

Zeiss LSM Image Browser Software

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Background

The Image Browser software (*version R3.2*) is an image database program that is available as a free download from the Zeiss website. The advantage of using the Image Browser is that the interface is identical to the one used on the LSM 510. In addition, many of the same functions are available for use “off-line”.

Once the software has been installed, the user is able to:

- Open databases of images created on the LSM 510 confocal microscope.
- Create new databases, as well as transfer images and all related information between databases.
- Access specific information about how their images were acquired (*settings, filters, etc.*).
- Annotate their image data with specific information about specimen preparation and/or interpretation.
- Export images to common file formats (*e.g., tif, bmp, gif, jpg*).
- Sort the database based on user-selected criteria.
- Quickly create data pages for lab notebooks using the multiprint feature.
- View stored 3D data (*slices and rotations*).
- Quickly and easily add scalebars and/or other types of drawing overlays to images.
- Change an image’s color look-up-tables.

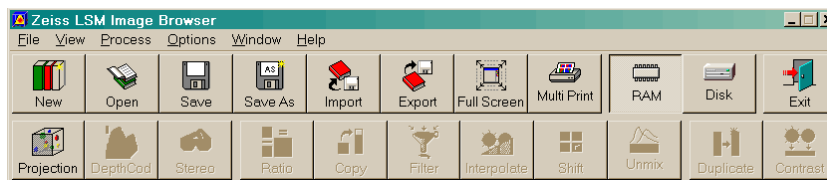
Installing the Image Browser software

We highly recommend that users get their software installation files from the FTP server on the LSM 510. To get the installation files using FTP, point your web browser to [Call to get the network address](#) and copy the contents of the folder to a directory on your local computer. A handout (*in Adobe Acrobat PDF format*) entitled “Installing the LSM Image Browser Software” is stored in this folder along with the installation files.

Note, the LSM Image browser software only runs on the Microsoft Windows operating system.

File Management

The image database files are stored on the LSM 510 computer in a folder (*directory*) that has the form of **PROJECT.MDB**. Inside the folder is a single **MDB** file (*this is the database file*) and several **LSM** files (*the images in the proprietary Zeiss format*). These files must stay together so that the acquisition information is available when the image is opened in the Image Browser software.



Zeiss Image Browser toolbar

To open a database, click the **NEW** button in the toolbar and browse to the directory that holds the data and double click on the **MDB** file. The database will load in the **FORM** view. To look at an image in its own window use either the **LOAD** button in the toolbar on the right side of the image, or just double click on the image.

In the FORM view there are several key areas of information:

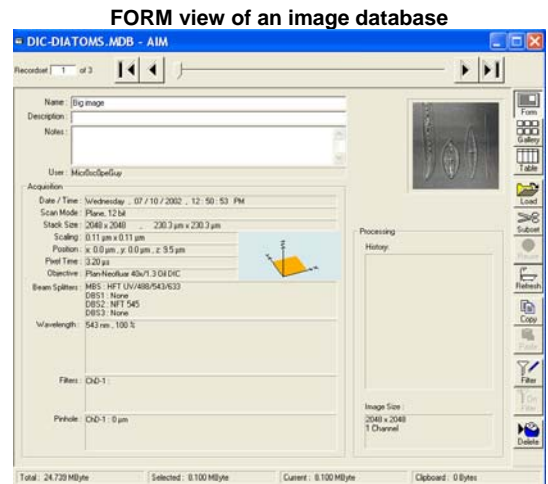
Recordset: The arrows allow the user to scroll through the database one image set at a time.

Name/Description/Notes: Users can change the file name, add a brief description or provide additional notes regarding the sample preparation.

Acquisition: Displays detailed information regarding the parameters used to acquire the image set.

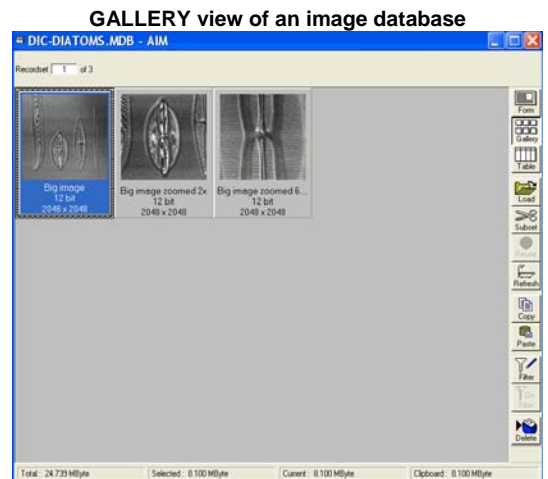
Processing/History: Stores any modifications to the original image that were done in the Zeiss software.

