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Applications and challenges of digital pathology and whole slide imaging

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Abstract

Virtual microscopy is a method for digitizing images of tissue on glass slides and using a computer to view, navigate, change magnification, focus and mark areas of interest. Virtual microscope systems (also called digital pathology or whole slide imaging systems) offer several advantages for biological scientists who use slides as part of their general, pharmaceutical, biotechnology or clinical research. The systems usually are based on one of two methodologies: area scanning or line scanning. Virtual microscope systems enable automatic sample detection, virtual-Z acquisition and creation of focal maps. Virtual slides are layered with multiple resolutions at each location, including the highest resolution needed to allow more detailed review of specific regions of interest. Scans may be acquired at 2, 10, 20, 40, 60 and 100× or a combination of magnifications to highlight important detail. Digital microscopy starts when a slide collection is put into an automated or manual scanning system. The original slides are archived, then a server allows users to review multilayer digital images of the captured slides either by a closed network or by the internet. One challenge for adopting the technology is the lack of a universally accepted file format for virtual slides. Additional challenges include maintaining focus in an uneven sample, detecting specimens accurately, maximizing color fidelity with optimal brightness and contrast, optimizing resolution and keeping the images artifact-free. There are several manufacturers in the field and each has not only its own approach to these issues, but also its own image analysis software, which provides many options for users to enhance the speed, quality and accuracy of their process through virtual microscopy. Virtual microscope systems are widely used and are trusted to provide high quality solutions for teleconsultation, education, quality control, archiving, veterinary medicine, research and other fields.

Key words: biological imaging, digital imaging, digital microscope, digital pathology, digital slide, image analysis, image resolution, microscope, pathology, virtual microscopy, virtual slide, whole slide imaging

The process of viewing, annotating, comparing and analyzing specimens on glass slides has, until recently, remained relatively unchanged for more than a century. Users stained the specimen on a slide, placed the slide under the microscope and made observations. Examiners could access several fields of view and indicate areas of interest, which they then could review at different magnifications to highlight detail or overall patterns of interest. If necessary, a photograph could be used to capture and document their findings.

Although traditional slide review is accurate in the hands of a skilled and capable professional, it can be highly subjective. It is possible for the same person to analyze a slide one day and to arrive at different conclusions the following week. In addition, the process is labor intensive and the number of glass slides that must be kept accessible, clean and protected creates additional storage and labor demands.

Today, the field has evolved and demands have increased. Today's users want to compare slides

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under different lighting and staining conditions, and using multiple biomarkers. They want to see multiple layers (depths) within a single tissue for easy analysis and to compare potential pathologies to images of known pathologies on file for more accurate diagnosis. There are now more efficient ways to capture and review microscopic images digitally in response to these demands. Even the most complex slides, with multiple fields or planes of interest, can be reviewed in multiple formats at different magnifications and under different lighting conditions using a variety of stains. These new techniques are referred to as virtual microscopy, digital pathology or whole slide imaging.

Virtual microscopy

A virtual microscope (whole slide imaging system) consists of software and hardware designed to be a substitute for a traditional microscope. These systems accurately digitize images from glass slides using common objective magnifications. A virtual slide is the digitally captured image of material on a glass slide; the virtual slide consists of numerous high quality images. While each image may depict only a small region of the overall slide, software is used to stitch the images together seamlessly so that one image can show all or any part of the entire slide (Fig. 1). Together, these digital images can provide the equivalent of the original glass slide on a microscope. A computer then is used to view, navigate, change magnification and focus through the virtual slide with speed and ease. Because virtual slides include an enormous amount of information, they typically are hundreds of megabytes to several gigabytes in size, depending on the area depicted and the required resolution of the objective used. Virtual microscopes have the potential to replace conventional microscopes for certain applications.

Scanner types

There currently are two methods for scanning a glass slide to create a virtual slide: area scanning and line scanning. Area scanners use a CCD-based camera and a square sensor consisting of at least 1360×1024 pixels; some instruments use sensors of up to 2456×2058 pixels for increased detail. Area scanners move over a specimen tile by tile, section by section. At each location they stop, capture an image, then re-position to the next location.

