4 Photography

Norman J. Barker, M.S., R.B.P.

Quality gross specimen photographs are an essential part of surgical pathology. Aesthetically pleasing 35-mm color slides, black-and-white prints, and digital images are used not only to document the diagnosis but also for conferences, presentations, teaching, and publications. Unfortunately, photographs are often not taken; or if they are taken, they are not useful because of underexposure, overexposure, inappropriate lighting, poor selection of background, or blood-stained or blood-smeared backgrounds. Fortunately, with care and a standardized system, you can produce consistent high-quality photographs. This chapter first describes how to set up a standard photographic system and then describes how to photograph specimens and trouble shoot a variety of problems.

Setting Up a Photographic System

Photographic Stand

A variety of camera stands are on the market. Pick one with a sturdy column. Sturdy columns eliminate camera vibration, which causes photographs to be out of focus. Probably the most versatile and easy to use system on the market is the Polaroid MP4, which consists of a heavy-duty stand, a 4 × 5-inch camera, and permanently mounted but adjustable lighting (Fig. 4-A). It also has an adapter for a 35-mm camera. With this system, Polaroid 4 × 5-inch black-and-white negative film can be used to produce an instant print and negative. The print serves as an instant record, documenting the size and condition of the specimen, and notes can be made directly on the print, providing a visual correlate of the gross description. The negative can be filed and prints for publication made from it at a future date.

Lens Selection

Most major camera manufacturers offer a choice of two types of macro lenses. The 60-mm macro lens is sufficient for 95% of routine work in the surgical pathology laboratory. The 105-mm macro lens allows for more working distance between the specimen and the front of the lens. This feature is helpful when doing close-up work. Both of these macro lenses are extremely useful because they can focus down to a point where the image on the negative is the same size as the specimen, and they will cover specimens ranging in size from a large colon down to small polyps. One of the scales that is not present on most ordinary lenses, but that is printed on macro lenses, is the reproduction ratio. You should be familiar with this scale because, as discussed later, it can be used to determine the appropriate exposure for a specimen (Fig. 4-B).

Background Selection

Before lighting the subject, background selection must be considered. The correct background will enhance and highlight the subject to be photographed. A background table can be easily made or commercially purchased. The latter is a good choice but is only good for medium to small specimens. The Aristo Box DA-17 (Fig. 4-C) has a cool rectangular, fluorescent light inside. The

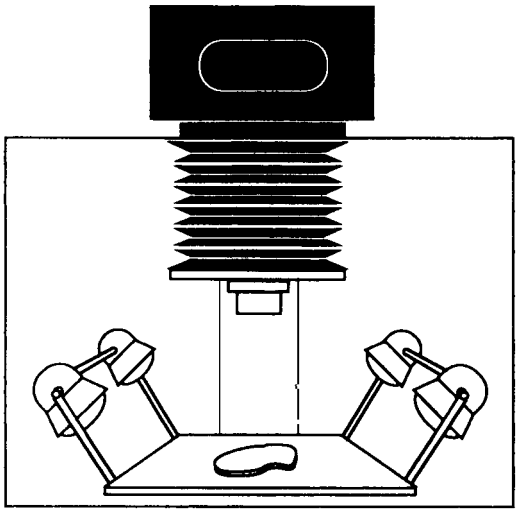

Fig. 4-A. The Polaroid MP4 system.

Film T-64 60-mm macro lens		
f-Stop	Time (s)	Reproduction Ratio
22 ½	¼	1 : 10
22	¼	1 : 7
16 ½	¼	1 : 3
16	¼	1 : 1

Fig. 4-B. Example of a gross stand exposure chart. Create a chart such as this one to calculate the f-stop when doing close-up work. The reproduction ratio value is printed on most lenses.

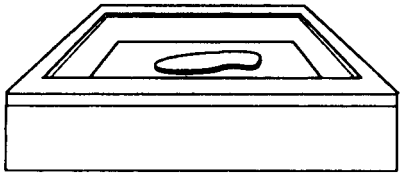

Fig. 4-C. Aristo DA-17 light box.

