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Review Article

Going fully digital: Perspective of a Dutch academic pathology lab

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Abstract

During the last years, whole slide imaging has become more affordable and widely accepted in pathology labs. Digital slides are increasingly being used for digital archiving of routinely produced clinical slides, remote consultation and tumor boards, and quantitative image analysis for research purposes and in education. However, the implementation of a fully digital Pathology Department requires an in depth look into the suitability of digital slides for routine clinical use (the image quality of the produced digital slides and the factors that affect it) and the required infrastructure to support such use (the storage requirements and integration with lab management and hospital information systems). Optimization of digital pathology workflow requires communication between several systems, which can be facilitated by the use of open standards for digital slide storage and scanner management. Consideration of these aspects along with appropriate validation of the use of digital slides for routine pathology can pave the way for pathology departments to go "fully digital." In this paper, we summarize our experiences so far in the process of implementing a fully digital workflow at our Pathology Department and the steps that are needed to complete this process.

Key words: Digital pathology, digital pathology workflow, digital scanning, telepathology, whole slide images

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INTRODUCTION

The current standard practice of examining histopathology slides is still under a conventional light microscope. Already in 1986 "telepathology" was made possible after the introduction of video cameras mounted on microscopes, making it possible for live images to be shared with people at different locations.[1,2] This allowed live teleconsultation and remote diagnosis of frozen sections,[3] although at relatively low resolution. In the last two decades, affordable digital cameras became available, allowing efficient capturing of still digital images at high resolution. In the last decade, digital slide scanners were introduced and slowly made their way into pathology labs as a "digital age" alternative to the conventional microscope. Digital slide scanners are now-a-days mostly table-top devices that take glass slides as input and produce whole-slide images as output, in a cost and time efficient manner, often automating all intermediate steps such as localization of the tissue and focus plane selection. The goal of whole-slide imaging (WSI),[1,2] coupled with whole-slide image viewers, is to simulate slide viewing by a conventional microscope on a computer screen. The last step toward a complete digital workflow, where as many as possible of the steps from placing an order at a pathology lab to the report of the pathologist are digital,

is the integration of WSI in the regular workflow, indeed replacing the conventional diagnosis procedure. This digital workflow is often referred to as Pathology 2.0.

During the last few years, WSI has become more affordable and widely accepted in pathology labs. There are numerous advantages to WSI, most of which stem from the fact that compared to glass slides, digital slides are very portable entities; thus, easily retrieved from a digital archive. They allow simultaneous viewing by multiple people at the same time and are accessible through a computer network from remote locations. Other advantage of WSI is that it can directly facilitate the use of tissue morphometry and other automated image analysis algorithms.[3] Digital slide viewers can offer an enriched user experience, for example, by showing an overview image along with the high-power view enabling better orientation or by showing two or more slides side by side.

For all the advantages they offer, digital slide representations have certain limitations when compared to conventional light microscopy. One disadvantage is that slide scanning adds an additional time delay in the tissue preparation process, unless it is carried out after the examination by a pathologist solely for archiving purposes. This is being addressed by scanner manufacturers, with newer models achieving short scan times at high magnification suitable for integration into the tissue preparation process. Addition of the scanning equipment as the last stage of the automatic slide staining process can significantly reduce the slide processing time and further enable a fully digital workflow. The creation of a fully digital pathology laboratory requires specialized IT infrastructure for storing and accessing the digital slides on top of the other IT facilities that are needed to optimize the workflow. Enterprise solutions and tiered management systems are needed in order to store this large amount of image data. Integration with the laboratory information and management system (LIMS) is needed in order to provide a better user experience. In relation to image quality, there is always a trade-off between file size and storage costs. Because of the large image sizes, lossy compression is often used, which can add compression artifacts to the images. Another disadvantage is that the time it takes for a pathologist to make a diagnosis is longer than using a traditional glass slide, which can initially be up to 60%.[4] A probably more important limitation is that most of the whole slide scanners routinely acquire the tissue only at a single focal plane; thus, providing a 2D image of a structure that is essentially 3D. This can be problematic as the slides are essentially a topological relief, and potentially important information can be lost in the imaging process. Example usage of digital slides in daily pathology practice includes remote consultation, quality assurance, education and research.[5-11] Currently, it is still under investigation whether digital slides are suitable for routine diagnosis and prognosis.

