Digital photography in anatomical pathology

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ABSTRACT

Digital imaging has made major inroads into the routine practice of anatomical pathology and replaces photographic prints and Kodachromes for reporting and conference purposes. More advanced systems coupled to computers allow greater versatility and speed of turnaround as well as lower costs of incorporating macroscopic and microscopic pictures into pathology reports and publications. Digital images allow transmission to remote sites via the Internet for consultation, quality assurance and educational purposes, and can be stored on and disseminated by CD-ROM. Total slide digitisation is now a reality and will replace glass slides to a large extent. Three-dimensional images of gross specimens can be assembled and posted on websites for interactive educational programmes. There are also applications in research, allowing more objective and automated quantitation of a variety of morphological and immunohistological parameters. Early reports indicate that medical vision systems are a reality and can provide for automated computer-generated histopathological diagnosis and quality assurance.

KEY WORDS: Digital photography, anatomical pathology, Internet, medical vision systems, education, computer-generated diagnosis, pathology reporting, quality assurance, remote consultation

Among the many functions of an anatomical pathologist are diagnosis, consultation, documentation, and education. Implicit in these activities is the need to record morphological findings both at the macroscopic and microscopic level.[1] Conventionally, this has been performed through the process of descriptive prose with inherent variations in the style, vocabulary and abilities of individual pathologists, which are often considerable and beset by idiosyncrasies. Comparisons and descriptions associated with food are commonplace with such confusing classics like "caseous necrosis" which describes a resemblance to cottage cheese and not cheddar cheese, "nutmeg liver" resembling the cut surface of a nutmeg seed and not the fruit, and "anchovy sauce" which may not be found on the dining tables in many countries. Common colours may become irreproducible when terms like "off white", "light grey", "creamy-white", "pale white", "grey-tan", "egg white" and "ice-white" may be used for the same colour.

Photographs documented the true appearances of the pathological changes and eliminated much of the inaccuracies resulting from the variation in descriptive prowess but there remained the inevitable delays of print production and cost restricted the number of photographs. Mostly black and white prints were employed in reports. Some institutions maintained elaborate photography set-ups that depended on trained staff to process and print these photos, and to develop coloured projection slides. The advent of the Polaroid film allowed instant prints and also immediate projection slides averting the problems of delays, but again costs were significant and quality was not optimal.

The development of digital photography and the rapidly falling costs of good quality digital cameras has made major inroads into our traditional way of documenting pathological findings at both the gross and microscopic level. Together with the expanding use of the Internet, digital photography paves the way for many new applications of image documentation in anatomical pathology. This review will be confined to the capturing of digital images and their current and potential applications in anatomical pathology.

Advantages of Digital Photography

There are several compelling reasons to convert to digital technology but the most persuasive is lower running costs and shorter image production turnaround times. Following the initial capital investment, additional costs are minimal. Memory cards for temporary storage of images are reusable and storage media such as CD-ROMs are inexpensive. Results are almost instantaneous and images can be edited, and the quality and composition improved. It is easier to catalogue, archive and disseminate/transmit a digital image than a print or 35-mm slide. Multiple digital copies in different formats are possible. Photographic laboratories can create glossy prints

that are virtually indistinguishable from those made from a chemical film.

In addition to improving current practice, the change to the digital medium opens up a new range of applications such as telemicroscopy/ telepathology, image processing and analysis, digital slide archiving and, eventually, automated machine vision systems. Another reason is necessity. As other disciplines of medicine and non-medical fields convert to the digital medium, lack of familiarity with digital imaging may eventually become a handicap and be viewed as a deficiency in practice.

The Digital Camera

Many of the factors important in chemical photography are not applicable to digital photography. Consideration of film speed and type are not relevant and factors such as colour, temperature and white balancing are considerably easier to compensate for with digital imaging. Focussing is much easier as many cameras have automatic fine focussing.

Three primary functions of the microscope are to resolve detail, increase contrast and magnify. Digital imaging can enhance the performance of the microscope through its ability to improve upon the latter two functions, the first being still dependent on optics. The variables influencing image quality are too numerous to discuss but good optics remains the single most important factor in the acquisition of high-quality digital images. Optical deficiencies revealed by chromatic variance and poor image clarity are far more noticeable when using a digital image sensor compared with 35-mm film.

There has been a proliferation of digital microscopy cameras in recent years and a short list of the commonly advertised cameras by the larger microscopy companies is provided in Table 1. Although such devices are generally maintenance-free, it is preferable to select a camera from a manufacturer, which reviews and updates its software drivers with the same degree of efficiency and frequency as a major computer peripheral manufacturer. This is particularly necessary given the rapid rate of evolution of computer operating systems.

