A Practical Guide to Whole Slide Imaging

A White Paper From the Digital Pathology Association

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• ***Context.***—**Whole slide imaging (WSI) represents a paradigm shift in pathology, serving as a necessary first step for a wide array of digital tools to enter the field. Its basic function is to digitize glass slides, but its impact on pathology workflows, reproducibility, dissemination of educational material, expansion of service to underprivileged areas, and intrainstitutional and interinstitutional collaboration exemplifies a significant innovative movement with far-reaching effects. Although the benefits of WSI to pathology practices, academic centers, and research institutions are many, the complexities of implementation remain an obstacle to widespread adoption. In the wake of the first regulatory clearance of WSI for primary diagnosis in the United States, some barriers to adoption have fallen. Nevertheless, implementation of WSI remains a difficult prospect for many institutions, especially those with stakeholders unfamiliar with the technologies necessary to implement a system or who cannot effectively communicate to executive leadership and sponsors the benefits of a technology that may lack clear and immediate reimbursement opportunity.**

Objectives.—**To present an overview of WSI technology—present and future—and to demonstrate several immediate applications of WSI that support pathology practice, medical education, research, and collaboration.**

Data Sources.—**Peer-reviewed literature was reviewed by pathologists, scientists, and technologists who have practical knowledge of and experience with WSI.**

Conclusions.—**Implementation of WSI is a multifaceted and inherently multidisciplinary endeavor requiring contributions from pathologists, technologists, and executive leadership. Improved understanding of the current challenges to implementation, as well as the benefits and successes of the technology, can help prospective users identify the best path for success.**

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Pathology as a practice vitally relies on the accurate interpretation of microscopic images in order to correctly diagnose patients and guide therapeutic decision making. With the advent of whole slide imaging (WSI), pathologists have begun to transfer the act of viewing glass slides from the microscope to the computer monitor. Using modern WSI systems, pathologists can navigate a virtual slide in much the same way they navigate Google Maps. This has led to a number of new opportunities not possible using conventional microscopes, including digital collaboration/telepathology, integration with electronic workflows and health records, and diagnostic support based on computational tools like artificial intelligence. The regulatory apparatus that governs this technology in the United States is just starting to come together, with US Food and Drug Administration (FDA) clearance having been granted only a year ago and accreditation agencies still working to establish comprehensive policies that ensure proper validation and use.[1] Nevertheless, its role in clinical diagnostics, education, and research has begun to materialize, and rapid adoption of WSI in pathology laboratories will likely follow.

This white paper outlines the current state of WSI in pathology, emphasizing the practical considerations for adoption and the regulatory guidelines that govern its use. Additionally, we present several areas of ongoing research and development poised to introduce transformative prognostic and predictive capabilities to pathology.

BASICS OF OPERATION

Whole slide imaging encompasses the digitization of entire histology slides or preselected areas. It was first

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described by Wetzel and Gilbertson in 1999.[2] The process of digitization includes 4 sequential parts: image acquisition (scanning), storage, editing, and display of images.[3] Whole slide imaging uses slide scanners that consist of 4 main components: light source, slide stage, objective lenses, and a high-resolution camera for image capture.[4–6] Scanning of other materials, such as cytology slides and other specimens (eg, plant material), is also possible, although not described in depth here.[7,8]

Whole Slide Scanning

Whole slide scanners capture images of tissue sections tile by tile or in a line-scanning fashion. The multiple images (tiles or lines, respectively) are captured and digitally assembled ("stitched") to generate a digital image of the entire slide.[9,10] When pairing scanners with slide staining techniques, WSI can be categorized as brightfield, fluorescent, and multispectral. Some scanners can accommodate more than one modality, for example enabling both brightfield and fluorescent scanning. Brightfield scanning emulates standard brightfield microscopy and is the most common and cost-effective approach. Fluorescent scanning is akin to fluorescent microscopy and is used to digitize fluorescently labeled slides (ie, fluorescent immunohistochemistry [IHC], fluorescent in situ hybridization). Fluorescent scanners always capture images as tiles.[9] Multispectral imaging captures spectral information across the spectrum of light. It can be applied to both the brightfield and fluorescent settings.[9,11,12]

Methods of focusing along the z-axis of a slide vary from focusing every individual tile or focusing on selected tiles to using a series of focus points.[9] Although focusing on every tile is the most time-consuming strategy, it also potentially creates the highest-quality results. To reduce image acquisition time, users may select to focus only on every *n*th tile. Line scanning exclusively uses focus maps; however, these can also be used with tile scanning. In these maps, a network of focus points is placed over the entire surface of the tissue, resulting in the fastest scan times at the cost of the highest potential error rate. Focus points can be chosen manually or can be automatically set, a function included in most currently available scanning software.[9,13] More recently, scanning processes have been developed that incorporate continuous automatic refocusing processes, which has further increased the quality of scans.[14,15] Most modern scanners have also incorporated tissue recognition features that allow for automatic detection of the histology specimen via a low-magnification overview scan, hence greatly increasing scanner efficiency.[14]

Scanning can occur at multiple magnifications. Scanning at ×20 magnification is usually acceptable for standard viewing and interpretation, including routine image analysis of hematoxylin-eosin (H&E) and IHC slides. For other applications, such as digitization of in situ hybridization slides, images should be acquired at ×40 magnification to resolve information that may be separated by distances less than about 0.5 μm.[16] Digitizing of tissue slides at even higher resolution (×60/×63 or ×100, under oil) is now available via select scanners, but is typically only recommended for specific use cases such as blood smears.[6,14,17] A select number of scanners are available that can accommodate both dry scanning and oil immersion during the digitization process.[4]

Different scanner models vary not only in their scanning modality, but also in their slide-loading capacity and scan time. High-throughput scanners have loading units that can hold up to 400 slides.[4,9] Scanning times per slide range mainly from 30 seconds to several minutes.[4,10,18] Scanning at higher magnification (ie, ×40 versus ×20), digitization of larger tissue sections, and digitization of fluorescently labeled images can further increase the scan time (as well as file size).[17] Specialized scanners that can digitize whole tissue mounts and larger glass slide sizes are available.[4]

Although it is paramount to view digitized slides on monitors with adequate resolution, the selection of WSI system is an important first step on the path to comfortable viewing of images. How a scan looks on a monitor is only in part determined by the size and number of pixels that the monitor can display. Just as important is the objective lens magnification used by the scanner and the size and number of pixels within the digital camera's sensor. Although the scanning magnification is determined by the objective used (eg, ×20 objective), the resolution is defined as the minimum distance at which 2 distinct objects can be identified as separate events. In a purely optical system, the resolution is determined by the numerical aperture of the objective used. In contrast, in a digital system, resolution is now also influenced by the camera sensor and the viewing monitor. If the camera sensor has a lower resolution than the objective's numerical aperture allows for, information is lost, and even the use of a high-resolution monitor will not increase resolution when displaying a scan produced by this system. Therefore, the quality of the capturing camera within a digital scanner should be taken into consideration when comparing different models.[19]

