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Artificial intelligence in histopathology: enhancing cancer research and clinical oncology

Artem Shmatko[1,2,7], Narmin Ghaffari Laleh[3,7], Moritz Gerstung[1,2] ✉ and Jakob Nikolas Kather[3,4,5,6] ✉

Artificial intelligence (AI) methods have multiplied our capabilities to extract quantitative information from digital histopathology images. AI is expected to reduce workload for human experts, improve the objectivity and consistency of pathology reports, and have a clinical impact by extracting hidden information from routinely available data. Here, we describe how AI can be used to predict cancer outcome, treatment response, genetic alterations and gene expression from digitized histopathology slides. We summarize the underlying technologies and emerging approaches, noting limitations, including the need for data sharing and standards. Finally, we discuss the broader implications of AI in cancer research and oncology.

Malignant tumors are complex, heterogeneous, multicellular ecosystems[1–3]. Understanding the interplay between different cell types and tumor evolution and ecology is key for effective cancer treatment[4,5]. In the last decades, genomic, transcriptomic and proteomic analyses have become a focus of cancer research[6–11] and subsequently began to enter clinical routine[12] (Fig. 1a). A tumor's histological phenotype is an additional key layer of data that complements the genome, transcriptome and proteome, which in combination are essential to cancer diagnosis. Computational pathology refers to applications of AI in computer-based image analysis in histopathology, which were developed and broadly adopted in the recent past. Computational pathology methods can extract information from the histological phenotype, thereby enabling a broad range of new research and diagnostic applications (Fig. 1b). This ability is the result of rapid technological advances in the fields of histopathology and molecular diagnostics, combined with methodological innovations in computer vision and bioinformatics over the last decades (Box 1 and Fig. 1c). The investigation of diverse tumor properties on a comprehensive molecular level has been made possible by new high-throughput technologies that produce an ever more precise and richer characterization of tumors and their microenvironment. Biologically and clinically relevant information, such as actionable driver gene mutations, biomarkers of cancer immunotherapy and prognostic scores, can be extracted from the resulting wealth of data using the tools of bioinformatics and data science. By contrast, the visual examination of histopathological cancer phenotypes, which is essential to diagnose solid tumors, is traditionally performed by human experts without much technological assistance beyond an optical microscope. Visual examination of tissue sections on histopathology slides is necessary to diagnose solid tumors. A typical histopathology section contains hundreds of thousands of cells. Thus, beyond aiding routine diagnostic work through automation, computer-aided quantification of such samples would yield a large amount of scientifically and medically valuable information. However, the use of computer-based image-analysis methods for the assessment of routine pathology slides has, until recently, mostly stayed at the level of research rather than clinical routine application[13].

Starting in the 1960s, publications on digital histopathology used predefined features such as nuclear size and their color intensity as well as algorithms for automatic detection of different geometrical shapes and patterns, such as curves or corners[14]. Around the turn of the millennium, visual feature descriptors, such as scale-invariant feature transform and speeded up robust features, were adopted for a range of digital pathology problems[15,16]. Machine learning algorithms, such as support vector machines or random forests, were subsequently used to learn associations between predefined image features and other variables of interest. By contrast, modern convolutional neural networks (CNNs) automatize both feature extraction and learning of associations. CNNs, a specific type of neural networks, are machine learning algorithms that are particularly suited for computer vision and image classification[17,18]. CNNs were designed to use multi-level image structure, where basic image features such as contours are defined by changes in neighboring pixel intensities (Fig. 2a) and larger patterns are effectively successive combinations of smaller ones. These patterns can be identified at each scale using mathematical operations termed convolutions (Fig. 2b), resulting in sets of feature maps that represent the image content at an increasingly abstract level (Fig. 2c). CNNs generalize the idea of using convolutions with predefined kernels (for example, for edge detection) by treating kernel values as learnable parameters that can extract a broad range of patterns relevant to a given task. This allows CNNs to ultimately make predictions directly from images without relying on manually engineered intermediate steps (Fig. 2d). CNNs can detect objects regardless of their location or size on the image, even though the latter property is of less relevance for histopathological applications with fixed magnification. While passing through CNN layers, an image is gradually transformed into a set of feature values that enable the algorithm to discriminate

[1]Division of AI in Oncology, German Cancer Research Center (DKFZ), Heidelberg, Germany. [2]European Molecular Biology Laboratory, European Bioinformatics Institute, Cambridge, UK. [3]Department of Medicine III, University Hospital RWTH Aachen, Aachen, Germany. [4]Medical Oncology, National Center for Tumor Diseases, University Hospital Heidelberg, Heidelberg, Germany. [5]Pathology and Data Analytics, Leeds Institute of Medical Research at St James's, University of Leeds, Leeds, UK. [6]Else Kroener Fresenius Center for Digital Health, Medical Faculty Carl Gustav Carus, Technical University Dresden, Dresden, Germany. [7]These authors contributed equally: Artem Shmatko, Narmin Ghaffari Laleh. ✉e-mail: moritz.gerstung@dkfz.de; jakob_nikolas.kather@tu-dresden.de

