n.jar Update it 🔚
plugins/3D Viewer.jar	Update it
plugins/AnalyzeSkeleton .jar	Update it
plugins/Anisotropic Diffusion	2D.jar Update it
plugins/Arrow_jar	Update it
plugins/Auto_Threshold.jar	Update it
plugins/BalloonSegmentation	.jar Install it
plugins/BeanShell_Interpreter	.jar Updateit
plugins/Bug_Submitter-2.0.0-S	NAPS Update it
plugins/Colocalisation_Analysi	s.jar Update it
plugins/Colour_Deconvolution.	jar Install it
plugins/CorrectBleachjar	Install it
plugins/Daltonizejar	Uninstall it
pluging/Descriptor based regi	etrat Install it
Apply changes Ad	vanced mode 🔰 🗌 Cancel

In general, it is safe to just click on "Apply" to install the updates (and new packages).

More information is provided by the "Advanced Mode"...

Updating FiJi - Advanced

× – 🗉 ImageJ Updater		
Search:		
View Options: View all files		▼ Details
Please choose what you want	t to install/uninstall:	SCRIPTS/MIJI.III
Name scripts/InstallJava3D.m scripts/IsJava3DInstalled.m scripts/Matlab3DViewerDemo_1.m scripts/Matlab3DViewerDemo_2.m scripts/Matlab3DViewerDemo_3.m scripts/Matlab3DViewerDemo_3.m scripts/Matlab3DViewerDemo_3.m scripts/Matlab3DViewerDemo_3.m scripts/Matlab3DViewerDemo_3.m scripts/Matlab3DViewerDemo_3.m scripts/Matlab3DViewerIntroduction scripts/Matlab3DViewerIntroduction scripts/Miji_Test.m scripts/Record_Desktop.py scripts/Record_Window.py scripts/bfopen.m images/icon.png macros/StartupMacros.fiji.ijm macros/StartupMacros.fiji.ijm macros/toolsets/Clear Custom Too macros/toolsets/Drawing Tools.txi macros/toolsets/Lookup Tables.txi luts/16_colors.lut install/update: 1 (344.5kB) Keep as-is Install	Status/Action Up-to-date Local-only Up-to-date Locally modified Locally modified Up-to-date Up-to-date	Release date: 09 Aug 2012 Description : Use this Matlab function to initialize MIJ Authors: Jacques Pecreaux, Johannes Schindelin Category: interoperability Dependency: jars/mij.jar Update site: Fiji
Manage update sites	Show changes	

Image Types I

Digital Images are tables of intensities, each element defining a local brightness value.

Steps depend on the number of levels used for quantization:

- 1 bit \rightarrow 0 or 1 \rightarrow 2 steps
- -2 bit = $2^2 \rightarrow 4$ steps

 $- 8 \text{ bit} = 2^8 \rightarrow 256 \text{ steps}$ - 16 bit = 2^16 = 65536 - 32 bit = 2^32 > 4 million

Special case "RGB" = 8 bit for each, Red Green and Blue (24 bit overall)



Image Types II

ImageJ's handling of image-data evolved over time

- Single (x,y) - Stack (x,y,c) / (x,y,t)
- Hyperstack: 4D / 5D
 - multi-channel timelapse
 - multiple positions

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not limited to just 3 channels

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z



Colors

Several ways of dealing with colors exist in ImageJ:

- No additional information \rightarrow greyscale intensities
- Multi-channel with colors assigned
 - "color" mode
 - "composite" mode
- RGB colors are no separated channels!

For visualization, ImageJ provides the option to assign pseudo-colors via LUT (Look-Up-Tables) \rightarrow see later.

The user interface

× -	O Fij	I								
File	Edit	Image	Process	Analyze	Plugins	Window			Hel	р
ЦC		> / ্ব	+ <mark>,</mark> ×, ,	A 🔍 🖑	🖋 🗷 🕨	ey Stik LUT	9 8	ঞ		♠
Fiji/Ima	Fiji/ImageJ 1.47d; Java 1.6.0_24 [64-bit]; 23MB of 9575MB (<1%)									

The main window has three parts:

- Menu bar
- Tools
- Status line

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- ImageJ & Java version
- Memory architecture & current usage
- Pixel positions and intensities while hovering on images
- Descriptions of toolbar entries

Opening Images

Via Menu "File"

- Open / Open Recent
 - TIFF, GIF, JPEG, PNG, DICOM, BMP, PGM, FITS
- Import
 - Sequences, Text Images, URL's, AVI's, LUT's, ...