When establishing a workflow for digital pathology, one needs to understand that in addition to the traditional histology workflow, additional steps are added when tissue sections are intended for digitization.[20] This includes the need for additional equipment and staff, proper training of personnel, additional quality control steps, equipment and software maintenance, adequate information technology infrastructure, and proper pathologist workstation setup. Once slides have been digitized, the quality of scans needs to be confirmed. Scanning artifacts can affect downstream results, and can be caused by improper cleaning of slides prior to scanning, poorly focused scans, compensation lines from improper stitching of lines or tiles, and other factors.[21]

Virtual Slides

Whole slide scanning generates digital representations of glass slides that with the proper tools can be navigated in an interactive manner. In order to reproduce the magnifications necessary for many diagnostic and research applications, the slide must be captured at sufficiently high resolution and with adequate color depth. Resolution is typically expressed by vendors in units of μm per pixel and color depth is expressed as the number of bits allocated to a pixel, which specifies the total number of distinct colors possible in the image:

$$\text{Bits per } \mu m^2 = \frac{\text{Bit Depth}}{\mu m^2 \text{ per Pixel}}$$

A typical whole slide image scanned at ×40 magnification has a resolution of about 0.25 μm per pixel and 24-bit color depth. As a result, the number of bits of information representing a 1-mm^2 area of the slide is 384 million, resulting in a file size of approximately 48 MB without additional steps taken to more efficiently manage the data.

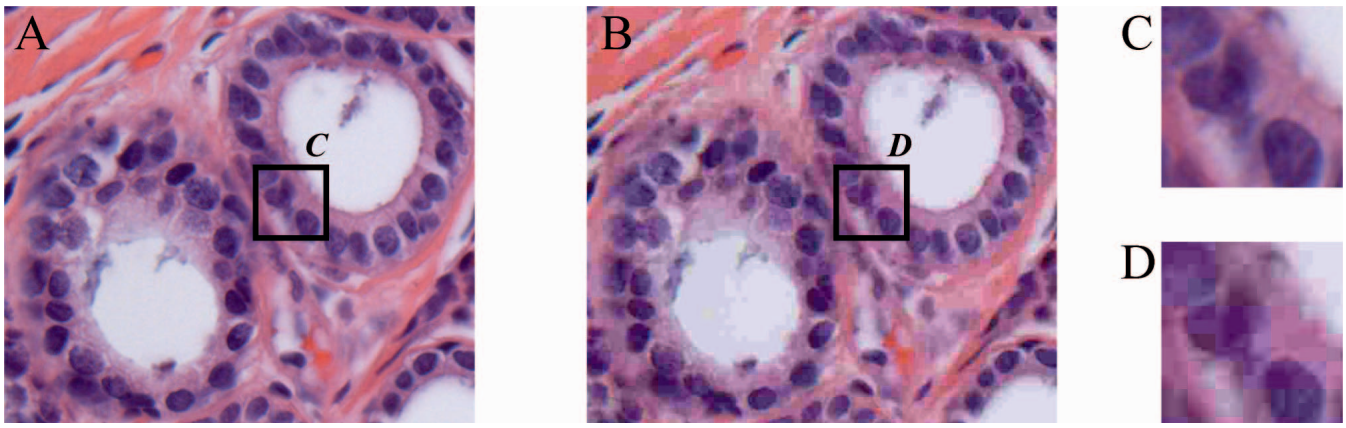

Figure 1. *Image quality following JPEG compression. A, A sample hematoxylin-eosin (H&E) image was compressed using a JPEG quality factor of 0.8, resulting in a reduction in file size by a factor of 15.9 (compared with the uncompressed image). B, The sample was compressed using a JPEG quality factor of 0.1, with compression ratio of 108.3, resulting in visible artifacts. C and D, Magnified views of the fields of view indicated in A and B, respectively, reveal substantial visible artifacts following compression (original magnification ×20 [A through D]).*

This value rapidly increases when an entire slide is considered or if multiple z-planes are captured, introducing a potential burden for storage and bandwidth that must be overcome in a way that provides a seamless experience for the user.

Image Compression

Methods to reduce file size using image compression are ubiquitous in WSI. Many vendors use JPEG, JPEG 2000, or LZW compression to reduce file size to manageable levels, often resulting in a reduction of file size by a factor of 7 or more.[22] Because JPEG is a lossy compression algorithm, information is lost in the conversion and cannot be recovered. For systems that enable users to specify the level of JPEG compression applied (usually expressed as a quality factor between 0 and 1), users are encouraged to select a value that optimally balances the tradeoff between image quality and file size, noting that excessive compression can introduce visible image artifacts (Figure 1, A through D). Although morphologic assessments appear to be less affected, densitometric assessments are increasingly sensitive to this loss of digital information.[10,23] For these reasons, users are also discouraged from applying JPEG compression successively on the same image, as each round of compression can result in degradation of image quality. An additional method commonly used in WSI to reduce file size is to discard blank regions of the slide altogether. Vendors have used this technique not only to reduce file sizes but also to reduce scan times by identifying regions in the initial macro snapshot that do not need to be scanned.

Pyramid Representation

Despite methods of reducing file size, a single whole slide image in practice often exceeds 1 GB in size. For a technology that promises enhanced digital collaboration over networks, files of this size can be prohibitive to download. Similarly, they can be difficult to load into memory for the purposes of display and navigation. Vendors have tackled this problem by noting the intrinsic relationship between image scale and field of view (Figure 2, A, black line). For large fields of view, resolution is limited by the computer monitor and therefore the image does not need to be loaded at the highest resolution. Conversely, when users examine tissue at high magnification, only a small field of view is visible on the monitor at any given time, and so the image does not need to be loaded in its entirety. These limitations in viewing provide an opportunity to more efficiently deliver images to users.

Whole slide images are stored at multiple resolutions to accommodate a streamlined method for loading images. For example, a sample whole slide image acquired at ×40 by the Aperio Scanscope whole slide scanner is accompanied by the same image downsampled at ×10, ×2.5, and ×1.25, as well as a thumbnail image that represents the entire tissue fit within a ~1-megapixel frame. These images are typically embedded within a single file, although this is not a requirement. This multi-resolution representation is commonly referred to as an image pyramid and enables more efficient data throughput by precomputing lower-resolution versions of the whole slide image. In this way, a viewer can retrieve a much smaller low-resolution component of the file when attempting to render large fields of view, therefore requiring less bandwidth to view the image (Figure 2, A, red line). In systems that allow the user to specify the number of levels in the pyramid, users are encouraged to select a value that balances the tradeoff between bandwidth and overall file size. More images in the pyramid result in incrementally larger file sizes (Figure 2, B) but allow viewing software to more efficiently select the scale at which to load data. A typical number of levels is 3 or 4.[24] Supporting metadata are frequently found in these files specifying image properties and the organization of the file. Additional images embedded within the file often include the slide label and a coarse snapshot of the slide in its entirety, but these are typically small and add a negligible amount to the file size.

