Article

Opening and Viewing Image Data Files

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In an ideal world, undertaking computer-based light microscopy would consist of sitting down at a computer, collecting the relevant image data sets and combining these to produce a magnificent picture. A few light microscopists appear to inhabit this Utopia but for most of us the process of capturing an image data set on a computer and then printing an image from it is not a quick or pleasant one. When the few residents of this Utopia are themselves metaphorically placed under the microscope they are invariably found to be knowledgeable about microscope image capture devices, computers, computer file formats, printers and printing devices, as well as being experts in the preparation of appropriate specimens. Whilst I would never claim to have achieved this level of knowledge I have made some progress through the purgatory that precedes this Utopia. In this article I collate and relate some of this information.

Using acquisition software

When image data files collected through a light microscope are to be viewed on the monitor screen of the computer that collected the data things are, hopefully, simple: use the program employed to acquire the data to display it using its default file format, which is frequently proprietary. Attempting to follow this advice can, however, lead to hidden complications. When the system used for acquiring the data is used heavily, the image data files are best examined at leisure on other computers dedicated to analysis. This means having two, or more, copies of software that can cost thousands of pounds a copy. In such cases it is essential that any negotiations pertaining to the number of copies to be supplied are carried out when the acquisition instrument is still to be purchased, when the vendors are at their most compliant.

Unfortunately, most of the programs that are used to acquire image data through a light microscope are not well suited to producing paper, overhead transparency or film-based images that depict as much of the information available as possible, especially when montages of several frames must be produced. Here, image data files must be created that permit the information to be exported to a suite of computer software that is capable of preparing final images more easily. Of course, these files must be in a format that is common to both suites of computer software, and there are a huge number of computer file formats that are dedicated to depicting bitmapped image data (if this means nothing to you see Entwistle, 2000 & Entwistle, 2001); a small sample is shown in Table 1. Very few file formats, however, were found to be common to both image data acquisition software packages (Table 2) and the packages of software that are commonly used to prepare image data files for display (Table 1). For the former types of computer software, TIFF and bitmap were found to be the most common formats (Table 2) and bitmap, JPEG, TIFF, GIF, Encapsulated PostScript and Kodak Photo CD were identified as common formats for the latter class (Table 1). The Amiga and Targa formats were also well supported by these software utilities. Moreover, these findings only apply when 8-bit mono-modal or greyscale and 24-bit tri-modal or colour image data files are considered. Whilst other bit depths

Table 1. Image data file formats common to more than one of four packages of computer software used for viewing 8-bit greyscale and 24-bit colour bitmap-based data.

Image data file format	Adobe Photoshop	Paint Shop Pro	Irfan View	Corel Photo Paint
Amiga IFF (.iff, .lmb)	Yes*	Yes*	Yes	
Autodesk Drawing		Yes		
Interchange (.dxf)				
Bitmap (.bmp, .rle)	**Yes***	**Yes***	**Yes***	**Yes***
Corel Draw (.cdr)		Yes		Yes
Corel Photo Paint (.cpp)			Yes	Yes"
Encapsulated Post Script	*Yes**	*Write**	*Yes*	*Yes**
(.eps, A13, A14 etc.)				
GEM Draw (.gem)		Yes		Yes*
GEM Paint (.img)		Yes*		Yes
GIF (.gif)	**Yes***	**Yes***	**Yes***	**Yes***
Graphics Metafile (.cgm)		Yes	Yes*	Yes
JPEG (.jpg, .jpe)	**Yes***	**Yes***	**Yes***	**Yes***
Kodak FlashPix (.fpx)		Yes*		Yes*
Kodak Photo CD (.pcd)	*Yes*	*Yes*	*Yes*	*Yes*
MacPaint (.mac)		Yes*		Yes
Micrografx Draw (.drw)		Yes		Yes
Paint Shop Pro (.psp)		Yes*	Yes	
PaintBrush (.pcx)	Yes*			Yes*
Photoshop (.psd, .pdd)	Yes*		Yes	Yes*
PICT FIle (.pct, .pic)	Yes*	Yes*		
Portable Network Graphics (.png)	Yes*	Yes*	Yes*	
Scitex CT (.sct, .ct)	Yes*	Yes*		
Sun Raster Images (.ras)		Yes*	Yes	
Targa (.tga, .vda) .	Yes*	Yes*	Yes*	
TIFF (.tif)	**Yes***	**Yes***	**Yes***	**Yes***
Windows Meta File (.wmf)		Yes*	Yes	
Zsoft Paintbrush (.pcx)		Yes*	Yes*	

*denotes that the package of computer software can also create image data files with that format.
Bold text indicates file formats that could be both read and written by all four suites of computer software
Italic text indicates file formats that could just be read by all four suites of software.

are often produced by the software which generates the image data files, these can only be read very occasionally by the software generally used for preparing the image data for printing.

