



**ARTIFICIAL INTELLIGENCE AND WHOLE-SLIDE IMAGING IN
CYTOPATHOLOGY: TECHNICAL CONSIDERATIONS, EVIDENCE, AND
WORKFLOW INTEGRATION**

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Abstract

Objective: To review the technical challenges, published evidence, validation considerations, and practical workflow integration of Whole-Slide Imaging (WSI) and Artificial Intelligence (AI) in gynecologic and non-gynecologic cytopathology.

Methods: A narrative review was undertaken using peer-reviewed literature on digital cytopathology, WSI, AI-assisted cytology, validation studies, and standardized reporting systems. Emphasis was placed on reported study populations, diagnostic performance, validation approaches, workflow effects, and limitations of current models.

Results: While digital histopathology has achieved widespread clinical adoption, digital cytopathology faces distinct physical constraints, including three-dimensional cellular overlapping, variable depth-of-field, liquid-based preparation variations, and massive digital file sizes. Recent developments in multi-layer z-stacking, fast volume scanning, and deep learning models have significantly advanced automated cell detection and classification across standardized reporting schemes (including Bethesda, Paris, Milan, Sydney, and TIS). However, issues regarding pre-analytic standardization, scanner focus tracking, laboratory information system (LIS) interoperability, and validation frameworks remain critical barriers to full routine deployment.

Conclusion: AI-assisted digital cytopathology demonstrates technological feasibility and promising research-level diagnostic performance, with selected applications showing emerging clinical utility. However, routine clinical implementation remains dependent on specimen and scanner standardization, laboratory-specific validation, interoperability, infrastructure, regulatory requirements, and continued cytopathologist oversight.

Keywords: Artificial Intelligence, Whole-Slide Imaging, Digital Cytopathology, Deep Learning, Z-Stacking, Workflow Integration.

1. Introduction

Digital pathology has transformed diagnostic medicine by enabling slide digitization, remote tele-consultation, automated image analysis, and seamless integration with electronic health records [27,30]. While digital workflows and Whole-Slide Imaging (WSI) have gained primary diagnostic regulatory approvals in surgical histopathology [29], cytopathology has historically lagged behind.

Cytological specimens present unique physical and optical challenges [10,28]. Unlike flat, uniformly cut 3-to-4-micrometer formalin-fixed paraffin-embedded (FFPE) tissue sections, cytological preparations, whether direct smears, liquid-based cytology (LBC), cytospins, or cell blocks are inherently three-dimensional [15,32]. Cells float at multiple focal planes, frequently aggregate in dense three-dimensional clusters, and exhibit fine nuclear details (e.g., chromatin texture, nuclear membrane irregularities, and intranuclear inclusions) that require high-power magnification and micro-focusing to interpret accurately [10,32].

Despite these physical limitations, recent technological breakthroughs in rapid multi-plane z-stacking, automated focus-tracking algorithms, and advanced Artificial Intelligence (AI) models, specifically Convolutional Neural Networks (CNNs) and Vision Transformers (ViTs), have advanced the technical feasibility of digital cytopathology [28,33,34]. This review provides a comprehensive synthesis of digital scanning technology, AI algorithm performance, technical validation requirements, and workflow integration strategies tailored for modern pathology laboratories.

2. Technical Specifications & Imaging Challenges in Cytopathology

2.1 The Focus and Depth-of-Field Dilemma: Single-Plane vs. Z-Stacking

The principal technical hurdle in digitizing cytological slides is depth-of-field [30,32]. Standard single-plane WSI captures images at a fixed focal depth, which routinely results in out-of-focus cellular clusters in non-gynecologic aspirates and thick smears [15,32].

To achieve diagnostic equivalence with conventional light microscopy, modern cytopathology scanners utilize two main methods [28,30]:

Multi-Plane Z-Stacking: The scanner captures multiple images of the same field of view across varying focal depths (typically 5 to 15 planes spaced 0.5 to 1.5 micrometers apart). The resulting file allows the user to manually "focus" up and down through the tissue volume on a digital display [32].

Focus-Extended (Focus-Stacking/Extended Depth-of-Field) Projection: An algorithm merges the in-focus regions from multiple z-planes into a single, composite two-

dimensional image [28,34]. While this reduces file sizes, it can occasionally introduce optical artifacts in overlapping cell groups.

