Original Article

The Importance of eSlide Macro Images for Primary Diagnosis with Whole Slide Imaging

Filippo Fraggetta[1], Yukako Yagi[2], Marcial Garcia-Rojo[3], Andrew J. Evans[4], J. Mark Tuthill[5], Alexi Baidoshvili[6], Douglas J. Hartman[7], Junya Fukuoka[8], Liron Pantanowitz[7]

[1]Department of Pathology, Cannizzaro Hospital, Catania, Italy, [2]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, [3]Department of Pathology, Hospital Universitario Puerta del Mar, Cádiz, Spain, [4]Department of Pathology, University Health Network, Toronto, Canada, [5]Department of Pathology, Henry Ford Health System, Detroit, Michigan, USA, [6]Laboratory for Pathology East Netherlands, The Netherlands, [7]Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA, [8]Department of Pathology, Nagasaki University Hospital, Nagasaki, Japan

Received: 26 September 2018 Accepted: 31 October 2018 Published: 24 December 2018

Abstract

Introduction: A whole slide image (WSI) is typically comprised of a macro image (low-power snapshot of the entire glass slide) and stacked tiles in a pyramid structure (with the lowest resolution thumbnail at the top). The macro image shows the label and all pieces of tissue on the slide. Many whole slide scanner vendors do not readily show the macro overview to pathologists. We demonstrate that failure to do so may result in a serious misdiagnosis. **Materials and Methods:** Various examples of errors were accumulated that occurred during the digitization of glass slides where the virtual slide differed from the macro image of the original glass slide. Such examples were retrieved from pathology laboratories using different types of scanners in the USA, Canada, Europe, and Asia. **Results:** The reasons for image errors were categorized into technical problems (e.g., automatic tissue finder failure, image mismatches, and poor scan coverage) and human operator mistakes (e.g., improper manual region of interest selection). These errors were all detected because they were highlighted in the macro image. **Conclusion:** Our experience indicates that WSI can be subject to inadvertent errors related to glitches in scanning slides, corrupt images, or mistakes made by humans when scanning slides. Displaying the macro image that accompanies WSIs is critical from a quality control perspective in digital pathology practice as this can help detect these serious image-related problems and avoid compromised diagnoses.

Keywords: Digital pathology, error, informatics, macro image, thumbnail, whole slide imaging

Introduction

A whole slide image (WSI) is acquired by digitizing a glass slide with a slide scanner. The WSI represents a digital counterpart of the glass slide and accordingly is intended to capture all of the material present on the slide that was scanned. Typically, different images are generated during the digitization process. These include (a) the macro (or slide label) image that is a low-resolution overview snapshot of the entire glass slide, (b) baseline tiled image often acquired at full resolution, (c) thumbnail which has smaller pixel dimensions, and (d) multiple intermediate images stacked in a pyramid. The macro image is acquired with a low-resolution macro or prescan camera that is different from the high-resolution camera(s) used to scan the entire slide.[1]

With a tiled organization of a WSI, the images are stored in squares or rectangular tiles and formatted as multiresolution pyramids.[2,3] In this pyramid model, the low-resolution images are stacked above images that are of greater resolution. Hence, the many intermediate images are sandwiched between the smallest thumbnail at the top and the largest baseline tiled image at the bottom. This allows each layer to be individually and quickly retrieved to facilitate rapid zooming of the virtual slide.[4] The baseline and intermediate images are often compressed. The sophisticated software used to preencode and assemble a WSI file is extremely reliable, and errors resulting in corrupted files are infrequent.

Access this article online

Quick Response Code:

Website: www.jpathinformatics.org

DOI: 10.4103/jpi.jpi_70_18

Address for correspondence: Dr. Filippo Fraggetta, Department of Pathology, Ospedale Cannizzaro Via Messina 829, 95126 Catania, Italy.
E-mail: filippofra@hotmail.com

How to cite this article: Fraggetta F, Yagi Y, Garcia-Rojo M, Evans AJ, Tuthill JM, Baidoshvili A, *et al*. The importance of eSlide macro images for primary diagnosis with whole slide imaging. J Pathol Inform 2018;9:46.

