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REVIEW

Digital imaging of haematological morphology

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Summary Microscopic images of haematological cells are now routinely photographed using digital cameras. Advances in technology mean that the quality of such digital images can now approach that viewed through a microscope. At the same time there is an emerging appreciation that such images can be used in many roles: digital images are now being used to construct digital 'virtual slides', or are being employed together with cell recognition systems for morphological screening. Additionally, an Internet-based viewing systems allow access to on-line annotation, as well as real-time data gathering and feedback. The process of viewing digital images differs from the viewing of glass slides through a microscope; however, such images can provide diagnostic equivalence, and have an emerging role in areas such as education, quality control and continuing professional development. This review explores some of the present strengths, weaknesses and future applications of digital imaging in haematology.

Keywords Digital morphology, virtual slide, morphology, EQA, continuous professional development

Introduction

Recent years have seen many major improvements in the technical aspects of digital microscopy. High-resolution digital camera systems produce detailed images of microscopic specimens that can be displayed using large monitor screens. Where 'live imaging' is required, the images are frequently refreshed on the screen, allowing smooth transitions between fields as the microscope pans around the specimen. Increasingly, microscope images are transmitted to local area networks, or to remote users via the Internet, or are stored on servers, allowing identical images simultaneously to be viewed by different individuals at distant sites and/or times. Additionally, image processing and annotation are being used to provide added features that extend the use of the digital image beyond that of the simple photographic representation. This is an exciting time, and a fast evolving field, in which the major strengths and shortcomings are being explored and addressed (Table 1). However, all too often, the images available on the Internet or presented at meetings do not fulfil the needs of haematologists. In this review, we explore the strengths and weaknesses of digital images in relation to haematology, looking at how weaknesses may be overcome and at how the advantages of digital imaging are being exploited.

The quality of the digital image of haematological cells

Morphological diagnosis in haematology depends on the interpretation of cytological detail; seeing that detail is dependant primarily on the ability of the microscope to detect individual features as separate elements (resolution). Additionally however, our brain interprets the microscopic image: detecting colour and contrast differences between individual cellular elements, and also forming a three-dimensional impression of different elements through the focussing capabilities of the microscope. For example, the separate granules of white blood cells (which measure 0.2–0.5 μm) can be detected using a standard light microscope equipped with a high-quality oil-immersion lens. Such structures are resolved at the optical limit of the lens system, but are clearly perceived as

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Table 1. Strengths and weaknesses of digital imaging in haematology

Strengths
Images are easily and cheaply reproduced and distributed
Identical images can be viewed by observers at distant sites or different times
Images can readily be archived and are not degraded on storage
Digital images are well suited to additional processing, annotation, or analysis
Images of rare morphological conditions can be widely distributed
Weaknesses
Viewing techniques are not the same as using a microscope
Quality not (yet) as good as a 'high-quality' microscope image
Optimal viewing requires high-quality equipment and some technical expertise
For optimal viewing, software/hardware require standardization

