A universal particle image correlation spectroscopy (UPICS) for the analysis of fast and densely diffusing particles

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With the advance of single molecule microscopy in medicine and biophysics there is growing demand for high accuracy analysis tools of fluorescent probes in tissue, cells, membranes or artificial media. Yet, the precision of diffusive parameters derived by conventional methods that rely on tracing the trajectories of molecules (single particle tracking, SPT) decreases with too high population numbers, thereby imposing limitations both on experimental design and statistics. To overcome these issues, particle image correlation spectroscopy (PICS) [1] has recently been introduced.
Building upon PICS we developed an algorithm applicable to all experimental situations without the need of approximations or any a priori input regarding potentially unknown system-inherent parameters. Theory and treatment of the correlation background yields high-output step length distributions and further provides information about the shape and particle distribution in the observed system.
By employing Monte Carlo simulations we compare UPICS to both classical and statistical SPT in a wide range of potential experimental situations. The results of the analyses show that UPICS works accurate to levels unreachable by SPT even when faced with blinking, bleaching and limitations by the size of the field of view.

Reference:
[1] S. Semrau and T. Schmidt, Biophys. J., 2007, 92(2):613-21