REVIEW

The ophthalmic pathology cut-up—Part 1: The enucleation and exenteration specimen

A.L. Forda,*, H.S. Mudhara, R. Farrb, M.A. Parsons\textsuperscript{a,b}

\textsuperscript{a}National Specialist Ophthalmic Pathology Service, Department of Histopathology, E Floor, Royal Hallamshire Hospital, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield S10 2JF, UK
\textsuperscript{b}Academic Unit of Ophthalmology and Orthoptics, University of Sheffield, O Floor, Royal Hallamshire Hospital, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield S10 2JF, UK

Summary
This is the first of two articles designed to facilitate the approach to cutting up ophthalmic pathology specimens. This article deals with the enucleation and orbital exenteration specimen. Ophthalmologists would have made a detailed examination of the eye in vivo with a slit-lamp microscope and imaging modalities. As such, they will expect high-quality pathology feedback to complete the clinicopathological loop. Both tumour and non-tumour pathology cases are discussed. This protocol should not be used for post-mortem enucleations in the setting of suspected non-accidental injury. The text does assume some basic knowledge of clinical ophthalmology terminology.

Specimen fixation

For all ophthalmic specimens, standard 10% neutral buffered formalin gives good results for routine paraffin processing.

Enucleations

These should be placed into at least 60 ml of 10% neutral buffered formalin and allowed to fix for at least 24 h prior to dissection.

• Formalin should not be injected into the globe or windows cut into the specimen before fixation. These processes are likely to damage intraocular structures, hindering diagnosis.

Orbital exenterations

These require a greater volume of fixative and longer fixation because they are larger.

• Fix for at least 48 h.
• Some place gauze or tissue paper between the eyelids during fixation; this is a personal preference and confers no particular advantage.
Equipment for macroscopic dissection

This consists of (Fig. 1):

- Stereo dissecting microscope with variable magnification and a fibre-optic light source.
- Fine watchmaker’s straight and curved forceps for careful handling of the specimen.
- Coloured Perspex sheets placed on the dissecting base of the microscope or bench give contrast when viewing light or dark specimens.
- Blades. We use razor blades for vortex vein dissection and removing the optic nerve margin, and long skin graft blades (snapped in half) or disposable microtomy blades for removing the caps of the eyeball and slicing an exenteration.
- An X-ray system for the identification of areas of calcification or foreign bodies within a sample.
- A digital camera with a live image set-up.
- Suitably designed proformas to assist macroscopic information collection, audit and research.

Macroscopic external description of enucleations

The main indications for enucleation are intractable pain (caused by end-stage glaucoma or in the setting of multiple operative procedures), trauma, malignancy or cosmetic.

The recognition of key anatomical landmarks is used to confirm a left or right globe.

- Long ciliary arteries—this posterior blue line indicates the horizontal plane (Fig. 2a–c).

Figure 1 Our standard set-up for the ophthalmic pathology cut-up in Sheffield. FO, fibre-optic light source; T, hand-held fibre-optic; P, Perspex sheet; B, blades; Pr, proforma; WK, waterproof keyboard; WM, microscope wall mount; DC, digital camera.

- The superior oblique muscle has a fine tendonous insertion into the superotemporal pole of the globe (Fig. 2a).
- The inferior oblique muscle has a thick muscular insertion into the posterotemporal side, just below the horizontal plane and below the temporal long ciliary artery (Fig. 2b).
- Cornea—this is longer horizontally than vertically.
- Optic nerve—lies closer to the nasal than the temporal limbus.

Once these landmarks have been identified, it is possible to determine laterality. When viewing the posterior aspect of the globe with the long ciliary arteries in the horizontal plane and the superior oblique uppermost, the inferior oblique will insert to the left of the optic nerve in a left eye and to the right in a right eye (Fig. 2b).

The globe is rinsed in water to remove excess formalin. It may be necessary to gently wipe away blood from the eye surface with a tissue to aid surface visualization.

- After laterality determination, assess whether the eyeball is intact, opened, collapsed or distorted.
- Measure the axial (anteroposterior), horizontal and vertical globe dimensions. The normal size of a globe is 22–23 mm axially, 22–23 mm horizontally and 22–23 mm vertically.

An increase in the axial dimension often indicates axial myopia or glaucomatous enlargement due to staphyloma formation. A decrease in axial dimension indicates shrinkage secondary to a prolonged decrease in intraocular pressure (hypotonia or atrophy bulbi) or age-related atrophy.

- Measure the corneal diameters (normally 12 mm horizontally, 11 mm vertically).
- Measure the length and diameter of the optic nerve (normal diameter 4 mm).

