

MINI-SYMPOSIUM: Computer- and Internet-Based Tools for Neuropathology in the 21st Century

Digital Pathology: A Tool for 21st Century Neuropathology

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The authors have no financial ties or interests with any of the websites, products or companies mentioned in this article. Dr. Judkins owns and uses slide scanners from Aperio and Bacus Laboratories, Inc. and has been an invited speaker at Visions 2008, a conference on digital pathology sponsored by Aperio.

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INTRODUCTION

Digital pathology refers to the relatively recent ability to create an electronic environment for performing pathologic analyses and managing the information associated with this activity. Digital pathology is the product of a series of technologic innovations driven by a number of companies, as well as by investigators who have harnessed this technology to enhance their research and clinical practice. One of the most familiar technologic changes is the introduction of digital cameras to capture still images, replacing film as the preferred medium for photomicroscopy. Hardly noticed now, this change introduced many pathologists to the benefit of capturing fields of interest in a digital format. This relatively modest change has afforded us the ability to use imaging information in new and innovative ways in clinical, educational and research endeavors.

Some of the key technology to produce dynamic, relatively high-resolution images of entire slides was introduced by Bacus Laboratories, Inc., beginning in the mid-1990s. The first generation of slide scanners capable of scanning whole slides became available around 2000. These systems were limited to a basic combination of scanning hardware and viewing software, the

Abstract

Digital pathology represents an electronic environment for performing pathologic analysis and managing the information associated with this activity. The technology to create and support digital pathology has largely developed over the last decade. The use of digital pathology tools is essential to adapt and lead in the rapidly changing environment of 21st century neuropathology. The utility of digital pathology has already been demonstrated by pathologists in several areas including consensus reviews, quality assurance (Q/A), tissue microarrays (TMAs), education and proficiency testing. These utilities notwithstanding, interface issues, storage and image formatting all present challenges to the integration of digital pathology into the neuropathology work environment. With continued technologic improvements, as well as the introduction of fluorescent side scanning and multispectral detection, future developments in digital pathology offer the promise of adding powerful analytic tools to the pathology work environment. The integration of digital pathology with biorepositories offers particular promise for neuropathologists engaged in tissue banking. The utilization of these tools will be essential for neuropathologists to continue as leaders in diagnostics, translational research and basic science in the 21st century.

latter allowing users to pan across the entire digital image and then zoom in (view at higher magnification) on areas of interest. This pan and zoom approach was designed to mimic the way a pathologist manipulates a glass slide at a microscope. Incremental improvements have been made in scanning technology to allow for robotic batch scanning of large numbers of slides, significant improvements in scanning speeds and the introduction of more sophisticated software not only to view virtual slides, but also to be able to integrate slides with other sources of digital information such as laboratory information systems (LISs), as well as to offer sophisticated image processing and analytic tools. It was only with the relatively recent addition of these tools that an electronic workspace or digital pathology environment could be said to exist. Even in its nascent state, digital pathology has introduced important changes in several areas of pathology practice and research, including consensus reviews, quality assurance (Q/A) programs, tissue microarray (TMA) analysis, education and proficiency testing, examples of which are discussed below. With continued technologic improvements and the introduction of fluorescent side scanning and multispectral detection, future developments in digital pathology offer the promise of adding powerful analytic tools to the pathology work environment.

The introduction of these new tools is occurring in a medical landscape that is rapidly being reshaped by trends in both the clinical and research environments within which neuropathologists operate. On the clinical side, neuropathology as a diagnostic specialty faces a constant challenge to embrace or develop tools in response to colleagues who demand rapid introduction of new diagnostic markers and molecular tests, ways to interact with the data resulting from this diagnostic work and integration of these data into an easily accessible digital medical record. In a relatively small field such as neuropathology with a limited number of specialists available for consultations, there is a constant challenge in finding ways to make these specialized services available in all the places where there is clinical demand. Some of these needs in neuropathology have been met through telepathology, discussed by Horbinski and Hamilton in this issue of *Brain Pathology*. On the research side, there is a burgeoning view of pathology departments as biorepositories to provide highly annotated clinical material to support basic and translational research programs, many of which are highly complex and involve multiple institutions. Development of new diagnostic paradigms and research efforts requires true quantitative assessment and evaluation of tissue with more complex image analysis.

The development of digital pathology offers neuropathologists the tools to meet these challenges and to develop new and innovative ways to practice their art in the 21st century. We will discuss some of the underlying technology that supports digital pathology and highlight areas in which digital pathology applications have particular relevance to neuropathology. Most of this work is currently being done in other pathology subspecialties. The greatest potential of digital pathology may be realized when pathologists choose to reconfigure their work environments to utilize this technology for routine clinical work in order to take advantage of computer-aided diagnosis and analytic tools to supplement traditional histopathologic assessment. We report on key lessons learned from the first phase of a project to implement these technologies at the Children's Hospital of Philadelphia (CHOP). Finally, some of the early promise of digital pathology to integrate complex data sets involved in biorepositories is being realized through innovative applications that have been developed at the Center for Childhood Cancer and the Biopathology Center (BPC) at the Research Institute at Nationwide Children's Hospital. Our analysis of the current state of the digital pathology suggests that the nature and pace of technologic change occurring within pathology are such that implementation of digital pathology technology and applications will be critical for neuropathologists to continue to take a leadership role in diagnostics and research in the 21st century.

CREATING, VIEWING, ANALYZING AND MANAGING WHOLE SLIDE IMAGES (WSIs)

Virtual microscopy is the ability to interactively view high-resolution WSI. Digital pathology applications are built around WSI. WSI has its origins in the work of Joel Saltz and colleagues on what they termed "Enhanced Field Microscopy," which utilized a robotic microscope to scan a large area of a glass slide and then combine the captured fields together to form a single large image (10). The first automated high-speed scanner for WSI was pro-

duced by Interscope Technologies and the University of Pittsburgh Medical Center (16). Subsequently, scanners to produce WSI have been produced by a number of different vendors. For a comprehensive review of available systems and technical details of their function, refer to Rojo *et al* (22). There are several fundamental principles common to various systems that use slide scanning to produce WSI. Glass slides are scanned either using a robotic microscope or an array-based scanner system. These systems typically utilize a high-resolution camera coupled with one or more high-quality microscope objectives to capture images of adjacent areas from a glass slide. The pattern of movement and exact type of fields captured in the scanning process vary between systems, but most utilize specialized software to reassemble images from these multiple individual fields in a single WSI. This image is then processed and stored in a format that allows viewing software to rapidly scan and zoom through a WSI, similar to how a pathologist views a slide through the microscope. Scanning is done at high resolution, often on the order of 0.25–0.5 microns/pixel and generates large amounts of data. For example, a scan of a typical 2 × 1 inch piece of tissue would represent 100 000 × 50 000 pixels or approximately 15 GB of uncompressed data. Image compression algorithms such as JPEG or JPEG2000 are typically used in order to reduce the typical file size for WSI to 0.5–1 GB (7).