Available FREE in open access from: http://www.jpathinformatics.org/text.asp?2018/9/1/46/248453

As the macro image represents a snapshot of the entire glass slide, it usually includes the slide label which contains identifiers (e.g., case accession number, barcode, text showing a patient name, and slide level or stain details) linking slides to specific specimens and patients. The barcode is often leveraged to automate image management and/or integration of the acquired WSI with a laboratory information system (LIS). The macro image also provides a low-magnification overview of all of the tissue pieces, marks (e.g., permanent pen markings such as hand-drawn arrows), and empty space present on the glass slide. The macro image serves mainly to guide the scanner's tissue detection system, focus-point selection, and subsequent high-resolution digitization of tissue recognized and/or manually selected by an operator. The term macro image and thumbnail are often used interchangeably. However, they are different. Unlike a macro image, the thumbnail forms part of the pyramid-encoded WSI file that is acquired with a high-resolution camera. The thumbnail corresponds only to the digitized region of the slide that was scanned.

A critical assumption in the use of WSI for patient care is that scanned slides represent completely accurate digital versions of glass slides. It is therefore of paramount importance that all tissue fragments present on glass slides are recognized and captured for review on the resulting digital slides. During scanning blank areas on the slide, where tissue is presumed to be absent, may be skipped if the scanner is programmed to do so. Indeed, certain scanners exploit the opportunity to skip areas on the slide devoid of tissue in order to speed up scan times and generate smaller file sizes. The thumbnail provides an overview of the tissue that was scanned and is typically displayed by default along with the high-resolution WSI where it can be used to guide pathologists as they navigate scanned slides.[5] The macro image provides an overview of the entire slide and indicates the area of the slide that was to be captured during the scanning process (e.g., with a region of interest or bounding box around the tissue). Unlike the thumbnail, the macro image is not necessarily displayed by default by all WSI vendors. Hence, laboratory personnel and pathologists alike need to be trained on how to find and use the macro image as part of basic quality control (QC). In the event of an error where the tissue detection mechanism fails to automatically identify a small pale piece of tissue, a user scanning a slide does not correctly select a region that adequately captures relevant material on the slide, or pre-/postscanning QC protocols are not followed, a potentially serious discrepancy between the tissue displayed in the macro and WSI (or thumbnail) images could result.

Digitizing an entire glass slide is a complex process that depends on the integration of state-of-the-art scanner hardware, robotics, and software. Producing good quality WSIs also depends on the skills of a well-trained operator (e.g., scan technologist) who guides the scanning procedure. Therefore, it is plausible that on occasion errors may occur due to the tissue on a slide being missed during scanning, technical glitches, or a WSI file that gets corrupted. To date, to the best of our knowledge, there has been no published series documenting such errors in digital pathology. Therefore, the aim of this study was to collect and categorize cases in which WSI errors occurred in different pathology laboratories worldwide.

Materials and Methods

Various examples of errors were accumulated that occurred during the digitization of glass slides. The aim of this study was not to report the incidence of such errors. Only errors in which the WSI (eSlide) differed from the macro image of the original glass slide were collected for this study. Examples were solicited from various pathology laboratories in the USA, Canada, Europe, and Asia. Each example submitted required a detailed explanation, if available images to document the error (e.g., screenshot), and any potential clinical impact that resulted. Examples of errors were attained from different types of scanners including an Aperio AT2 (Leica), Ultra-Fast Scanner (Philips), Pannoramic 250 (3DHistech), Nanozoomer (Hamamatsu), and iScan HT (Roche). Errors received were categorized into technical (scanner) and/or operator (manual) related causes and further evaluated for similarities and differences. Actual slide labels in some cases are displayed in order to illustrate the error that occurred; however, we believe that individual patients cannot be identified solely from these images.

Results

Nine different types of errors were compiled. Seven of these occurrences were due to technical problems, and in two instances, they were the result of a mistake made by an operator during scanning [Table 1]. In all cases, pathologists examining the macro image discovered these errors, and no case resulted in a clinical misdiagnosis.

