

Direct Single-Molecule Counting in Application to Diagnostic and Blood Screening Assays

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Single-molecule methods offer detailed information about molecular structure, dynamics or interactions that cannot be obtained in ensemble measurements. This is possible because of the ability to observe one molecule of interest at a time and/or to register spatially separated individual molecules rather than averaging over a group. Clearly, if individual molecules can be observed, then they can be counted, opening the possibility for a digital approach where the simpler requirement of distinguishing between ones and zeroes can reduce the relative detection noise. Using digital filters to minimize signal noise and thus improve assay sensitivity extends the limits and improves robustness of diagnostic and blood screening assays.

Sensitive assays are typically performed in the immunometric assay format requiring at least two antibodies binding the same analyte molecule and forming a sandwich. Usually, one of the antibodies is immobilized on a solid phase, e.g. microparticles, and the second antibody is conjugated with a signal-generating molecule(s). Using magnetic microparticles enables efficient reagent mixing/washing and empowers assay automation.

Super-bright conjugates, made by combining multiple phycoerythrin molecules with a dextran-BSA-antibody scaffold, enable imaging of individual antibody sandwiches directly on the microparticle surface using an epifluorescence microscope. It is also possible to elute signal generating molecules off the microparticles and transfer them to a glass surface for counting using single-molecule TIRF. Either method allows for a digital analysis of the binding data. Using different fluorophores in the imaging-based assays permits multicolor detection and multiplexing.

Using HIV P24 and HCV core antigen assays as examples, we show that single-molecule counting greatly improves the assay sensitivity and extends it to the low femtomolar range. Single-molecule detection techniques offer both sensitivity and flexibility, and thus, will benefit the fields of immunodiagnosics and blood screening.

These results will be presented in the larger context of a review of ultrasensitive detection methods including chemiluminescence, FCS-like flow/scanning, nanopores, and single-molecule ELISA.