Computerised image handling in pathology

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Introduction

Three of the major branches of clinical pathology—haematology, cytopathology and histopathology—depend on microscopy. Microscopic images are often recorded as transparencies or as colour or black and white prints, and then used for teaching, publication and research. The methods and equipment used for the preparation of photographic images have changed little in the past 30 years and serve their purpose well for most applications. However, a radically different approach to the gathering of microscopic images is now available to the pathologist, based on their conversion to digitised images in a microcomputer. Systems for processing, storage and retrieval of these images have been expensive in the past, but the steadily falling cost of microcomputers and of high capacity storage devices, and the availability of sophisticated image processing software, now make systems for high quality digital image processing, storage and retrieval a much more attractive proposition for the pathology laboratory.

In this review, we outline the considerable advantages of this approach to image handling, which is summarised schematically in fig 1. In the appendix, we give details of the hardware and software options which we have used over the past three years in our own department. Many digital imaging packages are available from scientific equipment manufacturers (typically as options for microscopes), but the purpose of this article is to describe a system built up of non-specialist components that are readily available and somewhat cheaper than an integrated system. There are many advantages in building a system in this way, the most important of which (after cost) is that one joins a much larger user base, ensuring better availability and maintenance of software. We believe that by the close of this century this new technology will be very widely used in all branches of clinical pathology, and we hope in this review to indicate that it is much more accessible than appears at first sight.

Advantages of electronic imaging

RAPIDITY

In conventional photography images captured on film require chemical development before the results can be visualised. "Instant" images can be obtained (using Polaroid sheet or transparency film), but they are relatively expensive compared with other options, and may not be of comparable quality. In contrast, electronically generated images are seen instantly (allowing unsatisfactory ones to be discarded), and print-outs can be obtained within a matter of seconds if needed.

EDITING

Conventional photomicroscopy is a relatively inflexible medium. Prints which are unsatisfactory in terms of contrast or colour values require reprinting. If multi-image numbered "collages", adorned with elements such as arrows and numbers, are required for publication or display, many hours of tedious work may be required with glue, scalpel and dry transfers. In contrast, electronic images can be almost infinitely manipulated. Images can be resized, cropped and rotated in the computer so that assembly of complex images is greatly facilitated. Lettering and arrows can be added (fig 2A), and these can be kept in separate "masks", to be added when desired for printing, so that the original image remains intact. Colour values can be coarsely or subtly adjusted (fig 2A) and, if nothing else, this can be used to correct instantly differences in adjacent images, or to correct for colour, temperature and exposure variations, a very common fault in published photomicrographs (as is evident from the pages of most pathology journals).

STORAGE

It is the authors' experience that photomicrographs, despite one's best intentions, are often stored (to put things optimistically) in a haphazard fashion. Retrieval of slides can be frustrating and time-consuming, and they are subject to attrition due to loan, loss or physical damage. Even if careful indexing is performed, the final archive often becomes increasingly cumbersome, and may need to be kept at a site away from the laboratory, adding further to the inconvenience of finding a particular image.

Electronically stored images, in contrast, can be kept together on a suitable storage format (for example, optical disk) and, even if the material is kept elsewhere, it can be accessed on the user's own microcomputer. As we outline below, linkage of microcomputers via networks means that images can be accessed almost as rapidly from another country as from the user's own microcomputer. Retrieval is easy through an indexed name search, a visual search of miniature representations of the stored images, or a combination of both.

FLEXIBILITY OF OUTPUT

Photomicroscopy conventionally yields colour reversal transparencies (for projection), or prints on black and white or colour paper. Electronic images can also be produced in these formats (fig 1), but their digital nature means that they can also, without modification, be displayed on the computer itself or on a TV

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Accepted for publication
20 December 1994

Figure 1 Schematic illustration of the many options for the acquisition, manipulation and output of digitised images.

monitor, projected onto a screen via a video projector, or exported for recording on video tape. Copies can be distributed on disk and the next few years will see the increasing use of compact disks (CD-ROM) as a means of distributing images. These disks have very high capacity and, provided they are of a suitable format, such as PhotoCD, can be displayed, using inexpensive equipment, on domestic TV monitors. Laser disks (designed originally as an alternative to video tape for home viewing of feature films) represent another TV compatible, high capacity storage medium, but our impression is that, at least in Europe, little use has so far been made of this technology for storing and viewing still images.

