

## REVIEW

# Digital imaging in pathology

K. Micklem\* and J. Sanderson†

\*Nuffield Department of Clinical Laboratory Sciences, University of Oxford, Level 4, Academic Block, John Radcliffe Hospital, Oxford and  
†Department of Cellular Pathology, Oxford Radcliffe Hospitals NHS Trust, Level 1, John Radcliffe Hospital, Oxford, OX3 9DU, UK

### KEYWORDS

pathology,  
photomicrography,  
diagnostic imaging,  
telepathology, image  
processing, computer-  
assisted, computers,  
human, support, non-US  
government

**Summary** We discuss the potential usefulness of digital imaging within the District General Hospital pathology department. We conclude that digital imaging has progressed to the point that there are no significant technical limitations to prevent it replacing traditional photography for most applications in pathology. The case for implementing a simple digital imaging system in the cut-up room and coupled to the microscope is presented. The state of the art in digital imaging applied to pathology is described including a review of current digital cameras and their applications. The advantages and disadvantages of digital image technology are considered, together with issues concerning the equipment and software necessary for creating a useful system.

© 2001 Harcourt Publishers Ltd

## INTRODUCTION

### Imaging in pathology

Cellular pathology cut-up, specimen preparation and reporting has changed little in the last century, and the final diagnosis is essentially based upon the written report of the pathologist as a summary for the end-user, be it the surgeon, GP or coroner. Nevertheless, we now live in a world of sweeping change: in particular the application of information technology and electronic communication. What then has the application of information technology to imaging to offer to the pathologist?

Routine pathology is naturally image-based and involves both macroscopic and microscopic inspection of tissues and cells. Describing the specimen using an image as an adjunct to the written report would improve on current practice, for example measurement of gross specimens at cut-up.<sup>1</sup> Conventional photography is not practicable on the grounds of speed and cost. Polaroid film has previously been used in an attempt to provide a fast macroscopic record<sup>2</sup> but this is not widely used. The economy and speed of digital imaging however, makes it possible to photograph every specimen, if so desired.

Dissection is necessarily destructive, so digital imaging can provide a sequential history through complex cases. For example, the value of a photographic record cannot be underestimated in cases of large bowel carcinoma: each case received in this hospital is already routinely photographed to document the resection margins and establish whether the tumour has spread to involve the radial margin or peritoneal surface. Digital imaging can also mimic the speed of frozen section work: an image can be presented immediately to the surgical team either alone or to corroborate a frozen section diagnosis.

In every case, illustrations can both support and amplify the classical written report.<sup>3</sup> This is particularly important where the increasing complexity of medical diagnosis leads the pathologist to be part of a wider team involved in patient care. For example, in certain cases macroscopic tissue abnormalities cannot be seen from inspection of an isolated biopsy, for example in a referred microscope slide. In this case an image of the biopsy site may assist the consulting pathologist.

### Changing to digital imaging

There is always initial reaction against adopting new technology. Some reasons for maintaining the status quo are:

- I don't really need it, so why bother.
- The technology is too problematic/there is no IT support on site.

- I don't have the time to learn how to use the equipment effectively.
- The surgical team won't use the online data: they want a single-page report.
- The money for start-up and maintenance costs is not available.
- We won't save time or money in the long term.
- Digital images at the microscopic level will limit information available on the H&E slide, which may be sidelined and forgotten.
- My images may be lifted and used by other clinicians out of context and without proper description or acknowledgement.

Nevertheless, set the objections of changing to a new technology and method of working against the advantages and disadvantages of digital imaging. The potential advantages, if used with forethought, clearly outweigh the relatively few disadvantages, and have been reviewed before.<sup>4,5</sup>

Digital imaging equipment is often more expensive than the photographic equivalent but the consumable costs are much lower. Many pieces of expensive equipment no longer have a part to play in digital imaging, such as photographic enlargers, and this lowers the overall cost compared with photography.

## DIGITAL IMAGES

### Fundamentals

Digital imaging is radically different from photographic imaging. Fundamentally the image is virtual, being stored as a series of numbers. It is seen only when displayed, and physically exists only when printed. The great advantage of digital images is the ease with which they may be manipulated. A useful analogy is with text. Word processors handle data in a digital form and have completely taken over from the typewriter, nowadays a rare item in the modern office.

The two characteristics of the digital image are the number of picture elements (pixels) comprising the image and the bit depth, being the number of binary digits available to encode the intensity. Colour images usually record 256 ( $2^8$ ) intensity levels for each red, green and blue data in 24 bits of information. For example an image may be described as having  $1200 \times 1000$  pixels, 1.2 million in total. Each pixel of such an image can have one of  $2^8 \times 2^8 \times 2^8$  or about 16 million different colours. The size of such an image is 3.6 megabytes (Mb), there being eight bits to a byte.

The size of the image when viewed, output to film or printed is governed by the resolution of the output device. This is usually defined in dots per inch (dpi). Typical values for computers screens are 72–96 dpi, for printers 300 dpi and film recorders 1300 dpi. Thus our

example image of  $1200 \times 1000$  pixels will just fit on a large computer monitor, will print out at  $4 \times 3.3$  inches or will half fill a 35 mm transparency frame.

