Research article

Introducing Digital Pathology in Fast-Frozen Section by Validating the Whole Slide Imaging Scanner Slideview VS200 Research Slide Scanner

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Background: The implementation and usage of digital pathology has undergone a huge development in recent years, However, the area of intraoperative consultation has not yet become digitized or fully investigated. The aim of this study was to explore the possibilities to digitize this area and evaluate the consistency between the diagnoses based on the digital slides versus the traditional microscopic review. The whole slide image scanner Olympus VS200 STL proclaimed that it would be able to scan glass slides with wet mounting glue.
Method: To find the optimal scanning profile a field of four different scan profiles were tested in different contexts. The chosen profile was used to generate 126 digital slides from 60 cases. These slides were assessed on three parameters; compliance between diagnosis of the digital and traditional method, compliance with the visual quality and could the scan time including operating time be completed within 180 seconds per slide.
Results: The overall result showed no deviation between diagnoses made with conventional microscope and the digital slide in 83% of the cases and the average operation time was 92.5 seconds.
Conclusion: Olympus VS200 STL has the potential to become implemented in a clinical pathology department for use in intraoperative diagnostics without affecting workflow, diagnostic accuracy, and demonstrates an acceptable time for review (180 seconds per slide).

Key words: Digital pathology, imaging, microscopy, pathology, whole slide image, fast-frozen section

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Introduction

Digital pathology has experienced a tremendous development in the last decade. Based on this evolution many pathology laboratories have or are in the process of exchanging traditional microscopes with whole slide imaging (WSI) scanners and digital microscopy. The labs are on the way to total digitization.[1-5] One area of pathology, intraoperative consultation (fast-frozen section), has the potential of becoming digitalized but this has not yet been fully investigated or well documented in the literature.[6-9] It has previously been shown that slides from frozen sections, can be scanned successfully and a correct diagnosis can be made from the scans.[6-9] However, only very few studies are based on glass slides where the mounting glue still is wet (wet slides) or within a time frame of minutes, which is the most realistic condition for working with fast-frozen sections in a clinical pathology setting. Therefore, there is little to no documentation to the best of our knowledge, on how WSI scanners handle the emergency function fast-frozen section, which can pose a problem as many pathology laboratories are going digital.

The fast-frozen section procedure is often required in many major hospitals which perform an extensive number of cancer-related surgeries. The surgeons need the diagnostic answer, presence of malignant cells or not, as fast as possible because the patient is still under anesthesia. Thus, the surgery can continue as soon as the diagnostic answer from the pathology department is available. Some of the reasons why the frozen section has not yet become digitized and implemented in the clinical pathology workflow are due to the WSI scanners available on the market. The scanners have not demonstrated the ability to perform successful scans on wet slides or perform the scan fast enough with satisfying results. Time and digital quality are of the essence in a clinical pathology department when working with fast-frozen sections, and a slide scan is an extra step in the workflow. Therefore, the operation of the WSI scanner must be easy and manageable, which also has been a challenge with the WSI scanners. The scanners have often been too complicated to use when a quick turnaround time is required for results.

The issue of digital quality, operation and scanning time in terms of being able to scan wet slides, is probably the most interesting for laboratories that perform many fast-frozen sections. This validation study was completed in the department of Pathology at Rigshospitalet, Copenhagen in 2020. The department performs about 75 to 80 urgent cryoslides per day and thereby this function constitutes a large part of the laboratory workload. In order to meet the urgent need for obtaining results, the maximum whole slide operation and scan time of a single wet slide should take no more than 180 seconds (sec.) per slide. This limit was set by experienced clinical staff at the pathology department at Rigshospitalet.

Therefore, it's important how these issues can be addressed and solved so that fast-frozen section can become digitized without compromising the diagnostic quality. Not only to achieve the obvious reasons by becoming digital, such as telemedicine and less transportation time but for the sole reason of becoming digital so future tools like artificial intelligence (AI) and machine learning (ML) can be used. However, pathology departments have been prevented from implementing digitization in this frozen section area, as there are very few validation studies on how to digitize a fast-frozen section laboratory. On the marked a new WSI scanner from Olympus; Slideview VS200 Research Slide Scanner (VS200 STL) holds potential and may fulfil the department's requirements.[10]

The aim of the current study was threefold. First, is the WSI scanner SLIDEVIEW VS200 Research Slide Scanner capable of scanning wet slides. Second, is the total operation and scan time within the limit of 180 sec. per slide, and finally, is the scanning quality high enough to contribute to a correct and credible diagnosis?

