THE BLACK AND WHITE OF BODIPY FLUORESCENT DYES

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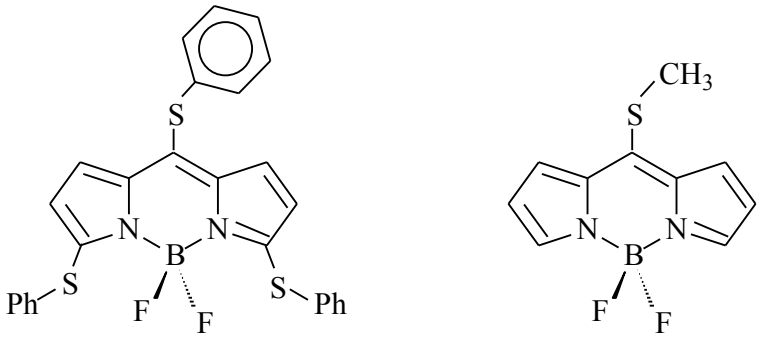
Life is all the more appealing thanks to the phenomenon of color. In general we understand color in the context of unabsorbed light reflecting from a surface; however, that may be only half the story. Many materials absorb light and the excited electrons relax by emission thereby giving rise to fluorescence and phosphorescence (Valeur & Beberan-Santos 2011). Many fluorophores are dyes and those with high absorption extinction coefficients and high quantum yield emissions in the visible range can exhibit two colors in solution, dependent upon the mode of viewing. One color results from the reflection or transmission of the light while the other color is associated with the dye's emissive properties associated with the excitation-relaxation process. Without the aid of a spectrophotometer, it is often difficult to determine the color corresponding to the reflectance or to the emission.

We illustrate a simple, elegant, methodology to illustrate the color associated with both the dye and it's fluorescence without the need for instrumentation; the only requirement is black and white paper. We use members the BODIPY family of fluorophores which possess both the high absorption extinction coefficients and high quantum yield emissions in the visible spectrum that are needed for the experiments described (Loudet et al. 2007).

Methods.–0.5% (m/v) solutions (10 mL) of two synthetically accessible BODIPY dyes, 3,5,8-tris-(phenylthio)$_3$-BODIPY (blue dye/red emission BODIPY, **1**, (absorption λ_{max} = 609 nm, emission λ_{max} = 634 nm) and 8-methylthio-BODIPY (yellow dye/green emission BODIPY, **2**, (absorption λ_{max} = 491 nm, emission λ_{max} = 532 nm) in dichloromethane were prepared (Fig. 1) (Goud et al. 2006; Roacho et al. 2015). The low concentration avoids any self-quenching effects (Magde et al. 2002). The solutions were placed on a watch glass under ambient laboratory light with two distinct backgrounds,

Recommended citation:
Saucedo, L.I., R.I. Roacho & K.H. Pannell. 2019. The black and white of BODIPY fluorescent dyes. Texas J. Sci. 71: General Note 5. https://doi.org/10.32011/txjsci_71_1_Note5.



1 **2**

Figure 1. blue/red BODIPY (**1**); yellow/green BODIPY (**2**).

white and black paper, and result in the observation of two distinct colors (Fig. 2). Other solvents may be used with equal success and a variety of containers, including sealed systems for a permanent exhibit, can be envisaged. A range of BODIPY and other fluorophore species are available from commercial houses where academic discounts are possible (Cuantico de Mexico, ThermoFisher Scientific).

Results & Discussion.–When the watch glass is placed upon a white paper background the various dyes exhibit the color associated with the normal transmission. Thus, blue for **1** and yellow for **2** (Fig. 2, center). Replacement of the white background with a black paper result in the transformation of the color observed to red (**1**) and green (**2**), (Fig. 2, left). A mixture of black and white background produces the bicolor schemes noted on the right of the figures.

The phenomenon of the discrete emission color from the sample placed upon the black background results from the absence of significant reflective light from that background reducing any transmission to an observer. On the other hand, the white background reflects the incident light and thereby illustrates the dye transmission color.