### Advantages

#### *Speed*

The image is viewable nearly instantaneously. This has the advantage of a high work rate, and sub-standard images can be rejected immediately. A new image can be re-acquired without setting up the specimen again.

#### *Low cost consumables*

The media upon which the digital images are stored is a consumable. The most useful storage media currently costs 50 pence for 200 images (see below). Output media is also relatively cheap: digital prints are cheaper than their photographic equivalents.

#### *Duplication*

Digital images can be copied exactly without loss since they are composed of sequences of numbers. Photographic duplication of an image always results in a sequential loss of information.

#### *No digitization loss*

Many image manipulation processes involve digitization, so it is a definite advantage to have a digital image to start with. This avoids any shortcomings arising from digitizing photographic material, such as dust and scratches. In addition the stored digital image does not deteriorate.

#### *Communication*

Digital images can be transported easily. Within seconds of creation they can be sent worldwide via telephone and satellite links. Hundreds of images may be transported on disk or on a portable computer.

#### *Manipulation*

The single most significant area in which digital imaging has had an impact is in manipulation and presentation of the image. Contrast and colour balance can be adjusted and the image presented in a variety of ways. Images can be inserted into the body of a written report with ease.

### Disadvantages

#### *Organization*

An electronic image can be easily misplaced or destroyed. Effective digital imaging requires planned

procedures and effective record keeping, even for personal images.

#### *Limitations*

The dynamic range (the ability to record extremes of light and dark) of film is superior to all but specialized high bit depth digital images (such as those used by radiologists). In practice the reduced range can be overcome by manipulation of the contrast.

There is no easy way of generating high resolution output onto 35 mm film. Digital film recorders are unable to expose film at much greater than 1300 dpi, whereas the resolving power of colour transparency film is over 2000 dpi. For this reason it is important not to submit digital images for publication as transparencies but only as prints, even if editors and publishers request otherwise.

#### *Medico-legal considerations*

Without a physical original, it is necessary to ensure the authenticity of an image if required. To be legally admissible, an expert witness must be able to document a standard operating procedure to guarantee authenticity. Typically such procedures include unalterable recording of the image and inclusion of information on image creation. Any alteration of an image must be standardised and not prejudicial. It is absolutely essential that good working practices should be applied which include patient consent for uses of the image other than diagnosis.

## DIGITAL CAMERAS

### Fundamentals

Digital cameras are usually based on the Charge Coupled Device (CCD), or less commonly a Complementary Metal Oxide Semiconductor (CMOS), sensor which are photosensitive devices that convert incident photons into electrons. An image focussed onto a rectangular array of such devices can be scanned to produce an electronic 'map' of the incident photons and used to recreate the image. The most important characteristic of a camera is the number of elements in the array which corresponds to the number of pixels in the resulting digital image and hence the image quality.

Sensor arrays can only detect brightness. Colour imaging is achieved by combining signals which have been collected through a mosaic colour filter overlying the array so that each element is sensitive to one of the three primary colours (red, green and blue). The usual form is the Bayer configuration, shown in the inset to Fig. 1A, with two green filters for each blue and red filter, to approximate the sensitivity of the eye. This has the effect

of reducing the effective resolution of the camera, but in practice this can be minimized by numerical interpolation of the raw output at the cost of reduced colour accuracy. One alternative strategy is to acquire three images sequentially through primary colour filters, avoiding compromising resolution or colour fidelity. This relatively slow method is only useful for stationary objects, for our purposes a microscopic image. Another approach is to use a dichroic prism to project the image onto three sensor arrays, one for each colour.

High sensitivity cameras designed for immunofluorescence microscopy are usually cooled to reduce noise in long exposure times. The most effective systems consist of monochrome cameras, acquiring multicolour images by sequential exposure with different excitation filters.<sup>6</sup>

### Macroscopic specimens

#### *Digital SLRs*

Digital single lens reflex (SLR) cameras are available which are designed to be a direct replacement for a conventional film camera. Typically they use identical camera bodies and share the same interchangeable lenses. For this reason they are very popular with users who wish to utilise their existing collection of lenses. In general they are excellent replacements and will perform well in pathology. An example image in Fig. 1A shows the quality obtainable.

