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Whole Slide Imaging Technology and Its Applications: Current and Emerging Perspectives

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Abstract

Background. 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. It utilizes virtual microscopy wherein glass slides are converted into digital slides and are viewed by pathologists by automated image analysis. Its impact on pathology workflow, reproducibility, dissemination of educational material, expansion of service to underprivileged areas, and institutional collaboration exemplifies a significant innovative movement. The recent US Food and Drug Administration approval to WSI for its use in primary surgical pathology diagnosis has opened opportunities for wider application of this technology in routine practice. *Main Text*. The ongoing technological advances in digital scanners, image visualization methods, and the integration of artificial intelligence-derived algorithms with these systems provide avenues to exploit its applications. Its benefits are innumerable such as ease of access through the internet, avoidance of physical storage space, and no risk of deterioration of staining quality or breakage of slides to name a few. Although the benefits of WSI to pathology practices are many, the complexities of implementation remain an obstacle to widespread adoption. Some barriers including the high cost, technical glitches, and most importantly professional hesitation to adopt a new technology have hindered its use in routine pathology. *Conclusions*. In this review, we summarize the technical aspects of WSI, its applications in diagnostic pathology, training, and research along with future perspectives. It also highlights improved understanding of the current challenges to implementation, as well as the benefits and successes of the technology. WSI provides a golden opportunity for pathologists to guide its evolution, standardization, and implementation to better acquaint them with the key aspects of this technology and its judicial use. Also, implementation of routine digital pathology is an extra step requiring resources which (currently) does not usually result increased efficiency or payment.

Keywords
whole slide imaging, telepathology, diagnosis, digital pathology, artificial intelligence

Introduction

Digital pathology (DP) utilizes whole slide imaging (WSI) tool wherein glass slides are converted into digital slides and the images are evaluated on a display, and image analysis may or may not be applied to be viewed by pathologists through automated image analysis.[1,2] It started in the 1960s with telepathology research and subsequent introduction of virtual microscopy and commercial WSI scanners in the 1990s.[3] However, technical requirements (scanner, storage, and network), high cost, and slow speed of scanning were major limiting factors for its broad dissemination. Over the last decade, the concept of DP has revolutionized as new, potent, affordable scanners and assisted technological advancement, and mass- or cloud-based storage technologies have developed bringing a paradigm shift in the field of pathology.[4] This has shown significant impact in dissemination of educational material, expansion of diagnostic services to underprivileged areas, real-time digital consultation services, virtual tumor board, and intra- and inter-institutional academic and research collaborations.[4,5]

WSI aids in the integration of electronic workflows and health records, and generation of diagnostic support based on computational tools like artificial intelligence (AI).[6] Although WSI has tremendous lucrative benefits in pathology, involving diagnostic and academic/research services,

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the complexities involved in the implementation as well as technical and logistic hindrances remain an impediment to its widespread and global adoption.[7] For example, current scanning technology does not satisfactorily accommodate thick smears and 3-dimensional cell groups in cytopathology.[8-11] With suboptimal tissue sections, scanners are currently not providing good results, when encountering tissue folds, air bubbles, and poor staining.[8] Unless significant modifications to the workflow are made centered around DP (eg, automation, continuous flow processes, and quality of the histology presented to the WSI devices), placing WSI systems in the clinical pathology laboratory has been shown to stress the system in terms of reliability, reproducibility, turn-around time, and throughput.[9]

History and some important milestones in digital and computational pathologies are as follows (Figure 1):

- 1950: Alan Turing conceived the idea of using computers to mimic intelligent behavior and critical thinking.
- 1956: John McCarthy coined the term artificial intelligence (AI).
- 1959: Arthur Samuel coined the term machine learning (ML) as "the ability to learn without being explicitly programmed."
- 1965: Computerized image analysis of microscopy images of cells and chromosomes by Judith Prewitt and Mortimer Mendelsohn.
- 1986: Term deep learning (DL) coined by Rina Dechter.
- 1988: Convolutional neural network (CNN) invented by Yann LeCun.
- 1990: Whole slide scanners introduced.
- 1998: Tripath becomes the first company with an automated PAP smear screening product to receive Food and Drug Administration (FDA) approval.
- 2003: Cytyc received FDA approval for their ThinPrep Imaging System.
- 2013: Development of photoacoustic microscopy imaging technique.
- 2014: Ian Goodfellow introduced generative adversarial network.
- 2016: Microscopy with UV surface excitation (MUSE) microscopy technique invented to enable high resolution imaging without tissue consumption.
- 2017: Philips receives approval for a digital pathology whole slide scanning solution (IntelliSite).
- 2018: FDA permits first medical device using AI to detect diabetic retinopathy in adults (IDx DR).
- 2021: FDA authorizes the first AI-based software to detect prostate cancer (Paige Prostate).

Technology

The process of digitization includes 4 sequential parts: image acquisition (scanning), storage, editing, and display of images.[11] WSI uses slide scanners that consist of 4 main components: light source, slide stage, objective lenses, and a high-resolution camera for image capture.[8,12-14]

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) are captured and digitally assembled to generate a digital image of the entire slide.[15,16] WSI can be categorized as bright field, fluorescent, and multispectral, and some scanners can accommodate more than 1 modality. Bright field scanning emulates standard bright field 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] and fluorescent in situ hybridization).[15,16] Multispectral imaging captures spectral information across the spectrum of light and can be applied to both the bright field and fluorescent settings.[15-19] Line scanning exclusively uses focus maps; however, these can also be used with tile scanning. More recently, scanning processes have been developed that incorporate continuous automatic refocusing processes, further increasing the quality of scans.[15-19] They have also incorporated tissue recognition features that allow for automatic detection of the histology specimen via a low-magnification overview scan.[16] Different scanners vary in their scanning modality, slide-loading capacity, and scan time with a capacity of holding 400 slides in high-throughput scanners.[15,20] Scanning times per slide range mainly from 30 s to several minutes.[21-23] If the camera sensor has a lower resolution than the objective's numerical aperture allows for, information is lost. Therefore, quality of the capturing camera within a digital scanner should be taken into consideration.[15-20] After digitization, quality of scans need to be assessed as scanning artifacts can affect downstream results, and can be caused by improper cleaning of slides prior to scanning, poorly focused scans, or compensation lines from improper stitching of lines or tiles.[24,25]

Virtual Slides

Whole slide scanning generates digital representations of glass slides that can be navigated in an interactive manner. The slide must be captured at sufficiently high resolution and with adequate color depth.