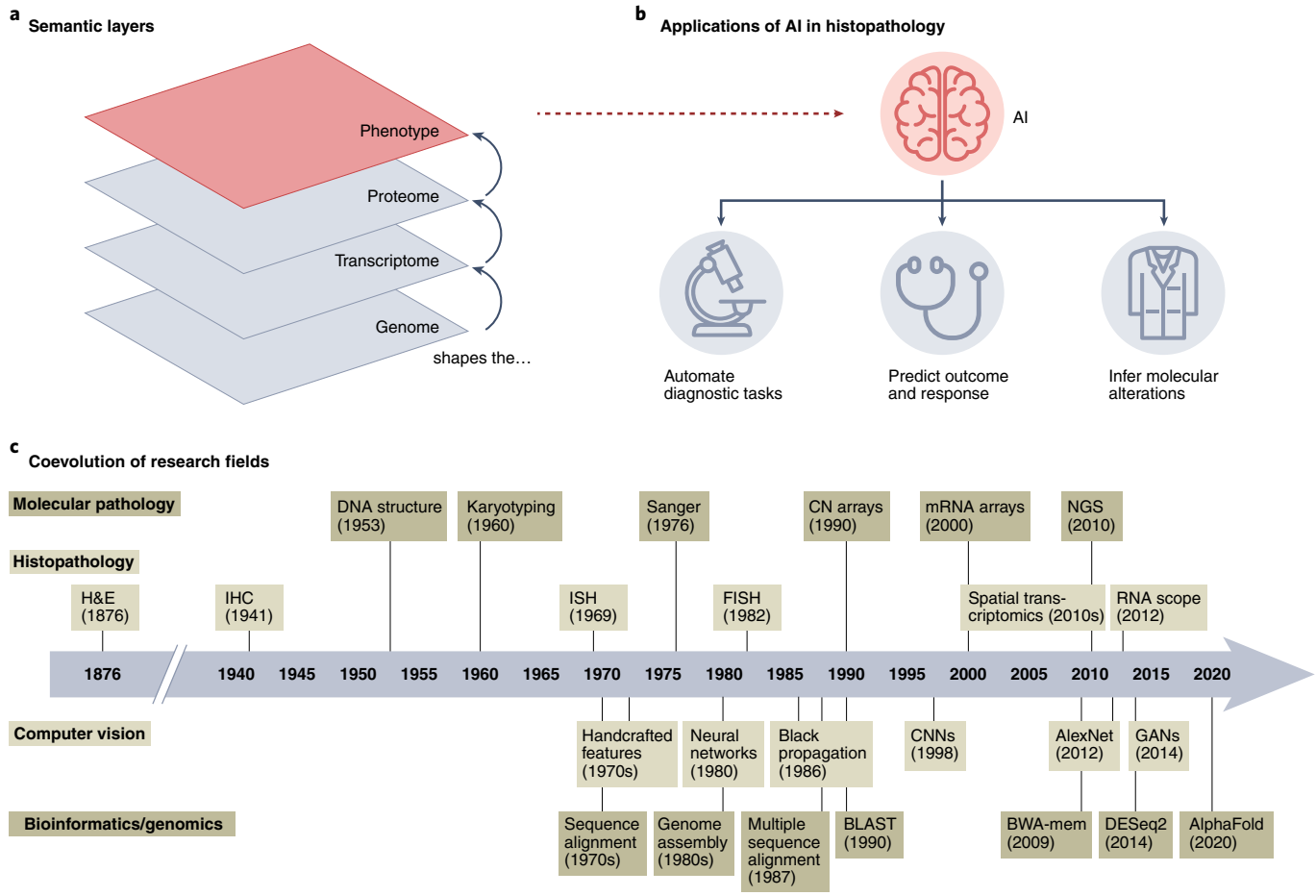

Fig. 1 | Clinical applications of AI-based computational pathology. a, Solid tumors can be conceptualized as being composed of multiple hierarchical layers. **b**, A hierarchy of established and emerging applications for AI in histopathology image analysis. **c**, Parallel evolution of distinct research fields culminating into computational pathology. Evolution of the research fields with a non-exhaustive list of milestones. IHC, immunohistochemistry; ISH, in situ hybridization; FISH, fluorescent in situ hybridization; CN, copy number; NGS, next-generation sequencing; BLAST, basic local alignment and search tool; BWA-mem, Burrows–Wheeler Aligner with maximal exact matches; DESeq2, differential gene expression analysis of RNA-seq data.

the labels used for training. In many cases, feature vectors obtained from a CNN trained for a particular task can also serve as predictors for other problems and datasets[19]. This approach of using a neural network pretrained on a large labeled dataset for feature extraction or fine-tuning the network by retraining on an additional dataset is called transfer learning and is widely used in scenarios in which the problem-specific dataset is not sufficient to train a network ab initio.

Although neural networks have existed since the 1980s, it was not until the early 2010s when more efficient algorithms for training very large and deep networks on ever-greater datasets were developed (Fig. 1c). The resulting deep learning algorithms form the backbone of many AI algorithms and have greatly improved computer vision with complex CNNs. This also holds great promise for cancer research, as, similar to traditional bioinformatics, computer algorithms can be used to extend human capabilities by sifting through large amounts of digitized cancer pathology slides and extracting scientifically and clinically relevant knowledge. This has led to the development of AI systems for automating diagnostic tasks, potentially alleviating the workload on pathologists when they are clinically adopted in the future. In addition, AI systems have expanded the types of information that can be systematically extracted from routine histopathology slides. AI can predict abstract categories directly from routine histopathology images of samples stained with hematoxylin and eosin (H&E). In particular, even though the training dataset only has labels for individual slides (Fig. 3a), it is possible to obtain spatially resolved predictions (Fig. 3b). Highly scoring image tiles can be visualized[19,20], allowing human experts to check the plausibility of AI systems and also discover new features with the help of AI[21,22] (Fig. 3c). Together, these methods enable various applications in research (Fig. 3d), including by improving the molecular characterization of tissue in research cohorts[19]. In clinical diagnostics, AI methods can predict outcome and treatment response and infer some genetic alterations directly from H&E-stained histopathology slides[13] (Fig. 3e). The abilities of AI are not restricted to image classification in which the task is to predict a certain state associated with the image, such as whether it is derived from tumor or normal tissue. Generative models reproduce the images themselves and offer new opportunities including efficient and secure training of models.

In the following sections, we provide an overview of key concepts and examples of AI use in digital pathology, focusing on its implications for research and clinical oncology.

Applications in cancer research and diagnostics

In this section, we describe how the adaptation of established computational methods is helping to address complex and clinically relevant questions from routinely available tumor tissue sections.

Box 1 | Definition of key concepts

Item	Definition
AI	An umbrella term for algorithms or machines capable of solving complex tasks, akin to human intelligence. In the field of computer vision and, in particular, computational histopathology, it is often a synonym for deep learning.
Machine learning	The field of computer algorithms capable of being trained by different datasets to fulfill particular tasks, such as classifying images of various kinds.
Computer vision	The field of computer science related to the analysis of images or videos.
Image classification	The algorithmic task within computer vision of deciding on a set of labels, such as tumor or normal tissue, based on an image.
Neural network	A type of machine learning algorithm in which information is passed through a network of basic mathematical operations termed neurons. The combination of multiple basic operations facilitates carrying out complex calculations.
Deep learning	The field of neural networks comprising many layers of neurons is termed deep learning. Due to their flexibility, deep neural networks are the basis of many AI algorithms.
CNN	A class of neural networks for computer vision that use mathematical operations combining information of neighboring pixels. These are successively applied at increasing fields of view, thereby extracting larger patterns and objects.
Transformer	A deep neural network architecture that uses a so-called attention mechanism to learn long-range patterns. Originally proposed for natural language processing, vision transformers have shown promise for computer vision, as length scales can be more flexibly learned by transformer neural networks than by CNNs.
Generative models	A machine learning model capable of reproducing (imaging) data, unlike classification models that only produce labels such as cancer or normal. A specific type are GANs, which are a combination of a generative and discriminative classification model for more efficient training.
Distributed learning	A technology that allows using multiple computers or data centers for large-scale training of a machine learning model, often involving the use of several independent datasets without sharing them between instances. This comprises federated learning with a central machine coordinating the learning progress and swarm learning in which different instances communicate directly with one another.
Bioinformatics	The interdisciplinary field developing computer algorithms for analyzing often complex and large biological datasets. Applications are often related, but not limited to, genomics.

Automation of routine histopathology workflows. The histopathological assessment of tumor samples involves fixation in formalin, cutting, embedding in paraffin, staining with H&E and subsequent visual characterization by a trained pathologist using a microscope. Pathologists investigate the presence, subtype and other histological characteristics of tumor tissue according to standardized criteria. In addition, for many tumor types, more quantitative analyses are performed, such as the assessment of grade, counting of mitotic cells and tumor-infiltrating lymphocytes, quantification of tumor budding and many other types of analyses depending on the tumor entity[23]. Many of these tasks are labor intensive and not perfectly reproducible by and between observers[24]. Additionally, many histopathology-related tasks require enduring attention to detail, which is likely to waver as a pathologist fatigues, unlike a computer. A decade ago, most image-analysis methods applied in digital pathology were aimed at automating such circumscribed repetitive tasks in which the ground truth was always clearly defined by a human expert or a panel of human experts[25]. CNNs have expanded the capabilities of computer-based image analysis for many routine histopathology tasks. These include tumor detection for breast[26], prostate[27] or esophageal cancer[28], usually in core needle biopsy specimens or in surgical resection. CNNs can also carry out subtyping of lung and kidney cancers[29,30]. Additionally, digital pathology is capable of classifying cancers of unknown primary origin, which can be difficult to diagnose and treat[31]. Specific advances have also been made in Gleason grading of prostate cancer, a task generally considered to have considerable interobserver variation[32–34]. Lastly, CNN-based approaches exist for labor-intensive tasks such as counting mitotic cells[35,36]. These are just a few examples of how AI can potentially reduce the workload for pathologists and enable a swift diagnosis for clear-cut cases, minimizing the undesired effects of interobserver variation for more challenging tasks. Progress and challenges of clinical adaptation are discussed in Toward clinical implementation.

