WHOLE SLIDE IMAGES

FOR PRIMARY DIAGNOSTICS IN PATHOLOGY

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The research presented in this thesis was performed at the Department of Pathology, University Medical Center Utrecht (UMC Utrecht) and Symbiant Pathology Expert Center, The Netherlands.

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(met een samenvatting in het Nederlands)

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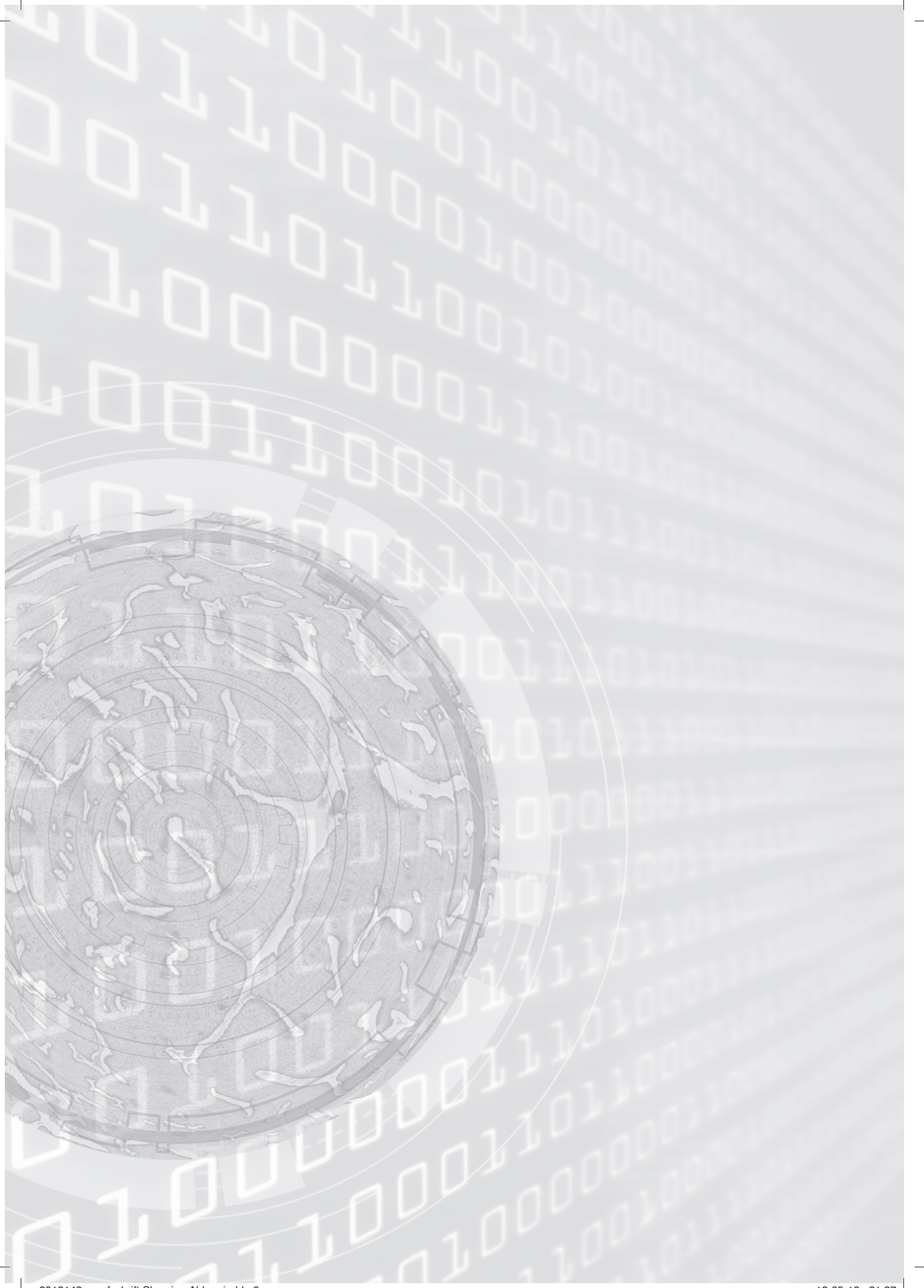


Chapter 1

General introduction

Introduction

For more than a century, examining the cellular morphology using glass slides and a conventional microscope is considered to be the basis of pathology practice and deemed the gold standard tailoring most of the therapeutic decisions within the clinical practice. However, development in imaging technology has revolutionized the way pathologists work by the introduction of slide scanners that enable the acquisition of pathology information from glass slides and translate this into a digital form commonly known as digital slides or Whole Slide Images (WSI). The discipline of pathology that studies the use of WSI for different purposes is called Digital Pathology. Interactive viewer software enables the pathologists to explore WSI on a computer screen in a manner basically mimicking conventional light microscopy, permitting an unlimited access to the entire specimen and seamless switching between different magnifications. Additional features are also provided by image viewers including the possibility to explore multiple digital slides simultaneously, the ability to share WSI among several users from different locations at the same moment and providing an overview image next to the high power view which aids in better orientation within the given slide. However, several disadvantages have been also seen when using WSI, concerned mainly with the time needed to upload and explore WSI in addition to the reduced image quality in some instances.

The ease of image accessibility and sharing has made WSI a feasible option in several settings within pathology, particularly for tele-consultation, revision, education, archiving, pathology panels and research. Moreover, the amenability of WSI to automated image analysis will definitely help in improving the objectivity and productivity of pathology practice. Despite of the several advantages of WSI, which have made them in some aspects superior to glass slides and a conventional microscope, adopting this novel technology in routine pathology practice is still in its initial stages. Taking the full advantage of WSI and digital pathology in routine diagnostics would necessitate setting up extensive validation studies evaluating the diagnostic performance of WSI for this purpose. The aim of this thesis was, therefore, to investigate the validity of WSI as a platform for primary diagnostics in pathology.

Outline of the thesis

In **Chapter two**, a detailed review of literature is presented, focusing mainly on the innovations, advantages and impact of this novel technology in the field of pathology.

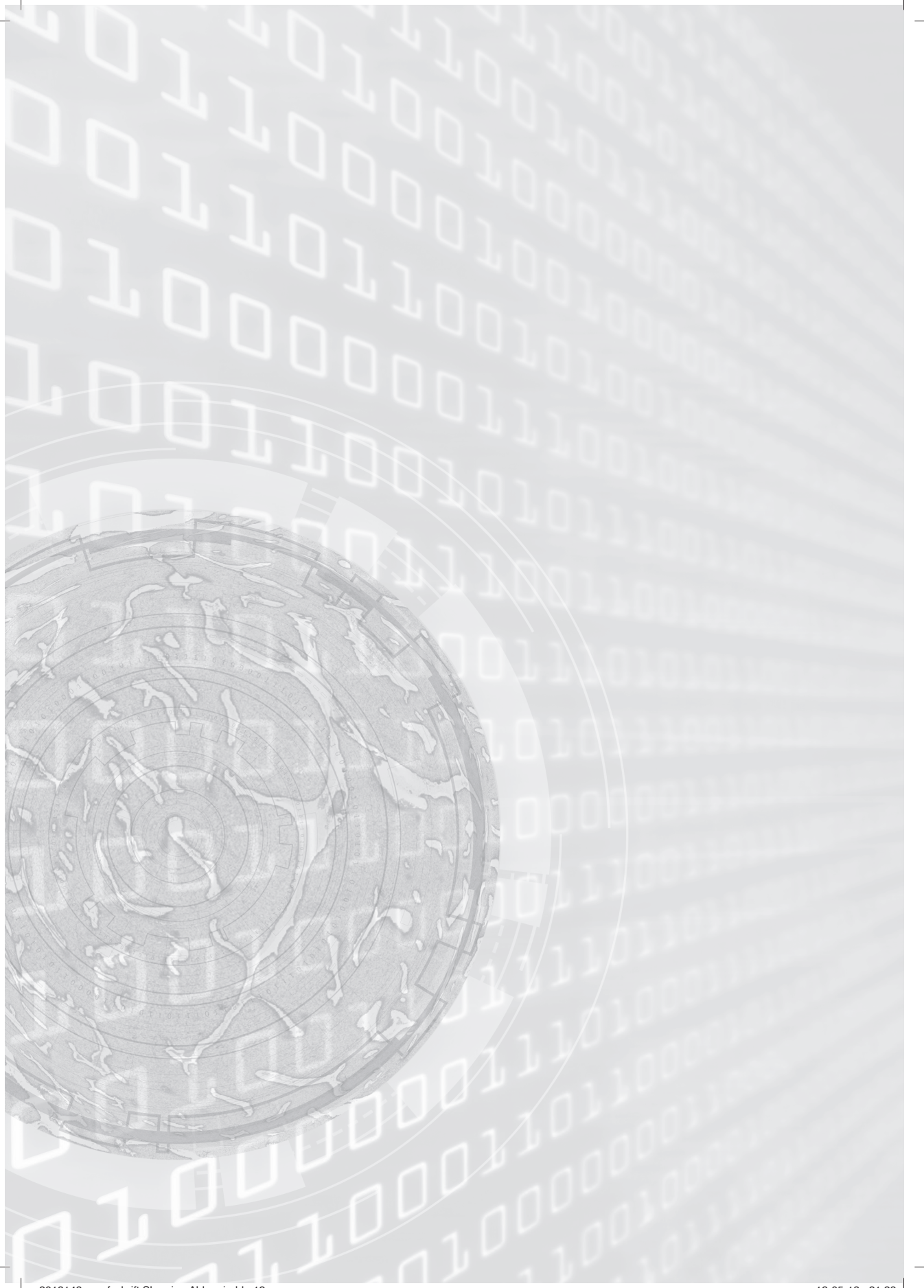
In the following **Chapters (3-7)**, the diagnostic performance using WSI was tested in several body systems including the gastrointestinal tract, dermatopathology, breast, pediatrics and finally the urinary system specimens. Five hundred cases (100 per system) were examined by a group of pathologist using a conventional microscope and WSI. The concordance and the discrepancies between pathology reports derived from the two diagnostics modalities were identified. Additionally, the discrepancies were extensively evaluated and the possible causes of discrepancies were further discussed.

The efficiency of WSI in evaluating specific cellular and nuclear details was further tested by conducting two other studies explained in chapters 8 and 9. The possibility of evaluating the Mitotic Activity Index (MAI) in breast cancer based on WSI was described in **Chapter 8** by evaluating MAI in 100 breast cancer specimens by three observers on two occasions; microscopically and digitally. The difference between mitotic scores obtained using different modalities was evaluated. In addition, inter- and intra-observers agreement between microscopic and digital mitotic counts and scores were assessed.

In **Chapter 9**, we explore the validity of WSI, scanned at 40x magnifications and on one focal plane, in assessing HER2 status for breast cancer specimens treated with the chromogenic *in situ* hybridization (CISH).

Chapter 10 discuses the experiences of implementing WSI in a routine pathology practice in a pathology laboratory in the Netherlands that has some years of experience in upfront digital diagnostics using WSI.

Finally, in **Chapter 11** we end this thesis with conclusions that can be drawn from these various chapters and a general discussion on the field of Digital Pathology.



Chapter 2

Digital pathology: Current status and future perspectives

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Abstract

During the last decade pathology has benefited from the rapid progress of image digitizing technology. The improvement in this technology had led to the creation of slide scanners which are able to produce whole slide images (WSI) which can be explored by image viewers in a way comparable to the conventional microscope. The file size of the WSI ranges from a few megabytes to several gigabytes, leading to challenges in the area of image storage and management when they would be routinely used in daily clinical practice.

Digital slides are used in pathology for education, diagnostic purposes (clinicopathological meetings, consultations, revisions, slide panels and increasingly for upfront clinical diagnostics), and archiving. As an alternative to conventional slides, WSI are generally well accepted, especially in education where they are available to a large number of students with full possibilities of annotations without the problem of variation between serial sections. Image processing techniques can also be applied to WSI, providing pathologists with tools assisting in the diagnosis making process. This article will highlight the current status of digital pathology applications and its impact on the field of pathology.

Introduction

Interpreting images of tissues and cells at a resolution higher than the naked human eye is the core of pathology. For a long time the microscope has been the only available instrumentation to this end, over centuries providing live images at increasing resolution through ever improving optics[1].

During the last decades, optical pathology has gradually changed[2] by the introduction of digital cameras producing still images, and microscope mounted video cameras that allow live examination of slides (dynamic images). These still or dynamic images can be transferred by the means of network connections to remote sites to be assessed by another pathologist, commonly called telepathology[3, 4]. This has found applications like teleconsultation and frozen section diagnosis[5].

Approximately a decade ago, further improvements of these techniques have resulted in the creation of digital slide scanners[6]. These slide scanners produce Whole Slide Images (WSI, also called digital or virtual slides) that combine the advantages of images from live cameras (whole slide access) and digital cameras (high resolution)[1].

WSI are explored using an image viewer, which enables the examination of digital slides in a manner comparable to the use of a conventional microscope in three aspects: First, WSI can be explored at different magnifications, with the additional advantage of in-between magnifications, if provided by the viewer software. Second, navigation of the slides in each direction is possible. Third, some scanners allow scanning more than one focus plane, thereby even allowing focusing up and down[7-11].

Furthermore, WSI have several virtues over conventional slides:

- Image viewers are able to show an overview image together with the high(er) power view, resulting in better orientation within the slide when viewing at high(er) magnification and more easy navigation to other regions of interest.
- Image viewers can display several slides side by side, so the examiner can compare structural details between slides or easily compare different stains of the same tissue area.
- WSI can be made available instantaneously to multiple examiners at the same time from all over the world through the internet without the need for a microscope.
- Focusing is carried out during scanning, necessitating less user interaction.
- The quality of WSI is constant over time.
- WSI can be used directly for automated image analysis and morphometry.
- WSI can be integrated within the electronic patient records, together with other images.

Figure 1 shows a screenshot of a WSI as it is seen with an image viewer.

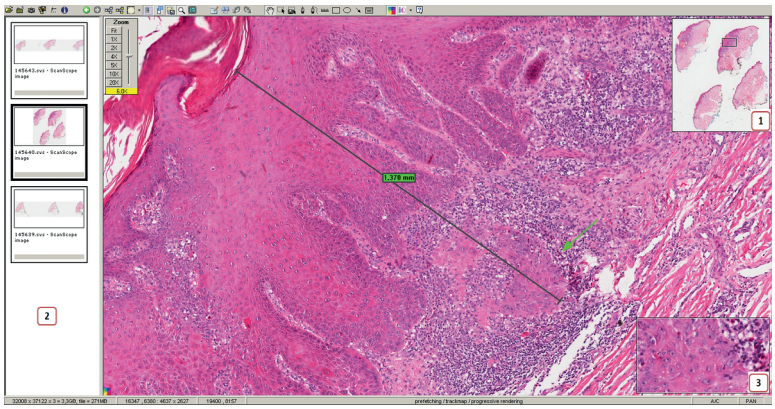

Figure 1. Screenshot from a whole slide image as seen in Aperio's ImageScope viewer application. The presence of a navigation (overview) in the upper right side (1) of the screen provides orientation within the shown slide. The other slides of the same case are presented in the panel on left side (2) of the screen which can be directly explored. Annotations can be placed on the slide (for example the arrow in the image presented above) and measurements can be easily performed (e.g. the line length shown in the image above, but also the area and lengths of boxes and circles which can be drawn on the slide can be measured). The current location of the cursor on the image is magnified further in the magnification window (3).

Slide scanners

There are major differences between the different manufacturers and types of slide scanners. One major difference is the capacity; some can be loaded with only one slide, others with several hundred slides per scanner load. They also use different acquisition techniques, the two major ones being line scanning which is done by continuous precise movement of a stage[1, 12] or by using a regular CCD camera that acquires square image tiles one by one[1, 13]. At the end of the scan, these lines or tiles are stitched together generating the final output image representing the slide[12, 14, 15].

Scanners are either supplied with one objective (further magnification is conducted by adding a 2x additional lens) or supplied with more objectives, having different magnifications and numerical apertures. Scanners with multiple objectives are supplied mainly with objectives of maximum magnification of 40x, although the DMetrix DX-40 is supplied with a 80x objective[16].

Table 1 shows a summary of some more scanner features and their different implementations between slide scanners. Some scanners are able to scan at multiple focus layers. By stacking those images together they provide a three-dimensional (3D) image stack. Although the scan time increases linearly with the number of layers, this can be beneficial for cytological specimens, frozen sections

and other thick specimens where the pathologist needs to inspect the cellular architecture at different planes. Further, mitoses recognition is easier when multiple focus layers are available.

Scanners equipped with special fluorescence illumination optics, light sources and more sensitive image acquisition sensors are provided by different vendors. These scanners are able to scan fluorescently labeled cell and tissue samples and convert it to high resolution color digital slides. Fluorescent digital imaging provides the opportunity to permanently store fluorescently stained slides, eliminating the problem of stains fading over time. These fluorescent WSI can also be utilized for automated image analysis, such as for fluorescence *in situ* hybridization (FISH).

There are several factors that determine the quality and usefulness of the final WSI as experienced by the end user [7, 13, 17]:

- The quality of the tissue itself (e.g. preservation state) and the technical quality of the original slide (e.g. leaked glue, scratches, tears, irregular mounting, the quality of staining, and the amount of text scribbling).
- The image acquisition technique of the slide scanner that is defined by the method of focusing, color management, white balancing and contrast.
- Post-processing of the scanned slides: the accuracy of stitching and degree of compression.
- Completeness of the scan (all tissue pieces on the original slide should be present on the WSI). To avoid scanning and storing unnecessary regions, some algorithm is often applied to scan only the area of interest.
- Image handling issues that are determined by the viewer (smooth scrolling, the ability to use various magnifications) or the information technology (IT) infrastructure (short access time).

Table 1. Essential slide scanner features and the extreme ends of implementation in slide scanners from different vendors and different types.

Feature	Alternative 1	Alternative 2
Available magnifications	One fixed objective (possibly with post-magnification)	Different objectives (sometimes even extendible)
Focusing technique	Placing different focus points on tissue areas	Continuously focusing
Image file format	Open format (can be standard, like JPEG 2000 or DICOM with JPEG (2000) compression)	Closed format (often proprietary)
Image acquisition technique	Linear scanning / line scanning	CCD camera
z-stack acquisition	Yes	No
Fluorescence	Yes	No

- Quality of the computer screen or projector used to display the images. Factors influencing the perception of digital slides include, but are not limited to, the resolution of the screen, the accuracy of color presentation, brightness, and contrast.

Because of the high resolution needed and the inherent color information present in each slide, the size of each scan is between a few megabytes up to several gigabytes, depending mainly on the amount of tissue present on the slide[1]. Different techniques exist to reduce this image size, for example reducing the scan area with algorithms to detect tissue areas, and compression of the final image[1, 18, 19]. The time needed to scan each slide is dependent on the size of tissue present on it, the time to handle the physical glass slide inside the scanner, speed of focusing, and processing of the output. For example, performing a whole slide scan (25x50 mm^2) at 20x takes 58 seconds for the Dmetrix (in ultra speed mode) scanner, while it takes 4 minutes for an Aperio ScanScope CS (as provided by the manufacturers)[16]. Performing scanning for slides areas of 15x15 mm at 40x will take between 9 and 80 minutes depending on the scanner type. A recently introduced scanner from Philips claims to scan a slide area of 15x15 mm at 40x in less than 50 seconds.

IT infrastructure

After a slide has been scanned it should be made available to the users, and the images should be linked in some way to a laboratory management or reporting system. To achieve this, barcodes on the slide are often used. Either 1-D barcodes or 2-D barcodes are suitable for this.

To store WSI, some type of storage infrastructure is needed. The total amount of required storage space is dependent on defined purposes of whole slide scanning. Storing a limited amount of WSI for consultation, research or educational purposes may not require mass storage capacity. However, large scale scanning, for example when routinely scanning all produced slides in a medium size laboratory already requires a huge storage environment up to 40 terabytes per year, excluding backup[1]. Depending on the retrieval characteristics of the end users, ultra fast fiber channel hard discs are required. Eventually (depending mainly on the pricing), flash based solid state drives will provide fast access, as they have a low access time and low latency. Because not all images are needed to be available instantaneously, older images might be archived to slower (but cheaper) storage media, like tape.

The quality of the displaying monitors affects digital slides examination significantly. The display resolution is the most important parameter, which determines the image quality and the size of the viewed field. For example,

monitors having a resolution of 1600x1200 pixels show only 21% of the corresponding field under the conventional microscope[20]. Other parameters like color calibration, contrast and brightness also have an effect on the perceived image quality.

The network speed is potentially limiting the speed of image retrieval and must be sufficient for continuous streaming of image files. Usually, 100 Mbit connections will be sufficient. Most image viewers incorporate efficient strategies for retrieving images, instead of downloading the complete image file, only the request area of interest and adjacent sections are fetched from file storage. This information is cached for fast retrieval in later requests. Also, some viewers first show low resolution tiles while fetching the high resolution tiles.

When the same WSI needs to be available to multiple users at the same time (e.g. for digital practical sessions or during slide courses), specially tuned accelerator servers may be required for even more strategic caching strategies.

At the time of writing, most slide scanner manufacturers use their own file format. Some are even proprietary; some are based on other standards, like JPEG 2000 (J2k). The former obviously is a big disadvantage to end users who are often forced to install multiple viewers when exchanging images, and hinders market penetration of digital microscopy. Some propose to use the JPEG 2000 format as a standard [18, 21]. In radiology, Digital Imaging and Communications in Medicine (DICOM) is the standard file format used for storing and exchanging images. The DICOM committee recently (August 2010) succeeded in finalizing a supplement to extend the DICOM standard to support WSI. This is an important development, which all vendors hopefully will take seriously and comply with.

Applications

One can think of many applications using digital slides in pathology, but they can basically be grouped into four different main applications: 1) education, 2) diagnostics, 3) research, and 4) archiving.

Education

Traditionally, education in the field of cell and tissue pathology has been based on glass slides and thus relied on conventional microscopy using double- or multiheaded microscopes[17, 22]. However, the multi-headed microscope limits the number of students able to access it. For a long time, next to live viewing of glass slides, static images in the form of diapositives have been used in presentations. The next stage was using static digital images that could be incorporated into teaching software, supplemented with annotations. Since WSI have become available, teaching was likely among the first applications of WSI[22, 23]. WSI provide exactly the same image to teacher and students, can be made available to an

unlimited number of students at the same time (even remote) and thereby function as a scalable multiheaded microscope[10], circumvent the unavoidable variation between serial sections from tissue blocks, provide full possibilities for annotations, links and incorporating questions, videos and sound clips. Taking full advantage of these virtues requires, however, a professional software environment such as PathXL (i-Path, Belfast, UK) or Digital Slidebox (Slidepath, Dublin, Ireland). Also complete training programs, including digital slides with annotations and questionnaires and online testing programs for pre- and post-graduates are provided by several companies.

The use of digital slides for education also has some disadvantages: students no longer learn to use the microscope[24, 25], which can however be learned later if necessary, and knowledge on the role of cells and tissues in disease is more important than the skill of handling a microscope. Further, digital education then depends fully on the well functioning IT infrastructure, and any failure or slow performance of the system will severely affect the teaching process. Lastly, the resolution provided by WSI from a decent scanner is lower than when eyeballing glass slides under a good microscope, but still more than good enough for teaching students.

Virtual microscope laboratories have been successfully applied in several universities around the world[26]. At the University Medical Center Utrecht, digital microscopy teaching was gradually implemented starting in 2007. The students quickly accepted WSI for teaching, liked it better than conventional microscopy, and their performance in examinations did not decrease with the use of WSI based teaching[25]. These results are comparable to those from other universities such as the University of Iowa and the University of Basel[7, 23, 24].

Digital diagnostics

With the availability of WSI, obstacles associated with the previous static and live systems (bias and error in selecting the images from microscopic fields for diagnosis in static system and low image resolution of the dynamic system) have been overcome[3]. The progress in image resolution of WSI[27], scanning speed, and user friendliness of the viewers, has made true digital slide based diagnostics feasible in several ways:

- Consultations for difficult or rare cases: digital consultation can be done within hours versus days to weeks for cases sent through regular mail. At the UMC Utrecht, we have implemented a server for digital consultation (www.slideconsult.com) where anybody having a WSI and an internet connection can upload a case for digital consultation with one of our pathologists. This server was implemented using mScope clinical software (Aurora MSC, Montreal, Canada). It is possible to discuss cases online, where one becomes the master

who can navigate through the image while the other participant(s) see these movements live on their screen.

- Slide conferences and panels, which is a special form of consultation. Super specialized pathologists in specific areas of pathology traditionally meet physically on a regular basis to discuss cases. Using the software as described above, panel members no longer need to travel to meet physically and can view and assess cases remotely by WSI, or participate in a virtual panel as described in the previous bullet-point. Several slide panels in The Netherlands exchange their images digitally and discuss them online through our server.

A screenshot from the pathology slide panel is shown in figure 2.

- Telerevision and quality assurance (QA): it is common practice to revise the relevant pathology material for referred patients. Again, shipping slides through regular mail is slow and slides may get lost or damaged. Conducting this digitally speeds up the revision process dramatically, and obviates the need of sending slides. Some hospitals perform digital QA conferences on daily bases to revise difficult, rare and new cancer cases from other hospitals. The experience from the University of Arizona Pathology Faculty showed that QA by WSI telepathology was very accurate and also allowed direct revision of the discrepant cases[28, 29]. Another study for assessing the usefulness of WSI for QA programs also showed that QA can be done efficiently with WSI[30]. For clinical trials where patient's material often needs to be revised by an expert pathologist before randomization this would also work very well. The same software system as described above can be used to accommodate this.
- Frozen sections diagnosis: Still or dynamic telepathology systems have been used to facilitate the evaluation of frozen sections for a long time, especially for hospitals without pathology department[5, 31-34]. The estimated average diagnostic accuracy of frozen section telepathologic diagnosis using old systems (especially dynamic, and hybrid) is about 95%-96%[35]. Using the WSI, pathologists were able to increase the diagnostic accuracy and reduce the time required to complete the diagnosis[36]. Another study showed that WSI were superior to a conventional dynamic telepathology system in term of usability and turnaround time. Reduction in the time of diagnosis and the better image quality were the main two causes for preferring telepathology using WSI[35].
- Image analysis: automated image analysis will enhance the diagnostic efficacy in histopathology. Since inspection of WSI is probably slightly more time consuming than conventional slides[37], the creation of programs for detection of regions of interest will be advantageous and speed up the work flow, especially if those areas of interest could be computed before the pathologist gets to see the image. To this end grid computing would probably be needed to be able to apply several algorithms to WSI (computing might take a long time because of

WSI resolution)[38]. Software for computerized quantification of immunohistochemically stained WSI to improve the objective assessment of the immunoreactivity is available from several scanner vendors. Such software estimates color intensity relative to control cells. Using this information they categorize the staining as 0+, 1+, 2+ or 3+. Examples of dedicated (non-scanner vendor) software packages for tissue quantification are Definiens TissueStudio (Definiens, Munich, Germany) and AQUA (HistoRx, Branford, Connecticut, USA). The most commonly seen application of image analysis based quantification of immunohistochemical stains is for HER-2/neu quantification[39, 40]. Some of these applications have clearance by the USA Food and Drug Administration (FDA) such as Automated Cellular Imaging System (ACIS III) which has approval for their Hercep test, oestrogen receptor (ER) and progesterone receptor (PR) applications[41]. Particularly for HER2 scoring in breast cancer it has been shown that WSI based image analysis provides a higher concordance rate with FISH than eyeballing and lowers inter-observer variability[42]. Other current applications include assessment of the percentage of estrogen receptor, progesterone receptor, and Ki67 positive nuclei.

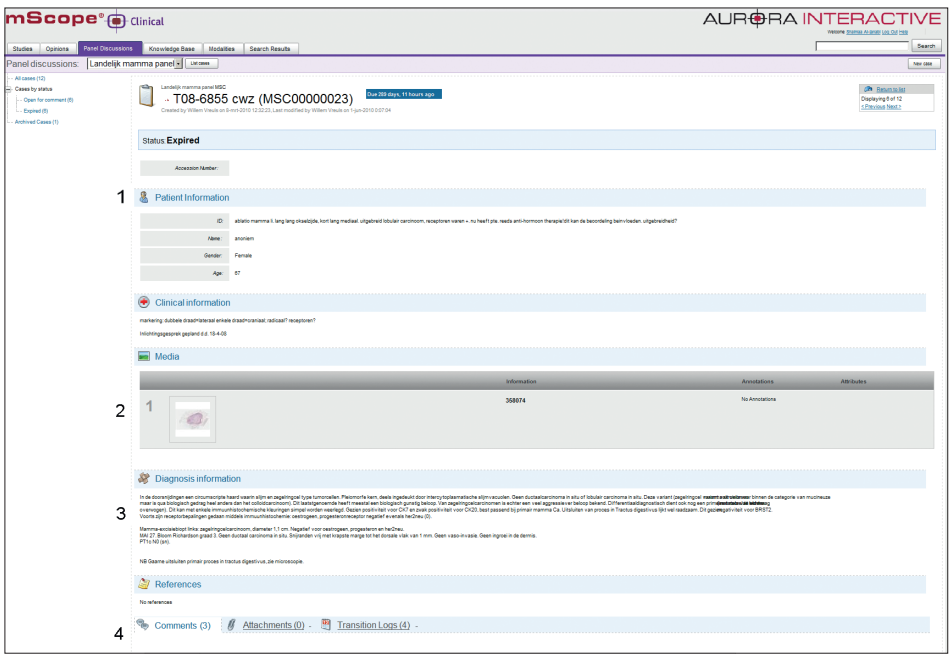

Figure 2. Screenshot from www.slideconsult.com. It shows slide the panel module, where the (registered) pathologists have access to digital slides and can render diagnosis from a distance. 1.Clinical information about this case, 2.The uploaded digital slide(s) for consultation, 3.Diagnosis of the specialist pathologist who submitted the case and 4.Comments from the other panel members.

The same principles can be applied to the quantitative assessment of Tissue Micro Arrays (TMA) where multiple tiny histological specimens are placed on the same slide to be assessed for immunoreactivity or gene amplification. Examples of scanners that are able to perform TMA analysis are ACIS, GenoMX, and Ariol SL-50 [43].

- Upfront digital diagnostics: the current state of technology already allows conducting upfront digital diagnostics. However, this is yet unusual, probably related to the fact that handling WSI still takes some more time than conventional slides, and the currently insufficient validation of WSI based diagnostics. Initial evaluation of the diagnostic accuracy on WSI showed high correlation with glass slide diagnosis in breast, pulmonary, gastrointestinal tract and prostate specimens [44]. Further validation is ongoing in different places in the world. WSI allows pathologists to work remotely, like from home or from any location around the world. Further, conglomerates of smaller pathology laboratories may start to superspecialize when cases are easily available through WSI.

Research

For research purposes digital slides can be used for viewing, storing annotations, measuring (most WSI viewers support measuring areas and lengths). Also image processing algorithms as described in the previous paragraph can be used, and many new ones are continuously being developed. Easy exchange of (annotated) images is a major advantage. Scoring TMAs can be easier, since the grid of the cores can be assessed and individual cores can then be presented as a perfect array and individually viewed and analyzed. Some biobanks systematically include WSI of banked cases for documentation (http://www.tubafrost.org).

Digital archiving

For many years the storage of the microscopic information in pathology has been in the form of glass slides. This is however not without problems such as the required large storage rooms with fortified floors, the fragile nature of the glass slide, fading of the stain over time, and finally the labor and logistic issues involved with ongoing storing and retrieving glass slides during which they regularly become misplaced[45]. Having a fully digital slide archive would have many advantages[1]:

- WSI are saved permanently with constant quality.
- Easy retrieval of cases for teaching, research, clinicopathological conferences, and quality assurance.
- The same case can be accessed by different observers at the same time.
- WSI can be integrated in the pathology report and the hospital information system.

The more widespread digital archiving across laboratories, the higher the potential gain would be for e.g. telerevision. Although local digital archives could be interfaced, there would certainly be economy of scale if larger (even nationwide) storage facilities would serve different laboratories. Archived digital slides are a huge data warehouse containing a lot of information, especially when linked to the original reports containing diagnostic information. Future developments in the area of automated image analysis and correlating this to, for example, clinical outcome, might give better insights into disease processes.

Future perspectives

During the last decade pathology has benefited hugely from the progress of information technology. The innovation of digital pathology has opened new challenges where whole slide examination on computer screens has become possible for several applications in pathology. The applications and use of WSI are expected to increase steeply over the next decade, also related to anticipated developments.

The large number of the slides for the daily diagnosis in pathology requires high speed scanners. Fortunately, new scanners are becoming available that can scan slides with a tissue area of 15x15mm at 40x in less than one minute.

Besides the required speed increase to facilitate upfront diagnostics, the image quality also needs to improve. Some vendors are currently selling scanners that have continuous focusing mechanisms which will prevent unfocused parts in the WSI. The option to do z-scanning to simulate focusing and scanning of fluorescent slides will likely become more common.

WSI have been used in many aspects of pathology and are generally well accepted. The use of digital slides for teleconsultation, telerevision, frozen section diagnoses and quality assurance is expected to increase over the next few years. Upfront WSI based diagnostics is currently validated in different centers and is expected to be successful, especially when viewers become more users friendly. Obtaining an FDA approval will definitely help, as well as standards for image storage and optimal IT infrastructure that support its routine use[20].

The validation of their use for daily pathology practice and the standardization of the image format will have great impact on pathology and health care system. In September 2010, an extension to the DICOM file format was accepted by the DICOM committee to support storing WSI. Adaptation to this standard by scanner vendors is now anticipated.

Hopefully, the use of WSI in education will yield generations of pathologists who are more familiar with the use of WSI. In addition, the application of digital

archiving is found to be the solution for the permanent slide storage with constant quality (especially for fluorescent slides) plus the advantage of easy retrieval for research purposes, education and revision. However, storage costs are still a limiting factor, but these are expected to drop steeply.
Progress in bandwidth of mobile connections may soon allow accessing WSI on PDAs, Apple's iPad or similar. Hopefully, software for this will soon be available.

Conclusion

We expect the next decade in digital pathology to bring several developments. First, we anticipate further improvements in scanning speed and image acquisition techniques, which will lead to scan speeds at 40x below 30 seconds. This will allow a setup where slides can be scanned before they leave the laboratory, and will also facilitate z-scanning without major impacts on performance. This is the way to go for upfront digital diagnostics, since the scanning delay can then be neglected and image analysis algorithms can be run in the background. Full integration of scanners into the laboratory workflow where e.g. a conveyor belt like setup takes slides through a stainer and coverslipper and then through the scanner would be a breakthrough. We expect improvements in compression algorithms (e.g. development of 3-D compression for reducing file size of z-scans), in storage solutions that will become faster and cheaper, and in software to access WSI on PDAs or Apple's iPad. Further, other research projects are focusing on the development of algorithms aiding in detection of e.g. mitotic figures, micro-organisms, metastases in lymph nodes, quantitative analysis of immunohistochemical stains and perhaps even automated "diagnosis" of the cases for e.g. QA, often called Computer Aided Diagnosis (CAD). Such algorithms can run on those images in the background and guide the pathologist to areas of interest (for example with a high mitotic count or possible metastases or microorganisms). Moreover, 3-D reconstruction of serial WSI may provide novel insights and better orientation within a given section. This has been tried for the colorectal biopsies which resulted in better detection of small intestinal polyps[46].

