DIRECT SINGLE-MOLECULE COUNTING FOR DIAGNOSTIC AND BLOOD SCREENING ASSAYS

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KEY WORDS: Single-molecule counting, digital immunodiagnostics, super-bright conjugates, smTIRF.

Every year over a hundred million units of donated blood undergo mandatory screening for HIV, hepatitis B, hepatitis C and syphilis worldwide. Often, donated blood is also screened for other pathogens such as HTLV, Chagas, Dengue and Malaria. Several billion diagnostic tests are performed annually around the world to measure more than four hundred biomarkers for cardiac, cancer, infectious and other diseases. Considering such volumes, every improvement in assay performance and/or throughput has a major impact.

Here we show that medically-relevant assay sensitivities and specificities can be improved up to 10-fold by direct single-molecule imaging using regular epifluorescence microscopes. In current microparticle-based assays, an ensemble of bound signal-generating molecules is measured as a whole. Instead, we acquire intensity profiles to identify and then count individual fluorescent complexes bound to targets on antibody-coated microparticles. This increases the signal-to-noise ratio and provides better discrimination over non-specific effects. It brings the detection sensitivity down to the zeptomolar (10^{-21} M) for model assay systems, and to the low femtomolar (10^{-16} M) for measuring analyte in patient samples. Transitioning from counting single-molecule peaks to averaging pixel intensities at higher analyte concentrations enables a continuous linear response from 10^{-21} to 10^{-5} M.

Emerging research for counting eluted labels in solution or on surfaces uses confocal or TIRF microscopy. Single-molecule ELISA also offers single-molecule sensitivity. However, these approaches require additional steps, microfluidic channels or nanowells. Direct imaging of single-molecules on microparticles is straightforward and thus more promising for clinical implementation. Moreover, single-molecule imaging-based detection simplifies the requirements for analyte capture and sandwich formation protocols. Highly sensitive assays can be performed faster with reduced or even with no washing steps because background and ambiguous peaks or clusters of pixels can be filtered out by image analysis algorithms. Additionally, our assays become insensitive to microparticle number and volume variations during the binding reaction, eliminating the main source of uncertainties in standard assays. Highly reproducible detection can be achieved by imaging fewer than two thousand microparticles. Altogether, these features allow for increased assay sensitivity, wide linear detection ranges, shorter incubation times, simpler assay protocols and minimal reagent consumption.