In this paper, we summarize our experiences so far in the process of implementing a fully digital workflow at our pathology department and the steps that are needed to complete this process. In the following sections, we first go through the different considerations regarding the choice of slide scanning equipment and scanner operation mode, with particular focus on image quality. We then move on to examine the required modifications of the pathology laboratory workflow in order to integrate digital imaging in a seamless manner. At the end, we give a short overview of the validation studies that have been performed at our department and give conclusions.

DIGITAL IMAGING IN PATHOLOGY

Digital slide scanners appeared as successors of early telepathology systems that facilitated transferring static and dynamic images via computer networks for remote consultation and second opinion.[1] Currently, there is a multitude of digital slide scanner manufacturers and models offered on the market.[12] The choice of slide scanning equipment is a crucial step in the design of a fully digital pathology lab. Several important aspects must be considered when analyzing the available options. This includes the quality of the produced digital slides (image quality), available magnifications, number of focus planes, scanning speed, level of automation, support of fluorescence imaging, support for z-stack scanning, ease of integration into the workflow and use of open standards. In addition, all these aspects and desired features have to be balanced against the cost of implementing digital slide scanning, which can be prohibitively high. Since 2007, the Pathology Department at the University Medical Center Utrecht scans all produced slides for tumor boards, archiving, and educational purposes. Our experiences with implementing a fully digital pathology archive are summarized in Huisman *et al*.[13]

Image Quality

The quality of the produced whole-slide images depends on a number of factors, such as tissue preparation, the optics of the digital slide scanner, tissue region selection algorithm, and autofocusing mechanism. Independent of the choice of scanning equipment, the quality of the tissue preparation process, such as the slice thickness, the placement of the tissue on the glass slides and the staining, can have a major impact on the resulting image quality. Thinner sections generally produce better quality images due to more successful autofocusing. Placing the tissue centrally on the glass slides can help avoid problems with incomplete scanning at the margins of the slide. Overstained slides result in images in which object and features are difficult to distinguish. Small changes of the tissue preparation process are likely to be needed in order to optimize the quality of the produced digital slides.[14]

In our experience, the most common source of image quality problems in digital slides is failed autofocusing resulting from either imprecise tissue detection or erroneous focus depth. This applies especially to cytological slides. Failed autofocusing is particularly manifested in thick slides or slides with faint immunohistochemical stainings. The most widely used autofocusing mechanism, is to determine the optimal focus for a number of automatically selected focus points and then extrapolate by triangulation to the entire slide area. This is carried out mainly because selecting the optimal focus for each capture unit (image tile or line, depending on the scanning mode) is prohibitively time consuming. Novel autofocusing techniques try to determine optimal focus in parallel with the image acquisition; thus, saving valuable time and enabling larger number of focus points to be used.[15] Another solution to this problem is to acquire images at multiple focal planes, so called z-stack acquisition. This, however, comes at the cost of lower scanning speed and increased storage requirements, the latter linear to the number of focus layers. The solution to this would be to couple the scanning process to a LIMS and automatically select if z-stack acquisition for that particular slide is relevant. Most current scanners do not support this kind of functionality. Ideally, only slides where fine tuning of the focus might be beneficial, for example when mitosis counting needs to be performed or for cytological slides, can be scanned at multiple focus planes and novel 3D compression techniques can be used to reduce the file size of the z-stacks.[16] In an ideal situation, the scanner would scan the barcode on the label and use this information to query the LIMS system and decide on meta-data in the LIMS system if z-stacking should be applied for this particular slide. An intermediate solution offered by some scanner manufacturers is extended focus, which scans the slide in different focus layers and recombines the information into a 2D projection with an optimal layer focus. The file size is significantly smaller than z-stack images but the scanning time remains the same.

