Dyes may be designated vital when they are used to stain living tissues or cells. In ophthalmology, vital dyes have become effective and useful surgical tools for identifying ocular tissues. Chromovitrectomy is a novel surgical technique for visualizing intraocular tissues during vitreoretinal surgery. This technique was introduced with the goal of avoiding ocular complications related to internal limiting membrane (ILM) peeling, inadequate removal of vitreous, and incomplete removal of the epiretinal membrane (ERM). Chromovitrectomy is credited with improving ILM peeling.1-10 Since 2000, this technique has become popular among vitreoretinal specialists.9

Vital dyes contain a variety of chemical structures, including chromophores, the moiety responsible for a molecule’s color.1-10 Although chromophores are highly important in organic chemistry, their identification in vital dyes relevant to chromovitrectomy has not been well studied. This field of research is important because it may be possible to separate the chromophore from other parts of the molecule, resulting in safer vital dyes for the retina.1-10

VITAL DYES

Triamcinolone acetonide. The state-of-the-art staining agent for identifying the vitreous is the white steroid triamcinolone acetonide.2 Its crystals bind avidly to the vitreous gel, enabling visualization of a clear contrast between empty portions of the vitreous cavity and areas in which vitreous fibers are still present.11,12

Triamcinolone acetonide is injected into the vitreous cavity toward the area to be visualized (0.1 to 0.3 mL, 40 mg/mL [4%] concentration). Injecting this steroid during vitrectomy for the management of retinal detachment may prevent fibrin reaction and proliferative vitreoretinopathy postoperatively.13,14,15,16 The steroid improves identification of tissue through the deposition of crystals, which helps the surgeon achieve complete detachment and removal of the posterior hyaloid and improves the results of primary vitrectomy for management of retinal
detachment and diabetic retinopathy in young patients (Figure 1).\textsuperscript{17,18}

**Indocyanine green.** Indocyanine green (ICG) and infracyanine green may be considered the gold standard dyes for staining and visualizing the ILM in surgical therapy for macular hole and diabetic macular edema. These dyes possess a great affinity for the matrix components of the ILM, such as collagen type 4 and laminin.\textsuperscript{2,19}

ICG-guided chromovitrectomy first gained worldwide popularity, and a number of studies showed ICG-guided peeling to be easier and less traumatic than surgery without ICG, demonstrating good clinical results in macular hole surgery. However, subsequent studies have revealed that ICG may be toxic to the retina. Clinical data showed that ICG can remain intravitreally or deposit persistently on the optic disc after surgery for macular hole. Studies also suggest that ICG can diffuse into the subretinal space through a macular hole, causing damage to the retinal pigment epithelium (RPE; Figure 2).\textsuperscript{20,21}

It has been postulated that the use of ICG at low concentrations in ILM peeling could be a safer alternative because lower rates of RPE abnormalities have been observed with ICG at a concentration of 0.5 mg/mL (0.05%) or less, and an osmolarity of approximately 290 mOsm.\textsuperscript{22}

There are many hypotheses about why and how ICG may induce retinal damage. Intravitreal ICG injections may change the osmolarity in the vitreous cavity, thereby damaging either the neurosensory retina or the RPE cells directly.\textsuperscript{23-26} Investigations in various animal models have shown that ICG may be hazardous to the RPE or neuroretinal cells. Moderate to high doses (2.5 [0.25%] to 25 mg/mL [2.5%]) of intravitreal ICG were toxic to retinochoroidal cells, and impairment of retinal function was described even at low doses of ICG (0.025 mg/mL [0.0025%]; Figure 3).\textsuperscript{26-28}

An ICG molecule has approximately 5% iodine in its final solution and no sodium or calcium.\textsuperscript{2,23,29} Nevertheless, it is has been suggested that removing sodium from the saline solution used for diluting the dye may decrease the risk of RPE damage.\textsuperscript{30} It has been speculated that ICG injection into the vitreous cavity may absorb light; this interaction may lead to a photodynamic effect that induces retinal damage. It was demonstrated that subretinal ICG injection plus light exposure in rabbits can result in functional retinal damage and RPE changes.\textsuperscript{2,21,28,29}

Figure 2. Autofluorescence and optical coherence tomography (OCT) images before macular hole surgery (A,C). Autofluorescence and OCT images after macular hole surgery and ILM peeling guided by 0.05% ICG staining (B,D). Note the absence of hyperautofluorescence image after surgery (B) and also the sealed hole by OCT (D). BCVA improved from 20/400 preoperative to 20/30 postoperative.
Once diluted in any solvent and exposed to light, ICG may undergo various chemical reactions by self-sensitized oxidation because it is chemically unstable; this phenomenon is called decomposition. It was demonstrated that, independent of light exposure, singlet oxygen (photodynamic type 2 reaction) is generated by ICG, leading to dioxetanes by cycloaddition of singlet oxygen. Furthermore, dioxetanes thermally decompose into several carbonyl compounds. Decomposition of ICG was blocked by sodium azide, a quencher of singlet oxygen. This supports the rationale for future use of quenchers in chromovitrectomy.