Image Compression

Many methods to reduce file size using image compression are available in WSI. Many vendors use picture formats like JPEG, JPEG 2000, or LZW compression to reduce file size, often resulting in a reduction of file size by a factor of 7 or more.[26] However, information is lost in the

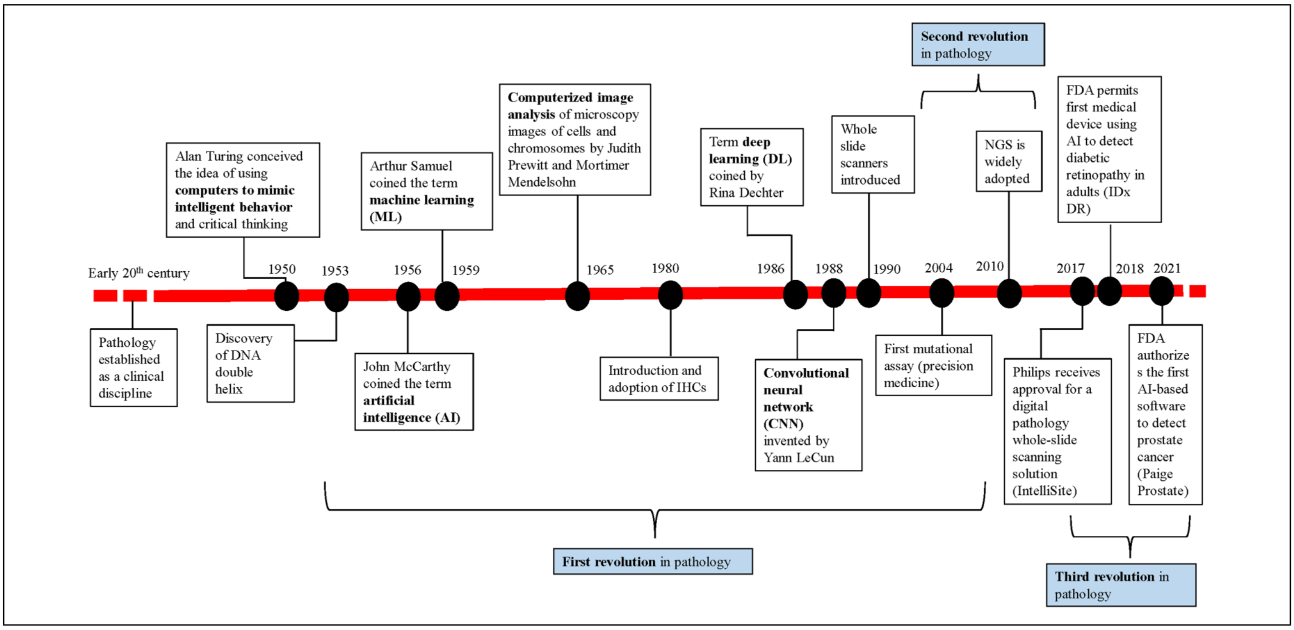

Figure 1. Milestones in digital and computational pathologies with depiction of 3 "revolutions" in the field.

conversion that cannot be recovered. Although morphologic assessments appear to be less affected, densitometric assessments are increasingly sensitive to this loss.[27] Thus, users are discouraged from applying JPEG compression successively on the same image, as it further degrades image quality. Discarding blank regions of the slide reduces file sizes as well as scan times by identifying regions in the initial macro snapshot that do not need to be scanned.[28]

Pyramid Representation

Despite methods of reducing file size, a single whole slide image in practice often exceeds 1 GB in size which can be prohibitive to download and load into memory. This problem can be tackled by noting the intrinsic relationship between image scale and field of view. 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. Whole slide images are stored at multiple resolutions to accommodate a streamlined method for loading images. This multi-resolution representation is commonly referred to as an image pyramid. 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.[23-28]

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. However, if retention is important, a complete backup strategy including off-site storage, redundant array of independent disks storage, or optical/tape storage may be used. Hybrid solutions that involve local and cloud-based storage and access, or hub-and-spoke models for multisite organizations, can also be effective strategies.[27-30]

Viewing and Managing Virtual Slides

WSI offers an opportunity to expand the tools available for users to include digital annotation, rapid navigation/magnification, and computer-assisted viewing and analysis.[30] For example, when whole slide images are used for educational purposes, access to a dedicated image viewer enables us to annotate images for quick identification and navigation to regions of interest in the slide.[30] 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, gross photos, and radiology).[31] For users who wish to apply image analysis algorithms to whole slide images, some of the viewers are packaged with algorithms that can detect cells, compute positive staining, perform regional segmentation, or perform nuclear segmentation in hematoxylin and eosin (H&E) images.[32] Viewers often support the ability to annotate images, save regions of interest, take snapshots of selected regions, and export images to other formats. These can be integrated into department's workflow in a seamless manner, providing on demand image analysis in conjunction with whole slide viewing[30,32,33] (Figure 2).

Image Management Systems

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 (LIS). Advanced features 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, human epidermal growth receptor 2 (HER2/neu 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.[28,30]

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[34] 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 s. 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. Additionally, color differences are commonly introduced by inter-display 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. 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. It begins by characterizing the whole slide scanner with a color calibration slide that contains a number of semi-transparent colored patches with known color attributes, such as that described by Yagi.[35] 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 and destination profile and automatically attached to subsequently scanned slides, providing a complete reference to describe the color transformation introduced by the digitization process.[36]

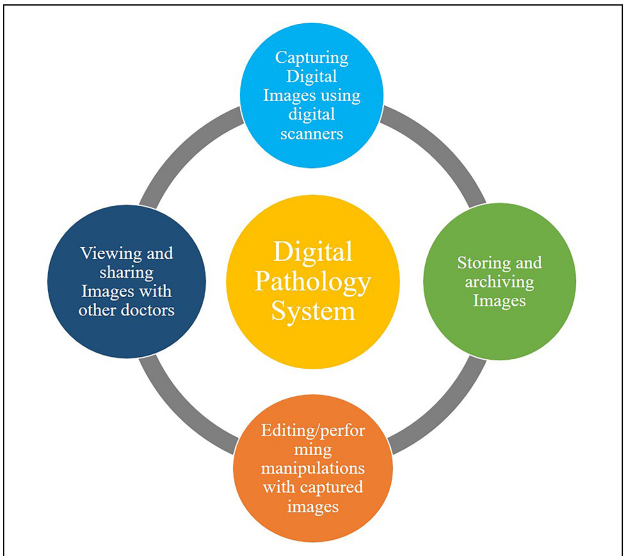

Figure 2. Overview of digital pathology implementation system.

