Digital Oncology 1

Digital pathology and artificial intelligence

Muhammad Khalid Khan Niazi, Anil V Parwani, Metin N Gurcan

In modern clinical practice, digital pathology has a crucial role and is increasingly a technological requirement in the scientific laboratory environment. The advent of whole-slide imaging, availability of faster networks, and cheaper storage solutions has made it easier for pathologists to manage digital slide images and share them for clinical use. In parallel, unprecedented advances in machine learning have enabled the synergy of artificial intelligence and digital pathology, which offers image-based diagnosis possibilities that were once limited only to radiology and cardiology. Integration of digital slides into the pathology workflow, advanced algorithms, and computer-aided diagnostic techniques extend the frontiers of the pathologist's view beyond a microscopic slide and enable true utilisation and integration of knowledge that is beyond human limits and boundaries, and we believe there is clear potential for artificial intelligence breakthroughs in the pathology setting. In this Review, we discuss advancements in digital slide-based image diagnosis for cancer along with some challenges and opportunities for artificial intelligence in digital pathology.

Lancet Oncol 2019; 20: e253–61

This is the first in a **Series** of two papers about Digital Oncology

Center for Biomedical Informatics, Wake Forest School of Medicine, Winston-Salem, NC, USA (M K K Niazi PhD, M N Gurcan PhD); **and Department of Pathology, The Ohio State University, Columbus, OH, USA** (A V Parwani MD)

Correspondence to: Dr Muhammad Khalid Khan Niazi, Center for Biomedical Informatics, Wake Forest School of Medicine, Winston-Salem, NC 27101, USA
mniazi@wakehealth.edu

Introduction

Digital pathology has a crucial role in modern clinical practice and is increasingly a technology requirement within the laboratory environment.[1,2] Advances in computing power, faster networks, and cheaper storage have enabled pathologists to manage digital slide images with more ease and flexibility than they could in the past and has enabled pathologists to share images for telepathology and clinical use. In the last two decades, digital imaging in pathology has seen the inception and evolution of whole-slide imaging, which allows entire slides to be imaged and permanently stored at high resolution.

In particular, whole-slide imaging serves as an enabling platform for the application of artificial intelligence (AI) in digital pathology. Until now, AI has been mostly used for image-based diagnosis in radiology and cardiology. Its application to pathology is an expanding field of active research with several research groups and dedicated companies. Images produced by whole-slide imaging are a great source of information; complexity is higher than in many other imaging modalities because of their large size (a resolution of 100k×100k is common), presence of colour information (haematoxylin and eosin and immunohistochemistry), no apparent anatomical orientation as in radiology, availability of information at multiple scales (eg, ×4, ×20), and multiple z-stack levels (each slice contains a finite thickness and, depending on the plane of focus, will generate different images). Clearly, this kind of visual information cannot be extracted as easily by a human reader.

With integration of digital slides into the pathology workflow, advanced algorithms and computer-aided diagnostic techniques extend the frontiers of the pathologist's view beyond a microscopic slide and enable true utilisation and integration of knowledge beyond human limits and boundaries.[2] AI already enables pathologists to identify unique imaging markers associated with disease processes with the goal of improving early detection, determining prognosis, and selecting treatments most likely to be effective. This allows pathologists to serve more patients while maintaining diagnostic and prognostic accuracy. This integration is especially important considering the increasing number of ageing patients, and that less than 2% of medical graduates go into pathology because of the information management, integration, and digital media accessibility aspect of the review and case sign-out process.[3]

Digital pathology and AI can have immense potential for oncology and precision medicine. Much like the evolution of efficiency and effectiveness in radiology, the pressure on pathologists to reduce turnaround time and develop more efficient workflows is trending towards digitalisation. This digital innovation has potential to change the way cancer diagnoses occur, with added benefits of shared images and data, increased efficiency and integrated diagnostics, modernisation of pathology workflows to improve patient care and safety, increased collaboration through multidisciplinary and disease-specific patient care conferences, improved accountability (on behalf of the physician, who makes the final clinical decision), and cost savings by optimising staff performance. By using AI algorithms many of the tasks that are manual and subjective can become more automated and standardised.

Although AI is slated to benefit many areas of clinical health sciences (eg, oncology and drug development), the focus of this Review is to highlight its use in digital pathology and whole-slide imaging, including education (eg, digital slide teaching sets), quality assurance (eg, second opinions, proficiency testing, and archiving), and clinical diagnosis (ie, telepathology). First, we explore how AI has advanced these areas of digital pathology, as well as specific use cases and applications of AI in research, image analysis, and computer-aided diagnosis; and discuss the techniques used, challenges, and

