Supplementary Information for

Automated Digital Microfluidic Platform for Magnetic Particle-based Immunoassays with Optimization by Design of Experiments

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Detector Characterization

The performance of the PMT in the automated system was evaluated against that of a well-plate reader using an on-chip homogeneous HRP assay with SuperSignal chemiluminescent substrate. Using digital microfluidics, one droplet each of HRP standard solution (0, 26, 52, 130, or 260 μU/mL in DPBS supplemented with 0.05% v/v L64) and chemiluminescent substrate (equal parts of H_2O_2 and Luminol-Enhancer) were dispensed from their respective reservoirs and merged together. The pooled droplet was mixed for 40 seconds, driven to the detection area, and the chemiluminescence was measured after 2 minutes using (a) the integrated PMT with the settings described in the experimental section, or (b) a commercially available well plate reader (Pherastar, BMG Labtech, Cary, NC), with the settings described previously.[1] Three measurements were collected for each HRP concentration, and were averaged and fitted to a linear equation. The limit of detection (LOD) for this assay was the concentration corresponding to the position on the curve of the average signal generated from blank measurements plus three times the standard deviation of the blank measurements.

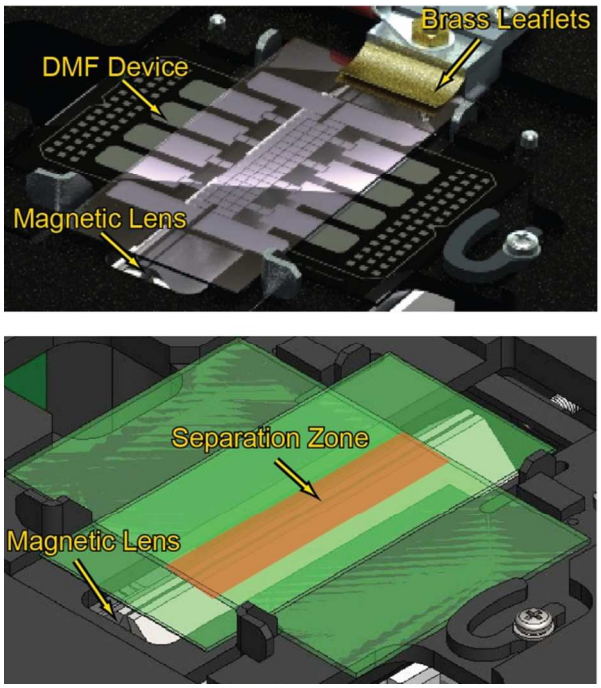

Figure S1: Top: Computer aided design (CAD) rendering of DMF device on device holder with top-plate connected to grounded brass leaflet and magnet is engaged. **Bottom**: Schematic showing magnet position relative to DMF device and the approximate separation zone (in orange) created by the magnetic lens.

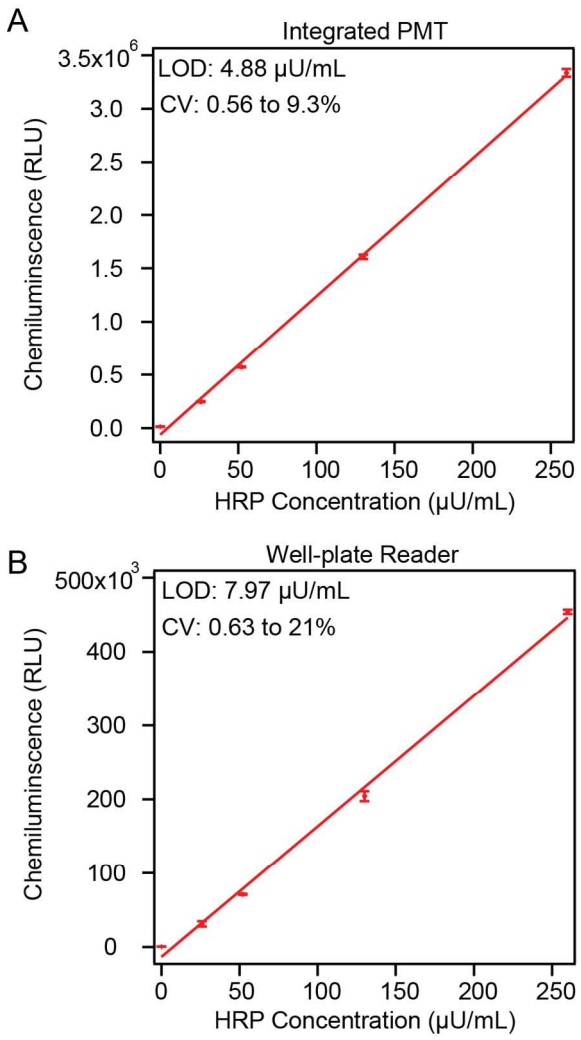

Figure S2: Calibration curves for homogenous HRP assay using H_2O_2/Luminol detected by (**A**) integrated PMT and (**B**) well-plate reader.

