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Analysis of application of digital image analysis in histopathology quality control

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ABSTRACT

Introduction: A correct histopathological diagnosis is dependent on an array of technical variables. The quality and completeness of a histological section on a slide is extremely prudent for correct interpretation. However, this is mostly done manually and depends largely on the expertise of histotechnician. In this study, we analysed the application of digital image analysis for quality control of histological section as a proof-of-concept.

Material and methods: Images of 1000 histological sections and their corresponding blocks were captured. Area of the section was measured from these digital images of tissue block (Digiblock) and slide (Digislide). The data was analysed to calculate DigislideQC score, dividing the area of tissue on the slide by the tissue area on the block and it was compared with the number of recuts done for incomplete section.

Results: Digislide QC score ranged from 0.1 to 0.99. It showed an area under curve (AUC) of 98.8%. A cut-off value of 0.65 had a sensitivity of 99.6% and a specificity of 96.7%.

Conclusion: Digiblock and Digislide images can provide information about quality of sections. DigislideQC score can correctly identify the slides which require recuts before it is sent for reporting and potentially reduce histopathologists' slide screening effort and ultimately turnaround time. These can be incorporated in routine histopathology workflows and lab information systems. This simple technology can also improve future digital pathology and telepathology workflows.

Introduction

Quality control (QC) is integral to any medical laboratory setup. Using automation, medical laboratories like haematology and biochemistry have overcome many challenges related to quality control. QC in histopathology had primarily been focused on the analytical aspects and staining.[1] However, tasks like tissue embedding and paraffin block section are still manually done, even in an advanced histopathology laboratory with automated instruments. Quality control in these steps is still largely subjective and dependent on the observation and expertise of the histotechnician. Presently, there is no objective method to evaluate these steps of histopathology workflow.

Histopathological diagnosis critically depends on the completeness of tissue present on the glass slide.[2] This becomes even more important in cases where the clinician submits smaller tissue bits, e.g., endoscopic, needle, and punch biopsies; there is a high probability of tissue loss during tissue processing. In such cases, the loss of even a tiny tissue area on the slide can lead to significant information loss about the tissue and recut is requested by the histopathologist. This can lead to delays in reporting and even repeat biopsies in such patients leading to delays in treatment.

In large histopathology workflows, it becomes difficult for a histotechnician to manually compare each slide with its block to check for the completeness of the section and the need for further sectioning of the block. Due to this, problems like suboptimal sectioning, section folding, and tissue loss during slide preparation and staining go unnoticed, and such slides get submitted for reporting.[3] Often, histopathologists have to ask for corresponding tissue block from the technicians to check if the tissue is adequately sectioned or not. This takes away the valuable time and effort of the pathologist and laboratory staff.

This is even more relevant for a future era of telepathology, digital pathology, and pathology artificial intelligence (AI) technologies. The slides will be scanned using a whole slide imaging (WSI) system, and the histopathologist may be reporting from a remote location. In such a situation, an objective method will be needed to check for the completeness of the biopsy section on the slide.[4,5]

An automated system can be incorporated at the stage before slide submission for reporting or WSI, which can record the images of the tissue block and corresponding slide. After this, an objective comparison of the tissue on the block with the tissue on the slide can be made to check the adequacy of the tissue on the final slide. This will help predetermine the need

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for re-sectioning before the slide is submitted for reporting or WSI, saving precious time and resources.

Only a few studies have highlighted the need for recuts of paraffin-embedded tissue to better represent tumor area, margins, and depth of invasion. A study by Manyam et al.[6] revealed that 47.5% of cases required deeper sectioning because initial tissue sections might not show complete features of the lesion. They identified superficial unrepresentative sections as one of the significant reasons for deeper cuts. They also emphasized that although deeper sections improve diagnostic accuracy but lead to delays in report dispatch.

