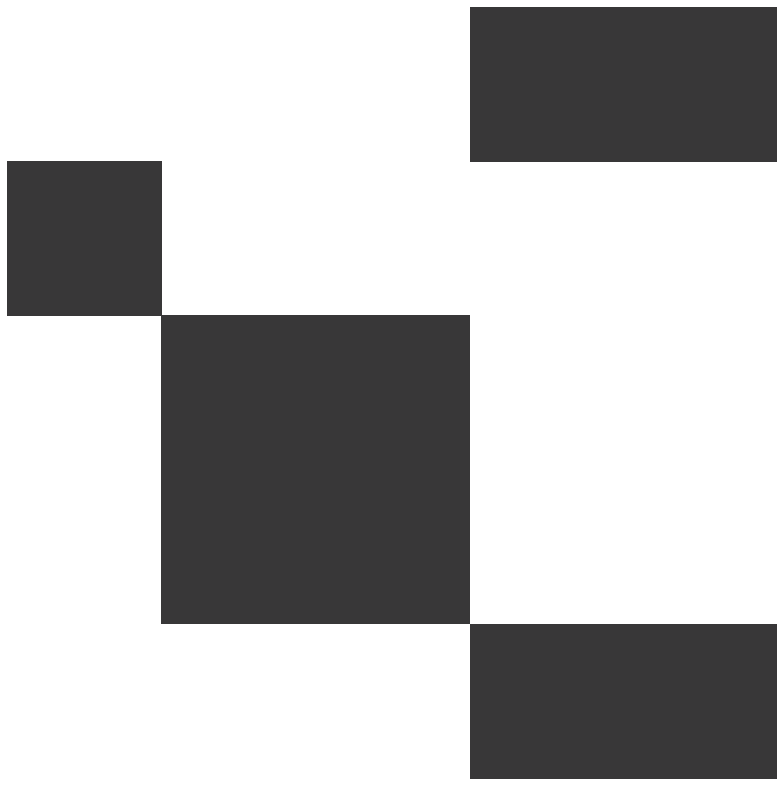


Seeing the Whole Picture: A Comparative Analysis of Digital Pathology Whole Slide Scanners

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CellCarta

INTRODUCTION

Digital pathology is gaining popularity in the field of pathology due to various reasons, including technological advancements, advanced brightfield, immunofluorescent multiplexing, the ability to perform special studies, and the ability to apply AI-driven algorithms to provide advanced output that has never been seen before. Another critical driver towards digital pathology is the issue of inter and intra-pathologist variation. Digitizing pathology images can help standardize readings and reduce subjective differences between pathologists or even the same pathologist at different times.

Despite the potential benefits of digital pathology, there lacks a standard whole slide image (WSI) system in the market. This lack of standardization can lead to high variation in color and quality between different WSI images, which raises questions about whether these images accurately represent what Pathologist can see directly under the microscope or the ground truth.

To address this issue, we propose to submit a poster that compares and contrasts the most common WSI imaging equipment and files in the market. The poster will provide an overview of each WSI format's key features and characteristics. This includes resolution, compression, color accuracy, and compatibility with different software and hardware platforms. Additionally, we will evaluate the impact of each WSI format on image quality, color accuracy, and reproducibility using a standardized set of pathological samples.

The poster aims to identify each WSI format's strengths and weaknesses and guide pathologists and researchers in selecting the most appropriate WSI format for their specific needs. This will also enable vendors and manufacturers to improve their WSI technology by addressing the limitations and challenges associated with each format.

By comparing and contrasting different WSI formats, our poster aims to contribute to the ongoing efforts to improve standardization in digital pathology. By providing guidance to pathologists and researchers in selecting the most appropriate WSI format, our poster aims to differentiate key features among leading scanners and their profile formats.

In conclusion, digital pathology offers many advantages in pathology, including the potential to reduce inter and intra-pathologist variation and advance analysis and outcome predictions. However, the need for a standard WSI system in the market is a significant challenge that needs to be addressed. Our poster aims to contribute to the ongoing efforts to improve standardization in digital pathology by comparing and contrasting the most common WSI files in the market and providing guidance to pathologists and researchers in selecting the most appropriate WSI format.

