35 Eye

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General Comments

The eye is a unique neurosensory organ. Much information can be gathered from the gross and microscopic examination of enucleated globes regarding the pathogenesis and manifestations of ocular and systemic diseases. In this chapter, we provide basic guidelines for the fixation and sectioning of ocular tissues, with emphasis on grossing the enucleated globe.

Enucleation Specimens

Fixation

The most commonly used fixative for ocular tissues is 10% neutral buffered formalin (4% formaldehyde), but even this standard fixative has its disadvantages. The relatively high osmolarity of 10% neutral buffered formalin may cause contraction of the anterior chamber and vitreous cavity, which may result in artifactual detachment of the retina. Moreover, formalin tends to dissolve water-soluble substances such as glycogen, resulting in shrinkage of fixed tissue. Thus, it is even a less suitable fixative when electron microscopy studies are required. The amount of 10% neutral buffered formalin required for adequate fixation varies with the size of the ocular specimen. For example, a small specimen such as a cornea or lid biopsy may require only 5 to 10 ml of formalin; an enucleated globe, 150 to 300 ml of formalin; and an orbital exenteration, 500 ml of formalin.

One alternative to formalin is glutaraldehyde 4%. This solution provides adequate fixation for both light and electron microscopy, while causing much less tissue shrinkage because of its lower osmolarity. For specimens less than 2 mm in diameter, glutaraldehyde 2.0% to 2.5% may be the preferred primary fixative for electron microscopy. Glutaraldehyde, however, causes tissues to become hard and brittle and may adversely affect the staining of tissues. For example, ocular tissues fixed in glutaraldehyde 4% stain less vividly with alcian blue and colloidal iron techniques, and they stain diffusely and nonspecifically with the periodic acid-Schiff (PAS) reaction. Yet another alternative is to combine fixatives to achieve optimal preservation for both light and electron microscopy. For example, a solution that combines 4% formaldehyde and 1% glutaraldehyde in phosphate buffer 0.1 mol/L can be used.

Fixation of ocular tissues requires patience. Practices that are designed to speed up the process (e.g., opening the globe, cutting windows into the sclera, or injecting fixative directly into the vitreous) are strongly discouraged because they are likely to induce artifactual disruption of the ocular tissues. The fixation of an enucleated globe in formalin generally requires 24 to 48 hours. After fixation, gently rinse the globe in running water for 16 hours, then place it in ethyl alcohol 60% for grossing.

Eyes that contain excessive calcium deposits or even bone formation may require special decalcifying agents in addition to routine fixatives. In these cases, first fix the globe and then decalcify it in a solution of sodium citrate and formic acid for 24 to 72 hours. When the specimen is soft enough to section, wash it overnight in running tap water to remove all traces of acid, and then place the specimen in ethyl alcohol 60% before

further processing. Because decalcification obscures histologic detail and interferes with staining, check the specimen daily while it is in solution to avoid excessive decalcification.

External Examination

Proper orientation of the eye is essential to document the location of a lesion within the eye. Although the eye is roughly spherical, careful attention to external landmarks allows one to orient this structure with respect to the horizontal median and nasal aspect. One such landmark is the cornea. The cornea occupies the anterior one sixth of the globe, measuring about 11 mm in its vertical plane and 11.5 mm in its horizontal plane. Thus, the longer axis of the cornea indicates the horizontal meridian. Other external landmarks are even more helpful in orienting the eye. The posterior ciliary arteries, for example, can be used to determine both the horizontal plane and the nasal aspect of the specimen. These vessels enter the sclera in the region of the optic nerve and then extend horizontally. Importantly, the nasal vessel is usually more prominent and therefore can be used to identify the nasal aspect of the eye. The nasal aspect can also be identified by measuring the distance between the limbus (the periphery of the cornea where it joins the sclera) and the optic nerve. This distance is shortest along the nasal aspect.