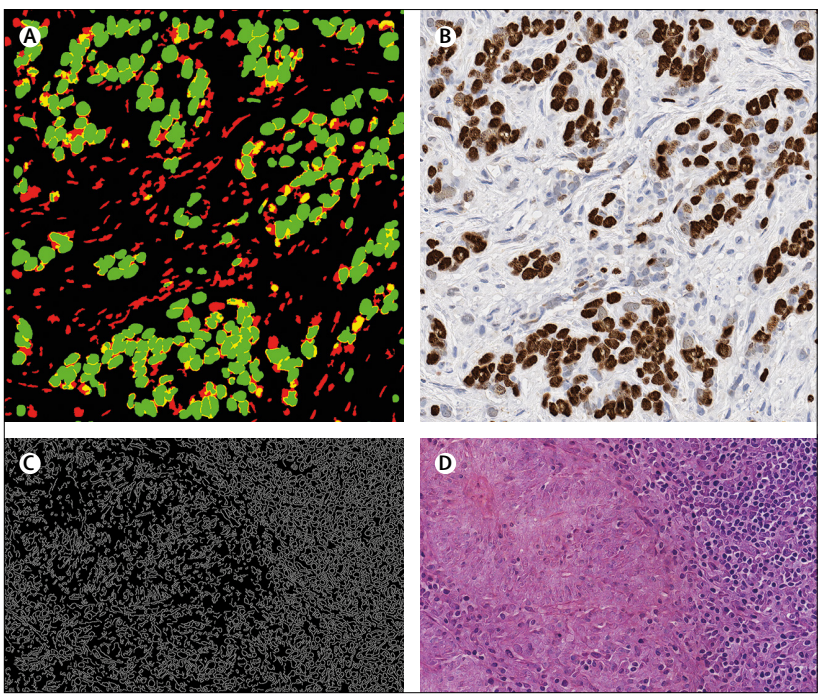

Figure 1: **Synthetic images**
(A) Input image with desired KI67 positive (green) and negative nuclei (red). (B) Synthetic (fake) image generated by conditional generative adversarial networks. (C) Randomly drawn lines. (D) Synthetic image generated from (C).

barriers.[1] Second, we discuss the ultimate goal of AI and whole-slide imaging—integration of pathological image information with clinical data— and its limitations. With whole-slide imaging as an enabling platform for AI, digital pathology will have meaningful and measurable effects on both clinical and research components of pathology workflow.[2,3]

AI and education

Whole-slide imaging is already used for teaching at conferences, virtual workshops, presentations, and tumour boards.[2] Equipped with whole-slide imaging, AI tools can help further training of the next generation of pathologists by providing on demand, standardised, and interactive digital slides that can be shared with multiple users anywhere, at any time.[2,3] Additionally, AI tools can provide automated annotations in the form of quizzes for trainees. With the help of these interactive tools trainees can view, pan, and zoom enhanced digital slides, which can provide tutoring in real-time and in a dynamic teaching environment. Our group has developed some of these approaches, specifically the generation of synthetic digital slides, which will be discussed herein.

In our first attempts at generating synthetic images, we extracted individual and clustered nuclei that were both positively and negatively stained from real whole-slide imaging images, and systematically placed the extracted nuclei clumps on an image canvas—a cut-and-paste approach.[4] These images were evaluated by four board certified pathologists in the task of estimating the ratio of positive to total number of nuclei. The resulting concordance correlation coefficients between the pathologist and the true ratio range from 0·86 to 0·95. In our follow-up study, we used the conditional Generative Adversarial Networks approach.[5] This method included two main components: the generator and the discriminator. Although the generator tries to create fake stained images, the discriminator tries to catch these fake images, each getting better at generating and detecting fake stained images in an iterative manner. The main idea is to force the generator to learn the underlying distribution of the images from the training data. The accuracy of five experts (three pathologists and two image analysts) in distinguishing between 15 real and 15 synthetic images was only 47·3% (±SD 8·5%). Generation of numerous synthetic histopathology images could be useful for educational purposes because it will give pathology trainees the opportunity to test their skills. Additionally, these approaches can be very useful for quality control and understanding the perceptual and cognitive challenges that pathologists face. Figure 1 shows examples of synthetic (fake) breast and colorectal cancer image generation.

AI and quality assurance

The development of automated, high-speed, and high-resolution whole-slide imaging has had a substantial effect on quality assurance. Digitised slides that are readily available to pathologists in the laboratory information system or on the intranet can be used for several quality assurance tasks, including teleconsultation, gauging inter-observer and intra-observer variance, proficiency testing, and archiving of slides. For example, the College of American Pathologists optionally sends whole-slide imaging in addition to glass slides of certain proficiency testing cases.

AI can have an important role in quality assurance. It is very difficult for pathologists and radiologists alike to be up to date with the new medical advances in all organ systems and cancer types. As with all disciplines, frequency of interactions builds confidence and skills, and helps keep practitioners current with evolving diagnostic tools. Additionally, by providing feedback manually or with intelligent deep learning and AI tools, a pathologist has the potential to keep improving on his or her performance. AI can be used as a supplement to these manual digital reviews in routine diagnostic workflow or as a complement to the more formal quality reviews that are part of a pathology laboratory's quality management process. AI can also provide a quality check on the diagnosis rendered by a pathologist by applying automated diagnostic algorithms prospectively or retrospectively. These methods can continue to serve as patient safety mechanisms to improve the quality of diagnosis and to prevent error.

AI for clinical diagnosis

Rendering routine pathological diagnoses using whole-slide imaging is a feasible approach. Several studies[6,7]

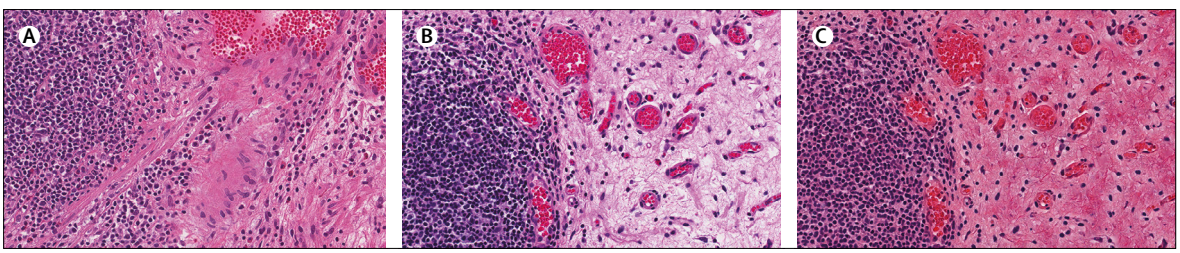

Figure 2: **Colour normalisation**
(A) Reference image of T1 bladder cancer. (B) Image to be colour normalised to (A). (C) Colour normalised image produced by conditional generative adversarial networks.

have been published comparing diagnostic interpretation using digital slides to diagnoses rendered using glass slides and a conventional light microscope. These studies show a range of concordance from 89% to 99% when comparing diagnostic interpretation using digital slides to diagnoses rendered using glass slides and a conventional light microscope. The range is wide and encompasses multiple organ systems and different types of specimen preparations. Although you will still need references for the studies and percentages. Some pathology laboratories have stopped using slides such as the General Pathology Laboratory at the Kalmar County Hospital in Kalmar, Sweden.[8] Others such as The Ohio State University have made substantial advances towards conversion to a digital pathology workflow.[9] Essential requirements for the integration of digital pathology into laboratory information systems include accurate digital reproduction of the scanned glass slide, running the slide scanner continually with little use of laboratory personnel and saving, archiving, and later retrieving the image without degradation. AI could improve on current solutions by detecting of out-of-focus areas and improving colour standardisation.

