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## An overview of some professional digital cameras and their use on the light microscope

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### Introduction

With the near continuous release of new cameras, coupled with the dynamic state of affairs the industry is presently in, discussing or writing about digital cameras seems to be an almost impossible undertaking. Specifics on the topic are all but meaningless. What is much more relevant and useful, are the criteria and considerations for choosing and using high-end digital cameras. In the final analysis, imaging goals and services provided should drive such a purchase.

The majority of my work is done at the light microscope. For this reason, many of the concerns I have revolve around those encountered working with this instrument (Figure 1). Some of the obvious concerns are chip resolution, image brightness and contrast, and spectral response, just to name a few. Given the difficulty in creating high quality photomicrographs using traditional methods, problems inherent to direct digital cameras would seem to only compound the challenge. Between the time I presented this paper orally in August of 1997 and the time of this writing (December 1997), I would guess that no fewer than ten new articles on the subject of digital cameras have appeared in the contemporary photography publications. Additionally, during that same time frame, no fewer than ten new digital cameras have been released, and industry projections forecast that close to seventy new models will be released

over the next year. (Many of those, though, will be of the less expensive, lower resolution variety.) Consequently, writing this piece has become like trying to catch an elusive dream—it's right there but always moving. It is the author's view that this is the case throughout the field of electronic imaging as a whole.

### Overview and a brief history

When the abstract for the original presentation was submitted, the issues I planned to discuss were primarily those that pertained to how different

cameras functioned in general, and how they performed on a light microscope specifically. This evolved, however, to one basic and very important question. Which camera would be the best choice for a particular situation and why?

I am quite fortunate in that having been a faculty member at RIT for 11 years, many of my former students work in positions that allow me to borrow cameras for demonstration and/or evaluation. For this reason, I am able to evaluate the pro's and con's of many cameras in side-by-side comparisons. Not so long ago, when direct digital



**Figure 1**—RIT senior Alex Forsythe is shown here working with an Olympus BHS photomicroscope fitted with a Fujix HC 1000 digital camera.

cameras were "still video" technology, resolution was so poor that the immediacy of image capture had minimal value to the Biomedical Photographer. It is also important to recall that this was not really that long ago. It has been said by many that electronic photography has done in seven years what it took silver halide technology 150 years to achieve. This brings about a dilemma when thinking about buying a digital camera because there is never a right time to purchase such a powerful tool. As each month brings something new, and better, and cheaper, one needs to use their best guess as to when to "jump in." These cameras do not come cheap either with the least expensive 35mm format "professional" camera starting in the \$6,000-\$7,000 range.

As mentioned above, one of the most important considerations for acquiring this technology is not only the capability of the camera but what you want to do with it. In the end analysis, products and services should drive hardware needs. As a consequence, one's entire computer operation needs to be integrated. What output devices are available, how the pictures will be incorporated into documents, at what resolution, and how they will be stored all factor into the analysis.

### Storage and digital files

One very important factor in considering whether or not to acquire a digital camera centers around image storage and retrieval issues. Access and storage of digital files is a continuing concern for photographers. Traditional 35mm 2 × 2 color slides are usually stored in plastic pages or cases with built-in illuminators. More often though, the slides leave the department with the customer. Only a few departments then, with the possible exception of such clinical areas as ophthalmology, pathology or dermatology, are overly concerned with storage. With the ability to create filmless images, the how, the where, and the who should store the files also warrants serious consideration, but that particular aspect is beyond the scope of this paper.

High-end digital cameras generate large files, often in the range of 5 to 25 MB and even larger. An integral part of the system then is the storage

media. Additionally, the hard drive space in the computer itself needs to be considered. Not so long ago computers came packaged with 40 MB hard drives, while now a 2-5 Gigabyte hard drive is common. Similarly, storage media has also come full circle. One of the very popular original leaders in storage media was SyQuest®, a disk that was capable of saving up to 44 MB. With uncompressed 25 MB files, only one such image could be archived on this type of disk. The Bernoulli Drive®, like so many products from the very recent past, have all but disappeared. Now SyQuest sells 88 and 200 MB drives. Many other products such as the magneto optical (mo) systems that stored 128 MB find themselves coming out with larger capacity storage abilities in the neighborhood of 650 MB. An extremely popular new storage media is the Zip Drive which will hold 100 MB while the Jaz drive will hold 1 GB. The Zip drive which sells now for less than \$100 is an excellent choice because of its relative low cost. Writable CD's are another very useful product for archiving and will hold approximately 650 MB of information.

