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ADVANCES IN MOLECULAR PATHOLOGY

Artificial Intelligence, Bioinformatics, and Pathology

Emerging Trends Part II—Current Applications in Anatomic and Molecular Pathology

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KEY POINTS

- Artificial intelligence technologies provide useful diagnostic decision aids which reconcile complexities in anatomic and molecular pathology.
- AI algorithms integrate information from burgeoning high-dimensional -omics and imaging datatypes, resolving ambiguity from evolving guidelines, and reducing the burden associated with tedious tasks.
- Whole slide images are hyperdimensional images analyzed by smaller subarrays. Graph neural networks capture large-scale architectural arrangements while detection neural networks identify constituent cells.
- Multimodal approaches integrate complementary information from -omics and imaging modalities and demonstrate improved performance compared to studying one datatype alone.
- The utilization of spatial and single-cell transcriptomic technologies presents an exciting opportunity to resolve cellular heterogeneity which can inform future clinical assays.

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INTRODUCTION

Pathology is rife with tedious and repetitive tasks, many of which are critical to patient care. There is no clear line between diagnosis and management—any failure or misrepresentation of the information presented (or not presented) can lead to disease mismanagement. Successfully delivering accurate information can, in many cases, prove challenging considering: (1) evolving grading and staging guidelines, where ambiguous criteria can lead to variable findings [1,2], (2) being presented with an overwhelming amount of information (eg, cytologic assessment of hundreds of thousands of cells, whole exomic sequencing assays) [3], and (3) performing such assessments all of the time (ie, on a nonstop basis), which can place a significant load on the pathology workflow and strain laboratory resources [4,5]. In addition, with the advent of complex molecular assays that can assess millions to billions of additional clinical variables, elucidating the disease pathogenesis to make timely and reliable decisions can prove daunting. These factors, combined, highlight the need to develop strategies that integrate information from a wide variety of sources, including genomics, transcriptomics, proteomics, and imaging assays, to better facilitate rapid and accurate diagnostic testing and prognostication.

Artificial intelligence (AI) technologies may be able to reconcile many of these complexities and provide reliable diagnostic decision aids [6]. These technologies can sift through and gather insights from vast quantities of data to form a comprehensive and nuanced understanding of the disease pathology. In a related review article (see Part I), we discussed the emerging role of machine learning (ML) technologies, which excels at such tasks and can greatly aid the practicing pathologist by pre-analyzing a given case and presenting a summary to be accepted or rejected by the practitioner. Most of the information that pathologists work with is very complex and requires ample time and effort to provide a comprehensive pathologic assessment. As such, ML can improve the efficiency of these assessments by automating the processing and examination of this high-dimensional data. One example use case involves next-generation sequencing (NGS), which infers RNA and DNA to facilitate the process of detecting germline and somatic mutations as well as aberrant expression patterns. As many predictors (eg, genes and genomic sites) and their interactions can be assessed, only a fraction of these predictors are actually relevant for the assessment. As such, these technologies learn to identify informative patterns and crucial risk factors by being presented with many examples, similar to how a medical trainee may require ample practice and repetition to comfortably provide the differential. In particular, deep learning technologies have recently come to the forefront with the adoption of graphics-accelerated hardware and these technologies are well-equipped to handle the explosive increase in the amount of biomedical data generated and used [7]. These algorithms, inspired by processes of the central nervous system through the use of artificial neural networks (ANNs), are better equipped to extract insights from imaging and time-stamped data through derivation of imaging filters (ie, shapes) and through retaining a working memory of events across space and time (eg, Long Short Term Memory [LSTM]). Curation of large, annotated datasets has improved the diagnostic accuracy of these algorithms, which can help make a pathologic decision-making more timely and cost-effective.

Although ML has applications across many medical subspecialties, they are especially pertinent for pathologists as a significant majority of data at most hospitals are generated in pathology laboratories. This includes (1) gigapixel images containing billions of pixels and up to millions of cells, (2) whole-genome sequencing assays to assess millions of point mutations, and (3) serologic assays (eg, assessing sodium), among many others (a more complete listing can be found in the related review article, Part I). In addition, many of these billions of clinical variables may interact with each other in unintuitive ways (eg, the prognostic value of cell-type differs by whether the patient has mismatch repair deficiency) [8]. Sometimes the impact of some clinical variables may become vary in a nonlinear way (eg, saturation and subsequent reductions of antibody titer). These additional complexities can be accounted for through the use of ML algorithms. Details of how such algorithms are able to accomplish these feats can be found in the related review paper, see Part I. In the following section, we will provide a comprehensive overview of AI technologies in digital pathology and example use cases to illustrate the type and scope of challenges in the domain of Pathology. Here, we will review a few select applications in both anatomic and molecular pathology.

APPLICATIONS IN MOLECULAR AND ANATOMIC PATHOLOGY

Select Applications of Artificial Intelligence in Anatomic Pathology

Analysis of biopsies is a critical component in the diagnosis of disease. Many diagnoses depend not only on

the presence of certain cytoarchitectural features but on their location within the tissue. Recently, several promising deep-learning diagnostic aids have been developed to classify digitized biopsy slides. These diagnostic aids hold immense potential for autonomous diagnosis, real-time surgical margin control, improved diagnostic accuracy, and reduced resource use (personnel, time, and reagents) for academic and commercial laboratories. The performance of deep-learning diagnostic tools depends heavily on the training data, ideally containing a large and diverse body of biopsy whole slide images (WSIs).

As the WSIs of histologic slides are prohibitively large for many ML approaches, images are often broken into subimages (smaller segments of the main image that contains objects of interest, eg, tissue, cells) [9]. Oftentimes, ML algorithms are used to tabulate statistics across each subimage (eg, number of lymphocytes within tumor), which are themselves tabulated across the WSI to associate with known outcomes (eg, SOX10 positivity in the dermis and potential for Melanoma metastasis). Specifically, segmentation and detection neural networks predict the presence and locations of histologically important substructures (eg, lymphocytes, ganglia, cells, and blood vessels) (Fig. 1A) [10–12]. Other downstream processing methods exist; for instance, subimages (eg, detected cells) may be connected to adjacent cells to form a graph that can represent intercellular communication patterns (Fig. 1B) [13,14]. Unsupervised techniques can segment the tissue into different tissue architectures based on histomorphological differences, obviating the need for explicit annotation/curation (Fig. 1D).

An additional consideration for WSI is stain normalization. Every institution runs slightly different protocols for creating the standard pathology stain hematoxylin and eosin (H&E). As such the intensity and quality of the resulting slides can differ (sometimes significantly) between institutions. The type of image scanner and format of stored images can also differentially affect WSI appearance. These differences can affect the performance of ML algorithms, especially if the model was trained on images with significantly different staining/scanning characteristics. Several strategies have been developed to normalize characteristic stains to a common reference (ie, representing hematoxylin with a common purple hue) to eliminate sources of variation that can confound these approaches [15].

In the following sections, we provide a few select examples, of many, which show application of ML methods to WSI as well as written pathology reports.

Prostate cancer grading through inference of Gleason score

Automated systems are being developed, which can predict the Gleason score from prostate core needle biopsies containing adenocarcinoma [16–18]. The researchers performed a clinical validation study that used the model as a second check on the manual pathologic examination. The model used gradient-boosting classifiers to localize tissue on the slide image and convolutional neural networks (CNNs) to localize the tumor within these regions. This system accurately predicted the Gleason score and additionally identified one case of cancer that was originally not identified by the clinician.

Surgical pathology

The surgical removal of tumorous tissue can be made more efficient and accurate through the use of AI technologies and automation [19]. One example application is Mohs surgery, which uses 100% real-time margin analysis (through assessment of intraoperative peripheral margins) to achieve cancer recurrence rates of 1% to 2% for the removal of common and simple skin lesions, which is far better than the 20% cancer recurrence through assessment of standard "breadloaf" excisions postoperatively (where only 1% to 2% of the true margin is examined). With 100% real-time analysis, the chance of postoperatively discovering missed or frankly positive margins is greatly reduced [20–23]. In most surgical contexts extending beyond Mohs, intraoperative assessment requires rapid coordination between the histotechnologist who stains and prepares tissue samples, the pathologist analyzing the tissue, and the surgeon who removes tissue. Such thorough analyses are generally difficult due to the limited time clinicians have, whereas a patient is under anesthesia and the size and complexity of the tumors that need to be removed.

