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Digital Photography: A Primer for Pathologists

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The computer and the digital camera provide a unique means for improving hematology education, research, and patient service. High quality photographic images of gross specimens can be rapidly and conveniently acquired with a high-resolution digital camera, and specialized digital cameras have been developed for photomicroscopy. Digital cameras utilize charge-coupled devices (CCD) or Complementary Metal Oxide Semiconductor (CMOS) image sensors to measure light energy and additional circuitry to convert the measured information into a digital signal. Since digital cameras do not utilize photographic film, images are immediately available for incorporation into web sites or digital publications, printing, transfer to other individuals by email, or other applications. Several excellent digital still cameras are now available for less than $2,500 that capture high quality images comprised of more than 6 megapixels. These images are essentially indistinguishable from conventional film images when viewed on a quality color monitor or printed on a quality color or black and white printer at sizes up to 11×14 inches. Several recent dedicated digital photomicroscopy cameras provide an ultrahigh quality image output of more than 12 megapixels and have low noise circuit designs permitting the direct capture of darkfield and fluorescence images.

There are many applications of digital images of pathologic specimens. Since pathology is a visual science, the inclusion of quality digital images into lectures, teaching handouts, and electronic documents is essential. A few institutions have gone beyond the basic application of digital images to developing large electronic hematology atlases, animated, audio-enhanced learning experiences, multidisciplinary Internet conferences, and other innovative applications. Digital images of single microscopic fields (single frame images) are the most widely utilized in hematology education at this time, but single images of many adjacent microscopic fields can be stitched together to prepare ""zoomable'' panoramas that encompass a large part of a microscope slide and closely simulate observation through a real microscope. With further advances in computer speed and Internet streaming technology, the virtual microscope could easily replace the real microscope in pathology education. Later in this decade, interactive immersive computer experiences may completely revolutionize hematology education and make the conventional lecture and laboratory format obsolete. Patient care is enhanced by the transmission of digital images to other individuals for consultation and education, and by the inclusion of these images in patient care documents. In research laboratories, digital cameras are widely used to document experimental results and to obtain experimental data. J. Clin. Lab. Anal. 18:91–128, 2004.

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INTRODUCTION

The digital camera offers unprecedented possibilities for improving pathology education, research, and patient service. Until recently, silver halide photography was the only means for pathologists to capture images of gross specimens and microscopic fields. The introduction of digital scanning technology in the mid-1980's

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permitted the digitization of photographic prints and 35 mm slides but did not eliminate the need for photographic film. Scanning 35 mm slides or photographic prints is also a slow, expensive, and inconvenient process that may produce less than optimal image quality. In contrast, digital images of diagnostic quality can be rapidly and conveniently acquired with modern high-resolution digital cameras without the high direct cost and environmental impact associated with photographic film. These digital images can be immediately transferred into print or digital reports or publications, emailed to other individuals, archived and later retrieved, annotated with descriptive text or audio files, or used in other ways.

Since their introduction in the early 1990's, consumer and professional digital cameras have become increasingly popular since the image quality has progressively improved and the cost has decreased (1–3). Approximately 3.8 million digital camera units were sold in the United States alone in 2000, generating over $1.8 billion in street revenue (4). By 2007, estimated worldwide low-end digital camera sales will reach 51 million units, generating approximately $11.8 billion in revenue (5). In the medical sciences, digital cameras have primarily been used for the capture of macroscopic images in fields such as oral diagnosis, forensic medicine, dermatology, and cosmetic surgery. In pathology, digital images of gross specimens and microscopic slides are widely available for medical education at this time, largely as web-based archives of digital images for medical students and pathology residents. However, the convenient and inexpensive capture and storage of relevant macroscopic and microscopic digital images for patient care is increasingly accepted because of the current emphasis on rapid accurate pathologic diagnosis and quality assurance. In fact, the technology for the capture, storage, and retrieval of digital images has became technically advanced to the point that an entire glass microscope slide can be digitized and the images restored on a computer screen, accurately simulating the original experience of visualizing the slide. This technique is referred to as "virtual microscopy." In addition to pathology education, high-resolution digital photography is essential for remote consultation and diagnosis via telepathology, and may open new avenues for computer-assisted diagnosis in the near future (6). The techniques and applications of digital photography will be explored in this article, with an emphasis on applications in pathology. Many recent reviews of digital photography and imaging have been published (7–15).

DIGITAL CAMERA THEORY AND TECHNIQUE

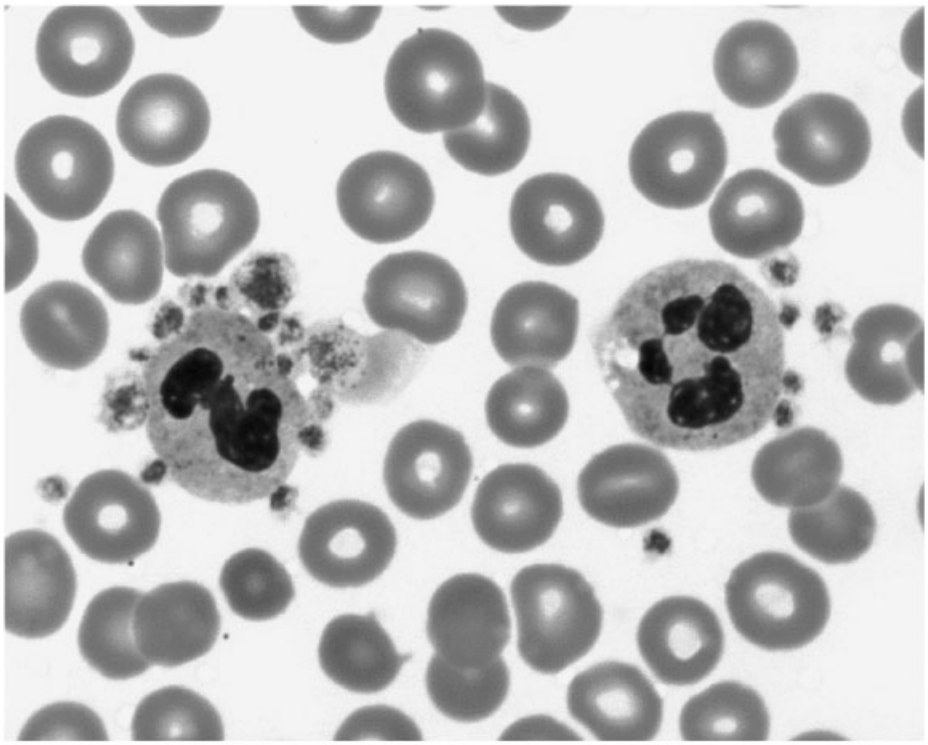
The camera consists of a series of lenses to capture and focus light energy, a device to convert the light energy into a digital format, and a device to record and store the captured information (9,16). Until recently, silver-halide film was used for both signal conversion and image storage. When an image is focused on silver-halide film, an emulsion on the surface of the film causes a chemical change specific for the color and intensity of the light. In contrast to conventional silver-halide "film" cameras, digital cameras ("digicams") use solid-state computer chips called image sensors to convert the light energy into a recordable form and a variety of magnetic and nonmagnetic devices to store the images. Portable self-contained digital cameras have removable storage devices to save the captured images as well as additional computer circuitry to display the images on a video monitor, print them, or transfer them to a laptop or desktop computer system for permanent storage or additional manipulation. Some studio and scientific digital cameras lack internal storage devices and are directly interfaced to a computer system. Digital still cameras capture single images, while digital video cameras capture multiple contiguous frames in a digital format. Scanners are special types of digital cameras used to convert printed material, photographs, or silver-halide film into a digital format.

Digital images are much more versatile than images on film because they are immediately available, and can be manipulated, transmitted to other people, or reproduced in a variety of formats. The major disadvantage of digital images is that conventional film cameras and associated equipment is commonplace and familiar to most individuals, while the more specialized equipment for capturing and displaying digital images is relatively more expensive and not as readily available. Image quality is not an issue with modern megapixel digital cameras and high-resolution printers, since the latest model digital cameras produce prints, 16" × 24" or larger, that rival or exceed the quality of conventional 35 mm film (Fig. 1). In addition, many digital cameras permit storage of the "raw" unprocessed data captured by the computer, so that the exposure, contrast, white balance, and other image parameters can be modified at a later date.

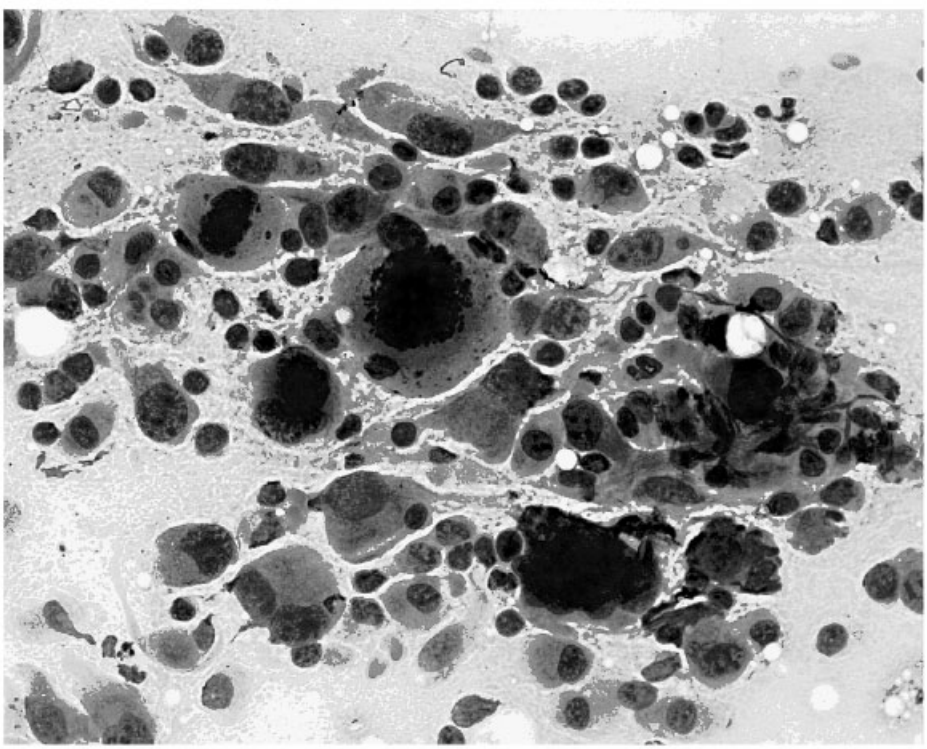
Fundamentals of Digital Imaging

Image sensors

The "heart" of the digital camera is the image sensor, a silicon semiconductor chip that measures and captures light. Presently, there two types of image sensors, the charge-coupled device (CCD) and the complementary metal oxide semiconductor (CMOS) image sensor. The CCD was developed in 1969 by two Bell Laboratory Scientists, Willard Boyle and George Smith, as a storage device for computer data (17). CCDs had limited

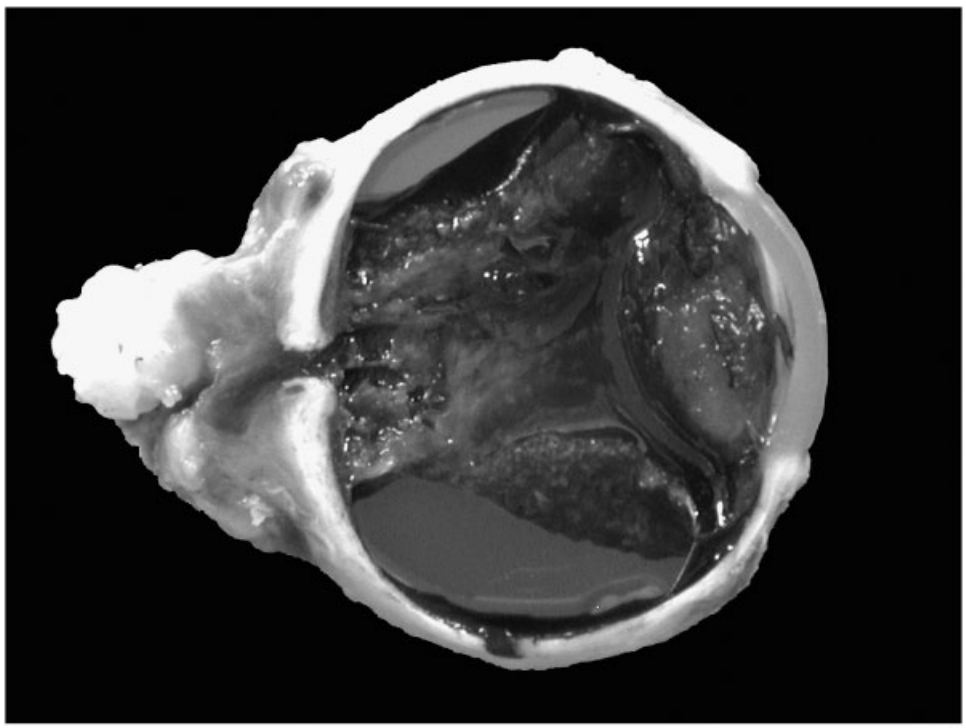


(A)



(B)

Fig. 1. Representative macroscopic and photomicroscopic images of pathology specimens. **A:** Peripheral blood smear with platelet satelliosis (Wright-Giemsa stain, 1000 ×, Nikon DXM1200 Digital Photomicroscopic System). **B:** Fine needle aspirate with malignant fibrous histiocytosis (Toluidine blue stain, 1000 × original magnification, Nikon DXM1200 Digital Photomicroscopic System). **C:** Eye, enucleation with retinoblastoma (Canon D30 digital camera).



(C)

Fig. 1. Continued.

usefulness for computer data storage, but they were incorporated into TV cameras and flatbed scanners in the mid-1970s, and into a solid state camera for laproscopy in 1982. Many scientific, industrial, and military applications of the CCD were developed in the 1980s and early 1990s; mass-produced, consumer-oriented digital cameras appeared in the mid-1990s. The CCD consists of a thin silicon wafer covered on one surface with an array of thousands to millions of microscopic (10–30 micron), light-sensitive silicon photodiodes (SPD's, photosites) placed at the focal plane of the lens system of a camera (17). Each photon of light striking a photodiode generates a small electric charge that is stored for read-out at the end of the exposure. The generation of electric charge is cumulative, so that the more photons striking s photodiode, the greater the voltage generated. This means that a photosite recording a highlighted area of the image will record a higher charge than a photosite recording a shadow. The analog voltage signals from each photodiode are stored until the exposure is complete, and then transferred row-by-row, in "bucket brigade" style, to a read-out register, an output amplifier, and finally to an analog to digital converter (ADC) chip, where the variations in voltage (i.e., brightness) are converted into discrete binary numbers (17,18) (Fig. 2). The process of converting the light information into numbers is termed photoelectric conversion. Some recently developed CCDs simplify the process by directly producing a digital output signal that is read by a computer. CCDs are relatively expensive since they are manufactured in dedicated and specialized factories.

CMOS technology is widely used in the computer industry for CPUs, memory modules, and other components. For example, the IBM Pentium III CMOS microprocessor (IBM, White Plains, NY) contains about 10 million active elements. Two types of CMOS image sensors are available. Passive-pixel CMOS image sensors simply consist of small photosites which generate an electrical charge and connectors which carry the generated charge to an amplifier and ADC. They are inexpensive ,but the image quality is limited due to background noise. Active-pixel CMOS image sensors incorporate on-chip noise reduction and other circuitry and can produce images of comparable quality to CCDs. One of the major advantages of CMOS image sensors over CCDs is their lower production cost since they are made in standard fabricating facilities that produce other computer semiconductor components. The incorporation of many other camera functions on the CMOS chip (very large scale integration, VLSI) also leads to smaller, more reliable, and less expensive cameras than their CCD counterparts. Other advantages of CMOS image sensors include low power

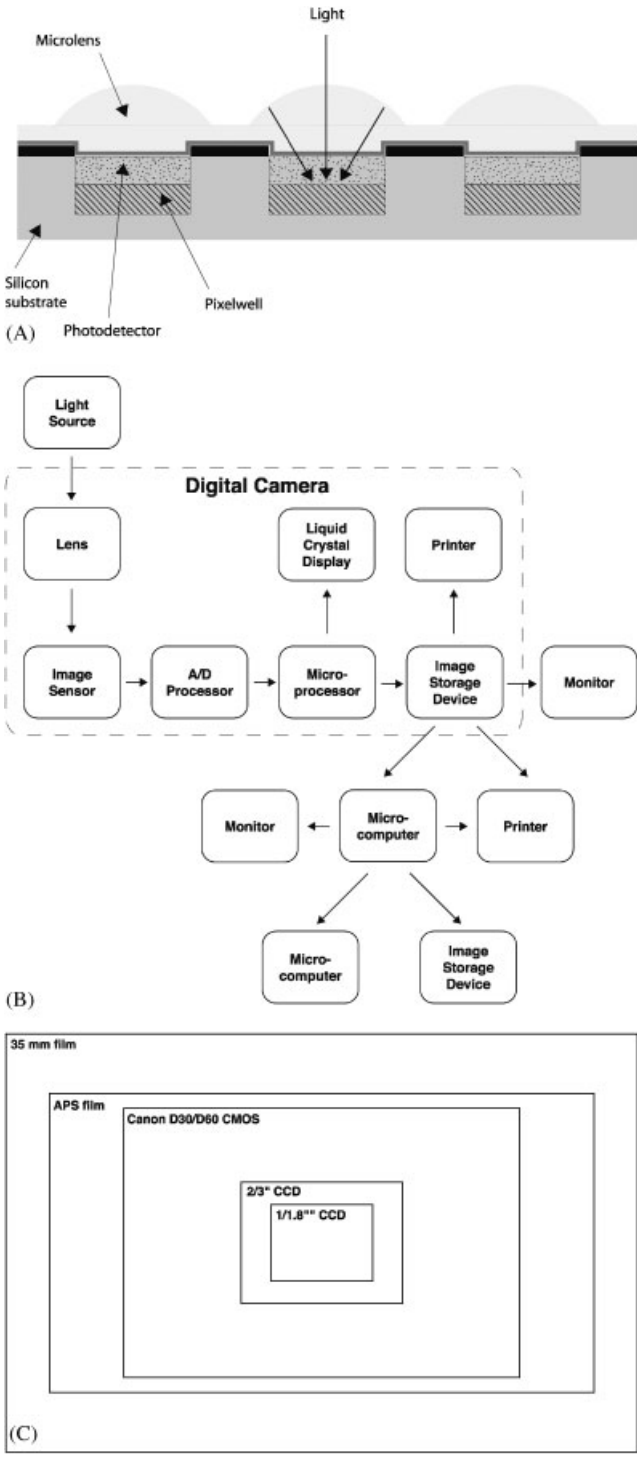


Fig. 2. Anatomy of the digital camera. **A:** Structure and function of the charge-coupled device. **B:** Optical and electronic components of a digital camera. **C:** Comparison of relative size of charge-coupled devices from a 35 mm film camera, an APS film camera, the Canon D30/D60/10D digital camera, and digital cameras utilizing a 1/1.8″ CCD.

