Review Article

"Slide less pathology": Fairy tale or reality?

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Abstract

Pathology practice is significantly advanced in various frontiers. Therefore, "slide less digital" pathology will not be a mere imagination in near future. Digitalization of histopathological slides (whole slide imaging [WSI]) is possible with the help of whole slide scanner. The WSI has a positive impact not only in routine practice but also in research field, medical education and bioindustry. Even if digital pathology has definitive advantages, its widespread use is not yet possible. As it is an upcoming technology in our field, this article is aimed to discussessential aspects of WSI.

Key Words: Digital pathology, medical education, telepathology, whole slide imaging

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INTRODUCTION

The current imaging modalities in pathology practice are static photomicrography of the slide, slide scanning by robotic microscope and whole slide imaging (WSI). Wetzel and Gilbertson developed automated WSI system in 1999 and since then methodology of imaging has been changing slowly but steadily.[1]

Whole slide image (virtual image, digital slides) denote a digitalized image of entire histopathology slide or a selected area of it.[2] These digital images have high-resolution and offer access to all areas on a slide. These images can be viewed on personal computers, laptops or iPhones in any magnification.[3]

DIGITALIZATION OF IMAGES

The digital imaging has four important steps: Image acquisition, storage, editing and display of images.[4] Digitalization is performed with the help of WSI scanners, which has a hardware and software component. Hardware component is for handling and scanning the slides, storing the images, transmission and display of the images to pathologists. Software part facilitates formatting, compressing and viewing the images. Other important requirements associated with WSI system is high-speed internet connection and security measures to protect patient information.[5]

Slide scanning can be done in tile or linear pattern. In tile pattern, the slide is scanned as a series of rectangular tiles [Figure 1a and b]. In linear pattern, image acquisition is done as long narrow strips [Figure 1c]. Focusing strategies can also vary among scanners. In "focus every field" method [Figure 1a], each field/tile is auto-focused and is imaged. It is an accurate but time-consuming method. In "focus every n^{th} field" method [Figure 1b], focusing occurs every n^{th} field. It is a faster and simpler method. "Focus map" method [Figure 1c] can be used with either tile or line pattern of scanning. Here, focus points

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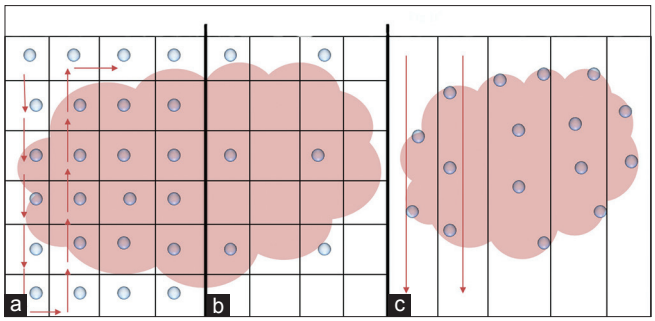

Figure 1: (a and b) Tile pattern of scanning. The arrows show direction of scanning. Blue dots in 1a indicate "focus every field" method. Dots in 1b indicate "focus every nth field" method. (Courtesy: Dr. Toby C. Cornish, PhD, Assistant Professor of Pathology, John Hopkins Medicine). (c) Linear pattern of scanning. Blue dots in 1c indicate "focus map" method (○ = focus point, → = direction of scanning)

are distributed over the tissue forming a surface. It is faster but less accurate method.[6,7]

WSI can be broadly divided into bright field, fluorescence and multispectral. Bright field WSI is the most common and least expensive type of method. Fluorescent WSI works on the same principle as a standard fluorescence microscope and only tile scanners are appropriate for fluorescence scanning. It is mainly used with immunofluorescence and fluorescent *in situ* hybridization (FISH).[8]