Storage and Access

The strategy for storing virtual slides is largely dependent on intended use. For applications with very few users and with no need for retention, local storage is often sufficient. For example, a research project may benefit from WSI to support quantitative analysis or to generate high quality images for publication but may have no particular need to share the image with collaborators or access it after the research project is complete. However, if retention is important, methods to ensure reliable archival should be explored. In this case, a complete backup strategy may be appropriate, which may include off-site storage, RAID

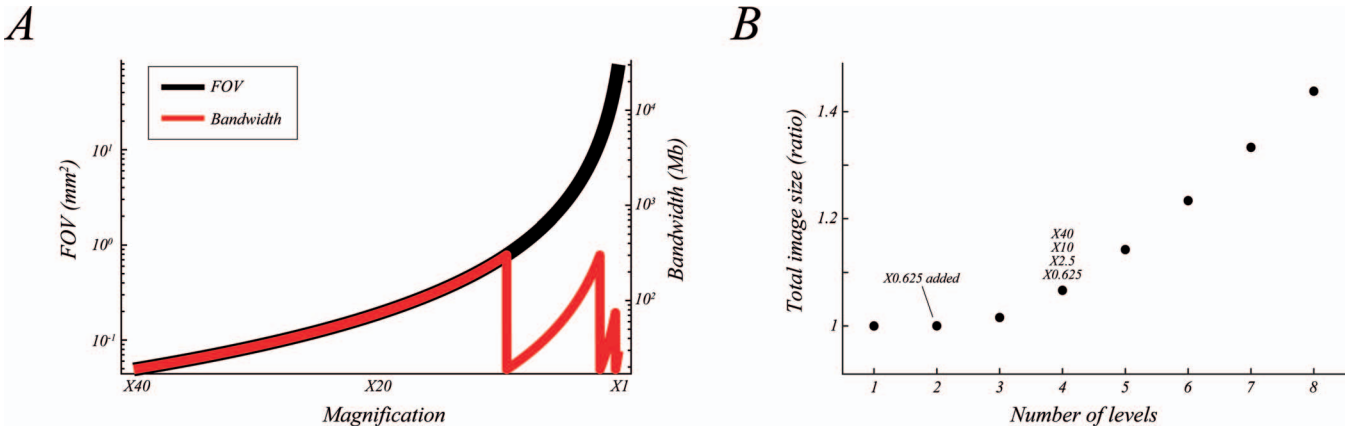

Figure 2. *Whole slide image structure and its impact on file size. A, At decreasing magnifications/resolutions, the field of view (FOV) represented within a 1024 × 768-pixel frame increases (black line). When the whole slide image is downsampled to also include ×10, ×2.5, and ×1.25 representations, the increase in bandwidth necessary for loading low-magnification views is reduced (red line, right axis). Base magnification: ×40. Pixel size: 0.25 μm. B, The addition of downsampled images in a whole slide image file is estimated based on the number of levels represented (compression is excluded from the analysis). An increasing number of levels results in larger overall file sizes, represented as the ratio of file size to the size of the original ×40 level. The magnification of each level was determined by the equation* $l(i) = \left(\frac{40}{0.625}\right)^{\frac{1}{N-1}} l(i-1)$, *which produces an exponentially distributed set of* N *levels with magnifications between the outer limits of ×40 and ×0.625.*

storage, optical/tape storage, or some combination. For applications where access for multiple users is required, network-based solutions are recommended, and can often be accomplished in parallel with a responsible backup strategy. Organizations must decide whether cloud-based or (local) server-based network access better suits the needs of the users and conforms to the information system protocols of the organization. For cloud-based solutions especially, a main consideration can be the cost that accompanies the bandwidth and data throughput necessary to achieve desired performance. Hybrid solutions that involve local and cloud-based storage and access, or hub-and-spoke models for multisite organizations, can also be effective strategies.

Viewing and Managing Virtual Slides

Whole slide imaging offers an opportunity to expand the set of tools available to users to include digital annotation, rapid navigation/magnification, and computer-assisted viewing and analysis. Typically, the intended use dictates the preferred method by which users will access whole slide images. For example, when whole slide images are used for educational purposes, instructors often need access to a dedicated image viewer that enables them to annotate images so that trainees can quickly identify and navigate to regions of interest in the slide. Similarly, the use of WSI to support clinical diagnostics is often aided by the ability to view images in association with the patient's clinical history, or alongside other slides or images that may have been acquired from the same patient (eg, serial sections, IHC, grossing photos, radiology). Some image viewing software supports advanced viewing tools, enabling users to simultaneously view multiple images in a single frame or overlay different stains from serial sections.

Many WSI systems include image viewing software that can be installed locally on user computers. Other vendors offer this ability as part of a larger software suite residing on network servers, enabling users to view whole slide images in their Web browsers. For users who wish to apply image analysis algorithms to whole slide images, some of the viewers provided by vendors are packaged with algorithms that can detect cells, compute positive staining, perform regional segmentation, or perform nuclear segmentation in H&E images. Viewers often support the ability to annotate images, save regions of interest, take snapshots of selected regions, and export images to other formats.

For users who require more sophisticated image analysis algorithms than their vendor provides, a number of software solutions have hit the market with exceptional capabilities. These can often be integrated into a department's workflow in a seamless manner, providing on-demand image analysis in conjunction with whole slide viewing. Many image analysis software vendors offer services in an a la carte fashion, whereas others provide tiered packages with different pricing options. A popular free alternative to these platforms is ImageJ,[25] a software tool made available by the National Institutes of Health that includes a number of common image analysis algorithms useful for histology image processing. A popular package that bundles a number of features and enhancements to ImageJ, called Fiji,[26] further extends the suite of image analysis algorithms available to researchers. Although these free software tools provide advanced image analysis capabilities with relevance to pathology, loading entire whole slide images in these platforms remains difficult. As described in the previous section, special considerations must be made to efficiently load and process these images, which may limit the utility of general image analysis tools like ImageJ to screenshots or exported snapshots. However, in 2017, the Centre for Cancer Research & Cell Biology at Queen's University Belfast, as part of research projects funded by Invest Northern Ireland and Cancer Research UK, developed a freely available open-source whole slide image viewer called QuPath,[27] which extends ImageJ-like functionality to a platform designed specifically for whole slide images. This tool offers whole slide viewing, annotations, image analysis, and automation, allowing researchers access to advanced open-source software tools.

