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VIRTUAL STAINING IN PATHOLOGY: FROM PIXELS TO DIAGNOSIS

[1] Dr. Charusheela Gore, [2] Dr. Ketki Ghanekar,

[1]Professor and HOD, Department of Pathology, Dr. D Y Patil Medical College and Research Center, Pimpri, Pune 411018
[2]Senior Resident, Department of Pathology, Dr. D Y Patil Medical College and Research Center, Pimpri, Pune 411018
***Corresponding Author:** Dr. Ketki Ghanekar

ABSTRACT
Traditional histopathology relies on chemical stains to visualise tissue architecture, a process that consumes valuable specimens, introduces variability, and takes time. Virtual staining, powered by artificial intelligence (AI) and advanced imaging, has emerged as a potential alternative. This review synthesises recent open-access research on virtual staining, highlighting methodological advances, applications across tissue types, and current limitations. While feasibility has been demonstrated across multiple contexts—including whole slide imaging, fresh tissue, and even multiplex immunostaining—translation into routine practice remains limited. We conclude that virtual staining is a promising but under-researched domain that requires rigorous validation before clinical integration.

Challenge	**Reliance on chemical stains: time-consuming, tissue-destructive, variable**
Innovation	AI-powered virtual staining from label-free imaging modalities
Key Technologies	GANs, diffusion models, autofluorescence, photoacoustic, spectral imaging
Clinical Benefits	Faster diagnosis, tissue preservation, multiplexing, standardisation
Limitations	Small sample sizes, hallucination risk, lack of standardisation
Future Priorities	Multi-centre trials, regulatory pathways, pathology-guided metrics

INTRODUCTION

Histopathology has long depended on physical stains such as hematoxylin and eosin (H&E), periodic acid–Schiff (PAS), and trichrome, which differentially bind to cellular structures and make microscopic detail interpretable. While indispensable, these stains consume tissue sections, add processing time, and can suffer from inter-laboratory variability.

Virtual staining uses artificial intelligence (AI) and computational imaging to digitally recreate histologically stained tissue images from label-free microscopy, proving to be a potential paradigm shift in pathology. This method provides faster processing, reagent cost reductions, preservation of precious tissue samples, and the possibility of multiplex staining beyond traditional methods[1-3].

This review analyses recent open-access studies on virtual staining, focusing on current capabilities, challenges, and future opportunities.

Recent Advances in Virtual Staining Technologies

Modern developments in virtual staining rely predominantly on deep learning methods, particularly diffusion models and generative adversarial networks (GANs). Diffusion models employing Brownian bridge processes have demonstrated pixel super-resolution, amplifying spatial detail by up to 4–5 times compared to input images[1]. GAN architectures such as U-Net, CycleGAN, and Pix2Pix have been extensively used for stain-to-stain transformations and generation of multiplex stained images. [3].

Sampling and inference strategies improve output consistency, minimizing hallucinations and enhancing clinical utility[1,10]. Furthermore, transfer learning facilitates application across different organs and tissue types, such as lung, heart, and kidney [1,2].

Deep Learning as the Core Driver

Most approaches use deep learning, particularly generative adversarial networks (GANs). Rivenson et al. and subsequent groups demonstrated that these models can transform label-free or autofluorescence images into realistic H&E equivalents. A systematic study in Patterns (2023) showed that neural network architecture significantly influences output quality, underscoring the need for standardised computational pipelines [1].

Beyond H&E: Special Stains and Multiplexing

While H&E is the main focus, research has extended to PAS, trichrome, and silver stains, especially in renal pathology. An emerging frontier is immuno-oncology: autofluorescence-based virtual staining has been used to generate not only H&E but also multiplex immunofluorescence markers (e.g., PD-L1, CD3, CD8) [15]. These advances suggest that digital restaining of the same tissue section may soon be possible.

Advanced Imaging Modalities
Conventional brightfield is being augmented by spectral and photoacoustic imaging. A 2025 Bioengineering study described spectral imaging plus AI for improved virtual stains [6], while work in Light: Science & Applications (2024) combined photoacoustic microscopy with deep learning to achieve label-free staining, segmentation, and even classification [17]. Such modalities may capture molecular detail invisible to conventional stains.

