COLLEGE of AMERICAN PATHOLOGISTS
Laboratory Quality Solutions

Practical Tips to Assist Implementation of Whole Slide Imaging

Contributors/Acknowledgements

Michael LaFriniere, HT(ASCP), Humberto E. Trejo Bittar, MD, FCAP, Marilyn M. Bui, MD, PhD, FCAP, Andrew J. Evans, MD, PhD, FCAP, Anil V. Parwani, MD, PhD, FCAP, Mansoor Nasim, MD, PhD, FCAP, Robert Lott, BS, HTL(ASCP), Liron Pantanowitz, MD, FCAP, Caitlin Routhier HT, QIHC (ASCP), Jamie Pert HTL, QIHC(ASCP)^{CM}, Antonia Valdez, BS, HTL (ASCP)

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Keywords: digital, imaging, microscopy, pathology, histology, whole slide images (WSI), digital pathology, quality control

Introduction

Whole slide images (WSI) are one of the newest technological developments in pathology review and are currently being implemented in histology laboratories worldwide. Over the next decade it is believed WSI will be the primary means of providing images to pathologists for diagnostic interpretation. It is believed the advent of WSI and implementation of its sister methodologies including automated or artificial intelligence (AI), and computational/deep learning processes will allow for increased accuracy in interpretation and revolutionize pathological diagnostics.[1] Due to its immense potential, we are being presented with advances in updated WSI technology and literature at a rapid rate, resulting in new and numerous opportunities that a laboratory's scanning program can implement at any time. Thus, the need to disseminate imaging technology knowledge, especially in general histology education and the overall training of histologists is at a most critical point in time. In the near future, diagnostic assessment using the current process of light microscopy review with standard glass slides will be considered archaic and the primary function of histologically stained tissue will be for scanning and computer imaging. Those who have not experienced the new world of WSI will be amazed at what it can do, how it has changed the ways in which practicing laboratories operate, and how it will change the fields of pathology and histology moving forward. As many of us are beginning the process of implementing the WSI process in our laboratories, some of the contributors to this article have been involved with FDA trials for implementing the approval of the use of imagers in the laboratory. In addition, many contributors are on committees in association with: The College of American Pathologists (CAP), National Society for Histotechnology (NSH) or the Digital Pathology Association (DPA).

The purpose of this article is to provide some experienced WSI guidance from the perspective of grossing to scanning outcomes in histological sections only. At this time, a discussion on cytological and haematological imaging will not be included due to potential inherent difficulties seen with focus, current scanner availabilities, and lack of FDA approval.[2,3] The information below should be utilized as an educational guide to WSI in histology and will include tips to assist you in imaging implementation and use while acknowledging some of the pros, cons and common problems that laboratories have experienced with its use.

Objective

The main objective of this article is understanding the preanalytical effects and how a laboratory's poor quality slide preparation is considered a primary limiting factor that may lead to significant impediment of successful WSI implementation. Imaging program success requires adaptation, with modifications and changes to current laboratory practices being of the utmost importance. This guide will provide a thorough understanding of common WSI pitfalls as they relate to slide preparation for scanning, and highlight current standards for routine microscopy acceptability, with particular focus on how current histological practices may not necessarily translate appropriately to the adoption of a digital scanning program in the modern laboratory.

A. Grossing

Grossing occurs post accessioning and is a process where a specimen comes to the laboratory for descriptive characterization and, if warranted, potential dissection into representative sections for histological processing. Appropriate execution of the grossing step is highly important, and care must always be taken to ensure an excellent final histology slide is produced. Handling and grossing techniques can differ from sample to sample, especially for larger specimens, requiring extensive experience to perform appropriately. Submitted specimens need to be sampled in a manner that will guarantee appropriate pathological interpretation of the final histology slide, both in physical glass and computer image formats. This is especially critical in cases of malignancy, where correct tumor staging and grading are key to understanding patient outcomes and potential treatment options. The following details WSI grossing recommendations that can improve the quality of a laboratory's histologic preparations in the digital age.

It is imperative to follow all general quality assurance guidelines. CAP checklist requirements acknowledge that common preanalytical factors can affect the quality of histologic preparations and needs to be taken into consideration when grossing any specimen. Regardless of if a slide is being made for physical microscopic examination or scanning, consistency must be maintained throughout the routine gross processes. This includes reviewing all patient information on the requisition and sample container to ensure appropriate identification prior to submission for grossing. It is also critical that only one specimen for one patient be handled at a time to prevent misidentification or cross contamination and that all cassettes generated be legible and labelled appropriately. The one major

difference in grossing for WSI is that the size of the tissue placed in the cassette must be strictly monitored. It is recommended that tissue submitted for processing should not be so large that its edges are touching the cassette walls. Similarly, when dealing with multiple pieces of tissue for one case, the total number added to a cassette needs to be taken into consideration and pieces should not crowd each other. In both these instances not only can fixation and processing be impaired, causing subsequent artifacts at microtomy, the sections can also end up too large for the glass slide. This, in turn, can affect coverslipping by not allowing full coverage of the tissue. Ultimately, it would significantly impair the quality of the scanned slide as, depending on the scanner's make and model, the tissue may end up being larger than the imaging box itself. Thus, portions of the tissue will fall outside of the scannable area and will not be represented in the digital image. This can be a serious artifact in digital pathology as it is fundamental that the scan be identical to the glass slide, with all submitted tissue components viewable. It is highly recommended and important that laboratory staff, and any scanning personnel in particular, maintain constant communication and feedback with the grossing staff. This ensures all appropriate guidelines are always followed and accurate glass slides for scanning are produced. In fact, the criticality of this issue has resulted in it being included as part of the 12 guideline recommendations for implementation of digital pathology developed by the CAP. It must be addressed as part of WSI validation and be mandatory as part of any quality improvement and assurance program.[4]

Grossing Tips in Review:

1. Size, thickness, and number of tissue samples going into a cassette needs to be appropriate.
2. Standard fixation times for specific tissues must be adhered to.

