Review paper

Generation of virtual stains: can artificial intelligence imitate chemistry?

Konstanty Korski

Data, Analytics and Research Imaging Domain, Diagnostics Division (RIS), F.Hoffmann-La Roche Ltd, Basel, Switzerland

Histological staining is the foundation of anatomic pathology, yet traditional wet-lab workflows are slow, consume irreplaceable tissue, and are prone to artifactual variability. As precision oncology increasingly demands comprehensive molecular testing from limited biopsy samples, conserving tissue while delivering rapid, accurate diagnoses is an urgent clinical challenge. This review explores "virtual staining" – the digital generation of routine and special stains directly from label-free tissue scans or primary hematoxylin and eosin slides. We examine the shift toward advanced artificial intelligence models that preserve overall tissue architecture, the development of "pathology-aware" quality metrics, and clinical applications like instant multiplexed immunofluorescence and 3D intraoperative biopsies. Ultimately, virtual staining allows pathologists to simultaneously evaluate multiple diagnostic stains without depleting the physical block or waiting for laboratory processing. By adopting rigorous quality controls and optimizing for time-sensitive clinical workflows, this technology is poised to transition from a research novelty into a fundamental tool for preserving invaluable patient tissue and accelerating diagnostic turnaround times.

Key words: virtual stain, artificial intelligence, digital pathology.

Introduction

For over a century, the microscopic evaluation of tissue has relied on the physical process of chemical staining – most notably hematoxylin and eosin (HE) – to impart contrast to otherwise translucent biological structures [1–3]. While this traditional workflow is robust, it is inherently resource-intensive, environmentally taxing due to the generation of toxic chemical waste, and subject to significant technical variability among different laboratories and technicians [4–6]. More importantly, standard histochemical staining is a destructive and irreversible process. A tissue section stained with HE cannot typically be re-stained for multiple targeted biomarkers, which presents a severe limitation in modern oncology where limited biopsy material must be conserved for downstream molecular sequencing and comprehensive spatial profiling [7, 8].

Virtual staining is a rapidly evolving field of digital pathology [2]. It represents a disruptive computational alternative that leverages deep learning to synthesize diagnostic-grade histological images without physical dyes. This short report covers the foundational label-free optical imaging modalities that capture endogenous tissue contrast, the evolution of the underlying artificial intelligence (AI) architectures (from Generative Adversarial Networks [GAN] to Transformers and Diffusion Models), and the expanding clinical applications ranging from stain-to-stain transformations to 3D volumetric pathology. Furthermore, it critically assesses the current state of clinical validation, the development of pathology-specific evaluation metrics, and the ethical paradigms necessary for clinical integration.

The timeliness of this topic cannot be overstated. Anatomic pathology laboratories are currently facing a "perfect storm" of rising case volumes, critical

staffing shortages, and escalating cost pressures [9]. Simultaneously, the rise of precision oncology demands deeper, spatially-resolved tissue insights at unprecedented speed. By bypassing the manual labor and chemical costs of the wet lab, AI-powered virtual staining offers a digital-first framework capable of redefining anatomical pathology workflows, democratizing access to specialized diagnostics, and preserving invaluable patient tissue for clinical decision-critical ancillary testing.

The optical input: capturing tissue detail without dyes

To digitally generate a stain, the software first needs a highly detailed map of the unstained tissue. Instead of relying on chemical dyes to create contrast under a standard brightfield microscope, virtual staining uses specialized imaging techniques that capture the inherent physical and biochemical properties of the raw tissue [2, 3]. One common approach is autofluorescence imaging, which captures the natural light emitted by native tissue elements (like collagen, elastin, and metabolic cofactors) to outline the microanatomy. Advanced techniques like fluorescence lifetime imaging microscopy further enhance this by capturing the decay rate of fluorescence, providing an extra dimension beyond intensity [10]. Another powerful tool is photon absorption remote sensing (PARS), which uses ultraviolet light to directly measure how different biomolecules absorb energy. For example, PARS can use specific UV wavelengths to capture dense DNA concentration (mimicking hematoxylin) while simultaneously capturing extracellular matrix proteins like collagen (mimicking eosin) [11]. When processed through deep learning, PARS data can generate multiple virtual stains – including HE, Masson's trichrome, and periodic acid-Schiff (PAS) – from a single scan [12]. Similarly, stimulated Raman scattering (SRS) microscopy measures the inherent molecular vibrational responses of lipids and proteins, enabling the rapid generation of virtual HE [13]. Because these microscopic techniques read the tissue's natural composition, they can distinguish structures without any chemical intervention. In dermatopathology, for instance, these label-free scans can accurately detect melanin within malignant melanoma cells or highlight red blood cells in capillary lumens [11]. In neuropathology and prostate biopsies, similar label-free techniques can penetrate thick, fresh-frozen tissue, bypassing the need to cut ultra-thin sections altogether [13].

