Bioinformatics, Digital Pathology, and Computational Pathology for Surgical Pathologists

Sambit K. Mohanty, Saba Shafi, and Anil V. Parwani

Contents

S. K. Mohanty
The Advanced Medical Research Institute and CORE Diagnostics, Bhubaneswar, Odisha, India

S. Shafi · A. V. Parwani (✉)
Department of Pathology, The Ohio State University, Wexner Medical Center, Columbus, OH, USA
e-mail: anil.parwani@osumc.edu

Introduction

- Surgical pathologists dedicate limitless hours in a structured training environment equipped with textbooks, scientific literature, principles, algorithms, and professional expertise to achieve proficiency in the diagnosis of disease and to interpret laboratory data
  - Based on their expertise and experience, they provide a report with the diagnosis, with or without prognostic factors
- With the emergence of genomics, proteomics, informatics, and associated metadata, in addition to the clinical, imaging, and laboratory data, surgical pathologists are now well equipped with managing, interpreting, and leveraging data of unmatched complexity

- **Bioinformatics** is a branch of science that refers to the management, acquisition, manipulation, and presentation of complex biological data sets, while clinical informatics is the application of information management in health care to promote safe, efficient, effective, and personalized care
- **Computational pathology (CPATH)** is the analysis of digitized pathology images with associated metadata, typically using artificial intelligence (AI) methods
  - Deep learning (DL) is a type of AI method, commonly used in CPATH that can 'learn' how to perform tasks based on examples
  - Training a typical supervised deep learning algorithm in CPATH involves large amounts of laboratory training data. Understanding the components of bioinformatics pipelines is also important for surgical pathologist
  - A few examples of bioinformatics-based computational pathology include predicting cancer outcomes from histology and genomics using convolutional networks and automated Gleason grading of prostate cancer tissue microarrays via deep learning

Bioinformatics, a Distinct Field in Pathology and Where Are We Now

- **Bioinformatics** is a blend of Computer Science, Statistics, and Biology. Several pathology laboratories, particularly the molecular pathology laboratories, have recruited specialists in dry laboratories who have skills in data management, statistics, computer programming, and data visualization that are needed for big-data applications in pathology, especially in the analyses of surgical pathology of cancers
- **Pathology Informatics (PI)** is a specialized branch of computational biology that deals with genetic and genomic information
  - Bioinformaticians/Pathology informaticians must have a strong background in population genetics, should have skills in computer programming, and must also have sufficient skills in information systems to set up and maintain computer-based algorithms required for genome-level data analyses and presentations
  - In essence, PI involves the collection, evaluation, reporting, and storage of large complex data sets derived from tests performed in clinical surgical pathology laboratories, and research laboratories to improve patient care and enhance our understanding of disease-related processes
  - PI is also ingrained in translational research tools and translates those into clinical practice required for timely, high-quality, accurate, regulated, contempo-

rary, and safe patient diagnostic, prognostic, and predictive applications
  - Researchers with a background in PI who are entering the field of clinical laboratory practice need additional training to fit themselves into the clinical environment. Also, scientists coming from a wet laboratories background require targeted training in computational and information technologies

Pathology Bioinformaticians and Their Role

- A clinical bioinformatician manages the complete suite of information and computational technologies that tracks a sample from intake through clinical report generation and delivery
- Additionally, they are the key managers of data aggregation and data sharing. These specialists typically develop and maintain laboratory information management systems so that samples coming to the laboratories can be tracked throughout the entire testing process and result data is properly linked to the case. For example, once the raw data is produced, bioinformaticians develop, manage, and operate analyses pipelines that synthesize the results into forms comprehensible to the laboratory staff tasked with reporting the results
- Bioinformaticians maintain and develop analysis information management systems which are also used to collect and monitor performance metrics and quality control
- Sequencing database calling algorithms (primary data analysis), alignment and variant calling (secondary analysis), and variant annotation and filtering (tertiary analysis) must all fit together—ideally in a set of linked methods that minimize manual processes such as data reformatting
- All these steps require the use of complex software that needs professional supervision to manage installation, versioning and updating, knowledge of error modes, ability to manage outputs, carry out statistical analyses of validation data, and maintain quality standards
- The latter is exceptionally important because the quality metrics for sequencing are complex and failure to understand them deeply leads to increased costs, slower turnaround, excessive requirements for confirmatory testing, and even interpretive errors

Specialized Training in Bioinformatics

- When a new clinical laboratory discipline begins to coalesce, only some members are scientists who are already practicing clinical bioinformatics

- Some of these scientists have mastered bioinformatics skills by self-instruction, while other laboratories recruit research bioinformatics specialists or have new trainees enter the diagnostic laboratories with a knowledge of computational biology
- A group of scientists is effectively putting together this new discipline, and in the process, they are creating a pronounced impact on clinical diagnosis
- The next step is the recognition of the need for training standards and a process for the certification of specialists
- Individuals who have already in practice should be offered the opportunity to certify under a credentialing program
- Scientists who are already practicing in this area should be immediately eligible for certification after passing a skill-and knowledge-oriented board examination
- Two-year fellowships for new trainee scientists entering the field would be ideal to ensure that they have mastered all the skills necessary for dry laboratories clinical diagnostics
- These individuals must have training in human, population, and medical genetics and genomics
- They must master the basic principles of human disease genetics, gain exposure to the real-world problems of genetic diagnosis, and get specific training in ethical clinical practice
- These individuals need exposure and competence in the programming languages used in routine laboratory practice and systems architecture, and the algorithmic basis for primary and secondary analyses and develop an approach for tertiary analyses and reporting

Definitions and Terminologies Related to Bioinformatics and Computational Pathology

Annotation

- Indication of the position and/or outline of structures or objects within digital images, usually produced by humans using a computer mouse or drawing tablet. Annotations may have associated metadata
- Annotations can be manually generated or can be established by algorithm tools

Artificial Intelligence (AI)

- A branch of computer science that deals with the simulation of intelligent behavior in computers
- Recently, there has been a momentous drive to apply advanced AI technologies to diagnostic medicine

- The introduction of AI has provided vast new opportunities to improve health care and has introduced a new wave of heightened precision in oncologic pathology
- The impact of AI on oncologic pathology has now become apparent, and its use with respect to oral oncology is still in the nascent stage

Black Box/Glass Box

- A neural network can be perceived as a black box that lacks a clear depiction of the image features used for a decision
- However, several methods can be employed to transform it into a glass box to understand the relationship between the input parameters and the output of the network

Cloud Computing

- The practice of using a network of remote servers hosted on the internet to store, manage, and process data, rather than a local server or a personal computer

Computational Pathology (CPATH)

- A branch of pathology that involves computational analyses of a broad array of methods to analyze patient specimens for the study of disease

Convolutional Neural Network (CNN)

- A type of deep neural network particularly designed for images. It uses a kernel or filter to convolve an image, which results in features useful for differentiating images

Deep Learning

- The subset of machine learning composed of algorithms that permit software to train itself to perform tasks by exposing multilayered artificial neural networks to vast amounts of data
- Data is fed into the input layers and is sequentially processed in a hierarchical manner with increasing complexity at each layer, modeled loosely after the hierarchical organization in the brain
- Optimization functions are iteratively trained to shape the processing functions of the layers and the connections between them

Data Augmentation

- Method commonly used in deep learning to increase the training data using operations such as rotating, cropping, zooming, and image histogram-based modifications
- This provides several advantages such as promoting positional and rotational invariance, robustness to staining variability, and improves the generalizability of the classifier

Digital Pathology (DP)

- A blanket term that encompasses tools and systems to digitize pathology slides and associated metadata, their storage, review, analyses, and enabling infrastructure

Gold Standard

- The practical standard that is used to capture the 'ground truth'
- The gold standard may not always be perfectly correct, but in general, it is viewed as the best approximation

Ground Truth

- A category, quantity, or laboratories assigned to a dataset that provides guidance to an algorithm during training
- Depending on the task, the ground truth can be a patient- or slide-level characterization or can be applied to objects or regions within the image
- The ground truth is an abstract concept of the 'truth'

Image Analysis

- A method to extract typically quantifiable information from images
- Image analysis can be applied to images of histology slides, but the term itself is broader and applies to the extraction of information from any image, biomedical or not

Machine Learning (ML)

- A branch of AI in which computer software learns to perform a task by being exposed to representative data

Metadata

- In the context of DP, the term metadata describes descriptive data associated with the individual, sample, or slide
- It may include image acquisition information, patient demographic data, pathologist annotation or classification, or outcome data from treatment
- Typically, metadata are entries that allow searches in databases
- Highly complex, large, multiple-time-point associated data, such as longitudinal image data (such as radiology) or genomic data, is not usually called 'metadata'

Supervised Machine Learning

- Supervised learning is used to train a model to predict an outcome or to classify a dataset based on a laboratory associated with a data point (i.e., ground truth)
- An example of supervised machine learning includes the design of classifiers to distinguish benign from malignant regions based on manual annotations

Unsupervised Machine Learning

- Unsupervised learning seeks to identify natural divisions in a dataset without the need for ground truth, often using methods such as cluster analysis or pattern matching
- Examples of unsupervised machine learning include the identification of images with similar attributes or the clustering of tumors into subtypes

Whole-Slide Image (WSI)

- Digital representation of an entire histopathology glass slide, digitized at microscope resolution
- These whole-slide scans are typically produced using slide scanners
- Slide scan viewing software enables inspection of the image in a way that mimics the use of a traditional microscope; the image can be viewed at different magnifications

Differences Between Traditional Image Analysis and Computational Pathology

- Traditional image analysis differs from the deep learning based computational pathology in the following ways (Table 2.1)

Table 2.1 Differences between traditional image analysis and deep learning-based computational pathology

Characteristics	Image analysis	Deep learning-based computational pathology
Definition and tasks performed	It is the extraction of meaningful information from the digital images by means of digital image processing techniques. It is the automation of repetitive tasks, e.g., cell counting, quantification of immune staining areas, etc.	It is the analysis of digitized pathology images with associated metadata, typically using AI methods, and the image features are correlated with the patient metadata that assist in disease diagnosis and predict therapy.
Tuning of the parameters used	Image features are manually tuned.	Automatic learning and extraction of a large number of features by computer-based algorithms.
Algorithm testing	A few marked regions of the stained slide.	The entire stained slide.
Computer unit used	Central processing unit.	Graphics processing unit.
Number of images required in the training set	It depends on the application, usually low.	Usually, very high images are required.