Extending conventional capabilities with deep learning. In many basic image-classification problems, the ground truth can be derived from the image data itself, either by laypersons or experts, with the algorithms ultimately reproducing human decision making. Yet it is also possible to use training labels recorded by other means, as is commonly done in oncological research and clinical applications, with the added potential to augment human skills. For example, AI systems have been used to predict the survival of patients directly from histology slides[37–39]. In this case, the ground truth is not derived from the imaging data themselves by a pathology expert but is defined by clinical follow-up. Similarly, AI has been used to predict genetic properties of tumors directly from pathology slides[40]. In the latter case, the labels for supervised prediction tasks are defined by next-generation sequencing or similar molecular biology methods. Finally, AI methods could potentially have a high clinical impact by predicting treatment response to a particular drug directly from routine pathology slides. Collectively, all AI approaches in which the ground truth is not defined by a human expert based on the pathology image have been summarized as 'advanced' prediction tasks[13]. Such tasks receive considerable interest from researchers in academia and industry[41] because they can potentially extract more quantitative information from pathology slides than human experts. Similar ideas have been explored in other medical imaging domains, such as prediction of survival from computer tomography images[42,43] and prediction of molecular markers from echocardiography images[44]. Collectively, in histopathology and beyond, these approaches are mostly constrained by the availability of suitable datasets with labeled image data. In the following sections, we will review such advanced prediction tasks in more detail.

Prediction of outcome and treatment response. Prognostic biomarkers allow forecasting of the natural course of a specific cancer. Histopathology image data harbor prognostically important information such as lymphocyte count[45,46], chromatin patterns[47]

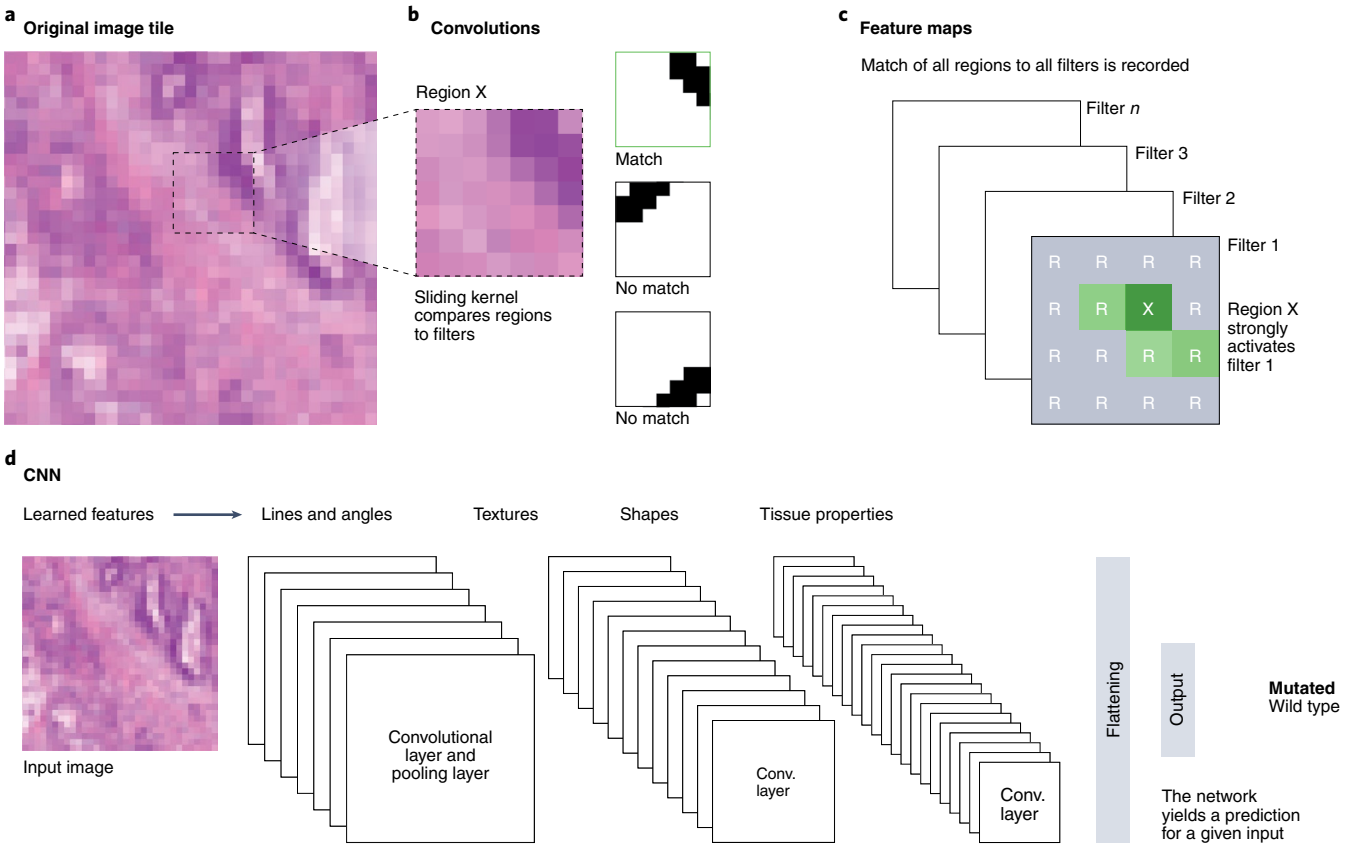

Fig. 2 | A CNN. a, A histological image tile is used as an input image. **b**, A kernel slides across the image, extracts smaller regions (for example, 7 × 7 pixels) and compares them to a set of filters. This process is called a 'convolution'. Filters are learned during training of the model. **c**, How well a specific region matches a specific filter is recorded in a set of feature maps. These maps are a more abstract representation of the image and contain its 'essence'. **d**, In a CNN, multiple layers of convolutions (conv.) are stacked, ultimately resulting in a prediction for a given image, for example, a categorical prediction of mutational status. Subsequent convolutions and implicit pooling layers condense ('shrink') the images but add more dimensions to the data. During training, the shallow layers of the network (close to the input) learn to detect simple shapes while the deep layers (close to the output) learn to detect abstract concepts. Histopathology image source: The Cancer Genome Atlas (https://gdc.cancer.gov/).

or proportions of tissue types[48], each of which are quantifiable by specific digital pathology approaches. End-to-end AI methods are not constrained to any predefined patterns but can piece together a multitude of subtle visual cues beyond those that are known beforehand. Such approaches can generate accurate risk scores in hepatocellular carcinoma[38], colorectal cancer[49,50], mesothelioma[51] and brain tumors[52] among other tumor types. Some studies have employed a pan-cancer approach, training AI models to predict survival in multiple tumor types using end-to-end[39] or transfer learning[19]. However, whenever such studies are limited to a single multicentric image database such as The Cancer Genome Atlas, naively trained survival prediction models have a high risk of bias and poor generalization[53]. This observation highlights that external validation is mandatory when developing prognostic AI biomarkers[54]. Whenever prognostic AI models are interrogated for relevant morphological patterns, they usually show that established morphological markers of prognosis are recovered[38], but, in some cases, previously unknown or underappreciated prognostic patterns are identified[21,22].