The quality of images produced by whole-slide imaging scanners has a direct influence on the readers' performance and their reliability of diagnosis.[10] Most modern scanners come equipped with autofocus optics system to select focal planes to accurately capture the three-dimensional tissue morphology similar to a two-dimensional digital image.[11] To account for varying thickness of tissue sections, autofocus optics systems determine a set of focus points at different focal planes. From these focal planes, scanners capture images to produce sharp tissue representation. However, whole-slide imaging scanners could still produce digital images with out-of-focus areas if the autofocus optics system erroneously selects focus points that lie in a different plane than the proper height of the tissue.[12] A naive solution would be to add several extra focus points, but that would be impractical because it would cause long delays in slide scanning. AI provides a better alternative by automatically identifying out-of-focus regions and allowing whole-slide imaging scanners to add a few extra focal points to those regions. AI achieves this by either feature engineering or via a representation learning approach. Lopez and colleagues[12] adopted a feature engineering approach by handcrafting texture features from grey level co-occurrence matrices and gradient information. These features were used in conjunction with decision trees to classify 200×200 pixel-sized regions as focused or blurred. Unfortunately, the method is only sensitive to a high level of blurriness, and it requires modifying programme parameters to adapt it to images acquired at different resolutions. Another approach called DeepFocus,[11] based on representation learning, automatically discovers features from the images to identify blurry regions. Because the DeepFocus programme automatically learns features at different levels of abstraction, it can generalise to different types of tissues and even to colour variations due to different types of staining, haematoxylin and eosin, and immunohistochemistry.

Standardisation of the colour displayed by digital slides is important for the accuracy of AI. Colour variations in digital slides are often produced because of different lots or manufacturers of staining reagents, variations in thickness of tissue sections, difference in staining protocols, and disparity in scanning characteristics. One such example of colour variation is shown in figure 2. These variations often impose obstacles to clinical diagnosis and prognosis done by humans, as well as machines.[13,14] Moreover, these variations are one of the main hurdles in generalisation of the machine learning algorithms to multiple sites. For this reason, the absence of colour normalisation in an AI pipeline could negatively affect the performance of machine learning algorithms.[14] For a long time, collecting colour statistics to perform colour matching across images has remained the main source of colour normalisation. However, progress in generative models has presented novel ways of colour normalisation. Zanjani and colleagues[15] used a generative adversarial network[16] for stain normalisation. Unlike conventional generative adversarial networks, which use noise as an input, this network requires the greyscale image as an input. This type of input enables the network to preserve the image structure while manipulating colour information. It also requires a colour system matrix (consisting of stain vectors that are similar to colour deconvolutional matrix) as an input for the generator in the network. This network can simultaneously learn the chromatic space of haematoxylin and eosin images and can normalise it to a template

image using the colour system matrix. The non-parametric nature of the network makes it applicable to a wide variety of histopathological images. In a similar effort, Bentaieb and colleagues[17] used the concept of style transfer[18] for colour normalisation. Their objective was to transfer the staining appearance of tissue images across different datasets to avoid colour variations caused by batch effects. When histopathological images are acquired in different experimental setups and tested on pretrained diagnostic models the prediction performance can suffer because of batch effects (ie, non-biological experimental variations such as age of sample, method of slide preparation, specifications of the imaging device, and type of software after processing).[19] Style transfer provides one plausible solution by finding image representations that independently model variations in the semantic image content and the style in which it is presented.[17] However, the method does not have a mechanism to standardise a dataset to a given slide without retraining the whole network. Shaban and colleagues[13] used the concept of cycle-consistent adversarial networks[20] to perform colour normalisation. CycleGAN, a variant of GAN, permits the unpaired image-to-image translation through cycle-consistency. CycleGAN allows the images to be mapped to a specified colour model and preserves the same tissue structure.

For the **The Cancer Genomic Atlas** see https://tcga-data.nci.nih.gov/docs/publications/tcga/?

AI and image analysis

Image analysis tools can automate and quantify with greater consistency and accuracy than light microscopy. Computer-aided diagnosis is widely used for oestrogen receptor, progesterone receptor, and HER2/neu assessments in breast cancer, Ki67 assessment in carcinoid tumours, as well as multiple other clinical and research stains. The reliability of these methods requires the standardisation of the image acquisition step, which has been discussed previously. The development of whole-slide imaging has facilitated large growth in numerous researchers and companies seeking to use computer-aided diagnoses to analyse whole-stain imaging and to develop new software tools to assist pathologists. Before whole-slide imaging, the field of image analysis was limited by the requirement of pathologists having to select regions of interest to be analysed. Because whole-slide imaging allows the entire slide to be available for analysis, field selection can be automated. The following section summarises various AI methods to enable this region of interest selection.

Nuclear segmentation in whole-slide imaging enables extraction of high-quality features for nuclear morphometrics and other analysis in computational pathology.[21] For this reason, automatic nuclei segmentation is among the most studied problems in AI.[22] AI efficiently enables a range of nuclear segmentation tasks, including segmenting of all nuclei from whole-slide imaging to identifying a subset of nuclei within specific anatomical regions.