As WSI are both large and likely to be a shared resource for many users, they are typically stored in a server. Some systems also offer specialized software to manage the storage and retrieval of WSI, as well as sharing of a single WSI simultaneously among several users for virtual slide conferencing. While the file size of WSI is quite large even with compression, a variety of different approaches are used by vendors of WSI systems to limit the amount of data that has to be moved from the server to the local computer for viewing of WSI. This is typically accomplished by only presenting the limited subset of data that is being viewed at any given time from the WSI, so-called pixel on demand. As the user pans and zooms, the system calls up the corresponding data from the WSI. In this way, the network load is minimized and software responsiveness is maximized to simulate working with an actual slide. The software for viewing WSI can be a Web-based or a stand-alone application and may offer a variety of basic tools for viewing, annotating and manipulating the basic image. Typically, this software provides the ability to export static images, just like conventional digital photomicroscopy.

The difference between virtual microscopy and digital pathology is the addition of tools to allow the pathologist not only to read and annotate an individual slide, but also to interface WSI data with existing LIS, perform image analysis and correlate pathology data in WSI with other imaging and test result data available for a given patient. Only recently have vendors begun to develop software to support this functionality for WSI. This development of an information management system that supports both the imaging application as well as a comprehensive support of workflow within the digital pathology workspace is analogous to the development of picture archiving and communication systems (PACS) in radiology. By supporting the entire workflow of radiology, the matured PACS technology has come to define the radiology digital workspace. In pathology, the components for such systems exist, and nascent digital pathology workspace environments are beginning to develop around vendor specific software applications such as Aperio's SpectrumPlus application (<http://www.aperio.com/>

pathology-services/SpectrumPlus-information-management.asp) or BioImagene's 3i and PATHIAM software (<http://www.bioimagene.com/index.html>), as well as user-developed applications such as Virtual Imaging for Pathology, Education & Research (VIPER) (see below).

Perhaps the greatest advantage offered by WSI is the ability to perform complex image analysis as an aid or adjunct to routine diagnosis. Neuropathologists have limited exposure to these methodologies in routine clinical practice, so examples from other areas of pathology are illustrative. One of the areas of pathology in which image processing technology has made the greatest impact in routine diagnostic work is in the examination of Pap smears in cytology. Beginning in the early 1990s, the Cytoc Corporation (Marlborough, MA, USA) started the development of the ThinPrep® Pap Test™. This test relies upon image processing software to automatically identify suspicious cells on specially prepared Pap smears. These abnormal cells are flagged for review. The introduction of the ThinPrep® Pap Test™ required changes in the cytopathology labs including preparation of special slides in which cells would be prepared as a monolayer, necessitating retraining of cyto-technologist screeners and laboratory workflow modifications. Working as a supplement to pathologist diagnosis, ThinPrep® Pap Test™ has improved detection of cervical abnormalities and has doubled the average reported cytotechnologist screening rate (5).

ThinPrep® Pap Test™ is based on the identification of atypical and malignant cells in specially prepared cytology samples. In a similar fashion, automated image analysis of routine histologic sections are now being used in surgical pathology for detection and quantification of HER2/neu overexpression/amplification in breast cancer, where it is associated with increased recurrence and worse prognosis (21). HER2/neu expression is also important because it predicts responsiveness to trastuzumab, which can increase survival and reduce risk of recurrence (21). Determination of HER2/neu status can be done by fluorescence *in situ* hybridization (FISH) for HER2 gene amplification or by immunohistochemical (IHC) staining to detect HER2 protein overexpression (4). Interobserver variability is a major challenge with standard IHC, but substantially improved correlation with HER2 gene amplification by FISH has been achieved with the use of automated image analysis (26). Currently, such analysis requires the capture of one or more static images from representative sections in which invasive tumor has been identified by the pathologists. WSI-based automated image analysis offers the ability to automatically perform these assays on selected regions, as well as in the future, automated detection of lesional areas. Currently, at least two vendors, Aperio (<http://www.aperio.com>) and BioImagene (<http://www.bioimagene.com/index.html>), offer United States Food and Drug Administration (FDA) approved *in vitro* diagnostic (IVD) algorithms for HER2, estrogen receptor (ER) and progesterone (PR) stained breast specimens on WSI. With these basic tools in place, the development of other specialized image analysis assays will continue to develop and will surely impact each subspecialty, including neuropathology.

There are also current examples of computer-based diagnosis for neuropathology applications similar to that used in the systems described above. For instance, a computer-assisted diagnosis system for grading astrocytomas has been developed and tested using digital images from hematoxylin and eosin (H&E) stained slides that were analyzed with imaging and learning algorithms

(14). Cases of astrocytomas that were diagnosed by histopathology as low grade (WHO grade II), high grade (WHO grade III and IV) and suspicious grade II–III lesions were collected, and selected images from representative areas were digitized. Image segmentation was performed on these selected images of tumor to identify nuclei and distinguish them from other structures using a probabilistic neural network pixel-based algorithm. On average, this algorithm correctly identified 86.5% of all the nuclei in these selected images. The morphologic features that were computed included measurement of the nuclear area, roundness and concavity. The data were processed, analyzed and separated in three categories: low, intermediate (suspicious) and high grade. One hundred and forty astrocytomas were included in the automated analysis. There was a 92.1% concordance between the computed and pathologist classifications. Low-grade lesions were accurately separated by the computed system in 95% of the cases. Of the high-grade lesions, 91% were correctly diagnosed and 83.3% of the suspicious cases were properly identified. Modifications in the classifier system and selection of different numbers of criteria have shown improvement in the computed system performance with an overall concordance as high as 97.8% (13). Whether or not a similar program could aid pathologists in the future in terms of assigning histologically borderline cases into either high- or low-grade categories will require additional studies and clinical follow-up. Another group utilized automated computed-based counts using public domain image analysis software to assess Ki-67 labeling indices in meningiomas. This approach showed a high correlation coefficient (0.98) in much less time than the conventional manual method (17). These assays are available for WSI from multiple vendors. Automated imaging analysis has also been applied to neurodegenerative diseases. Using a computer application that can be trained to classify various objects, Chubb *et al* demonstrated that automated image analysis could classify plaques and tangles with an accuracy comparable to manual methods and count neurofibrillary tangles for quantitative and comparative studies (3).

SPECIALIZED APPLICATIONS (CONSENSUS REVIEWS, Q/A, TMA ANALYSIS, EDUCATION AND PROFICIENCY TESTING)

WSI has been implemented in a number of specific niches within pathology since its introduction in 2000. In order to support the use of WSI for these purposes, studies have been performed to assess the performance of pathologists using WSI under a variety of conditions. A limited number of studies have been published that examine the performance of WSI in comparison to traditional microscopy in evaluating routine clinical material. For example, Gilbertson and colleagues compared the performance of three pathologists reviewing 25 specimens with multiple parts in a simulated environment and reported excellent concordance between the results obtained by WSI and microscopic analysis (12). In a carefully devised study, Fine *et al* evaluated the use of WSI to review IHC-stained sections of challenging prostate needle biopsies by pathologists at geographically disparate sites. Thirty cases where IHC was required to confirm or rule out cancer were identified and scanned to create WSI. Five pathologists, as well as an outside expert reviewer, were asked to review and score both the original glass slides and the WSI. Essentially, similar levels of interobserver

variability were identified between the WSI and glass slide reviews. In only one case did participants feel that the image quality of the WSI was worse than the original glass slide. The authors concluded that their findings could likely be generalized to other similar IHC applications outside of prostate biopsies (11).

