22 Digital pathology

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Key points

Whole slide imaging (WSI) allows the creation of digital images of entire histology and cytology slides with sufficient detail to allow viewing at varying magnifications comparable to that achievable with a conventional light microscope.

Acquiring digital WSIs requires dedicated hardware systems which create composite images of individually acquired tiles or lines. Such slide scanners typically include slide loading mechanisms, a motorized stage, a light path, image capture device and software to create the composite image. Although the data files which represent these images are large, software techniques enable these images to be viewed on screen in a manner analogous to how a slide may be viewed on a physical microscope.

Digital representation of slides enables histology images to be distributed, viewed and shared over computer networks rather than relying on physical handling of the glass slide. This is likely to represent the future of histology and cytology departments.

Introduction

Whole-slide imaging refers to the creation of a digital representation of the image presented by a glass histology slide, at a level of detail comparable to that seen with a light microscope. 'Digital pathology' is a broader term encompassing related processes which maximize the practical utility of such images, including the storage, viewing, annotation and use in applications including educational, research and clinical practice.

Benefits of digital images over physical glass slides

Digital images have several advantages over glass slides. Unlike a physical object, a digital image file can be moved from one physical location to another almost instantaneously. Indeed, an image file can be viewed at the same time by two, or more pathologists, in different locations and potentially separated by thousands of miles. With appropriate data-security arrangements, digital images should be more durable than glass slides which are physical, fragile and prone to fading. The overhead of sorting, filing, storing and retrieving glass slides is particularly burdensome on larger laboratories and a fully digital workflow has the potential to significantly reduce this. Finally, digital images are a prerequisite for automated image analysis.

Digital images

Images can be represented in numerical form in a variety of manners. The text on this page, for example, is ultimately represented by the printing software, not as letters and words but as collections of lines and curves. The commonest method for representing complex real world scenes, including histology images, is to consider the image as a grid of individual points, each with brightness and, for color images, hue. These individual points are referred to as pixels, the term pixel being a contraction of 'picture element' and the smallest resolvable detail. In all commonly encountered digital image formats pixels are square, although other shapes, in particular hexagons, are in principle possible. This approach represents images

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mathematically as a matrix of brightness and hue values. The perceived quality of a digital image of this type relates to the total number of pixels (resolution) and a parameter called pixel depth.

Resolution

The size of the smallest resolvable detail in a whole slide image is defined by the original absolute size of the area represented by each pixel. This is determined by the quality of the slide scanner optics and sensor. Although WSI system vendors often refer to image quality as 'x40 equivalent' or 'x20 equivalent', reference to microns/pixel is preferable since this is an unambiguous measure of image resolution, unaffected by downstream variables such as monitor resolution and viewing distance (Sellaro et al., 2013). Broadly however, when viewed under appropriate conditions, images in which each pixel represents a square of side 0.5 microns (0.5 µm/pixel) are regarded as providing an equivalent level of detail to that seen with a x20 objective on a high quality microscope, whilst 0.25 µm/pixel is regarded as comparable to a x40 objective.

Pixel depth

Image quality is not defined solely by resolution. The term 'pixel depth' refers to the extent to which subtly different colors can be distinguished. The crude but recognizable image in Fig. 22.1 has a pixel depth of 1, i.e. individual pixels represent either black or white. Only a single binary bit, 0 or 1, is required to capture this for each pixel. Most formats for the realistic representation of real life images use 24 bits, 8 to represent the intensity of each of the 3 colors red, green and blue. This enables 256 different intensity levels to be represented for each of these colors, a total of 16,777,216 different tones.

File size compression

The simplest format for representing a digital image is a matrix of pixels, each pixel represented by an appropriate number of bytes to capture the required color depth and the commonly used TIFF (tag image file format) is an example of this. A TIFF file representing an image of 1000 x 1000 pixels at 24-bit color depth, requires 3 million bytes, i.e. 3 MB.

Compressed image formats such as JPEG or JPEG 2000 reduce the required image file size using mathematical techniques to store the same data more efficiently, and through the identification of data which can be discarded with minimal impact on how the image is understood by a human observer. The term 'lossless compression' refers to techniques which allow for the extraction or 'decompression' of the exact original image with no loss of detail.

Conversely, 'lossy compression' techniques permanently discard information, aiming to do so only for information which is non-contributory to the overall appreciation of the image. Lossless compression techniques are typically only able to reduce image file sizes around 2-3 fold, whereas 'lossy' techniques can achieve 50-fold or higher compression ratios, albeit with noticeable artifact.

Histology as digital images

Whole slide images are distinguished by their sheer size. A typical 15 x 15 mm tissue section imaged at 0.25 µm/pixel at 24-bit pixel depth results in an uncompressed file size of just over 10 GB. The required file size may be compressed up to 30-fold using compression techniques without impact on diagnostic utility (Krupinski et al., 2012), resulting in file sizes of the order of tens to hundreds of megabytes. It is worth noting that discernable artifacts may be introduced into the images at lower levels of compression without necessarily impacting on the diagnostic utility of the image (Foran, 1997). Moreover, greater degrees of compression may be possible for non-H&E tinctorial stains than for H&E without undermining the practical utility of the image (Sharma et al., 2012).

The way in which pathologists interact with images also influences the way image data are stored. Many vendors of WSI systems use proprietary file formats, typically based around standard image compression techniques e.g. JPEG, JPEG 2000, but with additional features. Files may include the same image at multiple resolutions to support rapid zooming in and/or out as images are streamed over

