BMC Medical Imaging

SYSTEMATIC REVIEW

Open Access

Virtual histopathology methods in medical imaging - a systematic review

Muhammad Talha Imran[1], Imran Shafi[1], Jamil Ahmad[2], Muhammad Fasih Uddin Butt[3], Santos Gracia Villar[4,5,6], Eduardo Garcia Villena[4,7,8], Tahir Khurshaid[9*] and Imran Ashraf[10*]

Abstract

Virtual histopathology is an emerging technology in medical imaging that utilizes advanced computational methods to analyze tissue images for more precise disease diagnosis. Traditionally, histopathology relies on manual techniques and expertise, often resulting in time-consuming processes and variability in diagnoses. Virtual histopathology offers a more consistent, and automated approach, employing techniques like machine learning, deep learning, and image processing to simulate staining and enhance tissue analysis. This review explores the strengths, limitations, and clinical applications of these methods, highlighting recent advancements in virtual histopathological approaches. In addition, important areas are identified for future research to improve diagnostic accuracy and efficiency in clinical settings.

Keywords Dual contrastive learning, Image-to-image translation, Virtual histopathology, Medical image processing, Computational pathology

Introduction

Light microscopy applied to biopsies is one of the most common diagnostic tools of pathology since pathomorphological analysis of biopsies or resection margins remains one of the fundamental approaches to understanding disease patterns and the development of an effective treatment plan. Originally, this process entailed the use of dyes on tissues to make structures sharper and more easily distinguishable under the lens of a microscope. However, the conventional histopathology techniques have disadvantages; time time-consuming, involve human errors; and are dependent on the number of qualified pathologists [1]. Traditional histopathological analysis is, however, restricted by these challenges, and in the recent past, virtual histopathology has received a boost through the updates in digital image processing techniques, and artificial intelligence [2]. To this end, this introduction seeks to look at the antecedents, approaches, and possibilities that define modern virtual histopathology in medicine today.

Challenges of virtual histopathology

Even though virtual histopathology has matured into a very potent technique that has the potential to disrupt the field of medical imaging and diagnostics, many issues need to be resolved [3, 4]. Solving these problems will be

*Correspondence:
Tahir Khurshaid
tahir@ynu.ac.kr
Imran Ashraf
imranashraf@ynu.ac.kr
[1] College of Electrical and Mechanical Engineering, National University of Sciences and Technology (NUST), Islamabad 44000, Pakistan
[2] Department of Computing, Abasyn University Islamabad Campus, Islamabad 44000, Pakistan
[3] Department of Electrical and Computer Engineering, COMSATS University Islamabad, Islamabad 44000, Pakistan
[4] Universidad Europea del Atlantico, Santander 39011, Spain
[5] Universidad Internacional Iberoamericana, Campeche 24560, Mexico
[6] Universidade Internacional do Cuanza, Cuito, Angola
[7] Universidad Internacional Iberoamericana Arecibo, Puerto Rico 00613, USA
[8] Universidad de La Romana, La Romana, República Dominicana
[9] Department of Electrical Engineering, Yeungnam University, Gyeongsan 38541, Republic of Korea
[10] Department of Information and Communication Engineering, Yeungnam University, Gyeongsan 38541, Republic of Korea

essential for achieving the implementation of virtual histopathology in further practice.

Data standardization and quality control

The application of virtual histopathology has been considered to encounter one of the most significant drawbacks due to the absence of common datasets [5]. Histopathological images are extremely diverse concerning staining methods, the techniques used to acquire the images, and the preparation of the tissue samples. This variability can lead to over/under fitting of the overlearned models thus reducing there generalizability. Careful consideration of the issue and identification of important parameters for image acquisition and preprocessing enable the standardization of procedures followed in each dataset, thus ensuring reliability [6].

Annotation and labeling of training data

There is a need to have large sets of annotated data to train reliable machine learning models. However, the process of annotating histopathological images is quite a time-consuming task and needs prior specialized knowledge of the field. This impedes the formulation of models that may in return, be useless due to insufficient datasets that are labeled for this specific purpose [7, 8]. This is a major concern as the process of manually labeling a large dataset can be tremendously time-consuming and costly Crowdsourcing, semi-supervised learning [9], and active learning techniques can help make this more manageable as they enable the creation of high-quality labeling more efficiently [10, 11].

Interpretability and transparency of AI models

Clinically, the so-called 'hard-to-interpret' of many deep learning models becomes another difficult problem [12]. Clinicians have to implicitly trust the decision-making of Pathologists and thus, the same is required about the AI models. AI models that allow clinicians to understand the mechanism by which they arrived at the diagnosis are necessary for assuring medical professionals' trust and achieving viable application of virtual histopathology in practice [13, 14].

Importance of the review

Advancing histopathological diagnostics

It is thus pertinent to revisit the methods of virtual histopathology to improve diagnostic processes in histopathology. This research recognizes that conventional staining techniques while efficient, come with delays and hence result in shortening of valuable time when diagnosing patients [15–17]. Thus, virtual staining, especially in such procedures as dual contrastive learning, has a great enhancement in this regard because it simplifies the diagnostic process. It can be used to improve diagnostic outcomes in terms of time taken to arrive at the right verdict thus adding to the efficiency of clinicians' activities.

Improving diagnostic specificity and consistency

The review is tunned towards the direction of how virtual histopathology can increase the chance of a more specific and accurate diagnosis. Due to the application of such learning techniques, even if the images are virtually stained [17], it is possible to retain high-quality images that would not hinder or diminish diagnostic characteristics [11]. This leads to reproducibility and consistency in the diagnosis which is a drawback observed with the manual staining procedure [18].

Reducing resource dependency

Virtual histopathology methods can alleviate the highly dependent use of the physical or tangible resources commonly used in staining processes. Traditional approaches consist of the use of many materials, equipment, and professionals [19]. Virtual staining, in contrast, uses digital means to increase the contrast of the same images and does not require actual dyes, which makes Virtual staining most beneficial where the physical reagents are lacking most of the time. This can improve the availability of quality diagnostic equipment in areas that lack adequate medical care hence eradicating inequalities in health care provision [20].

Preserving precious tissue samples

The classical staining techniques are nondestructive of the tissue samples and may be a drawback when working with scarce or expensive tissues [21]. Secondly, VHP is a non-destructive technique, that can be done once or in the future multiple times on the same sample. The maintenance of tissue integrity is significant for the Maximal use of samples especially in research and Clinics where samples are scarce.

Supporting research and development

A comprehensive review of virtual histopathology [5] methods provides critical insights into the current technological landscape, revealing limitations and identifying areas for future development. This facilitates the continuous refinement of virtual staining algorithms and supports the broader adoption [22].

Objectives

Summarize current advances

Virtual histopathology aims to present a comprehensive overview of the latest advancements in the field. Virtual staining is a new technology that can be used by researchers and practitioners to improve histopathology.

Evaluate methodologies
It's important to analyze the different approaches used in virtual histopathology. Identify the crucial diagnostic methods that have achieved high diagnostic accuracy and pinpoint the areas that need to be improved. The review seeks to identify the advantages and disadvantages of these methods by identifying areas for improvement through research and practical use.

Assess diagnostic accuracy
A critical target is to assess the diagnostic precision of virtual histopathology [5] techniques. The assessment involves evaluating the degree of degree of preservation of tissue samples for diagnosis in comparison to conventional staining techniques. A review of virtual staining techniques to determine their suitability in clinical settings to maintain visual and structural coherence by examining their effectiveness.

Analyze computational efficiency
The review also intends to evaluate the computational complexity of virtual histopathology models [23]. I want to see if these models can be used in clinical templates and how they perform in terms of resource utilization and processing speed. Virtual staining technologies are a very efficient and computationally demanding diagnostic tool.

Explore clinical applications
Virtual histopathology [5] is also a focus in clinical applications. I want to see how these technologies can improve diagnostic procedures, increase the precision of diagnoses, and integrate with digital pathology systems. Aims to show how virtual histopathology can practically improve patient outcomes and streamline clinical workflows.

Identification of challenges and limitations
Future research should be based on identifying the limitations and difficulties of virtual histopathology techniques. The analysis is designed to identify specific shortcomings and suggest ways to address them. The review can address these issues and assist in the refinement and enhancement of virtual staining algorithms, leading to more effective and reliable histopathological diagnosis.

Structure of review paper
The first section after the Introduction is about the methodology which is described in Methodology section. The Literature Review section reviews, which Reviews the basic studies published in particular, those relevant to the past ten years of dual contrastive learning [24]. Analysis to Compare these frameworks in depth is used to study their performance stability usability, and other metrics. Followed by a conclusion and recommendation. Summarising the main things from their review giving practical tips for developers and organizations. All the references in this paper will be comprehensive enough. mentioned in the paper and at the end.

Methodology
Search strategy
A systematic literature review of existing works on virtual histopathology is carried out to ensure a thorough understanding of the advancements and challenges faced in this important field. In the first step, the scope of the review is defined which leads to the selection of appropriate online repositories/databases for searching relevant articles. For this purpose, appropriate keywords are selected using the common words used in the existing works on virtual histopathology. These articles were later filtered using the inclusion and exclusion criteria.

Expanded search strategy
To build a thorough understanding of virtual histopathology, a layered search strategy was employed, including broader assessment criteria for study relevance, recent developments, and interdisciplinary impact. The strategy details are outlined below:

i. **Iterative Development of Keywords:** The search began with broad keywords that were then refined to target studies across related fields. This involved testing terms like "digital slide analysis", "computational pathology", and "diagnostic imaging", allowing the identification of relevant studies from adjacent fields like image processing and computational diagnostics. This iterative approach helped capture unique terminologies within each field, ensuring comprehensive coverage of current and emerging terms in virtual histopathology.
ii. **Evaluating Quality and Influence of Studies:** Each study was assessed for quality based on its source, including high-impact journals and peer-reviewed conference proceedings. Citations were also used as an indicator of each study's impact on the field, helping to prioritize highly-referenced work. This selection emphasized studies with robust, validated methodologies and reliable metrics over purely theoretical research, thus aligning the review with evidence-backed research and practical insights.
iii. **Cross-Referencing with Existing Reviews:** To capture foundational work and identify new directions,

recent reviews in computational pathology and digital medical imaging were analyzed. By cross-referencing frequently cited or highly relevant articles within these reviews, key studies were included that contributed significantly to advancements in virtual histopathology. These reviews also highlighted areas needing further exploration, guiding the selection of research that addressed these gaps. This approach ensured both a well-rounded representation of established studies and coverage of recent innovations.

iv. **Trend Identification in Virtual Histopathology:** Analyzing research trends over the past decade, the search strategy aimed to capture high-growth areas, such as machine learning for diagnostic precision and virtual staining methods. Studies clustered around significant advancements were included to ensure the review covered both foundational and cutting-edge work. This method also allowed for the identification of recent methodologies, like contrastive learning and real-time imaging techniques, which are shaping current research and practical applications in digital pathology.

v. **Inclusion of Diverse and Regional Contributions:** To ensure global relevance, the strategy included research contributions from various regions and emerging research hubs. This expanded the range of sources to include studies capturing different datasets, healthcare practices, and methodological approaches worldwide. Regional studies brought unique perspectives, particularly in clinical applications, ensuring the review's findings were relevant across different healthcare environments.

Scope definition

Virtual histopathology techniques were the primary focus, with special emphasis on advanced image processing and machine learning approaches employed in this field. The objective was to analyze how staining can be made virtual and how it can be used to replicate traditional histopathological findings.

Database selection

The following databases were [25] chosen to cover a wide range of studies.

- **IEEE Xplore:** It is selected for its extensive range of engineering and technology publications.
- **Pubmed:** This repository is a biomedical journal that provides access to biomedical literature and research on medical imaging. It contains a large collection of articles published on virtual histopathology research.
- **Google Scholar:** Is expanding its collection to include more diverse academic articles, including those from interdisciplinary fields.

Search string

The decision on the search string influences the scope of the literature review conducted in the course of the systematic review. We used the following string to search for relevant articles:

"((Histopathology) *OR* (virtual histopathology)) *AND* ((deep learning) *OR* (machine learning) *OR* (medical imaging) *OR* (image analysis) *OR* (image processing) *OR* (virtual staining) *OR* (image-to-image translation) *OR* (contrastive learning))"

Besides the above-given search string, these keywords were used to search for articles that may be of interest to virtual histopathology, especially in the areas of enhancement in image processing and machine learning for medical image analysis. The articles had to be published between 2013 and 2023 while giving preference to peer-reviewed articles and conference papers focusing on the investigation of ML and DL in Virtual Histopathology.

"*Virtual histopathology*", "*histopathological deep learning*", "*machine learning*", "*deep learning*", "*virtual staining*", "*image-to-image translation*", and "*dual contrastive learning*"

Inclusion and exclusion criteria

The included studies were subjected to special scrutiny to confirm their quality and relevance. The following criteria are used to select relevant studies:

- Research published between 2013 and 2023,
- Conference papers and peer-reviewed studies are selected,
- Studies involving machine learning and deep learning for virtual histopathology investigations are selected only,
- Case studies that offered both quantitative and qualitative analyses of virtual staining techniques are also considered [26].

Besides using the inclusion criteria for selecting appropriate articles, exclusion criteria are also used to exclude irrelevant or redundant studies:

- Excluding those articles which are not available in English,
- Investigations using conventional histopathology without any computer-generated data are excluded,
- Studies that do not offer enough methodological details or evaluation metrics are also excluded,

Selection criteria

This research selected articles based on practices observed in highly cited review articles, using specific criteria to guide its selection process. Table 1 shows the article selection criteria.

Figure 1 shows the number of articles at each stage carried out to select appropriate articles concerning virtual histopathology.

Data extraction

To extract the main aspects the following standard approach was used: The extraction process involved several steps:

- **Abstract Evaluation:** Where the titles and abstracts of the papers obtained through the searching process above were first screened step to identify possibly relevant studies.
- **Journal Article:** So that all the papers selected can be put through a rigorous process to determine papers that contain only materials that are related and fall under the general umbrella of initiatives.
- **Interconnection of data:** To retrieve all of the data regarding a particular subject in everyone in each related investigation of the data, it is displayed in the form of tabular form.
- **Key aspects:** It is on this premise that this synthesis becomes useful to plot out many issues defining the area of digital innovation across these disciplines.

