LSM Reader 3.2d ImageJ Plug-In
Instructions Manual

Summary

1. ImageJ quick overview.

ImageJ is a free, open source, imaging software. For those who do not know ImageJ, it has similar features as the well-known software NIH Image. In fact, ImageJ can be considered as the exact copy of NIH Image, but written in Java language, thus available on any platform supporting java virtual machine including Windows, MacOs, and UNIX.

1.1. Features.

Features taken from ImageJ's web site (http://rsb.info.nih.gov/ij/) :

ImageJ Features

Data Types:
8-bit grayscale or indexed color, 16-bit unsigned integer, 32-bit floating-point and 32-bit RGB color.

File Formats:
Open and save all supported data types as TIFF (uncompressed) or as raw data. Open and save GIF, JPEG and ASCII. Open BMP, DICOM and FITS. Open GIFs, JPEGs and raw data using a URL.

Speed:
ImageJ is the world's fastest pure Java image processing program. It can filter a 2048x2048 image in 0.5 seconds. That's over 8 million pixels per second!

Plugins:
Extend ImageJ by developing plugins using ImageJ's built in command recorder, editor and Java compiler. Many example plugins are provided.

Toolkit:
Use ImageJ as a image processing toolkit (class library) to develop new applets and applications.

Image display:
Tools are provided for zooming (1:32 to 32:1) and scrolling images. All analysis and processing functions work at any magnification factor.

Regions of Interest:
Create rectangular, elliptical or irregular regions of interest (ROIs). Draw, fill, clear, filter or measure ROIs. Transfer an ROI to another image.

Image Enhancement:
Supports smoothing, sharpening, edge detection, median filtering and thresholding on both 8-bit grayscale and RGB color images. Interactively adjust brightness and contrast of 8, 16 and 32-bit images.

Geometric Operations:
Crop, scale, resize and rotate. Flip vertically or horizontally.

Analysis:
Measure area, mean, standard deviation, min and max of ROI or entire image. Measure lengths and angles. Use real world measurement units such as millimeters. Calibrate using density standards. Generate histograms and profile plots.

Editing:
Cut, copy or paste images or selections. Paste using AND, OR, XOR or "Blend" modes. Add text, arrows, rectangles, ellipses or polygons to images.

Color Processing:
Split a 32-bit color image into RGB or HSV components. Merge 8-bit components into a color image. Convert an RGB image to 8-bit indexed color. Apply pseudo-color palettes to grayscale images.

Stacks:
Display a "stack" of related images in a single window. Process an entire stack using a single command. Open a folder of images as a stack. Save stacks as multi-image TIFF files.

1.2. Installing ImageJ.

To get ImageJ's latest version go to the ImageJ's website and select "download vx.xx ". Simply follow the instructions that fits you're current platform.