B. Tissue Fixation and Processing

Preanalytics, or the fixation and processing steps that occur prior to histological staining, are key to ensuring an interpretable result at pathology review. It is widely understood that tissues with poor preanalytics will not perform well histologically and can especially negatively impact staining including the initial hematoxylin and eosin (H&E) stain as well as specialized downstream testing such as immunohistochemistry (IHC) and in-situ hybridization (ISH). Increased ischemic time or the time between surgical removal and immersion in fixative, inadequate total fixation time, and poor tissue processing are the primary causes of suboptimal stain quality. Ischemic time should be limited to less than 1 hour and fixation time for all clinical specimens should be no less than 6-8 hours and no more than 72 hours.[1] Additionally, thorough tissue processing with complete removal of unbound water is essential to ensure a specimen is properly prepped for microtomy and staining. In terms of WSI, the same qualities in embedding, microtomy, slide preparation, and staining that are important for overall good laboratory practice are also key to production of an optimal digital image and can be significantly handicapped if the pre-analytical steps in a laboratory are inappropriately executed. For example, of particular importance to microtomy and slide preparation are sample size (< 5 mm thick grossly), section thickness (3-5 µm), microtomy quality (absence of wrinkles, folds, knife lines, tears,

chatter, and holes), and staining. For any laboratory considering WSI, it is imperative that these practices be implemented across all tissue types.

Overall, it is fundamental to understand that proper grossing and processing is vital to producing the best quality H&E stain. Adhering to all established guidelines will ensure an optimal whole slide image is always produced.

Fixation and Processing Tips in Review:

1. Tissues with poor pre-analytics will not demonstrate appropriate staining and thus a poor WSI will be generated. All histological standards for fixation and processing must always be adhered to.

C. Embedding

Most institutions have guidelines for embedding routine tissue samples. However, when implementing digital pathology solutions into a laboratory workflow, additional recommendations should be established. It is good practice to develop a standardized process to ensure that all staff are embedding consistently and keeping potential translation to WSI in mind. Additionally, while you may not currently use digital pathology at your own institution, if sending slides off for pathology consultation at external laboratories the potential that they may have digital pathology analysis should be considered. Successful scanning and digital review at the embedding step largely depend on defining two key principles, sample arrangement and orientation. In terms of orientation, positioning of the tissue within the paraffin block can have a significant effect on the area to be scanned, the overall scan time and the resulting file size. Technicians should be conscious of the number of tissue pieces submitted and the mold size chosen for embedding. Trying to force too many sections into the wax and/or choosing a base too small can result in the tissue edges touching the sides of the resulting block, potentially leading to these areas being cut off by the scanner and thus missed at pathology review **(Figure 1)**. Similarly, it is best practice to make sure you have samples embedded in a row as it will create a higher quality slide that is easier to scan **(Figure 2)**. It is also imperative that all individuals within the histology laboratory follow identical post-embedding processes. Specifically, there should always be a defined standard as to how the cassette is placed in the base mold and whether the label is placed to the right or the left. This would save pathologists time as they won't have to define specific orientations for tissues based on key structures such as epithelium (skin, colon) using the physical glass slide and rotational tools in imaging software. It should be noted that how these orientations are defined can be scanner dependent and you will need to understand how your machine images a slide before you can establish appropriate embedding practices for your laboratory. For instance, while it may be standard practice to embed tissue at an angle for traditional pathology slide review, the same protocol may not actually be necessary when implementing a slide image analysis solution. Many times you'll find it is best to place tissues horizontally or vertically in the base mold depending on how the block is oriented into the microtome chuck when cutting.

Embedding Tips in Review:

1. Standardize embedding practices in your laboratory for both traditional pathology review and implementation of WSI solutions.
2. Select appropriate mold size so that the tissue in a given block does not touch the edges.
3. Place the tissue in its mold with appropriate orientation.
4. Limit the number of tissues per mold when multiple pieces require embedding.

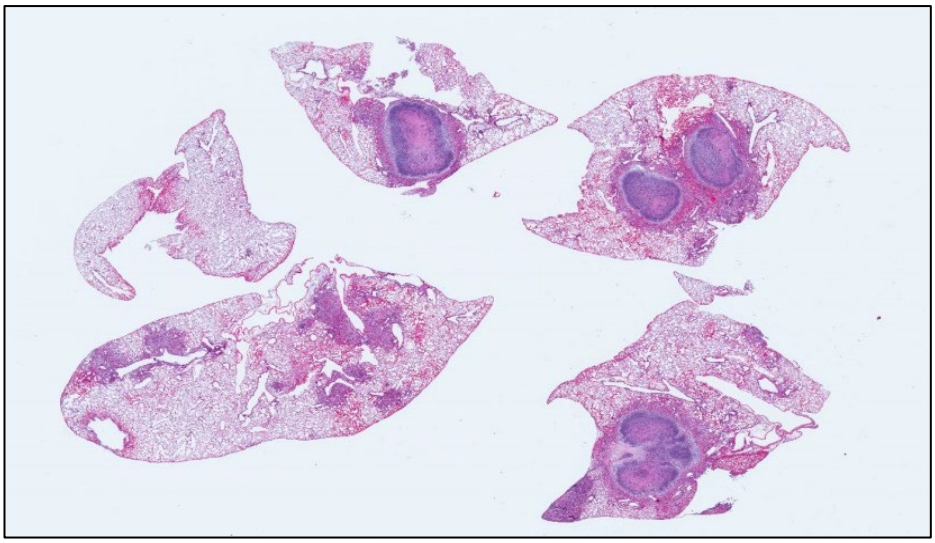

Figure 1: Image shows incorrect embedding orientation for WSI. Notice how close portions of the tissue are to the field edges.