Digitised images also lend themselves to time based display in sequence, an ideal medium for teaching. These "slide shows" can be interactive—that is, manipulated by selecting screen buttons or a via a keypad. For those who like that sort of thing, they can even be animated (for example, adorned with flying arrows, etc.) to increase their instructive value (and revive flagging viewer interest).

Requirements

The different ways in which digitised images can be fed to a computer and then output on different media are summarised in fig 1.

INPUT

Images can be digitised directly from the microscope, or scanned in digital format from an image already prepared through conventional photography (for example, a print or transparency). There are two parameters of resolution of the digital image: the number of pixels (the number of sampled points comprising the image) and the bit depth of each pixel (the maximum number of colours or tones available). In the systems we describe here the dimensions of the images are typically about 700 pixels wide by 500 pixels high, this being roughly the resolution of a 14" computer monitor. Each pixel is described by 8 bits for each red, green and blue signal giving a total of some 16 million colour variations. Whilst both these resolutions are relatively low compared with some integrated instruments and somewhat less than what is regarded as a minimum¹ they are adequate for most applications.

Direct video capture

When we began work in this field, we feared that video resolution images would be of insufficient quality as the resolution is so much lower than photography (approximately 4500×3000 pixels²). However, we have found that high quality video cameras are available (see appendix), which give images which are fully acceptable for most applications.

Figure 2 Illustrations prepared by digitised image manipulation compared with those created by conventional photography. (A) Transparencies were converted to digitised images (on PhotoCD disks) and then assembled into this collage. Minor changes were made (using Adobe Photoshop), including matching of colour values, cropping, and (in the picture of the patient) replacement of the background. Lettering and arrows were created in a separate mask, making it possible to add these to this print, while leaving the collage intact (rather as one can put lettering on a transparent sheet to overlay a conventional photograph). Conventionally applied lettering on photomicrographs may be difficult to read when it coincides with dark areas in the print and in this digitised collage we have placed "burnt out" boxes (with feathered edges) behind the lettering (most easily seen behind the words "Peripheral Blood" and "Tissue Biopsy").

Figure 2 (B) Three of the transparencies used to create the collage opposite are reproduced here without modification, as they would normally be handled by this journal. Comparison with the collage shows that some of these (notably the illustration of B and T cell staining in the blood smear) were greatly improved by digitised image manipulation.

A video camera mounted on the microscope feeds a video signal to the computer. In the computer a video digitiser “grabber” board converts the signal into digitised information. There are various types of board available that can be slotted into either a Macintosh (Apple) or an IBM PC compatible computer. Video images generated by video cassette recorders or video disk players can also be grabbed and digitised.

Scanning
Printed images—for example, a photographic print or an illustration from a journal, can

be acquired using a flat bed scanner. This resembles a conventional photocopier and versions are also available which will “read” transparencies. The Kodak PhotoCD format is also of interest here, because it allows transparencies to be digitised at very high resolution and stored on CDs. These are suitable for viewing on a microcomputer equipped with a CD drive, but can also be displayed on a TV screen from a suitable CD player.

IMAGE HANDLING
Hardware

A typical microscopic colour image will require about 1.2 megabytes when digitised. Manipulation of such information obviously requires a high speed processor (if the impatient user is not to be left waiting for long periods in front of a motionless screen). Details are given in the appendix of recommended microcomputer specifications.

Software
There are many scientific image processing and analysis programs available, but we prefer the widely used Adobe Photoshop program (see appendix). We do not attempt any analysis of the images.

IMAGE STORAGE
Image data files contain much more information than other types—for example, text files used for word processing, and so present a problem for storage. A single colour image is approximately equivalent to one high density, double sided floppy disk, and a high capacity hard disk within the computer for initial storage is mandatory. However, space on this in-built disk is rapidly consumed and other high capacity mass storage devices, preferably portable, are essential for backup and archiving purposes. The different options are summarised in the table and discussed in the appendix.