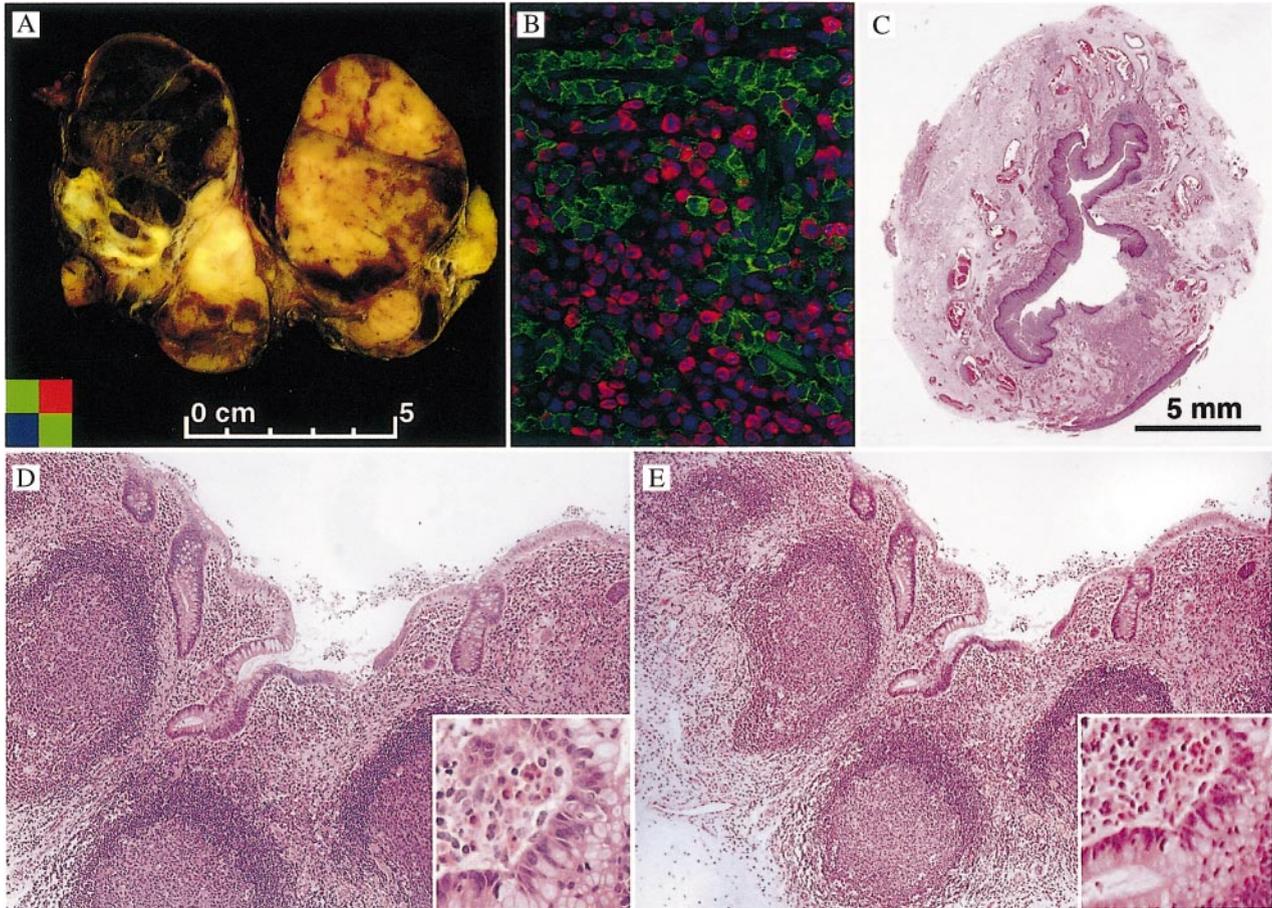
There is one important consideration however. Most CCD arrays are quite small compared with a 35 mm film frame and the pentaprism optics of a single lens reflex camera prevents the use of a lens to modify the field of view. This has been addressed to a certain extent by using large CCDs but for many cameras the field of view compared with 35 mm film is reduced. For general photographers this just means that they have to use a slightly shorter focal length lens to restore the field of view. In the cut-up room where much use is made of close focus macro lenses this may involve the purchase of new lenses.

#### *Consumer cameras*

High-end digital consumer cameras are available which are designed around small CCD sensors. They usually have a fixed zoom lens but many types have a range of close focus and teleconverter lenses to extend the basic range. Although less robust than digital SLRs they are much cheaper and can rival them in image quality. They have been used in pathology with some success.<sup>7,8</sup>

#### *Image integrity*

Image integrity is more of a problem in macroscopic images than in micrographs where a prepared



**Figure 1** Examples of digital images. (A) Gross macroscopic image of dissected human thyroid taken using a Nikon D1 SLR camera. The scale bar was added subsequently. The insert shows four cells of a 'Bayer' colour matrix. (B) Three-colour fluorescence photomicrograph of spleen taken using a Hamamatsu Orca camera. Sequential images were acquired for DAPI (blue), FITC labelled CD3 (green) and Texas Red labelled CD30 (red) and combined. (C) Transverse section of haematoxylin and eosin stained normal oesophagus. (D) Photomicrograph of a haematoxylin and eosin stained section of colon acquired using a Kontron ProgRes 3012 camera. The insert shows the image magnified four times. (E) The same area as (D) acquired with an Olympus Camedia 3030 consumer camera with a similar insert. The difference between the image quality is only visible in the inserts.

microscope slide is an authentic original. At the moment digital images are being used for forensic photography with little discussion of the unique problem of ensuring the integrity of a digital image. As the equipment for image manipulation has become commonplace there is a real risk that digital images will be perceived as less authentic than conventional photographs where there is a physical original. Security has to be applied within the camera; no system that operates after the image has left the camera can be considered verifiable. An invisible digital watermark ([www.webreference.com/multimedia/watermarks.html](http://www.webreference.com/multimedia/watermarks.html)) may be added to identify the origin of a particular image but this does not address the problem of authenticity.