Line scanners are of one of two types, either a linear array (4096 pixels) or a TDI CCD linear array ($> 64 \times 4096$ pixels). Linear arrays capture three lines at a time (red, green and blue) using three sensors and integrate them as the system sweeps the specimen. The TDI array integrates the signal 64 times so the illumination can be lower and the system can move through the process faster. Instead of starting and stopping, the user sees a continuous image created line by line in a manner similar to traditional fax machines. Each scanner has advantages and disadvantages.

The greatest benefits of a line scanner include its smooth, continuous movement and fast scanning; its greatest drawback is its lack of precision in fluorescence scanning and for Virtual-Z, which is used for collecting information at various depths (Khalbuss et al. 2011) The majority of line scanners use a $20\times$ objective because of its balance of resolution and depth of field. At higher magnifications, the depth of field is shallow and unforgiving if the specimen is not completely flat and results in an unfocused image. Line scanners that incorporate $20\times$ objectives typically use either a magnification changer to increase magnification or they use a 2×2 camera sensor binning area to simulate $20\times$ scanning and a non-bin area for $40\times$ scanning. Regardless of their ability to simulate higher magnifications, the optical resolution in these systems usually is limited to $20\times$.

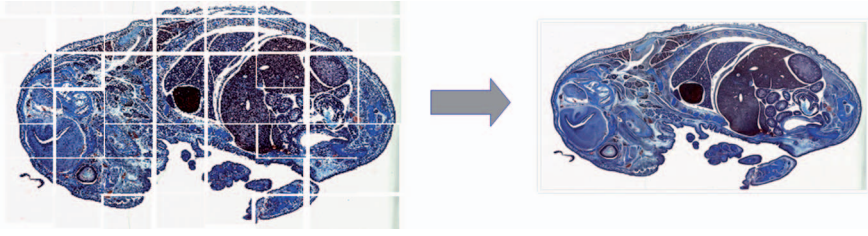

Fig. 1. A virtual slide is a digital representation of material on a glass slide. A number of high-quality specimen images are captured and assembled to create the virtual slide; a computer then is used to view, navigate, change magnification and focus through the virtual slide. Typically, virtual slides are hundreds of megabytes to a few gigabytes in size depending on the area of the specimen captured and the required resolution of the objective(s) used. All images courtesy of Olympus America Inc.

The greatest advantage of area scanners is their ability to achieve high resolution and capture images at multiple magnifications. They also can acquire multiple z-planes or different fluorescence channels with high precision at each location. The main drawback of most area scanners compared to line scanners is their lack of speed due to their stop-and-go acquisition process. An area scanner can concentrate on regions of interest at true 40, 60 or 100× oil magnifications to obtain fine resolution, but the scan time can be several times slower than for a line scanner. Purchasers must decide on an acceptable trade-off between speed and resolution/detail, and make the best decision for their application.

Virtual slide technologies

The creation of virtual slides is aided by a number of technologies. Because slide scanning creates such large amounts of data, methods for reducing unnecessary scanning are critical to a scanner's efficiency. Automatic sample detection achieves this by allowing the system first to capture a low magnification overview image of the entire slide. This overview image then can be used to designate regions to be scanned.

After tissue locations are identified, each system has its own way to create a focal map or other predictive focal mechanism. Because tissue does not lie flat on a glass slide, the system must autofocus continually or put together a sample focus map (Fig. 2). Although many typical pathology sections are 5 µm thick and one focal plane may be optimal, some samples can be greater than 50 µm thick or of different thickness in different areas, which makes it difficult to get the entire specimen in focus. In these situations, the system can either calculate an in-focus plane by acquiring multiple scan areas at different z-positions and creating a z-stack taking only focused areas of each focal plane into account, or it can acquire images at multiple planes and allow the user to virtually focus through the digital image as if they were using a real microscope.

Although most routine slide scanning applications use 20× objectives, additional magnifications can be helpful. Several layers can be scanned at many magnifications and stacked to combine many regions of interest within the final image. In addition to the tissue image on the display, a live overview image, which shows exactly where the current region of interest is located within the entire specimen, is displayed at all times. This live overview image helps users maintain their orientation throughout the review process. By scanning only specific regions of interest at higher magnifications, it is possible to decrease the overall image file size. Users can specify which regions they want scanned at each magnification.