Regulation and Validation

In the United States, federal regulations set forth in the Food, Drug and Cosmetic Act of 1938 and the Medical Device Amendments of 1976 provide the FDA with limited authority over medical devices. All the medical device manufacturers and distributors must register their organization with FDA to sell their devices. Unless it is granted by the FDA, all the establishments should be registered electronically using the FDA Unified Registration and Listing System (FURLS system). Within FDA, the Center for Devices and Radiological Health (CDRH) is responsible for ensuring the safety and effectiveness of medical devices and eliminating unnecessary exposure to radiation-emitting products. These regulations pertain primarily to manufacturers of whole slide digital imaging systems, and potentially also to laboratories that incorporate WSI in diagnostic services.[37-39] The FDA convened a panel hearing in October 2009 that focused on how best to regulate WSI systems that are to be used for primary diagnosis in surgical pathology.[6,35,37,38] While WSI systems are clearly medical devices subject to FDA regulation, there are a number of open issues the FDA will need to address before the regulatory environment is clarified. Table 1 lists some of the specific validation issues raised by WSI. Evaluators must consider a range of issues that include sample size and statistical power, separating pathologist performance issues from device performance issues, the scope of cases to include in a challenge set, whether the set should be "enriched" with difficult cases, washout (time interval before asking a pathologist to review the same diagnostic material), the time it takes pathologists to become facile with WSI instruments, and the setting in which validation is assessed.[11,36,39-41] Table 2 lists some key considerations for WSI validation.

VALID Act and FDA

At a high level, the VALID Act would create a new category of products that would be regulated by the FDA. Currently, in vitro diagnostics, including WSI and AI, are regulated under the medical device framework. VALID would create a new category called "in vitro clinical tests," which will apply to both commercial test as well as laboratory developed tests. By creating a risk-based framework, the act will require high-risk tests, like novel assays, to go through premarket review, while lower-risk tests could go to market after passing technological certification. This would be a significant change to the way in vitro diagnostics are regulated right now. Also, the technology certification review is a new approach that will allow in vitro diagnostics developers to submit a single representative test for FDA approval. If the FDA approves the developer's design and clinical validity and the test gets to market, the developer would be able to make test tweaks and develop new ones that rely on the same underlying technology. The goal is to allow for flexibility and if a high-quality developer has already demonstrated to the FDA that it can meet those standards and then technology certification allows continued innovation and test development to meet patient need without having to return to the agency for review. This has been proposed because diagnostics have a much more rapid cycle of innovation and modification compared to other devices, and it allows a developer to adjust a test or develop a new test based on emerging research. This means that the FDA should be able to demand and inspect clinical validation data, and adverse event reporting must be thorough.

Applications of WSI

Health care facilities are witnessing extensive digitization with inclusion of digital imaging connected to hospital information systems, LIS, picture archiving, and communication systems. Pathology laboratories equipped with WSI facility would fall into place well in such a setting with varied applications in diagnosis, education, and research.[11,34,37,42,43]

WSI in Education, Tumor Boards, Presentations, Quality Assurance, and Research

WSI has gained wide acceptance for education, at the tumor boards, and for presentations, research, and quality assurance (QA).[4,43-46] Digitized slides are more interactive compared to glass slides, can be easily shared anywhere at any time, and can help standardize training and research material. Many authors have highlighted its use in undergraduate medical education, pathology residents, and

Table 1. Issues to be Considered While Validating Whole Slide Imaging in Routine Diagnostic Application.

1	Separating the device from the practitioner
2	Pathologist experience (in practice and with the device)
3	Washout and validation setting
4	Types of data generated
5	Measuring accuracy
6	Measuring bias
7	Measuring precision (intra-rater, inter-rater, and inter-instrument)
8	Sample size
9	Generalization of the observations

Table 2. Preferences for Whole Slide Imaging Validation for Routine Diagnostic Application.

1	Measure intra-observer bias and precision
2	Use general pathologists with defined device experience
3	Utilize high-quality display
4	Enrich the case sample (stack with difficult cases)
5 (CAP guidelines, 2021)	A washout period of at least 2 weeks should occur between viewing digital and glass slides.
6	Analyze each parameter separately (eg, tumor type, tumor grade, etc).
7	80% power to detect 10% difference in bias or precision
8	Generalize to all specimens except hematology, cytology, and dermatopathology
9 (CAP guideline, 2021)	The validation process should include a sample set of at least 60 cases for one application, or use case, (eg, H&E-stained sections of fixed tissue, frozen sections, and hematology) that reflect the spectrum and complexity of specimen types and diagnoses likely to be encountered during routine practice. The validation should include another 20 cases to cover additional applications such as immunohistochemistry or other special stains if these applications are relevant to an intended use and were not included in the 60 cases mentioned above.
10 (CAP guideline, 2021)	The validation study should establish diagnostic concordance between digital and glass slides for the same observer (ie, intra-observer variability). If concordance is less than 95%, laboratories should investigate and attempt to remedy the cause.

fellow training.[45-48] Unlike glass slide teaching sets, digital slides do not fade, break, or disappear. Digital slides also offer the ability to standardize images, permit annotation, and can provide a wide case range for trainees.[48,49] Digital teaching sets can be accessed on a server over a network, are available to multiple users, and can be developed to contain test modules for trainees. WSI can also facilitate preparation and conduct of tumor boards through obviating the need of a multi-headed microscope or microscope with projection attachment or acquisition of multiple static images of a case.[50,51] This is because WSI offers higher quality images with annotation, greater educational value for clinicians, involves less preparation time than photographing cases, and permits real-time flexibility (eg, easy to add on cases, perform side-by-side viewing, and gives access to the entire slide which allows one to answer "on-the-spot" questions).[52] It is also useful in E-education, virtual workshops, and for proficiency testing.[53] The use of this technology in QA programs in surgical pathology and cytopathology can help in cost cutting and overcoming transportation difficulties, as also minimizing the potential second-reviewer bias by hiding the initial diagnosis.[10,30,54] Online WSI resources such as College of American Pathologists (CAP) virtual slide box, Digital Pathology Association hosted repository, and the Cancer Digital Slide Archive offer virtual slide sets for training and learning purposes. Virtual slides are also being used in pathology conferences and meetings to promote interactive learning and provide ease of visualization of multiple images of different stains in conjunction with relevant clinical material.[30] Electronic publication of text books and articles in scientific journals has also opened new panoramas of scientific communication.[55] Utilization of WSI-generated high-quality virtual images has proven to be the single most upgrade for pathology journals, thus empowering the readers to be involved in a scientifically based diagnostic approach to the lesion described[56] (Figure 3). Table 3 highlights the key clinical benefits of WSI.