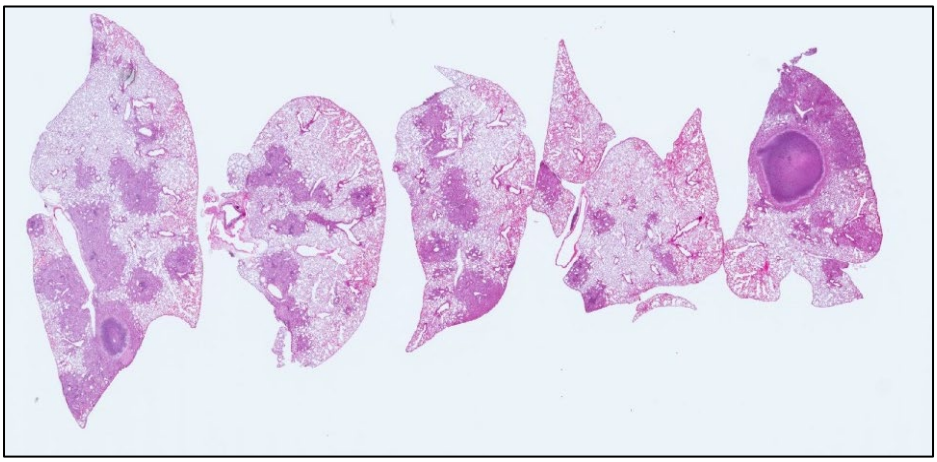

Figure 2: Image shows correct embedding orientation for WSI. Notice all pieces are aligned in a single row with edges centralized within the field of view.

D. Microtomy

Tissue sections cut for WSI purposes should generally be 4 µm in thickness, with some exceptions noted, such as lymph node which is better imaged at 3 µm.[1] It is imperative that the appropriate thickness is maintained for all sections to be scanned and that any potential thick and thin variability is not present. All sections should be placed in the center of the slide with sample orientation and rotational placement kept consistent. Staff should take as much care as possible to ensure a sample is not touching the slide edges. Ideally, all samples should end 2 mm from each side of the slide as some scanners are unable to reach beyond these areas. As mentioned previously, it is also best practice to limit the number of sections placed on a slide as the fewer tissue samples to image, the shorter the scanning time. Microtomy artifacts should also be avoided with technicians taking care to ensure no wrinkles, folders, holes, chatter, knife lines, or scratches are present.

Changes in Sectioning for WSI:

Histologists have become accustomed to picking up several consecutive sections “per level” on a slide. In terms of digital pathology implementation, these additional sections are considered unnecessary **(Figure 3)**. To increase scanner productivity, it may be helpful to place one tissue section for each level on a single microscope slide. In doing this the scanner no longer has to image unnecessary tissue, thus allowing the imager to run more efficiently **(Figure 4)**.

Figure 3: Image shows current practice for placement of consecutive levels on slides.

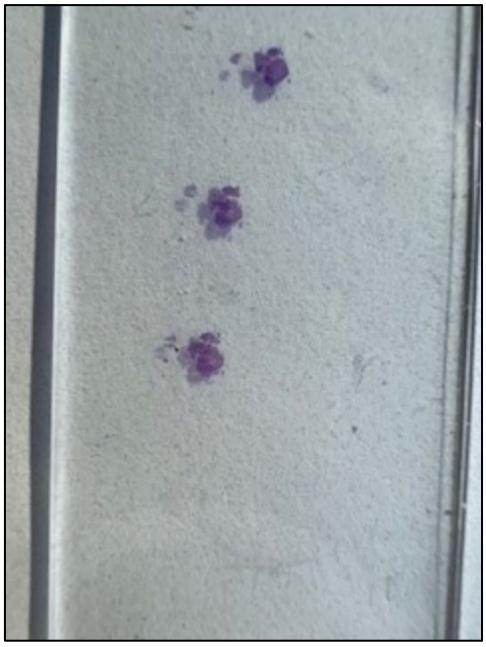

Figure 4: Image shows section placement adapted for use in WSI. Note that only 1 section per level is added.

E. Slide Labelling and Bar-Coding

Bar coding systems for slides commonly use a 2D option and should be set up to include significant information related to each case, such as accession number, tissue site or part, animal, dosage, group, etc. This allows for any digital slide scanned to be automatically placed into the correct section of its archival database. When combined with use of audit trails and role-based security access privileges, a tamper-proof chain of custody is then established for each sample.

Label placement is critical as it impacts the scanning process if not performed correctly. The following steps are suggested to ensure correct imaging always occurs. Labels should:

- be placed so the code orientation is either vertical or horizontal.
- not protrude from the edges of the slide.
- not cover tissue that may be close to the top of slide.

Using multiple and/or peeling labels can result in mishandling by a scanner, leading to malfunction during operation. It is important to note that individual scanning systems can handle labels differently, such as in instances where a slide may have two or three labels for consultation. As such, each laboratory will have to set specific parameters for their scanner that can account for these potential cases.

