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Feedback-driven tracking and trapping in confocal fluorescence microscopy[1]

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In comparison to wide-field microscopy, confocal fluorescence microscopy offers superior signal-to-noise as well as sub-nanosecond time-resolved capabilities for studies of single molecules in solution. However, Brownian diffusion limits the observation time of a molecule within the confocal volume; immobilization of a molecule to a surface alters its local environment and may stereo-chemically restrict interactions; and optical trapping of a nanoscale object requires intensities that may give perturbations due to heating. This talk reviews feedback-driven tracking and trapping, which entail real-time adjustment of the position or motion of the fluorescent target with respect to the confocal volume in response to a measurement of its position, and which largely avoid the aforementioned limitations. Such methods enable prolonged observations of an individual molecule or nanoparticle and can also record the spatial domain that is explored and monitor changes in the diffusional and/or directed motion. The talk will also discuss our recent work on trapping and tracking of nanoparticles in three dimensions, using astigmatic imaging or spatially and temporally modulated laser excitation to estimate the position of the nanoparticle and 3D-piezo or microfluidic manipulation to re-center the target in the confocal volume.

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