

## Independent Dual Sensor Scanning for Whole Slide Imaging

*Solving the throughput-quality trade-off for digital pathology*

Michael C. Montalto, PhD

Robert Filkins, PhD



Omnyx products have not been cleared or approved by the FDA for diagnostic use.



UPMC

Omnyx, LLC a joint venture of GE Healthcare and  
University of Pittsburgh Medical Center

30 Isabella Street,  
Suite 301  
Pittsburgh, PA 15212

800 Centennial Ave,  
Building 4  
Piscataway, NJ 08854

## SUMMARY:

Accurate focusing is a critical challenge of whole slide imaging, primarily due to inherent tissue topology variability. Traditional line scanning and tile-based scanning systems are limited in their ability to acquire a high degree of focus points while still maintaining high throughput. A novel system, referred to as Independent Dual Sensor scanning, decouples image acquisition from focusing resulting in rapid scanning while maintaining focus within each tile of a whole slide image and, thus, generates higher quality images at a fast rate.

## The Challenge with Autofocusing

Whole slide imaging requires the acquisition of multiple high resolution images that are subsequently aligned or stitched together to create a complete and seamless representation of the original whole tissue section. A fundamental challenge with whole slide imaging has been the ability to produce a high quality, in-focus image at fast speeds. To produce an in-focus image it is necessary to 'track' the topology variations (z dimension variation) that inherently exist in solid tissue samples. Topology variation can range from nanometers to several microns over a single millimeter (approximately a single field of view) in the x or y direction. **Figure 1** illustrates the variation of tissue topology in the z-dimension that exists in a typical 5  $\mu\text{m}$  thick tissue section. Standard microscopes allow the user to compensate for these variations by using the fine focus knobs in real time during viewing. However, such variation in topology can present dramatic challenges for whole slide imaging systems which attempt to frequently adjust focus automatically to compensate.

Figure 1 – A

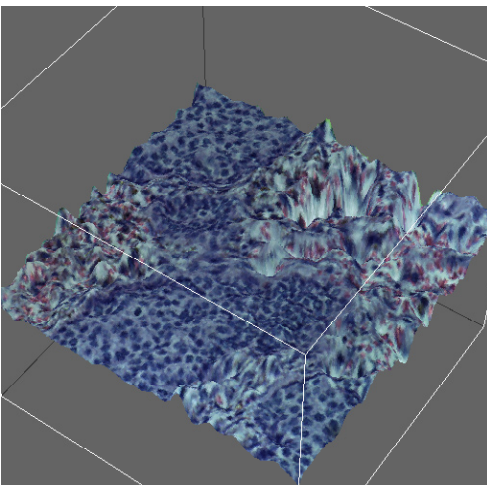
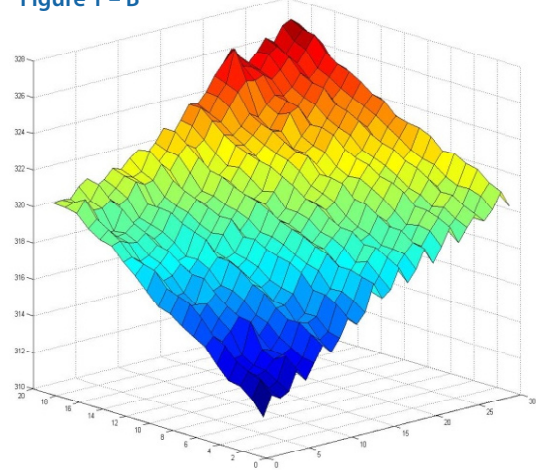


Figure 1 – B



**Figure 1. Variations in z-dimension of a whole slide image.** A) a topographical image of whole slide image illustrating the variations in z depth. Multiple z planes were acquired and composited to reconstruct the topology B) a graphical representation of z-dimension variations per tile of a whole slide image. Each acquired tile was stitched together to show the variation that occurs from tile to tile in the z-dimension. A single tile can vary over 1  $\mu\text{m}$  in the z-dimension from a neighboring tile. Stage tilt is easily observed (red to blue) which further contributes to variations in the z-dimension across a whole tissue section

