

Fluorescein dye as a novel cost-effective approach for staining raw specimens in ophthalmic pathology

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Purpose: To study the usefulness of sodium fluorescein dye for staining raw specimens in ophthalmic pathology. **Methods:** Laboratory-based observational study. Eye specimens received in the ocular pathology department of a tertiary eye care center in northeast India were included in the study after obtaining the informed consent. The study period was from 2016 to 2019. Specimens received were a corneal button, lid, orbital tissues, enucleated eyeballs, eviscerated eye, explanted intraocular lens (IOLs), optic nerve and ocular parasites. Sections of the gross specimens were stained with sodium fluorescein (C₂₀H₁₂O₅NA) dye. The average duration of tissue-stain contact time was 45 s. The sections were analyzed under the compound microscope. The intensity of illumination of the microscope was modulated to obtain high contrast digital photographs. **Results:** 26 corneal buttons with or without limbal tissue specimens were analyzed with fluorescein staining procedure; limbus with its pigmented cells were seen in the enucleated eyeballs. 33 enucleated eyes (retinoblastoma [RB] (*n* = 24), phthisical eyes (*n* = 4), choroidal melanomas (*n* = 2), and others (*n* = 3) were included in the study cohort. In these 33 enucleated eyes, vitreous were also examined for the presence of hyalocytes and other pathological cells. Retinal pigment epithelial cells were also seen (*n* = 11). RB seeds were seen with fluorescein stain and documented in 14 specimens. The RB seeds were mostly in vitreous (*n* = 9) and subretinal space (*n* = 5). Fat cells (*n* = 8) from orbital tissues and sebaceous cells (*n* = 5) from frozen section specimens were also observed and documented. **Conclusion:** This study highlights a novel method of rapid staining of gross ophthalmic pathology specimens.

Key words: Eye, fluorescein, microscope, pathology, staining

Eye pathology is unique as transparent structures are present in different parts of the eye.^[1-3] Cornea and limbus with different immunological cells, crystalline lens, vitreous, retina with retinal pigment epithelium (RPE), retinal vasculatures and optic nerve are very important structures to study during grossing and under high power compound microscope. The pathology of these structures has a lot of implications in describing the disease. The fluorescein dye used in the fluorescein angiography can be used to demarcate the arrangement of cells in tissue specimens, orientations of fibers, atrophic change, and tumor seeds inside the eye. The dye can be used to stain quickly the fresh specimens or formalin-fixed tissues during grossing without routine processing and another tissue staining.

Sodium fluorescein (C₂₀H₁₂O₅NA) solution (10%) containing 500 mg of fluorescein in 5 mL (Fluorescein Inj 3 mL, IP 20%w/v) was used in the study. Fluorescein is an orange-red crystalline hydrocarbon with low molecular weight (376.27 daltons). Its best working pH is 8.2–9.6 and it is known to diffuse readily through most of the body fluids and used in retinal angiography since 1958.^[2,3] It absorbs and then emits light in the visible spectrum and is used for angiography.^[2,3] The tissue staining procedure in this study was carried out before

processing the specimens. The color contrast properties of the fluorescein dye can be used to visualize the transparent ocular structures under the light source of the optical microscope. It is thus a cost-effective and quick tissue staining technique. We know that fluorescence or confocal microscopy can visualize the targeted cells using different fluorophores but these instruments are expensive and tissue samples need to be prepared before analysis.^[1-3]

Methods

Design: Laboratory-based observational study. Eye specimens received in the ocular pathology department of a tertiary eye care center in the northeast India were taken in the study after obtaining the informed consent. The study period was from 2016 to 2019. Specimens received were a corneal button, lid, orbital tissues, enucleated eyeballs, eviscerated eye, explanted intraocular lens (IOLs), optic nerve, and ocular parasites.

After the grossing of the specimens, small pieces of transparent or translucent tissues from different ocular sites

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Cite this article as: Das D, Deka P, Bhattacharjee H, Deshmukh S, Gupta P, Deka A, et al. Fluorescein dye as a novel cost-effective approach for staining raw specimens in ophthalmic pathology. Indian J Ophthalmol 2020;68:2175-8.