Ho *et al* used direct comparison of glass slides and WSI in a retrospective Q/A review of 24 complex genitourinary biopsies (comprising 47 diagnostic parts and 391 slides). Three pathologists reviewed these cases. Two pathologists were assigned WSI, and one received the original glass slides for each case; a standard Q/A form was used to evaluate both WSI and glass slides. Strong consensus was reported between WSI and glass slide reviews of these cases. However, in one case, a subtle but clinically significant discrepancy was identified between the WSI and glass slide, which suggested that technical issues in the WSI obscured a focal area of atypia. Overall, the authors reported that all study pathologists felt Q/A could be effectively performed using WSI (16). Significantly however, all participants also indicated that slide presentation and speed of WSI were inadequate to support routine case review based on WSI. Similarly, our own experience has been that this is a real limitation in the transition of digital pathology to daily clinical use. Future development of pathologist centric digital workspace environments will be essential for successful implementation.

Wan *et al* conceptually described the fundamental methods that underlie TMAs in 1987 (28). The technical approach currently used for TMAs was developed by Kononen *et al* in 1998 (18). Since that time, TMAs have become a widely used, even routine technique for IHC and *in situ* studies on large numbers of samples performed under uniform conditions. However, this same advantage of high tissue density translates into a somewhat cumbersome system for scoring and keeping track of individual cases. Because of the high throughput data generated by TMAs, WSI became an obvious application in order to capture individual cores in digital form, enhance the ability to retrieve, score and compare multiple results for each individual case side by side and perform image analysis for quantitative digital assessments.

By applying WSI to a large and diverse range of TMAs created over a wide range of specific research projects, the Stanford Tissue Microarray Database has captured over 200 000 stained and scored TMA images with associated annotations including both tissue descriptions and clinical data (20). One advantage of TMA-based studies is the ability to simultaneously examine large panels of markers over a wide population of samples. Using manual semi-quantitative scoring results uploaded to software originally developed for analyzing cDNA microarray results, it is possible to perform clustering analysis on these large data sets. In studies where TMA cores are drawn from well-characterized clinical populations with associated outcome data, this approach can yield cluster group designations based on IHC staining that show strong correlations with tumor grade, stage and cell type, while also being more reproducible and showing less interobserver variability than traditional histologic assessment of the same tumors (1).

A number of different vendors of WSI systems offer TMA packages with both data management and analytic tools; commercially available image analysis software can also be utilized to examine individual cores captured from TMA as static images. Automated image analysis is currently limited by weak tools to distinguish benign from malignant areas. In this regard, TMAs offer an ideally suited platform for automated image analysis as they are comprised

of areas carefully sampled to be representative of the lesional tissue. Alternatively, the addition of automated analysis for TMA scoring is particularly useful in studies involving large numbers of samples, as these are likely to eliminate human operator counting errors. For instance, the feasibility and utility of this approach has been established in a fully automated analysis of ER expression in a TMA containing 3484 invasive breast carcinoma cases with both treatment and outcome information, in which the fully automated analysis did not differ significantly from manual scoring of ER status by pathologists (27).

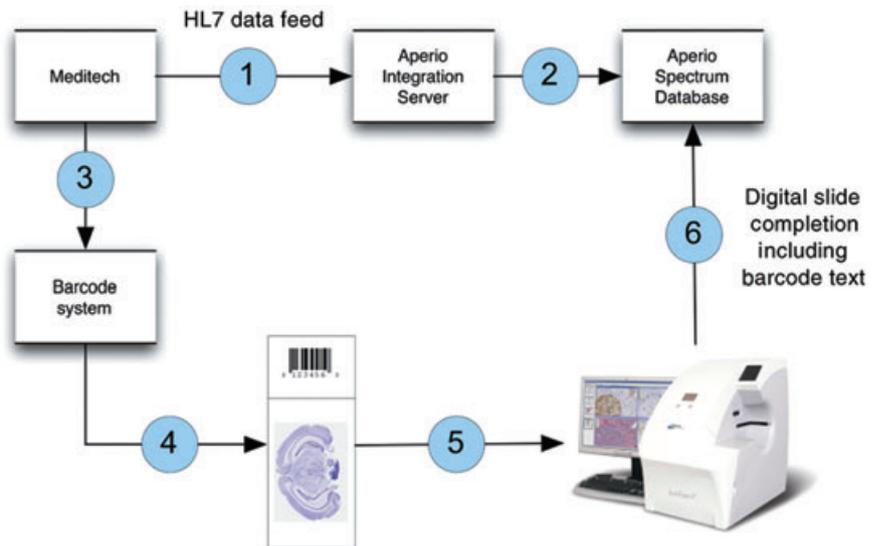
One area in which WSI has proven particularly useful is in education. A wide spectrum of applications have been reported, but particularly rapid growth has occurred in histology and pathology courses. A variety of published reports have documented good student satisfaction with educational materials prepared using WSI, as well as equivalent to sometimes improved performance on examinations (15, 19, 23). Many of the educational courses at national and international pathology meetings have started to adopt the WSI approach, both as a cost-saving tool and in order to broaden both the potential number of cases and participants. For instance, the blocks from small biopsies have often been insufficient to generate adequate numbers of teaching slides in the past, but WSI obviates that requirement. A good example of this utility in neuropathology is the diagnostic slide session at the annual American Association of Neuropathologists, Inc. (AANP) meeting. Limited needle biopsies from brain, muscle or nerve with rare disorders can now be submitted to the moderator as a single slide for scanning and presented to participants in the form of WSI. Similarly, popular educational courses using a WSI approach no longer need to cap the number of participants in order to match the limited number of slides obtainable from the average-sized paraffin block.

Coupled with education, assessment and proficiency testing are also areas where WSI has found significant use in pathology. Currently, the American Board of Pathology uses WSI in 15 of 75 of the microscopic slides for board examination (2). Particular consideration has been given to WSI as a means of proficiency testing in cytopathology. This interest stems from the national cytopathology proficiency-testing program that began in 2004. In the face of a need to create and distribute a large volume of well-characterized gynecologic cytology slides for proficiency tests, WSI has been viewed as a potential solution. Some groups have reported fairly good success with the use of WSI of ThinPrep® prepared samples (25). Finally, WSI can be used as a way to make rare or unusual samples of general interest accessible. An example of this related to neuropathology are two WSI of original brain sections used by Alois Alzheimer to describe the neurodegenerative disease named after him (<http://mirax.zeiss.de/alzheimerslides/show.aspx?slide1>).