Viewing single frame image data files

8-bit greyscale and 24-bit colour
For 8-bit greyscale and 24-bit colour image data files a sample of other computer programs was examined to see if they could also open files written using the more common formats described above. No single file format could be opened universally, although both the bitmap and TIFF formats came close to this and the JPEG and GIF formats, which compress image data irreversibly, were also well supported (Table 3). As none of the software tested in the creation of Table 3 could write Kodak Photo CD files the use of this format was not considered

Table 2. File formats used to record image data files using 8-bit greyscale or 24-bit colour formats by the software used to acquire the data.

Software	Image data can be saved as
Adobe Photo Deluxe	Proprietary, Photoshop, Portable Network Graphics, Bitmap, JPEG, PICT, TIFF, Encapsulated Post Script, Kodak Flash Pix
Bio-Rad SOM & Comos	Proprietary
Image-Pro Plus	TIFF, JPEG, Flat, PICT, Bitmap, Z Soft, Targa, Encapsulated Post Script
Kinetic Image Acquisition	Biorad Proprietary, Bitmap, Fenestra Proprietary, Kontron Proprietary, Micro Voxel Proprietary, Photometrics Proprietary, TIFF
Scion Image	Bitmap, TIFF
Zeiss LSM 510	Proprietary, Bitmap, TIFF, Z Soft, GIF Photoshop, Sun Raster Images, PICT, Targa, Portable Network Graphics, Encapsulated Post Script, JPEG.