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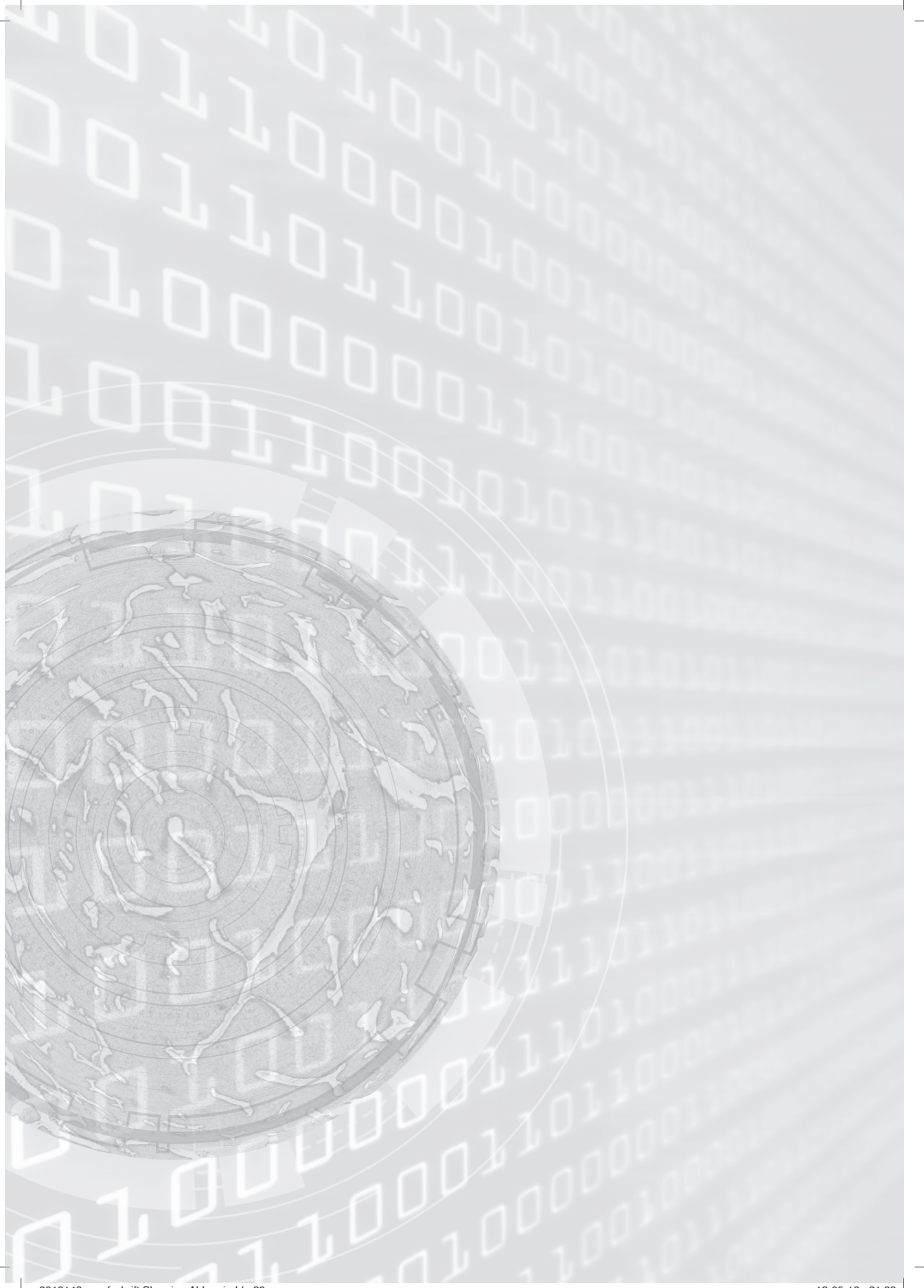

Chapter 3

Whole slide images for primary diagnostics of gastrointestinal tract pathology: A feasibility study

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Abstract

Introduction

During the last decade, whole slide images (WSI) have been used in many areas of pathology such as teaching, research, digital archiving, teleconsultation and quality assurance testing. However, WSI have as yet not much been used for upfront diagnostics because of the lack of validation studies. The aim of this study was, therefore, to test the feasibility of WSI for diagnosis of gastrointestinal tract specimens, one of the largest areas of diagnostic pathology.

Materials and methods

100 gastrointestinal tract biopsies and resections which had been diagnosed using a light microscopy one year before were re-diagnosed on WSI scanned at 20x magnification by 5 pathologist (all re-assessing their own cases), having the original clinical information available, but blinded to their original light microscopy diagnoses. The original light microscopy and WSI based diagnoses were compared and classified as concordant, slightly discordant (without clinical consequences) and discordant.

Results

The diagnoses based on light microscopy and the WSI based re-diagnoses were concordant in 95% of the cases. Light microscopy and WSI diagnosis in the remaining 5% of cases were slightly discordant, none of these were with clinical or prognostic implications.

Conclusion

Up-front histopathological diagnosis of gastrointestinal biopsies and resections can well be done on WSI.

Introduction

Traditionally, the examination of glass slides under a microscope has been common practice for both histopathology and cytology[1, 2]. Developments in imaging technology have led to the introduction of new ways of slide examination, such as digital snapshots and live telepathology[3-5]. For more than a decade, digital slide scanners that produce digital slides, also called Whole Slide Images (WSI)[6, 7], have been available.

These WSI can be examined on a computer screen by the aid of viewers that enable examination of the whole slide in a way comparable with conventional microscopy, navigating through the slide in any direction and at varying magnifications (obviously limited by the scanning magnification and the image resolution)[8-10]. Image viewers offer additional features such as an overview image to facilitate navigation within the examined slide, and examination of multiple slides at the same time, allowing side-by-side comparison of different stainings of the same specimen[8, 11, 12].

Other advantages of WSI could also be effectively used in daily pathology practice. Multiple people can open the same slide from different locations at the same time. This facilitates for example remote pathological consultation and will speed up the work flow, reducing the time needed for transferring the glass slide to remote places and improving the level of patient care[12, 13]. The use of WSI also facilitates pathology discussion panels through internet portals where participants can look at the slides from anywhere at any suitable moment and leave their comments[6, 12]. In addition, WSI eliminate the risk of slide breakage, loss and fading of stains. WSI have been used in many applications in daily pathology for example remote consultations, primary frozen section diagnosis, quality assurance, education and research [12-17]. However, WSI use in daily routine diagnosis is still a matter of debate, although some laboratories have performed local (smaller) validation studies and use digital slides for primary diagnostics. The most important factor that hinders their use is the lack of systematic validation in sufficiently sized studies, and in the United States, there is no approval from the Food and Drug Administration to use WSI for up-front diagnostics. Therefore, further validation of using WSI for upfront digital diagnostics remains necessary.

In previous articles we have described the setup of a workflow enabling routinely scanning and archiving all diagnostic slides in daily routine[6]. These scans are being used for clinicopathological meetings, comparison with new material, education and research. Furthermore we have reviewed the current status of the field of digital pathology and described our perspective on the future of digital pathology[12]. The aim of the present study was to evaluate the WSI for upfront routine digital diagnosis in gastrointestinal pathology practice, a major field in histopathology.

Materials and Methods

This study has been conducted in the Department of Pathology, University Medical Center Utrecht (UMCU), a medium size academic pathology laboratory in The Netherlands handling about 144,000 surgical pathology slides per year (from about 25,000 specimens), and 12,000 cytology slides each year. Since November 2007, all histopathology slides have routinely been scanned after they had been diagnosed by light microscopy. Scanning is performed on ScanScope XT scanners (Aperio, Vista, CA, USA). The whole process of scanning runs automatically (including selection of the area of the slide that contains tissue, placing focus points, calibration, etc.). The produced WSI are stored on a dedicated mass storage environment and linked to the pathology report[6], based on the recognized barcode on the slide label. WSI can be accessed through our pathology reporting system (U-DPS [Universeel Decentraal PALGA Systeem]; PALGA [Pathologisch Anatomisch Landelijk Geautomatiseerd Archief], Utrecht, The Netherlands), as well as other images, like gross images and scanned order forms.

For this study 100 cases from the gastrointestinal tract with a complete set of well focused WSI that had been diagnosed light microscopically by five pathologists in 2009 were selected, to guarantee a wash out period of 6-12 months. The same pathologists who did the initial diagnosis were asked to re-diagnose their own cases on WSI to exclude inter-observer variation as much as possible. The participating pathologists had varying but at least 3 years experience in using WSI, and numbers of cases varied among these five pathologists.

WSI were per case presented together with the original clinical information to the pathologists, blinded to the original report based on light microscopy evaluation. The selected cases consisted of biopsies and resection specimens from different parts of the gastrointestinal tract. Table 1 shows a summary of the study cases in relation to their origin from the gastro-intestinal tract and the type of the specimen (biopsy or resection). Table 2 shows an overview of the types of cases included in this study.

In total 100 WSI diagnoses were compared with 100 light microscopy diagnoses by five pathologists and concordance between WSI and light microscopy diagnoses was assessed by three independent pathologists (two of them sub-specialized in gastrointestinal pathology) as follows:

- Concordant; complete agreement between the first original signed out diagnosis and the diagnosis as drawn from the whole slide image
- Slightly discrepant; mild differences which would not have any clinical or prognostic implications
- Discrepant; differences with clinical and/or prognostic implications for the patient

The 95% confidence interval (CI) was calculated and the better one of the two diagnoses (light microscopy or WSI based) was noted.

Table 1. Overview of the anatomical site and the specimen type of 100 gastrointestinal cases re-diagnosed on WSI.

Types	Anatomical position	Number
Biopsies	Esophagus	9
	Stomach	10
	Duodenum	16
	Ileum	6
	Colon	47
	Sigmoid	2
	Rectum	5
Total biopsy		95
Resections	Colon	3
	Sigmoid	1
	Anus	1
Total resection		5

Table 2. Primary diagnosis of 100 gastrointestinal cases included in split up according to their origin from the gastrointestinal tract.

Organ	Benign		Preneoplastic	Neoplastic		No abnormality	Total
	Inflammation	Reactive		Benign	Malignant		
Esophagus	7	2					9
Stomach	4	5				1	10
Small intestine	5					17	22
Large intestine	21	9	13		1	15	59
Total	37	16	13		1	33	100

Results

From the 100 cases, 95 cases (95%) were concordant (95% CI 0.89-0.98), and 5 cases (5%) showed slight discordance between the digital and the light microscopy diagnosis. The percentage agreement between light microscopy and WSI diagnoses falls within the 95% confidence interval which is (0.89-0.98). Additionally, these five discrepancies were without any clinical or prognostic implications for the patient. Re-assessment of the glass slides and WSI by the reviewing pathologists showed that in two cases, the light microscopy diagnosis was the better one, and for three cases, the second (WSI) diagnosis was considered better. Table 3 details these five slightly discrepant cases.

The first case concerned a duodenal biopsy from a patient with iron deficiency anemia. The clinical question for this case was "evidence of celiac disease". The light microscopy diagnosis mentioned "hyperplasia of Brunner's glands without any increase in intraepithelial lymphocytes, no evidence of dysplasia or Giardia lamblia infection". This was confirmed by WSI re-diagnosis, but additionally, focally active inflammation was seen. Upon review, the light microscopy diagnosis was deemed the better one. The second case concerned colonic biopsies from a patient known to have ulcerative colitis. The light microscopy diagnosis was "chronic active inflammation without dysplasia". WSI diagnosis was "extensive chronic inflammation, consistent with inflammatory bowel disease without signs

Table 3. Original and WSI based diagnosis for the five slightly discrepant cases.

Specimen type	Site	Clinical data	Original diagnosis	Digital diagnosis	Preferred diagnosis
Biopsy	Duodenum	Iron deficiency anemia	Hyperplasia of Brunner's glands without any increase in the intraepithelial lymphocytes, no evidence of dysplasia or Giardia lamblia infection.	Same but with focally active inflammation	Original
Biopsy	Colon	Known ulcerative colitis	Minimal chronic active inflammation.	Extensive chronic inflammation, consistent with inflammatory bowel disease without signs of active inflammation	Original
Biopsy	Stomach	Stomach complaints, no macroscopic abnormalities	Minimal chronic inflammation, no evidence of H. pylori infection	Features of proton pump inhibitors; no evidence of H. pylori infection	Digital
Biopsy	Stomach	Stomach complaints	No inflammation or other specific changes, no evidence of H. pylori infection	Evidence of reactive gastropathy, no H. pylori infection	Digital
Biopsy	Colon	Diarrhea and constipation, microscopic colitis?	Pseudomelanosis, possibly due to long use of laxatives, no evidence of microscopic colitis	Elongation of crypts, with normal infiltrate, no other specific changes and no signs of microscopic colitis	Digital

of active inflammation". Upon review, the light microscopy diagnosis was viewed as the correct one. Figure 1 is a snapshot from the WSI of this biopsy showing the site of active inflammation that was missed on re-diagnosis.
The third case was a gastric biopsy from a patient with stomach complaints without any macroscopic abnormalities. The light microscopy diagnosis was "minimal chronic inflammation without H. pylori infection"; whereas the WSI diagnosis was "features of proton pump inhibitors without evidence of H. pylori infection". Revision confirmed the digital diagnosis to be the correct one.

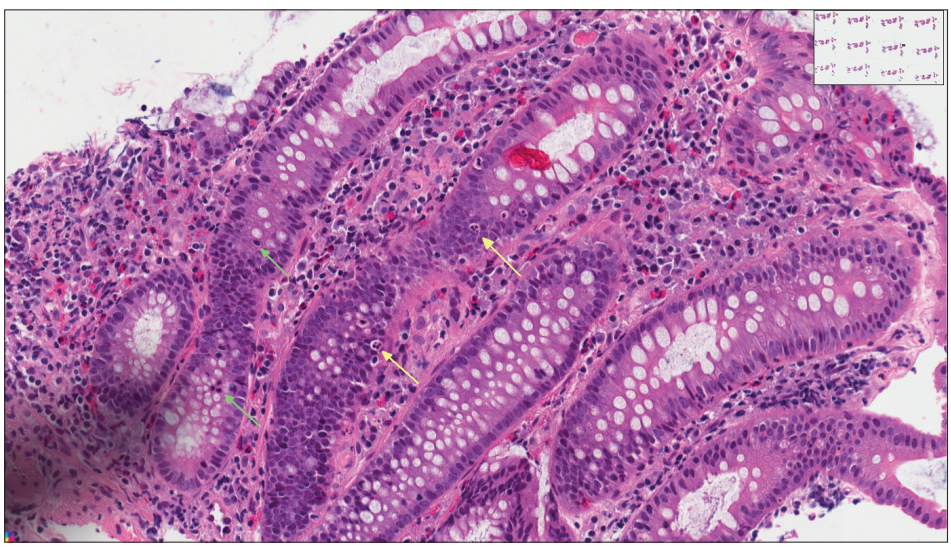

Figure 1. A snapshot from WSI of colonic biopsy showing the site of active inflammation in the colon of patient known with ulcerative colitis. Yellow arrows show polymorphic nuclear inflammatory cells (Neutrophils) within the glandular epithelium. Green arrows show intra-epithelial lymphocytes.

The fourth case comprised biopsies from the stomach for patient with "stomach complaints". The light microscopy diagnosis was "no inflammation or other specific changes, also no evidence of H. pylori infection". WSI diagnosis revealed "evidence of reactive gastropathy, no H. pylori infection". Reexamination of the glass slides confirmed that the epithelium showed some reactive changes, favoring the WSI diagnosis. Figure 2 is a snapshot from the WSI of this biopsy showing the reactive gastropathy.
The fifth case was a colon biopsy from a patient complaining of diarrhea on the bases of constipation, and the clinical question was "microscopic colitis". Light microscopy diagnosis was "pseudomelanosis coli as may be seen within the context of long use of laxatives, no evidence of microscopic colitis".

The WSI diagnosis was "elongation of crypts, with normal infiltrate, no other specific changes and no signs of microscopic colitis". Re-examination of the original glass slides denied the typical picture of pseudomelanosis coli, so the digital diagnosis was deemed to be the correct one.

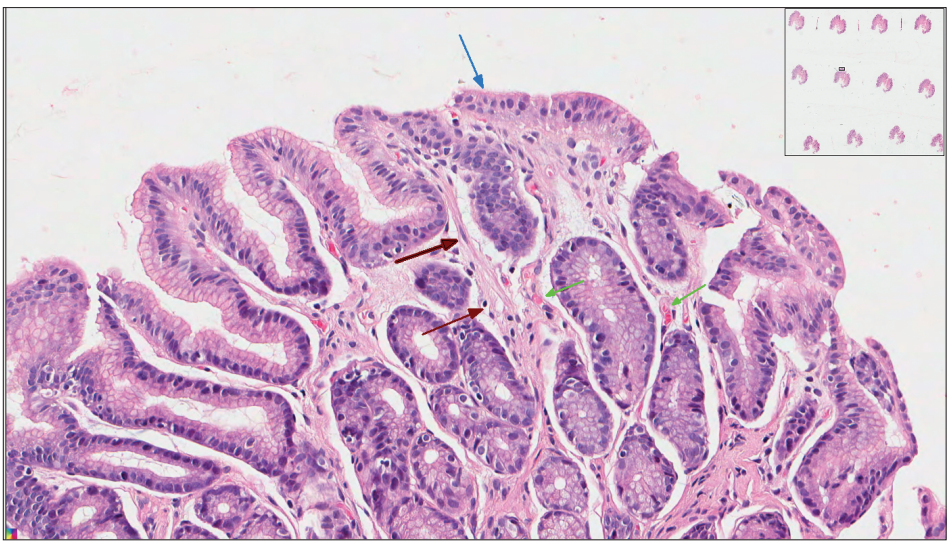

Figure 2. A snapshot from the WSI of this biopsy showing the reactive gastropathy. Blue arrows point to reactive superficial epithelium, green arrows show congested capillaries and the red arrows show smooth muscle fibers running in between gastric glands.

Discussion

The aim of this study was to test the validity of WSI for diagnosis of gastrointestinal tract specimens. In 100 biopsies and resections from the gastrointestinal tract, blinded re-diagnosed by the same pathologists on WSI was concordant with the light microscopy diagnosis in 95% of cases, the remaining 5% (all biopsies) being slightly discordant without clinical or prognostic implications. There were no major discrepant results, especially no discrepancies between benign, dysplasia, and malignant. It, therefore, seems that WSI may well be used for up-front histopathologic diagnosis of gastrointestinal tract specimens. This is underlined by the narrow confidence interval (95% CI 89-98%), which would not have been much narrower when we would have doubled the sample size (0.91-0.97).
We consider this rate of mild discrepancies within the range of generally observed (stochastic) intra-observer variability in pathology[18, 19], and a similar rate of discrepancies would likely be seen if cases were re-diagnosed by light microscopy instead of using digital images. In line with this, in three of the five slightly

discrepant cases the WSI diagnosis was preferred over the light microscopy diagnosis on review.

The 95% concordance rate is within the range of the few other validation studies which examined the performance of the WSI for the primary or secondary histopathologic diagnosis[3, 20-24], including one on gastrointestinal pathology[24]. The aim of the latter study was to evaluate WSI for primary histopathologic diagnosis of gastric and colonic biopsies. They included 103 specimens, and from each specimen, a single representative slide was selected. Histopathological sections and related clinical information were submitted to two independent pathologists to be assessed first by light microscopy and after a few weeks on WSI. Discordance between the WSI or light microscopy results and consensus diagnosis was found in 7.8% of the cases (eight cases). In five cases the light microscopy diagnosis was considered to be better and in the other three cases the WSI diagnosis was considered to be the better one. The authors concluded that the use of WSI for up-front diagnosis will be inevitable after further enhancement of scanning speed, image quality and storage requirements. The short time period between rendering diagnosis on the light microscope and by WSI is a limitation in this study since the study pathologists may have remembered their initial diagnosis in at least some cases. Moreover, selection of just one slide from each case may have limitations. We re-diagnosed all slides in the present study.

A limitation of the present study is the low percentage of neoplastic lesions, although we included 13 cases with preneoplastic transformation[25, 26]. This selection, however, reflects the case mix in our department and probably that of many other pathology laboratories. Nevertheless, further studies should perhaps be biased to include more neoplastic cases.

While performing the diagnosis digitally, the pathologists were comfortable in rendering the diagnosis at the applied 20x magnification. The fact that we checked the quality of all WSI (and rescanned when necessary) before showing them to the pathologists may have helped here. In real life, however, one would pose similar demands on WSI before feeling confident to make a digital diagnosis. We noticed, however, that the identification of microorganisms like Candida albicans, Helicobacter pylori and Giardia lamblia was sometimes difficult. The pathologists agreed that examination at 40x magnification would have given a more confident diagnosis of microorganisms, although no cases were digitally misdiagnosed in this respect. Scanning at higher magnification may be preferable and will likely be the future standard, but appears not to be very relevant for most cases while adding scanning time and necessitating significantly more storage.

Despite the advantages of WSI like the overview image, and the fact that the participating pathologists were comfortable using WSI, most still prefer to view slides under the microscope. This may be due to the fact that a mouse may not be

optimally suitable as a navigation tool for examining WSI. Other solutions that are available may allow easier handling of WSI during diagnosis, such as the Ergo Controller (Nikon, Melville, NY) or the iSlide input device (BioImagene, Tucson, Arizona, USA). These devices assist the pathologist to view the WSI in a way more comparable to the conventional microscope.

The general impression of the pathologists who participated in this study about performing diagnosis on WSI was that further advances in the field of digital pathology with regard to the user interface, viewing software and image resolution will help to accelerate the acceptance of WSI for the daily routine diagnosis in pathology.

Another potential advantage of WSI may be that they allow for image analysis and computer assisted diagnosis. Running e.g. algorithms on WSI to detect dysplastic/malignant cells or microorganisms before they are presented to the pathologist will save the time needed for diagnosis and may improve diagnosis and decrease inter-observer variability. Finding dysplastic and cancer cells within digital images of colonic biopsies was described by Hamilton et al. and Esigar et al. with acceptable accuracies[27, 28]. Automated analysis of WSI was also tried for diagnosis of inflammatory bowel disease and classification of gastric biopsies[29, 30]. 3-D Reconstruction of a stack of 2-D WSI provided better orientation of tiny intestinal polyps in one study[31].

In conclusion, histopathological diagnosis of routine gastrointestinal biopsies and resections can well be done on WSI acquired using today's scanning technology.

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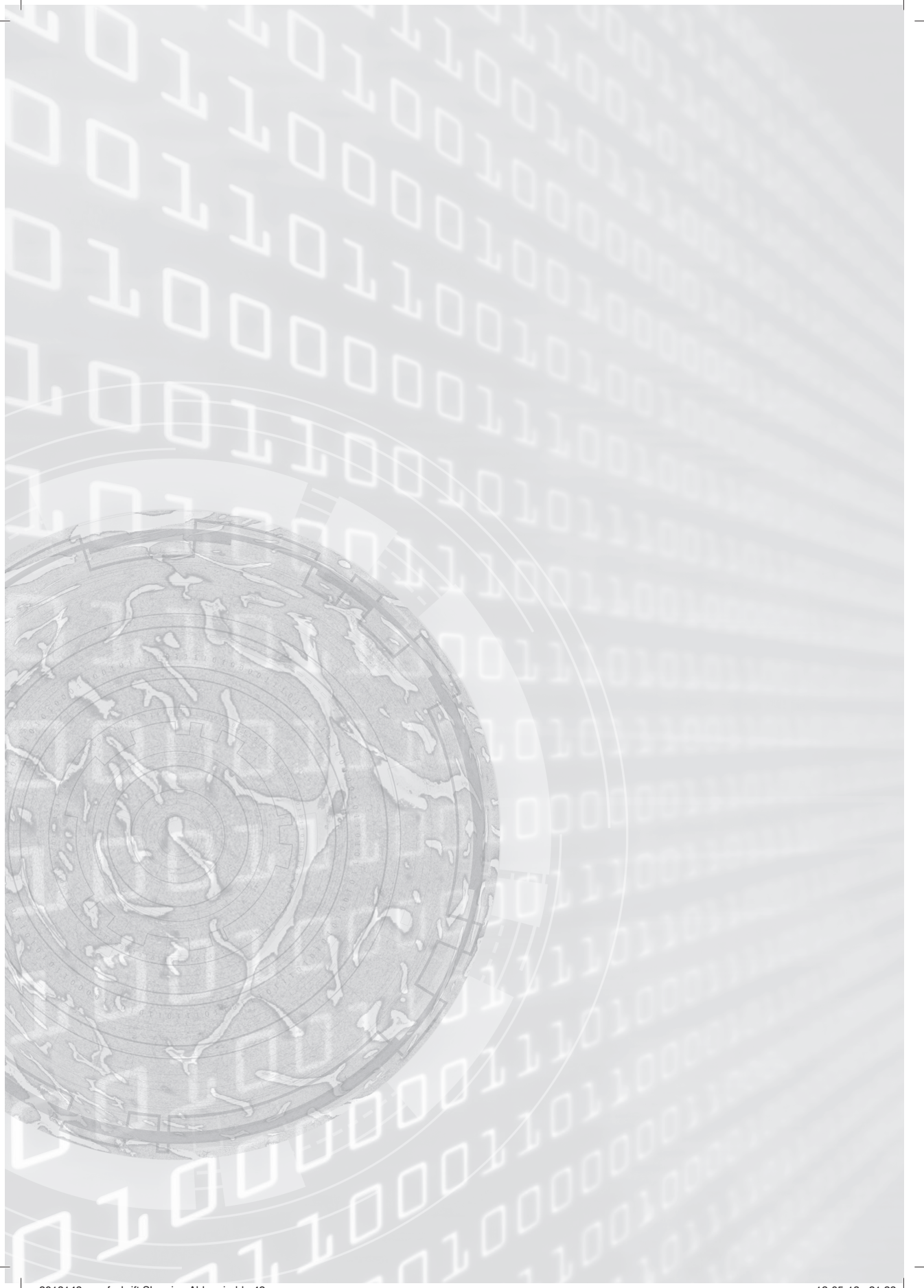

Chapter 4

Whole slide images for primary diagnostics in dermatopathology: A feasibility study

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Abstract

Introduction

During the last decade, whole slide images (WSI) have been used in many areas of pathology such as teaching, research, digital archiving, teleconsultation and quality assurance testing. However, WSI have as yet not regularly been used for routine diagnostic sign out, because of the lack of validation studies. The aim of this study was therefore to test the validity of using WSI for primary diagnostics of skin diseases.

Materials and methods

100 skin biopsies and resections which had been diagnosed light microscopically one year before were scanned at 20x magnification, and re-diagnosed by six pathologists (every pathologist assessed his own cases), having the original clinical information available, but blinded to the original diagnoses. The WSI diagnoses were compared to the initial light microscopy diagnosis and classified as concordant, slightly discordant (without clinical consequences) or discordant.

Results

The light microscopy and the WSI based diagnosis were concordant in 94% of the cases. The light microscopy and WSI diagnosis were slightly discordant in 6% of the cases. For one of the slightly discrepant cases the WSI diagnosis was considered better while the original diagnosis was preferred for the other five cases. There were no discordant cases with clinical or prognostic implications.

Conclusion

Primary histopathological diagnosis of skin biopsies and resections can well be done digitally using Whole Slide Images.

Introduction

Whole slide imaging is the process of digitizing glass slides by means of a dedicated slide scanner to present the acquired image on a computer screen[1, 2]. Digital slides or Whole Slide Images (WSI) can be explored by image viewers facilitating tissue examination in a way comparable to a regular microscope, also called virtual microscopy[3, 4]. However, additional features are often supplied by the image viewers, for example the ability to explore several slides at the same time and an overview image next to the high power view providing more orientation within the slide[5, 6]. Moreover WSI can be explored simultaneously by multiple viewers from different locations[7]. Multiple access of WSI by different users at the same time supports their use for many applications in pathology such as teleconsultation and education. Consultations using WSI will save the time needed for transferring glass slides to remote places for obtaining second opinions. In pathology education, having the opportunity to show all students exactly the same slide has many benefits over handing out consecutive sections[5, 8-10]. Other pathology applications such as frozen section diagnosis[11, 12], quality assurance testing [13], slide conferences and tumor boards[5] are examples where WSI can be used efficiently.

Despite the fact that some pilot studies suggest that WSI is as useful as conventional glass slides for rendering diagnosis[14-17], WSI based diagnosis has not been integrated within the routine pathology workflow until now (with a very small number of exceptions). There are several reasons why the use of WSI in daily routine work is still not common, among which are scanning speed, storage capacity (and pricing), software integration, and lack of systematic validation studies for their use for primary diagnosis[5, 18].

Many developments have taken place over the last few years which may help in reducing the impact of some of these issues, for example the tangible reduction of scanning time[2,5]. Second, the technique of image acquisition is also improved. Some scanners are able to scan in continuous auto-focusing mode instead of scanning in image stripes or capturing image tiles[1, 5]. This will minimize the focusing errors within the WSI. Third, suitable navigation instruments (replacing the ordinary computer mouse) for easy and more ergonomic handling of WSI in a way very similar to handling glass slide are also becoming available. Fourth, standardization according to the DICOM image format (also generally being used for storing images in radiology and radiotherapy imaging modalities) for storing WSI is a big step forward, which will ease the integration and exchange of images between different institutions and systems. Fifth, the increase of storage capacity with reduction of the cost per unit of storage is also expected in the near future, providing the opportunity to store more images for the same price. All of these factors will increase the acceptance of using WSI for primary diagnostics in pathology.

In previous articles we have described the setup of a workflow enabling scanning and archiving all diagnostic slides in daily routine[1]. These digital slides, which are routinely scanned in our laboratory now, are being used for clinicopathological meetings, comparisons with new material (digital archive), education and research. Furthermore we have reviewed the current status of the field of digital pathology and described our perspective on the future of digital pathology[5]. The aim of the present study was to evaluate the suitability of WSI for daily routine digital sign out in dermatopathology, generally a large part of the case mix in diagnostic histopathology.

Materials and Methods

This study was performed at the Department of Pathology, University Medical Center Utrecht (UMCU), a medium size academic pathology laboratory in The Netherlands. We handle about 144,000 surgical pathology slides per year (from about 25,000 specimens), and 12,000 cytology slides each year. Since November 2007, scanning was started on a daily basis for all histopathology slides after they had been diagnosed by light microscopy. Scanning is performed on 3 ScanScope XT scanners (Aperio, Vista, CA, USA). The whole process of scanning runs automatically (including selection of the area of the slide that contains tissue, placing focus points, calibration, etc.). Scanning slides of 15x15 mm on 20x took on average 2.5 minutes. The produced WSI are stored on a dedicated mass storage environment and linked to the pathology report[1], based on the recognized barcode on the slide label. WSI can be accessed through our pathology reporting system (U-DPS, PALGA, Utrecht, The Netherlands), as well as other images, like gross images and scanned order forms.

One hundred skin biopsies and resections with a complete set of well focused WSI that had been diagnosed light microscopically by six pathologists in 2009 were selected, to guarantee a period of 6-12 months between the first (light microscopic) and the second (WSI based) diagnosis. The same pathologists who did the initial diagnosis were asked to re-diagnose their own cases on WSI (the cases are therefore not equally distributed over the six pathologists). The participating pathologists had varying experience using WSI, but at least for 3 years.

WSI were presented to the pathologists per case together with the original clinical information, without showing the original report based on light microscopy examination. The WSI were displayed on standard consumer quality Samsung 245B (Samsung, Seoul, South Korea) displays of 24" (having a resolution of 1920 x 1200 pixels). The selected cases consisted of 46 biopsies and 54 resection specimens with different entities of skin diseases. Table 1 shows a summary of the

number of cases in relation to the type of the specimen (either biopsy or resection) and their diagnostic entity.

At the end of this experiment we have 100 WSI based diagnoses and 100 light microscopy based diagnoses rendered by six pathologists, each on their own cases. The original light microscopy and the WSI based diagnoses were compared by three independent pathologists to judge the concordance of both diagnoses as:

- Concordant; complete agreement between the first original signed out diagnosis and the diagnosis as determined on the WSI;
- Slightly discrepant; mild differences which would not have any clinical or prognostic implications;
- Discrepant; differences with clinical and/or prognostic implications for the patient.

The confidence interval was calculated and the preferred one of the two diagnoses (light microscopy or WSI based) was noted as well.

Table 1. Type of the specimen (biopsy or resection) and diagnostic category of 100 dermatopathology cases evaluated on whole slide images.

Diagnosis	Biopsy	Resection	Total
Dermatosis	11	1	12
Reactive changes	0	5	5
Benign epithelial tumor	9	9	18
Malignant epithelial tumor	11	13	24
Benign non epithelial tumor			
Nevus	3	17	20
Others	3	5	8
Malignant non epithelial tumor	0	0	0
Dysplastic lesion	8	4	12
No abnormality	1	0	1
Total	46	54	100

Results

For 94 out of 100 cases (94%) the original light microscopy and WSI based diagnosis were concordant, while the remaining 6 cases (6%) showed slight discordance between the digital and the light microscopy diagnoses. The percentage agreement between light microscopy and WSI based diagnosis falls within the 95%confidence interval which lies between (0.87- 0.97). Moreover none of these discrepancies were associated with clinical or prognostic implications for the patient.

Table 2. Overview of diagnostic features of 6 dermatopathology cases showing slight discrepancies when comparing diagnosis on conventional slides and whole slide digital images.

Specimen type	Site	Clinical data	Original diagnosis	Digital diagnosis	Preferred diagnosis
Biopsy	vulva	Patient known with differentiated VIN presented recently with hyperkeratosis, pre-malignant?	Lichen sclerosus	Lichen ruber	Digital
Biopsy	toe	Hyperkeratotic papule below the nail of the fourth toe.	Fibrokeratoma	Benign reactive verrucous lesion mostly verruca vulgaris	Original
Resection	cheek	Reexcision after melanoma in situ with a focus of invasive growing. Radical? Rest of melanoma in situ?	There is no evidence of remnant melanoma with presence of small nevus in slide 3	No remnant of melanoma, no other specific abnormality.	Original
Resection	anus	Condyloma, anal flap. Condyloma?	Skin resection with viral changes and moderate dysplasia (Anal intra-epithelial neoplasia grade II (AINII))	Anal intra–epithelial neoplasia grade III probably HPV.	Original
Resection	nipple	Family history of melanoma, clinical diagnosis is nevus. Benign?	1. Melanocytic lesion difficult to classify, best diagnosed as irritated junctional nevus. 2. Secondary diagnosis from the national panel was Spitz nevus	Skin excision nipple with dysplastic nevus	Original
Biopsy	unknown	Skin papule, DD: nevus, BCC	Benign lichenoid keratosis	Eczema	Original

Re-assessment of the glass slides and WSI by the reviewing pathologists revealed that in one case the WSI diagnosis was preferred over the diagnosis by conventional light microscopy. For the other five cases the original light microscopy diagnosis was considered to be better. Table 2 details these six slightly discrepant cases.