Technical errors

In one case, a H and E-stained glass slide of a gastric biopsy comprising multiple tissue pieces, when scanned at ×20 (Aperio AT2 scanner), was missing a small tissue fragment in the WSI [Figure 1]. The automated tissue finder tool did not detect and therefore initiate complete scanning of this tissue fragment. The missing piece of gastric tissue was readily identified in the accompanying macro image. A similar case was reported in which a glass slide scanned on an Ultra-Fast Scanner (Philips) showed several small tissue fragments in the macro image that were not present in the

Table 1: Different types of recorded errors related to whole slide imaging*

Technical errors	Human errors
Automated tissue finder failure	Failure to follow prescan quality control protocol
Skip blank stripes failure	
Mismatched macro with whole slide image	Failure to follow postscan quality control protocol
Failure in sensitivity of the scanner	

*Some errors may present as a combination of technical and human

WSI [Figure 2]. Another case was uncovered in which a much larger portion of tissue identifiable in the macro image was missed with scanning (Hamamatsu XR scanner) [Figure 3]. One laboratory reported an incident in which a glass slide of a prostate biopsy scanned using the skip blank stripes mode (Aperio AT2 Turbo scanner) not only skipped blank stripes but also skipped one of the core fragments present on one of two levels of the slide.

In another case where a glass slide was scanned at ×20 (Aperio AT2 scanner), a mismatch was noted between the macro image and corresponding WSI [Figure 4]. A related mismatched case was identified at another facility involving two cases that were scanned for immunofluorescent analysis (Pannoramic 250, 3DHistech) where the label image and accompanying WSI in one of the cases were incorrectly duplicated [Figure 5]. At a different medical center, when the same H and E-stained glass slide was scanned on different scanners, the WSI was missing an area of tissue [Figure 6], even though the macro image incorporated this region.

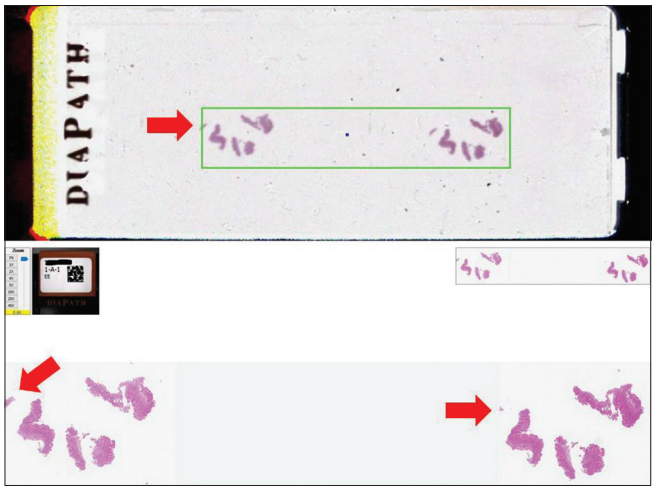

Figure 1: Whole slide image missing a tissue fragment. (Top image) Macro image showing a green box placed by the tissue finder tool on the region to be scanned. Arrows indicate the small piece of tissue on the edge that failed to adequately scan. (Bottom image) Thumbnail image showing that the small tissue fragment on the edge of the section was truncated (left part of image, red arrow) or missing (right part of image, red arrow) in the whole slide image

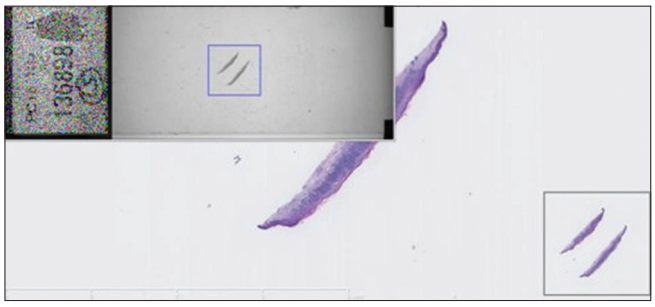

Figure 3: A scan error is shown where a slide of a shave biopsy with two portions of tissue, both seen in the macro image, only displayed one of the tissue pieces in the whole slide image

Operator errors

One hospital discovered an incident with a prostate biopsy attributed to scanner operator error and failure to follow pre- and postscan QC protocols. The pathologist involved received a set of prostate core needle biopsies where two cores are routinely procured from six anatomical sites of the prostate gland. The two cores from each biopsy site were submitted as separate specimen parts. The H&E-stained slides were all scanned using an Aperio AT2 Turbo device. The pathologist was expecting to see the usual two cores per site in the digital slides. However, for one part the pathologist immediately noted only one core was present and became suspicious. Review of the gross description confirmed that