The first useful in-camera system was the Kodak DCS Digital Image File Format which stores the raw image data from the camera, when the image was taken and the camera serial number. As the algorithm for generating a colour image from these data is so complex, any

attempt to reverse the process will be detectable as anomalies in the image.<sup>9</sup> Subsequently Epson has introduced its Image Authentication System (IAS) which is specifically designed to authenticate images on camera. It allows encrypted checksums of the image data to be included in a standard JPEG file. The file can be read in a normal way but can be verified at any time by Epson IAS software. The system can be applied to a variety of cameras including relatively inexpensive models ([www.epson.com](http://www.epson.com)). In the future it is expected that other manufacturers will produce their own systems.

### Digital photomicrography

High quality photomicrography is technically quite exacting and always very time consuming. Electronic imaging has so many advantages over conventional photography that it was this field that first changed from

photography, long before digital cameras were available.<sup>10</sup> The compound light microscope is the pathologist's essential tool and should be properly set up, whether used for digital imaging or not. Many scientific instruments simply will not function unless used properly. The light microscope is different, and will commonly give an image however it is set up and used. An explanation of aligning the light microscope is given in Evennett.<sup>11</sup>

### *Image resolution*

There are several factors which determine the detail resolved in a digital microscopic image: (1) the resolution of the image created by the objective lens which is a function of the numerical aperture of the objective and the wavelength of light; (2) the size of the individual sensor elements; (3) the number of sensors in the array, which in turn governs the size of the sensor and so the effective field of view of the camera. For example, a typical low resolution sensor of  $1280 \times 1024$   $6.8 \mu\text{m}$  elements is  $8.7 \text{ mm}$  across.

If we take a  $40 \times / \text{NA } 1.3$  apochromat objective as the highest optical index (the ratio of NA to magnification) in normal use, the minimum resolved distance in the primary image is  $10 \mu\text{m}$ . In order for the camera to match this resolution, it will have to sample this distance at least twice (the Nyquist theorem) and possibly more as it is using square pixels.<sup>12</sup> This gives a target value for the dimensions of an effective sensor pixel of  $4 \mu\text{m}$ . Of course we do not use such objectives all the time. The highest optical index objective a histopathologist may use is a  $10 \times / \text{NA } 0.25$  where the effective sensor pixel need only be  $6 \mu\text{m}$ . For a haematologist using a  $100 \times / \text{NA } 1.4$  oil objective the value is  $11 \mu\text{m}$ .

The low resolution sensor described above has a barely sufficient element size ( $6.8 \mu\text{m}$ ) and has a much smaller field of view than a  $35 \text{ mm}$  film camera which will have dimensions  $14 \times 9.5 \text{ mm}$  at the microscope image plane. If a camera is to sample the image at such a field of view it will ideally need from 2400 ( $10 \times$  objective) to 1300 ( $100 \times$  objective) pixels along the long axis. Modifications of the sensor field of view to match the image resolution are achieved by using an adapter, magnifying the image to reduce the field or vice versa.

Thus a haematologist or a cytologist using high power objectives may be able to work with relatively small digital images, the histologist using low power will often require very large digital images. Such images require high performance cameras which only recently have become available at an affordable price.

### *Practical designs*

*Low resolution.* Colour matrix CCD cameras suitable for general use at medium and high magnification with an

image size of  $1000 \times 800$  to  $1300 \times 1000$  pixels are readily available. The output from these camera produces a print  $8\text{--}11 \text{ cm}$  wide at full resolution on a typical printer. Beware of specifications where the pixels in the output image are greater than the elements of the CCD. This is a result of interpolating the data from a smaller original and is no substitute for an uninterpolated image.

These cameras are near the borderline of usefulness for microscopy (resolving  $6\text{--}8 \mu\text{m}$  in the image with the usual  $\times 1$  adapter) and a prospective purchaser must be aware of the limitations of the colour mosaic cameras. Accurate colour rendition is important for bright field microscopy and the compromises made by a camera designer for a typical image containing all colours may not be adequate for a histologist who often stains to give an image containing only red and blue (Haematoxylin and Eosin, for example). Typically the red and blue channels are at half resolution from a colour mosaic CCD.

If a low resolution camera is being purchased, then the advantages of sequential or three CCD cameras become significant and this type should be considered. These cameras do not compromise resolution in producing a colour image, each colour is at the same resolution. The sequential designs are very effective for fluorescent microscopy when used with 'Pinkel' filter sets ([www.omegafilters.com](http://www.omegafilters.com)): an example image is shown in Fig. 1B. Some use CMOS sensors which can give advantages over CCDs.