Each laboratory has its own standard operating procedure. A typical work flow for veterinary, educational and research pathology laboratories in the USA may include scanning each slide's barcode for identification, creating a 2× overview image for each slide, then capturing each slide at 20×. This process takes about an hour for a batch of 100 slides. These images may be pushed during scanning (or later) to a database from which they can be viewed locally using an internal network or anywhere in the world using the internet. In most cases, the original glass slides still are archived, but the pathologists review the virtual slides later on a computer monitor for analysis at various magnifications (Fig. 3). Because a virtual slide is a digital file, it does not deteriorate or bleach, it is more accessible and images can be managed easily. Multiple resolutions are possible for a single virtual slide and the laboratory staff can select whether the entire slide or just regions of interest must be captured. Images can be stored efficiently, shared, and annotated up to the maximum magnification at which they were scanned. Although mathematical algorithms

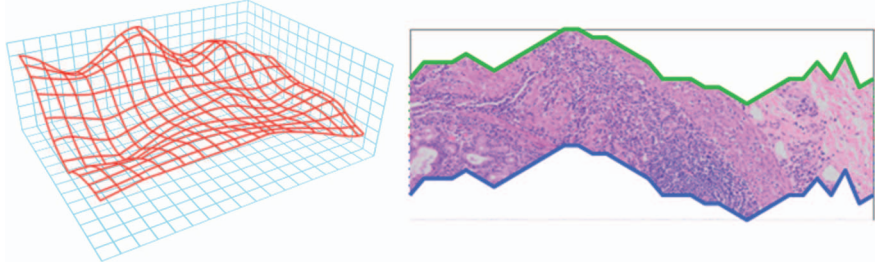

Fig. 2. Virtual Z acquisition. Left) Naturally occurring variation in Z (depth) of a typical specimen is depicted. Right) A focal map is sometimes created to illustrate the natural variation in Z (depth) of specimens; the map shown here corresponds to the specimen at left.

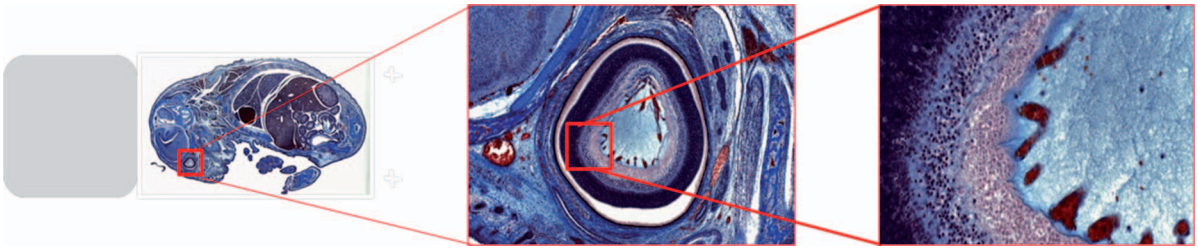

Fig. 3. Scanning and viewing. Multiple resolutions are possible on a single virtual slide. The entire slide, or a region of interest, can be captured. Since a virtual slide is a digital file, it can be viewed or sent anytime, anywhere through local-area or Internet networks. In addition, it will not deteriorate or bleach out, cannot be destroyed or lost if properly backed up, and is easily managed by databases.

can simulate higher magnifications than the scanning magnification, additional information cannot be created, so high magnification simulation is not usually recommended.

An advantage of virtual microscopy is the ability to review multiple images of the same or related tissues. The user can review the same specimen captured using different stains and view from two to 15 images side by side. This is useful for viewing morphology while reviewing what biomarkers reveal about the cells. Alternatively, the user can review hematoxylin and eosin (H & E) staining compared to immunohistochemistry double or triple staining, or one can look at each region of interest at both high and low magnifications simultaneously. When the user moves to any area of the slide, all the related images move synchronously (Fig. 4).

