ImageJ / FiJi Workshop 2012

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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 → source code available
- Java based → Win, Mac, Linux, ...
- Macros & plug-ins
- Huge potential, very active community
- Technical design limitations
  - 15 yrs. old, usability somewhat “aged”

→ 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

→ 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: http://imagejdocu.tudor.lu/

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

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

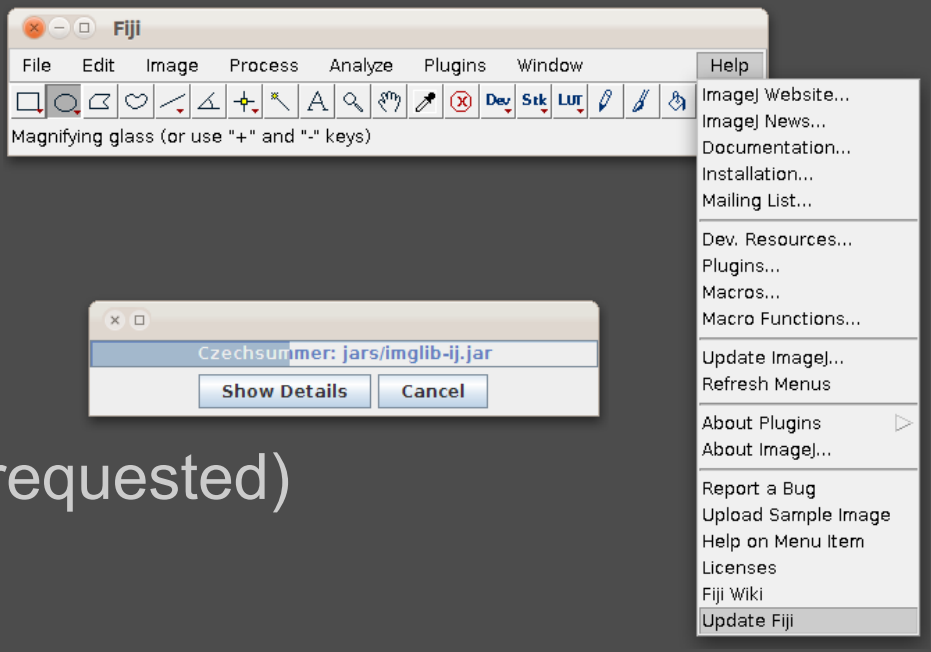
FiJi download: http://fiji.sc/wiki/index.php/Downloads

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

FiJi – getting started

First installation on Windows:

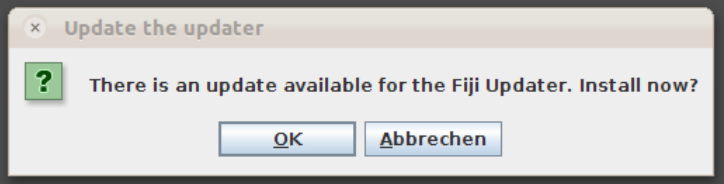
- Download from http://fiji.sc/
- If possible, use 64bit version
- Choose a writeable location
  - NOT “C:\Program Files\...” !
  - NOT on a network share (works, but slow!)
  - e.g. “D:\Tools\”
- Unzip
- Start & run updater
  - …

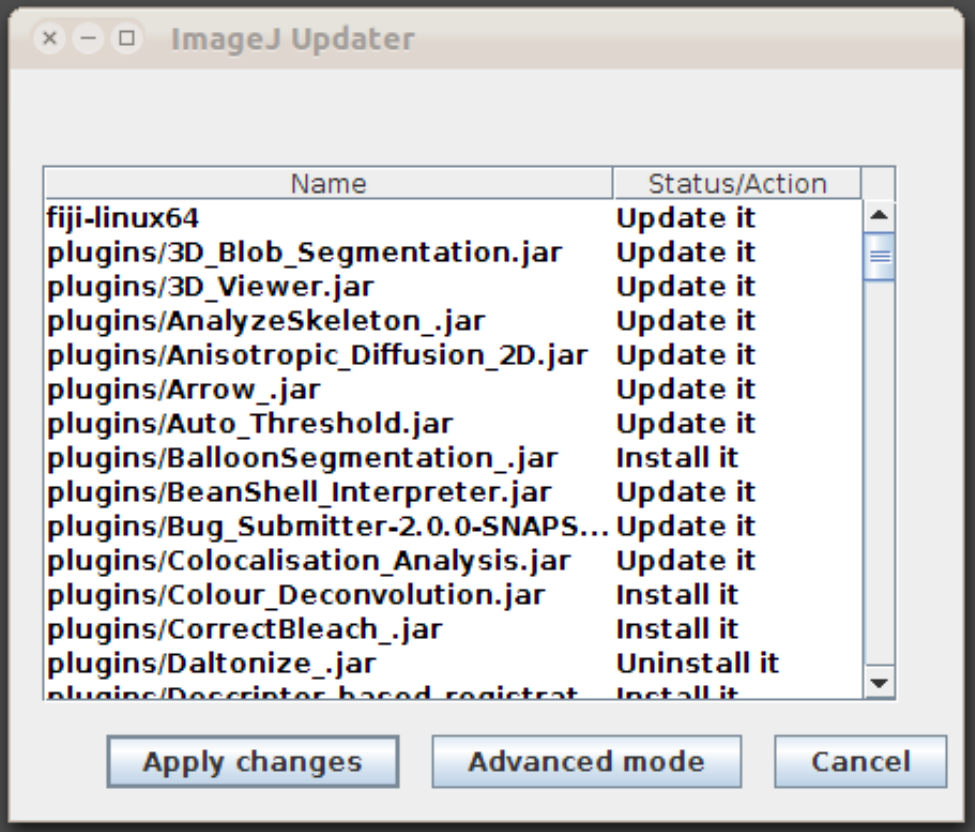


→ Do NOT use “Update ImageJ” (unless explicitly requested)

Updating FiJi

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





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

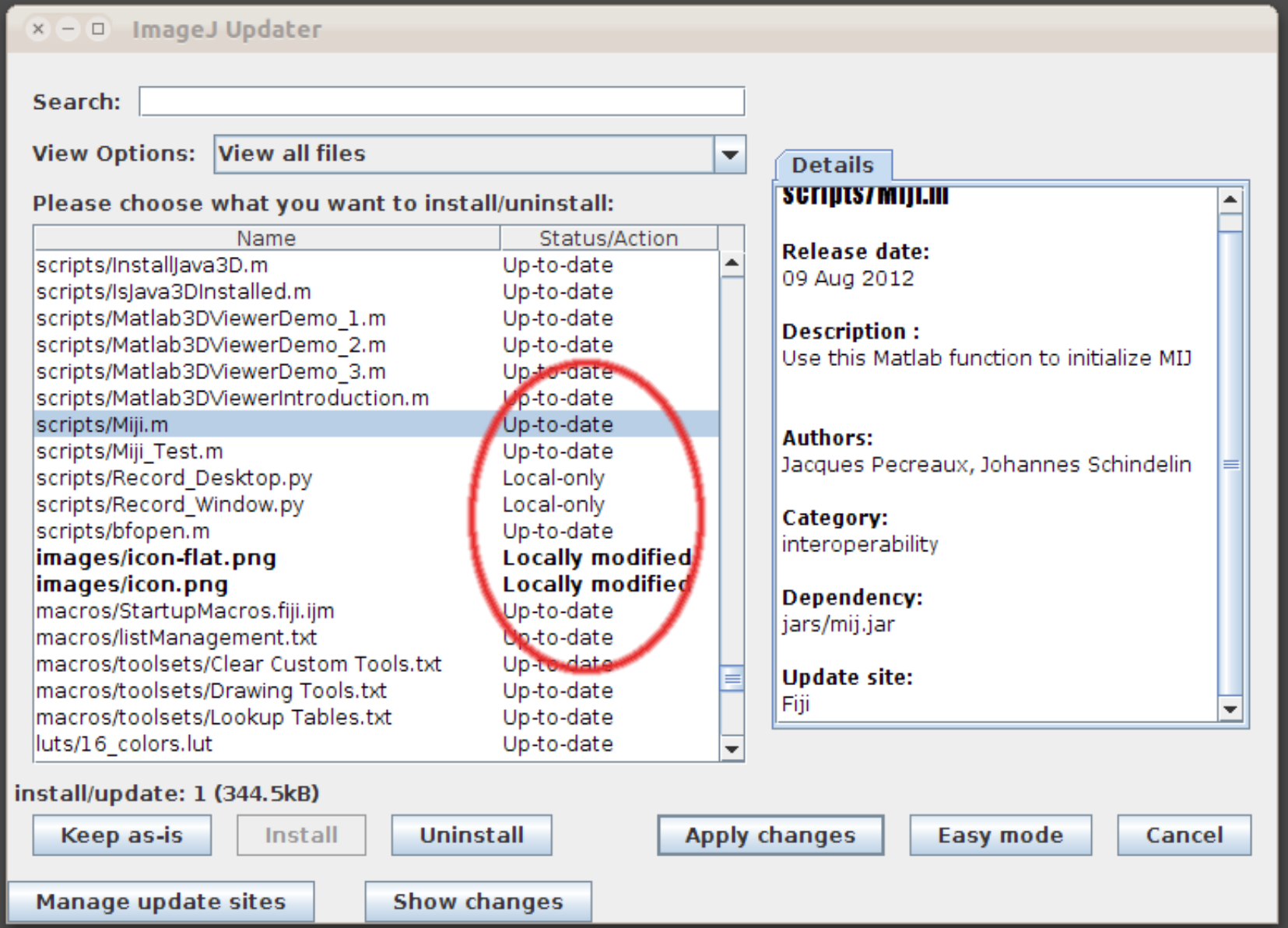
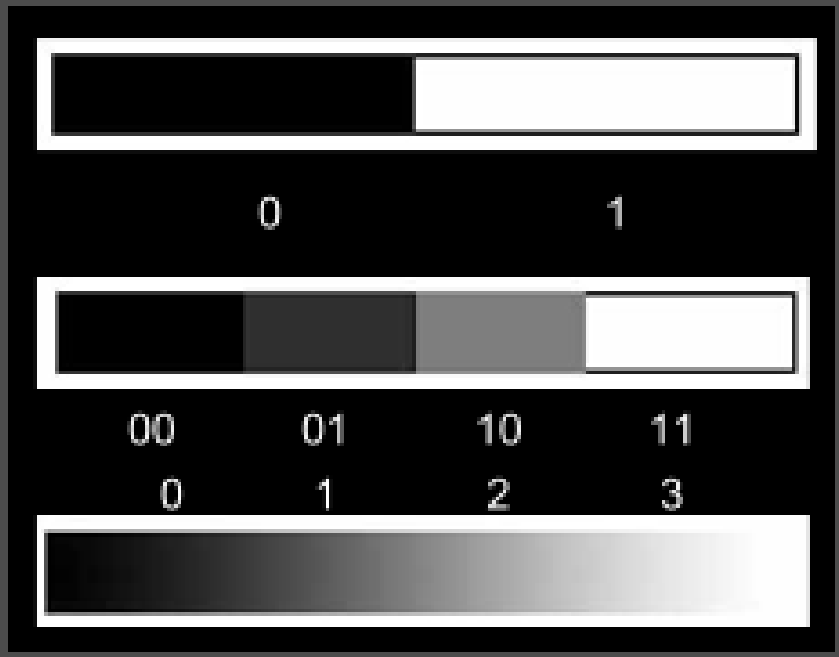