Even more clinically relevant than prognosis is the ability to predict the response to a particular treatment, thereby helping oncologists to make better treatment recommendations. Such models would require rigorous, ideally prospective[54], clinical validation, which has yet to be achieved for a predictive AI-based biomarker in histopathology. However, a number of proof-of-concept studies have demonstrated the capability of AI to predict response to immunotherapy[55] or targeted therapy[56] directly from pathology slides. An intermediate step is to predict established markers of treatment response, such as microsatellite instability (MSI) in colorectal[57–61], gastric[62] or endometrial cancer[63], which renders these tumors susceptible to immunotherapy with immune checkpoint inhibitors[64]. In addition, genetic surrogate markers of immunotherapy response, such as gene expression signatures, have been predicted from pathology images with AI[65]. Some researchers proposed to combine morphological features derived from routine pathology slides with other data types in multimodal AI models to improve outcome predictions[66–68]. Currently, data integration from multiple sources, including histopathology, radiology and genomics, is widely regarded as a prerequisite for the use of AI to improve patient outcomes in precision oncology[69,70].

Prediction of genetic alterations and gene expression. It is well established that a number of genetic alterations in cancer are associated with specific histopathological phenotypes. For example, tumors with MSI have long been known to be associated with specific patterns, such as a high density of tumor-infiltrating lymphocytes or mucinous differentiation[71,72]. However, in most tumor types, such genetic–morphological associations are not being used systematically. This may be partly because pathologists would need to be regularly trained on samples with a known mutation status to achieve robust detection of such mutations. A 2018 study described the AI-aided prediction of mutations in clinically relevant genes in

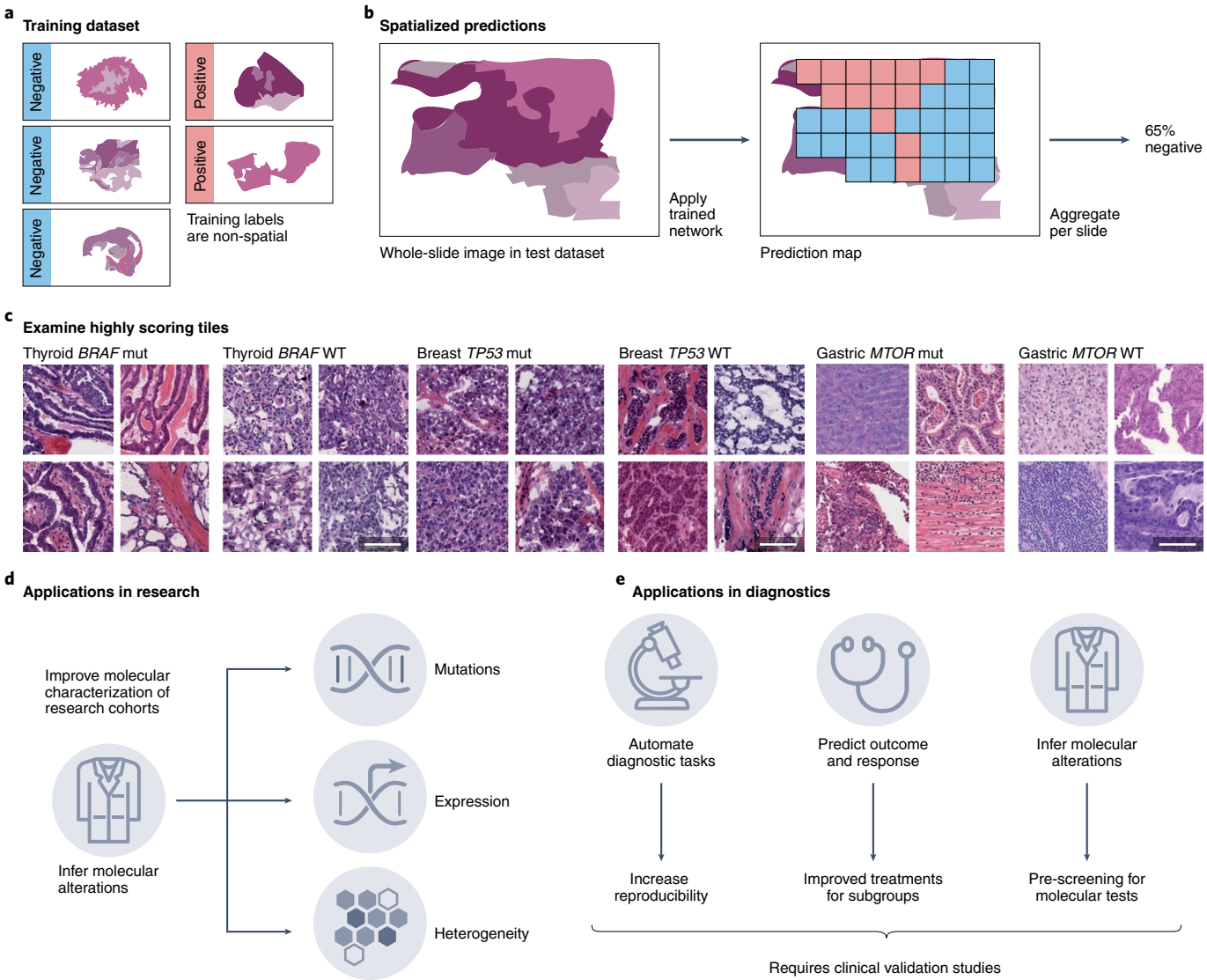

Fig. 3 | Implications of computational pathology for research and diagnostics. From training datasets (**a**), which are labeled on the level of slides, contemporaneous AI methods can generate predictions (**b**) that are spatially resolved as well as an averaged prediction for each slide in the test set. **c**, Examination of highly scoring image tiles by human experts allows them to investigate the plausibility and potentially identify new patterns in images. Scale bar, 100 µm. Mut, mutated; WT, wild type. This enables a range of research applications (**d**) and clinical applications (**e**), which, however, require clinical validation studies before routine use. Histopathology image source: Fu et al.[19] and Kather et al.[20].

non–small-cell lung cancer[40]. Although prediction performance did not reach clinical standards, this work provided seminal proof of principle and gave rise to follow-up studies on a number of solid tumor types, including breast cancer[73], prostate cancer[74] and gastrointestinal tumors[61,75,76]. In particular, AI was shown to predict MSI status from histology[57] in colorectal, gastric and endometrial cancer, which was subsequently validated in multiple larger follow-up studies[58,59,62,63,77–81]. Other studies have shown that dozens of clinically relevant genetic alterations can be inferred from H&E histopathology alone. A pan-cancer AI-based transfer learning model showed significant histopathological associations for many genomic alteration types including whole-genome duplication, copy number alterations and point mutations in 28 cancer types[19]. A separate paper showed that comparable results could be achieved using lightweight neural network models[20]. Yet another work focused on gene expression and provided significant predictions for more than 10^4 genes for various cancers, with results varying greatly across different datasets[79]. Other studies focused on established tumor subtypes defined by transcriptional signatures that could be recapitulated by AI from H&E slides alone, for instance, in lung cancer[82] and colorectal cancer[76]. Importantly, differences between known histopathological cancer subtypes displayed similar trends of molecular changes, but the level of association was usually found to be weaker, thus demonstrating that AI learns histopathological patterns that are not part of conventional classification[19].

