The Journal of Pathology, INVITED REVIEW

Software-assisted decision support in digital histopathology

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Potential conflict of interests: The authors declare no conflict of interest.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi:10.1002/path.5388

ABSTRACT

Tissue Diagnostics is the world of pathologists, and it is increasingly becoming digitalized to leverage the enormous potential of personalized medicine and of stratifying patients, enabling the administration of modern therapies. Therefore, the daily task for pathologists is changing drastically and will become increasingly demanding, in order to take advantage of the development of modern computer technologies. The role of pathologist has rapidly evolved from exclusively describing the morphology and phenomenology of a disease, to becoming a gatekeeper for novel and most effective treatment options. This is possible based on the retrieval and management of a wide range of complex information from tissue or a group of cells and associated meta-data. Intelligent and self-learning software solutions can support and guide pathologists to score clinically relevant decisions based on the accurate and robust quantification of multiple target molecules or surrogate biomarker as companion or complimentary diagnostics along with relevant spatial relationships and contextual information from digital H&E and multiplexed images. With the availability of multiplex staining techniques on a single slide, high-resolution image analysis tools and high-end computer hardware, machine and deep learning solutions now offer diagnostic rulesets and algorithms that still require clinical validation in well-designed studies. Before entering the clinical practice, the "human factor“ pathologist needs to develop trust into the output coming from the “digital black box of computational

pathology", including image analysis solutions and artificial intelligence algorithms to support critical clinical decisions which otherwise would not be available.

KEY WORDS:

Digital histology, computational pathology, image analysis, decision support, artificial intelligence

INTRODUCTION

Digitalization and intelligent data processing are playing increasingly important roles in the practice of histopathology. Consequently, traditional histopathology is gradually being transformed into a digital discipline whereby whole-slide scanners can capture images for further computer-assisted analyses. Algorithms can extract as much information from tissue and standardize the quantification of specific histopathological features, since it appears that it is no longer sufficient to exclusively classify cancers just on the basis of morphology and genomic profile. Rather, it is essential that the pathologist also accurately measures the quantity and dimensions of different critical components in the tissue and then links such parameters to all other patient's and available meta-data.

The comprehensive extraction of disease-related knowledge allows educated decisions on the precise individual prognosis and the selection of the best available treatment at that time. Today, open-source or proprietary software solutions allow pathologists not only to manage available information, but also obtain more actionable information and relevant insights when applying appropriate techniques. Machine learning will also guide future decisions in clinical histopathology as the computer itself also learns to exclude tissue- and image-based artefacts while including “regions of interest” (RoI) to answer relevant clinical questions.

Until today most commonly the clinically available software in a clinical histopathology laboratory is usually a validated information and management system (LIMS) that builds the communication interface between different clinical departments, outside contractors or analytical partners for tasks, performance and result management. The transition from a simple LIMS environment into a fully digital histopathology lab (maintaining the LIMS connectivity) requires the digitization of images as well as data and availability of significant virtual storage space. However, despite this disruption, digital histopathology allows entry into a new era of clinical decision making based on accumulation of big data to use advanced solutions, such as machine and deep learning tools generating novel clinical insights. Histopathologists have begun to resume different and more complex tasks that are almost impossible to achieve without software assistance.

The origin of digital pathology lies in the area of telepathology to share images across long distances for a remote second or expert opinions [1]. Pathologists have used telepathology effectively driving a microscope remotely for several decades [2], and now they are making further use of its applications. The field of digital histopathology is currently following technical advancements with its rapid transitioning from primarily a research tool into a viable clinical solution for patient primary diagnosis and the determination of prognosis. The use of such tools also allows for the discovery of novel features [3].

The success of digital pathology also relies on the availability technically sophisticated viewing devices and the entire infrastructure within the clinical setting according to regulatory standards. This includes whole-slide scanners, laboratory information systems, the digital archiving of specimens, and, of course, the willingness of histopathologists to quickly adopt clinically validated software.