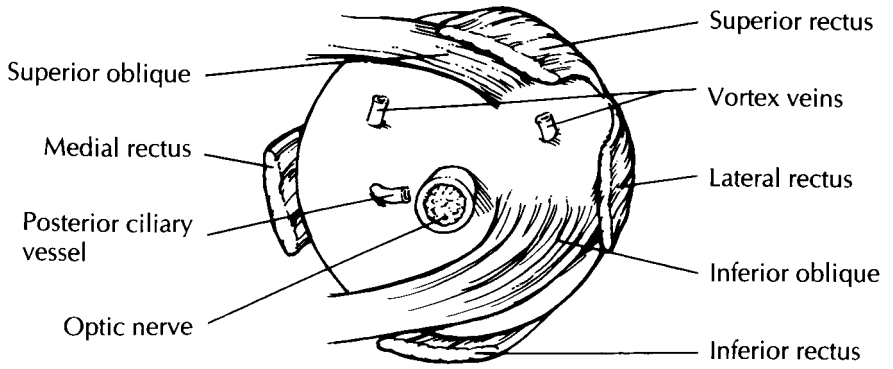
Perhaps the most reliable landmarks for orienting the eye are the insertions of the extraocular muscles. The use of these landmarks, however, requires a good understanding of ocular anatomy (Fig. 35-1). The tendon of the superior oblique muscle extends temporally from the trochlea in the nasal orbital wall to insert into the sclera superotemporally posterior and just temporal to the superior rectus insertion and superior to the optic nerve. The inferior oblique muscle extends temporally from the inferonasal orbital wall to insert into the sclera (as a muscular rather than as a tendinous insertion) just temporal to the optic nerve and posterior ciliary vessel. The insertion of the inferior oblique muscle overlies an area of the sclera corresponding to the macula inside the eye.

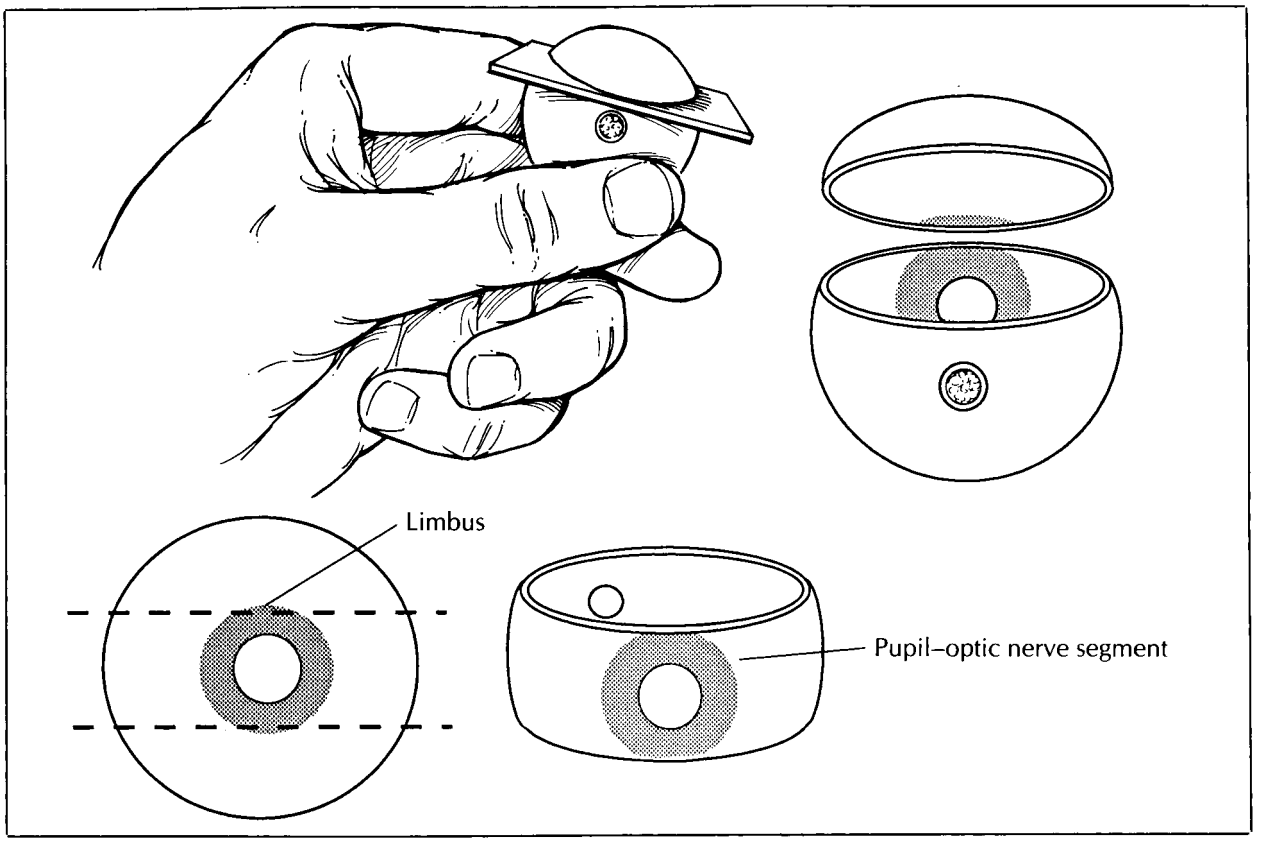
Once the eye has been properly oriented, measure its anteroposterior, horizontal, and vertical dimensions. Record all measurements in millimeters. Also record the dimensions of the cornea and the length of the optic nerve stump. Describe any abnormal external features such as corneal opacities and lacerations or wounds of the cornea and/or sclera. A careful external examination will often disclose important information regarding a history of eye pathology. Scars located at the superior limbus suggest prior surgery for cataracts or glaucoma, and the presence of a silicone band or sponge suggests prior surgery for retinal detachment. If these silicone bands and sponges are encountered, they need not be dislodged, because they will dissolve during processing. On the other hand, metal clips should be meticulously removed from any area submitted for histologic evaluation.

