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Information Management of Confocal Microscopy Images

Traditional Text-Based Databases and Image Gallery Databases

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1. Introduction

Imaging methods, whether they use film, videotape, or digital capture methods, are only of use if you are able to readily categorize and retrieve the information and images that are produced. Modern imaging techniques are capable of generating large numbers of images of various dimensions and content. Major obstacles confronting the confocal microscopist include how to keep records of (1) how the various images were collected, (2) where they are stored, (3) what they look like, (4) the important features of the images, and (5) how to keep track of modifications of these images. The digital nature of confocal images lends them well to the use of computer-based databases for information storage, classification and retrieval. This chapter deals with these problems and the application of simple databases for maintaining and tracking images and information of confocal image datafiles.

This chapter will outline a few useful and inexpensive database tools that prove of value in the specific context of confocal imaging. A brief introduction to the use of image databases for keeping track of confocal images was discussed in my chapter on Use of NIH-Image in Fluorescence and Confocal Microscopy. This chapter can be downloaded from my website at http://www-cajal.ucsd.edu. Confocal microscopists may find that chapter useful in dealing with transferring files between different computer systems, and for post-acquisition processing of confocal images using NIH-Image.

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1.1. What is a Database?

Databases are collections of information stored in an orderly manner, designed to sample and facilitate the retrieval of information based on logically structured queries. Databases are widely used in government, hospitals, industry, and laboratories. Every scientist relies heavily on databases such as that provided by the National Library of Medicine (Medline), the phone company directory, and the table of contents and index of a book. However, surprisingly few microscopists take advantage of modern computer based database tools for keeping track of their images.

1.2. How is a Database Organized?

A database File contains a grouping of Records. Each Record contains the information about a single image or series of images. A Record contains Fields. The various types of Fields may include designated categories of information, such as the particular microscope used, the magnification, laser lines used, dyes, location of file storage, animals, plane of section, a copy of the image itself, etc.

1.3. What Do I Want to Store in My Database?

The types of information of importance include information about the nature of the picture, the manner of its collection, and a gallery of images to allow rapid review and selection of particular images. An ideal database should also be integrated with the software used for image acquisition on the confocal microscope. The database should then be available for use on other computers, independently of the confocal microscope. Although some confocal microscope systems do provide this type of information, such as the new Zeiss LSM 510, it is not a standalone program, and cannot operate separately of the program specifically associated with the CLSM.

2. Two Types of Databases

I will deal with only two simple types of databases: (1) traditional text oriented databases with limited ability to display images and (2) Image Gallery databases.

Traditional text-based databases, can be used to store information about the nature of the images collected, the manner of collection, names of the files, date, comments and similar data, and a thumbnail image of the microscope image. There are a number of inexpensive programs that can be used for this purpose, including FileMaker Pro® (FileMaker Inc.) and Access® (Microsoft® Corp.). These types of programs are mainly used to store textual information, with limited ability to also store images. These programs have very limited

ability to simultaneously display large numbers of images. This type of database is particularly useful in providing tools for searching for specific textual information. They are described in further detail elsewhere in this chapter. These programs are most effective when used at the time of initial collection of confocal microscope images. Additional comments can subsequently be added to each record.

A sample Record of an image file is shown in **Fig. 1**. This contains information in a FileMaker Pro Template. The format of the Template is easily constructed and can be adapted to the preferences of individual users.

Individual Fields may include a group of predetermined values, such as various planes of section, as shown in the "Pull-down" menu shown in **Fig. 2**. This facilitates data entry, and establishes a rigorous protocol to ensure that all desired information is recorded by the user.

One of the most useful features of a program such as FileMaker Pro is the ease with which you can display the data in alternate formats. You can easily modify the layout, and instantaneously switch from one layout to another, in order to concentrate on a particular aspect of your data. You can make a layout that will provide a tabular summary that mainly emphasizes the Title, the date of collection, the plane of section, the species, the dye used, or any other feature. Several alternate columnar layouts are shown in **Figs. 3–5**. The lengths of the columns are only limited by the size of your computer monitor.

This type of database is mainly based on textual content. However, Fields for images can also be inserted into the Record. However, the nature of programs such as FileMaker Pro limits their suitability for gathering and storing large numbers of images. To limit the size of such files with many images, the original image is modified to produce a smaller scale "thumbnail" image. The process of generating thumbnails and then storing them in the correct location can be very tedious and time consuming.

Image Gallery databases are designed to present large numbers of "thumbnail" images on the computer to enable the viewer quickly find a desired image based on visual content, rather than on descriptive phrases. These programs automatically scan a disk for files containing specified types of images, such as Bio-Rad *.PIC files, then generate and store thumbnail images of files, the size of the file, the location and the format of the file, and various parameters pertaining to the original mode of collection of the images. The user can then view a "gallery" of thumbnail images from many files (*see* **Fig. 6**), zoom in on a selected file and examine it in detail, search for selected files based on keywords, date, file names, etc. This type of program typically is used hours or days after the original confocal image is collected. Most of these types of programs were developed in relationship to graphics arts as well as satellite imaging. However, Image Gallery databases provide only limited ability to also

