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Detection of membrane-bound and soluble antigens by magnetic levitation

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Abstract

Magnetic levitation is a technique for measuring the density and the magnetic properties of objects suspended in a paramagnetic field. We describe a novel magnetic levitation-based method that can specifically detect cell membrane-bound and soluble antigens by measurable changes in levitation height that result from the formation of antibody-coated bead and antigen complex. We demonstrate our method's ability to sensitively detect an array of membrane-bound and soluble antigens found in blood, including T-cell antigen CD3, eosinophil antigen Siglec-8, red blood cell antigens CD35 and RhD, red blood cell-bound Epstein-Barr viral particles, and soluble IL-6, and validate the results by flow cytometry and immunofluorescence microscopy performed in parallel. Additionally, employing an inexpensive, single lens, manual focus, *wifi*-enabled camera, we extend the portability of our method for its potential use as a point-of-care diagnostic assay.

1. Introduction

The use of smartphones as medical diagnostic devices has emerged as a new imaging and analysis platform. Due to compactness and portability, ease of use, and relative low cost of components, smartphone -enabled, -assisted, or -controlled medical diagnostic devices are gaining popularity over dedicated stand-alone technology, particularly for use in resource-poor settings as point-of-care (POC) devices. Numerous smartphone-based tests are now in

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Conflicts of interest

IG, AS and NS are co-inventors of the MELISSA technique, which is covered by a pending patent.

existence, such as smartphone-controlled ELISA for diagnosis of HIV and syphilis, smartphone microscopy of parasites such as malaria, helminths and tuberculosis, smartphone-based nucleotide detection systems, etc.^{1–7} As a result, World Health Organization and various other non-governmental organizations have increasingly promoted the development and testing of these medical diagnostic tests.^{8, 9}

We have previously described a magnetic levitation-based and smartphone-adapted technique that measures the specific mass and density of individual red blood cells (RBCs) by reporting the levitation height of cells as compared to a set of density reference beads¹⁰. We used the method for rapid separation and identification of old, new, iron-deficient, and sickled red blood cells. Digital image acquisition using a smartphone camera allowed us a rapid, portable and inexpensive generation of quantifiable output using unprocessed or minimally processed blood. The signal measured in our initial approach was generated by a change in the levitation height of cells that was indicative either of their activation status, in case of neutrophils, hemoglobin or water content in the case of red blood cells, or density in the case of circulating tumor cells.^{10–12}

Many detection methods in biomedical sciences rely on translating specific binding events, such as antibody-antigen interactions, into easy-to-detect, linear signals.¹³ These signals are based either on light (fluorescence, bioluminescence, chemiluminescence), color or color shift (colorimetric) of the probes, or changes in the radioactivity, electrical or light scattering properties of the targets. These methods usually require specialized detection instrumentation tuned specifically to the readout method, often involving complex assays that are difficult to employ in resource-poor settings.^{14, 15}

Herein, we introduce a novel magnetic levitation-based method for rapid identification of antigens, named “Magnetic-Linked Immunosorbent Assay”, or MeLISSA. The method allows for detection of cell-bound as well as soluble antigens using as positive signal shift in the levitation height of antigen-positive cells or antibody-coated beads. The new levitation height is easily imaged and analyzed using a microscope or mobile device, such as a smartphone or a tablet. The antigen-specific antibodies are linked, depending on the assay, on one or two reference beads with different densities from each other and from those of the interrogated cells. Following the interaction between the antibody-coated beads and the target antigen, the resulting complexes levitate in between the non-interacting beads and cells, at a height that depends directly on the new density of the bead-cell complex. The presence of the target antigen, therefore, can be specifically detected based solely on the altered density and levitation height the follows the interaction with the detection bead. When combined with a mobile camera with *WiFi* or Bluetooth capability, the device could potentially serve as a substitute for more sophisticated methods, such as flow cytometry, ELISA, or microscopy, as a tool for screening, detection and quantification of various blood antigens and pathogens in resource-poor settings.