Prediction of tumor clonality and spatial heterogeneity. When predicting genetic features directly from histopathology slides, the ground truth labels are usually obtained by bulk analysis methods, such as NGS of the DNA obtained from a bulk of tumor tissue. These molecular methods are usually not spatially resolved. However, processing the images at the level of small tiles rather than the whole slide yields spatially resolved predictions (Fig. 3b). Although this predicted intratumor heterogeneity could be seen as a mere artifact of complex processing pipelines, it has been shown to reflect genetic heterogeneity[79,83,84] as well as spatial patterns of

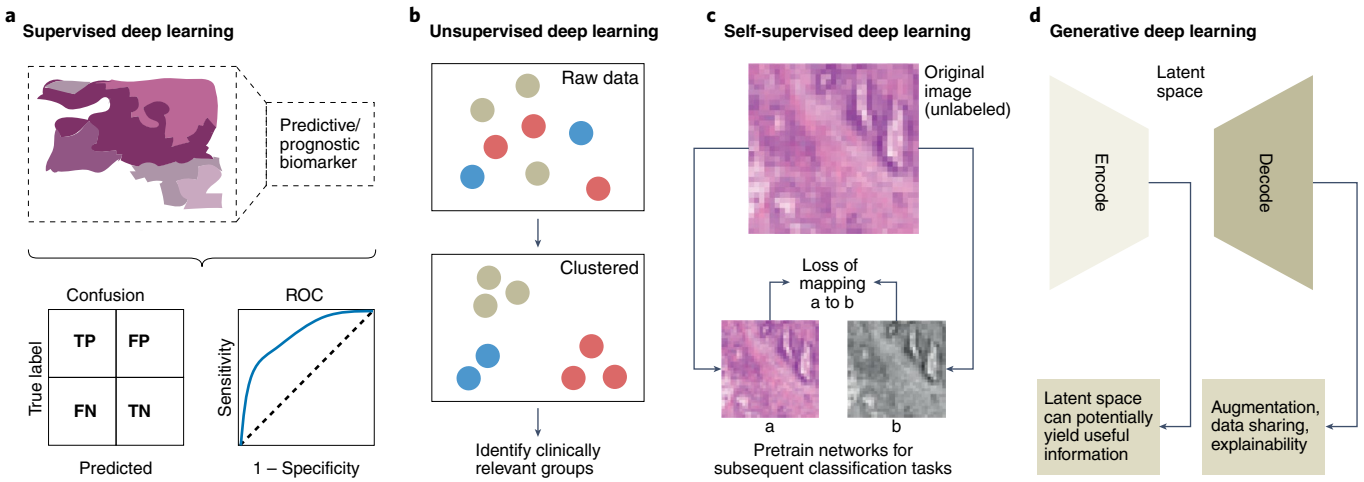

Fig. 4 | Types of AI applications and pitfalls in histopathology. a, Supervised deep learning is the most commonly used method in computational pathology. The objective is to predict a clearly defined label from an input image. Scale bar, 1 mm. TP, true positive; FP, false positive; FN, false negative; TN, true negative; ROC, receiver operating curve. **b**, In unsupervised deep learning, no labels are present and the objective is to structure the data, for example, by clustering similar instances. **c**, In SSL, the training objective is generated from the data itself. SSL is commonly used to improve the pattern-recognition capabilities of a neural network for subsequent use in supervised classification tasks. **d**, Generative deep learning models can synthesize images. This has applications in data sharing and augmentation of small datasets. Histopathology image source: The Cancer Genome Atlas (https://gdc.cancer.gov/).

transcriptomically defined cell types[19,79]. This ability of AI systems to learn spatial patterns and augment existing data information, despite only having been trained on bulk genomic, transcriptomic or prognostic data, could lead to new insights into tumor clonality and cellular heterogeneity[85]. This approach is widely used for tumor region detection on a whole-slide image[26,29]. AI models can be also used to investigate tumor heterogeneity on a cellular level[86]. Such approaches may benefit from newly emerging spatial transcriptomic[87–91] and genomic[92,93] technologies that enable detecting transcriptomic patterns and mutations in situ. Datasets of these types could provide spatially resolved ground truths for further refining AI algorithms that spatially deconvolve molecular data to generate spatially resolved predictions from histopathological data alone.

Methodological innovations

The development of new technologies is helping to continuously refine digital pathology approaches, making it possible to extract a wealth of hidden information from routinely obtainable tissue slides.

Reducing the need for labeled training data. AI in medical image analysis can be broadly categorized as supervised (Fig. 4a) and unsupervised (Fig. 4b). In a supervised approach, the aim is to predict the known label for a given input and evaluate the performance of the model. In case of categorical labels, this task is termed a classification problem[20,94–96]. A common classification problem in histopathology image analysis is predicting a clinical property of the tumor tissue from image data, for example, a good or poor prognostic phenotype, tumor grading or the mutational status of a gene of interest[19,20]. In case of continuous numerical labels, this task is termed a regression problem[97]. The key drawback of any supervised learning problem in digital pathology is that generating the labels is often laborious or expensive. Unsupervised approaches do not require labels but can extract knowledge relevant for tasks such as clustering, anomaly detection and dimensionality reduction by investigating patterns in the training data (Fig. 4b). Because they can be applied to raw data without ground truth labels, unsupervised methods are often useful to structure extremely large datasets[98–102]. A third approach, self-supervised learning (SSL), is related to unsupervised learning (Fig. 4c). Through SSL, models can learn morphological, geometrical and contextual content of images using unlabeled data[103]. Researchers can pretrain neural networks on an unlabeled dataset via SSL, allowing the networks to learn patterns that occur in many samples in the dataset. Subsequently, such a trained network can be applied to a supervised prediction task[78,103–105]. Such two-step pipelines have proven more powerful than classical single-step supervised workflows[78,97,106]. Lastly, the dramatic progress of natural language processing in conjunction with image analysis[107] has the potential to train digital pathology applications based on slide scans and existing associated pathology reports, which may reduce the need for further laborious annotation.

Generative models and synthetic data. Generative adversarial networks (GANs) are trained on collections of real images and subsequently synthesize new images that are similar but not identical to any particular input image[108] (Fig. 4d). Variational autoencoders and diffusion models achieve similar generative tasks and are sometimes easier to train than GANs. However, variational autoencoders are less versatile, and diffusion models require considerable computational resources. The ability to generate realistic synthetic data offers a range of applications[108,109]. For example, synthetic data can be used to augment original datasets and thus increase performance of AI models with limited training data[108]. This is similar to but more versatile and data driven than simple data augmentation, which implies applying small transformations, such as rotations or minor distortions, to the images to enrich the training dataset. A further important development is conditional GANs that generate images that have a set of defined attributes while being otherwise identical. In computational pathology, GANs can synthesize realistic image patches[110] that retain information about molecular alterations of cancer[111]. Furthermore, the underlying generative process can be designed to reflect interpretable histopathological states and subtypes; this is termed conditional image generation[111]. Conditional GANs have been used for stain color normalization and can convert H&E stains to mimic immunofluorescence stains. In addition, diffusion models yield high-quality histopathology images while being easier to train[109]. We anticipate that, in the next decade, modern generative models will be increasingly used in computational