The size of data files is an important consideration for virtual microscopy. For example, e-mailing multilayer images usually is not practical; each slide may comprise billions of pixels and even with compression the files may be impractical to display or download. In fact, the ability to grab intermediate planes and make them viewable without spending time to download the entire image is an important challenge for manufacturers of these systems. Viewing or image server software provides a more elegant way to make virtual slides viewable quickly. Many companies license patented technology developed by a company called Bacus (now owned by Olympus, Center Valley, PA) or Zoomify (Santa Cruz, CA) for retrieving intermediate frames without having to download the entire file structure (Fig. 5). This technology makes it practical to view an image at multiple resolutions over the internet, because the user does not have to wait for a gigabyte or more of image data to load each time he or she wants to retrieve a specific image field of view.

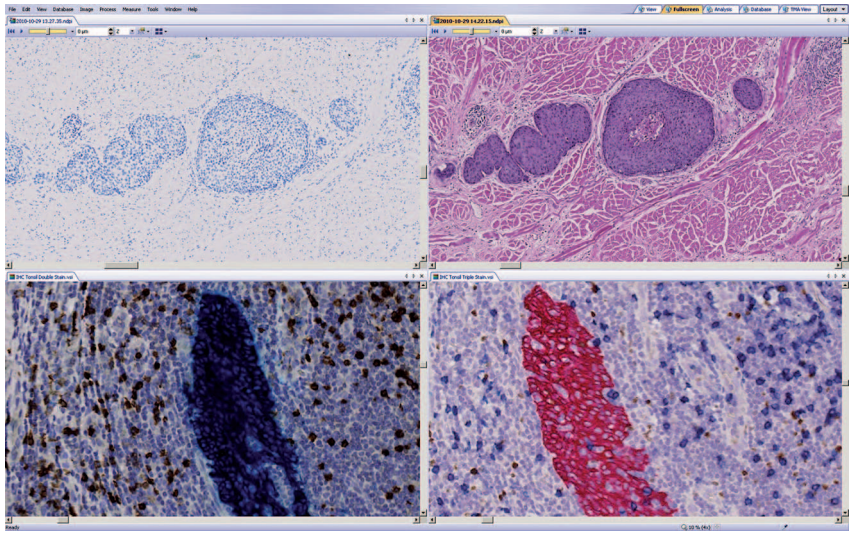

Fig. 4. Multiple slide images may be synchronized using virtual microscopy. At top, virtual images captured with eosin (left) and H&E (right) are arranged for comparative review. At bottom, double-stained (left) and triple-stained (right) images of a tonsil specimen are synchronized for comparison.

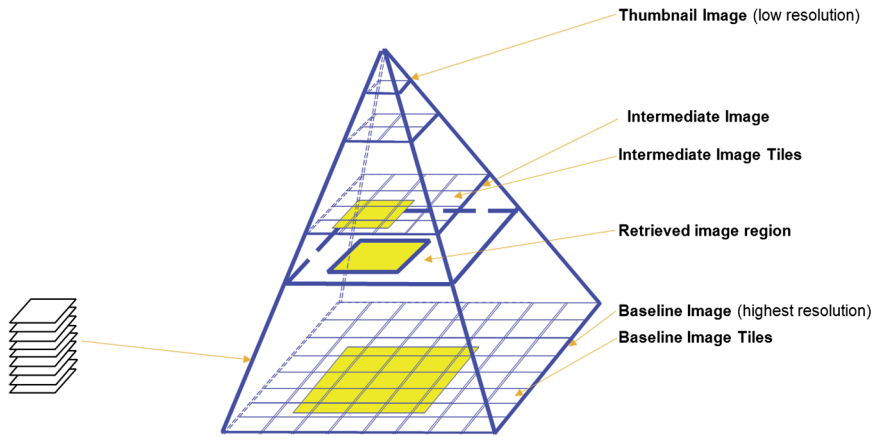

Fig. 5. A representation of a virtual slide, which can be described architecturally as a pyramid of separate data sets. Each "tile" of each level of each plane within the virtual slide corresponds to a single viewable image. Z-stacking allows the capture of multiple planes of focus at each location at the highest resolution needed for optimal viewing. All individual tiles, as well as combinations of tiled areas at various magnifications, are viewable. Pyramid adapted from DICOM Supplement 145: Whole Slide Microscopic Image IOD and SOP Classes, 2009.