2. Materials and Methods

2.1 Antibodies

Anti-human Siglec-8 (Biolegend, San Diego, Ca); Anti-human CD3 (Biolegend, San Diego, Ca); Anti-human CD35 (Bio-Rad, Hercules, CA); Anti-Human RhD and (Abcam, Cambridge, MA); IL-6 ELISA kit (R&D, Minneapolis, MN); anti-human CD47 (BD Biosciences Pharmingen, Franklin Lakes, NJ); anti-EBV (kind gift from Dr. Gordon Ogembo, University of Massachusetts, MA, USA). Mouse IgG, whole molecule (Jackson ImmunoResearch Labs, West Grove, PA, USA)

2.2 Preparation of red blood cells

The study was approved by the Beth Israel Deaconess Medical Center Institutional Review Board and all experiments were carried out in accordance with institutional guidelines on human subjects research. After obtaining informed consent, ten to 20 μL of fresh whole blood were obtained via finger prick from healthy volunteers. Cells were washed twice and re-suspended in HBSS with calcium and magnesium (HBSS^{++}) to a final concentration of 5×10^7 cells/mL, and used within 1 hour.

2.3 Preparation of peripheral blood mononuclear cells and polymorphonuclear cells

Ten mL of whole blood was drawn in syringes prefilled with 2.3 mL of 6% Dextran 500 (Sigma-Aldrich, St. Louis, MO) and 1 mL of 3.2% Sodium Citrate (Sigma-Aldrich, St. Louis, MO). After gently mixing the blood by tapping, the syringe rested in an upright position for 45 minutes. The RBC-free fraction was layered above 15 mL of Ficoll-Paque PREMIUM (density 1.077 g/mL, GE Healthcare Bio-Sciences) in a 50 mL tube and centrifuged at $500 \times g$ for 10 minutes. The peripheral blood mononuclear cells (PBMC) were located at the plasma-Ficoll interface and neutrophils at the bottom of the tube along with intact RBCs. The PBMC layer was collected and set aside for CD3 experiments. The polymorphonuclear cells (PMN) were resuspended in 0.5 mL of HBSS^{--} and transferred to a new 50 mL tube. Contaminating RBCs were lysed using hypotonic lysis. Twenty mL of 0.2% sodium-chloride (NaCl) were added to the PMN pellet for 45 seconds, followed by 20 mL of 1.6% NaCl. The cells were centrifuged at $500 \times g$ for 10 minutes after the lysing and the pellet was resuspended in 1.0 mL of HBSS^{--} . After isolation, PBMC and PMNs were washed twice and re-suspended in HBSS^{++} to a final concentration of 5×10^7 cells/mL.

2.4 Antibodies coupling

Two sterile Eppendorf microcentrifuge tubes were filled with 400 μL PBS and 100 μL goat anti-mouse IgG beads with a density of 1.05 g/mL (goat anti-mouse IgG-coated particles, 5.0% w/v, Spherotech). The control and the antigen specific antibody were added to separate tubes to a final concentration of 2 $\mu\text{g/mL}$ each. The bead-antibody solutions were then incubated for 30 minutes at 37°C and washed twice in 1 mL PBS. The pellet was resuspended in 100 μL PBS and prepared in 40 mM of gadobenate dimeglumine solution (MultiHance, Bracco Diagnostics, Monroe Township, NJ) (500 mM Gadolinium (Gd^{3+}) stock solution). The prepared cell suspensions were incubated for 30 minutes at room temperature, loaded in between the two magnets and viewed under the microscope.

2.5 Detection of soluble antigens

Polymethylmethacrylate microspheres (PMMA beads) were diluted in compound 2-(N-morpholino) ethanesulfonic acid (MES) buffer and incubated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) for 20 minutes at room temperature. The high-density beads were incubated with 1 $\mu\text{g/ml}$ anti-IL-6 (R&D Systems, Minneapolis, MN) capture mAb, and the low density beads with 1 $\mu\text{g/mL}$, anti-IL-6 detection mAb (R&D Systems, Minneapolis, MN) for 20 minutes. Beads were then washed and re-suspended in PBS buffer. Soluble IL-6 (R&D Systems, Minneapolis, MN) diluted in PBS was incubated with the high-density beads for 20 minutes at 37°C at a concentration of 1 pg/mL , 10 pg/mL , 100 pg/mL , and 1000 pg/mL . For the control arm, beads were incubated with PBS. The beads were then washed twice and incubated with the high-density beads for 20 minutes at 37°C. The samples were analyzed after being resuspended in 100 mM Gd^{3+} in HBSS⁺⁺.