2.2 Data Volume, Storage, and Bandwidth

Multi-plane z-stacking significantly increases file sizes [30]. A single high-power (40\times) single-plane slide scan averages 1 to 3 gigabytes (GB), whereas a 7-plane z-stack image scan can range from 5 to 15 GB per slide [28,32]. A medium-to-high-volume diagnostic laboratory scanning 200 cytological slides daily can generate up to 2 to 3 terabytes (TB) of data per day [30]. This requires high-performance local network bandwidth, image compression protocols (e.g., JPEG 2000, DICOM standard formatting), and tiered cloud/on-premise storage architecture [27,30].

3. Artificial Intelligence in Cytopathology: Architectures and Clinical Applications

3.1 Deep Learning Architectures

Modern AI algorithms in cytopathology rely on deep learning methodologies to perform tasks ranging from primary cellular detection to automated triage and risk stratification [28,33,34]:

Convolutional Neural Networks (CNNs): Architectures such as ResNet, EfficientNet, and DenseNet excel at localized feature extraction, identifying cellularity, nuclear-to-cytoplasmic (N/C) ratios, and nuclear membrane contours [33,34].

Vision Transformers (ViTs): ViTs process images as sequences of patches, leveraging self-attention mechanisms to analyze spatial relationships between distant cells and background contexts (e.g., necrotic debris, inflammatory background, or colloid) [28,34].

Multiple Instance Learning (MIL): Weakly-supervised frameworks where the AI processes an entire whole-slide image as a collection ("bag") of smaller image patches ("instances") without requiring labor-intensive cell-by-cell manual annotation [32,33].

Clinical Applications Across Standardized Reporting Systems

AI models have been tailored to enhance standardized diagnostic reporting systems across various organ sites:

- **Gynecologic Cytology (The Bethesda System):** Automated primary screening of liquid-based Pap tests for cervical dysplasia (LSIL, HSIL, atypical squamous cells) and detection of infectious organisms (e.g., Trichomonas, Candida, HSV, Actinomyces). Models act as automated triage tools to flag high-risk slides for cytopathology review.

- **Thyroid Fine-Needle Aspiration (The Bethesda System for Reporting Thyroid Cytopathology):** Segmentation and classification of follicular cells, Hurthle cells, and nuclear features (e.g., nuclear grooves, pseudoinclusions) indicative of Papillary Thyroid Carcinoma (PTC) or atypical thyroid lesions (AUS/FLUS).
- **Urine Cytology (The Paris System):** Quantification of high-grade urothelial carcinoma (HGUC) features, non-malignant atypical urothelial cells (AUC), and cell cluster morphometry in voided and washed urine samples.
- **Salivary Gland FNA (The Milan System):** Risk stratification of salivary gland lesions (e.g., pleomorphic adenoma vs. adenoid cystic carcinoma or high-grade carcinoma) by evaluating basaloid, oncocytic, and myoepithelial cell patterns.
- **Breast Cytology (The Yokohama System):** Classification of benign breast lesions, atypical ductal cells, and invasive carcinoma in resource-limited or high-throughput clinical settings.

3.2 Clinical Applications Across Standardized Reporting Systems

Standardized reporting frameworks provide essential, reproducible diagnostic criteria that form the ideal basis for AI algorithm development and clinical triage [10,14,19]:

The Bethesda System for Reporting Cervical Cytology: Automated pre-screening systems identify atypical squamous cells of undetermined significance (ASC-US), high-grade squamous intraepithelial lesions (HSIL), and viral cytopathic effects (HPV, HSV), highlighting high-risk fields of view (FOVs) for cytotechnologist review [28,34].

The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC): Machine learning models assess microfollicular architectural crowding and nuclear features (e.g., grooves, pseudoinclusions) to assist in triaging indeterminate Categories III (AUS) and IV (FN) [4,8,13,16]. Molecular integration (e.g., Afirma, ThyroSeq) further optimizes these indeterminate categories [3,17,18,24].

