

Review

The Quest for the Application of Artificial Intelligence to Whole Slide Imaging: Unique Prospective from New Advanced Tools

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Abstract: The introduction of machine learning in digital pathology has deeply impacted the field, especially with the advent of whole slide image (WSI) analysis. In this review, we tried to elucidate the role of machine learning algorithms in diagnostic precision, efficiency, and the reproducibility of the results. First, we discuss some of the most used tools, including QuPath, HistoQC, and HistomicsTK, and provide an updated overview of machine learning approaches and their application in pathology. Later, we report how these tools may simplify the automation of WSI analyses, also reducing manual workload and inter-observer variability. A novel aspect of this review is its focus on open-source tools, presented in a way that may help the adoption process for pathologists. Furthermore, we highlight the major benefits of these technologies, with the aim of making this review a practical guide for clinicians seeking to implement machine learning-based solutions in their specific workflows. Moreover, this review also emphasizes some crucial limitations related to data quality and the interpretability of the models, giving insight into future directions for research. Overall, this work tries to bridge the gap between the more recent technological progress in computer science and traditional clinical practice, supporting a broader, yet smooth, adoption of machine learning approaches in digital pathology.

Keywords: machine learning; digital pathology; whole slide imaging



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1. Introduction

Histopathology, based on the examination of tissue sections placed on a glass slide under a microscope, is generally considered the gold standard for the diagnosis of multiple human diseases, including cancer [1–3]. The goal of pathologists involved in research or clinical practice is the identification of numerous relevant features useful for the diagnosis and monitoring of human diseases. As a consequence of the complexity of the multiple and differently associated features that characterize human diseases, including multiple cancer types in different organs, only expert pathologists with years of training are able to perform a correct diagnosis, which is necessary for deciding on the correct therapeutic strategy to achieve patient health. Taken together, these observations show that histopathological analysis is a very complex process that requires in-depth knowledge of multiple diseases obtained through time-consuming training in different fields of human pathology. Moreover, studies on diagnostic concordance among pathologists regarding the interpretation of pathological specimens have revealed relevant interindividual variability in the interpretation of biopsy specimens [4,5]. In order to avoid these problems,

multiple algorithmic solutions have been developed in recent years, focused on finding new ways for digitizing histological specimens to produce high-resolution images. The aim of these studies was to aid histopathologists in performing reproducible, accurate, and efficient histopathological diagnoses [6]. The recent introduction of digital pathology into the clinical setting represents one of the most intriguing applications in the routine working environment of surgical pathologists involved in histopathological diagnosis [7]. The role of digital pathology in clinical practice has been underlined by the introduction of powerful whole slide scanners in pathology departments, which allow for the digitalization of tissue samples followed by whole slide analysis [8]. A milestone in this process was the validation of whole slide imaging for diagnostic purposes in pathology from the College of American Pathologists [9], followed by approval from the Food and Drug Administration (FDA) [10]. The introduction of digital pathology in clinical histopathology, with the automated analysis of tissue morphology, has immediately evidenced potential in decreasing the workload of pathologists (and also reducing the time required for the reporting of histopathological diagnoses), especially as a consequence of the automatic quantification of histopathological and immunohistochemical features and other biomarkers [11]. Because of the relevance of cancer diagnosis in surgical pathologist practice, the vast majority of studies based on the application of artificial intelligence were focused on different tumors, including colorectal cancer [12], with particular attention being paid to the automated analysis of tumor budding (epithelial-to-mesenchymal transition of tumor cells) [13] and outcome and prognosis prediction [14]. The aim of this review is to describe the various open-source machine learning solutions for digital pathology applications proposed in recent years, focusing specifically on those tools more useful for the extraction of diagnostic information from whole slide images, thus improving the productivity, accuracy, and reproducibility of pathological diagnoses. On the other hand, it is essential to acknowledge that several recent reviews opened up a more general discussion over the impact of the adoption of artificial intelligence in digital pathology [15–18].