PRACTICAL CONSIDERATIONS

While WSI has been introduced in numerous clinical, research and commercial settings, there is relatively little that has been reported about the use of WSI in an academic neuropathology setting. The Division of Neuropathology in the Department of Pathology and Laboratory Medicine at CHOP has made a long-term strategic commitment to implement digital pathology in clinical practice, as well as to support translational and basic research in pediatric brain

Figure 1. Sample workflow for the association of clinical data from the laboratory information system (LIS) (Meditech in this example), with whole slide image (WSI) (Aperio illustrated in this example). After clinical information has been properly mapped between the LIS and the WSI software (Spectrum in this example), the clinical information is sent by the LIS via an HL7 data feed (1) to an application to reformat these data for the WSI software. This allows the WSI of an individual slide from a particular case (4) to be generated (5) and then associated with the appropriate clinical information corresponding to that slide (2 and 6). Figure reproduced with permission from Mark Wrenn, Aperio.



tumors. Given the scope and complexity of such an undertaking, this project has three main phases: (i) developing standard operating procedures for scanning clinical material and integrating scanning to produce WSI into the normal clinical workflow for pediatric neuropathology cases at CHOP; (ii) implementation of WSI for the sign out of transient specimens such as consultation cases, where material is seen but not retained, and for autopsy neuropathology cases, which have longer turnaround time, affording greater flexibility for scanning; and (iii) implementation for general pediatric neuropathology diagnostic work. This work utilizes an Aperio (Vista, CA, USA) ScanScope® XT with a server containing 4 TB of direct attached storage.

In the process of accomplishing the objectives of phase 1 of this project, we have identified several practical considerations that illustrate some of the key steps and challenges in bringing WSI into an academic clinical environment. These fall into three key areas: training and resource utilization to support WSI production, interfacing existing LIS with WSI software to populate WSI with clinical information and storage for WSI. Specific issues arising from each of these areas are discussed below. Identifying and planning to address these kinds of issues are critical steps in the implementation of WSI in a clinical environment.

It is easy to underestimate the training and resource utilization required to support WSI even on a fairly modest scale. In order to support daily scanning of all new neuropathology cases at CHOP, approximately 3 months of full-time training and intensive scanning were required in order to train an operator and develop a workflow to support scanning. Multiple modalities were required for training including off-site training with the vendor, on-site training using clinical material and extensive use of technical support. By far, the most effective training is hands on using the clinical material and systems of the local institution. As interface issues become apparent, they can be rapidly addressed. Careful consideration should be given to the level of support offered by potential vendors in each of these areas. Our particular system can scan up to 120 slides in a single batch. An unanticipated source of resource utilization was created by the fact that up to 10% of slides coming from clinical labs (ours, or those of outside hospitals) had

to be returned to the lab or required additional handling prior to scanning. Most often, this was because of bubbles, misaligned coverslips or dirty slides, all of which interfered with producing high-quality WSI. An additional consideration if high-throughput scanning is required is what kind of slide handling pathway is used to feed individual slides into the scanning area. Certain slide handling systems are not well suited to wet slides that come directly from the lab, or the addition of slide labels may cause slides to jam. All of these can add additional time or prescan handling in the laboratory.

Interfacing the existing LIS with WSI software to populate WSI with clinical identifiers and complete pathology descriptions can be broken down into three key components. First, specimen identification for WSI systems can be greatly facilitated by bar coding. Systems that have the capacity to capture and decode information on slide labels allow for at least partial population of key slide identifiers such as case number, block or stain in the software that is used to populate and WSI information. In the absence of such a system, the scanning operator will have to manually enter these data for each WSI, a time-consuming and error-prone process. An even more important process is to carefully examine the way your existing LIS handles both demographic and clinical information, as well as the pathology-specific content in your cases. This information will need to be mapped into the software used to populate WSI information. Generally, the software used for WSI will have its own architecture with certain assumptions about how information should be handled and prioritized. Developing an appropriate data hierarchy, particularly without significant support from the LIS vendor can be a time-consuming process. However, this is essential for the final step in creating a working interface: obtaining and optimizing an HL7 feed by which information from your LIS can be transferred to your WSI system, properly formatted and containing the full pathology description desired. This process often requires support and even customization on both the LIS and WSI vendor sides. Pathologists can plan to spend a significant amount of time guiding this effort; this input is critical for a properly functioning system. Figure 1 shows a schematic view of the system developed at CHOP to accomplish these steps.

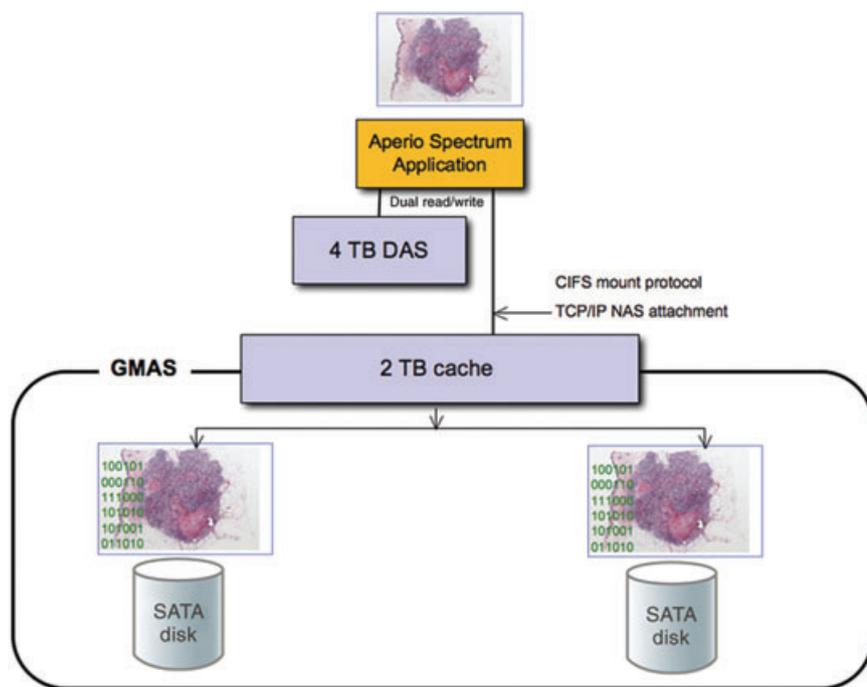


Figure 2. Schematic illustration of the potential interface between enterprise storage space and whole slide image (WSI) that is being developed at the Children’s Hospital of Philadelphia (CHOP). A direct mount to a large cache in the enterprise storage will allow for the WSI software (Spectrum in this example) to interface with remote storage. Modifications to the WSI software are required in order to directly interface with data in remote storage. Figure reproduced with permission from Thomas Rose, IBM. DAS = direct attached storage; TB = terabyte; GMAS = IBM Grid Medical Archive Solution, a vendor neutral technology for enterprise storage of medical imaging data; CIFS = common Internet file system; TCP/IP NAS = transmission control protocol/Internet protocol network attached storage; SATA = serial advanced technology attachment.