The concept of ‘personalized’ or ‘precision’ medicine, stratifying patients to the best available treatment according to diagnostic test results, has been built over the past decades. While genomic and gene expression analysis still represent a large proportion of “biomarker” tests, the analysis of tissue images is actually very well-suited to address complex biological questions especially in oncology.

Image mining software solutions can assist histopathologists in identifying RoI for further thorough exploitation, document the complete survey of the digitized image as part of a

certification-related quality measure, or to understand tumour heterogeneity in its entirety. In the dawning age of immunotherapies and complex combination treatments, along with cancer-specific scoring algorithms on different platforms that are linked to selected drugs and concordant molecular parameters, validated software solutions guide the decisions of the pathologist without depriving them of the role as the ultimate diagnostic authority.

There is still uncertainty amongst clinical histopathologists about the use of digital pathology, and perhaps there is a nervous perception that terminology and software applications in histopathology are difficult. Pathologists question what kind of solutions are of true value in the daily personal clinical practice and which are research-use only (RUO) applications. Has the tool or the solutions been validated or even approved by the authorities or notified bodies? Is the decision supported by the software or associated algorithms even more confusing or distracting when trying to conclude and sign-off a difficult clinical case, or are they indeed even misleading or plainly wrong? Is the software proprietary or an open-source product, and for what purpose and intended use? In the end, it is still the pathologist who signs out the case, and also takes full responsibility of any secondary action. The learning curve for pathologists and software engineers for the routine application of digital histopathology is still present and steep. One aim is to develop a mutually understandable language and nomenclature to agree to a common goal and the same meaning. A “solution” can have a different meaning for a clinician and for a software developer. In this publication, we follow the recommendation and suggestion of

the White Paper of members of the Digital Pathology Association (DPA) [4]. The term "computational pathology" (CPATH) is used in the context of digital pathology and goes beyond the simple digitization of stained slides and the inspection of tissue images on a computer screen. CPATH implies already an expansion of the normal viewing fields (e.g. via microscopy) because it allows the simultaneous inspection of various stains along with accompanying meta data, such as expression and/or sequencing data, or the tumour mutational burden which is currently under evaluation as a molecular surrogate to predict the clinical response to immune modulating agents.

THE CHALLENGES IN HISTOPATHOLOGY

Over the past 10 years, the pathology community experienced disruptive changes in the practice of histopathology and the consequences of its action. Although the US Food and Drug Agency (FDA) stated even in 2004 it would disapprove any novel drug without a biomarker assay under development or at least an existing biomarker strategy, we have only recently experienced the serious effects of this decision. Today, treatment decisions are still mainly achieved by histological investigations of individual cell populations. However, in the future, the study of networks of cells, their contextual relationship and even spatial genomics empowered by software solutions and AI-assisted algorithms will bring these decisions to a newer level. With the ability to measure and directly target checkpoint molecules, such as CTLA-4 and the PD-1/PDL-1 axis, to unleash a specific

anti-cancer immunity, the need for specific testing of the individual immune status in individual cancer types has become mandatory for most histopathology laboratories. But the complexity of measuring PD-1 and / or PD-L1 has by far excelled the testing for Her2/neu or any hormonal receptor in breast or prostate cancer, independent of the available staining platform or robust manual processes in place. Even expert histopathologists have to be trained on the correct reading and reporting of the different PD-1 / PD-L1 antibodies provided by different vendors, with the scores being dependent on the stage of disease and line of treatment. Sometimes it is “sufficient” to score the individual case “positive” in case of more than 25% PD-L1 positive cancer cells. In different indications, counting the absolute numbers of invasive cancer cells plus any PD-1 positive immune cells (sometimes even tumour-infiltrating lymphocytes versus macrophages or other immune cells) above 1% are considered a positive score. Consequentially, the accurate measure of checkpoint inhibitors in different tissue compartments (e.g. tumour-stroma) is of essential importance to select the best therapy option for an individual patient and might therefore warrant an automated PD-1 / PD-L1 scoring solution.