Figure 1: ImageJ's home page

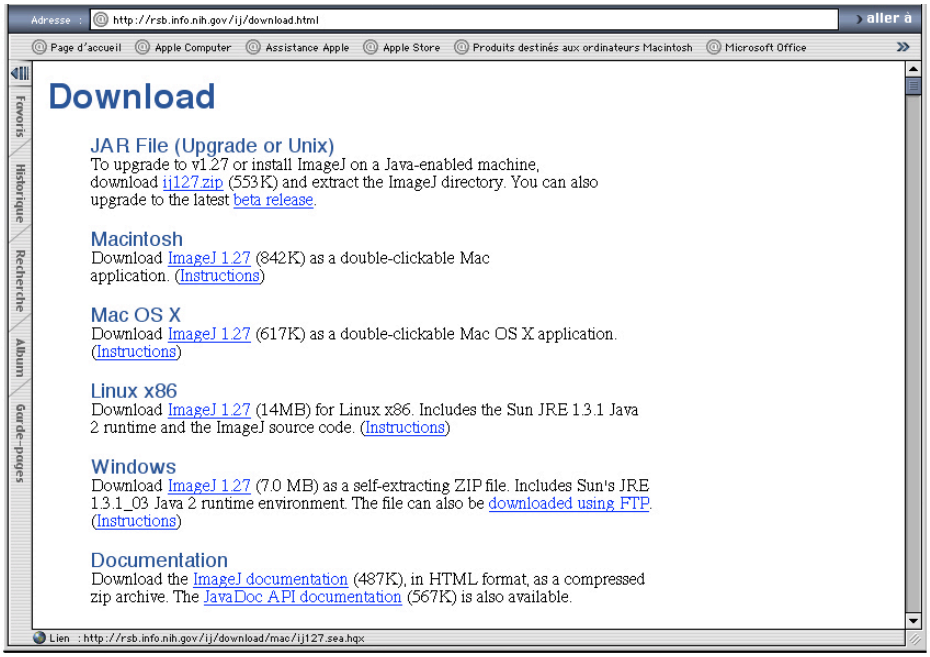

Figure 2 : Download page

1.3. Useful links.

ImageJ's web site:	http://rsb.info.nih.gov/ij/
NIH Image' web site:	http://rsb.info.nih.gov/nih-image/
CNRS-IBMP's microscopy pages :	http://ibmp.u-strasbg.fr/sg/microscopie/microscopie.html

2. LSM Reader 3.2d overview.

The LSM Reader 3.2d plug-in for ImageJ enhance ImageJ with the ability to open image files acquired with LSM 510 v3.2 from Carl ZEISS. It requires ImageJ version 1.30 or later.

Here are the LSM Reader 3.2d features:

LSM_Reader
Reads *.lsm files of version up to 3.2
Summarized CZInfo window
Detailed SCANINFO window
Recovers timestamps, z-stamps and lambda stamps, if present
Restores original acquisition color for each channel
Reads 8 bit and 12 bit datasets
Reads 4D datasets as Hypervolumes
Direct interaction with helper plugins Hypervolume Browser and LUT Panel
Compatibility with HandleExtraFiletypes plugin
Batch conversion of lsm files to jpg, bmp, or tif format