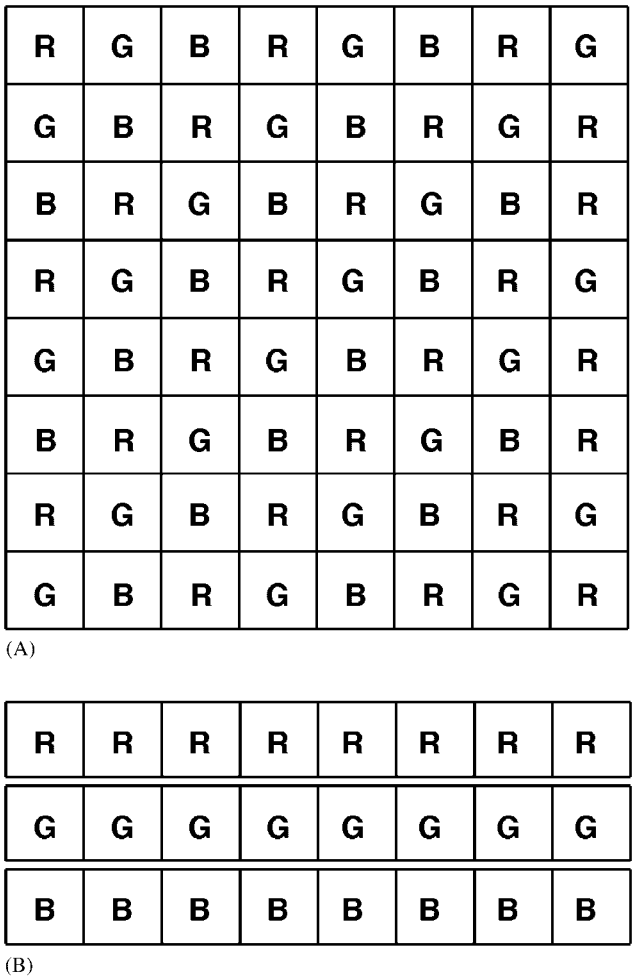


Fig. 3. Area (**A**) and linear array (**B**) digital cameras.

consumption, faster frame rates, few artifacts (smear and blooming) caused by charge transfer between adjacent pixels, and the ability to include ''higher-level'' camera functions such as image stabilization and wireless control. The major disadvantage of the CMOS image sensor, in comparison to the CCD, is lower light sensitivity caused by the presence of the other circuitry. The proportion of the CMOS pixel site that is active as a sensor (''fill factor'') is on the order of 30%, while CCDs typically have fill factors greater than 90%. Fortunately, increasing the size of the CMOS pixel partially compensates for the lower fill factor. Recent CMOS-based high-resolution digital cameras, such as the Canon D30 (Canon Inc., Tokyo, Japan), produce images equivalent to those of high-resolution CCD cameras.

Silicon photodiodes are inherently insensitive to color and capture only the brightness of the light focused upon them, in the form of a gray-scale range that ranges from pure white to pure black. Photodiode color

sensitivity is created by using red, green, and blue color filters. The number, type, and arrangement of the image sensors determines many of the properties of a digital camera. In present digital cameras, the image sensors are arranged in either a two-dimensional grid (''area array'') or in a row or column (''linear array'') (Fig. 3).

In an area array, the image sensors are arranged in a rectangular grid, and light from the entire scene reaches the sensor at one time. There are several types of area array cameras that differ in the number of sensors and the arrangement of color filters. ''One-chip, one shot'' digital cameras are the most common and least expensive. Tiny red, green, or blue filters are fixed over the individual photosites on an image sensor in a predefined pattern. During exposure, each photosite captures the brightness of its selected color at that point. After the exposure is complete, a computer process termed interpolation is used to calculate the two colors a sensor did not record from the color information of surrounding pixels. One-chip, one shot digital cameras are relatively inexpensive and can be used to record live action, but they are prone to an artifact termed color aliasing that results from inaccurate computer interpolation. The resolution of these cameras varies from poor to very good. The ''one-chip, three shot'' camera is a variation of the one-chip, one shot digital camera which has a liquid-crystal tunable filter or four-position rotating color wheel placed over the image sensor. The image is recorded with red, green, and blue filters, while a neutral filter position is used for composition and focusing. The resolution and color fidelity of one-chip, three shot cameras can be relatively high, but they can image only stationary objects in color. ''Three-chip'' cameras use a beam-splitting filter to generate three copies of an incoming scene that are simultaneously sent to separate image sensors optimized for red-, green-, or blue-light. Three-chip cameras can image moving subjects and have the highest resolution and greatest color, but they are bulky and expensive.

Scanning cameras use image sensors comprised of one or three linear arrays (''sticks'') of photosensors. If a single array of photosensors is present, the scene is scanned one row at a time sequentially with red, green, and blue filters, and the image is gradually accumulated. If three rows of sensors are present, each row is covered by a red, green, or blue filter. Although relatively slow and useful only for constantly illuminated and motionless subjects, scanning digital cameras are capable of very high resolution and color fidelity, since 10,000 or more pixels may be present in each array. This technique is widely used in scanners, high-end studio cameras for still photography, and in some specialized digital cameras for photomicroscopy. A recent variant of the scanning digital camera, the ''moving one-chip camera'' uses a one-chip, one shot image sensor that is moved in subpixel amounts in both X and Y directions to increase the resolution. The final image is dependent upon computer interpolation, but the resolution can be very high. Several high-quality digital cameras recently developed for photomicroscopy, including the Nikon DXM-1200 (Nikon Corporation, Tokyo, Japan) and the Carl Zeiss Axiocam (Carl Zeiss, Gottingen, Germany), use moving one-chip mechanisms.

Image characteristics

The brightness and color information recorded by a digital camera or scanner is used by a computer or printer to recreate an image of the original scene on the computer screen or paper. The data recorded by each photocell comprises a minute portion of the final image and appears on the screen as a tiny square termed a picture element or ''pixel.'' Since each pixel has a numbered address, and is stored in an area of memory called a bit-map, the process of creating a grid of pixels is termed ''bit mapping,'' and digital (raster) images are commonly referred to as ''bit-maps.'' The number and size of the pixels (spatial resolution) and the amount of brightness information (pixel depth, brightness resolution) largely determine how closely a bit-mapped image resembles the original scene, although dynamic range, noise, and other factors can affect the image.

The term ''resolution'' strictly refers to the ability of a lens to resolve close lines on a test chart, but this term has been widely used for the number of photocells comprising an image sensor and the number of pixels in a raster image. Most consumer digital cameras have maximum resolutions of 2 to 4 megapixels, and can produce very good color prints up to 8×10 inches. The maximum resolution for professional digital cameras is presently 16 megapixels. In comparison, the resolution of 35 mm color slide film is estimated at 10 to 20 megapixels, while the human eye has a resolution of approximately 120 megapixels. The optical resolution of a digital camera or scanner refers to the actual number of photosites, while interpolated resolution refers to the actual number of photosites plus those generated through interpolation. The ''resolution'' of a bit-map image is commonly expressed as the total number of pixels (i.e., 1,920,000 pixels or 1.92 megapixels), or by its dimensions in pixels (i.e., 1600×1200 pixels), where the first number is the number of pixels across the screen (columns) and the second number is the number of pixels down the screen (rows). The resolution of printers is commonly expressed in dots per inch (dpi), which varies from 300 dpi to 2400 dpi. A similar term, pixels per inch (ppi), is also applied to the resolution of

computer monitors. Presently, most monitors display images at the relatively low resolution of 72 ppi.

Pixel dimension, field of view, and optical magnification are major considerations for photomicroscopy of any type. Pixel dimension refers to the size of each pixel comprising the CCD array. For optimal image quality in digital photomicroscopy, each pixel comprising a CCD array should be between 6.5 to 7.5 microns in dimension. However, CCDs with pixel dimensions of 9–10 microns provide adequate image quality with optical magnifications above $20\times$, and their field of view is much larger than that of a CCD array with smaller pixels. For example, Hand (19) calculated that a 1.4 megapixel imager comprised of 6.5 micron pixels samples roughly half the field of view of an array with 9.5 micron pixels.

The amount of color information recorded by a digital camera is another important factor in determining the quality of a printed or monitor-displayed image. The color depth refers to the number of computer bits used to record information about each color. Eight bits per color (8 bits red, 8 bits green, and 8 bits blue; 24-bit color) is the minimum color depth for adequate color reproduction and permits the display of 256 shades/color, or 16.7 million total color values (256^3). Some consumer digital cameras offer 10 bits/color (1,024 shades/color, 30-bit color), while professional digital cameras and scanners commonly achieve 12 bits/color (4,096 shades/color, 36-bit color). The extra color information is usually downsized to 8-bits/color, since the human eye is theoretically incapable of differentiating more than 16.7 million colors.

The aspect ratio of an image is the ratio of its heighth to its depth. Although a 1.33:1 aspect ratio is common for image sensors and display monitors at common resolutions (640×480 pixels, 800×600 pixels, 1024×768 pixels), 35 mm film and 4×6 photo paper have 1.5:1 aspect ratios, while 8×10 inch photo paper has an aspect ratio of 1.29:1. Practically, this means that digitally acquired images may have to be adjusted (cropped) for different purposes. A few digital cameras permit user-adjustable aspect ratios.

Several mathematical algorithms (image formats) are available to store image sensor data in digital form (Table 1). Since each format has advantages and disadvantages, most digital cameras offer several user-selectable formats (i.e., JPEG, TIFF, or CCD RAW, etc.) (Table 2). The joint photographic experts group (JPEG) format is used almost universally by digital cameras and is also the most widely used format to store 24-bit color photographic images on the web. JPEG uses a ''lossy'' compression scheme that selectively removes information from the file, but the user can select the degree of compression. Low to intermediate compression of an original image produces dramatic reductions in file size with very little degradation of image quality. Tag image file format (TIFF) was originally developed for image transfer in the graphic arts and is widely used today in desktop publishing. There are a variety of TIFF formats, some of which use ''lossless'' image compression that does not cause image degradation. However, the file sizes of TIFF images are relatively large in comparison to those produced by the JPEG scheme. A few digital cameras permit image data to be saved in the raw, unprocessed form (CCD RAW, CRW) for later software modification on a desktop computer. This format produces relatively small files, decreases the time required to process and store images, and provides the option of later using software modification programs that may have advantages over those in the digital camera. In addition, the original image can be reprocessed at a future time to incorporate the latest advances in software technology.

Advantages and disadvantages of image sensors

Image sensors have several inherent advantages over conventional film cameras, including their light sensitivity, dynamic range, and linearity. The light sensitivity, or quantum efficiency, of a digital camera is the fraction of incident photons that are captured and converted into an electronic signal. The quantum efficiency of most high-quality CCDs is approximately 40%, although some special CCDs for scientific applications have quantum efficiencies as high as 90%. This compares to a quantum efficiency of approximately 20% for a conventional video camera, 3% for the human eye, and only a few percent for a film camera. Digital camera manufacturers utilize the film-equivalent International Organization for Standardization (ISO) number to rate the light sensitivity of image sensors in consumer and professional digital cameras. The more sensitive an image sensor is to light, the higher the ISO number. ISO numbers for image sensors used in present digital cameras vary from 80 to 3200. Some digital cameras have automatic or manual sensitivity adjustment, so that the sensitivity of the image sensor can be increased in low light conditions. Unfortunately, this increased sensitivity is usually achieved by amplifying the signal from the image sensor, which also increases the amount of noise and decreases the quality of the final image. The light sensitivity of the silicon photodiode extends across the visible wavelengths and into the near infrared.

The dynamic range (intensity resolution) of an image sensor is the variation in light level to which it is sensitive, or the ratio of the most intense signal to the smallest resolvable signal that can be generated by a digital camera. Under ideal conditions, the dynamic

Table 1. Features of common image storage formats

Format	Primary Application	Advantages	Disadvantages
EPS (encapsulated postScript)	Best file format for placing color images in page layout documents. Files include a low resolution preview image for screen display and image data written in Postscript.	Rapid screen rendering and high resolution printed output on PostScript compatible printers. Selectable preview display (none, 1-bit/8-bit TIFF, 1-bit/8-bit JPEG) and encoding (ASCII, binary, JPEG). PostScript color management.	Large files may be slow to print.
EXIF (exchangeable image file)	Format utilized by digital cameras to store image and camera-specific information in image file headers. Developed by JEITA (Japan Electronics and Information Technology Industries Association) Technical Standardization Committee	Flexible format, can record exposure information (shutter speed, aperture), the time and date the image was taken, and other information. Information is stored in file header and transferred with file. The new EXIF 2.2 standard includes information for printers to enable them to perform accurate image adjustment.	Specific application required to read EXIF data
FlashPix	Panoramic and ''zoomable'' images	Rapid display of zoomable images	Image display requires FlashPix format plug-in and Microsoft OLE
GIF (graphics interchange format)	Common on-line storage format for images with transparency effect (graphic type images, animated sequences) and small images with limited color data.	Support for alpha mask channel and transparency.	Limited to 8-bit (256 color) images.
JPEG (joint photographic experts group)	Universal image storage format in digital cameras and graphic software industry. A modified JPEG format (JPEG 2000) was recently developed by the Digital Imaging Group (DIG).	Efficient image compression format for digital images, level of compression is user-selectable. An 8- to 10-fold file compression is typically used without visible degradation of image quality.	''Lossy'' compression method removes ''extra'' image data and can cause image degradation and artifacts, especially if an image is recompressed. Detailed images do not compress effectively.
PhotoCD	Proprietary process of Kodak Corporation to place digitized files of photographs onto a CD-ROM. Images are adjusted for color and compressed to 4.5Mb.	Images can be opened to multiple sizes. A printed index sheet with thumbnails of the images is included.	Only specialized facilities with a Photo CD transfer station can produce PhotoCD CD-ROMs. Images cannot be opened by every graphics program.
PDF (portable document format)	Popular, flexible format for electronic publishing and prepress.	Independent of fonts and computer operating system, relatively small file sizes, high output resolution. Files can be opened and viewed with a freeware application (Adobe Acrobat Reader). Various levels of protection are available.	Specialized software is required to write PDF files.
PICT	Standard Macintosh image file format. Utilizes lossless Run Length Encoding.	Efficient file compression without image quality degradation.	Pixel size limitations, limited application in non-Macintosh computers. Best for images with large, flat areas of color.
PNG	Recently developed format for on-line images storage and display.	Efficient image compression, support for alpha mask channels, transparency, and 32-bit images.	Not widely utilized at present. Some graphic applications and older web browsers do not support PNG format.
RAW (CCD RAW)	File format utilized by professional digital cameras to store original, uncompressed file information with no in-camera processing (''digital negative'').	Files are stored at high resolution (10- or 12-bits), contain more information than JPEG or TIFF files, and are relatively small. Artifacts produced by in-camera processing or image compression are avoided.	RAW formats are specific for each digital camera manufacturer. Software (''acquire module'') must be available to open files. Image post-processing is required.
TIFF (tagged image file format)	Submission of images to labs and service bureaus. Most digital cameras utilize TIFF as the format for storing uncompressed (''lossless'') image.	Flexible, industry-standard image format read by most operating systems. Support for alpha channels and paths. ''Lossless'' compression (LZW compression) may be used.	Relatively large image file size. Byte order differs with computer operating systems.

