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Overcoming digital cytology barriers with an interactive imaging approach using Panoptiq™

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Modern cytology practices still face significant challenges with implementing digital cytology. Key barriers revolve around the inability to effectively image 3-dimensional (3D) cytologic structures, high cost of implementation, heavy storage requirements, and long imaging process. This report examines ViewsIQ's digital imaging system, Panoptiq™, as a solution to address some of the current challenges. Panoptiq's™ flexible imaging modality and integrated Z-stacking has the potential to overcome the current technological barriers with respect to sample three dimensionality. Panoptiq™'s region-of-interest imaging approach may introduce observer bias, limiting it's current applications in cytology proficiency testing. While the adoption of Panoptiq™ continues into different fields of utilization, its combination of high-magnification digital 3D imaging and easy integration into existing microscopy systems lends its particular application to cytology.

Current State

Technological innovation in recent years such as whole slide imaging (WSI), real-time image streaming, and increasingly higher capture resolutions has allowed sophisticated digital representation of glass slides to be accessible, propelling digital imaging to achieve practical relevancy. However, within practices such as cytology, where samples carry significant three-dimensionality (3D), the ability to capture cellular information along the Z-axis and readily observe multiple planes of focus remains a challenge[1]. This function, commonly known as Z-stacking, has been a technological barrier that prevents digital cytology from wide-spread adoption. Currently, standard two-dimensional imaging used in many modern WSI technologies is excellent for samples such as tissue sections with a defined plane of focus. However, as seen in **Figure 1**, it is prone to introducing unfocused regions that result in uncertainty and frustration for the observer when examining 3D structures of interest such as pleomorphic cell clusters during cytomorphological examination[2].

Since the introduction of the first whole slide scanner in 1999, improvements such as multi-focal plane WSI have been achieved to address some of the technical challenges with 3D samples. A number of modern imaging solutions allows users to predefine the number of planes and intervals between each plane prior to capture, automating the hardware to generate Z-stacks on every field of view. As a result, the multi-plane WSI is able to replicate a "zero bias digital slide environment". This type of WSI is particularly useful in proficiency testing as there is no concern of selection bias. However, this significantly increases the scanning time and storage requirements[1], as doing a 5-plane WSI is equivalent to capturing 5 times the amount of information. This also imposes a practical limitation to the number of planes that can be scanned with multi-focal plane WSI. Consequently, the current state continues to pose limitations for the effective use of WSI in cytological applications.

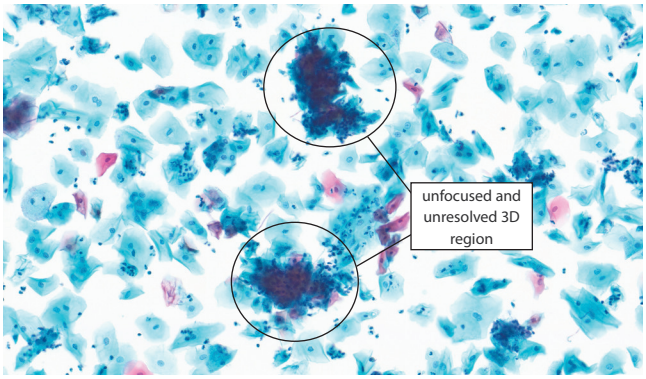

Figure 1. Single planar scans are prone to introducing unfocused regions in cytological samples due to the intrinsically 3D nature.

Panoptiq™ in Digital Cytology

Panoptiq™ is an interactive digital imaging system with a new approach to image acquisition. Using a high frame rate CCD camera attachment to any user's microscope, an image is captured and sent to a high-performance computer with the Panoptiq™ software. Unlike WSI scanners, where images are stitched together into a panoramic image in a predefined pattern until completion, the Panoptiq™ software dynamically stitches the images together in real-time as the observer scans around the sample with the microscope stage. Using the companion Z-module, as shown in **Figure 2**, Z-stacks can be captured and embedded into the scan as the observer finds a 3D region of interest. Captured as a high frame rate video with frame accurate playback at high resolution, the observer can digitally focus through the Z-stack to readily inspect the 3D planar details.