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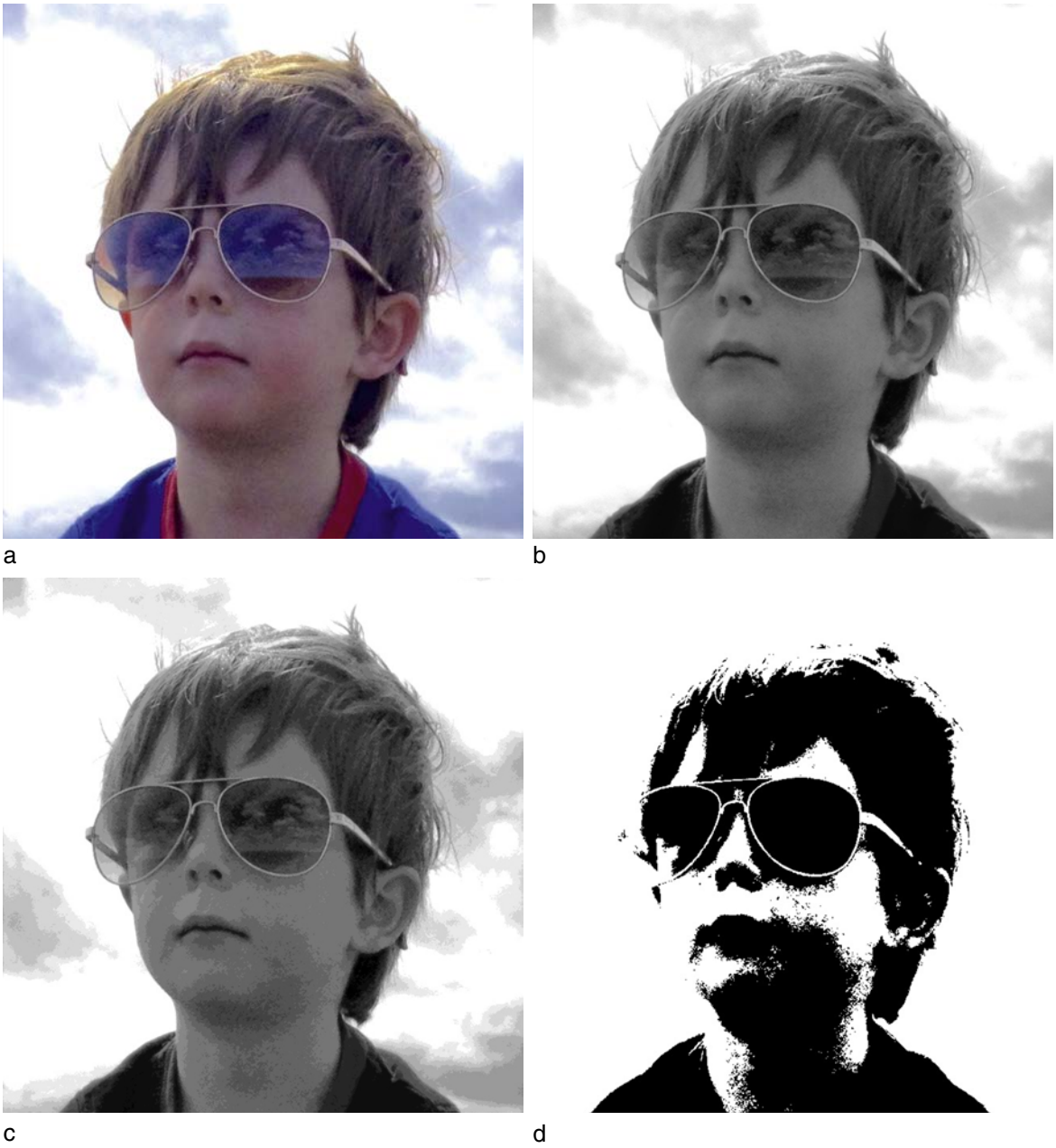

Fig. 22.1 When considering how images are represented 'perfect' fidelity may not be needed to convey meaningful information. The same original image is used throughout **a**-**d**. (**a**) A 'normal' full color image as produced by most conventional digital cameras. (**b**) The image modified to 256 shades of grey. (**c**) The image represented as only 16 possible shades of grey with a much reduced file size, but some discernible loss of image quality. (**d**) With only 2 possible values per pixel, the image is still recognizable and able to convey meaning, although it is significantly simplified and with a small fraction of the file size. (© Jonathan Bury)

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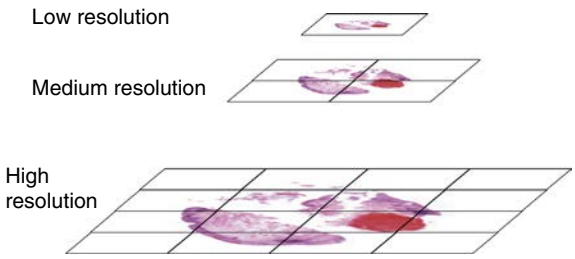

Fig. 22.2 Digital pathology file formats often use a pyramidal system. Multiple separate images are stored at different magnifications and resolutions. A pathologist viewing a low power overview image only needs to access the 'top' tier. As the magnification is increased, the relevant individual tiles from deeper tiers are displayed. This mechanism avoids the need to send entire image files across networks.

networks, i.e. pyramidal storage (Fig. 22.2). Files may also store metadata such as a timestamp, image file type, image size, pixel depth, make and model of the scanner and objective, and resolution data such as microns/pixel. Accurate metadata is particularly important if the WSI is going to be quantitatively analyzed, either manually or by an image analysis algorithm as all measurements are calculated from resolution data.

Image acquisition

A slide scanner essentially comprises an optical microscope with a mechanized stage control and focusing, coupled to a digital image capture device, usually a charge-coupled device (CCD) similar to that found in a digital camera. Additional hardware may typically include mechanical apparatus to sequentially load slides, operator controls, a visual display area and computer control hardware. The smallest single slide systems may have a footprint of only 500 x 500 mm, whilst high capacity scanners holding 200-300 slides may be 750 x 1200 mm or more. A typical 15 x 15 mm tissue area may take 30 to 60 seconds to scan using a 40x objective at 25 µm/pixel.

Systems typically use a standard 20x or 40x objective with a light path to a CCD camera. Prior to image acquisition, the slide scanner may register the sample number by reading a barcode printed on the glass slide. A scanner may also perform a low resolution overview scan to determine where the tissue is on a slide and only scan that area, minimizing overall scan time and therefore file size.

The slide is moved by the motorized stage and images are captured by the camera. Two commonly used methods of image acquisition are line scanning and tile scanning. In line scanning, the slide is moved in a linear fashion so that the camera captures strips of the image. In tile scanning, small squares of the image are captured by the camera. A strobe light source and high frame rate camera are typically employed to reduce movement, the blur artifact. With both methods, an 'image stitching' algorithm is then applied to assemble the strips or squares of image into a whole slide image (WSI). Reconstruction of a tile scanned image is computationally more complex but modern multi-core processors negate the effect this has on overall scan time.

The topography of tissue on a glass slide can vary by up to 20% of the tissue thickness over a distance of 1 mm. An image scanned using one focal plane would appear blurry in places, making it diagnostically useless. To counter this, slide scanners use an image based autofocus technique. This requires the generation of a focus map either by the operator or automatically from the overview scan image. The scanner could apply autofocus on a tile-by-tile basis in the case of tile scanning or at several points along a strip of image in line scanning. This near continuous autofocus would result in prohibitively slow image acquisition. Instead, in a trade-off between speed and image fidelity, the scanner generates a representative autofocus map, or focuses on every third or fifth tile of an image.

