Comparative Evaluation of Automated Coverslipping Film from Multiple Vendors

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Introduction

Automated film coverslipping is a widely adopted technique in high-throughput histopathology laboratories [1]. However, film performance can vary by vendor, affecting slide quality, instrument compatibility, and downstream imaging. This study compared three commercially available coverslipping films to identify optimal solutions for reliability, clarity, and operational efficiency as it pertains to pathology review and whole slide imaging.

Materials & Methods

A total of 300 H&E-stained slides (100 per vendor) were prepared and coverslipped using three different films including Sakura Finetek Tissue-Tek® Coverslipping Film on a Sakura Finetek Tissue-Tek Film® Automated Coverslipper with the following settings; 50mm film length and a Prime Level of 2 . All films were equilibrated 24 hours prior to use.

Slides were visually assessed on day 1 for compatibility, optical clarity, adhesion/sealing, drying time, flatness, and technician usability after coverslipping.

Post-coverslipping imaging was conducted at 20x on a Leica Biosystems Aperio AT2 whole slide scanner at the following time points - 1 day, 2 weeks, and 4 months post. The slides were not cleaned prior to scanning, and the bounding box was manual moved to include the entire coverslipped area. Although all slides were scanned, only the first 50 slides from each vendor were used for image analysis, performed in Indica Labs HALO™ software v4.0, to determine areas that were out of focus, large obstructions such as dust or hair particles, and edge artifacts.

Slides were scanned an additional time after 4 months with the automatic tissue detection (auto-ROI) mode. Image meta data was extracted, and file size and scan time were calculated [2].

Statistical analysis was done using GraphPad Prism version 10.6.0. A simple ANOVA followed by post-hoc tests were used for group comparisons with the significance threshold set to $P < 0.05$.

Results

Visual assessment of the coverslipping film demonstrated that all films performed acceptably for basic coverslipping needs, but differences emerged across evaluations over time. Sakura Finetek Film showed superior flatness with minimal drying issues. Films 1 and 2 exhibited greater surface waviness, especially along the edges of the film. Tension-related artifacts were also present with vendors 1 and 2 with uneven adherence, seen best by an oval area around the tissue appearing more adhered than further away from the tissue. Complete drying time for Sakura Finetek and vendor 2 was approximately 25 minutes; vendor 1 dried faster on the surface but showed persistent xylene pooling, resulting in faint visible lines on the slide.

All films seemed to have similar amounts of dust artifacts, which would not impact pathology review and did not worsen over time. The most common artifact was an edge artifact that appeared as air infiltration that worsened over time, particularly for vendors 1 and 2 (Figure 1). All vendors had comparable artifact areas 1 day post coverslipping, and all vendors saw a significant increase in artifact area 2 weeks post coverslipping. In addition, vendors 1 and 2 had significantly more artifact area than the Sakura Finetek Coverslipping Film at 2 weeks. At 4 months, the Sakura Finetek Tissue-Tek® Coverslipping Film did not change significantly in artifact area; however, both vendor 1 and vendor 2 saw another significant increase, again significantly greater than Sakura Finetek.

Sakura Finetek Tissue-Tek® Coverslipping Film had significantly less edge artifact than the other vendors, which had a notable impact when using auto-ROI. The auto-ROI included edge artifacts (Figure 2), and using metadata from the last scan, showed that this significantly increased the average scan time and file size (Figure 3).

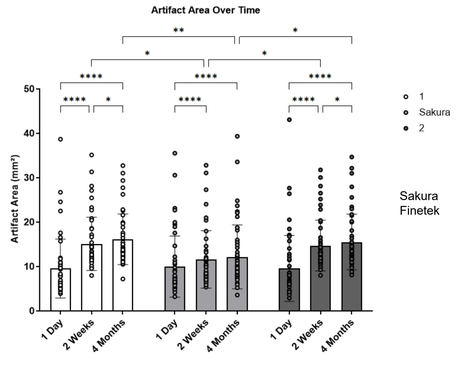

Figure 1. Each bar shows the total artifact area (mm²) for each slide (shown by each dot) for all three vendors. The horizontal lines indicate significant differences (* = $P \leq 0.05$, ** = $P \leq 0.01$, **** = $P \leq 0.0001$).
No significant differences were observed between vendors on day 1; however, differences appeared within groups and between groups by 2 weeks and 4 months, with Sakura Finetek appearing the most stable.

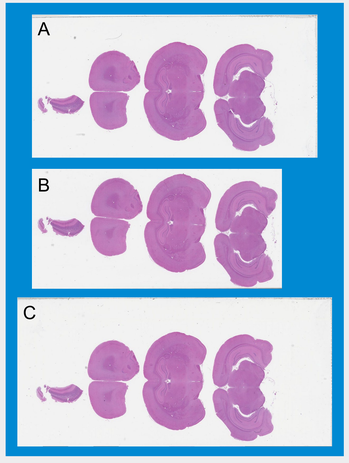

Figure 2. Scanning area for slide 15 with auto-selected region for all three vendors: vendor 1 (A), Sakura Finetek (B), and vendor 2 (C). All images were set to 0.25 magnification and the whole slide area was included in the picture. This demonstrates how edge artifacts increase scanning area and time.

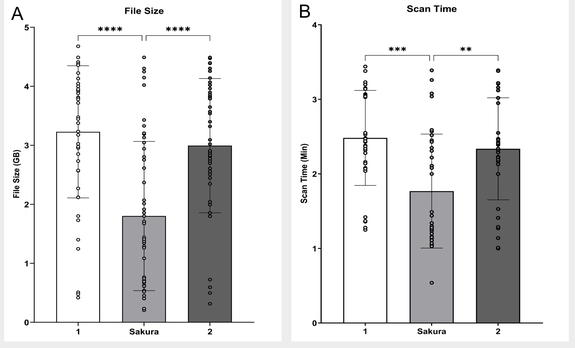

Figure 3. Graph A shows the average file size (GB) of each scanned slide after 4 months, with each dot representing a slide when using the auto-ROI mode. Graph B shows the average scan time (min) with each dot showing an individual slide. Sakura had significantly smaller file sizes and scan time due to less edge artifacts developing over time.

Conclusions

Among the three vendors evaluated, Sakura Finetek's Tissue-Tek® Coverslipping Film demonstrated the best overall performance across key parameters, particularly over time, making it a preferred choice for high-quality, reproducible slide preparation.

All films were adequate for pathology review; however, Sakura Finetek's Tissue-Tek® Film appeared to be best suited for digital pathology applications, especially if slides cannot be scanned immediately. Vendors 1 and 2 offer functional alternatives but may present limitations in flatness and archival integrity for high-resolution imaging workflows. This is especially true when using the auto-ROI as it will include edge artifacts within the scan region and result in larger file sizes and longer scan times.

For high volume laboratories, it is not feasible to clean and set the ROI for each slide. Artifacts, particularly edge artifacts, may have a large impact on total scan time and file size, which could impact overall workflow speed and storage capacity.

Since the data for scan time and file size was collected 4 months later, there may be minimal to no impact for laboratories scanning slides immediately after staining, but this would need to be confirmed with additional testing.

References

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