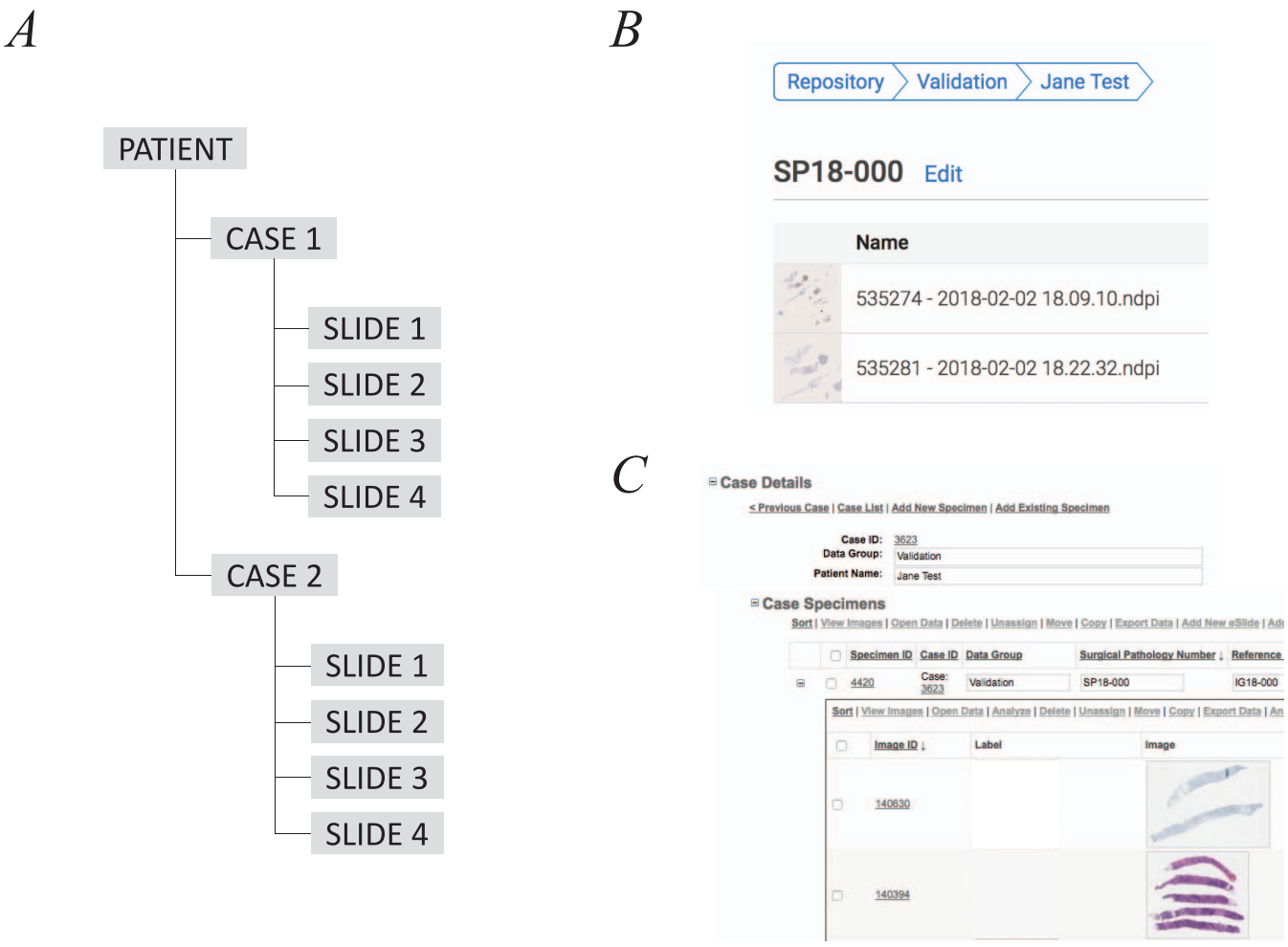

Figure 3. *A sample hierarchy for the organization of whole slide images in a clinical workflow (A). The top level is indexed by patient identifier and contains all cases and specimens for that patient. Individual whole slide images reside underneath the case level. Examples of this organization are shown for patient "Jane Test" with specimen identifier SP18-000 in the (B) PathcoreFlow (Pathcore, Toronto, Ontario, Canada) and (C) Aperio eSlideManager (Leica Biosystems, Wetzlar, Germany) image management systems.*

Direct Image Access

In research applications where automated quantitative processing is used, direct access to the image files is sometimes preferred. This enables computer algorithms to bypass the image viewer and directly access the data necessary for processing. A number of tools have been developed to enable direct access to WSI data. For instance, Matlab (Mathworks, Natick, Massachusetts) has built-in support for whole slide images using the *imread* function. This function has optional input parameters that allow users to specify the pyramid level to access and to restrict the region to load based on user-supplied coordinates. However, not all image formats and magnifications are supported. The OpenSlide[28] and Bio-Formats[29] libraries provide support for a greater number of image formats, and support integration with commonly used programming and scripting environments like Matlab and Python. OpenSlide and Bio-Formats each boast support for more than 8 proprietary whole slide formats, allowing users to use the same basic functionality for images across multiple vendors. This can facilitate multi-institution studies or analysis of public image repositories derived from multiple sources.

Image Management Systems

Although image viewing is a critical component of many WSI applications, in many cases image management holds just as important a role. Image management systems are software platforms that offer the ability to organize and access images using image metadata, patient information, or some other characteristic that can associate images into meaningful groups. For example, a common clinical workflow may organize slides in a hierarchy that provides users access to images in a manner not unlike laboratory information systems (Figure 3, A through C). Likewise, a research environment may be organized in a similar manner, where studies may be separated according to researcher, project, or clinical trial. Advanced features of image management systems often include integrated image viewers and analysis routines, the ability to save and recall slide annotations, integration with information systems, storage of computed data (eg, Her2 score), authentication and user management, and modules that provide reports of results. As a result, image management systems are often a central component of a WSI system. Alternatively, some hospital picture archiving and communication systems and laboratory information systems

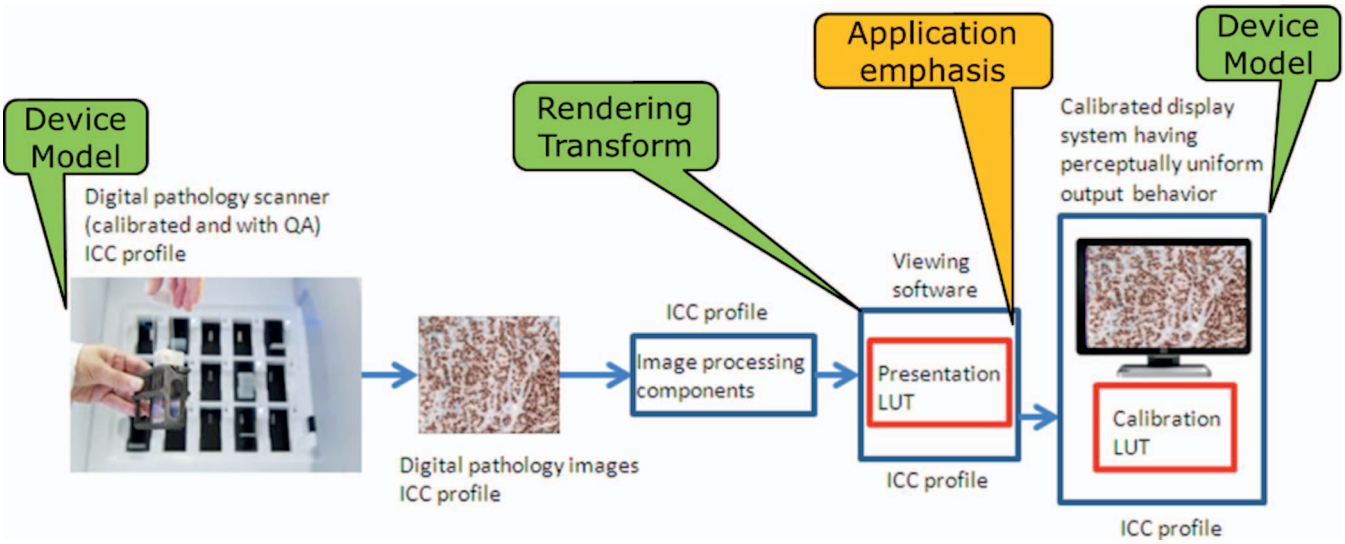

Figure 4. *Recommended color preservation pipeline for brightfield imaging using whole slide imaging and International Color Consortium (ICC) profiles. Abbreviations: LUT, lookup table; QA, quality assurance.*

may support image organization, although they typically have only limited support for the unique requirements of whole slide images.