Cameras fall into three main groups ranging from budget to expensive, and those for specific requirements, e.g. low-light imaging, multi-spectral imaging.[2] The cameras listed here are generally capable of reasonable imaging for transmission, presentation and print. The more expensive models mostly produce larger images of higher quality. Currently, the acquired image size is equated with quality but in the future, the crucial determinant will be the method by which the image is acquired at the sensor level as this has a greater influence. Not listed are cameras optimised for low-light photography and cameras capable of high-resolution, high frame-rate imaging. The latter have the advantage of using a technique of multiple-frame averaging to improve image quality. As such cameras have specific applications, additional expense can be avoided by ensuring that specialised features are not purchased if not required. High-end general microscopy cameras such as the ProgRes C14 and Nikon DXM1200 are capable of respectable low-light imaging.

There are essentially two groups of digital microscopy cameras – those designed for still image capture and those for 'live' image display. The latter gained popularity for clinical meetings where images are shown on a television. Recent developments have blurred the boundaries of there two types. Originally intended for use as a presentation tool, lower-priced analogue cameras combine all the visual information into a single video signal (composite video). Compared to a digital camera the quality is inferior; however, coupled with a video-capture unit ('imagegrabber'), it is a low-cost alternative. Video capture units accept analogue signals and 'capture' images at resolutions of up to 1500 x 1125 pixels through a technique based on oversampling the analogue signal. The resolutions claimed are spurious and those above the base camera resolution result in the magnification of electronic artefacts.

There are several cameras that are able to provide both functions. A high-end example is the Kontron/ Jenoptik ProgRes 3012 that is a dedicated microscopy camera capable of capturing still images up to 20 megapixels in size (5000 x 4000 pixels) and transmitting them via a digital connection to a computer. However, it also is capable of composite analogue video output and can be used for live image display. Similarly, the Nikon Coolpix cameras (950/990/995/5000/5700) are general consumer, still image cameras that store images up to 5 megapixels in size onto removable compact flash media. They also have composite video output that provides a live image view that is updated at 15 frames per second.

Dual still-image with live video output is slowly becoming the norm because even when camera and eyepieces are parfocal, it is still preferable for fine focusing to be performed automatically by the camera rather than through microscope eyepieces. The only way to provide this function is through some form of 'live' or rapidly updated camera view as found in the Nikon DXM1200, Kontron/ Jenoptik Prog Res 3012 or C14 that provide this within its image-grabbing software.

Table 1: Some typical digital microscopy cameras

High-end (>US$5000)	Mid-range (US$1000-5000)	Cameras which may optionally be microscope-mounted (US$500-5000)
Nikon DXM1200 ProgRes C14	Nikon DN100 Leica DC150, DC300	Olympus Camedia E-10, C-4040
Leica DC500 Zeiss Axiocam HR	Olympus DP12, DP50 Zeiss Axiocam MR	Nikon Coolpix 950, 990, 4500, 5000Nikon D100, D1, D1H, D1XOther digital SLRs

Note: This list is by no means comprehensive and represents the more readily available models through microscope vendors

'Live video' digital cameras remain in demand for applications where real-time image processing is required, such as in robotic telepathology where the live camera view is used for consultation or as the means by which the software-based autofocus is calculated. This area continues to develop with manufacturers using multiple image sensors to provide better image quality (so called 'triple-chip CCDs') and/or avoiding analogue artefacts through use of the relatively new IEEE1394 ('Firewire') digital interface. The Sony DFW series is an example of a range of microscope cameras that provide a pure digital live image of up to 1280 x 1024 pixels at 15 frames/second through an IEEE1394 interface.

Macroscopic Photography

In the past, the reliance has been on chemical film photography to produce black and white or more expensive colour prints to supplement the descriptive prose of the pathology report. The photocopier has also been employed for some specimens, particularly those with a flat cut surface, to produce instant, inexpensive prints that could be used to mark sites of block sampling and as a replacement for line drawings. Photomicrographs did not accompany such reports and were reserved for clinico-pathological conferences and published text.

Digital photography has changed the face of the pathology report significantly, allowing the incorporation of coloured prints of the gross specimen as well of relevant microscopic features; greatly enhancing the accuracy and reproducibility of the findings. As there are essentially no running costs involved, there is no limit to the number of images used so that it is possible to replace or supplement traditional, often lengthy, descriptive prose with high quality images that greatly improve the quality of the report. Coupled with synoptic texts, reports are more accurate and succinct[3] and lengthy block keys and hand-drawn diagrams can be replaced with digital photographs.