Although it has been shown that an experienced and well-trained histopathologist can generate correct scoring results manually without any technical assistance, it takes a considerable amount of time, and such cases will become even more complex in the future. Jerome Galon and his team already demonstrated with the “ImmunoScore” for Colon Cancer that only two different immune markers (CD3 and CD8) in different tissue compartments (invasive margin versus tumour centre) provide a significant progress on

the prognosis in limited versus advanced disease [5,6]. This was supported by a validated software solution that identified the different regions automatically and quantified the spatial ratio of immune cells in the different compartments [7].

Increasingly, software-assisted tools play a crucial role in the stratification of patients to certain therapies in the age of immunotherapies and beyond. The reasons are manifold: the increasing understanding of disease complexity and the so far underestimated role of tumour heterogeneity and its microenvironment. *Figure 1* schematically demonstrates the diagnostic challenges ahead in histopathology.

SOLUTIONS THROUGH SOFTWARE-ASSISTED IMAGE ANALYSIS

It was more than three decades ago, when pathologists were first offered assistance to count Ki-67-positive cells. At that time, there was still a significant gap between the capabilities of the available soft- and hardware compared to the intuitive competence and skill sets of well trained and experienced pathologists. The next challenge was to count mitotic figures, which not only had a prognostic value but could also determine malignancy with radical therapeutic consequences. Even today, software solutions still struggle to

reliably discriminate between a true mitosis or some wizened, clump of chromatin due to an activated apoptosis pathway or fixation artefact. This “mystery” is still not completely solved, independent of the use of sophisticated immunostainings related to cell cycle phases and different machine / deep learning attempts, which were subject to several grand challenges [9]

The success of the HER2/neu scoring “algorithm” as a first predictive digital biomarker about twenty years ago was rather an accomplishment of a community of pathologists who understood the need to optimally stratify patients with breast cancer and a single biopharmaceutical company that did their utmost to train and educate the practicing histopathologists. A HER2/neu scoring algorithm was the first to receive regulatory approval as an in-vitro diagnostic medical device. This strategy is still successfully deployed in the age of combination immunotherapies [10,11].

On the road to better diagnoses and combination therapies, the availability of different multiplex assays adds another mostly overwhelming level of insights but also complexity to the pathologists’ arsenal [12]. While tools like the molecular profiling or the mutational burden are very helpful to identify a plethora of potential diagnostic hints or therapeutic targets, they all lack spatial context and relevant contextual information that is often necessary to understand the complete tumour microenvironment. Software solutions as an integral part of image analysis tools can assist the pathologist to understand the multiplex

images or even virtually mount complex pictures to visualize the true nature of a tumour in a piece of tissue [13] (*Figure 2*).

SOFTWARE-ASSISTED ANNOTATION AND SEGMENTATION

The development of artificial intelligence (AI) has in part been shaped by the field of neuroscience and other non-medical applications. By understanding the human brain, scientists have attempted to build new intelligent machines capable of performing complex tasks. While the development of artificial intelligence algorithms has been fast paced, the actual use of most software algorithms in clinical practice is still markedly below its conceivably broader potentials also in histopathology. This is partly because for any algorithm to be incorporated into existing or future workflows, it has to stand the test of thorough scientific validation and robust clinical utility without causing any harm or confusing the human factor. In this context, there is much to be gained by combining AI and the human intelligence of experienced pathologists. Harnessing empirical knowledge, big data, computing power and storage capacities, and addressing clinical issues demand deploying expert knowledge in tandem with AI. Drug discovery and translational biopharmaceutical research will also gain from AI technology when humans fail to see pivotal next step and the next suitable application. Since the revolutionary success of the

deep learning architecture for ImageNet image classification in 2012, the computer vision community has seen an exponential growth in convolutional network methodologies, architectures and applications. This growth is fuelled by ever-increasing computational power, the availability of freely available open-source packages and the availability of robust big data [14,15].