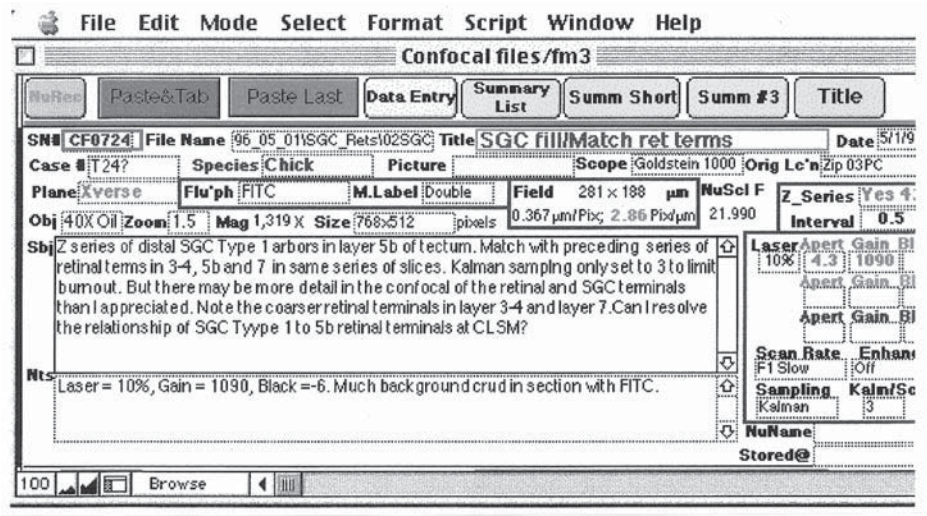


Fig. 1

Fig. 2

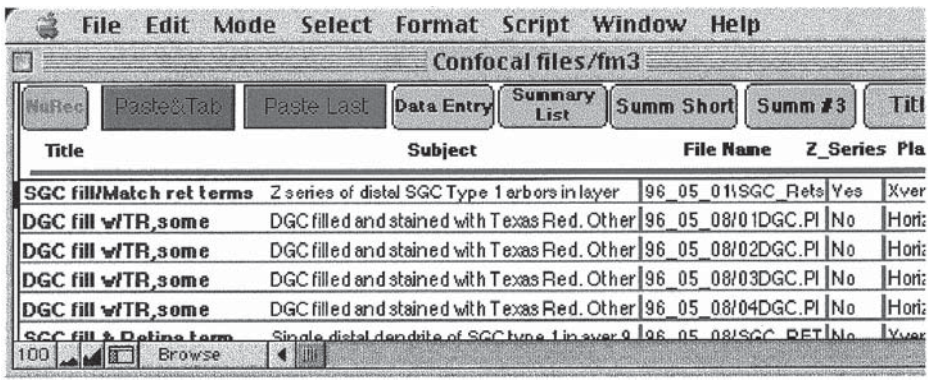


Fig. 3

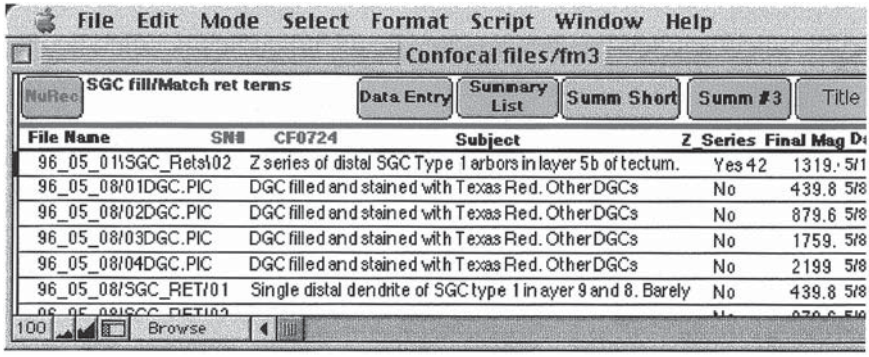


Fig. 4

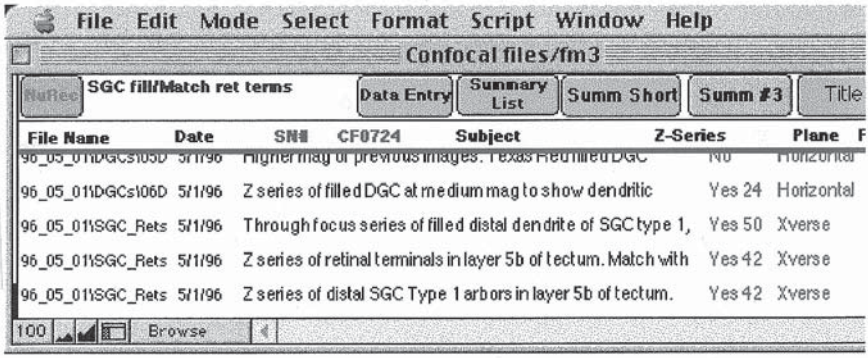


Fig. 5

store detailed information regarding method of data collection, detailed descriptions of content, relationship to other images, interpretation and analysis of the image content, etc. All such information is usually stored in a single field of very limited size. As in the first type of database, searching the database depends upon the use of text based descriptive features. Some representative programs of this type include Cumulus 4.0 (Canto Software), Portfolio™ 3.0 (Extensis Corp.), and ThumbsPlus® 3.1 (Cerious® Software, Inc.). Although all three of these Image Gallery programs perform similar operations, they differ is some important ways. The relative advantages and disadvantages of each of these programs are summarized later in this chapter.