Materials and Methods

To ensure the most standardized and valid method on how to validate WSI systems for diagnostic purposes in pathology the guidelines *Validating Slide Imaging for Diagnostic Purposes in Pathology: Guideline from the College of American Pathologists (CAP), Pathology and Laboratory Quality Center* from 2013 were utilized.[11] The study still fulfils the guideline recommendations from 2022.[12] It was also sought in the project that the virtual rendering of the digital slides mimicked what one might expect in the traditional microscope. It was also emphasized that assessments and decisions based on subjective judgement were made based on experience and professional knowledge.

In the first part of the validation of the slide scanner, it was essential to explore the best settings for the scanning profile, where magnification/resolution, autodetection and number of focus points were included. It was important that the process was fast and that the digital slides had the same morphological quality one could expect in the traditional microscope. A x20 magnification was used, which is the usual magnification used in the pathology department at Rigshospitalet. This magnification is also supported by Borowsky et al. as suitable.[2] Furthermore, the pre-program scan settings on autodetecting tissue on the VS200 STL were utilized. The selection included the amount of focus points needed for the best scan profile after a test of the four predefined settings by the company: *LOW, NORMAL, HIGH* and *EXTRA HIGH.*

The four options were tested on 26 randomly selected cryoslides, where 20 of them were wet slides. The slides were obtained from the workflow of the fast-frozen section laboratory at Rigshospitalet, where the freezing method used was Prestochill.[13] A quadviewer (Olyvia 3.1) was used for the histological assessment of the four possible focus point settings. The rating was blinded to avoid bias and performed by experienced laboratory personnel.

In the second part of the validation of the slide scanner, the slide scan time composed of the scanner operating and scanning time was evaluated, to assess the 180 sec. time limit per slide.

Measurements of the operating time were performed 20 times with two cryoslides at the same time. The operating time was defined as the time from mounting the glass cover to beginning the scanning of the slides. This part of the validation study aimed to investigate whether waiting time could occur and thereby create a potential possible bottleneck for the pathology department.

In the third part of the validation of the slide scanner, how the scanner handled different types of tissue was evaluated and if there would be any variation in the diagnoses made using a conventional microscope compared with diagnosis made using a digital scanning. The way in which it was investigated whether there was a discrepancy between the diagnoses (malignant or not), was performed independently by two senior consultant pathologists experienced in a clinical pathology laboratory setting performing diagnostics on fast frozen tissue for more than 20 years within gastrointestinal pathology, head and neck pathology and breast pathology, respectively. The samples were first assessed by using conventional microscope of the fast frozen slide, and after a washout period of more than 14 days. The pathologists reviewed the same slides again based on the digital scans. In fast frozen sectioning assessment, the pathologists distinguish tumors from other lesions and distinguish malignant from benign tumors as these can directly affect the patient's treatment decision. The diagnosis is primarily based on whether malignant cells are present or not.

Fresh tissue samples from breast, gastroenterological and otolaryngology surgery were included in the study. The tissue types had different textures, shapes, and sizes, but were not larger than 20x60mm. Samples originated from 60 different patients with a roughly equal distribution between men and

women. The age range was from 39 to 91 years. The data collection and storage were performed in compliance with the Danish Data Protection Agency.

By using different types of tissue samples, it was possible to explore how different pathology subspecialties will be affected by replacing the traditional microscope with WSI and whether there are differences between them. This also allowed the laboratory to get an indication of how tissue samples from e.g., otolaryngology, which receives a large proportion of freezing samples, could be affected by a possible WSI implementation. The type of tissue, number of cases and slides are listed in Table 1.

Table 1. Table of the tissue types within the three pathology subspecialties, as well as number of cases and number of slides.

