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REVIEW ARTICLE

Digital Pathology and Artificial Intelligence in Diagnostic Pathology

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Abstract

Currently, digital pathology is a profound transformation in the field of pathology. Numerous artificial intelligence (AI) algorithms have demonstrated significant potential for the improvement of diagnostic efficiency, morphometric analysis of biomarkers, and diagnostic screening. However, the application of AI in pathology is a matter of considerable worry among pathologists. Within this article, we provided a concise overview of the process of digital pathology and deep learning in diagnostic pathology. Additionally, we explored the advantages and uses, obstacles and constraints, and future potential of artificial intelligence in diagnostic pathology. The implementation of innovative AI-based methods in pathology laboratory processes will enhance the effectiveness of disease diagnosis, as the collaboration between pathologists and AI systems has demonstrated superior performance compared to both the individual pathologist and the system. Nevertheless, pathologists continue to be crucial in the finalisation of the diagnosis.

Keywords: Digital pathology, whole-slide imaging, diagnostic pathology, machine learning, artificial intelligence, AI, WSI

INTRODUCTION

Pathology, a branch of medical science dedicated to studying and diagnosing diseases, provides crucial diagnostic information to both patients and clinicians. Pathologists with the collaboration of allied professionals, such as technicians, to analyse tissues and organs through various tests and methods including histopathology, immunohistochemistry (IHC), and molecular biology.[1] Accurate and timely diagnosis is crucial for patient's treatment. Traditional pathology services examine tissue sections on microscopic glass slides under a microscope. However, the diagnosis relies on the pathologist's expertise and is sometimes apparently subjective. The variation among pathologists is the disadvantage of morphologic diagnosis, therefore, a technology that provides more reliable and consistent diagnosis is required to solve this problem. Additionally, sharing pathological microscopic slides for a second opinion can be a challenging and time-consuming process. Developing effective treatments has long been a challenge for medical practitioners. While traditional biopsy results are highly accurate, the process of finalising these results can be time-consuming.[2]

Artificial Intelligence (AI) is a field of science and technology focusing on developing intelligent systems that can replicate, emulate, or even surpass human intelligence in areas such as reasoning, learning, perception, and decision-making. AI algorithms and methodologies enable machines to process large amounts of data, recognise significant patterns, and make predictions or perform actions based on the analysis.[3] In recent years, advancements in computers, network connectivity, and digital technology have led to the growing integration of AI in pathology. This trend will significantly transform the practice of pathology, with an increased use of digital imaging. The developments in deep learning (DL), machine learning (ML), and AI together with the extraction of relevant information from image data and their computational analysis have given rise to a new field known as "digital pathology,"

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where pathologists interact with digital images on computer screens and conduct quantitative analyses to achieve diagnoses with accurate clinically relevant information, rather than relying solely on traditional microscopy.

Digital pathology refers to technologies and methods for digitising pathology slides and associated metadata and storing, interpreting, and assessing all information generated from a digitized glass slide. Whole-slide imaging (WSI) allows for the digitisation of glass slides, to create a single, comprehensive virtual image.[4] This virtual image accurately reflects the data on the glass slide. The digitised images of tissue samples are transferred to a computer, where advanced image processing and computer vision techniques are applied for analysis. By integrating the image with relevant clinical data, pathologists gain a comprehensive view of a patient's specific disease, enabling them to conduct further diagnostic procedures.[2]

Advantages of WSI over conventional microscopy include portability, ease of sharing and retrieving pictures, and job balance that allows pathologists to share digital images between hospitals and research labs for real-time analysis (telepathology) providing rapid on-site evaluation for delivering diagnoses, education, and consultation, ultimately enhancing workflow efficiency.[5] Thus, pathologists can leverage ML models to enhance analyses, improve the accuracy of diagnoses, and expand treatment options. Additionally, AI in pathology offers second opinions, significantly aiding practitioners in their routine tasks.[2,6-9]

Nevertheless, the use of AI in pathology is a concerning issue for pathologists despite the fact that many AI models have demonstrated significant potential in facilitating morphological diagnostics and quantitation of therapeutic targets. This article provides a concise explanation of the workflow of digital pathology and DL in diagnostic pathology, the benefits and applications, challenges and limitations, and future prospects of AI in diagnostic pathology.