*Consumer cameras.* A recent development has been the availability of microscope adapters for consumer digital cameras with fixed lenses. Due to the economies of mass production these cameras are cheap (less than  $\pounds 1000$ ) and have very good performance.<sup>7</sup> Currently three camera manufacturers, Nikon, Olympus and Kodak as well as third party vendors produce adapters. The Nikon Coolpix can be connected directly to some eyepieces.<sup>13</sup>

These cameras have quite large colour matrix CCD arrays, up to  $2048 \times 1536$  elements. They have high rate video output for focusing and in addition the Nikon CoolPix has a rotating split body allowing the built-in LCD display to be used for focusing. Some come with a remote control making it very easy to operate the shutter without shaking the microscope. All are independent of a computer with a variety of solid-state storage devices, as well as a computer interface for data transfer.

Consumer cameras are slightly more fiddly to use than instrument types but represent a very interesting alternative to a dedicated microscope camera. An example image is shown in Fig. 1E. This shows an entire image captured using an Olympus Camedia 3030. At low power the image quality is indistinguishable from a high resolution camera (ProgRes, Fig. 1D) when reproduced at this size. The difference in quality can only be seen when the image is magnified (insets) to the equivalent of a whole page.

Consumer cameras can of course be used as conventional cameras for recording macroscopic specimens. Noteworthy is the close focus performance of the Nikon CoolPix (20 mm).

Another alternative is to use one of the digital SLR camera bodies with a standard photomicroscope adapter. This option may be attractive if the microscope camera also has to be used to record macroscopic specimens. However digital SLRs are expensive and are not particularly high resolution. Whilst digital SLR cameras have revolutionized press photography, they are probably inappropriate for mounting onto a microscope.

*High resolution.* Ideally a histologist wants a camera capable of acquiring images of perhaps  $3000 \times 2000$  pixels with good colour fidelity. One can purchase cameras with CCD arrays this size but they are extremely expensive (£50 000). Studio photographers, who also require high resolution digital images, have for some time been using cameras which scan an image. These work in the same way as the familiar flatbed scanner or modern photocopier, mechanically scanning a linear (one dimensional) CCD array across the image. In fact, one studio camera, the Leaf Lumina, was modified for photomicroscopy. Although the images produced by such cameras are excellent<sup>12</sup> they have the limitation of slow operation. On a microscope a Leaf Microlumina can take up to 2 min to acquire an image. In addition the lack of high rate video output makes focusing quite difficult.

A solution to the problem of extracting high resolution from a small CCD was found by reducing the sensitive area of each CCD element by overlaying a mask with pinholes. To sample the image between the pinholes the whole CCD array is moved. As the distances involved are in the micron range this may be achieved using piezoelectric transducers which are fast and accurate. Even with a colour mosaic CCD, scanning algorithms which sample each part of the image with more than one colour give excellent colour resolution. This camera, the Kontron ProgRes, set a new performance standard.

In the last 2 years many of the main microscope manufacturers have adopted this scanning two-dimensional array strategy. Two new variants have emerged which involve using a diffraction device (Pixera and Olympus) and something similar to the original (Nikon) but clearly not covered by the Kontron patent. Zeiss have modified the original design under licence. These cameras have been integrated into digital imaging software systems which often include automatic microscope control. Although fairly expensive, such systems represent the state of the art for digital photomicroscopy. Such cameras are capable of producing images up to  $4000 \times 3000$  pixels. They are typically used with  $\times 0.63$  adapters to reduce the image size to match the sensor.

*Operation.* Some considerations will have to be addressed regardless of the sophistication of the camera finally selected. Workflow ergonomics and mode of use should be considered. Is there space for a microscope and computer side by side? Should the camera be independent of a computer, storing images internally until downloaded, or should the computer be attached? Is there room for a video monitor or should the camera have a LCD viewfinder? Should the camera have a remote control box or should it be personal computer controlled? Do you want to use a removable disk drive for direct storage? Various manufacturers have produced a good selection of cameras which can be operated in all these modes.

## DIGITIZATION OF EXISTING IMAGES

Existing photographic images can be digitized in a variety of ways. Most pathologists will have a large collection of 35 mm transparencies and these can be digitized by scanning the film. Either a film scanner can be purchased or the transparencies can be sent off to a bureau for scanning.

Consider only a high specification film scanner with a resolution of over 2500 dpi. Lower resolution film scanners and all but the most expensive ( $> \pounds 9000$ ) flatbed scanners are to be avoided. The Kodak PhotoCD scanning service available widely at different retail outlets is to be recommended. Check that the transparencies are clean before being sent for scanning.

Another use for a slide scanner is the direct scanning of specimens on microscope slides.<sup>14</sup> The slide is attached to a cut down transparency mount (a commercial version the PathScan Enabler, is available from Meyer Instruments, Inc., Houston, Texas, USA. [www.meyerinst.com](http://www.meyerinst.com)). This is equivalent to using a  $1 \times$  or  $0.5 \times$  objective on a microscope and can give very good results on large sections, for example Fig. 1C.