Several reports describe home-assembled digital photographic set-ups for routine use in surgical and autopsy pathology.[4-7] For an optimal set-up of gross photography the camera must be controlled remotely to avoid contamination, and downloading of images and specimen/accession number recognition should be automatic. While it is possible to obtain good digital images with many of the lower end cameras that produce 3-5 megapixel images, automatic downloading and remote control are much more difficult to achieve. One group has successfully developed software for these two functions employing the Coolpix 995 camera, spending about US$26500 on the system and achieving an annual savings of US$7500 in labour and film costs.[8]

We have previously described a prototype of an automated system,[9] which is now replaced by the more sophisticated MacroPath system (Milestone, Bergamo, Italy, retails at about US$18000). This system allows remote control of a video camera (with imagegrabber) through a foot pedal for zoom, capture and save features (Figure 1), auto focus, automatic downloading and the use and automatic recognition of specific specimen accession numbers. In addition, the requisition form may be photographed and stored, and the images can be stored to the laboratory information system, shared on the Intranet among pathologists or via floppy disk or CD-ROM. A useful feature of the system is the provision for measurements to be done on formalin-resistant screen. Prior calibration allows measurements to be made by drawing a line between points of interest on the image with a stylus or even finger. In a similar manner sites of sampling can be indicated with automatic labelling so that the use of lengthy conventional block keys and important aspect of report documentation can be dispensed with (Figure 2). Selected images can be incorpo-

Figure 1: The Milestone MacroPath system comprising a camera (black arrow), foot pedal control with zoom in, zoom out and freeze pedals (white arrow), formalin-resistant touch screen monitor with attached splash-proof computer (yellow arrow), infra-red bar code scanner (red arrow) and keyboard (broad arrow)

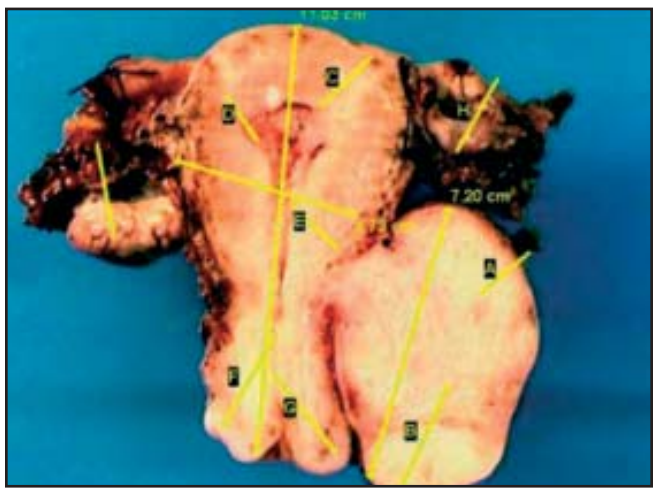

Figure 2: Digital image of uterus, bilateral tubes and ovaries with leiomyomas including a large subserosal leiomyoma. Measurements of the specimen made on the image and sites of sampling (block key) are shown. When supplemented by images of various cut surfaces, descriptive prose and block keys can be dispensed with.

rated into the report or exported through the Internet or appended as colour prints.

Microscopic Photography (Photomicrography)

A common mistake when making the transition to digital imaging is to be fixated upon the camera. Although it is the camera that converts the analogue image into a digital form, it is the interaction of all the components that determines the final outcome. Therefore, any weak component in the imaging chain can be detrimental to the final product. The minimum hardware components required for digital photomicrography are a digital camera, camera-to-microscope adaptor, microscope, and a computer with an adequate graphics card and monitor. Factors such as system Central Processing Unit speed, hard drive speed and capacity, and the nature of the removable storage medium are secondary concerns.

Dedicated digital microscopy cameras generally do not have a lens and attach to the microscope via a common connection, the 'C-mount', a 1-inch diameter x 32-threads/inch screw originally designed for 16-mm motion video cameras but now also adopted for lens- less video cameras. When a digital camera has a fixed lens, a feature of general consumer cameras such as the Nikon Coolpix series and Olympus D-3030 series, a 'C-mount adaptor' provides another layer of optics to compensate for the camera lens.[8]

The importance of good optics cannot be overemphasized, irrespective of the image sensor used. Noticeable improvement in digital image quality is achieved by upgrading objective lenses from fluorochromatic ('plan fluor') to apochromatic ('plan apo'). It is beyond the scope of this paper to discuss the relationship between pixel size and optical resolution. Suffice to say, each component in the imaging pathway should be calibrated to its optimum state. This includes Köhler illumination for the microscope, white balancing for the camera, and monitor calibration (contrast, brightness and gamma settings).

