Short Report

Evaluation of an Automated Tissue Sectioning Machine for Digital Pathology

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Abstract

Background: With the increasing workload in histology laboratories, a fully automated and digital workflow will be important for anatomic pathology in the future. While automated methods for tissue processing, embedding, staining, cover-slipping, and digitization have been achieved, tissue sectioning appears to be the biggest obstacle to a fully automated histology process. The aim of this study was to investigate an automated tissue sectioning machine for both clinical and research use.

Methods: A total of 77 tissue blocks embedded using clinical-standard paraffin, representing a variety of organs/sites from 41 previously signed out surgical resection cases, were sectioned automatically by the AS-410 (Dainippon Seiki Co. LTD., Japan) at 5 μm thickness using the vendor's default setting (Setting A). Ten slides were created per block and the last five slides were stained with H&E. All stained slides were digitized using a whole-slide scanner and then evaluated separately by an imaging scientist and a pathologist. The imaging scientist scored the images based on extent of imperfection (Evaluation I), while the pathologist scored the images based on suitability for clinical diagnosis (Evaluation II). Both scoring systems ranged from 1 to 5, with 1 being the worst and 5 the highest quality. Tissues with unsatisfactory sections were re-sectioned using modified parameters (Setting B) and evaluated again by the same imaging scientist and pathologist using the same scoring systems. The scores from the two different settings were compared. Auto-trimming and barcode reading and printing of the AS-410 were also evaluated.

Results: The AS-410 provided auto-trimming functionality by detecting exposed tissue on the cut surface of the block, accomplished by a camera coupled with imaging algorithms. It was also able to read and print barcodes, as well as system generated and custom input text, onto the glass slides. It produced good quality sections for most cases, with a median score of more than

4 in both Evaluations I and II using Setting A. When insufficiently sectioned tissue blocks were re-sectioned using Setting B, the quality of sections improved significantly. The importance of using the appropriate sectioning settings was also demonstrated using a small set of biopsy specimens.

Conclusions: The AS-410 automated tissue sectioning machine produces high-quality sections from clinical-standard paraffin tissue blocks from a variety of organs/sights when the proper sectioning parameters are used. The reliably good sectioning quality reveals that full automation is achievable for both the clinical and research histology lab.

Keywords: Automated tissue sectioning; Digital pathology; Automation; Histology
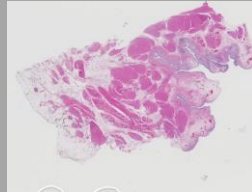
Virtual Slides:
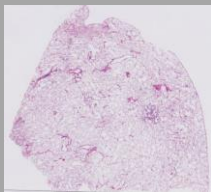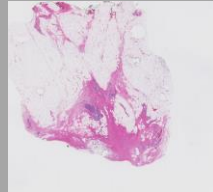
Lung   Breast   Prostate

Background

One of the current challenges in histology laboratories in the US is the draining supply of qualified histotechnicians, which is not keeping up with the increasing volume of tissue-based testing. Automated technology can minimize some of the tedious tasks required for preparation of tissue slides [1, 2]. Unlike the frequently utilized automations available for tissue processing, embedding, staining and cover-slipping, which can be performed on a single platform, automated microtomy appears to be the biggest barrier to a fully automated histology workflow. One of the reasons for this is its high dependence on skilled histotechnicians, more so than the other processes. Implementation of automated microtomy requires the abilities to: (i) align the tissue block with the blade; (ii) trim the block's excess paraffin and identify the right depth at which to commence the actual tissue sectioning; and (iii) pick up the sectioned tissues and carefully mount them onto glass slides. Indeed, these processes must be perfectly accomplished to produce good quality tissue sections that are suitable for clinical use.

Evaluation of an automated tissue sectioning machine (AS-200S Automated Tissue Sectioning System, Kurabo Industries, Osaka, Japan) was reported previously [2]. In that study, the authors compared quality of the sectioned tissues produced by the machine to those made manually by an experienced histotechnician using samples embedded in high melting point paraffin, i.e. 58°C. It was concluded that the quality of sectioned tissue produced by the automated sectioning

machine was superior to those made manually. Tissue quality is not only important for making clinical diagnoses, but also for a digital pathology workflow, especially in the application of image analysis algorithms and image data visualization, such as the 3D rendering of histologic images, which has been demonstrated in past studies [3-5]. Hence, an automated sectioning machine that ensures good quality tissue sections is vital for successful implementation into a digital workflow [5].

In the past, the AS-200S automated sectioning machine did not include certain features essential for seamless integration into a clinical histology workflow, especially in the USA. Firstly, the machine only worked well with high melting point paraffin, i.e. 58°C, which is not regularly used in the USA. Tissue blocks are also commonly tagged with bar codes, and tissue slides made from these blocks are labeled with similar barcodes, to allow for efficient Laboratory Information System (LIS) integration. However, the AS-200S neither had a built-in barcode reader nor a barcode printer. In addition, microtome blades of the AS-200S had to be replaced manually, e.g. every 100 cuttings. In this scenario, the user had to correctly note which group of tissue slides belonged to which tissue block and the timing at which the microtome blade needed replacement.