Special cases

Large blocks

These mega or jumbo blocks can also be scanned, but the scanner needs to be designed to accommodate larger slides. There is an increase in slide acquisition time and file size owing to the larger tissue area which needs to be scanned. Large slides may interrupt the workflow of a digital laboratory as they may require loading in separate batches to the standard size slides. One alternative to scanning

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mega blocks is to create composite blocks of a sample then scan these as standard sized slides. Image stitching software can be used to create a virtual mega block from the composite blocks.

Cytology preparations

The 3-dimensional nature of a typical cytology slide presents challenges. Using a conventional glass slide, a pathologist manually adjusts the microscope to bring different 'depths' of the preparation into focus. This is not possible on a standard digital image. This problem is addressed by acquiring multiple images of the cytology slide at different focal points which are treated as a stack of 2-dimensional images, a process called z-stacking, where the z refers to the z-axis of a 3-dimensional image (x, y, z). Images must be captured in several planes of focus, with consequent multiplication of the total file size and the time taken to capture the image. When viewing these composite z-stack images on a monitor, additional image processing is required to allow the smooth transition between virtual planes of focus.

Fluorescent slides

Acquiring digital images of slides stained with fluorescent stains will typically require additional hardware, particularly a suitable light source and filters. Some vendors supply the fluorescence modules, or a dedicated scanner may be required.

Measures to ensure good quality digital images

The quality of the virtual image depends upon the production of a high quality physical slide and quality control processes relating to the scanner itself. When using a conventional light microscope, a pathologist can work around artifacts including tissue folds, wax on the coverslip, air bubbles and tissue not covered by the coverslip. A slide scanner will faithfully reproduce all of these artifacts, potentially diminishing the quality of the scanned slide.

Standard laboratory quality control procedures should ensure the production of the highest quality slides and the presence of artifacts need to be audited. Additionally, the scanner operator should re-check and if necessary clean or re-coverslip slides prior to scanning. Attention should also be paid to minimizing vibration during the scanning process. If a laboratory is situated close to a major road or rail line or other source of vibration it may be prudent to consider installing a scanner on a vibration-proof table (Fig. 22.3).

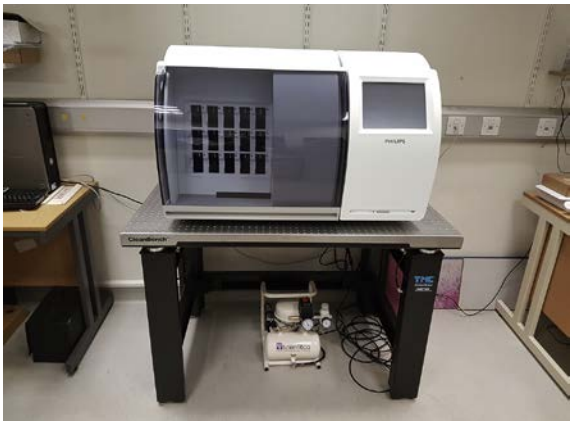

Fig. 22.3 This high-capacity high speed scanner (Philips UFS) has been installed on a vibration-damping table to minimize artifact from a railway line running very close to the laboratory. (Courtesy of Tristan Brain.)

The slide scanner should be regularly serviced and cleaned to ensure consistent lighting and focusing. A daily test slide should be scanned to assess the basic function of the scanner and detect major errors such as poor sample detection and abnormal color profiles. This procedure will also generate diagnostic information such as scanner temperature, time-to-focus and time-to-scan, all of which can create variance in digital image consistency and laboratory throughput. Color calibration of the scanner has been shown to increase diagnostic confidence and produce digital slides which are subjectively similar to slides viewed under a light microscope. This is achieved by scanning a standardized color patch affixed to a slide. The color values of this patch are known and can be compared with the on-board reference of the scanner. An adjustment to the color reproduction is then made by the scanner. This procedure is important to ensure day-to-day consistency of

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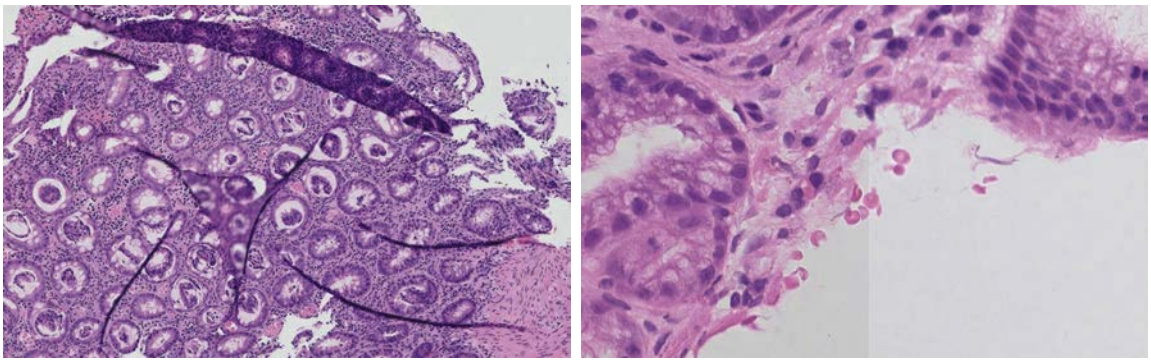

a b

Fig. 22.4 (**a**) Focusing artifact where the scanner has been unable to acquire a well-focused image of the entire tissue due to folds. Using a glass slide a pathologist may be able to compensate for this by focusing up and down through the depth of the tissue but such compensation is not possible on a digitally acquired image. (**b**) Striping artifact caused by inconsistent illumination across the slide. The scanner may require recalibrating.

color reproduction by the scanner and consistency between scanners in the same department (Fig. 22.4).

Accessing and viewing whole slide images

Image streaming

The sheer size of virtual slide images raises significant technical challenges around distributing image data over networks. When slides are being viewed, software techniques are used so that only the part of the image being viewed at any one time is passed over the network, requiring only a fraction of the data transfer.