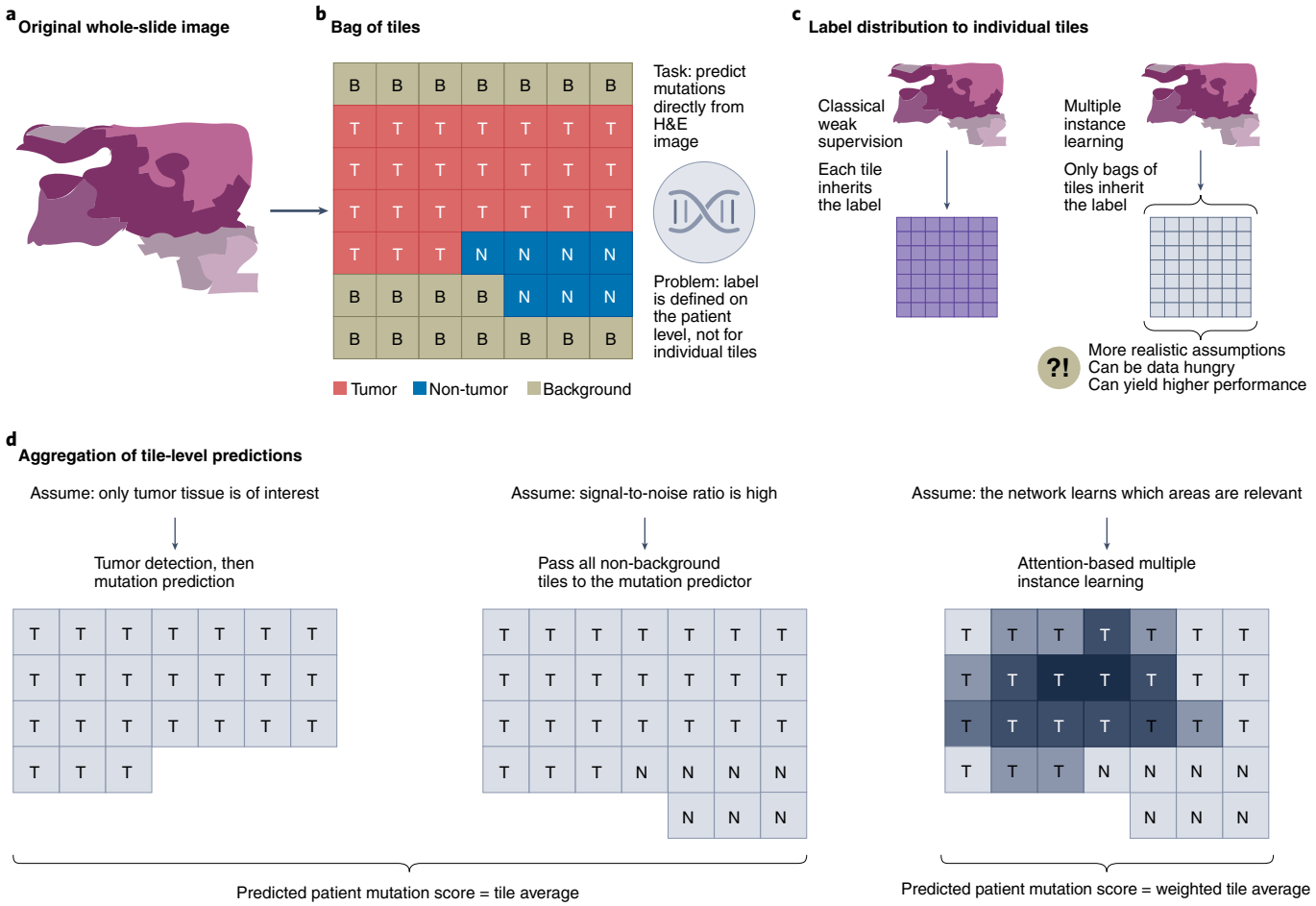

Fig. 5 | Principles of end-to-end weakly supervised prediction workflows. a, Histopathological whole-slide images are too large to be processed by a neural network in one go; hence, they are tessellated into image tiles. Scale bar, 1 mm. **b**, The bag of image tiles contains heterogeneous tissue types, not all of which reflect the underlying ground truth label, such as the mutational status of a tumor. B, background; N, non-tumor; T, tumor. **c**, To address this, two main processing strategies are used in research studies: classic weak supervision and MIL. **d**, In addition, different aggregation functions are used, that is, ways to pool tile-level predictions on the patient level.

pathology. As a positive side effect, they could also yield improved explainability of AI approaches in histopathology in general.

Learning spatially heterogeneous patterns. Most AI methods were originally developed for non-medical applications and were transferred to histopathology image analysis after some delay as this process is often not trivial given that histopathology whole-slide images are too large to be directly processed by CNNs. Virtually all computational pathology studies address this problem by extracting small patches, or tiles, from whole-slide images (Fig. 5a). However, this leads to a problem: for a supervised prediction task in which the mutational status of a given gene is the prediction target, the label is only defined for the whole-slide image but not for every single tile, making the supervised task a weakly supervised task. Often, tumor and non-tumor tissue are both present on the slide (Fig. 5b) and only the tumor tissue is linked to molecular alterations. This problem has been prominently solved in a study that used manual tumor outlines to generate tiles only from the tumor tissue[40]. The authors made the strong assumption that all individual image tiles generated from the tumor tissue would inherit the patient's ground truth label (Fig. 5c, left) and subsequently trained a CNN to predict the mutational status in each tile, followed by aggregation of tile-level predictions on a slide-level (Fig. 5d, left). Other studies have since adopted this protocol with slight changes, demonstrating its broad applicability to many clinically useful tasks and investigating multiple improvements[13,57]. Interestingly, even pipelines with relaxed assumptions showed high prediction performance[20]. In general, only the tumor tissue (and not tumor-adjacent, otherwise healthy tissue) should contain information about molecular alterations. However, in the average slide, the tumor content (that is, the signal-to-noise ratio) was high enough for the mutation prediction from image tiles to be feasible without any pre-selection of tiles[19,20,62] (Fig. 5d, center).

Recently, multiple instance learning (MIL) has come to dominate the field of computational pathology. In MIL, the hypothesis is that not all tiles generated from a given slide reflect the ground truth labels. Only the plurality (or 'bag') of tiles generated from a whole-slide image is associated with a specific label (Fig. 5c, right). As early as 2010, several groups experimented with MIL in pathology image analysis[112,113], but it took several years for these ideas to be more broadly used[114]. In 2019, this development culminated in one of the largest computational pathology studies to date, which analyzed images of over 10,000 patients with prostate cancer[27]. Empirically, however, simple MIL implementations have been shown to underperform in mutation-prediction tasks[115]. MIL can be coupled with an 'attention' approach in which the model learns how much each tile contributes to the final classification. In this case, the aggregation function is not a simple average but applies a weight

to each tile[29,31] (Fig. 5d, right). Empirically, all these approaches can yield good results, and it is presently unclear whether one of them is universally superior[115]. Variations of these commonly used approaches include the introduction of an explicit 'normal tissue' class in the mutational classifier[75] or clustering of tiles with subsequent expert-based rating of clusters[116]. Given the dynamic development of this research field, it can be assumed that the processing strategy will still evolve in the next decade.

