No difference in scan speed and stain quality comparing Film vs. glass coverslipped slides

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Introduction

Currently, there are two materials used to coverslip slides: Film and glass. Film is a xylene-activated adhesive tape while glass is a thin piece of glass that adheres to the slide using mounting medium.

The use of digital microscopy and Whole Slide Imaging (WSI) is accelerating rapidly. WSI scans the entire slide and creates a single high-resolution digital file, by taking a large number of small high-resolution images of the entire slide, then arranges the images into a picture of the slide. Digital images can be easily shared and analyzed quickly.

It is important to know if there is a difference in scanning speed and stain quality captured by imaging, when coverslipping slides with Film or glass.

Materials & Methods

This study included sixteen (16) microscopic slides prepared from archived formalin-fixed, paraffin-embedded, (FFPE), animal and human tissue blocks.

The blocks retrieved have porcine tissues (skin, kidney, gastrointestinal and lung) and human uterus. The blocks were used for sectioning, Hematoxylin and Eosin (H&E) staining followed by either glass or Film coverslipping.

All of the samples were processed on the Tissue-Tek VIP® 6 AI (Sakura Finetek USA) and were manually embedded on the Tissue-Tek® TEC™ 5 (Sakura Finetek USA) using the Tissue-Tek® Paraform® Processing/Embedding Medium (Sakura Finetek USA).

Twenty slides were prepared from each block using a new blade; sections of approximately 1 x 1 cm were cut at 4 microns using the Tissue-Tek AutoSection® Automated Microtome (Sakura Finetek USA).

Ribbons consisting of six (6) sections were collected from each block. Sections #2 through #5 were each placed on a Superfrost™ Plus slide (Thermo Fisher Scientific) and identified accordingly. Sections #1 and #6 were discarded to ensure that serial sections were consistent.

Materials & Methods Cont.

All slides were stained on the Tissue-Tek Prisma® *Plus* Automated Slide Stainer (Sakura Finetek USA) using the Tissue-Tek Prisma® H&E Stain Kit #1 (Sakura Finetek USA). Sections #2 and #4 were coverslipped using the Tissue-Tek Film® Coverslipper (Sakura Finetek USA) (xylene dispense level = 15) and Tissue-Tek® Coverslipping Film (Sakura Finetek USA), sections #3 and #5 were coverslipped using the Tissue-Tek® Glas™ *g2* Coverslipper (Sakura Finetek USA) (speed = 5, dispensing volume = 50), Tissue-Tek® Coverslips (Sakura Finetek USA) and Tissue-Tek® Mounting Medium (Sakura Finetek USA). Xylene from the same lot number was used for both coverslippers.

The fourteen (14) remaining slides from each block were kept for future reference.

Both sets of slides were checked for misalignment of coverslip, presence of large or small air bubbles or excess mounting medium. All coverslipped slides were allowed to dry at ambient temperature.

All slides were scanned using the Pannoramic® Scan 250 Flash III (3DHisTech) at 40x, and scan times and related data were collected. The resulting whole slide images were analyzed using the ImageDx™ Quantitative Image Analysis software (Reveal Biosciences). Digital stain isolation was performed by deconvolution to separate images into constituent hematoxylin and eosin stains. Average staining intensities were determined in nuclear (hematoxylin and eosin) and non-nuclear (eosin) tissue regions. The entire tissue area was analyzed and background whitespace was excluded from the analysis. Data was evaluated for differences using multi-factor ANOVA in the R software (The R Foundation).

Conclusions

For all of the parameters assessed, the observed differences between Film and glass were small in magnitude and considered not relevant. However, though small, the shorter scan time with Film may bring a cumulative benefit in throughput for high volume settings.

Results

For all of the parameters assessed, the observed differences between Film and glass were small in magnitude and considered not relevant. Scan time varied with tissue type ($p<0.001$) to accommodate different tissue sizes and on average was 13 seconds per slide or 3.7% shorter for Film ($p<0.001$) (Figure 1A). Average staining intensities in nuclear (hematoxylin and eosin) and non-nuclear (eosin) tissue regions also varied with tissue type ($p<0.001$), reflective of tissue-specific staining patterns (Figure 1B-D). Average hematoxylin intensity did not vary between Film and glass ($p=0.53$) (Figure 1B), while minimal differences were detected in nuclear eosin intensity (0.82% lower for Film, $p<0.01$, Figure 1C) and non-nuclear eosin intensity (0.08% higher for Film, $p=0.08$, Figure 1D).

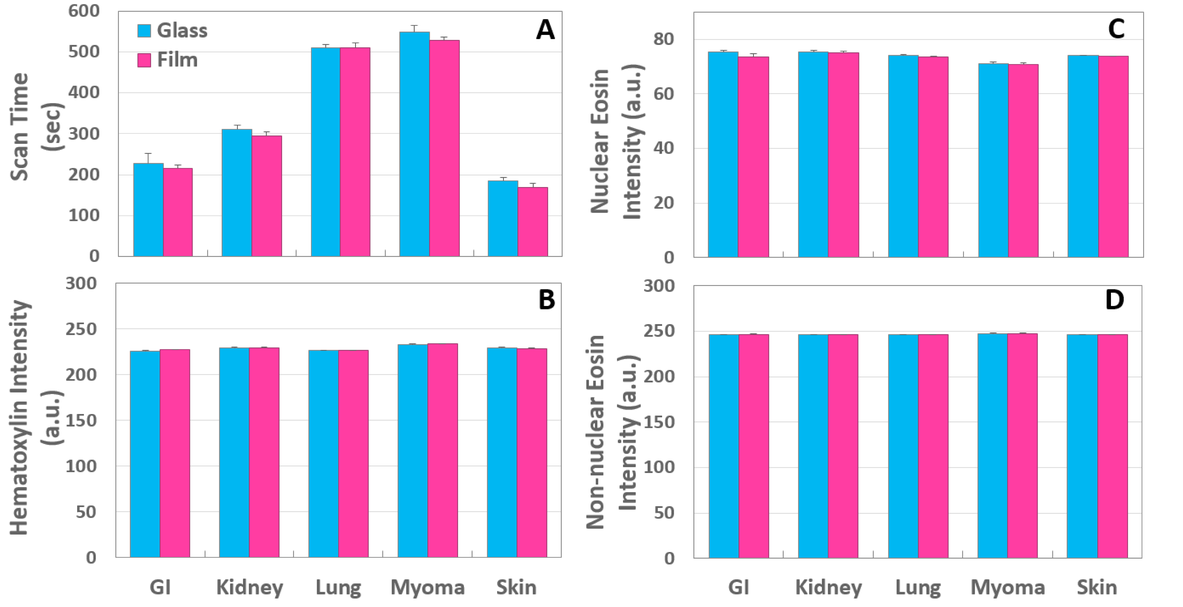

Figure 1: Scan time (A) and average staining intensities for nuclear hematoxylin (B) and eosin (C) and non-nuclear eosin (D) for slides coverslipped with Film and glass and scanned using the Pannoramic Scan 250 Flash III. Data presented as average ± standard deviation.

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