How Thin is a ThinPrep® Slide? Challenges of Achieving High Focus Quality on a Digital Whole Slide Imaging System

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Abstract

The emergence of digital whole slide imaging (WSI) systems is set to revolutionize the fields of pathology and cytology. The ability to obtain high quality whole slide images quickly will be a vital step in a successful clinical workflow, especially for high volume screening applications like the Pap test.

ThinPrep liquid-based cytology slides present a near monolayer visually to the reviewer, but cytology is inherently 3-dimensional. These slides can be challenging[1] for WSI due to the focal depth of closely juxtaposed material that can be an order of magnitude higher than the Depth of Field (DOF) of a high-power microscope objective. For this reason, cytology slides are more challenging to image than histologic tissue slides. Slides with film coverslips can also add to the scanning depth requirement due to curvature across the slide cell spot region. Most current WSI systems require repeated scans to cover multiple focal planes in order to acquire quality images, greatly increasing imaging time.

In this study, we will present the key challenges of obtaining high focus quality images for ThinPrep Pap test slides, including maps of focal depth and data on its statistical distribution.

We will also present our methods for efficiently scanning ThinPrep slides with a novel methodology using a digital imaging system in development by Hologic, including analysis and discussion of the advantages and limitations of these methods.

Objectives

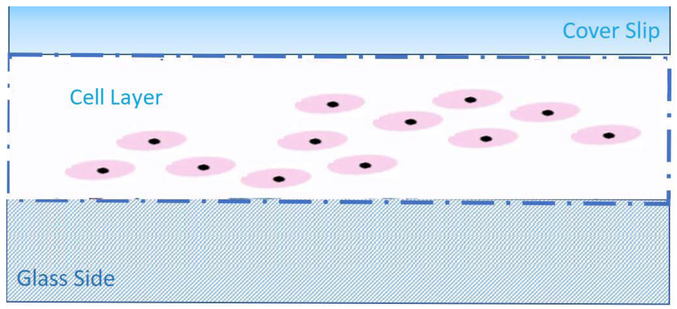
- List key challenges of obtaining high focus quality images of cytology slides.
- Measure the height distribution of cells on ThinPrep Pap slides.
- Present a method of efficiently scanning the required 3D volume.
- Discuss advantages and limitations of this technique.

What is ThinPrep Technology?

ThinPrep technology includes a state of the art cell separation and deposition technique that dramatically reduces problems[2] in the traditional Pap smear. It produces near monolayer slides for diagnostic review that significantly increase sensitivity of detecting high-grade squamous intraepithelial lesion (HSIL) and cancer (from 77.8% and 90.9% to 92.9% and 100% respectively)[2].

What are the Challenges?

Because cells may be suspended in the mounting medium and may also stack up, cytology slides are inherently 3-dimensional. Since a microscope objective has such a small depth of field (DOF), cells cannot all be captured in focus in a single image. In fact, individual cells may be thicker than a single DOF. A 40X microscope objective lens with NA 0.75 has a depth of field of less than 2 microns.



Cytology slides with flexible film coverslips can require even more scanning depth due to curvature across the cell spot region. To capture quality images of all cells on a ThinPrep slide, much wider ranges of focus are required.

Method

23 ThinPrep Pap slides were scanned on a computer-controlled microscope with a digital camera (Hologic ThinPrep Integrated Imager) to gather cell preparation depth data, using the following procedure:

- Scan the cell spot area using the computer-driven XY stage
- At each location, capture a stack of images at a wide range (> 40 microns) of Z height.
- Divide every image into small regions (35 microns square) and evaluate the Brenner focus score metric for every level in the Z stack. Determine the optimal focus for that tile.
- By focusing on the fiducial marks printed on the slide, determine the overall slide glass plane and subtract that from the focus data to determine relative heights of cell content.

References

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2. Camille Andy; Linda F.Turner. "Is the ThinPrep better than conventional Pap smear at detecting cervical cancer?" J Fam Pract. 2004 April; 53(4):308-331.
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4. Andrew J. Evans, Mohamed E. Salama, Walter H. Henricks, and Liron Pantanowitz."Implementation of Whole Slide Imaging for Clinical Purposes: Issues to Consider From the Perspective of Early Adopters". Arch Pathol Lab Med.2017;141:944–959.

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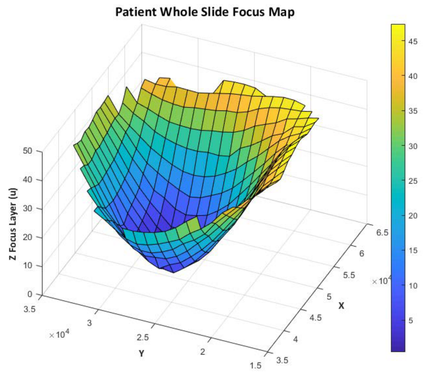
Results

Our data (Table 1) show the average cell depth of ThinPrep slides is 11.09 microns for slides with a glass cover slip and 23.6 microns for slides with film cover slips. In some cases, cell depth can can be greater than 40 microns, far exceeding some of the earlier findings.[3]

A surface plot focus map for each slide was created and reviewed. An example is shown below for a film-coverslip slide which shows the effect of curvature over the extent of the cell spot region.