AI technologies have shown the ability to automate and accelerate aspects of the tissue preparation and analysis and thereby make 100% real-time tissue analysis a more viable approach to other, nondermatological cancer surgery [19,24,25]. For example, three-dimensional (3D) modeling can be used to measure the size of the tissue and provide inking recommendations to the histotechnologist. Graph neural networks (GNNs) are a type of neural network that is especially helpful for summarizing findings across a whole WSI that can guide pathologic analysis by locating the tumor and detailing its relationship to the tissue margins (Fig. 1C). Finally, ML algorithms are

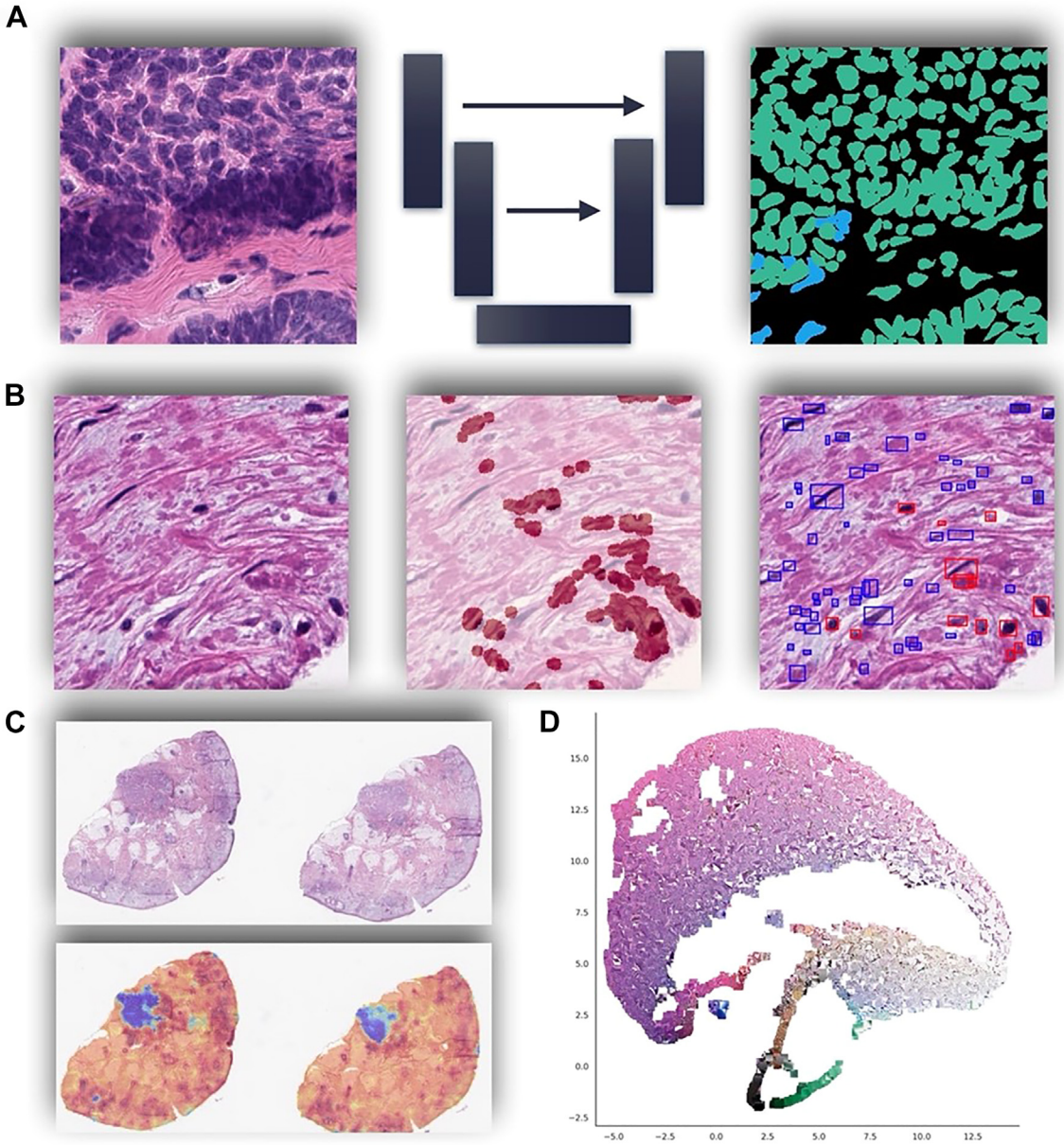

FIG. 1 Overview of AI technologies for anatomic pathology: (**A**) segmentation of nuclei in skin using a U-Net approach; (**B**) detection of immune cells within colon biopsies using detection neural networks and graph neural networks; (**C**) classification of distinct histologic structures within tissue slides (eg, tumor in skin specimen); and (**D**) unsupervised/self-supervised techniques cluster tissue with similar histomorphology.

used to "warp" the histologic findings back to the surgical tumor map in the correct anatomic position/orientation to facilitate the rapid removal of the tumor.

Applications in nuclei detection

Determining precise cytoplasmic/nuclei borders and the presence of abnormalities in nuclei is critical for the assessment of cytologic atypia in biopsy/cytology

slides. However, the heterogeneity of cytology slides and nuclei clumping (eg, overlapping nuclei borders) can make cytologic assessment difficult for even well-trained pathologists. Many attempts have been made to use ML algorithms to detect and segment nuclei in pathology slides [26–29]. The central challenge in nuclei detection lies in determining the shape as well as the spatial boundaries of overlapping nuclei/cytoplasmic borders. Although simpler approaches such as image thresholding (ie, defining a range of colors corresponding to nuclei) and clustering have been used with varying success, these approaches lack adaptability and generally underperform when presented with overlapping nuclei [30–32]. CNN-based algorithms that detect the pixel-wise presence of nuclei and cytoplasm (dubbed *segmentation* and *detection* algorithms) have emerged as a more successful method for segmenting overlapping and densely clustered nuclei than relatively simpler or purely statistical methods [33–36]. To determine locations of overlapping and unevenly stained nuclei, these neural networks are trained in a wide variety of imaging contexts. Nuclei detection in many cases can be achieved with significant accuracy, with scores ranging from 84% to 95.3% from some of the previously cited studies. Unsupervised learning techniques, such as using a Laplacian filter which studies structural imaging components [37], have also shown remarkable success, resulting in 98% accuracy in the cited study. However, these approaches are generally less time efficient and more challenging to implement.

Nuclei segmentation and classification have emerged as a rapidly growing application that could prove useful to clinicians and researchers for translational and basic science efforts including [32–39]: the automated scoring of immunohistochemistry (eg, ki67), quantitation of tumor purity in NGS specimens, and counting cells in a given region of tissue. Classification of specific nuclear features (eg, hyperchromasia) can aid in detection of distinct cellular populations for downstream metrics that rely on comparing the presence of specific cell populations in distinct macroarchitectures (eg, tumor-infiltrating lymphocytes) [40–43].

Applications in cytopathology

Several interesting applications of AI have emerged for assessing cytology specimens. Cytologic specimens are distinct from whole tissue-based specimens in that they are typically a random dispersion of cellular elements without architecture (Fig. 2B). Thus, the whole slide must be examined because a cell of interest can appear anywhere on the slide. This means that cytologic analysis is particularly tedious and prone to human weaknesses such as boredom, fatigue, and distraction. To address these issues, most cytologic diagnoses are made within the framework of consensus-derived scoring systems that aim to make the analysis process more rigorous and reproducible while still rendering a clinically useful diagnosis.

For instance, the Paris system for urine cytology is a semiquantitative scoring system for bladder cancer screening for urine cytology specimens. As these scoring systems evolve, they have become progressively simpler and more quantitative in nature. ML tools are being developed to more reliably quantitate features of a cytology slide, which are often too cumbersome to assess in their entirety, even for experienced cytotechnologists. Autoparis is an example of an AI-tool that can direct the cytopathologist's attention to relevant and potentially malignant urothelial cells to complement the traditional assessment method [35,44]. Such systems complement and may even inform the development of newer quantitative guidelines for cytopathology.

Cervical cancer screening (via Pap smear) is another area that can benefit from implementing AI and deep learning in cytopathology. Cervical cancer is the fourth most common and lethal cancer in women worldwide. In developing countries, it is the second most common cancer in women and the third cause of cancer-related death [45]. Several molecular events and cofactors trigger cervical cancer development; however, infection with high-risk human papillomavirus (HPV) is essential in transforming cervical epithelial cells [46]. Stratified squamous epithelial cells are particularly susceptible to infection, which leads to progressive dysplasia and eventually cancerous cells over a period of 10 to 20 years [47]. Cervical cancer gradually progresses from mild cervical intraepithelial neoplasia (CIN1) to severe neoplasia (CIN2) and microinvasive lesions (CN3). The natural slow progression of cervical cancer makes screening the first line of defense against the development of this malignancy.

The Papanicolaou-stained (Pap) smear is a screening tool that looks for changes in squamous cells in the cervix. The Pap smear has effectively reduced the incidence, morbidity, and mortality from cervical cancer in developed countries. However, cervical cancer screening has not had the same impact in developing countries [48]. Several factors contribute to these differences; for example, developed countries have organized screening programs that provide regular and frequent access to Pap smears [49]. Conversely, people in developing nations may have access to screening once or twice in their lifetime [48]. However, the frequency and regularity of

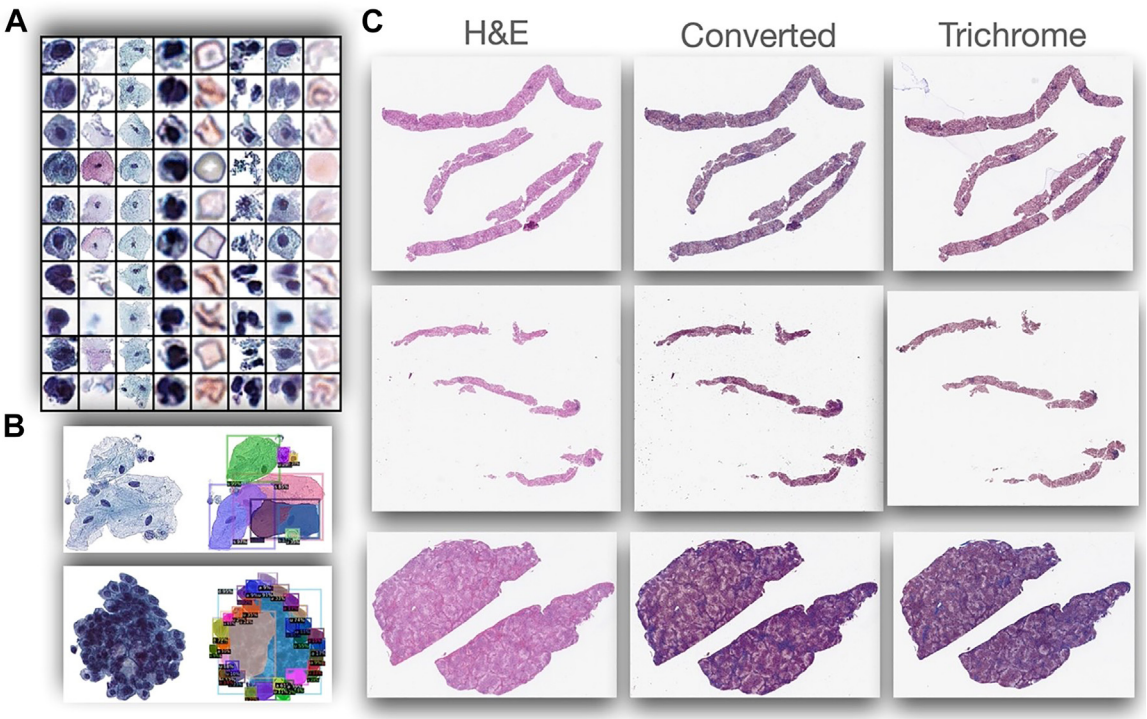

FIG. 2 Additional applications of AI in anatomic pathology: (**A**) generative adversarial network simulates and sorts various types of urothelial cells; (**B**) detection algorithms deployed across many cytopathology applications to isolate specific cells with overlapping cytoplasmic borders; and (**C**) virtual staining techniques digitally convert H&E-stained tissue into trichrome-stained tissue.

