Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Digital diffraction detection of protein markers for avian influenza

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Supplementary Figures

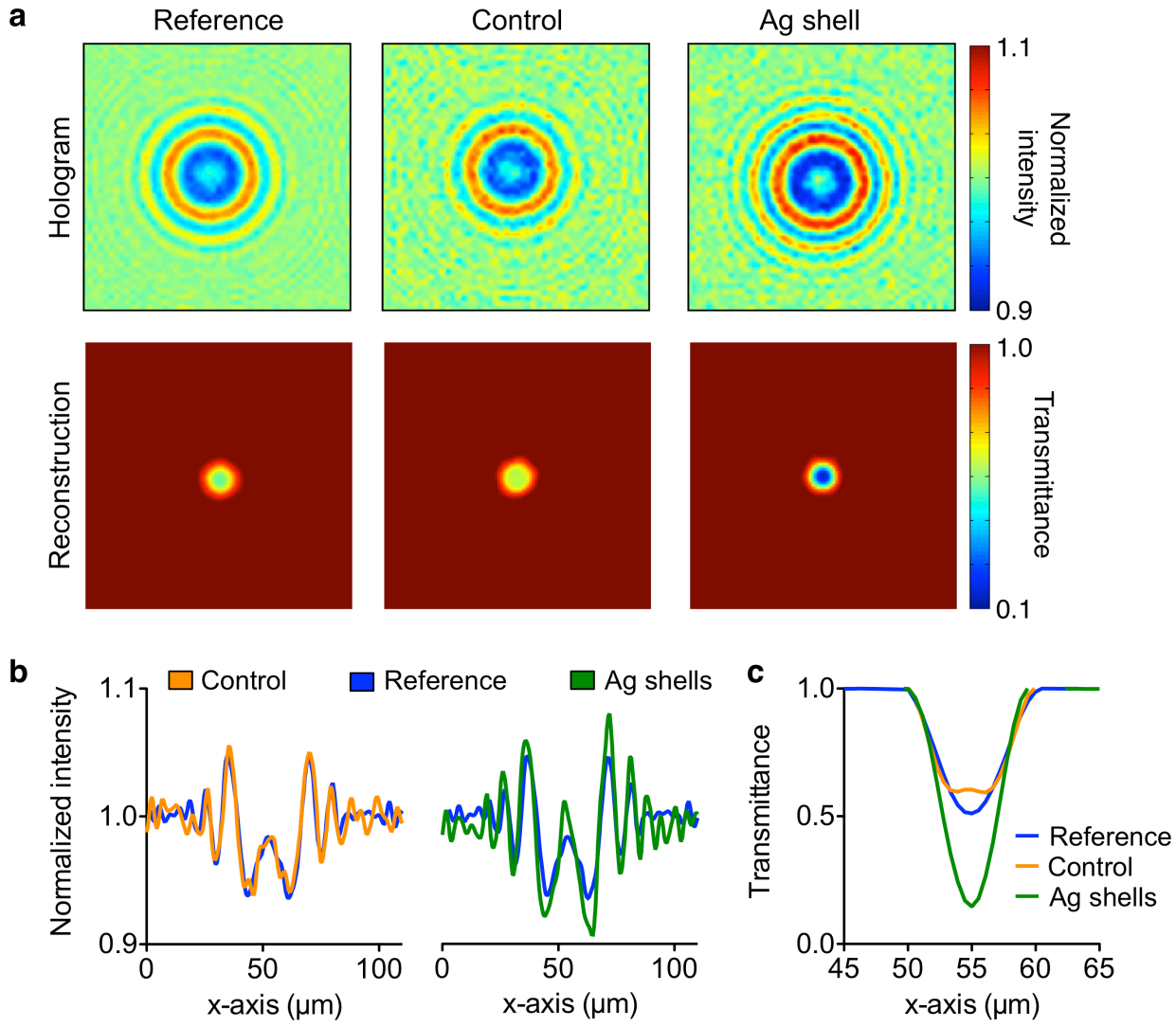

Figure S1. Holographic characterization of 5 *μ*m silica beads with and without Ag shells. (a) Ag-coated silica beads were distinguished from their hologram patterns while the change in a control bead, where non-complementary capture probes were used, was negligible. Corresponding cross-sectional profile of (b) hologram intensities and (c) transmittance of silica beads with and without Ag shells.

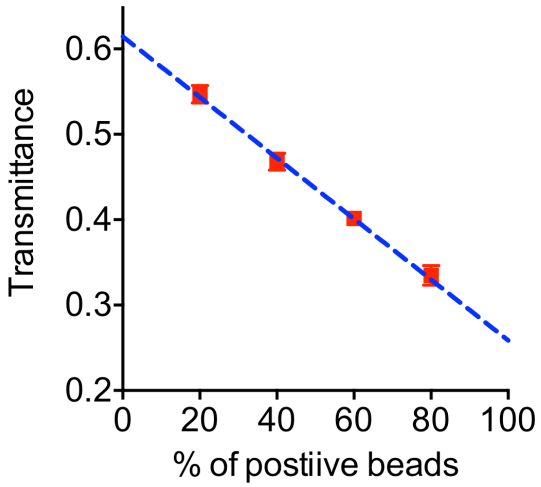

Figure S2. The mean intensities of the bead mixtures. As the proportion of Ag-coated microbeads (positive beads) increased, the mean intensities of the detected beads decreased because of the lower transmittance of Ag-coated beads.