Digitisation of Previous Collections of Projection Slides (Kodachromes)

The conversion to digital images makes previous collections of macroscopic and microscopic images on film obsolete. These can be scanned with a film scanner (if the chemical images are still in strips they can be scanned more expediently) and stored as digital images, but a more convenient and expedient method is to photograph the projection slide over an X-ray viewing box, a procedure that takes no more than a few seconds compared to the more laborious and lengthy scanning process. Some editing of the acquired image may be necessary. Similarly, the difficulties of acquiring low-power scanning images of an entire tissue section can be circumvented by photographing the tissue section over an X-ray viewing box.

Practical Aspects

Instead of mounting the camera via the C-mount it may be placed against one eyepiece of the microscope and images can be captured through this lens. This method is cumbersome and requires a steady hand. As previously discussed, it is preferable to allow the camera to perform automatic fine focussing rather than to focus through the microscope eyepiece even if the system is parfocal. Although one axiom with digital cameras is "what you see is the image you capture", uneven field illumination or vignetting may commonly be present in low-power images. While the view through the eyepieces may not reveal unevenness in the peripheral field, as the human eye may not detect it, the problem is clearly displayed in the digital image. Vignetting is the result of the coning of the light path between the microscope objective and the camera or may be due to an uneven light source. The pre-digital approach was simply to crop the image, decreasing the effective field. With digital imaging, retrospective correction of uneven illumination is possible through mathematical modelling of the illumination pattern.[10] An even simpler method is to take a blank reference image and subtract this from subsequent images. This reference frame corrects any uneven illumination present.[11] Vignetting can also be avoided to a large extent by using higher magnification objectives.

Most cameras provide both optical and digital zoom facilities. While optical zoom is useful, digital zoom should be avoided as the image tends to blur and the magnification is not true. Simple digital magnification or increasing the number of pixels in an image will not provide increased resolvable detail beyond the limits of the optics.

Three main variables influence the quality of a digital microscopy image. A combination of the objective lens, image sensor size, and the light path between the objective and the sensor determines the physical area imaged in a single field. As with the optical microscope, higher numerical apertures are associated with higher-grade objective lenses and therefore better quality digital images.[1] The numerical aperture, quality, and type of the microscope objective lens determines the accuracy in focusing light on the imaging sensor and therefore image quality and resolution. The image sensor in the digital camera determines how this light is converted into a digital signal and the number of pixels created.

Digital microscopy images are acquired for various purposes including teaching and clinical meetings, conference presentations, diagnosis, image analysis and archiving, each with slightly different requirements. Images for a clinical meeting are best projected live, in real-time, with no delays, and the image size requirements would be modest as the commonest mode of display is an analogue television set that has, at best, 550-600 horizontal lines. Thus, a 640 x 480 pixel image would suffice. On the other hand, an image for publication would require sufficient pixels to satisfy the publisher. A 4 x 3 inch image on a 300 dpi home printer would ideally require at least a 1200 x 900 pixel image. In PowerPoint presentations only 72 pixels per inch are required.

For telepathology purposes it seems that the price and availability of digital imaging equipment rather than scientific considerations of adequate sampling have guided pathologists.

Many studies have employed digital cameras with analogue video output. Such cameras typically have an image dimension in the vicinity of 768 x 576 pixels. Thus, some pathologists have based their clinical practice not upon a scientific measure but on an arbitrary figure set by camera manufacturers. Telepathology systems mostly employ images that are either in the region of 352 x 255 (video-conferencing size) or 768 x 576 pixels (typical framegrabber image size).

Image Storage

There are two aspects for consideration, the physical storage and the method of storage. Physical storage for daily work is commonly on fixed hard drives, which currently vary between 20-100 gigabytes in size. Portable hard drives offering similar performance through a Firewire (IEEE1394) or USB2 connection are increasingly popular; however, avoid the comparatively slower USB1 connections. Removable media such as Zip disks and optical disks are relatively expensive and USB media allow a cheap and convenient method of transferring data between computers. CD-R (650-700M) is the most economical form of near-permanent storage although DVD-R (4.6G) should become cost-effective in the near future.

Large image datasets are the norm when dealing with digitised slides and some form of data compression is often necessary. It is also necessary to compress images when the transmission bandwidth is narrow. The compromise for a smaller file is a decrease in quality. To the human eye, this may not be noticeable but poor quality digital compression such as JPEG or JPEG2000 inevitably introduces digital artefacts and alters the numerical values of the image. This may be significant if the image is to be used for image analysis. For printing, viewing and even interpretation, poor quality compressed images are acceptable provided the compression ratio is controlled.

Image Printing

Sales of general consumer digital cameras now exceed that of film cameras so that many film processing laboratories will now print digital images on glossy photo paper that are indistinguishable from those derived from 35-mm film. Submission of digital images for printing via the Internet is an acceptable and convenient route provided there is access to a good Internet connection and often, because of the size of the data, Broadband connection may be required.