Image Types I

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 → 0 or 1 → 2 steps
- 2 bit = 2^2 → 4 steps
- 8 bit = 2^8 → 256 steps
- 16 bit = 2^16 = 65536
- 32 bit = 2^32 > 4 million



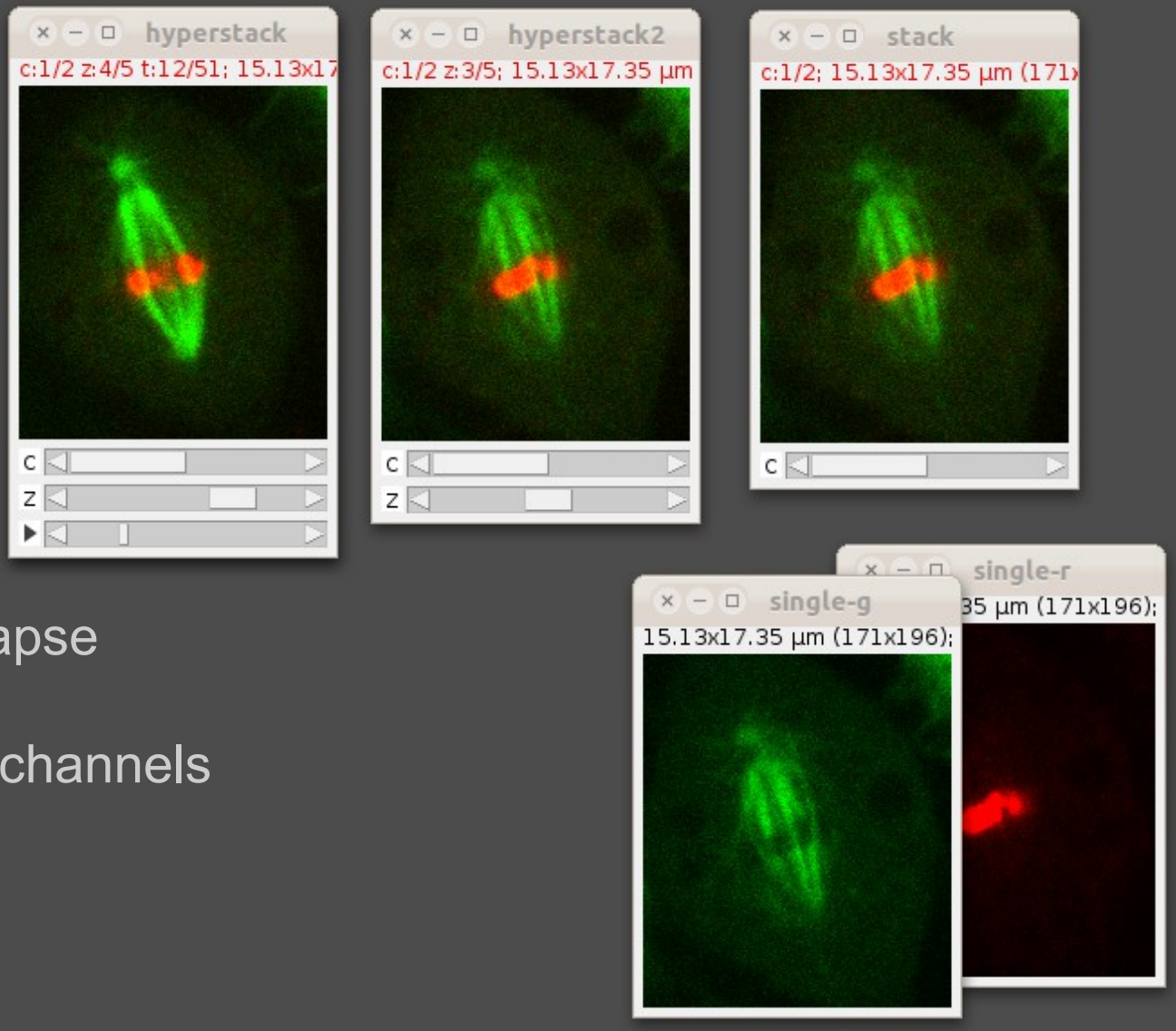
Special case “RGB” = 8 bit for each, Red Green and Blue (24 bit overall)

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
  – *not* limited to just 3 channels
  – ...



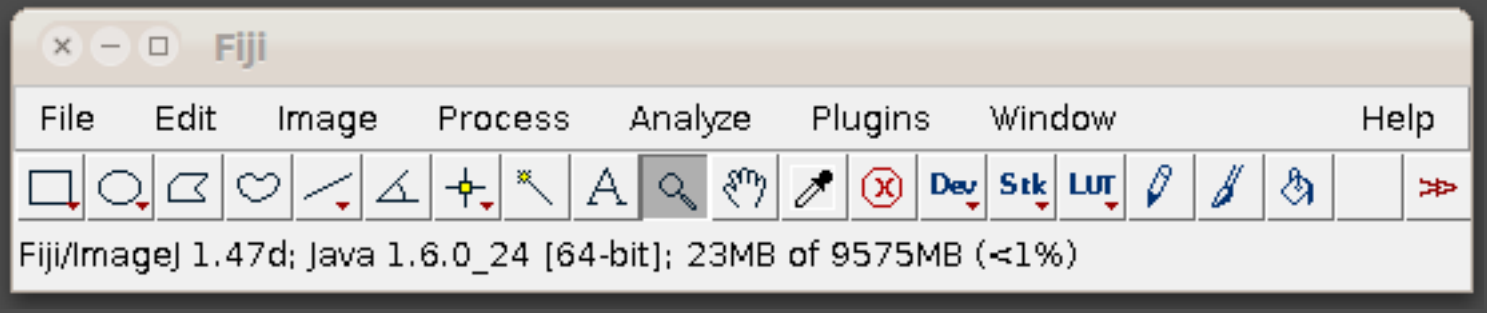
Colors

Several ways of dealing with colors exist in ImageJ:

- No additional information → 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) → see later.

The user interface



The main window has three parts:

- Menu bar
- Tools
- Status line
  - 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)

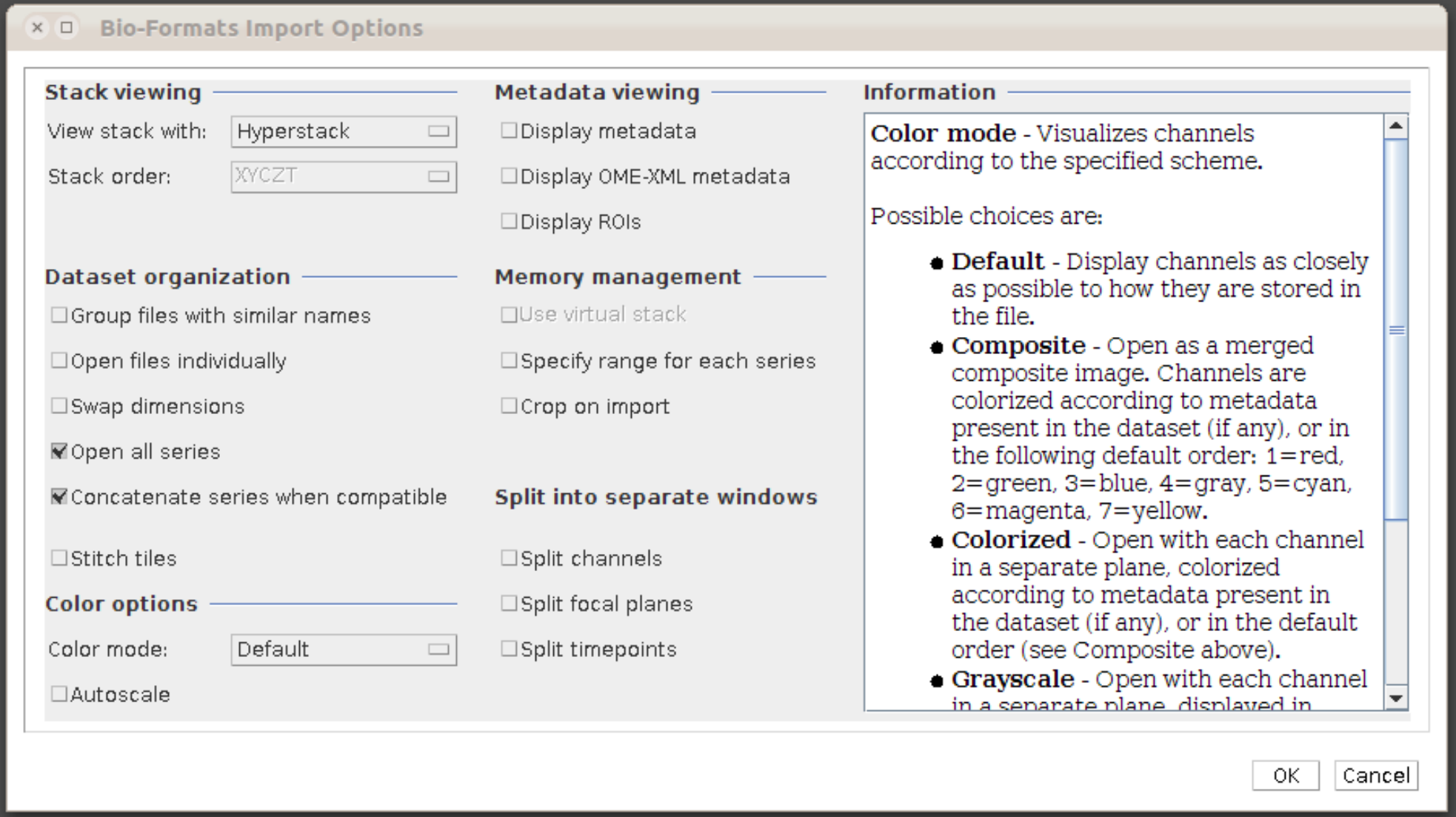
- Tries to guess the applicable reader (no choice)