Access this article online

Website:

www.ijo.in

DOI:

10.4103/ijo.IJO_2153_19

Quick Response Code:



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Received: 21-Nov-2019

Revision: 27-Dec-2019

Accepted: 26-Mar-2020

Published: 23-Sep-2020

with variable measurements were seen. The smallest being 1×1 mm, was selected for this staining procedure. Unprocessed tissues were placed on an uncoated glass slide and a drop of fluorescein dye was put on the tissue. The drop was delivered using a syringe and needle. The average dye-tissue contact time was 45 seconds. The stained tissue specimens were then immediately seen under different objectives of the compound microscope (Axioskop 40-microscope, camera-AxioCam MRc). While visualizing the tissues in $\times 5$ and $\times 10$ objectives, no cover slips over slides were used but when it was seen through $\times 40$ and $\times 100$ of the objectives, coverslips were put over the tissue with fluorescein. The intensity of illumination of the microscope was modulated for better contrast of the stain and tissues and to capture high-quality digital photographs. The microscope filter was changed for better pictures of the background and cells of interest.

On some occasions, dilution of fluorescein-tissue was done by adding a drop of tap water. Excessive fluorescein spread in the glass slides was wiped up by Millipore paper. After the procedure, all the glass slides were cleaned and disposed of as per the laboratory waste management protocol.

In RB cases, the lateral calottes were cut as per the bread loaf technique and the piece of the third calotte, which was smaller in size was stained with fluorescein stain according to

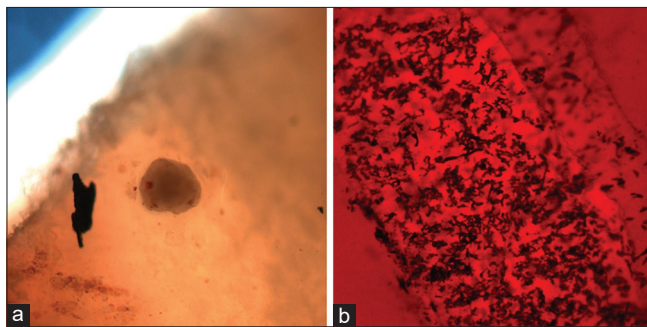


Figure 1: [a] Showing fluorescein stained gross photo of the cornea with keratic precipitate in an enucleated uveitic eye. [b] Showing part of limbus with pigmented cells ($\times 40$)

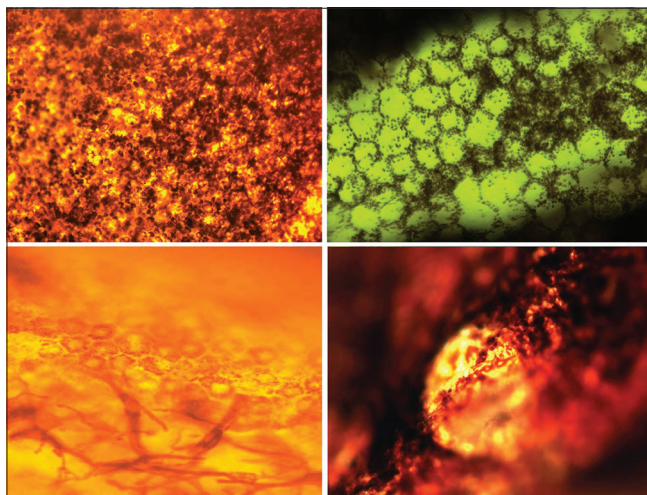


Figure 3: Showing retinal pigment epitheliums (RPEs) stained with fluorescein in gross RPE-retina complex ($\times 20$, $\times 40$)

designed protocol and all RB seeds were seen and documented rapidly. Utmost cares were taken not to distort the tissues. After the digital documentation, it was dipped gently in the same formalin to wash the excess fluorescein and transferred to the processing cassettes immediately. In all the cases, procedures were rapidly done and no drying of tissues was observed.

Results

Corneal button \pm limbal tissue ($n = 26$ specimens) were seen with fluorescein staining procedure; limbus with its pigmented cells were also seen in enucleated eyeballs [Fig. 1]. In lid tissue, sebaceous gland carcinoma (SGC) was studied specifically. Gross specimen, fluorescein stained lobules of SGC were compared with the frozen section and permanent section slides [Fig. 2]. 33 enucleated eyes (retinoblastoma [RB] ($n = 24$),