The manufacturing and marketing rights of the Auto Slide Preparation System were transferred from Kurabo Industries Ltd. to Dainippon Seiki Co., Ltd. in 2015. A new model AS-410 was introduced, which is the successor of the AS-200S. AS-410 is marketed to work well with standard paraffin used in surgical histology laboratories in the USA. It has the desired features that the AS-200S lacks - the barcode reader and printer, the auto-trimming functionality, and the ability to automatically replace the microtome blade when certain conditions are met. With these features, the AS-410 is more aligned with a clinical histology lab workflow. Our objectives in this study were to: (i) evaluate the AS-410 sectioned tissues embedded with USA-standard paraffin for clinical use; and (ii) demonstrate the importance of using the appropriate sectioning parameters to obtain the best quality tissue sections.

Methods

Automated Tissue Sectioning

We briefly describe here how the AS-410 automated sectioning machine works. The AS-410 machine has sample trays that can accommodate up to 96 tissue blocks. A robotic arm that is guided by a sensor, picks the tissue block to be sectioned from one of the sample trays and places it on the sample holder in the "tissue sectioning" area of the machine. The tissue block is first charged positively, cooled, and then humidified before it is sectioned. The tissue sectioned from the positively charged tissue block attaches to a negatively charged carrier tape that transports and deposits it on a glass slide moistened with water droplets that serve as a mini water bath to help spread the tissue. The tissue slide then glides to the machine's

"extension section" where heat is introduced to minimize, if not totally remove, wrinkles that might have been introduced in previous steps. Finally, the newly made tissue slide is brought to the drying chamber of the machine where it is dried for staining later.

While the basic functionalities of the AS-410 and the AS-200S automated tissue sectioning machines are the same, there are few essential differences, described below.

Type of paraffin

The AS-410 not only works with high melting point paraffin, i.e. melting point of 58°C (±0.5°C) as the AS-200S does, but it is also supposed to work with paraffin types that are regularly used in histology laboratories in the USA, which typically have a melting point of 56°C.

Auto-trimming

A camera and lighting have been incorporated into the AS-410 to capture the surface of the sample, and the ratio of exposed tissue is calculated by built-in imaging algorithms. When the ratio of exposed tissue to total block face surface area reaches a pre-set threshold, tissue sectioning will start. Otherwise, the machine will continue trimming until the threshold is reached.

Barcode reader and printer

The AS-410 has a built-in barcode reader and a printer. An installed camera captures the barcode printed on a tissue block. The acquired barcode, along with a system-generated sequential number that reflects the order in which the slides were made and other custom tags supplied by the user, are printed on the glass slide just before the sectioned tissue is deposited onto it.

Automated replacement of microtome-blade

The machine was also designed to accommodate the Feather A22 50-piece microtome-blade case. It has a special mechanism that automatically replaces the current blade with a new one from the case when the pre-set condition is met, i.e. maximum number of cuts allowed per blade.

Easy control of the machine's robotics

The user can control the robotics of the machine via a touch-screen display installed on its outer enclosure panel. When the machine is started up, this screen displays information, such as the remaining number of microtome blades in the case, number of slides that can be made with the

remaining amount of sectioning tape, how many slides are in the drying chamber of the machine, etc., that helps the user determine the appropriate setting before commencing the automated tissue sectioning process.

Paraffin-Embedded Tissue Blocks

This study was conducted with the approval of the institutional regulatory boards (IRB No. 2015P002724). An experienced pathologist selected a total of 41 surgical-resection cases from various organ types/sites, specifically breast, lung, prostate, soft tissue, and the gastrointestinal (GI) system, including esophagus, stomach and colon, as well as liver and gallbladder. We gathered a total of 77 surgical resection blocks by randomly selecting 1 tissue blocks from each case, or two blocks if the case had more than 5 blocks. Table 1 details the number of cases and blocks per organ/site.

Tissue Sectioning

Since the tissue blocks we used in the study were previously trimmed and sectioned for clinical purpose, we did not enable the auto-trimming function. However, to help ensure that the entire tissue section was exposed, especially since the tissue blocks were not new and the specimens could have shrunk, we set the rough cut to 200 - 250 μm and the waste cut to 50 μm. The rough cut removes excess paraffin while the waste cutting helps align the block face to the edge of the microtome blade. We set the AS-410 automated sectioning machine to cut 10 tissue slices at 5 μm in thickness from each tissue block, and for the microtome blade to be replaced automatically after every 100 sections cut. Sectioning duration was recorded to evaluate the productivity of the machine. The auto-trimming function of the AS-410 was evaluated using animal tissue blocks embedded with clinical standardized paraffin.

Parameter Settings

Part of the objective of this study was to demonstrate the importance of using the appropriate tissue-sectioning parameters to achieve the best quality tissue sections. The company's default sectioning parameters (Setting A) for the machine was recommended to start. The parameters were later adjusted (Setting B) to adapt to the paraffin embedded tissue blocks from a histology laboratory. Table 2 shows the variation in sectioning parameters between settings A and B.

H&E Staining and Whole-Slide Imaging

The dried tissue slides were brought to the histology laboratory of the Department of Pathology to be stained with hematoxylin and eosin (H&E) using the ST5020 Multistainer (Leica Biosystems,

Buffalo Grove, IL, USA). Whole slide images of the H&E stained slides, taken at a resolution of 0.46 μm/pixel, were acquired using the Nanozoomer 2.0HT (Hamamatsu, Japan) whole slide scanner.