To optimize printing, it is critical that routine maintenance is performed on your label printer. This includes cleaning the printer heads and rollers on a regular basis, even when printing in direct thermal mode. Developing a cleaning schedule is imperative and when standard maintenance is ignored various problems can be observed that can ultimately lead to scanning failures during WSI. As an example, when required maintenance is not performed, the barcode label (etched or printed) can be too faint for the scanner to read **(Figure 5)**. Also, when using labels smears or run-off errors can occur depending on the type of materials that were used for printing. These artifacts may result in a printed barcode becoming chemically “smudged.” Thus, it is generally beneficial to use chemically resistant labels or clean the printer regularly and carefully **(Figure 6)**.

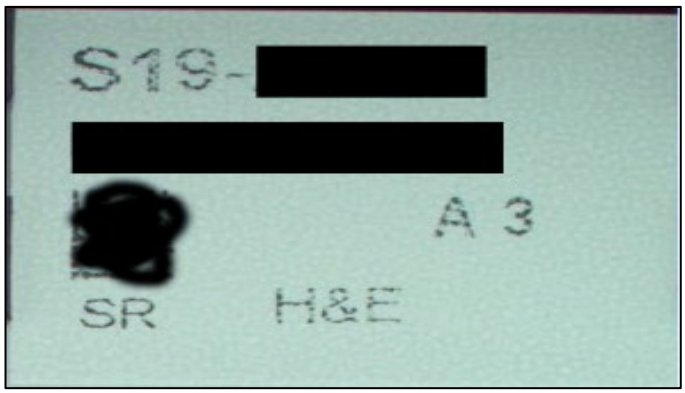

Figure 5: Image shows weak etching due to improper performance from lack of printer scheduled maintenance and cleaning.

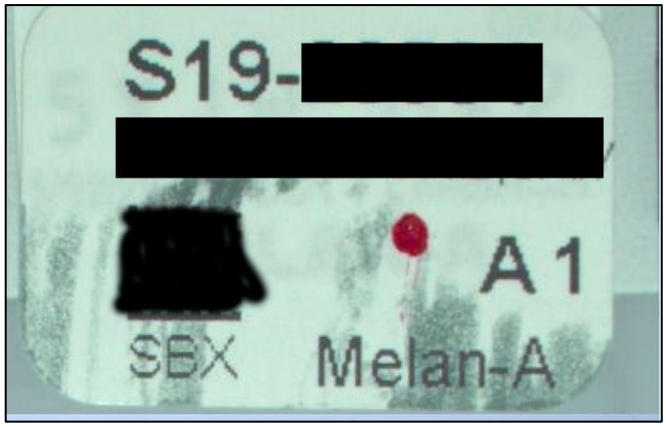

Figure 6: Image shows smudging artifact due to a poorly cleaned printer.

Microtomy and Slide Labelling/Bar-Coding Tips in Review:

1. Thickness of microtomy sections for WSI are best when cut at 3-4 µm, depending on tissue type.
2. Adapt sections for leveling to WSI practices by removing unnecessary consecutive sections and adding only 1 representative level to a single slide.
3. Set up a bar-coding system so it incorporates as much information as possible for easy archiving.
4. Ensure appropriate label placement on a slide always occurs. There should be no overhang and it should not cover any portion of the tissue itself.
5. Ensure appropriate printing of label or etching by maintaining a consistent cleaning and preventative maintenance schedule.

F. Staining

Regardless of the application (ie, H&E, special stains, IHC), sections stained for WSI should always be consistent by utilizing standard staining practices. There should never be any deviation from a laboratory's CAP validated standard operating protocol (SOP).[2] Many of the quality parameters outlined for fixation, processing, and sectioning for WSI imaging are also applicable to staining and, to a large degree, determine overall quality of the end-product. For example, one should see nuclei in H&E-stained sections that have well-defined nuclear membranes with clear open karyoplasm and scattered chromatin patterns easily visible. Eosin should always impart 3 distinct shades of pink, allowing for clear distinction between tissue structures such as muscle, connective tissue, and red blood cells. Analytical staining problems, like excessive background staining or understaining are generally associated with staining protocols that are not optimized or have been poorly optimized to a particular laboratory's SOP. Other problems such as variations in the color and intensity of a stain or stain/reagent binding to areas outside of the tissue itself (ie, glass of slide) must be corrected before WSI can be implemented as they can hamper the effectiveness of scanning and, eventually, qualitative and quantitative image analysis. Overall, in their respective laboratories, pathologists are

used to examining physical slides with particular tones and intensities for all stains, especially H&E. As a result, when digital pathology solutions are implemented, there should be no deviations between how a slide appears with standard microscopy and how its scanned image translates digitally.

Staining Tips in Review:

1. All staining protocols should be standardized when implementing digital pathology solutions. Appropriately validated stains will translate easily from light microscopy to scanned image review.

G. Coverslipping

Coverslipping refers to the adherence of a thin piece of glass or film, known as a coverslip, on top of a slide using mounting media to preserve and hold the tissue section in place. For the purposes of this section, we will refer only to permanently mounted slides, as dry and wet mounts are generally temporary procedures. While almost all glass slides for surgical pathology will eventually be coverslipped, there are examples of situations where a coverslip may not immediately be applied, such as intraoperative consultations. In these instances, a touch preparation, scrape, or smear may be made that can be stained and viewed immediately, thus not requiring a protective coverslip in the moment. The ideal coverslipping procedure should produce a slide where the glass slip fits precisely, does not protrude over the edge, is consistently clean and without fingerprints or contaminants, has no air bubbles trapped under it or excess mounting medium extending outside to the slide sides, and is placed without destroying both the tissue and slide label. In the histology laboratory, coverslipping can be performed manually or by means of automation. If manually coverslipping slides, it is important to ensure not too much mounting media is applied. The histologist should also not shake the bottle of mountant to avoid bubble formation in solution which can then easily translate to the slide. If any air bubbles can be seen on initial placement of the coverslip, gentle application of pressure can expel them out the sides of the slide. There are several coverslip types on the market, and they generally range in size and thickness (eg, 0.17 – 0.25 mm). They may be long enough to cover the entirety of a slide, or they may be a small square that covers only part of the slide surface. Overall, it is best to try and avoid extra-large, small, or square coverslips in favor of those that adequately cover the entire slide, as you are better able to guarantee the tissue you're coverslipping is fully protected and no part of it will dry out or fall off the slide. In terms of WSI, it is important that all tissue to be scanned are fully coverslipped as failure to do so may result in a digital image where tissue pieces may extend beyond the coverslip edges leading to areas that are blurry and often uninterpretable.