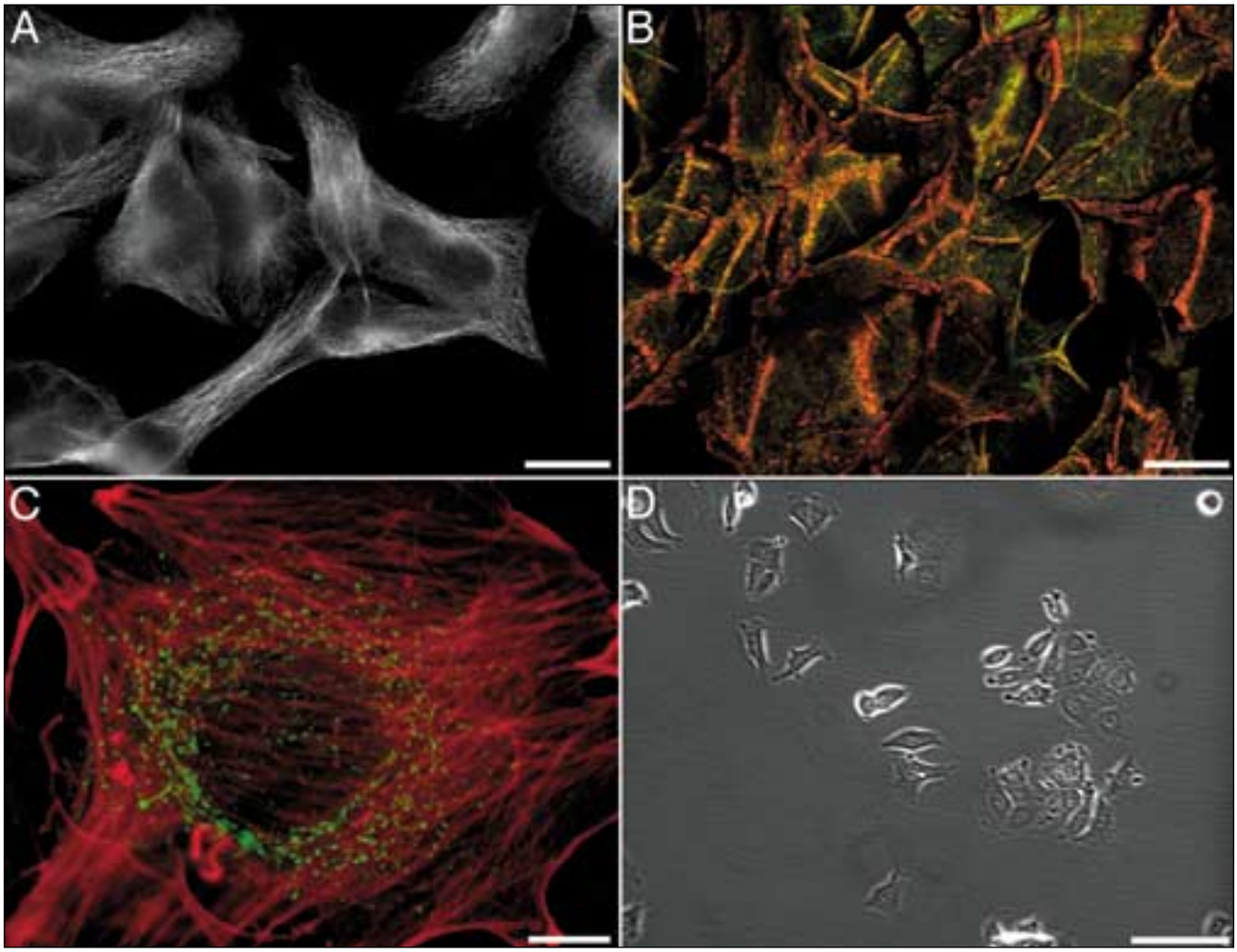

Fig. 1. With care, files can be moved without difficulty between suites of computer software using a wide variety of file formats. To illustrate this, an image data file depicting a montage of four different image data sets was created and subsequently extensively transposed between image data file formats. The original image data sets were collected as follows. (A) Microtubules (an almost universal structure-forming element in cells) were visualised in rhabdomyosarcoma cells by means of indirect immunofluorescent staining and the data recorded through a Coolview 12 cooled CCD camera (Photoinc Science) as a 12-bit monochrome data using Image Pro Plus. (B) A maximum intensity projection depicting air-dried human lung, fixed under inflation, visualised by means of a confocal laser scanning microscope as described elsewhere (Cookson & Entwistle, 2000). The specimen was prepared by the late Professor W.E. Whimster of the Department of Histopathology, King's College School of Medicine and Dentistry, the data were saved in the 36- (48-) bit colour .lsm format. (C) Actin filaments (another very common structure forming-element in cells), displayed in red, together with an unknown component of the Golgi system (responsible for much of the transport and export of proteins by cells), depicted in green. The image data sets were collected with a LSM510 confocal laser scanning microscope (Zeiss, Welwyn Garden City, UK) and also saved in the 36- (48-) bit .lsm format. (D) Phase contrast view of cells of the human breast line MCF7 grown in tissue culture by John Masters of the Institute of Urology, University College London. The original image data file was an 8-bit paletted multi-frame TIFF file. The original data files were all opened with Irfan View (in the case of the Multi-frame TIFF file a single frame was taken from the middle of the data stack) and saved as TIFF files (this operation converted all the files to 8-bit greyscale or 24-bit colour). The resulting TIFF files were opened with Adobe Photoshop, turned into a montage, annotated and saved as a multi-layered .psd image data file. This file was opened with Paint Shop Pro, flattened to a single layer and the product saved in the TARGA .tga format. The .tga file was opened with Microsoft Photo Editor and saved in the .pcx (PC Paintbrush) format. This was opened with Image Pro-Plus and saved as a bitmap. This was then opened with Confocal Assistant and saved as an AVI file. The AVI file was opened with Video Manager and saved in the Targa .tga format. This file was opened with Corel Photo Paint and saved in the Flash Pix .fpx format. Scale bars represent: (A) 20 μm, (B) 40 μm, (C) 5 μm, (D) 100 μm.

further. Few of the computer programs could read Encapsulated PostScript files that described image data, and where they could they tended to misinterpret the data, so this is a very poor format for interchanging bitmap-based files. The Amiga format was poorly supported and the Targa format was also far from being universal. For interchanging 8-bit greyscale and 24-bit colour image data files between computer programs, therefore, the bitmap and TIFF formats are those which can be read most widely. When some loss of information from the data sets can be tolerated, the JPEG and GIF formats offer the best prospects for exchanging data between computer programs. Since, of the two, the JPEG-based file format gives higher levels of compression of image data sets for comparable image quality (e.g. Entwistle 2001), this is the format preferred here.

16-bit greyscale and 48-bit colour

With devices such as cooled CCD cameras and laser scanning microscopes 8-bit data (256 grey levels) are not enough to record all the information that is available. Such devices can often generate data with a

Table 3. The ability of various computer programs to open image data files written using common 8-bit greyscale or 24-bit colour formats.

