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REVIEW

Synergies and Challenges in the Preclinical and Clinical Implementation of Pathology Artificial Intelligence Applications

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Abstract

Recent introduction of digitalization in pathology has disrupted the field greatly with the potential to change the area immensely. Digital pathology has created the potential of applying advanced quantitative analysis and artificial intelligence (AI) to the domain. In this study, we present an overview of what pathology AI applications have the greatest potential of widespread adoption in the preclinical domain and subsequently, in the clinical setting. We also discuss the major challenges in AI adoption being faced by the field of digital and computational pathology. We review the research literature in the domain and present a detailed analysis of the most promising areas of digital and computational pathology AI research and identify applications that are likely to see the first adoptions of AI technology. Our analysis shows that certain areas and fields of application have received more attention and can potentially affect the field of digital and computational pathology more favorably, leading to the advancement of the field. We also present the main challenges that are faced by the field and provide a comparative analysis of various aspects that are likely to influence the field for the long term in the future.

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Rapid digitalization of pathology is leading to new revolutionary developments in both clinical and nonclinical pathology settings. Digital and computational pathology (DCP) allows sharing and analysis of data online despite great geographical distances, enables quantitative assessment of data and application of advanced artificial intelligence (AI) techniques. It can also potentially lead to improvements in pathology by enhancing collaboration between the preclinical and clinical researchers.[1] In several preclinical studies, it has been safely concluded that certain compounds and naturally occurring fruits and vegetables help in treating some conditions, such as Alzheimer disease[2] and breast cancer,[3] and in cholesterol management,[4] but it still takes a significant amount of time and effort for translation of these findings into clinical scenarios. With the advent of digital pathology (DP) and proliferation of AI, there is an opportunity to accelerate the discovery of newer treatments and drugs, thus reducing the gap between clinical and preclinical domains.

Dunkle[5] in the early 2000s predicted that the use of machine learning and image informatics can help in the acceleration of drug discovery because 70% of the processes involved lead to an output image. Vamathevan et al[6] in a recent publication advocate the use of image-based AI for extraction of detailed quantitative information at various resolutions leading to the discovery of new biomarkers and allow for high-throughput analysis of digitized pathology images for accelerated drug development. Antolín[7] further elucidated the role AI can play in understanding the effects of drug administration, leading to personalized drugs and predicting disease-specific effects of a drug. Finally, Réda et al[8] advocate the automation of the drug discovery process for better

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ARTICLE HIGHLIGHTS

- This review presents the various application areas in pathology that are likely to see early applications of artificial intelligence.
- Survey of the literature is presented with a comparison of scanning and storage technology and a survey of important techniques presented in literature.
- Discussion of the main challenges in the mainstreaming of digital technology in pathology is also presented.

management of costs and enhanced success rates by combining pathologic phenotypes with gene expression analysis.

Although there are great benefits in digitalization of pathology, which can potentially lead to breakthroughs in cancer research and optimization of diagnostics and therapeutic decision making,[9] there still remain significant challenges in areas of slide scanning and standardization, processing power requirements for multimagnification analysis, handling of rare events not found in routine clinical and preclinical work, and management of uncertainties in diagnostics.[10] On the contrary, these issues can potentially be overcome by incorporation of quantitative pathology for standardization, employment of outlier detection techniques for detection of rare events, utilization of cloud infrastructure for multimagnification analysis, and incorporation of explainable AI for handling of diagnostic uncertainties. Explainable AI refers to the interpretability of the results provided by the AI algorithm in terms of their biological and diagnostic significance with respect to the medical field. Some applications, in our opinion, are likely to be addressed earlier than others with significant gains in time and efficiency, which include slide filtering into abnormal and normal, prioritization of cases, and content-based image retrieval.[11]