Evaluation of Tissue Sections

An experienced pathologist from the Department of Pathology and a trained imaging scientist from our laboratory evaluated section quality from the H&E stained whole slide images. The imaging scientist focused on the quality of the sectioned-tissue (Evaluation I) alone, while the expert pathologist focused on the clinical usability of the sectioned-tissues (Evaluation II) given their qualities. In both evaluations, a tissue slide is assigned a numerical evaluation score of 1 to 5. In Evaluation I, the scores indicate the extent of the imperfections that are mainly caused by tissue sectioning, e.g. tissue folding, tissue tearing or ripping. The scores in Evaluation II, on the other hand, indicate whether the quality of the sectioned tissue is sufficient for diagnosis. The scoring system we utilized in evaluating the sectioned tissues is explained further in Table 3.

Statistical Analysis

An average evaluation score for the 5 tissue sections evaluated from each block was calculated. Data are displayed using box and whisker charts showing distribution of the scores into quartiles, highlighting the median and outliers, with vertical lines indicating variability outside the upper and lower quartiles. Significant differences between evaluation scores using different sectioning parameters were tested using a paired t-test. A p value of ≤ 0.05 was considered statistically significant. Pearson correlation coefficient was calculated to compare consistency between evaluation scores made by the imaging scientist and experienced pathologist.

Results
Auto-trimming

Since the clinical surgical blocks had been previously trimmed and sectioned for clinical diagnosis, we demonstrated the machine's auto-trimming functionality using representative blocks of mouse tissues embedded in US standard paraffin. The images displayed in Figures 1C and 1D were acquired by the camera installed inside the machine, while the binary images in Figures 1A and 1B correspond to the detected tissue regions of 1C and 1D, respectively. Figure 1C shows the reflected shadow of all the embedded tissue, while Figure 1D corresponds to the block-face image after the auto-trimming exposed 60% of the entire tissue section. The user

may set the exposed tissue ratio to higher than 60%, for which case the machine will continue to trim the tissue block until the specified ratio is satisfied.

Barcode Reading and Printing

Figure 2 displays the typical information that is printed in the label area of the glass slide. The barcode of the tissue block, which encodes the patient ID, was captured and printed on the glass slide as part of its label. Aside from the barcode, the machine also printed the user's pre-defined text, e.g. "PICT". The slide number, e.g. 002, which reflects the order in which the slide was made, was automatically generated by the machine.

Productivity

The machine sectioned about 11 blocks per hour when producing 5 slides per block and about 7 blocks per hour when producing 10 slides per block. Based on vendor specifications, the system is expected to section 15 to 20 blocks per hour when producing 2 slides per block. The prolonged duration compared to manual sectioning (about 23 blocks per hour, by report) was due to tissue surface measurement and adjustment steps that can be easily performed by histology technicians but is strictly sequenced for the machine.

Evaluation of Stained Tissue Sections on Glass Slides

In this study, a total of 77 tissue blocks from 41 different cases encompassing the 5 organ/tissue types listed in Table 1 were first sectioned using the sectioning parameters specified for Setting A in Table 2. Because, in some cases, the first five of the ten sectioned tissues were not in their entirety, which was often due to the blade not yet reaching the appropriate depth, we only selected the last 5 tissue slides for H&E staining. A total of 385 H&E stained slides were scanned using a whole slide scanner at a resolution of 0.46 μm / pixel. The imaging scientist and the pathologist independently evaluated whole slide images of the H&E stained tissue sections. The average evaluation scores for the 5 stained tissues sectioned from each block were calculated and used to derive the box plots in Figure 3.

Technical Quality of Tissue Sections

The plot in Figure 3A demonstrates the results of the evaluation performed by the imaging scientist. An average evaluation score of greater than 3 signifies that the sectioned tissues from the tissue block experienced minimal sectioning defects, such as tissue folding and tearing. We

noted from the box plot that the majority of the cases have median evaluation scores of greater than 4, which generally indicates good quality tissue sections with minimal defects caused by sectioning. On the other hand, the box-plot shows that the soft tissue cases experienced the most variability in sectioned-tissue quality compared to other tissue types. The low evaluation scores for some soft tissue cases also indicate that the sectioning parameters used could potentially be modified to further improve the overall quality of the sectioned tissues.

Adequacy of Tissue Sections for Clinical Diagnosis

An experienced pathologist from the Department of Pathology also evaluated the whole slide images of the H&E stained tissue sections. The sectioned tissues were rated from 1 to 5, wherein 5 signifies great tissue quality for making clinical diagnosis, while 1 implies that the quality of the sectioned tissue is not sufficient for diagnostic assessment. The plot in Figure 3B shows that the median of the pathologist's evaluation scores for most tissue types is also greater than 4 implying that for most slides, the quality of the sectioned tissue is sufficient for making a diagnosis. There were few cases that did not receive favorable evaluation scores from the experienced pathologist based on suboptimal quality of the tissue sections, with highest variability seen for soft tissue and GI cases, similar to the evaluation made by the imaging scientist. In fact, the degree of correlation between the independent evaluations made by the trained imaging scientist and the expert pathologist was 0.96.