Software	Amiga	Bitmap	EPS	GIF	JPEG	Targa	TIFF
Adobe Photo Deluxe		**Yes**	Sometimes	Yes	Yes		**Yes**
Adobe Photoshop	Yes	**Yes**	Distorted	Yes	Yes	Yes	**Yes**
Canon Photo Record		**Yes**		Yes	Yes		**Yes**
Confocal Assistant		**Yes**		Yes	Yes		**Yes**
Corel Draw		**Poor**	Distorted	Poor	Poor		**Poor**
Corel Photo Paint		**Yes**	Distorted	Yes	Yes		**Yes**
Image Pro Plus		**Yes**			Yes	Yes	**Yes**
Imaging for Windows		**Yes**					**Yes**
Irfan View	Yes	**Yes**	Distorted	Yes	Yes	Yes	**Yes**
Kinetic Imaging		**Yes**					**Yes**
Acquisition							
Live Pix		**Yes**		Yes	Yes		**Yes**
Lucida		**Yes**				Yes	**Yes**
Microsoft Word		**Yes**	Distorted	Yes	Yes	Yes	**Yes**
Microsoft Paint		**Yes**					
Microsoft Photo Editor	**Yes**		Yes	Yes	Yes	**Yes**	
Microsoft PowerPoint		**Yes**	Distorted	Yes	Yes	Yes	**Yes**
Paint Shop Pro	Yes	**Yes**		Yes	Yes	Yes	**Yes**
Quick Time		**Yes**		Yes	Yes	Yes	**Yes**
Scion Image							**Yes**
Video Manager		**Yes**		Yes	Yes	Yes	**Yes**
LSM 510 (Zeiss)		**Yes**	Distorted	Yes	Yes	Yes	**Yes**

dynamic range that requires 12-bit (4096 grey levels) or even 16-bit (65536 grey levels) image data elements to accommodate it. The computer software that acquires such information has either to truncate it to 8 bits before it is written as a file or to save it in a format with a greater bit depth. As computer data are usually written as 8-bit bytes, the most common formats are those that allocate 2 bytes per image data element, or 65536 grey levels; these are 16-bit mono-modal or greyscale image data files and 48-bit tri-modal or colour image data files (Table 4). Software that is capable of reading these data sets is uncommon, and, of the programs that were used to produce final images examined here, only Adobe Photoshop supported this completely. Of the common file formats only the TIFF format appears to support 16-bit greyscale and 48-bit colour data. The other file formats available are either proprietary or consist of a header and the data, or simply the data. With these it is necessary to keep a separate record of the size of the frame of the image and some information about the structure (little endian or big endian (see Entwistle, 2000)) of the bytes used. When 16-bit greyscale and 48-bit colour image data sets are encountered the TIFF file format is that of choice and Adobe Photoshop becomes the software of choice for preparing image data sets for printing. File formats that compress true 16-bit image data efficiently are not generally available.

12-bit greyscale, 36-bit colour and bimodal data

When 12-bit mono-modal or greyscale data (36-bit tri-modal or colour data) are collected very great care must be taken in saving this to file. There is no official widely available file format that supports 12-bit (11/2 bytes per image element) data. Of the software tested here, Image Pro Plus could generate 12-bit greyscale files it calls TIFF (Table 4), but this structure is not supported formally in the definition of the TIFF format,

Table 4. File formats and software that create monochrome bit depths that are greater than 8 and colour bit depths that are greater than 24.

Software	12-bit greyscale	16-bit greyscale	48-bit colour
Image-Pro Plus	TIFF, Flat proprietary	TIFF, Flat proprietary	
Zeiss LSM	Claimed*	TIFF, proprietary	TIFF, proprietary
Biorad .pic		proprietary	
Kinetic Image Acquisition		TIFF	
Adobe Photoshop		TIFF, proprietary Raw (Macintosh & PC)	TIFF, proprietary Raw (Macintosh & PC)

*Zeiss 12 / 36-bit is really 16 / 48-bits deep.

Table 5. Software that can read monochrome bit depths that are greater than 8 and colour bit depths that are greater than 24.

Software	File formats read
Adobe Photoshop	16- & 48-bit TIFF, 16-bit Flat & Raw†
Irfan View	12-bit TIFF, 16- & 48-bit TIFF, 16-bit Photoshop††
NIH / Scion Image	16-bit TIFF, 16-bit flat*, Raw PC, Raw Macintosh**
Kinetic Image Acquisition	16- & 48-bit TIFF, 16-bit flat*, 16-bit raw PC, 16-bit raw Macintosh**

† Using 16-bit Macintosh orientation
†† Opened the top 8 bits only
* Using raw with 32-bit offset and Swap Bytes set
** Using raw with Swap Bytes set

although the program Irfan View could open these files (Table 5). In addition, Image Pro Plus has a flat format that supports the creation of 12-bit greyscale image data but these files could not be read with any of the other packages of computer software examined here. The Zeiss LSM 510 software has a 12- and 36-bit TIFF format but this still saves the data using 2 bytes, i.e. these are really 16- and 48-bit formats. Thus in general 12-bit greyscale and 36-bit colour image data files are unreadable except by the software that created them. Here, unless the image data can be truncated to 8- or 24-bit data, the best strategy is to convert it to 16-bit greyscale or 48-bit colour data. Were it not for a patent dispute that makes it difficult to determine which software is licensed to use this method it would be even better to save the 16- and 48-bit files using the widely supported, lossless Lempel-Ziv-Welch (LWZ) compression method, which generally reduces these types of file to a size equivalent to that obtained if the data really were saved in a 12- or 36-bit format. Unless

Table 6. The ability of various programs and suites of computer software to open a selection of multi-frame image files in three common formats.