Inkjet printing, when coupled with the appropriate paper can produce publication-quality results and it is not uncommon for professional photographers to exhibit their work in this manner. Colour laser printers are better suited for high volume output but are generally not appropriate for photographic-quality prints. The relationship between ink, paper and environment determines the quality and longevity of the print. Inkjet prints fade or show colour alteration, a phenomenon that usually takes weeks to months and is less likely to occur if the print is kept away from direct sunlight and behind glass, protected from environmental elements. 'Curing' the print immediately after production by placing it away from air and light, e.g. in an envelope, for a period of time, has been a suggested solution but there is no evidence to show that this improves subsequent colour stability. Matt photographic rather than glossy paper improves longevity but the more-familiar glossy finish may be preferred.

Production of monochromatic images is often necessary as colour plate publication is expensive. Until recently, many photo printers were unable to print black-and-white images without introducing some unsightly colour tint. A simple solution was to switch off the colour cartridge and use only black ink; however, the results were often slightly coarse particularly across smooth monochromatic tonal gradients. Recent generation photo printers such as the Epson 1270 are able to produce a neutral grey using a balanced mixture of black and colour inks. Even so, many prefer the greater control and tonal range offered by dedicated monochrome ink cartridges.

If the publisher accepts digital files, sending an uncompressed file (e.g., TIFF format, sRGB colorspace) on CD-ROM is always preferable to their attempt at scanning the printed image. This circumvents the need to print. (Although printed illustrations are still required in the initial review process they need not be of the best quality.)

Total Slide Digitisation and Archiving

The traditional glass slide collection of interesting cases remains an excellent teaching resource provided the collection is maintained. Unfortunately, such collections are fragile, will fade, cannot be replaced if damaged, are difficult to transport, and cannot be readily shared among many and never simultaneously. Additionally, they are not in a form for immediate presentation or publication. Digital image archiving provides the solution to many of these drawbacks. At the very least, multiple representative images may be stored and, provided they are properly catalogued, can be retrieved and disseminated far more easily and widely, and without fear of damage.

Sampling error and inadequacy of representative images are overcome by the development of total slide digitisation.[12] Systems from companies like Zem/Nikon,[13] BacusLabs,[14] Aperio,[15] and Interscope[16] can scan the entire tissue area on a slide at high-power (20x or 40x objective), and present the data in a manner that replicates the actual slide without loss of resolution. Such systems may form the basis of anatomical pathology laboratories of the future. When coupled with rapid tissue processing systems such as the microwave-stimulated system from Milestone,[17] which has the capability of ultra-rapid tissue processing from fresh tissue to paraffin-embedded block within 30-90 minutes, it will allow large processing "factories" to service pathologists located at distant sites. Specimens can be transported to the centralised "factory" where examination, photography of macroscopic specimens, ultra-rapid tissue processing and whole slide digitisation takes place. The images of gross specimens and total digitised scans of microscopic sections can be transmitted for reporting by pathologists located at distant laboratories, eliminating the need to incur the expense of maintaining such laboratories.[18]

The American Board of Pathology conducts its exam with virtual microscopic slides acquired through whole slide digitisation employing the BacusLabs system.[14]

Telepathology

Telepathology is practised to varying degrees worldwide and is primarily used for consultation.[19] This may be for primary opinion, frozen section consultation being an important application in Europe [20-22] or for second opinion.[23,24] There is some confusion in the terminology of the current methodologies available. "Simple store-and-forward" telepathology is the act of capturing several representative images beforehand and forwarding them to a remote site for review.[19,25] "Dynamic" telepathology has been used in the United States to describe real-time consultation where the pathologist views the live microscope image updated at least 15 frames per second.[26] This is commonly done through a video-conferencing codec.[27] However, elsewhere, users have adopted the term "dynamic" to describe any consultation occurring in real-time irrespective of the mode of image transfer.[28] In between these two modalities the terminology overlaps and other terms such as 'active' and 'passive'[21,29] have been introduced in an attempt to provide more clarity.

The introduction of digital slide scanning systems has further complicated terminology.[29,30] They can be regarded as extremely sophisticated store-and-forward systems but the difference between whole slide digitisation and a dozen representative pictures transmitted by email is considerable. We have suggested a classification for such telepathology systems.[2]

In recent times, reference immunohistology laboratories in the United States[31] and Australia[32] have employed the Internet to post images of stains performed on referred cases in order the circumvent the delays in courier/postal deliveries of the stained glass slides. While these can currently only be of selected representative fields, the advent of total slide digitisation will open new avenues of rapid slide transfer and examination.

