Whole-slide imaging: widening the scope of cytopathology

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Abstract

Whole slide imaging (WSI) is broadening the scope of cytopathology. Whole slide images are being used for telecytology, quality assurance activities (e.g. proficiency testing) and teaching (e.g. digital teaching sets and online virtual atlases). Progress in WSI technology that permits high resolution scanning, z-stacking, and hybrid robotic devices has encouraged the use of this imaging modality for cytology practice, education and research. However, widespread adoption in cytology still depends on overcoming barriers unrelated to cytology and challenges directly related to digitizing cytopathology slides. The aim of this article is to review WSI technology, applications and limitations specific to cytopathology.

Keywords cytology; digital pathology; informatics; proficiency testing; telecytology; whole slide imaging; z-stacking

Introduction

Whole slide imaging (WSI) has become an important modality in Digital Pathology. This technology allows entire glass slides to be digitized (scanned), producing an interactive digital image that can be examined in a manner simulating light microscopy. WSI has been vetted for several uses in surgical pathology including intraoperative consultations (frozen section), primary diagnosis, secondary consultation, image analysis, research and education. WSI has similarly broadened the scope of cytopathology where virtual slides (whole slide images) have been used for several purposes such as telecytology, quality activities (e.g. archiving and proficiency testing) and education (e.g. virtual atlases).[1] In addition, WSI has opened the door for image analysis and computer aided diagnoses in cytopathology. Several investigators have demonstrated WSI to be feasible for digitizing and interpreting both gynecological material (i.e. Pap tests) and non-gynecological cases.[2–4]

Rapid progress in WSI technology (e.g. high resolution scanning, z-stacking capability, hybrid robotic devices) has encouraged pathologists to explore the utility of this modality for cytology practice, education and research. However, widespread adoption in cytology is still impeded by several barriers; some unrelated to cytology (e.g. cost of scanners, regulations preventing primary diagnosis), whereas other challenges are directly related to the unique nature of cytopathology cases (e.g. 3D specimens that require z-stacking). The aim of this article is to review WSI technology, applications and limitations specific to cytopathology.

WSI technology

A WSI system includes a scanner (device to digitize glass slides), networked computer(s) and possibly a server or cloud solution for storage, display (e.g. computer monitor, tablet, etc.) and compatible software. The software is used for image viewing, and may include applications that facilitate image management, sharing and image analysis. A whole slide image, composed of thousands of pixels, is used to represent an entire scanned glass slide, or if desired a pre-selected region of interest (ROI) on the slide. Available WSI scanners differ in their capabilities including slide loading and handling, scanning method, scan time, microscope objectives (low and/or high magnification), remote robotic control, Z-stacking, and by the type of image files generated. Some of these features are particularly desirable when scanning cytology slides, such as the ability to scan glass slides with and without coverslips for rapid on-site evaluation (ROSE) of fine needle aspirations (FNA). For cytology it also important to be able to scan slides using a high magnification objective (e.g. 40x objective), allowing users to easily interpret fine cytological details. Z-stacking capability permits examination of cytological material at multiple focal planes.

Navigation, screening and annotation

In cytology, users are generally required to screen an entire slide (a task typically performed by a cytotechnologist) and then interpret cytological material (e.g. cells and background material). When screening all the material on a slide the user locates and marks ("dots", typically with a marker pen on glass slides) those areas (e.g. abnormal cells) that are important to make a diagnosis. In order to effectively screen slides, it is important for the user to easily navigate the slide (i.e. pan around in the x and y axes). However, using a computer mouse to screen slides is tedious and time consuming. This may account for some users' complaints in prior publications, where they reported lengthy times needed to read virtual cytology slides when compared with glass slides. In a prior slide exploration study, cytotechnologists were reported to take longer than cytopathologists when reading digital slides.[5] Although the cytotechnologists in this study had less experience with whole slide images, when tracking their movement around the digital slide they also screened a larger percentage of the slide at higher magnification (Figure 1).

When screening a cytology whole slide image it is important to establish a systematic review method. Screening can be facilitated by specific features in the viewing software, such as keyboard controlled navigation in conjunction with displaying a thumbnail image to confirm complete slide coverage.[6] With some viewers, built-in tracking tools that can record movements on a slide can be used to reassure users that they have covered all

