Virtual microscopy: a new effective tool in the laboratory

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Summary

The Swiss Cytometry Society (SCS) is a scientific society which acts as a privileged platform for exchanges, formation and development open to all cytometry professionals. It concerns both flow and image cytometry.
The annual meeting of the SCS was held on 17–18 November 2005 in Bern. The topic of this meeting was "Virtual Microscopy: Principles and Applications". The first day of the meeting was devoted to the principles of virtual microscopy and a workshop. Applications of virtual microscopy in education, remote expertise and external quality assurance were presented on the second day.

Principles

Virtual microscopy is an emerging technique which allows the complete digitization of a microscopic slide. The slide can then be analyzed on a computer in a manner that closely simulates observation with a real microscope.

To understand virtual microscopy, three levels should be considered: acquisition level, archival level and application level.

The acquisition level concerns the digitization of a whole slide, i.e. the transformation of the optical information contained in a slide into digital images. The most frequently used technique consists in capturing multiple small regions of the slide and storing the images into files (Fig 1). Acquisition is performed at one or more magnifications and in one or more focal planes. Three kinds of acquisition system are available on the market:

– Fully integrated systems which minimise intervention of the operator. These systems are very compact and easy to use.
– Systems based on a conventional microscope, a scanning stage and a slide loader. A slide loader is a system that automatically takes a slide from a rack and puts it under the microscope for analysis. These systems are designed to run automatically and process a large number of slides.
– Systems with a special optical bench designed to minimise the acquisition time. With such systems, a slide can be digitized in a few minutes.

The second level is the archival level. Virtual slides are stored into databanks which include not only the images but also associated data. Associated data are additional information which enriches the image. It includes all kinds of information types: texts, graphics, images, sounds, videos or even links to others sources of information.

The last level that should be considered is the application level. From the same core of information, i.e. the virtual slide and associated data, several applications can be developed either for local or remote access. These applications are in general Web-based applications. This means that the slide databanks are accessed through a server connected to the Internet. A simple browser is required and a fast Internet connection.

Following this theoretical part, participants experimented with virtual microscopy during a workshop using computers connected to the Internet. A set of useful links was proposed to ac-