Storage for WSI can represent a critical bottleneck and requires very careful consideration. As the goal of our long-term project is to develop a system that utilizes WSI within a clinical workflow for the diagnosis of clinical neuropathology material, we chose to scan and store our entire clinical neuropathology caseload including both in-house and consultation surgical and autopsy material. This choice was made so that we could determine for ourselves the storage requirements that would be encountered in routine clinical use of WSI in our environment. Over approximately 8 months, we scanned 10 351 slides. These slides required 5.2 TB of storage (additional disk storage space was added to our original 4 TB configuration). While a range of tissue sizes were present on the slides scanned, ranging from large sections in autopsy cases to small fragments in brain tumor biopsies, the average slide size for our neuropathology material, scanned at 20×, was 506 MB after JPEG compression. Based on these numbers, it would require approximately 8 TB of storage per year to store the WSI data from our case material. At an estimated cost of \$2600/TB, the cost of storage would be approximately \$20 800/year for our system. On a larger scale, the estimated storage required for all of CHOP pathology slide volume, around 55 000 slides per year, would be approximately 27 TB/year, at a cost of approximately \$70 000/year. At current rates, the cost of storage for WSI would be approximately \$1.27/slide for direct attached storage. This is on top of the estimated \$0.50/slide estimated cost for scanning a WSI (24). These cost estimates do not account for back-up, redundancy or disaster recovery, which are essential if digital pathology is part of the primary pathology work environment.

In addition to cost, the scale of storage required by WSI is currently prohibitive for most pathology departments. Long-term storage of WSI data in a manner similar to that currently done for glass slides requires pathologists to participate in enterprise-level shared storage arrangements. In many hospital environments, radi-

ology departments have already pioneered such arrangements to support digital pathology systems. Radiology as a specialty has largely completed a conversion to digital radiology, and today, most radiology images are digital from their creation. Institutional investments in shared storage have been made to support this change. Enterprise storage solutions also offer the additional advantage of back-up, redundancy and disaster recovery capabilities. In order for pathologists to have the infrastructure to pursue serious digital pathology initiatives, it is necessary to have a “seat at the negotiating table” and include WSI in the planning discussions around storage space and network bandwidth.

To be effective in discussions about enterprise storage, it is important to recognize that there are some critical differences between digital pathology and digital radiology. The relatively large file sizes involved in WSI are in marked contrast to digital radiology, where file sizes are far smaller. So, the addition of digital pathology content will rapidly expand the overall size requirements for any enterprise storage. A second challenge is that while enterprise storage is scalable and can likely meet the disk space requirements of large-scale WSI, it is considerably more expensive than directed attached storage, currently costing on the order of \$10 000/TB. These increased costs are related not only to disk storage, but also to associated network switches and other hardware needed to create these systems, multiple levels of redundancy that often includes off-site back-up systems that can rapidly be brought on line and customized software that manages flow of information into and out of these shared storage spaces. This means that the addition of capacity to accommodate digital pathology can be associated with some very significant costs.

Finally, it is important to recognize that, at the present time, most WSI systems are primarily designed to interact with a dedicated server with direct attached storage rather than remote enterprise storage. Partially, this reflects the specialized nature of software for

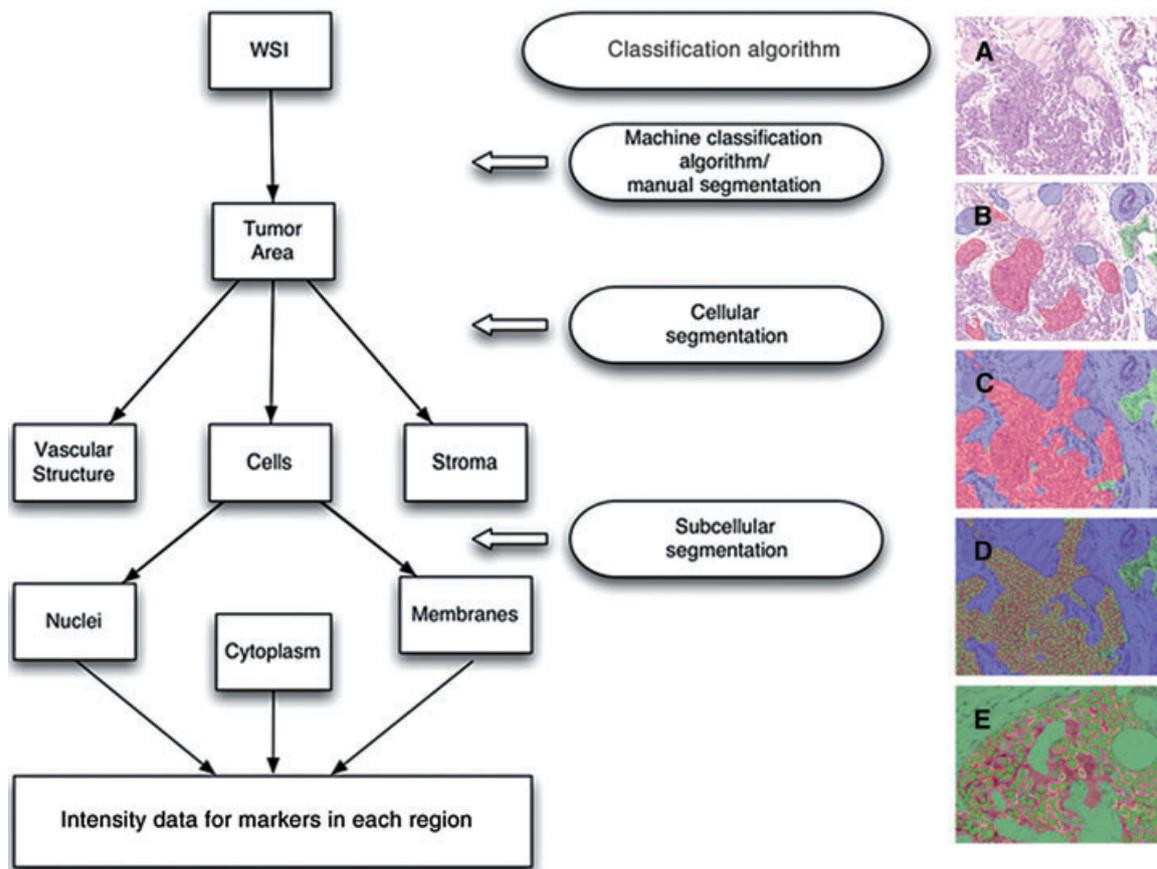


Figure 3. Schematic diagram highlighting the key steps in the analysis of a whole slide image (WSI) subjected to multispectral analysis. In this example, an invasive breast carcinoma has been stained with antibody for Vav and counterstained with hematoxylin (A). Representative fields of tumor (red), inflammation (green) and stroma/vascular structures (blue) are manually identified (B). Using these fields, the computer system is trained to recognize these regions within a sample. Based on this training, the computer classifies the sample into tumor (red), inflammation (green) and stroma/vascular compartments (blue) (C). Image

analysis algorithms can then be applied to specific areas of interest. In this case, the subcellular segmentation is applied only to the areas containing tumor (red). This identifies the nuclei (green) within this area (red) (D). Various subcellular compartments can be identified through this process including nuclei (green) and cytoplasm (light pink halos) with the tumor (red) (E). Intensity data for multispectral markers expressed in each of these regions can then be captured. (Acknowledgment: Michael Feldman MD, PhD, University of Pennsylvania Medical Center and Cliff Hoyt, CRI.)

displaying WSI. The scan and zoom approach described above is well suited to the large file sizes for WSI. By contrast, radiology and most other medical imaging systems are designed around the Digital Imaging and Communications in Medicine (DICOM) standard that uses quite a different store-and-forward access approach suited to relatively small image files and less dense image information. Because of these fundamental differences, WSIs are not currently compatible with the DICOM standard, although Working Group 26 (WG26) of the DICOM community is developing DICOM standards for virtual microscopy (8). The practical consequence of this is that software used to access and display WSI images will typically require some modification to access enterprise storage and that enterprise software to manage, share and display other medical imaging data typically will not work with WSI data. Figure 2 depicts of schematic illustration of the potential interface between enterprise storage space and WSI that is being developed at CHOP.