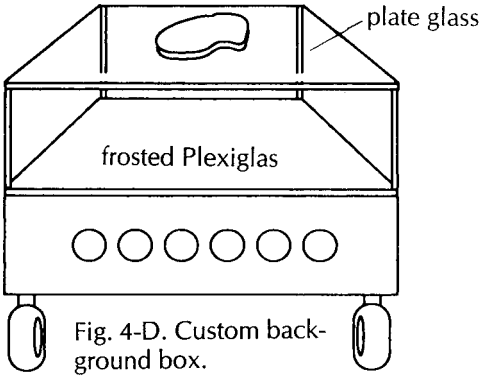

Fig. 4-D. Custom background box.

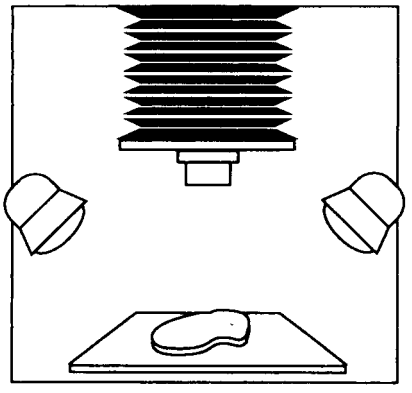

Fig. 4-E. Standard flat copy lighting.

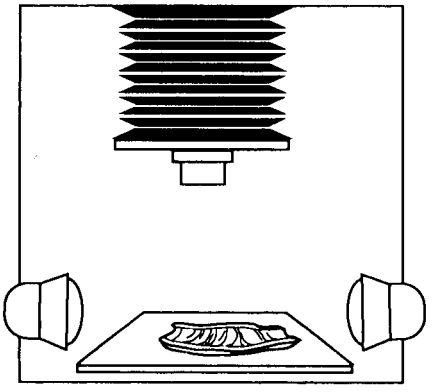

Fig. 4-F. Side lighting—texture.

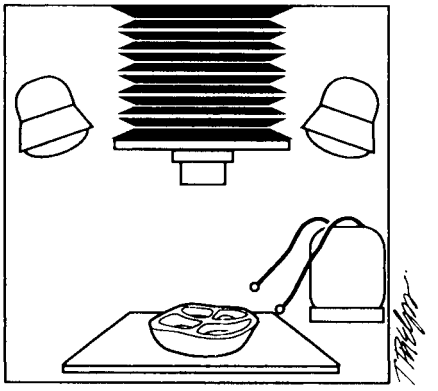

Fig. 4-G. Fiber-optic lighting—cavity.

box gives a flat shadowless illumination or, if turned upside down, a black background.

A more versatile system (Fig. 4-D) can be custom-made to fit space requirements. A custom background box should have even fluorescent lighting below a glass surface on which specimens can be placed. Immediately on top of the fluorescent lights is a place for opal glass or Plexiglas, which diffuses the light for an even background. Colored gels can then be placed on top of the Plexiglas for a variety of background selections. There is then a space of about 6 to 8 inches between the background and the specimen glass. This system eliminates shadows on the background.

If colored gels are used for backgrounds, great care must be taken. An ill-chosen background color can affect the color of the specimen in the photograph. For many specimens, red, yellow, and green are colors to avoid. Shooting an actual test photograph is the only way to tell for sure. One very simple type of background is a piece of black velvet available in fabric shops. This material will absorb all light that falls on it, yielding a pure black background. Therefore no shadows from the specimen will be seen. A solid black background also has the advantage of hiding liquids that have seeped out from a specimen. This makes a cleaner presentation in the final photograph.

Lighting

Important features of a specimen may be hidden when the lighting is too dim or obscured when the lighting is too bright. Appropriate lighting, on the other hand, will actually enhance the photographic demonstration of these findings. One of the many advantages of the Polaroid MP4 stand is the fact that the lights may be easily moved to highlight the features of a particular specimen. Lights placed in the standard 45° position (standard copy lighting) provide an even, flat illumination for the majority of specimens (Fig. 4-E).

The standard 45° positioning of the light source may not be optimal for every specimen. For example, when three-dimensional information is most important, the lights should be positioned at a lower angle (Fig. 4-F). This will provide shadows that will help suggest a relief or distinguish a form from its background. This positioning of the light source can help show texture as well. Another technique is to unplug the lights on one side so that a stronger contrast can be seen. The main thing to keep in mind is that lighting should not obscure information.

