VIRTUAL MICROSCOPY

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The last few years have seen the increasing incorporation of virtual microscopy systems in universities and biomedical laboratories as part of the evolving and dramatic changes associated with digital imaging. In many cases the traditional microscope is disappearing with the advent of the new imaging technologies (Coleman, 2009).

The four main areas involving virtual microscopy are:

1. **Telemedicine**, which allows transmission of high resolution histopathological images to a distant site to receive a second opinion. This has proved very effective when pathologists in isolated medical facilities can consult with distant experts in accredited centers of excellence. To date telemedicine has been the main application of virtual microscopy systems.
2. **Creating archives**. Pathology laboratories can now store all their scanned high resolution pathology slides in virtual archives, allowing instant access for consultation and slide-sharing with colleagues, locally or worldwide.
3. **Research studies**. There are major attempts to create and commercialize software programs for automatic diagnostic analysis of scanned histopathological slides. The aim is to create the means of automatic identification and analysis of specific diseases such as breast or prostate cancer from scanned digitized slides.
4. **E-learning**. This involves the replacement of microscopes as teaching tools in courses such as histology or histopathology.

Obsolescence in the digital world

In a very short time we have moved from analog to digital recording of images. Our image recording systems have rapidly become obsolete. Film and movies, 35mm cameras, transparencies, videotape, floppy disks, CDs and DVDs have become anachronistic and replaced by new media forms. In parallel, computing hardware and software have become much cheaper, more accessible and universally available. The new generation of students is enveloped in a digital environment, coming to class with laptops, music players and smartphones, which do far more than just send and receive vocal messages. In parallel the digital instrumentation has become increasingly miniaturized. Such dramatic changes have meant that the traditional microscopists and pathologists, despite the conservatism of the older generation, need to adapt to the new digital formats.

The benefits of virtual microscopy

The benefits of virtual microscopy include remote diagnostic viewing, remote consultation, sharing the same image with colleagues or students simultaneously, improved work ergonomics, digital archives, case-study libraries, tissue databanks, and image analysis. There are enormous economic advantages in the establishment of virtual slide collections for student education. After the initial investment in a virtual microscopy system, there are long-term savings in the annual budget of medical school administrators. It is no longer necessary to purchase individual student microscopes, or to deal with their maintenance, storage or repair, no worries about loss or damage of irreplaceable slides and no need to prepare and replace student slides. There is a reduced need for histology technicians to prepare and sort slides. There is considerable saving in both time and physical space in preparing and conducting laboratory classes. Access to virtual images and laboratory assignments can be done wherever it is convenient (at home, in the library, on the beach, in the middle of the night or while commuting). The virtual microscopy systems are great for annotating slides (indicating what to look for) and also for student examinations. Virtual microscopy can be a win-win situation if adopted correctly.

The basics of virtual microscopy

Virtual microscopy involves scanning prepared sections on microscope slides at high resolution, storing the images in a digital archive and allowing access for subsequent navigation and analysis. Moving from a traditional microscope system to virtual microscopy requires purchase of a microscope system with high resolution optics, a motorized scanning stage and the necessary computing hardware and software. Each scanned slide can occupy up to 2GB space on the hard disk or server. Slide scanning can be done by line or patch (tile) scanning with the line scanners being faster. Most systems use a sequence of scanned tiles, which are then "stitched together" using software making the seams invisible. The system involves autofocus for each patch as no section is perfectly flat. The images are stored at high resolution (TIFF or equivalent) and then the images are compressed to about 200MB. When you access an image from the archive you begin with a thumbnail image of the whole slide including the label or barcode and magnify (decompress) in the manner of Google Earth®. Students need a degree of patience while downloading and decompression stages are undertaken. Images can be navigated with a keyboard (up, down, right, left), joystick or games controller, touch pad, or with a finger (real digital) if the monitor has a touch screen.

All the major microscope manufacturers have their own instruments for virtual microscopy. They all come with high resolution optics, high precision scanning stages with automated software procedures for line scanning or tiling to ensure rapid and

accurate scanning, automated focus control, software for compression and decompression, navigation, access, image storage and image analysis.