Drag And Drop (on main window)

- Tries to guess the applicable reader (no choice)

Using the Bio-Formats library from the LOCI plugin collection:

- File > Import > Bio-Formats (at the bottom)
- Plugins > LOCI > Bio-Formats Importer

 \rightarrow Bio-Formats is the preferred way for most proprietary file-formats. <u>http://loci.wisc.edu/bio-formats/formats</u>

Opening Images with Bio-Formats

× Bio-Formats Import Options

Stack viewing -			Metadata viewing	Information			
View stack with:	Hyperstack		□Display metadata	Color mode - Visualizes channels			
Stack order:	XYCZT		□Display OME-XML metadata	according to the specified scheme.			
			□Display ROIs	Possible choices are:			
Dataset organia	zation		Memory management ——— —Use virtual stack	• Default - Display channels as closely as possible to how they are stored in the file.			
□Open files individually			□Specify range for each series	Composite - Open as a merged composite image. Channels are			
□Swap dimensions			□Crop on import	colorized according to metadata			
₩Open all series				the following default order: 1=red,			
■Concatenate series when compatible		ible	Split into separate windows	2=green, 3=blue, 4=gray, 5=cyan, 6=magenta, 7=yellow.			
□Stitch tiles			□Split channels	Colorized - Open with each channel in a separate plane, colorized			
Color options		□Split focal planes	according to metadata present in the default				
Color mode:	Default		□Split timepoints	order (see Composite above).			
□Autoscale				Grayscale - Open with each channel in a separate plane_displayed in			

OK Cancel

The image window



Header line showing information:

- Channels
- Slices
- Calibration
- Bit-depth
- Size (in memory!)

Sliders at the bottom to adjust:

- Active channel
- Z-slice
- Timepoint

Status bar in main window shows intensities of *current* channel.

The Toolbar

Selection Tools:

- Right click: choose variant
- Double click: properties
- \rightarrow select region(s) of interest



& pan

User-configurable toolsets

Image calibration

If you plan to do measurements on your images, check the calibration!

Image > Show Info

× – 🗆 Info for blobs.gif File Edit Font Title: blobs.gif Width: 256 pixels Height: 254 pixels Pixel size: 1x1 pixel ID: -17 Coordinate origin: 0,0 Bits per pixel: 8 (grayscale LUT) Display range: 0-255 No Threshold Uncalibrated URL: http://imagej.nih.gov/ij/images/blobs.gif No Selection

× 🛛 Fluorescen	tCell				
Channels (c): 3					
Slices (z): 1					
Frames (t): 1					
Note: c*z*t must ec	qual 3				
Unit of Length: P ^{ixe})				
Pixel Width: 1.00	000				
Pixel Height: 1.00	000				
Voxel Depth: 1.00	000				
Frame Interval: 0 se	ec				
Origin (pixels): 0,0					
Global					
OK Cancel					

Image > Properties

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Display Settings



Use Image > Adjust > "Brightness / Contrast" to set the display minimum and maximum values.

- → "Auto" allows for a minimal saturation usually sane setting for human perception.
- → These settings DO NOT alter your data, except when using the "Apply" button.

Color Modes

Use Image > Colors > Channels Tool to select the display mode and change the assignment of colors to the individual channels.





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Lookup Tables

LUT's are *filters* that are applied before the image is displayed on the screen.

- Useful to check for saturation
- Emphasize special intensities
- Take B&C settings into account!
- Not available for RGB images.

Image > Lookup Tables or Directly from the Toolbar

Characteristics & Editor:

- Image > Color > Show LUT
- Image > Color > Edit LUT



Histogram

Analyze > Histogram

Shows intensities distribution.

- use "Log" mode
- honors LUT
- Statistics
- Interactive





Niko Ehrenfeuchter

Imaging Core Facility

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Measuring

ImageJ provides plenty of information for selected regions.

To measure, just press "m" and the selected results will show up in a new window.

→ Types can be configured in Results > Set Measurements

 \rightarrow can be saved as txt or csv file



× :	- 0 R	esults			
File	Edit	Font	Result	s	
	Area	Mean	Min	Max	 1
1	4904	92.588	12	195	7
\square					2

× D Set Measurements

√ Area	🖬 Mean gray value					
□ Standard deviation	□Modal gray value					
🖬 Min & max gray value	Centroid					
□Center of mass	Perimeter					
□Bounding rectangle	□Fit ellipse					
□Shape descriptors	□Feret's diameter					
Integrated density	□Median					
Skewness	□Kurtosis					
□Area fraction	□Stack position					
Limit to threshold	Display label					
□Invert Y coordinates □	Scientific notation					
□Add to overlay						
Redirect to: None 🗆 Decimal places (0-9): 3						
[OK Cancel Help					

Distance / Profile Plots

Analyze > Plot Profile shows the intensity profile along a previously selected line.

E.g. useful to measure the diameter of an object for parts above a certain intensity only.

The Plot itself is again an image where, so measurements can be used on it again.