Image Size: Shows size of image in pixels for XY(Z), # of channels used



To open the GALLERY view, click the appropriate button on the right side toolbar. This view allows the user to preview all the image sets at one time. To look at an image in its own window highlight the thumbnail and use either the LOAD button in the toolbar on the right side, or double click on the thumbnail.

To open the TABLE view, click the appropriate button on the right side toolbar. This view allows the user to review a small spreadsheet of information about the files in the database. To look at an image in its own window highlight the image name/spreadsheet row and use either the LOAD button in the toolbar on the right side, or double click on the row.



Other tools available on the right side toolbar:

Subset: Allows the images to be loaded at a reduced resolution. This reduces the RAM requirements for large image datasets.

Copy/Paste: Image sets can be moved between database files with copy & paste. This can also be done in GALLERY mode using “drag & drop” by dragging the image set thumbnail from one database to the other. Copy can also be used with the multiprint feature located on the main Image Browser toolbar.

Refresh: Updates the database window.

Filter: Allows the database to be sorted by a specific criterion, such as words in the user-defined fields, specific filters or other acquisition parameters. The filter can be toggled on and off.

Delete: Deletes an image set from the database.

The ability to create new databases and to move files easily between databases gives users a powerful way to handle their data. A couple of noteworthy points to consider:

- Take advantage of the **Name/Description/Notes** fields in the database to annotate your data as you acquire it. In the software on the microscope you can use the menu **OPTIONS|OPTIONS** and the **SAVE** tab and click the **REMEMBER** checkbox. This will set up these fields so that most of the data from the previous file is repeated for the next file, leaving only minor modifications that need to be made each time (saves typing).
- Back up your **MDB** and **LSM** files. CD-R is still probably the best way to archive data.

Viewing, Annotating and Measuring Images

Opening an image from a database view launches a window that should be familiar to users of the LSM 510. The two right side toolbars contain many of the tools available on the microscope's computer. *Note: licenses for the full feature set of the LSM software are available from Carl Zeiss Inc, however, the license is quite expensive.*

For single images:

Zoom: Opens a separate toolbar to zoom the image in or out.

Channels: Opens a separate toolbar and allows the user to change the look-up-table for each channel.

Overlay: Opens a drawing toolbar, which allows the user to:

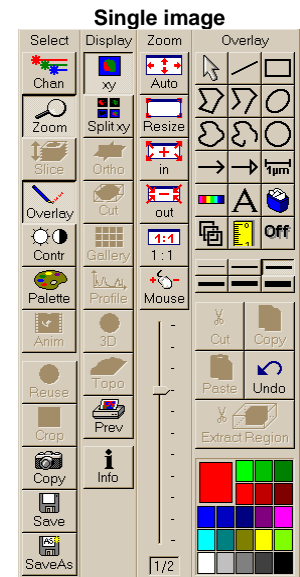
- Add a scalebar to the image. Users can change the color & weight of the scalebar.
- Add arrows, lines, circles, etc.
- Measure distances in microns.
- Extract a selected region of the image using the drawing tools.
- See pages 5-248 to 5-251 from the LSM manual (software ver 3.2) for additional information about each of these functions. These pages are available (Adobe Acrobat PDF file) via FTP in the same folder as the installation file.

Contr: Launches a window with brightness and contrast adjustment sliders (Caution: brightness and contrast are fine for minor adjustments, however they can cause image pixels to over- or under-saturate if larger adjustments are made.)

Copy: Copies the image in the window to the clipboard. Use this tool and the multiprint button on the Image Browser toolbar to create hardcopy documentation.

Info: Displays information about the image acquisition parameters.

Prev: Provides a "print preview" of the contents of the image window that can be sent directly to the user's printer. This is not as flexible as the multiprint feature available on the Image browser toolbar.



Additional tools are available for Z/time/lambda stacks:

Slice: Opens a separate toolbar to allow the user to move through the stack.

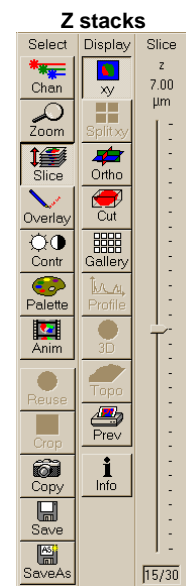
Ortho: Opens a separate toolbar and allows the user to view the optical section data (Z stacks) from the top and sides simultaneously.

Cut: Opens a separate toolbar, allows the user to create a new image at a new angle and rotation through a Z stack.

Gallery: View all the sections in a stack, label the sections with slice thickness/time stamp/wavelength information and/or extract a sub-set of the stack.

Anim: Allows the user to page through their stack at two different animation speeds. The software does not have the capability to export to movie files (note, this can be done with the software that is on the LSM 510).

