

# Viewing and editing of microscopy images acquired at CIAN

Note: This document summarizes some background information and short protocols for image manipulation of microscopy image files, excluding image analysis. For detailed instructions refer to the user manuals of the software packages.

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## I. Background on images acquired on the CIAN microscopes

All of the CIAN microscopes acquire images in an uncompressed format that includes metadata, i.e. information about the imaging parameters. **Always keep a copy of your images in the original acquisition format.**

Microscope	Acquisition software	Bit depth	File format
LSM1, LSM2	Zeiss AIM	8 or 12 bit	.lsm files inside an .mdb library (= directory)
SD2, SD3	Volocity	14 bit (SD2), 16 bit (SD3)	Volocity libraries; .mvd2 files
SD2, SD3	Metamorph	14 bit (SD2), 16 bit (SD3)	A variety of TIFF files that are tied together by file naming standards, plus small text files with metadata
OMX	Deltavision OMX	16 bit	.dv files plus text files as logs
Leica SP8	LAS AF	8, 12, or 16 bit	.lif files

Useful cross-reference for ImageJ, Metamorph, and Imaris:  
<http://www.mri.cnrs.fr/datas/fichiers/articles/75/398.pdf>

## II. Screen shots for quick reports

If you just want a quick representation of what you see on the computer screen, you can use screen shots. Note that the resolution is lower than with the true file export options, or with snapshot functions in the imaging software.

- arrange items on screen to your liking
- press “Print Scrn” button (in upper row of keyboard buttons) to put image of entire screen into clipboard; use “Alt+Print Scrn” to capture active window only
- paste image into **PowerPoint** or **Paint** document, or open clip board in **ImageJ/Fiji** (if installed on microscope computer) with “File/New/System Clipboard” for further use

### **III. LSM1 and LSM2: Zeiss LSM/AIM Software**

#### *File format*

Image data is stored in .lsm files, which are saved within an “MDB” database. LSM files can be handled and opened independently from the .mdb file.

#### *What can AIM software, LSM Image Browser do with the data?*

The Zeiss AIM software on the acquisition computers, and the freely available LSM Image Browser software (download from [http://www.zeiss.ca/microscopy/en\\_ca/downloads/lsm-5-series.html](http://www.zeiss.ca/microscopy/en_ca/downloads/lsm-5-series.html)) can open MDB databases and LSM files. Files can be viewed in orthogonal projection and cut view, but more advanced 3D rendering is slow and cumbersome.

#### *Opening LSM files in other post-acquisition software*

##### *ImageJ/Fiji*

LSM files can be loaded directly. MDB databases can also be opened, with a selection of which file(s) inside the database to open.

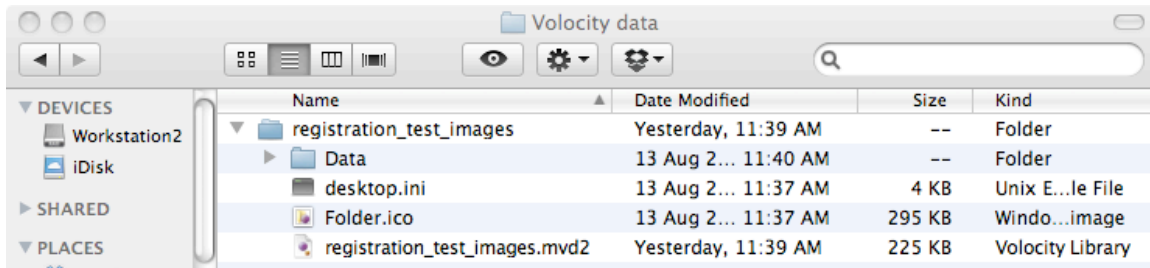
##### *Imaris, Volocity*

LSM files (not .mdb databases) can be opened directly.

## IV. SD2, SD3: Volocity

### *Volocity libraries*

Data is saved into a **library**. A library contains individual images, or **image sequences**, i.e. images that belong to multidimensional image stacks. The image sequences are also referred to as **items**.



The name of the **Volocity library** has no extension. In your file structure, you will see it as a folder/directory. It contains a data folder, and a file that has the library name with the extension .mvd2 (and possibly two files named desktop.ini and Folder.ico). **All of these elements need to stay together to maintain the integrity of the data.**

To open an existing library in Volocity, you open the library folder and select the .mvd2 file within the folder.

To delete individual image sequences/items within a library, open the library in Volocity, select the item, right-click on it, and select “Remove Item”.

To import data, also of other file types, create or open a Volocity library, then drag-and-drop the files, or use File/Import.

### *What can Volocity do with the data?*

Volocity is a feature-rich tool for manipulation and processing of multidimensional image data. Note that you might need to start in the “Visual class” (3D rendering) or “Restore Class” (deconvolution) configuration to make use of the advanced options.

### *Export of individual image sequences within Volocity*

To extract individual image sequences/items from a library with multiple items, they can be exported as Volocity library clippings. They can then be inserted into another (or a new) library.

- Select the image sequence(s)/item(s) in the library view on the left side of the screen
- File/Export:
  - o Define name, location
  - o Format: Item as Library Clipping
- The library clipping will show up as a file with the extension .acff
- To view and manipulate the data, open or create a library, then import the clipping into the library by drag-and-drop, or File/Import

### *Export into other file formats*

Image sequences (items) or the display of image sequences (views) can be exported into a number of scientific and presentation formats in the same way as described above by then selecting a different format.