Effect of Sectioning Parameter Adjustments

In this study, we also sought to determine the appropriate sectioning parameters that best adapts to the paraffin embedded tissue blocks. The settings we used in our experiments are presented in Table 1. Setting A was the default setting of the machine, while setting B represents the modified setting which we derived by adjusting the original sectioning parameters experimentally using non-clinical tissue blocks embedded at a histology laboratory in the US. The vendor's expert personnel guided us as to how the parameters should be adjusted and helped us resolve issues that we encountered during the course of this experiment.

To investigate the effectiveness of the modified sectioning parameters (setting B) we re-sectioned those blocks which initially had received poor evaluation scores. Five tissue slides were made from each block, H&E stained and then scanned with the Nanozoomer 2.0HT whole slide scanner. Both the imaging scientist and the experienced pathologist evaluated whole slide images of the new sets of stained tissue sections. The box plots in Figure 5 display the results of tissues sectioned from 7, 9, and 8 tissue blocks from prostate, soft tissue, and GI, respectively. Figure 5A corresponds to the evaluation scores given by the imaging scientist and Figure 5B to the scores given by the experienced pathologist. We can conclude from these plots that the

parameters in setting B are more appropriate for these tissue blocks. Specifically, results of the *t*-test statistical analysis of the evaluation scores given by the imaging scientist showed that the difference in the evaluation scores on the quality of tissues sectioned with setting A and B is significant, with $p = 0.003$, $p = 0.050$, $p = 0.001$ for prostate, soft tissue, and GI, respectively. Likewise, the difference in the evaluation scores given by the experienced pathologist on tissues sectioned with setting A and B is also significant, with $p = 0.043$, $p = 0.001$, and $p = 0.021$ for prostate, soft tissue, and GI, respectively.

Evaluation of Tissue Sections of Biopsy Specimens

We conducted another experiment to investigate how the AS-410 automated sectioning machine fares with biopsy specimens using different sectioning parameters. We found that out of tissues sectioned from 12 biopsy tissue blocks from a variety or organs/site (breast, lung, prostate, soft tissue, liver, colon and esophagus), only around 60% of the slides made with setting A contained tissue. However, with setting B all the sectioned tissues (100%) were successfully deposited onto the glass slides. Figure 6 illustrates a series of unstained biopsy tissue slides made with either setting A or setting B. These images clearly demonstrate the importance of using the appropriate setting when sectioning paraffin embedded tissue blocks with the AS-410 automated sectioning machine.

Conclusion

Histology workflow is composed of a series of processes of which some have already been successfully automated, e.g. staining, embedding, etc. A component of the histology workflow that has not yet been fully automated is the sectioning of tissues from paraffin embedded tissue blocks. An automated sectioning machine, the AS-200S, was introduced in [2] and was demonstrated to produce high-quality tissue sections. However, integration into the pathology LIS was not successfully pursued with that system, as it lacked the: (i) ability to intelligently trim the excess paraffin until a substantial amount of the entire tissue section is exposed for the actual cutting; (ii) capability to read the barcode labels of the tissue blocks and print them on the glass slides; and (iii) ability to change the microtome blade automatically. Fortunately, the new model AS-410 has these essential features.

In this study, we have in particular investigated the quality of the tissue sections produced by the AS-410. The results of the evaluation rendered by the expert pathologist showed that the quality of the tissue sections is sufficient for diagnostic purposes, and reliability of the sectioning quality supports deployment of the AS-410 automated tissue sectioning machine into a clinical setting. Furthermore, the strong degree of correlation between independent evaluations made by the trained imaging scientist and the expert pathologist (r = 0.96) suggests that clinical

usability of the tissue sections highly depends on their sectioning quality, i.e. absence of tissue fold, tearing, ripping, etc.

We have also demonstrated the importance of adjusting the sectioning parameters to obtain the best quality tissue sections. Examples of how the sectioning parameters affect quality of the tissue sections is illustrated in Figure 4 by the series of H&E stained images of representative tissues sectioned from prostate and soft tissue, with sectioning defects indicated by the arrows. Tissue sections produced with Setting A exhibited tissue folding (Figure 4A) and, in some cases, missing tissue (Figure 4B). In comparison, those slides produced after modifying the sectioning parameters (Setting B) exhibited great improvement in tissue quality for most tissue blocks. However, there were sporadic cases, in which the modified parameters did not work as expected. The series of H&E stained images in Figure 4C demonstrate this scenario, where we see no improvement in tissue quality with Setting B. Further experimentation is required to determine the best sectioning parameters for specific tissue types.

A digital pathology workflow involves a series of integrated steps from tissue processing to image acquisition and analysis, as well as data management. Previous studies have already demonstrated the importance of image quality in achieving reliable histology image analysis and visualization results [3, 4]. Improving the quality of tissue sections is core to obtaining quality histology images. In addition, a barcode reading and printing system that can retrieve sample information from an LIS, decide the appropriate sectioning protocol for the sample, and return the sectioning status to the LIS is also essential for a fully integrated and efficient digital pathology workflow [6]. An automated tissue sectioning machine, such as the AS-410, which produces high quality tissue sections and that also now incorporates a barcode reader and printer is advantageous for full implementation of a digital pathology workflow. The barcode reader and printer no longer requires manual labeling of the slides, which is error prone and introduces patient safety risks. While barcode designs come in various formats, currently, the AS-410 can only print a Quick Response (QR) barcode type. It is expected that future models of this machine will expand on this feature. The auto-trimming function of the machine has also been found to work well with the resection samples used. However, we suggest that this function be further evaluated for biopsy specimens, which pose a unique challenge because of their smaller size compared to general resection tissues. Also, it could be possible that the algorithm designed to detect amounts of exposed tissue can be sensitive to variations between the contrast of the paraffin and the actual tissue section. Further investigations need to be done regarding these aspects.