Scanning Magnification and Image Compression

The digital slides at our pathology department are routinely scanned at ×20 magnification, with sporadic use of ×40 magnification for research purposes. Our experiences show that ×20 magnification is sufficient for most diagnostic work,[17-19] although ×40 magnification is expected to be the standard in the near future. Since, storing digital slides in an uncompressed format or using lossless compression can result in very large files (in the order of several GB), a lossy compression technique is usually needed. For this, either the JPEG or JPEG 2000 image standards are most commonly used. Compared to JPEG, JPEG 2000 can produce better compression ratios while achieving the same or better quality, but at the cost of increased compression time, which reflects negatively on the throughput of the digital slide scanner (the scanners used at our department cannot continue with the next slide until the previous scanning and storage steps are completely finished). We find that using JPEG compression with compression quality factor 70 produces images with acceptable file size and unnoticeable compression artifacts for diagnostic purposes; although, this might compromise the future use of these slides for automated image analysis.

Image Analysis

The increased use of slide scanning in pathology labs has sparked an interest in development and use of automatic image analysis algorithms. The intended goal of these algorithms is to help pathologists with tasks that are notorious for their observer variability and/or are tedious and time consuming. Some example applications include quantification of immunohistochemical stainings, nuclear morphometry, mitotic figures counting, and detection of metastases. Some algorithms for quantification of immunohistochemical stainings already have approval by the USA Food and Drug Administration (FDA).[25] Our current research in this field focuses on development of methods for analysis of hematoxylin and eosin stained slides, since this is the standard stain in every pathology laboratory.[26,27]

STORAGE

Even when using lossy compression, the amount of storage needed for a fully digital pathology laboratory remains a significant obstacle. The current scanning protocol we employ results in file sizes of 350 MB on average. By scanning every produced slide, which for a medium sized pathology laboratory can be up to 500/day, the resulting daily storage needed is approximately 175 GB. Over the period of a month, that number can go up to 5 TB.

The current storage system consists of storage tiers with the top tier being a fast disk based solution and the lower tier a tape archive where all the digital slides are copied after a certain period of time. This solution is scalable and more economical than a fully disk based solution, but possesses several drawbacks, notably the access time of slides that have been copied to tape (approximately 2 min). Our department is currently migrating to a hospital wide object based storage solution, which is scalable to several petabytes, and is more than enough to accommodate for the needs of our own image archive. This solution [Figure 1] is a tiered system, which makes a distinction between storage in short-term, long-term and permanent. Every slide that is scanned initially is stored in the short term storage, which facilitates fast disk based solutions for less latency in writing and retrieving. After the slides have gone through the primary diagnostic round, if second opinions or external

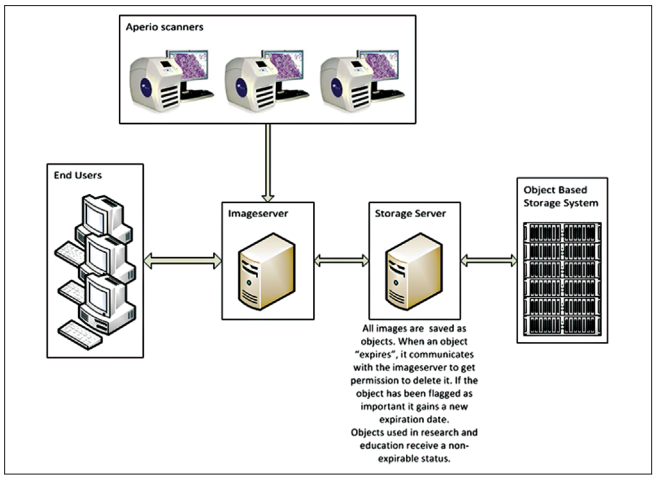

Figure 1: Architecture of digital image scanning

or inter-departmental consultations are needed, they are entered in the long-term storage. Slides that stay in short term storage "expire" after a period of time and are deleted. Slides, which end up in long-term storage expire after a longer fixed period of time and after the diagnostic procedure is concluded. Educational and research slides end up in the permanent storage where they are kept without an expiring time-frame. Object based storage offers complete redundancy of stored objects without the need to take incremental backups.