### The professional digital camera

The Digital Rev(s)olution is obviously here and becomes more apparent every day with increased resolution, overall quality of the pictures, and the capabilities of the computer to work on, and enhance the images. As a result, the serious photomicrographer who has a need for direct digital cameras has countless choices to look at and numerous issues to weigh before making a decision. Cost, speed, studio or portable and many other more subtle factors will be part of the decision making. High-end cameras can be categorized into three groups. The first includes cameras that work like scanners and need to be driven by a computer. The second are instantaneous capture cameras, and the third is multiple exposure instant capture. I have found that each group has advantages and disadvantages based on their intended use.

#### Linear array cameras

Scanning cameras produce the highest resolution of the three groups, and work similar to a film or flatbed

scanner. The camera is comprised of a single row of image sensors that form its CCD, or charged coupled device. The sensor moves, using a very fine stepping motor, across the image area from one side of the frame to the other. There are a number of cameras that work like this. Two of these are the Dicommed Studio Pro, Phase One and the Leaf MicroLumina. The backs on this type camera are all slightly different. Some fit onto a 4 × 5 camera similar to the way a cut film holder does, while others have a bayonet mount in the front that accepts a variety of lenses. The individual differences can easily be learned by referring to any distributor of digital products. Since the camera incorporates a single row of pixels over a fixed length (width), the camera is capable of very high resolution. The Leaf MicroLumina for example, has an array of 2700 pixels over a travel distance that comprises 3400 pixel points (length) which produces an image of 9 million picture elements and a file size of 26 MB. Additionally most of these cameras produce minimum 36-bit depth images. Unfortunately, Photo-shop 4.0 still only acquires 24-bit images. As a result, some of the camera's inherent high detail capability is lost even before it has ever been used.

Scanning cameras are typically slow in capturing images. For example, a hypothetical situation at a light microscope may require a  $\frac{1}{15}$  second exposure using Kodak EPY film. That same exposure might take 8-10 minutes using a scanning type camera. Because the sensor is moving, this type of camera cannot record moving subjects. It also needs to be tethered to a computer with a big hard drive and a minimum of 32 MB of RAM. One new studio camera requires a machine with a minimum of 256 MB of RAM. These cameras are low in sensitivity (response) and require high brightness levels. Electronic flash cannot be used because of its short duration while some tungsten filaments will experience "flicker" during the course of a long exposure. As a result, studios utilize HMI lights with this type camera because there is no flicker to cause different brightnesses as the sensor moves across the frame. The camera is solely a light sensor with the camera controls in the computer as a software interface. Camera access can often be

found as a Photoshop Plug in. These cameras almost universally have a pre-scan for initial set of the sensor based on the subject's requirements and the lighting. Image contrast can be adjusted, scan speed adjustments can be made, and many other features such as color response adjustments are available. Some of the cameras allow fine focusing during the prescan by having NTSC out that is concurrent with the still digital signal. The image sensors also respond best to flat lighting. Ratios that would be similar for transparency film or less seem to be ideal for CCD's.

This type of camera provided exceptional results at the microscope. Obtaining critical exposure was very important. With too much exposure, the sensor caused blooming, or unwanted exposure created in adjacent picture areas. This was evidenced as a color glow (exposure streaks) into adjacent shadow areas. As a result, making pre-scans that created a brightness value below 230 in the clearfield helped resolve this as long as the sample had adequate contrast. Flat subjects are especially problematic regardless of capture medium. With the scan speed determined, a white point could be set and the image corrected by the software. Another interesting discovery was that if the exposures were too short, the camera sensor often moved across the image field so quickly that vibration became a problem. Vibration in this type of camera is observed as bands or density differences across the width of the image. In the clearfield areas of a light microscope, this banding is very evident as density changes. At very low magnifications, where achieving Kohler illumination is difficult regardless of image capture method, the cameras produced obvious localized exposure differences as a result of the lamp filament. This was recorded as different pixel values as a result of the sensors being very responsive to localized brightness differences. The pixels enhance edges naturally so through the use of two diffusion filters, the lack of uniformity in the background was removed.

Accurate color reproduction seemed initially challenging but proved to be very accessible. The sensors from different cameras, like slide films, have different color responses. It

was interesting that the most accurate color was often achieved using lamps at maximum light intensity (approximately 3400°K). Neutral density filters should be used to reduce the intensity of the lamps to a normal working level. It was also my experience that in a situation that required an exposure longer than 1/4 second on film with 100 speed film, the digital camera (chip) would fail.