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

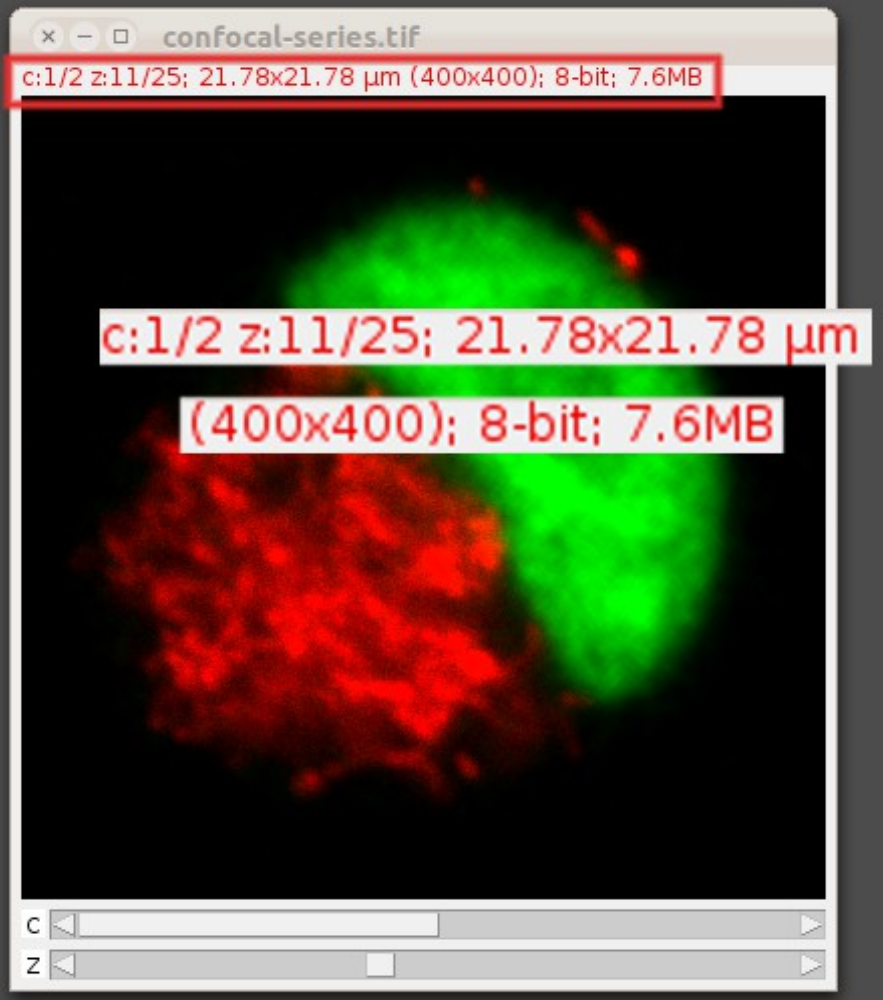
- File > Import > Bio-Formats (at the bottom)
- Plugins > LOCI > Bio-Formats Importer

→ Bio-Formats is the preferred way for most proprietary file-formats.
http://loci.wisc.edu/bio-formats/formats

Opening Images with Bio-Formats



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

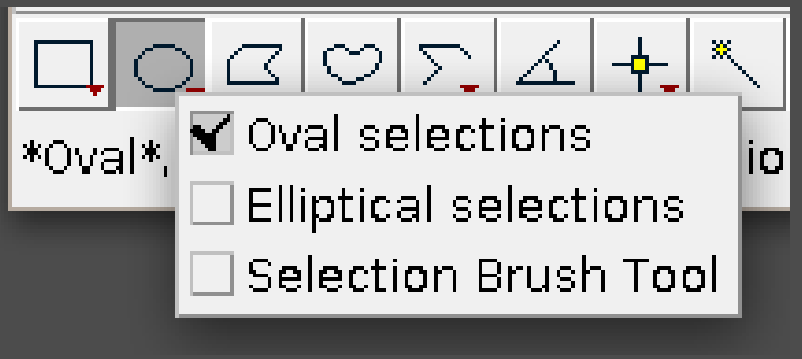
Status bar in main window shows intensities of ***current*** channel.

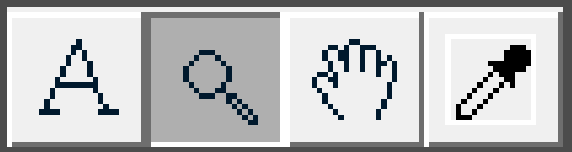
The Toolbar

Selection Tools:

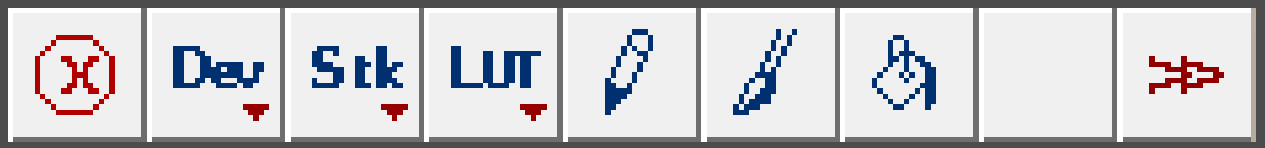
- Right click: choose variant
- Double click: properties

→ select region(s) of interest





- Text tool
- Zoom & pan
- Colorpicker

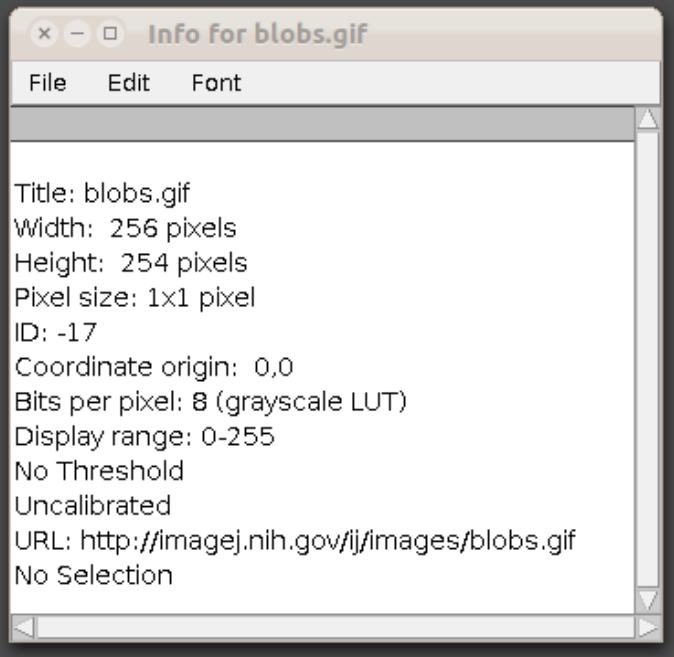


User-configurable toolsets

Image calibration

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

Image > Show Info



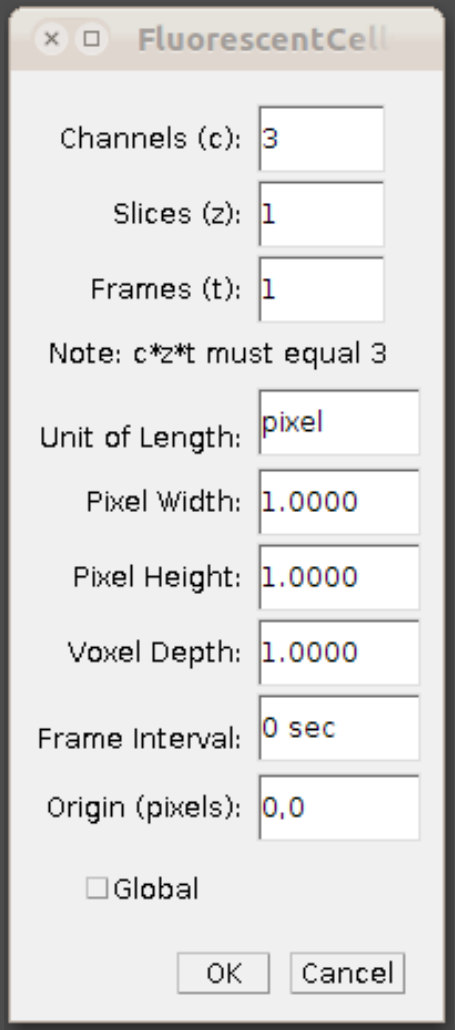
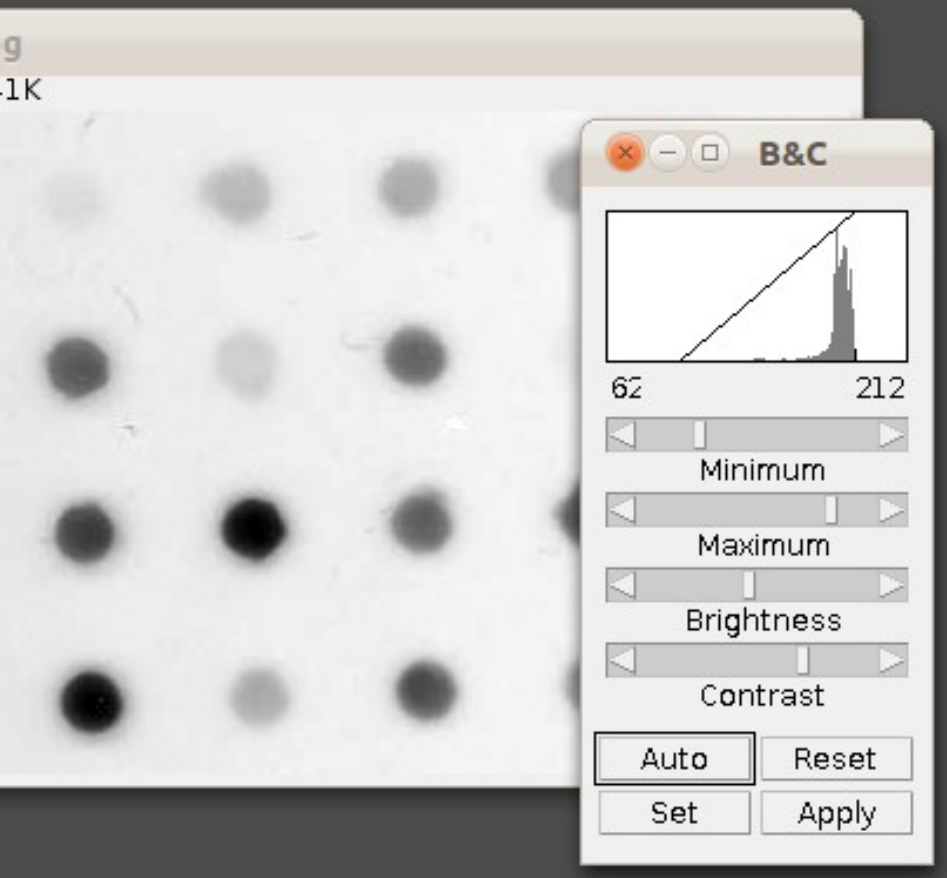


