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Revealing the Microscopic Landscape: A Review of Advanced Pathological Staining

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ABSTRACT: Because histochemical staining is the principal means by which the biochemical information that this approach produces is converted to comprehensible visual data, it remains central to clinical pathology. While H&E staining provides a relatively simple morphological reference, the diagnostic utility of these has limited insights on what specific molecular features are represented and fails to accurately detect subtle architectural features such as mild fibrosis or basement membrane thickening. The transition from routine histopathology techniques to ancillary stains such as Periodic Acid–Schiff (PAS), Masson's Trichrome and silver impregnation will be illustrated in this article. We focused here on the chemical logic behind these reactions ranging from outcomes of the oxidation mechanisms practiced by PAS to biological reduction met with metallic staining in silver stains, and modern use of immunohistochemistry (IHC) for diagnosis beyond a gross characterization. The classical specialized stains are "gold standards" as despite advances in genomic sequencing, they are both reproducible and cost-effective, providing the vital architectural context in assessing microbial infections and organ failure.

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Introduction

The Evolution of Histological Visualization

The existence of the modern pathology diagnostic landscape depends on a mid-19th century shift from empirical craft to scientific rigor. Innovators like Joseph von Gerlach and Paul Ehrlich remade rudimentary industrial dyes into precise tools, enabling pathologists to pierce the pale-yet-translucent outlines of unstained cells and uncover their inner cellular architecture [1].

The main purpose of stains is based on developing a contrast in particular areas that can exhibit the features of certain tissues and establishing standardized 'visual language'. However, ordinary protocols often come across "Gray Areas", where the information of the shape is not enough for a correct diagnosis. H&E has limitations, for example it cannot accurately tell the difference between early fungal infiltration and glycogen deposits or a healthy basement membrane from one thickened by disease. The especially chemical affinities crucial for subsequent labelling and imaging of carbohydrates, fibrillar connective tissue components or metallic deposits answer clinical challenges and required the establishment of specialized methods [1, 2].

The Histological Baseline: Hematoxylin and Eosin (H&E) Staining

H&E is the universal gold standard and provides the necessary differential for malignancy, inflammation and ischemia at a basic level. This technique is based on the dual principles of basophilia and acidophilic: Hematoxylin (basic dye) binds to acidic structures such as nuclei and lysosomes, while Eosin (acidic dye) binds to basic structures such as cytoplasm and extracellular matrix [3, 4].

Technical and Chemical Nuances

Hematoxylin, a natural product isolated from the logwood tree (*Haematoxylum campechianum*). As an academic histotechnologist, this should be born in mind. It has no inherent stain until it is converted through a two-step process of oxidation (either naturally

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or artificial in sodium iodide or mercuric chloride) into hematein, followed by metal mordanting. The mordant is the requisite link of hematein to tissue and without that chemical connection, the dye fails to fix in repeatable fashion resulting in the unpredictable outcome seen with inferior laboratory preparations (see Table 1) [5, 6].

Table 1: H&E Color Outcomes

Tissue Component	Stain Affinity	Color (H&E)
Nucleus (DNA/RNA)	Basophilic	Blue/Purple
Cytoplasm (Proteins)	Acidophilic	Pink/Red
Collagen	Acidophilic	Pink
Red Blood Cells	Acidophilic	Bright Red

Procedural Workflow:

H&E staining is a quick and multi-step high accuracy protocol:

1. **Fixation:** Formalin-based crosslinking is used to fix tissue specimens for the preservation of cellular morphology, thus also preventing protein and enzymatic degradation.
2. **Paraffin Embedding:** Tissue samples are embedded in paraffin to give support to the tissue in order to perform microtomy.
3. **Sectioning:** The specimens are cut into very thin slices (2–10 μm) in microtome and placed on glass slides.
4. **Deparaffinization and Rehydration:** The sections were first deparaffinized by immersing in xylene for 20 min and then rehydrated with graded alcohol (100% to 70%) followed by distilled water.
5. **Hematoxylin Staining:** 5–10 min in hematoxylin (stains cell nuclei)
6. **Bluing:** Hematoxylin-mordant complex is usually converted to a comparatively stable, bright blue color with treatment of the sections in dil. ammonium solution or lithium carbonate solution for contrasting staining of the nucleus.