For cases of suspected melanoma, carefully examine the outer surface of the specimen for tumor spread. Specifically, examine the vortex veins for engorgement by tumor, check the episcleral soft tissues for pigment deposition, and look for gross extrabulbar extension by the tumor. In cases of suspected retinoblastoma, carefully examine the optic nerve grossly, and take the surgical margin of the optic nerve for microscopic examination. A dissecting microscope is extremely helpful in identifying minute lesions, and it should be employed during the external and intraocular examinations.

Photography plays an integral part of the gross examination of ocular tissues. As discussed in Chapter 4, photographs are useful for documenting any abnormal features of the external globe and are very helpful in correlating these gross features with the clinical findings. Likewise, photographs should be taken of intraocular lesions after the eye has been opened. The best photographs are obtained with the specimen submerged in alcohol (60%) and with even illumination.

Transillumination of the globe plays an important role in the localization of intraocular tumors that cannot be directly visualized on external examination. To transilluminate the specimen, place the eye in front of a small intense light against a dark background. One method is to use a substage microscope lamp in a dark room. Rotate the globe over the light source, and look for areas of increased or decreased transmission of light. Increased transmission of light may be seen in defects of the iris as occur in pigmentary dispersion syndrome and following peripheral iridectomy or cataract surgery. Decreased transmission of light may be due to intraocular hemorrhage or intraocular tumors. Mark these transillumination