Image > Properties

Display Settings



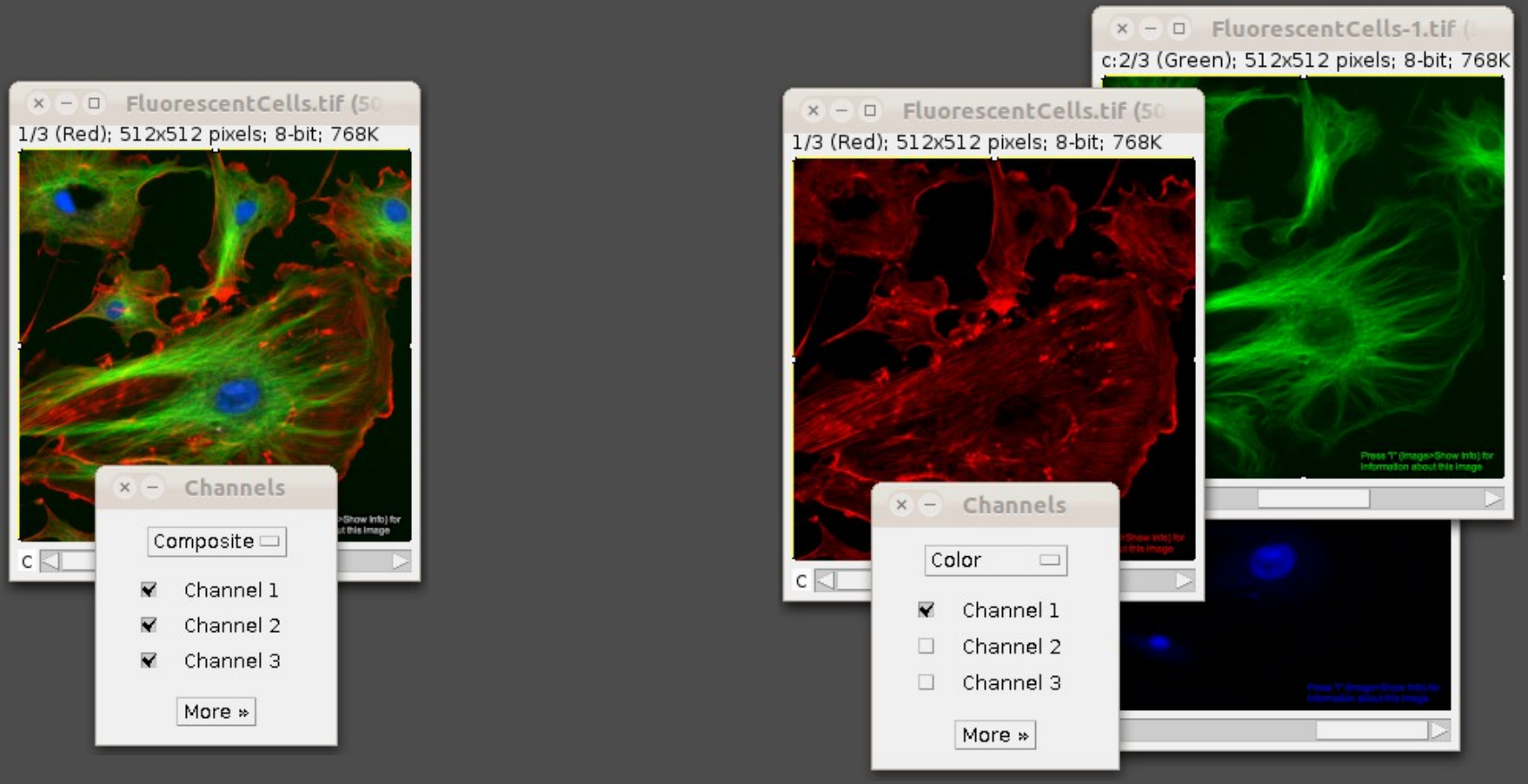
Use Image > Adjust > “Brightness / Contrast” to set the display minimum and maximum values.

→ “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.



Lookup Tables

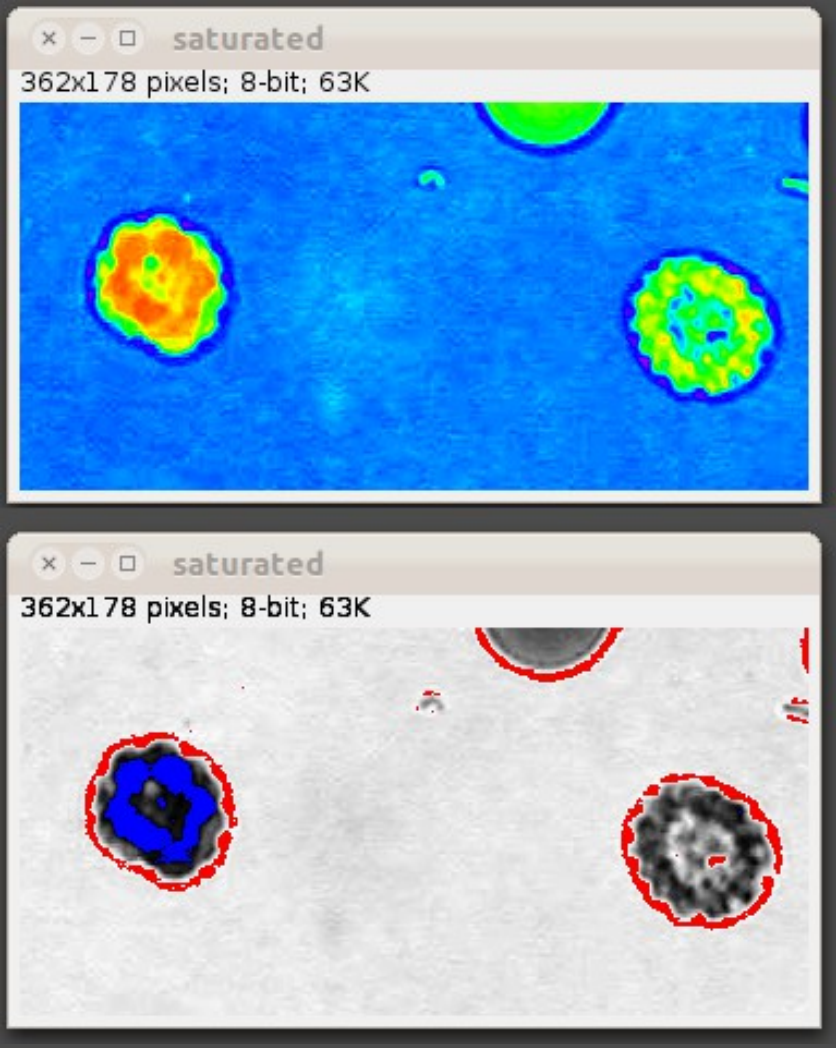
LUT's are ***filters*** that are applied before the image is displayed on the screen.

- 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

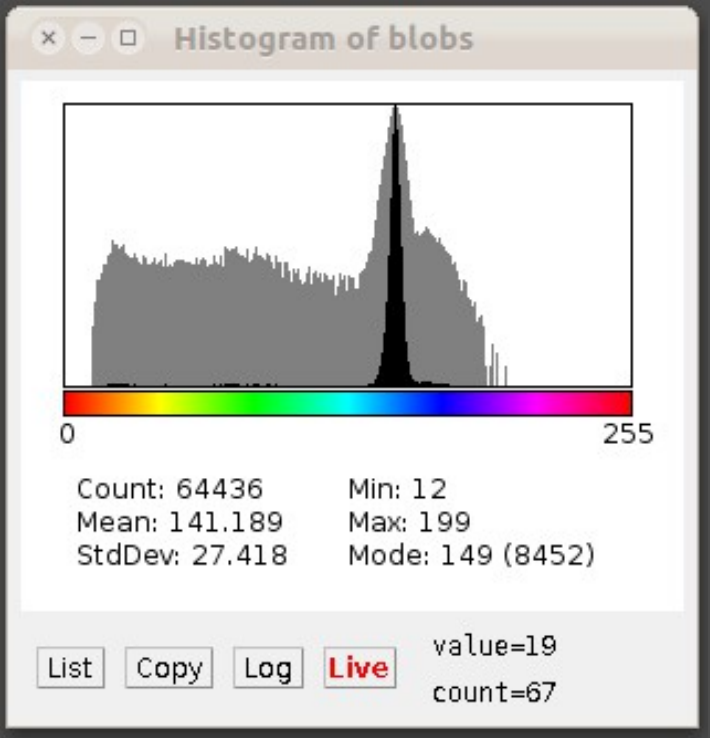


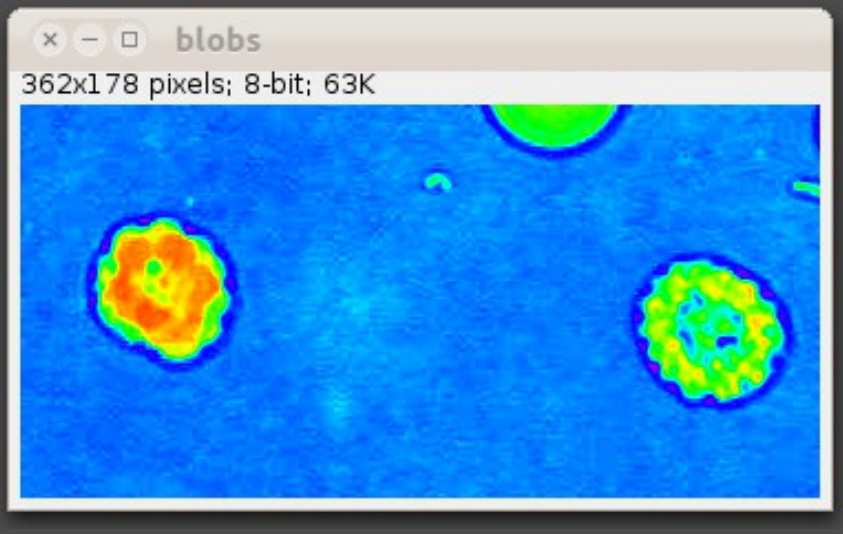
Histogram

Analyze > Histogram

Shows intensities distribution.

- use “Log” mode
- honors LUT
- Statistics
- Interactive



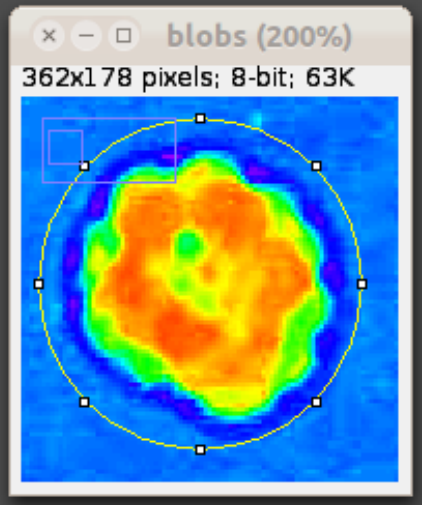


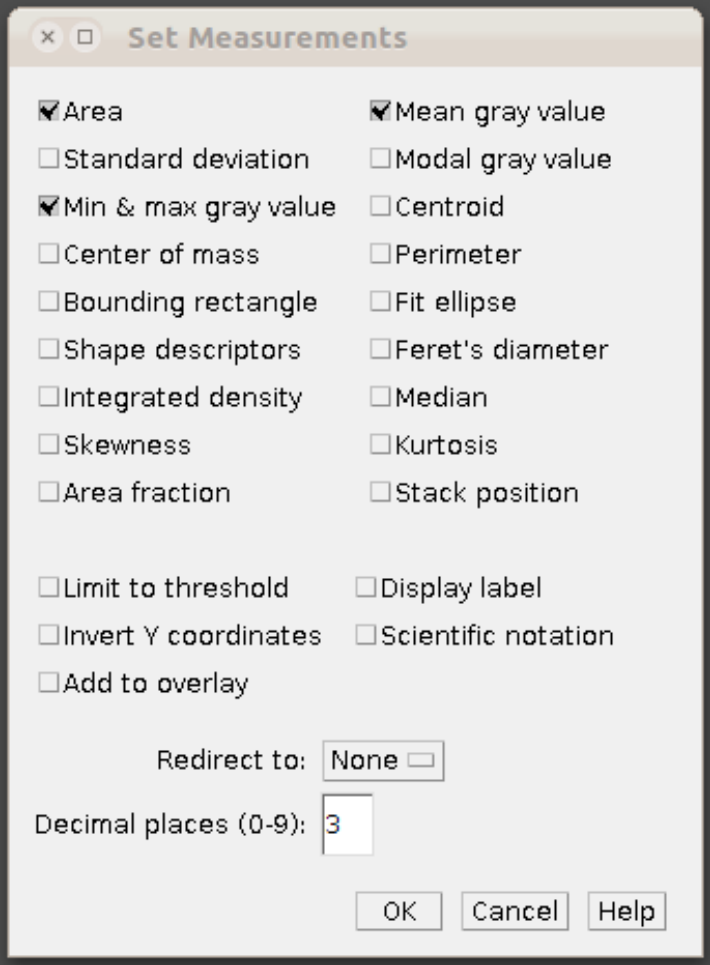
Measuring

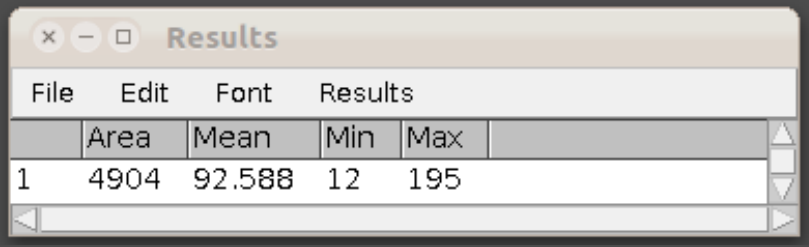
ImageJ provides plenty of information for selected regions.
To measure, just press “m” and the selected results will show up in a new window.