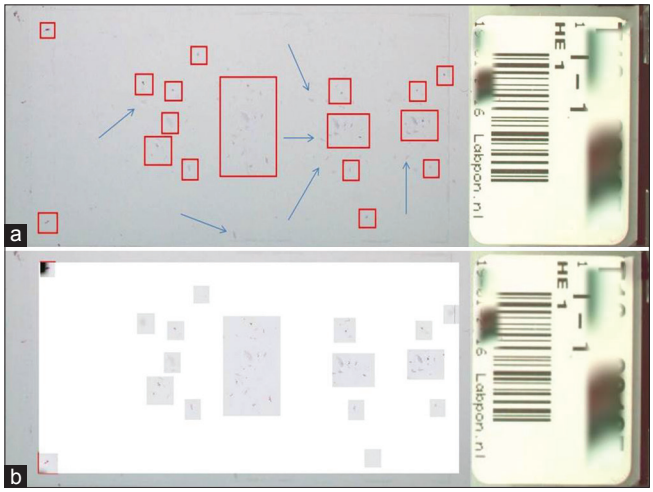

Figure 2: Whole slide image missing tissue fragments. (a) Macro image showing multiple red boxes containing pieces of tissue detected by the tissue finder algorithm, as well as extra tissue fragments (blue arrows) that were not detected. (b) Whole slide image thumbnail showing only those tissue fragments present within selected regions that were scanned

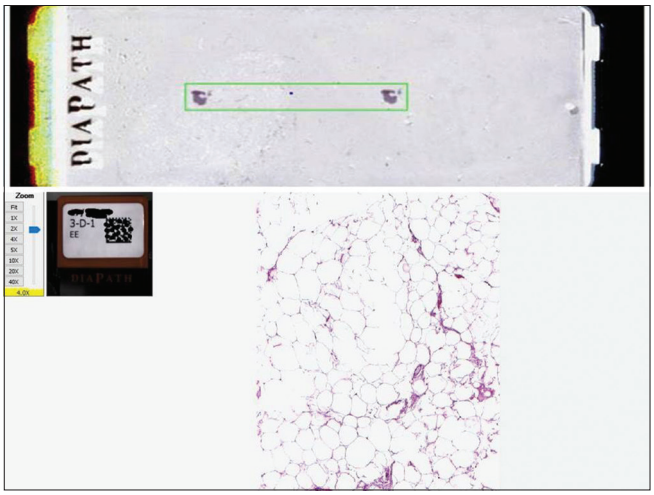

Figure 4: Mismatched macro and whole slide image. (Top image) The macro image accurately shows two H&E stained sections of a small fibromuscular tissue biopsy. (Bottom image) The whole slide image in this case unexpectedly shows adipose tissue that was not present on the glass slide

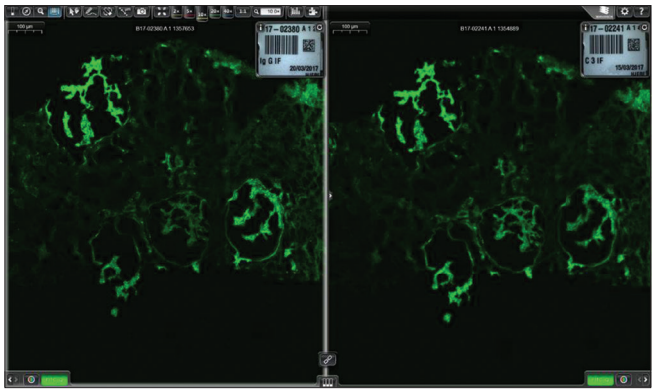

Figure 5: Mismatched label image and whole slide image. (Left) A scanned renal immunofluorescence slide showing the label (IgG stain) correctly associated with the whole slide image. (Right) A scanned slide of a skin biopsy showing the correct label (C3 stain) that is incorrectly associated with the wrong whole slide image from the renal immunofluorescence slide

two cores were received. This prompted a review of the macro image which showed the scan operator had placed a green box, defining the region to be scanned, on only a portion of one core [Figure 7]. In this case, the mandated prescan QC protocol established at this hospital to ensure complete capture of tissue on scanned slides was not followed, which stipulated that scanning personnel are to verify that the green bounding box captures all tissues on all slides before they get scanned. Immediate postscan QC review of the affected slides should also have identified the missing tissue, as prostate biopsies from this hospital contain two cores in each part almost without exception.