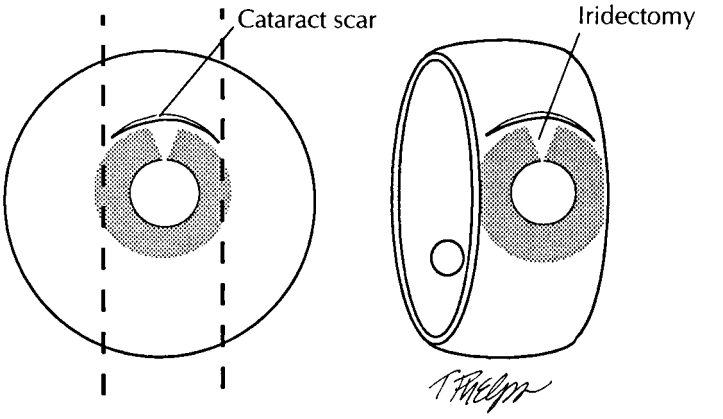


Figure 35-1 (Top). The posterior external aspect of a normal right globe.

Figure 35-2 (Middle). Routine sectioning of the globe with horizontal cuts. The pupil, optic nerve, and macula are all in the same plane (see anterior and posterior views).

Figure 35-3 (Bottom). Sectioning of the globe with vertical cuts to incorporate the cataract scar into the pupil–optic nerve segment.

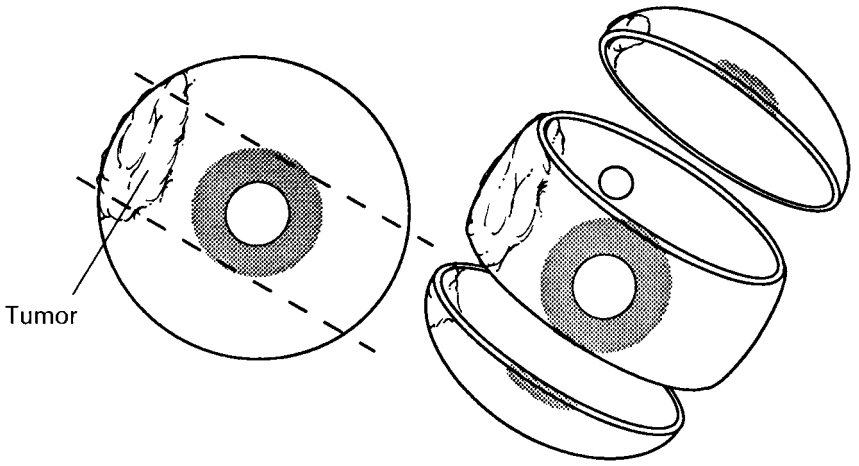
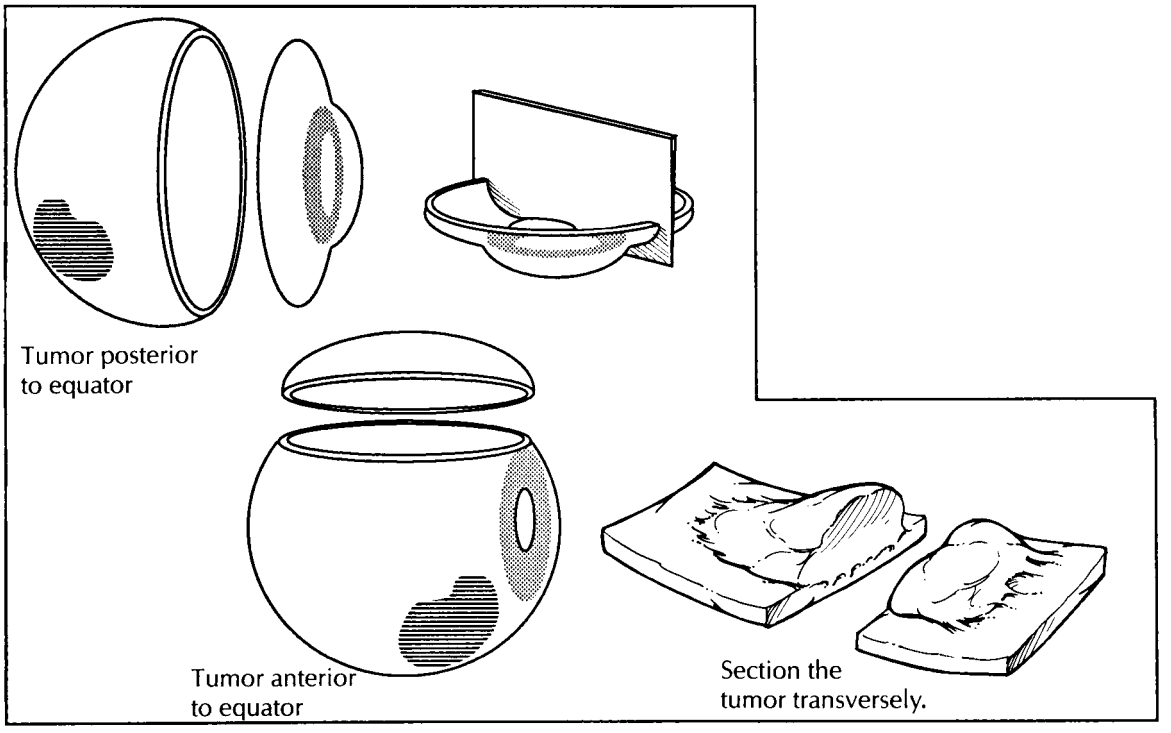
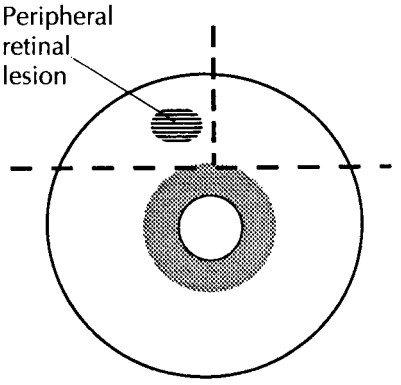