→ Types can be configured in Results > Set Measurements

→ can be saved as txt or csv file





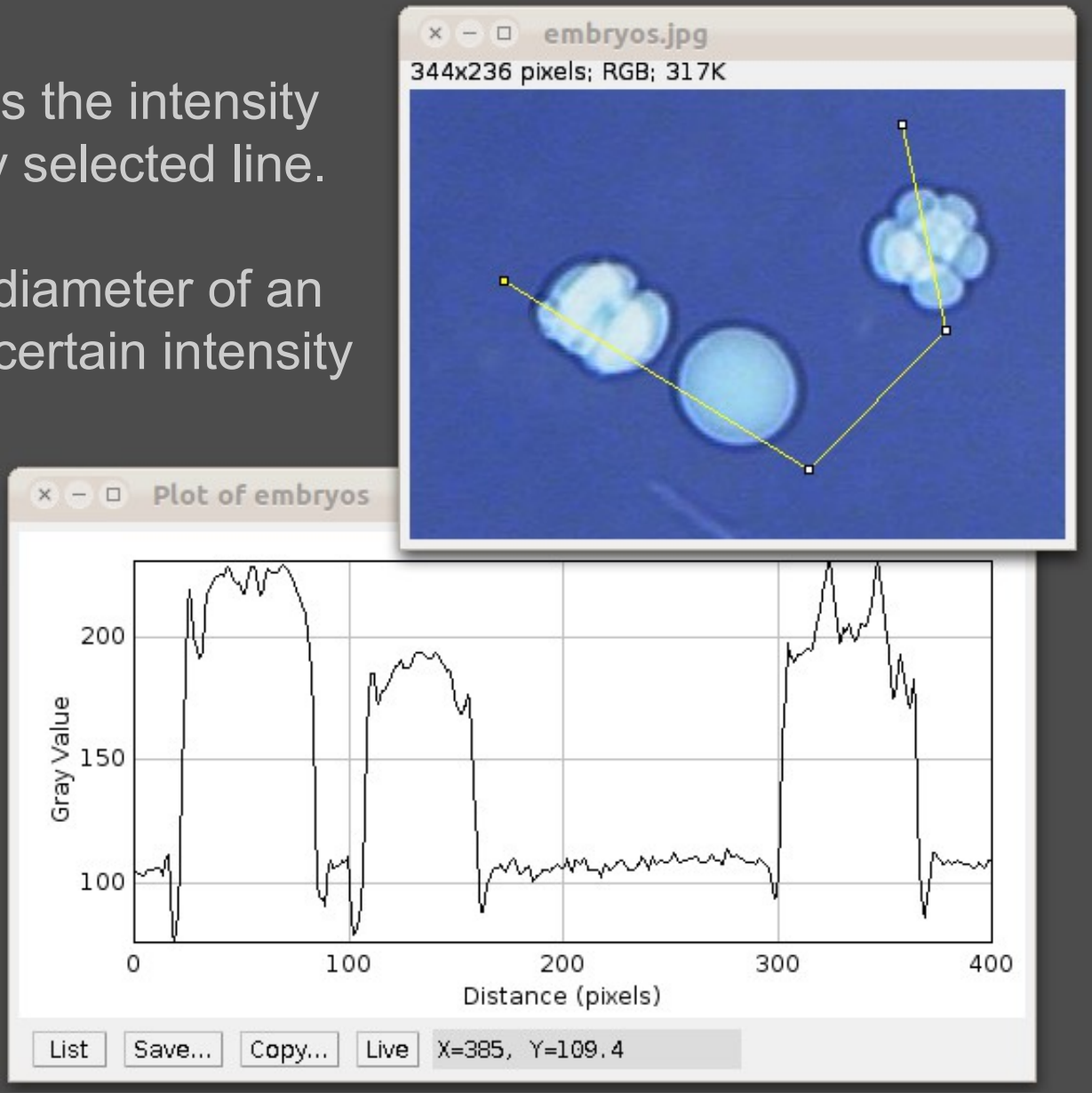


Distance / Profile Plots

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.

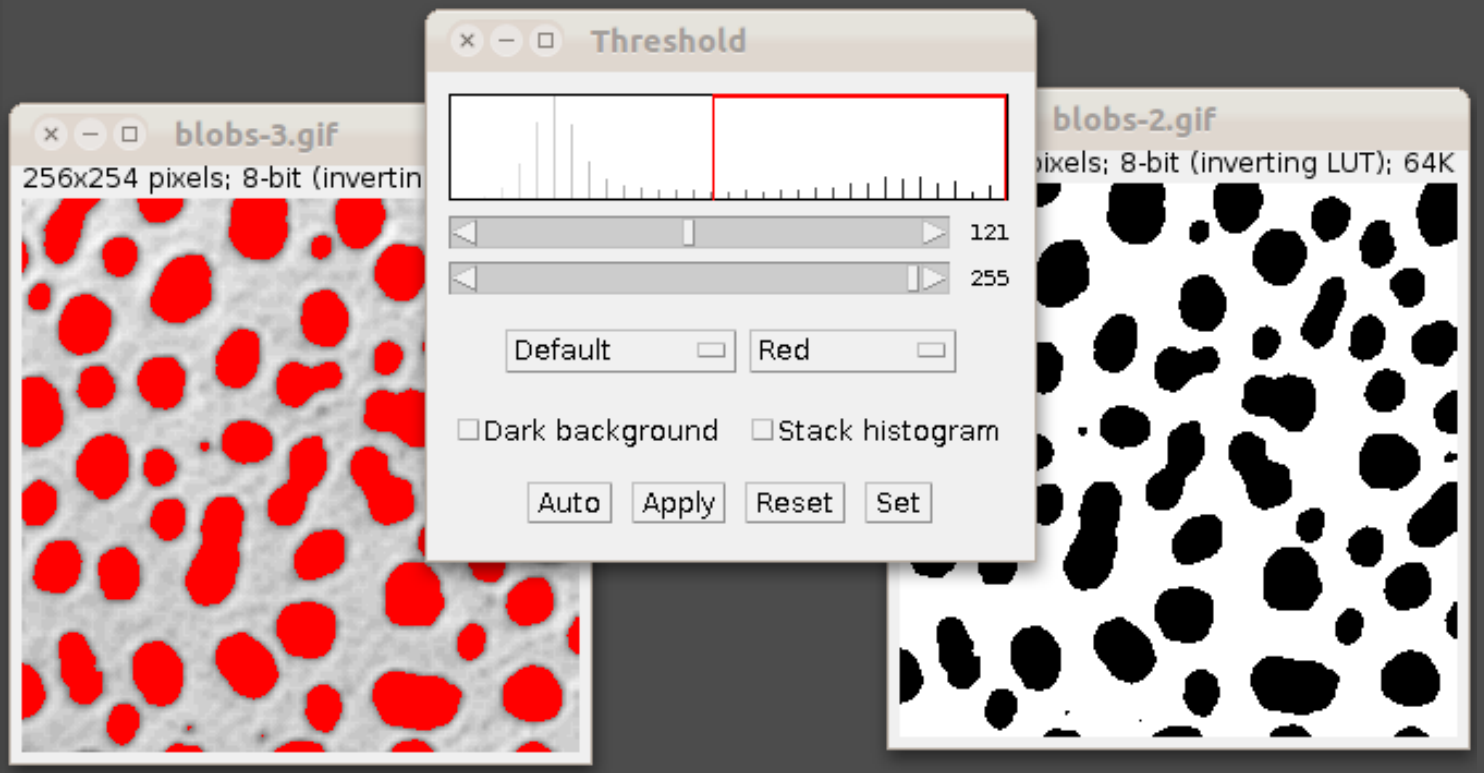
The Plot itself is again an image where, so measurements can be used on it again.



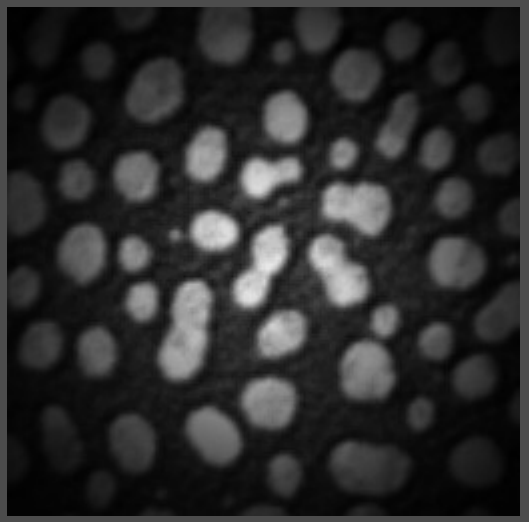
Thresholding

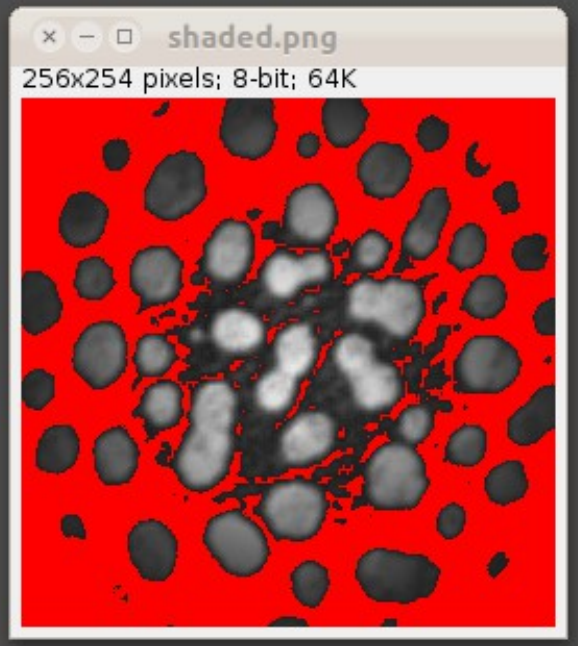
Simplest approach for segmentation:

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



Local Thresholding





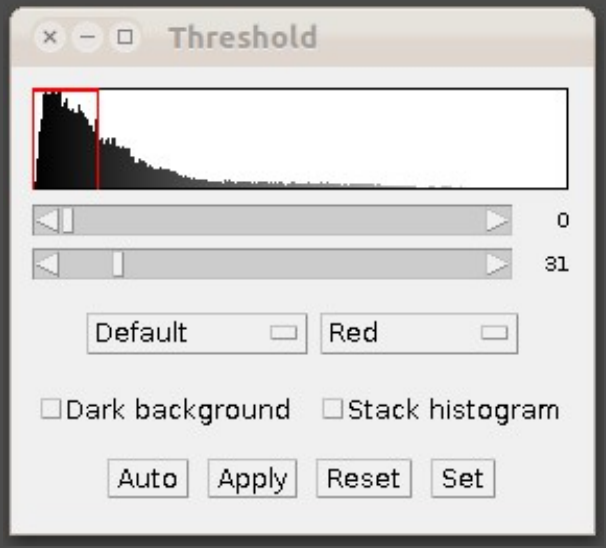
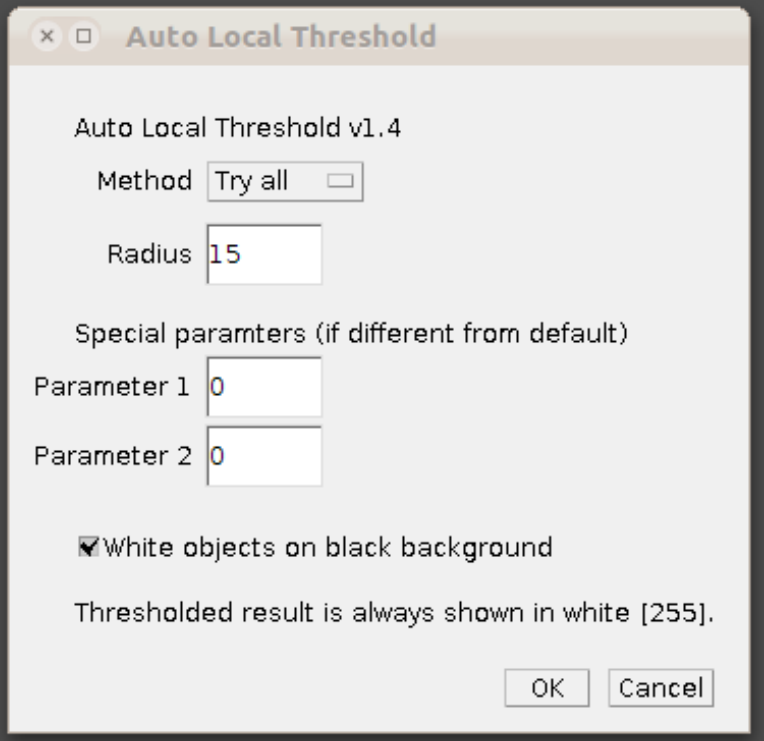


Image
> Adjust
> Auto Local Threshold

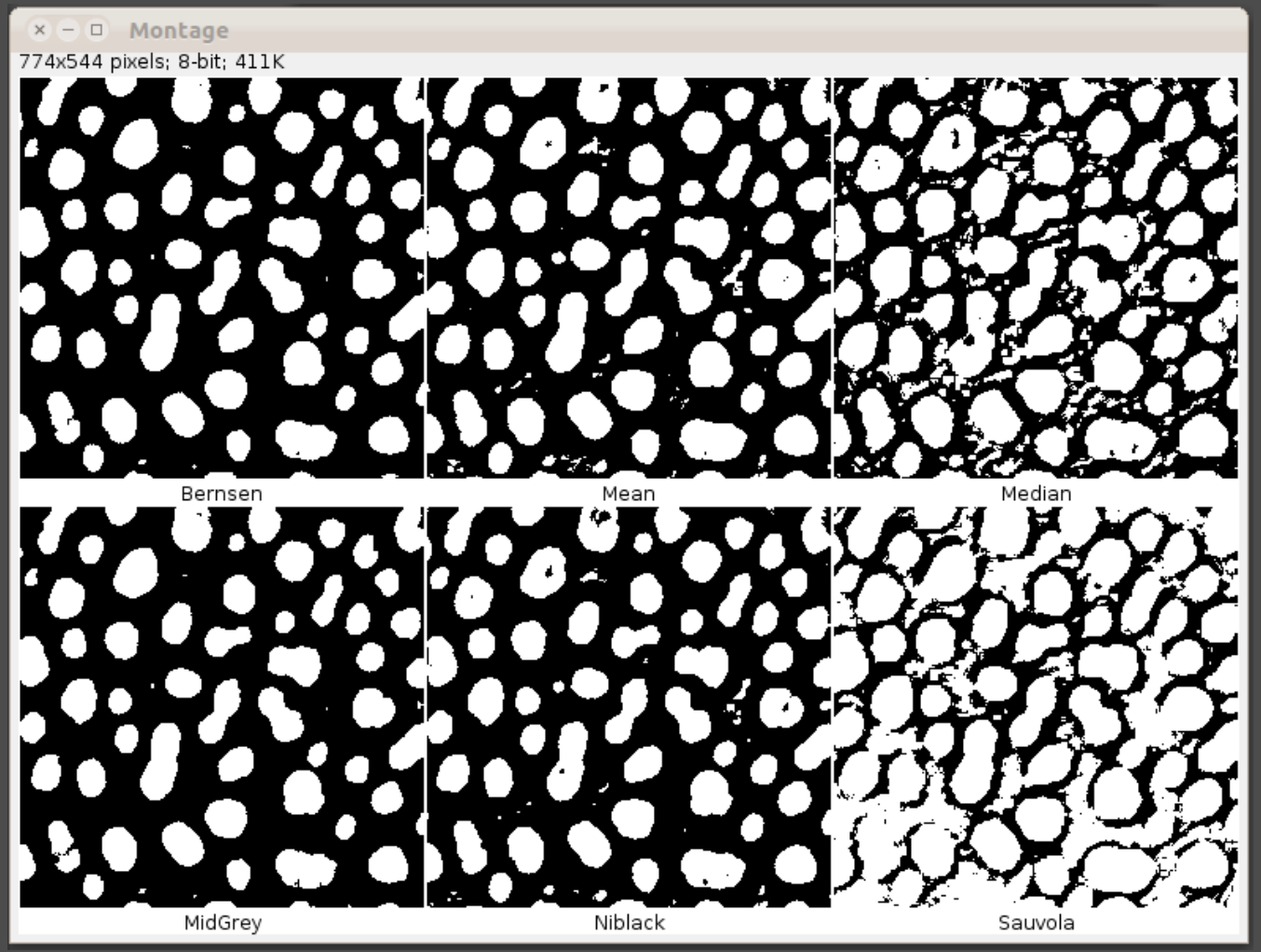


Local Thresholding

Displays results for all local thresholding methods.

→ Try various settings until results are good.

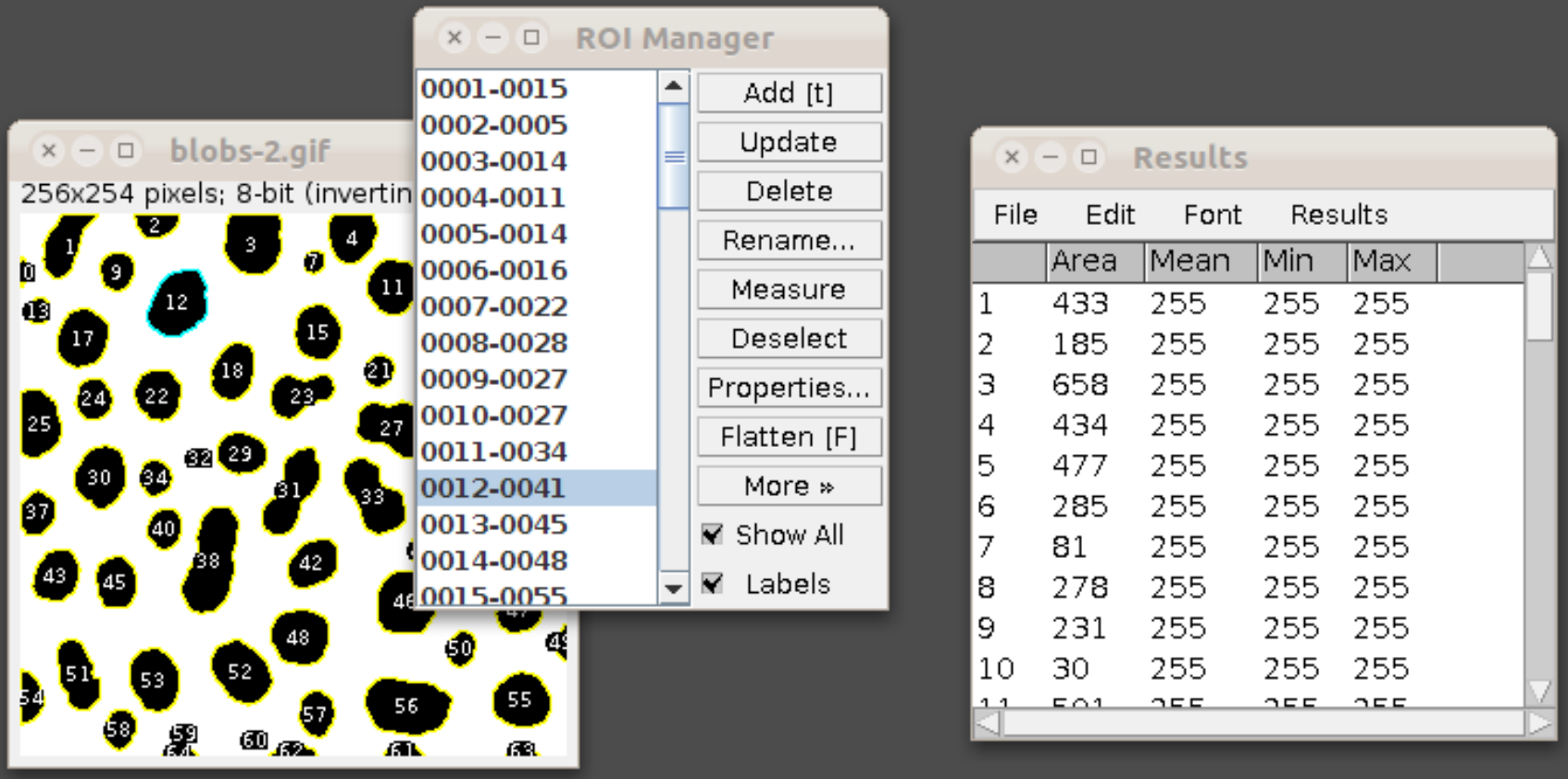
→ Run again and select the desired method instead of “all”.