Digital Pathology, Machine Learning, and Computational Pathology

- The development of slide scanners has made the process of virtualizing and digitalizing the whole glass slides possible
- DP involves the process of digitizing histopathology, immunohistochemistry, and cytology slides using whole-slide scanners and the interpretation, management, and analysis of these digitized whole-slide images using computational approaches
- The digital data of the slides can be stored in a central cloud-based space allowing for remote access to the information for manual review by a pathologist or automated review by a computer-assisted data algorithm
- This enables AI, a branch of computational biology that generates the data algorithms, to be applied to pathology
- Based on the degree of intelligence, AI can be divided into two major categories such as weak AI and strong AI
- Weak AI or artificial narrow intelligence refers to the classification of data based on a well-established statistic model that has already been trained to perform specific tasks, while strong AI or artificial general intelligence (AGI) can create a system, which can function intelli-

gently and independently by executing machine learning from any available normalized data
- Generally, ML is an AI process to allow a computer to automatically learn and improve from the data set by itself and to solve problems without being programmed during the process
- ML is an advanced branch of AGI using a large amount of initial data, training set, to build statistic algorithms to interpret and act on new data later
- Currently, various ML-based approaches have been developed and tested in pathology to assist pathologic diagnosis using the basic morphology pattern such as cancer cells, cell nuclei, cell divisions, ducts, blood vessels, etc.
- Deep learning, otherwise known as deep structured learning, is a subfield of ML that is based on artificial neural networks (ANNs) in which the statistic models are established from input training data
- Deep neural networks provide architectures for deep learning
- The ANNs can perform their own determination as to whether their interpretation or prediction is correct, resembling a biological complex neural network of the human brain
- ANNs are comprised of three functional layers of artificial neurons, known as nodes, which include an input layer, multiple hidden layers, and an output layer
- The artificial neurons are connected to each other in the ANNs, and the strength of their connections is known as weights
- The connections between artificial neurons in the ANNs are assessed using statistical methods, including clustering algorithms, K-nearest neighbor, support vector machines (SVM), and logistic regressions
- The involved artificial neurons, which are related to the output event, and their associated connections, which bear different weight, need to be trained by qualified big dataset to achieve an optimized algorithm for specific tasks
- The CNNs are a type of deep multilayer neural network particularly designed for visual image
- It employs convolutional kernels, a set of learnable filters, to build up a pooling layer that can effectively reduce the dimensions of the image data while still retaining its characteristics
- By flattening an image, removing, or reducing the dimensions, convolutional kernels act as a preprocess treatment that then allows for computer vision and machine vision models to process, analyze, and classify the digital images, or parts of the image, into known categories
- With slide scanning technology getting faster and more reliable, a larger volume of WSI data becomes available to train and validate CNN models

- In combination with clinical information, biomarkers, and multi-omics data, CPATH will become part of the new standard of care
- Computational pathology not only facilitates a more efficient pathology workflow, but also provides a more comprehensive and personalized view, enabling pathologists to address the progress of complex diseases for better patient care

Training Algorithm

Case Selection

- Patients' selection is the initial step in training the algorithm (Fig. 2.1)
- Both training set and validation set must include all sample types or variants which are related to the subject of diseases including stages, grades, histologic classification, complication, etc., to eliminate false-negative and false-positive scenarios
- Still very much a machine-driven process, algorithms have no way to recognize the variants that have not been included in the training set
- The criteria for the samples and subsequent slide selection for the learning set need to be established by experienced pathologists alongside a computational team
- Confounding variables must be isolated and removed. For example, the patients with other medical conditions which may interfere with the outcome should be eliminated
- In addition, inadequate slide preparation including blurred vision, over- or under-staining, air-bubbles, and folded tissue can produce inaccurate results and wrong algorithms
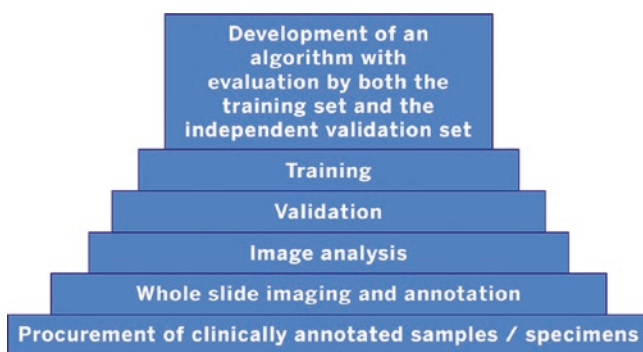

Fig. 2.1 The process of creating an algorithm is basically divided into the above steps. The initial step involves the procurement of clinically annotated samples/specimens, followed by the process of whole-slide imaging and annotation. Based on the image analysis data, an algorithm is developed and trained by both the training set and the independent validation set

- The comprehensive initial and follow-up clinical information, as well as laboratory results, should be collected and included
- The more relevant the information included, the more accurate the resulting algorithm

Whole-Slide Imaging

- Histopathology has been an integral part of the work of pathologists since the seventeenth century
- Today, histopathology largely remains a manual process in which pathologists examine glass slides using conventional brightfield microscopy
- Advances in digitization of glass slides in pathology occurred much later than the digital transformation witnessed in radiology, where digital sensors are widespread
- When histologic glass slides are digitized, they can be remotely viewed by a pathologist on a computer screen, or digitally analyzed using image analysis techniques
- At its inception, digital image analysis was predominantly used by researchers and often limited to individual fields of views, which was cumbersome and could introduce bias
- WSI allowed developments that have brought us from the application of traditional image analysis techniques on small manually selected regions of interest, to what is the current state-of-the-art in digital pathology: techniques that process the entire slide image automatically
- This allowed researchers to identify features not easily analyzed by visual evaluation alone
- Several slide scanning systems for WSI have been approved by the US Food and Drug Administration (FDA) to be used in clinical settings (Table 2.2)
- The first FDA-approved Ultra-Fast Scanner, the Philips IntelliSite Pathology Solution (PIPS), has a resolution of 0.25 μm/pixel, scanning speed of 60 s for a 15 × 15-mm scan area, and scanning capacity of 300 slides in one load
- The Aperio AT2 DX System from Leica Biosystems has 400 slide capacity for brightfield and fluorescent slides
- File sizes of digital images at applicable resolution vary depending on the scan area on the glass slides
- In general, pathology images are tremendously large, in the range of 1–3 GB per image
- Therefore, it requires a high capacity and fast digital working computer
- Furthermore, the number of slides needed to achieve a clinically accepted algorithm may vary by tissue type and diagnosis
- Campanella et al. showed that at least 10,000 slides are necessary for training to reach a good performance

Table 2.2 Various whole-slide imaging systems/devices

Name of the device	Vendor	Brightfield scanning technology	Fluorescence scanning technology	Slide loading capacity	Brightfield scanning speed	Fluorescence scanning speed	Highest throughput slides/h	Resolution (μm/pixel) × 20/×40 objective
VENTANA iScan HT	ROCHE	–	Integrated LED	160	Less than 2 min per slide	–	–	–
Aperio AT2	Leica	TDI line scan	No	400	72 s (20×)/3 min (40×)	No	50	0.50/0.25
VS200	Olympus	2/3-inch complementary metal oxide semiconductor, 3.45 × 3.45 μm pixel size	1-inch complementary metal oxide semiconductor, 3.45 × 3.45 μm pixel size	210	80 s	–	–	0.274/0.137
PathFusion	ASI	5 MP complementary metal oxide semiconductor color	–	99	–	–	–	–
TissueScope LE120	HURON digital pathology	0.75	No	120	Less than 1 min per slide	No	–	0.4/0.2
Philips IntelliSite pathology solution	Philips	Time delay and integration line scanning	–	300	60 s	–	–	0.25
3DHISTECH Pannoramic 250 FLASH III	Epredia	12 MP 12-bit camera with xenon flash illumination	Additional 4.2 MP 16-bit camera with 6-channel LED	250	35 s (20×)/1 min 35 s (40×)	5 min @ 31× 15 min @ 62×	60	0.242/0.121
ProScanner APro 5	AMOS	–	–	–	–	–	–	–
HLM8	Histo-line laboratories	–	–	–	–	–	–	–
Axiocam 208 color	ZEISS	No	1/2.1-inch Sony complementary metal oxide semiconductor, 1.85 × 1.85 μm pixel size	–	–	–	–	–
Axiocam 202 mDavidono	ZEISS	1/2.3-inch Sony complementary metal oxide semiconductor, 5.86 × 5.86 μm pixel size	No	–	–	–	–	–

Table 2.3 Basic principles for the development of supervised deep-learning algorithm development in computational pathology

Obtaining ground truth data
• Patient outcome data
• A field from the pathology report or laboratory information system
• A quantitative score assigned to the case
• Manually provided by a pathologist
• Considerations in acquisition
– Streamlined workflows
– Single common annotation tool
– Trade-off between quantity and accuracy
Good practices
• Training images must be representative of the image algorithm it is designed to be applied to
• Use a wide variety of data sources
• Use consistent preimaging steps
• Apply manual or automated image quality control processes
• Use larger and more representative training sets
• Calibrate algorithms for each laboratory prior to being used for clinical work
• Apply image preprocessing strategies such as color normalization
• Data augmentation to artificially add variation and increase (or balance) the training data
• Test developed models using a variety of test and validation sets to avoid overfitting

