Lauri Eklund

The Process of Digital Pathology and its Application in a Study

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Vastuuhenkilö: Prof. Pekka Taimen

UNIVERSITY OF TURKU

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Digital pathology saw its advent in the 60's with the introduction of telepathology and was brought into a brighter spotlight in the late 90's through the technological breakthrough in histopathological imaging, called whole slide imaging (WSI). With steady growth in interest among experts, the latest breakthrough in WSI happened in 2017, when both the US Food and Drug Administration and the European Union approved the use of WSI systems in primary diagnostics. So far, the adoption of digital pathology has been slower than many expected, but many laboratories around the world are looking to switch into a digital workflow.

In this text, I aim to describe the history and the technical basics of digital pathology and WSI, as well as discuss some of its most widely used and promising applications in education, research, telepathology, clinical work, and image analysis. To better illuminate the digital workflow, I describe the use of digital pathology in a study by Anttinen M et al., in which the author of this text participated in the form of digitizing the whole slide images used in the study.

With the advancements in digital pathology in the past two decades and with the regulation catching up, wider adoption WSI systems is to be expected. Many advantages can be associated with digital pathology e.g., better results in learning for students, cost reductions in clinical work, and the reduction in pathologists' workload due to automated image analysis methods.

Keywords: Digital pathology, whole slide imaging

Table of contents

Introduction

Background

The dawn of Digital Pathology (DP) in the latter half of the 20th century and its ever-accelerating development in the past two decades has brought the field of pathology to its next level. Due to the constant growth in modern computers' processing power, as well as the introduction of Whole Slide Imaging (WSI), the current advantages and the prospects of the digital workflow heavily steer the contemporary pathologist away from the microscope and towards the computer screen. These advantages include the ability to work or consult from anywhere with an adept workstation, the ability to teach and educate large groups at once with sharable, digital databases, and the possibility to incorporate computational methods e.g., Artificial Intelligence (AI), to research and clinical work in pathology.

In this review I aim to answer the following questions: (1) what is Digital Pathology and how does it work, (2) how DP has evolved and what were some of the pivotal points in its development, (3) how DP is used in contemporary pathology work, and (4) what are the main prospects and limitations of this technology and how to achieve and overcome them. To demonstrate some of the methods, I discuss their application in a study by Anttinen M et al. where the author of this review also participated. [60]

Technical Basics

The core of WSI revolves around four stages: image acquisition (scanning, capturing), storing the image (saving), manipulation of images (editing), and viewing, displaying, or sharing acquired images [13]. For the very basic task of capturing images, four components are essential: a light source, a slide stage, an objective lens, and a high-resolution camera. To achieve full operations, a computer with suitable software is needed for image creation, management, manipulation, and viewing. Since one of the great benefits of digitized slides is the ability to share and view them from a separate workstation (telepathology), a high-speed network connection is also highly recommended. [14][15][19]

WSI scanners capture images either in a tile or a linear pattern, where either small rectangular tiles or strips of the whole slide are captured, respectively. These smaller images are then digitally compiled to form a picture of the whole slide. Different focusing strategies can be implemented, others prioritizing superior image quality and others speed. Generally, the more focus points are applied, the longer the image capturing takes and the higher the quality of the captured image. The three main focusing strategies are (1) focusing every field, (2) focusing every n^{th} field, or (3) using focus maps where only the tissue regions are focused. Intuitively, the first method is the most accurate while taking the longest whereas the two latter methods are faster but compromise in quality. Linear scanning utilizes focus maps and tile scanning focus fields, although focus maps can also be applied to tile scanning but not vice-versa. Most scanners allow the user to manually select the focus points. This can be time-consuming, but it grants the user more control in terms of end-product [15][16][19].

Different variables in different WSI scanners leave the user with many options when purchasing a WSI system. These include, among other things, staining modalities (brightfield, fluorescent,

multispectral), slide capacity, scanning speed and different magnifications. For most applications of the final whole slide image e.g., viewing, and interpreting H&E and IHC slides, x20 magnification is sufficient [19][20]. Some applications requiring higher magnifications include cytology samples and in situ hybridization samples [25]. Most modern scanners offer modalities with higher magnifications, often at x40 and up to x100. Despite superior quality, these magnifications are not feasible in routine work because of their massive file sizes. These variables with numerous others can make the decision difficult when looking for the right system. The lack of standardization in terms of image capturing and post-processing make the choice even more difficult. Previously Farahani N et al. wrote an in-depth review about various features in WSI scanners [19]. Although the article is from 2014, the factors to be considered have mostly remained the same.

The magnification is only a part of the end-product quality. Other factors include lens-aperture, resolution of the camera as well as the resolution of the monitor. Any sub-optimal part produces a bottleneck where the image quality cannot be improved without addressing said part. Digital resolution is affected by the sensor which captures the information. If the sensor can capture, for example, 1 micron per pixel the smallest objects that can confidently be observed as separate must be at least 2 microns. Aperture is the numerical value of different angles from which the optics can collect light. If the aperture is too small, not enough information reaches the sensor and so the sensor cannot reach its full potential. Vice-versa, if the sensor is too small for the aperture, information is lost due to the sensor not being able to separate objects that are presented. If the end-product is then viewed on a low-resolution or otherwise unsuitable monitor, not all the captured information can be appreciated. Magnification does not change the resolution at which the image is captured but it decreases the minimum distance at which two objects can be seen as separate. This whole process is described in greater detail in Sellaro TL et al. [21]

In digital images each pixel contains the information from a fixed area based on the factors discussed earlier. This information is stored as color information, typically in 24-bit color. 24-bit color consists of three 8-bit RGB components creating a possible combination of 256x256x256 colors, so roughly 16.7 million possible combinations. If we assume a resolution of 1 micron per pixel and since each pixel contains 24-bits of information, a 1-mm^2 area would contain 24 million bits, or 3MB, of information if no compression is applied. Most commercially available scanners can capture features smaller than 0.50 microns per pixel at 20x magnification, so real-world examples would easily generate 10-fold amounts of data. [15][19]