As stated earlier, there are significant challenges in adoption of digitalization in pathology. An important factor is the pathologist's preference for the "more dependable tool, ie, the microscope" over whole-slide image (WSI), which is a rapidly evolving novel technology. To realize the potential of DP, Gauthier et al[12] asserted that a set of international "best practices" must be established, providing a basis for determining the requirements to be met by the equipment and the associated software. The European Society of Digital and Integrative Pathology has conducted a detailed analysis for the low adoption of DP and has made detailed policy recommendations for correct implementation of digital workflow in Europe.[13] Financial and economic benefit is of immense importance along with equivalence in terms of diagnostic efficacy between the WSI and microscope. Hanna et al[14] showed high intraobserver agreement between the WSI and the microscope-based diagnosis. Lujan et al[15] focused on the financial aspects and discussed various financial factors, elucidating the importance of a business plan in implementation of DP. In a study conducted at Memorial Sloan Kettering Cancer Center over 5 years, cost savings of $1.3 million were acquired using DP and 84% of the pathologists were happy in signing-out retrospective clinical cases, but only 54% were inclined to perform primary diagnosis using WSIs.[16] Hanna et al[17] claimed that the adoption of DP is essentially following the technology-adoption curve because the technology is highly disruptive. There are various other reasons for low adoption such as cost-effectiveness, which is difficult to achieve without a fully digital workflow; concerns pertaining offshoring of work; and drastic increase in the complexity of the profession because simpler cases would be automated.[17]

Aeffner et al[18] contended that the seamless and early adoption of DCP would occur earlier in the preclinical setting, leading to advances in drug development. This, in turn, would potentially lead to the transition of quantitative techniques from the preclinical to the clinical setting to serve as companion diagnostics. This review delves into the potential techniques and applications where breakthroughs and technological advancements in the preclinical domain can potentially affect the clinical domain. We explore how the advent of digitalization and leveraging of AI is likely to lead to developments of techniques in preclinical pathology that can be readily used in the clinical setting for societal and commercial benefit. We also aim to explore the main challenges in realizing the adoption of DP and AI in clinical and preclinical pathology. In the next section, we discuss the potential

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TABLE 1. Scanners and Image Formats Supported[a]

Scanners	WSI Viewer	Format
Philips	Philips IntelliSite Pathology Solutions (PIPS)	iSyntax
3D Histech	SlideViewer/CaseViewer	MRXS, JPG, JPEG2000
Digipath Pathscope	PathSuite	Joint Photographic Experts Group (JPEG)
Hamamatsu	NDP.view2	JPEG, NDPI, VMS, VMU, SVS, SCN, MRXS, CZI, VSI
Huron	HuronViewer	BigTIFF (DICOM Compliant)
Leica	Aperio ImageScope/ WebScope	TIFF (SVS), SCN
Olympus	VS200	VSI, TIFF
Sakura Finetek	VisionTek Viewer	BigTIFF, TIFF, JPG2000. SVSLIDE
Ventana	uPath	BIF, TIFF, JPEG2000
Optrascan	Optrascan Image Viewer	TIFF, JPEG2000

[a]BioImagene Image File (BIF), Big Tag Image File Format (BigTIFF), Carl Zeiss Image (CZI), Joint Photographic Experts Group (JPG/JPEG), MIRAX format (MRXS), NanoZoomer Digital Pathology Image (NDPI), ScanScope Virtual Slide (SVS), Leica Scan files (SCN), Virtual Microscope Image Standards (VMS, VMU) by Hamamatsu, Olympus VSI format (VSI)

application areas in pathology that are likely to see innovation (especially in AI) and have the greatest chance of adoption in routine pathology.

POTENTIAL TECHNIQUES IN THE PRECLINICAL DOMAIN WITH CLINICAL IMPORTANCE

In this section, we will present a review of the literature on techniques that have a potential for gaining widespread acceptance in the preclinical domain. These technologies can also be used in the clinical domain once they have reached an acceptable level of robustness and efficiency. The widespread adoption of these technologies in the preclinical domain would provide an environment for testing and further development of the techniques and would eventually lead to the development of best practices and standards, facilitating optimal adoption in the clinical domain.

Onscreen Multiresolution Viewing

In various publications discussed earlier,[15,16,18,19] the advantages of digitalization of pathology are enumerated, which range from efficient storage, retrieval, and sharing of slide data to more advanced developments such as personalized and AI-enabled diagnostics. The digitalization of pathology has been made possible only by the modern digital technologies for storage and viewing of WSIs.[20-22] Hence, the storage formats used to store WSIs and the viewers used to visualize the contents of WSIs are of paramount importance for the advancement of this technology. In this section we discuss the developments in these 2 main aspects of digital pathology.