WORKFLOW OF DIGITAL PATHOLOGY AND DL IN DIAGNOSTIC PATHOLOGY

The quality of WSI and annotations are the most fundamental and indispensable requirement in pathological AI model development, enhancing the accuracy of image classification and prediction to support pathologists in diagnostic decision-making. Annotation of pathological images must be performed by pathologists or a highly specialised pathologist in some cases. Key sequential steps in the digital pathology workflow include (1) WSI acquisition, (2) storage, (3) pre-processing, (4) DL pipelines, and (5) applications (FIG 1).[5]

(1) WSI Acquisition

Obtaining high-quality WSI is crucial for converting tissue slides into digital format. However, biopsies from patients with abnormal tissues often present limitations in morphology and tissue formation due to insufficient sample sizes for AI training. Even though the tissue on the slide may be large, the target tumour or disease could be small and/or dispersed. The slide may show normal tissue, necrosis, cystic gaps, and bleeding spots, along with the target tumour or lesion.[10]

The WSI acquisition process begins with the preparation of tissue samples, where patient specimens obtained through biopsy or excision are fixed in formalin to be processed for a paraffin tissue block. The formalin-fixed paraffin-embedded tissue block is sectioned into thin slices, typically 3-4 µm thick, and placed on glass slides. Haematoxylin and eosin (H&E)- stained slides are routinely produced for pathological diagnosis. Ancillary tests such as histochemistry, immunohistochemistry and fluorescent in situ hybridisation[11] are required for certain samples.

After the slide preparation, the prepared slides are digitised using a slide scanner. High-resolution slide scanners equipped with automated stage systems capture multiple adjacent fields of view (tiles) at various magnifications and planes.[12] Specialised software algorithms then stitch these tiles together to produce high-resolution WSIs. The choice of magnification and scanning techniques is largely dependent on the clinical or research application. The image acquisition process involves scanning entire histology glass slides of sectioned and stained tissue samples to generate high-resolution digital images. These images can be saved in various digital formats, such as .tiff, .jpeg, .raw, .bif, .vms, .vmu, .ndpi, .scn, .isyntax, .mrxs, .svslide, and .svs, depending on the WSI scanner used.[10] Open-source WSI viewers, such as QuPath, Cytomine, Orbit, ASAP, OpenSlide with OpenSeadragon, ImageScope and ImageJ, can be used to view these files. The choice of file type depends on the viewer and the file size, which impacts transmission and hardware performance when exchanging image files.[13]