Opsys & chip	Win 95 200 MHz			Win NT4 300 MHz			Mac OS9		
Reserved disk space	Little			Very large			Little		
(M Bytes)	(2 - 5 × 10^2)			(2 - 6 × 10^3)			(2 - 5 × 10^2)		
RAM (MBytes)	128			128			128		
Configured for	Images & Games			Images			Database work		
	File Format								
Software	MOV	AVI	MPEG	MOV	AVI	MPEG	MOV	AVI	MPEG
	Number of files (out of five) opened								
Animation Shop	0	4 (5)	0	0	5	0	NA	NA	NA
Confocal Assistant	0	3	0	0	3	0	NA	NA	NA
Corel Photo Paint	2 (5)*††	5*††	3 (4)*††	NA	NA	NA	NA	NA	NA
Hyper MPEG	0	0	5	0	0	5	NA	NA	NA
Internet Explorer	4‡‡	5‡‡	5‡‡	5‡‡	5‡‡	4‡‡			
Irfan View†	2	5	4	2	5	4	NA	NA	NA
Microsoft Power Point	0	5‡‡	4‡‡	2*‡‡	5‡‡	4‡‡	5‡‡	4‡‡	5‡‡
Sparkle	NA	NA	NA	NA	NA	NA	0á	0	3
Netscape Navigator	5‡‡	5‡‡	5‡‡	5*‡‡	5**‡‡	5†‡‡	0	0	5
Video Manager	0	5	0	0	5	0	NA	NA	NA
Quick Time	5‡‡	4‡‡	0	5‡‡	4‡‡	0	5‡‡	4‡‡	5‡‡
VMPEG	0	0	5	0	0	5††	NA	NA	NA
Windows Media Player	2‡‡	5‡‡	4‡‡	2‡‡	5‡‡	4‡‡	5‡‡	4‡‡	4‡‡

Values in parentheses indicate that truncated versions of the files could be opened but the full animation file was too large to play.
† Played multi frame TIFF files, as did Scion Image.
* All files slow to open.
** Large files slow to open
†† Small files play too quickly
‡ Would play sound but no image displayed
‡‡ Large files displayed but with obvious unacceptable discontinuities
NA indicates software neither present nor appropriate (compact, free software loaded anyway).

Table 7. The sizes of the animated image data files used to validate how easily they were opened and how well they displayed.

	MOV (MBytes)	AVI (MBytes)	MPEG (MBytes)
Animation No. 1, Air dried human lung	26.1	26.1	0.2 (26.1)
Animation No. 2, Video time lapse	94.1	94.1	1.3 (94.1)
Animation No. 3, Morphing	37.0	37.1	0.2 (37.1)
Different Commercial Animations	54.5 (869.2)	1.0	0.3
Different Commercial Animations	38.8	0.5	0.3

Values in parentheses indicate file size when uncompressed.

you are sure that the software you use is licensed correctly, the use of this compression method is probably best delayed until sometime in 2003, when the patent expires. Finally, bi-modal or two-channel data are occasionally generated. These are best converted to tri-modal, i.e. 24- or 48-bit colour image data, with the blue channel left empty of information prior to saving it in an appropriate format. Here too the use of the LWZ compression method will eventually be a sensible option to use routinely.

Viewing multiple frame image data files

With multi-frame image files there is a much smaller choice of common formats: the multi-frame TIFF format, the AVI format, the MOV format, the compressing animated GIF format and the highly compressing MPEG format. Programs that can read some of these some of the time include Video Manager, Corel Photo Paint, Irfan View, Animation Shop, Confocal Assistant, Windows Media Player, Quick Time, Microsoft PowerPoint, Internet Explorer and Netscape Navigator.

Of the formats available for recording uncompressed 8-bit greyscale and 24-bit multiple image data files the AVI format is the most widely supported and is therefore the best to use (Table 6) but the files can be very large (Entwistle, 2001 and Table 7). Two such suites of software for use on computers that use a Windows-based operating system are available free of charge, at least for non-commercial users, over the internet: Irfan View (Table 8) and Confocal Assistant (Table 8). The MOV format is also well supported, but of the software examined here only Corel Photo Paint could write in this format, making its use less than ideal. The multi-frame TIFF format is almost completely unsupported except in packages of software designed for capturing sequences of image data files (Table 7) and where possible is better avoided. When files in this format are encountered Irfan View and Image (another computer program which is free) can be used to view them (Table 8: NIH Image for Apple computers or Scion Image for Windows-based computers). Instructions for playing multi-frame TIFF files with these are available on the internet from

[http://128.40.242.20/DisplayFramer1.htm].