WSI Storage Formats. Digital slide scanners are used to acquire WSIs. The process of scanning a slide using digital slide scanners at 40× leads to 1600 megapixels, which is equivalent to a file of several gigabytes. This raises the issue of storing the data in a viable format. Digital Imaging and Communications in Medicine (DICOM) was designed as the standard format for medical images, allowing for interoperability of computer systems and sharing of data.[23] However, the DICOM standard did not have all required features; therefore, many DSS vendors have proposed their own proprietary image formats namely NanoZoomer Digital Pathology Image (Hamamatsu), SVS (Tiled TIFF-Tagged Image File Format), and iSyntax (Philips) (Table 1) etc. However, recently, DICOM standards have been able to store WSIs, leading to DICOM compliant sharing and viewing of WSIs by multiple vendors. There are still many challenges in successful integration of DICOM technology

TABLE 2. WSI Viewer Features and Comparison[a]

Viewer	Type	Regulatory Status	Platform	Features
PaigeAI FullFocus Viewer	Proprietary	FDA cleared, CE-marked IVD	Cloud	LIS/LIMS integration and AI support
QuPath	Opensource	None	Windows, MacOS X, and Linux	Stain estimation, reporting, color transformation, AI support, and annotation
Deciphex Patholytix	Proprietary	Nonclinical GLP compliant	Windows and Linux	GLP compliant workflow, AI support, image QC, WSI synced data capture, annotation, advanced visualization and multi-image display, and advanced quantification and measurements
Cytomine	Opensource	None	Web-based	Machine learning, object classification, scripting, annotation, user-behavior analytics, and multi-image display
Dynamyx	Proprietary	Research use only (US); CE-marked IVD	Web-based	LIS/LIMS integration, case management, collaboration, measure/quantify, annotate, coregister and multiple slides and view
Orbit	Opensource	None	Windows, Linux, and MacOS	Omero connectivity, spark integration, DL/ML ready, scripting support, object segmentation/classification, and annotations
ASAP	Opensource	None	Windows and Linux	Annotation, overlay-based visualization, ML support, and plugins
Sectra Digital Pathology Solution	Proprietary	FDA-cleared, CE-marked IVD	Web-based	LIMS/EMR integration, AI integration (any vendor), radiology PACs integration
Philips Intellisite Pathology Suite	Proprietary	FDA cleared	Web-based	Work-flow management, real-time collaboration, and measurement/annotation support
NDP.view2	Proprietary	None	Window and MacOS	Annotation, multiview of slides, case view, histogram, and LUT control

[a]AI, Artificial Intelligence; CE ,Conformité Européenne; DL, Deep Learning; EMR, Electronic Medical Records; FDA, Food and Drug Administration; GLP, Good Laboratory Practice; LIS/LIMS, Laboratory Information System and Laboratory Information Management System; LUT, Look-Up Table; ML, Machine Learning; PACS, Picture Archiving and Communication System; US, United States.

in pathology. Godinho et al[24] proposed a Picture Archiving and Communication System solution using DICOM technology for supporting DP.

Table 1 summarizes only how various manufacturers have handled the problem of image viewing and storage using different suitable image formats. It is by no means an exhaustive list. Table 2 compares the prominent WSI viewers available and tabulates how they compare in functionality and features. The capability of the different formats involved in the storage of images affects the quality of images and even their color profile. Lossy methods can lead to deterioration of the image quality, and it is important to assess how that would affect the diagnostics process. Although a lossy compression method such as jpeg reduces the size of the images to be stored greatly i.e. up to 10 times, but it reduces the quality of the image by removing some color information completely from the image. The information lost is unrecoverable. This is in contrast to lossless compression methods

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such as JPEG2000 (Joint Photographic Experts Group), which recovers the original information without loss decreasing the storage footprint of the image. Table 1 tabulates the various formats supported by different vendors of WSI scanners.[22]