When validating an iScan HT scanner (Roche) for digitization of immunohistochemical slides at an academic medical center, the department of pathology noted that for some of the slides being scanned a portion of the tissue was truncated or entirely missing in the WSI. Inspection of the macro image revealed that this problem arose when there was poor tissue placement on the slide (e.g., when tissue was too close to the slide edge), and as a result, the designated preset box to define the region of interest to be scanned did not include all of the tissue on the slide [Figure 8]. When identified, the error required human intervention by the operator to select a new more inclusive area to be scanned.

Discussion

Much progress has been made in the field of digital pathology. Many validation studies have adequately proven that WSI is noninferior to viewing glass slides with a conventional light microscope for diagnostic work.[6,7] In the United States, the Food and Drug Administration even approved the first WSI system in 2017 for primary diagnosis in surgical pathology.[8] Several pathology departments worldwide have reported their successful journey toward going fully digital.[9-11] As the adoption of WSI continues to expand, standardization and best practice guidelines will become increasingly important to promote safe implementation and application of these

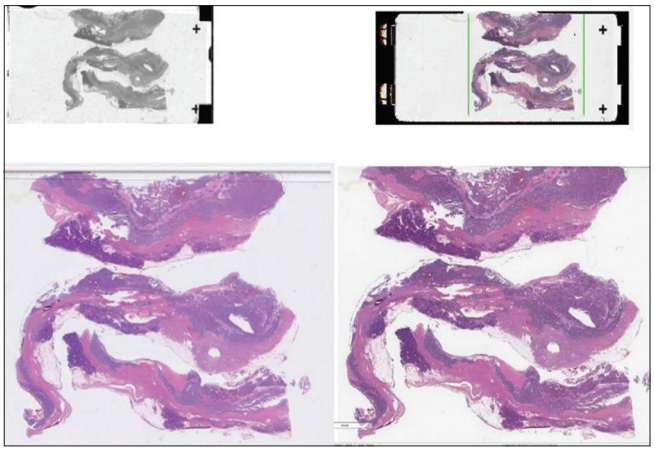

Figure 6: Whole slide image scanners with different scan coverage. An H and E stained slide with several large portions of tissue is shown in which one fragment at the top of the slide extends beyond the coverslip. (Left) The black and white macro image correctly shows all tissue pieces including the portion extending beyond the coverslip; the corresponding whole slide image accurately scanned all of the tissue (Hamamatsu HT2). (Right) The macro image is shown with a green box identifying the tissue region to be scanned, including the tissue extending beyond the coverslip; the corresponding scanned whole slide image is missing part of the tissue near the upper edge of the slide (Aperio AT2)

systems. These efforts should include measures to prevent and recognize potential errors related to scanning.

The present study highlights genuine failures and problems that have occurred when using WSI instruments to scan glass slides. These problems, due to either technical or operator errors, arose using different scanners in a number of pathology laboratories. Technical errors observed were related to either failure of automatic tissue finder algorithms to reliably detect and scan all pieces of tissue on a slide, inaccurate scan coverage thus missing or truncating tissue during scans, and mismatches ensuing between the label and WSIs. Mismatches with macro/label images and the WSI signify a serious technical glitch, as they imply a likely error with the software process of serially encoding a WSI file. Evidently, errors can also arise when human operators improperly preset or manually select a region of interest to be scanned. All of these errors could potentially result in a serious misdiagnosis, with negative clinical and legal consequences. Fortunately, in all of the cases we have shared the errors were caught when viewing the accompanying macro image. Unfortunately, such macro images are currently often only of low-resolution; therefore, it may not always be easy to distinguish missing small tissue fragments from dirt or other artifacts in these images.