Analyze Particles

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

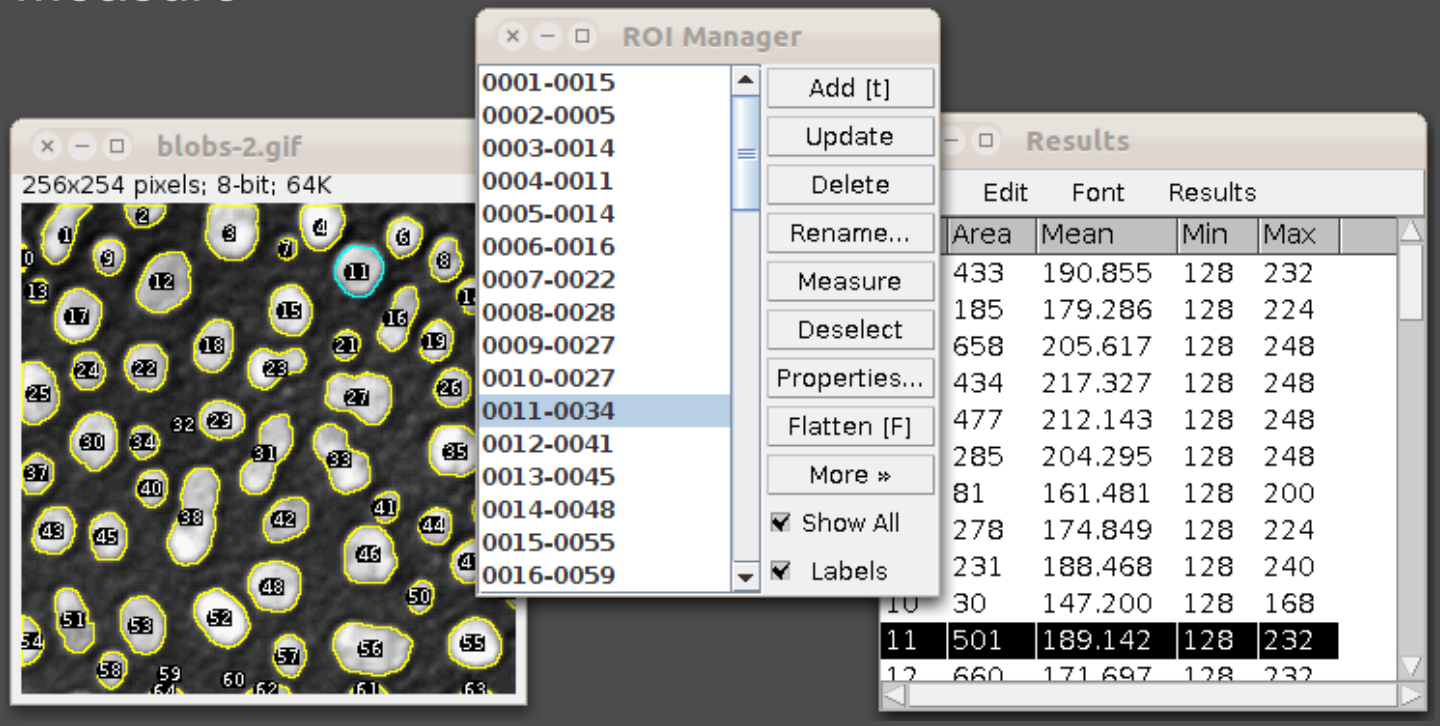
- Results and ROI Manager is shown



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

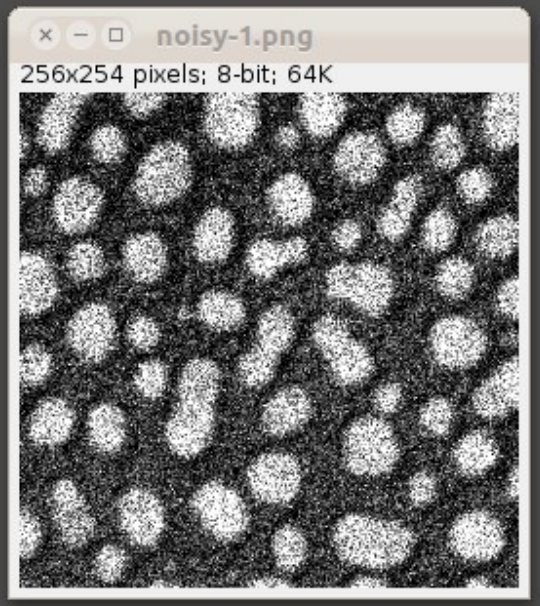


Touching Objects

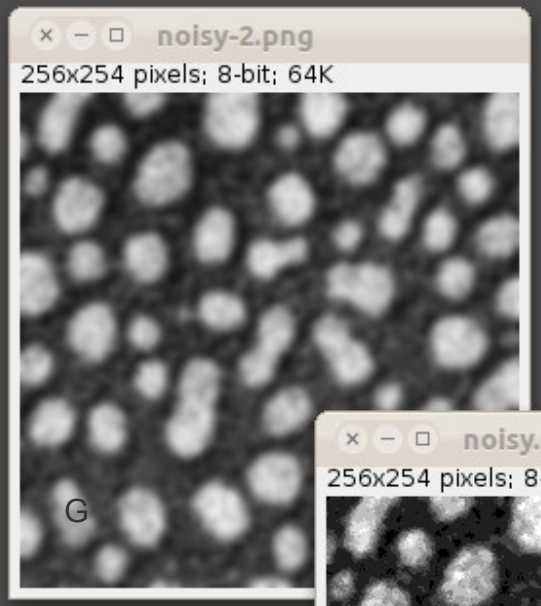
Use Process > Binary > Watershed to split touching objects in a binary image. Analyze Particles has to be re-run afterwards.