At the same time, Digital Pathology has been recognized as one of the most impactful application areas for AI, and headlines such as “Artificial intelligence could yield more accurate breast cancer diagnoses” [16] will certainly be followed by medical products implemented in daily clinical routine. The first systems have been already approved by the FDA, and Kapil *et al* published first results in Scientific Reports showing performance comparable to well-trained clinicians for an automated PD-L1 scoring solution for lung cancer therapy prediction [17].

The “correct annotation” to train any system is still the bottle neck, and the automated software solution should be as good as those human experts, who still define the “ground truth”. Most digital pathology systems are based on thousands and thousands of cells and annotated regions in histopathology images performed by human expert pathologists [18,19]. However, there is one way out: Yousefi *et al* presented in 2017 a scientific paper called “*Predicting clinical outcomes from large scale cancer genomic profiles with deep survival models*” [20]. It describes how to predict patient survival with very little biological

knowledge, and it opened the way for the research team to apply the published methods to whole slide images of our gastric and lung cancer patient cohorts. Other examples demonstrate how deep learning can be applied to classify lung cancer and predict mutations in lung cancer [21] or microsatellite instability in gastric cancer [22].

Machine learning, like deep learning, is a part of computer science and more precisely artificial intelligence that can learn from images and data without requiring the user to define explicit rules. Machine learning applications have made tremendous progress in the last decades, especially in the fields of image analysis, natural language processing and pattern recognition [23–25]. This development has been fostered by the availability of large data sets for training these systems as well as reasonably priced computing power, especially of graphics processing units (GPUs) [26]. Typical applications include, but are not limited to, the detection of RoI, automated tumour-stroma-separation or the detection, segmentation, and classification of objects, such as different cell types. Most of those tasks can often be achieved easily by humans (being an expert or becoming an expert after training). Therefore, the most common tasks in the analysis of digital pathology are the detection and segmentation of objects of interest, followed by an accurate measurement of staining or defining spatial relationships, which also requires image and data organization into training and test sets [27]. However, sometimes knowledge-based ("*heuristic model*s") approach like cognition network solutions [28] are not sufficient to

generate novel insights. Instead, reinforced (feature) learning solutions are warranted, and their applications are steadily growing (*Figure 3*).

The continues rise of the machines will trigger significant changes on how histopathologists analyse tissue images, profile patients, and derive medical conclusions. Besides image analysis, object classifications and well-trained machine-based algorithms, a fast-growing body of scientific literature and an increasing number of relevant clinical studies is another rich source of knowledge, which requires also emphasis on Natural Language Processing (NLP) plus text analysis to leverage all available information. There are 700 000 new articles on health care every year, which can no longer be comprehended by an individual or group of pathologists. Nowadays a stage is reached where, after expending significant effort in labelling (scientific) text, natural language sentences can be mapped to a logical form, which can be used in formalized reasoning mechanisms to work with these extracted formulas. However, the question remains whether the practicing pathologist needs to fully understand how any software comes to a certain conclusion coming from text, data, or images and how to trust the conclusion that comes from such a “black box”? [29]. Experts stress the need to become more transparent on the training of deep learning models and how to apply them at scale across increasingly more complex and diverse diagnostic tasks [30]. The path to efficiency will be led in part by steps of “small data” and the use of more unsupervised learning due to the scarcity of available tissue and good data. While clinical scientists try to make the clinical

trial formats even more efficient in designing basket, umbrella and platform trials, the number of relevant subjects might remain small. Sometimes there simply is not enough data available to feed a deep learning model. Since patients do not have the time to wait for that researchers are pushing to figure out ways to train systems on less data and are confident, they'll find a viable and clinically trustworthy solution. Current deep learning models require datasets that are not only massive but also representative of the relevant problem. "Supervised learning" largely relies on pathologists to do the labelling and the subject matter expert (e.g. expert pathologist) telling the system everything they know with relative certainty. On the other hand, unsupervised learning allows raw unlabelled data to be used to train a system with little to no human effort, and one might receive insights that are truly new and innovative. The relevance of the in- as well as out-put might be uncertain until it is clinically validated.