Core analysis

Technical approach

The central point in virtual histopathology is about using cutting-edge machine learning techniques in Deep Learning [27] and Generative Adversarial Networks [14] that have their key role in translating images of unstained tissue into virtually stained equivalents. In this respect, the model presented in the reviewed document, Dual Contrastive Learning Generative Adversarial Network [28], is such a model after considering the integration of

Table 1 Criteria for article selection

Criteria	Details
Publication year	Those studies which are published between 2013 and 2023
Relevance	Studies addressing neural stains, stain invariant, deep learning, cyclic attention, fabric GAN, and other related aspects
Type of study	Empirical studies (journal and conference articles) offering significant insights into the framework evaluation
Citations	Special preference is given to articles with a high number of citations

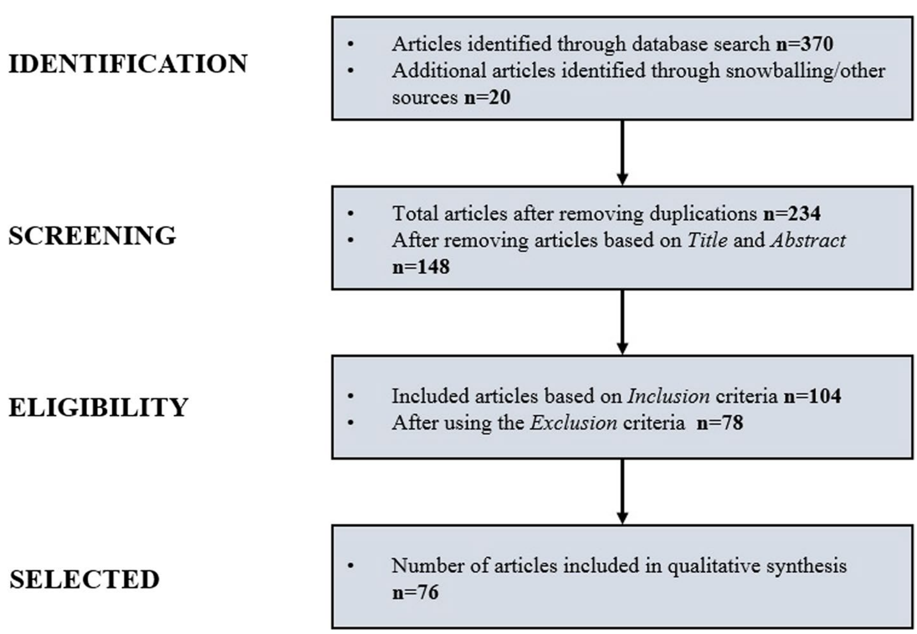

Fig. 1 Articles selection through various stages of review

dual generators with their corresponding discriminators. This method uses contrastive learning to make the virtually stained images as similar as possible to traditionally stained images [26].

Data and preprocessing

Data forms an important part in the training of most virtual [29] histopathology models. In this, models will usually require paired datasets of unstained and stained images of the same tissue samples. Other important steps include image normalization, resizing, and augmentation for the proper functioning of the learning model. All these preprocessing methods will standardize the input images to very minimal variability, therefore improving model performance and more robustness to a single set of test images [30].

Dataset and preprocessing issues in virtual histopathology

In virtual histopathology, the development and success of models using machine learning and deep learning depend mostly on the availability and quality of datasets. High-quality annotated datasets are needed for training robust models with a very accurate tissue classification, disease detection, or prediction. However, this is the greatest challenge: the unavailability of large, well-annotated datasets in the area. Images in histopathology are inherently complex and require expert annotation, which is time-consuming and expensive. In addition, variability in staining protocols, imaging equipment, and sample preparation methods is high, making the construction of standardized datasets quite challenging [26].

The other critical factor is the heterogeneity problem with the data itself. Histopathological images look very different due to differences in tissue processing and staining intensity, followed by differences in imaging conditions. These variations may further feed biases into the training data and result in models that don't generalize well on new, unseen data. For this, rigorous preprocessing steps have to be undertaken, including normalization of staining, aligning the images, and removing any artifacts within them. All these preprocessing steps by themselves are challenging and probably may change the biological information in these images, causing the loss of very important details for diagnosis.

Artificially increasing the dataset size using data augmentation techniques like rotation, flipping, and scaling is very common to improve model robustness. Some of these methods, however, at times introduce irrelevance in the real-world data, leading to very good results on augmented data but poor generalization on real clinical data. Therefore, it is key to the success of virtual histopathology that standardized preprocessing pipelines for such data are developed, and it is related to its improvement.

GAN challenges related to algorithm complexity in virtual histopathology

The reason GANs have gained great attention is because of the potential capability of generating high-resolution synthesized images that most nearly match real histopathological slides. Such synthetic images can be used for dataset augmentation, balancing data, or even virtual staining, whereby an unstained tissue would be digitally stained through a GAN. While GANs offer excellent potential, they also raise many challenges about algorithm complexity and reliability [26].

One of the major challenges to applying GANs in virtual histopathology is model complexity. Briefly, GANs consist of two neural networks, a generator and a discriminator, trained together in an adversarial process. The very fact that GANs contain dual networks sets them apart from other deep learning algorithms and makes them hard to train; it requires a balancing act so that neither network oversees the other in the training phase. Should the discriminator get too powerful, generating realistic images will then be hard for the generator, giving way to issues such as mode collapse. Here, the generator creates only limited types of images. On the other hand, when it is the opposite, that is if the generator is rather very strong against the discriminator, then it will generate unreal images that might at the same time fool the discriminator and, hence, reduce the quality of the synthetic data.

Another problem associated with GANs lies in their inherently unstable training process. One major issue with GANs is that they are notoriously hard to train because of vanishing gradients, convergence problems, and sensitivity to hyperparameters. These make the reproducibility of results difficult to attain, mostly while trying to create results that include high-fidelity histopathological images, which are virtually indistinguishable from real slides. Moreover, GAN training is computationally extremely intensive, making this model very hard to use in routine clinical practice.

These challenges form a great barrier to the wide application of GAN-based methods in virtual histopathology, in which accuracy and reliability are of concern. Several research studies are underway to develop more stable and efficient GAN architectures, such as Wasserstein GANs and CycleGANs, to sort out some of these problems. However, until these issues are fully addressed, the use of GANs in clinical settings is likely to remain limited.

Algorithm computational complexity

Advanced models, especially those that correspond to deep learning architectures, are computationally very expensive in virtual histopathology. Convolutional Neural Networks and generative adversarial networks have a high computational demand not only for training but also for inference [31]. This is due to the high volume of histopathological images processed at high resolution, which may be in the millions of pixels with multiple color channels.

Training these models typically requires the use of Graphics Processing Units (GPUs) or even better hardware like Tensor Processing Units (TPUs). These are high financial cost resources that may not be readily available in every clinical or research setting, more so in low-resource settings. Moreover, the training process for deep models is time-consuming, sometimes running into days or even weeks, depending on the size of a dataset and the model's complexity. This makes iterative model development and experimentation slow, reducing the pace at which progress is made within the field.

Moreover, even after training, the inference is computationally heavy in such models, especially considering WSIs in histopathology. In this context, WSIs are typically gigapixel images that need to be processed at high resolutions to avoid missing small-pathological features like an individual cancer cell. Additional challenges arise for latency and resource management when running such models in real time (e.g., during surgery or in an automated diagnostic workflow).

On the other hand, such computational complexity can be reduced using techniques like model pruning, quantization, and a new architecture of neural networks. All of these methods strive to reduce the size and computation of models without loss of performance as much as possible. Another way this could work is through cloud computing, which provides on-demand access to scalable computational resources. However, concerns about data security and patient privacy should be dealt with carefully concerning cloud-based solutions, especially where sensitive medical data is concerned.

Evaluation metrics

Quantitative metrics are considered a benchmark of the effectiveness of virtual histopathology models. Some of the key metrics include SSIM [20] and PSNR, which quantify the fidelity of the generated images relative to real ones. In addition, FID and KID quantify the quality of generated images concerning visual realism and preservation of content [26, 32, 33].

Clinical evaluation

Clinical relevance is paramount in virtual histopathology [5]. Most of the studies reviewed entail evaluations done by expert pathologists comparing the diagnostic utility between traditionally versus virtually stained images. These evaluations determine the extent of agreement between pathologists in diagnosis from virtual images and therefore establish the extent of applicability of the technology in clinical practice [26, 30, 34].

Applications and future directions

It has some very promising applications, including virtual histopathology. Virtual staining might considerably minimize the time taken for histopathological analysis and increase the speed of diagnosis, by providing better patient care. This minimizes dependence on dangerous chemicals; the virtual staining removes the use of chemical reagents involved in traditional staining and thus assures the safety of the process for the laboratory personnel and beings that are friendly to the environment.

Telepathology and remote consultation

Virtual staining encourages telepathology, as it increases the sharing and diagnosis of tissue material without staining by experts remotely.

Standardization and archiving

This methodology provides better consistency and standardization of histological images; it will be of great improvement in the long-term digital archiving and retrospective studies in medicine.

Research applications

Virtual staining can thus be applied to generate large synthetic H and E image datasets for training and developing other AI-empowered medical imaging instruments [26, 32].

While strengths brought to the field of virtual histopathology by ML, DL, and Visual Path are unique, the challenges each may pose are equally distinct. In that sense, ML brings versatility and predictive power, while DL uniquely offers very high image interpretation capabilities, and Visual Path enhances the quality of images with the potential for real time diagnoses. However, their successful application mandates careful attention to the challenges presented by operational concerns of data quality, computational demands, and ethical considerations. There is, therefore, the need to solve these challenges to realize the full potential of the application of these technologies in improving patient care with the continued development of virtual histopathology [26].

Literature analysis

Virtual histopathology has now given another frontier in medical imaging, wherein innovative computational algorithms fill an incorporated function by emulating the vast majority of traditional histopathological analysis devoid of the concomitant staining process. There is great interest in this approach due to its potentiality in workflow streamlining, reduction of chemical use, and increase in diagnostic accuracy. This paper presents an overview of virtual histopathology that unravels both the technical, clinical, and practical aspects evoked from the literature reviewed. Recent developments in computational methods have changed the face of medical imaging in terms of diagnosis and treatment. Among all these techniques, the ML, DL, and Visual Path techniques have emerged to be instrumental in improving the effectiveness of medical diagnoses. In this review, it is proposed to provide a critical analysis of the advantages and limitations of these approaches, and its focus is on virtual histopathology. Conducted based on the most current literature reviews involving new methods in tumor histopathological diagnosis, such as the deep learning-enabled virtual histological staining and other advancements in the approaches As such, this review is expected to provide a clear understanding of the present situation and scope for future enhancement. Figure 2 shows the graphical workflow of this analysis.

Machine learning approaches in virtual histopathology

Machine learning has become a cornerstone of modern medical imaging, particularly within virtual histopathology. Application of ML techniques in virtual histopathology involves training algorithms for the recognition of patterns and features in histological images that may

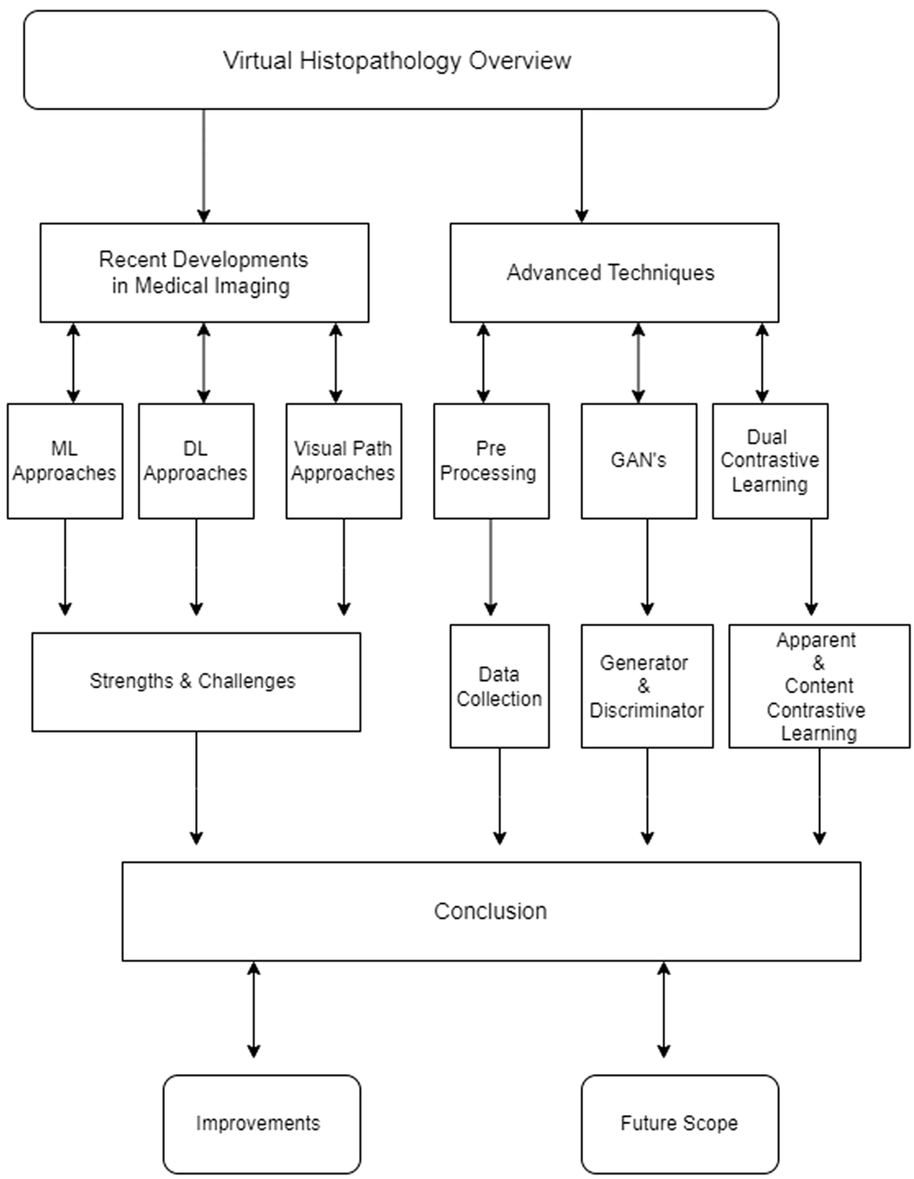

Fig. 2 Graphical workflow of literature analysis

help in diagnosis, grade tumors, and foresee patient outcomes, as shown in Fig. 3. One of the major strengths of ML in this regard has to do with its flexibility. In this context, ML algorithms have been applied to a multitude of imaging modalities, including traditional histology slides, images from immuno-histochemistry, and even very new methods for imaging, such as multi-photon microscopy.

Another key advantage of ML in virtual histopathology is that it enables predictive analytics. Algorithms of this nature can provide minute details regarding latent patterns correlating with disease progression or treatment response by analysis of huge amounts of data., as shown in Fig. 4. For example, in the diagnosis of cancer, ML models can be trained to perfectly distinguish benign from malignant cells with an accuracy degree sometimes higher than human pathologists [35].

However, virtual histopathology ML approaches do not come easy. The most prominent challenges in this field are related to the need for large, labeled datasets. To train ML models effectively, histopathological images need to be accurately annotated, which is time- and resource-intensive and requires expert opinion. Furthermore, it is the quality of training data that matters, since any biases or inconsistencies within the dataset may lead to skewed predictions and hence unreliable models. Of particular concern in this is medical applications, where misdiagnosis can result in grave consequences.

The second challenge relates to the interpretability of ML models. Many algorithms of ML, especially those using complex statistical methods, are often viewed to be of a "black box" nature, their inner decision processes remaining obscure. The problem in a virtual histopathology setting is the lack of transparency, whereby clinicians must understand and trust the model's predictions to come up with informed decisions about patient care. Indeed, interpretable ML models have been under development, but this has remained an area of ongoing research.