Coverslips are typically made of either glass or plastic film. Glass is commonly utilized because they are harder to scratch, remain flat over extended time periods, and can be cleaned with alcohol to remove markings. However, several laboratories now employ plastic film as part of instrumentation that is designed to automate the coverslipping process. Use of these types of coverslippers speeds up the process as the plastic is usually coated with a resin or cellulose that eliminates the need for

mounting medium, thus significantly reducing slide drying time. This type of coverslipping is highly beneficial for those laboratories looking to implement WSI as the lag time between slide staining and scanning is significantly reduced. Additionally, there are various types of mounting media available on the market for laboratories to choose from. Most are utilized due to their favourable properties including non-reactivity with tissue, ability to match the refractive index of glass (1.5 – 1.8), better preservation of slides for long-term storage, non-fluorescing, and possible prevention of photobleaching. It should be noted that after a slide is coverslipped with glass it will take time for the mounting media to dry. If a slide is manipulated before it can fully cure, the coverslip may not fully adhere to the slide and it can move around as a result, potentially leading to poor tissue scanning and even potential machine malfunction. Similarly, when wet slides are placed on a scanner, any excess mounting medium that may spill out onto the slide itself, can contaminate its internal components (eg, microscope stage, robotics, objectives). Over time, build-up of such residue can lead to possible scanner malfunction and ultimately result in slides being mishandled and even broken.[6] It is imperative to make sure the appropriate time is taken (eg, at least 1 hour) to ensure the mountant used has had a chance to thoroughly harden. Thus, several laboratories have adopted either pre-imaging incubation procedures (such as the use ovens and warming trays) or omitting the need for mountant altogether by replacing glass coverslips with plastic film. Both methods act to expedite the drying process for their recently mounted glass slides. Once fully dry and ready for imaging, coverslips should be checked and wiped down to make sure they are completely clean and free or dirt and/or marks (eg, pen marks and annotations). This is considered best practice and will ultimately produce a high-quality scan, as the slip is completely free of any artifact that may obscure the underlying tissue and interfere with automated focus and general scanning procedures.

Coverslipping Tips in Review:

1. Ensure the coverslip added to any slide appropriately fits the slide and does not protrude over the edge.
2. Always check for and remove any air bubbles that may have become trapped under the coverslip during mounting media application.
3. Always wipe down your coverslip to remove any fingerprints, contaminants and marking from annotations that may be present.
4. Always make sure your mounting media is fully cured and that no excess is extending outside the coverslip boundary prior to scanning.

H. Scanning

Prior to placing glass slides on a scanner, it is critical to first prepare them for imaging. This means they should be clean, or free of any dirt, dust, marks or coverslipping artifact. Failure to adhere to this practice will inevitably lead to defects in the resulting scanned image. Often, a dirty or dusty slide will result in out of focus or blurred areas in sections of the scanned image. **(Figure 7, 8)**

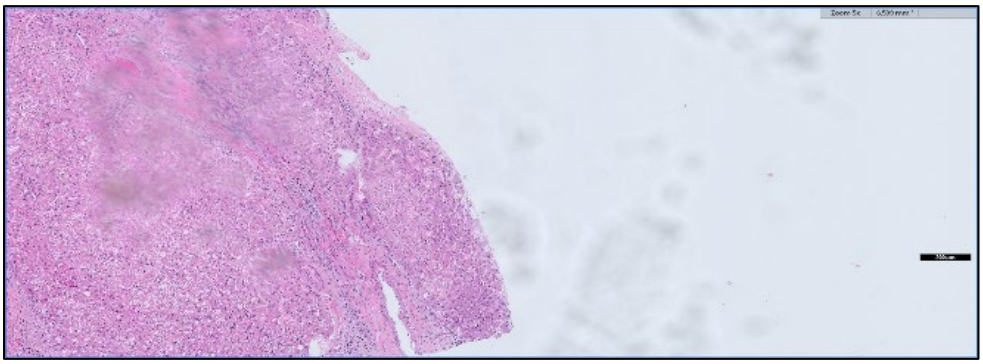

Figure 7: Image showing the results of a scanned “dirty” slide. Note the unfocused and blurry patches, possibly the result of poorly distributed mounting media.

