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Steady-state fluorescence anisotropy and lifetime measurements of fluorophores and fluorescent-dye-loaded microspheres JACOB A. COLE, SAM V. MIGIRDITCH, TYLER W. FOLEY, BROOKE C. HESTER, Appalachian State University — We perform steady-state fluorescence anisotropy and lifetime measurements via illumination of fluorophores with a continuous intensity beam of light. In steady-state fluorescence anisotropy, fluorophore molecules are excited when the polarization of the incoming excitation light is parallel to the excitation axis of the fluorophore. Following a delay known as the fluorophore lifetime τ a molecule will return to its rest state by emitting photons polarized along the instantaneous orientation of the molecule. The steady-state anisotropy r is defined as the average change in orientation of the sample weighted by the average intensity of each polarization axis. Experimental results are used to determine the anisotropy r and the lifetime τ of the samples: freely diffusing rhodamine as well as yellow-green fluorescent-dye-loaded microspheres with sizes ranging from 0.51 μm to 6.2 μm. Experimental outcomes confirm that the custom-made steady-state fluorescence anisotropy optical system and analysis software are properly engineered and optimized. These outcomes include the fluorescence lifetime τ for each fluorophore type, the fluorescence lifetime τ for each fluorescent bead size, and the anisotropy r at various temperatures.

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