#### Area Array cameras

The majority of cameras on the market seem to fall into two large groups, scanning and area array. Probably the largest group of cameras on the market are the area array type. Area array cameras, like film cameras, see the entire image simultaneously. Consequently all the camera controls found on a traditional film camera are the same on the digital camera. These cameras are available from many camera manufacturers such as Kodak, Nikon, Fuji, Canon and Minolta, just to name a few. So from the front, all the digital cameras look and operate the same as any film camera such as the Nikon N-90. As with film cameras, spot versus center weighted metering is just one of the many functions that are available. Behind the lens, though, is where the significant changes occur. A Charged Couple Device (CCD) is located where the pressure plate is in a conventional camera. As a sensor, it has a vertical and horizontal pixel array instead of film. The physical dimensions of the chip determine its resolution. A small chip might be 640 × 460 pixels while the highest resolution chip on the market boasts an image resolution of 2K × 3K. Many intermediate resolution cameras have an approximate chip size of 1K × 1.5K. Chips also vary in sensitivity. Most cameras seem to exhibit sensitivities (ISO potential) of 80 at the low end to 1600 as currently having the greatest response. The sensitivity for most cameras can be changed or is considered variable based on lighting requirements. As the ISO is increased, less quality is noticeable, even though proper exposure is achieved. This loss in quality is described as noise. Typically the higher the signal to noise ratio, the poorer the result. Conversely, when the ISO is lowered, the results are more superior.

Because chips have a physical di-

mension, digital cameras often see less of the field than do film cameras. As a result, the focal length of a lens used on a traditional film camera will not be identical to the lens used on its electronic counterpart. In order to capture what a 50mm lens sees on the Nikon N-90S with film, the Kodak DCS 420mm must use a 20mm lens. As a very general rule of thumb, the chips require one-half the focal length of the normal lens of a film camera that would be used with 35mm film. On a microscope, this often means using lower power objectives than one would ordinarily select. A field that can be easily observed with a 10× objective would now require a 4× objective to image the same field of view.

The CCD can only respond to differences in brightness not color. When using film, one can choose from many emulsions based on project requirements, CCD's however, must be modified to respond to color. As a consequence, color separation strategies are often required to create color vision. This is also true for scanning type detectors. One solution is to place a Red, Green and Blue filter over the sensor in a linear fashion. A long red filter, a long green filter and a long blue filter, typically 7µm in width, are run the length of the sensor. This divides the surface area into three equal areas that each render a different part of the spectrum. The computer software can then reprocess the signal and create a credible color display.

With area array cameras, the solution to the color vision problems varies, and as a result of proprietary information there is often a lack of specifics in this area of discussion. The Kodak cameras, for example, use colored filters on each pixel. So over the entire chip, each pixel is covered by either a red, green, blue or, in some cases, a subtractive filter such as cyan. Through the use of software interpolation, the image is written and color created from image processing. These cameras allow photographs to be made anywhere at anytime using a variety of light sources but will not operate at exposures longer than one second. This is because the chip gets too warm and fails to respond accurately, and sometimes the exposure is actually terminated.

Because the color is the result of

software interpolation, digital artifacts are sometimes produced. These color artifacts are often referred to as aliasing and are frequently observed in linear subjects that have high frequency patterns that span the length of several pixels. Aliasing is most evidenced in the medium resolution cameras and is easily correctable in Photoshop. The highest resolution camera on the market, the Kodak DCS 460, does not exhibit this aliasing. (Linear array cameras do not have this problem). Various options in Photoshop 4.0, specifically in Image and Color control commands can be used to change the radius and threshold of the pixel display all but eliminating the digital noise.

Another method uses three CCD's to create color. The image produced by the lens is separated by a prism and relayed through Red, Green and Blue filters to its own sensor. One camera that operates in this fashion is the Minolta RD-175. Each sensor is smaller than the chip as a whole, but the results are quite acceptable. As a basic response, the chip of the Minolta camera has an ISO of 800 which was very important when working in low light situations such as darkfield, fluorescence microscopy, and ophthalmic work.

Most CCD's are inherently sensitive to Infrared radiation, so using a "hot mirror filter" or IR cut-off filter is desirable, especially when using tungsten lights. The high response of the chip to IR energy often causes an unusual color response. When Kodak IR film was discussed in the older Kodak publications, a term false color was often used. Through the use of CCD's, false color is also possible without using an infrared cutoff filter.