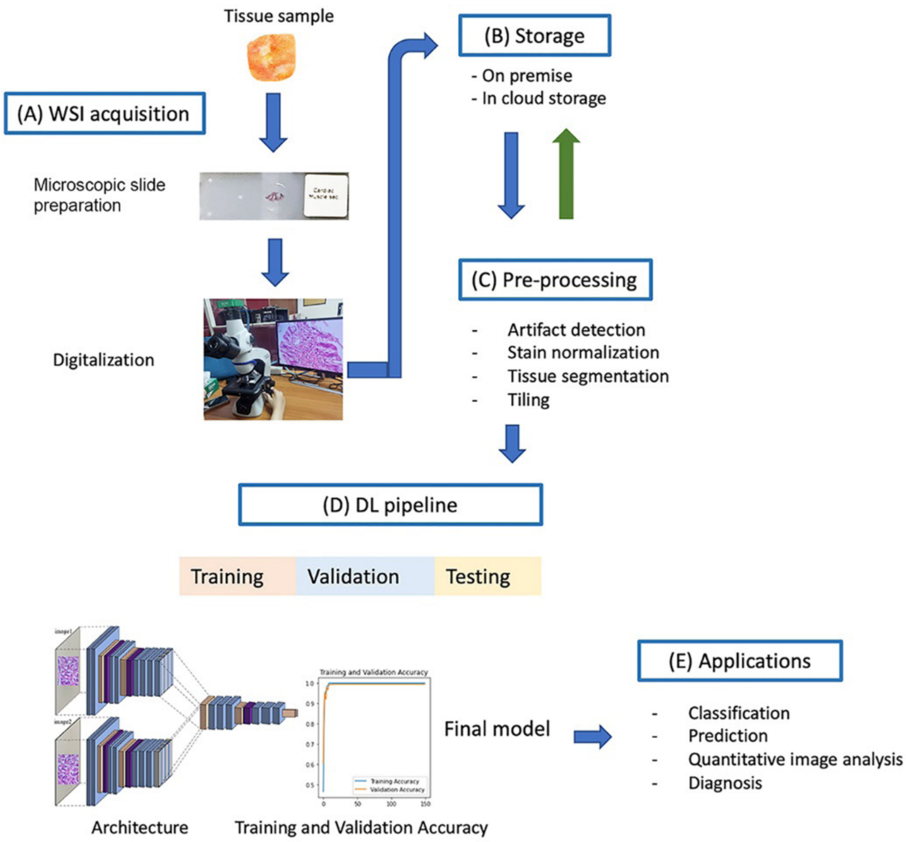

FIG. 1 Workflow of Digital Pathology and Deep Learning in Diagnostic Pathology: From Glass Slides to Applications. (A) WSI Acquisition: Involves tissue sample preparation, microscopic examination of glass slides, and digitisation using a scanner to produce whole-slide images. (B) Storage: WSIs are stored either on-premises or in cloud storage systems. (C) Pre-processing: Includes artifact detection, stain normalization, tissue segmentation, and tiling to prepare images for analysis. (D) Deep Learning Pipelines: Encompasses data splitting, model training, validation, and testing phases. (E) Applications: Utilisation of trained models for diagnostic, classification, prognosis prediction, and quantitative assessment purposes.

(2) Storage

After WSI, the scanner automatically pre-processes the virtual slide and stores it either on-premises or in cloud storage. This can be a centralised, vendor-neutral archive compatible with all types of medical imaging. Organising WSI within a digital pathology database adds considerable costs, which increase depending on the number of patients in a hospital. Another challenge is providing adequate storage space for the large volume of data generated by each digital slide. Additionally, server maintenance and security protection incur further expenses. Thus, before storage, a compression algorithm is often applied to reduce file size. However, searching the database for relevant WSI can be time-consuming and presents challenges for developers. The purpose of the study and the selection of the most appropriate histomorphological, histochemical, immunohistochemical, molecular, and oncological data play a crucial role in ensuring accurate diagnoses.[14]

(3) Pre-processing

The general purposes of pre-processing image data are to discard non-informative data, such as slide background or artifacts that are not pertinent to the histopathology of the individual patient, create a consistent dataset, and enable the processing of large WSI during downstream modelling. Image pre-processing involves various techniques, such as artifact detection, stain normalisation, tissue segmentation, and tiling.

Artifact detection and removal of artifacts are important techniques to limit the amount of non-informative in the downstream processing preventing automated diagnostic errors based on uninterpretable image information. Likewise, due to variations in tissue section thickness, staining methods, transparency, illumination conditions, cell formation, extracellular matrix composition, paraffin techniques, automated scanning, and viewer software, the stain normalisation technique reduces classification and prediction errors in models.[15] Traditional image processing clearly defines the steps involved, which are typically chosen from a wide range of established methods. For instance, processing an H&E image often begins with stain separation using colour deconvolution. This technique recombines the red, green, and blue values for each pixel into a weighted sum based on the stain colour. Difference kernels are then applied to the images in a process called convolution, followed by recombination through the addition or subtraction of relevant pixels.[16] Ultimately, this produces a binary image representing discrete objects that can be measured by distinguishing pixel values corresponding to structures of interest from all other pixels, usually via thresholding. Additional steps may be required to separate clustered objects or clarify boundaries.[17]

Other image-processing methods may integrate statistical and mathematical techniques to group different types of cell morphology. For greyscale operations, the values from each colour channel are averaged to produce a black-and-white image. The histogram graphically represents the frequency of each colour and its intensity, with colours ordered from least to most intense. Geometric transformations, such as rotation, scaling, cropping, and shifting, are also commonly used. During the process of training or making predictions using a deep learning model, it is necessary to tile the image and then reshape these tiles into an array that can be utilised by the algorithms.[10]

(4) DL pipelines

DL is a method in AI that enables machines to process data using deep neural networks to identify patterns, particularly in image datasets. Convolutional neural networks (CNNs) are frequently employed in medical image analysis. CNNs apply filters to an image and pass the filtered output to subsequent layers. These filters detect local patterns by performing a convolution operation, where the filter slides over the image's pixels, computing the product of the filter's parameters and the image. This process, known as parameter sharing, conserves memory by applying the filter to pixel neighbourhoods instead of every individual pixel. Additionally, pooling, a filtering method, reduces the image's dimensions by converting windows of pixels into a single-pixel output, leading to a smaller output size compared to the input.[10] The architecture of a DL model comprises the number of filters, layers, and connections between input and output. DL pipelines generally involve splitting the dataset into training, validation, and testing sets. The training set is used for initial model learning, while the validation set helps to fine-tune hyperparameters. The testing set is then used to assess the model's performance.[5] Given that many CNN architectures contain millions of parameters, they are susceptible to overfitting. To mitigate this, training is often halted when the model's performance on both the training and validation sets is optimised.[18]