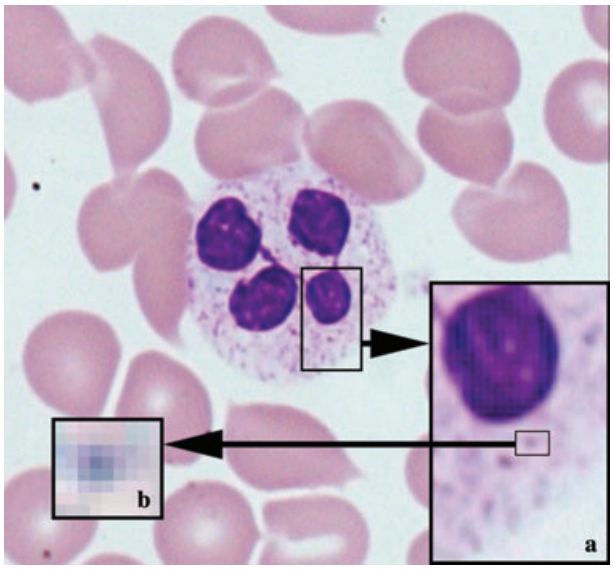

Figure 1. Digital image of a neutrophil showing typical granulation taken using a ×100 objective lens, the detail of the image is shown in the inset panels. Panel (a) shows a magnified image of an intermediate sized granule, magnified again in panel (b) to show how it is constructed from individual picture-forming elements (pixels). In this case the granule is constructed from 12 to 16 separate pixels. The figure illustrates how the colour and contrast is 'averaged' in each individual pixel resulting in a indistinct outline for the illustrated granule.

separate structures by our brains: first, because of the contrast and distinctive colour compared with the surrounding cytoplasm; secondly, because we (consciously or not) use the microscopes'abilities to focus 'up and down'; or use the 'panning' capability of our microscope to search 'for a good cell'. When the same structure is viewed by a digital system there are subtle differences that can make a significant difference to user perception. A digital camera detects the cellular detail using a 'charge-coupled device (CCD) chip': essentially this is composed of individual 'light-sensing units' arrayed as a grid of small squares, rectangles, or other shapes. The signal from each individual light-sensing unit will become a single 'picture element' (pixel) in the final digital image. In our laboratory, we employ a ×60 1.40 objective lens; using this lens, the smallest neutrophil granules (0.2 μm) are projected onto the surface of the CCD chip as a spot of diameter 12 μm (0.2 μm multiplied by the ×60 magnification of the objective). As the light-sensing unit of a modern CCD have a diameter of 7 μm or less, the granule will be detected by two to four separate light-sensing units. Therefore, even the smallest neutrophil granules will be detected by two (and for larger granules, up to 20) separate light-sensing units (and are therefore displayed in the final image as 2–20 individual pixels; Figure 1). The granule should therefore readily be perceived. However, unlike the image we see directly down the microscope, within each pixel of the digital image the colour is 'averaged'; this has the consequence, particularly at boundary points of altering colour and reducing contrast. This can make fine detail appear 'fuzzy' (Figure 1). This is made more acute when digital images are restricted to a single field, because the viewer cannot 'scout around' for additional 'good cells'; or improve resolution by focussing up or down through the cell.

These defects are of course only relative. When compared with traditional photomicroscopy using a camera and film, digital media offer a number of advantages: digital images now approach the detail of high-quality film, while exceeding that reproduced in text books. The images are cheap to acquire, and can be stored or shared as compressed images of just a few hundred 'KB' or in highly detailed bitmap or tagged image formats of over 10 MB. Acquired images can be instantly reviewed for quality, and can be adjusted to achieve optimal colour balance and contrast at the time of acquisition or subsequently. Moreover, quality is becoming very acceptable: those unused to modern digital systems should view images resolved by a modern dedicated camera system; or should access a web site showing digital morphology [for example, the UK NEQAS (H) web site offers annotated digitized images of those glass slides employed in recent surveys, http://www.ukneqas.org.uk (and follow links)]; you may be surprised at the quality of the images. Producing high-quality images inevitably requires specialist cameras and excellent microscopes and lenses, but very good quality is now possible. Moreover, digital microscopy is a fast-moving field – many of the defects discussed above

are being addressed either through technical advances, or through innovative software, while new applications are now addressing the strengths of the system (reviewed below).

'Live image viewing'

The capability for different individuals to view the same image has long been an important issue for haematologists: being an essential part of training and peer review sessions. Multiheaded microscopes offer a solution, but are expensive and make substantial demands on space. Unfortunately, the widely available video microscopy systems that many laboratories have used over the past decade offered poor image resolution and were not always easy to optimize for viewing; this widely (and correctly) led to a poor perception of recorded images at that time. However, digital systems are becoming increasingly attractive in this context for several reasons. First, advances in camera technology and computer hardware have greatly improved the convenience and performance of such systems. Secondly, the introduction of multidisciplinary meetings requires now that images are viewed not just from around the microscope, but also from different locations that may include different areas of the globe. Finally, the new capabilities and requirements of digital imaging have developed at a time when computer networking and the availability of high-quality Internet access has made remote access and sharing of images both feasible and attractive.