ML models hold the following advantages in the context of virtual histopathology:

- **Versatility and Predictive Power**: ML algorithms are adaptable to various imaging modalities, from traditional histology slides to advanced methods like multi-photon microscopy. They are particularly valuable in predictive analytics, offering insights into disease progression and treatment outcomes by identifying latent patterns in data.
- **Improvement in Diagnostic Accuracy**: ML models can achieve high accuracy in distinguishing between

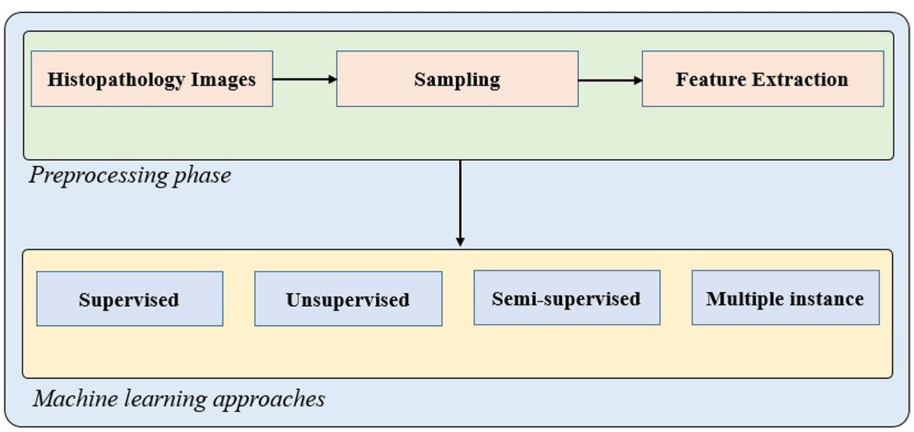

Fig. 3 Typical steps for machine learning in digital pathological image analysis. After preprocessing, various ML approaches can be applied

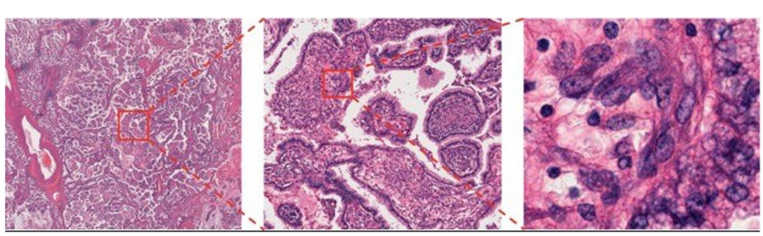

Fig. 4 Various magnification levels show different structures even for the same histopathological image; taken from [6]

benign and malignant cells, often surpassing human pathologists in specific tasks.

Despite their strengths and advantages, ML models face the following challenges:

- **Data Dependency**: The success of ML models heavily depends on the availability of large, accurately labeled datasets. The labor-intensive nature of data annotation and the potential for bias in training data pose significant obstacles.
- **Interpretability Issues**: Many ML models function as "black boxes", making their decision-making processes difficult to interpret. This lack of transparency is a significant barrier to clinical adoption, where understanding model predictions is crucial for informed decision-making.

Deep learning approaches in virtual histopathology

The latter are developments over the traditional ML techniques and provide more powerful tools for the analysis of such images. More importantly, mostly DL models, particularly CNNs, have registered very high performance in image recognition tasks and therefore are highly effective in virtual histopathology [36]. They can learn hierarchical features directly from raw data, so these technologies detect and classify tissue structures with minimal human intervention. A typical schematic of DL models for histological staining is provided in Fig. 5.

One of the great strengths of DL in virtual histopathology is its ability to manage massive high-dimensional datasets. Unlike traditional ML models requiring heavy feature engineering, the same is not required at the same level for DL models, which can autonomously learn features from data, an explicit reason for considerably less human curation and leading toward potentially discovering novel biomarkers. For example, DL-based algorithms have already demonstrated successful applications to tasks of tumor segmentation and classification of histopathological subtypes, down to genetic mutation prediction from histology images [37].

However, this is one of the strengths of DL, but instead, it challenges applications to virtual histopathology because of the high cost of computation for model training and inference. Typically, DL models require powerful hardware, such as GPUs, and large amounts of memory, which really can act as an impedance to their adoption in resource-limited settings. In addition, the process of training itself is quite time-consuming; it often requires, in the worst cases, even longer than a week or even a fortnight to attain optimal performance.

Another difficulty is susceptibility to overfitting, especially when working with small datasets [38]. This is quite a common issue with medical imaging data because medical datasets are often quite small in realistic clinical settings. When an overfitted classifier model is developed, it becomes too interested in the training set rather than generalizing over new, unseen examples. That is indicative of poor performance in real-world applications. This can be a serious limitation in virtual histopathology, where variability in tissue samples and staining protocols is common.

Not only that but also ethical considerations come into play in respect of the application of DL in virtual histopathology. Patient information privacy is paramount as DL models require access to voluminous sensitive medical data. The organization and protection of such data are of utmost importance concerning acting ethically. Besides, it must also work out the possibility of algorithmic bias since prejudiced models will equally perpetuate differences in health outcomes in healthcare.

DL models have the following advantages over other approaches:

- **High Performance in Image Recognition:** DL, particularly Convolutional Neural Networks (CNNs), has revolutionized image analysis in virtual histopathology. These models excel in identifying complex tissue structures with minimal human intervention.

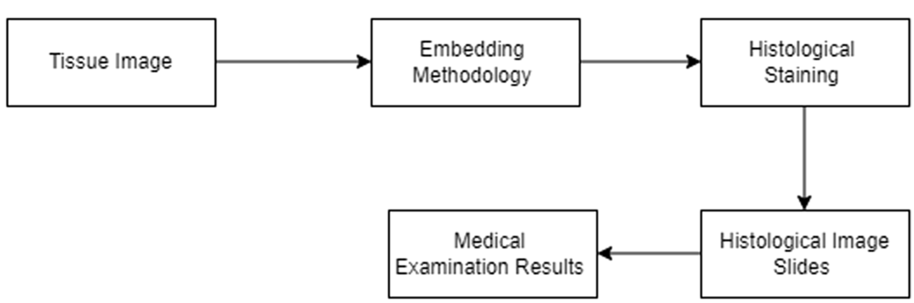

Fig. 5 Schematic of the standard histological staining and deep learning-based virtual staining

- **Handling High-Dimensional Data:** DL models can autonomously learn hierarchical features from vast datasets, reducing the need for extensive feature engineering. This capability has led to breakthroughs in tasks like tumor segmentation and mutation prediction from histology images.

The following are a few challenges related to DL approaches:

- **Computational Complexity:** DL models require substantial computational resources, such as GPUs or TPUs, for training and inference. This high cost can limit accessibility, particularly in resource-constrained settings.
- **Overfitting Risks:** DL models are prone to overfitting, especially with small datasets, leading to poor generalization in real-world applications. This is a significant concern in virtual histopathology, where dataset sizes are often limited.

Visual path approaches in virtual histopathology

The Virtual Path in virtual histopathology is based on the enhancement and analysis of histological images with a focus on better spatial resolution and image clarity. It can have applications in all those areas where correct visualization of tissue structures is critical like diagnosis of cancer or assessment of tissue morphology [37]. Visual schema of label-free virtual staining is provided in Fig. 6.

Probably one of the greatest strengths of the Visual Path approach is its potential to provide better image quality of histopathological images, wherein subtle features could become more visible and easier for analysis. Techniques such as super-resolution imaging, virtual staining, and image deconvolution could improve the resolution of digital slides very significantly. This would offer pathologists an opportunity to get a closer view of the tissues than they would have done using light microscopy. It can, therefore, result in increased accuracy in diagnosis and a finer understanding of the mechanisms behind diseases.

The Visual Path approach also holds a great deal of promise for monitoring and diagnosis in real-time. With improvements in imaging technology, systems that can capture images in real time can be developed and provide immediate feedback right at the procedure area during a surgical procedure or a biopsy examination. This would prove extremely valuable in situations whereby decisions have to be rendered within a very short time, such as during a surgery while identifying tumor margins.

Another strength of the Visual Path approach is its integration with other imaging modalities. Histopathological images can be combined with information from other modalities, like magnetic resonance imaging or positron emission tomography, for an ever more minute analysis of tissue. In this very multimodal approach, improved insight can be obtained into the underlying pathologies, hence improving diagnostic accuracy.

The challenges to the Visual Path approach are not few. Equipment and expertise in these advanced imaging techniques are fairly expensive and not available in most clinical settings. Besides, the quality of the imaging equipment is also very critical for the Visual Path

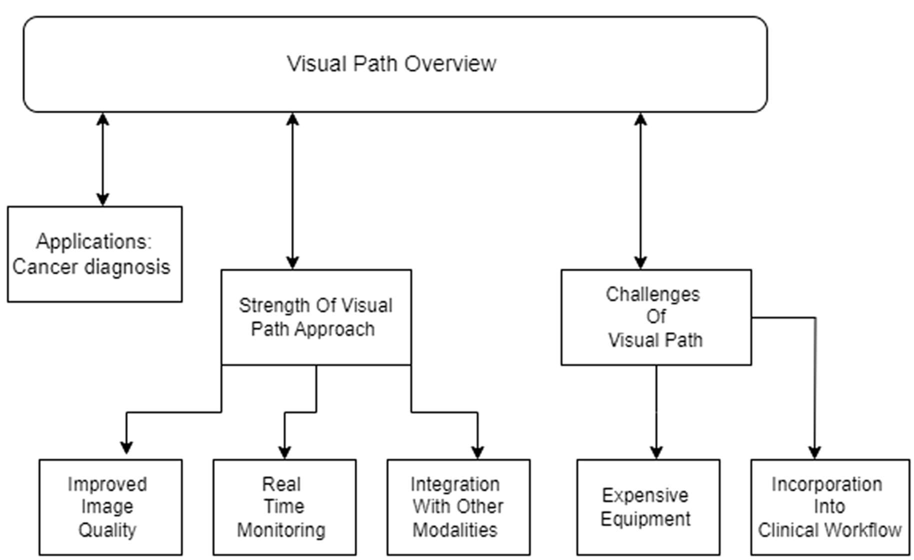

Fig. 6 Overview of steps involved in visual path approach for virtual histopathology

approach. This variability in imaging conditions, for example, because of different staining protocols or microscope calibration, already influences inconsistent results and thus limits the reliability of this approach when applied in routine clinical practice.

Another challenge involves incorporating these Visual Path techniques into the existing clinical workflow. The adoption of many new imaging technologies typically involves tremendous changes in well-established procedures, which might prevent their implementation. If these techniques are to be used at large scales, then it becomes very important that they are user-friendly and compatible with existing systems.

Visual Path approaches hold the following key advantages:

- **Enhanced Image Quality**: Techniques like super-resolution imaging and virtual staining have significantly improved the quality of histopathological images. This allows for more detailed tissue analysis, potentially leading to more accurate diagnoses.
- **Real-Time Diagnosis**: The Visual Path approach holds promise for real-time applications, such as during surgeries, where immediate feedback is critical. This capability could revolutionize intraoperative decision-making.

Even though Visual Path approaches have several key advantages, they are limited by the following factors:

- **Equipment and Expertise Requirements**: Advanced imaging techniques require sophisticated equipment and expertise, which may not be available in all clinical settings. This can limit the widespread adoption of the Visual Path approach.
- **Integration into Clinical Workflow:** Adapting these advanced techniques to fit into existing clinical workflows can be challenging. Compatibility and user-friendliness are essential for successful integration.

Comparison and future directions

Each approach, ML, DL, and Visual Path brings unique strengths to virtual histopathology, but they also come with distinct challenges. The continued advancement of these techniques will require addressing issues related to data quality, computational demands, and ethical considerations. Collaboration between researchers, clinicians, and regulatory bodies will be essential to overcoming these challenges and realizing the full potential of virtual histopathology in improving patient care (Table 2).

- **ML vs. DL:** While ML offers versatility and can work with various data types, DL provides superior performance in tasks requiring complex image analysis. However, DL's computational demands and risk of overfitting present significant challenges.
- **Visual Path's Unique Contribution:** The Visual Path approach stands out for its potential to enhance image quality and provide real-time diagnostic capabilities. However, its adoption is hampered by the need for advanced equipment and the complexity of integrating it into clinical practice.

Technical approach

Figure 7 shows the overview of the technical approach for virtual histopathology which involves generative adversarial networks (GANS), dual contrastive learning, and processing and handling of data. Each of these steps is further used for various tasks as shown in the figure.

Deep learning models find core applications in virtual histopathology in the image transformation by GANs [29] from images of unstained tissue into virtually stained images and the mimicking of their traditional stained specimen counterpart's visual characteristics. Key technical elements include:

Generative Adversarial Networks: They involve the generator and discriminator as two neural networks

Table 2 Approaches and results for the reference paper on various virtual histopathology

Reference	Approach	Results
Stain-invariant self-supervised learning for histopathology image analysis [39]	Utilizes self-supervised learning techniques to learn stain-invariant features from histopathology images. Focuses on extracting features that are robust to staining variations and enhances generalization across different datasets and staining protocols	Enhanced model generalization across various staining conditions and tissue types. Improved robustness and reliability in histopathological image analysis tasks such as segmentation and classification [40]
Deep learning-enabled virtual histological staining of biological samples	Implemented deep learning models to virtually stain biological samples. Utilized CNN and possibly other advanced architectures to simulate staining processes and generate virtual stains from unstained images	Successfully generated high-quality virtual stains with an accurate representation of tissue structures and biomarkers. Enabled cost-effective and scalable histopathological analysis without physical staining

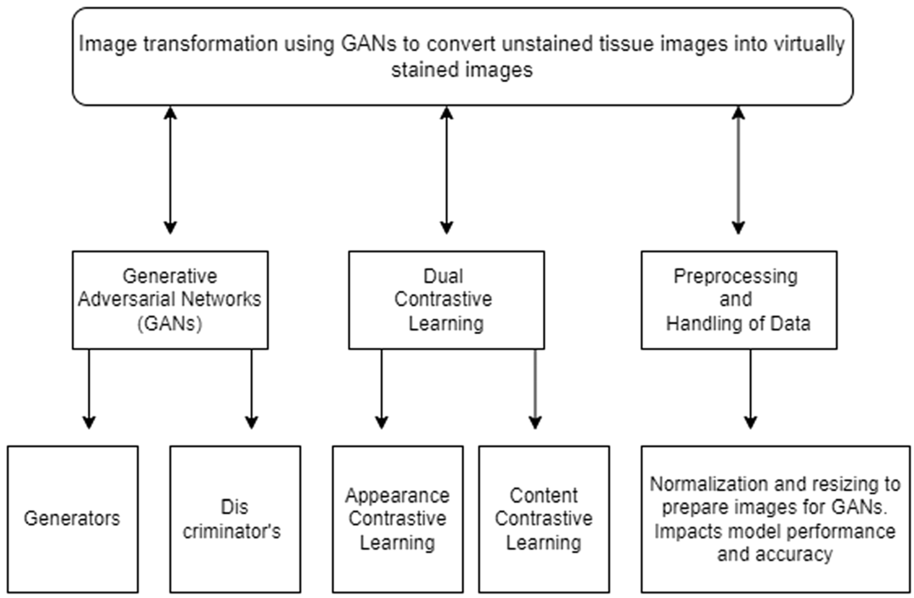

Fig. 7 Overview of technical approach for virtual histopathology

working together to offer the desired results. A generator creates images that look like the target domain, in this case, the stained images, while at the same time, a discriminator is required, which compares the authenticity of the images created. This therefore pushes the adversarial process to generate high-quality and realistic images [29, 41, 42].

Dual Contrastive Learning: This is a sophisticated methodology for the enhancement of realism and the diagnostic utility of virtual histopathology images. It includes the following two components:

Appearance Contrastive Learning: It ensures that the generated image will have very similar coloring and texture compared to the real image that is stained [43].

Content Contrastive Learning: This would preserve the integrity of the tissue structure and morphology, which is important for an accurate diagnosis [19, 28, 44].