Two frequently used models in digital pathology include classification models, such as VGG[19], ResNet[20], Inception[21], EfficientNet[22], and AlexNet[23], and segmentation models, such as UNET[24], FCN[25], and Fast-SCNN.[26] The commonly employed training modules for model training on image data in digital pathology are Tensorflow[27], PyTorch[28], CUDA[29], and GPUs.

(5) Applications

A large portion of the digital pathology infrastructure is designed to improve the precision and efficiency of pathologists' decision-making. The analytic pipeline's objectives determine the particular application that is built. Trained models have been applied in various areas of health care, such as diagnosis, classification, prognosis prediction, and quantitative assessment of abnormal microenvironments. DL has transformed quantitative image analysis (QIA) in digital pathology which offers sophisticated methods for analysing gigapixel histopathological pictures in order to find biomarkers and enhance cancer diagnosis. The automatic classification of individual cells using nuclear segmentation and morphology analysis is one common usage of DL in QIA. For example, oestrogen receptor status in breast cancer can be predicted using spatial and morphological information from cell nuclei in H&E whole slide images (WSIs).[30] This highlights how DL algorithms can unravel the complex cellular composition of tumours using routine histopathology images, allowing the extraction of human-interpretable features like cellular ratio and density, which can then be applied in downstream diagnostic and prognostic applications.[5]

BENEFITS AND APPLICATIONS OF AI IN DIAGNOSTIC PATHOLOGY

Over the past 10 years, digital pathology and AI models have offered benefits for pathologists, patients, and hospitals/clinical setting, especially in the fields of cancer diagnosis and treatment in many issues. Examples include (1) increased pathologist efficiency and precision of diagnosis; (2) savings in external consultations; (3) reduced cost of internal handling of glass slides; (4) savings in investment costs for large pathology laboratories.

(1) Increased pathologist efficiency and precision of diagnosis

Digital pathology and AI models assist pathologists in working faster and more efficiently in slide reviewing and image analysing. Mukhopadhyay *et al*. evaluated the diagnostic performance of digital images versus microscopic images in specimens from 1,992 patients with various tumour types, diagnosed by 16 surgical pathologists.[31] Their findings revealed that routine diagnostics using WSI were not inferior to those using light microscopy, with a major discordance rate of 4.9% for WSIs compared to 4.6% for microscopy.

Some algorithms assist pathologists by analysing tumour cell characteristics and structural changes in lesions. For instance, Raciti *et al*.[32] have shown that using an AI, Paige Prostate Alpha[33], during the evaluation of prostate needle biopsies stained with H&E by pathologists more often correctly classify smaller, lower grade tumours, and spent less time analysing each WSI. Another study regarding identification of prostate cancer in biopsy samples and detection of breast cancer metastasis in sentinel lymph nodes demonstrated the potential of AI to automatically distinguish between slides with prostate cancer and WSI of lymph nodes containing micro- and macro-metastases of breast cancer or benign and normal tissue, correctly identifying 30-40% of the cases as benign without the need for additional immunohistochemistry or human intervention.[34]

IHC enables pathologists to identify cellular markers indicative of specific diseases, such as cancer subtypes or infectious agents, thereby aiding in diagnosis, prognosis, and treatment planning across various medical specialties. AI algorithms can analyse IHC-stained digital slides with high accuracy.[35] Recent research has emphasised the crucial role of AI in quantitative IHC analysis, significantly improving the precision and efficiency of diagnostic procedures. For example, in prostate cancer, Ki-67 expression has been proven by conventional bright-field IHC to be a strong prognostic marker.[36] Blessin *et al*.[37] have demonstrated excellent agreement between the framework for automated Ki-67 labelling index (Ki-67 LI) assessment and the manual quantification in prostate biopsies from routine clinical practice. Although the Ki-67 LI is a powerful prognostic parameter that is applicable in routine clinical practice, in the case of multiple cancer-positive biopsies, the sole automated analysis of the worst biopsy is sufficient.[37]

