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Multi-photon Fluorescence Recovery After Photobleaching Applied to Systems Confined in One, Two or Three Dimensions[1] KELLEY D. SULLIVAN, EDWARD B. BROWN, University of Rochester — Multi-photon fluorescence recovery after photobleaching is a microscopy technique used to measure the diffusion coefficient of macromolecules in biological systems. As multi-photon fluorescence recovery after photobleaching is introduced into more systems in vivo, the need arises to adapt the technique for application to a wider range of physiological situations. In this talk, we present our findings into measuring diffusion coefficients in systems with boundary conditions on the order of the size of the focal volume. Using Monte Carlo methods, we model both diffusion and the fluorescence recovery process in systems where diffusion is limited in one, two and finally all three dimensions. We compare our calculations with experiments where diffusion is limited in one dimension by one and two boundaries. From our results, we can define where boundaries are sufficiently far away to apply multi-photon fluorescence recovery after photobleaching with the conventional analysis; where boundaries are too close to use the technique at all; and an intermediate range of boundary locations, where measurements of the diffusion coefficient are possible with minor modifications to the fluorescence recovery analysis.

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