Histology laboratories are gearing towards fully automating their workflow to increase productivity. To date, tissue sectioning is still done manually by skilled histotechnicians, albeit with the use of a semi-automated microtome. Together with automated tissue processing and embedding, an automated sectioning machine equipped with barcode reader and printer, such as the DNS-AS410, is ideal for implementation of a fully automated histology laboratory. While a barcode reader and printer are essential for interfacing with the pathology LIS, the capability

of an automated sectioning machine to produce good quality tissue sections that can be used for clinical purpose is also very important. The results of the evaluations by the trained imaging scientist and experienced pathologist demonstrate that the quality of the tissue sections produced by the AS-410 is useful for diagnostic purposes.

Competing Interests

References

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Table 1: Number of cases and tissue blocks per tissue type

| Organ/Tissue type	Number of surgical resection cases	Number of tissue blocks
Breast	9	18
Lung	9	17
Prostate	7	14
Soft tissue	9	17
GI*	7	11

*Includes representative sections of stomach, liver, colon, gallbladder and esophagus

Table 2: Tissue sectioning settings

Tissue sectioning parameters	Setting A	Setting B	
	Resection and Biopsy	Resection	Biopsy
Cutting speed (mm/s)	40	50	50
Tape moving (%)	92	96	96
Humidification (s)	4	2	2
Extension (s)	30	30	30
Cutting angle (°)	60	60	90
Putting position (mm)	50	50	45
Tape fall (mm)	21.5	29.5	20
Small size block	off	off	on
Change knife	*Every 100 sections*		