This process is referred to as streaming. The same approach is used in viewing films over the internet – rather than sending an entire movie file of perhaps 4 GB, only a fraction of the image data is sent at a time. A pyramidal image format assists with the image streaming process, with deeper subsections of the image transmitted as the pathologist increases magnification. A similar mechanism is used for the Google Earth image viewing system, in which a single low resolution image of the planet is first sent, with subsequent image data for higher magnification views of regions of interest sent in response to the user's selection of these areas. The rapid transmission of image data is achieved by only sending the area of the pyramid which is currently being viewed and potentially the surrounding image tiles. This allows smooth transition between magnifications and when panning without preloading the entire file, which would otherwise require a prohibitively large amount of network bandwidth.

Client software

This term refers to the software used by individual pathologists to request, view, navigate and manipulate WSIs, and distinguishes it from the 'server' software running on the central computer responsible for streaming the stored images to clients on demand. A key challenge in developing client software has been to provide zooming and panning in a manner which is intuitive for pathologists accustomed to physical microscopes. The speed at which the image can be refreshed is important, as a noticeable lag between operating the panning controls and the ultimate refreshing of the image will impact on the usability of the system.

Many manufacturers have developed interfaces which only require a web browser to view and navigate images, although relatively fast computers with capable graphics cards are required. Some interfaces incorporate sophisticated caseload management and

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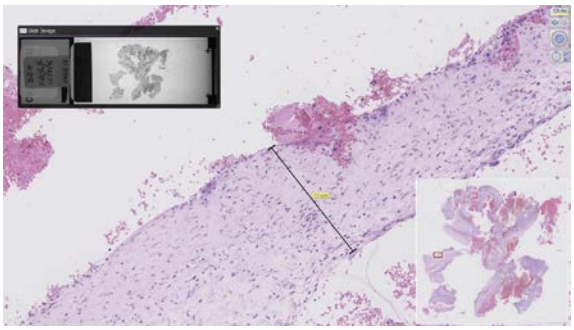

Fig. 22.5 This simple digital pathology viewing interface includes a slide overview (bottom right), as well as a view of the whole slide including the label (top left) so that identifiers on the slide label can be checked. Some image navigation controls are visible at the top right and tools for measuring, for example, can be invoked by keyboard shortcuts or mouse controls (Hamamatsu).

report writing tools which can be integrated with the laboratory information management system (LIMS). Others provide the core image viewing functionality only, which may be all that is required for many applications (Fig. 22.5).

Hardware installation and image file storage

Installation of a digital pathology system may be relatively straightforward for a small standalone scanner for which low slide volumes are anticipated. Even for such a simple arrangement however, adequate space, computer network access, a power supply (ideally dedicated and uninterruptible) and a robust table to accommodate the scanner will be required. The initial image scanning process generates particularly high data transfer requirements, far higher than the subsequent viewing of the images. For this reason, the network connection between the scanner hardware and server hardware should be as fast as possible (e.g. 1 Gb/s) and there may be a benefit to locating drives physically close to the scanner. Where higher slide volumes and large scale digitization is anticipated, multiple scanners may be needed to achieve the required throughput. Multiple units of rack-mounted server space, with fast networking connections, routers and adequate cooling will also be needed.

Operational problems with the system may be resolvable, or at least diagnosable, through remote network access from the supplier's technical support team. Arrangements for such access should be established in advance, taking into account institutional network security policies.

Image storage arrangements

Hard disc drives offer high capacity and rapid access. Tape drives have fallen somewhat out of fashion in recent years, but the current generation of tape storage systems offer high storage capacities at low prices. The LTO-7 (linear tape open, 7th generation) format provides 6 TB of uncompressed storage on a single cassette measuring 102 × 105.4 × 21.5 mm. The trade-off for tape systems is that files are inevitably slow to retrieve. One strategy for minimizing the costs of a large clinical slide archive whilst maximizing its performance is to specify different tiers of storage. The initial acquisition of a slide image requires the highest drive speeds as the entire image file is captured. Unreported images should be stored on high performance disks with the fastest available data-read times on servers able to deliver images to multiple pathologists at the same time. However, once reported, the images for these cases could be moved to a cheaper, less highly specified medium. The subset of slide images which need to be revisited, e.g. for multidisciplinary team/clinical review can be accessed, but with some minor lag in image availability.

If governance or regulatory frameworks mandate longer term archival storage of images, then tape drives may provide the most cost-effective solution. This is analogous to the arrangements for glass slide filing in most laboratories, where the most recent cases are in immediately accessible storage occupying valuable laboratory space, whilst older cases may be stored in cheaper offsite space, trading retrieval times for cost. Consideration should be given to the location of the data storage system. If it is accepted that a digital image must be held for a period of time for quality assurance purposes, then thought must also be given to how that storage is backed up. Primary storage is likely to exploit redundant array of interchangeable discs technology (RAID).

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In the RAID model a cluster of drives store multiple (2 or 3) copies of each image file across multiple individual disc drives. Should an individual disc fail, that unit can be replaced and the affected files restored from one of the other copies. This does not protect against catastrophic data loss due to, e.g. fire or flood. A robust approach to long-term storage is likely to involve multiple tape copies in different physical locations. The final cost will be considerably higher than the simple cost of drives and tapes. Any storage medium has a finite lifespan and real-world data storage solutions need to allow for regular migration of data onto new drives or tapes etc. to ensure data integrity.

Applications

Non-diagnostic applications

Education & training

Some of the earliest applications for WSIs were as educational resources. In an educational context, the speed of image acquisition is less critical than it may be in a clinical setting, so the longer scan times of early scanners (measured in minutes) are acceptable. Image quality may also be less critical. The ability to annotate images is particularly useful as significant features can be highlighted more clearly than using the traditional 'ink dot on the slide'. Many institutions have established large digital slide archives for educational purposes, and software can be used to enrich such resources with assessment questions, links to text etc.

Research, including image analysis

Digital pathology technologies can aid a research pipeline in two ways. Firstly, the file storage and viewing applications described above can be used to organize macroscopic digital photographs, whole slide images and tissue microarrays. This is useful in multicenter studies with central pathology review. Additionally, tissue banks can add a virtual slide bank to their resource, allowing easier collaboration. This approach has been pioneered by the Cancer Digital Slide Atlas, an element of the Cancer Genome Atlas (http://cancergenome.nih.gov/).

The second use of digital pathology in research is the development and application of image analysis techniques. Algorithms can group pixels within an image by similarity to their neighbors, identifying nuclei, stroma, architectural features or even specific cell types, e.g. lymphocytes. Further manipulation of these groups allows objective calculation of staining which can be useful in quantitatively assessing hyperchromasia in standard stained slides or antigen expression by immunohistochemistry. This creates the opportunity to measure binary or categorical features, e.g. low or high grade dysplasia, on a continuous scale with a potentially higher degree of precision and reproducibility.