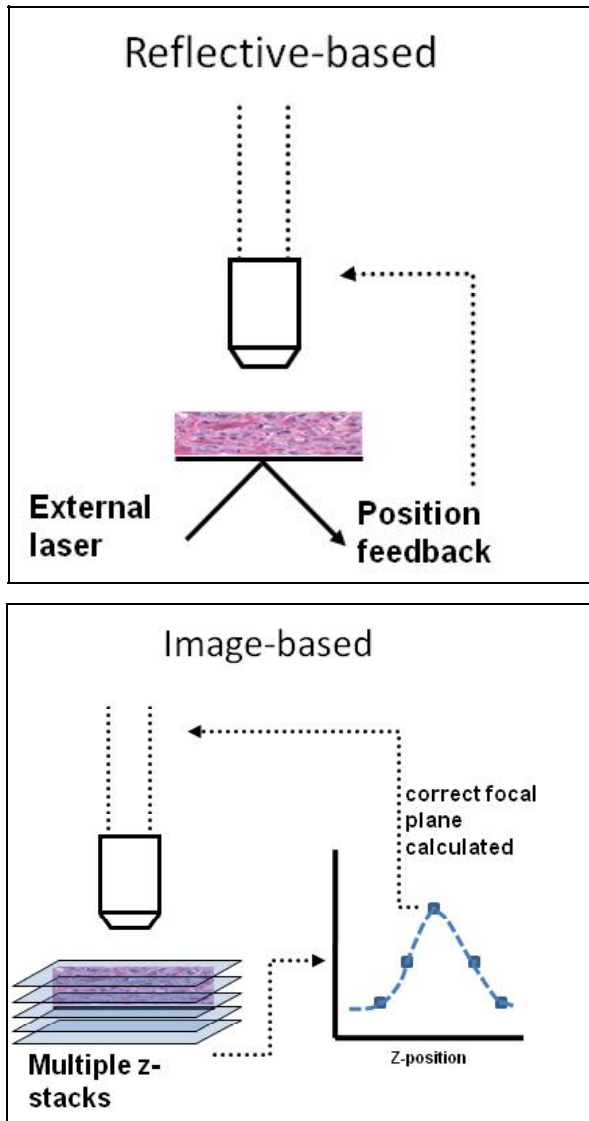
## Autofocus Methods

There are two main types of autofocus methods: 1) reflective based and 2) image based. (**Figure 2**) (Firestone, L.) The most common form of reflective based autofocus is a laser-based method based on tracking the angle of reflectance of a laser over a surface. This creates a single reference point to keep the objective at a constant distance from the sample. Laser-based systems are used effectively today for imaging samples whose topology is fixed or highly predictable. Laser-based focus systems rely on a reflective surface to establish a reference point from which an objective focus is set. However, as previously shown in Figure 1, the topology of an anatomic pathology sample is variable in z-dimension. While laser-based focus methods are fast, the requirement to accommodate variation in topology has led manufacturers of whole slide imaging devices to rely on image-based autofocusing methods rather than laser-based, or other reflective-based methods.

In contrast to laser autofocusing, image-based autofocusing, tracks the topology of tissue directly and not the surface of a glass slide, creating a more accurately focused image. This method requires multiple image stacks be acquired in

the z-dimension to calculate which focal distance is ideal. (Sun) A calculation based on the best z-dimension location, otherwise known as a figure of merit, is generated to select the ideal plane. Although this method is accurate, it has traditionally been slow and requires more time to acquire multiple images at each tile.

Figure 2



**Figure 2. Reflective vs. Image-based autofocus schemes.** Reflective-based approaches set focus a fixed distance above the reference surface (glass slide surface). Image-based approaches sample images at several different z-planes and apply a figure of merit calculation to determine the optimal focal plane.

## Depth of Field Impacts Focus

High numerical aperture (NA) objectives are typically used for standard microscopes and whole slide imaging systems because they allow for the highest resolving power. However, high NA lenses can exacerbate topology issues because they necessarily have a low depth of field (the z-range in which the focal plane exists). Although moving to lower NA objectives may help with topology issues by increasing the depth of field, this fix comes with a trade-off of lower resolution. Thus, automated microscopes are forced to compensate for focus variations in other ways.

## Autofocus: the speed vs quality trade-offs

The image quality of a whole slide image is dictated by several contributing factors including optics and illumination, camera/sensor specifications and methods for autofocus. Of these factors, autofocusing issues are the most challenging to overcome and are often cited by pathologists as the culprit for poor image quality. (Gilbertson) This is not because autofocusing per se is complex, but rather because of the desire to perform autofocus in as short a period of time as possible.

## Tiling and Line Scanning

There are two main image acquisition methods to generate whole slide images of anatomic pathology samples, namely a) line scanners and b) traditional tile scanning systems. (Garcia Rojo) Both methods are capable of adjusting focal planes on each acquired tile or line. This is done by moving the objective lens into correct position or moving the stage in z direction.