METHODOLOGY

In this study, a total of 10 formalin-fixed paraffin-embedded (FFPE) human tissue slides were utilized to accurately represent the prevalent color schemes employed in immunohistochemistry (IHC) staining procedures.

All slides Counterstained with hematoxylin to visualize nuclei. Analytes and their corresponding clone and chromogen listed in table 1.

No of slides	Analyte	Clone	Chromogen
2	CD3	mouse clone LN10	Diaminobenzidine (DAB)
2	PD-L1	mouse clone 22C3	DAB
3	GrB + Nkp46	mouse clone GrB-7 rabbit clone EPR22403-57	Vina Green Vulcan fast red
3	FoxP3 + CD3 + CD4	mouse clone 236A/E7 mouse clone LN10 rabbit clone EPR6855	Vulcan Red HIGHDEF® Yellow HIGHDEF® Blue

Table 1.

Each slide was subjected to scanning using distinct commercially available whole slide scanners, encompassing both 20x and 40x magnifications. These scanners are Leica AT2, Leica AT turbo, Leica GT450, Roche DP200, Roche DP600, and 3DHistech P250. The scanned slides underwent meticulous quality control (QC) to ensure the selection of the most precisely focused scans for inclusion in the study.

For slides scanned with the DP600, the Bif and Tif file formats were utilized, while the P250 scanner captured slides using both sRGB and cLIN color profiles.

Our board-certified Pathologists conducted a visual assessment of all images. Additionally, a quantitative color evaluation was conducted, relying on grey value data per pixel along a profile line. This analytical task was executed through the use of the ZEISS Zen lite software.

Region of interest (ROI) defined in each image to accurately portray relevant colors within each assay. A profile line was carefully drawn on each ROI, and the resulting graphs displayed the distribution of grey values along this line. The grey value represents the average pixel intensity derived from the red, green, and blue channels. Furthermore, the ICC (Intraclass Correlation Coefficient) value was calculated for various channels, based on one profile line per assay/scan profile combination. ICC value calculated for the different channels for one profile line per assay/scan profile combination.

ICC < 0.5 poor reliability
ICC between 0.5 and 0.75 moderate reliability
ICC between 0.75 to 0.9 good reliability
ICC > 0.9 excellent reliability1

Utilized a uniform computer system and display setup for all images. Discrepancies in focus levels may lead to variations in Y-values and peak sharpness. The manual placement of profile lines on the images could introduce minor discrepancies in peak positions along the X-axis in the graphs. Additionally, there may be variability in pixel size among different scanners.

Indica Lab's HALO 3.4 is employed for the generation of algorithms aimed at detecting these analytes. In parallel, HALO 3.4 is utilized to construct classifiers, which serve the purpose of categorizing objects and regions.

RESULTS

For the sake of simplicity in this poster, we have included only a select number of images and limited comparison data from Figure 1 to Figure 6.