Like other areas of pathology, deep learning algorithms are well known for their state-of-the-art performance on nuclei segmentation task.[22] In general, these algorithms estimate a probability map of the nuclear and non-nuclear (two-class) regions on the basis of learned nuclear appearances and rely on complex methods after processing to obtain the final nuclear shapes and separation between touching nuclei.[21] For example, Song and colleagues[23] used a multiscale convolutional network to generate a nuclear probability map. This map was subjected to graph partitioning to segment individual nuclei from the image. Similarly, Xing and colleagues[24] exploited the topology of the probability map using region growing[25] to segment the individual nuclei. Unfortunately, these methods require retraining for their generalisation to unseen datasets. Moreover, these methods also do not generalise if the training and test images belongs to different organs. To overcome these issues, there is a growing trend to train the nuclei segmentation methods on images taken from different organs.

Kumar and colleagues[21] created a well annotated database consisting of 30 whole-slide images of digitised tissue samples from several organs. The slides were taken from the publically available database The Cancer Genome Atlas.[26] The images were generated at 18 different hospitals, which adds to the diversity of this dataset in terms of variation in slide preparation protocols among laboratories. Over 21 000 nuclei were manually annotated to train a deep learning algorithm. Unlike former methods, the authors formulated the nuclei segmentation as a three-class problem. They considered the nuclei edges as a third class when generating the ternary probability map. This map was subjected to region growing to segment the individual nuclei. Mahmood and colleagues[27] adapted a generative model to do nuclei segmentation in images taken from different organs. They trained a generative model using images from four different organs to synthetically generate pathology images. These synthetically generated images were combined with real images to train convolutional neural networks to perform nuclei segmentation. Yousefi and colleagues[28] used a region convolutional neural network[29] for nucleus classification in histopathology images. Nuclei in these images were detected using class-agnostic models trained on small annotated patches. Li and colleagues[30] proposed a multistage deep learning framework for mitosis detection from haematoxylin and eosin images of breast biopsies. The method is a modified version of a Faster-Region Proposal Network.[29] The method consists of a deep segmentation network for generating mitosis region when only a weak label is given (ie, only the centroid pixel of mitosis is annotated), an elaborately designed deep detection network for localising mitosis by using contextual region information, and a deep verification network for improving detection accuracy by removing false positives.

During most pathological analysis, pathologists are interested in identifying a subset of nuclei in a particular anatomical region.[31] For example, in T1 bladder cancer, pathologists are interested in identifying the tumour nuclei within lamina propria.[32] Similarly, in breast and pancreatic neuroendocrine tumours, pathologists are interested only in the ratio of Ki67 tumour positive nuclei to total tumour nuclei within hotspots.[33,34] In follicular lymphoma, the analysis is limited to only the presence of centroblasts within follicles.[35,36] For these reasons, there is an increasing interest in developing AI algorithms that can identify a subset of cells within a certain anatomical region. Niazi and colleagues[34] adapted a transfer learning method[37] to identify positive and negative tumour nuclei from images of Ki67 breast cancer tissues (figure 3). In a similar effort, Coudray and colleagues[38] used transfer learning on whole-slide images obtained from The Cancer Genome Atlas to accurately and automatically classify them into adenocarcinoma, squamous cell carcinoma, or normal lung tissue. Tavolara and colleagues[39] designed a dual cGAN along a dictionary-learning framework[40] to identify tumour regions from non-tumour regions in patients with colorectal cancer.

Although far less established and routine than their use in clinical workflows in radiology, computer-assisted diagnoses is an active research area for tumour biopsies. Manual interpretation of these images often involves extremely laborious tasks such as cell counting. Moreover, these quantitative measures are far from exhaustive and typically consider only specific portions of biopsies (ie, hotspots)[41] and specific anatomical regions. Computer-assisted diagnoses offer increased efficiency, accurate quantification, and potentially novel subsensory features for analysis and interpretation of histopathological images, thus mitigating pathologist workload and inter-variability and intra-variability.

Most research in the automated analysis of digital tumour biopsies is on deep learning.[42] In the context of images, deep learning allows computers to mimic the process of human visual perception through a cascade of computational units that are layered and interconnected, which vaguely resemble biological neurons. Because of the sheer size (ie, file size) of digital biopsies and the computational demands and complexities of deep learning, research has focused mostly on physically smaller tasks, which look at small portions of the image like mitosis detection,[43] anatomical region identification,[44] and cancer identification.[45] However, recent research attempts to bypass these computational barriers through specialised deep learning methods, which take advantage of whole-slide information. These methods are whole slide because they use the entire tissue section and anatomical regions, which are not typically considered for diagnosis and decision making. Ehteshami and colleagues[46,47] developed a fully automatic method to detect ductal carcinoma in situ using whole-slide haematoxylin and eosin stained biopsies. By using their method, a whole slide is partitioned into superpixels on the basis of similarity at some magnification. Superpixels are grouped into anatomical regions (specifically epithelium) on the basis of graph clustering.[48] Finally, each cluster is classified as ductal carcinoma in situ or benign or normal on the basis of features extracted by deep learning.[47] Niazi and colleagues[34] used deep learning to identify tumour regions in pancreatic neuroendocrine tumours. Their method employed transfer learning, whereby the features of a pretrained deep learning algorithm (ie, trained on some other classification task) are fine-tuned and retrained to classify tumour and stroma regions. Both Ki67-positive and Ki67-negative tumour cells were used. Cruz-Roa and colleagues[49] developed a sampling method that is adaptive and automated for whole-slide images. Their method approached deep learning computational barriers by carefully picking regions of whole slides using quasi-Monte Carlo sampling.[50] Their method was able to detect invasive breast cancer with dice coefficient of 76% across multiple institutions, scanners, and preparation protocols. Finally, Niazi and colleagues[33] developed a novel region of interest selection method for hotspot detection in breast cancer to minimise magnitude of data transfer. Clearly, whole-slide image-based decisions are within reach and are preferred to methods utilising only portions of slides.