Other tools: the Image browser has a limited version of the PROJECTION tool on the toolbar. This allows a user to create a 3D projection image, at a single user-defined angle, from a Z stack.



Importing/Exporting Images

The LSM Image browser can be used to import images to take advantage of the database. The database does not maintain any specific acquisition parameters for imported images. The annotation features and most of the tools described above are available for use with imported images. The browser can only import images of the types described below.

The Image browser can export to several common and a few proprietary digital image file formats. Image formats that we do not recommend have a gray background in the table below:

LSM 4 – TIFF RGB Planar (*.tif)	Not recommended. Intended for compatibility with the LSM 410 database format only.
LSM 4 – TIFF Chunky (*.tif)	Not recommended. Intended for compatibility with the LSM 410 database format only.
TIFF 12-bit (*.tif)	Not recommended. Use 16bit tiff instead. Photoshop 6.0 opens these files, however they will appear black.
TIFF 16-bit (*.tif)	Use this format to save single layers of 12bit multichannel images to avoid the software automatically reducing the bit-depth from 12 to 8 bits on each channel. To simultaneously output all layers in a 12bit multichannel image as separate 12bit images, use the batch export utility on the LSM 510.
Attofluor 6.0	Proprietary format - compatible with AttoFluor Bioscience's fluorescence image analysis software
MetaFluor 3.0 (*.inf)	Proprietary format - compatible with Universal Imaging's MetaFluor fluorescence image analysis software
TIF – Tagged Image File (*.tif)	Standard 24bit tiff file format. The image will be 24bit color unless a single channel is exported as monochrome (8bit grayscale). Suitable for scientific data with three colors (RGB).
TIFLZW – Compressed TIFF (*.tif)	This format uses loss-less compression of the standard TIFF format. The file size is smaller, however some digital imaging programs cannot open this particular format.
BMP – Windows Bitmap (*.bmp)	This format supports 24bit color. In most cases TIFF would be a better choice.
GIF – Compuserve (*.gif)	This format supports only 256 total colors (8bit). Suitable for posting 8bit images to WWW pages.
JPEG – Best Quality (*.jpg)	Unsuitable for images that will analyzed at a later time since the compression algorithm can alter both xy location and intensity information. This format supports 24bit color. Suitable for posting images to WWW pages.
JPEG – Medium Quality (*.jpg)	Smaller file size than Best Quality JPEG, with some increase in common JPEG artifacts. See JPEG comments above.
JPEG – Highest Compression (*.jpg)	Smallest JPEG file, with noticeable increase in common JPEG artifacts. See JPEG comments above.

Additional file formats that can be exported only from the image browser on the LSM 510 computer include:

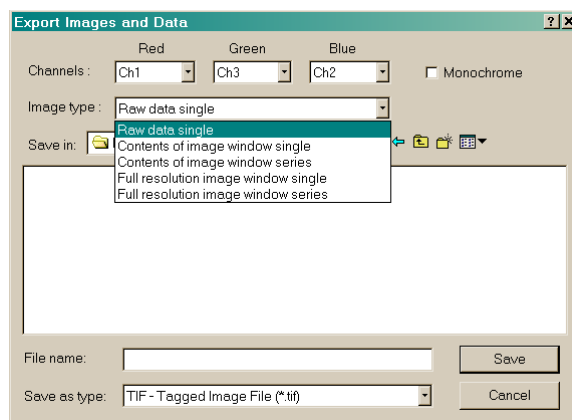
IMAGE FILES

BMP – OS/2 Version 1.X bitmap (*.bmp)
 BMP – OS/2 Version 2.X bitmap (*.bmp)
 PCX – Zsoft (*.pcx)
 PSD – Adobe Photoshop (*.psd)
 RAS – SUN Raster file (*.ras)
 PCT – Macintosh QuickDraw (*.pct)
 TARGA – Truvision (*.tga)
 PNG – Portable Network Graphics (*.png)

MOVIE FILES

AVI – Video for Windows (*.avi)
 Apple – Quicktime (*.mov)

To export files, have an image window open and select the **EXPORT** button on the Image Browser toolbar. The “Export Images and Data” window will open. This window has several options, starting from the top they are:



Channels: If your image has more than one channel (*images from different fluorescent dyes*), the software will suggest which channels should be assigned to the red, green, blue channels of a 24bit image. Users can change these assignments using the drop-down boxes. If there are only two fluorescence channels, one channel (*usually the blue*) will have “--” in the window, indicating that channel will not be used. If you are only exporting one channel, you can choose the color and/or export it as a smaller 8bit monochrome image (*use the check box*). This option is only available in the Raw Data Single image type.

Image Type: There are five choices in this drop-down list.