**Ideally, any imaging system will perform image-based auto focusing on every tile in a whole slide image.**

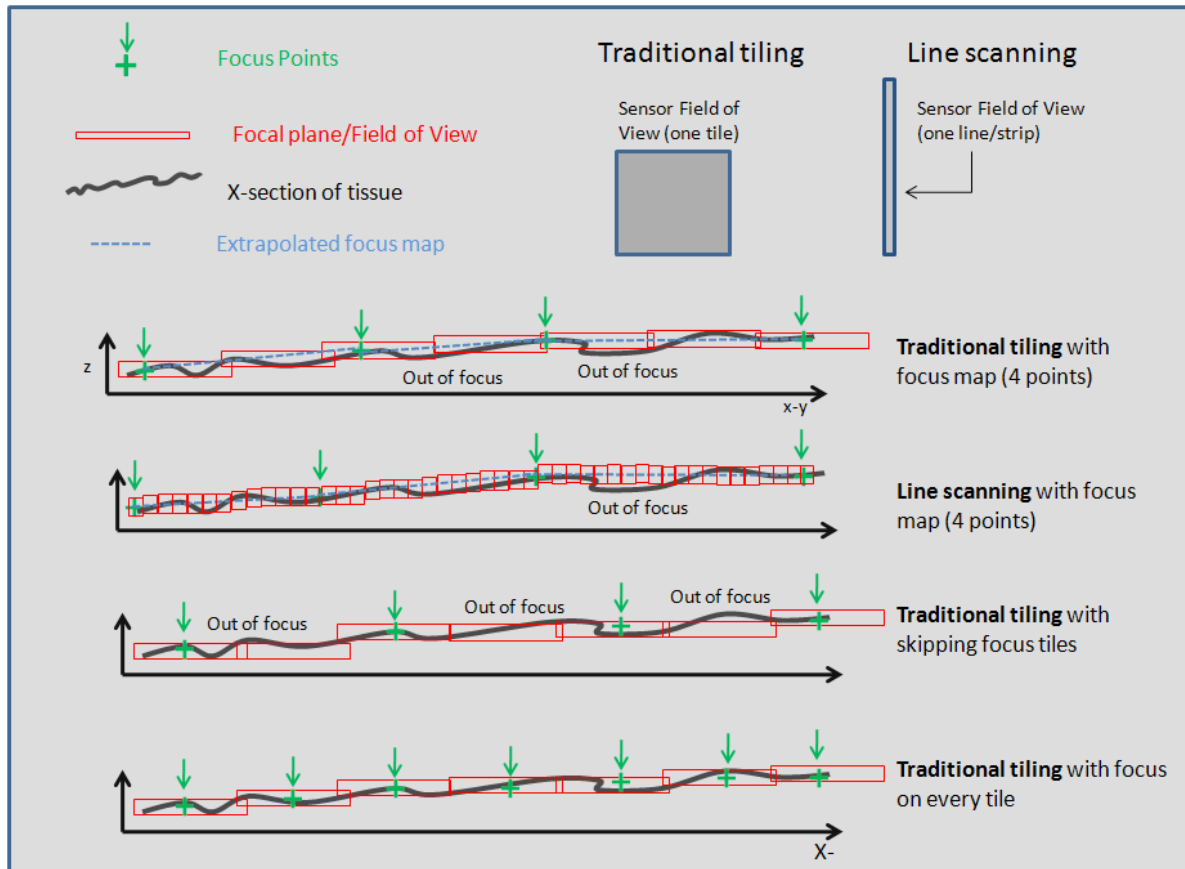
If this could be done the focus quality of the image will be high. However, as indicated above, this can take a significant amount of time. The time penalty is a result of the time required for deceleration, acceleration, settling and acquisition times at each tile. Further, acquisition time can vary based on the camera sensor size, stage speeds, light pulse speed, frame read out, etc. Assuming a rate of 100 milliseconds to acquire a single frame, surveying focus for each tile at five different focal distances would add an additional 0.5 second per tile. Thus, an image with 500 tiles (approximate for 20x) can take as much as 300 hundred seconds to acquire. This summarizes the historical challenges in generating high quality images in one minute.

