Digital Microscopy

Past, Present, and Future

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• ***Context.*—Digital viewing of histologic images is moving from presentations and publications to incorporation into the daily work of practicing pathologists. Many technologic limitations have been overcome recently, which should make widespread adoption more practical. The task now is for pathologists to become actively involved in its development and implementation, to ensure that the technology is developed with the intent to optimize workflow and to maintain diagnostic accuracy. An understanding of the basic precepts of digital imaging is required to make informed decisions related to hardware and software implementation and to collaborate with vendors and professionals outside of pathology (eg, regulatory agencies) as the technology rapidly develops.**

***Objective.*—To describe the state of digital microscopy as it applies to the field of pathology and to define specific issues related to adoption of whole slide imaging systems.**

***Data Sources.*—The information is derived from the experience of the author and review of the literature.**

***Conclusions.*—Digital microscopy is an important tool for surgical pathologists. It is currently an area of intense and rapid technologic development that will likely transform the workflow of many laboratories during the next several years.**

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THE PAST

Evolution From Kodachrome to Digital Photography

The evolution of photomicroscopy has been a continual process, as it has been necessary to share images whether in publications, presentations, reports, or education. While the technology has evolved, the need for high-quality, high-resolution images has not changed. Numerous improvements have been made to both hardware and software and now digital imaging is part of most pathologists' day-to-day case work and academic endeavors. The goal has never been to replace the microscope, but rather to reproduce what we see through the eyepiece as accurately as possible. This is not a trivial task. The amount of data in a single glass slide, typically viewed at magnifications of up to ×400, is staggering. The current storage requirements for pathology slides are relatively cheap when compared to a digital environment. The question that is now being asked focuses on the advantages of working with digital images. As applied to the field of telepathology, the advantages of digital microscopy are clear, since images have to be shared rapidly to remote locations. Telepathology has evolved significantly from remote robotic microscopes to remote viewing of digital whole slide images (WSIs) (reviewed in Weinstein et al[1]). For many pathologists, knowledge of digital microscopy relates to telepathology. The current digital microscopy discussion focuses on the transition to digital pathology and the many technical and practical issues that must be addressed to enable widespread adoption.

THE PRESENT

Storage

Since a typical slide scanned at ×20 magnification (0.5 microns per pixel) results in an uncompressed file size of greater than 3 gigabytes, the issue of storage is paramount. We have seen digital storage capacities rapidly increase from kilobytes to megabytes to gigabytes to terabytes and beyond. The cost per unit of storage has consistently decreased over time at an ever more rapid pace. Pathology has benefited directly from the Moore law (originally used to describe the increase in the number of transistors that could fit on a microchip) and its correlates, as storage capacity now doubles at a frequency of less than 18 months. The speed of access to data through wired and wireless connections has similarly increased. Software that ''streams'' image data has also improved, allowing standard desktops and laptops to access portions of images instead of downloading an entire slide. Most information in hospitals now resides in large servers, which are often off-site in ''server farms'' that can be readily expanded as necessary. The shift to electronic medical records has been a powerful driving force that will continue in the future; as radiology has made the digital transition, it is likely that pathology will as well. The main issue that is specific to pathology is the preparation of glass slides, whereas the primary mode of data acquisition in radiology is digital. Currently, there are no efforts to alter the requirement of long-term storage

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of glass slides if a scanned image exists. Data security and integrity are important issues that have to be addressed because they have a significant impact on cost, one of the many barriers to a complete digital transformation. We will see improved storage systems such as ''cloud'' storage become available for use as new business models, based exclusively on remote storage, continue to develop.