Stuart et al. studied the utility of additional sections in dermatopathology. They reported that additional sections in 9% of cases resulted in modification of diagnosis. They suggested that as smaller biopsies are not grossed and often embedded as whole, the initial section is usually less representative of the biopsied tissue. They found that the cost of prospective and retrospective deeper sections represented a 56% and 115% increase over the base cost, respectively.[7]

Shinde et al. reported that 52 out of 249 cases were sent for recutting due to folds in the tissue section on the slide, which led to an increase in the case turnaround time.[8]

Rabinowitz and Silvers surveyed 43 laboratories reporting dermatopathology cases. They found that a laboratory preparing more than 10 000 cases had a recut rate of 30%. They discussed the issue of non-diagnostic initial slide (NDIS) in dermatopathology practice. They have also discussed several ways to optimise time and lab resources and develop standards for recutting small skin punch biopsy specimens.[9]

L'Imperio et al. assessed the utility of capturing images of the surface of paraffin-embedded tissue blocks using the BlocDoc technology (SPOT Imaging, Sterlings Heights, USA) installed near the microtome. BlocDoc manually captures regular and polarised images of the block surface. These captured images were seen by the histotechnician, who compared them with the prepared slide to look for inconsistencies. If the final slide is adequate, the block surface image is approved. The system stored these images, accessioned case-wise to the linked laboratory information system (LIS). Later, these images were available for the reporting pathologist at his workstation. The BlocDoc images can be compared and matched with the macro-image from whole slide imaging system.[2]

In the present literature search, we did not find any study which measured and compared the area of tissue on paraffin block and its corresponding slide or utilised digital image analysis techniques for histopathology quality control.

In this study, we attempted to develop and evaluate a technology prototype for imaging histopathology tissue block surface and its slides and then apply digital image analysis methods to check the adequacy of tissue on the histopathology slide.

Material and methods

This was a cross-sectional observational study conducted in a tertiary care hospital and medical college under the ICMR-Short Term Studentship (STS) Program. One thousand tissue blocks, and their corresponding slides were taken for this study. In cases with a single slide containing more than one section of the same tissue block, the most appropriate section in terms of the maximum area was used for comparison. Biopsies/resections specimens for which more than 3 tissue blocks were made, only the first 3 tissue blocks were included, and the rest were excluded from the study.

A customised cardboard box was designed for capturing the images of blocks and slides. The box had a cut-out in the top for placement of the image capture device iPhone 13 Pro (Apple Inc, California, USA). The inner walls of the box were covered in white paper, and white light from an LED screen was used to evenly illuminate the area inside of the box without any shadows. Fixed slots were marked on the bottom of the box to place the block and slide. The dimensions of the box, the location of the block and slide, the distance between the object and camera and illumination type were kept fixed to prevent any bias due to these factors. A centimetre scale was fixed near the slots to calibrate the images for calculating the area of tissue on the block and slide. The same image capture device was used for capturing all the images (Fig 1). All images were saved in JPEG image format before image analysis.

Image capture

Tissue blocks and slides fulfilling the inclusion criteria were placed at the marked area at the bottom of the box. After the preparation of the initial or first cut slides, a digital image of the remaining block surface **(Digiblock)** and its corresponding slide (**Digislide**) was captured in a single image file.

Digital image analysis

Captured images were transferred to computer for further image analysis. Each image was analysed using the ImageJ 1.53K application (source: NIH, USA; open-source application available at https://imagej.nih.gov/ij/download.html) for measurement of the area of tissue in the block and tissue on the slide. The steps for images analysis are as follows.

1. Open ImageJ application
2. Open image to be analysed: File menu➔Open (ctrl + O)
3. Convert the image to 8-bit format: File menu➔Image➔Type➔ select 8-bit
4. Calibration for centimetre scale (Fig. 2A): File menu➔Line (select length on the cm scale)➔Analyse➔Set Scale➔enter value of selected length
5. Selection of desired area, tissue block or slide. (Fig. 2B): File menu➔Rectangle selection tool
6. Duplication: File Menu➔Image➔Duplicate (ctrl + shift + D)
7. Thresholding to mask the unwanted area and artefacts (Fig. 2C): File menu➔Image ➔Adjust Threshold (ctrl + shift + T)
8. Area measurement (Fig. 2D): File menu➔Analyse➔Measure (ctrl + M).

Data analysis

The area of Digiblock and Digislide was recorded in an excel sheet. For each sample, a **DigislideQC score** was calculated by dividing the area of tissue on the slide by the tissue area on the block**.** Data regarding the tissue samples/blocks for which recuts were asked by the histopathologist was

Fig. 1. Image capturing device for taking Digislide and Digiblock images.