Various lower cost programs have been developed for use with Photo CD storage. These programs do not seem to have the capability required for our purposes, but may be helpful when you are first familiarizing yourself with these types of programs.

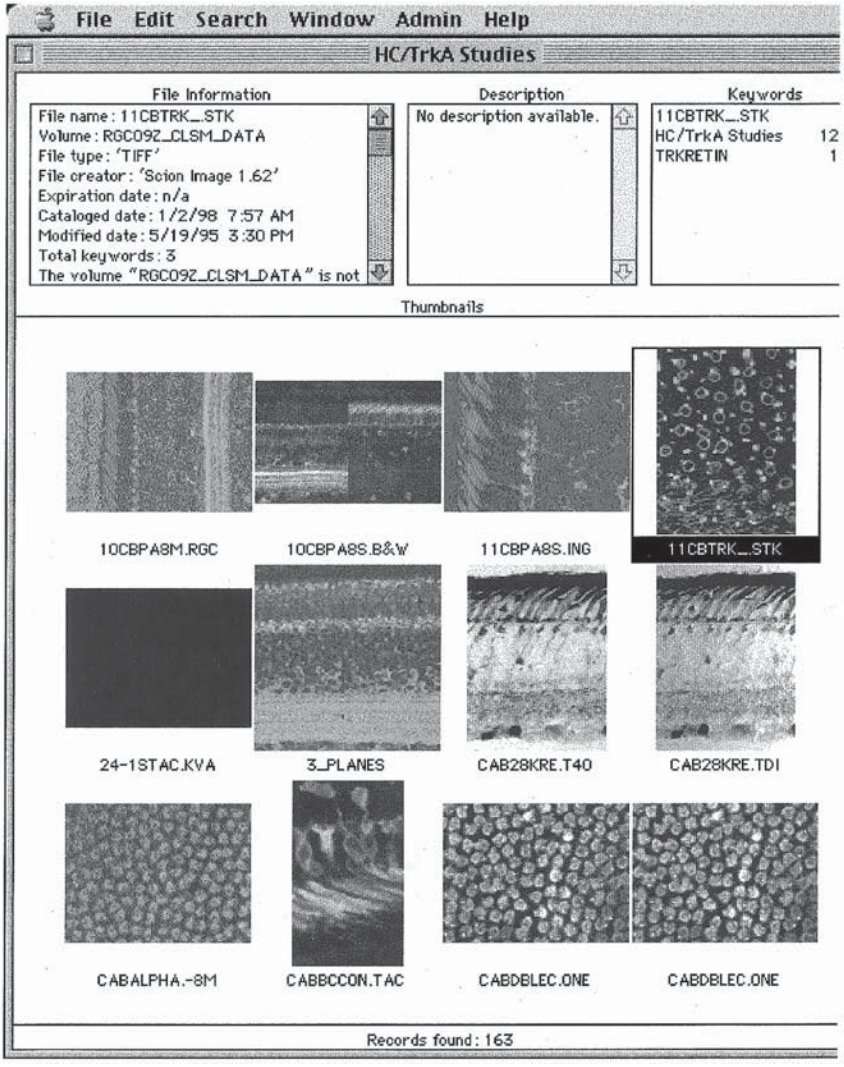


Fig. 6

3. Where Should Data About the Image be Stored: *With the Image, in a Separate Database, or in Both Places?*

Some of the software provided by confocal microscope manufacturers for collection and storage of images also includes the capability of storing a limited amount of information about the method of data collection and for comments about the image. This information is stored either in the same file as that containing the image itself, or in an associated file.

Bio-Rad includes some basic information about each image in the file itself. Leica stored similar information in a separate text field with a similar file name, but with a distinct three-letter extension indicating that it contained information about the collection parameters. When examining the file with Leica software, the file containing the text field is also displayed. However, if the image is viewed with other software, such as Adobe® Photoshop®, this information is not displayed. Olympus (FluoView) stores this information in the image data file as a specific TIFF field, although it is only available when the full data file is opened. Although the collection parameters were automatically stored with the file, most users do not include comments within the same file about the nature of the material, interpretation of the results, related experiments, etc. Perhaps they assume that they will write this down later, or that it will be self evident, though most commonly, I forget to enter such information.

The data in the original files may include information about the magnification, gain, offset, pinhole, resolution in micrometers/pixel, filters, look-up table (LUT), mode of accumulation (Kalman, accumulate to peak, etc.) and some limited textual information about items of note in the image. However, this information is generally only available as each file is individually opened. There is no simple means of examining all this information about all your images at a single time. Thus, you could not easily and rapidly search for all your images stained with a particular antibody, or of a unique preparation.

Various filters (a "filter" is software that translates a file from, e.g., Bio-Rad format to Photoshop format) have been developed that allow Bio-Rad, Leica, Zeiss, and Olympus files to be transferred to more general platforms (Mac, PC, and SGI) and more common file formats (Photoshop, NIH-Image). However, this transfer invariably resulted in loss of the collection parameters. Photoshop and NIH-Image do not allow hidden textual information to be stored in their files. Thus, if you transfer your file to either of these programs, the information is no longer associated with the file. Although you may be able to return to the original files to extract that information (see below), that is an extremely inefficient way of storing and comparing information.

