Video Article

Rapid Homogeneous Detection of Biological Assays Using Magnetic Modulation Biosensing System

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Abstract

A magnetic modulation biosensing system (MMB) [1,2] rapidly and homogeneously detected biological targets at low concentrations without any washing or separation step. When the IL-8 target was present, a 'sandwich'-based assay attached magnetic beads with IL-8 capture antibody to streptavidin coupled fluorescent protein via the IL-8 target and a biotinylated IL-8 antibody. The magnetic beads are maneuvered into oscillatory motion by applying an alternating magnetic field gradient through two electromagnetic poles. The fluorescent proteins, which are attached to the magnetic beads are condensed into the detection area and their movement in and out of an orthogonal laser beam produces a periodic fluorescent signal that is demodulated using synchronous detection. The magnetic modulation biosensing system was previously used to detect the coding sequences of the non-structural Ibaraki virus protein 3 (NS3) complementary DNA (cDNA) [2]. The techniques that are demonstrated in this work for external manipulation and condensation of particles may be used for other applications, e.g. delivery of magnetically-coupled drugs *in-vivo* or enhancing the contrast for *in-vivo* imaging applications.

Video Link

The video component of this article can be found at http://www.jove.com/video/1935/

Protocol

IL-8 assay:

1. The reaction mixture included the following four components in 100 µl final volume of assay buffer in their respective concentrations. 10 µl of magnetic beads with capture antibody at 100 beads/µl final concentrations, 2 µl of biotinylated IL-8 antibody at 1 nano-gram/µl final concentration, 1 µl of streptavidin fluorescent protein at 20 nano-gram/µl final concentration, and 1 µl of IL-8 target at 0.48 pico-gram/µl. The components are added one by one to the assay buffer and are then shaken for 30 minutes.
2. A control reaction is prepared the same way without the IL-8 target.
3. The reactions are later placed without any separation or washing step in the cuvettes and inspected using the MMB system.

MMB system:

1. Place the cuvette in its position between the two electromagnetic poles.
2. Operate the modulation current and wait 30 seconds to allow aggregation and condensation of the beads
3. Measure the signal of the lock-in amplifier using an oscilloscope.

Representative Results:

Visual inspection of the aggregated beads presented a distinct difference between the reaction with the target and the reaction without the target. In all the reactions with the target, the beads formed a single, dense aggregate that moved in and out the laser beam in a unite manner. However, in all the reaction without the target, the beads were less aggregated and their motion was less unite (see Figure 1).

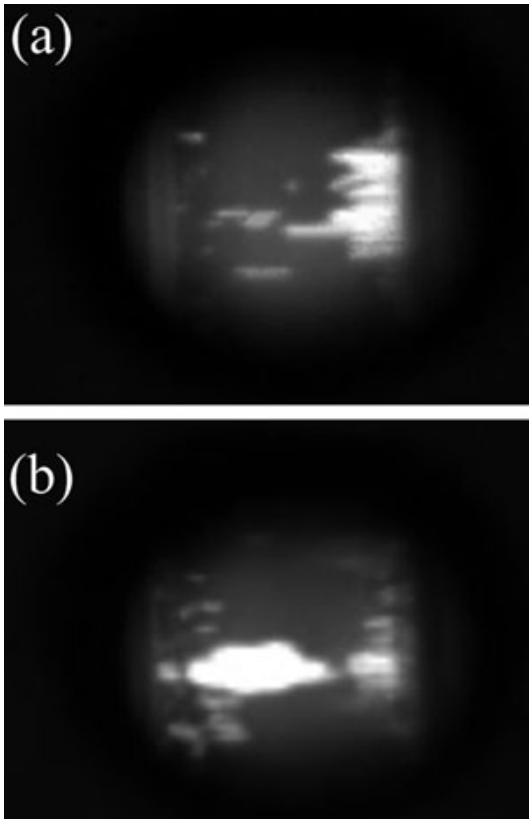

Figure 1: visual inspection of the sandwich' immunoassay (a) without the target IL-8 (b) With the target IL-8.

The pole modulation clock (yellow) and the PMT output signal (magenta) while detecting 0.48 pico-gram IL-8 target are presented in Figure 1(a). The modulation frequency for each pole is at 2 Hz. However, as it was theoretically expected, when the beads pass the laser beam, the PMT detects the fluorescent light and there is a peak in the PMT output voltage. Therefore, the demodulation frequency is at 4 Hz. When the PMT signal and the doubled-modulation clock (at 4 Hz) are fed to the lock in amplifier, the sensitive phase detector detects the synchronization and results with high voltage (see Figure 2).

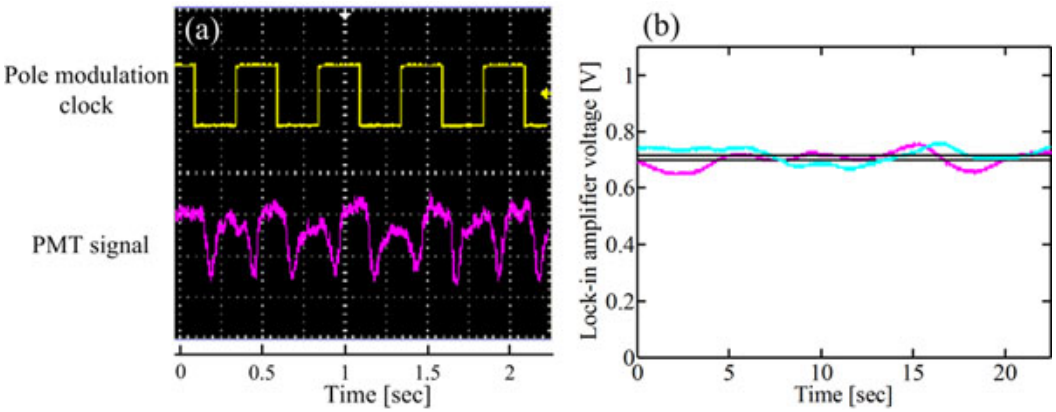

Figure 2: (a) the modulation clock (yellow) and the PMT signal (magenta) when detection 0.48 pico-gram IL-8 target. (b) The resulted lock in amplifier voltage at two different scans.

The lock in amplifier didn't detect any signal when the control sample was tested. This fact, together with the visual difference in aggregation suggests that the MMB system can clearly identify the presence of IL-8 target.

Discussion

In summary, we showed that the MMB system can be used to detect the presence of IL-8 target at low concentrations (0.5 pico-gram is the detection limit of the Bio-Plex Precision Pro cytokine assays [3,4]). The ability of the system is not limited to IL-8 and can be used to detect other

proteins. The advantages of the MMB system are the ability to detect the target rapidly and without any separation or washing step. Thus, it facilitates the detection process and allows the system to be used in field applications.

Acknowledgements

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References

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