To manage the vast amounts of data, a compression algorithm is applied. Popular algorithms include lossy compression JPEG, lossless LZW, and JPEG 2000 which houses both options in its design architecture. In lossy algorithms the information cannot be later recovered but the compression is usually more significant. Some WSI software allows for the user to choose the compression rate from uncompressed 1:1 rate to high compressions such as 128:1. In Krupinski EA et al. [22], the authors found that a lossy compression rate of 32:1 still wouldn't negatively impact the pathologists' ability to discriminate between benign and malignant breast tissue. However, it isn't clear what kind of impact this would have on computer assisted diagnostics. Lossy compressions should also never be applied multiple times on the same image to avoid

image degradation. Another way to reduce file size is to disregard blank regions in the image. [15]

When viewing a whole slide image on a monitor, the displayed image data must be retrieved form the file. If the virtual magnification is low and thus the field of view (FOV) is large e.g., the whole image, the monitor cannot utilize the full resolution of the image. Conversely, when the image is viewed at high magnification, only a small portion of the image must be loaded. Since the file sizes are enormous and the computing power needed to access these files is limited, many vendors have started utilizing image pyramids to bypass this issue. Image pyramids consist of multiple resolutions of the same image. This way, when the FOV is large, a lower resolution image can be loaded to reduce the necessary computing power. When zoomed in, the FOV becomes smaller, and a higher resolution image is loaded to accommodate monitor resolution. This makes the navigation smoother and drives down the system requirements. The trade-off is the WSI file size since multiple images must be saved. Some vendors allow the user to select the number of layers in these pyramids, allowing the choice between file size and accessibility based on their system specifications. [15]

So far, we've only discussed the basics of capturing the image. There are also considerations to be made in terms of storage and management, image access, viewing and manipulating the images, and post-processing. We will discuss these topics further in the context of different usages since they are usually the determining factors for different options.

History

Image analysis is nearly as old as the practice of microscopy itself. As described in Meijer GA et al. [1], image analysis is the term "reserved for a special discipline in pathology that aims to obtain diagnostically important information in an objective and reproducible manner—". While the first research around digitizing cytologic and histologic samples can be traced back to 1960s, the practice of digital imaging only became more prevalent in the late 90s when computer hardware could handle the vast amount of information stored in high-power digital images. Even after the required hardware became available, the lack of compatible and usable software hindered the development of the digital workflow. The first attempts at digitization systems included microscope-mounted cameras only producing static images. Soon after followed robotic microscopy where off-site controlled cameras would capture histologic images in real-time through light microscopes [18][19]. The inception of WSI software development started with spatial dataset research used in integrating spatio-temporal data from satellite imaging [3]. Interest for such technology quickly started gathering in the medical community and in 1997 Ferreira R et al. [2] published The Virtual Microscope, first software developed for such tasks. They described it as "—a software system that provides a realistic emulation of a high-power light microscope." This software could take single tile pictures of the histology sample at high magnification, but the tiles still had to be "stitched" together by hand. The use and development of these virtual microscopes has since been key in DP research and development. At the same time, the first commercial WSI scanner, called BLISS, was being developed by Bacus Laboratories Inc. They also filed the two first patents for WSI systems in 1997 and 1998. The patents weren't filed as Whole Slide Imaging systems, though. The term was only coined by

Wetzel and Gilbertson in 1999. Soon after followed many new system providers with steady improvements in each system, improving on the quality and speed of whole slide imaging. [18]

Tracing back from the 1990s when the first WSI scanners became commercially available, the systems would cost some $300,000 and took more than 24 hours to scan a single slide [3]. Nowadays, while the cost of high-end WSI scanners have not considerably come down, the entry level products are more accessible and manage similar tasks in minutes instead of hours. At the same time, vast improvements in photo-optics, image processing and digital storage systems as well as data transfer have made the use of WSI in everyday work more seamless and have led to the transition from classic light microscope imaging to digital imaging in multiple fields.

More recent advancements in the adoption of WSI in clinical and non-clinical use include several institutions switching to digital workflow [10][11][12]. This has been possible due to many studies indicating that WSI in primary diagnostics offer similar diagnostic value to conventional light microscopy, with numerous advantages compared to the latter [4][5][6]. An important milestone was reached in 2017 when the US Food and Drug Administration (FDA) approved the first WSI system for clinical use after a multi-site study proving non-inferiority of said system compared with conventional microscope [7][9]. This also changed the FDA classification of WSI systems from class III (highest risk) to class II (moderate risk with a predicate device on the market) and provided the manufacturers with clear paths for FDA approval which should accelerate system development for clinical use. As of Feb 2nd, 2022, two WSI systems have gained the FDA approval for primary diagnostics in clinical use: Philips IntelliSite Pathology Solution and Aperio AT2 DX System [8][9]. In EU the situation is different. The WSI systems in clinical use require a Conformité Européenne (CE) mark. New "in vitro diagnostic medical device regulation" (IVDR) EU 2017/746 regarding all in vitro medical devices came into effect May 25th, 2017. This also applies to WSI systems which according to the new IVDR should be considered Class C in vitro medical devices. The transition period ends on May 26th, 2022, but devices certified under the previous "in vitro diagnostic medical device directive" (IVDD) are still valid for two more years. Each member state must independently choose whether a diagnostic system falls under the scope of the new IVDR. [23][24]

Figure 1. Philips IntelliSite SG300. Philips IntelliSite was the first WSI system gaining FDA approval, paving the way for other manufacturers who wanted to seek the approval from FDA. https://images.philips.com/is/image/philipsconsumer/8db15a69ee5c4dcba0c4ad39009658f5?$jpglarge$&wid=840&hei=7

