

**Supporting Information for:**

**High-speed lens-free holographic sensing of protein molecules using quantitative agglutination assays**

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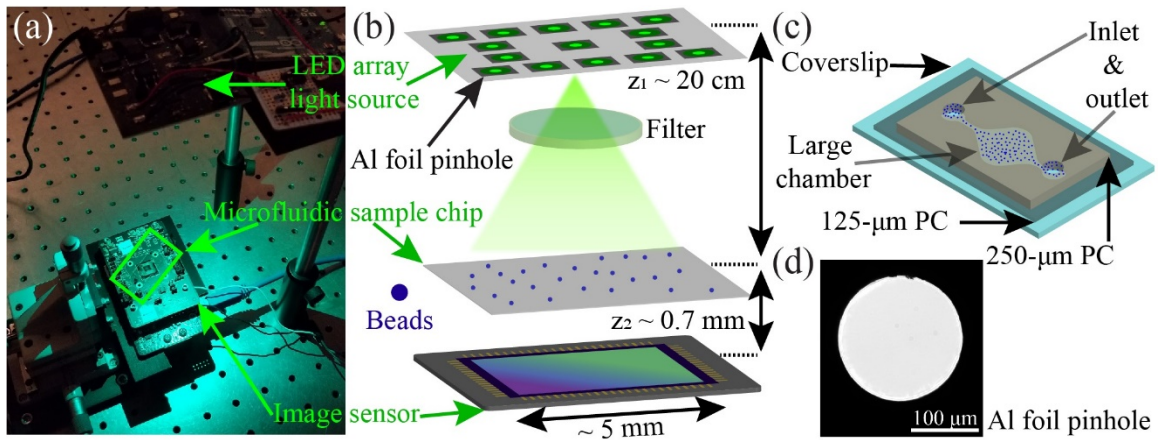


Figure S1: High-speed lens-free holographic microscope. (a) Photograph of our experimental platform. (b) Illustration of the hardware, including a custom LED array, a 3 nm bandwidth bandpass filter, custom aluminum foil pinholes, and a CMOS sensor. (c) Illustration of a MC consisting of two layers of laser-cut polycarbonate films and a glass coverslip. (d) Image of a hand-punched anodized aluminum foil pinhole under a 20X objective.