2. Whole Slide Imaging

The introduction of digital pathology technologies and, in particular, whole slide imaging has significantly improved the efficiency of modern clinical pathology departments by facilitating the storage, viewing, processing, and sharing of digital scans of tissue glass slides. The application of deep learning feature extraction methods for whole slide imaging (WSI) may improve, in the near future, the productivity, accuracy, and reproducibility of pathological diagnoses [19–25]. Whole slide imaging represents the digital equivalent of histological specimens scanned using digital scanners. High-resolution images are generated by digital scanners that can apply multiple magnifications and focal planes at different resolution levels. The workflow of WSI systems requires good histological technology to overcome practical challenges such as poor staining and tissue folds, which may negatively affect the quality of the scanned slides. In order to avoid these challenges, preparing glass slides for scanning requires a standardized approach to tissue sample fixation, embedding, sectioning, and staining [26]. Moreover, digital scanners may differ according to the methodology used to focus high-quality scanners on a range of depths across each tile, which is a time-consuming process [27]. From a practical point of view, surgical pathology slides require scanning at X 20 magnification, whereas cytology slides require scanning at X 40 magnification, which is necessary to ensure higher cytological diagnostic accuracy [28]. WSI-based analysis may be subdivided into four sequential steps as follows: (i) image acquisition, (ii) image storage, (iii) image processing, and (iv) image visualization.

3. Artificial Intelligence, Machine Learning, and Deep Learning in Digital Pathology

In the context of this review, it is crucial to make the terminology used around this recent topic clear to the reader. Understanding the distinctions and overlaps of these technical terms is essential for recognizing their roles in digital pathology. Artificial intelli-

gence encompasses all the applications aiming to create software (or machines) capable of performing tasks that typically require human intelligence. For example, in digital pathology, artificial intelligence is used to develop systems that can assist in computational histopathology analysis and AI-assisted diagnostics [15,29]. Machine learning is a subset of artificial intelligence, and it mainly focuses on the development of algorithms that enable computers to improve their performance on a specific task by learning directly from data. In digital pathology, machine learning algorithms are trained on large datasets of medical images and have been used to implement pattern recognition tasks and to predict disease states [30,31]. Deep learning refers to a specific subset of machine learning algorithms that make use of neural networks (typically organized into many layers) to model complex patterns in data (see Figure 1). Recently, in digital pathology, deep learning approaches have been shown to be effective for analyzing complex histopathological images and, in some specific applications, for providing accurate clinical-related insights [8,19,32]. In brief, both machine learning and deep learning are subsets of artificial intelligence, deep learning models represent a specific type of machine learning algorithms, and all machine learning approaches are part of the broader artificial intelligence systems. In conclusion, while artificial intelligence provides the overall goal of creating intelligent systems, machine learning offers methods for data-driven learning, and deep learning provides advanced techniques for the analysis of high-dimensional data. To date, all these technologies have played a crucial role in driving fundamental innovations in human digital pathology.

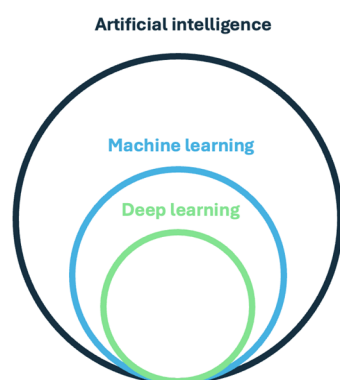


Figure 1. A schematic representation of the relationship among artificial intelligence, machine learning, and deep learning.

4. New Tools for Digital Pathology in the Era of Artificial Intelligence

As already stated, machine learning approaches have transformed the field of digital pathology, especially for whole slide image (WSI) analysis and processing. Traditional manual analysis is time-consuming and may also increase the risk of introducing errors [33]. Machine learning tools, specifically developed for WSI analysis, have been shown to enhance the whole diagnostic process and allow the achievement of more consistent and reproducible results. As we discuss in the following sections, these tools, offering relevant insights into a very complex diagnostic task, provide valid support for detecting patterns that may be missed by humans. The integration of these tools into standard workflows can reduce the workload of pathologists, allowing them to focus mainly on cases that are more complex. In our opinion, to date, the development and the introduction of these advanced tools into the practical clinical workflow is crucial and may represent a further step towards advancing precision medicine and eventually improving patient outcomes. In the last few years, several tools have been developed, allowing us to easily approach the application of machine learning algorithms to digital pathology. Here, we discuss some of the most used tools (see Table 1) and their more relevant applications in digital pathology.