WSI Viewers. As discussed earlier, it is important to analyze the variation in color and intensity profiles in the WSI images captured using different scanners. It is also essential to determine how viewing the same slide on different viewers affects the visual appearance of the slide. This goes along with ensuring that the slide preparation process meets the standard requirements as laid out in guidelines provided by scanner manufacturers.[25] In July 2021, the US Food and Drug Administration (FDA) cleared the FullFocus DP viewer for WSIs scanned with Philips Ultra Fast Scanner (Philips). Hence, the viewers and their integration with different scanners would be an important aspect in the foreseeable future. Cheng et al[26] compare different freely available viewers namely Sedeen (Pathcore), Automated Slide Analysis Platform (Radboud University Medical Center), and Qupath (Queen's University Belfast) with Nanozoomer Digital Pathology Viewer 2 (Hamamatsu). Images acquired using Hamamatsu Nanozoomer (Hamamatsu) compared at various magnifications showed clear differences in the color profile and quantitative comparisons using the Commission Internationale de l'Éclairage measure, indicating that Sedeen matches the factory specifications the best, whereas the other 2 freely available viewers show great variation. Hence, it can be concluded that not only the scanners but also the viewers are equally important in optimizing the visual appearance and achieving diagnostic efficiency in pathology practice. There is no detailed comparison available for the various scanners regarding viewing capabilities, and given the proprietary nature of the software, it remains a difficult task to perform because most commercial software is not readily available. However, Table 2 provides a comparison of various proprietary and open-source viewers available in the market and how they compare in terms of regulatory clearance and features provided.

Therefore, a very important area of innovation would be viewers that can be considered a standard across the domain of clinical and preclinical pathology. This would include development of tools and technologies that would enable viewing of slides in different International Color Consortium profiles and the capability of applying corrections in color and contrast to best suit the requirements of the experts. International Color Consortium defines how to correctly convert image files from one color space to another.

CASE PRIORITIZATION AND ABNORMALITY DETECTION

The great importance of saving pathologist's time by prioritizing cases has been established in several studies.[10,27-29] Prioritization of slides or regions of interest (ROIs) would help the pathologist focus on more important slides and regions. We present some recent research concerning abnormality detection and case prioritization that can potentially increase a pathologist's efficacy by directing their attention to the most important and pressing cases, leading to more efficient and timely analysis of data.

Ianni et al[30] proposed a system called pathology deep learning system, comprising 3 neural networks, for offering not only a class prediction for skin cancer pathology slides but also a confidence score indicating the degree of confidence of the predictions. The system is presented as a case prioritization tool and filtering system to enable a pathologist's review of more important cases. Somanchi et al[28] proposed a hierarchical linear time subset scanning method for analyzing a WSI and detecting ROIs. Pati et al[31] proposed a technique for automatic detection of tumor ROIs to enhance and expedite histopathology diagnostics. Tosun et al[32] presented HistoMapr that analyzes pairwise mutual information maps in a WSI, extracting structures and features of interest, and uses them to highlight ROIs with a confidence score, indicating the significance of an ROI. Different features correlate with different structural characteristics relevant to various diagnostic conditions and, hence, lead to explainable AI results. Although there is significant interest in the area and many novel techniques have been developed for the purpose, the application and acceptance of the technology by the broader community has been slow. However,

there is broad consensus that case prioritization can lead to better clinical and preclinical processes and improved patient outcomes and cost savings.

CONTENT-BASED IMAGE RETRIEVAL

Content-based image retrieval (CBIR) has the potential to greatly enhance the slide-reviewing process by providing online information concerning previously diagnosed cases and even provide results of the diagnostic decision made on a case-by-case basis. It can potentially provide statistical information concerning the diagnostic decision taken for different tissue morphologies and even the eventual patient outcome of the diagnostic process once integration with the health records has been achieved. Historical information can be used as a guide or even serve as a "secondary opinion" for a type of pathology. Content-based image retrieval automation can potentially lead to more reliable diagnostic decisions by clinicians.