Photographic prints, and indeed any flat original, can be scanned with a relatively inexpensive flatbed scanner. A resolution of 800–1200 dpi is sufficient for most purposes except 35 mm transparencies. In general scan at the same resolution as the output resolution but take into account enlargement or reduction, for example to produce a  $6 \times 8$  cm print on a 300 dpi printer from a  $3 \times 4$  cm original scan at 600 dpi ( $300 \times 6/3$ ). The use of a flatbed scanner to image gross specimens has been described.<sup>15</sup>

## DATA STORAGE

### On-camera storage

Digital images can either be stored on a storage medium within the camera or the camera is operated as

a computer peripheral and the images are immediately stored on the computer. Images stored within the camera can either be downloaded via a communication cable or the storage media is removed and placed in a reader connected to the computer. There are a variety of camera storage media available.

CompactFlash and SmartMedia are devices based on flash memory which requires no power to retain data. They are entirely solid-state, very robust and long lived. Capacities range from 4Mb to 320Mb. Smart Media is somewhat cheaper than CompactFlash but the maximum capacity is lower. The PC Card or PCMCIA Card is a standard for removable mass storage devices which include flash memory and hard disc drives. They have a range of capacities from 8 Mb to 1 Gb. They are used in high-end digital SLR cameras.

There are a variety of different reader devices available, several of which read more than one media type. Direct communication with the computer is either via a serial interface to transfer data or a Small Computer System Interface (SCSI) if the camera is computer controlled. The older RS232 serial interface is universal but very slow and so the newer, faster Universal Serial Bus (USB) interface is desirable.

## File type

Digital images are stored as sequences of numbers in data files. There are a variety of different file formats, some of which are generic and others, more limited in their application, are specific to particular software packages or branded hardware. It is important to recognize the differences between them. Failure to use an appropriate format may prevent its use without specific software or result in the image being damaged irrecoverably. In particular the repeated use of JPEG (Joint Photographic Experts Group) compression will result in degradation of the image.

Microsoft Windows operating systems identify file types by a three character suffix to the filename (e.g. image\_name.tif, image\_name.jpg). Apple Macintosh systems use another method not involving the filename but it is good practice to use the extensions in any case (and to use file names which work across all operating systems). We discuss the most common image file formats and compression algorithms below.

The tagged image file format (TIFF) is the most common image file type. It is common to find LZW compression (see below) used in TIFF formats.

JPEG is actually a compression standard rather than a file format. More correctly the files should be termed JFIF (JPEG file interchange format) files.

The graphics interchange format (GIF) is an image file format optimized for use in the world wide web. It uses a reduced colour palette and LZW encoding to efficiently

encode images. GIF works well on images with only a few distinct colours such as line drawings and simple cartoons. It is not appropriate for most digital imaging applications.

The Microsoft Windows operating system and the Macintosh operating system both have native image formats (BMP and PICT, respectively) but they are not widely supported on other systems.

Lempel-Ziv-Welch, or LZW compression replaces strings of characters with single codes, achieving compression where there is repetition and is applicable to all data types. It is occasionally used in TIFF files and is the basis of the GIF file format. LZW compression is lossless, that is decompression recreates the original data exactly with no loss of information.

JPEG takes advantage of the fact that small colour changes are perceived by the human eye less accurately than small changes in brightness. The algorithm selectively removes the high-resolution colour information. This method can achieve high levels of compression but it is not reversible. Grayscale images are less efficiently compressed as they, by definition, contain no colour information.

## Image file storage

The hard disk of the computer used for processing the digital images is the immediate storage medium. Hard disk capacity and value for money has kept pace with the increase in capacity required for imaging. 20 Gb or 40 Gb hard disks are fairly standard on most computers. The accumulation of image files is prevented by medium and long term storage on removable media. There are many types but only a few are recommended for image storage.

Digital tape is unsuitable on the grounds of life and access time although it has the lowest media cost.

Removable magnetic discs are cheap and widely available. In particular the 100 Mb Iomega Zip is a good choice due to its near universality. The cheapness per stored byte of the larger formats (250 Mb Zip and 1 Gb and 2 Gb Jazz) is offset by the consequences of failure of media containing such a large amount of data. They should be used for transport (where an original exists elsewhere) rather than as an archive.

Magneto-optical disks are cheap, reliable and have a very long life. Unfortunately they are difficult to use with the Macintosh operating system and this limitation has largely killed off this type of media which is becoming rarer on other operating systems.

Compact discs have two writable variants, write once (CD-R) and re-writable (CD-RW). Although re-writable disks may appear to be the most useful choice, the fact that write once disks do not change and so need be indexed only once, outweighs any other considerations.

Blank CD-R disks are so cheap it is cheaper to write a new CD three times than use a CD-RW. CD-R disks are our medium of choice for archiving image files although digital versatile disk (DVD) types may supersede them quite rapidly.

DVD uses similar technology to the CD but with higher capacity. There are two recordable types: DVD-R similar to CD-R with up to 4.7 Gb capacity, and DVD-RAM which is re-writable with capacities of 2.6 and 5.2 Gb. As DVD-R drives become widely available (DVD-R drives are now standard on high-end Macintosh computers) they will probably predominate.