FUTURE APPLICATIONS (MULTISPECTRAL ANALYSIS AND BIOREPOSITORIES)

One advantage of WSI is that it opens up possibilities for neuropathologists to supplement morphology-based tools with advanced image processing algorithms to support our roles as diagnosticians, translational researchers and basic scientists. In these roles, we increasingly confront the limitations of traditional morphologic approaches and manual grading. While significant interobserver variability and low sensitivity are well appreciated for IHC and immunofluorescent methodologies, another equally compelling problem is simple capacity. As the demand for clinical trial or animal model-related assessment of tissue samples grows, the limitation of any individual neuropathologist to directly evaluate large numbers of specimens becomes an absolute limitation.

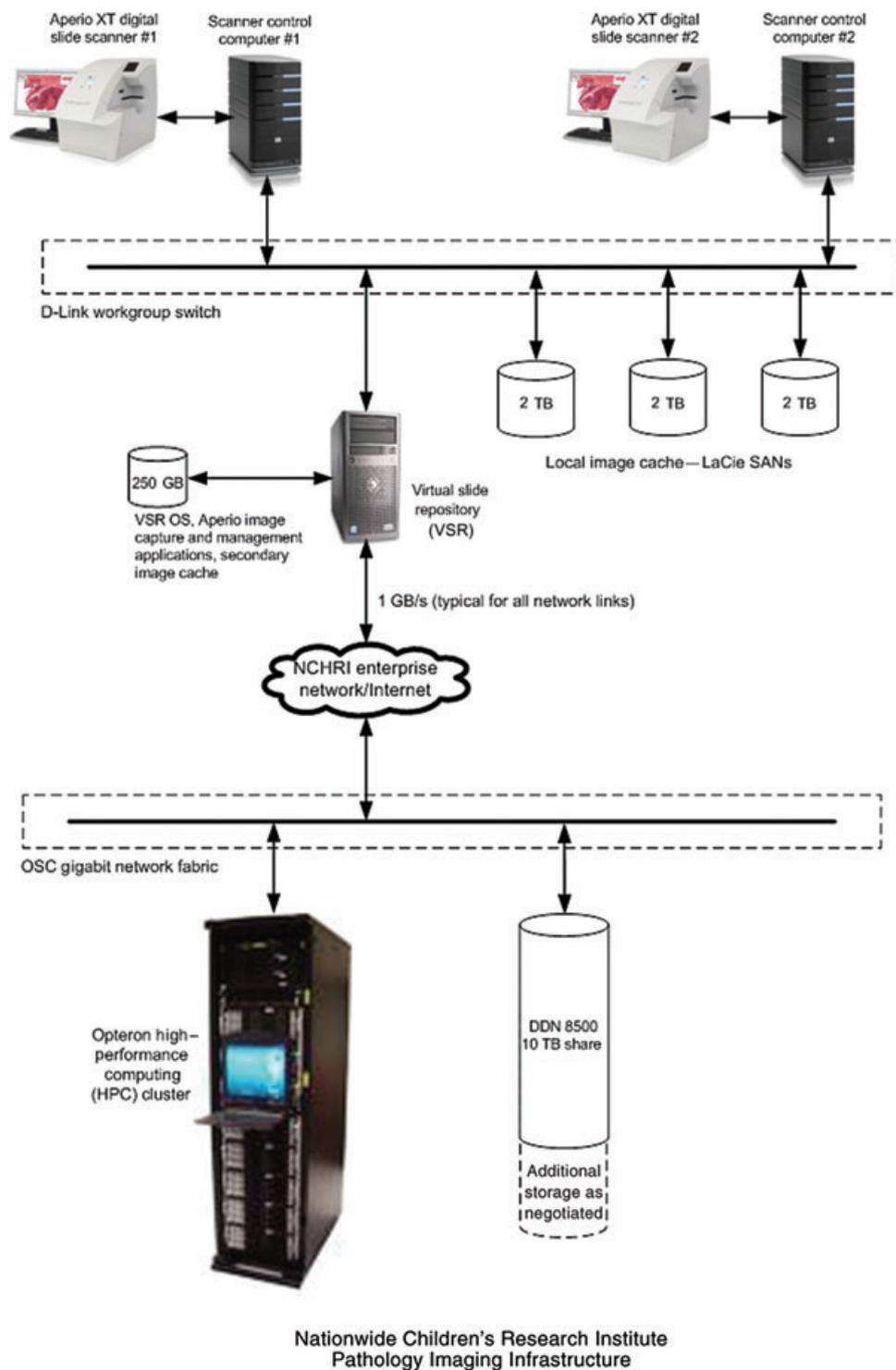


Figure 4. Architecture of system to capture and store whole slide image (WSI) data at the Nationwide Children's Research Institute. (Figure reproduced with permission of Thomas Barr and William Beyer, Biopathology Center at the Research Institute at Nationwide Children's Hospital.) OS = operating system; SANs = Storage Area Network; NCHRI = Nationwide Children's Hospital Research Institute; OSC = Ohio Supercomputer Center.

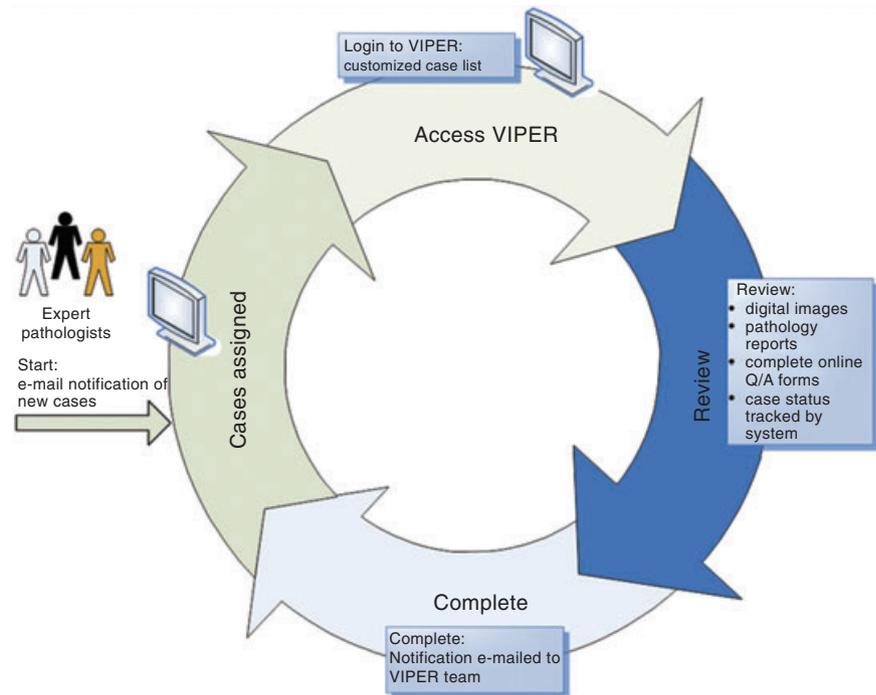


Figure 5. Virtual Imaging for Pathology, Education & Research (VIPER) promotes digital reviews by allowing pathologists and other researchers to view whole slide image (WSI), digital pathology reports and other necessary information. Q/A = quality assurance. [Figure reproduced with permission of Thomas Barr and William Beyer, Biopathology Center (BPC) at the Research Institute at Nationwide Children's Hospital.]