CBIR is one of the most studied subjects in pathology image analysis with a wealth of literature on the subject. As far back as 1998, Lowe et al[33] proposed a system for semantic indexing of medical data using both images and text-based data. Comaniciu et al[34] went much further and produced a complete computer system using bimodal input (mouse and voice) to communicate and acquire blood cell specimens based on an ROI. Almost 2 decades ago, Zheng et al[35] proposed a feature extraction—based approach to query pathology images from a server based on content similarity. Mehta et al[36] went a step further and acquired sub-ROIs from a WSI using scale-invariant features. Akakin and Gurcan[37] developed a method for content-based retrieval and for differentiating the different subtypes of pathology images, which are difficult to discriminate and classify within follicular lymphoma and neuroblastoma.

Quellec et al[38] proposed a wavelets-based approach for CBIR using wavelets-based signatures and distance measures, with a special mechanism to relate the distance measure to the medical interpretation of images. A decade earlier, Wang[39] proposed Pathfinder, a wavelets and integrated region matching distance—based algorithm, for searching high resolution pathology image libraries. Cai et al[40] reiterated the significance of retrieval of images that are medically relevant rather than those based only on visual characteristics. Zhou et al[41] proposed a method for extraction of cellular morphologic features for more meaningful retrieval of cases. Qi et al[42] proposed a method for retrieval of white blood cell images, with ranking of images based on similarity to the query image and the system learnt from observing human experts. Schaer et al[43] used Densenet to create a content-based visual retrieval system within WSI viewers for visual similarity—based searching in previously reviewed cases, incorporating the biomedical knowledge base. Sridhar et al[44] proposed a features-extraction approach for CBIR where visual features are weighted based on their significance using boosted spectral embedding. Although a lot of interesting literature and techniques have been published in the area of CBIR, a real-world application of CBIR is yet to be seen, and the efficacy of the various techniques in the real world is yet to be confirmed.

CELL DETECTION AND COUNTING

Cell counting by pathologists for diagnostic and prognostic purposes has been known to be inaccurate for some time now.[45] This leads to problems in estimating the extent of the tumor. Xing and Yang[46] provided a comprehensive review of different techniques used for cell detection and counting, ranging from transforms-based techniques to morphologic operations to supervised deep learning, covering many decades of research. Schüffler et al[47] provided an active learning—based free software toolkit (TMarker) for cell detection and counting and showed that the performance of the technique is similar to that of human experts. However, older methods involving image processing such as segmentation with ellipse fitting[48] have now been replaced by multiscale convolutional neural networks providing very high accuracies on multiple data sets.[49] Kumar et al[50] presented an annotated data set and a convolutional neural network-based technique for detection of cells and compared the technique with openly available software applications namely Cell Profiler (Cimini Lab-Broad Institute) and Fiji (ImageJ). Transfer learning has also been

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TABLE 3. Comparison of Various Cell Detection Algorithms.

Method	Application (cells of interest)	Image type	Reference
Image processing	Various	Various	Xing and Yang,[46] 2016
Deep learning	Various	Various	Serag et al,[53] 2019; Alom et al,[54] 2019
CNN based	Breast cancer	H&E	Janowczyk and Madabhushi,[55] 2016
Three-class CNN	Generalized	H&E	Kumar et al,[50] 2017
CNN based	Breast mitoses	H&E	Pantanowitz et al,[52] 2020
Darknet-53	Blood cell	—	Ren et al,[51] 2021

CNN, convolutional neural network; H&E, hematoxylin and eosin.

used more recently by Ren et al[51] for accurate cell detection.

Recent work by Pantanowitz et al[52] showed that cell counting using AI-based analysis greatly improved the accuracy while reducing the time required to perform the procedure by more than 25%. Serag et al[53] presented cell detection and counting, particularly in immunohistochemistry, as an important application of AI in pathology. Alom et al[54] compared different state-of-the-art techniques on several different DP image analysis problems such as nuclei segmentation. Janowczyk and Madabhushi[55] presented a software suite that can be used to perform a variety of pathology image analysis tasks such as cell detection and counting. This application can potentially lead to higher efficacy and efficiency in the pathology laboratory whether clinical or preclinical.

Table 3 summarizes the latest AI-based approaches proposed for various cell detection and counting applications proposed in recent years. The data sets used for development and validation of the solutions are mainly being derived from clinical use cases. Nevertheless, many approaches are transferable between tissue types and different stains. Because the data used and techniques developed are not available openly, it is difficult to compare and assess the performance of the techniques involved.