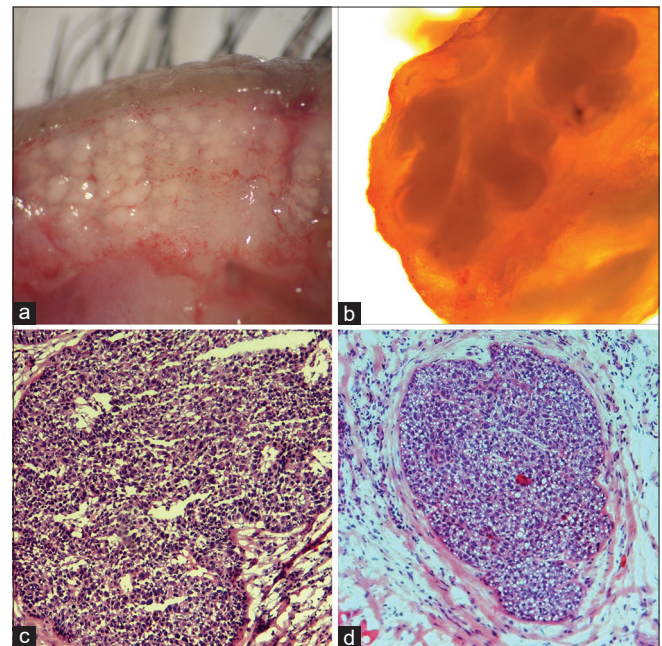


Figure 2: Showing a case of sebaceous gland carcinoma in grossing [a] ($\times 5$); fluorescein stained [b] ($\times 20$); compared with frozen [c] (H and E, $\times 20$) and permanent section [d] (H and E, $\times 20$)

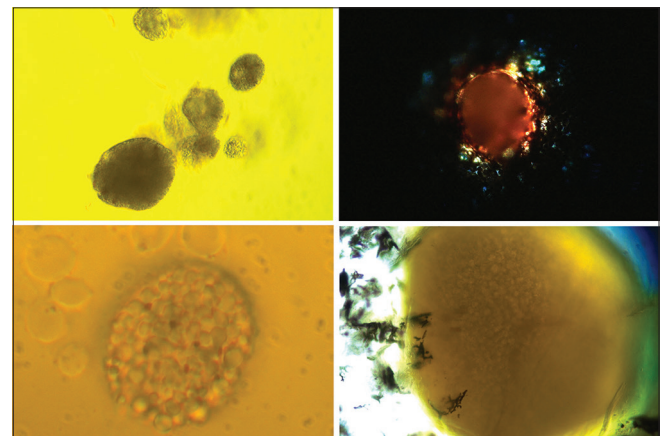


Figure 4: Showing retinoblastoma vitreous seeds in yellow to orange color with fluorescein stain. Seeds had intra-seed honeycomb appearance unlike drusens ($\times 40$)

phthisical eyes ($n = 4$), Choroidal melanomas ($n = 2$), and others ($n = 3$) were also included in the cohort. In these 33 eyes, vitreous was also examined for normal hyalocytes and other pathological cells.

RPE cells were seen in 11 specimens [Fig. 3]. RB seeds were seen in 14 specimens stained with fluorescein stain and documented [Fig. 4]. Seeds were mostly in the vitreous ($n=9$) and subretinal space ($n = 5$). A transparent retina with blood vessels showed stain positivity and they were documented ($n = 7$). Fat cells ($n = 8$) from orbital tissues and sebaceous cells ($n = 5$) from frozen section specimens were also observed and documented. IOL optics and haptics were also stained in a similar way to elicit different pathologies ($n = 6$). Drusens were seen in two enucleated eyes [Fig. 5].

Whole cells were seen under background orange to red or green color. Fungal hyphae were also visualized in two of the eviscerated specimens of fungal endophthalmitis which were confirmed in the permanent section by Grocott's methenamine silver (GMS) staining [Figs. 6 and 7]. The selection criteria of our study included the corneal pathology in infectious cases particularly with a history of suspected fungal endophthalmitis

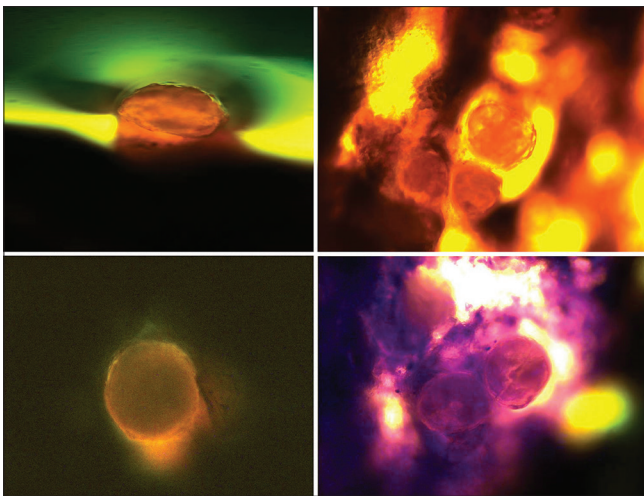


Figure 5: Showing drusens in the retina-RPE-choroid complex dissected out in an enucleated eyeball during grossing. Drusens are sub-RPE and seen in age-related macular degeneration and also other degenerative intraocular conditions ($\times 40$)