Rendering an accurate diagnosis using WSI rests on the premise that a WSI represents an accurate digital reproduction of the scanned glass slide. If diagnostic material present on a glass slide is missing in the digital image, this could result in a misdiagnosis. In the published guideline from the College of American Pathologists for validating WSI for diagnostic purposes one of the recommendations for pathology laboratories

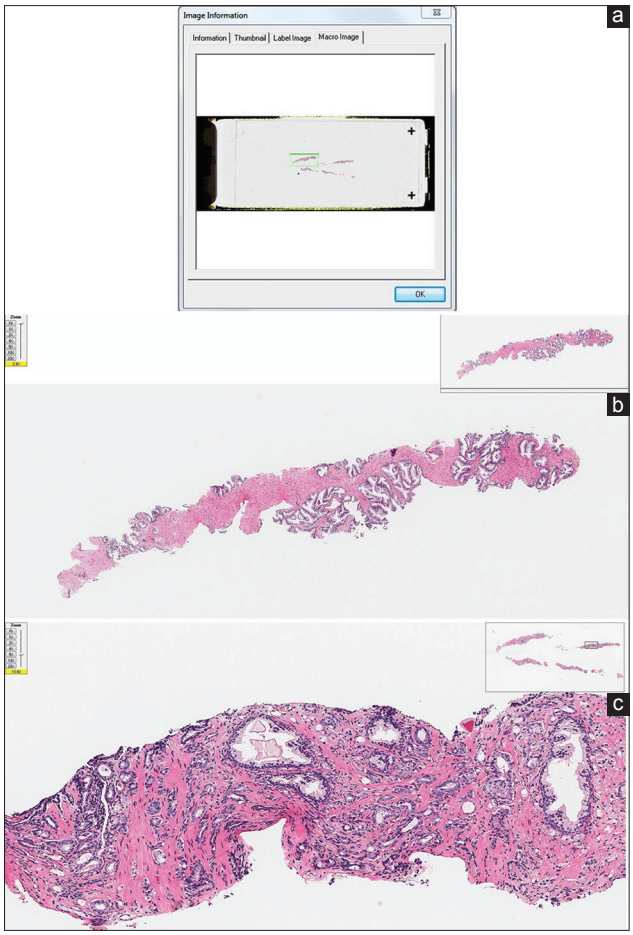

Figure 7: Prostate biopsy tissue erroneously excluded from scanning. (a) The macro image shows two core biopsies and that the operator has placed a green box around only part of one core biopsy. (b) The accompanying whole slide image of this partially scanned core biopsy was benign. (c) When the same glass slide was scanned again, this time with all cores included, the new digital slide contained all tissue fragments, one of which showed prostate adenocarcinoma

is to confirm that all of the material present on a glass slide to be scanned is included in the digital slide.[12] However, there have been no recommendations offering a practical method on how to address this issue. Based on the findings in our study, we recommend employing macro images for this purpose. Routinely displaying macro images, and not just thumbnails, can help detect serious image-related problems such as missing pieces of tissue. Vendors of WSI systems are accordingly encouraged to always display the macro overview of an entire slide and label in their workflow and/or case management software. In the Pathology Department at Cannizzaro Hospital in Italy where all histopathology glass slides routinely get scanned, a virtual slide tray showing the macro image of all eSlides is made available within the LIS for this exact reason.[11] Practice procedures, such as proper placement of appropriately sized pieces of tissue and limiting scanning to only relevant tissue-containing areas or skipping areas that appear to be blank, may need to also be carefully evaluated in order to avoid accidental errors where tissue can be missed during scanning.

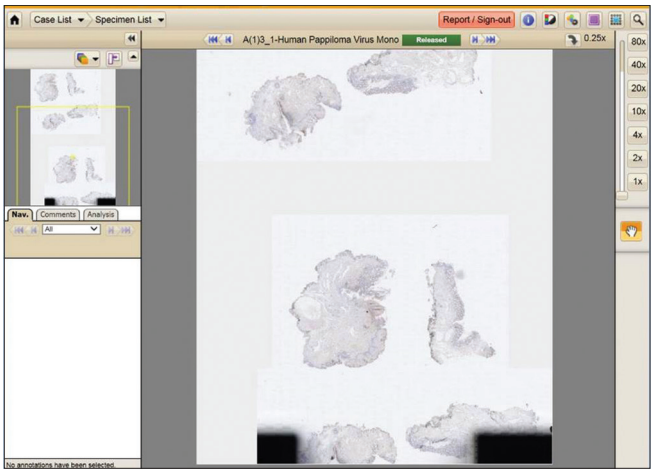

Figure 8: Missed tissue during scanning. The small macro image (left) shows that the predefined yellow box in this case does not include all of the tissue. The whole slide image (right) is missing those pieces of tissue located outside the selected region to be scanned