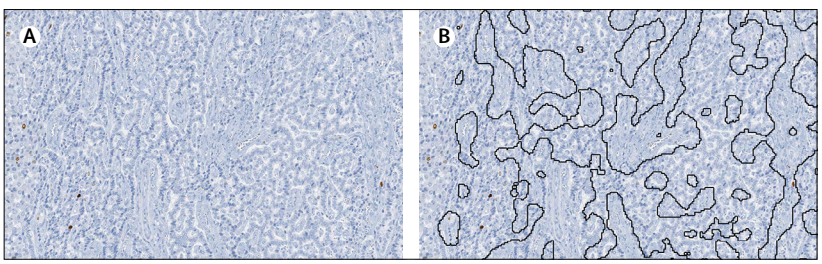

Figure 3: **Example of tumour identification**
(A) Image cropped from Ki67 slide of patients with a pancreatic neuroendocrine tumour. (B) The non-tumour regions are automatically outlined by a deep learning algorithm.

Integration of AI with other clinical data

Histopathological image analysis is not only limited to visual analysis; several other sources of data need to be included coming from omics, clinical records, and patient demographic information.[51,52] Clinical data (eg, demographic information, medical history, and laboratory and clinical results) are mostly in unstructured free-text reports. Natural language processing technologies can be used to extract relevant information and tie those to the information in histopathological slides.[53] Natural language processing has also started to benefit from deep learning-based AI technologies. AI will be essential to filter these disparate sources of information and help pathologists make the best clinical decisions for patients. AI will be able to discover more complicated or subtle connections than a human would. AI treatment

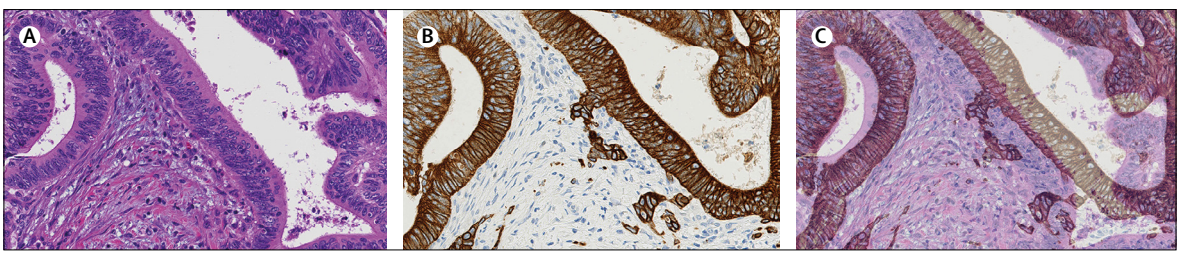

Figure 4: **Adjacent tissue section deformation**
(A) Haematoxylin and eosin image. (B) Adjacent tissue section of (A) stained for pan-cytokeratin. (C) Haematoxylin and eosin and pan-cytokeratin overlaid to depict the non-linear deformation between tissue sections.

needs to include not only images, but also clinical and outcome data, enabling high-dimensional analysis that is beyond what the human brain alone can accomplish. Rich sources of data could transform pathology from a clinical science to an informatics science in which the tissue would be only one of the sources of data.

In anatomical pathology, immunohistochemistry staining has a profound role in diagnosis by helping doctors to determine the biological characteristics of a wide variety of tumours,[54] to make a prognosis,[55] and select appropriate systemic therapies for patients with cancer.[4,56] As the use of genomic technologies increase, DNA-level and transcriptional-level features obtained from homogenised tissues will be evaluated for their utility in creating new subclassifications of malignancies to predict future disease behaviour and treatment response. Understanding the relationships between genomic features and quantitative immunohistochemistry features will be essential for further illuminating protein-genomic associations, and for creating improved molecular classifications that combine nucleotide-resolution information generated at exome scales with spatial and subcellular protein-level information. As a first step towards this process, AI helps to perform a cell-level registration among adjacent sections of the tumour tissue.[57] Image registration is required to compensate for the non-linear geometric deformation induced by the staining process. Figure 4 shows an example of such non-linear deformation.

Image registration enables medical professionals to study the behaviour of different biomarkers within the same cell.[58,59] It is also helps to better understand the tumour microenvironment. Moreover, image registration enables the combination of information from different modalities.[60] However, image registration is still one of the relatively unexplored frontiers of digital pathology. The aim of combining pathology with omics or other computational modalities hinges on the development of reliable image registration methods. With the advent of deep learning, substantial progress in this area is expected in the near future.

Perception and limitations of AI

Many AI approaches, particularly systems based on deep learning, are criticised for not being able to explain how they arrive at their decisions, hence the label black boxes. Although these algorithms will still offer benefits, clinical, legal, and regulatory issues need to be sorted out going forward. At the same time, there is active research to make the algorithms easier to interpret by humans and provide insight on how they work (eg, by providing some of the features that the algorithm is focusing on, or by dividing the AI algorithm execution into steps, both of which would make more logical sense to a human). These strategies will provide some transparency behind AI algorithms, but will often come at the expense of performance hit as a tradeoff. On the regulatory side, there could be restrictions: for example, the EU's new General Data Protection Regulation stipulates that "the data subject shall have the right not to be subject to a decision based solely on automated processing"[61] whose medical implications should be carefully reviewed. The financial and economic implications of AI are still being discussed and many of the discussions are speculative. It is not known at this stage whether current economic mechanisms will be put in place (eg, taxing AI), once these systems are shown to be safe and effective. Similarly, these developments can benefit or negatively affect low-income and middle-income countries (regions with the largest burden of disease), although the hope is that AI will benefit pathologists from these countries with faster and more accurate diagnoses, as well as faster screening.