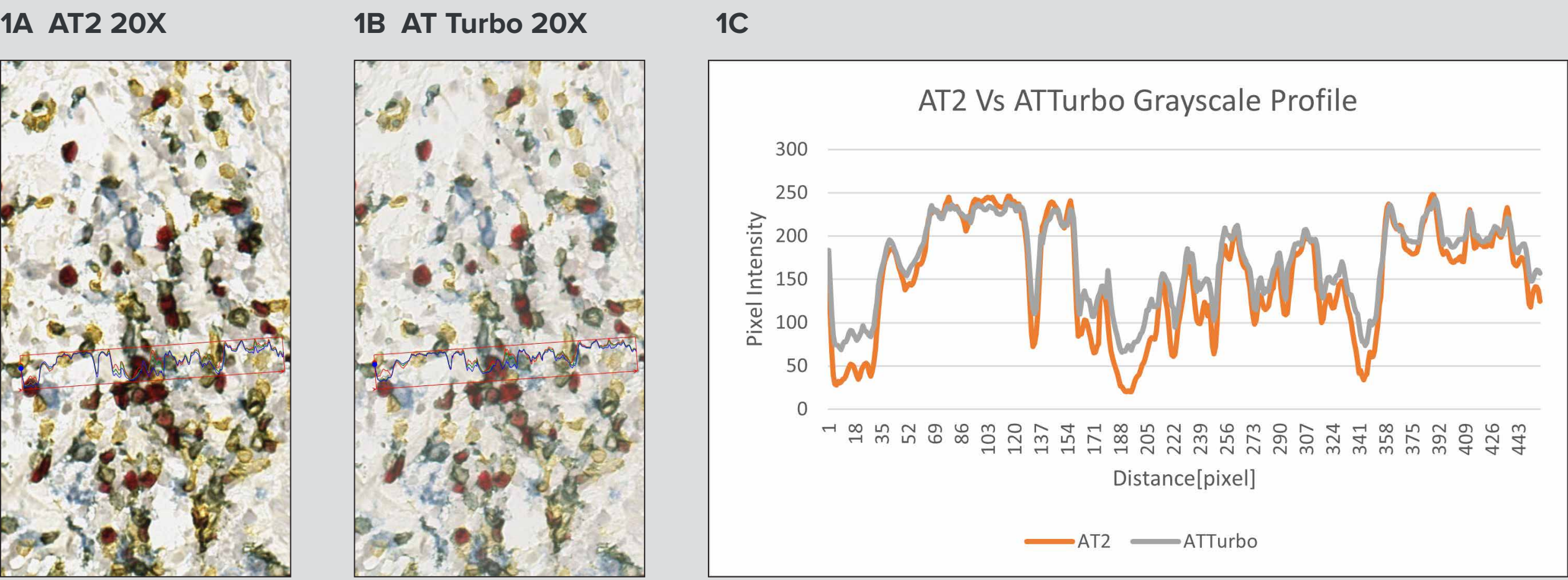


Figure 1. Comparative assessment of Images between AT2 and AT Turbo Scanners. (A) Illustrates a chosen ROI captured at 20x magnification using the AT turbo scanner, featuring a profile line illustrating RGB values against intensity. (B) Demonstrate identical ROI was captured at 20x magnification using the AT2 scanner, showing a profile line depicting RGB values against intensity. (C) The profile lines of both images were subsequently transposed into a scatter chart, utilizing color intensities from the grayscale. In this representation, the x-axis corresponds to the length of the profile line in pixels, while the y-axis signifies color intensity. D) Color channels with calculated ICC values and concordance between two scanner images.

