

Image analysis found no statistically significant differences in image quality of tissue sections on slides stained by H&E and coverslipped with Film and glass.

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Introduction

Imaging and image analysis of tissue sections have both substantially increased over the past years. This study compared two distinct coverslipping methods in terms of the most important image-quality parameters of tissue slides scanned at true 40x: brightness, sharpness, and contrast. This study does not promote any in-vitro diagnostic use of imaging and image analysis.

Materials & Methods

350 serial sections from an FFPE tissue microarray consisting of normal skin, normal colon, colorectal carcinoma, normal liver, oncocytoma and normal spleen were stained with H&E using one staining system solution including a Tissue-Tek Prisma Plus Stainer and a Tissue-Tek Prisma H&E Stain Kit #1 (Sakura Finetek USA). 66 were randomly selected where 33 slides were coverslipped with a Tissue-Tek Film Coverslipper and Tissue-Tek Coverslipping Film (Film), and 33 slides were coverslipped with a Tissue-Tek Glas g2 Coverslipper. All 66 slides were scanned using an Olympus SLIDEVIEW VS200 research scanner (Evident) at 40x with a UPLXAPO40X objective (NA 0.95: 0.137 $\mu\text{m}/\text{pixel}$). The two distinct sets of 33 slides each were analyzed using the imageDx ColorQC and Focus QC modules (Reveal Biosciences) first by identifying tissue (foreground) and background regions in the slides, and then by extracting a total of 9916 and 8949 foreground tiles from the film-coverslipped and glass-coverslipped slides, respectively. From each set of the foreground tiles, every tile's mean and standard deviation of brightness of pixels, as well as the mean of the Laplacian-filter responses, were aggregated in the corresponding histograms of brightness, contrast, and sharpness. Also, every tile's standard deviation of pixel brightness values was aggregated in the histogram of contrast. The resulting three pairs of histograms for the three image-quality features of the Film- and glass-coverslipped slides were compared by using two standard statistical tests: a chi-squared distance and the null-hypothesis paired t-test.

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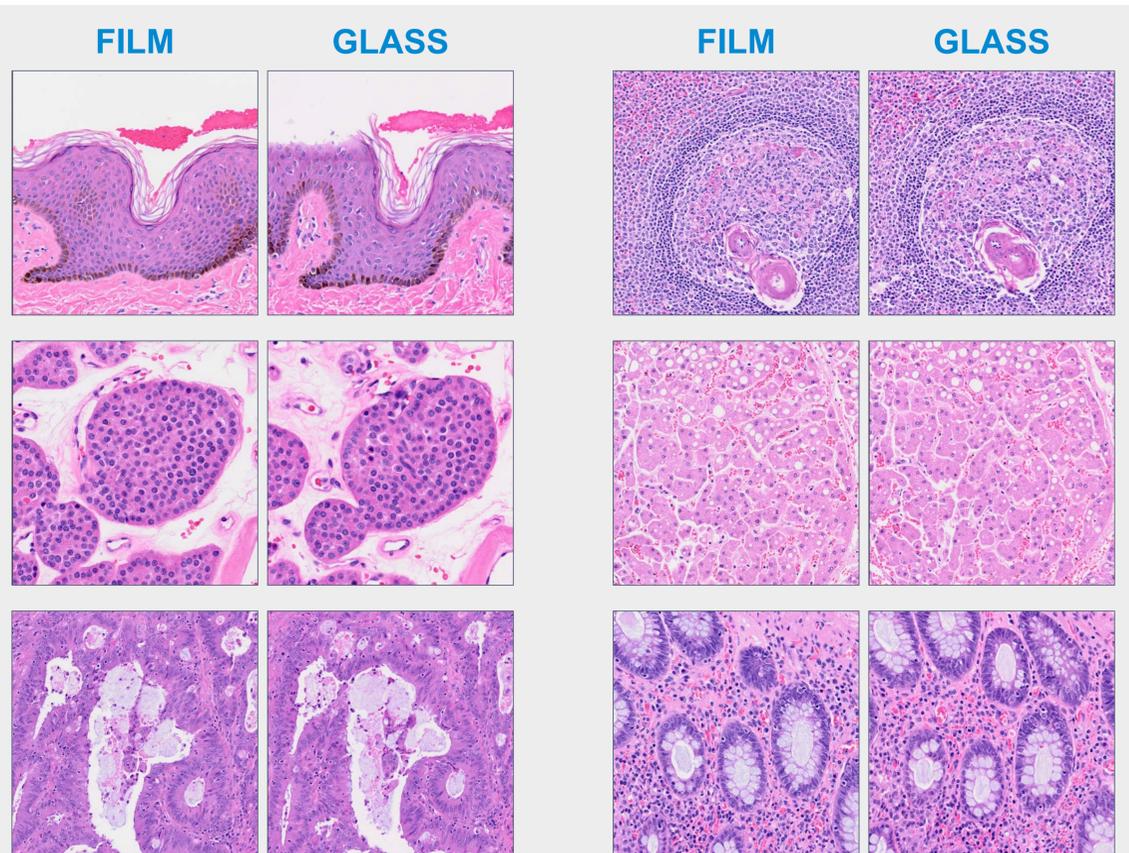