The first case concerned a skin biopsy from a patient with previous history of lichen sclerosus and differentiated Vulvar Intraepithelial Neoplasia (VIN), presenting with features of hyperkeratosis. The clinical question was if there was any evidence of premalignancy. Both the light microscopy and the WSI based diagnosis agreed about the absence of signs of malignancy, but disagreed about the type of lichenoid reaction seen within the biopsy. On WSI it was considered as "lichen ruber", while using light microscopy it had been considered as "lichen sclerosus". After revision of the glass slides and WSI, the diagnosis "lichen ruber" was deemed best. Figure 1 shows a snapshot of the WSI showing the lichenoid inflammation within this vulvar skin biopsy.

The second slightly discrepant case concerned a skin biopsy of a papule below the nail of the fourth toe. The original light microscopy diagnosis was "fibrokeratoma" while the diagnosis after reviewing the WSI was "verruca vulgaris". After revision, the light microscopy diagnosis was considered to be better. Figure 2 is a snapshot of a part of the WSI showing the microscopic features suggesting fibrokeratoma.

The third case was a skin re-excision assessing the status after resection of melanoma. Both light microscopy and WSI based diagnosis agreed that there was no remnant of melanoma, but additionally a small nevus was detected on light microscope which has been missed on the WSI. Figure 3 is snapshot of a section of the WSI showing the overlooked nevus.

The fourth case concerned an anal resection with condyloma. Both of the diagnoses on light microscopy and WSI agreed on the presence of features suggesting viral infection and Anal Intraepithelial Neoplasia (AIN), but the diagnoses disagreed about the grade of the lesion. On light microscopy it was considered as grade II,

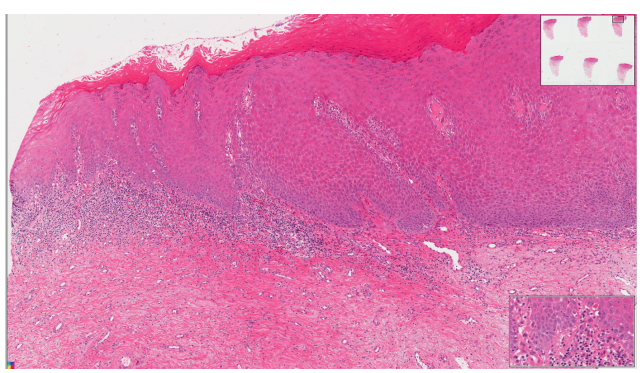

Figure 1. Snapshot of WSI showing lichenoid inflammation within vulvar skin biopsy. This case was misdiagnosed conventionally as "lichen sclerosus" but correctly classified as "lichen ruber" on WSI.

but it was considered to be grade III on WSI. On review, the reviewing pathologists agreed with the original light microscopy diagnosis. Figure 4 is a snapshot from an area of the WSI of this specimen showing the site of condyloma with AIN II.
The fifth case concerned a skin resection from the nipple from a patient with a strong family history of malignant melanoma. This skin lesion was diagnosed clinically as ”nevus”, a biopsy was taken to confirm the benign nature. This lesion has been diagnosed using light microscopy by two pathologists as irritated junctional nevus with the presence of some atypical hyperchromatic melanocytic cells. Later on, it was referred to the Dutch Melanoma Panel who suggested the diagnosis of “Spitz nevus”. This resection has been diagnosed as “dysplastic nevus” on WSI. The cause of this discrepancy was not because of the used diagnostic method but because of the interpretation of the pathological changes seen in this difficult lesion. Figure 5 is a snapshot from an area of interest from the WSI of this skin resection.

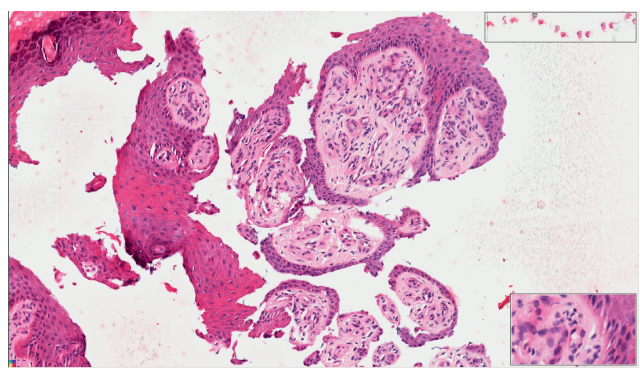

Figure 2. Snapshot of WSI of skin biopsy showing the microscopic features suggestive of “fibrokeratoma”. This case was erroneously classified as “verruca vulgaris” on WSI.

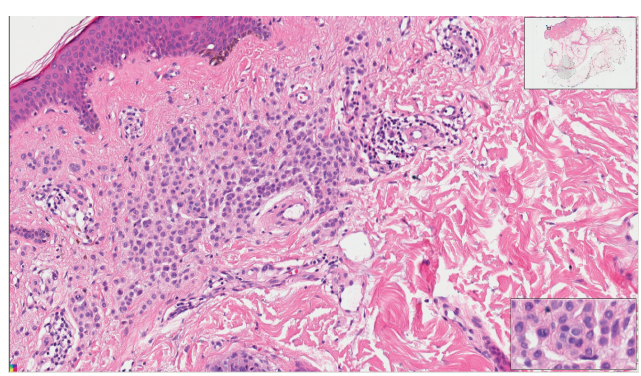

Figure 3. Snapshot from WSI of skin resection showing a small nevocellular nevus in a re-excision specimen for previous melanoma, which was overlooked while performing the diagnosis using WSI.

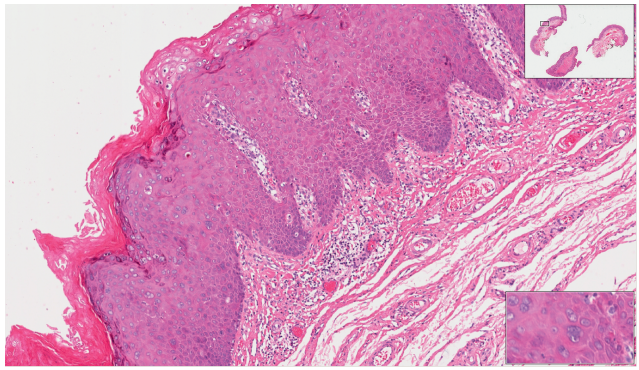

Figure 4. Snapshot from WSI of an anal skin specimen showing a condyloma with AIN II, graded as AIN III on WSI.

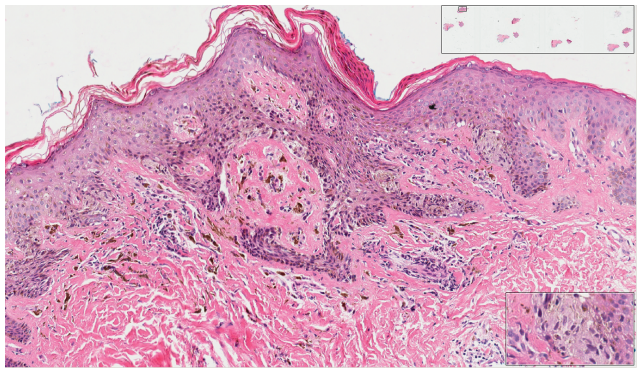

Figure 5. Snapshot from WSI of a skin resection specimen showing a "Spitz nevus", diagnosed finally as such after consultation of the Dutch Melanoma Panel. Originally, it was deemed an atypical nevocellular nevus. On WSI, it was diagnosed as dysplastic nevus.

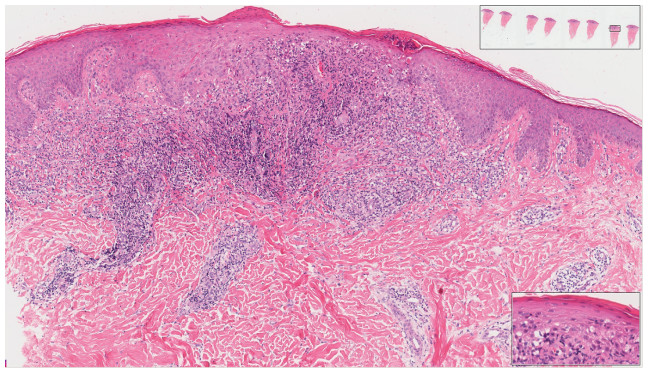

Figure 6. Snapshot from WSI of a skin biopsy showing "benign lichenoid keratosis", misdiagnosed as "eczema" on WSI.

The sixth case was a biopsy of a skin papule. On light microscopy it was diagnosed as ”benign lichenoid keratosis” while it was diagnosed as “eczema” on the WSI. Revision of the clinical presentation and the pathological changes supported the original diagnosis. Figure 6 is a snapshot from an area from the WSI of this skin biopsy showing the microscopical features of the lesion.

Discussion

The aim of this study was to test the feasibility of using WSI for the diagnosis of skin specimens. From the archive we selected 100 skin biopsies and resections received in 2009 which were blindly re-diagnosed using WSI (only clinical information was presented) at 20x magnification. The re-diagnosis was done by the same pathologist who did the initial diagnosis to avoid inter-observer variations. The re-diagnoses were concordant with the original conventional diagnosis in 94% of cases. Interestingly, this is irrespective of the fact that pathologists would have used the 40x magnification on and off during the original diagnostic process, as no restrictions applied at the time. The remaining 6% (3 biopsies and 3 resections) were slightly discordant, without clinical or prognostic implications. This indicates that WSI may well be used for performing primary histopathologic diagnosis of skin specimens. This is supported by the high percentage agreement between the diagnoses performed by the two diagnostic modalities and the narrow confidence interval (0.87- 0.97). The sample size is sufficient, since theoretically the confidence interval would not get much narrower if we would even double the sample size (0.91-0.97).

We consider this rate of mild discrepancies within the range of generally observed intra-observer variability in pathology[19, 20], and a similar rate of discrepancies would likely be seen if cases would be re-diagnosed microscopically instead of using digital slides. The discrepancy rate is in line with the fact that for one of the six slightly discrepant cases the WSI was in the end considered to be the better one. Additionally, none of the discrepancies was related to the perceived magnification or WSI quality, but mostly to different interpretation of difficult or borderline cases. In two of the cases, overlooking diagnostically important areas were the cause of discrepancy. In one case overlooking one of the skin fragments present on the WSI led to missing the correct diagnosis, while in the other case overlooking a small area in a one slide from a series of 13 WSI led to the discrepancy. This may be related to the lack of experience in reviewing WSI.

The 94% concordance rate is within the range of other validation studies which examined the performance of using WSI for primary or secondary histopathologic diagnosis [21-24]. These results are also comparable to the other validation studies

which were specific to skin pathology[14, 25-27]. Okada et al. and Leinweber et al. assessed the diagnostic accuracy of WSI based teledermatology. Their studies showed a high concordance rate between a diagnoses based on WSI and diagnoses based on light microscopy. However, these latter two studies focused only on the diagnosis of melanocytic tumors [25, 26]. In a study of Gilbertson et al. assessing the validity of WSI diagnosis in 25 genitourinary and dermatology specimens it was concluded that WSI produced by current slide scanners contain sufficient information for rendering diagnosis. A limitations of that study is the low number of cases and the fact that also some WSI with focal areas of poor image quality were included which were responsible for some discrepant results[27]. The validity of using WSI for routine diagnosis of skin tumors was studied by Nielsen et al. on 96 skin biopsies and shaves from which one glass slide was created for each case. The participating pathologists diagnosed the first on WSI and after a few weeks by light microscopy. Then they compared the two new diagnoses with the gold standard diagnosis from a highly experienced dermatopathologist. The diagnostic accuracy was 89.2% and 92.7% for WSI and glass slides respectively. The limitation of this study is that the participating pathologists were blinded to the clinical information which could affect the diagnostic outcome and also none of them had previous experience with using WSI. In addition, the short time period between performing the diagnosis by the two modalities was another limitation, since pathologists may have remembered the cases[14].

During digital evaluation, the pathologists did not have many difficulties in rendering the diagnosis at the applied 20x magnification. The fact that we checked the quality of all WSI (and rescanned when necessary) before showing them to the pathologists may have helped here. When routinely using WSI for primary diagnostics one would also demand adequate quality of the scanned slides for a confident diagnosis. Scanning at higher magnification may be preferable in general to avoid any possible issues related to the lack of resolution and routinely scanning at 40x will become possible in the near future when scanners are faster and storage price has come down. On the other hand, a higher magnification seems not to be relevant for most cases, and for now saves scanning time and storage requirements.

Despite advantages of WSI and the fact that the participating pathologists were comfortable using WSI, most of them still prefer to view slides under the microscope. This may be due to the fact that the WSI based diagnostic process was perceived to be slower (although no formal timing was performed) and a mouse may not be optimally suitable as a navigation tool for examining WSI. Devices allowing easier handling of WSI during diagnosis are currently available, such as the Ergo Controller (Nikon) or the iSlide input device (BioImagene), although they are specific for use together with image viewers specific for the slide scanners from

these vendors. These devices assist the pathologists to view the WSI in a way more comparable to the conventional light microscope, which may minimize errors resulted from improper navigation of WSI. Also better monitors with resolutions up to 6 megapixels having a very small picture pitch and with sRGB calibrated colors (like the ones used in radiology) will help, but current viewers first need to be optimized to handle these high resolutions.

A potential advantage of WSI is that it becomes possible to perform image analysis and eventually also computer assisted diagnosis, possibly improving the diagnosis and decreasing inter-observer variability. This may also assist in the objective diagnosis of aggressive skin tumors such as malignant melanoma[28-30]. Tissue counter analysis of dermatoscopic[31] and microscopic images have been investigated for differentiation of molluscum contagiosum from normal skin and also for classification of melanocytic skin tumor with acceptable results[32-35]. In another study on malignant melanomas and its correlation with patient survival, the authors concluded that automated measurement of cross sectional areas of malignant melanoma on digital slides can help in assessing patient prognosis[36].

Some legal issues arise from the use of WSI for primary diagnosis, related to image quality, image presentation (monitor quality), storage space, adequate backup, document transfer, patient confidentiality and the confidence of the pathologist to sign-out a pathology report depending on WSI. We expect that most of these issues will be settled in the near future. Several digital pathology vendors are currently seeking approval from the US Food and Drug Administration (FDA) for using WSI in primary diagnosis which will definitely encourage the general use of WSI in primary diagnostics after conditions for the above issues have been defined. One aspect of FDA approval is systematic validation of WSI for primary diagnosis in sufficient sized populations like this study does for skin tissue. In Europe, The Royal College of Pathologists in its August 2003 guidelines" Code of practice for pathologists participating in remote reporting of Histopathology or Cytopathology" declared the necessity of remote reporting services especially when no regional pathologist is available. However, the remote pathologist should take in consideration that all the necessary data (clinical, laboratory feedback, contact with clinician) are available to guarantee a good quality of the pathology report (Code of practice.pdf). This issue has been also reported in its guidelines for the year 2005 where they stated that "The conclusions of that report are relevant whether the remote reporting is achieved by transfer of microscopic slides or by telepathology" (Telepathology-May05.pdf). The Dutch Society of Pathology already considers WSI an alternative for stored glass slides (Dutch guidelines.pdf). In September 2010 an extension to the DICOM file format has been accepted by the DICOM committee to support storage and exchange of WSI, which is an important development in the field of digital pathology[5]. Further systematic

validations in addition to solving some practical issues will help the adoption of WSI for primary pathology diagnosis.
WSI diagnosis did not have a major clinical impact in the current study in terms of patient management, and we feel that the discrepancies between conventional and WSI based diagnosis are within the ranges of generally inter- and even intra-observer variation. Therefore, we do not expect WSI based diagnosis to affect cancer registries and incidence rates in general.
In conclusion, it seems that primary histopathological diagnosis of skin biopsies and resections can well be done on WSI acquired using today's scanning technology.

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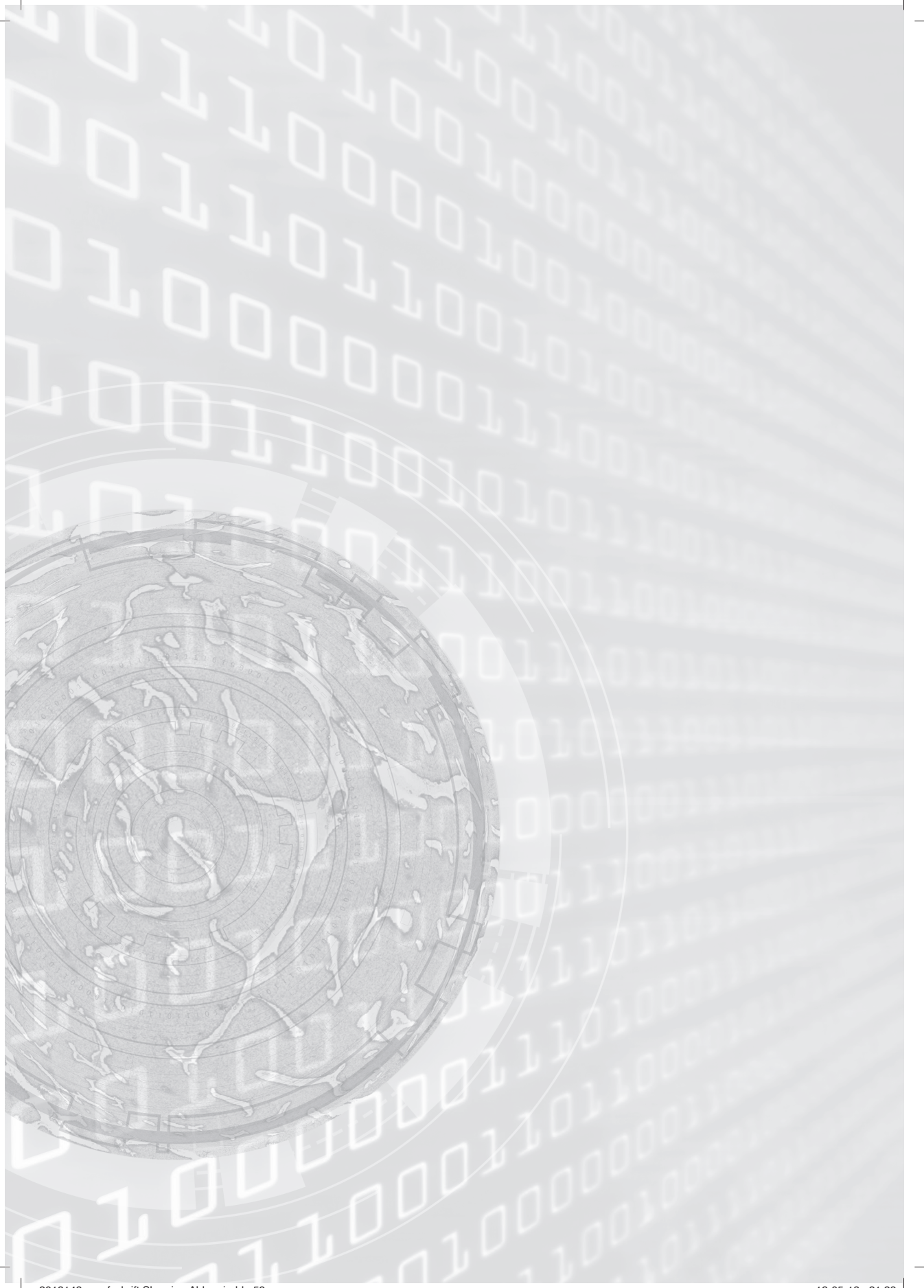

Chapter 5

Digital slide images for primary diagnostics in breast pathology: A feasibility study

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Abstract

Introduction

Digital Slide Images (DSI) have been used in many areas of pathology such as teaching, research, digital archiving, teleconsultation and quality assurance testing. However, they have as yet not much been used for upfront diagnostics. The aim of this study was therefore to test the feasibility of DSI based diagnosis of breast specimens.

Materials and methods

Sections of 100 breast specimens which had been diagnosed conventionally previously were scanned and re-diagnosed on DSI by the same pathologist who performed the initial light microscopy based diagnosis. The DSI diagnoses were compared to the light microscopy diagnoses and classified as concordant, slightly discrepant (without clinical or prognostic consequences) or discrepant.

Results

The original light microscopy and DSI based diagnoses were concordant in 93% and slightly discrepant in 6% of cases. There was only one discrepant case with clinical or prognostic implication to the patient. However, for this case, no final agreement could be achieved. For four out of the six slightly discrepant cases, DSI diagnosis was considered the better one while the original diagnosis was preferred only in one case. In addition for one case which was categorized as slightly discrepant, both the DSI and conventional diagnosis were imperfect according to 2 reviewing breast pathologists.

Conclusion

Upfront histopathological diagnosis of breast biopsies and resections can reliably be done on DSI.

Introduction

Advances in imaging technology have led to the introduction of new ways of slide examination and rendering diagnosis in pathology, such as on digital snapshots or dynamic telepathology[1, 2]. Since more than decade, digital slide scanner have been available that produce Digital Slides Images (DSI), also called Whole Slide Images[3, 4] which improved the limitations of the live imaging based telepathology[5, 6].

These DSI can be examined on a computer screen with the aid of image viewers that enable examination of the whole slide in a way comparable with conventional microscopy, navigating through the slide in any direction and at varying magnifications[7, 8]. Image viewers offer many additional features such as an overview image to facilitate navigation within the examined slide, examination of multiple slides at the same time, allowing side-by-side comparison of different stainings of the same specimen and a continuous zoom functionality, as opposed to the discrete steps that are possible using a regular light microscope[7, 9, 10].

Other properties of digital slides are also beneficial in daily pathology practice. For example, DSI can be explored by multiple examiners from different places facilitating their use for teleconsultation, speeding up the workflow and reducing the time needed for transferring the glass slide to remote places[10, 11]. Instantaneous multiple access of DSI facilitates also their use in many pathology applications such as quality assurance testing (QA), frozen section diagnosis, running clinicopathologic conferences, slide expert panels, and in education[10, 12-14]

As to the latter, many universities in the USA and Europe have substituted DSI for light microscopy in teaching pathology to medical students and residents, using the full potential of DSI by incorporating annotations, multimedia and questionnaires as a better means of education. DSI also enable the creation of online digital teaching atlases. An example of this is the online atlas for breast pathology (www.webmicroscope.net/breastatlas). On this website about 150 DSI of different breast pathologies has been uploaded which can be used for all levels of pathology education and review[11, 15].

Despite of all the advantages of DSI and the presence of pilot studies which suggest that DSI may be as useful as glass slides in rendering histopathological diagnosis[16-18], it has not yet been published that DSI are being used for up-front diagnostics in pathology. One of the important factors that hinder the use of DSI for this purpose is the lack of systematic validation in sufficiently sized study groups, and in the United States there is no approval from the Food and Drug Administration (FDA) to use DSI for primary diagnostics. Therefore, systematic validation of using DSI for upfront digital diagnostics remains necessary.

In a previous article we have described the setup of a workflow enabling routinely scanning and archiving all diagnostic slides in daily routine[3]. These scans are being used for clinicopathological meetings, histological comparison with new patient material, education and research. Furthermore we have reviewed the current status of the field of digital pathology and described our perspective on the future of digital pathology[10]. The aim of the present study was to evaluate DSI for upfront routine digital diagnosis in breast pathology practice. This study is part of a larger project in which we systematically validate the use of DSI for different areas of pathology.

Materials and Methods

This study was performed at the Department of Pathology, University Medical Center Utrecht (UMCU), a medium sized academic pathology laboratory in The Netherlands. We handle about 144,000 surgical pathology slides per year (from about 25,000 specimens), and 12,000 cytology slides each year. Beginning in November 2007, scanning was started on a daily basis for all histopathology slides after diagnosis by light microscopy using 3 ScanScope XT scanners (Aperio, Vista, CA, USA). The whole process of scanning runs automatically (including selection of the area of the slide that contains tissue, placing focus points, calibration, etc.). The produced DSI are stored on a dedicated mass storage environment and linked to the pathology report[3], based on the recognized barcode on the slide label. DSI can be accessed through our pathology reporting system (U-DPS, PALGA, Utrecht, The Netherlands), as well as other images, like gross images and scanned order forms.

Breast biopsies, resections and mammoplasty specimens that had been diagnosed using light microscopy in the years 2008-2010, were selected to guarantee a wash out period of at least 6 months. These hundred cases were all consecutively diagnosed by one breast pathologist. After case selection, the quality of the DSI was checked and slides were rescanned if necessary (e.g. when poorly focused or for missing DSI in the digital archive). Cases without clinical history (from external consultation or revision) or having more than twenty slides per case were excluded, leaving 100 cases. The same pathologist (having experience in using DSI for at least 3 years) who did the initial diagnosis was asked to re-diagnose his own cases on DSI.

DSI were presented to the pathologist per case together with the original clinical information, without showing the original light microscopy based report. The standard viewer provided with the Aperio ScanScope XT scanners was used for visualizing the images. They were displayed on standard consumer quality Samsung 245B (Samsung, Seoul, South Korea) 24-in displays (having a resolution

of 1920 x 1200 pixels). The selected cases consisted of 84 biopsies, 14 resections, and 2 mammoplasty specimens with different breast disease entities (including benign, high risk, premalignant, and malignant breast lesions).
After digital re-diagnosis of the 100 breast specimens, the light microscopy and DSI based pathology reports were compared by two independent reviewing pathologists to judge the concordance of both diagnoses as:

- Concordant: complete agreement between the first original signed out diagnosis and the diagnosis as determined from the whole slide image;
- Slightly discrepant: mild differences which would not have any clinical or prognostic implications;
- Discrepant: differences with clinical and/or prognostic implications for the patient.

The preferred diagnosis of both ones obtained (light microscopy or DSI based) was noted by the reviewing pathologists and the 95% confidence interval (CI) of the percentage concordance was calculated.

Results

For 93 out of 100 cases (93%, 95% CI 86-97) the light microscopy and the DSI based diagnosis were completely concordant. For the other seven cases, six showed slight discordance between the digital and the light microscopy diagnoses without any clinical or prognostic implications for the patient. However, in one case the discrepancy would have clinical implications for the patient.
Re-assessment of the glass slides and the DSI for the discrepant cases by the two reviewing pathologists revealed that in four slightly discrepant cases the DSI diagnosis was preferred over the diagnosis based on light microscopy whereas the original light microscopy based diagnosis was preferred only in one slightly discrepant case. For the sixth slightly discrepant case both original and DSI based diagnoses were considered imperfect. For the one discrepant case encountered in this study, no consensus diagnosis was reached. Table 1 details these seven discrepant cases.
The first case concerned a breast biopsy of a Breast Imaging-Reporting and Data System (BIRADS) III lesion with architectural abnormalities on ultrasound and mammography. This lesion was diagnosed using light microscopy as “columnar cell metaplasia and ductal hyperplasia” whereas on DSI it was diagnosed as “usual ductal hyperplasia and in one duct atypical ductal hyperplasia”. These two different diagnoses would influence the chosen treatment for this patient, warranting excision in the latter. However, for this borderline lesion no consensus diagnosis could be reached, emphasizing the difficulty of diagnosing this case.

The second case (slightly discrepant) concerned an excision of a breast tumor, clinically considered to be a fibroadenoma. This lesion was diagnosed by light microscopy as "benign phyllodes tumor" while diagnosed as "fibroadenoma" on DSI. Figure 1 is a snapshot from DSI of this breast tumor. Because the lesion had been completely removed there were no consequences for the patient.

Table 1. Original and Digital slide image based diagnosis for the seven discrepant breast cases.

Specimen type	Clinical data	Original diagnosis	Digital diagnosis	Type discrepancy	Preferred diagnosis
1. Biopsy	BIRADS III, architectural abnormality of breast tissue on ultrasound and mammography	Columnar cells metaplasia and ductal hyperplasia.	Usual ductal hyperplasia with one focus of atypical ductal hyperplasia.	Discrepant	Equivocal
2. Excision	Tumor of the breast, fibroadenoma?	Benign phyllodes tumor	Fibroadenoma	Slightly discrepant	Digital
3. Excision	Excision of palpable swelling. Fibroadenoma?	Fibroadenoma	Benign phyllodes tumor	Slightly discrepant	Digital
4. Biopsy	Recurrent mastitis. Differential diagnosis: inflammation/DCIS	Invasive ductal carcinoma	Invasive ductal carcinoma and lymphatic invasion	Slightly discrepant	Digital
5. Biopsy	Vaguely palpable lesion.	Mucinous DCIS grade I, lobular neoplasia.	Mucinous DCIS grade I, lobular neoplasia and ductal hyperplasia. On edge of biopsy loose tumor fragments. suspicious of invasive carcinoma	Slightly discrepant	Digital
6. Excision	Lumpectomy for a breast carcinoma. Radical?	Infiltrative ductal carcinoma, radically removed. PR + ve, ER + , HER2 – ve	Infiltrative ductal carcinoma, radically removed. PR -ve, ER +ve, HER2-ve	Slightly discrepant	Original
7. Biopsy	Microcalcifications	DCIS III without signs of invasion	DCIS III with suspicion of invasion	Slightly discrepant	Both imperfect

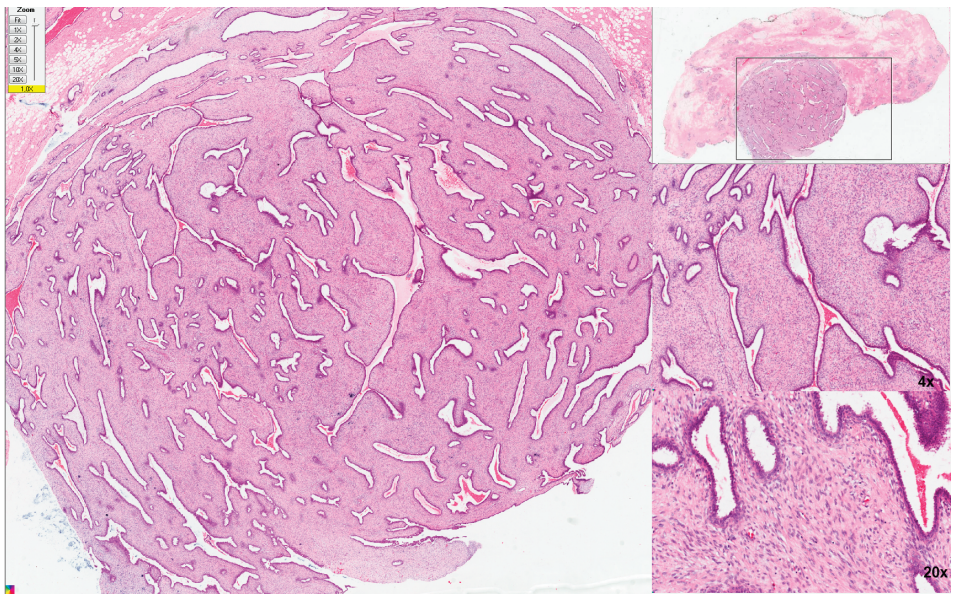

Figure 1. Snapshot from DSI of highly cellular fibro-epithelial breast tumor stained with H&E stain. This tumor was difficult to classify because of features of both fibroadenoma and benign phyllodes tumor, eventually diagnosed as fibroadenoma.

The third case concerned an excision of palpable tumor of the breast, clinically diagnosed as a fibroadenoma. This tumor had been diagnosed using light microscopy as "fibroadenoma" but considered as "benign phyllodes tumor" on DSI. Figure 2 is a snapshot from an area of interest from the DSI of this resection. Both the second and the third cases were fibroepithelial lesions that showed both slight increase in stromal cellularity and some stromal overgrowth. Therefore, both lesions were difficult to classify because they contained features of both fibroadenoma and benign phyllodes tumor. For this reason they were referred to the Dutch Breast Panel and for both lesions the DSI diagnosis was deemed best. However, initially there was no full consensus on the second case, illustrating the difficulty of its differential diagnosis.

The fourth case was a breast biopsy from a patient with clinically recurrent mastitis. It was diagnosed as "ductal adenocarcinoma" on light microscopy. On DSI it was diagnosed as "ductal adenocarcinoma with lymphatic invasion". Review of the glass slides and the DSI favored the DSI diagnosis. Figure 3 is a snapshot from the DSI of this breast biopsy showing the site of lymphatic invasion.

The fifth case concerned a breast biopsy because of a vaguely palpable lesion. Both light microscopy and DSI rendered a diagnosis of mucinous ductal carcinoma in situ (DCIS) grade I and lobular neoplasia. In addition, suspicion of invasion was

suggested on DSI which was not mentioned in the initial light microscopy diagnosis. After examining the DSI and the glass slides by the reviewing pathologists agreed about the presence of features suggestive for invasion. Figure 4 is a snapshot from an area from the DSI of this breast biopsy showing the site suspected for invasion.

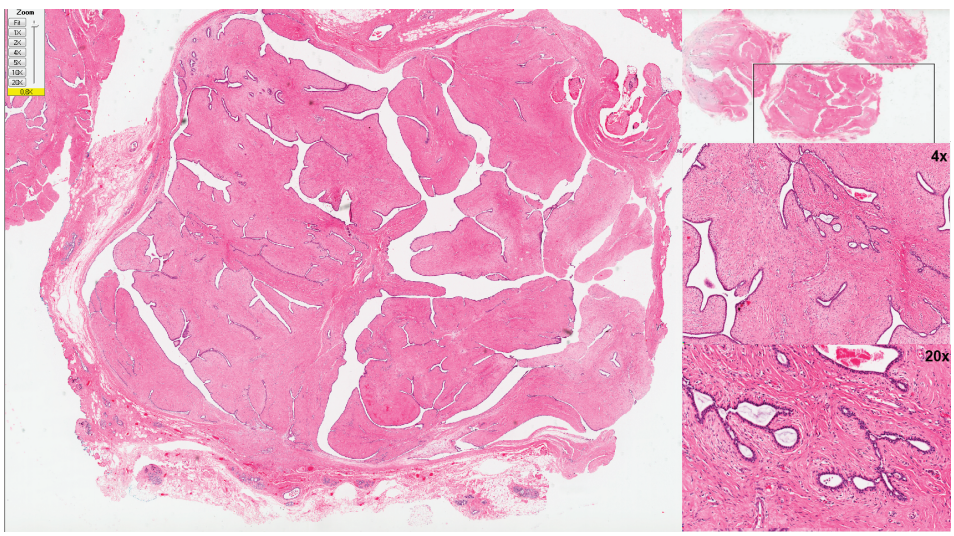

Figure 2. Snapshot from DSI of highly cellular fibroepithelial breast tumor stained with H&E stain which was difficult to classify because of features of both fibroadenoma and benign phyllodes tumor, eventually diagnosed as benign phyllodes tumor.