screening are critical as Pap tests can miss 50% of high-grade precancerous and cancerous lesions in a single screening [50]. Other factors include limited access to trained professionals and technology (eg, liquid-based cytology [LBC] preparation instruments).

Pap smear processing, screening, and follow-up require highly specialized professionals such as cytotechnologists, cytopathologists, colposcopy specialists, and pathologists. Developing nations often do not have broad access to these trained professionals, significantly decreasing Pap smears availability. Furthermore, developed countries have improved screening by implementing better cytology techniques [48]. LBC is a newer way of preparing samples for cytology analysis. It consists of making a cell suspension from the sample to produce a cellular monolayer in a restricted zone on the slide [51](whereas traditional pap smears cover most of a slide surface in a layer of variable thickness, which effects analysis). LBC not only allows for faster and more accurate Pap smear results but also testing for HPV using the same Pap test sample [48]. Unfortunately, LBC implementation/instrumentation is complex and expensive; thus, developing countries continue to use conventional methods that provide low-quality cytology smears and do not allow for simultaneous testing for HPV [52] with a single swab. HPV testing is crucial as it has increased sensitivity for the detection of CIN2 compared with cytology and has a high negative predictive value, which leads to longer intervals between required screenings [53].

Therefore, developing countries are disproportionately affected by cervical cancer due to a lack of resources and technology. Nonetheless, the implementation of deep learning in cytopathology has the potential to bridge the gap by allowing developing countries to implement effective and organized screening programs with less resource utilization. Currently, ThinPrep Imaging System and the FocalPoint GS Imaging System are two cytology algorithms that allow for semi-automated screening of Pap smears. Specifically, they apply proprietary ML algorithms to screen every cell on a given slide and define fields of view

(FOV) that contain the most atypical cells [54]. A cytotechnologist reviews these FOV and will either directly verify benign cases or triage the case to a cytopathologist for final diagnosis. These cytology algorithms increase accuracy and decrease processing time, thereby allowing cytotechnologists to screen more slides per day while remaining below legally proscribed daily screening limits. In developed countries, this technology has allowed cytology departments to remain solvent while being asked to screen more slide with less resources. Implementing similar technology in developing countries would be hugely beneficial for obvious reasons. Unfortunately, ThinPrep and Focal Point both require LBC, special reagents, and complex scanning/screening instruments that are costly and require regular maintenance from expert technicians.

Consequently, there is an immense need for cytology algorithms capable of analyzing traditional Pap smears. By reducing resource utilization, this technology could potentially increase the frequency, and regularity of Pap smears in countries that do not currently offer organized screening programs. There are many creative ways such a system could be deployed. For example, mobile versions of these algorithms could be used in self-screening scenarios or drop-off clinics.

Improving HPV screening is also extremely important as it has been shown that a single round of HPV screening could reduce the incidence and mortality of cervical cancer by approximately 50% [55]. However, given that HPV screening is a molecular test, there is a significant associated cost. One could envision AI algorithms being trained to infer the presence of HPV infection from Pap smear images and using this information to triage high-risk patients for molecular HPV testing.

Virtual staining and generative adversarial networks in digital pathology

Stained human tissue samples are the basis of histologic analysis and are a gold standard for assessing several diseases by highlighting specific nuclear, cytoplasmic, or microarchitectural features relevant for diagnosis/prognosis (eg, invasion, fibrosis, and edema). Visual analysis of histology requires the physical application of chemical stains. The backbone of pathology is built upon centuries of cultural experience interpreting these stained slides in the context of various disease states [56].

The routine stain ––H&E––is the most common histologic stain applied to almost all clinical cases. Dozens of other specialized histologic stains with varying properties (ie, special stains) are used by pathologists to highlight various additional tissue features. For instance, connective tissue can be viewed using the Masson's trichrome (MT) [57] stain and the glomerular architecture can be visualized using the Jones Methenamine Silver (JMS) stain [58]. These traditional stain workflows are time-intensive and require the sufficient laboratory infrastructure and trained personnel. H&E staining is typically performed by an automated mechanized process in bulk, whereas obtaining special stains may require significant effort and time and running small batches or even single slides. This is inefficient and time-consuming compared with H&E staining. Moreover, it is cumbersome to perform these steps while under pressure to obtain an urgent diagnosis or assessment (eg, as is done for standard excisions for melanoma intraoperatively). Recently, AI methods have been developed which take as input a WSI of an H&E-stained slide and output a transformed image that predicts what a wholly different chemical stain would look like. These algorithms learn from pairs of slides wherein the H&E slide is scanned, destained, restained with another chemical agent, scanned, and compared with the original image. The algorithm thus learns which features of H&E slides predict the results of other stains. Deep learning methods have also been developed which perform the same task on unstained tissue sections by using hyperspectral imaging [59], autofluorescence [60,61], and quantitative phase imaging [62]. However, this requires specialized equipment not present in even very large academic pathology departments.

Generative modeling techniques have proven beneficial in this domain. The core technology for these research applications is called generative adversarial networks (GANs) [63], which is a special type of deep learning architecture that has revolutionized algorithms for several applications, including image enhancement [64–68], natural language processing (NLP) [69–71], object detection [72–74], and medical image analysis [75–78]. GANs are composed of two neural networks: a generator and a discriminator. The GAN aims to train the generator $G(x)$ to produce images such that the discriminator $D(y)$ cannot distinguish between those images and the actual data (Fig. 2A; Fig. 3A). The generator influences the discriminator to believe that the produced images are accurate; thus, the discriminators are provided with the real data to give feedback to the generator so that the produced images can be enhanced, and both networks aim to improve the other's robustness. Conditional GANs (Fig. 3B) aim to improve GANs by conditioning both the networks on auxiliary

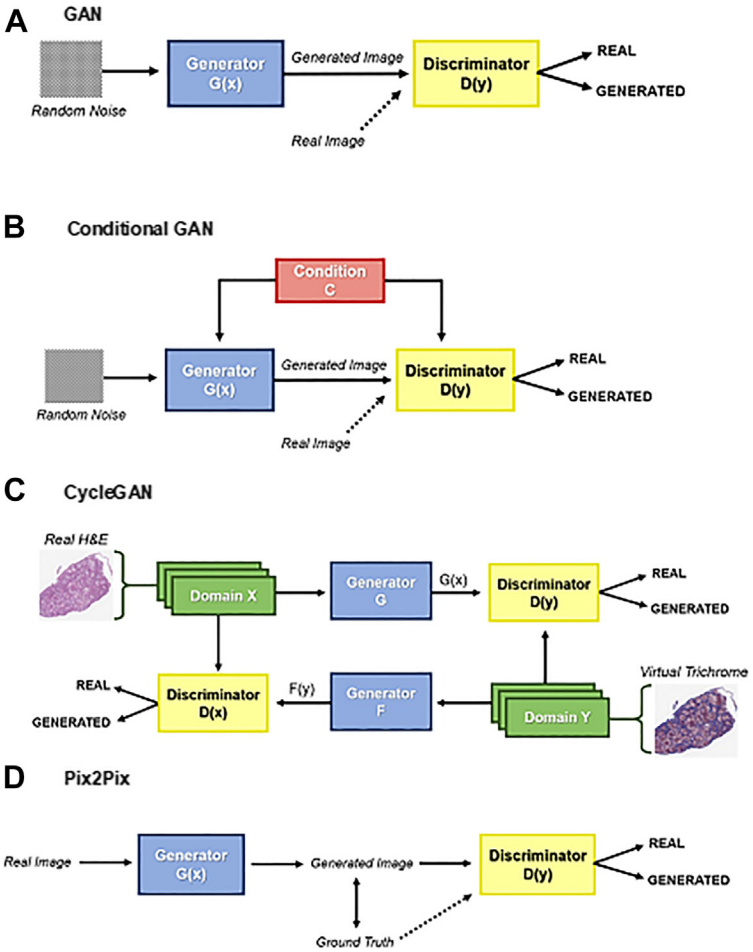

FIG. 3 Depiction of several types of generative adversarial networks: (**A**) generative adversarial network (GAN); (**B**) conditional GAN; (**C**) CycleGAN; and (**D**) Pix2Pix.

information (eg, the type of tissue, eg, tumor vs portal region) to direct the data generation process [79].