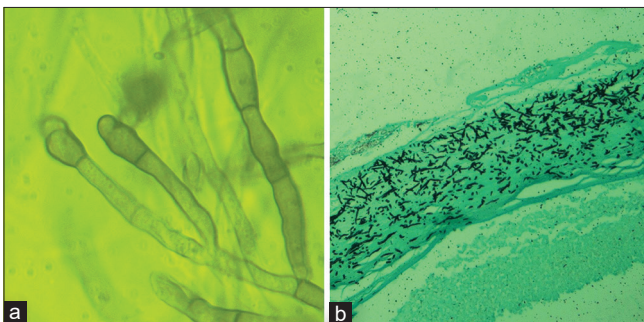


Figure 7: [a] Showing *Aspergillus* in fluorescein stained slide ($\times 40$). [b] Section of the corneal button was positive for filamentous fungus in Grocott's methenamine silver-stained slide in the permanent section ($\times 10$). Please note: This corneal button is a part of eviscerated fulminant fungal endophthalmitis case

in the eviscerated specimen. In one index case, we could observe the *Aspergillus* fungus on fluorescein staining in the raw specimen and then it was processed and stained with hematoxylin-eosin (H and E) for the findings and GMS stain showed filamentous fungus. Previously submitted specimens in microbiology also confirmed *Aspergillus niger* in the culture. Two parasites were documented using this methodology [Fig. 8].

All specimens were processed finally for the permanent sections and reported by the pathologists.

Discussion

Light and its properties have long been used in optical microscopy in laboratory science.^[1-9] In our study, we documented the use of fluorescein dye for rapidly staining the ophthalmic tissue specimens. The eye is the only organ in the body where transparent tissues are seen which can be visualized under the objective of the microscope in a fresh and formalin fixated tissue without even normal processing with routine and specialized staining. Fluorescein dye meant for angiography was used in this study in raw ocular tissues to obtain the simple but important findings in a rapid way for a possible diagnosis.^[2,3] Fluorescein solution (10%) containing 500 mg of fluorescein in 5 mL was used in this study.^[2,3] Fluorescence microscopy which uses fluorochrome or immune fluorescence is an expensive instrument which labels the antibodies in different excitation and emission mode.^[1] Whereas in the light microscope, the property of light is used to see the fluorescent dye directly with the change of filters and illumination.^[1,4,7-9] Our query on basic pathology

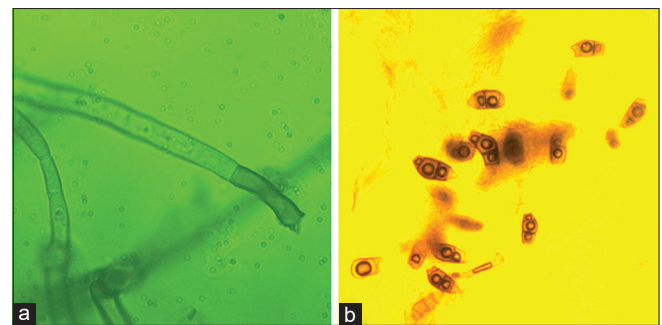


Figure 6: [a] Showing *Aspergillus* fungal hyphae. [b] Spores of *Curvularia* with fluorescein stain (here, the contact time were less than 15 seconds); ($\times 40$)

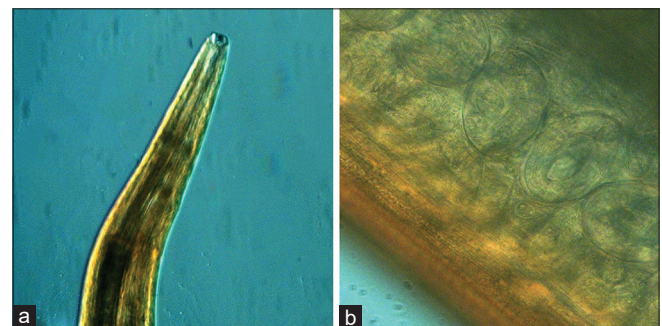


Figure 8: [a] Showing fluorescein stained parasite *Thelazia*. [b] Baby worms in the abdomen of the parasite with cross striations ($\times 20$, $\times 100$)