Resolution

The evolution of commercial and home digital cameras has been steady, such that a 1-megapixel digital camera can now be found in a child's toy. As high-resolution charge-coupled devices capable of 12-megapixel images and beyond are standard, the demand for increasing resolution has decreased. Since WSIs are routinely stitched by digital slide scanners that use a line or grid method to acquire images, these high resolutions are not necessary. By combining a series of smaller images, a large single image can be created. One of the major issues in this process is consistent focus. Since a slide of tissue or cells is not entirely flat or of uniform thickness, it is necessary to define multiple focus points to be able to maintain a consistently focused high-quality image across the entire slide. The number of focus points is often not fixed and will depend on the size of tissue. For some tissues, such as decalcified bone, this is a particularly problematic issue because the bone in the section is often thicker than the surrounding tissue.

Slides are typically scanned with an ×20 objective and some systems can scan at ×40 or even at ×100 with proportionally larger file sizes. The goal is to reproduce the standard microscopic experience as faithfully as possible. Although a digital image can be viewed at any magnification, enlarging an image beyond the true resolution will result in pixelation or create an image that may appear to be out of focus. Plasma cells on a bone marrow aspirate smear appear out of focus when scanned with an ×20 objective (Figure 1, A), but a sharper image is produced when the same slide is scanned at either ×40 (Figure 1, B) or ×100 (Figure 1, C). A persistent theme, however, is that of trade-offs—in this case, between file size and sufficient image detail—to make an accurate diagnosis. In fact, another less commonly thought of issue is the resolution at low power, which requires a reverse digital zoom that—because of the limited number of pixels that can be displayed by a monitor or projector—cannot reproduce what is seen through an eyepiece.

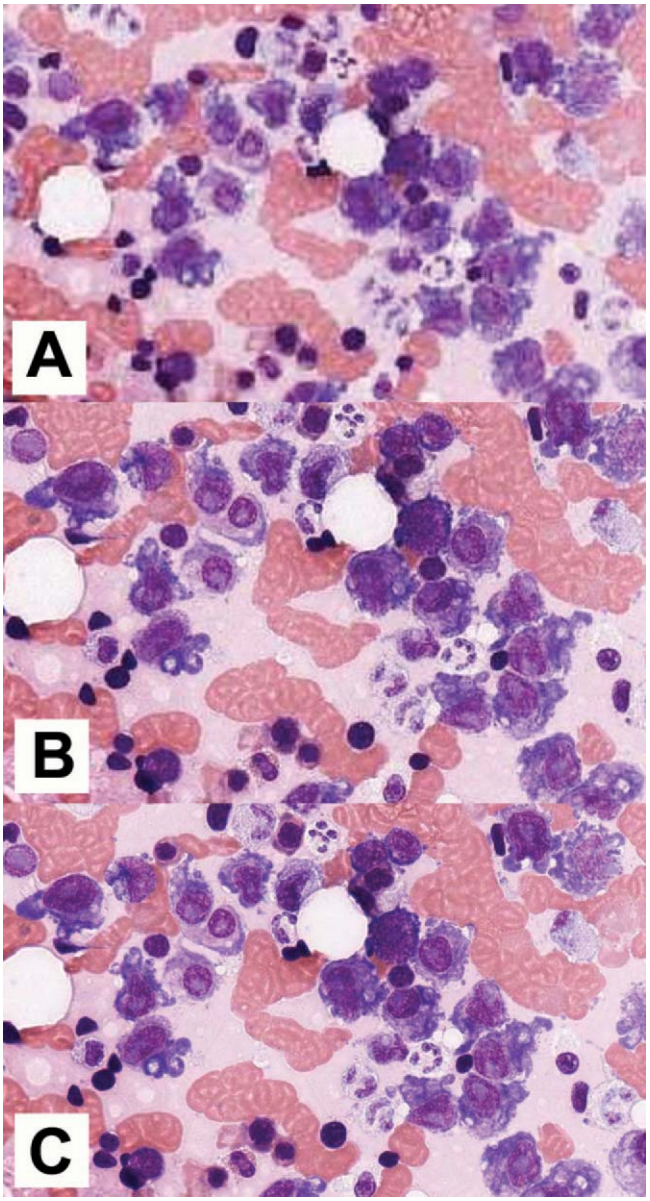

Figure 1. *Effect of scanning magnification on resolution. A bone marrow aspirate smear stained with Giemsa was scanned with an ×20 (A), ×40 (B), and ×100 oil immersion (C) objective. The images are all displayed at an equivalent of ×400 magnification or 0.25 microns per pixel.*