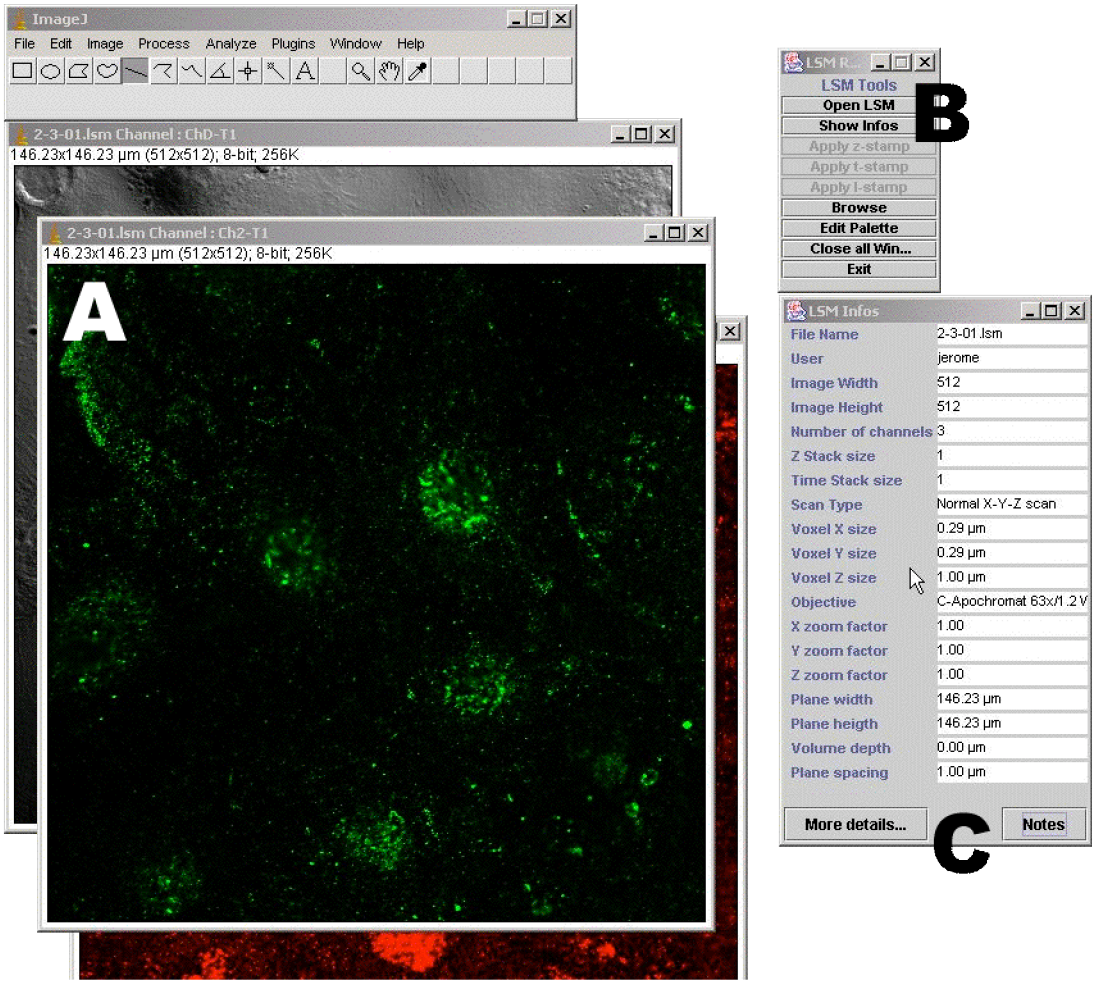

Figure 3: LSM_Reader v3.2d, A : Channels visualization windows, B : LSM Tools Panel, C: Summarized scan infos window.

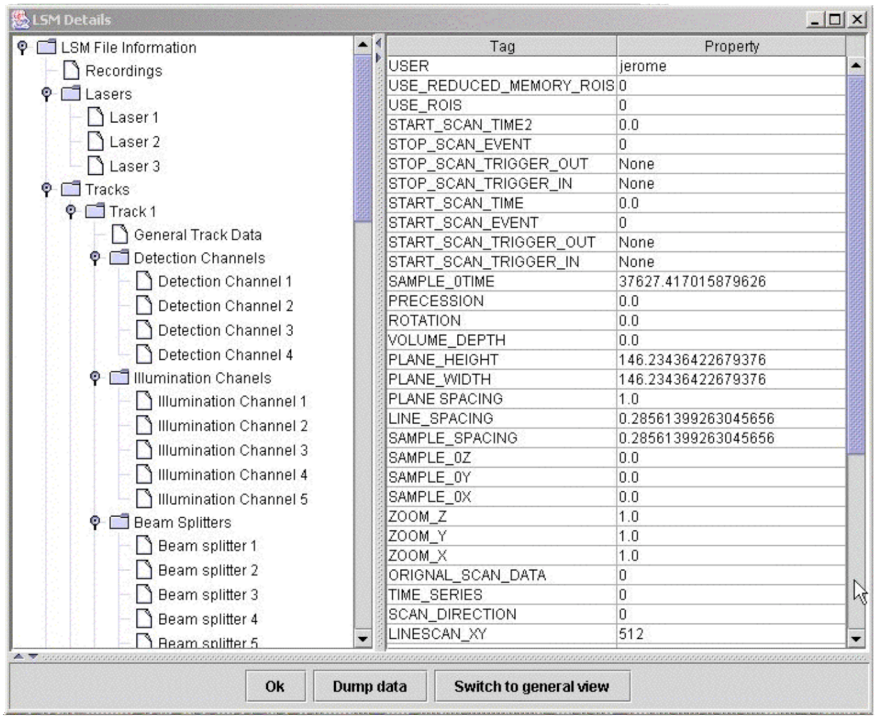

Figure 6: Detailed scan infos window.

