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## Single Molecule Confocal Fluorescence Lifetime Correlation Spectroscopy for Accurate Nanoparticle Size Determination

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For characterization of individual nanoparticles (NPs) and molecules, electron microscopies such as scanning electron and transmission electron microscopy and scanning probe microscopies such as atomic force microscopy and scanning tunneling microscopy have been employed for structural analysis at the nanometer and sub-nanometer spatial resolution. A variety of analytical spectroscopy tools such as X-ray photoelectron spectroscopy, secondary ion mass spectroscopy, and nuclear magnetic resonance have also been instrumental to assess NP chemical compositions and structural details. However, the sensitivity of these techniques is limited to ensemble-averaged measurements, and samples need to be immobilized on a substrate or in a thin film for the measurement. On the other hand, optical measurements such as dynamic light scattering and fluorescence correlation spectroscopy allow for the non-invasive assessment of the physico-chemical properties of single molecules and NPs in solution. We report on an experimental procedure in confocal single molecule fluorescence lifetime correlation spectroscopy (FLCS) to determine the range of excitation power and molecule concentration in solution under which the application of an unmodified model autocorrelation function is justified. This procedure enables fitting of the autocorrelation to an accurate model to measure diffusion length and diffusion time of single molecules in solution. This procedure determines a set of experimental parameters with which the Stoke-Einstein equation accurately measures the hydrodynamic radii of spherical nanoparticles, enabling the determination of the particle size range for which the hydrodynamic radius by the S-E equation measures the real particle radius.