OUT-OF-FOCUS AREAS DETECTION

One problem that has been seen in almost all scanners is that they often produce images that have artifacts, as discussed by Barisoni et al.[56] One important artifact that is frequently seen in WSIs is the out-of-focus (OOF) areas. Out-of-focus is the main artifact in the scanning process and, hence, most relevant to the digitization process. Other artifacts such as tissue folds and coverslipping faults may lead to a poor scan but are due to problems in slide-preparation steps, which occur before the scan and should be handled in the prescan quality check. Quality control in scanners is likely to focus on OOF areas because it remains the most important aspect of the WSI scan. It is extremely important to detect OOF areas and quality check the slides to see whether they are fit for review by pathologists because ignoring them could potentially lead to great delays in diagnostic reviews.

Many techniques involving machine learning and deep learning have been developed to detect OOF areas. Campanella et al[57] provide a comparison of feature-based and deep learning–based approaches for detecting blurriness with both producing very high accuracies. Janowczyk et al[58] in their study have introduced HistoQC (opensource), which is a tool that can be used to detect blurred areas within a slide along with other artifacts and localize them, which can speed up the process of reviewing and quality checking of the slides, hence improving the workflow. Kohlberger et al[59] developed a convolutional neural network called ConvFocus to exhaustively localize and quantify the OOF areas on a slide. Haghighat et al[60] presented a novel technique for profiling prostate WSIs, providing a measure of their usability and evaluate multiple openly available databases. They also compared the technique called PathProfiler with HistoQC and

handcrafted features-based techniques and showed that PathProfiler performs much better in comparison.

Development of quality control tools such as OOF detection is an area of fervent activity. Many scanner manufacturers and computational pathology software providers have already started integrating tools and algorithms for detection of OOF areas on scanned slides. There is likely to be room for third parties to provide fast and accurate tools for dependable quality control of WSIs.

BIOMARKERS AND AI

AI applications may potentiate the usage of biomarkers with automated assessment of prognostic biomarkers, such as *Ki-67* (for expansion of gene symbols, use search tool at www.genenames.org) in breast cancer or quantification of immune cell biomarkers.[61,62] There is significant interest in predicting molecular biomarker status based on morphological features in WSI, potentially for gene expression profiling and predicting microsatellite instability, mutational status, and copy number alterations.[63] This may extend into predicting treatment response, survival, and other clinical outcomes. It has been proposed that the integration of transcriptomic analysis, clinical information and AI-based image analysis, in effect integrative AI, can help health care professionals make improved treatment decisions in cancer owing to improved therapeutic stratification of patients.[64]

CHALLENGES

Although there is great potential and interest in digitalization and application of AI and image processing in pathology, there remain some important challenges that must be addressed. We address some of these issues in the further sections in light of the relevant literature and techniques published in the past decade or so.

Reproducibility Across Laboratories

Reproducibility of results is a significant factor affecting the confidence of the community on the state-of-the-art algorithms. Li and Chen[65] discussed this issue in their article for a well-known neural network and openly available database and stated that enough detail is not provided in articles to reproduce the results. Cui and Zhang[66] asserted the significance of standardization of slide preparation and digitization and data preprocessing and algorithm development so that the same algorithms can be used across different laboratories. Bussola et al[67] proposed "histolab," a python library that can be used to preprocess data so that data leakage can be avoided, and robust algorithms can be developed that work well in a cross-section of DP domains with greater reproducibility.

However, the problem of reproducibility of deep learning results remains an important issue for medical image analysis. The problem is also seen in related medical domains such as radiology, as discussed by Renard et al,[68] who provided recommendations to address the issue of variability of image segmentation. Bizzego et al[69] discussed the significance of reproducibility of results in reference to the DAPPER framework (FDA's MicroArray Quality Control project), which is designed to evaluate and identify the causes of variation in predictive biomarkers. Acs et al[70] reiterated the importance of reproducibility of AI algorithms for precision pathology. However, the reproducibility of algorithms in DP remains difficult to assess overall as discussed by Wagner et al[71] because of the majority of the research in the area failing to provide mechanisms and data for independent evaluation. However, some researchers such as Cruz-Roa et al[72] showed how their algorithm works consistently well on openly available data because efforts were made to share all relevant code and optimal parameters. Beam et al[73] presented the challenges faced by machine learning in health care and adhering to standards is deemed as extremely important for acquiring, comparing, and verifying results.