The notion that AI will replace pathologists is just a speculation at this point. Expert and AI combination will yield results that are more accurate, consistent, and useful than what an expert can do alone. Although AI will continue to make decisions in narrow fields, humans can take several factors into account and are better at synthesising information to arrive at decisions than machines. AI will be trained to extract information and connect it with other complementary sources of information. For example, we evaluated multiple regions of single slides and multiple sections from different patients' tumours using computational histologic analysis and semi-quantitative proteomic profiling of neuroblastoma slides.[62] We found that both approaches showed that intertumour heterogeneity was greater than intratumour heterogeneity and both techniques can supplement a pathologist's review of neuroblastoma for refined risk stratification. It is important to keep in mind

Search strategy and selection criteria

We used Google Scholar and PubMed to find relevant manuscripts. We restricted our search to papers published in English between Jan 1, 2013, and Feb 25, 2019. We used the following terms in different combinations: "WSI", "deep learning", "AI", "digital pathology", "GAN", "histopathologic image analysis", "nuclei segmentation", "whole slide imaging", "artificial Intelligence and digital pathology", "digital pathology and deep learning", "histopathology and deep learning", "GAN and histopathology", "nuclei segmentation histopathology", "nuclei segmentation deep learning", and "cGAN histopathology".

that different AI platforms can lead to different conclusions as different doctors might follow the same protocol yet reach different conclusions. Although there is a need for proper regulatory control (ie, these methods and devices need to be shown to be safe and effective), there could be more than one method to solve a particular problem.

Some exciting developments in AI haven not been applied to medicine yet. For example, one shot learning is learning from only a small number of training samples as opposed to a large number of samples. It is done typically by means of transferring knowledge from other domains or models or extracting information from their particular context. This could be particularly useful in pathology in which deep truthing (the annotation of images at the sub-cellular, cellular, and regional scale to produce ground truth) is challenging because of the complexity of the images and their sheer size. In reinforcement learning, algorithms are trained to reach complex goals by comparing the immediate actions with long-term outcomes. As in human learning, the algorithm must wait until final outcomes to find out whether particular actions have led to success or failure. Testing algorithms in this way could be particularly useful in training some algorithms to make complex decisions in pathology in which the outcome might be known yet what particular biological factors or treatment options led to that outcome might not be completely apparent.

Conclusion

Pathology is rapidly transitioning to digital methods with new developments in AI. Therefore, bringing the disciplines of pathology and AI together could result in exciting changes to health care, although a large number of technical, ethical, and legal questions still need to be answered.[63,64] Combining digital pathology and AI will lead to an improved workflow and advanced diagnostics, enabling researchers and clinical teams to share and review images instantly, and use computational algorithms to assess and contribute valuable insights that can ultimately lead to a more informed and detailed cancer diagnosis. This integration will help advance the future of precision oncology and can result in personalised care plans for each patient.

Contributors

All authors were involved in and contributed equally to the literature search, study design, data collection, and writing if the Review. MKKN produced the figures.

Declaration of interests

The authors declare no competing interests.

Acknowledgments

This project described was funded in part by the National Cancer Institute and The Ohio State University Comprehensive Cancer Center Intramural Research (Pelotonia) Award: R01CA134451 given to Metin N Gurcan (co-principal investigator) and Gerard Lozanski (co-principal investigator); U24CA199374 given to Metin N Gurcan, Anant Madabushi, and Anne Martel (all co-principal investigators); and U01 U01 CA220401 given to Lee Cooper (principal investigator), Metin N Gurcan (co-principal investigator), and Chris Flowers (co-principal investigator). The content of this Review is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health. The authors are not employed by the National Institutes of Health.

References

1 Tizhoosh HR, Pantanowitz L. Artificial intelligence and digital pathology: challenges and opportunities. *J Pathol Inform* 2018; **9:** 38.

2 Farahani N, Parwani AV, Pantanowitz L. Whole slide imaging in pathology: advantages, limitations, and emerging perspectives. *Pathol Lab Med Int* 2015; **7:** 23–33.

3 Zarella MD, Bowman D, Aeffner F, et al. A practical guide to whole slide imaging: a white paper from the digital pathology association. *Arch Pathol Lab Med* 2019; **143:** 222–34.

4 Niazi MKK, Abas FS, Senaras C, et al. Nuclear IHC enumeration: a digital phantom to evaluate the performance of automated algorithms in digital pathology. *PloS One* 2018; **13:** e0196547.

5 Mirza M, Osindero S. Conditional generative adversarial nets. 2014. https://arxiv.org/abs/1411.1784 (accessed Dec 12, 2018).

6 Tabata K, Mori I, Sasaki T, et al. Whole-slide imaging at primary pathological diagnosis: validation of whole-slide imaging-based primary pathological diagnosis at twelve Japanese academic institutes. *Pathol Int* 2017; **67:** 547–54.

7 Loughrey MB, Kelly PJ, Houghton OP, et al. Digital slide viewing for primary reporting in gastrointestinal pathology: a validation study. *Virchows Arch* 2015; **467:** 137–44.

8 Thorstenson S, Molin J, Lundström C. Implementation of large-scale routine diagnostics using whole slide imaging in Sweden: Digital pathology experiences 2006-2013. *J Pathol Inform* 2014; **5:** 14.

9 Lloyd M, Kellough D, Shanks T, et al. How to acquire over 500 000 whole slides images a year: creating a massive novel data modality to accelerate cancer research. United States and Canadian Academy of Pathology Annual Meeting (USCAP); Vancouver, BC, Canada; March 20, 2018. Abstract 1647.

10 Shrestha P, Kneepkens R, Vrijnsen J, Vossen D, Abels E, Hulsken B. A quantitative approach to evaluate image quality of whole slide imaging scanners. *J Pathol Inform* 2016; **7:** 56.

11 Senaras C, Niazi MKK, Lozanski G, Gurcan MN. DeepFocus: detection of out-of-focus regions in whole slide digital images using deep learning. *PloS One* 2018; **13:** e0205387.