The major technical details of imaging systems are beyond the scope of this review, and are well described on the web sites of major microscope manufacturers. Briefly however, manufacturers are developing systems to meet the specific needs of users: automated correction of exposure and colour balance, allow cameras rapidly to respond to changes of lens or field of view, refresh rates exceeding 10 frames/s allow smooth transitions as field of view changes; for live imaging pixel number is balanced to screen size (for example, a 1.3 megapixel camera makes optimal use of a monitor using 1280/960 screen resolution for most lenses). Increasingly, cameras allow platform-independent networking: allowing images to be viewed remotely through local area networks or the Internet, using standard web browsers or dedicated software. Images can be viewed as still images updated at user-defined intervals, or as streaming video images that allow observers to follow 'live' microscopy. Changes in monitor style have also enhanced viewing: modern flat-panel liquid crystal displays (LCD) or plasma screens are larger and lighter than the 'traditional' cathode ray tube (CRT) monitor, allowing them be placed in areas where CRT screens were not suitable; and using large screen size allowing greatly magnified images that can be readily viewed by audiences without the need to 'crowd around' the monitor to view cell detail.

The outcome is that live digital imaging is now a reality in many laboratories, and one that with increasing technical advances, and with the development of specialist equipment and software, is likely to improve more rapidly with time.

Digital slides

Microscopes do not simply view single static images, the skill of microscopy includes: appropriate field selection, panning within an area to recognize and review essential diagnostic elements, and the use of focussing to enhance perception of detail. One area of great interest in digital imaging is the attempt to extend the digital image to allow these essential skills to be used or tested. The technical aspects of the technique have been very well reviewed by others (Weinstein *et al.*, 2001; Lee, 2005), and will only be discussed in outline here. Essentially a digital slide attempts to reproduce either partly or completely the features of a glass slide. The precise methods by which such slides are constructed can vary, most commonly involving either continuous scanning of the slide, or the 'stitching together' of multiple high-power fields. The outcome is a very large field of view, but with a high level of detail present in the digital image. Therefore, slides can be scanned at low magnification (the equivalent of a low-power field), then individual areas can be examined at high magnification (equivalent to a high-power field) where required (Figure 2). At its simplest level, a digital slide may consist of a small number of high-power fields selected to show important features and stitched into a single image that can be viewed through a range of nonspecialized software viewing systems. At a more sophisticated (and expensive) level, the electronic image may represent the entire slide, offer the ability to magnify up or down within the plane of the image through the use of multiple image layers in different focal planes (*z*-stacks), and images can be viewed using specialist software designed to reproduce microscopic skills – this type of viewing may be better termed 'virtual microscopy' because many features of a microscope are reproduced. While the large digital slide with consecutive horizontal planes has great appeal, it places significantly greater demands on hardware and on storage/processing, as well as providing far more information than might be required for most users. The advantages and limitations of the different approaches are presented in Table 2 below, and are discussed in more detail elsewhere (Lee, 2005).