Preservation of Color Through the WSI Pipeline

Differences in color can have an influence on the diagnostic performance of pathologists. In a set of experiments examining the effect of a computer display's age on color, Avanaki et al[30] found that aging reduced the color saturation and luminosity of the display and produced a shift in the color point of white. Consequently, they found that the average time for pathologists to score digital slides increased from 41 to 50 seconds on aged displays. In addition, the ease of reading reported by subjects on a scale from 1 (hard) to 10 (easy) dropped from 9.7 to 8.7. Intersession percentage agreement of diagnostic scores for slides shown on a non-aged display was about 20% higher than that of aged slides. These findings indicate that preventing the degradation of color in the digitization and display process is important for optimal results.

Every laboratory has its own protocols for processing samples at each step, including tissue acquisition, fixation, processing, and staining. This is a first source of perceived color differences; an agreement on standardized tissue handling protocols and stain standardization may be useful at the preimaging stage.[31] Whole slide imaging will even further highlight the color differences among slides as more components that can introduce differences are added to the visualization chain. For example, a slide scanned by one scanner but interpreted with 2 different viewing applications may exhibit visible color differences if presented using 2 different presentation lookup tables. A similar effect can be observed when using 2 different scanners to acquire images from the same slide. The color rendition of the scans can be very different between the 2 scanners.

An additional element in the imaging chain is the display. The display is a key component for the visualization of slides and has an enormous influence on how colors are perceived. Color differences are commonly introduced by interdisplay differences, which in turn can cause differences in color perception when the same slide, acquired by the same scanner and visualized in the same viewer application, is viewed on different displays. Another potential source of color difference occurs when the color gamut of the whole slide scanner does not match the color gamut of the display. For example, the most saturated red that can be acquired by the scanner may not correspond to the most saturated red of the display. This may cause a different color sensation than intended by the scanner manufacturer.

Absolute color calibration can be achieved with the use of the International Color Consortium (ICC) framework, an open, vendor-neutral, cross platform color management protocol. The procedure, as shown in Figure 4, begins by characterizing the whole slide scanner with a color calibration slide. This slide contains a number of semitransparent colored patches with known color attributes, such as that described by Yagi.[32] After scanning the calibration slide, the relationship between the original color attributes of the reference patches and the values produced by the scanner is determined. This can then be characterized in the ICC source profile and automatically attached to subsequently scanned slides, providing a complete reference to describe the color transformation introduced by the digitization process. Similarly, a destination ICC profile for the display can be derived by establishing the relationship between the input values to the display and the measured colors on the panel of the display. Viewer applications that adopt this strategy can use the source and destination ICC profiles to match the color gamut of the scanner and the display, thereby creating a consistent representation of color independent of the scanner and display used.[33]

Although absolute color representation based on ICC is an optimal option for brightfield imaging, it may result in a reduction of color gamut, contrast, and luminance.[34] In fluorescence imaging, it may be advantageous to instead maximize relative color visibility[35] in order to maximize tissue contrast. This can be accomplished using the color standard display function, a perceptually uniform calibration that makes color differences appear equal in strength. The color standard display function has been shown to increase the perceived contrast of clinically relevant features by about 40% compared with absolute color calibration.[36] By using

the full range of the display device color space, the overall color contrast presented to the viewer is maximized, and by perceptually distributing the shades, color differences that may be present in the scanned image are no longer unnecessarily compressed. The effect on color demonstrates that a WSI system must be considered in its entirety, from slide to display.

USE CASES

Whole slide imaging represents a substrate from which glass slides can enter the digital domain. This process enables microscopic images of tissue to be analyzed by advanced digital tools or to be rapidly communicated to multiple users at once. Although many of the functions that we envision becoming available to pathologists in the future are not yet mature, a number of uses for WSI presently exist that serve to enhance traditional workflows in pathology practice, medical education, and biomedical research.

Slide Archival

Maintaining an archive of glass slides requires physical space and effort, and the slides are prone to breakage, mislabeling, misfiling, or being misplaced. Additionally, environmental factors such as light, heat, gravity, and moisture tend to degrade slides over time, which may result in adverse consequences such as shifting of the tissue section, fading of the stains, and separation of the coverslips. This may cause a loss of important diagnostic information[37–39] that may render a slide unusable for research or education purposes. Creating a virtual slide archive allows an institution to digitally preserve the slide while the H&E, IHC, immunofluorescent, and other stains are fresh. This maintains image quality and enables digital analysis even after years of storage.[38,40,41] Images from a digital archive provide a readily accessible backup for cases and, if connected to a hospital information system, can be merged with the electronic medical record of the patient.[38] Furthermore, WSI enables institutions to retain digital copies of cases sent for consultation, which ensures that the images are available while the glass slides are transported back and forth. Similarly, a permanent record of the slide can be kept when the tissue from a slide is needed for molecular testing. Slides can also be archived for medicolegal and forensic purposes.[41]

Remote Consultation and Telepathology

Remote intraoperative consultation was one of the first use cases for digital pathology and arguably one of the most widely cited reasons for the adoption of WSI.[42] This allows for pathologists to be located in a centralized location in order to support peripheral sites (subject of course to licensing requirements), for example, in subspecialized sign-out, where intraoperative consultations are exclusively performed by subspecialized pathologists.[43,44] Remote consultation for routine cases is an additional use case that has been demonstrated in a few specific scenarios. At the University of Pittsburgh Medical Center, remote consultations have been performed since 2012, and that institution's experience during 3 years has been described.[45] The University of California at Los Angeles has also created a successful international remote consultation relationship.[46] International remote consultation represents a major time savings for the sending country as well, enabling the sender to retain the human tissue within the originating country without need for international shipping considerations. Guidelines for the use of telepathology have been released by the American Telemedicine Association.[47]

In-Line Scanning

Following the approval of WSI for primary diagnosis, the prospect of using digital pathology for sign-out is likely to gain significant traction. This type of scanning can be referred to as in-line scanning, as the process of scanning slides is integrated into the histology laboratory workflow. In-line scanning represents the optimal method for the deployment of WSI because glass slides are never more pure (ie, no fingerprints, dotting pen marks, etc) than when they have first been made. Significant evaluation and considerations should be undertaken before deciding to use WSI for primary diagnosis.[48] The first consideration is the time needed to scan a slide after it has been made. Ideally, the time it takes to scan should be less than or equivalent to the delivery time that it takes for slides to reach the pathologist (assuming the pathologist processes the case as soon as it is received). It is important that laboratories determine the throughput time for the whole slide scanner so that they can have an understanding of expected performance once the system is up and running. Delivery time and distance are components that can be overcome with WSI because the images are readily available across vast distances once scanned.

Although in-line scanning represents a more difficult implementation, this deployment potentially represents the largest return on investment from an organizational standpoint. However, the costs associated with this approach can be significant for laboratories not already prepared for this undertaking. In addition to the immediate instrumentation and implementation costs, process workflows must be developed to integrate scanning into the practice, which may require additional employees or infrastructure. Costs such as these, in conjunction with the lack of added reimbursement to perform sign-out using WSI in the current environment, may be difficult for some laboratories to absorb.