2.6 Magnetic levitation

Cells (concentration 5×10^7 cells/mL) suspended in HBSS⁺⁺ or HBSS⁻⁻ (for eosinophil granulocytes only) were mixed with gadolinium solution (Gd^{3+}). Cells were levitated in a final 40 mM Gd solution for all the experiments. The goat anti-mouse IgG (FC) particles mixed with cells were diluted 1:200. The levitation was performed in square capillary tubes with the dimensions, 1×1 mm cross-section, and 5 cm in length (VitroCom, Mountain Lakes, NJ). Critoseal (Fisher Scientific, Pittsburgh, PA) applied to the ends prior to loading to prevent drifting of cells during imaging. N52-grade NdFeb magnets with dimensions 1×5×50 mm (K&J Magnetics, Pipersville, PA) and surface field 0.375 T, placed at 1 mm distance, with same poles facing each other (Fig. 1C) were used for levitation.

2.7 Microscopy setup

We used an Olympus Provis AX-70 microscope mounted on the side as previously described¹⁰, also depicted in Fig. 1.A, to capture images in side-view of the capillary tube. With this setting, the gravitational, magnetic and buoyant forces accountable for the levitation of the cells were appropriately aligned. In order to adjust for the length and the vertical orientation of the capillary tube with respect to the objective and focus while maintaining proper Kohler illumination, micromanipulation X-Y and Z- stages were used (Thorlabs, Newton, NJ)(Fig. 1.B). This setup enabled imaging of levitating cells and beads using medium-high magnification objectives such as 40×0.70 NA and 60×0.70 NA. Most of the images were acquired using the 10×0.30 NA or 20×0.45 NA to allow the recording and analysis of large number of cells in one frame. Images were acquired using a QImaging Retiga EXi Blue cooled CCD camera controlled by Image-Pro Plus 7.0 (Media Cybernetics, Inc., Rockville, MD), precisely 15 minutes after inserting the capillary tube between the magnets.

2.8 Image and statistical analysis

Images captured were cropped to only show beads, cells and intermediate bead-cell complexes (BCC). The 16 bit .tiff files were inverted and binarized, such that black was assigned 0 value, and white 1. The bitmaps were exported to Excel (Microsoft, Redmond,

WA), where an auto-sum was performed for each column of pixels. Waves were created in Igor Pro (Wavemetrics, Portland, OR). Gaussian fits were performed for all the peaks. Next, the area under the Gaussian fit corresponding to the BCC was calculated in the XY table of the Gaussian fit, the X value increased in uniform increments. Since the area under the curve is $\int f(x) dx$, dx (the increment) is constant and $f(x)$ is the Y value, the expression became

(increment) * $\sum_{x_i}^{x_f} f(x)$. The borders x_f and x_i of the peak were at each end of the peak and represented the unbound beads and cells respectively. The distance between the peaks was calculated from the midpoints of each Gaussian fits. The peak shift, indicative of bead: cell or bead: bead complexes, was computed by measuring the difference between each adjacent peaks. Unpaired, *t*-test was used to determine if the difference between various populations was significant.

2.9 Flow cytometry

Cells were incubated for 30 min at 37° with corresponding primary antibodies, washed and re-suspended in HBSS⁺⁺. Cells were then labelled using AlexaFluor 488-conjugated goat anti-mouse IgG (H+L) for 15 mins at room temperature, washed twice, resuspended in buffer, and analyzed on a FACSscan flow cytometer within 10 minutes from the last wash step. Results were then analyzed using Flowjo 10.1r5 software (Tree Star).

