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Single-image diffusion coefficient measurements of proteins in free solution SHANNON KIAN ZAREH, MICHAEL DESANTIS, JONATHAN KESSLER, YAN MEI WANG, Washing University in St. Louis, Physics department — Diffusion coefficient measurement of biomolecules is important for particle size determination, reaction rate characterization, and molecular dynamics investigation. Here we present a simple and fast method for determining diffusion coefficient of nanometer- and sub-nanometer-sized fluorophores, such as GFP, in free solution by analyzing their single fluorescence images with sub-millisecond exposure times. In this method, the standard deviation (SD) of a diffusing molecule's intensity profile is used to determine its diffusion coefficient. Our SD vs. diffusion coefficient expression is consistent with our simulation and experimental measurement results, rendering this sub-millisecond-long method to be an improvement of at least 100-fold in temporal resolution over current diffusion coefficient measurement methods, such as single-particle-tracking and FCS.

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