Preprocessing and Handling of Data: Thus, effective techniques in preprocessing, such as normalizing images and resizing, are applied to the data to get it GAN-ready. Model performance and accuracy are thus impacted by the quality of the input data. Table 3 shows the approaches and results of reference papers.

Clinical evaluation

Virtual histopathology clinical applicability assessment, done by various metrics and expert evaluations, includes the following:

Visual Quality and Realism: This generally involves visual fidelity assessment between generated images and traditional images of tissues, which are normally dyed with different dyes like hematoxylin [5]. Usually, visual quality and the degree of structural preservation of the images are quantified using metrics such as SSIM and PSNR [20], which provide a score for the similarity in

Table 3 Approaches and results for the reference paper on various virtual histopathology

Reference	Approach	Results
Dual Contrastive Learning-Based Image-to-Image Translation of unstained Skin tissue into Virtually Stained H&E Images	Utilizes dual-stream contrastive learning to translate unstained skin tissue images to virtually stained H&E images. Maintains visual appearance and structural coherence of tissue samples simultaneously	Achieved high diagnostic accuracy comparable to traditional H&E staining. Efficient generation of virtual stains suitable for clinical integration. Significant reduction in time and resource requirements compared to manual staining
Neural Stain-Style Transfer Learning using GAN for Histopathological Images	Employs GANs for stain-style transfer in histopathological images. Learns to convert images from one staining style to another while preserving tissue structures and relevant features	Demonstrated effective stain- style transfer, enabling adaptation of stained images to different staining protocols or enhancement of specific staining characteristics. Improved visual quality and interpretability of histopathological images

appearance between the virtual and real histopathology slides.

Diagnostic Accuracy: The diagnosis against that made by the traditional method is used to measure the diagnostic utility of the virtually stained images [45]. High concordance between these methods would infer the potential for virtual histopathology applications to replace traditional staining procedures in clinical practice.

Clinical Workflows Efficiency: Virtual histopathology can save considerable time spent on staining procedures, thereby accelerating the diagnosis and, therefore, the treatment of patients. Exposure to toxic chemicals is also minimized while enhancing safety and sustainability for the environment. By analyzing these key thematic areas, the literature analysis will provide a comprehensive understanding of images suitable for clinical use.

Machine Learning In Medical Imaging: Machine Learning has shown remarkable versatility across various imaging modalities, making it a valuable asset in medical imaging. One of its primary strengths lies in its capability to perform predictive analytics, enabling the early detection and monitoring of disease progression. Moreover, ML algorithms excel at extracting meaningful features from complex datasets, facilitating the identification of patterns that may not be evident to human observers. However, ML also faces significant challenges, particularly the need for large, labeled datasets to train models effectively. The quality of the data is crucial, as poor-quality data can introduce biases and reduce the reliability of predictions. Furthermore, ML models often suffer from interpretability issues, with many functioning as "black boxes" where the decision-making process is not easily understood, posing challenges in clinical settings where transparency is essential.

Deep Learning In Medical Imaging: Deep learning has considerably outperformed the results in image recognition tasks, convincingly dominating the supremacy of medical imaging tasks. Compared with traditional ML, DL models can learn hierarchical features from raw data itself; hence, they offer an automated way of feature engineering and can probably discover new biomarkers. However, these strengths come with notable challenges: DL models are computationally intensive and need high computational resources for training and deployment. This can act to prevent diffusion in resource-limited settings [46]. Moreover, DL models have the fatal flaw of overfitting, which can be very strong when trained only on small datasets. Generalization in real-world applications can be very poor. Other ethical concerns exist relative to patient data privacy and algorithmic bias that could arise, thus potentially acting to further the disparities that already exist in health care.

Visual Path Approach: Where medical imaging through the Visual Path approach makes a difference is in spatial resolution and clarity. This approach better visualizes tissue structures and, therefore, significantly assists histopathological studies. Other advantages of this technique are real-time monitoring and diagnosis, whereby the clinician can know the results immediately during the procedure. The integration with other modalities of imaging provides strength to the potential by providing a more holistic approach to analyzing medical conditions. The complications, however, kick in with the implementation of the techniques from Visual Path. That is complex and calls for specialized knowledge and equipment to integrate these methods with existing systems. Moreover, the efficiency of Visual Path techniques depends much on the quality of imaging equipment, and variability in image conditions may result in inconsistent results, hence limiting reliability in clinical practice.

Comparative Analysis of Approaches: Comparing ML to DL, one could say that there exist different plus sides and minus sides for these approaches, as discussed in Table 4. If the former is less resource-intensive and has applicability in a much wider range of tasks, then the latter provides superior performance in image interpretation, especially for more complex scenarios. At the same time, DL has an immense requirement for computational resources and large datasets, which are its major limitations. Compared to ML and DL, the Visual Path focuses on quality and resolution but leaves the interpretation of the data to the physicians. It is therefore more specialized, with strengths in real-time application and integration with other modalities; nevertheless, it has a higher dependence on high-quality imaging equipment and may turn out to be less flexible than either ML or DL approaches.

Applications

Improved workflow efficiency

Virtual histopathology eliminates the need for physical staining, allowing labs to conduct more rapid diagnosis.

Safety and environmental impact

The less use of dangerous staining chemicals means that the lab is safer and the environment is cleaner.

Telepathology and remote diagnostics

Virtual histopathology can help ensure consistent diagnosis across laboratories and can help make staining results more standardized and reproducible [47]. Moreover, it enables the electronic preservation of tissue samples, facilitating the storage of them for extended periods and conducting retrospective examinations.

Table 4 Comparative analysis of various approaches

Criteria	ML	DL	GAN	CNN
Meaning	Traditional algorithms with feature extraction and classification	Advanced algorithms that learn features automatically	Generative model for creating new data samples	Specialized neural networks for image analysis
Accuracy	Moderate; dependent on feature selection	High; learns complex patterns from data	High for generating realistic images, moderate for classification	High for image classification and object detection
Train Time	Generally shorter, depends on feature complexity	Longer, requires extensive data and computational power	Very long involves training two networks	Moderate to long, depending on depth and complexity
Algorithm Complexity	Low to moderate, simpler models with manual feature extraction	High; complex architectures with many layers	Very high; involves two networks with adversarial training	High; involves multiple convolutional layers and pooling
Feature Extraction	Manual, requires domain knowledge	Automatic, learned from data	Automatic, can generate new features	Automatic, optimized for spatial hierarchies
Data Requirements	Moderate, may need preprocessed features	High, requires large volumes of labeled data	High, requires diverse and high-quality data for realistic generation	High, large, labeled datasets improve performance
Scalability	Limited, may not handle large datasets effectively	Excellent, scales well with data size	Excellent, can generate a wide range of data variations	Excellent, scales well with data size and complexity
Interpretability	High; models and features are often understandable	Low, complex models can be hard to interpret	Low; outputs are often less interpretable due to generative nature	Moderate; some interpretability with techniques like Grad-CAM
Application in Histopathology	Useful for simpler classification tasks	Effective for complex image analysis, including automated feature extraction	Useful for generating synthetic histopathology images for training	Highly effective for automated image analysis and classification tasks
Advantages	Simpler and faster for specific tasks, easier to understand	Can model complex patterns and relationships, self- improving	Can generate high- quality synthetic data, useful for data augmentation	Excellent for spatial feature extraction, strong performance in image-related tasks
Disadvantages	May require extensive feature engineering, less effective with large datasets	Computationally intensive, requires large datasets	Training can be unstable and resource-intensive, complex setup	Requires large datasets and computational resources, can be complex to tune

Standardization and archiving

Computational pathology can be advanced by utilizing large datasets of synthetic images to support the training and development of other AI-powered diagnostic tools.

Execution

It aims to address the challenges associated with the variability in staining techniques used in histopathology. This variability can hinder the performance of machine learning models due to differences in color and texture that are not related to the biological tissue being analyzed. Here's a summary of the performance and impact of such methods

i. **Stain Normalization**:

- Stain normalization is crucial for reducing variability and improving the robustness of machine learning models [48].
- GANs are employed to learn the mapping between different staining styles, producing images that appear as if they were stained using a standard protocol [49].

ii. **Generative Adversarial Networks (GANs)**:

- GANs consist of a generator and a discriminator network.
- The generator creates images that resemble the target staining style, while the discriminator attempts to distinguish between real and generated images.
- Through this adversarial training process, GANs can produce high-quality, realistic images in the desired staining style.

The Performance Evaluation is as follows:

i. **Visual Quality and Realism**:

- GAN-based methods, particularly those using advanced architectures like CycleGAN [28] or StarGAN [21], have been shown to produce visually convincing stain-normalized images.
- Evaluations often involve expert pathologists who assess the realism and usability of the generated images for diagnostic purposes.

ii. **Quantitative Metrics**:

- Structural Similarity Index (SSIM) and Peak Signal-to-Noise Ratio (PSNR) [20] are commonly used metrics.
- Studies have reported improvements in these metrics, indicating better preservation of tissue structure and higher image quality after stain normalization.

iii. **Impact on Downstream Tasks**:

- Classification: Improved stain normalization leads to better performance of classifiers trained on histopathological images [50].
- Segmentation: Enhanced consistency in staining improves the accuracy and reliability of segmentation algorithms.
- Detection: Object detection models, such as those identifying cancerous cells, benefit from the reduced variability introduced by different staining techniques [51].

iv. **Adaptability and Generalization**:

- GANs [8,20] trained on diverse datasets tend to generalize better across different staining protocols and types of histopathological images [52].
- Methods like CycleGAN [21, 28, 52], which can learn without paired examples, are particularly effective in handling diverse and unpaired datasets.

User experience

User experience is also a critical factor in the development of ML models for medical image analysis. This becomes even more important when advanced techniques such as GANs [28, 29, 53] are used. GANs are powerful tools for tasks like stain-style transfer learning in histopathological images [5, 20], but their complexity can impact user experience regarding implementation and practical application [54].

However, implementing GANs [21] can be resource-intensive and complex. The training process for GANs requires significant computational power and large datasets. Despite these challenges, the benefits in terms of image quality and model performance are substantial. For example, CycleGAN [21, 28]] uses unpaired datasets to learn the transformation between different staining styles, making it highly adaptable and effective for diverse histopathological images.

The user experience with GANs is also influenced by the availability of resources such as pre-trained models and open-source code. These resources can greatly enhance the practical usability of GAN-based methods. Researchers have found that when such resources are available, the adoption and implementation of these advanced techniques become much more feasible.

User feedback is crucial for evaluating the effectiveness of stain normalization methods. Studies have shown that pathologists and medical professionals tend to prefer the quality of images processed using GANs over those processed by traditional methods. This preference is due to the higher fidelity and consistency provided by GAN-based normalization.

Feedback from users regarding methods for virtual histopathology [55] has been quite positive, generally more so on the quality and consistency of the images produced. The quality of image fidelity is very high, especially for digitally stained images, reflecting the reality that pathologists and medical professionals could efficiently realize accurate and reliable diagnoses. On top of this, some researchers also pointed to considerable benefits arising from the availability of open-source code and pre-trained models, which can help both researchers and practitioners adopt and implement such advanced techniques.

Although certain challenges exist in virtual histopathology, it does give way to attendant benefits, among them an increase in diagnostic accuracy, greater efficiency, and resource optimization in medical diagnostics. It underlines show that a positive user experience with this technology allows for transformative potential in the histopathological [19] practices toward better patient outcomes.

In conclusion, while traditional methods for stain normalization in histopathological images [5] are easier and cheaper to implement, they often fall short in terms of quality and consistency. GAN-based methods [28], although more complex and resource-intensive, provide superior results that are highly valued in medical image analysis [56]. This suggests that the user experience with GANs, despite their complexity, is generally positive due to the significant improvements in image quality and model performance.

Performance

Visual quality and stain invariance

- **Robustness to Variability:** Self-supervised learning (SSL) techniques focus on learning robust [25, 57] representations from data without the need for manual annotations. When applied to histopathology images, stain-invariant SSL methods effectively handle the variability in staining protocols [45].
- **Consistency:** These methods produce consistent and high-quality features regardless of the staining variations, which are critical for accurate downstream analysis.

Quantitative metrics

- **Accuracy and Sensitivity:** Studies have demonstrated that stain-invariant SSL [45] models achieve high accuracy and sensitivity in classification tasks. The learned representations capture relevant tissue features while being invariant to stain differences.
- **Comparison with Supervised Methods:** SSL methods [45] often approach or even exceed the performance of traditional supervised learning methods, particularly when large labeled datasets are not available. Metrics such as F1-score, precision, and recall show significant improvements over baseline models trained on stained data.

Impact on downstream tasks

- **Classification:** Improved stain invariance leads to better performance in classifying histopathological images into different categories (e.g., cancerous vs. non-cancerous) [58]. Models trained with SSL [59] exhibit enhanced generalization across various staining conditions [60].
- **Segmentation:** Stain-invariant features enhance the accuracy and robustness of segmentation algorithms, leading to the precise delineation of tissue structures, [55] which is vital for diagnostic purposes.
- **Detection:** Object detection tasks, such as identifying specific cellular structures or anomalies, benefit from the invariant features learned through SSL [45], resulting in higher detection rates and reduced false positives.

Adaptability and generalization

- **Cross-Dataset Performance:** SSL [45] methods show strong generalization across different datasets and staining protocols. This adaptability is crucial for deploying models in diverse clinical settings where staining variability is common.
- **Unsupervised Pre-training:** By leveraging large amounts of unlabeled data, SSL [45, 61] models can be pre-trained and later fine-tuned on smaller, labeled datasets. This approach significantly boosts performance and reduces the dependency on extensive annotated data.

Computational efficiency

- **Training Time:** While SSL [45] methods can be computationally intensive during the pre-training phase, the benefits in terms of reduced need for labeled data and improved model robustness often justify the initial computational cost.
- **Inference Speed:** Once trained, these models typically maintain efficient inference speeds, making them suitable for real-time or high-throughput analysis in clinical environments.

Table 5 Performance comparison of methodologies for histopathology

Techniqie	Performance
Image To Image Translation [63]	Good performance
Transfer Learning [64]	Good performance
Stain Invariant Self Supervised Learning [8, 39]	Good performance
Deep Learning [48, 50]	Moderate performance, depends on model
Unsupervised Stain Normalization [65]	Low performance
Fabric GAN [66, 67]	Good performance
Cyclic Attention GAN [62, 68]	Good performance

Technical approach

At the core of this research is a very advanced architecture the model with generators and discriminators to do image translation based on contrastive learning. The generators map images of unstained tissues to their corresponding virtually stained images, while the discriminators estimate their realness. The contrastive learning strategy ensures a high-fidelity translated image, which is a real virtual-stained image, by highlighting the changes between the paired samples in the source dataset.

Evaluation metrics

Quality evaluation for the virtually stained images generated by the model utilizes quantitative metrics. Some of the key metrics are the Frechet Inception Distance and [44] Kernel Inception Distance [62], which reflect a more realistic similarity between the images generated and real virtual images. Such metrics provide a numerical basis for the assessment of how much the virtual images most nearly approximate traditional staining [42] on samples so that processes of virtual staining meet high standards of accuracy and realism. Table 5 shows the performance results of various histopathology studies.