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7. **Differentiation:** The sections are immersed in acid alcohol for short time to remove excess hematoxylin (if the nuclei are overstained) and improve nuclear details.

8. **Eosin Staining:** The final step involves immersing the sections in eosin for a time ranging from 1 to 3 minutes in order to stain cytoplasm and extracellular matrix on top of nuclei that are stained with hematoxylin.

9. **Dehydration and Clearing:** Samples are dehydrated through a series of increasing concentrations of alcohol, followed by clearing in xylene to make the tissue transparent.

10. **Mounting:** Sections are then mounted in a synthetic resin (e.g., DPX) and overlaid with coverslip for microscope viewing [3, 4].

Technical Limitations

While hematoxylin and eosin (H&E) staining remains the bedrock of histopathological diagnosis, it suffers some significant drawbacks. Its major limitation is that it does not identify specific molecular biomarkers such as HER2, estrogen receptors, progesterone receptors or other immunohistochemistry targets required for disease categorization and targeted treatment. Lastly, due to its lack of specificity, H&E staining fails to depict specific tissue components such as elastic fibers, reticular fibers, basement membranes and lipids; thus, special histochemical stains or immunohistochemical methods are needed for a better evaluation. Additionally, long-term storage of H&E-stained slides may lead to eventual fading or maxing out of the staining intensity and morphological detail necessary for accurate diagnosis [5–7].

Visualizing Carbohydrates and Glycogen: Periodic Acid-Schiff (PAS)

The PAS (periodic acid-Schiff) stain is thought of as a gold normal for staining carbohydrate-rich buildings together with mucins, fungal organisms and basement membranes [8].

Chemical Mechanism and the "Lison" Caveat

Therefore, periodic acid oxidizes hydroxyls of sugar molecules followed by producing aldehydes [9, 10]. Receptively, these aldehydes interact with Schiff's reagent (a colorless

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mixture of basic fuchsin (Fuchsine), HCl and sodium metabisulfite) to produce a durable magenta compound [11] It is, therefore, crucial that histotechnologists control this very closely because other than aldehydes or any fixing agent could also re-stain Schiff's reagent as shown by **Lison** and attachment of iodate or periodate on tissue components may yield false-positive results [12].

Standard PAS Staining Protocol

1. Through xylene and graded alcohols, deparaffinize and hydrate.
2. **Oxidation:** Periodic acid at 0.5% for a period of five minutes
3. **Schiff Reaction:** This extract is mixed with Schiff reagent In the dark for 15–30 minutes (the solution must turn light pink before proceeding).
4. **Washing:** Develop the magenta color with lukewarm tap water in 5–10 minutes.
5. **Counterstain:** Hematoxylin, Mayer (1 minute for nuclear exposure)
6. **Dehydrate, clear, and mount** [13, 14].

Differentiating Connective Tissues: Masson's Trichrome (MT)

Masson's Trichrome is used to measure dermal wound healing and tissue fibrosis because it clearly differs between blue collagen, pink muscle (which both stain equally on H&E) (see Table 2) [15].

Table 2: Masson's Trichrome Color Differentiation

Tissue Component	Color (Masson's Trichrome)	Diagnostic Significance
Nuclei	Blue/Black	Cellular identification
Muscle and Cytoplasm	Red	Distinguishing parenchyma from stroma
Collagen/Fibrous Tissue	Blue	Mapping extent of fibrosis/remodelling
Erythrocytes	Red	Vascular assessment

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MT is commonly used to assess fibrotic deposition of excessive extracellular matrix (ECM) within the liver, heart and lungs [16, 17]. Although Picrosirius red is more specific for collagen types when viewed under polarized light, MT remains the gold standard clinically because it can be performed in laboratories without a polarizing microscope [18, 19]. The protocol uses phosphomolybdic acid to stain nuclei with Iron Hematoxylin, cytoplasm with Biebrich Scarlet-Acid Fuchsin, and collagen with Aniline Blue [20].