Open or closed-box scanning instruments

You can have systems for bright-field and also for fluorescence. The purchaser must decide the configuration according to the intended use. The virtual microscopy microscopes fall into two basic categories. There are "open" systems and "closed box" systems. The open systems, such as those of Olympus (dot.Slide, VS110, VS120) look like regular research microscopes with a series of objectives. However, most of the commercial virtual microscopy instruments belong to the “closed box” category and do not look like microscopes at all (Aperio, Pannoramic 3D Histech, Leica, Hamamatsu, MicroScan D2) with no user access to the mechanical or optical components of the system. Each category has its advantages and disadvantages. The open systems allow more flexibility (range of objectives and illumination) than the closed box systems. The "closed box" systems typically incorporate a single objective lens, the most popular being x20 plan-achromatic objectives with an NA 0.80 (such as the Olympus U Plan S APO2 or Zeiss plan-achromatic). In a very competitive commercial world, the virtual microscope manufacturers battle each other at the European scanning microscopy contests. In the resolution category the Olympus VS120 x40 objective lens system recently won the best image award (0.16µm/pixel). Most hematological studies on smears require an oil immersion lens. In such cases the open systems are more convenient, though some specialized "closed box" systems incorporate an oil immersion lens for such smears. It is important to remember that any virtual microscopy system needs good quality flat sections with adequate staining and contrast. You will probably encounter problems in scanning total preparations, where the range of focus exceeds the limits of the virtual microscope configuration. Any faults in sections (folds, tears, dirt, staining artefacts) become very apparent in scanned slides, though all the systems allow cropping to remove the faulty areas of the section prior to scanning and image storage.

The market for virtual microscopy systems

The main adoption of virtual microscopy systems has so far been the hospital laboratory involved in analysis of large numbers of histopathological slides and subsequent archiving. Olympus Corporation has now sold over 350 of their VS systems worldwide. Many virtual microscopy systems can be purchased with automatic loaders. These are useful in hospital environments where they can perform automatic loading and scanning of 150 to 400+ slides during the night. These robotic slide loaders use software for automated tissue recognition, focusing, and calibration. The scanning time for each slide depends on the specimen size and the objective used. Improvements in computing power in recent years have reduced scanning times to 2-6 minutes depending on the size of the section. The Hamamatsu

NanoZoomer 2.0 has reduced the time for scanning slides to approximately 1 minute 40 seconds, while the Aperio ScanScope AT pathology slide scanner can process about 30 slides per hour in its automated loading mode. The automated loaders are ideal where you need to image and record large numbers of slides, e.g. blood smears, PAP smears etc. The pathologist can speed up diagnosis using these scanned images and suffers less discomfort than the traditional examination of slides in a microscope. On the other hand, such automatic loaders are rarely needed in research laboratories in universities and the standard manual loading holders of 5 to 12 slides suffice.

Diagnostic histopathology

There is considerable innovation in the virtual microscopy market, especially for histopathological diagnostics. It is possible to create automatic diagnostic analysis programs using histomorphometric data such as the structure and staining characteristics of nuclei (size, shape, optical density, texture). Unfortunately the success rate for such automated analyses is still relatively poor and it is doubtful in the near future if the pathologist will feel threatened by robotic analysis. Despite the current relatively low success rate for automated diagnostic analysis, enormous efforts are being undertaken with anticipation of substantial financial rewards for successful innovative analytical software. Royal Philips Electronics of the Netherlands joined forces with NEC Corporation in April 2011 to develop integrated pathology slide scanners and are developing systems for grading breast and prostate cancer. At first sight it is hard to recognize that their digital pathology automated slide system is really a highly sophisticated virtual microscope. A different approach to diagnostic analysis has involved the marriage of spectral imaging with virtual microscopy systems. Olympus (Europe) in May 2012 joined forces with Applied Spectral Imaging to market such a spectral analysis capability for improved automated imaging platforms for genetic and pathological analysis.

Several commercial virtual microscopy companies have developed the software for virtual microscopy of tissue microarrays, which have become increasingly popular in diagnostic studies in recent years. TMA slides, with 16 or more different samples, can be scanned, explored, analyzed at high resolution and archived. They can be imaged in a range of illumination conditions: brightfield, darkfield, fluorescence and for FISH studies and archived in image banks.

Photomacrography

Virtual microscopy has revolutionized the preparation of low power high resolution histology images. Traditional macrophotographers encountered major problems in recording images of whole sections of embryos. Difficulties involved limited field of view, recognizing focus, uneven illumination, vignetting and poor resolution.