GANs have enabled significant improvements to digital pathology workflows. Specifically, GANs have been used for critical histopathological image processing applications, including color normalization [80–82], data augmentation [83–85], and image enhancement [86–88]. For instance, a CycleGAN model [89] was deployed for converting H&E stain to trichome stain for the staging of liver fibrosis (Fig. 2C) [90]. Moreover, Pix2Pix and CycleGAN models (Fig. 3C, D) were used to convert H&E stain to SOX10 IHC stain [31]. After some algorithmic fine-tuning, the CycleGAN-based conversion of H&E stain to trichome stain was used to stage nonalcoholic steatohepatitis (NASH) [91] for fibrosis in a large-scale observational study. GANs have also been used to transform wide-field autofluorescence images of unlabeled tissue sections into histologically stained [60] bright-field images. An improved stain-to-stain transformation network [92] based on CycleGAN has also been proposed to efficiently transform H&E stains to the special stains––periodic acid-Schiff, MT, and Jones silver stain using kidney needle core biopsy tissue sections. Virtual staining has clear benefits in terms of cost and speed but more investigation is necessary to determine what applications are appropriate in the clinical context.

More recent, yet underrecognized, applications of GANs include calculating nuclear to cytoplasm ratios of cells in urine cytology and generating nucleus masks

in immunohistochemically stained WSIs [90,93]. Effective preprocessing of the histopathological WSIs is imperative to enhance the performance of digital pathology algorithms. ML algorithms can be very sensitive to changes in input data. Unprocessed WSI from multiple institutions on multiple different scanners can therefore effect algorithm performance in unpredictable and often detrimental ways. Many techniques have therefore been developed to account for these differences and attempt to normalize input images to a shared, idealized color-space. For example, a conditional GAN with a stain-style transfer approach was used for color normalization of lymph node samples [94]. Similar techniques have used to standardize digital tissue staining properties for colon adenocarcinoma tissue, breast histology, and ovarian carcinoma images [81]. Self-attentive (ie, a neural network that explicitly models relevant parts of an image) adversarial networks have also been leveraged for color normalization in duodenal biopsy samples [82].

Concerns with privacy and cost have made the acquisition of large-scale pathologic datasets difficult at best and intractable at worst. Thus, efficient data augmentation strategies are immensely helpful and in some cases absolutely necessary. Combining GAN-generated synthetic images with the training set has been shown to improve classification performance for digital pathology applications [95]. For instance, to generate cervical histopathology images [84], a conditional GAN with a specific filtering mechanism that controlled the feature quality of the synthetic images was implemented. Separately, a CycleGAN [27] model was used to localize nuclei through the generation of synthetic H&E patches from different tissue types.

AI image enhancement techniques have also been developed to improve the quality of contrast, color, and brightness of input images. Noise elimination from breast histopathological images has been performed using Super-Resolution GANs (SRGANs) [87]. A CycleGAN-based approach was implemented to remove ink marks from human melanoma tissues while preserving the tissue structure [88]. The generated clean WSIs (ie, after ink marks were removed) were indistinguishable from the original clean WSIs (ie, before ink marks were applied; where the presence of ink could confound image analysis techniques by indicating the tumor's location, etc.). GANs can also remove natural (not just artificial) discolorations of H&E-stained histologic tissues [90].

Indeed, GANs play a crucial, multi-faceted role in digital pathology.

Graph neural networks in digital pathology

The traditional CNNs use convolutional kernels to extract and relate microarchitectural tissue features, yet are often challenged in their ability to capture larger tissue architectures, thus limiting their performance in capturing global contextual information [96]. Graph CNNs have been designed to encode global relational information (ie, macroarchitecture).

A graph is represented as G = (V, E, and W), such that V is the set of vertices with n nodes, E is the set of edges connecting those n nodes, and W is the weighted adjacency matrix that assigns importance to the connections between nodes. Graph representations are used in digital pathology by describing a histology image as an entity graph, denoting the biological entities (ie, image subarrays, cells) as nodes, and assigning the inter-entity interactions as the edges.

Several efforts toward leveraging graph deep learning in digital pathology have been made. graph convolutional networks (GCNs) have been used to classify WSIs [97] such that a graph depicts the spatial adjacency between its constituent cells, where local image descriptors were used as vertices (eg, nuclear morphology), and the gland formation features were incorporated as edges. Similar approaches that model histology tissue as graphs use mathematical techniques known as *attention* to dynamically weight important information across a slide [98]. In this specific application, clusters of nuclei with a low *attention* score (ie, deemed not importance) were pruned for the model to capture more relevant information. A hierarchical GNN, which operates on tissue features at multiple levels––microarchitectural (ie, cells) to macroarchitectural (ie, whole tissue regions), was used to incorporate morphology and tissue topology to map recurring tissue structures to relevant tissue subtypes [99]. This method yielded better performance and aggregated classification than CNNs, whose pixel-based processing does not explicitly capture histologically meaningful structures (eg, cells, glands, and tissue types) [99]. More broadly, similar methods (eg, hierarchical cell-to-tissue [HACT]) have been used to better quantify the relationship between tissue representation and tissue functionality [99]. A GCN was also applied to histology-based cancer survival outcome predictions [100]. For heterogeneous cancers, such as glioma, augmenting CNNs with GCNs in Pathomic Fusion (an integrated framework that combines histopathological data with genomic data) is more clinically relevant than CNNs alone in delineating survival curves of lower stage (ie, less aggressive) tumors [100]. These GCNs bridged the gap

between genomic (eg, copy number variation (CNV), mutations, translocations, RNA-seq) and histopathological (eg, tumor microenvironment) data in the prognostic staging of cancer. Significantly, these GCNs may be used either *in addition to* or *in conjunction with* CNNs when extracting morphologic features and multimodal fusion.

It has been shown that GCNs are more effective than conventional patch-based CNNs for detecting malignancy and invasiveness in H&E-stained breast histology WSIs [97]. The advantage of GCNs (compared with CNNs) is that GCNs do not require a discrete Cartesian grid to facilitate geometric deep learning [97]. GCNs have been used to address challenging clinical endeavors, such as the prediction of lymph node metastasis in colorectal cancer [97]. In this study, GCNs were used in bag level representation and classification of histopathological WSIs, which showed superior performance to other state-of-the-art methods [97]. The graph attention multi-instance [101] learning framework considers spatial relationships to predict colorectal tumor node metastasis staging and separately, a GCN framework was proposed to [13] determine local tumor invasiveness from histology images.

The intuitive interpretability of deep learning predictions is essential to assess the reliability of the deployed algorithms and to increase the adoption of these methods by clinicians. Graph representations are effective not only for diagnostic predictions but also for providing improved explanations. For instance, such a framework [102] was introduced for breast-cancer subtyping. In this context, GNNs function as conceptual analogues of 2D convolution and (unlike CNNs) acknowledge inherent relationships contained in these slides. Reinforcement learning based XGNN [103] was also proposed to provide model-level explanations. Furthermore, PGExplainer [104] was implemented and incorporates a generative probabilistic model to explain each instance with a global view of the GNN model.

Undoubtedly, GNNs will play an instrumental role in the development of nascent image analysis techniques for digital pathology [96].

Natural language processing for pathology notes

NLP has also received significant attention from researchers. Although patient information is often structured (eg, one value per column/clinical variable) within electronic health records (EHR) systems, clinical text is unstructured and sometimes cannot conform to these reporting systems. However, this unstructured information can provide insight into the patient's clinical narrative to elucidate less studied risk factors as well as areas of operational inefficiency [105–107].

Common tasks include: (1) disease subtype prediction tasks (classification) [108–110], (2) health report auto-completion tasks (text generation) [111], (3) extracting synoptic medical reporting information through named entity recognition (NER) [112–115], etc. Modern deep learning technologies do not require substantial training on a domain-specific corpuses, thereby limiting the number of pathology reports required for training purposes. This is because these models contain large information registries that have been exposed to vast quantities of textual information. For example, researchers in tangentially related fields (eg, NLP at Google) have trained massive language models that are freely available for *transfer learning* (ie, initializing a model based on information from one domain to yield significant performance improvements in another domain; eg, clinical discharge notes to pathology reports) [116,117]. Although the text these models learned on may be nonmedical in nature (eg, extraction of patterns from Wikipedia), they are highly adaptable to the medical domain due to the shared syntactic patterns common to any language. In addition, many models which have been explicitly trained on medical text are freely available online. "Fine-tuning" (ie, updating parameters on domain-specific knowledge) these models can reduce time and computational resource expenditures necessary to train a more specific model on local medical text [118,119]. The language-comprehension capabilities of these models are already primed with parameters initialized through training on large external text corpuses. Using these parameters, it is sometimes possible to achieve optimal performance on specialized domain texts and tasks, even if no further adjustments are made. This performance can be further increased if the model is retrained and fine-tuned on text examples specific to the task at hand. However, not all pre-trained text models will achieve optimal or even acceptable performance (even with retraining on specific text) and better performance can be achieved based on alignment to the target domain (eg, they are all medical-related data, which contain similar medical terminology).

Limitations of deep learning models for pathology reports include the challenges associated with labeling clinical text. In part, this is related to the specialized nature of medical text, which require domain expertise that are in high demand. These reports are often filled with nuanced medical jargon, requiring professional interpretation. As expert time is fairly expensive as

compared with annotation for other standard text tasks, it is difficult to annotate medical texts [120].