Multispectral imaging is a specialized form of digital microscopy for capturing spectral information across the visible range of light to near infrared bands. Multispectral systems are capable of working with fluorescence or bright field-based analysis.[1] Spectral imaging helps fluorescent imaging to overcome problems created by autofluorescence in tissue specimens.[1,9] The main disadvantage is its slow working pattern and complexity of the sample preparation.[9]

Scanning of slides occurs at multiple planes (x, y and z-axis) and magnifications.[5] Finally, small images are digitally "knits" into a single large image. Many scanners have auto loading facility where up to 200 slides can be loaded in the racks instead of one slide at a time.[3]

WHOLE SLIDE IMAGING FILE SIZE AND FORMAT

The file size of digital images is large, which comes in gigabits. As it is difficult to store such large files, they are usually compressed using Joint Photographic Experts Group (JPEG) or JPEG 2000 algorithms.[10,11]

APPLICATIONS OF WHOLE SLIDE IMAGING

Different sectors of pathology are aided by WSI. Applications of WSI are summarized in Table 1.

Table 1: Applications of whole slide imaging

Whole slide imaging		
Pathology clinics	**Education**	**Research and bioindustry**
Primary[12]/remote Frozen section Diagnosis[4,5]	Pathology training/ E-learning[8,10]	Tissue banking[8]
Teleconsultation[4,5]	Publications[4,10]	Biomarker-development[14]
Virtual immunohistochemistry[5]	Teleconferencing[4,10]	Image analysis[4,5]
Archival of cases[4]	Virtual tracking and workshops[10]	POS* for immunotherapy[15]
Tissue transplant assessment[13]	Tumor boards[10]	Human genome project[14]
Automated cytology smear and blood film analysis[14]		Dosing studies[14]
		The Food and Drug Administration clearances[14]
		Computer-assisted screening techniques[14]

*POS: Personalized oncology suite

WHOLE SLIDE IMAGING IN ROUTINE PRACTICE AND TELEPATHOLOGY

Digital slides are currently entering into day-to-day pathology practice. Adoption of WSI for routine diagnostic procedures has been done only in few centers.[4,5,16] Digital slides offer several advantages over glass slides such as portability and ease of sharing.[4,5]

According to Jen *et al.*, digital slides are reliable for the evaluation of renal allograft biopsies.[13] Krishnamurthy *et al.* compared digital slides with optical microscopy for interpretation of breast carcinoma tissues. They concluded that both methods have similar accuracy, precision and reproducibility in interpretation.[12]

Good correlation between standard glass slide and digital slides are noted with gastrointestinal,[17] pulmonary[18] and prostate[19] specimens.

Telepathology system includes a digital imaging station, telecommunications network to transmit images and monitor or screen to remotely view the digital images. WSI telepathology has several advantages over telepathology using precaptured still images. Accessibility to an entire digital slide, the ability to choose automated or manual scanning, high-resolution of images and the option to use software for image analysis are main advantages of WSI telepathology.[4]

Digitalization combined with telepathology is helpful when there is a shortage of pathologists, when the hospital is in remote location with large number of difficult cases/frozen sections, which require rapid interpretations.[5,20] The University Health Network Canada found this technology safe, accurate and reliable.[5] According to Piccolo *et al.*, telepathology for dermatologic diagnosis appears to be reliable.[21]

WHOLE SLIDE IMAGING FOR MEDICAL EDUCATION AND RESEARCH

Many medical schools accepted digital slides for undergraduate and resident training.[22,23] According to Pantanowitz, this is the best possible application for pathology education.[4] At the Poznan University of Medical Sciences in Poland, WebMicroscope (online interactive teaching and examination platform) facilitate accessibility to pathology for dental students.[24]

Digital slides are easy to handle in many ways.[3] In newly established medical and dental colleges, there will be shortage of histopathological slides and microscopes. Digital slides can encourage self-study as it can view on laptop or iPhones.[25] Online slide-sharing services such as PathXchange, Slide2Go provide accessibility to online virtual teaching sets.[10,26] Case presentations can be made more effective and interactive with help of digital images. It takes less preparation time than photographing the histopathological details. Salient pathological features can be marked on digital slides for teaching purpose and presentations.[3]