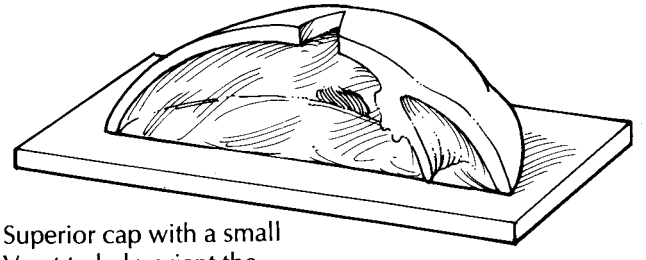
Figure 35-4 (Top). Sectioning of the globe with oblique cuts to incorporate the area of a transillumination defect into the pupil–optic nerve segment.

Figure 35-5 (Middle). Alternate examination techniques for an eye with a melanoma anterior and posterior to the equator.

Figure 35-6 (Bottom). Sectioning of inferior or superior cap for histopathologic examination of a lesion in the peripheral retina.







Superior cap with a small V cut to help orient the tissue during embedding

defects on the corresponding sclera with a pencil. The location of these transillumination defects will determine how the eye should be sectioned.

Sectioning

The planes of section through the globe depend on the presence and location of a lesion. Begin by cutting off the distal 3-mm portion of the optic nerve, and submit this portion on end for histologic examination. If no focal lesions are apparent after external examination, transillumination, and review of the clinical and surgical findings, open the eye in a horizontal plane parallel to the center of the optic nerve and macula. The ultimate aim is to provide a median section along the pupil and optic nerve axis—the pupil–optic nerve (P-O) section—which includes the pupil, optic nerve head, and macula in the same section. Approaching the eye from its posterior aspect, place a razor blade 5 mm superior to the center of the optic nerve. Do not aim the blade toward the center of the pupil, as the lens is a hard structure that is easily dislodged if you try to cut through it. Instead, avoid the lens by aiming 0.5 mm central to the limbus. Once the razor blade is engaged, turn the eye, and view it from the side to help maintain the proper plane of sectioning. Using a sawing motion, continue the section all the way through the limbus (Fig. 35-2).

