

Scientific Principle of Simoa™ (Single Molecule Array) Technology

Traditional ELISA (analog) readout systems require large volumes that ultimately dilute reaction product, requiring millions of enzyme labels to generate signals that are detectable utilizing conventional plate readers. Sensitivity is therefore limited to the picomolar (i.e., pg/mL) range and above. Single-molecule analysis provides a resolution that simply cannot be obtained with bulk ensemble measurements. Single molecule measurements are digital in nature: Each molecule generates a signal that can be counted. It is much easier to measure the presence or absence of signal than to detect the absolute amount of signal—that is, counting is easier than integrating.

Quanterix has developed an approach for detecting thousands of single protein molecules simultaneously. Using the same reagents as a conventional ELISA, this method has been used to measure proteins in a variety of different matrices (serum, plasma, cerebrospinal fluid, urine, cell extracts, etc.) at femtomolar (fg/mL) concentrations, offering a roughly 1000-fold improvement in sensitivity. This approach makes use of arrays of femtoliter-sized reaction chambers, which are termed **single-molecule arrays** (Simoa™), that can isolate and detect single enzyme molecules. Because the array volumes are approximately 2 billion times smaller than a conventional ELISA, a rapid buildup of fluorescent product is generated if a labeled protein is present. With diffusion defeated, this high local concentration of product can be readily observed. Only a single molecule is needed to reach the detection limit (Fig. 1).

In the first step of this single-molecule immunoassay, antibody capture agents are attached to the surface of paramagnetic beads (2.7 μm diameter) that will be used to concentrate a dilute solution of molecules. The beads typically contain approximately 250,000 attachment sites, so one can think of each bead as having a “lawn” of capture molecules. The beads are added to the sample solution such that there are many more beads than target molecules. Typically 500,000 beads will be added to a 100-μL sample.

Adding so many beads confers two advantages. First, at a roughly 10:1 bead-to-molecule ratio, the percentage of beads that contain a labeled immunocomplex follows a

Poisson distribution. At low concentrations of protein, the Poisson distribution indicates that each bead will capture either a single immunocomplex or none. For example, if 1 fM of a protein in 0.1 mL (60,000 molecules) is captured and labeled on 500,000 beads, then 12% of the beads will carry one protein molecule and 88% will not carry any protein molecules. Second, with so many beads in solution, the bead-to-bead distance is small, so that every molecule encounters a bead in less than a minute. At this time scale, diffusion of the target analyte molecules, even large proteins, occurs quickly, and in theory all the molecules should have multiple collisions with multiple beads.

In this manner, the slow binding to a fixed capture surface is avoided and the efficiency of binding increases dramatically. The beads are then washed to remove nonspecifically bound proteins and incubated with biotinylated detection antibody and then with β-galactosidase-labeled streptavidin. In this manner, each bead that has captured a single protein molecule is labeled with an enzyme. Beads that do not capture a molecule remain label-free.

Rather than an ensemble readout, beads are loaded into arrays of 216,000 femtoliter-sized wells that have been sized to hold no more than one bead per well (4.25 μm width, 3.25 μm depth) (Fig. 2). Beads are added in the presence of substrate, and wells are subsequently sealed

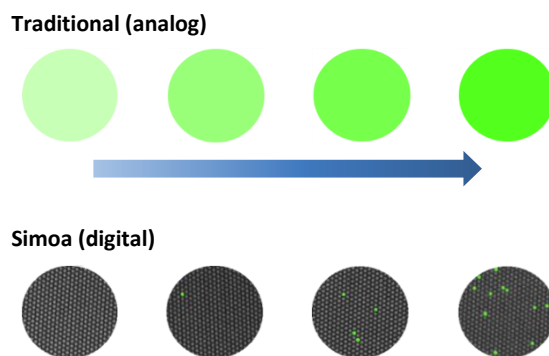


Figure 1. Top, Analog measurements give increasing intensity as the concentration increases. Bottom, In contrast, digital measurements are independent of intensity and simply rely on a signal/no signal readout.