- The authors also observed the discrepancy in the prediction between Leica Aperio and PIPS and found that brightness, contrast, and sharpness affect the prediction performance
- Whole-slide image analysis techniques are now routinely utilized for basic and translational research, drug development, and clinical diagnostics including laboratory-developed tests and in vitro diagnostics
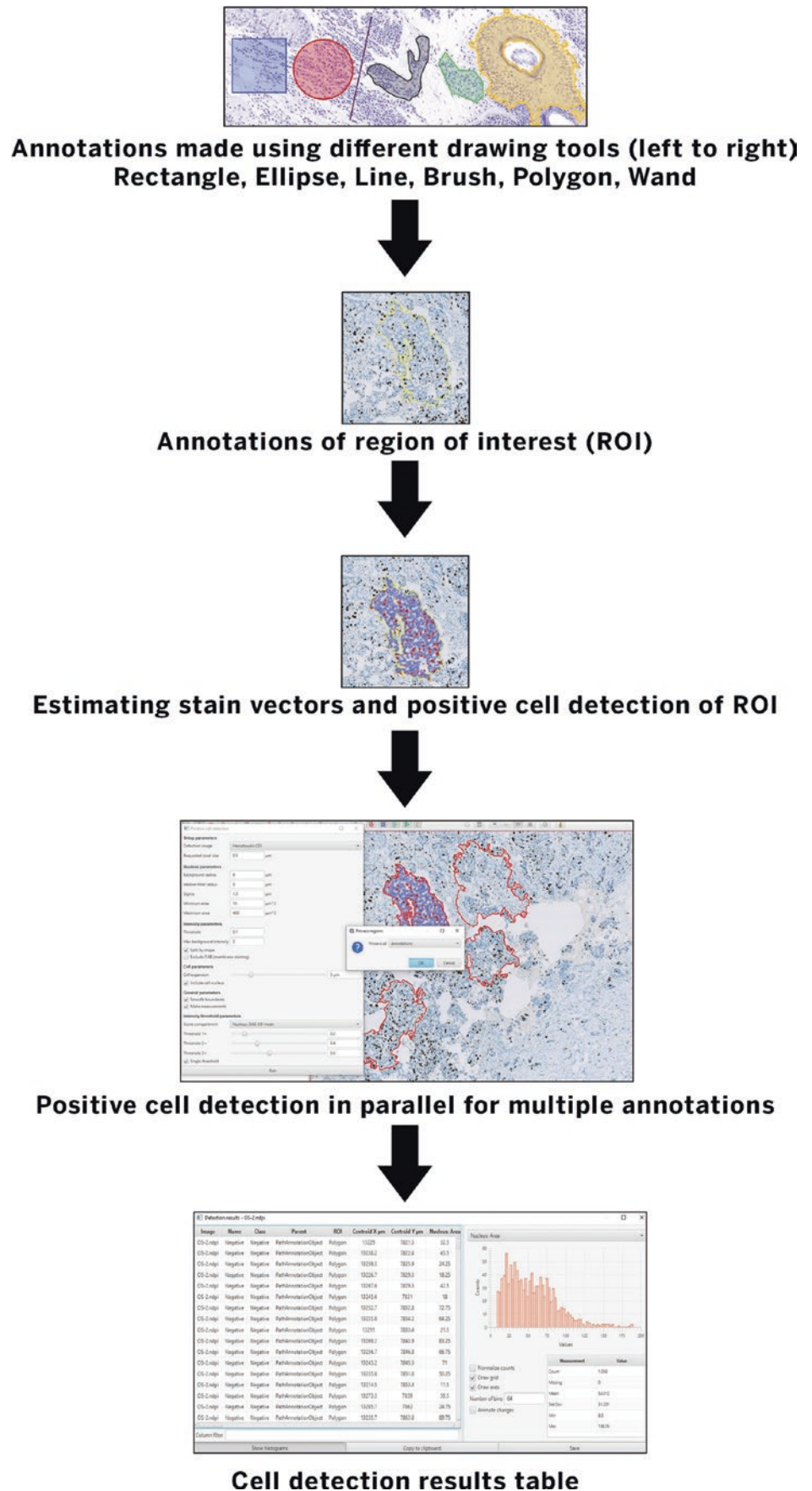
Traditional Image Analysis Enhanced by Machine Learning

- Traditional digital image analysis focuses on three broad categories of measurements: localization, classification, and quantification of image objects
- This method is an iterative process where typically a few parameters are manually tuned, built into an algorithm, and often tested only on a region of the slide image

- Aspects that fail a quality control review are tweaked until the algorithm performance meets predetermined analysis criteria
- ML has facilitated significant advancements within the field of image analysis, as it often allows the generation of more robust algorithms that need fewer iterative optimizations for each dataset, compared with methods where parameters are manually tuned
- Supervised ML techniques, in which an algorithm is trained using ground truth laboratories, are particularly effective in image segmentation (detection of specific objects) and classification (such as tumor diagnosis) tasks
- The ground truths may be a category or laboratories assigned to a dataset that provides guidance to an algorithm
- The capabilities of ML have dramatically expanded in the last decade due to the developments in deep learning, an approach that enables an algorithm to automatically discover relevant image features that contribute to computer vision tasks
- One of the first uses of deep learning in histopathology was the work of Ciresan et al. in the International Conference on Pattern Recognition (ICPR) challenge in 2012, which focused on fully automated recognition of mitotic figures in hematoxylin and eosin (H&E)-stained breast cancer tissue
- Using CNNs, the authors were able to generate results that far exceeded those of the competition
- These early studies applied ML to histopathology using small, manually selected regions of interest, but later research showed that these techniques could work equally well on whole-slide images
- In DP, ML-enhanced image analysis is now widely employed by researchers and implemented in several commercially available image analysis software products (Table 2.3)
- For digital slide analysis, Senaras et al. described a novel deep-learning framework, called DeepFocus, which enables the automatic identification of blurry regions in digital slides for immediate re-scan to improve image quality for pathologists and image analysis algorithms

- Janowczyk et al. presented an open-source tool called HistoQC to assess color histograms, brightness, and contrast of each slide and to identify cohort-level outliers (e.g., darker or lighter stain than other slides in the cohort)
- These methods play an essential role in the quality control of whole-slide images to standardize the quality of images in computational pathology
- Due to improvements in various smart image-recognition algorithmic discriminators, based on high-capacity deep neural network models, the pathologist can be released from extensive manual annotations for each whole-slide images at the pixel level so that they can focus on other parts of the clinical workflow
- The patch-based whole-slide images (224 × 224 to 256 × 256) have been widely used in many machine learning domains to train classifiers for diagnostic or prognostic tasks
- For example, Campanella et al. employed multiple instance learning (MIL) approaches with 'bag' and 'instance' based on convolutional neural networks and recurrent neural networks to classify the prostate cancer images of H&E slides
- Kapil et al. applied deep semi-supervised architecture and auxiliary classifier generative adversarial networks, including one generator network and one discriminator network, to automatically analyze the PD-L1 expression in immunohistochemistry slide of late-stage nonsmall cell lung cancer needle biopsies

- Barker et al. revealed an elastic net linear regression model and weighted voting system to differentiate glioblastoma and lower-grade glioma with an accuracy of 93.1%
- Several machine learning tools such as QuPath, Halo, Visiopharm, Image J are readily available for automatic quantification of various biomarkers
- Of these, QuPath is an open-source digital pathology tool that has been used for the objective assessment and scoring of several markers like Ki67, KLF4, SOX2, etc.
- QuPath performs WSI analysis through tissue and nuclei segmentation and automatically computes a series of features with various algorithms
- Briefly, stain vectors are identified using QuPath tools, then applied to ROI using the 'tissue detection' command (Fig. 2.2)
- After a step of evaluable tissue selection, excluding all nonassessable areas and regions without tumors, the 'cell detection' tool is used to segment nuclei
- If necessary, it is possible to then proceed to draw further annotations around areas of interest
- These can be processed one-by-one by running 'positive cell detection' on an annotation when it is selected, or else they can be processed all together (in parallel)
  - This has been used for the assessment of the proliferative compartment of solid tumors on H&E-stained sections as well as for an objective determination of Ki67 in cancers

Fig. 2.2 The relationship between different levels of artificial intelligence and important hurdles. The four challenges are experienced computational experts who can develop algorithms for particular issues, hardware limitations, qualified applicable data, and ethical issues

Annotations made using different drawing tools (left to right) Rectangle, Ellipse, Line, Brush, Polygon, Wand

Annotations of region of interest (ROI)

Estimating stain vectors and positive cell detection of ROI

Positive cell detection in parallel for multiple annotations

Cell detection results table

Pathologist-Centered Medical System

- Although most AI research is still focused on the detection and grading of tumors in digital pathology and radiology, computational pathology is not limited to the detection of a morphological pattern
- It can also contribute to the complex process of analysis and judgment using demographic information, digital pathology, −omics, and laboratory results
- Therefore, AI has the potential to contribute to nearly all aspects of the clinical workflow, from more accurate diagnosis to prognosis, and individualized treatment
- Multiple sources of clinical data are incorporated into mathematic models to generate diagnostic inferences and predictions, to enable physicians, patients, and laboratory personnel to make the best possible medical decisions
- For example, deep neural networks have been applied to automated biomarker assessment of breast tumor images, such as HER2, ER, and Ki67
- Hamidinekoo et al. created a novel convolutional neural network-based mammography–histology–phenotype–linking–model to connect and map the features and phenotypes between mammographic abnormalities and their histopathological representation
- Mobadersany et al. developed a genomic survival convolutional neural network model to integrate information from both histology images and genomic data to predict time-to-event outcomes and demonstrated that the prediction accuracy surpassed the current clinical paradigm for predicting the overall survival of patients diagnosed with glioma
- As electronic health record (EHR) systems enable us to collect medical data such as age, race, gender, social history, and clinic history, applying these data as independent factors of a particular disease to an appropriate mathematic algorithm becomes feasible
- These integrated data allow pathologists to gain deeper insights and to switch between different algorithms of treatment at different stages of the disease and/or for different statuses of the patient
- As the health-related apps on mobile devices and smart personal trackers become popular, direct access to continuous real-time health information, such as temperature, heart rate, respiratory rate, electrocardiogram, body mass index, blood glucose, and blood oxygen content, can be recorded into individual health data
- These data can then be incorporated into the EHR and laboratory information systems (LIS) to reintegrate into a virtualized and digitalized person, which was not possible previously and was beyond what the human brain alone can accomplish
- This new system of data-driven care requires the pathology, as a cornerstone of modern medicine, to integrate data, algorithms, and analytics to deliver high-quality and efficient care
- The combination of computational pathology and big-data mining offers the potential to create a revolutionary way of practicing evidence-based, personalized medicine

Global Pathology Service Model

- Three essential advancements happened in recent years: the possibility to store a great amount of data from network-attached storage to cloud storage, the growing speed of network from WIFI-6 to 5 G, and high-performance central processing unit (CPU) and graphics processing unit
- These technological improvements not only enhance people's daily life, but also have a great impact on medicine, especially digital and computational pathology (Fig. 2.3)
- Together with the surging development of network and information technology, these technologic improvements allow for the centralization of medical and computing resources—with the benefit of larger sample data volume for optimization of algorithms
- Furthermore, the central cloud-based AI laboratory and data bank of digital and computational pathology make the global network of computational pathology possible
- In local laboratories or centralized scanning centers, histology slides can be converted to whole-slide images and numerical data
- This data can then be transferred to the central laboratory together with EHR data and multi-omics data for further analysis
- Patients in different geographic areas around the world can benefit from more efficient and effective diagnosis, treatment, and follow-up
- In the meantime, pathologists can access the information they need to care for patients or to collaborate with specialists anytime and anywhere
- Deep-learning platforms have the potential to facilitate the discovery of more complicated or subtle connections and to help pathologists make the best clinical decisions to meet every patient's needs