Table 2. Hardware-related limitations in digital photography

Problem	Cause and characteristics	Possible solutions
Bad pixels	Photodetectors that produce inaccurate data. May appear as consistent bright or dark spots in an image. The most common form of bad pixel is the "stuck pixel" or "hot pixel", which appears as a fixed colored spots in images recorded at long exposure times.	Software interpolation of images or subtraction of a "dark frame". Some newer digital cameras include software to "map out" bad pixels.
Blooming ("light spill-over") ("streaking")	Results when the charge in a photosite exceeds its storage capacity ("oversaturation") and overflows to an adjacent photosite. Appears as bright vertical streaks, white halos, or spots in an image with extreme exposure values.	Modern image sensors incorporate anti-blooming gates and overflow wells to eliminate excess electrons. Sensitivity and dynamic range may be reduced by anti-blooming measures.
Colour aliasing artifacts ("Christmas tree lights")	Inaccurate averaging between adjacent pixels. Most apparent in enlarged images and diagonal lines.	Software correction may be possible.
Fringing	Wavelength-dependent, wave-like patterns in a digital image due to reflections of incident light in the CCD or associated filters. Most obvious when the incident light source contains a strong component at a single wavelength. A particular problem in astronomy.	Software correction using a flat field with a wavelength corresponding closely to the image.
Nonlinearity	Exceeding the linear range ("over-saturating") a photodiode so that a further increase in light intensity does not cause a corresponding increase in signal intensity.	Decrease light intensity or combine multiple short exposure into a single image
Pixelation	Blocks of color most apparent as jagged diagonal lines in an image. Caused by over-compression of an image with a "lossy" technique, over-enlarging an image, or capturing a detailed image with a low-resolution CCD.	Using a camera with a higher resolution CCD. Reduce the level of compression or capture the image in TIFF or CCD RAW format.
Pixel sensitivity	Small variations in light sensitivity of individual photodiodes due to manufacturing imperfections. Significant in astronomy or scientific applications where quantitative data is acquired.	Calibration of the image sensor with an image of an evenly illuminated light source (flat field).
Read-out signal noise and bias	Electronic noise generated during amplification of the photodiode signal into analog voltage. Bias is an offset, false signal generated during signal amplification.	Design of CCDs and amplification circuitry. Bias is removed with data from bias strips and bias frames.
Stepping	Lines of different density and color, particularly in shadowed regions of scanned images. May result from light source variations or electronic noise.	Upgrade scanner.
Thermal noise	Random, thermally-dependent noise produced by image sensors in the absence of light. Principle limitation of dynamic range in digital cameras. Appears as random white dots in images, most visible on images taken at long exposures.	Some digital cameras utilize electronic dark noise subtraction to minimize problem. Image sensor cooling by forced air, thermoelectric, or cryogenic techniques is required to minimize thermal noise in digital cameras for many scientific applications. Image sensor design can reduce problem.
Vignetting	Dimming of objects at the edge of an image due to mechanical interference. A particular problem with digital photomicroscopy utilizing fixed lens digital cameras.	"Zoom-in" with the digital camera (with a reduction in the field of view), find an appropriate camera/microscope adapter.

range of current high-quality CCDs is theoretically 10^5, or an order of magnitude of 1450:1. However, the dynamic range of consumer digital cameras is much less, on the order of 450:1 to 500:1. For comparison, the human eye has a potential dynamic range of 10^9, and a dynamic range of about 30,000:1 under normal conditions. The dynamic range of photographic film is approximately 10^3, or 7.5 magnitude. Practically, the dynamic range of an image sensor is usually expressed as a gray-scale resolution (i.e., 8-bit, 12-bit, 16-bit, 24-bit, etc.). The dynamic range is one of the most critical characteristics of an image sensor for scientific applications, such as quantitative image analysis, where it is important to measure very small differences in light levels. Fortunately, the dynamic range and sensitivity of most image sensors is linear across the visible wavelength of light and range of light intensities.

At the present time, the disadvantages of image sensors include their smaller physical size relative to 35 mm film and their tendency to generate instrumental artifacts. The size of an image sensor is presently limited by cost, manufacturing capability, and the availability of electronic and computer technology to rapidly read and process millions of signals. Unfortunately, image sensors also generate noise and other artifacts that can limit their application in science and other fields. Some of the instrumentation-related problems in digital photography are listed in Table 2.

Thermal noise (dark current) is thermally-dependent electronic noise that is generated by an image sensor even if no light is present (20). Dark current noise, which appears as ''hot pixels'' or white dots in images taken at room temperature with a long exposure time, is primarily responsible for limiting the dynamic range of image sensors in low light conditions. Since dark current effects are temperature-dependent and double for each 10°F increase in the ambient temperature, they can be reduced by cooling the image sensor. Active cooling to reduce thermal noise is especially necessary for digital photography in dim light conditions, such as astronomy and certain forms of photomicroscopy, including dark-field and fluorescence microscopy. Some astronomers use liquid nitrogen to cool their CCDs, but Peltier-cooled digital cameras are more common in the biological sciences. Peltier coolers are electrically-driven ''heat pumps'' or ''thermal cyclers'' based on a principle discovered in 1834 by French physicist Jean Charles Athanase Peltier. Peltier found that passing current along a circuit containing dissimilar materials results in a refrigerating effect, in which heat is absorbed at one junction of the two materials and is released at the other junction. Modern Peltier coolers consist of paired semiconductors sandwiched between two ceramic plates. When current is passed through the semiconductor, heat from one side of the Peltier cooler moves to the other, creating a temperature differential of up to 120 degrees. Exposure times of up to 30 sec are usually possible if the CCD is cooled to 0°C. Digital cameras equipped with image intensifiers (''intensified digital cameras'') are also available for low-light imaging. Photon noise and fixed pattern noise are also generated by the image sensor.

Amplification of the stored charge on each photodiode into analog voltage also generates noise (20) . This ''read-out'' noise has been substantially reduced by recent advances in CCD design and manufacture but is still a consideration in high-resolution scientific photography. Read-out noise increases in proportion to speed and is lowest in the slow-scan digital cameras. Other forms of noise that develop during amplification and digitization of the analog signal include reset noise, I/f noise, and quantization noise (20). The amount of electronic noise is expressed by the ''signal-to-noise'' ratio, which varies from about 200:1 (46 dB) in a typical video camera to greather than 100,000:1 (100 dB) in a very high-resolution digital camera.

Bad pixels are photodetectors that return incorrect signals. Among the millions of photodetectors comprising a modern image sensor, a few inaccurate pixels are not unusual. Unfortunately, since data is read in a ''bucket brigade'' style, a single bad pixel can occasionally compromise an entire row or column. Digital image processing software can sometimes recognize a bad pixel and replace the data by interpolation from surrounding photosensors.

Imperfections in CCD formation result in small wavelength-dependent, random variations in the sensitivity of the photodiodes comprising an array. The variation in pixel sensitivity is inconsequential for most digital photography applications but can be calibrated for special applications if needed. The calibration is performed by imaging an evenly illuminated source (flat field) and measuring variation in individual pixels. ''Fringes'' are wavelength-dependent, wave-like patterns in a digital image caused by multiple reflections of the incident light in the CCD or associated filters. Fringing is most obvious when the incident light contains a strong, single-wavelength component and is corrected with a flat field illuminated under the same wavelength conditions.

Image storage

A major advantage of the digital camera is the ability to accumulate and temporarily store large numbers of images in the camera for later transfer to a computer. The images are usually stored on removable reuseable storage media that can be plugged into a computer or printer (Table 3). The number of images that can be

Table 3. Characteristics of digital camera removable memory storage devices ("digital film")

Format	General features	Dimensions	Advantages	Disadvantages
CompactFlash I/II	Popular, matchbook-sized cards developed by SanDisk Corp in 1994. Uses ATA architecture which emulates hard disk drive. CompactFlash I and II cards differ only in size and storage capacity.	CompactFlash I – 43 × 36 × 3.3 mm CompactFlash II - 43 × 36 × 5.5 mm	Widely utilized in digital cameras and portable electronic devices. Reusable, sturdy and reliability. High storage capacity (up to 1 GB). Fast data transfer (up to 2.4 MB/sec) be used directly in a PCMCIA slot with an adapter.	Requires a computer with USB or USB card reader for efficiency. High capacity CompactFlash cards are relatively expensive.
IBM MicroDrive	Small hard drives which use CompactFlash Type II format.	43 × 36 × 5.5 mm	Reusable, high storage capacity (up to 1 GB), relatively inexpensive.	Slower and more fragile than other memory cards with a higher power consumption. Heat generation can be a problem in some digital cameras. Not supported by some digital cameras.
MemoryStick	Small ("chewing gum stick-sized") flash memory cards developed by Sony Corp. and used in Sony digital cameras and other Sony portable electronic devices.	50 × 21.5 × 2.8 mm	Small, sturdy, erasure prevention switch.	Usable only with Sony equipment, relatively expensive, limited storage capacity (up to 128 MB)
Multimedia Card	Developed by SanDisk Corporation and Siemens AG/Infineon Technologies AG in November 1997 for digital communication devices.	24 mm × 32 mm × 1.4 mm	Small, reliable, lightweight format with present storage capacity up to 64 MB.	Recently developed format, not widely used in digital cameras at present.
PCMCIA Cards	Storage cards which utilize the PCMCIA slot in laptop computers.	Type I – 85.6 × 54 × 3.3 mm Type II – 85.6 × 54 × 5 mm Type III – 85.6 × 54 × 10.5 mm	Compact, rugged, and reusable with large storage capacity (up to several GB) and long data retention life.	Expensive, size limits use to large professional digital cameras. Laptop computer or PCMCIA adapter required to download data.
Smartmedia	Popular, small, thin, lightweight cards based on ATA architecture. Do not contain controllers or supporting circuitry. Originally developed as the "Solid State Floppy Disk Card" (DDFDC).	45.0 × 37.0 × 0.76 mm	Small, thin, and relatively inexpensive. Widely utilized in digital cameras and portable electronics. Relatively high capacity. Floppy disk drive adapter is available computer data transfer.	Controller functions must reside in the camera. New cards may not be compatible with older cameras. Not as sturdy as other cards.

stored on each storage device depends on the capacity of the storage device (presently 8 MB to 2 GB), the number of pixels comprising the final image (300,000 to 16 million), the type of storage format chosen by the user, and the degree of compression applied. However, present high-resolution (2 to 3 megapixels) images stored at typical compression ratios each require 500 K to 1 Mb of storage space. When the memory on a storage device is exhausted, it can be removed and replaced with an empty one, so that a digital photographer's storage capacity is practically limited only by his/her budget. Professional digital cameras that are directly linked to a computer do not have the limitations imposed by removable storage media, but the camera is restricted to one location.

Small inexpensive flash memory devices are presently the most popular storage media for portable devices such as digital cameras, as well as cellular phones, voice recorders, handheld computers, and portable music players. Flash memory devices are solid-state chips similar to the RAM chips in a computer, but they use no battery power and do not lose stored information when the recording device is turned off. The chips are enclosed in a plastic case with the necessary connectors, and the entire device is usually smaller than a matchbook. Unfortunately, there are several different flash memory formats that are incompatible with each other, although some digital cameras have slots to use more than one format. Table 3 provides information on several popular image memory devices.

Image processing, storage, and manipulation

The images acquired by a digital camera must be transferred, or downloaded, into a computer for long-term storage and further manipulation. During the downloading process, images are transferred from the camera storage device into the storage device in the computer. This can be accomplished by connecting the digital camera to a computer and directly downloading the images from a storage device, or by removing the storage device from the camera and using an adapter connected to the computer to transfer the images. Since most digital cameras include internal serial, parallel, USB (Universal Serial Bus), or IEEE 1394 (Firewire) port, downloading can be accomplished most simply by linking the camera port to the corresponding computer port by a cable, and then using a computer program to view, select, and transfer the images. The speed of image transfer varies from 0.12 Megabits per second (Mbps) for serial devices to 25 Mbps for Firewire ports. Some digital cameras also incorporate wireless (infrared or cellular) transfer technology that eliminates the need for a physical connection between the camera and computer. Card readers or adapters are popular for the frequent transfer of images into the computer, since they are usually faster and eliminate the need for constantly connecting and disconnecting the digital camera to the computer. The PC Card slot of a laptop computer provides a convenient and rapid means of transferring images into these computers. Adapters are available for most popular types of flash memory that permit insertion of the memory card into the PC card slot. A variety of card readers are available for desktop computers without PC card slots. These utilize the serial, parallel, USB, or Firewire port and have reading slots for one to several types of flash memory cards. A special adapter is also available for Smartmedia cards that utilize the 3.5" floppy disk slot of a computer.

An almost endless number of software manipulations can be performed on captured digital images to remove artifacts and optimize them for special purposes, such as printing. Image manipulation and printing is discussed below.

SELECTING DIGITAL PHOTOGRAPHY EQUIPMENT

Digital photomicroscopy of the peripheral blood smear requires a digital camera, a high-quality microscope, a mount for attaching the camera and microscope, and computer hardware and software for processing and storing the digital images. Fortunately a multitude of digital camera, microscope, microcomputer, and software options are available for the pathologist interested in digital photography. The selection of the appropriate components of a digital camera system is dependent upon a number of factors, including existing microscopic resources, the anticipated applications of the digital camera, specimen types, the expected application of the digital images, the availability of funds, and the knowledge and prior experience of the individuals involved. Both digital still cameras and digital video cameras are suitable for photomicroscopy, although the features of each type of camera is different (Table 4). A professional SLR-type digital camera with a removable lens, or a prosumer digital camera with good close-up capability is recommended for brightfield photography if the camera will also be removed from the microscope and utilized for gross photography or general photography. In contrast, most digital video cameras are permanently fixed to the microscope but permit a "live" high-resolution image to be displayed on a high-resolution monitor(s) for simultaneous viewing by multiple individuals. Digital video cameras also permit imaging at low light levels and are required when morphometric measurements are performed. Digital camera models with high light

Table 4. Comparative features of selected digital still cameras

Camera, model, manufacturer	Image and color resolution	Optical/computer interface or image storage mechanism	Comment
Digital cameras dedicated to digital photomicroscopy			
AxioCam Digital Microscope Camera (Carl Zeiss, Inc., Thornwood, NY)	2/3'' CCD, 1.4 megapixels 3900 × 3090 pixels, 71 MB maximum file size, 14-bits/pixel	C-mount PCI interface card Win 95/98, Win NT 4.0 (Mac under preparation)	Single stage Peltier cooling for high dynamic range (>2000), color-co-site sampling for color accuracy, real-time, on-line images at 20 images/sec.
DN100 Digital Camera for Photomicrography (Nikon Corporation, Tokyo, Japan)	1/2.72'' high density CCD; 1.3 million pixels 1280 × 960 pixels (max); SXGA equivalent	Direct attachment to LAN/WAN or internet via a 10-base-T cable with RJ-45 connectors; sharing information in real time is possible. Type 2 CompactFlash card slot also present. Firmware operated using mouse or control panel.	Multiple exposure control options (adjustable program AE, shutter-priority AE, manual); center-weighted or average metering; 1/1000 to 60 sec exposure time, electronic zoom; draw on image; SXGA/XGA resolution display; split screen; noise reduction; shading correction; electronic zoom; auto scale; auto exposure; time lapse. 15 frames/sec (max).
DP-12 Digital Microscope Camera (Olympus Optical Co., Ltd., Tokyo, Japan)	3.34 megapixel CCD 2048 × 1536 pixels, 1/1.8" (~0.55") interlace scan CCD	C-mount adapter Smartmedia storage slot and USB computer interface.	Operated from handset control unit with high resolution, tilting 3.5", 200,000 pixel color LCD. User selectable ISO rating and white balance, automatic or manual exposure modes, 1/3 stop exposure adjustment, selectable metering (1% spot or 30% average), and 2X electronic zoom. Unique features include focusing indicator and calibrated scale bar that can be superimposed horizontally or vertically on the displayed image.
DP-70 Digital Microscope Camera (Olympus Optical Co., Ltd., Tokyo, Japan)	2/3", 1.45 megapixel, single chip color, progressive scan CCD, Piezo shifted, Peltier-cooled, 4080 × 3072 pixels, 12-bit color	C-mount adapter. PCI computer interface, IBM PC-AT compatible, Pentium 4, 1.3 GHz or more recommended.	12.5 megapixel cooled high-resolution, high-sensitivity digital color camera. Fast frame rate (15 frames/sec) for easy focusing and framing, automatic exposure for fluorescence images, binning function for dim fluorescence images, scale bar feature, time lapse function, multiple image merging for fluorescence and DIC images, movable spot focus.
DS-5M-L1 Digital Sight Camera System (Nikon Corporation, Tokyo, Japan)	2/3", 5 million pixel (6.45 micron square pixels) color Bayer mask CCD detector, 2560 × 1920 pixels, 12 = bit color	C-mount adapter. Integral CompactFlash (Type I, Type II) storage. 10/100 Base-TX network connection for PC/Mac web browser support is also available for live image displays and camera control. USB 1.1/2.0 port for file transfer to PC/Mac.	Self-contained digital camera system that can be used independent of a PC or controlled through a PC. Includes separate CCD detector and 6.3". LCD monitor and camera control unit operating in progressive or interlaced mode. Software includes four live display modes and one-touch pre-programmed image mode buttons are available for standard biological and industrial applications, including dark field and phase-contrast images. Several special