× – □ embryos.jpg 344x236 pixels; RGB; 317K

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Thresholding

Simplest approach for segmentation:

- Image > Adjust > Threshold
- Choose variant, background and value
- Result is a binary image

Í	× – D Threshold	
× – D blobs-3.gif 256x254 pixels; 8-bit (invertin	121 255	blobs-2.gif pixels; 8-bit (inverting LUT); 64K
	Default 🗆 Red 🗆	
	Dark background Stack histogram	
	Auto Appiy Reset Set	

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Local Thresholding



256x254 pixels; 8-bit; 64K
× – D Threshold
× - D Threshold
× - • Threshold
× - D Threshold
 Threshold 0 31 Default Red
 Threshold Threshold 0 31 Default Red Dark background Stack histogram

Image > Adjust > Auto Local Threshold

× Auto Local Threshold
Auto Local Threshold v1.4 Method Try all 🗖 Radius 15
Special paramters (if different from default)
Parameter 1 0
Parameter 2 0
▼White objects on black background
Thresholded result is always shown in white [255].
OK Cancel

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Local Thresholding

Displays results for all local thresholding methods.

- → Try various settings until results are good.
- → Run again and select the desired method instead of "all".



Analyze Particles

Analyze > Analyze Particles finds connected components in a thresholded (binary) image.

Results and ROI Manager is shown



ROI Manager

Analyze > Tools > ROI Manager: provides possibilities to handle multiple regions of interest, for example:

- Edit, delete, label
- Save, load to and from files
- Reapply to another image
- Measure

	× – 🗆 ROI Manager							
	0001-0015	-	Add [t]					
	0002-0005		Undota		Desculto			
× – 🗆 blobs-2.gif	0003-0014	=	Opuare		Results			
256x254 pixels; 8-bit; 64K	0004-0011		Delete	Edit	Font	Result	5	
	0005-0014		Rename	Area	Mean	Min	Max	
	0006-0016			422	100.955	120	222	
	0007-0022		Measure	433	190.600	120	232	
	0008-0028		Deselect	185	179.286	128	224	
	0009-0027			658	205.617	128	248	
	0010-0027		Properties	434	217.327	128	248	
32 🚳 👝 💥 👗	0011-0034		Flatten (F)	477	212.143	128	248	
a 🕺 🗳 👘 🚮 🚳	0012-0041			285	204.295	128	248	
P a a C V V V	0013-0045		More »	81	161.481	128	200	
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	0015-0055			231	199.469	120	240	
	0016-0059	•	M Labeis	231	100.400	120	240	
			10	30	147.200	128	168	
	5)		11	501	189.142	128	232	_
59 59 60 62 61	2		12	660	171 697	128	232	-M

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Touching Objects

Use Process > Binary > Watershed to split touching objects in a binary image. Analyze Particles has to be re-run afterwards.



Filtering

Located in Process > Filters



× – □ noisy-2.png 256x254 pixels; 8-bit; 64K



Gaussian Blur

× – D noisy.png 256x254 pixels; 8-bit; 64K



Median

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3D Viewer





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Plugins > Shortcuts > List Shortcuts...

× – 🗆 Keyboard Short c	uts	
File Edit Font		
Hot Key	Command	Δ
Close Bracket	Show All	
Open Bracket	Script Editor	
1	Select First Lane	
2	Select Next Lane	
3	Plot Lanes	
4	Original Scale	
5	View 100%	
9	*recent commands	
A	Select None	
В	Blobs (25K)	
с	Brightness/Contrast	
D	Duplicate	
E	Restore Selection	
F	Flatten	
G	Capture Screen	
н	Orthogonal Views	
1	Invert	
К	Color Picker	
м	Install	
N	Text Window	
0	Open Next	∇
	· · · · · · · · · · · · · · · · · · ·	\sum

Undo limitations – just ONE step:

 There used to be a "multi undo" option, unfortunately it doesn't show up in recent FiJi / ImageJ versions.

 While generating a work flow for image analysis use the "Duplicate" (Shift-D) command to work on a copy.

While "playing" it is easy to get confused which operations you used, especially when trying to <u>reproduce</u> an intermediate result.

 \rightarrow The "Recent commands" window shows them in reverse order.

× □ Recent Commands				
Recent Commands:				
Find Commands				
Close				
3D Viewer				
T1 Head (2.4M, 16-bits)				
List Shortcuts				
Remove				
Update LOCI Plugins				
About ImageJ				
Frequently used Commands:				
Close				
Find Commands				
Channels Tool				
Rename				
List Shortcuts				
Duplicate				
Bio-Formats Importer				
RGB Color				
OK Cancel Options				

The LOCI tools provide a "shortcut" window containing buttons for the individual tools – very useful if you need to access them often in your work flow.

Open the window in Plugins > LOCI > LOCI Plugins Shortcut Window



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Instead of navigating through the tedious menus, ImageJ provides the "Control Panel" which shows the complete menu structure in a collapsable tree view – sub-menus stay open an can be undocked for quick access.

Open it via Shift-Ctrl-U or Plugins > Utilities > Control Panel.

A very fast and handy way is to use the command finder. Just press "L" on the keyboard and start typing right away:



A more sophisticated way to adjust ImageJ to your needs is provided by the "ActionBar" Plugin that creates graphical toolbars with big icons:



While this is a very nice tool it unfortunately doesn't have a nice way to create or modify the Action Bars – this requires manually editing the definition text file of a bar.

http://imagejdocu.tudor.lu/doku.php?id=plugin:utilities:action_bar:start

Hands-on session