Photomacrography set-ups were complex, prone to vibration and exposure problems. Many of the older generation recall the frustrations of macrophotography of slides placed on light-boxes with frosted glass and SLR cameras with enormous bellows. Nowadays you can use any virtual microscope system to image large embryonic sections with perfect illumination at high resolution. This is proving a boon for the teaching of embryology. Leica Microsystems with their SCN400 instrument have taken this a stage further with the capability of scanning Jumbo slides (113x76mm), which will also be widely appreciated by neuroscientists.

Commercial software packages for diagnostics and e-learning

Although there are many commercial companies producing software packages for diagnostic pathology, one of the leaders is undoubtedly Definiens (Munich, Germany) that specializes in image analysis and data mining solutions for quantitative digital pathology. They have developed software for automated stereology from virtual microscopy images, which has the advantage of obtaining quantitative data about 3D features from 2D tissue sections.

Although most of the software packages for virtual microscopy have been developed for telemedicine, there are now several commercial packages for e-learning. Unfortunately these are typically very expensive. Two outstanding leaders in the field of diagnostic pathology and e-learning are SlidePath of Dublin, which is adopted by Leica Microsystems in their SCN400 instrument and PathXL of Belfast, recommended by Olympus. The software of both these companies is compatible with all the various virtual microscopy systems. QuestionMark software is widely used especially with the Aperio instruments in the USA. Owing to the very high cost of these comprehensive virtual microscopy software programs, increasing numbers of medical schools are producing their own software. Many medical schools have evaluated the economics of purchasing their own virtual scanning stage microscope and reached the conclusion that after initial scanning of their slide collection, these instruments would sit around gathering dust. This was the fate of many of the Nikon Coolscope II instruments located in pathology laboratories. The instrument manufacturers and the software companies all provide commercial services to scan your slide collections. Alternatively institutions can purchase a comprehensive collection of virtual slides from these companies, but read the small print carefully regarding restrictions of use (licensing agreements).

Recent developments

It is now possible to publish virtual microscopy images. The journal Diagnostic Pathology (BioMed Central) allows authors to upload their virtual microscopy images, which readers can access and navigate using software from SlidePath,

DiagnomX and Leica Microsystems. Leica also has introduced an application for downloading digital pathology images to iPad and iPhone.

If you want a really special virtual microscopy experience, Multitouch of Finland market an instrument with a giant touch screen (minimum 46 inch). By touching this screen the users can navigate a virtual microscopy slide, rotate the images and zoom 1000 fold to show cellular and subcellular detail (http://tinyurl.com/6zbbypq).

The main forum for the exchange of information on Virtual Microscopy is the biennial international congress devoted to telepathology and virtual microscopy. The 11th European Congress on Telepathology and 5th International Congress on Virtual Microscopy were recently held in Venice, Italy (June 6-9, 2012). The leading plenary lectures are subsequently published in Diagnostic Pathology.

Archives and National Image Repositories

Although there are several major repositories for microscopy images, these are not specialized for virtual microscopy. Such image repositories include the Wellcome Foundation and the NASA sponsored Virtual Microscope Laboratory Initiative in the University of Illinois at Urbana-Champaign.

The future for virtual microscopy archives for teaching and research involves software and "cloud computing". Smart Imaging Technologies (Houston, Texas) market the necessary commercial technology. You upload your virtual slides, which can be accessed, viewed, annotated, measured, and navigated simultaneously from any computer or tablet using technology similar to that of Google Maps (www.live.simagis.com).

With the increasing use of digitized virtual microscopy images replacing traditional microscopy images, the next stage of evolution is the establishment of archives of such images for educational and research purposes. In the first instance these archives are typically local (at university or hospital level), however there is now considerable movement to encourage collaboration between various centers to create inter-university, national and international repositories or virtual image banks. Such facilities will allow access, retrieval and navigation of infinite organized collections of virtual microscopy images. These large image repositories, funded by national science councils, will operate using a system similar to that of Google Earth or Google Maps with the virtual images stored in a “cloud” (large distant server) allowing wide access to authorized individuals. Establishing national repositories need clear ground-rules including resolving questions about copyright of images and restrictions on commercial use. In Israel we are currently trying to establish a national repository for virtual microscopy, which is intended to provide educational resources for an enormous population of potential users in the medical, nursing and

biology sectors. Maybe the Royal Microscopical Society, the Science Museum or the Wellcome Foundation could be the instigator of a virtual microscopy image collection.