Unfortunately, the various confocal microscope manufacturers have been unable or unwilling to develop a single uniform standardized file format for dealing with these problems. Different confocal microscope manufacturers have chosen to use different data file formats.

These formats vary depending upon several factors:

Are the collection parameters and users notes stored in the same file as the image data itself?

1. Do the image files also contain data about collection parameters, your comments, scale bars as separate from the actual image (versus a scale bar that has been written directly on the image with resultant loss of the underlying data)?

2. The nature of the image (single, double or triple label, Z-series of single label, Z-series of double label, Z-series of triple label, etc.)

In view of this deficiency, I strongly recommend that information about each image also be stored in a separate database file containing information about all your images.

4. External Databases, Both Image Gallery Type and Traditional Records/Fields Types to Store all Collection Parameters

The second way of handling this information is to record the same information in a traditional database, consisting of multiple Records with multiple Fields. Each single record is used to store information about each image set. Each Field contains distinct categories of information pertaining to each record, e.g., an alternate title that more suitably describes the content of the file, the lens used, the filter, plane of section, stain, and so forth. Users can easily add additional Fields to their records, reflecting their personal needs and preferences. Modern database software also provides means of storing one or more images in each Record, several formats for displaying the same data, sorting the data, searching for specific information, etc.

A well-designed database template will also impose some discipline on your record keeping, and prompt you to insert all required information.

5. Text-Based Database

Using either FileMaker Pro or Microsoft Access, you should make a template reflecting the type of information you find important for your research. A sample record is shown in **Fig. 1**.

5.1 What File Name Shall I Call this Image or Set of Images?

It is often easier to take a good picture than to think of a file name for it. This is particularly bothersome when you have to use a DOS based file naming convention (8 + 3 = filename.ext), but also taxes my imagination even when using a Mac- or Windows NT®-based system with the option of long file names.

If you rely heavily on the file name to indicate the content there are potentially serious disadvantages. If you capture a triple labeled immunofluorescent stained section of the peripheral retina from animal NR12345, at 725× magnification, and only have a limited number of files, you can make up some name on the spur of the moment, such as "triplRet.tif," and hope that you remember what filename you used, what it contains, and where it is stored. As the number of images increases, you may find your creativity in designing file names is severely taxed. You can have a nonspecific descriptor, but a file name such as MX4bixi.tif is not much better. If you take one image a month, you can live

with either approach. If confronted with a confusing group of names on your computer, you will likely consider shifting to hand drawn representations of your slide. Each person must develop their own strategy. My current solution is heavily oriented toward computerized databases. It solves some problems, but not all of them.

6. Using a Database to Designate a File Name

In addition to a Field that automatically assigns a serial number to each image, a database program can generate a formal file name (e.g., BR805689.TIF), or you can choose a less anonymous title, such as CalRet01.tif to indicate that this is an image containing a stain for calretinin, and is the first image in the series.

Image Title: This is a longer and generally far more comprehensible title that conveys more information about the file content, e.g., "Monkey retina stained for calretinin and calbindin: Z-series in transverse plane."

Several additional fields may consist of Pull-down lists that include commonly used values, as shown in **Fig. 2**. These may include: plane of section (transverse, horizontal, sagittal); objective lens used (10X, 20X Plan Apo, 40X oil, etc.); fluorescent dyes used; the number of sections in a Z-series and their spacing, scanning speed, PMT, gain, offset, special filters, pinhole diameter, laser power, etc. Some of this information may be redundant of information also stored in the original image file, but is far more readily retrieved from this database. In addition, you can also insert a thumbnail image of the original confocal image to help identify the content of the file.

The most valuable Field in the Record, however, is that containing a detailed description or commentary on the image, its relevance to your work, comments about unusual aspects of the manner of preparation, why this particular image was saved, what you learn from it, etc. You can also add technical comments regarding errors in data collection, irregularities in specimen quality, etc. This capability is particularly important, and requires that the Database program support variable length fields. All Fields in FileMaker Pro are variable length fields, and are fully indexed, so you can continue to add comments into a field without constraints on their length. Once indexed, you can then search for particular phrases or words. Many database programs, including Microsoft Access 97, require that the user define the length of each field, frequently limiting it to 255 bytes. Only a single variable length field is permitted in each record, and the contents are often not indexed. Thus searching for particular words in this field cannot be accomplished. This is a severe constraint when you want to enter lengthy comments about an image.

However, many users may find that both FileMaker Pro 4.0 and Microsoft Access 97 are effective for maintaining records of data collection. While both

also provide Relational Database tools, I suggest that initially you keep things simple and use a flat-file, nonrelational organization. FileMaker Pro has the advantage that the files are fully cross-platform compatible; i.e., the same file can be examined without difficulty on both a PC and a Mac PowerPC. Microsoft Access is not presently available in a PowerPC version. If you only work on a Windows NT platform, either program should prove satisfactory.