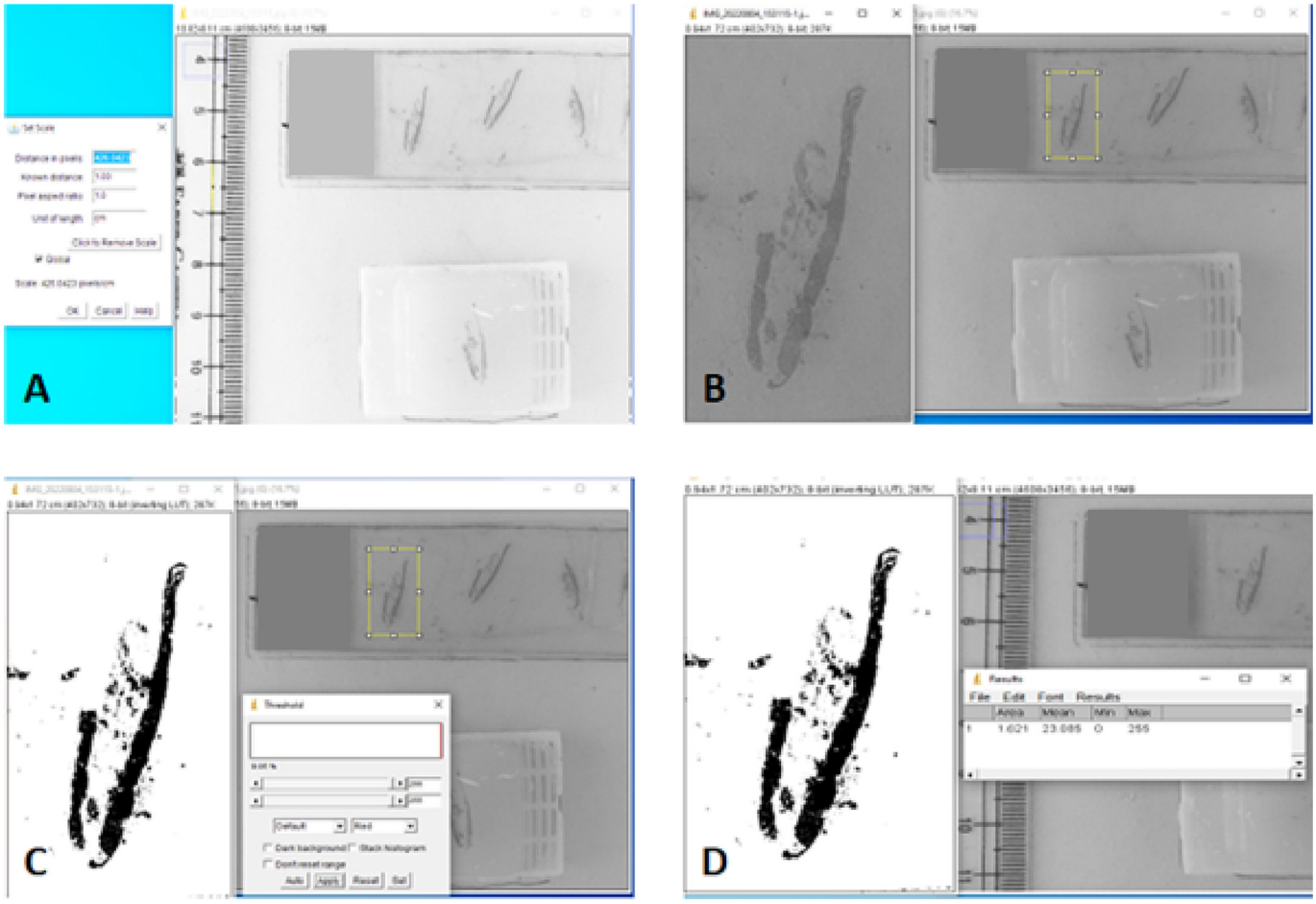

Fig. 2. Conversion of the image to 8-bit format and calibration for centimetre scale (A), selection of desired area (B), thresholding to mask the unwanted area and artefacts (C) and area measurement (D).