INTEGRATION WITH LAB MANAGEMENT AND HOSPITAL INFORMATION SYSTEMS

Most of the scanners on the market include 1D or 2D barcode scanners, which help the integration with LIMS systems. In this way, the image management system can store relevant meta-data (e.g., the staining name) together with the image and scan meta-data. Our department has already implemented a connection between the reporting system used internally "Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief" (PALGA) and the image management system to display digital slides linked to every case number. It retrieves metadata from the laboratory management system (such as staining and block identification) and displays it along with macroscopic gross images.

In terms of image management, a vendor neutral archive Picture Archiving and Communication System (PACS) could be used for pathology images along with radiology, forming an institution-wide common imaging infrastructure. This solution although can be physically separate from a Radiology PACS solution would be integrated on an application level in a common system to easily facilitate inter departmental collaboration. PACS solutions are mature in terms of image management having a proper way to handle study/patient ID, anonymization, retrieval, and image queries and also protocols for accessing images remotely using the web access to Digital Imaging and Communications Committee (DICOM) Objects (WADO).[20] To optimize the digital pathology workflow, communication between several systems is needed: Reporting software, image management, speech recognition, LIMS, digital slide scanner, etc., The use of open standards for both digital slide storage and scanner management can accelerate the acceptance of digital pathology. The DICOM has recently extended the DICOM standard for storing and exchanging medical images to support digital slides.[20,21] In addition, efforts like the development of Openslide, a library that provides interface to already establish file formats can help achieve further vendor independence.[22] Other standards are Health Level 7 (HL7) and terminology standards for encoding the findings like Systematized Nomenclature of Medicine – Clinical Terms or International Classification of Diseases (ICD-10). The international organization Integrating the Health-care Enterprise (IHE) enables the discussion between health-care providers and vendors to describe use cases and find existing standards, like the ones mentioned before, to solve their issues. Those solutions are described in so called integration profiles. For pathology, there is an international workgroup in IHE as well as general purpose integration profiles or integration profiles developed for other domains, like radiology, which might be applicable to pathology. One of the interesting examples to mention is Cross-Enterprise Document Sharing for Imaging (XDS-i), which describes how images can be exchanged in a vendor neutral way between different systems (for example for consultation).

SLIDE VIEWERS

One of the largest benefits of implementing digital pathology is that digital slide viewing can offer an enriched user experience. Digital slides viewers can show an overview image along with the high-power view enabling better orientation. Showing two or more slides side by side is a feature of many slide viewers, which can be useful for examining the same tissue stained with different staining's. Most slide viewers are provided as stand-alone applications by the scanner manufacturer, but cloud storage and viewing solutions are becoming more common. Within slide viewers, measurements and annotations can be made, which can be added to the pathology report or saved for a future reference. It has been found that the pathologists of our department find that the standard desktop monitors offer sufficient quality for case reviewing although there are several medical grade diagnostic monitors available throughout the department. Several slide scanner vendors offer different input devices for working with digital slide viewers such as emulating the microscope stage, using the multitouch pads

or screens (http://www.webmicroscope.net/)[23] or even using the game controllers. Such systems increase the adoption of digital slide viewing applications and the ease of use. In the case of the system that used game controllers, pathologists report being able to use the system comfortably after only 15 min of training.[4] Another interesting digital slide viewer implementation uses a combination of high resolution monitors and virtual reality technology to construct a digital pathology workstation, that can perform as well as the conventional light microscope diagnosis.[24] The workstation combines three 27 inch monitors with a combined resolution of 11 megapixels to create an equivalent field of view of 0.07 mm^2 at ×40 magnification, which is larger than the field of view of a typical microscope at 0.03 mm^2 on the same magnification. The difference in time of diagnosis between the virtual reality microscope and a conventional microscope was shown to be not significant.

DIGITAL PATHOLOGY WORKFLOW

Presently, the typical workflow begins with the procedure performed on the patient, most commonly a biopsy or a resection. The material is then sent to a pathology department accompanied by an order (ideally in a digital way), along with the relevant clinical information. This information usually comes out of the local electronic health records, together with localization and clinical data of the material. When the material is received in the Pathology Department, it is registered in the local laboratory information system before undergoing the necessary procedure in order to be processed to glass slides. Then, the glass slides are examined under a light microscope in order to produce the pathology report.