Table 4. continued

Camera, model, manufacturer	Image and color resolution	Optical/computer interface or image storage mechanism	Comment
			functions, including distance measurement, scale calibration, count-mark function, and superimpose function.
DXM1200F Digital Camera for Photomicrography (Nikon Corporation, Tokyo, Japan)	2/3" high density, 1.5 megapixel CCD, 3840 × 3072 pixels, 26.1 MB files (RGB)	C-mount adapter. PCI computer interface, PC compatible.	High S/N digital circuit technology, high-speed data transfer (12 frames/sec), integrated software with white balance, focus indicator, automatic sequential imaging. High-sensitivity CCD can capture fluorescence images. Low-profile, wide field 0.63X C-mount adapter. Exposure time – 1/12,000 to 170 sec.
Q-Color5 TM and the Q-Color3 Digital Cameras (Olympus Optical Co., Ltd., Tokyo, Japan)	Q-Color5: 5 megapixel, 2/3" Bayer color sensor, 2580 × 1944 pixel resolution. Q-Color3: 3.2 megapixel, 1" × 1.8" (0.55") Bayer color sensor, 2080 × 1542 pixel resolution	C-mount adapter. Firewire computer interface, Mac and PC compatible.	30/24 bit digitization and preview options, color binning (up to 4 × 4) for rapid previews and focusing; live histogram for optimizing dynamic range; live gain, offset, and zoom capabilities; auto white balance or live color balancing of individual red, green and blue color channels; optional Peltier cooling to 10°C below ambient.
High resolution digital still cameras adaptable to digital photomicroscopy			
Coolpix 5000	5.24 megapixel, 2/3" CCD, 2560 × 1920 pixels	Fixed-lens digital camera, USB 1.1 interface, integral Compact Flash Type I/II slots, NTSC/PAL video outputs.	Prosumer digital camera with 3 × Nikkor zoom lens (28–85) plus 4 × stepless digital zoom, 7,123-step autofocus, 256 element matrix metering, matrix auto white balance with TTL control, 50 step manual focus plus macro focus as close as 0.8", flip-out and twist LCDs.
Olympus C-3040z Digital Camera	3.34 megapixel 1/1.8" solid-state CCD (2048 × 1536 pixels)	USB and serial computer interfaces, SmartMedia and CompactFlash slots, NTSC/PAL video outputs TM.	F1.8 multi-element aspherical glass 3 × zoom lens, 4.5 cm/1.8" color TFT LCD monitor, Olympus lens 6.5–19.5mm f 2.8, eight elements in six groups (equivalent to 32–96 mm lens on 35 mm camera), continuous 1–2.5 × digital zoom, digital "ESP" matrix metering system and spot metering, 16 sec–1/800 sec, ISO equivalent 100, to 400 sensitivity range, "iESP" TTl system autofocus (contrast detection system)
Nikon Coolpix 995 Digital Camera	3.34 megapixel 1/1.8" solid-state CCD (2048 × 1536 pixels)	High speed USB interface, CompactFlash Type I/II slot, NTSC/PAL video outputs	4 × Nikkor zoom lens (38-152) plus 4 × stepless digital zoom, 7,123-step autofocus, 256 element matrix metering, matrix auto white balance with TTL control, 50 Step manual focus plus macro focus as close as 0.8" 1.8" color LCD display

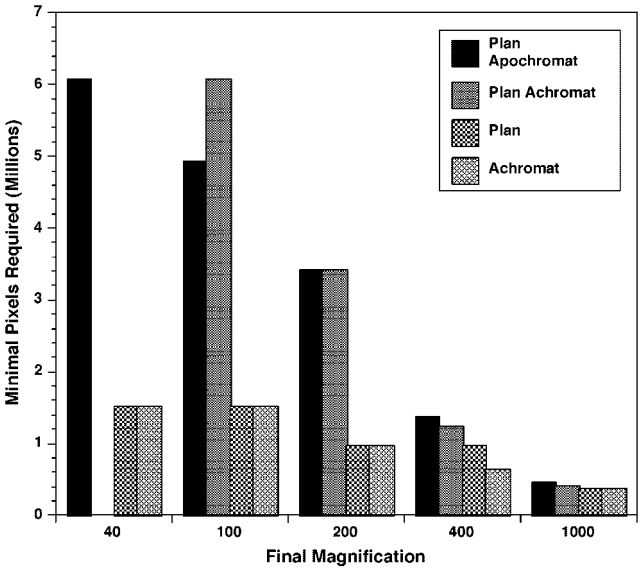


Fig 4. Minimum CCD pixel density required to capture maximal optical data through a microscope using plan apochromat objectives. The calculations are based on an average illumination wavelength of 580 nanometers. Data from Brian O. Flynn and Michael W. Davidson (http://www.microscopyu.com/tutorials/flash/pixelcalc/index.html)

sensitivity, short integration times, and Peltier cooling are required for the acquisition of fluorescence or darkfield images, where imaging times of greater than one second are common.

The required field of view, pixel dimension, objective image magnification, and objective numerical aperture are factors that determine the necessary resolution of the digital camera. CCDs with a pixel dimension between 6.5 and 7.5 microns are optimal for photomicroscopy, although larger pixels will increase the field of view. Because of the field of view, CCDs with higher resolution are required for low magnification objectives than for higher magnification objectives (Fig. 4). At a given magnification, increasing the number of pixels beyond the minimum increases the color accuracy and field of view but not the spatial resolution (21).

Digital Still Cameras

Digital still cameras include both integral-lens, ''prosumer,'' ''point-and-shoot'' types and ''single lens reflex (SLR)'' types a with removable interchangeable lens. The major advantages of these cameras are their relatively low cost and versatility. The cameras can be adapted to existing microscope systems and easily removed from the microscope for macrophotography or other purposes. The image resolution of integral lens digital cameras varies from 2 to 8 megapixels, and the cost of the cameras is less than $1,000. The Nikon Coolpix models (Nikon Corp., Tokyo, Japan), Olympus C models (Olympus Corp., Tokyo, Japan), Kodak (Eastman Kodak Co., Rochester, NY), and other digital camera models have been successfully adapted for digital photomicroscopy.

For occasional noncritical photomicroscopy, the front lens of the fixed-lens digital camera may be set for infinity focus, manually placed at the focal point of the microscope eyepiece, held in a steady position, and the shutter tripped (22). The LCD monitor of the camera can be used for fine composition and evaluation of the final image. However, using an adapter to temporarily couple the camera to a microscope eyepiece eliminates camera movement and permits much easier composition of the image. The LE-Adapter (LensPlus, Redding, CA) is the simplest and least expensive of these devices. The LE-Adapter consists of a threaded coupling ring and a lens adapter ring. The coupling ring is threaded into the filter threads of the digital camera lens, and the eyepiece is inserted into the lens adapter and secured by turning three sizing screws. Finally, the digital camera/LE-adapter assembly is inserted into the sleeve of the microscope eyepiece. A special relay lens (Nikon MDC Lens) for the Nikon Coolpix 950, 990, and 995 digital cameras was recently released by the Nikon Corporation. This consists of a $10\times$ eyepiece with male 28 mm threads compatible with the female mounting thread of Nikon Coolpix cameras. The relay (projection) lens is also inserted into the sleeve of the microscope eyepiece. Recent adapters were also introduced by Nikon and Olympus to mount the Nikon Coolpix 950/990 or the Olympus C2020/C3030/C3040 digital cameras on the trinocular camera tube of a microscope. The Digital Camera Coupler (Optem, Fairport, NY) can be adapted to any integral lens camera with 28 mm, 37 mm, or 43 mm mounting threads. The Kodak MDS 290 Universal Optical Adapter is available for the Kodak MDS 290 digital camera. Digital camera adapters for various prosumer digital cameras are also available from Micro Tech Lab (Graz, Austria), Zarf Enterprises (Spokane, WA), ScopeTronix (Cape Coral, FL), MicroTech Microscope (Mendota, IL), Microscope World (Encinitas, CA), and other sources. Adapters can also be constructed for a specific camera by an optical facility if care is taken to provide a stable support for the camera, avoid damaging the optics of the camera and microscope, and completely eliminate extraneous light. Bennin (23) has described a practical, very inexpensive mount for a Coolpix 950 digital camera using velcro and a garden hose clamp.

''Professional'' digital still cameras are high quality single-lens reflex (SLR) cameras that have been adapted for digital photography. These cameras (DSLRs) presently offer image resolutions of 3 to 14 megapixels and are available from Nikon Corporation, Kodak

Corporation, Canon Corporation, and Fujifilm in a price range of $1,500 to $8,000. The image quality of these cameras is much higher than that of prosumer cameras due to their physically larger CCDs, more sophisticated image processing circuitry, and the capability to obtain 16-bit images in RAW format. These cameras are mounted to a microscope with a "T-mount," and can be easily removed from the microscope for high-quality macrophotography, copy work, or other uses. Furthermore, these cameras utilize existing Nikon (D1X, D2H, Fujifilm S3 Pro (Fujifilm, Tokyo, Japan), Kodak DCS14n) or Canon (10D, 1Ds) lenses, flash units, and other SLR camera accessories that may already be available in the pathology laboratory. These cameras provide a live "through-the-lens" image through the optical viewfinder that can be used for focusing just as with an SLR film camera. However, a low power ($2\times$ or $4\times$) eyepiece magnifier or auxiliary focus finder is recommended with these cameras to reduce focusing errors at low magnification. Although these cameras do not provide live video output, a high resolution (480 lines horizontal), relatively inexpensive color video camera is available ("LiveView" Remote Viewing System, Microptics, Inc., Asheville, NC) that attaches to the viewfinder of the still camera and displays a color at 30 fps into a video monitor or personal computer screen. The image quality of the DSLR and microscope can be further enhanced through the use of flash illumination.

Several dedicated digital photomicroscopic cameras provide the high resolution, flexibility, and ease of use for critical photomicrography. The Nikon DXM1200F digital camera system contains a 2/3-inch, 1.4 million pixel, high-density CCD but utilizes a special interpolation process termed "Inter Pixel Stepping" that brings the final maximum resolution to 12 million output pixels (3840×3072). Furthermore, fluorescence photomicroscopy and long exposure times (1/12,00 to 170 sec) are possible without Peltier cooling due to the high signal-noise ratio. The DXM1200F has an integral C-mount for mounting to a microscope, and interfaces with a Pentium III 500 MHz or faster computer through a dedicated PCI bus interface card with a single cable. High-speed data transfer at 12 frames/sec provides a preview of the image in live, still image, or thumbnail format. The shooting conditions are displayed on the computer monitor, and the integral software provides a wide variety of control, including focusing, white balance adjustment, automatic sequential shooting, edge enhancement, optimization of contrast, brightness, gamma, and other image parameters. A video output signal is not provided. The Carl Zeiss Axiocam uses a 1.3 million pixel CCD with a 6.7 micron pixel dimension and patented technology to provide a real resolution (i.e., without a method of interpolation) in an area of between 1300×1030 and 3900×3090 pixels in color. Color accuracy is assured through "color co-site sampling" at 14 bits/color channel, and the camera provides a live on-line monitor image updated at 20 images/sec. The camera has single stage Peltier cooling for fluorescence and darkfield microscopy, a C-mount interface to a microscope, and a PCI computer interface card with thin fiber optic cable for data and control lines at 200 Mbit/sec. Advanced computer functions are available for image acquisition, adjustment, archiving, and reporting. A feature termed "extended focus" overcomes the problem of limited depth of focus by automatically combining images taken at sequential focus planes. There is no provision for a video output signal. The Olympus DP-70 digital camera is similar in design, utilizing a Piezo-shifted, 2/3-inch, 1.45 million pixel Peltier-cooled CCD to produce a 12-bit 12.5 million pixel image. Special features include advanced automatic exposure for fluorescence images, binning function, fast frame rate for rapid preview and easy focusing, time-lapse function for the collection of sequential images, and multiple image merging. Similar high-resolution digital cameras for photomicrography include the Penguin 600L (5.8 megapixels; Pixera Corp., XXX), Micropublisher 5.0 (5 megapixels; QImaging, Burnaby, British Columbia, Canada), SPOT-RT (Diagnostic Instruments, Inc., Sterling Heights, MI), and Q-Color5 (Olympus America, Inc., Melville, NY).

The Olympus DP12 digital microscope camera is a self-contained photomicroscopy system that incorporates a 3.34 megapixels (2048×1536 pixels) progressive-scan CCD and storage media slot that accepts Smart-Media cards. The DP12 is attached to a compatible microscope via a C-mount and has a separate control unit with an integral 3.5" tilting 200,000 pixel color LCD monitor for vibration-free image capture and easy access to camera functions. Manual control of white balance, exposure, color temperature, image storage quality, ISO rating, and picture sharpness is available, and there is direct "live" video output for viewing images on a standard analog video monitor. Focusing is aided by a focusing indicator and $2\times$ electronic zoom feature. The unit also features automatic and manual exposure modes, white balance selection, and selectable exposure metering (1% spot or 30% average). A calibrated scale bar can be superimposed on the image in either a horizontal or vertical orientation. The DP-12 control unit can be interfaced to a personal computer through a USB port for rapid image transfer, and optional software permits control of the camera through the computer. The Nikon DS-5M-L1 is an alternative "stand alone" digital camera featuring a 2/3-inch, 5 megapixels high density CCD, 6.3 inch LCD monitor,

image superimposition, point-to-point distance measurement, and full-scale calibration. In addition to CompactFlash storage, the DS-5M-L1 has a USB 1.2/2.0 USB port for direct PC viewing and transfer of stored images, and 10/100 Base Tx Ethernet port with http, telnet, FTP Server/Client supportand DHCP compatibility for data transfer up to 100 Mbps.

There are presently three slide scanning systems that provide rapid automated digitization of entire microscope slides. The BLISS slide scanner (Baccus Laboratories, Inc., Lombard, IL) is a state of the art integrated system with a fully automated computerized microscope which can rapidly digitize an entire microscope slide, or any part of a slide, at multiple objective levels with an adjustable depth of field and create a distributable virtual microscope slide, termed a WebSlide. In addition to microscope slides, the BLISS system has the capacity to scan tissue micro array slides and provide a very high-resolution wide field image of each core. WebSlides can be distributed on internet or intranet servers, CD-ROMs, DVDs, or other storage devices, and viewed using special software termed WebSlide Browser Baccus Laboratories, Lombard, IL. With this software, the images can be navigated, the magnification and focus changed, area measurements performed, and annotation performed with translucent overlay grids, arrows, or lines. Multiple users can share a WebSlide, each using their own individual computer complete with pointer sharing and a chat system, to create a virtual double-headed microscope. The Scan Scope (Aperio Technologies, Inc., Vista, CA) is a self-contained virtual microscopy system based on an ultrafast, microscopic slide scanner that digitizes an entire slide at 54,000 pixels/inch with a $20\times$ objective lens at 54,000 pixels/inch or with a $40\times$ objective lens at 108,000 pixels/inch. The individual images are stored in JPEG2000 format across a local area network (LAN) on a PC configured as a file server. Virtual slides can be viewed from another computer on the LAN with ImageScope software. Additional software provides the capability to place virtual slides on a web site for visualization with a web browser. The Dmetrix (DMetrix, Inc., Tucson, AZ) is an automated, completely self-contained microscope-slide scanner with a slide loader and image server that scans a slide at high resolution and permits serving of stored slide images over any TCP/IP connection. Since the data from each scanned slide occupies approximately 12 GB, the standard configuration for the unit includes one terabyte of storage space.

Digital Video Cameras (CCTV)

Digital video cameras offer the advantage of high-resolution, ''real-time'' what you see is what you get (WYSIWYG) output for situations such as video conferencing where a good video image display is necessary (Table 5). Video cameras with three-chip technology offer good to excellent color resolution and adequate to good low light sensitivity. In general, adaptation of these cameras to an existing microscope system is more difficult than with digital still cameras. Since a computer interface is required, compatibility of the computer software and image processing board is often a critical issue. The optical interface with the microscope can also be complex, and these cameras are prone to image display problems such as RF interference and color balancing problems. Some digital video cameras will accept video or SLR camera lenses, but their bulk and requirements for a computer interface are limiting factors for applications other than studio photography and photomicroscopy.

Some manufacturers have modified existing video technology to provide high-resolution photomicroscopic digital images (24). These cameras are directly connected with a computer, either through an image transfer board or through a conventional computer interface (serial, parallel, USB, Firewire), so that an image processing board is not required. The most sophisticated of the digital imaging cameras are the video hybrid imagers (19,25). These cameras provide high-resolution 3-CCD images with complete WYSIWYG functionality, TWAIN compliance (Twain Working Group, San Jose, CA), and ''real time'' image viewing for image composition, focusing, or small-group conferencing. Most of these cameras are physically small and easy to mount to a microscope trinocular head. Fluorescence photomicroscopy is possible with hybrid video cameras because of their excellent light sensitivity, relatively cool operation, and short duration integration times. Peltier cooling is available in some hybrid cameras adapted for imaging of low light level fluorescence images. Some recent models also offer ''real time'' color correction and image enhancement. The camera resolution in TV lines, the type of output (i.e., RGB, S-video, composite) and the signal-to-noise ratio are major factors to consider in selecting a video camera.