Algorithmic evolution: from digital artifacts to diagnostic fidelity

The software that translates these label-free scans into familiar HE or immunohistochemistry (IHC) images has improved dramatically. Early virtual staining programs – based on architectures like Convolutional Neural Networks and GAN and their iterations such as contrastive unpaired translation and dual contrastive learning GAN – often struggled with tissue alignment and were prone to "hallucinations" – meaning the software might artificially insert a nucleus where none existed, blur the cytoplasm, or completely miss important features like fungal hyphae [5, 11, 14, 15]. Today's advanced AI models (like vision transformers and diffusion models) are designed to understand the "global context" of a whole-slide image rather than just looking at isolated pixels [16, 17]. By evaluating how different tissue structures relate to one another across the entire biopsy, these modern models maintain strict architectural and cytological fidelity. For a pathologist, this means the software is now capable of preserving critical diagnostic criteria. For example, in squamous cell carcinoma, the AI accurately replicates complex features like keratin pearls, intercellular bridges, and true nuclear pleomorphism [18]. In basal cell carcinoma, it successfully maintains the peripheral palisading of basaloid nests without blurring the surrounding fibromyxoid stroma [18]. Crucially, newer models have significantly improved their ability to accurately depict mitotic figures and nuclear atypia – features that earlier software routinely distorted [17].

Expanding the diagnostic repertoire: instant special stains and multiplexing

Perhaps the most significant clinical advantage of virtual staining is the ability to generate multiple different stains from a single physical tissue section [19]. Because the physical tissue is either left completely unstained or stained only once with a routine HE, the AI can digitally infer (deduce) additional stains, saving the physical tissue block for critical downstream molecular testing (like next-generation sequencing). This AI-enabled transformation of one existing chemical stain into another is a process known as stain-to-stain transformation [20]. This capability has immediate practical applications across subspecialties. In renal pathology, from a single label-free scan, the AI can simultaneously generate an HE, a PAS stain to highlight glomerular basement membranes, a Masson's trichrome to evaluate interstitial fibrosis, and a Jones methenamine silver stain to assess reticular fibers. This allows pathologists to instantly correlate mesangial expansion across all four stains without waiting for the histotech to cut new levels [11, 21]. In hepatology, for staging metabolic dysfunction-associated steatohepatitis, AI can digitally generate a virtual Masson's trichrome directly from an HE liver biopsy slide to accurately score fibrosis, lobular inflammation, and hepatocyte ballooning, completely bypassing the wet-lab special stain [22]. In immuno-oncology,

in lung cancer, AI can take an unstained slide and generate a highly multiplexed panel (e.g., PanCK, PD-L1, CD3, CD8, and DAPI) alongside a virtual HE. This allows the pathologist to instantly classify a tumor as "immune-inflamed" or "immune-desert" and calculate a tumor proportion score for PD-L1 without depleting the tissue block [7]. Furthermore, AI-driven virtual staining is paving the way for 3D computational pathology. Standard 2D histopathology severely under-samples tissue volumes, examining less than 1% of a specimen. By pairing non-destructive, open-top light-sheet microscopy or thick-section SRS with virtual staining, researchers can generate continuous, volumetric HE representations of entire biopsies [23]. Deep-learning triage models based on multiple-instance learning can then automatically assess these massive 3D datasets, highlighting high-risk regions for the pathologist and fundamentally solving the spatial heterogeneity problem of conventional 2D sectioning [24].