Table 1. A list of tools analyzed in the present review. The number of citations refers to that available in Google Scholar on 20 May 2024. The tools are listed and discussed in chronological order, considering the publication date.

Name	Link	License	Citations	Original Work
QuPath	https://qupath.github.io/ (accessed on 21 May 2024)	Open source	4731	
HistomicsTK	https://github.com/DigitalSlideArchive/HistomicsTK (accessed on 21 May 2024)	Open source	152	
HistoQC	https://github.com/choosehappy/HistoQC (accessed on 21 May 2024)	Open source	246	
MONAI	https://monai.io/ (accessed on 21 May 2024)	Open source	246	
PathML	https://pathml.org/ (accessed on 21 May 2024)	Open source	15	
Histolab	https://github.com/histolab/histolab (accessed on 21 May 2024)	Open source	15	
SlidDL	https://github.com/markowetzlab/slidl-tutorial (accessed on 21 May 2024)	Open source	3	
SISH	https://github.com/mahmoodlab/SISH (accessed on 21 May 2024)	Open source	62	

4.1. QuPath

QuPath is one of the most widely used open-source bioimage analysis software. It was developed to visualize and analyze whole slide images comprehensively, offer pathologists a useful tool for the identification of human tumors, and provide histopathologists with batch-processing and scripting functionalities. Moreover, QuPath is an extensible platform with which pathologists can share algorithms to analyze complex images. According to this original flexible design, to date, QuPath appears to be a suitable tool for a wide range of additional image analysis applications over multiple biomedical research fields [34]. The impact of QuPath in delivering highly impactful research is summarized in a recent review [35] specifically dedicated to this software. Probably the most relevant key point of QuPath is linked with its inherent flexibility, which is demonstrated by its application to a wide range of human pathology research and clinical areas. QuPath has been successfully utilized for computer-assisted tumor grading, PD-L1 scoring, and quantification of CD8-positive immune cell density in urothelial carcinoma [36] and to detect and measure cell morphology. Another study [37] used QuPath to identify MLH1-deficient inflammatory bowel disease-associated colorectal cancers from tissue microarray. In this case, the software demonstrated high sensitivity and specificity in detecting MLH1 expression and improving diagnostic accuracy (100% sensitive and 98.25% specific). QuPath was also used to analyze whole slide images for the identification of islets of Langerhans [38] and define their composition and essential morphological characteristics, showing that QuPath can identify immune cell populations and accurately quantify immune infiltrates in the pancreas.

4.2. DSA and HistomicsTK

The DSA (Digital Slide Archive) is an open-source web-based platform for digital pathology that was developed for the management, integration, and analysis of histological images. The tool includes cell segmentation, stain color deconvolution, and normalization and augmentation [39] for cancer research. HistomicsTK is a companion library of image analysis algorithms providing image analysis capabilities for the DSA. As reported in the original study, the DSA and HistomicsTK provide cancer researchers with a free digital pathology platform, avoiding the need for costly commercial software, which is sometimes also expensive to scale. HistomicsTK was used for the extraction of pathomic features in a study on tumor-infiltrating lymphocyte classification in breast cancer (the best performance was obtained by Random Forest with an ROC AUC of 0.86) [40]. Another study used HistomicsTK in the context of image analysis for renal allograft evaluation and fibrosis quantification [41]. In 2022, a study [42] used HistomicsTK for nucleus classification and segmentation in breast cancer to manage data and annotations. The positive pixel counting

function included in this tool was used to correlate age-related cognitive impairment with neuropathologic features using WSI images (a mean AUC of 0.63 and a mean balanced accuracy of 0.59 were found in the hippocampus) [43]. Moreover, nuclear size, shape, staining intensity, boundary complexity, edges (chromatin clumping), and texture were extracted using HistomicsTK to investigate a digital histologic biomarker for the prognosis of invasive breast cancer [44].