Most common problems encountered with fluorescent labels, especially in sensitive research areas such as genomic FISH, are small signal size and fading of fluorescent signals which can leads to false negative results. WSI reveals these small signals by scanning several focal planes through the sample (z-stacking) and these digital slides act as permanent records. Therefore, WSI is an accurate method to assess quantification and location of gene/chromosome.[8] Tissue microarray (TMA) has been extensively used for biomarker screening and validation studies in cancer research and toxicology testing. TMA image acquisition is a tedious procedure with optical microscopy. Digital TMA is more ergonomic and accurate.[8,27] Digital pathology can also assist virtual tumor banks such as the European human frozen tumor tissue bank.[28]

WHOLE SLIDE IMAGING FOR IMAGE ANALYSIS

WSI image analysis assesses cytomorphometry, antibodies detection and quantification of biomarkers.[5]

Slodkowska *et al*. tried to detect accuracy of Ki-67 assessment in brain tumors with computer-assisted image analysis using WSI. They proved that computer-assisted image analysis using WSI can be an effective alternative.[29] Virtual immunohistochemistry is done routinely for human epidermal growth factor receptor 2, estrogen receptor and progesterone receptor analysis.[4,5,30] Biomarker quantification in TMA can be more accurately done with the automated image analysis options.[8] Multispectral image analysis is valuable tool in cytopathology to differentiate the similar lesions.[31]

VALIDATION OF WHOLE SLIDE IMAGING SYSTEM

The Food and Drug Administration included WSI in Class III (highest risk) medical devices. Class III devices require quality system regulation and premarket approval.[32] Therefore, validation of WSI is vital to ensure its diagnostic performance. Currently, there are no standard guidelines for validation of WSI.[2]

The College of American Pathologists's (CAP) Pathology and Laboratory quality center proposed certain guidelines for validation of WSI.[2] CAP guidelines are summarized in Table 2.

ADVANTAGES OF WHOLE SLIDE IMAGING

Digital slides have several advantages over conventional method. Digitalization provides high-resolution digital images within relatively short time span. Redondo *et al*. compared digital slides with photomicrographs taken with help of optical microscope. Better image quality in term of color and contrast was noted with digital slides. Sharma *et al*. reported that some attributes of digital images might be better than conventional methods such as portability, archiving, sharing and performing image analysis.[22] Ramey *et al*. reported positive results in terms of concordance when virtual slides of frozen sections were reviewed on a mobile device (iPad).[33,34]

Easy retrieval of archival images is another advantage. There will not be any issue related to slide breakage and fading of stains. Ability to make use of computer-aided image analysis becomes possible by the advent of digital images. Quality assurance and testing can be done easily with the use of digital slides. It can also act as a permanent record of sent-out slides, slides sent for medico legal cases and digital image analysis.[4,5]

DISADVANTAGES OF WHOLE SLIDE IMAGING

However, the cost for infrastructure, additional human resources and validation of the process cannot be affordable for institutions/laboratories in developing countries.

The pathologist, histotechnologists and information technology staffs who form the backbone of telepathology team should be available at the center. Continuous supply of power during overnight scanning, uninterrupted internet services and high-resolution monitors are other difficult areas.[5] Technical problems such as scanning difficulties, hardware and software problems are main limitations for WSI telepathology.[4]

Large size of the digital file would be an issue while digital archiving of slides. Z-scanning of glass slides increases scanning time and file size. Few currently available scanners can afford

Table 2: The College of American Pathologists guidelines regarding validation of whole slide imaging