with oil and imaged. Simoa permits the detection of very low concentrations of enzyme labels by confining the fluorophores generated by individual enzymes to extremely small volumes (~40 fL), ensuring a high local concentration of fluorescent product molecules. If a target analyte has been captured (that is, an immunocomplex has formed), then the substrate will be converted to a fluorescent product by the captured enzyme label (Fig. 3). The ratio of the number of wells containing an enzyme-



Figure 2. Simoa disc containing 24 array assemblies arranged radially. Each array contains 216,000 femtoliter-sized wells, which can contain individual beads with or without an associated immunocomplex.

labeled bead to the total number of wells containing a bead corresponds to the analyte concentration in the sample. By acquiring two fluorescence images of the array, it is possible to demonstrate an increase in signal, thereby confirming the presence of a true immunocomplex, and beads associated with a single enzyme molecule (an “on” well) can be distinguished from those not associated with an enzyme (an “off” well). The protein concentration in the test sample is determined by counting the number of wells containing both a bead and fluorescent product relative to the total number of wells containing beads. Because Simoa enables concentration to be determined digitally rather than by measurement of the total analog signal, this approach to detecting single immunocomplexes has been termed **digital ELISA**. The ability of digital ELISA to measure much lower concentrations of proteins than conventional ELISA derives from two effects: (1) the high sensitivity of Simoa to enzyme label and (2) the low level of background signal that can be achieved by digitizing protein detection. For antibodies of given affinity, the sensitivity of the immunoassay will be determined by the assay background. The high label sensitivity and decreased label concen-

tration help reduce nonspecific binding to the capture surface, resulting in much lower background signal.

The Simoa technology at the heart of the platform developed by Quanterix will enable the detection and quantification of biomarkers previously difficult or impossible to measure, opening up new applications to address significant unmet needs in life science research, biopharma, and in-vitro diagnostics. For example, fewer than 150 proteins having FDA approval are in use today, yet the human proteome contains over 2,500 secreted proteins. Most of the “missing” proteins are simply below the detection limit of the best ELISAs. Consequently, more sensitive measurements will likely result in earlier cancer detection, earlier detection of infectious disease, and identification of a host of new biomarkers with utility for in vitro diagnostics and companion diagnostics.

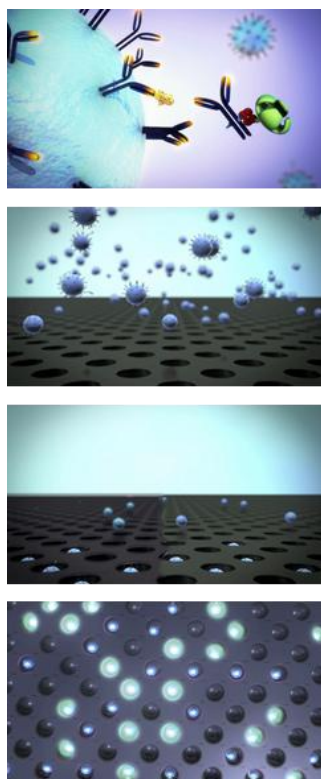


Figure 3. Loading, sealing, and imaging of single paramagnetic beads in arrays of femtoliter-sized wells. (A) Beads, a fraction of which are associated with captured and enzyme-labeled protein molecules, are introduced into the array. (B) Beads settle by gravity onto the surface of the array, and a fraction of them fall into microwells. The remainder lie on the surface. (C) Oil is introduced into the channel to displace the aqueous medium and excess beads and seal the wells. (D) Sealed wells are imaged. Fluorescent signals are generated in sealed wells that contain beads associated with captured and enzyme-labeled protein molecules.

For more information, visit www.Quanterix.com or email us at Sales@Quanterix.com.