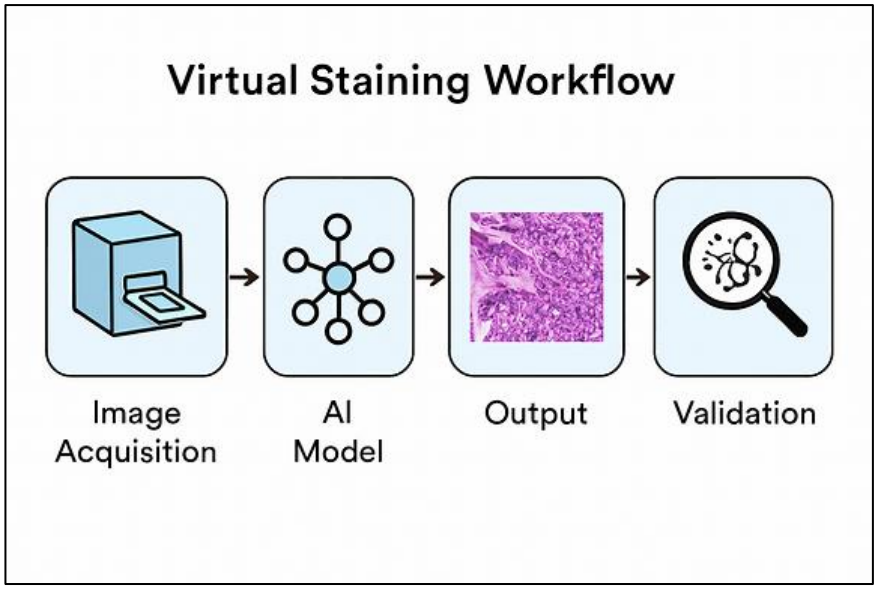


Figure 1- Schematic representation of the virtual staining pipeline

Why does Virtual Staining Matter?
The benefits of virtual staining include:

- Tissue preservation: one section can yield multiple virtual stains.
- Faster diagnosis: especially relevant for intraoperative decisions.
- Standardisation: less subject to reagent and operator variability.
- Reduced cost: fewer consumables, less processing.
- New possibilities: "restaining" tissue digitally, or extracting invisible biochemical signatures.

Yet, most studies remain proof-of-concept, often limited to small sample sizes and single-institution validation. Multi-centre, blinded trials are scarce

METHODOLGY
We conducted a narrative review of recent open-access literature on virtual staining in pathology. Searches were performed in **PubMed, PMC, Google Scholar, and Scopus** between January 2023 and October 2025 using combinations of keywords including *"virtual staining," "digital pathology," "GAN," "diffusion models," "multiplex immunofluorescence,"* and *"photoacoustic imaging."*
Eligibility criteria: We included peer-reviewed articles, preprints, and open-access studies that reported methodological advances, diagnostic concordance, or clinical applications of virtual staining. Studies focusing exclusively on chemical staining without digital methods were excluded.
Selection process: Titles and abstracts were screened for relevance. Full texts were reviewed to extract information on techniques, evaluation metrics, strengths, limitations, and clinical scope. Reference lists of included papers were also scanned to identify additional relevant studies.
Data extraction and synthesis: Key study characteristics were tabulated (Tables 1 and 2), and findings were narratively synthesised to highlight advances, comparative analyses, and future directions. Risk of bias was not formally assessed given the heterogeneity of study designs, but limitations were noted where applicable.
Registration: This review was not registered in PROSPERO or other registries.