Fig. 2.3 A computational pathology team includes pathologists to identify a clinically relevant issue, data scientists to develop and train the algorithm, and engineers to support the operating environment. Subsequently, during the actual clinical practice, the pathologists play an important role in applying and monitoring the algorithm and relay feedback to the developers for optimization
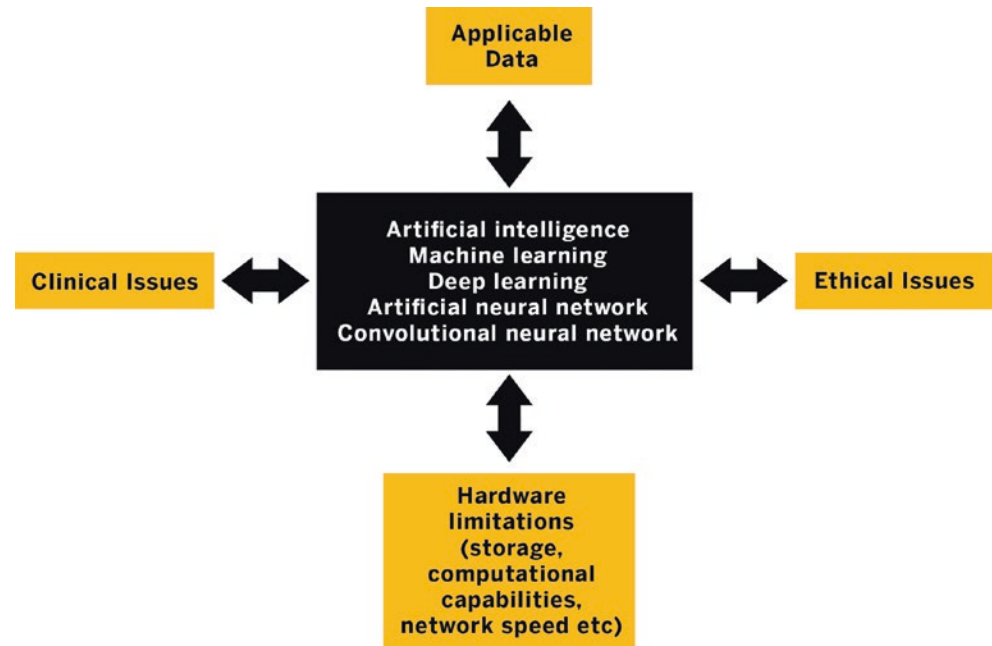
Examples of Bioinformatics and AI-Driven Pathology Workflow

- Increasingly, AI detection is being applied to different subspecialties with various sample types
- Early reports on accuracy have shown to be promising and that the AI-assisted systems have the potential to classify accurately at an unprecedented scale and lay the foundation for the deployment of computational pathology in nearly all subspecialties

Prostate Cancer

- Campanella et al. validated a high-capacity deep neural network-based algorithm to analyze image classification and categorization of 44,732 whole-slide images across three different cancer types, including prostate cancer, basal cell carcinoma, and breast cancer metastases to axillary lymph nodes
- In terms of whole-slide images, they found that ×5 magnification has higher accuracy
- They trained a statistic model with MIL-based tile classifier for each tissue type and achieved area under receiver operating curve (AUC) above 0.98 for all cancer types
- Its clinical application would allow pathologists to exclude 65–75% of slides while retaining 100% sensitivity
- Wildeboer et al. discussed deep learning techniques based on different imaging sources including magnetic resonance imaging, echogenicity in ultrasound imaging, and radio density in computed tomography as computer-aided diagnostic tools for prostate cancer

- They found that the algorithm of convolutional neural network architecture performed equal or better than SVM or random forest classifiers in machine learning
- As usage of AI for diagnosing prostate cancer in biopsies are limited to individual studies, they lack validation in multinational settings
- The PANDA challenge, the largest histopathology competition to date, joined by 1290 developers, has been organized to catalyze development of reproducible AI algorithms for Gleason grading using 10,616 digitized prostate biopsies
- They validated a diverse set of submitted algorithms that reached pathologist-level performance on independent cross-continental cohorts, fully blinded to the algorithm developers
- In United States and European external validation sets, the algorithms achieved agreements of 0.862 (quadratically weighted κ, 95% confidence interval (CI), 0.840–0.884) and 0.868 (95% CI, 0.835–0.900) with expert uropathologists
- Successful generalization across different patient populations, laboratories, and reference standards, achieved by a variety of algorithmic approaches, warrants evaluating AI-based Gleason grading in prospective clinical trials

Colorectal Cancer

- Korbar et al. developed multiple deep-learning algorithms, modified version of a residual network architecture, which can accurately classify whole-slide images of five types of colorectal polyps, including hyperplastic,

- sessile serrated, traditional serrated, tubular, and tubulo-villous/villous polyps
- Among 2074 images, 90% of them were used for model training and the remaining 10% of images were assigned to the validation set
- The overall accuracy for classification of colorectal polyps was 93% (confidence interval (CI) 95%, 89.0–95.9%)
- Bychkov et al. combined CNNs and recurrent neural network architectures to predict colorectal cancer outcomes based on tissue microarray (TMA) samples from 420 colorectal cancer patients
- Their results show that the AUC of deep neural network-based outcome prediction was 0.69 (hazard ratio, 2.3; CI 95%, 1.79–3.03)
- For comparison, pathology experts performed inferiorly on both TMA samples (HR, 1.67; CI 95%, 1.28–2.19; AUC, 0.58) and whole-slide level (HR, 1.65; CI 95%, 1.30–2.15; AUC, 0.57), which implied that deep neural networks could extract more prognostic information from the tissue morphology of colorectal cancer than an experienced pathologist

Breast Cancer

- Wang et al., the team of winner of competitions in the CAMELYON16 challenge, used input 256 × 256-pixel patches from positive and negative regions of the whole-slide images of breast sentinel lymph nodes to train various classification models including GoogLeNet Patch, AlexNet, VGG16, and FaceNet
- The patch classification accuracy is 98.4, 92.1, 97.9, and 96.8% separately
- Among the algorithms, GoogLeNet has the best performance and is generally faster and more stable, which achieved AUC of 0.925 for whole-slide images classification
- With the assistance of deep learning system, the accuracy of pathologist's diagnoses improved significantly as the AUC increased from 0.966 to 0.995, representing ~85% reduction of human error rate
- Furthermore, the open resource of a data set of annotated whole-slide images for CAMELYON16 and CAMELYON17 challenges enable testing of new machine learning and image analysis strategies for digital pathology

Cancer Cytopathology

- Martin et al. applied convolutional neural networks for classifying cervical cytology images into five diagnostic categories, including negative for intraepithelial lesion or malignancy, atypical squamous cells of undetermined sig-

nificance, low-grade squamous intraepithelial lesion, atypical squamous cells cannot exclude low-grade squamous intraepithelial lesion and high-grade squamous intraepithelial lesion, and achieved accuracies of 56%, 36%, 72%, 17%, and 86% separately, which implies convolutional neural networks are able to learn cytological features
- In another cytopathology study, the authors used morphometric algorithm and semantic segmentation network based on VGG-19 to classify urine cytology whole-slide images according to Paris System for Urine Cytopathology and achieved a sensitivity of 77%, false-positive rate of 30% and AUC of 0.8

Challenges and Limitations

- Despite the promises of CPATH and advancement in ML with promising results and benefits, most algorithms used in current clinical practice are limited to traditional image analysis of immunohistochemical stains, which do not employ advanced ML techniques such as deep learning
- In this section, we address the many barriers to implementing CPATH for clinical use, and potential strategies to overcome them (Fig. 2.4)

Infrastructure Considerations

- Implementation of CPATH may require a significant investment in IT infrastructure
- In general, data to be analyzed is captured as images of tissue sections, often scanned at 20× or 40× objective magnification
- In clinical practice, pathology images are commonly larger than 50,000 by 50,000 pixels
- As a benchmark, this can translate into estimated file sizes ranging from 0.5 to 4 GB for 40× images, depending on the size of the scan area and image compression type
- The large size of these images may present a problem for evaluation, storage, and inventory management
- The primary computing obstacles that users face are processor speed and memory requirements of local workstations, data storage requirements, and limitations of the network
- For CPATH to perform effectively, it is important that there are safeguards to ensure that images are fully loaded, and that the analysis algorithm is not interrupted due to insufficient bandwidth, processing power or memory
- Additional considerations when running deep-learning algorithms include, but are not limited to, the number of intended users, flexibility of the server or cloud configuration to accommodate new algorithms or caseloads, cybersecurity, and associated costs

