Improving Digital Image Quality Through the Enhancement of Whole Slide Imaging Protocol

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The Biopathology Center (BPC), located in Columbus, Ohio, functions as a biorepository under the guidance of The Research Institute at Nationwide Children's Hospital in collaboration with the Ohio State University Medical Center. The fundamental purpose of the BPC is acquisition and storage of cancer, normal and diseased biospecimens. The Biomedical Imaging Team (BIT) is a multidisciplinary team within the BPC which provides imaging services to enhance biomedical research for several national organizations including the Children's Oncology Group (COG), the Gynecologic Oncology Group (GOG), the Cooperative Human Tissue Network (CHTN) and The Cancer Genome Atlas (TCGA). In addition to providing their services to these national organizations, the BIT works closely with internal departments at Nationwide Children's Hospital, including the Department of Molecular Genetics and the Morphology Core Laboratory. Through additions in equipment, the BIT generated 9,859 images in 2010. To date, the BPC's current digital archive stands at over 72,000 total images, currently housed at the Ohio Supercomputer Center (OSC) and made available through the Virtual Imaging for Pathology, Education & Research (VIPER) application.

In the last decade, whole slide imaging (WSI) and virtual microscopy (VM) have seen tremendous growth in the functionality and utility of their services. An important educational tool and capable of image sharing with a quick turnaround time, virtual microscopy has appealed to clinicians and researchers alike.

Challenges in WSI exist, particularly due to the lack of a standard protocol for this developing field. Variations in slide preparation and scanning procedures contribute to inconsistencies in slide image quality such as blurring, stitching errors, staining issues, etc. These irregularities emphasize the need for standardization of the entire imaging process from receiving the fresh tissue to image analysis. Tissue folding is one such issue which can affect image quality, compromising usefulness for researchers and computational image analysis. However, the blurring and stitching effects caused by folded tissue can often be identified and mitigated with adjustments in protocol. Through further development, this standard protocol will continue to enhance image efficiency while conserving image quality.

METHODS

Proper slide preparation is critical for obtaining quality images. Folds can occur during cutting of a frozen section in the cryostat. Different tissues cut optimally at different temperatures which can range from -5C to -50C. Anti roll plates, glass plates attached near the cutting edge, are normally used to help avoid folding issues. A Peak Optical Lupe can be used to check for folds before the slide is stained.

After receiving them, the imaging team must prepare the slides for scanning. The slides are then loaded into the Aperio™ XT Scanners and snapshots are taken. This step includes naming the image, annotating the region of interest, and applying focus points to that region. The slides are then scanned and the images are reviewed for quality control by imaging technicians.

Finalized WSI can be utilized by image analysis algorithms to gather statistics and metadata, annotate regions of interest, assist in diagnosis, and make review by researchers and doctors less time consuming and less expensive. While the images that come off most scanners are in a proprietary format, several provide a framework in which to open images, write analysis algorithms, and add annotations to the images. Several open-source frameworks are also available

To demonstrate how a potential folding issue can be mitigated by adjusting the protocol, a slide with folded tissue was scanned in a way in which serious blurring issues were present. Adjustments were made to the way the slide was scanned, namely the placement of focus points, in an attempt to improve image quality. In addition, an algorithm for the detection of potential tissue folds within the WSI was written in the Aperio™ Algorithm Framework for execution within Aperio™ Imagescope (Figure 1). The algorithm searches for large areas of over-stained tissue, which is the typical appearance at the point of overlapped tissue.

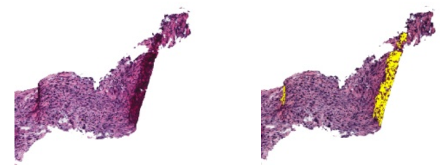

Figure 1. Fold detection algorithm identifies potential folded tissue regions in (a) and annotates these folds in yellow in image (b).