Validating the virtual stain: pathology-aware quality control

For virtual stains to be adopted in routine sign-out, they must be diagnostically equivalent to chemical stains. Historically, computer scientists used metrics that evaluated images like standard photographs (checking for pixel noise or generic structural similarity), which often failed to capture the nuances of pathology. Today, researchers use "pathology-aware" evaluation frameworks (e.g. pathology-aware perceptual image similarity [PaPIS] or histology-specific fidelity index [HSFI]) [5, 25]. New grading algorithms specifically evaluate the virtual slides based on clinical imperatives: Is the chromatin texture accurate? Is the epidermal stratification correct? Are the tumor margins cleanly delineated? Algorithms such as autonomous quality and hallucination assessment (AQuA) have been developed specifically to detect artifacts in virtual staining. Operating without access to a histochemically stained ground truth, AQuA achieves 99.8% accuracy in differentiating acceptable from unacceptable virtually stained tissue, serving as an automated gatekeeper to prevent faulty AI images from misleading diagnosticians [26]. In blinded "Turing tests," expert pathologists frequently cannot reliably distinguish a virtual HE from a chemical HE. In a recent validation on skin cancer excisions, pathologists demonstrated a 95.5% agreement in primary diagnoses between virtual and chemical HE slides, easily distinguishing between benign nevi, squamous cell carcinoma, and basal cell carcinoma [11]. Furthermore, automated quality-control software acts as a gatekeeper, autonomously flagging any virtual slide that contains unacceptable digital artifacts before it ever reaches the pathologist's screen.

Workflow integration and practical challenges

Integrating virtual staining into a busy anatomic pathology lab promises to eliminate batch-staining delays, reduce the need for recuts, and drastically cut reagent costs. It also holds tremendous potential for rapid onsite evaluation and intraoperative consultations [13]. By scanning thick, fresh tissue directly, pathologists can obtain permanent-section-quality virtual HE images in minutes, entirely avoiding the freezing artifacts and tissue folds that plague traditional frozen sections. Despite these benefits, practical hurdles remain. Virtual staining algorithms can currently be confused by routine pre-analytical variations, such as the blue surgical margin ink used during grossing, which the AI might mistakenly colorize as red blood cells or eosinophilic cytoplasm. The software can also struggle if the tissue has a significant cautery artifact or if there is out-of-focus scanning [11]. Additionally, models trained in one specific hospital may suffer a drop in quality when applied to slides processed at a different institution due to variations in fixation times or scanner hardware (domain shift) [17]. Widespread clinical adoption will require rigorous multi-center validation to ensure these tools are robust enough to handle the daily realities and imperfections of the routine histology bench.

Perspective/future directions

The core motivation: empowering patients and clinicians

The ultimate motivation driving the implementation of virtual staining is the profound impact it can have on patient care: delivering high-quality, timely diagnostics while providing clinicians with reliable, comprehensive information to guide precision treatments. By transitioning to a digital-first computational framework, laboratories can overcome the prolonged processing times, physical tissue exhaustion, and technical variability that plague traditional wet-lab workflows (Figure 1). For the practicing pathologist, this technology translates to an unprecedented ability to rapidly evaluate the tumor microenvironment, confidently assess surgical margins in real time, and ensure that invaluable biopsy material is conserved for critical downstream molecular analyses and next-generation sequencing.

The huge potential: from stain-to-stain transformations to biomarker prescreening

The potential of this technology is staggering, offering capabilities that transcend the physical limitations of conventional chemistry [27]. Deep learning algorithms can now perform high-fidelity stain-