Fig. 2.4 Cell detection by QuPath involves annotation of ROI, estimation of stain vectors, followed by positive cell detection, which gives a tabulated result and thus removes subjectivity in pathology
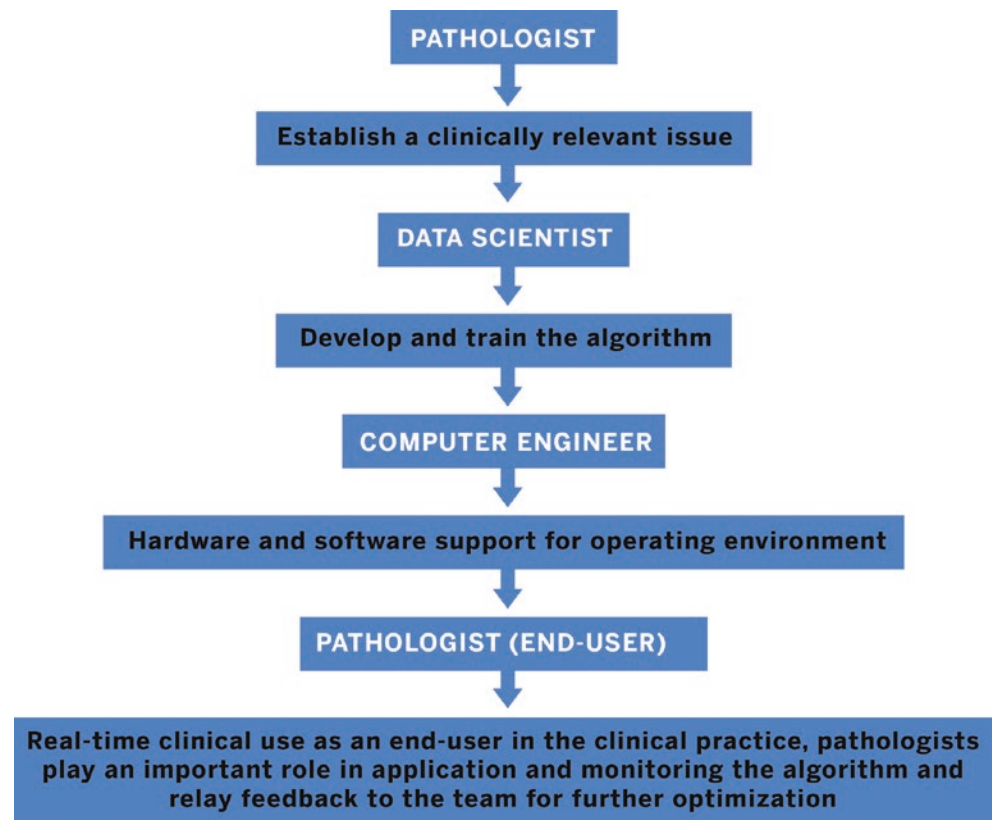
Processor Speed and Sources

- The performance of any image processing is highly dependent on processor speed
- Deep learning is best performed using graphics processing units (GPUs), which can provide significant performance enhancement over central processing units (CPUs)
- Most computers are designed to perform computations on their CPU and use the GPU simply to render graphics
- It may be necessary to purchase a more powerful GPU designed for deep learning; these are generally more expensive and tend to generate more heat
- Some laboratories may therefore elect to dedicate high-performance workstations strictly for deep learning
- However, some vendors offer the ability to perform image analysis at the server or cloud level, which may provide significantly more resources and can potentially distribute deep learning capabilities to a much larger user base

Network Limitations

- For implementations in which either data is stored remotely, or image processing is performed remotely, network bandwidth becomes an important consideration

- The large size of whole-slide images presents a potential hurdle for efficient processing in environments that lack sufficient bandwidth
- Depending on the network implementation, there are several data transfer considerations
- First, digital slide data from the whole-slide scanner must be transferred to its network storage location, which requires the file in its entirety
- Second, the digital slide must be transferred from its network storage location to the image analysis environment (which may reside locally, elsewhere on the network, or in the cloud), which can often be accomplished in a more efficient manner, since the entire image is unlikely to be analyzed at once
- Training a deep learning network on an entire slide image at full resolution is currently very challenging, so it usually operates on a smaller tiled image or patch
- Downscaling (reducing resolution) of these images is one possible approach, but this may lead to loss of discriminative details as using small, high-resolution tiles may lose tissue context
- The optimal resolution and tile size for analysis are highly case-dependent
- If only small regions of interest are to be processed, or if the processing can occur at a reduced magnification,

smaller portions of the virtual slide file need to be transferred due to the pyramid structure of most WSI file formats

Acquiring Training Data

- Deep learning is generally extremely data-hungry, especially compared with traditional image analysis where 'important features' are manually selected, as it must automatically identify these features
- For supervised learning, in addition to the raw image sets, a ground truth must be included in the dataset to provide appropriate diagnostic context
- Algorithms can then be trained to predict or characterize an image guided by the ground truth provided
- The ground truth may be derived from patient outcome data, a field extracted from the pathology report or laboratory information system (e.g., histologic grade), a quantitative score assigned to the case (e.g., molecular testing), or it can be a factor manually provided by a pathologist reviewing the case specifically to support the algorithm training
- Obtaining clinical ground truth data suitable for algorithm development is often time-consuming and challenging
- It usually takes a long time to generate enough survival data from clinical patients, and clinical data are generally locked into an unstructured format within one or more disparate electronic medical records
- The data must be manually or automatically curated before being incorporated into an algorithm
- Furthermore, training may also require manual annotations applied to the digital slide, including the designation of specific areas of interest, for instance identifying cancer from benign tissue
- Obtaining adequately annotated datasets for deep learning by a trained expert can be difficult due to the amount of time required, associated expenses, and the tedious nature of the task
- The use of streamlined workflows and a single common annotation tool with an intuitive user interface can make the task of creating and sharing manual regional annotations considerably easier
- Web-based tools may be ideal in sharing annotations between different research groups, as they avoid the need to install specific software on multiple systems
- In addition, research has shown that, for some tasks, annotations of expert observers (i.e., pathologists) may not always be necessary
- Yet there is generally a trade-off between quantity and accuracy
- Also, training images must be representative of the images that the algorithm is designed to be applied to, and appropriately 'balanced'; for example, contain approximately similar numbers of examples for different objects it is intended to identify

Data Variability

- It is important that supervised algorithms are developed using a wide variety of data sources, to handle variations more robustly when exposed to other datasets
- A pertinent consideration is to implement prospective review using retrospective data during development, and/or verification or validation
- When algorithms are developed using limited datasets supplied by only one or few pathology laboratories, the algorithms may not have incorporated all the variations and artifacts encountered across different labs, including preimaging, imaging, and postimaging steps within the WSI workflow
- This is in part because, in surgical pathology, there is currently no accepted global standard for tissue processing, staining, and slide preparation
- Even digital acquisition may introduce variability. As such, an algorithm designed to perform well on one set of WSIs may not perform equally well when generalized and used around the world by many laboratories
- This could be somewhat alleviated by implementing consistent preimaging steps, applying manual or automated image quality control processes, using larger and more representative training sets, and calibrating algorithms for each lab prior to being used for clinical work
- It is also possible to apply image preprocessing strategies such as color normalization to reduce the impact of stain and processing variability, and data augmentation to artificially add variation and increase (or balance) the training data to make it more representative of the application data
- Other best practices include testing developed models using a variety of test and validation sets to avoid overfitting, and clearly reporting characteristics of the patients used to build a model, since additional training data may be required for it to perform well on other populations
- One may consider the addition of prospective real-world data collection to monitor and optimize performance

Public Sources

- There are currently only limited publicly available datasets with annotated images and associated nonimage patient data that are required for CPATH
- This may be one of the greatest factors limiting progress in the field of CPATH

- However, some initiatives to overcome these hurdles are described below
- The Cancer Genome Atlas (TCGA) has performed comprehensive molecular profiling on approximately 10,000 cancers (National Cancer Institute, The Cancer Genome Atlas, https://cancergenome.nih.gov/)
- In addition to the collection of molecular and clinical data, TCGA has collected WSI data from a subset of its participants
- Some other examples of public digital slide datasets are the breast cancer images used for the CAMELYON competition; the Medical Image Computing and Computer Assisted Intervention Society (MICCAI) 2014 brain tumor digital pathology challenge for distinguishing brain cancer subtypes; and the Tumor Proliferation Assessment Challenge (TUPAC16), which includes hundreds of cases
- The Grand Challenge website (https://grand-challenge.org/challenges/) maintains a list of all challenges that have been organized in the field of medical image analysis
- Some of these challenges offer developers additional pathology digital datasets for CPATH
- However, care must be taken as the quality of public samples may be variable, so they should be carefully tested before use

Crowdsourcing

- An alternative for obtaining large-scale image annotations is crowdsourcing, in which this function is outsourced to an undefined and generally large group of nonexpert people in the form of an open call
- Crowdsourced image annotation has been successfully used to serve a diverse set of scientific goals, including the detection of malaria from blood smears, and estrogen receptor classification
- Compared with public sources or pathologist annotations, crowdsourcing may be cheaper and quicker, but it has the potential to introduce noise
- It is possible that this noise can be compensated by a sufficiently large body of training data, and by having multiple people annotate the same slide to achieve consensus
- But it is imperative to ensure that all annotators are taught to perform the task in the same way

Active Learning

- Active learning is considered semi-supervised learning, which may reduce the size of required training data
- In active learning, the algorithm interactively queries for expert assistance to obtain annotations for ambiguous data points

- Essentially, the algorithm uses a sampling strategy to select small sets of data iteratively for experts to label, only when it has trouble determining the outcome
- For each iteration, the classifier is updated, and then all unlabeled data are re-evaluated for their ability to further improve the classifier
- Thus, active learning offers a solution to the problem of limited data annotations in pathology by having the pathologist engage actively with the algorithm, which evolves through continuous learning

Quality Control and Reliability of the Algorithm

- It is currently difficult to establish strict quality control steps for deep learning algorithms, especially in segmentation problems, for various reasons
- A general principle in training any machine learning algorithm is to split the annotated data into 'training' and 'test' datasets and ensure that these sets are independent when assessing performance
- The algorithm should be trained on the training set and applied to the test set, then the results should be compared with the 'ground truth' associated to the test set
- However, quality control for the segmentation step may suffer from the 'gold-standard paradox'
- This paradox arises from histopathological assessments by the pathologist being considered the gold standard, but the algorithm data may in fact be more reproducible than human assessment
- This may be partially overcome by comparing the algorithm data to patient outcome, to see whether it is better able to predict outcome compared with manual pathology assessment/scoring
- Still, the best methods to determine the reliability of an algorithm applied to novel datasets are an area of active debate
- In addition, local regulations apply to legally market any clinical-grade software solution
- In the USA, such an algorithm should be developed under the Food and Drug Administration's existing Quality System Regulation (QSR, 21 CFR Part 820), and Good Machine Learning Practices (GMLP; https://www.fda.gov/media/122535/download), which are currently being discussed

Understanding Algorithms

- A principal concern with the use of deep learning is that it is very difficult to understand some of the features and neural pathways used to make decisions

- When deep learning is used to automatically extract features from an image that are directly correlated to clinical endpoints, without including a segmentation step where image objects are first extracted (see the section on correlating images to patient response), it is particularly challenging to understand why the algorithm reached its conclusions
- Artificial neural networks have accordingly been described as a 'black box'
- This has led to several concerns: difficulty in correcting an underperforming algorithm; lack of transparency, explicability, and provability for humans who may not trust how an algorithm generates reliable results; and regulatory concerns because, unlike traditional image analysis, in deep learning the image features are abstracted in a way that is very difficult for a human to understand
- In response, there have been efforts to convert deep-learning algorithms into a 'glass box' by clarifying the inputs and their relation to measured outputs, making it more interpretable by a human using a variety of techniques
- By providing information to the reviewing pathologist about the histopathologic features used by the algorithm in a particular instance, trust in the algorithm can be fostered, and synergy between pathologist and machine can be achieved that may exceed the performance of either AI or pathologist alone

