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Boundary effects in FRAP recovery in the confined geometries of animal, plant and fungal cells J. KINGSLEY, — WPIFluorescence Recovery After Photobleaching (FRAP) has been an important tool for cell biologists to study diffusion and binding kinetics of proteins, vesicles and other molecules. In FRAP, a laser is used to photobleach a target area, and the transport of fluorescent molecules into the bleached area is used determine their diffusion coefficient and bound fraction. While many FRAP models have been developed to assist in analysis, the influence of complex boundaries and optical effects has been largely neglected. Here, we developed a three-dimensional computational model of the FRAP process, incorporating particle diffusion, cell boundary effects, and the optical properties of the microscope, and validated this model using thetip- growing cells of Physcomitrella patens. We show that these effects confound FRAP analysis, affecting the apparent bound fraction and number of dynamic states of a fluorescent protein. Finally, we illustrate how existing theoretical and computational models perform in each of these scenarios, and provide guidelines on how to use FRAP quantitatively in prohibitive geometries.

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