Before sectioning further, stop to examine the intraocular components. This examination should be performed with a dissecting microscope. Systematically evaluate the lens, iris, ciliary body, vitreous, choroid, retina, and optic nerve head. Note the size and location of any lesions. If an intraocular tumor is present, document its location using the ora serrata, optic disk, and macula as points of reference. Record its size in all dimensions. Try to determine which ocular structures are involved. Specifically note the relationship of the tumor to the optic nerve.

Once the intraocular examination is complete, finish sectioning the eye. Place the cut surface of the globe on a flat surface, and then cut the eye in a plane parallel to the initial section. Again, the razor blade should enter the posterior aspect of the eye 5 mm from the center of the optic nerve, and it should exit the anterior surface of the eye through the periphery of the cornea (i.e., 0.5 mm central to the limbus). The completion of this second cut will result in two caps (or callottes) and the P-O segment.

For eyes with focal lesions, the eye can be sectioned vertically or obliquely to include the lesion in the P-O plane. A few common examples are worthy of specific description. If evidence of prior cataract surgery is present, cut the globe vertically in a plane perpendicular to the wound (Fig. 35-3). If a corneal laceration is present, cut the globe perpendicular to the long axis of the lesion. If a transillumination defect (e.g., melanoma) is present, cut the globe so that the center of the tumor is in the plane of the P-O segment (Fig. 35-4).

An alternative technique for sectioning an eye with melanoma has been designed to permit direct visualization of the tumor from the perspective of the clinician and thus enhance clinicopathologic correlations.[17] For melanomas located posterior to the equator, the anterior segment (cornea, iris, lens, and pars plicata) is removed en bloc by a coronal cut through the pars plana just anterior to the ora serrata. For melanomas that extend anterior to the equator, a cap is removed by a cut along the P-O plane. Either of these cuts permits the examiner to look into the globe and directly visualize the apex of the tumor (Fig. 35-5).

Tissue Sampling

In most cases, only the P-O segment is submitted for histologic evaluation. Important exceptions are when a cap contains the macula (as when the P-O segment is taken in the vertical plane) or a lesion. If a lesion is present in the cap, then the cap may be further sectioned into superonasal, superotemporal, inferonasal, and/or inferotemporal segments, as necessary, and submitted for processing. A mark on the tissue section and a note to the histotechnologist may help guide proper orientation of the tissue during embedding. For example, a small V may be cut into the segment opposite the margin of interest with instructions provided to ''embed V up'' (Fig. 35-6). The pathologist may wish to supervise the embedding of the tissue to ensure proper orientation. Store any remaining tissue in formalin, taking care to designate the tissue properly.

Cataract Specimens

The handling of cataract specimens varies from institution to institution. In general, cataracts

are difficult to evaluate by light microscopy. Hence, the histopathologic examination of cataract specimens is performed only for special indications (such as with congenital cataracts and pseudoexfoliation syndrome). If the submission of cataract specimens is required, document the color, diameter, and thickness of the lens nucleus.

Intracapsular cataract extraction (ICCE) is a surgical technique that allows for the best evaluation of the lens, as the lens is removed in its entirety (capsule, cortex, and nucleus). ICCE was the procedure of choice for cataract extraction for many years; however, this technique is used today only in rare circumstances (such as in conditions with zonular weakness).

Phacoemulsification is a type of small-incision extracapsular cataract extraction (ECCE) that was initially introduced in 1967. Recently, this technique has gained popularity over the more traditional technique of ECCE. With phacoemulsification, there is a smaller incision, less induced astigmatism, and perhaps a faster recovery of vision. The lens nucleus is emulsified ultrasonically; therefore, no cataract specimen is submitted.