Tissue type	Number of cases	Number of slides
Breast tissue		
- Sentinel lymph node	20	40
Gastrointestinal tissue		
- Esophagus	3	6
- Pancreas	5	12
- Peritoneum	3	6
- Ductus hepaticus	4	8
Liver	2	4
- Other types of tissues from gastroenterology	3	7
Otolaryngology		
- Tonsil	5	12
- Tongue	2	4
- Plica aryepiglottica	2	4
- Other types of tissues from Otolaryngology	11	23
Total	60	126

The total amount of 126 cryoslides fulfil the College of American Pathology (CAP) guideline recommendations.[11,12] The diagnostic results are obtained by comparing the original fast-frozen diagnosis (conventional microscopy) to the diagnoses based on the WSI. If discrepancies arose between the two diagnoses, this would either be confirmed or not by means of the verification slide, which was considered as the true value of the diagnosis. A formalin-fixed paraffin-embedded (FFPE) slide, made from the tissue, post the fast-frozen workflow, was used as the verification slide (golden standard) in this study.

There was a distinction between major and minor discrepancies. Major discrepancies were defined as where the discrepancy in the diagnosis would be significant for the sample's further examination in the pathology department or whether the patient should undergo alternative analyzes, examinations or treatments. Minor discrepancies would not cause a change for the sample's further examination in the pathology department or changed patient treatments if the alternative diagnosis had been made e.g., identification of inflammation. This way of assessing minor or possibly major inconsistencies has been used before in other studies.[2,6]

Results

The results of the assessment of the four possible scan profiles *LOW* and *EXTRA HIGH* were opted out either because of demonstrated low morphological quality or unacceptable scanning time extension in relation to morphological quality achieved. An illustration of how the quadviewer presented the four scan profiles (*LOW, NORMAL, HIGHT* and *EXTRA HIGH)* is shown in Figure 1. Based on the results, the best scan profile was chosen to be the focus point setting *HIGH*. This profile had an average scan time of 95.5 sec. per slide (the shortest scan time was 46 sec. per slide, and the longest scan time was 189 sec. per slide), based on 20 wet slides and therefore there was a presumption that the time limit of 180 sec. per slide could be met. Furthermore, the *HIGH* profile showed no area where the slides were out of focus or blurred compared to using both *LOW, NORMAL* and *EXTRA HIGH* profile.