The aim of this study was to underscore the importance of potential errors that may occur with WSI and not to report their incidence. Nevertheless, in one of the author's (FF) laboratories, they initially experienced problems related to tissue detection in 2% of their digitized cases, but after making adjustments to the tissue finder on their scanner, this error rate dropped to below 0.5% of eSlides. The findings described herein must also be interpreted within some context. First, these are rare occurrences especially if proper attention is paid to QC and quality assurance (QA) measures pertaining to slide preparation as well as during and after implementation of WSI systems for diagnostic purpose. Their rarity is exemplified by the fact that the authors had to approach eight laboratories with extensive experience in the use of WSI in order to collect the aforementioned examples. Nonetheless, these errors can have devastating consequences for patients, pathologists, clinicians, and the field of digital pathology as applied to patient care. They illustrate the need for continuous vigilance on the part of laboratory personnel involved in scanning slides and the pathologists who view them in diagnostic settings. While some of the issues described in this report are unique to WSI and the slide scanning process, the concept of shared responsibility for QC and QA by technical staff and pathologists applies equally to traditional diagnostic workflow based on glass slides and light microscopy. However, manual quality checks should not become too overburdensome, especially since adoption of digital pathology is intended to promote efficiency and automation. Finally, it would be reassuring to see advancements from the manufacturers of these devices to help address these errors such as increasing the resolution of macro images, improving automated tissue finders, and introducing alerts when tissue fragments are missing or too close to the border.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Pantanowitz L. Whole slide imaging. In: Pantanowitz L, Parwani AV, editors. Digital Pathology. Chicago, USA: ASCP Press; 2017. p. 59-76.
2. Daniel C, García Rojo M, Bourquard K, Henin D, Schrader T, Della Mea V, *et al.* Standards to support information systems integration in anatomic pathology. Arch Pathol Lab Med 2009;133:1841-9.
3. Singh R, Chubb L, Pantanowitz L, Parwani A. Standardization in digital pathology: Supplement 145 of the DICOM standards. J Pathol Inform 2011;2:23.
4. Soenksen DG. A fully integrated virtual microscopy system for analysis and discovery. In: Gu J, Ogilview RW, editors. Virtual Microscopy and Virtual Slides in Teaching, Diagnosis, and Research. Boca Raton: CRC Taylor & Francis; 2005. p. 35-47.
5. Thrall MJ, Rivera AL, Takei H, Powell SZ. Validation of a novel robotic telepathology platform for neuropathology intraoperative touch preparations. J Pathol Inform 2014;5:21.
6. Williams BJ, DaCosta P, Goacher E, Treanor D. A systematic analysis of discordant diagnoses in digital pathology compared with light microscopy. Arch Pathol Lab Med 2017;141:1712-8.
7. Mukhopadhyay S, Feldman MD, Abels E, Ashfaq R, Beltaifa S, Cacciabeve NG, *et al.* Whole slide imaging versus microscopy for primary diagnosis in surgical pathology: A Multicenter blinded randomized noninferiority study of 1992 cases (Pivotal study). Am J Surg Pathol 2018;42:39-52.
8. Abels E, Pantanowitz L. Current state of the regulatory trajectory for whole slide imaging devices in the USA. J Pathol Inform 2017;8:23.
9. Stathonikos N, Veta M, Huisman A, van Diest PJ. Going fully digital: Perspective of a Dutch academic pathology lab. J Pathol Inform 2013;4:15.
10. Cheng CL, Azhar R, Sng SH, Chua YQ, Hwang JS, Chin JP, *et al.* Enabling digital pathology in the diagnostic setting: Navigating through the implementation journey in an academic medical centre. J Clin Pathol 2016;69:784-92.
11. Fraggetta F, Garozzo S, Zannoni GF, Pantanowitz L, Rossi ED. Routine digital pathology workflow: The Catania experience. J Pathol Inform 2017;8:51.
12. Pantanowitz L, Sinard JH, Henricks WH, Fatheree LA, Carter AB, Contis L, *et al.* Validating whole slide imaging for diagnostic purposes in pathology: Guideline from the college of American Pathologists Pathology and Laboratory Quality Center. Arch Pathol Lab Med 2013;137:1710-22.