Quality Assurance and Educational Programmes

The use of representative digital images or entire digitised slides will eliminate the need to section and disseminate multiple glass slides, besides ensuring that all recipients see exactly the same section.[33,34] This circumvents the time and costs involved for slide distribution and is particularly important in cytology quality assurance programmes where only limited smears are available.[25] Digital images will also eliminate the need for cumbersome storage of educational and quality assurance glass slides that fade with time and take up space. Furthermore, digital images can be edited and improved in quality and can be made available on websites for educational purposes after completion of the quality assurance exercise.[25,34] The same advantages apply to both— digital microscopic images and images of gross specimens from autopsy or surgical biopsy examination.[9]

Digital images circulated on CD-ROM will also gradually replace the traditional glass slides that are used for the teaching slide seminar, and colour images can also be made available as a component of the handout for such seminars and lectures.

Digital Pathology Images in Education

The idea of using image databases as educational resources is not new. There are several websites that provide pathology-related images, the most well-known being Webpath.[35] However, the quality of the images is often poor, both in diagnostic sophistication and quality, and in the number of macroscopic and histological images. A major problem has been the lack of standard in terms of image size. Many older databases began collating images before high-quality digital cameras were readily available. Total slide digitisation now replaces the need to store representative images of each teaching case as they virtually contain all the data in the tissue section.

At a macroscopic level, three-dimensional digital simulations of organs provide an alternative to plastination or formalin-filled pathology pots. Digital representations are not subject to the legalities of organ and tissue retention; neither do they require maintenance or physical space for storage. There are several methods of creating a three-dimensional specimen. Contemporary rendering software has capabilities of reconstructing faces using only two different views. Rendering diseased human organs is a more difficult task due to the more complex shapes and textures. At Oxford University, we used a lower cost but more labour-intensive method of photographing the organ from at least 12 angles and reconstructing it into an interactive rotatable object, deliverable through a web interface as a Quicktime VR object.[36] Importantly, both gross and microscopic digital images allow a greater scope for interactive teaching programmes that can be posted on websites or distributed on CD-ROM.

At a microscopic level, visualisation of histological image data in three dimensions does not significantly improve diagnostic accuracy but can be a useful exercise in understanding the micro-anatomical growth and arrangement of structures. For example, the impact and influence a tumour has on its surrounding microvasculature may provide clues to the diagnosis and understanding of its growth pattern. Image data derived from confocal microscopy can be rendered into a three-dimensional image by computer software. It is also possible to do this at a lower cost using serial sections and normal microscopy images, as we have done with tubular carcinoma of the breast.[37]

Medical Vision Systems

Automated cytology screening machines have made major inroads into pathology practice where they address the shortage of cytology screeners and reduce the false-negative detection rate of the human screener.[38,39] It is unlikely that computer diagnosis will completely replace human interpretation. However, computer-assisted systems can enhance human performance by identifying highly suspicious areas or cells in cytological preparations, thus acting as a safety net or quality assurance for the pathologist. We are engaged in the preliminary

development of such systems.[37] The day may come when it will be regarded as negligent in a court of law if the reporting pathologist does not employ such a system for back-up or quality assurance. Fortunately, as yet, there are no such systems available.

The attempt to teach machines to recognise histological conditions is a worthy pursuit with the intermediate benefit of the objectification of histological interpretation. Given an appropriate training set, it is possible to derive objective criteria to describe a histological condition and it is also possible to statistically rank the features in terms of diagnostic strength.[37] Work in several areas including colonic dysplasia,[40] grading of cervical intraepithelial neoplasia,[41] endometrial hyperplasia,[42] and prostatic biopsy diagnosis[43,44] has made this a reality.

Other Applications of Digital Imaging in Pathology

Quantitative pathology may be viewed as an instrument of research. Its penetration into daily clinical practice is low, despite the existence of many aspects of histopathology, which would benefit from some form of quantification— mitotic counting, assessment of tumour volume and extent, morphometry and immunohistological densitometric quantitation being some examples. Quantitation of digital images will be much more reliable than assessment by eyeballing, using poorly defined criteria that cannot be reliably reproduced. Image analysis may render tumour grading to be more accurate and reproducible, and automation will make quantitation more acceptable in routine practice.

Some critics of digital imaging cite the technique as an invitation to scientific fraud.[45] In fact, the opportunity to be fraudulent existed long before the advent of digital imaging and there are certainly easier ways of falsifying scientific data. It is impossible to have an image that is completely free of some form of processing. For single-chip CCD cameras, the presence of a colour filter array means that there is complex data interpolation being employed to create the final image. Additionally, there is further processing of image data to improve the signal to noise ratio.