When file formats that can compress data are employed there are advantages to using the MPEG format for displaying sequences of image data collected through a light microscope. These were described earlier in this series of articles (Entwistle, 2001) and this remains the format of choice. Moreover, the display of this format is also widely supported and more than one package of free computer software is available on the Internet, e.g. Irfan View, described above, and VMPEG, written by Stefan Eckart (Table 8). Further, computer software that can convert AVI files into MPEG files, AVItoMPEG, is readily available at no cost through the Internet (Table 8), and instructions for setting up and using this software are similarly available from

[http://128.40.242.20/AVI2MPEGFramer1.htm].

It was found that when 12- or 16-bit greyscale and 48-bit colour multiple frame image data files had to be archived, in addition to proprietary file formats, the multi-frame TIFF had to be used as it was the only format examined which could accommodate data of this type. Here the proprietary file formats and the software that created the files are the best to use for viewing them.

Unlike the display of single frame image data sets, problems can arise when multi-frame files are displayed; small data sets can be displayed too rapidly, sudden and obvious discontinuities can arise when large data sets are displayed or, worse, the files will simply not open at all (Table 6). In addition, the quality of reproduction of animated sequences of image data files displayed using the same computer programs can vary on different computers and computer monitor screens. The computer itself can create problems if it is too slow or has insufficient memory, insufficient space on its hard disk drive or if the operating system is designed inappropriately or configured poorly. The interaction between the computer and the monitor or other display devices such as video projectors can cause problems if they are poorly matched or if the data cannot be sent for display quickly enough. Lastly, the monitor can cause problems if it is too small or cannot depict enough colours to display the data correctly.

Table 8. Computer software used to create this article.

Software	From	Version used here	Cost
AVItoMPEG	http://www.mnsi.net/~jschlic1.	1.7	Free
Confocal Assistant	Todd Clark Brelje ftp://ftp.genetics.bio-rad.com/Public/confocal/cas	4.02	Free
Corel Draw	Corel Corporation, Ottawa, Canada http://Corel.com (Can try Corel Draw 9)	7.4	£££
Hyepr MPEG	Hong Seungwook http://ftp.vse.cz/pub/msdos/SAC/pc/graph/	0	Free
Internet Explorer	Microsoft Corporation, WA, USA http://windowsupdate.microsoft.com/	4.72	Free
Image Pro Plus	Media Cybernetics, MD, USA http://www.mediacy.com/ippage.htm	3.01	££££
Irfan View	Irfan Skiljan http://stud1.tuwien.ac.at/~e9227474/	3.25	Free
NIH Image	ftp://rsbweb.nih.gov/pub/nih-image/	NA	Free
Netscape Navigator	http://cgi.netscape.com/cgi-bin/su/intro.cgi	4.07	Free
Paint Shop Pro	http://www.jasc.com/download_4.asp (30 day trial)	5.01	££
Photo Editor	In Microsoft Office Suite	8.0	£££
Photo Paint	In Corel Draw	7.4	£££
PowerPoint	In Microsoft Office Suite	8.0	£££
Photoshop	Adobe Systems Inc., CA, USA http://www.adobe.com/products/photoshop/main.html (Can try Photoshop 6)	4.01	£££
Quick Time	Apple Computers Inc., CA, USA http://www.apple.com/quicktime/download/	4.1	Free
Scion Image	Scion Corporation, MD, USA http://www.scioncorp.com/	3b	Free
Sparkle (for Apple)	Maynard Handley ftp://ftp.ncsa.uiuc.edu/Mac/Mosaic/Helpers/sparkle-245.hqx	NA	Free
Tracking	Kinetic Imaging, Mersyside, UK http://www.kineticimaging.com/products.htm		£££
Video Manager	Stoik Software Ltd., Moscow, Russia http://www.stoik.com/products/videoman/vm_demo.htm (15 Day trial)	1.1	£
VMPEG	Stefan Eckart ftp://ftp.shu.ac.uk/pub/windows/viewers/mpeg/ (v1.2a, DOS/Win9*/NT) ftp://ftp.staffs.ac.uk/PC_Software/Video/ (v1.7 Win95/98/NT)	1.2a	Free
Windows Media Player	Microsoft Corporation, WA, USA http://www.microsoft.com/windows/windowsmedia/en/download/default.asp	6.4	Free
Word	In Microsoft Office Suite	8.0	£££
Zeiss LSM510	Carl Zeiss ,Welwyn Garden City, UK http://www.zeiss.co.uk/uk/home.nsf/allBySubject/Launch+-+Zeiss-engl+JavaNavigator	2.01	££££
Zeiss LSM Browser	Carl Zeiss, Jena, Germany http://www.zeiss.de/de/home_e.nsf/allBySubject/Launch+-+Zeiss-engl+JavaNavigator	2.3	Free