The Fujix HC 2000 is a very interesting area array camera that differs from other area array cameras. The Fujix 2000 is a studio area array camera that has the capability of producing both a video signal and digital output. This feature allows for precise composition, focusing and exposure determination directly from a second monitor, (one in addition to a computer workstation). This camera created images with file sizes of around 5 MB. It was excellent for use at the microscope, on a gross specimen stand and even for portraits. The camera is tethered to a computer so it is not portable.

It requires an ENG mount for adapting to a microscope while any small video lens mount worked for studio applications. The ENG mount needed to be par focalized to the eyepieces but this was not difficult to accomplish.

### PCMCIA Cards

Images captured with most area array cameras are written to small removable hard drives sometimes referred to as film cards in the amateur market. These removable hard drives are also called PCMCIA cards in professional circles and are available in variable storage capacities. Cards are classified by their physical size as either Type 1, 2 or 3. Often the Type 3 card is the largest and has the greatest storage capacity. The storage capability of these cards has been growing steadily since the introduction of these products. The Kodak DCS 460, for instance, is often sold with at least a 230 MB card. This information is readily available from any supplier of such cameras. It has been suggested as a rule of thumb that these cards sell for \$100 per 5 MB of storage.

Images are captured and then written to these miniature hard drives for storage and retrieval. Once the camera's shutter has been activated, the camera's hard drive is also activated. The term "waking up the hard drive" has evolved to describe this process. Once activated, the camera records the image and then writes it to the drive. In most cameras, an LED or some other indicator lets you know that the drive is writing. Some cameras respond faster than others. For this reason, shooting events that have the potential for pictures that might be needed at a moments notice, in the field of photojournalism for instance, require the photographer to continually keep the camera awake (ready). Additionally, each camera differs in its capability to hold several intermediate images before writing to the drive. Each camera, therefore, will have a different burst rate or ability to record more than one image at any time. The lower end cameras can only perform one function at a time—activate, capture, then write, while the higher end cameras designed specifically for photojournalistic work, might be able to record up to five images before needing to write. Cards are removable from the

camera but should never be removed while they are writing. Removing a card during writing will permanently damage it.

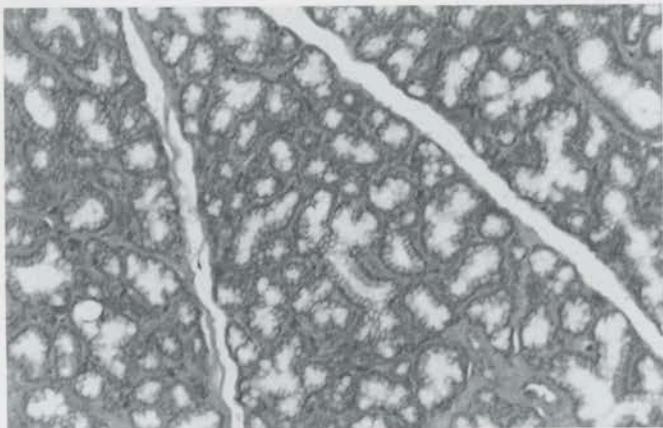
After shooting, images from area array cameras will need to be moved from the camera/card to your workstation. This can be accomplished in several ways. The camera itself can be tethered to the computer with a SCSI cable or the card can be ejected from the camera and the file accessed through the use of a card reader. A card reader is nothing more than a very small drive designed specifically for PCMCIA cards. These products vary in price and drives can be addressed through the Photoshop Plug in for that particular camera brand. Historically, each camera had its own file format which has proven to be problematic. Formats such as DCS, HD, LDR MDR and others are a few of the many proprietary formats that have caused problems for users thus far. Recently though, manufacturers have been releasing cameras that save and export JPEG or TIFF image file formats which has greatly simplified working at various locations in the digital image chain.

Here are a few of the cameras I have worked with:

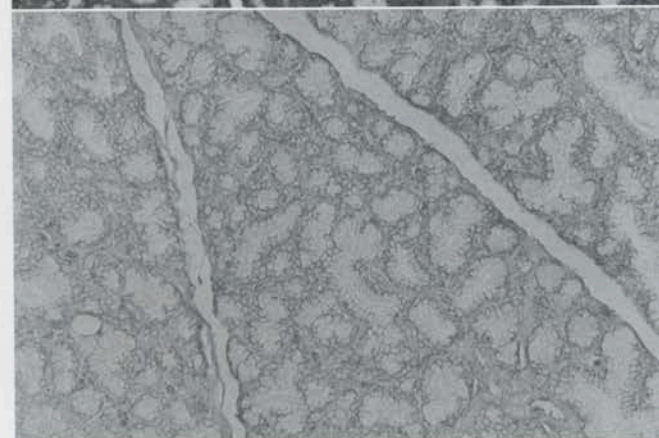
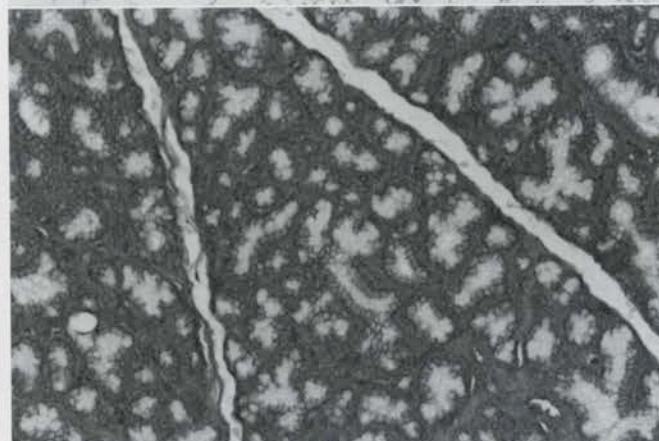
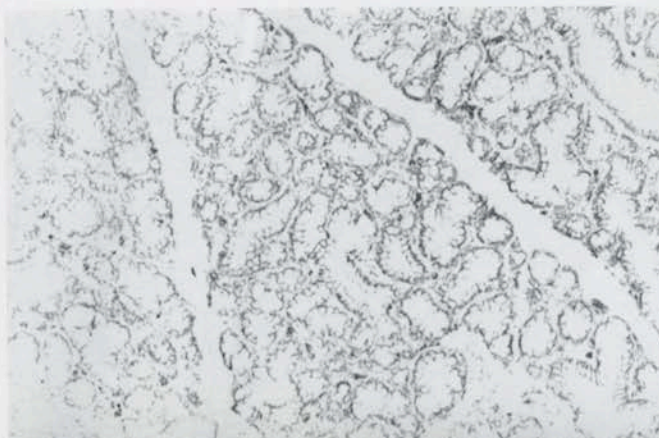
#### **KODAK DCS 460** (Figures 2, 3, and 4)

- Nikon N90 body
- area array chip
- 2K by 3K pixels
- PCMCIA variable storage film card
- Portable or Tethered
- Chip speed ISO 80
- Camera/Images accessed through Photoshop Plug in

This camera accepts all Nikon lenses and is capable as well of being coupled to the Nikon Bayonet mounts found on the Nikon Eclipse or older OptiPhot microscopes. The camera does not have a mirror lock up for use with higher magnification objectives where the potential for vibration is greatest. The camera produces 18.4 MB DCS files that have 36-bit color depth. Additionally because of the chip's inherent red sensitivity, a Tiffen Hot filter (IR removal) is recommended for use with tungsten illumination. It is also recommended that an 80A filter be used in the system to provide a 5500K illuminant and import images using the day-



**Figure 2**—Digital cameras may be prone to many of the same problems as film cameras. This photomicrograph of a tooth exhibits vibration from the mirror.



**Figure 3**—These photomicrographs of a tooth cross section demonstrate the different tonal response of the Kodak DCS 460 camera when using tungsten illumination alone (bottom) and with an 80A filter (top). Both photographs were made using the Tiffen IR filter as well.

light option. So as to not lose valuable data, images were saved as JPEG medium files reducing them to approximately 1mB. This camera is less prone to image aliasing than the DCS 420. The chip is nearly equal to 35mm film in physical size so the viewfinder is close to accurate. The use of a di-

dymium filter enhanced the way H & E samples were recorded by the chip as might be expected.