Ethics

- In the new era of computation-driven decision-making processes based on AI and machine learning, computational pathology will involve more complicated interactions of massive information from clinical history, omics data, living environment to social habits
- It is very likely that the experts involved in these decision-making processes will no longer be exclusively pathologists
- Instead, the decision-making panel will include other experts such as data statisticians and bio-informaticians, which may raise ethical concerns
- A continuous massive, sensitive health data transfer among clinics, laboratories, and data banks can enable higher precision medicine but, at the same time, increases the security vulnerability
- Policies around the strict protection of patient privacy and personal data create an obstacle for computational pathology to access the health databases need to create more comprehensive training data sets
- General Data Protection Regulation was enacted in May 2018 in Europe to impose new responsibilities on organizations that process the data of European Union citizens for scientific research

- This concept highlights the proportionate approach to regulate computational pathology-related security and ethical issues while not limiting innovation unduly, which is difficult but critical

Cyber-Security

- Cyber-security concerns of CPATH primarily stem from storing large amounts of medical data in cloud-based systems that can be accessed via the Internet
- To minimize a data breach, it is prudent to decouple CPATH data (i.e., digital images) from patient data (i.e., personal identifiers such as medical record number and date of birth)
- Several cloud service providers now offer Health Insurance Portability and Accountability Act (HIPAA) compliant solutions
- In addition, the FDA has created guidelines for cyber-security (U.S. Food and Drug Administration, Postmarket Management of Cyber-security in Medical Devices, 2016)
- The European Union's general data protection regulation (GDPR) imposes similar security requirements on those who process personal data

Future Directions

- Technological innovation in health care is growing at an increasingly fast pace and has been integrated into both our daily lives, such as smart healthy tracker, and diagnostic algorithm in medical practice
- With the rapid development of digital pathology, molecular pathology, and informatics pathology, computational pathology is increasingly involved in many subspecialties such as pulmonary, renal, gastrointestinal, neurology, and gynecology pathology
- We believe the initial phase of AI will start with specific tasks such as the diagnoses of particular cancers and classification of tissue types, which require limited and simple criteria
- For example, the common subtypes and variants of benign and malignant neoplasm in prostate should be included in the training and validation to ensure the feasibility of daily pathology practice
- As a result of more data collection and more powerful computing capacity over time, the clinical applications of AI will be broader, and the number of nonspecific cases in the gray zone or with red flags classified by AI for manual review will be decreased
- The growing medical data, including genomics, proteomics, informatics, and whole-slide images, is expected

to integrate to become a data-rich pathomics and lead to rapid development and prosperity of an AI-assisted computational pathology

- Although many challenges remain, computational pathology with the deployment of digital pathology technology and statistic algorithm will continue to improve clinical workflows and collaboration among pathologist and other members of the patient care team

- The improved infrastructure of the network environment, the enhanced computing capacity, and broad integration of informatics has ushered in new horizons for both computational pathology and collaborative pattern, which makes data travel and cloud-based central laboratory and data bank to deliver better care for patients at lower costs possible

Conclusions

- In the new era of deep learning-assisted pathology, data banking, integration, and cloud laboratory are becoming an essential part of daily practice of pathology

- Furthermore, pathologists, data scientists, and industry are starting to incorporate genomics, proteomics, bioinformatics, and computer algorithms into a large amount of complex clinical information

- Through this process, computational pathology can contribute valuable insights to the diagnosis, prognosis, and, ultimately treatment of disease

- Although many technical and ethical challenges need to be addressed, computational pathology as a synergistic system will lead to an integrated workflow, enabling clinical teams to share and analyze image data in a broader platform

- Currently, deep learning has been applied to solve more and more specialized tasks in medicine

- Several studies discussed above show that algorithm assistance has the potential to not only improve the sensitivity and accuracy of the diagnoses but also improve turnaround time

- Moreover, around 75% of pathologists across 59 countries in the world are interested and excited about using AI as a diagnostic tool

- Finally, despite the challenges and obstacles, the potential of computational pathology will change and improve the current healthcare system in promising and exciting ways

Further Reading

Abels E, Pantanowitz L, Aeffner F, Zarella MD, van der Laak J, Bui MM, Vemuri VN, Parwani AV, Gibbs J, Agosto-Arroyo E, Beck AH, Kozlowski C. Computational pathology definitions, best practices, and recommendations for regulatory guidance: a white paper from the digital pathology association. J Pathol. 2019;249(3):286–94. https://doi.org/10.1002/path.5331.

Acs B, Pelekanou V, Bai Y, Martinez-Morilla S, Toki M, Leung S, Nielsen TO, Rimm DL. Ki67 reproducibility using digital image analysis: an inter-platform and inter-operator study. Lab Invest. 2019;99(1):107–17. https://doi.org/10.1038/s41374-018-0123-7.

Aeffner F, Wilson K, Martin NT, Black JC, Hendriks CLL, Bolon B, Rudmann DG, Gianani R, Koegler SR, Krueger J, Young GD. The gold standard paradox in digital image analysis: manual versus automated scoring as ground truth. Arch Pathol Lab Med. 2017;141(9):1267–75. https://doi.org/10.5858/arpa.2016-0386-RA.

Aeffner F, Zarella MD, Buchbinder N, Bui MM, Goodman MR, Hartman DJ, Lujan GM, Molani MA, Parwani AV, Lillard K, Turner OC, Vemuri VNP, Yuil-Valdes AG, Bowman D. Introduction to digital image analysis in whole-slide imaging: a white paper from the Digital Pathology Association. J Pathol Inform. 2019;10:9. https://doi.org/10.4103/jpi.jpi_82_18. Erratum in: J Pathol Inform. 2019;10:15.

Allen TC. Regulating artificial intelligence for a successful pathology future. Arch Pathol Lab Med. 2019;143(10):1175–9. https://doi.org/10.5858/arpa.2019-0229-ED.

Amgad M, Elfandy H, Hussein H, Atteya LA, Elsebaie MAT, Abo Elnasr LS, Sakr RA, Salem HSE, Ismail AF, Saad AM, Ahmed J, Elsebaie MAT, Rahman M, Ruhban IA, Elgazar NM, Alagha Y, Osman MH, Alhusseiny AM, Khalaf MM, Younes AF, Abdulkarim A, Younes DM, Gadallah AM, Elkashash AM, Fala SY, Zaki BM, Beezley J, Chittajallu DR, Manthey D, Gutman DA, Cooper LAD. Structured crowdsourcing enables convolutional segmentation of histology images. Bioinformatics. 2019;35(18):3461–7. https://doi.org/10.1093/bioinformatics/btz083.

Barker J, Hoogi A, Depeursinge A, Rubin DL. Automated classification of brain tumor type in whole-slide digital pathology images using local representative tiles. Med Image Anal. 2016;30:60–71. https://doi.org/10.1016/j.media.2015.12.002.

Bera K, Schalper KA, Rimm DL, Velcheti V, Madabhushi A. Artificial intelligence in digital pathology—new tools for diagnosis and precision oncology. Nat Rev. Clin Oncol. 2019;16(11):703–15. https://doi.org/10.1038/s41571-019-0252-y.

Bulten W, Kartasalo K, Chen PC, Ström P, Pinckaers H, Nagpal K, Cai Y, Steiner DF, van Boven H, Vink R, Hulsbergen-van de Kaa C, van der Laak J, Amin MB, Evans AJ, van der Kwast T, Allan R, Humphrey PA, Grönberg H, Samaratunga H, Delahunt B, Tsuzuki T, Häkkinen T, Egevad L, Demkin M, Dane S, Tan F, Valkonen M, Corrado GS, Peng L, Mermel CH, Ruusuvuori P, Litjens G, Eklund M, PANDA Challenge Consortium. Artificial intelligence for diagnosis and Gleason grading of prostate cancer: the PANDA challenge. Nat Med. 2022;28(1):154–63. https://doi.org/10.1038/s41591-021-01620-2.

Bychkov D, Linder N, Turkki R, Nordling S, Kovanen PE, Verrill C, Walliander M, Lundin M, Haglund C, Lundin J. Deep learning-based tissue analysis predicts outcome in colorectal cancer. Sci Rep. 2018;8(1):3395. https://doi.org/10.1038/s41598-018-21758-3.

Campanella G, Hanna MG, Geneslaw L, Miraflor A, Werneck Krauss Silva V, Busam KJ, Brogi E, Reuter VE, Klimstra DS, Fuchs TJ. Clinical-grade computational pathology using weakly supervised deep learning on whole slide images. Nat Med. 2019;25(8):1301–9. https://doi.org/10.1038/s41591-019-0508-1.

Candido Dos Reis FJ, Lynn S, Ali HR, Eccles D, Hanby A, Provenzano E, Caldas C, Howat WJ, McDuffus LA, Liu B, Daley F, Coulson P, Vyas RJ, Harris LM, Owens JM, Carton AF, McQuillan JP, Paterson AM, Hirji Z, Christie SK, Holmes AR, Schmidt MK, Garcia-Closas M, Easton DF, Bolla MK, Wang Q, Benitez J, Milne RL, Mannermaa A, Couch F, Devilee P, Tollenaar RA, Seynaeve C, Cox A, Cross SS, Blows FM, Sanders J, de Groot R, Figueroa J, Sherman M, Hooning M, Brenner H, Holleczek B, Stegmaier C, Lintott C, Pharoah PD. Crowdsourcing the general public for

large scale molecular pathology studies in cancer. EBioMedicine. 2015;2(7):681–9. https://doi.org/10.1016/j.ebiom.2015.05.009.

Chang HY, Jung CK, Woo JI, Lee S, Cho J, Kim SW, Kwak TY. Artificial intelligence in pathology. J Pathol Transl Med. 2019;53(1):1–12. https://doi.org/10.4132/jptm.2018.12.16.

Chen J, Qian F, Yan W, Shen B. Translational biomedical informatics in the cloud: present and future. Biomed Res Int. 2013;2013:658925. https://doi.org/10.1155/2013/658925.

Chico V. The impact of the general data protection regulation on health research. Br Med Bull. 2018;128(1):109–18. https://doi.org/10.1093/bmb/ldy038.