Compression

Most are familiar with the concept of lossy or lossless compression, as pathologists must often work with digital image editing software. There are of course trade-offs between file size and resolution when choosing a particular file format. Even with a format such as JPEG, there are varying levels of compression that can be chosen, which will affect the image quality. As file sizes decrease with greater compression, the image will look, at some point, either out of focus or pixelated. Manufacturers of whole slide scanners will often choose some level of compression that seems to maintain the image quality while creating a file of manageable size. Several studies[2] have examined the effect of image compression on the accuracy of pathologic diagnoses, although there are no clearly accepted standards. As depicted in Figure 2, an image can be compressed several-fold without any perceived loss of image quality. If even a fraction of the slides routinely prepared in a pathology laboratory were to be scanned on a daily basis, the storage savings provided by compression would become increasingly important. Also, the reduced file sizes should also improve the image browsing experience, especially if the user is viewing the digital slide remotely through a sub-broadband connection.

Speed

Again, pathology is a benefactor of the Moore law, not only because faster desktop computers allow us to sign out our cases faster, but also because the computers and servers that are connected to digital slide scanners are significantly more powerful, such that they can process

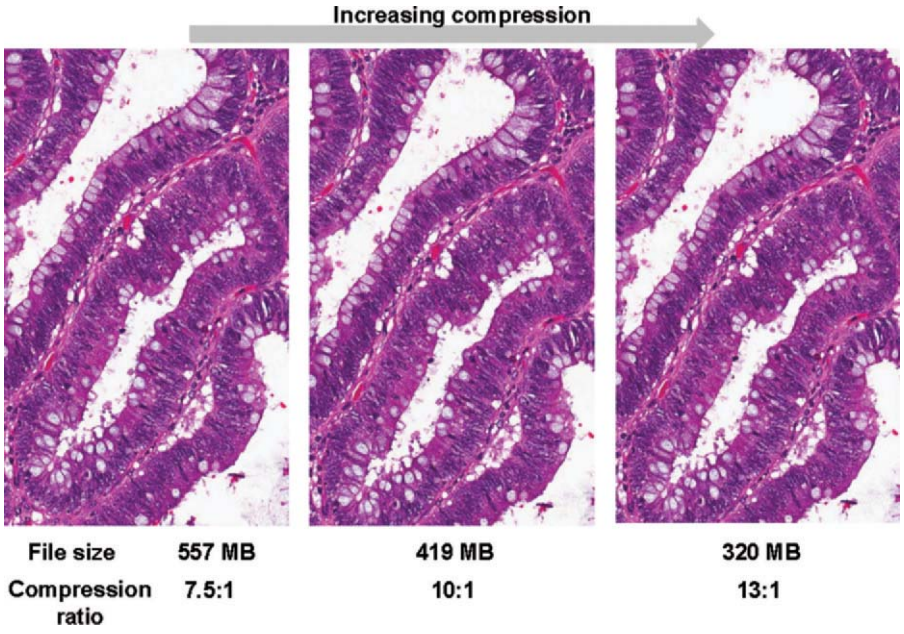

Figure 2. *Effect of compression on image quality. A typical tissue section from an adenomatous polyp of the colon was scanned at ×20 with varying compression levels. A representative field is displayed at ×200 magnification. The compression ratio and file size for the whole slide image are shown.*

