Application Note

Digital Pathology

Agilent Dako

Evaluating the Robustness and Functionality of an Open, Agnostic, End-to-End Digital Pathology Solution

A proof-of-concept study

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Abstract

This proof-of-concept study aims to substantiate the readiness, reproducibility, and effectiveness of a multimanufacturer, open, agnostic digital pathology solution scaled for use in high-volume pathology laboratories.

Two hundred and one coverslipped glass slides, stained on Agilent Dako Omnis and Agilent Dako CoverStainer, were used to represent typical high-volume workflows. Slides were scanned once daily over three days and processed through Agilent's end-to-end digital pathology solution, with performance metrics and quality control tracked and reported.

The results showed: consistent 40-second scanning times per slide, a 100% success rate, and no scanner errors. The system accurately detected tissue and assigned patient data, with precise AI overlay visualization. The entire process—from scanning to final viewing—was completed within a single workday for all 201 slides. These findings highlight the system's expected efficiency and reliability, even under demanding conditions.

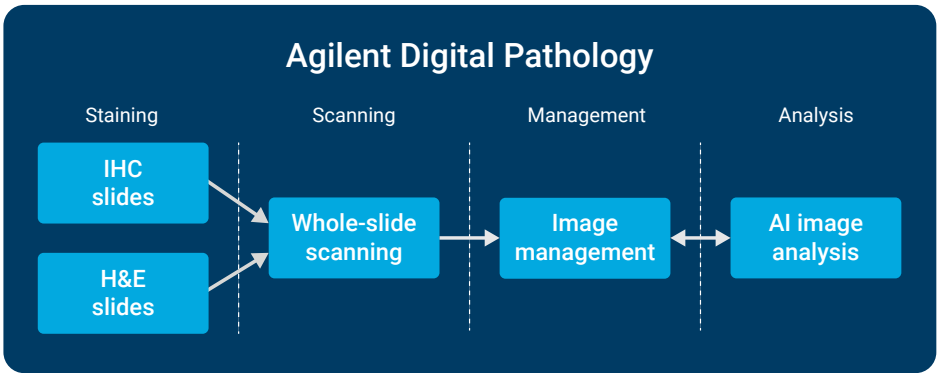

Figure 1. Agilent digital pathology end-to-end workflow from staining to AI image analysis.

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Introduction

The increasing incidence of cancer and the complexity of testing[1,2] are putting significant pressure on pathology laboratories, further worsened by a declining number of pathologists.[3,4] Digital technologies offer solutions to these challenges, transforming pathology into a more efficient, remote-friendly process.

Digital technologies have proven effective in overcoming resource and complexity challenges, and pathology is beginning the transition into a digital process. These technologies not only alleviate common issues and provide better opportunities for remote work but also facilitate faster and easier peer review of cases. Additionally, digital pathology improves efficiency and may help attract new pathologists.[5-9] However, the scalability of end-to-end digital pathology workflows, from staining to AI analysis, still needs thorough investigation. This study examines an open, agnostic digital pathology workflow designed for high-volume laboratories (see Figure 1).

Study

Staining

The study comprised a total of 201 coverslipped glass slides. Of these, 141 slides were stained with IHC protocols on Dako Omnis and 60 were H&E stained on Dako CoverStainer. All slides were coverslipped and labeled with barcodes.

Scanning

Scanning was performed in the Agilent Dako Academy laboratory in Copenhagen using a Hamamatsu NanoZoomer S360MD. All slides were checked for misaligned coverslips and labels.

All slides were of good quality and no additional preparation was required. A single scan profile was used to scan all slides in Auto Mode (40x) with a predefined scanning profile to capture whole-slide images (WSI). All slides were scanned once each day over three days.

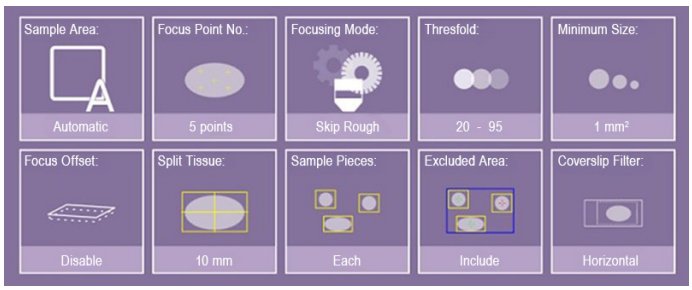


Figure 2. Hamamatsu S360MD parameters used for scanning all slides.

Image management system (IMS)

Anonymized patient profile metadata was generated for each unique barcode, including details such as name, accession date, specimen type, date of birth, case ID, and medical record number.

