Supporting Information

The potential of backscattering interferometry as an *in vitro* clinical diagnostic tool for the serological diagnosis of infectious disease

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Instrumental Setup:

BSI utilizes a red helium-neon (HeNe) laser (λ = 632.8 nm) to illuminate the microfluidic channel, etched in glass, in a simple optical train (Figure S1). Specifically, the laser is coupled to a collimating lens through a single-mode fiber, producing a 100 μm diameter beam and yielding probe volumes in the 300 picoliter range. Subsequently, when the laser beam impinges the channel and interacts with the fluid contained in the channel, a set of high contrast interference fringes is produced and monitored in direct backscatter region at relatively shallow angles using a linear CCD array. The spatial position of these fringes depends upon the refractive index (RI) of the fluid within the channel. The change in fringe position is monitored in near real-time using a Fourier analysis,[1-3] enabling the quantification of this positional shift as a change in spatial phase, calculated in the Fourier domain. The Fourier transform provides a way to decompose the fringe pattern into its constituent frequencies; revealing periodicities in the fringes as well as the relative strengths of any periodic components. For the fringe pattern created by BSI, there is a single dominant frequency observed. The use of a Fourier transform provides a means to lock in on a specific frequency and extract the phase information. As

the fringes shift, there is no change in the frequency of the pattern; therefore it is possible to calculate the amount of the spatial shift of the fringe pattern as a change in the phase of that frequency.

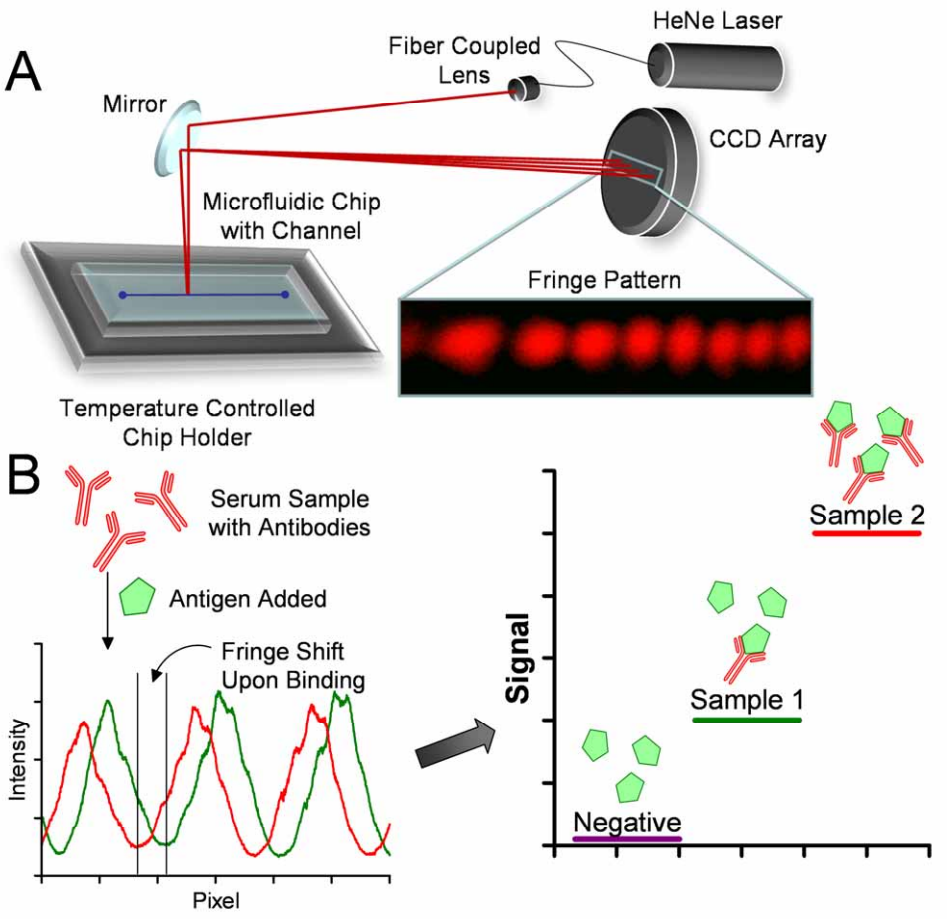

Figure S1: A. Block diagram of Backscattering Interferometry (BSI). B. Cartoon representation of the experiments.