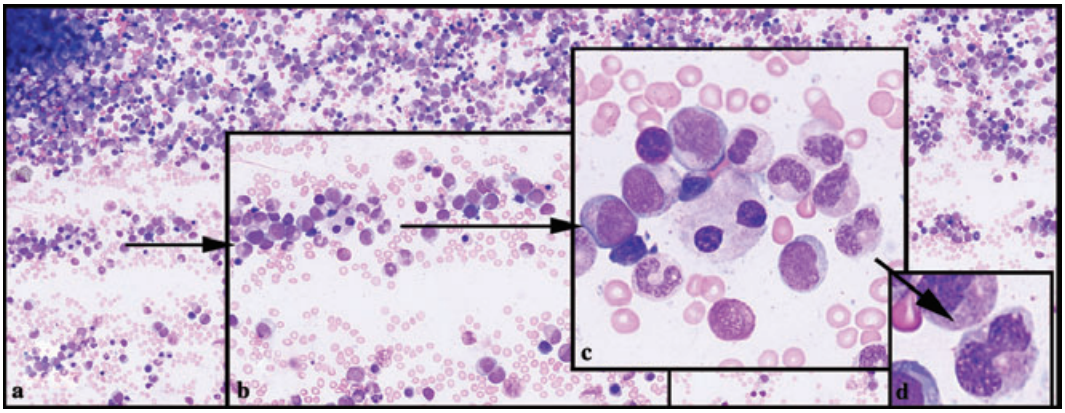

Figure 2. The background panel shows a digital slide prepared from 60 separate ×60 oil-immersion microscopic fields. Panels (b and c) show magnified images of details from the main slide, illustrating the cytological detail of a micromegakaryocyte, several blast cells and dysplastic maturing myeloid cells. Panel (d) shows a dysplastic neutrophil at high magnification. Please note this image is a printed reproduction and does not therefore precisely reproduce the quality of the original image.

Table 2. A summary of the advantages and limitations of different digital approaches

Very large digital slides or 'whole' slides in digital format
No selection-bias as photographer does not determine fields of view
All features available for review by viewer
Large size [up to 60 GB (or more) for a full slide]
Larger file sizes requires specialist formats for viewing (e.g. jpg2000)
Construction requires automation (technically difficult using oil-immersion lenses)
Time-consuming to construct
Limited slides of selected microscope fields
Small files readily distributed in standard formats
Can be constructed by nonspecialist with standard equipment
Viewed using nonspecialist viewers: cheap but not fully featured for microscopy
Selection-bias introduced by photographer
Introduction of multiple focal planes to simulate focussing through specimen
Allows focussing up or down within digital image (*z*-planes)
Increases file size
Requires dedicated viewing software
Not quite the same as focussing

Once slides are constructed and viewing systems are in place; however, the advantages of the digital slide are significant. When compared with single digital images, the digital slide tests essential skills of cell selection and identification of important morphological features, while the wide field of view restricts 'selection-bias' introduced by the photographer. When compared with glass slides, the digital equivalent can readily be stored and distributed, allowing identical images to be viewed by different individuals and at different sites. Moreover, access via an Internet-based system allows interaction between the user and the provider, allowing rapid data collection and feedback (see 'New roles', below). Therefore, while clearly not the equivalent of glass slides, the digital format has a significant role in contexts such as teaching, continuing professional development and in quality assessment. Digital slides have been widely tested in histopathology and cytology applications (Leong *et al.*, 2001; Steinberg & Ali, 2001). However, their introduction and testing in haematology has been comparatively slow, although the Swedish Quality Control Group (EQUALIS) conduct a survey using single-field digital images. The slow introduction of digital images in haematological practice is partly due to the requirements for highly detailed cytological features discussed earlier; but in addition, technical difficulties with acquiring large slides using oil-immersion mean that high-quality virtual slides are not yet a reality in haematology. Nonetheless, large digital images can be acquired and used.

Recently, the authors institution in collaboration with UK NEQAS (H), have conducted a pilot study of the use of digital slides in haematological EQA, with encouraging results (Burthem *et al.*, 2005). Participation in this voluntary study was high [nearly 40% of UK NEQAS (H) participants took part] suggesting levels of Interest, access and familiarity with electronic resources; participants reported download speeds and viewing to allow acceptable reporting of NEQAS surveys. And, although the digital slides were limited in scope (40 high-power fields) the majority of participants felt the electronic 'film area' provided was sufficient to form a diagnostic impression. This conclusion was supported by the outcome of the assessment: the results of digital slide interpretation were