This filtered view displays only active scan parameters.

Switching to general view will show all present scaninfo in the lsm file, regardless of their activation upon acquisition. This probably means lots of useless values.

Dumping data to a text file lets you make a comprehensive printout of all present acquisition parameters. This might be a useful to pass over to a colleague or to a Zeiss technical advisor to assess a given microscope setup configuration.

3. LSM Reader 3.2d installation instructions, step-by-step.

First of all you have to get the plug-in from ImageJ's plug-in page (http://rsb.info.nih.gov/ij/plugins/index.html). Alternatively, you can get it from the IBMP's Confocal Microscopy Platform pages (http://ibmp.u-strasbg.fr/sg/microscopie/microscopie.html).

The plug-in file can be found in *.jar format containing the source code and all necessary classes.

Step 1

- Download LSM_Reader.jar file from ImageJ's plug-in web pages into your local ImageJ's plug-in folder.

Step 2

- Restart Image J and select LSM_Reader from the Plugin list.

Congratulations! You have correctly installed the LSM Reader 3.2d plug-in. Now please have a look on the "LSM Reader usage " section.

4. LSM Reader 3.2d usage.

After having correctly installed the plug-in, you will have a new LSM_Reader command in the "Plug-In" menu; or in the menu specified during the plug-in installation process. Start LSM_Reader with this menu command or with the shortcut created during the plug-in installation process.

At the lower right hand side corner of your screen, the LSM Tools Panel is displayed.

Figure 7: The MacOsX LSM Tools Panel.

The buttons labels are self-explanatory.

Open LSM	Starts a file opening dialog to select an *.lsm file to open
Show Infos	Shows the scan informations window for the active lsm window
Apply z-stamp	Applies z-axis stamp to the image planes, at selection
Apply t-stamp	Applies time stamp to the image planes, at selection
Apply l-stamp	Applies lambda stamp to the image planes, at selection
Browse	Starts HyperVolume Browser plugin, if present. This option displays a modified Image window, with 1 slider per dimension.
Edit Palette	Starts LUT Panel plugin, if present
Batch Convert	Extracts image data from lsm files into jpg, bmp, or tif format
Close all windows	Closes all ImageJ windows, but this one
Exits	Exits LSM_Reader

Now you can simply use any of ImageJ's many features on the images or image stacks newly opened.

5. Advised plug-ins.

"HandleExtraFileTypes" lets you open lsm images by using standart File/Open menu item, or by drag and drop. However, in this case associated textual image info will not be retrieved.

"LUT Panel" lets you edit the color palette for your 8 bit datasets.

"Hypervolume Browser" gives you a second slider in your Stack winows. E.g. one for z-axis, and one for time for your 3D timelapse datasets.

As this plug-in can open many image stack windows (one per channel) , it will rapidly become hard to handle all the frames, and brings quite a mess on the desktop. To ease the use of this plug-in it is strongly advised to use another plug-in together with this one. This plug-in is called " **Synchronize Windows** " and it is available on ImageJ's plug-in page.

You will also welcome a plug-in that allows you to render a 3d image of the image stacks you open. Again, there is a plug-in to do that, called "**VolumeJ**", and available on ImageJ's plug-in page.

For the moment, that plug-in does not exists but one that could be interesting is a toll that allows you to merge images or image stacks. Check ImageJ's plug-in page weekly, because I think this one will appear soon as it should deserve a lot of people.

Finally, don't forget that the way of thinking of ImageJ is to bring you a minimal set of tools, to ensure that all of them will fit any user's needs. If you search a particular tool for your own purpose, just check the plug-in page, and you will probably find it. If not, why don't you write a new plug-in for yourself, it's easy!

6. Authors infos.

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