The Paris System for Reporting Urinary Cytology (TPS 2.0): AI algorithms measure exact nuclear area and calculate N/C ratios, providing quantitative thresholds to differentiate Negative for High-Grade Urothelial Carcinoma (NHGUC) from Atypical Urothelial Cells (AUC) and High-Grade Urothelial Carcinoma (HGUC) [5,25].

The Milan System for Reporting Salivary Gland Cytopathology: Deep learning models help distinguish low-grade basaloid/oncocytic lesions from high-grade malignancies, aiding in the stratification of Neoplasm of Uncertain Malignant Potential (SUMP) [21,22], bolstered by immunocytochemical and translocation markers [12,23].

The Sydney System for Reporting Lymph Node Cytopathology: Automated feature extraction assists in sorting reactive lymphoid populations from atypical lymphoproliferative disorders [2,11], working alongside rapid on-site evaluation (ROSE) and flow cytometry workflows [26].

The International System for Reporting Serous Fluid Cytopathology (TIS): Algorithms assist in distinguishing reactive mesothelial cells from metastatic adenocarcinoma [6,20], utilizing specialized ancillary panels such as BAP1, MTAP, and Claudin-4 [7,9].

Resource-Limited Deployments: Standardized systems paired with digital triage offer diagnostic utility in low-resource settings [1].

Published studies provide quantitative evidence of promising performance, but they also demonstrate why claims of clinical readiness should be interpreted cautiously. Examples include multicentre cervical cytology studies, thyroid cytology research, and digitized urine cytology validation studies. The studies differ in specimen preparation, diagnostic endpoints, reference standards, and external validation, limiting direct comparison across models. Representative evidence is summarized in Table 2 [36,38-40].

3.3 Quantitative Evidence from Selected AI Cytopathology Studies

Table 1. Selected quantitative evidence from AI-assisted cytopathology studies.

Study/application	Sample size	Task validation approach	Reported performance	Clinical relevance / limitation
Xue et al., 2026 – cervical cytology	1,920 women; 4 non-expert cytopathologists	Multicentre randomized crossover; DL-assisted vs manual reading; 4-week washout	Sensitivity 85.7% vs 71.3%; specificity 86.5% vs 85.1%; reading time 31 vs 175 s	Prospective clinical workflow evidence; improved sensitivity and efficiency, but evaluated a defined cervical LBC setting.
Elliott Range et al., 2020 – thyroid cytology	908 slides	CNN-based benign vs malignant classification	Sensitivity 92.0%; specificity 90.5%; AUROC 0.932	Large slide-level study; retrospective design limits direct inference to routine prospective deployment.
Dov et al., 2021 – thyroid cytology decision support	109 slides	Assessment of pathologist performance with/without AI decision support	Pairwise weighted kappa 0.924	Shows reproducibility/decision-support potential; sample size was relatively small and context-specific.
Urine cytology AI system, 2024	535 consecutive slides (181 development; 39 cell-level test; 315 slide-level test)	Deep-learning prediction of histologic HGUC from digitized urine cytology	AI specificity 83% vs pathologists 89%; AI accuracy 74% vs 68%; AI evaluation time 139 s	Separate development and test sets; supports assistance rather than replacement of expert interpretation.
AIxURO urine cytology study, 2026	200 cases; 98 with biopsy follow-up	AI-assisted vs microscopy; consensus cytology and biopsy correlation	Sensitivity 85.0–88.3% vs 79.3%; specificity 85.7–82.3% vs 94.3%; review time reduced 66–78%	Sensitivity/efficiency gains occurred with a specificity trade-off; further multicentre validation remains important.

4. Workflow Integration, Validation, and Regulatory Compliance

4.1 Laboratory Workflow Options

Integrating WSI and AI into routine cytopathology requires selecting an appropriate operational model [27,28]:

Pre-Screening Triage Model: The AI analyzes whole-slide images overnight or immediately post-scanning, flagging high-risk areas and sorting normal/benign cases to the bottom of the worklist [28,32]. Cytotechnologists and cytopathologists focus their initial review on AI-selected target regions.

Interactive Concurrent Model: The clinician reviews the digital slide with AI running in the background, drawing real-time bounding boxes around suspicious cell clusters and displaying automated morphometric data on demand [32,33].