Pathology reports contain text material relevant to patients' diagnosis, prognosis, and medical treatment processing, and can also serve as an auditable trial of a patient's clinical pathway. Textual patterns have been found to influence clinical endpoints and biomarker information using NLP pipelines, which include rule-based or machine-learning analytics. For example, given that deep learning methods have risen to the forefront of NLP, there have been few comparisons between their performance and that of other machine-learning methods in extracting key information for the prediction of medical procedural information. Furthermore, the value of aggregating and sorting data from numerous report subfields versus solely using the diagnostic field for predicting Current Procedural Terminology (CPT) codes, which are also called CPT codes, and signing pathologists is under investigation [121]. Standardizing reports can improve hospital revenue by better identifying instances of misbilling. The authors of one approach for CPT code identification used advanced topic modeling to identify broad topics from pathology reports. Leveraging both the diagnostic text alone and text from all subfields, this work compared the effectiveness of the XGBoost, SVM, and BERT (Bidirectional Encoder Representation from Transformers) techniques for the prediction of primary CPT codes (eg, complexity codes) as well as 38 ancillary CPT codes. They used similar methods to characterize text from a group of 20 pathologists who signed the most pathology reports as means to identify nonstandard text lexicon. Finally, they used model explanation techniques to find relevant report subcomponents.

Other pertinent applications of ML to EHR data include prospective identification of various malignancies from time-stamped EHR information (eg, risk of Pancreatic cancer from prior diagnostic codes, lab measurements, and clinical characteristics) [122]

Select Applications of Artificial Intelligence in Molecular Pathology

Emerging applications have been applied to numerous biological phenomena, each playing crucial roles in co-regulation and governing crucial biological pathways (Fig. 4) [123]. In the following sections, we provide high-level overviews of a few emerging applications:

Genomic polymorphisms

Inferring the presence of single-nucleotide polymorphisms/variants (SNP) and disease risk attributed to potential subsequent genomic dysregulation was once accomplished through targeted micro-array gene panels, though has transitioned to whole genome/exomic sequencing technologies (see Fig. 4B). Deep neural networks have proven incredibly adept at calling SNPs [124,125]. Once detected, variants may act in concert with one another to modify the risk of disease for prognostically important subsets of patients. ML techniques (eg, elastic net, random forest, XGBoost, multifactor dimensionality reduction) are tools that can be used to mine important survival-associated *epistatic* interactions (eg, gene-gene, SNP–SNP), which are traditionally nearly impossible to curate [126–128].

DNA methylation

DNA methylation (DNAm) represents an important chemical modification to the DNA which is heavily implicated in transcriptional repression/activation without alteration to the DNA itself. As an upstream epigenetic regulator of transcription (see Fig. 4B), DNAm alterations are intimately tied to cellular processes governing differentiation, aging, and pathogenesis and is commonly used as a marker to study environmental impacts on healthy development [129–132]. Microarray technologies are commonly used to accurately measure the proportion of methylated alleles at specific sites in the genome, though whole genome sequencing technologies do exist [133]. Although traditional analysis approaches, epigenome wide association studies, measure differential DNAm between groups, ML models are well-equipped to capture residual heterogeneity. For instance, one population application of ML models for DNAm is to infer subject age through development of a biological clock [134]. The residual between the subjects predicted ("biological") age and chronologic age is known as age acceleration and has been the subject of attempts to identify modifiable disease risk factors for prevention/intervention efforts or targeted therapies to slow or reverse aging. Of relevance to Pathology, ML models have proven incredibly adept at inferring presence of disease survival/recurrence, concurrent metastasis, diagnostically/prognostically relevant brain cancer subtypes, among many other applications [135–139]. As often phenotypic differences may be driven by constituent cellular proportions (eg, neutrophil to lymphocyte ratio), ML models that consider this heterogeneity are well positioned to uncover and interpret novel tumor biology [140–142]. Distilling findings into lower cost assays present opportunities to realize the translational potential of these technologies by spurring widespread adoption.

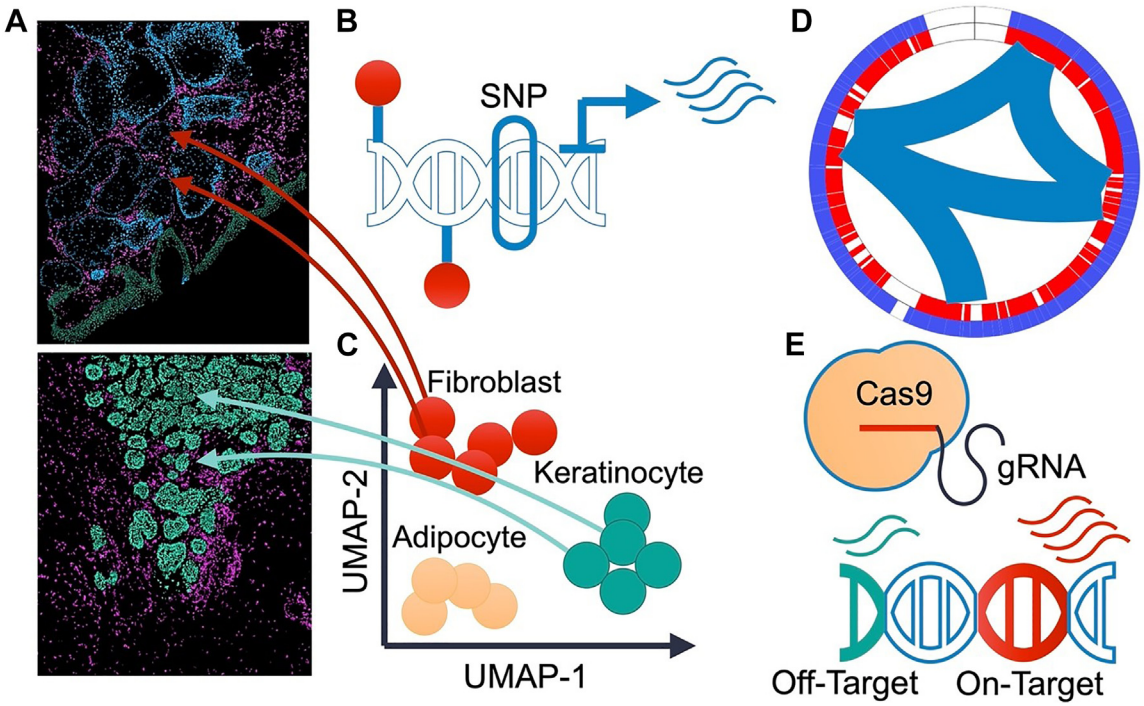

FIG. 4 Applications of AI in molecular pathology: (**A**) molecular information is assayed from tissue specimens and is profiled using techniques that report bulk expression or at the single-cell/spatial resolution; (**B**) many machine-learning techniques aim to estimate the impact of DNA methylation (*red*), single-nucleotide polymorphisms, and other genetic/epigenetic marks on expression and health/disease; (**C**) single-cell mRNA profiles delineate molecular alterations in distinct cellular populations within the specimen are mapped back to the specimen via paired spatial omics information; (**D**) machine-learning methods can characterize genetic diversity within/between specimen based on DNA sequences; and (**E**) machine-learning technologies can guide the selection of guides (*red*) to avoid the potential impact of off-target effects (*blue*).

RNASeq

The ability to quantify the abundance of RNA transcripts for tens of thousands of protein coding and nonprotein coding genes have led to the discovery of vitally important diagnostic and prognostic molecular markers, yet interrelationship and co-regulation in molecular pathways has not been well explored. ML models implicitly capture these complexities and may prove useful in exploring unknown disease biology. As an example, early ML applications attempted to classify molecular profiles corresponding to scleroderma patients into intrinsic subtypes who would respond well to therapy [143–145]. Classifiers trained on mRNA data have also been used to identify patients with various viral conditions, including tuberculosis (TB) patients, human immunodeficiency virus (HIV) and coronavirus disease-2019 (COVID-19) [146–150]. Hierarchical clustering is commonly used to partition molecular profiles and genes by their similarities and differences through generation of a tree or dendrogram (where subjects on similar branches have similar expression patterns) [151]. It is not uncommon to see such diagrams along with heatmaps denoting gene expression across many patients/genes in many works that leverage such data. Network-based approaches such as Weighted Gene Correlation Network Analysis identify groups or modules of genes related to specific phenotypes [152]. Micro-RNAs and long noncoding RNAs are profiled similarly and serve as epigenetic regulators of mRNA expression. ATAC-Seq profiling establishes chromatin accessibility of specific genes for transcription. Pairing this information with other epigenetic regulators (eg, inferred through paired Hi-C, CHIP-Seq, ATAC-Seq data) builds a more informative model of coregulation [153–157].

Machine-learning approaches for single-cell and spatially resolved omics

Most RNASeq studies profile bulk cell aggregates. Such information is non-specific to the slide location and cell type. Thus, emerging molecular profiling methods can disaggregate tissue slides into constituent cell populations and their locations [158,159]

Although methods such as immunohistochemistry covered in the previous sections can localize specific cell populations, such methods are not highly multiplexed and may miss important disease-associated markers of interest. Emerging technologies for spatial analysis include the Nanostring GeoMX Digital Spatial Profiler and the 10x Genomics Spatial Transcriptomics platform [158,160,161]. Both platforms currently offer or will soon offer both joint proteomic and whole transcriptome profiling ($\sim$18,000 genes). The protein assays are more targeted and allow for the assessment of up to approximately one hundred proteins. ML methods for spatially localized omics information can make similar inferences as bulk RNA expression, though can localize such inferences to distinct pockets within the tissue slide (see Fig. 4A; Fig. 5) (eg, comparing markers of metastasis within the tumor and at the tumor immune interface at the primary site). One study applied such methods to high-dimensional omics data, accounting for molecular variation commonly attributed to patient and batch. As ML models can more effectively combine information from disparate modalities, this model was able to report markers of metastasis that varies across different demographic factors (eg, effect of CD20 by age) [159]. Other applications include cell-type deconvolution, inference of spatially variable genes and inference of cellular interactions [162,163], among others.