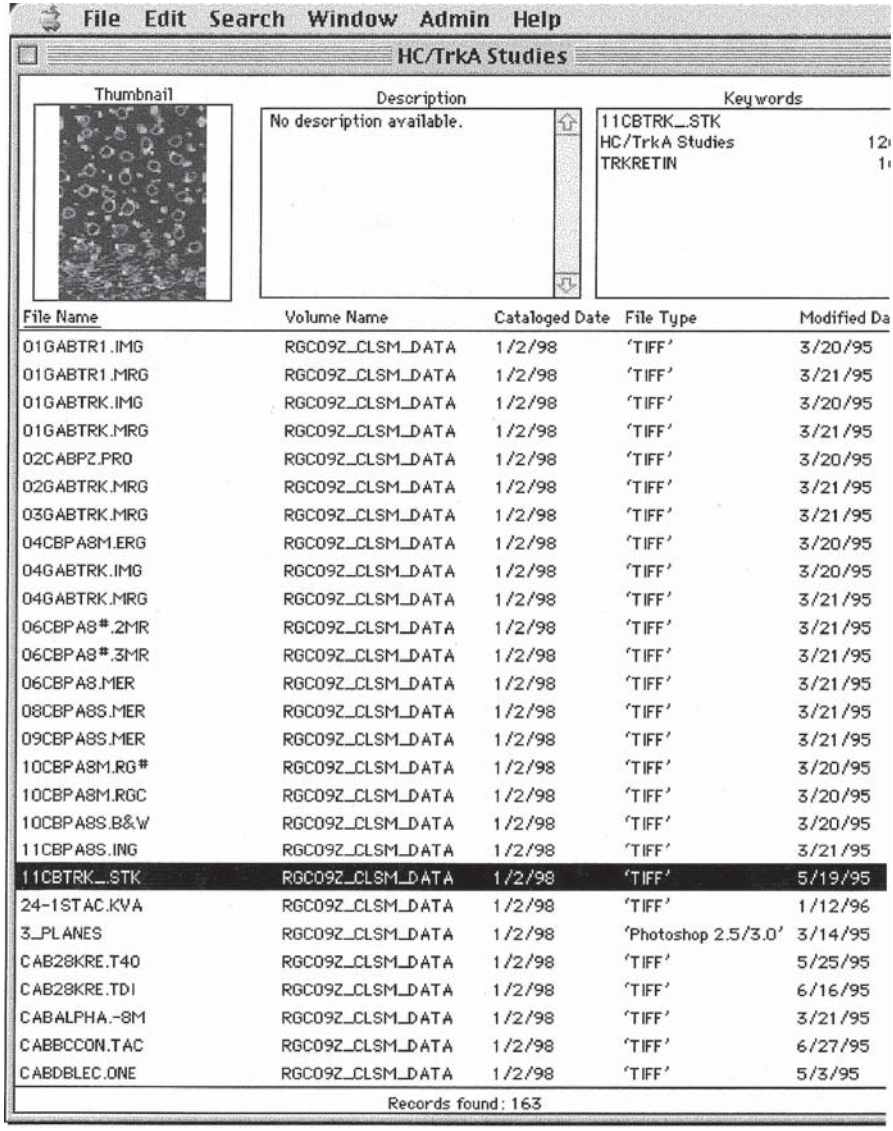
I suggest that you keep a copy of the Database Program open as you collect your original images, and update your file as you collect each image or set of images.

7. Image Gallery Databases

As mentioned previously, there are several image database programs specifically designed to store, display, and retrieve images. They all have limited text display capability, but provide a useful "Gallery" display of thumbnail images. FileMaker Pro does not provide this type of utility. However, you can generate Thumbnail images that can then be pasted into FileMaker. One of the most useful qualities shared by all the Image Gallery Programs, including ThumbsPlus, Cumulus, Portfolio, and Multi-Ad Search® (Multi-Ad Services), is their ability to automatically scan your hard disk and generate thumbnail images of each file. Each of these programs also allows you to store images contained on removable media, such Jaz, Zip, and Mag-Optical disks.

In addition, Canto Cumulus permits insertion of the thumbnail image into a FileMaker Pro database. The new editions of Extensis Portfolio and ThumbsPlus promise to provide similar functions in the PowerPC version. All of these programs also allow users to view a full screen image of the thumbnail, and to directly open the original file in the program that produced the original image. These programs also provide information about the size of the files, its current storage location, file format, image dimension, and keywords. None of these programs will generate a simple Z-projection of an extended Z-series. The thumbnail of a Z-series is invariably the first image in a Z-series. Because the first image is usually at the margin of the most interesting data, the thumbnail is of little value. If your set of images have been transferred to an image processing program, such as NIH-Image, I suggest that you make a Z-projection of the stack of images in a Z-series, and place that as the first image in the stack. Label the image so that you not confuse it with the main part of the stack when you perform further operations on the Z-series. The resulting thumbnail image will be that of the first image in the stack, containing the Z-projection.

In addition to the Image Gallery display of large numbers of thumbnail images, these programs also permit the user to view a List of images, and the thumbnail image of a single file at a time, as shown in **Fig. 7**.