Figure 1. Side-by-side comparison of digital images scanned from slides coverslipped with film vs. glass for the following tissue types: skin (top left), spleen (top right), oncocytoma (middle left), liver (middle right), colon carcinoma (bottom left), and colon (bottom right).

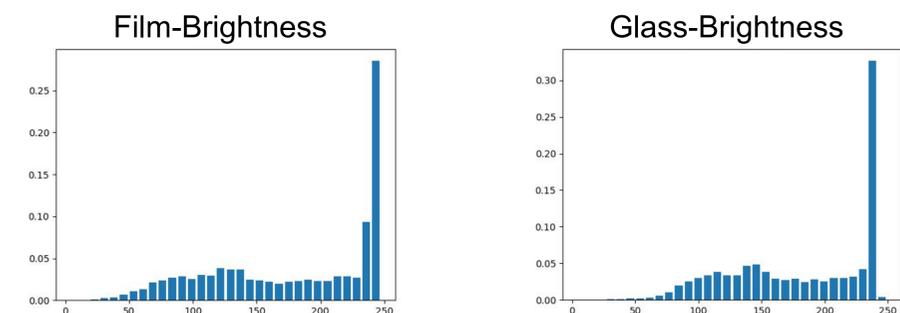
Conclusion

No statistically significant differences in brightness, sharpness, and contrast between Film- and Glass-coverslipped slides were found.

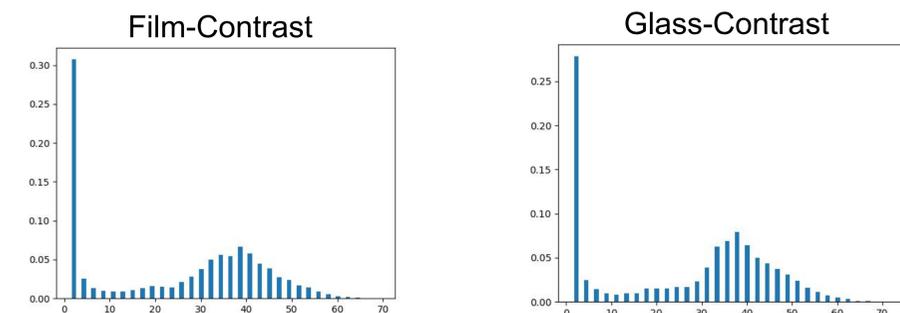
Acknowledgements

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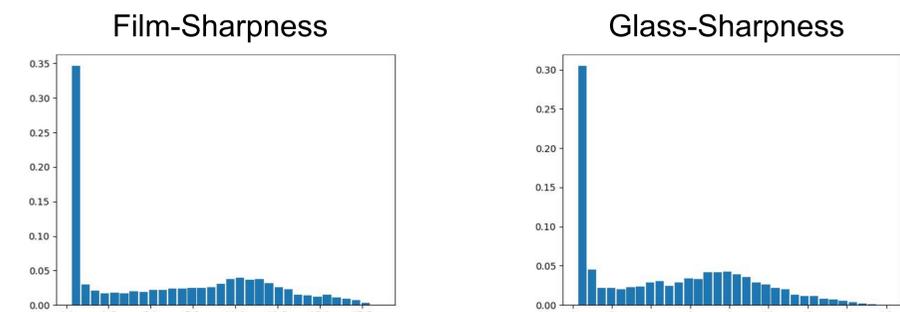
Histograms of Image-Quality Features from the Film and Glass Image Sets



- Alternative Chi² distance (Chi²=0.92) showed very low brightness distribution differences
- Two sample t-test (P-Value = 1.0) showed no statistically significant difference in brightness between the two groups (Film vs glass)



- Alternative Chi² distance (Chi²=0.03) showed very low contrast distribution differences
- Two sample t-test (P-Value = 1.0) showed no statistically significant difference in contrast between the two groups (Film vs glass)



- Alternative Chi² distance (Chi²=0.08) showed very low sharpness distribution differences
- Two sample t-test (P-Value = 1.0) showed no statistically significant difference in sharpness between the two groups (Film vs glass)