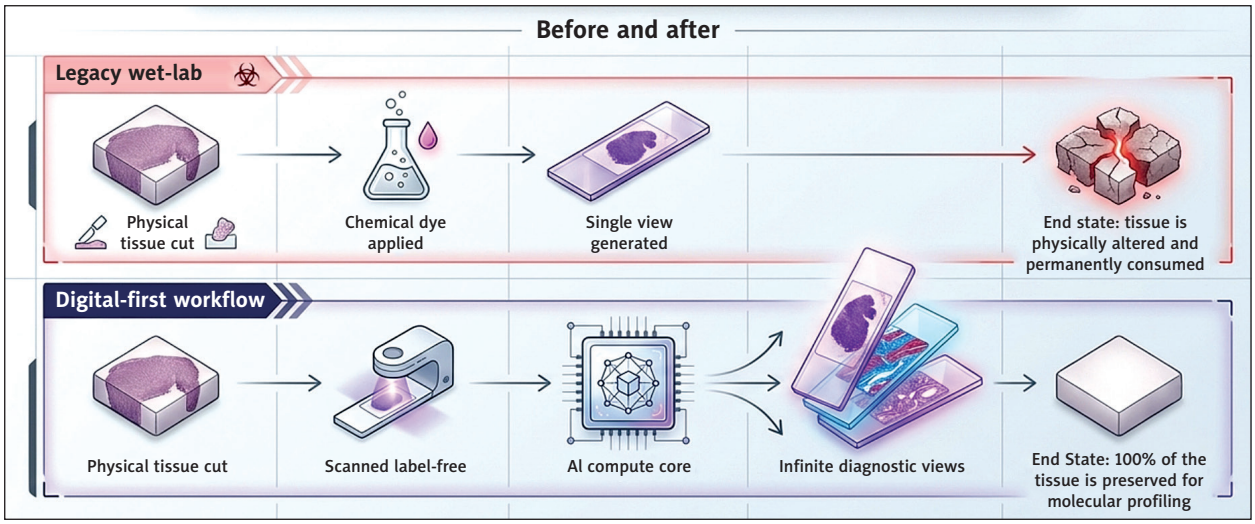

Figure 1. Paradigm shift: synthesizing stains without chemical dyes

Virtual stain uses advanced deep learning to synthesize diagnostic-grade histological images directly from label-free tissue scans or primary HE slides.

to-stain transformations, such as converting a standard HE slide into highly specific IHC stains or special stains (e.g., Masson's trichrome, PAS) in a matter of minutes. Furthermore, virtual multiplexed immunostaining can simultaneously generate multiple clinical markers – such as identifying endothelial cells and epithelial tumor cells to assess vascular invasion – from a single label-free tissue section.

Beyond visualization, AI-driven virtual models hold immense promise for biomarker prescreening. "Class B" AI tools can act as indirect proxies for molecular tests, predicting the presence of molecular alterations like epidermal growth factor receptor mutations or microsatellite instability directly from routine HE slides. This prescreening capability can rapidly identify patients who urgently need targeted therapies and streamline the genomic testing workflow, radically shrinking the time from biopsy to treatment initiation [28].

Critical risks still to be mitigated

Despite its transformative potential, the clinical deployment of virtual staining must be approached with a balanced understanding of its current limitations. The persistent risk of generative "hallucinations" – where an AI model invents nonexistent morphological structures or drops out critical diagnostic features – remains a major barrier to primary diagnostic use. Furthermore, these models are highly susceptible to "domain shift"; an algorithm trained on slides from one specific scanner or laboratory protocol may suffer severe performance degradation when exposed to variations in tissue handling or alternative hardware at a different institution. There is also the "black box" ethical dilemma regarding accountability: if an AI tool misidentifies a critical feature, the liability between the software developer, the institution, and the pathologist remains complex and legally ambiguous [29].

The role of the pathology community and regulatory agencies

To safely mitigate these risks and transition virtual staining to the routine histology bench, the active leadership of the global pathology community is paramount. Generic computer-vision metrics must be abandoned in favor of "pathology-aware" evaluation frameworks (such as PaPIS or HSFI) that rigorously penalize AI models for distorting critical diagnostic imperatives like nuclear atypia or mitotic figures. Furthermore, autonomous quality-control algorithms (like AQuA) must be deployed as digital gatekeepers to intercept hallucinated artifacts before they reach the pathologist.

The successful clinical integration and ethical governance of these AI tools will rely heavily on the collaborative efforts of major professional societies. Organizations such as the Digital Pathology Association (https://digitalpathologyassociation.org/), the European Society of Pathology (https://www.esp-pathology.org/), the United States and Canadian Academy of Pathology (https://uscap.org/), and the European Society for Digital and Integrative Pathology (https://www.esdipath.org/) are essential for establishing the foundational guidelines for AI implementation. These institutions must champion multi-center validation trials, curate diverse and standardized whole slide image datasets, and intensively promote AI literacy in medical training so that pathologists can critically assess algorithmic outputs. Concurrently, regulatory agencies must enforce strict classification and validation frameworks, mandating continuous post-market surveillance to monitor for data drift over time.