Challenges for virtual microscopy

Virtual microscopy faces several important challenges. One of the greatest of these is the lack of a universal format for virtual slide data. (Daniel et al. 2011, Pantanowitz et al. 2011, Weinstein et al. 2009, Yagi and Gilbertson 2005). Each manufacturer has a proprietary format that is optimized for its own software. There are professional groups working to create a digital imaging and communications (DICOM) standard for virtual slides for medical use, but for now, selecting a company means being locked into a particular format for a period of time.

Each manufacturer also has its own method of managing technical challenges. One such challenge is dealing with the focus setting for images with low contrast, such as adipose tissue. The focus setting also can be compromised if the edges of the coverglass or user's markings on the original slide are recognized as samples, if the samples are elongated or if there are multiple samples on a single slide.

In addition to focus setting, each manufacturer handles color contrast and exposure differently (Yagi 2011). Some scanners adjust contrast at acquisition, while others have a default for collecting what the microscope actually sees and allowing the user to perform contrast adjustment later. This option allows the investigator to collect maximum data from the sample and to adjust for personal preference in the slide-review stage. Because our eyes do not perceive light the way image capture devices do, gamma correction often is used to "translate" between the light sensitivity of the imaging device and that of the human eye. In addition, what an individual sees as proper color and brightness often is a matter of personal taste. For example, some microscope users prefer a very white background in their slides. Collecting enough data to make the background perfectly white, however, can sometimes wash out an image, and it is hard on the eyes to view large areas of white for long periods. Therefore, collecting maximum information at the time of acquisition, then making it possible for the user to adjust the gamma to reflect personal viewing preference may be a good alternative for many facilities, especially those with multiple users (Fig. 6).

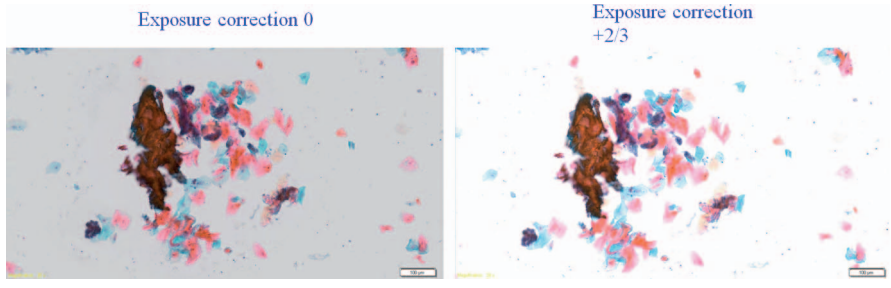

Fig. 6. Sample pair of images demonstrating the benefit of collecting maximum data at the time of exposure; later adjustments in brightness can be made by individual users to enhance image quality for their needs.

Another challenge is balancing contrast and resolution. Many turnkey virtual microscope systems are designed for throughput with a fixed condenser aperture, fixed objective and a simple magnification changer. These systems cannot achieve what a pathologist does, i.e., manually adjust the settings to optimize contrast and resolution to obtain the best image quality. The turnkey systems, however, can move quickly through a stack of slides to collect data. Other systems act more like a traditional microscope with the potential to open or close the condenser aperture to fine tune the quality of the images. Depending on the volume, a laboratory may have separate instruments for high volume, low volume and specialty microscope slides.

The challenge of deciding what magnifications may be needed to capture images is another determinant for deciding what instrument is best for a given purpose. When comparing image quality and resolution, users should look at images at different magnifications as captured by instruments from different companies. If a 20× capture is sufficient for a facility's needs, then there is flexibility. Digitally enhancing a 20× capture to simulate 40, 60 or 100×, however, may not deliver the same resolution or image quality as a true high magnification optical capture (Fig. 7). If a user collects images of specimens with hard to find or hard to measure features, it may be best to consider systems that provide a higher resolution.