Contemporary Use

Digital Pathology Workflow

Digital pathology workflow starts with its implementation. There are in-depth articles regarding the implementation of WSI systems [25][26] as well as multiple documented examples of laboratories adopting DP workflow [10][11][12][25][40]. Since all laboratories are different and DP can be used in a variety of ways, these guidelines and examples will not cover all aspects of adopting DP workflow but may provide much needed guidance. The implementation starts with recruiting the affected personnel e.g., pathologists, lab technicians, IT support, to create a team of experts in their respective fields. Any concerns should be thoroughly discussed, and any previous inefficiencies corrected before implementing a new system. A robust quality control system and a validation process should be prepared before transitioning to the digital system. The laboratory information system (LIS) plays an integral role in digital pathology. With fully integrated LIS, the pathology specimen can be tracked from macroscopic examination to the retrieval and manipulation of the WSI. This also allows the linking of event logs with the case, helps with quality control, and automatically links the WSI with the patient information. An integrated LIS is highly recommended if WSI is planned for primary diagnostics. For a recent, in-depth review of the implementation process see Fraggetta F et al. (2021) [26].

The first step is to prepare the macroscopic specimen. Choosing the correct slide size, fitting possible fragments close to each other in the paraffin block, and fitting the specimen so that all areas are scanned need to be considered. Microtomes that ensure uniform tissue thickness are advised. Automated staining and mounting solutions, such as Tissue-Tek Prisma Plus® & Film®, are recommended for faster and more predictable staining in the end-product. The possibility of slide racks compatible with both the staining system and the slide scanners should also be considered for a more streamlined production. In some implementations, the subsequent glass slides are dried in 60 °C from 5 to 60 minutes to avoid them sticking to the slide racks during scanning. [12][25]

Once the glass slides are prepared, they are loaded into the WSI scanner according to manufacturer specifications. Some WSI systems allow the user to adjust different parameters such as magnification, color, and the amount of focus points. As discussed earlier, magnification and focusing strategies should depend on the WSI application, but often a 20x magnification is sufficient. If the system is color calibrated the user should refrain from adjusting color parameters. We will discuss this topic further in the next section in the context of validation process. The subsequent WSI files are transferred into their assigned storage, usually the computer hard drive or a local server. Integrated LISs allow the automatic allocation of final WSIs with their respective patient data but often this step needs to be done manually. [15][25][40]

The scanning process is automated but is prone to issues, which can considerably slow down the operation. Some of these issues during the scanning include the slides sticking to the slide racks or the slide stage, dropped slides on the stage, or software problems. Due to these issues, time

sensitive WSIs should be scanned with a trained lab technician available to correct any mistakes as soon as possible. Should the scanning process complete with seemingly no issues, the end-product might still not be up to par. Frequent mistakes can happen especially in focusing the images, which may require re-scanning of one or more slides with manually applied focus points. Fraggetta et al. [12] reported a scan fail rate of around 1%, and it seems newer systems are less prone to failures. Many of the focusing issues can be tracked back to the slide preparation stage. Slide dimensions, especially thickness, need to be considered. Thick tissue samples as well as coverslips may affect the total dimensions. Some WSI systems allow for size calibration, reducing the number of possible issues. Other issues with dimensions may arise from poor coverslip alignment or excess adhesive use, although these problems can be mostly fixed with automating the mounting process and sufficient staff training. Focusing issues may also derive from slide preparation. Excess glue on the coverslip, excess mounting medium creating air bubbles, or tissue folding can all create difficulties in image focusing. Attention should be paid to make sure the glass slides are clean before scanning. [12][40]

Once the final WSI is captured and saved, the pathologist needs to access it. This step varies based on where the image was stored, what viewing software the pathologist is using, and whether the system is integrated. In Eloy C et al. [25], at IPATIMUP, the laboratory implemented an integrated system, where the WSIs were first stored in a local disk and automatically gathered and stored into a 3DHISTECH CaseCenter server and transferred to the corresponding patient's file on the LIS. Reportedly, the images would be available for the pathologist to see, on average, only 30s after they finished scanning. Understandably, manually transferring the images onto a separate external hard drive and physically transporting them for the pathologist to use is much more time consuming and prone to error.

The viewing software is usually provided by the WSI system vendor, although there are some popular free alternatives, like QuPath, ImageJ, and OpenSlide, that support a variety of image formats and offer diverse tools for viewing and image manipulation [27][28][29]. Often the selected software is based on the WSI application. For example, for educational purposes ease of navigation, the ability to annotate the WSI, and the ability to capture and share regions of interest is wanted. Some software offer basic image analysis algorithms, such as cell or mitosis detection and counting. If used for research and development, it is recommended that the software can use direct image access to access the data while forgoing the viewer. [15]

Validation process

The increased adoption of DP solutions at varying levels has created the need for guidelines in implementing these systems. A meta-analysis of 25 validation studies by Azam AS et al. showed a clinical concordance of 98.3%, with major discordance factors including the assessment of nuclear atypia, and grading of dysplasia and malignancy [30]. So far, no strong evidence on different solutions exists but some guidelines and recommendations have been published. Probably the most widely cited and used are the guidelines by College of American Pathologists (CAP) created in 2013 and updated in 2021 [14][31]. The new guidelines offer 3 strong recommendations and 9 good practice statements (weak recommendations), with the 3 strong recommendations dealing with validation set size, the diagnostic concordance between digital

microscope (DM) and light microscope (LM), and the washout period between DM and LM sets. The evidence quality for strong recommendations is stated to be moderate.