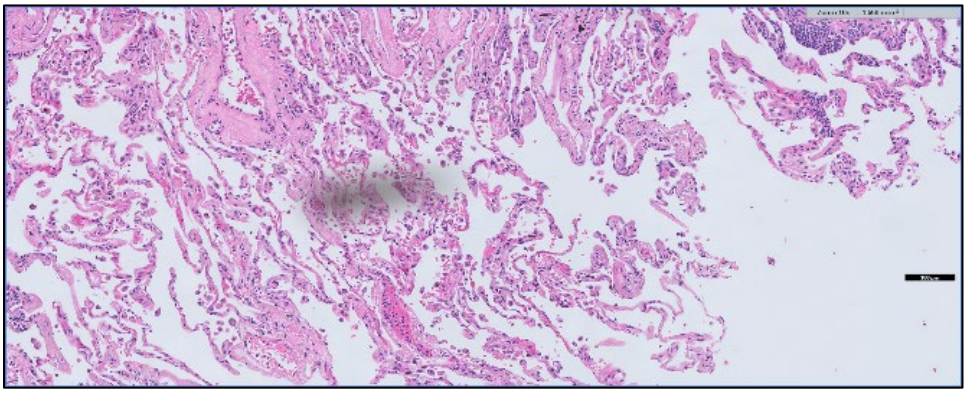

Figure 8: Image showing a slide that was not properly wiped down prior to scanning. Dust particles remain on the glass which, upon scanning, result in shadowed and unfocused areas directly overlying the tissue.

Other types of artifacts that can also cause scanning issues are ink marks from annotations made during pathology review. These can often interfere with scanning algorithms and how they process information, even creating inappropriate macro image or thumbnails. Additionally, the scanner can also pick up on any excessive debris that has become trapped underneath the coverslip and embedded in the mounting media. This can lead to dark and uninterpretable or blurry patches scattered on top of and around the tissue image. **(Figure 9, 10)**

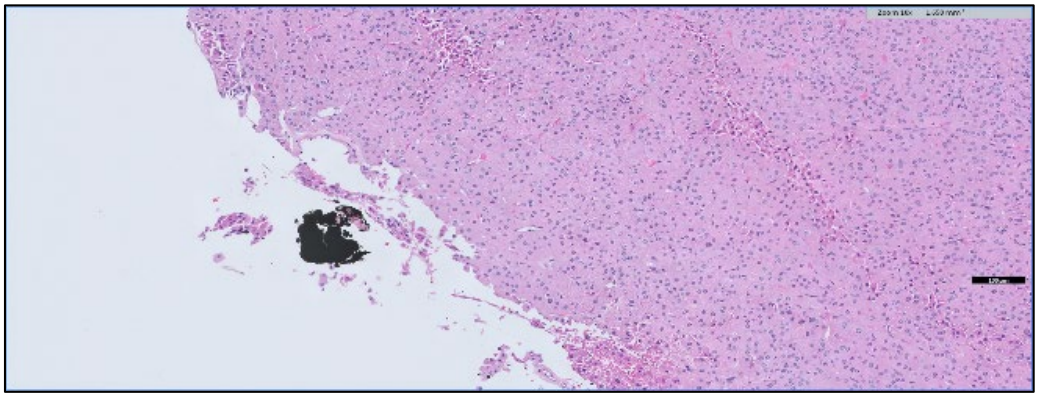

Figure 9: Image shows scanned debris that has become trapped underneath the slide coverslip.

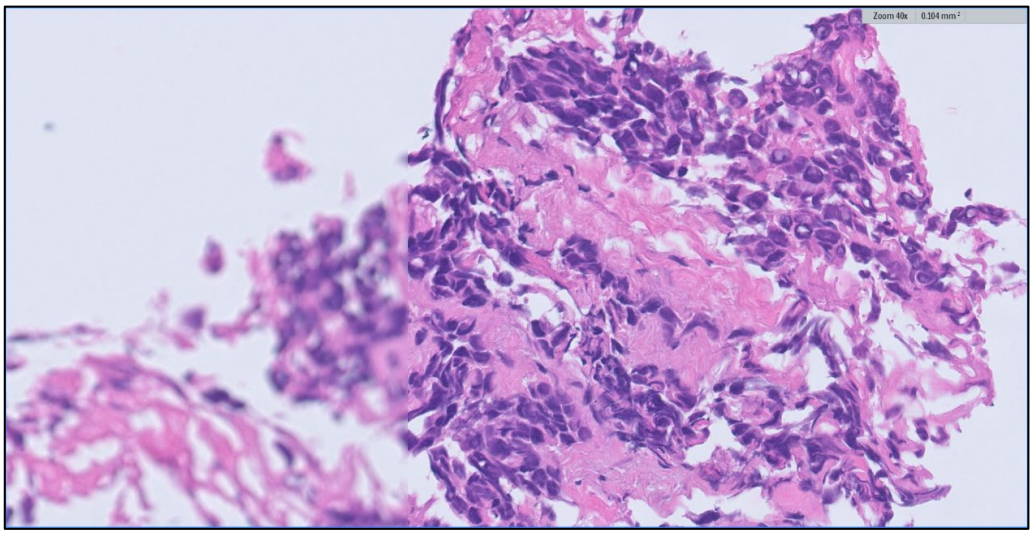

Figure 10: Image shows a scanned slide with excessive debris trapped underneath the coverslip. This caused several tiles to become out of focus during imaging, leading to blurry, uninterpretable patches.

Use of Macro Images:

A macro image is a snapshot or thumbnail that shows the entirety of the glass slide. Macro images will typically include the slide label which will contains key identifiers such as patient name, accession number, barcode, and specified stain details. Some vendors will provide details to improve your macro image result by placing tissue in a “bounding box,” or predefined, bordered area surrounding the full glass slide. These borders are included as a method of mitigating issues with the tissue being cut off from the scan as they fall outside the box area **(Figure 11)**. A rarer type of artifact that can occur with macro images is when sunlight shines directly into the scanner. This will result in what is called “solar flare” that is picked up by the scanner and imprinted on the resulting image. Overall, this affect is often considered minor and doesn’t generally have any impact on WSI **(Figure 12)**.

