Virtual slides: an introduction

Darren Treanor

Abstract

This review is a basic summary of virtual slides and assumes no prior knowledge of the area. It describes how virtual slides are produced and the technical issues underlying them. The advantages and disadvantages of virtual slide are highlighted. Finally, the use of virtual slides in education, training and diagnosis is discussed, and the technical and organizational challenges they present are described.

Keywords histopathology; informatics; microscopy; virtual slide

Introduction

Virtual slides have been around in a usable form since the early 1990s and, as a technology, are increasing in maturity. They have many innovative uses in education and training, and are becoming an essential tool in research practice. However, their use in routine diagnosis has yet to come. This review aims to give a basic introduction to virtual slides, assuming no prior knowledge of the subject, and to discuss the issues around virtual slides in diagnosis.

What is a virtual slide

A virtual slide is a digital image produced by scanning a glass pathology slide at high resolution. Typically, virtual slide scanners use standard microscope lenses and light sources to obtain a microscopic image of the tissue. A robotic system sequentially moves the lens or slide so that the entire slide is scanned. The resulting image is captured electronically with an image capture device similar to those seen in digital cameras (a so-called CCD or CMOS chip) to record all of the image data from the slide. The effective resolution of virtual slide scanners is similar to that of a microscope: of the order of 0.2–0.5 μm per pixel. Put another way, they scan at 100,000 dots per inch (a desktop paper scanner scans at 200–600 dots per inch). As they capture high-resolution colour images of the entire slide, virtual slides tend to be large images – gigapixels in size – which, when saved as a computer file, can be hundreds of megabytes or even several gigabytes in size. In practical terms, many virtual slide scanners have robotic systems to allow several slides to be scanned at once – ranging from 5 to more than 150.

***Darren Treanor** BSc(Computing) MB FRCPath is a Consultant Pathologist/ Honorary Senior Lecturer, Pathology and Tumour Biology at the Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK.*

Virtual slides differ from telepathology, although the end result may appear the same to the user. In telepathology, a microscope is remotely driven by the pathologist, and the image from the microscope is transmitted to them live. For virtual slides, however, the image acquisition and viewing need not happen at the same time. Virtual slides are only feasible due to the improvements in computer processing and storage capabilities of the last one to two decades; before these, the idea of scanning and transmitting gigapixel images would have been unthinkable.

Producing a virtual slide

Virtual slides differ from the image seen down the microscope in several ways due to the methods applied to acquire and store them. A summary of the important steps in producing virtual slides follows.

Image focusing

Histopathology and cytopathology specimens are three-dimensional (3D); virtual slides tend to be two-dimensional (2D) images captured from the 3D object. This can be seen both as an advantage or disadvantage. On the one hand, a virtual slide can be seen as an optimally focussed 2D capture of a lumpy glass slide − typically, virtual slide systems adjust the focus rapidly (tens or hundreds of times) during scanning to maintain a focused image in the final virtual slide. On the other hand, there may be important diagnostic information in the 3D slide that is lost by this approach. This is particularly true in cytopathology. Virtual slide vendors have addressed this by offering partial or whole slide 3D scanning – a significant drawback of this is that the already large digital image is made even larger when multiple scans in the third dimension (producing a so-called z-stack) are made.

Stitching or tiling of images

As virtual slide scanners may acquire hundreds or thousands of individual images to make one virtual slide, the images must be joined to make one large image. Vendors differ in whether they stitch together strips of images or tile rectangular images together, but both methods require computer processing by the scanner to ensure that the joins are not visible in the final virtual slide.

Image processing

The image undergoes a variable degree of processing (alteration) during and after acquisition. For example, the image may be automatically sharpened (to enhance the definition of edges in the image) or the colour and brightness may be adjusted. This is standard practice in digital imaging (and can be used with conventional digital cameras to enhance photomicroscopy), but it is important to remember that the digital image is a processed digital reproduction of the captured image.

Image compression

For reasons of practicality, the image files produced by virtual slides are usually compressed before the image is stored on a computer. For example, tissue on a glass slide measuring 3 × 2 cm scanned at 0.2 μm per pixel produces a colour image with

120,000 × 80,000 pixels (9.6 gigapixels). Uncompressed, this would require an image file of 28 GB to be stored on a computer. Image compression uses computer algorithms to reduce the storage space required. Various algorithms are used but they can be broadly divided into (1) lossless algorithms, which reduce the image size without losing any image information (e.g. Lempel-Ziv-Welch (LZW)) and (2) lossy algorithms which remove image detail (e.g. Joint Photographic Experts Group (JPEG)). The latter are commonly used in virtual slide scanning, as they can reduce image sizes significantly; for example, the 9 gigapixel image above could be compressed to 1 GB or less with JPEG compression, depending on the amount of data discarded during compression. Obviously, if lossy compression is excessive there is a danger that diagnostic information is lost from the slide – work needs to be done to establish what is an acceptable degree of compression.