Ching T, Himmelstein DS, Beaulieu-Jones BK, Kalinin AA, Do BT, Way GP, Ferrero E, Agapow PM, Zietz M, Hoffman MM, Xie W, Rosen GL, Lengerich BJ, Israeli J, Lanchantin J, Woloszynek S, Carpenter AE, Shrikumar A, Xu J, Cofer EM, Lavender CA, Turaga SC, Alexandari AM, Lu Z, Harris DJ, DeCaprio D, Qi Y, Kundaje A, Peng Y, Wiley LK, Segler MHS, Boca SM, Swamidass SJ, Huang A, Gitter A, Greene CS. Opportunities and obstacles for deep learning in biology and medicine. J R Soc Interface. 2018;15(141):20170387. https://doi.org/10.1098/rsif.2017.0387.

Cireşan DC, Giusti A, Gambardella LM, Schmidhuber J. Mitosis detection in breast cancer histology images with deep neural networks. Med Image Comput Comput Assist Interv. 2013;16(Pt 2):411–8. https://doi.org/10.1007/978-3-642-40763-5_51.

Cong L, Feng W, Yao Z, Zhou X, Xiao W. Deep learning model as a new trend in computer-aided diagnosis of tumor pathology for lung cancer. J Cancer. 2020;11(12):3615–22. https://doi.org/10.7150/jca.43268.

Evans AJ, Bauer TW, Bui MM, Cornish TC, Duncan H, Glassy EF, Hipp J, McGee RS, Murphy D, Myers C, O'Neill DG, Parwani AV, Rampy BA, Salama ME, Pantanowitz L. US Food and Drug Administration approval of whole slide imaging for primary diagnosis: a key milestone is reached and new questions are raised. Arch Pathol Lab Med. 2018;142(11):1383–7. https://doi.org/10.5858/arpa.2017-0496-CP.

Farnell DA, Huntsman D, Bashashati A. The coming 15 years in gynaecological pathology: digitisation, artificial intelligence, and new technologies. Histopathology. 2020;76(1):171–7. https://doi.org/10.1111/his.13991.

Fuchs TJ, Buhmann JM. Computational pathology: challenges and promises for tissue analysis. Comput Med Imaging Graph. 2011;35(7–8):515–30. https://doi.org/10.1016/j.compmedimag.2011.02.006.

Gabril MY, Yousef GM. Informatics for practicing anatomical pathologists: marking a new era in pathology practice. Mod Pathol. 2010;23(3):349–58. https://doi.org/10.1038/modpathol.2009.190.

George MR, Johnson KA, Gratzinger DA, Brissette MD, McCloskey CB, Conran RM, Dixon LR, Roberts CA, Rojiani AM, Shyu I, Timmons CF Jr, Hoffman RD. Will I need to move to get my first job? Geographic relocation and other trends in the pathology job market. Arch Pathol Lab Med. 2020;144(4):427–34. https://doi.org/10.5858/arpa.2019-0150-CP.

Granter SR, Beck AH, Papke DJ Jr. AlphaGo, deep learning, and the future of the human microscopist. Arch Pathol Lab Med. 2017;141(5):619–21. https://doi.org/10.5858/arpa.2016-0471-ED.

Hamidinekoo A, Denton E, Rampun A, Honnor K, Zwiggelaar R. Deep learning in mammography and breast histology, an overview and future trends. Med Image Anal. 2018;47:45–67. https://doi.org/10.1016/j.media.2018.03.006.

Hanna MG, Parwani A, Sirintrapun SJ. Whole slide imaging: technology and applications. Adv Anat Pathol. 2020;27(4):251–9. https://doi.org/10.1097/PAP.0000000000000273.

Harmon SA, Tuncer S, Sanford T, Choyke PL, Türkbey B. Artificial intelligence at the intersection of pathology and radiology in prostate cancer. Diagn Interv Radiol. 2019;25(3):183–8. https://doi.org/10.5152/dir.2019.19125.

Hassell LA, Blick KE. Training in informatics: teaching informatics in surgical pathology. Surg Pathol Clin. 2015;8(2):289–300. https://doi.org/10.1016/j.path.2015.02.008.

Hassell LA, Blick KE. Training in informatics: teaching informatics in surgical pathology. Clin Lab Med. 2016;36(1):183–97. https://doi.org/10.1016/j.cll.2015.09.014.

Hou L, Samaras D, Kurc TM, Gao Y, Davis JE, Saltz JH. Patch-based convolutional neural network for whole slide tissue image classification. Proc IEEE Comput Soc Conf Comput Vis Pattern Recognit. 2016;2016:2424–33. https://doi.org/10.1109/CVPR.2016.266.

Houssami N, Kirkpatrick-Jones G, Noguchi N, Lee CI. Artificial intelligence (AI) for the early detection of breast cancer: a scoping review to assess AI's potential in breast screening practice. Expert Rev. Med Devices. 2019;16(5):351–62. https://doi.org/10.1080/17434440.2019.1610387.

Hughes AJ, Mornin JD, Biswas SK, Beck LE, Bauer DP, Raj A, Bianco S, Gartner ZJ. Quanti.us: a tool for rapid, flexible, crowd-based annotation of images. Nat Methods. 2018;15(8):587–90. https://doi.org/10.1038/s41592-018-0069-0.

Irshad H, Montaser-Kouhsari L, Waltz G, Bucur O, Nowak JA, Dong F, Knoblauch NW, Beck AH. Crowdsourcing image annotation for nucleus detection and segmentation in computational pathology: evaluating experts, automated methods, and the crowd. Pac Symp Biocomput. 2015;2015:294–305. https://doi.org/10.1142/9789814644730_0029.

Jang HJ, Cho KO. Applications of deep learning for the analysis of medical data. Arch Pharm Res. 2019;42(6):492–504. https://doi.org/10.1007/s12272-019-01162-9.

Janowczyk A, Zuo R, Gilmore H, Feldman M, Madabhushi A. HistoQC: an open-source quality control tool for digital pathology slides. JCO Clin Cancer Inform. 2019;3:1–7. https://doi.org/10.1200/CCI.18.00157.

Jovanović J, Bagheri E. Semantic annotation in biomedicine: the current landscape. J Biomed Semantics. 2017;8(1):44. https://doi.org/10.1186/s13326-017-0153-x.

Kapil A, Meier A, Zuraw A, Steele KE, Rebelatto MC, Schmidt G, Brieu N. Deep semi supervised generative learning for automated tumor proportion scoring on NSCLC tissue needle biopsies. Sci Rep. 2018;8(1):17343. https://doi.org/10.1038/s41598-018-35501-5.

Komura D, Ishikawa S. Machine learning approaches for pathologic diagnosis. Virchows Arch. 2019;475(2):131–8. https://doi.org/10.1007/s00428-019-02594-w.

Komura D, Ishikawa S. Machine learning methods for histopathological image analysis. Comput Struct Biotechnol J. 2018;16:34–42. https://doi.org/10.1016/j.csbj.2018.01.001.

Korbar B, Olofson AM, Miraflor AP, Nicka CM, Suriawinata MA, Torresani L, Suriawinata AA, Hassanpour S. Deep learning for classification of colorectal polyps on whole-slide images. J Pathol Inform. 2017;8:30. https://doi.org/10.4103/jpi.jpi_34_17.

Krempel R, Kulkarni P, Yim A, Lang U, Habermann B, Frommolt P. Integrative analysis and machine learning on cancer genomics data using the cancer systems biology database (CancerSysDB). BMC Bioinformatics. 2018;19(1):156. https://doi.org/10.1186/s12859-018-2157-7.

Landau MS, Pantanowitz L. Artificial intelligence in cytopathology: a review of the literature and overview of commercial landscape. J Am Soc Cytopathol. 2019;8(4):230–41. https://doi.org/10.1016/j.jasc.2019.03.003.

LeCun Y, Bengio Y, Hinton G. Deep learning. Nature. 2015;521(7553):436–44. https://doi.org/10.1038/nature14539.

Levine AB, Schlosser C, Grewal J, Coope R, Jones SJM, Yip S. Rise of the machines: advances in deep learning for cancer diagnosis. Trends Cancer. 2019;5(3):157–69. https://doi.org/10.1016/j.trecan.2019.02.002.

Li C, Wang X, Liu W, Latecki LJ, Wang B, Huang J. Weakly supervised mitosis detection in breast histopathology images using concentric

loss. Med Image Anal. 2019;53:165–78. https://doi.org/10.1016/j.media.2019.01.013.

Lin JC, Fan CT, Liao CC, Chen YS. Taiwan biobank: making cross-database convergence possible in the big data era. Gigascience. 2018;7(1):1–4. https://doi.org/10.1093/gigascience/gix110.

Litjens G, Bandi P, Ehteshami Bejnordi B, Geessink O, Balkenhol M, Bult P, Halilovic A, Hermsen M, van de Loo R, Vogels R, Manson QF, Stathonikos N, Baidoshvili A, van Diest P, Wauters C, van Dijk M, van der Laak J. 1399 H&E-stained sentinel lymph node sections of breast cancer patients: the CAMELYON dataset. Gigascience. 2018;7(6):giy065. https://doi.org/10.1093/gigascience/giy065.

Litjens G, Kooi T, Bejnordi BE, Setio AAA, Ciompi F, Ghafoorian M, van der Laak JAWM, van Ginneken B, Sánchez CI. A survey on deep learning in medical image analysis. Med Image Anal. 2017;42:60–88. https://doi.org/10.1016/j.media.2017.07.005.

Louis DN, Feldman M, Carter AB, Dighe AS, Pfeifer JD, Bry L, Almeida JS, Saltz J, Braun J, Tomaszewski JE, Gilbertson JR, Sinard JH, Gerber GK, Galli SJ, Golden JA, Becich MJ. Computational pathology: a path ahead. Arch Pathol Lab Med. 2016;140(1):41–50. https://doi.org/10.5858/arpa.2015-0093-SA.

Louis DN, Gerber GK, Baron JM, Bry L, Dighe AS, Getz G, Higgins JM, Kuo FC, Lane WJ, Michaelson JS, Le LP, Mermel CH, Gilbertson JR, Golden JA. Computational pathology: an emerging definition. Arch Pathol Lab Med. 2014;138(9):1133–8. https://doi.org/10.5858/arpa.2014-0034-ED.