4.3. HistoQC

HistoQC is a Python-based algorithm developed for the quality control of whole slide images that also enables the detection of artifacts and the identification of batch effects (such as slides with darker staining compared with the other slides), making use of the inspection of color distribution, edge, and smoothness detectors. Moreover, HistoQC provides an interactive and user-friendly interface, which allows for the exploration of data [45]. HistoQC was used to assess the role of tissue quality in the application of deep learning approaches in computational pathology [46]. HistoQC was also used to automatically detect image artifacts in comparison with a manual review of WSI images in the context of renal histomorphometry [47]. Another study [48], aiming to quantify peritubular capillary attributes using conventional and whole slide images, used HistoQC to identify WSI images that represented outliers within a cohort and to identify artifact-free regions (the normalized peritubular capillary aspect ratio was below 0.6; a 0.1 increase in the normalized aspect ratio was significantly associated with disease progression, with a hazard ratio of 1.28).

4.4. MONAI

MONAI (Medical Open Network for Artificial Intelligence) is an extension Python library that was developed as a framework for performing deep learning on medical images. It supports the tiling of whole slide images and model evaluation metrics, as well as providing support for neural network architectures, optimizers, and AI-assisted annotation [49]. MONAI was used for a variety of different applications on medical images, including deep learning segmentation of fetal brain MRI [50], evaluation of the diagnostic performance of traditional and deep learning CAD systems in children trained with adult data for the detection of lung nodules on chest CT scans [51], and segmentation of the liver from publicly available CT scan [52].

4.5. PathML

This is a tool specifically designed for the application of machine learning in cancer research. PathML is a Python library that can support the tiling of whole slide images and implement classical approaches to artifact and foreground detection. PathML also provides a variety of pre-processing methods, including stain normalization. Nevertheless, PathML was not designed to perform any post-processing steps [53]. PathML was used to understand how mesenchymal cell states mediate prostate cancer progression [54] and investigate the mechanisms of acquired resistance to immune checkpoint inhibitors in patients with non-small cell lung cancer [55].

4.6. Histolab

Histolab is a Python library that provides a set of functions for classical image analysis and facilitates tissue detection and the removal of artifacts. Histolab also allows for the tiling of whole slide images and the random extraction of tiles according to tissue detection scores [56]. Histolab has been successfully used to perform rapid artifact removal and H&E-stained tissue segmentation [57] and to investigate the cross-scale association between radiological scans and digitalized pathology images for immunotherapy-treated non-small cell lung cancer patients [58].

4.7. SliDL

SliDL is a Python toolbox developed for processing whole slide images using deep learning methods. It is characterized by excellent performance and aims to reduce the workload of pathologists drastically. This tool makes whole slide image data handling easy and bridges the gap between standard histological image analysis and whole slide image analysis. Moreover, SliDL provides tile extraction for model evaluation and tissue extraction [59]. SliDL was used for the early detection and risk prediction of esophageal cancer [60].

4.8. SISH

SISH (Self-supervised Image Search for Histology) is an open-source tool developed in the Pathology Department of Brigham and Women's Hospital in Boston, which requires only slide-level annotations for training and allows for a fast and scalable search for whole slide images. Recently, SISH was evaluated on very large databases of over 22,000 patients, including a vast set of disease subtypes and rare cancer types. SISH also provides solutions to many open challenges in whole slide imaging, such as accuracy, speed, and scalability, and it shows strong performance on diverse datasets [61]. It has been used in rare diseases that are often difficult to diagnose, making predicting the best course of treatment challenging for clinicians [62]. It has also been used to search for similar images in archives of histology and histopathology images [63].

4.9. Other Tools

PathEX [64] is a framework that integrates intersection over tile (IoT) and background over tile (BoT) algorithms for tile image extraction at the boundaries of annotated regions while excluding blank tiles within these regions. It can be used to set the thresholds for IoT and BoT to facilitate tile image extraction, providing valuable guidance for tile image extraction in digital pathology applications. SVPath [65] uses deep learning to extract and analyze the stria vascularis and its associated capillary bed from whole temporal bone histopathology slides. Slideflow [66] is a deep learning library for histopathology, supporting a wide variety of deep learning approaches, that includes a whole slide interface for deploying trained models. HistoMIL [67] is a Python package for training multiple instance learning models on histopathology slides designed to streamline the implementation, training, and inference process of MIL-based algorithms for computational pathologists.