Learning distal patterns with transformers. CNNs have been the workhorse for computational pathology since 2018. They have enabled massive improvements for computational image analysis but are limited by their rigid structure in which information from nearby pixels is integrated in defined steps, whereas long-range interactions across the whole image are not always learned by the model. In this respect, transformers, originally developed for natural language processing[117], are more flexible because they can learn distal patterns (also called long-range interactions) in the input data. In the context of natural language processing, transformer architectures have already replaced classical neural network architectures as the state of the art in almost all applications. In 2020, vision transformers were prominently introduced by teams at Google[118] and Facebook[119]. Shortly after their emergence, transformers outperformed CNNs in many computer vision tasks and proved more robust against adversarial attacks[120,121]. Historically, very large pretraining datasets were thought to be a prerequisite for using transformers; however, recently developed training strategies were shown to partially eliminate this dependence[122]. Transformers pretrained on the ImageNet dataset in particular outperform other architectures in various computational histopathology tasks[115]. It is thus likely that transformer-based architectures will prove useful for computational pathology, especially for analysis of larger-image regions in which distal patterns are even more relevant than in smaller-image tiles.

Privacy preservation: co-training of AI models without data exchange. An essential requirement for training-improved AI algorithms is the availability of high-quality training data. Size and variety of training data are key to train robust, unbiased and high-performing AI models in histopathology[27,58]. In some cases, practical and legal issues are precluding institutions from sharing data at scale. Although no personally identifiable information is encoded in digital pathology images themselves, it can be present in clinical or genomic data (metadata), which are used together with the images. In these situations, co-training AI models without sharing any data could be a solution, both preserving privacy and combining computing power of different institutions. Two main approaches have been explored for distributed learning in histopathology. In federated learning[123], several models are trained independently, each on a separate dataset (Fig. 6b). During the training process, participants exchange model updates using a centralized server without revealing the data itself. The server merges the model weights and sends back the updated model to each participant. Similarly, in swarm learning[124,125], multiple parties co-train a model, but, in this case, they are coordinated by blockchain-based communication. Thus, this approach eliminates the need for a central server, allowing exchanging model updates directly using a peer-to-peer network (Fig. 6b) and making it more robust to the loss of a single party. In addition, in swarm learning, the control over the network is distributed rather than being concentrated in a single actor. We anticipate that, in the future, open-source projects for distributed learning will implement new variations of swarm learning, potentially leading to secure, egalitarian and privacy-preserving co-training of AI models in histopathology and beyond. Alternatively to distributed systems, generative AI is a possible solution for the data-sharing problem in histopathology[126] (Fig. 6c). If a GAN is trained on a large dataset (several thousands of images with associated metadata), it is almost impossible to extract any private information from newly synthesized data or the model itself; however, there is ongoing research on developing new attack methods, some of which are variations of classical 'membership-inference attacks'[127].

Toward clinical implementation

AI is now widely used in cancer research and has expanded our quantitative understanding of the tumor phenotype. In addition to these research applications, AI algorithms are moving toward clinical deployment, as evident by a growing number of commercial enterprises that are active in this field. However, clinical translation of a research biomarker is not trivial and requires extra precautions, as we will detail in this section.

Quality control and robustness. For an algorithm to be clinically applicable, it must perform accurately and do so consistently across a range of datasets expected in different clinical environments. Unfortunately, when deploying histopathology AI solutions to different datasets, a so-called 'domain shift' is often observed, meaning that the properties of histology images and/or distribution of prediction scores are different. Such differences include technical, digital and compositional biases of the training data[53] or even minor artifacts such as dust, scratches or fingerprints on slides[128]. Without mitigation, this can adversely influence performance of AI systems and lead to erroneous predictions, often exacerbated by a degree of overconfidence. Because the ultimate aim is to use AI systems in clinical routine, this is highly undesirable. One way to mitigate this problem is to ensure that a robust quality-control procedure is in place, enabling the algorithm to detect unusual input data[129]. Another approach is to use 'domain adaptation' methods that allow extending AI models to new datasets in an unsupervised manner, without the need to retrain models from scratch or collect new labeled data[130]. In addition, AI systems should be trained or at least validated on the target population before routine use.

Biases in AI systems. In addition, AI systems can reproduce biases inherent in the datasets that they are trained on and analyze. This is problematic because it can propagate sexism, racism and other types of discrimination inherent in many medical datasets[131,132]. Some of these problems can be addressed by improved quality control, ensuring that only high-quality and appropriate data are used as an input for AI models. Alternatively, AI systems themselves can be made more resilient with respect to variations in input data. As a simple way to achieve this, some studies have proposed to train these systems on large multinational cohorts with broad variability[58,62]. However, aggregating such large datasets is often hampered by practical and legal barriers. In parallel, technical improvements have enabled researchers to train more robust AI models with improved generalizability even on smaller datasets[29,61]. We anticipate that, in the next decade, this trend will continue and newer, more robust AI architectures for histopathology will become available for research use. To mitigate the problems associated with AI in computational pathology, several hardening measures are being and should be investigated (Fig. 6a).

Explainability and plausibility of AI systems. AI systems are often referred to as 'black boxes' because their decision-making process is frequently opaque. The general consensus is that this lack of inherent explainability is problematic as this produces the aforementioned biases, creates difficulties in detecting false positives and negatives and also hides potential insights that may be derived from AI[133]. However, others have argued that a well-performing model does not necessarily have to be explainable to be useful[134]. The visualization of prototypical images associated with an algorithm's decision can be a useful way to grasp the underlying