Concerning the longevity of recorded CD-R and DVD-R disks the manufacturers specify that long term storage in cool, dry and dark conditions will maximise the life of the media, estimated at 50–200 years. Avoid handling either surface to prevent marking and store in opaque enclosures. Detailed information on CD-R can be found at [www.fadden.com/cdrfaq](http://www.fadden.com/cdrfaq).

In conclusion, a workflow of camera – hard disk – 100 Mb Zip – CD-R or DVD-R is recommended. It cannot be stressed too highly that image files be properly stored (in duplicate) to prevent loss. It is recommended that all archive discs are indexed immediately they are made. Indexing software is widely available and varies in price according to capability.

## COMPUTERS AND SOFTWARE

The choice of computer operating system can be difficult. Decide on the availability of software: if the program you like only runs on a Windows machine then that is what you should get. In general the issues are ones of support and local knowledge. For most purposes the choice is between Windows and Macintosh operating systems. To generalize, Windows expertise is more widespread but the Macintosh is more geared to imaging.

The essential requirements of a digital imaging software package are to read/write in various different file formats, perform basic contrast and colour manipulation and provide text annotation. Some packages permit more operations, such as image analysis.

To avoid degradation from repeated compression only lossless compression should be used to save images whilst processing. Cameras often use JPEG format; immediately save the image in a lossless format, usually TIFF, or the software's proprietary format. Do not leave the file in JPEG format whilst it is being processed. Once the image has been edited, it can be archived in a compressed format.

There is an enormous choice of generic image manipulation software. Here are a few examples: Adobe PhotoShop ([www.adobe.com](http://www.adobe.com)) is the de facto standard image manipulation software. It provides comprehensive manipulation and editing facilities. Most directly

connected imaging devices, including digital cameras and scanners operate as plug-in modules to the basic Photoshop program. Although expensive it can be obtained under an educational discount. Photoshop Limited Edition with reduced capabilities is an alternative and is perfectly adequate for most purposes. It is available for both Windows and Macintosh. Jasc Paint Shop Pro ([www.jasc.com](http://www.jasc.com)) is an image editing package that has most of the features of PhotoShop at a lower price. It is only available for Windows. Equilibrium Debabelizer ([www.equilibrium.com](http://www.equilibrium.com)) is a useful batch processing program available for both Windows and Macintosh. The shareware program GraphicConverter (available from shareware archives) is a useful Macintosh-only alternative. The free image analysis program NIH Image ([rsb.info.nih.gov/nih-image](http://rsb.info.nih.gov/nih-image)) is worthy of mention. It is capable of sophisticated analysis and automated processing. The original program is for the Macintosh but Java and Windows versions, Scion Image ([www.scioncorp.com](http://www.scioncorp.com)), are available.

Of course each microscope manufacturer and imaging system supplier has their own software dedicated to their products. Whilst expensive they are designed specifically for medical imaging. It is most important to test complete systems, either ones built up from components or 'off-the-shelf' systems, before purchase.

There is an enormous amount of excellent information available on the Internet. In particular the following URLs are recommended:

Image basics: <http://www.epi-centre.com/basics/>

Compression: [www.faqs.org/faqs/compression-faq/](http://www.faqs.org/faqs/compression-faq/)

File types: [www.ora.com/centers/gff/](http://www.ora.com/centers/gff/)

Consumer digital cameras: [www.steves-digicams.com](http://www.steves-digicams.com)

[www.shortcourses.com](http://www.shortcourses.com) and [www.imaging-resource.com](http://www.imaging-resource.com)

Camera adapter theory is covered in the Diagnostics Instruments site: [www.diaginc.com](http://www.diaginc.com)

## OUTPUT

### Print

There are several colour printing processes available for digital image printing including laser, inkjet, solid ink and dye sublimation. Each method has particular advantages but the inkjet process predominates in the printing of digital images. In the past when inkjets were lower resolution there was a problem using inkjet prints as originals for publication. The continuous tone dye sublimation process was used but is now more expensive and lower resolution than inkjet. The six-colour printers readily available from several manufacturers are currently the best method of printing images. Glossy papers produce a print which looks similar to a photograph but they are much more expensive than

matt prints which are just as good as originals for reproduction.

### 35 mm transparency

Digital images can be output on 35 mm transparency film with a digital film recorder. These are expensive items costing several thousand pounds. They are used extensively in medical illustration departments for producing slide presentations from software such as Microsoft Powerpoint. As noted earlier, the resolution is limited and so transparencies are unsuitable for use as originals for reproduction.

### Electronic (presentations/web)

Micrographs can be presented on screen or projected with a digital projector. A particular strength is that multiple images can be presented simultaneously allowing subtle differences to be illustrated. Images can be inserted into Microsoft Powerpoint presentations but beware of adding an inappropriately large image; at most a full-screen image should be no more than 2 Mb and 1 Mb is fine for most purposes: re-sample a copy of the image to the appropriate size before insertion. Bear in mind that image-laden presentations will be too big to fit on a floppy disk and may require a high performance computer to display them at a sensible speed.