Augmentation of data preparation

The CAGAN [58] model is designed to be very similar to a dual-cycle architecture but with added attention mechanisms aimed at improving the quality of the generated images [69]. The generators translate in both directions: that from unstained to H and E-stained images [5, 70] and that from H and E-stained [5] to unstained images. The attention module in each generator ensures that crucial areas in tissue samples are focused on by the network for fine details to be reproduced. The discriminators are used to distinguish between the real and the generated images, whilst the cyclic loss enforces consistency in translation.

The CAGAN model is trained using a diverse database consisting of images of skin tissue that are unstained as well as their corresponding H and E stained [71] images. In data preparation, there is a lot of care taken in preprocessing, the image's main attributes such as size, brightness, and contrast, are normalized and standardized. These steps are important for applying the model to various conditions in a sample and having more generalizable results. The augmentation techniques like rotation, flipping, and color jittering assist in preparing a dataset for training that helps the model to work better.

Pathologist validation

To perform a clinical validation, pathologists look at the reference images under the bright-field microscope and compare them with the Fabric GAN [21, 28] generated IHC stained images. Completely, the comparison will be made regarding diagnostic capability in terms of accuracy and interpretability of the key histological features and their practical usefulness in routine clinical settings. Such feedbacks inform the improvements made to the model and final preparations for its deployment for practical use. The dataset used to train the Fabric GAN contains images from both unstained and stained IHC tissue sections. Basic image processing includes normalization of the image, resizing the image, and also augmentation of the images used before feeding the data into the computer. Processing is done in a way that gains better contrast of the image and lessens the presence of noise Signals such as contrast adjustment and noise reduction are used to improve the training data so that the model can mimic the staining process accurately [72].

To rate the presented Fabric GAN model, several indices are applied: Some of these are the Frechet Inception Distance (FID) and Peak Signal-to-Noise Ratio (PSNR) [20] that measures the similarity of the generated images. Higher results in the above-mentioned parameters describe that the virtual stained images look like actual stained images to a greater extent in terms of color and texture.

Role of deep learning and CNN in medical histopathology

Machine learning is a broader concept of making machines [73] learn without being explicitly programmed, and a subcategory of it is known as deep learning [59] where neural networks are employed to mimic important patterns of data. In medical imaging these approaches have redefined how histopathological images are analyzed and classified for identification of tumors or cancerous tissues [74–77].

Convolutional Neural Networks (CNNs) are considered a base for deep learning particularly in image processing. In histopathology, CNNs can learn to establish correlations [78, 79] between tissue patterns and colorectal cancer possibilities independently. Structural functionalism like VGG, ResNet, and Inception have been used in increasing diagnostic accuracy. VGG, ResNet, AlexNet, GoogLeNet (Inception), and MobileNet are among the most widely applied CNN-based models for medical histopathology [80–82].

Freezing and fine-tuning models for histopathology has been useful as most of the time labeled data in histopathology is scarce. When these models are trained on histopathological images, researchers [1] have learned that fine-tuning the latter models enhances the performance further, while shortening training times in the process. In the case of powerful deep learning models, data preparation plays a significant role that results in very high-quality datasets. They gathered histopathological tissue examples in what were normal light fields, taken at x200 and x400 magnification, labeled by pathologists, and preprocessed. Standard preprocessing procedures may involve normalization, augmentation, and stain normalization to help overcome the issue of staining on the tissues.

Role of DL and ML virtual histological staining

The conventional staining technique is based on the use of chemical reagents and involves the application of dyes on biological tissues to enhance the visibility of the structures that are useful in diagnosis and investigation. Nevertheless, it can take relatively a long time and may also change the ensuing histological appearance of the tissue sample. Recent advances in deep learning offer a novel approach: virtual staining refers to the procedure in which histochemical staining is performed virtually without a human staining agent.

Higher-level architectures such as CNNs [78] have demonstrated superb performance in the stain inference process from label-free microscope images to virtual stains. These models learn to map the features of unstained samples to the features of the stained tissues by mimicking the pattern and nature of stains or features that have been previously provided.

The effectiveness of virtual staining models is dependent on the comparison made between the virtual section of the stained tissue and the physically stained section of the same tissue. Structural similarity index (SSIM), peak signal-to-noise ratio (PSNR) [20] and other benchmark studies which are complemented by the examination and scrutiny of the results by expert pathologists are some of the measures that are used in this regard. Training is conducted using the input of pairs of unstained and stained images to map them; backpropagation and optimization are involved [83]. Mean squared error (MSE) and perceptual loss have been used in predicting the stained images with the object of evaluating the difference between the predicted and the actual stained images within the learning process of the model. Table 6 shows various uses of CNN architectures.

Dual contrastive learning models

Dual contrastive learning models are a cutting-edge approach in virtual histopathology, leveraging the power of deep learning to enhance the accuracy and efficiency of histopathological analysis. These models use contrastive learning techniques to improve the differentiation between various histopathological features, leading to more precise diagnostic outcomes. Table 7 provides a summary of the advantages of dual contrastive learning. It has the following advantages.

- **Enhanced Feature Extraction:** Dual contrastive learning models excel at extracting intricate features

Table 6 Use of CNN architectures for virtual staining

Architecture's	Methodologies
U-Net	Known for its effectiveness in biomedical image segmentation and also it is popular for generating detailed and accurate virtual stains [84]
GAN's	GAN's, particularly like Pix2Pix and Cyclic GAN, are employed to create high fidelity-stained images from label-free inputs [49, 66, 67]
Autoencoders	These networks compress and reconstruct images, learning the essential features required for virtual staining [60]

Table 7 Advantages and limitations of dual contrastive learning model

	Description
Advantages	
Enhanced Feature Extraction	Extracts intricate features, aiding in detailed analysis
Improved Diagnostic Accuracy	Reduces misclassification rates
Robustness To Variations	Handles variations in staining and image quality effectively
Automated Learning	Requires minimal manual intervention
Limitations	
High Computational Cost	Requires significant computational resources
Data Dependency	Performance is highly dependent on training data quality and quantity
Complex Implementation	Needs expertise in deep learning and medical imaging for setup and tuning
Interpretability Issues	Difficult to interpret model decisions

from histopathological images, facilitating more detailed and accurate analysis.

- **Improved Diagnostic Accuracy:** By contrasting different image pairs, these models reduce misclassification rates, leading to higher diagnostic accuracy.
- **Robustness to Variations:** They are more robust to variations in staining, image quality, and other inconsistencies commonly found in histopathological data.
- **Automated Learning:** These models require less manual intervention during the training process, making them highly efficient and scalable.

Dual contrastive learning suffers from the following limitations:

- **High Computational Cost:** Training dual contrastive learning models requires significant computational resources, including high-performance GPUs.
- **Data Dependency:** The performance of these models is heavily dependent on the quality and quantity of the training data.
- **Complex Implementation:** Setting up and fine-tuning these models can be complex and requires expertise in deep learning and medical imaging.
- **Interpretability Issues:** Like many deep learning models, dual contrastive learning models can act as a "black box", making it challenging to interpret how decisions are made.

Case studies and applications

Case Study 1:Breast Cancer Detection:

In research conducted at the National University of Sciences and Technology, dual contrastive learning models were applied to histopathological images of the breast for adequate cancer detection [85, 86]. This showed a great deal of improvement in the differentiation of malignant from benign tissues, which arises at an accuracy rate of 94 percent. This improved accuracy can be ascribed to how the model learns slight differences in morphology.

Case Study 2: Liver Disease Classification

A group of researchers from the University of California used contrastive learning models for the classification of various liver diseases against the background of histopathological slides [87]. The accuracy of classification was improved and the analysis time was reduced by these models [88]. According to the study, diagnostic speed increased by 20 percent, hence making the process much more efficient for pathologists. Results of case studies are given in Table 8.

Applications

- **Cancer Diagnosis:** Dual-contrastive learning models show very good performance in the diagnosis of cancer, where differentiation between malignant and benign tissues is of paramount importance.
- **Automated Pathology:** These models help in automated pathology workflows, hence decongesting the workload of pathologists, increasing the throughput [89].

Table 8 Case studies on dual constrastive learning models

Case study	Application	Results
Breast cancer detection	Differentiation of malignant and benign tissues	94% accuracy in detection
Liver disease detection	Classification of various liver diseases	20% increase in diagnostic speed

- **Research and Development:** They are very instrumental tools in medical research, as they aid in the development of new diagnostic techniques and the finding of treatments [90].

Image-to-image translation approaches

Image-to-image translation serves as a significant tool in virtual histopathology, consisting of the process of image transformation from one domain to another with the view of preserving essential characteristics. This approach is particularly useful in tasks like virtual staining, where an image of unstained tissue is translated into its stained counterpart image, thus enabling more accurate and efficient analysis. An overview of image-to-image translation approaches is illustrated in Fig. 8.

Generative adversarial networks

On image-to-image translation, GANs are very popular. There exist two neural networks: one generating images and the other discriminating between the synthetic images. In other words, this struggle of the two networks against each other will come up with highly realistic translations.

CycleGANs

CycleGANs are specifically designed GANs aimed at an image-to-image translation task when paired training data is not available. They work by forcing a cycle-based consistency loss, which is usually implemented to ensure that translating an image in another domain and then back again brings back the original image.

Pix2Pix

Pix2Pix is a framework for conditional GANs and relies upon paired images during training. It learns a mapping from input to output images and a loss function to train this mapping. It is especially good when paired datasets are available.

Architectures based on U-Nets

One of the major reasons U-Net architectures are vastly applied in medical imaging originates from the fact that image segmentation tasks are applied with elevated

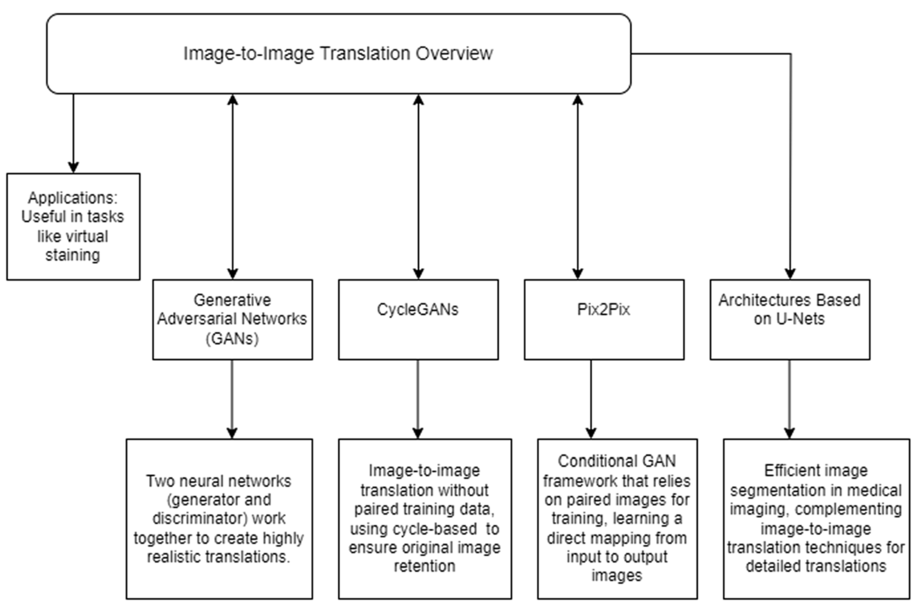

Fig. 8 Overview of image-to-image approaches for virtual histopathology

Table 9 Current methods in image-to-image translation

Method	Description
Generative Adversarial Networks	Consists of a generator and discriminator for creating and evaluating synthetic images
CycleGANS	Uses cycle consistency loss to translate images without paired training data
Pix2Pix	Relies on paired images for training, learning a direct mapping from input to output images
U-Net Based Architectures	Combines U-Net efficiency in segmentation with translation techniques for high-quality images

efficiency. Further complemented by image-to-image translation techniques, they provide a fineness to the details of the translated images. Table 9 provides a summary of image-to-image translation approaches.

Comparative analysis

All these techniques have some pros and cons that make them quite suitable for different applications of virtual histopathology.

i. **Generative Adversarial Networks**

- **Advantages:** Yield high-quality synthesized images, thus yielding high quality generated output images; can be used on a wide number of translation tasks.
- **Disadvantages:** Need huge computational resources; can get very unstable and hard to train.

ii. **CycleGANs**

- **Advantages:** Work quite well on unpaired datasets; high retention field-of-view through cycle consistency loss.
- **Disadvantages:** Might not retain fine details; computationally quite very expensive as it is a cyclic process.

iii. **Pix2Pix**

- **Advantages:** Is very accurate if paired datasets are used. Direct and Efficient Mapping from Source to target.
- **Disadvantages:** Require paired training data which is usually not available in medical imaging. Less effective with unpaired data.

iv. **U-Net Based Architectures**

- **Strengths:** Preserve well fine details. Good efficiency for segmentation tasks.
- **Disadvantages:** It might need further modification to work better for translation tasks. Has not been as widely used for translating images-to-images, unlike GANs.

Comparative analysis of image-to-image translation methods is given in Table 10.

Applications

- **Virtual Staining:** Image-to-image translation methods abundantly apply to virtual staining in the domain of converting images of unstained tissue into their virtually stained counterparts for easier analysis.
- **Artifact Removal:** Artifacts from histopathological images can be removed by the following to improve the quality of the image and improve diagnostic accuracy.
- **Modality transformation:** This is the process of transforming images from one imaging modality to another, such as from MRI to CT scans, etc. so that all images can be analyzed comprehensively.

Training and education for pathologists

As methods of virtual histopathology keep improving, there is a necessity for training and education to enable pathologists to make proper use of innovative technologies. Hands-on proper training programs would be immensely instrumental in bridging the knowledge gap in using virtual histopathology tools.

Bridging the knowledge gap

The incorporation of virtual histopathology into clinical routine requires knowledge on the part of pathologists in terms of both theory and practice concerning these technologies. Three major elements for bridging this knowledge gap are as follows:

Table 10 Comparative analysis of image-to-image translation methods

Methods	Strengths	Weaknesses
GANS	High visual fidelity	High computational cost
CycleGANS	Effective with unpaired data	Struggles with fine details, computationally intensive
Pix2Pix	High accuracy with paired data, efficient mapping	Requires paired data, and is less effective with unpaired data
U-Net Based Architecture	Excellent detail preservation, efficient for segmentation	May need modification for translation tasks, not as widely used in this context

Theoretical training

That means educating rationalists about the basic foundations of virtual histopathology, such as machine learning algorithms, techniques for image analysis, and digital pathology workflows [91].

Technical knowledge

This should ensure that pathologists have the requisite experience in using virtual histopathology software and hardware, from image acquisition and processing to analysis tools.

Clinical applications

Demonstrate the application of virtual histopathology within a clinical environment using case studies and practical examples. Table 11 provides a few components to bridge the knowledge gap.

Hands on training programs

Though bridging the knowledge gap primarily would require hands-on training programs, these should be designed to enable hands-on experience with arrangements for an inbuilt deep understanding of the methods of virtual histopathology.

i. **Workshops and Seminars:** There can be a continuous series of workshops and seminars on specific aspects of Virtual Histopathology. Such workshops and seminars should have an interactive segment mandatorily in the form of live demonstrations and an ordered Q and A session.
ii. **Simulation-Based Training:** The use of virtual simulation platforms would provide a risk-free environment for pathologists to practice and enhance their skills. Realistic scenarios may also be simulated with practice performance feedback.
iii. **Mentorship programs:** Setting up less-experienced pathologists with more senior experts in the area of virtual histopathology can make knowledge transfer easier and arrange for continued support and advice on this matter.
iv. **Online Courses and Certifications:** Full-fledged online certification programs in courses can quite reasonably offer flexible learning opportunities to pathologists for learning about virtual histopathology at their convenience.
v. **Collaborative Learning:** This training experience will be greatly enhanced by the much-needed collaborative learning built through group projects and peer-to-peer interactions, fostering community among pathologists.