Tumor Board

Using WSI in place of static images, multi-headed microscopes, and projection microscopes for presentation in multidisciplinary tumor boards can improve the experience for both the presenter and the audience.[41,49] Traditionally, using static photomicrographs for case presentation requires approximately 4 days' worth of preparation, whereas, with virtual microscopy, that time can be reduced in half.[50] This reduction is achieved because certain steps, such as pulling the required glass slides from a physical archive, printing reports, photographing pertinent regions under a microscope, and highlighting those areas in a transferable presentation, can be eliminated. Furthermore, with WSI, the presenter is no longer limited by the quality and magnification of the static images or by the possibility of inadequate representation of the required areas. Whole slide imaging does not require a multi-headed microscope or a microscope connected to a projector in the presentation venue, which may not always be present in a conference room. Instead, most venues are usually equipped with an audiovisual presentation system, often with network access, which can conveniently display digital images.[7] Presenters can access additional archived images and other patient

electronic records on demand, making the experience more interactive for the audience.[49]

Education

Digital imaging and whole slide scanning have revolutionized histopathology education. In the United States, medical schools, dental schools, and veterinary schools have increasingly used digital slides for histology and pathology courses.[51] For pathology trainees in residency and fellowship programs, there is a need to standardize pathology education so that the core material will be taught to all trainees regardless of the size, type, and location of the training programs. Collections of carefully curated and organized cases, some of which are freely available in public repositories and others accessible via subscription, can provide the core material for trainees to build knowledge and skills. Collections such as these can also be used for assessment of progressive improvement.[52] During a 1-day, computer-based anatomic pathology examination administered by the American Board of Pathology, 120 static digital images and 25 virtual slides are used.[53]

For practicing pathologists, continuing education is critical to maintain competency to deliver quality patient care. Virtual slides are now ubiquitously used in pathology meetings to provide interactive learning and simulate real-life practice. In order to improve proficiency and quality, pathologists participate in competency assessment programs such as the College of American Pathologists proficiency survey, which has incorporated a WSI component into many of its programs. There are also peer review services that connect experts with clients using digital pathology. Online collections of virtual slides are becoming more abundant, including the Digital Pathology Association–hosted repository.[54] Online training with virtual slides is proving beneficial for vendors to certify pathologists to interpret prognostic and predictive biomarkers correctly. Journals are also slowly using WSI.[55] Recently, College of American Pathologists Press published the 2nd edition of *Color Atlas of Hematology*,[56] containing more than 10 videos and 100 virtual slide links to peripheral blood smears that provide the reader a one-of-a-kind interactive experience.

Accessibility and convenience to WSI educational material is important. Smartphone and personal mobile platforms have become more desirable.[57] Ultimately, by combining the visual information of WSI with audio to create multimedia presentations, we will improve automated distance learning for pathologists.[58]

Research

For biotechnology and pharmaceutical companies, the goal of many of the early quantitative biomarker assays is to develop an assay that will ultimately be used in clinical studies to help drive the treatment paradigm (eg, as a patient selection or predictive biomarker assay). Because of the tumor morphology and anatomy that is preserved in histology, WSI can be used to better understand the spatial relationship of various biological phenotypes. For example, in immuno-oncology, the immune phenotype (eg, presence of various immune cells) has been shown to be an important potentially predictive and prognostic biomarker.[59,60] Whole slide imaging–based technologies can be used to measure the immune phenotype and its relationship to the tumor microenvironment (eg, tumor versus stroma).

Biotechnology and pharmaceutical companies have made major investments in digital pathology technologies during the past decade. These investments include automation technologies for histology (tissue storage, sectioning, staining), slide scanning, image management, and image-analysis software. A major focus has been the development of IHC-based biomarkers that can be used to characterize drug effects and outcomes in early discovery, translational, and clinical studies. In early discovery, quantitative IHC biomarker assays can be used to confirm target engagement and explore downstream biological pathway effects. For translational studies, these assays are used to support a project's clinical development plan. One example is target expression profiling, which can identify tumor indications where the drug may prove efficacious, leading to a potential target patient population. As these efforts increasingly become reliant on digital tools, WSI will play a pivotal role.

REGULATORY AND VALIDATION CONSIDERATIONS

The past several years have seen significant technical advancement in the ability to image large numbers of slides automatically, rapidly, and at high resolution. The ability to routinely digitize entire slides allows pathologists to better apply computer power and network connectivity to the study of pathologic morphology and ultimately use these images for primary diagnosis. In the United States, the vendors who market WSI for clinical use are regulated by the FDA. Recently, the regulatory field for WSI advanced significantly when it was recommended that manufacturers of WSI devices for primary diagnosis in surgical pathology submit their applications to the FDA through their de novo process.[61] This was accomplished by close collaboration between the Digital Pathology Association and the FDA.

Prior to the collaboration between the Digital Pathology Association and the FDA, the FDA had designated WSI systems as class III medical devices. The FDA uses the class III categorization to label devices as "highest risk." Class III devices are therefore the most highly regulated of all medical devices, requiring not only general controls (eg, quality system regulation and good manufacturing procedures) but also premarket approval. In the last decade, many companies that market WSI systems had hoped that the FDA would declare WSI systems as class II (moderate-risk devices that already have a predicate device on the market) or class I (no premarket notification required). An important and pivotal breakthrough was accomplished when the FDA allowed the first system, the Philips IntelliSite Pathology Solution, to market its WSI device for primary diagnostic use of surgical pathology slides in the United States. This included slides that were prepared from biopsied tissue as well as resection specimens. The FDA published in their classification order that this device, and substantially equivalent devices of this generic type, should be classified as class II devices. This clearance and down-classification to class II has now given digital pathology vendors a clear and less difficult pathway toward FDA approval.

Laboratories also need to validate the WSI systems that they implement. The College of American Pathologists recently convened a panel of pathologists with expertise in the use of WSI to produce recommendations for the validation of WSI systems for diagnostic purposes in pathology.[5] The panel reviewed 767 articles on digital pathology published broadly between 2000 and early 2012. Based on rigorous inclusion criteria, 23 articles qualified as acceptable as bases for the panel's recommendations. This working group, using evidence from the literature and

expert opinion on WSI validation, drafted 13 recommendations for laboratories to follow to validate WSI systems. In addition, the College of American Pathologists currently has several accreditation program checklists for WSI validation. These recommendations and checklists are important for laboratories to consider as they move toward adoption.

PREPARING TO PURCHASE A SYSTEM

When selecting a system to purchase, a rigorous evaluation process is often used. For some organizations, a formal request for proposal process is required. The more formal request for proposal process establishes timelines and creates uniform expectations. However, it can be viewed as unnecessary bureaucracy and paperwork. In order to maximally inform the decision-making process, an evaluation of the organization should be performed.

Defining Your Needs

A given organization/department has complex unique features that will be important to understand in order to inform the procurement process. It may be wise to inquire within the organization (particularly with the radiology department) about their experience with digital storage needs and how they interact with digital material. Depending on the size of the organization, it may make sense to combine resources between pathology and radiology departments, although this will require some cross-department negotiation. Of note, several editorials have suggested the creation of diagnostics departments that combine radiology and pathology departments.[62]

Determining the use cases for your department will impact the needs for your infrastructure. For example, scanning at ×40 magnification versus ×20 magnification, or scanning slides with z-stacks, will require greater storage needs. In general, storage costs have become less of a barrier to adoption, but planning for them will still require understanding the intended use cases. The necessary access for the virtual slides is also a consideration when planning for infrastructure. If the images are to be used for clinical diagnostic purposes, they must be readily accessible across different sites of the department. This includes not only access to the storage location for the digital slides but also suitable network bandwidth so that the virtual slides can be reasonably rendered by the users. Depending on the institution and use cases, it may make sense to have the virtual slides integrated with the pathology laboratory information system. This integration can take the form of a direct connection with the laboratory information systems or could be connected through single sign-on solutions.