Panoptiq's™ interactive imaging approach addresses several of the challenges faced in digital cytology. First, with the high frame

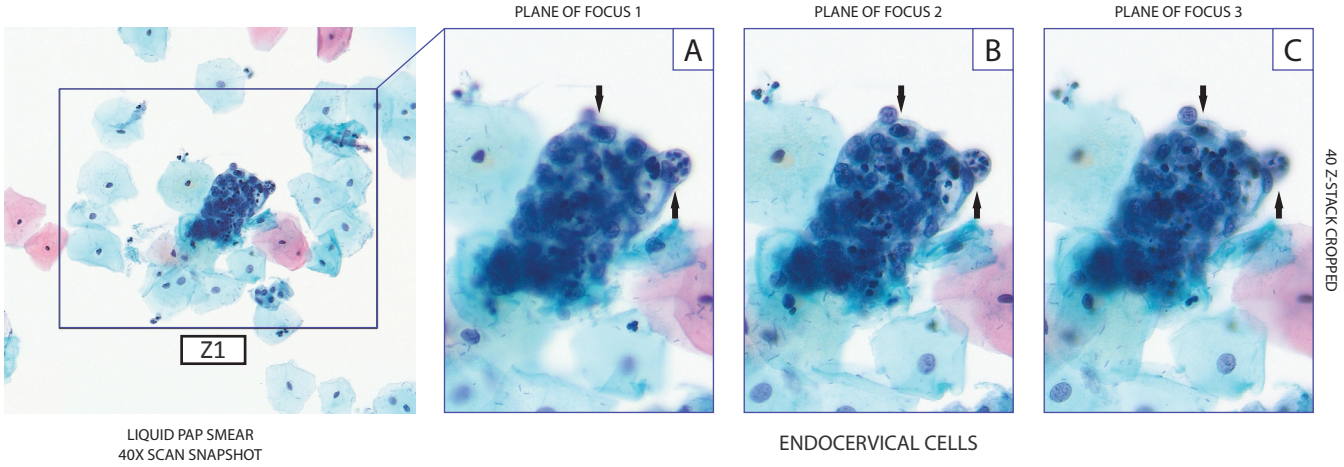

Figure 2. Panoptiq's™ Z module allows Z-stacks to be embedded into the 2D scan at regions-of-interest, in real-time, to allow for rapid multi-objective and multi-planar imaging. As demonstrated with an endocervical cell cluster, the 40X Z-stack snapshot at the "bottom" (A), "middle" (B), and the "top" (C) of the cluster show significantly different details due to the change in planar focus. Refer to the black arrows for example.

rate format, the digital focusing of the Z-stack is smooth without the loss of information that occurs in conventional modalities using predefined planes of focus. This allows users to maximize captured cellular information along the Z-axis. Second, the ability to scan, embed Z-stacks, and annotate as needed facilitates efficient region of interest imaging. This form of Z-stack acquisition has significantly lower storage requirements as the 3D planar information is only captured at relevant regions. In addition, the option of imaging only what users dictate and not the entire slide can also decrease both time and storage demands. The archival features also allow users to quickly navigate to regions of interest during collaborative reviews. Finally, with the help of web-conferencing software, the real-time and interactive digital imaging approach allows immediate live consultations such as adequacy evaluation during fine needle aspiration sampling - something that has previously been unachievable via WSI.

Education Applications

The interactive imaging approach of Panoptiq™ has the potential to enhance various aspects of cytology education. Traditionally, digital cytology has been utilized to good effect as a teaching tool[1-3]. Using basic camera attachments with single fields of view, cells of interest can be captured as single snapshots or videos for use in Power Point presentations. Alternatively, WSI technology has introduced vastly enhanced fields of view that adds an extra layer of realism as students access annotated slides through school networks and practice their diagnostic skills using improved digital formats closer to that of traditional glass slides. In laboratory situations, having such reference materials allow students to reaffirm their cell/pathology identifier skills. In addition, the emergence of sophisticated internet infrastructure provides many options for accessing digitized slides and course material online.