Table 3: Scoring system used in the evaluation of the sectioned tissues

| Score	Type of evaluation	
	Evaluation I (Sectioned tissue imperfection)	Evaluation II (Clinical usability)
1	Imperfection >50%	poor quality, not sufficient for diagnosis
2	Imperfection >25% but ≤50%	significant defect but still sufficient for diagnosis
3	Imperfection >10% but ≤25%	moderate defect but still sufficient for diagnosis
4	Imperfection ≤10%	mild defect, sufficient for diagnosis
5	0% Imperfection	great quality, sufficient for diagnosis
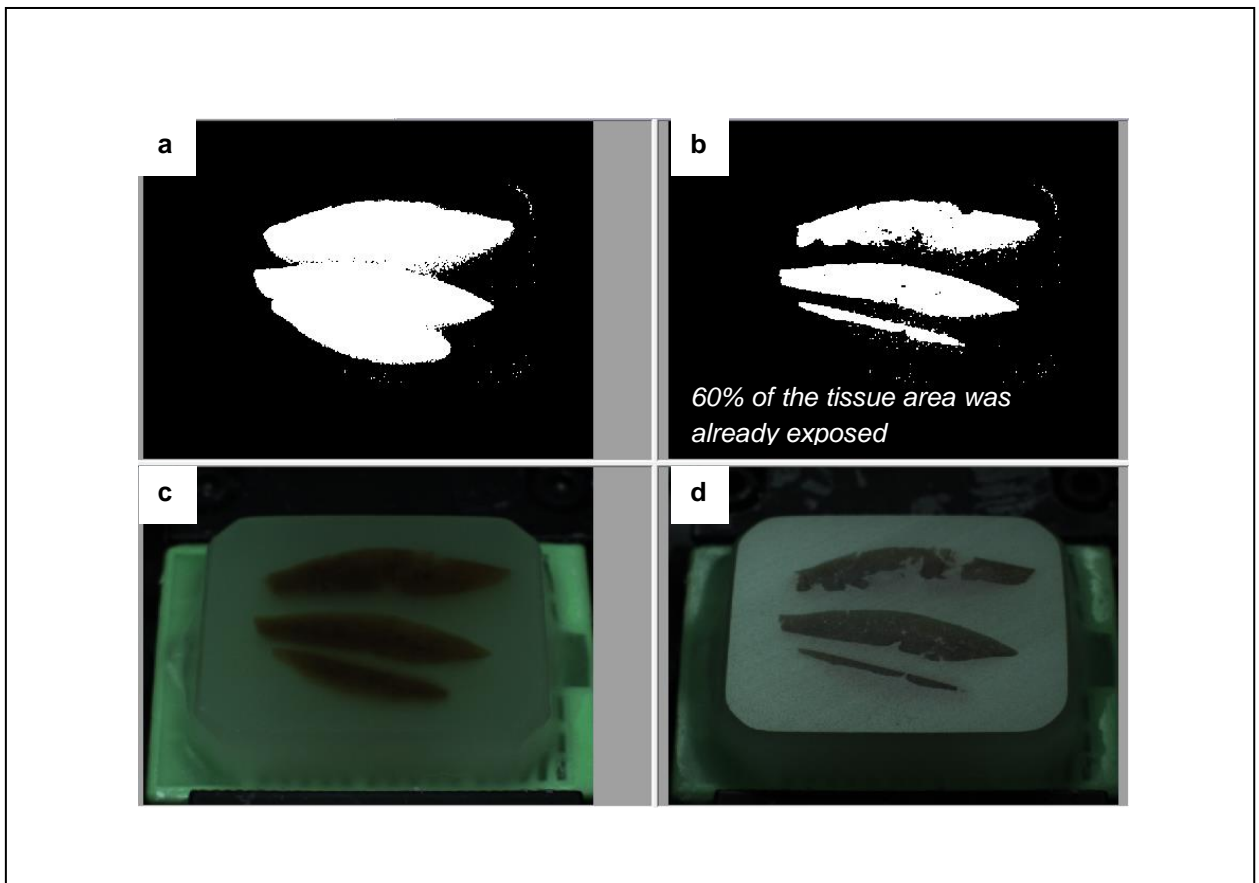
Figure 1: Calculation of tissue exposure ratio. C and D. Representative images of sample blocks acquired by the camera installed inside the machine. **A and B.** Binary images corresponding to the detected tissue regions of 1C and 1D, respectively. In 1B, 60% of the entire tissue section is exposed on the block face, which is calculated by the machine by dividing the area of tissue in 1B by the area of tissue in 1A.