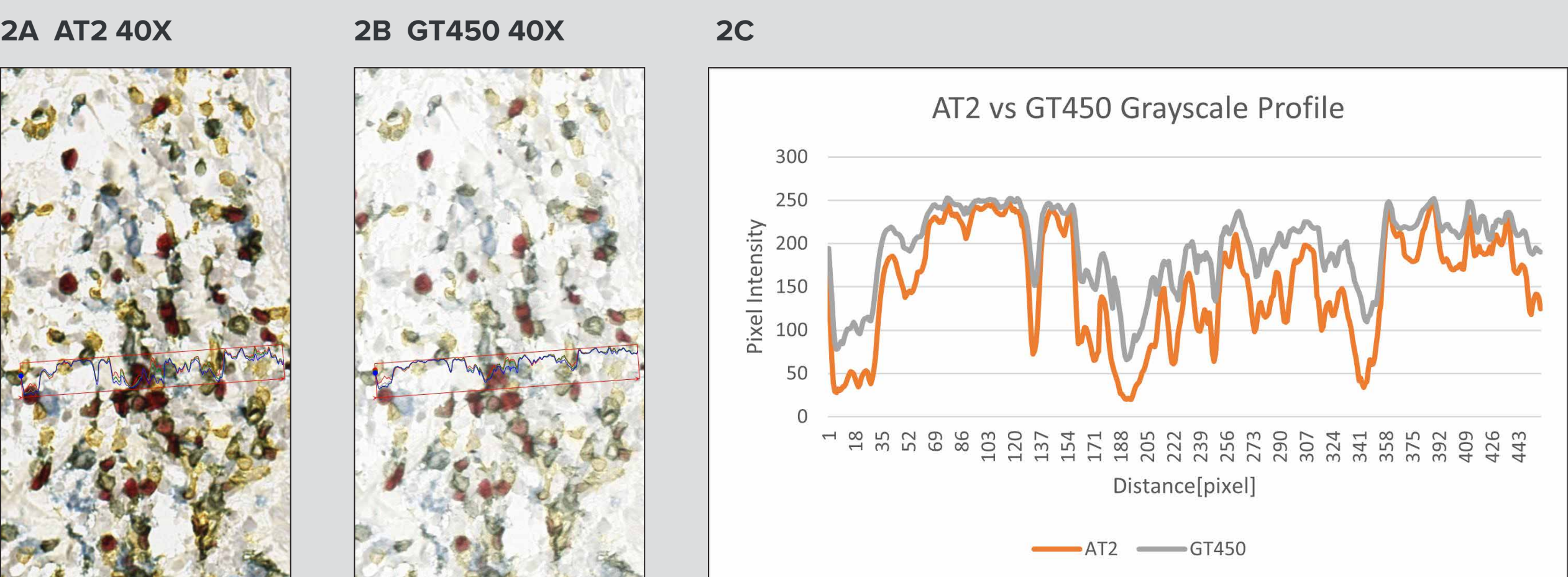


Figure 2. Comparative assessment of Images between AT2 and GT450 Scanners. (A) Illustrates a chosen ROI captured at 40x magnification using the AT2 scanner, featuring a profile line illustrating RGB values against intensity. (B) Demonstrate identical ROI was captured at 40x magnification using the GT450 scanner, showing a profile line depicting RGB values against intensity. (C) The profile lines of both images were subsequently transposed into a scatter chart, utilizing color intensities from the grayscale. In this representation, the x-axis corresponds to the length of the profile line in pixels, while the y-axis signifies color intensity. D) Color channels with calculated ICC values and concordance between two scanner images.