Filtering

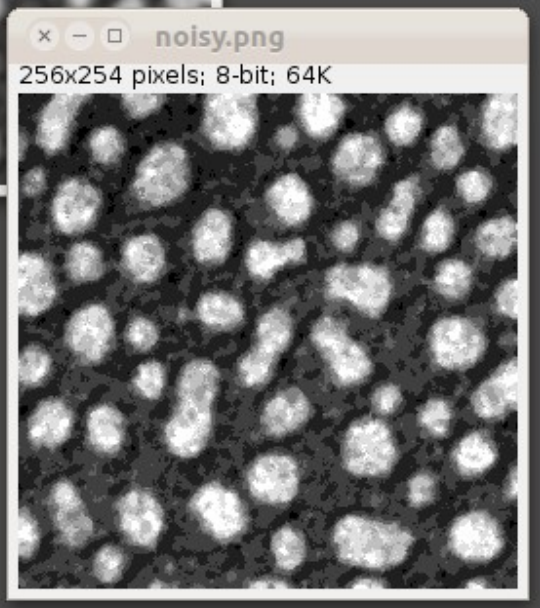
Located in Process > Filters



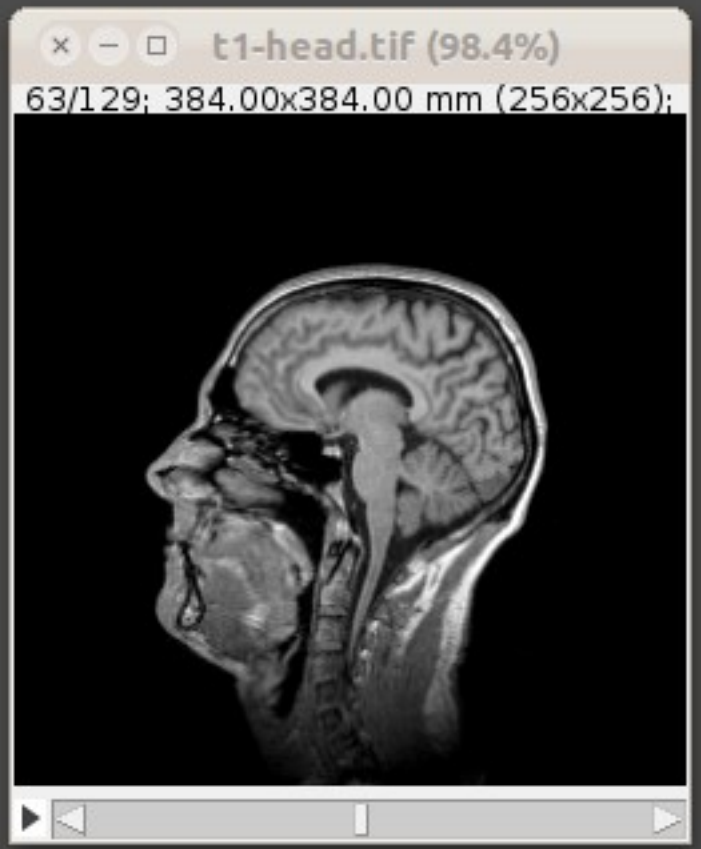
Gaussian Blur

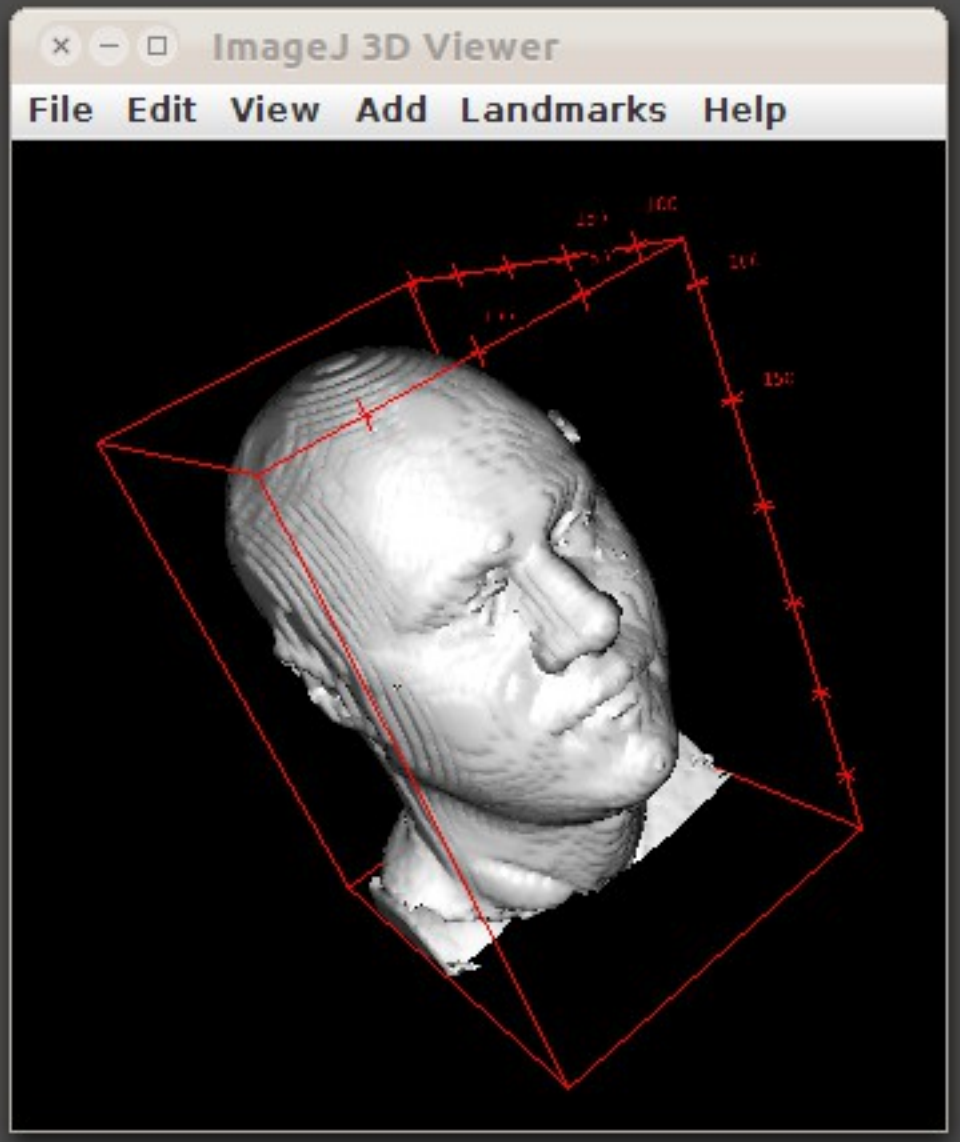


Median



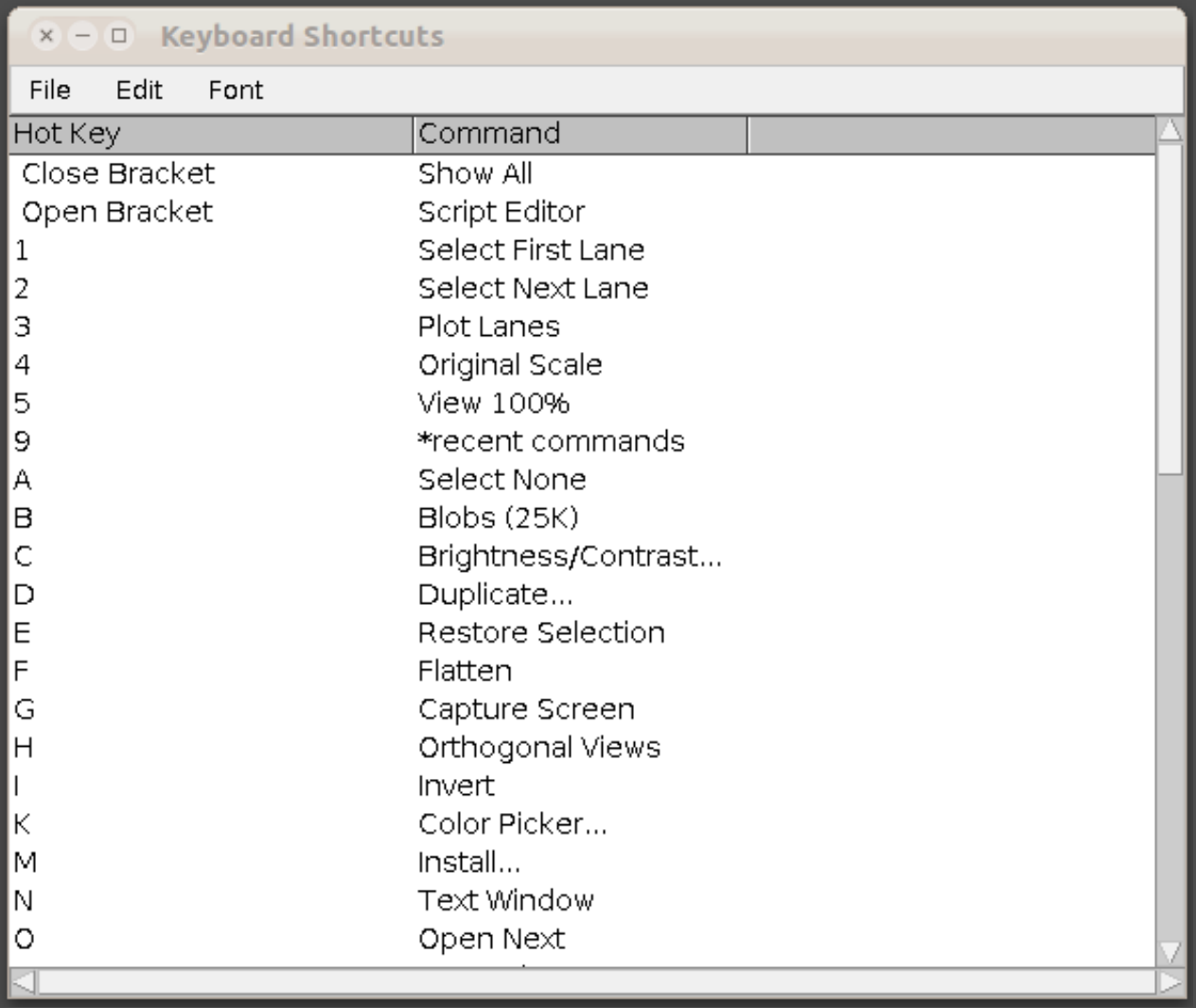
3D Viewer


t1-head.tif (98.4%)
63/129; 384.00x384.00 mm (256x256);


ImageJ 3D Viewer
File Edit View Add Landmarks Help

Usability

Plugins > Shortcuts > List Shortcuts...



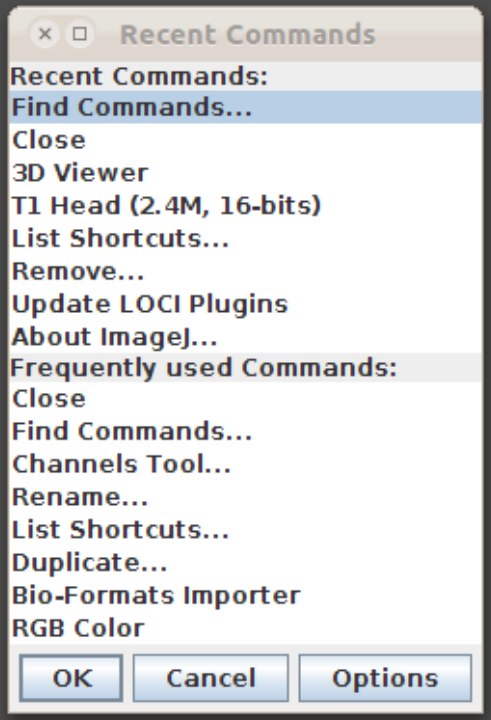
Usability

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 reproduce an intermediate result.

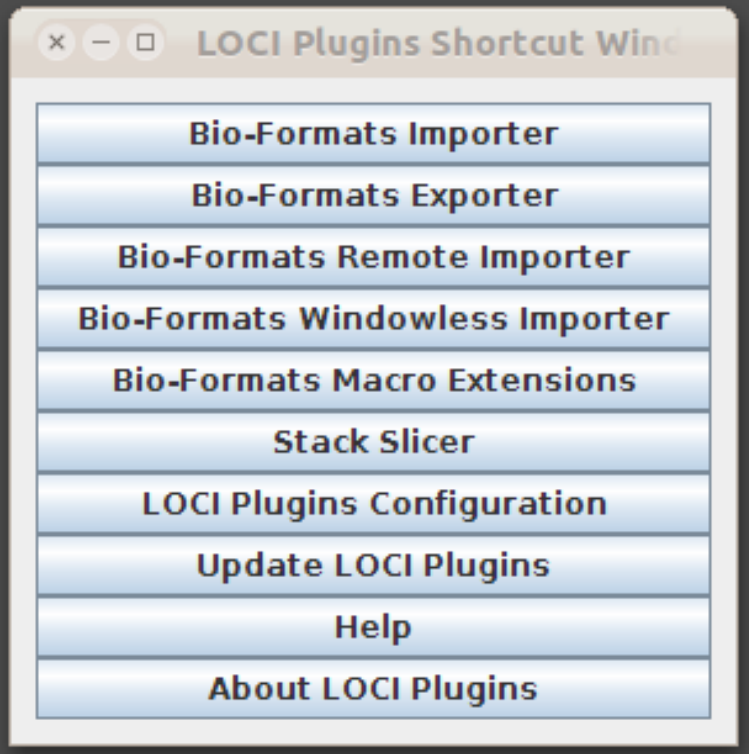
→ The “Recent commands” window shows them in reverse order.



Usability

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

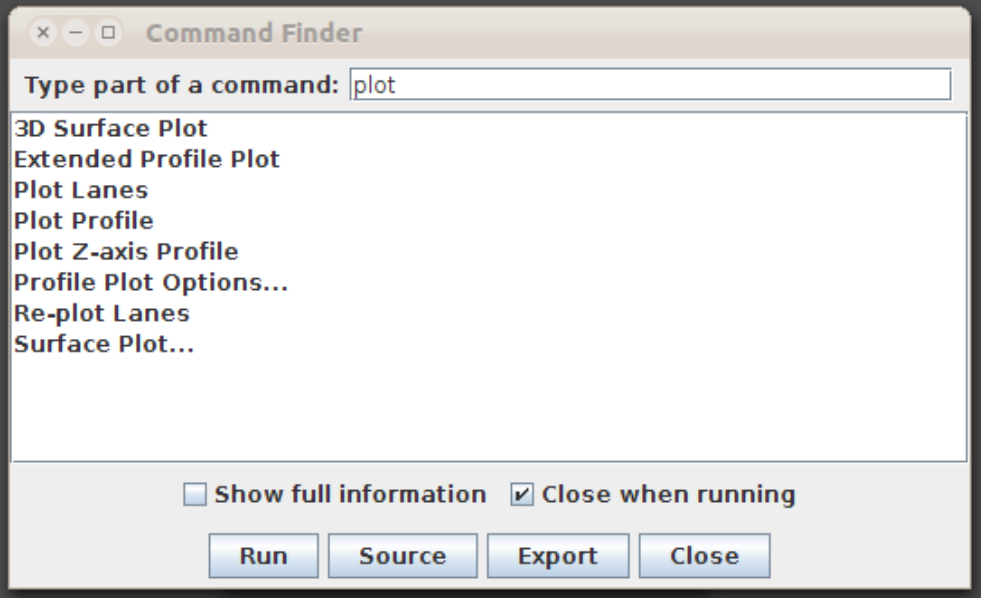


Usability

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.

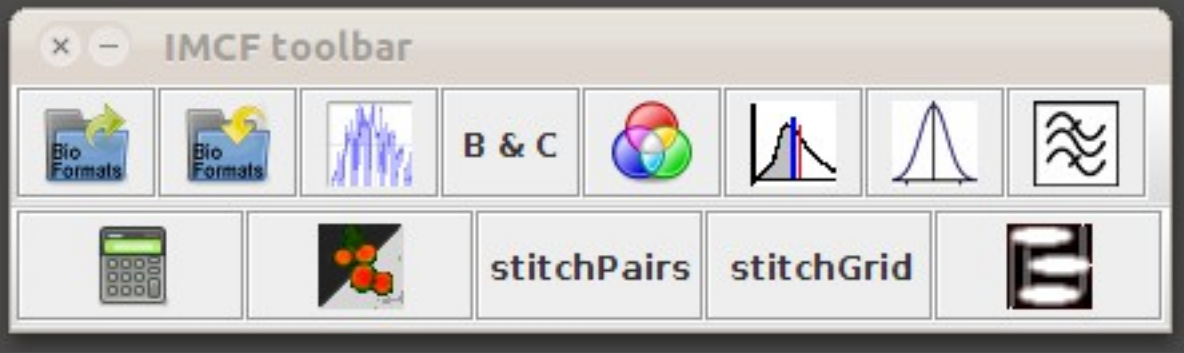
Open it via Shift-Ctrl-U or Plugins > Utilities > Control Panel.

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



Usability

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