The following structures are examined systematically and the following points noted:

- Conjunctiva—usually a rim remains around the cornea. Look for congested vessels, sentinel vessels, pigmented and non-pigmented tumours, sutures, fibrosis, cysts, pterygium and pingueculum.
- Cornea—formalin fixation will result in a mild degree of cloudiness that may obscure some features. Limbal vessels do not normally pass into the cornea. Look for arcus senilis, bullous...
Figure 2  (a) An enucleation viewed from behind. Arrowheads mark the location of the horizontal long ciliary arteries. Note the tendonous superior oblique muscle (so) on, optic nerve. (b) Arrowheads mark the horizontal long ciliary arteries. Note the thicker muscular insertion of the inferior oblique muscle (io). This inserts to the left of the optic nerve (on), therefore a left eye. (c) Arrowheads show the horizontal long ciliary arteries. The vortex veins (arrows) lie at approximately 45° angles from the optic nerve. (d) Transillumination of this eye with a fibre-optic light source (fo) reveals an anterior shadow in the ciliary-choroidal area. (e) The shadow in (d), marked out with a pen. (f) Penetration of the cornea 1–2 mm inside the limbus by the blade (arrow). This allows infiltration of the anterior chamber by wax, stabilizing the anterior chamber and cornea during sectioning. Arrowheads point to tumour permeation of the drainage angle, within the anterior chamber. (g) The shadow seen in (d) corresponds to a solitary ciliary-choroidal black lesion (arrow). Histology showed a uveal melanoma. L, lens. (h) Lateral (Plate a) and anterior (Plate b) views of the eye show the ideal parallel cuts (in this case, horizontal cuts). The resulting ring of tissue (Plate c) that includes the pupil and optic nerve is processed. In tumour cases (Plate d), the cuts may be oblique. In this case, the standard parallel cuts incorporate a ring of tissue comprising the tumour, optic nerve and pupil.
keratopathy, band keratopathy, ulceration, perforation, pigmentation, stromal deposits, scarring, thinning and vascularization with or without associated lipid keratopathy. Look for crescent-shaped scars at the superior limbus and sclera; these are the result of previous cataract or glaucoma surgery, respectively. Phakoemulsification scars are not always obvious. Look for remnants of suture material along scars. Observe for iridocorneal adhesions.

- Anterior chamber—normally this is deep and contains clear colourless aqueous humour. Look for a shallow chamber, blood (hyphaema), pus (hypopyon) and tumour (pseudohypopyon in retinoblastoma).
- Angle—the normal open iridocorneal angle is 45°. A narrowed angle is usually 5–30°. A closed angle is where there is obliteration of the iridocorneal gap, via adhesions called peripheral anterior synaechiae.
- Pupil—measure size and position. Any distortions in shape, size and position are noted.
- Iris—look for ectropion uveae, entropion uveae, ruberosis iridis, atrophy, iridectomy sites and tumour.
- Episclera and sclera—look for sentinel vessels, staphylomas, trauma wounds, fibrosis, thinning, surgical intervention, sutures and transcleral spread of an intraocular tumour. Previous surgery could include vitrectomy ports, plastic indentation bands, explants, valves, Seton tubes, local tumour biopsy/resection scars or tantalum discs from previous proton beam therapy for intraocular melanoma. Describe the shape, size and location of these in relation to clock hours and in relation to fixed anatomical points (limbus, optic nerve). For sutures, record the type, colour, number and status. If the enucleation has been performed to remove a tumour, a sclerotomy may be present, representing tumour sampling prior to fixation.
- Vortex veins—these are located approximately 6–8 mm from the optic nerve and arranged in the four quadrants of the eye at 45° to the vertical/horizontal planes (Fig. 2c). They tunnel out of the sclera at an oblique angle. They are sampled by cutting flush with the sclera. If veins are not obvious but their scleral exit point can be identified, a sample of the scleral tunnel is taken as an alternative. Intraocular tumours such as uveal melanoma can undergo extraocular spread (a poor prognostic feature) via vortex vein invasion.
- Optic nerve—normally measures 4 mm in diameter. Look for atrophy with associated meningeal redundancy, demyelination, infarction and tumour. Leave a small remnant of optic nerve after sampling. This prevents inadvertently causing a hole in the posterior pole of the globe if the optic disc is severely cupped. Secondly, leaving a remnant behind allows identification of the optic nerve head during microtomy. For intraocular retinoblastoma, the optic nerve should always be sampled first. This prevents friable retinoblastoma contaminating the surgical optic nerve margin. This allows trouble-free assessment of prognosis, as the extent of optic nerve involvement by tumour determines survival.