Binding in Buffer:

Affinity-purified total IgG from a rabbit inoculated with *Treponema pallidum* was obtained from the Centers for Disease Control and Prevention (CDC). Increasing

concentrations of total IgG were prepared in phosphate buffered saline (PBS), and each concentration was mixed with either a recombinant treponemal antigen r17 (Genway Biotech, Inc.) or PBS to serve as a calibrator. The final concentrations of the total IgG were 0 – 50 μg/mL and the final antigen concentration was 100 μg/mL. The BSI signal was measured for each sample by introducing them sequentially into the channel and monitoring the response for thirty seconds. The signal from the calibration (IgG with PBS added) was subtracted from the total binding signal (IgG with r17 antigen added) in order to give the specific binding signal. This subtration was designed to exclude the bulk refractive index contributions of the IgG and any RI changes due to nonspecific binding of the IgG. The remaining specific binding signal is the signal arising from the antigen-antibody binding and was plotted versus IgG concentration.

Binding in Spiked Serum:

A nonreactive commercial serum sample obtained from the TrepSure enzyme immuno assay kit (Phoenix Bio-Tech Corporation) was mixed with increasing concentrations of total IgG. Each concentration was then mixed with either r17 antigen or PBS. The final mixtures contained a 1:40 dilution of serum and an r17 antigen concentration of 100 μg/mL with IgG concentrations ranging from 0 to 50 μg/mL. The BSI signal was measured for each sample and the bulk contributions of IgG, serum, and r17 antigen were subtracted in order to obtain the signal created during the binding event. This signal was plotted versus total IgG concentration.

Evalution of Clinical Samples:

Human serum samples previously characterized according to reactivity in the Treponema pallidum passive particle agglutination assay (TP-PA) and the nontreponemal rapid plasma regain (RPR) test were provided by the CDC. The serum samples were first diluted with PBS and were then mixed with antigen (r17 antigen or one of three cardiolipin-based antigens) to yield a solution with a final serum dilution of 1:20 and a final antigen concentration of 5 μg/mL. The binding signal of the interaction, calculated in the same manner as for the calibration experiments, was measured and plotted versus the serum classification. An example time trace of one trial of the binding of the clinical samples to the synthetic MAPS – cardiolipin antigen is shown in Figure S2. For the experiments measuring the interaction of human samples with the r17 antigen, the phase change was normalized to the sample with the highest signal.

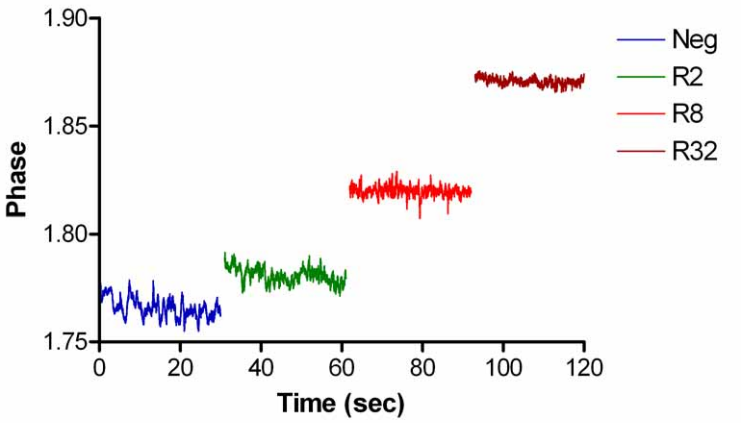

Figure S2: Real-time trace of one trial of the measurement of the binding of the clinical samples to the synthetic MAPS – cardiolipin antigen.

1 D. J. Bornhop, J. C. Latham, A. Kussrow, D. A. Markov, R. D. Jones, and H. S. Sorensen, *Science*, 2007, **317**, 1732.

2 D. Markov, D. Begari, and D. J. Bornhop, *Analytical Chemistry*, 2002, **74**, 5438.

3 D. A. Markov, K. Swinney, and D. J. Bornhop, *Journal of the American Chemical Society*, 2004, **126**, 16659.