more efficiently the high-level data stream as the slide is scanned and a large image file is stored. What once might have taken 30 minutes or more can now be accomplished in less than 2 minutes by many systems or likely even in less than 30 seconds in the near future. As the concept of a digital environment crystallizes in the minds of pathologists and business, throughput becomes an important issue. If a pathology laboratory stains thousands of slides per day, the requirement that these slides be scanned the same day becomes a limiting factor. The number of scanners needed to scan the slides at an individual laboratory is not currently clear. Most WSI systems have auto-loaders that can handle more than 100 slides at a time. However, if the volume is 1000 slides at a rate of 2 minutes per slide or 2000 minutes (33 hours), the scan time would be more than the length of a day. Adding to this the time necessary to load the instrument and assuming a 10% rescan rate (a low estimation in my opinion), then the need for several scanners becomes clear. Of course, the cost of the digital transformation will then increase significantly. Perhaps with a continuous slide-loading system and a scan rate of 30 seconds per slide (120 slides per hour), a low number of scanners per laboratory seems feasible.

Image-Viewing Browser

Continuing the recurring theme of technologic advancement is the experience with viewing digital images, which has rapidly improved from the use of 15-inch cathode ray tube monitors to high-resolution, 30-inch liquid crystal display flat screens to computers that support multiple displays at high resolution and at a relatively low cost. The contrast ratios and screen brightness have also increased, so that even in a bright light environment or at indirect viewing angles, the displays can more accurately reproduce the original images. Although slide navigation has not evolved from control with a PC (personal computer) mouse, newer versions of this device do feature wireless capabilities and laser and optical features to make the experience more fluid. Other navigation devices (eg, trackball and joystick) have been tried, but have not been widely adopted. Browsing software has been optimized to efficiently stream image data and minimize the lag time during rapid navigation of a large image file, but other features such as integration into existing laboratory information systems (LISs) has been limited. Typically, the slide images are stored in a database that is entirely separate from the LIS, such that data associated with each slide must be entered manually. In an ideal system, a link would exist to all the digital slides for each specimen during the viewing of a patient record in the LIS. Efforts are ongoing to enable communication between the LIS and image databases; however, an integrated system is not currently available. A typical browser includes multiple tools to navigate and annotate images and mark regions of interest for image analysis or to segment tissue microarray slides, but features that would help with primary review of working clinical cases are generally lacking.

Standardization

Image standards in pathology do not currently exist. Working groups in radiology have progressed significantly further than in pathology, with adoption of imaging standards and the formation of the DICOM (digital imaging and communications in medicine) medical imaging standard, which is now at version 3.0. To be able to share images between institutions, as we currently do with glass slides, we will need access to a minimum amount of metadata associated with each WSI to know how the slide was scanned (resolution, image label, etc). Vendors must also cooperate so that WSIs from one scanner can be read with a viewer from a different vendor. Efforts are underway to develop universal viewers, but without cooperation from all scanner manufacturers, this will not be possible.

Cost

Although storage costs have significantly decreased, the typical price of a slide scanner has not. Although many of the components of these instruments have improved, the total cost of hardware and software has increased in many circumstances, as many businesses attempt to increase