CAPTURING DIGITAL IMAGES

Photomicrography

The major requirements for optimal digital photomicrography are the same as silver halide photography; namely, a well-aligned and evenly illuminated microscope with clean, high quality lenses. Vetter (26) and others have extensively reviewed the basic principles of microscopy optimization for photomicrography; the manufacturer's manual should also be consulted for particular microscope models (23,26–38). Special

Table 5. Comparative features of selected digital video cameras

Camera, Model, and Manufacturer	Image and color resolution	Optical/computer interface or image storage mechanism	Comment
ColorView II Soft Imaging System (GmbH, Munster, Germany)	7.1 × 5.3 mm (1/1.8") Peltier-cooled 3.34 megapixel CCD (2048 × 1536 pixels), 12-bit × 3 (RGB).	C-mount adapter FireWire (IEEE 1394) computer interface fully controlled by analysis image processing software.	Compact design, noise reduction (digital correlated double sampling), wide choice of exposure times (1 ms to 100 sec), color binning, high frame rate (25 fps search, 5 fps high resolution)
MicroLumina Digital Camera (Leaf Systems, Southborough, MA)	2700 element, tri-linear, RGB CCD. 2700 × 3380 pixels, 36-bit, 26.1 Mb.	Nikon "F" bayonet mount, SCSI-2 interface, Macintosh and PC compatible. Photoshop plug-in.	Can accept Nikon compatible lens, many optional accessories, including slide illuminator.
Pixera Professional High Resolution Digital Camera	1/3", 25,000 pixel CCD, 1024 × 960 pixels, 24 bit color	C-mount adapter PCMCIA or PCI interface Mac and PC compatible	S/N ratio: 46 dB. Variable focus C-mount adapter.
Sony DKC-ST5 Digital Video Camera	3-CCD prism system with diagonal spacial offset, 2/3" progressive scan 4.2 megapixel CCD (2560 × 2048 pixels). 15 MB image files.	Bayonet lens mount (2/3"). SCSI II (db50) digital interface. Video, Composite, S-Video (Y-C), RGB monitor outputs.	Sensitivity selectable between ISO 20 and 160. Shutter speed from 1/10,000 to 4 sec. Can accept Nikon compatible lens.
Sony DKC-5000 CatsEye Digital Video Camera	Three 1/2" CCD chips, totaling 1.3 million pixels. 1544 × 1120 pixel image files (5.2 MB).	Bayonet lens mount (1/2 inch, 38mm). SCSI digital interface, Macintosh and PC compatible.	Functions through control box with remote control. Control box has memory for up to 10 images. Zoom, focus and aperture can be adjusted manually or by remote control, adjustable gamma and strobe synchronization. Shutter speed from 1/10,000 to 4 sec. Can accept Nikon compatible lens.
SV Micro Digital Camera Electron Microscopy (Sciences Sound Vision, Framingham, MA)	3-shot digital camera. 1" format CMOS chip. 1000 × 800 pixel sensor resolution. 2.2 or interpolated 8.8 Mb files, 30-bit color.	C-mount adapter Parallel or SCSI interface, Macintosh and PC compatible. Operates as PhotoShop plugin.	Active pixel CMOS sensor technology. Rotating three-shot filter wheel. Live video focusing and preview. Shutter speed – 1/1000 to 1 sec.
HC-300Z Digital Microscope Camera	Mosaic image CCD. 2/3", 1.4 megapixel CCD 1,280(H) × 1,000(V), 8 bits per RGB channel.	2/3" C-mount adapter. Image storage on DOS-formated Zip disks via control box and attached zip drive. SCSI interface image-grabbing card and computer software available. Mac and PC compatible.	Square pixel video camera. Functions through control box. Shutter speed – 1/2,000 to 1/4 sec.
Magnafire (Optronics, Goleta, CA)	SONY ICX085AL 2/3" Interline Transfer CCD (1280 × 1024 pixels) with 6.7 micron square pixels.	C-mount optical interface, IEEE 1394 (Firewire) computer interface 400 megabits/sec.	Sealed gas, Peltier Cooled CCD to 40°C below ambient (Magnafire SP version without Peltier cooling). Exposure times – 0.00017 sec to 1560.0 sec. in normal mode. Re-programmable EEPROM permits field updates of camera control firmware. Lucis™ DHP (differential hysteresis process) image feature extraction. LIVE image processing includes: brightness, intensity detect, contrast, histogram equalize, stretch intensity, and manual exposure. Turbo™ Image Gain reduces exposure duration; eliminates the requirement for binning while maintaining

			full image resolution at a wide range of exposures. 10–24 images per sec. Automatic "live" mode, via Firewire™, with auto white balance, auto exposure, Image-Pro® Express software.
DEI-750D (Optronics, Goleta, CA)	Three 1/2" Power-HAD CCDs with 750 lines of horizontal resolution (1280 × 1024 pixels). Digital Download at selectable resolutions.	Proprietary full frame digital image transfer using parallel port, Windows® 95/98/NT, 2000 mHz processor. C-mount optical interface with 10-foot cable to processor. RS232 serial interface is provided for remote computer control of camera functions. Video outputs: composite color, Y/C, RGB, monochrome.	Three-chip color camera with direct digital output for brightfield, darkfield and fluorescence applications. Real-time digital image processing. Patented continuous ratchet auto exposure algorithm with three selectable light weighting modes. High low light sensitivity (.03 lux @ 0 dB gain). 4 sec to 1/10,000 of a second shutter speeds. Remote camera controller. Built-in image memory. Multiple color and B/W video outputs allows simultaneous delivery of true-color images using RGB, Y/C (S-Video), and composite outputs. Also includes a black and white (RS170) output for easy system integration. Ideal for darkfield, DIC, fluorescence and confocal imaging.
LE-Digital (Optronics, Goleta, CA)	1/2" interline transfer CCD (1280 × 1024 pixels) with 6.4 mm × 4.8 mm pixel dimensions; four color mosaic filter. Horizontal resolution: 470 TV lines.	Digital outputs: Parallel port, Twain 32 compliant (Windows® 95/98/NT). C-mount optical interface with parfocal adjustment. Video I/O: NTSC or PAL, composite color, Y/C, RS-170 monochrome, RS232, keyboard.	High resolution CCD color with real time digital image enhancement and direct digital capture. Software control of all major camera functions. Patented microprocessor controlled continuous ratchet auto exposure algorithm with three selectable light metering. Optional 6" head-mounted LCD color display for easy subject viewing conveniently mounted on the microscope. 1/10,000 – 4 sec shutter speed.
SPOT RT COLOR (Diagnostic Instruments Inc.) (Sterling Heights, MI)	Three shot CCD. Kodak KAI – 2000 11.1 × 7.9 mm, 1.92 megapixel (1600 × 1200) CCD with 7.4 micron square pixels, 24 = or 36 = bit color.	Nikon "F" bayonet mount optical interface, PCI bus computer interface card (supplied, PC and Mac compatible).	CCD cooling (37 degrees differential from room temperature), live image window, image capture window, auto exposure, auto white balance, image setups, sequential imaging, zoom and pan window. Color video output at 533 × 400 resolution, 12 frames/sec. 24/36 = bit color or 8/12 = bit monochrome image editing; undo last, resize, rotate, flip and crop. Adjust in RGB, HSL or HSV for hue, saturation, brightness, contrast, gamma, histogram and auto bright-dark stretch.

attention should be taken to isolate the microscope system from vibrations. The system should be placed on a sturdy table with four legs, and the end of each leg should be embedded in a small container of sand (39). Special vibration-dampening equipment and techniques have also been described (40,41).

Once the microscope is optimized for photomicrography, digital photomicrography with most prosumer integral lens digital cameras is performed with the camera in the aperture priority mode and the focus adjusted to an infinity setting. The aperture is adjusted for the lowest F-number, and the zoom setting is adjusted to assure the desired image field of view while avoiding vignetting. An AC adapter is preferred over internal batteries to reduce weight and provide a convenient and continuous power supply. The metering, image adjustment, contrast, sharpening, and other functions of the camera should be adjusted to the users preference. The principles of digital photomicrography with interchangeable lens SLR-type digital cameras are similar to their film-based SLR counterparts except for the availability of digital software functions, the ability to immediately view captured images on the LCD monitor, and the special problems imposed by digital photomicrography (see below). The operation of video cameras and digital still cameras dedicated to photomicrography is variable depending on the equipment manufacturer and not further addressed in this article.

The magnification and physical diameter of the microscope-to-camera relay lens, the optimal placement of the camera lens, the camera aperture, and the lens magnification (''zoom factor'') must be carefully balanced to optimize field size and image brightness, while miminizing vignetting. Using the ''auto'' setting on a digital camera for digital photomicroscopy usually results in a dark image since the white balance is not optimized for the tungsten or halogen illumination systems of microscopes, and the camera averages the scene for a gray-scale image. Therefore, it is important to manually set the white balance for the camera on a blank, fully illuminated microscope field prior to obtaining images. The level of illumination should not be changed after setting the white balance, since the color temperature of the light will change, and the white balance will be altered. For convenience, many microscopes have a ''photography'' illumination setting that is helpful to assure consistency between images. Proper white balance is especially important for specimens with scattered cells and much ''open'' background, such as peripheral blood smears, bone marrow aspirates, and cytopathology specimens. ''Spot metering'' on individual cells will also produce correct exposure values in these specimens, as well as exposure compensation. In general, an exposure compensation of $+1$ to $+2$ is necessary for these specimens. Although many exposure and color balance problems can be corrected in an image analysis program, it is much less time consuming to assure proper exposure at the time of imaging.

There are many causes of suboptimal digital photomicrography. Many of the problems common to silver halide photography apply, as well as problems unique to digital microscopy. According to Vetter [28], the problems most commonly encountered in film photomicrography include uneven illumination, out-of-focus images at low magnification, loss of important image detail, poor color rendition, and inconsistent exposures. Additional problems unique to digital photomicrography include vignetting, a small field of view, and a dark image background. Solutions for these and other problems are described in Table 6. A major advantage of the digital camera is that captured images can be viewed immediately, permitting the easy recognition and correction of imaging problems.

Vignetting is the appearance of dark rounded edges on a digital image. This problem results because a rectangular digital image is obtained through a circular lens, and occurs when the lens diameter of the digital camera is greater than the diameter of the microscope eyepiece opening. Vignetting is eliminated by using a relatively small camera aperture and a properly seated relay lens with sufficient magnification to fill the diagonal of the film frame. Cameras with zoom capability can also be ''zoomed in'' to fill the film frame, although only the central portion of the lens area will be recorded in the final image. The relatively small size of the CDD, in comparison to 35 mm film, also contributes to the small field size of the digital photomicrograph.

Focusing can be a major problem with digital camera systems, particularly at low magnification. This occurs because low power objectives ($1\times$, $2\times$, $4\times$) have a very small depth of focus (0.03 to 1.4 microns) at the image plane. In contrast, focusing is not as critical for higher power objectives with a much larger depth of focus of (20×-4.75 microns, 40×-12.0 microns) (19,25). Furthermore, the optical viewfinders on integral lens digital cameras cannot be utilized for focusing because they are of the ''rangefinder'' type and do not reveal the ''through-the-lens'' microscopic image. A ''live'' image is displayed on the integral LCD monitors of most integral lens prosumer digital cameras, but these monitors are also suboptimal for focusing with the unaided eye because they are small (1.8'' to 2.0'' diagonally) and of relatively low quality. The best solution to this problem is to connect the camera to a television monitor with a video cable and use the ''video-out'' feature of the camera. Most digicams with this

Table 6. Common problems in digital photomicroscopy

Problem	Cause	Solution
Uneven illumination	Microscope is misaligned for Kohler illumination is not present or microscope design does not conform to Kohler illumination. Common causes of misalignment include an off center substage condenser or lamp, improper use of the diaphragms, and and out of focus condenser.	Realign microscope optics to ensure Kohler illumination.
Out-of-focus images at low magnifications ($2\times$, $4\times$)	Shallow depth-of-focus of low power objectives; inability of eye to accommodate objects at different planes of focus.	Use low power ($4\times$) auxiliary focus finder; use parallax focusing; focus with higher magnification objective ($40\times$) then return to low power objective without refocusing.
Loss of image detail	Improper setting of aperture diaphragm (lack of contrast with excessively large aperture diaphragm, diffraction and refraction with excessively small aperture diaphragm); vibrations and "camera shake;" poorly focused image; low digital camera resolution.	Adjust aperture diaphragm to achieve best balance of detail and contrast; use "digital zoom" or focusing aids; use remote shutter release; place microscope/camera system on a sturdy surface; acquire a digital camera with higher resolution.
Vignetting	Relatively small diameter of relay lens; narrow camera aperture.	Set camera to "aperture priority" mode and set sperture to largest lens opening (smallest f-number); "zoom-in" obtain relay lens with wider diameter.
Small field of view	Relatively small physical dimensions of CCDs in comparison to 35 mm slide or print film.	Zoom-out" to maximize field of view, while avoiding vignetting.
Dark images	Fast camera shutter speed.	Increase illumination; use camera manual settings to increase exposure time.
Dark image background	Improper white balance.	Manually set white balance prior to obtaining images; use exposure compensation; use spot metering.
Out of focus areas, particularly at image periphery	Nonplanar microscope objectives, lens aberrations in camera or microscope.	Try different objectives and relay lens, realign optical path of microscope.
Reflections, bright spots, or concentric rings on digital images	Internal reflections from multiple lens surfaces and/or noncoated lens surfaces.	Set camera to "aperture priority" mode and set sperture to largest lens opening (smallest f-number); try different objectives and relay lens; realign optical path of microscope.
Dark spots on digital images	Dust and debris on microscope, camera lens, or CCD.	Carefully clean all lenses in the optical path, use a microscope dust cover when system not in use. Clean CCD (professional digital cameras only).

feature will display images in either the American (NTSC) standard or European (PAL) standard. The live image displayed on the monitor is the same as that seen on the camera LCD display. The live image is in a low quality "viewfinding" format that is inadequate for videoconferencing but adequate for focusing and scene composition. Alternately, commercially available video-capture adapters are available that permit the importation of S-Video or Composite video signals into the USB port or PCMCIA Type II slot of a laptop or desktop computer at 30 frames/sec.

If a larger monitor or laptop computer is not available, a $2\times$ or $4\times$ magnifying glass or a special LCD magnifying viewer such as the Xtend-a-View Pro™ (Photosolve, Saratoga, CA) can be used to magnify the image on the LCD monitor for more accurate focusing, or the monitor image can be enlarged with the digital zoom feature of the camera, focused, and then be reduced to the original dimensions for imaging. The remote controls available for most digicams make this "zoom-focus" technique relatively easy to accomplish. Photography with low power objectives (i.e., $2\times$, $5\times$, $10\times$) is best avoided with digital cameras due to the problems with focusing. If an image with a large field of view and low magnification is needed, it is best to "stitch" together several images taken at medium power (i.e., $20\times$, $40\times$).

A 35 mm digital slide scanner can also be used for the creation of low-power photomicrographs from stained tissue sections on conventional microscope slides. Some manufacturers offer modified 35 mm film slide mounts or slide scanners with special slots for placing microscope slides. Tissue sections can also be mounted on 2" × 2" glass slides and scanner in the same manner as a 35mm slide. An area of up to 24×36 mm can be covered, or the "preview" function of the scanner software can be used to select a specific region of the tissue section to scan. Several investigators reported that images prepared by this technique provide excellent sharpness, even illumination, and superior color reproduction as compared to conventional images taken with a camera or microscope (42–46).

Macroscopic Photography

The optimal photography of gross specimens requires extensive experience, high quality equipment, and a detailed knowledge of camera operation and the principles of photography. Most prosumer digital cameras with integral lenses have excellent "close-up" or "macro" capability; these cameras can be utilized for macroscopic pathology without further modification, or a variety of lens attachments can be applied for special purposes. The excellent macrophotography lenses, lighting equipment, and other equipment manufactured by Canon Inc. (Tokyo, Japan) or Nikon Corporation (Tokyo, Japan) can be used with several still SLR-type digital cameras, although the "multiplier effect" or "magnification factor" must be considered. The multiplier effect arises because many SLR-type digital cameras utilize preexisting camera bodies with a CCD mounted at the film plane. Since the optics of these cameras are optimized for 35 mm film, and existing CCDs are smaller than a 35 mm film frame, the image projected on the CCD is physically larger than the size of the CCD and only the center portion of the image is captured. Practically, this multiplies the effective focal length of any mounted lens, so that a conventional 50 mm lens mounted on a digital camera with a multiplication factor of 1.5 behaves like a 75 mm lens and a 100 mm lens behaves like a 150 mm lens. The magnification factor does not apply to several recently developed SLR digital cameras that utilize a "full frame" CCD equal in size to a 35 mm frame (24×36 mm). These cameras include the Canon 1Ds and the Kodak 14n.

Modification of existing camera stands and other equipment may be necessary in a surgical pathology or autopsy suite to accommodate this difference. The immediate availability of digital macroscopic images is advantageous for the surgical pathology or autopsy laboratory, since they can be printed, annotated with descriptive text, or used in other ways to facilitate diagnostic evaluation of the corresponding microscopic sections, quality assurance, or conferencing. Hard copies of complex specimens, especially those with resection margins, are commonly used in the gross room to "map out" the areas sampled for microscopic examination.

The flatbed scanner can be used to capture gross images of pathological specimens. As described by Mai et al. (47), the specimen was placed on the surface of the scanner bed that was covered by transparent sheet of plastic or projector transparency film. With proper lighting and the use of background material, they were able to obtain high-resolution, shadowless images for surfaces with a depth up to 30 mm. Matthews and Denney (48) described a "wet scanning" technique to improve the quality of images with wet highlights. They mounted a Perspex box with a clear 3 mm base on a scanner, partially filled the box with distilled water, immersed the specimens in the water, and scanned the specimen using conventional PC hardware and software (48). A slide scanner can be used to prepare digital images of gross ocular specimens from glass slides (49). An advanced, digitally based workstation for gross pathology specimens has been described by Leong et al. (50).