THE HUMAN FACTOR PATHOLOGIST

Pathologists usually struggle with unsupervised learning methods or AI solutions. During their course of training they are used to learning from other experienced pathologists, slide seminars and scientific conferences. While in recent years most novel biological and clinical insights have come from molecular pathology and deep sub-typing of different cancer types, now also software solutions enter the perception of histopathologists.

An automated software solution for histopathology that achieves a performance of 90 percent may appear to outperform human pathologists. But the conditions are usually not quite the identical, so “pathologist versus AI” may not really be the right comparison. Instead, pathologists and computers need to work together, each performing to their own strengths and controlling each other.

Asking the pathologist to determine the percentage of cells (to be evaluated against a pre-determined threshold, for example, >25 percent) of a certain cell type (for example, all invasive tumour cells) that have any positive staining (for example PD-L1) in a certain cell compartment (e.g. tumour versus stroma) that is above an absolute threshold is not an uncommon task nowadays, and it seems obvious that manual scoring will lead to high inter and intra-pathologist variabilities unless the histopathologists was thoroughly trained and maintains a significant level of routine. The pathology practice involved in immuno-oncology today has to deal with even more stains in different tissue contexts and apply more complex scoring schemes (*see above*). The level of complex analysis required is becoming almost impossible for a pathologist using just a microscope. A computer, on the other hand, would complete many of these tasks with little difficulty if the used algorithm has been designed, trained, and validated properly. With the increasing adoption of digital pathology, which enables computers to analyse images of histology slides, it is time to allow software to assist and support the pathologist.

One of the key problems for any automated system in pathology is the variation between samples, the pre-analytical inconsistency. No two examples of a disease look the same – even in similar patients under similar conditions. Human pathologists know that only too well. The best way is to develop a standard clinical workflow as the perfect intermediate step between manual microscopy and automated software solutions. This also requires as a first step the integration of true or virtual multiplex staining and software solutions into a standardized laboratory environment where possible and suitable (*Figure 4*).

SOFTWARE-ASSISTED DECISIONS IN CLINICAL PRACTICE

Digital Pathology is already now rapidly translating into the clinical practice, facilitating multiple advantages compared to traditional histopathology today [31]. Regulatory approval and advances in associated technologies including high power computing and data storage capabilities along with whole-slide image (WSI) scanners allow large batch image capture and the application of deep learning to make informed decisions in histopathology [32] Despite all progress in the genomic and post-genomic era [33], the importance of spatial localization of gene expression ("spatial genomics") has been recognized as a missing link that provides order to the conundrum of cancer biology. This not only allows the translation into clinical development and practice, but also to train the future generation of young pathologists and tissue experts [34]. The next generation of

domain experts is eager to use the support of software-based solutions especially to error-prone problems, like Gleason Scoring [35,36] which usually require substantial training or identify biomarker signatures that are not too obvious [37,38]. But it still to the discretion of the pathology on how and to what extent accept or dismiss the results of a software-based image and data analysis solution (*Figure 5*).

OUTLOOK

Digital pathology is currently proving itself to be a reliable tool in the clinical practice and is also becoming a part of the education and training of histopathologists, as well as a critical in translational tissue-based research. Although still in its infancy, computational pathology in general, and especially software-assisted decision support systems, are here to stay and are 'waiting at the front door' to enter routine histopathology, given all the challenges ahead of pathologists [39,40]. While there are still regulatory and psychological barriers, there are multiple reasons for the clinical adoption of this technology, including technical advances in the digital technology and the availability of cognitive computing. No histopathologist will be replaced by software, but the way to practice histopathology will change; digital pathology would be there to augment diagnostics only, as one of the many tools used by histopathologists in their diagnostic tool repertoires. The question remains when to enter the routine use of clinical software support. Other clinical disciplines have

already embraced this ‘disruptive’ technology as the digital interface with the global community of domain experts [41], and to allow the real-time accessibility of all available data and best solutions for comprehensive patient profiling and optimal therapy matching [42, 43].

