

Intracellular fluorescence lifetime measurements of a molecular rotor for determination of apparent microviscosity

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We have used a commercially available fluorescent molecular rotor, dicyanovinyljulolidine (DCVJ) to measure apparent microviscosity in cells. We have employed fluorescence lifetime imaging microscopy (FLIM) using time-correlated single photon counting (TCSPC) detection. We have calibrated the fluorescence lifetime of DCVJ as a function of viscosity in methanol/glycerol solutions between 60 cP and 600 cP. A natural killer cell line and a HeLa cell line were incubated with solutions of DCVJ and the uptake of the dye was measured using confocal microscopy and multiphoton microscopy. Both cell lines exhibited a punctate spatial arrangement of the dye within the cells with the nucleus remaining essentially unstained. The DCVJ fluorescence decay profiles can be adequately fitted to a biexponential model. The FLIM images show a uniform distribution of both fluorescence lifetimes across the dyed regions of the cells. The presence of two lifetimes is indicative of two environments being sampled by the dye. These results demonstrate the benefits of using time-resolved fluorescence lifetime data for measurements of viscosity using molecular rotors.