File Name	Volume Name	Cataloged Date	File Type	Modified Da
01GABTR1.IMG	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/20/95
01GABTR1.MRG	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/21/95
01GABTRK.IMG	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/20/95
01GABTRK.MRG	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/21/95
02CABPZ.PRO	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/20/95
02GABTRK.MRG	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/21/95
03GABTRK.MRG	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/21/95
04CBPA8M.ERG	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/20/95
04GABTRK.IMG	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/20/95
04GABTRK.MRG	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/21/95
06CBPA8#.2MR	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/21/95
06CBPA8#.3MR	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/21/95
06CBPA8.MER	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/21/95
08CBPA8S.MER	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/21/95
09CBPA8S.MER	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/21/95
10CBPA8M.RG#	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/20/95
10CBPA8M.RGC	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/20/95
10CBPA8S.B&W	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/20/95
11CBPA8S.ING	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/21/95
11CBTRK_.STK	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	5/19/95
24-1STAC.KVA	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	1/12/96
3_PLANES	RGC09Z_CLSM_DATA	1/2/98	'Photoshop 2.5/3.0'	3/14/95
CAB28KRE.T40	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	5/25/95
CAB28KRE.TDI	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	6/16/95
CABALPHA.-8M	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	3/21/95
CABBCCON.TAC	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	6/27/95
CABDBLEC.ONE	RGC09Z_CLSM_DATA	1/2/98	'TIFF'	5/3/95

Fig. 7

8. Where Shall I Store My Images?

Most confocal microscopes are purchased for multiuser environments. The images generated in a single day can occupy several gigabytes or more. This would quickly overload even the largest hard disk, and most confocal facilities provide a means of storing data on removable media or, via a network, on

remote computers located in the individual user's lab. Moving the new data off the original collection site should be a standard practice on all computers, in order to free up space for other users. DO NOT ERASE THE ORIGINAL FILES UNTIL YOU ARE CERTAIN THAT YOU HAVE A VALID COPY ON ANOTHER COMPUTER. We now routinely transfer copies of the original files to a CD-ROM for long term stable storage. Your database files should indicate where the file is currently stored, and alternate backup storage sites, such as CD-ROM disks.

9. Lack of Standard File Format: Comments on Image Software

All the Image Gallery software described in this chapter will provide useful image galleries and thumbnails of common file formats, such as TIFF/tif, JPEG, PICT, and any file that includes it's own "Preview" image, such as Illustrator®, Photoshop, Canvas™ (Deneba Software), and others.

However, the nature of the file format generated by various confocal microscopes differs, depending upon the software written for each microscope. Thus, Bio-Rad uses a unique file format, Leica a simple TIFF format, the older Zeiss format was also TIFF, whereas the newer formats of Zeiss vary depending upon the manner in which the data was collected. The recently released Zeiss LSM 510 provides optional choice of 8-bit or 12-bit collection. But it is not yet clear if the 12-bit images are immediately converted to 8 bits, or can be stored as 12 bits. Preliminary information from Zeiss indicated that they have not yet released their file specifications. They presently are apparently using a proprietary format, but are reportedly considering shifting to TIFF 6.0 standards, with Multi-TIFF format. The new Olympus FluoView collects images in 12-bit/channel, but permits saving the files in either 12-bit/channel Multi-TIFF, in 8-bit TIFF or in 24- (RGB) bit (8-bits/channel). The NORAN confocal is based on the SGI and stores files in 8-bit format. To add further to the confusion, a Z-series of sections may be stored in a single file or as a series of individual files. A double or triple labeled Z-series can be stored in a single large file, or with each labeled Z-series as a separate file, or each section of each Z-series as separate files.

The lack of a uniform data file format is a continuing source of confusion, and hopefully, with the maturation of the confocal microscope industry, will eventually lead to a more widely used format, as is now happening in the field of radiology (DICOM) and satellite imaging (HDF).

This variation in file structure imposes special problems on both the user and on the manufacturer of both Image Processing and Image Gallery Database software. The confocal microscope community represents only a small number of users, thus most authors of Image Gallery software designed for a

mass consumer market, have not found it worth their effort to write software to meet the specialized needs of Bio-Rad, Olympus, and others.

Recently, however, some of these software companies are indicating interest in attracting confocal microscopists as customers. The most notable amongst them, to date, is ThumbsPlus (Cerious Software), which now supports 12/16 bits/channel and can directly read the Olympus Multi-TIFF files, and Bio-Rad *.PIC files. Extensis Portfolio and Canto Cumulus have indicated that they may support the Bio-Rad format in future editions, but are unwilling to state their plans at the present time.

Part of the problem derives both from the often nonstandardized format (as in Bio-Rad) or the limited number of likely customers interested in 16-bit Multi-TIFF files. The use of specialized file formats, such as those containing only two channels, or a red and green channel with a gray scale nomarski image, poses problems that Extensis and Canto refuse to address. Fortunately, Cerious Software, Inc (ThumbsPlus) has been extremely helpful in this regard.

10. Three Important Strategies When Collecting Images

10.1. Use Folders (Directories) and SubFolders (Subdirectories) to Cluster Files

Give the folder a name indicating the experiments contained within, e.g., calbindin_retina. Subfolders might include: Monkey Calbindin, Pigeon Calbindin, etc. Within the Monkey Calbindin folder, I would have additional folders representing each experiment.