## Focus Maps and Skipping Tiles for Speed

Clearly, focusing on every tile is time consuming. Thus, to alleviate the time burden, line scanners and traditional tiling systems will either create a “focus map” prior to scanning or survey focus points every-n-tiles or lines, in effect skipping areas to save time. Focus maps require a detailed survey of focus at different points on the tissue. Then, each ideal focus point is triangulated to recreate a theoretical map of the surface of the tissue, in effect filling in the blanks. Delaunay triangulation is a typical method for focus mapping, and has also been used for other medical imaging applications. Line scanners perform better than traditional tiling approaches that use focus maps because line scanners can change focus throughout their field of view (i.e., at shorter

intervals). (Figure 3)(Wrazidlo) An alternative approach for tiling systems is to skip every 3 or 4 tiles in which to focus in order to preserve speed. The assumption with skipping tiles is that a neighboring FoV will have nearly the same z position as its other neighbors. However, this is not true and two adjacent tiles can vary by more than 1 $\mu$ m in Z. (Figure 1) Nonetheless, a focus map or skipping tiles allows the system to be in continuous motion while scanning, at the expense of not surveying each and every tile/line during scanning for the perfect focal point. Many scanning systems will let the user select the number of focus points used to create a map. More focus points will naturally increase the accuracy of the overall focus quality. Yet more focus comes only at the expense of decreasing speed, since it necessarily takes time to survey more focus points.

Figure 3



**Figure 3. Image-based auto-focusing approaches.**

A cross section of a piece of tissue is shown. Green cross hairs represent the focus points used to calculate a focus map. The blue dotted (focus map) line is the calculated focal plane that is interpolated between focus points. Red boxes represent the focal plane for each field of view (i.e. each line or line or tile). Each red box/focal plane can be adjusted in z position during the scan. Line scanners can have more opportunity to adjust the z depth during scanning. Both line scanners and traditional tiling systems can incorrectly predict focus between focus map points. Focusing on every tile increases chances of having correct focus throughout the scan.

## Solution: Independent Dual Sensor (IDS) Scanning

Line scanning and traditional tiling systems use a single sensor to both survey focus and acquire the image. It is important to realize that during acquisition there is a certain amount of “dead time” while the camera is reading out an image to memory. During this time, the acquisition camera cannot be used to survey focus. In a continuous scanning system, this leads to gaps in focus sampling or it necessitates a slower speed to literally stop during read out. A better approach that provides quality and speed advantages are to use a second sensor that is independent from the first which can survey focus in parallel. In this dual sensor concept one camera acquires the image and the other simultaneously surveys focus. It is important to also try to minimize the number of focus points surveyed while still generating enough data to calculate an accurate focal plane. (Yazdanfar) After the correct z-depth is calculated, this information is used to position the stage in time for the acquisition camera to move a single tile and acquire the next image. In this concept, the focus camera operates independently, in parallel and at a much faster frame rate than the acquisition camera to ensure a survey of multiple image planes during the same time that the main camera is reading out.

Unlike traditional tiling or line scanning, IDS scanning technology is capable of surveying focus on every field of view, or tile. This equates to thousands of survey focus points as opposed to ten or twenty for other systems that do focus mapping.

IDS scanning technology brings every tile into focus, similar to a pathologist using the fine focus control as they follow tissue topology from one field of view to another at high magnification

The clear advantage to surveying such a large number of focus points is the ability to track the true nature of tissue topology more accurately and select an optimal z location. (Figure 3) This inherently leads to better image quality. Obviously, another major advantage to IDS scanning is speed of acquisition.

Although saving tens or hundreds of milliseconds from a single tile may seem to be a small advantage, it adds up to significant time when one considers that a standard 15x15mm<sup>2</sup> image at 20x contains 600 tiles and at 40x contains 2400 tiles. Thus, it is possible to perform rapid scanning while still taking orders of magnitude more focus points.

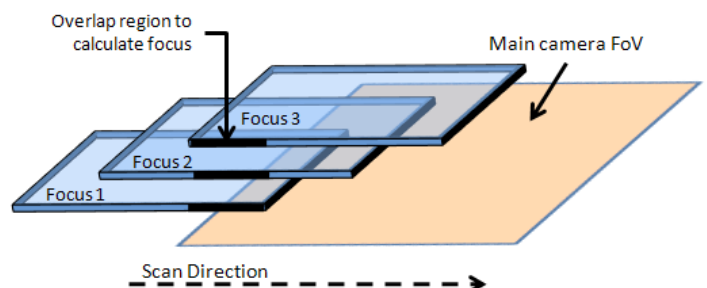
Since the acquisition camera does not need to wait at any time for focusing, the whole scan can occur more quickly.

It is also important to realize the IDS scanning does not require user intervention to set points for pre-focus mapping. This eliminates a labor intensive step during scanning and allows for scans to be more standardized by eliminating variability in focus point selections.