Silver Impregnation and Metallic Stains

Silver staining is a very sensitive detection technique with nanogram-level detection limits (detection of proteins, nucleic acids). The mechanism relates to the reduction of ionic silver into metallic silver at particular biochemical sites (including basic residues like lysine and histidine, in addition to sulfur-containing amino acids) [21, 22].

Technical Specifics and Specific Stains

Silver staining is not a linear method; the intensity of silver bands do not necessarily correlate to protein concentration and are greatly influenced by their local redox microenvironment and even conformation [23, 24].

- ***Warthin–Starry (WS):*** Mainly for Helicobacter pylori and spirochetes Although WS is effective, IHC is now the gold standard as WS may miss isolated organisms that IHC detects using specific antigen-antibody binding [25].
- ***Grocott-Gomori's Methenamine Silver (GMS):*** GMS is well-known for fungal detection, but it is also positive for different bacteria and mycobacteria. TTCC oxidizes polysaccharides within cell walls and lowers silver to obtain a black-on-pale-green contrast. It is also an essential tool for the diagnosis of aspiration pneumonia as it visualizes plant cell walls in aspirated food particles [26, 27].

Clinical Correlations: From Histochemistry to Immunohistochemistry (IHC)

From a morphology-based histochemistry, modern pathology has progressed to molecularly targeted immunohistochemistry (IHC). Special histochemical stains improve visualization of particular chemical/structural constituents within tissues while immunohistochemistry provides a "molecular fingerprint" due to identification of specific

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protein biomarkers that can help in comprehensive tumor classification, subtyping and diagnosis (see Table 3) [28, 29].

Table 3: Common IHC Markers and Diagnostic Utility

Marker	Expression Pattern	Diagnostic Use
CK7 / CK20	Cytoplasmic	Origin of adenocarcinoma (Lung vs. Colon)
TTF-1	Nuclear	Primary lung adenocarcinoma
p63	Nuclear	Squamous cell differentiation
S-100 / HMB-45	Cytoplasmic	Melanoma
CD45	Membranous	Lymphoma/Leukemia

Identifying Early Cellular Damage and Ischemia

Such staining techniques are indispensable for the diagnosis of early coagulative necrosis, especially in silent myocardial ischemia where morphological characteristics on Hematoxylin and Eosin (H&E) stain can be minor or difficult to appreciate [30].

- ***PAS:*** First evidence of the rapid depletion of glycogen, as an early marker of metabolic damage in ischemic cardiomyocytes.
- ***Silver Stains:*** Mark reticulin fibers that remain intact during early necrosis and can help differentiate true coagulative necrosis from postmortem autolysis [27].
- ***IHC Markers:*** The identification of markers of cellular injury such as cleaved caspase-3 (a marker of apoptosis) and the loss of mitochondrial proteins corroborates histochemical findings while providing a more complete picture of cellular dysfunction and tissue injury [29].

Conclusion

Molecular diagnostics have progressed rapidly in recent years, but classical special stains continue to play an important role as part of the pathology diagnostic toolbox. Periodic Acid–Schiff (PAS), Masson Trichrome, and silver stains are rapid, reliable, inexpensive methods with standardised protocols that have excellent tissue architecture and chemical composition visualisation capabilities to ensure even the foundational structures are preserved between molecular assays and staining. They have the power to expose

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glycogen, basement membranes, collagen, reticular fibres and microorganisms that continue in turn allowing appropriate diagnosis of a wide spectrum diseases. An understanding of the qualitative and quantitative chemical principles underlying these histological staining methods ranging from hematein–tissue interactions through silver impregnation chemistry empowers pathologists to confidently interpret histologic observations, enabling color-based histochemistry to continue serving as a bedrock template in modern diagnostics.