A few important components of the hands-on training program are provided in Table 12.

Implementation strategy

This is the strategy that can be adopted to successfully implement training and education programs about pathologists:

i. **Needs Assessment:** A needs assessment has to be carried out to identify the specific needs of the pathologists concerning their training in different settings.

Table 11 Components of bridging the knowledge gap

Component	Description
Theoretical training	Education on principles of virtual histopathology including machine learning and image analysis
Technical proficiency	Training in software and hardware for image acquisition, processing, and analysis
Clinical applications	Case studies and practical examples demonstrating clinical applications

Table 12 Components of the hands-on training program

Components	Description
Workshops and Seminars	In-depth training sessions with interactive components
Simulation-based Training	Virtual simulations providing practice in a risk-free environment
Mentorship Programs	Pairing with experienced experts for guidance and support
Online Courses and Certifications	Flexible learning opportunities with structured courses and certifications
Collaborative Learning	Group projects and peer-to-peer interactions for enhanced learning

ii. **Curriculum Development:** A detailed curriculum on both the theoretical and practical aspects of virtual histopathology.
iii. **Resource Allocation:** Ensure adequate availability of resources in trainers, equipment, and funds to support the training programs.
iv. **Evaluation and Feedback:** Checking the effectiveness of the training programs at regular intervals, incorporating feedback for continual improvement in the process of training.

Table 13 provides steps needed for the implementation strategy for training and education.

By addressing the knowledge gap and providing hands-on training, pathologists can effectively adopt and utilize virtual histopathology methods, ultimately improving diagnostic accuracy and patient outcomes.

Future research directions in virtual histopathology

With the continuous development of virtual histopathology, some areas are ripe for future research. Those provide huge potential for improvements in the field of medical imaging, diagnostic accuracy, and most importantly, improvements in patient outcomes. The unexplored areas in virtual histopathology are enumerated below:

Real-time image processing

- **Description:** Algorithm and system development for real-time processing of histopathological images.
- **Potential Impact:** Immediate diagnostic feedback in surgery leads to improved patient outcomes.

Multi-modal image integration

- **Description:** Integration of multi-modality images, like MRI, CT, and PET images, with histopathology images.
- **Potential Impact:** It gives the minute details of tissues, hence enhancing diagnosis accuracy.

Personalised medicine applications

- **Description:** Use of virtual histopathology in individual treatment planning concerning a single histopathological profile.
- **Potential Impact:** The treatments will be given according to the sufferer's characteristics of the disease, hence its effectiveness is increased and side effects are reduced.

Advanced AI techniques

- **Description:** Investigation on the application of cutting-edge AI techniques, such as reinforcement and federated learning, toward virtual histopathology.
- **Potential Impact:** Enhanced accuracy and robustness of diagnostic algorithms.

A few important unexplored areas in virtual histopathology are discussed in Table 14.

Case study: clinical application of virtual histopathology

One of the notable applications of virtual histopathology has been in the diagnosis of breast cancer. In a study conducted by researchers at the University of California, a deep learning-based virtual staining technique was implemented to distinguish malignant from benign breast tissue samples. The team employed a convolutional neural network (CNN) model to create virtually stained slides from unstained tissue images, accurately simulating the appearance of traditional histochemical stains used in breast cancer diagnostics.

Clinical Impact This virtual staining technique demonstrated a high diagnostic accuracy comparable to that of

Table 13 Implementation strategy for training programs

Steps	Description
Needs Assessment	Identifying specific training requirements
Curriculum Development	Creating a comprehensive curriculum covering theoretical and practical aspects
Resource Allocation	Ensuring availability of trainers, equipment, and funding
Evaluation and Feedback	Regular evaluation and incorporation of feedback to improve training programs

Table 14 Unexplored areas in virtual histopathology

Area	Description	Potential Impact
Real-time Image Processing	Developing algorithms for real-time image processing	Immediate diagnostic feedback during surgeries
Multi-modal Image Integration	Integrating multiple imaging modalities with histopathological images	Comprehensive tissue view for more accurate diagnoses
Personalized Medicine Applications	Utilizing virtual histopathology for personalized treatment planning	Tailored treatments for improved efficacy and reduced side effects
Advanced AI Techniques	Exploring reinforcement and federated learning in virtual histopathology	Enhanced accuracy and robustness of diagnostic algorithms

traditional methods, with a reported accuracy of over 94 percent. This allowed pathologists to assess the same visual detail and cellular structure without requiring physical dyes. The virtual process reduced diagnostic time significantly, enabling more rapid treatment decisions. In clinical practice, the time efficiency and reduction in chemical reagents also contributed to lower operational costs and enhanced safety for laboratory staff.

Broader Implications The success of virtual histopathology in breast cancer diagnostics has sparked further research into its use for other cancers, such as prostate and lung cancer. Additionally, studies have begun to explore integrating these techniques into telemedicine and remote diagnostics, which could make histopathological assessment more accessible in underserved regions.

Academic and industry partnerships

i. **Joint Research Initiatives**

- **Description:** Academic-industry collaborative research projects
- **Impact:** Integrates to link academic heft with industry resources to drive faster innovation and application.

ii. **Technology Transfer Programs**

- **Description:** Programs that facilitate technology and knowledge transfer from academia into industries.
- **Potential Impact:** Fosters Commercialization of Research Done at the Academic Level; Real-world Applications Driven.

iii. **Industry-sponsored Fellowships**

- **Description:** Funding from the industry sponsors for researchers in virtual histopathology
- **Impact:** Integration of business heft with academic, leading to accelerated innovations and applications. item **Potential Impact:** Provides financial support and sector access to researchers to innovate.

iv. **Collaborative Networks and Consortia**

- **Description:** A special kind of network of consortia consisting of several stakeholders coming together in a joint research setup.
- **Potential Impact:** Helps in knowledge sharing and pooling of resources, particularly the big ones.

Table 15 provides guidelines concerning industry and academia partnerships and their overall impact.

Table 15 Academic and industry partnerships

Partnership	Description	Potential Impact
Joint research initiatives	Collaborative projects between academia and industry	Accelerates innovation and application
Technology transfer programs	Facilitating technology and knowledge transfer from academia to industry	Promotes commercialization of research
Industry-sponsored fellowships	Fellowships funded by industry to support researchers	Provides financial support and industry connections
Collaborative networks and consortia	Networks bring together multiple stakeholders	Enhances knowledge sharing and resource pooling

Discussion of challenges

Discussions

Enhanced diagnostic accuracy

A huge step forward in diagnostic accuracy is the development of dual contrastive learning models that can generate virtual stains that are as accurate as traditional staining. Models can be used to identify and diagnose problems with tissue samples and preserve both the visual and structural integrity of the samples.

Efficiency and scalability

Improved computational efficiency enables the quick generation of quality virtual stains, thereby reducing the time and resources consumed by manual staining and analyses. This streamlining improves workflow efficiency and supports timely decision-making in clinical settings.

Resource optimization

Virtual staining is much more efficient and resource-efficient than staining with physical reagents and skilled workers. Computational methods make diagnosing easier and cheaper.

Advantage of traditional machine learning approaches

In light of the aforementioned challenges associated with deep learning models, traditional machine learning approaches present a viable alternative, particularly when combined with intelligent data preprocessing strategies. Studies such as Kumar et al. [92], Guo et al. [93], and Shehab et al. [94] have demonstrated that traditional machine learning algorithms can outperform deep learning models in specific medical imaging tasks, achieving higher accuracy, sensitivity, and specificity. These approaches typically require less computational power and offer greater interpretability, facilitating their adoption in diverse clinical contexts.

Challenges

Integration into clinical practice

This will require attention to some regulatory considerations, protocol standardization, and healthcare personnel training to be seamlessly integrated into routine clinical practice. Broad diffusion and effective use of virtual histopathology methods call for pragmatic steps.

Opportunities for improvement

Handlings of advanced neural network architectures, such as generative adversarial networks [95] and self-supervised learning [96], are likely to realize future developments in virtual histopathology, further improving quality, robustness, and applicability of the envisioned techniques of virtual staining [12].

Collaborative efforts

Multidisciplinary collaboration between machine learning experts, pathologists, and biomedical engineers forms the bedrock of innovation in virtual histopathology. This will ensure that the models being developed are relevant clinically, robust, and implemented efficiently to benefit a patient [64, 78, 97].

Finally, while the virtual histopathology methods herald exciting opportunities for better diagnostics and smoothening healthcare workflows, continued research efforts, collaboration, and technological advances are needed to surmount the challenges brought forward by these cutting-edge methodologies and to bring about their full clinical potential.

Dataset and preprocessing issues in virtual histopathology

The availability and quality of datasets are paramount for the successful deployment of machine learning and deep learning models in virtual histopathology. High-quality, well-annotated datasets enable models to perform accurate tissue classification and disease detection. However, obtaining such datasets is challenging due to the complexity of histopathological images and the extensive expert annotation required, which is both time-consuming and costly. Furthermore, variations in staining protocols, imaging equipment, and sample preparation methods introduce significant heterogeneity, complicating the standardization of datasets and potentially affecting model performance.

To mitigate these issues, rigorous preprocessing steps such as stain normalization, image alignment, and artifact removal are essential. While these processes aim to enhance data consistency, they also risk altering critical biological information necessary for accurate diagnosis. Data augmentation techniques like rotation, flipping, and scaling are commonly employed to artificially increase dataset size and improve model robustness. However, these methods may introduce unrealistic variations, leading to models that perform well on augmented data but poorly on real-world clinical data. This underscores the need for developing standardized and biologically informed preprocessing pipelines to ensure data quality and model generalizability.

GAN challenges related to algorithm complexity in virtual histopathology

Generative Adversarial Networks (GANs) have shown promise in generating high-resolution synthetic images that closely resemble real histopathological slides, offering potential applications in data augmentation and virtual staining. Despite their capabilities, GANs present significant challenges related to algorithmic complexity and training stability. The adversarial training process between the generator and discriminator networks is delicate and prone to issues such as mode collapse and convergence instability. Additionally, GANs are sensitive to hyperparameter settings and require substantial computational resources, limiting their accessibility and practical implementation in clinical settings.

Recent advancements, including the development of architectures like Wasserstein GANs and Cycle Gans, aim to address some of these stability and efficiency concerns. However, further research is necessary to fully resolve these challenges and facilitate the reliable application of GANs in routine clinical practice.

Algorithm computational complexity

Advanced deep learning models, particularly convolutional neural networks (CNNs) and GANs, demand significant computational resources for both training and inference. Processing high-resolution whole slide images (WSIs), which can contain billions of pixels, exacerbates this computational burden. Access to high-performance hardware such as GPUs or TPUs is often required, posing financial and logistical barriers, especially in resource-limited settings. Prolonged training times also impede iterative model development and experimentation.

Techniques such as model pruning, quantization, and the exploration of more efficient neural network architectures offer potential pathways to reduce computational complexity without substantially compromising performance. Additionally, leveraging cloud computing resources can provide scalable computational power, though concerns regarding data security and patient privacy must be carefully managed in compliance with relevant regulations.

Problems and difficulties in virtual histopathology methods

There are many technical problems and challenges that come into play with regard to the efficiency and acceptance of virtual histopathology techniques in medical imaging.

Another major technical challenge is data variability and quality. Images in histopathology are very sensitive to variations in sample preparation, staining, and imaging conditions. This may introduce errors and reduce the reliability of the data. For example, variations in the strength of staining or the thickness of tissue sections may drastically alter the characteristics of the image, by which the analysis and understanding become pretty challenging. Such variability requires the following proper stages of standardization and pre-processing, which are time- and power-consuming.

Another important issue is image resolution and scale. Some forms of analysis will view cellular structure, which means that high resolution is necessary. However, high-resolution images will be large, and storage- and processing-intensive. This may be a subsequent decrease in scalability of the various forms of virtual histopathology, more in resource-restricted environments. Efficient solutions for storing images and optimized algorithms for processing will be needed to address these concerns.

This presents additional complexities: In histopathology, images produced using different modalities, such as histological and molecular, are often combined. Data for each type may be of variant formats and variant resolutions, and it is thus challenging to align and correlate them accurately. These kinds of complications call for sophisticated techniques in the integration of multimodal data for harmonizing and analyzing several datasets, which may be quite technically demanding.

Another key aspect is that of computational resource demands. Modern methods, most of which operate on deep learning algorithms, require heavy computational resources to train and predict. The requirement for powerful computing systems is sometimes an issue, especially for less endowed institutions or labs. Improving algorithms towards better efficiency and the use of cloud computing resources are possible solutions to help with this challenge.

Algorithm strength is very important. Many algorithms, particularly those that use machine learning and deep learning, are very good at specific datasets but sometimes perform poorly on new or different data. The problem may be due to overfitting or poor generalization. Therefore, ensuring the strength of algorithms to work well with multiple datasets and situations is paramount for their applicability in real-life scenarios. In turn, other techniques such as cross-validation and augmentation enhance the strengths of these algorithms.

Problems with data privacy and security are very important: Histopathological data is very sensitive, and dealing with this data requires strict privacy and security rules. Making sure that the data is anonymized and kept safe from unauthorized access is crucial for protecting patient confidentiality and following regulatory standards.

Another issue is model visualization transparency, particularly in deep learning. Sometimes, deep learning models are pejoratively referred to as "black box" because the way these models make decisions is obscure. Model interpretability, using visualization techniques or feature attribution methods, is the key to gaining the trust of doctors in carrying these methods out to real medical situations.

There are several challenges in integrating virtual histopathology methods into the clinical workflow. Doctors need to trust and accept these methods to ensure they meet the medical standard and can be used easily daily. This can be resolved by working in collaboration with doctors and through rigorous validation studies.

AI-driven histopathology methods are faced with challenges related to regulatory and ethical concerns. It would be necessary to abide by rules and conduct oneself ethically, to check if AI systems are fair and transparent. Rules are followed and regulatory bodies are collaborated with for compliance and ethical integrity during the task.

Finally, the requirement for training and expertise makes the application of these methods difficult. Generation, utilization, and maintenance of sophisticated algorithms require special types of knowledge and abilities. Providing training and resources to professionals for the use and understanding of these methods can help in closing this gap and promoting their use. These are technical problems and challenges that need to be fixed to improve virtual histopathology methods and to make sure that they work well in medical practice. Research and development will continue, and the teamwork of the researchers, doctors, and regulatory groups will be very critical in surmounting these roadblocks to advance virtual histopathology in medical imaging. Table 16 provides a comparative analysis concerning the strengths, weaknesses, and potential applications of different virtual histopathology approaches.

Future challenges and issues in virtual histopathology methods

As virtual histopathology methods continue to evolve, several future challenges and issues are expected to arise, influencing the trajectory of this field.

One of the future outstanding challenging issues is the scalability and generalization of the algorithms. As virtual histopathology systems are more widely deployed, there will be the need to ensure that the algorithms can handle diverse and large-scale datasets from various sources or populations.

Models perform well on some datasets but are thrown off by different imaging conditions, tissue types, and patient backgrounds; hence, strong algorithms that could adapt to such differences without large requirements for retraining will be important for their broader applications.