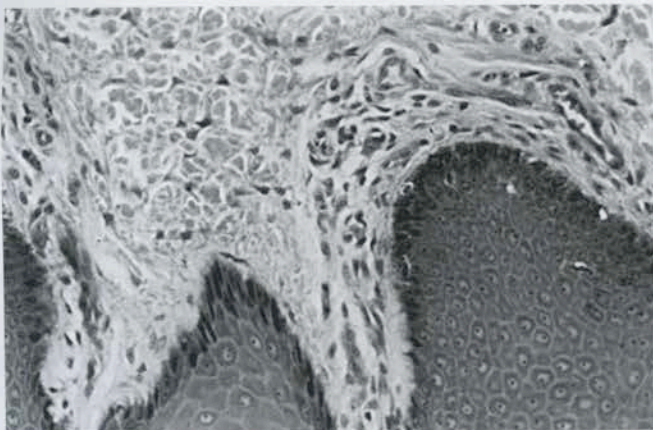
**Kodak DCS 420 (Figure 5)**

- Nikon N90 body
- area array chip
- 1K by 1.5K pixels

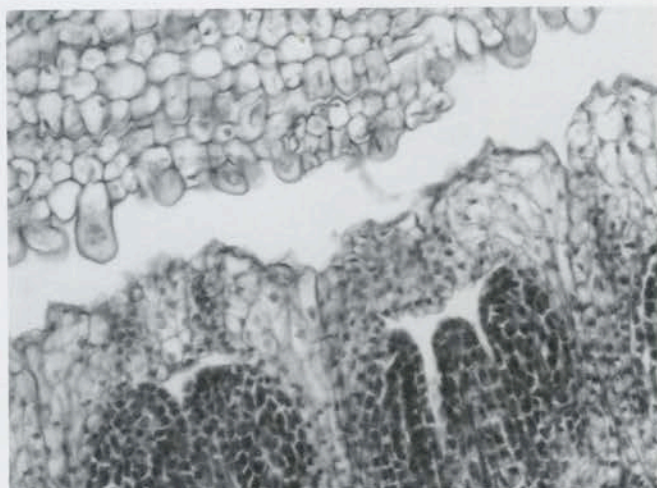
- Variable storage film card
- Portable or Tethered
- Chip Speed ISO 100, variable 200 and 400
- Camera/Images accessed through Photoshop Plug in

This camera operates similarly to the DCS 460 in coupling to lenses or micro-

**Figure 4**—Tooth cross section in Figure 3 showing the Kodak DCS 460 camera's color response as seen by each of its RGB channels. Notice the red (top) channel's response as compared to other channels. The overexposure of the red channel can cause problems when performing such manipulations as sharpening.



**Figure 5**—This photomicrograph of cornified human skin revealing the germinative layer was taken with Kodak DCS 420 camera using a Tiffen IR filter.



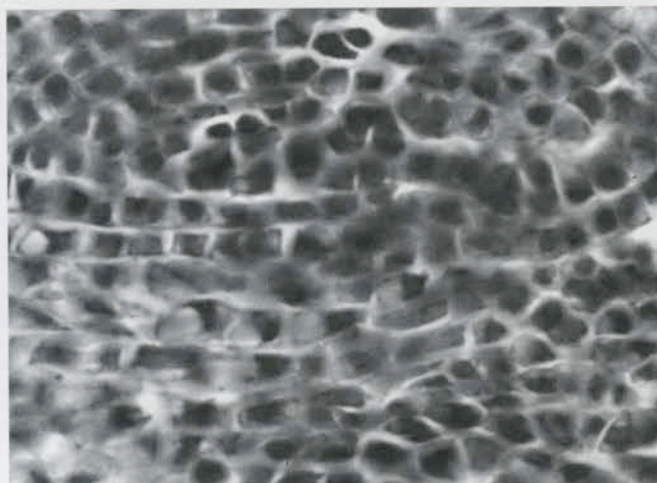
**Figure 6**—This photomicrograph of a dandelion cross section stained with fast green and Safrinin was taken with the Minolta RD-175 without an IR filter.

scopes. The Nikon N 90 camera does not have mirror lock up. The camera produces a 5.4 MB DCS file that has 24-bit color depth. Additionally, because of the chip's inherent red sensitivity, a Tiffen Hot filter is also recommended for use in this application or other situations where tungsten is the illuminant. It is also recommended that an 80A filter be used and that you import images using the daylight option. This camera is sometimes prone to some aliasing as a result of the filter and color vision strategies used. The viewfinder has a black line to define what is captured on the chip which is approximately one half of what the viewfinder sees.

#### **Minolta RD 175** (Figures 6 and 7)

- area array 3 CCD camera
- 1K by 1.3K pixels
- Variable storage film card
- Portable or Tethered
- Camera/Images accessed through Photoshop Plug in
- Chip Speed ISO up to 800 and variable

This camera is very interesting in that it uses three chips rather than the filter over the pixels approach used in other brands. As a result there is no aliasing. With a higher ISO potential, this camera worked well in low light situations such as darkfield as well as in normal brightfield applications with neutral density filters. The images produced were slightly lower in resolution than with the Kodak DCS 420 camera but appeared equally as "crisp." The camera has a fresnel lens for focusing in the viewfinder which made focusing low contrast subjects difficult, though



**Figure 7**—This photomicrograph of a dandelion cross section stained with fast green and Safrinin was taken with the Minolta RD-175 without an IR filter.

achievable with some care. Under higher magnifications with more shallow depth of field, focusing was even more difficult. The viewfinder represented the full image capture area with no cropping of what the chip actually sees. The images can be acquired through a Photoshop Plug in and is simple to accomplish, although this process is not instant by any means. This camera produces 5 MB images.