Ultimately I anticipate international collaboration in virtual microscopy image collections. Maybe a major philanthropic donor or concern could provide the means to establish an international forum for such a world-wide virtual microscopy archive (Microscopy Society of America? European Microscopy Society? National Institutes of Health? NASA? Bill Gates?). Google has all the facilities already in place, but an image archive needs structure and not a disorganized collection of images such as in Google Images. Google is quite correctly identified as a "search engine", as any request instantly leads to thousands or hundreds of thousands of links, but the lack of systematic order means that the scientist has to "search" through the links and hope to get lucky.

In 2001 the Proceedings of the RMS, the precursor of Infocus Magazine, published a review article entitled Virtual Microscopy by Colin Eberhardt (Proc RMS 36/3 September 2001). This today makes very interesting reading and seems like prehistory. Who could have imagined only a decade ago the enormous progress from the very early days of remote control of microscopes and simulated microscopes and the creation of the first image databases to the current state-of-the-science.

Reference

Coleman R. (2009). Can histology and pathology be taught without microscopes? The advantages and disadvantages of virtual histology. Acta histochemica 111, 1-4.

Disclaimer: The instruments and associated materials described in this article do not indicate endorsement or recommendation by the author.

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Raymond Coleman received his Ph.D. in the University of Leeds in 1967 and held an appointment as Assistant Lecturer and Lecturer in the Department of Zoology,

Bedford College, University of London from 1967-1973. He was awarded a Royal Society-Israel Academy Fellowship in Tel Aviv University from 1973-1975. Since 1975 he has been teaching histology in the Rappaport Faculty of Medicine of the Technion-Israel Institute of Technology with an appointment as associate professor since 1989. He is currently involved in establishing a virtual histology and histopathology program for e-learning in the Technion and also on a larger scale is prime mover in the efforts to establish an Israel National Repository for Histology and Histopathology (virtual microscopy image archive). He serves as the Editor-in-Chief of Acta histochemica (Elsevier) and is on the Executive Committee of the International Federation of Societies of Histochemistry and Cytochemistry (IFSHC). At the 14th International Congress of Histochemistry and Cytochemistry, Kyoto, Japan (August 26-29, 2012), he is co-organizer of the session on virtual microscopy in conjunction with the Japanese Society for Telepathology. An historic claim to fame is his contemporary schooling with Paul McCartney and George Harrison at the Liverpool Institute High School for Boys. More recently he is proud that two of his colleagues in the Technion Faculty of Medicine (Abraham Hershko and Aaron Ciechanover) received the Nobel Prize of Chemistry in 2004 for their pioneering studies on ubiquitin and more recently for Dan Shechtman's Nobel Prize in 2011 for his discovery of periodic quasicrystals based on his EM studies.

Photographs attached with possible captions:

1. Traditional classes in histology and histopathology as seen here are being replaced with virtual microscopy. No further need for individual microscopes or slide sets.
2. The Olympus dot.Slide is an open-system. This instrument is located in the Pathology Department of the Rambam Medical Center, Haifa, Israel.
3. Another view of the Olympus dot.Slide. This is clearly recognizable as a microscope.
4. Nikon have both closed systems (such as their Coolscope II) and open systems as seen in the following configuration used for research in Life Sciences in Tel Aviv University, Israel.
5. Olympus VS110 is an open system.
6. Olympus VS110 features are demonstrated by its project manager, Dr. Daniel Göttel, Olympus Soft Imaging Solutions, Berlin.
7. Aperio have been one of the leading pioneers of closed-box virtual microscopy. Here their Scanscope AT is seen with automatic loaders.
8. Leica SCN400 is a closed box system and like most VM systems can be configured for both bright-field and fluorescence. It incorporates the slide.Path software.
9. The 3D Histech Pannoramic Midi instrument in the Technion-Faculty of Medicine. This has a 12 place slide holder.
10. The Philips-NEC unit for hospitals with an automatic loading of up to 400 slides. This closed box does not look like a microscope.
11. View of Poznan, Poland virtual microscopy laboratory for histology and histopathology funded by the European Union.
12. Poznan, Poland virtual microscopy laboratory. An alternative to fixed computer modules is downloading directly to computer laptops or tablets.
13. Application of spectral cubes to virtual microscopes provides an added dimension for histopathological diagnostics.

14. The author (Raymond Coleman) is on the left with Professor Agnieska Malinska, Head of Histology and Embryology, Poznan University of Medical Sciences, where the author was an invited speaker on virtual microscopy at the Polish Society for Histochemistry and Cytochemistry and the III Conference on “Digitalization of Microscopic Images in Medicine”, May 2012.