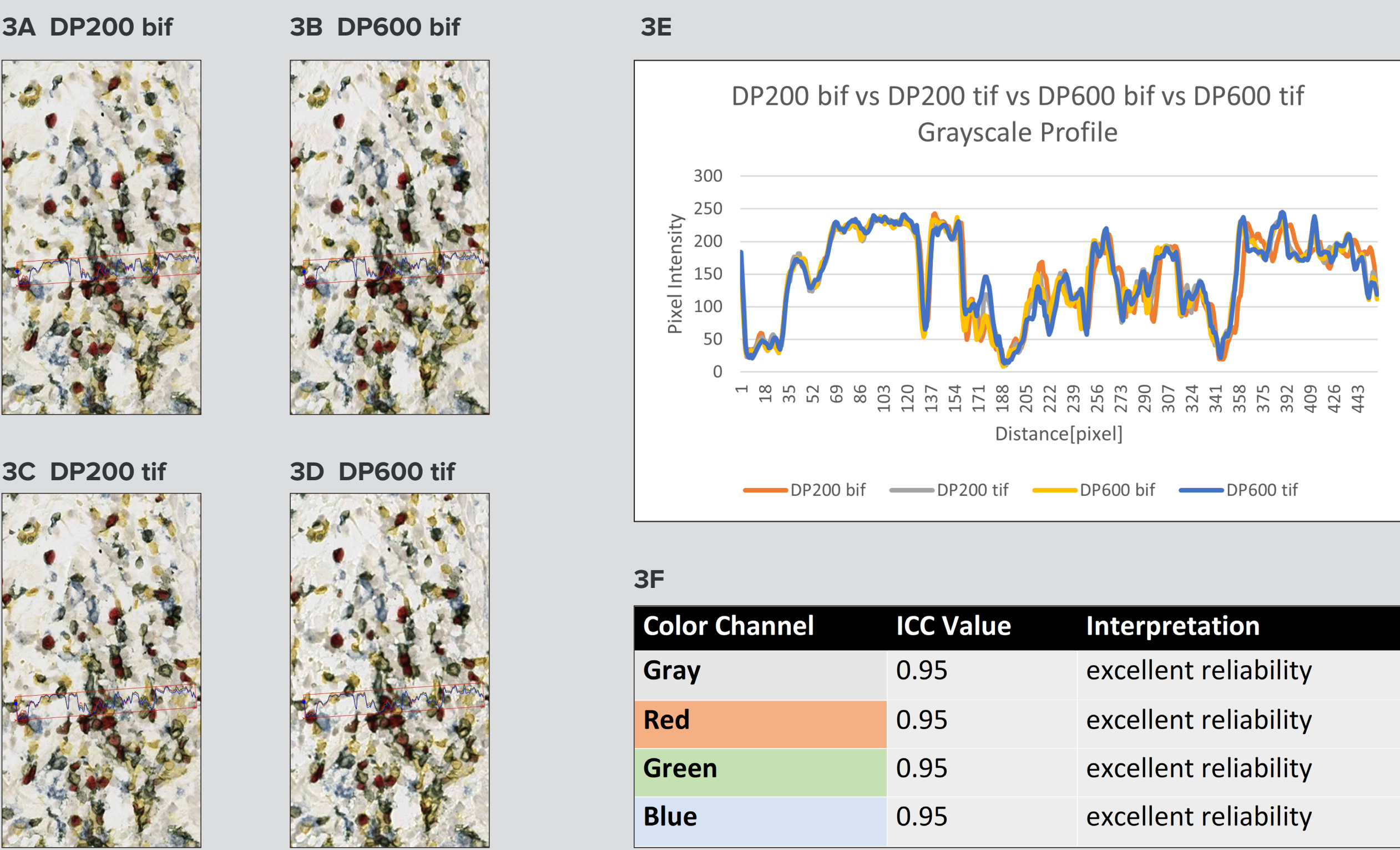


Figure 3. Comparative assessment of Images between DP200 and DP600 Scanners with both bif and tif file formats. The following images showcase selected regions of interest (ROI) captured at 20x magnification using both the DP200 and DP600 scanners, with two different file formats (bif and tif). Each image includes a profile line that highlights the RGB values in relation to intensity (A)DP200 with bif file format. (B) DP600 with bif file format. (C) DP200 with tif file format. (D) DP600 with tif file format. (E) The profile lines of all 4 images were subsequently transposed into a scatter chart, utilizing color intensities from the grayscale. In this representation, the x-axis corresponds to the length of the profile line in pixels, while the y-axis signifies color intensity. D) Color channels with calculated ICC values and concordance between two scanner images.