5. Discussion

5.1. Main Findings and Limitations

From this review, it emerges that machine learning algorithms have improved diagnostic accuracy by providing an excellent tool for the identification of anomalies in whole slide images (WSIs) that may be missed, in some cases, by human pathologists. For example, recent studies have demonstrated improved accuracy in tumor grading and biomarker scoring, such as PD-L1 and CD8+ cell density measurements in cancer tissues [36,37]. The adoption of these tools drastically reduced the time for manual review, allowing pathologists to focus on those cases that are inherently more complex [37,38].

The most important aspect concerns the fact that machine learning algorithms simplified the quantitative analysis of complex histopathological images, also providing more consistent and reproducible results and reducing inter-observer variability [38,39]. Finally, these tools have shown good performance in detecting and classifying various cell types and tissues, which is of paramount importance for understanding diseases and their progression. Despite these undeniable positive aspects, there is a list of limitations that need to be discussed.

First, the performance of machine learning algorithms is strongly affected by the quality and quantity of data. Obtaining such big datasets can be challenging because of manual annotation (which is time-consuming) and variability in image quality. Moreover, for

deep learning approaches, high-performance computing infrastructures are an important prerequisite, and this issue can represent an obstacle for several institutions.

Second, there are issues related to the need for pathologists to adapt to new technologies despite the fact that these tools may indirectly hinder traditional clinical practices. In our opinion, considering that the internal mechanism of these tools is usually hidden from the user, the most relevant limitation is probably related to interpretability, which makes it difficult to understand how final decisions are made [68]. In fact, in some machine learning systems, such as linear models and decision trees, it can be relatively straightforward to explain the algorithm's outcomes based on the image features used. However, the most effective algorithms for medical image analysis today are often based on deep learning models. These models are considered "black boxes" because their decision-making processes are typically hidden (even to their developers). Even the specific image features extracted by these algorithms are not always clear, making it difficult to understand why a particular decision was made. This lack of interpretability is particularly concerning in medicine, as it is crucial for the physician to comprehend the rationale behind algorithmic decisions, both to avoid errors and ensure patient safety.

Third, an effect to consider when using machine learning models, especially deep learning models, is system bias. Bias in deep learning models refers to the tendency to produce skewed or unfair outcomes because of underlying biases in the training data. This effect can result from unbalanced or non-representative training data, leading to unequal performance in medical image characterization and potentially exacerbating healthcare disparities. A typical example is when a demographic group is overrepresented in the training dataset, causing unequal performance across different demographic groups and further contributing to healthcare disparities [69,70].

5.2. Comparison with Traditional Methods and Impact on Pathologists

The adoption of artificial intelligence in pathology departments worldwide and the routine creation of large repositories of whole slide images in different fields of human pathology will represent a revolution in surgical pathology, with a complete transformation of daily pathology practice. Digital pathology technologies, including routine digitalization of tissue glass slides, i.e., classical histological specimens, are significantly improving all aspects of modern clinical practices, ranging from storing to reviewing, processing, diagnosing, and sharing digital scans or better whole slide images. In short, machine learning approaches are forcing the automation of significant parts of pathology workflows, thus improving the clinical abilities of histopathologists, cytopathologists, biologists, and technicians involved in clinical practice and research. The WSI-based approach is characterized by the following goals: (i) high speed in searching databases of digitized pathology reports, allowing the identification of WSIs with morphological features similar to those of the case of interest; (ii) accuracy in the interpretation of histological and immunohistochemical images, finding cases with similar morphology and learning how their mentors would diagnose the case of interest [71]; (iii) strong performance in reaching a "good" diagnosis in different fields of human pathology, identifying tumors that share specific features useful for clinical correlations and predicting outcome, including rare diseases; and (iv) fusing histopathology and genomic features, allowing for the identification of genomic changes associated with peculiar morphologies, useful for cancer diagnosis, therapy and prognosis [72]. In short, deep learning methods applied to the analysis of WSIs will allow pathologists to perform excellently in all fields of human pathology, with a substantial reduction in their daily workload. One of the most important applications of this new vision for pathologists working without a microscope at hand is the training of young pathologists. Pathologists in training might utilize the large repositories of WSIs to computationally identify images with similar morphological and immunohistochemical features and easily learn the fundamentals from the interpretation of biopsies, thus ending with the correct interpretation of biopsies and a correct and fast diagnosis of the case of interest. This new approach should require marked modifications in supervised training. The speed