An area of active research and development in pathology informatics is computer-aided detection and related technologies that will likely form a layer of ancillary techniques that it is useful to consider as potential “pathology helpers” (9). Researchers are actively developing techniques to query multiple cellular compartments simultaneously in a single tissue section, and then use multi-spectral imaging to capture large data sets and perform what is in essence, “slide-based histocytometry” (9). The starting point in this process is acquisition of WSI data that can then be subjected to classification algorithms to identify regions of interest, for example areas of tumor. In its simplest form, this process can rely on manual segmentation of WSI by a pathologist selecting regions of interest. More elegantly, this process can be automated around machine classification algorithms that take advantage of the inherent properties of WSI. Recently, systems using the latter approach based on WSI data have been described (9).

Key steps in this process are outlined in Figure 3. Once regions of interest are identified, automated algorithms for cellular and subcellular segmentation can be applied to separate out cells, nuclei, membranes and cytoplasm. This segmentation can then be applied to multispectral data to identify the precise location of simultaneous events in multiple cellular compartments in WSI. The use of such an approach allows for evaluation of complex pathways and events in whole tissue sections and does so in ways that are highly accurate and reproducible. WSI in particular affords the technologic possibility for “machine learning space” for the development of customized algorithms for various tissues and diseases. Proof of concept for this approach has already been reported for prostate tissue and Gleason pattern recognition (6, 9). It is only through the development of tools that marry histopathology with highly quantifiable, automated and reproducible staining that neuropathologists, working on everything from neurodegenerative

disease to brain tumors, will be able to support the growing demand associated with clinical trials and animal model systems.

Neuropathologists have long taken a leadership role in tissue procurement and banking to support both basic and translational research. Significant advances in LIS have improved considerably the annotation and tracking of specimens in tissue repositories. While annotation of clinical information in tissue repositories has become quite rich, along with accompanying molecular and genetic data, pathology information available to users is often considerably less robust, typically comprised of diagnosis, limited pathologic description and scoring information. Tissue analysis restricted to the review of glass slides performed on microscopes has been a significant obstacle to expanding pathology content in biorepositories.

WSI offers several possible advantages for these applications. First, they are remotely accessible, offering the possibility of scanning and then storing glass slides as a deep repository, with first-line access being through WSI stored on a server that can be accessed by repository staff and users. WSI also offers the possibility for multiple users to conduct a simultaneous review from remote locations via digital slide conferencing. Software to support this varies by vendor, but in general, WSI on a single server is accessed by multiple users who can dynamically pass control over the slides among themselves and can see digital markings and annotations added to the WSI. With appropriate institutional information systems (IS) support, this technology can be an effective supplement or replacement for multiheaded scope reviews or off-site consensus conferences. Perhaps, the most exciting for users of tissue repositories is the promise that WSI can be combined with the database that stores and presents covariate data as well as the original pathology, pathologic description and any annotations or content that was added at the time of diagnosis or subsequently.



Figure 6. Virtual Microscope to Microarray (VM2M) allows researchers to search covariate patient data including tissue and cell type, sex, age, disease type and stage and then view both whole slide image (WSI) and expression microarray data within the application. [Figure reproduced with permission of Thomas Barr and William Beyer, Biopathology Center (BPC) at the Research Institute at Nationwide Children’s Hospital.]

With the proper procedures for sharing of information and collaborative interactions, the ability to annotate tissue in repositories offers great promise for neuropathologists both as leaders and users of tissue-banking repositories.

Pioneering advances in the addition of pathology content to tissue repositories in the form of WSI have been made by the Biomedical Imaging Team (BIT) of the Center for Childhood Cancer and the BPC at the Research Institute at Nationwide Children’s Hospital (<http://imaging.nchresearch.org/>). The BPC (<http://www.biopathologycenter.org>) at the Research Institute serves as a biorepository for the Children’s Oncology Group (COG), the Gynecologic Oncology Group (GOG) and the pediatric division of the Cooperative Human Tissue Network (CHTN). Through these interactions, the BPC receives specimens from over 500 institutions for review and research and distributes material worldwide. The BIT has integrated WSI into BPC operations and developed specialized applications that take advantage of WSI data to support users of the BPC.

Once specimens are processed and slides are available, the BIT uses two Aperio ScanScope XT scanners to generate high-quality WSI of the material. Utilizing a partnership with the Ohio Supercomputer Center (OSC), the BIT can provide long-term storage and Internet-based access to these WSI; see Figure 4. To support the pathology review function of the Biospecimen Core, the BIT

has developed a custom application called VIPER; see Figure 5. VIPER allows participants to remotely view WSI of case material submitted to the BPC. Users are able to see a customized case list, perform pathologic reviews by calling up WSI stored at the OSC, review submitted pathology reports and complete internal evaluation and Q/A forms.

While VIPER is purpose built, its elements represent a road map for a future digital pathology workspace environment. As the workflow that VIPER is designed to support makes clear, the individual components of such a program could readily be modified to create a digital clinical workspace environment in which information from the LIS, hospital electronic medical record, medical imaging and advanced molecular testing data such as FISH or single nucleotide polymorphism (SNP) results, could all be presented along with the corresponding WSI of a biopsy. The BIT has demonstrated the feasibility of one major component that would be required for such an approach—the integration of WSI data and expression microarray data in their application, Virtual Microscope to Microarray (VM2M). The main components of VM2M are WSI of a tumor, microarray data for the same tumor, covariate data (patient demographics, etc.) and analytic software, data storage and network access to facilitate their simultaneous display. WSIs created by the BIT are paired with molecular expression data created by Dr. Timothy

Triche at Children's Hospital Los Angeles; see Figure 6. VM2M has been developed as a diagnostic platform and may represent a critical first step toward creating the digital pathology workspace environment of the 21st century.