almost identical to those when the same material was sent as part of a glass slide survey (even where reported features were not compatible with the known diagnosis). When participants were asked about the potential future role of electronic slides 71% of respondents believed that the technique has a role in EQA/continuous professional development (CPD), and that electronic images had a wider relevance with high levels of agreement for a role in education, or in the illustration of 'expert opinion'. Particular enthusiasm was held for the ability to demonstrate uncommon appearances, or rare samples. These findings suggest a significant acceptance and enthusiasm for the use of digital slides as a resource in haematology, and it is likely that we will see a more widespread application of the technology over the next few years.

New roles

A range of new applications of digital slide technology will undoubtedly emerge in haematology and other areas of pathology over the coming years. However, already in haematology, several new applications are presently being explored, and new techniques are being introduced. In particular, the ease with which digital images can be transmitted, shared and evaluated makes them a very attractive medium for teaching and assessment. Increasingly, the additional capability to link images to analysis, annotation and feedback is being explored. Furthermore, software developers are now exploring systems that will allow data submission via the Internet to be linked to 'real-time' feedback including cumulative statistical analysis of responses to be available 'on-line' to participants. More sophisticated tools that allow users or assessors to track the examination of a specimen, e.g. allowing assessors to view the time spent looking at individual areas of a slide or film are also being developed, and may prove invaluable in assessing how successful or unsuccessful diagnoses are reached. With the need to prove that Biomedical Scientists, and indeed all staff responsible for morphology, are achieving satisfactory standards, improving quality of images and the development of appropriate computer software has potential to offer a variety of educational and assessment opportunities. Schemes to provide images for CPD are now being piloted [e.g. the UK NEQAS (H) Digital Morphology Pilot Scheme for Biomedical Scientists], using online image viewing, expert comment and analysis.

Another role in which the digital image is playing a major emerging role, involves automated cell recognition systems. Already companies such as CellaVision™ (Lund, Sweden) and AMS™ (Advanced Measurement Systems, Tirat Carmel, Israel) provide digital computer-aided microscope systems to laboratories providing automated and semiautomated image analysis. Such systems locate and preclassify nucleated cells (predominantly white cells) against a database of known cell characteristics using measurements of cell size, area, shape, density and colour. The white cells can then be viewed in a number of ways, e.g. by classification groups, enabling the user to verify large numbers of cells quickly and to reclassify if required. Some systems also include automated slide handling and continuous slide feed to enhance capacity. Such facilities may be highly attractive to laboratories with low-staffing levels, or to centres screening large numbers of relatively normal smears. By increasing the reference database the systems become more able to classify immature cell types. The challenge for imaging systems has been developing consistent analysis for red cell morphology where the lack of granules or regular inclusions places limits on the reference points available for image comparison but improvements are already being introduced. The role of an automated smear scanner has yet to be fully explored however, and may only gain support if able to reliably report on abnormal cells.

Conclusion

Digital imaging may never match the detail, and 'feel', we have when viewing a glass slide through a high-quality microscope. However, the advantages offered by the digital medium must also be recognized: in particular, the ease with which classical or rare cases can be selected, produced using high-quality microscopes, then copied and distributed, is a major benefit; add to that and the capability to provide an integrated commentary and interpretation, and we have a very powerful new medium. How many people have been put off microscopy for life after viewing poor slides, through the low-quality instruments available at toyshops and many schools? How many of us would value the ability to navigate around, and to view slides of microscopic specimens that we would not normally see in our laboratories? Would we value the opportunity to do this with expert commentary? In the 1980s, the arrival of the videocassette recorder was predicted to destroy the film industry. Videos lacked the depth, feeling and sense of occasion of a visit to the cinema. But, by making films more accessible, a whole new generation became able to appreciate the value of the medium, and attendances at cinemas steadily increased. Digital imaging is there to be used, directed and to complement the microscopic skills we use in our day-to-day work.

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