Additionally, AI models can integrate WSI of oncologic patients with diverse and complex data from multi-omics approaches, such as transcriptomics, proteomics, radiomics, radiogenomics, and electronic health records, thereby advancing precision oncology and predicting disease aggressiveness, patient outcomes, and therapeutic response. For example, Coudray *et al*.[38] have trained DL models on WSI of adenocarcinoma of the lung (LUAD) to predict the ten most mutated genes in LUAD and found six biomarkers, STK11, EGFR, FAT1, SETBP1, KRAS, and TP53, that can be predicted from the pathology images suggesting that DL models can help pathologists in detecting cancer subtypes or gene mutations. Ning *et al*.[39] have proposed a cross-modal feature-based integrative framework trained on features extracted from computed tomography scans, WSI, and transcriptomic profiles of clear cell renal cell carcinoma. The proposed model can stratify high- and low-risk subgroups with a significant difference and outperform the predictive performance of those models based on single-modality features in the independent testing cohort. It suggests that the integrative framework can be used to predict prognosis in patients with clear cell RCC.

(2) Savings in external consultations

By remotely sharing digital slides with other specialists, the turnaround times for external consultations are greatly reduced. This also eliminates the expenses related to transporting, packaging, and labelling glass slides, and the risks of breakage or loss, which is a significant potential area for cost savings. Pathological diagnosis requires a thorough review of all medical and past pathological data. AI-based models in pathology facilitate the comprehensive analysis of medical and historical pathological data, allowing for seamless integration into the diagnostic process and the collection and management of extensive pathological data. Moreover, this comprehensive pathological data can be used as precious instructional material for training different AI diagnostic models essential for pathological diagnosis.[40]

(3) Reduced cost of internal handling of glass slides

The adoption of digital pathology significantly decreases the need for the management of the glass slides by support staff. Staff no longer need to manually sort slides, deliver them to pathologists, or retrieve previous cases from archives, resulting in a substantial reduction in administrative workload.

(4) Saving in investment costs for large pathology laboratories

AI has the potential to create more cost-efficient pathology workflows, resulting in significant savings for pathology laboratories. Ho *et al*.[41] reported that in a top-tier pathology laboratory in the United States, which handles more than 210,000 cases each year, can save around USD 18 million over a span of 5 years.

CHALLENGES AND LIMITATIONS OF AI IN DIAGNOSTIC PATHOLOGY

Although AI in digital pathology has been successfully improved in the diagnosis of diseases, it still encounters various obstacles. The primary challenges include (1) poor image quality and resolution; (2) the need for large histologically annotated data sets; (3) shortage of clinical and technical expertise; (4) data dimensionality and hardware constraints; (5) model generalisability; (6) lack of transparency.

(1) Poor image quality and resolution

The quality of WSIs is directly influenced by the quality of tissue sample preparation. The process of preparing these slide images is complex, involving steps such as embedding, cutting, staining, and scanning tissue samples. Quality of sample processing, as frozen and formalin-fixed specimens have unique histologic artifacts, and biopsy and resection require different approaches.[42] The intricate preparation process can lead to distorted slide samples, where artifacts, such as dust and air bubbles might go unnoticed. Also, quality of the glass slides and inconsistent staining can lead to variations in colour, intensity, and contrast. These limit the availability of high-quality datasets for effective training. Additionally, training an AI model requires large-scale and fine annotations of WSI. These raise concerns about their reliability.[11,43]

(2) The need for large histologically annotated data sets

One of the significant hurdles in model training and validation is the need for large, annotated data sets. Creating these data sets is both time-consuming and labour-intensive, requiring expert pathologists to review and annotate images. Centralising and sharing publicly available data sets will greatly support the development and validation of DL models and other applications.[11]

(3) Shortage of clinical and technical expertise

Developing an AI model requires collaboration between experts in clinical-grade computational pathology, statistics, AI, and medical professionals.[44] The process includes clinical data collection and preparation, annotation, model training, and validation. Each expert plays an essential role, from guiding data analysis to building robust models and validating outcomes. However, assembling such a diverse team is both time-consuming and costly, adding to the complexity of the development process.