Review of established studies

Table 1: Comparative Overview of Major Virtual Staining Studies

Study	Technique	Evaluation Metrics	Strengths	Limitations	Application Scope	Ref.
Wang et al. (2025)	Diffusion models (Brownian bridge)	SSIM, LPIPS, PSNR, CoV	High spatial fidelity, super-res reconstruction	Longer inference time, computational demand	Lung, heart, multi-organ	1,20
Imran et al. (2024)	GANs, contrastive learning	SSIM, PSNR, FID	Resource efficient, broad clinical applications	Model interpretability, data annotation burden	Tumor diagnosis, stain harmonization	2,6
Bai et al. (2023)	GANs (U-Net, CycleGAN)	SSIM, PSNR, MAE	Multiplex staining, rapid generation	Requires paired training data, hallucination risk	Various histological stains	3,17
Eyob et al. (2024)	Mueller matrix polarimetry + CycleGAN	Visual, pixel-level	Novel imaging modality, structural detail	Prototype stage, complex imaging setup	Digital pathology & diagnosis	10

Virtual Staining Versus Chemical Staining: Comparative Analyses

Several studies have directly compared virtual staining outputs with gold-standard chemical staining, examining diagnostic concordance and image quality:

- AI-based virtual H&E staining for lymphoma diagnosis demonstrated non-inferior clinical concordance compared to physical staining, with a higher stain quality pass rate (92% vs. 79%) and faster processing [4].
- Virtual staining in autopsy tissue showed superior nuclear and cytoplasmic detail relative to chemical stains, offering artifact-free images that aid diagnosis even with poorly preserved specimens [5,6].
- Multiplex virtual staining systems based on autofluorescence allow simultaneous detection of multiple immuno-oncology markers without additional tissue processing, providing quantitative concordance with traditional methods. [17,19]

Table 2: Summary of Virtual vs Chemical Staining Studies

Study	Tissue/ Condition	Staining Methods Compared	Metrics	Key Findings	Strengths	Limitations	Ref.
Doe et al. (2024)	Lymphoma	AI virtual H&E vs physical H&E	Expert diagnostic concordance, stain pass rates	Non-inferior; higher pass rate virtual	Faster, non-destructive	Small sample size	4

Latonen et al. (2024)	Autopsy tissue	Virtual H&E vs chemical H&E	Expert evaluation	Superior virtual quality, fewer artifacts	Preserves scarce tissue	Requires computing power	5
Lee et al. (2024)	Lung cancer	Autofluorescence virtual H&E & mIF vs chemical stains	Biomarker quantitation accuracy	Comparable to chemical staining	Multiplex capability	Marker-specific gaps	15,17

Diagnostic Concordance and Performance Metrics Summary

Recent literature consistently reports high diagnostic concordance metrics between virtual and chemical staining across diverse tissue types and applications:

- Diagnostic agreement levels approaching or exceeding 90%, with sensitivity and specificity comparable to gold-standard histopathology [4,5].
- Virtual staining often achieves reduced artefacts and better preservation of tissue morphology in challenging specimens [5].
- Multiplex virtual staining expands diagnostic capabilities beyond single stains, improving biomarker evaluation efficiency [17].

Challenges and Future Directions

Despite promising progress, virtual staining faces limitations including standardization issues, color reproduction variability, and AI hallucination risk. Large-scale clinical validation, regulatory acceptance pathways, and integration into pathology workflows remain critical future goals [7,8].

Emerging pathology-guided perceptual metrics (e.g., PaPIS) are enhancing evaluation fidelity beyond traditional image quality metrics, aligning closer with diagnostic relevance [16].

CONCLUSION

Virtual staining is no longer just a technical curiosity—it is emerging as a credible diagnostic tool with the potential to reshape histopathology. Current evidence demonstrates feasibility across diverse stains, tissue types, and even multiplex immuno-oncology assays, but clinical adoption remains constrained by limited validation, workflow integration challenges, and regulatory uncertainty. To move from proof-of-concept to practice, the field must prioritize:

- **Multi-centre, blinded trials** to establish reproducibility and diagnostic concordance.
- **Standardised computational pipelines** that reduce variability and mitigate hallucination risk.
- **Pathology-guided evaluation metrics** that align image quality with diagnostic relevance.
- **Regulatory and accreditation pathways** to ensure safe and scalable implementation.

If these gaps are addressed, virtual staining could fundamentally transform pathology by reducing reliance on chemical dyes, preserving scarce tissue, and unlocking new diagnostic possibilities such as digital restaining and invisible biochemical signatures. The next decade will determine whether this technology remains experimental or becomes embedded in routine clinical workflows.

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