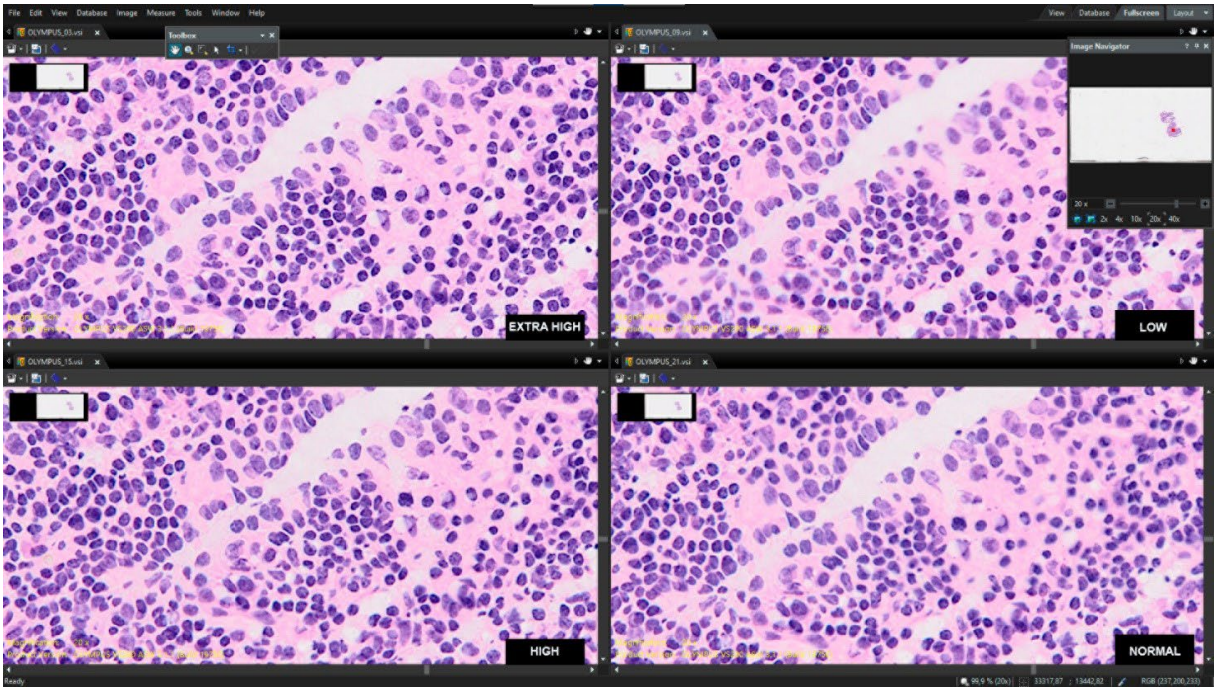

Figure 1: Random placement of the four scan options (LOW, NORMAL, HIGH and EXTRA HIGH) in the quadviewer from Olyvia 3.1 to illustrate how the assessment was performed.

Table 2. The average scanning time distributed within the three subspecialties. The calculated average total slide scan time/slide is also included.

Tissue type	Number of slides	Average scanning time/slide	Average operation time/slide	Average total slide scanning time/slide
Breast tissue	40	170.6 sec	26.4 sec	197.0 sec
Gastroenterology	43	146.5 sec		172.9 sec
Otolaryngology	43	100.0 sec		126.4 sec
All three tissue types	126	137.8 sec		164.2 sec

This assumption was confirmed as the average scanning time for all the 126 cryoslides was 137.8 sec. per slide (Table 2). The result of the additional task in the validation study, about how long it took to operate VS200 STL had an average time of 26.4 sec. per slide.

Based on golden standard assessment of the cases, the total count of 19 malignant cases and 41 benign cases with an overall total of 60 cases. Diagnosis with either minor or major deviations was compared to the diagnosis made from the FFPE tissue slide using conventional microscopy (the verification slide). The overall result showed no major discrepancies between the two diagnoses made by using the conventional microscope compared by using the digital slide in 50 out of 60 cases, this corresponds to 83% of the cases. An overview of the results can be seen in Table 3.

In total 7 minor diagnostic deviations were identified. Of minor diagnostic deviations, 1 out of 7 made by the conventional microscope was not in alignment with the verification slide. The 6 minor diagnostic deviations were due to additional findings of ulcerations and acute/chronic inflammation in the digital slide.

In total 3 major deviations were identified. Of major diagnostic deviations, 2 out of 3 made by the conventional microscope were not in alignment with the verification slide due to the finding of 1) ulceration and 2) adenocarcinoma (digital microscopy) versus the finding of 1) radiation damages and 2) no sign of squamous

Table 3. Description of the five types of discrepancies that could be judged between the traditional microscope and the digital slides. The number of cases is stated as well as in percentage.

Description of compliance between the fast frozen diagnosis using a traditional microscope and the digital slide	Cases	Percentage
No deviation between the two diagnostic methods	50	83%
Minor deviation and in accordance with the verification slide	1	2%
Minor deviation which is not seen in the verification slide	6	10%
Large deviation and in accordance with the verification slide	2	3%
Large deviation which is not seen in the verification slide	1	2%

squamous cell carcinoma (digital microscopy) versus the finding of a suspect lymphoma (conventional microscopy).

Discussion

The overall conclusion of the results is that there will be no change in the treatment of the patient in 98% (59:60) of the cases, where the diagnosis is made on a digital slide instead and compared with the verification slide.