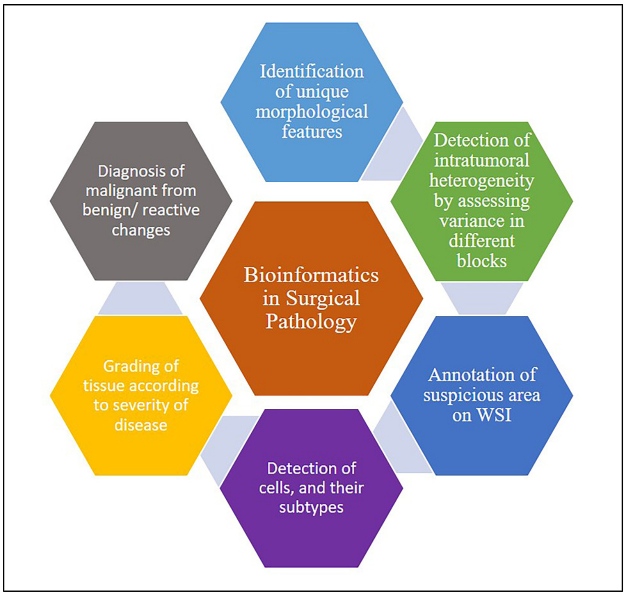

Figure 3. Bioinformatics and WSI in education, tumor boards, presentations, quality assurance, and research.

WSI in Primary Frozen Section/ Intraoperative Consultation/Diagnosis

Over the last few years, WSI has been utilized in primary frozen section diagnosis and secondary/tertiary teleconsultation.[43,51,57-59] The advantages include access to an entire digitized slide or even an entire case (set of slides), automated scanning, the high resolution of images available for review, rapid interpretation time, and the ability of teleconferencing.

A high concordance rate between WSI-based frozen section and permanent section diagnosis or on-site interpretation has been demonstrated in several studies.[11,37,60] However, further studies on a range of different pathologies are required to validate the utility and limitations of WSI. Successful implementation requires: effective planning and communication, a willingness to adjust old routines without compromising quality, and histo-technologists who are able to provide consistently high-quality frozen section slides.[41,61,62]

WSI in Routine Pathological Diagnosis

WSI is increasingly being used in the daily practice of surgical pathology, particularly for teleconsultation and certain quality assurance practices, such as obtaining second opinions.[31,63,64] However, it raises a question whether WSI will be utilized for making routine pathologic diagnoses, ushering in the era of the "slideless" laboratory, especially after the COVID-19 pandemic.[60,65,66] The adoption of DP has been somehow slower than in radiology

Table 3. Key Clinical Benefits of Whole Slide Imaging.

1	Whole slide imaging of selected or all slides from the cases submitted for consultation
2	Directly enhances patient care through the availability, portability, and permanence of the images for patient care conferences eg, tumor board
3	Provision of a quality assurance function
4	Whole slide imaging of slides that will be destroyed by ancillary testing
5	Whole slide imaging of slides that will be sent out to another institution
6	Whole slide imaging of medicolegal cases
7	Whole slide imaging of cases for digital image analysis for further study or biomarker validation

partly due to the fact that in pathology, digital data is acquired in a slightly different manner from that in radiology.[31,67,68] Although both fields require an imaging modality to collect primary data, in radiology, images begin as digital data whereas pathology images have to be converted from an analog substrate into a digital format and secondly, as pathologists, we are doing the equivalent of exposing a plain film, developing them film, and then scanning the developed film into a machine rather than direct-to-digital[67-69] (Figure 4).

Rendering routine pathologic diagnoses using WSI is feasible if the images truly represent an accurate digital reproduction of the scanned glass slide which can be saved, archived, reviewed, and later retrieved without any degradation.[70] Moreover, apart from integration with the LIS, the routine use of WSI requires seamless connectivity over broadband networks, efficient workstations, cost-effective storage solutions, and standards-based informatics transactions for integrating information.[63,71] Discrepancies between digital and glass slide diagnoses may be attributed to inadequate clinical data, missed tissue on the digital slide, and the pathologists' lack of experience using a WSI system.[72] One study demonstrated that using a virtual slide system, correct diagnosis was made in 66% of cases without clinical data provided compared to a correct diagnosis of 76% when clinical data was provided.[72] Therefore, in order for WSI to become an accepted diagnostic modality, the provision of adequate medical information (eg, gross pathology description, prior pathology reports, clinical history, imaging and other relevant laboratory parameters, etc) will need to be weaved into the imaging system.[63,73,74]

Digital slides offer several advantages in terms of fidelity of the diagnostic material, portability, ease of sharing, retrieval of archival images, and ability to make use of computer-aided diagnostic tools (eg, image algorithms).[37,62,75,76] WSI has also permitted new business models of care in pathology;[77] for example, virtual IHC service provided by large national laboratories. After the remote reference laboratory performs staining and slide scanning services, the referring pathologist is provided with full access to these IHC slides for interpretation or referral to a teleconsultant.[43] In the near future, the adoption of standards, validation guidelines, automation of workflow, creation of new revenue streams, and nuances of clinical digital practice will likely dictate a new standard of care for primary pathologic interpretations[11,77-80] (Figure 5).

WSI and Immunohistochemistry and Electron Microscopy

WSI offers advantages in enhancing objectivity in the interpretation of IHC used in tumor diagnosis, prognosis, and evaluation of biomarkers for targeted therapy.[1,81,82] A study reported a concordance of 90% between WSI and glass slides of HER2/neu expression in breast cancer.[81] Application of automated image analysis with algorithm-based scoring for the prognostic markers can assist in improving the scoring protocols and thereby enhance the efficacy of targeted therapies.[82] Also in electron microscopy, a virtual ultrathin slide allows the pathologists to navigate the slide in their office while noting the exact location of the specific features. Apart from this, WSI technology can be valuable for obtaining consultation on ultrathin sections from experts located in higher centers.[83]