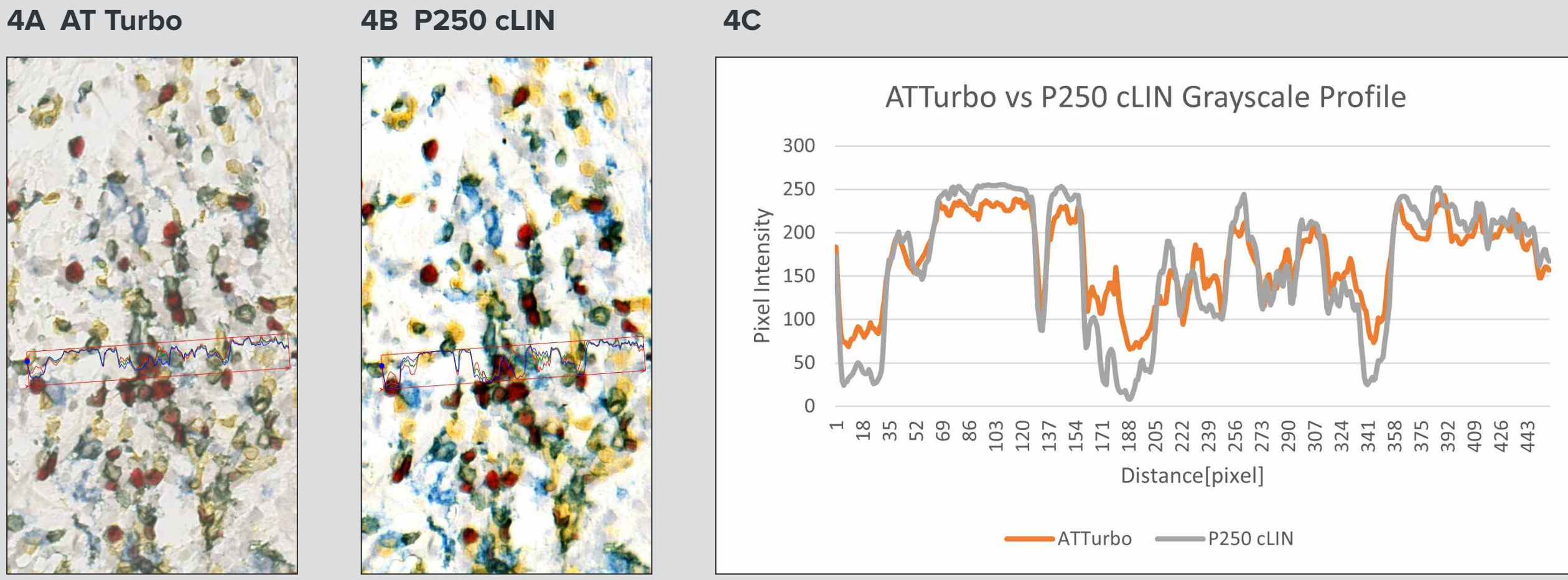


Figure 4. Comparative assessment of Images between AT Turbo and P250 Scanner with cLIN color profile. (A) Illustrates a chosen ROI captured at 20x magnification using the AT Turbo scanner, featuring a profile line illustrating RGB values against intensity. (B) Demonstrate identical ROI was captured at 20x magnification using the P250 scanner with cLIN profile, showing a profile line depicting RGB values against intensity. (C) The profile lines of both images were subsequently transposed into a scatter chart, utilizing color intensities from the grayscale. In this representation, the x-axis corresponds to the length of the profile line in pixels, while the y-axis signifies color intensity. D) Color channels with calculated ICC values and concordance between two scanner images.

We harnessed Indica Lab's HALO 3.4 to formulate an algorithm dedicated to the detection of PD-L1 (mouse clone 22C3) positivity. In this assay PD-L1 stained with DAP and nuclei counterstained with hematoxylin. We exclusively employed AT2-scanned images at 20x magnification to construct an AI driven algorithm and classifier for object and region categorization. Demonstrating the algorithm's unwavering consistency, we applied it to the same image in five separate instances, consistently yielding 100% equivalent results as evidence of its reliability. Our Pathologist agreed upon its detection percentages and the classifiers performance for accuracy.

Subsequently, we broadened the utilization of these algorithms to encompass images scanned at various magnifications using different scanners showcased in Figure 6.