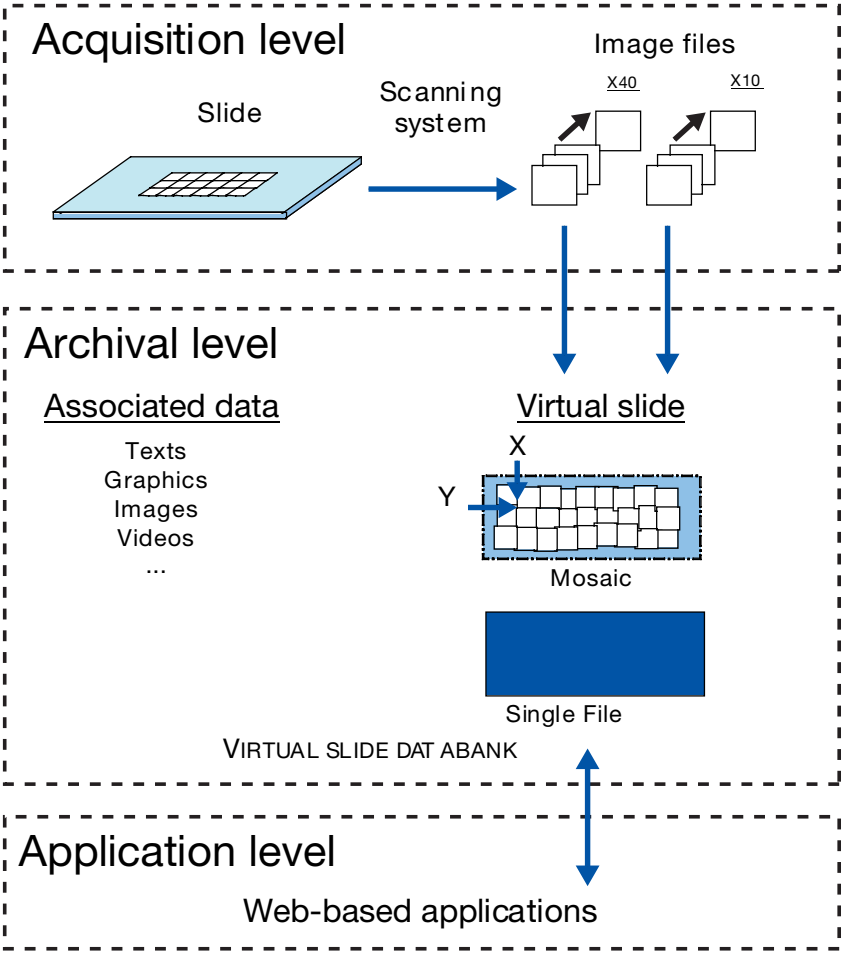

Figure 1.
The three main levels in a virtual microscopy application.

cess applications of virtual microscopy in education, remote expertise and external quality assurance.

Applications: education

The first application concerned education in pathology. It has been developed at the University of Basel. Technical considerations were first presented. These emphasised the enormous amount of data generated by the acquisition of virtual slides: approximately 10 Gb per slide depending on the resolution. In a routine environment, a slide databank could require up to one petabyte (10^{15}) of storage space leading to major costs and backup problems. But it should be noted that the storage capacities of media are increasing yearly while costs are decreasing.

The University of Basel proposes the vMic application program. This is an Opensource software allowing the viewing of virtual slides. The screen is horizontally divided into two parts. The upper part contains an image of the slide at low resolution and the lower part the image at a higher magnification. The magnification can be selected using buttons. The displacements in the slide are very simple and are effected by moving the cursor in either the upper or lower image.

Three slide databanks are accessible online and concern pathology, oral histology and mineralogy. In the pathology slide databank, cases can be selected by organ system. Additional information is given including the diagnosis, a short description, the contributor and the dimension of the slide.

An interesting issue of this presentation was the choice of a virtual slide system. It was mentioned that it may vary according to the intended utilisation. The criteria that should be considered are speed, usability, image quality, open standards and price. For example, a teaching application will support images with a lower resolution than that required for diagnosis. Appropriateness of the system to needs is important. For example, demanding images with higher resolution implies more expensive systems, longer acquisition time and larger storage space than medium resolution images.

The second presentation concerned a virtual slide databank developed at the University of Geneva. This databank includes more than 150 virtual slides of varying origins such as liver, colon, bone and blood.

The first page of the Web site includes a gallery of all available slides in the databank. The slides can be sorted according to multiple criteria such as organ, staining or diagnosis. Once a slide is selected, the image of a portion of the slide is displayed. The user can then scan the slide from contiguous fields to contiguous fields or move to any position in the slide by simply clicking on the reduced image of the whole slide. The magnification can be changed, allowing the user to display details at high resolution or to observe macro structures. This is one very effective advantage of virtual microscopy, to break the barrier of magnification, i.e. the possibility of rapidly switching from a high resolution and small field of view to a large field of view at lower resolution.

An interesting feature is the possibility of tracking the displacements in the slide, i.e. to mark the location that has been viewed. Thus, checking whether the whole slide area has been scanned is done by a glance at the reduced image.

Annotations can be added on the images. They include lines, contours and texts. A quiz can be constructed and proposed to students for auto-evaluation.

Applications: remote expertise

The next topic concerned the usability of virtual microscopy for remote expertise. This presentation reported an evaluation carried out at Geneva Hospital.

In haematology as in other domains, virtual microscopy is considered a powerful tool for education and research. For routine work, there is a great interest in communicating and sharing expertise between physicians (haematologists, oncologists, etc.). and the university hospital. The proposed scheme includes a virtual slides databank at the hospital and local network or Internet access by physicians from inside or outside the hospital. Sharing of information could also be easily extended to national or international experts.

Emphasis was placed on the specificities of virtual microscopy applied to routine work in general and to haematology in particular. First, routine work requires fast acquisition of virtual slides. This implies systems that are able to scan slides in a few minutes. Second, diagnosis is based on high-resolution images. Whereas ×20 or ×40 magnification objectives are sufficient in histology examination, ×100 magnification is often necessary in haematology. This constraint was convincingly illustrated by images of red blood cells infected with Plasmodium falciparum. Third, for effective and rapid analysis of a case, the user interface has to be user-friendly and allow rapid displacement and zooming in the slide.