A good addition to a lighting system is a fiber-optic light source (Fig. 4-G). Manipulable fiber optic wands provide an excellent source of illumination when close-ups are required. They are particularly useful in illuminating cavities and crevices. When used in combination with other light sources, fiber-optic lights are also effective in ''filling in'' otherwise darkened areas. Keep in mind that when lighting changes are made, some changes in exposure may also be necessary.

Thirty-Five–Millimeter Slides

Not only can 35-mm slides be used for projection at conferences, they can be used for publication as well. The slides also can be scanned into a computer, and letters, numbers, or arrows may be added. Other slides can be scanned and combined together, such as a gross and microscopic view on one slide. A 35-mm slide may be converted to a black-and-white print for publication.

Color slides are best obtained with a 35-mm single-lens-reflex camera with through-the-lens light metering. The best results are achieved with fine-grain films. A film should be selected for accurate color rendition, and it should match the Kelvin temperature of the lights used. Most gross specimen photography is done with tungsten halogen lamps (3200 K) as opposed to an electronic flash (5500 K). Unlike the transient illumination of the electronic flash, tungsten lamps provide constant illumination so that the lighting can be critically evaluated before the photograph is taken. Kodak Ektachrome tungsten EPY-64 is a good choice of film. This film is balanced for tungsten lamps, it has a high resolution, tends to match the true specimen colors, and is a user-processed film. (User-processed film can be processed at most hospital in-house photographic departments, usually on a same-day basis.) Films such as Kodachrome, on the other hand, tend to enhance red and yellow colors and must be sent to outside laboratories for processing.

Standardized Exposure Determination

The amount of light that reaches the film or digital camera can be controlled in two ways. One is

to change the aperture. The *aperture* refers to the diameter of the lens diaphragm, and this can be adjusted by changing the f-stop. Each f-stop setting changes the amount of light passing through the lens by a factor of 2. The smaller the f-stop number, the larger the aperture and the more open the lens. For example, an f-stop of 2 will let more light into the camera than an f-stop of 22. The aperture also controls the *depth of field*, which refers to the zone of sharpness in front of and behind the point of focus. With large apertures (smaller f-stop numbers), everything outside the plane of focus abruptly becomes blurred. At very small apertures (such as an f-stop of 22), objects remain in focus over a greater depth of field. Therefore, optimal clarity is best achieved using a smaller aperture (higher f-stop).

The amount of light hitting the film is also controlled by the shutter speed. The *shutter speed* refers to the length of time that the lens is open. Each shutter speed, like the f-stop, doubles or halves the exposure at the next setting. Standard speeds on modern shutters are 1, 1/2, 1/4, 1/8, 1/15, 1/30, 1/60, 1/125 second, etc. Keep in mind that both the aperture and shutter control the amount of light falling on the film.

You need to identify the best f-stops and shutter speeds for your particular system by running a series of exposure tests for each different specimen magnification. To do this, you need a light meter. An incident light meter works best because it measures the light falling on the specimen. The reflected light meters that are found in most cameras can be ''fooled'' and give incorrect readings because of the light coming from the background. For example, reflected light meters may read too much of the background and not enough of the specimen when a lightly colored specimen on a black background is being photographed. Once the exposure is approximated with the hand-held incident light meter, a series of test exposures should be made at different magnifications (reproduction ratios). Most manufactured lenses have reproduction ratios listed on the lens barrel for each magnification. In making this test run, choose a standard exposure such as 1/4 second, and then vary the aperture of the camera by one half of an f-stop at each magnification. This exposure time will enable you to use small apertures, which result in a better depth of field and translate into better specimen focus. The best aperture then can be determined for a specific image magnification, and a chart can be made listing the correct aperture that matches each magnification (Fig. 4-B). While this system is easy to use, it may be necessary to modify the exposure for very dark blood-red specimens or very light white specimens.