Single cell methods enable comprehensive molecular characterization of alterations in relevant pathologic cellular subsets [14]. As whole transcriptomic signals are related to individual cells, the data is highly sparse and, in some cases, uninformative. Autoencoding neural networks and dimensionality reduction techniques can help visualize relationships and developmental trajectories for these cells (see Fig. 4C) [164]. Several methods have been developed which are able to project or infer the location of the single-cell RNA profiles across tissue slides to improve the resolution of the spatial information while further uncovering the pathogenesis of various diseases based on spatial migration of specific cell types [165–167]. Lately, single cell RNAseq information has been combined with high-speed imaging of the same cell to depict morphologic underpinnings and to develop better means to flow sort cells for sample purification [168]. ML algorithms leveraged for joint single cell RNA-Seq, ATAC-Seq and CRISPR profiling will enable unprecedented characterization of novel gene target perturbations [169].

Characterizing genetic diversity within species

Given a metagenomic sample—potentially containing thousands of individual genomes—separating genetic information by species is a challenging task [170,171]. Binning is the process of partitioning genetic information based on the species assignment and provides insights into microbial communities, sometimes leading to the discovery of novel genomes [172]. Methods of binning can be broadly classified as supervised or unsupervised, based on their utilization of known or unknown genetic information (see Fig. 4D) [170,173–175].

In supervised binning methods, reads or contigs are aligned to reference genomes. Alternatively, detection of marker genes in reads or contigs can be used to classify genetic fragments. In general, most of the reads and contigs from a metagenome do not contain marker genes; thus, the marker gene method often leaves most of the metagenomic fragments unassigned [176,177]. Because supervised binning methods rely on matching genetic fragments to labeled genetic material, supervised methods are unable to bin previously unsequenced genomic sequences.

Unsupervised binning methods generally first group reads/contigs/scaffolds using various techniques based on a set of extractable genomic features. For example, the frequencies of tetranucleotides and GC-content (along with other metrics, eg, presence of specific k-nucleotide long oligonucleotides, ie, kmers) can be used to group together contigs/scaffolds. Read coverage provides another method for grouping together contigs–contigs from the same genome may have similar sequencing depth. When contig coverage is tracked across multiple samples, this metric becomes even better for grouping genetic material (differential coverage binning––comparing contig frequency across multiple samples) [178]. Depending on the complexity of a sample, it can be challenging to classify genetic material into species; in some binning methods, contigs are sorted into broader taxonomic groups (in some cases based on predicted gene expression). Unsupervised binning methods lessen the risk of false positives—if constructed genetic scaffolds correspond to a known species genome, there is a high likelihood that species is present in the sample. Unsupervised binning methods generally require high coverage on each

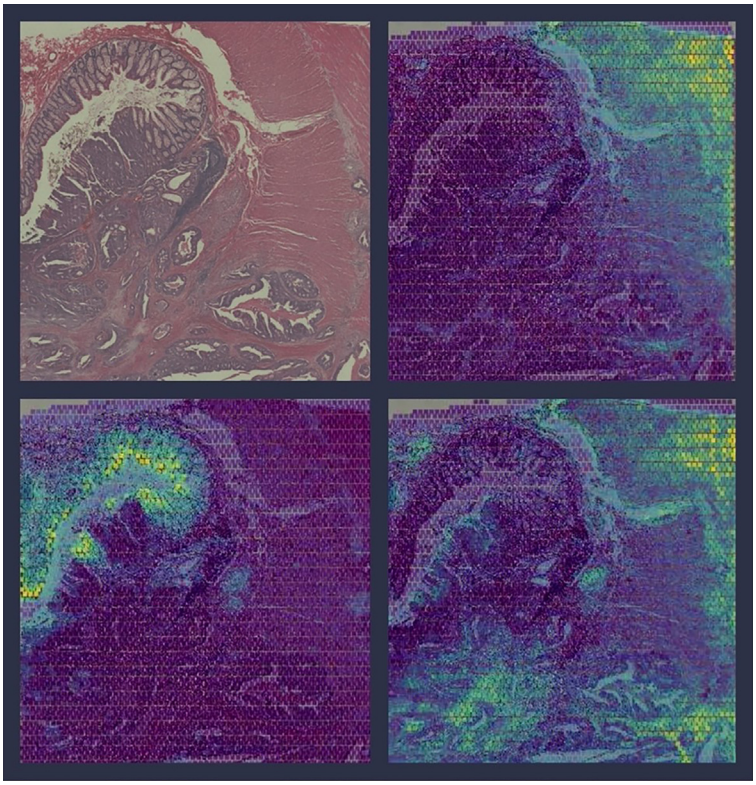

FIG. 5 Spatial whole transcriptomic expression data collected on colon specimen.

organism for detection and are often computationally expensive. Example computational methods/software for taxonomic assignment have been included in the appendix (Appendix, section "Taxonomic Binning Software," Supplementary Table 1) [176,179–187].

Characterizing microbiome diversity between patients

Determining the species composition of microbiome and metagenomic samples are highly relevant for identifying novel factors of disease pathogenesis. For instance, measures of biodiversity are key to distinguish prognostically important subcommunities, or in the context of a study that may arise from a set of individuals (eg, gut microbiome and its relation to health; infant microbiome shared with mother and impacted by environmental contaminants; obesity, inflammation and the microbiome). In particular, beta diversity (also read as β-diversity) measures the difference of species composition between two samples/communities [188], and thus is a staple tool in many microbiome studies by identifying major components of taxonomic variation as a clinically actionable biomarker.

A popular method of delineating beta diversity lies in Principal Coordinate Analysis (PCoA) plots. PCoA plots represents the taxonomic distances between samples on a 2D scatterplot to visually represent similarities or differences/distances between samples (ie, the degree to which two patients share the same set of microbiota via species counts, etc.) after application of Multidimensional Scaling (MDS) (Fig. 6). Common dissimilarity metrics include Bray-Curtis or UniFrac distances [189]. If samples are distinctly different, they may be clustered into clinically relevant subgroups, as shown in the example figure below. In the supplementary materials (Appendix, section "PcoA Software"), we have included a section on software for generating PcoA plots. Several studies have leveraged taxonomic assignment information and species diversity to establish novel disease associations [190–192].

Clustered regularly interspaced short palindromic repeats assay

It's difficult to write any contemporary article without mentioning the impact CRISPR-Cas has in biomedical science. CRISPR-Cas (Clustered Regularly Interspaced

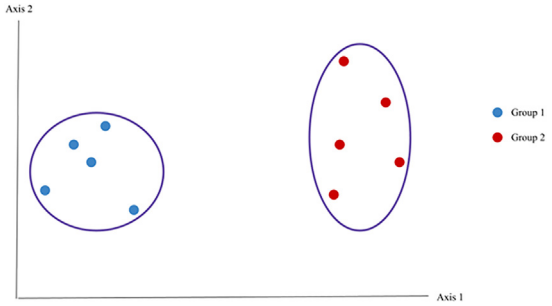

FIG. 6 A sample PCoA plot showing two different sample groups and their clusters based on presence of different microbiota. It should be noted that distinct clustering is not guaranteed. However, this figure shows how diversity among different samples can be visually represented. From a pathologic standpoint, this method can distinguish different pathogens in different samples, or represent dissimilarity between other pathologic samples.

Short Palindromic Repeats ––CRISPR-associated sequences) was first identified in 1987 and later characterized functionally in the mid-2000s as an innate immune system identified in both archaea and bacteria. Today, this programmable system is able to identify, edit, and change any gene of choice, making it a revolutionary tool in medicine.

Many recognize CRISPR as a method to edit genes in the context of therapeutics. CRISPR-Cas has been proposed as a viable method for eradicating anything from Lyme disease to cancer. To date there are roughly 100 clinical trials, mostly in phase I, that are harnessing the power of CRISPR-Cas to fight disease. Furthermore, the impact of CRISPR has also revolutionized disease characterizations and diagnostics. This was shown during the rise of the SARS-CoV-2 when CRISPR-Cas diagnostic methods were both approved and implemented via emergency US Food and Drug Administration (FDA) approval as a fast and reliable method for detecting SARS-CoV-2 [193]. The rise of spatial and single cell omics have also led to the development of multiplexed assays that allow for perturbation on the single-cell level to characterize concomitant morphologic and molecular changes.

As a result of the gene-editing revolution, the need for analyzing and predicting genomic data has also increased in demand. Two broad areas of bioinformatics development in the context of CRISPR can be defined as (1) the characterization of novel CRISPR systems, and (2) the prediction and quantification of gene editing efficiency [194]. Although the discovery of new CRISPR systems is nonetheless important, CRISPR efficiency will be expanded on here.

A subject of concern is how often CRISPR mistakenly edits a sequence that is not the intended target sequence (see Fig. 4E). These unintended cut sites are called "off-targets." For example, in a clinical trial using CRISPR to edit HIV, CRISPR would be programmed with a custom gRNA (guide-RNA) sequence that guides the Cas enzyme to a specific HIV sequence. The guide RNA contains a 20-nucleotide sequence "arm" that is identical to the HIV sequence to be cut. The selection of this sequence is critical to the recognition and cutting of Cas. If the gRNA design is not specific enough to the target, errors can occur when Cas cuts another gene in the human genome if the sequence is similar to the guide itself. Understandably, it is of great importance that researchers and clinicians are aware of the potential off-target sequences, and where in the genome they reside. A deletion in an important coding region may have a detrimental impact.