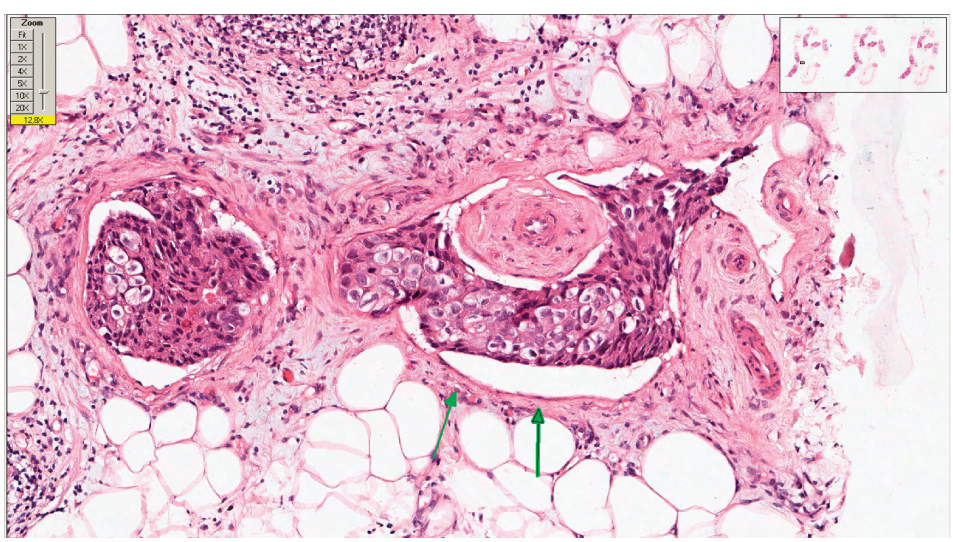

Figure 3. Snapshot from the DSI of H&E stained breast biopsy showing lymphatic invasion (green arrow) which was missed on conventional microscopy.

The sixth case was a lumpectomy from a patient known with breast cancer after core needle biopsy. Both light microscopy and DSI based diagnosis agreed on primary histopathological diagnostic features, but they disagreed on the scoring of the immunohistochemical progesterone receptor (PR) stainings. PR was regarded as negative on DSI, while a distinct part of the lesion was clearly PR positive which has been missed on DSI. Figure 5 is snapshot of a section of the DSI showing the overlooked area which was PR positive.

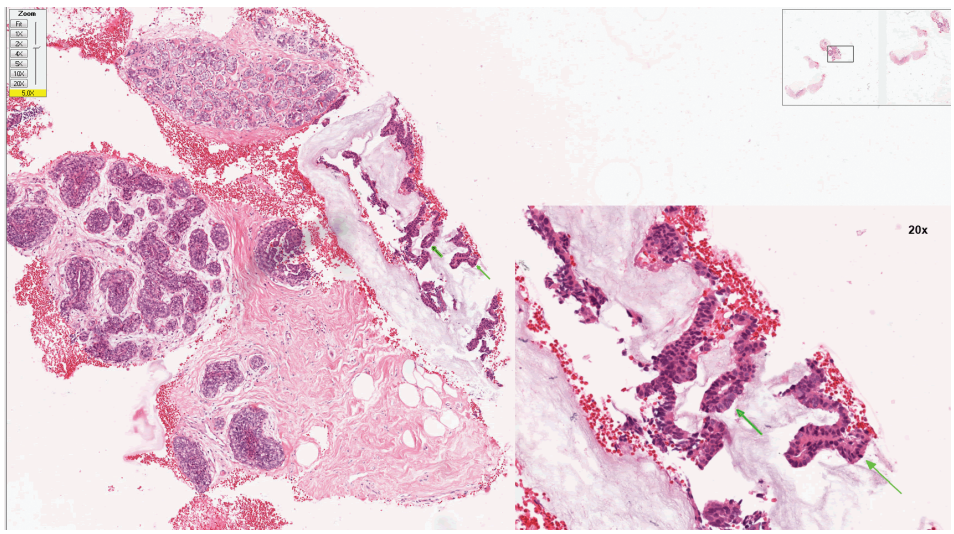

Figure 4. Snapshot from an area from the DSI of H&E stained breast biopsy showing site suspected for invasion (green arrows).

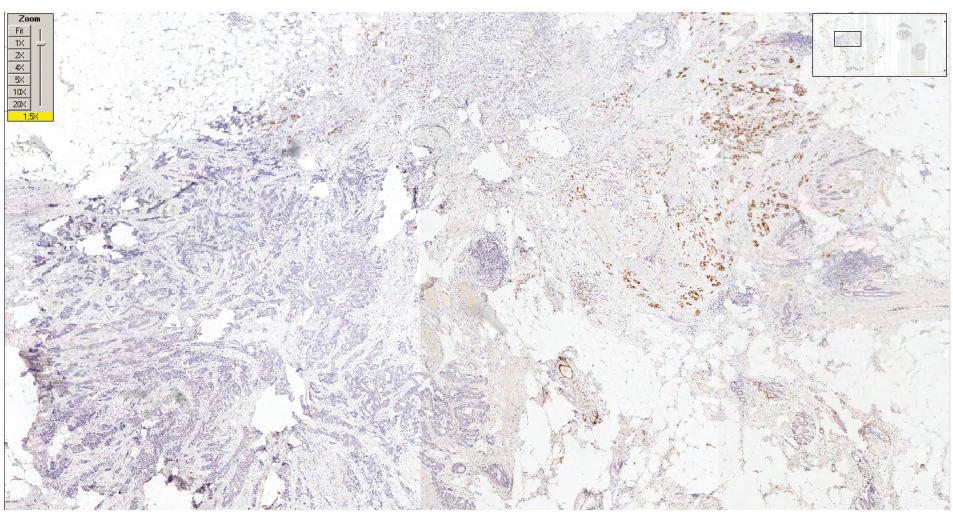

Figure 5. Snapshot of a section of the DSI showing immunohistochemical stains of breast biopsy diagnosed as invasive ductal carcinoma. The lesion stained negatively for PR stains except in the upper right part which was positively stained.

The seventh case concerned a biopsy from a patient with calcification on the mammogram. Both the light microscopy and the DSI based diagnosis agreed about the presence of DCIS grade III, but invasion was proposed only by DSI. After reviewing the glass slides and the DSI, both diagnoses were considered to be imperfect and "invasion cannot be excluded" would have been the best phrasing since there was no definite invasion in this case, but only some loose tumor fragments on the edge of the biopsy.

Discussion

The aim of this study was to test the feasibility of using DSI for the diagnosis of breast specimens. From the archive we selected 100 breast biopsies, resections and mammoplasty specimens received between 2008-2010 which were blindly re-diagnosed using DSI. The re-diagnosis was done by the same pathologist who did the initial diagnosis to avoid inter-observer variations. The re-diagnoses were concordant with the original light microscopy diagnosis in 93% of cases (95% CI 86-97). There were slight discrepancies between the light microscopy and the DSI diagnosis in 6% of the cases (3 biopsies and 3 resections), without clinical or prognostic implications to the patients, and one discrepant case which would result in clinical implication to the patient. In this discrepant case no consensus diagnosis was achieved due to borderline nature of this lesion. These results show that DSI may well be used for performing primary histopathologic diagnosis of breast specimens.

This rate of mild discrepancies falls within the range of generally observed intra-observer variability in pathology[19, 20]. According to literature we would expect a very similar rate of discrepancies if the cases would have been re-diagnosed on light microscopy instead of using DSI[16, 21-23]. Moreover, in four of these slightly discrepant cases DSI diagnosis was considered the better one. The possible causes of the discrepancies in this study were mostly due to different interpretation of difficult or borderline cases, which were also illustrated by the lacking of consensus for case one and the initial lack of full consensus for case two when reviewed by an expert panel of breast pathologists

Performing diagnosis on DSI scanned on 20x magnification was not perceived to be difficult. The fact that we checked the quality of all DSI (and rescanned when necessary) before showing them to the pathologist may have helped here. When routinely using DSI for primary diagnostics one would also demand adequate quality control being integrated in the scanning process for a confident diagnosis. Scanning at higher magnification may be preferable in general to completely avoid any possible issues related to the lack of resolution. At the moment scanning at

higher magnification would increase scanning time and storage requirements by a factor four.

An important advantage of DSI is the presence of a complete overview image that helps to navigate the slide systematically, decreasing the risk of skipping or overlooking tissue fragments. Indeed, most viewer applications allow marking the regions that have already been visited. The problem of overlooking a tissue area with consequences for the diagnostic outcome was encountered only once in this study. This overlooked area concerned a PR positive part of a largely PR negative tumor.

The 93% concordance rate is within the range of other validation studies which examined the performance of using DSI for primary or secondary histopathologic diagnosis[21, 24-26]. The validity of DSI based diagnosis of breast biopsies was studied by Weinstein et al.[17] Thirty breast biopsies (16 benign and 14 malignant) were retrospectively selected, from each case a single representative slide was scanned using a DMetrix ultrarapid virtual slide scanner. In that study glass slides and DSI were examined by four pathologists on two occasions a few weeks apart. From 120 DSI based diagnoses, only three diagnoses were incorrect. These discrepancies in diagnosis were not related to the quality of DSI but more to rendering diagnosis of borderline cases. The drawbacks of that study might be that the reviewing pathologists were not supplied with clinical data and that only a single slide was selected from each case. These factors could have affected the diagnostic outcome[17].

The increasing incidence of breast cancer[27, 28] necessitates realizing intensive programs for early detection, proper diagnosis and treatment. DSI technology can play an important role in speeding up the workflow and improving the level of patient care. The Sharing of DSI by different examiners in different places is unique for digital pathology. Images can be uploaded through an internet portal to be examined by the consultant pathologist without the need of transferring the glass slide to remote places. This will decrease the time needed for consultation while minimizing the risk of slide loss or damage. At the UMC Utrecht, a server for digital consultation has been implemented (www.slideconsult.com) using mScope clinical software (Aurora MSC, Montreal, Canada). Through this server, digital slides can be uploaded for digital consultation. This software system can be used also for performing telerevision, quality assurance and tumor boards[10].

Daily DSI based telerevision and quality assurance of difficult or borderline breast cases has been applied successfully at the University of Arizona showing a high concordance rate of 90.3% between diagnoses using glass slide and DSI. Additionally, it allows rapid revision of the cases and rendering diagnosis[29].

In line with the fact that DSI exploration turns out to be slightly more time consuming than glass slide examination, the creation of programs for computer assisted diagnosis or automated detection for region of interest might be a valuable

tool, which may also decrease inter-observer variation with respect to grading or counting for example[10, 30]. To this end high performance computing resources would probably be needed to be able to apply several algorithms to DSI in the background, before the images are presented to the pathologist[31]. Software for computerized quantification of immunohistochemically stained DSI for objective assessment of the immunoreactivity is available from several scanner vendors. Some of these applications have clearance by the USA Food and Drug Administration (FDA) such as Automated Cellular Imaging System (ACIS III) and Aperio software that has approval for their Hercep test, ER and PR applications. Pathology Image Analysis and Management software from Bioimagene also received a FDA approval for semi-quantitative assessment of HER2 staining[32]. Especially for HER2 scoring in breast cancer it has been shown that DSI based image analysis provides higher concordance rate with fluorescence in situ hybridization (FISH) than eyeballing and lowers inter-observer variability[33]. Similar promising results were obtained for scoring the intensity of immunofluorescent stains[34] .

Other experiments are ongoing in digital pathology, for example stitching of scanned serial sections to each other resulting in three dimensional orientation within the given specimen which may give better insight for invasive breast carcinoma[10, 11].

In conclusion, we propose that primary histopathological diagnosis of breast biopsies and resections can well be done on DSI.

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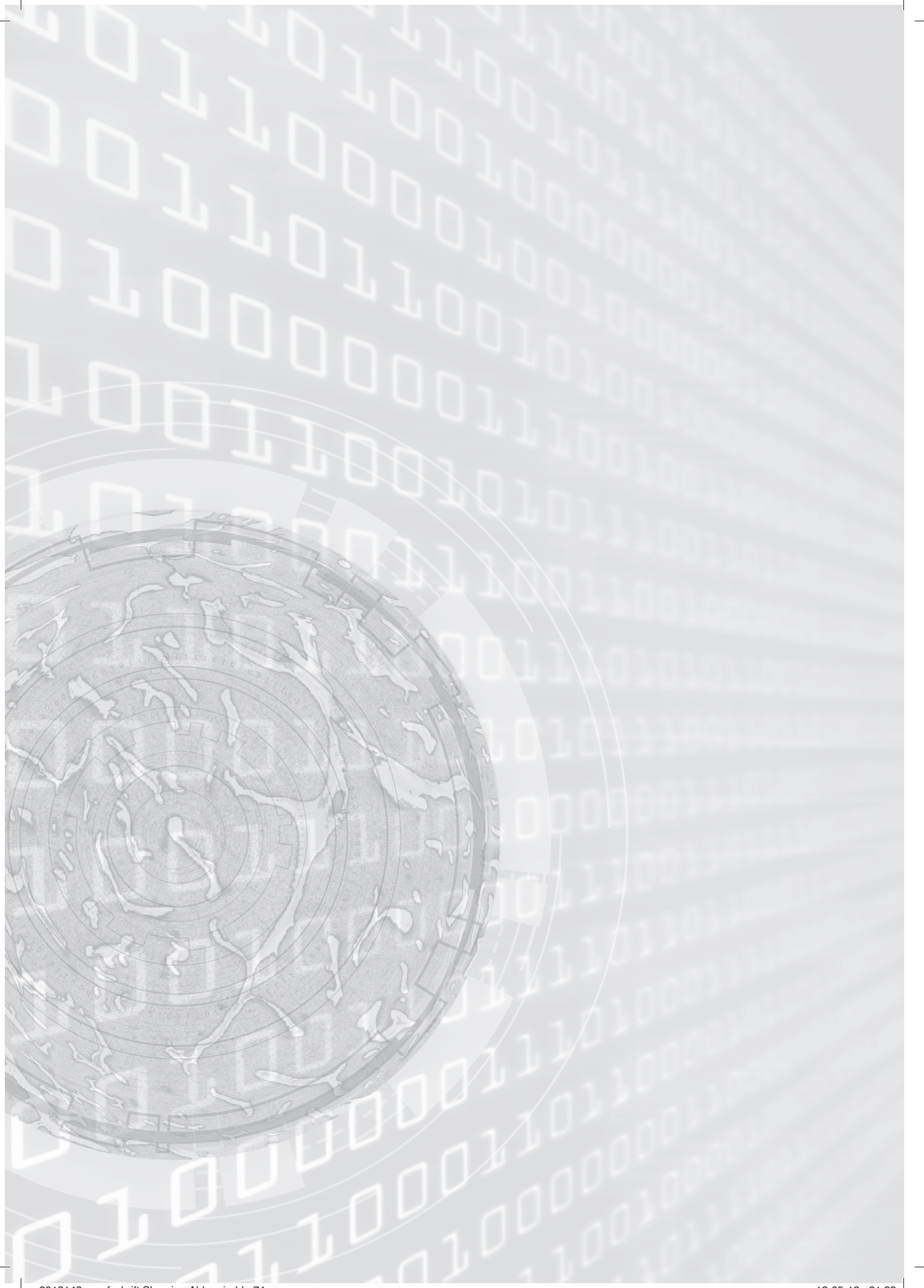

Chapter 6

Whole slide images for primary diagnostics of pediatric pathology specimens: A feasibility study

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Abstract

Introduction

whole slide images (WSI) have been used in many pathology applications such as teleconsultation, teaching and research, but not in primary diagnostics. The aim of this study was to test the feasibility of using WSI in primary diagnostics of pediatric pathology specimens and placental tissue.

Materials and methods

Eighty consecutive tissues biopsies and resections from patients under 18 years old were selected, as well as twenty placentas. These cases had been diagnosed in the year 2009 by a single pathologist. The same pathologist who had performed the original diagnosis based on light microscopy was asked to re-diagnose these 100 cases on WSI scanned at 20x magnification as well as by light microscopy having the original clinical information available, but blinded to the original light microscopic diagnoses. The original diagnoses were compared with WSI based diagnoses and rediagnoses by light microscopy and classified as concordant, mildly discordant (without clinical consequences) and discordant (with clinical consequences).

Results

The original diagnoses were concordant with WSI and light microscopic diagnoses in 90% and 93% of cases respectively, which was not significantly different. Digital reassessment yielded 8 mild discrepancies and 2 discrepant cases (2%) where the difference in diagnoses could have clinical implications for the patient. Light microscopic reassessment showed 7 mild discrepancies. It turned out to be difficult to identify nucleated red blood cells on WSI, even when scanned at 40x.

Conclusion

Primary diagnostics of pediatric tissue biopsies and resections can generally well be done on WSI. However, some difficulties were encountered in examining placenta tissue where the identification of nucleated red blood cells may need higher resolution or even scanning at multiple focus depths, which is well possible on most current slide scanners.

Introduction

Imaging technology has revolutionized the field of pathology by the introduction of new ways of tissue examination and rendering diagnosis[1, 2]. This is achieved by automatic digitization of the complete glass slides, producing what is commonly referred to as Digital Slides or Whole Slides Images (WSI). The current technology allows the examination of tissue section on a computer screen by the aid of viewers enabling the examination of the complete slide in a way comparable to conventional microscopy, navigating through the slide in any direction and at varying magnifications[3-5]. Exploration of the same digital slide by multiple examiners from different locations, examination of multiple slides at the same time (allowing side-by-side comparison of different staining of the same specimen), the presence of an overview image facilitating the navigation within the digital slides, easy integration of annotations into WSI are all supplementary features provided by image viewers[1, 3, 6]. The afore mentioned criteria have supported the use of WSI in different application in pathology for example remote consultations, primary frozen section diagnosis, quality assurance, education and research[1, 7-11]. However, WSI for upfront diagnostics are the least practiced application of digital pathology. Nevertheless, they have been used for upfront diagnostics in some laboratories after performing their local validation studies, for example in Atrium Medical Center Heerlen, Heerlen, The Netherlands[12] and Kalmar County Hospital, Kalmar, Sweden [13].

The integration of WSI for daily routine diagnostics is accompanied by many challenges such as controlling image quality, standardization of image storage and retrieval, integration of WSI into software systems in place and legal aspects, which are crucial issues that need to be discussed before WSI for primary diagnostics become common practice in pathology. Moreover, WSI have not been approved yet by Food and Drug Administration (FDA) for primary diagnostics. Gaining FDA approval will definitely encourage the general use of WSI for upfront diagnostics, especially in the United States, but it may also warrant extensive validation studies to test the feasibility of WSI for this purpose, especially because the FDA has classified whole slide scanners as Class III medical devices (Slide scanner classification).

The aim of the present study was to evaluate the use of WSI for upfront diagnostics of placental tissue, and biopsies and resection from different body systems of patients under 18 years of age. To our knowledge this is the first article examining the validity of WSI for upfront diagnostics in pediatric pathology. This study is part of a large project aimed for systematic validation of WSI for upfront diagnostics in different areas of pathology[14-16] .

Materials and Methods

This study has been conducted in the Department of Pathology, University Medical Center Utrecht (UMCU), a medium size academic pathology laboratory in The Netherlands handling about 144,000 surgical pathology and 12,000 cytology slides each year. Since November 2007, all histopathology slides have routinely been scanned after they had been diagnosed on a light microscope. Scanning is performed on ScanScope XT scanners (Aperio, Vista, CA, USA). The produced WSI are stored on a dedicated mass storage environment and linked to the pathology report, based on the recognized barcode on the slide label.

Primary pediatric pathology diagnostics on WSI

For the first part of this study 80 consecutive tissue (67 biopsies and 13 resections) from patient under the 18 years and 20 placentas with a complete set of well focused WSI (20x magnification) were selected. These cases had been diagnosed by light microscopy by one pathologist in 2009. The same pathologist who did the original diagnosis was asked to re-diagnose his own cases blinded to the original diagnosis on two other occasions; first digitally and then microscopically. The wash out time between rendering diagnosis by each modality was more than one year. Table 1 gives an overview of the anatomical site and the specimen type of the 100 cases included in this study. Digital and new microscopic diagnoses were compared with the original diagnoses by three independent pathologists and categorized as in our previous articles into [14-16]:

- Concordant; complete agreement between the original diagnosis and second diagnoses (digital and microscopic)
- Mildly discrepant; mild differences between original and second diagnoses (digital and microscopic) which would not have any clinical or prognostic implications

Table 1. Overview of the anatomical site and the specimen type of 100 pediatric pathology cases re-diagnosed on WSI.

Site	Biopsy	Resection	Grand Total
Gastrointestinal tract	64	2	66
Genitourinary tract	1	7	8
Respiratory tract	1		1
Skin	1	3	4
Placenta			20
Tonsil		1	1
Grand Total	67	13	100

- Discrepant; differences with clinical and/or prognostic implications for the patient

The percentage agreement, 95% confidence interval (CI) and the level of significance (using Fisher's exact test) was calculated using SPSS software.

Identification of Nucleated Red Blood Cells on WSI

The second part of the study tested if the identification of nucleated red blood cells (NRBCs) in placental WSI scanned at 40x would work better than on 20x, which was found to be difficult in the first part. Six months after the 20x digital diagnosis, the placenta cases were presented for the second time to the same pathologist to be examined by light microscopy first and 3 months later on WSI scanned at 40x. The pathologist was asked only to note the presence or absence of NRBCs; for which the light microscopic and 40x digital diagnoses were compared.

Results

Primary diagnostics on WSI

WSI based diagnoses and microscopic diagnoses were concordant with the original diagnoses in 90 % (95% CI 0.84-0.96), 93% (95% CI 0.88-0.98) of cases respectively (P=0.144).

Digitally there were ten discrepancies, eight (8%) of them showed mild discordance between digital and original diagnoses without clinical or prognostic implications for the patient. However, in two cases (2%) the difference in the diagnosis could be associated with clinical implications. Only 7 mild discrepancies were seen between original and second light microscopic diagnoses. Table 2 shows the rates of concordance between digital, microscopic and original diagnosis in different body systems.

Out of 100 cases, there were about 66 cases from different regions of the gastrointestinal tract (GIT) for which WSI based diagnosis and light microscopic diagnoses were concordant with the original diagnosis in 93.9 % (62/66, CI: 0.88-0.99) and 98% (65/66, CI: 0.95-1.0) of cases, respectively.

Digitally there were 4 discrepancies between original and WSI based diagnosis. Two of them (cases 1, 2, Table 3) were mild while the other two (cases 3, 4, Table 3) were discrepant with potential effects on the patient. Case number 3 concerned a biopsy from the rectum. The clinical history in this case was suggestive of M. Hirschsprung (Morbus Hirschsprung). The report from WSI confirmed the absence of ganglion cells and presence of hyperplastic nerve tissue bundles in three stains (H&E, ACE, SDH) but the presence of one ganglion cell was suspected on the NADH stained slide. Based on the latter stain the diagnosis M. Hischprung was

rejected. This case had been diagnosed before as M. Hirschsprung on light microscopy. On revision, the light microscopy diagnosis was deemed the better one. The other case (case 4) concerned a small intestinal resection. The light microscopy diagnosis was "Small intestinal resection with perforation and ulceration with evidence of candidiasis" while WSI based diagnosis was "Mild reactive changes with ischemia, ulceration and necrosis. No candidiasis". On revision, Candidiasis turned out to have been missed while performing the digital diagnosis. Figure 1 is a snapshot from an area of the WSI from this resection showing the site with Candidiasis.

Light microscopically there was only single mild discrepancy where the microscopic descriptions were similar but the final interpretation differed. Table 4 details the discrepancies between original and light microscopic diagnoses.

A lower concordance rate (70%, CI: 0.48-0.92) between original, WSI based diagnoses and microscopic diagnoses was encountered in the placenta cases. Out of the 20 placenta cases included in this study, there were six mild discrepancies (digitally and microscopically) where the pathologist missed the presence of inflammation either in the villi, chorion or umbilical cord. These discrepancies were mild without any further effect on patient treatment and prognosis (Tables 3, 4).

In cases from the skin, tonsil, genitourinary and respiratory system there was 100% agreement between the original and the second diagnoses (digital and microscopic) (Table 2).

Table 2. Overview of discrepancies between original light microscopy based diagnosis and digital re-diagnosis on WSI of pediatric pathology cases.

Site	Sort diagnosis	Agreement			Total	Percentage agreement
		Concordant	Mild discrepant	Discrepant		
Gastrointestinal tract	Digital	62	2	2	66	93.9%
	Microscopic	65	1		66	98%
Genitourinary tract	Digital	8			8	100%
	Microscopic	8			8	100%
Placenta	Digital	14	6		20	70%
	Microscopic	14	6		20	70%
Respiratory tract	Digital	1			1	100%
	Microscopic	1			1	100%
Skin	Digital	4			4	100%
	Microscopic	4			4	100%
Tonsil	Digital	1			1	100%
	Microscopic	1			1	100%
Total	Digital	90	8	2	100	90%
	Microscopic	93	7		100	93%

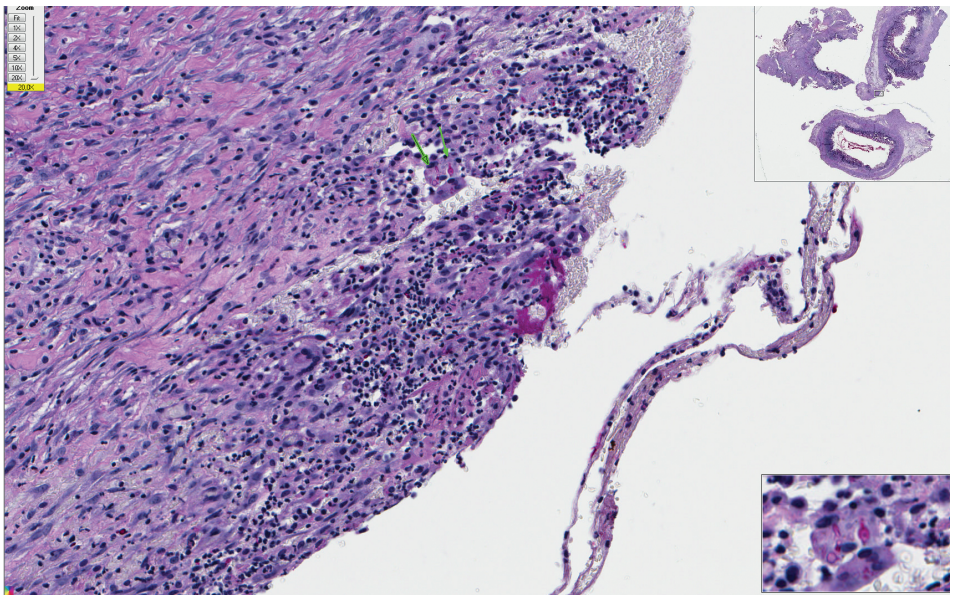

Figure 1. Snapshot from an area of the WSI from small bowel resection showing the site of Candida infection (green arrows) which was missed on WSI based diagnosis.

In further analysis by side-by-side comparison of both glass slides and WSI of the 10 discrepant cases, nine original diagnoses were considered better and in one case the WSI diagnosis was deemed the better one (Table 3)

Identification of NRBCs on WSI

For the second part of the study with 40x digital placenta sections, 16/20 cases were positive for NRBCs by light microscopy and 4 cases were negative for NRBCs. On WSI, the pathologist reported 10 cases as positive for NRBCs and 3 cases as negative for NRBCs, while in 7 cases the pathologist could not confidently give a diagnosis. WSI diagnoses in regard to NRBCs were thus concordant with light microscopy diagnosis in only 65% of the cases (CI: 0.42-0.88). Figure 2 is a snapshot of WSI from placenta section showing nucleated RBCs.

Discussion

The aim of this study was to test the validity of WSI for primary diagnostics of pediatric pathology. WSI based and light microscopic rediagnoses were concordant with the original diagnosis in 90% and 93% of cases, respectively, which was not significantly different. There was a mild discrepancy between original and WSI

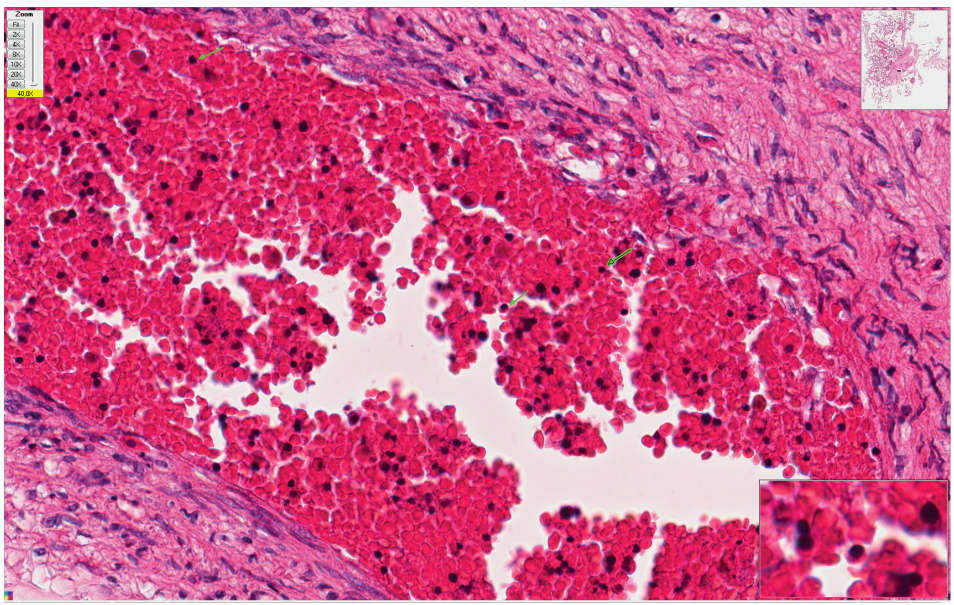

Figure 2. Snapshot of WSI from placenta section showing nucleated RBCs (green arrows).

based diagnoses in 8% of cases without clinical or prognostic implications for the patient, and 2% discrepant cases where the difference in the diagnosis could affect patient treatment and prognosis. All of the discrepancies in the second round of light microscopy were mild without clinical implication for the patient. We would therefore consider the WSI discrepancy rate to fall within the range of the intra-observer variability in light microscopic pathology as shown in the present study and by others[17, 18]. The 90% concordance rate between the original and WSI based diagnoses is also within the range of that in several other validation studies which examined the performance of the WSI for primary or secondary histopathologic diagnosis[19-24]. In line with this, for one case the WSI based diagnosis was even considered to be the better one.

Digitally, there were two discrepant cases with clinical implication to the patient. In case 3 (see Table 3), the pathologist rejected the diagnosis of M. Hirschsprung because of the suspected presence of one ganglion cell in one stain. In routine practice, if the pathologist is not sure about the diagnosis of M. Hirschsprung and clinical features were suggestive of the disease, contact between the pathologist and the pediatrician should take place to make a plan to arrive at a more certain diagnosis. However, such contact was missing within the research context described in this paper. We assume that if WSI are adopted in the routine work, the same working standards for routine pathologic diagnosis based on conventional

Table 3. Original and WSI based diagnosis of the ten discrepant cases digitally.

Case	Site	Clinical data	Original Diagnosis	Digital Diagnosis	Discrepancy type	Preferred Diagnosis
1.	Stomach	Suspicion of reflux, red stomach	Without specific changes	Very minor chronic changes	Mild	WSI
2.	Terminal ileum	Prednisone resistance. Morbus Crohn?	Active chronic inflammation with ulceration	Mild active inflammation, no granuloma.	Mild	Original
3.	Rectum	Suspicion of Morbus Hirschsprung?	Morbus Hirschsprung	No morbus Hirschsprung	Discrepant	Original
4.	Small intestine	Perforation with free air 7 days after resection. Leakage?	Small intestinal resection with perforation and ulceration with evidence of candidasis.	Mild reactive changes with ischemia, ulceration and necrosis. No candidiasis	Discrepant	Original
5.	Placenta	Mother with history of epilepsy without medication. Baby born with lethal kidney abnormality. Placental abnormality?	Severe chorioamnionitis, funiculitis, chronic villitis	Severe chorioamnionitis, mild chronic villitis	Mild	Original
6.	Placenta	Intrauterine Growth Retardation (IUGR), placental abnormality?	Mild chronic villitis, diffuse ischemia and infarction	Mild ischemic changes, mild chorioamnionitis, moderate chronic villitis and mild delay in the maturation	Mild	Original
7.	Placenta	Premature delivery, placental abnormality?	Extensive chorioamnionitis especially in the chorionic plate, and features suggestive of chronic abruption oligohydro amnios sequence (CAOS).	Presence of some acute inflammatory cells, and features of placental abruption	Mild	Original
8.	Placenta	Fetal distress, meconium. Placental insufficiency?	Chorioamnionitis, funiculitis and chronic villitis with unknown origin, delay in maturation	Mild ischemia and mild delay in the maturation, very mild chronic villitis and chorioamnionitis of unknown origin	Mild	Original
9	Placenta	Dichornioic diamniotic twin pregnancy, IUGR.	Mild chorioamnionitis and chronic villitis from unknown etiology.	Very mild chorioamnionitis, no villitis	Mild	Original
10.	Placenta	Diabetic mother, Placental abnormalities?	Paranchymal changes suggestive of Diabetes Mellitus (DM). mild chronic villitis of unknown origin	Very mild ischemic changes, slight delay in maturation compatible with DM	Mild	Original

Table 4. Original and light microscopic diagnosis of the light microscopically discrepant cases.

Case	Site	Clinical diagnosis	Original light microscopic diagnosis	Second light microscopic diagnosis	Discrepancy type
1.	Esophagus	History of eosinopilic esophagitis, eosinophilia? Inflammation?	Eosinophilic esophagitis	Massive eosinophilic infiltration, not enough for eosinophilic esophagitis but consistent with reflux.	Mild
2.	Placenta	Missed abortion, trophoblastic changes or other abnormality?	Parts of first trimester pregnancy with degenerative changes and signs of Intrauterine Fetal Death (IUFD) and parenchyma changes suggestive of chromosomal (CHX) abnormality.	First trimester parenchymal tissue with a signs of IUFD.	Mild
3.	Placenta	Premature delivery, placental abnormality?	Extensive chorioamnionitis especially in the chorionic plate, and features suggestive of CAOS.	No inflammation, no ischemia, no villitis, some degenerative changes in the membranes, iron deposition in chorionic plate. The picture is consistent with mild COAS	Mild
4.	Placenta	Spontaneous abortion, Abnormalities?	35 weeks placenta with less than 5% micro-infarction, no other abnormality seen.	Grade one chronic villitis, ischemia and small infarction, normal maturation (close to term)	Mild
5.	Placenta	Premature delivery. Chorioamnionitis?	Placenta with normal weight and first signs of chorioamnionitis	Normal for gestational age (GA), no inflammation, no funiculitis, no chorioamnionitis	Mild
6.	Placenta	Dichorionic diamniotic twin pregnancy, IUGR.	Mild chorioamnionitis and chronic villitis from unknown etiology.	Bichorionic placenta. The first part shows some increase in the maturation. The second placenta shows more advanced maturation and mild chronic villitis.	Mild
7.	Placenta	Diabetic and hypertensive mother, preeclampsia? Infarction?	Placenta, mild chorioamnionitis and infarction	Recent and old infarction, no signs of maternal diabetes	Mild

microscopy will be adopted. This fact has been supported by of Royal College of Pathologist in their 2003 guidelines 'Code of practice for pathologists participating in remote reporting of histopathology or cytopathology'. In this report, it has been stated that the remote pathologist should take into consideration that all the necessary data (clinical, laboratory feedback, contact with clinician) are available to guarantee a good quality of the pathology report irrespective to the diagnostic modality (whether based on glass slides or telepathology system) (Remote reporting pdf).