**Infra*cyanine green**. Iodine and its derivates may be toxic to the RPE. Therefore, infra*cyanine green (IFCG), a dye free of iodine in its formulation either as free ion or as part of the dye moiety, is believed to have less potential for RPE toxicity than ICG. With this presumably safer profile, IFCG may represent an alternative to ICG during ILM peeling in chromovitrectomy due to the lack of sodium iodine in its formulation and physiologic osmolarity.

**Brilliant blue**. In humans, brilliant blue causes adequate ILM staining in an isoosmolar solution of 0.25 mg/mL (0.025%) to 0.50 mg/mL (0.05%) with good clinical results and no signs of toxicity on multifocal electroretinogram. This stain has become a good alternative to ICG and IFCG in chromovitrectomy because of its remarkable affinity for the ILM. Toxicity data regarding its application are limited, so further investigations to confirm these observations are warranted. We have recently demonstrated that subretinal migration of brilliant blue may cause atrophic changes to the RPE. Therefore, we strongly suggest avoidance of brilliant blue exposure to the RPE during chromovitrectomy.
However, we consider this dye the best one for ILM peeling in macular hole surgery. It is also used worldwide despite the fact that there are no clinical trials to support its use (unpublished data). This dye may be used without fluid-air exchange; additionally, no dilution in glucose is necessary.

**Trypan blue.** This dye may not enable ILM visualization as well as ICG, but this blue dye remains an alternative. In order to enhance the staining properties of trypan blue, the dye may be injected into the posterior pole after fluid air exchange, or it may be mixed with glucose 5% to 10% to create a “heavy” trypan blue, which is denser than balanced salt solution. However, higher glucose concentrations should be avoided because glucose 50% has a highly toxic osmolality of 2020 mOsm/L. It is recommended that trypan blue be used mainly for ERM staining. Trypan blue has an affinity for epiretinal glial tissues such as the ERM. Therefore, we consider trypan blue the best dye for staining the ERM. It is suggested to mix 0.3 mL of trypan blue with 0.1 mL of glucose 10%, resulting in a 1 mg/mL (0.1%) solution with an osmolarity of 300 mOsm.

**DOUBLE STAINING TECHNIQUE**

The double-staining technique (Figures 4 and 5) is an elegant procedure that may facilitate the identification of the posterior hyaloid and ILM. In this technique, a dye with a high affinity to the vitreous is injected to enable vitreous removal, followed by a second injection of a dye such as ICG, ICG, trypan blue, or brilliant blue, to stain and peel preretinal membranes. As an alternative technique, two dyes may be injected at once before both peeling procedures.

Mauricio Maia, MD, PhD, is Assistant Professor of Ophthalmology, Vitreoretinal Surgery Unit at the Department of Ophthalmology, Vision Institute, Federal University of São Paulo, Brazil. He states that he receives funding from the Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) and the Pan-American Association of Ophthalmology (PAAO)/Pan-American Ophthalmological Foundation (PAOF). Dr. Mauricio Maia may be reached at retina@femanet.com.br.

Eduardo B. Rodrigues, MD, is a Professor of Ophthalmology at the Department of Ophthalmology, Vision Institute, Federal University of São Paulo, Brazil, Department of Ophthalmology, Federal University of São Paulo in São Paulo, Brazil. He states that he receives funding from FAPESP and the PAAO/PAOF. Dr. Rodrigues may be reached at edubrodriguess@yahoo.com.br.

Michel Eid Farah, MD, PhD is Associate Professor of Ophthalmology, Vitreoretinal Surgery Unit at the Department of Ophthalmology, Vision Institute, Federal University of São Paulo, Brazil. He states that he receives funding from FAPESP and the PAAO/PAOF. Dr. Farah may be reached at mefarah@uol.com.br.

André Maia, MD, is Professor of Ophthalmology, Vitreoretinal Surgery Unit at the Department of Ophthalmology, Vision Institute, Federal University of São Paulo, Brazil. He states that he receives funding from FAPESP and the PAAO/PAOF. Dr Andre Maia may be reached at maia@retina.com.br.

Acácio Lima, PhD, is Chief of Ocular Pharmacology Unit at the Department of Ophthalmology, Vision Institute, Federal University of São Paulo, Brazil. He states that he receives funding from FASEP and the PAAO/PAOF. Dr Lima may be reached at acaciolima@gmail.com.

Octaviano Magalhães Jr, MD, is Professor of Ophthalmology, Vitreoretinal Surgery Unit at the Department of Ophthalmology, Vision Institute, Federal University of São Paulo, Brazil. He states that he receives funding from FAPESP and the PAAO/PAOF. Dr Magalhães may be reached at octavianomj@terra.com.br.