2.10 Mini-MeLISSA

We used a Yi Action Camera, Full HD 1080p videos, 16 MP photos, as base for our portable device. The original objective lens of the camera was removed and replaced with a high NA (0.72) aspheric lens (Edmund Optics, Barrington, New Jersey), identical with the lens using for the microscope-attached setup (Figure 1). The frame for the camera was designed in Adobe Illustrator (Adobe Systems Incorporated, San Jose, CA), and parts were cut on a laser cutter (Fig 5.A, B). The magnets used were identical to those used for the microscope setup. The broadcasted images from this Wi-Fi-enabled captured images can be viewed/captured live on any device with a Wi-Fi connection and access to an application store. A separate LED module was installed in front of the camera to prevent the dependence of the image acquisition on the ambient light. Images were captured via the App YI action for this experiment on an iPhone 6.

2.11. Statistical analysis

Results were analyzed using ANOVA test followed by post-hoc analysis (Tukey test). Results were considered significant for $P < 0.05$

3. Results

3.1 Magnetic levitation focuses objects based on density

Magnetic levitation focuses objects with different densities at specific heights following a non-linear relationship between magnetic field density, concentration of gadolinium solution, density of the objects and their paramagnetic properties.¹⁰ In a given setup, objects with negligible magnetic properties will rest at a levitation height that will depend exclusively on the object density. The levitation height of a complex of two beads with

different densities will depend on their resulting average density. In Fig. 1D, the two interacting beads that levitate between the top and bottom rows of beads, have an average density of 1.125 g/mL, due to the fact that both beads have the same diameters (10 μ m), thus the same volumes, and densities of 1.05 and 1.2 g/mL, respectively. Therefore, the final levitation height of the bead complexes is located directly between the top and bottom bead. In addition, the magnetic levitation setup is sensitive enough to detect density gradients within the levitating objects, as seen in Fig. 1D and 1E, where beads with higher density always point downwards and beads with lower density upwards. We used this principle to detect and visualize specific binding events between antibody-coated beads and cell-bound and soluble antigens as a POC-friendly alternative to microscopy, flow cytometry and ELISA.

3.2 Magnetic levitation detects antigens on white blood cells

To evaluate the ability of magnetic levitation to detect different antigens on the surface of white blood cells (WBC), we isolated PBMC and PMC from buffy coat from blood collected from self-declared healthy donors. To test the limits of the system, we chose to detect an abundant WBC, the T-cell, and a relatively scarce WBC, the eosinophil granulocyte. A schematic drawing of the overall approach is shown in Fig. 2A. Primary antibodies were attached to capture antibody-coated beads following the protocols described in the methods. CD3+ T-cells were detected by magnetic levitation using anti-CD3 (Fig. 2B) in the PBMC layer compared to the IgG control (Fig. 2D).

For analysis of the results, we used area under the curve as shown on the graphs located on the right side of each microscopy images. The peak between the beads and cells rows, shown in Fig. 2B, represents the bead-cell complexes. In the control arm of the experiment, PBMC were incubated with control IgG-coated beads and shown no discernable binding between cells and beads. Flow cytometry analysis performed in parallel confirms a second population of cells with a higher fluorescence (MFI=130, 27.4% of population – Fig. 2C) than that of the IgG control (MFI=21.6, 0.29% of population – Fig. 2E). To test the lower detection limit of this approach, we chose a cell type, the eosinophil granulocyte, that makes up only 2–3% of a healthy donors nucleated blood cells, and are therefore considered relatively rare compared to other white blood cells.¹⁶ Fig. 2F, shows the presence of eosinophils bound to anti-Siglec-8-coated beads, and lack of binding in the control arm of the experiment (Fig. 2H). In average, we identified 3 to 4 BCC in one full capillary tube containing beads coated with anti-Siglec-8, while no BCC were seen in the control tube. Moreover, the eosinophil-specific granules are clearly visible, further confirming the specificity of the binding of anti-Siglec-8 coated beads.¹⁷ The flow cytometry results shown in Figs. 2G and I, also confirm the specific identification of eosinophil population by siglec-8 (MFI= 9.75, 3.0% of total event) vs IgG-control (MFI= 7.25, 0.29% of total events).