The problems of establishing an effective system for storing and retrieving images are not trivial, but a detailed discussion is outside the scope of this review. There are, however, essentially two options. One involves using one of the many general purpose personal computer databases, entering information on each image under categories such as tissue, disease and keywords, and incorporating details of where each image is located (for example, on PhotoCD, on hard disk, etc.). Some databases—for example, Filemaker Pro and Hypercard permit the creation of reduced “thumbnail” copies of images, which can help during retrieval.

An alternative is to use a dedicated image archiving program. The demands of markets such as the graphic art world mean that here too there is a substantial choice, including the Fetch, Pick Bank, Image Browser, and Cumulus programs. We currently use the Shoebox program, which was developed by Kodak specifically for PhotoCD archiving.

Mass storage devices and their approximate cost

Device	Capacity	Cost (in pounds sterling)	
		Drive	Media
Removable hard disk	44 megabytes	100	30
CD-ROM	650 megabytes	230	120
PhotoCD	100 images	230	80
Magneto-optical disk	128–2000 megabytes	600–2000	20–200
Digital audio tape	1200 megabytes	1000	50

OUTPUT

Once an image has been captured, it is temporarily stored on the computer hard disk, and modification or enhancement may be performed at this stage. Multiple images may be combined to form a single composite illustration. The image may now be output in a number of formats (see appendix and fig 1), the choice depending on the final use for the image.

Film recorders

The image may be sent to a film recorder to yield conventional 35 mm transparencies for projection. These can also be used to produce colour prints on Cibachrome paper. Alternatively, the images can be recorded on colour negative film and prints then made cheaply on colour printing paper. The quality of such prints is lower than that of dye-sublimation prints so that they could not be used in preparing large collages for publication but they are adequate for many other applications—for example, posters.

Printers

The image can be sent to a conventional laser printer but the result is rarely of an adequate standard for purposes other than checking layout and obtaining a rough indication of the final appearance. Colour prints can be produced by printers which rely (in increasing order of cost) on inkjet, thermal transfer or dye-sublimation technology. Colour photocopiers are available which can also accept digital input and print images. Dye-sublimation printers yield prints essentially indistinguishable from conventional photographic prints. Their high cost (£7–12 000) means that normally one would use a print bureau. Images can also be printed as colour separation films from an image setter to make plates for offset printing and, although we have no experience of this, it is likely to be used increasingly by publishers in the future.

Applications—now and the future

Digital image processing finds many applications in laboratory practice.

TEACHING

High quality digital images can be integrated into computer assisted teaching programs. Furthermore, images saved in FilmStrip format can be used to make animated sequences. These, and automated slide shows, make teaching/learning sessions more stimulating.

In the future, it is hoped that digitised equivalents of atlases of pathology will appear, as these contain pictures of great value in teaching and training. It is clear that publishers will increasingly publish in electronic form, and it is probable that much more copious illustration—for example, multiple colour pictures rather than one or two black and white illustrations for each condition, will be possible. It is also likely that many pathology laboratories

with responsibility for teaching will become their own publishers via the creation of digital image databases which can be accessed locally or globally through computer networks such as the Internet. Many computers around the world are already connected via the Internet, and some readers of this article may already have found how easily one can down-load images (for example, of a satellite land image showing the next weather system to reach these isles) from distant databases. The ability to distribute images in this way, based on clinical pathology photomicrographs, would be of obvious value for teaching.

RESEARCH

Writing grant proposals, preparing reports, giving presentations and writing papers for publication are part and parcel of the researcher's life today. The approach described in this review finds application in all these areas. Illustrations to accompany written progress reports, slides for presentations, and records of the results of experiments (for example, microscopic images, electrophoretic gels) are but some of the examples. We believe that in many instances the quality of this material can make a crucial difference between success and failure.

ROUTINE SERVICES

Pathologists daily translate pictures into words, whether for reporting haematology, cytopathology or histopathology samples, for describing an electrophoresis result, or for recording a stab wound. In practice it is impractical to archive digitally every image of this sort (because of cost), but with the increasing trend towards keeping medical records in multimedia format, representative pathology images may appear more widely in patients' records in the future.