Image analysis algorithms can be combined to measure multiple features in an image. Many of these features cannot easily be assessed by eye, e.g. the orientation (in degrees) of nuclei relative to one another, the distance between nuclei, the precise percentage of tumor and stroma, the degree of nuclear membrane irregularity and the absolute nuclear area. Each of these features can be measured on a continuous scale and given varying weight in statistical models which predict clinical variables, including response to treatment, survival and tumor recurrence (Beck et al., 2011). This technique has been called 'tissue-omics', suggesting a kinship with other big data techniques such as genomics, proteomics and metabolomics. It is the combination and integration of data from these sources which provide the greatest potential for important research findings. However, an enormous amount of data is generated in each of these approaches and storage, transmission and analysis become the limiting factors.

Quality assurance

Diagnostic external quality assurance (EQA) schemes involve the circulation of sets of slides to pathologists to ensure diagnostic uniformity. Where large numbers of pathologists are involved, multiple sections from the same tissue blocks may be required precluding the use of small biopsies or cases with only focal pathology. The use of digital slides avoids this problem allowing integration with web-based systems for collating diagnoses. Digital slides are now widely used and accepted in diagnostic EQA schemes in the UK.

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The technology is currently enabling, e.g. approximately 500 pathologists to regularly participate in the UK Bowel Cancer Screening Programme scheme, with participants in the UK, Eire, Slovenia and the Netherlands.

Diagnostic applications

The improvement in scan times and image quality is allowing digital pathology to be used as a viable option for the primary diagnosis in routine clinical practice. As noted at the start of this chapter, digital pathology advantages include the ability of the technology to seamlessly, near-instantaneously move images from one location to another, and the positive impact on slide filing, archiving and retrieval. The balance of cost against benefits therefore favors applications where it is important to move slide images rapidly and/or where a digital approach tangibly reduces the need for filing and retrieval.

Remote intraoperative diagnosis

Intraoperative cryostat (frozen section) diagnosis has been one of the first applications where digital pathology has reached clinical practice. This has been driven by geographical arrangements, specifically the scenario where laboratories are not located at the same site as the operating theatre. Early approaches to this problem employed remote microscopes, i.e. a video camera linked to a microscope, with stage controls operated by a distant pathologist, or transmission of still images of areas of interest taken by an on-site technician and, e.g. emailed to the pathologist (Fig. 22.6).

Whole slide imaging avoids the complexity and time-lag of a remote microscope and enables the entire slide to be seen rather than a pre-selected region of interest. Studies have demonstrated comparable accuracy to that achieved using light microscopy (Bauer et al., 2015; Evans et al., 2010). One practical point to note is that ensuring uniform section thickness and avoiding tissue folds is particularly difficult with cryostat preparations, and this can lead to problems acquiring a well-focused image. Z-stacked images capturing multiple focal planes help overcome this but may significantly increase scan times which can be unacceptable in the intraoperative context. Care must also be taken to avoid excess mountant coming into contact with the scanner objective lens (Fig. 22.7).

Fig. 22.6 This Hamamatsu scanner is used for capturing images for intraoperative frozen section diagnosis. The system holds just 6 slides but offers fast scan times which is appropriate for this application.

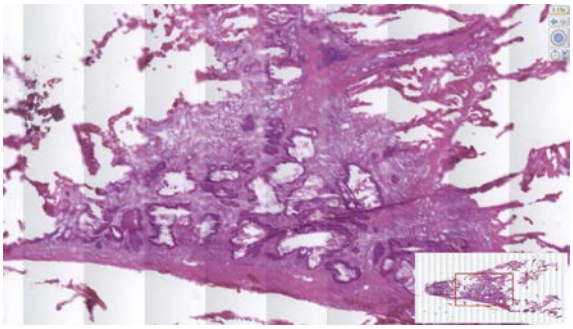

Fig. 22.7 This image is part of a whole slide image acquired intraoperatively. During the scanning process the objective came into contact with excess mountant on the slide and the resultant image is unsuitable for diagnosis.

It is also necessary to have scientific staff at the site of surgery. These personnel can examine and then dissect the specimens. This means for example, breast duct or bronchial resection margins are dissected and oriented appropriately, or tumor masses within lung wedges are sampled confidently.

Second opinion

The ability to swiftly and easily seek a second opinion is an often-cited application where digital pathology may be of benefit. As above, the speed at which the digital image is viewable by a pathologist

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is an advantage. There are also savings on packing and postage. Digital image transmission also avoids the situation where a slide is needed for clinicopathological review and discussion, but is instead in-transit to/from the second opinion site. Beyond these logistical benefits, digital slides can be annotated on screen to draw attention to particular areas of interest or diagnostic relevance. It is also possible for geographically separate pathologists to confer by telephone whilst looking at the same image on screen, rather than needing to be together at a multi-headed microscope.

Multidisciplinary team (MDT) meetings and clinicopathological conference (CPC)

Locating, retrieving and refiling slides for regular MDT/CPC meetings, places a considerable burden on many laboratories. In a conventional 'glass slide' laboratory, it may be possible to selectively scan slides from cases likely to be discussed if they are identified as such by the reporting pathologist, or flagged on receipt in the laboratory. This selective scanning however may simply introduce additional operational complexity and delay. Using digital pathology to support MDT/CPCs may be more efficient within the context of a fully digital laboratory process. Once flagged as being needed, digital images allow easy annotation of histological features of interest which can speed up case review and enable salient features to be easily displayed to the clinicians. This digital data handling is already well established in radiology.

Clinical quantification

Measurements of dysplasia, immunohistochemical staining, percentage of tumor/stroma and glandular complexity are examples of features which a subjective observer, the pathologist, must objectively ascribe to recognized categories of histopathological features. This leads to measurable intra- and inter-observer variation. At a clinical level, image analysis can perform tasks which pathologists do already but with the promise of greater precision, reproducibility and efficiency.

Many image analysis systems exist which are aimed at improving the accuracy and reproducibility of HER2 scoring in breast cancer. At least one of these is FDA approved for clinical use. Early work suggested that an image analysis approach may resolve equivocal HER2 scores reducing the need for secondary FISH analysis. The problems with validation and reproducibility between laboratories however, have hampered widespread use largely due to the variation in staining intensity between, and even within the laboratory. This requires frequent recalibration and validation of algorithms which ameliorates time and efficiency savings, introducing the possibility of error in a supposed 'objective' system. Additionally, the handing over of such an important task to a 'black box' may be viewed with unease by clinicians and patients alike.