Secondary Quality Assurance (QA) Model: The AI re-evaluates all cases signed out as negative or non-diagnostic, flagging potential false-negative cases for mandatory secondary human re-review [28,33].

4.2 Technical Validation and Regulatory Considerations

Validation should be laboratory-specific and linked to the intended use, specimen types, scanner, display environment, software version, and applicable regulatory requirements. CAP guidance provides a framework for WSI validation in pathology, but published evidence indicates that cytopathology has historically had limited representation in these recommendations; therefore, the requirements should not be presented as universal cytopathology thresholds [31,37].

Validation dataset: The number and composition of cases should be justified according to intended use, specimen type, diagnostic spectrum, and local workload. A fixed universal number of 60–100 cytology cases per preparation type should not be presented as a mandatory requirement [31,37].

Review sequence and washout: Validation studies should minimize recall and order effects by using appropriate randomization and, where applicable, washout periods. The duration should follow the design of the validation study rather than being stated as a universal 2–4-week requirement [31,37].

Concordance and reproducibility: Agreement between glass-slide and digital interpretations should be assessed using predefined, clinically appropriate endpoints. A universal >95% concordance threshold should not be assumed across all cytopathology

applications because acceptable performance depends on the diagnostic task and intended use [31,37].

5. Comparative Matrix: WSI and AI Implementation in Cytopathology

Table 2 summarizes the technical, clinical, and operational characteristics of major cytology specimen types when integrated into digital workflows:

Specimen Type	Scanning Mode	Avg File Size	Primary Application	AI	Key Technical Bottleneck
Cervical (LBC)	Pap Single-Plane EDOF	+ 1.5 – 2.5 GB	Automated Dysplasia & Organism Detection	Triage	Cellular overlapping in dense clusters
Cervical (Smear)	Pap Z-Stack layers)	(5-7 8.0 – 15.0 GB	Squamous Cell Atypia & Malignancy Screening		High background debris & variable thickness
Thyroid FNA	Z-Stack layers)	(7-10 12.0 – 20.0 GB	Nuclear Feature Analysis (Grooves, Inclusions)		3D hypercellular microfollicular clusters
Urine Cytology	Single-Plane EDOF	/ 1.0 – 2.0 GB	Urothelial Cell Atypia & Nuclear Quantitation		Low cellularity & degenerative changes
Body Fluids (Effusions)	Single-Plane EDOF	+ 2.0 – 4.0 GB	Mesothelial Adenocarcinoma Discrimination	vs.	Inflammatory background & reactive atypia [6,15,22,25,28,32].

6. Future Directions and Challenges

Despite significant technological advances, several challenges must be addressed before universal implementation can be achieved [27,30]:

Pre-Analytic Standardization: Staining variability across laboratories, differences in coverslip thickness, and liquid-based preparation inconsistencies can impact AI algorithm accuracy [15,32]. Standardized quality control procedures are essential.

LIS Interoperability and DICOM Adoption: Universal adoption of open DICOM standards for digital cytopathology slides is critical to enable seamless communication between image management systems (IMS), AI engines, and electronic health records [27,30].

Cost and Infrastructure: High capital expenditure for multi-plane scanners, robust data storage systems, and cloud computing infrastructure remains a significant barrier for lower-resource healthcare settings [1,30].

Algorithm Interpretability (The "Black Box" Problem): AI models must incorporate explainable AI features such as heatmaps, target overlays, and explicit morphometric measurements, to ensure transparency and maintain clinician trust [28,33].

7. Conclusion

Whole-slide imaging and artificial intelligence have demonstrated substantial technological feasibility and promising research-level performance in cytopathology. Evidence from cervical, thyroid, and urine cytology indicates that AI can improve selected measures of sensitivity, efficiency, reproducibility, or triage; however, performance is heterogeneous and often derived from retrospective or institution-specific datasets. Cytopathology also presents distinctive challenges related to three-dimensional cellular arrangement, depth-of-field, specimen preparation, and image quality. Accordingly, routine clinical implementation should be viewed as an emerging, application-specific process rather than a universal state of clinical readiness. Laboratories considering deployment should undertake intended-use-specific validation, standardize pre-analytic and scanning procedures, ensure LIS/interoperability and data-management capacity, and retain cytopathologist oversight.

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