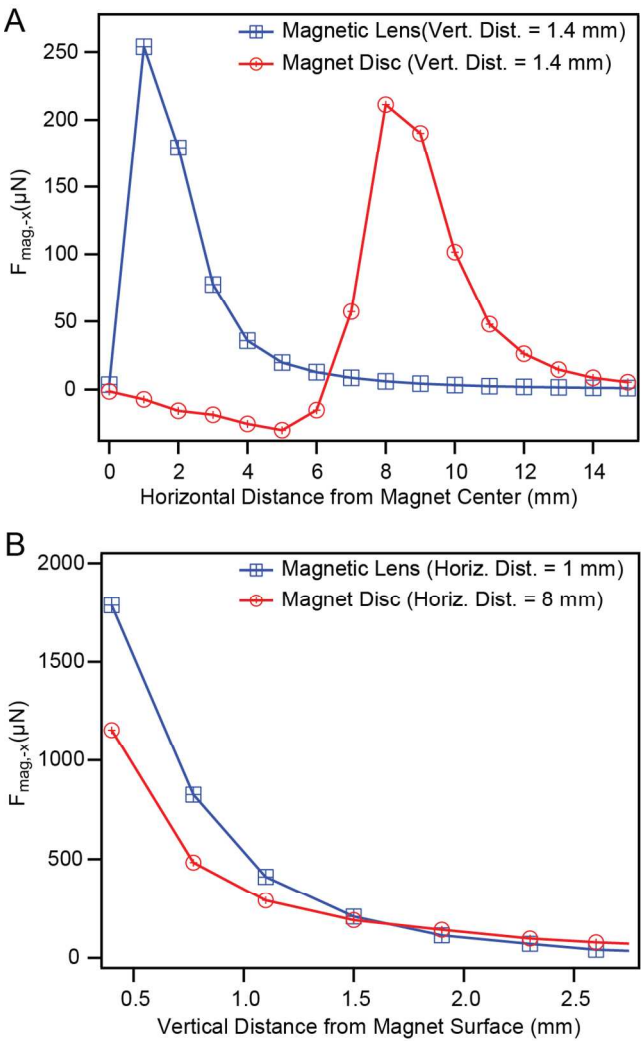

Figure S3: Numerical simulations (blue squares: magnetic lens, red circles: magnetic disc used previously[1]) of force on particle pellet (directed horizontally towards magnet center) as a function of (**A**) horizontal position from magnet center and (**B**) vertical position from magnet surface.

Table S1: Comparison of prototype setup and the new integrated platform

Prototype Setup[1]		New Integrated Platform Reported Here	
Property	Effect	Property	Effect
Manual particle separation using magnet disc	-Two separations per magnet -Labour intensive to manually move magnet -Separation failure caused by undesired magnet positioning	Computer controlled particle separation using magnetic lens	-Eight separations per magnet (easily scaled up) -Automated magnet movement -Precise magnet positioning ensured by motor and sensor
Detection by commercial well-plate reader	-Device position in well-plate is prone to variability (CV: 0.63- 21%), causing reduced sensitivity (LOD: 7.97µU/mL HRP)	Detection by a dedicated PMT integrated in the automation system	-Improved reproducibility (CV: 0.56-9.3% CV) and sensitivity (LOD: 4.88 µU/mL HRP)
Droplet actuation using handheld high voltage probes	-Labour intensive and prone to variability	Droplet actuation using computer controlled 90 pogo pin interface	-Automated and reproducible droplet movement from pre-programmed sequences, allowing continuous mixing and control of larger droplet volume

References

(1) Ng, A. H. C.; Choi, K.; Luoma, R. P.; Robinson, J. M.; Wheeler, A. R. *Anal. Chem.* **2012**, *84*, 8805-8812.