However, the aforementioned methods still lack an effective way to dynamically encapsulate 3D information with an element of interactivity to promote experiential learning for students. Currently, existing methods of digital imaging lack key control elements such as fine/coarse focus adjustments and stage navigation that are crucial for building a student's foundational knowledge in microscopy use. In addition, WSI implementation in education is met with familiar issues regarding high integration cost, heavy storage demands, and time required to scan single or muti-plane WSI[1,3]. With Panoptiq's™ integration with a lab's existing microscope, combined with embedded Z-stacks, students may utilize the microscope as they normally would, with the advantage of real-time digital capture and examination of 3D structures of interest with relevant annotations. This allows students to experience a digital environment that closely replicates physical microscopy, captured using a microscope. In addition, the modality of real-time scanning allows students to observe diagnostic pathways from an experienced operator. In an environment where regions of interest are often educationally sufficient, Panoptiq's™ ability to allow users to dictate the scanning regions is advantageous. This allows flexibility on the users' part with respect to time and storage requirements. Currently, Panoptiq™ has been adopted in a number of medical laboratory technology programs, where said applications have been applied with significant success.

Clinical Applications

Within clinical cytology, there also exist multiple applications for a real-time imaging system such as Panoptiq™. Modern clinical practices have an established recognition of cytopathology, specifically fine needle aspirations (FNA), as a cost effective and safe diagnostic technique with competitive accuracy compared to biopsies[4-6]. With the goal of using cytomorphological analysis to reach

a final diagnosis, the use of FNA is more averse to the complications seen in biopsies due to a less invasive procedure[7]. For optimal diagnostic accuracy, studies have recommended the presence or tele-consultation of a cytopathologist during FNA sampling which has shown to significantly decrease downstream errors due to improved sampling adequacy[7]. Additionally, inexperience at the cytopathologist level can lead to false positive diagnoses, requiring further peer consultation to prevent erroneous results[8]. Via the real-time scanning and Z-stacking abilities of Panoptiq™, this adequacy consultation amongst cytopathologists can occur immediately after sampling either on-site or remotely through tele-conferencing software. Using a real-time remote consultation system can significantly decrease the intra-facility travel time of personnel and thus maximize efficiency. Most importantly, the aforementioned flexible Z-stacking approach also prevents the observer uncertainty and frustration seen in 2D imaging technology[1]. As telecytology studies have shown, the lack of 3D analysis when working with static data such as WSI can result in lower accuracy or diagnostic agreements when compared to traditional microscopy[9,10]. However, Raab et al. notes that observer experience and familiarity with digital imaging is an important consideration as performance can vary between individuals[9]. Incidentally, another study observed that while the lack of 3D analysis did not prohibit accurate diagnosis, the time required to reach the diagnosis significantly increased due to the focal ambiguity of 2D WSI[11]. Ultimately, the flexibility and immediacy of Panoptiq™ offers a new method of satisfying consultation needs that lends credence towards Panoptiq's™ potential as a new tele-consultation solution.

Limitations and Considerations

With all systems, it is important to consider the technical limitations and considerations. Panoptiq's™ current emphasis on interactivity does require manual user operation. Consequently, the option to do multi-focal plane WSI is not feasible without automation as such operation using Panoptiq™ would require the manual capture of hundreds to thousands of Z-stacks. Therefore, Panoptiq's™ current application within cytology proficiency testing remains limited due to the inherent presence of selection bias introduced from region of interest imaging. Additionally, Panoptiq™ does not yet have an option for automation, which would limit its applications in high throughput laboratories. Observer experience is also a risk factor with digital imaging technologies in general, as the lack of clinical experience could lead to false negatives due to erroneous selection bias such as capturing Z-stacks at structures of little diagnostic relevance (eg. Polies). This poses a strong case for the supervision, consultation, and/or operation of an experienced observer such as a cytotechnologist or cytopathologist. Finally, consideration of how such systems will be integrated and/or impact a laboratory's workflow must be assessed.

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