To test the ability of magnetic levitation-based capture assay to identify specific cells by cell surface markers, we initially used biotin/streptavidin-based system. Similar to the previous reports,¹⁸ we have found that the approach generated significant background, thus rendering the biotin-streptavidin system unsuitable for detection of low abundant events. With the current approach, the efficiency of binding and identifying of eosinophils was around 10–

20%, judging by the number of eosinophils identified compared to their expected number in the PBMC population present in a capillary tube. We believe that manipulation of the cells during loading and the short time of incubation with antibody-coated beads could explain the observed sensitivity of detection. While increase detection efficiency is possible by altering the protocol, for our immediate purpose of testing the suitability of magnetic levitation to detect and visualize specific cells from a PBMC population, the reported sensitivity is sufficient.

3.3 Detection of membrane-bound antigens on red blood cells

We next investigated whether magnetic levitation could identify not just the presence, but also distinguish between various expression levels of cell surface antigens. We chose CR1 (CD35, complement receptor 1), an exclusively human protein, which is expressed on circulating red blood cells (RBC) in a genetically determined fashion, with individuals expressing either 90 CR1 copies/RBC, 500 CR1/RBC, or 1200 CR1/RBC.¹⁹ The individuals expressing high levels of CR1 on RBC membranes, are protected against late-onset Alzheimer's disease and septic shock²⁰, while low CR1 expressers are protected against malaria, as CR1 serves as docking protein for *Plasmodium falciparum*.²¹ We incubated RBCs from previously identified low and high CR1-expressers with beads coated with anti-CR1 or IgG control.^{22–25} The overall approach for this experiment is shown schematically in Fig 3.A. Our results illustrated in Figs. 3B and D show that a high CR1 expression level promoted an increase in the number of RBCs per anti-CR1-coated bead interaction, compared to low CR1 expression. Moreover, in the high CR1/RBC samples, the BCC were not only larger, but also levitated at a lower level when compared to the BCC from low CR1/RBC expressers, signifying higher densities of these complexes due to the presence of more RBCs/BCC. The peaks, representing the average distance between the capture beads and unbound beads confirm the observed results, with a Y-peak value of 88.8 [83.0;95.5] for high CR1, and only 58.5 [58.0;59.0] with a $P=0.002$ and a 95%-confidence interval [18.2;42.3]. We next compared the magnetic levitation results to flow cytometry, a method currently accepted and widely used for detection of expression levels of cell surface markers. Results in Fig. 3C and 3E, show the relative shifts in fluorescence for low and high CR1 expression as detected by flow, that followed the same pattern as the one observed by MeLISSA.

Rapid detection of circulating RBCs with unique antigenic signatures, such as Rh(+) in Rh(−) pregnant woman, are beneficial in POC settings for early detection of high risk pregnancies.²⁶ We therefore tested the sensitivity of our approach to identify Rh(+) RBCs spiked in an Rh(−) blood sample. RBCs from a known Rh+ healthy donor were mixed with RBC from a Rh(−) donor at a 1:100 ratio for 5 minutes and then incubated with either anti-RhD-coated or IgG control beads. The results are shown in Figs. 3F and 3G, where the histogram profile of the control only shows two peaks, one corresponding to the beads and other to RBCs, in the experimental arm there was a significant population in between the two reference peaks, indicative of BCC formation. Although the number of RhD molecules per RBC is in the range of 10,000 to 30,000, significantly more than the number of CR1 from high expressers, the number of Rh(+) RBCs captured was lower, and the size of BCC smaller compared to those seen with RBC from high CR1 expressers. One explanation for