Software Modification of Digital Images

Software manipulations of digital images are performed to: 1) adjust the tonal balance, hue, saturation, sharpness, or other image properties to more closely resemble the original image; 2) remove image flaws and artifacts such as noise, dust, scratches, etc.; 3) change the size (resample), format, resolution, or orientation of an image for a publication, email, print, 35 mm slide, web page, or other specific purpose; 4) crop an image to remove extraneous information or emphasize the key area; 5) add text, arrows, and/or voice annotation to an image to clarify or add additional information; 6) extract information to perform further analysis, such as image enhancement, image analysis, or measurement; and 7) merge multiple single images together to create a panoramic image, three-dimensional (3D) stereo image, montage, or animated display (10,51–55). Modern photo-editing (''electronic darkroom'') computer programs such as Photoshop (Adobe Systems Inc., San Jose, CA), Paint-Shop Pro (JASC Software, Eden Prairie, MN), Corel PhotoPaint (Corel Corp., Ottawa, Ontario), and many others make these and other enhancements easy to accomplish, even for users with no previous experience (Table 7). Some image analysis and enhancement programs, such as nik Sharpener Pro! (TECHnik Group, San Diego, CA), Silverfast DC (LaserSoft Imaging, Inc., Longboat Key, FL), Quantum Mechanic (Camera Bits, Portland, OR) , iCorrect Professional, (Pictographics International Corp., Burnsville, MN), Intellihance Pro (Extensis, Portland, OR), and AutoEye (Auto FX Software, Birmingham, AL) can greatly assist in removing noise, sharpening, and color correction with minimal time for the user. As yet, pathologists have not embraced other professional image storage and retrieval formats, such as DICOM. DICOM was developed through collaboration of the American College of Radiology (ACR) with medical industry companies and the National Electrical Manufacturers Association (NEMA). Other software programs, termed image content managers, function as ''digital light boxes'' or ''image databases,'' and permit images to be displayed in a variety of ways, organized, renamed, assigned keywords, printed, and stored in collections on hard drives, CD-ROM media, tape, or other storage devices. Most of these programs permit complex searches of image collections for specific files.

The brightness dynamics of an image are referred to as its tonal balance (55). Each channel (i.e., red, green, blue) of an 8-bit color digital image consists of multiple gray spots that can vary in 255 levels from black (level 0) to white (level 255). Image detail can be enhanced in many cases by adjusting the brightness without altering the color composition. The brightness and contrast controls of Photoshop or another image processing programs can be used for this purpose, but some information may be lost in the process. However, both the levels control and the curves control avoid information loss and also permit more control of the enhancement process. The bright, midrange, and dark values of an image can be adjusted separately with the levels control, which also provides a visual representation of brightness values in the form of a histogram (Fig. 5). Brightness values (0–256) are represented on the horizontal axis of the histogram, while the number of pixels at each brightness value are plotted on the vertical axis. Adjusting the midrange values to increase or decrease the brightness of an image without changing the shadow or highlight extremes or color composition is the only manipulation required for many images. This is analogous to changing the ''gamma'' on a computer monitor. If necessary, the shadow or highlight values can also be changed to reveal image detail lost in the black or white areas of the image. This can be accomplished by moving sliders below the histogram or by using special ''eyedroppers'' to select the darkest region of an image for assignment as ''black'' and the lightest region for assignment as ''white.'' A third histogram manipulation that can be performed is termed ''histogram equalization.'' Images that have underutilized gray-scale values (i.e., large valleys) often show more detail when some of the gray level ''peaks'' are reassigned and the valleys compressed.

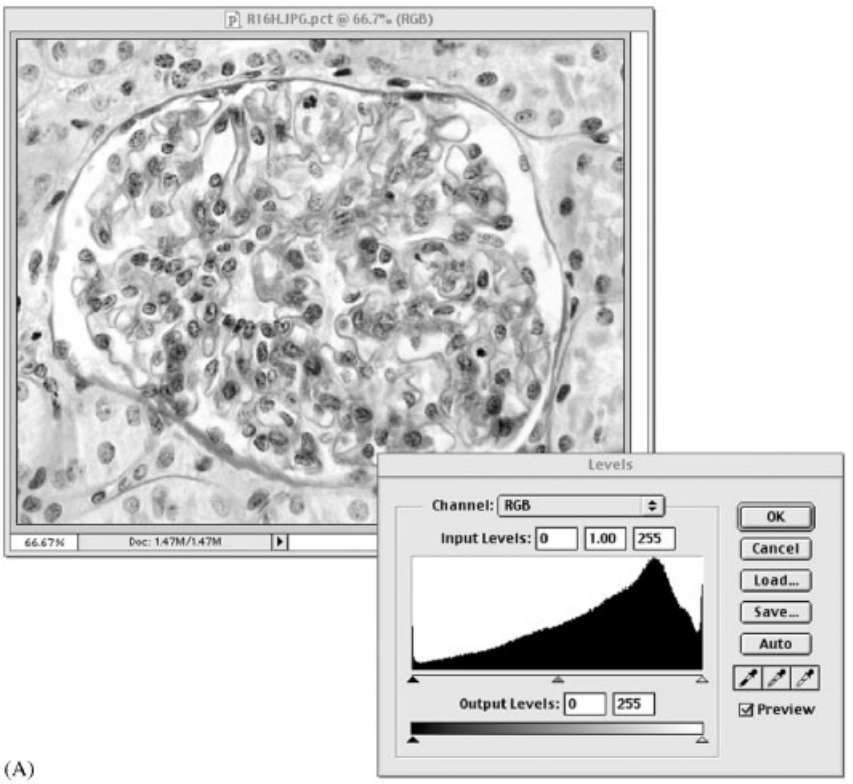
The curves control is more complex but also permits more precise tonal correction, since any point along the 255-level tonal scale of each channel can be changed (Fig. 6) (55). The curves control of an original, unaltered image consists of a two-dimensional line graph with a slanted line running through it at an angle of 45°. The horizontal and vertical axes of the graph represent tonal values in vs. tonal values out. Clicking on the graph creates a point with the line flowing through it. Moving the point changes the brightness of the image similar to moving the midrange point of the levels control. A new point can be placed on the graph with another click. With practice, curves can be created that thoroughly optimize the brightness and contrast of an image. Furthermore, curves can be saved, reloaded, and applied to other images.

Color adjustment can be performed with the hue and saturation controls of image manipulation programs. Hue adjustment of an image or selected color channel is analogous to a move around the color wheel. Saturation refers to the ''purity'' of a color, and a change in saturation represents a move across the radius of the color wheel. The hue and saturation values of images from high-resolution digital cameras rarely require adjustment. Small image defects such as dust and

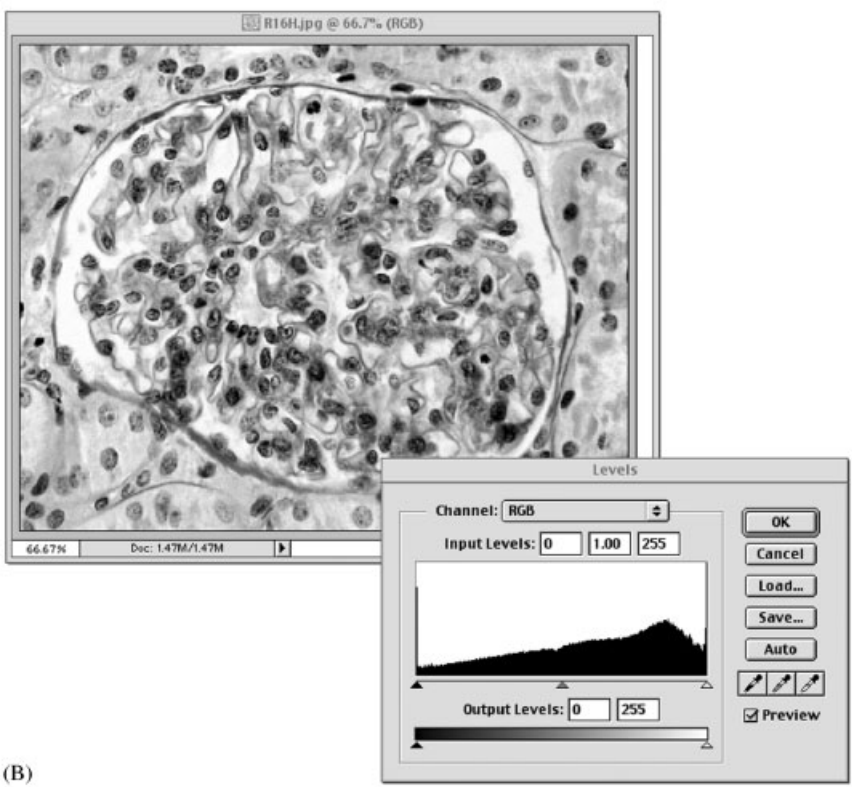
Table 7. Software applications for scientific imaging

Name	Company	Platform/operating system	Comment
AnalySIS	Soft Imaging System (GmbH, Munster, Germany, http://www.soft-imaging.de)	Stand alone application.	Advanced software for image acquisition, archiving, processing, and analysis. Available in three expansion levels with different feature sets. Modular architecture to permit customization and expansion. Unique features include the use of an intelligent viewpoint image display system, advanced networkable image archival and retrieval system, page layout report system, differential contrast optimization, automatic or manual montaging of laterally displaced images into a single image, conditional filters to reduce noise without blurring edges, multi-phase threshold setting, shading correction, image geometry and arithmetic, and many other features. "Extended focal imaging" permits construction of a single image from multiple images acquired at different focal depths.
AutoDeblur	AutoQuant Imaging Inc. (Watervliet, NY, http://www.aqi.com/)	Stand alone application, Windows NT 4.0, or Windows 98.	Advanced software program that uses blind deconvolution technology is used to restore out-of-focus areas in digital images.
Fovea Pro/Image Processing Tool Kit (IPTK)	Reindeer Graphics (Ashville, NC, http://www.reindeergraphics.com/)	Adobe Photoshop plug-in Macintosh and Windows.	Fovea Pro is an advanced, comprehensive suite of 195 utilities for image adjustment, color manipulation, Fourier processing (including deconvolution), image morphology, image measurement (including automatic classification), and surface analysis. All functions and values can be automated. IPTK features 175 utilities.
ImagedPath	eVirchow (http://www.evirchow.com/imagedpath.htm)	Stand alone application, Windows and Macintosh.	Comprehensive ODBC-compliant relational image database designed for anatomic pathology. Includes layout program for report design and generation with references, and graphic elements such as prognostic charts. Permits plotting of PSA values and incorporation of graphical representations of Gleason and Scarff-Bloom Richardson scoring.
Image-Pro Plus	Media Cybernetics, Inc. (Silver Spring, MD, http://www.mediacy.com/)	Stand alone application, Windows.	Comprehensive networkable database for image acquisition, processing, and analysis. Advanced image measurement and analysis features. Optional modules for automated microscopy, acquisition of fluorescent images, and analysis of gel.
Lucis	Image Content Technology LLC (Glastonbury, CT, http://www.imagecontent.com/lucis/)	Stand alone application, Windows 95, 98, 2000 or NT.	Image enhancement program based "differential hysteresis processing." Enhances fine contrast variations detail in shadows and highlights simultaneously to repair both underexposed and overexposed images.
Microtome	VayTek (Fairfield, IA, http://www.vaytek.com/decon.htm)	Stand alone application, Macintosh and Windows.	Advanced deconvolution software that includes seven methods for deconvolution.

			An "image space version" of the deconvolution algorithms works with measured or calculated point spread functions.
NIH Image Scion Image for Windows	Research Services Branch (RSB) or National Institute of Mental Health (NIMH) (http://rbs.info.nih.gov/nih-image)	Stand alone application, Macintosh, Windows.	Versatile public domain image processing and analysis program. Features image acquisition, display, editing, enhancement, and animation. Permits image analysis with spatial calibration and density calibration.
OpenLab	Improvision, Inc. (Lexington, MA, http://www.improvision.com/default.lasso)	Stand alone application, Macintosh.	Modular imaging program specifically designed for multiuser scientific imaging. The core program is designed for image acquisition, measurement, presentation and publication. Separate Modules are available to control hardware or add additional software capability such as time lapse imaging, ratio imaging, deconvolution and 3D imaging.
Optipix	Reindeer Graphics (Ashville, NC, http://www.reindeergraphics.com/)	Adobe Photoshop plug-in, Macintosh and Windows.	Suite of nine utilities for image enhancement, including autocontrast, blending exposures, image averaging, image nudging and alignment, edge enhancement, sharpening, and noise removal.
PAX-it! Digital Image Management	MIS, Inc. (Franklin Park, IL, http://www.paxit.com/PAX-it_html/paxit.asp)	Stand alone application, Windows.	Comprehensive modular ''electronic filing system'' with standard ODBC database interface, flexible input and output, report generation, annotation tools, measurement, networking, web-based image sharing, and realtime videoconferencing
Phyllum	Improvision, Inc. (Lexington, MA, http://www.improvision.com/default.lasso)	Stand-alone program, Macintosh and PC.	Digital image management application designed to manage, organize and find archived digital images quickly and easily.
Purple De-Haze	Intelligent Imaging Innovations, Inc. (Denver, CO, http://www.intelligent-imaging.com/products.html)	Adobe Photoshop plug-in, Macintosh, Windows under development.	Economical no-neighbor deconvolution software.
Slidebook	Spectra Services Inc. (Webster, NY, http://SpectraServices.com/analysis/index.html)	Stand alone application, MacOS and NT/2000/XP workstations.	Modular image processing software package, specifically designed for multidimensional digital microscopy. Includes an image database as well as modules for image capture, deconvolution, 3D visualization, and FURA2 ratio imaging. Program is designed around a slide model, which keeps all images collected from a single slide in a single file.
ZEM Still/ZEM Dynamic /ZEM VirtualSlide	ZEM Technology (The Netherlands, http://www.zem.com/telemicroscopy.html)	Stand alone application, Windows (95,98, NT, 2000) and Mac OS.	ZEM Still – Telemicroscopy application for archiving and sharing still microscopic images. Includes relational database with picture gallery. Permits multiple types of annotations, video-conferencing. AEM Dynamic adds real-time telemicroscopy, including remote microscope control. ZEM VirtualSlide permits construction of digital representations of microscope images that can be viewed at all magnifications.



(A)



(B)

Fig. 5. Peripheral blood photomicrographs with histograms drawn by Adobe Photoshop. **A:** Prior to histogram equalization. ''Peaks'' in the histogram correspond to the more common brightness values which often identify particular structures present in the image. ''Valleys'' between the ''peaks'' indicate brightness values that are less common in the image. The flat regions at both ends of the histogram show that no pixels have those values indicating that the image brightness does not cover the full 0-255 range available. **B:** After histogram equalization.

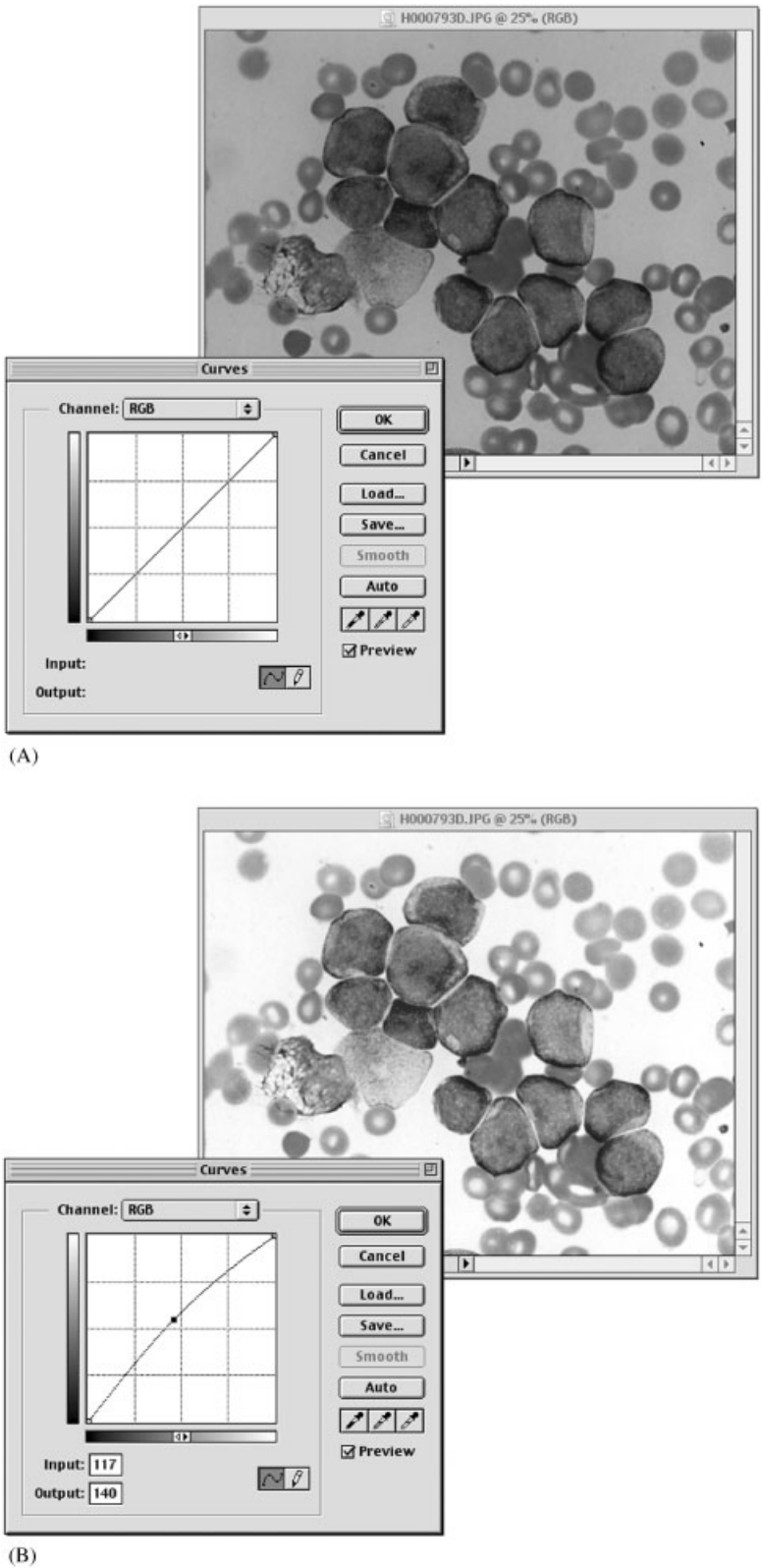


Fig 6. **A,B:** Example of curves control.

scratches can be removed with special subroutines (''filters'') in Photoshop or other programs, or by manually replacing the defective areas with pixels from the surrounding region.