References

1. Valent P, et al. Paul Ehrlich (1854-1915) and His Contributions to the Foundation and Birth of Translational Medicine. J Innate Immun. 2016;8(2):111-20.
2. Alturkistani HA, et al. Histological Stains: A Literature Review and Case Study. Global Journal of Health Science. 2016;8(3).
3. Ussi UH, et al. Assessment and Comparison of the Efficacy of Clove Oil versus Xylene... Journal of Biosciences and Medicines. 2026;14:407-427.
4. Gurina TS, Simms L. Histology, Staining. StatPearls. 2026.
5. Hasan MI, et al. A better and copacetic protocol for histopathological slide preparation using H&E stain: A review. Journal of Fisheries, Livestock and Veterinary Science. 2021;02(01):57-67.
6. Morrison LE, et al. Concurrent Viewing of H&E and Multiplex Immunohistochemistry in Clinical Specimens. Diagnostics (Basel). 2025;15(2):164.
7. Lorsuwannarat N, et al. Optimizing Re-staining Techniques for the Restoration of Faded Hematoxylin and Eosin-stained Histopathology Slides... J Histochem Cytochem. 2024;72(11-12):733-742.
8. Al Drees A, et al. Histological and Immunohistochemical Basis of the Effect of Aminoguanidine on Renal Changes Associated with Hemorrhagic Shock... Acta Histochem Cytochem. 2017;50(1):11-19.

 DOI: *10.5281/zenodo.22053967*

9. Greene J, et al. State-of-the-Art Methods for Skeletal Muscle Glycogen Analysis in Athletes... Biosensors (Basel). 2017;7(1):11.

10. Gurina TS, Simms L. Histology, Staining. StatPearls. 2026.

11. Shedge AS, et al. Periodic Acid Schiff (PAS) Staining: A Useful Technique for Demonstration of Carbohydrates. KIMS Deemed to be University.

12. McManus JF. Histological and histochemical uses of periodic acid. Stain Technol. 1948;23(3):99-108.

13. Knoten A, Jain S. PAS Stain. protocols.io. 2019.

14. Vyas M, et al. PAS (Periodic acid-Schiff). PathologyOutlines.com.

15. Suvik A, Effendy AWM. The Use of Modified Masson's Trichrome Staining in Collagen Evaluation in Wound Healing Study. Malaysian Journal of Veterinary Research. 2012;3(1):39-47.

16. Islam MA, Kumar S. Masson's Trichrome Staining Technique to Evaluate Tissue Fibrosis. Methods in Molecular Biology. 2026;2983.

17. Van De Vlekkert D, et al. Analysis of generalized fibrosis in mouse tissue sections with Masson's trichrome staining. Bio Protoc. 2020; 10:3629.

18. Husain O, et al. Quantitative Histomorphometric Analysis of Collagen Bundles in Masson's Trichrome Stained Rat Skin... Health Sci Rep. 2026;9(3): e71998.

19. López De Padilla CM, et al. Picrosirius Red Staining: Revisiting Its Application... Journal of Histochemistry & Cytochemistry. 2021;69(10):633–643.

20. Chooi KF, et al. The Dimethylnitrosamine Induced Liver Fibrosis Model in the Rat. J Vis Exp. 2016;(112):54208.

21. Merril CR. Development and Mechanisms of Silver Stains for Electrophoresis. Acta Histochem. Cytochem. 1986;19(5).

22. Piavchenko G, et al. A combined use of silver pretreatment and impregnation with consequent Nissl staining... Front Neuroanat. 2022; 16:940993.

 DOI: *10.5281/zenodo.22053967*

23. Uchihara T. Silver diagnosis in neuropathology: principles, practice and revised interpretation. Acta Neuropathol. 2007; 113:483–499.

24. Fonseca Mata JC. Brain tissue (Silver staining). Wikimedia Commons. 2014.

25. Farouk WI, et al. Warthin-Starry Staining for the Detection of Helicobacter pylori in Gastric Biopsies. Malays J Med Sci. 2018;25(4):92-99.

26. Carmen M. Exploring Clinico-pathological Features Through Immunohistochemical Analysis... J Cytol Histol. 2024; 15:748.

27. Jayagopal D, et al. Immunohistochemistry as a diagnostic tool in pathology. Bioinformation. 2025;21(10):3595-3598.

28. Pragnya C, Patil V. Immunohistochemical Expression of MLH1 and MSH2 in Colorectal Carcinoma... Cureus. 2026;18(3): e105200.

29. Battaglin F, et al. Microsatellite instability in colorectal cancer: overview of its clinical significance... Clin Adv Hematol Oncol. 2018;16(11):735-745.

30. Shams P, et al. Silent Myocardial Ischemia. StatPearls. 2026.

 DOI: *10.5281/zenodo.22053967*