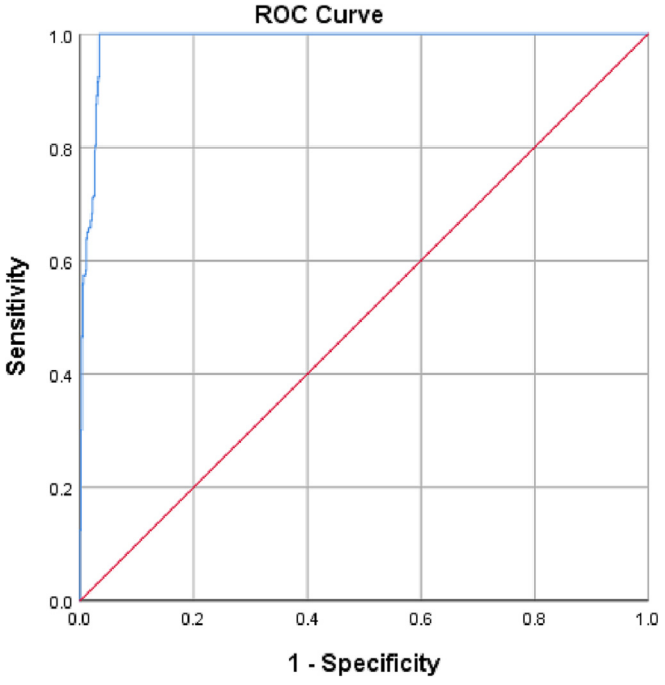

Fig. 3. ROC curve depicting area under curve (AUC) of 98.8%.

retrieved from the histopathology recut register. Histotechnician and reporting histopathologist were blinded for these steps.

Statistical analysis

The data of the area of Digislide and Digiblock was entered in an MS Excel sheet, and analysis was done using the SPSS version 23.0 (Armonk, New York, USA). A paired *t*-test was applied to compare the area of tissue on block with that of the slide; a *P*-value < .05 was considered significant. The performance of the Digislide QC score for recuts and deep cuts of the sections was estimated by the receiver operating characteristic curve (ROC), and the area under curve (AUC) was calculated for the best possible cut-off. (Fig. 3)

Results

One thousand tissue blocks, and corresponding slides were analysed during the study period. Of these, 249 (24.9%) tissue blocks were sent for recutting. The mean area of the Digiblock was 7.12 cm^2 (SD 2.35 cm^2) and ranged from 0.59 to 13.43 cm^2. The mean area of Digislide was 4.99 cm^2 (SD 1.68 cm^2) and ranged from 0.19 to 9.44 cm^2. We found significant differences between the size of tissue in the block and slide ($P < .0001$).

The Digislide QC score ranged from 0.1 to 0.99. Considering the frequency of recuts, the ROC curve plotted for the DigislideQC score showed an area under curve (AUC) of 98.8%. A cut-off value of 0.65 had a sensitivity of 99.6% and a specificity of 96.7%.

Discussion

Rabinowitz and Silvers found that a dermatopathology lab preparing more than 10 000 cases had a recut rate of 30% and it was due to non-diagnostic initial slide (NDIS).[9] In the present study, we had a recut rate of 24.9%. This may be due to the reason that in the present study we included samples from small biopsy to larger resection specimens.

L'Imperio et al. have reported that almost all samples were affected by tissue inconsistencies among the block surface and the obtained glass slide. They divided the errors into 2 groups, systematic error, due to the 3-dimensional structure and occasional error, due to the inexperience of the histotechnician and tiny specimens; it was found that only 50% of the systematic errors and <0.1% of the occasional errors was detected manually by the histotechnician leading to increased number of blocks being sent to recut increasing the time and cost. However, BlocDoc, a digital tool, was able to identify almost 100% of the systematic and occasional errors.[2]

Digiblock is a digital record of the surface of paraffin-embedded tissue block similar to the BlocDoc system. However, compared to BlocDoc, it's different in its approach. The histotechnician supervises BlocDoc to check for the final adequacy of the slide and approval.[2] Digiblock can be made automated and can process and store the block images in bulk. In this study, we used a centimetre scale to calibrate the ImageJ software to measure the tissue area on the block. The mean area for Digiblock was 7.12 cm^2 (SD 2.35 cm^2) and ranged from 0.59 to 13.43 cm^2. This variation is because of tissue blocks of small as well as larger resection specimens that were included in the study. As compared to BlocDoc, measurement of the tissue area on Digiblock provides a more objective approach to check the adequacy of the tissue on the slide.