Most natural scenes consist of an almost infinite gradation of color and tone. Conversion of a scene into a digital image comprised of a finite number of ordered pixels invariably results in a loss of detail, which is perceived as blurring or softening. Fortunately, a computer process termed unsharp masking can restore much of the lost detail. Most high quality digital cameras incorporate unsharp masking routines that perform this process automatically during image acquisition; repeated unsharp masking may be required if the image is resampled. Unsharp masking routines work by finding pairs of adjacent pixels that differ in brightness by a specified amount (e.g., edges) and increasing the contrast of these pixels and any others that fall within a certain radius. The brightness difference (threshold) can be specified, as well as the percentage by which the contrast of the edge pixels is increased (amount) and the number of pixels around each edge that are sharpened (radius). The optimal setting for these parameters depends on the type of image and the resolution of the final output device (i.e., on-screen display, ink-jet printer, laser printer, etc.).

The hue of an image can be changed by controls that effectively rotate all of the colors in the image around the color wheel. Saturation, or color purity, can be changed by a separate control in image manipulation programs. The hue and saturation do not normally require adjustment in images acquired by better digital cameras. Small artifacts can be removed from digital images with the ''dust and scratches'' control of most image manipulation programs. This task can also be performed manually by carefully replacing the undesired areas with pixels copied from an adjacent area. Although photographic images are commonly stored in the Photoshop native, JPEG, BMP, TIFF, or PICT formats, there are more than 145 available image formats. All image manipulation programs and content managers permit images in a variety of formats to be opened and then saved in a different format. Some programs specialize in the conversion of images from one format to another. If a limited portion of an image contains information of interest, this area can be selected with ''cropping'' tools and resaved as a separate image. Alternatively, ''masking'' tools permit desired objects, such as a cell or a person, to be selected and transferred to another image or the undesired parts of the image can be removed. Masking is especially useful for removing bloody or unattractive backgrounds from digital images of gross pathology specimens. Information, such as symbols, arrows, text, or even voice annotations, can be added to digital images with most image manipulation, word processing, and web site creation/management programs.

The capability to manipulate, or even to dramatically alter a digital image with electron darkroom software, is a major ethical and legal concern because these images can play an essential role in patient care and scientific research (56–61). However, the problem, as Suvarna and Ansary (62) emphasize, is not the software, but rather the ''intention of the individual to deliberately falsify an image.'' The problem of fraudulent images is likely to multiply as digital images become even more widely accepted for medical and scientific applications. Possible solutions to this problem for publishers is to randomly audit individuals submitting papers or to require authors to submit both the original and modified versions of digital images.

Panoramic, Continuous, and Object Photography

The creation of panoramic images for medical education is one of the most promising applications of digital photography. Panoramas are ''images with unusually wide fields of view that extend far beyond a single camera snapshot'' that convey more of a sense of realism than images of normal width (63). Circular and spherical panoramas are popular at this time and widely used to present immersive views or ''virtual tours'' of landscape photographs, real estate, and architecture. However, flat or planar panoramas can also be prepared from a mosaic of single images of a microscope slide, high-altitude photographs, maps, or similar data. Panoramic pictures are prepared by merging or ''stitching'' multiple detailed overlapping images into a larger image. When panoramas are saved in Quicktime VR (Quicktime VR, Apple Computer, Inc., Cupertino, CA) movie or similar format, the viewer can view the entire image, ''zoom-in'' for a high-resolution view of a particular area, or move horizontally or virtually. The term ''virtual microscopy'' has been applied to panoramic images of microscope slides, since the viewing experience is analogous to using a microscope in real time. Panoramic images can be prepared with Photoshop or similar image editors by manually merging pictures together, but they are much easier and less time-consuming to prepare with special computer programs written for this purpose, termed ''stitching software,'' or ''image stitchers.'' These programs require multiple (two to hundreds) images of a scene that overlap by 30 to 50% (Fig. 7). Some programs will only prepare mosaics from images taken at the same horizontal plane (single-row panorama), while other programs will stitch images from mosaics or multiple rows (multi-row panorama), or even from a sphere (spherical panorama). Many

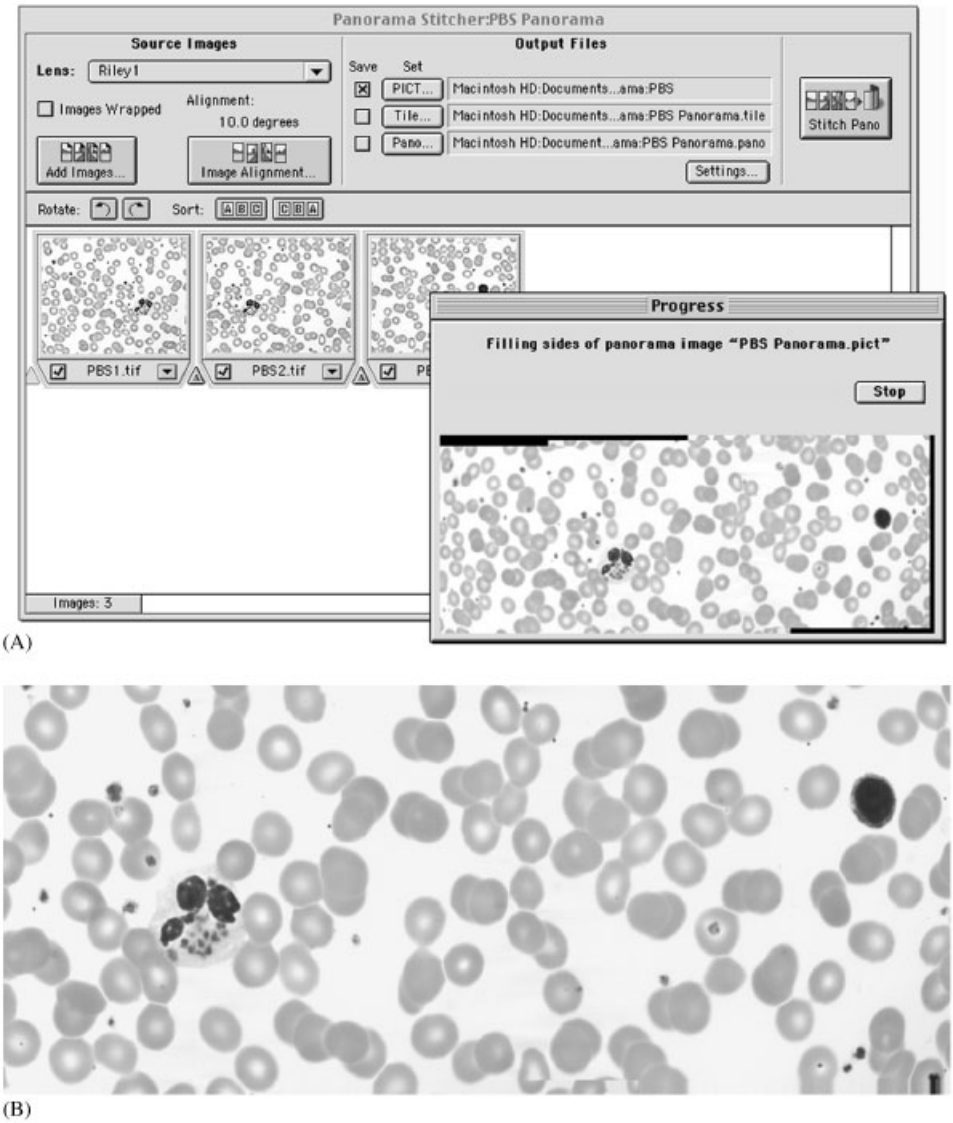


Fig. 7. Single-row panoramic image of a peripheral blood smear. **A:** Stitching process. **B:** Final image.

stitching programs will not only seamlessly stitch digital images, but will remove artifacts in the original photographs and those created by the stitching process. To prepare circular or spherical panoramas of landscapes or building interiors, a camera mounted on a tripod is required, and the camera must be positioned so that one of the nodal points of the lens is centered above the point of camera rotation on the tripod to avoid an optical artifact termed ''parallax.'' Special tripod heads (''panoramic heads'') are available for this purpose. Parallax is not a problem with mosaic panoramas prepared from flat objects such as microscope slides, although a mechanized means to move and properly position the slide simplifies the process of taking multiple overlapping images.

In continuous photography, a sequential series of pictures are combined into a movie or animated gif to show an action sequence. Some digital cameras permit time lapse photography by taking a series of pictures at specified intervals. Object photography is performed by taking multiple images of a stationary object at specified angles around its periphery. With the proper software, a view at any angle can be displayed, or the images can be combined into an animated gif or movie so that the object appears to rotate in space. Small objects are usually photographed on a special turntable termed an object rig. The object is mounted in the center of the turntable, which is rotated a precise number of degrees, as photographs are taken from a digital camera at a fixed position.

Displaying, Printing, and Sharing Digital Images

Electronic publishing

Electronic publishing is "distributing information via computer instead of paper" (64). Following the computer revolution of the past two decades, high-quality electronic publishing hardware and software is available to nearly every profession and to much of the general public in the developed countries. With electronic publishing software and hardware, it is possible to distribute digital images and written documents not only on the World Wide Web but also through e-mail messages and locally via intranets and CD-ROM. The major advantage of electronic publishing is speed and flexibility. Documents can be shared in seconds rather than hours or days, and document creation is not dependent upon dictating machines, secretaries, or printers! Electronic publishing is presently standardized around two major formats, HyperText Markup Language (HTML) and Adobe Portable Document Format (PDF). HTML is most widely utilized for internet-based publications, but it can also be used for other forms of document sharing. HTML is extremely flexible because documents can vary greatly in complexity and include formatted text, hypertext links, images, and animations. PDF documents are mainly used to share documents via CD-ROM and e-mail. Since the PDF format is based on the Adobe Postscript language used by some printers; a document prepared in any software program can be compactly stored in its original form as a PDF file, and then reproduced on screen or in printed format by anyone with a free viewing program termed the Adobe Acrobat Reader. PDF documents can also include interactive and multimedia components such as hypertext links, text searching, and "fill-in-the-blank" forms.

Sharing images on the web must involve consideration of the wide audience that may access the web site. Since not all users have large monitors or fast internet connections, the web creator must strive for good to excellent image quality with conservative file sizes (i.e., <100 kilobytes) and images no larger than 800×600 pixels. Most users will not tolerate long download times or poor image quality and will simply move to another web site if these are encountered. The key to good image quality is to start with a high quality image. Editing images for the web should include downsizing, sharpening, and removal of any scanning or imaging artifacts. An image editing program such as Photoshop or Paintshop Pro (Jasc Software, Eden Prairie, MN) is indispensable for this process, as well as several supplemental plug-ins.

The worldwide availability of original texts and images via the internet and CD-ROM has made copyright issues more important than ever, and this issue must be considered by creators of documents for electronic publishing. In the United States, writers, inventors, artists, playwrights, musicians, and other creators of original works are protected by copyright protection by the U.S. constitution (Article I, Section 8), in order to "To promote the progress of science and useful arts, by securing for limited times to authors and inventors the exclusive right to their respective writings and discoveries." Duplication of all, or any portion of a work, without permission from the creator is a violation of copyright law and subject to fines and prosecution, with the exception of materials which are no longer protected because of time or applications that meet the doctrine of fair use, defined as "...the fair use of a copyrighted work, including such use by reproduction for purposes such as criticism, comment, news reporting, teaching, scholarship, or research." Material that is not copyrighted is considered to be in the "public domain" and can be freely duplicated provided credit is given to the original author. In spite of the existence of the copyright law, many photographers apply additional protection to their works by using digital watermarks. Visible watermarks are translucent overlays applied to an image with the authors name and other information. Invisible watermarks are imbedded information in the file itself which can be displayed by the appropriate software.

Printing digital images

Digital imaging, computer graphics, and technological innovations in output technology have revolutionized the world of printing (65). Today, the world of printing encompasses a number of output options, including home and small office printing, film recording, service bureau printing, and commercial printing (66).

In spite of the recent emphasis on producing graphics for the World Wide Web, pathologists may need to output digital images to a physical media for patient care reports, handouts for a clinical case conference, or educational documents for students, residents, or fellows. In addition, some professional journals do not presently accept digital images, and the academic pathologist may be required to print high-resolution images for publication. The output of digital images to 35 mm slide film is also required in some circumstances because a multimedia projector is not available for every presentation.

At this time, a variety of high quality and relatively inexpensive color printers and black-and-white laser printers are available for the home and small business office, although liquid inkjet printers are the most common. High-quality ("photo-quality") inkjet printers are available for less than $400 and produce a full-color

letter-sized photo-realistic print in several minutes for about $1.00. These printers spray small "microdroplets" of liquid ink on a paper surface by a mechanical or piezoelectric process as the print head travels across and down on a sheet of paper. At each of several million points on each page, the printer can print a dot of color, print several dots on top of each other, or leave the dot blank (white). The liquid dye is stored in small, user-replaceable cartridges. In addition to the quality of dye and the number of spray nozzles, print quality is dependent on the type of paper used. Since liquid dye soaks into ordinary printer paper, high-resolution printing requires specially treated glossy paper. Most inkjet prints fade with time, although special dyes and papers were recently introduced that can last 30 years or longer. Although "desktop" inkjet printers cannot match the resolution, color accuracy, or longevity of a color printing press or photographic print, the performance/price ratio continues to increase with rapid technical innovations in the field.

Inkjet printers have a multitude of educational uses and can be used to proof documents for submission to a service bureau. The major disadvantage of personal color printers is that they reproduce a smaller range of color, or have smaller color gamuts, than color monitors, photographic film, or other types of color printers (66). Fortunately, there are several ways to optimize the printed output. Calibration of the color monitor to the printer is an absolute requirement so that the behavior of the printer can be accurately predicted from the appearance of an open file on the screen. If adjustments are needed to produce more accurate print output, the parameters of the printing program can be changed to send different instructions to the printer, or the parameters of a copy of the original file can be altered. Electronic darkroom software programs, such as Photoshop or PaintShop Pro, are usually used to print digital images, but there are a large number of supplemental software "plug-ins" which greatly expand the capability of these programs. For example, Test Strip (Vivid Details, Ojai, CA) permits multiple file parameters to be changed and printed as small segments on the same page. Once a satisfactory image has been printed, Test Strip permits the original file to be saved with embedded instructions for later printing if needed. Qimage Pro (Digital Domain Inc., Finksburg, MD) is a unique, inexpensive application for Microsoft Windows-compatible computers that provides high quality printing and a wide variety of image editing and enhancement functions. nik Sharpener Pro! (TECHnik Group, San Diego, CA) is a Photoshop plug-in that utilizes automatic image analysis and adjustment capabilities to provide optimal image sharpening for monitor display, web utilization, or printing on different types of printers.

Other types of color printers include dye sublimation (dye-sub), solid ink-jet, thermal-wax, thermo autochrome, snapshot, Fujix Pictrography (Fujifilm, Tokyo, Japan), Iris inkjet printers (Iris Graphics, Inc., Bedford, MA), and Fiery color servers (Xerox Corp., Stamford, CA). Generally, these are much more expensive than inkjet printers but are capable of extremely high resoluton. High-quality photographic prints for publication, insurance documentation, or other uses can be economically obtained from on-line service bureaus with digital photographic printers (67).

Film recorders are devices that render digital images to color or black and white film, either as a film negative or transparency at resolutions of 2,000 to 8,000 lines of resolution (66). In a film recorder, the output file is displayed on a small cathode ray tube (CRT) that is used to expose a frame of film in a line-by-line fashion. Film recorders are available in pathology departments, university multimedia support departments, and commercial slide imaging service bureaus. A 35 mm slide film is the most common output media for film recorders, although larger output film recorders are available that can image 4×5-inch film to produce poster-sized prints, or 8×10-inch overhead transparencies for presentation. To assure accurate color reproduction, the gamma of the monitor used to develop the slide must match the gamma of the film recorder's CRT. The output file must also have the proper aspect ratio and be of the proper size. If the slides include text, font compatibility between the computer of origin and the film recorder is crucial.

Image Archiving, Storage, and Management

Digital images must be readily available for later retrieval. Since image collections rapidly increase in size, advance planning is critical. The appropriate system depends on the number of images, the number of users, and the available resources. Individuals with relatively modest needs usually rely on a personal computer-based archiving system that includes image management software, two hard drives, and a CD-R/-RW or DVD-R/-RW drive. A wide variety of commercial image management software is available, including ACDSee (ACD Systems Ltd., British Columbia, Canada), Canto Cumulus (Canto Software, Inc., San Francisco, CA), Cerious Thumbsplus (Cerious Software Inc., Charlotte NC), FotoStation Pro (FotoWare AS, Oslo, Norway), iView Multimedia Pro (iView Multimedia Ltd., London, UK), Photodex CompuPic Pro (Photodex Corporation, Austin, TX), PhotoMechanic (Camera Bits, Inc., Portland, OR), Phylum (Improvision Inc., Lexington, MA), and Portfolio (Extensis, Portland, OR). These programs permit image viewing, searching, cataloging, managing,

storing, sharing, and printing images on Windows and/or Macintosh personal computers, and some also have additional functions for image manipulation, correcting, and processing. Automated backup software can be used to maintain a working copy of the primary image collection on another hard drive, while a permanent archive of the image collection is maintained on compact disks or DVDs. Online image storage and file sharing is another option, supported by a growing number of companies. Academic and commercial employees should consult their information technology specialist, since most pathology information managers now include advanced image storage and retrieval.