Palpation transillumination and retro-illumination of the globe

- Gently palpate the globe with the thumb and fingers. Assess the tension of the globe surface (globally firm in glaucoma or intraocular calcification, globally soft in atrophia bulbi, focally firm with focal mass lesions, focally soft in areas of staphylomas).
- Transillumination detects the presence of intraocular mass lesions. Position a bright light source (such as a fibre-optic light) adjacent to the cornea, within a darkened room. The eye will glow red due to the highly vascularized choroid. Any thickened areas (usually intraocular tumours) cast a shadow (Fig. 2d). Mark the shadow out on the outer scleral surface with a permanent marker (Fig. 2e); this will assist the orientation of the first cut into the specimen. Any areas of thinning (e.g. staphyloma) allow the passage of increased light and appear brighter.
- Retro-illumination is where the light source is placed alongside the optic nerve on the sclera. It is used to detect iris atrophy and to observe pupillary alterations.

Sectioning of an enucleation

- Tumour enucleations are sectioned to include the main site of the tumour, with the pupil and optic nerve included (Fig. 2g and h, Plate d); the plane of sectioning is dependent on the position of the tumour, as determined by prior transillumination (Figs. 2d, f and h, Plate d).
- For non-tumour globes, vertical cuts are used for cataract and glaucoma surgical sites as these are located superiorly. Horizontal cuts will reveal macular pathology. Oblique cuts may also be necessary to reveal other areas of interest. Any
Examine the following systematically:

- Cornea—see above.
- Anterior chamber—see above.
- Angle—examine to see if closed or open. If open, look for blood, abnormal blood vessels, pigment deposits and tumour.
- Iris—see above.
- Lens—note whether present or absent. If present, whether it is the natural lens or an artificial one (intraocular lens prosthesis—IOL). The latter should not be removed because it will dissolve during the processing, leaving an outline for assessment. With natural lenses, look at the size, shape and colour and assess for the presence of cataracts. For an IOL, look for decentration, opacification and a laser capsulotomy site.
- Ciliary body—look for effusions, cryotherapy marks indicative of ciliary body ablation for intractable glaucoma and cyclic membrane.
- Vitreous—look at consistency and texture. Normal vitreous is a gelatinous colourless substance. Age-related degeneration called syneresis appears liquid and this type of vitreous will run out of the eyeball on initial slicing. Look for haemorrhage, vitreous condensation bands, retinal detachment surgery tamponade agents, such as silicon oil or heavy liquids, and opacities that may indicate infection, lymphoma or disseminated tumour.
- Retina—look to see if attached or detached. A detached retina may be artefactual as a result of handling and fixation. A true retinal detachment shows pigment disturbance under the neural retina, secondary to proliferation and migration of retinal pigment epithelial cells. Secondly, a gelatinous yellow-brown proteinaceous exudate may be present beneath the detached portion, representing subretinal fluid. Look for retinal tears and holes and features of retinal detachment surgery, such as encircling bands, cryotherapy scars and intraocular tamponade agents (see above).
- Look for retinal haemorrhages, yellow exudates and whitish cotton wool spots indicative of retinal ischaemic states (commonly diabetes and retinal vein occlusive conditions). Look for pigment disturbances that may indicate previous radio, laser or cryotherapy, photoreceptor dystrophies or trauma. Examine for retinal surface white band-like or confluent white areas with irregular edges, with or without brown pigment, associated with wrinkling of the retinal surface; these changes indicate epiretinal membranes. Examine the vessels for silver wiring, emboli, ghost profiles and perivascular flushing indicative of a vasculitis. The peripheral retina may show degenerative changes (i.e. microcystoid degeneration that appears as a grey honeycomb and paving stone degeneration similar to the areas of anterior-placed cryotherapy).
- Macula—located two optic disc diameters temporal to the optic disc in the horizontal plane. A useful external feature is the insertion of the inferior oblique muscle that lies opposite to the macula. Look for macular oedema, exudates, holes, haemorrhage, disciform scars and drusen.
- Optic disc—examine for papilloedema, atrophy, cupping and vascularization.

Macroscopic internal examination of an enucleation

Examine the following systematically:

- It is useful to mark the position of the first cut onto the external surface of the globe with a marker pen. The cut should begin 2 mm from the external optic nerve edge and end 2 mm inside the rim of the cornea (Fig. 2f). Corneal breach ensures solution penetration of the anterior chamber during processing.
- Commence the first cut with a skin graft or microtomy blade using a gentle, smooth action and not a sawing motion.
- The cut should ideally travel from the posterior pole towards 2 mm inside the corneal rim; this prevents dislocation of the lens. However, it is equally effective to cut from side to side across the edge of the cornea (Fig. 2f). Watch out for a prosthetic intraocular lens (IOL). This will catch on the blade and will impede dissection. If there is suspicion of an IOL, it is prudent to keep the cut outside the corneal rim.
- The first piece removed is called a cap. This may also need processing if it contains interesting pathology. If so, the cap is cut into strips after processing. Cutting into strips before processing will lead to separation of the retina and choroid. Scissors are used to cut the cap as it hardens during processing.1
- If any obstruction is encountered during the sectioning of the globe, stop and X-ray it to look for calcification and foreign bodies. If the globe is calcified, decalcify it with standard media before slicing further.

