

# Timing of Color Fundus Photographs and Intravenous Fluorescein Angiography

## Letter To The Journal

Patrick J. Saine, B.S., Jerald A. Bovino, M.D., Daniel F. Marcus, M.D., and Philip T. Nelsen, M.D.  
*Retina Unit, St. Vincent Medical Center, 2213 Cherry Street, Toledo, OH 43608*

Color fundus photographs are frequently taken at the time of intravenous fluorescein angiography. Traditionally they have been taken prior to dye injection. Justice<sup>1</sup> advises taking fundus photographs before dye injection stating that leaking fluorescein can obscure fundus detail. Schatz, Burton, Yanuzzi, and Rabb<sup>2</sup> suggest taking color photographs after dye injection and before the late stage but mention that abnormal fundus fluorescence can produce false, greenish discoloration. To determine the optimal timing of fundus photography we used color densitometry to investigate color changes in fundus photographs after intravenous fluorescein.

Fundus photography was performed prior to fluorescein angiography in 40 patients using a Zeiss Fundus Flash III and Kodachrome 25 film. Three to seven minutes after the dye injection (2 cm<sup>3</sup> of 25% sodium fluorescein) color fundus photographs were repeated. Color densitometry was performed using a Brumac TRD-8S color densitometer on 40 pairs of photographs taken before and after dye injection. Red, green, blue, and black densities were measured in three areas of each slide: 1) over the blood vessels at the disc, 2) over the fundus where no major blood vessels were present, and 3) over the fundus where one or more blood vessels were present. Sampled areas were identical within each pair. The resulting changes in density were compared using the T-test of matched pairs of observations (Table).

There was no significant change in the density of the red sensitive layer of color film after dye injection. The absorption and emission characteristics of fluorescein dye influence wavelengths which are shorter than those recorded by the red sensitive layer of Kodachrome 25<sup>3-5</sup> (see figure). The stability of this layer served as a control for external factors, such as processing procedures and minor emulsion variations.

The green sensitive layer of the color film exhibited a statistically significant negative density shift between the pre-injection and post-injection photographs. Prior to the injections, green density was a result of green light reflecting from the fundus. After the injection, the green density was a result of both green reflectance and the peak emission of green wavelengths by excited fluorescein. This additional exposure served

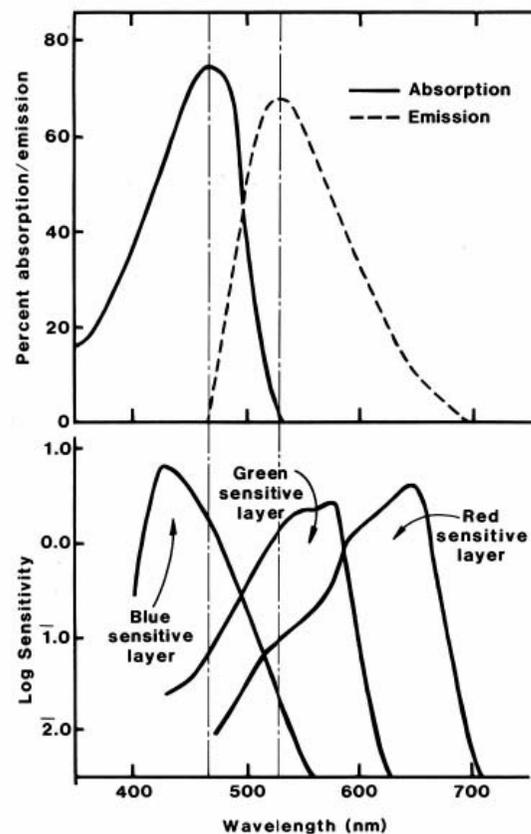


Figure: The relationship between spectral sensitivity curves of Kodachrome 25 film and *in vitro* absorption/emission spectra of aqueous sodium fluorescein eye. *In vitro* spectra are used for comparison because *in vivo* spectra are based on induced fluorescence with a B4 (450-500 nm) filter.

Saine, P., Timing of  
Color Photographs...

Table  
Changes in Film Density After  
Injection of Intravenous Fluorescein Dye

Layer of Film Measured	Site	Mean Change in Measured Densities (Density Units)	Range of Change in Measured Densities (Density Units)	P
Red Sensitive	Disc	-.01	-.30 - +.24	.30
	Fundus without vessels	-.02	-.59 - +.39	.25
	Fundus with vessels	+.01	-.39 - +.40	.39
Green Sensitive	Disc	-.06	-.72 - +.35	.04
	Fundus without vessels	-.10	-.91 - +.43	.02
	Fundus with vessels	-.09	-.70 - +.59	.01
Blue Sensitive	Disc	.00	-.32 - +.35	.50
	Fundus without vessels	-.05	-.43 - +.33	.02
	Fundus with vessels	-.04	-.44 - +.43	.04
Black (All)	Disc	-.04	-.42 - +.22	.04
	Fundus without vessels	-.05	-.60 - +.32	.04
	Fundus with vessels	-.05	-.47 - +.41	.04

to comparatively overexpose the green sensitive layer, decreasing its density.

The blue sensitive layer displayed a statistically significant negative density change after dye injection when the choroid was included in the measurements. There was no significant difference in the density of the blue sensitive layer when only the fluorescein in the retinal blood vessels was measured. The emission characteristics of fluorescein are within the absorption spectrum of the blue sensitive layer, however, the overlap is small (see figure). The extent of the overlap is not significant when the amount of fluorescein is small but becomes significant when a large amount of fluorescein is involved.

The overall negative density change (black) can be directly attributed to the decrease in the density of the green and blue sensitive layers.

This study demonstrates a statistically significant overexposure of the blue and green sensitive layers of Kodachrome 25 film when color fundus photographs follow an injection of 25% sodium fluorescein. We recommend taking color fundus photographs prior to the injection of fluorescein dye if accurate color representation is required.

## References

- Justice, J.: Fluorescein angiography, *Int. Ophthalmol. Clin.* 16:33, 1976.
- Schatz, H.; Burton, T. C.; Yanuzzi, L. A.; Rabb, M. F.: *Interpretation of Fundus Fluorescein Angiography*. St. Louis, C. V. Mosby, 1978, p. 43.
- Emmart, E. W.: Observations on the absorption spectra of fluorescein, fluorescein derivatives, and conjugates, *Arch. Biochem. Biophys.*, 73:1, 1958.
- Eastman Kodak Company: *Kodak Color Films for Professional Use*, 7th ed. Rochester, Eastman Kodak Company, 1977, p. 26.
- Delori, F. C.; Ben-Sira, I.: Excitation and emission spectra of fluorescein dye in human ocular fundus, *Invest. Ophthalmol.*, 14:487, 1975.

\* This paper is published with permission from the American Journal of Ophthalmology. Copyright by the Ophthalmic Publishing Company. *Am. J. Ophthalmol.*, 97:783-785, 1984.

Presented in part at the 14th annual meeting of the Ophthalmic Photographers Society in Chicago, Illinois on November 2, 1983.  
Send inquiries to: Jerald A. Bovino, M.D., Retina Unit, St. Vincent Medical Center, 2213 Cherry Street, Toledo, OH 43608.