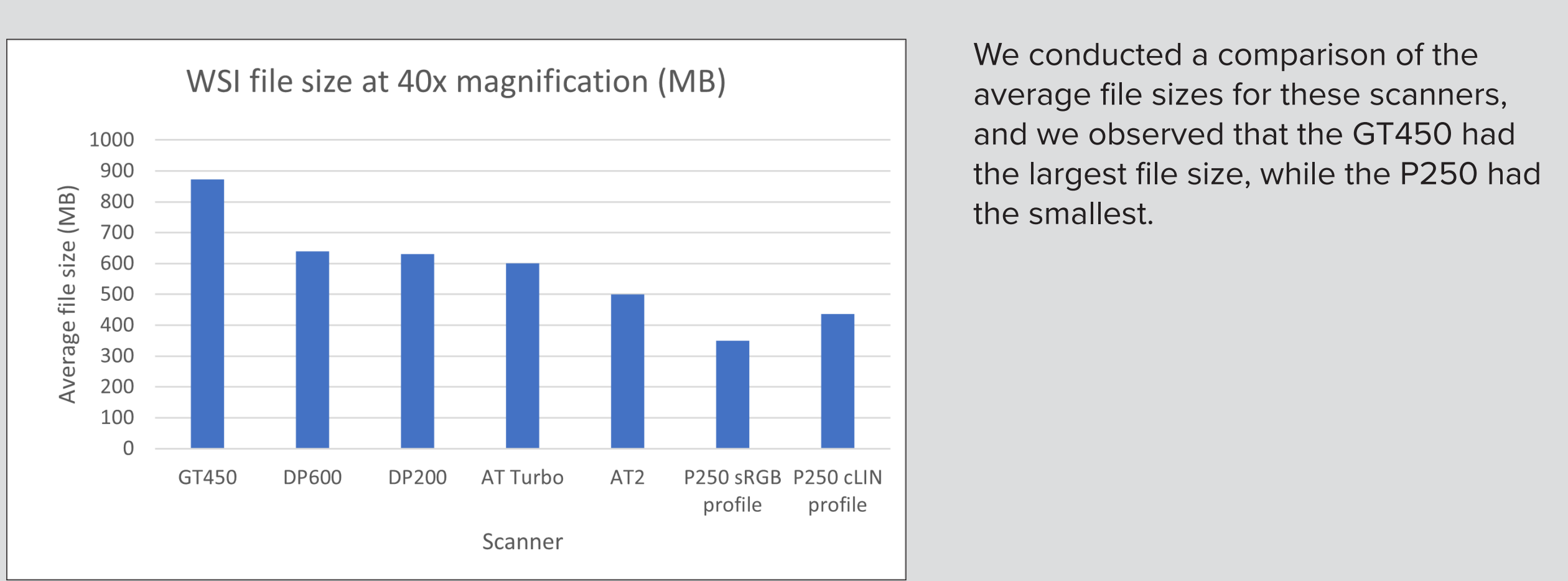
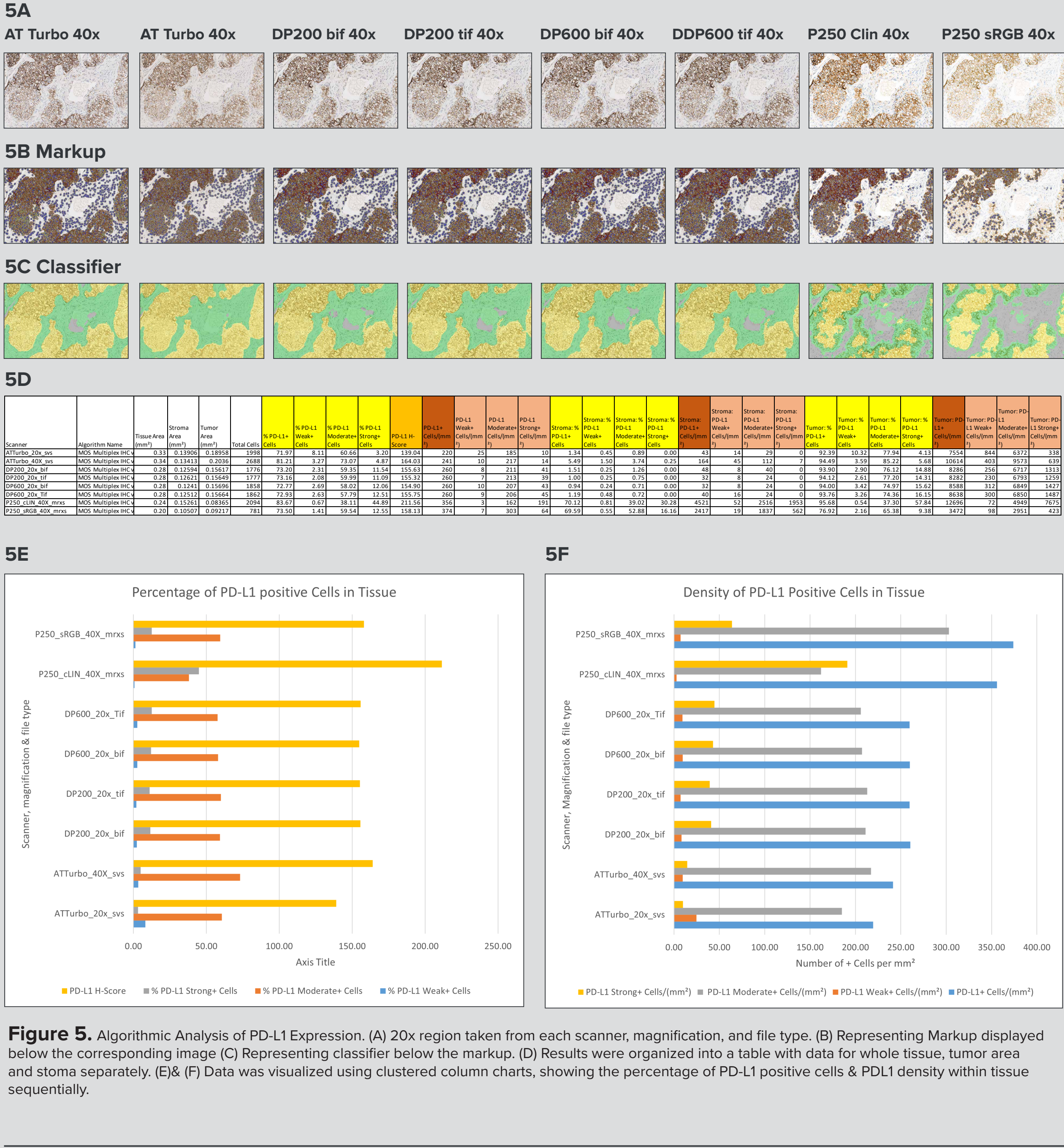


