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Sir,
**Successful retrieval of internal limiting membrane
specimens**

Internal limiting membrane (ILM) peel is undertaken during pars plana vitrectomy for diabetic macular oedema,^{1,2} as an adjuvant to epiretinal membrane peel^{3,4} and in macular hole surgery.^{5,6} There is some controversy concerning the benefits of the procedure, and histological

investigations are often required in the course of attempts to establish the usefulness of ILM peel. For example, histopathological assessment of the tissue removed is important to ascertain surgical cleavage planes and the presence or absence of neural tissue on the retinal side of the peeled ILM, as well as to confirm the nature of the excised tissue.

It is notoriously difficult to retrieve ILM tissue specimens for histology because of their small size and transparent character. These features render the tissue easily lost between removal from the eye and embedding within a histological medium such as wax.^{7,8} Many methods have been employed to improve the recovery rate of these tiny specimens, including retrieval into small volumes of fixative, the application of tiny amounts of ink or dye to the specimen-containing fluid (in order to stain and hence visualise the ILM) and 'sandwiching' the specimen in folded paper.^{7,8}

We have recently adopted two further modifications to these methods that we have found has increased our rate of successful retrieval of ILM specimens to approaching 100% of excised ILM specimens. The first modification involves placing the specimen on a 1 × 1 cm wet instrument gauze (BD Visiwipe, USA) directly from the retinal forceps that were used to peel the ILM. The microporous nature of the wet instrument gauze tends to 'suck' the specimen to its surface, thereby keeping it secure and flat as can be seen in the case of ILM removed with the aid of Indocyanine Green (ICG) (Figure 1a and b). Then the gauze with attached specimen is transferred to a specimen bottle containing 20 ml of 10% formalin. It is important to transfer the specimen to the specimen bottle containing formalin quickly as the microporous nature of tissue gauze tends to withdraw water from the specimen and could make it brittle.

The second modification is conducted in the laboratory. Even when such intra-operative dyes as ICG or Trypan blue have been employed to remove the tissue, by the time the specimen reaches the laboratory it has usually regained its transparency. Therefore, in the laboratory, 1–2 drops of 1% alcian blue in 3% of acetic acid (1:10 diluted, pH 2.5) are added to the specimen bottle and left for 2 h to re-stain the specimen. Alcian blue is a group of polyvalent dyes that are water soluble. The blue colour is due to the presence of copper molecules and it stains mucopolysaccharide of epithelial cells and connective tissue mucins, although it will stain every tissue component over a larger period of time. The contents of the bottle are then poured in a boat dish and the now intensely blue stained specimen is easily retrieved to a tissue wrap and processed further for light or electron microscopy (Figure 1b).

In our experience, the histology of the peeled ILM is unaltered by these modifications to their retrieval

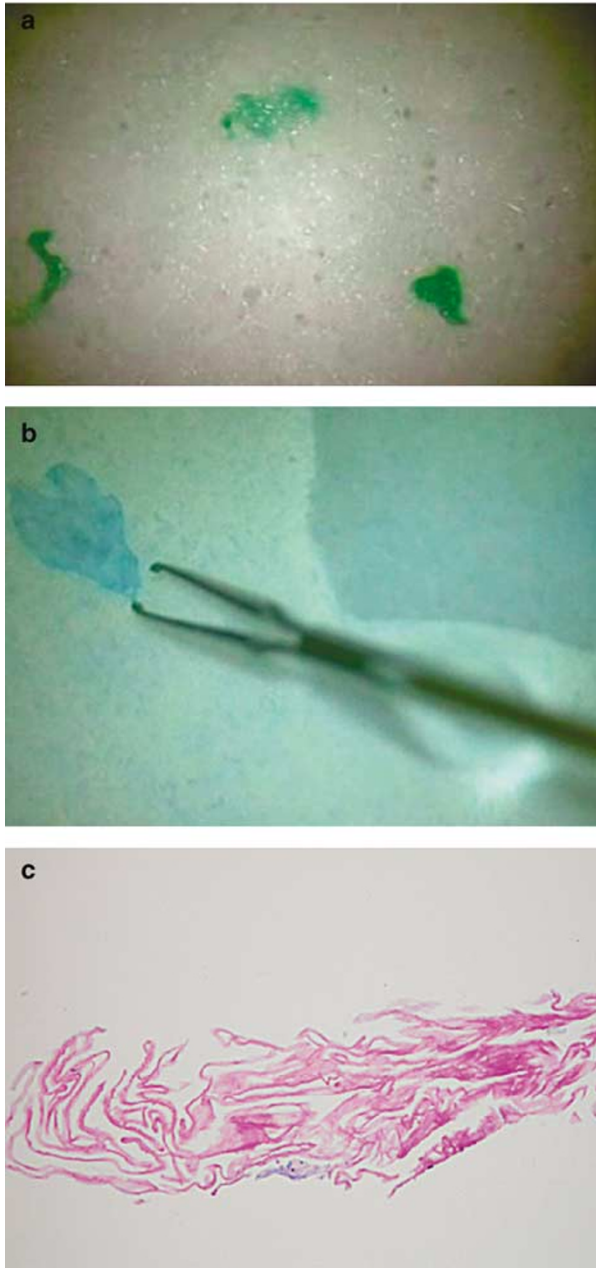


Figure 1 (a) Three pieces of ICG-stained ILM on a wet instrument gauze. (b) Alcian blue-stained ILM specimen. (c) Histological assessment of ILM.

(Figure 1c). We are currently evaluating the procedure to determine whether the modifications change the immunohistochemical properties of the ILM.

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Sir,

Dye-assisted small incision cataract surgery in an eye with cataract and coexisting corneal scarring and epithelial disease

We read with interest the article by Titiyal *et al*¹ on 'Dye-assisted small incision cataract surgery (SICS) in eyes with cataract and coexisting corneal opacity'. The authors describe the use of trypan blue to aid visualisation of the anterior lens capsule in the presence