Electronic Quantification of Protein Biomarkers Based on Bead Aggregate Sizing

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Current microbiological techniques applied for protein biomarker detection, involved time consuming methods based on sandwich immunoassays [1]. Here, we use our electronic biochip for the rapid detection and quantification of protein biomarkers electronically. We successfully demonstrate proof of concept based on detection of streptavidin-biotin binding.

Figure 1 shows the basic device. Our assay operates by immobilizing beads of different sizes with complementary antibody pairs. The presence of the target antigen will result in binding of the beads of different sizes. The use of electrical impedance cytometry allows for differentiating between unbound beads and beads which are bound to each other. Thus, this technique can be used for biomarker detection, as the number of peaks of bead aggregtes is proportional to concentration of target antigen in the test sample. This electronic biochip (Fig 2a and Fig 2b) consists of two microelectrodes on a glass substrate with the channel above it, which were all formed in a PDMS cover. Electrodes were patterned lithography, gold evaporation, and lift-off processing. For proof of concept, 3um biotin (Fig. 3a) beads and 7um (Fig. 3b) streptavidin beads were diluted and mixed off chip. A portion of the beads will bind together, resulting in effectively larger beads, (Fig. 3c). The beads are then injected into the micro-channel. As the beads passing through the electrodes which are connected to an AC voltage source and lock-in amplifier, there will be a drop in the ionic current. The amplitude of the current peak is a function of the bead's size. Larger size of beads results in bigger current drop compared to smaller size beads.

The assay was confirmed electronically, where the beads in the channel were quantified by the number of peaks and we also record the peak size. The peak were detected by our electronic system which is consists of electrodes and our lock-in amplifier (Zurish Instrument HF2 series). The Frequency and amplitude of the input AC voltage are 300k Hz and 1V. The gain is 1k. The Probability distribution measured as a function of peak amplitude is shown in (Fig 4a) which confirms that bead aggregates can be differentiated from both 7 um and 3um beads. This is evident both from looking at the probability distribution (Fig. 4a) and also the average amplitude of the various bead types with their associated error bars (Fig. 4b).

This platform is capable of continuous, label-free electrical detection, and quantification of target biomarkers. Though, for proof of concept, we focused on streptavidin-biotin binding, we emphasize that this device and method is applicable to all protein biomarkers. Ultimately we envision that electronic detection will enable this to be ultimately used as a wearable miniaturized device.

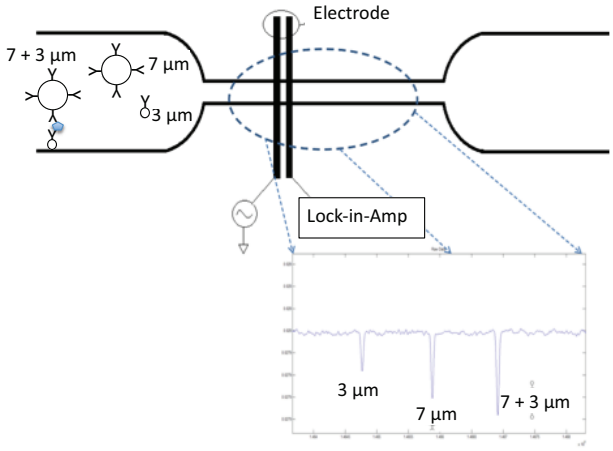

Figure 1: Schematic of microfluidic biochip. Presence of target protein biomarker results in 7um and 3um bead binding together and aggregating. Impedance based sizing allows differentiation between 3 um, 7 um, and 7 + 3 um beads. Proportion of 7+3 um beads quantifies protein biomarker.