Slides that have been used in the primary diagnostic round are then sent to be scanned. The produced digital slides are stored on the storage system and registered in the image management system where they are connected to the pathology report. The system that is currently in place has the barcode scanning function integrated; by scanning the barcode of a case number or a glass slide, the pathology report can be retrieved along with all the digital slides and gross images produced. Via the same interface, images can be added to a meeting worklist, which can be used for intra or inter-department meetings within the hospital. For external consultations and panel meetings digital slides are uploaded to pathoconsult.nl, which is an online image viewer platform maintained by our department. Slides uploaded to this online platform can be shared with other persons or departments for online meetings, eliminating the need for a physical meeting using glass slides.

After the report is carried out, it is sent both to the local Electronic Health Record system (Chipsoft Ezis) and to the PALGA - a national registry of pathology reports for both research and clinical purposes (stored completely separated). In The Netherlands, all the reports are sent daily to PALGA. Tumor registries have a connection with PALGA to retrieve a note on each tumor case. Further clinical information is collected by personnel of the tumor boards who retrieve this per hospital. The electronic health record system has an Inbox function for clinicians where new reports are brought to their attention. Satisfaction of the "customers" of the pathology department is periodically reviewed. Pathologists participate in all kinds of tumor boards, which is seen as a review procedure, and the concordance of the revisited material is reported in the reporting system.

The archived material (paraffin blocks, freezed sections, cytology slides) are managed by the local management system, which keeps track of their status, position and quantity along with relevant clinical data.

The current workflow incorporates digital pathology as an added service, which complements the pathologists' normal workflow by providing additional tools and the ease of a fully digital archive. Time spent retrieving glass slides from the archive or panel meetings around multiheaded microscopes are greatly reduced.

The implementation of such systems assists the department to achieve faster turnaround times, make inter-departmental meetings more accessible and increase the reliability and reproducibility of reports. In the future, our department aims to switch the workflow to a fully digital one, meaning that the computer monitor instead of the conventional microscope will be the primary diagnostic device. All findings will be annotated within the digital slides, which can be later attached to the final report. The annotations will serve as valuable clinical information, which can then be used for research and/or educational purposes.

Switching the current workflow to a fully digital one would require glass slides to be scanned prior to sending them to the pathologists, which can add cumulatively to the overall diagnosis time. This can be addressed by using faster scanners and integrating the scanning with the cover slipping and staining process. On the other hand, the current physical distribution of glass slides can be eliminated. However, that would require a change in physical slide management along with handling procedures. This includes slides being properly dried before being placed in the digital scanner, compatible slide racks between lab and scanner and dedicated personnel responsible for the archiving and scanning. After a case is available digitally, a workflow manager should guide the diagnostic process presenting the pathologists with personalized worklists and cases that need to be reviewed, sorted by priority. The manager should have an integrated image viewer giving the ability to view a case jointly with other specialists or just sending a link via E-mail to remote colleague to seek a second opinion. Users of these systems should not worry about manually copying data and sending them to colleagues, but instead a seamless solution must be provided to

handle the transactions between interested parties.

Currently, the task of adding extra scanners from different manufacturers involves the integration of separate image formats (usually proprietary) and the addition of separate image viewers with separate image management systems. Different WSI formats handle image metadata in a different way requiring custom integration solutions to register images in an existing image management system using the correct metadata. Ultimately, this can be solved by adding middleware to handle communication from different digital scanners to the storage and management solution.

VALIDATION OF DIGITAL SLIDES FOR UPFRONT DIGITAL DIAGNOSTICS

As we have mentioned in the previous sections, the scanning of slides at our pathology department is currently done for tumor boards, reviewing, and archiving, education, and research purposes only. The digital slides are viewed mainly after the pathologist has already examined the glass slide. There are two main reasons for this, the first one being that the technical challenges for seamless integration into the laboratory workflow are still being addressed. The second and arguably more important reason is that in order to go "fully digital," appropriate validation of the use of digital slides for routine pathology diagnostics and prognostics needs to be performed.