10.2. Use a Database Program as You Collect Images

Using a simple, low cost database program, such as FileMaker Pro 4.0, information about each image is entered as a separate Record in the FileMaker Pro database. The program automatically assigns a serial number to each record. The Record of that image contains a Field where you insert the name of the Folder/subfolder containing the original data. A Z-series is treated as a single record (This is OK for Bio-Rad, when a Z-series is stored as a single file. May have to work out a different strategy for Leica or those optional Bio-Rad images, where a Z-series is stored as a series of single files). The Automatic Serial Number allocation is now used as the file name for storing the original images. Just to be safe use serial numbers with no more than five characters in the file name if you frequently collect extended Z-series. Bio-Rad, Leica, Zeiss, and Olympus may all overwrite the characters 8, 7, and 6 of the filename, when storing a lengthy Z-series. Thus, you might have thoughtfully called your file "myfilenw.tif." But if you have multi-label image or a Z-series, it will be renamed as "myfile01.tif, myfile02.tif, myfile03.tif...myfilexx.tif."

10.3. Use an Image Gallery Database on Completion of a Work Session

Once you have completed a worksession collecting images on the confocal microscope, and transferred files to their final storage media or location, you should immediately generate thumbnail images with a program such as ThumbsPlus. It will also facilitate your being able to recall critical features of those images, and insert suitable keywords into the correct thumbnail.

11. Comparison of Various Image Gallery Software

A number of Image Gallery software programs are suitable for use in conjunction with confocal microscopy. This is only a partial listing of such programs. The selection reflects only my personal experience, and is not intended to be comprehensive. The major criteria determining the choice of programs included: (1) cross-platform compatibility, as many microscopists work on both PC and Mac computers; (2) ability to work across a network of computers; (3) ability to work with large number of file types, including Bio-Rad, Olympus, Zeiss, and Leica; (4) current or potential ability to read 12/16 bits/channel images; and (5) ability to read data collection parameters stored in the original files.

The only program currently able to directly read Olympus FluoView 12-bit files and Bio-Rad native files is ThumbsPlus. Extensis and Canto have been less emphatic about such plans for the future. In view of the widespread use of Bio-Rad confocals, and their unique file format, this is a serious limitation on the use of Portfolio 3.0 and Cumulus 4.0. However, if you routinely convert your Bio-Rad files to NIH-Image, the resultant 8-bit TIFF file can be cataloged by all of these programs. ThumbsPlus has some limited ability to display all the images in a Z-series, although with somewhat limited performance. Cumulus, Portfolio, and ThumbsPlus indicate that they may soon be able to read and display a movie in PICS format.

One of the valuable operations provided by ThumbsPlus is that which allows the user to crop and change the brightness, contrast and color balance of the thumbnail image, without having to modify the original data file.

Based on these several considerations, there is no single program that fully meets all current needs of the confocal microscopist (*see* **Table 1**). The one that comes closest is ThumbsPlus, as it is presently the only one able to read Bio-Rad 8 bit files, Olympus 12/16 bits/channel Multi-TIFF images, can read the collection parameters of both confocal data files, and works with Windows® 3.11, as well as MacOS, Windows NT, and Windows 95®. The ability to work with Windows 3.11 is of value for users of Bio-Rad's Lasersharp 1024 which operates under IBM's OS/2. OS/2 supports simultaneous processes under Windows 3.1, but not NT or 95. The Olympus FluoView operates under Windows

Table 1
Comparisons of Image Databases

	Portfolio 3.0	Search 3.1	Canto Cum 3.0	ThumbsPlus 3.1	Confocal system
Mac	Y	Y	Y	Y	
Windows 3.11	N	N	N	Y	FV, BR (OS/2)
Windows 95	Y	N	Y	Y	
Windows NT	Y	N	Y	Y	FV, Z, L
Networkable	Y	N	Y*	Y	
8-bit TIFF	Y	Y	Y	Y	BR, Z, L, N, MD
12/16-bit TIFF (FV)	N?	N	N?	Y (Excellent)	FV, (BR?), Z
Bio-RAD *.PIC	N	N	N	Y (Mac version)	BR
Make thumbnails	Y (Fixed sizes)	Y	Y (Fixed sizes)	Y (Variable size)	
Modify thumbnails	N	N	?	Y (Excellent)	
Editing software	N	N	N	Y (Good)	
Read CLSM parameters	?	N	?	Y (Excellent)	FV, BR
Image gallery	Y (Fair)	Y (Good)	Y (Good)	Y (Good)	
List® view	Y (Good)	Y (Good)	Y (Good)	Y (Fair)	
Linkage to full database	N	N	Y (FM Pro)	ODBC?	
Full path of file	Y	Y	Y	Y	
Web enable	?	N	Y	Y	
Drag & drop thumbnail	Y/N	Y/N	Y	Pending?	
AppleScript	?	N	Y	N	
OLE	?	N	Y?	Y*	
Price	$100	$100	Basic = $100 Apple Script = $600 Network = $1,900	Single user = $70 Multi-User = $250	

Abbreviations used to indicate manufacturer of different confocal microscope systems: FV = Olympus FluoView; BR = Bio-Rad; Z = Zeiss; L = Leica; N = Nikon; MD = Molecular Dynamics.