this apparent discrepancy could be that the number of available Rh (+) RBCs for cell-bead interaction in the spiked samples was low, thus preventing the formation of large BCC. Therefore, we next tested whether a large number of antigenic determinants on the cell surface would negatively impact the imaging or analysis efficiency. We compared detection of CD47, a critical molecule involved in blocking the phagocytosis of RBCs during the immune transfer process by interacting with its cognate receptor on tissue macrophages, SIRP- α .²⁷ The expression level of CD47 on human circulating RBCs is approximately 50,000 molecules/RBC.²⁷ Fig. 3H and 3I show that the highly abundant CD47 generate large BCC complexes, which could be challenging from a data processing and analysis standpoint. Therefore, lowering of the concentration of detection antibody immobilized on capture beads, and/or decreasing the number of target cells would lower the binding efficiency between the beads and the cells, thus overcoming the aforementioned limitations. As it is with other detection methods such as, ELISA or flow cytometry, it is necessary to have previous knowledge of the antigen density, presentation, and availability on the cell membrane, as well as specific antibody affinity.

3.4 Detection of cell-bound viruses using MeLISSA

In humans, circulating RBCs continuously clear blood pathogens and foreign particles by transferring them to the reticulo-endothelial system through a process called immune-adherence clearance.²⁸ During early stages of an infection, most viral (HIV, EBV, Dengue, Zika, Chikungunya, HAV, HBV s.a) and bacterial pathogens become opsonized with complement components C3b, C4b, MBL, and then circulate for days bound to RBCs, using RBC CR1 as the docking protein.^{29–31} We tested whether the approach used in the experiments delineated above (Fig. 4A) would allow the detection of RBC CR1-bound virions. Figs. 4B and 4C show that incubation of RBCs isolated from an Epstein-Barr virus (EBV) positive donor with anti-EBV beads, formed BCC, whereas incubation of RBCs isolated from non-infected donors with the same beads generated no detectable binding events. We confirmed these results both by using immuno-fluorescence microscopy and flow cytometry. We labelled the EBV genome using Syto16, a nucleic acid specific fluorescent dye (Ex 488nm/Em 519nm), and label RBC CR1 by incubating RBCs with an anti-CR1 reporter antibody followed by Alexa 594-labeled - antibody. Fig. 4D shows a differential interference contrast (DIC)/Nomarski image of one of the RBC fields investigated, with the inset focusing on the cellular location of CR1 molecules (red), EBV virions (green) and co-localization between the CR1 and EBV (yellow). The lack of complete co-localization between CR1 and EBV signals may be due to the fact that some circulating EBV virions lack genetic material (empty virion) thus being Syto16 negative, while some CR1 molecules may already be occupied by immune complexes, therefore inaccessible to EBV virions, but accessible to the reporter anti-CR1 antibody. The flow cytometry (Fig. 4E) analysis of RBCs isolated from a EBV positive donor show small shift in fluorescence signal (MFI= 14.7) compared to control IgG (MFI= 7.4) confirming the presence of EBV on the circulating RBCs. Our results demonstrate that MeLISSA can readily detect RBC bound virus particles, simply by incubating antibody-coated beads with whole blood and detecting the presence of newly formed BCC.

3.5 Detection of soluble antigens

We next tested the ability of MELISSA to detect soluble antigens using an approach similar to sandwich ELISA³²(Fig. 5A). We chose IL-6 as target molecule as its plasma concentration in healthy individuals is low, around 1.4 pg/mL, and increases several logs in diseases such as sepsis and myocardial infarction.^{33–37} For this approach, a positive signal is generated when the antibody-coated high-density beads (1.2 g/mL) bind to the antibody-coated low-density beads (1.05 g/mL) through a bridge formed by the targeted antigen. The new bead-bead complex therefore levitates in-between the capture and detection beads due to the change in its density, now intermediate of the two parent beads. We coated the beads with a set of anti-IL-6 monoclonal antibody recognizing two non-overlapping epitopes in the IL-6 molecule. Our cumulative results in Fig. 5B show that while the control sample lacking IL-6, contained few bead-bead complexes (3.32 ± 1.7 /field of view,) there were increasing number of these complexes that matched the increase in the concentration of soluble IL-6 in the experimental arm: 4.15 for 1 ng/mL ($p=0.103$, ns), 8.68 for 10 pg/mL ($p<0.05$); 25.6 for 100 ng/mL ($p<0.001$) and 14.2 for 1000 pg/mL ($p<0.001$). The decrease in number of bead-bead complexes observed at the highest concentration is likely due to the binding of IL-6 molecules to both the capture and detection antibodies thus preventing the formation of the antibody-antigen-antibody sandwich (the high-dose hook effect)³⁸, a phenomenon commonly seen in immunoassays. The comparative results obtained from a standard IL-6 ELISA (R&D) when testing the same concentration range is shown in supplemental sFig. 1. Our data show that similar to the MeLISSA approach, detection of 1pg/mL of IL-6 was outside the sensitivity range of the ELISA (buffer OD = 0.056 ± 0.001 , 1pg/mL IL-6 OD, 0.062 ± 0.001), with similar sensitivity for the rest of the tested concentrations.³² The Hook effect is not typically seen with such two-step assays if extensive washing is possible to remove the excess of antigen before incubating with the detection antibody. Such a step is not possible in one-step MeLISSA.