Digislide is a digital record of the tissue on the slide. This is similar to the macro-slide image on a WSI system. In the present study, the mean area for Digislide was 4.99 cm^2 (SD 1.68 cm^2) and ranged from 0.19 to 9.44 cm^2. It was found to be significantly lower than the mean area for Digiblock (*P*-value <.0001). This highlights that for initial sections, the tissue on the slide is less as compared to the tissue on the block. The sections are sent for recuts to get more representative tissue on the slide. Although, this also depends on the expertise of the histotechnician and diagnostic query for each case. We did not find any previous study measuring the area of tissue on the block and slide.

Digislide can be superimposed on Digiblock to identify its adequacy. It can be further used to measure the tissue area, cutting artefacts, folds, and stain quality. In cases where multiple serial cuts are done on the same slide, Digislide can individually measure the area for each cut. It can also label the best tissue section with respect to the area for examination. This can be helpful in WSI and AI applications.[10]

Customised universal tissue cassettes and glass slides can be made with pre-printed scales to standardise the calibration and measurement. Modern technologies like 3D imaging and photogrammetric methods can also be applied to measure areas.[11]

DigislideQC Score is the ratio of the area of Digislide to Digiblock. Its value should usually be less than 1. This is because Digiblock is captured after the preparation of slides. Hence, in cases where the tissue block is superficially cut, the area of tissue on the slide is always less than that of the tissue block. While for the cases where tissue remaining on the block is less, the value of the DigislideQC score can be more than 1. In the present study, we got a DigislideQC score in the range of 0.10–0.99. The actual recut data was considered, to calculate the best possible cut-off value. We found that Digislide QC score cut-off value of 0.65 had a sensitivity of 99.6% and specificity of 96.7%. It can predict the need for recuts in 99.6% of cases before these are submitted for histopathology reporting. This can ultimately reduce the turnaround time of samples needing recuts.

There are many factors for suboptimal tissue on the final prepared slide. Technical aspects like inadequate trimming of the tissue block, improper handling of the tissue strip, the temperature of the floatation water bath, use of uncharged slides, and improper handling of prepared slides and folds in the tissue. These factors not only lead to tissue loss during slide preparation but can also lead to tissue loss during staining procedures. Tissue samples for which the staining steps require more manual handling or encounter excessive water currents are more prone to loss of section. This is a common issue with IHC staining, especially when antigen retrieval is done by manual methods. These slides land up for reporting and recuts are usually asked. This leads to an increase in turnaround time. DigislideQC score can be validated for different staining methods for quality control.

The pathologist or histotechnician if needed can manually superimpose and compare the Digiblock and Digislide having a QC score and label the artefacts, viz. tissue folding, tissue loss fragmentation, poor sectioning etc. This data can further identify the most frequent quality control issues in tissue sectioning and staining. This can guide a more focused approach to improve the section quality.

In this study, we have created a simple tool to record the images of tissue blocks and corresponding slides. We recommend that Digiblock and Digislide be captured for all the tissue samples and that the DigislideQC score also be calculated. This should be integrated with LIS and available for the pathologist while reporting to check the adequacy of the tissue section. Each lab can identify its own cut-off values to determine the need for recut before reporting. This can also be divided as needed for several types of tissue bits, be it small, large, chips, cores etc. All slides with a DigislideQC score below a certain threshold should automatically be sent for recutting. For slides above that threshold, the pathologist or histotechnician may digitally compare the slide and block with DigislideQC score to manually decide on recutting if needed depending on the diagnosis of the case, viz. to measure the distance of tumour from margin.

Conclusions

In the present study, we found that the DigislideQC score can correctly identify the slides which may require recuts before it is sent for reporting. DigislideQC score had a sensitivity of 99.6% and specificity of 96.7%, area under the curve 98.8% for a cut-off value of 0.65. This can prevent the submission of suboptimal slides for reporting and can potentially reduce histopathologists' slide screening effort and ultimately turnaround time. It can be easily incorporated with routine histopathology workflows and lab information systems. Digiblock and Digislide images can be integrated into the telepathology workflows and provide the reporting histopathologist with information about the adequacy and quality of sections. This simple technology can benefit future digital workflows, reporting, and telepathology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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