WSI and Cytopathology

The role of WSI in cytopathology has been increasing but there are certain obstacles such as the inherent complexity of scanning, higher scanning time, and storage costs.[10,84-87] The scanning of cytology smear is difficult as well as complex because of its 3-dimensional character.[85] Consequently, it is essential to integrate z-stacking or multiplane scanning feature into the systems intended for use in cytopathology.[87] Alternative approach includes the conversion of z-stacks of images into video frames and storing the stack as a high-efficiency video coding file(s). Subsequent video compression has demonstrated to exceed the JPEG compression with comparable image quality.[88] A comparison of conventional glass slides and WSI in 10 cervical and 20 non-gynecologic cytology cases showed similar diagnostic concordance between the 2 modalities among the reviewing cytopathologists.[89] Another recent study comparing WSI with glass slides of thin-layer cervical specimens demonstrated 95.3% concordance rates, paving the way for WSI use in routine cytologic diagnosis.[86] A study by Wright et al evaluated the efficiency of WSI in cervico-vaginal cytology highlighting issues such as a lack of familiarity with the technology, difficulty in detecting few abnormal cells in the smears, problems with hyperchromatic nuclei, dark and crowded groups of cells, and massive image file size leading to increased duration of scanning.[90] Certain problems encountered while using WSI in cytology smears compared to the histology sections include (a) presence of dense overlapping tissue fragments making it difficult for scanners to focus on the cells; (b) red cell contamination of the smear and/or background acellular material(s) leading the scanner to focus on red cells and/or the background material rather than the cells of interest; (c) smears with scant cellularity making z-stacking difficult; and (d) need to remove the screening marks/dots before scanning (for which keeping a photographic record of the diagnostic screening marks is recommended).[91] Papanicolaou- and H&E-stained smears often have cells in multiple planes due to wet fixation, and thus require z-scanning to obtain a crisp, high quality image. On the other hand, air-dried Romanowsky-stained smears can be scanned with only x and y-axes, as air drying flattens the cells thus minimizing the requirement of z-stacking.[91,92]

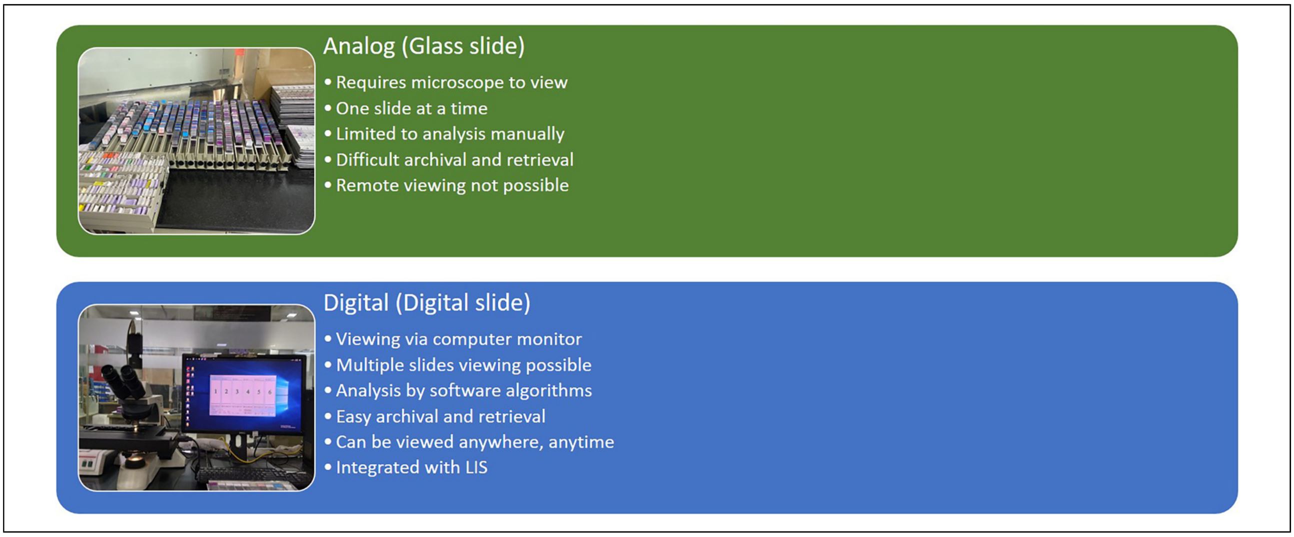

Figure 4. Implementation of WSI in routine pathological diagnosis.

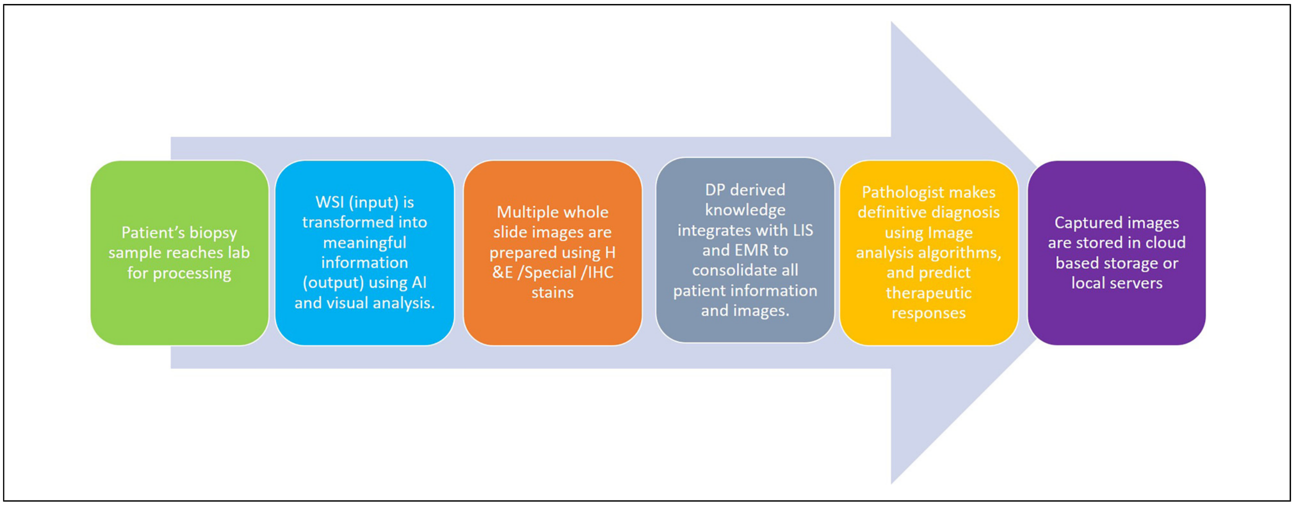

Figure 5. The adoption of standards, validation guidelines, automation of workflow, creation of new revenue streams, and nuances of clinical digital practice will likely dictate a new standard of care for primary pathologic interpretations.