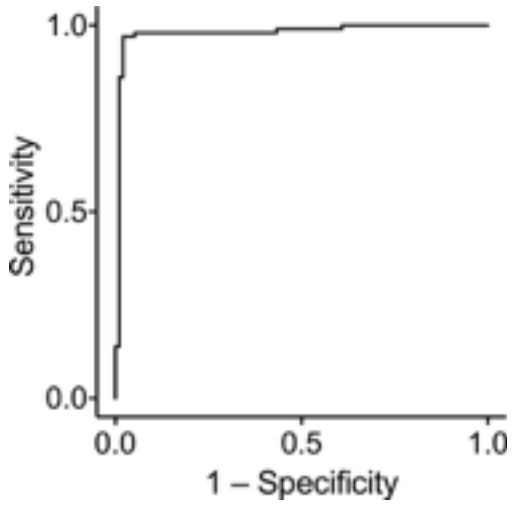

Figure S3. Determination of the transmittance cutoff. A receiver operating characteristic (ROC) curve was generated using transmittance values of Ag-coated (*n* = 97) and non-coated (*n* = 101) We determined the transmittance cutoff (0.45) that maximized both the sensitivity and the specificity.

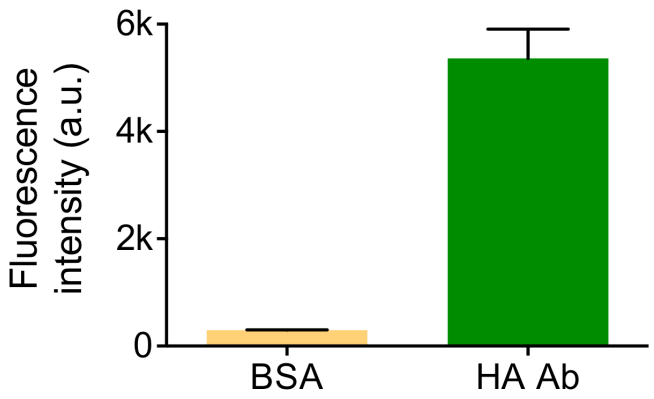

Figure S4. Detection of antibody to avian influenza using the conventional plate reader. Streptavidin-labeled fluorescence dyes were used instead of Au nanoparticles for detection.

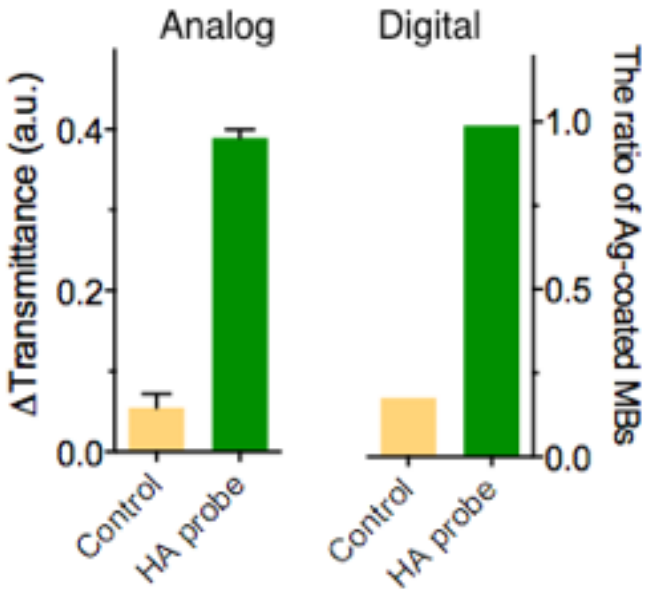

Figure S5. Comparison of analog and digital D3 readouts for the HA-antibody detection. The analog readout is based on the measurement of mean transmittance intensity while the digital readout is based on the bead count of Ag-shell coated beads determined by the threshold transmittance intensity. Both analyses can be performed using the same system.

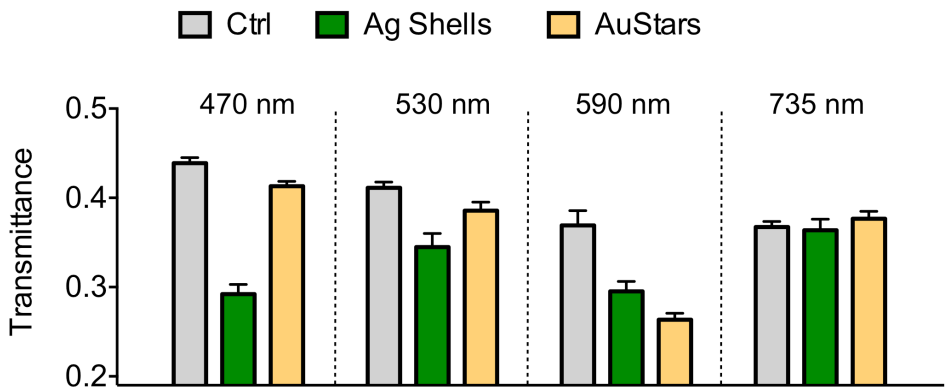

Figure S6. Wavelength optimization for silica beads coated with Ag shells and star-shaped Au nanoparticles. Among the wavelengths tested (470, 530, 590 and 735 nm), the transmittance difference between silica beads and Ag-shell coated beads was the greatest at 470 nm while the difference between silica beads and Au nanoparticle coated beads was the greatest at 590 nm. The difference in the optimal wavelength could be due to the plasmonic resonance properties of Ag shells and star-shaped Au nanoparticles.

Supplementary Tables

Step	Procedure	Time
1	Mix peptide-coated beads with samples	10 min
2	Secondary antibody labeling	10 min
3	AuNPs conjugates	15 min
4	Ag shell growth	5 min
5	Measurement and analysis	3 min
		43 min

Table S1. List of D3 assay procedure and time.