

Multiparameter single-particle motion analysis for homogeneous digital immunoassay

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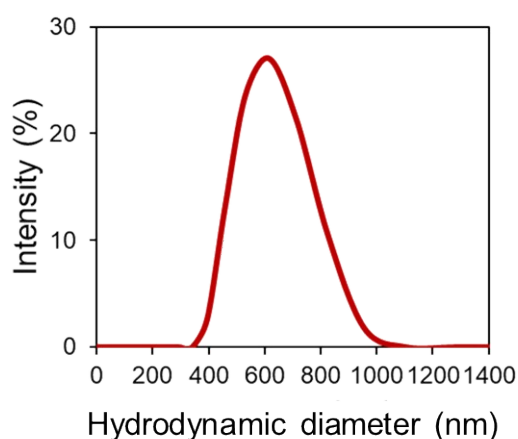


Figure S1. □ The size distribution of magnetic nanoparticles measured with zetasizer (ZSP, Malvern).

Supplementary Text 1

To evaluate the performance of multiparameter analysis for clinical test, we measured and analyzed the serum samples.

A PSA-free human serum was purchased from Sunfco Ltd (Tokyo, Japan). The PSA spiked into the dilution buffer or human serum was prepared as a sample solution. After the spike, all samples were stored for over 3 days at 4 °C. A total of 80 μL of the bead solution containing 1.0×10^6 beads, which were coated by anti-PSA antibodies, was added to 10 μL of the sample solution and incubated at 25 °C for 1 h. A 90 μL volume of the mixed solution of the sample and beads was introduced into the flow cell, which was constructed by assembling FRAD. Then, the flow cell was placed on a magnet, and fluorocarbon oil (FC40) was introduced into the flow cell to flush out the excess aqueous solution and seal the individual reactors. For the antigen–antibody reaction in fL reactors, the flow cell was incubated at 25 °C for 5 min. The beads encapsulated in the FRAD were observed under an inverted microscope (IX83; Olympus, Tokyo, Japan) equipped with a sCMOS camera (Zyla4.2; Andor) and a 40× objective lens (UPLSAPO40 × 2; Olympus, Tokyo, Japan). The sequential bright-field images were acquired at 10 ms time resolution with 10 ms interval time (50 frames in total). The total FOVs was 32, and the total time for imaging of 32 FOVs was within 1 min.

□ The acquired bright-field images were analyzed using ImageJ software. First, an algorithm identified the reactors captured aggregated beads or more than one beads by image thresholding and then eliminated them from the images. Next, the bead tracking analysis was conducted using the TrackMate plugin of ImageJ. At each frame of the sequential images, the centroid coordinates of each bead were calculated by two-dimensional Gaussian fitting. Then, the trajectory of each bead was determined from the centroid coordinates in subsequent frames, and some trajectories with a lack of spots were removed. From centroid coordinates, RMSD and aspect ratio (AR) of tracks were calculated.