High-order image correlation spectroscopy for fluorescent nanoparticle microscopy

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High-order image correlation spectroscopy (HICS) [1] or fluorescence cumulant analysis (FCA) [2] are the variants of correlation spectroscopy (FCS or FFS) that utilises emission signal moments and cumulants of high-order, (i.e., *n*th order central moment μ_n and cumulant κ_n) to extract information on dynamics of aggregate- or hetero-species [1]. Such techniques have seen application in detecting cell-signalling at the surface via receptor aggregation, localising the signalling pathways, or accurately quantifying nanoparticle uptake in cells [1,2]. While proven useful, its experimental validity has been limited to fluorophores with well-defined quantum yield (QY) of emission. Fluorescent nanoparticles such as silicon, gold, or semiconducting nanoparticles have large dispersion in the emission QY even at small variation in size, which makes correlation spectroscopy based on these emitters difficult and unpredictable. In order to address this issue, a modified theory of HICS and FCA are provided here, in which the emission QY for hetero-species is allowed to distribute within probabilistic bounds (utilising central moments and cumulants of the QY distribution). These values can be readily measured from a batch of sample. The modified new theory of HICS is tested against randomly positioned scattering plasmonic nanoparticles with monomeric and dimeric species with distributed emission QY. Numerical simulations of randomly positioned nanoparticle microscopic images show that the modified theory of high-order moment/cumulant analysis is able to accurately predict the number of particles while the original HICS formulation could not (Fig.1). In this paper, we validate the theory extensively through numerical and experimental means (gold nanoparticles). This new modified theory can be extended to any emissive species with distributed QY.

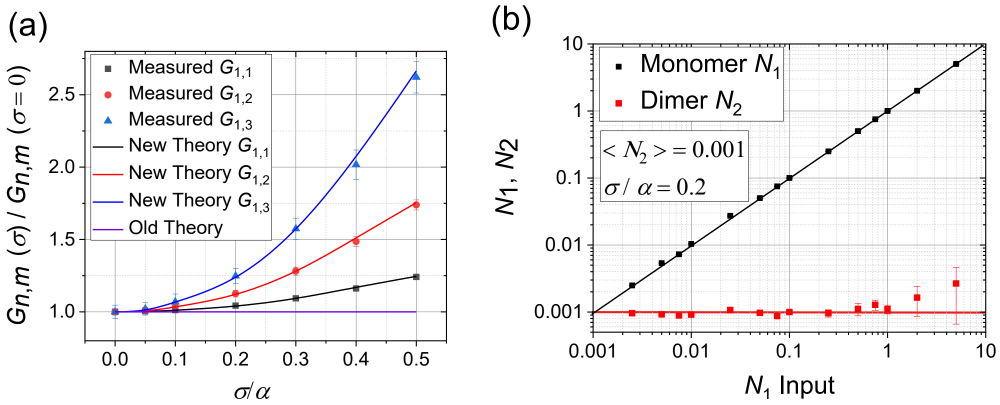

Figure.1 (a) Correlation function $G_{n,m}$ of high-order microscopy images of gold nanoparticles with spread in QY (normalised standard deviation of emission σ on mean emission intensity α, in comparison to the new modified HICS. The modified theory lines are overlaid and show good agreement. (b) Measure of monomer and dimer numbers of gold nanoparticles within an image using the new modified HICS theory, in comparison to input simulation values, showing good agreement.

[1] D. Katoozi, A. H. A. Clayton, D. J. Moss and J. W. M. Chon, *Biomed. Opt. Express* **100**, 187 (2020).

[2] J. D. Mueller, *Biophys. J.* **86**, 3981 (2004)