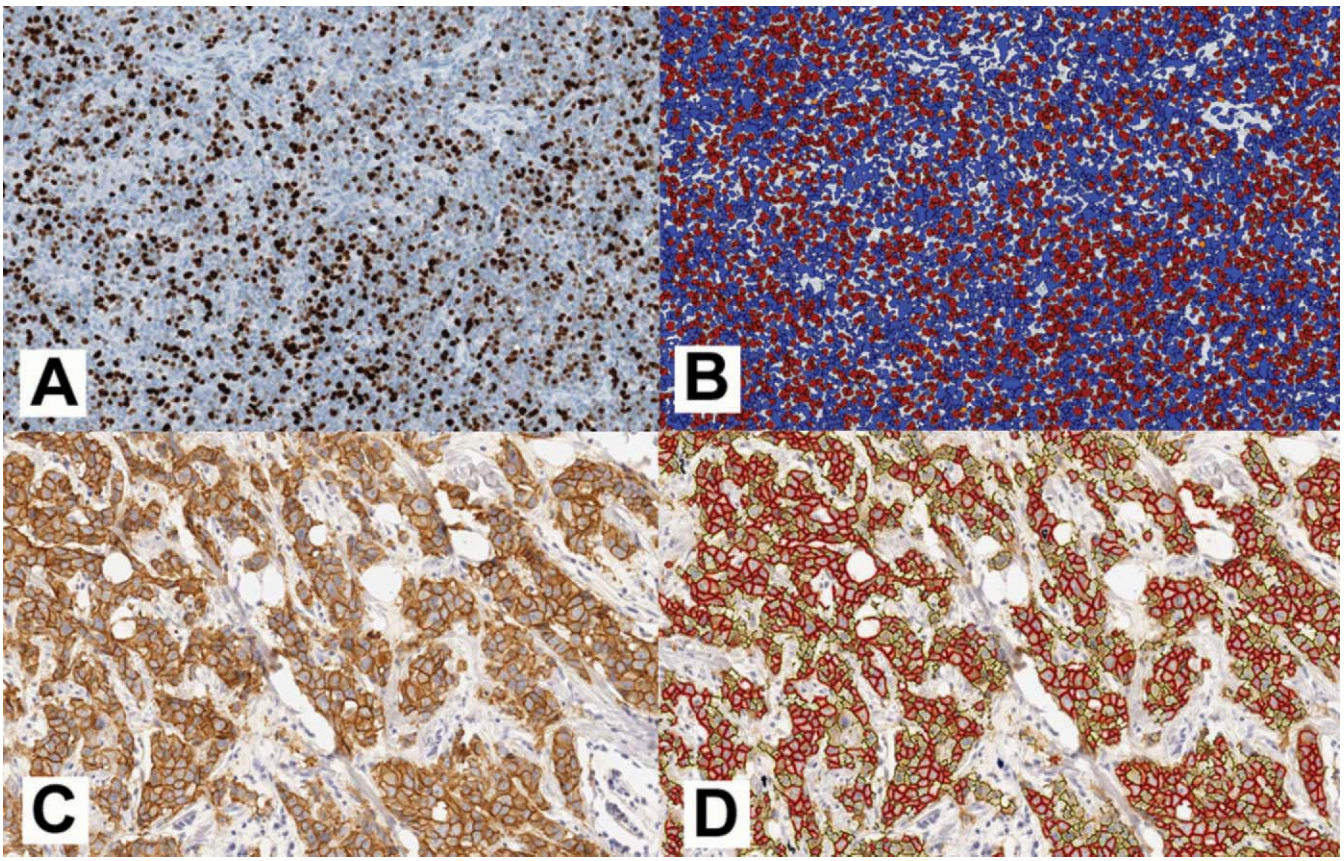

Figure 3. *Example of image analysis results. A, A section of a mantle cell lymphoma was stained for Ki-67. B, The scanned slide was analyzed with an algorithm that recognizes brown 3,3′-diaminobenzidine–stained nuclei (indicated in red) and blue (hematoxylin)–stained nuclei (indicated in blue). C, An invasive breast ductal carcinoma was stained for HER2/neu. D, The image analysis results are displayed, with red indicating 3+ staining, orange 2+, and yellow 1+, based on the intensity of stain and membrane completeness (original magnifications ×200).*

revenues. It is unlikely that costs associated with acquisition of new systems and maintenance will be reduced in the near future, although increased competition among the growing number of manufacturers may change this trend.