Many *in silico* tools have been developed to characterize and predict these errors. Early off-target prediction tools include CHOPCHOP, COSMID, and Cas-OFFinder [195–197]. Each use a different scoring method to rate the specificity of each sequence to on-target cutting. Although the number of mismatches, insertions and deletions between the gRNA and DNA sequence can impact the propensity of cutting, it has been shown the position of the mismatch in off-target sequence can also impact the vulnerability to cutting as well [198]. Some models use a simpler approach using alignment and sequence similarity to predict off-targets, whereas other, more advanced methods, leverage ML to predict off-targets by incorporating a multitude of factors such as, nucleotide position, GC content, chromatin accessibility, gene expression profile, melting temperature and free energy [199].

As details begin to emerge about different factors that affect CRISPR binding affinity, more ML models have also started to surface. These models have proved to be more accurate than earlier developments. A recent tool, CRISPRon, trains CNNs from binding energy of gRNA to DNA obtained from experimental data to predict off-target sites [200]. This tool has been shown to outperform all currently off-target prediction tools. Another program close in competition uses off-target sequencing results compiled from multiple datasets and research groups to train a CNN and displays highly accurate predictions in comparison to tools that do not employ deep learning [201].

Other ML-based programs such as CRISPRater, CRISPRScan, CRISTA and TUSCAN have been developed based on linear, logistic regression or random forest models are easy-to-train models but fail to give

reliable results with small-batch gRNA predictions. Poor performances in the models have been attributed to the scale and complex relationship to some features [7]. In a comparative analysis of all off-target models using ML, elevation, a multilevel model using Gradient Boosting Regression Tree, Boost Regression Tree, and Logistic Regression outperformed all other tested models [7]. This further supports the complexity of predicting genomic interactions and the need for a dynamic approach that ML can provide.

ML is beginning to play an important part in CRISPR design. However, there are still many areas that have shortcomings and need to be developed such as expanding off-target training data for lesser-known Cas systems and for broadening the scope of target species [7].

Integration of Anatomic and Molecular Pathology

Slide imaging as a quality control mechanism for tumor purity

Typically, pathologists macrodissect and sequence tumors to identify mutations/molecular alterations suggestive of potential malignancies. However, tumor purity (ie, the percentage of cells within the dissected region that are malignant) often confounds many genetic or epigenetic studies and is a measure commonly controlled for through multivariable regression modeling or restriction (ie, exclude if fails to surpass a threshold) [202]. Furthermore, identification of regions to macrodissect is often marked through inking patterns that encircle the tumor. Tumor purity is often overestimated as malignant cells often present larger than benign infiltrates and imprecise inking of slides can further bias these estimates. AI models are being developed which simultaneously: (1) precisely define the tumor region to improve microdissection, and (2) count the number of benign and malignant cells as a functional tumor purity estimate [203]. With this information (tumor area and purity), information can be provided to the practitioner to assess the number of slides required to optimize DNA yield.

As an aside, inking is highly relevant for predicting regions to macrodissect and optimizing DNA yield yet is both imprecise and can potentially introduce endogeneity (ie, confounding factor related to target of inference––location of tumor) into the study design if improperly implemented. For example, if all training slides contained ink, the model may require the presence of ink to highlight tumors during inference on new slides––such a model may fail to generalize to specimens not marked with ink. If the algorithm associates ink discoloration as a feature of malignancy, this in turn may bias/skew tumor purity estimates. The ideal model should adjudicate tumor purity information based on the microarchitecture of each tissue patch on the WSI. Ink removal from histopathology images is very challenging because tissue samples may contain inter and intra-slide texture variabilities. In addition, ink markings are permanent/opaque and once converted into a digital slide, it is difficult to effectively remove it from these tissues themselves [204].

Several methods have surfaced for mitigating the impact of ink as an external factor. As an example, several groups have opted to use a color transformation approach to resemble the surrounding tissue, but the high opacity ink annotations and texture variation render such methods ineffective. GANs have shown the capacity for ink removal; in particular, a deep learning algorithm based on the Pix2Pix model, achieved a 20% increase in structural similarity index compared with previous methods of ink removal. Furthermore, the resulting images from the generator model displayed a 30% higher signal-to-noise ratio and doubled the image clarity based on a measurement of visual information fidelity (VIF) score. Another GAN algorithm, which performs image-to-image feature translation based on two given image datasets, was used to remove marker ink and H&E stains from WSIs, and achieved similar results, obtaining optimal performance as assessed by ResNet image recognition model. However, generative models such as the GAN are not perfect; naive applications, especially involving unclear boundaries between background and foreground ink removal or low-quality images, can further confound findings by reducing clarity in the underlying tissue histomorphology [88,205,206].

Multimodal deep learning in pathology

Biological processes are naturally interconnected, as one process is impacted by and influences many others. This phenomenon can be observed by considering DNA methylation as an example––binding of methyl-groups to the DNA of gene promoters islands can make them inaccessible to transcription factors thus inactivating transcription, potentially manifesting in phenotypic effects (eg, microsatellite instability in Colorectal cancer tumors) [207]. To reflect these types of complex interactions across modalities, many applications of deep learning for pathology have used multimodal approaches to leverage complementary histomorphological, molecular, and patient demographic characteristics. The overarching goal of these methods is to effectively extract relevant information

from each individual data type, as well as from interactions across different types of pathologic findings. This is important, because a given type of data may contain clues that only it can represent, such as the arrangement of tumor-infiltrating lymphocytes visible in histopathology slides which may influence cancer treatment response. Concurrently, multimodal interactions may also be present, such as how the relationship between gene expression and clinical outcomes may differ depending on the presence of constituent cell types and tissue architectures, also seen in those same slides. Neural networks extract relevant information from each modality (eg, gene expression and WSIs) in the form of numerically derived features (stored in a vector; eg, activation of certain pathways, the relationship between mucosa and tumor, etc.) known as *embeddings*. To predict clinical outcomes such as survival and recurrence from this data, these *embeddings* are combined or "fused" together (Fig. 7). The specific method for fusing embeddings from different modalities is an area of ongoing research. For example, embeddings can be simply concatenated together (similar to adding additional columns to a computer spreadsheet), added using learned weights, or combined in a specific manner dependent on the values in the embeddings. Furthermore, it is imperative that all multimodal pathology AI solutions adopt interpretability, including an understanding of which modalities are being prioritized in different cases and how information across modalities is jointly attended to (eg, relevant WSI regions). If unimodal deep learning models are considered black box, multimodal models without such interpretability features are effectively black holes and end users (especially medical professionals) will struggle to trust a system they cannot understand especially as it grows more complex.

An early work in this niche was *Pathomic Fusion*, which integrated histology, mutation data, CNV, and gene expression, to predict patient survival [100]. The authors modeled interactions across modalities using a Kroekcner Product-based method, following a "late fusion" approach where embeddings are extracted individually for each modality using individual neural networks, followed by fusion and downstream prediction using additional layers. Further works from other investigators researched (1) additional fusion methods, (2) modeling approaches to induce learning of features shared across modalities as well as those distinct to each modality, (3) interpretation and explanation of neural networks, and (4) experiments to determine best practices for training multimodal networks for biomedical data [100,208,209]. To achieve clinical benefit and implementation, future studies should prioritize learning from diverse heterogeneous datasets to mimic real-world data. Interpretation of neural network decision-making should also be validated by medical practitioners to assess whether these models capture rational interactions as well as potentially new biomarkers indicative of clinical outcomes (eg, new imaging/molecular tumor microenvironment predictors).

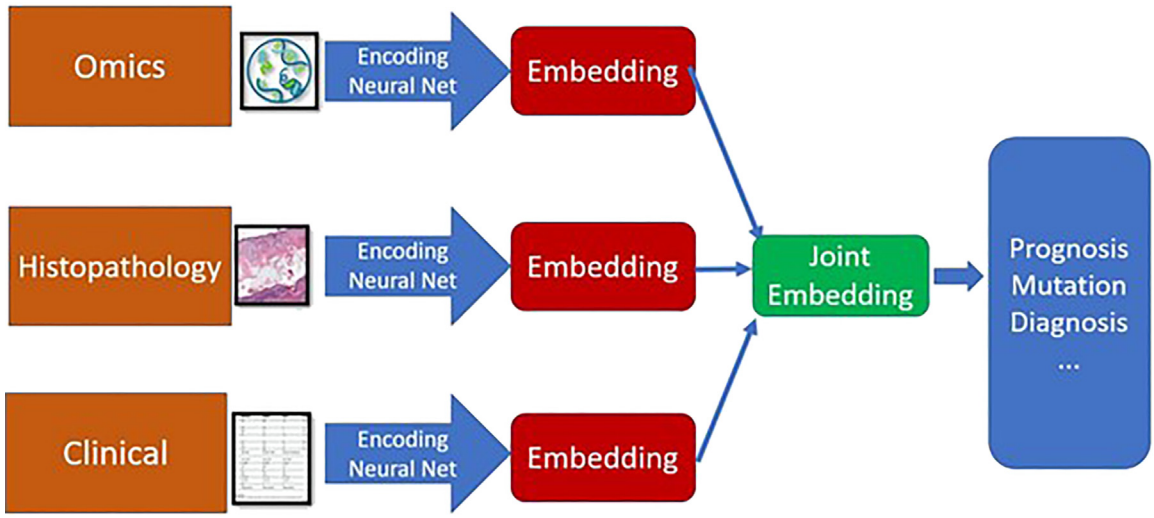

FIG. 7 A generalized pathology multimodal deep learning workflow; each modality is encoded, to extract embeddings (numerical representations); the embeddings are fused together, and used to predict downstream targets such as patient prognosis, mutation status, and diagnosis.