Cross-Scanner Variation and Display Variation

The scanning and visualization of WSIs is a very important aspect of DCP. Variation in scanning and display technology has been seen to lead to considerable variation in appearance of the slides. Summit on Color in Medical Imaging in May 2013[74] highlighted the requirement for a gold standard in WSI colors. Krupinski et al[75] performed a comparison between a color-calibrated and an uncalibrated monitor, demonstrating that there was no benefit in color calibration in

diagnostic accuracy, but there was significant improvement seen in diagnosis time. Kimpe et al[76] indicated that color and luminance stability increases diagnostic accuracy and interpathologist agreement and leads to decreased reading time. However, Hanna et al[77] argued that the effect of display color calibration on diagnostic accuracy is minimal. Clarke and Treanor[78] discussed the issue of standardization of color in DCP in detail, differentiating between the internal screening and external scanning color calibration. Moreover, the importance of color calibration is elucidated by Shrestha and Hulsken,[79] who showed that interscanner color differences can be reduced using color calibration.

Inoue and Yagi[80] discussed the 5 major reasons of color variation: specimen thickness, staining, scanners, viewers, and display devices, and stressed on the importance of standardization. Yagi[81] in a previous article highlighted the major reasons for color variation and some remedial processes, for example, standardization in the slide creation process. The Royal College of Pathology in their document on best practices for implementing digital pathology noted that there are differences between microscopes and WSI systems and different scanners technology regarding, for example, lenses and light source technology. Hence, tests for color accuracy should be performed when acquiring scanners.[82] Cheng et al[83] compared 2 commercial WSI scanners with a hypothetical monochrome scanner, and a multispectral imaging system was used to determine the color truth. The authors concluded that there is still great room for improvement for the modern WSI scanners. Jahn et al[19] reasserted the importance of accurate color rendition and recommended the correction of the color deviations using software profiles. The importance of color standardization for medical imaging is discussed in detail by Badano et al[84] based on the discussions at the Color Summit and its significance in various fields of medical imaging, especially histopathology. Bradley and Jacobsen[85] discussed the importance of color fidelity, which is the accuracy and consistency of color regarding the acceptance of WSI technology in toxicologic pathology, and encouraged the practice of using International Color Consortium color profiles to calibrate devices. Cross-scanner variation is also important for machine learning applications because Leo et al[86] showed that scanner variation leads to features instability, which affects classification accuracies. As the industry develops and more agreement is attained regarding standardization of slide scanning and image representation, the field of DCP is likely to benefit and develop further.

Challenges in Adoption of Digitalization

The great advantages of digitalization of pathology slides include easy sharing of data for teleconsultation, robust and dependable storage of data,[87] and advanced image analysis. However, the universal digital workflow in pathology still remains a pipe dream. Although the efficacy and validity of digital review of slides has been proven repeatedly,[88-91] the adoption still remains slow; however, there has been an increase in adoption since Covid-19 pandemic. Before the pandemic, strict US federal regulations prevented the adoption of DP, however, to allow the pathologist to work from home, the enforcement of regulations were relaxed, leading to much greater adoption.[92] Similarly in the National Health Services, United Kingodm, adoption of DP allowed the maintenance of the practice during the pandemic and, according to a survey by Browning et al,[93] has led to other benefits such as facilitating second opinion and double reporting and ease of access. Moreover, many studies have shown that DP-based analysis of slides is equivalent to a microscope in most instances[14] and can even lead to speed ups in immunohistochemistry.[94]

The various challenges that stand in the way of adoption of digitalization range from limitations in staff training to issues in standardization of scanning technology. Various authors have highlighted these aspects. Cheng and Tan[95] highlighted the requirement of specialized services referred to as DP service management to fulfil the technical and training needs of the laboratory personnel. García-Rojo[96] presented the various guidelines and technical specifications advocated by various pathology associations and bodies across America, Europe, and Australia and suggested that it remains important that good best practices and guidelines are developed across the discipline so that the adoption of DP is facilitated. Clunie[97] asserted the importance of standardization of image formats to overcome the issues

of scalability and interoperability and recommended the adoption of DICOM-Tagged Image File Format to overcome all issues. Jarkman et al[98] discussed the importance of generalization in adopting AI technologies in pathology and highlighted the fact that even a small change in data set can lead to high variation in accuracies.