Figure 6. Average file size with different scanners.

DISCUSSION

This study undertakes a critical exploration of a burgeoning field in medical science - digital pathology whole slide scanners (WSI). As the demand for digital pathology continues to rise, the need for standardized WSI systems becomes increasingly evident.

Key Findings and Implications:

Variability in Scanner Performance: This study showcasing variations in image quality, color accuracy, and reliability. Notably, certain scanners performed better in terms of reliability for specific color channels, while others demonstrated poor performance. Some scanners showed poor reproducibility. This highlights the importance of selecting a scanner that aligns with the specific needs of pathologists and researchers.

Algorithmic Analysis: The inclusion of artificial intelligence algorithms to detect specific markers like PD-L1 showcases the potential for automation and objective assessment in digital pathology. This finding suggests that the integration of AI into pathology workflows can enhance the efficiency and accuracy of diagnoses.

Recommendations for Best Practices: The study rightly emphasizes the need for pathologists and institutions to assess image quality and consult with experts when choosing a WSI scanner. The suggestion to develop and validate algorithms tailored to specific scanners is a critical recommendation, as it ensures that the transition between scanners does not compromise data integrity. This is particularly relevant for institutions planning to upgrade or expand their digital pathology capabilities.

Universal Hardware: The call for universal hardware for digital pathology handling is a practical recommendation. Standardizing hardware can simplify processes and improve compatibility across different systems, reducing the potential for errors or inconsistencies in digital pathology workflows.

Future Directions:

While this study provides valuable insights into the performance of various WSI scanners, there are potential avenues for further research:

Expanded Sample Cohort: As mentioned in the conclusion, conducting studies with a larger cohort of samples and a broader range of WSI scanners could further validate and refine the findings.

Longitudinal Studies: Investigating the performance of WSI scanners over time, considering factors like maintenance, calibration, and software updates, could shed light on the durability and reliability of these systems.

AI Integration: Expanding research into the integration of AI algorithms with different WSI scanners could provide insights into the adaptability of AI solutions across various hardware platforms

Standardization Efforts: Collaboration with industry stakeholders and organizations involved in digital pathology to establish standardized WSI formats and quality control measures could contribute to resolving the standardization challenges highlighted in the Study.

CONCLUSION

- The output from scanners varies in terms of file types and image quality. Each facility should assess image quality and consult with a pathologist to select the most suitable scanner to meet their specific needs.
- It is crucial that algorithms are developed for specific scanners and not interchangeably used. If there is a need to switch from one scanner to another, a validation study must be conducted to demonstrate that Scanner A with algorithm A produces statistically similar results to Scanner B with algorithm B. Among the scanners used in this study, only DP200 and DP600 can be interchangeably used, as they produce identical results if you lock the file type.
- The use of universal hardware for handling digital pathology is recommended.
- Further studies with a larger cohort of samples and WSI scanners are needed for comparison.

REFERENCES

- Koo, T. K., & Li, M. Y. (2016). A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. Journal of Chiropractic Medicine, 15(2), 155-163. <https://doi.org/https://doi.org/10.1016/j.jcm.2016.02.012>

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