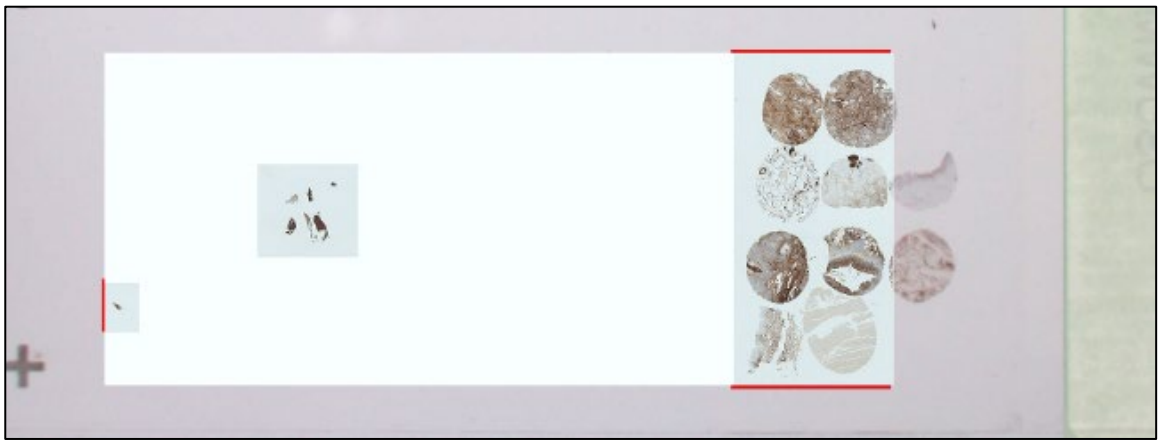

Figure 11: Image shows an example of a tissue micro array control where two sections of its tissues fall outside the bounding box (top right) and thus are not captured in the region of interest for scanning.

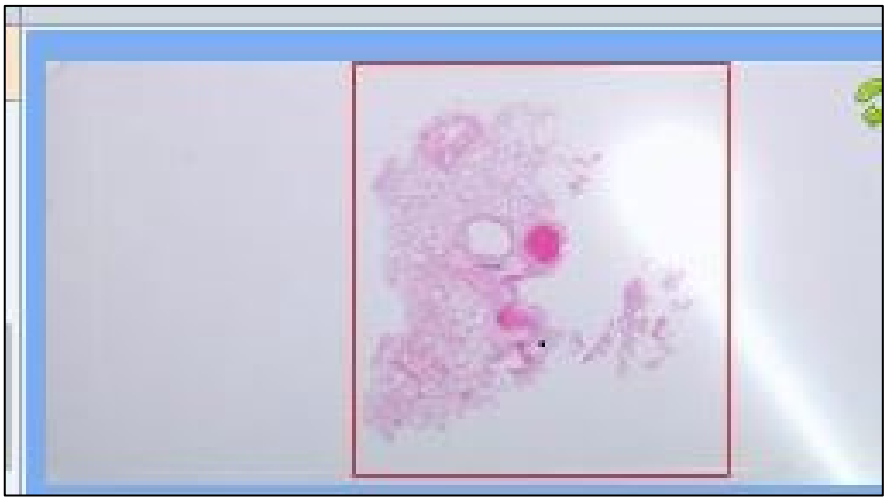

Figure 12: Image demonstrating “solar flare” artifact in a scanned macro image. Note the patch of white light overlying the glass and portions of the tissue to the right.

Scanning Tips in Review:

1. Ensure cleanliness of all physical slides prior to scanning is of utmost important.
2. Ensure cleanliness of all physical slides prior to coverslipping. This is key to keeping debris from getting trapped under the glass and in the mounting media.
3. Slides should be free of any annotations or ink marks prior to scanning to ensure the digital algorithm will work appropriately.

I. Quality Assurance and Quality Control

All laboratories should have standardized quality assurance and quality control procedures already well defined and in place for any routine histological practice. These standards should be set at the beginning of the preanalytical stage as quality histology is very much dependent on how the patient

specimen is handled initially. There are up to 60 potential variables that can influence results of downstream processes such as IHC. As such, optimization and standardization of these steps are of prime importance.[3] The CAP offers multiple proficiency testing (PT)/external quality assessment (EQA) programs and practice guidelines that can assist in addressing these issues and provide guidance on result improvement. In general, laboratories should always be striving to achieve national benchmarks for quality for all stained slides whether they are examined manually or digitally. However, it should never be assumed that standard histology processes implemented for manual reading of H&E and IHC slides will automatically translate appropriately to whole slide imaging and digital diagnosis. In fact, digital slides generally require additional preanalytical steps to ensure the image rendered by the scanner is of the highest quality. To help laboratories assess and improve the quality of their digital images in all facets of the histological process, the CAP developed a Histotechnology Quality Improvement Program (HistoQIP) in 2019 specifically addressing digital pathology (HQWSI). This addition to the HistoQIP programs offered surveys common areas of potential concern such as tissue size, placement, and fixation and processing problems as they relate to WSI. More information can be found at cap.org.

Discussion

Digital images of histological tissue sections are a wealth of information that can help a pathologist forge a deeper understanding of patient cases than what manual review alone has offered previously. The advent of digital pathology allows pathology to review more efficiently, break down features of a tissue sample by particular stain, tissue structure, and even individual cell type in terms of morphology and/or phenotype. Thus, it is essential that sections intended for WSI use be optimized to a laboratory's specific digital pathology program, ensuring all slides submitted for scanning will be free from artifacts that may be caused by common preanalytic and analytic processes.