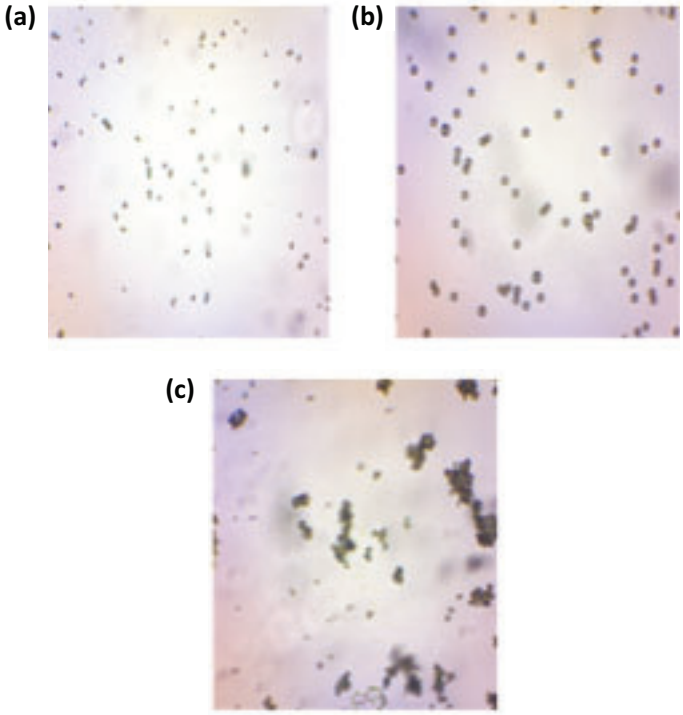

Figure 3: *(a) 3um beads (b) 7um beads (c) 3+7 beads.*

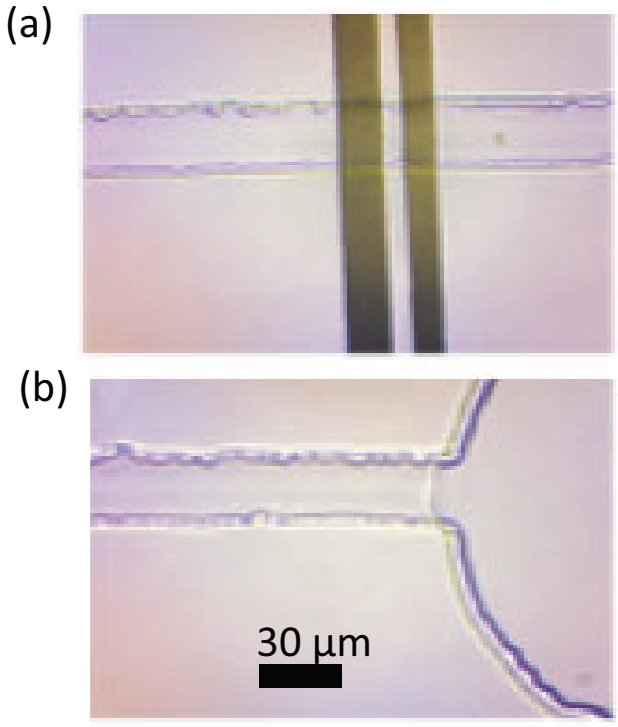

Figure 3: a) The electrodes of the device b) The entrance of the channel.

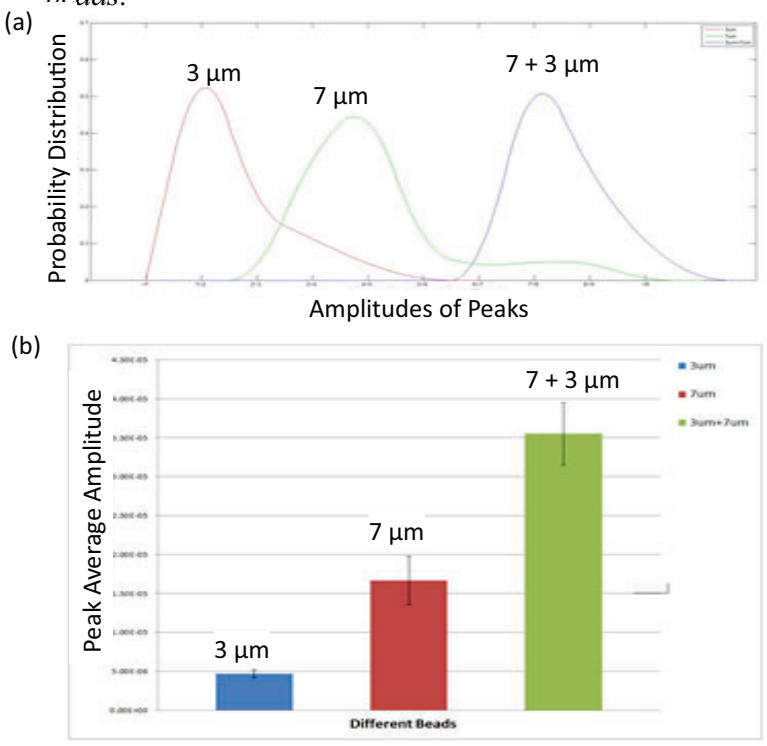

Fig. 4: a) Probability distribution as a function of Peak amplitude for 3 different size beads. We can see lager beads has a larger amplitude
b) Average amplitude of current peak with respect to the beads' size