Abstract
Recent breakthroughs in optical microscopy are allowing chemical analysis to be carried out with unprecedented spatial resolution (in fL volumes) and sensitivity (at the single-molecule level). To investigate the chemistry of small suspended particles in liquids, 'optical tweezers' are combined with Raman-scattering microscopy for single-particle chemical analysis. Sub-micrometer particles can be trapped and observed for hours allowing changes in chemical structure to be followed over time. Adaptation of this method to measuring the structure and contents of individual lipid vesicles will be presented, with applications in sensitive detection in very small volumes. High-throughput optical microscopy can also be applied to fluorescence imaging, where detection limits have reached the single-molecule level. This capability allows quantitative analysis of molecular populations to be based on counting single molecules and characterizing individual molecular binding events. Discrete binding of single molecules can be observed in 'movies' of reversible chemical interactions. Binding isotherms and equilibrium constants are determined by counting bound molecules, without need for signal calibration. Rates of unbinding are measured directly from histograms of off-times in fluorescence images. An application of this concept to investigation of the kinetics of signaling-peptide binding to lipid bilayers will be discussed.