### *Use of Volocity libraries in post-acquisition software other than Volocity*

#### ImageJ/Fiji

Libraries can be opened if the LOCI Bio-Formats plugin is present. Drag and drop the .mvd2 file on the ImageJ/Fiji icon, or use File/Open..., which will open a dialog window with display options. Can open one or more items from a library with multiple items.

#### Imaris

Libraries cannot be opened directly. Export individual image sequences/items into OME TIFF, or Openlab LIFF formats (not “TIFF for publication”!), which can then be opened in Imaris.

#### Metamorph

Libraries cannot be opened directly. Export image sequences/items into Multiple TIFF, which will create a folder that contains one Tiff image for each slice/color/timepoint, with the names of the files incremented accordingly.

## V. SD2, SD3: Metamorph

### *Metamorph files*

The files generated by Metamorph are Tiff images. These come in different flavors:

MetaSeries Single/Multi-Plane TIFF (.tif)	single- or multi-plane (z-stack) images; default format
MetaMorph TIFF File Format (.tif)	single-plane images; legacy format
MetaMorph Stack File Format (.stk)	multi-plane images; legacy format

### *Files from MDA*

The Multi-Dimensional Acquisition (MDA) interface automatically saves data as the MetaSeries **Single/Multi-Plane Tiff**, with **one file per z-stack**, i.e. separate files for multiple channels, time points, or stage positions. Information about the channel, time point, or position is appended to the base file name.

Example: “BaseFileName1\_w11. GFP confocal\_t2.TIF” is one single or multi-plane image of an acquisition with the name “BaseFileName”, using the illumination path 11 (GFP confocal), second time point.

In addition, the MDA creates a **file with the “.nd” extension**. This text file contains important information about your multidimensional data, so do not delete it! It can be used to open the set in some software, as described below.

### *What can Metamorph do with the data?*

Metamorph can manipulate and analyze multi-dimensional data. The interface is less intuitive and graphics-driven than in Imaris or Volocity, but there is more control over parameters, and scripts (journals) can be used to automate tasks.

### *Opening existing files in Metamorph*

Single Tiff images can be opened with File/Open.

Using File/Open on the .nd file of an MDA data set will open only the first time point, but combine all channels and z-slices for that time point into one stack. To open the full set, use File/Open in 4D Viewer... on the .nd file.

### *Opening Metamorph files in other post-acquisition software*

ImageJ/Fiji, Volocity, Imaris

Can open Tiff files and ND files directly.

## VI. OMX: Deltavision OMX/SoftWorx

### *Deltavision files*

The OMX super-resolution microscope generates image files in the Deltavision format, the extension is .dv.

When processing the raw data in Softworx (on the Linux workstation), files of the same type are generated. The file name (not the file extension) is changed to reflect what kind of processing was performed.

...\_SIR.dv    structured illumination reconstruction  
...\_ALX.dv    OMX image registration  
...\_D3D.dv    deconvolution  
...\_PRJ.dv    projection (e.g. maximum intensity projection)

For each processing step, a log file is generated. Keep these logs to document the processing parameters. They are not needed when working with the data in another program.

### *What can SoftWorx do with the data?*

Softworx can display, manipulate, and process the data. Softworx is the only tool we have to do the SI reconstruction, and the xyz alignment of the channels, including rotation. 3D rendering tools are somewhat awkward to use and slow.

### *Opening Deltavision files in other post-acquisition software*

#### ImageJ/Fiji

With the LOCI Bioformats plugin, Deltavision files can be opened directly. The plugin is part of Fiji. If you don't have the plugin in your ImageJ installation, you can get information and download instructions here: <http://loci.wisc.edu/bio-formats/imagej>

#### Imaris, Volocity

Deltavision files can be opened directly in Imaris and Volocity.

#### Metamorph

Save .dv file as Tiff (16 bit gray, no scaling), generating a folder containing one Tiff file per color and Z-slice (grid positions are treated as Z-slices in raw data), then open each color into a stack: Open Special/Build Stack/Quick, identify first image of one wavelength, generate stack; repeat for other wavelengths

## VII. Leica SP8: Leica Application Suite (LAS AF) software

### *File format*

Image data are stored in .lif files, which can contain many images or image sequences/stacks.

### *What can LAS AF software do with the data?*

The LAS AF software on the acquisition computer has a full complement of image editing and quantification tools, and can do spectral unmixing and channel dye separation. Z Stacks can be viewed in orthogonal projection or as galleries, but we don't have the 3D rendering module available in the facility.

The freely available LAS AF Lite software is available from this site:

<http://www.leica-microsystems.com/products/microscope-software/software-for-life-science-research/las-af-4-advanced-fluorescence/#modules>

It can open .lif files, and display the images within. Colors can be edited, intensities measured, and individual images can be moved to different experiments (existing or new .lif files).

### *Opening SP8 files in other post-acquisition software*

#### *ImageJ/Fiji*

With the LOCI Bioformats plugin, Leica files can be opened directly. The plugin is part of Fiji. If you don't have the plugin in your ImageJ installation, you can get information and download instructions here: <http://loci.wisc.edu/bio-formats/imagej>

#### *Volocity*

Leica .lif files can be opened directly or imported into existing libraries.

#### *Metamorph, Imaris*

Leica .lif files can be opened directly