Output on the world wide web is quite straightforward. Information on web page construction can be obtained from many sources e.g. [www.webreference.com](http://www.webreference.com). In general JPEG images give the best quality and, as they will usually not be used as reproduction originals, high levels of compression may be used. In any case, images should be limited in file size to a few hundred kb at most to avoid long download times. Strategies such as the use of clickable thumbnail images and progressive image formats can be used. An application such as Adobe ImageReady makes image preparation very easy.

### CONCLUSION

We consider that digital images are a useful adjunct to the classical written report. Once even a simple image acquisition system has been set up, it is possible to streamline routine workloads, and increase the information available to the wider clinical team. The impact of the world wide web on hospital pathology for deriving database-stored information has recently been reviewed by Rashbass<sup>16</sup> and Wyatt;<sup>17</sup> various image databanks are already available for reference. New developments may arise from the use of digital imaging in particular fields such as image analysis,<sup>18</sup> quality control,<sup>19</sup> and remote microscopy ('Telepathology').<sup>20</sup> Already advances in

automated pattern recognition have been particularly useful in cytology.<sup>21</sup> The power and flexibility of digital imaging cannot be ignored and, used wisely, can only serve to facilitate the communication of information and benefit patient care and management.

### ACKNOWLEDGEMENTS

We would like to thank our colleagues amongst the consultant pathology staff at the John Radcliffe Hospital for helpful discussions. We would also like to thank Alison Lockhart from Olympus (UK) for the loan of an Olympus Camedia 3030 and Margaret Jones for providing the fluorescence image.

### PRACTICE POINTS

- Digital imaging is now a practical alternative to photography in routine pathology.
- Electronic storage and manipulation of images has significant advantages over photographic methods
- The effective introduction of digital imaging requires planning and organization not previously required for photography.

### REFERENCES

1. Leong A S, Visinoni F, Visinoni C, Milios J. An advanced digital image-capture computer system for gross specimens: a substitute for gross description. *Pathology* 2000; 32: 131–135.
2. Heatley M K. The value of Polaroid photography in reporting cervical cone and large loop excision specimens. *J Cell Pathol* 2000; 5: 7–10.
3. Papier A, Peres M R, Bobrow M, Bhatia A. The digital imaging system and dermatology. *Int J Dermatol* 2000; 39: 561–575.
4. Cheong S K, Micklem K, Mason D Y. Computerized image handling in pathology. *J Clin Pathol* 1995; 48: 796–802.
5. Furness P N. The use of digital images in pathology. *J Pathol* 1997; 183: 253–263.
6. Mason D Y, Micklem K, Jones M. Double immunofluorescence labelling of routinely processed paraffin sections. *J Pathol* 2000; 191: 452–461.
7. Tse C C. Anatomic pathology image capture using a consumer-type digital camera. *Am J Surg Pathol* 1999; 23: 1555–1558.
8. Belanger A J, Lopes A E, Sinarid J H. Implementation of a practical digital imaging system for routine gross photography in an autopsy environment. *Arch Pathol Lab Med* 2000; 124: 160–165.
9. Shipman W R. Regarding the Integrity of Data as Captured in the Kodak DCS Digital Image File Format. Kodak Global Customer Service & Support, 1999.
10. Shotton D M. Robert Feulgen Prize Lecture 1995. Electronic light microscopy: present capabilities and future prospects. *Histochem Cell Biol* 1995; 104: 97–137.
11. Evennett P J. Image analysis in histology: conventional and confocal microscopy. In: Wootton R, Springhall D R, Polak J M (eds). *Light Microscopy* Cambridge: Cambridge University Press, 1995: 134–150.
12. Entwistle A A comparison between the use of a high-resolution CCD camera and 35 mm film for obtaining coloured micrographs. *J Microsc* 1998; 192: 81–89.

13. Evensett P. The new photomicrography. *Proc Roy Microsc Soc* 2000; 35: 253–256.
14. Ventura L, Leocata P, Colimberti P. Digital scanning of histologic sections. *Am J Surg Pathol* 1999; 23: 1435.
15. Beer T W. Scanning of gross specimens. *Am J Surg Pathol* 2000; 24: 1170–1171.
16. Rashbass J. The impact of information technology on histopathology. *Histopathology* 2000; 36: 1–7.
17. Wyatt J. Knowledge and the Internet. *J R Soc Med* 2000; 93: 565–570.
18. Meijer G A, Belien J A, van Diest P J, Baak J P. Origins of ... image analysis in clinical pathology. *J Clin Pathol* 1997; 50: 365–370.
19. Rashbass J, Vawer A. A networked computer program for managing a national external quality assurance scheme in cytopathology. *Cytopathology* 1996; 7: 377–385.
20. Leong F J W-M, Graham A K, Gahm T, McGee J O D. Telepathology: Clinical utility and methodology. In: Underwood J (ed). *Recent advances in histopathology*. London: Churchill Livingstone 1999; 217–240.
21. Mango L J, Radensky P W. Interactive neural-network-assisted screening. A clinical assessment. *Acta Cytol* 1998; 42: 233–245.