The influence of the computer on viewing image data files

Size and speed

To reproduce a sequence of image data as an animation, each frame must be extracted from the data file and sent to the display device. Ideally the system will have the capacity to carry out this operation up to 25 times a second, a frame every 40 ms. Consider two sets of multi-frame image data:

- 8-bit monochrome information obtained with a standard video camera, about 430 kbytes per frame
- frames of 1024 × 1024 pixels each recording three channels of fluorescent emission (48-bit colour) of the sort often collected with confocal laser scanning microscopes, 6 Mbytes of data per frame.

If these are displayed on a computer that is not very old (166 MHz clock speed, 4 ms seek time for the portion of the hard drive that contains the data which describes the frame and 15 ns to read each 8-bit byte of data from the disk, giving 11 ms per frame), and an up-to-the-minute computer configured for playing large computer games (1000 MHz clock speed, 2 ms seek time, 6 ns read time, 38 ms per frame) respectively, they can keep up with the demands of the monochrome example.

The older computer could not, however, keep up with displaying the colour data even if it were truncated from 48-bit to 24-bit colour (49 ms per frame). When a machine of this type is presented with this sort of data there are four obvious possibilities:

- the computer can simply fail to display the data;
- the computer can first copy the image data file into the random access memory (RAM) of the computer, where it can be accessed far more quickly, and then display it. Here, unless the computer has more than 64 MBytes of RAM, which is unlikely, it will not even be able to cope with a total of 20 frames of data, which is quite a short movie (< 1 s);
- the computer can skip some of the frames. This strategy solves the problem of handling the data but it often causes sudden, unacceptable discontinuities to appear in the final display. It does allow any accompanying audio commentary to be reproduced accurately;
- the computer can display the frames more slowly; this, at least with data collected through light microscopes, is generally completely acceptable although with other types of animated data set this distorts any accompanying audio commentary.

In practice, the first, third and fourth approaches are the ones used most commonly. There is little the individual can do to alter this, at least in the short term, except to be aware which strategy the different programs use and switch to a program of a different type when problems that generate an unacceptable display are encountered. Of the software examined here Corel PhotoPaint, HYMPEG, Video Manager and VMPEG slow down the rate of display but show all the frames, Irfan View, Microsoft Media Player, Microsoft PowerPoint and Apple Quick Time all miss out frames but run at video rates, Confocal Assistant loads as many frames as possible into RAM and then displays the frames stored in RAM quickly followed by the frames left on the hard drive far more slowly. A potential fifth possibility, reducing the number of pixels in each frame, would not work here. The blocks of unread data lie between the blocks of data that are needed and still have to pass through the system even though they are ignored.

Other components

Factors other than the rate at which data can be read from the hard disk drive can also determine how quickly animated sequences of image data can be displayed. For example, the various sub-components that collectively comprise the computer: the hard disk drive, the video display card, the central processing unit etc., communicate with each other through a centralised channel, the BUS, of the computer, and the speed at which this can move data from one component to another can easily limit the rate of display. In addition, computer data files are not always stored as a single run of data but can be saved as a series of blocks of data widely dispersed over the hard disk; they can be fragmented. When this is the case more than one relatively slow seek operation may be needed to extract each frame, reducing the rate at which frames can be displayed significantly. This problem is most likely to become a serious one if the hard disk drives are filled or almost completely filled with data on a regular basis. For this reasons hard disk drives should never be allowed to become very full (>95%). In addition, hard drives should be monitored for fragmentation regularly and defragmented as necessary. In this respect it is worth noting that even the new super-fast computer described in the illustration above could no longer display its chosen file at video rates if just three seek operations per frame were required.

When multi-frame image data sequences are being displayed some programs open one frame at a time, while others have to open the entire multi-frame image data file before they will display it. In the latter case, and also when very large montages of single frame image data sets are created, a temporary duplicate copy of the image data must be stored somewhere, generally in one of three places:

- the random access memory of the computer, if it has enough
- in a temporary folder on the hard disk reserved for the program
- in the temporary folder on the hard drive reserved by the operating system.