In addition to network resources, there are also considerations for the local resources that will be used to access the virtual slides. A dedicated scanning technician may be required for some implementations; the Digital Pathology Association has recently partnered with the National Society for Histotechnology to develop an online certificate program to aid in technician training and assessment. In addition, as previously discussed, computer monitors may be one of the more important considerations, as the quality of the monitor will directly impact the viewing experience regardless of the scanned image. Of note, FDA regulation is focused on the so-called pixel pathway from the glass slide to the pathologist's display. Therefore, vendors are likely to specify recommended hardware that should be used with their products. This may require significant replacement of departmental computer resources.

Building Buy-in

Creating enthusiasm for a WSI implementation requires a series of buy-ins from different levels of stakeholders. Importantly, there must be a champion within the pathology informatics leadership. This person must have a stake in implementing WSI and supporting the project through to completion. The first level of buy-in needed is with senior leadership. At this level, the overall goals of the project will need to be explained. Also, at this level, the budget for the project will be established. The business use cases will influence the goals of the project as well as the budget for the project and should provide an idea of the return on investment. Once the senior leadership has bought into the project, the selection of vendors can be performed. During this selection process, the leader of the project should include all of the stakeholders for the implemented WSI solution. Possible stakeholders to include in the decision-making process include senior leadership within the department, the technical staff for the department (histology laboratory and administrative staff), pathologists, and the information technology team that will be supporting the WSI solution. Other possible stakeholders may be unique to a department's implementation/business use plans.

Once the WSI system has been chosen, there are several methods by which it can be implemented. A scaled/staggered implementation of the system is likely to be beneficial to identify and solve minor issues that might arise without too much interruption of others using the system. These early adopters need to be encouraged and empowered to be champions for the digital pathology solution. Of course, a well-functioning system will also encourage these early adopters to spread the usefulness of the new digital pathology solution to the rest of the department. It is important to include both the pathologists who might be using the system and the technical staff. By including key stakeholders along the process, buy-in throughout the department should be easy to achieve.

Funding Strategy

Determining a funding strategy for the digital pathology solution will be influenced by the return on investment. Although there may be departmental resources dedicated to funding WSI, it is more likely that it will need to be funded from outside the department. This funding may come from institutional resources or from institutional collaborations that may be facilitated by this technology. From within the department, there may be some cost savings related to centralization or workload balancing that can be used to justify the cost for the system. By following a formal process (whether it is a request for proposal or a simple software acquisition process), the department should be able to calculate how the implementation will supply the proposed return on investment.

THE FUTURE OF WSI

Three-Dimensional Reconstruction

Three-dimensional (3-D) imaging describes a more general process by which sensors and detectors are used to traverse objects, surfaces, or body parts to obtain stacks of images (also known as a *z-stack*) and convert them into a 3-

D model or representation.[63] Historically, WSI has been marketed and used mainly as a tool for 2-dimensional analysis, mimicking existing histopathology workflows, which are inherently designed around conventional 2-dimensional light microscopy and staining with specific dyes or antibodies.[64] However, examination of 3-D structures, like tumors or normal tissue, in 2 dimensions generates a well-known information gap between recorded observations and the true state of the original tissue.[65] As such, 3-D reconstruction of whole slide histologic data is becoming more relevant and has demonstrated value in both tissue visualization and clinical diagnosis.[63,66] High-resolution 3-D histopathologic imagery is especially advantageous in discovering diagnostic patterns because of the improved correlation among imaging modalities such as magnetic resonance imaging, conventional computed tomography, and WSI.[67–70]

The initial step in 3-D reconstruction of virtual slides involves generating serial, ultrathin tissue sections, which are then mounted to glass slides. These serial sections can be obtained in either a manual or an automated process. For example, automated robotic microtomes may be used to trim and section blocks, which is particularly useful for the 3-D reconstruction process.[71] Benefits afforded by automated sectioning compared with traditional manual sectioning include the near-uniform thickness of sections, uniform alignment of sections, and fewer sectioning artifacts.[72] These factors are critical players in facilitating the interpolation of structures between sections and thus are highly correlated with the generation of high-quality 3-D reconstructions.[63]

Serial sections of tissue are typically acquired at a thickness of 4 to 6 μm, which falls within the range of the conventional 2-dimensional light microscopy workflow. These serial sections are then mounted on glass slides, ideally in a coherent and consistent fashion to facilitate the 3-D reconstruction process. Next, serial sections are stained using either routine histologic and/or IHC techniques. Stained serial sections are then scanned using WSI, which generates a z-stack of digital images, each corresponding to a different scanned level of the tissue block. Serial whole slide images are then run through commercially available or custom software to generate 3-D models (Figure 5). In general, most 3-D reconstruction software involves several image processing steps, including image registration, segmentation, interpolation, and volumetric rendering.[63]

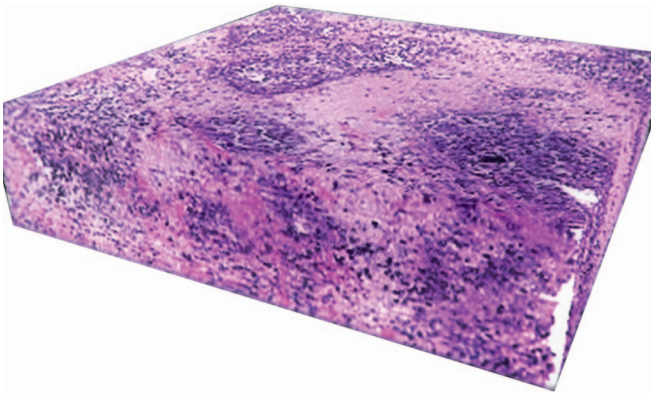

Figure 5. *Three-dimensional reconstruction of lung adenocarcinoma from serial 2-dimensional whole slide images. Hematoxylin-eosin–stained glass slide–derived images were processed using custom software to generate a 3-dimensional model from a 2-dimensional z-stack.*

Multispectral WSI

Whole slide imaging is rapidly evolving into a substrate for more advanced microscopic techniques to flourish. A growing area of interest has been dedicated to multispectral imaging, which endeavors to acquire images of tissue at different wavelengths in order to accurately characterize the chromatic properties of stains or markers. This is often considered a superior option over standard broadband acquisition paradigms that combine chromatic information into a single representation (eg, RGB) where the chromatic information cannot be recovered. Multispectral imaging can therefore disentangle the complex chromatic attributes of stained tissue, with the potential to support multi-labeling studies,[73–77] improve color-based classification,[78] and provide more detailed chromatic information that may carry its own inherent prognostic significance.[79–81]

Implementing multispectral imaging in WSI is difficult because it typically requires collecting and storing images at each wavelength examined, which results in much longer scan times and much larger file sizes. As data storage continues to become more affordable, routine storage of multispectral whole slide images becomes a more realizable goal. Vendors must place additional emphasis, however, on continuing to improve whole slide scan times to accommodate the multiple passes often necessary to collect images at different wavelengths. Alternatively, some groups have begun to develop robust tools to rapidly acquire multispectral images without requiring multiple passes,[82,83] which could be harnessed in WSI systems to improve scan time and optical throughput. With continued development, multispectral acquisition and storage may become ubiquitous in WSI, resulting in an overall expansion of analysis capabilities available to pathologists.