Algorithms have also been developed which either assess the stroma: tumor ratio or count tumor nuclei. These are important tasks when submitting formalin fixed paraffin wax embedded (FFPE) blocks for mutation analysis, e.g. in lung cancer. Currently these algorithms have only been used for quality control and benchmarking when performed by a pathologist. In this situation, the image analysis software produces an objective measure against which pathologists can be assessed. By increasing the use of digital slides and a digital workflow, it can be seen how automated results from digital image analysis could be integrated into a report. In this situation image analysis may become a tool which can be deployed on demand and augment the performance of pathologists and the laboratory.

Whole laboratory digitization

Many pathologists have gained familiarity with digital images through involvement in research, education and quality assurance schemes, but despite the advantages and maturity of the technology there are few examples of entire laboratories which have adopted a fully digital approach. There has been a tendency to attribute this lack of adoption to pathologists being unwilling to work from digital images. This is to some extent reasonable, as some early systems offered suboptimal image quality and unresponsive user interfaces. The lack of penetration into routine practice however, may have less to do with the images themselves, but instead that there is little benefit to simply digitizing images for the sake of it.

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A fundamental change occurred with the emergence of pathologist workflow management systems. Contemporary digital pathology clinical interfaces borrow heavily from lessons learnt in radiology, and offer far more functionality than simply the ability to view a digital slide image. The user interface can be tightly integrated with the LIMS, meaning previous reports and images for a patient under consideration can be accessed. Cases can be assigned to individual pathologists with varying levels of prioritization and cases can be flagged for MDT, educational discussion etc. The availability of these features makes digital pathology a far more compelling proposal than viewing images on-screen for no reason other than it being technically possible. Digital workflow has demonstrated measurable improvements in productivity (Haroske and Moerz, 2016). The barrier to adoption is now more to do with the high capital costs of scanners and data storage than the acceptability to pathologists. At the time of writing a typical high-speed, high capacity scanner may cost between $100,000-150,000. Nevertheless, some laboratories are already scanning high proportions of their workload (Thorstenson et al., 2014; Baidoshvili, 2015). The practical issues surrounding the transition to a fully digital workflow are discussed in the 'Future development' section at the end of this chapter.

The digital pathology workstation

The digital pathology workstation comprises at least two computer monitors with separate interfaces on each. The first monitor displays a virtual microscope which allows familiar controls such as panning and zooming. This monitor should be of medical grade and calibrated to consistently reproduce histologic color profiles. Calibration with a standardized test object has been shown to improve diagnostic confidence and produce color profiles subjectively closer to equivalent glass slides. The virtual microscope allows improvements in workflow efficiency. Dedicated software, e.g. that used with the Leeds Virtual Microscope (Randall et al., 2014), will move pixels quickly enough so the whole slide image can be panned and zoomed without artifacts or pixelation occurring. Small 'thumbnail' images of each slide in a case allows ease of movement between slides and rapid comparison of non-sequential slides. Virtual microscope software also allows slide annotation to identify regions of interest (ROI), which are useful for teaching sessions or consultation with a colleague. These regions can also be extracted and appended to a report.

The second monitor of the workstation provides an interface for the LIMS or image management software (IMS). At its simplest level, this displays a list of cases and their status, e.g. to be viewed, report in progress or report authorized. When scaled up over an entire department this interface facilitates distribution of work and real-time monitoring of case turnaround time. Additional functionality can include instant sharing of cases and the tagging of cases with labels which automatically add the case to an MDT meeting agenda or a teaching set.

The virtual microscope and the LIMS/IMS interfaces have the potential to improve efficiency and safety within a pathology department but their design must facilitate ease of use (Fig. 22.8).

Validation and regulatory issues

The validation of a WSI system is essential prior to its use for routine diagnostic work. A validation study aims to demonstrate that a diagnosis from a digital slide has at least the same accuracy as that from a glass slide. The Digital Pathology Association and the College of American Pathologists have both published guidelines on the conduct of validation studies. These guidelines state that the WSI system should be validated in its entirety in a real-world clinical setting and separately validated for each new clinical application. Each slide is viewed by the same pathologist using a light microscope and a digital microscope. A washout period, the time between digital and glass slide review, of up to four weeks is recommended to reduce recall bias. It has been suggested (Campbell et al., 2015) that a longer washout period may be necessary as effective recall of cases is common, even after a period of months, between glass and digital slide review.

A sample size of between 60-100 cases is recommended for each validation study. Most high quality recently published studies have greatly exceeded this number. The largest study to date used a sample

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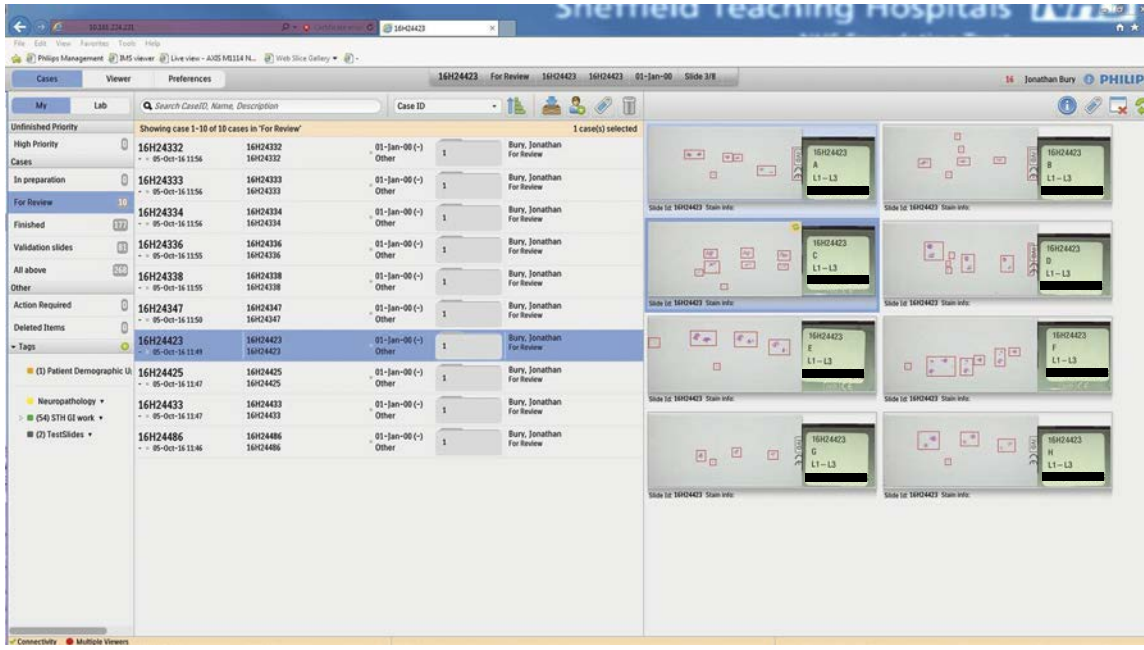

