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Analytical methods based on single particle counting with microscopic imaging

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Single-particle based counting is but one approach to achieve high sensitivity hopefully without the aid of target amplification. In the efforts towards single particle counting based biosensing, we have developed algorithm for dark-field microscope imaging based gold nanoparticle counting. By calibration using reference gold nanoparticles, the counting accuracy was improved, and the versatility of the established counting platform was demonstrated with applications for nucleic acids and biomarker detection. With very simple experimental implementation with target or signal amplification, high sensitivity with a limit of detection as low as femtomolar was achieved for nucleic acid detection. In many situations such as diagnosis and therapy of human diseases, the sensitive multiplexed detection of targets in a single sample by a simple manner is most desirable. To extend the counting strategy to multiplexed detection, an automatic fluorescent nanoparticle (FNP) counting platform was developed with a common fluorescence microscopic imaging setup for non-amplification multiplexed detection of nucleic acids. Quantification for multiplex nucleic acids, including DNAs, microRNAs (miRNA), as well as the DNA and miRNA mixture was achieved with the limit of detection down to 5 amol (5- L detection volume), and the 81–115% spike recovery from different biological sample matrices. We further developed a multiplex SNV detection method that combines the masking tactic with FNP counting based on the sandwich design. With the discrimination factor ranged from 204 to 1177, as low as 0.05% abundance was successfully identified for most cases. The single nanoparticle counting with microscopic imaging shows potentials for simple and highly sensitive biosensing applications. Efforts are under the way to further improve the counting fidelity and sensitivity by working on the counting algorithm, nanoparticles and strategies.

Biography

Na Li is a Professor at the College of Chemistry and Molecular Engineering, Peking University. She obtained her BS (1988), MS (1991) and PhD (1994) degrees from Peking University. Her current research interests are optical sensing methods and applications with the major interest in nanoparticle-based spectroscopy (fluorescence, localized surface plasmon resonance absorption or light-scattering) and the application in biosensing.

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