3.6 Mini-MeLISSA produces images similar to those obtained using a standard microscope

There is an immediate need for low-cost POC assays in resource-poor settings that are easy to operate and inexpensive to maintain. While employing a cell phone would be very advantageous for imaging, data collection and analysis, the adaptor necessary to connect the diagnostic device to the phone would likely be brand and model specific. We here introduce the Mini-MeLISSA, an inexpensive (less than USD 100) camera that is compact and sturdy, captures images with a simple manual focus comparable to the quality of a laboratory microscope, and broadcasts it to any smartphone or tablet in the vicinity from its built-in WiFi. Fig. 6 shows the device and its parts (Fig. 6A and 6B) and the image broadcasted to a smartphone (Figure 6.C) using its own WiFi signal. We used anti-CR1 beads to demonstrate the resolution of YI-camera, which, using a single lens with an NA of 0.71, is able to detect BCC as well as the number of RBCs and detection beads for each cluster (insets). Mini-MeLISSA extends the portability of our method of antigen detection, obviating the need for a laboratory microscope, allowing for direct use of MeLISSA technique in the field.

4. Conclusions

We have developed a magnetic levitation-based method for measurement of density of living cells and demonstrated its utility in detecting cell membrane-bound and soluble antigens using antibody-coated beads. The basis of this approach is translation of antigen-antibody binding events that lead to the formation of bead-cell complexes and alteration of the levitation height. This approach does not require any background subtraction operations and can provide results in as little as five minutes. By using capture beads of different colors, shapes or densities, this approach can be multiplexed to interrogate a blood sample for more than one virus or one soluble or membrane-bound antigen. This assay can be used both in laboratory as well as at the bed-side, providing binary as well as quantitative measurements without the need for sophisticated scientific equipment or dedicated training. Further studies are needed in order to determine a suitable range for specific soluble and membrane-bound antigens when using magnetic levitation-based detection methods, and to validate this technology at the point-of-care.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Magnetic levitation setup and the concept of average density

A. An Olympus Provis AX70 microscope was placed on its side to capture the images of the capillary tube. The microscope has three micro-manipulators, allowing for both horizontal and vertical search of the capillary tube. **B.** Magnetic levitation setup showing the top and bottom magnet with the capillary tube mounted on an adjustable stage. **C.** Schematic of the capillary tube mounted between two magnets. **D.** An example of the detection of binding events with beads of different densities spiked with minimum number of cells. Upon cell-bead interaction, a bead-bead complex levitates between the high and low density beads. **E.** Schematic drawing of D.