The identification of microorganisms like Candida albicans, Helicobacter pylori and Giardia lamblia was sometimes difficult. Scanning at 40x magnification would probably have given a more confident diagnosis of microorganisms. Scanning at higher magnification may therefore be preferable and will likely be the future standard, but appears not to be very relevant for most cases while adding scanning time and necessitating significantly more storage. Missing microorganisms happened in only one case in the present study where the pathologist was not sure about the presence of microorganisms (case 4; Table 3) which resulted in a discrepant diagnosis.

A high concordance rate of 100% was seen in cases from skin, tonsils, genitourinary and respiratory system. The number of cases for these systems was however low which could be a limitation of the present study.

Digital diagnosis of cases from the placenta was slightly problematic. First, exploration of placental WSI was felt to be more time consuming than light microscopy, although no formal time measurements were performed. This might be related to the fact that a computer mouse is not the optimal tool for exploring WSI. Presenting WSI on multi-touch screen of high resolution probably with horizontal format (SurfaceSlide)[25] where navigating WSI is allowed in a simple and intuitive way may lead to better and faster exploration of WSI. Comparable solutions are available like Ergo Controller (Nikon) and iSlide input device (BioImagene). Second, placental sections require careful search for inflammatory cells and NRBCs in addition to other possible abnormalities which was shown to be easier under the microscope. In the present study, the pathologist indeed missed the inflammation either in the umbilical cord or in the villi or chorion in a few cases on WSI, but similar discrepancies were also seen when rediagnosing light microscopically (table 4). Using advanced image viewers that aid in better image presentation or assist in tracking the examined areas may aid in better digital diagnostic outcomes. Third, WSI scanned at 20x were insufficient for the identification of NRBCs in placental slides. Thus, it was decided to rescan the placenta slides at 40x magnifications to test if a higher magnification helped, and this indeed worked for some cases of the placenta with low density of erythrocytes in the vessels. However, in 7/20 cases the pathologist could still not reliably

establish the presence or absence of NRBCs due to thick preparations with cells crowding in vessels. Scanning at multiple focus levels as some scanners allow may further facilitate more confident diagnosis of NRBCs[26]. However, such technology will cost more storage and scanning time, which is for the time being inconvenient for routine diagnostics[27]. Running image analysis algorithms in the background to identify inflammatory cells, NRBCs and/or microorganism in scanned tissue sections before being presented to the pathologist will definitely help in increasing the productivity and accuracy of pathology reports.

In conclusion, histopathological diagnosis of biopsies and resections can generally be done well on WSI acquired using today's scanning technology. However WSI scanned at 20x magnification were not optimal for exploring placental tissue. A higher resolution may be necessary for more confidant identification of NRBCs and inflammatory cells in placental tissue, and also multilayer scanning may be required in some cases.

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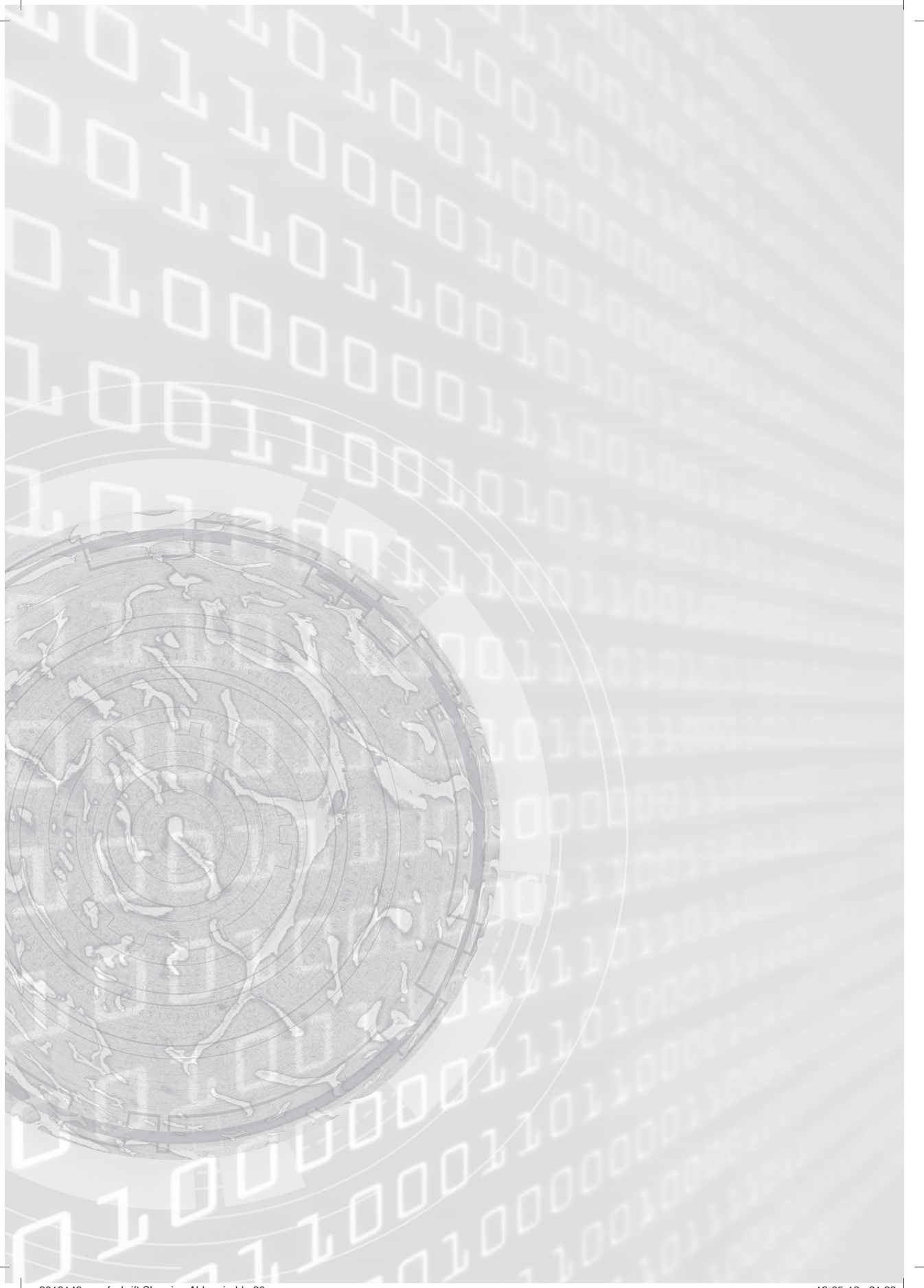

Chapter 7

Whole slide images for primary diagnostics of urinary system pathology: A feasibility study

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Abstract

Introduction

During the last decade, whole slide images (WSI) have been used in many areas of pathology such as teaching, research, digital archiving, teleconsultation and quality assurance testing. However, WSI have as yet not much been used for upfront diagnostics because of the lack of validation studies.

The aim of this study was to test the feasibility of WSI for primary diagnosis urinary tract pathology.

Materials and methods

100 consecutive urinary tract biopsies and resections which had been diagnosed conventionally between the years 2008-2009 were scanned at 20× magnification, and rediagnosed by two pathologists on WSI, having the original clinical information available, but blinded to the original diagnoses. Original and WSI diagnoses were compared and classified as concordant, slightly discordant (without clinical consequences) and discordant.

Results

Original and WSI based rediagnosis were concordant in 87% of the cases. Original and WSI diagnosis were slightly discordant in 8% of cases. Major discrepancies with clinical or prognostic implications were founded in only 5 cases. However, for 6 out of the 13 discrepancies, WSI based diagnoses were considered to be better than the original diagnoses.

Conclusion

Primary diagnostics of urinary tract specimens can be reliably done on WSI. Further improvements of image resolution may help to increase diagnostic accuracy and WSI acceptance in routine pathology.

Introduction

Whole slide imaging technology allows automatic digitization of the entire glass slides, producing what is commonly referred to as Digital Slides or Whole Slides Images (WSI). WSI are usually examined on a computer screen by the aid of image viewers enabling the examination and manipulation of the whole tissue section in a way comparable to a conventional microscope.
Easy image annotation, accessibility, sharing as well as the possibility of capturing static images for documentation, insertion of comments, and subjection of automated image analysis are all additional features intimately bound to WSI making them superior to using glass slides and a conventional microscope for several applications within the pathology workflow. Simultaneous viewing of WSI by different examiners from different places makes WSI more suitable for education, teleconsultation, pathology panels and revision. WSI can be digitally archived and retrieved minimizing the time and effort needed for preparing slides for revision or conferences. Moreover, the possibility of linking WSI to a patient's complete medical record could increase the diagnostic accuracy and decrease the errors resulting from the lack of clinically relevant information. To this end, linking WSI and patients' information to a central storage will facilitate teleconsultation and telerevision resulting in enhancing patients' care.
Despite all the advantages of digital pathology and WSI, unfortunately their use as a tool for primary diagnostics is still not widespread. One of the factors hindering WSI integration in routine pathology practice is that they have not yet been approved for primary diagnostics by the Food and Drug Administration (FDA) in the USA and the scanners to acquire WSI have been classified as class III medical devices. This makes the approval process very time consuming and expensive for scanner vendors. One of the required steps will be to setup collaborations with multiple pathology laboratories for large scale multicenter validation studies aimed for systematic validation of WSI for primary diagnostic purposes. The aim of this study was to evaluate the feasibility of primary pathology diagnosis of urinary system specimens using WSI by comparing this to the performance of using conventional microscopy. This study is a part of a larger study aimed for systematic validation of WSI for primary diagnostics in a number of different organ systems[1-4].

Materials and methods

For this study 100 cases (50 from kidney and 50 cases from other parts of the urinary system) with a complete set of well focused WSI that had been

conventionally diagnosed by two pathologists in 2008-2009 were selected. The same pathologists who did the initial diagnosis were asked to re-diagnose their own cases on WSI to exclude inter-observer variation as much as possible. The time period between the primary microscopic diagnosis and re-diagnosis on WSI ranged from 6 months to one year to guarantee wash out. The participating pathologists had varying but at least 1 years experience in using WSI for secondary diagnostics (tumor boards, education, reviewing archived slides, etc.).

WSI were per case presented to the pathologists together with the original clinical information, blinded to the original report. The selected cases consisted of 89 biopsies and 11 resections from kidney and other parts of the urinary system. Table 1 summarizes these cases in relation to their origin and the type of the specimen (biopsy or resection). Table 2 and 3 detail the diagnostic entities of cases included in this study.

The original and WSI based diagnoses were compared by three independent pathologists to judge the concordance between the two diagnoses as before [1-4] as:

- Concordant; complete agreement between the first original signed out diagnosis and the diagnosis as drawn from the whole slide image
- Slightly discrepant; mild differences which would not have any clinical or prognostic implications
- Discrepant; differences with clinical and/or prognostic implications for the patient

The better one of the two diagnoses (original or WSI based) was noted.

Results

For 87 out of 100 cases (87%, 95% CI 0.80-0.94), the light microscopy and the WSI based diagnosis were concordant. Of the other 13 cases, eight showed slight discordance between the digital and the light microscopic diagnoses without any

Table 1. Specimen type and origin in the urinary tract of cases included in this study.

Organ	Specimen type		Total
	Biopsy	Resection	
Kidney	45	5	50
Bladder	41	2	43
Ureter		1	1
Urethra	3	3	6
Total	89	11	100

clinical or prognostic implications for the patient, while in five cases the discrepancy could have an effect on patient treatment and prognosis.
Re-assessment of the glass slides and the WSI for the discrepant cases by the three reviewing pathologists revealed that in six cases the WSI diagnosis was preferred over light microscopy diagnosis while the original light microscopy based diagnosis was preferred in five cases. However, in two cases (one discrepant and one slightly discrepant) both diagnoses gave imperfect description to the problem. Table 4 details these discrepant cases.
In the subgroup of 50 biopsies and resections that originated from the kidney, the original microscopic diagnoses were concordant with WSI based diagnoses in 42 cases (84%, 95% CI: 0.73-0.95). In five cases, there were major discrepancies with possible clinical implications on the patient treatment and prognosis. Reassessment of the glass slides for these discrepant cases by the reviewing pathologists revealed that WSI based diagnoses were preferred in two discrepant cases and the original light microscopic diagnoses were preferred for two cases as well.

Table 2. Primary diagnoses of fifty cases originating from the kidney.

Disease category	Type kidney		Total
	Native	Transplant	
Vascular		6	6
Glomerular	11	2	13
Tubulointerstitial	2	13	15
Tubulointerstitial and vascular		6	6
Tubulointerstitial, vascular and glomerular		1	1
Developmental anomaly	2		2
No specific abnormality		5	5
Carcinoma	2		2
Total	17	33	50

Table 3. Primary diagnoses of fifty cases non-kidney cases included.

Location	Diagnosis entity			Total
	Benign	Neoplastic	Normal	
Bladder	22	16	5	4 3
Ureter	1			1
Urethra	4	2		6
Total	27	18	5	50

Table 4. Details of all cases with discrepancies between light microscopic and digital diagnoses.

	Tissue type	Microscopic diagnosis	Digital diagnosis	Type discrepancy	Preferred diagnosis
1.	TK	Acute cellular tubulointerstitial rejection and suspicion of vascular rejection	Chronic damage with reactive inflammatory infiltrate. Insufficient evidence for acute rejection or toxicity	Discrepant	Both imperfect
2.	TK	Acute vascular rejection (Banff IIA) with thrombotic microangiopathy	(Sub-)acute thrombotic microangiopathy	Discrepant	Original
3.	TK	Kidney biopsy with an acute borderline cellular tubulointerstitial rejection	Less than 5 % IFTA, slight ischemic changes in the glomeruli. Insufficient evidence for rejection	Discrepant	Original
4.	TK	Slight acute tubular damage. No signs of rejection or ATN	Calcineurin inhibitor toxicity. Insufficient evidence for rejection	Discrepant	Digital
5.	TK	Acute borderline cellular tubulointerstitial rejection	Calcineurin inhibitor toxicity. Insufficient evidence for rejection	Discrepant	Digital
6.	TK	Biopsy with heavy inflammation and signs of acute tubulointerstitial rejection (Banff grade IA) BK –ve	Severe acute tubulointerstitial rejection, with heavy inflammatory infiltrate with apparent disruption of tubular basement membrane, suggestive of Banff grade 1B acute tubulointerstitial rejection	Mildly discrepant	Original
7.	TK	Granulomatous TIN. Drug induced? Acute cellular tubulointerstitial rejection cannot be excluded.	Antibody mediated rejection (capillaritis). Acute cellular tubulointerstitial rejection with Granulomatous reaction and destruction of tubules consistent with Banff grade IB rejection	Mildly discrepant	Digital
8.	TK	Tubulointerstitial and vascular rejection (Banff grade IIA) Suspected antibody mediated rejection component	Tubulointerstitial rejection (Banff grade IA)	Mildly discrepant	Digital
9.	Bladder	TUR with small location of transitional cell carcinoma grade 3 without evidence of invasive growth in addition to the presence of loose group of cells which is strongly atypical	Grade 3 transitional cell carcinoma, invasive in lamina propria	Mildly discrepant	Both imperfect

Table 4 continued

10.	Bladder	Erosive active chronic inflammation with the presence of loose atypical tissue fragments which cannot be good assessed	Necrosis and moderate chronic inflammation, insufficient for CIS diagnosis	Mildly discrepant	Original
11.	Bladder	Mechanical tissue damage with papillary transitional cell carcinoma grade 3. The picture is suspicious for superficial invasive growth but no definite diagnosis	Papillary transitional carcinoma grade 3, focally invasive in lamina propria with well circumscribed CIS	Mildly discrepant	Original
12.	Bladder	Large fragment of muscular tissue without malignancy with the presence of superficial fragments of transitional cell carcinoma, no invasion	Loose tumour cells (transitional cell carcinoma) and muscles fragments. Invasion cannot be assessed	Mildly discrepant	Digital
13.	Bladder	Bladder biopsy without specific abnormality	Chronic inflammation	Mildly discrepant	Digital

TK: transplanted kidney, TUR: Transurethral resection, TIN: Tubulointerstitial nephritis, ATN: Acute tubular necrosis, BK: virus, CIS: carcinoma in situ, IFTA: interstitial fibrosis and tubular atrophy

For one discrepant case, both digital and light microscopic diagnoses gave imperfect description of the underlying pathology. In this case, tubulointerstitial rejection and suspicion of vascular rejection had been stated microscopically which was not confirmed digitally (case 1, table 4). On revision by conventional microscopy, the presence of tubulointerstitial rejection was confirmed but evidence of vascular rejection was considered insufficient.
For renal specimens, discrepancies were mostly related to over- or underestimation of rejection. In addition, there were about 3 other mildly discrepant cases where the difference between conventional microscopy and WSI would not have an effect on patient treatment and prognosis.
In the subgroup of 50 cases that originated from the other parts of urinary system, the WSI based diagnoses were concordant with the light microscopic diagnoses in 90% of the cases (95% CI: 0.81-0.99). All of these discrepancies were mild without further clinical implication. Reassessment of the glass slides by the reviewing pathologists, revealed that the WSI based diagnoses were preferred in two cases and the original light microscopic diagnoses were preferred again in two cases, whereas in one case both diagnoses were imperfect (case 9, table 4). In this resection, invasion was proposed digitally but could not be confirmed microscopically. On revision, both diagnoses were considered to be imperfect and "invasion cannot be excluded" was concluded to be the best description of the lesion.

Discussion

The aim of this study was to test the feasibility of using WSI for primary diagnosis of tissue biopsies and resection specimens originating from the kidney and other parts of the urinary tract. One hundred cases received between 2008 and 2009 were retrospectively collected and blindly re-diagnosed by two pathologists on the bases of WSI after a wash-out period of at least six months. The re-diagnosis was done by the same pathologists who performed the initial diagnosis to avoid inter-observer variations due to e.g. difference in experience. The re-diagnoses were concordant with the original light microscopy diagnosis in 87% of cases (95% CI 0.80-0.94). There were mild discrepancies between the light microscopy and the WSI based diagnoses in 8% of the cases, without clinical or prognostic implications to the patients. However, in 5 cases (5%) the pathology reports obtained by the two diagnostic modalities were discrepant with potential impact on the patient's treatment.
The concordance rate of 87% and the mild rate of discrepancies are within the range of previously observed inter- and intra-observer variability in microscopic

pathology in general[5-7] and in renal pathology specifically[8-13], and is in line with previous similar studies by us in other organ systems[1-4]. Furthermore, in 6 out of 13 discrepancies the WSI diagnoses were deemed better. These results indicate that WSI may reliably be used for primary diagnostics of urinary system specimens. Despite the fact that several studies have emphasized the benefits of WSI in different pathology applications and also in primary diagnostics, integrating WSI in the routine workflow will probably not be achieved unless pathologists are convinced that the diagnostic performance on WSI is not inferior to a light microscopy based diagnoses based on glass slides[14]. This requires solid evidence obtained from well-designed validation studies genuinely reflecting the reliability of digital pathology.

WSI based diagnostics offers a seamless and reliable medium for revising cases and providing pathology diagnostic services especially to remote hospitals lacking an on-site pathologist. This fact has been illustrated in a study of Furness et al. where the adequacy of WSI as a medium for internet-based telepathology was evaluated by multiple examiners in the context of The National Renal Pathology External Quality Assurance scheme in the UK[15]. Their results have shown no significant difference between the diagnostic accuracy of the pathology reports derived from WSI and conventional microscopy; this could endorse the frequent use of this technology in the quality assurance programs.

The results in the present study are comparable to other studies that evaluated the validity of WSI for primary diagnostics of renal specimens. In a study by Ozluk et al., three pathologists scored 11 pathologic criteria derived from the Banff classification of renal transplant in 40 renal biopsies and eventually constructed the final conclusion of acute rejection or transplant glomerulopathy. Each biopsy was examined by each observer independently on four occasions; twice microscopically and twice on WSI with at least 3 weeks time in between each diagnostic modality. Their results revealed good intraobserver reproducibility of Banff scoring system using WSI as well as glass slides. Moreover, there was no significant difference in evaluating acute rejection using both diagnostic methods. Glomerulopathy score was the most reproducible feature with almost similar accuracy between glass slides and WSI[16]. The drawback of this study is the multiple readings within a relatively short time, as the pathologists might have remembered the diagnosis in some of the cases.

Jen et al. investigated the validity of WSI in evaluating renal allograft biopsies. Six pathologists assessed the presence of certain morphologic features and acute rejection in 25 renal biopsies using conventional microscope and WSI with at least a period of two weeks in between the two diagnostics. Their results showed substantial agreement between glass slides and WSI based diagnoses in assessing specific morphologic criteria and acute rejection. Moreover, the interobserver

agreement was shown to be comparable between the two diagnostic modalities[17]. The low number of cases included in that study and the short time between the examinations of the cases are however limitations of their study.

The resolution of WSI scanned at 20x was perceived to be on the low side for rendering diagnostics of renal specimens. Evaluating the status of transplanted kidney and the possibility of transplant rejection requires careful assessment of fine morphologic features among which is the presence of inflammation, in particular tubulitis, fibrosis and subtle changes in glomeruli, blood vessels and tubules. This task was found to be slightly more difficult and time consuming on 20x WSI than in conventional microscopy. Moreover, with the digital readings in this study, clinical information provided on transplant biopsies was generally less extensive than with the original microscopic evaluation, and also feedback from interdisciplinary discussion was lacking, which all might have contributed to discrepancies in 5 cases when comparing digital with conventional readings. However, issues related to lower resolution scan are expected to be solved in the near future especially with the presence of high throughput scanners which are able to scan the whole slide in less than one minute.

Rendering diagnosis on 20x WSI for biopsies and resections from the other parts of the urinary tract was considered to be relatively easier. This was reflected by the higher concordance rate of 90% and the mild discrepancies with minimal clinical impact on patient.

One of the limitations hindering the use of WSI for primary diagnostics of urinary system specimens is the time needed for image exploration. Examining WSI was perceived to take considerably more time than evaluation by conventional microscope (although no formal timing has been conducted). This has also been noted in the study of Jen et al. where exploring WSI cost 1.4 longer time than using glass slides and conventional microscope. Relative lack of routine, still limited image resolution and suboptimal navigation tools might all have contributed to this difference. We expect that the impact of time factor will be reduced when a high resolution scan becomes a common standard in pathology and with the introduction of more user-friendly interfaces where exploring WSI can be done in simple intuitive way as using efficient tools for navigating through the image instead of the mouse[18].

Implementing WSI in primary diagnostics will enhance pathology practice especially for sub-specialties such as transplantation pathology. With the aid of WSI, problematic or difficult cases can be efficiently shared immediately with one or more experts within suitable time constrains sparing the time required for shipping glass slides to far places. Integrating WSI into a patient's medical report will allow the pathologists to work within an integral environment including the clinical information, pathology data besides the pathology specimens which will

eventually permit comparing new and old patient's materials to evaluate the progress in the patient's condition. WSI can also be electronically archived and retrieved decreasing the time spent on searching for glass slides for consultation, conferences, teaching and research purposes. Furthermore WSI can be subjected to automated image analysis which is believed to improve the productivity and objectivity in daily diagnostics.

The above mentioned features may encourage considering WSI as platform for primary diagnostics in pathology. Nevertheless, integrating WSI in routine practice may still require investing the efforts for step-wise conversion from conventional to digital practice.

In conclusion, primary diagnostics of urinary system specimens can overall be reliably done on WSI scanned on 20x. However higher resolution scans may be required especially in assessing the status of renal transplants.

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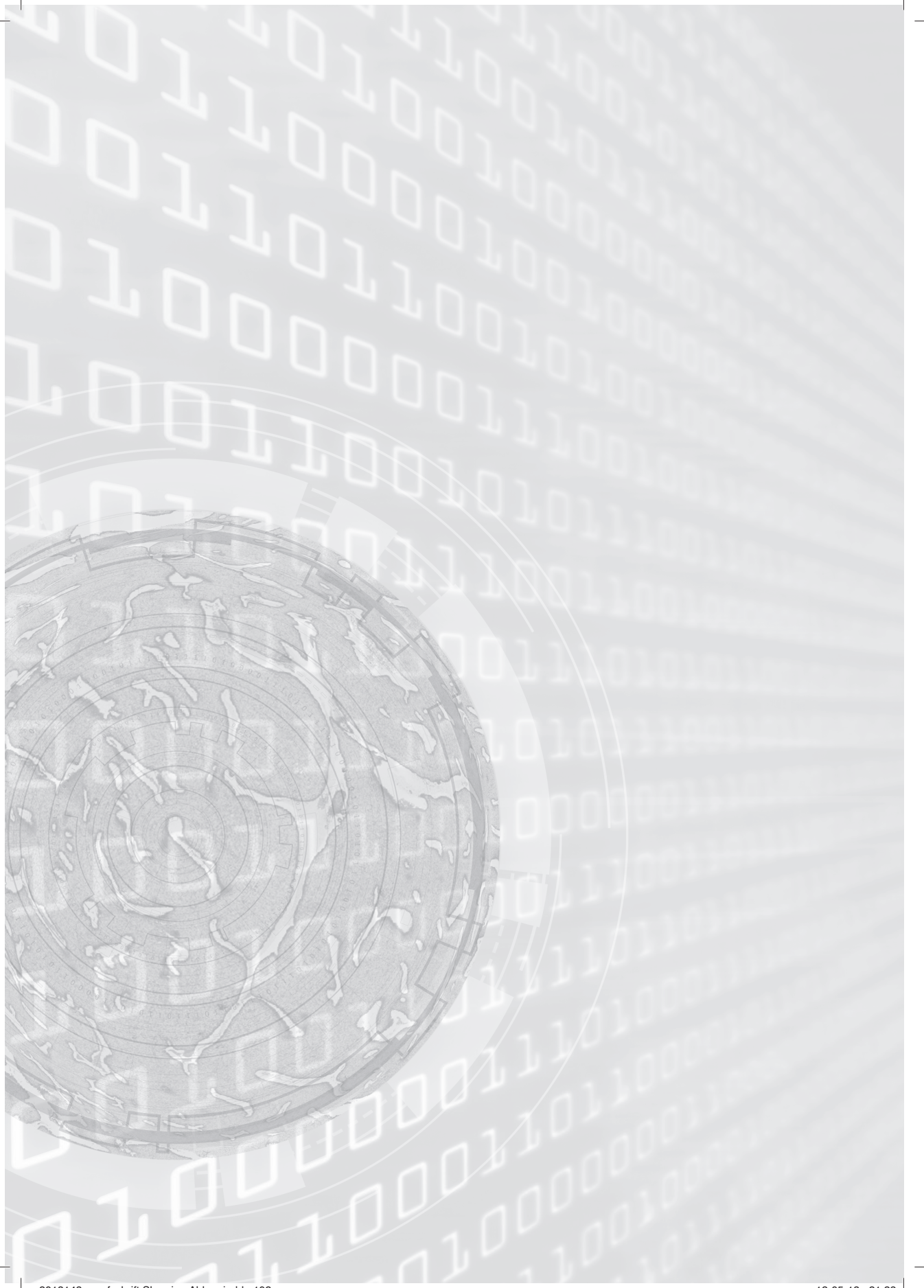

Chapter 8

Evaluation of mitotic activity index in breast cancer using whole slide digital images

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Abstract

Introduction

Mitotic Activity Index (MAI) is an important independent prognostic factor and an integral part of the breast cancer grading system. Thus, correct estimation of this prognostically relevant feature is essential for guiding treatment decision and assessing patient prognosis. The aim of this study was to validate the use of high resolution Whole Slide Images (WSI) in estimating MAI in breast cancer specimens.

Materials and methods

MAI was evaluated in 100 consecutive breast cancer specimens by three observers on two occasions, microscopically and on WSI with a wash out period of 4 months. MAI was also translated to mitotic scores as in grading. Inter- and intra-observer agreement between microscopic and digital MAI counts and scores was measured.

Results

Almost perfect inter-observer agreements were obtained from counting MAI using a conventional microscope (intra-class correlation coefficient (ICCC) 0.879) as well as on WSI (ICCC 0.924). K coefficients reflected good inter-observer agreements among observers' microscopic mitotic scores (average kappa 0.642). Comparable results were also observed among digital mitotic scores (average kappa 0.635). There was strong to perfect intra-observer agreements between MAI counts and mitotic scores for the two diagnostic modalities (ICCC 0.716-0.863, kappa 0.506-0.617). There were no significant differences in mitotic scores using both diagnostic modalities.

Conclusion

Scoring mitoses using WSI in breast cancer seems to be just as reliable and reproducible as when using a microscope. Further development of software and image quality will definitely encourage the use of WSI in routine pathology practice.

Introduction

More than a decade ago, the practice of pathology began changing, with the introduction of slide scanners which enable the acquisition of pathology information from glass slides and translate it into a digital form commonly known as digital slides or Whole Slide Images (WSI). WSI provide the possibility of viewing and manipulating pathology samples on a computer screen in a way comparable to a conventional microscope[1,2]. Moreover, WSI boast many advantages over glass slides and a conventional microscope; including easy image accessibility, sharing, annotating and amenability to automated image analysis which is believed to improve the objectivity and productivity within pathology practice. These features facilitated WSI integration in different pathology applications, mainly used for education, consultation, frozen section diagnosis, quality assurance, clinico-pathological conferences and research[3-7]. Despite the fact that several validation studies have shown that the diagnostic performance using WSI is comparable to that of a conventional microscope[8-19], implementing WSI in primary diagnostics is still in its infancy. However, WSI have been used for this purpose in some pathology laboratories after carrying out their own local validation studies[20,21]. One of the possible factors hindering WSI integration in routine pathology practice is that they have yet to be approved for primary diagnostics by the Food and Drug Administration (FDA)[22]. Additionally, the FDA has classified whole slide scanners as Class III medical devices (Slide scanner classification) necessitating extensive systematic validation studies and premarket approval before WSI can become a platform for primary diagnostics[23].

From our previous studies concerning the validating WSI for primary diagnostics of different body systems[10-13], we concluded that WSI contain sufficient information for rendering most of the diagnostics within pathology. Nevertheless, we would expect that examining fine cellular details such as cellular division (mitosis) on WSI, scanned at one focal plane could pose some diagnostic difficulties. Thus, testing the validity of WSI in assessing this theoretically difficult but clinically relevant feature is crucial.

In breast cancer, tumor proliferation is one of the most important independent prognostic factors and is an integral part of tumor grading system which has also an impact on the determination of patient treatment[24,25]. Different techniques may be used to estimate proliferation[26-29]; the most widely applicable method used in the common practice is the estimation of the mitotic activity index (MAI). MAI is defined as the numbers of mitotic figures in a given area of tumor[30]. Traditionally, MAI is scored on glass slides and light microscopy where mitosis is counted in 10 high power fields (40x magnification) or per unit area (2 mm^2) in the most active part of the tumor[27,30,31]. Scoring MAI under a microscope requires the differentiation

of true mitoses from similar figures such as apoptotic bodies, dark nuclei and tissue artifacts, for which a three-dimensional view and a fine microscopic focusing is required. Missing the z-axis and the ability of fine microscopic focusing on WSI scanned at one focal plane, may lead to under or overestimating MAI scores on WSI. To our knowledge, this is the first multi-observer study concerned with validating the scoring of MAI in breast cancer on the bases of WSI and digital microscope.

Materials and methods

This study was performed at Symbiant Pathology Expert Center in The Netherlands, consisting of pathology laboratories at three different locations serving 6 hospitals in the province of North Holland with a population of about one million people.

One hundred consecutive breast cancer cases which have been previously assessed for their proliferative activity were included in this study. These concerned 6 biopsies in cases undergoing neo-adjuvant chemotherapy and 94 resections from two laboratories. From each case one representative slide was selected by two pathologists to be used for evaluating the Mitotic Activity Index (MAI). In addition, the regions for mitosis counting were marked beforehand. This study was performed in two phases. First, MAI was scored by three observers on the same marked area on the selected glass slides using light microscopy. Thereafter, the glass slides were scanned and after a wash out period of at least 4 months WSI were presented to the same observers to recount mitosis. Table 1 details the cases included in the study.

Microscopically, only cells with very evident morphology of mitosis were counted as defined before[32,33] by absence of the nuclear membrane, clearly visible hairy

Table 1. Overview of cases included for comparing mitoses counts on glass slides and whole slide images.

Diagnostic entity	Specimen type		Total
	Biopsy	Resection	
Invasive ductal carcinoma	6	76	82
Invasive lobular carcinoma		13	13
Mucinous carcinoma		1	1
Papillary carcinoma		3	3
Tubular carcinoma		1	1
Total	6	94	100

extension of nuclear material (condensed chromosome), either clotted (beginning metaphase), in plane (metaphase/anaphase), or in separate clots (telophase). Doubtful cells with a hyperchromatic nucleus or cells suspected of apoptosis were excluded. The above mentioned criteria have been adopted in counting mitoses using a conventional microscope as well as WSI.
The time needed for scoring mitosis was recorded for the first ten cases in this study. Additionally, tissue quality (as poor, acceptable or good) and scan quality (as hazy, acceptable with some indistinct regions, acceptable or good) were assessed by all observers.

MAI assessment on glass slides by conventional microscopy
Two pathologists marked the regions for mitoses counting on the H&E slides. These regions were selected at the most cellular area of the tumor, mostly located at the peripheral invasive part of the tumor as before[31]. Areas with necrosis or Ductal Carcinoma In Situ were excluded. Counting mitoses was performed at 400x magnification using a Leica light microscope equipped with 10x ocular and 40x (0.85 N/A) objective (having a field diameter of about 540μm) in 9 consecutive fields with a total surface area of 2.06 mm^2. The total number of mitoses in those 9 fields was taken as the MAI.

MAI assessment on WSI
Glass slides were scanned using a Leica Scanner SCN400 at 40x. The standard image viewer for Leica Scanner "Digital Image Hub" was used for annotating and exploring WSI. WSI were displayed on high resolution 30" Barco Pathology Displays (Barco, Brussels, Belgium) having a resolution of 6 MP. Examining WSI on 40x, in an area of 2 mm^2, about 7 screen fields fitted into the same 2 mm^2 area annotated before on the glass slides. Each observer was asked to annotate all the mitotic figures that he could detect within this area. Afterwards mitoses annotations were counted for each observer separately. Figure 1 is a snapshot from a WSI of an invasive breast cancer showing annotated mitotic figures within a 2 mm^2 area.

Direct comparison of mitoses on glass slides and WSI
Perception of mitotic figures might be more difficult on WSI scanned at one focal plane than in conventional slides where one can perceive the 3-D structure by focusing. For this purpose and in order to gain insight into the differences in appearance using the two diagnostic modalities, mitotic figures from 15 cases were identified under a microscope and compared instantaneously with the corresponding object on WSI. Digitally, these mitoses appeared as dark nuclei with very fine projections (early metaphase), in plane with irregular margins (late metaphase), mitoses with individually dispersed chromosomes and dark ring-like

shapes (anaphase) or as separated parallel dark clots (telophase). Figure 2 shows snap shots from several WSI showing these different forms of actual mitotic figures. Figure 3 show snap shots from WSI showing different mitosis-like figures.