The development of image analysis algorithms geared toward scanned images for specific applications or stains such as ISH (chromogenic or fluorescent) or IHC, adds another layer of importance in terms of fully standardizing laboratory practices to fit newly implemented WSI solutions. Specifically, it is important to keep in mind that similar preanalytical and analytical factors can significantly impact image analysis outcomes, with results potentially varying widely depending on quality discrepancies in tissue processing, staining, and scanning.

Several factors related to slide preparation can contribute to accuracy when using image analysis. Below you will find a list of these laboratory practices with tips to ensure that your final slides for scanning are of the highest quality, thus leading to more accurate and reliable quantitation and scoring when employing algorithms.

- Tissue fixation: The tissue should be properly fixed to preserve its morphology. Timing and volumes optimized to each tissue type will help preventing shrinkage or distortion which can make algorithm use difficult.

- Tissue processing: The tissue should be processed and embedded, depending on tissue type, using standardized processes previously established for WSI and sectioned at a uniform thickness.
- Microtomy: Artifacts such as tissue folds can make it difficult for algorithms to correctly segment tissue structures such as glands and individual cellular components such as nuclei and membranes.
- Slide staining: High quality staining protocols that are consistent and reproducible should be standardized for all tissues that will undergo potential image analysis. Care should be taken to make sure all staining is artifact free and even. Uneven staining can be especially difficult to overcome when implementing algorithms on scanned images as it can make it difficult for them to accurately identify and classify cells.
- Coverslipping: Coverslips should be mounted using a media that will produce a flat and even surface. Care should be taken to make sure the glass is free of dust, debris and annotation marking. Any air bubbles present should also be expelled prior to scanning and analysis as algorithms can misclassify them as cells or other tissue structures.

Additional Resources

Manual review of individual scanned slides is onerous and slows down throughput in high-volume scanning operations. Janowczyk et al. have developed an open-source tool called HistoQC that can perform rapid quality control checks on scanned slides to identify artefacts and outliers (eg, stains that are too dark or light).[5] Bautista and Yagi developed an enhancement technique using color saturation and luminance to improve the detection and visualization of tissue folds from scanned slides.[6] Moreover, several authors have developed methods to correct and/or normalize staining in digital images to accommodate potential and inevitable variations that will arise.[5,6,7] Incorporating these tools into routine practice could soon offer us an automated quality control option that will allow for detection and correction of artifacts, thus improving the overall quality of digital slides.

Frozen section: Tissue folds on frozen section slides for telepathology presented a diagnostic challenge.[8] Other artifacts that compromise diagnosis include uneven section, air bubbles, and sticky mounting medium.

References:

1. Lott R, Tunnicliffe J, Sheppard E, et al. Practical Guide to Specimen Handling in Surgical Pathology. College of American Pathologists. https://cap.objects.frb.io/documents/practical-guide-specimen-handling.pdf. Version 11.0. Published September 2023.
2. Kumar N, Gupta R, Gupta S. Whole Slide Imaging (WSI) in Pathology: Current Perspectives and Future Directions. *J Digit Imaging*. 2020;33(4):1034-1040.
3. Wilbur DC. Digital cytology: current state of the art and prospects for the future. *Acta Cytol*. 2011;55(3):227-238.
4. Engel KB, Moore HM. Effects of preanalytical variables on the detection of proteins by immunohistochemistry in formalin-fixed, paraffin-embedded tissue. *Arch Pathol Lab Med*. 2011;135(5):537-543.
5. Janowczyk A, Zuo R, Gilmore H, Feldman M, Madabhushi A. HistoQC: An Open-Source Quality Control Tool for Digital Pathology Slides. *JCO Clin Cancer Inform*. 2019;3(3):1-7.
6. Bautista, PA., Yagi Y. Staining Correction in Digital Pathology by Utilizing a Dye Amount Table. *J Digit Imaging*. 2015;28:283-294.
7. Victory J, Couture HD, Thomas NE, et al. Appearance Normalization of Histology Slides. *Comput Med Imaging Graph*. 2015;43:89-98.
8. Pradhan D, Monaco SE, Parvani AV, Ahmed I, Duboy J, Pantanowitz L. Evaluation of panoramic digital images using Panotiq for frozen section diagnosis. *J Pathol Inform.* 2016;4(7):26.
9. CAP Validating Whole Slide Imaging for Diagnostic Purposes in Pathology: Guideline Update 2021.
10. Hartman DJ, Pantanowitz L, McHugh JS, Piccoli AL, OLeary MJ, Lauro GR. Enterprise Implementation of Digital Pathology: Feasibility, Challenges, and Opportunities. *J Digit Imaging*. 2017;30(5):555-560.

Contributor Affiliations

Michael LaFriniere, HT(ASCP) – Capitol Choice Pathology Laboratory

Humberto E. Trejo Bittar, MD – Moffit Cancer Center

Marilyn M. Bui, MD, PhD – Moffit Cancer Center

Andrew J. Evans, MD, PhD – University of Rochester Medical Center

Anil V. Parwani, MD, PhD – Ohio State University Wexner Medical Center

Mansoor Nasim, MD, PhD – New York University Langone Health

Robert Lott, BS, HTL(ASCP) – Roche Diagnostic Solutions (RDS)

Liron Pantanowitz, MD, PhD – University of Pittsburgh Medical Center

Antonia Valdez, BS, HTL (ASCP) – Sagis Diagnostics

Caitlin Routhier, HT, QIHC(ASCP) – Mersana Therapeutics

Jamie Pert, HTL, QIHC(ASCP)^{CM} – Corewell Health William Beaumont University Hospital