There are significant challenges in adoption of digitalization and advanced technologies such as quantitative pathology and AI when it comes to pathology. However, with the rising number of imaging modalities and staining techniques and the higher health care burden in the industrialized world, it is imperative that new efficiency-increasing technologies such as DCP are adopted.

EXPLAINABLE AI MODELS

Recently, the European General Data Protection Right has introduced the clause of "right to explanation," requiring the AI algorithm's results to be interpretable. Interpretability of AI results is relevant in preclinical and clinical setting. Explainable AI,[99] often abbreviated as xAI, is relatively new to computational pathology, but it has important legal and regulatory implications. However, there has been a lot of effort recently in leveraging the power of AI models interpretability and explainability in wider domains.[100] Morales et al[101] contended that explainable AI is likely to gain attention and become an important aspect of DCP.

As stated earlier, the concept of explainable AI is likely to gain more traction and relevance owing to legal and practical implications. A practitioner would have more confidence in a technique and its findings if there is a rationale that is medically relevant. However, deep learning techniques are almost always difficult to explain regarding both the results and their internal working. There are now techniques available to visualize the features or regions in an image that caused the neural network to reach a particular decision. These may be aligned with previously known medical knowledge and morphologies. Moreover, new features commonly known as biomarkers may be discovered, which are not known previously and are not easy for the naked eye to observe. Fitzgerald et al[64] discussed the important role AI can play in personalized medicine and biomarker evaluation. It is imperative that pathology departments worldwide pay attention to this area of research in collaboration with academia and industry because it would eventually enable effective use of AI in DCP. Heinz et al[63] presented a survey of pathologists from industry and academia pertaining to what applications of AI in pathology are likely to be most useful. The prediction of treatment response remained the most popular, followed by analysis by subgroups and age and finally prediction of genetic alterations, expression, and survival directly from pathology slides. Explainable AI would be very important for such applications. However, there will always be techniques that produce very good results but would be difficult or impossible to explain. Their use would be limited by regulatory approvals and may only be used as part of active research into previously unknown medical facts.

AI poses regulatory and ethical challenges.[102,103] For medical device software products, key regulatory requirements and application of technical standards must be met to achieve FDA clearance in the United States or Conformité Européenne certification in Europe. The level of regulatory requirements applicable to a medical device is generally proportional to the level of risk associated to the device, where, for example, the FDA may classify software algorithm devices to assist users in DP as class II.[104] Generally, performance evaluation includes several key aspects to verify and validate the product for its intended use in the clinical setting, particularly scientific validity (eg, association between the software output and the targeted condition), analytical performance (accurate and reliable output), and clinical performance showing the output meets the intended purpose in a clinical context and for the target population. Additional regulations are expected to come into place in the coming months and years, such as the European Union AI Act and standards and guidance in relation to AI.

CONCLUSION

In this review, we presented various techniques and applications that can potentially be perfected in the preclinical setting and then can eventually find their way into the clinical domain. In addition, we discussed

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the various challenges that are likely to hamper smooth progress toward digitalization in pathology and adoption of advanced image processing and AI. However, as the technology develops and advancements are made in scanning and WSI data processing and analysis, DCP is likely to become the standard in pathology practice, leading to increased efficiency and efficacy and improved standardization in diagnosis.

POTENTIAL COMPETING INTERESTS

There are no conflicts of interest in the submission and publication of this article.

Abbreviations and Acronyms: **AI,** artificial intelligence; **CNN,** convolutional neural network; **DCP,** digital and computational pathology; **DICOM,** Digital Imaging and Communications in Medicine; **DP,** digital pathology; **FDA,** Food and Drug Administration; **OOF,** out of focus; **ROI,** region of interest; **WSI,** whole-slide image

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