The CAP guidelines use the GRADE approach in creating the guidelines. The first strong recommendation is regarding the validation set size. Their recommendation based on literature review is a minimum set size of 60 samples for each imaging modality (H&E stain, frozen sections, hematology) with additional 20 samples if further applications within sets are included, such as immunohistochemistry. The authors emphasize that these guidelines are not meant to be rigid but rather help those wanting to implement their own WSI system. It is also noted that the validation sets should represent real-world cases. [31]

The second strong recommendation states that intraobserver concordance between DM and LM samples should be established and that the concordance should be no less than 95%. If this cannot be achieved, the team participating in the study should investigate and correct the underlying cause. The 95% figure was based on 33 studies reviewed by the authors, where the weighted mean concordance rate was 95.2%. The ground truth diagnosis in most studies is the one done on LM and the most common study design is to show non-inferiority of DM compared to LM's "ground truth". [31]

The third strong recommendation states that "a washout period of at least 2 weeks should occur between viewing digital and glass slides". This is to reduce recall bias among pathologists reviewing and diagnosing the cases in validation sets. The authors note that studies specifically designed to identify an optimal washout period duration are nonexistent, although one study by Campbell et al. [32] compares the difference of 2- versus 4-week washout period and the pathologists' ability to identify previously seen cases. At 2 weeks the pathologists could reportedly recall 40% of cases correctly. In their review of 14 studies, CAP observed no influence in the concordance rate based on the washout period duration. Due to these findings, longer washout periods cannot be recommended. [31]

The 9 good practice statements differ in that they aren't evidence based. They simply have "high level of certainty that the recommendation will do more good than harm". These statements include recommendations that each laboratory should carry out their own validation studies when implementing new DP solutions despite previous validations of similar systems, validation studies should look to emulate real-world scenarios, and they should include specimens that are relevant in their intended application. Other recommendations deal with staff training, documentation, and the scope of the study and how it should be carried out. [31]

Difference in color presentation can lead a multitude of problems including reduced speed in diagnosis, increased difficulties in reading the images, and even reducing the interobserver agreement in diagnoses [33]. First major differences in color can form in tissue and slide preparation e.g., tissue staining. Differences between scanners can lead to different color representation in the same sample, and different viewing software can display even the same image with noticeably differing colors. The same image on the same viewing software can also be perceived vastly different on two separate displays. [15]

A solution proposed by Shrestha P et al. [34] is to introduce color calibrating phantom slides to translate scanner specific colors to the standard RGB color space and thus reduce the inter-scanner color variability. Their technique follows the International Color Consortium (ICC) standards, with the standardized reference "IT8.7/1 target", 28 greys/264 colors. After scanning the calibration slides their reference values can be compared with values produced by scanner to ensure accurate representation and to create an ICC profile, which corrects the color deviations, for that particular scanner. Similar procedure can be applied to the display as well, comparing the input and output values. Many articles also discuss the importance of sufficient displays for DP workflow [15][26][35], noting that monitor resolution, brightness, color depth, contrast, fidelity, and profiles should be considered.

Digital Pathology in Education

The use of WSI in teaching and education has been shown to have multiple advantages over LM in several studies [36], including improved test results in undergraduate teaching, overall more positive attitude towards teaching in students [37], reduced time and cost in examinations [38], and increased interactivity during teaching [36][39]. In their review article, Saco A et al. [36] broadly portray different advantages as well as some disadvantages in using WSIs for teaching purposes. DP can also be used at all stages of education from undergraduate to certified pathologist and can be utilized with less restrictions than a LM.

Advantages of WSI can be divided into 3 categories: (1) equipment related, (2) viewer related, and (3) slide related. Equipment related advantages deal with the differences in DM and LM. It has been well documented that the contemporary student feels more comfortable with computer-based tools than using a conventional microscope [37]. Because there is no need to learn using a new tool i.e., the LM, students start learning about the anatomy and histology faster. It also helps that whole slide images are always in focus. The versatility and ease of access, due to requiring only a computer with internet access, supports learning by allowing the students to study anywhere and anytime. It also enables teaching in a normal computer class with no special equipment needed. The equipment used in a computer class are also considerably cheaper to acquire and maintain than those with conventional microscopes. The ability to share a case to innumerable people, only limited by server bandwidth, also entails homogeneity in learning and encourages interaction between students and teachers. [36][37][39]

Viewer related advantages include the specifications unique to the viewing software compared with a LM. The ability to view a thumbnail picture on the screen and the ability to zoom out while navigating the sample helps with orientation. The ability to view multiple slides, for example H&E stains and immunohistochemistry, side by side simultaneously allows the viewer to better understand their relations. One can also include other vital clinical data, such as patient history, macroscopic images, and radiology images and reports. Another excellent feature over conventional microscope, for teachers and students alike, is the ability to annotate regions of interest and share their markings. This promotes interactivity and learning, and there's some evidence suggesting that students who use annotations score higher on their examinations. [36][37]

Slide related advantages have to do with the differences between physical and digital histology samples. Digital slides have the advantage of never losing quality, unlike physical slides that can break or deteriorate over time. One digital slide can be shared with as many people as one would like, whereas physical slides would need additional histological sections to accommodate larger audiences. This reduces the costs of teaching and homogenizes the material used for teaching, so no student would suffer due to lower quality slides. There are many large databases of histological samples available so even rarer cases can be taught with ample material. Preparing the material for a teaching session can also be considerably faster if the slides are previously digitized and can be accessed from a remote location. [36][37]

Some of the disadvantages of WSI compared with LM are the initial investment required to purchase and operate a WSI system, the need for large digital storage units and high-speed internet, and not learning to use the conventional LM. For the initial cost, a possible solution could be to use readily available histological databases, so there's no need to purchase a WSI system. Most education regarding histology happens at universities in co-operation with university hospitals, where the WSI system cost can also be divided among many users. Digital storage space is becoming cheaper and more accessible year by year and so the costs can be expected to decrease over time. There is ever-growing evidence that digital microscopy and learning overshadows the traditional light microscope in terms of results and versatility. This will undoubtedly lead to more universities replacing LM with WSI in certain parts of their curriculums. [15][36]

Digital Pathology in Clinical Use

WSI has been associated with multiple benefits in clinical use but so far only few laboratories around the world have gone fully digital [41][42]. This is despite strong evidence indicating non-inferiority compared to LM [30], validation studies for different organ systems in primary diagnostics [4][5][6][7], and reports of reduced costs, reduced turnaround time, and an improvement in worker satisfaction [43]. The slow adoption has been associated with, among other things, high overhead costs [41][42]. Many laboratories have adopted some level of DP solutions and the interest towards increasing digital workflow is high among pathologists. It is to be expected that many laboratories will start implementing WSI systems into their routine work in the coming years.