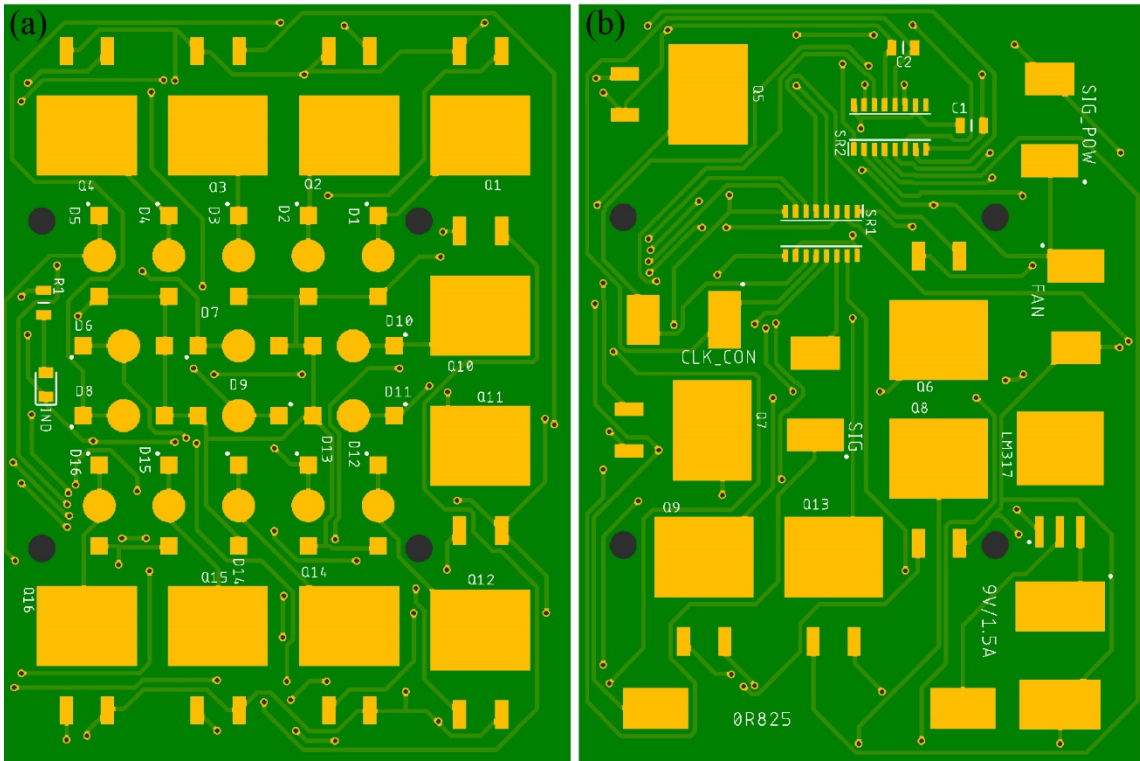


Figure S2: Aluminum-core printed circuit board previews. (a) Front side where high-power LEDs are soldered. (b) Back side where shift registers and other components are soldered. The board dimensions are 67 mm x 89 mm.