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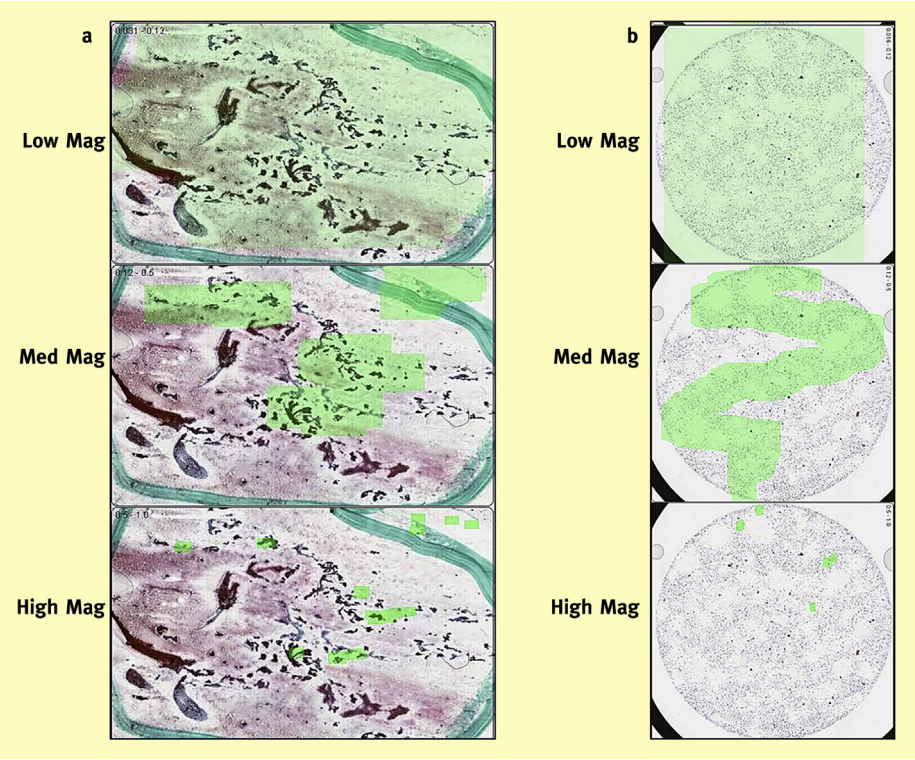

Figure 1 Search maps are shown for cytotechnologists at low, medium and high magnification of (**a**) a FNA smear and (**b**) ThinPrep slide. The light green area represents the examined area covered by the user.

areas of a virtual slide. For example, screened areas can be indicated with a color change on the image. In fact, with some viewers the intensity of the color may even be proportional to the magnification at which that area was screened.[6] The use of other methods to better navigate cytology slides (e.g. trackballs, touch pads, gaming station controls, touchscreens) has not been well studied. Some of these methods have proven to be beneficial when navigating histopathology slides.[7]

Imaging software can be used to also annotate important areas of a digital slide, akin to "dotting" a glass slide. Available image viewers permit a wide variety of annotations (e.g. colors and shapes) that can be saved with an image. This software feature facilitates effective communication between screeners and final reviewers.[6] The built in annotation capability can also be used for teaching and testing trainees, where hidden annotations can be revealed later. Annotating images is preferred to keeping actual dots on glass slides before they get scanned, because pen markings on glass slides may affect focusing and/or cause image artifacts with some scanners (Figure 2).[8]

Magnification and resolution

For surgical pathology, scanning glass slides using a 20× objective will usually suffice. However, when digitizing cytology material most users prefer to scan slides using a microscope objective lens with at least 40× magnification. This generates whole slide images of greater resolution, which offers good image quality without too much pixelation when zooming in at higher "magnification" to examine cells. Moreover, objectives with greater numerical aperture (NA) can be used to further enhance resolution. While optical resolution depends on the objective lens and its NA, it is important to be aware that digital resolution also depends on the scanner's digital camera sensor (charge coupled device) and the monitor on which the image is displayed.[9] Magnification alone can be misleading, because some devices can scan at 20× but at different resolutions (e.g. by pixel binning to get faster times and smaller file size). Moreover, scanning a glass slide at 40× with low resolution (e.g. 0.275 micron/pixel) would be comparable to an image generated by scanning at 20× with 0.275 micron/pixel and then digitally zooming to 40×. In one study, a higher diagnostic accuracy and lower interpretation time were obtained when scanning at 40× (with 0.75 NA and 0.23 micron/pixel resolution) compared to slides scanned at 20× (with 0.5 NA and 0.46 micron/pixel resolution).[2] Therefore, when evaluating WSI scanners for cytology instead of looking at their ability to scan at 40× one should evaluate the micron/pixel parameter for each objective to determine the instrument's actual image resolution.

Z-stacking

Glass slides with histology tissue sections (e.g. 4-6 micron in thickness) have mild variation in topology. Thus, scanning these glass slides with one focal plane is usually sufficient in order to produce a digital image with two dimensional (2D) features that are in focus. Proper evaluation of a digital cytology slide requires that all cellular material is in focus. However, scanning cytology slides can be problematic for thick smears or cytology specimens (e.g. Pap tests and fluids) that contain three dimensional (3D) cell groups (Figure 3). Making sure all cells and cell groups are in