Thus far, we have performed several internal validation studies at our department and in collaboration with other institutions in The Netherlands. In four separate validation studies[14,17-19] for primary digital diagnostics in gastrointestinal tract pathology, dermatopathology, pediatric and breast pathology, overall more than 90% of the diagnoses were found to be concordant with classical light microscopy. The majority of the remaining cases were found to be only slightly discordant with no clinical implications, and in some cases the diagnosis based on digital slides was even preferred over the original diagnosis. Discordant cases with possible clinical implications were found only in the pediatric pathology diagnoses (2% of the total number of patients). In a pilot implementation of a fully digital diagnostic workflow at the Atrium Medical Center in Heerlen, The Netherlands, it was found that primary diagnostics of breast biopsies can be performed using the digital slides in more than 80% of the cases.[14] Image quality and logistic problems were found to be the reason for the failure to produce diagnosis for the remaining of the cases. Specifically, 5% of the cases had problems with scanning artifacts, such as blurry images and incomplete slides. In 1.5% of the cases logistic problems were encountered-either the scans could not be located or there were network problems. The technical problems encountered in 1.2% of the cases had to do with bad staining, bad positioning and tissue folding. These pitfalls are expected to be addressed in the near feature by use of more advanced scanning equipment and better and more integrated IT solutions in order to avoid logistics problems. We have also performed a small pilot study to examine if digital slides are suitable for the task of mitosis counting for prognosis of invasive breast carcinoma patients. We observed quite a reasonable reproducibility with a slight underestimation of the mitotic count for the patients with high proliferation of the tumor, but the data sample was too small to draw any definite conclusions [Figure 2]. This small pilot experiment will be followed by a larger study to further investigate if digital slides are suitable for these tasks.

Some vendors have started large multi-center validation studies for submission to the FDA to show that their solutions are ready for primary diagnostic purposes. This will be very important before full adoption to digital pathology will become mainstream, particularly for the US market. In Europe we see that many labs are performing internal validation studies. These studies are limited because of small sample sizes and usually not covering all of specialties. Although there are many variations between laboratories with respect to the staining, slide thickness, etc., it might be a good idea to collaborate between labs and perform multi-center studies for a certain setup. In addition, more formal validation is needed so that the users become is familiar with working on a computer monitor instead of a microscope. This is crucial because the novelty of the working setup might hinder their diagnostic performance and increase the time needed to come to a diagnosis.

CONCLUSION

Pathology laboratories are increasingly switching parts of

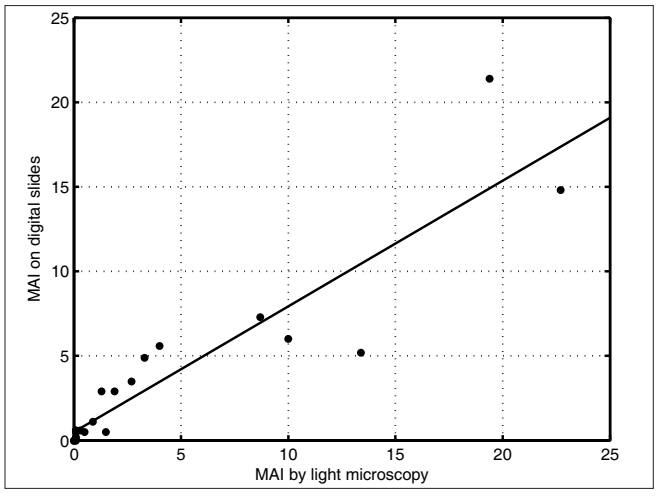

Figure 2: Scatter plot of the mitotic activity index as estimated by light microscopy and on digital slides (R = 0.92)

the workflow (from the placement of the order and the diagnosis of the cases, to the distribution of the reports and the revision) to the digital domain, with the end goal of going "fully digital." The demand for standardized infrastructure to facilitate and expedite communication within a pathology department as well as with external parties is higher than ever. Investment in standardized storage solutions with a future outlook in communicating to other systems is necessary as well as provision for future growth. The validation of digital slides coupled with digital slides viewers as a primary diagnostic tool is still being investigated, but looks very promising.

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