Kinetic fingerprinting of DNA binding at single-molecule level with single-base-mismatch specificity by interferometric scattering microscopy

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While single-molecule optical techniques overcome limitations of ensemble-averaged assays, their sensitivity is compromised by background noise and signals arising from nonspecific binding events. Here, we present a single-particle kinetic fingerprinting (SPKF) platform based on interferometric scattering microscopy (iSCAT) for digital, ultrasensitive detection of single-stranded DNA at the single-molecule level. By combining gold nanoparticle–enhanced scattering, oblique illumination to reduce background noise, controlled microfluidic flow to accelerate mass transport, and a two-step kinetic fingerprinting strategy to discriminate nonspecific interactions, the SPKF assay enables direct visualization and massively parallel counting of individual specific binding events. Specifically, the algorithm first isolates diffusing particles via correlation-time gating, then discriminates specific from nonspecific interactions through dynamic dwell-time analysis, thereby achieving a limit of detection (LOD) of ~40 aM and single-base-mismatch discrimination. Collectively, this works establishes a general platform for ultrasensitive and highly specific biosensing, leveraging high spatiotemporal resolution of iSCAT and kinetic analysis at the single-molecule level.