Divisions in the guidelines	Key points in the guidelines
CAP recommendations[2]	Validation should be appropriate for and applicable to the intended clinical use
	Validation study should closely emulate the real-world clinical environment in which the technology will be used
	Validation study should encompass the entire WSI system
	A pathologist(s) adequately trained to use the WSI system must be involved in the validation process
	The validation process should include samples of at least 60 cases for one application
	The validation study should establish diagnostic concordance between digital and glass slides for the same observer (i.e., intraobserver variability)
	Digital and glass slides can be evaluated in random or nonrandom order during the validation process
	A washout period of at least 2 weeks should occur between viewing digital and glass slides
CAP suggestion[2]	Validation study should establish diagnostic concordance between digital and glass slides for the same observer
Expert consensus opinions[2]	All pathology laboratories implementing WSI should carry out their own validation studies
	Revalidation is required whenever significant change is made to any component of the WSI system
	The validation process should confirm that all of the material present on a glass slide to be scanned and included in the digital image
	Documentation should be maintained recording the method, measurements and final approval of validation for the WSI system to be used in the clinical laboratory

CAP: The College of American Pathologists, WSI: Whole slide imaging

slides from large tissue blocks.[35] It was also observed by some investigators that the time required to review a virtual slide took longer than that needed to examine a glass slide.

Other issues limiting the use of digital pathology include the perception among pathologists that WSI systems are inferior in terms of performance and legal issues related to teleconsultation across states and internationally.[1,5]

Quality of virtual slides depends on the quality of the original slide to a certain extent. The glass slides to be scanned have to be free of artifacts such as folds, knife marks, air bubbles and stain deposits. Current scanning technology does not satisfactorily accommodate thick smears and three-dimensional cell groups in cytopathology.[3] Inadequate clinical data (gross pathology description, prior pathology reports, clinical history), missed tissue on the digital slide, pathologists' lack of experience using a WSI system can lead to erroneous diagnosis.[36] Table 3 summarizes the advantages and disadvantages of WSI.

HOW TO OVERCOME LIMITATIONS OF WHOLE SLIDE IMAGING?

The main limitations of WSI system are its high cost and lack of proper training for the use of the new technology. Discrepancies in diagnoses between digital and glass slides were reported in few publications.[12]

Discrepancies in diagnoses between digital and glass slides were attributed to poor image quality, rarely missed tissue on the digital image, inadequate clinical data and pathologists' lack of experience using the WSI system. Increased time taken for digital slide analysis is perhaps because of the inherent learning curve associated with the new technology.[12] Proper training of pathologists and technicians could overcome these problems.

Table 3: Advantages and disadvantages of whole slide imaging

Advantages	Disadvantages
High-resolution images	High cost of scanner
Relatively short time for scanning	Need of uninterrupted internet and power supply
Easy image portability and retrieval	Extra time and effort for validation
Support online teaching and self-learning	Process and training to pathologists and technicians
Easy to handle	Difficulty to manage large digital files
Fast image analysis	Difficulty to scan large tissue sections and thick smears
Act as permanent record even if the glass slides are sent out for any other purposes	Need of special data protection system

It is still difficult to scan thick smears using WSI. This can be overcome by multiplane scanning along multiple z-axes or intercalation of scanned images along different focal points.[4]

WSI was perceived to be more time-consuming than optical microscopy.[12] Independent dual sensor scanning is helpful to reduce the time for image acquisition.[7] According to the study conducted by Yagi and Gilbertson, thinner tissue sectioning (by automated tissue sectioning) significantly reduces slide scanning times and improve image quality.[37]

The main limitation, i.e., cost of scanners, is expected to be decreased by invention of new technologies for economic manufacturing. This may lead to adoption of WSI even in developing countries.

CONCLUSION

Until now, microscopes remain to be the cornerstone of pathology practice. However, in near future, slide scanners can share that position. More accurate and speedy diagnosis of lesions is the ultimate goal of pathology practice regardless of

the methods/instruments used. A state of mind that accepts and adapts to the better changes is the most important tool to overcome any limitation.

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