APPLICATIONS OF DIGITAL IMAGES IN PATHOLOGY

Pathology is an extremely visual science. Representative high quality images of gross and microscopic specimens are essential for teaching and selected areas of patient care and research. With the recent availability of high quality digital images, many of the past limitations of film photography have disappeared, and a new era in pathology has began. The means for optimally utilizing digital images in pathology are still being explored, but the possibilities are nearly endless (3,61,68,69).

Pathology Education

Digital imaging is an important tool of the pathology educator (70). Physicians, medical students, dental students, residents and fellows, medical technologists, and other health professionals are no longer restricted to using cumbersome pathology books, atlases, and slide sets but can access high quality teaching material at any time via the internet or CD-ROM (tele-education) (71,72). In addition, digital presentations are increasingly common for general medical education, since they are relatively inexpensive, easy to prepare and update, and can be immediately provided as supplemental material for a lecture or presentation. Several institutions provide large, high-quality Web-based digital atlases for medical education. Notable examples include the Internet Pathology Library for Pathology Education of Florida State University College of Medicine and the University of Utah (http://medlib.utah.edu/webpath/webpath/html), the American Society of Hematology Slide Bank (http://www.ashtimagebank.org), the Histology Atlas of the National Institutes of Health (http://histology.nih.gov/), and the Urbana Atlas of Pathology (http://www.med.uiuc.edu/pathatlast/titlepage.html) of the University of Illinois College of Medicine at Urbana-Champaign (73,74).

Although static digital educational material is readily available, the creation of highly advanced user-interactive multimedia programs for pathology education is within the reach of every pathology educator. One example of the innovative use of digital education is a Web-based tutorial on prostatic biopsy interpretation developed by Kronz and et al. (75) at the Johns Hopkins Hospital, Baltimore, MD. The tutorial consists of 20 pretutorial images for grading, 24 tutorial images, and the same 20 posttutorial images for Gleason grading. In 2.5 months, the 151 residents completed the tutorial and showed significantly improved grading in 11 of 20 images (75). Another example is an interactive web site for Continuing Medical Education (CME) developed by Landman et al. (76) at the University of Pittsburgh Medical Center. The web site features multiresolution, Flashpix-based digital images and an interface designed with Javascript, Java, and Common Gateway Interface tools to simulate microscopic analysis (76). A complete software-based multimedia pathology self-education and individual examination program for medical students and dental students at one university has been described (77). Several other examples of Web-based pathology education have been described (78,79). Conventional 35 mm film technology will gradually disappear as teaching becomes totally based on digital technology (80).

There is great interest in the use of panoramic, object-based, and 3D gross and panoramic microscopic photography in medical education. For example, Ikeda et al. (81) utilized stitched photomicrographic images of tissue sections to create widefield microscopic views of skin lesions. Object photography and QuickTime (Apple Computer, Inc., Cupertino, CA) virtual reality (QTVR) software were utilized by Nieder et al. (82) to create a photo-realistic virtual reality program on the anatomy of the human skull. A digital panorama of the heart has been described, as well as a web-based 3D reference library of pathological organs (83,84). A technique termed ''macroscopic cryosectioning'' has been described to produce digital 3D image databases for use in veterinary anatomy (85). The technique uses a modified circular saw that cuts serial cryosections at a minimal thickness of 1 mm without gaps from any tissue. The sections are imaged by a high-resolution digital camera and merged together.

A potentially important application of digital photography is the documentation and teaching of medical procedures and techniques. With the ease and relatively low cost of digital photography, photographs or video clips can easily supplement written procedure manuals and quality assurance documents (Fig. 8). This should reduce training time, increase the efficacy of the education process, and ultimately reduce medical errors.

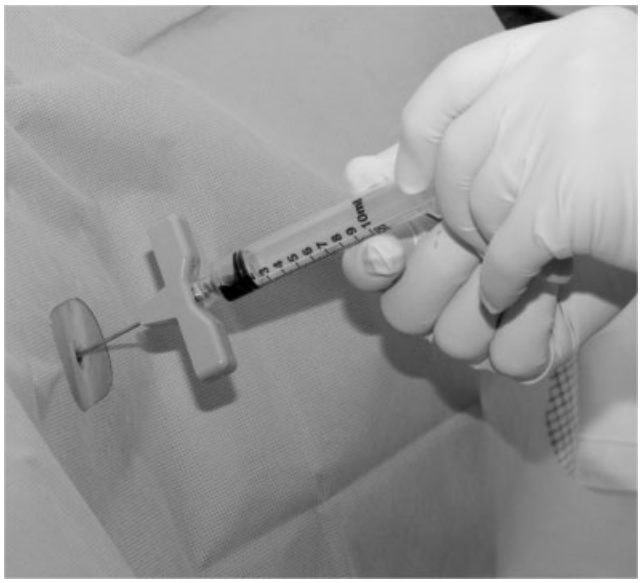


Fig. 8. Digital photograph of a bone marrow aspiration. The image is part of a online document used to train residents and fellows the technique of diagnostic bone marrow procurement.

In addition, digital cameras are used by laboratory administrators to prepare advertising and promotional material.

Patient Care

The issues encountered in using pathology images for patient care are more complex than those in pathology education, and only rudimentary progress has been made in this area. At the present, most interest in this area is concentrated on telepathology and value-added image-enhanced specimen reporting.

Telepathology

Telepathology is the sharing of microscopic images via a telecommunication device for remote primary diagnosis, expert consultation and concensus diagnosis, case conferencing, quality assurance, or education (3,6,59,86–94). The simplest form of telepathology is sending selected digital images of an interesting or perplexing case to an expert pathologist as an email attachment or to a group of colleagues or residents for educational purposes (static telepathology). At its most complex, telepathology involves interactive imaging, where data is continually transmitted, and microscope functions at the transmitting site are remotely controlled by the consultant (dynamic telepathology). Static telepathology systems are used when telecommunication resources are limited or under circumstances where it is not desired to give the remote viewer unlimited access to the specimen. Although valuable as an educational tool, static telepathology has limited usefulness in service pathology because a few selected views of a specimen are generally inadequate for diagnosis. On the other hand, dynamic telepathology systems are more expensive and require a telecommunications link with high bandwidth (>128 kbit/sec). Hybrid telepathology systems permitting simultaneous transmission of both real-time microscopy and static imagery have also been described (95).

Dynamic telepathology was first demonstrated in 1980, but was of little interest to most pathologists because it required a microwave transmission link and special hardware and software (94). Since that time, the

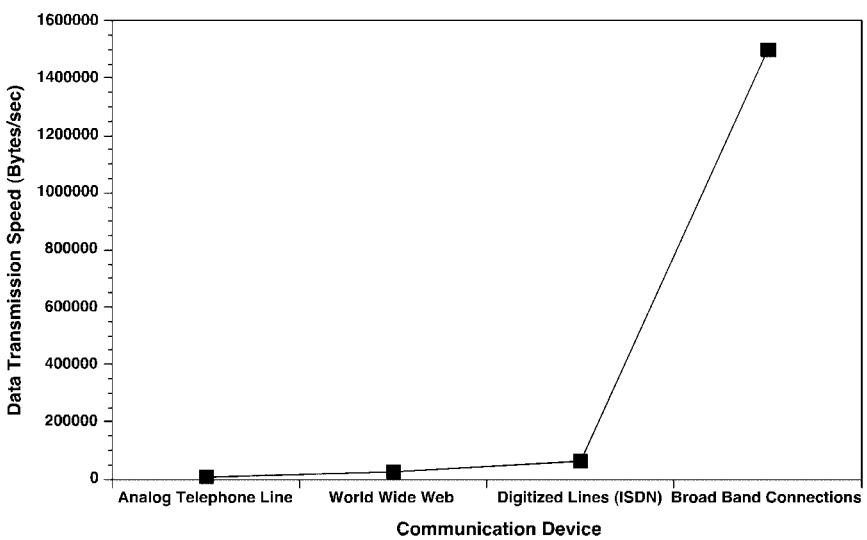


Fig. 9. Speed of data transmission (bandwidth) Telecommunication speed. analog telephone lines (9.2 kbaud), digitized lines (ISDN, 64 kbaud), broad band connections (1.5 Mbaud) and the World Wide Web (28 kbaud).

advent of the personal computer, advanced software engineering, the world wide web, high bandwidth telecommunication devices, high-resolution digital cameras, and microscopes with improved optical quality have greatly enhanced the technical aspects of telepathology (Fig. 9). As a result, several institutions in the United States and other countries have reported using dynamic telepathology for the remote diagnosis of frozen sections and surgical pathology specimens, while static image transmission is widely used for expert consultation, quality assurance, and pathology education (91,93,96–99). Unfortunately, the issues of medical licensing and credentialing, patient confidentiality, billing, insurance, malpractice liability, and other medicolegal problems have not been addressed and will limit the widespread use of telepathology in the near future (57,60,90,92,100).

The widespread adoption of telepathology may change the economics of pathology in the future. With the perfection of remote diagnosis and consultation, regionalization, or even globalization, of pathology services may occur (91). Several investigators have specifically examined the economic feasibility of telepathology. Agha, Weinstein, and Dunn compared on-site pathology at a small hospital to dynamic robotic telepathology and a conventional courier method for the diagnosis of routine and frozen section specimens at a regional medical center. They concluded that the courier method is the most economical technique at the present time, although a further reduction in the cost of telepathology pathology equipment and telecommunications will favor telepathology. Della Mea et al. (101) evaluated methods for pathologic diagnosis at a small hospital and concluded that a courier method was the most economical at a case load of 73 frozen sections per year, while telepathology was less economical at higher case loads. Time is a major economic consideration for intraoperative consultations because hospitals without an on-site pathologist must rely on a courier service for specimen transportation or travel of a pathologist from a nearby facility. Under these conditions, Battmann et al. (102) reported a time saving of approximately 45 min per case for a community hospital located 100 km from a university hospital. In this study, the time for intraoperative consultation with telepathology varied from 4 to 25 min. For intraoperative consultation, Winokur et al. (103) found no statistical difference between conventional light microscopic and telepathology diagnosis in a prospective study of 99 frozen sections.

Telepathology has been utilized for gross diagnosis as well as microscopic pathology. For example, Tennstedt et al. (104) sent static gross images of hearts from 10 autopsy cases with congenital cardiac malformations to five experts in four countries for a second opinion. Between three and seven images were sent per case in the form of Microsoft PowerPoint (Microsoft Corp., Redmond, CA) presentations. Between 1 and 2 hr per case was required for preparation of the files. The experts judged the quality of the images as very good, and a correct diagnosis was made in all cases, although the submission of additional material was required in eight cases.

Value added pathology

Value-added pathology is the enhancement of the conventional written pathology report and with supplemental data made possible through the use of computer technology (105,106). In addition to digital images, value-added enhancements include prognostic correlations, trend graphs, links to previous reports, expert review and opinion, clinical pathway information, and links to literature reports and internet sites. Although anatomic pathologists have not traditionally been concerned with information technology, the increasing competitiveness of the market, pressure from technologically sophisticated colleagues in other fields, and other factors have led them to embrace image-enhanced reports, telepathology, and other modern technological innovations to improve patient care (69). Although the integration of a few selected images into a report is of dubious value for patient care, the availability of large-scale, automated, bar-coded, multi-resolution imaging of entire slides into a networked database could dramatically alter the microscope-centered world of the surgical pathologist and greatly improve their efficiency. Essentially freed from the microscope, pathologists could simultaneously view multiple slides, compare different parts of the same slide at the same time, perform image analysis, annotate the slides with graphics or text, adjust the color, brightness, and other parameters of the slides to their preference, and perform other manipulations (69). Current and previous patient data would be immediately available for clinical consultations, patient care, educational conferences, and research applications throughout the institution without the need for the repeated archival and retrieval of glass slides. Peer review and professional consultations could be efficiently obtained, and external "send-outs" could be rapidly performed without the need for expensive recutting and transportation of glass microscope slides (69). The importance of the existing large biomedical databases and image atlases (i.e., PUBMED, On-Line Mendelian Inheritance in Man, DXplain, Pathfinder, PAPNET, NORTESS, etc.) has been elaborated (99).

Digital cameras have been utilized in the hospital autopsy suite, forensic pathology laboratory, and

surgical pathology gross room to document pathologic lesions and supplement verbal descriptions (107–109). In an academic autopsy, Belanger and et al. (110) found the digital camera to be practical, reliable, and cost-effective. With digital photography, the number of images taken increased nearly twofold per case, and the technology was readily accepted by both pathology residents and the technical staff (110). A user-friendly, high-resolution gross image capture system with report generation and telepathology capability has been described (50). Computer video capture cards have been used to obtain high-quality digital images from analog scanning electron microscopes (111).

Quality assurance and quality control

Quality assurance in pathology is presently documented by peer review of glass microscope slides. In order to decrease the extra work load associated with maintenance of the slides, Cruz et al. (112) evaluated the use of digital images for quality assurance. In their study, the referring pathologist captured up to 12 static digital images per case at an appropriate magnification that were archived for review by another pathologist. Image quality was inadequate in only 2.8% of the cases, but the amount of time required by the referring pathologist (4.5 min/case) was the major disadvantage of the study (113). McClanin (113) evaluated semiautomated imaging of patient histopathology slides with bar coded labels as a technique to reduce the error rate in pathology. The corrected report rate was reduced from 0.2% for conventional reports to 0.04% with the inclusion of images, presumably because the diagnostic errors were more obvious on proofreading and signout. The potential uses of telepathology for quality assurance and quality control in pathology was recently reviewed by Kayser et al. (114).

Digital images have not been widely utilized for proficiency testing in pathology, in spite of the potential advantages of high reproducibility, durability, and ease of duplication for mass distribution (6).

Research and Scholarly Activity

Digital photography has greatly enhanced the ability of researchers to obtain many types of research data, as well as to prepare papers for presentation or publication. The advantages of the digital camera in research are similar to other applications and include rapid and cost-effective digital acquisition and the ability to immediately preview the results. In addition, the related techniques of quantitative image analysis provide powerful new previously unavailable techniques of image analysis. For example, Bornfleth et al. (115) used a one-chip true-color CCD camera with a triple-bandpass filter for comparative genomic hybridization imaging on metaphase chromosomes through the simultaneous registration of the three dyes Texas red, FTTC, and DAPI (115). A standardized model for stain recognition and analysis using RGB data captured from immuno-double-stained tissue sections by transmitted light microscopy was developed by van Der Laak et al. (116). In this technique, RGB image data and optical density values from the tissue sections is obtained. The RGB data is transformed to a hue-saturation-intensity (HIS) color model, which is applied to the optical density values to obtain data regarding the density of each stain (hue-saturation-density transform). Digital images and automated image analysis has been used for the identification of fungal species growing on agar plates and for the automated identification of tubercle bacilli in sputum (117,118). Ernsting et al. (119) used image analysis of digital pictures of the conjunctiva to accurately predict hemoglobin concentration, while Vrolijk et al. (120) were able to detect in situ hybridization spots in interphase nuclei using centromere-specific probes with a noncooled video-rated CCD camera. Numerous other examples of the innovative use of the digital camera can be found in scientific literature.

Digital photography also facilitates the creation of scientific publications and presentations. Many journals accept digital images in file format, and inexpensive high-resolution prints can be prepared from those which do not accept files. Chen et al. (121) found that the average cost of a 5×7 inch black-and-white publication-quality print could be reduced from \$8.50 to \$1.00 with a printer, and a 30-panel, 4×8 foot (1.2×2.4 m) standard-sized poster, could be produced for approximately \$100. Frank et al. (122) evaluated the megapixel digital camera for producing publication-quality illustrations. The impact of file size on quality for the production of publication quality black and white images of histopathologic specimens was evaluated by Barker et al. (123). The authors recommended a minimum file size of 2.8 Mb for a publication-quality 5×7 inch print at $\times 100$, and a 1.7 Mb file for a similar image at $\times 460$.

THE FUTURE OF DIGITAL PHOTOGRAPHY IN PATHOLOGY

The digital revolution will continue to bring continued technical innovations and changes to the profession of pathology, as well as the other health sciences. Since pathology is a highly visual science, the advantages of the digicam over the conventional film camera have already been well documented in the classroom and conference room and, more recently, in the gross room and frozen section suite. In the near future, the digital

camera will be an essential part of every pathology gross and microscopic workstation, and further developments in the engineering of laboratory management systems will thoroughly integrate digital images into specimen reporting and quality assurance. There will be continued advances in the technology of image enhancement, and various forms of quantitative image analysis will be used routinely by the pathologist (124). In addition, globalization of pathology will lead to large network-based digital histology atlases and the rapid availability of expert assistance. Digital storage technology will continue to develop with the development of large, centrally located, professionally-maintained file servers (125). Advanced digital pattern recognition systems will ultimately be available to supplement the diagnostic abilities of the pathologist.

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