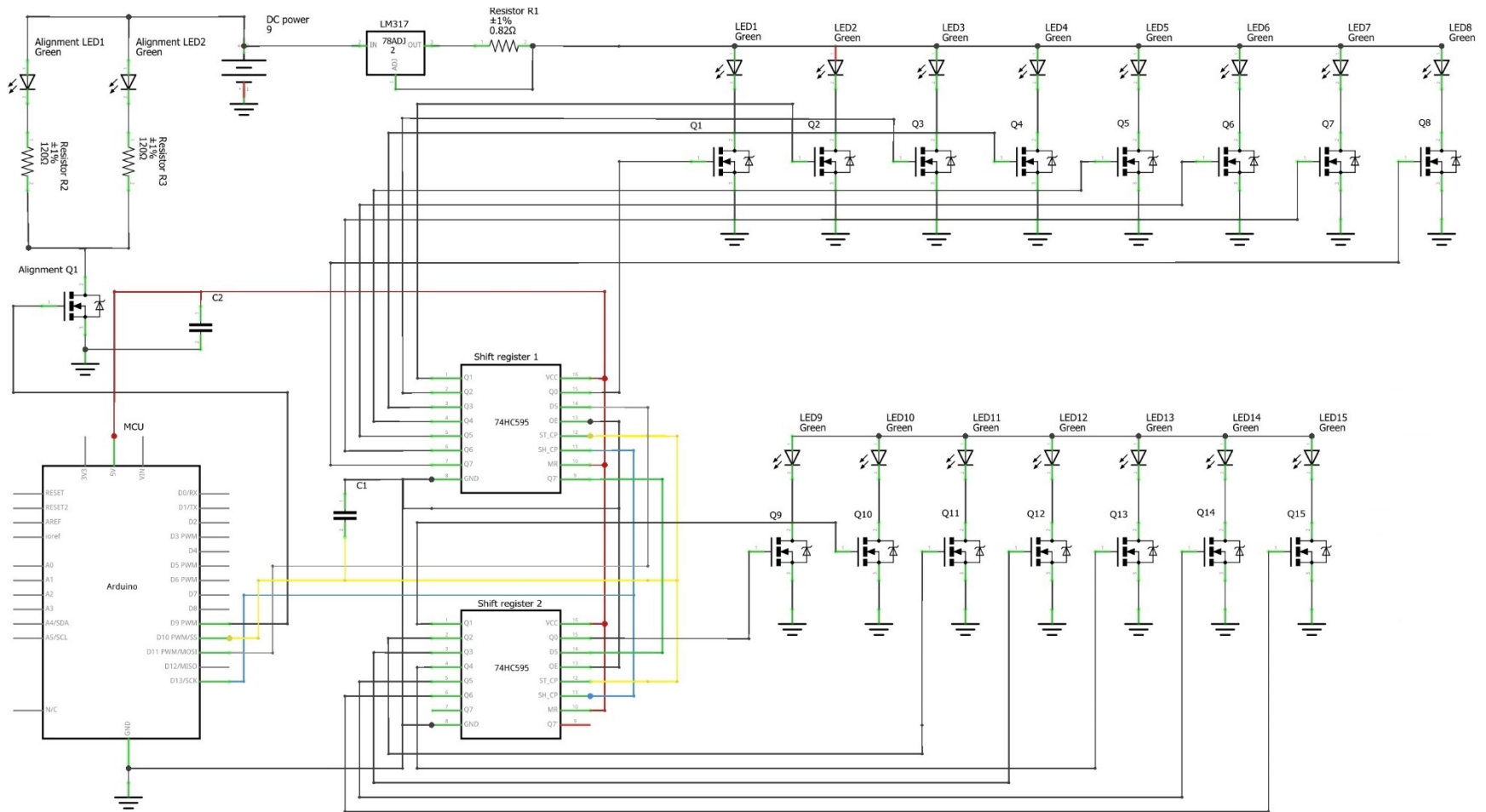


Figure S3: The schematic for our custom high-speed high-power LED array. An LM317 voltage regulator is used with a power resistor R1 to serve as a precision current-limiter circuit, which serves as a 1.5 A current source. An Arduino Leonardo microcontroller is used to communicate with two cascaded 8-bit shift registers (74HC595) via serial peripheral interface. Since 15 LEDs are switched on and off sequentially, the current source is shared among all LEDs. Two cascaded 8-bit shift registers are used to digitally switch 15 LEDs with 15 MOSFETs (STMicroelectronics STP40NF03L). For example, sending 0x02 from Arduino would turn on only LED2.

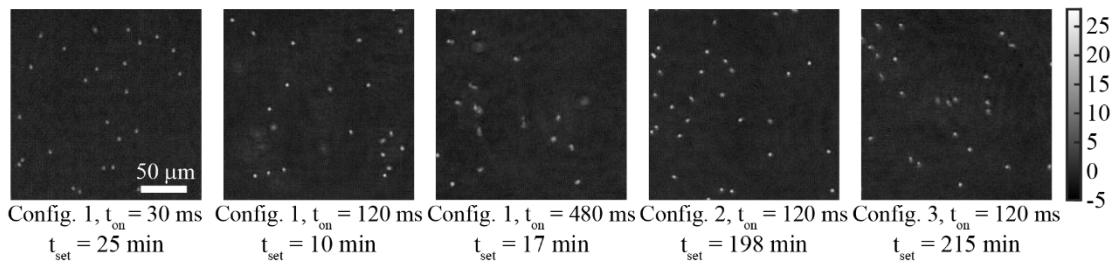


Figure S4: Amplitude reconstructions of 2- $\mu\text{m}$  beads under various conditions. The colorbar is in terms of unitless SNR.