Data evaluation

MAI values were transferred into mitotic scores as for grading as follows: Score 1: 0-6 mitosis/2mm^2, Score 2: 7-12 mitosis/2mm^2, Score 3: 13 mitosis or more/2mm^2. Agreement was assessed between observers using the same diagnostic modality, and for each observer using the two different modalities. Intra- and inter-observers agreement for the continuous MAI was assessed using Intra Class Correlation Coefficient (ICCC), scores 0-0.2, 0.3-0.4, 0.5-0.6, 0.7-0.8, >0.8 indicating poor, fair, moderate, strong and almost perfect agreement, respectively. For mitotic scores, kappa statistics (K) were calculated to estimate inter- and intra-observer agreement[34-37], kappas < 0.20, 0.21-0.40, 0.41 - 0.60, 0.61 - 0.80, and 0.81 - 1.00 indicating poor, fair, moderate, good and perfect agreement, respectively.

The level of significance was calculated using the Wilcoxon signed-rank test. Systematic differences between microscopic and digital MAI values and mitotic scores were read from the Wilcoxon signed rank test and scatter plots.

The possible effects of tissue and scan quality on differences between the conventional and digital MAI assessments were evaluated using the Mann-Whitney test.

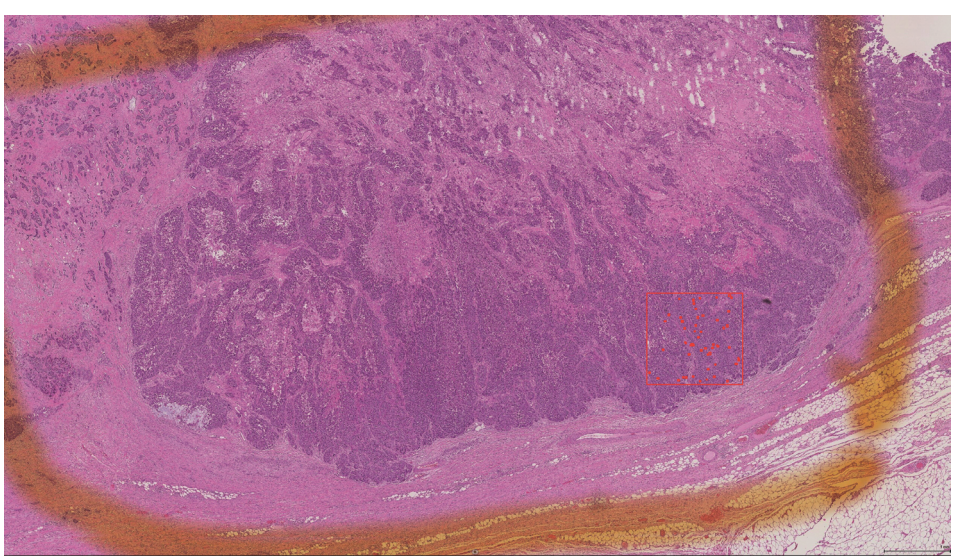

Figure 1. Snapshot from a WSI showing an area of an interest for counting mitosis.

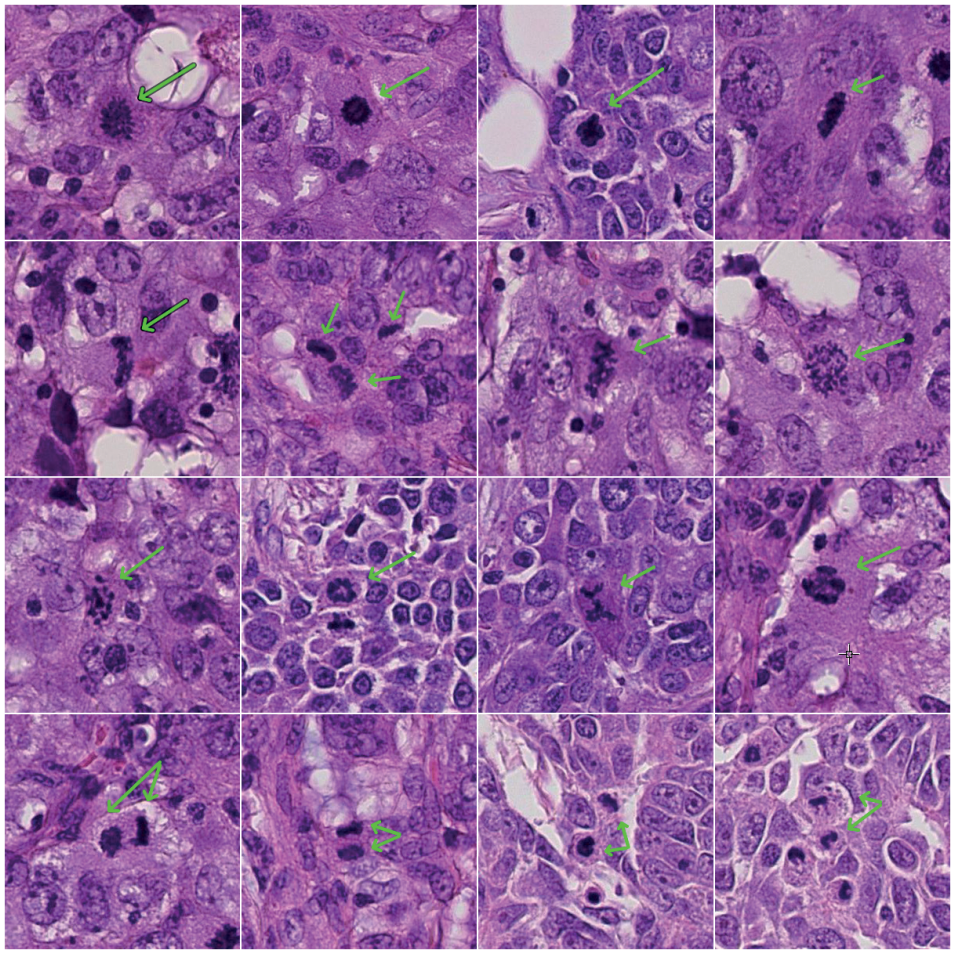

Figure 2. Snapshots from several WSI showing different appearances of mitotic figures.

Results

For all observers, tissue quality did not have a significant effect on the differences between conventional and digital mitotic scores (P = 0.836, 0.187 and 0.225 for observers 1, 2, and 3, respectively). Per observer, there was no significant effect of scan quality on the differences in scoring mitosis conventionally and on WSI (P 0,328, 0,275 and 0.266 for observer 1, 2 and 3 respectively. Counting mitoses on WSI was more time consuming than on glass slides. The average amount of time needed to count mitoses on glass slides ranged from 3-5 minutes versus 10-12 minutes for WSI.

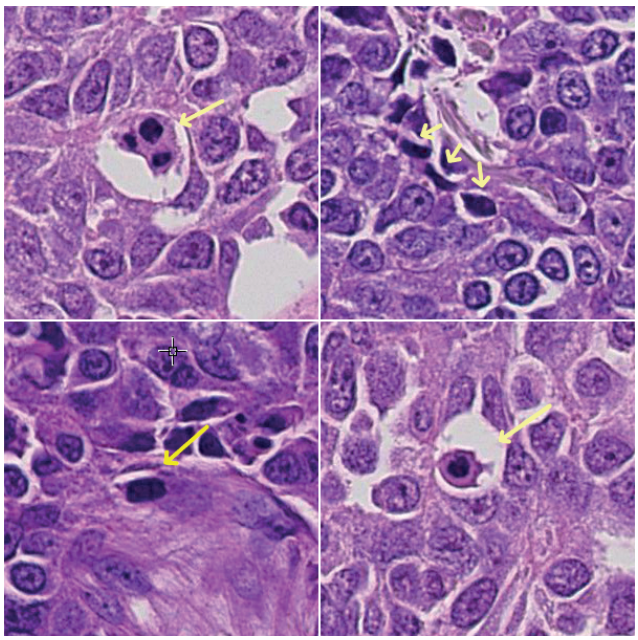

Figure 3. Snapshots of WSI showing mitosis-like figures.

Inter-observer agreement for the same diagnostic modality

There was almost perfect inter-observer agreement among all observers in assessing MAI using a conventional microscope (ICCC 0.879) and on WSI (ICCC 0.924). Mitotic scores again yielded a good inter-observer agreements among all observers using a conventional microscope (average kappa 0.642 (K1=0.645, K2=0.667, K3=0.615)), and WSI (average kappa 0.635 (K1= 0.756, K2=0.584, K3=0.565)). Table 2 gives an overview of stepwise kappa statistics between observers.

Intra-observer agreement for microscopic vs. WSI based mitoses counting

There was strong to perfect intra-observer agreement in counting mitoses when comparing both diagnostic modalities with ICCC of 0.863, 0.716, and 0.773 for observers 1, 2 and 3, respectively. In general, there was a noticeable trend towards underestimating mitotic counts on WSI if compared to microscopic mitotic counts as shown in figure 4 per observer. Moderate to good intra-observer agreement was observed between mitotic scores using both methods with kappa values of 0.617, 0.617, and 0.506 for observers 1, 2 and 3, respectively. Digital MAI scores were lower in 43/300 pairs of scores (microscopic and digital) and higher in 30/300 pairs (P 0.683, 0.086 0.590 for the three observers respectively). Table 3 details the results per observer.

Table 2. An overview of stepwise kappa statistics between observers.

Interobserver agreement using Kappa statistics between observers scoring mitosis on the bases of conventional microscope					
		Observer 2			Total
		1	2	3	
Observer 1	1	51	7	0	58
	2	7	5	3	15
	3	1	2	24	27
Total		59	14	27	100
K1 0.645 CI: 0.506 - 0.784					
		Observer 3			Total
		1	2	3	
Observer 1	1	47	9	2	58
	2	3	9	3	15
	3	0	3	24	27
Total		50	21	29	100
K2 0.667 CI: 0.536 - 0.797					
		Observer 3			Total
		1	2	3	
Observer 2	1	46	11	2	59
	2	4	7	3	14
	3	0	3	24	27
Total		50	21	29	100
K3 0.615 CI: 0.476 - 0.753					

Interobserver agreement using Kappa statistics between observers scoring mitosis on the bases of Whole Slide Images (WSI)					
		Observer 2			Total
		1	2	3	
Observer 1	1	59	3	0	62
	2	3	6	0	9
	3	1	6	22	29
Total		63	15	22	100
K1 0.756 CI: 0.631 - 0.879					
		Observer 3			Total
		1	2	3	
Observer 1	1	46	16	0	62
	2	3	5	1	9
	3	0	5	24	29
Total		49	26	25	100
K2 0.584 CI: 0.442 - 0.724					
		Observer 3			Total
		1	2	3	
Observer 2	1	46	16	1	63
	2	3	8	4	15
	3	0	2	20	22
Total		49	26	25	100
K3 0.565 CI: 0.420 - 0.708					

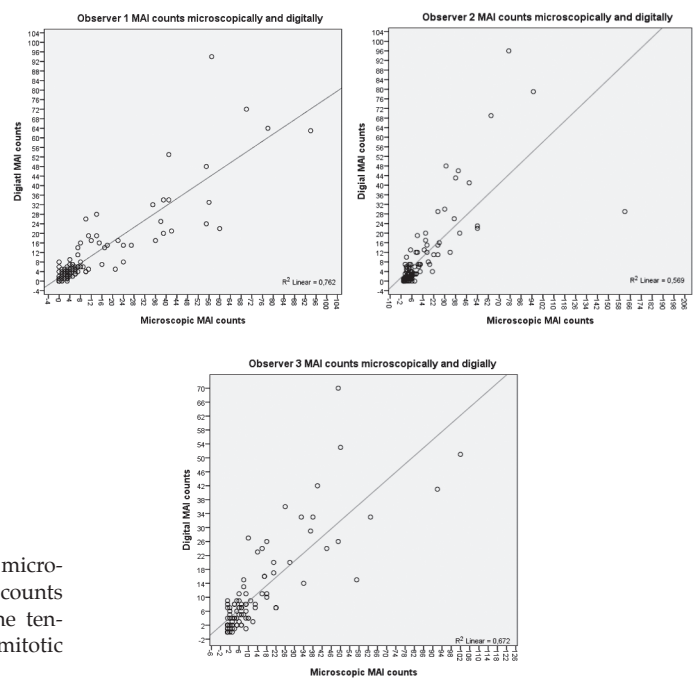

Figure 4. Scatter plots of microscopic versus digital MAI counts per observer indicating the tendency to underestimate of mitotic counts on WSI.

Table 3. Concordance rate of each observer for scoring Mitotic Activity Index (MAI) using WSI and conventional microscopy.

Statistics			Observer 1	Observer 2	Observer 3
Agreement of digital and microscopic MAI counts		ICCC	0.863	0.716	0.773
Agreement of digital and microscopic MAI scores		Kappa	0.617	0.617	0.506
		Kappa CI	0.47- 0.76	0.47- 0.76	0.36- 0.65
Wilcoxon test P value			0.683	0.086	0.590
Scores estimation	Digitally underestimated*		11	15	17
	Digitally overestimated*		10	6	14
	Same score Digitally and conventionally		79	79	69

* Compared to gold standard conventional counting

Discussion

The aim of this study was to validate the use of WSI in evaluating the MAI in breast cancer cases. MAI is an integral part of the breast cancer grading system and eventually gives an estimation of the degree of aggressiveness of the tumor and guides treatment protocols[38]. Correct evaluation of this prognostically relevant criterion is crucial since under- or overestimating mitosis scores could have important clinical implications for the patient[28].

100 breast cancer biopsies and resections were subjected to mitosis counting by three observers on two occasions; first using a conventional microscope and then after a wash-out period of at least 4 months on WSI scanned at 40x. There was almost perfect inter-observer agreement in assessment of the MAI on the bases of the conventional microscope (ICCC 0.879) and on WSI (ICCC 0.924). There was also a good inter-observer agreement among three observers in scoring MAI using either a conventional microscope or WSI with average kappa values of 0.642 and 0.635, respectively. The results of this study are comparable to other studies that examined inter-observer agreement of scoring mitoses and grading of breast cancer cases by conventional microscopy only[33,39-41].

There was a tendency to slightly underestimating the number of mitoses on WSI, but when transferring mitoses counts to mitotic scores as in grading, WSI based scores did not significantly differ from scoring mitosis using glass slides and a conventional microscope (Table 3). This indicates that scoring mitosis in breast cancer cases can be reliably done on WSI scanned at 40x magnifications and at one focal plane without influencing prognostic impact of mitotic counts.

Inter-observer agreement of the digital mitotic counts (ICCC 0.924) was slightly higher than microscopic mitotic counts (ICCC 0.879). This might be due to the fact that digital mitotic counts were performed precisely in the same annotated area

of 2 mm^2 whereas this was not the case for microscopic counting. Selection of different areas for estimating MAI and tumor heterogeneity[28] might explain the slightly lower observer agreement in counting mitosis microscopically.

Fine microscopic focusing can be helpful for differentiation of actual mitoses from mitotic-like bodies. Losing the ability of fine focusing on WSI scanned at one focal plane may theoretically impede mitosis identification. Scanning glass slides on multiple focal planes providing a z-axis to WSI may facilitate the digital evaluation of mitotic figures but increases scanning time and storage requirements which is yet impractical for routine pathology work. With the continuous improvement of scanning speed and reduction in storage cost, we expect that such limitations will be solved in the near future. Improving inter- and intra-observer reproducibility in counting mitoses can possibly be achieved by following a strict scoring protocol[33] as well as practicing more digital MAI scoring[3,9].

Counting mitoses on WSI turned out to be more time consuming than its conventional counterpart, mainly due to cumbersome software that requires 5-6 mouse clicks to annotate one mitotic figure, and counting the total number of annotations at the end. However, annotating each mitotic figure was important for the context of this study but might not be necessary in routine practice. Adjusting the next versions of the software for research purposes to include more features such as one click annotation, an option for an automatic mitotic annotation counter and applying a 2 mm^2 grid has been discussed with the vender. These additional features will definitely decrease scoring time and risks of error in counting mitoses and may eventually increase reproducibility. Furthermore, running automated MAI scoring on WSI would be a step forward and will assist in the objective determination of mitotic activity and hence tumor grading. Automatic detection of cancerous epithelial cells on imprint cytology slides created from breast cancer specimens[42], automated measurement of nuclear size in breast cancer[43], has already been tried with acceptable results.

The quality of WSI was generally good and adequate for use in estimating the MAI. The most frequently used level of magnification for mitosis perception was 80x digital magnification since this level of magnification offers the observer a field of view most comparable to 40x under a microscope. Also, keyboard shortcuts, which provide a more user friendly and optimal navigation within WSI, were used to move WSI in order to explore a 2 mm^2 surface area.

Tissue quality was not optimal for every case included in this study and this might be the reason behind the discrepancies in mitotic scores. This leads to extra difficulty in counting mitosis either microscopically or digitally. Such cases were not excluded from this study as they reflect the routine mix in this pathology centre. Since poor tissue morphology can have an effect on counting mitoses[28,33] and can rapidly compromise the quality of WSI for MAI scoring, further studies

testing the effect of proper tissue morphology on digital mitosis scoring are important. However, the quality of the tissue sections included in this study did not have significant effect on the difference in scoring mitosis microscopically and digitally.

Despite of the fact that the quality of the currently produced WSI is sufficient to perform most of the diagnostics within pathology as has been approved by several validation studies, primary diagnostics based solely on WSI requires improvement of many issues such as scanning speed, image quality, software solutions and navigation interface will definitely guarantee the successful integration of WSI routine pathology.

In conclusion, counting mitoses in breast cancer can reliably be done on high resolution WSI scanned at one focal plane. Further improvement in the software characteristics, scanning speed, and image quality will definitely encourage the use of WSI in routine practice.

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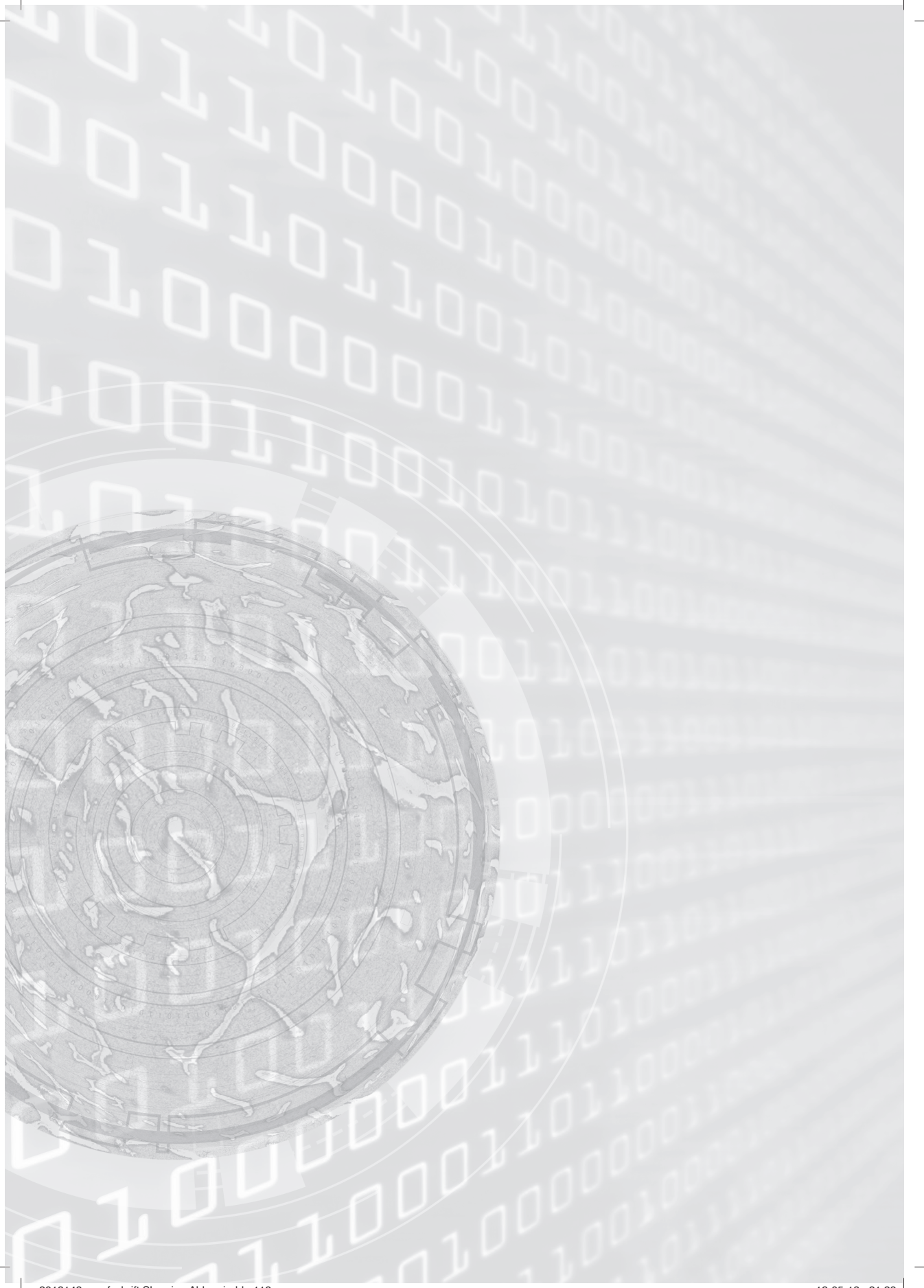

Chapter 9

Validity of whole slide images for scoring HER2 chromogenic in situ hybridization in breast cancer

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Abstract

Introduction

Since their introduction, Whole Slides Images (WSI) have stimulated a paradigm shift from conventional to digital pathology in several applications mainly for teleconsultaion, education and research. However, WSI are not yet widely incorporated into routine diagnostics because they have not yet been FDA approved for this purpose. Thus validating their use for different diagnostic purposes is still mandatory. The aim of this study was to test the validity of WSI in assessing HER2 status in breast cancer specimens using chromogenic in situ hybridization (CISH).

Materials and methods

Fifty HER2 CISH slides were scored by the same observer on a light microscope (400x viewing magnification) and on WSI (40x scanning magnification, one focus plane) with a minimum of six weeks wash out period. The concordance between digital and microscopic HER2 scores was assessed.

Results

Digitally, 47/50 cases could be assessed (94%). The average time needed to digitally evaluate HER2 status in these 47 cases was about 2.8 minutes per WSI. Microscopic and digital evaluation of HER2 amplification status were concordant in 44/47 cases ((94%, 95% CI: 0,863-1.00), Kappa 0.819). Two discrepant cases were considered to have low-level amplification using conventional microscopy but were regarded as non-amplified on WSI, and one case was microscopically assessed as low-level amplified but was highly amplified on WSI. When comparing the number of spots counted, digital HER2 CISH scores indicating a tendency to underestimate the average scores on WSI: lower in 31 and higher in 5 cases.

Conclusion

In general, HER2 amplification status by CISH seems to be well assessable on WSI. However, there was a noticeable tendency toward underestimating the number of HER2 spots on WSI leading to missing low level HER2 amplification in 2/47 cases. Scanning at multiple focus planes could offer a better resolution for improved CISH spot counting.

Introduction

Whole slide imaging is the process of scanning glass slides and converting them into a digital form commonly known as Digital Slides or Whole Slide Images (WSI)[1]. WSI are usually explored with the aid of image viewers which allow the manipulation of the entire tissue section in any direction and at any magnification. Image viewers offer added benefits such as the ease of accessing, sharing, annotating, storing and retrieving images; making the use of WSI in some aspects more convenient than using a glass slide and a conventional microscope. As a result, WSI stimulated a paradigm shift from a conventional to a digital mode in several applications within pathology, particularly in teaching, tele-consultation, clinico-pathologic conferences, frozen section diagnosis and research. However, the use of WSI for upfront diagnostics is still uncommon possibly because in the United States WSI have not yet been approved for this purpose by the FDA (Food and Drug Administration). Several validation studies evaluating the efficiency of WSI for primary diagnostics of different pathology specimens have shown a good concordance between digital and conventional diagnoses[2-9]. Most of the available studies have assessed the validity of WSI for H&E stained tissue sections[10] while the current pathology work relies not only on H&E stained tissue sections but also on immune stains and additional molecular techniques.

In breast cancer it is of great importance to assess the status of specific genes or receptors as they may influence patient's prognosis and response to therapy[11]. Human epidermal growth factor receptor 2 (HER2) is a trans-membrane glycoprotein receptor with a tyrosine kinase activity which has shown to be over-expressed in 10-20% of breast cancer cases[12,13]. This protein is encoded by a gene located on chromosome 17 commonly called ERBB2 or neu[14-16]. Assessing HER2 gene amplification and/or protein over-expression at diagnosis is recommended for all breast cancer patients since a positive HER2 status is commonly associated with a poor prognosis, resistance to conventional chemotherapy[17-19] and response to treatment with the recombinant humanized monoclonal anti-HER2 antibody trastuzumab.

HER2 protein over-expression is usually determined by immunohistochemistry (IHC), whereas assessing HER2 gene amplification on DNA level is usually done by conducting one of the following tests: FISH (Fluorescent In Situ Hybridization), CISH (Chromogenic In Situ Hybridization) or MLPA (Multiplex Ligation-Dependent Probe Amplification)[20,21].

CISH is a morphologic test that allows the evaluation of HER2 gene by assessing small nuclear signals within tumor cells using a glass slide and a bright field microscopy. The ease of scoring, image sharing and documentation of annotation

on WSI encouraged us to start a study aimed at evaluating the feasibility of using high resolution WSI in routine assessment of HER2 status using CISH.

Materials and methods

Fifty randomly selected breast cancer cases (18 biopsies and 32 resections) on which CISH had previously been performed in the Symbiant Pathology Expert Center in The Netherlands were included in this study. Table 1 shows tumors and specimen types of cases selected for HER2 evaluation.

CISH assay was performed using the ZytoDot SPEC HER2 Probe Kit (ZytoVision, Bremerhaven, Germany) according to the manufacturer's instructions. The enzymatic reaction from this test yields prominent brown nuclear signals which can be easily visualized under a microscope at 40x magnification. No correction for chromosome 17 copy number was conducted as true polysomy 17 is now believed to be a very uncommon event in breast cancer[22].

HER2 amplification was assessed in at least 30 cells in the invasive part of the tumor. Only nuclei with distinct nuclear borders were evaluated, areas with necrosis or overlapping of nuclei were excluded. Samples with an average of < 5 spots/nucleus were considered non-amplified whereas samples with >10 spots/nucleus were considered to be highly amplified. An average number of spots ranging between 5-10 spots/nucleus was considered to represent low-level amplification.

The 50 glass slides were scanned at 40x using a Leica slide scanner SCN400. WSI were presented on high resolution 30" Barco Pathology Displays (Barco, Brussels, Belgium) having a resolution of 6 megapixels.

An experienced molecular technician scored the slides microscopically at 400x magnification and on WSI with a wash out period of at least six weeks. The time needed to score 30 representative cells on WSI was noted.

Table 1. Basic data on tumor type and type of specimen for 50 breast cancer cases subjected to CISH scoring by light microscopy and on WSI.

Tumor type	Specimen type		Total
	Biopsies	Resections	
Invasive ductal carcinoma	15	27	42 (84%)
Invasive lobular carcinoma	3	4	7 (14%)
Mucinous carcinoma		1	1(2%)
Total	18	32	50

Statistics

Statistical analysis was performed using statistical software SPSS 20. Microscopic and digital HER2 classes (normal/low level/high level amplified) were compared using the concordance coefficient Kappa (K) as suggested by Landis and Koch[23]. A K value of 0.00-0.20 suggests a slight agreement, 0.21-0.40 a fair agreement, 0.41-0.60 a moderate agreement, 0.61-0.80 a substantial agreement and 0.81-1 a perfect agreement. The percentage agreement with its 95% Confidence Interval (CI) were also calculated. Systematic differences between the two scoring modalities were evaluated using linear regression analysis and Wilcoxon's signed rank test. P- values < 0.05 were considered statistically significant.

Results

Digitally, observers could elaborate HER2 status in 47 cases (94%). However, in 3 cases the observer could not establish HER2 status. Non-interpretation and case deferral was either due to poorly prepared specimens with partial detachment of the tissue from the glass slide, making such cases subsequently difficult to be optimally scanned or because of the inability to perceive distinct cell borders and/or clear nuclear signals. The average time needed to digitally evaluate HER2 status in the 47 eligible cases was 2.8 minutes per WSI. Table 2 shows the concordance of HER2 status assessed by conventional microscopy and on WSI. Figure 1 presents snapshots of WSIs showing different levels of HER2 CISH amplification (a) normal, (b) low-level amplification (c) high-level amplification.

Microscopic and digital evaluation of HER2 status were concordant in 44/47 cases ((94%, 95% CI: 0.863-1.00), Kappa 0.819, P 0.564). Two discrepant cases were considered to have low-level amplification using a conventional microscopy but were regarded as non-amplified on WSI, and one case was microscopically assessed as low-level amplified but highly amplified on WSI. Overall, HER2 CISH scores were digitally lower in 31 and higher in 5 cases.

Table 2. Concordance between HER2 CISH scoring of 47 breast cancer cases using conventional microscopy and WSI.

Microscopic scoring	Digital scoring			Total
	Normal	Low level amplification	High level amplification	
Normal	36	0	0	36
Low level amplification	2	1	1	4
High level amplification	0	0	7	7
Total	38	1	8	47

This tendency to underestimate the average spot counts on WSI despite very good correlation (R=0.898) is illustrated by the scatterplot of the linear regression analysis in figure 2 revealing an intercept of -0.418.

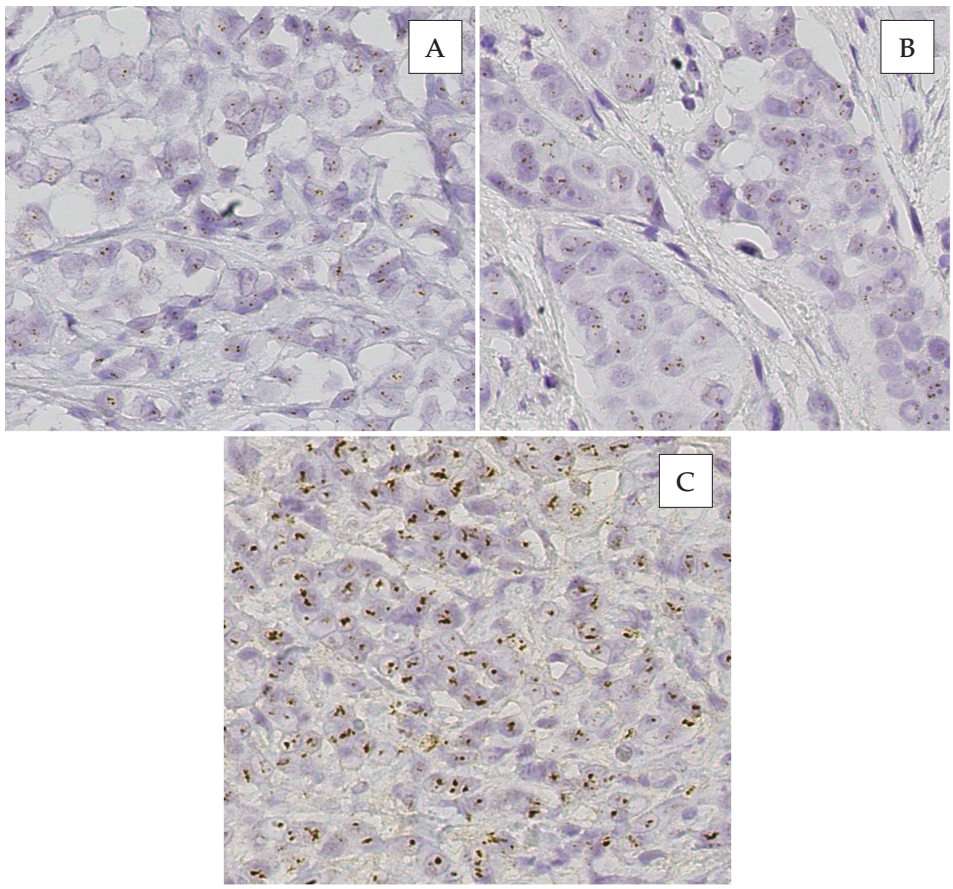

Figure 1. Snapshots of WSIs showing different levels of HER2 CISH amplification (a) normal, (b) low-level amplification (c) high-level amplification.

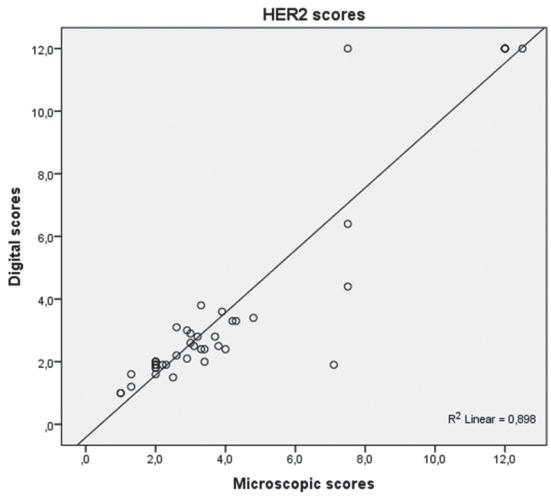

Figure 2. Scatter plot of microscopic versus digital HER2 CISH spot counts indicating the tendency to underestimate of the average scores on WSI (intercept -0.418) despite very good correlation (R=0.898).

Discussion

Altered HER2 status is usually associated with poor prognosis, shortened disease free periods and decreased overall survival times in patients diagnosed with breast cancer. More importantly, it predicts eligibility for trastuzumab therapy[24-27]. Thus correct estimation of HER2 gene amplification and /or protein over-expression at the time of diagnosis is a pivotal prerequisite to support treatment decisions.

CISH allows the evaluation of HER2 gene amplification by manual scoring of small nuclear signals using glass slides and a conventional microscope[28,29]. High resolution WSI are considered to be a novel alternative to glass slides which enable exploring pathology specimens on a computer screen in a way comparable to a microscope. Additionally, the flexibility derived from the ease of accessing and sharing of WSI has led to an inevitable and gradual conversion toward the digital era within pathology where WSI have been broadly incorporated into several applications mainly in education and tele-consultation[30-33].

This study aimed, therefore, to investigate the validity of WSI in assessing HER2 status in patients with breast cancer using CISH. Fifty breast cancer biopsies and resections were evaluated microscopically and on WSI by one observer with a wash-out period of at least 6 weeks. The amount of time needed to score WSI was deemed acceptable by the observer.