Given the ongoing need for a cytologic diagnosis, the trend of using WSI in cytopathology may possibly increase in the future as minimally invasive procedures to obtain material for genetic/molecular analysis are used. The possibility to scan whole slides and to organize them in structured databases accessible via the internet would represent a powerful educational resource. The examples of rare cases can be shared without the risk of stain fading or loss or breakage of slide(s). A selected list of websites, with public or restricted access, including cytopathology teaching resources are as follows: http://www.bsccp.org.uk/, http://www.eurocytology.eu, http://www.cytest.eu/, http://www.cytology-asc.com/, http://www.cytology-iac.org/, http://www.cytologyiac.org/educationalresources/virtual-slide-library, http://www.cytologystuff.com/, http://www.cytopathology.org/, http://www.cytology.cloud/gk/, http://www.papsociety.org/index.html, http://www.tasteproject.eu/, http://www.uscap.org/, http://www.viewsiq.com, https://bethesda.soc.wisc.edu/, http://nih.techriver.net/, http://icytology.wordpress.com/, http://pathhsw5m54.ucsf.edu/introduction.html, http://pathorama.ch/, http://screening.iarc.fr/, http://www.virtualpathology.leeds.ac.uk/slides/, and http://137.189.150.85/cytopathology/

WSI in Artificial Intelligence

Education

Equipped with whole slide imaging, AI tools can help further training of the next generation of pathologists by providing on demand, standardized, and interactive digital slides that can be shared with multiple users anywhere, at any time.[92,93] Additionally, AI tools can provide automated annotations in the form of quizzes for trainees. With the help of these interactive tools, trainees can view, pan, and zoom enhanced digital slides, which can provide tutoring in real-time and in a dynamic teaching environment. For the purpose of generating synthetic images, researchers extracted individual and clustered nuclei that were both positively and negatively stained from real whole slide imaging images, and systematically placed the extracted nuclei clumps on an image canvas—cut-and-paste approach. These images were evaluated by trained pathologists in the task of estimating the ratio of positive to total number of nuclei. The resulting concordance correlation coefficients between the pathologist and the true ratio range from 0.86 to 0.95.[94] The main idea is to force the generator to learn the underlying distribution of the images from the training data. Generation of numerous synthetic histopathology images could be useful because it will give pathology trainees the opportunity to test their skills, besides being useful for quality control and understanding the perceptual and cognitive challenges that pathologists face[75,94] (Figures 6 to 8).

Quality Assurance

The development of automated, high-speed, and high resolution WSI has a substantial effect on QA. Digitized slides that are readily available to pathologists in the LIS or on the intranet can be used for several QA tasks, including teleconsultation, gauging inter-observer and intra-observer variance, proficiency testing, and archiving of slides.[8,39,95,96] For example, CAP optionally sends whole slide images in addition to glass slides for certain proficiency testing cases. AI can have an important role in QA. By providing feedback manually or with intelligent deep learning and AI tools, a pathologist has the potential to keep improving on his or her performance. AI can be used as a supplement to these manual digital reviews in routine diagnostic workflow or as a complement to the more formal quality reviews that are part of a pathology laboratory's quality management process. AI can also provide a quality check on the diagnosis rendered by a pathologist by applying automated diagnostic algorithms. These methods can continue to serve as patient safety mechanisms to improve the quality of diagnosis and to prevent error.[75]

Pathological Diagnosis

WSI and AI have been increasingly used for routine pathological diagnosis. Several studies have shown a concordance of 89% to 99% when comparing diagnostic interpretation using digital slides to diagnoses rendered using glass slides and a conventional light microscope.[19,32,43,70,92,97] The quality of images produced by WSI scanners has a direct influence on the readers' performance and their reliability of diagnosis. Most modern scanners come equipped with autofocus optics system to select focal planes to accurately capture the 3-dimensional tissue morphology similar to a 2-dimensional digital image.[43] To account for varying thickness of tissue sections, these systems determine a set of focus points at different focal planes from which scanners capture images to produce sharp tissue representation. However, still digital images with out-of-focus areas may be produced if the autofocus optics system erroneously selects focus points that lie in a different plane than the proper height of the tissue.[19,43] To overcome this, AI automatically identifies out-of-focus regions allowing WSI scanners to add a few extra focal points to those regions by either feature engineering or via a representation learning approach. Another approach called DeepFocus, based on representation learning, automatically discovers features from the images to identify blurry regions. Because the DeepFocus program automatically learns features at different levels of abstraction, it can generalize to different types of tissues and even to color variations due to different types of staining, H&E, and IHC.[10] Standardization of the color displayed by digital slides is important for the accuracy of AI. Color variations in digital slides are often produced because of different lots or manufacturers of staining reagents, variations in thickness of tissue sections, difference in staining protocols, and disparity in scanning characteristics. For this reason, the absence of color normalization in an AI pipeline could negatively affect the performance of machine learning algorithms. For a long time, collecting color statistics to perform color matching across images has remained the main source of color normalization. However, progresses in the generative models have presented novel ways of color normalization[98] (Figures 6 to 8).

Image Analysis

Image analysis tools can automate and quantify with greater consistency and accuracy than light microscopy.[81,99] Computer-aided diagnosis is widely used for estrogen receptor, progesterone receptor, and HER2/neu assessments in breast cancer,[100,101] Ki67 assessment in neuroendocrine neoplasms,[102,103] PD-L1 as immune checkpoint molecules in various solid organ malignancies, as well as multiple other clinical and research stains. The reliability of these methods requires the standardization of the image acquisition step. AI methods aid in enabling the regions of interest selection.[104,105] Nuclear segmentation in WSI enables extraction of high-quality features for nuclear morphometrics and other analysis in computational pathology.[94] For this reason, automatic nuclei