of tissue staining is that; first, why does any tissue stain? Second, why all parts of the same tissue do not stain in the same way? Uptake of a dye in tissue depends on the affinity and rate of absorption factors.^[8,9] The stain is considered as a marker and attractive forces bind the dye to a tissue.^[8,9] We see that when fluorescein dye meant for angiography is injected in a vein, it immediately moves to the target sites in the variable time period.^[2,3] In raw tissue, Coulombic attraction is anticipated as a part of electrostatic attractive ionic force where colors of the stain in tissue get reflected.^[8,9] One special thing about some of the ocular structures is that they are thinner and translucent and we know thinner specimen stain faster than a thicker and opaque tissue.^[8,9] The average time in our study for fluorescein-raw tissue interaction was less than a minute. Fluorescein stained tissue appeared orange, green, or grey colored. Grey-colored contrast was seen when white balance was clicked in the photography mode of the computer. Image formation in the compound microscope is a simple one compared to the advanced microscopes. In a visual microscope, the lens brings together the refracted rays to a single focus where the clear image is formed.^[8,9] When light composed of all spectral colors pass through a simple lens, it is refracted to a different extent depending on the wavelength of the light, blue color in one end and red color in the other end.^[8,9] The variation of color was externally controlled with illumination adjustment in our study. In our cases, orange, red or green color refracted from the background and cells were seen in various morphological patterns and this helped in immediate pattern recognition of the underlying pathologies. In our study, corneal pathology, crystalline lens, IOLs, RPEs, vitreous pathology, RB seeds, optic nerve, and drusens provided significant information. Drusens could be differentiated from RB seeds as seeds had honeycomb appearance unlike drusens in high power objective of a microscope. Limitation of the procedure is that the finer inner details of the cells (nuclear/cytoplasmic findings), lamellar details, fibers, nuclei, etc. could not be elicited in this methodology. The permanent section is still a gold standard for pathologists to report.

Conclusion

In conclusion, this study though small sample-sized, put forward a novel method of rapid staining of gross ophthalmic pathology specimens. Moreover, some pathology is always missed in the permanent section where the only visualization of one plane is possible. In our study, three-dimensional visualization of the RPE cells in flat preparation gave a wider view of pathologies such as drusens, RPE defects,

etc. Microscopic pathological findings in three-dimensional eyeballs can be elicited with rapidity while doing tissue grossing itself and this cost-effective staining procedure can also help the general pathologists to use this idea in the pathology of synovial space in peripheral joints, cyst walls and even in staining the cartilages. However, this procedure needs to be standardized in the future on a much wider scale.

Acknowledgments

Dr. Kasturi Bhattacharjee MS, DNB, FRCS; Dr. Manab Jyoti Barman DO, DNB; Dr. Bidhan Chandra Das MD; Dr. Jayanta Kumar Das DO, DNB, Ph.D.; Dr. Ganesh Chandra Kuri MS and Miss Bhaswati Talukdar M.Sc.

Sri Sankaradeva Nethralaya, Guwahati, Assam, India.

Kanchi Sankara Health and Educational Foundation, Guwahati, Assam, India.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Eagle RC Jr. Eye Pathology: An Atlas and Text. 2nd ed. Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins; 2011.
2. Yannuzzi LA, Rohrer KT, Tindel LJ, Sobel RS, Costanza MA, Shields W, *et al.* Fluorescein angiography complication Survey. *Ophthalmology* 1986;93:611-7.
3. Hang S, Fu AD, Johnson RN, McDonald HR, Jumper JM, Cunningham Jr ET, *et al.* Fluorescein angiography: Basic principles and interpretation. In: Schachat AP, ed. *Ryan's Retina*. 6th ed. Vol 1. Philadelphia, PA: Elsevier; 2018:1-45.
4. Van Noorden CJF, Butcher RG. 1991. Quantitative enzyme cytochemistry. In: Stoward PJ, Pearse AGE, editors. *Histochemistry, theoretical and applied*, vol. 3: enzyme histochemistry, 4th ed. Edinburgh, UK: Churchill Livingstone; p. 355-432
5. Bennion PJ, Horobin RW. Some effects of salts on staining: The use of the Donnan equilibrium to describe staining of tissue sections with acid and basic dye. *Histochemistry* 1974;39:71-82.
6. Chayen J, Bitensky L. *Practical Histochemistry*. 2nd ed. Chichester: Wiley; 1991.
7. Berkow JW, Flower RW, Orth DH, Kelley JS. *Fluorescein and Indocyanine Green Angiography- Technique and Interpretation*. San Francisco: American Academy of Ophthalmology; 1997.
8. Bancroft JD, Gamble M. *Theory and Practice of Histological Techniques*. 5th ed. Churchill Livingstone; 2002.
9. Solem JC, Baldwin GC. Microholography of living organisms. *Science* 1982;218:229-35.