In three of these cases the observer felt uneasy making an assessment on WSI. In the remaining 47 cases, microscopic and WSI based evaluation of HER2 status were concordant in 44 cases (94%). Of the three discrepant cases, two were considered to have low-level amplification using conventional microscopy but

were regarded as non-amplified on WSI, and one case was microscopically assessed as low-level amplified but highly amplified on WSI. Therefore, HER2 amplification status by CISH overall seems to be well assessable on WSI. These results are in line with our previous studies[6-8] and similar studies[3,5,34-36] evaluating the suitability of WSI for upfront diagnostics which have indicated that the diagnostic performance of WSI is comparable to that of a glass slide and a conventional microscope. Further, the results may be in line with previous studies assessing the reproducibility of HER2 CISH scoring by light microscopy[37]. However, missing low level amplification on WSI may deny patients trastuzumab so this may be serious. The difference between a classification of low level and high level amplification seems to be clinically less relevant.

Further, there was despite an excellent correlation coefficient (0.898) an obvious tendency toward underestimating HER2 spot counts on WSI (31 cases were underscored digitally versus only 5 cases with over-scoring, intercept on linear regression analysis -0.418). Underscoring HER2 nuclear signals on WSI may indicate an inability to visualize fine nuclear signals which were able to be perceived using a conventional microscope. Scanning tissue sections at one focal plane as in the present study may compromise visualizing fine nuclear spots not completely lying in the chosen focus plane. In general, we expect that scanning at multiple planes (Z-stacking) could offer a better resolution for identifying fine cellular and nuclear details. Although Z-stacking is still not affordable for routine diagnostics as it demands a long scanning time and necessitates significantly more storage, it may be required for optimal assessment of HER2 CISH.

As WSI are highly amenable to automated image analysis, they aroused a growing interest in creating various algorithms for performing different diagnostic tasks. Algorithms assessing HER2 immune tests have already been created and some of them have been approved by FDA such as the Automated Cellular Imaging System III (ACISIII) and PATHIAMTM IVD from Bioimagene[38]. Similar algorithms for quantitative assessment of FISH[39] and CISH are also available from Bioimagene and Visiopharm. Using automated image analysis may contribute to objective assessment and increase the productivity in pathology[40,41].

In conclusion, in general HER2 amplification status by CISH seems to be well assessable on WSI. However, there was a noticeable tendency toward underestimating the number of HER2 spots on WSI leading to missing clinically relevant low level HER2 amplification in 2/47 cases. Scanning at multiple focus planes could offer a better resolution for improved CISH spot counting.

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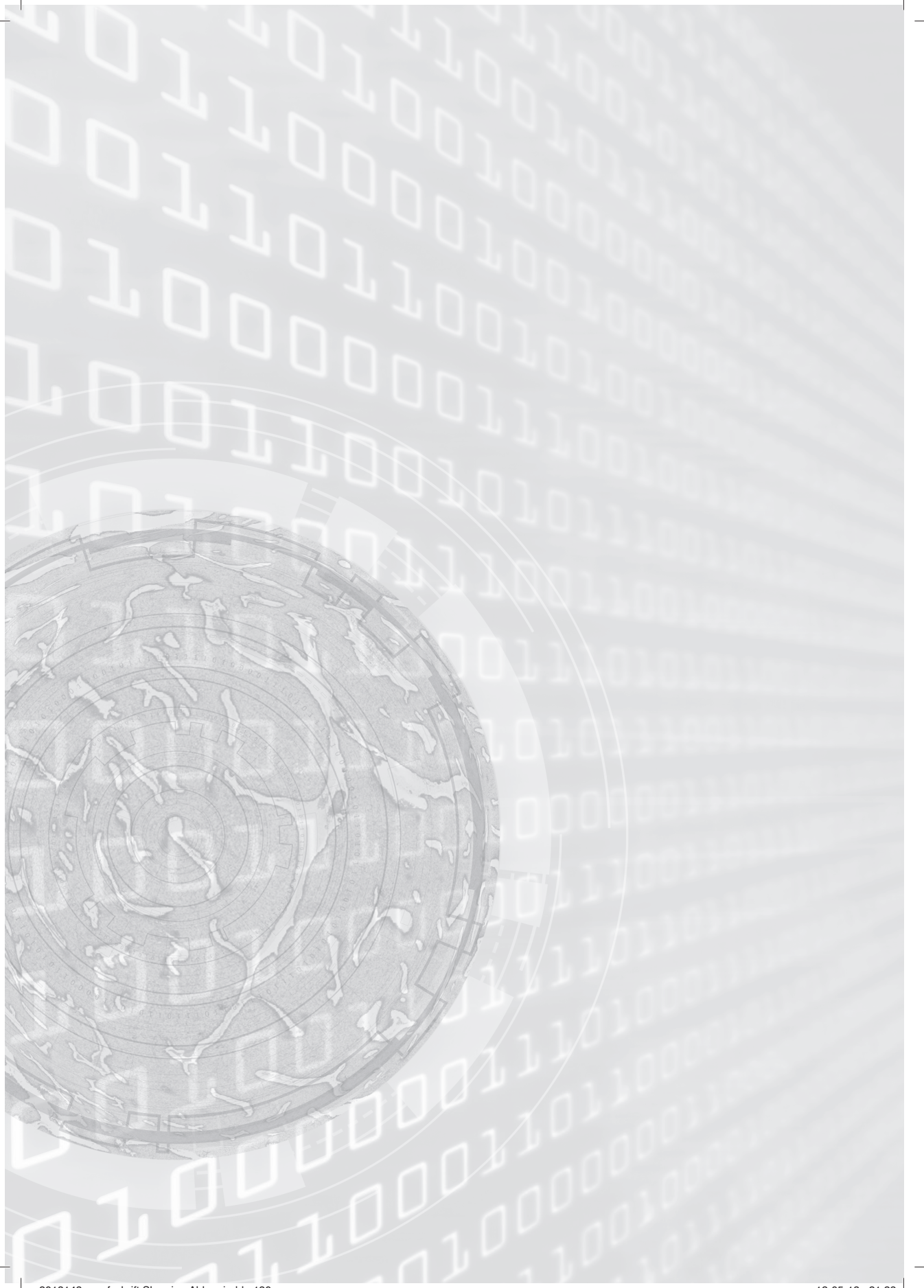

Chapter 10

Whole slide images as platform for initial diagnostics in histopathology in a medium-sized routine laboratory

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Abstract

Introduction

Whole slide imaging is the process of digitizing glass slides and the creation of Whole Slide Images (WSI), which enable the examination of pathology samples on a computer screen in a manner comparable to light microscopy. WSI have been used for different applications in pathology but their use for primary diagnostics is still limited. Implementing WSI for primary diagnostics would be a turning point necessitating extensive validation to unravel pitfalls and difficulties that could be encountered within the routine workflow. This article is aimed to describe the gradual integration of WSI into routine pathology diagnostics in a medium-sized routine pathology laboratory.

Materials and methods

This project was started with optimizing the digital work environment including the setting up of validation studies, scanning preferences, storing WSI and the implemented adjustments to the workflow for the laboratory and the pathologist. Afterwards scanning glass slides was initiated in the department of pathology at the Atrium Medical Center, Heerlen, The Netherlands, for performing primary diagnostics of breast biopsies. Later this was extended to other specimen types including resections.

Results

The validation studies yielded a high concordance rate between WSI and conventional diagnoses. Routine primary WSI based diagnosis was possible in 82.1% of cases. Failure of digital diagnosis was mainly related to poor image quality and logistic problems.

Conclusion

The quality of the currently produced WSI is sufficient for primary diagnostics in 82.1% of the cases. Improving image quality, adequate retrieval and controlling scanning errors will definitely encourage the wide adaptation in routine diagnostics.

Introduction

Looking at glass slides through the conventional microscope has been the standard way of working for diagnostic histo- and cytopathology for a long time[1]. Over the last decades, new methods for rendering diagnosis have emerged for pathology practice[2]. Rendering diagnosis depending on static or dynamic images transferred through network connections to remote places for second opinion and teleconsultation, commonly called telepathology, is widely accepted nowadays[3]. These systems have been followed by more sophisticated methods of image acquisition, called whole slide imaging (or virtual microscopy) where the whole glass slide is converted into a digital form (Whole Slide Images, WSI) allowing the examination of pathology specimen on a computer display with the aid of an image viewer[4, 5]. Scanners dedicated to scan glass slides and the creation of WSI became available more than a decade ago[6, 7]. WSI combine the features of both previous telepathology systems (static and dynamic), providing high resolution images with unlimited access to the entire pathology specimen at different magnification[8, 9]. Remote access of WSI by different examiners from different places at any time is unique for WSI, which supports their use for different applications in pathology, especially for teleconsultation[10, 11], telerevision, and education[12-14]. Examination of multiple digital slides simultaneously is also a property supplied by the image viewer, which allows comparison of different stains and sections. The above-mentioned features not only support the use of WSI for teleconsultation, frozen section diagnosis, clinical conferences, research and image analysis but also daily routine diagnostics, although WSI have not widely been applied for this purpose until now.

A major bottleneck in hindering the use of whole slide images in diagnostics is the time needed for scanning and exploring WSI. Moreover, the integration of WSI into daily routine practice is also accompanied by many challenges regarding workflow in the lab and for the pathologist. The presence of scanners with a more acceptable scanning speed (2-4 minutes per slide on 20x magnification), improved image quality and the ongoing reduction of storage costs encouraged some medical institutes to perform WSI scanning on a daily bases, either for the entire routine work or for selective cases. In The Netherlands, scanning the complete daily production of histopathology specimens to build up a digital archive is being done by the University Medical Center Utrecht (UMCU) only[7]. Scanned slides are used for clinicopathological conferences, revision, consultation, teaching and research but not yet for daily routine diagnostics. Scanning of a fixed number of cases on daily bases aimed for performing primary diagnostics of histopathological specimens has been implemented in the Atrium Medical Center, Heerlen (AMCH). According to our knowledge the AMCH was the first hospital in The Netherlands

that uses WSI for primary diagnostics in pathology. The aim of this article is to share the experience in setting up primary WSI based diagnosis in a medium sized routine pathology lab.

Materials and methods

The department of pathology of the AMCH handles about 21,000 histology and 16,000 cytology specimens per year. In 2006 it was decided to start a project on digital pathology, with one of the goals to use WSI for primary diagnostics. At the time the project started there were scanners that would be able to handle a sufficient part of the workload of the AMCH with adequate quality to start a pilot to validate WSI for primary diagnostics.

For the scanner, criteria were formulated regarding scanning speed, loading capacity and price. After testing the available scanners in the market at that time (from Aperio, Zeiss, Hamamatsu and Olympus), the Mirax Scan (3DHistech, Budapest, Hungary) was selected as the most suitable scanner to meet the goals of this project. This scanner was able to load and automatically scan 150 slides in one run, divided over multiple cassettes. Scanning standard glass slides at 20x took between 2.25 and 6.45 minutes depending on the size of the specimen[15]. After registration during several weeks of the objectives used with conventional microscopy, pathologists agreed that 20x would be adequate for diagnosing most cases. The slides were labeled with a 2D barcode, containing the specimen number, to allow easy image retrieval and a smooth workflow. WSI were stored on external hard disks with a total capacity of 750 Gigabyte accessible over the network. The images belonging to one specimen were grouped in folders with the specimen number based on the barcode.

Preliminary experiments revealed that several steps had to be optimized to arrive at proper scans for digital diagnosis. This concerned the size of the section that had to fit underneath the coverglass. For this, dissection was modified and technicians were instructed to extra carefully position the sections centrally on the glass slides. Section thickness was standardized at 4 μm. The processing of small biopsies was also changed from putting three consecutive sections from the same level on three rows to putting three sections from different levels on one row when it was noticed that additional information came from additional levels rather than from additional sections at the same level.

During digital diagnosis making, pathologists were asked to note for each case if it was possible to make the diagnosis based on the WSI. When unable to make the diagnosis on the digital image, they noted the cause of diagnostic difficulty (image quality, technical problems or other causes).

Diagnostic validation studies

The first retrospective validation study concerned digital re-diagnosis of 100 breast needle core biopsies that had been routinely diagnosed conventionally. Five pathologists from three different countries (Greece, Anna Batistatou, Hungary, Janina Kulka and The Netherlands, Marius Nap, Nathalie Van de Vijver and Paul Theunissen) participated in this study. The degree of agreement between the five pathologists was calculated using the kappa statistic.

The second prospective validation study concerned the diagnosis of 85 cases from different body systems by WSI and conventional light microscopy. During a period of several months, about five cases were scanned and digitally diagnosed every week. Each time, the participating pathologists were asked to render the diagnosis on WSI only. Afterwards, another pathologist was asked to re-diagnose these cases by light microscopy blinded to the WSI based diagnosis. If the WSI diagnosis matched with the light microscopy based diagnosis, the report was signed out immediately. If not, the report was adjusted based on the information derived from light microscopy diagnosis. In this way, the pathologists could closely monitor to which extent WSI can be used for primary diagnostics. At the end of the study the agreement between WSI based and conventional diagnoses was assessed.

Implementation of WSI in the routine pathology diagnostic workflow

Scanning and rendering diagnosis on WSI for all breast needle core biopsy specimens were initiated at the end of 2007. Afterwards, it was decided to dedicate one day per week for digital diagnosis of all tissue biopsies and resection specimens. Since four pathologists participated in this project, each pathologist performed digital diagnosis for all his routine work one day per month. Pathologists were supplied with the usual clinical information and were free to request additional histochemical- or immunostains. From January 2009 onwards, on average eight cases per day were diagnosed digitally by two pathologists who enjoyed doing diagnostics digitally. The number of scans per year from 2007 to 2010 is illustrated in Table 1.

Results

Retrospective WSI validation study

Comparing the light microscopy based diagnosis and WSI based diagnosis of the 100 breast needle core biopsies yielded a very high agreement between the five pathologists with a kappa score of 0.97.

Table 1. Total number of histopathology cases and the total number of scanned cases per year.

Years	2007	2008	2009	2010
The total number of cases	22085	22432	22395	23495
Total number of scanned cases	1353	2415	4123	4654
Breast cases	377	559	543	589

Prospective WSI validation study

The results were comparable to the retrospective study with almost perfect agreement between the light microscopic and WSI based diagnosis as published on the website of the department at the time (Year report 2009).
Unfortunately at the time these experiments were done, we had no intention to use this for publication and the results of this validation were not stored for formal statistics. Repeating the experiment at a later stage would not be logical since the conditions of individual experience have changed too much to give a realistic impression.

WSI based diagnosis in routine workflow

The total number of scanned cases for primary diagnostics was 3923 in 2010, from which 3222 cases were completely digitally diagnosed and 701 cases were not digitally signed out. Table 2 shows the number of scanned cases per pathologist sorted by the diagnostic modality. There were four major causes for failing digital diagnosis (see Table 3):

Table 2. Total number of scanned cases per pathologist and numbers of cases successfully diagnosed digitally.

Pathologist	Total no. of scanned cases	# of cases successfully digitally diagnosed	% of cases successfully diagnosed digitally
1	1829	1655	90.4% 95%CI(0.891-0.918) 99% CI(0.887-0.922)
2	287	143	49.8% 95% CI(0.44-0.56) 99% CI (0.42-0.57)
3	1522	1346	88.4% 95% CI(0.868-0.900) 99% CI(0.863-0.905)
4	285	78	27.3% 95% CI (0.221- 0.325) 99% CI (0.205-0.342)
Total	3923	3222	82.1% 95%CI(0.809-0.833) 99%CI(0.805-0.836)

1. Scanning problems: unsharp images, incomplete scanning.
2. Logistic problems: scan could not be located, network problems, scans were not available in time to fit in the routine work schedule.
3. Technical problems: bad (H&E) staining, bad positioning and tissue folding.
4. Extra procedures required: additional sections, immunohistochemical stains, or internal consultation. Table 4 summarizes the exact causes of extra procedures.

The major cause of failing digital diagnosis (Table 3) was related to image quality (N=209), with completely or partly unsharp WSI or incompletely scanned tissue, followed by logistic problems (N=61), extra procedures required (N=56), and (laboratory) technical problems (N=49), for the other cases (N=326) the cause remained unknown where the pathologist didn't specify the causes of case deferral and non digital diagnosis. Thus primary WSI based diagnosis was possible in 82.1% of the scanned cases where the estimated 99% CI is (0.805-0.836)

Table 3. Reasons for failure of digital diagnosis per pathologist.

	Scanning problems / image quality	Logistic problems	Technical problems	Extra requests	Other causes	Total
Path 1	124	29	2	18	1	174
Path 2	10	8	3	5	118	144
Path 3	74	24	44	33	1	176
Path 4	1	0	0	0	206	207
Total	209	61	49	56	326	701

Discussion

The aim of this article is to share the experience of using WSI for primary diagnostics in a medium routine pathology practice. The integration of WSI in the routine workflow was performed in a stepwise manner starting with minor adjustments of specimen handling, followed by two validation studies, finally resulting in implementing primary WSI based diagnostics for part of the routine work.

Studying the validity of WSI was a crucial point. The results of the two validation studies were very promising and encouraged the pathologists to start with primary WSI based diagnostics as part of the routine work. This was applied at first for breast needle core biopsies and later extended to other body systems. The gradual introduction of WSI based diagnosis in the daily routine was a very important step that revealed difficulties and problems associated with WSI based diagnostics, and allowed for timely finding solutions.

Table 4. Summary of extra request per pathologist.

Extra requests	Pathologist 1		Pathologist 2		Pathologist 3		Total
	Sort cases	No	Sort cases	No	Sort cases	No	
Special or immune pathology stain	Dermatofibroma Nevus Melanoma in situ Skin inflammation Trichoepithelioma Liver chronic inflammation	1 2 1 1 1 2			Breast adenosis Basosequamous ca (eye) Dermatofibroma Dermatosis Follicular keratosis Helicobacter Pylori Liver Bx (hepatitis) Liver Bx (fatty liver) Malignant lymphoma Neurofibroma Skin scar	1 1 1 1 1 11 1 1 1 1 1	29
Consult	Nevus Chondroid syringomas	2 1	Soft tissue atrophy Annular inflammation (sigmoid) Sclerosing adenosis (breast)	1 1 1	Lentigo solaris Liver Bx (fatty liver) Melanoma Nevus Hemangioma	1 1 1 3 1	13
Difficult on digital slides			Axillary needle Bx suspect malignancy Normal breast biopsy	1 1			2
Birefringence	Epulis (gingiva) Skin infection	1 1					2
Deeper	LN carcinoma Nevus Radicular cyst (mouth) Skin infection Inflammation (appendix)	1 1 1 1 1			Bowen disease Melanoma in situ Nevus Osteosarcoma	1 1 2 1	10
Total		18		5		33	56

This trial showed that about 82.1% of scanned cases could be digitally signed out successfully. The main reasons for not being able to sign out the remaining cases were image quality, followed by logistical and technical problems. Image quality is expected to improve when focusing algorithms become faster and better, allowing for focusing on maybe every pixel instead of a few fields scattered over the section. Another aspect that we had not expected to influence the process was the variation of thickness of the object glass. Unpredictable solitary or groups of slides appeared to have a thickness just under or above the tolerance of the automatic focusing range of the scanner. Although this variation could not be managed by the glass slide manufacturer, in newer versions of the hardware this problem has been solved. Some adjustment to the laboratory work, especially central placing of the tissue on the glass slides, will help to avoid incomplete scans. Also in tissues with low optical density the parameters for scanning can be adjusted, resulting in a higher sensitivity and complete images. Guaranteed network access and speed will probably require available Information technology (IT) support, which may not be easy to arrange for smaller laboratories. For a better flow of the routine work, WSI should be stored in an appropriate way so that the pathologist will be able to access them without delay preferably linked to the pathology report and stored in a sustainable digital archive for later referral if needed. The current work approach is that the pathologist accesses over the network the WSI stored on an external hard disk on which digital slides from the same case are stored in one a folder carrying the case number. For the time being the existing storage system and accessibility are adequate for the department work. Nevertheless, there were about 61 cases with logistic problems where the pathologist could not locate the WSI or there were network problems which were responsible for case deferral. Performing scanning of all the tissue specimens on a daily basis would require a larger storage environment and easier access (e.g. by using a storage area network). To this end, linking WSI to the pathology report would be of great advantage. It will not be necessary for the pathologist to open different files (the current work approach) to access WSI but can smoothly open the same pathology database where the patient history, macroscopic images and WSI are stored together. Linking of WSI with the patient history and pathology report has been successfully implemented in the UMC Utrecht, which facilitates image retrieval for revision, clinico-pathologic conferences and research[7]. The same or very similar approach will be adopted in AMCH in the near future to overcome logistic problems.

Some technical laboratory errors and the need of extra stain where responsible for case deferral in 105 cases. The same could be encountered if the diagnosis was performed by glass slides and conventional microscope. Rescanning of glass slides with technical errors would be an option to enrich and complete the department's

digital archive. However, this would not serve the primary goal of having WSI available in a timely and complete manner to perform WSI based routine diagnostic analysis in a medium sized routine pathology lab. Most of the cases which required extra procedure were from skin lesions where the pathologists need extra information to complete the diagnosis either by asking for extra stain, deeper sections, internal consult or even asking for the glass slide when the digital image information does not satisfy the diagnostic needs. In this study we have only four cases where the presence of glass slide was necessary to complete the diagnosis. Two of them to check the double refraction in gingival and skin infection as this feature cannot be supported in WSI. Perhaps the easiest category to start primary digital diagnosis with may be Gastro-intestinal (GI) and breast biopsies [16, 17]. Table 4 details these cases.

There was a remarkable variation (27-90%) between the 4 pathologists in successfully diagnosing cases digitally. Although information as to the exact reasons for deferring cases was incomplete, this shows that adaptation of digital diagnosis may differ between pathologists when starting routine digital diagnosis. Scanning of the complete daily production of pathology routine work will become possible shortly with the introduction of scanners which are able to scan standard glass slides at 40x in less than one minute. Primary diagnostics on WSI will facilitate pathology routine workflow through easy image sharing and retrieval and it will not be time consuming anymore to ask for a second opinion for difficult cases. Storing microscopic information in a digital from has also many advantages over storing physical slides since WSI can be stored permanently with constant quality. These images can be used for different applications such as teaching, research and revision. Performing quality assurance (QA) and teleconsultation based on WSI is less time consuming obviating the time needed to send glass slides to far places. This will be more efficient if a national storage or image exchange facility would be present where pathologists could look up digital slides from different institutes for teleconsultation and telerevision. Furthermore, WSI can be used for image analysis which will likely improve diagnostic accuracy and productivity [9, 18].

Primary diagnostics based solely on WSI was applied also in Kalmar County Hospital, Sweden. In this hospital digitization of the whole daily production of histopathological specimens was initiated around the same time as in AMCH and has resulted in a situation where 75% of the diagnostics are performed digitally in addition to teleconsultations for frozen sections from other hospitals without local pathologists. Assessing the feasibility of Primary WSI based diagnostics is ongoing currently in University of Pittsburgh Medical Center, USA [5], University Medical Center Utrecht, The Netherlands [16, 17, 19] and University Medical Center Nijmegen in collaboration with AMCH (manuscript in preparation). In addition,

Digital Pathology Association (DPA) has presented two papers at the Pathology Vision conference 2011 discussing the high level validation approach of WSI for primary diagnostics. The continuous efforts aimed for validating WSI for primary diagnostics will help eventually in wide use and acceptance of WSI for this purpose. A recent review (via Medscape) has discussed the legal aspects of primary diagnostics using WSI. In this review it was stated that according to the available documentation it would be sufficient to have local descriptions of the workflow process and to monitor the performance of the individual pathologists, an approach very similar to that for monitoring the quality of conventional microscopy.
Quality assurance (QA) and quality control has not been worked out for primary digital pathology diagnosis, but is clearly an important issue. Guidelines are e.g. needed as to the desired resolution, completeness of the scans, color depth, compression ratios, quality of focus, and duration and quality of storage. When starting primary digital diagnosis, it seems wise to have an initial period of full QA of about 50 cases in major diagnostic areas for every pathologist, and then to do random QA in about every 50th case.
In conclusion, the quality of the currently produced WSI is sufficient for primary diagnostics in histopathology. Large scale scanning of the whole daily routine production in histopathology should be accompanied by some modifications regarding adequate and timely image retrieval and controlling scanning errors. Solving theses issues is necessary before complete replacement of glass slides within pathology work could be achieved. Still, with currently available technology, it seems that at least 82.1% of routine pathology can be signed out digitally.

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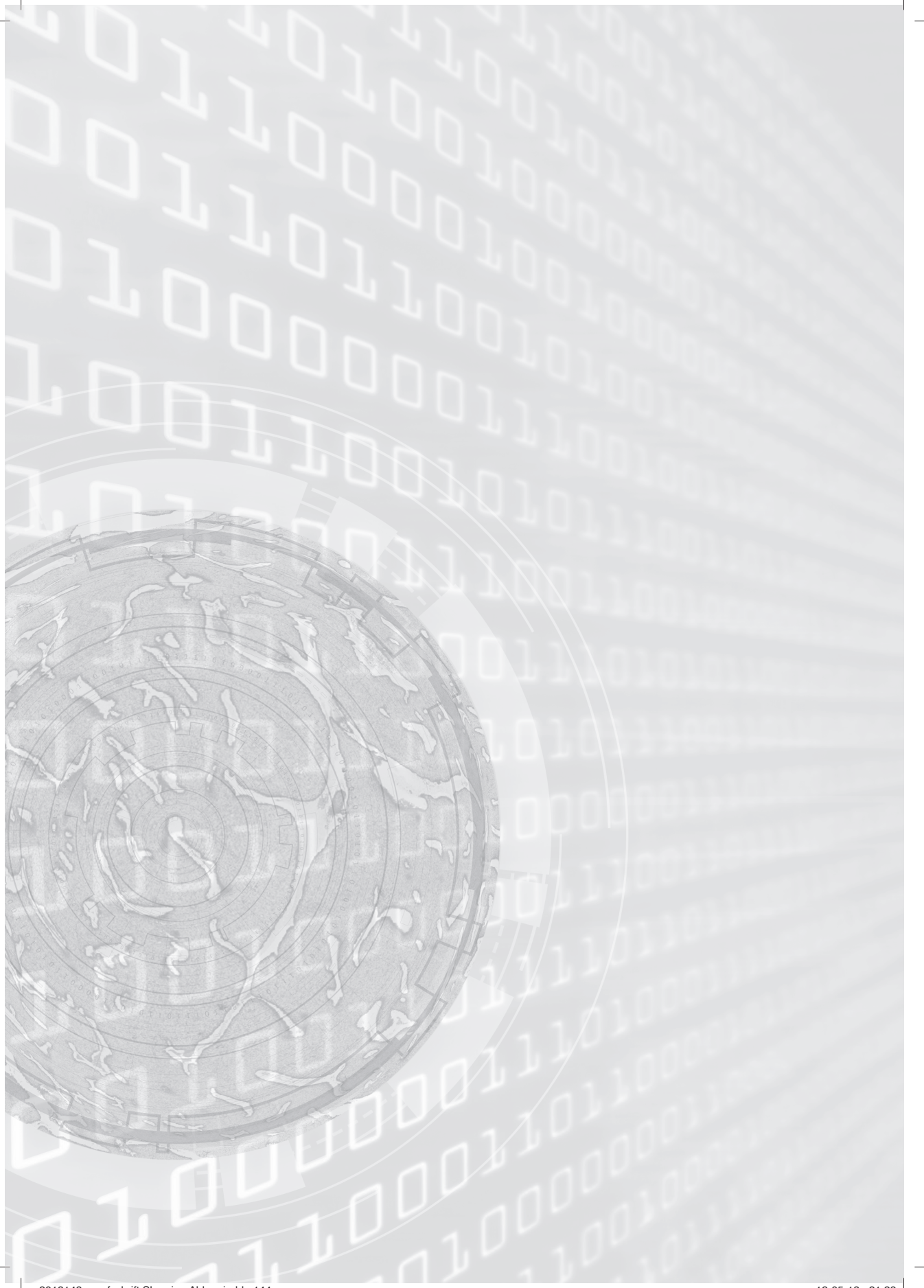

Chapter 11

General discussion and conclusions

General discussion and conclusions

Whole slide imaging is the process of digitizing glass slides resulting in the creation of Whole Slide Images (WSI). WSI are usually explored with the aid of an image viewer in a manner that closely simulates examining glass slides with a conventional microscope, permitting the manipulation of an entire tissue section in any direction and at any magnification. WSI have been incorporated into several applications within pathology. Nevertheless their use in primary diagnostics is still limited, possibly because it is not approved yet from the Food and Drug Administration (FDA) for such purposes. The main aim of this thesis was therefore to assess the validity of using WSI as a platform for primary diagnostics in pathology. Below, you will first find a summary per Chapter and after that some general conclusions that can be drawn by combining the data from the different Chapters.

In **Chapter 2**, we review the currently available literature covering the field of digital pathology. WSI lend numerous advantages over a conventional microscope in terms of easy slide annotation, accessibility, sharing by multiple observers at the same time from different locations as well as the ability to apply automated image analysis. Due to the aforementioned virtues, WSI are regarded as a flexible alternative to glass slides and a microscope in several applications within pathology, particularly education, tele-consultation and research. The general tenet of automated image analysis will eventually extend the capabilities beyond subjective diagnostics to a more objective and productive pathology practice. More details about WSIs characteristics, advantages, applications and the future of digital pathology are presented in this review.

Chapters 3-7 present five different studies aimed at the validation of WSI for primary diagnostics of different body systems; comprising the gastrointestinal tract, dermatopathology, breast, pediatrics and urinary system specimens. Five hundred biopsies and resections (100 per system) were assessed by a group of pathologists on two occasions, microscopically and on WSI scanned at 20x. Different concordance rates were observed between microscopic and digital diagnoses in different body systems. The highest concordance rates were encountered in cases originating from the gastrointestinal tract and dermatopathology with percentages agreement of 95% and 94%, respectively. In both systems, the encountered discrepancies were mild without expected clinical or prognostic implications for the patients. Concordance rates of 93%, 90% and 87% were observed between microscopic and WSI based diagnoses of breast, pediatrics and urinary system cases, respectively. Discrepancies with possible clinical implications for therapy were noted in these three systems but at low frequencies. Table 1 shows an overview of the concordance rate per system for the five validation studies.

Table 1. An overview of the concordance rate per system for the five validation studies.

System	% concordance	Discrepancies without clinical consequences	Discrepancies with clinical consequences	WSI diagnosis was preferred
Gastro-intestinal tract	95%	5	0	3
Dermatopathology	94%	6	0	1
Breast	93%	6	1	4
Pediatrics	90%	8	2	1
Urinary system	87%	8	5	6
Total / 500 cases	92%	33	8	15

We consider these low rates of discrepancies to be within the range of inter- and intra-observer variation in pathology if these cases would be reexamined with the aid of glass slides and a conventional microscope. Pathologists did not encounter many difficulties in rendering diagnostics using WSI scanned at 20x magnifications. In some cases, however, a higher resolution was preferred for more confident diagnosis such as in cases suspected for microorganism infections, renal specimens and placentas. Scanning at a high resolution is generally preferable to avoid any problems relating to the lack of resolution and this is anticipated to be the future standard. However, scanning slides at 40x seems not necessary in most cases while still increasing scanning time and storage requirements, although it will soon become the standard anyway. The main conclusion that can be drawn from these five validation studies is that primary diagnostics in pathology can in general be reliably performed on WSI acquired using today's scanning technology.

Our validation studies aimed mainly at investigating the intra-observers variability when using different diagnostic modalities (inter-modality variability); namely a conventional microscope and WSI. During the study, cases which were diagnosed previously on the bases of glass slides and a conventional microscope were re-diagnosed with the aid of WSI by the same pathologist who did the initial diagnosis to avoid inter-observer variation as much as possible. Such study setup is crucial at this initial phase to discover mainly the feasibility but also the advantages, possibilities and the drawbacks of the field of digital pathology for primary diagnostics. Different study designs assessing the inter-observer reproducibility using different modalities (WSI versus microscopic) would be less beneficial because it would be difficult to analyze the reasons of discrepancies which can be caused either by the impact of using different methodologies or by the differences in pathologists' experience.

Simulating routine practice, we have included all the slides belonging to the same case and did not select a so called representative slides as other studies have done.

Additionally, multiple systems and different specimen types as well as diverse diagnostic entities covering a broad spectrum of surgical pathology problems were included in our validation studies. Furthermore, in one study (**Chapter 6**) we have also assessed the statistical differences in rendering the diagnostics using different modalities (digital and microscopic).

Further validation studies covering specific diagnostic entities, such as melanocytic, inflammatory skin lesion, various borderline lesions as well as the possibility to evaluate different types of micro-organisms and scoring various kinds of immunohistochemical stains on WSI would be valuable. It would also be interesting to investigate the intra-observer variability using WSI and compare it to that of a microscope.

The validity of WSI for examining fine cellular details was tested further in chapter 8 and 9. The reliability of evaluating the Mitotic Activity Index (MAI) in breast cancer cases using WSI was thoroughly investigated in **Chapter 8**. One hundred breast cancer biopsies and resections were subjected to mitosis counting by three observers on two occasions; microscopically and on WSI scanned at 40x. A "perfect" inter-observer agreement was obtained from counting mitosis on the bases of the conventional microscope (intra-class correlation coefficient (ICCC) 0.879) and on WSI (ICCC 0.924). Similar good inter-observer agreement with average kappa values of 0.642 and 0.635 was shown between MAI scores using a conventional microscope and WSI. There was strong to perfect intra-observer agreement between MAI counts and mitotic scores per observer when using the two diagnostic modalities (ICCC 0.716-0.863, kappa 0.506-0.617). However, there was an obvious tendency to slightly underestimate the number of mitoses on WSI, but when transferring mitosis counts to mitotic scores as in grading, WSI based scores did not significantly differ from scoring mitosis using glass slides and a conventional microscope. These results indicate that scoring mitoses in breast cancer cases can be reliably done on WSI scanned at 40x magnifications and at one focal plane, probably without influencing prognostic impact of mitotic counts. However, the latter remains to be formally studied.

Chapter 9 focuses on the possibility of assessing HER2 amplification in breast cancer cases by scoring HER2 chromogenic in situ hybridization (CISH) stained slides on WSI scanned at 40x magnification. 50 HER2 were scored by an experienced observer microscopically and on WSI. The results revealed an overall high concordance between digital and microscopic assessment of HER2 CISH, but there was a noticeable tendency toward underestimating the number of HER2 spots on WSI leading to missing low level HER2 amplification in 2/47 cases. Scanning at multiple focus planes may therefore be necessary for optimal HER2 CISH spot counting.

Chapter 10 presents the experience of implementing WSI in routine pathology diagnostics in a medium-sized pathology laboratory. At the Atrium Medical Center

Heerlen in The Netherlands, WSI have been integrated in a stepwise manner into routine diagnostics. This trial was started with minor adjustments of specimens' handling, followed by the performance of two validation studies, ended with successful conversion from conventional to digital diagnostics for part of the routine work. A few of their pathologists digitally do the primary diagnostics on screen now because of the good results of local validation studies, first restricted to breast biopsies and extended later to include different body systems. The gradual introduction of WSI based diagnosis in the daily routine was a very important step to reveal difficulties and problems associated with digital diagnostics, and allow for timely finding solutions.