12 Lopez XM, D'Andrea E, Barbot P, et al. An automated blur detection method for histological whole slide imaging. *PloS One* 2013; **8:** e82710.

13 Shaban MT, Baur C, Navab N, Albarqouni S. StainGAN: stain style transfer for digital histological images. 2018. https://arxiv.org/abs/1804.01601 (accessed Dec 12, 2018).

14 Komura D, Ishikawa S. Machine learning methods for histopathological image analysis. *Comput Struct Biotechnol J* 2018; **16:** 34–42.

15 Zanjani FG, Zinger S, Bejnordi BE, van der Laak JA, de With PH. Stain normalization of histopathology images using generative adversarial networks. 2018. https://www.researchgate.net/publication/325522633_Stain_normalization_of_histopathology_images_using_generative_adversarial_networks (accessed Dec 12, 2018).

16 Goodfellow I, Pouget-Abadie J, Mirza M, et al., eds. Generative adversarial nets. In: Ghahramani Z, Welling M, Cortes C, Lawrence ND, Weinberger KQ, eds. Advances in neural information processing systems 27. NY, USA: Curran Associates, Inc., 2014: 2672–2680.

17 Bentaieb A, Hamarneh G. Adversarial stain transfer for histopathology image analysis. *IEEE Trans Med Imaging* 2018; **37:** 792–802.

18 Gatys LA, Ecker AS, Bethge M, editors. Image style transfer using convolutional neural networks. 2016. https://www.cvfoundation.org/openaccess/content_cvpr_2016/papers/Gatys_Image_Style_Transfer_CVPR_2016_paper.pdf (accessed Nov 25, 2018).

19 Kothari S, Phan JH, Stokes TH, Osunkoya AO, Young AN, Wang MD. Removing batch effects from histopathological images for enhanced cancer diagnosis. *IEEE Trans Med Imaging* 2014; **18:** 765–72.

20 Zhu J-Y, Park T, Isola P, Efros AA. Unpaired image-to-image translation using cycle-consistent adversarial networks. 2017. https://arxiv.org/abs/1703·10593 (accessed Nov 25, 2018).

21 Kumar N, Verma R, Sharma S, Bhargava S, Vahadane A, Sethi A. A dataset and a technique for generalized nuclear segmentation for computational pathology. *IEEE Trans Med Imaging* 2017; **36:** 1550–60.

22 Xing F, Yang L. Robust nucleus/cell detection and segmentation in digital pathology and microscopy images: a comprehensive review. *IEEE Rev Biomed Eng* 2016; **9:** 234–63.

23 Song Y, Zhang L, Chen S, Ni D, Lei B, Wang T. Accurate segmentation of cervical cytoplasm and nuclei based on multiscale convolutional network and graph partitioning. *IEEE Rev Biomed Eng* 2015; **62:** 2421–33.

24 Xing F, Xie Y, Yang L. An automatic learning-based framework for robust nucleus segmentation. *IEEE Trans Med Imaging* 2016; **35:** 550–66.

25 Tremeau A, Borel N. A region growing and merging algorithm to color segmentation. *Pattern Recognition* 1997; **30:** 1191–203.

26 Weinstein JN, Collisson EA, Mills GB, et al. The cancer genome atlas pan-cancer analysis project *Nature genetics* 2013; **45:** 1113.

27 Mahmood F, Borders D, Chen R, et al. Deep adversarial training for multi-organ nuclei segmentation in histopathology images. 2018. https://arxiv.org/abs/1810.00236 (accessed Nov 25, 2018).

28 Yousefi S, Nie Y. Transfer learning from nucleus detection to classification in histopathology images. *BioRxiv* 2019: published online Jan 24. https://doi.org/10.1101/530113 (preprint).

29 Ren S, He K, Girshick R, Sun J. Faster R-CNN: towards real-time object detection with region proposal networks. In: Cortes C, Lawrence ND, Lee DD, Sugiyama M, Garnett R, eds. Advances in neural information processing systems 28. NY, USA: Curran Associates, Inc., 2015: 91–99.

30 Li C, Wang X, Liu W, Latecki LJ. Deep mitosis: mitosis detection via deep detection, verification and segmentation networks. *Medical image analysis* 2018; **45:** 121–33.

31 Niazi MKK, Tavolara TE, Arole V, et al. Automated T1 bladder risk stratification based on depth of lamina propria invasion from H&E tissue biopsies: a deep learning approach. *SPIE Medical Imaging* 2018; **10581:** 105810H1–H9.

32 Niazi MKK, Tavolara T, Arole V, Parwani A, Lee C, Gurcan M. MP58–06 automated staging of T1 bladder cancer using digital pathologic H&E images: a deep learning approach. *J Urol* 2018; **199:** e775.

33 Niazi MKK, Lin Y, Liu F, et al. Pathological image compression for big data image analysis: application to hotspot detection in breast cancer. *Artif Intell Med* 2018; published online Sep 25. DOI:10.1016/j.artmed.2018.09.002.

34 Niazi MKK, Tavolara TE, Arole V, Hartman DJ, Pantanowitz L, Gurcan MN. Identifying tumor in pancreatic neuroendocrine neoplasms from Ki67 images using transfer learning. *PloS One* 2018; **13:** e0195621.

35 Kornaropoulos EN, Niazi M, Lozanski G, Gurcan MN. Histopathological image analysis for centroblasts classification through dimensionality reduction approaches. *Cytometry A* 2014; **85:** 242–55.

36 Sertel O, Lozanski G, Shana'ah A, Gurcan MN. Computer-aided detection of centroblasts for follicular lymphoma grading using adaptive likelihood-based cell segmentation. *IEEE Trans Biomed Eng* 2010; **57:** 2613–16.