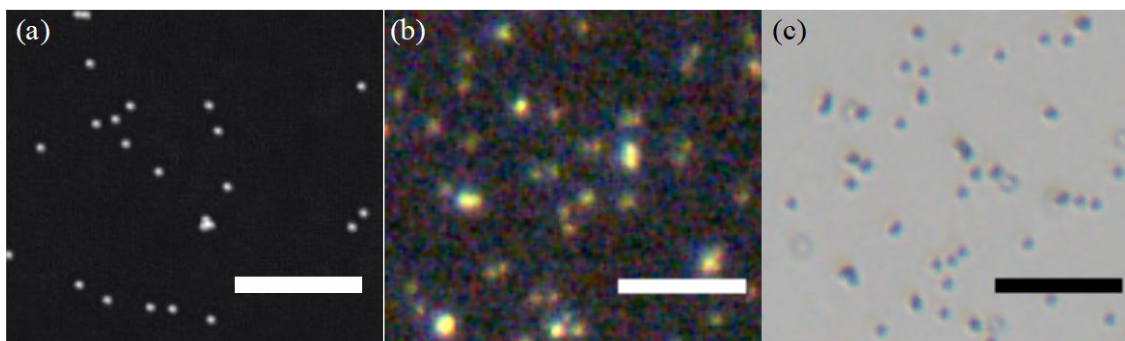


Figure S5: Representative images of 0.01% 2- $\mu\text{m}$  B-beads with 60 ng/mL of NA in PBST using different imaging modalities. (a) LFHM image captured using the QLAB sensor. (b) Darkfield image and (c) brightfield image captured using a high-end Olympus BX-53 microscope with a 4 $\times$  objective. Images captured with LFHM are visibly more resolved and have a significantly higher SNR than those captured by the Olympus microscope. Scale bars are all 50  $\mu\text{m}$  long.

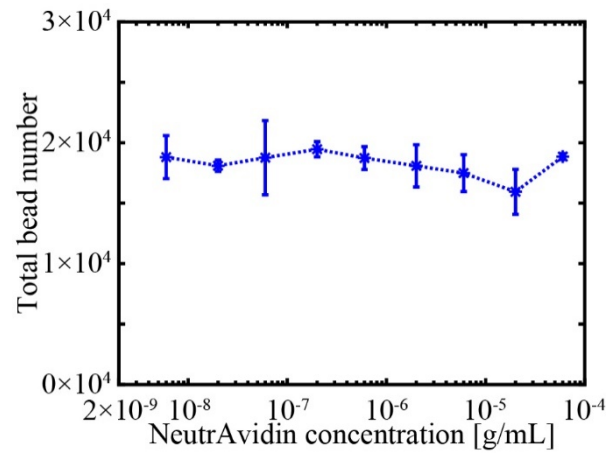


Figure S6: Total counted bead numbers for all NA concentrations in NA batch-to-batch repeatability experiments. The consistency in bead count indicates the repeatability of the process. The coefficient of variation in bead number over all experiments plotted is 9.1%.



Figure S7: Full FOV reconstruction for a sample containing 2  $\mu\text{g}/\text{mL}$  of NA and 0.01% 2- $\mu\text{m}$  B-beads in PBST. This sample has 18268 2- $\mu\text{m}$  B-beads exhibiting a bound ratio of 80.39%.



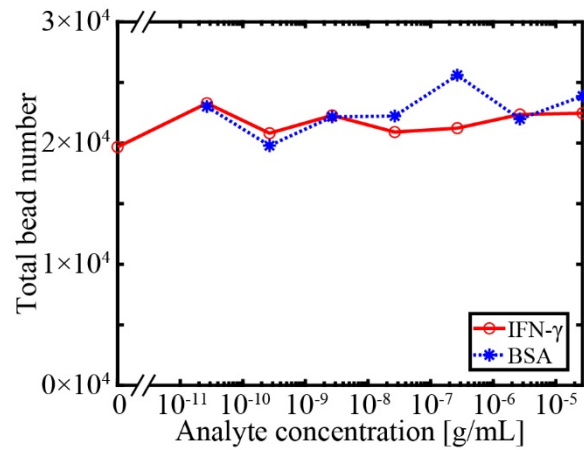


Figure S8: Total bead counts for IFN- $\gamma$  sensing with BSA as a negative control. The consistency across experiments indicates the repeatability of the process. The combined coefficient of variation in bead number for both IFN- $\gamma$  and BSA experiments is 6.9%.