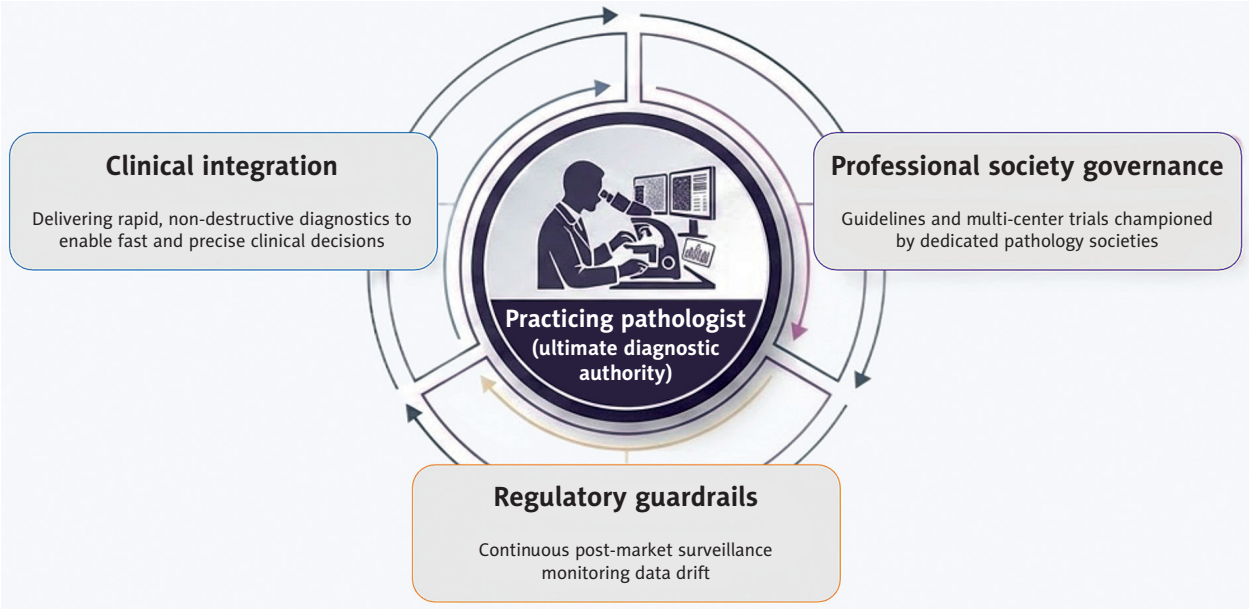

Figure 2. The human-in-the-loop paradigm

Closely monitored and officially regulated, virtual stain has a potential to become a tissue-saving precision diagnostics that enables practicing pathologists to make faster and more accurate diagnostic decisions.

Conclusions

Ultimately, virtual staining must be regulated and adopted under a strict "human-in-the-loop" paradigm – serving as an advanced, assistive decision-support tool where the practicing pathologist retains ultimate diagnostic oversight and legal accountability (Figure 2). As these community-led and regulatory milestones are achieved, virtual staining will securely transition into a fundamental pillar of modern pathology, delivering faster, richer, and highly precise diagnostics to the patients who need them most.

Disclosures

1. Institutional review board statement: Not applicable.
2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

References

1. Bancroft JD, Gamble M (eds.). Theory and practice of histological techniques. Elsevier Health Sciences 2008.
2. Pillar N, Ozcan A. Virtual tissue staining in pathology using machine learning. Expert Rev Mol Diagn 2022; 22: 987-989.
3. Abraham TM, Levenson R. Current landscape of advanced imaging tools for pathology diagnostics. Mod Pathol 2024; 37: 100443.
4. Pillar N, Li Y, Zhang Y, Ozcan A. Virtual staining of nonfixed tissue histology. Mod Pathol 2024; 37: 100444.
5. Hussain MA, Waris MA, Akram MU, Khan MJ, Asaf MZ, Javaid A, et al. VISGAB: Virtual staining-driven GAN benchmarking for optimizing skin tissue histology. Sci Rep 2025; 15: 42430.
6. Ounissi M, Sarbout I, Hugot JP, Martinez-Vinson C, Berrebi D, Racoceanu D. Scalable, trustworthy generative model for virtual multi-staining from H&E whole slide images. PLoS Comput Biol 2025; 21: e1013516.
7. Loo J, Robbins M, McNeil C, Yoshitake T, Santori C, Shan C, et al. Autofluorescence virtual staining system for H&E histology and multiplex immunofluorescence applied to immuno-oncology biomarkers in lung cancer. Cancer Res Commun 2025; 5: 54-65.
8. Fanous MJ, Pillar N, Ozcan A. Digital staining facilitates biomedical microscopy. Front Bioinform 2023; 3: 1243663.
9. PathAI. Transforming histology: how AI-powered virtual staining could unlock lab efficiency and redefine anatomic pathology workflows. PathAI Blog 2024. Available from: https://www.pathai.com/blog/transforming-histology-how-ai-powered-virtual-staining-could-unlock-lab-efficiency-and-redefine-anatomic-pathology-workflows (accessed: 05.2026).
10. Wang Q, Akram AR, Dorward DA, Talas S, Monks B, Thum C, et al. Deep learning-based virtual H&E staining from label-free autofluorescence lifetime images. NPJ Imaging 2024; 2: 17.
11. Tweel JE, Ecclestone BR, Tummon Simmons JA, Boktor M, Fieguth P, Reza PH. Label-free whole slide virtual multi-staining using dual-excitation photon absorption remote sensing microscopy. NPJ Imaging 2026; 4: 22.
12. De Haan K, Zhang Y, Zuckerman JE, Liu T, Sisk AE, Houwen MFPC, et al. Deep learning-based transformation of H&E stained tissues into special stains. Nat Commun 2021; 12: 4884.
13. Falahkheirkhah K, Mukherjee SS, Gupta S, Herrera-Hernandez L, McCarthy MR, Jimenez RE, et al. Accelerating cancer histopathology workflows with chemical imaging and machine learning. Cancer Res Commun 2023; 3: 1875-1887.
14. Tweel JE, Ecclestone BR, Boktor M, Tummon Simmons JA, Fieguth P, Reza PH. Virtual histology with photon absorption remote sensing using a cycle-consistent generative adversarial network with weakly registered pairs. arXiv 2023. Available from: https://doi.org/10.48550/arXiv.2306.08583.
15. Ma J, Li W, Li J, Xu Z, Zhang Y, Wei B, et al. Generative AI for misalignment-resistant virtual staining to accelerate histopathology workflows. Nat Commun 2026; 17: 4494.
16. Klöckner P, Teixeira J, Montezuma D, Oliveira J, Caspers J, Schierholz R, et al. H&E to IHC virtual staining methods in breast cancer: an overview and benchmarking. NPJ Digit Med 2025; 8: 384.