#### **Leaf MicroLumina** (Figure 8)

- Studio camera/scan back technology
- Tri-linear array
- 2200K by 3800K pixels
- Requires computer interface
- Images and camera accessed through Photoshop Plug in

- Chip speed Sensitivity (ISO) low
- Many camera software controls available such as White and Black point setting

This camera accepts all Nikon lenses and is capable of coupling to the Nikon Bayonet mounts found on the Nikon Eclipse or older OptiPhot microscopes. This is a linear area camera so capturing an image at full resolution is slow but very good. The camera functions as only a detector with all the controls located as a Photoshop Plug in. There is a control to change the response curve to handle subjects with challenging contrast. The camera produces full resolution 18mB files that are easily imported into Photoshop. One interesting feature is the camera's ability to

be set up by the manufacturer to record IR. As with other linear array cameras, if the scanning speed is too fast the motor can cause image vibrations, so slower scan speeds are desirable. A didymium filter enhanced the way this camera recorded H & E stained specimens. Low light situations such as those associated with fluorescence could not be photographed with this camera.

**Fujix HC-1000** (Figure 9)

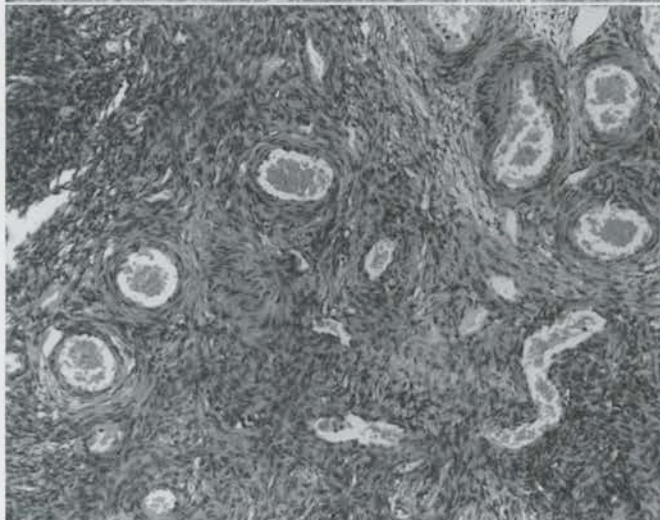
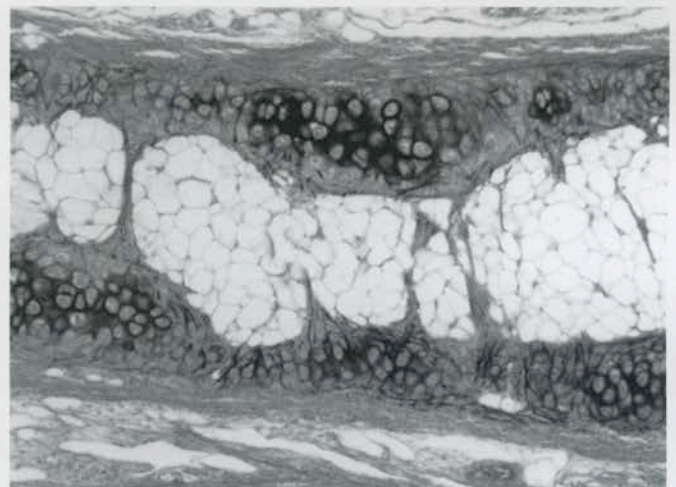
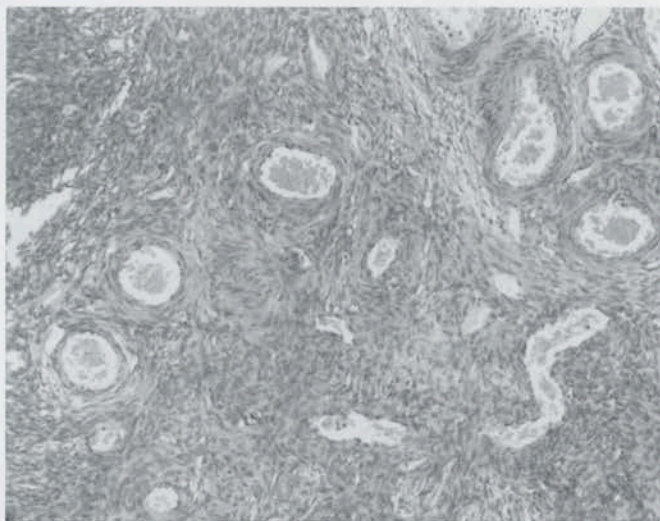
- 3 chip CCD area pixel array RGB camera
- Requires computer interface and video monitor for NTSC preview signal used for composition and focusing
- Tethered studio camera
- Images and camera accessed through Photoshop Plug in