(4) Data dimensionality and hardware constraints

WSI generates gigapixel resolution images, which are approximately 1000 times larger than X-ray images and 100 times larger than CT images.[45] These large files can burden storage resources and slow image processing and analysis. DL algorithms are typically trained on much smaller images, around 250 × 250 pixels, due to hardware constraints.[46] Consequently, images are often downscaled before being input into the model, which can result in the loss of critical information and a subsequent decline in model performance. In other words, low-resolution images may lack sufficient detail for accurate diagnosis, while excessively high-resolution images can lead to large files and slow processing times. To mitigate this, image compression techniques are employed to reduce file sizes without significantly compromising image quality. The choice of appropriate lossless compression algorithms, such as LZW or JPEG2000, and suitable file formats is crucial for efficient data storage and management without sacrificing diagnostic accuracy. Additionally, consistent illumination is vital for maintaining uniform brightness and contrast across the WSI, therefore, accurate autofocus is essential to ensure alignment between the focal plane and the tissue section.[47] To address these challenges, scanner manufacturers must optimize system performance through rigorous testing and quality control procedures.

(5) Model generalisability
The generalisability of digital pathology processes across different laboratories or institutions is an important challenge. Variations in hardware, tissue handling and staining protocols, image acquisition, and annotation methods in different laboratories or institutions lead to differences in image preprocessing can lead to quality inconsistencies that ultimately impact the performance of DL models. Approaches like data augmentation, transfer learning, and domain adaptation can help address this challenge by enhancing training data diversity, thereby improving the generalisation ability of the models.[48-50]

(6) Lack of transparency
Another challenge is the lack of transparency. Despite their success in various fields, deep learning algorithms are often considered "black box" models, providing limited insight into how their results are derived.[6] The difficulty in understanding the rationale behind the predictions of "black box" models can impede their acceptance by pathologists, clinicians, and patients. In medical contexts, transparency and accountability are crucial. Practitioners need to justify and explain their decisions, but since AI models often lack clear reasoning, building trust in their outcomes can be challenging. Developing DL techniques that incorporate interpretable decision-making processes is crucial for enhancing transparency and building trust among users. One promising approach is attention-based multiple instance learning, which can identify tissue regions with high diagnostic relevance.[51]

FUTURE PROSPECTIVE

Several advancements are anticipated in the future to enhance the clinical utility of AI applications in diagnostic pathology, including:

1) The integration of diverse AI-based algorithms into routine workflows, such as combining WSI pathology algorithms with radiology AI algorithms and potentially incorporating omics data from the same patients to produce a comprehensive final report.
2) The expansion in capabilities of AI algorithms to perform complex tasks, such as identifying various lesion types for cancer diagnosis.
3) The development of multiparameter algorithms capable of assessing multiple features to generate comprehensive, stand-alone pathology reports without the need for pathologist input.
4) The creation of large databases containing rare and challenging lesions, enabling AI algorithms to assist pathologists in handling complex and uncommon cases, thus reducing the need for specialist consultations.
5) The development of digital pathology platforms with modules that seamlessly integrate with laboratory information systems, ensuring a continuous data flow. This integration would allow pathologists to easily access patient records, case histories, and other critical data.

CONCLUSIONS

Overall, digital pathology tools and AI assist pathologists in working faster and more efficiently in slide reviewing and image analysing, significantly reducing diagnosis and treatment errors. Internal and external consultations are easier and faster resulting in shorter turnaround times. Additionally, adopting digital pathology leads to a significant reduction in the cost of internal handling of glass slides and laboratory and office space savings as pathologists will have the opportunity for remote work. For patients, digital pathology offers benefits including shorter waiting times, increased precision of diagnosis, and faster treatment resulting in increasing therapy success rates and chances of patient recovery and survival. Moreover, AI in digital pathology also provides benefits in saving investment costs for large pathology laboratories as hospitals can be relatively easily quantified to build a business case and justify the investment in digital pathology. For patients, benefit from digital pathology includes shorter waiting times, increased diagnosis precision, and faster treatment, resulting in increased success rates and chances of therapy, recovery, and survival. Moreover, AI in digital pathology not only saves investment costs for large pathology laboratories, but also makes it relatively easy for hospitals to build a business case and justify their investment in digital pathology. However, challenges and limitations of AI in diagnostic pathology, such as the need for the availability of high-quality datasets from different regions/countries and standardisation of WSI file format, hardware limitations, and the lack of transparency of AI models remain to be addressed. Instigating novel AI-based approaches into pathology laboratory workflows will improve the efficiency of disease diagnosis as the synergy between pathologists and AI systems shows outperforming both the individual pathologist and the system. However, pathologists still play an important role in making final decisions for the diagnosis.

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