Pathologists often first evaluate the digital image by glance to ensure the image quality and asses its properties. Then they will quickly focus on regions of interest such as suspected malignant regions. There have been studies showing that high-resolution monitors can speed up this stage by making malignant regions easier to find on low magnification. Some viewer software offer image analysis assistance that can mark these suspicious regions for an even faster evaluation on the slide and to make sure smaller regions are not missed. [53]

Viewer integrated measurement and annotation tools that can easily be exported to pathology reports can also make the pathologists life easier. Some AI based tools can help with tedious or challenging quantifiable markers in tissue, including immunohistochemical Ki-67 or PD-L1 evaluation, residual cancer burden after chemotherapy, or detection of prostate cancer. One form of assisting software has been introduced on the Phillips IntelliSite platform called Galen^{TM}

which can be configured to analyze prostate biopsies and alert of discrepancies between its findings and the pathology report before sign-out. [53]

In Rajaganesan S et al. [44] a comparison between LM and different digital pathology systems was made following CAP guidelines [31]. The authors pitted the systems against each other in 4 specimen categories: (1) biopsies, (2) resection specimen, (3) cytology samples, and (4) frozen sections. They found that all WSI systems performed as well as the LM i.e., differences were statistically insignificant, in all categories except in cytology samples. All systems had variable levels of difficulties in focusing on the samples and some couldn't complete the task even when rescanned. Their findings are well in line with the existing literature [4][7][45], although, there are some studies suggesting that WSI is feasible in diagnosing cytology, but such evidence still seems lacking [46].

Tumor boards and clinicopathological meetings are an obvious application for WSI. One of the major advantages is being able to use a computer with an audio-visual system found in most conference rooms, instead of a multiheaded microscope or a projector. The ability to view other patient data in conjunction with the digital slide or the ability to annotate during the meeting with no additional tools can help make the experience more dynamic as previously discussed in educational context. [15][36].

Telepathology

Telepathology was arguably the first application in digital pathology having its roots precede the creation of WSI by 30 years [47]. The term was coined by Ronald Weinstein in 1986, and it is described as "the practice of pathology at a distance" in an overview article of the subject by Farahani N et al. in 2015 [48]. It has revolutionized the aspects of consultations and second opinions and can be used as a valuable tool in quality control, education, and research. Telepathology can be divided into static telepathology, robotic telepathology, and WSI based telepathology, of which WSI based is the latest and offers greatest potential for future. [48]

Telepathology has been successfully used in all fields of pathology, although the analysis of cytology specimens has historically had some issues [49]. Telepathology allows for intraoperative diagnostics through frozen sections in hospitals with no on-site pathologist. It also enables expedited consultations between pathologists e.g., in difficult cases, between a general pathologist and a sub-specialist, or if patient requests a second opinion. Accessing the image over internet rather than physically sending the slide has many advantages, including sped-up process and not having to send the unique physical slide that can get lost or break during shipping. Telepathology also allows easier co-operation between experts in tumor meetings or research settings. The potential to offer pathology services to developing countries has also been explored. [48]

The disadvantages associated with telepathology include potentially high initial costs, potentially longer time to diagnosis compared to glass slides, being prone to technological issues, requiring additional maintenance, potentially lower image quality, and the difficulty to handle certain cases. There has also been some resistance from experts to adopt telepathology. Many of the

listed disadvantages can be overcome with proper implementations and the eventual benefits outweigh the disadvantages e.g., proven cost-benefits over time. [48][50]

Digital Pathology in Research

In a survey study among UK pathology institutions, research was the most popular use case for DP [42]. The interest in DP and research related with it can be observed through the explosive increase in publications containing the words "Digital Pathology", "Telepathology" and "Whole Slide Imaging", which have been used interchangeably in the past [51]. The potential use of WSI cases in research vary from simple illustrative snapshots to thorough data mining of the images for deep learning applications. Through this potential, WSI has gathered the interest of many public research centers e.g., universities, as well as biotechnology and pharmaceutical companies that have made major investments in DP. The goals of this research are to develop new methods and algorithms for clinical work to improve patient selection, prognosis assessment, and ultimately to aid in choosing the right treatment option. [15]

According to Betmouni S [51], the majority of DP publications (30%) are technical in nature, dealing with Artificial Intelligence (AI), Augmented Reality (AR), image management systems, image analysis, color standardization, or archiving. Two of the second largest groups (14%) consist of case studies of deploying DP services for clinical practice and evaluation, validation, and concordance studies which we have discussed previously in this text. Other fields of publications include telemedicine, reviews, practical guides in DP, DP utilization in education, research, and international collaborations.