All images were managed in Proscia's Concentriq AP software, which was set up on an Amazon Web Services (AWS) cloud environment according to standard specifications. A connector was configured to automatically upload slides from the local scanner storage for ingestion into Concentriq AP. Each scanned slide was matched to its corresponding case using the barcode.

AI image analysis

In total, 543 out of 603 WSIs were analyzed with the corresponding Visiopharm AI application. The remaining 60 slides had no corresponding AI application. WSIs were analyzed by the Visiopharm applications HER2, Ki-67, ER/PR, PD-L1, and metastasis detection (H&E). The barcoded metadata determined the specific IHC or H&E protocol for each slide, which was then automatically matched to the proper Visiopharm AI application for analysis. The analyzed slides were automatically returned to Concentriq AP for viewing with embedded AI results.

Repeatability and robustness

To test the repeatability and robustness of the workflow, each step was performed in three runs on the same slides. Functional and timing assessments were conducted in Concentriq AP through the REST API.

Results

Scanning

Across three independent experimental repeats, a total of 603 slides were successfully scanned with a 100% success rate. No slides were mishandled, no scanner errors occurred, and no maintenance was required on the scanner between days.

All tissue was detected on all scans in a fully automated manner, meaning no human interaction was needed to adjust any scan settings during the run.

Table 1. Consistent scanning data from 201 slides scanned on each of the three days.

	Day 1	Day 2	Day 3
Total Scanning Time	2 h 46 min	2 h 45 min	2 h 44 min
Average Scan Time per Slide (sec)	40	40	40
Average Time per Slide, Incl. Loading (sec)	50	49	49
Throughput (slides/hour)	72	73	73
Average Scan Area (mm)	16 x 14	16 x 14	16 x 14
Average Focus Score	96	96	96
Average File Size	0.83 GB	0.83 GB	0.83 GB
Slide Barcodes Scanned (%)	100	100	100

Data captured by the scanner showed a significant linear correlation (r=0.99, p<0.01) between scan area and scan time (Figure 3).

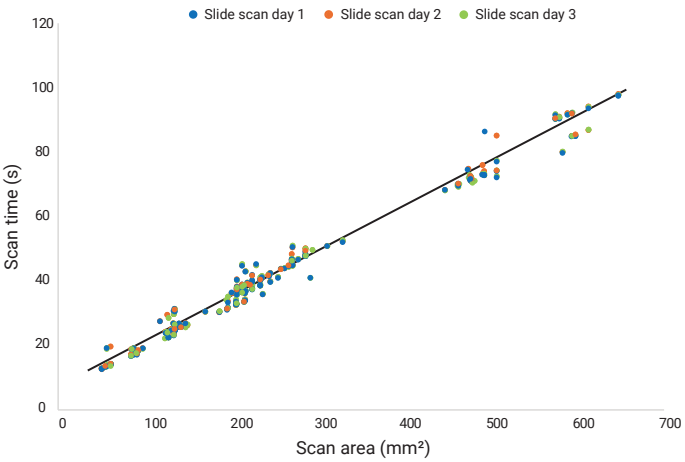


Figure 3. Significant linear correlation between scan area and scan time.

The Hamamatsu scanner repeatedly scanned a slide containing an air bubble with no impact on image quality or focus (Figure 4).

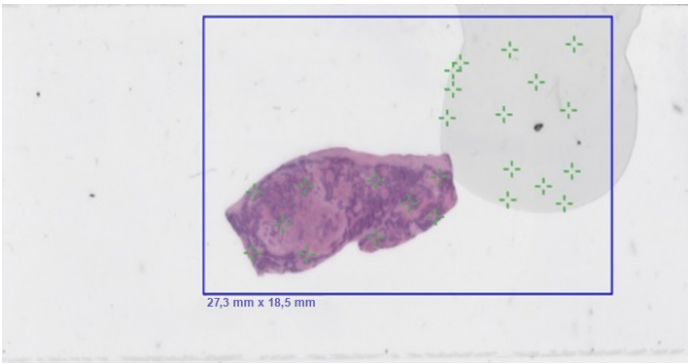


Figure 4. The air bubble (top right) increased the scan area unnecessarily (purple area, lower left), but had no impact on image quality.

The large air bubble underneath the coverslip (top right) increased the number of focus points, which increased the scan area. This observation demonstrates that the scanner can handle imperfect slides; however, high-quality slides improve scanning speed and throughput.

Image management system

Concentriq AP IMS demonstrated robust performance in handling large-scale slide ingestion. All 603 WSIs were seamlessly uploaded from the local scanner and ingested without errors. IT resources were scaled to complete ingestion of one slide every 30 seconds, with an average actual ingestion time of 28 seconds. Following ingestion, WSIs were automatically organized into their respective cases using the barcode data.