Scale

While many find scales and labels unnecessary distracters in an otherwise aesthetic photograph, at least one of the specimen photographs should include a scale along with the specimen identification number. A scale helps the viewer orient the specimen and provides a benchmark for the perception of size, while specimen labeling ensures that the photographs will not be lost or misidentified later. Commercially prepared plastic rulers are available that can be made into various sizes to accommodate different specimens. Small adhesive labels with the specimen identification number can be attached directly to the ruler. When placing the scale in a photograph, be sure to place it in the anatomical inferior position. The scale should be positioned at the level of the specimen so that it is in plane of focus.

Digital Photography

When it comes to photographing gross specimens, digital images offer several advantages over conventional 35-mm photography. First and foremost is the ease with which an image, once captured in digital format, can be edited, organized, catalogued, and stored. Second, a digital system permits immediate review of the image captured. Most digital cameras have a video ''out'' port that allows for the image to be captured and displayed on a video monitor in real time. Some digital cameras are also equipped with small screens for reviewing the image captured. Third, digital imaging is cost-effective. For laboratories that routinely use photography as a component of their gross dissections, digital image photography can reduce film and processing costs.

Digital imaging technology is advancing at breakneck speed. Each month seems to bring an updated digital camera that is less costly and of higher quality than the previous model. There are literally hundreds of digital cameras now on the market, and choosing the best one is a formidable task. When selecting a digital camera, one

of the most important features to keep in mind is the resolution of the image sensor. Resolution has to do with the ability to appreciate fine detail in an image. Digital photographs are made up of thousands to millions of picture elements known as pixels, and the quality of an image depends, in part, on the number of pixels used to create the image. High pixel numbers enhance detail, sharpen edges, and provide a more meaningful record of the specimen. Conversely, low pixel numbers obscure fine detail and result in images that have less value for teaching, publication, and documentation of the gross findings. Keep in mind that a high-resolution digital camera is not a substitute for good judgment and technique. No matter how good the camera, informative images still require careful attention to specimen orientation, lighting, and exposure.

Some Pointers on Photographing Specimens

General Principles

A few simple steps will improve the aesthetic quality of your photographs. First, photograph the cut surface of the specimen. A photograph of the external surface of a specimen is seldom informative. Section the specimen using a fluid sweeping motion to create a cut surface that is smooth and unruffled. Take the photograph before gouging out tissue for frozen section evaluation or tumor collection; or if these studies are urgently needed, take the tissue from an area that will not be shown in the photograph. Gently rinse blood and fluid from the surface of the specimen, and then blot the surface of the specimen dry so that fluid does not seep across the field of view.

Second, decide whether the pathology is best demonstrated in the specimen before or after it is fixed. Color is best seen when the specimen is photographed fresh, while fine structural details are sometimes better appreciated in fixed specimens, which reflect less light.

Third, position the specimen so that (1) the area of interest is centered in the field of view; (2) its long axis is oriented along the long axis of the frame; and (3) the specimen fills at least 75% of the frame. For bivalved specimens, there is no need to photograph both halves of the specimen. Instead, fill the frame with a closer view of just one of the two halves. To point out a focal area of interest, use a clean and unassuming probe or pointer (not a finger).

Fourth, make sure the background is clean and free of distracters. Remember that your work is not over once the photograph is taken. Remove the specimen, and clean the background so that it is ready for the next user.

Dark Specimens

Many fresh specimens and bloody specimens tend to produce dark images on film. As a general rule, you can compensate by opening the lens by one f-stop (decrease the f-stop number). This will lighten the specimen in the final photograph.

Light Specimens

Photographs of fixed specimens can sometimes be bleached white with little or no color information. Try taking one photograph at the normal exposure. Then take several more photographs while increasing the f-stop number in half increments (e.g., f-stop 16, 16½, 22, etc.) This intentional closing of the lens and consequent underexposure should provide more detail in the very light areas.

Large Specimens

Large specimens generally require at least two sets of photographs: one of the entire specimen and the other a close-up of the area of interest. The close-up photograph will demonstrate the finer details of a lesion, while the overall view will show the relationship of the lesion to the rest of the specimen.