Aside from aggregating information across a slide in tandem with related molecular information, methods to integrate spatially localized transcriptional information with tissue histology are generating excitement as means to further infer molecular information across a slide or jointly profile morphologic and molecular information with improved resolution. Popular approaches include GNNs, which take advantage of co-localized histologic and molecular patterns. There exist numerous methodologies to predict highly-multiplexed spatial mRNA patterns from WSIs using co-registered Visium spatial transcriptomics data [158] to reveal morphologic correlates while providing the means to study spatial biology on held-out samples where appropriate. Alternatively, integrating spatial expression patterns with co-localized image patches can reveal nuanced spatial biological variation.

DISCUSSION

In this work, we illustrated potential applications of ML technologies for the study of anatomic and molecular pathology. As hyperdimensional imaging and sequencing assays gain widespread utilization in modern pathology laboratories, the ability of algorithms to study and make sense of the staggering amount of data has lagged behind. The advent of modern deep learning techniques has accelerated the rate of development of novel assessment methodology to match the pace of these novel profiling methods. There are many exciting opportunities for innovation in digital pathology, but it is going to be very difficult to temper hype and expectations with the reality of what can actually be accomplished using these up-and-coming technologies.

To spur the adoption of these technologies, the rush to apply AI to pathology will be balanced by respect for the viewpoints of those who assume ultimate responsibility for clinical decision-making. For example, although a preponderance of AI-powered clinical decision aids has been developed for pathology and dermatology, very few, if any, have shown sufficient clinical efficacy, utility, and value to warrant inclusion in the clinical workflow. Only a small handful of technologies have been properly validated and cleared by the FDA for nonautonomous usage across all medical disciplines [210]. For instance, *Paige Prostate* was the first device to receive such approval in 2021 to suggest regions in a prostate examination that warrants follow-up by the practicing subspecialist [211]. None of the devices thus far have been approved for nonautonomous decision-making [212]. There are a few considerations worth addressing to spur the adoption and implementation of these technologies. We have enumerated some of these concerns as follows:

1. Technological superiority or noninferiority: Development of technologies to maximally show noninferiority to standard practice requires additional algorithmic fine-tuning, data collection, creation/adoption of novel methods, and selection of realistic clinical outcomes (eg, staging) and scenarios for validation to accurately communicate and not overstate results. Simulated clinical outcomes (eg, surgical resection speed and completeness) should be as close to their real-world counterparts as possible. An interventional randomized controlled trial would be the logical next step pending successful and realistic demonstration of noninferiority [213].
2. Data collection and annotation: To show the viability of an AI technology, sufficiently diverse data should be collected to train these models. The collected data should attempt to account for realistic edge cases (eg, bacterial infection of urothelial cells impacting estimation of the NC ratio) [214]. This may require substantial data curation and annotation. As expert time is significantly limited and expensive, this may require exploration into time and cost-efficient data collection strategies (eg, public datasets). Data generation has become a somewhat contentious issue as institutions scramble to define policies over data ownership given the significant time and resources expended by clinicians and stakeholders into data generation [215–218].
3. External applicability: ML models can be deployed in settings where these models have been well tested/validated in. As the aspirations for these technologies are to deploy them nationally and/or globally, models should be tuned to handle a wide variety of specimen preparation and slide scanning techniques with the appropriate caveats stated where such testing has not been done. These challenges may require multi-institutional agreements to carry out multi-center clinical trials [219,220].
4. Attitudes, beliefs, and cost-effectiveness: Performing cost-benefit and comparative effectiveness analyses with comparison to gold-standard laboratory automation technologies (eg, auto-staining), including surveys of attitudes and beliefs about these AI technologies can help identify and incorporate design elements to maximize adoptability. For instance, using Likert scale surveys (eg, strongly disagree, disagree, agree, strongly agree) may help show subjective preference for clinical usefulness as assessed

by relevant stakeholders. This may require the formation of interdisciplinary teams who aspire to realize the translational potential of AI technologies by better understanding worthwhile clinical applications that are often overlooked by computational researchers. For example, although several flashy AI algorithms are being developed (eg, augmented reality microscope that can spot tumors), algorithms that are not informed through clinician input are less likely to be effective when deployed (eg, a pathologist can spot the tumor in a fraction of the time required to operate the AI-augmented microscope) [221,222]. Illustrating the cost-effectiveness over other competing laboratory automation technologies (eg, auto-stainers) will provide a compelling rationale for adoption. This may require the identification of CPT codes that are likely to be used by the new diagnostic tool.

5. Explainability and reliability are two barriers that have been persistently identified for facilitating trust and adoption in decision-making. Explainable ML models can indicate to practitioners how these models came to their decision [223]. These model explanations can identify systematic sources of error that can be ameliorated in the design, collection, and analysis stages of the project (eg, using one type of slide scanner for malignant cases or at a specific time of day). Medical professionals are also keenly interested in how models communicate uncertainty in the model findings, which can inform decision-making [224]. For instance, although an ML algorithm may classify a case as a Melanoma, the model may only be 30% confident in its assessment (reliability––ie, indicating that slight modifications to the input data, eg, rotation, may produce widely different results, etc.), warranting a re-review from the dermatopathologist [225].
6. Bias, fairness, and ethics: The current way that AI technologies are developed can lead to bias, with their impact being limited by (1) who provides the source information for algorithm development (ie, cohort demographics), (2) who acquires the data (ie, what is the approach of the specific domain expert), and (3) who develops/implements the algorithm (ie, who is the quantitative researcher, and what do and don't they bring to the table) [213,226–228]. There is ample evidence to suggest that in these three areas, crucial input is missing from practitioners and participants from underserved populations, which has led to algorithmic behavior that negatively biases historically underserved groups and can ultimately result in higher under-diagnosis and misdiagnosis rates in already underserved patient populations [229,230].
7. Collaboration with privacy and security: Coordinating feasibility studies showing the application of federated learning technologies, which allow for decentralized training and assessment of ML models from the safety of each institution's firewall, and other privacy-preserving tools, may facilitate collaboration and trust between multi-institutional partners [231]. Collaborating among institutions requires the identification of a unique set of barriers and stakeholders for each participating institution.
8. Simplicity and elegance: Above all, newly proposed technologies may benefit through simplification of design specifications and key innovations. Complicated designs, even if efficacious, may ultimately prove to be too disruptive to the existing workflow and challenging to implement. For instance, multimodal technologies which integrate information across WSI and a multitude of omics modalities for prognostication often perplex practitioners who may prefer prognostic tests that assess specific immune components of the tumor microenvironment (eg, immunoscore) [232].

A likely explanation for the lack of adoption is that many of the proposed technologies may be disconnected from their core users. Consensus from experts has indicated that technologies should be informed through user-centered design processes and interviews targeting utility, cost savings, trust, explainability, and reproducibility. It has been suggested that validation strategies should be established early and revisited frequently [233]. This has prompted experts and decision-makers to rethink strategies for adopting nascent technologies and the systems of care they are poised to influence. This may require the adoption of rigorous delivery science frameworks that use scientific methodologies to assess how well the process of planning and coordinating with stakeholders, forming teams, etc., was carried out (eg, capacity to identify and resolve additional adoption barriers). This may complement strategies to spur effective engagement (eg, receptiveness to feedback) with both hospital leaders and key stakeholders (eg, histotechnicians and pathologists). Placing informatics teams with sufficient expertise in data analysis, ML and knowledge of day-to-day clinical operations (ie, well-integrated with stakeholders) within the hospital infrastructure (ie, clinicians) may facilitate interaction between these

groups, reducing the potential for disconnection. Although these challenges are paramount for the successful integration of AI technologies into the pathology workflow, several mature technologies have been developed which embody these design and engagement principles that, if properly assessed, present opportunities to realize the potential of these informatics technologies.

SUMMARY

AI technologies will continue to have a transformative impact on health care delivery by augmenting clinical decision-making through the automation of tedious, repetitive tasks, which will allow pathologists to serve more patients, reduce barriers to specialized care, and devote more resources to more challenging biomedical issues (eg, studying the immunomodulatory implications of the Tumor Immune Microenvironment). There exist numerous applications of AI in anatomic and molecular pathology, which are poised to significantly impact the pathology workflow. Adoption of mature technologies will require critical consideration of implementation strategies and effective engagement with and feedback from key stakeholders. Although it is unclear what innovative digital pathology technologies are in store, collaborative teams of clinician-scientists and quantitative researchers will work together to identify the problems worth addressing.

CLINICS CARE POINTS

- Aberrant use of chemical staining reagents and variable image quality can compromise the integrity of these machine learning algorithms. Care should be taken to ensure application of staining reagents has been standardized through autostainers. Additional differences can be resolved through stain normalization, an algorithmic approach for standardizing tissue stains.
- Virtual staining technologies can digitally convert between two different types of staining reagents (e.g., H&E and Trichrome), permitting rapid reflexive staining which can strengthen pathology laboratory infrastructure at a low cost. Two approaches to generate synthetic stains are through generative adversarial networks or identifying and segmenting cells tagged by co-registered IHC/mIF WSI. Establishing clinical concordance is crucial for validation of these technologies.
- Natural language processing can detect erroneous billing in pathology reports and identify sources of ambiguity and discrepancies in reporting. Reports should be deidentified to ensure patient privacy prior to fitting the machine learning workflows. Validation of these algorithms in practice requires close coordination with the billing staff.
- Organizing genes in processing layers based on their molecular pathways can aid neural networks in molecular analyses to identify pathways associated with outcomes of interest, such as survival.
- Whole slide imaging algorithms can also be used as quality control for molecular genomics through accurate estimation of tumor purity. This requires localizing the area of tumor to inform microdissection and accurate count of malignant cells within that area.

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SUPPLEMENTARY DATA

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