a

b

Fig. 22.8 A clinical workflow oriented interface. As well as enabling individual images to be viewed, connection to LIMS enables a variety of pathologist 'workflow' functions to be accessed. A split screen is provided. The workflow (**a**) shows those cases which are assigned to a particular pathologist and different priorities can be assigned. Cases can be assigned to different folders, e.g. cases of educational value or cases for team discussion. Cases are marked as 'finished' once reported. The image (**b**) displays the slide currently being viewed. Measurement and annotation tools are available and a slide overview is provided (top right). Documents associated with the case, e.g. the request form or macroscopic photographs may be accessed through the same interface. Reports may also be typed directly into this user interface and authorized (Philips).

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size of over 3,000 cases, totaling 10,000 slides. Ideally, each slide should be reviewed in digital and glass format by two pathologists, allowing calculation of inter/intra-observer agreement respectively. In practice this often creates an unfeasible volume of work in addition to routine reporting, and a hybrid of inter- and intra-observer agreement is used to give overall concordance between the glass and digital diagnosis. Any non-concordant cases should have a ground truth diagnosis made by consensus.

Examples of validation studies

Following the development of whole slide scanners in the late 1990s, the first validation studies of digital pathology took place in the early 2000s (Gilbertson et al., 2006). A recent systematic review found 38 validation studies (5312 total cases) from 2006-2015 (Goacher et al., 2017). The weighted mean percentage diagnostic concordance was 92.4% with a kappa diagnostic agreement of 0.75 indicating substantial agreement between digital and glass diagnoses. A trend towards higher quality study design over time was observed which may reflect the introduction of the College of American Pathologists (CAP) and Digital Pathology Association (DPA) validation study guidelines in 2013.

Subsequent to this systematic review, Snead et al. (2016) showed digital pathology (DP) diagnosis to be of equivalent accuracy to light microscope (LM) diagnosis in a large single center validation study. A clinically significant discrepancy between DP and LM occurred in 0.7% of cases. It is worth noting that in nearly half of these cases the ground truth diagnosis, arrived at by consensus, lay with the DP diagnosis. The authors also commented on potential benefits to workflow when using digital pathology, e.g. easier measurement of margins and tumor size, and easy access to and sharing of cases. Several smaller validation studies demonstrated similar equivalence between LM and DP in individual specialty areas such as gynecological and colorectal pathology.

Regulatory frameworks and standards

Whole slide scanners are classified as medical devices and are subject to regulatory frameworks. Several vendors have been granted the Conformité Européenne (CE) mark for digital slide scanners which enables these to be used within the European Union for all relevant applications, including primary diagnosis. A similar situation exists in Canada where a Class II Medical Device License has been granted to two digital pathology vendors. This classifies scanners as moderate risk devices and allows these systems to be used for primary diagnosis.

In the USA, digital slide scanners are currently classified as Class III medical devices by the Food and Drug Administration (FDA). A class III device is considered to be of substantial importance in preventing impairment of human health or present a high risk of causing injury or illness. This means that pre-market approval (PMA) is required before a scanner can be used for primary diagnosis. Following negotiation between the Digital Pathology Association and the FDA, vendors may now make a de novo application for a scanner to be classified as a class II device. This pathway is shorter and less costly than the PMA route and may pave the way for the use of digital pathology in primary diagnosis in the USA.

File storage

The UK's Royal College of Pathologists (RCPath) currently recommend that glass slides are held for 10 years, whilst paraffin wax blocks must be held for 30 years. This is to ensure that the pathology remains available should subsequent review be required. The RCPath's 2014 guidelines recommend that digital images used for primary diagnosis are retained for audit purposes for 10 years, or at least two cycles of laboratory accreditation, effectively 8 years under the 4 yearly clinical pathology accreditation (CPA) regime or potentially just 2 years under the annual ISO system.

A laboratory producing 1000 slides per day may choose to store digital images for 1 month for initial diagnosis and MDT/CPC review, prior to deletion. Storing each digital image file for 10 years from the date of creation however, would increase the storage requirement by a factor of 120, pushing the storage requirements of a large laboratory towards petabyte (10^{15} bytes) volume, with significant cost implications.

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Future development

Image quality and scan speeds have reached the point where routine digitization of all slides, even in a large laboratory can be contemplated. At the time of writing, digital slide scanners are still relatively novel pieces of equipment and they are certainly not routine capital purchases for the vast majority of laboratories. Nevertheless, akin to desktop computers, it is likely that they will/must become commonplace pieces of laboratory hardware.

The typical scanner size, in terms of the number of slides which can be loaded at once, may evolve as experience directs the balance between the convenience of a single large machine versus the resilience and flexibility of using a pool of smaller machines. The requirement for fluorescence and jumbo slides will also need to be catered for. There is a possibility to integrate staining, cover-slipping and scanning processes into a single piece of hardware. These changes are perhaps best seen as refinements and evolution, rather than fundamental changes.

More significance will be attached to the software and technical infrastructure which stores and distributes images and supports the pathologist's workflow practice. Vendors are less likely to highlight the optical quality or scan speed which will be taken for granted. Greater emphasis will be placed on demonstrating how software tools can improve the workflow both of individual pathologists and the laboratory as a whole. There may be convergence with other medical image handling systems as ultimately, whole-slide images are just one more form of image data handled in healthcare, alongside radiology images, scanned documents and endoscopic photography.

A fully digital pathology department fundamentally changes how a pathologist interacts with their workload. For example, trays of slides no longer need to be collected or delivered to an individual pathologist's office, slides from historical cases do not need to be retrieved from archival storage and cases required for MDT meetings or teaching sessions cannot be lost or left in an obscure location. Additionally, digital slides can be viewed simultaneously by multiple doctors allowing synchronous review by trainees and consultants, or by two consultants in cases which require double reporting or second opinions. These changes in workflow require an interface which facilitates the viewing and reporting of digital slides.