37 Szegedy C, Vanhoucke V, Ioffe S, Shlens J, Wojna Z, editors. Rethinking the inception architecture for computer vision. 2016. https://www.cv-foundation.org/openaccess/content_cvpr_2016/papers/Szegedy_Rethinking_the_Inception_CVPR_2016_paper.pdf (accessed Nov 19, 2018).

38 Coudray N, Ocampo PS, Sakellaropoulos T, et al. Classification and mutation prediction from non–small cell lung cancer histopathology images using deep learning. *Nature Medicine* 2018; **24:** 1559.

39 Tavolara T, Niazi MKK, Chen W, Frakel W, Gurcan MN. Colorectal tumor identification by tranferring knowledge from pan-cytokeratin to H&E. *SPIE Medical Imaging* 2019; **10956:** 1–9.

40 Mairal J, Ponce J, Sapiro G, Zisserman A, Bach FR. Supervised dictionary learning. Advances in neural information processing systems. 2009. https://papers.nips.cc/paper/3448-supervised-dictionary-learning (accessed Nov 1, 2018).

41 Niazi MKK, Hartman DJ, Pantanowitz L, Gurcan MN. Hotspot detection in pancreatic neuroendocrine tumors: density approximation by α-shape maps. *SPIE Medical Imaging* 2016; **9791:** 97910B1–B8.

42 LeCun Y, Bengio Y, Hinton G. Deep learning. *Nature* 2015; **521:** 436.

43 Albarqouni S, Baur C, Achilles F, Belagiannis V, Demirci S, Navab N. Aggnet: deep learning from crowds for mitosis detection in breast cancer histology images. *IEEE Trans Med Imaging* 2016; **35:** 1313–21.

44 Niazi MKK, Tavolara TE, Arole V, Parwani AV, Lee C, Gurcan MN. Automated T1 bladder risk stratification based on depth of lamina propria invasion from H&E tissue biopsies: a deep learning approach. *SPIE Medical Imaging* 2018; **10581:** 105810H1–H9.

45 Litjens G, Sánchez CI, Timofeeva N, et al. Deep learning as a tool for increased accuracy and efficiency of histopathological diagnosis. *Sci Rep* 2016; **6:** 26286.

46 Bejnordi BE, Zuidhof G, Balkenhol M, et al. Context-aware stacked convolutional neural networks for classification of breast carcinomas in whole-slide histopathology images. *J Med Imaging* 2017; **4:** 044504.

47 Bejnordi BE, Balkenhol M, Litjens G, et al. Automated detection of DCIS in whole-slide H&E stained breast histopathology images. *IEEE Trans Med Imaging* 2016; **35:** 2141–50.

48 Schaeffer SE. Graph clustering. *Comp Sci Rev* 2007; **1:** 27–64.

49 Cruz-Roa A, Gilmore H, Basavanhally A, et al. High-throughput adaptive sampling for whole-slide histopathology image analysis (HASHI) via convolutional neural networks: application to invasive breast cancer detection. *PloS One* 2018; **13:** e0196828.

50 Caflisch RE. Monte carlo and quasi-monte carlo methods. *Acta Numerica* 1998; **7:** 1–49.

51 Natrajan R, Sailem H, Mardakheh FK, et al. Microenvironmental heterogeneity parallels breast cancer progression: a histology–genomic integration analysis. *PLoS Med* 2016; **13:** e1001961.

52 Heindl A, Nawaz S, Yuan Y. Mapping spatial heterogeneity in the tumor microenvironment: a new era for digital pathology. *Lab Invest* 2015; **95:** 377.

53 Louis DN, Gerber GK, Baron JM, et al. Computational pathology: an emerging definition. *Arch Pathol Lab Med* 2014; **138:** 1133–38.

54 Zaha DC. Significance of immunohistochemistry in breast cancer. *World J Clin Oncol* 2014; **5:** 382.

55 Niazi MKK, Downs-Kelly E, Gurcan MN. Hot spot detection for breast cancer in Ki-67 stained slides: image dependent filtering approach. *SPIE Medical Imaging* 2014; **9041:** 9041061–68.

56 Das H, Wang Z, Niazi MKK, et al. Impact of diffusion barriers to small cytotoxic molecules on the efficacy of immunotherapy in breast cancer. *PloS One* 2013; **8:** e61398.

57 Sertel O, Dogdas B, Chiu CS, Gurcan MN. Muscle histology image analysis for sarcopenia: registration of successive sections with distinct atpase activity. Biomedical imaging: from nano to macro, 2010 IEEE International Symposium; Rotterdam, Netherlands; April 14–17, 2010.

58 Johnson S, Brandwein M, Doyle S. Registration parameter optimization for 3D tissue modeling from resected tumors cut into serial H&E slides. *SPIE Medical Imaging* 2018; **10581:** 105810T.

59 Yigitsoy M, Schmidt G. Hierarchical patch-based co-registration of differently stained histopathology slides. *SPIE Medical Imaging* 2017; **10140:** 1014009.

60 Chappelow J, Bloch BN, Rofsky N, et al. Elastic registration of multimodal prostate MRI and histology via multiattribute combined mutual information. *Medical Physics* 2011; **38:** 2005–18.
61 Intersoft Consulting. Automated individual decision-making, including profiling. https://gdpr-info.eu/ (April 15, 2019).
62 Niazi MKK, Chung JH, Heaton-Johnson KJ, et al. Advancing clinicopathologic diagnosis of high-risk neuroblastoma using computerized image analysis and proteomic profiling. *Pediatr Dev Pathol* 2017; **20:** 394–402.
63 Price I, Nicholson W. Artificial intelligence in health care: applications and legal implications. 2017. https://repository.law.umich.edu/cgi/viewcontent.cgi?article=2932&context=articles (accessed Nov 1, 2018).
64 Dignum V. Ethics in artificial intelligence: introduction to the special issue. *Ethics Inf Technol* 2018; **20:** 1–3.