Martin V, Kim TH, Kwon M, Kuko M, Pourhomayoun M, Martin S. A more comprehensive cervical cell classification using convolutional neural network. J Am Soc Cytopathol. 2018;7(5):S66. https://doi.org/10.1016/j.jasc.2018.06.156.

Martino F, Varricchio S, Russo D, Merolla F, Ilardi G, Mascolo M, dell'Aversana GO, Califano L, Toscano G, Pietro G, Frucci M, Brancati N, Fraggetta F, Staibano S. A machine-learning approach for the assessment of the proliferative compartment of solid tumors on hematoxylin-eosin-stained sections. Cancers. 2020;12(5):1344. https://doi.org/10.3390/cancers12051344.

Mazzanti M, Shirka E, Gjergo H, Hasimi E. Imaging, health record, and artificial intelligence: hype or hope? Curr Cardiol Rep. 2018;20(6):48. https://doi.org/10.1007/s11886-018-0990-y.

Miller DD, Brown EW. Artificial intelligence in medical practice: the question to the answer? Am J Med. 2018;131(2):129–33. https://doi.org/10.1016/j.amjmed.2017.10.035.

Mintz Y, Brodie R. Introduction to artificial intelligence in medicine. Minim Invasive Ther Allied Technol. 2019;28(2):73–81. https://doi.org/10.1080/13645706.2019.1575882.

Mobadersany P, Yousefi S, Amgad M, Gutman DA, Barnholtz-Sloan JS, Velázquez Vega JE, Brat DJ, Cooper LAD. Predicting cancer outcomes from histology and genomics using convolutional networks. Proc Natl Acad Sci U S A. 2018;115(13):E2970–9. https://doi.org/10.1073/pnas.1717139115.

Mohanty SK, Parwani AV, Crowley RS, Winters S, Becich MJ. The importance of pathology informatics in translational research. Adv Anat Pathol. 2007;14(5):320–2. https://doi.org/10.1097/PAP.0b013e3180ca8a79.

Moscatelli M, Manconi A, Pessina M, Fellegara G, Rampoldi S, Milanesi L, Casasco A, Gnocchi M. An infrastructure for precision medicine through analysis of big data. BMC Bioinformatics. 2018;19(Suppl 10):351. https://doi.org/10.1186/s12859-018-2300-5.

Murphy JFA. The general data protection regulation (GDPR). Ir Med J. 2018;111(5):747.

Nakata N. Recent technical development of artificial intelligence for diagnostic medical imaging. Jpn J Radiol. 2019;37(2):103–8. https://doi.org/10.1007/s11604-018-0804-6.

Niazi MKK, Parwani AV, Gurcan MN. Digital pathology and artificial intelligence. Lancet Oncol. 2019;20(5):e253–61. https://doi.org/10.1016/S1470-2045(19)30154-8.

Pantanowitz L, Sinard JH, Henricks WH, Fatheree LA, Carter AB, Contis L, Beckwith BA, Evans AJ, Lal A, Parwani AV, College of American Pathologists Pathology and Laboratory Quality Center. Validating whole slide imaging for diagnostic purposes in pathology: guideline from the College of American Pathologists Pathology and Laboratory Quality Center. Arch Pathol Lab Med. 2013;137(12):1710–22. https://doi.org/10.5858/arpa.2013-0093-CP.

Paparella ML, et al. Quantitative analysis of KLF4 and SOX2 expression in oral carcinomas reveals independent association with oral tongue subsite location and histological grade. Cancer Biomark. 2021;32:37–48.

Parwani AV. Next generation diagnostic pathology: use of digital pathology and artificial intelligence tools to augment a pathological diagnosis. Diagn Pathol. 2019;14(1):138. https://doi.org/10.1186/s13000-019-0921-2.

Parwani AV. Preface. Pathology informatics. Surg Pathol Clin. 2015;8(2):xi–xii. https://doi.org/10.1016/j.path.2015.04.001.

Rashidi HH, Tran NK, Betts EV, Howell LP, Green R. Artificial intelligence and machine learning in pathology: the present landscape of supervised methods. Acad Pathol. 2019;6:2374289519873088. https://doi.org/10.1177/2374289519873088.

Robertson S, Azizpour H, Smith K, Hartman J. Digital image analysis in breast pathology-from image processing techniques to artificial intelligence. Transl Res. 2018;194:19–35. https://doi.org/10.1016/j.trsl.2017.10.010.

Roux L, Racoceanu D, Loménie N, Kulikova M, Irshad H, Klossa J, Capron F, Genestie C, Le Naour G, Gurcan MN. Mitosis detection in breast cancer histological images an ICPR 2012 contest. J Pathol Inform. 2013;4:8. https://doi.org/10.4103/2153-3539.112693.

Roy S, Coldren C, Karunamurthy A, Kip NS, Klee EW, Lincoln SE, Leon A, Pullambhatla M, Temple-Smolkin RL, Voelkerding KV, Wang C, Carter AB. Standards and guidelines for validating next-generation sequencing bioinformatics pipelines: a joint recommendation of the Association for Molecular Pathology and the College of American Pathologists. J Mol Diagn. 2018;20(1):4–27. https://doi.org/10.1016/j.jmoldx.2017.11.003.

Roy S. Molecular pathology informatics. Clin Lab Med. 2016;36(1):57–66. https://doi.org/10.1016/j.cll.2015.09.007.

Roy S. Molecular pathology informatics. Surg Pathol Clin. 2015;8(2):187–94. https://doi.org/10.1016/j.path.2015.02.013.

Saltz J, Gupta R, Hou L, Kurc T, Singh P, Nguyen V, Samaras D, Shroyer KR, Zhao T, Batiste R, Van Arnam J, et al. Spatial organization and molecular correlation of tumor-infiltrating lymphocytes using deep learning on pathology images. Cell Rep. 2018;23(1):181–193.e7. https://doi.org/10.1016/j.celrep.2018.03.086.

Sarwar S, Dent A, Faust K, Richer M, Djuric U, Van Ommeren R, Diamandis P. Physician perspectives on integration of artificial intelligence into diagnostic pathology. NPJ Digit Med. 2019;2:28. https://doi.org/10.1038/s41746-019-0106-0.

Senaras C, Niazi MKK, Lozanski G, Gurcan MN. DeepFocus: detection of out-of-focus regions in whole slide digital images using deep learning. PLoS One. 2018;13(10):e0205387. https://doi.org/10.1371/journal.pone.0205387.

Shen D, Wu G, Suk HI. Deep learning in medical image analysis. Annu Rev. Biomed Eng. 2017;19:221–48. https://doi.org/10.1146/annurev-bioeng-071516-044442.

Smaïl-Tabbone M, Rance B, Section Editors for the IMIA Yearbook Section on Bioinformatics and Translational Informatics. Contributions from the 2019 literature on bioinformatics and translational informatics. Yearb Med Inform. 2020;29(1):188–92. https://doi.org/10.1055/s-0040-1702002.

Steiner DF, MacDonald R, Liu Y, Truszkowski P, Hipp JD, Gammage C, Thng F, Peng L, Stumpe MC. Impact of deep learning assistance on the histopathologic review of lymph nodes for metastatic breast cancer. Am J Surg Pathol. 2018;42(12):1636–46. https://doi.org/10.1097/PAS.0000000000001151.

Stoeklé HC, Mamzer-Bruneel MF, Frouart CH, Le Tourneau C, Laurent-Puig P, Vogt G, Hervé C. Molecular tumor boards: ethical issues in

the new era of data medicine. Sci Eng Ethics. 2018;24(1):307–22. https://doi.org/10.1007/s11948-017-9880-8.

Tappeiner E, Pröll S, Hönig M, Raudaschl PF, Zaffino P, Spadea MF, Sharp GC, Schubert R, Fritscher K. Multi-organ segmentation of the head and neck area: an efficient hierarchical neural networks approach. Int J Comput Assist Radiol Surg. 2019;14(5):745–54. https://doi.org/10.1007/s11548-019-01922-4.

Tizhoosh HR, Pantanowitz L. Artificial intelligence and digital pathology: challenges and opportunities. J Pathol Inform. 2018;9:38. https://doi.org/10.4103/jpi.jpi_53_18.

Vaickus LJ, Suriawinata AA, Wei JW, Liu X. Automating the Paris system for urine cytopathology-a hybrid deep-learning and morphometric approach. Cancer Cytopathol. 2019;127(2):98–115. https://doi.org/10.1002/cncy.22099.

Wang DY, Khosla A, Gargeya R, Irshad H, Beck AH. Deep learning for identifying metastatic breast cancer. arXiv. 2016. https://arxiv.org/abs/1606.05718.

Wang S, Yang DM, Rong R, Zhan X, Fujimoto J, Liu H, Minna J, Wistuba II, Xie Y, Xiao G. Artificial intelligence in lung cancer pathology image analysis. Cancers (Basel). 2019;11(11):1673. https://doi.org/10.3390/cancers11111673.

Wen S, Kurc TM, Hou L, Saltz JH, Gupta RR, Batiste R, Zhao T, Nguyen V, Samaras D, Zhu W. Comparison of different classifiers with active learning to support quality control in nucleus segmentation in pathology images. AMIA Jt Summits Transl Sci Proc. 2017;2018:227–36.

Wildeboer RR, van Sloun RJG, Wijkstra H, Mischi M. Artificial intelligence in multiparametric prostate cancer imaging with focus on deep-learning methods. Comput Methods Prog Biomed. 2020;189:105316. https://doi.org/10.1016/j.cmpb.2020.105316.

Yagi Y. Color standardization and optimization in whole slide imaging. Diagn Pathol. 2011;6(Suppl 1):S15. https://doi.org/10.1186/1746-1596-6-S1-S15.

Zarella MD, Feldscher A. Laboratory computer performance in a digital pathology environment: outcomes from a single institution. J Pathol Inform. 2018;9:44. https://doi.org/10.4103/jpi.jpi_47_18.

Zarella MD, Quaschnick MR, Breen DE, Garcia FU. Estimation of fine-scale histologic features at low magnification. Arch Pathol Lab Med. 2018;142(11):1394–402. https://doi.org/10.5858/arpa.2017-0380-OA.

Zarella MD, Yeoh C, Breen DE, Garcia FU. An alternative reference space for H&E color normalization. PLoS One. 2017;12(3):e0174489. https://doi.org/10.1371/journal.pone.0174489.

Zhang DY. An outline of office-based bladder and prostate biopsy pathology. Hauppauge, NY: Nova Science Publishers; 2019.