Virtual slide scanners are relatively simple devices which can be placed in a laboratory workflow just after the coverslipping machine. Image acquisition is automated or semi-automated, requiring an operator to load the images and check the scan quality. Quality control procedures are usually necessary to ensure that slides are in focus and captured in total.

A more significant challenge to implementation of virtual slides, however, is the information technology knowledge and support required to maintain a system. Used regularly, a scanner will quickly generate thousands of gigabytes of data. The images must be catalogued in a professionally designed database, stored and backed up safely. This is a task that requires professional IT knowledge and assistance.

Viewing virtual slides

Virtual slides are viewed using specialized software (Figure 1); most virtual slide vendors have custom built software for their own slides. All of the viewing systems have similar basic features reminiscent of software to navigate maps – the image is displayed in a window; a small thumbnail of the whole slide displays the current 'location' on the slide; the pathologist can zoom in and out by using buttons on the screen or keyboard, and can pan by using similar controls or by 'dragging and dropping' the image. A key benefit of virtual slides is the ability to rapidly pan around a slide by clicking anywhere on the thumbnail image – something that is impossible with a conventional microscope. Various other functions are added to the viewing software, such as the ability to take snapshots, make annotations or measurements, and image analysis features.

Despite their large size, virtual slides can be viewed on relatively basic personal computers. This is made possible by the use of so-called client–server technology. Briefly, the pathologist has virtual slide viewing software on their computer (the 'client') which requests images from the computer on which the virtual slides are stored (the 'server'). Rather than downloading the entire virtual slide (which could take several days depending on the speed of the network connection between the client and server), only the currently displayed view of the slide is displayed. So, only one 'screenful' of information is requested at a time. In practice, this means that – provided a reasonably fast modern connection is available – virtual slides can be viewed over the internet.

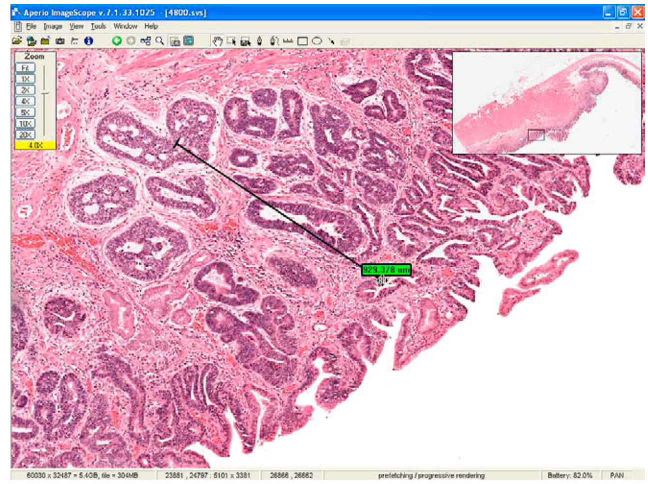

Figure 1 Virtual slide viewing software. A case of gastric cancer stained with H&E is examined using Aperio's ImageScope software (Aperio Technologies Inc, San Diego, CA, USA). A thumbnail (top right) shows an overview of the tissue, with a black rectangle indicating the current position on the slide. Buttons on the left of the screen allow a choice of fixed magnifications; the user pans around the image by 'dragging and dropping' with the mouse on the main image, or by clicking on a region of interest in the thumbnail. Other controls in the toolbar at the top of the screen allow snapshots to be taken, text/drawing annotations to be made, or measurements to be made. In this case the depth of tumour invasion is being measured on screen.

Advantages and disadvantages of virtual slides

The advantages and disadvantages of virtual slides are listed in Table 1.

Uses of virtual slides in education and training

Undergraduate teaching

With an increasing trend in medical schools away from microscope based tutorials, there is a risk that teaching of basic pathology will suffer. Several medical schools have developed e-learning based approaches to teaching microscopy – for example, see the excellent WebPath site.[1] While this is undoubtedly of benefit, viewing static images does not offer the same interactivity as using a microscope. However, medical students tend to dislike using microscopes as they take practice to use them. Virtual slides allow students to examine normal and diseased tissue in an interactive way, but without the complexity and logistic needs of a microscope laboratory. In addition, virtual slides can be integrated into e-learning material such as tutorials or online questionnaires, and can be annotated to guide students to areas of interest. In this context, virtual slides have already been successfully implemented in medical education at several institutions.[2,3]

Postgraduate training

Familiarity with the microscope is not a problem in postgraduate training, but access to material is. During their training, pathologists are expected to combine reading with practical experience of interpreting slides. However, departmental and personal slide collections are often incomplete, can only be accessed by a small