When very large image data files must be opened those using the program must know where the temporary copies of the files are kept and ensure that there is sufficient free space to accommodate the duplicate files, bearing in mind that the program will almost certainly decompress or inflate any compressed file, expanding it to its full bitmap size. If this is not done, some versions of the Apple operating system will continue to attempt to open the file until they run out of any sort of free space and then will simply cease to respond to any stimulus, necessitating shutting them down completely and restarting or re-booting them. In most other cases the program will either fail to open the file, open the first frame of the file but fail to display it as an animated sequence, or continue to open the file until it runs out of space and then fail to open the file. The allocation of memory is dependent upon the primary use of the computer; it often varies with different computer operating systems and must be resolved on a case by case basis, taking it beyond the scope of this article.

Some programs designed to project a series of illustrations and animations as the accompaniment to a talk or presentation, for example Microsoft PowerPoint, contain another hidden danger that relates to the management of files. They do not necessarily include multi-frame image data files as part of the final presentation file. Instead they retain the first frame of the animation as an image and keep a pointer to the absolute location of the original file. If the location of the file changes then the software managing the presentation can no longer locate the file and it will not display the animation. PowerPoint is not, therefore, a good package of software for displaying animated sequences of image data if they are to be transferred from one computer to another.

The image display device and the computer

If image data files are to be viewed the computer and the display device it controls must be co-configured correctly. There are three parameters that can be adjusted: the size of the frame, the number of colours used and the rate at which the view on the screen is updated, the refresh rate. As an example of this, unless the display size is set to 800 × 600 or 1024 × 768 pixels, many video projectors will simply fail to display any sort of image at all.

To change the setting:

- With most Windows computers find the control by clicking the Start button found in one corner of the desktop, choose Settings in the pop-up menu and opt for Control Panel in the sub-menu. Locate Display in this window, double click on this and the Display Properties window opens. In this window click on the Settings tab to see information about the Display Area, the Color Palette and the refresh rate.
- With Apple computers click on the Apple icon in the top left corner of the screen, choose Control Panels in the drop-down menu, Monitors in the pop-out menu box, and the window that opens gives information about the set up of the screen.

The Color Palette is best set to 24-bit or 32-bit colour and if absolutely essential can be set to 16-bit colour. 256 colour options should not be used, especially when compressed image data sets are to be viewed, as the colours displayed can become completely distorted. Once the colour has been set the monitor Display area should be set to be as large as possible. Here the amount of video memory can become a limiting factor: video memory is usually a separate allocation of RAM called V (video) or D (display) RAM. As a rule of thumb, this needs the size of the display frame times the (8-bit) byte depth of the colour to be projected, and so 24-bit colour with a Display area of 1280 × 1024 needs 4 MBytes of special display card memory. In general, the refresh rate will take care of itself, although this should be at least 25 Hz or 40 frames per second. Where the viewer has control of this parameter it should be set as high as possible without compromising the other settings. Here too it may be necessary to configure the operating system of the computer for the individual type of monitor to get the very best from it, a topic which is again beyond the scope of this article. If many relatively inexpensive computers are to be used extensively for viewing image data files they may need to have a separate image data card fitted in them (I have found both ATI [http://www.ati.com/na/pages/na_index.html] and Matrox [http://www.matrox. com/] cards to be excellent and have no experience with any others) but their installation can be tricky and you may need professional help with this.

Summary

Saving single frame uncompressed 8-bit greyscale and 24-bit colour image data files in the TIFF or bitmap formats make them most accessible. When the data sets can be compressed, JPEG-based formats are the best choice. The use of 12-bit greyscale, 36-bit colour and 2 channels with any bit depth for saving image data files is better avoided. When a bit depth greater than 8 is required the 16-bit greyscale and 48-bit colour TIFF formats are the best ones to use. On Windows 95/98 and NT-based computers the most useful software packages for opening and viewing single frame image data files were Irfan View (free, Table 8), Paint Shop Pro (££, not 16/48 bit), Adobe Photoshop (£££) and Corel PhotoPaint (if you already have Corel Draw). The latter two are also excellent choices for use on Apple computers. For UNIX computers (and Windows 95/98 and NT) there is a free software suite, GIMP [http://www.gimp.org], which has many ardent fans, although I did not find it very easy to use and have not pursued this.

With multi-frame 8-bit greyscale and 24-bit colour image data files that cannot be compressed, saving them in the AVI format makes them most accessible. When they can be compressed the use of MPEG-based formats is recommended. Here it is advisable to have access to several computer programs that can read them. On Windows 95/98 and NT-based computers VMPEG, the most recent version of Windows Media Player and Irfan View (all available at no cost through the Internet, Table 8) should all be present. On Apple computers Quick Time and Sparkle (also available at no cost, Table 8) should be available. Finally, especially when viewing multi-frame image data files, do ensure that you are getting the best from your computer.

References

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