This study indicates that the quality of the currently produced WSI is generally sufficient for primary diagnostics in histopathology. Nevertheless, a large-scale scanning of the complete daily production in histopathology would require some modifications regarding adequate and timely image retrieval and controlling scanning errors. With currently available technology, however, it seems that over 80% of routine pathology can be signed out digitally.

The conventional microscope has long been considered the gold standard for upfront diagnostics in pathology. Thus, assessing the diagnostic concordance of WSI with that of a conventional microscope is considered to be an indirect measurement of the adequacy of WSI for primary diagnostics. The main conclusion to be drawn from this thesis is that WSI contain sufficient information for rendering most of the diagnostics within pathology which is confirmed by the comparable diagnostics performance achieved by the two modalities. There are several advantages of digital pathology over the conventional way of practicing pathology. The ease of accessing and sharing can only be translated in a flexible way of rendering diagnostics. With the aid of WSI, problematic or difficult cases can be efficiently shared with an expert within suitable time constrains sparing the time required for sending glass slides to faraway places asking for a second opinion. The digital nature of WSI allows their integration into a patient's medical report which will permit pathologists to work within an integral environment that includes the clinical information, pathology data and pathology specimens. WSI can also be electronically archived and retrieved, decreasing the amount of time spent searching for glass slides for consultation, conferences, teaching and research purposes. Furthermore WSI can be subjected to automated image analysis which is believed to improve the productivity and objectivity in daily diagnostics.

The previously mentioned features and the results of the validation studies would undoubtedly encourage the adaptation of WSI as platform for primary diagnostics in pathology. However, several issues need to be addressed before the transition to digital pathology can be achieved. The main issues currently are:

1. The overall image quality besides the presence of inadequately scanned regions

in a portion of the scanned slides arouses concerns over the safety of this technique in routine practice. Adopting WSI in primary diagnostics would necessitate the presence of adequate control of the image quality and resolution matching the diagnostics requirement.

2. The key to successful scan is the good quality of glass slide. In some instances, poor image quality might be traced back to inadequate preparation of the glass slide (Chapters 9, 10). Factors such as uneven tissue thickness, tissue folding, inadequate staining and air bubble formation during coverslipping could negatively affect the scanning process (mainly the focus quality) and consequently WSI quality. In our study, it was difficult to gain a good scan quality in few cases (even with rescanning) because of poor glass slide preparation which has led eventually to case deferral and non digital diagnosis (poorly prepared HER2 CISH slides, Chapter 9). Upfront diagnostics depending solely on WSI would thus necessitate the optimization of glass slide preparation[1-3] for scanning purposes.
3. Over the last few years a dramatic improvement in scanning speed has been seen. Fast and robust scanners which are able to scan a tissue area of 15x15mm at 40x magnifications in one minute were recently introduced from different scanner vendors. With the currently available scanners, timely scanning of the whole daily production of a medium sized pathology laboratory would generally require the presence of multiple scanners operated round the clock to fulfill the scanning requirement. Introducing faster scanners which are able to scan the whole tissue section at 40x in about 30 seconds would encourage more institutes to move beyond merely scanning important or rare cases to large-scale scanning for the complete daily production since slides could be scanned before they leave the lab. This necessitates integration of scanning into the workflow of the pathology lab (e.g. like a conveyor belt). This development would in turn encourage frequent use of WSI in routine work.
4. Exploring WSI was found to be more time consuming than examining a glass slide under the microscope[4]. This might be due to the fact that a computer mouse is not a very suitable navigation tool to explore WSI. Suitable navigation instruments for easier and more efficient exploration of WSI are becoming available.
5. Issues concerning the creation of convenient data management systems to store and retrieve images, capable of handling large amounts of data images need to be dealt with. Also, efficient technical support needs to be adequately arranged.
6. One of the factors hindering WSI integration in routine pathology practice is that the use for this purpose has not yet been approved for primary diagnostics by the Food and Drug Administration (FDA) in the USA. Moreover, the FDA

did not announce any formal guide or a validation path to get such approval. This would make the approval process a time consuming and expensive for scanner vendors. Nevertheless, several topics concerning validating WSI for primary diagnostic purposes have been discussed during the meeting of Pathology Visions 2011(San Diego, CA) suggesting that validation studies should include a large sample size to provide an adequate statistical power and should be specimen specific rather than diagnoses specific. In addition, validating diagnostics on WSI should simulate the routine practice by including all the slides from each case and not only representative slides[5]. As the FDA approval for using WSI in primary diagnostics is not yet issued, the results obtained from this validation study as well as from similar studies in the literature can not be generalized to all pathology laboratories. Using WSI in routine pathology practice in different institutes should always be accompanied by preliminary validation studies in place reflecting the reliability and safety of this technology for their routine practice.

With the growing interest in the field of digital pathology, we anticipate that all of these issues will be solved in the near future leading to the inevitable and radical conversion from conventional to digital practice within pathology.

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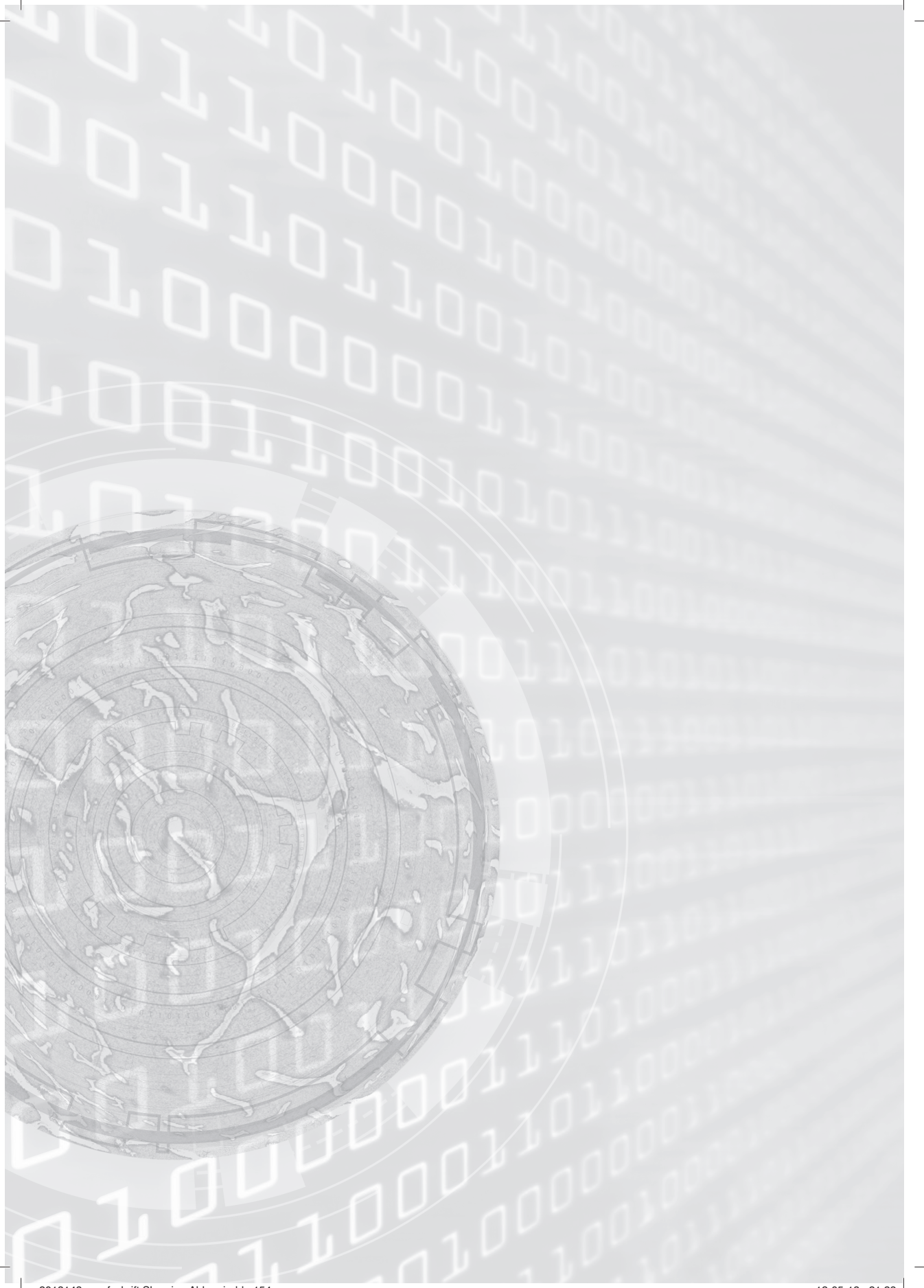

Chapter 12

Nederlandse samenvatting

Nederlandse samenvatting

Whole slide imaging is het proces van het digitaliseren van weefselcoupes resulterend in Whole Slide Images (WSI). WSI worden meestal bekeken met behulp van beeld viewers op een manier die vergelijkbaar is met het bekijken van weefselcoupes onder een conventionele microscoop. Zo bieden beeld viewers de volledige toegang tot de hele coupe en kan hier op in- en uit gezoomd worden en bieden deze viewers allerlei extra functionaliteiten aan.
WSI worden gebruikt voor verschillende toepassingen binnen de pathologie. Niettemin is het gebruik ervan in de primaire diagnostiek nog steeds beperkt, mogelijk omdat het nog niet is goedgekeurd door de Amerikaanse Food and Drug Administration (FDA) voor dergelijke doeleinden.
Het belangrijkste doel van dit proefschrift was om de validiteit van het gebruik van WSI als platform voor primaire diagnostiek binnen de pathologie te onderzoeken. Hieronder vindt u eerst een overzicht per hoofdstuk en daarna een aantal algemene conclusies die kunnen worden getrokken na het combineren van de gegevens uit de verschillende hoofdstukken.
In **Hoofdstuk 2** wordt de bestaande literatuur op het gebied van digitale pathologie besproken. WSI hebben talrijke voordelen boven een conventionele microscoop, waaronder het eenvoudig kunnen plaatsen van annotaties, toegankelijkheid door meerdere gebruikers tegelijkertijd vanuit verschillende locaties en de mogelijkheid om geautomatiseerde beeldanalyse toe te passen. Door de hiervoor genoemde voordelen kunnen WSI worden beschouwd als een flexibel alternatief voor glascoupes en een microscoop voor een aantal toepassingen binnen de pathologie, met name onderwijs, teleconsultatie en onderzoek. Het algemene principe van automatische beeldanalyse kan uiteindelijk leiden tot een verschuiving van subjectieve diagnostiek naar een meer objectieve pathologie met wellicht zelfs kortere doorlooptijden. De kenmerken, voordelen en toepassingen van WSI en de toekomst van digitale pathologie worden in meer detail beschreven in de review in dit hoofdstuk.
In de **Hoofdstukken 3 tot en met 7** worden vijf verschillende studies beschreven. Deze studies zijn gericht op de validatie van WSI voor primaire diagnostiek van de volgende subspecialisaties: maag-darmstelsel, huid, borst, kinderpathologie en de urinewegen. Vijfhonderd biopten en resecties (100 per subspecialisatie) werden beoordeeld door een groep van pathologen met twee diagnostische modaliteiten, namelijk microscopisch en op WSI gescand op een 20x vergroting. De mate van overeenstemming tussen de microscopische en digitale diagnoses was verschillend per subspecialisatie. De hoogste overeenkomsten werden aangetroffen in casus afkomstig van het maag-darmstelsel en de huid met overeenstemmingspercentages van respectievelijk 95% en 94%. De gevonden

discrepanties waren in beide systemen zonder verwachte klinische of prognostische gevolgen voor de patiënt. Concordanties van 93%, 90% en 87% werden aangetroffen tussen diagnoses gebaseerd op microscopische en WSI beoordeling van casus afkomstig van respectievelijk de borst, kinderpathologie en de urinewegen. Discrepanties met mogelijk klinische consequenties voor therapie werden in deze drie systemen wel gezien, echter in lage aantallen. Tabel 1 laat een overzicht zien van de overeenkomstpercentages per orgaansysteem.

We gaan ervan uit dat deze lage discrepanties binnen de range van inter- en intraobserver variatie van de pathologie beoordeling in zijn algemeenheid vallen. Het uitvoeren van de diagnostiek op basis van WSI gescand op een 20x vergroting, was niet moeilijke volgens de pathologen. Echter in sommige gevallen gaf men de voorkeur aan de hogere (microscopische) resolutie om een diagnose met zekerheid vast te stellen; zoals in casus met verdenking op aanwezigheid van micro-organismen en in casus afkomstig van de nieren en de placenta.

Wanneer men scant op een hogere vergroting, bijvoorbeeld 40x, bevatten de scans veel meer informatie. Naar verwachting zal dit de standaard voor de toekomst worden. Echter, scannen op 40x blijkt voor de meeste casus niet noodzakelijk en vergt daarnaast een lange scantijd en meer kostbare opslagruimte. De belangrijkste conclusie die uit deze vijf validatiestudies getrokken kan worden is dat de primaire diagnostiek in de pathologie over het algemeen kan worden uitgevoerd met behulp van WSI geproduceerd met de huidige scan technologie.

Onze validatiestudies zijn vooral gericht op het onderzoeken van de intra-observer variabiliteit bij het gebruik van verschillende diagnostische modaliteiten (inter-modaliteit variabiliteit), namelijk een conventionele microscoop en WSI.

Tijdens de studie werden alle casus opnieuw beoordeeld met behulp van WSI door dezelfde patholoog die in eerste instantie de diagnose gaf op basis van glazen coupes en een conventionele microscoop om interobserver variatie zoveel mogelijk te vermijden. Een dergelijke studieopzet is cruciaal in deze beginfase om zowel

Tabel 1. Overzicht van overeenkomstenpercentage per orgaansysteem.

Systeem	% overeenkomst	Discrepanties zonder klinische consequenties	Discrepanties met klinische consequenties	WSI was beter
Maag-darmstelsel	95%	5	0	3
Huid	94%	6	0	1
Mamma	93%	6	1	4
Kinderpathologie	90%	8	2	1
Urinewegen	87%	8	5	6
Totaal/500 casus	92%	33	8	15

de validiteit van digitale pathologie voor primaire diagnostiek als de voor- en nadelen ervan te kunnen beoordelen.
Een studieopzet waarbij de inter-observer reproduceerbaarheid tussen verschillende modaliteiten (WSI versus microscopisch) bestudeerd wordt maakt het onderzoek veel complexer, omdat het lastig te analyseren is of de discrepanties veroorzaakt werden door het verschil in ervaring tussen de waarnemers of door het gebruik van verschillende modaliteiten.
Om de routine diagnostiek zoveel mogelijk na te bootsen hebben we in de vijf validatiestudies alle coupes behorende tot één casus opgenomen en niet alleen een representatieve coupe van elke casus, zoals in andere studies is gedaan.
Bovendien hebben we verschillende pathologische subspecialiteiten en diverse diagnostische entiteiten onderzocht die het brede spectrum van klinische pathologie beslaan. Daarnaast hebben we in **Hoofdstuk 6** de statistische verschillen onderzocht tussen het uitvoeren van de diagnostiek met de verschillende modaliteiten (digitaal versus microscopisch). Verdere validatiestudies gericht op specifieke diagnostische entiteiten, zoals melanocytaire en inflammatoire huidlaesies, en borderline laesies, en de mogelijkheid om de aanwezigheid van verschillende micro-organismen op basis van digitale coupes te beoordelen zijn ook van belang. Ook is het interessant om de intra-observer variatie bij het gebruik van WSI te beoordelen en te vergelijken met die van microscopische beoordeling.
De validiteit van digitale coupes om fijne cellulaire details te beoordelen is verder getest in **Hoofdstuk 8 en 9**. De betrouwbaarheid van WSI voor het beoordelen van de Mitotische Activiteits Index (MAI) is grondig onderzocht in **Hoofdstuk 8**. De MAI was beoordeeld door drie waarnemers in honderd borstkanker casus middels twee modaliteiten: onder de microscoop en met behulp van digitale coupes gescand op 40x. Een zeer goede inter-observer overeenstemming werd gezien wanneer mitosen werden geteld met behulp van de microscoop (intra-class correlation coefficient (ICCC) 0,879) alsmede met WSI (ICCC 0,924). Inter-observer overeenkomsten waren vergelijkbaar voor MAI scores die verkregen waren met behulp van de microscoop en WSI met gemiddelde kappa waarden van respectievelijk 0,642 en 0,635. Er was een sterke tot perfecte intra-observer overeenkomst tussen het aantal mitosefiguren en MAI scores per observer met de twee diagnostische modaliteiten (ICCC 0,716-0,863, kappa 0,506-0,617). Er was een duidelijke tendens om het aantal mitosefiguren op basis van digitale coupes licht te onderschatten, maar wanneer het aantal mitosen werd omgezet in een gradering bleek er geen significant verschil tussen microscopische en digitale MAI scores te zijn. De resultaten van deze studie geven aan dat het scoren van mitosen op digitale coupes, gescand op 40x en op één focal plane, betrouwbaar is en waarschijnlijk geen invloed heeft op de behandeling en prognose voor de patiënten. Dit laatste zal echter nog wel goed bestudeerd moeten worden in vervolgstudies.

Hoofdstuk 9 richt zich op de mogelijkheid om HER2 amplificatie in borstkanker casus te bepalen door middel van het scoren van HER2 chromogene in situ hybridisatie (CISH) op WSI gescand op een 40x vergroting. Vijftig HER2 coupes werden gescoord door een ervaren analiste met behulp van de microscoop en digitale coupes. Uit de resultaten blijkt dat er een hoge concordantie was tussen microscopische en digitale HER2 bepalingen. Echter er was een zichtbare tendens om het aantal HER2 kopieën op digitale coupes te onderschatten met als gevolg dat twee gevallen met een lage amplificatie werden beoordeeld als niet geamplificeerd. Scannen op meerdere focusvlakken kan daarom noodzakelijk zijn voor het optimaal kunnen tellen van het aantal CISH kopieën. Ook dit heeft als nadeel dat de scantijd langer is en de beelden groter worden.

Hoofdstuk 10 presenteert de ervaringen omtrent de implementatie van WSI in de routine diagnostiek binnen de pathologie van een middelgroot pathologie laboratorium. In het Atrium Medisch Centrum Heerlen zijn WSI stapsgewijs geïntegreerd in de routine diagnostiek. Deze proef begon met kleine aanpassingen in de behandeling van het weefsel, gevolgd door twee validatiestudies, eindigend met een succesvolle omschakeling van conventionele naar digitale diagnostiek voor een deel van het routine werk. De goede resultaten van de twee validatiestudies hebben ertoe geleid dat een paar van hun pathologen met primaire digitale diagnostiek zijn begonnen. Primaire digitale diagnostiek was in eerste instantie beperkt tot borstbiopten en werd later uitgebreid naar andere weefsels. De geleidelijke invoering van WSI in de dagelijkse routine was een zeer belangrijke stap om moeilijkheden en problemen in verband met digitale diagnostiek tijdig te kunnen ontdekken en oplossen.

Deze studie geeft aan dat de kwaliteit van de momenteel geproduceerde WSI, in het algemeen, voldoende is voor het gebruik in routine primaire diagnostiek in histopathologie. Desondanks, om het op grote schaal scannen van de volledige dagelijkse productie binnen de histopathologie te kunnen realiseren, zijn er enkele aanpassingen nodig met betrekking tot de optimalisatie van ICT systemen die de beelden opslaan en weer moeten presenteren aan de gebruiker. Daarnaast is het belangrijk om kwaliteitscontrole van ingescande coupes goed te regelen. Echter, met de huidige kwaliteit van digitale coupes kan meer dan 80% van de diagnostiek digitaal uitgevoerd worden.

De conventionele microscoop wordt beschouwd als de gouden standaard voor het uitvoeren van de diagnostiek in de pathologie. Het bepalen van de diagnostische concordanties van WSI met een conventionele microscoop kan dus beschouwd worden als een indirecte meting van de geschiktheid van WSI voor primaire diagnostiek. De belangrijkste conclusie die uit dit proefschrift getrokken kan worden is dat WSI voldoende informatie bevatten om het grootste deel van de diagnostiek binnen de pathologie uit te voeren. Dit feit wordt bevestigd door

de vergelijkbare diagnostische prestaties van beide modaliteiten.
Er zijn duidelijke voordelen van digitale pathologie boven conventionele pathologie. Het gemak waarmee men toegang heeft tot digitale coupes en waarmee ze gedeeld kunnen worden, maken digitale pathologie een flexibele manier om diagnostiek uit te voeren. Met behulp van WSI kunnen problematische of moeilijke gevallen makkelijk met een expert worden gedeeld zonder dat er tijd verloren gaat die anders nodig zou zijn voor het verzenden van de glazen coupes voor een second opinion. De digitale aard van WSI maakt integratie van digitale beelden in de medische rapporten van patiënten mogelijk. Hierdoor werken pathologen in een integrale omgeving die de klinische informatie, de pathologiegegevens en de pathologiebeelden omvat. Daarnaast kunnen WSI ook elektronisch worden gearchiveerd en opgevraagd vanuit bijvoorbeeld een EPD of tijdens een multidisciplinair overleg, waardoor de hoeveelheid tijd die normaal besteed wordt aan het zoeken naar glazen coupes voor overleg, conferenties en onderwijs- en onderzoeksdoeleinden enorm afneemt. De mogelijkheid om automatische beeldanalyse toe te passen zal waarschijnlijk de productiviteit en de objectiviteit van de dagelijkse diagnostiek verbeteren.
De eerder genoemde kenmerken en de resultaten van dit validatieonderzoek moedigen zonder twijfel de implementatie van WSI als een platform voor primaire diagnostiek in de pathologie aan. Er zijn echter verschillende aspecten die aangepakt moeten worden voordat de digitale pathologie definitief de conventionele microscoop vervangt.
De belangrijkste punten op dit moment zijn:

1. De algehele beeldkwaliteit, de aanwezigheid van slecht gescande gebieden in de coupe en het feit dat soms niet al het weefselmateriaal op de coupe gescand wordt, geeft aanleiding tot bezorgdheid over de veiligheid van deze techniek in de dagelijkse praktijk. Het kunnen gebruiken van WSI voor primaire diagnostiek vereist adequate controle van de beeldkwaliteit en de resolutie die noodzakelijk is voor diagnostiek.
2. Goede kwaliteit van de oorspronkelijke coupe is cruciaal voor een goede scankwaliteit. Kwalitatief onvoldoende glazen coupes kunnen leiden tot een slechte beeldkwaliteit (**Hoofdstukken 9,10**). Bovendien kunnen factoren zoals ongelijke weefseldikte, vouwen in het weefsel, vlekken en luchtbelvorming tijdens het afdekken van een glazen coupe een negatieve invloed hebben op het scanproces (voornamelijk de focus kwaliteit) en daarmee de kwaliteit van WSI. In onze studies was het in enkele gevallen moeilijk om een goede scankwaliteit te krijgen (zelfs na herhaaldelijk scannen). De oorzaak daarvan was slechte kwaliteit van de glazen coupes resulterend in slechte kwaliteit van de digitale coupes. Dit heeft in deze gevallen uiteindelijk geleid tot het uitstellen van de diagnose en niet-digitale beoordeling (slecht vervaardigde HER2 CISH

coupes, Hoofdstuk 9). Het uitvoeren van de diagnostiek uitsluitend op basis van digitale coupes vereist de optimalisatie van de vervaardiging van glazen coupes. Uiteraard is de mate waarin deze effecten optreden afhankelijk van de scanner die gebruikt wordt.

3. De afgelopen jaren hebben we een dramatische verbetering in de scansnelheid gezien. Snelle en robuuste scanners die in staat zijn om een weefselgebied van 15x15 mm op 40x vergroting binnen een minuut te scannen worden door verschillende leveranciers aangeboden. Met de momenteel beschikbare scanners zouden voor het tijdig scannen van de volledige dagelijkse productie van een middelgroot pathologielaboratorium toch nog meerdere scanners vereist zijn die 24 uur per dag functioneren. Introductie van een snellere scanner die het hele weefsel binnen 30 seconden kan scannen zou meer instellingen aanmoedigen om te beginnen met digitalisatie van hun laboratoria en over te gaan naar grootschalig scannen in plaats alleen moeilijke en zeldzame gevallen selectief te scannen. Integratie van het scanproces binnen de workflow van het lab (zoals een transportband tussen de verschillende apparaten die betrokken zijn bij het productieproces van een coupe en het scannen hiervan) zou frequent gebruik van digitale coupes in de routine pathologie stimuleren.
4. Een diagnose stellen met behulp van digitale coupes kost in het algemeen iets meer tijd dan het bekijken van glazen coupes onder microscoop. Dit kan te wijten zijn aan het feit dat een muis geen geschikt navigatiemiddel is. Geschikte navigatie-instrumenten die een eenvoudigere en efficiëntere manier van navigeren kunnen bieden komen nu langzaam aan beschikbaar.
5. Kwesties met betrekking tot het inrichten van geschikte beeld- en datamanagement systemen om digitale beelden soepel te kunnen verwerken moeten opgelost worden. Een efficiënte (en betaalbare) ICT infrastructuur, zowel de hardware voor bijvoorbeeld beeldopslag als de software, zal goed geregeld moeten worden. De kosten voor het opslaan van beelden zijn voor veel laboratoria momenteel een probleem, waardoor het alleen mogelijk is om een selectie van de coupes te scannen en/of te bewaren.
6. Eén van de factoren die de integratie van digitale coupes in de dagelijkse diagnostiek belemmeren is dat WSI nog niet zijn goedgekeurd door de Amerikaanse FDA voor primaire diagnostiek. Ondanks dat regelgeving hierover in de Verenigde Staten anders is dan in Europa (hier volstaat een relatief makkelijk te verkrijgen CE markering) vertraagt dit het ontwikkelproces en motivatie van leveranciers. Bovendien is er vanuit de FDA geen duidelijke of formele richtlijn hoe ze willen dat leveranciers dit proces oppakken. Dit maakt het validatieproces tijdrovend en duur voor leveranciers die hier inmiddels toch mee gestart zijn. Niettemin heeft de FDA op het Pathology Visions congres in 2011 (San Diego, CA) wel bepaalde onderwerpen die te

maken hebben met de validatie van WSI besproken, waarbij ook iemand van de FDA aanwezig was: de validatie studies moeten een grote steekproefomvang bevatten om voldoende statistische power te geven en de validatiestudies moeten monster gericht en niet diagnose gericht zijn. Bovendien moeten van elke casus alle coupes bekeken worden en niet alleen representatieve coupes. De resultaten van deze studie alsmede van vergelijkbare studies kunnen niet gegeneraliseerd worden naar alle pathologie laboratoria omdat de experimenten gedaan zijn binnen een bepaalde context die de resultaten beïnvloedt. Het gebruik van digitale coupes in andere instituten moet altijd voorafgegaan worden door een lokale validatiestudie om de betrouwbaarheid van digitale coupes voor diagnostiek te bewijzen in die specifieke labcontext.

Met de groeiende belangstelling voor digitale pathologie, wat leidt tot meer concurrentie en daarmee betere producten voor een lagere prijs, verwachten we dat deze problemen in de nabije toekomst zullen worden opgelost. Dit zal leiden tot de onvermijdelijke en radicale omschakeling naar het breed gebruiken van digitale coupes in plaats van de conventionele microscoop binnen de pathologie voor primaire diagnostiek.

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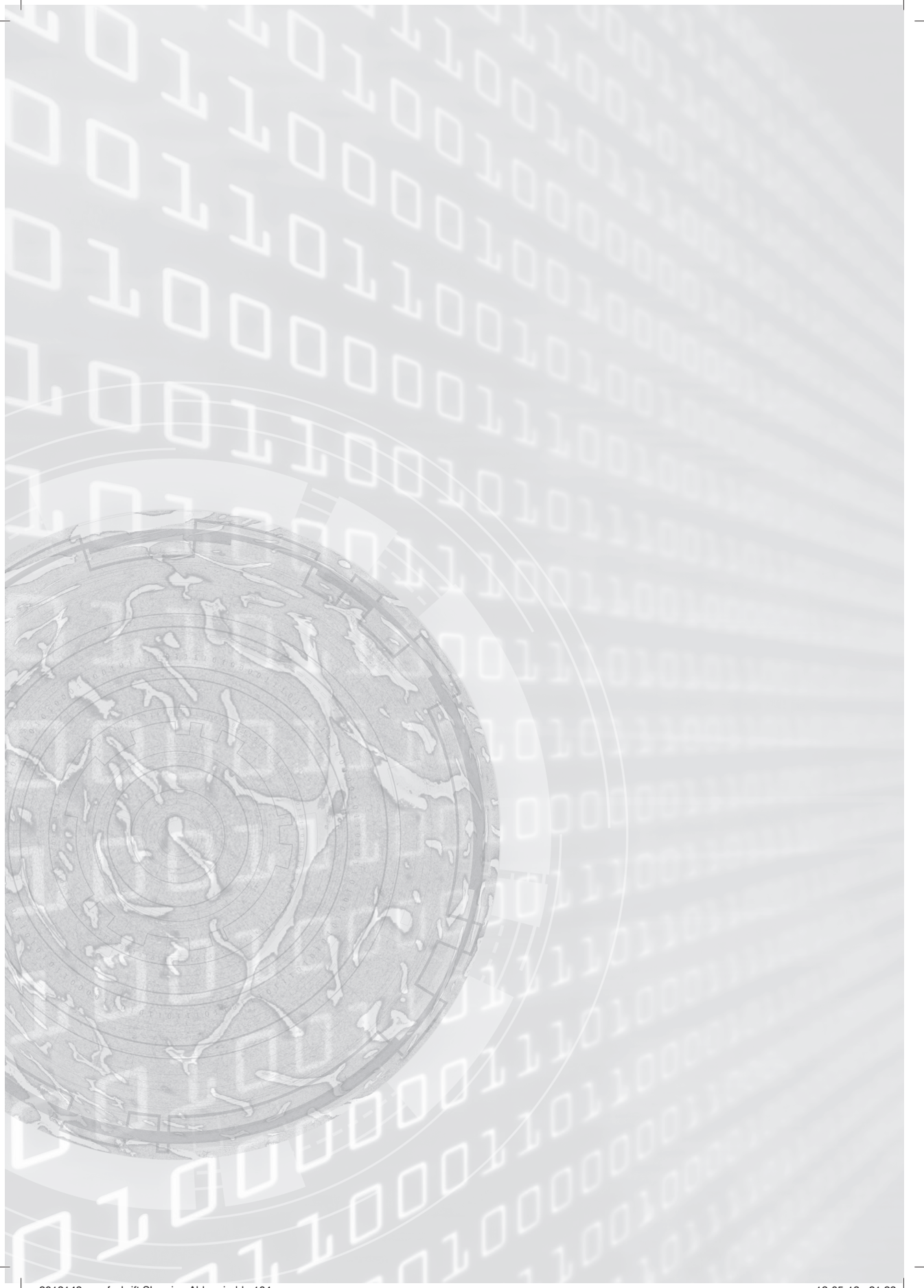

Appendix

Acknowledgements
Curriculum Vitae
Publications
Abbreviations

Acknowledgements

This thesis is the result of a research project that was conducted at University Medical Center Utrecht (UMCU) and Symbiant Pathology Expert Center. The success of any research project requires a lot of guidance and support from many people. Fortunately, I was surrounded by such supportive people without whose help it would not have been possible to write this doctoral thesis. Here, I would like to take the opportunity to express my appreciation and to thank all the people who have actively contributed to this research and helped to finish this book, starting with some special words to my supervisors.

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It is very common in The Netherlands if you pronounce one or two Dutch words with a foreign accent that you get a reply in English. It was the other way around in our room at Symbiant /Alkmaar, where even when I spoke English, I got the answer in Dutch, which was a very good way for me to improve my Dutch. Dear Chantal and Matilda, thank you for improving my Dutch lately.

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Curriculum Vitae

The author of this thesis, Shaimaa Al-Janabi was born on March 3rd, 1978 Baghdad, Iraq. Upon finishing secondary school in 1995 she was immediately accepted as a medical student at the Al-Nahrain University (Baghdad-Iraq), where she obtained her medical degree in the summer of 2001. After graduation she worked as a general practitioner at Al-Kadhmyia Teaching Hospital in Baghdad for a period of two years.

In 2003 she decided to further her studies and was accepted as a resident in the Pathology Department at Al-Nahrain University and was allowed to join a two-year master program, at the end of which, she received her Master's degree in Pathology in November of 2005.

In November of 2009, she started her PhD at the Department of Pathology at the University Medical Center Utrecht (UMCU). In the first two years of the research period she worked at the UMCU where she had the opportunity to conduct her research project. In 2011 she started her work as an Arts onderzoeker at Symbiant Pathology Expert Center to finish her PhD and to gain more experience in starting a Digital Pathology unit in a medical institute.

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Evaluation of Mitotic Activity Index in breast cancer using whole slide digital images.
Submitted for publication

Kuijpers CCHJ, Moelans CB, van Slooten HJ, Horstman A, Hinrichs JWJ, **Al- Janabi S**, van Diest PJ, Jiwa NM.
Added Value of HER-2 Amplification Testing by Multiplex Ligation-dependent Probe Amplification in Invasive Breast Cancer.
Submitted for publication.

Abbreviations

ACE	acetylcholinesterase
ACIS	automated cellular imaging system
ADH	atypical ductal hyperplasia
AIN II	anal intraepithelial neoplasia grade II
ATN	acute tubular necrosis
BCC	basal cell carcinoma
BIRADS	Breast Imaging-Reporting and Data System
BK	virus
CAD	computer-aided diagnosis
CAOS	chronic abruption oligohydramnios sequence
CHX	chromosomal
CI	confidence interval
CIS	carcinoma in situ
CISH	chromogenic in situ hybridization
DCIS	ductal carcinoma in situ
DD	differential diagnosis
DICOM	Digital Imaging and Communications in Medicine
DM	diabetes mellitus
DSI	digital slide images
ER	estrogen receptor
FDA	Food and Drug Administration
FISH	fluorescent in situ hybridization
GA	gestational age
GI	gastro-intestinal
H&E	hematoxylin and eosin stain
H. pylori	helicobacter pylori
HER2	human epidermal growth factor receptor 2
HPV	human papillomavirus
ICCC	intra-class correlation coefficient
IFTA	interstitial fibrosis and tubular atrophy
IHC	immunohistochemistry
IT	information technology
IUFD	intrauterine fetal death
IUGR	intrauterine growth retardation
K	kappa
MAI	mitotic activity index
MLPA	multiplex ligation- dependent probe amplification
NADH	nicotine amide diamine hydrogenase

NRBCs	nucleated red blood cells
PALGA	Pathologisch Anatomisch Landelijk Geautomatiseerd Archief, (Pathologic Anatomic National Automated Archive)
PR	progesterone receptor
QA	quality assurance
SDH	succinic dehydrogenase
TIN	tubulointerstitial nephritis
TK	transplanted kidney
TMA	tissue microarrays
TUR	transurethral resection
U-DPS	Universeel Decentraal PALGA Systeem, (Universal Decentralized PALGA system)
VIN	vulvar intraepithelial neoplasia
WSI	whole slide images

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