Image Analysis

I have focused the discussion exclusively on the viewing of standard brightfield images of slides; however, one significant advantage of working with a digital image is the ability to perform a variety of analyses.[3] Whether stained with hematoxylin-eosin or an immunohistochemical stain, sophisticated tools are available to quantify specific features. For example, the number of cells in a region of interest can be automatically counted or the intensity of a chromogen, whether in the nucleus, membrane, or cytoplasm, can be quantified. One advantage of this technique is that it is reproducible. If a specific region is analyzed multiple times, the same result will be achieved. If, on the other hand, separate observers score the same slide, an exact interobserver or intraobserver agreement is not likely. In our study of Ki-67 staining in mantle cell lymphoma (Figure 3, A and B), the correlation coefficient was relatively high when estimating the percentage of positively staining cells (interobserver, 0.90–0.92; intraobserver, 0.90–0.95; author's unpublished data, 2010); however, interpretation of intensity of staining is typically more subjective and likely to show greater variability. The consistency of analysis of immunohistochemical staining has been applied in several studies of HER2/*neu* expression in breast cancer.[4,5] Since HER2 overexpression by immunohistochemistry correlates with gene amplification, as detected by fluorescence in situ hybridization analysis, the accuracy of image analysis can be determined. In addition, an objective, reproducible technique for HER2 interpretation is particularly valuable, since the result leads to an important treatment decision. Several software developers have applied for and received US Food and Drug Administration (FDA) approval for digital-assisted analysis of HER2/*neu* staining and other stains including estrogen receptor and progesterone receptor. An example of HER2 image analysis of breast cancer is shown in Figure 3, C and D. FDA approval and the ability to bill for this service has been one of the main driving forces for acquisition of digital imaging systems in many institutions. One significant limitation of most current image analysis systems is the inability to recognize particular cell types (eg, tumor versus normal). The pathologist must mark the region of interest that is to be interpreted and verify the image analysis result. Vendors have recognized this limitation and are developing algorithms to recognize specific cellular subpopulations that will facilitate this process further.

THE FUTURE

One Niche at a Time

Where is this technology currently making the greatest inroads? For specific applications, the advantages of digital microscopy outweigh the disadvantages signifi-

cantly enough that adoption is already underway. Telepathology, including remote viewing of frozen sections, is the first area to experience widespread use of digital systems. Many studies have validated the accuracy of remote viewing of digital images either in a consultation setting or in a distributed environment where 1 pathologist covers more than 1 hospital.[6–8] For these applications, limitations such as storage, speed, and lack of laboratory information system integration do not prevent the use of this technology. Like many other technologies, there are penalties for early adopters. As slide scanners are improved, new systems will have to be acquired to take advantage of advancements in scan speed, image quality, and workflow efficiencies. Software can often be updated; however, the hardware must often be replaced at significant cost.

For research applications, the power of automated analysis of digital images is the driving force behind digital microscopy utilization. Software for analyzing digital images has long been used in the research setting to quantify morphometric features or intensity of staining. FDA approval and the ability to bill for digital interpretation of certain immunostains have led to adoption of dedicated imaging systems for that purpose. Whole slide images and the development of powerful, inexpensive desktop computers that can efficiently handle large image files have enabled analysis of large image data sets with increasingly sophisticated algorithms that are currently undergoing rapid technologic advancements.

From pathology examinations to medical schools, education has benefited from digital microscopy. With an Internet connection, multiple viewers can study a single slide simultaneously from any location. Online pathology image archives for teaching purposes now exist and are continuing to develop as a useful resource. In the future, extensive catalogs of disease entities, either as stand-alone tools or as companions to a textbook, will certainly increase in frequency and probably become expected features of any new text.

Will Pathologists Ever Be Replaced by Computers?

The evolution of digital image analysis software has been relatively slow.[9,10] The concept is not new; however, market penetration has been limited. An increasing number of vendors in this area have pushed the field forward somewhat, and new applications are emerging. Computer-aided diagnosis (CAD) is now developing at a noticeable pace. Initially used for rescreening of Papanicolau smears, it is now being developed for tissue analysis. Developers are marketing this type of software for quality assurance purposes, but as the algorithms become more sophisticated, one can envision a future in which primary diagnoses will be made by such an instrument, if proven to be accurate. In the meantime, these pattern recognition algorithms can be used to improve immunohistochemical quantitation algorithms, such that only tumor cells or a particular cell type are quantified, obviating the need to specifically designate regions of interest. The sensitivity and specificity of these algorithms remain to be proven, and they will need to undergo numerous iterations and advances before a computer program can begin to unravel the morphologic complexity of human disease.

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