## Predictive Autofocusing

Since an IDS scanning system is in constant motion while scanning, there is an offset for the focus field of view compared to the acquisition field of view. Further, each focus image is offset with respect to each other. However, in the case of the focus camera, the data used to calculate the best focal plane is sampled only from the overlapped region of the focus fields. (Figure 4) This allows for the most accurate calculations of focus within the sampled areas. Additionally, although there is an offset between the acquired field of view and the focus field of views, once the correct focal point is set, the main camera has only moved approximately half a tile. Thus, much of the data used to calculate focus was contained within the main camera’s field of view, and therefore, the predictions are very close to perfect.

Figure 4



**Figure 4. Predictive auto-focusing in IDS scanning.** The focus sensor acquires three images in different z planes while in motion and uses the overlapping region to calculate the best focal plane. The main camera waits until the z stage moves into the correct focal plane and acquires the main image. This allows for focus calculations within each acquired image tile.

## Time is Money: Time to first image vs. total throughput

“Scan speed” is a term that has different meanings to different users and it is important to define this term. The industry has unofficially settled on a 15mm x 15mm area and a 20x magnification as the standard size and magnification to measure speed. Time to first image is the total time between loading a single slide and when that slide is available for viewing by the pathologist at high resolution. Time to first image includes any post acquisition steps (color balance, sharpening, compression, transmission, etc) that needs to occur prior to image viewing. The time to first image value does not have huge significance for routine anatomic pathology, save for the single slide frozen section where seconds indeed count. However, if digital pathology is thought of as a general laboratory device for routine samples, then the time to first image is less relevant. However, the overall throughput speed is very relevant.

When imaging a multiplicity of slides, the steps involved in post acquisition of one slide can occur in parallel to acquiring the image from subsequent slides. Thus, the total throughput should be faster than the time to first image. As an example, if ten slides are imaged and it takes sixty seconds to acquire an image and thirty seconds to compress and transmit, the total time to first image is ninety seconds. However, all ten slides should take approximately eleven minutes (first slide is ninety seconds; the next nine are run through at sixty seconds with parallel computing). Thus, the overall throughput for ten slides is close to sixty seconds per slide.

The difference between ninety and sixty seconds may seem like splitting hairs, but it is the throughput value that can have significant cost impact on a laboratory that is implementing digital imaging solutions. For example, a laboratory that plans to run 1000 slides per day (equates to nearly 500,000 slides per year) will require two scanners at sixty second per slide throughput. For every thirty seconds more per slide, the lab will require an additional scanner. Thus at a ninety second throughput, that lab needs three scanners and at 120 seconds per slide the lab needs four scanners... and so on.

## Conclusion

In summary, accurate focusing is a critical challenge of whole slide imaging, primarily due to inherent tissue topology variability. Traditional line scanning and tiling scanning systems are limited in their ability to acquire a high degree of focus points while still maintaining high throughput. A novel system, referred to as Independent Dual Sensor Scanning, decouples image acquisition from focusing and allows for parallel processing resulting in rapid scanning while focusing within each tile of the whole slide image and generating higher quality images.

## References:

- Firestone L., et al., Comparison of autofocus methods for automated microscopy, *Cytometry* 12, 195-206 (2004)
- Garcia Rojo M., et al., Critical comparison of 31 commercially available digital slide systems in pathology, *Int. J. Surg. Pathol.*, 14, 4 285-305 (2006)
- Gilbertson, JR et al., Primary histologic diagnosis using automated whole slide imaging: a validation study. *BMC Clinical Pathology*, 6:4, 1-19 (2006)
- Sun, Y. et al., Autofocusing in computer microscopy: Selecting the optimal focus algorithm, *Microsc. Res. Tech.*, 65, 139-149 (2004)
- Wrazidlo W., et al., An alternative method of three-dimensional reconstruction from two-dimensional CT and MR data sets. *Eur J Radiol.* Jan-Feb;12(1):11-6. (1991)
- Yazdanfar S., Kenny K.B., Tasimi K., Corwin A.D., Dixon E.L., and Filkins R.J., Simple and robust image-based autofocus for digital microscopy, *Opt. Express* 16, 8670-8677 (2008)



30 Isabella Street,  
Suite 301  
Pittsburgh, PA 15212

800 Centennial Ave,  
Building 4  
Piscataway, NJ 08854

**For more information contact:**

**Michael Montalto,**  
**VP Instrument Research & Development.**  
**[michael\\_montalto@omnyx.com](mailto:michael_montalto@omnyx.com)**

Omnyx products have not been cleared or approved by the FDA for diagnostic use.



UPMC

Omnyx, LLC a joint venture of GE Healthcare and  
University of Pittsburgh Medical Center