Digital Imaging and Communications in Medicine

Most WSI vendors use proprietary file formats to store image data and associated metadata. These often include arbitrary headers, nonstandard levels, different compression algorithms, and different file organization. This proprietary nature can make it a challenge to use tools from other vendors to organize, archive, view, or analyze images acquired from different scanners without explicit support for those file formats and their requirements. This can potentially impact interoperability and scalability, and may ultimately limit the capabilities of the overall digital pathology workflow. Digital Imaging and Communications in Medicine is a standard widely used in other specialties that seeks to mitigate many of these challenges. Digital Imaging and Communications in Medicine is composed of a set of file format and communication protocols that provide a universal vendor-neutral language for image capture devices, image management systems, image viewers, and analytical tools to seamlessly and efficiently communicate with one another.

Digital Imaging and Communications in Medicine Supplement 145 lays out the general framework by which adoption of the standard may occur in WSI[84] but, at present, it has not yet been implemented by vendors in commercial products. However, in 2017, the Digital Pathology Association hosted a Connectathon at its annual Pathology Visions meeting that challenged WSI device vendors to generate Digital Imaging and Communications in Medicine files and WSI image management vendors to parse these files and

display the images. This effort was largely successful and demonstrated vendor-neutral interoperability, but a number of pitfalls were noted and recommendations for further refinements were made.[85] Nevertheless, participation by device and software vendors alike indicates a willingness to explore partnerships in interoperability that may be achieved by adopting Digital Imaging and Communications in Medicine as a standard.

Image Analysis

Whole slide images of tissue sections are highly rich in information that includes color, tissue morphology, cell morphology, and complex cell phenotypes. These complex phenotypes can be due to the pathology of the tissue or the pathology of the disease. In order to assess these complex tissue and cellular phenotypes, a pathologist develops specific expertise through years of training and experience evaluating case studies and participating in peer review sessions. However, visual assessment by pathologists can be influenced by inherent cognitive and visual biases.[86,87] To overcome many of these challenges, there are a number of technologies that will enable the extraction of information from images that is cumbersome, error prone, or generally difficult for the human visual system to assess. This is a rapidly evolving field, and new analysis approaches, software tools, and companies continue to enter this space.

A typical image analysis workflow involves 2 key steps: identification of a region of interest and cellular analysis. Most tissue samples (biopsies, resected samples) contain a mix of host tissue, target tissue, blood vessels, stroma, tumor, organ compartments, and so forth. It is important to delineate the target compartment in the most relevant region, as the biomarker of interest may exist in other tissue compartments that are not relevant to the current study.[21] Once the region of interest is defined, the analysis algorithm is configured and tested to optimize accurate segmentation of cells and measurement. Common user-configurable parameters include color, threshold, categorical thresholds, and object size (eg, to split adjacent or overlapping nuclei if the algorithm has an expectation of nuclear size).[88] Optimization of image analysis algorithms are often iterative processes; the user adjusts the parameters, runs the algorithm on representative images, assesses, and reoptimizes if necessary.

Image analysis algorithms are often developed for specific applications or stains such as in situ hybridization (chromogenic or fluorescent), nuclear/cytoplasmic/membrane biomarkers, and neurite outgrowth. It is also important to keep in mind that image analysis results can vary depending on a number of factors such as staining quality, image capture quality, and tissue quality.[89] It is important to develop appropriate quality control measures to assess potential artifacts throughout the IHC and image analysis workflow. Some of the most common image analysis artifacts include segmentation errors (eg, oversegmentation or undersegmentation of nuclei) and classification errors (eg, classifying tumor as stroma and vice versa). Therefore, it is important that a pathologist be involved in the image-analysis workflow, which should include at least one quality control step where the pathologist reviews all aspects that can influence image analysis results.[21]

Artificial Intelligence and Machine Learning

The field of artificial intelligence, which was founded in the late 1950s, has produced a wide variety of technologies that can simulate human reasoning, becoming increasingly proficient in the performance of tasks previously considered too difficult for computers to tackle (eg, medical diagnosis).[90,91] Similarly, the field of computer vision began in earnest in the early '80s. At that time, a majority of computer vision research was concerned with how to derive features thought to be correlated with a particular imaging target or disease state. As these techniques become more commonplace, the use of supervised machine learning (ML) techniques has grown. Supervised ML specifically refers to the ability of computers to develop models by exposure to a ground truth without the need to explicitly provide programmed instructions. These techniques allow for the automatic discovery of patterns in images that can then be used to make predictions and derive additional insights. There has also been recent interest in unsupervised ML techniques,[92] which attempt to identify natural divisions in a data set without the need for a ground truth.

As WSI adoption and utilization grow, larger amounts of digital tissue data are becoming available for use by ML and other artificial intelligence–derived methods. For example, numerous computer algorithms for H&E-stained pathology images have been developed in recent years to aid pathologists with diagnosis and prognosis.[92,93] Pathology-centric ML approaches include cellular heterogeneity and stromal feature extraction algorithms, which have proven to be quite useful for prognosis in the setting of breast carcinoma.[93,94] Similarly, ML approaches to analyze tissue-specific morphologic features have also demonstrated utility for prognosis in the setting of lung carcinoma.[95]

More recently, deep ML methods like convolutional neural networks have become more popular in the biomedical setting. These represent a fusion of traditional computer vision approaches with modern ML optimization, where the computer selects both the intermediate features that are extracted and the learning applied to those features within a single model. Thus far, deep ML techniques have been used for image segmentation, object classification, object recognition, and clinical outcomes prediction. More specifically, in the setting of pathology, deep ML methods have been extensively researched and applied to H&E-stained whole slide images for tumor region identification, detection of metastatic foci, tumor classification, and prediction of gene mutations.[96,97] Standardized frameworks exist that, for a suitably annotated body of reference data, will meet or exceed in an automated fashion the performance of any one pathologist.[98] As such, artificial intelligence techniques, and artificial intelligence–derived methods including computer vision, ML, and deep ML, promise to provide pathologists with a number of useful tools, beginning with mechanisms for automated case review and eventually leading to computer-aided diagnosis. These tools, which will undoubtedly enhance pathology workflows, will ultimately play a larger role in improving patient outcomes.

CONCLUSIONS

As WSI continues to evolve and its regulatory and validation considerations become resolved, this technology will undoubtedly begin to play a larger role in pathology. It is important for pathologists, technicians, researchers, and trainees to stay informed about advances in the technology and new commercial products available. Those interested in adopting WSI in their institutions should assess the precise

role that WSI will play and formulate a strategy for adoption, while being mindful of the future potential that it may be able to provide to their users. Key stakeholders should clearly communicate the advantages of WSI to executive leadership and users, and work closely with information technology departments and vendors to integrate into their departments a digital pathology solution that includes WSI.

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