Recommendations: If you want control over the output to the RGB channels, use “Raw Data Single”. If you want to export an image or images that include overlay data at the XY resolution that they were acquired on the microscope, use “Full Resolution Image Window”. If you want to export galleries and/or split screens with overlays use “Contents of Image Window...” (*see comments below*).

- Raw Data Single – Exports a single image with all selected channels at the same resolution and intensities (8bit) as when the image was acquired. This setting will not export a scalebar or other overlay information. To export a DIC (*grayscale*) image with this choice, use the monochrome check box for just the DIC image and re-assemble with the other colored channels (*exported separately*) later in Photoshop.
- Contents of Image Window Single – Exports a single image representing what is displayed in the image window at the resolution the image is displayed on the monitor. This choice is completely dependent on the size at which the image is displayed on the user’s monitor. If the image is large on the monitor, the file will be large. If the image is small on the monitor, the file will be small and there is the potential that the image will be very pixelated if it is enlarged at a later time. This choice also includes features like zoom, split screen, galley, overlay information (*e.g., scalebar, arrows, etc.*). Users do not have control over the channels that are exported.
- Contents of Image Window Series – See comments for “Contents of Image Window Single”. This choice exports a series of images (*such as: Z, Time or Lambda*).
- Full Resolution Image Window Single – Exports a single image at its full resolution (*including any overlays such as scalebars, arrows, etc*). Users do not have control over the channels that are exported.
- Full Resolution Image Window Series – See comments for “Full Resolution Image Window Single”. This choice exports a series of images (*such as: Z, Time or Lambda*)

Save in: Allows the user to browse to the folder/directory where they would like to save their image(s).

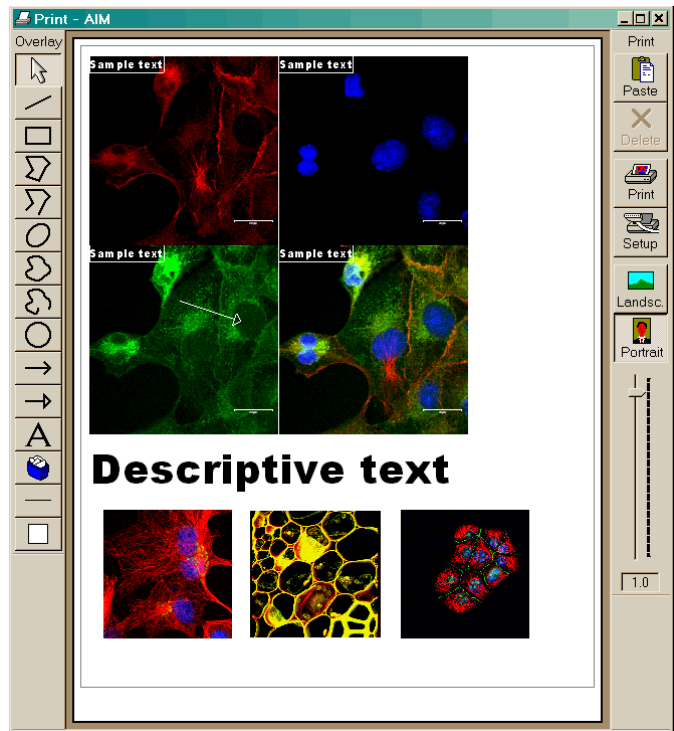
File name: Allows the user to name the image(s) they are exporting.

Save as type: Displays the different image file formats which are described above. For most images TIFF should be used (*note: using TIFF means that 12bit images will be rescaled to 8bit*).

Printing Images

The LSM Image Browser provides a feature called multiprint which allows users to quickly and easily assemble a page of images that can be sent to a printer. Multiprint is activated on the Image Browser toolbar when the user has an image open in an image window.

The user can add text and a scalebar to an image in the image window using the overlay feature (*right side toolbar*) and then use the copy button in the image window. By switching to the print window they can paste the image onto a page, align it, resize it, add additional text, add arrows, and change the color of text and/or arrows. The page can then be sent to any printer that the user can access with their computer.



Macintosh users should see the "Installing the Zeiss LSM Image Browser Software" handout for more information on the available options for Macintosh users.

Resources

- LSM 510NLO-Meta System Manual - (ver 3 & 3.2), Carl Zeiss Inc.
- Troy Tholen & Sebastian Tille, Advanced Imaging Microscopy, Carl Zeiss Inc, One Zeiss Drive, Thornwood, NY 10594 (1-800-233-2343)

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About the author

Mr. Cromey is the manager of the Cellular Imaging Core, a service that provides training & technical expertise to SWEHSC investigators interested in using microscopy and scientific imaging in their research. The SWEHSC is funded by the NIEHS, grant # ES06694. The Cellular Imaging Core is also host to *Microscopy & Imaging Resources on the WWW*, located at: <http://swehsc.pharmacy.arizona.edu/exppath/>



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