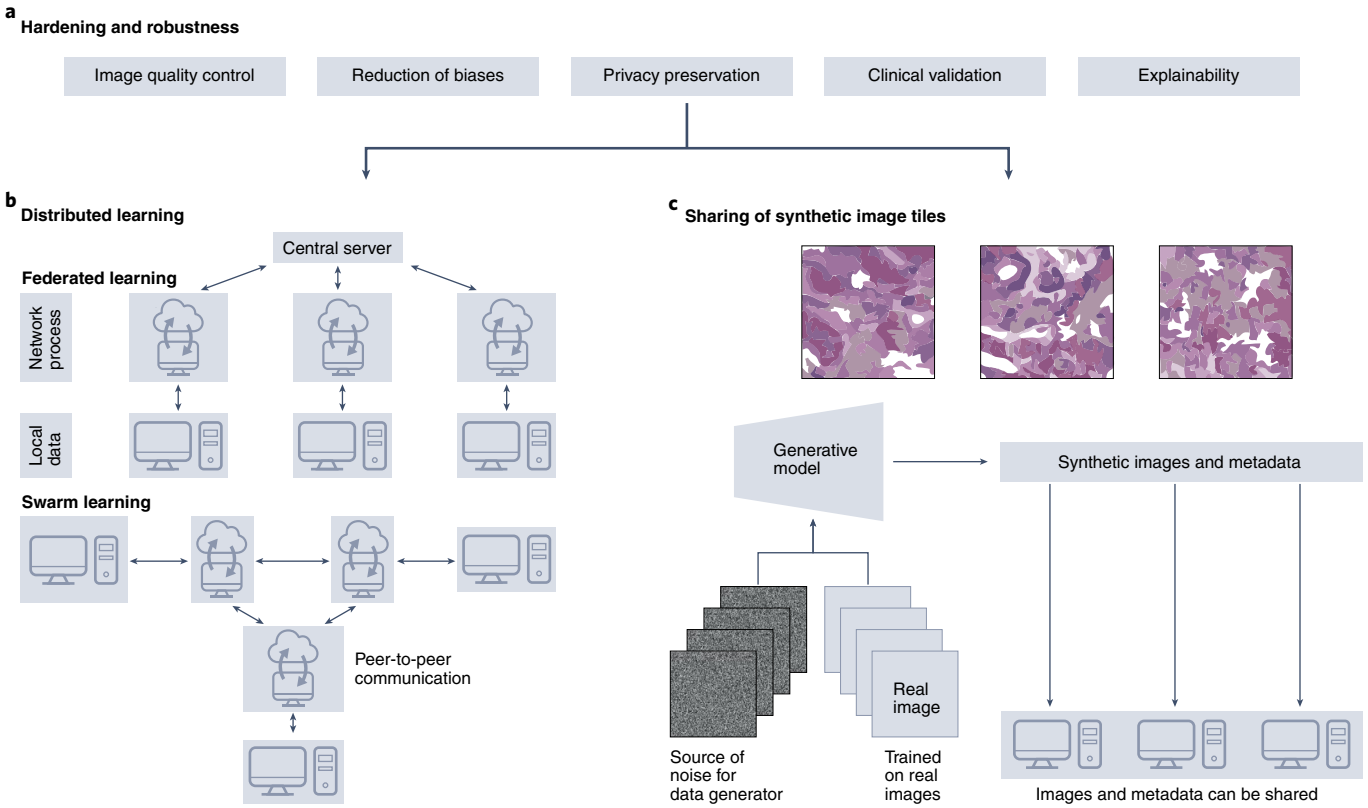

Fig. 6 | Robust computational pathology with focus on privacy-preserving deep learning. a, Progress and clinical translation of AI methods is made possible by a number of enabling technologies, especially precautions for hardening and robustness of AI systems. **b**, Distributed AI training can help multiple participants to co-train a model without exchanging data. In federated learning, a central process coordinates model updates while in swarm learning, this role is taken by the decentralized network via a blockchain mechanism. **c**, In principle, generative AI models can also be used for data sharing. In some cases, precautions against privacy leaks are required for peace of mind. Other potentially important applications of generative AI models are their potential use for explainability, quality control and teaching.

features. This can be especially insightful when the learning task is not based on a visually defined trait such as mutations[21,22]. Such overviews can also make use of *t*-SNE (*t*-distributed stochastic neighbor embedding) or UMAP (uniform manifold approximation and projection) plots of the underlying image features to reveal which images the algorithm considered similar[19,135]. Furthermore, it is possible to inspect specific parts of an individual image, for example, by extracting highly predictive image regions[19] and prediction heatmaps[40]. A similar, more model-centric approach is through model attention heatmaps[29] or deep dream images[48] that can reveal informative image features. These visualizations and newer methods for explainability such as activation atlases[136] or identification of specific multimodal neurons[137] allow human experts to partly recapitulate the algorithm's decision by offering some level of perceptive and mathematical interpretability[138]. However, visualization does not equal explanation and such methods are still far from providing true explainability of the inner mechanisms of AI models. This may change with the development of explainable AI (XAI) algorithms that also learn explicit abstractions, rules or even natural language descriptions of their decision making[139]. The leaps made by AI in connecting images and text[107,140] are of particular relevance to histopathology, in which algorithms may not only learn from but also learn to generate basic histopathology reports. Hence, we anticipate that, in the next decade, biomedical researchers will adopt these methods and apply them to cancer research, mirroring past developments in which new techniques developed in non-medical fields were adopted by biomedical researchers with a similar delay[13,141].

Clinical AI workflows. As the field of computational pathology matures, more and more high-level programming packages become available for academic use. For end-users without programming skills, the open-source software QuPath[142] remains a powerful way to analyze images. For researchers with basic proficiency in the programming language Python, multiple packages allow easy application of end-to-end workflows, for example, CLAM[29], DeepMed[143], TIAToolbox[144], PathML[145] and slideflow[146]. However, these pipelines are currently only available for research use, whereas developing software for clinical application is an entirely different feat. In addition, any diagnostic, prognostic or predictive biomarker that is used in clinical routine should undergo rigorous testing and ideally prospective validation[54]. Rigorous standards should already be applied to preclinical and early clinical work; and indeed this is increasingly mandated by AI-specific reporting guidelines for scientific studies[147,148].

Despite the great advances described above, clinically mature adaptations are still rare. Beyond demonstrating sufficient accuracy and utility, achieving generalizability of a given image-analysis system to evaluate its performance in rigorous trials and to embed it into real-world practice remains a key issue[54]. Blinded, prospective trials of diagnostic AI systems have successfully been performed[149] and should also be performed in the future to enable clinical deployment of AI biomarkers. In addition, regulators have recommended guiding principles for AI development, such as the 'Good Machine Learning Practice' (ref. [150]). A critical point that is highlighted in these guidelines is the topic of human–computer interactions, which describes how pathologists will use AI models in the real world and

requires elements of explainable AI. Few applications have passed this bar yet. Detection and grading of prostate cancer has received broad attention[27,33,34] due to the high prevalence of this disease and the typically large interobserver variation in histopathology assessment. In 2021, the US Food and Drug Administration granted marketing authorization to a prostate cancer-detection software by the company Paige[151].

Nevertheless, current diagnostic routine still involves visual assessment of even the simplest quantification tasks by pathologists. One of the key reasons for the lag in pathology AI systems is that, unlike many other medical imaging technologies, pathology routine workflows are rarely fully digital[152]. However, the benefits of digital pathology applications are expected to lead to the digitization of histopathology workflows in many pathology departments over the next decade. This is especially the case given the potential to automate existing diagnostic tasks, augment current workflows and reduce the workload of pathologists, highly trained professionals who are in global shortage, a fact that is especially problematic in light of the rising global cancer cases in an aging population.

Conclusion

Combining the rapid advances in computer vision, molecular pathology, genomics and bioinformatics, computational pathology allows researchers and clinicians to quantify a cancer's histopathology at unprecedented detail and scale. AI algorithms provide the framework for distilling biologically and clinically relevant information from vast amounts of molecular and histopathological data. In addition to diagnostic and prognostic tasks, a series of publications have demonstrated that AI algorithms reveal diverse histological patterns indicative of a broad range of molecular and genomic alterations. In some cases and when sufficient training data are available, the underlying genetic alterations may even be predicted from standard histopathology slides with accuracy comparable to that of molecular tests as in the case of MSI. Although it is unlikely that AI-based predictors will replace genomic profiling altogether, they may act as a readily available first diagnostic tool, offering the ability to assess whether genomic alterations of unknown importance produce the expected phenotypes and providing spatial contextualization. The latter is especially important as tumors are not homogeneous but rather constitute ecosystems of genetically diverse cancer cells that interact with a broad range of normal cell types. Understanding such patterns of cellular phenotypes and interactions through AI will provide new insights into cancer biology and the basis for identifying new clinical biomarkers.

As the first AI-based histopathology algorithms find their way into the clinic, a number of roadblocks need to be overcome. International data sharing will help train more robust and more accurate algorithms for clinical practice. This will need to be supported by more resilient AI algorithms and innovative distributed learning methods that will help to overcome barriers generated by data protection and will enable individual parties to benefit from shared data storage. Achieving this will require data-sharing frameworks and standardization similar to those set in the field of genomics by the Global Alliance for Genomics and Health (https://www.ga4gh.org/) or the International Cancer Genome Consortium—Accelerating Research in Genomic Oncology (https://www.icgc-argo.org/). Creating community standard datasets, such as ImageNet, for routine objects will not only spur faster development of new algorithms but also allow objective evaluation of new approaches.

Similar to the emerging field of genomic medicine that is driven by affordable sequencing technologies and reliable algorithms to inform oncological therapies, AI-supported computational pathology is likely to transform how cancers are diagnosed, studied and treated in the future.

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Additional information

Correspondence should be addressed to Moritz Gerstung or Jakob Nikolas Kather.

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