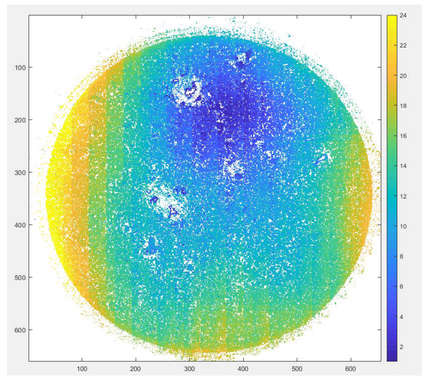


Table 1

Slide Number (Glass Cover Slip)	ΔZ Focus Depth (in μ)
1	8.4
2	11.3
3	11
4	13.7
5	9
6	8.8
7	13.2
8	11.85
9	12.6
MEAN ± STD	11.09 ± 1.969

Slide Number (Plastic Cover Slip)	ΔZ Focus Depth (in μ)
1	11.8
2	22
3	14.38
4	24.2
5	15
6	22.86
7	47
8	28
9	15.7
10	27
11	24.48
12	23.68
13	19.9
14	29.6
MEAN ± STD	23.6 ± 8.96

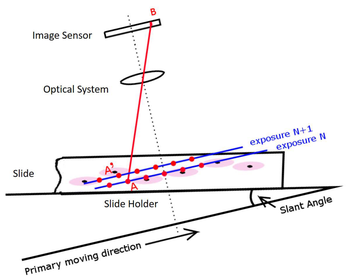
Table 1 presents a summary of the data. Note that even with glass coverslips, the local variations in cell height within a slide amount to as much as 7 times the depth of field of the microscope objective.

Volumetric Scanning

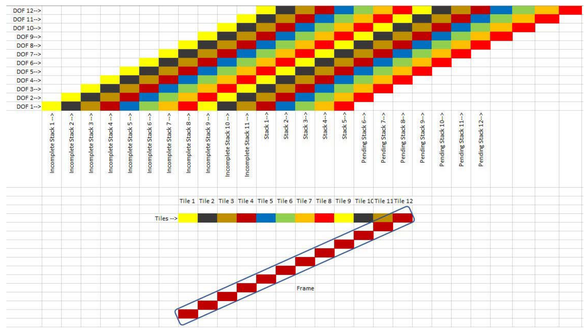
Most current WSI systems scan a single focal plane at a time. A typical scanner completes a 15 x 15 mm scan in 1 minute.[4] At this rate, scanning a circular ThinPrep cell spot region to a depth of 14 focal planes would take at least 26 minutes.

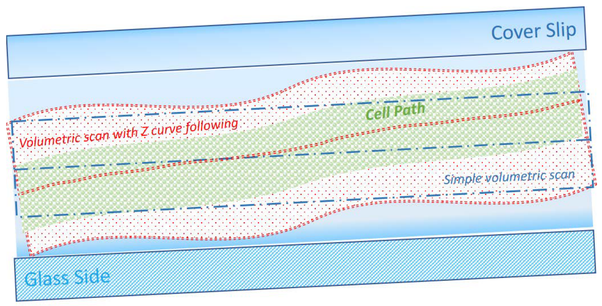
Using a tilt-plane volumetric scanning method (in development) significantly decreases the acquisition time for scanning the full cell content area. A ThinPrep Pap slide can be completed in approximately 2.5 minutes.

The imaging optics and camera are tilted with respect to the slide. The region of the image at one edge of the camera frame acquires images closer to the slide glass than the region at the other edge of the camera frame (see drawing below). A tilt angle of 48 milliradians and an image frame width at the slide of 0.5mm provides a scan depth of 0.5 x sin(0.048), which is 24 microns.



As the camera moves continuously, it is triggered to obtain a new image every time it has moved 1/14 of its own width. A very high speed (> 100 fps) camera is used. These overlapping images can be sliced and reassembled to obtain the 14 focal plane images.(see Figure right)

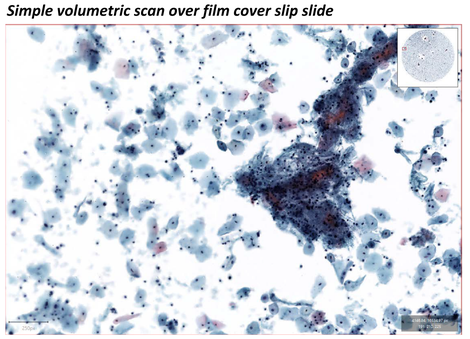




To optimize storage space, the focal planes are combined into a single extended depth of field image by selecting in-focus pixels from the various planes. The image processing is done in real time using GPU hardware acceleration.

To handle the larger total cell depth range found on slides with film cover slips, the imaging optics can be driven in the Z axis to follow the curvature. This method works because the local cell depth in the area the size of the camera field of view is within the tilt-plane scan depth. It is only over longer distances that the larger variation comes into play.

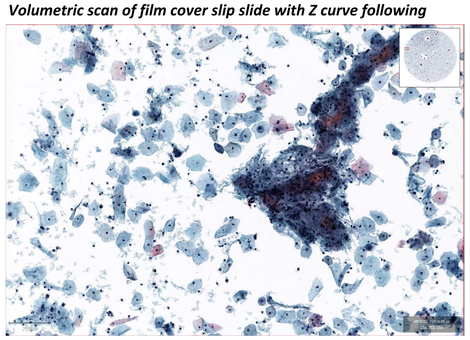
Simple volumetric scan over film cover slip slide



A curve-following scanning method minimizes local focus errors, providing higher quality WSI images (Right).

This slide has a Z focus depth over 40 microns.

Volumetric scan of film cover slip slide with Z curve following



Discussion

One of the key challenges in moving to digital cytology is obtaining high focus quality WSI images in the time frame demanded by today's high throughput lab workflow. The volumetric scanning system described provides a practical solution meeting the needs of digital cytology and pathology WSI, where a ThinPrep Pap slide can be scanned and processed within 2.5 minutes. The speed of slide scanning is limited primarily by the camera rate and image processing speed. As hardware and software technologies advance, still faster slide scan rates will become possible.