Concerning the quality of the images, comparisons were made with images acquired with a conventional microscope, a TV camera and using the virtual microscopy technique. The conclusion was that virtual microscopy images closely approached the quality of those observed under the microscope. Attention should be paid to colour rendering.

Applications: external quality assurance

This last presentation concerned experience of virtual microscopy at the University of Tampere in Finland. The slide databank includes more than 1,500 virtual slides representing more than 1.5 terabytes of compressed data. Applications were experimented with in multiple domains such as basic education, specialist training, slide seminars, tools in research, external quality assurance.

This talk provided convincing evidence of the major capacity of virtual microscopy.

First, the application domains of virtual microscopy are numerous. The Finnish slide databank includes various cases in histology, cytology, haematology, immunohistochemistry, tissue microarrays, karyotyping and FISH.

Second, virtual microscopy offers new functionalities that are not available in conventional microscopy. For example, the superimposition of two virtual slides obtained from the identical slide with different staining is possible. This was illustrated with a prostatic needle

biopsy stained with haematoxylin and eosin. After acquisition, the slide was destained and then immunostained with a cocktail of AMACR and p63 antibodies. Two new virtual slides were acquired. With the viewer, it was then possible to observe the H&E-stained cells and to progressively switch to the immunostaining. A direct correlation between morphology and immunostaining is thus possible.

Third, taking into account the Z dimension is realistic. In a slide using the FISH technique dots are distributed in several focal planes. Using virtual slide acquisition in several focal planes, dots were clearly visible by simply moving a slider. It is also possible to use an alternative technique called extended focus projection, which combines the images in all the focal planes into one single image.

Concerning quality assurance (QA), the University of Tampere has developed several programs in teaching and self-evaluation. For external quality assurance, virtual slide technology has been used. The main application fields are HER-2 immunohistochemistry, gynaecological cytology, urine cytology and haematology. The principle is simple. Participants apply to a QA program. The Web site of the QA centerprovides a list of slides with clinical information. Using a browser, the virtual slide is screened. The magnification and the focal plane can be changed. Predefined regions of interest can be displayed for guided exploration. The diagnosis is then recorded on a form and sent to the QA center. Additional remarks on diagnosis (specific elements, etc.) or comments can be added. It should be noted that users of this new mode of slide analysis have to overcome a "psychological" barrier. But the experience reported in this presentation demonstrated a high degree of satisfaction for those who overcame this obstacle.

Working groups

The role of the SCS is to promote exchanges between members. For that purpose, two working groups were created in 2005: a Clinical Flow Cytometry (CFC) working group and Virtual Microscopy (VM) working group.

The main objective of the CFC group is to improve communication and collaboration between the various laboratories in Switzerland performing clinical flow cytometry. The 1st business meeting of this group was held during the SCS meeting. Subjects for collaboration were discussed and meetings planned for 2006, including participation to the 2006 SCS meeting.

Three main points were discussed during the 1st business meeting of the VM group: assessment of existing applications and needs, definition of common interests and new applications, exchange, diffusion and sharing of information. Two practical actions were undertaken. First, a survey on standardisation of virtual microscopy, image format, interfaces to slide databank in particular. Second, the creation of a directory of existing virtual slides and associated data accessible from the Internet.

Conclusion

In conclusion, virtual microscopy requires further development and improvements, in particular acquisition of high resolution images, increased acquisition speed, management of the focus, faster image retrieval and display on the Internet.

But at the present time virtual microscopy already appears to be an effective tool in the laboratory which complements conventional microscopic evaluation of slides. In the medium term it should replace the use of the conventional microscope, being more flexible and allowing easier communication and sharing of information. It is also a mandatory step in integrating cytometry in the routine laboratory-work by offering easy-to-use and efficient tools for manual or automatic quantification of cellular components of biological samples.

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Links

http://www.scsnet.ch/PageVM1.htm
http://vslwww.unige.ch/
http://vmic.unibas.ch/index.html
http://www.webmicroscope.net/demo/BernSCSslides.asp

Information
Swiss Cytometry Society, http://www.scsnet.ch