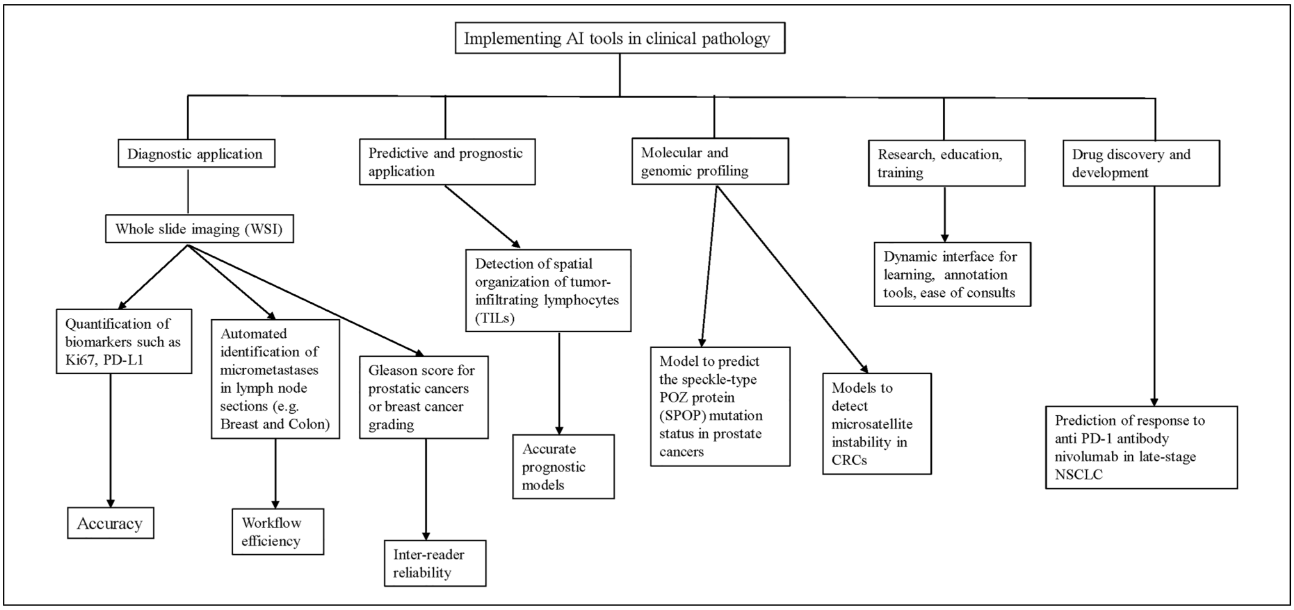

Figure 6. Schematic representation of the how artificial intelligence can be applied in the education and practice of pathology.

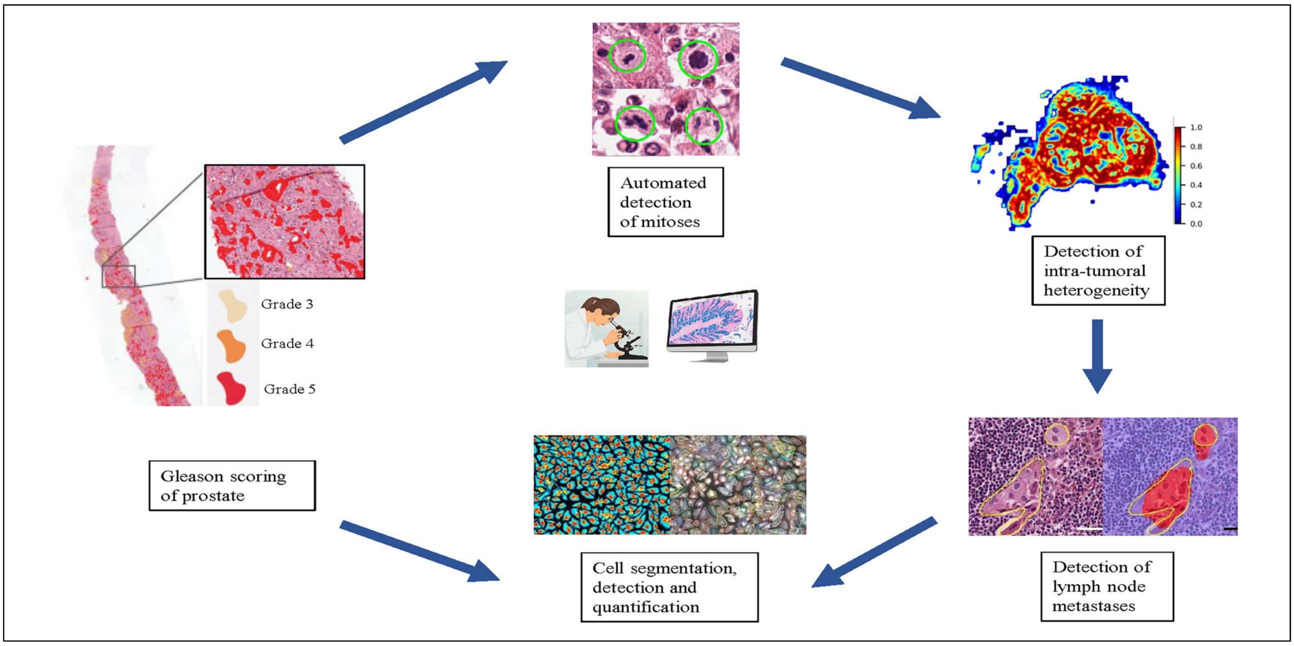

Figure 7. Depiction of artificial intelligence and machine learning approaches currently used by pathologists to analyze images from tumors.

segmentation is among the most studied problems in AI. In general, these algorithms estimate a probability map of the nuclear and non-nuclear (two-class) regions on the basis of learned nuclear appearances and rely on complex methods after processing to obtain the final nuclear shapes and separation between touching nuclei.[106] Kumar et al[107] have created a well-annotated database consisting of 30 whole slide images of digitized tissue samples from several organs. The slides were taken from the publically available database The Cancer Genome Atlas. The images were generated at 18 different hospitals, which adds to the diversity of this dataset in terms of variation in slide preparation protocols among laboratories. Over 21 000 nuclei were manually annotated to train a deep learning algorithm. Unlike former methods, a nuclei segmentation as a 3-class problem was created. They considered the nuclei edges

Process involved in integration of AI tools in pathology	Challenges and roadblocks
Identification of needs	• Incorrect assessment of end-user needs and demands • Small market size for AI usage • Lack of awareness of possibilities of use
Collaborative inter-disciplinary efforts	• Lack of coordination between different players • Discordance in goals of participants
Study concept, design	• Scientific background/rationale • Funding • Ethical approval
Development of algorithmic models	• Pre-analytical, analytical factors • Lack of objective ground truth
Optimization, validation and standardization	• Lack of appropriate validation dataset • Overfitting
Interpretability	• Lack of interpretability and generalizability • Black-box issue
Data curation	• Difficulty in obtaining well-curated, annotated data
Regulation/approval	• Lack of clear-cut regulatory guidelines
Installation	• Pathologists' resistance to changes in old workflow • IT infrastructure investment and overhead costs
Accreditation	• No external quality assurance scheme • Unestablished audit cycles
Reimbursement	• Lack of dedicated procedure codes
Clinical adoption	• Lack of FDA approval for use of AI • Skepticism among pathologists and oncologists
Computational system, data storage	• Need for powerful, high specification hardware • Cost-benefit ratio considerations

Figure 8. An overview of the challenges and roadblocks encountered during various steps of using artificial intelligence tools in pathology workflow.

as a third class when generating the tertiary probability map. This map was subjected to region growing to segment the individual nuclei.