17. Hussain MA, Waris MA, Akram MU, Khan MJ, Asaf MZ, Javaid A, et al. ViT-stain: vision transformer-driven virtual staining for skin histopathology *via* global contextual learning. PloS One 2026; 21: e0341311.
18. Ecclestone BR, Tweel J, Li K, Gaouda H, Bell K, Dinakaran D, et al. Exploration of photoacoustic remote sensing virtual histology in skin malignancies. Proceeding SPIE 2023; PC12352: PC1235201.
19. 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.
20. Al-Qudah R, Bala A, Almuhajri M, Zakaria K, Suen CY. A systematic review of machine learning for digital stain processing in pathology. Neurocomput 2026; 676: 133064.
21. Ju W, Border S, Afsari F, Seth S, Rezapourdamanab S, Renteria R, et al. Validation of virtual trichrome stains for kidney fibrosis evaluation using dual-mode emission and transmission microscopy. medRxiv 2025.
22. Balaha HM, Ali KM, Mahmoud A, Aboudessouki A, Azam MT, Giridharan GA, et al. From hematoxylin and eosin to masson's trichrome: a comprehensive framework for virtual stain transformation in chronic liver disease diagnosis. Diagnostics (Basel) 2026; 16: 764.
23. Zhang Y, Kang L, Yu W, Tsang VTC, Wong TTW. Three-dimensional label-free histological imaging of whole organs by microtomy-assisted autofluorescence tomography. iScience 2022; 25: 103721.
24. Gao G, Yan R, Song AH, Hsieh HC, Barner LAE, Wang F, et al. Deep-learning triage of 3D pathology datasets for comprehensive and efficient pathologist assessments. bioRxiv 2025.
25. Wang Q, Tweel JED, Reza PH, Layton A. Pathology-guided virtual staining metric for evaluation and training. arXiv 2025.
26. Huang L, Li Y, Pillar N, de Haan K, Zhang Y, Bai B, et al. A robust and scalable framework for hallucination detection in virtual tissue staining and digital pathology. Nat Biomed Eng 2025; 9: 2196-2214.
27. Imran MT, Shafi I, Ahmad J, Butt MFU, Villar SG, Villena EG, et al. Virtual histopathology methods in medical imaging – a systematic review. BMC Med Imag 2024; 24: 318.
28. Aldea M, Salto-Tellez M, Marra A, Umeton R, Stenzinger A, Koopman M, et al. ESMO basic requirements for AI-based biomarkers in oncology (EBAI). Ann Oncol 2026; 37: 414-430.
29. Chauhan C, Gullapalli RR. Ethics of AI in pathology: current paradigms and emerging issues. Am J Pathol 2021; 191: 1673-1683.

Address for correspondence

Konstanty Korski, MD, PhD
F.Hoffmann-La Roche Ltd
Grenzacherstr. 124 (Bld. 1/20)
4070 Basel, Switzerland
e-mail: konstanty.korski@roche.com