The expectation for the validation of slide scanner VS200 STL was that with only small variations it would be possible to make the same diagnosis on the digital slides that had previously been made by using the traditional microscope. In a study by Bauer et. al a validation of a WSI scanner for use in the fast-frozen section had set a limit of 4% for large discrepancies in the variations between the diagnosis.[3] However, a final limit on how much discrepancy can be accepted between the two diagnostic methods is difficult to decide. The difference may be due to intra- and interpersonal variations and variations may also occur in different tissue types and specialties. As a result of this study, the 4% limit was also accepted as it is supported by the study from Borowsky et.al.[2]

In addition, the 126 cryoslides were dry when scanned. This prevented disrupting the routine procedure in the fast-frozen section laboratory because patients were in real time surgeries and there were surgeons waiting for diagnostic responses (presence of malignancy or not). Therefore, the process could not be delayed in any way, by scanning the slides before the diagnosis has taken place. One potential method to scan the 126 cryoslides without delaying the diagnostic work could have been to cut an extra slide on the specific tissue sample. This could have been problematic when the two diagnostic answers had to be compared as the extra slide would not have been 100% identical to the first diagnostic slide. It was also impossible to scan the 126 cryoslides after the diagnosis was released as the mounting glue would have been completely dry at this point. The second reason for not using wet slides, was the results obtained during the preliminary testing were completed to find the most optimal settings for the Scanning profile. During this testing both wet and dry slides were used. In these tests, the wet slides were presented as superior in quality to the dry slides. The reason for this was that no glue artefacts were present in the slides until the slides dried. As for the expectation of messy work with having wet glue in a scanner, this did not seem to be the case with this scanner. With the slides being locked in position the provided racks, and the racks always staying in a horizontal position, the scanner was never actually in contact with any of the slides and therefore the wet glue.

The study hypothesis was that it would be possible to scan slides and then diagnose subsequently, but it was unknown at what speed or what image quality would be possible. It is a matter of finding the point where the image quality is adequate and maintain the time frame of 180 sec. per slide. The possibility of this improvement could be achieved by examining the VS200 STL autodetection mechanism in more detail.

The diagnostic results obtained in the study on the digital slides had great compliance with the diagnoses obtained by the traditional method and even though the approach was

very close to how the clinical work usually proceeds, the limitation of the study removed the psychological pressure from the pathologists during the process. In this study, the pathologists could review and determine a diagnosis from the digital slides without time pressure and with the knowledge that the patient was already in treatment, so the results were without consequences. According to the CAP guidelines, the optimal validation, conventional versus digital assessment should have been performed in random order.[11,12] However, this was not possible as surgeons were waiting for a diagnostic answer.

The way the verification slide was used in the study was not optimal. It was only used when there was a discrepancy between the two diagnoses from the fast-frozen section made using digital slide and traditional microscopy. It could be interesting also to examine the verification slide when the two diagnoses were consistent. Since this has not been investigated, there is no information as to whether there were any undetected irregularities or if there would have been a different result. Another possible shortcoming was that gynecology was not included in the validation study, although Borowsky et.al. included it, where it was referred to that it can be complicated and with major deviations to place diagnoses on gynecology. However, the tissue types included in this study were a good representation of what is being treated and examined in a pathology department. Although the tissue samples from breast surgery were too monotonous, it was the fact that only the sentinel node was included and no other type of breast tissue. A reason for this was that the cases were not specifically selected for the study. Instead, a date was chosen, further back than the necessary washout period, and from there the last 20 cases that were handled by the participating pathologists were selected within the chosen subspecialties. Tissues from neurology were unfortunately not included. It would have been interesting to include to see if it would be as easy to diagnose as the other tissue types included in the validation study.

Conclusions

The results of this study, within the set limits, demonstrates that wet slides from fast-frozen sections could be handled (scanner's operation time) and scanned within a time limit of 180 sec. It was also demonstrated that reliable and credible diagnoses (95 % sensitivity) could be achieved. It was also demonstrated that reliable and credible diagnoses based on whether malignant cells were present or not could be achieved as well on the digital slides as by using the traditional microscope. Overall, the study showed that implementation of a whole slide imaging scanner (e.g., Slideview VS200 research Slide Scanner, Olympus) has the potential of being implemented in a clinical pathology department for use in the fast-frozen intraoperative diagnostic section without affecting the laboratory workflow and maintaining the diagnostic accuracy.

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