Once digital images are accessible on computer networks, one can hold image based conferences for consultation on difficult cases, and this can be done on a global scale.

Problems

Any pathologist embarking on digitised image handling is immediately faced by the difficult question of what computer to use. The choice is essentially between a Macintosh and one of the many IBM PC compatible instruments. Our own preference, based on factors such as speed and the wide choice of off the shelf graphics programs, is for Macintoshes, but it should be noted that the disk formats used by Macintosh and IBM clones for high capacity removable storage media (for example, magneto-optical disks) are not readily compatible. However, small images can be transferred between the two types of computer on floppy disks and all images are exchangeable via networks. For example, the Photoshop program when run on a Macintosh offers the user more than a dozen different formats for reading and saving files, covering both standard formats

(for example, GIF, JIF, TIFF, and bitmap) and proprietary formats (for example, Amiga IFF, PIXAR, Kodak PhotoCD, and Targa).

With versatile software that permits easy image editing, the question arises as to how extensively one is justified in modifying an illustration. A number of our colleagues, on seeing this technology for the first time (and in particular the remarkable manipulation capabilities of the Photoshop program) smile disapprovingly (or furtively) and say "So you could move the band on the gel" or "You don't need antibodies—you can just paint the cells in the computer". These fears (or hopes), which were the subject of a recent article in *Science*,³ imply that conventional photographic reproduction is near perfect, whereas computer based imaging techniques belong in the realm of the counterfeiter. However, it is evident on a moment's reflection that a photograph, whether it shows an electrophoretic gel or a tissue section, is a highly artificial rendering of the original image. It is dependent on the histological or protein stain used, and on the photographer's choice of film paper and developing method. It may be non-representative either because an expert photographer has obediently enhanced or "lost" elements in the picture, or because an unskilled photographer has failed to capture the image well. In our experience computer processed images usually provide a more accurate rendition of the original than the average photograph, and it is often the old technology rather than the new that is producing deceptive results.

Another potentially controversial topic concerns the use of images prepared in another laboratory. This becomes increasingly relevant as remote image transmission and exchange is used more widely. Plagiarism may cause problems, particularly if some kind of unjustified modification or enhancement is made, and ethical and legal issues are posed which require careful consideration if users are to avoid infringing copyright. However, it is possible to exaggerate problems of this sort and they should not become an obstacle to the wide use of the new technology.

Conclusion

Desktop digital image processing has now come of age and is within the grasp of many pathology laboratories in terms of affordability and simplicity of operation. The authors have found the facility immensely useful in the setting of our research and teaching department and believe that its wider use will, in addition to benefiting the laboratory itself, also improve outside perceptions of clinical pathology and raise the profile of this medical specialty.

This work was supported by the Leukaemia Research Fund.

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Appendix

In this section we give technical data on the hardware and software options of which we have personal experience. However, this is a rapidly growing field and we cannot pretend to have carried out a "Best Buy" survey of all available options. The one rule to follow is to evaluate any system at first hand, ideally in one's own laboratory, before making a final choice.

INPUT

Microscopy

We have used a JVC KY-F30 colour camera (which contains three CCD chips) for three years and obtained excellent results. Single chip cameras, however, are substantially cheaper although they produce slightly lower resolution images. A new generation of cameras providing digital signal processing within the camera has appeared and they provide even better results.

Any high quality video digitiser board accepting RGB signals is suitable. We have used the Kingfisher and Falcon boards (Graphics Unlimited, Cambridge, UK) because the manufacturer, the designer and the software authors are locally available.

Flat bed scanners

Many scanners currently available in the market yield high quality images, and selection is very much a matter of personal preference. Colour scanners are desirable, although a grey scale scanner may be used for monochrome images (electron micrographs, electrophoretic gels). For best results images on x ray film must be acquired with a scanner fitted with a trans-illuminator, or by video grabbing from a camera.