Such whole laboratory digitization presents considerable technical challenges. Substantial investment in servers, network infrastructure and suitable viewing stations are required. The amount of data generated each day may be in the order of tens or hundreds of gigabytes, an order of magnitude higher than even the largest of radiology departments. A single high capacity scanner will take time to scan a full load of approximately 300 slides, even at 30 seconds to 1 minute per slide, introducing a delay. An alternative configuration may be to use two or more medium capacity scanners, perhaps each holding 120-150 slides and operating in parallel. This arrangement also has the advantage of affording some resilience in the event of scanner breakdown. The manpower required to load and unload slides to and from a scanner may be offset by the time saved distributing slides around a laboratory and their subsequent collection.

Realizing the benefits of a digital workflow

From the description above it should be clear that the technology required to enable a digital slide workflow across a laboratory's entire operation is available. However, simply investing in the equipment is unlikely to be sufficient to realize the potential benefits. The successful deployment of new technology into any environment requires not only that the technology be a reasonable 'fit', but also that some modifications will be needed to that environment so that the potential of the new technology is best exploited. Some specific issues of relevance to a whole-laboratory digitization program may be as follows.

Integration with existing laboratory management systems

As the pathologist's interaction with histological images moves from the microscope to the computer,

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one useful concept is the distinction between a LIMS driven workflow and an IMS driven workflow. In a LIMS driven approach, a pathologist's primary point of interaction is with the standard hospital information system which may present the pathologist with a worklist of cases for reporting, tools for viewing previous patient reports and a mechanism for creating a report on the case currently under consideration. This interface could be expanded to trigger the presentation of a whole-slide image as required.

The LIMS is the pathologist's primary point of interaction, much as it might be in a non-digital environment, and it is this which 'drives' the presentation of digital slide images. Conversely, it may be that the image management software instead serves as the pathologist's primary point of interaction. Worklists, or other case management tools, can be created in the IMS, which may communicate 'behind the scenes' with the laboratory information system enabling reports to be created and authorized (signed-out), or other patient information such as previous reports to be accessed.

A pathologist's workflow involves selection of a case of interest, review of the clinical details, macroscopic description, viewing the slide(s) and then the drafting and authorization of a report. Additional steps may include the review of previous reports for that patient, or review of a previous slide, as well as ordering additional stains. Cases may be shared with colleagues or set aside for educational purposes and MDT meetings etc. One model of workflow integration would use the LIMS as the main focus of the pathologist's interaction, with the digital image server prompted by the LIMS to display images of interest. An alternative model places the digital image management system (IMS) at the forefront of the pathologist's interactions, with messages passed to and from the LIMS in the background as required. These models are referred to as LIMS driven and IMS driven workflows respectively. The choice of approach is to some extent arbitrary. Laboratories with a limited LIMS interface may prefer an IMS driven model, which may also be appropriate where pathologists in one department are required to review material from various source laboratories with different LIMS. Laboratories with a well-developed and operationally efficient LIMS may opt to retain that interface at the center of their workflow.

Barcoding of slides

This may require investing in new labelling equipment and other adjustments to the laboratory processes. The barcode must contain sufficient information to uniquely identify each slide. A variety of barcode formats exist. One-dimensional barcodes comprise a series of parallel black and white lines, whilst 2-dimensional formats, e.g. the quick response (QR) code is an array of black and white squares (Fig. 22.9).

Report creation

If pathologists are interacting directly with slide images on screen, it may be logical for them to enter and sign-out the final report concurrently where possible, perhaps using voice recognition software with a standardized 'canned text' phrase or a reporting template. In many laboratory workflows however, entry of the specimen details, clinical details and the macroscopic description elements of the final report does not take place until the dictated microscopy and final interpretation is typed. Entry of clinical details, macroscopic description

Fig. 22.9 This slide bears a 2-dimensional barcode. This encodes data which uniquely identifies the slide and includes patient identifiers and details of the stain used. Use of such barcodes on slides is essential in high volume slide scanning applications to avoid time-consuming and error-prone manual image annotation.

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etc. would have to be rescheduled so that it preceded microscopic reporting if pathologists were to be able to sign-out as soon as the slide images are inspected.

Adoption of paperless requesting and reporting

The benefits of digital distribution of slides are lost if paper request forms must be distributed to pathologists to enable them to report, e.g. to see the clinical details, etc. It may be that the initial clinician's request can be made electronically, or that paper request forms are scanned on receipt with the scanned request form image presented to the pathologist as slides are viewed. Establishing institution-wide paperless requesting or routine scanning of paper request forms by the laboratory are both significant undertakings.

Staff training and reprofiling

Whole-laboratory digitization offers savings in terms of staff time spent on sorting, filing and retrieving slides as well as potential saving on slide storage. However, this will be offset by the requirement to load and unload slide scanners and there will be a need for equipment troubleshooting and maintenance with significant IT support.

Pathologist workstations

Routine institutional software updates, e.g. security and software upgrades may conflict with the requirements of digital slide viewing software, raising the possibility that the digital pathology workstations need to remain separate from the pathologists' standard PC used for email and other routine office tasks.

Pathologist training

This chapter has not addressed whether reporting from on-screen images is acceptable to pathologists, although the large validation studies cited above all suggest that the technology is effective. Training in the use of the software chosen will be required, and it may be for accreditation purposes that this training should be documented and repeated at intervals.

Application of specific validation studies

As noted above, validation is required for each application of a digital slide, e.g. general gastrointestinal pathology or neuropathology. Ongoing audit of each case type where digital reporting may pose specific challenges, e.g. grading of dysplasia or identification of organisms such as *Helicobacter* may be required. Separate validation studies may also be required for special stains, immunohistochemistry, frozen sections and cytology preparations.

Summary

Although the technical principles of digital image handling are well established in other fields (e.g. radiology), adoption of the technology in histopathology will inevitably be challenging. Existing laboratory processes will need to be adapted so that the full potential of the new technology can be made. Guidance on governance arrangements for instance on slide retention will need to be updated and pathologists and biomedical scientists will see changes to the nature of their work. There is uncertainty surrounding how such extensive changes can take place, particularly given the high costs of the equipment involved. Nevertheless, digital pathology is likely to fundamentally impact on histopathology laboratory operations over the next decade.

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