Figure 2. Detection of Eosinophil and T cell-specific antigens by magnetic levitation

A. Schematic drawing of the formation of a bead-cell complex. **B.** Magnetic levitation of PBMCs with anti-CD3 antibody-coated beads showing formation of bead-CD3 positive T-cell complexes. The panel on the right shows the Gaussian fit of the whole image from the bitmap analysis (applies for all magnetic levitation images). **C.** Flow cytometry of the anti-CD3 antibody treated PBMCs identifying CD3 positive T-cells as approximately 27.4% of total cells. **D.** Magnetic levitation of PBMCs with IgG-control beads showing the absence of bead-cell complexes. **E.** Flow cytometry of PBMCs with IgG control **F.** Magnetic levitation of PMNs with anti-Siglec-8 antibody-coated identifying eosinophils. Inset showing

magnified image of a bead-cell complex. **G.** Flow cytometry of the anti-Siglec-8-treated PMNs identifying eosinophils as approximately 3.0% of total cells. **H.** Magnetic levitation of PMNs with IgG-control beads showing the absence of bead-cell complexes. **I.** Flow cytometry of PMNs with IgG control. The experiments were performed twice.

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Figure 3. Detection of red cell membrane antigens by magnetic levitation

A. Schematic drawing of formation of an antibody-coated bead-RBC complex. **B–E.** Magnetic levitation of RBCs with anti-CR1-coated beads from a low-CR1 expresser (**B**) compared to a high-CR1 expresser (**D**) with bitmap analyses differentiating the low and high CR1 expression levels. Flow cytometry of RBCs with anti-CR1 antibody performed in parallel confirms low (**C**) and high (**E**) CR1 expression levels. **F.** Magnetic levitation of Rh(–) RBCs spiked 1:100 with Rh(+) cells with anti-RhD antibody-coated beads showing the formation of bead-Rh(+) cell complexes. **G.** Magnetic levitation of Rh(+) RBCs with IgG control beads showing absence of bead-cell complexes **H.** Magnetic levitation of RBCs

with anti-CD47-coated beads showing formation of bead cell complex. **I.** Magnetic levitation of RBCs with IgG control beads showing the absence of bead-cell complexes. The experiments were performed 3 times with two different sets of CR1 high and low expressers.

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Figure 4. Detection of RBC-bound Epstein-Barr viral particles

A. Schematic drawing of detection of EBV bound to RBC antigen CR1 with anti-EBV antibody-coated beads. **B.** Magnetic levitation of RBCs with IgG control beads showing the absence of bead cell complex. **C.** Magnetic levitation of RBCs with anti-EBV antibody-coated beads showing the formation of bead cell complex. Inset showing magnified image of this interaction. **D.** The left frame shows bright field image of RBCs. The right frame shows magnified image of the square (upper left; DIC technique), TxRed-labeled CR1 (upper right), FITC-labeled EBV (lower left) and merged image (lower right). Arrows show co-localization (yellow) of CR1 and EBV. The white line represents 25 μM . **E.** Flow cytometry of EBV-negative (red) and EBV-positive (blue) RBCs performed in parallel. The experiment was performed four times.

Figure 5. Detection of soluble antigens by magnetic levitation

A. Schematic drawing of the interaction between a soluble antigen with detection antibody-coated 1.05 g/mL goat anti-mouse bead and capture antibody-coated 1.2 g/mL PMMA bead.

B. Line graph showing the dose-response relationship between IL-6 concentration and the number of bead-bead complexes. **C. Left panel:** Control arm showing background binding of the capture and detection beads in the absence of IL-6. **Right panel:** Experimental arm showing the formation of bead-bead complexes in the presence of 10 pg/mL IL-6. The experiment was performed twice.

Figure 6. Detection of RBC CR1 antigen using Mini-MeLISSA

A. The compact Mini- MeLISSA **B. Left panel:** Deconstructed Mini-MeLISSA showing the two module pieces. The left is the light module that has a built-in 2.5 V LED light. The right is the camera shell module, housing the WiFi-enabled camera and the magnetic levitation setup with the two magnets and room for a squared 1×1 mm capillary tube, as described above. The specialized lens is placed behind the magnets with a fixed focus. **Right:** Upper-side-view of the two-piece module showing the light module to the left and the camera on the right. **C.** A cell phone image of anti-CR1 antibody-coated bead and RBC complexes from a high CR1 expresser taken with Mini-MeLISSA. Magnified image below shows bead cell complexes further enhanced to the right. Magnetic levitation of RBCs from the same donor with IgG control beads shows the absence of bead-cell complexes.