Kodak PhotoCD

In 1990 Kodak introduced a new approach to the storage and display of 35 mm photographic images, based on scanning (at a range of different resolutions) and writing them to CDs. We are in the process of transferring many of the transparencies which we have acquired in the past to this format. The scanner and CD writer which records these images are expensive so that film has to be despatched to a processing centre, which will charge approximately £50 for a disk holding 100 images. Images stored in this format can then be viewed on a conventional television screen using a PhotoCD player, or on a computer monitor from a CD-ROM drive. Each CD has a catalogue facility, including miniature "thumbnail" images to facilitate viewing and retrieval.

IMAGE HANDLING

We prefer to use Apple Macintosh computers, because they are very well suited to image processing and have a user friendly operating

system. We typically use a Macintosh Quadra 700 with 20 megabytes of RAM (a figure which represents a minimal requirement).

For processing, we use Adobe Photoshop (Adobe Systems Inc., Mountain View, California, USA). This is a pixel based image editing program which endows, among many features, the ability to crop images, to rotate them, to adjust their intensity, contrast and colour values, and to make any alteration required in their size. Most importantly the program is modular, allowing the addition of "Plug Ins" to acquire images directly from input devices such as video grabbers and scanners. This allows us to use just one program for all image processing rather than the software provided with the different devices. Modules are also available to read a variety of image formats, including compressed images from Kodak PhotoCDs; to write in non-Apple formats—for example, CompuServe GIF (used widely on the Internet); and for specific "filtering" operations which modify an image.

IMAGE STORAGE

Removable hard disk

SyQuest 44 disks are relatively fast and can store 44 megabytes. Whilst these now represent technology developed some years ago, they are still the most widely used method of transferring data, especially to desktop publishing bureaux.

Magneto-optical disk

These are available with capacities ranging from 128 to 1200 megabytes. As they can store a high volume of data and are small and robust, they represent our choice at present for medium term storage.

Compact disk—read only memory (CD-ROM)

These disks are identical to the compact disks widely used for musical recording. With a storage capacity of 650 megabytes, each disk can store 100 to 6000 images (depending on resolution). CD-ROM drives are relatively inexpensive and provide the best option for long term storage of image data. CD recorders are becoming cheaper, making this a very good method of archiving.

Digital audio tape

This is an inexpensive approach to image archiving. Images are stored sequentially and retrieved through an indexed name list. The process is considerably slower than disk based forms of storage, so that it is not recommended other than for archiving.

Image compression

Though mass storage media such as magneto-optical disks have come down in price, the ever expanding nature of any image database means that the cost of storage may pose problems. Some kind of file compression strategy is desirable to reduce images from their working format to a more manageable size. For a full frame 24 bit colour image, the industrial standard for image compression is JPEG (Joint Photographic Expert Group). This method can reduce the file size by a factor of 20:1 with little effect on appearance.⁴ Not only does this save space, but image transmission times over computer networks are also reduced. Compression should be used with care, as successive steps of compression and decompression for viewing cause image degradation.

OUTPUT

File formats

The image may be converted and saved in a variety of different formats depending on use. We routinely save images in the native Apple PICT format for transfer to other applications. TIFF format is used for transfer to IBM PC compatible computers. Postscript formats are not suited to large bitmapped images and should be avoided except for desktop publishing.

Film recorders

There are a wide range of 35 mm film recorders available and at present we use an Agfa Slide-writer. We have found that 4000 line resolution gives distinctly better results than 2000 line resolution, suggesting that the former may be a useful minimum standard. Higher resolution recorders will only provide better results when recording onto film formats larger than 35 mm.

Dye-sublimation printers

There are only a few printers available at this level. We have found that bureaux equipped with the Kodak XLT7720 printer provide the best results, even though its specified resolution (203 dots per inch) is lower than some other printers. This is the means by which fig 2A was prepared for printing.

FURTHER ILLUSTRATIONS

Additional examples of digitised images from the authors' department can be accessed via the World Wide Web Internet, site URL <http://phoenix.jr2.ox.ac.uk>. A set of computer generated transparencies (illustrating the REAL lymphoma classification) are available from the authors (e-mail: david.mason@cellular-science.ox.ac.uk; fax: 01865 63272).