RESULTS

Figure 2a shows a snapshot of the tissue and placement of the focus points prior to scanning. Figure 2b is the resulting image when positioning the focus points in the folded region. Note the inconsistency of the image along with significant blurring that can be found throughout the entire WSI in this worst case scenario.

Figure 3a shows an identical snapshot with the placement of the focus points moved away from the folded region. Note the sharp focus in Figure 3b, the resulting image. By placing focus points in an area with no tissue folds, a high quality resolution was maintained throughout the tissue.

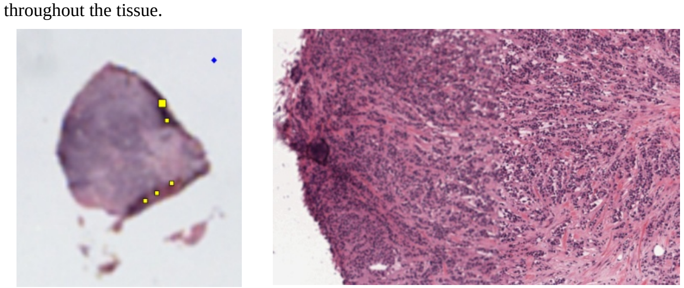

Figure 2. Placement of focus points (in yellow) on the folded regions (a) and subsection of resulting scan (b).

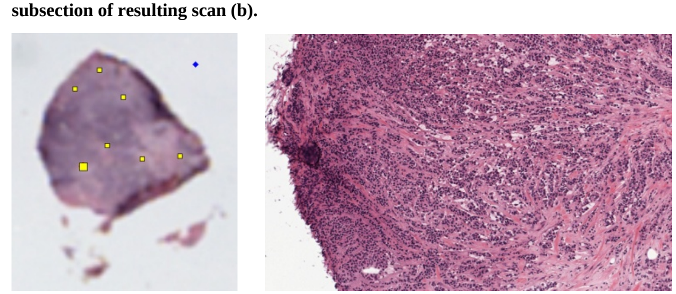

Figure 3. Placement of focus points (in yellow) away from folded regions (a) and subsection of resulting scan (b).

DISCUSSION

The protocol for creating a slide, at least at Nationwide Children's Hospital, has reached its upper threshold with regards to minimizing folding issues. In other words, while folds cannot be avoided in every situation due to tissue types and other factors, no additional steps or alterations would be beneficial in creating a significantly higher quality digital image.

Within the imaging workflow, placement of the focus points is the most critical step in ensuring acceptable image quality and can be affected by both tissue thickness and the presence of folds. While these issues are also present in traditional glass slide pathology, it is much easier for the pathologist to navigate to other portions of the tissue in order to bypass the affected regions. In digital pathology however, the presence of a single tissue fold or an area of tissue thickness has to ability to compromise the quality of the entire tissue if the focus points are not properly placed. If the scanner is in semi-automatic mode, then focus points will be placed throughout the region of interest and those values will be averaged together during the calibration step of the process. Understanding the way in which the scanners function and the purpose of the focus points, gives the technicians a way to troubleshoot and improve the quality of the image by just merely rearranging the placement of these points. Thus, the placement must be done in such a way that it best cancels out the focus points on the fold or thicker tissue with those on the unaffected tissue. In the example illustrated in Figures 2 and 3, the folded region was along the edges of the tissue, and thus the best placement pattern was to move the focus points away from the folds. However, if folded tissue is present throughout the slide, one such pattern that has shown positive results is to have a focus point on the fold alternating with one directly adjacent to the fold. Through the rearrangement and placement of the focus points, reflecting an understanding of the way they function, the image quality was significantly improved.

Algorithms like the fold detection algorithm demonstrated in Figure 1 could be included in a series of Quality Control (QC) algorithms, helping to reduce the amount of manual review performed by Imaging Technicians. Within the automated QC workflow, this particular algorithm could notify the user that folded tissue is present on the region of interest, and of the issues that are commonly associated with digitally imaging folded tissue. As demonstrated, folded tissue can create issues in WSI that can limit effectiveness in both human and computational analysis.

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