Eyelid Excisions

Basal cell carcinoma accounts for 85% to 95% of all malignant epithelial tumors of the eyelids. Most excised specimens with basal cell carcinoma are elliptical. If the long axis of the specimen is less than 10 mm, the specimen should be bisected perpendicular to its long axis through the center of the tumor. This technique allows for the evaluation of three surgical margins (two skin margins and the deep margin). If the ellipse is larger than 10 mm, the specimen can be cut in a cruciate configuration, resulting in a 3- to 4-mm central portion and two end portions. The two end portions are bisected in a plane perpendicular to the central portion. Separately label and submit the central portion and end sections. With this technique, all five surgical margins (e.g., deep, lateral, medial, superior, and inferior) are evaluated.

Special Techniques

Given the easy accessibility of the eye, small tissue samples for diagnostic purposes are readily obtained using a variety of methods. Although these specimens do not require complex dissections, they do require careful handling to preserve cellular detail. Scrapings of the cornea and conjunctiva can be processed as wet-fixed smears. This technique is useful in cases of conjunctival intraepithelial neoplasia. Rapid fixation of the slides in 95% ethyl alcohol is essential in this technique. Do not allow the specimen to air dry. After fixation, the slides may be stained with a modified Papanicolaou technique. Air-dried smears are often useful to look for infectious agents in cases of suspected exogenous or endogenous endophthalmitis. Drops of the specimen are placed on the center of three or more slides and allowed to air dry. The slides can then be fixed in 100% methanol for 5 minutes. Microorganisms can then be detected using Gram, Giemsa, periodic acid-Schiff (PAS), and Papanicolaou stains.

The Millipore filter preparation technique can be used to examine ocular fluid specimens in cases of vitreous hemorrhage, proliferative vitreoretinopathy, and suspected intraocular tumors. This procedure provides for excellent cytologic preservation. The specimen may be received in a plastic syringe or a vitrectomy cassette and must be fresh and unfixed before filtration. After filtration, the cells are fixed with 95% ethyl alcohol. Do not allow the filter to air dry at any time during the procedure. If a specimen is very cellular, divide it among several filters. During filtration, direct the washings along the sides of the funnel to avoid disturbing the cells on the filter. Fix the filters for a minimum of 15 minutes in a Petri dish with 95% ethyl alcohol. A modified Papanicolaou technique, Gomori's stain for iron, and the PAS stain are routinely used to stain the Millipore filter. Absolute propylalcohol rather than absolute ethyl alcohol should be used during staining to avoid dissolution of the filter. The stained Millipore filters are then mounted on glass slides for microscopic examination.

The celloidin bag technique is useful for the retrieval of tissue fragments and cellular material suspended in a fluid. Place 10% neutral buffered formalin and the specimen into a centrifuge-tube lined by a celloidin bag. Centrifuge for 10 minutes. Decant the supernatant, remove the celloidin bag, and tie the bag with a string just above the pellet. Fix the specimen again in formalin for at least 30 minutes. The specimen may

now be submitted for routine paraffin processing and sectioning.

Important Issues to Address in Your Surgical Pathology Report on the Eye

- What procedure was performed?
- Is the specimen a right or left eye? What is the size of the eye? (What are the anteroposterior, horizontal, and vertical dimensions? What is the length of the optic nerve? What are the horizontal and vertical dimensions of the cornea?)
- What is the status of the anterior segment (surgical incisions, corneal opacification, iris or lens abnormalities)?
- Are any transillumination defects present? What are the measurements of these defects, and where are they in relation to external landmarks?
- Note the condition of the iris, ciliary body, and lens. Is an intraocular lens present? If so, is it in the anterior or posterior chamber? If in the posterior chamber, is it in the capsular bag or in the sulcus (between the ciliary body and the root of the iris)?
- Is there a posterior vitreous or retinal detachment? Is any hemorrhage present in the vitreous or retina? Is the choroid thickened? Is the optic nerve head cupped or swollen?
- Is an intraocular tumor present? Describe its type, location, size, color, margins, and consistency. Is any associated hemorrhage or necrosis present? What ocular structures are involved? Does the tumor extend into the optic nerve? Is the tumor present grossly at the cranial or surgical margin of the optic nerve?