AI image analysis

Figure 5. Concentriq AP IMS with Visiopharm PD-L1 TPS results visualized.

WSI data and required metadata were automatically shared (tile-based) from within the Concentriq AP system, and results were presented visually without user interaction. Results included structured text (for example, PD-L1 TPS score) and the cellular overlays created by the Visiopharm software. Out of 543 WSIs triggered for AI processing, 542 (99.8%) were successfully analyzed automatically by the Visiopharm applications.

To investigate the repeatability of scanning and image analysis, a 'technical' and a 'significant' metric for each marker were defined. The technical metric for continuous variables (PD-L1, Ki67 and ER/PR), was defined as a strict 1% change rule. For HER2 and lymph node metastases, the categorical scores (0, 1+, 2+, and 3+), and 1% relative change in the estimated largest metastases, respectively, were used. Significant repeatability considered the specific application guidelines, with change to the scoring category between runs being significant. This metric intends to capture the clinical impact of any variability in the AI analysis between runs. Technical and significant repeatability measurements were

calculated for each marker (see Table 2). For the latter, one PD-L1 case was between 49.3 and 50.5%, hence considered significant around the 50% cut-off (borderline case).

Table 2. The results show the automated image analysis workflow is effective (99.8%) in analyzing test cases. Additionally, it shows strong repeatability (99.9%) on multiple repeated scans of the same samples on both IHC and H&E applications.

App	n (scans)	Repeatability (technical)	Repeatability (significant)
HER2	105	100%	**100%**
Ki-67	102	99%	**100%**
ER/PR	96	100%	**100%**
Metastases Detection (H&E)	60	95%	**100%**
PD-L1	180	97%	**99%**
Overall	543	99%	**99.9%**

Conclusions

Scanner

The Hamamatsu NanoZoomer S360MD scanner performed as intended. A notably high throughput of slides per hour and a 100% success rate in scanning was achieved. This success is largely attributed to the high-quality slide preparation. Tissue detection was precise, with no missed tissue. Even the presence of an air bubble on one slide, did not adversely affect the image quality. This study highlights that high-quality slides allow for optimal scanner operation, positively impacting throughput and reliability.

Transfer and IMS

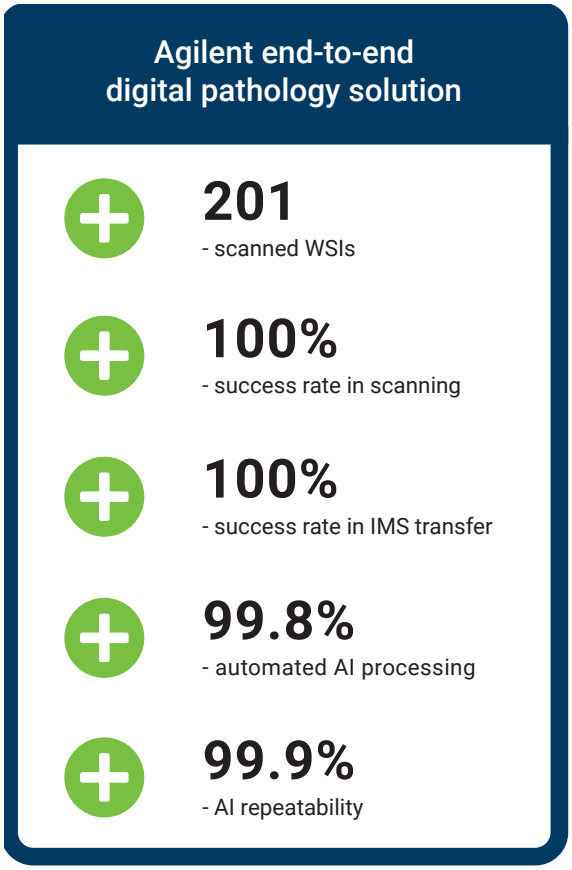
Concentriq AP IMS demonstrated robust performance in handling large-scale slide ingestion. It took an average of 28 seconds to transfer one image from the local scanner to the IMS, with no errors encountered. Slides were automatically and accurately sorted into cases based on the barcode metadata.

AI image analysis

All but one WSIs (99.8%) triggered for AI processing were successfully analyzed automatically by the Visiopharm applications with an average processing time per slide of 4.2 min. In 99.9% of the cases, the AI-assisted scoring was the same, based on significance, on multiple repeated scans of the same samples.

End-to-end digital pathology solution

The study demonstrated that the used setup fits perfectly into high-volume pathology labs staining over 200 slides in a single workday. Based on the set test criteria, we conclude the following was achieved:



This was achieved with minimal human intervention.

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This study is for proof of concept. This Application Note does not imply any clinical functionality.
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