Small Specimens

Most normal lenses will not focus when moved very close (i.e., within 2 feet) to the specimen. A special macro lens is needed for very small specimens or for close-up photographs to show fine detail. The 105-mm macro lens is especially suitable for these purposes. It can focus to a point where the image on the negative is the same size as the specimen, while maintaining a comfortable working distance between the front of the lens and the specimen. Remember that focus is critical

in close-up work. Even small specimens can have depth, so avoid focusing only on the top of the specimen by focusing on a point about one third of the way down from the top of the specimen. Also, use a small camera aperture (an f-stop of 22) for increased depth of field.

Oddly Shaped Specimens

Oddly shaped specimens are a nuisance to photograph when they cannot be maintained in the correct position. A simple solution is modeling clay, which can be used to prop up the specimen. Mold the clay into shape, and use it as a base to hold the specimen. Before taking the picture, be sure to look through the viewfinder to make sure that the clay will not show up in the final photograph. Small fishhooks with nylon cord and an attached weight can be used to hold areas open. For example, this technique can be useful when photographing the interior of heart valves.

Cavities

Under standard lighting conditions, the walls of a cavity cast shadows that obscure the base of the cavity. To circumvent this problem, place the lights as high as possible, so that they illuminate the depths of the cavity. This type of vertical illumination will help reduce shadows as well as light the entire cavity. A separate fiber-optic light source can also be of great help. Make sure that you are focused on the area of interest because depth of field can be a problem.

Three-Dimensional Structures

Side lighting is the best lighting to demonstrate surface detail or to show the three-dimensional quality of a tumor. The lower the angle of the light, the more surface relief will be seen. Shadows give the form shape, depth, and contrast. Be careful not to set the lights too low, as this can create harsh shadows that obscure detail.

Troubleshooting

Reflection

If you are using a piece of glass to support the specimen, watch out for reflections. A valuable photograph can be ruined when overhead (ceiling) lights, the photographer's hand, or the photographer's face is seen reflected in the background. Always use a cable release (the extender cable that allows the photographer to trigger the shutter from a distance), not only to keep the camera still but also to avoid reflections in the glass. Fixed specimens reflect much less light than fresh specimens. If the specimen is fresh, reflected light can be reduced by drying the surface of the specimen with a paper towel. Before tripping the shutter, look through the viewfinder and study the field. Make sure that the lighting and arrangement best demonstrate the pathology of interest.

Exposure

Once an exposure test has been made and an exposure chart posted, the camera should consistently produce uniform high-quality exposures. A photograph that is too light is likely due to overexposure. The simple solution is to close the aperture. (For example, an f-stop setting of 11 can be changed to a setting of 16 or 22.) If the photograph is too dark, simply open the aperture so that the film receives more light. With a little practice and a standard system, you should be well on the way to top-quality specimen photographs.

Maintenance of the Photography System

Not surprisingly, a system designed for use by many users is particularly susceptible to abuse and neglect. Proper maintenance of a photography system is a daily task that is less likely to be neglected if assigned to one person. This person should be responsible for: maintaining clean lenses and viewfinders as well as a fresh supply of film; checking that the cameras are loaded with film; and checking that the film is delivered for processing in a timely fashion. There is nothing worse than spending valuable time in photographing an important specimen and afterward finding out that there was no film in the camera!

Things to Remember when Photographing a Specimen

- Always make sure the background is clean. A spray bottle and towels should be part of the photography setup.
- Orient the specimen. Mark the specimen with a proper identification tag, and include a scale in the plane of focus.
- Always use a cable release to eliminate unwanted camera vibration and reflection.
- Double-check the focus and exposure settings.
- Verify that the lighting shows what you want. Take great care to avoid shadows that obscure features of particular interest.

Commercial Products/ Equipment Vendors

1. Aristo DA-17 Light Box: Aristo Grids Lamp Products, 65 Harbor Rd., P.O. Box 769, Port Washington, NY 11050.
2. Polaroid MP4 Camera: Polaroid Corp., 575 Technology Square, Cambridge, MA 02139.
3. Nikon Inc., 1300 Walt Whitman Dr., Melville, NY 11747-3064 (www.NIKONUSA.com).
4. 150-mm Rulers (Cat. No. 09-016): Fisher Scientific Co., 711 Forbes Ave., Pittsburgh, PA 25219.
5. Photodyne Technologies, Inc., 19441-134 Business Center Dr., Northridge, CA 91324 (www.Photodyne.com).