Many techniques such as spectral imaging and confocal microscopy are dependent upon processing of the image data to produce a viewable image. Photography of immunofluoresence is not optimal without some degree of contrast and brightness adjustment afterwards in an attempt to have the digital image match what was viewed through the microscope eyepieces. The closest equivalent to a raw negative are the unprocessed data files that some cameras such as the Nikon D1 series and Coolpix 5700 can produce. This is image data in a proprietary format that requires software to assemble it into a digital image.

In some situations, image processing can assist interpretation. We all are aware of inter- and intra-laboratory variations in histochemical and immunohistological staining. The human eye adapts and compensates for these variations. However, when sections fade with time or sun-exposure, re-staining is often necessary. With digital image processing it is possible to rescale the distribution of values in a faded image to provide clarity equivalent to re-staining the section.

Conclusion

While digital imaging is relatively new in pathology, it is rapidly being adopted in many areas because of cost savings, rapidity and convenience of usage, and the many new applications it offers. The use of digital images enhances the quality and accuracy of pathology reports and whole slide digitisation has the potential to completely change the way pathologists examine and store tissue sections. In addition, the applications in education are numerous and include conference presentations, publication, undergraduate and postgraduate teaching as well as remote consultation through telepathology, and quality assurance programmes. Digital images also have many other applications that are yet to be exploited including morphometric and densitometric quantitation and in medical vision systems with the ultimate aim of computer-generated histological diagnosis, either as a primary diagnostic tool, as a back-up system to aid the pathologist in diagnosis, or in quality control. Rapidly falling costs and extensive applications makes the adoption of digital imaging in anatomical pathology laboratories an essential consideration.

References

1. Leong AS, James C, Thomas A. Handbook of surgical pathology. London: Churchill Livingstone; 1996. p. 43-100, 177-92.
2. Leong FJ, Leong AS. Digital imaging applications in anatomical pathology. Adv Anat Path 2003;10:88-95.
3. Leong AS. Synoptic/checklist reporting of breast biopsies: Has the time come? Breast J 2001;7:271-4.
4. Schubert E, Gross W, Sideritis RH, Deckenbaugh L, He F, Becich MJ. A pathologist-designed imaging system for anatomic pathology signout, teaching and research. Sem Diagn Pathol 1994;11:263-73.
5. Cruz D, Seixas MA. A surgical pathology system for gross specimen examination. Proc AMIA Symp 1999;236-40.
6. Belanger AJ, Lopes AE, Sinard JH. Implementation of a practical digital imaging system for routine gross photography in an autopsy environment. Arch Pathol Lab Med 2000;124:160-5.
7. Marchevsky AM, Dulbandzhyan R, Seely K, Carey S, Duncan RG. Storage and distribution of pathology digital images using integrated Web-based viewing systems. Arch Pathol Lab Med 2002;126:533-9.
8. Park R, Eom J, Park P, Lee K, Joo H. Automation of gross photography using a remote-controlled digital camera system. Arch Pathol Lab Med 2003;127:726-31.
9. Leong AS, Visinoni F, Visinoni C, Milios J. An advanced digital image-capture computer system for gross specimens: A substitute for gross description. Pathology 2000;32:131-5.
10. Likar B, Maintz JBA, Viergever MA, Pernus F. Retrospective shading correction based on entropy minimization. J Microsc 2000;197:285-95.
11. Leong FJ, Brady M, McGee JO. Correction of uneven illumination (vignetting) in digital microscopy images. J Clin Pathol 2003;56:619-21.
12. Leong FJ, McGee JO. Automated complete slide digitization: a medium for simultaneous viewing by multiple pathologists. J Pathol 2001;195:508-14.
13. Nikon UK. http://www.nikon.co.uk and http://www.nikon.co.uk/inst/flashframeset.htm. Accessed September 2003.
14. BacusLabs. http://www.bacuslabs.com/. Accessed September 2003.
15. Aperio Technologies. http://www.aperio.com/home.asp. Accessed September 2003.
16. Interscope. http://www.interscopetech.com/index-products.htm. Accessed September 2003.
17. Visinoni F, Milios J, LEONG AS-Y, Boon ME, Kok LP. Ultra rapid microwave/variable pressure-induced tissue processing-description of a new tissue processor. J Histotechol 1988;21:219-24.
18. Leong AS-Y, Leong FJW-M. Telepathology-the system for Australia. National Telepathology Conference, Sydney, October 5-6, 2001.
19. Leong FJW-M, Graham AK, Gahm T, McGee JOD. Telepathology: Clinical utility and methodology. *In*: Underwood J, ed. Recent Advances in Histopathology. London: Churchill Livingstone;1999. p. 217-40.
20. Eide TJ, Nordrum I. Frozen section service via the telenetwork in Northern Norway. Zentralbl Pathol 1992;138:409-12.
21. Kayser K. Telepathology in Europe. Its practical use. Arch Anat Cytol Pathol 1995;43:196-9.
22. Wellnitz U, Fritz P, Voudouri V, Linder A, Toomes H, Schmid J et al. The validity of telepathological frozen section diagnosis with ISDN- mediated remote microscopy.