CONCLUSION

The history and development of digital pathology provide a useful guide to the likely future of these technologies and their impact on the practice of neuropathology in the 21st century. Approximately 3 years elapsed from the first introduction of acquisition techniques for WSI in the late 1990s to the advent of the first generation of scanners or robotic microscopes capable of producing WSI by 2001. In terms of technical capacity, at least two subsequent generations of scanners have developed since then, and a number of vendors have entered the marketplace. In the last 3 years, significant advances in software for storing, annotating and analyzing WSI have been made, creating for the first time the possibility for a true digital pathology workspace. In parallel to these developments, computer-aided image analysis has gained rapid acceptance for specific applications in other areas of anatomic pathology including both cytopathology and surgical pathology. The rapid development of these technologies, as well as the continuously growing demand for specialized neuropathology services in clinical, translational and basic research applications, suggests the need for neuropathologists to embrace and guide the implementation of these technologies. The tools that digital pathology offers will become essential for the practice of neuropathology in the 21st century in both clinical and research arenas. Familiarity with digital pathology, as well as broader trends in the medical environment, leaves little doubt about the development of a robust digital pathology environment in the not too distant future. As we seek to engage these technologies, we can build on the work that has been done in other areas of pathology. Most critically, however, neuropathologists need to begin to exercise a leadership role in the introduction of these technologies so that we are able to shape the development of the emerging digital pathology workspace around our skills and needs. It is only by doing this that we will be able to ensure our ability to continue to exercise the historic leadership role of neuropathology in diagnostics and research in the 21st century.

REFERENCES

- Alkushi A, Clarke BA, Akbari M, Makretsov N, Lim P, Miller D et al (2007) Identification of prognostically relevant and reproducible subsets of endometrial adenocarcinoma based on clustering analysis of immunostaining data. *Mod Pathol* **20**:1156–1165.
- Bennett BD (2006) Certification from the American Board of Pathology: getting it and keeping it. *Hum Pathol* **37**:978–981.
- Chubb C, Inagaki Y, Sheu P, Cummings B, Wasserman A, Head E, Cotman C (2006) BioVision: an application for the automated image analysis of histological sections. *Neurobiol Aging* **27**:1462–1476.
- Ciampa A, Xu B, Ayata G, Baiyee D, Wallace J, Wertheimer M et al (2006) HER-2 status in breast cancer: correlation of gene amplification by FISH with immunohistochemistry expression using advanced cellular imaging system. *Appl Immunohistochem Mol Morphol* **14**:132–137.
- Dawson AE (2004) Can we change the way we screen?: the ThinPrep Imaging System. *Cancer* **102**:340–344.
- Doyle S, Rodriguez C, Madabhushi A, Tomaszewski J, Feldman M (2006) Detecting prostatic adenocarcinoma from digitized histology using a multi-scale hierarchical classification approach. *Conf Proc IEEE Eng Med Biol Soc* **1**:4759–4762.
- Eichorn O (2004) Hello, world. *The Daily Scan*. p. Aperio's daily news and views about Digital Pathology. Available at: <http://blog.aperio.com/2004/03/page/2/> (accessed 1 December 2008).
- Eichorn O (2008) Storing whole slide images in DICOM. *Digital Pathology Blog: A Weblog for the Digital Pathology Community and Laboratory Professionals*. Available at: <http://www.tissuepathology.typepad.com/weblog/2008/10/storing-whole-s.html> (accessed 1 December 2008).
- Feldman MD (2008) Beyond morphology: whole slide imaging, computer-aided detection, and other techniques. *Arch Pathol Lab Med* **132**:758–763.
- Ferreira R, Moon B, Humphries J, Sussman A, Saltz J, Miller R, Demarzo A (1997) The virtual microscope. *Proc AMIA Annu Fall Symp* 449–453.
- Fine JL, Grzybicki DM, Silowash R, Ho J, Gilbertson JR, Anthony L et al (2008) Evaluation of whole slide image immunohistochemistry interpretation in challenging prostate needle biopsies. *Hum Pathol* **39**:564–572.
- Gilbertson JR, Ho J, Anthony L, Jukic DM, Yagi Y, Parwani AV (2006) Primary histologic diagnosis using automated whole slide imaging: a validation study. *BMC Clin Pathol* **6**:4.
- Glotsos D, Kalatzis I, Spyridonos P, Kostopoulos S, Daskalakis A, Athanasiadis E et al (2008) Improving accuracy in astrocytomas grading by integrating a robust least squares mapping driven support vector machine classifier into a two level grade classification scheme. *Comput Methods Programs Biomed* **90**:251–261.
- Glotsos D, Tohka J, Ravazoula P, Cavouras D, Nikiforidis G (2005) Automated diagnosis of brain tumours astrocytomas using probabilistic neural network clustering and support vector machines. *Int J Neural Syst* **15**:1–11.
- Goldberg HR, Dintzis R (2007) The positive impact of team-based virtual microscopy on student learning in physiology and histology. *Adv Physiol Educ* **31**:261–265.
- Ho J, Parwani AV, Jukic DM, Yagi Y, Anthony L, Gilbertson JR (2006) Use of whole slide imaging in surgical pathology quality assurance: design and pilot validation studies. *Hum Pathol* **37**:322–331.
- Kim YJ, Romeike BF, Uszkoreit J, Feiden W (2006) Automated nuclear segmentation in the determination of the Ki-67 labeling index in meningiomas. *Clin Neuropathol* **25**:67–73.
- Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S et al (1998) Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med* **4**:844–847.
- Krippendorf BB, Lough J (2005) Complete and rapid switch from light microscopy to virtual microscopy for teaching medical histology. *Anat Rec B New Anat* **285**:19–25.
- Marinelli RJ, Montgomery K, Liu CL, Shah NH, Prapong W, Nitzberg M et al (2008) The Stanford Tissue Microarray Database. *Nucleic Acids Res* **36**(Database issue):D871–D877.
- Nanda R (2007) Targeting the human epidermal growth factor receptor 2 (HER2) in the treatment of breast cancer: recent advances and future directions. *Rev Recent Clin Trials* **2**:111–116.
- Rojo MG, Garcia GB, Mateos CP, Garcia JG, Vicente MC (2006) Critical comparison of 31 commercially available digital slide systems in pathology. *Int J Surg Pathol* **14**:285–305.
- Scoville SA, Buskirk TD (2007) Traditional and virtual microscopy compared experimentally in a classroom setting. *Clin Anat* **20**:565–570.

24. Soenksen D (2008) Digging their way in: digital pathology systems. In: *CAP Today*. SL Rice (ed). College of American Pathologists: Northfield, IL.
25. Stewart J 3rd, Miyazaki K, Bevans-Wilkins K, Ye C, Kurtycz DF, Selvaggi SM (2007) Virtual microscopy for cytology proficiency testing: are we there yet? *Cancer* **111**:203–209.
26. Tawfik OW, Kimler BF, Davis M, Donahue JK, Persons DL, Fan F et al (2006) Comparison of immunohistochemistry by automated cellular imaging system (ACIS) versus fluorescence in-situ hybridization in the evaluation of HER-2/neu expression in primary breast carcinoma. *Histopathology* **48**:258–267.
27. Turbin DA, Leung S, Cheang MC, Kennecke HA, Montgomery KD, McKinney S et al (2008) Automated quantitative analysis of estrogen receptor expression in breast carcinoma does not differ from expert pathologist scoring: a tissue microarray study of 3,484 cases. *Breast Cancer Res Treat* **110**:417–426.
28. Wan WH, Fortuna MB, Furmanski P (1987) A rapid and efficient method for testing immunohistochemical reactivity of monoclonal antibodies against multiple tissue samples simultaneously. *J Immunol Methods* **103**:121–129.