Another critical challenge is the integration of new technologies. The rapid development of linked technologies such as genomics and proteomics brings both opportunities for deeper understanding and added complication. Such integration of heterogeneous data from multiple sources maintaining accuracy and efficiency will require sophisticated approaches and cross-discipline collaboration. Doing so in a seamless manner, mining useful information without consuming excessive computer resources, will be the challenge.

Data privacy and security will be of prime concern with the increased usage of virtual histopathology methods. Thus, strong measures of data protection will be paramount as the sharing and creation of sensitive medical data becomes more frequent. This will require future changes to adhere to regulations, like GDPR and HIPAA, that include advanced encryption and access control systems securing patient information and ensuring these are properly followed.

Explain and understand complex models: Large models will present a big challenge. As deep learning models get more and more complex, it will be incumbent that their decision-making is understood to create trust and facilitate their use in healthcare. New ways of explaining models will need to be designed by researchers to ensure that their predictions are understood clearly, especially in critical healthcare situations.

This also entails logistical and operational challenges in the inclusion of virtual histopathology methods within regular clinical practice. As such, if it hopes to secure successful acceptance, the system needs to ensure the component of ease of use and fitting within the existing

Table 16 Comparative summary of strengths and weaknesses of various histopathology approaches

Approach	Strengths	Weaknesses	Potential applications
Machine learning	Versatile, high predictive accuracy in simpler tasks, adaptable to different imaging modalities	Requires large, labeled datasets; often lacks interpretability	Diagnostic classification, data augmentation
Deep learning	High accuracy in complex image recognition; learns features autonomously	High computational cost, risk of overfitting with small datasets, "black box" interpretation	Tumor segmentation, mutation prediction, disease progression.
Visual path	Enhances spatial resolution and image clarity; enables real-time applications in some cases	Dependent on advanced equipment, integration challenges with existing clinical workflows	Intraoperative diagnostics, high-resolution imaging

workflow. As such, it paves the way for tackling issues in training medical professionals, standard procedure development, and validation and quality control processes that are compatible with the clinical setting.

In the future, the issue of ethics and bias in AI models will only increase, as more of these technologies are utilized for a varied population. Algorithms must, therefore, be made to be unbiased and fair, not continuing the gaps in healthcare currently. Mechanisms will have to be developed in the future for the identification and reduction of biases in train data and algorithms for ensuring fairness in output.

In the final analysis, the practicality and affordability of using advanced virtual histopathology on a large scale will become an important issue. Creating, testing, and maintaining complex systems can be very expensive for some institutions. Demonstration of benefits through carefully done clinical studies and cost-benefit analyses, and the finding of affordable solutions, may make more people begin using these methods. These future challenges will be faced by providing continuous research, collaboration from different fields, and a forward-looking approach to anticipate and minimize potential issues. Overcoming these challenges will help push the field of virtual histopathology toward better, fairer, and more commonly adopted solutions in medical imaging.

Technical issues and challenges for future research

One of the most critical issues is data variability and quality. Histopathological images are highly sensitive to variations in sample preparation, staining, and imaging conditions. These factors can introduce errors and reduce the reliability of data, requiring extensive standardization and pre-processing, which are both time- and resource-intensive. Additionally, the resolution and scale of images pose significant challenges. High-resolution images, necessary for detailed cellular analysis, are large and demand substantial storage and processing resources, which can limit scalability, especially in resource-constrained environments.

The integration of different data modalities, such as histological and molecular images, adds another layer of complexity. Aligning and correlating multi-modal data accurately is technically demanding and requires sophisticated techniques, particularly when the data are in variant formats and resolutions. Computational demands are also a major concern. Modern DL algorithms require powerful computing systems, which may not be accessible to all institutions. Improving algorithmic efficiency and leveraging cloud computing are potential solutions.

Algorithm robustness is another critical factor. ML and DL algorithms often perform well on specific datasets but may struggle with new or different data due to issues like overfitting. Techniques such as cross-validation and data augmentation are essential to enhance algorithm robustness and ensure their applicability in real-world scenarios.

Privacy and security of data are paramount, given the sensitive nature of histopathological information. Ensuring data anonymization and protection against unauthorized access is crucial for maintaining patient confidentiality and complying with regulatory standards. Additionally, the "black-box" nature of DL models can hinder their acceptance in clinical settings. Improving model interpretability through visualization techniques or feature attribution methods is essential for gaining the trust of healthcare professionals.

The integration of virtual histopathology methods into clinical workflows presents logistical and operational challenges. Collaboration with clinicians and rigorous validation studies are necessary to ensure these methods meet medical standards and are easy to use. Furthermore, as AI-driven histopathology methods become more prevalent, addressing ethical and regulatory concerns will be critical to ensuring fairness and transparency.

Looking forward, the scalability and generalization of algorithms will become increasingly important as virtual histopathology systems are more widely deployed. Ensuring that algorithms can handle diverse and large-scale datasets without extensive retraining will be crucial. The integration of new technologies, such as genomics and proteomics, will require sophisticated approaches and cross-disciplinary collaboration to maintain accuracy and efficiency.

In summary, while the strengths of ML, DL, and Visual Path approaches in virtual histopathology are well-established, addressing the technical challenges they present is essential for realizing their full potential. Continuous research, collaboration, and a forward-looking approach will be necessary to overcome these challenges and advance the field of virtual histopathology in medical imaging.

Emerging techniques for data privacy and computational efficiency

As virtual histopathology relies on processing sensitive medical data, ensuring data privacy and managing computational demands are critical. The following are some of the recent non-AI techniques gaining traction:

i. **Federated Learning for Distributed Data Analysis:** Federated learning enables multiple institutions to collaborate on model training without sharing the raw data, thus safeguarding patient privacy. Each institution's data remains within its local system, with only model parameters being shared and updated globally. This decentralized approach minimizes the risk of data breaches, making it particularly useful in clinical environments.

ii. **Homomorphic Encryption for Secure Data Processing:** Homomorphic encryption allows computations to be performed on encrypted data without decrypting it first. This enables sensitive patient data to be securely processed by external servers or cloud resources while maintaining strict confidentiality. Applications include secure diagnostic processing and analysis in centralized research facilities.

iii. **Model Compression Techniques to Improve Efficiency:** Model compression methods, such as pruning and quantization, reduce the complexity of computational models by trimming less relevant data and shrinking model size. These techniques enable faster model inference and lower energy consumption, making virtual histopathology feasible in lower-resource settings, such as rural clinics or mobile health units.

iv. **Data Anonymization and De-identification Protocols:** To comply with data protection regulations, de-identification techniques remove or mask identifiable patient information in histopathological data. Coupled with anonymization practices, these protocols allow data sharing for research and diagnostic development without compromising patient privacy.

Conclusion

Contrarily, virtual histopathology techniques are a quantum leap in medical imaging that empowers investigators to realize very new and unprecedented capabilities for the examination and interpretation of tissue samples. The methods of digitization and computational analysis of histological slides have innovations such as higher diagnostic accuracy, increased workflow efficiency, and the discovery of new mechanisms of diseases.

These innovative technologies have allowed for the development of scanners and AI-driven machine learning algorithms to make higher-resolution, more accurate, and subjective measures for identifying subtle changes in tissue morphology and pathology, best supporting the clinician's decision and promoting further collaborative research efforts that convey greater benefits to patients.

Virtual histopathology techniques are based on digital slides and computational analysis, offering a broad range of new opportunities in tissue sample examination. Better image acquisition through AI algorithms identifies tiny changes in the tissues most correctly, enabling then a finer diagnosis. In addition, digitization enables faster workflows. It follows, by natural extension, how there will be greater efficiency through analysis and collaboration. Another important aspect is discovery potential. Virtual analysis helps the discovery of new mechanisms of diseases that will allow for the discovery of better treatments. These benefits extend beyond the confines of the laboratory itself.

Telepathology also needs to be considered in this regard. Virtual slides can enable remote consultation and second opinions at a minimum on a global scale, especially in resource-limited areas. Moreover, ensuring the responsible use of validation and ethics is of great importance. Validation is a continuous process and adherence to ethics needs to be kept up so that these technologies retain trustworthiness and are responsibly used clinically.

Virtual histopathology also offers excellent potential for applications in telepathology, which includes remote consultancies and second opinions, particularly at locations that have limited access to specialized expertise. Keeping in view the continuous development of these technologies, a sine qua non would always be further validation studies and following ethical protocols that ensure their inbuilt efficacy, reliability, and ethics regarding use in clinics. Virtual histopathology techniques singlehandedly drive a very hard paradigm shift toward individualized medicine, base-driven care, and population health-diagnostic and therapeutic standards are foreshadowed to be rewritten soon.

Acknowledgements
Not applicable.

Code availability
Not applicable.

Authors' contributions
MTI conceived the idea, performed formal analysis, and wrote the original manuscript. IS conceived the idea, performed data curation, and wrote the original manuscript. JA designed the methodology and performed formal analysis and data curation. MFUB dealt with software, performed visualization and project administration. SGV acquired funding and performed investigation and project administration. EGV performed investigation and visualization and dealt with software. TK designed methodology, performed validation and formal analysis. IA supervised the work, performed validation, and edited the manuscript. All authors reviewed the manuscript.

Funding
This study is supported by the European University of Atlantics.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 16 July 2024 Accepted: 12 November 2024
Published online: 26 November 2024

References

1. Wang J, Qin L, Chen D, Wang J, Han BW, Zhu Z, et al. An improved Hover-net for nuclear segmentation and classification in histopathology images. Neural Comput Appl. 2023;35(19):14403–17.
2. Bahadir CD, Omar M, Rosenthal J, Marchionni L, Liechty B, Pisapia DJ, et al. Artificial intelligence applications in histopathology. Nat Rev Electr Eng. 2024;1(2):93–108.
3. Hamed EAR, Salem MAM, Badr NL, Tolba MF. A Deep Learning-Based Classification Framework for Annotated Histopathology Lung Cancer Images. In: International Conference on Advanced Intelligent Systems and Informatics. Switzerland: Springer; 2023. pp. 86–94.
4. Varghese J, Saleema J. Machine learning techniques for automated nuclear atypia detection in histopathology images: a review. In: Inventive Computation and Information Technologies: Proceedings of ICICIT 2022. Singapore: Springer; 2023. pp. 717–40.
5. Asaf MZ, Rao B, Akram MU, Khawaja SG, Khan S, Truong TM, et al. Dual contrastive learning based image-to-image translation of unstained skin tissue into virtually stained H &E images. Sci Rep. 2024;14(1):2335.
6. Bai B, Yang X, Li Y, Zhang Y, Pillar N, Ozcan A. Deep learning-enabled virtual histological staining of biological samples. Light Sci Appl. 2023;12(1):57.
7. Kajala A, Jaiswal S. Classification of Breast Cancer Histopathology Images Using EfficientNet Architectures. In: Advances in Information Communication Technology and Computing: Proceedings of AICTC 2021. Singapore: Springer; 2022. pp. 639–53.
8. Chen T, Kornblith S, Swersky K, Norouzi M, Hinton GE. Big self-supervised models are strong semi-supervised learners. Adv Neural Inf Process Syst. 2020;33:22243–55.
9. Lan X, Mei J, Lin R, Chen J, Zhang Y. A Weakly Supervised Semantic Segmentation Method on Lung Adenocarcinoma Histopathology Images. In: International Conference on Intelligent Computing. Singapore: Springer; 2023. pp. 688–98.
10. Hu J, Shen L, Sun G. Squeeze-and-excitation networks. In: Proceedings of the IEEE conference on computer vision and pattern recognition. New York City: IEEE; 2018. pp. 7132–41.
11. Xing F, Cornish TC, Bennett TD, Ghosh D. Bidirectional mapping-based domain adaptation for nucleus detection in cross-modality microscopy images. IEEE Trans Med Imaging. 2020;40(10):2880–96.
12. Ren Y, Li G, Liu S, Li TH. Deep spatial transformation for pose-guided person image generation and animation. IEEE Trans Image Process. 2020;29:8622–35.
13. Shen D, Wu M, Zheng S, Chen J, Chen Y, Chen Y, et al. Unsupervised Domain Adaptation with Dual U-DenseTransformer Generation. In: 2022 IEEE International Conference on Bioinformatics and Biomedicine (BIBM). New York City: IEEE; 2022. pp. 1170–7.
14. Rukmangad A, Deshpande A, Jamthikar A, Gupta D, Bhurane A, Meshram NB. Classification of H &E Stained Liver Histopathology Images Using Ensemble Learning Techniques for Detection of the Level of Malignancy of Hepatocellular Carcinoma (HCC). In: Advances in Artificial Intelligence-Empowered Decision Support Systems: Papers in Honour of Professor John Psarras. Switzerland: Springer; 2024. pp. 89–108.
15. Alturkistani HA, Tashkandi FM, Mohammedsaleh ZM. Histological stains: a literature review and case study. Global J Health Sci. 2016;8(3):72.
16. Javaeed A, Qamar S, Ali S, Mustafa MAT, Nusrat A, Ghauri SK. Histological stains in the past, present, and future. Cureus. 2021;13(10):e18486.
17. Horobin RW. Theory of histological staining. Bancroft's Theory and Practice of Histological Techniques, 8th ed.; E-Book. 2018. p. 114–123
18. Stacke K, Eilertsen G, Unger J, Lundström C. Measuring domain shift for deep learning in histopathology. IEEE J Biomed Health Inform. 2020;25(2):325–36.
19. Unger M, Kather JN. Deep learning in cancer genomics and histopathology. Genome Med. 2024;16(1):44.
20. Damania K, Angel Arul Jothi J. Graph Convolutional Neural Networks for Nuclei Segmentation from Histopathology Images. In: International Conference on Soft Computing and its Engineering Applications. Switzerland: Springer; 2022. pp. 158–69.
21. Ghahremani P, Li Y, Kaufman A, Vanguri R, Greenwald N, Angelo M, et al. Deep learning-inferred multiplex immunofluorescence for immunohistochemical image quantification. Nat Mach Intell. 2022;4(4):401–12.
22. Cho H, Lim S, Choi G, Min H. Neural stain-style transfer learning using GAN for histopathological images. 2017. arXiv:1710.08543.
23. Sridhar N, Elad M, McNeil C, Rivlin E, Freedman D. Diffusion models for generative histopathology. In: International Conference on Medical Image Computing and Computer-Assisted Intervention. Switzerland: Springer; 2023. pp. 154–63.
24. Cechnicka S, Ball J, Reynaud H, Arthurs C, Roufosse C, Kainz B. Realistic Data Enrichment for Robust Image Segmentation in Histopathology. In: MICCAI Workshop on Domain Adaptation and Representation Transfer. Switzerland: Springer; 2023. pp. 63–72.
25. Khaled M, Hammouda MA, Ali H, Elattar M, Selim S. Efficient Semantic Segmentation of Nuclei in Histopathology Images Using Segformer. In: Annual Conference on Medical Image Understanding and Analysis. Switzerland: Springer; 2023. pp. 81–95.
26. Vorndran J, Neuner C, Coras R, Hoffmann L, Geffers S, Honke J, et al. A deep learning-based histopathology classifier for Focal Cortical Dysplasia. Neural Comput Appl. 2023;35(17):12775–92.
27. Kumar Shukla R, Kumar Tiwari A. Comparative analysis of machine learning based approaches for face detection and recognition. J Inf Technol Manag. 2021;13(1):1–21.
28. Runz M, Rusche D, Schmidt S, Weihrauch MR, Hesser J, Weis CA. Normalization of HE-stained histological images using cycle consistent generative adversarial networks. Diagn Pathol. 2021;16:1–10.
29. Patnaik R, Rath PS, Padhy S, Dash S. Histopathological Colorectal Cancer Image Classification by Using Inception V4 CNN Model. In: International Conference on Robotics, Control, Automation and Artificial Intelligence. Singapore: Springer; 2022. pp. 1003–14.
30. Wei J, Suriawinata A, Ren B, Liu X, Lisovsky M, Vaickus L, et al. A petri dish for histopathology image analysis. In: Artificial Intelligence in Medicine: 19th International Conference on Artificial Intelligence in Medicine, AIME 2021, Virtual Event, June 15–18, 2021, Proceedings. Switzerland: Springer; 2021. pp. 11–24.
31. Shafi I, Din S, Khan A, Díez IDLT, Casanova RDJP, Pifarre KT, et al. An effective method for lung cancer diagnosis from ct scan using deep learning-based support vector network. Cancers. 2022;14(21):5457.
32. Lijo J, Janardhanan Subramanian S. LP norm regularized deep CNN classifier based on biwolf optimization for mitosis detection in histopathology images. Int J Inf Technol. 2024;16:1–20.
33. Ge Y, Zhu F, Chen D, Zhao R, et al. Self-paced contrastive learning with hybrid memory for domain adaptive object re-id. Adv Neural Inf Process Syst. 2020;33:11309–21.
34. Liang M, Zhang Q, Wang G, Xu N, Wang L, Liu H, et al. Multi-scale self-attention generative adversarial network for pathology image restoration. Vis Comput. 2023;39(9):4305–21.
35. Komura D, Ishikawa S. Machine Learning Methods for Histopathological Image Analysis. Front Oncol. 2023;13. https://www.frontiersin.org/articles/10.3389/fonc.2023.830645/full.
36. Fatima A, Shafi I, Afzal H, Mahmood K, Díez IdlT, Lipari V, et al. Deep learning-based multiclass instance segmentation for dental lesion detection. Healthcare. 2023;11(3):347.
37. Bai B, Yang X, Li Y, Zhang Y, Pillar N, Ozcan A. Deep learning-enabled virtual histological staining of biological samples. Light Sci Appl. 2023;12:57.
38. Shafi I, Sajad M, Fatima A, Aray DG, Lipari V, Diez IDLT, et al. Teeth lesion detection using deep learning and the Internet of Things post-COVID-19. Sensors. 2023;23(15):6837.
39. Tiard A, Wong A, Ho DJ, Wu Y, Nof E, Goh AC, et al. Stain-invariant self supervised learning for histopathology image analysis. 2022. arXiv:2211.07590.
40. Yurttakal AH, Erbay H. Segmentation of Larynx histopathology images via convolutional neural networks. In: Intelligent and Fuzzy Techniques: Smart and Innovative Solutions: Proceedings of the INFUS 2020