Finally, how image analysis is handled is an important issue among various systems (Pantanowitz 2010). There are software packages that offer repeatable image analysis of large batches of slides, which saves the user time and resources. Some programs conduct additional reviews over time, which creates better output control and the development of stronger analytical algorithms. Such image analysis enhancements may be beneficial in both the clinical and research settings by reducing time frames for introducing drugs and helping investigators make better informed decisions about which therapies may be most effective.

Conclusion

The tradeoffs between speed and throughput on the one hand, and flexibility and resolution on the other should be considered before committing to virtual microscopy. Does the user need the highest image quality with the widest variety of parameters? Is moving the maximum number of slides through the review process the primary goal? Are fluorite objectives sufficient or does the application require apochromat objectives (Abramowitz 2003)? Are there multiple users who may have different requirements? All of these issues contribute to the complexity of virtual microscopy use. Perhaps one day we will have standard requirements for virtual images for a variety of applications.

In the interim virtual microscopy currently is widely used without incident for many important applications including teleconsulting, archiving, research and pathology education/quality control

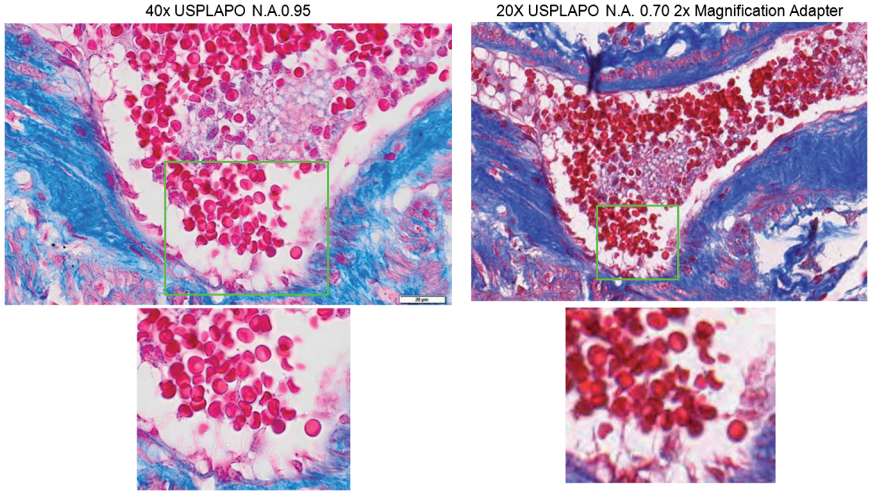

Fig. 7. Comparison of blood cell images captured using different methods. Images on the left were captured using a true 40× objective; images on the right were captured using a high-quality magnification changer – a method of digital enhancement that allows the production of a 40× image from a 20× capture. Because there is a difference in image quality, users who require magnifications of 40× or higher sometimes choose to consider enhanced-resolution systems.

(Leong and McGee 2001). Teleconsultation is widely used in Canada and Europe, and it is used in the USA for veterinary pathology. Tissue banks and other database managers are archiving specimens digitally for large scale data analytics and to keep records of unique cases. In pharmaceutical and biotechnology research, virtual microscopy has helped shorten the time required to bring new medicines and treatments to market compared to manually reviewing thousands of glass slides. In education, there is an opportunity to change the way doctors are taught (Foster 2010); more than 50 US medical schools already use digital pathology images for part of their physician training programs. We already are benefiting from this technology in areas as varied as distance learning, clinical research and data mining. The possibilities of using digital specimen files for a wide variety of research and other applications are nearly unlimited. While hurdles remain for both manufacturers and users, virtual microscopy is a tool with the potential to make work more efficient, provide more consistent and accurate data for analysis, and to help people share resources and knowledge around the world for the good of all.

Declaration of interest: The author reports the following interest: the author, who teaches widely in the field of virtual microscopy, is an employee of Olympus America Inc. Olympus' parent company, Olympus Corp., provides hardware and software for virtual microscopy. The content of this paper was delivered as an educational presentation; the author was requested by the Biological Stain Commission to publish this paper. Material accompanying this article is not for clinical diagnostic use.

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