DP offers great prospects in automating tissue analysis, reducing pathologists' workload and assisting in choosing the correct treatment option. In this type of research, WSI is a tool to transform the initially analog, organic information into a digital format that different algorithms and machine learning tools can utilize. Some of the potentially revolutionary research in WSI, in addition to image analysis, includes 3D reconstruction of tissues, multispectral imaging, and deep learning. [15]

Digital Pathology in Image Analysis and Computational Pathology

We have briefly touched on image analysis and different computational methods, such as Artificial Intelligence (AI), in digital pathology in previous sections. Computational Pathology (CP) refers to a large variety of IT-assisted analysis methods designed to aid in diagnostics of histological images. It covers computer assisted applications such as image enhancing, measuring, quantification, heatmapping, and ultimately fully automatic diagnostics. In this section I aim to highlight some of the more widely used applications of CP as well as touch on the cutting-edge technologies currently available. [53]

Because there is a significant intrinsic subjectivity embedded in visual interpretation of tissue characteristics, one of the most interesting and well researched categories of DP is image analysis. Hamilton PW et al. [52] have done an overview of the subject and recognized 7 different use cases for image analysis in WSI: (1) nuclear morphology, DNA content and augmented visualization, (2) measuring tissue architecture, (3) quantitative immunohistochemistry, (4) tissue microarray analysis, (5) tumor heterogeneity, (6) fluorescence

imaging, and (7) quantitative biomarker discovery. Some of the more notable implementations of these analytical methods include convolutional neural network (CNN) for detecting invasive tumors in breast cancer whole slide images [54], a CNN for mitosis detection in H&E slides based on PHH3 reference [55], image analysis of HER2 expression in breast cancer [56], and image analysis of Ki-67 in breast cancer [57]. These are just some examples with many more already available and even more under development all the time. [58]

With deep learning tools, such as CNN, it is possible to recognize more tissue features than a human is capable of, and correlate these "hidden" features with patient prognosis. Even in cases where the feature is visible to the naked eye, it may be impossible for a human to assign prognostic value of such feature, unlike for a deep learning algorithm. One example of such features is a deep learning algorithm that was capable of assessing ductal carcinoma in situ - grade based on a stromal feature next to the malignant proliferation in breast tissue [59]. If one of these features is discovered, a tool can even be developed for the pathologist to extract this single piece of information and combine it with other relevant data to aid clinical work. [58]

There are still some limitations with CP. Many of the deep-learning algorithms are trained with a relatively small set of images from a single WSI scanner. The features preferred by the algorithm may not apply to other datasets, which is why standardization of image quality, color, and formatting is paramount, especially in deep learning research and applications. Novel features and foreign bodies may also prove difficult, as well as problems in image quality such as tissue folding, air bubbles or out-of-focus regions. Increasing need for processing power and the need for high quality datasets can prove difficult or expensive to acquire. So called supervised learning requires "ground truth" which is usually based on a pathologist's interpretation of a tissue sample and may introduce the same biases to the algorithm that the pathologist had. Other concerns are more ethical in nature. The decisions that a deep learning algorithm makes and the features it uses can be impossible to understand, even for the people who developed it. This, along with other issues, raises the question of who is responsible for the diagnosis and subsequent treatment options. Currently, from a legal point of view, it is the doctor in understanding with the patient's wishes, as aforementioned algorithms do not have the capacity for bearing responsibility. [53][58]

Future Prospects and Limitations

It is likely that the majority of pathology laboratories will adopt WSI at some levels in the near future. Wider adoption will further expedite the research in DP since a wider audience can be reached, thus increasing the potential for financial gain for manufacturers and vendors. The lack of standards needs to be addressed, though. Proprietary file formats, arbitrary headers, as well as different compression algorithms and file organizations reduce the potential for interoperability and scalability. Other medical spaces, such as radiology, use standard Digital Imaging and Communications in Medicine (DICOM) guidelines to operate and maximize interoperability between systems. DICOM supplement 145 introduced the groundwork for such standards but the wider adoption among vendors is still lacking. [15][53]

The potential for WSI, especially with AI, has the possibility of reducing the pathologist's workload. Increasingly, the strenuous tasks of measurements and quantifications can be automated. To which degree AI will replace the pathologist remains to be seen. It seems that in more challenging situations, both the pathologist and the AI still struggle. In these cases, the ability to integrate information will matter more. It is possible that AI will indeed reduce the workload in simpler tasks, leaving the expert with the more complex cases. Overall, it is projected that professions requiring medical expertise are at low risk of being automated. [53][58]

The lack of transparency in some deep learning methods is attempted to be addressed through a subfield of AI called explainable AI. It is the study of exposing unexplainable deep learning models in a systematic and interpretable manner. Since this research is still at its first stages, and no complete transparency and understanding can be achieved, it is unlikely that deep learning diagnostics will see wide implementation soon. A robust regulatory system for such technologies must be developed as well. It seems likely that the supervised learning algorithms and image analysis will be the first to be accepted into routine clinical work. [53]

Use of WSI in a Study: An Example

In Anttinen M et al. [60], 6 patients with MRI-visible, biopsy concordant prostate carcinoma (PCa) lesions underwent lesion-targeted transurethral ultrasound ablation (TULSA) treatment, followed by robot-assisted laparoscopic prostatectomy 3 weeks post-treatment. The purpose of this study was to characterize the immunohistochemical profile of thermofixated tissue after TULSA treatment, and thus prove that thermofixated tissue is no longer viable, although it may appear as such in H&E staining. In this section I aim to describe the digital pathology process used for this particular project, with the understanding that these methods might not be suitable for every situation.

Before the scanning, the prostatectomy specimens were prepared according to laboratory standards. They were fixed in 10% formalin and cut using free hand method into approximately 5 mm sections. The apex and the base were cut in coronal plane, the seminal vesicles in sagittal plane, and the mid-gland in transverse plane perpendicular to the long axis of the urethra. All the sections were embedded in paraffin in whole mount cassettes. From each block, two 5µm sections were cut for H&E staining. Additional sections were cut from blocks where the thermally ablated regions would appear as though viable or remained ambiguous in H&E stain i.e., if thermofixated regions were present. For the IHC stain, CKCam5.2, p16, and androgen receptor (AR) were used to evaluate the prostate glandular epithelium. AMACR was used to distinguish benign glands from malignant ones. Ki-67 was used to evaluate proliferation activity. Factor VIII antibody (von Willebrand, vWF VIII) was used to assess the damage done to blood vessels in the ablated region. The additional IHC sections were prepared using BenchMark XT and ULTRA IHC/ISH automated slide staining instruments (Ventana Medical Systems, Arizona, USA).