Conference, Istanbul, Turkey, July 21-23, 2020. Switzerland: Springer; 2021. pp. 949–54.

41. Ai S, Li C, Li X, Jiang T, Grzegorzek M, Sun C, et al. A State-of-the-Art Review for Gastric Histopathology Image Analysis Approaches and Future Development. BioMed Res Int. 2021;2021(1):6671417.
42. Öztürk Ş, Akdemir B. Effects of histopathological image pre-processing on convolutional neural networks. Procedia Comput Sci. 2018;132:396–403.
43. Jose L, Liu S, Russo C, Nadort A, Di Ieva A. Generative adversarial networks in digital pathology and histopathological image processing: a review. J Pathol Inform. 2021;12(1):43.
44. Ren J, Hacihaliloglu I, Singer EA, Foran DJ, Qi X. Unsupervised domain adaptation for classification of histopathology whole-slide images. Front Bioeng Biotechnol. 2019;7:102.
45. Singh PD, Bhavsar A, Harinarayanan K. Histopathology Whole Slide Image Analysis for Breast Cancer Detection. In: Biomedical Signal and Image Processing with Artificial Intelligence. Switzerland: Springer; 2022. pp. 31–56.
46. Jabbar A, Liaqat HB, Akram A, Sana MU, Azpíroz ID, Diez IDLT, et al. A Lesion-Based Diabetic Retinopathy Detection Through Hybrid Deep Learning Model. IEEE Access. 2024;12:40019–36.
47. Ahmed HK, Tantawi B, Magdy M, Sayed GI. Quantum optimized AlexNet for histopathology breast image diagnosis. In: International Conference on Advanced Intelligent Systems and Informatics. Switzerland: Springer; 2023. pp. 348–57.
48. Majeed T, Aalam SW, Ahanger AB, Dar RA, Masoodi TA, Macha MA, et al. Unleashing the Potential of Deep Learning for Precise Nuclei Segmentation and Classification in H &E-Stained Whole Slide Images. In: International conference on soft computing for problem-solving. Singapore: Springer; 2023. pp. 97–110.
49. Gong X, Chang S, Jiang Y, Wang Z. Autogan: Neural architecture search for generative adversarial networks. In: Proceedings of the IEEE/CVF International Conference on Computer Vision. New York City: IEEE; 2019. pp. 3224–34.
50. Xu J, Zhou C, Lang B, Liu Q. Deep learning for histopathological image analysis: Towards computerized diagnosis on cancers. In: Deep learning and convolutional neural networks for medical image computing: precision medicine, high performance and large-scale datasets. 2017. pp. 73–95.
51. Kurmi Y, Chaurasia V, Kapoor N. Histopathology image segmentation and classification for cancer revelation. Signal Image Video Process. 2021;15(6):1341–9.
52. Roy B, Gupta M. Macroscopic Reconstruction for Histopathology Images: A Survey. In: Computer Vision and Machine Intelligence in Medical Image Analysis: International Symposium, ISCMM 2019. Singapore: Springer; 2020. pp. 101–12.
53. Khan S, Rahmani H, Shah SAA, Bennamoun M, Medioni G, Dickinson S. A guide to convolutional neural networks for computer vision. Synthesis Lectures on Computer Vision. 2018. https://doi.org/10.1007/978-3-031-01821-3.
54. Aitazaz T, Tubaishat A, Al-Obeidat F, Shah B, Zia T, Tariq A. Transfer learning for histopathology images: an empirical study. Neural Comput Appl. 2023;35(11):7963–74.
55. Kumar S, Sharma S. Sub-classification of invasive and non-invasive cancer from magnification independent histopathological images using hybrid neural networks. Evol Intel. 2022;15(3):1531–43.
56. Kosaraju SC, Hao J, Koh HM, Kang M. Deep-Hipo: Multi-scale receptive field deep learning for histopathological image analysis. Methods. 2020;179:3–13.
57. Yang P, Hong Z, Yin X, Zhu C, Jiang R. Self-supervised visual representation learning for histopathological images. In: Medical Image Computing and Computer Assisted Intervention–MICCAI 2021: 24th International Conference, Strasbourg, France, September 27–October 1, 2021, Proceedings, Part II 24. Switzerland: Springer; 2021. pp. 47–57.
58. Zeiler MD, Fergus R. Visualizing and understanding convolutional networks. In: Computer Vision–ECCV 2014: 13th European Conference, Zurich, Switzerland, September 6-12, 2014, Proceedings, Part I 13. Switzerland: Springer; 2014. pp. 818–33.
59. Chollet F. Xception: Deep learning with depthwise separable convolutions. In: Proceedings of the IEEE conference on computer vision and pattern recognition. New York City: IEEE; 2017. pp. 1251–8.
60. Kingma DP, Welling M. Auto-encoding variational bayes. 2013. arXiv:1312.6114.
61. Şengöz N, Yiğit T, Özmen Ö, Isık AH. Importance of preprocessing in histopathology image classification using deep convolutional neural network. Adv Artif Intell Res. 2022;2(1):1–6.
62. Jabbar A, Li X, Omar B. A survey on generative adversarial networks: Variants, applications, and training. ACM Comput Surv (CSUR). 2021;54(8):1–49.
63. Isola P, Zhu JY, Zhou T, Efros AA. Image-to-image translation with conditional adversarial networks. In: Proceedings of the IEEE conference on computer vision and pattern recognition. New York City: IEEE; 2017. pp. 1125–34.
64. Mehmood S, Ghazal TM, Khan MA, Zubair M, Naseem MT, Faiz T, et al. Malignancy detection in lung and colon histopathology images using transfer learning with class selective image processing. IEEE Access. 2022;10:25657–68.
65. Vasiljević J, Feuerhake F, Wemmert C, Lampert T. Towards histopathological stain invariance by unsupervised domain augmentation using generative adversarial networks. Neurocomputing. 2021;460:277–91.
66. Cheng J, Yang Y, Tang X, Xiong N, Zhang Y, Lei F. Generative adversarial networks: A literature review. KSII Trans Internet Inf Syst (TIIS). 2020;14(12):4625–47.
67. Wang K, Gou C, Duan Y, Lin Y, Zheng X, Wang FY. Generative adversarial networks: introduction and outlook. IEEE/CAA J Autom Sin. 2017;4(4):588–98.
68. Li Y, Swersky K, Zemel R. Generative moment matching networks. In: International conference on machine learning. New York: PMLR; 2015. pp. 1718–27.
69. Deo BS, Pal M, Panigrahi PK, Pradhan A. Supremacy of attention-based transformer in oral cancer classification using histopathology images. Int J Data Sci Analytics. 2024;1–19.
70. Madusanka N, Jayalath P, Fernando D, Yasakethu L, Lee BI. Impact of h &e stain normalization on deep learning models in cancer image classification: performance, complexity, and trade-offs. Cancers. 2023;15(16):4144.
71. Paul Wilson K, Srinivasa Reddy M, Karthik SC, Anudeep VVK, Giri Babu K. Histopathology Cancer Detection. In: Microelectronics, Circuits and Systems: Select Proceedings of Micro2021. Singapore: Springer; 2023. pp. 251–61.
72. McCann MT, Ozolek JA, Castro CA, Parvin B, Kovacevic J. Automated histology analysis: Opportunities for signal processing. IEEE Signal Process Mag. 2014;32(1):78–87.
73. Mathew T, Kini JR, Rajan J. Computational methods for automated mitosis detection in histopathology images: A review. Biocybernetics Biomed Eng. 2021;41(1):64–82.
74. Davri A, Birbas E, Kanavos T, Ntritsos G, Giannakeas N, Tzallas AT, et al. Deep learning on histopathological images for colorectal cancer diagnosis: a systematic review. Diagnostics. 2022;12(4):837.
75. Dabass M, Dabass J. Preprocessing techniques for colon histopathology images. In: International Conference on Advanced Communication and Computational Technology. Singapore: Springer; 2019. pp. 1121–38.
76. Benomar ML, Settouti N, Xiao R, Ambrosetti D, Descombes X. Convolutional neuronal networks for tumor regions detection in histopathology images. In: International Conference on Digital Technologies and Applications. Switzerland: Springer; 2021. pp. 13–23.
77. Martínez-Arboleyda DN, Cruz-Guerrero IA, Campos-Delgado DU. Tumor Tissue Classification in Hyperspectral Histopathology Images Through Individual and Ensemble of Machine Learning Algorithms. In: Congreso Nacional de Ingeniería Biomédica. Singapore: Springer; 2023. pp. 100–11.
78. Mulrane L, Rexhepaj E, Penney S, Callanan JJ, Gallagher WM. Automated image analysis in histopathology: a valuable tool in medical diagnostics. Exp Rev Mol Diagn. 2008;8(6):707–25.
79. Girshick R. Fast r-cnn. In: Proceedings of the IEEE international conference on computer vision. New York City: IEEE; 2015. pp. 1440–8.
80. Belsare A, Mushrif M. Histopathological image analysis using image processing techniques: An overview. Signal Image Process. 2012;3(4):23.
81. De Matos J, Britto Jr AdS, Oliveira LE, Koerich AL. Histopathologic image processing: A review. 2019. arXiv:1904.07900.
82. Hossain MS, Armstrong LJ, Abu-Khalaf J, Cook DM. The segmentation of nuclei from histopathology images with synthetic data. Signal Image Video Process. 2023;17(7):3703–11.

83. Saldanha OL, Quirke P, West NP, James JA, Loughrey MB, Grabsch HI, et al. Swarm learning for decentralized artificial intelligence in cancer histopathology. Nat Med. 2022;28(6):1232–9.
84. Ronneberger O, Fischer P, Brox T. U-net: Convolutional networks for biomedical image segmentation. In: Medical image computing and computer-assisted intervention–MICCAI 2015: 18th international conference, Munich, Germany, October 5-9, 2015, proceedings, part III 18. Switzerland: Springer; 2015. pp. 234–41.
85. Saxena S, Shukla PK, Ukalkar Y. A shallow convolutional neural network model for breast cancer histopathology image classification. In: Proceedings of International Conference on Recent Trends in Computing: ICRTC 2022. Singapore: Springer; 2023. pp. 593–602.
86. Altuntaş TS, Toyran T, Arıca S. Categorization of breast carcinoma histopathology images by utilizing region-based convolutional neural networks. Arab J Sci Eng. 2024;49(5):6695–705.
87. Aatresh AA, Alabhya K, Lal S, Kini J, Saxena PP. LiverNet: efficient and robust deep learning model for automatic diagnosis of sub-types of liver hepatocellular carcinoma cancer from H &E stained liver histopathology images. Int J Comput Assist Radiol Surg. 2021;16:1549–63.
88. Roy S, Das D, Lal S, Kini J. Novel edge detection method for nuclei segmentation of liver cancer histopathology images. J Ambient Intell Humanized Comput. 2023;14(1):479–96.
89. Janowczyk A, Madabhushi A. Deep learning for digital pathology image analysis: A comprehensive tutorial with selected use cases. J Pathol Inform. 2016;7(1):29.
90. Esteva A, Kuprel B, Novoa RA, Ko J, Swetter SM, Blau HM, et al. Dermatologist-level classification of skin cancer with deep neural networks. Nature. 2017;542(7639):115–8.
91. De Matos J, Ataky STM, de Souza Britto Jr A, Soares de Oliveira LE, Lameiras Koerich A. Machine learning methods for histopathological image analysis: A review. Electronics. 2021;10(5):562.
92. Kumar A, Bi L, Kim J, Feng DD. Machine learning in medical imaging. In: Biomedical Information Technology. Amsterdam: Elsevier; 2020. pp. 167–96.
93. Guo JL, Januszyk M, Longaker MT. Machine learning in tissue engineering. Tissue Eng Part A. 2023;29(1–2):2–19.
94. Shehab M, Abualigah L, Shambour Q, Abu-Hashem MA, Shambour MKY, Alsalibi AI, et al. Machine learning in medical applications: A review of state-of-the-art methods. Comput Biol Med. 2022;145:105458.
95. Ganin Y, Ustinova E, Ajakan H, Germain P, Larochelle H, Laviolette F, et al. Domain-adversarial training of neural networks. J Mach Learn Res. 2016;17(59):1–35.
96. Kalavathi R, Swamy Das M. A Review on Deep Learning Approaches for Histopathology Breast Cancer Classification. In: Proceedings of Fourth International Conference on Computer and Communication Technologies: IC3T 2022. Singapore: Springer; 2023. pp. 367–75.
97. Elazab N, Soliman H, El-Sappagh S, Islam SR, Elmogy M. Objective diagnosis for histopathological images based on machine learning techniques: Classical approaches and new trends. Mathematics. 2020;8(11):1863.

Publisher's Note