ACKNOWLEDGEMENTS

We apologize to all those authors whose excellent work could not be mentioned or discussed here. Digital or computational pathology, the field of artificial intelligence and software development including novel hardware solutions are so rapidly evolving that any article on this subject must have its limitations and many excellent key references cannot be cited.

AUTHOR CONTRIBUTIONS STATEMENT

Both authors wrote and revised the manuscript and approved its final version.

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Figure legends

Figure 1. Today's diagnostic challenge in histopathology is the understanding of the entire tumour heterogeneity and the complex immune interplay of different stakeholders in various compartments. Different groups have shown that it is of pivotal importance to precisely locate and accurately quantify immune cells inside ("hot tumour") and outside ("excluded") and correlate it with the entire area or provide percentages to start doing the math. It is an erroneous belief that tumours are only hot, cold or excluded. They can be a mixture of all activation states, which might change after treatment or in a metastatic setting. It could also be demonstrated that the topology of the spatial relationship of activating and modulating cells have a significant influence on different treatment modalities and their possible combinations. Only the use of a robust digital image analysis and mining solutions allow for the correct assessment of the true complexity and enable the proper diagnosis [adapted from 5–8].

Figure 2. Most current problems in routine histopathology can be solved with software-guided image analysis tools. The increasing emergence of complex multiplex analysis allow the consideration of the tumour heterogeneity and the spatial relationships of various markers (manual attributions are a challenge in terms of robustness and accuracy). If single slide multiplexing is not possible due to inconsistent antigen-retrieval or disparate

staining protocols, virtual multiplexing is also an option. Novel Artificial Intelligence (AI) solutions allow to reveal even novel and unique signatures from H&E slides which are available in abundance. The required hardware such as whole slide imaging (WSI) and related scanning devices are currently under regulatory scrutiny and have received approval in some instances.

Figure 3: Simplified scheme on the use of different AI-tools (*e.g. cognition network language, Bayes network, fuzzy logic, Random Forest, End-to-end solutions*) in Machine Learning (ML) and related disciplines. Initial INPUT is usually provided by expert knowledge from pathologists supported by training data such as digitized images. With different level of supervision, active learning algorithms build mathematical models that provide OUTPUT solutions, which can be continuously improved by several iterations (*reinforced learning*) until the result becomes explainable ("*Explainable AI*") and clinically validated. The transition from heuristic models to feature learning should be seamless, depending on the problem to be solved.

Figure 4. Adaption of a single mono- or multi-plex assay as a “lab-developed-test” in a CAP / CLIA (accredited according to the College of American Pathologists / Clinical Laboratory Improvement Amendments) histopathology lab environment and consecutive ruleset development, which still requires clinical validation. This allows the transfer of research solutions to enter the diagnostic market in a regulated environment. Alternatively, an already validated IVD (*in vitro* diagnostic) assay can be deployed or manual reading is still an option for the expert pathologist to sign out the case. By any means, it is still dual path forward for the pathologist to still read the stained slide(s) manually under the microscope but also to utilize the computer screen with its cloud-based or locally installed software solution to gain trust and perform an analytical and clinical plausibility check.

Figure 5. Software-guided image analysis [4] is still embedded into a network of quality control measures and decision making before a diagnostic recommendation is accepted and an individual case is signed out. The recommended decision provided by the software (SW) has to be plausible to the experienced pathologists [1]. The domain expert can dismiss any digital recommendation [2] if a decision is clearly wrong or based on inadequate input data or an inappropriate analytical process. The use of any pre-existing ruleset or computer algorithm has be carefully assessed for the particular histopathological solution prior to any clinical application [3]. It might even require a clinical validation step. Different

free open-source or commercial proprietary software solutions are readily available to complete different tasks with more or less IA components [39, 40].