The subsequent whole mount slides were first evaluated by a certified pathologist, with a sub-specialty in uropathology, using a conventional light microscope. The whole mount slide with the best representation of tissue architecture from each of the 6 cases was chosen for scanning. Each whole slide mount was prepared for scanning, checking first for misaligned coverslips and excess glue or any obvious air bubbles that could lead to focusing issues. No such issues were found, and the slides were cleaned of any pen markings using rubbing alcohol (95% vol) and afterwards cleaned using microfiber cloth to ensure no fingerprints or such remained. The H&E whole mount slides were digitized using NanoZoomer S60 (Hamamatsu Photonics, Hamamatsu, Japan) with NDP.scan software (version 3.2.12) at 20x magnification (0.46 microns per pixel). Automated focusing strategy was used. No issues were recorded during the scanning and the 6 whole slide images had sufficient image quality, so no rescanning was needed. Further 7 whole slide images were scanned after the IHC staining of one case was completed. The preparation process and imaging modalities remained the same and no problems presented during the scanning. All the whole slide images were first saved into a local hard drive, since the computer that the scanner was connected to had no internet access, and no integrated LIS was used at the laboratory at that time. The images were eventually moved onto an external hard drive for later use and storage.

The whole slide images were used for quality control of the LM findings as well as to illustrate the findings in higher quality for the readers. The images were evaluated and annotated by the author of this review using NDP.view2 software. At that time, the author had completed all pathology courses required for a medical degree in Finland, as well as voluntary studies in pathology. The goal was to annotate 3 thermal damage boundaries: (1) the outer boundary of complete necrosis, (2) the outer limit of thermal injury (OLTI), and (3) the boundary of thermofixation. Outside the OLTI, no visual evidence of thermal damage should remain. The annotations were done using a free-hand tool at varying levels of magnification, with the focus on including all the ROIs, rather allowing small amounts of healthy tissue to be included. The zone inside the boundary of complete necrosis was defined as coagulation necrosis zone (CNZ), and the region between boundary of complete necrosis and OLTI as margin zone (MZ). The annotations were examined by the same uropathologist who had done the initial evaluation with LM, and necessary corrections were made using the same tool. Reference pictures are shown in Figure 2.

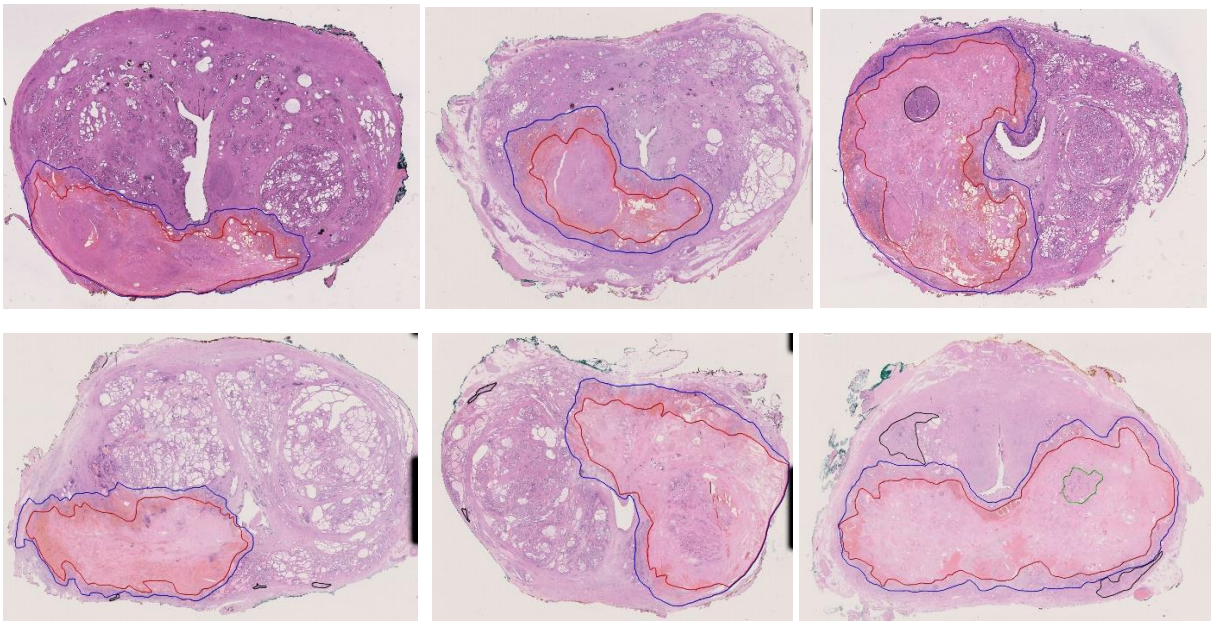

Figure 2. The snapshots of the whole slide images in JPG. The blue line denotes the outer limit of thermal injury (OLTI). The red line denotes the outer boundary of complete necrosis. The black line indicates malignant tissue still appearing viable in H&E stains. The green line indicates incomplete necrosis inside the coagulation necrosis zone (NCZ), with some nuclear structures still visible.

From the 6 fully annotated representative whole slide images, snapshots were taken at the lowest magnification to display the whole cross-section of the prostate as well as the size and orientation of the ROIs. The snapshots were saved in JPG format to reduce the file size for the eventual publication. One case presented with thermofixated area. Further snapshots were taken from 2 regions in each staining modality: (1) benign region (control), and (2) suspected thermofixated region (case). The regions in H&E stains were matched with their corresponding regions in the IHC stains and snapshots were taken at 10x magnification, again saved in JPG format. Annotations were added to the low magnification H&E images to better illustrate the regions and help the reader orient themselves. Reference pictures are shown in Figure 3.