A second illustration of the same bicolor observation can be achieved using an overhead projector, one of many uses of such

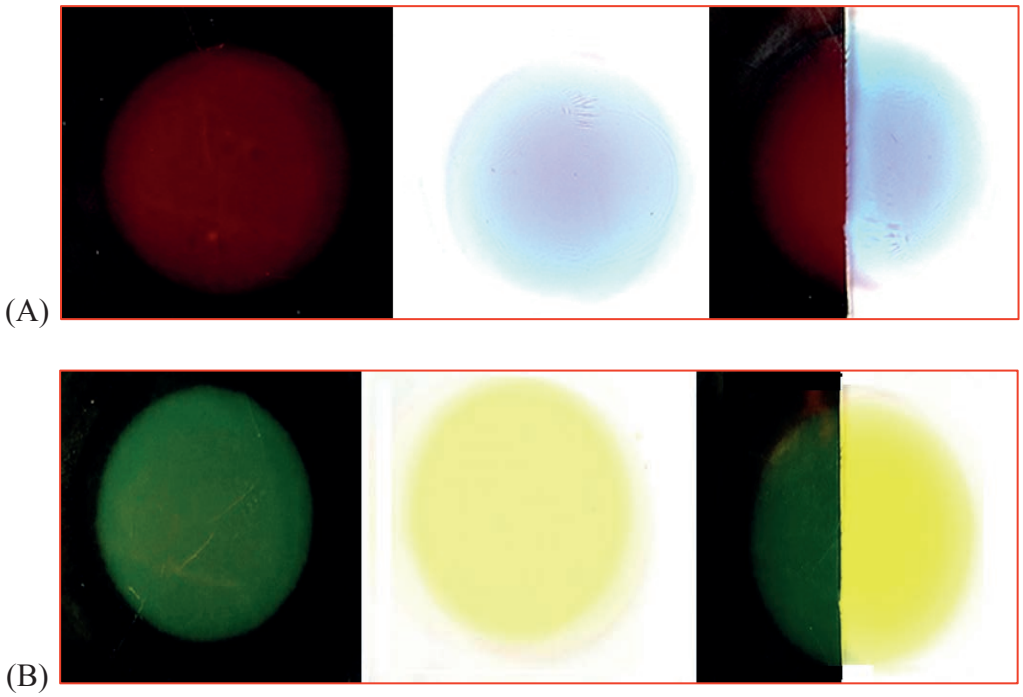


Figure 2. (A) 3,5,8-tris(phenylthio)$_3$-BODIPY (**1**) solution on black (left), white (center) and black/white (right) backgrounds; (B) 8-methylthio-BODIPY (**2**) solution on black (left), white (center) and black/white (right) backgrounds.

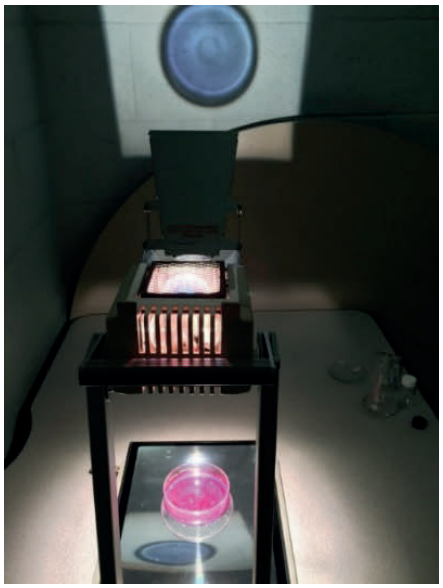


Figure 3. Blue/red BODIPY **1** and its projection.

equipment (Goodman 1995; 2000). Using the same dye/fluorophore solutions, the lamp from the projector acts as the energy source for the excitation process and the emission from the dye solution on the watch glass can be directly observed, red. However, the image projected, is associated with only the transmission through the solution, blue (Fig. 3).

Addition of small quantities of the fluorophores to nujol on a microscope slide creates a mull which exhibits a similar black and white background phenomenon that remains viable for long periods of time, to date well over 1 year. Such samples, and the demonstrations noted above, initiate much conversation with students, faculty and coworkers about the nature of color.

Acknowledgments.–We thank The Welch Foundation, Houston, TX for support *via* Grant AH-0546. Laura Saucedo acknowledges the NIH-Maximizing Access to Research Careers (MARC) program at the University of Texas at El Paso, via grant T34GM008048.

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