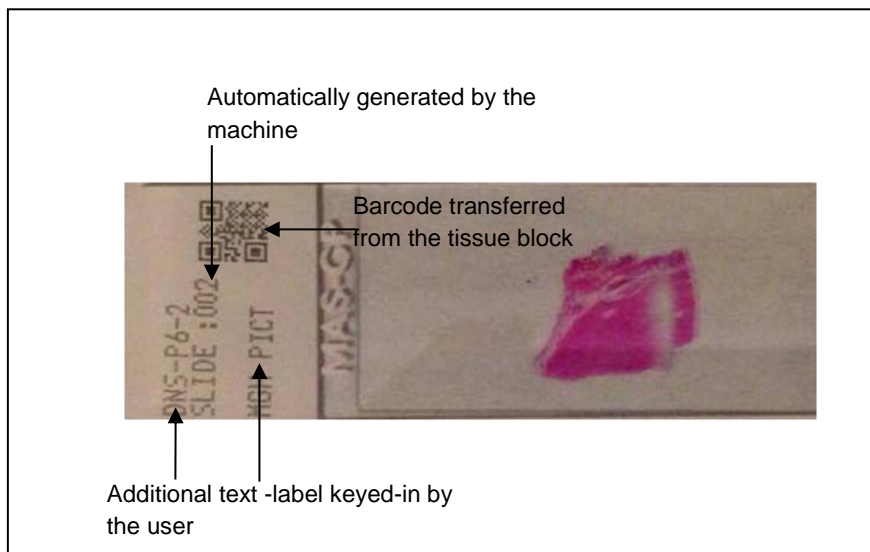
Figure 2: An example of slide labeling. The AS-410 prints a barcode image, which includes sample information, as well as keyed-in text by the user and an automatically generated slide order number.
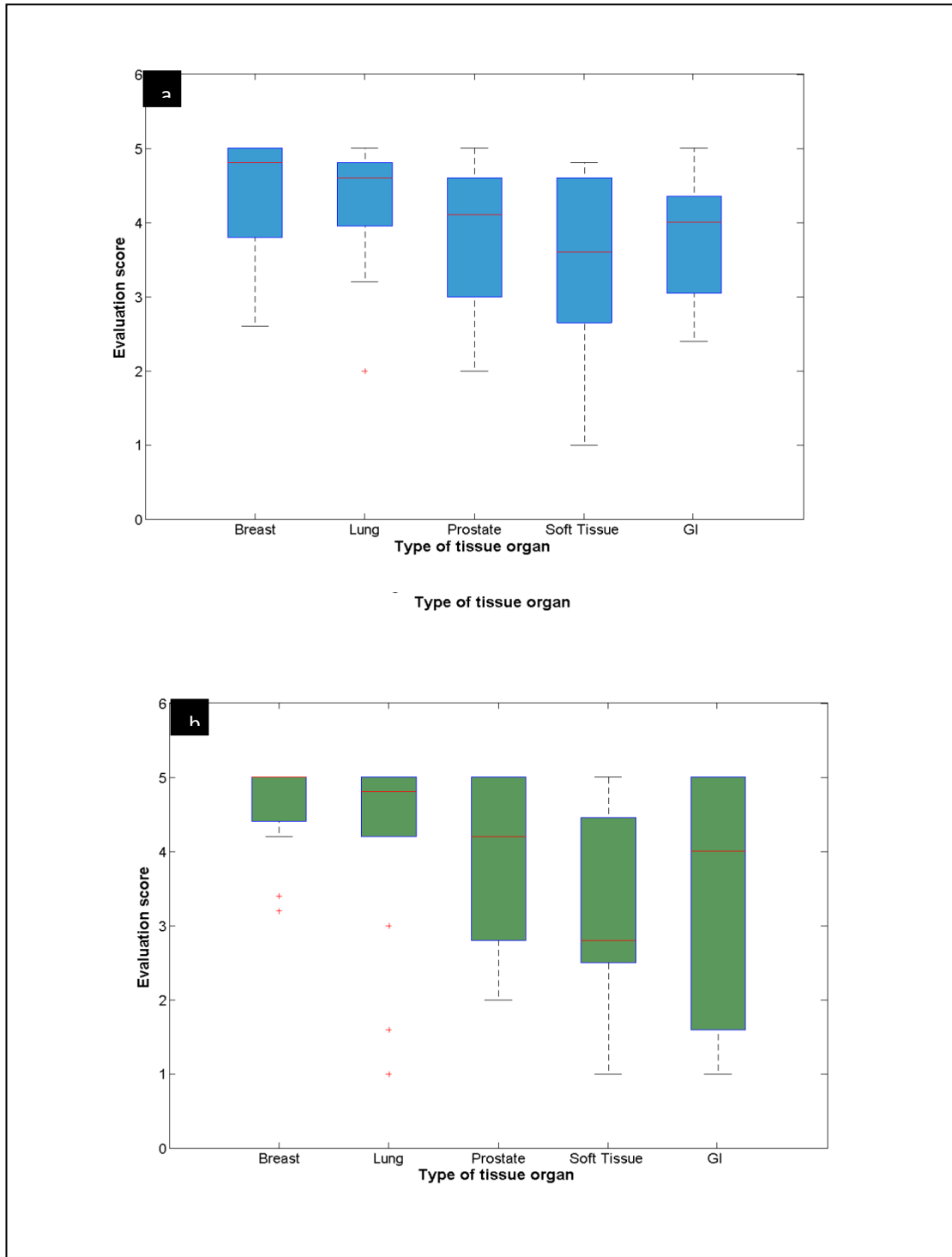
Figure 3: Evaluation of section quality. A. Box plots of average evaluation scores given by the imaging scientist for various organs/tissue sectioned with the default setting. **B.** Box plots of average evaluation scores given by the experienced pathologist for various organs/tissue sectioned with the default setting
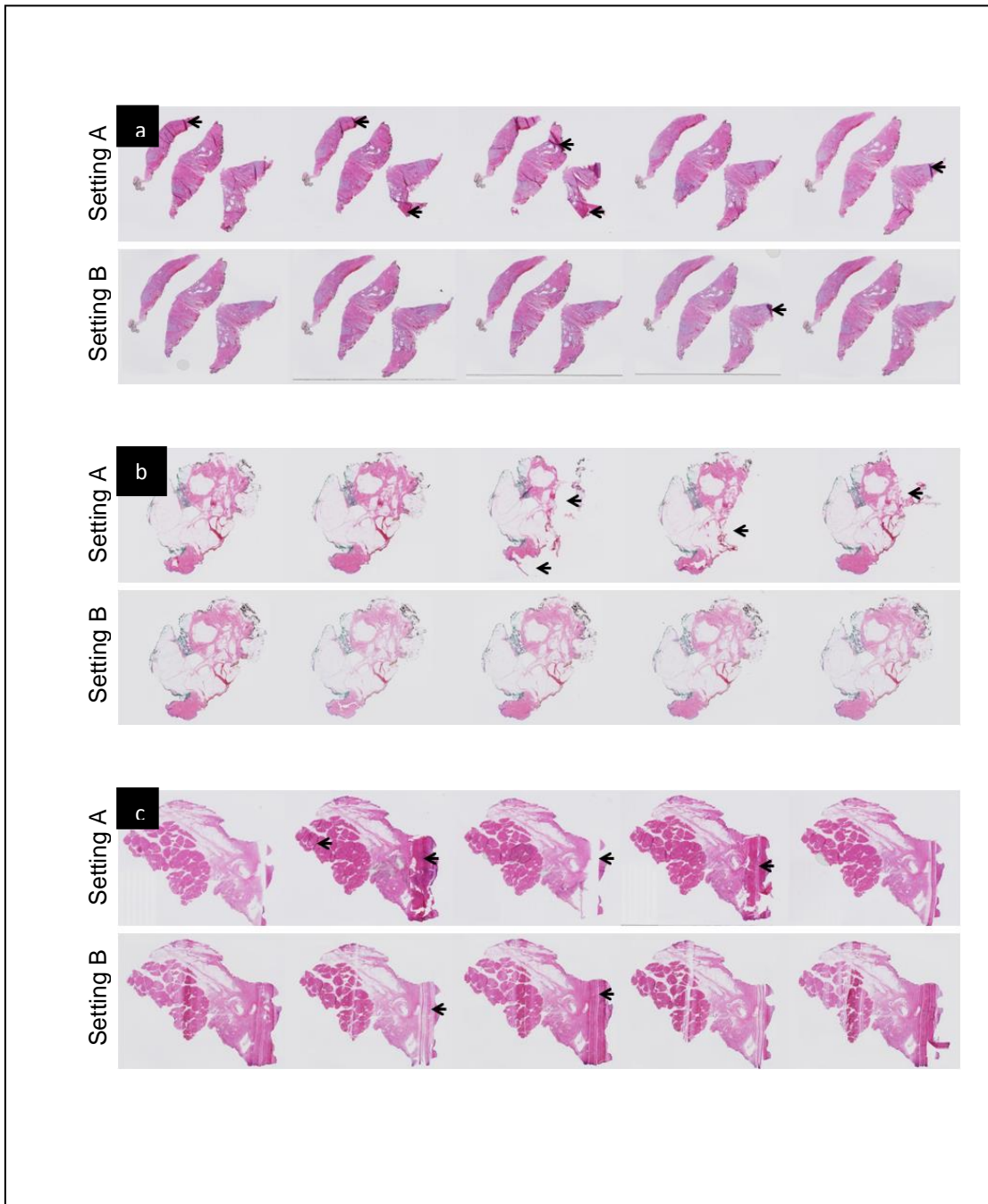
Figure 4: Representative images of H&E stained slides sectioned with setting A and setting B.