- Many camera software controls available such as White and Black point setting, focusing and other camera functions can be controlled from the workstation as well

This is a very easy to use camera as a function primarily of the video signal generated along with the still digital image. Precise exposure, framing and other critical adjustments can be made on screen prior to capture. The camera's interface allows for white point as well as black point settings—a nice feature for challenging subjects that require altered contrast treatments. An 80A filter is required because the chip is calibrated to a daylight response. The images produced were around 5 MB before saving and can be exported directly into PhotoShop in the acquire mode. Because there is no

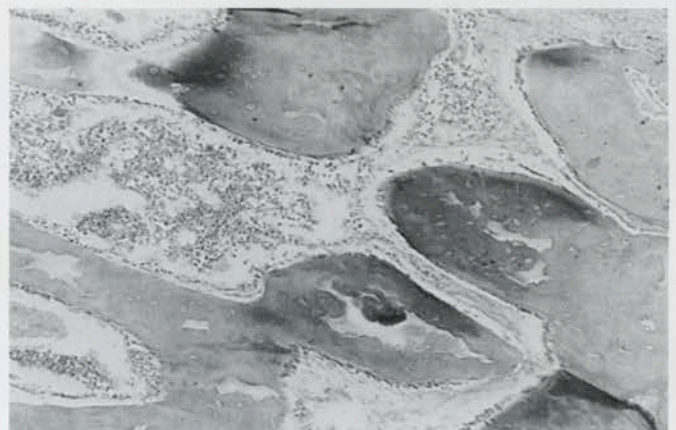
mirror, there is no problem with vibration and no aliasing was evidenced. The camera, although not portable, has potentials well beyond the microscope such as studio work.

There are obviously countless other cameras on the market, each having their own distinct advantages and disadvantages based on applications. I have also worked with, but not extensively, with other cameras such as the Leaf DCB, the Canon Kodak EOS, the Kodak DCS 410, as well as the Phase One and Dicomed scanning backs. Recently, I was most fortunate to work with the Kodak DCS 520 before its release (Figure 10). As with film cameras, it would be next to impossible to personally use all the cameras available on the market today.



**Figure 8**—These photomicrographs demonstrate the tonal response of the Leaf MicroLumina digital camera. Specimen is an ovary cross section stained with H & E photographed with (bottom) and without (top) a didymium filter.

**Figure 9**—This photomicrograph of elastic cartilage was taken with the Fujix HC-1000 digital camera with an 80A filter.



**Figure 10**—Fetal bone stained with Tri-Chrome taken with Kodak DCS 520 camera with a built-in IR filter.



## Conclusion

As with all new technologies, there will always be waves of acceptance and the field of Biocommunications is clearly in a phase in which the majority of photographers are, or will be adopting digital systems very soon. Digital cameras will continue to be produced more cheaply and deliver higher resolution. For now, the digital camera market will not be stable, as new technology, such as active pixel c-moss, begins to change the way chips are manufactured and integrated into cameras. The comment made some years ago by a participant at a RIT symposium still holds much truth, "the only thing for certain that we can say

about the future is that there will be change." The advantages of using digital cameras in certain applications is becoming quite evident, and it is certainly easy to project many more without too much trouble. Prior to purchase, adequate research is essential to evaluate the many options available. Additionally long term goals should be considered in setting a direction away from film with an integrated approach. Buying a digital camera will cause consumable expenses to go down, but the initial hardware investment will be high. The life expectancy of such an investment is also a factor. Remember when you realized ten years use from say a Nikon F-3 pur-

chase? Well the acquisition of a digital camera and computer system will never achieve that life expectancy. Welcome to the age of rapid change. What started out as simple curiosity on my part, blossomed into a long and complicated project. With the cameras that are available today, producing digital photomicrographs is no longer a technique that is "not quite there."

**About the author:** Michael Peres is a Registered Biological Photographer and a Fellow of the BCA. He is an Associate Professor and Chairman of the Biomedical Photographic Communications Program at the Rochester Institute of Technology.