of algorithms when searching WSIs with similar morphologies compared to the case of interest will indicate the field to which the differential diagnosis should be restricted, facilitating the diagnostic process by the pathologist in training [73]. Artificial intelligence-based pathology has been shown to help pathologists in multiple fields of complexity in daily clinical practice, such as the identification of origins for cancers of unknown primary [74]. The supervision of expert pathologists might be restricted to the diagnosis of complex cases or to rare cases of human pathology not included in the algorithm yet. Scalability is a critical challenge for the adoption of the WSI-based approach in the daily practice of pathology departments worldwide. WSIs are characterized by a computationally prohibitive size, and WSI retrieval systems need a growing number of slides, each consisting of billions of pixels and measuring several gigabytes in size. This represents a challenge for the adoption of machine learning methods applied to histopathological image analysis [75].

6. Conclusions

Different from other fields of medicine, such as radiology and clinical laboratory, which have been characterized by a recent technical revolution in terms of automatization, surgical pathology has been characterized by the persistence of old techniques and approaches. Nowadays, histopathological diagnosis, the gold standard for the correct diagnosis and therapy of human diseases, is based on the manual evaluation of colored tissue sections mounted on glasses under an optical microscope. The manual evaluation of a large number of biopsies using a microscope is time-consuming and, on some occasions, error-prone, particularly when facing rare and complex pathologic entities, for which long training is often mandatory. Consequently, pathologists involved in clinical practice are at risk of possible errors in diagnosis, and patients are affected by a delayed or erroneous diagnosis, with negative consequences on their health status [76]. The recent introduction of low-cost scanners and the possibility to obtain whole slide images from each tissue section represents a real revolution in the field of surgical pathology, with significant advantages in the evaluation of histopathological images. The medical image algorithms here reported, and the new ones that will be developed in the further months, will aid pathologists in obtaining more rapid, reliable, expert, and validated histopathological and cytological diagnoses in different fields of human pathology. Thanks to the application of machine learning techniques to pathology, pathologists in all hospitals of the world will gain access to large volumes of histopathological images previously diagnosed and validated, which could be compared with the case of interest, allowing for the quick identification of the disease class the histological specimen under examination fits with. Moreover, medical imaging algorithms could disclose details and correlations that may be undetected even by an expert's naked eye. The high resolution and multiscale nature of WSIs allow for identifying the local texture and spatial context within each histological specimen, representing a very rich data source, at a much higher resolution than that obtained using a microscope with the naked eye, which can be correlated with histopathological diagnosis and patient prognosis [32]. Based on these data, we believe that there is room for the establishment of teams of engineers specialized in AI and pathologists involved in clinical practice that can drive the development of AI technologies in pathology and develop reliable and accurate algorithms that will become clinical tools for enhancing the diagnostic accuracy of human diseases. In this project, both computer science experts and pathologists may play key roles. The role of engineers is of utmost importance, thanks to their ability to offer plenty of opportunities and explore alternative new methods, like convolutional neural networks, which have shown outstanding performance on image classification tasks [77] and improved automated classification of tissue sections. On the other hand, the role of pathologists is also of utmost importance, as they are early adopters of the algorithms and participate in the validation phase of new algorithms, ultimately ending with the development and accreditation of tools for a better diagnosis and treatment of human diseases. In conclusion, while machine learning algorithms have recently shown great promise in enhancing WSI

analysis in digital pathology, in our opinion, it is crucial to address their current limitations for the effective implementation of these tools in clinical practice.

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