Virchows Arch 2000;437:52-7.
23. Milosavljevic II, Spasic P, Mihailovic D, Kostov M, Mijuskovic S, Mijatovic D, et al. Telepathology-second opinion network in Yugoslavia. Adv Clin Path 1998;2:156.
24. Della Mea V, Beltrami CA. Telepathology applications of the Internet multimedia electronic mail. Med Inform (Lond) 1998;23:237-44.
25. Lee ES, Kim IS, Choi JS, Yeom B, Kim HK, Ahn GH, et al. Practical telepathology using a digital camera and the Internet. Telemed J E Health 2002;8:159-65.
26. Dunn BE, Choi H, Almagro UA, Recla DL. Combined robotic and nonrobotic telepathology as an integral service component of a geographically dispersed laboratory network. Hum Pathol 2001;32:1300-3.
27. Vazir MH, Loane MA, Wootton R. A pilot study of low-cost dynamic telepathology using the public telephone network. J Telemed Telecare 1998;4:168-71.
28. Afework A, Beynon MD, Bustamante F, Cho S, Demarzo A, Ferreira R, et al. Digital dynamic telepathology—the Virtual Microscope. Proc AMIA Symp 1998:912-6.
29. Kayser K, Syzmas J, Weinstein R. Telepathology-Telecommunication, electronic education and publication in pathology. Berlin: Springer-Verlag; 1999.
30. Demichelis F, Barbareschi M, Dalla Palma P, Forti S. The virtual case: a new method to completely digitize cytological and histological slides. Virchows Arch 2002;441:159-64.
31. PhenoPath Laboratories. http://www.phenopath.com/. Accessed September 2003.
32. AIP Laboratory Zoom-in system. http://www.aiplab.com/online/login.mv [Accessed February 2003].
33. Leong FJ, Graham AK, Schwarzmann P, McGee JO. Clinical trial of telepathology as an alternative modality in breast histopathology quality assurance. Telemed J E Health 2000;6:373-7.
34. Leong FJ, McGee JO. Robotic interactive telepathology in proficiency testing/quality assurance schemes. Electr J Pathol Histol 2001;012-01.
35. Webpath. http://www-medlib.med.utah.edu/WebPath/webpath.html. Accessed October 2003
36. Oxford University Laboratory Medicine Course. http://www.ndcls.ox.ac.uk/LMC. Accessed September 2003.
37. Leong FJ. An automated diagnostic system for tubular carcinoma of the breast-a model for computer-based cancer diagnosis. DPhil Thesis: Oxford University;2002.
38. Cenci M, Nagar C, Giovagnoli MR, Vecchione A. The PAPNET system for quality control of cervical smears: validation and limits. Anticancer Res 1997;17:4731-4.
39. Patten SF Jr, Lee JS, Wilbur DC, Bonfiglio TA, Colgan TJ, Richart RM, et al. The AutoPap 300 QC System multi-center clinical trials for use in quality control re-screening of cervical smears: II. A prospective intended use study. Cancer 1997;81:337-42.
40. Hamilton PW, Bartels PH, Thompson D, Anderson NH, Montironi R, Sloan JM. Automated location of dysplastic fields in colorectal histology using image texture analysis. J Pathol 1997;182:68-75.
41. Keenan SJ, Diamond J, Glenn McCluggage W, Bharucha H, Thompson D, Bartels PH et al. An automated machine vision system for the histological grading of cervical intraepithelial neoplasia (CIN). J Pathol 2000;192:351-62.
42. Morrison ML, McCluggage WG, Price GJ, Diamond J, Sheeran MR, Mulholland KM, et al. Expert system support using a Bayesian belief network for the classification of endometrial hyperplasia. J Pathol 2002;197:403-14.
43. Bartels PH, Thompson D, Bartels HG, Montironi R, Scarpelli M, Hamilton PW. Machine vision-based histometry of premalignant and malignant prostatic lesions. Pathol Res Pract 1995;191:935-44.
44. Montironi R, Bartels PH, Hamilton PW, Thompson D. Atypical adenomatous hyperplasia (adenosis) of the prostate:development of a Bayesian belief network for its distinction from well-differentiated adenocarcinoma. Hum Pathol 1996;27:396-407.
45. Suvarna SK, Ansary MA. Histopathology and the 'third great lie'. When is an image not a scientifically authentic image? Histopathology 2001;39:441-6.