A. Slides of prostate sectioned with setting A (top) and setting B (bottom). **B**. Slides of soft tissue sectioned with setting A (top) and setting B (bottom). **C**. Slides of soft tissue sectioned with setting A (top) and setting B (bottom). Arrows indicate sectioning defects.
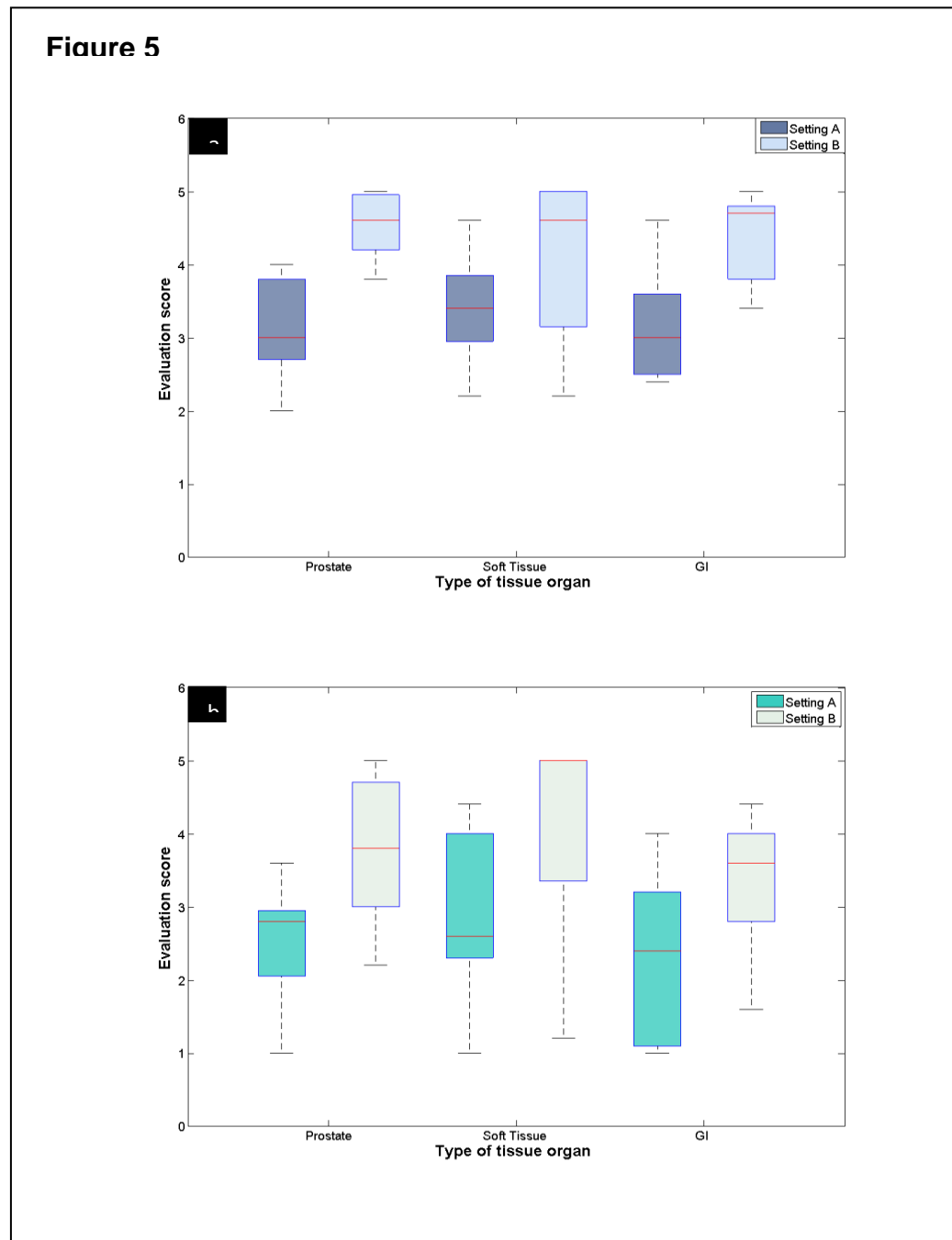
Figure 5: Improved sectioning quality with modified sectioning parameters. A. Comparison of evaluation scores given by the imaging scientist on slides sectioned with setting A and setting B for various tissue/organ types. **B**. Comparison of evaluation score given by the experienced pathologist on slides sectioned with setting A and setting B for various tissue/organ types

Figure 6: Representative images of sections from biopsy samples. Unstained slides from a liver biopsy block sectioned using setting A (top) and setting B (bottom).