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- Choroid—look for haemorrhage, exudates, bone formation (in a phthisical eye) and solid white/grey areas indicating inflammation or tumour.
- Tumour—its basic organization (solitary/multiple), shape, colour, texture, size, location and position in relation to other structures (distance from optic nerve head and pars plicata) should be recorded. The effect on surrounding structures such as iris rubeosis, retinal detachment, haemorrhages or areas of invasion should also be noted.
- After this description, the second cap should be removed parallel to the first. This provides a central band of tissue, including the optic nerve, pupil and area(s) of interest (Fig. 2h, Plate c).
- The central block of tissue should be of uniform thickness and not a wedge shape; this helps to maintain the orientation of the plane of sectioning during microtomy (Fig. 2h, Plate c).
- The removal of the second cap is much more difficult than the first, as the stability of the structure has been lost. It is easier to lay the globe, cut-side down, onto a flat surface, to steady it during this process and then to make the second cut, travelling in a posterior to anterior direction or from side to side. Outlining

Figure 3 (a) Anterior view of a complete left exenteration. Note the upper lid (UL) with prominent lashes (La), the palpebral fissure (PF) and the lower lid (LL) with fewer and shorter lashes. The yellow arrows denote how the measurement of the lid skin is made. (b) Posterior view of (a). Note the optic nerve (on), location of the lacrimal gland (arrows) and orbital fat (of). (c) Eversion of the lower eyelid shows the lower punctum (circled) at the medial aspect of the lid margin and exposes the caruncle (arrow). Eversion of the upper eyelid will show the upper punctum in a similar location. (d) Bread-slicing the specimen in the vertical plane using a large skin graft blade. The first cut just avoids the globe. (e) A sliced exenteration with a tumour arising from the superior tarsal conjunctiva (arrow). PO, pupil—optic nerve block. Numbers indicate the order in which the slices were made: 1 = lateral, 5 = medial.
the second cut with a marker pen helps to maintain the neatness of the cut. Alternatively, scissors can be employed. If the second cap contains features of interest, process it as for the first cap (see above).

**Macroscopic external examination of an orbital exenteration specimen**

Indications for an exenteration are principally uncontrolled or advanced malignant neoplasms of the eyelid skin and conjunctiva (melanoma, sebaceous carcinoma and squamous carcinoma) and advanced orbital tumours (lacrimal and non-lacrimal). Adjacent bony structures may be included in the specimen if involved by malignancy.

Exenterations may be complete or partial.

- A complete exenteration involves removal of the globe, upper and lower eyelids, optic nerve, extra-ocular muscles, orbital fat, periosteum and lacrimal gland (Fig. 3a and b).
- The anterior surface will usually be eyelids. The upper eyelid is longer in the vertical plane, exhibits the superior lid crease and shows lashes that are more numerous and longer than those of the lower lid (Fig. 3a).
- The medial aspect is identified by the presence of the caruncle and upper and lower puncta (Fig. 3c). The proximal nasolacrimal duct lies embedded in the medial soft tissues (not shown).
- The posterior aspect will display the optic nerve stump (Fig. 3b).
- State whether a complete or partial exenteration. Measure the specimen in the axial (anterior-posterior), vertical and horizontal planes.
- Measure the eyelid skin (Fig. 3a) and the optic nerve length and diameter. The shape, colour, size, consistency and relationship of visible tumour should be noted. This can be assisted by prising open the eyelids.
- Describe the structures from the conjunctiva to the pupil and optic nerve as for an enucleation specimen—see above.

**Sectioning of an exenteration**

- Paint with two different coloured tissue dyes to distinguish superior and inferior parts of the specimen.
- Take the optic nerve margin. If bone is included, remove it and decalcify.
- Sample the nasolacrimal duct resection margin. This can be assisted by probing the puncta. Melanoma and sebaceous carcinoma can involve this margin via pagetoid spread.
- If the cornea can be visualized, it can be used as guide for slicing the specimen. The basic approach is to vertically bread-slice the specimen, ending up with a series of approximately 10–15-mm-thick slices, including a block with the pupil, optic disc and main pathology included (Fig. 3d and e). The medial and lateral slices of the specimen should be further horizontally sliced to examine the distance of the lesion to the medial and lateral borders.

**Macroscopic internal examination of an exenteration**

- Describe the lesion’s colour, shape, texture, size, location, relationships and margin status.
- Any intraocular pathology should be examined as for an enucleation specimen—see above.

**References**