3.11, although there is some expectation that a Windows NT version will be available in the near future. The only shortcoming of ThumbsPlus is the need for further development of the List View interface and the need for support of Drag and Drop on the Mac. It is fully cross platform compatible.

ThumbsPlus, Portfolio, and Cumulus are able to work across a network, although with varying reliability and great variations in cost. ThumbsPlus and Portfolio include this capability for approximately $100 (U.S.). Canto Cumulus charges an additional $1800 for this capability. Although Cumulus is an excellent program, you will have to decide if the robust industrial level performance of Cumulus justifies the enormous added expense. However, Cumulus 4.0 has a number of excellent functions, including very smooth interactions with FileMaker Pro, ability to handle vast numbers of images at very high speed, and the most sophisticated search engine of this group of programs. Portfolio 3.0 is a program that has been on the market for a long time, under different names. Initially marketed by Multi-Ad as Search 1.0, then sold to Adobe and marketed as Fetch 2.0 and now marketed by Extensis as Portfolio 3.0. The major change in Portfolio is the introduction of cross platform compatibility, increased numbers of file filters for reading a broader range of file types, and ability to work over a network. The program is very easy to learn, generates thumbnails very quickly, and is supported by a major corporation. Hopefully, Extensis plans to maintain an interest in this excellent, but frequently orphaned, product.

12. How Many Versions of the Image Should I Save?

There is no perfect image of a specimen. This is all the more true in the case of a confocal image. The number of variables can be overwhelming, and include selection of the field, orientation of the image, noise in the images, fading, depth of focus of the picture, change in contrast, brightness, color balance, etc. As a result, you may find that you have several different images of the same preparation, each showing slightly different features. Each such sample adds additional burden to your storage and retrieval capability.

Once you have collected an image, however, you invariably find it desirable to perform various procedures on the dataset that are collectively referred to as "Image Processing."

Image processing may consist of a range of operations varying from simple change in brightness or contrast, to cropping an image, generating stereo views, animating a sequence, reslicing the stack of images in alternate planes, changing colors, selectively editing parts of the image to heighten detail, and then saving the new set of images in a location associated with the original file. This requires massive amounts of storage capacity. This is manifest at three different points in the use of CLSM: (1) data collection, (2) image processing and analysis, and (3) preparation of final image for publication.

Inventing file names for such images will again tax your imagination. I suggest that they should be only slight modifications of the name of the original file, or else you may not be able to keep track of their heritage, in the event that you want to go back to the original file to modify or resample the image that provided the interesting variant image.

Each new file can either be associated with a new Record in your master database, or you can dedicate a Field in that record to indicate what Image Processing operations you performed on the original dataset, and where it is stored. My own preference is to keep this information in the same Record as a history of this image set. This strategy will help track related images as you prepare for final publication.

13. Conclusions

13.1. Why do You Need a Database?

Most scientists are told of the importance of taking careful notes from their first visit to a laboratory. Some of them even do so! Some people are naturally inclined to planning out every detail of their lives in advance, including how they will store their collections of rocks, stamps and confocal images even before they start saving them.

But very few scientists seem to have planned out a global strategy for their data storage. I start collecting rocks, mix in some stamps in the same box; and add in photos, books, and miscellaneous other lifetime accumulations. When the box becomes too full, I worry about separating the items into more sensibly organized groupings. How many new boxes do I need? How shall I label them? Do I have to take everything out before I reclassify them?

In the case of complex digital images, it is important to have some means of self imposed discipline to be sure that you record all relevant information at the time of image collection. It is equally important to decide in advance where you will store the files and how they should be grouped.

To deal with the large numbers of big files associated with confocal microscopy, and to be able to find things when you need them, I strongly urge the reader to use the widely available and inexpensive database programs now available for the personal computer.

13.2. Using a Database to Help Name, Sort, Identify, and Retrieve Images

Several confocal microscopy software packages already provide a means of storing specific details about each image, such as collection parameters, file size, magnification, resolution of image, and extensive comments about the image and its scientific significance. This information, however, does not

readily allow you to organize the information in a manner commensurate with your personal preference for grouping information, or for comparing the contents of large numbers of files. In addition, most software provided with confocal microscopes has very limited capacity for searching for unique or related files, e.g., "show all the calbindin containing images of the monkey and mouse retinae in transverse section." Specialized Image Database Programs, such as Portfolio, ThumbsPlus, and Cumulus provide excellent means of automatically scanning a disk, generating and displaying many images in thumbnail format, with some limited information about each image, mainly limited to some key words, location of the file, etc. Image Database programs have advantages for displaying large numbers of thumbnail sketches. However, they have limited ability to categorize multiple types of information, and generally only are useful for generating thumbnail images and comments well after you have finished collecting a large number of images. They are not useful as a means of assigning filenames, storing extensive comments, setting designated fields of the nature of the material, species of animal, fluorescent dyes used, special filters, laser power, confocal apertures, etc.

Traditional database programs, such as FileMaker Pro 4.0 and Microsoft Access provide a range of such essential functions, can be used simultaneously while collecting confocal images, but are far less effective in dealing with large numbers of images. Individual thumbnails can be displayed in these programs, but they cannot automatically scan a disk and generate thumbnails, nor can they display them readily in gallery format.

I anticipate that in the near future, these two formats will converge in a single database design.