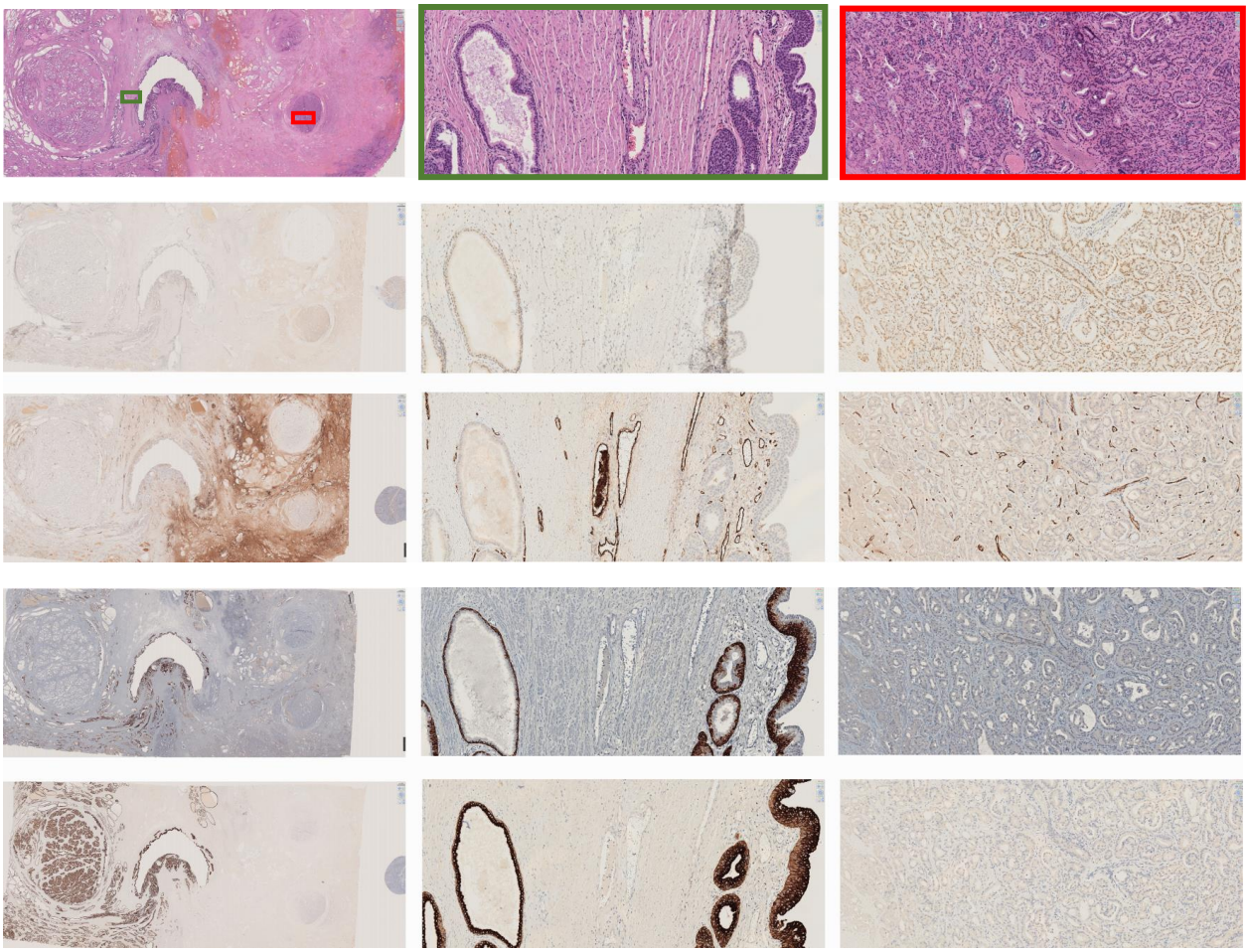

Figure 3. Snapshots of all the staining modalities with the case containing thermofixated area. Left to right are: 2x magnification of the whole slide image with color coded areas for adjacent images, 10x magnification of benign region (control, green), and 10x magnification of thermofixated region (case, red), respectively. Top to bottom are: H&E stain, androgen receptor (AR), von Willebrand Factor VIII (vW FVIII), AMACR + CK5/6, CKCam5.2, respectively.

Conclusions

WSI is already in the process of changing pathology. For the first time since microscopy was invented, a revolutionary new technology has been introduced that can change the landscape and the practice in pathology. With the development of telepathology, international collaborations should become more common. The utility of DP in education is undeniable, allowing the students to learn more quickly and with better interactivity. Already some laboratories have switched to fully digital workflow, with many others waiting to follow. With advanced, AI based image analysis methods, the workflow can be radically changed, and the workload seriously reduced. Lastly, the potential for WSI in research seems nearly limitless at this point, with many applications being developed around the world.

The Turku University Hospital has already implemented DP into its workflow, having more than 90% of all histological material evaluated first with DM rather than LM. Cytology samples are still assessed using conventional LM, as well as some technically challenging breast tissue whole mounts. The laboratory is using a Phillips WSI solution.

There are still considerable limitations. While the regulation has finally started to catch up with the progress in DP, allowing laboratories to confidently switch to digital workflow, many more concerns still need to be addressed. More work still needs to be done to guide and standardize the emerging technologies, and more research needs to be done to validate the current systems for different samples. The ethical problems brought by the eventual widespread use of machine learning algorithms in DP are still not adequately addressed, with more problems in the medico-legal field when the human component can be eliminated in the medical process. These problems can be overcome, though, and the potential in DP far outweighs the limitations.

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