During most pathological analysis, pathologists are interested in identifying a subset of nuclei in a particular anatomical region. For example, in T1 bladder cancer,[108] pathologists are interested in identifying the tumor nuclei within lamina propria. Similarly, in breast and neuroendocrine tumors, the pathologists are interested in the ratio of Ki67 tumor positive nuclei to total tumor nuclei within the hotspots. In follicular lymphoma, the analysis is limited to only the presence of centroblasts within the neoplastic follicles. For these reasons, there is an increasing interest in developing AI algorithms that can identify a subset of cells within a certain anatomical region. Also, whole slide is partitioned into superpixels on the basis of similarity at some magnification. Superpixels are grouped into anatomical regions (specifically epithelium) on the basis of graph clustering. Finally, each cluster is classified as ductal carcinoma in situ or benign or normal on the basis of features extracted by DL[104,109,110] (Figures 6 to 8).

Caveats and Challenges of WSI

In order to integrate WSI into routine clinical pathology practice, an infrastructure needs to be developed in the pathology department.[37,60,66,75,111] This infrastructure consists of: (i) hardware for scanning slides; storing the scanned images, transmission of the images to pathologists, and the interfaces necessary to display the images and report interpretations; and (ii) the software to facilitate the workflow of the image movement, display, and reporting of the results. Additionally, for remote teleconsultation, features like security of protected patient information, process validation, as well as regulatory, medicolegal, and billing issues need to be added. However, there are many unresolved issues, as outlined below, which still need to be addressed before WSI finds its place in routine application across the wide specialty of pathology.[37,51]

Cost

The cost of procurement, implementation, and operational costs of WSI may be prohibitive, especially for small pathology laboratories due to huge initial cost of the scanners and additional hidden costs of training of staff and pathologists, technical support, digital slide storage systems, and regulatory or licensing costs.[43,112] Technological support for telepathology further compounds these costs. A recently published cost-benefit analysis at a large-volume academic center with slides in excess of 1.5 million showed a projected $1.3m savings over a 5-year period.[113] Another study showed that the projected 5-year total cost savings for our large academic-based health care organization upon fully implementing a DPS was approximately $18m. If the costs of DP acquisition and implementation do not exceed this value, the return on investment becomes attractive to hospital administrators. Furthermore, improved patient outcome enabled by this technology strengthens the argument supporting adoption of an enterprise-wide DP solution.[42,52] Another main component of cost savings is supporting improved patient outcomes by reducing interpretive errors and thereby eliminating costs for unnecessary treatment or progression of a disease and its related extra costs. However, these costs may be overestimated due to the limitations in the various models described where WSI is only applied to cancer cases and by nonsubspecialists. The abovementioned models possibly overestimate the cost savings due to improved patient outcomes as a result of reduced interpretative errors by nonsubspecialists within the pathology department. However, the same analysis needs to be undertaken for smaller laboratories and low-resource settings.

Technological Issues

Scanning the whole slide/smear is a tedious and time-consuming process at present. Scanning times can vary from 1 to 5 min for a small biopsy to 5 to 20 min for a surgical specimen and 3 to 5 min for a liquid-based cytology smear.[58] Another limitation with currently available scanners is the requirement of massive data storage capacity. Scanning at ×40 magnification of a 1 mm^2 area results in a file size of 48 MB. Hence, the majority of the WSI systems incorporate image compression algorithms (JPEG, JPEG 2000, and LZW) to reduce the file size, however that introduces image artifacts. Some scanners offer the ability of multi-resolution representation (pyramid representation) where the field of view on the screen is inversely proportional to the magnification being viewed.[43] The majority of the WSI systems utilize a content management system with specific programming in order to display the virtual slides in a consistent and specific manner.[43] Currently, there are vendor-dependent limitations with WSI systems. Some vendors use proprietary modules with limited scope of cross-browser compatibility or seamless execution on multiple devices.

Professional Barriers

Unlike radiology where digital systems obviate the need of making films, WSI in pathology does not reduce the laboratory's workload since glass slides still need to be prepared to be scanned. However, WSI does allow for streamlined navigation of the slides at various magnifications without the fear of accidentally breaking a slide at the microscope. The current WSI systems allow for batch-wise scanning of slides, thus improving the efficiency of the laboratory.[8,43,113]

Other issues include available bandwidth of the network at the pathologists' workplace, security issues related to information technology, and installation of compatible browsers. However, with progress in information technology, the systems shall continue to be upgraded for improved speed and compatibility with browsers.[60]

The FDA approval of WSI in primary surgical pathology diagnosis does open up the issue of legal implications for the reporting pathologists. The relevant regulatory agencies (such as CLIA) need to put forth their guidelines in light of the expected changes with adoption of WSI by pathologists.

Regulatory Issues

Though FDA has accorded its approval for use of WSI in surgical pathology practice in 2018, the other subspecialties of pathology still have a long path to tread towards this goal. Validation is a CAP Laboratory Accreditation Program (LAP) requirement for laboratories using WSI for diagnostic purposes. While the LAP checklist does not specify how to validate WSI, it is very clear that validation must be performed and documented. Regulations also need to be put in place regarding the archiving, retrieval, and access rights of the virtual slide library so formed.[8,38,113]

Conclusion

WSI is an exciting and promising technology with various advantages and a few challenges. The future of WSI lies in having an ideal vendor-neutral archive wherein a single software–hardware solution allows single viewing, storage, and retrieval with no barriers of the data source. This coupled with the promise of AI, pathology is poised for new discoveries and solutions.[81,92]

Future of WSI

1. Availability of high-resolution 3-dimensional imaging, especially for tumors, would improve the use of this technology with correlation between radiologic imaging and WSI.[64]
2. Multispectral imaging, when applied to WSI, would offer the ability to characterize chromatic properties and support color-based classification and multi-labeling studies.[58]

3. Refinement of AI and machine learning algorithms would allow the pathologists contribute in a larger role in improving patient management and outcomes.[51]

Virtual microscopy using whole slide scanning is an area of profound and rapid technologic development with numerous applications in the field of pathology. Despite its several advantages and claims of it being equivalent to conventional microscopy, the adoption of this technique has been rather slow even in the developed nations. The barriers referred to in this paper currently preclude the wide application of whole slide scanning in the resource constrained medical institutions of the developing world. Apart from the technical and cost-related issues, regulatory and validation requirements also need to be adequately addressed, especially for the developing nations. Nevertheless, WSI does provide a golden opportunity for pathologists to guide its evolution, standardization, and implementation by playing a key role in defining/refining guidelines, designing the resource specific DP laboratories, and propagating standardized educational modules to train the next generation of virtual pathologists.

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